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ORIGINS AND SHORT-TERM SEDIMENTARY FATE OF GLOBALLY DISTRIBUTED BIOLOGICAL MARKER HYDROCARBONS

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ORIGINS AND SHORT-TERM SEDIMENTARY FATE OF

GLOBALLY DISTRIBUTED BIOLOGICAL MARKER

HYDROCARBONS

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TO MY FAMILY

ORIGINS AND SHORT-TERM SEDIMENTARY FATE OF GLOBALLY DISTRIBUTED BIOLOGICAL MARKER HYDROCARBONS

by

Simon John Hird

ABSTRACT

Nearly thirty C_{20}, C_{25} and C_{30} highly branched isoprenoid (HBI) hydrocarbons have been detected, sometimes in high concentrations, in recent freshwater, estuarine, coastal and hypersaline sediments, and water column particulate matter from numerous locations worldwide. The parent structures have been proved but only a few of the double bond positions have been established. The assignment of C_{21}, C_{22} and C_{26} homologues and other C_{20} and C_{25} isomers, remains tentative. A wide body of evidence suggests that the compounds are biogenic in origin, with algae and possibly bacteria the most likely source organisms. A few of the compounds have been identified in field samples of algae but none have been reported in laboratory cultured biota.

The alkenes with more than two double bonds appear to be rapidly removed from the hydrocarbon fraction in most sediments, whereas the alkanes and monoenes seem to be more resistant to biodegradation and hence occur in some more ancient sediments and oils. There is evidence that some of the alkenes react rapidly with sulphur to form either S-containing HBI heterocycles or become bound within macromolecular aggregates both found in sediments and some oils. The compounds, both as hydrocarbons and S-containing analogues, may prove useful environmental indicators once the sources and exact structures of more of them have been established.

In the literature the structural elucidation of C_{25} and C_{30} HBI alkenes has been based mainly on the analysis of their hydrogenation products. However, some authors concluded that the alkenes are cyclic since some could not be fully hydrogenated. The structure of a C_{25} HBI diene was proven to be acyclic by hydrogenation studies and GC and GC-MS analyses which showed the HBI compound to be fully saturated.

The isolation and characterisation of synthetic alkenes resulted in the assignment, or partial assignment, of structures to four C_{20}, six C_{25} and four C_{30} monoenes. The formation of novel monoenes via isomerisation reactions has also been achieved. The compounds form a valuable database of chromatographic and spectroscopic information for the assignment of sedimentary alkenes but the importance of isolation and micro-ozonolysis has been emphasised.

Synthetic HBI alkenes were used to assign structures and partial structures to naturally occurring HBI hydrocarbons in three sediments. Other monoenes (both with methylene double bonds) were isolated from the sediments and characterised using spectroscopic and micro-ozonolysis data.

The widespread occurrence of C_{20} and C_{25} HBI hydrocarbons in Tamar sediments and associated algae (macrophytes and diatoms), the large variation in isotopic composition evident for the C_{20} monoene, and the seasonal sedimentary distribution all suggest two possible sources for the HBI hydrocarbons; microalgae and/or heterotrophic bacteria.

Investigation of the distribution of hydrocarbons from the Peru upwelling area confirmed the rapid decrease in concentration of C_{25} HBI alkenes with depth. A mixture of C_{25} HBI monoenes was successfully incorporated into melanoidins but not detected in the humic acid pyrolysate which implied that incorporation of HBI alkenes into accreting humic substances was not a major mechanism of diagenesis of HBI alkenes.

This study has extended present knowledge of the structures of HBI monoenes and has suggested two possible biological sources. There is still much to be learned about HBI polyenes and the subject is proving to be a fruitful area for further research into biomarker potential. Some possible future approaches are suggested.

Parts of this work have been published [Rowland et al. (1990), Org. Geochem. 15: 215-218; Hird et al. (1992), Mar. Chem. 37: 117-129].

ACKNOWLEDGEMENTS

PREFACE

This thesis is presented in eight chapters. Each chapter is divided into subsections (*e.g.* 1.1, 1.2 ... *etc.*). Further subdivisions are similarly numbered sequentially and, where necessary, by the use of italics. Compound structures are assigned unique numbers (*e.g.* **1**, **2** ... *etc.*), generally in chronological order of appearance in the text and are presented at the end of each chapter.

Chapter 1 provides an introduction and general background to the research described herein. Chapters 2-6 describe the research into the characterisation, distribution and fate of highly branched isoprenoid (HBI) hydrocarbons. Suggestions for further work are given in Chapter 7, whereas Chapter 8 covers the experimental and analytical procedures used.

ABBREVIATIONS USED IN THE TEXT

HBI	highly branched isoprenoid
DCM	dichloromethane
Na_2SO_4	sodium sulphate
$LiAlH_4$	lithium aluminium hydride
Mg	magnesium
$CeCl_3.7H_2O$	cerium chloride heptahydrate
Et_2O	diethyl ether
THF	tetrahydrofuran
NaOH	sodium hydroxide
$AgNO_3$	silver nitrate
KCl	potassium chloride
$Na_4P_2O_7$	sodium pyrophosphate
DMF	dimethylformamide
CS_2	carbon disulphide
BuLi	butyllithium
TsOH	toluene-p-sulphonic acid
HOAc	acetic acid
$NaHCO_3$	sodium hydrogen carbonate
$PtO_2.H_2O$	platinum (IV) oxide monohydrate
TOC	total organic carbon
ODP	Ocean Drilling Project
TLC	thin layer chromatography
HPLC	high performance liquid chromatography
GC	gas chromatography
MS	mass spectrometry
PY	pyrolysis
NMR	nuclear magnetic resonance
FTIR	fourier transform infra red spectroscopy
RI	retention index
LRMS	low resolution mass spectrometry
IRMS	isotope ratio mass spectrometry
McL	McLafferty rearrangement

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CHAPTER ONE

INTRODUCTION

The occurrence of widely distributed acyclic isoprenoid hydrocarbons (C_{20}, C_{25} and C_{30}) with highly branched structures, in sediments and biota is reviewed. The compounds occur as alkanes and alkenes with from one to at least five double bonds in young aquatic (both marine and lacustrine) sediments from many parts of the globe (e.g. Peru, Antarctica, Gulf of Suez, North Sea and Atlantic). Sometimes found in high concentrations in surface sediments (e.g. 40 μgg^{-1}) the compounds rapidly disappear in older sediments, possibly due to biodegradation and reaction with sedimentary sulphur. The sources of this group of hydrocarbons remains largely unknown, though evidence points to algae (possibly diatoms) as one possibility. The identification of related sulphur-containing compounds promises to extend the number of reports of these compounds still further, and to increase their importance as environmental biological markers.

1.1 INTRODUCTION

One of the consequences of the large number of studies of hydrocarbon pollution in sediments has been the coincidental reporting of nearly thirty, non-pollutant, highly branched isoprenoid (HBI) alkanes and alkenes.

The occurrence, identification and distribution of HBI in sediments and biota has been comprehensively reviewed by Rowland and Robson (1990) and the present study therefore summarises the main findings of that publication and reviews subsequent related research.

Such hydrocarbons have been variously termed "cycloalkenes" (*e.g.* Farrington *et al.*, 1977), "multibranched olefins" (*e.g.* Albaigés *et al.*, 1984ab), "multibranched acyclic hydrocarbons" (*e.g.* Bates *et al.*, 1984; Prahl and Carpenter, 1984), "three-pronged C_{20} alkane" (Mackenzie, 1984), "highly branched hydrocarbons" (*e.g.* Rowland *et al.*, 1985), "7-isopranyl-farnesenes" (Robson and Rowland, 1986; 1988ab), "the GX series" (Comet and Eglinton, 1987; Thomas, 1990), and "highly branched isoprenoids" (HBI) (*e.g.* Sinninghe Damsté *et al.*, 1989a). They occur as C_{20}, C_{25} and C_{30} alkanes and alkenes with from one to five double bonds and have parent structures 1-3. The saturated compounds have been unambiguously identified by synthesis (Yon *et al.*, 1982; Robson and Rowland, 1986; 1988a) and mass spectra are shown in Figure 1.1. The carbon skeletons of the alkenes have been inferred on the basis of hydrogenation to the parent alkanes (*e.g.* Gearing *et al.*, 1976; Barrick *et al.*, 1980; Prahl *et al.*, 1980).
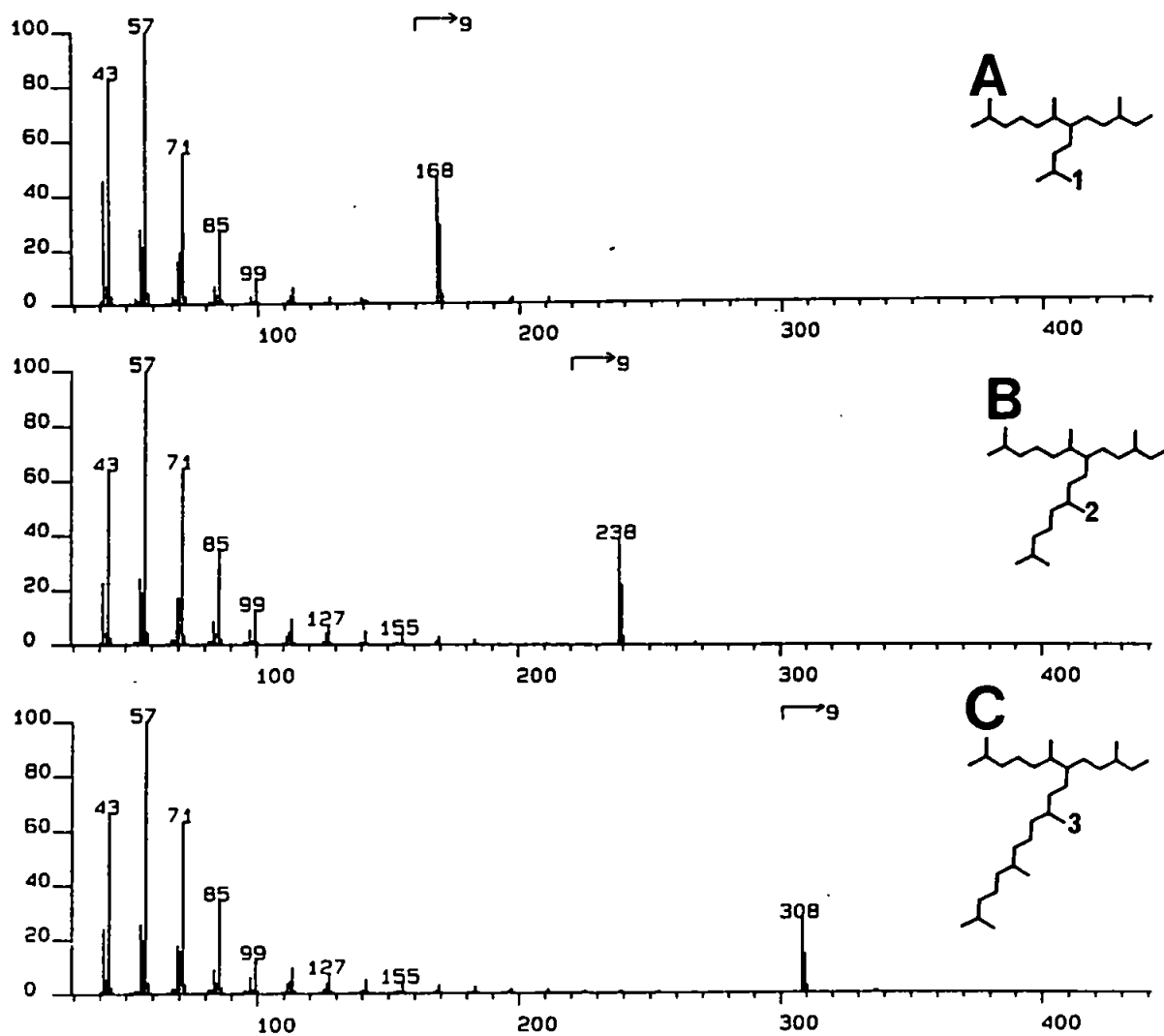
FIGURE 1.1 ELECTRON IMPACT (EI) MASS SPECTRA (40 eV, 250°C):
(A) 2,6,10-trimethyl-7-(3'-methylbutyl)dodecane (*1*)
(B) 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadecane (*2*)
(C) 2,6,10,14,18-pentamethyl-7-(3'-methylpentyl)nonadecane (*3*)
Kratos MS25 double focusing magnetic sector mass spectrometer
(Robson and Rowland, 1986)

Those of the sulphur-containing analogues, HBI thiolanes and thiophenes were assigned by Raney nickel desulphurisation and subsequent hydrogenation to yield HBI alkanes 1-3 and the structures of the some of the original thiolanes and thiophenes have been confirmed by synthesis (Sinninghe Damsté *et al.*, 1989a; Kohnen *et al.*, 1990a).

HBI have been identified in young aquatic (lacustrine, marine and hypersaline) sediments from all over the globe. In many cases, the compounds are the most abundant hydrocarbons reported, especially in unpolluted coastal and estuarine sediments. In total, nearly thirty HBI have been detected (Tables 1.1 and 1.2) sometimes in high surface concentrations (*e.g.* 40 μgg^{-1} dry sediment; Smith *et al.*, 1983a). However, HBI concentrations often decrease with increasing sediment depth. Biodegradation (Rowland *et al.*, 1985; Robson and Rowland, 1988; Volkman *et al.*, 1983), accretion into humic substances during diagenesis (Volkman *et al.*, 1983) and intra- or intermolecular incorporation of inorganic sedimentary sulphur to form alkylthiophenes, alkylthiolanes and sulphur-containing high molecular weight substances (*e.g.* Sinninghe Damsté *et al.*, 1987; 1988ab; 1989ab; 1990a; Kohnen *et al.*, 1990ab; 1991ab; 1992) have all been offered as explanations for this decrease.

Most identifications of the HBI compounds have been made by gas chromatography (GC) and/or gas chromatography mass spectrometry (GC-MS) and Figure 1.2 shows typical gas chromatographic retention positions. Alkanes 1-3 have retention indices (GC RI) of 1707_{ov_1}, 2107_{ov_1} and 2524_{ov_1} respectively and the related alkenes have GC RI close to n-C_{17}, n-C_{21} and n-C_{25} alkanes respectively on nonpolar GC stationary phases.

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TABLE 1.1 CONCENTRATIONS OF HBI HYDROCARBONS IN RECENT SEDIMENTS (from Rowland and Robson, 1990)

Location	Type of environment	Major HBI hydrocarbon	Surface sediment concentrations	Reference
Buzzards Bay, USA	Recent/estuarine	c25:2:2; 2075_{FFAP}[a]	0.6[b] (50% of total hydrocarbons)	Farrington et al., 1977
Rhode Island Sound, USA	Recent/estuarine	c25:2:2; 2080_{OVI}[a]	1.55[b]	Boehm and Quinn, 1978
Mid Narrangansett Bay	Recent/estuarine	c25:2:2[a]	1.32[b]	Wade and Quinn, 1979
Narrangansett Bay, USA	Recent/estuarine	c25:2:2[a]	4.2[c]	Hurtt and Quinn, 1979
Southern Californian Bight, USA	Recent/marine	$C_{25}H_{48}$; 2074_{OVI}[a] (probably br25:2)	0.14[b]	Venkatesan et al., 1980
Puget Sound, Washington State, USA	Recent/landlocked marine	br25:3; 2090_{SP2100}	14.0, 8.6[c]	Barrick et al., 1980
Narrangansett Bay, USA	Recent/estuarine	c25:2:2; 2097_{SE30}[a]	87.2[c]	Requejo and Quinn, 1983a
Peru continental shelf (Upwelling region)	Recent/marine	br25:3; 2092_{SE52}	10.1 (surface)[b] 0.41 (16 cm depth)	Volkman et al., 1983
Peru continental shelf (Upwelling region)	Recent/marine	br25:4	40[b]	Smith et al., 1983a
Pettaquamscutt River, Rhode Island, USA	Recent/estuarine	c30:2:2[a]	1.9 (surface)[b] 0.1 (30 cm depth)	Requejo et al., 1984
Alfacs Bay, Spain	Recent/marine	br25:1; 2112_{OVI}	15.0 ngl⁻¹ particulate matter	Albaigés et al., 1984b
Washington coastal sediments, USA	Recent/marine	br25:3	27.0[c]	Prahl and Carpenter, 1984
Shark Bay, Australia	Recent/marine/ hypersaline	br25:1; 2112_{MS}	0.16[b] (18% total hydrocarbons)	Dunlop and Jefferies, 1985
Round Swamp, Narrangansett Bay, USA	Recent/salt marsh	br25:3; 2091_{SE30}	3.2[b]	Requejo and Quinn, 1985
Great Barrier Reef,	Recent/marine	$C_{25}H_{48}$	0.0005[b]	Coates et al., 1986

Key: [a] cyclic designation made in original reference, [b] μgg⁻¹ dry sediment, [c] μgg⁻¹ organic carbon)
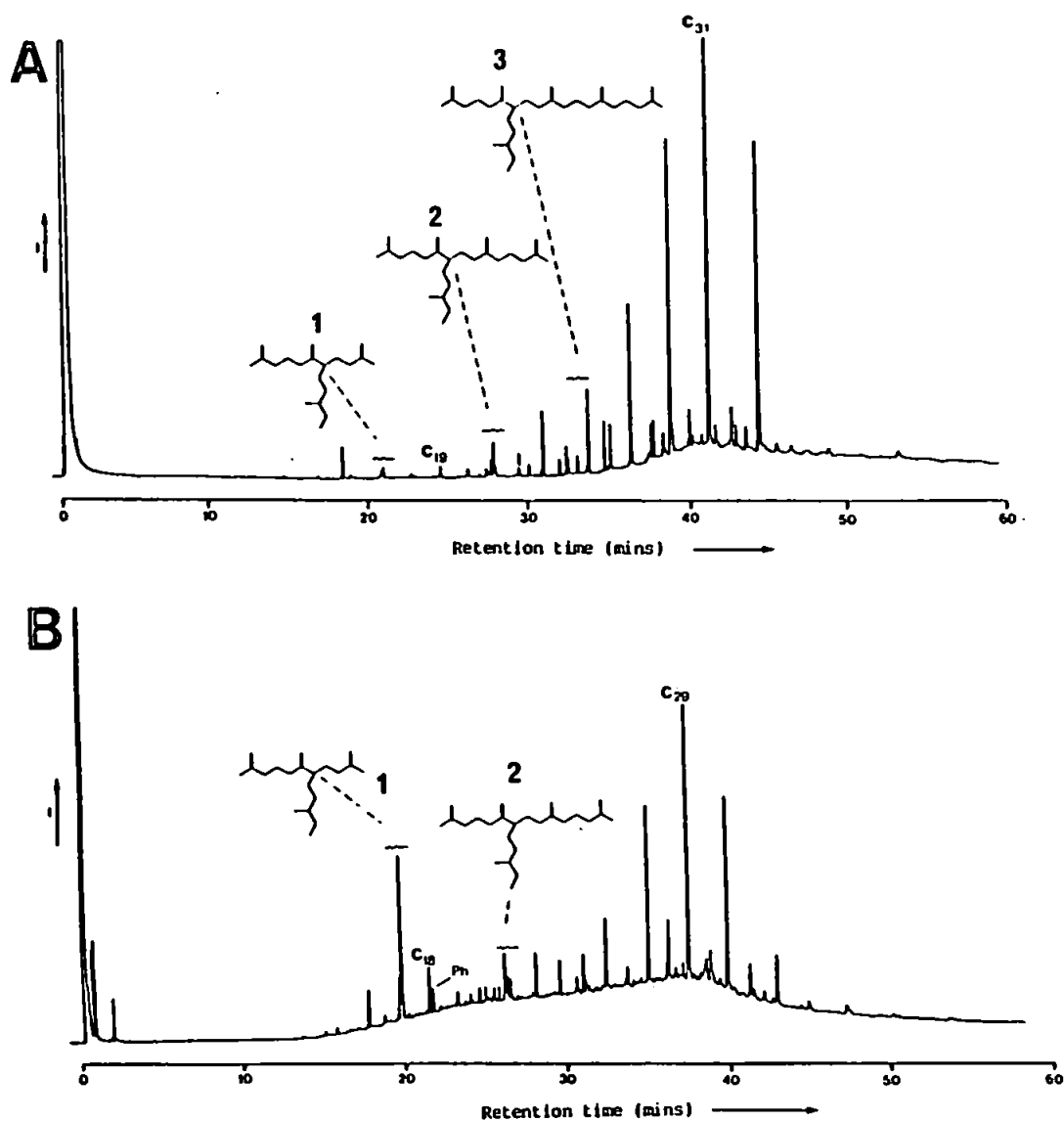
FIGURE 1.2 GAS CHROMATOGRAMS SHOWING TYPICAL SEDIMENTARY DISTRIBUTIONS AND RETENTION POSITIONS OF C_{20}, C_{25} AND C_{30} HBI HYDROCARBONS

(A) Gluss Voe, Shetland Isles
(B) Tamar Estuary, UK

Numbers refer to carbon chain length of n-alkanes. Peaks represent hydrocarbons with carbon skeletons of 2,6,10-trimethyl-7-(3'-trimethylbutyl)dodecane (1), 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadecane (2) and 2,6,10,14,18-pentamethyl-7-(3'-methylpentyl)nonadecane (3), respectively. GC conditions: Carlo Erba 4160, 25m x 0.32mm i.d. OV1 (GC^2), 40-80°C @ 10°Cmin^{-1}, 80-290°C @ 6°Cmin^{-1}, H$_2$ carrier gas (Rowland and Robson, 1990).

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For brevity, unknown compounds will be referred to herein by GC RI and by denoting suspected branched compounds by *br* and cyclic compounds by *c*, in addition to the carbon number and degrees of unsaturation, after the method of Barrick *et al.* (1980). Thus, an acyclic branched diene with a retention index of 2082 on OV-1 stationary phase is referred to as br25:2; 2082_{OV1} and a suspected bicyclic triene as a c30:3:2.

A wide body of evidence suggests that the compounds are biogenic in origin, with algae and possibly bacteria the most likely source organisms but nothing definite is known (Rowland and Robson, 1990) and is an obvious area for future research.

1.2 THE OCCURRENCE OF C_{20} HBI HYDROCARBONS IN MARINE AND LACUSTRINE SEDIMENTS.

In addition to the numerous reports of C_{20} hydrocarbons summarised by Rowland and Robson (1990; Table 1.2), Smith and workers (1986) identified the highly branched C_{20} alkane, 2,6,10-trimethyl-7-(3'-methylbutyl)dodecane (br20:0, 1) in small amounts (15-20 ngg^{-1}) in core sections from a Recent Sapropel from the Hellenic Outer Ridge, Eastern Mediterranean Sea. This assignment was made on the basis of comparison of GC relative retention time and mass spectral data with that of the authentic compound synthesised by Yon (1982).

A related C_{20} HBI monoene (br20:1; no RI given) was reported in surface sediments of the Peru Upwelling Area at 15°S (Farrington *et al.*, 1988). This alkene dominated the trace amounts of n-C_{17}, pristane and other compounds in the alkane-alkene fraction in the n-C_{15} to n-C_{20} molecular weight range. The concentration of

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br20:1 was reported as within the μgg^{-1} dry weight mass range at the surface section of the two cores examined as compared to 10-100 ngg^{-1} in surface sediments previously reported (Barrick *et al.*, 1980, Bayona *et al.*, 1983, Dunlop and Jefferies, 1985). The mass spectrum was said to be identical to that published by Rowland *et al.* (1985) for the br20:1 alkene occurring in *Enteromorpha*, a green alga. Only trace amounts of 1 were reported. This is in contrast to other reports where the unsaturated compound is usually a minor component compared to 1, n-C_{17} or n-$C_{17:1}$.

Concentrations ranging from 1.6 to 10.9 ngg^{-1} dry weight of a C_{20} HBI monoene (br20:1; no GC RI given) were observed by Volkman *et al.* (1988) in estuarine sediments from the D'Entrecasteaux Channel near Hobart, Australia. The alkene was identified by GC-MS (M^+ at m/z 280 and major fragment ions at m/z 210, 196, 140 and 126).

Alkane 1 has more recently been reported in late Tertiary to Quaternary sediments from the Tyrrhenian Sea (ODP Leg 107; Holes 652A and 654A) (Emeis *et al.*, 1990) and in surficial sediments from the Baltic Sea (Pihlaja *et al.*, 1990) In both cases it was identified by comparing the mass spectrum with that reported by Yon *et al.* (1982) for synthetic 1.

The occurrence of a novel and previously unreported C_{20} HBI monoene (br20:1; 1716_{DBS}) was reported by Porte *et al.* (1990) in bivalve samples collected in the Todos os Santos Bay, Bahia, Brazil. This alkene was converted upon hydrogenation to a C_{20} HBI alkane assigned as *sic* 2,8,12-trimethyl-5-(isopropyl)-tetradecane (br20:0; 1738_{DBS}, 4) by interpretation of the mass spectrum. That of the original precursor alkene (br20:1; 1716_{DBS}) exhibited a molecular ion at m/z 280 and enhanced fragment ions at m/z 181 and m/z 224. From these data this isomer was

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tentatively assigned as 5. Alkane 1 ($br20:0$; 1704_{DB5}) was also present in some samples.

There have also been a number of reports of C_{20} HBI hydrocarbons in hypersaline environments rich in organic matter. For example, the hydrocarbon composition of the carbonate domain of a model evaporitic environment (a saline circuit) at Santa Pola, Spain, described by Barbé *et al.* (1990) was dominated by a mixture C_{20} HBI alkenes (up to 20 μgg^{-1}). No further assignment of structure was made. This report demonstrates how the lack of GC RI data (apparent in many publications) can hinder the structural characterisation of HBI hydrocarbons. Although the assignment of structures by GC RI alone is not recommended, their use does allow for the comparison of RI data with known compounds and others even if the structures of the latter components may be unknown. In this case, it is not possible to compare Barbé's data with any recorded in the literature.

Kenig *et al.* (1990) investigated the origins of types of organic matter in carbonate lagoon and sabkha sedimentary environments of Abu Dhabi, United Arab Emirates. HBI alkane 1 was observed to be the major compound in surface and buried lagoonal sediments containing seagrass and those containing microbial mat but was only a minor component in the modern microbial mat and mangrove palaeosoil. In addition, the co-occurrence of C_{21} and C_{22} HBI alkanes was noted and their structures tentatively identified as 6 and 7 according to their mass spectra (Figure 1.3), GC RI (not cited) and comparison with previously published data (Dunlop and Jefferies, 1985). Monounsaturated homologues of the C_{20} and C_{21} HBI alkanes and an unknown saturated C_{21} isomer, tentatively assigned as 8, were also detected as minor components in some of the samples studied (Kenig, 1991). The mass spectrum

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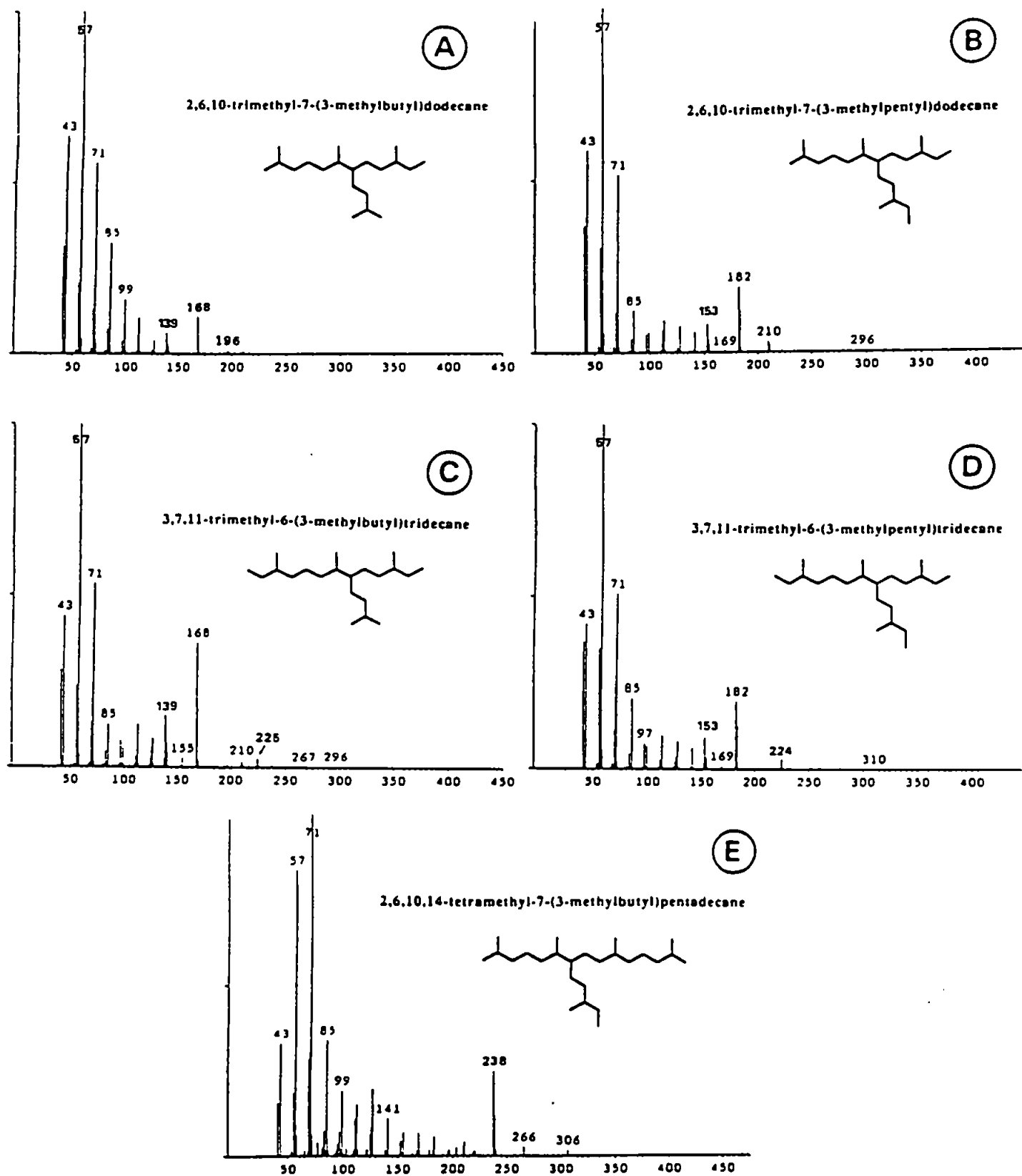
FIGURE 1.3 EI MASS SPECTRA OF HBI ALKANES AS RECORDED BY
KENIG (1991) IN SEDIMENTS FROM ABU DHABI

of the C_{20} monoene was similar to those published for HBI alkenes br20:1; 1696_{ovi} and 1702_{ovi} (*e.g.* Robson, 1987) with a molecular ion at *m/z* 280 and characteristic fragment ions at *m/z* 126, 196 and 210.

In addition to the reports of 1 and related HBI monoenes in marine sediments a few authors have noted their occurrence in freshwater lacustrine sediments. Cranwell and workers (1978; 1982; 1987; 1988) showed that the alkane and related monoenes occur in sediments of mesotrophic and oligotrophic upland lakes *e.g.* Priest Pot (*ca.* 600 ngg^{-1}) and Robinson *et al.* (1987) recorded the same compound in sediments from an oligomesotrophic lake, Coniston Water. The surface sediment of Lake Kinneret, Israel, a relict lake from the Neogene, contained a relatively large amount of the alkane 1 (1880 ngg^{-1}) but was absent from deeper sediment (15 cm) (Robinson *et al.*, 1986). Robson (1987) identified both the HBI alkane br20:0; 1707_{ovi} and related monoene br20:0; 1702_{ovi} in the aliphatic hydrocarbon extract (urea non-adduct) from a eutrophic freshwater lagoon, Loe Pool, Cornwall.

Kurakolova *et al.* (1991) reported the occurrence of 1 in sediment from hypersaline and freshwater lakes in West Siberia.

De las Heras (personal communication) showed that the aliphatic hydrocarbon fraction of sediments from the Ribesalbes Basin, Spain, an ancient lacustrine environment was dominated by 1. The presence of a series of C_{20} monoenes was demonstrated by mass chromatography although comparison of GC RI and mass spectral data with that of synthetic HBI monoenes would help to confirm a highly branched carbon skeleton for these alkenes.

The C_{20} HBI alkane 1 proved to be the dominant hydrocarbon in many of the organic facies of Holocene carbonates in North Stromalolite Lake, South Australia

(Hayball *et al.*, 1991). This hydrocarbon was most abundant in sapropels of organic-rich mudstone which were derived from a density-stratified lacustrine paleoenvironment.

In conclusion, the existence of **1** and two related C_{20} HBI monoenes which exhibit similar mass spectra (Figure 1.4) but are differentiated by their GC RI (*e.g.* 1698_{SP2100} and 1702_{SP2100}; Barrick *et al.*, 1980), is now known. In addition, the identity of a monoene (br20:1; 1716_{DB5}; **5**) with a different carbon skeleton has been inferred on the basis of hydrogenation to a parent alkane **4** and that of C_{21} and C_{22} homologues **6-8** by comparison with previously published data. As only the structure of **1** has been confirmed by synthesis and the position of one double bond located by ozonolysis (**9**; Dunlop and Jefferies, 1985), these other assignments based soley on GC RI and mass spectral data must remain tentative awaiting further characterisation and synthetic studies.
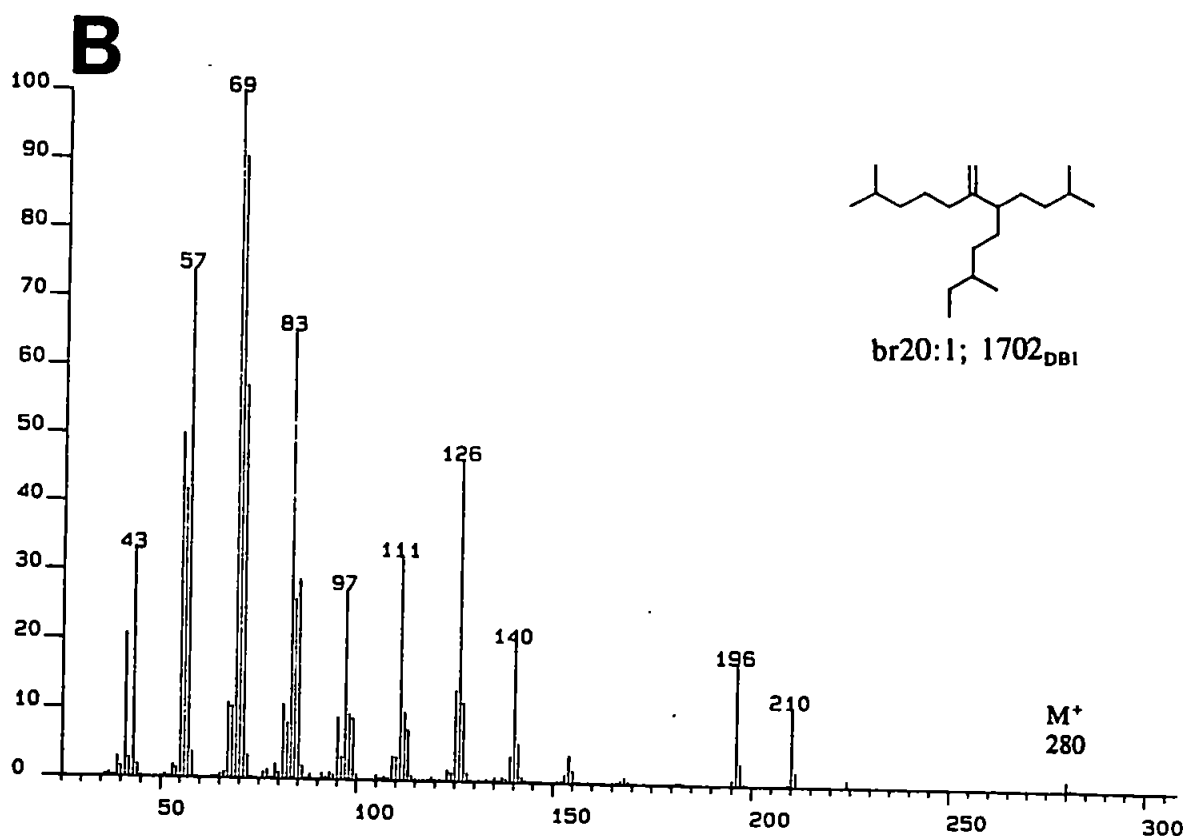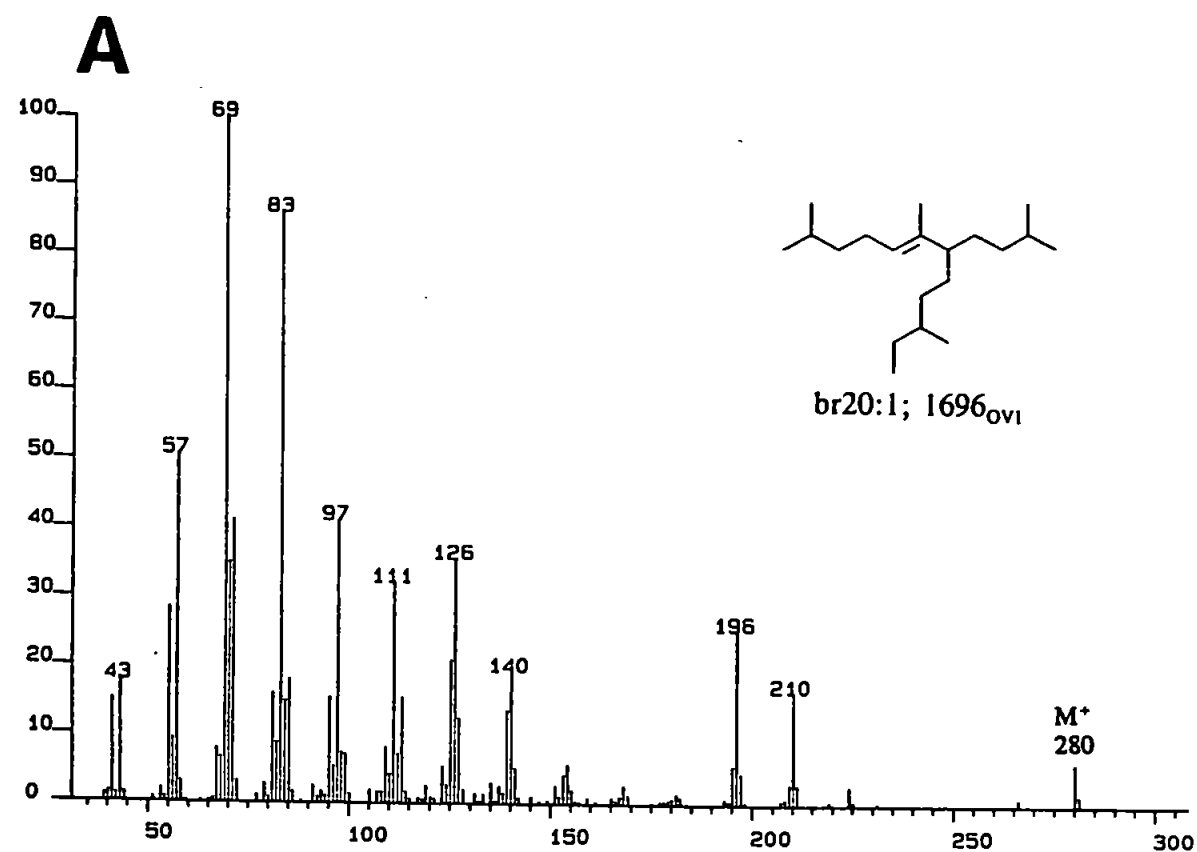
FIGURE 1.4 EI MASS SPECTRA OF SEDIMENTARY C_{20} HBI MONOENES

(A) br20:1; 1696_{OVI}

(B) br20:1; 1702_{DBI}

Structural assignments based upon mass spectral data (A; Robson, 1987) and ozonolysis (B; Dunlop and Jefferies, 1985).

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1.3 THE OCCURRENCE OF C_{25} HBI HYDROCARBONS IN MARINE AND LACUSTRINE SEDIMENTS

Perhaps the greatest advance in the identification of these compounds up to this time was made by Robson and Rowland (1986). In an extremely thorough piece of work confirmation of the structure **2** was made by synthesis of the reference alkane (via a mixture of six C_{25} monoenes). This synthetic compound was used to identify conclusively for the first time the presence of **2** and related mono-, di-, tri-, and tetraenes in various sediments (reviewed by Rowland and Robson, 1990). Previous to this work numerous incorrect assignments have been made based purely upon mass spectral and GC RI data. For example, Crisp *et al.* (1979) reported the occurrence of four C_{25} alkenes in sediment trap particulates off the coast of Southern California. These alkenes ($C_{25}H_{48}$; 2072_{OV101}, $C_{25}H_{46}$; 2044_{OV101}, $C_{25}H_{44}$;2073_{OV101}, $C_{25}H_{44}$;2078_{OV101}) were proposed as cyclic with 1-3 double bonds. However, Robson (1987) showed by hydrogenation and examination of the products that three of these hydrocarbons can now be correctly assigned the acyclic carbon skeleton of **2**. Previous reports of biogenic polyolefinic hydrocarbons in this region include the presence of unknown components eluting at approximately RI 2080_{OV101}. Many of these acyclic C_{25} HBI compounds have been previously misidentified as cyclic because they had not been fully hydrogenated to **2** (see Rowland *et al.*, 1990). This emphasises the requirement for the synthesis of HBI alkenes to act as references for the comparison of analytical data with compounds detected in the environment and thus enable the full characterisation of such biogenic alkenes.

Subsequent to the reports reviewed by Rowland and Robson (1990) of the

occurrence of HBI in Antarctica, unknown C_{25} HBI dienes were the most prominent

hydrocarbons reported by Matsumoto *et al.* (1990c) in sediment samples from

Lützow-Holm Bay. They were not, however, detected in Antarctic lakes or soils

(Volkman *et al.*, 1986; Matsumoto, 1989; Matsumoto *et al.*, 1979; 1989; 1990ab).

Such a predominance of biogenic compounds in unpolluted Antarctic sediments has

been recorded previously (*e.g.* Clarke and Law, 1981; Venkatesan and Kaplan, 1987;

Venkatesan, 1988; Cripps and Priddle, 1990).

Gomez-Belinchan *et al.* (1988) described the occurrence of saturated and

unsaturated HBI hydrocarbons in extracts of particulate matter from various

environments of the Ebro Delta, Spain. However, no further characterisation was

made and the data was not included in the cross-correlation study of the multivariate

dataset resulting from the distribution of lipid components.

Volkman *et al.*, (1988) reported the co-occurrence of five polyunsaturated C_{25}

HBI alkenes in those sediments (from Hobart, Australia) which also contained the C_{20}

HBI monoene br20:1. These alkenes were converted to 2,6,10,14-tetramethyl-7-(3'-

methyl-pentyl)pentadecane (br25:0) 2 by hydrogenation but no retention indices of the

alkenes were given. The major hydrocarbon in these sediments was the

polyunsaturated alkene n-$C_{21:6}$ (heneicosahexaene).

Summons *et al.* (1989) showed that the occurrence of C_{25} HBI alkenes in

microbial mats from Hamelin Pool, West Australia, was limited to the permanently

submerged (subtidal) environments, including the surfaces of actively growing

stromatolites. These were colonised by communities mainly consisting of diatoms,

unicelluar cyanobacteria and flagellated green algae. Differences in the distribution

of C_{25} HBI alkenes were evident in the shallow and deep subtidal environments. For

example, in the shallow regions a monoene (br25:1) was dominant, whereas a diene (br25:2) was relatively more abundant in deeper areas. The structure later assigned to the C_{25} HBI diene (10) was determined by detailed NMR and mass spectral analysis (^{13}C and ^1H NMR, MS and MS-MS), as well as chemical degradation (ozonolysis) (Summons *et al.*, 1992).

Alkane 2 and two unsaturated isomers (a HBI monoene and diene) were reported by Kenig (1991) as very minor components in samples of sediment from Abu Dhabi which were dominated by the C_{20} homologue (Kenig *et al.*, 1990).

Kohnen *et al.* (1990a) identified a number of C_{25} HBI polyenes with three and four double bonds in extracts from a Recent Black Sea sediment.

In many of the Baltic Sea sediments studied by Pihlaja *et al.* (1990) in which 1 was identified, the presence of two C_{25} HBI dienes was also reported. Their mass spectra correspond to those presented by Rowland *et al.* (1985) and Nichols *et al.* (1988) for br25:2; 2082_{OVI} and br25:2; 2088_{MS} respectively. In addition an unknown cyclic compound with the formula $C_{25}H_{48}$ was observed. This component was tentatively identified as a alkylcyclohexane with a double bond in the ring (*i.e.* c25:1:1). This assignment was based upon the GC RI and mass spectral characteristics of the compound. It is interesting to compare the relatively late retention index of this component (*ca.* 2500) compared to other *sic* cyclic alkenes (RI 2000-2100) which have been proven later to be HBIs (*e.g.* Robson, 1987).

Porte *et al.* (1990) described a number of hydrocarbons which had GC RI *ca.* 2100_{DB5} in extracts from bivalves living in the more pristine areas studied and in which the C_{20} homologue had been identified. After hydrogenation all these components were converted to an alkane with a mass spectrum and retention index

identical to those previously published for **2** (*e.g.* Bayona *et al.*, 1983; Albaigés *et al.*, 1984ab; Robson and Rowland, 1986). These alkenes were identified as a C_{25} HBI diene (br25:2; 2068_{DB5}), trienes (br25:3; 2044, 2091, 2107 and 2156), tetraenes and pentaenes based upon interpretation of their mass spectra. Four pairs of geometric isomers were identified (Table 1.3). Two of these pairs, br25:4; 2086 and 2133, and br25:5; 2144 and 2169, and two other pentaenes br25:5; 2124 and 2183 had not been previously reported. The other tetraenes, br25:4; 2079 and 2126 had been previously reported (Barrick *et al.*, 1980). As all of the mass spectra of the pentaenes exhibited molecular loss of *m/z* 69 corresponding to isoprenyl fragments it was assumed that each isoprenoid unit contained one double bond. Tentative structures for the pentaenes (br25:5; 2124, 2144, 2169 and 2183) were proposed (**11** and **12**). A tentative structure (**13**) was also assigned to a new saturated C_{25} HBI isomer by comparison of the mass spectrum with the C_{20} homologue **4**. This parent carbon skeleton was also assigned to a diene isolated from diatomaceous microbial communities of Shark Bay, Western Australia (Summons *et al.*, 1992).

C_{25} HBI alkenes were important constituents of particulate matter and sediment from the sediment-water interface collected in the Cariaco Trench (Wakeham, 1990). In particular two pairs of C_{25} HBI trienes and tetraenes (br25:3 and br25:3', and br25:4 and br25:4') were abundant hydrocarbons at various depth zones in the water column. However, the parent C_{25} HBI alkane and C_{20} homologues were absent. The GC RI (not actually cited) and mass spectra of the C_{25} HBI trienes and tetraenes were similar to components reported in surface sediments of Dabob Bay and Puget Sound, USA (Prahl *et al.*, 1980; Barrick *et al.*, 1980) and the Peru upwelling area (Volkman *et al.*, 1983). Two additional C_{25} HBI alkenes were detected, one a minor component

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in the particles, had a mass spectrum similar to that of br25:2' in Narragansett Bay

and Pettasquamscutt River sediments (Requejo and Quinn, 1983a; Requejo, *et al.*,

1984). The second was the most abundant hydrocarbon in the sediment and had a

spectrum similar to that reported for a bicyclic diene c25:2:2 in a variety of sediments

(Farrington *et al.*, 1977; Barrick and Hedges, 1981; Requejo and Quinn, 1983a;

Volkman *et al.*, 1983). More recently Robson and Rowland (1986) suggested that the

component previously referred to by other workers as a cyclic diene c25:2:2, was

probably acyclic (*i.e.* br25:4).

TABLE 1.3 C$_{25}$ HBI ALKENES IDENTIFIED IN EXTRACTS FROM BIVALVES (Porte *et al.*, 1990)

Compound	RI$_{DB5}$	Molecular weight	Reference (previous reports)
br25:2	2068	348	Albaigés *et al.*, 1984a
br25:3*	2044	346	Barrick *et al.*, 1980
br25:3*	2091	346	Barrick *et al.*, 1980
br25:3 ·	2107	346	Albaigés *et al.*, 1984b
br25:3	2156	346	
br25:4*	2086	344	
br25:4*	2133	344	
br25:4*	2079	344	Barrick *et al.*, 1980
br25:4*	2126	344	Barrick *et al.*, 1980
br25:5	2124	342	
br25:5*	2144	342	
br25:5*	2169	342	
br25:5	2183	342	

Key: Isomeric components are indicated with asterisks

During a study of bioconcentration factors for anthropogenic and biogenic hydrocarbons in mussels from Port Philip Bay, Victoria (Australia) Murray *et al.* (1991) noted the presence of "biogenic alkenes ...mainly C_{25} and C_{30} skeletons with varying degrees of unsaturation" in most water and mussel samples collected but HBI structures were not confirmed.

The first tentative determination of the double bond position in a highly branched polyene by derivatisation and mass spectrometry was described by Yruela *et al.* (1990). A C_{25} HBI diene (br25:2; 2085_{CPSIL8CB}) was shown to elute as a prominent peak in the hydrocarbon fraction from Guadalquivir Delta (Spain) sediments. An epoxide formation technique using *m*-chloroperbenzoic acid was found to be adequate for the derivatisation of a HBI diene. Interpretation of the mass spectrum of the epoxy derivative of the above diene allowed a tentative assignment of the double bond positions (14).
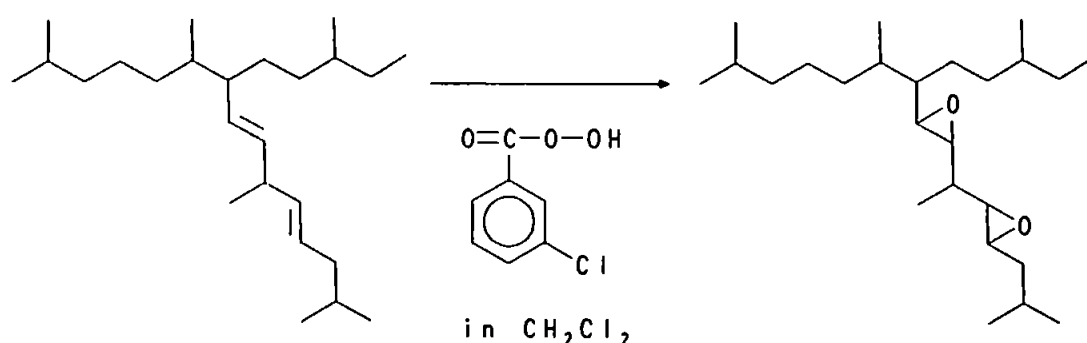
A study of the organic matter in surface sediments collected in the lagoons of two atolls of the Tuamotu Archipelago (French Polynesia) was undertaken by Poupet et al. (1991). Two C_{25} HBI alkenes were reported as major components (up to 250 ngg^{-1} dry weight of sediment) in surface sediments (0-2 cm) from Tikehau atoll, with lower amounts of the C_{30} HBI compounds. The mass spectra of the C_{25} compounds exhibited molecular ions at m/z 350 and m/z 348 indicating that the compounds were br25:1 and br25:2 respectively. No retention indices were reported, however, the mass spectrum of the monoene exhibited intense ions at m/z 210 and m/z 266 whereas the diene displayed characteristic fragments at m/z 266 and m/z 320.

Cranwell (1987; 1988) demonstrated that the HBI alkane 2 and related alkenes were only trace constituents in the deepest sections from lacustrine sequences examined. This contrasts with the report by de las Heras (personal communication) who identified the parent C_{25} HBI alkane 2 and a series of possibly related monoenes in ancient lacustrine sediment sequences from the Ribasalbes Basin, Spain.

Kurakolova et al. (1991) presented a study of HBI ocurring in recent sediments from West Siberia. C_{25} HBI hydrocarbons were identified in five freshwater and hypersaline lakes. Little change in hydrocarbon composition was apparent after hydrogenation (Kurakolova, personal communication) which infers the presence of 2 in the sediments. The same compound was liberated from carbonates upon treatment with acid and found in peat/sapropel samples taken from freshwater lakes. A novel C_{26} HBI compound was found to occur in significant amounts (no concentration values given) in degraded low-moor peat samples. No molecular ion at m/z 366 was apparent in the mass spectrum but major fragment ions were apparent at m/z 182/183, 210/211 and 281 with relative intensities of 21:3:1. The retention indices recorded

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were $2270_{Apiezon L}$ and $2300_{SP2100/OV101}$. The higher GC RI value relative to that expected from a C_{26} HBI alkane with the tertiary centre of branching at C7 suggested a more symmetrical structure. The alkane was assigned from its mass spectrum and retention index as 2,6,10,14-tetramethyl-11-(3'-methylpentyl)hexadecane **15**. This compound was previously reported by Vorobjeva *et al.* (1986) together with C_{20}, C_{25} and C_{30} HBI hydrocarbons in recent sediments and C_{20} and C_{25} homologues in Russian oils. The structure of **15** is analogous to the C_{21} HBI **6** reported by Dunlop and Jefferies (1985) and Kenig *et al.* (1990).

Given the considerable number of C_{25} HBI compounds reported to occur in the environment (Table 1.2), it is suprising that so few have been fully characterised. In addition to the identification of the parent structure **2**, the position of double bonds in related alkenes has only been established for two compounds; in the methylene position of the monene **16** (Dunlop and Jefferies, 1985) and located in the diene **12** (Yruela *et al.*, 1990). Robson (1987) was also able to restrict the double bond position in two C_{25} HBI monoenes to either **17, 18** or **19** by comparison with GC RI of synthetic compounds. Summons *et al.* (1992) more recently formulated structure **10** for a psuedohomologous C_{25} HBI diene. Thus, the only 'established' double bond positions in the sedimentary C_{25} HBI alkenes are **10, 13** and **16-19**. The importance of such characterisation has been emphasised by the work of Sinninghe Damsté *et al.* (1989a) who proposed a relationship between the number and positions of double bonds in C_{25} HBI alkenes and the formation of related HBI thiophenes and thiolanes via early diagenetic sulphur-incorporation. The hindered nature of some double bonds has prevented the derivatisation of particular HBI alkenes in the laboratory (Rowland, unpublished data; Robson, 1987; Nichols *et al.*, 1988) and has led to the

misassignment of some alkenes as cyclic based upon their non-hydrogenation. These features emphasise the need for further studies involving synthesis of the alkenes and the importance of establishing the positions and geometry of the double bonds in more of the sedimentary alkenes.

1.4 THE OCCURRENCE OF C_{30} HBI HYDROCARBONS IN MARINE AND LACUSTRINE SEDIMENTS

As noted by Rowland and Robson (1990), reports of C_{30} HBI hydrocarbons related to 3 have remained far more scarce than those of the C_{20} and C_{25} compounds. Several C_{30} HBI alkenes were identified in bivalves from the Todos os Santos Bay, Bahia, Brazil assigned as bicyclic dienes, c30:2:2; 2444_{DBS} and c30:2:2; 2498 and one acyclic tetraene br30:4; 2530) from their GC RI and mass spectral characteristics and by comparison with similar reports in the literature (Porte *et al.*, 1990). The bicyclodiene c30:2:2; 2498 has been previously reported in estuarine sediments (Requejo and Quinn, 1983a; Barrick and Hedges, 1981). Upon hydrogenation, the acyclic tetraene br30:4; 2530 yielded the highly branched C_{30} alkane br30:0; 2514 3 identified by GC-MS.

Rogers (1988) reported the occurrence of C_{30} HBI alkenes in sediments near Hobart, Australia at lower levels than the C_{25} homologues reported by Volkman *et al.* (1988). No further characteristics were described. More recently a similar distribution was described by Poupet *et al.* (1991) for lagoonal sediments from Tikehau atoll, French Polynesia.

Polyunsaturated hydrocarbons were the major compounds isolated from a Recent Black Sea sediment (Kohnen *et al.*, 1990). They mainly comprised compounds

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tentatively identified as C_{30} HBI polyenes with four, five or six double bonds. These assignments were confirmed by hydrogenation of the alkenes which afforded the corresponding alkane **3** the mass spectrum and GC RI of which compared favourably with that reported by Robson and Rowland (1986; 1988a) for synthetic **3**. The mass spectra of the alkenes exhibited molecular ions at m/z 410, m/z 412 and m/z 414 for the hexaenes, pentaenes and tetraenes respectively (Kohnen, personal communication)

The first report of a C_{30} HBI monoene was recently made by Kenig (1991). This was isolated from sediment containing microbial mat. Kenig noted that the fragment at m/z 210 in the mass spectrum of this compound was characteristic of all the HBI monoenes detected in the carbonate sediments from Abu Dhabi (C_{20}, C_{21}, C_{25} and C_{30}) (Figure 1.5; Kenig, 1991; Kenig *et al.*, 1990).
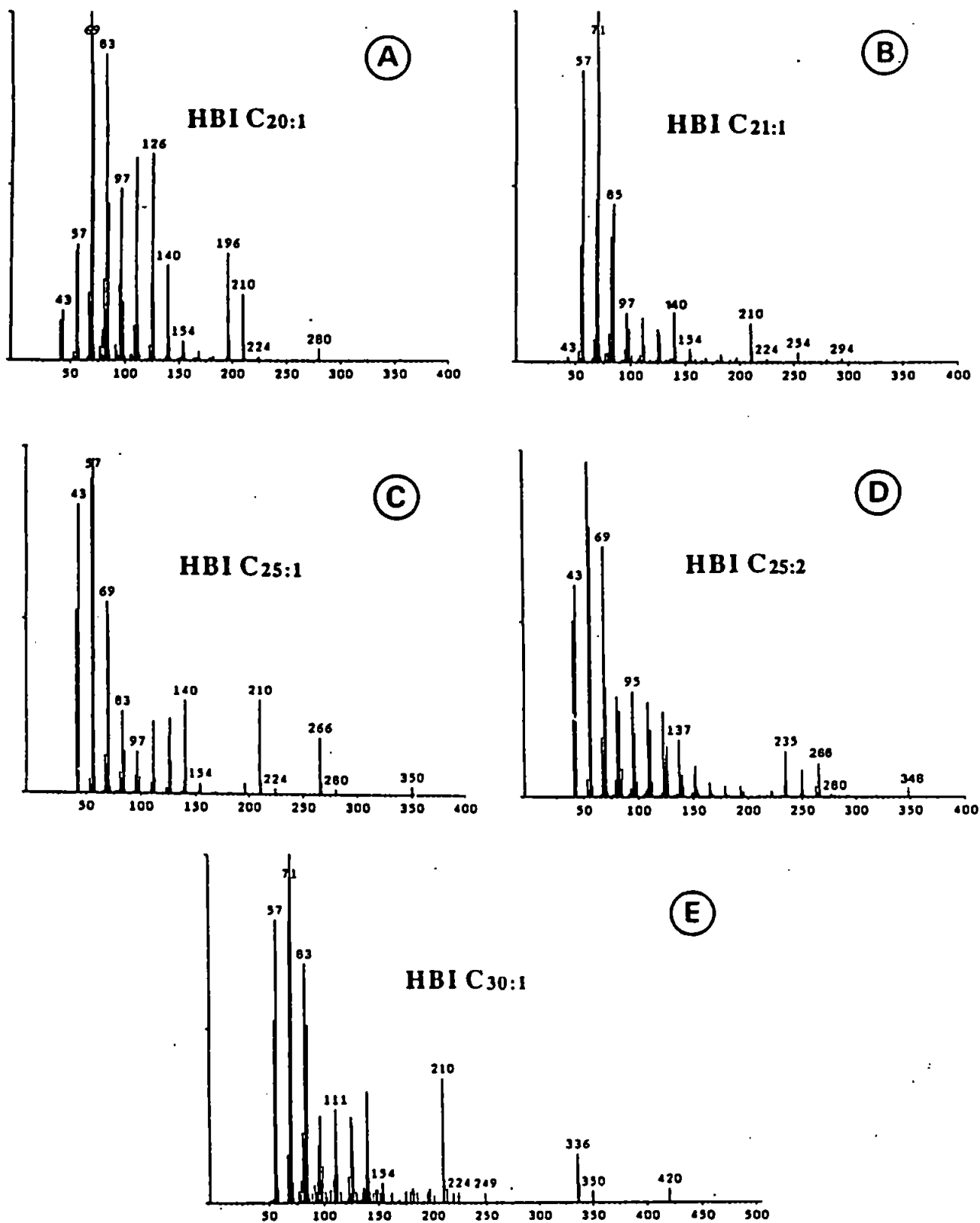
FIGURE 1.5 EI MASS SPECTRA OF HBI ALKENES AS RECORDED BY
KENIG (1991) IN SEDIMENTS FROM ABU DHABI

Based upon differences in mass spectra, it appears that there are both cyclic and acylic C_{30} alkenes present in marine sediments and that the situation is generally more complex than for the C_{20} and C_{25} alkenes which mostly have the acyclic structures 1 and 2. For example, the mass spectrum of the hydrogenation product of the major C_{30} alkene in Narrangansett Bay sediments (Requejo and Quinn, 1983a) had a base peak ion at m/z 193 which is indeed similar to the mass spectra of several bicyclic alkanes (Noble, 1986). The occurrence of the C_{30} HBI skeleton 3 was firmly established by Robson and Rowland (1988a) who synthesised the alkane 3. Other C_{30} acyclic structures also exist as two highly branched C_{30} alkanes with virtually identical mass spectra to 3 were found in the Maoming oil shale (Brassell *et al.*, 1986d). However co-chromatography showed neither was identical to 3 (Robson and Rowland, 1988a).

1.5   SOURCES OF C_{20}, C_{25} AND C_{30} HYDROCARBONS

Since most of the C_{20}, C_{25} and C_{30} HBI hydrocarbons reported contain various degrees of unsaturation, most authors have assumed them to be of natural, biological origin rather than to be pollutants. Some workers have used the sedimentary distribution patterns of the compounds to try to define the sources (*e.g.* Gearing *et al.*, 1976; Boehm and Quinn, 1978) sometimes in conjunction with ^{13}C isotope studies (Requejo and Quinn, 1983a). Others have used a more direct approach by screening organisms associated with the sediment (*e.g.* Rowland *et al.*, 1985) or the water column (*e.g.* Nichols *et al.*, 1988) for the presence of the hydrocarbons (Table 1.4).

TABLE 1.4 OCCURRENCES OF HBI HYDROCARBONS IN BIOTA

Location	Biota	Compounds	Reference
Narrangansett Bay, U.S.A.	*Artica islandica* (bivalve)	$c25:2:2$; 2080_{OV101} $c25:1:1$; 2025_{OV101} $c25:4:1$; 2170_{OV101}	Farrington *et al.*, 1977
Dabob Bay, U.S.A.	mixed phytoplankton	$c30:4:1$; 2509_{SP2100} $c30:3:2$; 2558_{SP2100} RI 2563_{SP2100}	Prahl *et al.*, 1980
Coast of Kuwait	*Pinctada margaretifera* (bivalve)	various C_{25} alkenes	Anderlini *et al.*, 1981
Sandyhaven and Mumbles Head, Wales	*Enteromorpha prolifera* (green alga)	$br20:1$; 1700_{OV1} $br20:0$; 1705_{OV1} $br25:2$; 2082_{OV1}	Rowland *et al.*, 1985
Great Barrier Reef, Australia	*Holothuria* (sea cucumber)	$C_{25}H_{48}$ diene	Coates *et al.*, 1986
McMurdo Sound, Antarctica	mixed diatom communities	$br25:2$; 2088_{MS}	Nichols *et al.*, 1988
Todos os Santos Bay, Brazil	various bivalves e.g. *Anomalocardia brasiliana*	various C_{20}, C_{25} and C_{30} HBI	Porte *et al.*, 1990
Port Philips Bay, Australia	blue mussels e.g. *Mytilus edulis*	C_{25} and C_{30} HBI alkenes	Murray *et al.*, 1991
Lake Karachi, Siberia	unnamed heterotrophic bacteria	C_{26} HBI alkane	Kurakolova *et al.*, 1991

Direct evidence for the biological source of the these compounds is scant. Rowland et $al.$ (1985) identified the C_{20} HBI alkane 1, a related monoene br20:1; 1702_{ovi}, and a psuedohomologous C_{25} HBI diene br25:2; 2082_{ovi} in field samples the green alga $Enteromorpha$ $prolifera$. Nichols et $al.$ (1988) reported a C_{25} HBI diene br25:2; 2088_{MS} as a major hydrocarbon in natural populations of sea-ice diatom communities. Both these authors also suggested that the occurrence of this diene in field samples of $Enteromorpha$ $prolifera$ may be due to the presence of epiphytic microalgae or bacteria. Further discussion is given in the review by Rowland and Robson (1990) but it is fair to say that up to that time no clear source organisms were known. Some new evidence has subsequently been discovered and this is summarised below.

Barbé et $al.$ (1990) found that the occurrence of C_{20} HBI alkenes in sediments of varying salinity was limited to samples taken from the carbonate domain of a model evaporitic environment (a saline circuit). The ecology of this environment consisted of diatoms, cyanobacteria and green algae. However, the analysis of pure cultures of $Cladophora$ sp. (the main green alga), diatom and cyanobacterial species isolated from these ponds showed no trace of such hydrocarbons in any of these organisms. The authors concluded that the majority of the sedimentary material accumulated in the salt pond circuit was deposited in the calcite sediment (carbonate domain) and that this lipid material, including the HBI, was related to algal/cyanobacterial debris.

Kenig et $al.$ (1990) found the occurrence of C_{20}, C_{21} and C_{22} highly branched alkanes was not limited to lagoonal sediments containing seagrass ($Halodule$ sp.) but that the compounds were also prominent in buried microbial mats.

The occurrence of HBI in bivalves from a tropical bay in Brazil was attributed by Porte *et al.* (1990) to particular environmental conditions. These included the oxygen content at the sediment-water interface and the water temperature, because these parameters control the degree of unsaturation of other bacterial and algal lipids (Tornabene *et al.*, 1979; Brassell *et al.*, 1986ab). The presence of such biogenic components in bivalves was related to their feeding habits.

An upper water column algal source for two pairs of acyclic C_{25} HBI alkenes (br25:3 and br25:3', and br25:4 and br25:4') was suggested by Wakeham (1990) based upon the high amounts of these compounds in surface water particles and their decreased abundance with increased water depth. However, Wakeham did acknowledge that there had been no reports of these alkenes in specific pelagic phytoplankton to confirm their origin.

An algal origin for the C_{25} and C_{30} HBI alkenes was presumed by Murray *et al.* (1991) because the alkenes were generally associated with particles in the water column. In the one case where the alkenes were classified as "dissolved" (*i.e.* passed through the filter and retained by a XAD-type resin column), it was suggested that alkene-bearing algae at that site were small enough to pass through the filter and thus trapped by the resin column. It is interesting to note that the qualitative distribution of alkenes at this site was different to that found at the other stations.

Poupet *et al.* (1991) discovered a positive correlation between the C_{25} HBI alkenes and accepted algal tracer compounds such as polyunsaturated and some monounsaturated fatty acids ($C_{16}:1\omega7$, $C_{18}:1\omega9$ and $C_{17}:1\omega8$) and inferred an algal source for the C_{25} HBI alkenes detected in the sediments. Low molecular weight alkanes and alkenes also showed good correlation as algal markers. No

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polyunsaturated alkene n-$C_{21:6}$ (heneicosahexaene), which has been associated with some diatoms (*e.g.* Blumer *et al.*, 1971) was found in the surface sediments.

No C_{20}, C_{25} or C_{30} HBI hydrocarbons have been found in marine bacteria (Tornabene and Oró, 1967; Oró *et al.*, 1967; Han *et al.*, 1968; Han and Calvin, 1969; Albro, 1976; Albro and Dittmer, 1970; Holzer *et al.*, 1979; Tornabene, 1976; 1981; Tornabene *et al.*, 1978; 1979; 1982; Nes and Nes, 1980; Brassell *et al.*, 1981; Langworthy, 1982; Risatti *et al.*, 1984; Taylor, 1984; de Rosa *et al.*, 1986ab; de Rosa and Gambacorta, 1988; Franzman *et al.*, 1988; Gossens *et al.*, 1986; 1989a). However, a C_{26} HBI pseudohomologue 13 (RI 2300_{OV101}) reported in the hydrocarbons of peat by Kurakolova *et al.* (1991), was also identified in unidentified heterotrophic bacteria isolated from a sediment core from the hypersaline Lake Karachi (West Siberia).

The biogeochemistry of recent laminated microbial mats was investigated by de Wit *et al.* (1991) to determine the influence of environmental conditions upon the microbiology. In an elegant piece of work, where fine millimetric lipid structure was described, the concentration of a C_{20} HBI monoene was shown to maximise (70 μgg^{-1} dry weight) at a depth of 12-18 mm, characterised by high activity of anaerobic heterotrophic microorganisms.

1.5.1 COMPOUND-SPECIFIC ISOTOPE ($\delta^{13}C$) ANALYSIS (CSIA)

Molecular isotopic analysis provides information on the biological origin of individual compounds. Relative abundances of the stable carbon isotopes vary systematically in sedimentary organic compounds. Isotopic compositions of geolipids approximate those of their biological precursors which are, in turn, determined by the isotopic composition of the carbon assimilated by the organism and the biogeochemical processes by which they are synthesized (Hayes *et al.*, 1990). The isotopic composition of geolipids are likely to be close to those of their precursor biochemicals. Isotopic fractionations during diagenetic processeses (*e.g.* loss of functional groups) are considered to be small since the chemical reactions occur at specific sites within the biolipid. Isotopic abundances at those sites may shift as reactions occur, but other portions of the molecule will be unaffected and their isotopic constancy will buffer the effects of isotopic shifts at the reaction sites (Hayes *et al.*, 1990).

Compound-specific isotope analysis by gas chromatography combined with isotope-ratio mass spectrometry (GC-IRMS) enables the precise determination ($\pm$ 0.0003 atom percent) of the ^{13}C contents of individual peaks in high resolution gas chromatograms (Matthews and Hayes, 1978; Freeman *et al.*, 1990; 1991; Hayes *et al.*, 1990). An important function of such analyses is the resolution of the isotopic composition of material derived from primary sources (photosynthate) from that of secondary inputs. Isotopic analyses of biological marker compounds enable elucidation of secondary (mostly bacterially-mediated) processes that can influence the generation and preservation of organic matter within a depositional environment (Hayes *et al.*,

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1990; Schoell *et al.*, 1992). Isotopic analyses of individual compounds from units representing a range of paleoenvironments demonstrated that compounds with common biological origins have similar isotopic compositions (Freeman *et al.*, 1990). Conversely, the isotopic composition of a geolipid can also reveal information about the different source organisms and environments of biosynthesis of one particular compound type. For example, molecular isotopic results have suggested that the origin of long-chain *n*-alkanes (*i.e.* C_{26} to C_{33}) is not exclusive to land plants (Freeman *et al.*, 1990; 1991; Hollander *et al.*, 1991; Kohnen *et al.*, 1991b; 1992). The results of CSIA carried out on HBI compounds are summarised in Table 1.5.

TABLE 1.5 COMPOUND-SPECIFIC ISOTOPE ANALYSIS OF HBI HYDROCARBONS[a]

Carbon skeleton	Surface water particulates, Cariaco Trench[b]	Diatomaceous microbial communities Shark Bay[c]	Sediment (Marl-2) from the Messinian Vena del Gresso basin[d]			
			Hydrocarbon fraction	Alkylthiophene fraction[e]	Alkylsulphide fraction	Polar fraction
C_{20} HBI	—	-11.0	-17.7±0.6	—	—	—
C_{25} HBI	-24	-12.0[f] -12.8[g]	—	-27.3±0.9	-23.4±0.6	-23.9±0.6[h]
For comparison						
PME[j]	-28	—	-25.8±1.6	—	—	—
Lycopane	-30	—	-25.3±1.0	—	—	—
C_{29} steradiene	—	-12.8	—	—	—	—

Key: [a]The carbon isotope data are presented in $\delta^{13}C$ values (‰; see Kohnen *et al.*, 1991b);
[b]Freeman *et al.* (1991);
[c]Summons *et al.* (1992)
[d]Kohnen *et al.* (1991b, 1992); Schouten *et al.* (1991);
[e]For the OSC the isotope analysis are performed on desulphurised compounds;
[f]br25:1 (**16**)
[g]br25:2 (**10**)
[h]Compound exclusively derived from macromolecularly sulphur-bound moieties;
[j]2,6,10,15,19-pentamethyleicosane.

Freeman *et al.* (1991) presented a means of identifying the biological sources of hydrocarbons based upon predicted values for the ^{13}C content of plankton biomass. An expression for the predicted isotopic composition of autotrophic biomass, δ^{*}_{p}, calculated from the composition of dissolved CO_2, was derived from published data on isotopic fractionation by marine phytoplankton. The C_{25} HBI alkenes previously reported to occur in surface water particulates from the Cariaco Trench (Wakeham, 1990) were shown to have a summed δ ^{13}C value of -24 ‰. A planktonic source for the C_{25} HBI was suggested by comparing δ^{*}_{p} (surface water; -24 ‰) and the observed lipid δ value. Other hydrocarbon "phytoplankton biomarkers" classified in this way included lycopane (δ -30 ‰) and pentamethyleicosane (δ -28 ‰).

The δ ^{13}C value for a related C_{25} highly branched isoprenoid thiophene (HBIT) 20 in sediment from Vena del Gesso (Italy) was recorded as -27.3±0.9 ‰ [1] which was consistent with a diatomaceous source for the precursor HBI alkene (Kohnen *et al.*, 1991b; 1992; Schouten *et al.*, 1991). This hypothesis was supported by the similarity of the isotopic signature of the algal derived steranes (*e.g.* cholestane; -26.3±0.3 ‰) in the same sediment. The macromolecularly S-bound C_{25} HBI carbon skeleton was assigned a different precursor as reflected by its different mode of occurrence and isotopic composition (-23.4±0.8 ‰ and -23.9±0.6 ‰). This demonstrates a possible multiple origin for the C_{25} HBI carbon skeleton.

In contrast, the δ value for the C_{20} HBI alkane 1 was -17.7±0.6 ‰. This compound was reported to be derived from alga(e) which were periodically blooming and causing a significant drop in the concentration of CO_2 in the water. Consequently, the biosynthesized algal biomass became enriched in [13]C. Kohnen *et al.* (1991b)

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[1] Isotope analyses were performed on desulphurised compounds

concluded that the isotopic signature of these HBI, the specific source of which is still unknown, has provided information concerning the habitat of their biological source. It is also noteworthy that compound-specific isotope analyses may help to establish genetic relationships between different lipids. For example, the C_{20} HBI showed an isotopic composition which was similar to that of macromolecularly S-bound n-C_{31} carbon skeleton (-17.6±0.3 ‰). This similarity in $\delta^{13}C$ values, which were relatively unique in the sediments analysed, justified the proposal of a common source for their precursors (Kohnen *et al.*, 1991b).

In the benthic microbial community sample from which C_{25} HBI diene **10** was isolated, the $\delta^{13}C$ values for the C_{20} HBI alkane **1**, the C_{25} HBI monoene **16**, and **10** were -11.0, -12.0 and -12.8 ‰ respectively (Summons *et al.*, 1992). Some sterene hydrocarbons, biomarkers for eukaryotic algae, had similar "heavy" carbon isotopic signatures with a co-occurring C_{29} steradiene having a value of -12.8 ‰.

Considering the widespread occurrence of HBI hydrocarbons in sediments and particulate matter it is suprising that the source of this group of hydrocarbons remains largely unknown. The main reasons for this anomaly lie with the inherent difficulties in isolating pure, epiphyte-free, biological specimens in the field together with the failure to identify HBI hydrocarbons in axenic cultures in the laboratory. However, compound-specific isotope analysis by GC-IRMS has provided a tool with which to study the carbon cycle at the molecular scale. This has enabled the comparison of $\delta^{13}C$ of compounds from specific biota with those from HBI hydrocarbons in the environment. Indeed, such analyses produced information concerning the habitat of the biological source of HBI (*i.e.* water hardness) even though the specific source was not known.

1.6 DIAGENETIC FATE OF HBI HYDROCARBONS

In common with many organic geochemical studies of biological marker compounds, considerable interest has been shown, not only in the origins of the HBI hydrocarbons, but also in their longer term geological fate. Although detailed interpretations of downhole diagenetic fate have been hampered by the incomplete structural characterisation of most of the HBI compounds, several studies have attempted to follow their fate in a general way both in sediments and in the water column. Most of these studies have been discussed by Robson and Rowland (1988b) and Rowland and Robson (1990).

Numerous studies have shown that high concentrations of C_{25} HBI alkenes are typically only present in surface sediments and decrease quite rapidly with increasing sediment depth and in the water column (*e.g.* Farrington *et al.*, 1977; Boehm and Quinn, 1978; Hurtt and Quinn, 1978; Wade and Quinn, 1979; Barrick *et al.*, 1980; Venkatesan *et al.*, 1980; Brault and Simoneit, 1989). Barrick and Hedges (1981) showed that the C_{30} alkenes also decreased with depth. Various proposals have been made to expain this decrease including geochemical alteration with time (*e.g.* a reaction involving the double bonds) (Wade and Quinn, 1979), microbial oxidation or polymerisation (Venkatesan *et al.*, 1980) and non-selective mineralisation in the sediment (Prahl and Carpenter, 1984). A number of authors have proposed an *in situ* degradation (Requejo and Quinn, 1983a); Volkman *et al.* (1983) favoured a microbial degradation process and the incorporation into accreting polymeric material via cross-linking involving double bonds whereas Barrick *et al.* (1980) invoked an *in situ* chemical degradation. More recently, Wakeham (1990) suggested a microbially mediated degradation process to explain the decrease in concentration with water

column depth of C_{25} tri- and tetraenes. In a few sediments HBI alkene concentrations maximise a few centimeters below the surface (Requejo *et al.*, 1984). In such cases some workers have proposed that the hydrocarbons are *in situ* bacterial products (Requejo *et al.*, 1984).

There are few reports of the HBI hydrocarbons at depth in freshwater lacustrine sediments. Robinson *et al.* (1986) showed that the C_{20} HBI alkane 1 was present (1880 ngg^{-1}) only in surface sediment of Lake Kinneret, Israel (a section 2-5 cm thick). This suggested that there may have been a change in the ecology of the lake and thus a difference in the input of lipids to the sediment between the times of deposition of the sediment samples. By contrast, in sediments from Coniston Water the alkane 1 became more abundant with increasing depth (*ca.* 600 ngg^{-1} dry weight sediment at 0-3 cm increasing to *ca.* 1000 ngg^{-1} at 8-12 cm; Robinson *et al.*, 1987). This might indicate *in situ* formation under anoxic conditions by bacteria. Even though microbial activity is at a maximum in the upper most section of the lake, differences in bacterial populations in the three sediment sections were recognised. In both cases the *n*-alkanes were shown to decrease in concentration with increasing depth.

Some aspects of the depth profiles of the HBI compounds are markedly different even in replicate neighbouring cores of sediments. Farrington *et al.* (1988) discussed the different depth profiles evident for a C_{20} HBI monoene apparent in two box cores from Peru surface sediments (Figure 1.6). The depth profile of concentration (for dry sediment weight) for one core (SC6) was essentially exponential (if smoothed) with a few minor fluctuations of concentration with depth. The other (SC4) exhibited a subbottom maximum which interrupted an otherwise

A
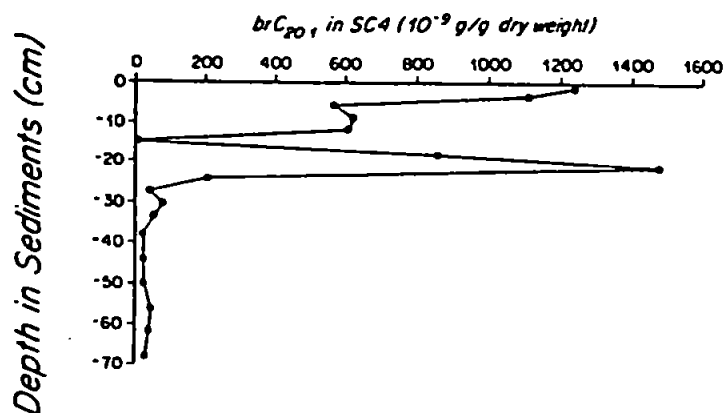
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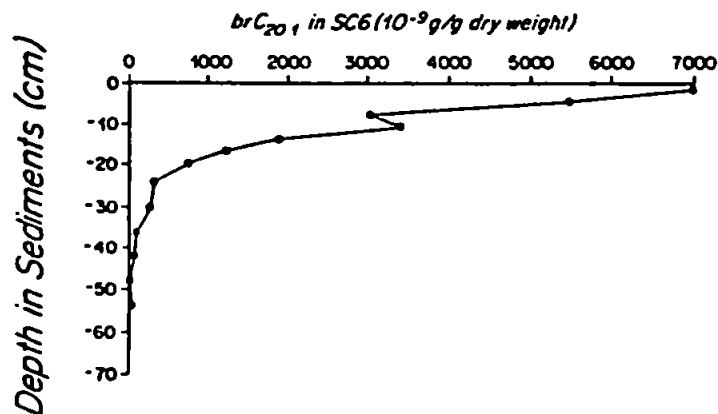
FIGURE 1.6 CONCENTRATION OF C_{20} HBI MONOENE IN SURFACE
SEDIMENTS OF THE PERU UPWELLING AREA
(Farrington *et al.*, 1988)
(A) Box Core SC4, 90 m water depth
(B) Box Core SC6, 268 m water depth

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fairly uniform exponential decrease of concentration with depth. This subbottom maximum for br20:1 was said to represent a high flux of organic material with appreciable concentration of br20:1. Similar subbottom maxima were evident for n-C_{37} alkenones nomalised against organic carbon concentration. A substantial deposition of planktonic detritus under low oxygen conditions at the sediment-water interface was invoked to account for the preservation of the maximum.

Several authors have shown that C_{25} HBI alkenes , in general, decrease quite rapidly in concentration with increasing sediment depth (*e.g.* Farrington *et al.*, 1977). For example, Requejo and Quinn (1985) reported the highest concentrations of HBI alkenes near the sediment-water interface (*e.g. ca.* 0.5-4.0 μgg^{-1} dry weight; New England Salt Marsh) which rapidly decreased to low constant values (<0.3 μgg^{-1} dry weight) by approximately 20 cm, below which they were seen to decrease only slightly. In this core the organic carbon profile was shown to decrease concurrently with the HBI alkenes, probably due to decreases in benthic primary productivity with depth. This was in contrast to some Narragansett Bay sediments (Requejo and Quinn, 1983a) where the organic carbon profiles exhibited no change in concentration over the same depth interval but where the HBI alkene concentrations generally decreased. The subsurface decrease in Narragansett Bay sediments was attributed to a degradation of the alkenes after burial rather than a recent increase in surface input or *in situ* production. In contrast, the sedimentary profiles of HBI alkene concentrations relative to organic carbon from New England Salt Marsh were consistent with a bacterial source for these compounds. By assuming that anaerobic decomposition of organic matter in marsh sediments proceeded primarily by bacterial sulphate reduction and that the zone of maximum sulphate reduction in such sediments

was limited to the upper 10 cm, the authors proposed a correlation between this process and the high alkene concentration in surface sediments *i.e.* the simultaneous decreases of HBI alkene and organic carbon concentrations suggested that a diminishing supply of recently-produced organic matter (and thereby microorganisms active in its remineralization) may be of greater importance in determining HBI alkene depth profiles in salt marsh sediments than in Narragansett Bay.

Sub-surface maxima of highly branched and cyclic (*sic*) alkenes have been less frequently observed. (*e.g.* Requejo and Quinn, 1983a; Requejo *et al.*, 1984; Farrington *et al.*, 1988). For example, variations in the subbottom concentration of an unidentified C_{25} alkene (RI 2081_{DB5}; no molecular ion evident) in a core from an ancient sediment from the Pigmy Basin (DSDP Site 619) was described by Requejo *et al.* (1988). The concentration increased to a maximum at 65 m subbottom (130-150 cm sediment depth) below which it decreased to low and constant values (*ca.* 10 ngg^{-1} dry weight). An inverse relationship was evident between the subbottom profile for the sulphate content of pore water and this compound. The depletion of sulphate in the pore water suggested the presence of oxygen-depleted water which permitted the preservation of hydrocarbons including the C_{25} alkene. The organic carbon concentration was relatively invariant with depth (range 0.66-1.02%) with one exception (0.09% at *ca.* 100 m below the surface).

1.6.1 BIODEGRADATION OF HBI HYDROCARBONS

Another posible mechanism for changes in concentration of HBI hydrocarbons with depth is biodegradation. The only laboratory-based degradation studies carried out on the HBI hydrocarbons (Robson and Rowland, 1986; Robson, 1987; Robson and Rowland, 1988b; Gough *et al.*, 1992) showed alkanes 1-3 to be more resistant to degradation by *Psuedomonas aeruginosa* under aerobic conditions than *n*-alkanes and regular acyclic isoprenoids with the same molecular weights. In addition it was demonstrated that synthetic br20:1 and br25:1 alkenes were more resistant to degradation than the n-C_{17} and n-C_{20} alk-1-enes. The C_{25} HBI monoenes were more slowly degraded than the C_{20} isomers but no difference was observed within the isomers. This last point is in agreement with observations made by Prahl and Carpenter (1984) who noted that the ratio within some C_{25} HBI trienes remained constant even though a decrease in total alkenes with depth was evident. However, the C_{25} HBI isomers used in the laboratory studies have been infrequently reported in the environment. A comparison of degradation rates of naturally occurring HBI polyenes under realistic environmental conditions has yet to be accomplished.

1.6.2 THE FORMATION OF HBI ORGANIC SULPHUR COMPOUNDS

An alternative mechanism for the depletion of these highly branched alkenes with increasing sediment depth (age) comes from the analysis of related organic sulphur compounds (OSC) reviewed by Sinninghe Damsté and de Leeuw (1990), Orr and Sinninghe Damsté (1990) and Kohnen *et al.* (1990a). Sinninghe Damsté *et al.* (1989b) proposed that inorganic sulphur is abiotically incorporated into unsaturated lipid precursors during early stages of sediment diagenesis or even in the water

column. This sulphur enrichment of organic matter ("quenching") would thus remove the labile fuctionalised precursors from the geochemical record, while still preserving their carbon skeletons and information on the sites of their functionality (*e.g.* double bond position in the case of alkenes).
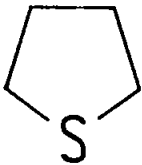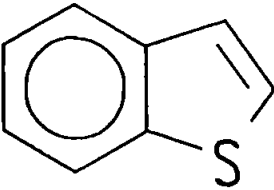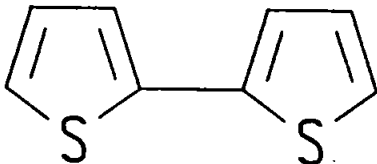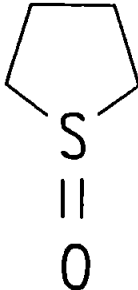
The modes of occurrence of carbon skeletons including OSC has been recently reviewed by Kohnen *et al.* (1992). Those occurring as OSCs can be divided into two subcategories, one comprised of compounds containing S-heterocyclic rings and having molecular weights (MW) up to *ca.* 500 dalton, the other comprised of aggregates in which diverse carbon skeletons are linked by sulphide bridges. These aggregates range in size from MW *ca.* 500 dalton to macromolecular.

Sulphur-containing heterocycles with carbon skeletons **1**, **2** and **3** occur with thiophene, benzo[b]thiophene, thiolane and bithiolane ring systems in sediments and oils from different geographical locations which range from Pleistocene to Cretaceous (Table 1.6). A number of thiophenes with carbon skeletons **1** and **2** (HBIT) have been identified in consolidated sediments and immature oils (20-30; Sinninghe Damsté *et al.*, 1987, 1989ab; **31** unpublished results). Thiophenes **21** and **20** were identified by reference to synthetic compounds. The occurrence of C_{20} HBIT is restricted to Rozel Point seep oil and West Rozel oil (Sinninghe Damsté, 1989abc) whilst that of C_{25} HBIT is more widespread (Sinninghe Damsté, 1989abc, Kohnen *et al.*, 1990a; ten Haven *et al.*, 1990ab). This parallels the relative distributions of the C_{20} and C_{25} hydrocarbon precursors. Indeed, C_{25} HBIT are sometimes the major constituents of "aromatic hydrocarbon" fractions from deep sea sediments (Kohnen *et al.*, 1990a, b; ten Haven *et al.*, 1990ab). The C_{20} and C_{25} HBIT **21** and **20** occur as a pair of diastereoisomers which are separable by GC. Corresponding C_{25} saturated and

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unsaturated HBI thiolanes (**32** and **34**) have been tentatively assigned in deep sea sediments (ten Haven *et al.*, 1990ab) and the identification of the saturated thiolane **30** has been confirmed by synthesis (Kohnen *et al.*, 1990a). Compound **32** occurs as a complicated mixture of diastereoisomers since it has six chiral centers (Kohnen *et al.*, 1990a). C_{25} unsaturated HBI thiolanes with one to two double bonds in the long alkyl side chain (**34**) and structurally related C_{30} psuedohomologues possessing two to four double bonds respectively (**35**) have been identified in a recent Black Sea sediment (Kohnen *et al.*, 1990a). The exact positions and stereochemistry of the double bonds in the side chains remains unknown. Related sulphoxides were also present in this sediment sample (Kohnen *et al.*, 1990a; **36** and **37**).

A C_{25} HBI benzo[*b*]thiophene assigned as **38**, was present in Jurf ed Darwish oil shale (Kohnen *et al.*, 1990ab). This identification was based on the comparison of the mass spectral data with known fragmentation patterns of alkyl benzo[*b*]-thiophenes (Perakis, 1986, Sinninghe Damsté *et al.*, 1987). This tentative assignment awaits further confirmation by comparision of retention index and mass spectrum with those of an authentic standard.

TABLE 1.6 ORGANIC SULPHUR COMPOUNDS (OSC) WITH HBI CARBON SKELETONS

HBI Carbon Number	Thiolane	Thiophene	Benzothiophene	Bithiophene	Sulphoxide
20	------	3, 4	------	1	------
25	5, 7	5, 6, 7	7	------	6, 7
30	5, 7	------	------	------	6, 7

C_{25} and C_{30} HBI carbon skeletons also released by desulphurisation of polar resin fractions (macro S-bound) 5, 6, 7.

Numbers refer to references: (1) Schimid (1986); (2) ten Haven *et al.* (1988); (3) Sinninghe Damsté *et al.* (1988); (4) Sinninghe Damsté *et al.* (1989); (5) ten Haven *et al.* (1989); (6) Kohnen *et al.* (1990a); (7) Kohnen *et al.* (1990b)

Recent developments in the characterisation of organically-bound sulphur present in the macromolecular substances *i.e.* kerogen, "protokerogen", asphaltenes and high molecular weight fractions of crude oil and bitumen (Schmid, 1986; Sinninghe Damsté *et al.*, 1988ab, 1989cd, 1990b; Rullkötter and Orr, 1990; Kenig and Huc, 1990; Kohnen *et al.*, 1990a, 1991ab) show that the sulphur-containing moieties in these substances are formed in a similar way to the low-molecular weight OSC (Sinninghe Damsté *et al.*, 1989c). HBI alkanes **2** and **3** have been identified in desulphurisation mixtures of resin fractions of recent sediments (Cenozoic age) from the Peruvian upwelling area (ten Haven *et al.*, 1990ab) and the Black Sea (Kohnen *et al.*, 1990a) and in Miocene sediments from the Monterey formations (Sinninghe Damsté *et al.*, 1990b). This shows the HBI carbon skeleton to be present in macromolecules bonded by one or more sulphur bridges.

It has been postulated that the formation of these HBI sulphur compounds is initiated by incorporation of hydrogen sulphide and/or polysulphides into di- and/or poly-unsaturated HBI alkenes during early diagenesis (Sinninghe Damsté *et al.*, 1989b) but this has yet to be proven. Berner (1980; 1984; 1985) invoked bacterial sulphate reduction as the mechanism of OSC formation. Such OSC may be formed either by intramolecular incorporation of sulphur into HBI alkenes, intermolecular sulphur incorporation into (poly)unsaturated HBI OSCs, or by intramolecular incorporation of sulphur into macromolecularly bound HBI alkenes. It is assumed that because inorganic sulphur reacts with alkanes only at temperatures higher than those to which Recent sediments have been subjected (*e.g.* Veno del Gesso; Sinninghe Damsté *et al.*, 1988b) that the carbon skeletons found in the sulphur-containing fractions must derive from functionalized biolipids that furnished sites (mainly double

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bonds) suitable for attack by sulphur species (*e.g.* hydrogen sulphide and polysulphides). The formation of OSC can be considered in terms of a two-step process. Initially, attack by inorganic sulphur forms a C-S bond and yields a reactive intermediate (a thiol). In the second step, the reactive intermediate is stabilised by formation of a second C-S bond. The second bond-formation reaction can be *intra-* or *inter*molecular. If intramolecular, a cyclic product is formed. Intermolecular reactions yield aggregations of carbon skeletons.

Kohnen *et al.* (1990a)

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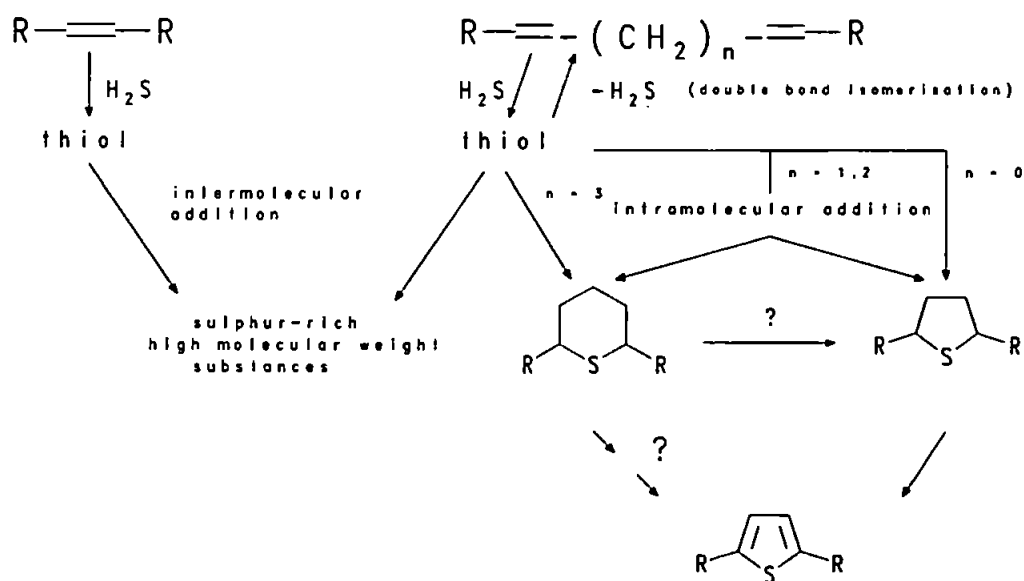
It is evident from the range of HBI sulphur-containing heterocycles isolated by Sinninghe Damsté and others that intramolecular cyclization occurs only when two double bonds (sites suitable for C-S bond formation) are separated by fewer than four sp^3-hybridized carbon atoms (Sinninghe Damsté *et al.*, 1989b). When this condition is not met, intermolecular S linkages are formed. If the carbon skeletons linked in this way contain additional reaction sites, further S linkage may form. A continuum of products exists, ranging from two carbon skeletons bound by one sulphide link to macromolecular materials in which multiple carbon skeletons are linked by S bridges (Kohnen *et al.*, 1990a; 1992). The carbon skeletons within those aggregated can be identified only after the S bridges have been broken, and this is accomplished by treatment with Raney Ni and analysis of the resulting hydrocarbons.

Hence HBI alkenes with two or more double bonds may undergo sulphide addition reactions. If the double bonds are separated by 0 to 3 sp^3-hybridised carbon atoms, sulphur incorporation may lead to restricted number of isomers. The HBIT formed thus seem to be limited because the positions of sulphur attachment are determined by the positions of double bonds in the precursor HBI diene. For example, the widespread occurrence of C_{25} HBIT **20** in combination with the absence of other C_{25} HBIT was explained by sulphur incorporation into a C_{25} HBI diene with double bonds at C1'-C6' of the carbon skeleton (Sinninghe Damsté *et al.*, 1989b). HBI dienes with mass spectra consistent with double bonds in these positions have frequently been reported in recent sediments and sedimenting particles (Requejo and Quinn, 1983a; Albaigés *et al.*, 1984b; Requejo *et al.*, 1984; Requejo *et al.*, 1985; Venkatesan and Kaplan, 1987; Venkatesan, 1988) and also in a field sample of sea-ice diatoms (Nichols *et al.*, 1988). Rowland *et al.* (1990) proposed that one of the double

bonds in C_{25} dienes br25:2; 2082_{DBS} and 2088_{DBS} was in the 6(17) position and thus amenable to intramolecular sulphur incorporation.
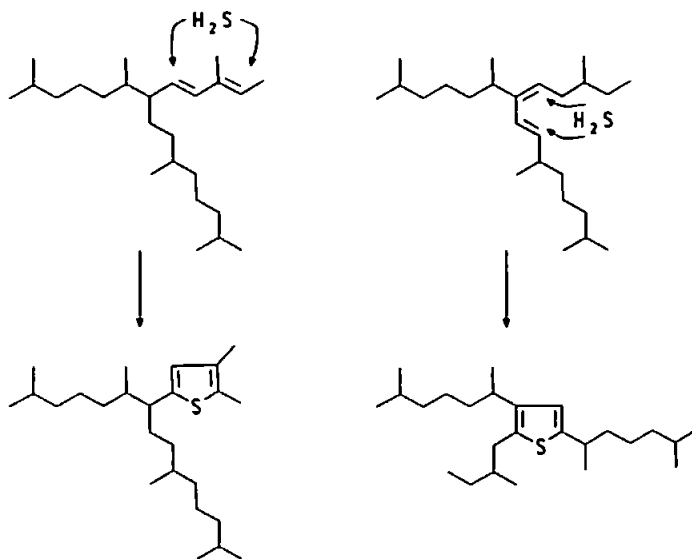
In contrast, it is of interest to note that Yruela *et al.* (1990) assigned the double bond position in a C_{25} HBI diene (br25:2; $2085_{CPSIL8CB}$) as C8-C15 of the carbon skeleton. Intramolecular incorporation of sulphur into this structure using the scheme proposed by Sinninghe Damsté (1989b) can only proceed via a substituted thiane which could be reduced to a thiophene.
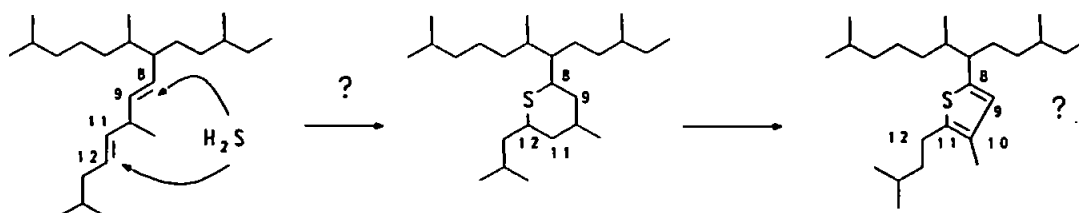
Such compounds, however, have yet to be detected in recent sediments or oils. So, preceding the intramolecular incorporation of sulphur at such positions the double bonds would have to be shifted to more favourable positions. This would involve double bond isomerisations via secondary carbocations thought unlikely to occur in recent sediments (de Leeuw *et al.*, 1989). Alternatively, this C_{25} HBI diene may be incorporated into the sulphur-rich macromolecular fraction.

HBI alkenes with more than two double bonds similarly undergo sulphide addition reactions to form thiolanes with double bonds in the long alkyl side chain (unsaturated thiolanes).

When the double bonds are not separated by 0 to 3 sp^3-hybridised carbon atoms a sequence of sulphur addition and elimination reactions has been proposed (Sinninghe Damsté, 1989b), ultimately modifying the stereochemistry of the compound so that the position of two of the double bonds allow formation of a thiolane with double bonds in the alkyl side chain if the number of double bonds exceeds two. The resulting HBI OSC mixture from this second set of sulphide

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addition/elimination reactions is characterised by a higher amount of structural isomers caused by the preliminary rearrangement of alkene double bonds.

Alternatively, reactions may occur in competition with those above by which the alkene becomes part of sulphur rich high molecular weight substances via intermolecular S linkages.

Thus intra- and intermolecular incorporation of inorganic sulphur species into HBI alkenes (Figure 1.6) may explain the rapid decrease in concentration of these alkenes with depth reported in many recent marine sediments (see review by Rowland and Robson, 1990).

The C_{25} and C_{30} unsaturated HBI thiolanes identified in Recent Black Sea sediments (Kohnen *et al.*, 1990a) possessed two double bonds less than their precursors, indicating that the formation of a thiolane ring requires the presence of two double bonds. It would seem that not only the number of double bonds is important but also the stereochemistry as only a limited number of all possible OSC structural isomers are formed. For example, only three of the 17 possible C_{25} HBIT isomers have been detected in sediment samples studied (*e.g.* Sinninghe Damsté *et al.*, 1986; 1987; 1989ab; 1990a; Kohnen *et al.*, 1990a; 1991ab; ten Haven *et al.*, 1990ab). This suggests that formation of HBI OSC might be limited by the stereochemistry of the original alkene, previously ignored by most workers.

FIGURE 1.6 DIAGENETIC SCHEME SHOWING THE POSSIBLE ORIGINS AND PRESUMED PATHWAYS OF C_{25} HBI ALKENES AND C_{25} HBI OSC AND MACROMOLECULARLY BOUND C_{25} HBI SKELETONS ENCOUNTERED IN OILS AND SEDIMENT EXTRACTS (Kohnen *et al.*, 1990a)

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Sedimentary sulphur incorporation into C_{20}, C_{25} and C_{30} HBI alkenes may act as an adventitous confirmation of the acyclic structures of the HBI hydrocarbons and may also aid the identification of the position of the original double bond(s).

Although the C_{20} HBIT have, to date, only been observed in two immature oils (Sinninghe Damsté, 1989ab) whereas the C_{25} HBIT are widespread in sediments and oils (Sinninghe Damsté, 1989ab; ten Haven *et al.*, 1990ab) more structural isomers have been identified for the C_{20} homologues (nine) and conversely the absence of particular stereochemistries noted (Sinninghe Damsté *et al.*, 1986; 1987; 1989ab). All but one of the C_{20} HBIT present in Messinian marl had a structure consistent with sulphur incorporation into C_{20} HBI mono-6(14)ene (br20:1; 1702_{ov1}; **9**) characterised by Dunlop and Jefferies (1985) in sediment from Shark Bay, Australia, or by limited isomerisation of this alkene. The concept of limited isomerisation was proposed for the early diagenetic pathway of steroids; double bond isomerisations occur in nature only via tertiary carbocations and not via secondary carbocations (de Leeuw *et al.*, 1989). Limited isomerisation of HBI mono-6(14)ene during early diagenesis would thus only yield mono -5(6)-, 6(7)-, 7(8)- and 7(1')-enes **39-42**. However, the mechanism of formation of C_{20} HBIT from these alkenes remains unclear because it is thought that the presence of at least two double bonds in the precursor is a prerequisite for the formation of thiolanes or thianes by abiotic sulphur incorporation (Sinninghe Damsté *et al.*, 1989b); further dehydration is necessary for the formation of thiophenes. For the major part of the OSC identified in sediments and oils (Sinninghe Damsté and de Leuuw, 1990) naturally occurring precursors exist for OSC which are in agreement with the model of sulphur incorporation proposed by Sinninghe Damsté (Table 1.7).

TABLE 1.7 RELATIONSHIPS BETWEEN OSC PROPOSED BIOLOGICAL MARKER PRECURSORS AND BIOLOGICAL SOURCES (Sinninghe Damsté and de Leeuw, 1990)

Organisms	Functional lipid(s)	OSC
Eubacteria	bacteriohopanetetrol	thiophene hopanoids
algae, higher plants	phytol, phytadienes	C_{20} isoprenoid thiophenes
photosynthetic sulphur bacteria	$\Delta^{2,6}$phytadienol, phytadienes	C_{20} isoprenoid thiophenes
Archaebacteria	geranyl geraniol	C_{20} isoprenoid thiophenes
diatoms	C_{25} HBI alkenes	C_{25} HBI thiophenes
prymnesiophyte algae	C_{37} and C_{38} unsaturated ketones & corresponding alkenes	C_{37} & C_{38} 2,5-dialkylthiolanes, -thiophenes and 2,6-alkylthianes
algae, higher plants *etc.*	sterols	S-containing steroids
bacteria	squalenes	C_{30} isoprenoid thiophenes
algae, higher plants *etc.*	carotenoids	bicyclic terpenoid sulphides

No polyunsaturated C_{20} HBI alkenes have been reported. Thus, if the C_{20} HBIT are derived from sedimentary C_{20} HBI monoenes (Rowland and Robson, 1990) via thiol formation (Sinninghe Damsté *et al.*, 1989b), an alternative mechanism for the loss of hydrogen to allow thiolane ring closure would be required. It is possible that the C_{20} HBIT are formed by intramolecular sulphur incorporation into C_{20} polyunsaturated HBI alkenes which yet have to be reported in sediments. Conversely, it could be argued that the absence of C_{20} HBI polyenes in recent sediments is caused by rapid facile OSC formation during very early diagenesis.

Thus, using the mechanism proposed by Sinninghe Damsté *et al.* (1989b) sedimentary HBI alkenes with only one double bond (*i.e.* monoenes) are not likely to serve as precursors to OSC through intramolecular sulphur incorporation. However, they may become part of sulphur rich high molecular weight substances (*e.g.* resins, asphaltenes and kerogen) via the addition of H_2S hydrogen sulphide and subsequent intermolecular addition of the resulting thiol (Figure 1.6). The presence of many more C_{20} than C_{25} and C_{30} HBIT isomers may be a function of, or reflect the different modes of formation, namely a less stereospecific intermolecular amalgamation of macromolecular organic matter for the C_{20} HBIT isomers. The C_{25} and C_{30} HBIT isomers having numerous precursor alkenes with 1-5 double bonds (Rowland and Robson, 1990) may be preferentially formed by intramolecular incorporation via specific stereochemical pathways.

Although the actual agent/mode of sulphur "quenching" has yet to be proven various mechanisms and diagenetic pathways for OSCs involving H_2S (HS^-), elemental sulphur (S_8) and/or polysulphides have been proposed (Brassell *et al.*, 1986c; Kohnen *et al.*, 1989; Sinninghe Damsté *et al.*, 1989b). The reactions of

polysulphides in the formation of OSC has been recently reviewed by LaLonde (1990). A number of attempts have been made to simulate the incorporation of inorganic sulphur into organic matter in the laboratory (Boelens *et al.*, 1974; Schwab *et al.*, 1976; Mango, 1983; LaLonde *et al.*, 1987; Moers *et al.*, 1988; Al-Lihaibi and Wolff, 1991; Fukushima *et al.*, 1992; Rowland *et al.*, 1992). This approach has recently yielded promising results. Modes of formation of C_{20} alkylthiophenes have recently been demonstrated by Al-Lihaibi (1991), Fukushima *et al.* (1992) and Rowland *et al.* (1992). Al-Lihaibi (1991) reported the successful incorporation of elemental sulphur (S^0) into phytadienes, at low temperature (45°C), under basic conditions (in the presence of trimethylamines known to occur in recent sediments; Abdul-Rashid *et al.*, 1991). Fukushima *et al.* (1992) demonstrated the likely formation reaction of the C_{20} alkylthiophenes from chlorophyll-derived phytol and hydrogen sulphide via phytadiene intermediates. Phytenic aldehydes, which may be significant components in recent marine sediments (Rontani *et al.*, 1990; Rowland and Maxwell, 1992), were also shown to be possible important precursors of such isoprenoid thiophenes (Rowland *et al.*, 1992). These results provide substantial evidence for a mild reaction to produce the limited number of C_{20} alkylthiophene isomers which occurs during very early stages of diagenesis confirming the hypothesis of Sinninghe Damsté *et al.* (1989b) and Kohnen *et al.* (1990a) that sulphur incorporation occurs during very early diagenesis. These laboratory experiments using synthetic phytol and phytadienes as precursors for the formation of C_{20} isoprenoid thiophenes demonstrates another requirement for the synthesis of HBI alkenes for laboratory-based reaction in H_2S-saturated waters.

To summarise, HBI polyenes may undergo either intra- or intermolecular

incorporation of inorganic sulphur or both leading to the formation of sulphur rich HMW substances with units also containing intramolecularly incorporated sulphur (HBI OSC connected to each other by sulphur bridges). The number and position(s) of the bonds in the HBI alkenes will control their ultimate mode or occurrence: alkylthiophene *vs.* macromolecularly sulphur-bound.

Several authors have suggested that a sequence of reactions from thiolanes/thianes via thiophenes to benzo[*b*]thiophenes and finally dibenzothiophenes is related to increasing diagenesis (Perakis, 1986; Sinninghe Damsté and de Leeuw, 1990; Sinninghe Damsté *et al.*, 1987, 1989ab; Kohnen *et al.*, 1990a; Figure 1.6).

1.6.3 THE FATE OF HBI HYDROCARBONS IN THE WATER COLUMN

The occurrence of the HBI alkenes has also been reported in particulate organic matters in the water column and in sediment from the sediment-water interface. Samples recovered from sediment traps or via filtration have been collected at various locations over the world's coastal and oceanic regions; California (Crisp *et al.*, 1979), Dabob Bay, U.S.A. (Prahl *et al.*, 1980), Alfraques Bay, Spain (Bayona *et al.*, 1983), Kiel Bight (Osterroht *et al.*, 1983), Peru upwelling area (Volkman *et al.*, 1983), Ebro Delta, Spain (Albaigés *et al.*, 1984b), Puget Sound, U.S.A. (Bates *et al.*, 1984), Eastern North Pacific (Matsueda and Handa, 1986a; Matsueda *et al.*, 1986), the Antarctic Ocean (Matsueda and Handa, 1986b) and the Cariaco Trench (Wakeham, 1990). The HBI composition of sinking particules has been shown to change with increased sampling depth (*e.g.* Matsueda and Handa, 1986ab; Wakeham, 1990) and the regional variability of this vertical change has also been investigated

(*e.g.* Matseuda and Handa, 1986b). Bathymetry (water depth) is an important control on the organic matter content of marine sediments since the proportion of organic matter surviving sedimentation decreases with increasing residence time in the water column (Tyson, 1987). Indeed, Matseuda and Handa (1986a) showed that the vertical flux of the sum of C_{25} HBI tri- and tetraenes (br25:3; 2047_{SE52}, br25:4; 2083_{SE52}, br25:3'; 2092_{SE52}) decreased rapidly with depth throughout the stations sampled in the Eastern North Pacific Ocean. The C_{25} HBI alkenes exhibited a slower rate of decomposition than more labile components (short-chained *n*-alkanes and heneicosahexaene [n-$C_{21:6}$]) but was more rapid than the longer chained *n*-alkanes (C_{21} to C_{32}). These differences were attributed to biological sources of the different compounds, namely phytoplankton for the linear hydrocarbons and zooplankton/ bacteria for the C_{25} HBI alkenes. However, it is more likely to be related to structure; short-chained *n*-alkanes are more susceptible to microbiological attack than longer-chained *n*-alkanes in natural aquatic environments (Giger *et al.*, 1980) and heneicosahexaene (n-$C_{21:6}$) is readily biodegraded (Youngblood *et al.*, 1971; Youngblood and Blumer, 1973).

The regional differences observed in the vertical profiles of the summed concentrations of the C_{25} HBI alkenes were not caused solely by microbial decomposition and zooplankton grazing. The size of the particles, which is closely related to their sinking rate, was reported to influence the extent of the C_{25} HBI decay (Matsueda and Handa, 1986b). Thus, it is likely that at the stations where sinking rates are greater (larger particle size), the organic matter (including C_{25} HBI alkenes) is transported through the water column more rapidly (hence less degraded) and relatively high concentrations are observed at depth.

The concept that the size (and hence the sinking rates) of particles is a primary factor in controlling hydrocarbon composition of particulate organic matter was investigated by Wakeham (1990). The suspended matter in the water column of the Cariaco Trench was reported to contain different distributions of hydrocarbons depending on the particle size ($<$53 μm vs. $>$53 μm) and depth at which they were collected (oxic water column vs. anoxic water column). Two pairs of C_{25} HBI trienes (br25:3 and br25:3') and tetraenes (br25:4 and br25:4') were shown to be abundant in both $<$53 μm and $>$53 μm particles in the upper oxic water column ($<$150 m; e.g. br25:3' 1.0 ngl^{-1} at 50 m in the $>$53 μm size fraction). This was in contrast to heneicosahexaene (n-$C_{21:6}$) which though abundant in the 50 m $<$53 μm size fraction was only a minor component in the $>$53 μm material at the same depth (no actual values cited). The C_{25} HBI alkenes were reported as relatively minor components at greater depths ($<$0.05 ngl^{-1}). The concentration of one C_{25} HBI (br25:3') was again shown to decrease rapidly with depth both in terms of dry weight of sediment and normalised against particulate organic carbon (POC) content.

The hydrocarbon distribution in the sediment floc (the upper 2-3 mm of flocculant material at the sediment-water interface) were markedly different from those of the suspended particles from the water column. The most abundant compound in the C_{25} HBI alkene group was another tetraene (br25:4*) previously assigned as a bicyclic diene, c25:2:2 (e.g. Requejo and Quinn, 1983a). It is interesting to note that whilst this compound was a minor constituent of the $<$53 μm particles in the anoxic zone it was absent from particles from the oxic zone. The enrichment of br25:4* (and br25:2) in the $<$53 μm particles from the anoxic zone and the sediment floc could be explained if the HBI had an anaerobic microbial origin

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for these compounds as proposed by Requejo *et al.* (1983; 1984) and Wakeham (1990). However, a number of alternative mechanisms are plausible, namely an *in situ* diagenetic production from other more labile C_{25} HBI alkenes by isomerisation or partial hydrogenation of double bonds or the delivery of material to the sediment by near-bottom lateral currents, thereby not present in the water column (and thus not sampled by the WHISPS). Alternatively, these isomers might in fact more stable to microbial degradation and survive diagenetic changes in the water column to be concentrated in the surface sediments.

1.6.4 THE OCCURRENCE OF HBI HYDROCARBONS IN CRUDE OILS

During an investigation of immature oils from Eastern Sakhalin and Western Kamchatka (Siberia), Bazhenova and Arefiev (1990) noted the presence of high concentrations of the C_{25} HBI alkane 2 (no concentration values given) in oils and bitumens from the Oligocene Pileng Series at the Okruzhnoye field. The immature oils in this region are associated with argillaceous-siliceous sediments. This was only the second recorded occurrence of the HBI alkanes in oils and the first report of the C_{25} HBI alkane. Previously, only the C_{20} HBI alkane 1 has been reported, in Rozel Point oil (Yon *et al.*, 1982; Sinninghe Damsté *et al.*, 1986). Indeed, further examination of various oils and bitumens from silica and "clayey silica" strata in the Okruzhnoye field in the Eastern Sakhalin (Bazhenova, personal communication) revealed the presence of all three HBI alkane homologues (1, 2 and 3). The presence of the C_{25} HBI alkane in two oils from the Okruzhnoye and Nihokvazhnoe fields, Eastern Sakhalin was confirmed by comparison of the mass spectrum of the component in the oils with the synthetic 2 and by co-injection studies using the latter

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(Hird, unpublished results).

The oils from the Okruzhnoye field are light (density 0.82-0.86) with a low sulphur content (0.17-0.45%) with abundant asphaltenes and resins (8.6-24%) and a high pristane and phytane content.

In a further study, Bazhenova and Arefiev (1991) investigated the role of the bitumenous component during early generation of hydrocarbons to determine concrete genetic precursors for immature oils. The C_{25} HBI alkane shown to be present in both the oil and free hydrocarbon fraction from the (source rock) organic matter , *i.e.* the bitumen, was absent from the thermolysis products from the kerogen and from the asphaltene fraction. The absence of this compound from the kerogen matrix was explained by assuming such structures were formed independently of the kerogen matrix; *i.e.* the hydrocarbons in the oil and bitumens were not genetically related to kerogen which had undergone thermolysis. An alternative explanation given was that they were released from the kerogen during early catagenesis.

Among the oils occurring within the Palaeogene marine deposits of the Fergana depression (Hankis) there are some distinguished by the presence of the C_{20} HBI alkane in amounts comparable with the content of regular isoprenoids (Punanov *et al.*, 1991). The occurrence of alkane 1 was restricted to oil fields sourced from either one limestone bed located in the Middle Eocene roof or from two adjacent beds of the Upper Eocene (depth 1700 to 5600 m). The deposition of these sediments occurred in lagoons under conditions of high salinity as demonstrated by the prevalence of gypsum and gypsiferous clays. In addition, after further examination, the presence of the C_{25} homologue 2 was revealed (Bazhenova, personal communication) but it is not yet known whether the occurrence of this compound is

restricted in the same way as that of alkane **1**.

1.7 POTENTIAL USE OF HBI HYDROCARBONS AND OSC AS BIOLOGICAL MARKER COMPOUNDS

Biological markers chemicals (geochemical markers, biomarkers or chemical fossils) are sedimentary organic compounds whose basic skeletons suggest an unambiguous link with known contemporary natural products, and were synthesised by biota present at the time of the deposition of the sediment. These compounds are commonly used to assess palaeoenvironmental conditions of deposition of Recent and ancient sediments (de Leeuw, 1986; Brassell *et al.*, 1987; Philp and Lewis, 1987; ten Haven *et al.*, 1988; Volkman *et al.*, 1988; Poynter and Eglinton, 1991). Saturated hydrocarbons are relatively easy to analyse and may contain much geochemical information, and are therefore traditionally the most widely used class of biomarkers in palaeoenvironmental reconstruction (Mackenzie, 1984; Philp, 1985; Johns, 1986; Brassell and Eglinton, 1986; Volkman and Maxwell, 1986; Philp and Oung, 1988; Philp *et al.*, 1991).

The unusual structures **1-3** and their widespread sedimentary occurrence (Tables 1.1 and 1.2) representing a range of depositional environments throughout the maturity window of organic matter confers great biomarker potential on the HBI hydrocarbons and their sulphur-containing anologues. However, advancement in this area has been hindered by misidentification and non-identification. The HBI alkenes with more than one/two double bonds appear to be more rapidly removed from the hydrocarbon fraction of most sediments than the alkanes and monoenes which seem to be more resistant to degradation. Thus the HBI polyenes may prove useful

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indicators of the source of organic matter in recent/Recent sediments whereas the HBI alkanes, monoenes and OSC occurring in some more ancient sediments and immature oils might prove to be useful empirical indicators of environmental conditions of deposition.

The biosynthetic hierarchy forms a convenient starting point for discussion of biological marker potential. Most of the great range of compounds and biopolymers found in sediments (*e.g.* Brooks *et al.*, 1987) are generated by four main biosynthetic pathways. The mevalonate pathway leads to the formation of multiple, regularly branched carbon compounds known as isoprenoids. Both cyclic and acyclic compounds are known (*e.g.* tetrahymanol **43** and phytol **44**). In the vast majority of cases of the latter, the five-carbon isoprene units/segments are attached by a "regular" or head-to-tail fusion (1',4 linkage) and numerous naturally occurring terpenes have been identified that contain this 1',4 linkage (*e.g.* phytane **45**). Isoprene units joined by "irregular" or non-head-to-tail bonds are encountered less frequently (see the review by Poulter, 1990). Examples of "irregular" isoprenoids include squalene **46** and botryococcane **47**.

The 1',4 linkage (head-to-tail) is generated during the fundamental polymerisation reaction of isoprene metabolism where successive molecules of isopentyl diphosphate are attached to growing allylic diphosphate polyisoprene chains (Poulter and Rilling, 1981). Branching of the isoprenoid chain at C(7) (*i.e.* addition of an isoprene unit at C7 - C5') produces the unusual HBI structures. For example the C_{20} HBI alkane **1** has a carbon skeleton composed of four isoprene units linked in a nonlinear fashion.

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to sedimentary organic matter relies on the information from the lipid composition of appropriate organisms. However, in the case of the HBI hydrocarbons such information is scant. Indeed and chemotaxonomic description of organisms using HBI hydrocarbons is lacking as little information has been obtained from the analysis of natural populations of mixed species for these hydrocarbons and no successful determination has been achieved using either laboratory cultures or natural populations of individual species. However, HBI hydrocarbons possess structures that retain obvious links to biosynthetic components (isoprene units). It remains unclear whether these compounds are direct biosynthetic products or are generated by the diagenetic transformation of an unidentified precursor(s).

An early use of a HBI alkene as a non-diagnostic marker compound was demonstrated by Boehm and Quinn (1978) who labelled a *sic* cycloalkene (later proven by Robson [1987] to be acyclic br25:3; 2079_{ovi}) of molecular weight 344 ($C_{25}H_{44}$) a marker of "normal biogenic and/or diagenetic activity". The basis for this assumption was a significant covariance with organic carbon in most sediments analysed.

The limited occurrence of the C_{20} HBI alkane 1 in Ancient geological samples (*e.g.* Rozel Point crude oil) suggested to Yon *et al.* (1982) who were the first to correctly assign the structure of the compound, that it might have potential as a useful input biological marker.

Although the data on biological occurrence of HBI hydrocarbons is limited to a few compounds in two reports, some authors cite HBI hydrocarbons as biological markers for phytoplankton or green algae in general. For example, both Brassell and Eglinton (1986) and Cranwell (1987, 1988) include the C_{20} HBI alkane as possibly

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diagnostic of green algae whereas Comet and Eglinton (1987) proposed the presence of the highly branched 'GX' series to be an indicator of aquatic phytoplankton organic matter. Based upon the suggestion of Barrick et al., (1980) that a particular $C_{25:3}$ alkene was derived from a phytoplanktonic source, Brassell et al., (1987) used the above HBI alkene as a possible biological marker compound for phytoplankton in organic matter from Middle America Trench sediments.

A different conclusion was made by Matsueda et al., (1986abc) who reported that C_{25} HBI alkenes (11 to 18% of total hydrocarbons from sinking particles in the Antarctic) were a group of biological markers characteristic of the particulate matter excreted by zooplankton (i.e. of zooplankton fecal pellets).

These unfortunate uses of the C_{20} and C_{25} HBI hydrocarbons as biological marker compounds by authors who have incorrectly assumed the biological sources, detract from the potentially very useful specific structures and stereochemistries of the compounds. In a review of biological markers of marine productivity the position has been adequately summarised by Prahl (1992) who discounted the use of the C_{25} HBI diene (br25:2; 2082_{ovi}) as a specific indicator (for diatoms) because of the scant information concerning the biological source. He questioned the validity of the reports of the biological occurrences in green algae and diatoms by Rowland et al. (1985) and Nichols et al. (1988) and emphasised that no occurrence of this compound has yet been reported in specimens of the proposed algal sources grown under laboratory conditions free from other types of biological contamination. Indeed, he concluded that there was no unequivocal biomarker to identify organic carbon contributions made by diatoms to sediments. In contrast to HBI, other compounds assigned as diatom markers sic do not even appear to survive the early stages of sedimentation.

Thus, the extreme diagenetic sensitivity of such compounds (*e.g.* heneiecosahexaene or fucoxanthin) and the apparent survival of the carbon skeleton of HBIs through early diagenesis, and the occurrence in Ancient sediments and oils, emphasise the importance of screening organisms (especially species of algae) for the presence of these hydrocarbons and hence the potential of HBIs as biological marker compounds.

Sinninghe Damsté and others have discussed the potential applications of sulphur-containing HBI (OSC) as molecular indicators for the assessment of sources of organic matter and reported the use of OSC as "geochemical tools for palaeoenvironmental and stratigraphic assessment" (Sinninghé Damste *et al.*, 1989c, 1990a; Kohnen *et al.*, 1990b; 1991ab; de Leeuw and Sinninghé Damste, 1990). Significant variations in the depth profiles of specific sulphur compounds (related to certain organisms) including HBI OSC were shown to reflect changes both in sources of organic matter and the physical conditions of the depositional environment. Sinninghe Damsté *et al.* (1989ab) suggested that C_{25} HBI thiophenes (HBIT) are biomarkers for diatoms. However, in the case of the HBI carbon skeleton, the use of the C_{25} HBIT as biological marker compounds for diatoms is based upon the incorporation of sulphur into HBI alkenes not yet proven to be biosynthesised by certain diatom species. In addition, in order for sulphur incorporation into sedimentary organic matter to take place, conditions of anoxia (with low amounts of available iron) must prevail. However, evidence for the use of C_{25} HBIT as "diatom markers" is circumstantial as their concentrations in sediment facies have been shown to parallel P_2O_5 concentrations in Jurf ed Darwish oil shale. This was consistent with the fact that phytoplankton tend to be dominated by diatoms and that phosphatic sediments are often deposited in upwelling environments and that this facies was also

reported to contain trace amounts of fragmented diatom frustules (Kohnen *et al.*, 1990b; Sinninghe Damsté *et al.*, 1990a). Two C_{25} HBI thiophenes **20** and **30** (and sometimes the saturated thiolane anologue **32**) have been shown to be ubiquitous in other diatomaceous sediments, oil shales and oils deposited in upwelling regions; the bitumen of the Monterey formation (Miocene) (Sinninghe Damsté *et al.*, 1990a), immature (Pleistocene) sediments from the Gulf of California (Rullkotter *et al.*, 1982), samples of Cenzoic age off southern California and Baja California (ten Haven *et al.*, 1990b). However, despite the abundance of diatoms in the Peru margin sediments, or off the coast of Namibia, the two C_{25} HBI thiophenes were only observed at trace levels and no HBI alkenes were detected in the sediment (ten Haven *et al.*, 1990ab). However, the Peru sediment was shown (ten Haven *et al.*, 1990a; Kohnen *et al.*, 1991a) to contain a considerable amount (220 mgkg⁻¹) of C_{25} HBI carbon moities bound via a sulphur bridge to a macromolecule released by desulphurisation using Raney nickel which has been shown to cleave C-S bonds selectively and quantitatively (Sinninghé Damste *et al.*, 1988b). The latter example demonstrates the potential use of OSC in the "bound" form as biomarker compounds in situations where the labile hydrocarbon and/or oxygenated biomarkers have been removed during early diagenesis as has been shown to occur in many cases with the HBI alkenes (see section **1.6**). The biases from natural sulphurisation in paleoenvironmental reconstruction based on hydrocarbon biomarker distributions was recently discussed by Kohnen *et al.* (1991a). Erroneous conclusions can be reached on the basis of hydrocarbon biomarker distributions alone (de Leeuw and Sinninghé Damste, 1990). The C_{25} HBI alkenes are striking examples in this respect as they are sometimes absent in the hydrocarbon fraction (including thiophenes in the 'aromatic'

fraction) but are major compounds in the desulphurised resin fraction.

Kohnen *et al.* (1991b; 1992a) has attempted to improve upon the use of molecular sulphur to recognise paleochemicals by utilising the carbon isotopic composition of sedimentary lipids. In this way the mode of occurrence and carbon isotopic composition of HBI was used to retrieve information concerning the biotic community present in the depositional environment. The δ ^{13}C value for the C_{25} HBIT **20** was recorded as -27.3 $\pm$ 0.9 ‰ and that for the C_{25} HBI alkane released by desulphurisation of macromolecular organic matter was -23.9 $\pm$ 0.6 ‰ confirming a diatomaceous source for the precursor HBI alkene and thus the presence of diatoms in the paleoenvironment.

Schouten *et al.* (1991) used this assumption to demonstrate that the contribution of diatoms, as determined from the concentration of the C_{25} HBIT **20**, decreased rapidly during the deposition of the marl layer in samples from the Vena del Gesso Basin, Northern Italy.

It is clear that unambiguous markers are necessary to identify accurately sources of organic material, but unfortunately some chemical structures are common to several types of living organism, both terrestrial and marine. Moreover, many inventories of biolipids are incomplete, often limited to selected species and often non-extendable to natural environments, particularly when they have been obtained from *in vitro* experiments or from cultures performed far from natural conditions. Although the specific source of HBI hydrocarbons remains unclear, the use of HBI isotopic signatures has provided information concerning the habitat of their biological source and hence paleoenvironment.

1.8   SUMMARY

Nearly thirty C_{20}, C_{25} and C_{30} HBI hydrocarbons have been detected, sometimes in high concentrations, in recent freshwater, estuarine, coastal and hypersaline sediments and water column particulate matter from numerous locations worldwide (Tables 1.1 and 1.2). The parent structures have been proved (1-3) but only a few of the double bond positions have been established (9, 14, 16-19). The assignment of C_{21}, C_{22} and C_{26} homologues (6-8, 15) and other C_{20} (4) and C_{25} (10, 13) isomers, remains tentative until the structures are confirmed by synthesis as proved possible for 1-3. A wide body of evidence suggests that the compounds are biogenic in origin, with algae and possibly bacteria the most likely source organisms. A few of the compounds have been identified in field samples of algae but none have been reported in laboratory cultured biota.

The alkenes with more than two double bonds appear to be rapidly removed from the hydrocarbon fraction in most sediments, whereas the alkanes and monoenes seem to be more resistant to biodegradation and hence occur in some more ancient sediments and oils. There is evidence that some of the alkenes react rapidly with sulphur to form either S-containing HBI heterocycles (20-38) or become bound within macromolecular aggregates both found in sediments and some oils.

The compounds, both as hydrocarbons and S-containing anologues, may prove useful environmental indicators once the sources and exact structures of more of them have been established.

1.9   SCOPE AND FRAMEWORK OF THE THESIS

The general objective of the research described in this thesis is to further

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assess the potential of HBI hydrocarbons as molecular indicators of paleoenvironments. In order to meet this objective a better knowledge of the structures, sources and short-term fate of HBI alkenes is a prerequisite. Comparison of spectroscopic, GC retention and ozonolysis data of synthetic monoenes with sedimentary compounds facilated correct structural assignments herein. Comparison of the distribution of HBI hydrocarbons in sediments and biota and the determination of molecular isotopic signatures has indicated likely biological sources for the sedimentary compounds.

Chapter 2 demonstrates the great care required during the identification of such alkenes as cyclic purely on the basis of hydrogenation behaviour and mass spectra interpretation of the hydrogenation products. Some HBI alkenes resistant to hydrogenation have been assigned previously as cyclic. This chapter provides compelling evidence that one C_{25} HBI diene present in Antarctic sediments is acyclic. These features emphasise the need for further studies involving synthesis of the alkenes and the importance of establishing the positions and geometry of the double bonds in more of the sedimentary alkenes.

Chapter 3 describes the attempted synthesis of HBI alkenes and the successful isolation and characterisation of a number of previously synthesised monoenes (Robson, 1987). Several novel compounds are produced via isomerisation of previously synthesised monoenes. Some of these have also been isolated and identified. Isolation of pure isomers or isomeric pairs was made using argentation chromatography (HPLC and TLC). Structural assignments based on spectroscopic examination (*i.e.* GC-MS, IR and ^{1}H NMR) and micro-ozonolysis studies are discussed. The relationships between homologues of synthetic alkenes are confirmed.

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Chapter 4 describes the use of the synthetic monenes to assign structures and partial structures to naturally occurring hydrocarbons in three sediments. Isolation of pure isomers from sediments was made using normal and argentation chromatography (TLC). Structural assignments based on chromatographic and spectroscopic examination (*i.e.* GC RI, GC-MS and ^{1}H NMR) and micro-ozonolysis studies are discussed.

Chapter 5 describes the distribution of C_{20} and C_{25} HBI hydrocarbons in recent estuarine sediments and in related biota. The isotopic compositions of alkane 1 and a related monoene, conclusively identified in Chapter 4, are determined. The results suggest likely sources for the sedimentary HBI hydrocarbons. The spatial and temporal distribution of sedimentary HBI hydrocarbons is reported and the implications discussed.

One of the reasons proposed for the removal of HBI alkenes from the hydrocarbon fraction in certain sediments is that they are accreted into humic substances during diagenesis (Volkman *et al.*, 1983). Pyrolysis-GC-MS of humic substances from Peru upwelling zone sediments and incorporation experiments using a mixture of synthetic C_{25} monoenes and melanoidins, acidic polymeric products of amino acid/sugar condensation reactions proposed to be model humic acids (Larter and Douglas, 1978), are described in Chapter 6. The results demonstrated that although pyrolysis of the spiked melanoidins released the recognisable isomeric monoene mixture, no HBI alkenes or recognisable fragments were produced by pyrolysis of the sedimentary humic substances. The implications of these results are discussed.

STRUCTURES

CHAPTER ONE
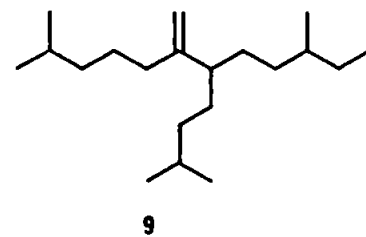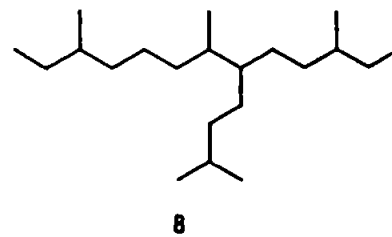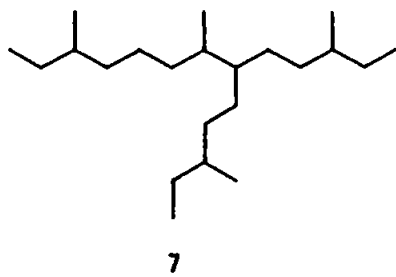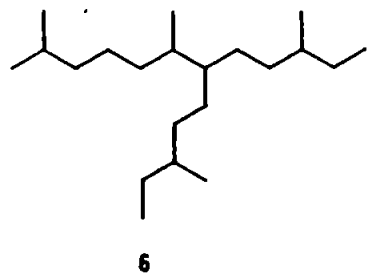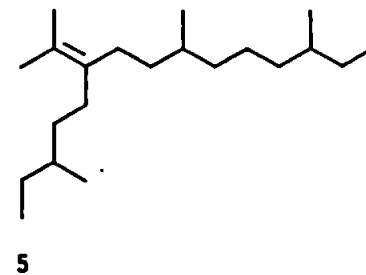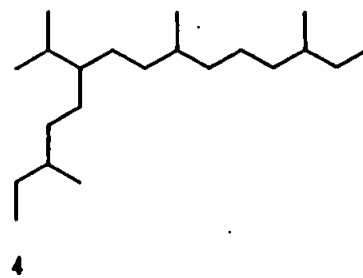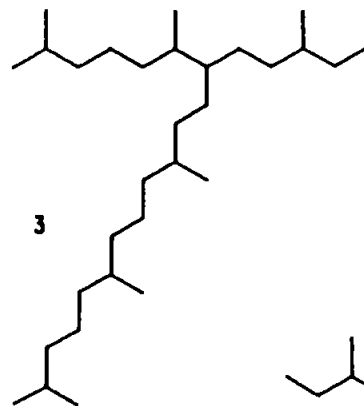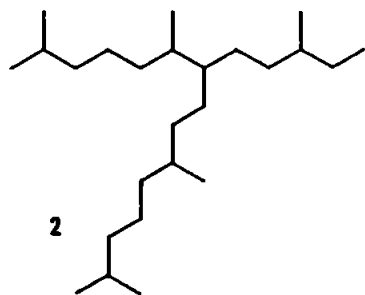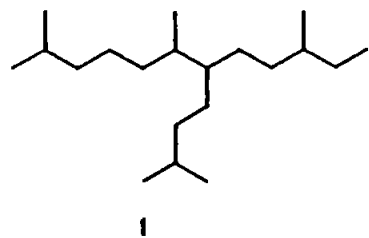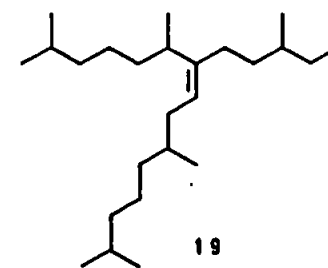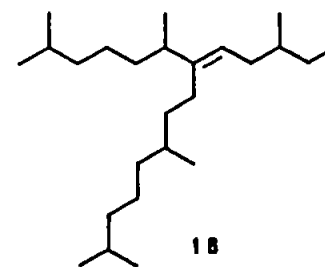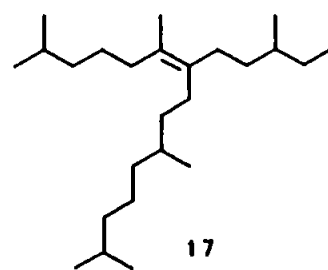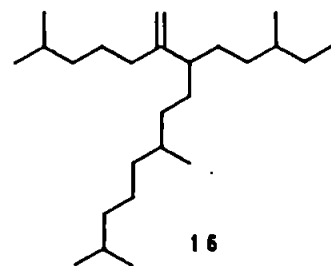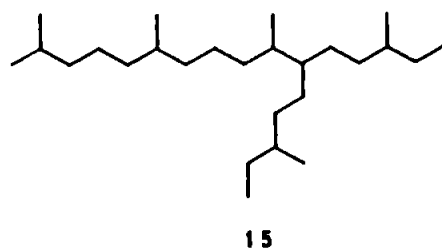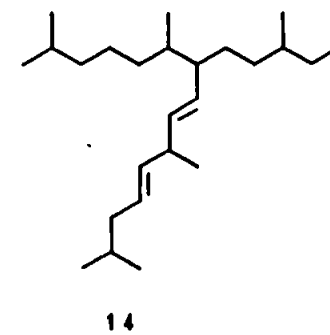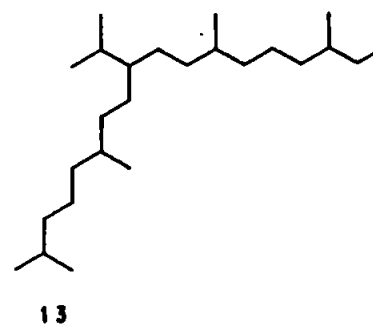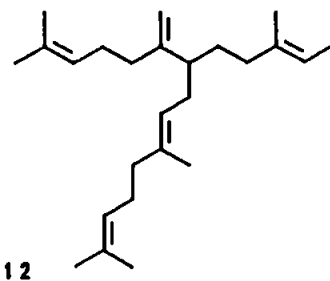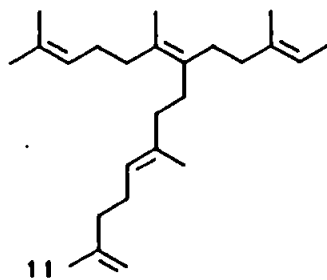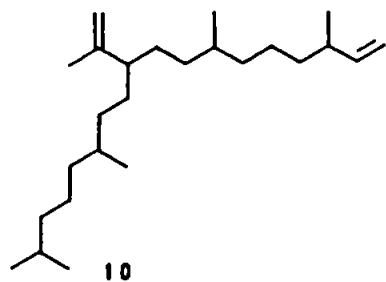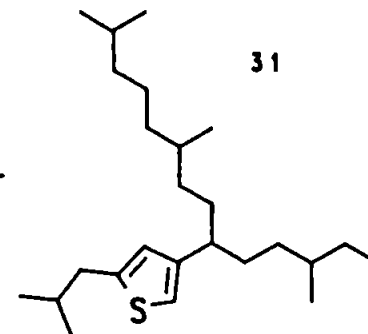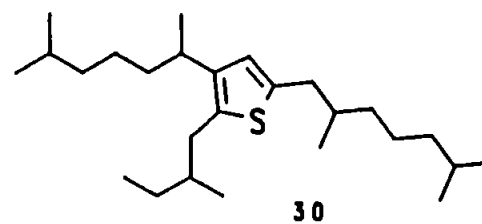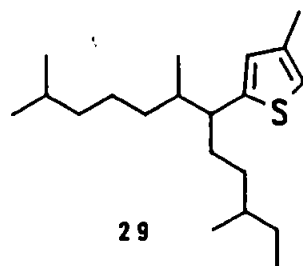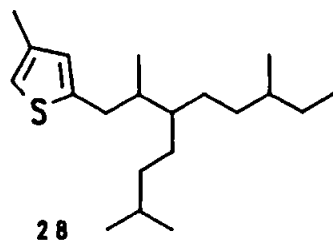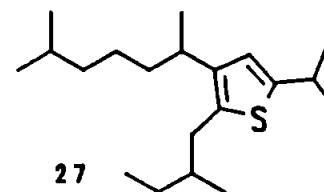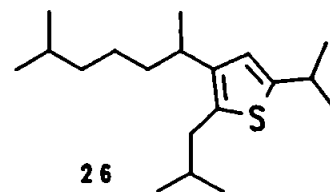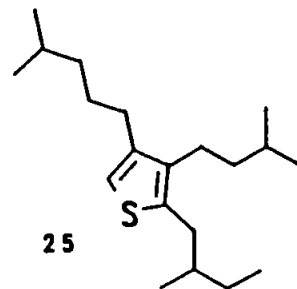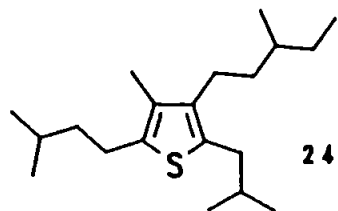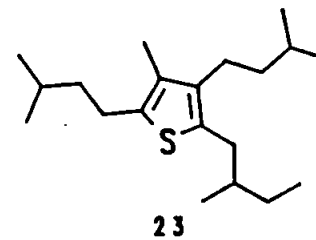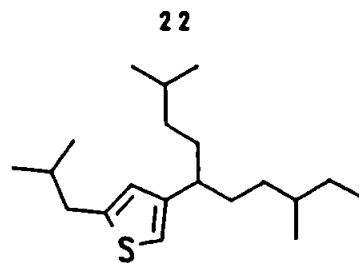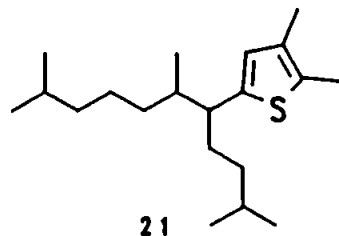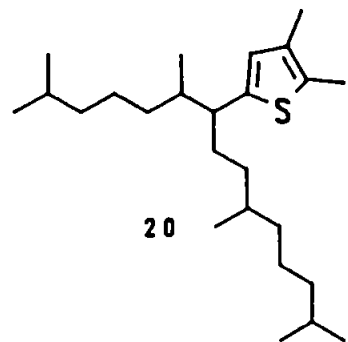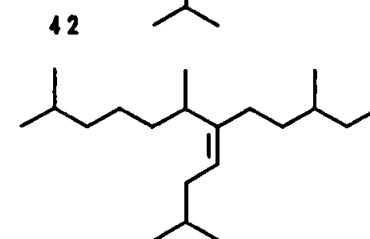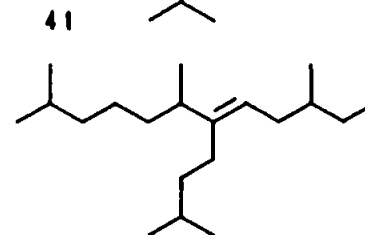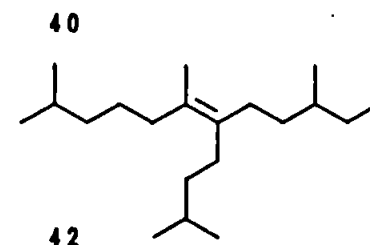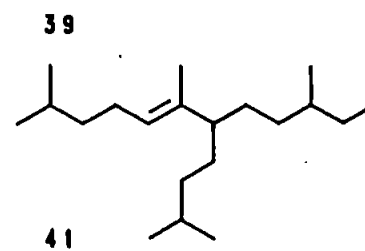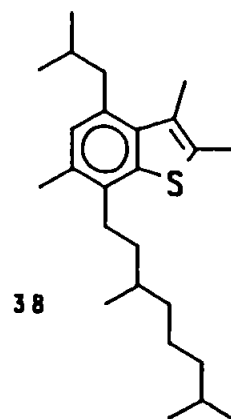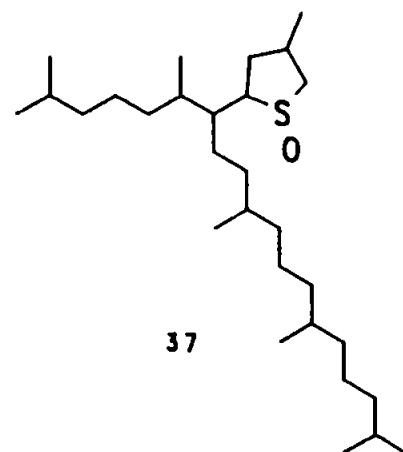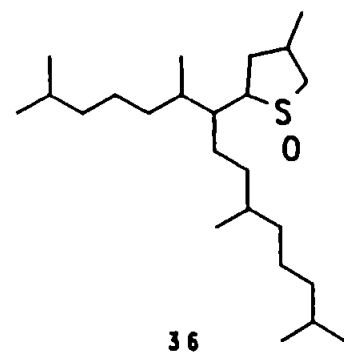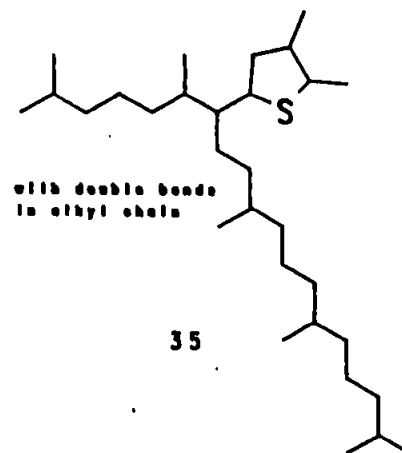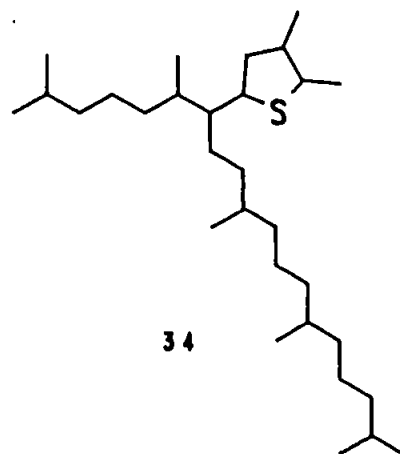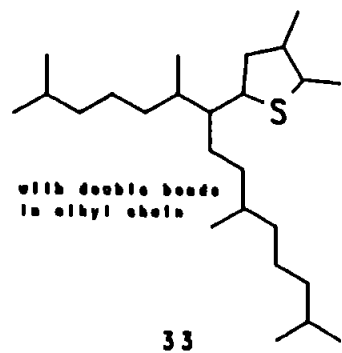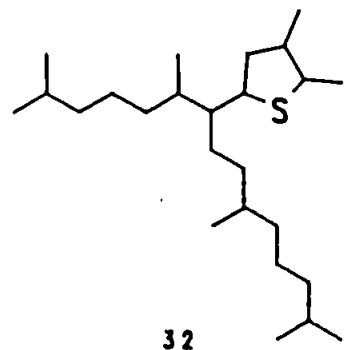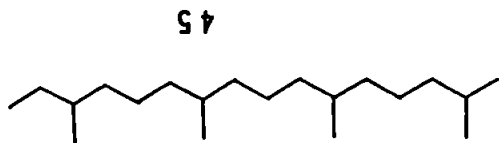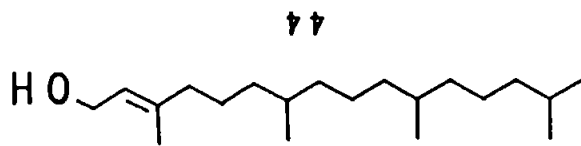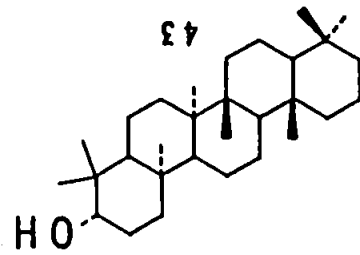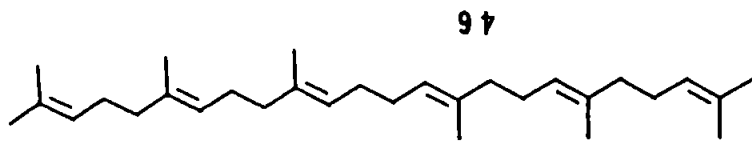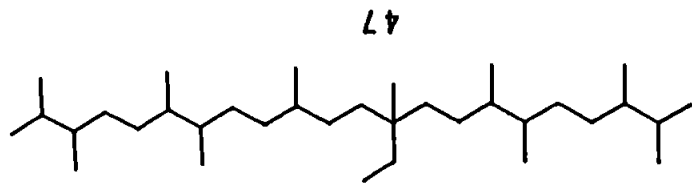
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with double bonds
in alkyl chain

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35
with double bonds
in alkyl chain

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HO

HO

CHAPTER TWO

HYDROGENATION BEHAVIOUR OF A C_{25} HIGHLY BRANCHED DIENE FROM AN ANTARCTIC MARINE SEDIMENT

In the literature the structural elucidation of C_{25} and C_{30} HBI alkenes has been based mainly on the analysis of their hydrogenation products. However, some confusion has been generated since some of these alkenes could not be fully hydrogenated, and it has been concluded by some authors that the alkenes are cyclic. This chapter clarifies the confusion in the case of the C_{25} HBI alkenes. Analysis of such hydrogenation products by GC and GC-MS revealed a mixture of alkane and monoene which could only be separated using a polar GC stationary phase. Further hydrogenation and analyses showed the HBI compound to be fully saturated. The formation of a $M^{+}-2$ ion under particular MS conditions, which added to the confusion, was investigated using MS-MS.

2.1 INTRODUCTION

The foregoing chapter emphasised that whilst alkanes 1-3 have been identified by synthesis, the identifications of sedimentary C_{20}, C_{25} and C_{30} HBI alkenes has often been less rigorous. Indeed, most alkene assignments have been made either on the basis of complete hydrogenation of the alkenes to 1-3 or have been even more perfunctory and based entirely on mass spectral interpretation. Considerable confusion has resulted from this approach since some alkenes can only be fully hydrogenated with difficulty, and some workers have concluded erroneously that partially hydrogenated products are cyclic.

For example, in a discussion of the geochemistry of C_{25} and C_{30} biogenic alkenes of Narrangansett Bay estuary Requejo and Quinn (1983a) noted a compound, RI 2079_{SE30}, which exhibited a mass spectrum almost identical to that of an alkene identified as the HBI diene br25:2; 2084_{SE30} and yet they proposed that the compound was a monocyclic monoene (c25:1:1; 2079_{SE30}) because of the molecular ion at m/z 350 observed in the spectrum of the hydrogenation product (presumed to be c25:1:0; 2104_{SE30}). However, an alternative explanation is that c25:1:1; 2079_{SE30} could contain a double bond which was resistant to hydrogenation, and simply be a branched positional or geometric isomer of br25:2; 2084_{SE30}. Similarly, Prahl *et al.* (1980) reported a suspected C_{30} monocyclic tetraene c30:4:1; 2509_{SP2100} in their study of Dabob Bay sediments even though hydrogenation produced a compound with a retention index of 2524_{SP2100}, the mass spectrum of which had none of the particular fragment ions characteristic of cyclic alkanes (*e.g. m/z* 123; Noble, 1986). Barrick and Hedges (1981) later decided that the compound was acyclic and that one bond was hindered to hydrogenation.

Such reports, several of which are summarised in Table 2.1, introduce an element of confusion into the firm assignments where HBI alkenes (*e.g.* br25:2; 2083_{OVI}; Robson and Rowland, 1986) have been attributed to acyclic skeletons 1-3 by comparison with synthetic compounds. A number of these studies require re-examination, as detailed in the following example.

The hydrocarbon chemistry of Antarctica has been reviewed by Cripps and Priddle (1991). In sediments from McMurdo Sound and Bransfield Strait, Antarctica, Venkatesan (1988), Venkatesan and Kaplan (1987) and Brault and Simoneit (1988) identified C_{25} dienes (RI 2082_{DBS}; RI 2088_{DBS}) with identical mass spectra which when hydrogenated by "passing hydrogen at a rate of 38-40 cm^3 for 45 minutes to a stirred suspension of PtO_2 in hexane" (Venkatesan, 1988), produced compounds RI 2106_{DBS} and 2101_{DBS}, the mass spectra of which contained an apparent molecular ion m/z 350 ($C_{25}H_{50}$) (Figures 2.1). The authors noted that this incomplete saturation could be due to the presence of a highly double bond in the original diene which could not be hydrogenated under the above conditions but they rather favoured the monocyclic structure (Venkatesan, 1988).

An attempt is made here to clarify this confusion.

A

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0-2 cm
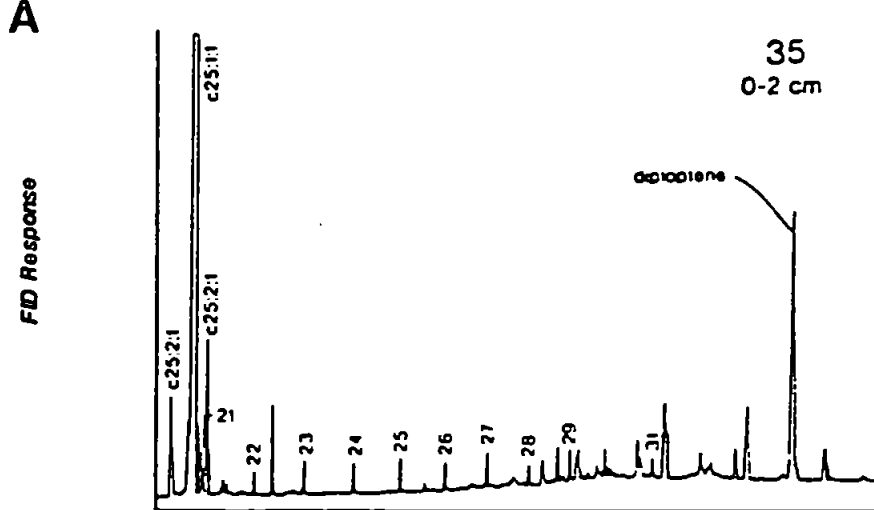
GC conditions; HP 5840, DB5 (30m x 0.25mm i.d., 0.25μm), 35-290°C @ 4°Cmin⁻¹·
MS conditions; Finnigan 4000; 70eV; 50-500amu @ 2 secscan⁻¹

B   *c25:1:1*

C   *hydrogenated compound c25:0:1*
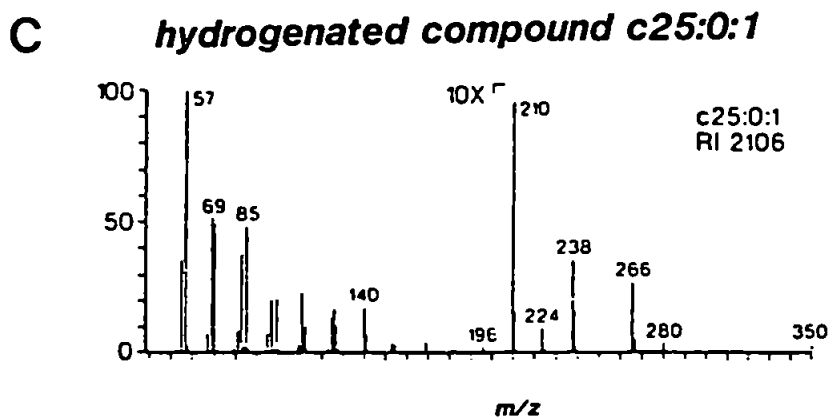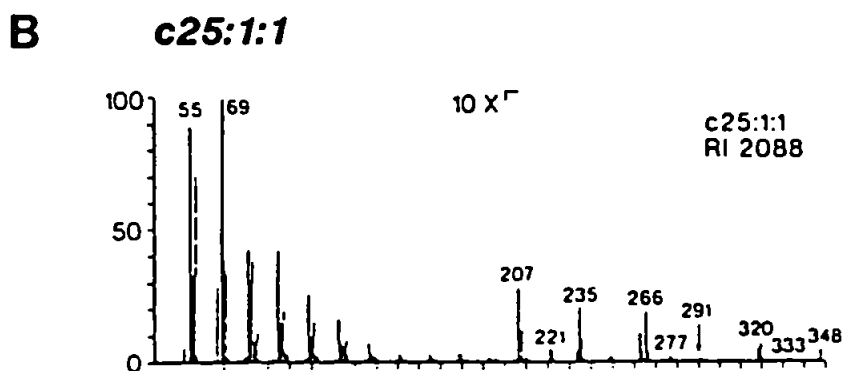
FIGURE 2.1 (A) Partial gas chromatogram of aliphatic hydrocarbons from McMurdo Sound sediment
(B) EI mass spectrum of *sic* c25:1:1
(C) EI mass spectrum of the hydrogenation product *sic* c25:0:1
(From Venkatesan, 1988).

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TABLE 2.1 OCCURRENCES OF SOME ACYCLIC C_{25} AND C_{30} ALKENES DESIGNATED CYCLIC STRUCTURES IN THE LITERATURE

Reference	Notation used	Characteristic ions (m/z)	Presumed hydrogenation product	Characteristic ions (m/z)	Comments
Venkatesan and Kaplan, 1987	c25:1:1; 2088$_{DBS}$	207,235,266,320,348	c25:0:1; 2101$_{DBS}$	210,238,266,350	monoene br25:1; 2110$_{OVI}$ (2101$_{DBS}$) hydrogenated to C_{25} HBI alkane 1 by Robson and Rowland, 1986
Requejo et al., 1984	br25:2; 2088	207,235,266,320,348	br25:0; 2111$_{SE30}$	210,238,266	
Nichols et al., 1988	br25:2; 2088$_{MS}$	207,235,266,320,(no M$^+$)	br25:0; 2111$_{MS}$	210,238,266	
Venkatesan, 1988 Robson and Rowland, 1986	c25:1:1: 2083$_{DBS}$ br25:2; 2083$_{OVI}$	207,235,266,320,348 207,266,320,348	c25:0:1; 2106$_{DBS}$ br25:0; 2109$_{OVI}$	210,238,266,350 238,266/7	br25:1; 2107$_{OVI}$ hydrogenated by Robson, 1987
Requejo and Quinn, 1983a	c25:1:1; 2079$_{SE30}$ br25:2; 2084$_{SE30}$	207,235,266,348 207,235,266,320,348	c25:0:1; 2104$_{SE30}$ br25:0; 2111$_{SE30}$	210,238,266,350 210,238,266	mass spectra of RI 2079 and 2084 almost identical
Prahl et al., 1980	c30:4:1; 2509$_{SP2100}$	203,231,299,357,412	c30:0:1; 2524$_{SP2100}$	211,225,308,420	C_{30} HBI pentaene with one hindered double bond
Barrick and Hedges, 1981	br30:5; 2509$_{SP2100}$	203,231,299,357,412	br30:0 + br30:1	211,225,308,420	

2.2 RESULTS AND DISCUSSION

McMurdo Sound aliphatic hydrocarbons isolated by Venkatesan (1988) were supplied by Dr. Venkatesan (personal communication).

The hydrogenation products of the aliphatic hydrocarbons from McMurdo Sound (Venkatesan, 1988) sediment were re-examined by GC and GC-MS on DB5 and DB1 stationary phases. Examination of the chromatogram of these hydrogenation products contained a major peak (RI 2101_{DB5}; 2110_{DB1}) which coeluted with synthetic **2**. This was in contrast to Venkatesan (1988) who recorded the RI of the hydrogenated compound at 2106_{DB5}. The EI mass spectrum (40eV, 250°C source temperature; Figure 2.2A) contained an apparent molecular ion m/z 350 and ^{13}C isotope ion at m/z 351 for a C_{25} monoene. Under the same conditions a mixture of synthetic C_{25} monoenes **4** (Robson, 1987) produced a similar m/z 350 and m/z 351 (Figure 2.2B). Much of the remainder of the spectrum of the McMurdo Sound sample resembled that of synthetic **2** (viz. m/z C_nH_{2n+1}, 85, 99... Robson and Rowland, 1986; Figure 2.2C). Indeed, computer subtraction of the spectrum of synthetic **2** from that obtained for the McMurdo sample produced a spectrum (Figure 2.3) similar to that of the C_{25} HBI monoene **5** identified in Shark Bay, Western Australia, and which had a similar retention index (RI 2110_{DB1} McMurdo; RI 2112_{MS} **5**; Dunlop and Jefferies, 1985). This suggested that the hydrogenation product from the McMurdo Sound sediment was in fact a coeluting mixture of **2** and a C_{25} monoene (**5**?). This would adequately explain the mass spectrum. This was confirmed when chromatography on CPWAX52 phase produced a separation of the suspected mixture into two components of approximately equal concentration (RI $2065_{CPWAX52}$; RI $2092_{CPWAX52}$; Figure 2.4A) with only the former coeluting with synthetic **2**.
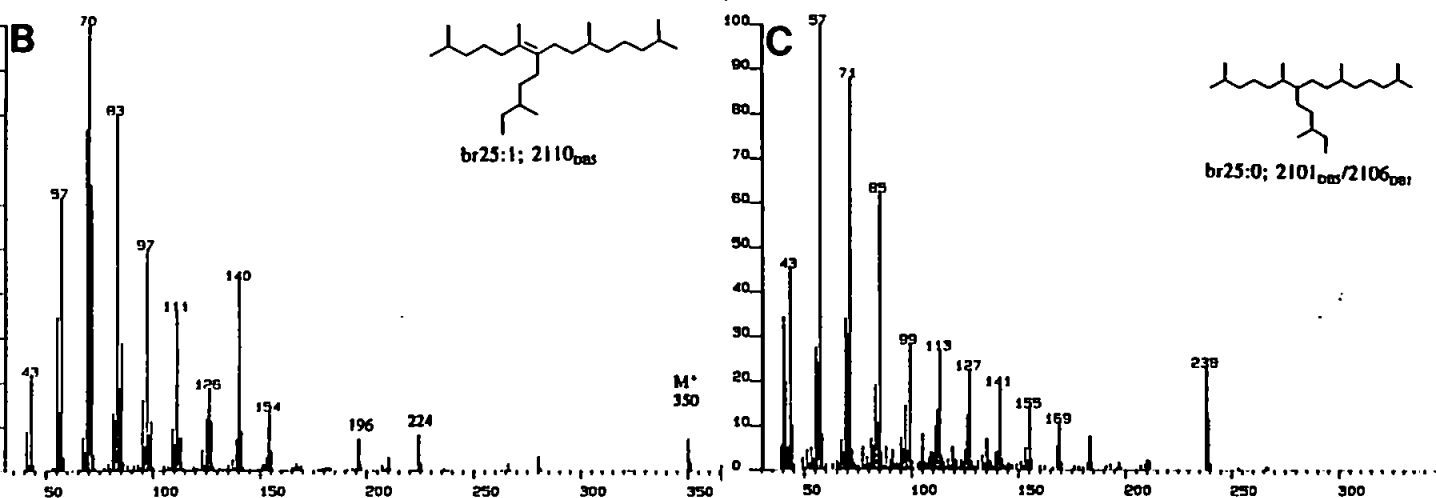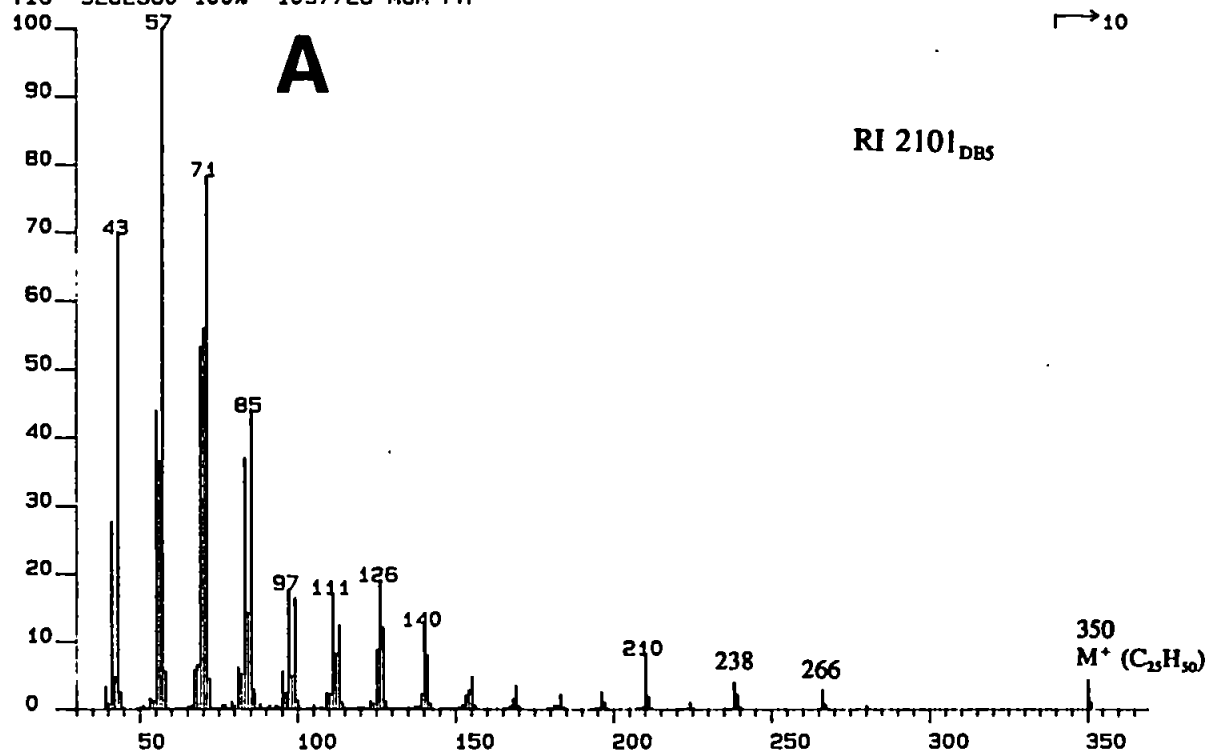
FIGURE 2.2 EI MASS SPECTRA OF
(A) partial hydrogenation product
(B) synthetic C_{25} HBI monoene
(C) synthetic C_{25} HBI alkane

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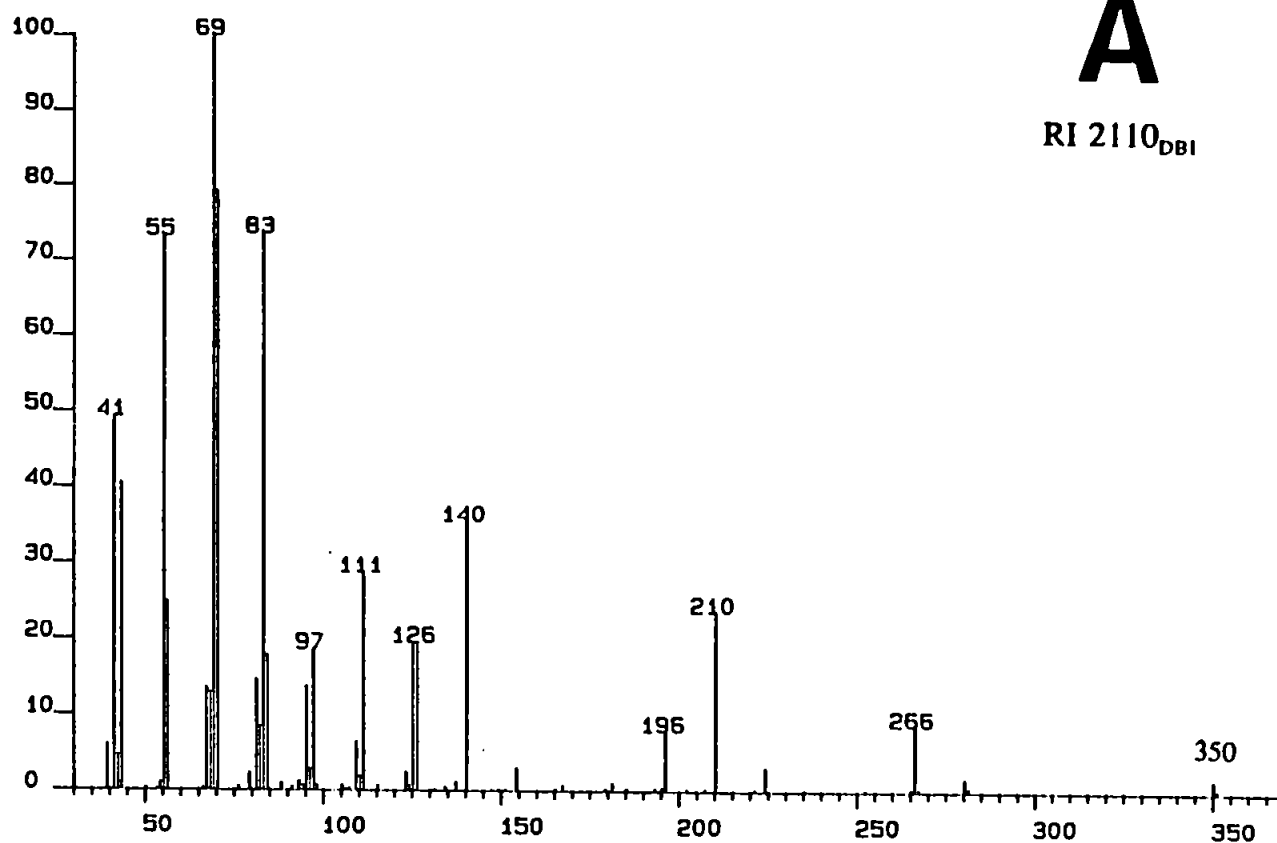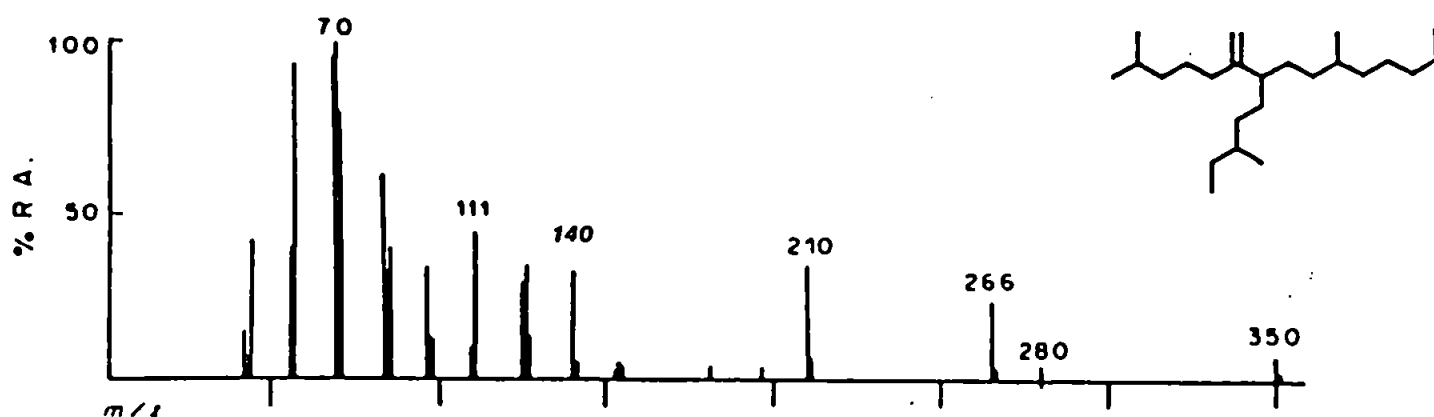
FIGURE 2.3 (A) Computer-generated mass spectrum produced by subtraction
of the spectrum of synthetic C_{25} HBI alkane from that of the
mixture of partial hydrogenation products
(B) Mass spectrum of the C_{25} HBI monoene from Shark Bay,
Australia

82
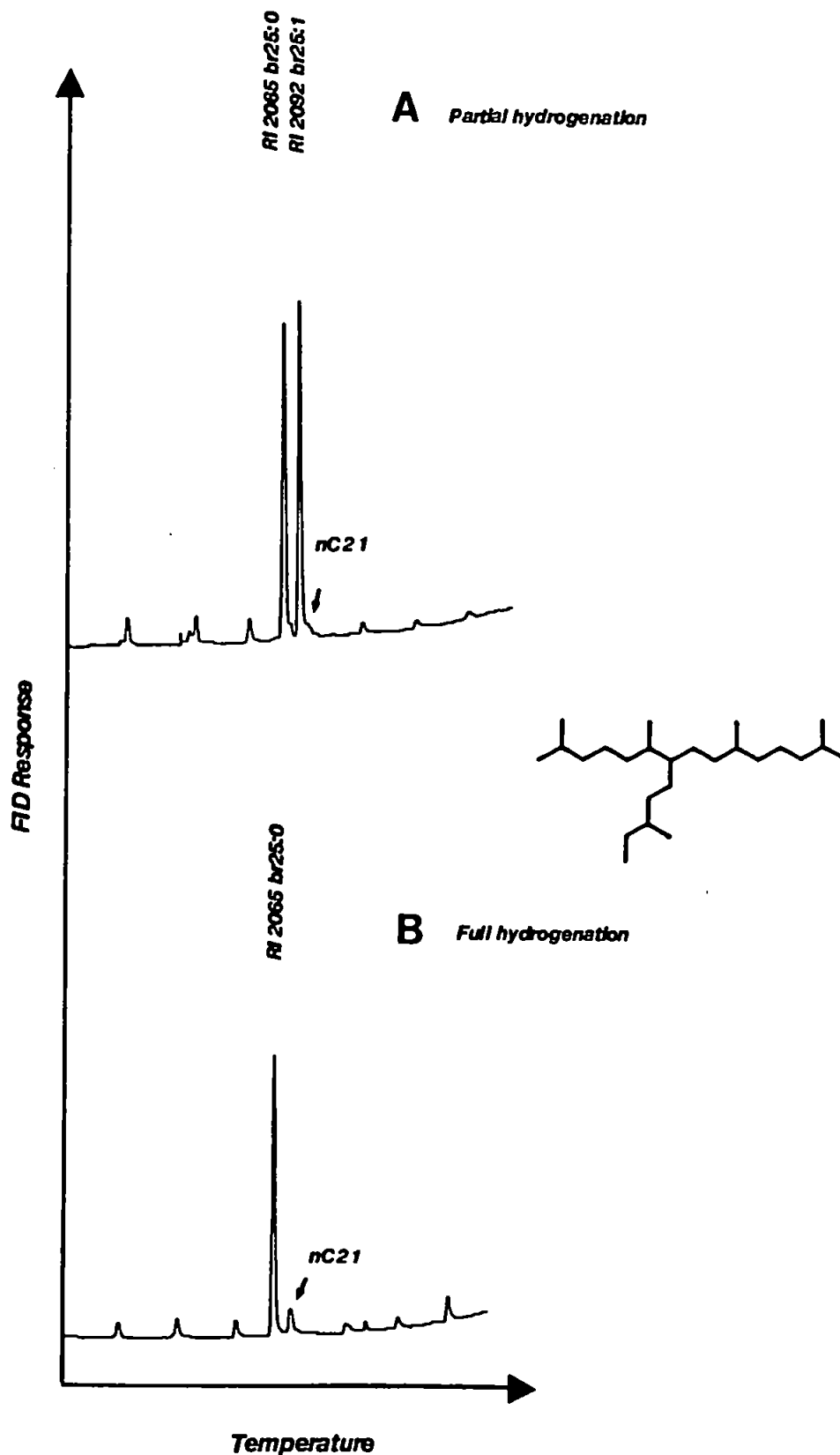
FIGURE 2.4 PARTIAL GAS CHROMATOGRAMS (CPWAX52) OF McMURDO SOUND ALIPHATIC HYDROCARBONS AFTER
(A) Partial hydrogenation
(B) Full hydrogenation

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Further hydrogenation of the McMurdo Sound sample (60 minutes; $PtO_2.H_2O$),

GC on CPWAX52 and the coinjection of synthetic **2**, showed that the second

component (RI $2092_{CPWAX52}$) had now been completely converted to **2** (RI $2065_{CPWAX52}$;

Figure 2.4B). Whilst this reaction could not be observed on the apolar GC stationary

phases where the two compounds were not separable (Figure 2.5), nevertheless the

mass spectrum (Figure 2.6) after further hydrogenation was identical to that of **2**

under the same conditions; notably no M^+ ions at m/z 350 or m/z 352 were present

in either.

These data show that the C_{25} monoene, partial hydrogenation products from

McMurdo Sound sediments, and hence the C_{25} diene (RI 2082_{DB5}) from which it

originated was not monocyclic but acyclic (*viz.* skeleton **2**). Indeed, the diene

therefore has the same carbon skeleton as the diene (br25:2; 2088_{MS}) isolated from

mixed sea-ice diatom communities in McMurdo Sound (Nichols *et al.*, 1988) and

many other sediments (Rowland and Robson, 1990). The hydrogenation conditions

used by Venkatesan (1988) simply did not produce complete saturation of all the

diene. Complete hydrogenation requires conditions similar to those used by Nichols

et al. (1988) where hydrogen was bubbled through a suspension of the hydrocarbons

and PtO_2 (Adams catalyst) for 3 hours, a procedure similar to that used during the

present study (H_2, $PtO_2.H_2O$, 60 min.; section **8.3**).
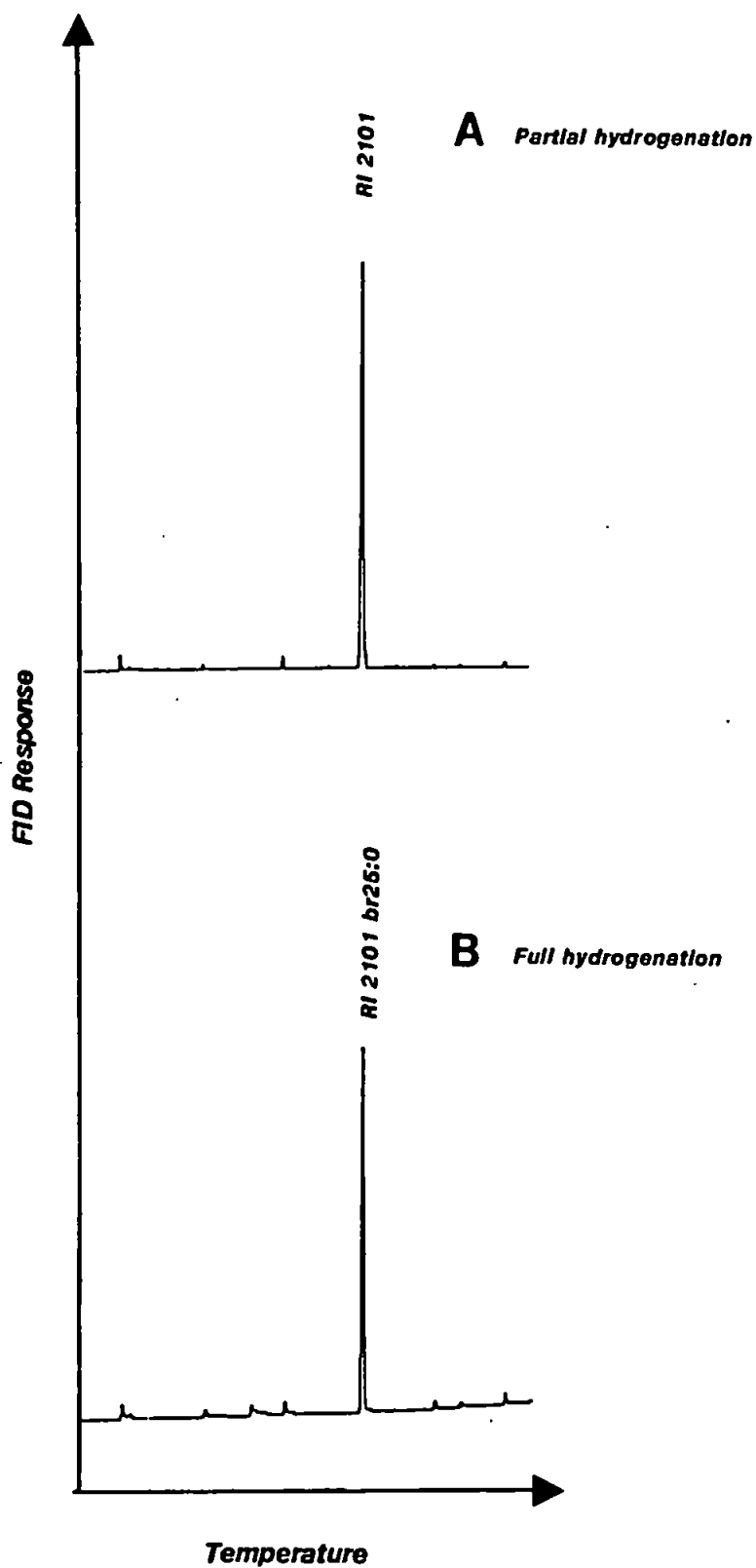
FIGURE 2.5 PARTIAL GAS CHROMATOGRAMS (DB5) OF McMURDO SOUND ALIPHATIC HYDROCARBONS AFTER
(A) Partial hydrogenation
(B) Full hydrogenation

85

A

RI 2101$_{DB5}$

B

br25:0; 2101$_{DB5}$/2106$_{DB1}$
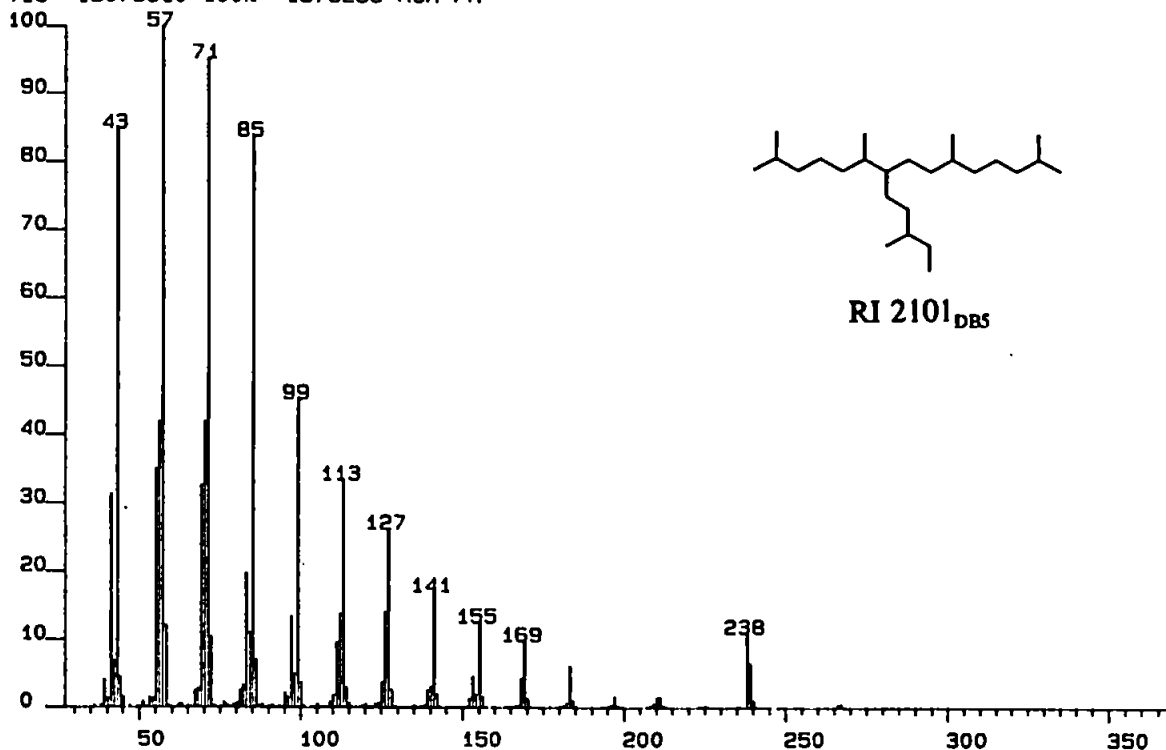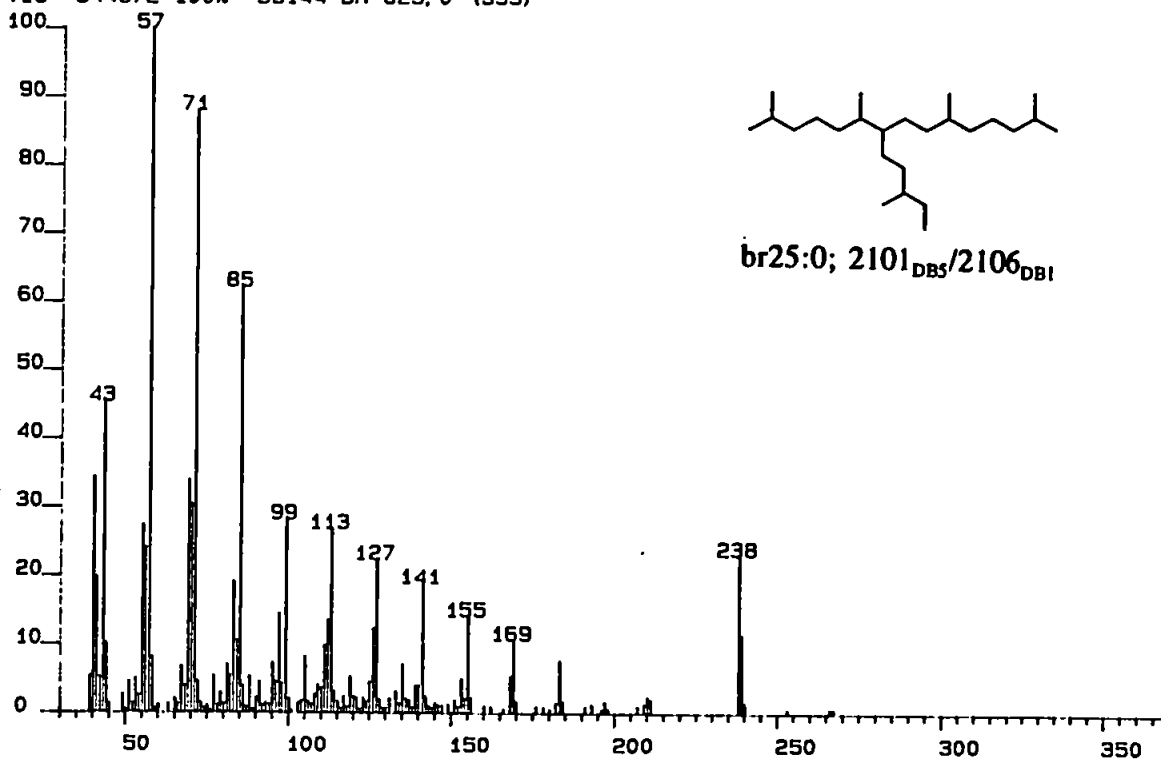
FIGURE 2.6 EI MASS SPECTRA (40eV; 250°C) OF
(A) Product of full hydrogenation
(B) Synthetic C$_{25}$ HBI alkane

To obtain further evidence that the complete hydrogenation product from McMurdo Sound, (RI 2110_{DBI}; 2101_{DBS}; $2065_{CPWAX52}$) was a C_{25} HBI alkane, both it and synthetic 2 were examined at mass spectral operating conditions which are less favourable to fragmentation (20eV; 200°C source temperature) and which were thought to be more favourable to the formation of the molecular ion. The molecular ion m/z 352 was observed for both under these conditions (Figure 2.7). However, quite unexpectedly, a very small m/z 350 ion was also observed for both. That synthetic 2 was fully saturated was confirmed by ^{13}C and ^1H NMR, HRMS and IR spectroscopy (Robson, 1987; Robson and Rowland, 1986) so it was suspected that m/z 350 was an M^+-2 ion from m/z 352.

A number of worker have also observed such M^+-2 ions in the mass spectra of isoprenoid and HBI hydrocarbons (Sinninghe Damsté, Volkman and Summons; personal communications). Summons and Capon (1991) reported that the EI mass spectrum of synthesised botryococcane showed no molecular ion although the M^+-2 species was prominent. The phenomenon is evident in the mass spectra of other botryococcanes (Metzger et al., 1985; 1988) and was originally reported by Maxwell et al. (1968). The M^+-2 ion seems likely to be an artefact ion rather than the result of unsaturated or moncyclic impurities given the rigorous characterisation of synthetic HBI C_{25} alkane described by Robson and Rowland (1986; 1988a) and botryococcane by Summons and Capon (1991).
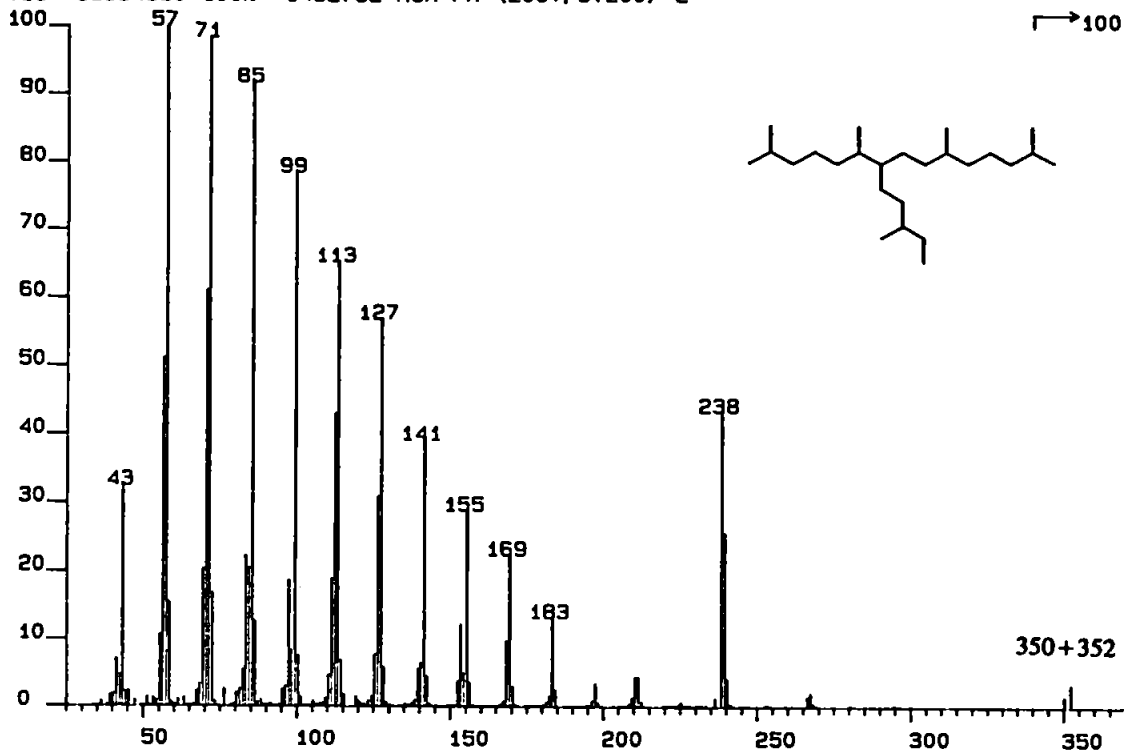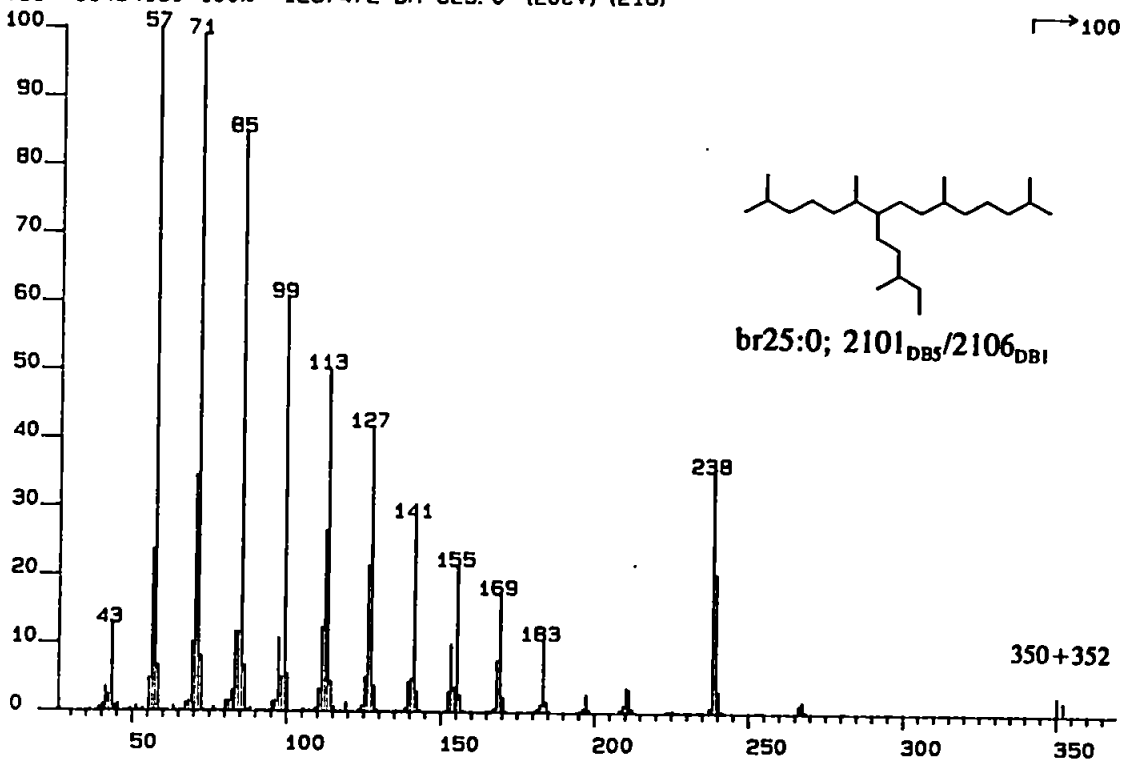
FIGURE 2.7 EI MASS SPECTRA (20eV; 200°C) OF
(A) Product of full hydrogenation
(B) Synthetic C$_{25}$ HBI alkane

In an attempt to prove the M^+-2 association of *m/z* 350 and *m/z* 352 both were examined by tandem mass spectrometry (MS-MS) under EI source conditions which produced the two ions of interest (40eV; 130°C source temperature). Using a tandem quadrupole mass spectrometer, the first quadrupole (Q1) provides parent (precursor) ion selection from the source and the third quadrupole (Q3) provides daughter (product) ion selection from the collision cell. The second quadrupole (Q2) is not a mass filter, but provides nonmass selective ion containment for the low-energy collisionally activated dissociation (CAD) process. The following procedure was applied to establish any association of ions *m/z* 350 and *m/z* 352. A daughter scan provided a spectrum of all the daughter ions produced by fragmentation of the selected ion induced by CAD using argon gas. This was obtained by setting Q1 to pass *m/z* 352 and scanning Q3 for all the fragment ions. A parent scan was the spectrum of all the parent ion masses that produced the particular daughter mass which was obtained by scanning Q1 with Q3 set for the daughter ion at *m/z* 350. However the experiment failed to show that *m/z* 350 was a daughter ion of *m/z* 352, or that *m/z* 352 was a parent of *m/z* 350 (Figure 2.8).

It seems likely that *m/z* 350 is produced by dehydrogenation of **2** within the EI ion source by some unusual thermal or catalytic process that does not occur in the collision cell of the tandem quadrupole MS-MS instrument (TSQ) *i.e.* was not induced by low energy CAD (40-4eV). This finding does not detract from the conclusion that the sedimentary alkenes are acyclic, but it does emphasise the great care necessary in such studies.
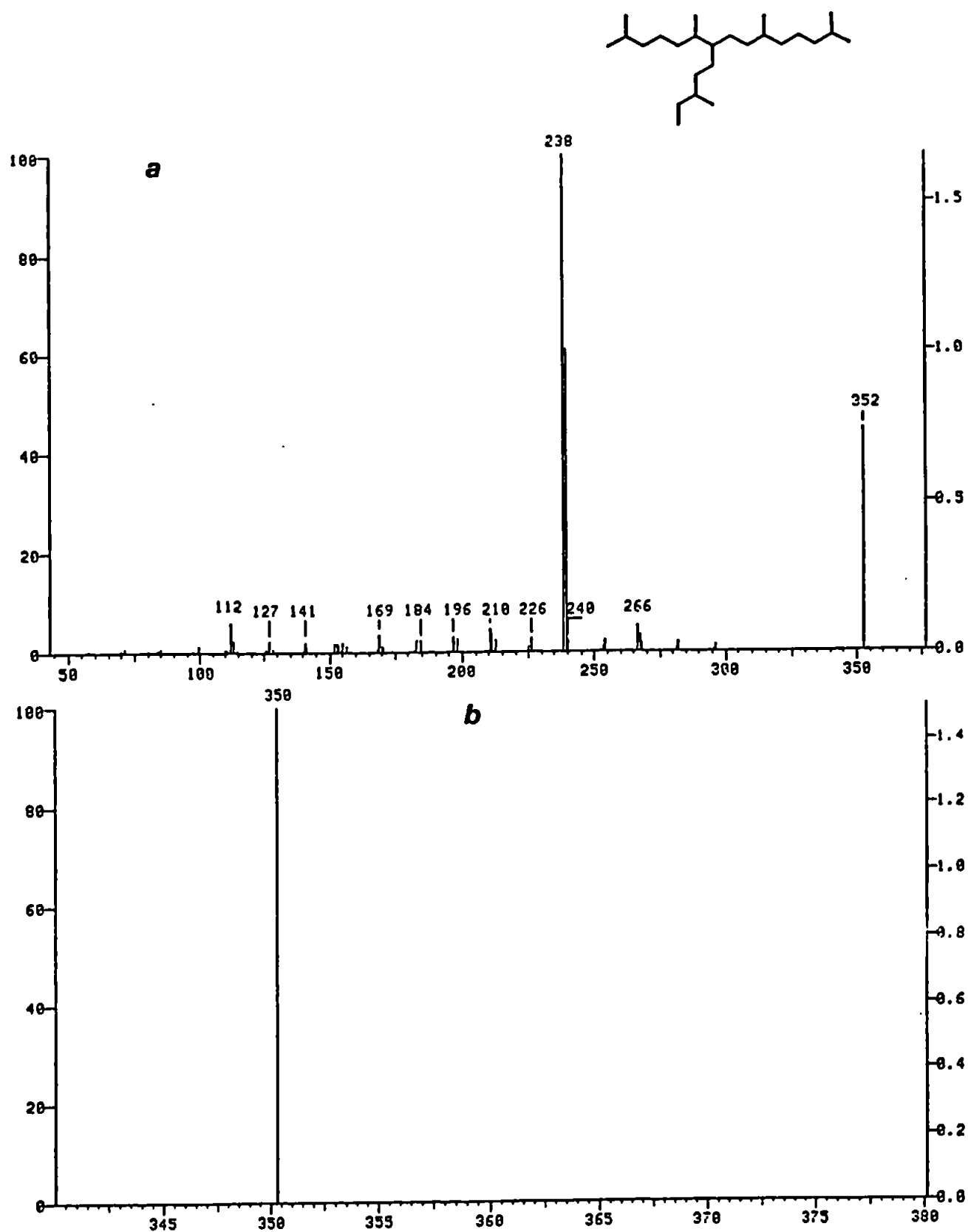
FIGURE 2.8 EI MASS SPECTRA OF SYNTHETIC C$_{25}$ HBI ALKANE
(a) Daughter ions of m/z 352
(b) Parent ions of m/z 350

2.3 CONCLUSIONS

It is clear that great care must be taken with the identification of HBI alkenes. The non-hydrogenation behaviour of some compounds and ease of hydrogenation of others makes identification tortuous. It seems possible that changes in the positions and/or geometry of double bonds may be taking place even in the presence of hydrogenation catalysts. Several studies probably bear re-examination in the light of these results. For example, the data of Requejo and Quinn (1983a) for *sic* c25:1:1; 2079_{SE30} and its hydrogenation product (*sic* c25:0:1; 2104_{SE30}) and of Barrick and Hedges (1981) for "HC412" (*viz.* suspected C_{30} monoene). The mass spectrum of c25:0:1; 2104_{SE30} is similar to that of the partially hydrogenated C_{25} diene from McMurdo Sound and may represent a similar mixture of **2** and **5**.

The occurrence of the C_{30} skeleton psuedohomologous to **2** in recent sediments was also firmly established by Robson and Rowland (1986; 1988a) who synthesised the C_{30} HBI alkane **3** which was shown to have a mass spectrum and retention index (2524_{OV1}) very similar to that of the hydrogenation product of the C_{30} pentaene noted by Prahl *et al.* (1980) and Barrick and Hedges (1981). The latter exhibited an apparent molecular ion at *m/z* 420, whereas no molecular ion was observed for synthetic **3**.

Circumstantial evidence for the acyclic nature of C_{30} polyenes present in Black Sea sediments was presented by Kohnen *et al.* (1990a). Catalytic hydrogenation of the corresponding unsaturated C_{30} HBI thiolanes yielded exclusively compounds showing a molecular ion at *m/z* 452, which was consistent with a C_{30} thiolane with an acyclic carbon skeleton. Raney Ni desulphurisation and subsequent hydrogenation of these OSCs yielded the C_{30} HBI alkane **3** and a minor amount of a related monoene. Catalytic hydrogenation of the C_{30} polyenes yielded the fully hydrogenated

3 and a related monoene. The latter displayed a mass spectrum similar to that of the monoene obtained upon desulphurisation of the C_{30} HBI OSCs namely an apparent molecular ion at m/z 420 and a number of enhanced fragment ions at m/z 196, 210, 224, 266 and 280. Therefore, Kohnen *et al.* (1990a) concluded that the C_{30} HBI polyenes present in the Black Sea sample were indeed acyclic.

Thus, there is evidence that the apparent molecular ion at m/z 420 in the mass spectrum of the product of hydrogenation of the C_{30} pentaene (Prahl *et al.* ,1980; Barrick and Hedges, 1981) was either an M^{+}-2 ion from m/z 422 or M^{+} of a very small amount of unhydrogenated monoene containing a hindered double bond.

In contrast, the mass spectra of some C_{30} alkenes have been reported to exhibit particular fragment ions which could be associated with bicyclic alkanes. For example, the mass spectrum of the hydrogenation product of the major C_{30} alkene identified by Requejo and Quinn (1983a) in sediment from Narrangansett Bay had a base peak at m/z 193 which is similar to the mass spectra of several bicyclic alkanes (Noble, 1986). It appears therefore, that there are indeed both cyclic and acyclic C_{30} alkenes present in marine sediments and the situation is generally more complex than for the C_{20} and C_{25} alkenes which mostly have the acyclic structures 1 and 2.

The results from these analyses demonstrate an obvious need for further synthetic HBI compounds in order to more fully characterise the HBI hydrocarbons in the environment.

STRUCTURES

CHAPTER TWO
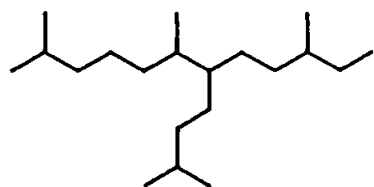
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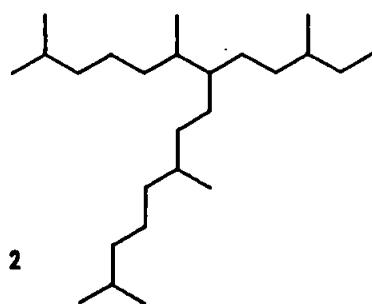
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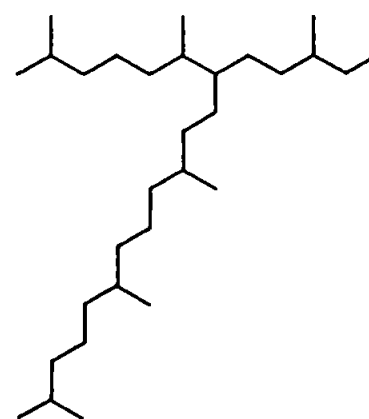
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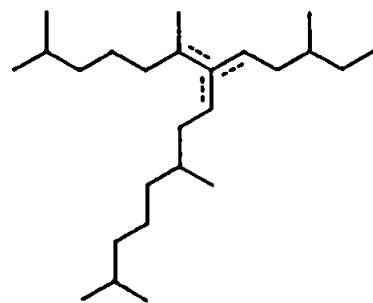
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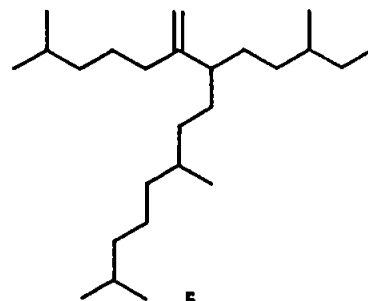
5

CHAPTER THREE

ISOLATION AND CHARACTERISATION OF SYNTHETIC HBI ALKENES

This chapter describes the attempted synthesis of C_{20} and C_{25} HBI monoenes and the successful isolation and characterisation of several C_{20}, C_{25} and C_{30} monoenes synthesised previously. The formation of novel monoenes via isomerisation reactions is also described. Isolation of pure isomers or isomeric pairs from the mixtures was made using argention chromatography (HPLC and TLC). Structural assignments based on spectroscopic examination (i.e. GC-MS, IR, ^1H NMR) and micro-ozonolysis studies are discussed. The relationships between homologues of synthetic alkenes are confirmed.

3.1   INTRODUCTION

The need for authentic samples of synthetic HBI alkenes for the identification

of sedimentary alkenes has been amply demonstrated in the preceding chapters just

as the successful identification of the HBI alkanes was greatly aided by the synthesis

of 1-3 (Yon et al., 1982; Robson and Rowland, 1986; 1988a). The number and

position of double bonds in the HBI alkenes has been shown to influence the fate of

these compounds including their transformation into sulphur-containing HBI, which

are potentially useful biological markers. Although GC-MS is a common analytical

technique applied to the monitoring of hydrocarbons in the environment, electron

impact (EI) mass spectrometry is not very helpful for the location of double bond

positions because of the ease of electron-induced isomerisation ($< 10^5$ seconds; see

review by Mackenzie, 1970; Borchers et al., 1977). In chemical ionisation mode

(CI), this problem has been approached by selection of reagent gases producing low

exothermic ion-molecule reactions and/or formation of adduct ions with the olefinic

double bonds (e.g. Hunt and Harvey, 1975; Budzikiewicz and Busker, 1980; Vine,

1980; Chai and Harrison, 1981; Doolittle et al., 1985; Einhorn et al., 1985; Scribe

et al., 1990). Such CI methods have proved useful for the structural elucidation of

individual unsaturated molecules, although, in some cases, the adducts produced are

not specific to alkene functions (see review by Attygalle and Morgan, 1988). They

are, however, inadequate for the analysis of complex mixtures containing branched

unsaturated compounds at the nanogram level and do not provide information on the

geometry of the double bonds.

The synthesis and full characterisation of authentic reference HBI compounds

has proved invaluable during the previous investigations of unknown sedimentary HBI

compounds (Yon *et al.*, 1982; Robson and Rowland, 1986; 1988a; Sinninghe Damsté,

1989ab; Kohnen *et al.*, 1990a). The present chapter, therefore, describes attempts to

synthesise such compounds. Unfortunately, these attempts failed and reasons are

discussed. In view of this, individual components were isolated from previously

synthesised mixtures (Robson and Rowland, 1986) and the individual isomers

characterised by spectroscopy and ozonolysis.

Previous syntheses of HBI alkenes resulted in the production of mixtures of

isomers (*e.g.* C_{25} HBI monoenes **4-6**) from the dehydration of the appropriate tertiary

alcohols (Robson and Rowland, 1986; 1988a).

WHERE R = CH_3,

Robson (1987) reported that these mixtures chromatographed on both apolar

and polar GC columns as five peaks with some partial coelution (Figure 3.1).

Classical derivatization techniques (*e.g.* methoxymercuration) failed to confirm the

double bond position, probably due to the hindered nature of the double bonds. In

addition, the isomers could not be separated by argentation TLC, however, it was

shown that monoenes with similar GC RI did exist in sediments (*e.g.* br25:1;

2078_{OVI}; Robson and Rowland, 1986).

FIGURE 3.1 GAS CHROMATOGRAMS OF THE SYNTHETIC ISOMERIC MIXTURES (Robson, 1987)
(A) 2,6,10-trimethyl-7-(3'-methylbutyl)dodecenes (br20:1)
(B) 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadecenes (br25:1)
(C) 2,6,10,14,18-pentamethyl-7-(3'-methylpentyl)nonadecenes (br30:1)
GC conditions: OV1 (GC^2), 40-80°C @ 10°Cmin^{-1}, 80°C-290°C @ 6°Cmin^{-1}.

It was considered that the identification of the double bond position in sedimentary HBI alkenes could best be proven by the introduction of the double bond in known positions, or failing this, in limited mixtures of isomers by synthesis. Confirmation of double bond position could be made by ozonolysis and NMR and geometry by FTIR. In designing protocols for the synthesis of the C_{20} and C_{25} HBI monoenes, it was considered beneficial if the scheme allowed the incorporation of synthons made available by previous syntheses (Robson, 1987; Robson and Rowland, 1986; 1988a).

3.1.2 SYNTHESIS OF A C_{25} HBI MONOENE

For the synthesis of one of the C_{25} HBI monoenes (*viz* 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadec-7(1')-ene, 6) a C_{19} isoprenoid ketone 7 was available and could, in theory, be conveniently coupled with a C_6 phosphonium bromide 8, to insert the double bond in the 7(1') position (Figure 3.2). The scheme was based on the Wittig reaction in which an aldehyde or ketone is treated with a phosphorus ylide (also called a phosphorane) to give an alkene (see reviews by Gosney and Rowley, 1979; Bestmann and Vostrowsky, 1983).
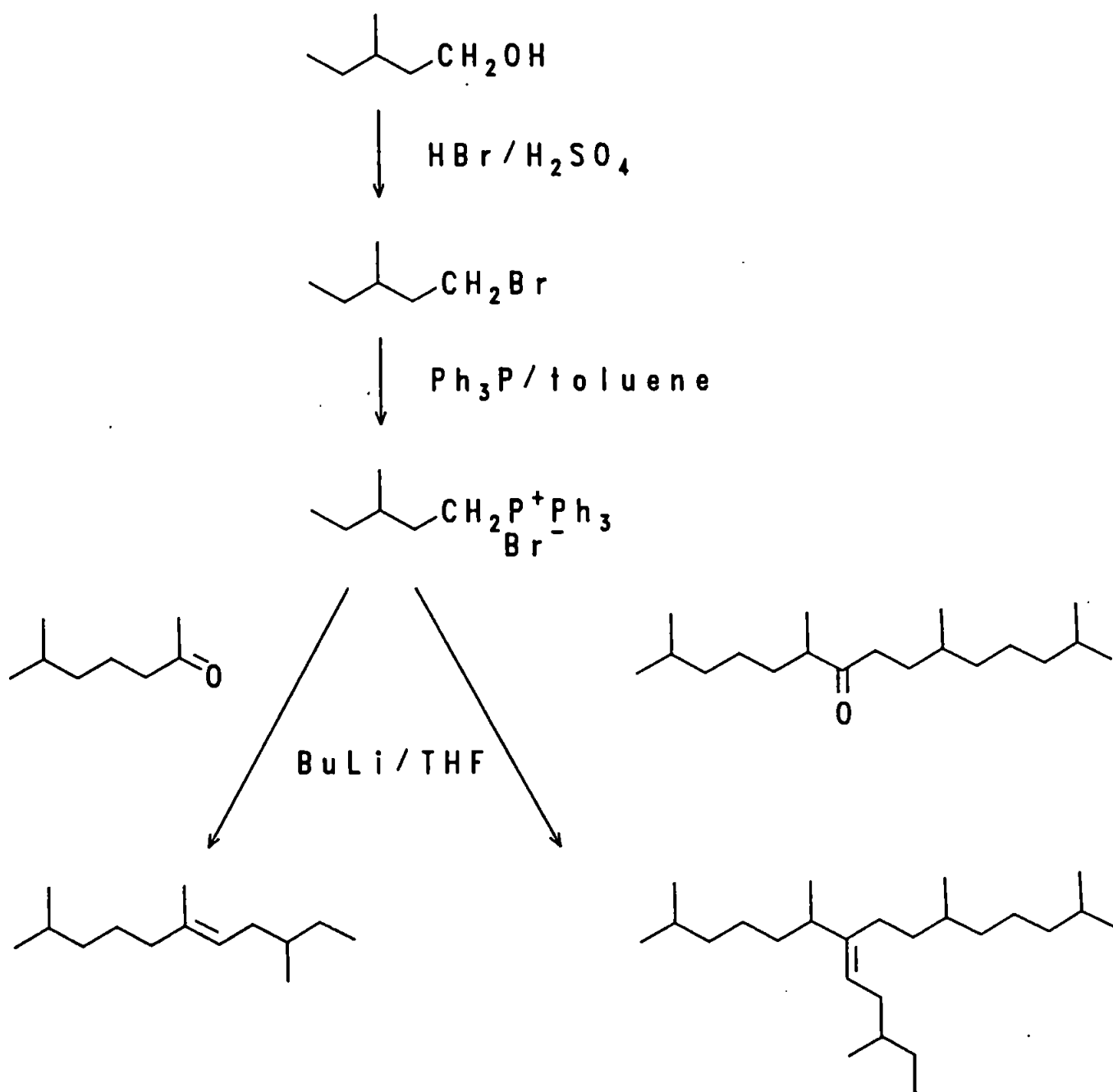
FIGURE 3.2 GENERAL SYNTHETIC SCHEME FOR THE
STEREOSELECTIVE PREPARATION OF A C_{25} MONOENE

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The preparation of an alkene by the Wittig reaction involves three stages. Firstly, the phosphonium salt must be prepared, usually from triphenylphosphine. The preparation of phosphonium salts has been comprehensively reviewed (Bestmann, 1965; Johnson, 1966; Beck, 1972). In the second stage of the reaction the salt (I) is treated with a base to convert it into the ylide (II) which is then, third, allowed to react with the carbonyl compound (III) to give the olefinic product (VIII) and triphenylphosphine oxide (IX) via the intermediacy of the oxaphosphetane-betaine complex (IV-VII).

$$Ph_3P^+Br^-CH_2R^1 \rightarrow Ph_3P{=}CHR^1$$
$$I \phantom{CH_2R^1 \rightarrow Ph_3P{=}}II$$
$$Ph_3P{=}CHR^1 + R^2R^3C{=}O \rightarrow Ph_3P^+CR^1CR^2R^3O^-$$
$$\phantom{Ph_3P{=}CHR}II III \phantom{R^3C{=}O \rightarrow Ph_3P^+}IV{\text-}VII$$
$$Ph_3P^+CR^1CR^2R^3O^- \rightarrow R^1HC{=}CR^2R^3 + Ph_3PO$$
$$VII \phantom{R^2R^3O^- \rightarrow R^1HC{=}}VIII IX$$
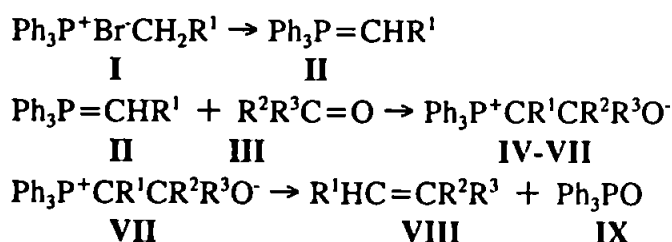
The mechanism was examined by Bestmann and workers (1980; 1983) who suggested that ylide (II) and ketone (III) combine to give betaine/oxaphosphetane (IV) in which the oxygen atom occupies an apical position on the pentavalent phosphorus. Cleavage of the ylide phosphorus-carbon (C-P) bond necessary for alkene formation requires a ligand rearrangement process (pseudorotation), which brings this bond into the apical position (V). The opening of the C-P bond to give VI occurs during, or after, the conversion to the trigonal bipyramidal structure V. The electronic nature of the substituents R^{1-3} of IV-VII determines the lifetime of this zwitterionic species, and thus, the olefinic products. The alkyl substituents (R^1 and R^2) of the ketone and any steric hindrance caused by bulky groups, however, may

III

$O=C-R^2$ with R^1

II $Ph_3\overset{+}{P}-\overset{-}{C}-R^3$ with H

IV

$Ph_3\overset{+}{P}$ ---- with R^1, R^2, $\overset{-}{O}$, H, R^3

V

$Ph_3\overset{+}{P}$ ---- $\overset{-}{O}$, R^2, R^1, H, R^3

VI

$Ph_3\overset{+}{P}$, O, R^2, R^1, $\overset{-}{H}$, R^3

VII

Ph_3P, O, R^2, R^1, H, R^3

VIII

H, R^3, R^2, R^1

100

also influence the stereochemistry and usually a mixture of (E) and (Z) isomers is obtained.

The phosphorus ylide (Cadogan, 1979) can be considered as a unique form of carbanion, the charge of which is modified by possible $d\pi$-$p\pi$ bonding.

$$Ph_3P = CHR_1 \leftrightarrow Ph_3P^+ - \overline{C}HR_1$$
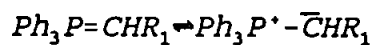
(a)   (b)

The contributing dipolar ylide form (b) gives the ylide a nucleophilic character which is further modified by the nature of the group R^3. Thus, groups which are strongly electron-donating, (e.g. alkyl) will destabilise the carbanion, confer abnormal instability and produce "reactive" ylides.

3.2 ATTEMPTED SYNTHESIS OF 2,6,10,14-TETRAMETHYL-7-(3'-METHYLPENTYL)PENTADEC-7(1')-ENE

Before coupling the small amount of available isoprenoid ketone, 2,6,10,14-tetramethylpentadecan-7-one **7** (synthesised previously by Robson, 1987; Robson and Rowland, 1986) with the isoprenoid phosphonium bromide **8**, it was considered prudent to verify the synthetic method using a similar, but widely available C_8 ketone (*viz.* 6-methylheptan-2-one; **9**) to produce a C_{14} monoene (3,6,10-trimethylundec-5(6)-ene; **10**). In this way the reactivity of the bromide could be monitored and the reaction conditions optimised.

3.2.1 PREPARATION OF C_6 ALKYL BROMIDE

$$HBr / H_2SO_4$$
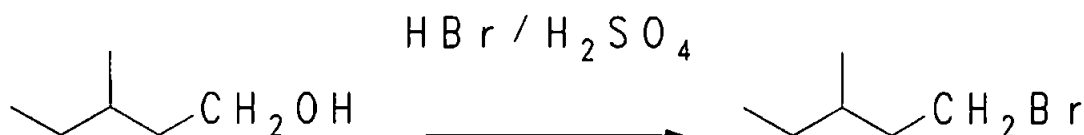
3-methylpentanol **11** was converted to 1-bromo-3-methylpentane **12** by the method of Kamm and Marvel (1960). The mass spectrum of the product exhibited the isotopic ratio (^{79}Br:^{81}Br; *ca.* 1:1), molecular ion, and fragmentation pattern of the expected C_6 bromide. The weak molecular ion, with loss of the halogen to give carbenium ions and hydrocarbon-like spectra, is typical of the mass spectra of bromides (Lambert *et al.*, 1987).

3.2.2 PREPARATION OF C$_6$ PHOSPHONIUM BROMIDE

$$\text{(3-methylpentyl)}-CH_2Br \xrightarrow[\text{toluene}]{Ph_3P} \text{(3-methylpentyl)}-CH_2\overset{+}{P}Ph_3 \; Br^-$$
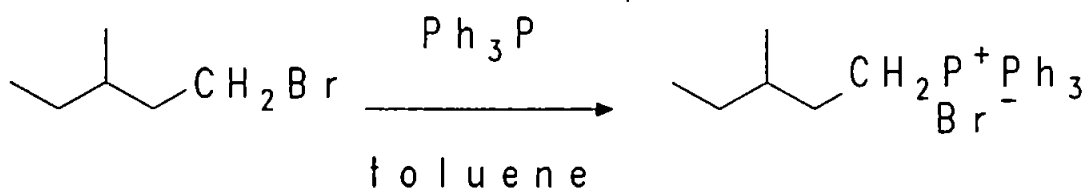
The conversion of tricoordinate phosphorus via nucleophilic attack on an electrophile to give tetracoordinate phosphorus is the most widely used process leading to phosphonium compounds. The quaternization of alkyl halides with triphenylphosphine has been described with, and without, the use of a solvent (Jerchel *et al.*, 1950; Friedich and Henning, 1959; Koster *et al.*, 1970; Sonnet *et al.*, 1974; Bestmann *et al.*, 1975). Normally solvent is used when the alkyl halide is a solid, but, in the present case a solvent was employed even though the bromide was a liquid because of the small scale of preparation. The phosphonium salt was washed with Et$_2$O to remove unreacted bromide and triphenylphosphine. The melting point of 200-202°C was constant for all batches of 3-methylpentyl-triphenylphosphonium bromide. It did not prove possible to confirm the identity of this phosphonium salt by conventional GC or GC-MS because of its ionic properties.

A complex IR spectrum was obtained with a number of overlapping bands (Figure 3.3). The phenyl ring attached directly to the phosphorus atom displays a sharp and relatively strong absorption at 1435 cm^{-1} (Miller and Willis, 1969; Silverstein *et al.*, 1974; Pouchert, 1975; Williams and Flemming, 1987; Lambert

ν   stretch

δ,   symmetrical in plane bending or scissoring

δ(oop)   out of plane bending or wagging

m.s.   monosubstituted

sk.   skeletal

aromatic combination
and overtone bands, m.s.

ν(P-CH₂)
1440-1405

610
overlapping
ν(C-P)

ν(O-H) hydrogen
bonded, polymeric
(due to H₂O in KBr)

910

780

substitution pattern
broken up into a number
of strong absorptions

1580
ν(C=C)
sk.
(=C-H)

1465

δ,(CH₂)

1150

2780
due to shift
of the CH₂ ν(C-H)
stretch caused by
attachment of
P-atom

990

1490
ν(C=C)
sk., m.s.

ν(C-H) aryl and alkyl

ν(C-C) aryl

1000

740

ν(C=C) sk., m.s.

1601 (poly)

750

720

690

1425

1100

δ(=C-H)
oop m.s.
aryl

ν(P-Ph)

ν(P⁺-Ph)

TRANSMISSION %

40

20

0

4000   WAVENUMBER (CM⁻¹)   2500   2000   1800   1600   1400   WAVENUMBER (CM⁻¹)   800   600

FIGURE 3.3 IR SPECTRUM OF 3-METHYLPENTYLTRIPHENYLPHOSPHONIUM BROMIDE

et al., 1987), but this and the other ν(Ph-P) bands are common for both triphenylphosphine and 3-methylpentyltriphenyl-phosphonium bromide **8**. The phosphonium salt however, did exhibit a strong, sharp absorption near 1100 cm^{-1}, which is characteristic of a quaternary phosphorus atom attached to a benzene ring. Miller and Willis (1969) state that there appears to be no evidence to support assignments of bands characteristic of P^{+}-Ph, and it would appear that these cannot be distinguished from normal, tervalent P-Ph compounds. Other useful bands were those indicating the presence of an aliphatic moiety, such as the CH_3 and CH_2 stretching absorptions <3000 cm^{-1}, C-H bends *e.g.* $\delta_s(CH_2)$ 1465 cm^{-1} and the CH_2 rock at 720 cm^{-1}. A weak but sharp band at 2780 cm^{-1} was due to the shift of the CH_2 carbon-hydrogen stretch caused by its attachment to the phosphorus atom. The substitution pattern between 770-665 cm^{-1} was broken up into a large number of strong absorptions. Other bands characteristic of P-CH_2- (1440-1405 cm^{-1}) and ν(P-C) (910-650 cm^{-1}) tend to be overlapped by other bands in those regions.

3.2.3 ATTEMPTED SYNTHESIS OF 3,6,10-TRIMETHYLDODEC-5(6)-ENES

BuLi / THF
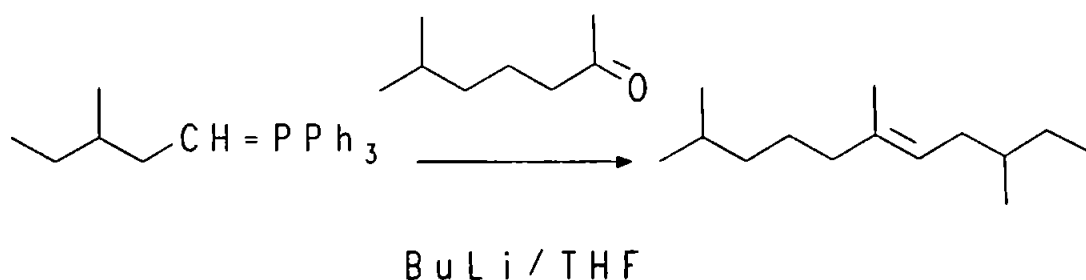
The alkyltriphenylphosphonium salt reacted smoothly with butyllithium (BuLi) in hexane giving the expected yellow colour change (Johnson, 1966). Gas chromatography (GC) of the products of the initial attempt at Wittig coupling (method of Adlercreutz and Magnusson, 1980) of 3-methylpentyltriphenylphosphonium bromide 8 and 6-methylheptan-2-one 9, however, indicated the presence of competing side reactions. Hydrolysis of the phosphorane, leading to the production of triphenylphosphine, and aldol condensation between two molecules of ketone with subsequent dehydration, was shown to have occurred.

In the case of sterically hindered, or highly basic phosphoranes, enolisation of the carbonyl component with concomitant aldol condensation has been reported frequently (Adlercreutz and Magnusson, 1980). To avoid this, the above workers used repeated additions of stoichiometric amounts of water to regenerate the ketone. This procedure was difficult to implement at the small scale of synthesis practised herein.

H
H–C–H ⟶ :B

H₂C

⟶

H_2C –

CH_2

H_3C δ+

O δ⁻

O
OH

⟶ –H₂O

O
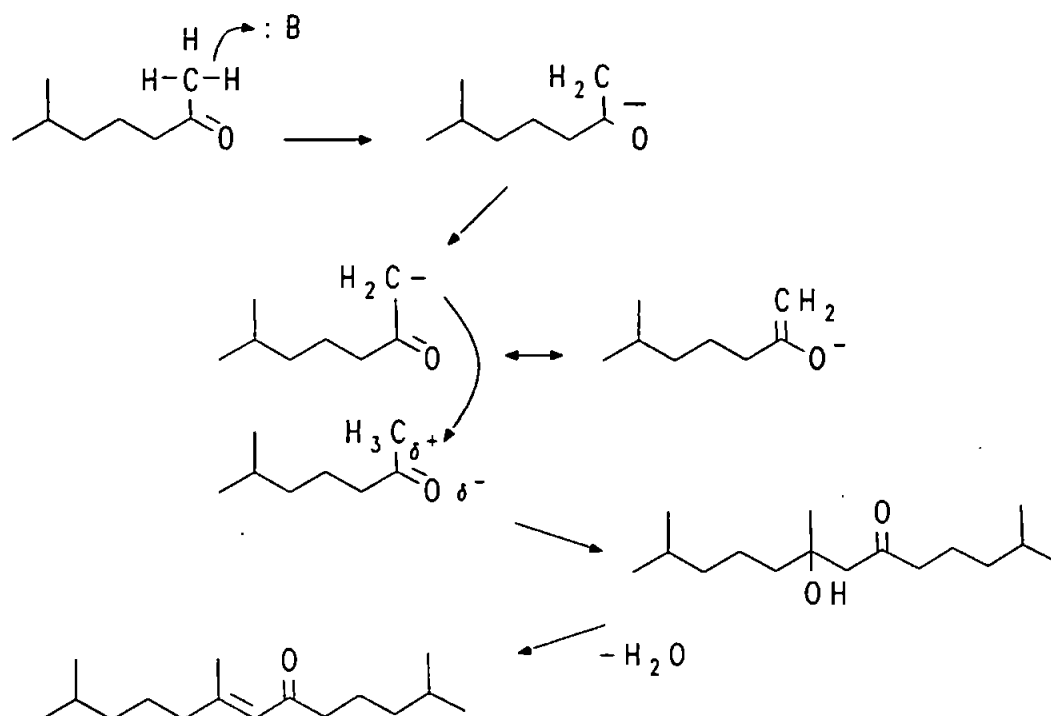
During aldol condensation the α-carbon of one ketone molecule adds to the carbonyl carbon of another resulting in dimerisation (Nielson and Houlihan, 1968). 6-methylheptan-2-one **9** is an enolisable ketone some of which is converted to the corresponding enolate ion by base abstraction of an acidic α-hydrogen. The acidity of the hydrogen atoms attached to the α-carbons is strengthened by delocalisation of the negative charge of the carbanion formed (resonance through participation of the carbonyl group). The enolate ion acts as a nucleophilic donor and adds to the electrophilic carbonyl of the acceptor component, in this case another molecule of 6-methylheptan-2-one. Asymmetrical ketones condense on the side that has more hydrogens (March, 1985); *i.e.* the more acidic α-carbon (Carey, 1987). In addition, with respect to this compound, condensation is more likely to take place on the methyl, rather than the methylene group, as the latter carbanion would prove less

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stable due to the positive inductive effect of the alkyl substituent.

The products were ß-hydroxy ketones which dehydrated spontaneously to form a new double bond in conjugation with the carbonyl bond. The preferred product is highly substituted and hence is the most stable alkene possible. A mixture of two α,β-unsaturated ketones (enones), assigned 13 and 14, was formed.

The mass spectra (Figure 3.4) of each were very similar and exhibited strong M^+ (m/z 238), an (M^+-CH_3) ion (m/z 223) and an (M^+-43) from loss of isopropyl group (m/z 195). Rupture of the bond allylic to the carbonyl group is more favoured by one isomer (II) which furnishes an ion a m/z 153 species as the base peak. Cleavage α to the carbonyl group is evident in the spectra of the other aldol product (I) producing an (M^+-15) at m/z 223. The α-cleavage process in α,β-unsaturated ketones occurs ß in preference to α with respect to the carbon-carbon double bond (Bowie, 1970). The acylium ions produced undergo secondary fragmentations. The more highly substituted ion undergoes allylic cleavage with hydrogen transfer (m/z 168, m/z 153, and m/z 95). Double bond migration seems to be more favourable in the second, more substituted acylium ion followed by allylic cleavage (m/z 95). This ion could also arise through allylic cleavage and simultaneous loss of a terminal isopentyl group (induced by the delocalisation of electrons between the $C=O$ and $C=C$ double bonds). Neither acylium ion seems to fragment with the loss of CO. The double bond tends to be stabilised by conjugation with the carbonyl group and the alkyl substitution and thus, isomerisation through double bond migration is unlikely. McLafferty rearrangement does not occur for α,β-unsaturated substituents, nor when the only available γ-hydrogen atom is attached to a double bond.
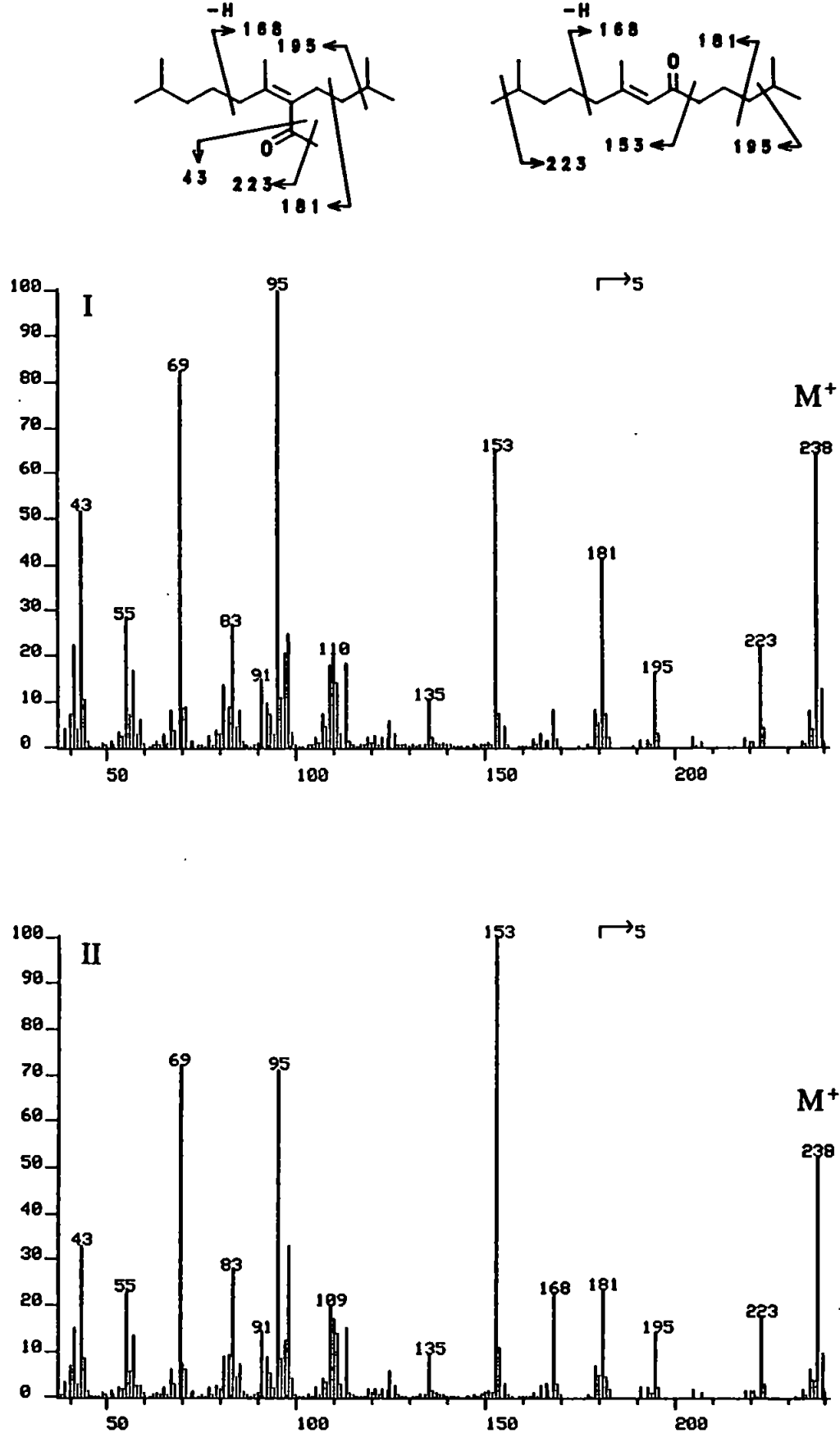
FIGURE 3.4 EI MASS SPECTRA OF PRODUCTS OF ALDOL CONDENSATION FROM THE ATTEMPTED SYNTHESIS OF C_{14} ALKENES

3.2.4 SYNTHESIS OF (E/Z)-3,6,10-TRIMETHYLUNDEC-5(6)-ENES

The failure of the initial attempt to synthesise 3,6,10-trimethylundec-5(6)-ene, the reaction was repeated using BuLi standardised by titration (Kofron and Baclawski, 1976).

The total ion current (TIC) chromatogram (Figure 3.5A) of the hydrocarbon products of the repeated Wittig reaction indicated that isomeric mixtures of two alkenes had now been produced. The mass spectra of the C_{14} alkenes, **I** and **II** in the chromatogram, prepared by the repeated Wittig reaction are shown in Figure 3.5. The spectra are very similar and exhibit the M^+ ion (m/z 196) of the expected C_{14} alkene **10**. McLafferty (1973) reported that cis and trans isomers have identical mass spectra. Therefore, the spectra indicate that the monoenes **I** and **II** are geometric isomers. The prominent fragmentation in the mass spectra of substituted alkenes is γ-hydrogen rearrangement (ß-cleavage with H-transfer) which give rise to the m/z 56, m/z 70 and m/z 126 ions. The same bond is ruptured in this rearrangement as in simple allylic cleavage (ß to the C=C double bond) (m/z 41, 55, 69, 83). Alkene ions show a strong tendency to isomerise through migration of the double bond. Thus alkenes are characterised by clusters of peaks representing C_nH_{2n-1} and C_nH_{2n} fragments, produced by multiple allylic cleavage of migrating double bonds. McLafferty (1973) reported that the spectra of branched unsaturated alkenes $RCH=C(CH_3)CH_2R^1$ and $RCH_2C(CH_3)=CHR^1$, show abundant RCH_2^+ ions which appear to arise by initial migration of the double bond away from the position of branching (m/z 57 and m/z 71).
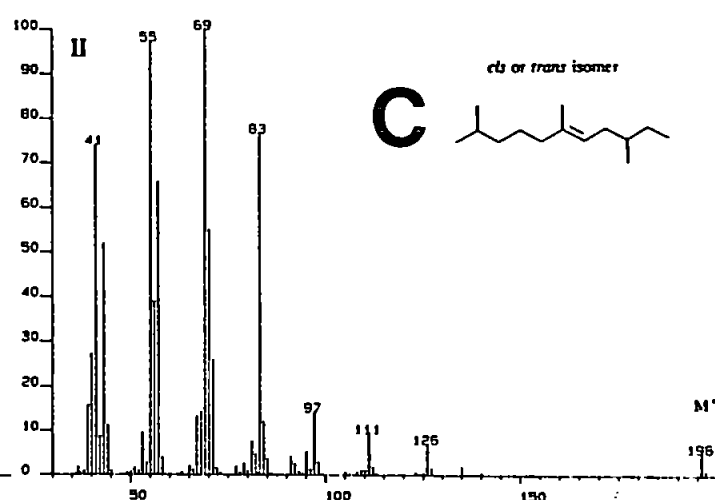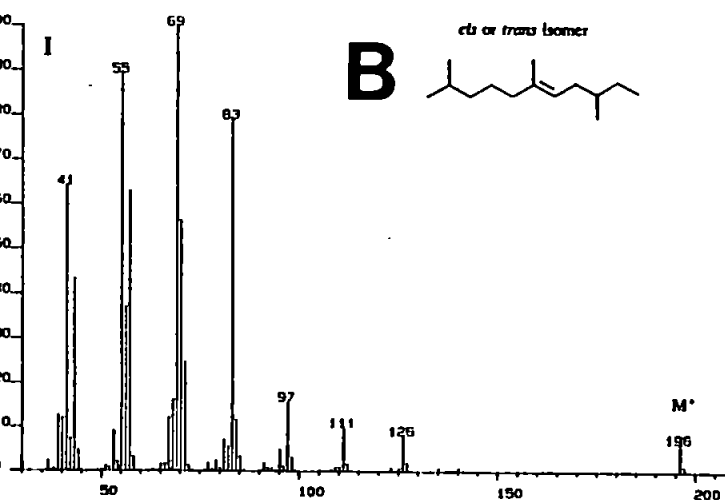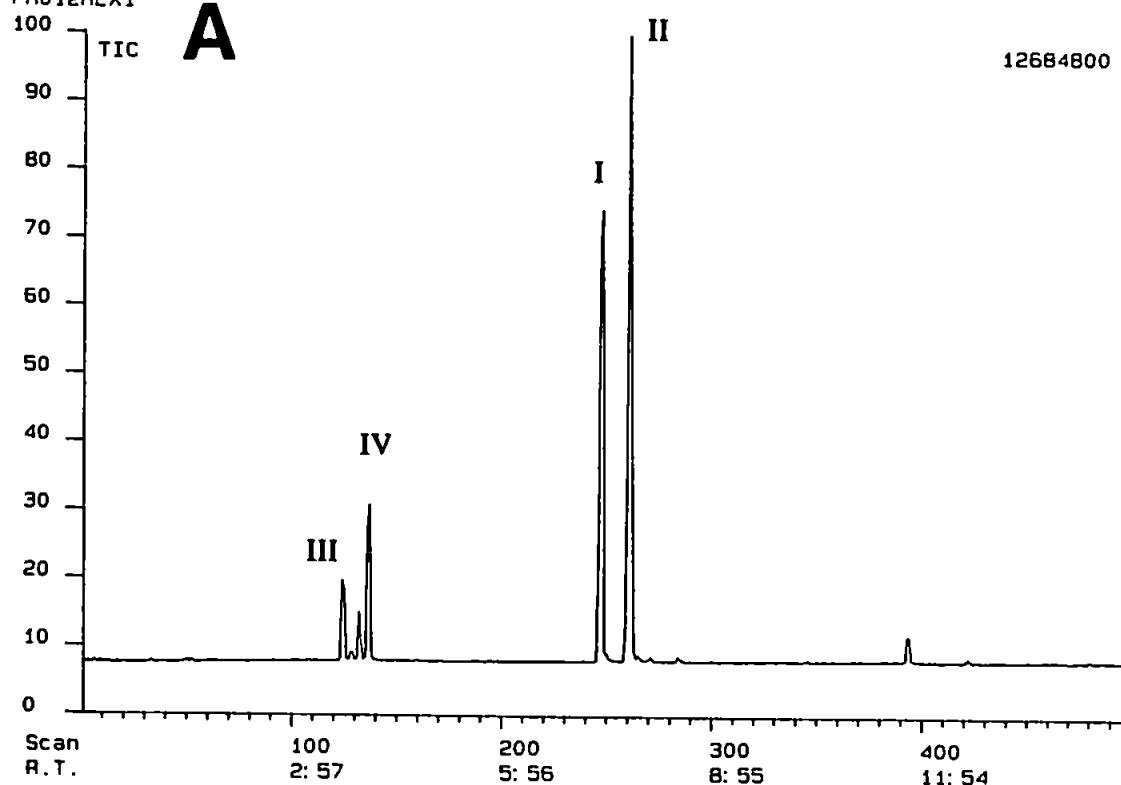
FIGURE 3.5 (A) TIC OF THE PRODUCTS FROM THE SYNTHESIS OF
3,6,10-TRIMETHYLUNDEC-5(6)-ENES
(B) AND (C) EI MASS SPECTRA OF (E/Z)-3,6,10-
TRIMETHYLUNDEC-5(6)-ENES

The mass spectra of compounds **III** and **IV**, also formed during the Wittig reaction, again showed much similarity and exhibited the M^+ ion (m/z 168) of C_{12} alkenes (Figure 3.6). The formation of these compounds can be rationalised by the ease of oxidation of the reactive C_6 alkylidene phosphorane **15**. Symmetrical alkenes are made by simply oxidising the solution of ylide with air (Bestmann and Stransky, 1974). Oxidation leads initially to triphenylphosphine oxide and a carbonyl; the latter undergoes a Wittig reaction with unoxidized ylide to form the symmetrical alkene in which both halves have come from the alkylidene phosphorane (Bestmann, 1960; Bestmann and Kratzer, 1962).

The mass spectra of the C_{12} alkenes contain the same ions but these differ in relative distributions. This argues against the possibility that the compounds are *cis-trans* isomers and it would appear that isomerisation of 3,8-dimethyldecen-5(6)-ene **16**, formed by oxidation of the C_6 phosphorane has occurred during the reaction, possibly by base-catalysed protropic rearrangements (see review by Mackenzie, 1970). Location of the double bond for each isomer by EI mass spectrometry is difficult because of its facile migration in the fragments. The $C_4H_9^+$, $C_4H_8^+$ and $C_4H_7^+$ ions are dominant in most of the spectra due to the loss of a stabilised butyl group at a point of branching activated by allylic cleavage.

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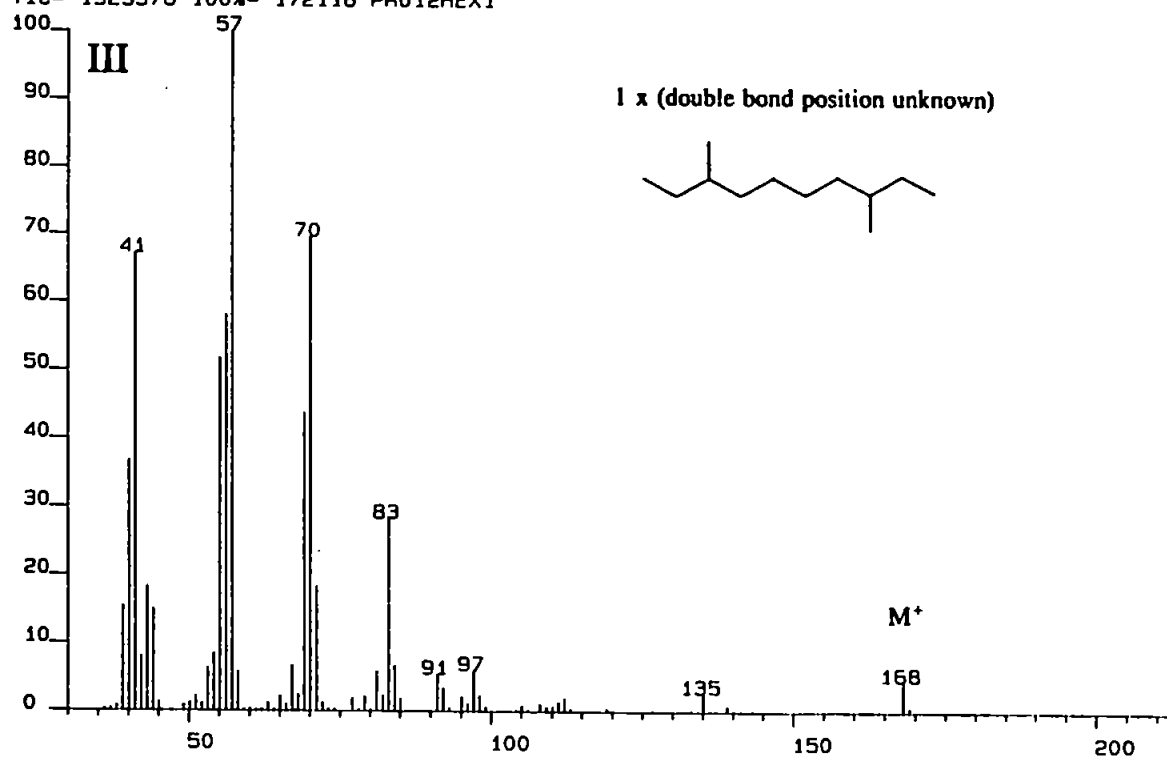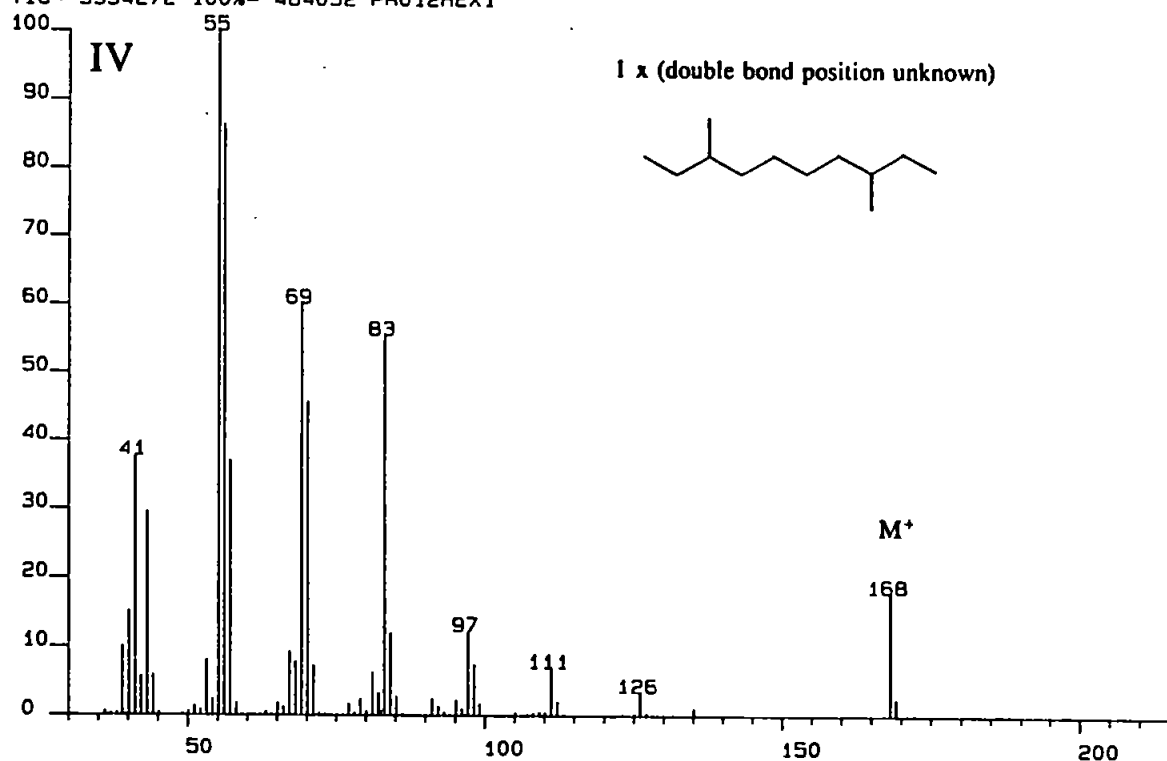
FIGURE 3.6 EI MASS SPECTRA OF C_{12} BRANCHED ALKENES

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3.2.5 ATTEMPTED SYNTHESIS OF 2,6,10,14-TETRAMETHYL-7-(3'-METHYLPENTYL)PENTADEC-7(1')ENE
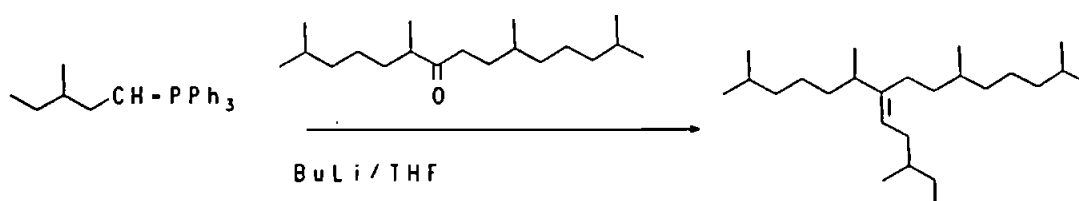
The successful Wittig procedure (modified to ensure no oxidation or hydrolysis of the ylide solution), was used twice with 2,6,10,14-tetramethylpentadecan-7-one **7** (less likely to be enolisable due to the lack of stability of the enolate ion and decrease in acidity of the α-hydrogens). The results from GC and GC-MS indicated, however, that despite observation of the expected colour changes, no reaction had taken place: only reactants were recovered. The failure of this Wittig reaction may have stemmed from the more hindered nature of the ketone, as demonstrated in Figure 3.7 which shows a computer-constructed, space-filled molecular model (Alchemy II; Tripos Associates Inc.) which emphasised the inacessibility of the carbonyl carbon. More extreme experimental conditions may have been required to complete the formation of betaine-oxaphosphetane intermediates and elimination of the alkene (*e.g.* increased temperature, pressure and reaction time).

OXYGEN

CARBON

HYDROGEN
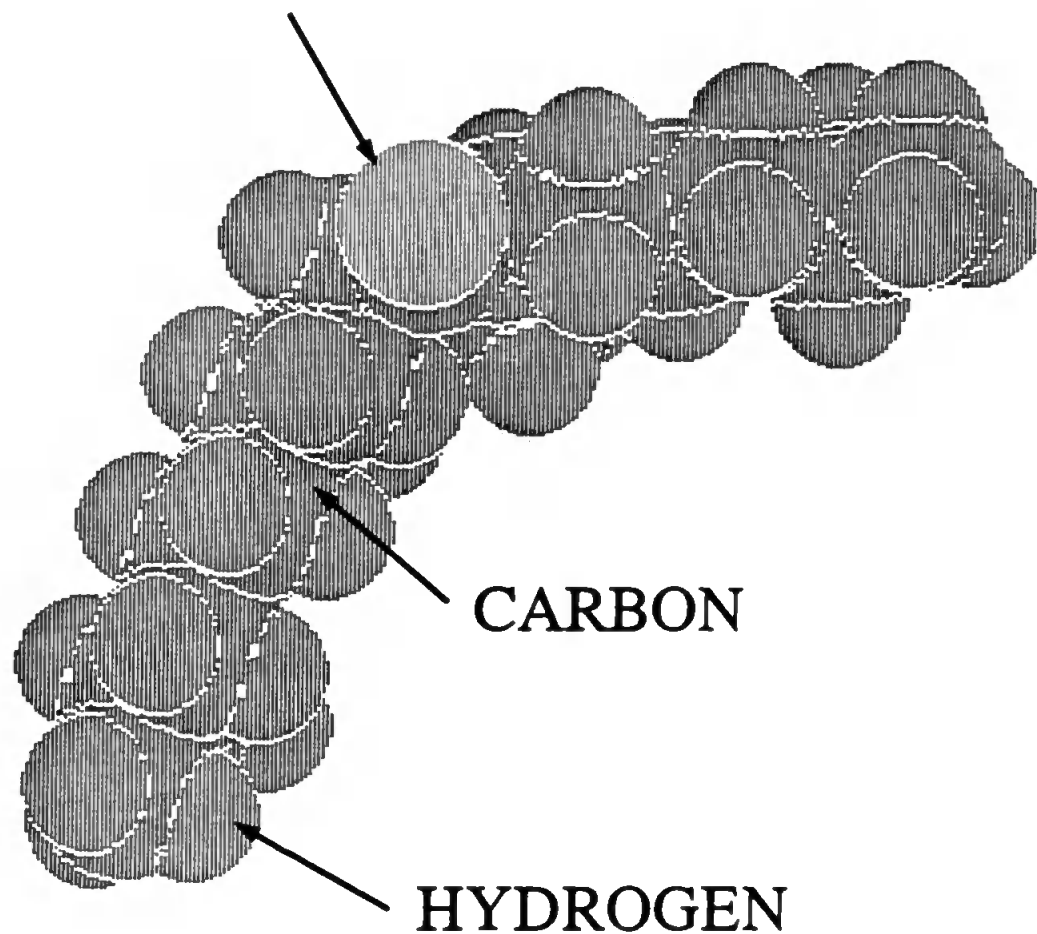
FIGURE 3.7 A "SPACEFILL" REPRESENTATION OF
2,6,10,14-TETRAMETHYLPENTADECAN-7-ONE (Alchemy II)

3.3 ATTEMPTED SYNTHESIS OF 2,6,10-TRIMETHYL-7-(3'-METHYLBUTYL)DODECENES

Given the unsuccessful nature of the Wittig reaction in producing the required C_{25} monoene stereoselectively, alternative routes to the HBI alkenes were sought. Although dehydration of tertiary alcohols usually produces mixtures of alkenes, if enough isomers are synthesised, GC allows each to be identified on the basis of GC retention index (GC RI). For example, Robson (1987) dehydrated 2,6,10-trimethyl-7-(3'-methylbutyl)dodecan-7-ol to give a mixture of E/Z 6(7), 7(1') and 7(8) C_{20} HBI monoenes (Robson and Rowland, 1986).

$$POCl_3 \xrightarrow{\quad} pyridine$$
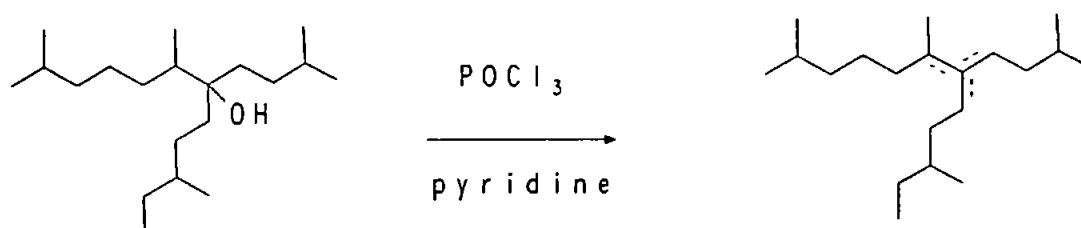
The GC RI of these are known and none proved to be C_{20} sedimentary alkenes. Dunlop and Jefferies (1985) had previously identified the 6(14) alkene by ozonolysis and recorded the GC RI as 1703_{MS} (*i.e.* br20:1; 1703_{MS}). Thus the only remaining position for the sedimentary C_{20} HBI monoene br20:1; 1698_{OV1}, detected in sediments worldwide (Rowland and Robson, 1990) consistent with the mass spectrum recorded, was 5(6). If a mixture of monoenes containing the 5(6), 6(14) and 6(7) isomers could be synthesised, and compared to the previous data, the 5(6)

compound could be easily identified by the GC RI. For this reason the synthetic route to the C_{20} monoenes (Figure 3.8) was chosen whereby dehydration of 2,6,10-trimethyl-7-(3'-methylbutyl)-dodecan-7-ol **17** would yield just such a mixture. The route utilised the presumed coupling of a C_{12} secondary bromide with a C_8 ketone via the Grignard reaction.

3.3.1 CHARACTERISATION OF C_{12} ALCOHOL SYNTHON

The mass spectrum of 2,8-dimethyldecan-5-ol **18**, synthesised previously (Kim, 1988) exhibited ions at m/z 186 (M^+), m/z 185 (M^+-1), m/z 184 (M^+-2), and m/z 168 (M^+-H_2O). McLafferty (1973) reported that in mass spectrometry, alcohols often undergo thermal and catalytic reactions and electron-impact induced fragmentations which give rise to spurious peaks such as (M^+-1), (M^+-2) and (M^+-18). The ions at m/z 101 and m/z 115 were generated by α-cleavage either side of the hydroxy bearing carbon to form oxonium ions. The ions at m/z 55, m/z 69, m/z 83, and m/z 97 were fragments with one degree of unsaturation ($C_nH_{2n-1}^+$) caused by secondary fragmentations (allylic cleavage) of the unsaturated ions formed by dehydration of the secondary alcohol. The ion at m/z 139 was also formed in this manner by loss of the terminal ethyl group, whilst m/z 43 was derived from cleavage of a terminal isopropyl moiety.

The IR spectrum of the C_{12} alcohol was consistent with that of a saturated secondary alcohol.
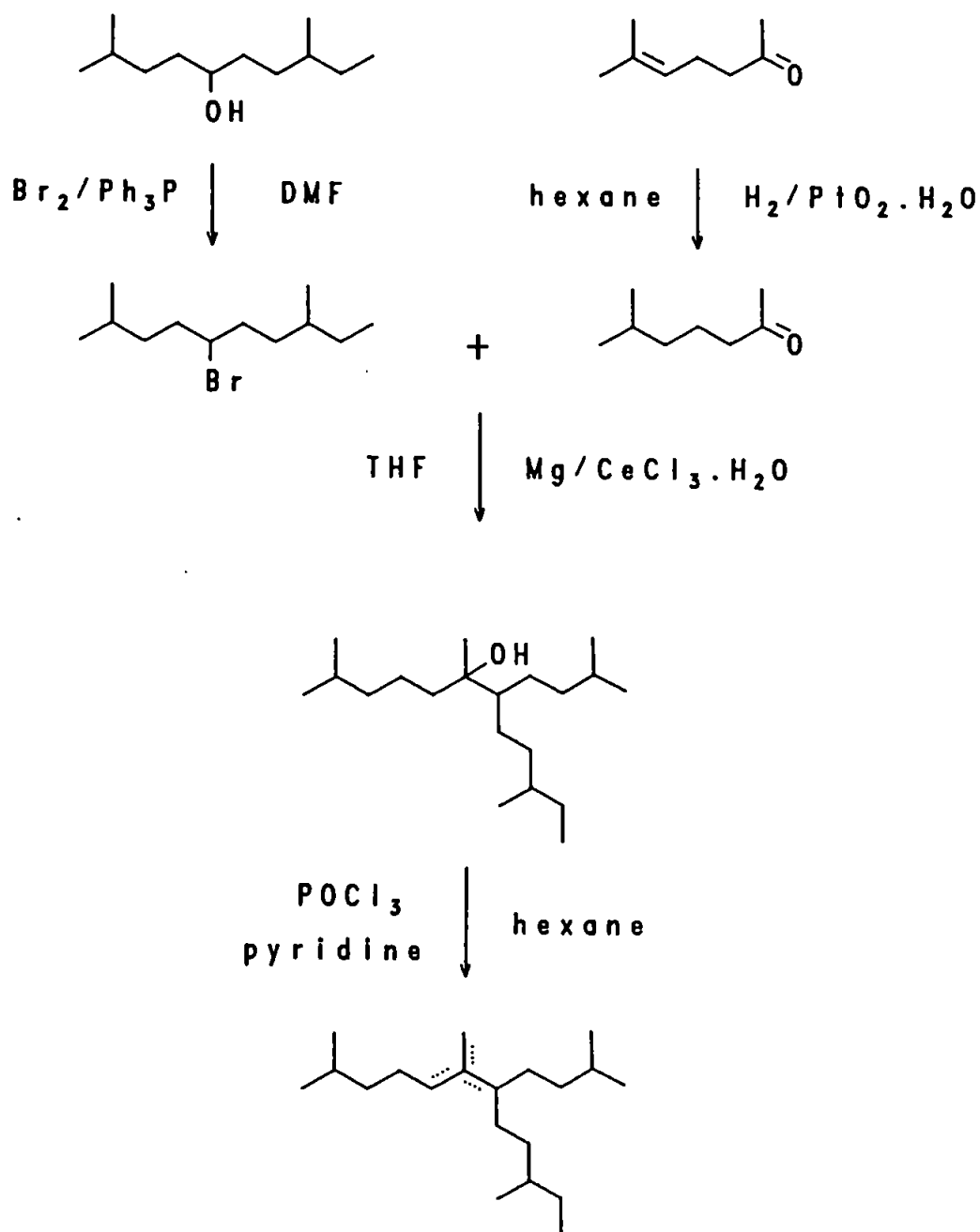
FIGURE 3.8 GENERAL SYNTHETIC SCHEME FOR THE PREPARATION OF ISOMERIC C$_{20}$ MONOENES

3.3.2 SYNTHESIS OF 5-BROMO-2,8-DIMETHYLDECANE

$Br_2 / PH_3P / DMF$
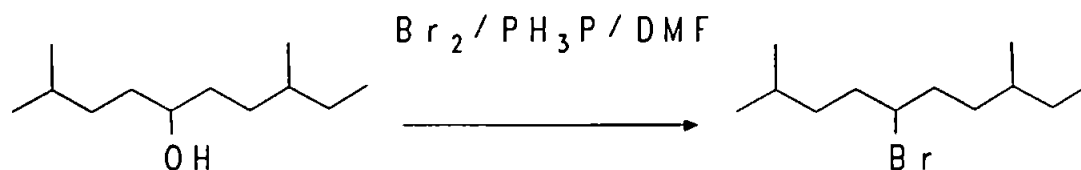
The corresponding C_{12} bromide 19 was prepared by the method of Wiley et $al.$ (1964). The molecular ion, M^+ (m/z 248), and the isotope ^{81}Br peak $M^+ + 2$ (m/z 250), were absent from the mass spectrum but ions at m/z 247/9 ($M^+ - 1$) were apparent. Bromine can stabilise a positive charge to form a halonium ion $R(R')C = Br^+$. α-Cleavage to form the $RC = X^+$ ion is not favourable in branched secondary bromides but trace ions were observed at m/z 163/5 and m/z 177/9. Carbon-bromide bond cleavage produced two ion series derived from the ions at m/z 169 ($M^+ - Br$) and m/z 168 ($M^+ - HBr$); the former was characteristically decomposed further by losses of C_nH_{2n} $e.g.$ m/z 43, 57, 71, 85, 99, 113, and 127, while the latter was accompanied by secondary ions characteristic of an alkene $e.g.$ m/z 41, 55, 69, 83, 97, and 111. The formation of a secondary bromide from the appropriate alcohol could not be accomplished by the addition of hydrogen bromide since the reaction is acid-catalysed and dehydration to alkenes is favoured. Dehydration and rearrangement was avoided by the use of trialkylphosphine dihalides (Wiley et $al.$, 1964). The reaction of alcohols with triphenylphosphine and bromine proceeds via the formation of the reactive tertiary phosphine dihalide. Production of the alkyloxytriphenyl-

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phosphonium halide intermediate followed by its slow decomposition to alkyl halide and triphenylphosphine oxide proceeds by way of a S_N2 displacement (Kaplan, 1966). The alcohol-to-halide conversion is frequently most troublesome with secondary alcohols because a nucleophilic bimolecular displacement is retarded by steric hindrance, and formation of carbonium ion intermediates may become a serious competing reaction. The low yield of 5-bromo-2,8-dimethyldecane was attributed to the slow rate of decomposition of the alkoxide intermediate. A longer reaction time may be required to ensure greater yields. In addition, it should be noted that distillation is preferred as the means of isolation of the bromide since the presence of the alkoxide presents a problem in terms of solvent solubility with respect to the partition and chromatography steps of purification.

3.3.3 PREPARATION OF 6-METHYLHEPTAN-2-ONE
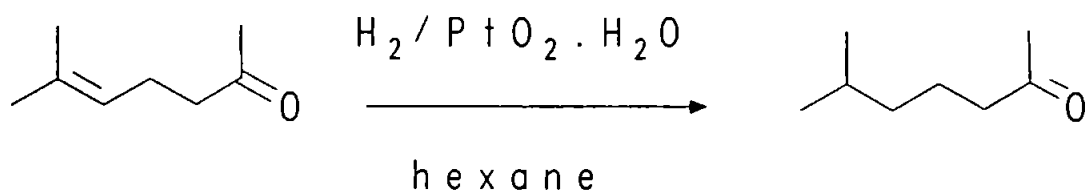
Commercial 6-methylhept-5-en-2-one was smoothly hydrogenated over Adams catalyst ($PtO_2.H_2O$) to produce 6-methylheptan-2-one **9** which was assigned by GC-MS and IR. The most notable feature of the mass spectrum of the saturated ketone was the McLafferty rearrangement ion which gave the base peak at *m/z* 58.

3.3.4 ATTEMPTED SYNTHESIS OF 2,6,10-TRIMETHYL-7-(3'-METHYLBUTYL)DODECAN-6-OL
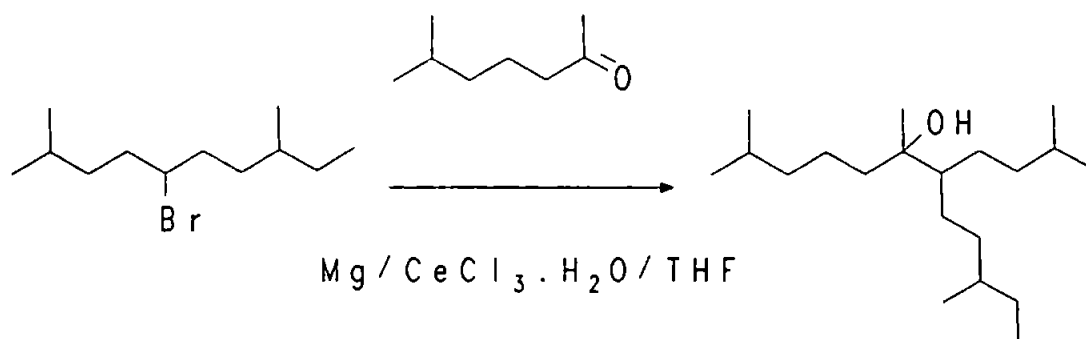
Figure 3.9A displays the TIC chromatogram of the crude total reaction products of the Grignard addition (method of Imamato *et al.*, 1985) of 5-bromo-2,8-dimethyldecane to 6-methylheptan-2-one. The desired alcohol product was absent. The two major components **I** and **II** were unreacted ketone **9** and an alkane the mass spectra of which are given in Figures 3.9B and 3.9C. Minor components proved to be two C_{16} unsaturated ketones and a C_{12} alkene. Aldol condensation (and subsequent dehydration) of the ketone may have occurred during the hydrolysis step of the procedure, which produced the unsaturated ketones. The alkane was tentatively assigned as the C_{24} alkane **20** representing the Wurtz-coupling product of 5-bromo-2,8-dimethyldecane. Any Grignard reagent formed apparently underwent elimination to give the alkene. The failure of the Grignard coupling step may have been due to the difficulty in the production of a secondary Grignard reagent.
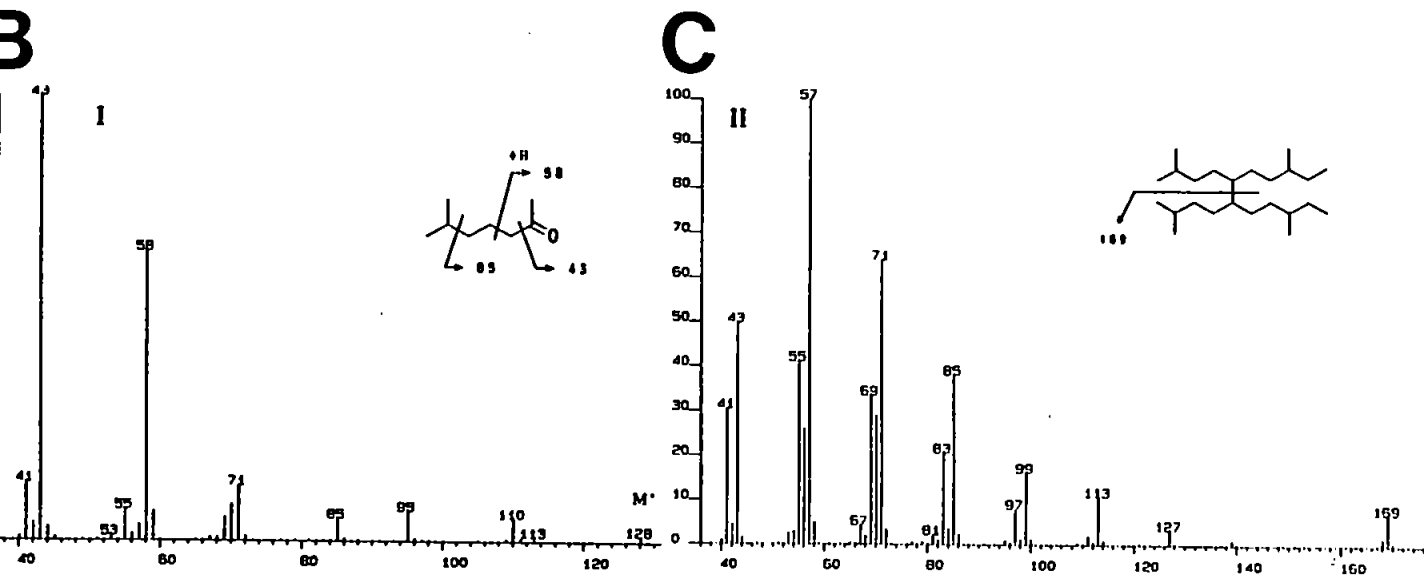
FIGURE 3.9
(A) TIC of products of Grignard addition of C_{12} bromide to C_8 ketone
(B) and (C) EI mass spectra of major products I and II.

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3.4   ISOMERISATION OF EXISTING MIXTURES OF C_{20}, C_{25}, AND C_{30} MONOENES

WHERE R = CH_3,   OR
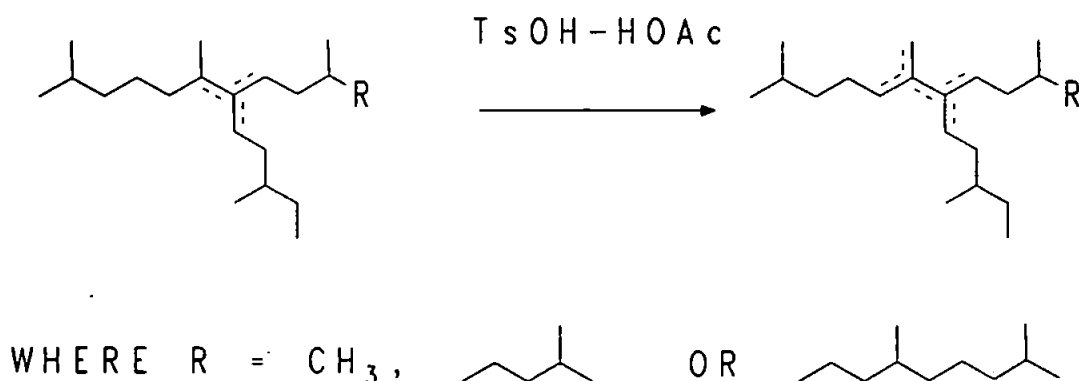
Given the unsuccessful nature of the Grignard reaction, an alternative route involving further isomerisation of existing synthetic mixtures of HBI-isomers was chosen in order to provide more and novel C_{20}, C_{25} and C_{30} isomers. The synthetic mixtures of monoenes, each of six isomers (*viz* 4-6), originally produced as intermediates during the previous syntheses of alkanes 1-3 (Robson, 1987; Robson and Rowland, 1986; 1988a), were treated with tosic acid in a procedure which was known to induce double bond movement through tertiary carbocation formation. This method has been successfully used by Peakman and Maxwell (1988) for the isomerisation of sterenes. Using this method, the formation of isomers with double bonds in positions away from the tertiary centre of branching was envisaged, which would be more amenable to separation and isolation by argentation chromatography. In the present study, the result in each case (C_{20}, C_{25} and C_{30}) was the production of mixtures containing one major "new" GC peak (see Figure 3.10). Time course experiments using C_{25} HBI monoenes (5) with a C_{25} highly branched alkane internal

standard (*n*-7-hexylnonyldecane), showed that all original isomers were reduced in concentration as a result of the formation of a new isomer assigned the RI 2109_{DBI} (RI 2101_{DBI}) by GC analysis. The changes in isomer distribution over the period of the experiment are illustrated in Figure 3.11, which serves to emphasise the rapid nature of the isomerisation. Other isomers were also produced as minor products of the acid-catalysed rearrangement and details of GC RI data and the mass spectra of all isomers produced are summarised in Table 3.1. The mass spectra of all the isomers retained the expected molecular ion at m/z 350, and intense ions presumably derived from allylic cleavage with H-transfer of original double bonds and from resonance intermediates resulting from migration of the double bond from the tertiary point of branching. These fragmentations (*e.g.* br25:1; 2076_{DBI} [m/z 280, 266, 224, 196]) have been shown to be characteristic of acyclic HBI monoenes (Robson, 1987).

a. Original mixture (Robson and Rowland, 1986)

2076
2085
2092
2115 2124

b. Isomerisation mixture (tosic acid)

2109

N.b. "New" peak

Time (mins)
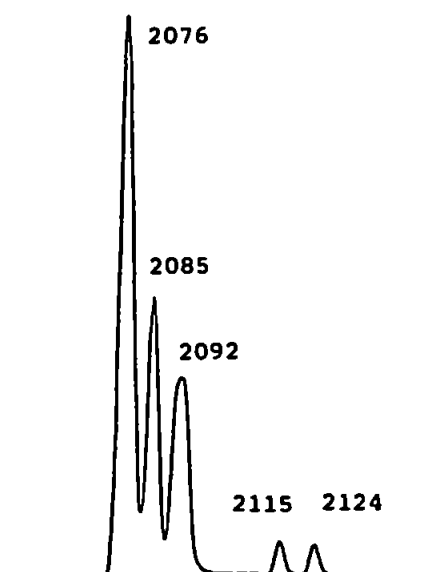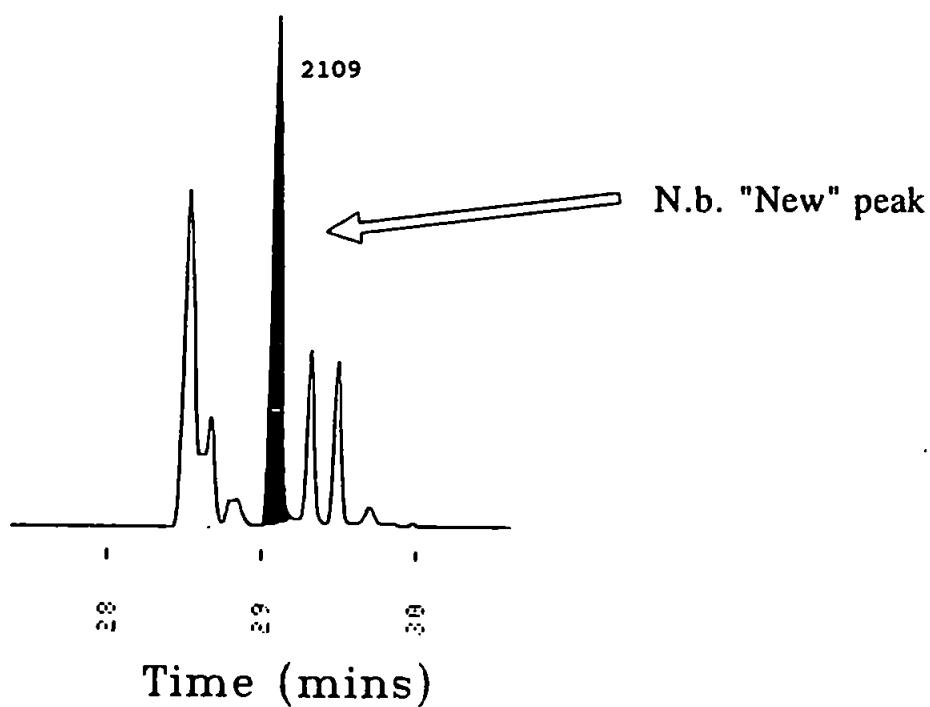
FIGURE 3.10 PARTIAL GAS CHROMATOGRAMS OF C_{25} HBI MONOENES
Numbers refer to GC RI. GC conditions: Carlo Erba Mega, 30m x 0.32mm i.d.
DB1 (J&W), 40-80°C @ 10°Cmin^{-1}, 80-300°C @ 6°Cmin^{-1}, H_2 carrier.
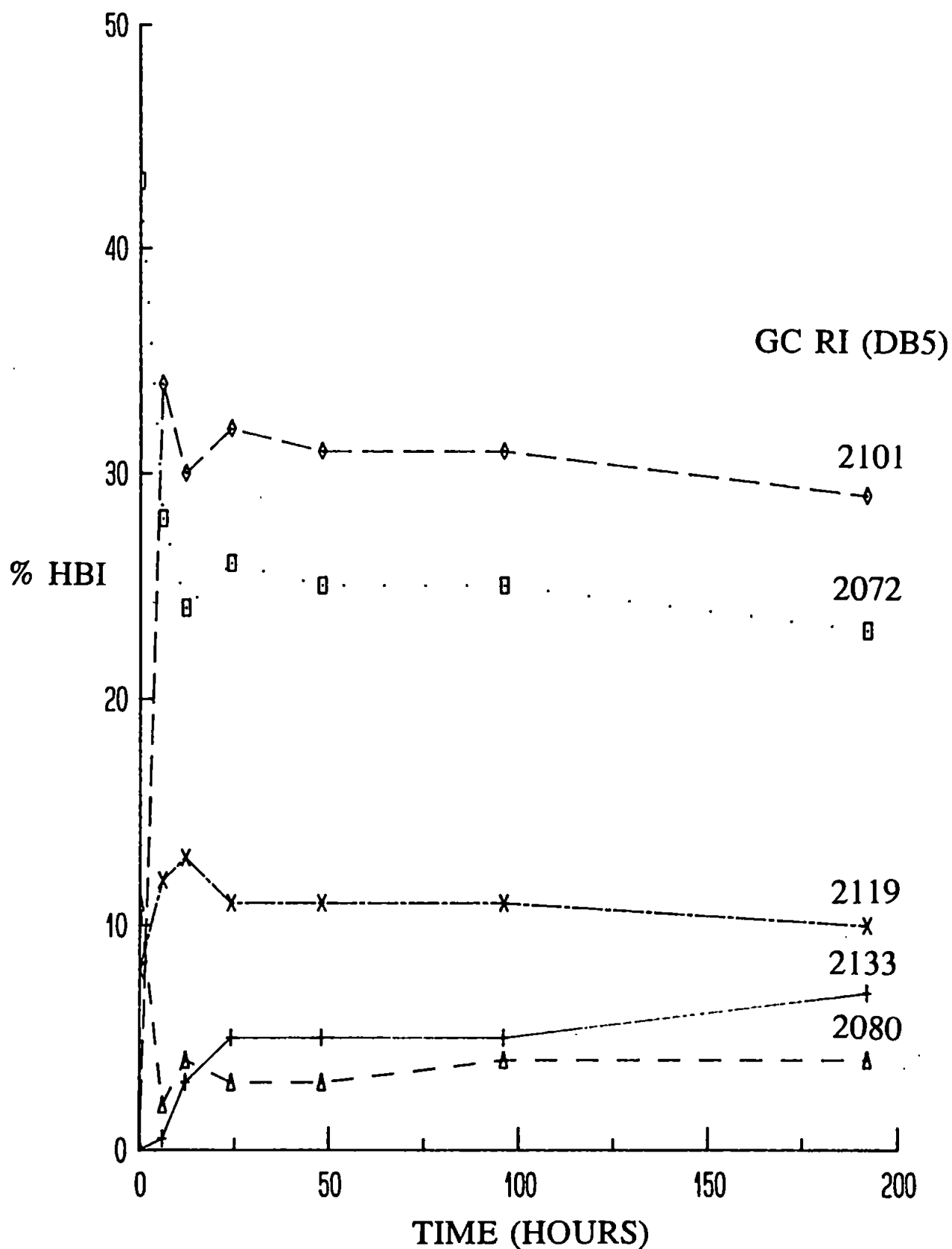
FIGURE 3.11 GRAPH ILLUSTRATING THE CHANGES IN THE DISTRIBUTION OF C_{25} HBI MONOENES DURING ACID-CATALYSED REARRANGEMENT

TABLE 3.1 (A) GC RI and mass spectral data for 2,6,10-trimethyl-7-(3'-methylbutyl)dodecenes (br20:1)

(B) GC RI and mass spectral data for 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadecenes (br25:1)

A

ION INTENSITY %

DB1	DB5 (GC RI)	DBWAX	Formula m/z	$C_{20}H_{40}$ 280	$C_{16}H_{32}$ 224	$C_{15}H_{30}$ 210	$C_{14}H_{28}$ 196	$C_{11}H_{22}$ 154	$C_{10}H_{20}$ 140	$C_{9}H_{18}$ 126	$C_{5}H_{9}$ 69
1678	1675	1643		37	11	37	50	18	52	80	99
1686	1683	1653		32	30	8	28	23	84	42	100
1690	1687	1659		44	4	40	24	20	46	100	94
1697	1693	1670		19	4	40	56	8	28	43	97
nd	1705	nd		10	4	33	50	10	33	85	100
1711	1706	1688		60	12	21	11	21	60	65	90
1714	1709	1700		56	12	21	15	23	56	65	89
1725	1728	1714		20	2	27	42	10	25	42	58
1733	1736	1727		20	10	33	23	11	32	33	73
1739	1739	1731		22	10	32	30	11	33	34	86
1742	1744	1734		20	2	25	15	15	30	77	64

B

ION INTENSITY %

DB1	DB5 (GC RI)	DBWAX	Formula m/z	$C_{25}H_{50}$ 350	$C_{20}H_{40}$ 280	$C_{19}H_{38}$ 266	$C_{16}H_{32}$ 224	$C_{15}H_{30}$ 210	$C_{14}H_{29}$ 196	$C_{10}H_{20}$ 140	$C_{9}H_{18}$ 126	83	(Base ion)
2076	2072	2023		10	9	10	10	11	13	46	30	98	100(69)
2085	2080	2035		5	5	10	4	19	10	28	30	98	100(69)
2092	2086	2044		6	1	3	13	3	5	50	16	80	100(70)
2109	2101	2063		3	1	10	3	30	6	23	25	100	100(83)
2115	2110	2074		10	3	1	10	4	9	43	19	80	100(70)
2125	2119	2083		12	6	2	10	3	11	50	21	85	100(70)
2134	2133	2105		5	1	4	8	13	3	33	11	48	100(57)
2141	2142	2118		5	4	5	5	9	7	32	19	53	100(69)
2146	2148	2127		6	9	2	10	1	13	23	28	49	100(70)

The double bonds of alkenes have also been shown to shift upon treatment with acids (*e.g.* Turner *et al.*, 1957; Blunt *et al.*, 1969; Peakman and Maxwell, 1988; Peakman *et al.*, 1988). In many cases equilibrium mixtures are obtained and the most thermodynamically stable isomer predominates. The reaction, for which the term prototropic rearrangement is often used (March, 1985), is an example of electrophilic substitution with accompanying allylic rearrangement. In this case, the proton donated by the acid acts as an electrophile and attacks the π bond of the alkene. The electrons in the π bond are exposed because the π orbital has considerable p character. The proton uses the π electrons to form a σ bond to one carbon of the alkene. The carbocation so formed then combines with a proton at the position which will give the most stable alkene isomer resulting in the loss of a proton.

The stability of alkenes, mostly based upon heats of hydrogenation, combustion and formation, has been shown to be related to the degree of substitution. The greater the number of attached alkyl groups, *i.e.* the more highly substituted the carbon atoms of the double·bond, the greater the stability of the alkene. The reasons for the relative stabilities of substituted alkenes are still the subject of much debate and appear to be interpreted on the basis of relative bond strengths and the stabilising interaction termed hyperconjugation (orbital overlap between the C-C π bond and a properly oriented C-H σ bond on a neighbouring substituent; McMurray, 1984) or hybridization effect (Streitwieser and Heathcock, 1985). Part of the explanation can be given in terms of the electron-releasing effect of the alkyl groups, an effect that satisfies the electron-withdrawing properties of the sp^2-hybridised carbon atoms of the double bond. The more alkyl substituents present, the more opportunities exist for hyperconjugation, the more effectively the developing positive charge from the alkyl

groups is delocalised, and therefore, the more stable the alkene.

In the case of the C_{25} HBI monoenes, there are two adjacent carbons which are trisubstituted, 6(7), which would thus provide sites for the formation of such stable tertiary carbocations. Isomerisation of the original mixture of alkenes was likely to preferentially form trisubstituted alkenes with the double bond in the 5(6) position rather than the vinylidene double bond in the methylene position, 5(17), or disubstituted alkenes produced by isomerisation through secondary carbocations [*e.g.* 4(5)].
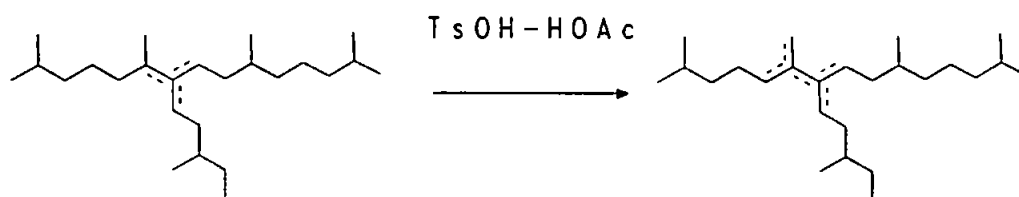
Thus, the acid-catalysed rearrangement was thought to have resulted in an equilibrium mixture consisting of tetra- and trisubstituted C_{25} HBI monoenes. The hindered structures of these HBI monoenes (4-6), however, is likely to introduce steric factors into the formation of the reaction products and the formation of unlikely double bonds, such as in the methylene position, 5(17), cannot be discounted.

3.5 ATTEMPTED CHARACTERISATION OF ISOMERIC MIXTURES OF C_{25} HBI ALKENES BY GC-FTIR AND FTIR

Both sets of C_{25} monoene isomers, original (*viz* that of Robson, 1987) and tosic acid isomerised, were analyzed by FTIR. Only the former was analysed by GC-FTIR. This was the first such analysis of HBI alkenes by FTIR. Absorptions arising from carbon-hydrogen bending vibrations of alkenes occur in the 600-1000 cm^{-1} region of the IR spectrum. The exact location of these peaks can often be used to determine the nature and configuration of a double bond. GC-FTIR involved the separation of monoenes prior to obtaining a snapshot IR spectrum of each component. No diagnostic CH out-of-plane (oop) bending (wagging [γ] or twisting [τ]) deformations were evident and in many cases even the C=C stretch (ν) was either weak or absent. Such spectra (*e.g.* Figure 3.12) are indicative of tri- and tetrasubstituted alkenes of which the isomeric mixture was known to comprise. However, the GC-FTIR technique does suffer from the disadvantage of sensitivity related to the maximum loading on the capillary GC column, the split injection used and the short scan time available (sample residence time in IR cell).

Because the diagnostic IR band (γoop(CH); 850-790 cm^{-1}) for trisubstituted alkenes, especially HBI monoenes with hindered, highly substituted double bonds were suspected from these results to be weak, the compounds were analysed retrospectively as mixtures before and after isomerisation by FTIR. The rearrangement of double bonds within the HBI carbon skeleton was thought likely to have produced tri- and disubstituted alkenes. Such compounds were thought to have distinguishing features in IR spectra (*e.g.* -CH=CH- *cis*; sharp CH oop deformation at 980-955 cm^{-1}).

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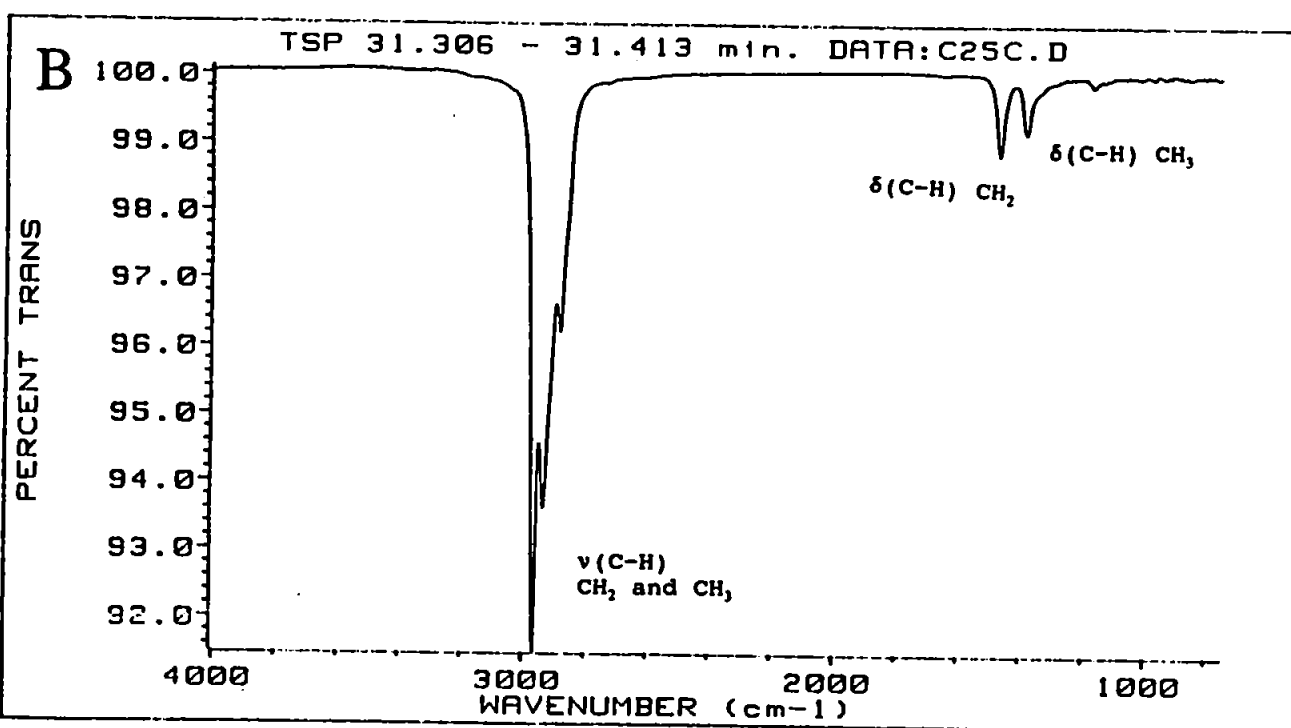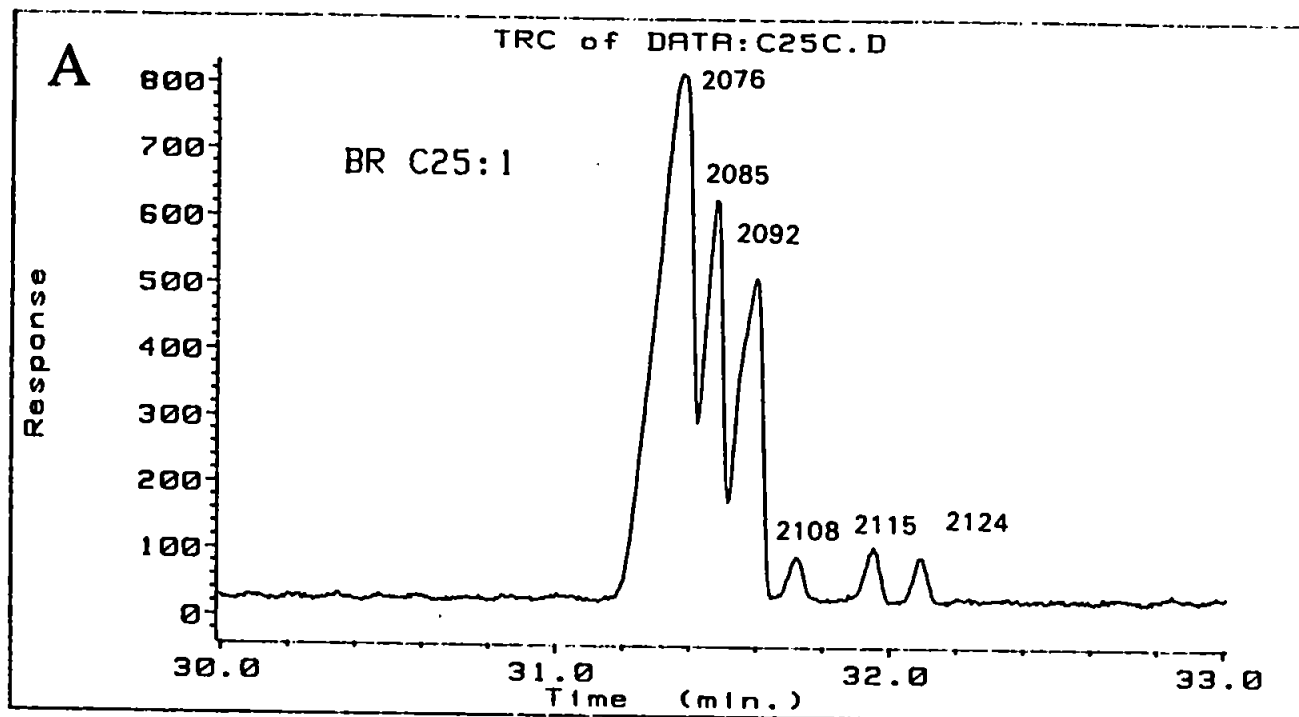
FIGURE 3.12   (A) TOTAL RESPONSE CHROMATOGRAM (TRC) OF THE ISOMERIC MIXTURE
(B) SNAPSHOT IR SPECTRUM OF ONE C_{25} HBI MONOENE (RI 2076_{OV1})

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The IR spectra of C_{25} HBI monoenes prior to and after isomerisation are shown in Figure 3.13. In the spectrum of the original mixture of tri- and tetrasubstituted alkenes no absorption bands corresponding to the =CH stretch, C=C stretch or CH out-of-plane bending were detected. The spectrum of the isomerised mixture was similar. A weak and broad absorption was observed at 1750 cm⁻¹ but this was thought unlikely to be the C=C stretch. Although a number of signals were observed in the absorbance region of 900-500 cm⁻¹ these could not be reliably assigned to γoop CH or CH_2 bending or wagging deformations. To aid the interpretation of the HBI spectra three authentic alkene standards, n-tetradec-1-ene, *trans* n-tetradec-7-ene and 2,6,10,15,19,23-heptamethyl-2,6,10,14,18,22-tetracosahexaene (squalene), containing vinyl, vinylene and trisubstituted double bonds, were analysed under the same conditions. These spectra are shown in Figure 3.14 for comparison with those of the C_{25} HBI monoene mixtures (Figure 3.13). The vinyl double bond displayed absorptions at 3077 cm⁻¹ [ν(=CH₂)], 1642 cm⁻¹ [ν(C=C)] and deformations at 992 cm⁻¹ [γoop(CH)], 909 cm⁻¹ [γoop(CH₂)], whereas the vinylene compound exhibited no signal from C=C or =CH stretching but strong absorptions at 965 cm⁻¹ and 724 cm⁻¹. Since the double bond was known to be all *trans* configuration, it was interesting to note the presence of γoop(CH) for both *cis* (965 cm⁻¹) and *trans* (724 cm⁻¹) isomers. The spectra of the trisubstituted polyene, squalene, also showed absorptions from ν(CH) (3050 cm⁻¹) and ν(C=C) (1668 cm⁻¹) as expected, and a number of bands in the 1200-600 cm⁻¹ range, including γoop(CH) at 835 cm⁻¹. Comparison of these data with those from the isomerised HBI alkenes strengthens the evidence for the assignment of the absorptions at 801 cm⁻¹ and 723 cm⁻¹ as possible γoop(CH) from trisubstituted and *trans* disubstituted double bonds

133
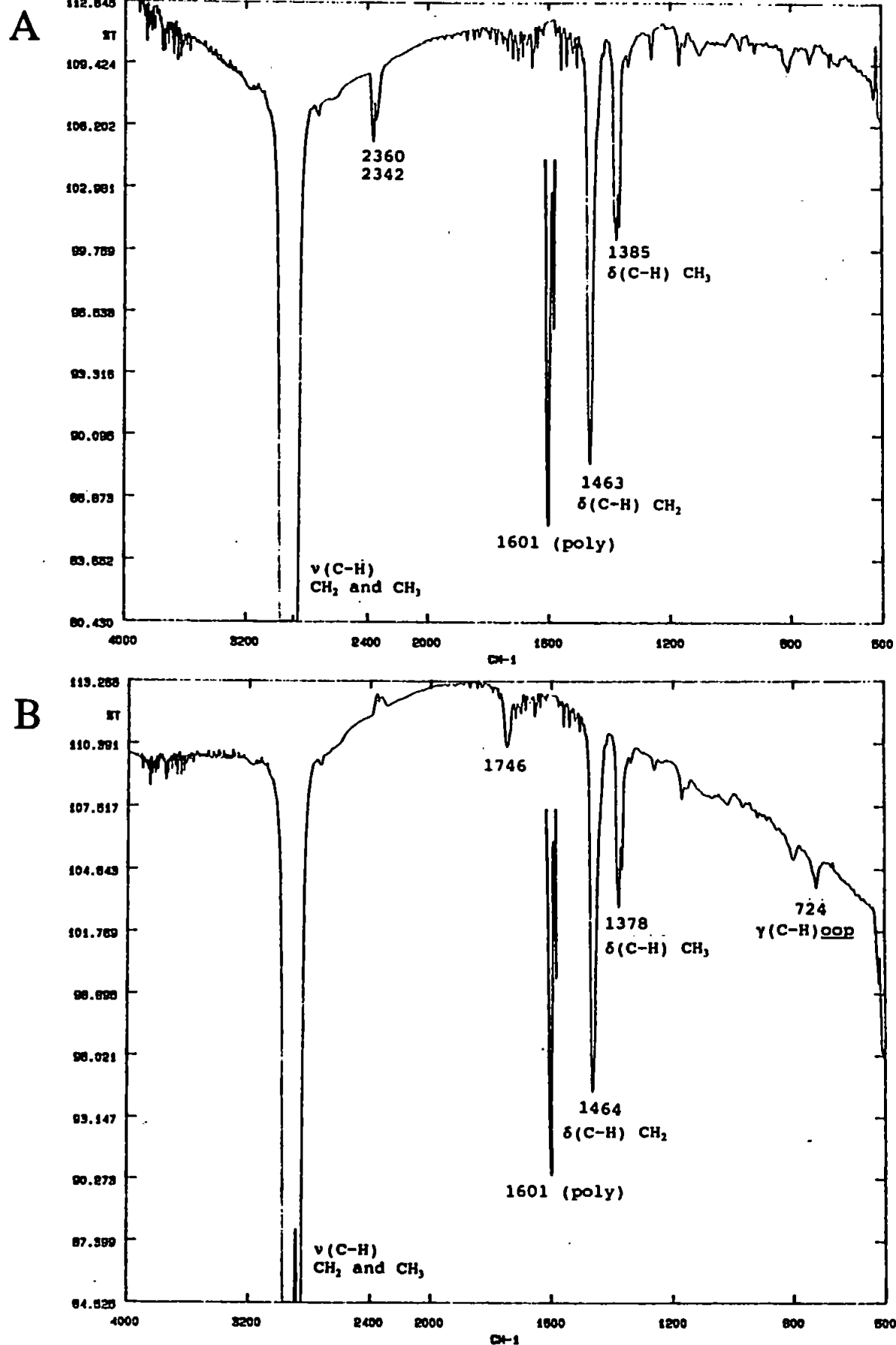
FIGURE 3.13 IR SPECTRA OF ISOMERIC C$_{25}$ HBI MONOENES
(A) Original mixture (Robson and Rowland, 1986)
(B) Isomerisation mixture
poly = polystyrene

134
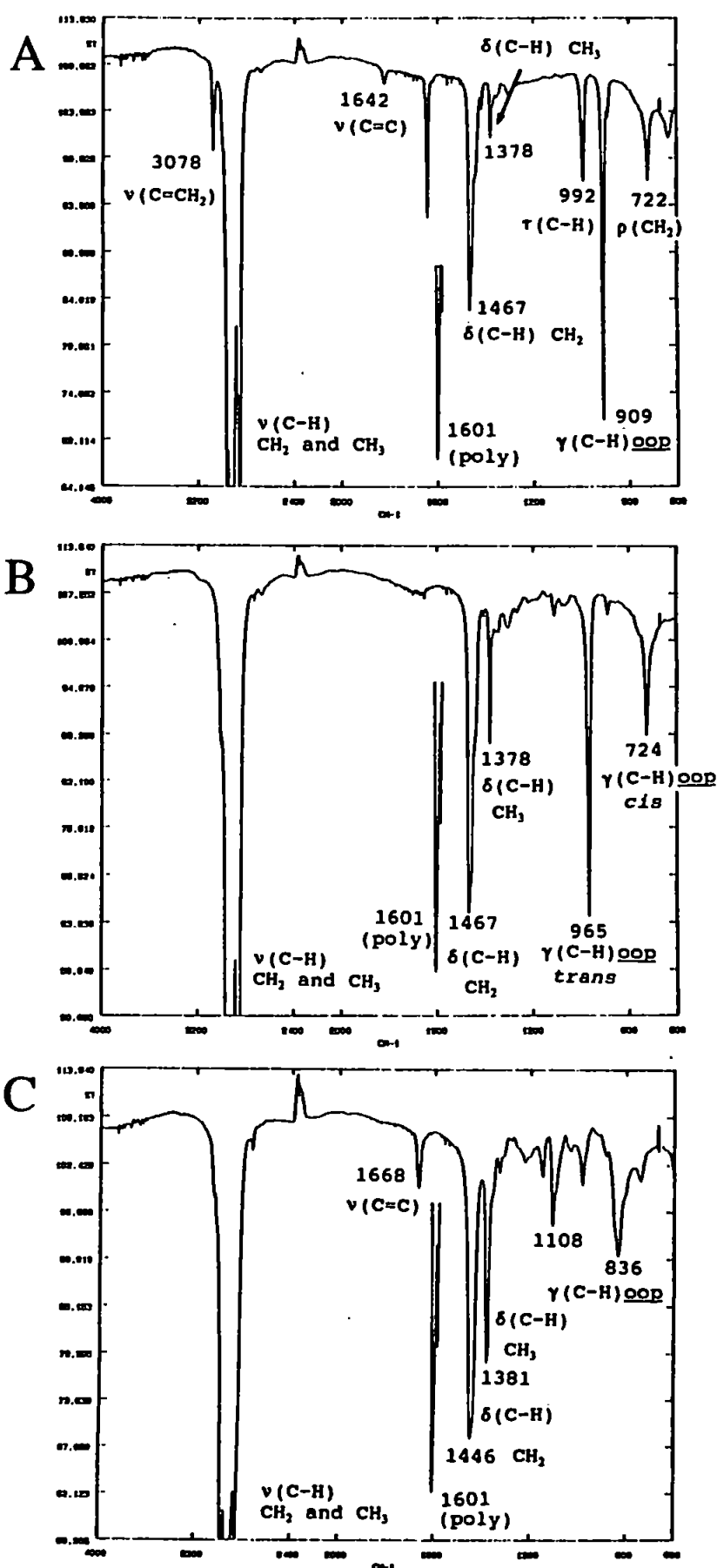
FIGURE 3.14 IR SPECTRA OF

(A) *n*-tetradec-1-ene,(B) *trans* *n*-tetradec-7-ene,(C) squalene
poly = polystyrene

135

not detected in the original mixture.

In summary, FTIR did not prove useful for the determination of the class of double bond, either from single GC peaks, or by the analysis of mixtures. It is known that internal double bonds generally absorb in the infrared more weakly than terminal double bonds due to pseudosymmetry. This phenomenon was probably exacerbated by the hindered nature of the double bonds in the synthetic HBI monoenes (4-6) located about the tertiary centre of branching (C7). The tentative assignment of the presence of novel tri- and disubstituted double bonds must await the isolation of isomers and confirmation by FTIR analysis on pure HBI compounds as described for the reference alkenes used for comparison during the experiment.

3.6 ISOLATION OF SYNTHETIC HBI MONOENES

Preparative Ag^+ chromatography afforded sufficient quantities (and in adequate purity) of some of the original isomers and those produced by isomerisation, for further characterisation.

Although C_{20}, C_{25} and C_{30} HBI monoenes produced as intermediates during the previous syntheses of 1-3 (Robson, 1987; Robson and Rowland, 1986; 1988a) were not separable by preliminary Ag^+ TLC experiments (Robson, 1987), when examined by Ag^+ HPLC or TLC in the present study, slight separation of each group of isomers was observed (Figure 3.15). This was in contrast to the good separation of n-alkanes and n-alkenes used during development of the Ag^+ HPLC technique. Complete resolution of alkane and monoenes with terminal and internal double bonds was achieved (Figure 3.16). In order to obtain even partial resolution of HBI alkenes, the mobile phase flow rate was substantially reduced and very fine control of fraction collection employed to achieved isolation of pure compounds.

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a. Original mixture (Robson and Rowland, 1986)
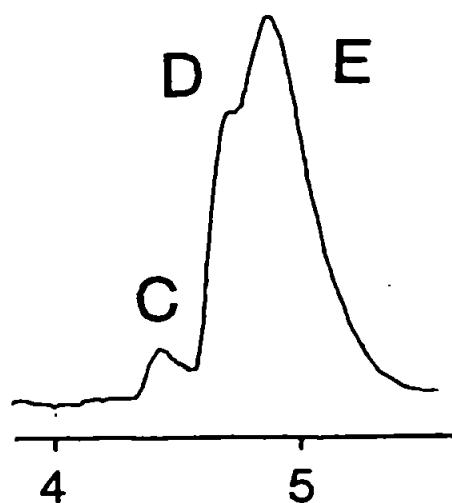
b. Isomerisation mixture (tosic acid)
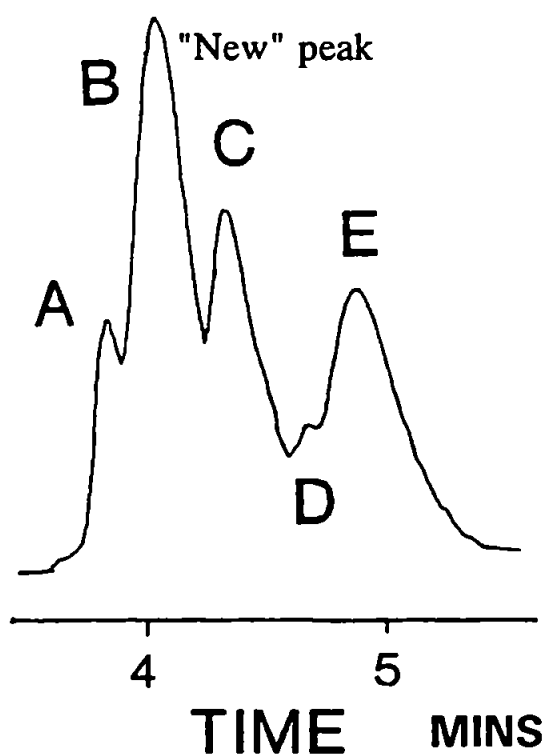
FIGURE 3.16   Ag$^+$ HPLC CHROMATOGRAMS OF C$_{20}$ MONOENES

a. n-tetradecane
b. n-tetradec-1-ene
c. (E)-n-tetradec-7-ene
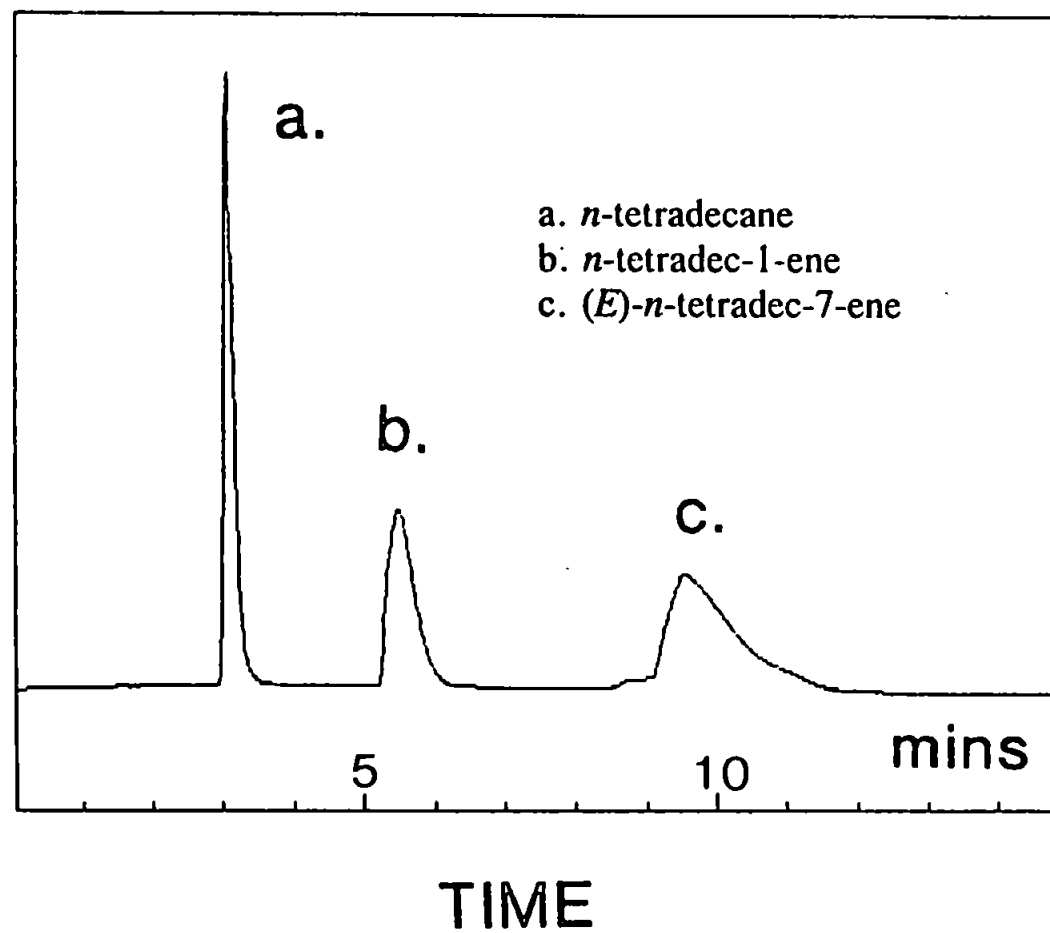
FIGURE 3.17   **Ag⁺ HPLC CHROMATOGRAM OF C$_{14}$ HYDROCARBONS**

This is the first report of the successful separation and isolation of branched alkenes by a Ag$^+$ HPLC technique. Dimitrova *et al.* (1979) reported difficulties in obtaining such separations.

3.7 CHARACTERISATION OF ISOLATED HBI ALKENES

Careful preparative scale HPLC or TLC allowed reasonably pure samples of some isomers of the C_{20}, C_{25} and C_{30} monoenes to be collected (Table 3.1). These were examined by GC, GC-MS, and some by ^{1}H NMR. In addition, all were micro-ozonolysed and the ozonolysis products examined by GC and GC-MS. Ozonolysis was found to be a particularly useful technique for the location of double bonds (*e.g.* Davison and Dutton, 1966; Nickell and Privett, 1966). Its development as a microchemical technique owes much to the work of Beroza and Bierl (1966, 1967). The ozone generator and equipment used during the present study was similar to that described by Beroza and Bierl (1969) and the apparatus is illustrated in Figure 3.16. The method has been applied successfully in the fields of insect chemistry (see review by Attygalle and Morgan, 1988) and the chemistry of other natural products (*e.g.* Barr *et al.*, 1989).

TABLE 3.2 CHROMATOGRAPHIC AND SPECTRAL DATA FOR ISOLATED SYNTHETIC HBI ALKENES

Alkene	Structure	GC retention index			Characteristic ions (*m/z*)	GC purity (%)	^{1}H-NMR[2] (δ ppm)	Identification method
		DB1	DB5	DBWAX				
br20:1[1]	39 or 40	1711	1706	1688	280, 210, 196	34[1]	-	O$_3$
br20:1[1]	39 or 40	1714	1709	1700	280, 210, 196	38[1]	-	O$_3$
br20:1	47 + 48	1697	1693	1670	280, 210, 196	67	-	O$_3$
br25:1[1]	23 or 24	2115	2110	2074	350, 224, 196	42[1]	-	O$_3$
br25:1[1]	23 or 24	2125	2119	2083	350, 224, 196	44[1]	-	O$_3$
br25:1	25 + 26	2076	2072	2023	350, 280, 266, 224, 196	88	5.1 (t)	O$_3$, NMR
br25:1	44 + 45	2109	2101	2063	350, 266, 238, 210	83	5.1, 5.2 (t) 1.4, 1.5 (s)	O$_3$, NMR
br30:1	34 + 35	2492	2494	----	420, 350, 266, 224, 210, 196	90	-	O$_3$
br30:1[1]	32 or 33	2524	2527	----	420, 350, 266, 244, 196	42[1]	-	O$_3$
br30:1[1]	32 or 33	2541	2540	----	420, 350, 266, 244, 196	55[1]	-	O$_3$
br20:1	41 or 42	1677	1674	1643	280, 210, 196	85	-	Comparison with structural homologue

[1]Isolated as pairs of geometric isomers
[2]Vinylic protons
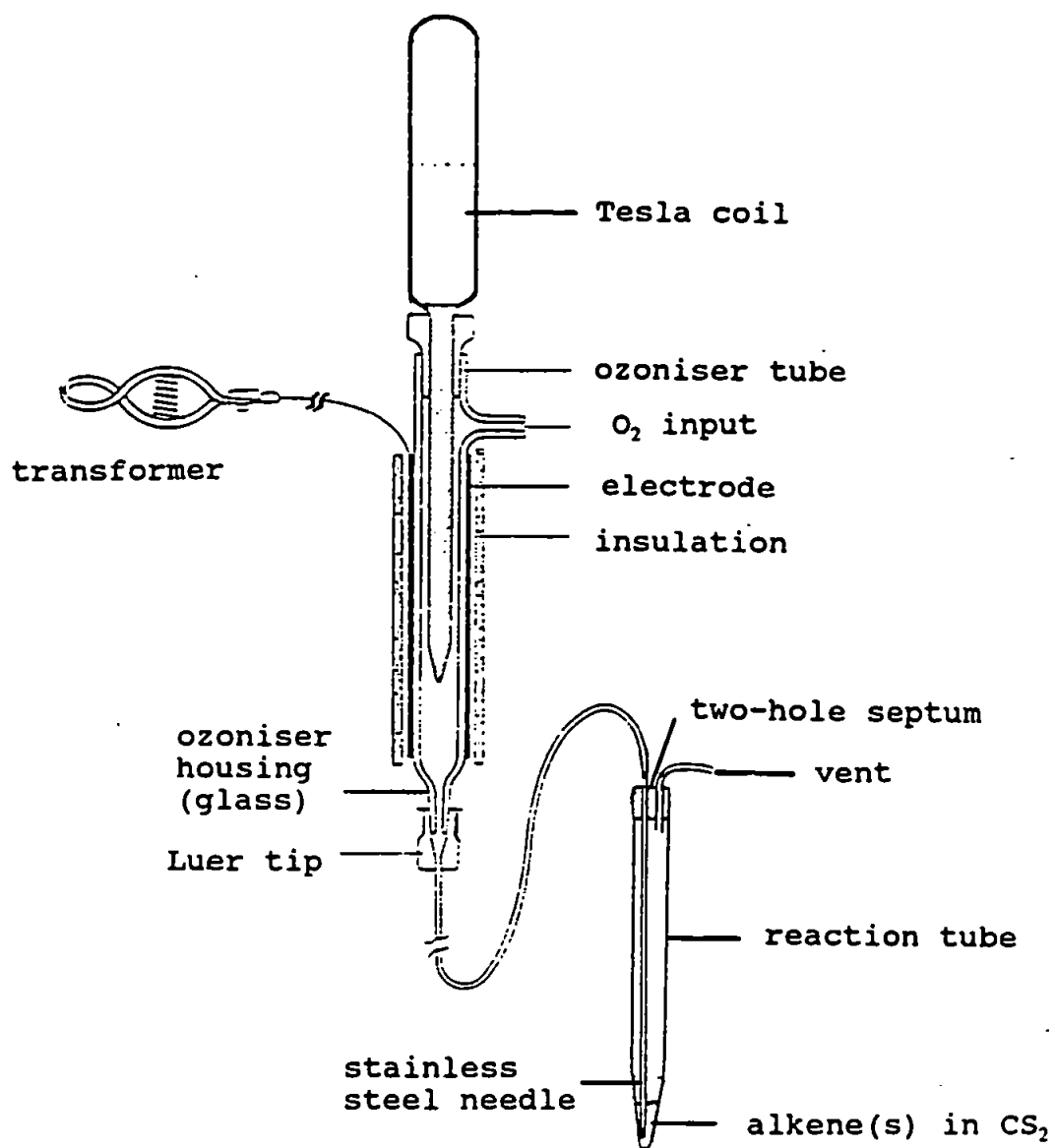
FIGURE 3.16   MICRO-OZONOLYSIS APPARATUS

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Although a large amount of work has been accomplished concerning the mechanism of ozonization (formation of ozonides: see review by Criegee, 1975; March, 1985), but not all the details are known. It has been established, however, that when compounds containing double bonds are treated with ozone, they are converted to compounds called ozonides which can be isolated or reduced to two moles of aldehyde, two moles of ketone or one mole of each, depending on the groups attached to the alkene.
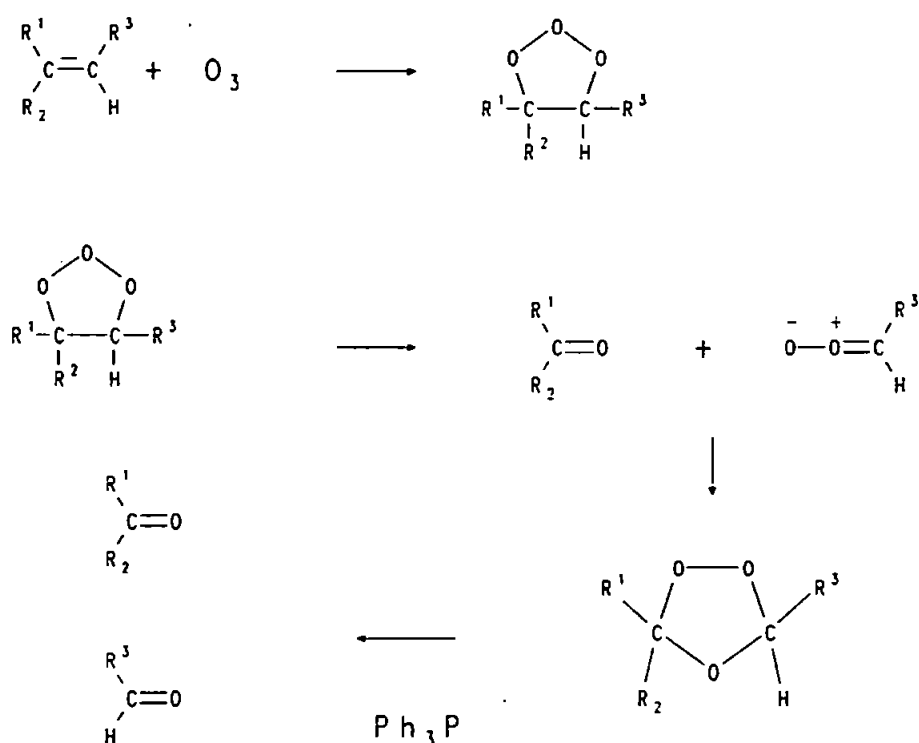
The micro-ozonolysis studies described here allowed the positions of the double bonds in several of the synthetic monoenes to be established (Table 3.2).

3.7.1 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadec-6(7)-enes
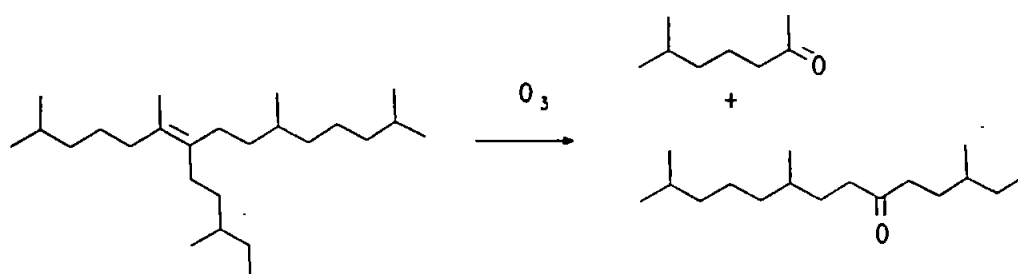
Isomers br25:1; 2115_{DB1} and 2125_{DB1} produced, on ozonolysis, only ketones 21 and 22. Ketone 21 was identified by comparison of the mass spectrum (Figure 3.18A) with that of the synthetic compound 9 (synthesised via method of Robson and Rowland, 1986). The most notable feature of the mass spectrum of 6-methylheptan-2-one 21 was the McLafferty rearrangement ion which gave the base peak at m/z 58:
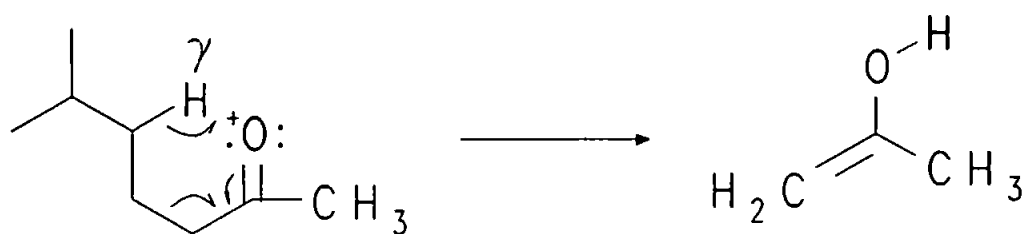
The mass spectrum of 3,9,13-trimethyltetradeca-6-one (22) (Figure 3.18B) exhibited a M^+ at m/z 254, ions at m/z 169 and m/z 113 which arise from α-cleavage either side of the carbonyl group and an ion at m/z 95, from loss of water from the latter acylium ion. The alkenyl ion at m/z 126 is derived from the loss of the McLafferty rearrangement ion from M^+.
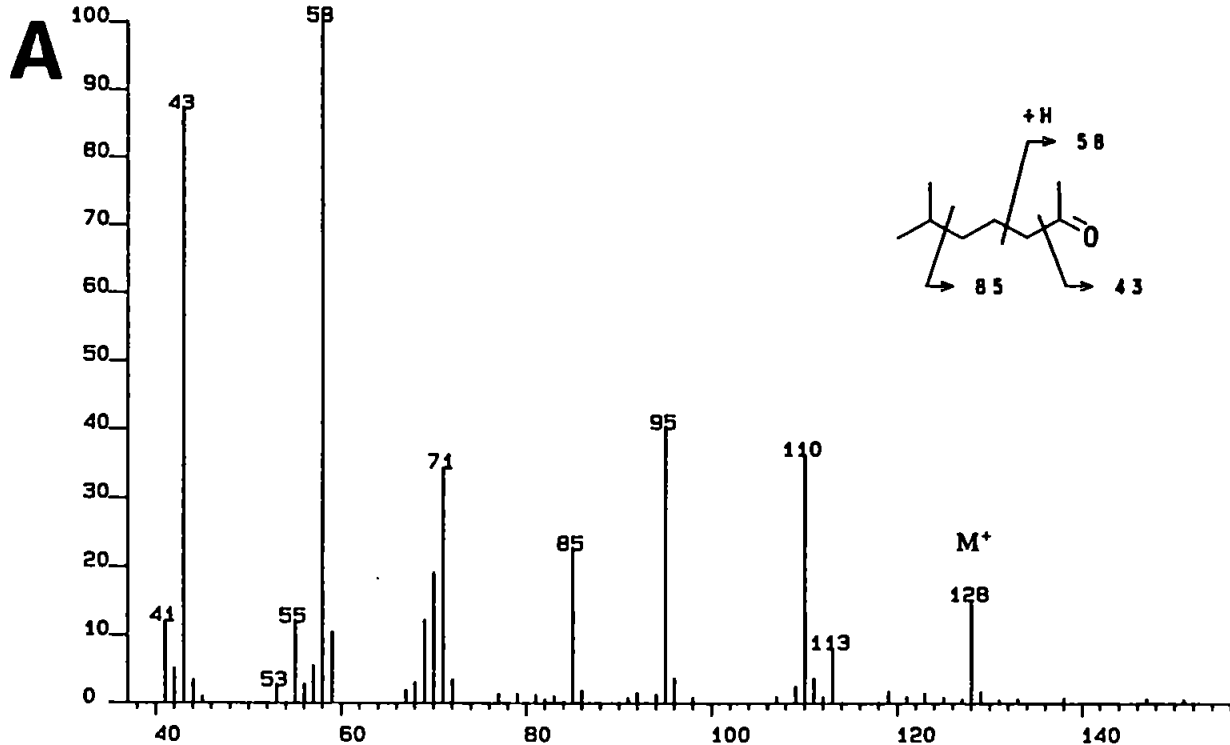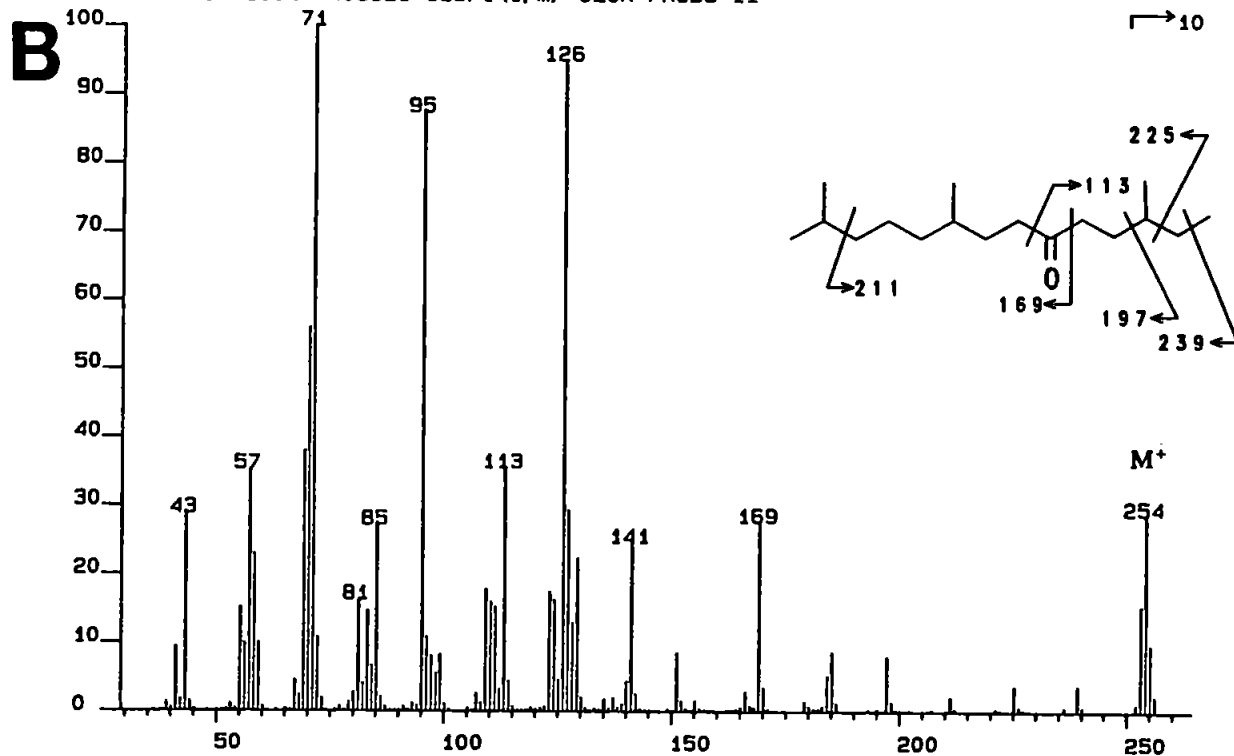
FIGURE 3.18   EI MASS SPECTRA OF
(A) 6-methylheptan-2-one
(B) 3,9,13-trimethyltetradecan-6-one

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The usual double McLafferty rearrangement does not seem favoured in this molecule possibly due to steric hinderance caused by the presence of methyl groups γ to the carbonyl carbon. The presence of the double rearrangement ion without any H-transfer, which gave the base peak at *m/z* 71, was also noted:
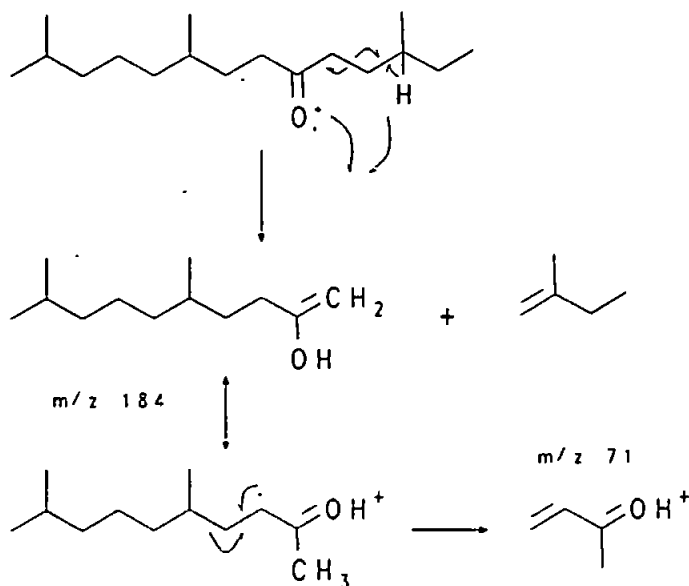
This identified the double bond as 6(7) so alkenes br25:1; 2115 and 2125 are

E/Z isomers (**23** and **24**). Examination of the mass spectral data for these isomers (Table 3.1; Figure 3.19) demonstrates that they are identical, as reported for other geometric isomers (McLafferty, 1973).
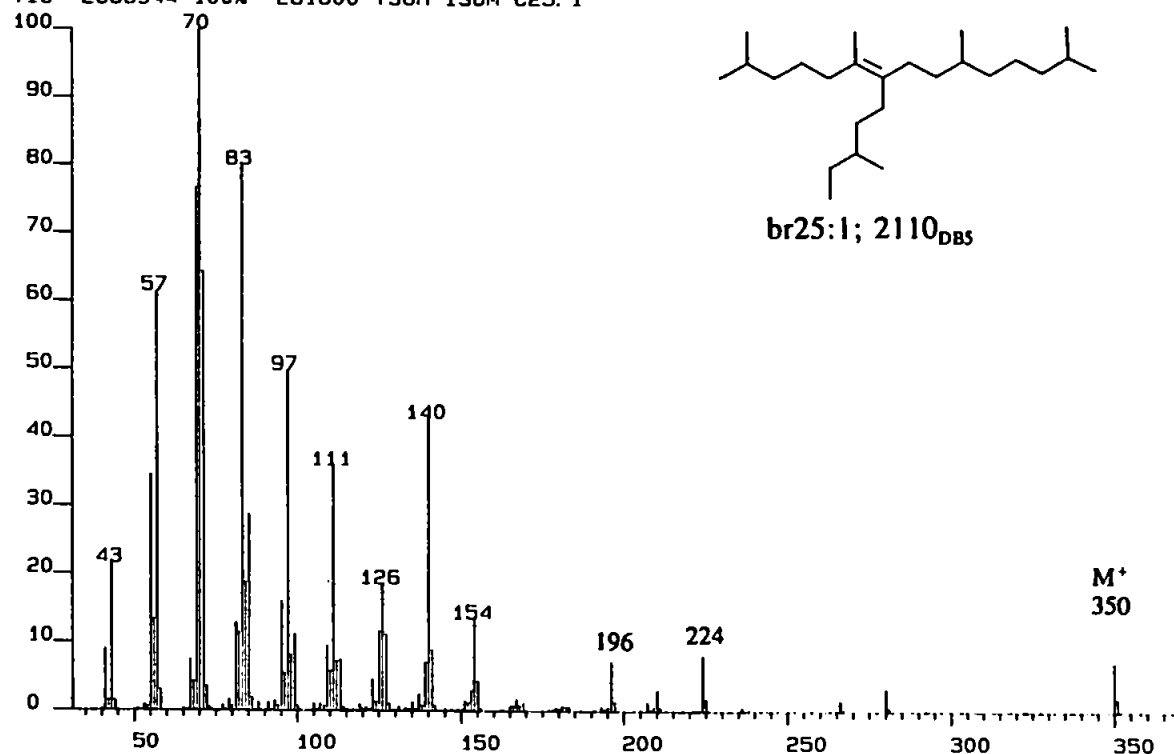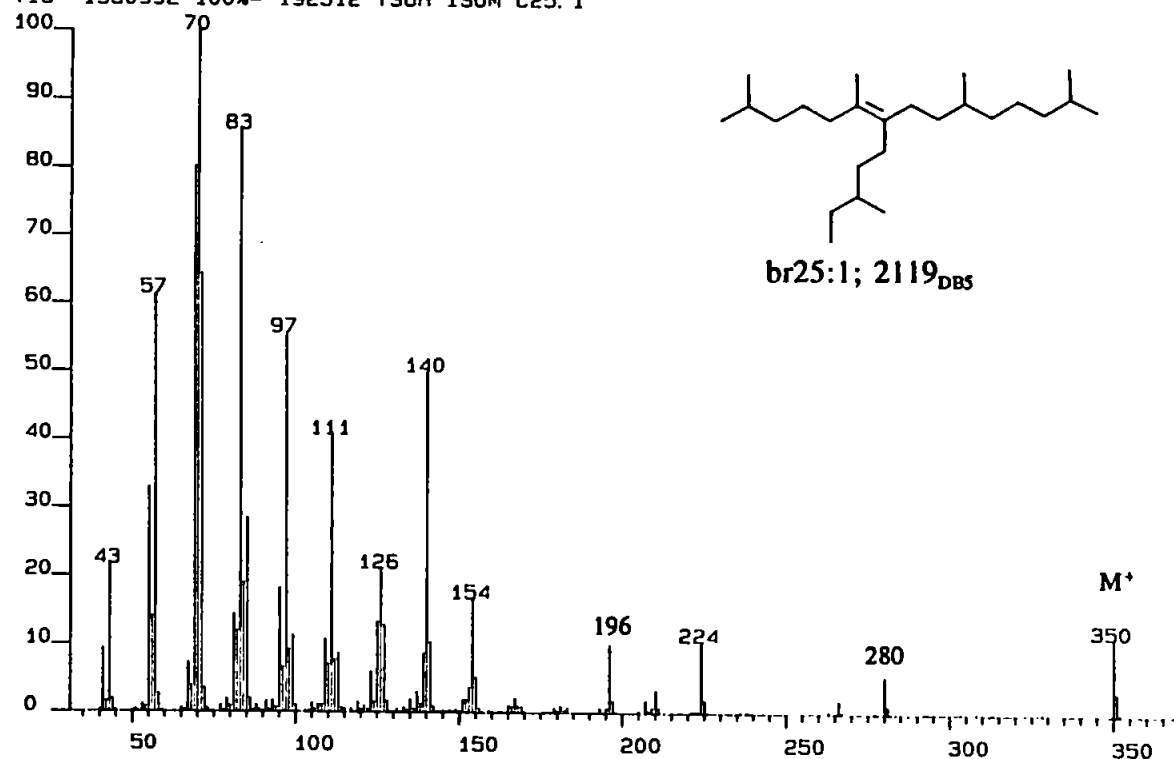
FIGURE 3.19 EI MASS SPECTRA OF THE GEOMETRIC ISOMERS OF
2,6,10,16-tetramethyl-7-(3'-methylpentyl)pentadec-6(7)-ene

3.7.2 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadec-7(8)- and -7(1')-enes
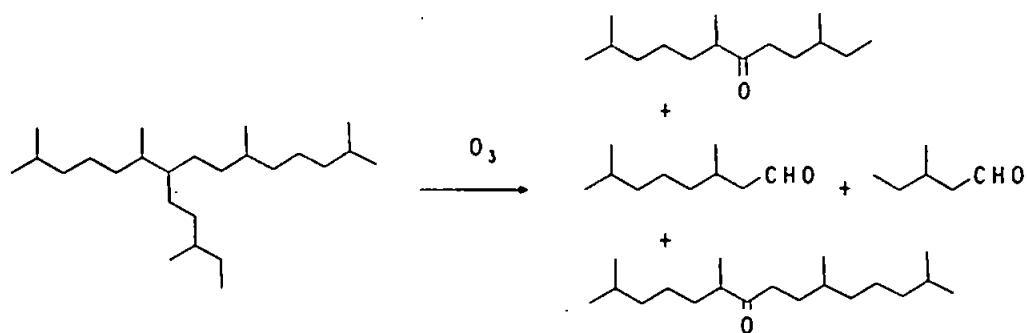
Alkene peak RI 2076_{DB1} proved to be a mixture of two alkenes (viz **25** and **26**). The products from ozonolysis included ketones **27** and **28**, identified by mass spectral comparison with synthetic **7** (Robson and Rowland, 1986) and **28**, produced by oxidation of **2** (Yon, 1981). The mass spectrum (Figures 3.20A) of 2,6,10,14-tetramethylpentadecan-7-one (**27**) exhibits a M^+ at m/z 282, an (M^+-15) ion at m/z 267, McLafferty rearrangement ions at m/z 198 and m/z 156 and the presence of a double McLafferty rearrangement ion at m/z 72:

m/z 156

m/z 72
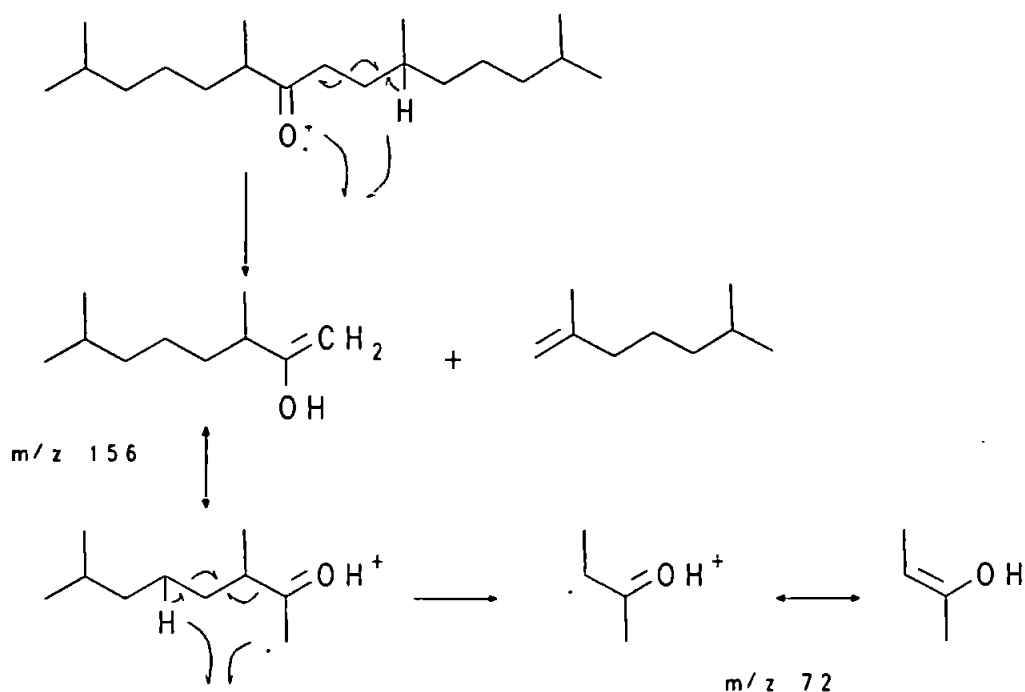
Obviously a similar secondary rearrangement exists for the other primary McLafferty rearrangement ion (*m/z* 198). Also evident in the mass spectrum are the ions at *m/z* 169 and *m/z* 141, arising from α-cleavage either side of the carbonyl group, and the associated losses of water and CO.

The mass spectrum of the other ketone produced by ozonolysis, 3,7,11-trimethyldodecan-6-one (**28**), is shown in Figure 3.20B. The mass spectrum exhibits a M⁺ at *m/z* 226, an (M⁺-15) ion at *m/z* 211 and McLafferty rearrangement ions at *m/z* 156 and *m/z* 142. Additionally the presence of an ion at *m/z* 72 could be attributed to secondary rearrangement (double McLafferty) of both primary McLafferty rearrangement ions (*m/z* 156 and *m/z* 142):

149

m/z 156

m/z 72
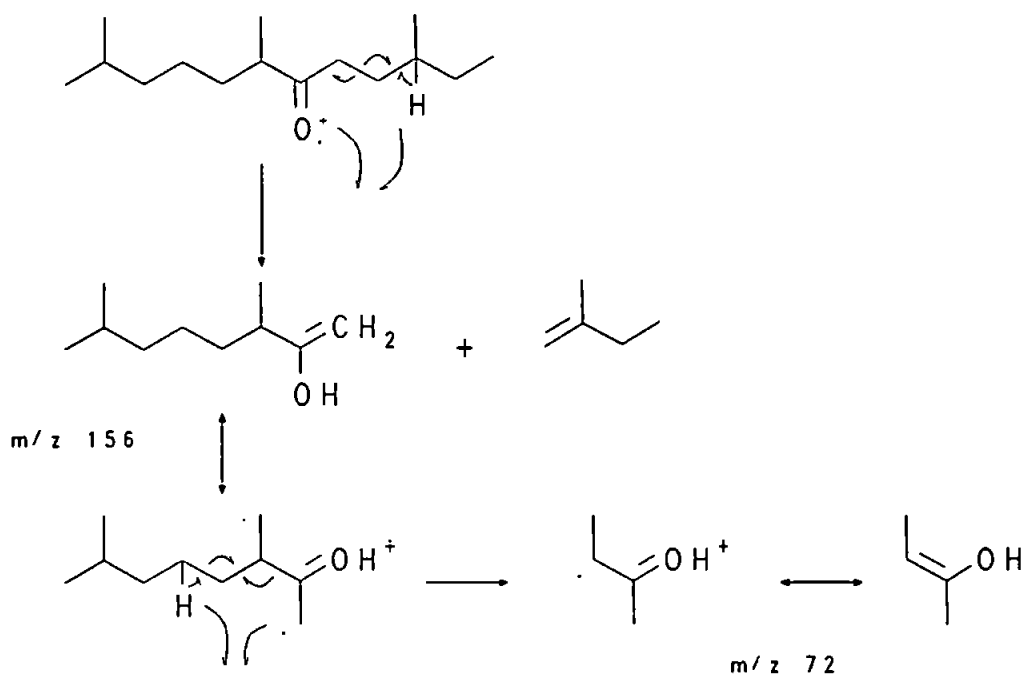
Another major ion is observed at m/z 113 (α-cleavage) with the corresponding

ion for the loss of water at m/z 95.

Other ozonolysis products included the aldehydes **29** and **30**, identified by

interpretation of their mass spectra (Figures 3.21A and 3.21B). Although no M^+ was

apparent in the mass spectrum of 2,6-dimethyloctanal (**29**), ions at m/z 138 and m/z

123, corresponded to loss of water (M^+-18) and then a methyl group (M^+-18-15).

The dominant ions in the mass spectrum derive from fragmentation of the acyclium

ion $C_9H_{19}CO^+$ (*e.g.* m/z 71, $C_3H_7CO^+$; m/z 69, C_5H_9; m/z 56, C_4H_8) and the loss of

the McLafferty rearrangement ion at m/z 112.

The mass spectrum of 3-methylpentanal (**30**) shows the M^+ at m/z 100 and

expected loss of the McLafferty rearrangement ion at m/z 56 (base peak).

150

FIGURE 3.20   **EI MASS SPECTRA OF**
(A) 2,6,10,14-tetramethylpentadecan-7-one
(B) 3,7,11-trimethyldodecan-6-one

151

FIGURE 3.21   EI MASS SPECTRA OF
(A) 2,6-dimethyloctanal
(B) 3-methylpentanal

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As only one triplet due to vinylic protons was present in the ^{1}H NMR spectrum of **25/26** (δ ppm 5.1; Figure 3.22) it was confirmed that **25** and **26** were either both E or both Z.

By inference the remaining two alkenes of the original synthetic mixture, br25:1; 2085_{OVI} and 2092_{OVI} (Robson, 1987) must also be both E (or both Z) isomers of **25** and **26**.

The C_{20} and C_{30} alkenes were isolated and characterised in the same manner as the C_{25} homologues, but the C_{20} alkenes were only obtained in sufficient purity for one pair of C_{20} isomers (br20:1; 1711_{DBI} and 1714_{DBI}) to be characterised. The ketones and aldehydes produced by ozonolysis of C_{20} and C_{30} HBI alkenes displayed similar fragmentation patterns described for the C_{25} homologues.
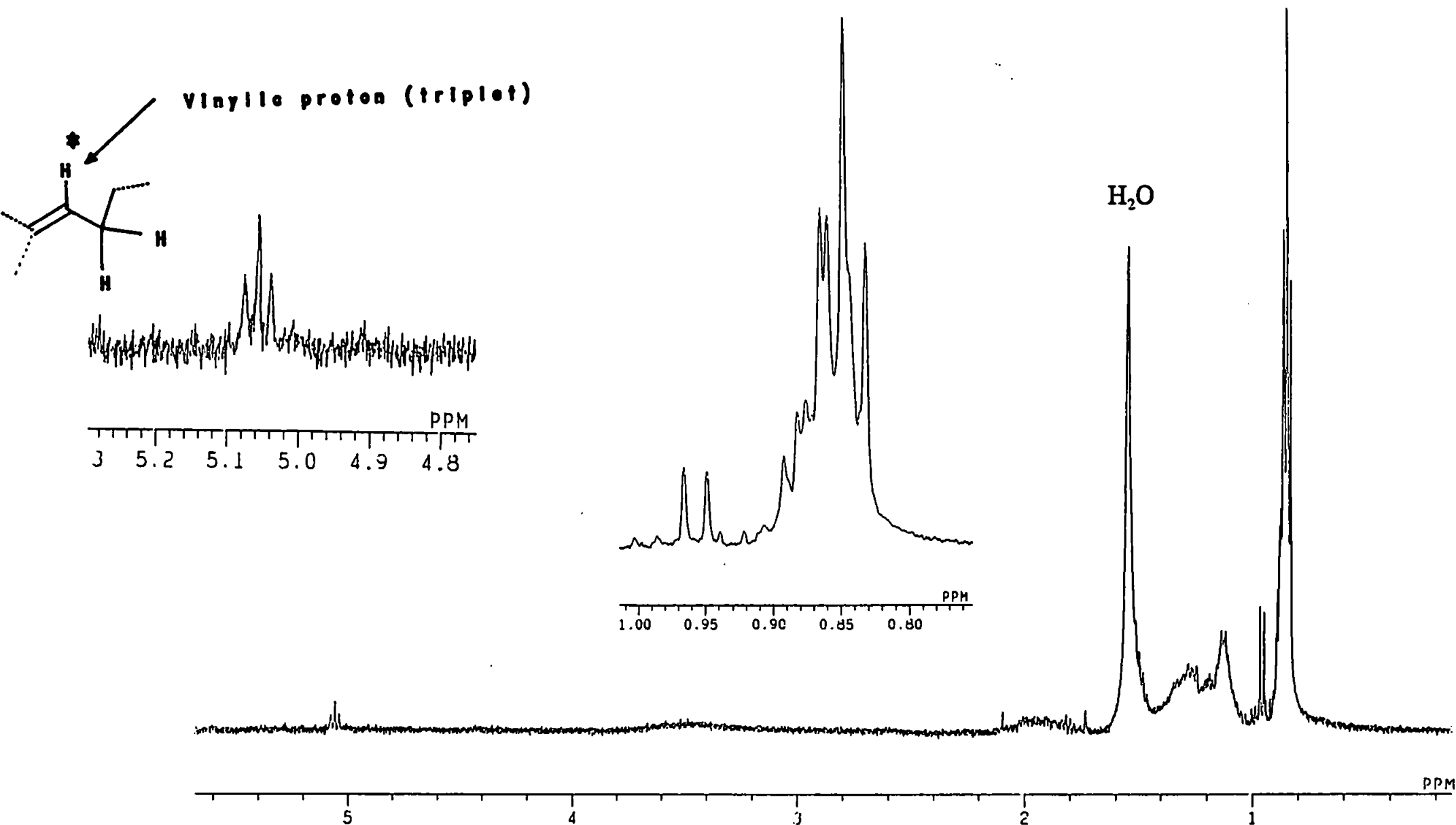
FIGURE 3.22 ¹H NMR SPECTRUM OF A MIXTURE OF 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadec-7(8)- and -7(1')-enes

3.7.3 2,6,10,14,18-pentamethyl-7-(3'-methylpentyl)nonadec-6(7)-enes
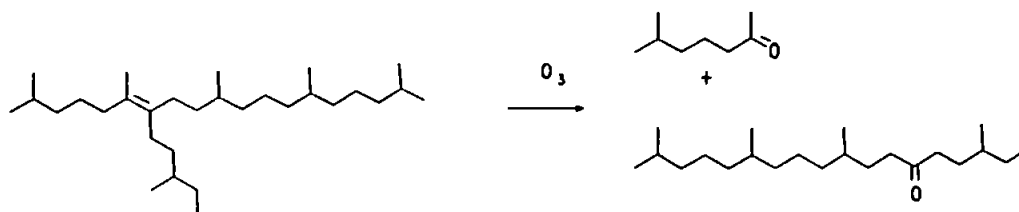
Isomers br30:1; 2565_{DB1} and 2579_{DB1} produced, on ozonolysis, only ketones **21** and **31**. Ketone **21** was identified by comparison of the mass spectrum with that of the synthetic compound **9** (Robson and Rowland, 1986) described above **(3.4.1)** and **31** by interpretation of the mass spectrum (Figure 3.23; M^+ 324, 323 [M^+-1], 239 [18%, α-cleavage$\rightarrow C_{15}H_{31}CO^+$], 196 [40%, M^+-McL or M^+-Double McL$\rightarrow C_{14}H_{28}$], 141 [30%], 129 [25%, Double H-transfer + β-cleavage], 126 [30%, C_9H_{18}], 113 [40%, α-cleavage$\rightarrow C_6H_{13}CO^+$], 95 [58%, α-cleavage-H_2O], 85 [40%, α-cleavage-CO], 71 [100%, McL + γ-cleavage$\rightarrow C_4H_6OH^+$ and/or C_5H_{11}]). This identified the double bond as 6(7) so alkenes br30:1; 2565 and 2579 are *E/Z* isomers (**32** and **33**).
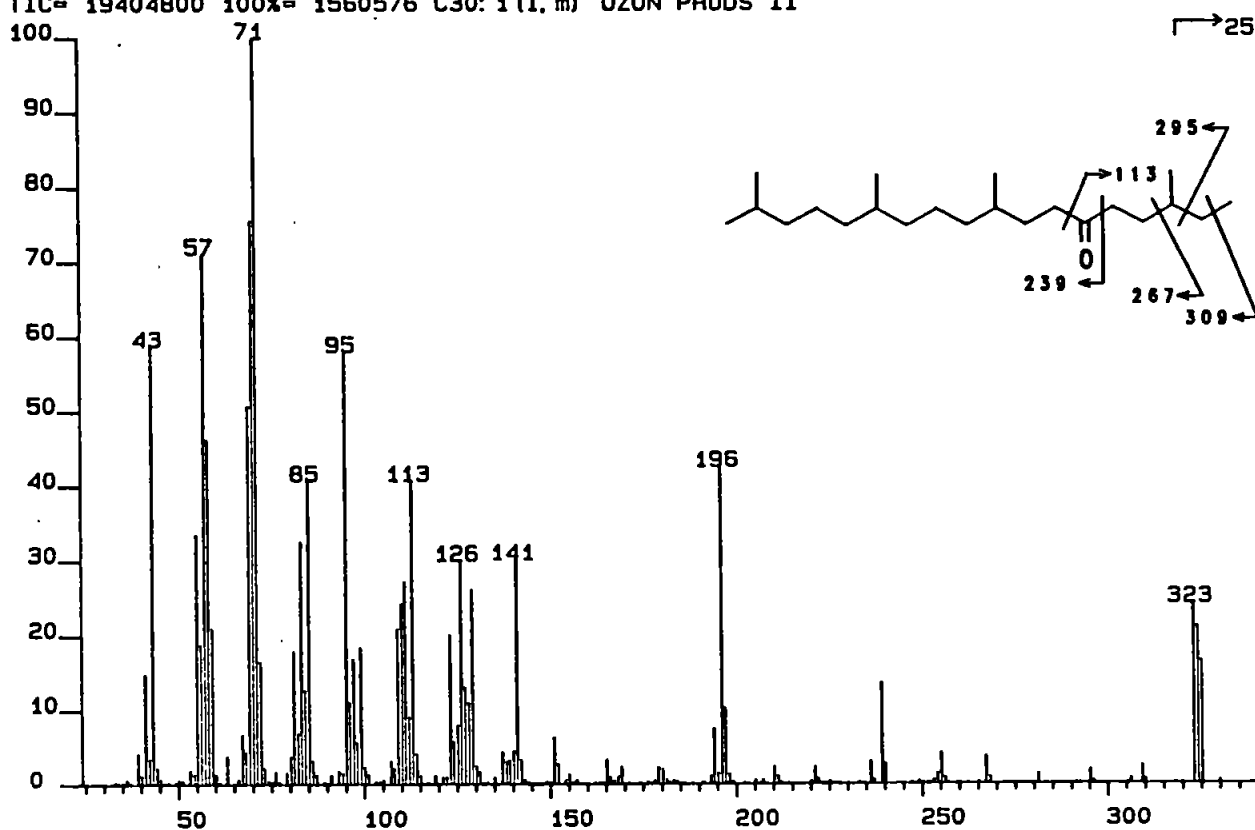
FIGURE 3.23   **EI MASS SPECTRUM OF**
3,9,13,17-tetramethyloctadecan-6-one

156

3.7.4 2,6,10,14,18-pentamethyl-7-(3'-methylpentyl)nonadec-7(8)-and-7(1')-enes
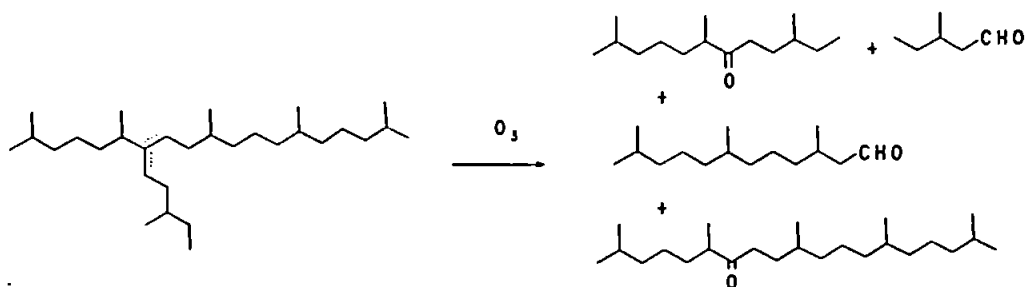
Alkene peak RI 2527$_{DB1}$ proved to be a mixture of two alkenes

(viz **34** and **35**). The products from ozonolysis included ketones **36** and **28**, identified

by mass spectral comparison with synthetic **36** (Robson and Rowland, 1986; 1988a)

and **28**, produced by oxidation of **2** (Yon, 1981). The mass spectrum of **28** was

described earlier (**3.4.2**), whereas that of **36** is shown in Figure 3.24 (M$^+$ 352, 337

[10%, M$^+$-CH$_3$], 239 [45%, α-cleavage$\rightarrow$C$_{15}$H$_{31}$CO$^+$], 196 [80%, C$_9$H$_{18}$], 156 [45%,

McLafferty], 157 [30%, Double H-transfer], 141 [20%, α-cleavage$\rightarrow$C$_8$H$_{17}$CO$^+$], 126

[35%, C$_9$H$_{18}$], 113 [30%, α-cleavage-CO], 95 [45%, α-cleavage-H$_2$O-CO], 85 [80%,

McL+γ-cleavage$\rightarrow$C$_5$H$_8$OH$^+$ and C$_6$H$_{13}$], 72 [100%, Double McL]. Other ozonolysis

products included the aldehydes **30** and **37** identified by interpretation of their mass

spectra (**30**: see **3.4.2**; **37**: M$^+$ absent, 182 [25%, M$^+$-McLafferty], 126 [40%,

C$_9$H$_{18}$], 112 [30%, C$_8$H$_{16}$], 97 [55%, C$_7$H$_{13}$], 81 [42%], 71 [100%, C$_5$H$_{11}$ and

C$_3$H$_6$CHO].
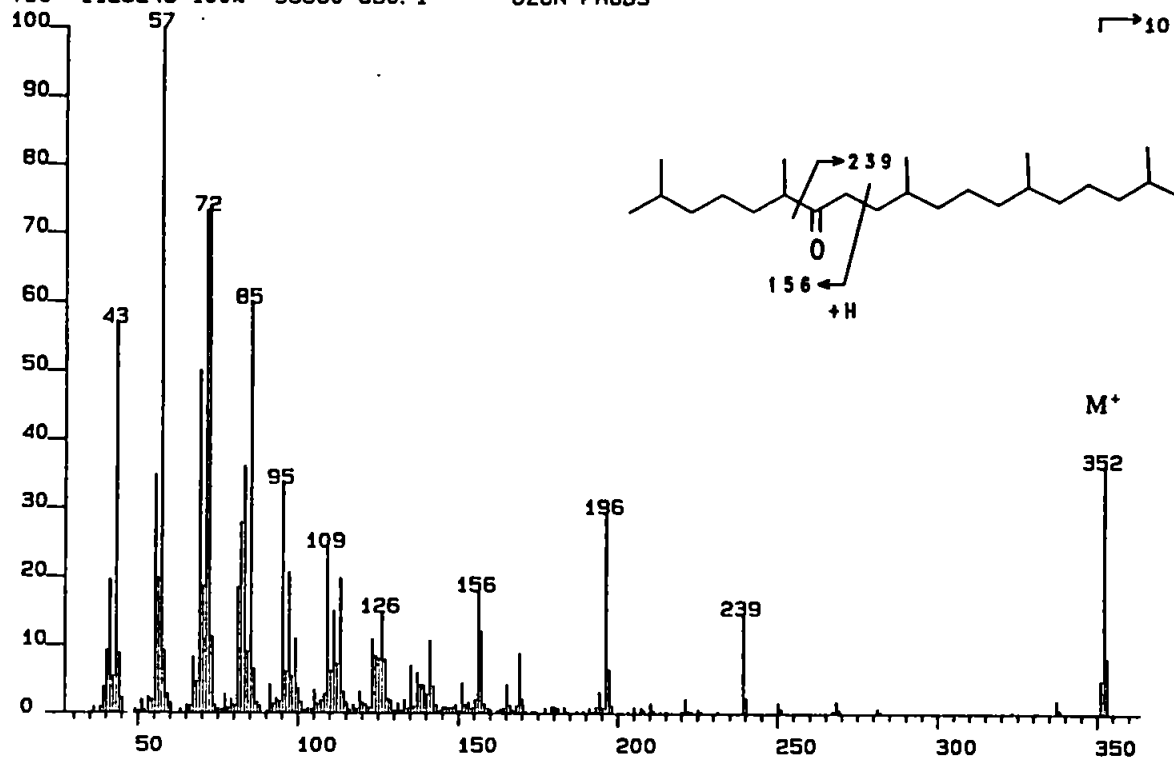
FIGURE 3.24   **EI MASS SPECTRUM OF**
2,6,10,14,18-pentamethylnonadecan-7-one

158

3.7.5 2,6,10-trimethyl-7-(3'-methylbutyl)dodec-6(7)-enes
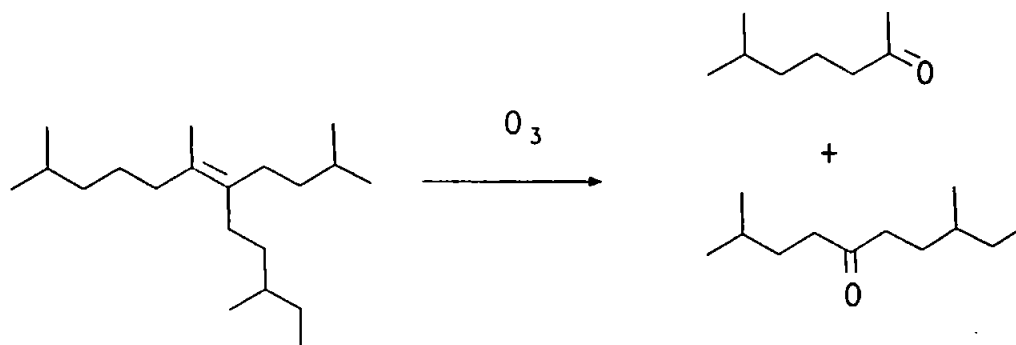
Isomers br20:1; 1711_{DB1} and 1714_{DB1} produced, on ozonolysis, only ketones **21** and **38**. Ketones **21** and **38** were identified by comparison of the mass spectrum of **21** with that of the synthetic compound **9** (synthesised via method of Robson and Rowland, 1986) described above (**3.4.1.**) and **38** with that of **38** produced by oxidation of **2** (Yon, 1981) and interpretation of the mass spectrum (Figure 3.25; M^+ 184, 128 [20%, McLafferty], 114 [25%, McLafferty], 113 [60%, α-cleavage$\rightarrow C_6H_{13}CO^+$], 99 [70%, α-cleavage$\rightarrow C_5H_{11}CO^+$], 95 [86%, α-cleavage-H_2O], 81 [65%, α-cleavage-H_2O], 71, [100%, α-cleavage-CO and/or C_5H_{11}], 58 [85%, Double McL]). This identified the double bond as 6(7) so alkenes br20:1; 1711 and 1714 are *E/Z* isomers (**39** and **40**). Examination of the mass spectral data for these isomers (Table 3.1; Figure 3.26) demonstrates, as shown for the C_{25} homologues above (**3.4.1**) that the spectra are identical.

FIGURE 3.24   EI MASS SPECTRUM OF
2,8-dimethyldecan-5-one

160
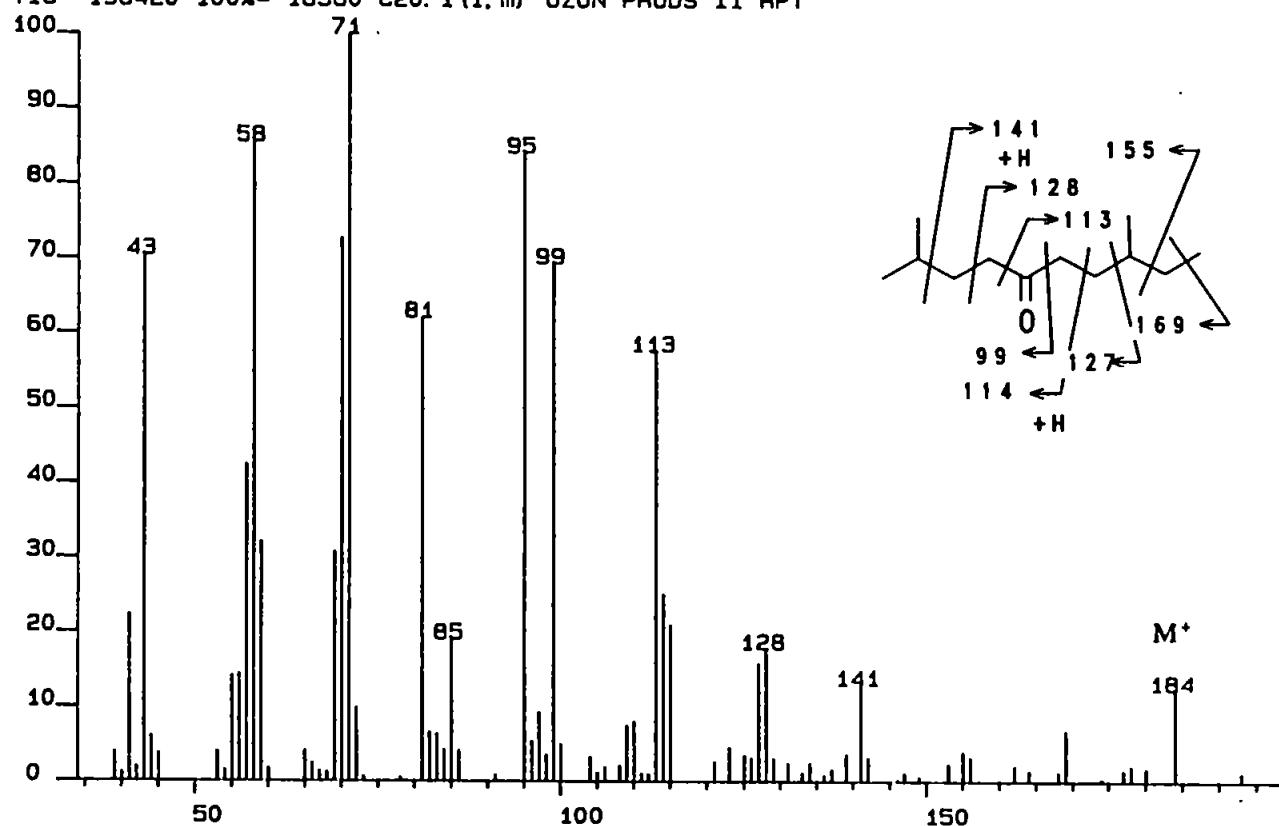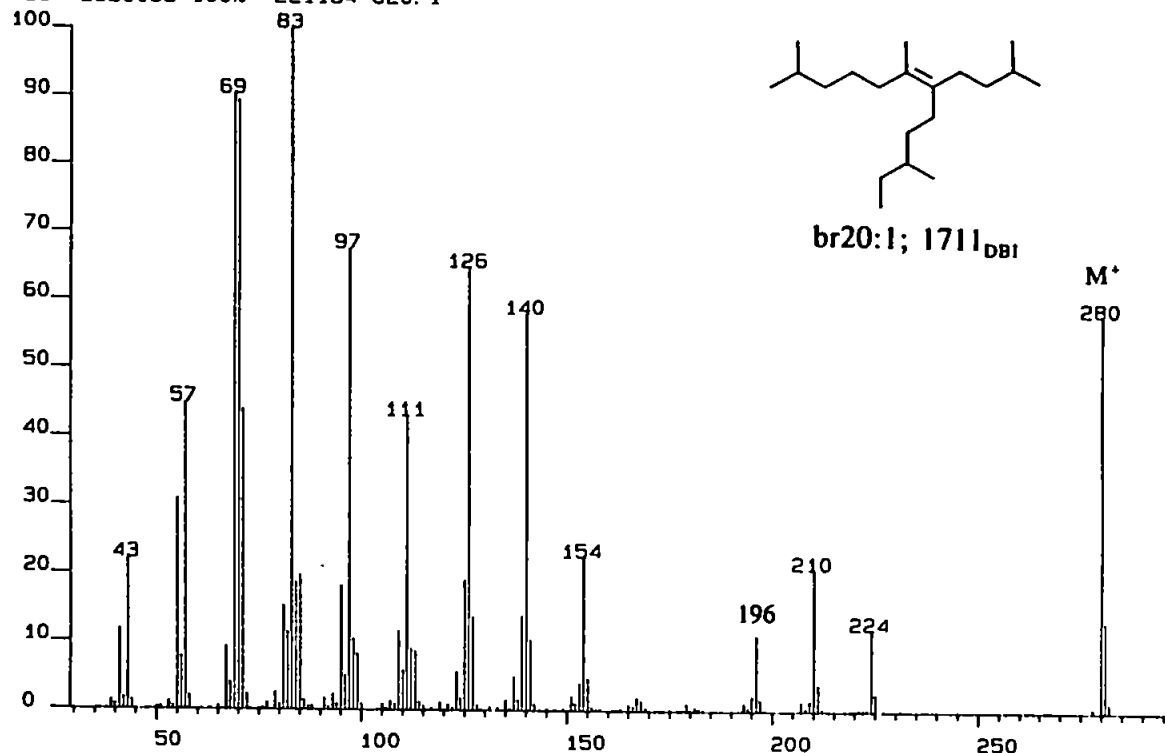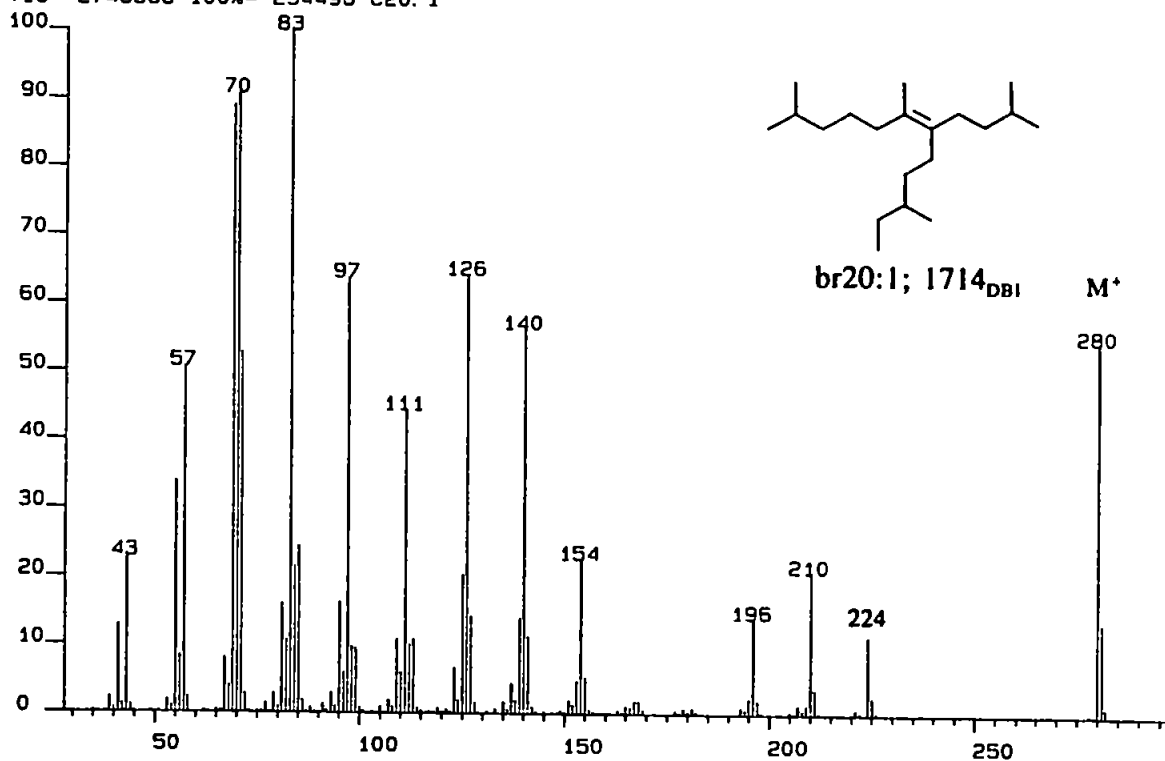
FIGURE 3.25 EI MASS SPECTRA OF THE GEOMETRIC ISOMERS OF
2,6,10,-trimethyl-7-(3'-methylbutyl)dodec-6(7)-ene

161

3.7.6 Other C_{20} HBI monoenes

The assignments of double bond positions for the other C_{20} HBI compounds (41 and 42) were made solely by comparison of the GC elution orders between the C_{20} and C_{25} alkenes. The parent alkanes have been shown to plot co-linearly on a graph of RI *versus* carbon number, as expected for structural homologues (Robson, 1987). These assignments, based upon structural homologues, remain tentative until sufficient material can be isolated for ozonolysis.

3.8 HBI MONOENES PRODUCED BY ISOMERISATION

Preparative Ag^+ chromatography of the C_{20} and C_{25} mixtures containing the new isomers produced by acid-catalysed rearrangement (tosic acid), afforded components in sufficient purity for ozonolysis and one, the C_{25} isomer br25:1; 2110_{DB1}, in sufficient quantity for ^1H NMR.

3.8.1 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadec-5(6)-ene

The ^1H NMR spectrum of C_{25} HBI alkene br25:1; 2109_{DB1} is shown in Figure 3.26. Two triplets assigned to vinylic protons were observed in the NMR spectrum (δ ppm 5.1, 5.2) and two singlets assigned to the allylic methyl (δ ppm 1.4, 1.5). These suggested that both E and Z isomers were present in a ratio E/Z, $Z/E = 1.6$; no GC separation was achieved on any stationary phase used (DB1, DB5, DBWAX or CPWAX52).

(two triplets)

vinylic proton

? DCM

PPM
5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8

PPM
0.95 0.90 0.85 0.80 0.75

H₂O

? DCM

PPM
5   4   3   2   1
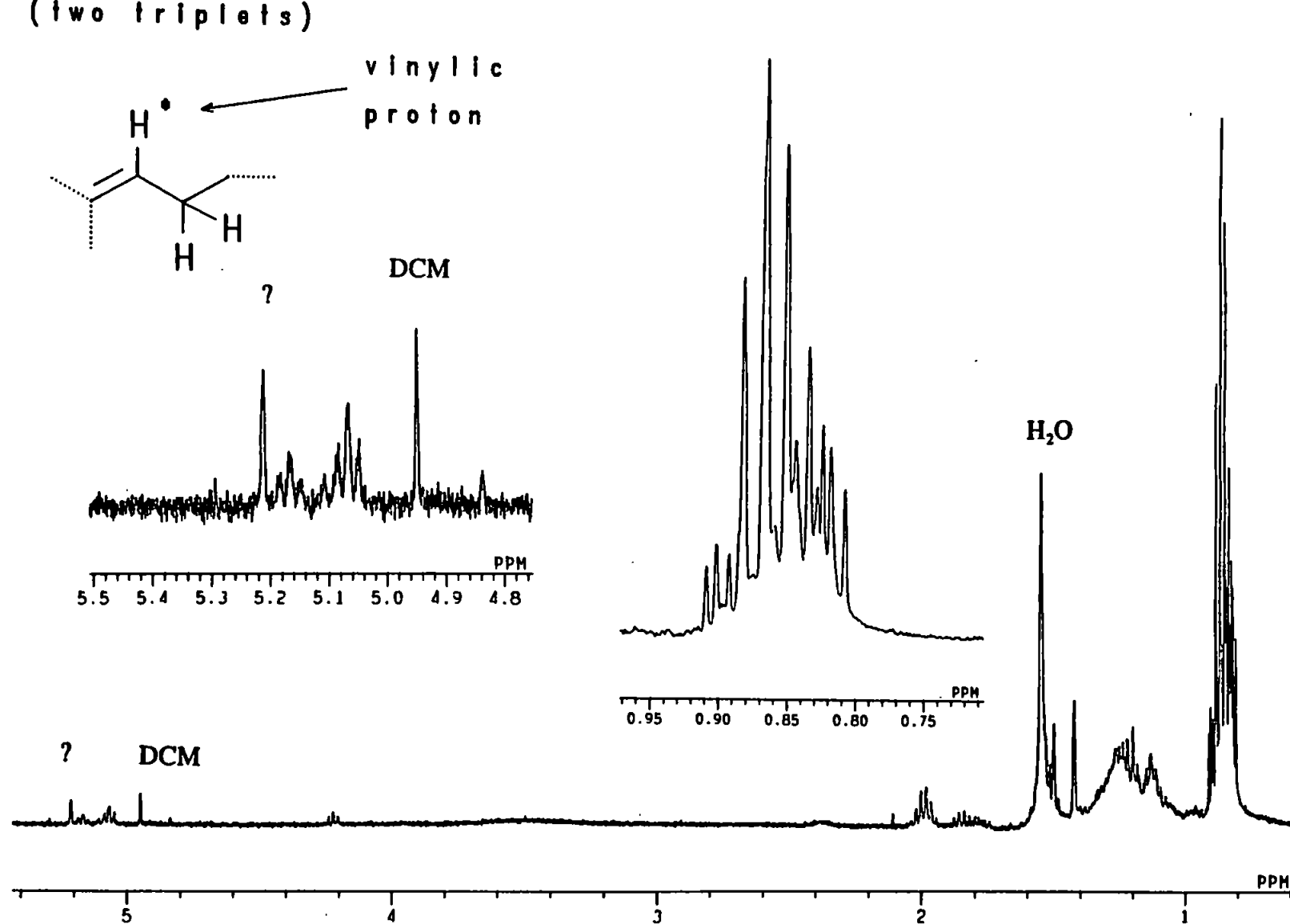
FIGURE 3.26 ¹H NMR SPECTRUM OF 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadec-5(6)-ene

Ozonolysis was consistent with these assignments since a C_{19} compound, assigned **43** from the mass spectrum (Figure 3.27), was the only ketone detected in the products.
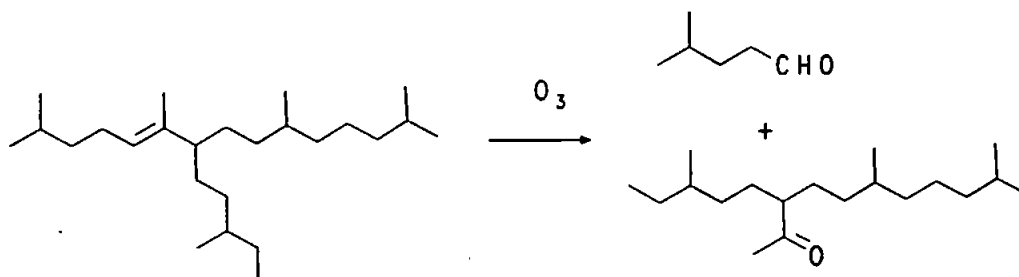
The mass spectrum of 6,10-dimethyl-3-(3'-methylpentyl)-undecan-2-one exhibits a M^+ at m/z 282, an (M^+-29) ion at m/z 253 and McLafferty rearrangement ions at m/z 198 and m/z 142 with corresponding losses of water at m/z 180 and m/z 124. Further rearrangement of the McLafferty ions was evident from the ion at m/z 71, produced by γ-cleavage of the primary McLafferty rearrangement ions:

m/z 198
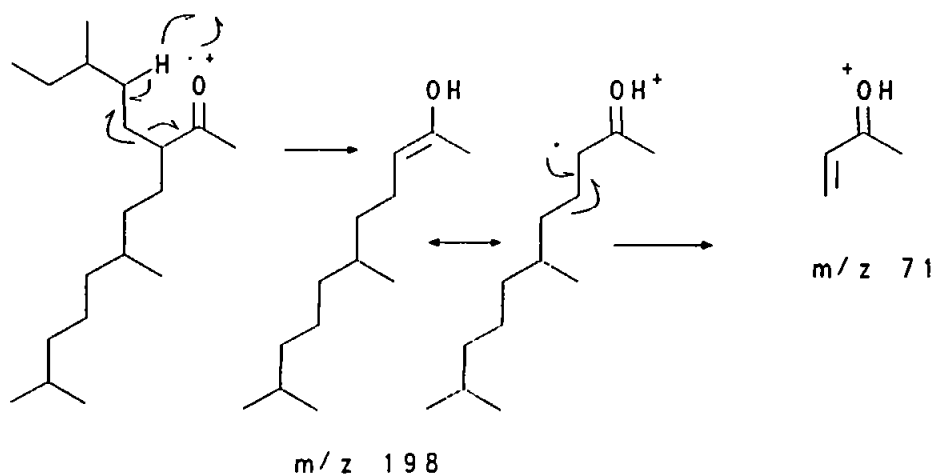
This identified the double bond as 5(6) so alkene 2109_{DB1} was a mixture of (E/Z) isomers (**44, 45**).
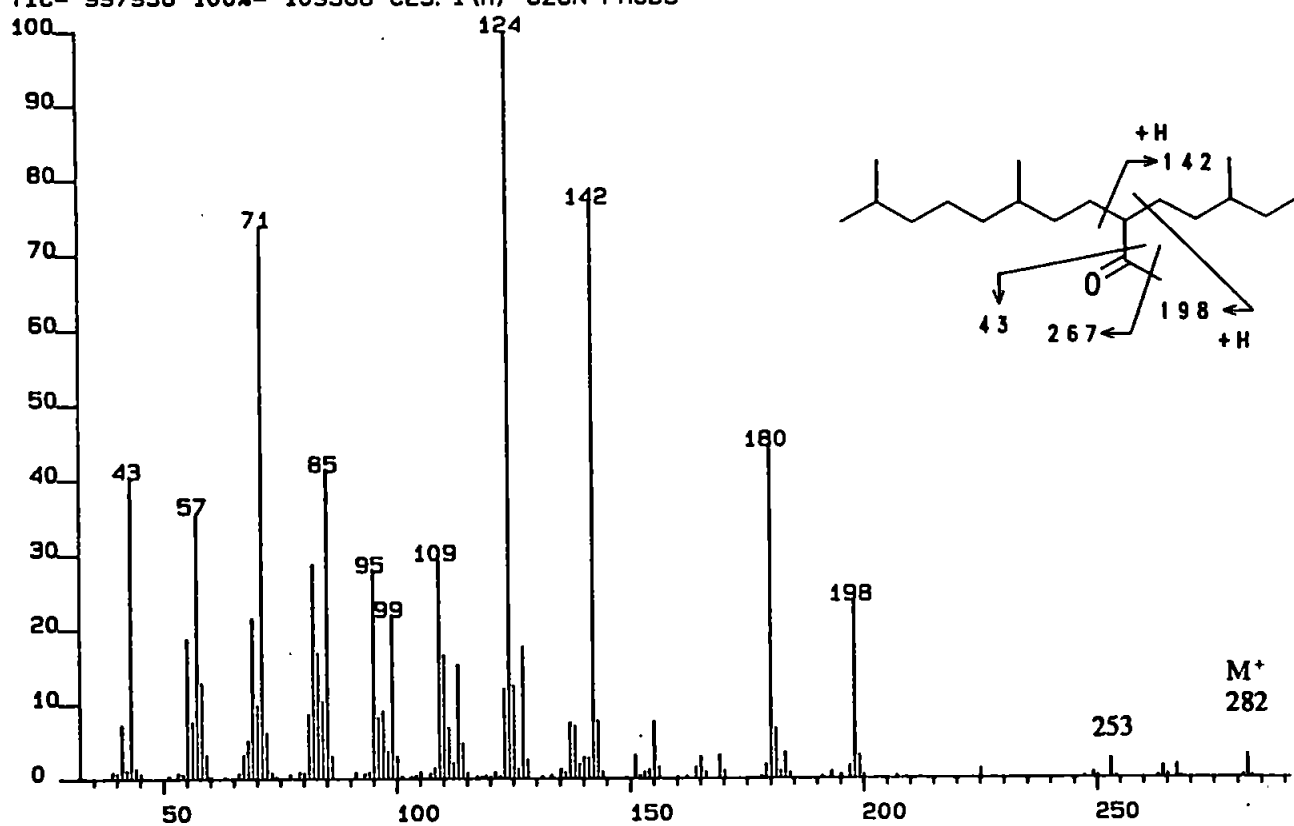
FIGURE 3.27   EI MASS SPECTRUM OF
6,10-dimethyl-3-(3'-methylpentyl)-undecan-2-one

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3.8.2 2,6,10-trimethyl-7-(3'-methylbutyl)dodec-5(6)-ene
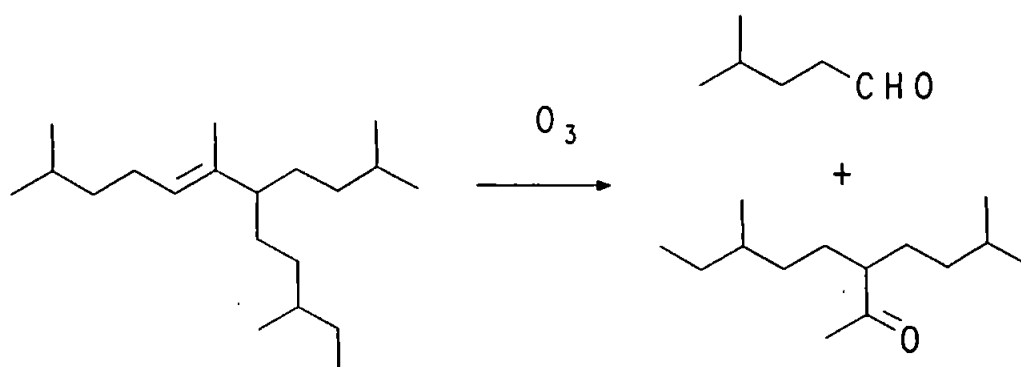
Ozonolysis of br20:1; 1697_{DBI} was consistent with the assignment of the C_{25} homologue since a C_{14} ketone, assigned **46** by comparison with the synthetic compound (Yon, 1981), was the major product. The mass spectrum of 6-methyl-3-(3'-methylbutyl)octan-2-one **(46)** shown in Figure 3.28, displays similar fragmentations to that of **46** above (M^+ 212, 197 [2%, M_+-CH_3], 194 [2%, M_+-H_2O], 183 [3% M_+-C_2H_5], 142 [30%, McLafferty], 128 [50%, McLafferty], 124 [45%, McL-H_2O], 110 [65%, McL-H_2O], 71 [85%, McL+ γ-cleavage$\rightarrow C_4H_6OH^+$]). This identified the double bond as 5(6) so alkene 1697_{DBI} was **47/48**.
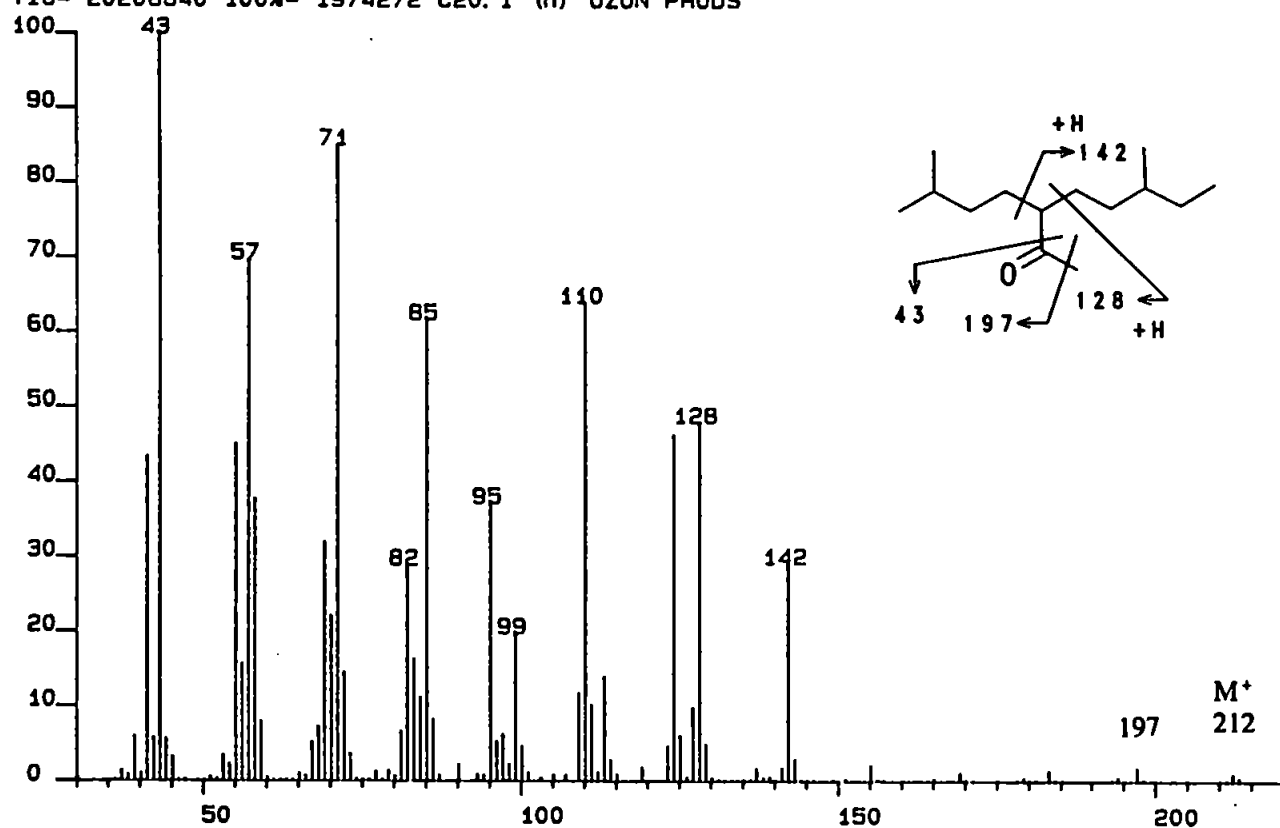
FIGURE 3.29   EI MASS SPECTRUM OF
6-methyl-3-(3'-methylbutyl)octan-2-one

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3.9   SUMMARY

The isolation and characterisation of synthetic alkenes resulted in the assignment, or partial assignment, of structures to four C_{20} (**39, 40, 47** and **48**), six C_{25} (**23-26, 44** and **45**) and four C_{30} (**32-35**) monoenes. Other tentative assignments have been made by inference (**25, 26, 34** and **35**) and on the basis of the existence of structural homologues (**41** and **42**). The compounds form a valuable database of chromatographic (GC RI) and spectroscopic (NMR, MS) information for the assignment of sedimentary alkenes. However, as will be seen in the following section (Chapter 4), care should be taken when using GC retention indices alone as a basis for structural assignments of these alkenes and this emphasises the importance of the micro-ozonolysis data.

STRUCTURES

CHAPTER THREE
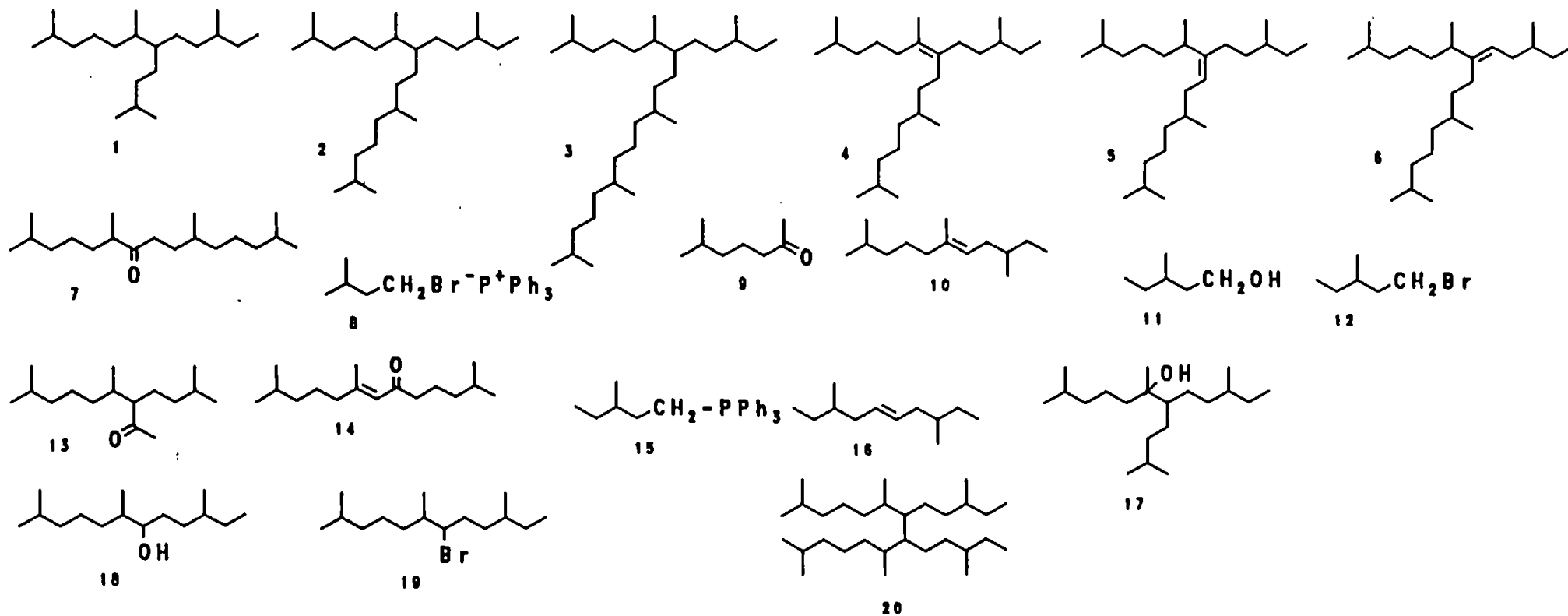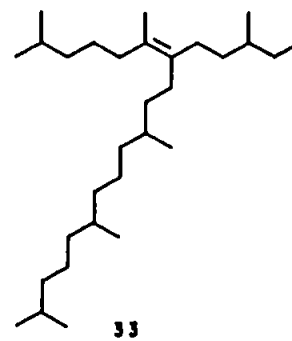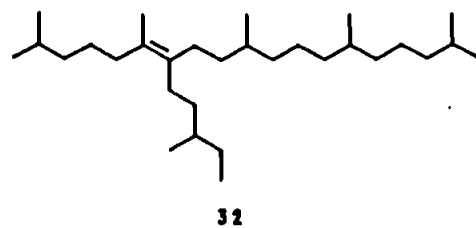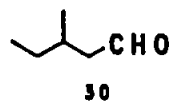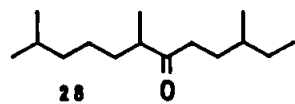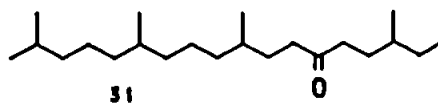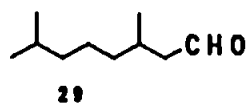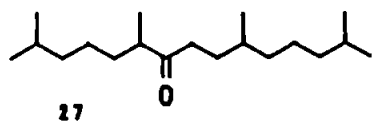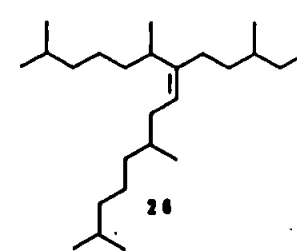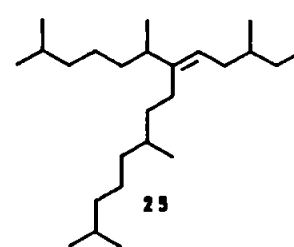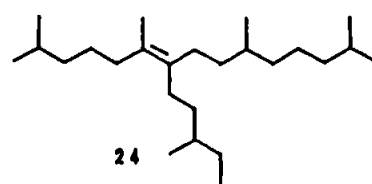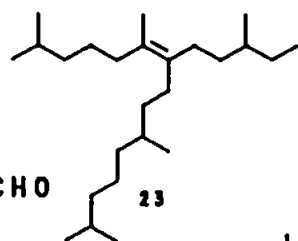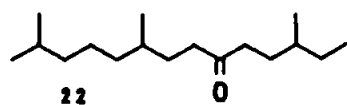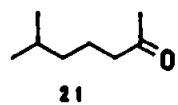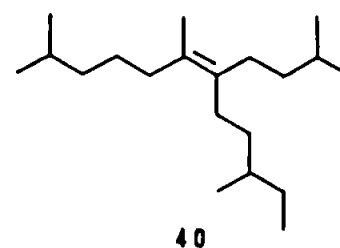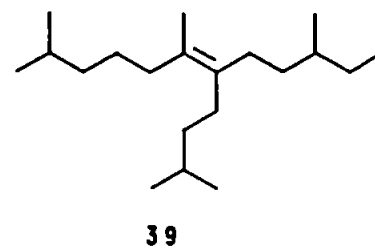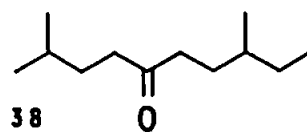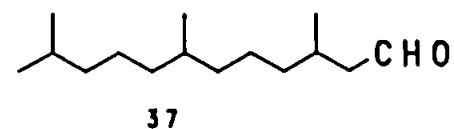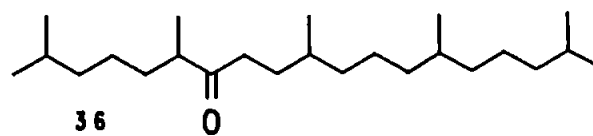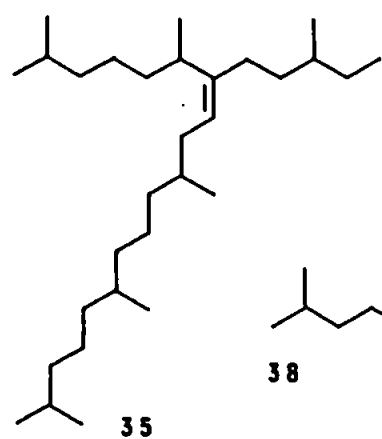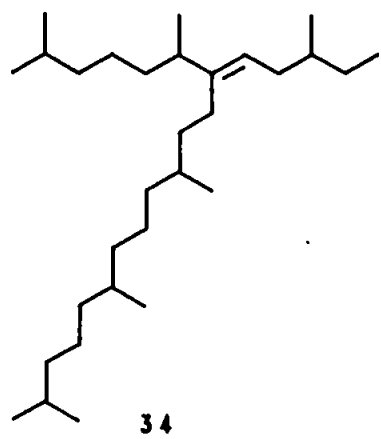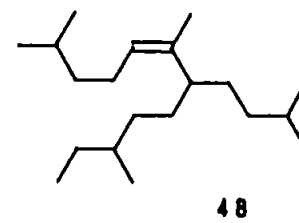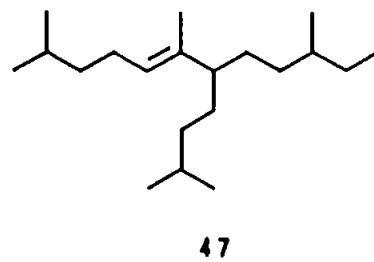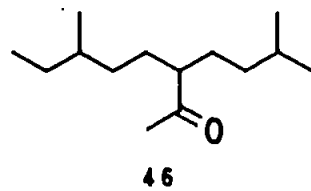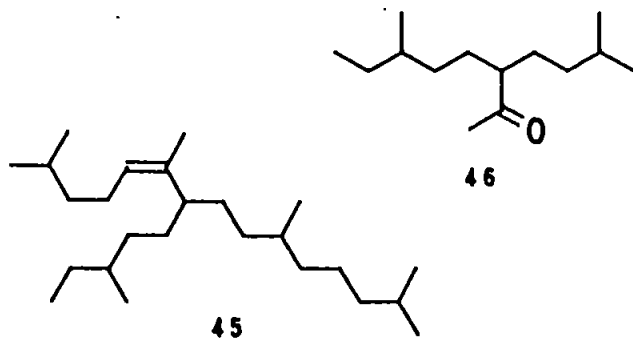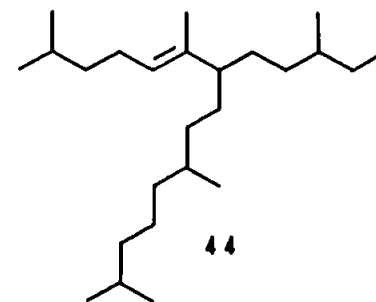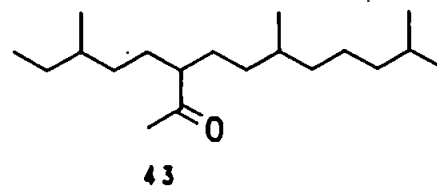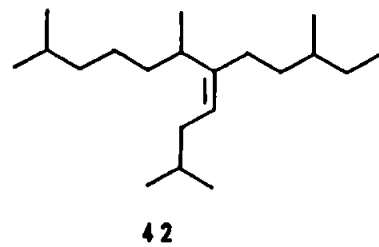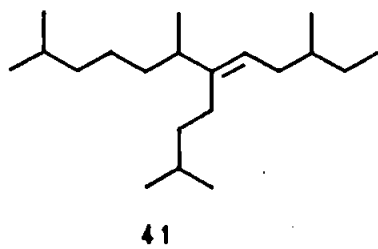
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8 CH₂Br⁻P⁺Ph₃

9

10

11 CH₂OH

12 CH₂Br

13

14

15 CH₂-PPh₃

16

17 OH

18 OH

19 Br

20

21

22

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CHAPTER FOUR

ISOLATION AND CHARACTERISATION OF SEDIMENTARY

C_{20} AND C_{25} HIGHLY BRANCHED ALKENES

This chapter describes the use of synthetic HBI alkenes to assign structures or partial structures to naturally occurring HBI hydrocarbons in three sediments. Isolation of pure isomers from sediments was made using normal and argentation chromatographic techniques (TLC). Structural assignments based upon chromatographic and spectroscopic examination (i.e. GC RI, MS and ^1H NMR) and micro-ozonolysis are discussed.

4.1 INTRODUCTION

The C_{20} and C_{25} highly branched isoprenoid (HBI) hydrocarbons, with carbon skeletons 1 and 2, which are of interest in the present study, have been shown to be widely distributed in Recent sediments in coastal regions all over the globe including many estuaries (Tables 1.1 and 1.2). It is interesting to note that whilst the C_{25} HBI alkenes occur as monoenes through pentaenes, only two C_{20} HBI monoenes have been reported and no polyenes (Rowland and Robson, 1990). The position of double bonds in C_{20} and C_{25} HBI alkenes has only been established in alkenes from hypersaline and mesohaline sediments (Shark Bay, W. Australia and Guadalquivir Delta, SW. Spain). This chapter details the further characterisation of HBI hydrocarbons in sediments from temperate and cold environments.

To avoid the ambiguity associated with many previous studies, the synthetic monoenes isolated and characterised as detailed in the previous chapter (3.4, 3.5; Table 3.2) have been used to confirm the identities of the sedimentary compounds. Also, they have provided a useful database for the identification of sedimentary HBI monoenes in the future.

In the present chapter, the double bond positions in a C_{20} HBI monoene, previously detected in sediment from Gluss Voe, Shetland Islands (Robson, 1987), in a C_{20} and two C_{25} HBI monoenes from Tamar estuary (U.K.) sediments and in a C_{25} HBI monoene (partial hydrogenation product of a diene) from McMurdo Sound, Antarctica (Venkatesan, 1988; Rowland et al., 1990) are reported.

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4.2   GLUSS VOE, SHETLAND ISLANDS (U.K.)

Sullom Voe, in the Shetland Islands, is the site of a large oil terminal and hence the hydrocarbon chemistry of sediments from the Shetland Islands, including Gluss Voe, is monitored at least annually. In sediments collected in 1985, Robson (1987) identified two C_{20} HBI monoenes with parent skeleton 1 (br20:1; 1696_{OV1} and 1702_{OV1}). The former had a very similar GC retention index (GC RI) and mass spectrum to synthetic monoene 3, isolated and identified herein (Figure 4.1) and therefore, this structure is tentatively assigned to the sedimentary alkene. A C_{20} HBI monoene with a similar retention index was detected in sediments of Puget Sound, U.S.A. by Barrick et al. (1980). A more rigorous assignment must await isolation and characterisation of the sedimentary alkene by ozonolysis and/or NMR as was possible for the other C_{20} HBI monoene br20:1; 1702_{DB1} which was isolated from Tamar sediment as is discussed in the next section.

4.3   MILLBROOK, THE TAMAR ESTUARY (U.K.)

Robson (1987) reported the presence of C_{20} and C_{25} HBI monoenes and a C_{20} HBI alkane in Millbrook sediments and was able to show, by hydrogenation and GC co-injection, that the parent structures were identical to synthetic 1 and 2. The double bond position in br20:1; 1702_{OV1} however, was not assigned and that in the two C_{25} isomers was limited to one of three positions (i.e. 4). Isolation and elucidation of C_{25} synthetic alkenes 5-8 in the present study by argentation chromatography methods, discussed in Chapter 3 (3.3.7) and comparison of the GC RI with those reported by Robson (1987) (viz br25:1; 2076_{OV1} and 2091_{OV1}), allowed rejection of 5 and 6 as possible structures, reducing the possibilities to E and/or Z isomers of 7 and/or 8.

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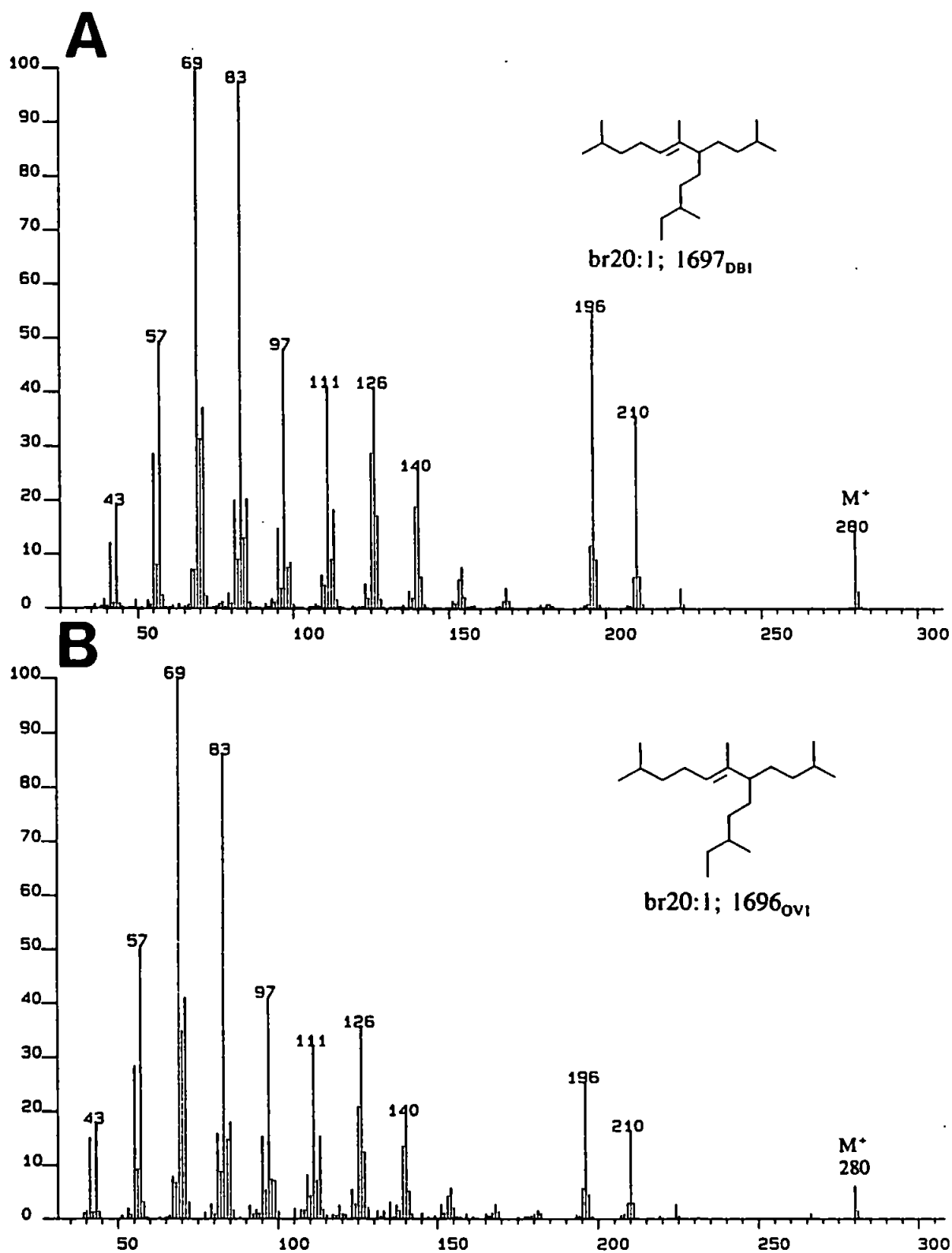
FIGURE 4.1 EI MASS SPECTRA OF
(A) (E/Z)-2,6,10-trimethyl-7-(3'-methylbutyl)dodec-5(6)-ene
(B) br20:1; 1696_{ovi} identified in Gluss Voe sediments
(Robson, 1987)

A more rigorous assignment must await isolation and ozonolysis of the sedimentary

C_{25} alkenes, as proved possible for the corresponding C_{20} monoene.

Extraction and isolation of the C_{20} HBI monoene (br20:1; 1702_{OVI}, 1699_{DBS})

afforded enough of the compound in sufficient purity for ozonolysis.
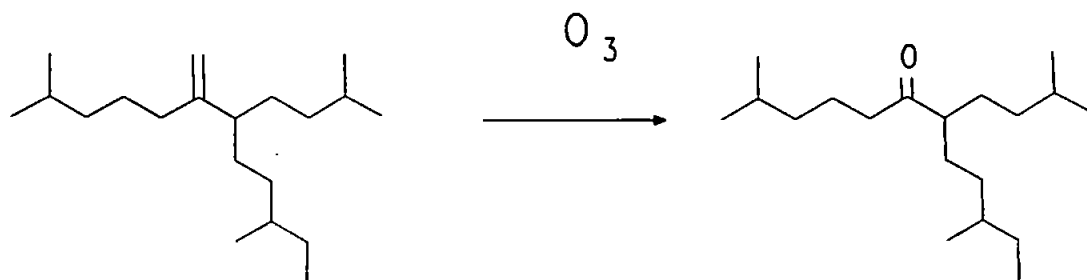
This showed that the alkene had structure **9**. The only ozonolysis product was

the C_{19} ketone, assigned **10** by interpretation of the mass spectrum and by comparison

with that of **10** reported by Dunlop and Jefferies (1985).

The mass spectrum of the C_{19} ketone is shown in Figure 4.2. The mass

spectrum of 2,10-dimethyl-7-(3'-methylbutyl)dodecan-6-one exhibits a M^+ at m/z 282,

an (M^+-15) ion at m/z 267 and McLafferty rearrangement ions at m/z 212 and m/z

198 with corresponding losses of water at m/z 194 and m/z 180.
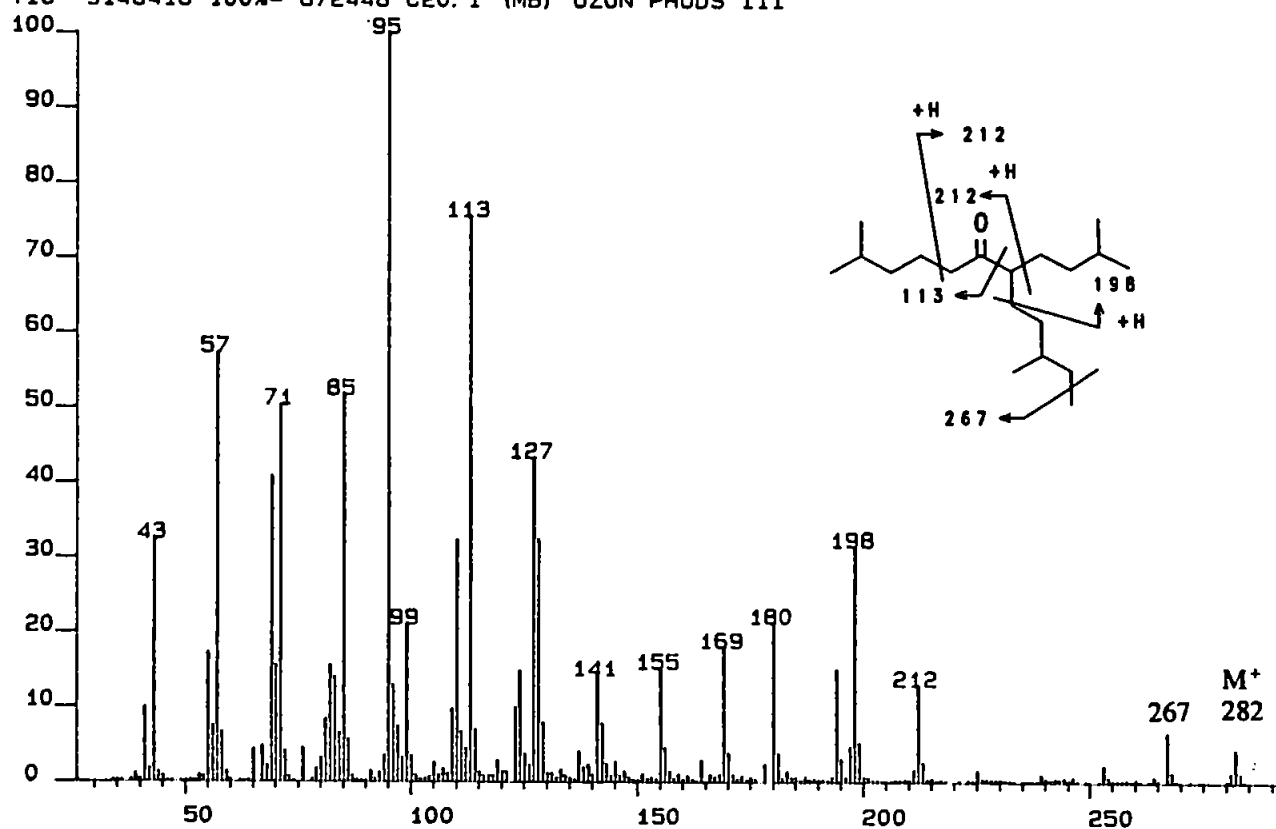
FIGURE 4.2 EI MASS SPECTRUM OF
2,10-dimethyl-7-(3'-methylbutyl)dodecan-6-one

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Additionally the presence of further rearrangements with (double McLafferty;

m/z 142 and *m/z* 128)

m/z 212

m/z 128   m/z 142
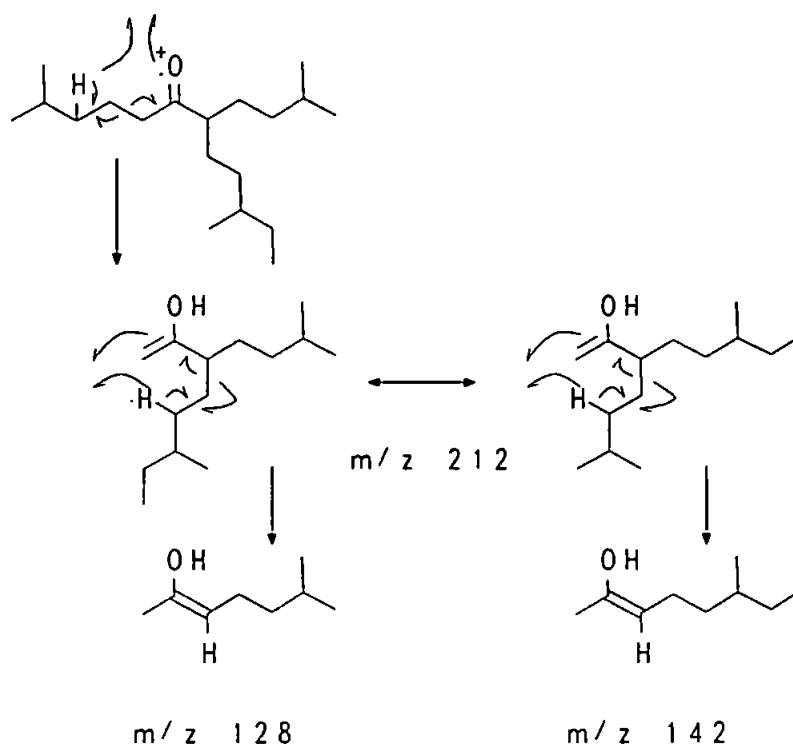
and without H-transfer (McL→β-cleavage; *m/z* 141 and *m/z* 127) is noted:
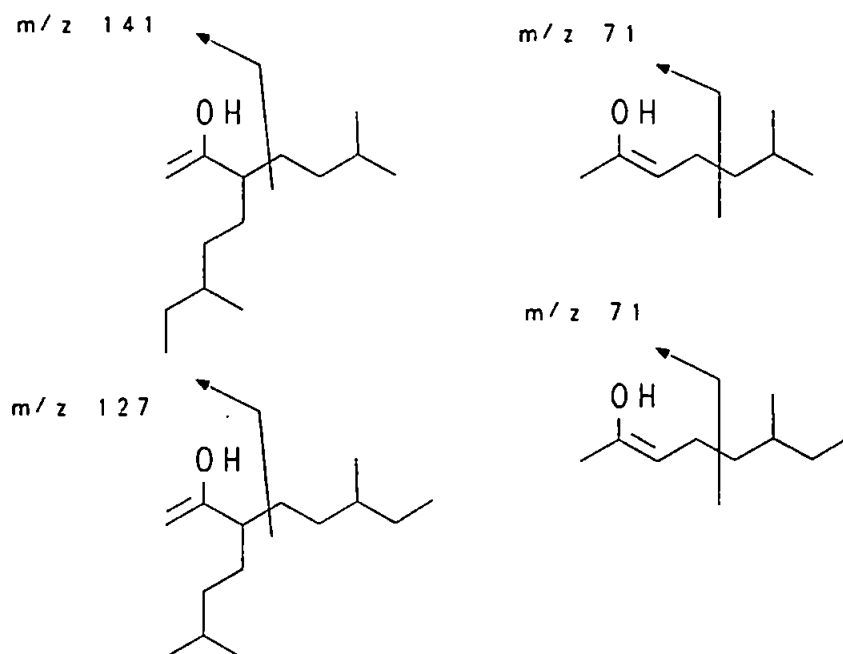
Also evident in the mass spectrum of the C_{18} ketone is the m/z 113 ion which arises from α-cleavage without H-transfer at the carbonyl carbon and the corresponding loss of water and CO at m/z 95 and m/z 85.

The partial ^{1}H NMR spectrum of the same C_{20} monoene (br20:1; 1702_{DB1}), but isolated from sediment at Cargreen in the Tamar estuary, is illustrated in Figure 4.3. Although one singlet (δ 5.3 ppm) due to vinylidene ($R_1R_2C=CH_2$) protons is evident and the triplet (δ 1.9 ppm) was assigned to the three allylic protons in **9**, the doublet (δ 4.71 ppm) and multiplet (δ 5.38 ppm) suggests the presence of impurities, possibly external and internal vinylic protons respectively from n-heptadecenes (Goodloe and Light, 1982) or another C_{20} HBI isomer (*e.g.* **9**) although no allylic protons within the expected range (δ 1.4-1.5) could be assigned. The ^{1}H NMR was not performed on the same isolate as the ozonolysis since this exhausted the supply of pure monoene from Millbrook. The C_{20} HBI monoene (*ca.* 90% purity by GC), which was subjected to NMR (and GC-IRMS), was isolated from Cargreen sediment in April .

Although one of the synthetic C_{20} HBI monoenes, (*viz* **3**; RI 1697_{DB1}) had a similar GC RI to **9** (RI 1702_{DB1}; Table 4.1), the expected ozonolysis products from this alkene (see **3.5.2**) were not observed for the sedimentary C_{20} HBI alkene. This emphasises the care needed in making assignments by GC RI alone. Thus the C_{20} HBI monoene in these temperate zone intertidal sediments is the same as that found in hypersaline sediments in Western Australia (Dunlop and Jefferies, 1985) whereas the C_{25} HBI monoenes (at least at certain times of the year) are different to those found in hypersaline Shark Bay.
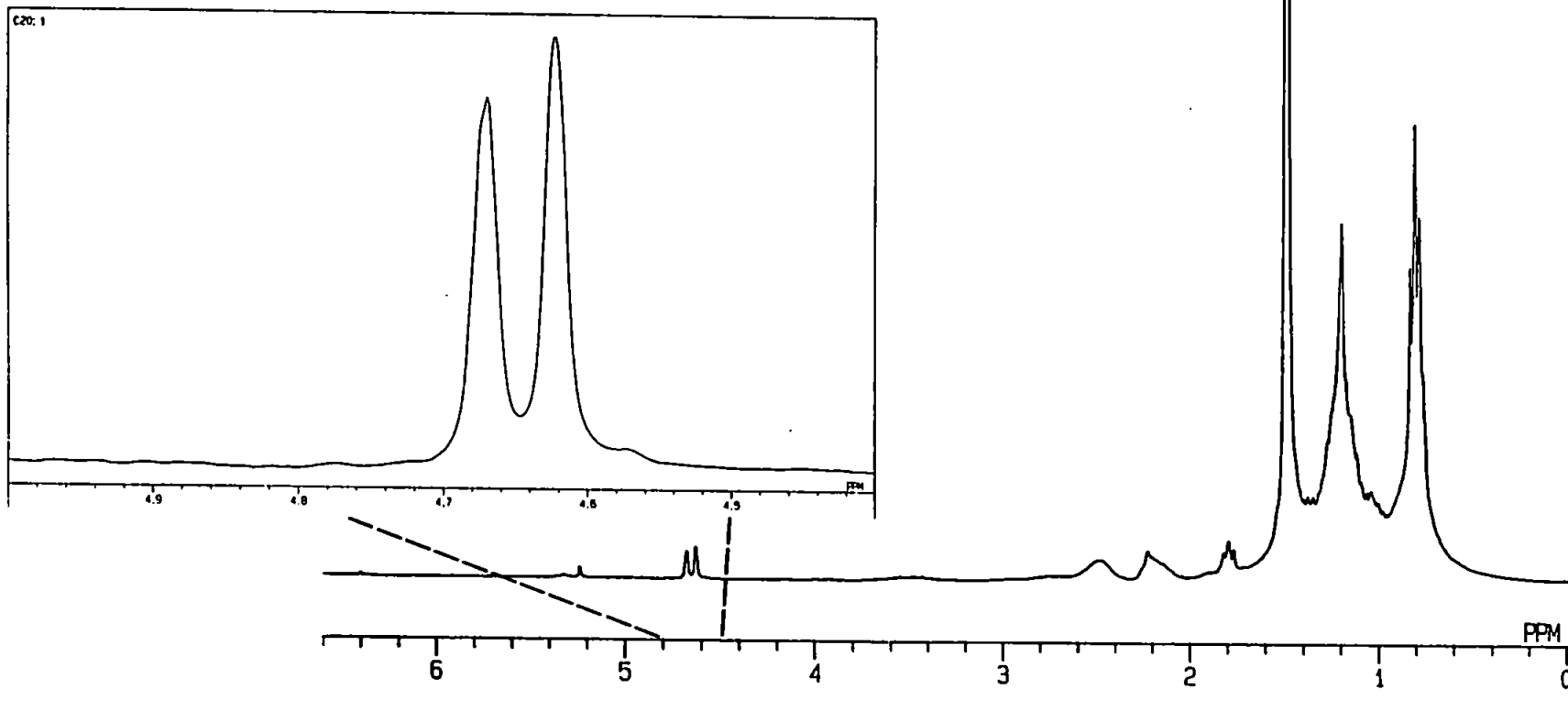
FIGURE 4.3 ^{1}H NMR SPECTRUM OF 2,6,10-trimethyl-7-(3'-methylbutyl)dodec-6(13)-ene (isolated from Cargreen sediments in April, 1990).

TABLE 4.1 CHROMATOGRAPHIC AND MASS SPECTRAL DATA FOR ISOLATED SEDIMENTARY HBI ALKENES

Alkene	Structure	GC retention index			Characteristic ions (m/z)	GC purity	Identification method
		DB1	DB5	DBWAX			
Tamar							
br20:1	**9**	1702	1698	1659	280, 210, 196	95	O_3, ^{1}H NMR
br25:1	**7 or 8**	2076$_{OV1}$	--		350, 280, 266, 224, 196	--	GC vs. synthetic
Gluss Voe							
br20:1	**3**	1696$_{OV1}$	--		280, 210, 196	--	H_2, GC vs. synthetic
McMurdo Sound							
br25:1[1]	**11**	2110	2101	2077	350, 210, 196	--	H_2, O_3, GC vs. synthetic

[1]product of partial hydrogenation of HBI diene br25:2; 2088$_{DB5}$ (Venkatesan, 1988)

4.4 MCMURDO SOUND (ANTARCTICA)

The organic geochemistry of McMurdo Sound region in Antarctica has been studied by Venkatesan and coworkers (*e.g.* see Chapter 2 and Venkatesan, 1988). Venkatesan and others (Brault and Simoneit, 1988) reported the presence of a C_{25} diene in McMurdo Sound sediments (RI 2082_{DB5}) which has been shown in Chapter 2 by hydrogenation experiments to have the acyclic HBI parent skeleton **2**. Partial hydrogenation of the diene (Venkatesan, 1988) however, produced a mixture comprising **2** and an unknown monoene ($C_{25:1}$; 2101_{DB5}). The monoene was tentatively identified in Chapter 2 as **11** by comparison of the retention index (2110_{DB1}) and mass spectrum with that of **11** (br25:1; 2112_{MS}), identified by ozonolysis by Dunlop and Jefferies (1985). Ozonolysis of the C_{25} monoene in the partial hydrogenation products produced the C_{24} ketone **12**.
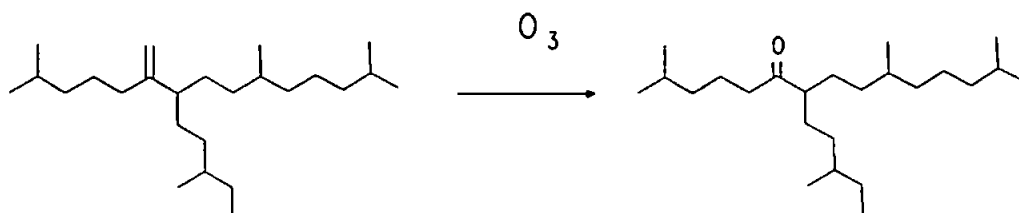
The mass spectrum of the C_{24} ketone 2,10,14-trimethyl-7-(3'-methylpentyl)pentadecan-6-one which exhibited similar fragmentations to the C_{19} ketone above (**4.3**) is shown in Figure 4.4 (M^+ 352, 337 [5%, M^+-CH_3], 268 [10%, McL], 250 [18%, McL-H_2O], 212 [17%, McL], 194 [20%, McL-H_2O], 127 [70%, C_9H_{11}], 113 [55%, α-cleavage$\rightarrow C_6H_{13}CO^+$], 95 [100%, α-cleavage-H_2O]).
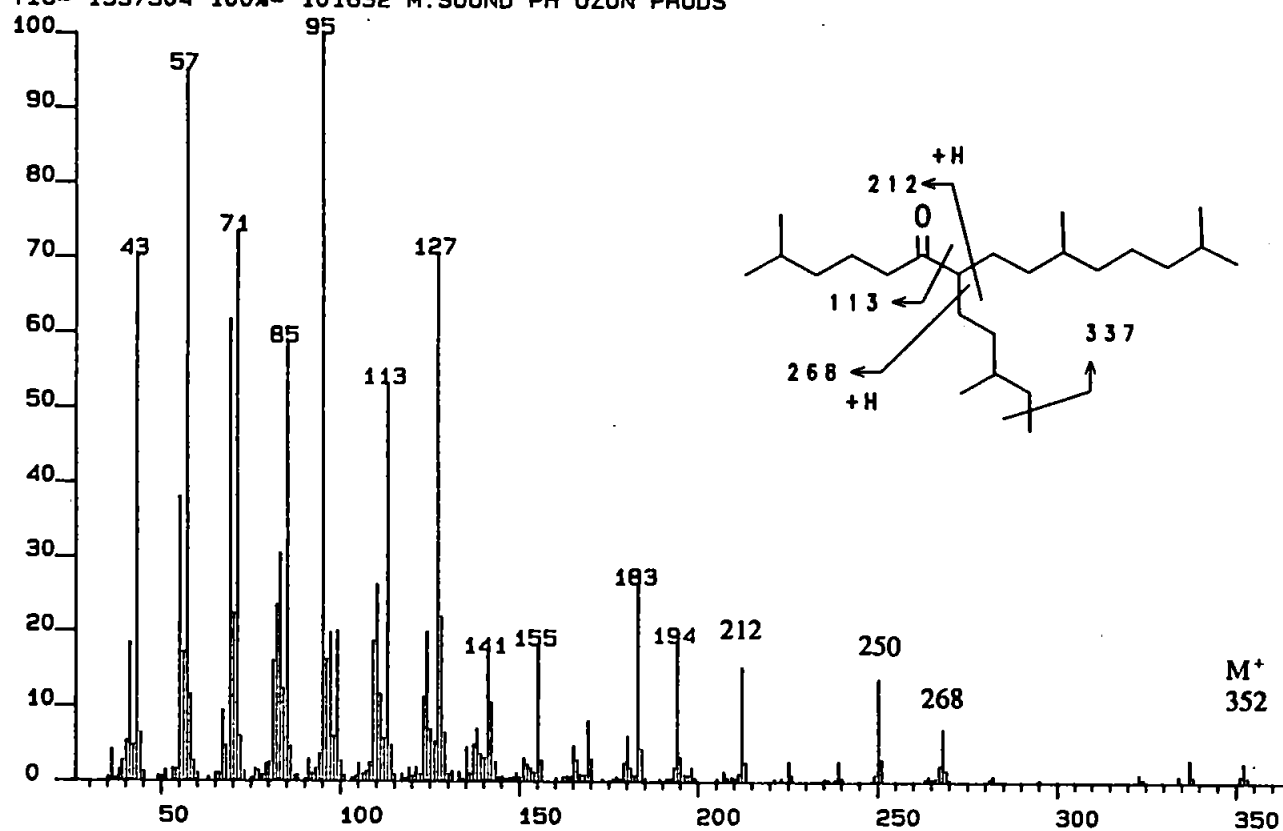
FIGURE 4.4 EI MASS SPECTRUM OF
2,10,14-trimethyl-7-(3'-methylpentyl)pentadecan-6-one

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The ozonolysis confirmed the structure of the alkene as **11** and established the position of one of the double bonds in the sedimentary C_{25} HBI diene (and by inference in the C_{25} HBI diene observed in sea-ice diatoms from McMurdo Sound [Nichols *et al.*, 1988]) as C6(17). This contrasts with the double bond positions found recently in the C_{25} HBI diene (br25:2; $2085_{CPSil8CB}$) isolated from a highly eutrophic mesohaline lagoon, Guadalaquivir Delta, south-west Spain (*i.e.* **13**) which were established by epoxidation with *m*-chlorobenzoic acid (Yruela *et al.*, 1990).

4.5 DISCUSSION OF THE BIOGEOCHEMICAL IMPLICATIONS OF THE POSITIONS OF DOUBLE BONDS IN HBI ALKENES

Differences in double bond positions in HBI alkenes are intriguing and possibly have important implications for the biochemistry of HBI alkenes, for their diagenetic fate and ultimately, for their potential use as biological marker compounds.

The use of the structure specifity of biolipids has been hindered by the limited knowledge of lipid composition of organisms and biochemical uses of such lipids. The nature and position of functional groups, attached to the carbon skeleton increases the specifity of biolipid. The size and nature of the carbon skeleton, including its stereochemical features, in combination with the type(s) and position(s) of functional groups(s) are dictated by its enzyme-controlled biosynthesis (Kohnen *et al.*, 1992). The unusual carbon skeleton of HBI compounds lends itself to a specific biochemical function, as yet unknown. It seems possible that many organisms which possess the mevalonate-isoprenoid pathway could be capable of biosynthesis of HBI compounds but the exact biosynthetic pathway as well as the source organism(s) is not known. Precursor biolipids with the HBI carbon skeleton containing functional groups other

than double bonds (*e.g.* hydroxyl) have yet to be reported. This suggests that the difference in the number of double bonds found in the C_{20} and C_{25} homologues, the number and position of double bonds in the numerous C_{25} HBI alkenes, as well as the existence of saturated homologues, may be related to biochemical reactions, possibly involving the hydrogenation of polyenes. Such a control on the degree of unsaturation in molecules may reflect differing growth conditions (Risatti *et al.*, 1984) or be due to the physiological state of the cells (Tornabene *et al.*, 1979).

The biochemical role of unsaturated biolipids has been investigated. For example, Tornabene *et al.* (1978) speculated that the role of squalene in archaebacteria (*Methanobacterium thermoautotrophicum*) was to act as a hydrogen sink, accepting and donating protons in a reversible manner, and proposed that archaebacteria controlled their internal reduction potential by varying the degree of unsaturation of C_{30} compounds with the squalane skeleton (Tornabene *et al.*, 1979). One use of this property may be in controlling the membrane permeability of divalent cations (Amdur *et al.*, 1978). It was also shown by laboratory culture experiments that when halophilic archaebacteria (*Thermoplasma* and *Sulfolobus*) were grown under varying oxygen tensions, the proportion of squalene and hydrogenated derivatives shifted dramatically (Tornabene, 1978). In particular, high oxygen tensions produced greater amounts of squalane whereas low oxygen tensions increased the proportion of tetrahydrosqualene.

The number of double bonds in another group of unsaturated hydrocarbons of geochemical significance, long-chain alkenes, alkenones, and alkenoates has been related to the contemporary water temperature at the time of biosynthesis (Brassell *et al.*, 1986; Prahl *et al.*, 1988). The degree of unsaturation was shown to decrease

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as growth temperature increased (Marlowe *et al.*, 1984; Prahl and Wakeham, 1987); a physiological response characteristic of classical membrane lipids (Harwood and Russell, 1984, and references therein). Rechka and Maxwell (1989) showed by synthesis and GC coinjection studies that the alkenones in the alga *Emiliania huxleyi* had the unusual and unexpected *E* configuration. This example demonstrates how characterisation of the stereochemistry of unsaturated compounds can be of use in palaeoenvironmental studies.

Although some authors have proposed the use of HBI compounds as "estuarine chemoenvironmental indicators" (*e.g.* Porte *et al.*, 1990) in a similar way to the alkenones in oceanic waters (Brassell *et al.*, 1986) this is rather premature whilst the exact source of HBI compounds remains unknown.

In such biogeochemical investigations, information on predominant source inputs and early diagenetic processes has been obtained from the structural elucidation of unsaturated groups (number, position and stereochemistry). For example, in water particulates the relative input of zooplankton, phytoplankton and bacteria has been estimated from the composition of $18:1\omega9$, $16:1\omega9$ and $18:1\omega11$ fatty acids (Wakeham *et al.*, 1984; Wakeham and Canuel, 1988; see the review by Saliot *et al.*, 1991). The use of fatty acids also emphasises the importance of bond geometry since it appears that both bacteria and marine invertebrates may be sources of $16:1\omega10$ *cis*, whilst $16:1\omega10$ *trans* has been found in a large variety of marine animals (Nichols *et al.*, 1989). It is likely that such discrimination will prove important if HBI alkenes are to be used as successful biogeochemical marker compounds.

It has been shown that the concentration of HBI alkenes generally decrease quite rapidly with increasing depth in sediments and the water column (*e.g.* Requejo

and Quinn, 1983a; Matsueda *et al.*, 1986abc). In many sediments, the parent alkanes, monoenes and possibly dienes, at least in the case of the C_{25} compounds, appear to be somewhat resistant to degradation (*e.g.* Barrick *et al.*, 1980; Dunlop and Jefferies, 1985) whereas HBI polyenes, with three or more degrees of unsaturation, are readily removed. No C_{20} HBI polyenes have been identified to this date as only the parent C_{20} alkane and two related monoenes have been reported (see review by Rowland and Robson, 1990). The results of biodegradation studies are consistent with this hypothesis as Robson and Rowland (1988b) showed the parent alkanes 1-3 and two mixtures of monoenes (*i.e.* 4 and 5-8) to be resistant to aerobic degradation under conditions where the corresponding *n*-alkanes were rapidly degraded. Gough *et al.* (1992) have shown the C_{25} HBI alkane to be slightly more resistant to aerobic degradation than regular isoprenoids. This is in contrast to the apparently facile removal of HBI polyenes from the water column and with depth of sediment (*e.g.* Volkman *et al.*, 1983). Thus, it would seem that the number of double bonds exerts some control on the diagenetic fate of HBI compounds. Although the exact influence of double bonds upon biodegradation or upon the process of incorporation of such lipids into accreting polymeric material (humic substances) is not known, some problems encountered during the analysis of these compounds have indicated differences in physicochemical behaviour. Some double bonds in particular isomers (*e.g.* br25:2; 2083_{OVI}: Rowland, unpublished results; br25:1 4-8: Robson and Rowland, 1986; br25:2; 2088_{MS}: Nichols *et al.*, 1988) appear hindered as derivatisation of these compounds (*e.g.* methoxy-mercuration) was not successful. Other isomers were only partially hydrogenated under mild conditions (*e.g.* br25:2; 2088_{DBS}: Requejo *et al.*, 1984; Venkatesan and Kaplan, 1987; br25:2; 2083_{DBS}:

Venkatesan, 1988) which again indicates the presence of a highly hindered double bond. The steric hinderance of specific structures and stereochemistries may also influence their biodegradation and ultimate diagenetic fate.

The C_{25} monoene 11 proved resistant to hydrogenation by the method used by Venkatesan and Kaplan (1987) and Nichols *et al.* (1988) were unable to accomplish derivatisation of the precursor diene br25:2; 2088_{DB5}. Hence, it appears that the double bond in the 6(14) methylene position may be sterically hindered.

The number and position(s) of double bonds in HBI compounds is likely to control their diagenetic fate and thus ultimate mode of occurrence in sediments or even oils: hydrocarbon, alkylthiophene or macromolecularly sulphur-bound (Kohnen *et al.*, 1992). The apparent rapid decrease in concentration of C_{25} HBI polyenes with depth in sediment and the water column could be due to the incorporation of reduced sulphur species, both in an intra- and intermolecular fashion into the HBI carbon skeleton (Kohnen *et al.*, 1990a). The C_{25} HBI alkenes with multiple double bonds accumulate as saturated (diene precursor) and unsaturated (>2 double bonds in precursor) alkylthiolanes, alkylthiophenes, or as macromolecularly sulphur-bound moieties. The presence of numerous reaction sites makes the formation of at least one intermolecular S-linkage likely, despite the fact that the double bonds may be separated by less than four sp^3-hybridised carbon atoms, favouring intramolecular incorporation of sulphur. Hence, both HBI hydrocarbon and sulphur compounds are bound via a sulphur bridge to a macromolecule. The survival of alkanes 1 and 2, and related monoenes in sediments, and the presence of 1 and 2 in selected immature oils (Yon *et al.*, 1982; Sinninghe Damsté *et al.*, 1986; Bazhenova and Arefiev, 1990), can be rationalized by the inability of the alkanes to react with inorganic sulphur species

and the low probability of monoenes to form a sulphur bridge to a macromolecule. The mechanism proposed by Sinninghe Damsté *et al.* (1986, 1989b) does not yet account for the formation of C_{20} HBI thiophenes, even though their occurrence is limited. A prerequisite for the intramolecular incorporation of inorganic sulphur species into the C_{20} carbon skeleton, is the existence of di- and/or poly-unsaturated C_{20} HBI alkenes, but only two monoenes with structures 3 and 9 have been identified to date.

4.6   SUMMARY

Isolation and characterisation of a C_{20} HBI monoene from sediments of the Tamar estuary and of a C_{25} HBI monoene (hydrogenation product of a diene) from McMurdo Sound sediments, showed that they both contained methylene double bonds, identical those found previously in monoenes from Shark Bay (Western Australia). Comparison of the GC and GC-MS data of synthetic monoenes with those obtained from a sedimentary C_{20} HBI monoene from Gluss Voe and two C_{25} HBI monoenes from the Tamar, showed that the double bonds in these compounds, although non-methylenic, are all trisubstituted and 0-2 sp^3-hybridised carbon atoms away from the methylene position. This implies that these structures (3 and 9; 7, 8 and 11) may be interconverted by limited isomerisation via tertiary carbocations (C-6 and C-7) during early diagenesis. The absence of any other HBI monoenes from sediments worldwide to date, and the lack of isomerisation in immature sediments via secondary carbocations, suggests that at least one of these double bond positions has a biosynthetic origin and physiological function.

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STRUCTURES

CHAPTER FOUR

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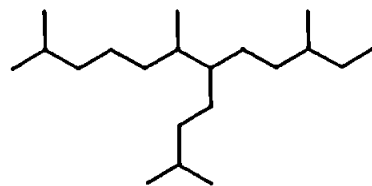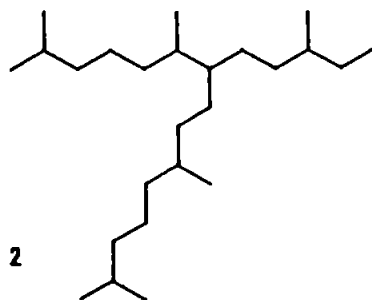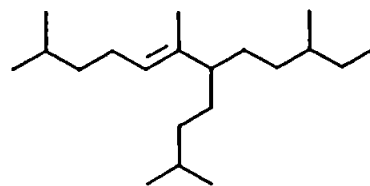
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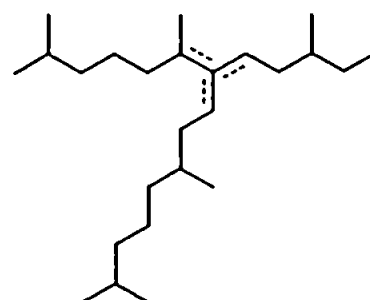
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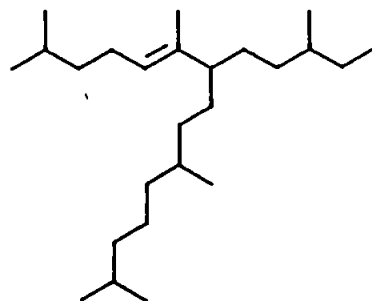
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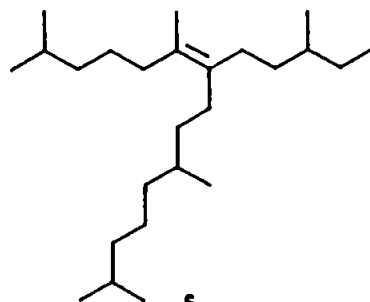
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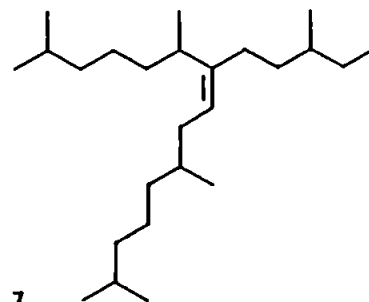
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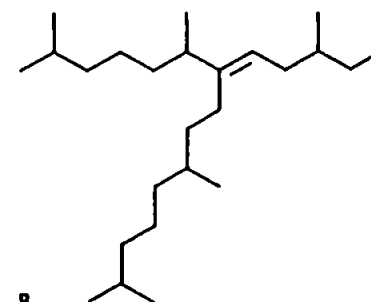
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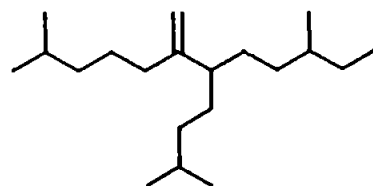
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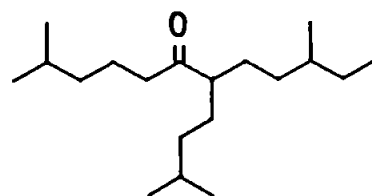
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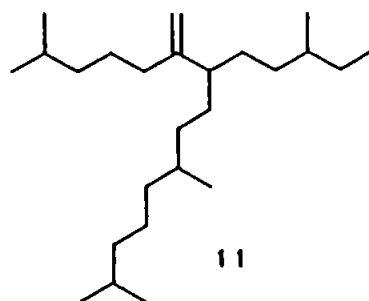
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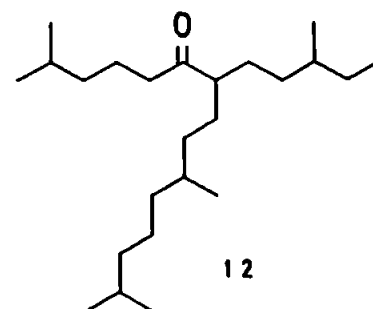
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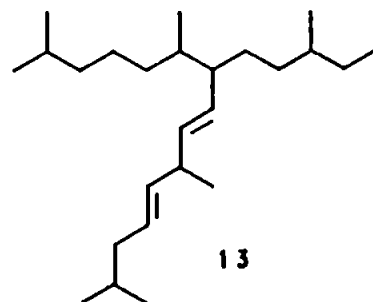
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CHAPTER FIVE

INVESTIGATIONS INTO THE SEDIMENTARY OCCURRENCE AND

BIOLOGICAL SOURCES OF C_{20} AND C_{25} HBI HYDROCARBONS:

TEMPORAL AND SPATIAL DISTRIBUTIONS IN THE TAMAR ESTUARY

This chapter describes the distribution of C_{20} and C_{25} HBI hydrocarbons in recent estuarine sediments and in related biota. The isotopic composition of alkane 1 and a related monoene 4, are determined. The results suggest possible sources for the sedimentary HBI hydrocarbons. The spatial and temporal distribution of HBI hydrocarbons in sediments is reported and the implications are discussed.

5.1 INTRODUCTION

The conclusive identification of several sedimentary HBI monoenes for the first time described in the preceding section also allowed an investigation of the temporal and spatial distributions of HBI hydrocarbons in some recent sediments in the Tamar Estuary. Potential biological sources of these compounds in the estuary were also examined. The hydrocarbon assemblage of the sediments and the biota are briefly discussed initially and followed by an overall discussion of the occurrence of the HBI hydrocarbons in biota and sediment and the relevance to the biological origin of the compounds of interest.

Estuaries constitute a major interface between land and ocean. Biogeochemical processes occurring in such environments greatly affect the fate of material originating upstream (Head, 1976; Reuter, 1981). This influence is particularly important for organic material; depending on estuarine conditions, production of autochthonous material or degradation of allochthonous matter will dominate. In estuarine systems, such as the Tamar estuary, the allochthonous organic material may derive from the river or ocean, or from anthropogenic release of sewage into the estuary. The autochthonous organic material originates mainly from phytoplankton production. Outputs of organic matter are essentially the degradation of the most labile organic products, grazing activities of animals and net export to the ocean.

Although the majority of this organic fraction consists of chemically ill-defined humic materials, the use of organic biogeochemical markers has proved to be a valuable tool in differentiation of organic inputs to estuarine sediments. Such markers include extractable lipids which can be readily isolated from the particulate matter or sediment and which have intrinsic structural features that are indicative of their

biological origins. These biolipids, especially hydrocarbons, are less labile and less susceptible to significant modification in the environment than other biological organic components such as proteins and carbohydrates. The use of lipid biogeochemical markers has been widely developed for tracing the sources of inputs of organic matter in estuarine systems (see general review by Saliot *et al.*, 1991 and more specifically for sediments, Requejo and Quinn, 1984; Readman *et al.*, 1986ab; and for water and particulate material, Readman *et al.*, 1982; Albaigés *et al.*, 1984b; Saliot *et al.*, 1984; 1988; Tronczynski *et al.*, 1985; 1986).

The estuarine sediments studied here receive large inputs of detrital organic matter consisting primarily of decaying vascular plants and macrophytes native to the intertidal sediment. Benthic microalgae and their decomposing remains also comprise a significant fraction of the autochthonous organic matter from such sediments (*e.g.* Nixon and Oviatt, 1973; Lytle *et al.*, 1979; Riblelin and Collier, 1979). In addition, Tamar estuary sediments derive much allochthonous organic matter from plankton or terrigenous (including anthropogenic) sources (*e.g.* Readman *et al.*, 1986a).

5.2   ENVIRONMENTAL SETTING OF THE TAMAR ESTUARY

The geographical locations of the sediments are shown in Figure 5.1. Sample descriptions and selected organic biogeochemical parameters are shown in Table 5.1.

The Tamar estuary is located in south-west England. Its physical, chemical and biological characteristics are well known (Butler and Tibbets, 1972; Morris *et al.*, 1978; 1981; 1982; Loring *et al.*, 1982; 1985; Readman *et al.*, 1982; 1986ab; Uncles *et al.*, 1983; Reeves and Preston, 1989). The estuary extends for approximately

32 km from its seaward boundary with Plymouth Sound to limit of saline intrusion at Gunnislake weir. Normally the limit of salt water intrusion is located 5-15 km seaward of the weir. It is characterised by extensive intertidal mudflats, particularly at its lower reaches (Figure 5.1A). The estuary receives considerable nutrient inputs from both sewage effluent and agricultural fertilizers run-off which have increased substantially over the last 20 years. Levels of nitrates and phosphates are such that extreme growth of opportunistic green algae are present in the lower estuary from May to October. These are principally of the *Enteromorpha* spp. (*E. prolifera, E. intestinalis, E. compressa, E. linza*), with *Ulva lactuca, Chaetomorpha linum* and *Cladophora* spp. also present in some more sheltered areas). These mat-forming algae develop rapidly in the spring and may persist at high density for several months before disappearing in late autumn (Hull, 1987). Much of the mat is buried *in situ* to provide an annual organic input to the sediment (Levinton, 1985).

Water depths are typically *ca.* 20-25 m at the entrance to Plymouth Sound and grade to *ca.* 3-4 m in mid estuary. The river Tamar is the major freshwater input, accounting for *ca.* 50-70% of the net discharge to Plymouth Sound. At low discharge with mean spring tides, vertically mixed conditions prevail over most of the estuary, but during average runoff the estuary generally conforms to the partially mixed type with vertical stratification (Upstill-Goddard *et al.*, 1989). Water temperatures reach a maximum of 18-21°C in August and decline *ca.* 3-5°C during February (Morris *et al.*, 1982). There is a well-defined turbidity maximum in the vicinity of the freshwater/brackish interface (Morris *et al.*, 1982; Uncles *et al.*, 1983). Suspended loads within the turbidity zone range from 50-1000 mgdm^{-3}. There is a marked seasonal migration of sediment in which particles are gradually accumulated in the
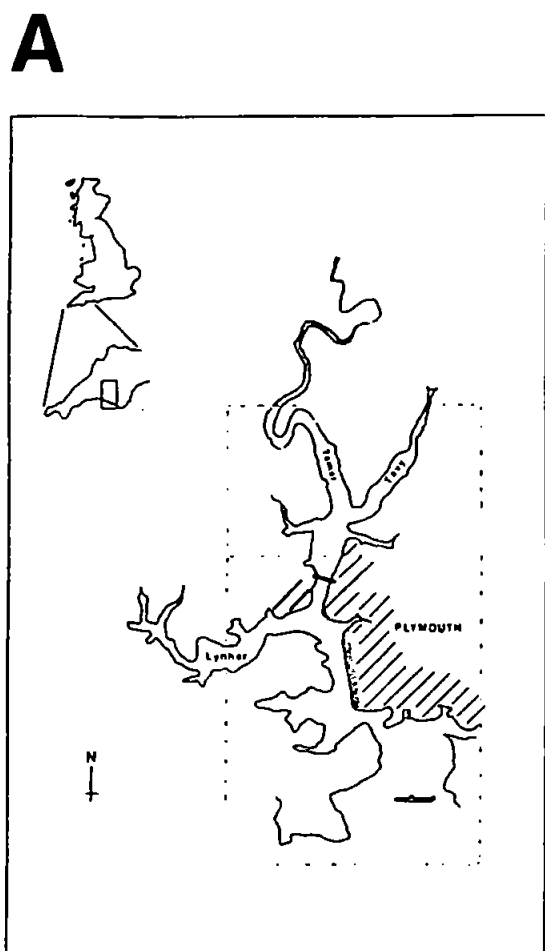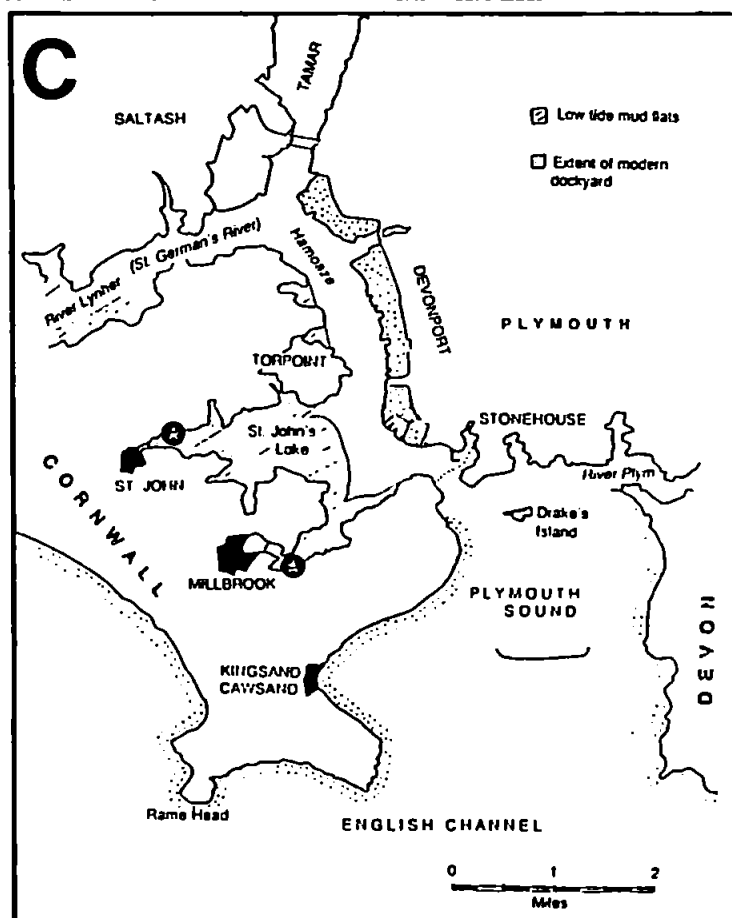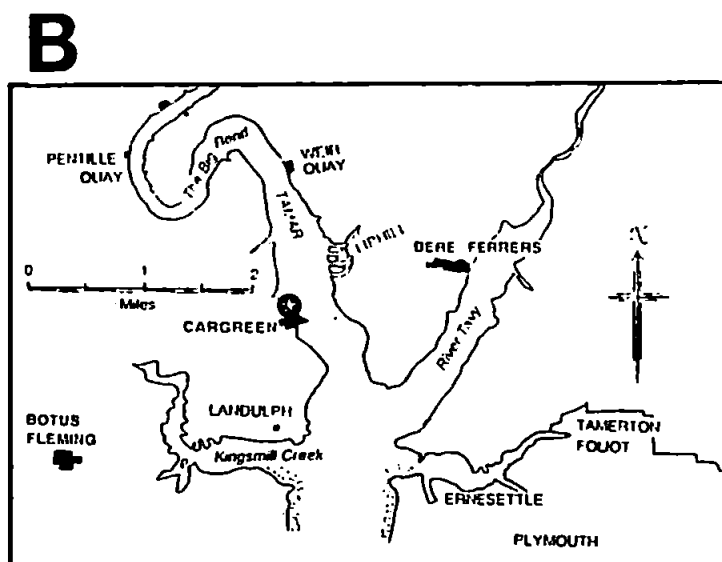
FIGURE 5.1 MAP OF TAMAR ESTUARY SHOWING LOCATION OF SEDIMENT AND ALGAE SAMPLING SITES

upper estuary throughout the summer to be redispersed down-estuary when river flow increases during autumn and winter (Bale *et al.*, 1985). The physical hydrography of the estuary is described by George (1975).

The locations of the sediment study sites are shown in Figure 5.1. The intertidal sediments in the estuary are largely unpolluted (*e.g.* Robson, 1987), chemically homogenous (Alexander, 1985) and predominantly dark-brown (khaki) in colour, but grading to black with depth. They are overlain by a light-brown, presumably oxidised, cap varying in thickness from a few millimetres (St. Johns Lake) to *ca.* 3-4 cm (Cargreen). Typical organic carbon contents by weight are *ca.* 1.5% at St. Johns Lake (Upstill-Goddard, 1985), *ca.* 3% at Millbrook and 3%-7% at Cargreen. Readman *et al.* (1986a) recorded a range in organic carbon content of 1.1% to 4.4% in sediments through the Tamar Estuary.

There are significant intersite differences in sedimentation regimes. St. Johns Lake sediments are generally characterised by long-term stability, as evidenced by ^{210}Pb chronology, which yielded sedimentation rates *ca.* 1 cmyr^{-1} (Clifton and Hamilton, 1979). Nevertheless, small perturbations of the uppermost 1 cm of the sediment column occur in response to tidal oscillations (Bale *et al.*, 1985).

In contrast, the sediments at Cargreen are more fluid, reflecting significant tidal reworking in this region of the estuary. Because of the dynamics of the estuarine circulation, the uppermost 20 cm of the sediments are periodically resuspended (Bale *et al.*, 1985).

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TABLE 5.1 SELECTED PHYSICAL AND CHEMICAL PROPERTIES OF THREE TAMAR SEDIMENT SAMPLES COLLECTED IN JUNE, 1989 (All organic-rich muds)

Sediment sample site	Environment (all intertidal estuarine)	Dominant flora	Total aliphatic hydrocarbons[a] μgg^{-1} dry sediment	mgg^{-1} OC	Organic carbon content (%TOC)	Corg/N	normal alkanes[a] (n-C_{20} to C_{36}) μgg^{-1} OC	(CPI)[b]
Millbrook	sheltered harbour	macrophytic green algae	550	15.3	3.6	6	560	(5.1)
St. Johns Lake	sheltered bay, stable muds	epipelic microalgae	470	10.0	4.7	10	550	(3.5)
Cargreen	riverside, unstable muds	epipelic microalgae	420	11.4	3.7	18	560	(5.3)

Key: [a] by integration of total FID response of aliphatic hydrocarbons or n-alkanes and direct reference to internal standard. [b] CPI is the Carbon Preference Index, measured from C_{20} to C_{36}. $CPI = 0.5*(C_{n+2}+...+C_m)+(C_n+...+C_{m-2})/(C_{n+1}+...+C_{m-1})$ where n to m is the desired range.

The sampling station at Millbrook is situated on the upper reaches of Millbrook Lake, a spur off the main channel of the River Tamar (Figure 5.1C). The sheltered nature of the river at Millbrook has allowed the build-up of more consolidated sediment of a greater range in particular size from silt to pebbles. The site was characterised by the presence of freshwater streams running across the shore and although macroalgae such as *Cladophora* occurred sporadically in the silt, they were more likely to be anchored to pebbles (rock debris) or shells buried in the sediment. In addition, the shoreline was subjected periodically to freshwater released from a sluiced lagoon upstream. Thus, it might be expected that the intertidal flora be adapted to low salinities (*e.g.* the genus *Enteromorpha*).

At the other sites investigated (St. Johns Lake and Cargreen; Figure 5.1AB), little growth of macrophytes was noted although the hydrocarbon distributions were very similar to that recorded at Millbrook. Tidal mudflat biocoenosis is extremely complicated and requires an introduction.

At the microphytic and micro- and macrofaunal levels of trophodynamics thousands of individuals and hundreds of species interact in very localised patches or assemblages that make up the community mosaic. Benthic microalgae are much less well understood ecologically than planktonic species. The benthos is more diverse than the plankton, both in terms of numbers and the lifeforms present (Round *et al.*, 1990).

Microassemblages that live freely on and in sediments are termed epipelic and endopelic (Round, 1979). These tend to be motile species which migrate up and down in the topmost sediment in relation to environmental cycles (*e.g.* light-dark cycles, in some instances modified by tidal disturbance). Diatom and non-diatom members

of the epipelon exist, the latter comprising of desmids (order *Zygnematales*), blue-green algae (*Cyanophyta*), golden algae (*Chrysophyta*), euglenoids (*Euglenophyta*), dinoflagellates (*Pyrrhophyta*) and cryptomonads (*Cryptophyta*). These microalgae, including the diatoms, may act as hosts for endosymbiotic organisms such as bacteria. Detrital particulates held together by algal extracelluar mucilage are often colonized by bacteria and can include complex assemblages of autotrophs and heterotrophs.

Epipelic diatoms frequently comprise the majority of these photosynthetic microorganisms in intertidal estuarine sediments (Hopkins, 1963; 1964; Palmer and Round, 1965; Fenchel and Straarup, 1971; Round, 1979; Joint, 1981) and can often be seen as a brown slime on the sediment surface (*e.g.* Aleem, 1950). Indeed, diatoms have been reported as the most important primary producers on tidal mudflats (Admiraal, 1984). Extensive brown patches on the surface of the Tamar sediments were common at many periods of the year and abundant during the spring and summer months. No green coloration characteristic of euglenoids and/or blue-green algae was observed. Mills *et al.* (1986) suggested that the presence of such brown and green patches on tidal flats of various British estuaries may have indicated a high standing crop of benthic microalgae under certain polluted (eutrophic) conditions. The great majority of algae in sediments have the ability to move and several forms have been shown to carry out vertical movements in response to light and tidal changes (*e.g.* Fauré-Fremiert, 1951; Taylor, 1964; Hopkins, 1966ab; Admiraal, 1977).

TABLE 5.2 A SUMMARY OF TAMAR SEDIMENT AND ALGAL SAMPLES COLLECTED FOR ANALYSIS

Sample	Location	Time of collection
Sediment homogenate	Millbrook	July, 1989
Sediment homogenate	St. Johns Lake	July, 1989
Sediment homogenate	Cargreen	July, 1989
Semi-buried macroalgal mat (*Cladophora*)	Millbrook	July, 1989
Epiphytic algae	Millbrook	July, 1989
Sediment under algal mat	Millbrook	August, 1990
Sediment clear of mat	Millbrook	August, 1990
Cladophora sp.	Millbrook	August, 1990
Cladophora sp. (2 more specimens)	Millbrook	May-August, 1990
Ulva lactuca	Millbrook	May-August, 1990
Enteromorpha linza	Millbrook	May-August, 1990
Enteromorpha spp. (2 more specimens)	Millbrook	May-August, 1990
Algal debris associated with *Enteromorpha* spp.	Millbrook	May-August, 1990
Fucus distichus	Millbrook	October, 1990
Sediment homogenates	Cargreen	December, 1989 to November, 1990 (monthly)
Epipelic diatoms (mainly *Navicula* spp.)	Cargreen	August, 1990
Laboratory degraded *Cladophora* sp.	orginally Millbrook	August, 1990-1991

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5.3 INTERSITE VARIABILITY OF HBI HYDROCARBONS WITHIN SEDIMENTS OF THE TAMAR ESTUARY (JULY, 1989)

The sediment study sites shown in Figure 5.1 were chosen to investigate the variability in HBI hydrocarbon distribution and concentration within the Tamar estuary.

Figure 5.2 shows gas chromatograms of the 'aliphatic hydrocarbon' extracts from Millbrook, Cargreen and St. Johns Lake. These are similar to those presented by both Readman *et al.* (1986ab) and Robson and Rowland (1986) for hydrocarbon extracts of intertidal sediments from the Tamar Estuary. Peak assignments and approximate concentrations of various compounds including the acyclic C_{20} and C_{25} HBI hydrocarbons of interest in these Tamar sediments are summarised in Table 5.3. The major chromatographic peaks of interest corresponding to the C_{20} and C_{25} HBI hydrocarbons of significance to this study elute between at RI 1695-1710 and RI 2000-2200 (Robson, 1987). Robson (1987) reported the presence of C_{20} and C_{25} HBI alkenes and a C_{20} HBI alkane in similar sediments (Cargreen) and was able to show by hydrogenation and GC co-injection that the parent structures were identical to synthetic 1 and 2. However, as mentioned previously, the double bond position in br20:1; 1702_{ovi} was not assigned and that in the two C_{25} isomers, br25:1; 2076_{ovi} and br25:1; 2091_{ovi}, was limited to one of three positions (*i.e.* 3).
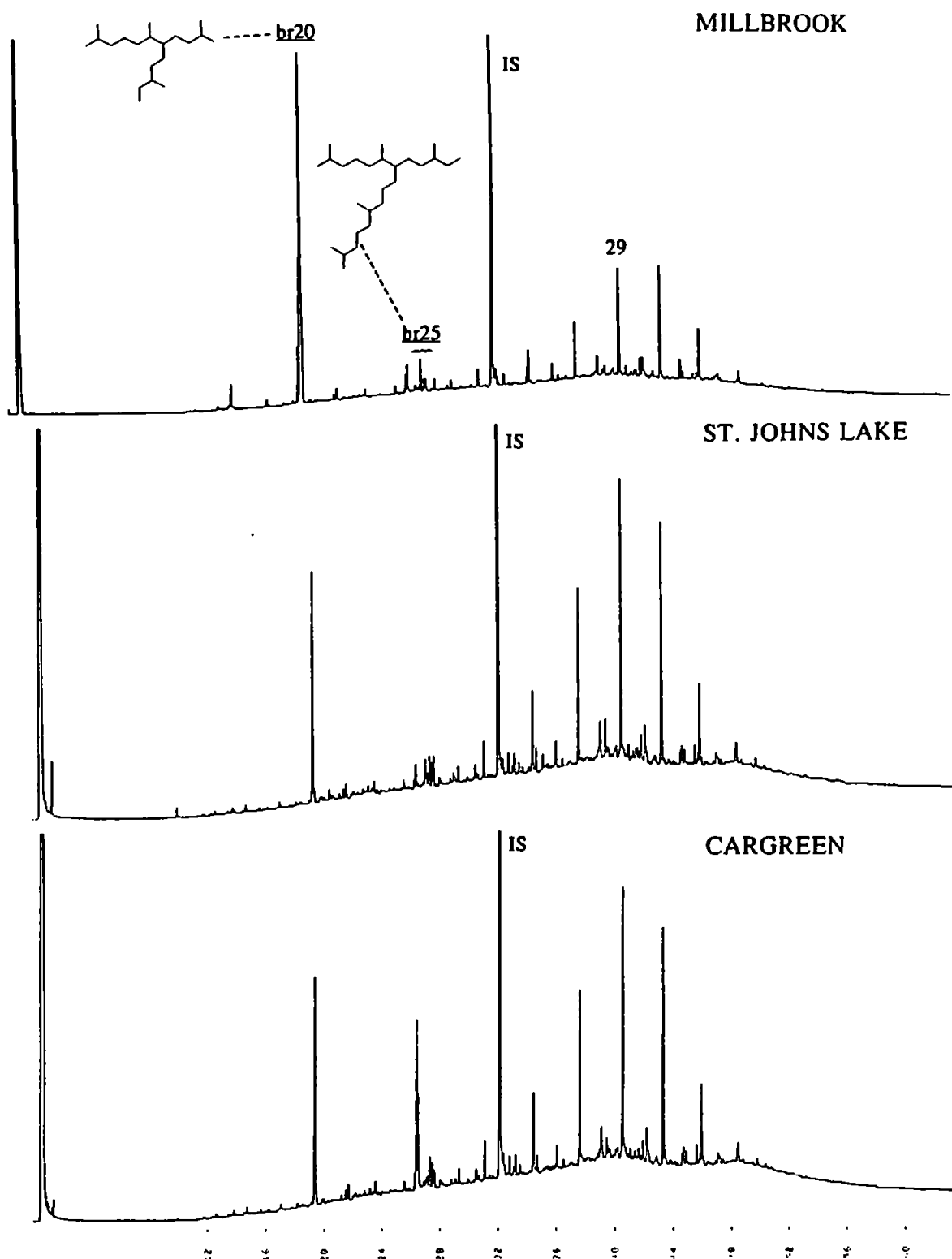
FIGURE 5.2 GAS CHROMATOGRAMS OF THE ALIPHATIC HYDROCARBONS ISOLATED FROM SEDIMENTS AT THREE SITES WITHIN THE TAMAR ESTUARY.

Peaks labelled hr20 and hr25 represent hydrocarbons with the carbon skeleton of 2,6,10-trimethyl-7-(3'-methylbutyl)dodecane and 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadecane respectively. For conditions see text; DB5 (J&W).

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5.3.1 C_{20} HBI HYDROCARBONS

The chromatogram of aliphatic hydrocarbons isolated from Millbrook sediment was dominated by a peak at RI 1700_{DB5}; 1707_{DB1} which had a mass spectrum and RI identical to that of synthetic alkane 1. RI $1700_{DB5}/1707_{DB1}$ also co-chromatographed with synthetic 1 on three different stationary phases (DB1, DB5 and DBWAX). The mass spectrum of RI 1698_{DB5}; 1702_{DB1} (Figure 5.3) contained a molecular ion at m/z 280 and characteristic fragments at m/z 126, 196, and 210 and was proposed by Rowland and Robson (1986) to be a C_{20} monoene (br20:1; 1698_{DB5}; 1702_{OV1}). Isolation and ozonolysis, as described in section 4.1.3, showed that the alkene had structure 4. A hydrocarbon with an identical mass spectrum and retention index to RI 1702_{DB1} has been reported in numerous sediments (e.g. Barrick et al., 1980) and Dunlop and Jefferies. The shoulder peak on br20:0; 1707_{DB1} was shown to be pristane (RI 1711_{DB1}) by co-chromatography and mass chromatography. The aliphatic hydrocarbons isolated from sediment from Cargreen and St. Johns Lake sites were also dominated by HBI 1 and related monoene 4.

5.3.2 C_{25} HBI HYDROCARBONS

Examination of the mass spectra of the chromatographic peaks RI 2000-2200 revealed the presence of a number of C_{25} HBI alkenes in all the Tamar sediment samples, varying in degree of unsaturation as indicated by the values of the respective molecular ions (e.g. br25:3; 2089_{DB5}; 2091_{DB1}; m/z 346). The C_{25} monoenes (br25:1; 2076_{OV1} and 2091_{OV1}), which were evident in Tamar sediment in 1985 (Robson, 1987) were absent from all those sampled 1989-1990.
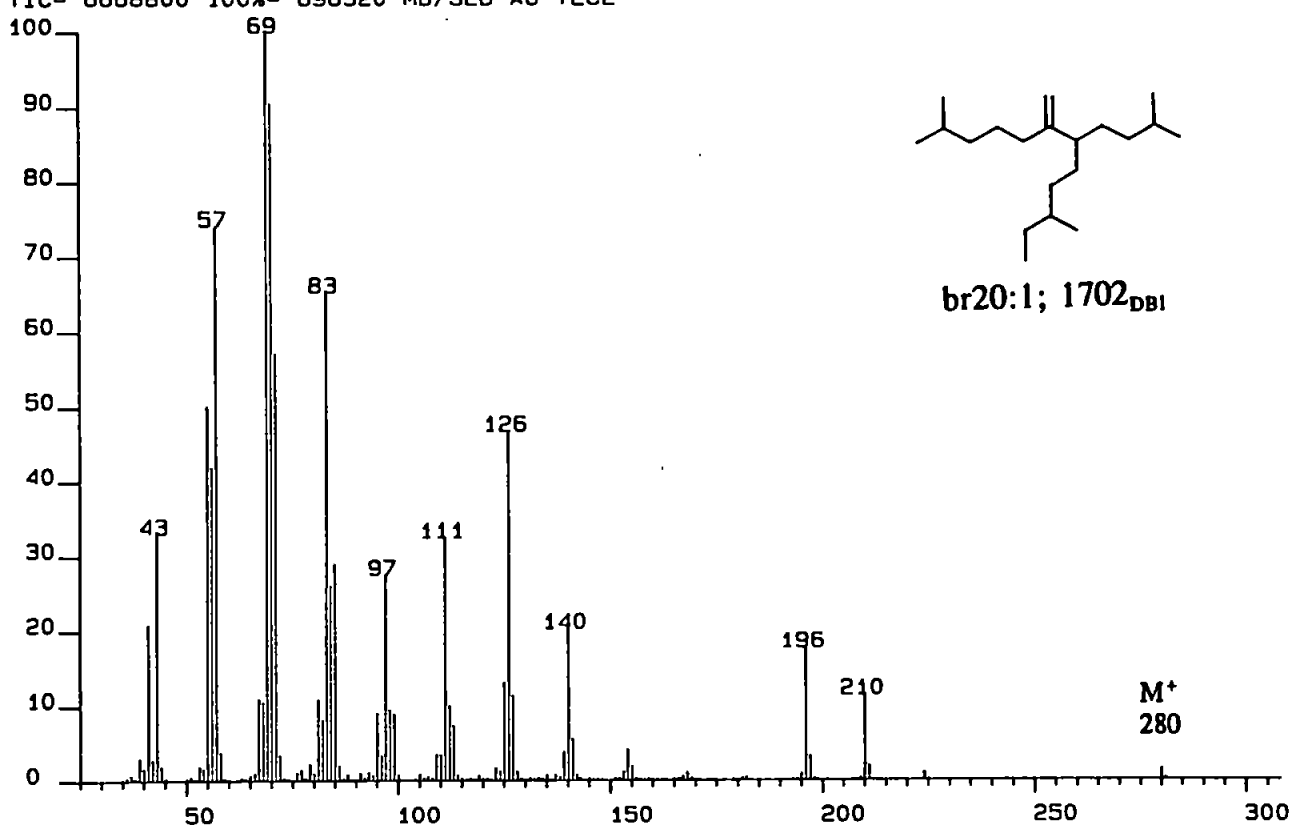
FIGURE 5.3 MASS SPECTRUM OF br20:1; 1702$_{DBI}$ ISOLATED FROM TAMAR SEDIMENT.
Structure assigned from spectral and ozonolysis data (see text)

Some inter-site differences in the distribution of C_{25} HBI alkenes are evident

from examination of the gas chromatograms in Figure 5.2. These are illustrated in

Figures 5.4 and 5.5, and Table 5.3. The C_{25} HBI alkenes seem more abundant at the

Millbrook site. The mass spectra (Figure 5.6) of RI 2042, 2089 and 2107 displayed

molecular ions at m/z 346 suggesting that they were C_{25} trienes. Similar mass spectra

and RI to br25:3; 2042_{DB5} and br25:3; 2089_{DB5} were presented for two isomeric

acyclic C_{25} trienes by Barrick *et al.* (1980) and others (*e.g.* Volkman *et al.*, 1983;

Robson, 1987). Requejo and Quinn (1985) noted that a cluster of C_{25} HBI alkenes

were only partially resolved within this region of the chromatogram (RI 2080-2095)

and that the presence of relatively large amounts of one component masked the

presence of another. For example, one GC peak normally attributed to br25:3;

2091_{SE30} in fact displayed a mass spectrum similar to that expected from a mixture of

br25:3 and a diene br25:2; 2088_{SE30} (including two apparent molecular ions at m/z 346

and m/z 348). In Tamar sediments no molecular ion at m/z 348 was detected in the

mass spectrum of RI 2089_{DB5} which indicated the absence of the latter.

Chromatography on a methylsilicone GC stationary phase (DB1) facilitated a better

separation of these isomers (br25:2; 2088_{DB1} and br25:3; 2091_{DB1}) and confirmed

br25:3 to be the dominant component. However, no attempt was made to quantify the

relative amounts of each. In addition, use of DB1 facilitated a more reliable

identification of the HBI triene br25:3; 2040_{DB1} which was shown to coelute with one

n-C_{21} polyene (see **5.3.3** below) on the GC stationary phase routinely used for the

analysis of C_{25} HBI alkenes during this study (DB5). This emphasises the care

required when identifying isomers within the complex distribution of C_{25} HBI

hydrocarbons indigenous to many sediments and the need for chromatography on

TABLE 5.3 CONCENTRATION OF HBI AND OTHER HYDROCARBONS IN TAMAR SEDIMENTS IN JULY, 1989 (mgkg^{-1} dry sediment)

Compound (RI)	Millbrook	St.Johns Lake	Cargreen
n-C$_{17:1}$; 1690$_{DB1}$	1.3	nd	nd
n-C$_{17}$	nm	nm	nm
br20:1; 1702$_{DB1}$	3.5	3.4	0.83
br20:0; 1707$_{DB1}$	9.7	12	3.3
Σn-C$_{21}$ polyenes	1.2	nd	16.4
br25:3; 2042$_{DB5}$	2.1	0.13	0.28
br25:2; 2070$_{DB5}$	nd	0.26	0.21
br25:3; 2089$_{DB5}$	2.3	0.19	0.71
br25:3; 2107$_{DB5}$	1.2	0.39	0.45
br25:4; 2128$_{DB5}$	0.31	nd	0.45
br25:2; 2140$_{DB5}$	0.92	0.38	nd

Key: nd = not detected, nm = not measured

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C20 HBI and n-C17 hydrocarbons
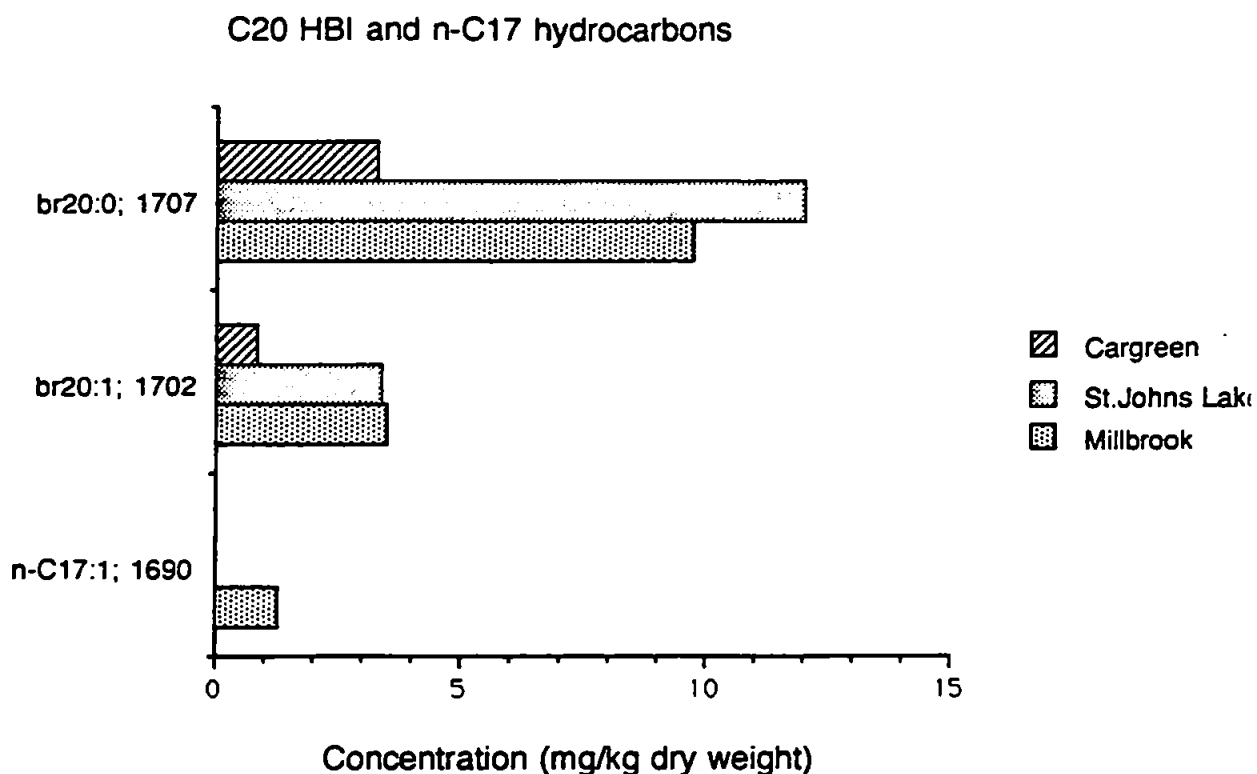
FIGURE 5.4   **CONCENTRATION OF C_{20} HBI HYDROCARBONS IN SEDIMENTS AT THREE SITES WITHIN THE TAMAR ESTUARY**

C25 HBI and n-C21 polyenes
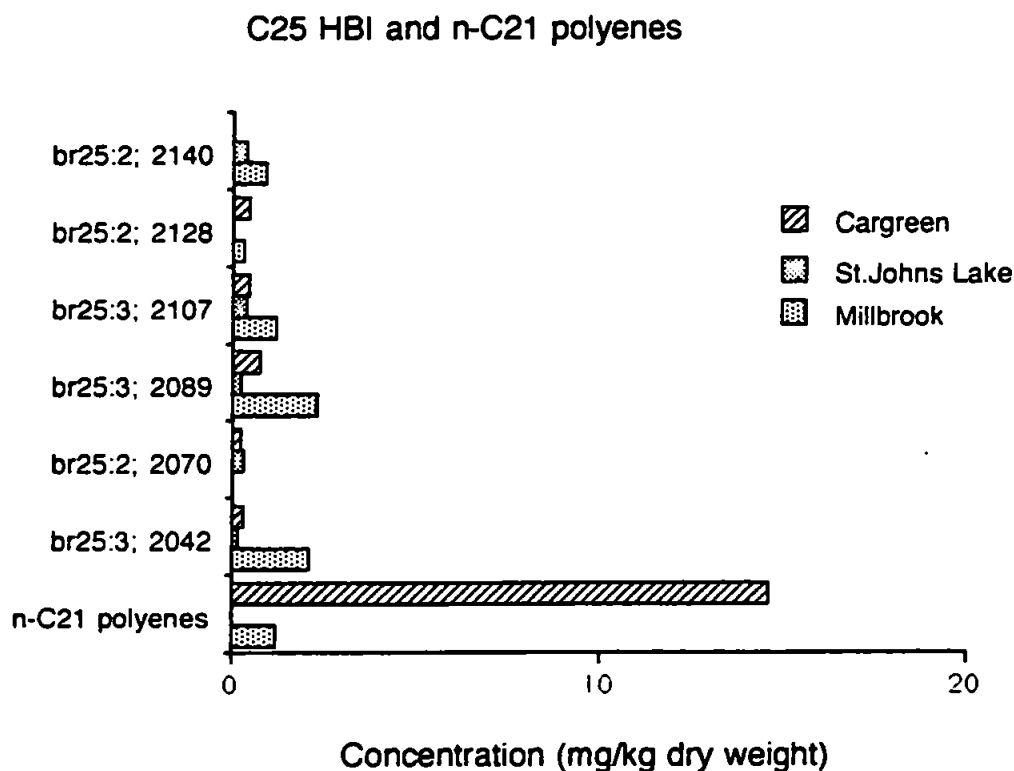
FIGURE 5.5   **CONCENTRATION OF C_{25} HBI ALKENES IN SEDIMENTS AT THREE SITES WITHIN THE TAMAR ESTUARY**
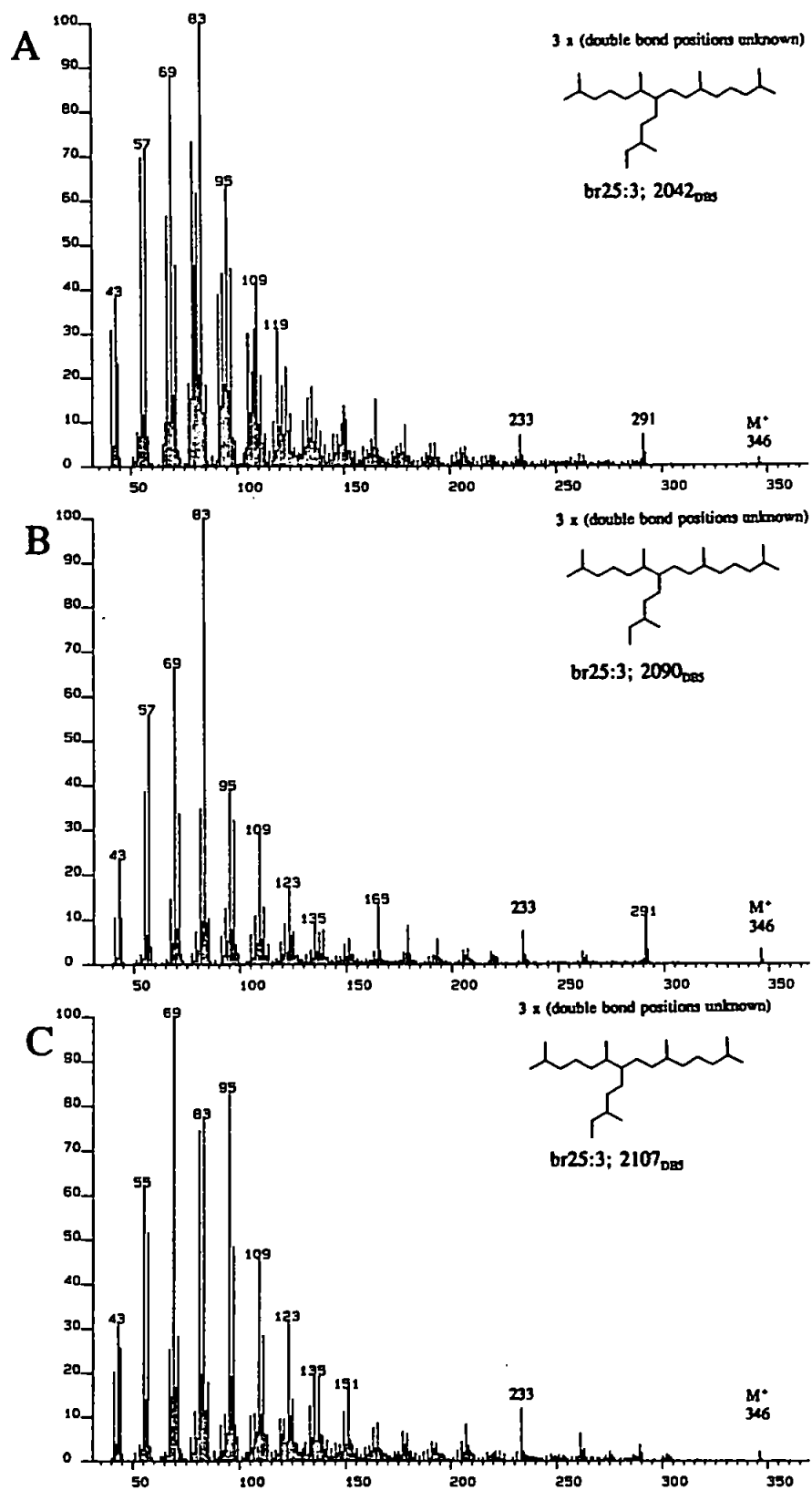
FIGURE 5.6   **MASS SPECTRA OF**
(A) br25:3; 2042_{DB5}
(B) br25:3; 2089_{DB5}
(C) br25:3; 2107_{DB5}

more than one GC stationary phase.

The mass spectrum and retention index of br25:3; 2107_{DBS} was similar to that reported by Albaigés *et al.* (1984a) and Porte *et al.* 1990 (a molecular ion at *m/z* 346 and fragment ions at *m/z* 191, 223 and 261). The mass spectrum (Figure 5.7A) for the alkene at RI 2140_{DBS} was identical to that of a hydrocarbon first proposed to be a monocyclic C_{25} monoene by Requejo and Quinn (1983a; c25:1:1; 2140_{SE30}) and Albaigés *et al.* (1984b; c25:1:1; 2139_{SE30}) as it was converted upon hydrogenation to a *sic* monocyclic C_{25} alkane (*i.e. m/z* 350; c25:0:1; 2156_{SE30}). However, Robson and Rowland (1986) showed that RI 2140 may represent either a diunsaturated analogue of the HBI alkane 2 namely br25:2; 2139_{OVI}, or an acyclic alkene with an unknown carbon skeleton. The mass spectra both contained ions at *m/z* 308/309 but differed in the absence of an ion at *m/z* 350 in the hydrocarbon (RI 2154_{OVI}) reported by Robson (1987). A cyclic structure was dismissed by Robson (1987) as the fragmentation pattern exhibited by RI 2154_{OVI}, the presumed hydrogenation product of RI 2139_{OVI}, was consistent with that of an acyclic alkane (prominent odd mass fragment ions of the C_nH_{2n+1} series). Moreover, such a hydrocarbon was reported by Porte *et al.* (1990) as a hydrogenation product of aliphatic hydrocarbons isolated from bivalves. This compound exhibited a spectrum similar to that presented by Robson (1987) but displayed an apparent molecular ion at *m/z* 352 indicating the complete reduction to an alkane. A tentative assignment of the carbon skeleton of this hydrogenation product, br25:0; 2158_{DBS}; 2154_{OVI}; 5 was made based upon interpretation of the mass spectrum of the HBI alkane. Interestingly, no HBI alkene in the lipids of the bivalves was assigned as the precursor to this novel C_{25} HBI alkane which demonstrates the difficulty in assigning precursors to the products of hydrogenation in complex
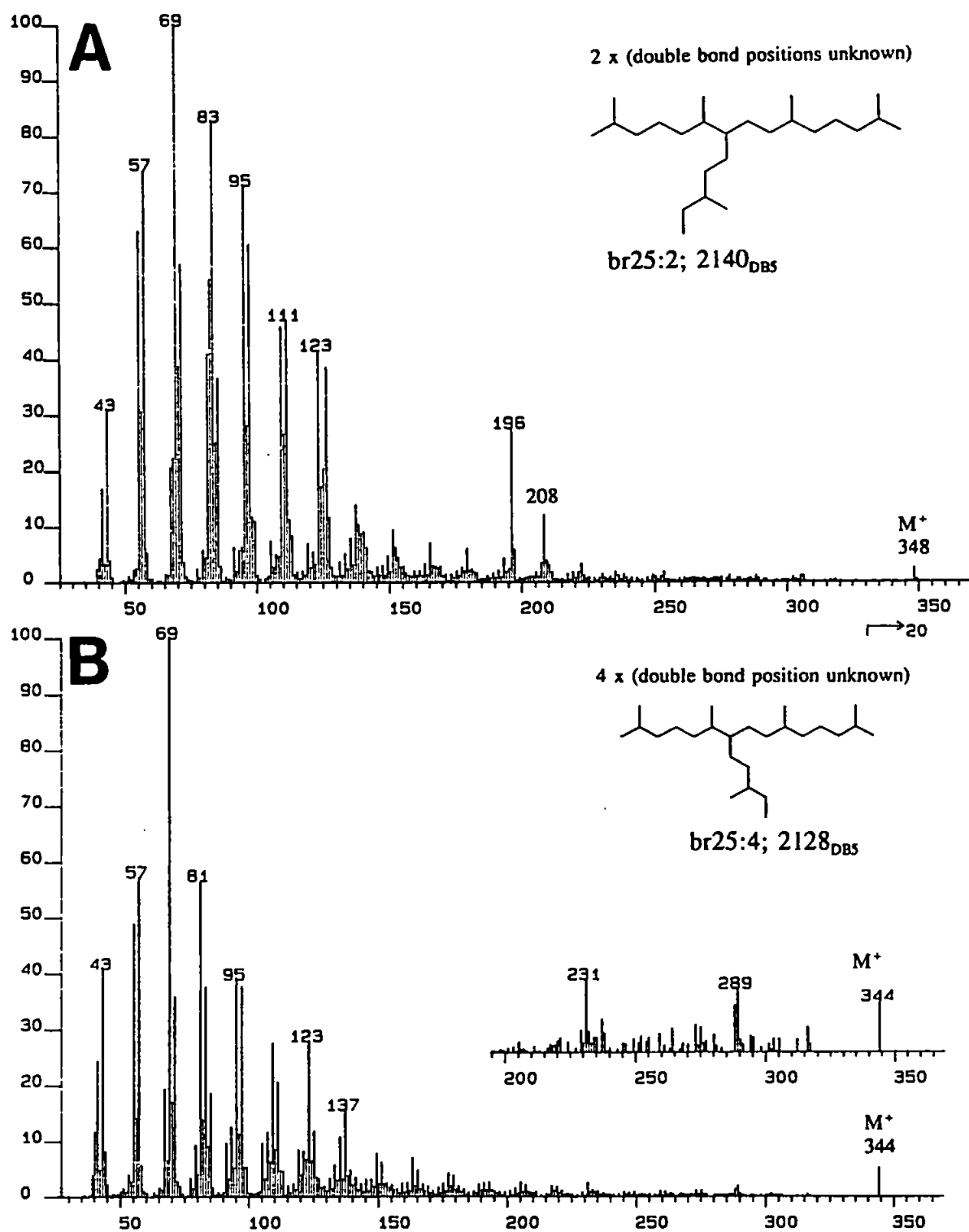
FIGURE 5.7   MASS SPECTRA OF
(A) br25:2; 2140$_{DBS}$
(B) br25:4; 2128$_{DBS}$

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samples. Thus, upon hydrogenation, the C_{25} HBI diene br25:2; 2139_{OVl} could have been converted by Robson (1987) to either of the parent HBI alkanes, br25:0; 2107_{OVl} or br25:0; 2158_{DB5}; 2154_{OVl} depending on the carbon skeleton of br25:2; 2140_{DB5}.

The presence of a C_{25} tetraene br25:4; 2128_{DB5} in the sediment was confirmed by comparison of the mass spectrum (Figure 5.7B) with that reported for a pair of C_{25} HBI tetraenes by Barrick et al. (1980; br25:4; 2078_{SP2100} and br25:4'; 2124_{SP2100}; M^+ at m/z 344 and fragment ions at m/z 163, 205, 231 and 289).

5.3.3   STRAIGHT CHAIN ALKENES AND ALKANES

Straight chain alkenes were found in all sediments. The mass spectrum of RI 1690_{DB1} isolated from sediment at Millbrook contained a molecular ion at m/z 238 and fragmentation typical of a normal alkene which is proposed to be a n-C_{17} monoene, n-heptadecene (n-$C_{17:1}$; 1690_{DB1}). Minor amounts of heptadienes (e.g. n-$C_{17:2}$; 1659_{DB1}) were also present in some of the sediments. The n-C_{17} monoene was apparently absent from the samples from Cargreen and St. Johns Lake, the mudflats which seemed devoid of macroalgal mats but which exhibited a "diatom slime" (Thompson and Eglinton, 1976; 1979) at particular periods depending upon the state of the tide, weather conditions and time of year. Compounds RI 2038, 2043 and 2048 were found in all sediments the mass spectra of which exhibited apparent molecular ions at m/z 288, 286 and 284 respectively which suggested molecular formulae of $C_{21}H_{36}$, $C_{21}H_{34}$ and $C_{21}H_{32}$. Hydrogenation of RI 2038_{DB5}, 2043_{DB5} and 2048_{DB5} produced a single compound at RI 2100 and had an identical mass spectrum to n-heneicosane. These compounds were thus assigned as C_{21} n-alkenes with four, five and six double bonds respectively. The major alkene was the polyunsaturated alkene heneicosahexaene

(n-$C_{21:6}$; 2048_{DB5}). Gas chromatographic analyses of the sedimentary hydrocarbons on a DB1 stationary phase produced a single peak at RI 2048_{DB1} which corresponded to the coelution of n-C_{21} polyenes.

Another dominant feature included a series of high molecular weight n-alkanes (*i.e.* n-C_{23} to C_{33}) with a predominant odd carbon number preference (*e.g.* CPI > 4) characteristic of natural vascular plants (Eglinton *et al.*, 1962; Eglinton and Hamilton, 1963; 1967; Riely *et al.*, 1991a and references therein), and thus the input of terrigenous organic matter (Cranwell, 1973; Brassell *et al.*, 1978; Simoneit, 1978; Thompson and Eglinton, 1978). The presence of an unresolved complex mixture (UCM) suggests contamination of the sediment by petroleum hydrocarbons (Brassell *et al.*, 1978; Brassell and Eglinton, 1986; Jones *et al.*, 1986).

The occurrence of n-heptadecenes in sediments has been related to marine algal sources, as n-$C_{17:1}$ is the major hydrocarbons of green algae (*Chlorophycea*) *e.g.* *Ulva lactuca* and *Enteromorpha compressa* (Youngblood *et al.*, 1971; Youngblood and Blummer, 1973; Shaw and Wiggs, 1979), *Cladophora* sp. (Requejo and Quinn, 1983b) and *Chlorodesmis fastigiata* (Murray *et al.*, 1976; Coates *et al.*, 1986).

Heneicosahexaene (n-$C_{21:6}$) has been reported as abundant in many algae including some marine plankton (Blumer, 1970; Lee *et al.*, 1970; Lee and Loeblich, 1971; Blumer *et al.*, 1971; Volkman *et al.*, 1980a; Osterroht and Petrick, 1982; Osterroht *et al.*, 1983) and has been related to primary productivity as it is only found in the photosynthetic species. The n-$C_{21:6}$ polyene has also been isolated from benthic marine algae (Thompson and Eglinton, 1976; 1979). This component is rapidly decomposed in the presence of oxygen, so its presence in the top (0-2 cm) of

Tamar sediment may indicate that living or intact cells are important sources of organic matter to these sediments. In sediments sampled at greater depth, n-$C_{21:6}$ was absent suggesting that it was rapidly removed even under anoxic conditions (e.g. Volkman et al., 1986). Small amounts of the related alkenes heneicosapentaene (n-$C_{21:5}$; 2043_{DBS}) and heneicosatetraene (n-$C_{21:4}$; 2038_{DBS}), of presumed algal origin (Youngblood et al., 1971; Lee and Loeblich, 1971), were also found in some of the top sediments. In contrast, intertidal sediments of Kachemak Bay, Alaska contained only the penta- and tetraene (n-$C_{21:5}$; $1.1 \mu gg^{-1}$ and n-$C_{21:4}$; $0.03 \mu gg^{-1}$) but n-$C_{21:6}$ was not detected (Shaw and Wiggs, 1980). An unknown alkene with the formula $C_{21}H_{36}$ which exhibited a similar mass spectrum to n-$C_{21:4}$ was identified in top sediments from the Southern California Bight (Venkatesan et al., 1980) and from the Baltic Sea (Pihlaja et al., 1990). The presence of these n-C_{21} polyenes in sediments from the Tamar estuary represented a direct input of organic matter from microalgae.

The sediments from all three sites sampled in the Tamar Estuary in 1989 were dominated by HBI alkane 1 and related monoene 4 with some differences in the distribution of C_{25} HBI alkenes (Table 5.2; Figures 5.4 and 5.5). The presence of a n-$C_{17:1}$ isomer and C_{21} polyenes, together with n-alkanes having a distribution maximum at n-C_{29}, and a UCM suggested organic inputs to these sediments from algae, vascular plants and anthropogenic sources.

5.4 EXAMINATION OF MACROALGAE AS A POTENTIAL SOURCE OF HBI HYDROCARBONS TO THE SEDIMENT IN TAMAR ESTUARY

In order to investigate the reports of Rowland *et al.* (1985) and Nichols *et al.* (1988) that HBI hydrocarbons may be derived from algae, several potential algal sources of organic matter to the Tamar sediment were examined.

5.4.1 MACROALGAL MAT AT MILLBROOK (JULY, 1989)

At the same time as the sediment described above was collected at Millbrook (July, 1989), a sample of decayed, semi-buried, filamentous "macroalgal mat" was taken. Microscopic examination of the mat revealed that it consisted primarily of the filamentous green alga of the genus *Cladophora*. No further identification of the species was possible. The amount of sediment in this sample was relatively high and organic carbon content of the mat was remarkably similar to that of the surrounding sediment (TOC: mat, 3.1%; *cf.* sediment 3.6%). Indeed, comparison of the gas chromatograms of the aliphatic hydrocarbons showed little qualitative difference (Figure 5.8). However, the absolute concentrations of HBI hydrocarbons were different (Table 5.4; Figures 5.9 and 5.10). Interestingly, the relative contribution of n-$C_{21:6}$ was elevated in the macroalgal mat, possibly together with one of the C_{25}HBI trienes (br25:3; 2042_{DBS}; 2040_{DB1}) compared with the sediment. The major C_{25} HBI alkenes in the macroalgal mat were the trienes br25:3; 2042_{DBS}, br25:3; 2089_{DBS} and br25:3; 2107_{DBS}.
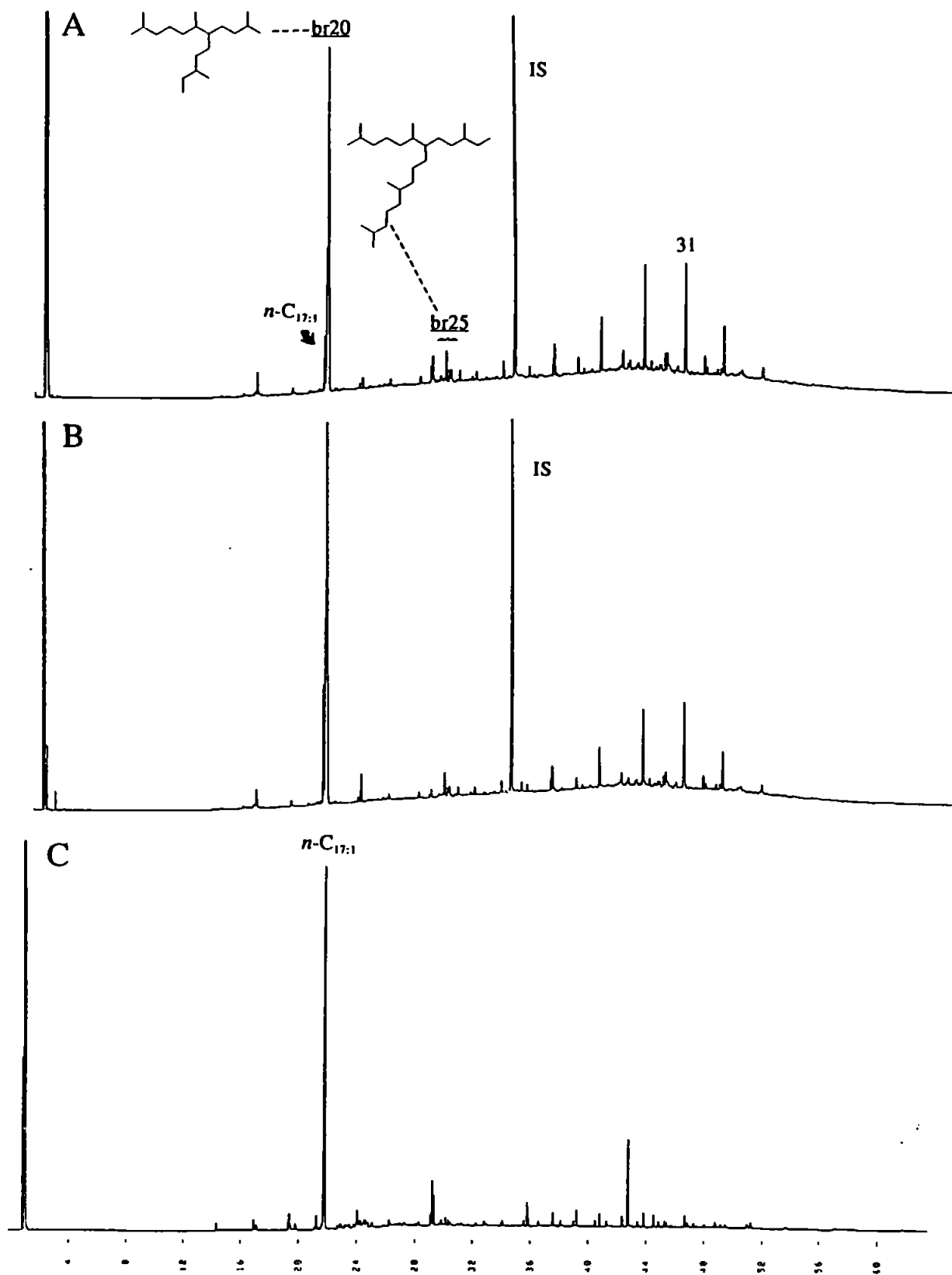
FIGURE 5.8 GAS CHROMATOGRAMS OF ALIPHATIC HYDROCARBONS
ISOLATED FROM
(A) Millbrook sediment
(B) Semi-buried algal mat
(C) Epiphytic microalgae from the mat
For conditions see text; DB5 (J&W).

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TABLE 5.4 CONCENTRATION OF HBI AND OTHER HYDROCARBONS IN SEDIMENT AND ALGAE AT MILLBROOK IN JULY, 1989 (mgkg^{-1} dry weight)

Compound (RI)	Sediment	Semi-buried algal mat	Epiphytes
n-$C_{17:1}$; 1667_{DBI}	nd	nd	+
n-$C_{17:1}$; 1690_{DBI}	1.3	6.4	+
n-$C_{17:1}$; 1697_{DBI}	nd	nd	+ (70%)
n-C_{17}	nm	nm	+
br20:1; 1702_{DBI}	3.5	18	+
br20:0; 1707_{DBI}	9.7	50	+
Σn-C_{21} polyenes	1.2	6.0	+
br25:3; 2042_{DB5}	2.1	11	+ (+br25:4)
br25:2; 2070_{DB5}	nd	nd	+
br25:3; 2089_{DB5}	2.3	12	+ (+br25:1)
br25:3; 2107_{DB5}	1.2	6.0	+
br25:1; 2110_{DBI}	nd	nd	+
br25:4; 2128_{DB5}	0.31	0.9	+
br25:2; 2140_{DB5}	0.92	4.5	+

Key: nd = not detected, nm = not measured, + = detected

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C20 HBI and n-C17 hydrocarbons, Millbrook, 1989

br20:0; 1707

br20:1; 1702

☑ Semi-buried mat
▦ Sediment

n-C17:1; 1690

0   10   20   30   40   50

Concentration (mg/kg dry weight)
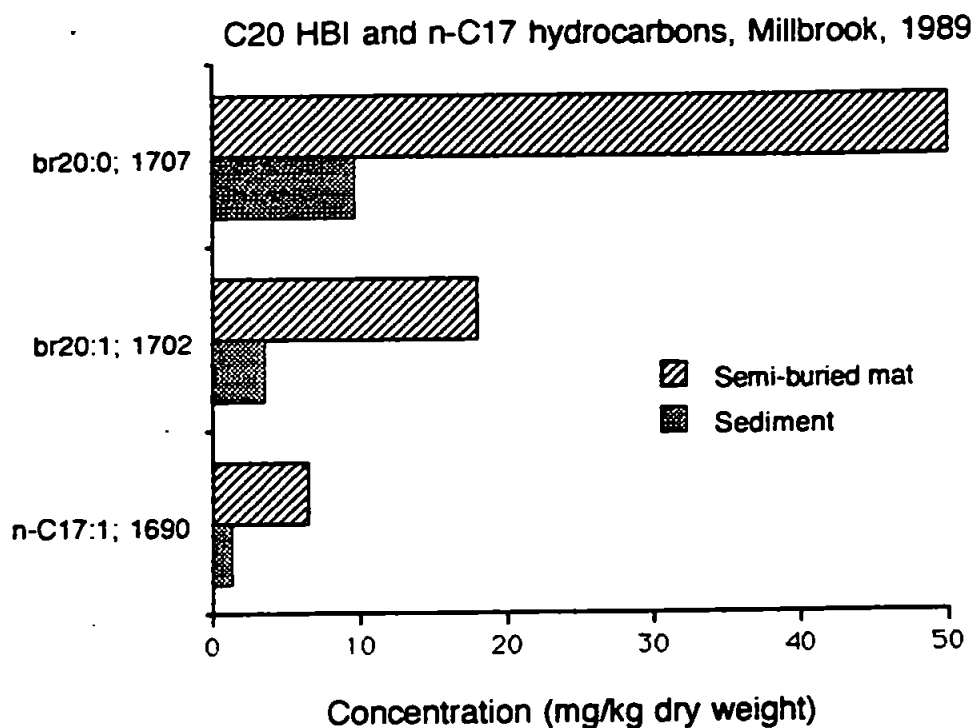
FIGURE 5.9 **CONCENTRATION OF C_{20} HBI HYDROCARBONS IN SEDIMENT AND SEMI-BURIED ALGAL MAT AT MILLBROOK IN JULY, 1989**

C25 HBI and n-C21 polyenes, Millbrook, 1989

br25:2; 2140

br25:2; 2128

☑ Semi-buried mat
▦ Sediment

br25:3; 2107

br25:3; 2089

br25:3; 2042

n-C21 polyenes

0   5   10   15

Concentration (mg/kg dry weight)
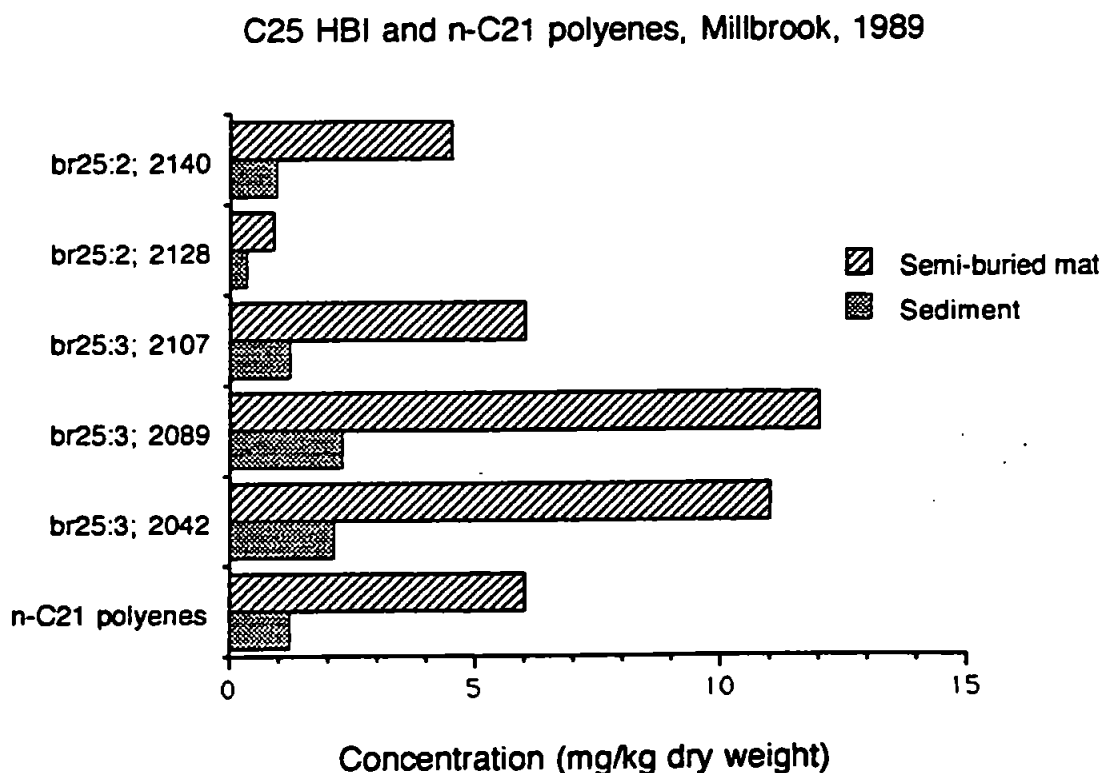
FIGURE 5.10 **CONCENTRATION OF C_{25} HBI ALKENES IN SEDIMENT AND SEMI-BURIED ALGAL MAT AT MILLBROOK IN JULY, 1989**

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The presence of n-$C_{21:6}$ in the mat material may indicate the presence of either epiphytic and/or trapped epipelic microalgae. Although common in some microalgae, this polyene has also been detected in specimens of benthic green macroalgae (*e.g.* Youngblood *et al.*, 1971). Microscopic examination did reveal the presence of diatoms. An attempt was made to physically remove a proportion of these organisms from the macroalgal mat. The modified method of Moss and Eaton (1968) as used in similar studies by Thompson and Eglinton (1976; 1979) was used to isolate motile and light-sensitive phytobenthic organisms from the macroalgal mat.

5.4.2 MICROALGAE ISOLATED FROM THE MACROALGAL MAT AT MILLBROOK (JULY, 1989)

The distribution of hydrocarbons in the gas chromatogram of the 'aliphatic' extract from this sample was shown to be less complex than those of the sediment and macroalgal mat (Figure 5.8) and was dominated by an isomer of n-heptadecene (n-$C_{17:1}$; 1697_{DBI}; 70% total aliphatic hydrocarbons)[1]. Other relatively minor components present included n-C_{17} alkane and alkenes (n-$C_{17:1}$; 1667_{DBI} and n-$C_{17:2}$; 1689_{DBI}) and the C_{20} HBI alkane **1**. The shoulder peak on n-C_{17} proved to be the C_{20} monounsaturated HBI homologue **4**. However, it was noted that the lens tissue used had trapped strands of filamentous algae (presumably derived from fresh growth of the macroalga) and may account for the dominance of the n-$C_{17:1}$ isomer which was probably derived from *Cladophora* spp.. Examination of the chromatographic peaks RI 2000-2100 revealed the presence of n-$C_{21:6}$ which suggested that the isolation

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[1]Analysis of microalgae isolated via the tissue lens technique could not be made quantitative due to the difficulty involved in determining the weight of algae attached to the tissue.

method did concentrate diatomaceous microalgae containing the n-C_{21} polyene. However, a macroalgal source for this compound cannot be excluded in this case because of the presence of the *Cladophora* strands. In contrast to the total aliphatic hydrocarbons, comparison of the distribution of the C_{25} HBI alkenes in the sediment, the partially buried mat and the isolate on the lens tissue shows a great similarity. However, the occurrence of the C_{25} monoenes br25:1; 2100_{DBS} (2110_{DB1}) and br25:1; 2087_{DBS}, the mass spectra of the former which is shown in Figure 5.11, was limited to the epiphytic/epipelic isolate. Comparison of the GC RI of these algal compounds with those of C_{25} synthetic and sedimentary monoenes isolated and characterised during the present study (sections **3.3-3.5** and **4.1.3**) allowed tentative assignment of br25:1; 2100_{DBS} as either **6** or **7**, and br25:1; 2087_{DBS} as either **8** or **9**. However, a more rigorous assignment must await isolation and ozonolysis of the algal alkenes, as proved possible for sedimentary monoenes **4** and **6**.

The results suggest that the HBI hydrocarbons are not solely associated with the sediment but perhaps with macroalga or motile epiphytic or epipelic organisms, living within the top layer of sediment or upon organic debris (Table 5.4; Figures 5.9 and 5.10). There was little evidence of vascular plant input in either the semi-buried mat or microalgal isolate which helps to discount the possibility of transfer of sediment through the lens tissue barrier or the presence of exogenous lipids (either trapped on the filamentous strands or in the mucilage extruded by the microalgae). The example of the alkene, n-$C_{21:6}$ highlights the difficulties inherent in the assignment of source to individual organic compounds isolated from complex biological communities. In addition, many compounds have multiple potential sources. For example, the presence of n-$C_{17:1}$ in the sediments at Millbrook may be attributed
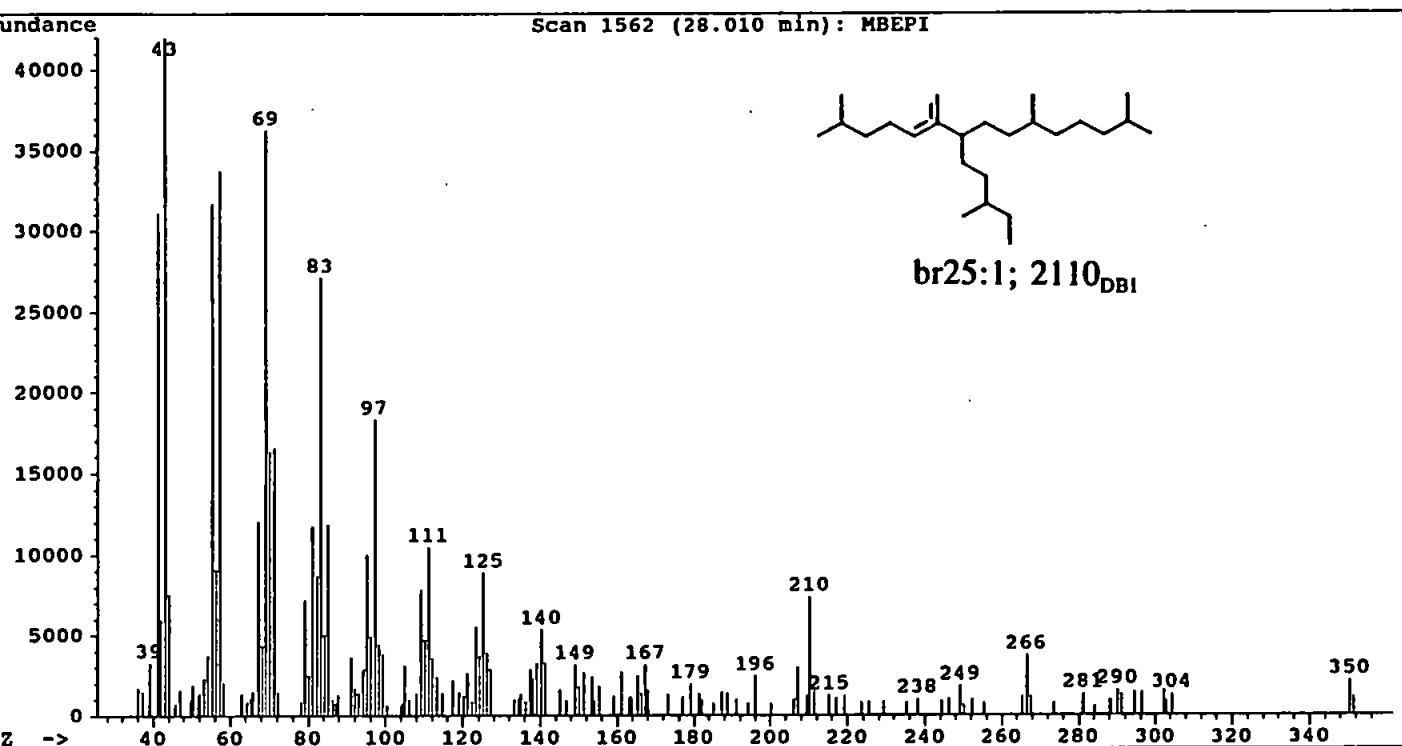
FIGURE 5.11   MASS SPECTRA OF
br25:1; 2110$_{DBS}$
Conditions: HP 5970 MSD; *m/z* 35-400, 1.5 scan/sec.

to a contribution of organic matter from autochthonous algal detritus possibly including the macroalga *Cladophora*. However, the occurrence of *n*-heptadecenes is not restricted to algae of the genus *Cladophora*.

Given the problems encountered during the analysis of macroalgal mat in 1989, the hydrocarbons from other potential algal sources of organic matter to Tamar sediments were examined in order to investigate further the hypothesis that HBI hydrocarbons may be derived from algae.

5.5 SEDIMENTS AND MACROALGAL MATS AT MILLBROOK (AUGUST, 1990)

In retrospect, the hydrocarbons extracted from the sample of macroalgal mat taken in June 1989 proved to be not simply derived from the macroalga *Cladophora* as the mat was partially buried possibly by storm action or during the process of decay. Thus further field samples of fresh macroalga were taken on occasions when 'clean' specimens were observed. An attempt was made to sample particular species when it was a dominant type growing on the sediment. In addition, samples of sediment were taken both underneath the algal mats and from clear sites at two locations in the Tamar Estuary. The aim of this particular study was to determine the hydrocarbon distribution extracted from the algae and to compare them with that of the sediments.

5.5.1 SEDIMENTS AT MILLBROOK (AUGUST, 1990)

Chromatographic profiles of hydrocarbons isolated from sediment lying beneath fresh algal mats and that of surficial sediment from areas away from macroalgal growth are shown in Figure 5.12. The results of quantitative analyses are presented in Table 5.5, and Figure 5.13 and 5.14.

Both sediments were dominated by the presence of the C_{20} HBI alkane 1 and the related monoene 4 previously shown to be the principal hydrocarbons in sediment collected at Millbrook, in July, 1989. Similarly, the three trienes, tetraene br25:4; 2128_{DBS} and diene br25:2; 2140_{DBS}, comprised the principal C_{25} HBI alkenes detected in the sediment samples.
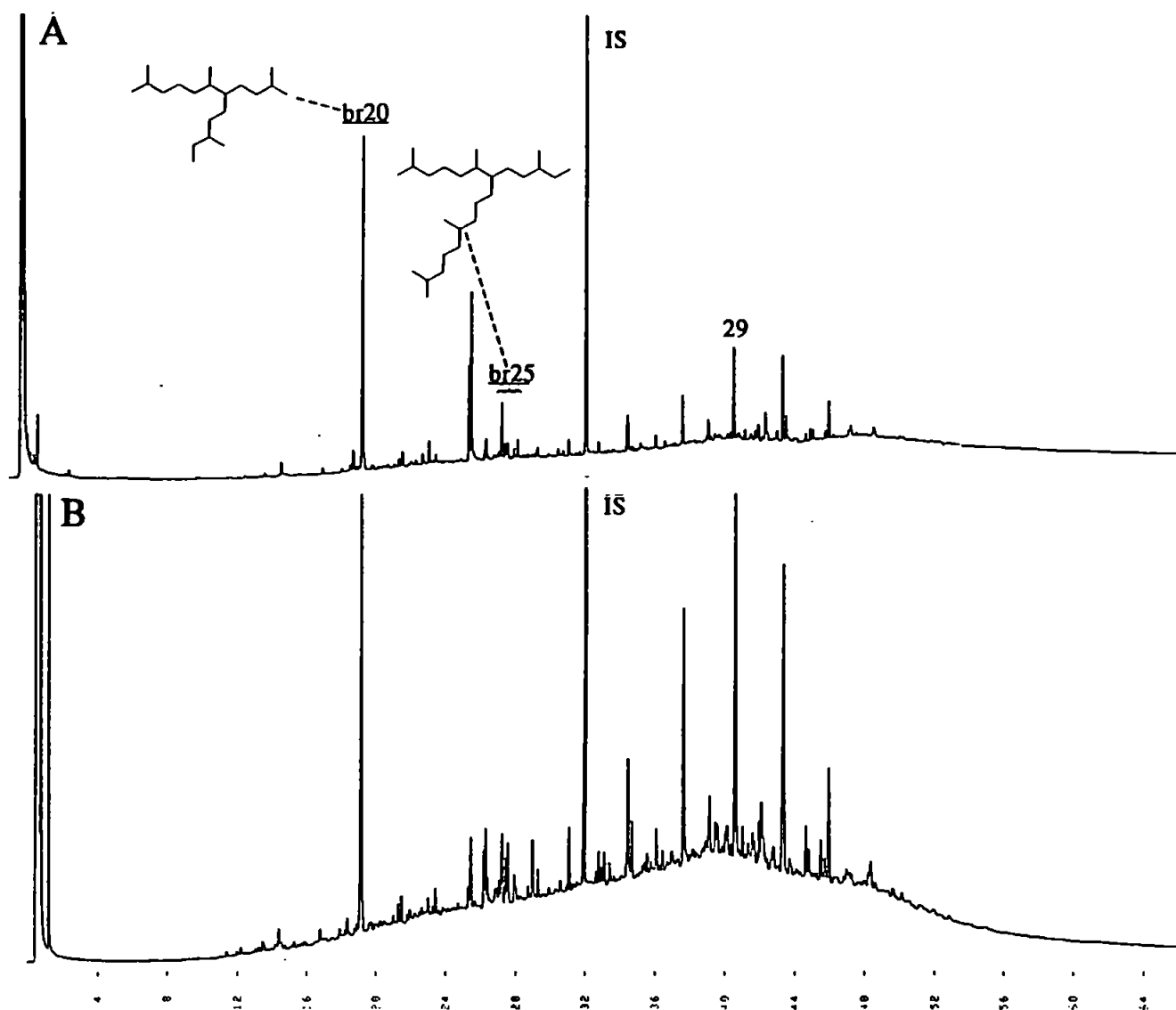
FIGURE 5.12   GAS CHROMATOGRAMS OF THE ALIPHATIC HYDROCARBONS ISOLATED FROM SEDIMENTS AT MILLBROOK IN AUGUST, 1990
(A) Sediment under algal mat
(B) Bare sediment
For conditions see text; DB5 (J&W).

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TABLE 5.5 CONCENTRATION OF HBI AND OTHER HYDROCARBONS IN SEDIMENTS AT MILLBROOK IN AUGUST, 1990 (mgkg⁻¹ dry weight)

Compound (RI)	Bare sediment	Sediment under algal mat
n-$C_{17:2}$; 1659_{DB1}	nd	0.31
n-$C_{17:1}$; 1678_{DB1}	nd	0.13
n-$C_{17:1}$; 1691_{DB1}	1.2	nd
n-$C_{17:1}$; 1695_{DB1}	nd	0.11
br20:1; 1702_{DB1}	4.2	2.0
br20:0; 1707_{DB1}	10	3.3
n-$C_{20:1}$; 1990_{DB1}	0.21	1.4
n-$C_{20:0}$	0.52	1.9
Σn-C_{21} polyenes	1.1	0.19
br25:3; 2044_{DB5}	2.1	0.47
br25:3; 2090_{DB5}	2.4	0.91
br25:3; 2107_{DB5}	1.1	0.31
br25:4; 2128_{DB5}	0.15	0.09
br25:2; 2140_{DB5}	0.85	0.27

Key: nd = not detected, nm = not measured

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C20 HBI and other hydrocarbons
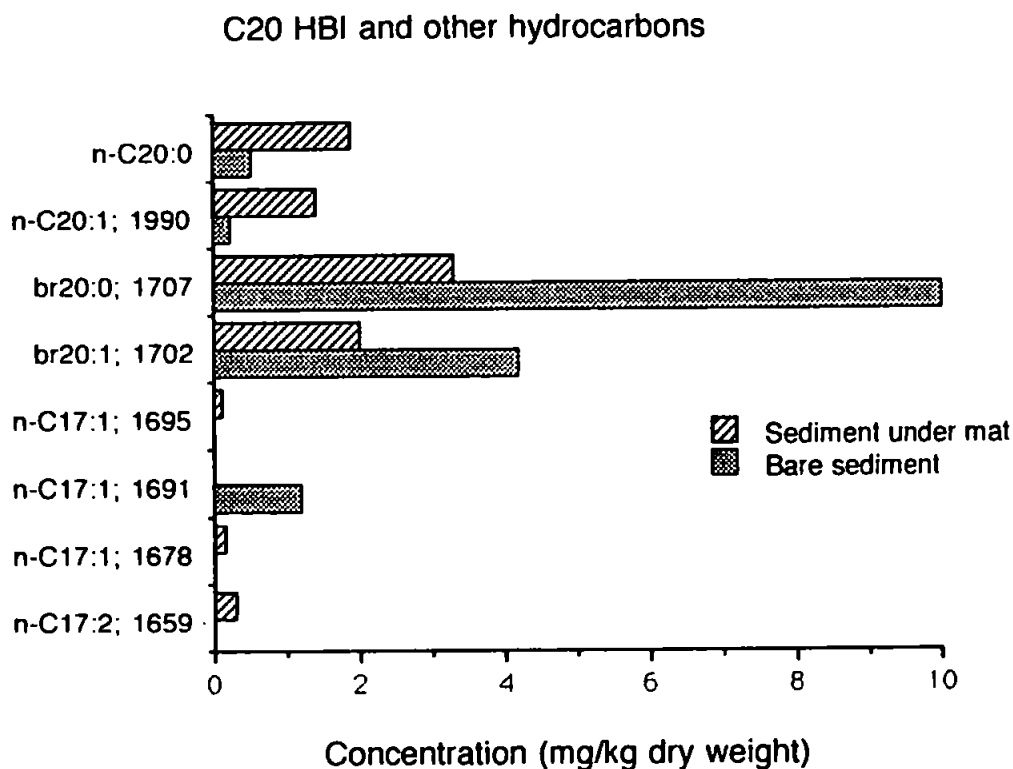
FIGURE 5.13   CONCENTRATION OF C_{20} HBI HYDROCARBONS IN SEDIMENTS AT MILLBROOK IN AUGUST, 1990

C25 HBI and n-C21 polyenes
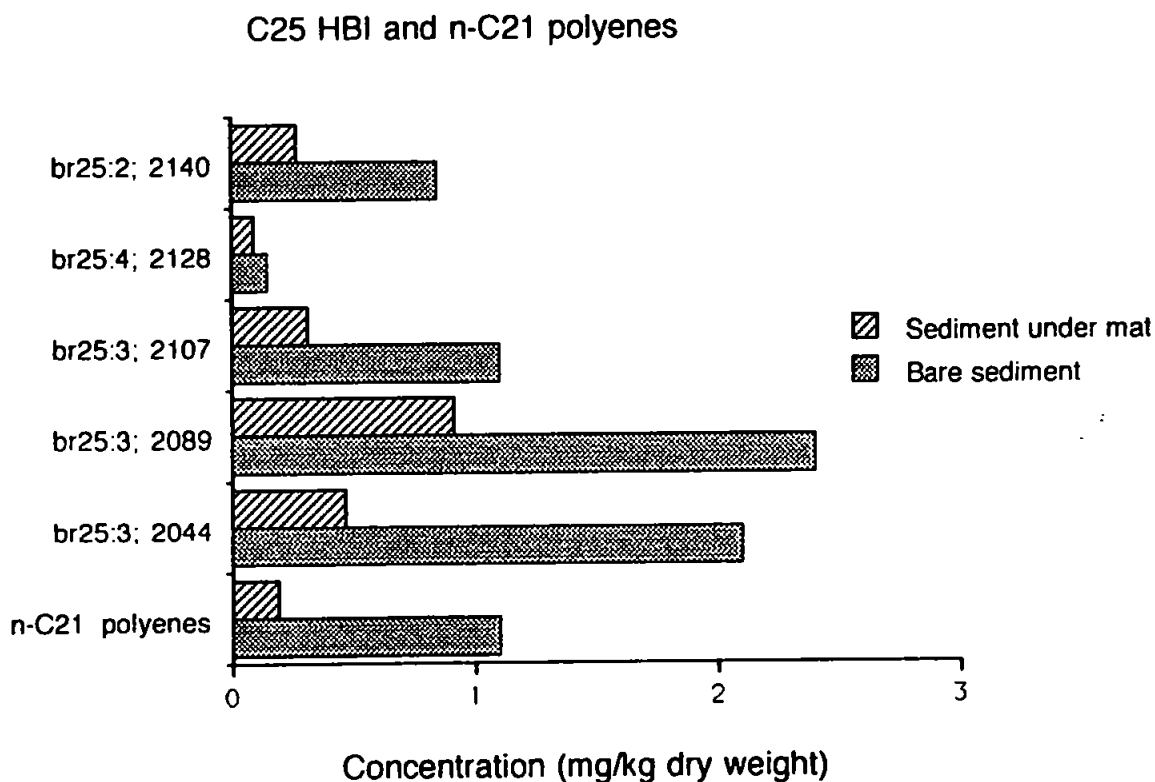
FIGURE 5.14   CONCENTRATION OF C_{25} HBI ALKENES IN SEDIMENTS AT MILLBROOK IN AUGUST, 1990

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Similar to many other recent intertidal sediments (*e.g.* Brooks *et al.*, 1976; 1977; Thompson and Eglinton, 1976; 1979; Shaw and Wiggs, 1980; Rowland *et al.*, 1985; Robson and Rowland, 1986; Requejo and Quinn, 1985; Volkman, 1980b), in addition to those described earlier, the hydrocarbon fractions of the sediments are characterised by a distribution of *n*-alkanes (*n*-C$_{15}$ to *n*-C$_{35}$). The majority of the *n*-alkanes in both sediment samples were in the range C$_{25}$ to C$_{33}$ with a high odd carbon number predominance (CPI >4) which suggests an input from epicuticular waxes of vascular plants (Eglinton *et al.*, 1962; Eglinton and Hamilton, 1963; 1967; Riely *et al.*, 1991a and references therein). In many sediments, a bimodal distribution of *n*-alkanes maximising at *n*-C$_{17}$ and *n*-C$_{27}$ is recorded (*e.g.* Cranwell 1978; Giger *et al.*, 1980; Smith *et al.*, 1986; Pihlaja *et al.*, 1990). The abundance of *n*-heptadecane (*n*-C$_{17}$) is attributed to a contribution from autochthonous algal detritus (Albaigés *et al.*, 1984a; Pihjaja *et al.*, 1990). However, in the case of the intertidal sediments at Millbrook, on the River Tamar, only a trace of *n*-C$_{17}$ was detected which suggested either that *n*-heptadecane was metabolised more quickly in the aquatic environment than the higher *n*-alkane homologues (C$_{23}$-C$_{35}$), the short-chain alkanes being more susceptible to microbial attack than the longer-chain compounds (Giger *et al.*, 1980; Cranwell, 1981; 1984; Gossens *et al.*, 1989b; Riley *et al.*, 1991a), or that an algal input of organic matter to the sediments is infrequent related to hydrographic and ecological parameters including the tides, current, season, water temperature, the allochthonous supply of nutrients, turbidity and salinity. This is in contrast to the more constant input of higher plant waxes via both autochthonous (*e.g.* runoff) and allochthonous (*e.g.* wind-driven) sources. Leaf fall from the surrounding trees provided a direct input of vascular plant material which is transported

downwards along the gradual slope of the mudflats. Plant leaves are, compared to algae, relatively inaccessible to decomposing microorganisms and are less readily resuspended into the oxic water during tidal scouring. As a consequence, the sediment might have become selectively enriched with vascular plant material.

Comparison of the gas chromatograms of the 'aliphatic hydrocarbon' extracts from the two sediments revealed other, less subtle differences in hydrocarbon distribution (Figure 5.13). Unlike the bare sediment the hydrocarbons of which contained only one n-heptadecene monoene (n-$C_{17:1}$; 1691_{DBI}), that from under the mat consisted of two isomers (n-$C_{17:1}$; 1678_{DBI} and n-$C_{17:1}$; 1695_{DBI}) and one diene (n-$C_{17:2}$; 1659_{DBI}).

5.5.2   MACROALGAL MATS AT MILLBROOK (AUGUST, 1990)

In August 1990, extensive growths of mat made up of *Cladophora* spp. were again observed at the Millbrook site. This material seemed fresh and, for the most part was not buried by sediment. Examination of the hydrocarbons from the *Cladophora* mat showed that n-C_{17} alkenes dominated the gas chromatogram (Figure 5.15; 60% total aliphatic hydrocarbons). Other components present in the alga included heptadecane (n-C_{17}) and related dienes (n-$C_{17:2}$; 1659_{DBI} and n-$C_{17:2}$; 1690_{DBI}), and the C_{20} HBI alkane, br20:0; 1707_{DBI}. A shoulder peak on n-C_{17} proved to be the monounsaturated homologue br20:1; 1702_{DBI}. Quantification of this C_{20} HBI monoene proved not possible in the algal hydrocarbons due to the large amount of n-C_{17} present, relative to the HBI alkene. In such proportions, co-injection studies on two GC stationary phases demonstrated the co-elution of the n-alkane (RI 1700) and HBI alkene (RI 1702_{DBI} and 1698_{DBS}). A better separation from n-C_{17} was obtained using

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a polyethylene glycol stationary phase (br20:1; 1659_{DBWAX}). In situations where n-C_{17} was present only in trace quantities, usually in sediments, the quantification of br20:1; 1702_{DBI} proved possible (*e.g.* see Table 5.3).

The partial TIC chromatogram of the hydrocarbons isolated from *Cladophora* mat (Figure 5.16) shows a number of components eluting between n-C_{20} and n-C_{22} on the nonpolar GC column used. Analysis by GC-MS detected a number of peaks which corresponded to C_{25} HBI alkenes. The GC RI and mass spectra of the compounds of interest in this study were similar to those previously reported for C_{25} HBI alkenes in surface sediments and particulate matter worldwide (see Rowland and Robson, 1990) and in the sediment at the Millbrook site, on the River Tamar, in this study. The characterisation of trienes, br25:3; 2044_{DB5}, br25:3; 2091_{DB5} and br25:3; 2107_{DB5} and diene br25:2; 2140_{DB5} have been described earlier. The hydrocarbon at RI 2157_{DB5} displayed a mass spectrum (Figure 5.17A) indicative of another C_{25} HBI triene br25:3; 2157_{DB5} previously unreported. Although a triene with a similar retention index (2156_{DB5}) was presented by Porte *et al.* (1990), comparison of the mass spectra revealed substantial differences in fragmentation patterns. The spectrum of the component from the *Cladophora* mat exhibited a molecular ion at *m/z* 346 and prominent fragment ions at *m/z* 193 and 233. That presented by Porte *et al.* (1990) was dominated by an uncharacteristic ion at *m/z* 163 (60% relative intensity) with other ions at *m/z* 191, 261 and 289.

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FIGURE 5.15   GAS CHROMATOGRAM OF ALIPHATIC HYDROCARBONS ISOLATED FROM CLADOPHORA
For conditions see text; DB5 (J&W).
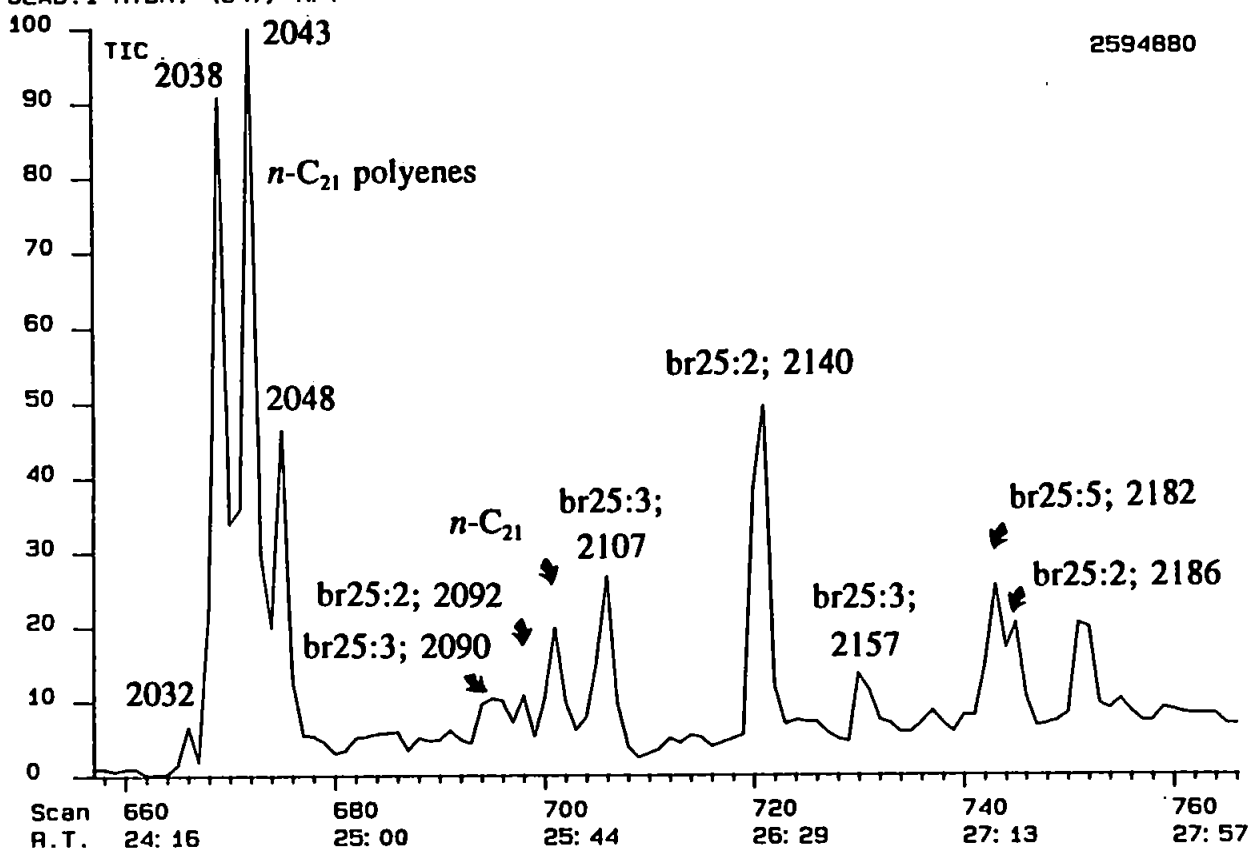
FIGURE 5.16   PARTIAL TIC CHROMATOGRAM OF ALIPHATIC HYDROCARBONS ISOLATED FROM CLADOPHORA

For conditions see text; DB5 (J&W).

A

B
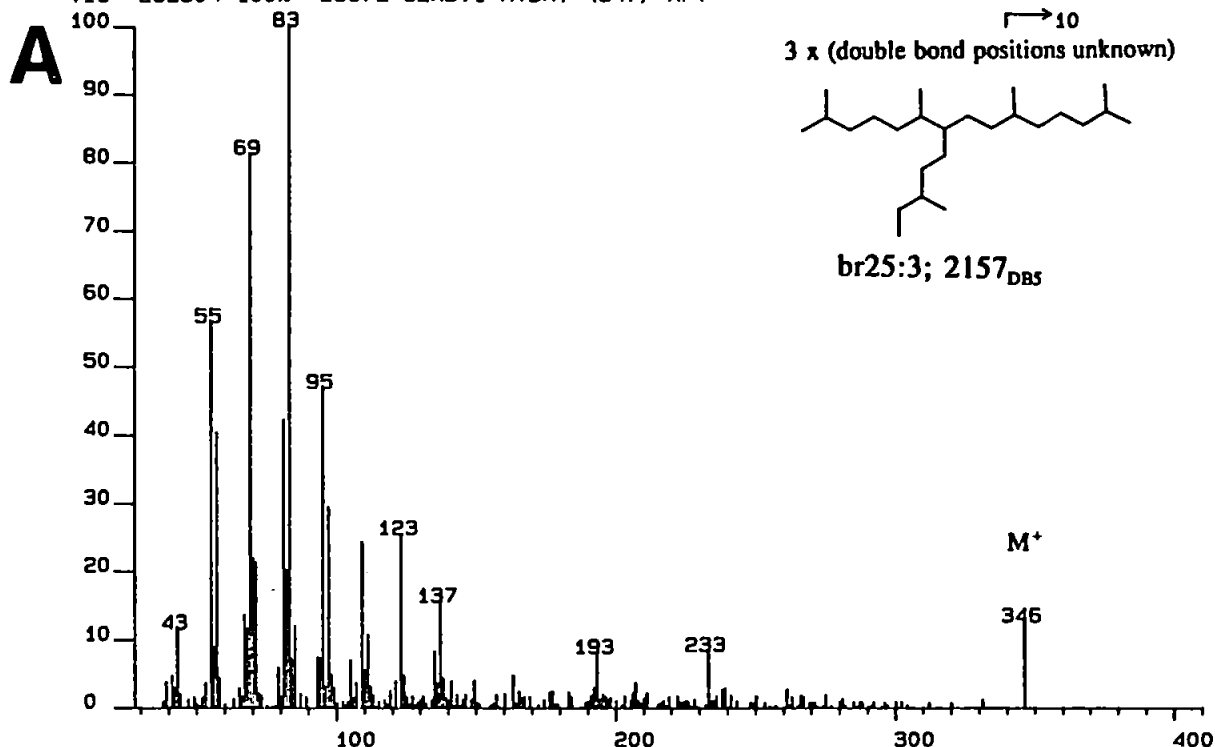
FIGURE 5.17   MASS SPECTRA OF
(A) br25:3; 2157_{DBS} (background subtracted)
(B) br25:5; 2182_{DBS}

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Examination of the mass spectrum of RI 2182_{DBS} (Figure 5.17B) revealed principal ions at m/z 205 and 273 but two apparent molecular ions at m/z 344 and 342. The spectrum of a pentaene br25:5; 2183_{DBS} was recorded by Porte $et\ al.$ (1990) which was almost identical to that in Figure 5.17B but no psuedomolecular ion at m/z 344 was evident. Moreover, a C_{25} HBI tetraene br25:4; 2183_{DBS} has yet to be reported in the literature. A tentative structure, **10** for this pentaene br25:5; 2183_{DBS} was proposed by Porte $et\ al.$ (1990) based solely upon the mass spectrum.

Other samples of *Cladophora* were taken over an interval of two months (August to September, 1990) to investigate variability between specimens. Differences in both hydrocarbon distribution and concentration were apparent in the field specimens analysed (Table 5.6; Figures 5.18 and 5.19). In particular, the relative abundance of the various n-C_{17} alkenes and parent alkane was seen to be subject to large variation. However, in each case the C_{20} HBI alkane and related monoene were detected.

TABLE 5.6 CONCENTRATION OF HBI AND OTHER HYDROCARBONS IN SPECIMENS OF *CLADOPHORA* COLLECTED AT MILLBROOK IN AUGUST, 1990 (mgkg^{-1} dry weight)

Compound (RI)	Specimen 1	Specimen 2	Specimen 3
$n\text{-}C_{15}$	nd	nd	1.8
$n\text{-}C_{17:2}$	1.1	nd	0.68
$n\text{-}C_{17:1}$; 1681_{DBI}	140	19	40
$n\text{-}C_{17:2}$; 1684_{DBI} $n\text{-}C_{17:1}$; 1684_{DBI}	15	0.45	0.68
$n\text{-}C_{17:1}$; 1692_{DBI}	2.6	1.8	4.5
$n\text{-}C_{17}$	3.5	0.73	2.6
br20:1; 1702_{DBI}	nm	nm	nm
br20:0; 1707_{DBI}	1.8	1.5	1.6
$\Sigma n\text{-}C_{21}$ polyenes	6.3	5.5	15
br25:3; 2044_{DBS}	nm	nm	nm
br25:3; 2090_{DBS}	0.25	nd	0.23
br25:2; 2092_{DBS}	0.13	nd	nd
br25:3; 2107_{DBS}	0.59	0.59	0.21
br25:2; 2140_{DBS}	1.2	0.31	0.14
br25:3; 2157_{DBS}	0.29	nd	nd
br25:5; 2182_{DBS}	0.50	nd	nd
br25:2; 2186_{DBS}	0.40	nd	nd

Key: nd = not detected, nm = not measured

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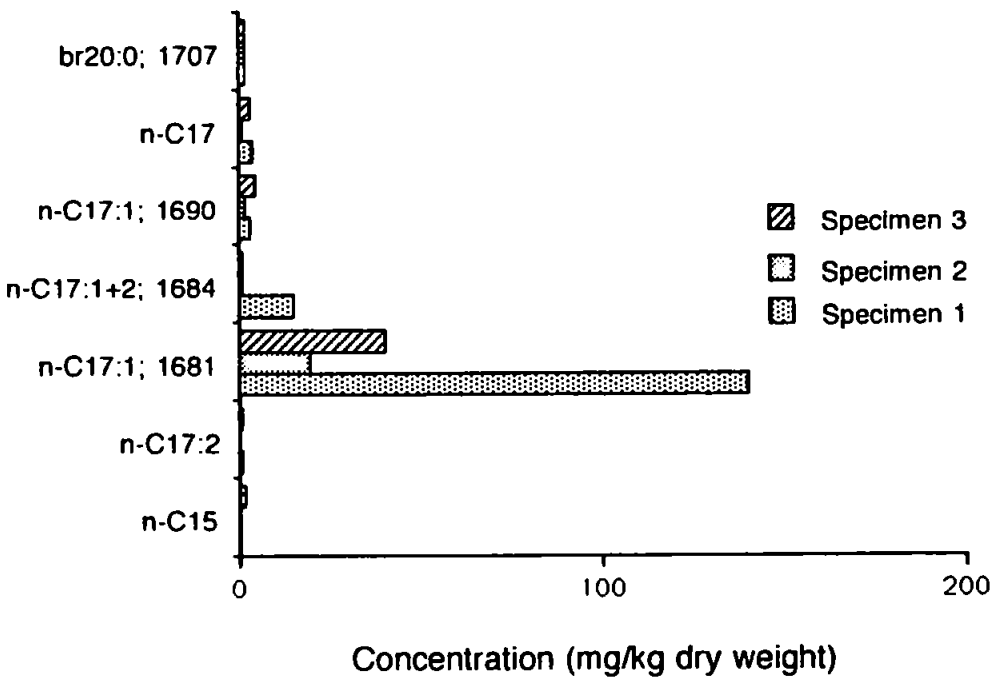
FIGURE 5.18   CONCENTRATION OF C_{20} HBI HYDROCARBONS IN
CLADOPHORA AT MILLBROOK IN AUGUST, 1990

C25 HBI alkenes in Cladophora spp.
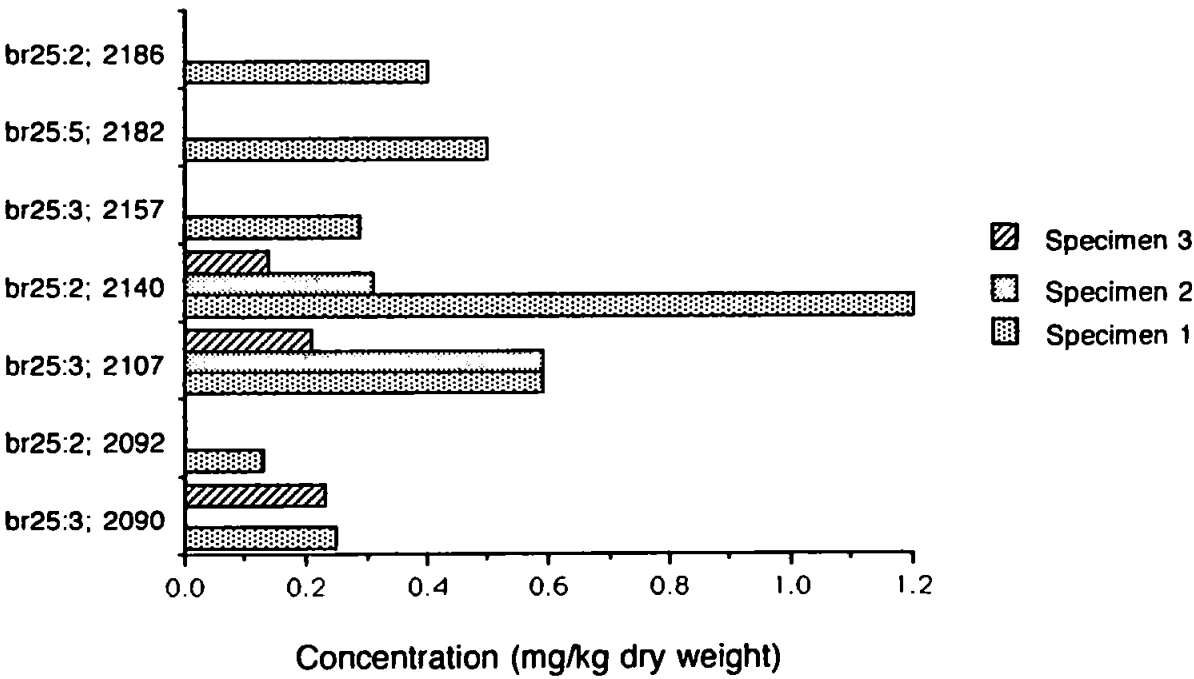
FIGURE 5.19   CONCENTRATION OF C_{25} HBI ALKENES IN
CLADOPHORA AT MILLBROOK IN AUGUST, 1990

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5.5.3 OTHER CHLOROPHYTA MACROALGA AT MILLBROOK (MAY-AUGUST, 1990)

During the same period of the same year (May to August, 1990) the occurrence of further members of the division of *Chlorophyta* (green algae) from the order *Uvales* were observed at the Millbrook site. A number of plants of the *Enteromorpha intestinalis-compressa* complex and specimens of the related *Enteromorpha linza* and *Ulva lactuca* were taken. As there is evidence that *Ulva* and *Enteromorpha* are congeneric (Bonneau, 1977) a similarity in hydrocarbon distribution was expected. The distribution of hydrocarbons in the various algae collected are summarised in Table 5.7 and Figures 5.20 and 5.21. The aliphatic hydrocarbons of *Ulva lactuca* were dominated by a heptadecene isomer (n-$C_{17:1}$; 1678_{DBI}) which made up 87% of the total. Although, n-heptadecene (n-$C_{17:1}$; 1674_{DBI}) was also the most abundant single component in *Enteromorpha linza*, n-pentadecane proved the principal hydrocarbon in specimens of the *Enteromorpha intestinalis-compressa* complex (35% to 60% total aliphatic hydrocarbons). All the *Ulvacea* species did contain a substantial amount of n-heptadecene and trace quantities of the C_{20} HBI alkane br20:0; 1707_{DBI} and related monoene br20:1; 1702_{DBI}, as previously shown for the *Cladophora* samples in this study.

The distributions of C_{25} HBI alkenes in the lipids of *Ulva lactuca* was similar to that observed in collections of *Cladophora* during this study and comprised two trienes br25:3; 2089_{DB5} and br25:3; 2107_{DB5}, and the diene br25:2; 2138_{DB5}. The major C_{25} HBI alkene in the specimens of *Enteromorpha* spp. proved to be the triene br25:3; 2089_{DB5}. However, another component RI 2070_{DB5} detected in the lipids of *Enteromorpha* spp. was absent from all the other algae examined here.

TABLE 5.7 CONCENTRATION OF HBI AND OTHER HYDROCARBONS IN SPECIMENS OF OTHER ALGAE COLLECTED AT MILLBROOK, 1989-90 ($mgkg^{-1}$ dry weight)

Compound (RI)	Ulva sp.	E. linza	Enteromorpha sp.	Enteromorpha sp.	Fucus distichus
n-C_{14}	1.1	0.70	nd	nd	nd
n-C_{15}	0.9	11	92	120	11
n-$C_{17:2}$; 1659_{DB1}	7.2	nd	8.0	1.3	nd
n-$C_{17:1}$; 1678_{DB1}	110	120	50	52	0.25
n-$C_{17:1}$; 1686_{DB1}	nd	nd	nd	nd	0.44
n-$C_{17:1}$; 1690_{DB1}	1.7	nd	9.1	0.67	0.74
n-C_{17}	1.8	tr	19	1.4	5.4
br20:1; 1702_{DB1}	nm	nm	nm	nm	nd
br20:0; 1707_{DB1}	0.32	0.24	0.92	0.84	nd
Σn-C_{21} polyenes	7.3	2.5	nd	nd	6.5
br25:2; 2070_{DB5}	nd	0.22	0.32	0.21	nd
br25:3; 2090_{DB5}	0.26	0.35	0.49	0.32	nd
br25:3; 2107_{DB5}	0.10	nd	nd	nd	nd
br25:2; 2140_{DB5}	0.48	nd	nd	nd	nd

Key: nd = not detected, tr = trace, nm = not measured

C20 HBI and other hydrocarbons in macroalgae, Millbrook, 1990
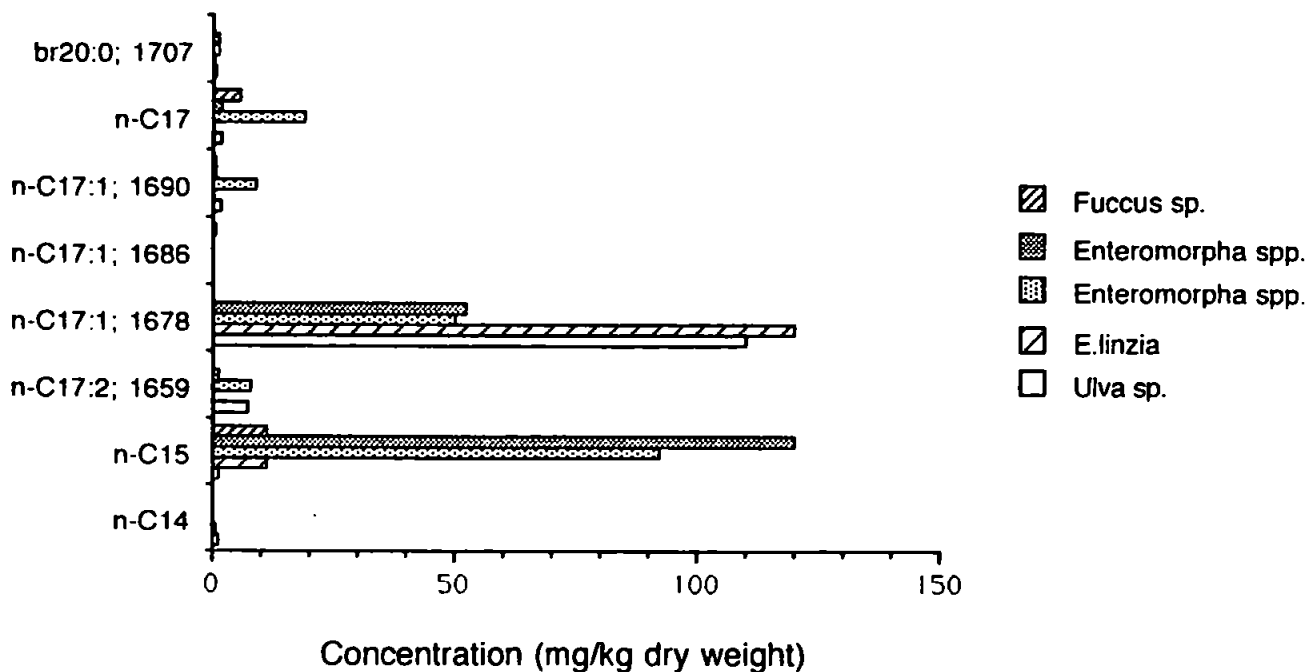
FIGURE 5.20   **CONCENTRATION OF C_{20} HBI HYDROCARBONS IN MACROALGAE AT MILLBROOK, 1989-1990**

C25 HBI and n-C21 polyenes in macroalgae, Millbrook, 1990
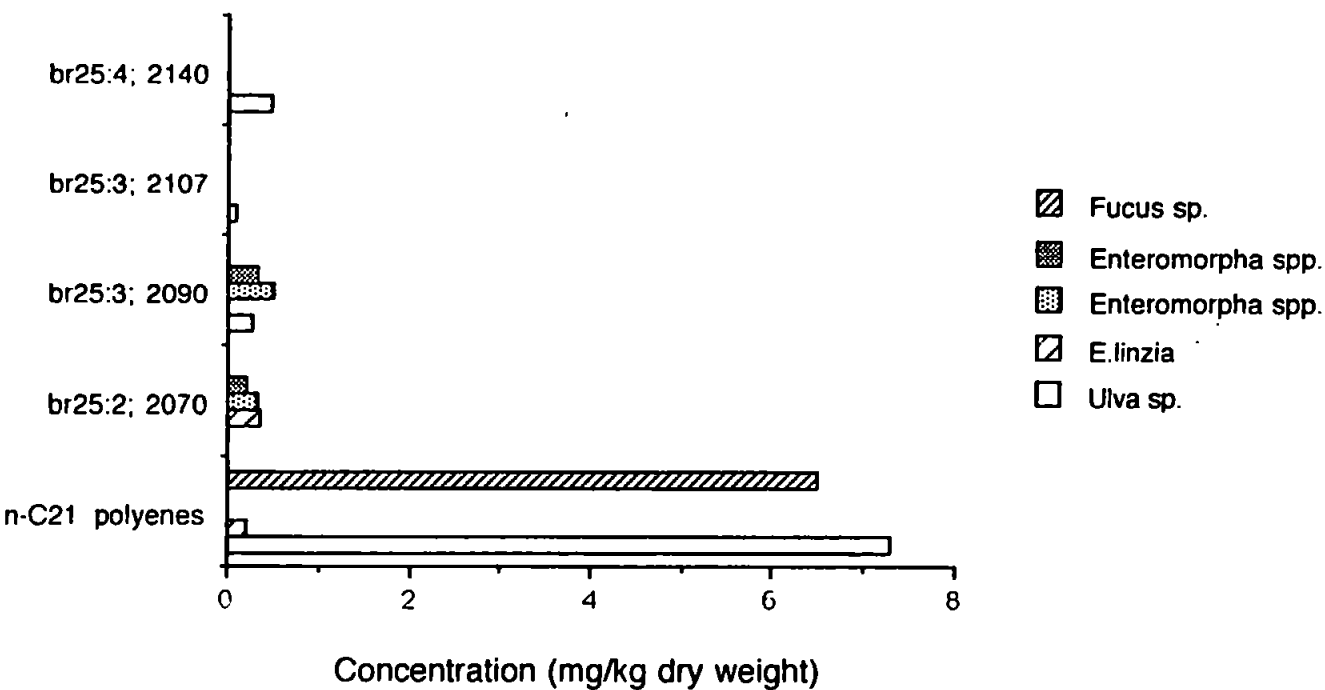
FIGURE 5.21   **CONCENTRATION OF C_{25} HBI ALKENES IN MACROALGAE AT MILLBROOK, 1989-1990**

The mass spectrum (Figure 5.22) and RI value of this HBI alkene was similar to that of a diene br25:2; 2070_{SE30} reported by Requejo *et al.* (1984), Albaigés *et al.* (1984a), Robson and Rowland (1986) and others (see Rowland and Robson, 1990). A molecular ion was evident at *m/z* 348 and the spectrum exhibited the characteristic fragment ion at *m/z* 264. The C_{25} HBI diene isomer, br25:2; 2082_{OV1} identified by Rowland *et al.* (1985) in *Enteromorpha prolifera* was absent from the lipids of all the *Enteromorpha* spp. plants examined here. In contrast to the C_{20} homologues, which seemed indigenous to all *Chlorophytae* examined, some inter- and intraspecific differences in distribution of C_{25} HBI hydrocarbons were apparent (Tables 5.6 and 5.7; Figures 5.18-5.21). Although the presence of br25:3; 2090_{DB5} was common to the hydrocarbons from all the green algae analysed, the occurrence of other alkenes was less specific. For example, br25:2; 2070_{DB5} was only detected in the species of *Enteromorpha*. The occurrence of br25:2; 2140_{DB5} and br25:3; 2108_{DB5} was limited to *Ulva* and *Cladophora*. Within the collections of *Cladophora*, one specimen exhibited a more complex distribution of C_{25} HBI alkenes than was associated with other plants examined. The occurrence of br25:2; 2094_{DB5}, br25:3; 2157_{DB5}, br25:5; 2182_{DB5} and br25:2; 2188_{DB5} was restricted to the one sample.

Although the *n*-C_{21} polyenes (*n*-$C_{21:4}$; 2037_{DB5}, *n*-$C_{21:5}$; 2042_{DB5} and *n*-$C_{21:6}$; 2048_{DB5}) were abundant in *Ulva lactuca*, only a trace of *n*-$C_{21:6}$ (2.5 μgg_{-1}) could be detected in the *Enteromorpha linza*, and *n*-C_{21} polyenes were not present in the other specimens of *Enteromorpha* collected. Microscopic examination of tissue from all the macroalgae did not indicate the presence of epiphytic diatoms in large numbers.
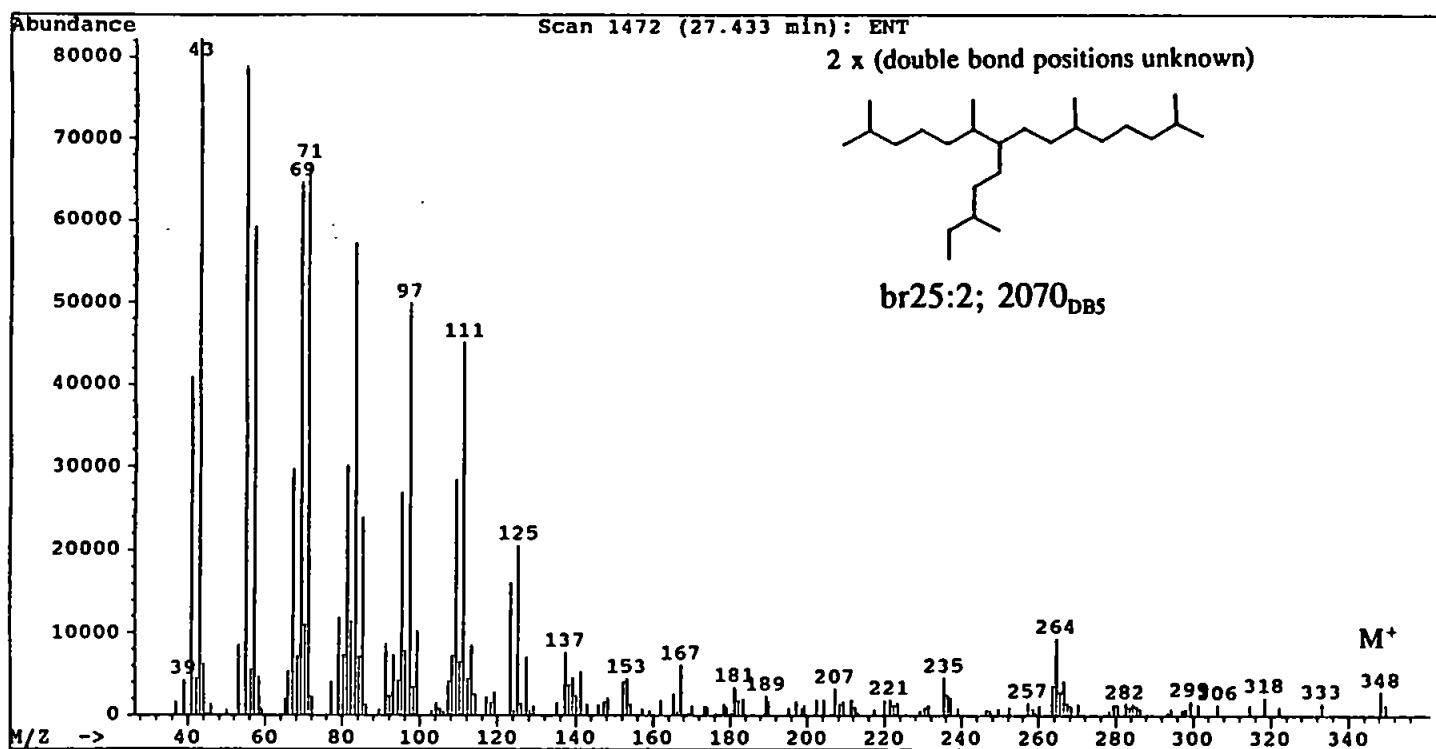
FIGURE 5.22   **MASS SPECTRUM OF br25:2; 2070$_{DBS}$ ISOLATED FROM**
***ENTEROMORPHA* (background subtracted)**
Conditions: HP 5970 MSD; *m/z* 35-400, 1.5 scan/sec.

5.5.4 PHAEOPHYTA MACROALGA AT MILLBROOK (OCTOBER, 1990)

In contrast to the green algae, the hydrocarbons of the one specimen of brown alga (*Phaeophtya*; *Fucus distichus*) collected (October, 1990) were dominated by *n*-pentadecane (*n*-C_{15}) and *n*-C_{21} polyenes which constituted over 50% of the total aliphatic hydrocarbons. A chromatogram (Figure 5.23) more complex than that associated with the hydrocarbons from most of the *Chlorophytae* displays a series of *n*-alkanes (C_{11}-C_{40}) with no apparent carbon number preference for the range C_{20} to C_{40} (CPI=1.3).

Whilst distributions of *n*-alkanes (C_{11}-C_{40}) with no apparent carbon number preference for the range C_{20} to C_{40} have been observed previously in *Fucus* spp. and other algae (Clark and Blumer, 1967; Youngblood *et al.*, 1971; Shaw and Wiggs, 1979), other materials from the marine environment have been found to contain similar components (Barbier *et al.*, 1973; Goutx and Saliot, 1980; Gassman, 1982; Lee *et al.*, 1983; Saliot *et al.*, 1983; Nishimura and Baker, 1986; Qui *et al.*, 1991). In the case of Shaw and Wiggs (1979), of the sixteen collections of *Fucus distichus* examined only one contained such a *n*-alkane distribution. Based upon this apparently sporadic occurrence they suspected a bacterial source for these hydrocarbons. Although this study was limited to only one specimen of *Fucus*, the absence of a UCM from the hydrocarbon distribution in the chromatogram suggested at most a minor contribution from petrogenic sources. The low carbon preference index (CPI) may therefore be interpreted as representing either endogenous products of algal biosynthesis (Youngblood *et al.*, 1971), direct incorporation of bacterial lipid residues (Bird and Lynch, 1974; Goutx and Saliot, 1980), or microbial reworking of algal

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and/or terrestrial lipids (Johnson and Calder, 1973; Simoneit and Kaplan, 1980; Fevrier *et al.*, 1983). Re-examination of the hydrocarbons of the *Chlorophyta* revealed the presence of saturated hydrocarbons that might have also been associated with exogenous coatings of the plants. For example, a specimen of *Cladophora* showed traces of *n*-alkanes from C_{19} to C_{33}, with a marked dominance of odd carbon chain lengths (CPI > 4) associated with terrigenous plant materials (Eglinton *et al.*, 1962; Eglinton and Hamilton, 1963; 1967; Riely *et al.*, 1991a and references therein). These compounds are characteristic of the intertidal muds of the area.

A number of *n*-C_{21} polyenes (RI 2031_{DB5}, 2037_{DB5} and 2044_{DB5}) were detected in the brown alga. The mass spectra of these hydrocarbons were consistent with polyunsaturated alkenes but molecular ions were absent. Comparison of RI values with those *n*-C_{21} polyenes identified in the *Chlorophyta* suggested that two of the components could be assigned as *n*-$C_{21:5}$; 2044_{DB5} and *n*-$C_{21:4}$; 2037_{DB5}. Previous reports of hydrocarbons in *Fucus distichus* (Youngblood *et al.*, 1971; Shaw and Wigg, 1979) recorded *n*-$C_{21:6}$ as an abundant component. Heneicosapentaene (*n*-$C_{21:5}$) was only reported as present in other related brown algae (*Agarum cribosum*, *Alaria* sp. and *Cymathere triplicata*).

No trace of C_{20} or C_{25} HBI hydrocarbons was detected in either endogenous or exogenous lipids from *Fucus distichus* (*Phaeophyta*).
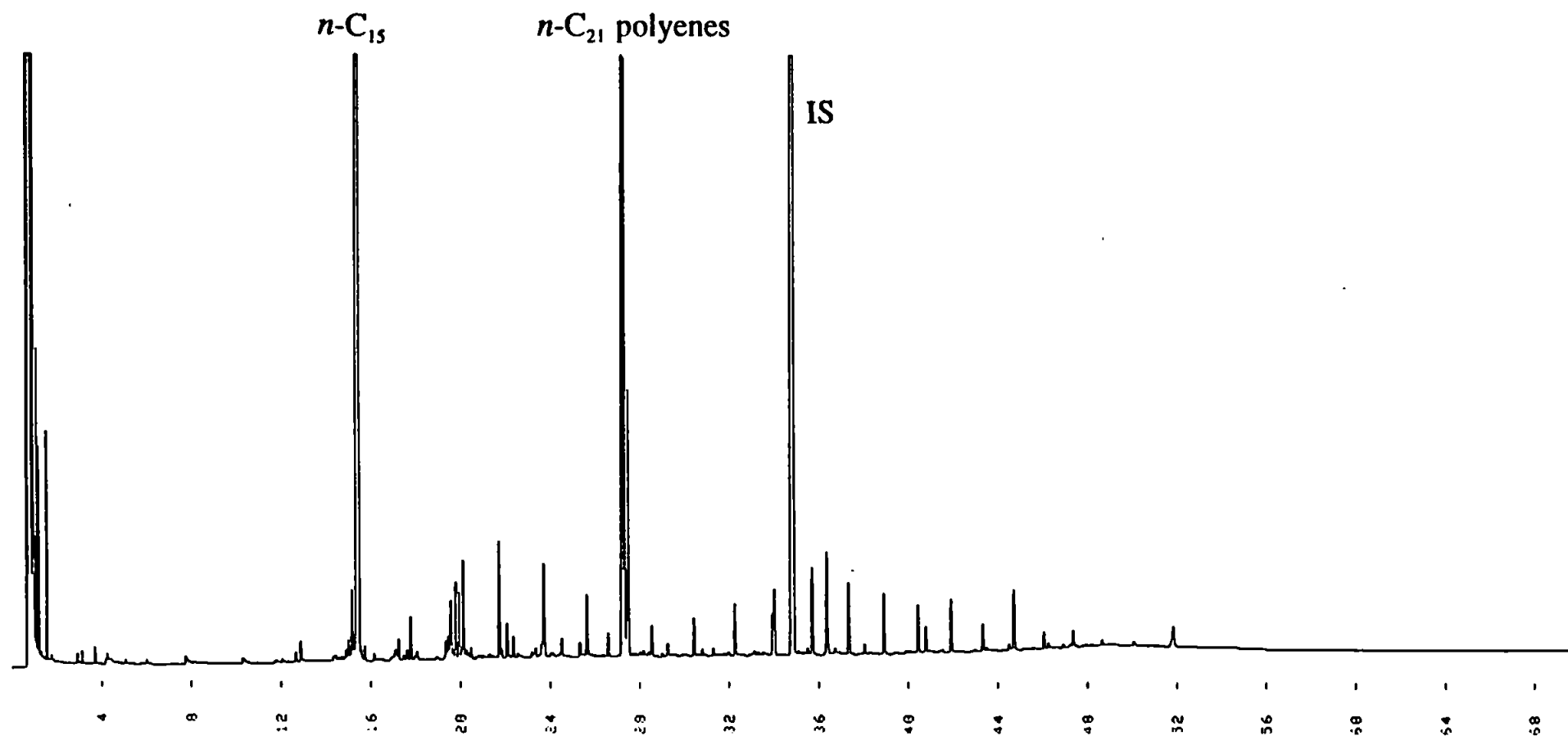
FIGURE 5.23 GAS CHROMATOGRAM OF ALIPHATIC HYDROCARBONS ISOLATED FROM *FUCUS* AT MILLBROOK IN OCTOBER, 1990

For conditions see text; DB5 (J&W).

5.6 ENDOGENOUS AND EXOGENOUS ALGAL HYDROCARBONS

Based upon previous reports of hydrocarbons in marine intertidal algae (Youngblood et al., 1971; Youngblood and Blumer, 1973; Lytle et al., 1979; Shaw and Wiggs, 1979), it was assumed that the principal hydrocarbons, n-pentadecane (n-C_{15}), n-heptadecane (n-C_{17}) and related n-alkenes (n-$C_{17:1}$ and n-$C_{17:2}$), detected in the lipids from the various macrophytic algae during the present study, are products of endogenous biosynthesis of the algae via decarboxylation of the corresponding fatty acid (McInnes et al., 1980). The scarcity of previous reports of the occurrence of HBI hydrocarbons in such algae and their ubiquitous presence in collections of *Chlorophyta* sampled during the summer period prompted the examination of hydrocarbons which appear to be exogenous in origin. To investigate the possibility that the HBI hydrocarbons detected may have been associated with exogenous coatings on the plant material, the water-washings of some of the plant collections were analyzed. In addition, tissue from the algal specimens and the detritus recovered from the original washing of the plant material with copious volumes of distilled water, were subjected to microscopic analysis.

Similarities were evident in the hydrocarbon distribution isolated from the algal detritus, a light brown floc, washed from *Enteromorpha* and both the algal tissue and surrounding sediment (Table 5.8; Figures 5.24-5.26). The chromatogram (Figure 5.24) of aliphatic hydrocarbons isolated from this algal debris contained the hydrocarbons n-pentadecane (n-C_{15}) and a n-heptadecene (n-$C_{17:1}$; 1674_{DB1}) of algal origin.

TABLE 5.8 CONCENTRATION OF HBI AND OTHER HYDROCARBONS ASSOCIATED WITH *ENTEROMORPHA* AND SURROUNDING SEDIMENT AT MILLBROOK, 1989 (mgkg⁻¹ dry weight)

Compound (RI)	*Enteromorpha* sp.	Algal debris	Sediment
n-C_{15}	92	+	0.51
n-$C_{17:2}$; 1659$_{DB1}$	8.0	+	nd
n-$C_{17:1}$; 1678$_{DB1}$	50	+	nd
n-$C_{17:1}$; 1686$_{DB1}$	nd	+	nd
n-$C_{17:1}$; 1690$_{DB1}$	9.1	+	1.2
n-C_{17}	19	nd	nd
br20:1; 1702$_{DB1}$	nm	+	4.2
br20:0; 1707$_{DB1}$	0.92	+	10
Σn-C_{21} polyenes	nd	+	1.1
br25:3; 2044$_{DB5}$	nd	+	2.1
br25:2; 2070$_{DB5}$	0.32	+	nd
br25:2; 2083$_{DB5}$	nd	+	nd
br25:3; 2089$_{DB5}$	0.49	+	2.4
br25:3; 2107$_{DB5}$	nd	+	1.1
br25:4; 2128$_{DB5}$	nd	nd	0.15
br25:2; 2140$_{DB5}$	nd	+	0.85

Key: + = detected, nd = not detected, nm = not measured
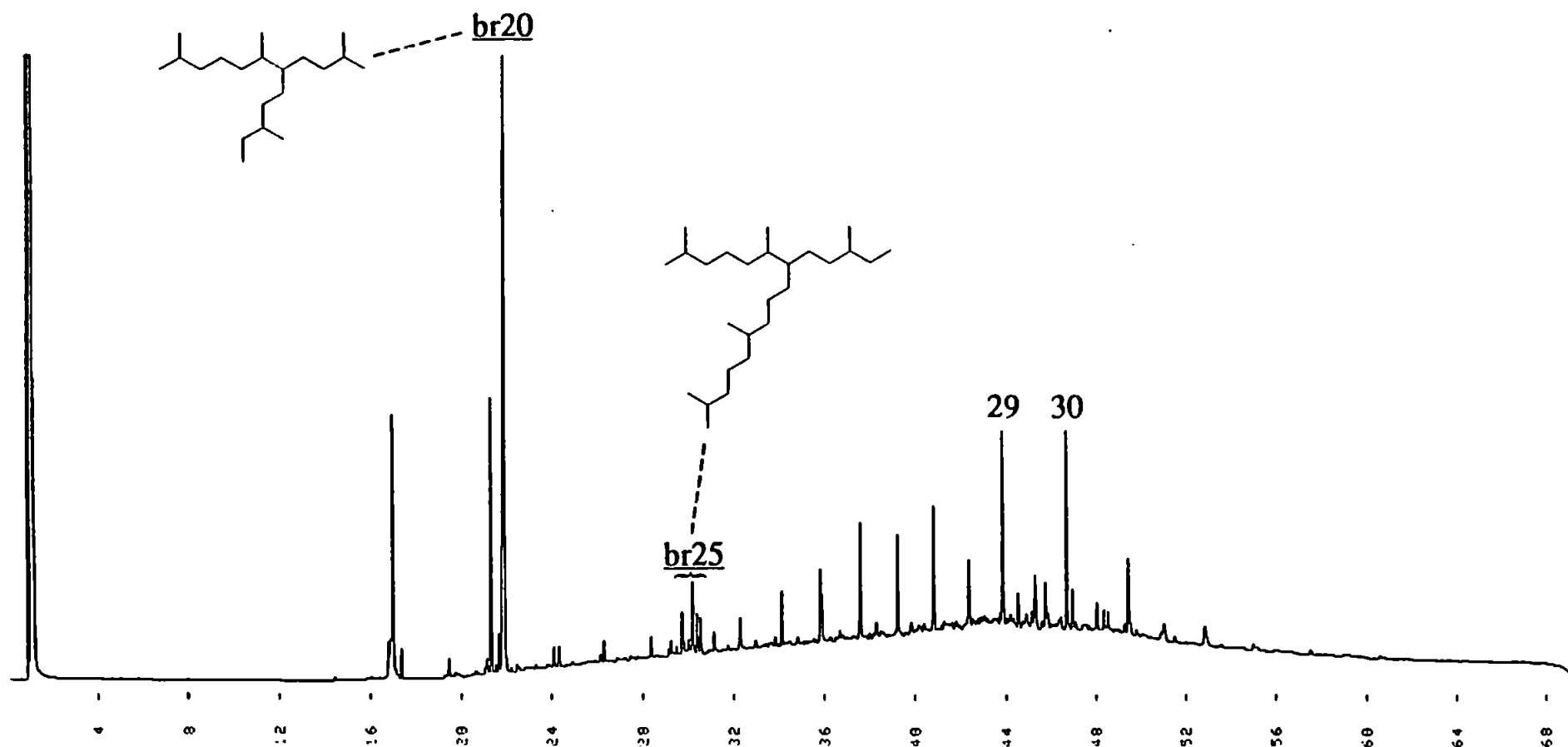
FIGURE 5.24   GAS CHROMATOGRAM OF ALIPHATIC HYDROCARBONS ISOLATED FROM DETRITUS WASHED FROM *ENTEROMORPHA*

For conditions see text; DB5 (J&W).

C20 HBI and other hydrocarbons, Millbrook, 1989
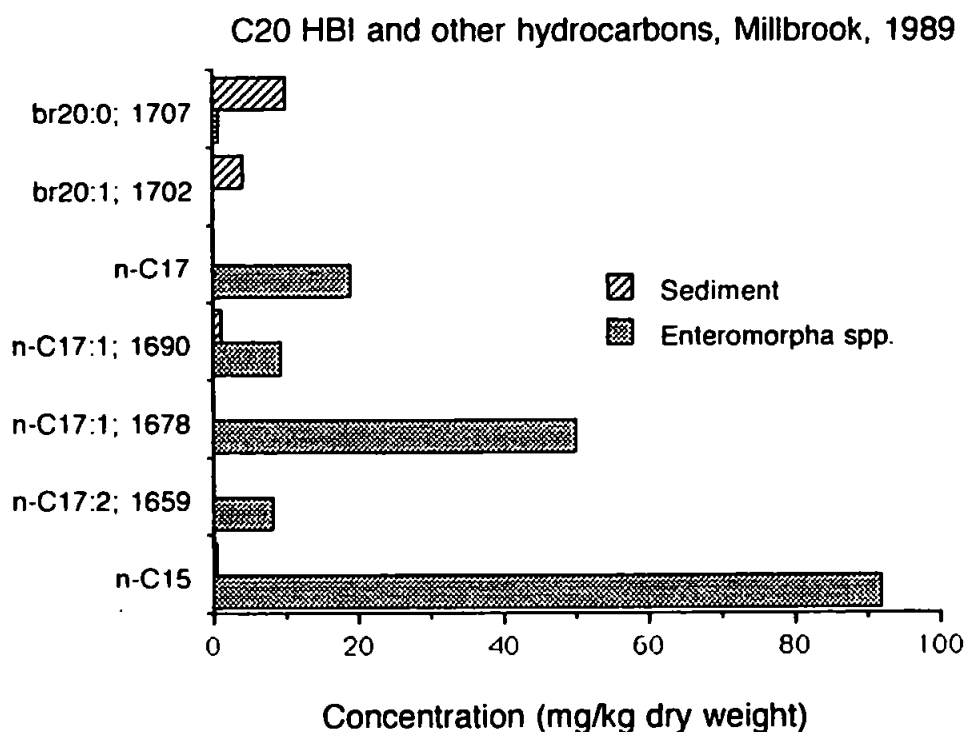
FIGURE 5.25   CONCENTRATION OF C_{20} HBI HYDROCARBONS ASSOCIATED WITH *ENTEROMORPHA* AT MILLBROOK, 1989

C25 HBI and n-C21 polyenes, Millbrook, 1989
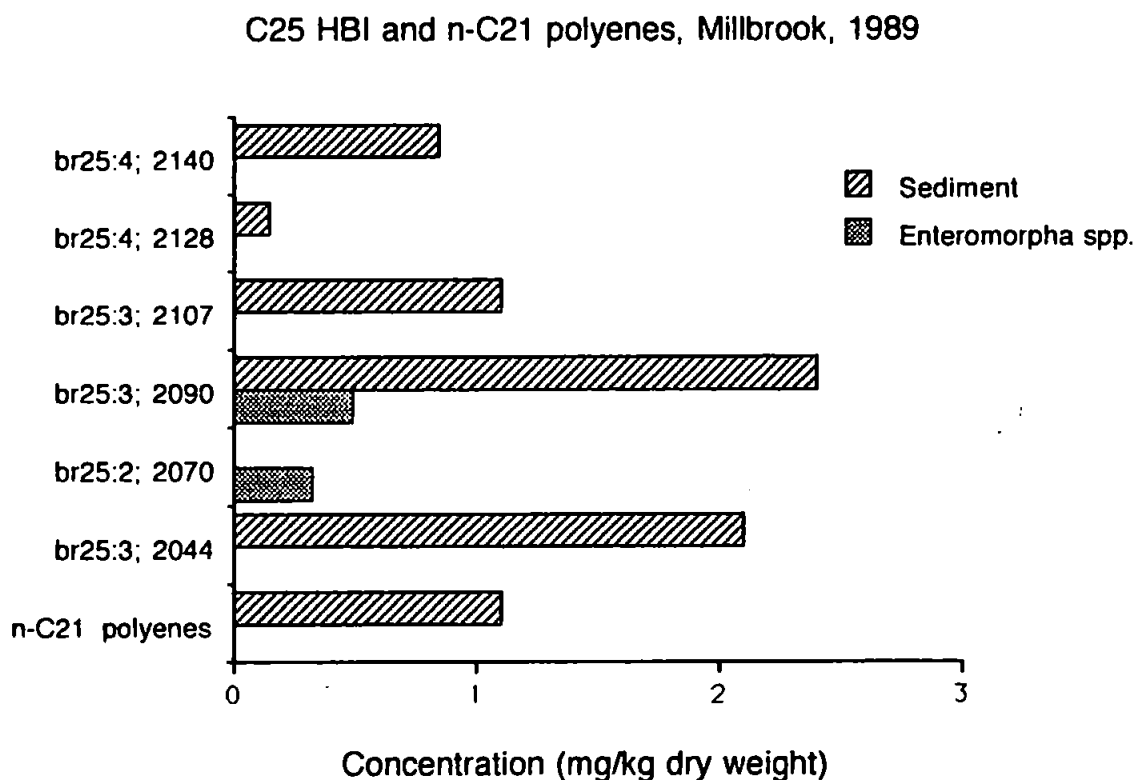
FIGURE 5.26   CONCENTRATION OF C_{25} HBI ALKENES ASSOCIATED WITH *ENTEROMORPHA* AT MILLBROOK, 1989

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However, in contrast to the algal lipids where the above hydrocarbons were principal components, the hydrocarbons of the detrital lipids were dominated by the C_{20} HBI alkane 1 as seen in the sediment[2]. Unlike the algal lipids but similar to the sediments, the detritus did not seem to contain n-C_{17} in significant quantity but the C_{20} HBI monoene 4 was identified in the algal debris. Some other n-C_{17} alkenes indigenous to algae (n-$C_{17:1}$; 1686_{DBI} and n-$C_{17:2}$; 1663_{DBI}) were also detected in the detrital sample.

The pattern of C_{25} HBI alkenes in the detrital hydrocarbons was more complex than that reported earlier for the source alga, *Enteromorpha*. Although compounds br25:2; 2070_{DB5} and br25:3; 2090_{DB5} indigenous to the alga were abundant, the co-occurrence of other components was apparent from examination of the chromatogram. The mass spectrum of RI 2043_{DB5} was not consistent with that of a n-C_{21} polyene. Indeed, no n-C_{21} polyenes (including n-$C_{21:6}$) were detected in either the macroalga or detritus. Microscopic examination of both algal tissue and detritus revealed a minimal contribution of diatom frustules or tests to the debris and a complete absence of diatoms living on the algal fronds.

The mass spectra and retention indices of RI 2043_{DB5}, 2090_{DB5}, 2107_{DB5} and 2140_{DB5} were consistent with those of the C_{25} HBI alkenes identified in Millbrook sediments during the present study. However, Figure 5.27 shows the mass spectrum of RI 2083_{DB5}, present solely in the detrital hydrocarbons. A molecular ion was evident at m/z 348 which suggested the diene br25:2; 2083_{DB5}. A hydrocarbon with almost identical mass spectrum (prominent fragment ions at m/z 207, 235, 266 and 320) and retention index has been reported not only in sediments worldwide

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[2]Quantification of hydrocarbons in the algal debris was not performed because of the difficulty in weighing the small amount of detritus available.
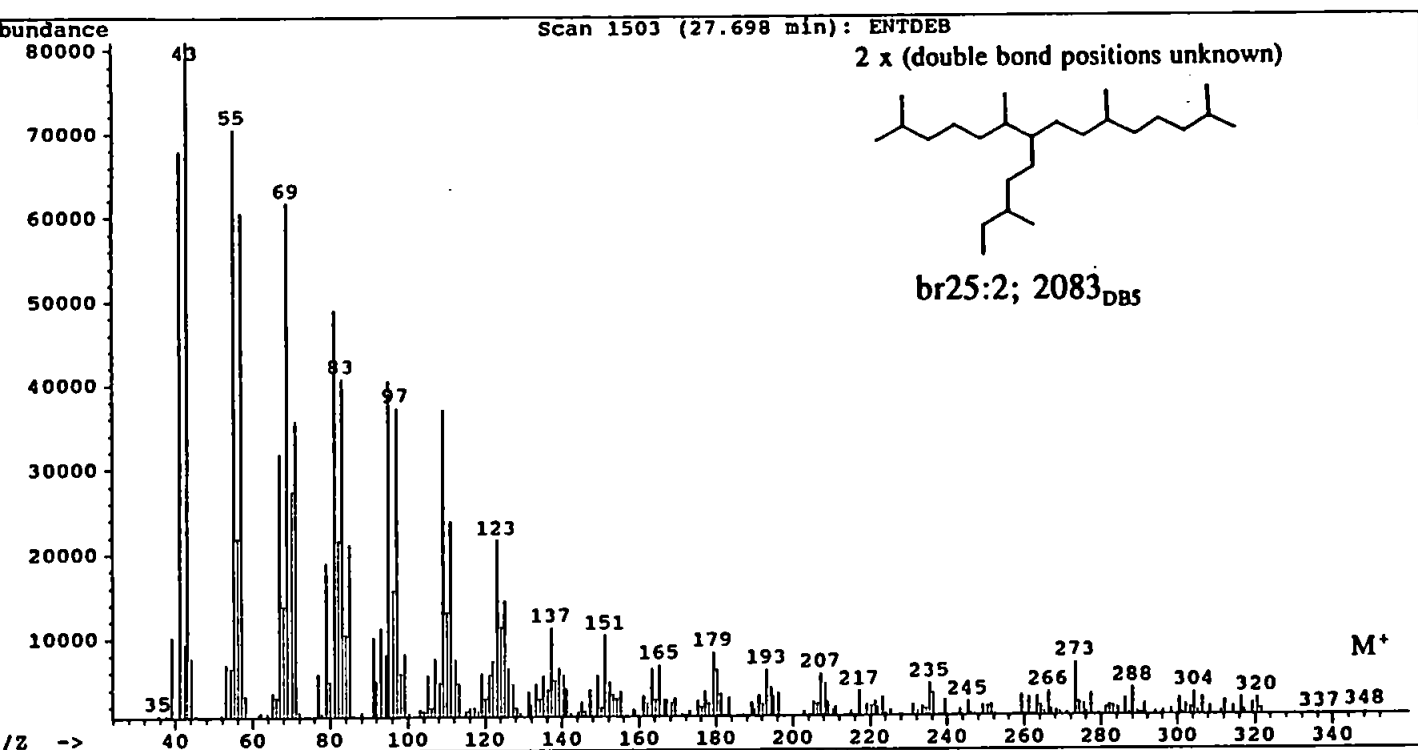
FIGURE 5.27   MASS SPECTRUM OF br25:2; 2083$_{DBS}$ ISOLATED FROM DETRITUS WASHED FROM *ENTEROMORPHA*

Conditions: HP 5970 MSD; *m/z* 35-400, 1.5 scan/sec.

(*e.g.* Requejo and Quinn, 1983a; see Rowland and Robson, 1990) but also in the *Enteromorpha prolifera* examined by Rowland *et al.* (1985). The absence of br25:2; 2083_{DBs} in specimens of *Enteromorpha* spp. examined during the present study, and the occurrence in a detrital sample suggested that its occurrence may not be derived from endogenous algal biosynthesis. Moreover, it could be argued that all the C_{20} and C_{25} HBI hydrocarbons detected in the algae and related samples were not indigenous to the algae but were isolated from detrital matter bound in some way to the algal tissue (fronds) which had escaped complete removal by water washing. Brown (1970) demonstrated the steady removal of epiphytes from a plant of the genus *Equisetum* during three water washings. Although a large amount of *Oedogonium* and *Bulbochaete* was removed by washing, an even greater amount was firmly attached which was removed only by a final scraping with a scalpel.

The ecology of the epiphyton of macroalgae can be very complex. The diatom population structure of a salt-marsh epiphytic community growing on *Enteromorpha intestinalis* was studied by Lee *et al.* (1975) throughout one summer season. A total of 218 species of varieties were recognised. The distribution of many species in the community was found to be seasonal and possible nutritional relationship between *Enteromorpha* and its epiphytes was established. Chudyba (1965; 1968) found a total of 220 species of epiphytic algae on *Cladophora glomerata* in a river and of these 176 were diatoms.

Lipids themselves may collect on the surfaces of algal tissue. Pavoni *et al.* (1990) elucidated that algal fronds are in part constituted by spongy materials which act as lypophile traps. In contrast, macrophytic algae themselves have been shown to release large amounts of photoassimilated but as of yet largely uncharacterised carbon

onto external membranes and into the surrounding environment (Rashid and Prakash, 1972 and references therein; Bell *et al.*, 1974; Jensen and Sønderyaard, 1982; Zutic *et al.*, 1981; Hoyer *et al.*, 1985). Perhaps this mucilage might contain hydrocarbons as well as some lipid-soluble vitamins, quinones and steroids present in the exudate from *Enteromorpha*. Lee *et al.* (1975) concluded that many organic substrates including the *sic* n-C_{17} cyclopropane, later suggested by Rowland *et al.* (1985) to be a C_{20} HBI alkene, were recycled amongst the members of the epiphytic community growing on *Enteromorpha*. The role of this mucilage extruded on the surface of the algae would seem to be either as a substrate to promote the growth of symbiotic epiphytes or as an anti-bacteria/fungal agent.

The growth of bacteria on algae is certainly very common, if not universal, on macroscopic genera, but very little is known of the ecology of these associations. All natural populations of these algae have epiphytic bacteria associated with their surfaces either in a casual manner degrading dead and excreted material or as specialised symbiotic epiphytes (Round, 1984). For example, the filamentous genus *Leucothrix mucor* seems to have a worldwide distribution on marine algae (Johnson *et al.*, 1971). The bacterial population on the surface of the algae is likely to be more concentrated than in the surrounding water.

Re-examination of the chromatograms of hydrocarbons isolated from the algae collected during this study revealed components other than endogenous products of algal biosynthesis. For example, Figure 5.28 shows the hydrocarbons isolated from *Enteromorpha* sp. which exhibits a n-alkane distribution ($> C_{20}$) with an even carbon
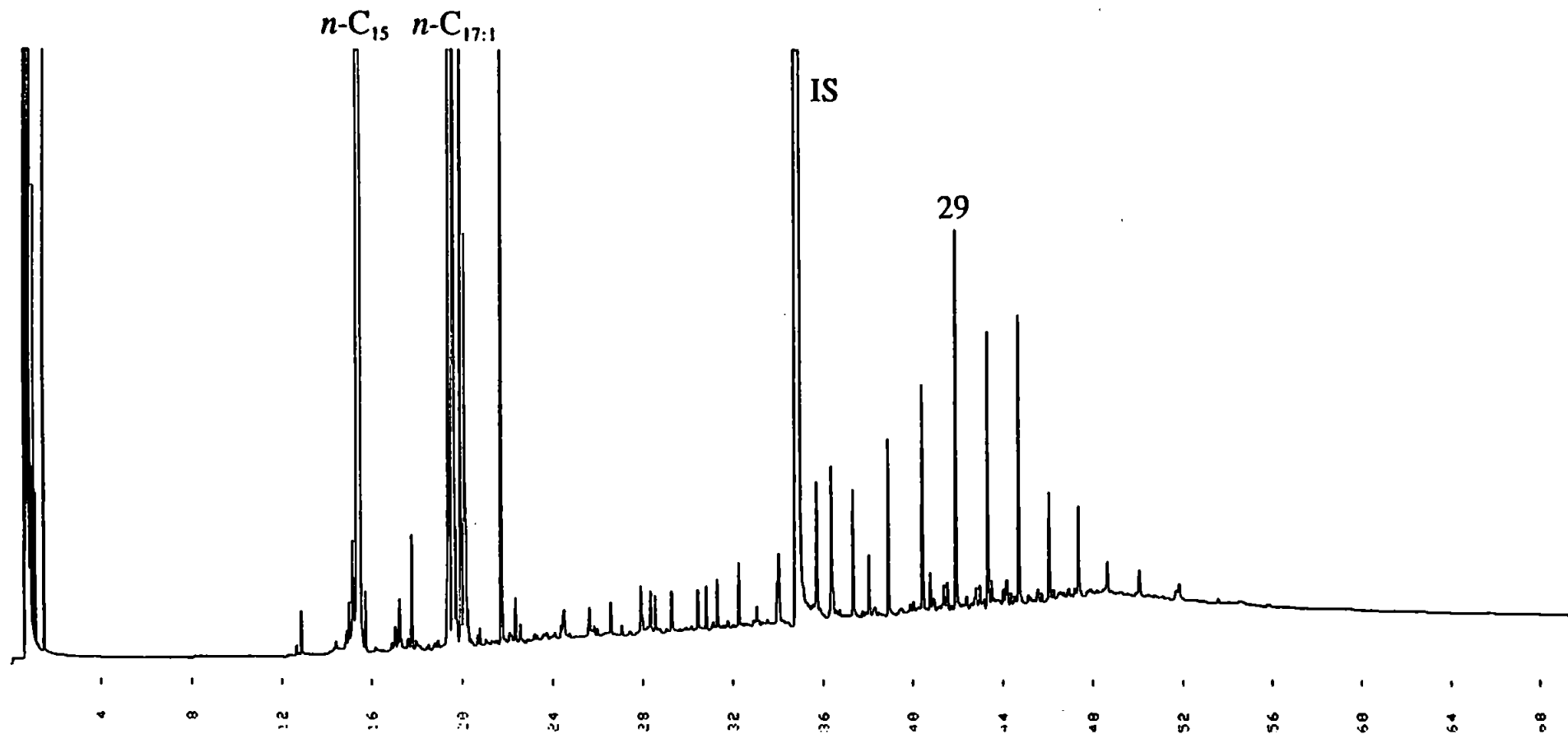
FIGURE 5.28   GAS CHROMATOGRAM OF ALIPHATIC HYDROCARBONS ISOLATED FROM *ENTEROMORPHA* AT MILLBROOK, 1989

For conditions see text; DB5 (J&W).

number preference (CPI=0.75; n-C_{20}-C_{34}) which may have resulted from microbial decomposition of autochthonous material or from a direct bacterial input. Distributions of n-alkanes showing no odd-even predominance and even carbon number preference over a wide range in molecular weight have been reported to occur in certain bacteria (Davis, 1968; Han and Calvin, 1969; Albro and Dittmer, 1970; Albro, 1976). Although distributions of n-alkanes similar to that observed in Figure 5.28, attributed to a microbial origin, have been reported previously in salt-marsh sediment (Johnson and Calder, 1973), such a distribution was not characteristic of the intertidal muds of the area studied herein which were dominated by vascular plants. Cranwell (1976) showed that such a distribution of n-alkanes was only observed in the "bound" fraction of algal detritus unlike the solvent-extractable n-alkanes, which, in blue-green algal detritus, consisted almost entirely of heptadecane.

5.7   DECOMPOSED MACROALGAL MAT

Since the detritus formed by microbial attack on algal populations has been shown to constitute a large part of the input of autochthonous material to sedimentary organic matter (*e.g.* Cranwell, 1976; Khandekar and Johns, 1990ab), a sample of flora collected from Millbrook intertidal sediment was allowed to decompose aerobically. The aim of this study was to assess whether the process of microbial decomposition of algal detritus might be an important source of the HBI alkenes detected in the algae and sediments at that site. In addition to the possible diagenetic modification of algal organic matter, it has been proposed (Requejo, 1983; Requejo and Quinn, 1983a; Requejo *et al.*, 1984) that HBI alkenes may be produced

by *de novo* synthesis by microorganisms although Requejo and Quinn (1983b) were unable to induce synthesis during anaerobic laboratory decompositions of either algal or vascular plants.

In the present study, algal material, composed largely of *Cladophora* sp. was allowed to decompose open to the atmosphere and light for twelve months, a period after which immediate microbial degradation had previously been shown to be almost completed during a similar study (Fukushima *et al.*, 1982). During the incubation the structure of the plant completely disintegrated to give a green residue in the form of a disc approximately 10 mm deep. A rust-coloured crust and white patches were observed on the surface of the decomposed mat which presumably indicated the presence of photosynthetic bacteria (Fenchel and Staarup, 1970; Hirsh, 1977ab; Liebezeit *et al.*, 1991). A chromatogram of hydrocarbons isolated from the decomposed mat is shown in Figure 5.29. Little change from the original alga was observed in the distribution of hydrocarbons (Table 5.9; Figures 5.30 and 5.31) except that a series of phytenes (*e.g.* i-20:1; 1843_{DBI}), phytadienes (*e.g.* i-20:2; 1835_{DBI}) and n-alkenes (*e.g.* n-$C_{18:1}$; 1787_{DBI}), identified from their mass spectra, were evident in the decomposed sample. The isoprenoid compounds are thought to be derived from diagenesis of chlorophyll. Although present in the lipids of methanogenic bacteria (*e.g.* Risatti *et al.*, 1986), such a source is considered unlikely in this case. An alternative explanation is that phytol-derived isoprenoids were released from chlorophyll by the extensive saponification procedure required to breakdown the decomposed mat for solvent extraction. No further structural characterisation of this series was attempted and the identity of some components remains uncertain.

TABLE 5.9 CONCENTRATION OF HBI AND OTHER HYDROCARBONS IN ALGAL MATS ($mgkg^{-1}$ dry weight)

Compound (RI)	Original mat	Decayed mat
n-$C_{17:2}$; 1673_{DB1}	1.1	3.2
n-$C_{17:1}$; 1680_{DB1}	140	204
n-$C_{17:2}$; 1684_{DB1} + n-$C_{17:1}$; 1684_{DB1}	15	tr
n-$C_{17:1}$; 1692_{DB1}	2.6	tr
n-$C_{17:1}$; 1697_{DB1}	nd	12
n-C_{17}	3.5	13
br20:1; 1702_{DB1}	nm	nm
br20:0; 1707_{DB1}	1.8	2.5
n-$C_{17:1}$; 1710_{DB1}	nd	0.43
Σn-C_{21} polyenes	6.3	nd
br25:3; 2042_{DB5}	nd	0.09
br25:2; 2083_{DB5}	nd	0.12
br25:3; 2090_{DB5}	0.25	0.26
br25:2; 2092_{DB5}	0.13	0.38
br25:3; 2107_{DB5}	0.59	0.36
br25:2; 2140_{DB5}	1.2	0.82
br25:3; 2158_{DB5}	0.29	nd
br25:5; 2182_{DB5}	0.50	nd
br25:2; 2186_{DB5}	0.40	nd

Key: tr = trace, nd = not detected, nm = not measured
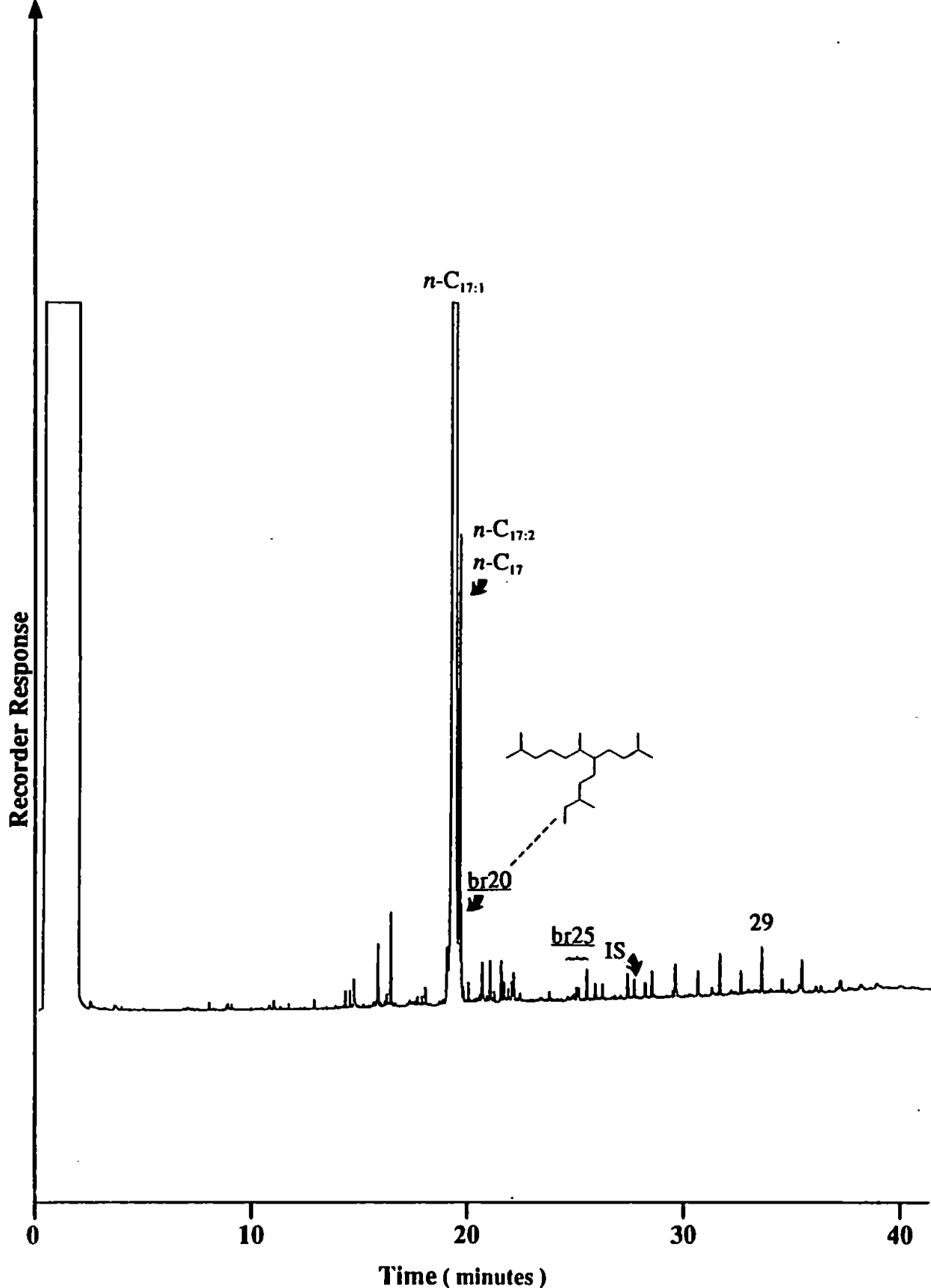
FIGURE 5.29 GAS CHROMATOGRAM OF ALIPHATIC HYDROCARBONS ISOLATED FROM LABORATORY-DECAYED ALGAL MAT

GC conditions: Carlo Erba 4160, 25m x 0.32mm i.d. Rt$_x$-1 (Restek), 40-340°C @ 8°Cmin^{-1}, He carrier gas, internal standard 2,21-dimethyldocosane (20 μg).

The chromatogram (Figure 5.29) was again dominated by the presence of a

n-heptadecene (n-$C_{17:1}$; 1680_{DBI}), n-pentadecane (n-C_{15}), n-heptadecane (n-C_{17}) and

related dienes (n-$C_{17:2}$; 1673_{DBI} and n-$C_{17:2}$; 1697_{DBI}) indigenous to mat and sediment.

The C_{20} HBI alkane 1 and related monoene 4 were detected in both fresh and

laboratory decomposed algal mats. The concentration of n-C_{17} increased relative to

the C_{20} HBI alkane br20:0; 1707_{DBI} in the decomposed mat (Table 5.9; Figure 5.30).

Examination of the chromatogram of algal hydrocarbons after degradation

revealed little qualitative change in C_{25} HBI alkene distribution although the

concentrations had been reduced over the period of decay. In contrast, no trace of any

n-C_{21} polyenes which were abundant in the original mat (Σ n-C_{21} polyenes; 6.3 μgg^{-1}

dry weight) was detected, consistent with their rapid degradation under oxic

conditions. The C_{25} HBI hydrocarbons br25:3; 2090_{DB5}, br25:3; 2108_{DB5} and br25:2;

2140_{DB5} remained the principal components. The absence of n-C_{21} polyenes facilitated

the identification of another HBI alkene at RI 2040_{DB5} the mass spectrum of which

exhibited a molecular ion at m/z 346 and prominent fragment ions at m/z 223 and m/z

261, which was assigned as the triene br25:3; 2040_{DB5}, presumably an isomer of

br25:3; 2090_{DB5} also detected in the decomposed mat.

No difference in degradation rates between C_{25} HBI alkene isomers was

observed during degradation of the mat as was reported by Robson and Rowland

(1988b) and Robson (1987) for a mixture of isomeric C_{25} HBI monoenes.

Little change from the original alga was observed in the distribution of

hydrocarbons as was recorded by Fukushima *et al.* (1982; 1987) during incubation

experiments using submerged plants (*Hydrilla verticillata* and *Myriophyllum*

spicatum). No production of novel isomers either by *de novo* biosynthesis or

C20 HBI and n-C17 hydrocarbons in algal mats
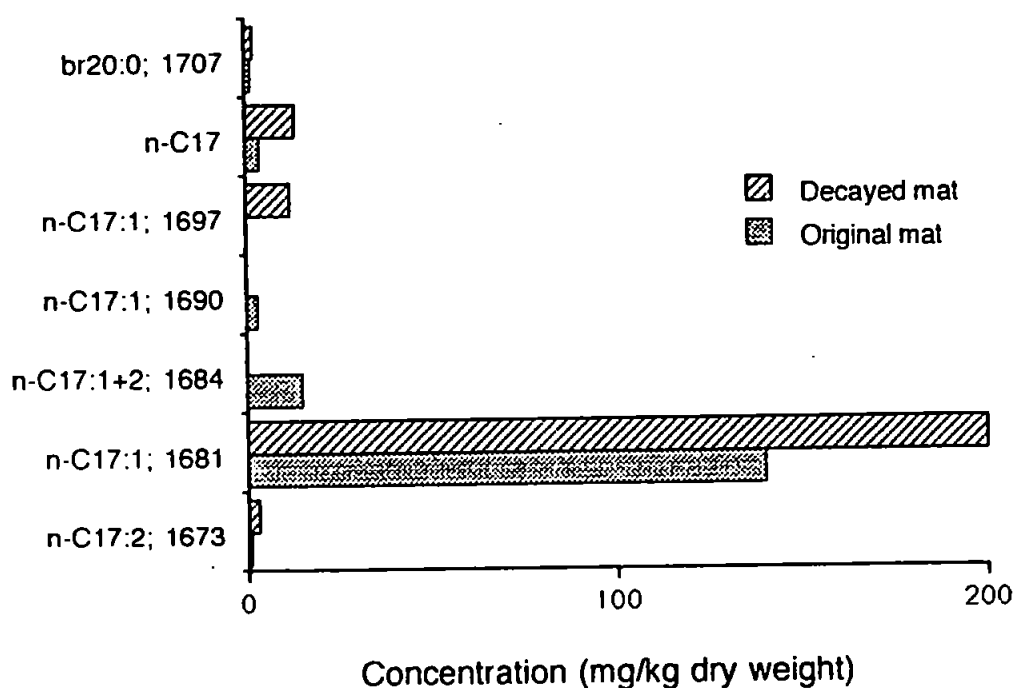
FIGURE 5.30   CONCENTRATION OF C_{20} HBI HYDROCARBONS IN
CLADOPHORA MATS

C25 HBI alkenes in algal mats
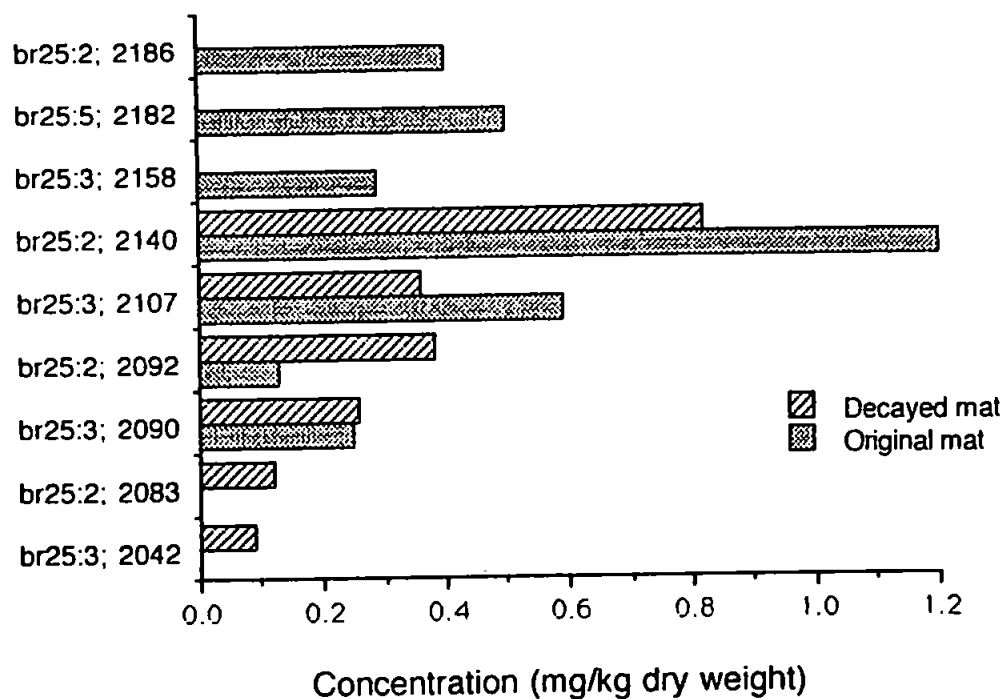
FIGURE 5.31   CONCENTRATION OF C_{25} HBI ALKENES IN
CLADOPHORA MATS

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diagenetic change of original isomers present in the *Cladophora* mat was observed during the present study. In contrast to Requejo and Quinn (1983b), a series of isomeric *n*-alkadienes (*n*-nonadecadienes and *n*-heneicosadienes) were not observed. Their formation during the decomposition of a *Cladophora* mat mixed with sediment was presumably limited to the anaerobic conditions employed by Requejo and Quinn (1983b). Patches of purple photosynthetic bacteria (genus unknown; possibly sulphurbacteria) were observed at the illuminated sides of the beaker and surface of the mat during decomposition as indicated by the rust-coloured crust and white patches on the surface of the mat. Photosynthetic bacteria are known to move in response to light as well as chemical gradients (Pfenning, 1967; Hirsh, 1977b). The presence of such bacteria was also tentatively recorded by microscopic examination of algal material sampled during decomposition. Even aerobic photosynthetic bacteria prefer reducing conditions and as photosynthetic bacteria are unable to use water as the source of hydrogen for photosynthesis, sulphur bacteria use H_2S whereas non-sulphur bacteria utilise an organic substrate under anaerobic conditions. These organisms are therefore, in many cases restricted to below the surface of the mat.

Robson and Rowland (1988b) showed that the C_{20} HBI alkane was resistant to degradation by the aerobe *Pseudomonas aeruginosa* under conditions were the corresponding *n*-alkane (*n*-eicosane) was rapidly degraded. Hence a relative enrichment of the C_{20} HBI alkane in the decayed algal mat would be expected. However, as *n*-heptadecane (*n*-C_{17}) has also been reported as a principal component in the lipids of some bacteria (*e.g. Vibrio marinus*; Oró *et al.*, 1967), the increase in concentration of *n*-C_{17} relative to the C_{20} HBI alkane could be attributed to a bacterial input of the *n*-alkane.

Contributions of hydrocarbons characteristic of bacteria, other than the ubiquitous n-C_{17}, were either not detected or minor relative to the amount originally present. The failure to discern a distinct bacterial contribution to the hydrocarbons of the decayed matter could have been due to an initial abundance of algal hydrocarbons, which obscured the small bacterial component. Alternatively, the failure could have arisen because the only significant bacterial contribution was to the n-C_{17} alkane already present in the alga (Cranwell, 1976). Some algal constituents may be sufficiently stable to microbial attack to act as markers of algal input to sediments. For example, the faster rate of aerobic degradation of n-heptadec-1-ene (n-$C_{17:1}$) than the HBI alkanes and monoenes was suggested by Robson and Rowland (1988b) to explain the lack of abundance of n-$C_{17:1}$ in sediments relative to the HBI hydrocarbons even though n-$C_{17:1}$ is a major hydrocarbon in algae. In the present study, little difference in rates of degradation between algal n-C_{17} monoenes and HBI hydrocarbons was observed.

However, the inability to induce HBI alkene synthesis during anaerobic and aerobic laboratory decompositions of algal and vascular plants, in conjunction with the failure of other researchers to detect HBI hydrocarbons among lipids of bacterial genera examined (see the reviews by Albro, 1976; Nes and Nes, 1980; Tornabene, 1981: other studies by Davis, 1968; Han and Calvin, 1969; Oró et al., 1967; Albro and Dittmer, 1970; Lechevalier, 1977; Holzer et al., 1979; Goldfine, 1982; Langworthy, 1982; Taylor, 1984; Gossens et al., 1986; 1989ab; Sheia et al., 1991), leaves the exact origin of these compounds uncertain. Studies of the lipid composition of cyanobacteria (blue-green algae; Cyanophyta) and related microbial mats (Robinson and Eglinton, 1990; Sheia et al., 1990; 1991 and references therein) have shown HBI

hydrocarbons to be absent from all samples examined to date even though monomethyl-, dimethyl- and multibranched alkanes have been identified.

5.8 EPIPELIC DIATOMS ISOLATED FROM THE SEDIMENT AT CARGREEN (AUGUST, 1990)

Epipelic diatoms were harvested by laying lens tissue on surface sediment, allowing them to migrate up through it, and then removing the tissue from the sediment (Eaton and Moss, 1966; Palmer and Round, 1967; Thompson and Eglinton, 1976; 1979). The species of diatoms found in the samples, which were fairly typical of an unpolluted, brackish-water site, consisted of 25 taxa dominated by the genus *Navicula* (*N. gregaria*, *N. phyllepta* and *N. salinarum*). The Cargreen site was void of all macrophytes of the genus *Chlorophyta*, such as *Enteromorpha* spp. throughout the year although sparse colonies of *Fucus* sp. were present during autumn.

The hydrocarbon distribution of the epipelic diatoms isolated from the sediment at Cargreen, in August 1990, is shown in Figure 5.32. The sedimentary hydrocarbons at the same site are given in same figure for comparison. The hydrocarbons isolated from the mixed epipelic community were dominated by n-C_{21} polyenes (RI 2040_{DB5}, 2045_{DB5}, 2050_{DB5}; 2026_{DB1}) with minor contributions from n-heptadecane, related monoenes (n-$C_{17:1}$; 1675_{DB1} and n-$C_{17:1}$; 1695_{DB1}) and the C_{20} HBI alkane 1 and related monoene 4, br20:1; 1702_{DB1}. The chromatogram also exhibited an n-alkane distribution ($> C_{20}$) with little carbon number preference (CPI=1.5), as observed for the hydrocarbons of some macroalga, which may have resulted from microbial decomposition of the microalgal autochthonous material. Distributions of n-alkanes showing no odd-even predominance over a wide range in

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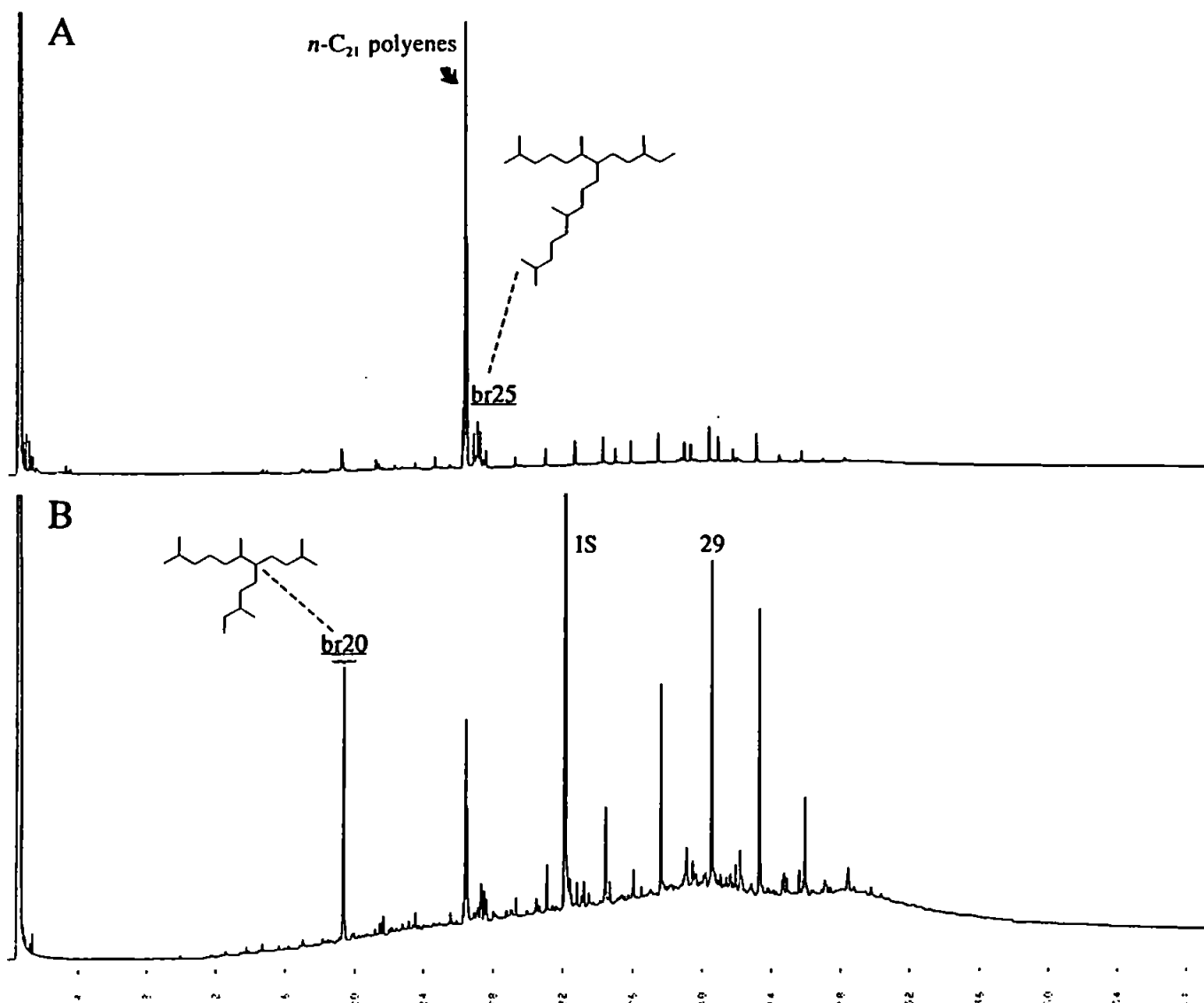
FIGURE 5.32   GAS CHROMATOGRAMS OF ALIPHATIC
HYDROCARBONS ISOLATED FROM
(A) Epipelic diatoms (mainly *Navicula* spp.)
(B) Surrounding sediment at Cargreen in August, 1990
For conditions see text; DB5 (J&W).

molecular weight have been reported to occur in certain bacteria (Davis, 1968; Oró *et al.*, 1967; Han and Calvin, 1969; Albro and Dittmer, 1970; Albro, 1976; Tornabene, 1981; Sheia *et al.*, 1991).

The distributions of C_{25} HBI alkenes in the isolated epipelic microalgae comprised two trienes (br25:3; 2090_{DBS} and br25:3; 2108_{DBS}), one diene (br25:2; 2082_{DBS}) and compound RI 2072_{DBS} (Table 5.10). Examination of the mass spectrum (Figure 5.33) of RI 2072_{DBS} in the algal hydrocarbons revealed a molecular ion at *m/z* 350 and prominent fragment ions at *m/z* 196, 210, 224, 266 and 280 which suggested a C_{25} HBI monoene br25:1; 2072_{DBS}. Isolation and elucidation of synthetic alkenes in the present study (Chapter 3) and comparison of the retention indices on two GC stationary phases (2076_{DB1} and 2072_{DBS}), has reduced the possible positions of the double bond in this monoene to an *E* or *Z* isomer of **8** or **9**. However, a more rigorous assignment must still await isolation and ozonolysis of the algal alkene. Another C_{25} HBI monoene br25:1; 2087_{DBS}, detected in microalgae isolated from *Cladophora* (**5.4.2**), was also tentatively assigned **8** or **9**.

TABLE 5.10 CONCENTRATION AND DISTRIBUTION OF HBI AND OTHER HYDROCARBONS IN CARGREEN SEDIMENT AND ASSOCIATED EPIPELIC DIATOMS (AUGUST, 1990) (mgkg⁻¹ dry weight)

Compound (RI)	Sediment	Epipelic microalgae
n-C$_{17:1}$; 1675$_{DB1}$	nd	+
n-C$_{17:1}$; 1695$_{DB1}$	nd	+
n-C$_{17}$	nm	+
br20:1; 1702$_{DB1}$	0.98	+
br20:0; 1707$_{DB1}$	4.2 ·	+
Σn-C$_{21}$ polyenes	5.3	+
br25:3; 2044$_{DB5}$	0.32	nm
br25:2; 2070$_{DB5}$	0.15	(br25:1; 2072$_{DB5}$)
br25:2; 2083$_{DB5}$	0.21	+
br25:3; 2090$_{DB5}$	1.1	+
br25:3; 2107$_{DB5}$	0.32	+
br25:4; 2128$_{DB5}$	0.11	nd
br25:4; 2175$_{DB5}$	0.12	nd
br25:5; 2182$_{DB5}$	0.10	nd

Key: nd = not detected, + = detected, nm = not measured

Exact Nominal Multiplet Ref / Lock Exc / Half Significant Saturated
DS90 SJH3S1.701 RT= 25:12 +EI SLRP 4-Jul-92 14:52 Sub
TIC= 616736 100%= 52992 EPI (AUG90) (921)
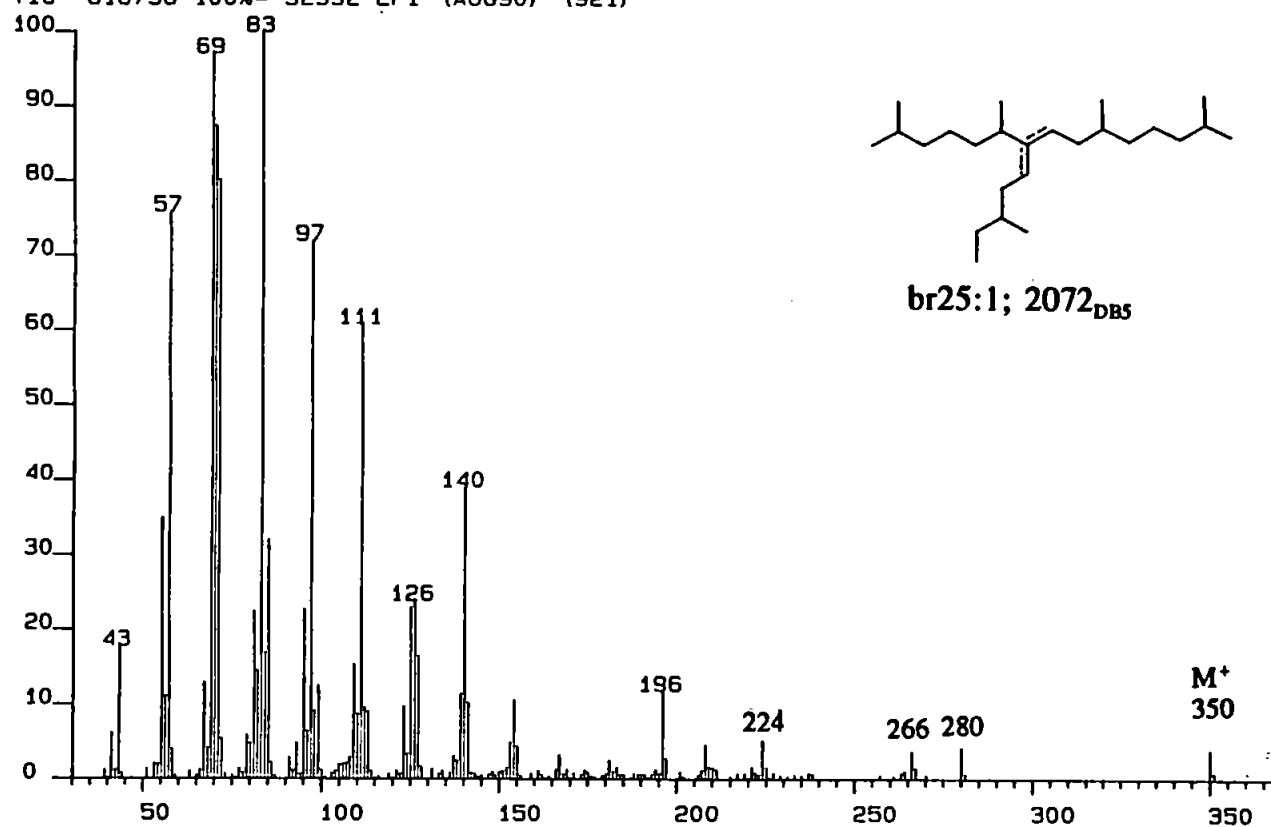
br25:1; 2072$_{DB5}$

FIGURE 5.33   MASS SPECTRUM OF br25:1; 2072$_{DB5}$ ISOLATED FROM EPIPELIC DIATOMS AT CARGREEN, 1990

Conditions: HP 5970 MSD; *m/z* 35-400, 1.5 scan/sec.

5.9 HBI HYDROCARBONS IN ALGAE: DISCUSSION

This is the first reported occurrence of the C_{20} HBI alkane and related monoene br20:1; 1702_{DBI} isolated from the green macroalgae of the genera *Cladophora* and *Ulva*. Previously only linear saturated and unsaturated hydrocarbons mainly n-C_{17} monoenes (*e.g. Cladophora*; Requejo and Quinn, 1983b) have been detected. Only Rowland *et al.* (1985) have reported the occurrence of these C_{20} HBI hydrocarbons in macroalgae in two field-collected specimens of the green alga *Enteromorpha prolifera*. The latter specimens also contained heneicosahexaene (n-$C_{21:6}$; 2048_{OVI}), an alkene associated with diatoms, so, as Rowland and Robson (1990) indicated, epiphytic diatoms may also have been present, as well as naturally occurring bacteria. These authors also reported that the analytical data recorded by Youngblood *et al.* (1971) for a compound tentatively identified as a C_{17} cyclopropane could also be interpreted to be due to a C_{20} HBI alkene with the carbon skeleton 1. The double bond in this alkene must have been either in a position resistant to hydrogenation or the mild conditions used were not sufficient to reduce the compound. Rowland *et al.* (1990) discussed the problems of assignment of the cyclic/acyclic nature of hydrocarbons based upon their hydrogenation behaviour.

Reports of the occurrence of C_{25} HBI hydrocarbons in algae are limited to two related dienes, br25:2; 2082_{OVI} in the green alga *Enteromorpha prolifera* (Rowland *et al.*, 1985) and br25:2; 2088_{MS} in natural populations of mixed diatom-dominated sea-ice communities (Nichols *et al.*, 1988).

Although the distribution of hydrocarbons in macroalgae, including HBI alkenes, may reflect phylogenetic relationships, as demonstrated by the qualitative similarities in C_{20} and C_{25} HBI alkene distributions displayed by the various

collections of *Chlorophytae* examined (Tables 5.6 and 5.7), intraspecific variation largely obscured any chemotaxonomic relationships which may exist. The primary cause of this variation is probably differences in plant age or vigour. Young rapidly growing plants and tissues have been shown to have higher hydrocarbon concentrations than mature ones (Youngblood and Blumer, 1973). During their growth period macrophytes constitute a temporary storage system. The source of interspecific qualitative differences may also be derived from either the presence of epiphytes (microalgae or bacteria) or poor sampling of field specimens as differences in other hydrocarbon distributions have been observed in morphologically different parts of the plant (Youngblood *et al.*, 1971; Youngblood and Blumer, 1973). The limited number of species analysed here, together with the difficulty in excluding exogenous hydrocarbons from microbial and detrital sources, and the dependence of hydrocarbon composition upon age and morphology of the algae subjected to the analysis, precludes the use of HBI hydrocarbons detected in macroalgae as biological markers of distinct algal species.

Saturated and unsaturated n-C_{17} hydrocarbons exist in a variety of marine and freshwater algae, including greens (*Chlorophyta*), browns (*Phaetophyta*), diatoms (*Bacillariophyta*) and other phytoplankton (Calvin and Han, 1969; Gelpi *et al.*, 1970; Blumer *et al.*, 1971; Lee and Loeblich, 1971; Youngblood *et al.*, 1971; Lytle *et al.*, 1979; Shaw and Wiggs, 1979; Tornabene *et al.*, 1980). A number of polyunsaturated n-C_{21} (polyenes) hydrocarbons, with from two to six double bonds were detected in the samples of macroalgae collected at the Millbrook site, including in the lipids of *Cladophora*. Some authors (*e.g.* Nichols *et al.*, 1988) have interpreted the presence

of such n-C_{21} polyenes, especially heneicosahexaene (n-$C_{21:6}$), in hydrocarbons isolated from macroalgae, as an indication of the presence of epiphytic diatoms. However, it is important to note that the occurrence of n-$C_{21:6}$ is not restricted to diatoms but has been reported in other microalgae and macrophytes (*e.g.* Tornabene *et al.*, 1980; green algae [*Chlorophyta*], diatoms [*Bacillariophyta*], golden algae [*Chrysophyta*], dinoflagellates [*Pyrrhophyta*] and coccolithophorides [*Prymnesiophyta*]), albeit at lower concentrations. For example, Lee and Loeblich (1971) showed that in diatoms (*e.g.* *Chaetoceros curvisetus*), the hydrocarbon n-$C_{21:6}$ accounted for 1-15% of the lipid whereas it accounted for less than 1% of the lipids of dinoflagellates (*e.g.* *Peridinium sociale*; Ackman *et al.*, 1968; Lee and Loeblich, 1971). In contrast, the lipids of nonphotosynthetic diatoms, cyanobacteria (blue green algae; *Cyanophyta*) and most photosynthetic bacteria, all prokaryotic organisms, contain no n-$C_{21:6}$.

5.10   SEASONAL VARIATION IN ABUNDANCE OF HBI HYDROCARBONS IN SEDIMENTS AT CARGREEN (1989-1990)

Having determined that the epipelic microalgal community, dominated by diatoms (and possible epiphytic bacteria) constituted at least one source of C_{20} and C_{25} HBI hydrocarbons to the sediments at Cargreen, the seasonal distribution of those hydrocarbons was investigated over the period of one year. Changes in the distribution of the HBI hydrocarbons of interest in this study may be related to the advent of ecological succession of various microbiological (algal and bacterial) communities of the intertidal mudflats of the Tamar estuary. These changes in benthic ecology and productivity are stimulated by numerous environmental variables and

physical processes such as water temperature, turbidity, light, nutrients, salinity, hydrographic conditions and the rate of decomposition of organic matter. Sedimentation of detritus plays an important role as linkage between the biosphere and geosphere. Organic matter which has been adsorbed on inorganic particles, originating partly from terrestrial runoff may be deposited in the benthos of the estuary by a series of depositional mechanisms, involving gravitational settling, which operate over each tidal cycle. In this way, enrichment of HBI hydrocarbons in the sediment over particular periods of the year might reflect an increased contribution of the source organism(s) to the biomass of the phytobenthos. The relative stability of the HBI hydrocarbons relative to other hydrocarbons derived from known algal or bacterial sources (*e.g.* n-C_{17}) can only enhance the potential of HBI hydrocarbons to reflect ecological change in the surface sediment.

Topmost sediment samples were obtained for 12 months at Cargreen and thus only broad inferences with respect to the source and diagenetic fate of HBI hydrocarbons can be drawn. In addition no consideration has been given here to the affect of tidal scouring of the sediment surface or storm action which may have disrupted any seasonal trends.

The aliphatic hydrocarbon content of the sediments at Cargreen ranged from about 200 mgkg^{-1} in March to 1700 mgkg^{-1} in June. The principal component of these hydrocarbons each month was an unresolved complex mixture (UCM) which constituted *ca.* 80% to 90% of the total. The ratio of unresolved to resolved components (U/R) remained relatively constant (Table 5.11) throughout the year. The presence of a UCM and a minor n-alkane series (C_{15}-C_{20}) lacking an odd carbon predominance reflected petroleum contamination of the sediments. The chief resolved

TABLE 5.11 SELECTED CHEMICAL PROPERTIES OF CARGREEN SEDIMENTS COLLECTED DEC. 1989 TO NOV. 1990.

Month	Total organic carbon (TOC %wt)	Total aliphatic hydrocarbons[a] μgg^{-1} dry weight	mgg^{-1} OC	UCM[a] mgg^{-1} OC	U/R[b]	n-alkanes[a] $(C_{25}-C_{34})$ μgg^{-1} OC	CPI[c]	Corg/N[d]
December[e]	3.3	890	27	24	8.3	1500	5.1	13
January	5.5	570	10	9.1	6.9	850	6.7	13
February	4.9	1200	24	22	8.7	950	4.5	16
March	6.2	190	3.1	2.7	7.4	180	6.5	15
April	6.6	1100	16	14	6.0	1000	8.2	16
May	4.4	910	21	17	4.7	1400	4.7	16
June	4.8	1700	35	31	8.8	1700	7.7	20
July	5.1	420	8.2	7.3	8.6	570	6.8	18
August	3.5	670	19	17	7.8	940	5.1	22
September	3.8	430	11	10	8.3	560	4.8	18
October	4.4	420	9.6	8.4	7.7	590	7.7	12
November	4.0	1200	29	25	6.3	1500	5.5	10

Key: [a] by integration of total FID response of aliphatic hydrocarbons or n-alkanes and direct reference to internal standard. [b] total unresolved/total resolved aliphatic hydrocarbons. [c] CPI is the Carbon Preference Index, measured from C_{25} to C_{34}. $CPI=0.5*(C_{n+2}+...+C_m)+(C_n+...+C_{m-2})/(C_{n+1}+...+C_{m-1})$ where n to m is the desired range. [d] TOC/nitrogen ratio. [e] 1989 (remainder 1990).

components in the chromatograms (*e.g.* Figure 5.34) remained long chain *n*-alkanes the distribution of which maximised at *n*-C_{29} and exhibited a marked odd carbon predominance (CPI 4.5-8.2) which were thought to be derived from the epicuticular waxes of vascular plants and thus indicative of the input of terrigenous organic material (Riely *et al.*, 1991a and references therein). The concentration of these *n*-alkanes (C_{25}-C_{34}) ranged from 11 $mgkg^{-1}$ in March to 81 $mgkg^{-1}$ in June. Squalene, an isoprenoid hydrocarbon with six double bonds, is a highly significant biological compound as it is a precursor of sterols. It has been identified in phytoplankton (Paoletti *et al.*, 1976; Volkman *et al.*, 1980a), microorganisms (Han and Calvin, 1969; Holzer *et al.*, 1979) and in marine suspended particles (Crisp *et al.*, 1979). Squalene was detected in the sediments at various times of the year in addition to a number of shorter chain *n*-alkenes which were not fully characterised.

The C_{20} HBI alkane 1 and related monoene br20:1; 1702$_{DB1}$ 4 were recorded as dominant components in the sediment throughout the year consistently more abundant than *n*-heptadecane (*n*-C_{17}). The seasonal maximum in concentration of C_{20} HBI hydrocarbons (April-June) shown in Figure 5.35A was not simply a function of a total organic carbon (TOC) maximum over the same period. Plotting the ratio of · concentration of C_{20} HBI hydrocarbons to TOC still revealed an early-mid summer maximum (Figure 5.35B).

The concentration of organic carbon (TOC) at the Cargreen site ranged from about 3.3% in December to a maximum of 6.6% in April (Table 5.11). This range in values was similar to those reported previously for sediments in the Tamar estuary (Upstill-Goddard, 1985; Readman *et al.*, 1986ab; unpublished data; Preston and Reeves, 1989). Seasonal fluctuations in TOC content of the sediments at Cargreen

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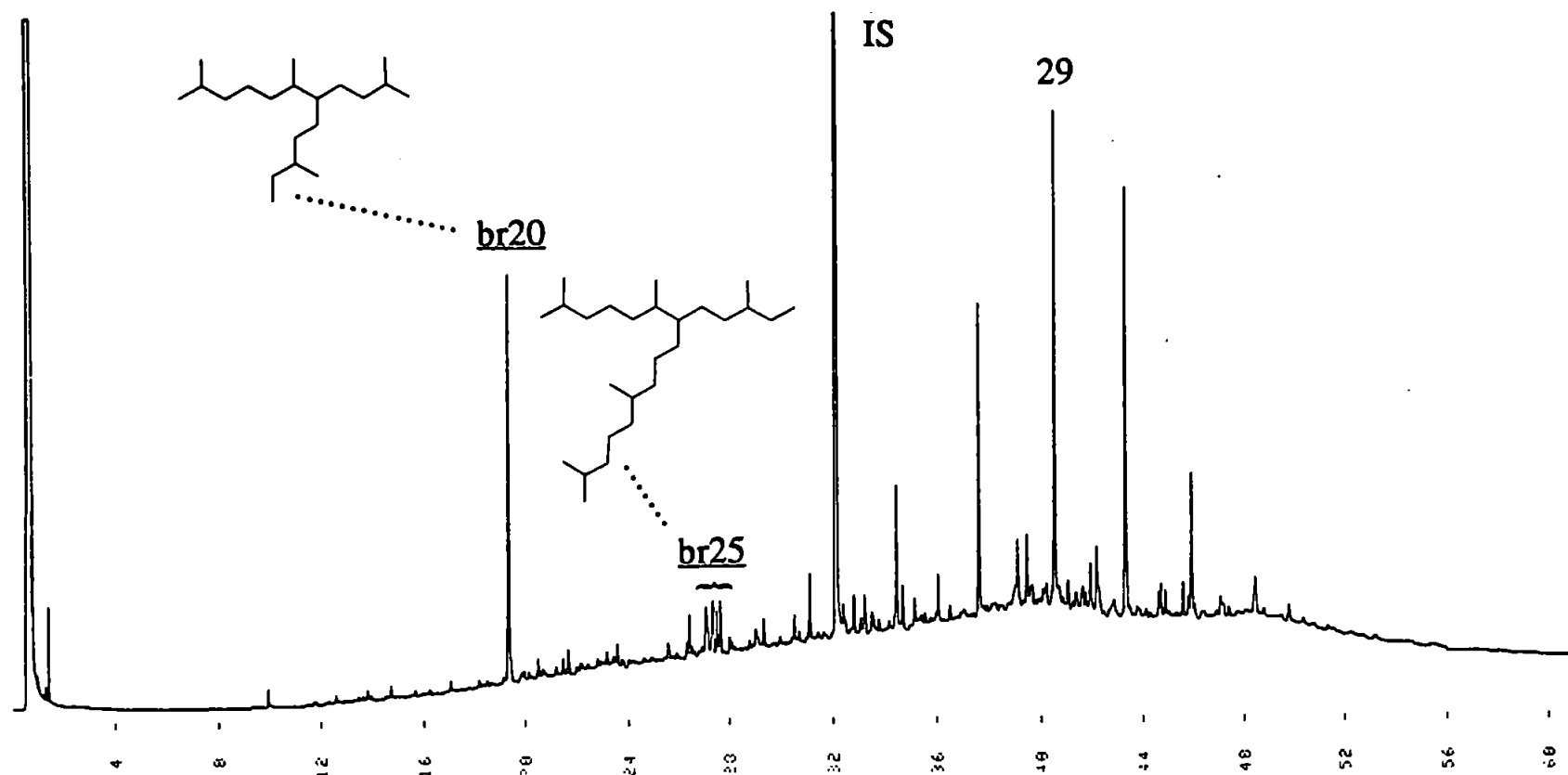
FIGURE 5.34   **GAS CHROMATOGRAM OF ALIPHATIC HYDROCARBONS ISOLATED FROM SEDIMENTS AT CARGREEN IN JULY, 1990.**

Conditions see text; DB5 (J&W)
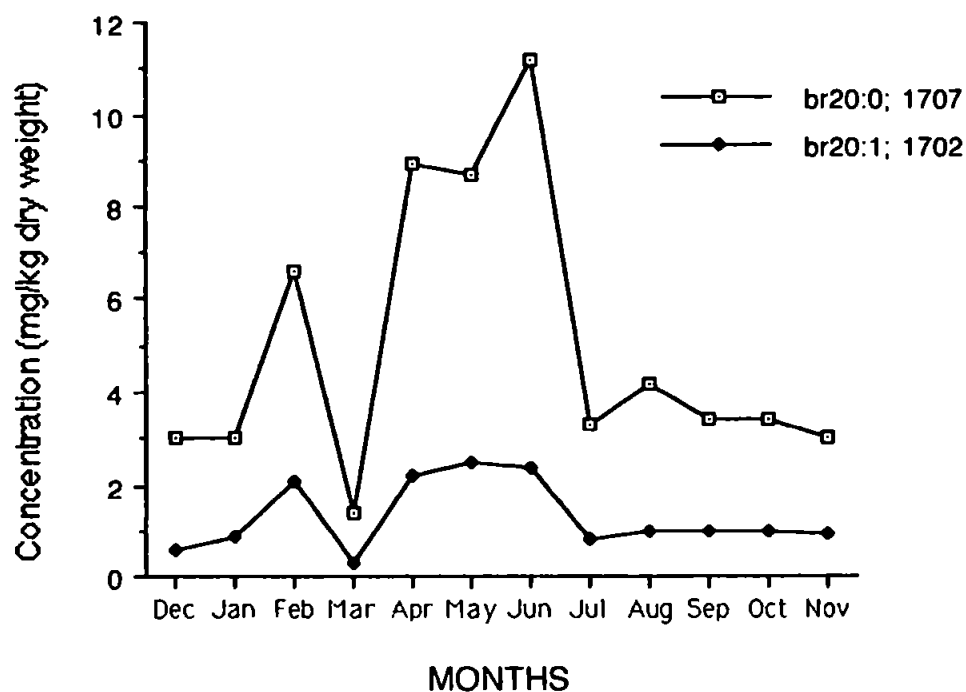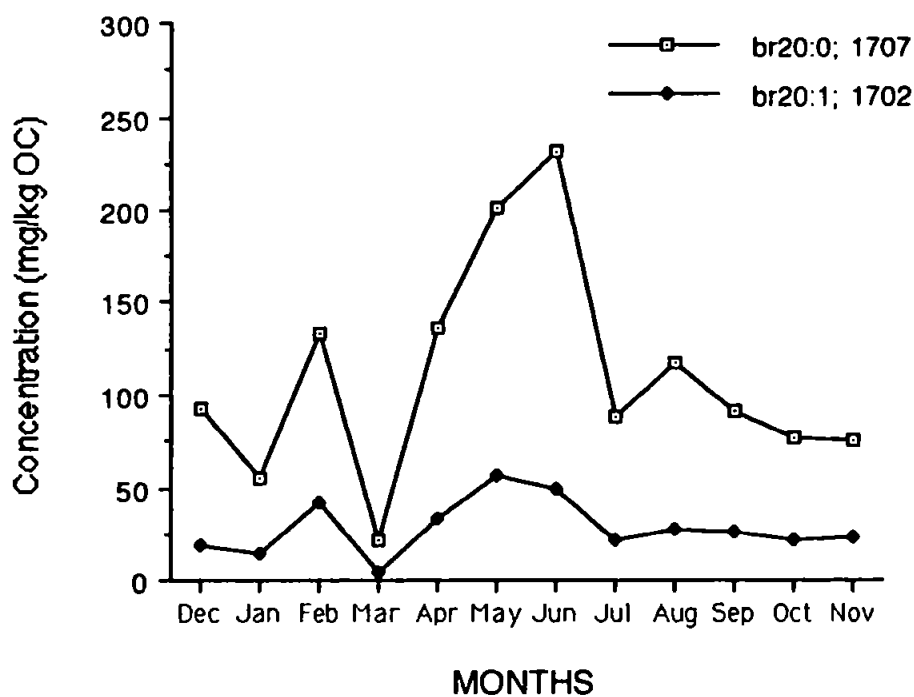
FIGURE 5.35   CONCENTRATION OF C_{20} HBI HYDROCARBONS IN SEDIMENTS AT CARGREEN
(A) normalised against dry weight of sediment
(B) normalised against organic carbon content

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cannot be presumed to be a reflection of solely benthic productivity of the epipelic community of the mudflats. Primary productivity in the overlying water column as well as within the topmost sediment is not the only control on TOC content of the intertidal sediments. Seasonal fluctuations occur in the relative amounts of allochthonous (terrigenous and marine) particulate organic matter in the estuarine water column some of which is deposited in the benthos of the intertidal mudflats. Although estuaries are considered areas of high biological productivity (*e.g.* Moss, 1968; Head, 1976; Malcom and Stanley, 1982; Relexans *et al.*, 1988) driven by the allochthonous supply of nutrients from the river and effluent outfalls and this algal productivity constitutes a large amount of organic material entering the sediments, although a higher proportion of organic matter present in upper estuary sediments is likely to be terrigenous in origin. This is partly due to the direct input of vascular plant material in the form of leaf fall from the trees bordering the river and leaf and soil-derived material entering the estuary via the many streams and rivers in the Tamar catchment area, the flux of which is increased during periods of high rainfall. In addition, the preferential preservation of terrigenous organic matter/lipids in the water column and sediment has been reported (Cranwell, 1981; 1982; Prahl and Carpenter, 1984; Brassell and Eglinton, 1986; Haddad and Martens, 1987; Kenig *et al.*, 1990). The enhanced stability of terrigenous organic matter can be explained in terms of packaging as the organic material is contained within spores or inside waxy coatings which protect the compounds from heterotrophic attack and thus is not mineralized. As very few organisms possess the necessary enzymes to hydrolyse the structural polysaccharides and other polymers (*e.g.* lignin) which compose macrophyte tissues, they are refractory and can survive long enough to be potentially

widely distributed throughout the estuary. Another portion of terrigenous organic matter is derived from soil which is reported to consist of nonmetabolisable carbon (Hedges, 1988ab).

Other factors reported as controlling TOC in marine sediments (Tyson, 1987) such as sediment texture (especially grain size), water depth and the rate of sediment accumulation were considered unlikely to greatly influence the seasonal change in TOC content of the intertidal sediment at Cargreen.

If the biomass of the organisms producing C_{20} HBI hydrocarbons was governed by the amount of organic carbon deposited or incorporated into the sediments some covariance of the seasonal profiles of hydrocarbons with TOC would be expected. Such plots showed a reasonably good correspondence (Figure 5.36). However, the TOC was found to have maximised during March-April (1990) whereas the HBI compounds were shown to be most abundant later during the year (April-June, 1990). Comparison of the profiles suggested either an increased contribution of source organism(s) to the biomass of the phytobenthos during a period of reduced organic carbon content of the sediment or the preferential consumption of other more labile components of the carbon pool relative to HBI hydrocarbons. This suggested a relationship between the supply of recently produced organic matter and thereby microorganisms active in its mineralisation and the seasonal profile of C_{20} HBI hydrocarbons, possibily indicating a bacterial source for the C_{20} HBI hydrocarbons.
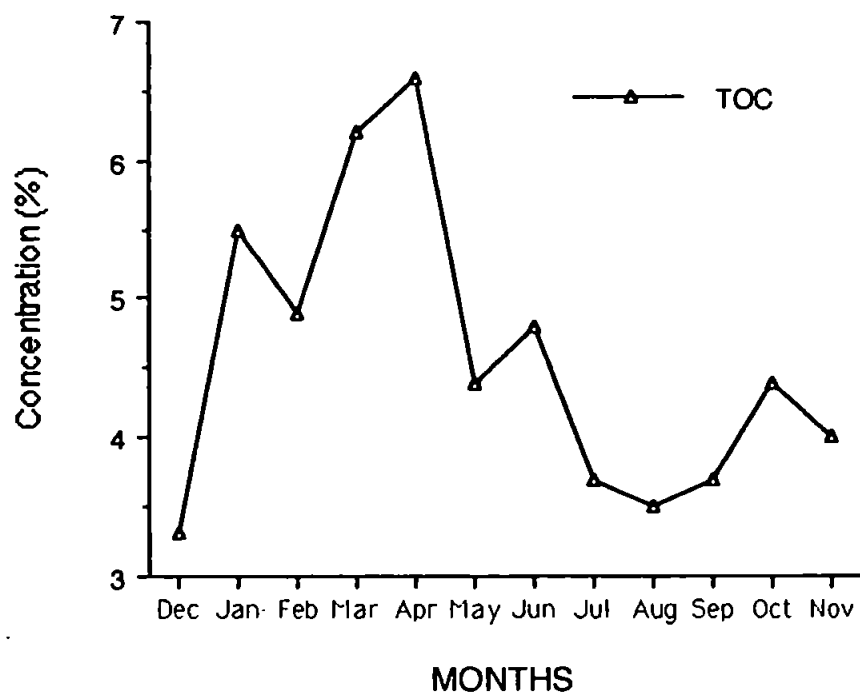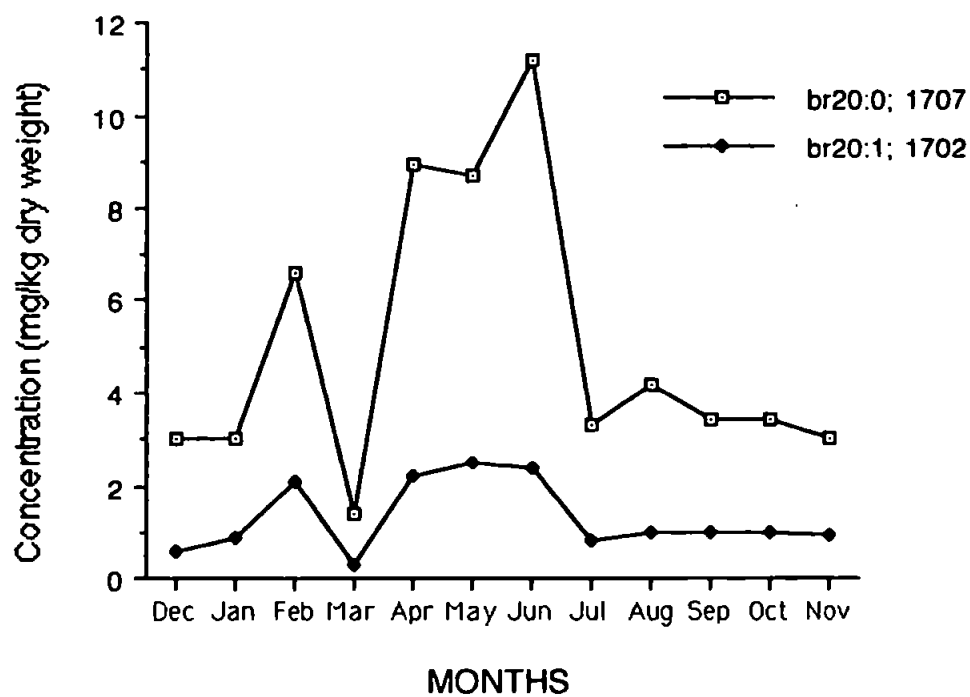
FIGURE 5.36   **SEASONAL CHANGES IN CONCENTRATION OF**
(A) TOC
(B) C_{20} HBI hydrocarbons
AT CARGREEN, 1990

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This was complete contrast to the seasonal distribution of n-C_{21} polyenes (Figure 5.37), thought to reflect the abundance of microalgae, probably diatoms (*Navicula* spp.), within the epipelic community. The occurrence of these polyenes, abundant in Cargreen sediments only in August, has been related to the growth phase of diatoms (*e.g.* Ackman *et al.*, 1964). Less n-$C_{21:6}$ was present in the lipids of younger cultures of *Skeletonema costatum* which suggested that the enzyme responsible for decarboxylation of the corresponding 22:6ω3 fatty acid was either not active in young cultures or that the alkene was synthesised during the later part of the exponential growth phase. Blumer *et al.* (1970; 1971) reported that unsaturated hydrocarbons decreased as cultures of planktonic algae made the transition from the period of rapid growth to the stationary phase. Hence, although the occurrence of n-C_{21} polyenes in sediments is not restricted to diatoms (Tornabene, 1981), the relative abundance of the n-C_{21} hexaene in Cargreen sediments in August, compared to any other month, reflected a summer bloom of epipelic diatoms. Any n-C_{21} polyenes derived from phytoplankton would be unlikely to survive diagenesis through the water column and would not be strongly imprinted in the sedimentary hydrocarbons.

An early summer (May-June, 1990) maximum was also observed for the seasonal concentrations of vascular plant-derived n-alkanes (C_{25}-C_{34}) (Figure 5.38). However, the profile was bimodal the second maximum occurring during the winter months. The disparity between this second enrichment, controlled by processes which deviated from those which controlled total organic matter deposition, and the TOC profile was considered to reflect the differential preservation of bulk organic matter and the n-alkanes during a period of low productivity and the relative importance of
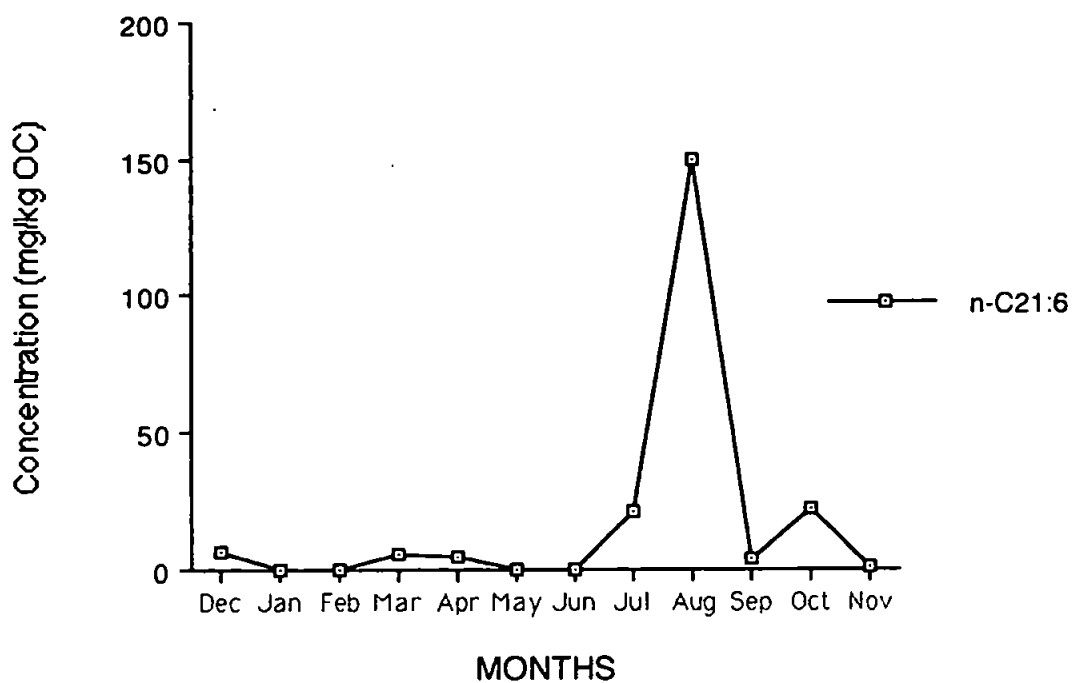
FIGURE 5.37   SEASONAL CHANGES IN THE CONCENTRATION OF
n-C$_{21:6}$ POLYENE IN SEDIMENTS FROM CARGREEN,
1990.
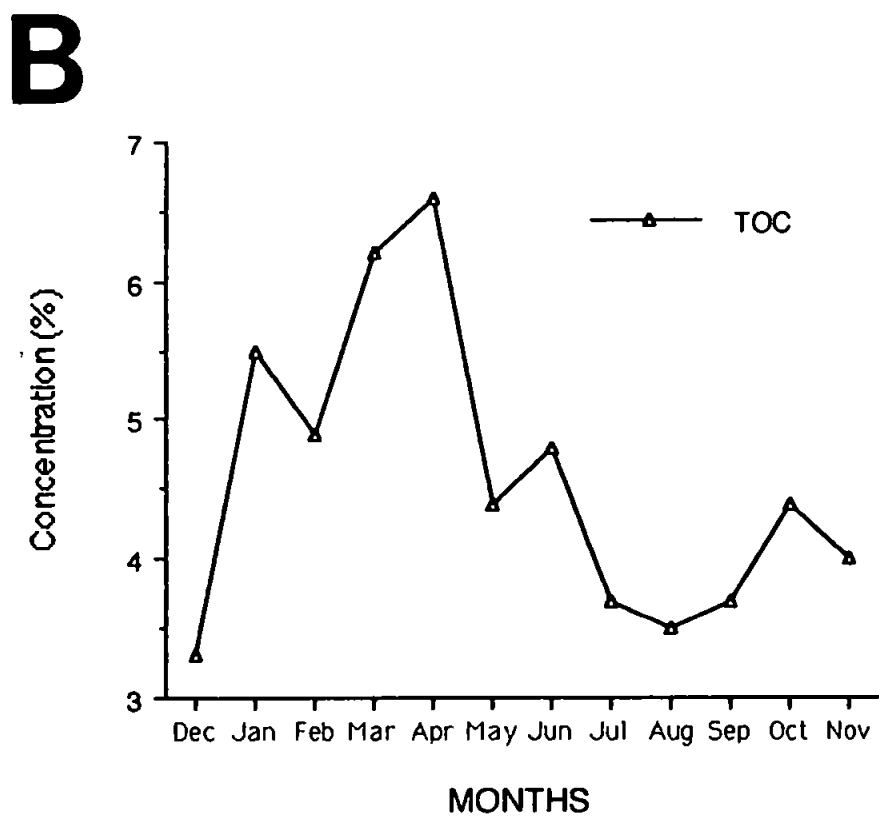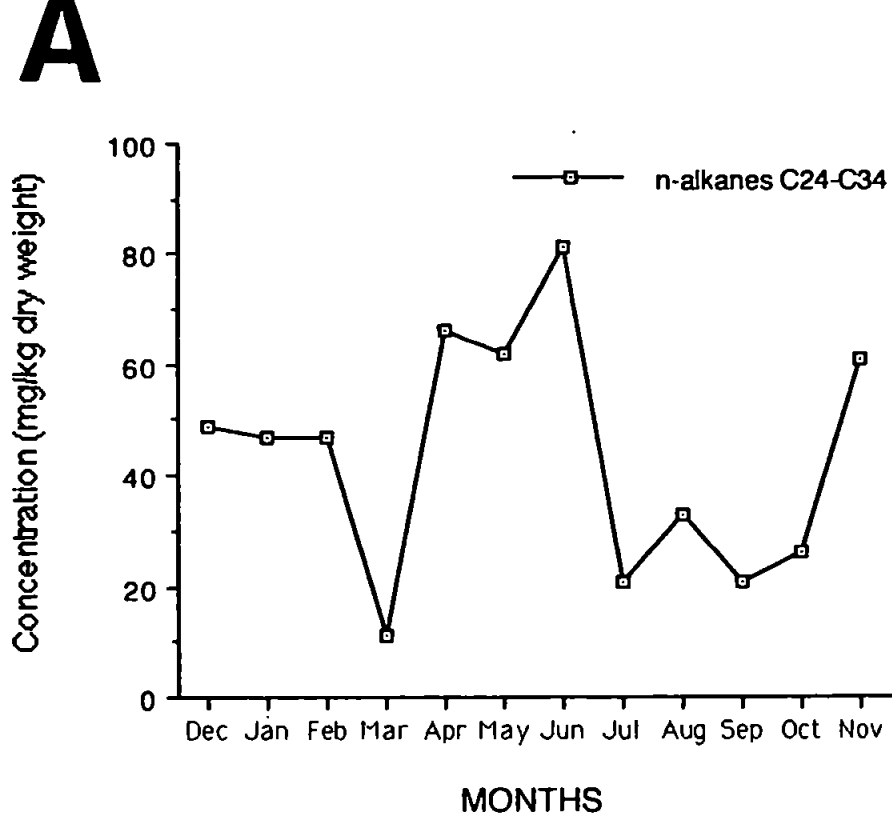
FIGURE 5.38   **SEASONAL CHANGES IN CONCENTRATION OF**
(A) *n*-alkanes
(B) TOC
AT CARGREEN, 1990

allochthonous terrigenous sources to the sedimentation of organic matter in the estuary over the winter months when autochthonous biological activity was presumed to be reduced and terrestrial run-off high. Although no such analyses were carried out during the present study, previous work within the Tamar estuary (Readman *et al.*, 1986a; Morris *et al.*, 1982) using pigments has indicated that biological activity in the water column in the estuary was found to be substantially reduced during the winter months and the periods of highest primary productivity were reported as spring and summer. Cursory microscopic examination of the sediments at Cargreen revealed the presence of large quantities of finely dispersed vascular plant remains including plant cuticles and woody debris. Estuarine intertidal sediments receive carbon from both terrigenous and marine sources. Terrigenous organic carbon is a mixture of vascular plant debris and highly oxidised humified soil organic carbon. In newly deposited, contemporary sediments, this vascular plant debris is highly refractory (Hedges and Mann, 1979). Terrigenous carbon may be especially significant in restricted estuarine environments. Organic carbon to nitrogen elemental ratios (Corg/N) are useful in differentiating sources of organic matter since marine organisms are enriched in nitrogen compared to terrestrial vascular plants. (Prahl *et al.*, 1980; Khalil and Labbé, 1982; Malcolm and Stanley, 1982; Premuzic *et al.*, 1982; Prahl and Carpenter, 1984; Hedges *et al.*, 1986). The range of values for the Corg/N ratio for the sediments at Cargreen was 10-22 (Table 5.11). This compared with a range of 6-16 determined from Readman *et al.* (1986a) for middle channel, subtidal sediments transversing the length of the Tamar estuary. Thus, the sedimentary organic matter at this site in the upper estuary was due largely to the input and preservation of terrigenous organic material as reflected by sediment

Corg/N ratios of greater than 10 (Pocklington, 1976; Pocklington and Leonard, 1979). Marine plankton and bacteria are characterised by Corg/N ratios of <8 (Redfield *et al.*, 1963; Hamiliton and Hedges, 1988). The observed fluctuation in the Corg/N ratios through the year reflected differing relative contributions of marine, estuarine and terrigenous organic matter. However, this signature is strongly influenced by the extent of degradation of organic matter. Microbial degradation prior to and after burial results in the more rapid metabolism of organic nitrogen than the organic carbon (Rosenfield, 1979). The amount of carbon relative to nitrogen has been shown to increase in the organic matter during degradation as the nitrogen-rich marine material is more easily mineralized than the refractory carbon-rich terrigenous detritus and thus the signal from terrigenous organic matter is enriched through selective preservation. Nitrogen-rich humic substances in the soil may well be preferentially retained during degradation of surface plant litter and passage of leachate through the soil (*e.g.* Cronan and Aiken, 1985). Thus values of 10-12 for Corg/N ratios may be characteristic of soil-derived terrigenous organic matter (Hedges *et al.*, 1986) whereas higher values (>15) can be explained in part by elevated levels of vascular plant debris (Corg/N = 20-300; Hedges *et al.*, 1985; 1986) or by the selective removal of nitrogen relative to carbon during microbial degradation either in the water column or *in situ* in the sediment (Rosenfield, 1979). It should be noted, however, that high Corg/N ratios (>20) have also been recorded in particulate organic matter with significant concentrations of photosynthetic pigments characteristic of phytoplankton or periphyton (Liaw and MacCrimmon, 1977). Moribund planktonic and epipelic material undergoes microbial attack and thus refractory organic substances/compounds may be accumulated over a long period after

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the degradation of labile organic constituents.

The covariance of the seasonal maximum in abundance of C_{20} HBI hydrocarbons with the early summer maximum of vascular plant-derived n-alkanes suggested a relationship between the preservation of refractory terrigenous organic matter and the seasonal C_{20} HBI hydrocarbon profile. This indicated that the C_{20} HBI seasonal profile was influenced by remineralisation of organic matter. This phenomenon was confirmed by the increases in the Corg/N ratio values (>15) over the period of maximum abundance of HBI hydrocarbons and n-alkanes (Figure 5.39). The significant maximum in abundance of n-$C_{21:6}$ from Cargreen sediments in August, reflecting a summer bloom of epipelic diatoms (*Navicula* spp.) also corresponded to a secondary maxima of C_{20} HBI hydrocarbons (Figure 5.40).

Some covariance was observed between the seasonal fluctuation in abundance of various C_{25} HBI alkenes normalised to organic carbon (TOC) content of the sediments (Table 5.12). HBI hydrocarbons concentrations were normalised to organic carbon rather than to sediment dry weight to compensate for varying ratios of organic to inorganic material in texturally dissimilar samples. Three C_{25} HBI compounds br25:2; 2083_{DBS}, br25:3; 2090_{DBS} and br25:4; 2128_{DBS} displayed the same early-mid summer maximum (May-June, 1990) previously described for the C_{20} HBI homologues (Figure 5.41A). However other isomers showed maxima at other stages of the year. For example, HBI trienes br25:3; 2107_{DBS} and br25:3; 2044_{DBS} were most abundant in July, a month later than the three C_{25} HBI isomers described above (Figure 5.41B). A second less significant maxima for br25:3; 2090_{DBS} and br25:4; 2128_{DBS} was apparent during late summer (August-September, 1990). In contrast, two other isomers displayed maxima outside this summer period. The C_{25} HBI pentaene
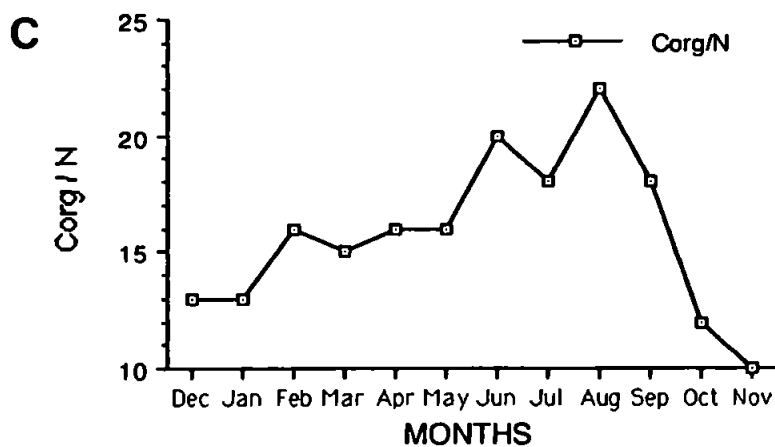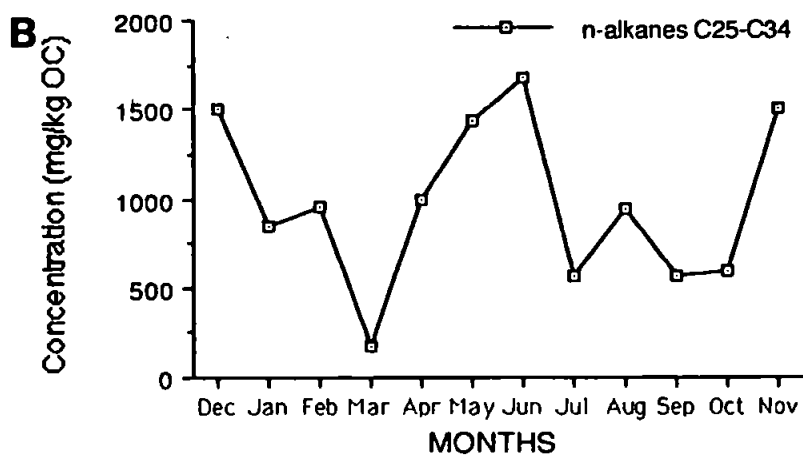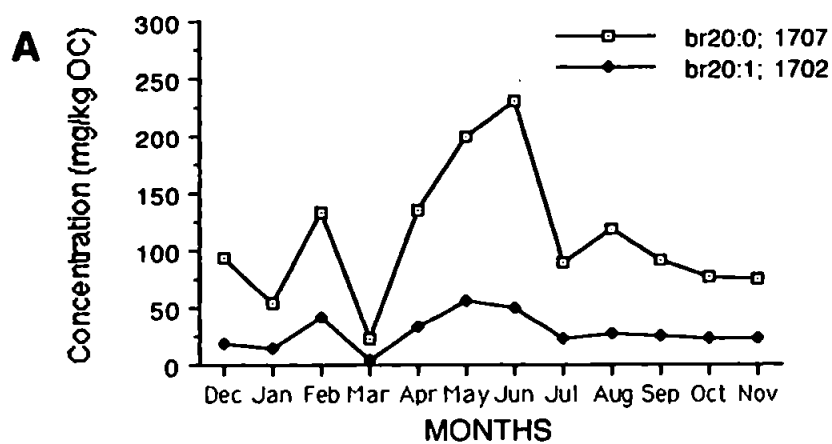
FIGURE 5.39   SEASONAL CHANGES IN THE CONCENTRATION OF HYDROCARBONS AND "C/N RATIO" IN SEDIMENTS AT CARGREEN, 1990

(A) C_{20} HBI hydrocarbons, (B) n-alkanes and (C) ratio of organic C to N.

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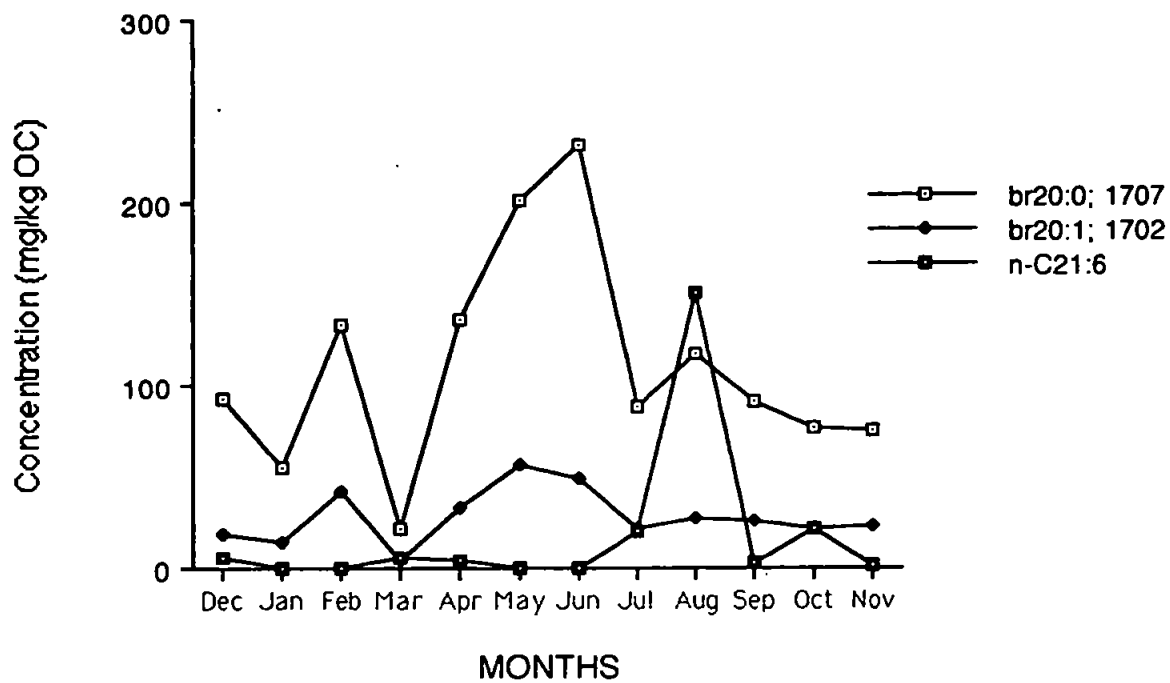
FIGURE 5.40   SEASONAL CHANGES IN THE CONCENTRATION OF C_{20} HBI AND $n\text{-}C_{21:6}$ HYDROCARBONS IN SEDIMENTS FROM CARGREEN, 1990.

TABLE 5.12 CONCENTRATION OF HBI HYDROCARBONS IN SEDIMENTS AT CARGREEN, 1990 (μgg^{-1} OC)

Compound/Month[a]	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov
br20:1; 1702_{DB1}	19	15	42	4.3	34	57	49	22	28	26	22	23
br20:0; 1702_{DB1}	93	55	130	22	140	200	230	89	120	91	77	75
$\Sigma n\text{-}C_{21}$ polyenes	6.4	nd	nd	5.6	4.4	nd	nd	21	150	3.5	22	1.2
br25:3; 2044_{DB5}	1.2	0.72	nd	0.32	4.6	4.1	6.2	23	9.1	6.4	6.8	6.0
br25:2; 2070_{DB5}	nd	nd	nd	0.32	nd	nd	4.8	5.1	4.3	3.8	5.0	6.0
br25:2; 2083_{DB5}	1.9	1.1	3.0	0.48	nd	9.9	15	6.2	6.0	3.7	2.5	1.5
br25:3; 2090_{DB5}	18	8.0	19	1.8	18	30	54	21	30	21	20	13
br25:3; 2107_{DB5}	1.9	0.72	3.4	nd	1.2	4.6	4.0	12	9.1	5.3	2.3	1.5
br25:4; 2128_{DB5}	5.9	2.4	nd	0.32	nd	nd	13	nd	3.1	2.4	nd	nd
br25:4; 2175_{DB5}	2.5	1.8	8.9	0.64	nd	nd	nd	nd	3.4	nd	nd	nd
br25:5; 2182_{DB5}	nd	nd	nd	nd	nd	nd	nd	3.2	2.9	2.9	36	26

Key: [a] 1989 (remainder 1990)

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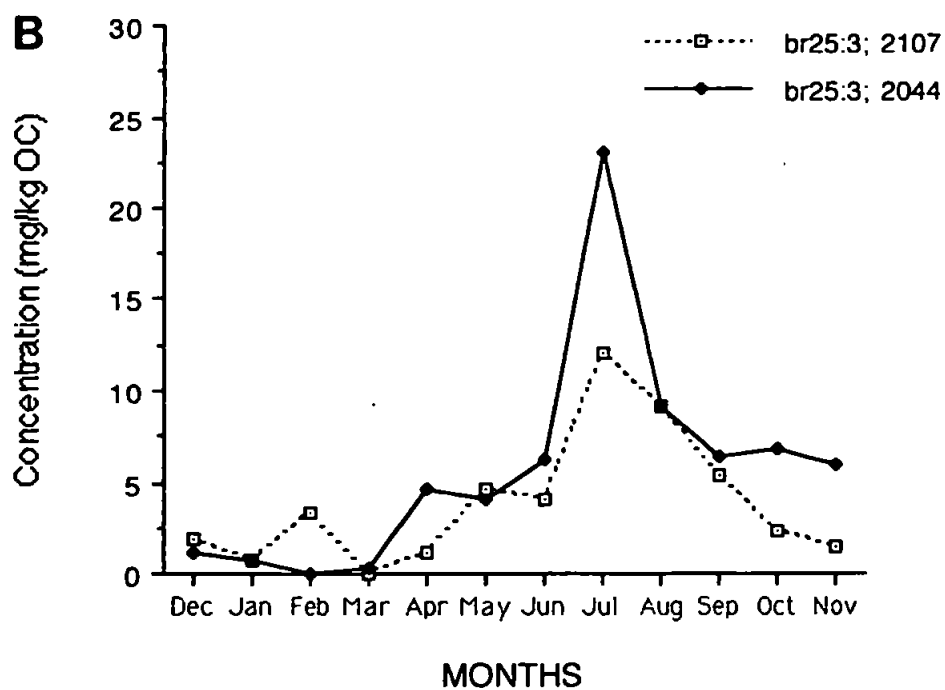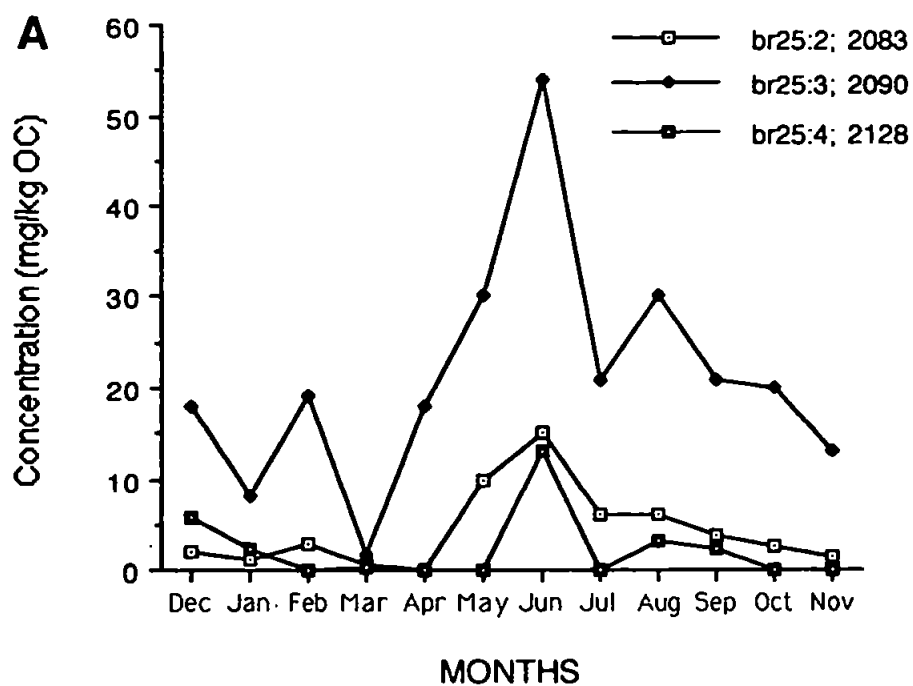
FIGURE 5.41 **CONCENTRATION OF C_{25} HBI ALKENES IN SEDIMENT AT CARGREEN, 1990**

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br25:5; 2182_{DBS} was apparently absent from the sediment at Cargreen throughout most of the year (December 1989 through to June 1990) and was only detected at significant levels in the autumn (October-November, 1990) whereas br25:4; 2175_{DBS} was generally limited to the early part of the year maximising in February 1990 (Figure 5.42). The occurrence of the C_{25} HBI diene br25:2; 2070_{DBS} was limited to the late part of 1990 (May-November) (Figure 5.42).

Although this is the first extensive report of seasonal variation of the abundance of HBI hydrocarbons in sediments, other authors have described the predominance of various HBI compounds in particulate matter. Prahl *et al.* (1980) identified the C_{25} triene br25:3; 2090_{SP2100} in sediment trap samples collected in autumn (September-November) in Dabob Bay, U.S.A and in a sample of mixed phytoplankton collected in November. In agreement with Prahl, Osterroht *et al.* (1983) observed the same compound solely in autumn particulates from the Kiel Bight which suggested that the br25:3; 2090_{SP2100} was derived from planktonic species appearing late in the yearly succession. This was in contrast to the present sediment study where the occurrence of the same compound, the principal C_{25} HBI alkene in the sediment, maximised during June and was present in the sediment through out the year. The abundance of br25:3; 2090_{SP2100} in trapped material from Dabob Bay in autumn showed good covariance with the flux of organic carbon, total chlorophyll and pristane over the same time period which suggested a marine planktonic source of organic matter and the C_{25} HBI triene (Prahl *et al.*, 1980). This was confirmed by Corg/N ratios of 8.3-8.8 and $\delta^{13}C$ values of -22.9 (September-October) and -21.7 (October-November) which reflected a high relative contribution of marine to terrestrial organic matter. The early summer seasonal maximum in abundance of
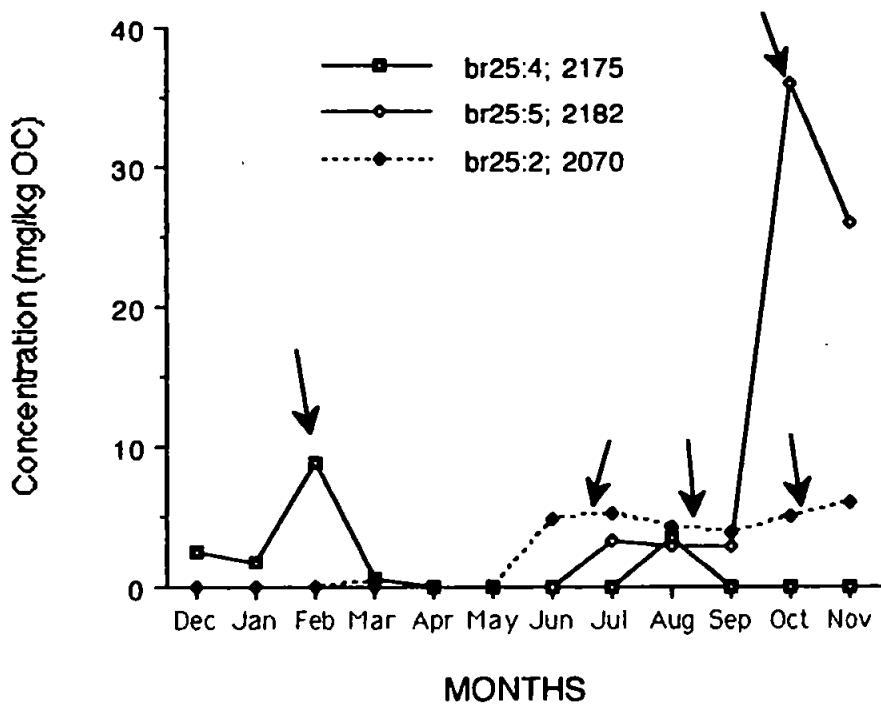
FIGURE 5.42   **CONCENTRATION OF C$_{25}$ HBI ALKENES IN SEDIMENT AT CARGREEN, 1990.**
Arrows indicate periods of maximum abundance

br25:3; 2090_{DBS} in Cargreen sediments showed a maximum during March-April. The Corg/N ratio corresponding to the HBI triene maximum was 20 which indicated that the organic material was highly degraded.

In particulate matter collected from Alfacs Bay, Spain, Albaigés *et al.* (1984b) showed that the HBI diene br25:2; 2082_{SES2} which had reached a maximum in concentration during the autumn had disappeared from the water column by winter. In the summer the triene br25:3; 2119_{SES2} was the principal HBI component. The reason for the relative predominance of these HBI compounds, of which only br25:3; 2082 was detected at Cargreen, was attributed to changes in biological productivity and environmental conditions in the bay. Interestingly, the n-C_{21} polyene, heneicosahexaene (n-$C_{21:6}$) was shown to be consistently present in the water column and little seasonal variation was reported. The absence of this highly unsaturated compound from sediments in Alfac Bay was ascribed to its rapid diagenetic degradation and/or metabolism. In Cargreen sediments a significant maximum in abundance of n-$C_{21:6}$ was recorded in August. This maximum did not correspond exactly to that of any of the HBI hydrocarbons (Figure 5.43).

Although the epipelic community, dominated by diatoms of the genus *Navicula*, isolated from the topmost sediment in August, did contain HBI hydrocarbons, only one compound, br25:3; 2090_{DBS} exhibited an increased abundance during August, which corresponded to the secondary maximum of the HBI compound over the year (Figure 5.43A). Another C_{25} HBI alkene, br25:1; 2070_{DBS}, prominent in the algae, was absent from the sediment not only in August but throughout the year. Thus, the bloom of epipelic diatoms, as indicated by the maximum of n-$C_{21:6}$ could not be related to seasonal variation in abundance of the majority of C_{25} alkenes.
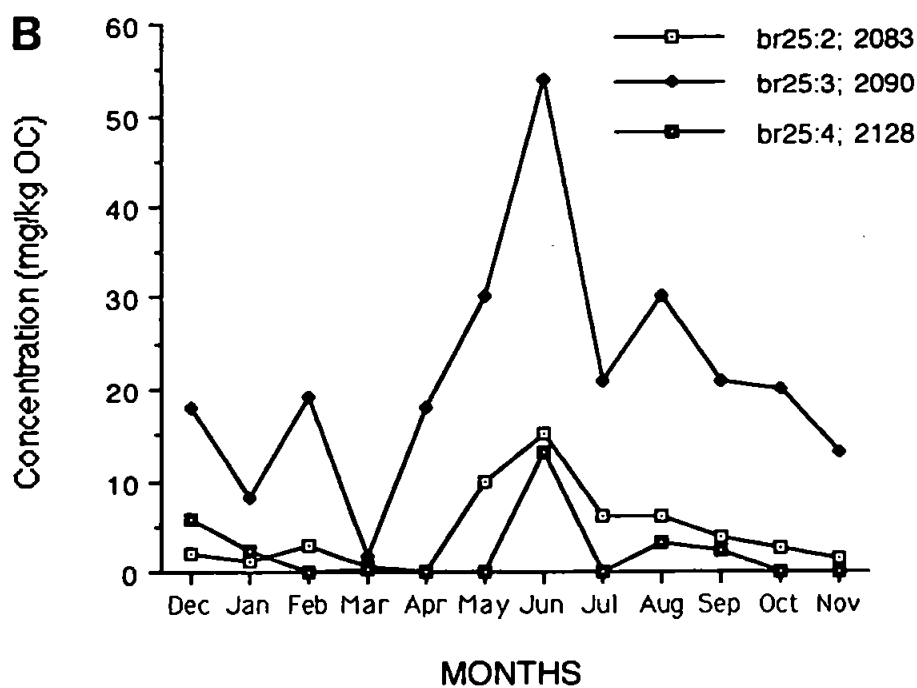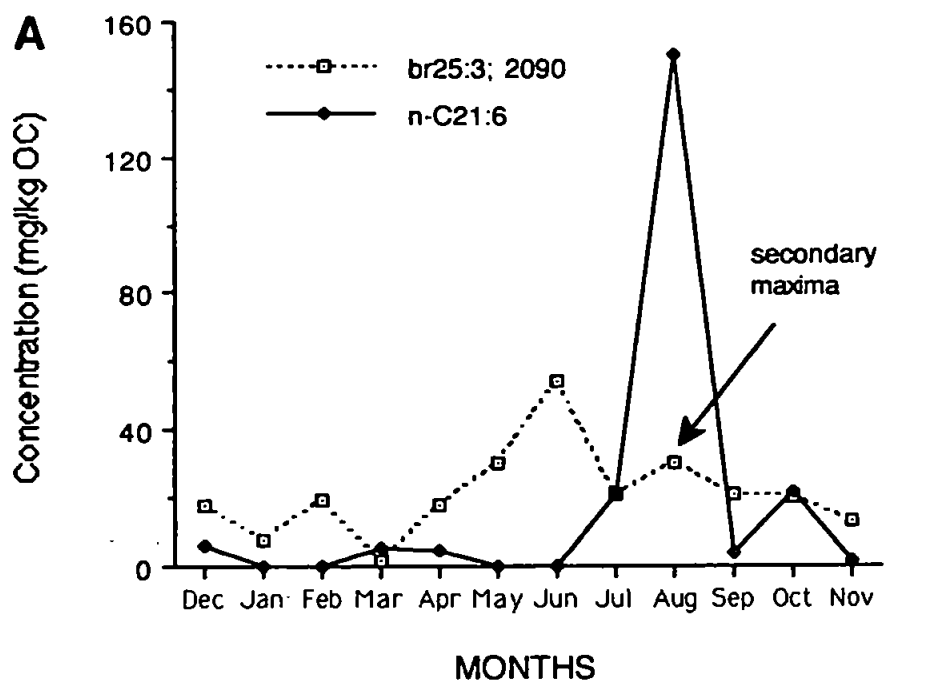
FIGURE 5.43 **SEASONAL CHANGES IN THE CONCENTRATION OF
C_{25} HBI AND n-$C_{21:6}$ HYDROCARBONS IN SEDIMENTS
FROM CARGREEN, 1990.**

5.11 THE ISOTOPIC COMPOSITION OF C_{20} HBI HYDROCARBONS ISOLATED FROM SEDIMENT LOCATED IN THE TAMAR ESTUARY

Structural elucidation of new potential biological markers such as HBI hydrocarbons and sulphur-containing analogues has invariably increased the need to search for the biological source for these HBI compounds. Given the problems encountered with discerning between an algal and/or bacterial source for HBI hydrocarbons, from screening sediment and biota (5.2-5.10), "Compound Specific Isotopic Analysis" (CSIA) was used to provide more information on the biological origin(s) of HBI hydrocarbons. Recent applications have shown CSIA provides a useful method for determining the origins of novel biomarkers. For example, Freeman *et al.* (1990) and Hayes *et al.* (1990) applied this technique to demonstrate that hopanes in the lacustrine Eocene Messel shale have multiple bacterial origins, while Moldwan *et al.* (1991) concluded from isotopic signatures that hopanes and rearranged hopanes in a Prudhoe Bay oil were derived from cyanobacteria or heterotrophic bacteria.

Molecular isotopic analysis provides information on the biological origin of individual compounds. The carbon isotope composition of living organisms is determined by the isotope composition of carbon which is initial for biosynthesis and by the isotope fractionation during biosynthesis. Biological (autotrophic) carbon fixation proceeds by a limited number of assimilatory pathways that transfer carbon dioxide (CO_2), bicarbonate ion (HCO_3^-) and carbon monoxide (CO) from the inorganic carbon reservoir to the biosphere (see review by Schidlowski *et al.*, 1983). The relationship between $\delta^{13}C$ of organic matter (marine plankton) and the concentration of molecular CO_2, [$CO_2(aq)$] in ocean surface water has been the

subject of much investigation (see review by Rau *et al.*, 1991). The inorganic carbon compounds, notably CO_2, are primarily fixed as C_3 compounds (phosphoglycerate, pyruvate, phosphoenolpyruvate), C_4 compounds (oxaloacetate) and C_2 compounds (acetate, acetyl coenzyme-A). Since the bulk of primary production in the biological carbon cycle is due to light-powered conversion (photoreduction) of CO_2 to organic matter, biological carbon fixation is, in essence, fixation of CO_2 by green plants and photoautotrophic protists (*e.g.* algae) and prokaryotes (photosynthetic bacteria including cyanobacteria). The biochemistry of carbon isotope fractionations during CO_2 uptake and metabolism has been extensively reviewed (*e.g.* Vogel, 1980; O'Leary, 1981; Schidlowski *et al.* 1983; Popp *et al.*, 1989). Carbon isotope discrimination during photosynthesis is mainly due to enzymatic reactions, which catalyse the initial carboxylation step (Fischer, 1991).

Although the isotopic composition of heterotrophs reflects that of particles ingested, some variation can occur depending on metabolic processes. During respiration, carbon retained as biomass becomes enriched in ^{13}C relative to that respired, the difference being 1.0-1.5 ‰ (DeNiro and Epstein, 1978; McConnaughy and McRoy, 1979). Hence, the average isotopic shifts *per trophic level* are expected to be less than 1.0-1.5 ‰ (Hayes *et al.*, 1990). Fermentative and chemoautrophic bacteria use biochemical processes that are markedly different from those in respiring heterotrophs. For example, aerobic methanotrophs use isotopically light methane as a carbon source (Freeman *et al.*, 1990). The presence of a methane cycle is revealed by the appearance of lipids strongly depleted in [13]C (*ca.* 50%; Freeman *et al.*, 1990). In green sulphur bacteria anaerobic phototrophic CO_2 fixation takes place *via* the reverse-TCA cycle which results in biomass with an anomalously heavy carbon

isotopic composition (Quandt *et al.*, 1977; Sirevag *et al.*, 1977; Summons and Powell, 1986; 1987).

Relative abundances of the stable carbon isotopes vary systematically in sedimentary organic compounds. Isotopic compositions of geolipids approximate those of their biological precursors which are, in turn, determined by the isotopic composition of the carbon assimilated by the organism and the biogeochemical processes by which they are synthesized (Hayes *et al.*, 1990). The isotopic composition of geolipids are likely to be close to those of their precursor biochemicals since isotopic fractionations during diagenetic processes (*e.g.* loss of functional groups) are considered to be small since the chemical reactions occur at specific sites within the biolipid. Isotopic abundances at those sites may shift as reactions occur, but other portions of the molecule will be unaffected and their isotopic constancy will buffer the effects of isotopic shifts at the reaction sites (Hayes *et al.*, 1990).

In this study, the C_{20} HBI monoene **4** was isolated from Millbrook sediment in July by the TLC techniques described in Chapter 4 and the carbon isotopic composition (δ ^{13}C ‰) of the alkene determined. For comparison, phytol was isolated from the same sediment using similar chromatographic techniques and δ ^{13}C ‰ determined in the same manner. These compounds were also isolated from Cargreen sediment in April and were subjected to analysis by GC-IRMS together with the saturated aliphatic hydrocarbon fraction isolated from the same sediment.

The results of these analyses are summarised in Table 5.13. Comparison of the δ ^{13}C values for the isolated C_{20} HBI monoene **4** (-26.5 ‰) and phytol (-28.5 ‰) reveal a difference of 2 ‰ with phytol being relatively depleted in ^{13}C. No results could be obtained for phytol using GC-IRMS because of technical difficulties.

Surprisingly when the fraction isolated from Cargreen sediment containing **4** was analysed by GC-IRMS the $\delta^{13}C$ value recorded for the C_{20} monoene peak was very different to that recorded for the Millbrook July sediment by the conventional IRMS technique (-18.3 ‰; Δ = 8.2 ‰). This difference is far in excess of that reported by Hayes *et al.* (1990) during a comparison of isotopic composition of individual *n*-alkanes and isoprenoids analysed by GC-IRMS and by conventional, combustion, dual inlet, IRMS using purified individual compounds (Δ = 0-0.6 ‰).

Therefore, this disparity can be attributed to real differences in the carbon isotopic composition of HBI the sediments. The $\delta^{13}C$ of the corresponding alkane **1** (-19.1 ‰) in Cargreen April sediment was similar to that of the alkene by GC-IRMS. In contrast the range of values recorded for four *n*-alkanes (*n*-C_{25}, *n*-C_{27}, *n*-C_{29} and *n*-C_{31}; $\delta^{13}C$ -26.6 ‰ to -30.8 ‰) were more negative being depleted in ^{13}C. These latter values are consistent with bulk values for higher plants following the C3 metabolic pathway (Troughton, 1979).

It is not unusual for the ^{13}C contents of primary products to vary significantly. Factors that have been reported to influence the isotopic fractionation associated with photosynthetic carbon fixation include variation in atmospheric CO_2 concentration ($[CO_2]$) seasonally as well as the progressive increase in the last 200 years resulting mainly from fossil fuel combustion (Griffiths, 1991). Fluctuations in atmospheric $[CO_2]$ are also dependent on environmental parameters such as light intensity. In aquatic habitats isotopic fractionation may be related to the concentration of dissolved CO_2, $[CO_2(aq)]$ (Popp *et al.*, 1989) and rate of growth of the organism (Deuser, 1970). If HCO_3^- is the inorganic carbon source accumulated by plants, $\delta^{13}C$ values reflect both the equilibrium fractionation in favour of ^{13}C as well as variations in $\delta^{13}C$

TABLE 5.13 A SUMMARY OF ISOTOPIC COMPOSITION OF C_{20} HBI HYDROCARBONS AND OTHER COMPOUNDS FROM TAMAR SEDIMENTS

Compound	$\delta^{13}C$ value[a]	Location	Month	Method
br20:1; 1702$_{DB1}$ **4**	-26.5±0.1	Millbrook	July	Ag$^+$ TLC, IRMS
phytol	-28.5±0.1	Millbrook	July	TLC, IRMS
br20:0; 1707$_{DB1}$ **1**	-19.1	Cargreen	April	GC-IRMS
br20:1; 1702$_{DB1}$ **4**	-18.3	Cargreen	April	GC-IRMS
n-C$_{25}$-C$_{31}$ alkanes	-26.6 to -30.8	Cargreen	April	GC-IRMS

Key: [a] $\delta = 10^3[(R_x-R_s)/R_s]$ in ‰, where R = $^{13}C/^{12}C$, x designates sample, s designates PDB standard.

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of the carbon source (Raven *et al.*, 1987; Berry, 1989) It has also been reported that particular types of algae tend to be enriched in ^{13}C (*e.g.* diatoms) with the range in differences exceeding 10 ‰ (Deuser, 1970). Elevated plankton $\delta^{13}C$ values are often found in areas or times of high plankton productivity (*e.g.* Degens *et al.*, 1968ab; Deuser, 1970; Cifuentes *et al.*, 1988; Goering *et al.*, 1990; Fry and Wrainwright, 1991; Fischer, 1991). As shown *in vitro*, phytoplankton $\delta^{13}C$ can vary significantly in response to water turbulence (Degens *et al.*, 1968a; Smith and Walker, 1980), nutrient limitation (Descolas-Gros and Fontugne, 1990; Fry and Wainwright, 1991), and changes in species composition (Wong and Sackett, 1978; Falkowski, 1991; Fry and Wrainwright, 1991). These factors may ultimately influence or reflect changes in carbon transport to, and transport and demand within phytoplankton, thus affecting their biomass $\delta^{13}C$ and the isotopic signature of sedimentary organic compounds.

A summary of the $\delta^{13}C$ composition of some plankton derived from the literature is shown in Table 5.14 whereas isotope data for other potential HBI source organisms, extant plants and autotrophic microorganisms, are given in Table 5.15. These data serve to demonstrate the large range in $\delta^{13}C$ values rcorded for different organisms.

The separation between compounds of different biological origins however, is often not simple. Schoell *et al.* (1992) recently demonstrated that compounds of very diverse origins such as 1,1-biphytane (archaebacterial), C_{27}-sterane (planktonic), C_{29}-C_{35} hopanes (bacterial) and C_{18}-C_{20} isoprenoids (algal or bacterial) vary isotopically in a narrow range of about 3 ‰ (Figures 5.44 and 5.45).

The $\delta^{13}C$ value recorded for **4** (-18.3 ‰) and that of the corresponding alkane **1** (-19.1 ‰) in Cargreen sediment in April was similar to that reported for **1** in immature sediment (Marl-2) from the Messinian Vena del Gesso basin, Italy (-17±0.6 ‰; Kohnen *et al.*, 1991b; 1992; Schouten *et al.*, 1991). Whereas that of **4** in sediment from Millbrook in July (-26.5 ‰) and phytol (-28.5 ‰) were similar to a homologous C_{25} HBI thiophene after desulphurisation from the same Marl-2 (-27.3±0.9 ‰; Kohnen *et al.*, 1991b; 1992; Schouten *et al.*, 1991). Freeman *et al.* (1991) recorded a mean value of -24 ‰ for C_{25} HBI alkenes in suspended particulates from the Cariaco Trench.

TABLE 5.14 ISOTOPIC COMPOSITION OF SOME ALGAE

Type	$\delta^{13}C$ value	Reference
marine plankton	-20	Galimov, 1977
fluvial plankton	-24 to -30	
eukaryotic algae	-12 to -35	Schidlowski, 1986
marine algae	-18 to -31	
benthic diatoms	-16.2 to -17.9	Haines, 1976
benthic and macroscopic algae	-18.4	Fry *et al.*, 1977
seagrass epiphytes	-15.4	
offshore plankton	-22.3	
marine macroalgae	-8 to -17	Craig, 1953
subtropical plankton: Mississipi Sound	-23 to -28	Sackett and Thompson, 1963
open Gulf of Mexico with		
20% nearshore plant forms	-17	
50% nearshore plant forms	-13	
plankton: high latitude T= -1.8°C	-30	Sackett *et al.*, 1965
low latitude T= 25°C	-20	
Antarctic plankton	-18 to -34	Fischer, 1991
coastal plankton	-20 to -23	Gearing *et al.*, 1984
Black Sea plankton: surface	-25	Freeman *et al.*, 1991
oxic/anoxic interface	-40	
Cariaco Trench plankton: surface	-24	
oxic/anoxic interface	-29	

12 ‰ variation in plankton $\delta^{13}C$ observed globally in the present ocean (Rau *et al.*, 1982)

TABLE 5.15 ISOTOPIC SIGNATURES OF OTHER ORGANISMS

Type	$\delta^{13}C$ value	Reference
Cyanobacteria	-8 to -22	Schidowski, 1986
Anaerobic photosynthetic bacteria:		Schidowski, 1986
Species using the Calvin cycle	-30 to -36	
Purple sulphur bacteria	-26.6 and -29.5	
Purple nonsulphur bacteria	-19.4 and -27.5	
Green sulphur bacteria	-9.5 to -21	
Chlorofexus sp.	-17.8 and -19.4	
Thioploca spp.	-21.8	McCaffrey *et al.*, 1989
chemoautotrophs	<-40	Schidowski, 1986
seagrasses	-3 to -13	Blair and Carter, 1992 (and references cited therein)
higher C_3 plants	-23 to -34 (mean = -27)	Schidowski, 1986
higher C_4 plants	-6 to -23 (average -12 to -14)	
CAM plants	-11 to -33	
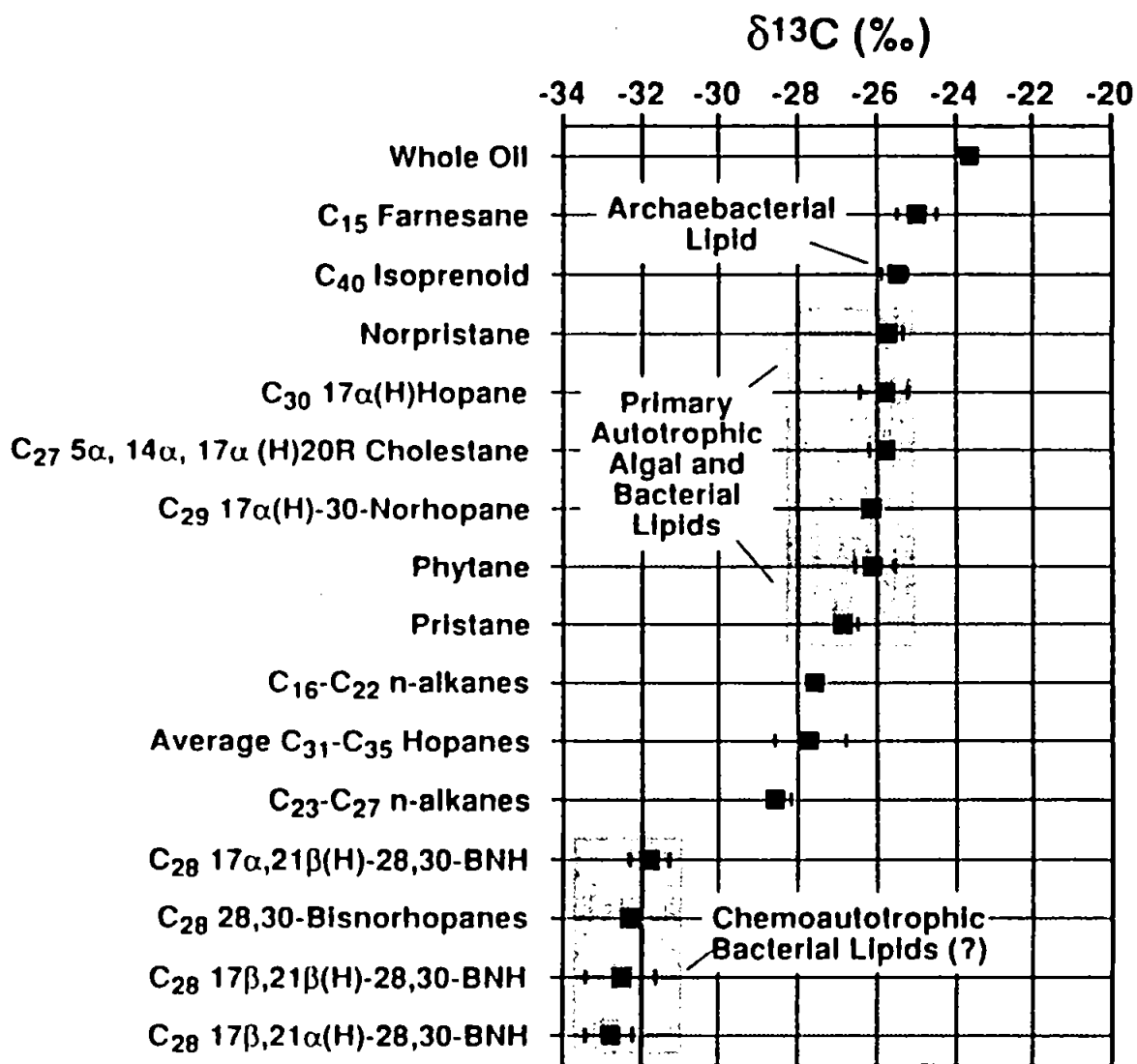
FIGURE 5.44   ISOTOPIC COMPOSITION OF VARIOUS COMPOUNDS
IN THE FREE LIPID SATURATE FRACTION OF A
LOW-GRAVITY, IMMATURE MONTEREY OIL AND
PROPOSED PRECURSOR ORGANISMS
(Schoell *et al.*, 1992).

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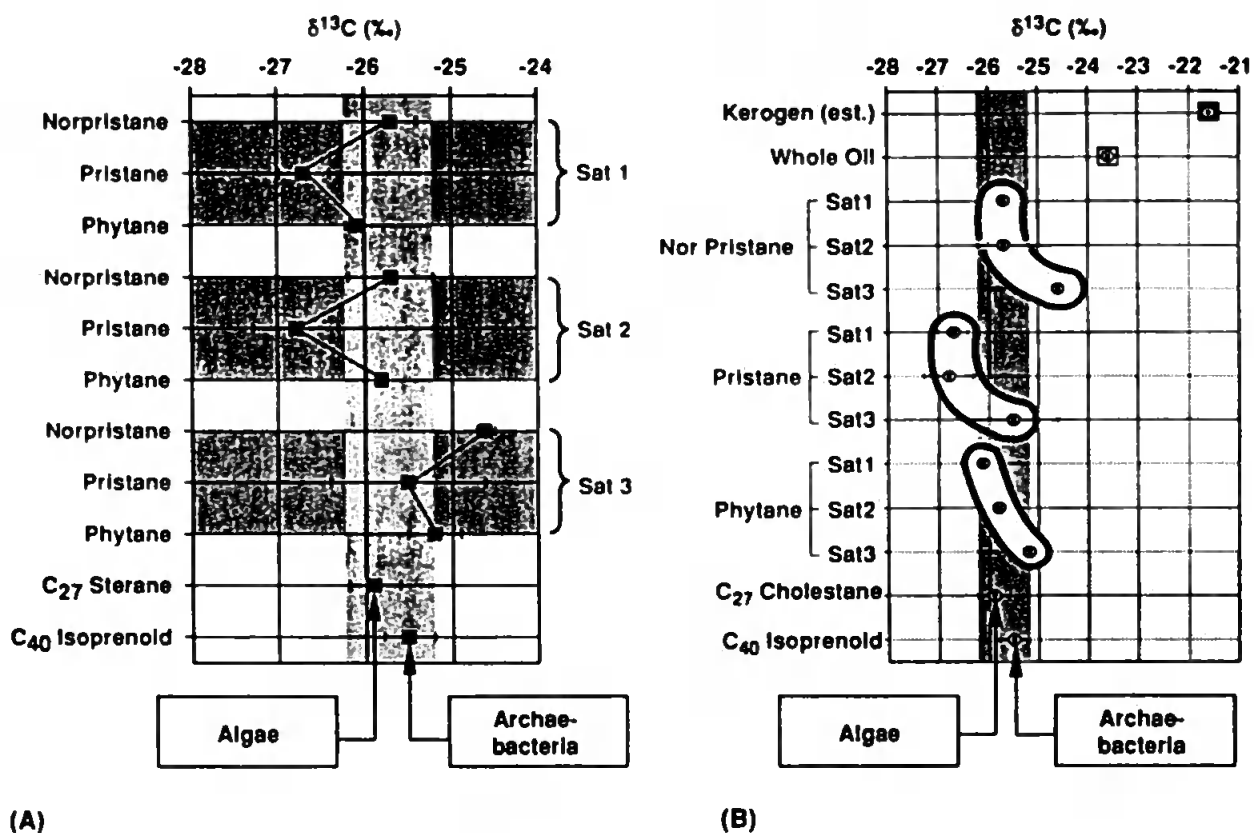
FIGURE 5.45 ISOTOPIC PATTERN OF C_{18}-C_{20} ISOPRENOIDS IN A MONTEREY CRUDE OIL.

Sat 1 denotes isoprenoids in the free lipid fraction; Sat 2 and Sat 3 are isoprenoids in the saturate fraction liberated by desulphurisation of the aromatic maltene and the polar maltene fractions, respectively. The isotope values for lipids of potential precursor organisms of the isoprenoids are shown for comparison: (a) Depiction of the isotope patterns in the free lipid and sulphur bound fractions. (b) Isoprenoids are arranged so that the isotopic difference between a free lipid isoprenoid compared to the same sulphur-bound isoprenoid becomes apparent (Schoell *et al.*, 1992).

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5.12 SOURCES OF HBI HYDROCARBONS IN TAMAR SEDIMENTS: DISCUSSION

Similarities were evident in the distribution of HBI hydrocarbons isolated from the sediments and green macroalgae (*Chlorophytae*) at the Millbrook site (Table 5.16). Algal lipids may have been directly released into the surrounding sediment from the filaments of the living algae or may have resulted from microbially mediated decomposition of senescent (possibly buried) algal cells. The relative predominance of the C_{20} HBI alkane 1 and the related monoene 4 in sediment relative to *n*-heptadecane and *n*-heptadecenes dominant in the various algae examined (*e.g.* 60% total aliphatic hydrocarbon of *Cladophora* spp.) was possibly caused by preferential removal of the labile linear compounds as observed by Robson and Rowland (1988b) when biodegradation of the C_{20} HBI alkane and a mixture of related monoenes by *Pseudomonas aeruginosa* in the laboratory showed that the HBI hydrocarbon was more resistant than *n*-eicosane and *n*-heptadec-1-ene.

Of the C_{25} HBI alkenes detected in Millbrook sediments in August 1990, all but two were isolated in specimens of the macroalgal mat prevalent at the time (consisting largely of *Cladophora* spp.). The C_{25} HBI trienes br25:3; 2090_{DB5}, br25:3; 2107_{DB5} and diene br25:2; 2140_{DB5} were detected in all three algal specimens collected (*e.g.* br25:3; 2107_{DB5}; 0.59 μgg^{-1} dry weight) and also in sediment underlying that mat (br25:3; 2107_{DB5}; 0.31 μgg^{-1} dry weight) and from sediment clear of the macroalgal growth (br25:3; 2107_{DB5}; 1.2 μgg^{-1} dry weight). Thus the biological source of these isomers was considered, at least in part, to be the green alga *Cladophora* and/or epiphytic microalgae or bacteria associated with the macrophyte. Another C_{25} HBI triene br25:3; 2042_{DB5} detected in the sediments (*e.g.* sediment under the mat;

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TABLE 5.16 A SUMMARY OF THE OCCURRENCES OF C_{20} AND C_{25} HBI HYDROCARBONS IN SEDIMENTS AND ASSOCIATED ALGAE IN THE TAMAR ESTUARY

Sample / Compound	SJL89	CG89	MB89	SBAM	EPH	MB90A	MB90B	CLD1	LDAM	CLD2	CLD3	UL	ENTL	ENT1	ENT2	AD	FD	CGEP	CG90
br20:1; 1702 (4)	3.4	0.83	3.5	18	+	4.2	2.0	nm	nm	nm	nm	nm	nm	nm	nm	+	nd	+	0.98
br20:0; 1707 (1)	12	3.3	9.7	50	+	10	3.3	1.8	2.5	1.5	1.6	0.32	0.24	0.92	0.84	+	nd	+	4.2
Dn-C_{21} polyene	nd	16	1.2	6.0	+	1.1	0.19	6.3	nd	5.5	15	7.3	2.3	nd	nd	+	6.5	+	5.3
br25:3; 2042	0.13	0.28	2.1	11	+	2.1	0.47	nd	0.09	nm	nm	nm	nm	nm	nm	+	nd	nm	0.32
br25:4; 2044	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
br25:2; 2070	0.26	0.21	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	0.22	nd	0.21	nd	nd	+	0.15
br25:1; 2072(8/9)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd
br25:2; 2083	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	+	0.21
br25:1; 2087(8/9)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
br25:3; 2090	0.19	0.71	2.3	12	+	2.4	0.91	0.25	0.26	nd	0.26	0.35	0.35	0.49	0.32	+	nd	+	1.1
br25:2; 2092	nd	nd	nd	nd	nd	nd	nd	0.13	0.38	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
br25:3; 2107	0.39	0.45	1.2	6.0	+	1.1	0.31	0.59	0.36	0.59	0.21	0.10	nd	0.32	nd	+	nd	+	0.32
br25:1; 2101(6/7)	nd	nd	nd	nd	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	+	nd
br25:3; 2123	nd	0.45	0.31	0.90	+	0.15	0.09	nd	nd	nd	nd	nd	nd	nd	nd	+	nd	+	0.11
br25:2; 2140	0.38	nd	0.92	4.5	+	0.85	0.27	1.2	0.82	0.31	0.14	0.48	nd	nd	nd	+	nd	+	nd
br25:3; 2157	nd	nd	nd	nd	nd	nd	nd	0.29	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
br25:4; 2175	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.12
br25:5; 2182	nd	nd	nd	nd	nd	nd	nd	0.50	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.10
br25:2; 2186	nd	nd	nd	nd	nd	nd	nd	0.40	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Key: SJL89 = St. Johns Lake sediment 1989, CG89 = Cargreen sediment 1989, MB89 = Millbrook sediment 1989, SBAM = semi-buried algal mat, EPH = epiphytic algae, MB90A = Millbrook bare sediment 1990, MB90B = Millbrook sediment under mat 1990, CLD1 = Cladophora specimen 1, LDAM = Laboratory decayed algal mat, CLD2 = Cladophora specimen 2, CLD3 = Cladophora specimen 3, UL = Ulva lactuca, ENTL = Enteromorpha linza, ENT1 = Enteromorpha spp. specimen 1, ENT2 = Enteromorpha spp. specimen 2, AD = algal debris, FD = Fucus distichus, CGEP = Cargreen epiphytic algae, CG90 = Cargreen sediment August 1990; GC RI: DB1 for C_{20} HBI and DB5 for C_{25} HBI hydrocarbons.

(0.47 μgg^{-1}) could not be detected in any of the algae examined. It is possible that the presence this isomer in the algae was masked by the large amount of n-C_{21} polyenes (n-$C_{21:5}$; 2043_{DBS} in particular). The tetraene br25:4; 2128_{DBS} detected only in the sediment underlying the algal mat (0.09 μgg^{-1} dry weight) was not isolated from the mat itself nor from any of the other algal specimens examined. Given this evidence, a macroalgal source (together with associated epiphytics) for this isomer seems unlikely.

Comparison of the distribution of C_{25} HBI alkenes in the hydrocarbons from the epipelic algae and sediment at Cargreen (Table 5.16) revealed the following; the tetraenes br25:4; 2128_{DBS}, br25:4; 2171_{DBS} and br25:4; 2184_{DBS} were absent from the algae, trienes br25:3; 2090_{DBS} and br25:3; 2108_{DBS} were present in both and the occurrence of diene br25:2; 2083_{DBS} and monoene br25:1; 2072_{DBS} was restricted to the microalgae. This suggests that the epipelic algae (dominated by *Navicula* spp.) may be a source of only the trienes br25:3; 2090_{DBS} and br25:3; 2108_{DBS} in the Cargreen sediment. Hence, the C_{25} HBI tetraenes may be derived from an alternative biological source or be the products of early diagenesis within either the water column or top most sediment.

The presence of HBI hydrocarbons in sediments from areas both covered with and void of macrophytic algae indicates that macrophytes are not the major source. Their presence in epipelic microalgae, dominated by diatoms, suggests this is a likely source as such microalgae, epiphytic or epipelic in nature, were common to both environments. However, HBI hydrocarbons were not identified in any temperate microalgae species in axenic culture screened during this study (Table 5.17), or previously (see reviews by Weete, 1976; Tornabene, 1981; Borowitzka, 1988).

TABLE 5.17 AXENIC CULTURES OF MICROALGAE SCREENED FOR THE PRESENCE OF HBI HYDROCARBONS

Species	Division	Common name
Thalassiosira weissflogii	Bacillariophyta	Diatoms
Thalassiora pseudonana	Bacillariophyta	Diatoms
Chaetoceros mulleri	Bacillariophyta	Diatoms
Nitzschia seriata	Bacillariophyta	Diatoms
Skeletonema costatum (constant and exponential growth phases)	Bacillariophyta	Diatoms
Dunaliella tertiolecta	Chlorophyta	Green algae
Tetraselmis tetrathele	Chlorophyta	Green algae
Olisthodiscus luteus	Chrysophyta	Golden algae
Cryptomonas maculata	Cryptophyta	Cyptomonads
Gonyaulax tamarensis	Pyrrhophyta (Dinophyceae)	Dinoflagellates
Scrippsiella trochoidea	Pyrrhophyta (Dinophyceae)	Dinoflagellates
Eutreptiella sp.	Euglenophyta	Euglenoids
Chrysochromulina kappa	Chrysophyta (Prymnesiophyceae)	Coccolithophores
Emiliania huxleyi	Chrysophyta (Prymnesiophyceae)	Coccolithophores
Isochrysis galbana	Chrysophyta (Prymnesiophyceae)	Coccolithophores
Phaeocystis pouchetti	Chrysophyta (Prymnesiophyceae)	Coccolithophores

Nichols *et al.* (1988) observed a C_{25} diene br25:2; 2088$_{MS}$ in sea-ice diatoms dominated by *Amphiprora*, *Nitzschia* and *Berkeleya* spp.. However, the authors could not identify any temperate diatom species in culture that contained the alkene. Although Nichols *et al.* (1988) dismissed bacteria as a source of C_{25} HBI alkenes in marine sediments, Grossi *et al.* (1984) hypothesised that microalgae stimulate bacterial growth in sea-ice possibly by providing the bacteria with organic substrates. Sullivan and Palmisano (1984) measured the distribution and abundance of sea-ice bacteria around McMurdo Sound, Antarctica in 1980 and found a correlation between bacterial numbers and chlorophyll *a* concentrations. They also reported a morphologically diverse epiphytic flora associated with diatoms of the genus *Amphiprora* (Sullivan and Palmisano, 1981). The number of bacteria in annual sea ice were shown to increase directly with the number of algae during the spring sea-ice diatoms bloom in McMurdo Sound. During December, 1981, the same month in which the sea ice diatom communities were to be collected by Nichols *et al.* in 1985 (1988), the bacterial coverage of most species was extensive. Filamentous and prosthecate bacteria dominated the epiphytes associated with *Amphiprora* in December but fusiform cells, cocci, and short and long rods were also abundant. *Amphiprora* sp. were reported by Nicohols *et al.* (1988) to be dominant in the mixed sea-ice diatom communities collected in 1985 and shown to contain the C_{25} HBI diene br25:2; 2088$_{MS}$. The authors considered the maximum bacterial carbon contribution estimated by Grossi *et al.* (1984) as insignificant (0.05%) compared to the average carbon contribution by algae and thus Nichols *et al.* (1988) considered diatoms to be the principal source of carbon and therefore lipids in sea-ice microbial communities.

Studies on algal-bacterial interactions in aquatic ecosystems are few (*e.g.*

Caldwell and Caldwell, 1978; Sullivan and Palmisano, 1981; 1984; Cole, 1982; Grossi *et al.*, 1984; see the discussion by Round, 1984). Studies are limited by the difficulties of plating and observing the bacteria. They have been reported to occur embedded in the mucilage of cyanobacteria (Kessel, 1975) or attached directly to the cell surface (Paerl, 1975; 1976) using a holdfast (Hirsh, 1972). Although the colonization of microalgae (including diatoms) has been shown to be prevented by the production of antibacterial substances (*e.g.* Bell *et al.*, 1974), physical and chemical alteration to provide a favourable surface and the release of extracellular organics may succeed in stimulating bacterial growth. Bacteria have been shown to persist as contaminants of microalgae in culture (Berland *et al.*, 1969) which may influence the hydrocarbon distribution isolated from axenic cultures grown to investigate the lipid composition of microalgae.

As scanning electron microscopy and/or bacteria counting was beyond the scope of the present study, no evidence can be provided for the bacterial colonization of epipelic diatoms at Cargreen or of macrophytic and epiphytic algae at Millbrook. Yet, as bacterial growth has been shown to be stimulated by the succession of microalgal growth throughout the year and is enhanced by dark conditions and in the presence of senescent algal cells, as apparent within the topmost sediment at Cargreen, an epiphytic bacterial source of HBI hydrocarbons in this case cannot be discounted.

As organic matter in sediments is ultimately derived from photosynthetic organisms, the sedimentary $\delta^{13}C$ values recorded for the C_{20} HBI hydrocarbons during this study reflect the mixing of $\delta^{13}C$ values of abundant plant species in the tidal mudflat environment (*i.e.* benthic epipelic diatoms and macroalgal epiphytes). The

variability of $\delta^{13}C$ values, recorded at different sites and times, indicates that the isotopic compositions of the source organism(s) in the Tamar may have varied significantly, either because of the existence of different source organisms, or due to ecological (*e.g.* blooming; Deuser, 1970) or physiological (*e.g.* bicarbonate pumping; Popp *et al.*, 1989) factors, as suggested by Kohnen *et al.* (1992). It is acknowledged that epipelic diatoms may be one source of HBI hydrocarbons in Tamar sediments (see 5.8) and that the isotopic compositions of the C_{20} HBI hydrocarbons recorded in Cargreen sediment in April, compare well with that of benthic diatoms in a salt marsh (-16.2 ‰ to -17.9 ‰; Haines, 1976) and in coastal sediments (-18.4 ‰; Fry *et al.*, 1977). The difference in $\delta^{13}C$ values between April and July could be attributed to diatom communities with different carbon demands. Hence the $\delta^{13}C$ value recorded for **4** (-18.3 ‰) and that of the corresponding alkane **1** (-19.1 ‰) could have been caused by growth during a period of limited CO_2 concentration in the topmost sediment or possibly in the water column resulting in isotopic disequilibria and a preferred ^{13}C incorporation. Such values (*ca.* -18 ‰) were recorded in highest biomasses in sea-ice cores dominated by pennate diatoms in the Antarctic during the more or less closed sea-ice system (Fischer, 1991). Measurements of $\delta^{13}C$ of other sea-ice pennnate diatoms released from melting ice elsewhere in the Antarctic revealed values around -28 ‰, similar to those values obtained for **4** and phytol from Millbrook sediment in July. It is assumed that diatom growth occurred during the summer period at Millbrook under non-limiting CO_2 conditions resulting in $\delta^{13}C$ values more depleted in ^{13}C than in April at Cargreen. It has been suggested (*e.g.* Fischer, 1991) that differences in $\delta^{13}C$ values may linked to productivity, high productivity being linked to ^{13}C enrichments in phytoplankton carbon. High internal

carbon demands as a result of rapid carbon fixation may cause isotopic disequilibria in and/or around the cells, reducing the magnitude of the isotopic fractionation (Degens *et al.*, 1968; Deuser, 1970; Estep *et al.*, 1978; Smith and Walker, 1980; O'Leary, 1981; Kerby and Raven, 1985). However, no general relationship between the $\delta^{13}C$ of particulate organic carbon and primary production has been found when production is low (Fontugne, 1983; Descolas-Gros and Fontugne, 1990).

The ^{13}C-depletion of lipids in estuarine sediments (Parker, 1964) and a wide range of biological samples (Abelson and Hoering, 1961; Parker, 1964; Degens *et al.*, 1968; DeNiro and Epstein, 1977; Monson and Hayes, 1982ab) has also been attributed to isotope effects associated with the biosynthesis and cycling of the lipid precursor, acetyl CoA (DeNiro and Epstein, 1977; Monson and Hayes, 1982ab; Blair *et al.*, 1985). Given the possibility of a bacterial source for the HBI **4** expressed earlier, ^{13}C enrichment by heterotrophic bacteria recycling autochthonous organic matter via such a route cannot be discounted. Indeed, the 2 ‰ enrichment of ^{13}C in the HBI monoene **4** relative to phytol does indicate a heterotrophic source for **4** in Millbrook sediments in July, using algal detritus as a food source. However, it would be very difficult to distinguish between the lipids of either autotrophic microalgae or heterotrophic bacteria involved in the degradation of planktonic algal matter in the water column or top-most sediments by comparison of isotopic composition alone as it is acknowledged that additional phytol in the sediment may be derived from non-algal sources, namely higher plant chlorophylls and/or bacteriochlorophyll *a*. Even so, the isotopic composition of phytol has proved a useful comparison during this study. This compound, also a C_{20} monounsaturated isoprenoid, mainly originates from the phytyl side-chain of chlorins (chlorophylls *a*, *b* and *d*, and bacteriochlorophyll *a*;

Didyk *et al.*, 1978; Gillian and Johns, 1980) and the major contributor of phytol to the top-most oxic sediment or water column is likely to be limited to autochthonous input from the degradation of algal chlorophyll. A higher plant origin for phytol in these sediments is thought unlikely because of the facile and rapid degradation of chlorophyll prior to reaching the surface of the mudflats (Hendry *et al.*, 1987). Although isotopic fractionation during carbon flow through biosynthetic pathways has been reported (O'Leary, 1981; Griffiths, 1991) and resulting isotopic heterogeneities in different compounds classes recorded (Blair and Carter, 1992), the difference in $\delta^{13}C$ values for HBI **4** and phytol in July sediment (Table 5.13) is unlikely to be derived from such a source as it is anticipated that both compounds are biosynthesised via the same mevalonate pathway. Assuming the phytol in Millbrook sediment in July was derived from algal chlorophyll, this suggests that the source of **4** at this time, was not photosynthetic organisms as was assigned previously for the alkane **1** and related monoene **4** in Cargreen sediment in April, but more likely to be heterotrophic bacteria feeding upon the benthic algae. It is acknowleged, however, that the lipids of photoautotrophic bacteria, also a potential source of phytol to the sediment, use dissolved CO_2 as their carbon source and may be isotopically similar to lipids derived from autotrophic algae living in the same environment. Hence, given the narrow range in isotopic variation between compounds of different biological origins (*e.g. ca.* 3 ‰; Schoell *et al.*, 1992), the separation of compounds of algal and bacterial origin is not simple. Confirmation of an algal source of phytol in the sediments could be provided by further CSIA of the compound isolated from Cargreen sediment in April, if it proved to be as enriched with ^{13}C as the HBI monoene **4** (-18.3 ‰) and alkane **1** (-19.1 ‰).

The distribution of C_{20} HBI hydrocarbons, in the sediments at Cargreen, showed that the abundance of 1 and 4 was not at a seasonal maximum at the time of the summer epipelic diatom bloom (as reflected by the maximum sedimentary n-$C_{21:6}$ concentration in August). This suggests that the biosynthesis of 1 and 4, known to be present in some epipelic diatoms isolated from Cargreen sediment (*Navicula* spp.), may be related to changes in the stages of algal growth. The increased production of n-$C_{21:6}$ by epipelic diatoms via decarboxylation of n-C_{22} polyunsaturated fatty acids, corresponds to the later part of the expontential growth phase (Ackman *et al.*, 1964). Heavy isotopic compositions of C_{20} HBI hydrocarbons (-18.3 ‰ 4 and -19.1 ‰ 1) were recorded in April, when C_{20} HBI hydrocarbons were seen to be abundant in the sediment. As such enrichment in ^{13}C is thought to reflect high photosynthetic productivity (Fry and Wainwright, 1991; Fischer, 1991), epipelic diatoms could synthesis C_{20} HBI hydrocarbons during the beginning of a period of rapid growth. It is difficult, however, to preclude heterotrophic bacteria as the source of the C_{20} HBI hydrocarbons in these sediments as their population, as well as isotopic composition, are likely to mirror those of an algal carbon source.

The distribution of C_{25} HBI alkenes was shown to be as widespread in sediments and algae as the C_{20} HBI hydrocarbons described previously. The variation in seasonal abundance of the C_{25} isomers, precludes a single biological synthesis since HBI concentrations in the sediment peak at different times of the year. This may, however, be a reflection of differences in rates of removal by degradation and sulphurisation caused by the number, position and stereochemistry of the double bonds. The covariance of the abundance of some C_{25} HBI alkenes with both vascular plant n-alkanes and n-$C_{21:6}$, suggested a relationship between remineralisation of

terrigenous and algal organic matter and the HBI distribution and hence, possibly heterotrophic bacteria feeding on the organic matter, as a source of HBI hydrocarbons.

5.13 SUMMARY

The widespread occurrence of C_{20} HBI hydrocarbons in Tamar sediments and associated algae (macrophytes and diatoms), the large variation in isotopic composition evident for the monoene **4**, and the seasonal sedimentary distribution all suggest three possible sources for the HBI hydrocarbons at the two sites; a microalgal origin (but biosynthesised under somewhat different conditions of growth at the particular sites and times of year), a dual source derived from microalgae blooming in April at Cargreen and heterotrophic bacteria in July at Millbrook, or from heterotrophic bacteria, the population and isotopic composition of which is dependent upon that of the carbon source (*i.e.* the stage of algal growth).

STRUCTURES

CHAPTER FIVE
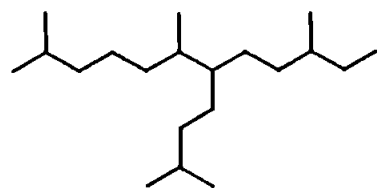
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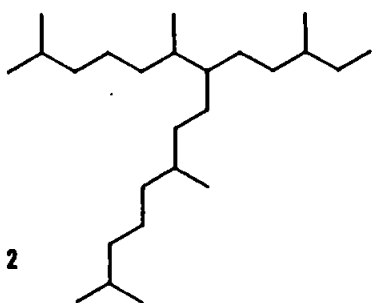
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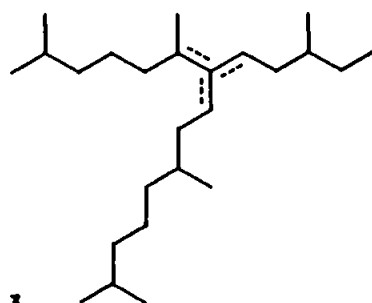
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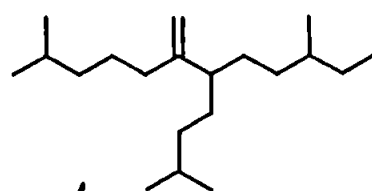
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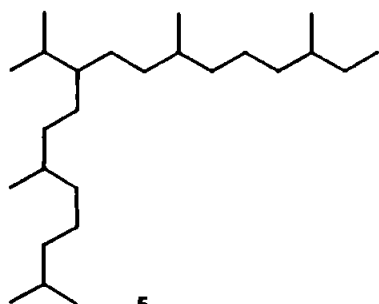
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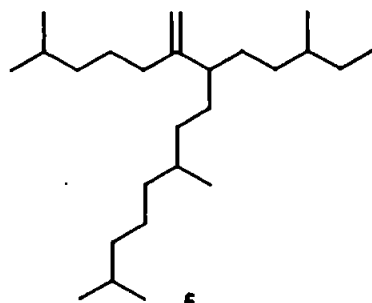
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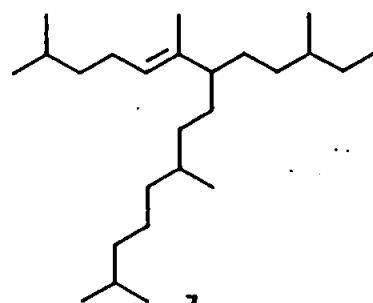
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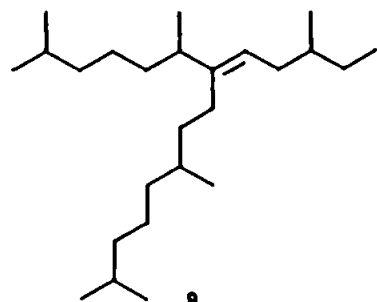
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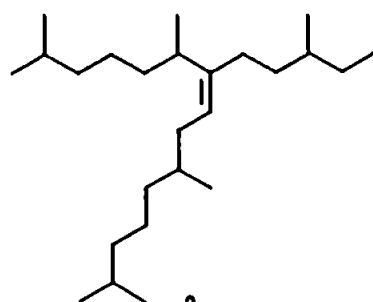
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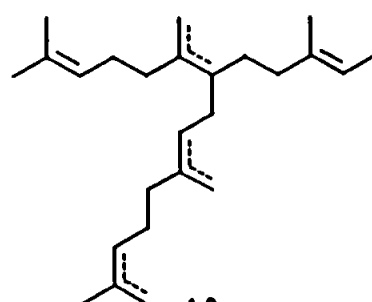
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CHAPTER SIX

THE DIAGENETIC FATE OF C_{25} HBI ALKENES
IN SEDIMENTS FROM THE PERU UPWELLING REGION

This chapter describes the distribution of hydrocarbons from Peru upwelling area. The composition of sedimentary humic acids and synthetic melanoidins are compared. A mixture of C_{25} HBI monoenes are successfully incorporated into the melanoidins but not detected in the humic acid pyrolysate. The implications of these results are discussed.

6.1 INTRODUCTION

In off-shore Peru, high sedimentary organic carbon contents are a direct consequence of the extremely high primary productivity (*ca.* 1000g carbon $m^{-2}yr^{-1}$; Reimers and Suess, 1983) which, in turn, is supported by the upwelling of nutrient-rich waters near the coast. Diatoms represent the major phytoplankton type and give rise to sediments dominated by biogenic silica and planktonic organic matter. The remineralisation of this large flux of organic matter to the bottom waters and sediments results in oxygen depletion over large areas of the shelf which, in turn, promotes organic carbon preservation in the underlying sediments. Sulphide from sulphate reduction is prevalent in the bottom waters (Fossing, 1990) and with a limited availability of iron (due to the dominant biogenic input coupled with a very low influx of detritral sediments) the excess sulphide is available for reaction with organic matter. As a result high organic sulphur concentrations are found in the sediments (Mossman *et al.*, 1990; Patience *et al.*, 1990).

The coastal Peru upwelling region is believed to be a modern analogue to the depositional environments of petroleum source rocks such as the Miocene Monterey Formation of the California Borderland (Soutar *et al.*, 1981). Because organic matter alteration pathways in surface sediments utimately influence kerogen type and eventual petroleum yield, there has been interest in characterising surface sediments such as those off-shore Peru. In addition, the excellent preservation of climatic records in the constituent organic matter of the sediments has resulted in extensive organic geochemical study of the Peru upwelling area. These studies have addressed both water column particulate matter (Gagosian *et al.*, 1983ab; Repeta and Gagosian, 1983; Wakeham *et al.*, 1983; 1984) and the underlying sediments (Smith *et al.*,

1983ab; Poutanen and Morris, 1983; Reimers and Suess, 1983; Volkman *et al.*, 1983; 1987; Henrichs and Farrington, 1984; Henrichs *et al.*, 1984; Rowe and Howarth, 1985; Cooper *et al.*, 1986ab; Repeta and Gagosian, 1987; Farrington *et al.*, 1988; McCaffrey *et al.*, 1989; 1990; 1991; Farrimond *et al.*, 1990; ten Haven *et al.*, 1990a) and have been directed to a wide variety of molecular components (sterols, fatty acids, ketones, hydrocarbons, carotenoids, amino acids and humic acids). Most of these scientists discussed the biological marker distribution of surface sediments in the Peru upwelling area, the probable biological sources of the organic matter, and its early diagenetic modification. However, studies of the macromolecular components of the sediments have been less extensive (Poutanen and Morris, 1983; Patience *et al.*, 1990; 1992; Eglinton *et al.*, 1991; Aplin *et al.*, 1992; Rees, unpublished data).

The process by which biopolymers, the remnants of living organisms in the water column or buried in sediments, are degraded and rearranged into insoluble geopolymers is usually referred to as diagenesis. The conventional view of the processes involves the microbial degradation of biological macromolecules into smaller components; condensation of these small highly functionalised compounds into geopolymers such as humic acids, fulvic acids and less functionalised humin residues; and insolubilisation of these condensed structures via elimination of hydrophilic functional groups to form insoluble kerogen (Tissot and Welte, 1984; Figure 6.1). Marine humic substances have been suggested to derive from sugar-amino acid condensation. These geopolymeric products have been referred to as melanoidins which may also be produced by a condensation reaction under laboratory conditions (Larter and Douglas, 1980; Wilson *et al.*, 1983; Boon *et al.*, 1984; Rubinsztain *et al.*, 1984). One of the compound classes thought to be incorporated into accreting
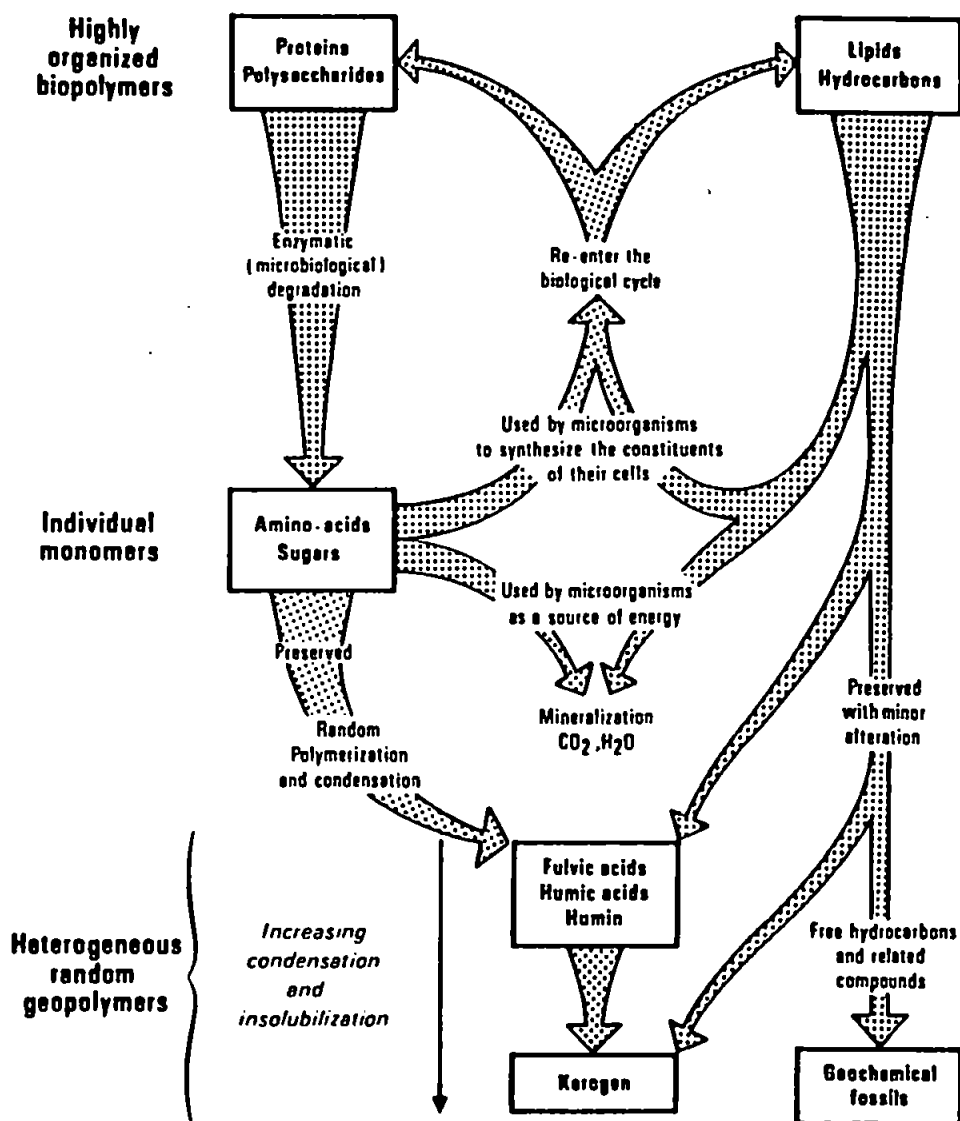
FIGURE 6.1 FROM BIOMASS TO KEROGEN - A SUMMARY OF THE
CONVENTIONAL VIEW OF PROCESSES INVOLVED IN THE
TRANSFORMATION OF BIOLOGICAL MATTER TO
KEROGEN AND GEOCHEMICAL FOSSILS (from Tissot and
Welte, 1984)

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kerogens are olefinic lipids (Cane and Albion, 1973; Van der Berg *et al.*, 1977; Larter *et al.*, 1983).

Analytical pyrolysis is the simplest tool for the degradation of macromolecular fossil organic matter into smaller units and their subsequent on-line identification, commonly by gas chromatography, mass spectrometry or a combination of both (see the reviews by Philp, 1982; Larter and Horsfield, 1990). Pyrolysis gas chromatography (PYGC) or pyrolysis gas chromatography-mass spectrometry (PYGC-MS), pyrolysis mass spectrometry (PYMS), and more recently pyrolysis gas chromatography-atomic emission detection (PYGC-AED), have been shown to be powerful discriminatory tools for the "typing" of organic matter including biopolymers, humic substances and kerogens (Wilson *et al.*, 1983; Nip *et al.*, 1986; Larter and Douglas, 1980; 1982; Philp, 1982; Eglinton *et al.*, 1991; Sinninghe Damsté *et al.*, 1989d, 1990b). Pyrolysis of all five ODP Leg 112 sediments produced very complex distributions comprising over 400 different pyrolysate products ranging from gases (*e.g.* methane and carbon dioxide) to compounds up to C_{30} (Patience *et al.*, 1990; Rees, unpublished data). Comparison of the PYGC-MS TIC and PYGC-AED carbon emission line chromatograms showed that all the samples were similar to one another, as expected for sediments which have received a fairly uniform input of organic matter (Rowland *et al.*, 1992b). Such distributions were characteristic of sedimentary organic matter and not living organisms (Patience *et al.*, 1990). Aplin *et al.* (1992), using PYGC-MS, showed that the organic matter, even in the topmost Peru sediment, was significantly altered from the composition of bacteria and algae.

Nichols *et al.* (1988) and Sinninghe Damsté *et al.* (1989ab) have suggested that C_{25} HBI alkenes are derived from diatoms which are the dominant phytoplankton

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off the coast of Peru. In surface sediments (0-2 cm) from the upwelling area, the C_{25} HBI alkenes, with carbon skeleton 1, were far more abundant than either n-alkanes of higher plant origin or isoprenoid hydrocarbons (*e.g.* pristane and cholestenes) thought to derive from algal inputs (Volkman *et al.*, 1983). The high concentrations of these alkenes in solvent extracts (26 mgkg^{-1}) from surface sediments decreased rapidly with increased sediment depth (<3 mgkg^{-1}; 19 cm). Few explanations have been given for the rapid removal of C_{25} HBI alkenes from the hydrocarbon fraction. Laboratory-based studies conducted by Robson and Rowland (1988b) and Gough *et al.* (1992) showed the parent alkane 1 and a mixture of related monoenes 2, to be more resistant to aerobic biodegradation than n-alkanes, n-alkenes and branched alkanes of the same molecular weight. Therefore, biodegradation cannot be considered as a major mechanism causing the observed trends since, for instance, Volkman *et al.* (1983) observed a slight increase in the concentration of n-alkanes with depth, which was in sharp contrast with the profile of HBI alkenes. It has been postulated that the decrease in sedimentary concentrations with depth, in the Peru upwelling area, may be due to incorporation into accreting humic substances, perhaps following any biodegradation (Volkman *et al.*, 1983).

A coastal marine sediment from the Peru upwelling region (112-679D-1-1, 25-35 cm) was examined to determine the distribution of C_{25} HBI alkenes in both free lipid and humic acid fractions, in order to investigate the fate of such sedimentary HBI compounds during early diagenesis.

6.2   RESULTS AND DISCUSSION

The locations of the ODP Leg 112 sites, Peru margin and shelf, are shown in Figure 6.2. Full discussion of the organic geochemistry of the organic matter in the Peru upwelling sediment cores is given in Suess *et al.* (1990). Sample 112-679D-1-1, 25-35 cm, a dark, olive green diatomaceous/foraminiferal ooze, was from lithological Unit I of Holocene-Pleistocene age.

6.2.1   HYDROCARBONS

Examination of the hydrocarbons (Figure 6.3A) isolated from sediment confirmed that C_{25} HBI alkenes were present in the solvent extract at this site. As expected at this depth, the alkenes were only minor components relative to, for example, n-alkanes ($\Sigma br25$; 0.2 mgkg^{-1}). Furthermore the distribution was somewhat different to that obtained at 19 cm by Volkman *et al.* (1983; Figure 6.3B).
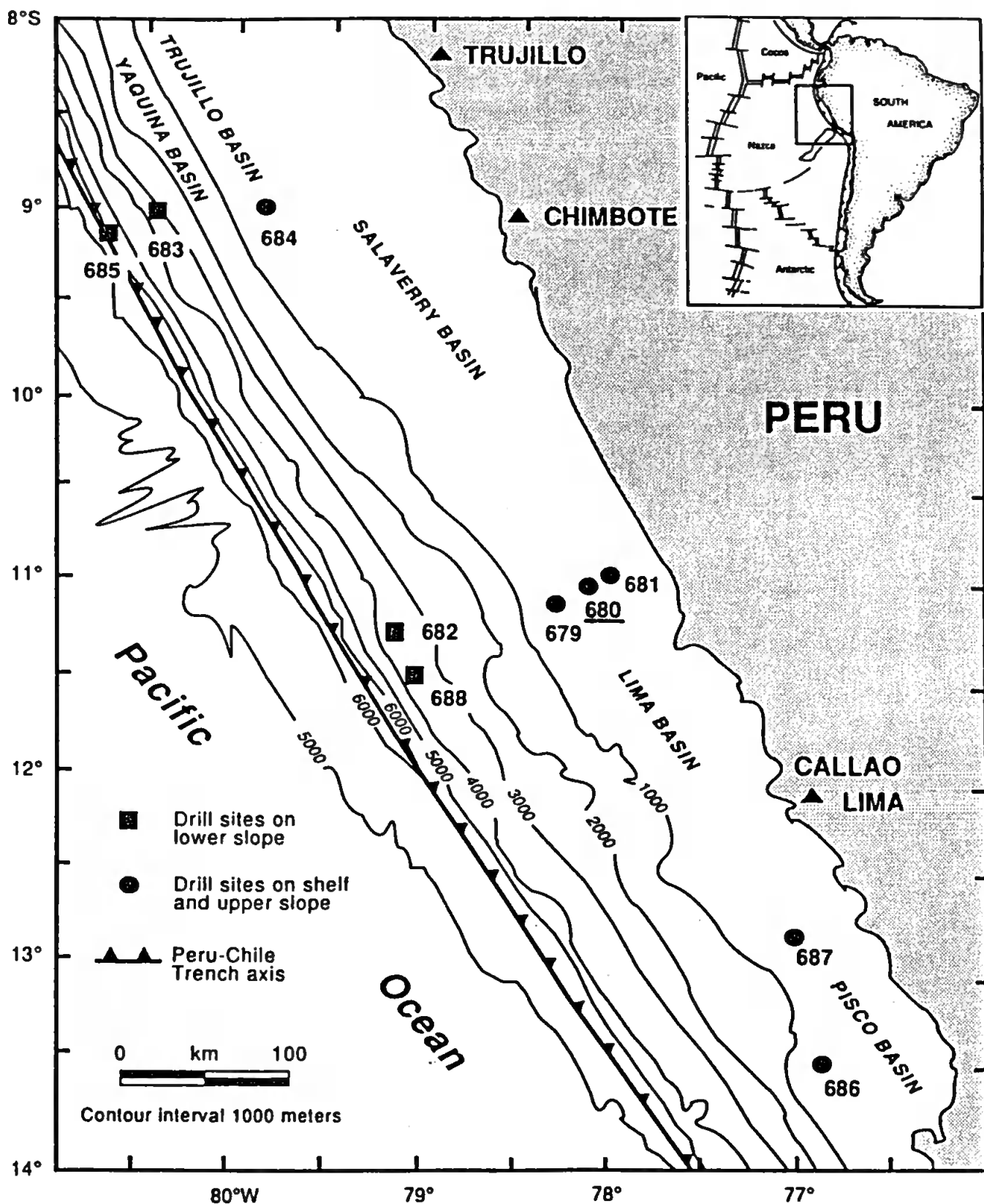
FIGURE 6.2 MAP SHOWING SITE LOCATIONS, LEG 112, PERU MARGIN AND SHELF (Suess *et al.*, 1990)

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A PERU
ODP 112, 679D-1-1, 25-35 cm
HYDROCARBONS
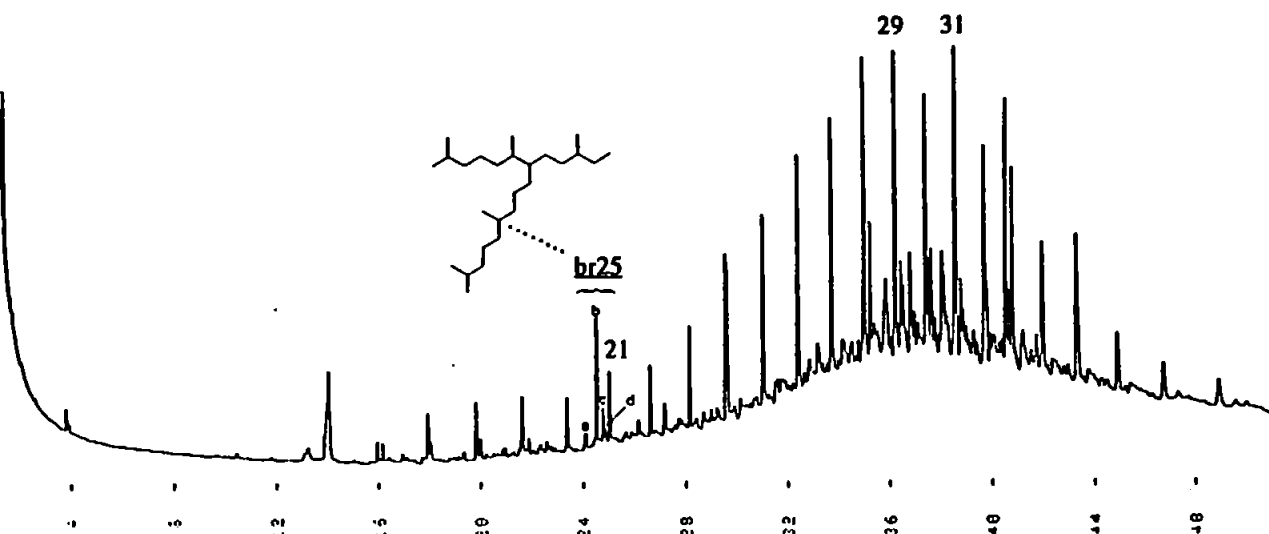
B PERU
BC 7 (0-1) cm
HYDROCARBONS
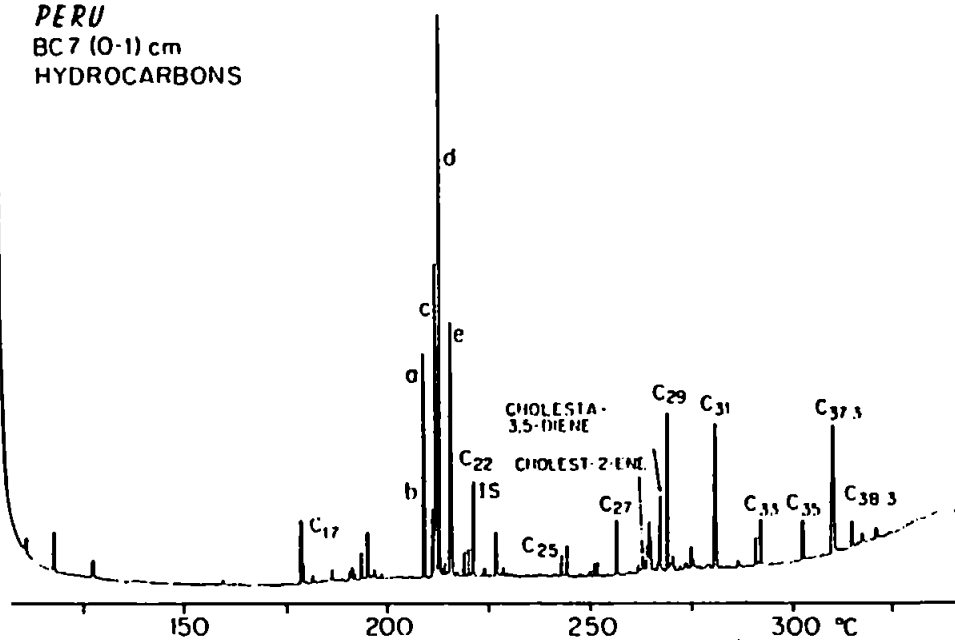
Gas chromatogram of total hydrocarbons in BC7 surface sediment; 20 m × 0.30 mm i.d. WCOT SE-52 capillary column programmed from 80 to 330 °C at 4 °C/min.

FIGURE 6.3 GAS CHROMATOGRAMS OF HYDROCARBONS FROM
(A) ODP Leg 112, 679D-1-1, 25-35 cm
(B) BC 7 (0-1) cm (Volkman *et al.*, 1983).
Key: a br25:3; 2044, b br25:2; 2070, c br25:4; 2084, d br25:3; 2090
Conditions see text; DB5 (or equivalent).

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6.2.2 HUMIC ACIDS

Flash pyrolysis of the humic acids isolated from the sediment was carried out to observe whether the alkenes, or more likely, fragments of the alkenes could be released. The total ion current (TIC) chromatogram of the pyrolysis products recorded by PYGC-MS showed no C_{25} HBI alkenes or recognisable fragments to be present (Figure 6.4). This was confirmed by gas chromatography of the hydrocarbons isolated from the trapped pyrolysate. A mixture of synthetic C_{25} HBI monoenes 2 was also subjected to the same PYGC-MS procedure which showed that the HBI carbon skeleton did not fragment during pyrolysis under the conditions used (610°C for 20 seconds; Figure 6.5). The humic acid fraction comprised abundant aromatic hydrocarbons (e.g. toluene, alkylbenzenes, napthalenes and alkyl-indenes), nitrogenous compounds (e.g. alkylpyridines, alkylpyrroles and alkyl-indoles), and alkyl phenols; all typical products of the pyrolysis of proteins (e.g. Klok et al., 1984). Contributions from alkylthiophenes, alkylfurans and homologous n-alkane/1-alkene doublets, derived from sulphur-containing macromolecular substances, carbohydrates and lipid-rich terrigenous organic matter respectively, were all minor. The absence of substituted methoxyphenols was evidence that lignin was not a major contributor to the organic matter of this sediments. In general, these data, summarised in Table 6.1, are consistent with results of other analyses carried out on whole sediments from the Peru upwelling region (Patience et al., 1990; 1992; Whelan et al., 1990; Aplin et al., 1992; Rees, unpublished data; Rowland et al., 1992b). Patience et al. (1992) and Rowland et al. (1992) used the selectively of atomic emmission detection (PYGC-AED) to reduce the complexity of the pyrolysis chromatogram to monitor nitrogen- and sulphur-containing pyroproducts respectively.
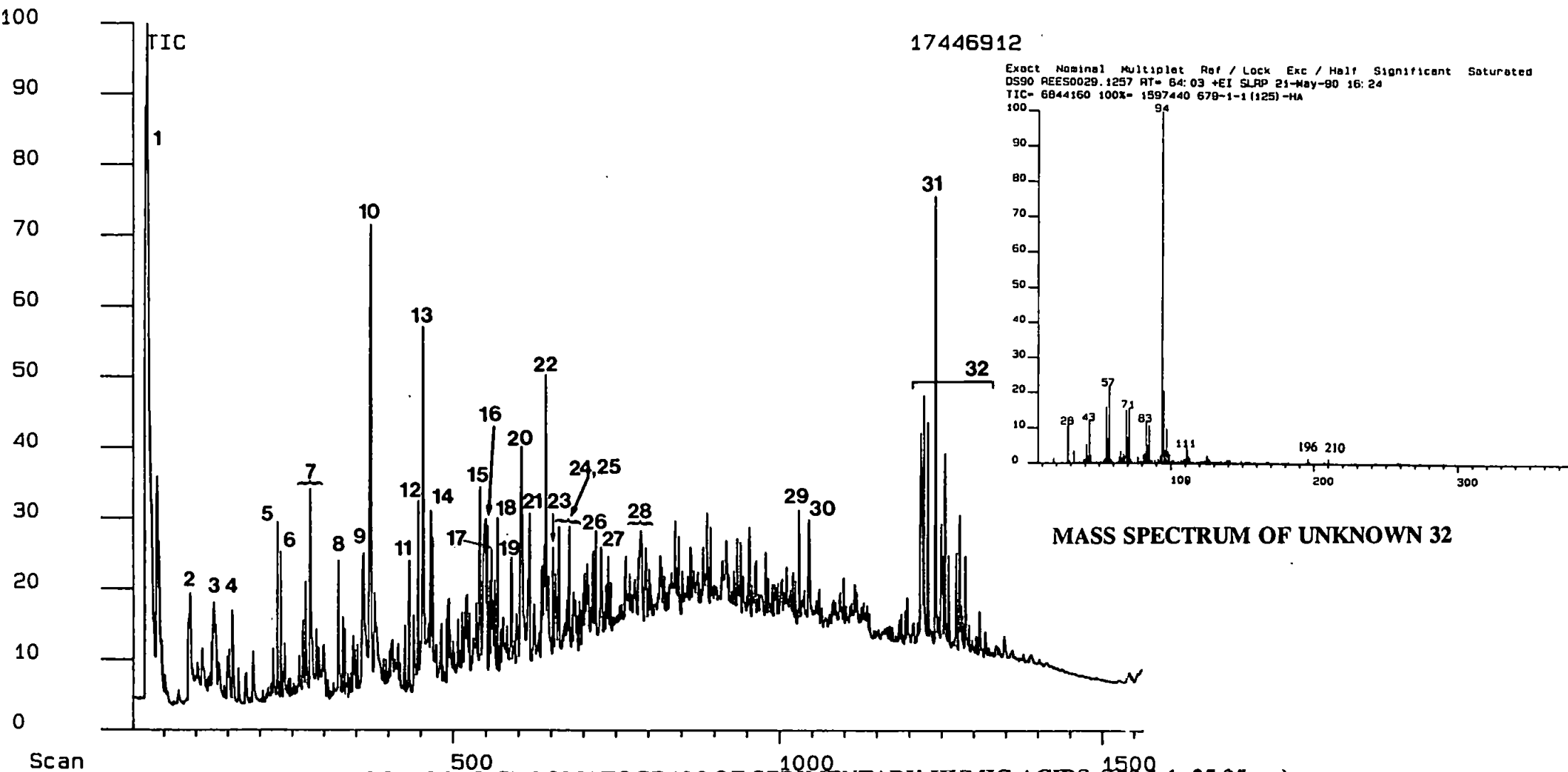
FIGURE 6.4 PYGC-MS TIC CHROMATOGRAM OF SEDIMENTARY HUMIC ACIDS (679-1-1, 25-35 cm)

MASS SPECTRUM OF UNKNOWN 32

TABLE 6.1   SUMMARY OF PEAK ASSIGNMENTS FROM PYGC-MS OF HUMIC ACID FRACTION (679-1-1, 25-35 cm, HA)

NUMBER	PEAK ASSIGNMENT
1	H_2S, CO_2, CH_4 and COS
2	acetonitrile
3	a pentadiene + ?
4	propanitrile
5	hexene
6	a methylfuran
7	benzene + thiophene + pyrrole
8	heptene
9	pyridine
10	toluene + methylthiophene
11	methylpyrroles
12	ethylbenzene
13	m-/p-xylenes
14	styrene + o-xylene
15	C_3-benzene
16	phenol
17	C_3-benzene
18	indene
19	o-cresol
20	m-/p-cresols + ethylstyrene
21	undecene
22	benzothiophene
23	naphthalene
24	dodecene
25	dodecane
26	indole
27	methylnaphthalenes
28	dimethylnaphthalenes
29	dibutylphthalate
30	hexadecanoic acid
31	dioctylphthalate
32	cluster of isomeric unknowns
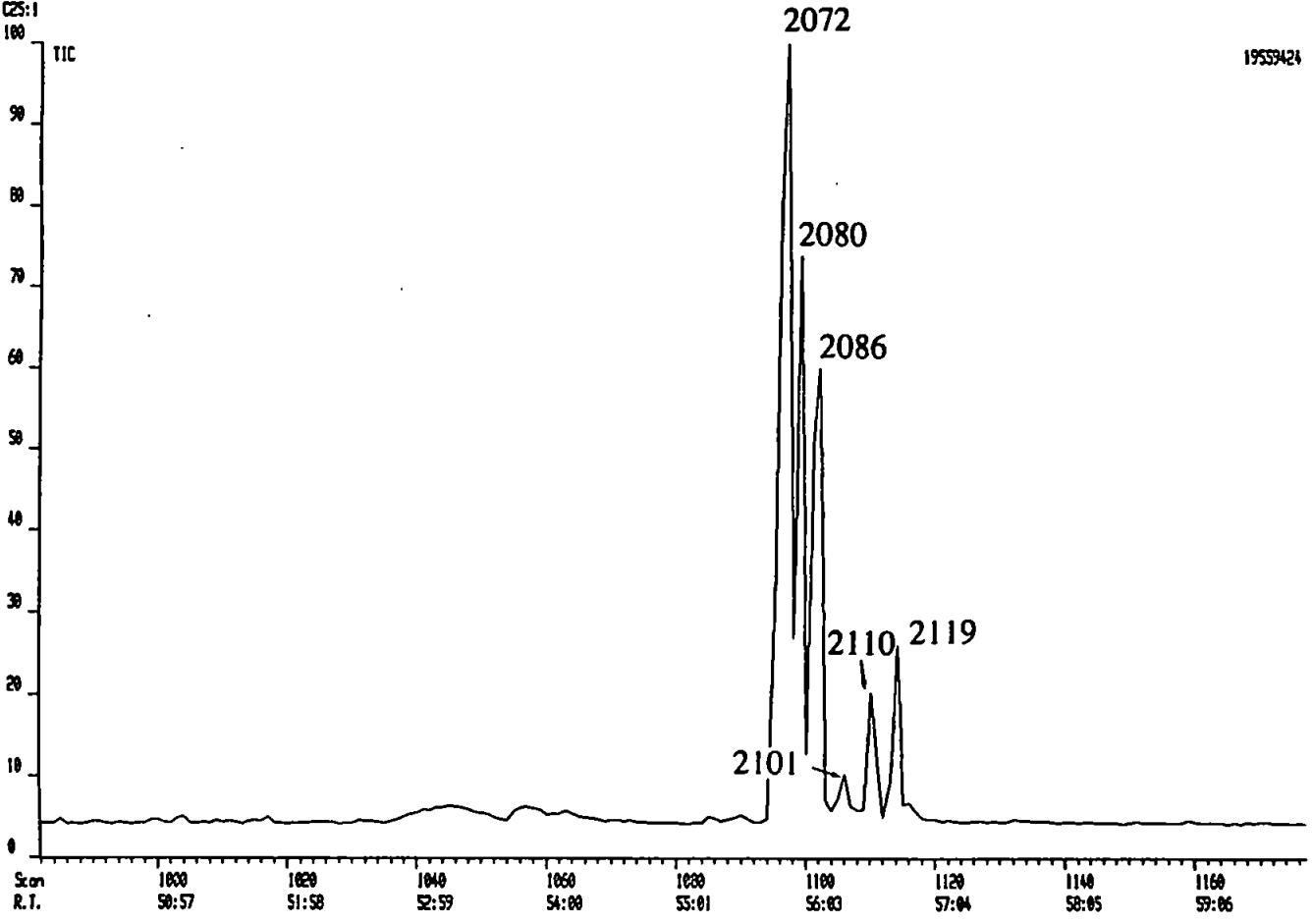
FIGURE 6.5 PYGC-MS PARTIAL TIC OF C_{25} HBI MONOENES
(numbers are GC RI_{DB5})

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The major sulphur products included H_2S, methanethiol, carboxysulphide and carbon disulphide with minor amounts of thiophene and C_1-C_3 alkylated homologues whereas the nitrogen compounds could be divided into amino, pyrrole, pyridine and (tentatively) quaternary structures. However, a cluster of high-molecular peaks were evident in the chromatogram, the mass spectra of which were very similar (Figure 6.6) dominated by an ion at m/z 94. Such compounds were not reported by other workers during pyrolysis analysis of whole sediments from the area, hence they may be artifacts of the humic substance isolation procedure. Another component which eluted within the cluster was identified as dioctylphthalate, a common plasticiser.

6.2.3   MELANOIDINS

Melanoidins, acidic polymeric products of amino acid/sugar condensation reactions, have been shown to have many of the properties of humic acids (Larter and Douglas, 1980; Rubinsztain *et al.*, 1984) and can bind lipid molecules into high molecular weight complexes; thus have been proposed as model humic acids (Boon *et al.*, 1984). Pyrolysis of synthetic melanoidins, spiked with synthetic C_{25} HBI monoenes 2, released the recognisable mixture of isomers (Figure 6.7). This was confirmed by gas chromatography of the hydrocarbons isolated from the trapped pyrolysate (2% w/w of melanoidin; 5% w/w of spiked alkenes). The melanoidin pyrolysis products (Figure 6.8; Table 6.2) also comprised aromatic hydrocarbons (*e.g.* toluene, alkylbenzenes, napthalenes and alkyl-indenes), nitrogenous compounds (alkylpyridines and alkylpyrroles), and alkyl phenols; all typical products of the pyrolysis of proteins and amino acids. However, the most abundant compound type were alkylfurans, presumably derived from the glucose. Although there did seem to

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FIGURE 6.7 PYGC-MS TIC CHROMATOGRAM OF MELANOIDINS SPIKED WITH C_{25} HBI MONOENES

DS90 Chromatogram report Run: REES0030,   21-May-90 19:28
MEL.HLK
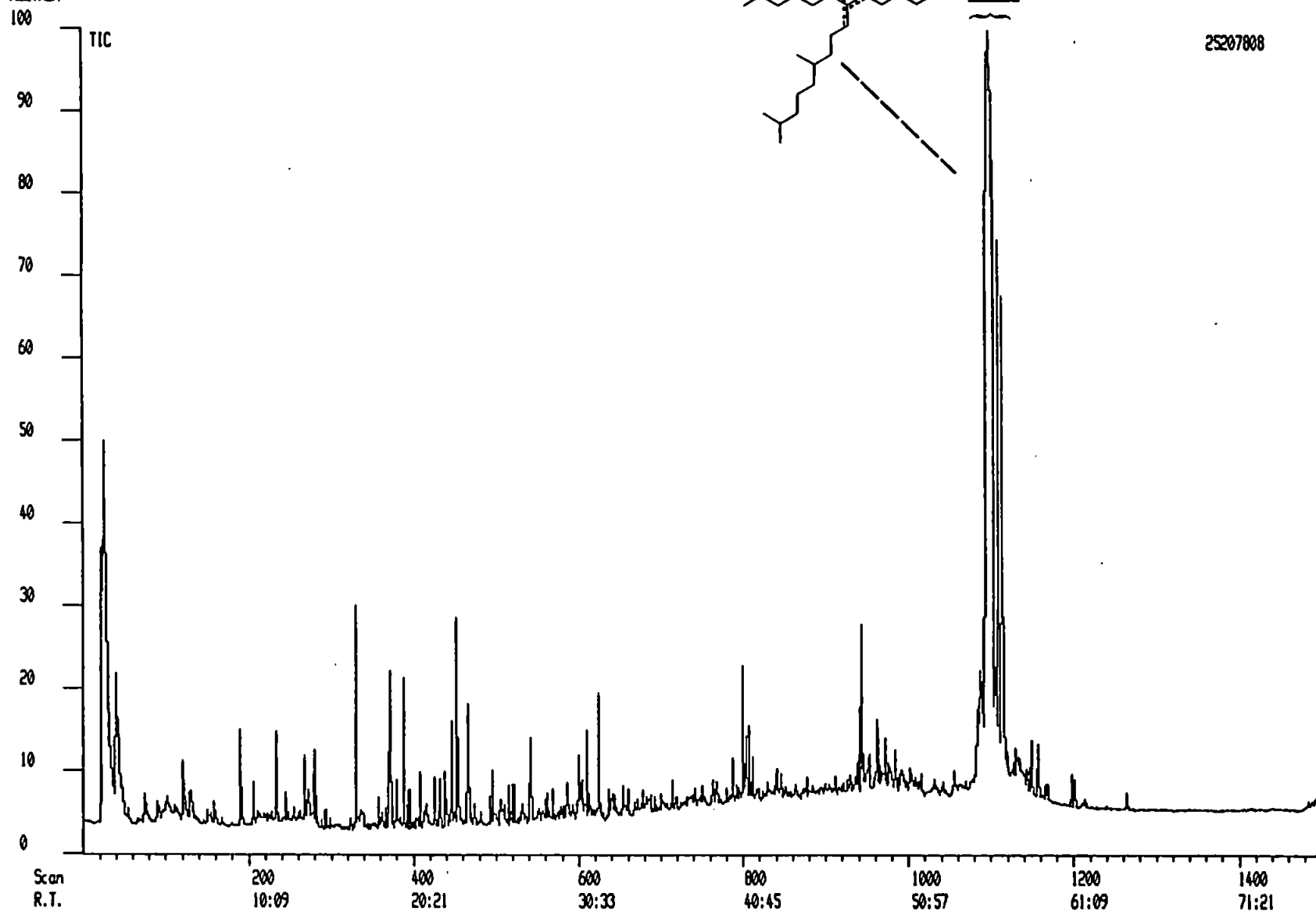
FIGURE 6.8 PYGC-MS TIC CHROMATOGRAM OF MELANOIDINS

DS90 Chromatogram report   Run: REES0028.   21-May-90 13:56
MEL
14903808
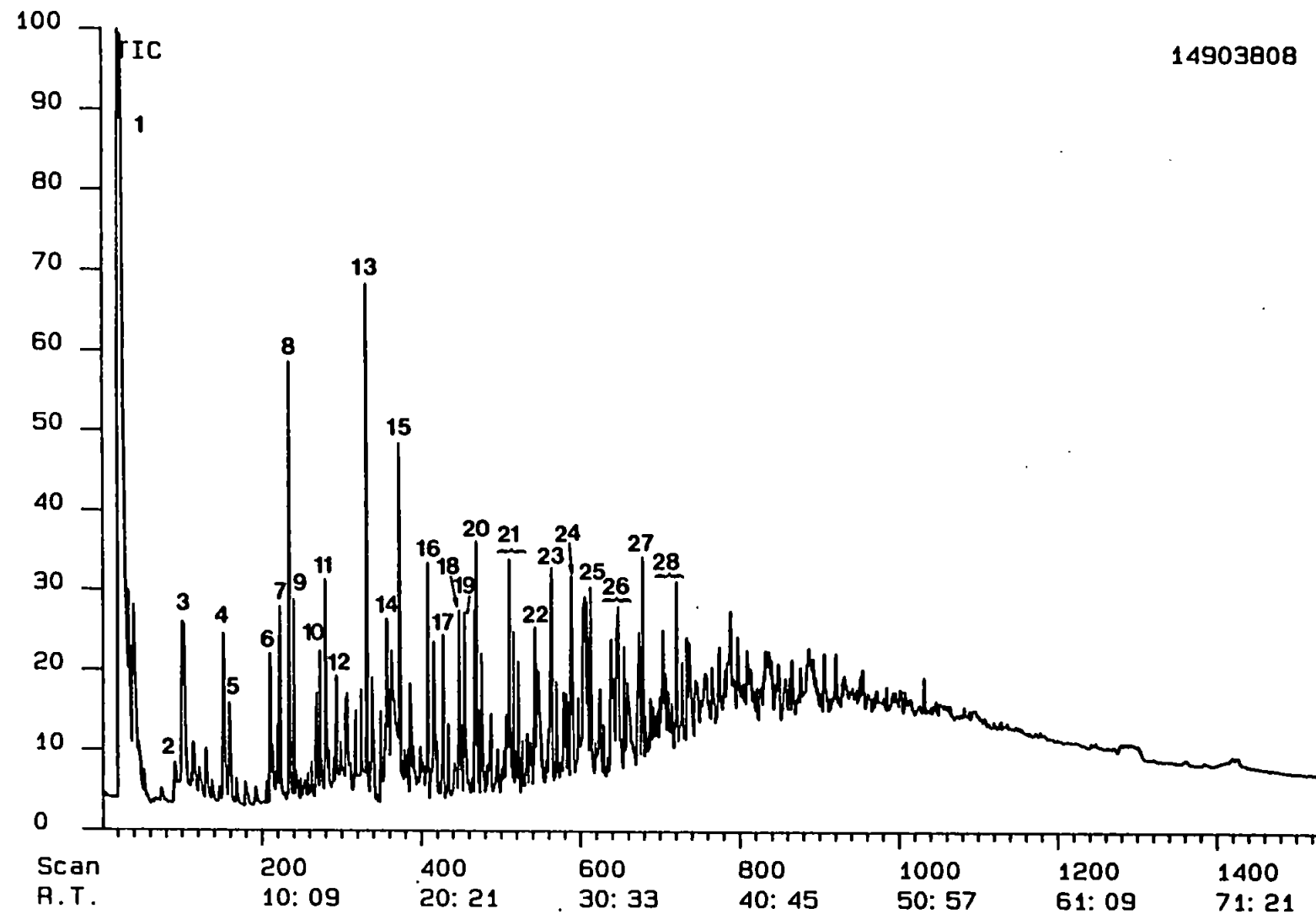
TABLE 6.2 SUMMARY OF PEAK ASSIGNMENTS FROM PYGC-MS OF MELANOIDINS

NUMBER	PEAK ASSIGNMENT
1	H_2S, CO_2, CH_4 and COS
2	acetonitrile
3	2-propanone
4	dichloromethane
5	propanitrile
6	2-butenone
7	2-butanone
8	a methylfuran
9	3-pentanone
10	cyclohexadienes
11	benzene + pyrrole
12	thiophene
13	a dimethylfuran
14	pyridine
15	toluene + methylthiophene
16	C_2-furans?
17	methoxybenzene?
18	ethylbenzene
19	m-/p-xylenes
20	styrene + o-xylene
21	C_3-furans
22	phenol
23	C_4-furans
24	o-cresol
25	m-/p-cresols + ethylstyrene
26	C_2-phenols
27	dimethylbenzofuran
28	methylnaphthalenes

be some similarity between the PYGC-MS TIC chromatograms, a more detailed examination revealed the dominance of alkylfurans in the melanoidin pyrolysate as the main difference between the sedimentary humic acids and synthetic melanoidins (Figure 6.5; *cf.* Figure 6.8).

6.3 CONCLUSIONS

Even though alkene-melanoidin interaction has been shown to occur, no C_{25} HBI compounds or fragments were released by pyrolysis of the sedimentary humic substances. During PYGC-MS of the spiked melanoidins, it was noted that more C_{25} HBI monoenes were released from the melanoidin matrix by thermal desorption (250°C for 5 min.) than during pyrolysis. As the spiked melanoidin mixture was extensively solvent extracted (dichloromethane) prior to analysis, release of the bound HBI alkenes by thermal desorption suggests a relatively weak physical interaction between alkene molecules and melanoidins rather than covalent chemical bonds cleaved by pyrolysis. The HBI alkenes may have become physically occluded within the matrix during the formation of the melanoidins and were released upon internal bond movement, similar to the swelling of pores of dust particles, during heating. Alternatively, a weak dipole-dipole interaction may have occurred involving the π bond electrons of the monoenes and electrophilic groups within the melandoidins.

The discovery of the insoluble, non-hydrolysable, highly aliphatic biopolymers in extant organisms and geological samples has led to a reappraisal of the processes involved in kerogen formation (de Leeuw and Largeau, 1990; Tegelaar *et al.*, 1989). In the modified scheme (Figure 6.9; *cf.* to Figure 6.1), more emphasis is placed on the selective preservation of biopolymers. Hence, it becomes evident that simulation

of kerogen formation by heating, *e.g.* amino acids and sugars (melanoidin formation) may no longer be the optimal approach (Rullkötter and Michaelis, 1990). It has yet to be seen whether the formation of humic substances is related to kerogen formation or how closely melanoidins relate to marine sedimentary humic substances.

Sinninghe Damsté and de Leeuw (1990) have shown that the reactions of inorganic sulphur species with specific functionalised lipids may play an important role in the selective preservation and, hence, enrichment of specific lipids, including HBI carbon skeletons, which otherwise are prone to microbial transformation or mineralisation. If these lipids are incorporated into high-molecular-weight substances via sulphur linkages, the preserved lipids may be released again during diagenesis, resulting in relatively high amounts of the corresponding hydrocarbons.

ten Haven *et al.* (1989) and Kohnen *et al.* (1991ab) provided evidence to support this hypothesis. They reported the absence of C_{25} HBI alkenes, thiolanes or thiophenes from ODP Leg 112 sediments but the C_{25} HBI alkane was detected (1200 mgkg^{-1}) after desulphurisation (Raney nickel) of the maltene/polar fraction from sediment at site 679, at a sediment depth of *ca.* 1 m.
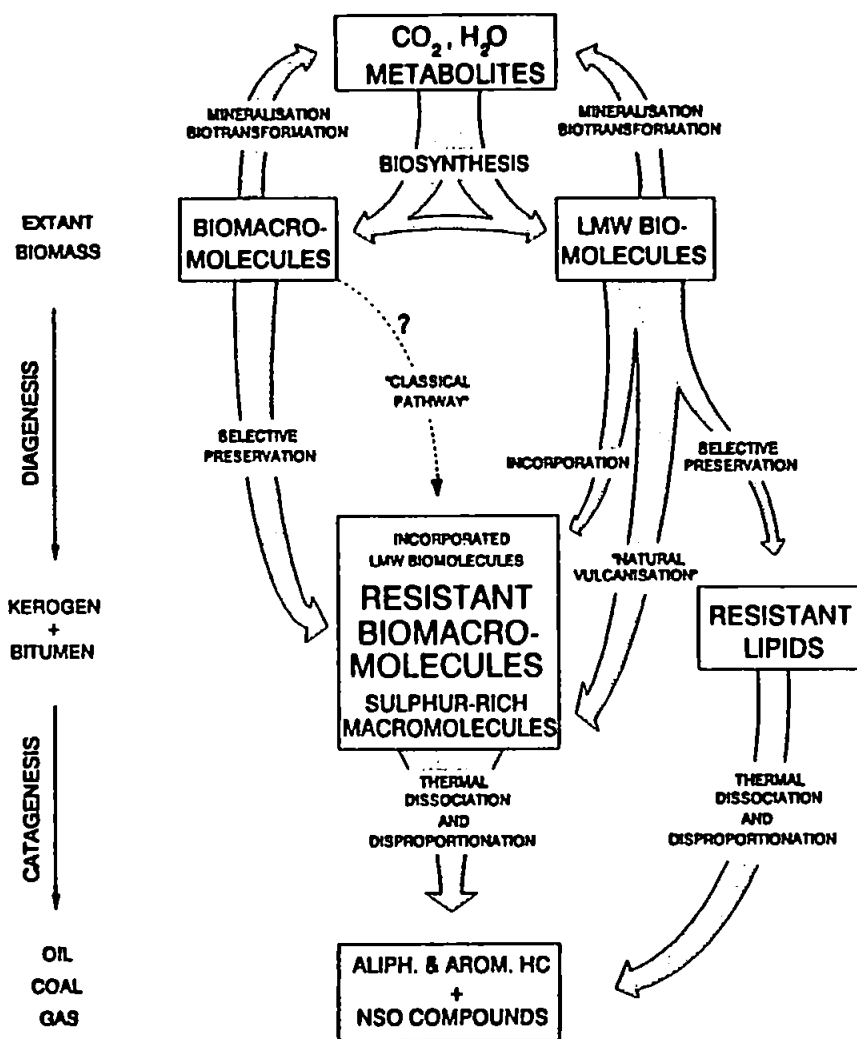
FIGURE 6.9 REVISED MECHANISM FOR KEROGEN FORMATION BASED ON THE CONCEPT OF SELECTED PRESERVATION OF (HIGHLY ALIPHATIC) RESISTANT BIOPOLYMERS (from Tregelaar *et al.*, 1989)

6.4 SUMMARY

High concentrations of C_{25} HBI alkenes are typically only present in surface sediments from the Peru upwelling region and decrease rapidly with increasing sediment depth. It has been shown that a mixture of synthetic C_{25} HBI monoenes were bound into the structure of melanoidins but the C_{25} HBI carbon skeleton was not released by pyrolysis of humic acids isolated from sediment. The results suggest that incorporation of C_{25} HBI alkenes into humic substances during diagenesis is unlikely to be a factor controlling sedimentary distributions of these widespread and abundant compounds. The widespread occurrence of HBI OSC, their early disgenetic formation and the fact that bacterial sulphate reduction leading to formation of hydrogen sulphide is a common phenomenon in Recent marine organic-rich sediments suggest that either intra- or intermolecular incorporation of sulphur into HBI alkenes may explain their apparent rapid decrease in surface sediments (Kohnen *et al.*, 1991a). Indeed, the abiogenic reactions of inorganic sulphur species with specific functionalised lipids, such as C_{25} HBI alkenes, has been proposed to lead to a selective removal of precursors of hydrocarbon biomarkers (Kohnen *et al.*, 1990b). Thus, although the relevance of C_{25} HBI alkenes to palaeoenvironmental interpretation seemed reduced with increased sediment depth, the biomarker potential is not lost, but bound up as sulphurised lipids, released by simple desulphurisation.

STRUCTURES

CHAPTER SIX

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2
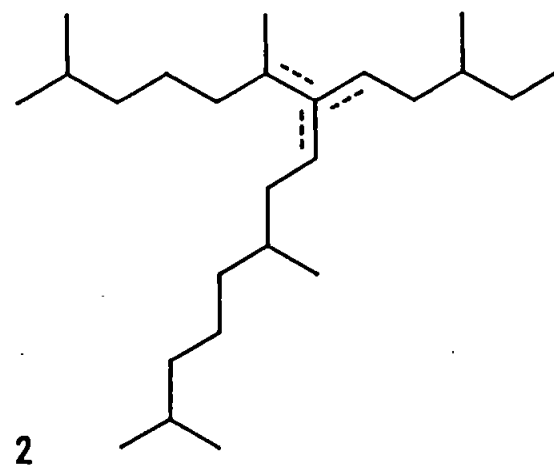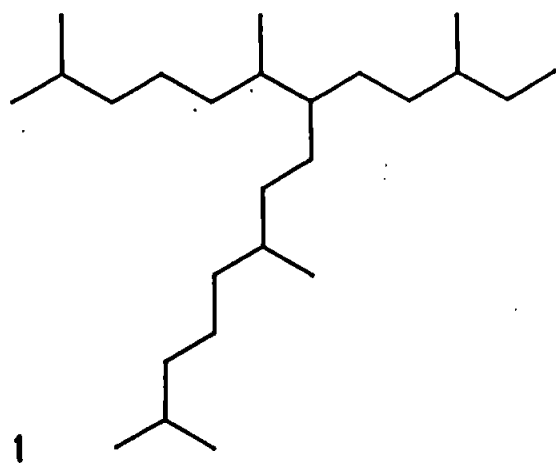
CHAPTER SEVEN

FUTURE RESEARCH

This study has extended present knowledge of the structures of HBI monoenes and has suggested two possible biological origins. There is much to be learned about HBI polyenes and the subject is proving to be a fruitful area for further research into biomarker potential. Some possible future approaches are suggested.

PROPOSALS FOR FUTURE WORK

The synthetic HBI alkenes isolated and characterised during this study have formed a valuable database of chromatographic and spectrometric information for the assignment of naturally occurring HBI hydrocarbons. Although C_{20} HBI monoenes are widely distributed, the occurrence of C_{25} homologues seems more restricted. In many other sediments, C_{25} polyenes, with two to five double bonds, are more abundant. Isolation and characterisation of sedimentary alkenes may reveal the significance of the methylene double bonds assigned in monoenes during the present study. The distribution of C_{25} HBI alkenes in sediments is sometimes dominated by one compound (*e.g.* McMurdo Sound) which could simplify isolation procedures. Parallel studies could include the synthesis of C_{25} polyenes, such as a diene (Yon, 1981) to help identify the likely positions of double bonds in natural, widespread HBI alkenes and perhaps to confirm the structure of isolated compounds.

The isolation of individual HBI alkenes has proved possible using careful argentation chromatography. The capacity of the Ag^+ HPLC technique used successfully during this study can be increased by switching to a preparative-scale column. These techniques can now be applied to hydrocarbons in a range of sediments and biota, to obtain pure HBI alkenes, in sufficient quantity for characterisation by spectroscopic (NMR) and chemical degradation (ozonolysis). An alternative degradation technique (epoxidation) for the determination of the positions of double bonds has been employed successfully by Yruela *et al.* (1990). Analysis of monoenes, assigned during this study, by the epoxidation technique, will prove an interesting comparison.

Acid-catalysed isomerisation has proved to be a useful reaction forming novel isomers. As isomerisation was shown to be complete almost immediately, under the experimental conditions used, it is sugested that the reaction is repeated at room temperature and products carefully monitored. Other isomerisation intermediates so formed may be isolated and characterised. Although not separated on the GC stationary phases used here, analysis of isomeric pairs isolated during this study on chiral or cyano bonded phases may prove more successful.

Individual compounds, either synthesised or isolated and characterised, could then be used as models in experiments designed to investigate the diagenetic fate of HBI hydrocarbons. These might include the incorporation of sulphur, anaerobic biodegradation and isotopic fractionation during the recycling of autothonous organic matter by heterotrophic bacteria.

The seasonal monitoring of sediments and biota from the Tamar estuary has yielded much information. For this approach to be succesful, however, expansion of the analytical program is required. Although GC-IRMS has shown promise as a useful tool for the identification of the biological source of HBI hydrocarbons, the need for the parallel analysis of other compounds, of known biological origin, has proven paramount. This can be applied in general to other work concerning the source of HBI hydrocarbons. For example, the co-occurrence of bacterial or algal markers in the sediments, with the abundance of HBI alkenes, may suggest precursor organisms. Three compound types, alcohols, fatty acids and pigments, could be targeted. Another area for expansion of the analytical program is the identification of OSC HBI in contemporary sediments to investigate how early in terms of diagenesis, sulphur incorporation may occur. Screening aromatic fractions from previous years (GC-FPD)

could be a starting point. Various forms of sulphur-rich macromolecular material can also be investigated as a mechanism for the removal of HBI hydrocarbons from the sediments.

The need for interdisciplinary study within this research was emphasised by the importance of identifying particular HBI hydrocarbons in epipelic algae. Collaboration with paleolimnologists, ecologists and microbiologists could help to bridge the gap of understanding between organic biogeochemistry and the living world. One particular area of interest is the culturing of microalgae and bacteria from sediments and macrophytes. Once isolated and identified, these organisms can be screened for the presence of HBI compounds. Ecological changes over time may be reflected in the palaeoliminology of sediment cores. Comparison of such data with the HBI distribution over the same time period, incorporating HBI isotopic compositions, may help determine the biolological significance of HBI in marine and lacustrine environments.

CHAPTER EIGHT

EXPERIMENTAL DETAILS

This chapter describes the analytical and synthetic procedures used in this study

EXPERIMENTAL DETAILS

8.1 GENERAL PROCEDURES

Glassware was cleaned in chromic acid and/or Decon-90, rinsed in doubly-distilled/millipore-grade water, oven dried (200°C; overnight) and finally rinsed with dichloromethane immediately before use. The glassware used in sensitive synthetic procedures was assembled whilst hot and immediately placed under inert atmosphere (argon or nitrogen). .

General purpose solvents (*e.g.* hexane, dichloromethane, methanol) were distilled in all-glass apparatus prior to use. Alternatively HPLC-grade solvents (dichloromethane and methanol; Rathburn) were found to be of adequate purity. Solvent purity was checked by evaporation (rotary evaporator) of 100 cm^3 of the solvent to dryness, transfer of any residue to a vial, followed by analysis of a 0.5 mm^3 aliquot from 100 mm^3 by gas chromatography (GC). Diethyl ether (Et$_2$O), tetrahydrofuran (THF) and chloroform (CHCl$_3$) were purified by elution through basic alumina (BDH; 10 g per 100 cm^3 solvent). The ethers were distilled over LiAlH$_4$ and stored over 4Å molecular sieve. The chloroform was distilled as for general purpose solvents above and kept in the dark with NaOH pellets to prevent phosgene formation. Dimethylformamide was initially purified by azeotropic distillation with benzene to remove the benzene:water azeotrope. Residual solvent was shaken with powdered barium oxide, filtered and distilled under nitrogen at reduced pressure. Redistilled DMF was stored over 4Å molecular sieve.

The silica gel (BDH; 60-120 mesh) and alumina (BDH; Grade 1; neutral) used as adsorbents in column chromatography were Soxhlet extracted with dichloromethane

(48 hours) before activation (185°C and 450°C respectively; 12 hours). Deactivated silica gel and alumina were prepared by shaking (8 hours) the absorbent with the appropriate quantity of millipore grade water and stored (50°C and 120°C; 12 hours). Thin-layer chromatography (TLC) plates were prepared on solvent-washed 20 x 20 cm or 20 x 10 cm glass plates with a coating of 0.25 mm (analytical) or 0.5 mm (preparative) silica gel (Merck Kiesel gel type 60G). Argentation TLC plates were prepared from slurries of silica gel made up in an aqueous solution of 10% w/w $AgNO_3$. Following drying (120°C; 1 hour) all plates were predeveloped in ethyl acetate and used after activation (120°C; 12 hours).

Anhydrous sodium sulphate (anhydrous Na_2SO_4), cotton wool, water (H_2O), hydrochloric acid (0.2M), aqueous solutions of sodium chloride (NaCl; brine), potassium chloride (KCl), sodium hydrogen carbonate ($NaHCO_3$), and glacial ethanoic acid, and activated copper were all extracted with dichloromethane before use.

Activated copper for the removal of elemental sulphur from the geological samples was prepared according to the method of Blumer (1957). Copper sulphate (M&B Pronalys°Ar) (*ca.* 45 g) was placed in a beaker (500 cm³) containing ice-cold deionised water and hydrochloric acid (2M; 25 cm³). In another beaker (1 dm³) a thick slurry of powdered zinc (Aldrich; 15 g) in 25 cm³ deionised water was prepared. To aid the wetting of the zinc powder, acetone (BDH; Analar; 1 cm³) was added as a 'wetting agent'. The copper solution was then added to the rapidly stirred zinc slurry. Stirring was continued until effervescence ceased and the colour of the copper turned from a bright red to a dark red-brown. The supernatant was decanted, allowing the finer particles to be removed. The copper was washed with ice-cold deionised water repeatedly, until all traces of dark particulate matter were removed.

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Any excess copper not used immediately was covered in ice and stored in a freezer.

The activated copper was packed into columns of various dimensions and water removed by washing with acetone (BDH; Analar). After several washings the solvent was changed to hexane prior to use.

8.2 EXTRACTION AND FRACTIONATION OF BIOLOGICAL SAMPLES

The general protocol used for the isolation of hydrocarbons from various biological samples is illustrated in Figure 8.1.

8.2.1 SAMPLE COLLECTION AND SOLVENT EXTRACTION

8.2.1.1 *Macroalgae*

Various field samples of macroalgae were collected at one of the sites of sampling of Tamar sediment, Millbrook. Samples of underlying sediment from 0-2 cm depth were also taken (see geochemical samples 8.3). Macroalgae fronds and/or filamentous mats were sampled with metal pliers/tweezers and washed with estuary water. Trapped particulate matter, small snails, sand hoppers and other visible parasites and other non-algal material, were carefully removed and stored as necessary. The samples were further washed (Millipore grade water) and macerated using a scalpel in the laboratory. Aliquots were taken for total organic carbon (TOC) analysis (see elemental analysis 8.5.1) and the remainder stored in Petri dishes/sealed beakers and frozen.

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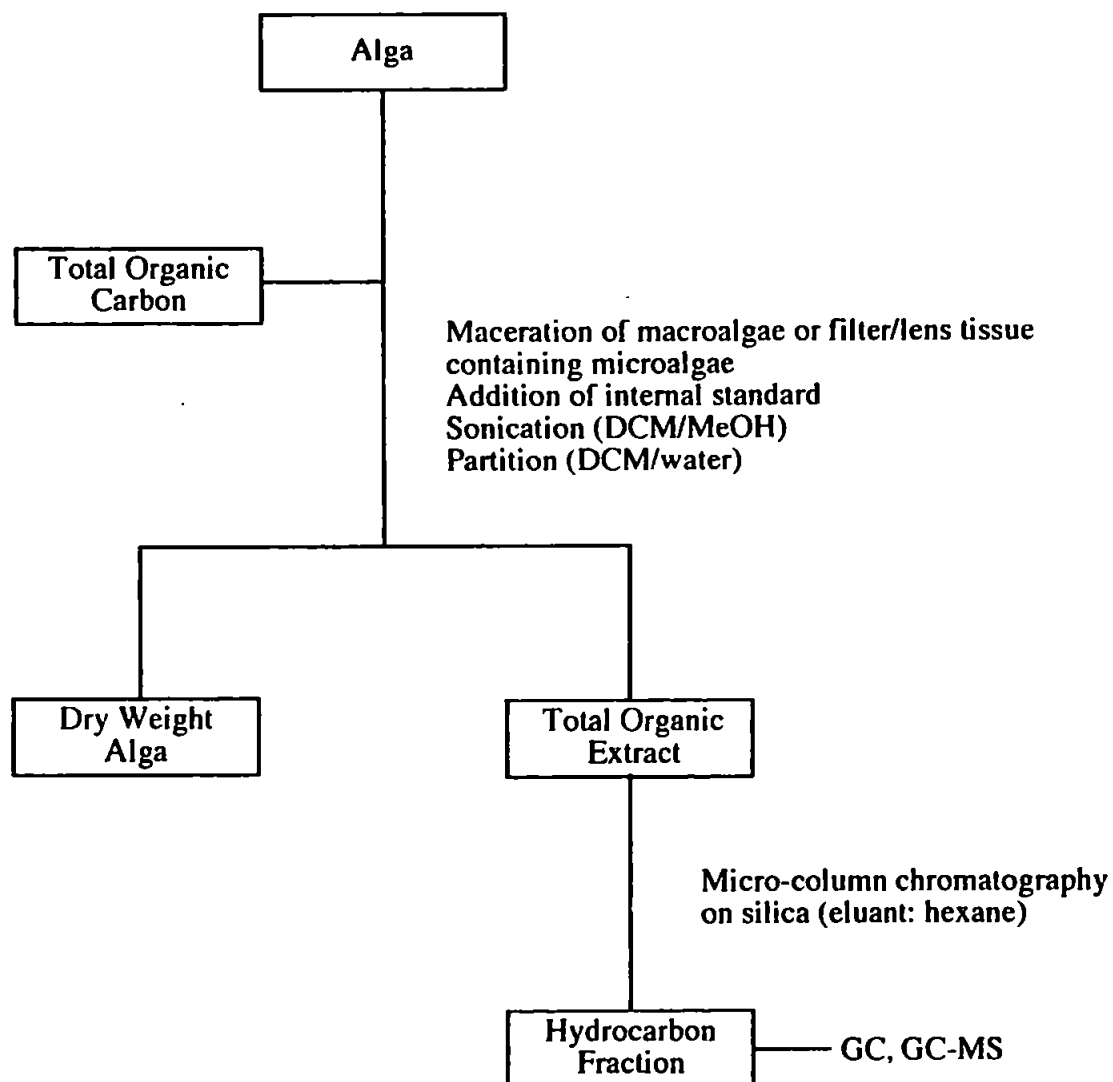
FIGURE 8.1 ISOLATION AND ANALYSIS OF ALIPHATIC HYDROCARBONS FROM ALGAE

The macroalgae (approximately 20 g wet weight) were allowed to thaw and sequentially extracted with methanol (20 cm^3), dichloromethane/ methanol (3:1 v/v; 20 cm^3) and dichloromethane (20 cm^3) by ultrasonication (3 x 5 min.) with cooling (ice bath). The organic extract was separated by centrifugation (3 x 15 min.) and decanted. The combined extracts were shaken (separating funnel) with water (15 cm^3) and the lower organic layer collected, along with the dichloromethane washings (3 x 10 cm^3) of the aqueous layer. Solvent was removed (Buchi; 30°C), the extract dried (anhydrous Na$_2$SO$_4$) and the total organic extract transferred quantitatively to a vial and weighed.

8.2.1.2   *Collection of epipelic algae*

An area of brown algal 'slime' was removed from the fine muddy sediment with a thin, flat, metal spatula. Samples of sediment from 0-2 cm depth were also taken (see geochemical samples **8.3**).

8.2.1.3   *Separation of epipelic algae*

Epipelic algae were harvested using the method of Thompson and Eglinton (1976). Algal 'slime' was spread as thinly as possible in Petri dishes with lids and covered with two sheets of lens tissue (Whatman grade 105) which had been pre-extracted with dichloromethane and methanol. A few drops of estuary water were used to further moisten the lens tissue, and the Petri dishes were kept at ambient temperature in the light. The top sheet of lens tissue with adhering algae was removed *ca.* 24 hours later. Small parts of each sheet were set aside for microscopic examination. The remainder was extracted.

8.2.1.4 *Solvent extraction of epipelic algae*

The lens tissue in which the algae were entrained was suspended in water (2.5 cm^3) and dichloromethane (3 cm^3) was added, followed by methanol (6 cm^3). The suspension was stirred and subjected to utrasonication (5 min.; Soniprep 150-probe) with cooling (ice bath). Dichloromethane (3 cm^3) was added, followed by a further period of ultrasonication (5 min.). Addition of water (3 cm^3) and further ultrasonication (5 min.) was followed by centrifugation (15 min.; 1800rpm). The aqueous layer was removed/discarded and the lower organic layer aspirated. Solvent was removed (Buchi; $30°C$), the extract dried (anhydrous Na$_2$SO$_4$) and the total organic extract transferred quantitatively to a vial and weighed.

8.2.1.5 *Collection and solvent extraction of epiphytic algae*

This was attempted by applying above methods (see collection and solvent extraction of epipelic algae **8.2.1.2/3**) to a partially buried *Cladophora* mat as the substrate. In addition, the debris washed from a sample of *Enteromorpha* sp. was extracted as above (solvent extraction of epipelic algae **8.2.1.4**).

8.2.1.6 *Euxinic algal cultures*

Various cultures of phytoplankton were grown under controlled nutritional and thermal conditions in filtered sea water. Prior to collection of biomass/cells/algae the filters (Whatman glass microfibre GF/F; 4.7 cm diameter) were ashed ($300°C$), washed with dichloromethane/methanol ($2:1$ v/v; 100 cm^3), dried and weighed. Collection of biomass/cells from the culture was made using a Millipore vacuum filtration system. The filters were immediately placed in petri dishes, sealed and

frozen until extraction.

Filters containing cultured algae (2 or 3) were allowed to thaw and sequentially extracted with methanol (3 x 10 cm^3), dichloromethane/ methanol (3:1 v/v; 3 x 10 cm^3) and dichloromethane (3 x 10 cm^3) by ultrasonication (9 x 5 min.) with cooling (ice bath). The organic extract was separated by centrifugation (9 x 15 min.) and decanted. The combined extracts were shaken (separating funnel) with water (15 cm^3) and the lower organic layer collected, along with the dichloromethane washings (3 x 5 cm^3) of the aqueous layer. Solvent was removed (Buchi; 30°C), the extract dried (anhydrous Na$_2$SO$_4$) and the total organic extract transferred quantitatively to a vial and weighed.

8.2.2   FRACTIONATION OF ALGAL TOTAL ORGANIC EXTRACTS

Hydrocarbons were isolated by microcolumn chromatography (146 mm x 10 mm o.d. columns) on deactivated (4%) silica (dry packed; *ca.* 5g) using hexane (*ca.* 2 cm^3) as the mobile phase. The solvent was evaporated under nitrogen and the hydrocarbon extract stored in dichloromethane (100 mm^3).

8.3 EXTRACTION AND FRACTIONATION OF GEOCHEMICAL SAMPLES

8.3.1 TAMAR ESTUARY, UK

The general protocol used for the isolation of hydrocarbons from Tamar sediment samples is illustrated in Figure 8.2.

8.3.1.1 *Sample collection and solvent extraction*

The location of the sample sites is shown in Figure 5.1. Surface sediment (0-2 cm depth) was collected from a number of sites at Cargreen to ensure a homogenous sample. The homogenates were collected by metal spatula, transferred to clean aluminium cans and frozen immediately. Single and homogenate sediment samples were taken at Millbrook, from sediment covered with macroalgal mats and from sediment clear of such growth. Other sediment samples were taken at St. Johns Lake. Aliquots of many were taken for organic carbon (TOC) analysis (see elemental analysis **8.3.1**). The thawed samples were solvent extracted using the method of Douglas *et al*. (1981). Sediment (approximately 40 g wet weight) was extracted with methanol (40 cm^3) by ultrasonication (5 min.; Soniprep 150-probe) with cooling (ice bath). The organic extract was separated by centrifugation (20 min.; 1800 rpm) and decanted. This procedure was repeated using dichloromethane/methanol (7:3 v/v), dichloromethane/ methanol (4:1 v/v) and dichloromethane. The combined extracts were shaken (separating funnel) with water (Millipore grade; 30 cm^3) and the lower organic layer collected, along with the dichloromethane washings (3 x 15 cm^3) of the aqueous layer. Solvent was removed (Buchi; 30°C) and the total organic extract
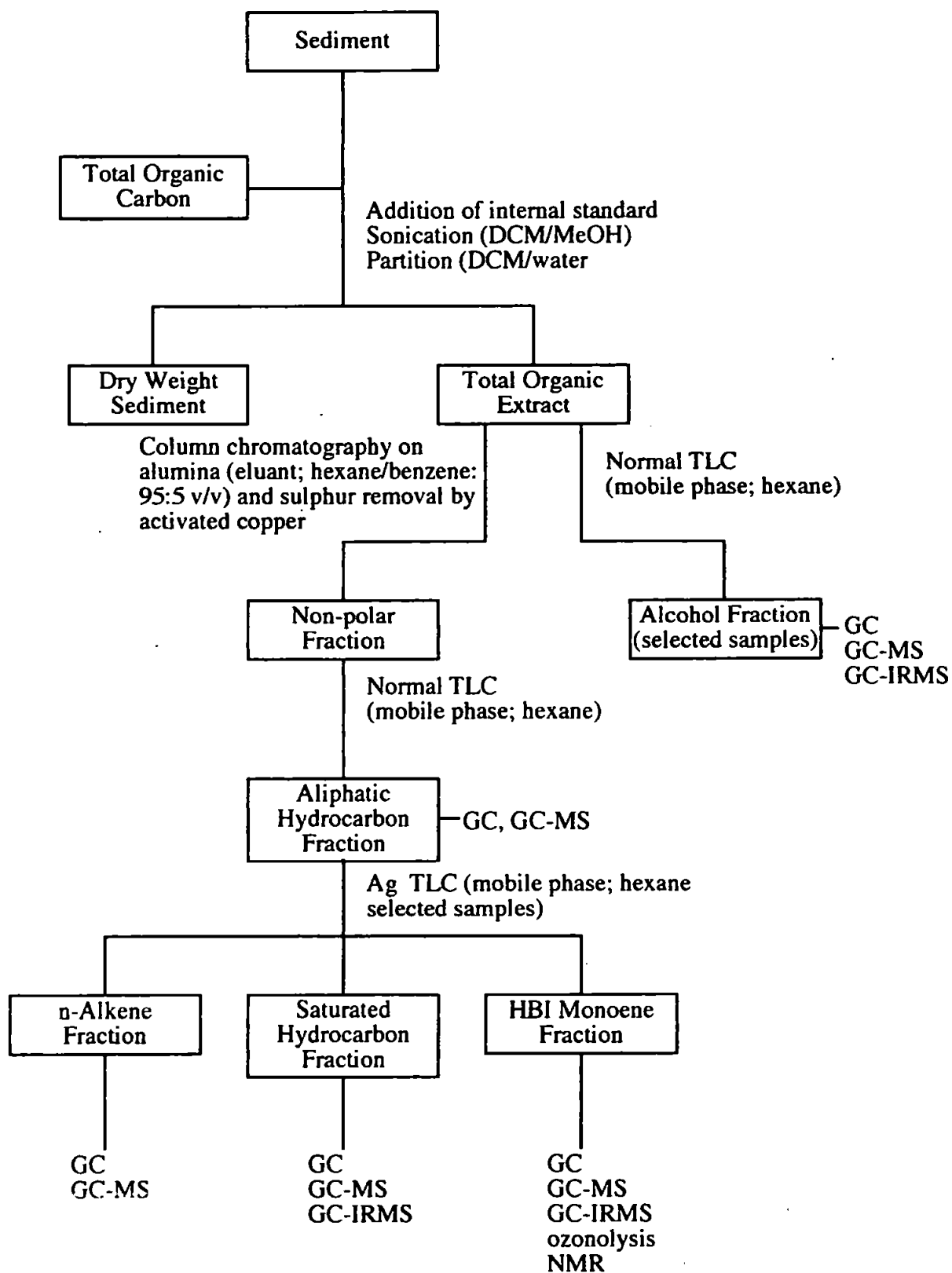
FIGURE 8.2 ISOLATION AND ANALYSIS OF ALIPHATIC HYDROCARBONS FROM TAMAR SEDIMENTS

transferred quantitatively to a vial and weighed. If water was still present after solvent removal, dichloromethane (20 cm^3) was added and the mixture transferred to a small separating funnel, where the lower organic layer was carefully removed, reconcentrated and weighed.

8.3.1.2   *Fractionation of total organic extract*

The extract was pre-absorbed onto alumina (*ca.* 100 mg) and applied to a short column (20 cm x 1.0 cm o.d.) containing alumina (5% deactivated; 1 g) over activated copper powder (0.2-0.5 g w/w) and eluted with hexane/benzene (95:5 v/v; 5 cm^3). This column procedure removed most of the polar, nonhydrocarbon organic material (*e.g.* pigments) and elemental sulphur prior to TLC. The column eluate was evaporated to dryness, weighed and dichloromethane (100 mm^3) added. The hydrocarbons were isolated by TLC.

The sample (hydrocarbons and nonpolar pigments) (usually less than 15 mg) was spotted 2 cm from the bottom of the plate which was then developed with hexane. The different bands were visualised by spraying the plate with a methanolic solution (0.5%) of Rhodamine G (in some cases dichlorofluororscien was used) and then viewing under ultra-violet light (365 nm). On each plate, reference compounds (a mixture of *n*-eicosane, *n*-eicos-1-ene, squalene and anthracene) was used. The "hydrocarbon" band, corresponding to a R_f value of 0.35-0.92), was removed and the rest of the plate, divided into two fractions corresponding to R_f values of 0-0.08 and 0.08-0.35 was removed separately. The latter fraction contained aromatic components and carotenoid-type pigments. The hydrocarbons were recovered from the silica gel by desorption with hexane/dichloromethane (60:40 v/v; *ca.* 5 cm^3) using a Pasteur

pipette containing a bed of alumina and the eluates were collected in vials. After removal of the solvent the extracts were weighed and stored in dichloromethane (approximately 100 mm^3) at 4°C.

In cases where the analysis of phytol and HBI hydrocarbons was required, both were isolated by TLC. The total organic extract was spotted 2 cm from the bottom of the plate which was then developed with hexane:diethylether (95:5 v/v). The different bands were visualised by spraying the plate with a methanolic solution (0.5%) of Rhodamine G and then viewing under ultra-violet light (365 nm). On each plate, reference compounds (a mixture of n-eicosane, n-eicos-1-ene, squalene and phytol) was used. The "hydrocarbon" band, corresponding to a R$_f$ value of 0.7-1.0, and "alcohol" band, corresponding to a R$_f$ value of 0.0-0.1 was removed. The hydrocarbons were recovered from the silica gel by desorption with hexane/dichloromethane (60:40 v/v; $ca.$ 5 cm^3) using a Pasteur pipette containing a bed of alumina and the eluate was collected in a vial. The alcohol band usually required further purification by TLC as above but using dichloromethane as the mobile phase. The band coresponding to the phytol reference standard was removed and phytol recovered by desorption with dichloromethane ($ca.$ 5 cm^3). After removal of the solvent the extracts were weighed and stored in dichloromethane (approximately 100 mm^3) at 4°C prior to analysis by GC and GC-MS.

Certain hydrocarbon fractions were further separated into saturated and unsaturated components by silver ion TLC (10% AgNO$_3$/silica gel w/w) using hexane as the mobile phase. The plate was visualised (UV light, 365 nm; 0.5% Rhodamine 6G in methanol) and the saturated, HBI unsaturated and other unsaturated aliphatic hydrocarbon bands, corresponding to R$_f$ values of 0.7-1.0, 0.4-0.7, and the rest of

the plate, were removed as three fractions. The hydrocarbons were recovered from the silica gel by desorption with hexane/dichloromethane (60:40 v/v; *ca.* 5 cm^3) using a Pasteur pippette containing a bed of alumina and the eluates collected in vials. After removal of the solvent the extracts were weighed and stored in dichloromethane (approximately 100 mm^3) at 4°C prior to analysis by GC and GC-MS and further characterisation (ozonolysis and NMR).

8.3.2   PERU CONTINENTAL MARGIN UPWELLING REGION (ODP LEG 112)

The procedure used for the separation of humic, fulvic, and solvent extractable substances from the bulk sediment is illustrated in Figure 8.3.

8.3.2.1   *Solvent extraction*

The freeze dried sediment (*ca.* 0.5 g) was weighed into a centrifuge tube, suspended in water (5 cm^3) and sequentially extracted with chloroform (3 x 6 cm^3) and methanol (3 x 6 cm^3) using ultrasonication and centrifugation (15 min, 3500 rpm). Brine (1 cm^3) was added to aid flocculation of colloidal mineral material. The chloroform layer was aspirated and solvent removed (Buchi; 30°C) and the total organic extract transferred to a vial for storage.

8.3.2.2   *Fractionation of total organic extract*

Elemental sulphur was removed from the extract by passing the extract dissolved in hexane (100 mm^3) through a Pasteur pipette (146 mm x 10 mm o.d.) packed with activated copper ("spongy copper"; *ca.* 0.5 g w/w) according to the method of Blumer (1957). Hydrocarbons were then isolated by microcolumn chromatography on deactivated (4%) silica (dry packed; *ca.* 1 g) using hexane (*ca.* 5 cm^3) as the mobile phase. The solvent was evaporated under nitrogen and the hydrocarbon extract stored).
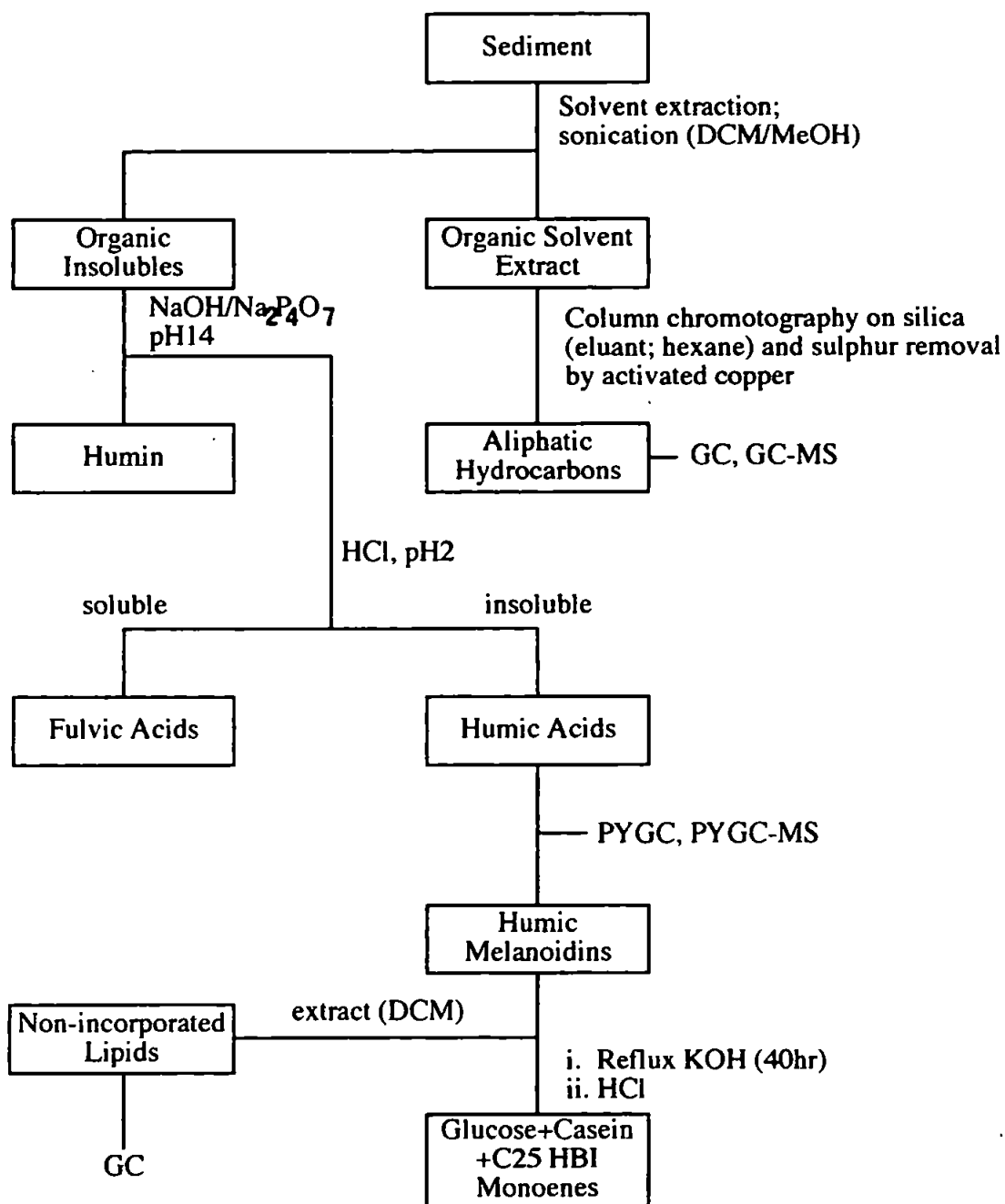
FIGURE 8.3 ISOLATION AND ANALYSIS OF HYDROCARBON AND HUMIC ACID FRACTIONS FROM PERU UPWELLING SEDIMENT AND THE SYNTHESIS OF MELANOIDINS.

8.3.2.3   *Extraction of humic substances*

Humic compounds were recovered hydrolysis and extraction according to the method of Munier-Lamy *et al.* (1986). The extracting solution (1% $Na_4P_2O_7$) was prepared from $Na_4P_2O_7$ (1 g) and NaOH (100 cm^3) and pre-extracted with dichloromethane (20 cm^3). Extraction (9 x 2 cm^3) of the soluble organics from the humin was carried out as above. The combined supernatants were transferred to another centrifuge tube and clays were removed by flocculation on the addition of KCl (1%; 2 cm^3) followed by centrifugation. The supernatant was transferred to another tube and humic acids precipitated by the addition of HCl (0.2M; pH 2). After further centrifugation, the supernatant was removed and stored as fulvic acids and the humic acids transferred to vial and stored under nitrogen.

8.3.2.4   *Production of melanoidins*

The melanoidin mixture was prepared (Larter and Douglas, 1980), by dissolving δ-glucose (65 mg), an amino acid mixture (7 mg) and the C_{25} HBI monoene mixture (Robson and Rowland, 1986; 5.5 mg) in distilled water (400 mm^3). The amino acid mixture was a protein (casein) hydrolysate and contained all the common acids. The mixture was adjusted to pH 8 (KOH) then refluxed (*ca.* 40 hours). The cooled mixture was acidified to pH 2 (HCl) the filtered precipitate being washed copiously with water. The melanoidin was then solvent extracted with dichloromethane (250 mm^3) to remove non-incorporated lipids which was transferred to a vial for storage. The residual humic melanoidin-lipid mixture was transferred to a vial and stored under nitrogen gas (3.8 mg; 5% yield).

8.4 MICROSCALE HYDROGENATION OF ALIPHATIC HYDROCARBONS

The "hydrogenation products" of the aliphatic hydrocarbons from McMurdo Sound sediments as obtained by Venkatesan (1988) were hydrogenated using the following procedure. Hydrogenation was effected by bubbling hydrogen for 60 minutes through the extract dissolved in hexane (1 cm^3; 20°C) containing activated $PtO_2.H_2O$ (10 mg). A mixture of synthetic C_{25} HBI alkenes (Robson and Rowland, 1986) was subjected to the same procedure. The products were carefully filtered and dried (anhydrous Na_2SO_4) and transferred to vials for storage.

8.5   ANALYSES

8.5.1   ELEMENTAL ANALYSIS

The organic carbon (TOC) content of algal specimens and carbonate-free sediment was determined by high temperature combustion with a Carlo Erba model 1106 elemental analyser. Sediments were acidified to remove carbonate using the method described by Boehm and Quinn (1978).

8.5.2   GAS CHROMATOGRAPHY (GC)

Hydrocarbons were examined on a Carlo Erba Series 5300 Mega gas chromatograph fitted with fused silica columns (0.32 mm i.d.) of various lengths and phases (mainly DB1 or DB5; J&W; see text) using flame ionisation detection and on-column injection. Using DB1/DB5, the column oven was programmed from 40-80°C at 10°C min.$^{-1}$, 80-300°C at 5°C min.$^{-1}$ and held at the final temperature for 20 minutes. Hydrogen was used as the carrier gas at a flow rate of 2 cm^{3}min.$^{-1}$ (set at 250°C) supplied at a pressure of 0.4 kgcm^{-2}.

Certain solvent extracts were also analysed using a 25 m column coated with CPWAX52 (Chrompack, Holland) or a 15 m column coated with DBWAX (J&W) using flame ionisation detection and on-column injection. The carrier gas was hydrogen (2 cm^{3}min.$^{-1}$) and the oven temperature programmed from 40-80°C at 10°C min.$^{-1}$, 80-240°C at 6° cmin.$^{-1}$ and held at 240°C for 10 minutes.

Retention indices (GC RI) were calculated according using the following formula:

$$RI = 100z + 100 \frac{t_R(unknown) - t_R(z)}{t_R(z+1) - t_R(z)}$$

where t_R is the net retention time and z represents an n-alkane with z carbon atoms. A known alkane mixture was added to the hydrocarbons where appropriate.

Quantitation of individual hydrocarbons was accomplished by measurements of GC peak area using a Shimadzu CR3-A recording integrator. These were then compared to the response of known concentration of internal standard, usually n-7-hexylnonadecane except where stated in the text (*ca.* 5 mgkg^{-1}), added prior to solvent extraction.

8.5.3 GAS CHROMATOGRAPHY-MASS CHROMATOGRAPHY (GC-MS)

Analysis of hydrocarbon extracts was performed on a Carlo Erba Series 5160 Mega chromatograph coupled to a Kratos MS25 double focusing magnetic sector mass spectrometer. A 30 m fused silica column coated with either DB1 or DB5 (J&W) was introduced directly into the ion source of the mass spectrometer. On-column injection and helium carrier gas were used and the column oven programmed as for GC. Mass spectrometer operating conditions were; ion source temperature 250°C, 40 eV ionising energy and a filament emission current of 400 μA. On a number of occasions the ionising energy was reduced to 20 eV and the source temperature to 200°C. Spectra (m/z 40-532) were collected every 1.5 seconds using a DS90 data system.

8.5.4 TANDEM MASS SPECTROMETRY (MS-MS)

MS-MS analyses were performed using a Finnigan TSQ 70 triple quadrupole mass spectrometer. Samples were introduced into the ion source via the direct insertion probe (DIP). Conventional EI data was recorded scanning Q3 from 50-400 daltons in 1 second with 70 eV electron energy and 200 μA emission. Tandem MS data was obtained with a pressure of 0.2 mTorr of argon in the second quadrupole region and a collision energy of -5 eV.

8.5.5 $\delta^{13}C$ ISOTOPE MEASUREMENTS ON INDIVIDUAL COMPOUNDS

8.5.5.1 GAS CHROMATOGRAPHY-ISOTOPE RATIO MASS SPECTROMETRY (GC-IRMS)

The GC-IRMS was performed using a VG Isochrom II isotope ratio mass spectrometer attached to a Hewlett-Packard 5890A gas chromatograph (Fina Research, Belgium). The Isochrom II GC-IRMS standard containing four n-alkanes was crosschecked using a VG SIRA II dual inlet IRMS instrument.

8.5.5.2 ISOTOPE RATIO MASS SPECTROMETRY (IRMS)

Isotope analyses on isolated compounds were carried out by Database Ltd using a conventional combustion, dual inlet IRMS technique.

$^{13}C/^{12}C$ ratios are expressed in the δ notation and refer to the international PDB standard (Craig, 1957) calculated with reference to the NBS 22 standard. The δ value is given in per mil (‰) and is defined as

$$\delta^{13}C = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

where R represents the $^{13}C/^{12}C$ isotope ratio.

8.5.6 PYROLYSIS GAS CHROMATOGRAPHY (PYGC) AND PYROLYSIS GAS CHROMATOGRAPHY-MASS CHROMATOGRAPHY (PYGC-MS)

PYGC and PYGC-MS was carried out on both sedimentary and synthetic humic substances (*ca.* 0.1 mg) in both off-line and on-line modes. For the former pyrolysis system, a C.D.S. 120 pyroprobe with a platinum coil, directly inserted into the heated (200°C) modified injection port of the GC. Pyrolysis occurred for 10 seconds at a maximum temperature of 600°C. The pyrolysate was trapped in glass capillary tubes cooled by liquid nitrogen and the compounds desorbed by extraction with dichloromethane. The hydrocarbons were isolated by column chromatography on deactivated (4%) silica (*ca.* 1g; dry packed) using hexane as the mobile phase (*ca.* 5 cm³). The pyrolysate hydrocarbons were then analysed by GC and GC-MS as above (8.5.2/3).

For the on-line PYGC-MS system, the pyroprobe was directly inserted into the heated (250°C) injection port of the GC. Thermal desorption of the sample was carried out for 5 minutes prior to pyrolysis and these products condensed onto the GC column at 40°C. The column temperature was then raised at a rate of 15°Cmin.$^{-1}$ to a maximum of 300°C during which the thermal desorption products were monitored by GC-MS. Pyrolysis occurred for 20 seconds at a maximum temperature of 610°C. The GC oven was programmed from -40°C (held for 5 minutes) using a liquid carbon

dioxide cryogenic cooling system (Carlo-Erba Cryo 520), to 300°C at 5°Cmin.$^{-1}$, and held at 300°C for 15 minutes. A mixture of C_{25} HBI monoenes were subjected to similar pyrolysis treatment (insertion port 150°C).

8.5.7 COMPOUND IDENTIFICATION

Individual hydrocarbons were identified by co-chromatography with authentic compounds on GC columns of different polarities and by comparison of gas chromatographic retention indices (GC RI) with literature data. Additional information was provided by GC-MS: the recognition of components from their mass spectra was made by comparison with the spectra of authentic compounds, published spectra or by spectral interpretation, as indicated in the text.

8.5.8 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR)

The ^{1}H NMR spectrum of a sedimentary C_{20} HBI monoene was recorded in a $CDCl_3$ solution using a Jeol EX270 (270 MHz; Polytechnic South West) high resolution FT-NMR spectrometer. Chemical shifts were measured on the δ scale using tetramethylsilane (TMS) as an internal standard. Peaks are described as singlet (*s*), doublet (*d*), doublet of doublets (*d* of *d*), triplet (*t*), doublet of triplets (*d* of *t*), quartet (*q*) or multiplet (*m*).

8.6   CHARACTERISATION OF SEDIMENTARY HBI MONOENES

8.6.1   MICROSCALE OZONOLYSIS OF ISOLATED SEDIMENTARY HBI MONOENES

Ozonolysis was employed in the elucidation of the positions of double bonds in various HBI alkenes. The "Micro-Ozonizer" (Supelco Inc., U.S.A; a modification of the design of Beroza and Bierl, 1969) generated ozone which was passed into a sealed vial containing the isolated alkene(s) dissolved in CS_2 within a limited volume insert (100 mm^3) at -70°C for about 5 minutes. After the reaction was completed an aliquot (1 mm^3) of the solution of aldehydes, and/or ketones (produced by cleavage of the ozonide) were analysis by GC and GC-MS and the position of the double bond determined from identification of the cleavage products. Prior to the analysis of HBI alkenes, the technique was validated using simple *n*-alkenes (tetradec-7-ene and heptadec-1-ene) which were succesfully ozonolysed to *n*-1-heptanal and *n*-1-hexadecanal respectively.

8.6.2.1   *Partial hydrogenation product from McMurdo Sound sedimentary hydrocarbons*
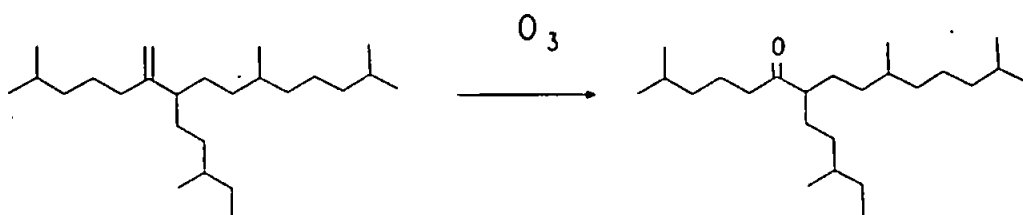
2,10,14-trimethyl-7-(3'-methylpentyl)pentan-6-one

GC-MS m/z : M$^+$ 352, 337 [5%, M$^+$-CH$_3$], 268 [10%, McL], 250 [18%, McL-H$_2$O], 212 [17%, McL], 194 [20%, McL-H$_2$O], 127 [70%, C$_9$H$_{11}$], 113 [55%, α-cleavage$\rightarrow$ C$_6$H$_{13}$CO$^+$], 95 [100%, α-cleavage-H$_2$O].

2,10-dimethyl-7-(3'-methylbutyl)dodecan-6-one

GC-MS *m/z* : M$^+$ 282, 267 [10%, M$^+$-CH$_3$], 212 [20%, McL], 198 [45%, McL],

180 [30%, McL-H$_2$O], 128 [42%, Double McL], 127 [52%, McL + γ-cleavage],

113 [90%, α-cleavage→C$_6$H$_{13}$CO$^+$], 95 [100%, α-cleavage-H$_2$O].

C_{20} HBI monoene isolated from Cargreen (Tamar) sediment in April, 1990

¹H NMR (270 Mhz) δ ppm:
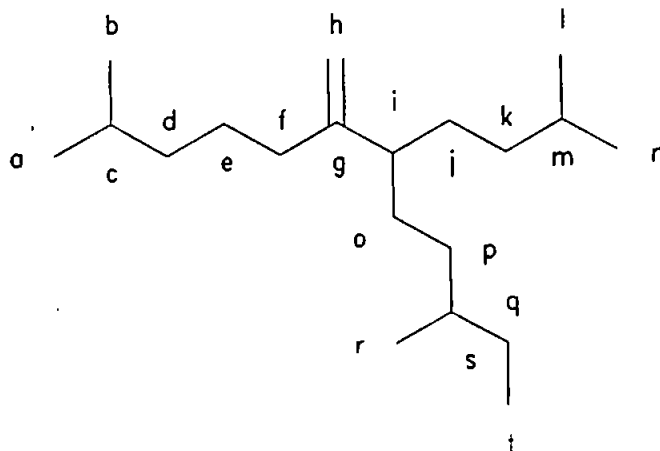
0.89 (*m*; 18H; a, b, l, n, r, and t)

1.25 (*m*; 15H; d, j, k, o, p, s and -CH c, m, q)

1.80-1.95 (*t*; 3H; f, i)

4.71 (*d*; external vinylic proton; contaminant)

5.30 (*s*; 2H; h)

5.38 (*m*; internal vinylic proton; contaminant)

SYNTHESES

8.7 INSTRUMENTATION

8.7.1 GAS CHROMATOGRAPHY (GC)

Synthetic reaction mixtures were examined first by the use of a Varian Series 1400 gas chromatograph fitted with packed stainless steel column (6' x ¼" o.d), OV1 and Carbowax stationary phases, septum vaporising injector and a flame ionisation detector. Further examination was made using Carlo Erba Series 4160 and 5300 instruments fitted with fused silica columns (25-30 m x 0.25-0.32 i.d.), Grob split vaporising or on-column injector and flame ionisation detector. The column phase and temperature programme employed varied for each analysis. The carrier gas was typically hydrogen at a flow rate of 2 $cm^3min.^{-1}$ (measured at an oven temperature of 250°C) supplied at a pressure of approximately 0.6 $kgcm^{-2}$. Chromatograms were recorded using a Shimadzu CR3-A integrator.

8.7.2 LOW RESOLUTION MASS SPECTROMETRY (LRMS)

Low resolution electron impact mass spectra of synthetic reaction products were recorded with a Carlo Erba 5160 Mega gas chromatograph coupled to a Kratos MS25 mass spectrometer (GC-MS). The operating conditions were as GC-MS above but the temperature of the oven was typically programmed from 40-300°C at 6°C $min.^{-1}$. Spectra of pure compounds were also recorded by use of a direct insertion probe.

8.7.3   INFRARED SPECTROMETRY (IR)

Infrared spectra were recorded as either liquid films, KBr discs or solutions (in CCl_4) either on Perkin Elmer 298 or 1330 IR spectrometers or a Perkin Elmer Series 1720X FTIR spectrometer. For GC-FTIR, a Hewlett-Packard 5890A gas chromatograph (split injection) coupled to a HP 4965A infrared detector (IRD) was used (BP Research, Sunbury). The 1603 cm^{-1} [aryl ν(C=C)] stretch in the spectra of polystyrene was used as a reference in all cases.

8.7.4   NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR)

^{1}H NMR spectra were recorded in $CDCl_3$ solutions using either a Jeol GX400 (400 MHz; Bristol University), Jeol GX270 (270 MHz; Jeol Ltd, U.K.) or Jeol EX270 (270 MHz; Polytechnic South West) high resolution FT-NMR spectrometers. Chemical shifts were measured on the δ scale using tetramethylsilane (TMS) as an internal standard. Peaks are described as singlet (s), doublet (d), doublet of doublets (d of d), triplet (t), doublet of triplets (d of t), quartet (q) or multiplet (m).

8.7.5   PREPARATIVE LIQUID CHROMATOGRAPHY

8.7.5.1   HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

An HPLC method for separation of structural isomers of unsaturated hydrocarbons using small particle size silver nitrate impregnated silica as a stationary phase was adapted from Dimitrova (1979). A liquid chromatograph was equipped with a Perkin Elmer Series 410 2C Pump, a Knauer differiential refractometer, a Rheodyne injector incorporating a 20 mm^3 sample loop, and Pharmacia FRAC-100

fraction collector. Chromatograms were recorded using a chart recorder and Perkin Elmer Nelson PC Model 2100 integrator installed on an Amstrad personal computer. Silica gel (Hypersil Shandon; 5 μm) was used as a support for silver nitrate impregnation. The silica gel (5 g) was preactivated by heating at about 100°C *in vacuo* for about 30 minutes. A solution of silver nitrate (BDH Analar; 0.5 g) in acetonitrile (BDH HiPerSolv; 50 cm^3) was added and the contents of the flask homogenised by ultrasound. The acetonitrile was removed under low pressure (Buchi; 100°C). As photodecomposition of AgNO$_3$ occurs readily, all manipulations were carried out in blacked-out flasks and darkened laboratories. The column (25 cm x 0.5 cm) was slurry-packed under pressure using chloroform as the solvent. The column was then equilibriated using heptane (BDH HyPerSolv). Prior to the analysis of HBI alkenes, the technique was validated using a mixture of normal alkanes and alkenes. Partial separation of HBI isomers was only achieved using very low flow rates (*e.g.* 0.2 cm^{3}min^{-1} of heptane) and fractions (20 mm^3) collected. This procedure was repeated until sufficient material was collected to enable further characterisation. After removal of the solvent the extracts were weighed and stored in dichloromethane (approximately 100 mm^3) at 4°C. Each fraction was analysed by GC and GC-MS to confirm the identity of HBI alkenes isolated in each fraction and those of sufficient purity and quantity characterized further by micro-ozonolysis and, in some cases, ^{1}H NMR.

8.7.5.2   THIN-LAYER CHROMATOGRAPHY (TLC)

Certain fractions were separated by silver ion TLC (10% AgNO$_3$/silica gel w/w) using hexane as the mobile phase. The plate was visualised (UV light, 365 nm;

0.5% Rhodamine 6G in methanol) and six bands corresponding to R_f values between 0.4 and 0.7 about 0.05 R_f units wide, were removed. The alkenes were recovered from the silica gel by desorption with hexane/dichloromethane (60:40 v/v; *ca.* 5 cm^3) using a Pasteur pippette containing a bed of alumina and the eluates collected in vials. After removal of the solvent the extracts were weighed and stored in dichloromethane (approximately 100 mm^3) at 4°C. Each fraction was analysed by GC and GC-MS to confirm the identity of HBI alkenes isolated in each fraction and those of sufficient purity and quantity characterized further by micro-ozonolysis and, in some cases, ^{1}H NMR.

8.7.6   MICROSCALE OZONOLYSIS

Ozonolysis was employed in the elucidation of the positions of double bonds in various HBI alkenes. The "Micro-Ozonizer" (Supelco Inc., U.S.A; a modification of the design of Beroza and Bierl, 1969) generated ozone which was passed into a sealed vial containing the isolated alkene(s) dissolved in CS$_2$ within a limited volume insert (100 mm^3) at -70°C for about 5 minutes. After the reaction was completed an aliquot (1 mm^3) of the solution of aldehydes, and/or ketones (produced by cleavage of the ozonide) were analysis by GC and GC-MS and the position of the double bond determined from identification of the cleavage products. Prior to the analysis of HBI alkenes, the technique was validated using simple *n*-alkenes (tetradec-7-ene and heptadec-1-ene) which were succesfully ozonolysed to *n*-heptanal and *n*-hexadecanal respectively.

8.7.7   SILYLATION OF ALCOHOLS

Simple alcohols were derivatised by silylation with *bis*(trimethylsilyl)trifluoroacetamide (BSTFA). BSTFA (100 mm^3) was added to the dry alcohol (*ca.* 1 mg) and heated (60°C; 5 min.).

8.8 SYNTHESIS

2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadec-7(1')-ene

8.8.1 STARTING MATERIALS

The authenticity of all starting materials used in the synthesis of 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadec-7(1')-ene was confirmed by GC, LRMS and IR spectroscopy.

2,6,10,14-tetramethylpentadecan-7-one

GC purity : 95%.

LRMS m/z : 282 [0.5%, M^+], 267 [0.3%, M^+-CH_3], 198 [2%, M^+-C_6H_{12}], 169 [11%], 156 [9%, M^+-C_9H_{18}], 126 [23%], 72 [60%, $CH_3CH=C(CH_3)OH$], 57 [81%], 43 [100%].

IR (liquid film) : ν(C=O) 1715 cm^{-1}, δ_s(CH$_3$) gem dimethyl 1385 cm$_{-1}$ 1370 cm^{-1}, ν(C-CO-C) 1175 cm^{-1}.

3-methylpentanol

GC purity : 96%

LRMS (TMS ether) m/z : 159 [11%, M^+-CH_3], 103 [32%, $CH_2=OSi(CH_3)_3$], 73

[100%, (CH$_3$)$_3$Si$^+$].

IR (liquid film) : ν(O-H) 3320 cm^{-1} intermolecularly bonded, δ_s(CH$_2$) 1460 cm^{-1}, δ_s(CH$_3$) 1375 cm^{-1}, ν(C-O) 1055 cm^{-1} primary saturated.

Triphenylphosphine

Melting point : 80-82°C

IR (KBr disc) : ν(C-H) aryl 2980-3030 cm^{-1} multiple, 1820-1980 cm^{-1} aromatic overtones/combinations, ν(C=C) aryl 1580 cm^{-1}, ν(P-Ph) 1425, 1090, 1025 cm^{-1}, δ(=C-H)oop aryl, 750-760 cm^{-1}, δ(C-C)oop aryl 700-705 cm^{-1}.

6-methylheptan-2-one

GC purity : 97%

LRMS m/z : 128 [25%, M$^+$], 113 [10%, M$^+$-CH$_3$], 110 [47%, M$^+$-H$_2$O] 95 [50%, M$^+$-H$_2$O-CH$_3$], 85 [28%], 71 [40%], 58 [100%, McL].

IR (liquid film) : ν(C=O) overtone 3420 cm^{-1}, ν(C=O) 1720 cm^{-1}, δ_s(CH$_3$) gem dimethyl 1385 cm^{-1} 1370 cm^{-1}, ν(C-CO-C) 1170 cm^{-1}.

8.8.2   1-bromo-3-methylpentane

$$\text{(CH}_3\text{)(C}_2\text{H}_5)\text{CH-CH}_2\text{-CH}_2\text{OH} \xrightarrow{\quad HBr / H_2SO_4 \quad} \text{(CH}_3\text{)(C}_2\text{H}_5)\text{CH-CH}_2\text{-CH}_2Br}$$
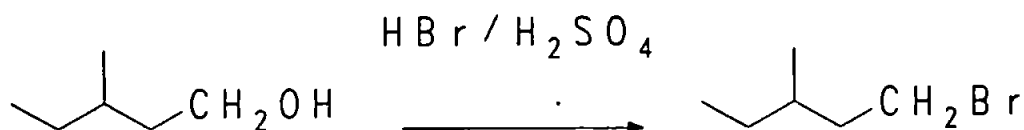
The synthesis was performed using a modification of the method of Kamm and Marvel (1960). Concentrated H_2SO_4 (BDH Analar; 3.0 g) was added carefully with stirring to 48% HBr (BDH Analar; 10.5 g). Following the dropwise addition of 3-methylpentanol (4.4 g; 43 mmol) and a further aliquot of concentrated H_2SO_4 (0.5 g), the reaction mixture was gently refluxed (24 hours). When cool, hexane (25 cm³) and H_2O (25 cm³) were added and the brown-coloured mixture transferred to a separating funnel (250 cm³). The upper dark brown, organic layer containing some suspended solids, was removed and combined with the washings (4 x 15 cm³; hexane) of the aqueous layer. The organic extract was dried (anhydrous Na_2SO_4) and solvent removed by distillation. The crude bromide was purified by column chromatography on deactivated (4%) alumina (100 g). Elution with hexane (200 cm³) and subsequent solvent distillation afforded 1-bromo-3-methyl- pentane (4.42 g).

GC purity : 99%.

Yield   : 62%.

LRMS *m/z* : 164/166 [6%, M$^+$], 135/137 [7%], 107/109 [8%], 85 [100%], 57 [95%].

IR (liquid cell): δ_s(CH$_3$) 1380 cm^{-1}, ω(CH$_2$Br) 1255 cm^{-1}, ν(C-Br) 645 cm^{-1}.

8.8.3   3-methylpentyltriphenylphosphonium bromide
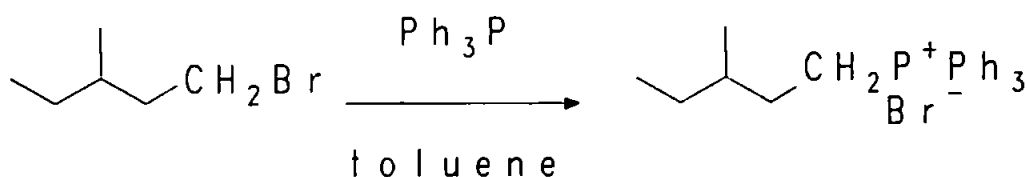
Triphenylphosphine (BDH GPR; 0.8 g) was added to a RBF (25 cm^3) containing toluene (5 cm^3). The contents of the flask were sonicated to dissolve the triphenylphosphine. 1-bromo-3-methylpentane (0.52 g; 3.1 mmol) added and the mixture was stirred and gently refluxed (36 hours) under a pressure of dry nitrogen. Stirring was maintained and the RBF cooled using an ice bath. The white solid (0.91 g) formed was washed with Et$_2$O (10 cm^3) and dried *in vacuo* (P$_2$O$_5$).

Melting point   : 200-202°C

Yield : 70%

IR (KBr disc) : ν(C-H)[CH$_2$] 2780 cm^{-1}, ν(C-H) aliphatic 2780-2980 cm^{-1}, ν(C-H)

aryl 3000-3100 cm^{-1} multiple, ν(P-CH$_2$-) 1425 cm^{-1}, ν(P-Ph) 1435, 1110, 995 cm^{-1},

ρCH$_2$ 720 cm^{-1}, Ph-P$^+$ 1100 cm^{-1}.

8.8.4 3,6,10-trimethylundec-5-enes
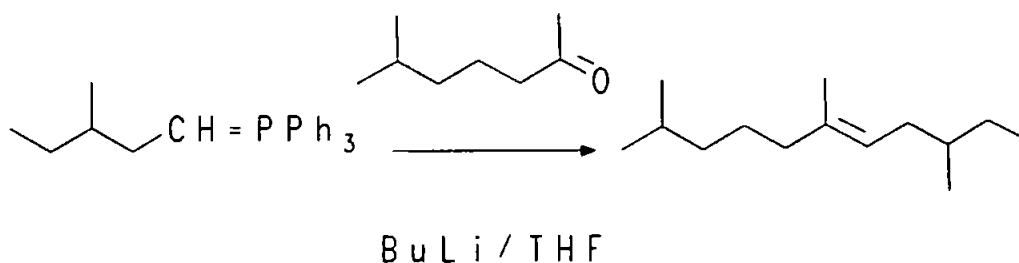
The Wittig reaction of 3-methylpentylphosphonium bromide and a ketone was

first tested by coupling the phosphonium salt with a model compound, 6-

methylheptan-2-one to produce the trisubstituted alkene, 3,6,10-trimethylundec-5-ene.

3-methylpentylphosphonium bromide was pulverised and an aliquot (800 mg;

1.88 mmol) was added to a RBF (100 cm^3) in a suspension of Et$_2$O (20 cm^3) at room

temperature. BuLi (1.6M; 1200 mm^3) in hexane, was added with stirring and in an

inert atmosphere of dry nitrogen gas. On addition of the BuLi a canary-yellow

suspension was formed. The suspension of 3-methyl-pentylenetriphenylphosphorane

was transferred by syringe, under nitrogen, in aliquots (2 cm^3) to a second RBF

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(50 cm^3) which contained the ketone, 6-methylheptan-2-one (240 mg; 1.88 mmol). The mixture was stirred at room temperature and 5 equivalents (5 x 3.4 mm^3) were injected into the flask over a period of 30 minutes. The mixture was transferred to a separating funnel (100 cm^3) and combined with the flask washings (Et$_2$O, 10 cm^3; H$_2$O, 10 cm^3), and the organic layer removed. The aqueous phase was washed with Et$_2$O (3 x 20 cm^3) and the extracts combined with the organic layer. The organic extract was dried (anhydrous Na$_2$SO$_4$) and solvent removed by distillation. The crude products were examined by analytical TLC (0.5 mm silica gel; hexane mobile phase) to determine the compound class composition of the mixture. Visualisation (365 nm; dichlorofluoroscein, 0.1% in IMS) enabled bands at R$_f$ 0.2-0.3 (Ph$_3$P) and 0.7-0.8 (hydrocarbons) to be identified by comparison with authentic compounds. The bulk of the crude product was purified by column chromatography on deactivated (4%) alumina (30 g). Elution with hexane (200 cm^3) and subsequent solvent removal (Buchi; 30°C) afforded a mixture which was further analysed by GC and GC-MS.

Most of the recovered material proved to be triphenylphosphine. Other components included ketol condensation products.

The Wittig reaction was repeated using the same ketone, 6-methyl-heptan-2-one (120 mg; 0.94 mmol) but the procedure modified. The BuLi was first standardised using diphenylacetic acid titration (Kofron and Baclawski, 1976). On addition of the required equivalent of BuLi, the canary-yellow suspension was replaced by a dark red solution. The reaction then proceeded as above and the hydrocarbons produced were analysed by GC and GC-MS. The product, 3,6,10-trimethylundec-5-ene, was recovered in low yield (6%).

GC purity : 87%

LRMS *m\z* : 196 [11%, M⁺], 126 [11%], 111 [15%], 97 [20%], 83 [95%], 69 [100%].

8.8.5   **2,6,10,14-tetramethyl-7-(3'-methylpentylpentadec-7(1')-enes**
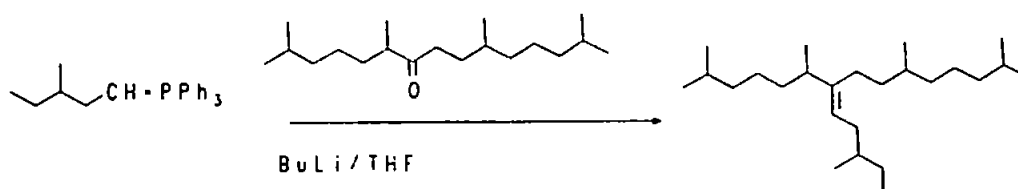
Pulverised 3-methylpentyltriphenylphosphonium bromide (59 mg; 0.14 mmol) was added to the RBF (25 cm³) in a suspension of Et₂O (10 cm³) at room temperature under a pressure of dry nitrogen gas. BuLi (1.6M; 80 mm³) was added dropwise via syringe under nitrogen to the flask (-10°C; ice/NaCl bath) and the contents stirred. The temperature was allowed to rise gradually to room temperature (45 min.). On addition of the BuLi, the formation of a canary-yellow suspension and then a dark red solution was observed. Aliquots of Et₂O were injected to maintain the volume of the solution.

The phosphorane was transferred dropwise under nitrogen to another RBF (10 cm³) by the use of a dry double-ended needle, and added to the ketone,

2,6,10,14-tetramethylpentadecan-7-one (40 mg; 0.14 mmol). The flask temperature was raised from -50°C to room temperature and the contents stirred (45 min.). The reaction products were then decanted into a separating funnel (50 cm³). Water was added (15 cm³) until two phases separated. The organic layer was collected and combined with the washings (Et₂O; 4 x 5 cm³) of the aqueous layer. The extract was dried (anhydrous Na₂SO₄) and solvent removed (Buchi;30°C). The crude products (40 mg) were purified by TLC (0.5 mm silica gel; hexane). Following visualisation (365 nm; dichlorofluoroscein) bands at R$_f$ 0.05-0.5 (2,6,10,14-tetramethylpentadecan-7-one; 23 mg), 0.5-0.6 (triphenylphosphine) and 0.8-0.9 (hydrocarbons; 2.6 mg), were removed and the compounds recovered from the silica gel by desorption with dichloromethane (20 cm³). Solvent was removed (Buchi; 30°C) and the fraction transferred to vials for storage. Analysis by GC and GC-MS showed no formation 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadec-7(1')-ene, the major components being unused ketone, and triphenylphosphine.

The Wittig was repeated coupling 2,6,10,14-tetramethylpentadecan-7-one (23 mg; 0.08 mmol) with 3-methylpentyltriphenylphosphonium bromide (35 mg) using a modified procedure. The solvent used was THF and the RBF temperature reduced to (-80°C; liquid N₂) for the addition of the BuLi. The temperature was allowed to rise gradually and the contents stirred at room temperature (24 hours). The ketone was added dropwise in THF (2 cm³), via syringe under nitrogen to the RBF (-80°C) and stirred (48 hours) under dry argon gas. The crude products were extracted, purified and analysed as previously the major component again proved to be unreacted 2,6,10,14-tetramethyl-pentadecan-7-one.

8.9   SYNTHESIS

2,6,10-trimethyl-7-(3'-methylbuty)dodecenes

8.9.1   STARTING MATERIALS

6-methyl-5-hepten-2-one

GC purity : 94%

LRMS *m/z* : 126 [10%, M^+], 111 [22%, M^+-CH_3], 108 [32%, M^+-H_2O], 93 [14%], 69 [58%], 55 [62%], 43 [100%].

IR (liquid film) : ν(C=O) 1715 cm^{-1}, ν(C-CO-C) 1172 cm^{-1}, ν(C=C) 1625 cm^{-1}, δ_s(CH_3) gem dimethyl 1380 cm^{-1} 1370 cm^{-1}, δ(C-H) oop 840 cm^{-1}, 810 cm^{-1}.

2,8-dimethyldecan-5-ol

GC purity : 75%

LRMS *m/z* : 184 [5%, M^+-2], 168 [10%, M^+-H_2O], 115 [55%], 97 [100%], 83 [95%].

IR (solution cell; CCl_4) : ν(O-H) 3610 cm^{-1} free, ν(O-H) 3440 cm^{-1} intermolecularly bonded, ν(C-O) 1070 cm^{-1} saturated secondary, δ_s(CH_3) gem dimethyl 1390 cm^{-1}

1375 cm^{-1}, ν(C=O) 1735 cm^{-1} (due to saturated aldehyde contaminant).

8.9.2 · 5-bromo-2,8-dimethyldecane
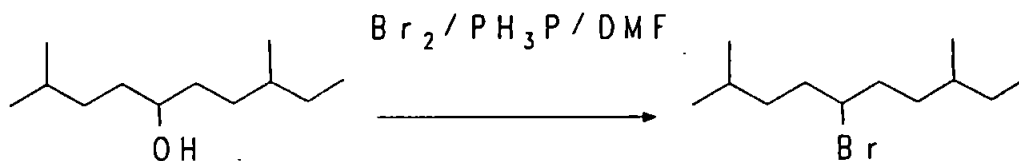
The secondary alcohol was first purified by column chromatography on deactivated (10%) silica (30 g). Elution with hexane:Et$_2$O (90:10; 100 cm^3) removed the aldehyde and 2,8-dimethyldecan-5-ol (with a GC purity of 96%) was afforded by elution, and subsequent removal of (Buchi:30°C) dichloromethane (200 cm^3).

The method used to prepare 5-bromo-2,8-dimethyldecane was a modification of the method of Wiley *et al.* (1964). Triphenylphosphine (BDH, GPR; 350 mg; 1.3 mmol) was dried (P$_2$O$_5$) under argon, *in vacuo*, overnight. Dry DMF (stored over molecular sieve; 5 cm^3) was added to a RBF (25 cm^3) and the contents of the flask stirred and cooled (ice bath; 0°C). An excess of dry bromine (Aldrich; 100 mm^3) was injected dropwise until an orange-brown suspension formed. 2,8-dimethyldecan-5-ol (250 mg; 1.3 mmol) was added dropwise in DMF (3 cm^3) and the ice bath removed. The brown solution produced was allowed to reach room temperature whilst stirring was continued. The temperature was then gently increased (40°C; 3 hours). The

reaction mixture was then transferred to a separating funnel (150 cm³) containing cold

water (50 cm³). A yellow suspension was immediately formed. More water (25 cm³)

was added until the suspension dissolved and then Et₂O (25 cm³) was added.

The aqueous phase was extracted (Et₂O; 4 x 10 cm³) until the water was

colourless. The organic extract was dried (anhydrous Na₂SO₄) and solvent removed

(Buchi; 30°C). The crude products were purified by column chromatography on

deactivated (4%) silica (30 g). Elution with hexane (200 cm³) and subsequent solvent

removal (Buchi; 30°C) afforded 5-bromo-2,8-dimethyldecane.

GC purity : 90%

Yield : 22%

LRMS m/z : 248/50 [tr, M⁺], 247/49 [tr, M⁺-H], 219/21 [tr], 205/07 [tr], 169 [5%,

M⁺-Br], 168 [13%, M⁺-HBr], 127 [8%], 113 [20%], 99 [30%], 85 [65%], 57

[100%].

IR (solution cell; CCl₄) : δ_s(CH₃) gem dimethyl 1375 cm⁻¹ 1390 cm⁻¹, absence of;

ν(C=C) 1650-1675 cm⁻¹, δ(=C-H)oop 950-980 cm⁻¹ (*trans*), 650-750 cm⁻¹ (*cis*). NB.

ν(C-Br) shifted beyond 600 cm⁻¹; secondary bromide.

8.9.3   6-methylheptan-2-one
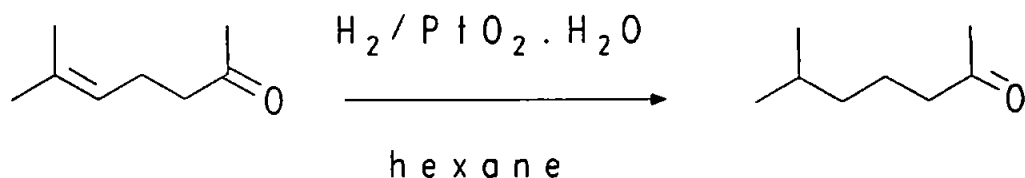
The saturated ketone was prepared by hydrogenation of 6-methylhept-5-en-2-one. 6-methylhept-5-en-2-one (Aldrich; 10.0 g; 80 mmol) was added to rapidly stirred hexane (150 cm^3) containing preactivated (30 min.) $PtO_2.H_2O$ (Adams catalyst; 0.15 g). Hydrogen uptake was monitored and the reaction stopped upon the uptake of the stoichiometric quantity (1790 cm^3). Following filtration and drying (anhydrous Na_2SO_4), solvent was removed (Buchi; 30°C) and the crude product purified by column chromatography on deactivated (4%) neutral alumina (100 g). Elution with hexane (200 cm^3) and subsequent solvent removal (Buchi; 30°C) afforded 6-methylheptan-2-one (9.1 g; 71 mmol).

GC purity : 97%

Yield : 90%

LRMS m/z : 128 (25%, M$^+$], 113 [10%, M$^+$-CH$_3$], 110 [47%, M$^+$-H$_2$O], 95 [50%, M$^+$-H$_2$O-CH$_3$], 85 [28%], 71 [40%], 58 [100%, McL].

IR (liquid film) : ν(C=O) overtone 3420 cm^{-1}, ν(C=O) 1720 cm^{-1}, δ_s(CH$_3$) gem dimethyl 1385 cm^{-1} 1370 cm^{-1}, ν(C-CO-C) 1170 cm^{-1}.

8.9.4   2,6,10-trimethyl-7-(3'-methylbutyl)dodecan-6-ol
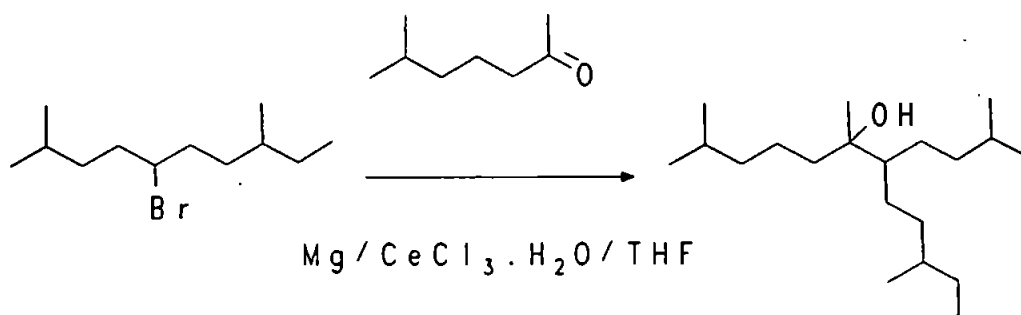
"Cerium chloride promoted Grignard" synthesis of 2,6,10-trimethyl-7-(3'-methylbutyl)dodecan-6-ol was performed according to the method of Imamato *et al.* (1985) as employed by Robson and Rowland (1986, 1988a) for similar compounds.

Part i : 5-bromo-2,8-dimethyldecane (70 mg; 0.28 mmol) in THF (5 cm^3; redistilled from LiAlH$_4$) was added dropwise over the period of 1 hour to an excess of Mg scrapings (30 mg; freshly prepared from Mg ribbon) a drop of dibromoethane having been initially added. During the course of the addition a cloudy white precipitate appeared and disappeared and much of the Mg scrapings were consumed. Reflux (1 hour) completed the preparation of 2,8-dimethyldec-5-magnesium bromide.

Pàrt ii : CeCl$_3$.7H$_2$O (Aldrich; 107 mg) was quickly and finely powdered in a mortar and placed in a RBF (25 cm^3). The water of crystallisation was removed by heating *in vacuo* (0.45 mg Hg; 12°C; oil bath) for 1 hour, adding a magnetic stirrer

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and then stirring *in vacuo* at the same temperature for a further hour. Whilst still hot, argon (dried over activated molecular sieve) was introduced, the flask cooled and THF (10 cm^3) added. Following stirring, the suspension was cooled (20°C; ice bath) and the previously prepared Grignard reagent (part i) transferred carefully by the use of a dried double-ended needle into the flask. A further period of stirring (0°C; 1 hour) was followed by the rapid addition of 6-methylheptan-2-one (30 mg; 0.23 mmol) in THF (2 cm^3). After stirring (0°C; 1 hour) the mixture was transferred to a separating funnel (50 cm^3) and treated with aqueous glacial ethanoic acid (4%; 25 cm^3). The organic layer was removed and the aqueous layer re-extracted with Et$_2$O (2 x 10 cm^3). The combined organic extract was washed successively with NaHCO$_3$ (saturated solution; 15 cm^3), brine (15 cm^3) and H$_2$O (10 cm^3). After drying (anhydrous Na$_2$SO$_4$), solvent was removed (Buchi; 30°C) and the crude product purified by TLC (0.5 mm silica gel; hexane:Et$_2$O [95:5] mobile phase). Following visualisation (365 nm; dichlorofluoroscein) bands at R$_f$ 0.05-0.15 (corresponding to unreacted ketone), 0.20 and 0.25, and 0.65-0.90 were removed and the compounds recovered from the silica gel by desorption with dichloromethane (20 cm^3). Solvent was evaporated (Buchi; 30°C) and each fraction quantitatively transferred to a vial for storage. Further analysis by GC and GC-MS of all the fractions showed no 2,6,10-trimethyl-7-(3'-methylbutyl)-dodecan-6-ol to be present.

8.10 ACID-CATALYSED REARRANGEMENTS

8.10.1 STARTING MATERIAL

Anhydrous toluene-*p*-sulphonic acid-acetic acid reagent

Toluene-*p*-sulphonic acid (TsOH) was prepared from the monohydrate (Matheson, Coleman and Bell) by recrystallisation from ethyl acetate and dried under vacuum (50°C). Anhydrous toluene-*p*-sulphonic acid-acetic acid (TsOH-HOAc) was prepared by heating TsOH (1.0 g) under reflux in HOAc (35 cm³) and cyclohexane (10 cm³) in a distillation apparatus until the temperature reached 117°C. The remaining solution was allowed to cool and used as required.

8.10.2 REARRANGEMENT OF SYNTHETIC HBI MONOENES
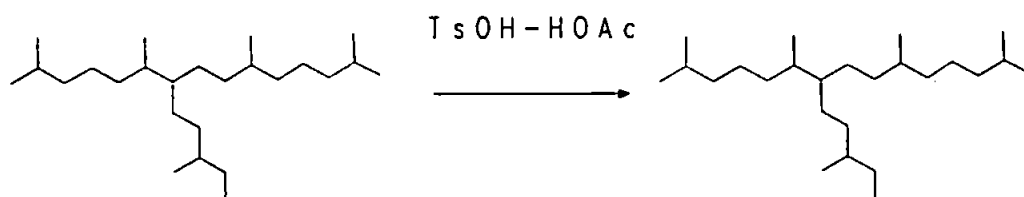
8.10.2.1 *Isomerisation of C₂₅ HBI monoenes*

The acid catalysed isomerisation of 2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadecenes was performed initially on a pilot scale using the

procedure of Peakman and Maxwell (1988).

Anhydrous TsOH-HOAc (500 mm^3) was added to the alkenes (*ca.* 1 mg) in a Reacti-vial (1 cm^3) and heated (heating block; 70°C) for 7 days. The reaction mixture was diluted with water (500 mm^3) and extracted with hexane (3 x 100 mm^3). The combined organic extracts were washed with NaHCO$_3$ (saturated solution; 1 cm^3), dried (anhydrous Na$_2$SO$_4$) and filtered. Solvent was evaporated under a stream of nitrogen gas. The remaining aqueous solution was further extracted with dichloromethane (300 mm^3). The isomeric mixture (0.1 mg; *ca.* 10% yield) was examined by GC and GC-MS. The GC RI and mass spectrum of each compound were recorded.

The acid-catalysed rearrangement was repeated on a larger scale and transformations monitored with time. Anhydrous TsOH-HOAc (500 mm^3) was added to one Reacti-vial (1 cm^3) containing the C$_{25}$ HBI alkenes (5 mg) and 7-*n*-hexylnonadecane (1 mg) as an internal standard. The contents were heated (70°C) and sampled at various intervals. After 10 days the temperature was raised to 150°C. A second Reacti-vial (1 cm^3) containing only TsOH-HOAc (500 mm^3) was heated (10 days 70°C; 2 days 150°C) as a procedural blank. Aliquots (50 mm^3) were taken from the sample vial using a syringe and worked up as previously.

Various fractions were examined by IR (Perkin Elmer Series 1720X FTIR) and argentation TLC. The former was carried out on the isomeric mixture of C$_{25}$ HBI alkenes prior to, and after the acid-catalysed rearrangement.

Argentation TLC (0.25 mm silica gel (10% w/w AgNO$_3$): hexane mobile phase] afforded 3 spots. Following visualisation (Rhodamine 6G; 365 nm), spots at Rf 0.60-0.70 (7-*n*-hexylnonadecane), 0.45-0.50 and 0.28-0.39 (both alkenes) were

removed and the compounds recovered from the silica gel by desorption with dichloromethane (1.5 cm^3). These TLC fractions were examined by GC and GC-MS.

8.10.2.2   *Further isomerisation reactions using* C_{20}, C_{25} *and* C_{30} *monoenes*

The TsOH-HOAc rearrangement was repeated further using C_{20}, C_{25} and C_{30} HBI alkenes (5 mg) heated at 70°C for 2 days. After work up, the alkenes produced were examined by GC and GC-MS. The GC RI and mass spectrum of each isomer were recorded. The isomers were then separated by either argentation HPLC or TLC (as described above; **8.7.5**). Bands at R$_f$ 0.40-0.45, 0.45-0.53, 0.53-0.60, 0.60-0.67 and 0.67-0.74 were removed and the compounds recovered from the silica gel by desorption with dichloromethane (1.5 cm^3). Solvent was evaporated and the individual fractions collected by HPLC and TLC examined by GC and GC-MS. Those containing single isomers of sufficient purity were combined and were assigned by micro-ozonolysis and some by ^{1}H NMR (400 MHz and/or 270 MHz) as described earlier (**8.7.4** and **8.7.6**).

8.11   CHARACTERISATION OF ALKENE FRACTIONS ISOLATED BY Ag$^+$ PREPARATIVE CHROMATOGRAPHY

8.11.1   *2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadec-6(7)-enes*

Isomers br25:1; 2115$_{DBI}$ and 2125$_{DBI}$ produced, after ozonolysis, only two ketones.

2-methylheptan-6-one

M$^+$ 128, 110 [38%, M$^+$-H$_2$O], 95 [40%, M$^+$-H$_2$O-CH$_3$], 85% [22%, M$^+$-C$_3$H$_7$], 58 [100%, McL].

3,9,13-trimethyltridecan-6-one

M^+ 254, 169 [9%, α-cleavage$\rightarrow$$C_{10}H_{21}CO^+$], 126 [30%, M^+-McL], 95 [40%, α-cleavage-H_2O], 71 [100%, McL+ γ-cleavage^1$\rightarrow$$C_4H_6OH^+$ and/or C_5H_{11}].

8.11.2   *2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadec-7(8)-*

and -7(1')-enes

The products from ozonolysis included two ketones.

2,6,10,14-tetramethylpentadecan-7-one

M^+ 282, 267 [5%, M-CH_3], 198 [10%, McL] 169 [55%, α-cleavage$\rightarrow$$C_{10}H_{21}CO^+$], 126 [80%, C_9H_{18}], 95 [85%, α-cleavage-H_2O-CO], 72 [95%, Double McL], 57 [100%].

2,6,10-trimethyldodecan-7-one

M^+ 226, 211 [4%, M^+-CH_3], 169 [6%, M^+-C_4H_9], 156 [20%, McL], 142 [45%, McL], 113 [90%, α-cleavage$\rightarrow$$C_6H_{13}CO^+$], 95 [100%, α-cleavage-H_2O], 72 [95%, Double McL].

Other ozonolysis products included two aldehydes.

2,6-dimethyloctanal

M^+ absent, 138 [1%, M^+-H_2O], 123 [10%, M^+-H_2O-CH_3], 112 [60%, M^+-McL], 71 [95%, α-cleavage$\rightarrow$$C_3H_7CO^+$], 69 [70%, C_5H_9], 56 [100%, C_4H_8].

3-methylpentanal

M^+ 100, 82 [5%, M^+-H_2O], 71 [15%, C_5H_{11}], 56 [100%, M^+-McL].

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^{1}The product of McLafferty rearrangement may undergo further rearrangement with (Double McL) and without H-transfer. To distinguish between the two, the latter is termed γ-cleavage.

¹H NMR (400 Mhz) δ ppm:
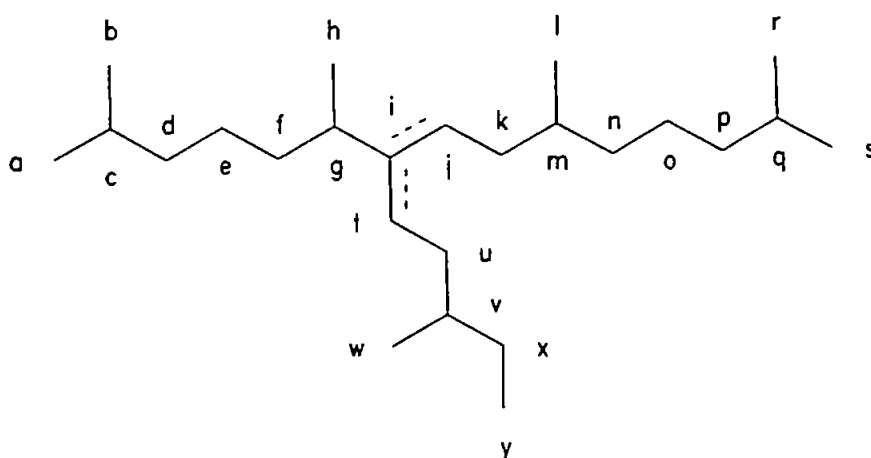
0.849 (*m*; 21H; a, b, l, r, s, w and y)

0.958 (*d*; 3H; h)

1.10-1.40 (*m*; 20H; d-f, k, n-p, x and -CH from c, m, q or v)

1.65-2.05 (*m*; 5H; g, j and u)

5.05 (*t*; 1H; t)

8.11.3   *2,6,10,14,18-pentamethyl-7-(3'-methylpentyl)nonadec-6(7)-enes*

Isomers br30:1; 2565$_{DB1}$ and 2579$_{DB1}$ produced, after ozonolysis, only two ketones, one of which was 2-methylheptan-6-one (see **7.10.1**).

3,9,13,17-tetramethyloctadecan-6-one

M$^+$ 324, 323 [M$^+$-1], 239 [18%, α-cleavage→C$_{15}$H$_{31}$CO$^+$], 196 [40%, M$^+$-McL or M$^+$-Double McL→C$_{14}$H$_{28}$], 141 [30%], 129 [25%, Double H-transfer + β-cleavage], 126 [30%, C$_9$H$_{18}$], 113 [40%, α-cleavage→C$_6$H$_{13}$CO$^+$], 95 [58%, α-cleavage-H$_2$O], 85 [40%, α-cleavage-CO], 71 [100%, McL + γ-cleavage→C$_4$H$_6$OH$^+$ and/or C$_5$H$_{11}$].

8.11.4   *2,6,10,14,18-pentamethyl-7-(3'-methylpentyl)nonadec-7(8)-*

and -7(1')-enes

The products from ozonolysis included two ketones one of which was 2,6,10-trimethyldodecan-7-one (see **8.11.2**).

2,6,10,14,18-pentamethylnonadecan-7-one

M^+ 352, 353 [M^++1], 337 [10%, M-CH$_3$], 268/9 [10%, McL/Double H-transfer], 239 [45%, α-cleavage→C$_{15}$H$_{31}$CO$^+$], 196 [80%, C$_9$H$_{18}$], 156 [45%, McL], 157 [30%, Double H-transfer], 141 [20%, α-cleavage→C$_8$H$_{17}$CO$^+$], 126 [35%, C$_9$H$_{18}$], 113 [30%, α-cleavage-CO], 95 [45%, α-cleavage-H$_2$O-CO], 85 [80%, McL + γ-cleavage→C$_5$H$_8$OH$^+$ and C$_6$H$_{13}$], 72 [100%, Double McL].

Other ozonolysis products included two aldehydes one of which was 3-methylpentanal (see **8.11.2**).

2,6,10-trimethyldodecanal

M^+ absent, 182 [25%, M^+-McL], 126 [40%, C$_9$H$_{18}$], 112 [30%, C$_8$H$_{16}$], 97 [55%, C$_7$H$_{13}$], 81 [42%], 71 [100%, C$_5$H$_{11}$ and C$_3$H$_6$CHO].

8.11.5 *2,6,10-trimethyl-7-(3'-methylbutyl)dodec-6(7)-enes*

Isomers br20:1; 1711_{DBI} and 1714_{DBI} produced, after ozonolysis, only two ketones one of which was 2-methylheptan-6-one.

2,8-dimethyldecan-5-one

M^+ 184, 128 [20%, McL], 114 [25%, McL], 113 [60%, α-cleavage$\rightarrow C_6H_{13}CO^+$], 99 [70%, α-cleavage$\rightarrow C_5H_{11}CO^+$], 95 [86%, α-cleavage-H_2O], 81 [65%, α-cleavage-H_2O], 71, [100%, McL + γ-cleavage$\rightarrow C_4H_6OH^+$ and/or C_5H_{11}], 58 [85%, Double McL].

8.11.6 *2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadec-5(6)-ene*

Only one ketone was detected in the ozonolysis products.

2,6,10-trimethyl-(3'-methylpentyl)undecan-2-one

M^+ 282, 267 [2%, M^+-CH_3], 264 [2%, M^+-H_2O], 253 [3% M^+-C_2H_5], 198 [25%, McL], 180 [40%, McL-H_2O], 142 [95%, McL] 124 [100%, McL-H_2O].

H NMR (270 Mhz) δ ppm:
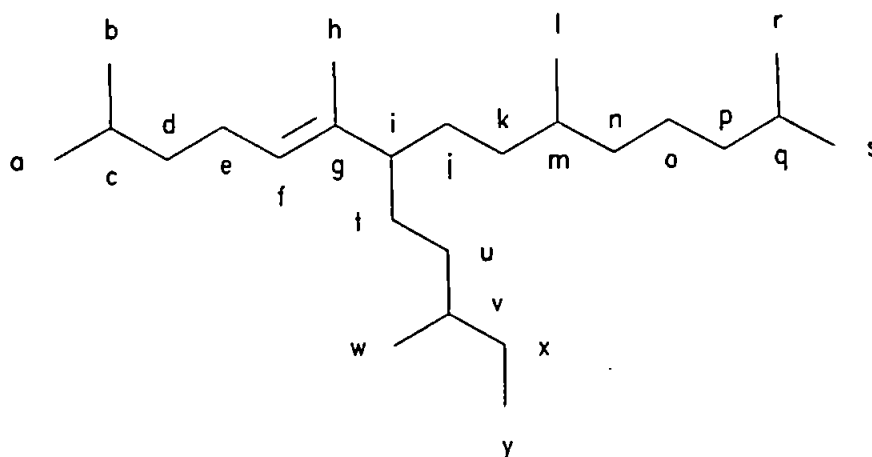
0.867 (*m*; 21H; a, b, l, r, s, w and y)

1.05-1.38 (*m*; 22H; d, j, k, n-p, t, u, x and -CH c, m, q, v)

1.42 (*s*; 3H; h)

1.50 (*s*; 3H; h)

1.85-1.96 (*m*; 2H; e)

1.98 (*m*; 1H; i)

5.07 (*t*; 1H; f)

5.21 (*t*; 1H; f)

8.11.7   *2,6,10-trimethyl-7-(3'-methylbutyl)dodec-5(6)-ene*

Only one ketone was detected in the ozonolysis products.

6-methyl-(3'-methylbutyl)octan-2-one

M^+ 212, 197 [2%, M^+-CH_3], 194 [2%, M^+-H_2O], 183 [3% M^+-C_2H_5], 142 [30%, McL], 128 [50%, McL], 124 [45%, McL-H_2O], 110 [65%, McL-H_2O], 71 [85%, McL + γ-cleavage→$C_4H_6OH^+$].

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i) CONFERENCES

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1st British Organic Society meeting, June, 1988, Bangor, U.K.

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ii) POSTERS

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iii) ORAL PRESENTATIONS

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iv) PUBLICATIONS

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Isolation and characterization of sedimentary and synthetic highly branched C_{20} and C_{25} monoenes

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ABSTRACT

Hird, S.J., Evens, R. and Rowland, S.J., 1992. Isolation and characterization of sedimentary and synthetic highly branched C_{20} and C_{25} monoenes. *Mar. Chem.*, 37: 117–129.

Argentation chromatography (TLC, HPLC) followed by gas chromatographic (GC), spectroscopic (GCMS, and in two cases, NMR) and degradative (ozonolysis) analyses of pure isolates, has allowed the double bond positions of several synthetic highly branched C_{20} and C_{25} monoenes to be established.

A similar isolation and characterization of a C_{20} monoene from sediments of the Tamar estuary (UK) and of a C_{25} monoene (hydrogenation product of a diene) from McMurdo Sound (Antarctica) sediments, showed that they both contained methylene double bonds, identical to those previously found in monoenes from Shark Bay (Western Australia). Comparison of the GC and GCMS data of the synthetic monoenes with those obtained for a sedimentary C_{20} monoene from Gluss Voe (Shetland Isles, UK) and two C_{25} monoenes from the Tamar estuary, showed that the double bonds in these compounds were probably in non-methylenic positions.

These findings may have important implications. The differences in double bond positions may reflect contributions of alkenes from different source organisms, or from the same organisms living under different environmental conditions. In time the compounds may prove to be useful biological markers of recent and palaeoenvironments. Also, since it has been suggested that reactions between the alkenes and sedimentary inorganic sulphur species may be controlled by the position and extent of unsaturation, a knowledge of the double bond positions will further our understanding of the diagenetic fate of these unusual compounds.

INTRODUCTION

C_{20}, C_{25} and C_{30} alkenes with highly branched isoprenoid parent structures (1–3) occur widely in recent sediments (see review by Rowland and Robson, 1990). Although interest in these compounds is still largely academic, their unusual structures, widespread occurrence in a variety of sediments, their occurrence in field samples of algae (Rowland et al., 1985; Nichols et al., 1988) and the detection of the parent alkanes and related sulphur analogues in sed-

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iments, oil-shales and oils (Sinninghe Damsté et al., 1989) has provoked several suggestions that the alkenes may be useful markers of biological contributions of organic matter to sediments (e.g. Yon et al., 1982; Dunlop and Jefferies, 1985; Kenig et al., 1990). However, although the parent structures of the hydrocarbons have been proved by synthesis (Yon et al., 1982; Robson and Rowland, 1986, 1988) the position of the double bond(s) has only been reported in two studies (for a C_{20} and C_{25} monoene and a C_{25} diene; Dunlop and Jefferies, 1985; Yruela et al., 1990). A more complete knowledge of the position and geometry of the double bonds may prove of value for elucidation of the biological origin, biosynthesis, biochemical function and diagenetic fate of the hydrocarbons. For example, it is possible that the mechanism of sedimentary sulphur incorporation (Sinninghe Damsté et al., 1989) is partly controlled by such structural features.

In the present study we report the double bond positions in a C_{20} monoene from Gluss Voe (Shetland Islands, UK) sediments, in a C_{20} and two C_{25} monoenes from the Tamar estuary (UK) sediments, and in a C_{25} monoene (partial hydrogenation product of a diene) from McMurdo Sound (Antarctica) sediments. The identifications have been accomplished by use of argentation chromatography (TLC and HPLC), gas chromatography (GC), GC mass spectroscopy (GCMS) and micro-ozonolysis. Isolation and characterization of mixtures of C_{20} and C_{25} monoenes (mixtures synthesized previously, Robson, 1987; Robson and Rowland, 1986) by GC, GCMS, NMR and micro-ozonolysis has provided a useful database for these and probably also for future identifications.

EXPERIMENTAL

Extraction and fractionation of sediments

Gluss Voe
The isolation and fractionation of hydrocarbons from Gluss Voe sediments has been described previously (Robson, 1987).

Tamar estuary
Surface sediments (0–2 cm depth) from a number of randomly selected sites at Millbrook were combined. The samples were collected by metal spatula, transferred to a clean aluminium can and frozen immediately. The thawed sample was extracted using the method of Douglas et al. (1981). Briefly, sediment (approximately 40 g wet weight) was extracted with methanol (40 cm³) by ultrasonication (5 min Soniprep 150-probe) with cooling (ice bath). The organic extract was separated by centrifugation (20 min; 1800 r.p.m.) and decanted. This procedure was repeated using dichloromethane/methanol (7:3 v/v), dichloromethane/methanol (4:1 v/v) and dichloromethane. The

combined extracts were shaken (separating funnel) with water (Millipore grade; 30 cm^3) and the lower organic layer collected, along with the dichloromethane washings (3×15 cm^3) of the aqueous layer. Solvent was removed (Buchi; 30°C) and the total organic extract transferred quantitatively to a vial and weighed. Where water was still present after solvent removal, dichloromethane (20 cm^3) was added and the mixture transferred to a small separating funnel, where the lower organic layer was carefully removed, reconcentrated and weighed. The extract was presorbed on to alumina (ca. 100 mg) and applied to a short column (20 cm $\times$ 1.0 cm o.d.) containing alumina (5% deactivated; 1 g) over activated copper powder (0.2–0.5 g w/w) and eluted with hexane/benzene (95:5 v/v; 5 cm^3). This column procedure removed most of the polar, non-hydrocarbon organic material (e.g. pigments) and elemental sulphur, prior to TLC. The column eluate was evaporated to dryness, weighed and dichloromethane (100 mm^3) was added. The sample (hydrocarbons and non-polar pigments, less than 15 mg) was spotted 2 cm from the bottom of a silica gel TLC plate which was then developed with hexane. The bands were visualized by spraying the plate with a methanolic solution (0.5%) of Rhodamine G (in some cases dichlorofluoroscein was used) and then viewed under ultraviolet (UV) light (365 nm). A reference mixture of n=eicosane, n-eicos-1-ene, squalene and anthracene was also used. The hydrocarbon band corresponding to an R_f value of 0.35–0.92, was removed and the rest of the plate (divided into two fractions corresponding to R_f values of 0–0.08 and 0.08–0.35) was removed separately. The latter fraction contained mainly aromatic hydrocarbons. The hydrocarbons were recovered from the silica gel by desorption with hexane/dichloromethane (60:40 v/v; ca. 5 cm^3) through a Pasteur pipette containing a bed of alumina and the eluates collected in vials. After removal of the solvent, the fractions were weighed and stored in dichloromethane (approximately 100 mm^3) at 4°C.

The non-aromatic hydrocarbon fractions were further separated into saturated and unsaturated components by silver ion TLC (10% AgNO$_3$/silica gel w/w) using hexane as the mobile phase. The bands on the plate were visualized (UV light, 365 nm; 0.5% Rhodamine 6G in methanol) and the saturated and unsaturated aliphatic hydrocarbon bands, corresponding to R_f values of 0.65–0.85 and 0.30–0.65, and the rest of the plate, were removed as three fractions. The hydrocarbons were recovered in the normal way.

High performance liquid chromatography (HPLC)

A high performance liquid chromatography (HPLC) method for separation of structural isomers of unsaturated hydrocarbons using small particle size, silver nitrate-impregnated silica as a stationary phase was adapted from Dimitrova (1979). A liquid chromatograph was equipped with a Perkin Elmer Series 410 2C Pump, a Knauer differential refractometer, a Rheodyne

injector incorporating a 20 mm³ sample loop, and a Pharmacia FRAC-100 fraction collector. Chromatograms were recorded using a chart recorder and Perkin Elmer Nelson PC Model 2100 integrator installed on an Amstrad personal computer. Silica gel (Hypersil Shandon; 5 μm) was used as a support for silver nitrate impregnation. The silica gel (5 g) was preactivated by heating at about 100°C in vacuo for about 30 min. A solution of silver nitrate (BDH Analar; 0.5 g) in acetonitrile (BDH HiPerSolv; 50 cm³) was added and the contents of the flask homogenized by ultrasound. The acetonitrile was removed under low pressure (Buchi; 100°C). All manipulations were carried out in blacked-out flasks and darkened laboratories. The column (25 cm × 0.5 cm) was slurry-packed under pressure using chloroform as the solvent. The column was then equilibrated using heptane (BDH HyPerSolv). Partial separation of isomers was achieved using flow rates of 0.2 and 0.5 cm³ min⁻¹ and fractions (20 mm³) collected. Solvent was evaporated and the individual fractions examined by GC and GCMS. Those containing single isomers of sufficient purity were combined and assigned by micro-ozonolysis and some by ¹H NMR.

Acid-catalysed rearrangement of synthetic C₂₀ and C₂₅ monoenes

Mixtures of C₂₀ and C₂₅ synthetic monoenes (Robson and Rowland, 1986; Robson, 1987) were isomerized to produce further isomers by the method of Peakman and Maxwell (1988). Briefly, toluene-p-sulphonic acid (TsOH) was prepared from the monohydrate by recrystallization from ethyl acetate and dried under vacuum (50°C). Anhydrous toluene-p-sulphonic acid–acetic acid (TsOH–HOAc) was prepared by heating TsOH (1.0 g) under reflux in HOAc (35 cm³) and cyclohexane (10 cm³) in a distillation apparatus until the temperature reached 117°C. The remaining solution was allowed to cool and used as required.

Anhydrous TsOH–HOAc (500 mm³) was added to the alkenes (ca. 5–10 mg) plus 7-n-hexylnonadecane internal standard (1 mg) in a reactivial (1 cm³) and heated (heating block; 70°C) for 2 days. The reaction mixture was diluted with water (500 mm³) and extracted with hexane (3 × 100 mm³). The combined organic extracts were washed with NaHCO₃ (saturated solution; 1 cm³), dried (anhydrous Na₂SO₄) and filtered. Solvent was evaporated under a stream of nitrogen gas. The remaining aqueous solution was further extracted with dichloromethane (300 mm³). The isomeric mixtures (ca. 10% yield of internal standard) were examined by GC and GCMS. The retention index (RI) and mass spectrum of each isomer was recorded. Essentially this reaction produced one 'new' C₂₀ and one 'new' C₂₅ isomer. Argentation TLC (0.25 mm silica gel (10% w/w AgNO₃): hexane mobile phase) of the reaction products afforded three bands from which, following visualization (Rhodamine 6G; 365 μm), the 'new' isomers were recovered (R_f 0.45–0.50) by

desorption with dichloromethane (1.5 cm^3). The 'new' alkene isomers were examined by GC, micro-ozonolysis and (C$_{25}$ only) ^{1}H NMR.

Microscale ozonolysis

Ozonolysis was employed in the elucidation of the positions of double bonds in the various alkenes. The 'Micro-Ozonizer' (Supelco, Inc., USA), a modification of the design of Beroza and Bierl (1969), generated ozone which was passed into a sealed vial containing the isolated alkene(s) in a limited-volume insert (100 mm^3) at $-70°$C for about 5 min. After the reaction was completed an aliquot (1 mm^3) of the solution of aldehydes, and/or ketones (produced by cleavage of the ozonide) was analysed by GC and GCMS and the position of the double bond determined from identification of the cleavage products.

Gas chromatography (GC)

Hydrocarbons were examined on a Carlo Erba Series 5300 Mega gas chromatograph fitted with fused silica columns (0.3 mm i.d.) of various lengths and phases (mainly DB-1 or DB-5; J&W) using flame ionization detection and on-column injection. Using DB-1/DB-5, the column oven was programmed from 40 to 80°C at 10°C min^{-1}, and from 80 to 300°C at 5°C min^{-1} and held at the final temperature for 20 min. Hydrogen was used as the carrier gas at a flow rate of 2 cm^3 min^{-1} (set at 250°C) supplied at a pressure of 0.4 kg cm^{-2}. Certain solvent extracts were also analysed using a 25 m column coated with CPWAX52 (Chrompack, Holland) and/or 15 m DBWAX column (J&W) using flame ionization detection and on-column injection. The carrier gas was hydrogen (2 cm^3 min^{-1}) and the oven temperature programmed from 40 to 80°C at 10°C min^{-1}, and from 80 to 240°C at 6°C min^{-1} and held at 240°C for 10 min.

Gas chromatography mass spectrometry (GCMS)

Analysis of selected hydrocarbon extracts was performed on a Carlo Erba Series 5160 Mega chromatograph coupled to a Kratos MS 25 double focussing magnetic sector mass spectrometer. A 30 m fused silica column coated with DB-5 (J&W) was introduced directly into the ion source of the mass spectrometer. On-column injection and helium carrier gas were used and the column oven programmed as for GC.

Mass spectrometer operating conditions were; ion source temperature 250°C, 40 eV ionizing energy and a filament emission current of 400 μA. On a number of occasions the ionizing energy was reduced to 20 eV and the source temperature to 200°C. Spectra (m/z 40–532) were collected every 1.5 s using a DS90 data system.

Compound identification

Individual hydrocarbons were identified by co-chromatography with authentic compounds on columns of different polarities and by comparison of gas chromatographic retention indices with literature data. Additional information was provided by GCMS: the recognition of components from their mass spectra was made by comparison with the spectra of authentic compounds, published spectra or by spectral interpretation as indicated in the text.

Infrared spectrometry (IR)

Infrared spectra were recorded as either liquid films, KBr discs or solutions (in CCl_4) using a Perkin Elmer Series 1720X FTIR spectrometer.

Nuclear magnetic resonance spectroscopy

^{1}H NMR spectra were recorded on a 400 MHz Jeol spectrometer in $CDCl_3$ solutions.

RESULTS AND DISCUSSION

Synthetic monoenes

Previous syntheses of *1* and *2* produced, as intermediates, mixtures of C_{20} and C_{25} monoenes (*4* and *5*; Robson and Rowland, 1986; Robson, 1987). The isomers were not separable by preliminary Ag^+ TLC experiments (Robson, 1987) and the double bond positions could not be confirmed by derivatization techniques such as bis-hydroxylation (OsO_4) or thiolation (Robson, 1987).

However, when examined by Ag^+ HPLC in the present study, slight separation of each group of isomers *4* and *5* was observed and careful preparative scale HPLC allowed reasonably pure samples of some isomers of the C_{20} and C_{25} monoenes to be collected (Table 1). These were examined by GC, GCMS, and some (*10/11, 14*) by ^{1}H NMR. In addition, all were micro-ozonolysed and the ozonolysis products examined by GC and GCMS. These studies allowed the positions of the double bonds in several of the synthetic monoenes to be established (Table 1). For example, isomers *6* and *7* produced, on ozonolysis, only ketones *8* and *9*. Ketone *8* was identified by comparison of the mass spectrum with that of the synthetic compound (synthesized via the method of Robson and Rowland, 1986) and *9* by interpretation of the mass spectrum ($M^+ \cdot 254$, 169 (30%, $C_{10}H_{21}CO^+$), 126 (95%), 95 (88%), 71 (100%). This identifies the double bond in *6* and *7* as 7(8). Alkenes *6* and *7* are E/Z isomers.

Alkene peak *10* proved to be a mixture of two alkenes (namely *10* and *11*). The products from ozonolysis were ketones *12* and *13* identified by mass

TABLE I

Chromatographic data for isolated synthetic and sedimentary alkenes

Alkene	Structure[a]	GC retention index[b]			GC purity (%)	Identification method
		DBI	DB5	DBWAX		
Synthetic						
$C_{20:1}$	*21*	1677	1674	1643	85	O_3
	22	1697	1693	1670	67	O_3
$C_{25:1}$	*6, 7*	2115, 2154	2110, 2119	2074, 2083	42, 44	O_3
	10, 11	2076	2072	2023	88	O_3, NMR
	14	2110	2100	2062	83	O_3, NMR
Tamar estuary						
$C_{20:1}$	*17*	1702	1698	1659	95	O_3, NMR, FTIR
$C_{25:1}$	*10, 11*	2076	–	–	–	GC vs. synthetic
Gluss Voe						
$C_{20:1}$	*22*	1696	–	–	–	GC vs. synthetic
McMurdo Sound						
$C_{25:2}$[c] (monoene hydrogenation product)	*19*	2110	2100	2077[d]	–	H_2, O_3

[a]See Fig. 1.
[b]For GC conditions see text.
[c]Position of second double bond unknown.
[d]RI on CPWax 52 = 2092.

spectral comparison with synthetic *12* (Robson and Rowland, 1986) and *13* produced by oxidation of *1* (Yon, 1981). (*12*; $M^+ \cdot 282$, 267 (5%, $M-CH_3$), 198 (10%, McLafferty), 169 (55%, $C_{10}H_{21}CO^+$), 126 (80%), 57 (100%). *13*; $M^+ \cdot 266$, 156 (20%, McLafferty), 142 (45%, McLafferty), 113 (90%), 95 (100%)). Only one triplet due to vinylic protons was present in the ^{1}H NMR spectrum (δ ppm 5.1) and we assume that *10* and *11* are either both E or both Z. By inference the remaining two alkenes of the original synthetic mixture (Robson, 1987) must also be both E (or both Z) isomers of *10* and *11*. The C_{20} alkenes were isolated and ozonized in the same manner, leading to the assignments in Table 1. The similarity in GC elution orders between the C_{20} and C_{25} alkenes (Robson, 1987) also supported the assignments.

To provide further C_{20} and C_{25} isomers, the original synthetic mixtures, each of six isomers, (namely *4* and *5*) were treated with tosic acid in a pro-
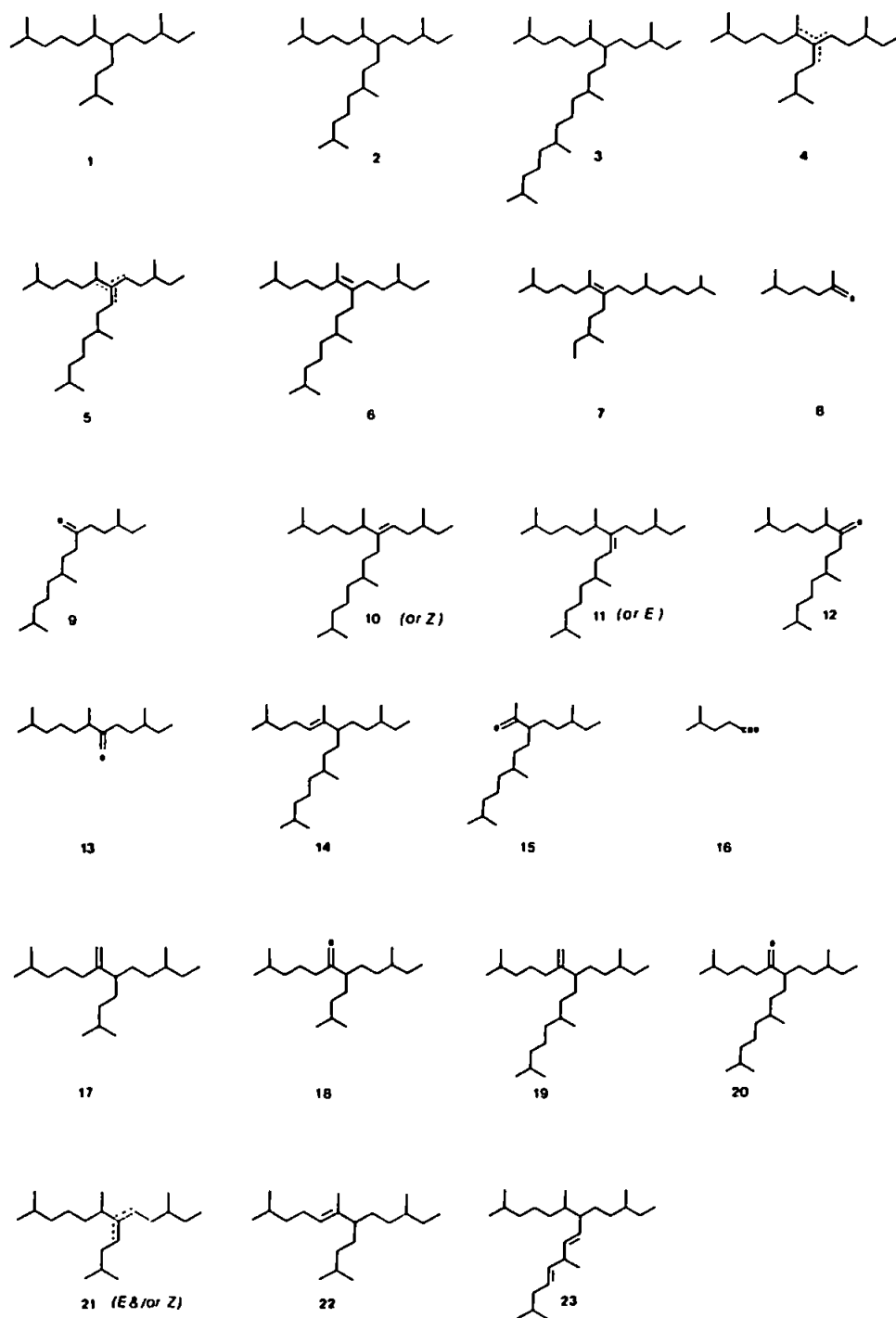
Fig. 1. Structures of compounds referred to in the text.

cedure that is known to induce double bond movement through tertiary carbocation formation. This has been successfully used by Peakman and Maxwell (1988) for the isomerization of sterenes. In the present study the result in each case (C_{20} and C_{25}) was the production of mixtures each containing one 'new' GC peak. Time course experiments with an alkane internal standard, showed that all isomers 5 (and 4) were reduced in concentration as a result of the formation of 14 (and 22). Preparative Ag^+ chromatography afforded 14 (and 22) in sufficient purity for ozonolysis and 14 in sufficient quantity for ^1H NMR.

For 14 two triplets assigned to vinylic protons were observed in the NMR spectrum (δ ppm 5.1, 5.2) and two singlets assigned to the allylic methyl (δ ppm 1.4, 1.5). These suggested that both E and Z isomers were present in a ratio E/Z, Z/E = 1.6; no GC separation was achieved on any phase. Ozonolysis was consistent with these assignments since only a C_{19} ketone (assigned 15; $M^+ \cdot 282$, 267 (2%, M–CH$_3$), 264 (2%, M–H$_2$O), 253 (3% M–C$_2$H$_5$), 198 (25%, McLafferty), 180 (40% McL–H$_2$O), 142 (95%, McLafferty), 124 (100%, McL–H$_2$O)) and C_6 aldehyde (assigned 16 by comparison with the synthetic compound (Yon, 1981)) were produced.

To summarize, the isolation and characterization of synthetic alkenes resulted in the assignment, or partial assignment, of structures to two C_{20} and five C_{25} monoenes. This is a valuable database of chromatographic (e.g. GC retention indices) and spectroscopic (NMR, MS) information for the assignment of sedimentary alkenes. However, as will be seen in the following section, care should be taken when using retention indices alone as a basis for structural assignments in these alkenes and this emphasizes the importance of the micro-ozonolysis data.

Sedimentary alkenes

The highly branched C_{20} and C_{25} alkenes are widely distributed in young sediments in coastal regions all over the globe including many estuaries; a few occurrences have also been noted in non-marine environments (reviewed by Rowland and Robson, 1990). However, it is interesting to note that whilst the C_{25} alkenes occur as monoenes through pentaenes, only two C_{20} monoenes have been reported and no higher polyenes. So far the position of the double bonds in C_{20} and C_{25} alkenes has only been established in alkenes from hypersaline and mesohaline sediments (from Shark Bay, Western Australia and the Guadalquivir delta, southwest Spain). We therefore decided to examine the hydrocarbons in sediments from other environments.

Gluss Voe, Shetland Islands (UK)
The hydrocarbon chemistry of the sediments of the Sullom Voe region of the Shetland Islands (including Gluss Voe) is monitored at least annually

because Sullom Voe is the site of a large oil terminal. In sediments collected in 1985, Robson (1987) identified two C_{20} monoenes with parent skeleton *1* ($C_{20:1 OV-1}$ 1696 and 1702).

The former had a very similar RI and mass spectrum to synthetic *22* isolated and identified herein and we, therefore, tentatively assign this structure to the sedimentary alkene. A $C_{20:1}$ alkene with a similar RI was detected in sediments of Puget Sound, USA by Barrick et al. (1980). A more rigorous assignment must await isolation and characterization of the sedimentary alkene by ozonolysis and/or NMR (see below).

Tamar estuary, UK

The Tamar estuary (UK) has been widely studied (see organic geochemistry review by Readman, 1982). Robson (1987) reported the presence of C_{20} and C_{25} monoenes and a C_{20} alkane in these sediments and was able to show by hydrogenation and GC coinjection that the parent structures were identical to synthetic *1* and *2*. However, the double bond position in the $C_{20:1}$ was not assigned and that in the two $C_{25:1}$ isomers was limited to one of three positions (i.e. *5*).

Isolation and elucidation of synthetic alkenes *6, 7, 10* and *11* in the present study (see Experimental; Table 1) and comparison of the retention indices with those reported by Robson (1987) (namely $C_{25:1}$ 2076 and $C_{25:1}$ 2091) allows us to reject *6* and *7* as possibilities, reducing the possibilities to E and/or Z isomers of *10* and/or *11*. However, a more rigorous assignment must still await isolation and ozonolysis of the sedimentary alkenes, as proved possible for the corresponding C_{20} monoene.

During a monthly monitoring of the abundances of the C_{20} and C_{25} alkenes in the Tamar estuary sediments, S.J. Hird and S.J. Rowland, (unpublished results, 1990) noted that sediments collected in June 1989 contained only the C_{20} compounds (*1* and a monoene). Extraction and isolation of the hydrocarbons followed by Ag^+ TLC afforded the monoene in sufficient purity for ozonolysis. This showed that the alkene had structure *17*. The only ozonolysis product was the C_{19} ketone *18* identified by comparison of the mass spectrum with that of *18* reported by Dunlop and Jefferies, (1985).

Although one of the synthetic C_{20} monoenes, (namely *22*; RI 1697$_{DB1}$) had a similar GC RI to *17* (RI 1702$_{DB1}$; Table 1), the expected ozonolysis products from this alkene were not observed in the sedimentary C_{20} alkene. This emphasizes the care needed in making assignments by GC RI alone. Thus the C_{20} monoene in these temperate zone intertidal sediments is the same as that found in hypersaline sediments in Western Australia (Dunlop and Jefferies, 1985) whereas the C_{25} monoenes (at least at certain times of the year) are different from those found in hypersaline Shark Bay. Details of our monthly analysis of the concentrations and distributions of these alkenes in Tamar estuary sediments will be published separately.

McMurdo Sound, Antarctica

The organic geochemistry of McMurdo Sound and region in Antarctica has been studied by Venkatesan and coworkers (e.g. Venkatesan and Kaplan, 1982, 1987; Venkatesan, 1988). These workers and others (Martine and Simoneit, 1988) reported the presence of a highly branched C_{25} diene in McMurdo Sound sediments $C_{25:2DBs}$ 2082 which Rowland et al. (1990) were able to show (by hydrogenation experiments) had parent skeleton 2. However, partial hydrogenation of the diene (Venkatesan, 1988) produced a mixture comprising 2 and an unknown monoene ($C_{25:1DBs}$ 2101) which Rowland et al. (1990) tentatively identified as 19 by comparison of the RI ($C_{25:1}$ $_{OV-1}$ 2110) and mass spectrum with that of 19 ($C_{25:1}$ $_{OV-1}$ 2112) identified by ozonolysis by Dunlop and Jefferies (1985). In the present study, ozonolysis of the $C_{25:1}$ partial hydrogenation product also produced the C_{24} ketone (20), confirming the structure of the alkene as 19 and establishing the position of one of the double bonds in the sedimentary C_{25} diene (and probably also in the C_{25} diene observed in sea-ice diatoms from McMurdo Sound (Nichols et al., 1988)). This contrasts with the double bond positions found recently in the C_{25} diene ($C_{25CP\,Si18\,CB}$ 2085) isolated from a highly eutrophic mesohaline lagoon, the Guadalquivir delta in southwest Spain (i.e. 23) which were established by epoxidation with m-chlorobenzoic acid (Yruela et al., 1990).

CONCLUSIONS

Isolation and characterization of synthetic and sedimentary C_{20} and C_{25} monoenes has shown that:

(1) The C_{20} monoene in the Tamar estuary sediments (in June 1989) contained a C6(14) methylene double bond. This is the same as that observed previously in the C_{20} monoene from hypersaline sediments of Shark Bay, Western Australia, but different from that in sediments of Gluss Voe, Shetland Isles, where the double bond has been tentatively assigned to the 5(6) position on the basis of RI. These differences may reflect differences in the source organisms, or in the environments, or both.

(2) The C_{25} monoenes (two isomers) in the Tamar estuary sediments (in 1985) contained double bonds assigned by RI to C7(8) and/or C7(1') positions. This is different from that observed in the Shark Bay C_{25} monoene.

(3) The C_{25} monoene produced by partial hydrogenation of the diene in McMurdo Sound, Antarctica sediments contains a C6(17) methylene double bond. This is the same as that observed in the Shark Bay monoenes. By inference this also establishes the position of one of the double bonds in the C_{25} diene in Antarctic diatoms. The C_{25} diene found in the Guadalquivir delta sediments (Spain) did not contain a methylene double bond. These differences in double bond positions are intriguing and possibly have important

implications for the fate of these alkenes in sediments (e.g. by sulphur incorporation) and for the unusual distributions of the parent alkanes and related thiolanes and thiophenes in crude oils.

ACKNOWLEDGEMENTS

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NOTE

Hydrogenation behaviour of two highly branched C_{25} dienes from Antarctic marine sediments

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C_{25} hydrocarbons with skeleton (1) are widely distributed in young sediments (reviewed by Rowland and Robson, 1989). Alkane (1) and alkenes with one to four double bonds have been identified in sediments, and dienes have been found in field samples of the green alga, *Enteromorpha prolifera* (RI 2082_{OVI}; Rowland *et al.*, 1985) and mixed sea-ice diatoms from McMurdo Sound, Antarctica (RI 2088_{MS}; Nichols *et al.*, 1988). The gas chromatography (GC) and mass spectrometry (MS) data obtained after hydrogenation of these alkenes compare well with these of synthetic (1) (Robson and Rowland, 1986).

However, in sediments from McMurdo Sound and Bransfield Strait, Antarctica, Venkatesan (1988), Venkatesan and Kaplan (1987) and Martine and Simoneit (1988) identified C_{25} dienes (RI 2082_{DB5}; RI 2088_{DB5}) which when hydrogenated by passing hydrogen at a rate of 38–40 cm^3 for 45 min to a stirred suspension of PtO_2 in hexane (Venkatesan, 1988), produced a compound (RI 2101_{DB5}), the mass spectrum of which contained an apparent molecular ion m/z 350 ($C_{25}H_{50}$). This led the authors to conclude that the hydrogenation product was probably a monocyclic compound although it was acknowledged that the alkene may contain one double bond which could not be hydrogenated under the above conditions (Venkatesan, 1988). This suggestion follows similar statements by Requejo and Quinn (1983) who also observed some of the products of hydrogenation of a $C_{25}H_{48}$ compound (so-called c25:1:1, RI 2079_{SE30}) to contain one degree of unsaturation. These reports introduce an element of confusion into the firm assignments made by Robson and Rowland (1986) where the alkenes were attributed to acyclic skeleton (1) by comparison with synthetic (1) and a synthetic alkene mixture (2). In order to clarify this confusion the McMurdo Sound and Bransfield Strait aliphatic hydrocarbons isolated by Venkatesan (1988), and Venkatesan and Kaplan (1987) were examined by the hydrogenation and GC–MS proce-

dures of Robson and Rowland (1986, 1988). Hydrogenation was effected by bubbling hydrogen for 60 min through a solution of the alkenes in hexane in the presence of $PtO_2 \cdot H_2O$. The catalyst was first activated (black colouration) by bubbling hydrogen through the hexane/$PtO_2 \cdot H_2O$ for *c.* 3 min. GC–MS was carried out on a Kratos MS25 double focusing instrument fitted with a Carlo Erba Mega Series 5300 gas chromatograph and a variety of fused silica GC columns (typically 30 m × 0.2 mm) as indicated in the text. Probe MS–MS analyses were performed using a Finnigan TSQ 70 MS instrument under EI conditions.

Venkatesan's hydrogenation products of the aliphatic hydrocarbons from McMurdo Sound and Bransfield Strait sediments (Venkatesan, 1988; Venkatesan and Kaplan, 1987) were re-examined by GC–MS on DB5 and DB1 stationary phases. This did indeed reveal a major peak (RI 2101_{DB5}; 2110_{DB1}) which coeluted with synthetic (1) as expected from previous reports. The mass spectrum of the McMurdo Sound component (40 eV, 250°C source temp.) shown in Fig. 1, contained an ion m/z 350 and ^{13}C isotope ion at m/z 351. Under the same conditions synthetic C_{25} monoenes (e.g. 2) produced similar ions at m/z 350 and m/z 351. However, it was noticed that much of the remainder of the spectrum (Fig. 1) of the McMurdo product resembled that of synthetic (1) (viz. m/z C_nH_{2n+1}, 85, 99 . . .; Robson and Rowland, 1986). Indeed, computer subtraction of the spectrum of synthetic (1) from that obtained for the McMurdo sample produced a spectrum similar to that of the acyclic monoene (3) identified by ozonolysis in Shark Bay, W. Australia, and which had a similar retention index (RI 2110_{DB1} McMurdo; RI 2112_{MS} (3) Dunlop and Jefferies (1985). It was suspected, therefore, that the monocyclic (*sic*) partial hydrogenation product was in fact a mixture of (1) and a C_{25} monoene (3?). This was confirmed by further chromatography on CPWAX52 phase which
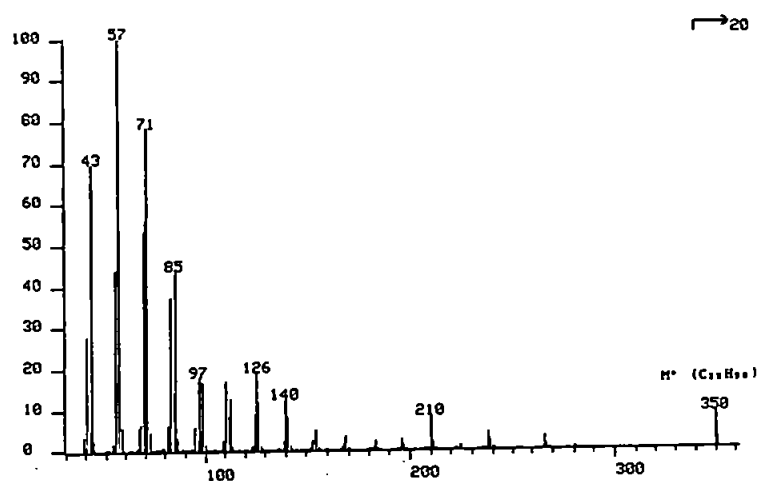
Fig. 1. EI mass spectrum of partial hydrogenation product of McMurdo Sound aliphatic hydrocarbons (RI 2101$_{DBs}$) 40 eV, 250°C source temperature.
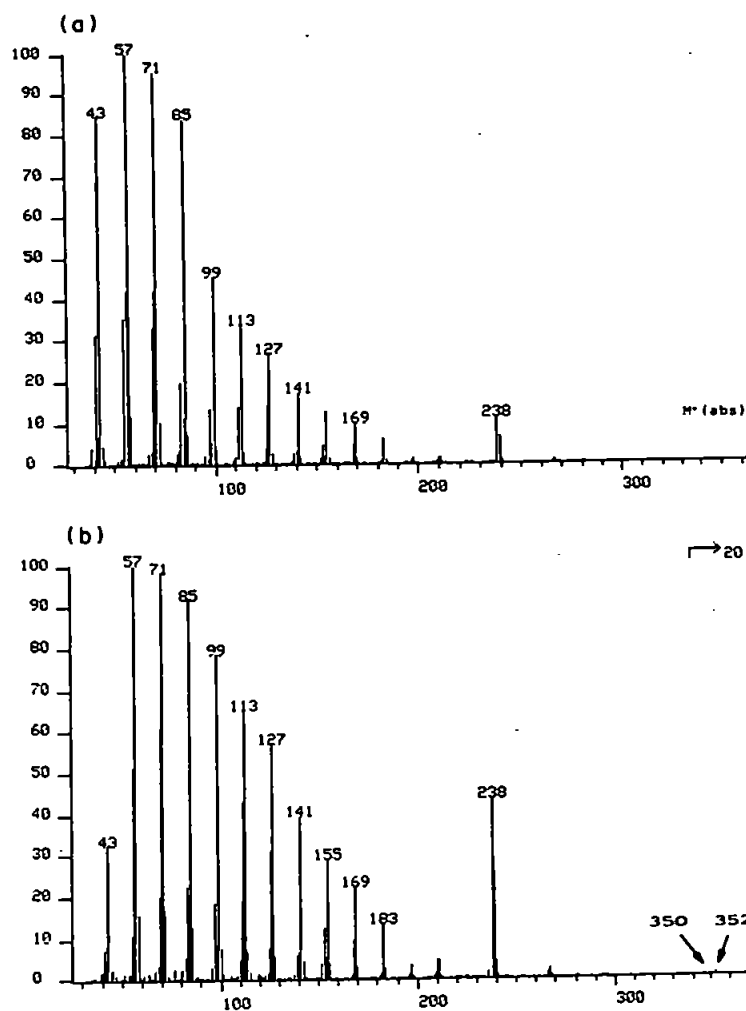

Fig. 2. EI mass spectra of partial hydrogenation product of McMurdo Sound aliphatic hydrocarbons (2101$_{DBs}$) after further hydrogenation at (a) 40 eV, 250°C source temperature and (b) 20 eV, 200°C source temperature.
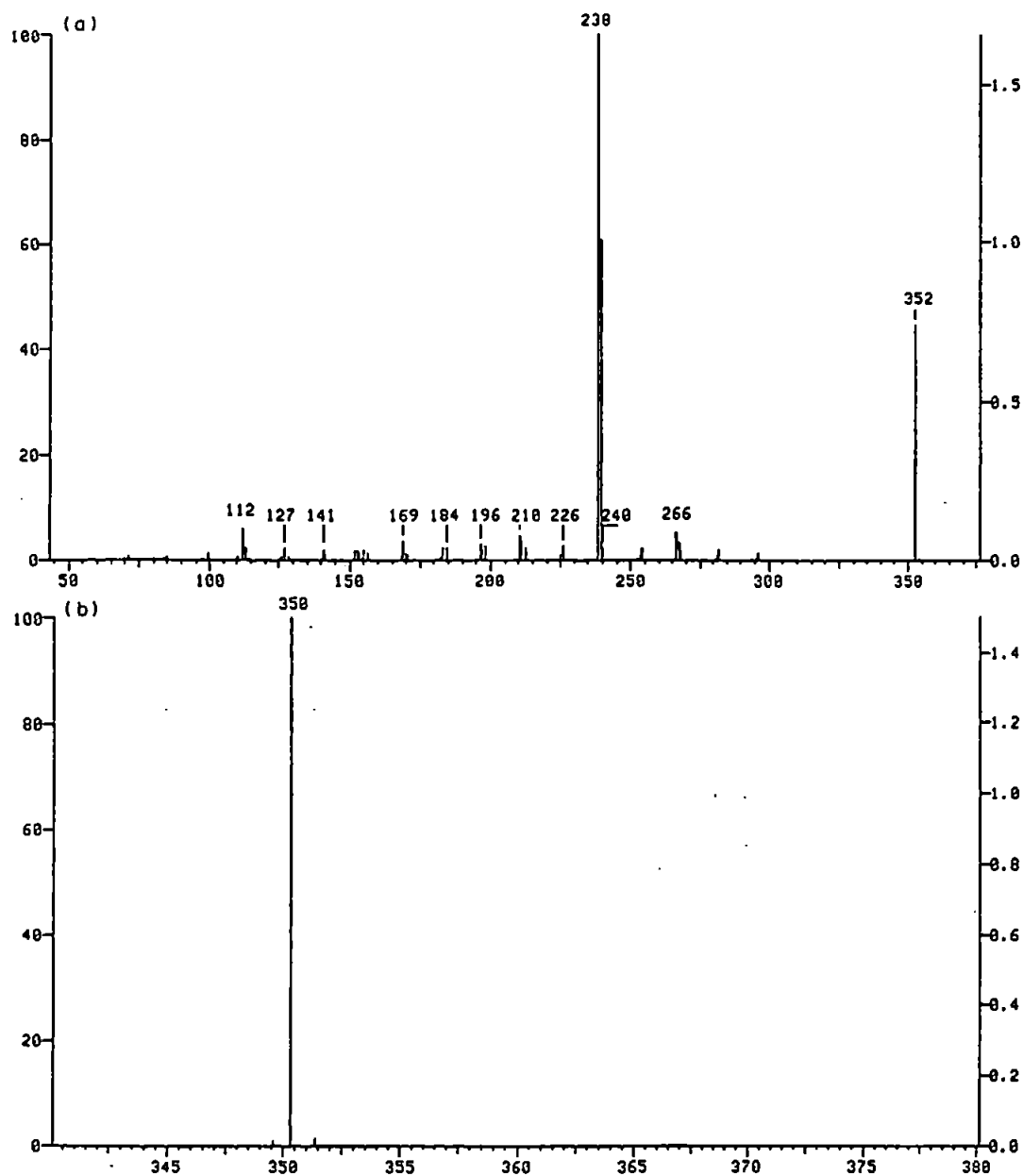
Fig. 3. EI mass spectra of synthetic (1) (40 eV, 120°C source temperature). (a) Daughter ions of m/z 352.
(b) Parent ions of m/z 350.

enabled separation of the suspected mixture into two components of approximately equal concentration (RI 2065; RI 2092). The former coeluted with synthetic (1).

Hydrogenation of this mixture and GC on CPWAX52 showed that the monoene (RI 2092) had been converted to the alkane (1) (RI 2065). As expected, no shift was observed on apolar GC phases but the mass spectrum [Fig. 2(a)] was identical to that of (1) under the same conditions; notably no M^{+} ions at m/z 350 or m/z 352 were present in either spectrum. The same results were obtained for the Bransfield Strait sample.

These data show that the C_{25} monoene, partial hydrogenation product from McMurdo Sound and Bransfield Strait sediments, and hence the C_{25} dienes (RI 2082$_{DB5}$ and RI 2088$_{DB5}$) are not monocyclic but acyclic (viz. skeleton 1) and have the same carbon skeleton as the diene in McMurdo Sound diatoms (cf. Nichols et al., 1988) and many sediments (Rowland and Robson, 1989). The hydrogenation conditions used by Venkatesan and Kaplan (1987) apparently resulted in incomplete saturation of some of the dienes.

To obtain further evidence that our hydrogenation product was a C_{25} alkane, we examined the mass
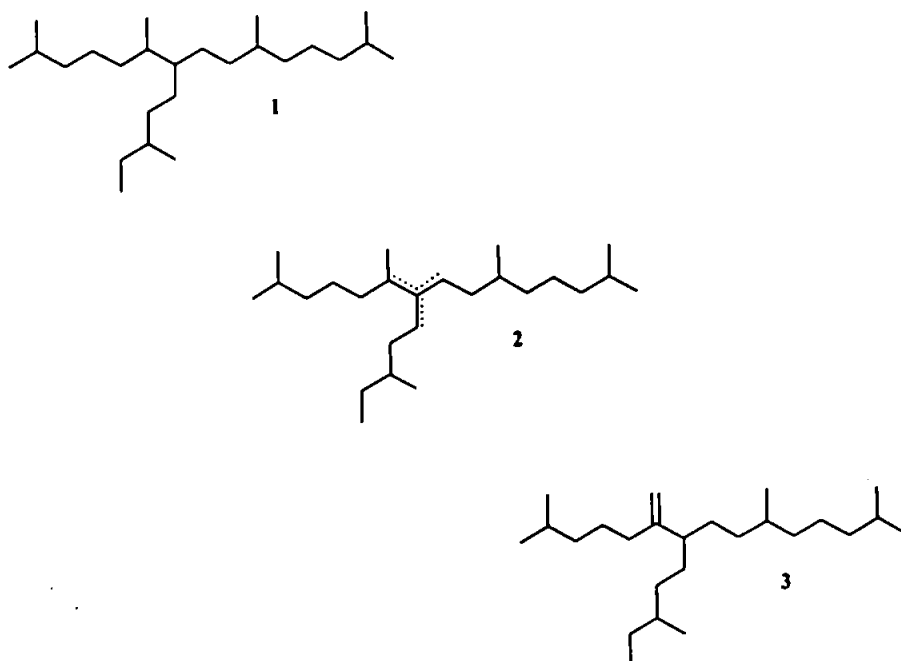
Scheme 1

spectrum of both it and synthetic (1) under different operating conditions (20 eV; 200°C source temp.) whereupon the molecular ion m/z 352 was observed for both [Fig. 2(b)]. However, quite unexpectedly, an ion at m/z 350 was now also observed for both. It was suspected that m/z 350 was either an $M^{+\cdot} - 2$ ion from m/z 352 or $M^{+\cdot}$ of a very small amount of unhydrogenated monoene.

Analysis by MS–MS (40 eV; 120°C source temp.) of synthetic (1) failed to show that m/z 350 was a daughter ion of m/z 352, or that m/z 352 was a parent of m/z 350 [Figs 3(a) and 3(b)].

We are forced to conclude therefore, either that even our hydrogenation method does not fully reduce the alkenes (2) or that m/z 350 is produced by dehydrogenation of (1) within the ion source by a process that is not reproduced by collision-induced dissociation of m/z 352 in the collision cell of the TSQ mass spectrometer. This finding does not detract from the conclusion that the sedimentary alkenes are acyclic, but it does emphasise the care necessary in such studies.

Indeed, the data of Requejo and Quinn (1983) for "c25:1:1" (sic) and its hydrogenation product ("c25:0:1") and of Barrick and Hedges (1981) for HC412 (now suspected to be a C_{30} monoene), probably require re-examination in the light of these results. The mass spectrum of "c25:0:1" (RI 2104_{SE30}) is similar to that of the partially hydrogenated C_{25} diene from McMurdo Sound and may represent a similar mixture of (1) and (3?).

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