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SYNTHETIC AND BIODEGRADATION STUDIES

OF SOME SEDIMENTARY ISOPRENOID HYDROCARBONS

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Nature is often hidden; sometimes overcome;

seldom extinguished.

Francis Bacon - 'Of Nature in Men'

1561 - 1626

TO MY PARENTS, SISTER

and

102 YEAR OLD GRANDMOTHER

SYNTHETIC AND BIODEGRADATION STUDIES OF SOME NOVEL SEDIMENTARY ISOPRENOID HYDROCARBONS

J.N. ROBSON

ABSTRACT

Over the past twenty years there have been many published reports of a group of abundant C_{20}, C_{25} and C_{30} highly branched acyclic alkanes and alkenes in surface sediments. Synthesis and spectral and chromatographic characterisation of 2,6,10-trimethyl-7-(3-methylbutyl) dodecane (C_{20}), 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (C_{25}) and 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane (C_{30}) in the present study showed that these were the parent skeleta of the unknown hydrocarbons. Only the C_{20} hydrocarbon has been synthesised previously. Intermediates in the synthetic routes were also fully characterised.

The synthetic alkanes and alkenes were used to assign structures to hydrocarbons in several sediments from Europe and Africa and a comprehensive overview of the sedimentary occurrences of these new 'biomarker' hydrocarbons is made.

Biodegradation of the synthetic alkanes and alkenes using pure cultures of the bacterium Pseudomonas aeruginosa indicated that the high abundance of the hydrocarbons relative to normal alkanes in certain environments is related to their resistance to biodegradation.

A group of bicyclic C_{25} and C_{30} hydrocarbons have often been reported to co-occur with the acyclic hydrocarbons. Mass spectra of these compounds suggest that they possess 'extended-drimane' carbon skeletons. Synthesis of two bicyclic hydrocarbons, $8\alpha\beta$(H) $9\alpha\beta$(H) 11-tetrahydrogeranyldrimane (C_{25}) and $8\alpha\beta$(H) $9\alpha\beta$(H) 11-hexahydrofarnesyldrimane (C_{30}), and comparison of these with the sedimentary hydrocarbons indicated that the compounds were similar but not identical. However, from the data presented more likely structures are proposed.

The synthesis of certain other geologically-occurring 'irregular' acyclic isoprenoid alkanes is described.

ACKNOWLEDGEMENTS

ABBREVIATIONS

The major abbreviations used (all introduced in the text) are as follows:

cc	column chromatography
DCM	dichloromethane
Et_2O	diethylether
Gc	Gas chromatography
Gcms	Gas chromatography-mass spectrometry
Hplc	High performance liquid chromatography
HRMS	High resolution mass spectrometry
I	Intensity
IR	Infra-red spectroscopy
LRMS	Low resolution mass spectrometry
NMR	Nuclear magnetic resonance spectroscopy
THF	Tetrahydrofuran
tlc	thin layer chromatography

Note added in ADDENDUM

For Entereomorpha read Enteromorpha

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CHAPTER ONE

INTRODUCTION TO SEDIMENTARY

ACYCLIC AND BICYCLIC ISOPRENOID HYDROCARBONS

1.1 THE CONCEPT AND USES OF BIOLOGICAL MARKERS

Within Organic Geochemistry, the term biological marker (biomarker) or geochemical marker is defined as "any compound present in the geosphere whose carbon skeleton can be traced to an obvious biogenic precursor" (Eglinton and Calvin, 1967). Examples of important biomarkers are given in Fig. 1:1. In Ancient sediments and petroleums, biological markers such as the hopanes and steranes have been used as maturity indicators (Seifert and Moldowan, 1978; MacKenzie et al., 1980, 1981; review by Tissot, 1984), in oil/oil and oil/source rock correlations (Philp, 1983; Seifert and Moldowan, 1978, 1981) and in reconstructing the thermal histories of sedimentary basins (MacKenzie and McKenzie, 1983). Within Recent (including present day) sediments, distributions of biological markers have been used to indicate the presence of oil pollution (reviewed by Jones, 1986) and numerous studies have related sedimentary biological marker distributions to biogenic source input (see Brassell and Eglinton, 1983; Brassell et al., 1983a; Volkman, 1985). It has also been suggested that biological markers can be used as indicators of paleoenvironmental parameters such as water column temperature (Brassell et al., 1986b,d) and paleoclimate (Kawamura and Ishiwatari, 1981).

The above examples clearly demonstrate the importance of the study of biological marker distributions in both Recent and Ancient sediments. Amongst these biological markers, isoprenoid hydrocarbons have emerged as being particularly useful. These compounds are formed biosynthetically from 3(R) - mevalonic acid (Fig. 1:2) and are formed of linked units of 2-methylbutane (1), the so-called isoprene unit (reviewed by Nes and McKean, 1978). Both cyclic and acyclic hydrocarbons are known (e.g. cholestane, 2; pristane, 3). The

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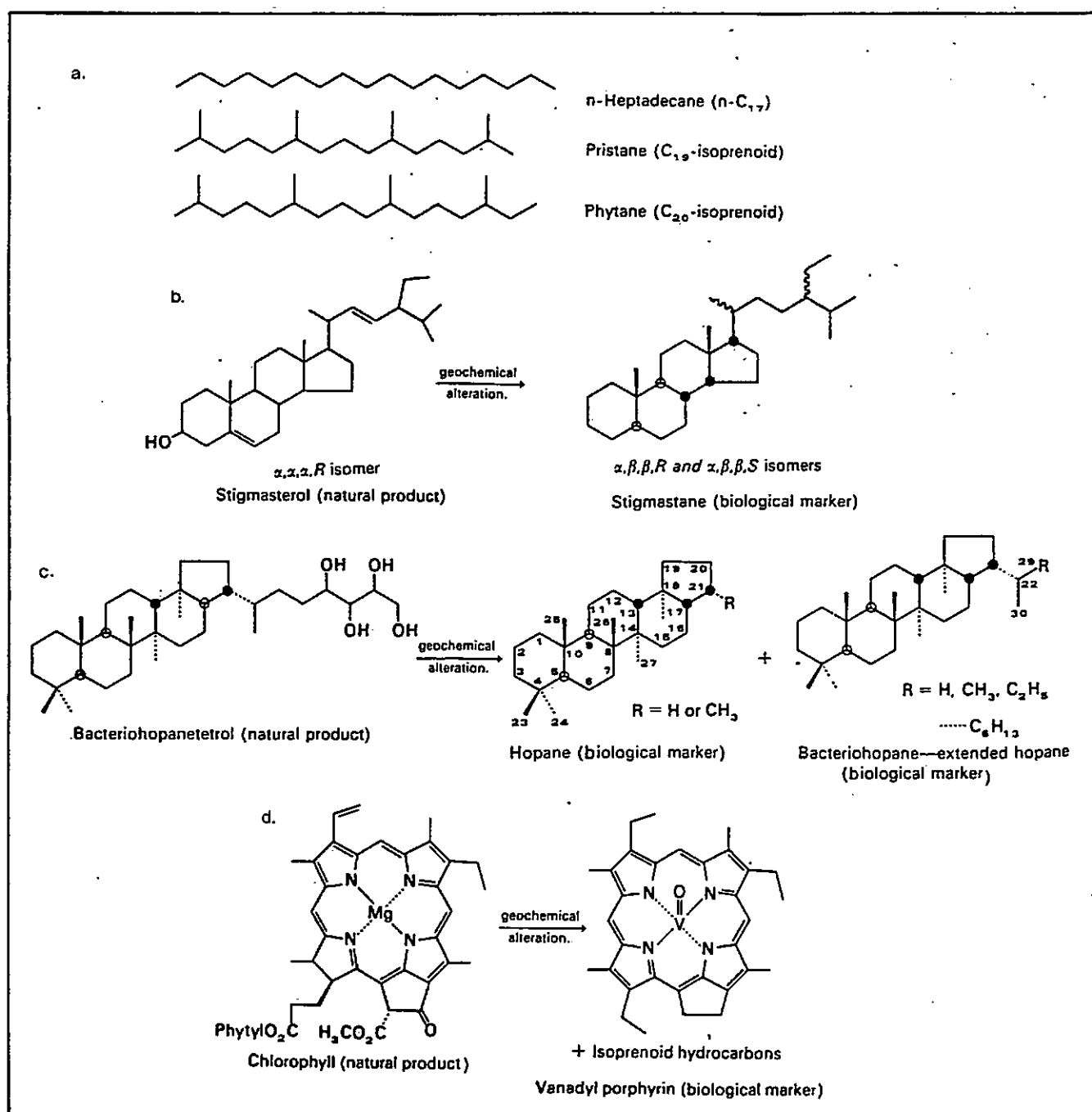
Fig. 1:1   The structure of some biological marker compounds and their
           postulated biolipid precursors (after Brooks, 1983). Open and
           solid circles represent α(H) and β(H) configurations of alicyclic
           chiral centres respectively. Dashed and reinforced lines denote
           α and β substituents respectively, while sinuous lines represent
           substituents of uncertain or mixed (α and β) stereochemistry.

OH CH₃

HOOC — CH₂OH   3(R) – mevalonic acid

C_5 **B**   hemiterpenoids
CH₂OPP

A   CH₂OPP   PP = pyrophosphate [–P=O(OH)OP=O(OH)₂]

C_{10} CH₂OPP   monoterpenoids (e.g. geraniol)

CH₂OP·P

C_{15} CH₂OPP $\dfrac{\text{(t–t) linkage}}{\text{of 2 × } C_{15} \text{ units}}$ → squalene

sesquiterpenoids (e.g. farnesol)

CH₂OPP

cyclisation

steroids
triterpenoids

C_{20} CH₂OPP $\dfrac{\text{(t–t) linkage}}{\text{of 2 × } C_{20} \text{ units}}$ → carotenoids

diterpenoids

CH₂OPP

C_{25} CH₂OPP   sesterterpenoids

CH₂OPP

polyisoprenoids

Fig.1:2   A simplified scheme for the biosynthesis of isoprenoid
hydrocarbons. 3(R)-Mevalonic acid is converted to
isopentenyl pyrophosphate and dimethylallyl pyrophosphate.
Addition of isopentenyl pyrophosphate (A) to dimethylallyl
pyrophosphate (B) produces monoterpenoids; further
consecutive additions of isopentenyl pyrophosphate produces
C_{15}, C_{20}, C_{25} and longer chain isoprenoids.
(Adapted from Nes and McKean, 1977).

geochemistry of sedimentary isoprenoids, which include many functionalised compounds, has been reviewed by many authors (e.g. Lough, 1973; Cranwell, 1982; Brassell et al., 1983; Volkman and Maxwell, 1986).

The present introduction therefore focuses primarily on the occurrence, distributions and sources of acyclic and bicyclic isoprenoid hydrocarbons, i.e. the particular groups of biomarkers that are pertinent to the present study.

1.2 ACYCLIC ISOPRENOID HYDROCARBONS

The structures of acyclic isoprenoids are characterised by a linked chain of methyl branched C_5 'isoprene' units (1). In organic geochemistry, the term 'regular' isoprenoid defines a carbon skeleton, irrespective of total number of carbons, in which the isoprene units are linked head to tail. Examples of head to tail (h - t) isoprenoids include 2,6,10,14-tetramethylpentadecane (pristane, 3) and 2,6,10,14-tetramethylhexadecane (phytane, 4). Other acyclic isoprenoid skeleta are termed 'irregular' and include head to head (h - h; e.g. 2,6,9,13-tetramethyltetradecane; 5) and tail to tail (t - t; e.g. 2,6,11,15-tetramethylhexadecane; 6) linked 'isoprene' units.

1.2.1 'Regular' acyclic isoprenoid hydrocarbons in the geosphere

The widespread occurrence of certain 'regular' acyclic isoprenoid hydrocarbons in the geosphere is shown by selected references summarised in Table 1:1. Of these compounds, the most frequently reported are pristane (3) and phytane (4) although, as can be seen from Table 1:1, C_9 - C_{45} hydrocarbons do occur. Since phytane was first identified in an Iranian crude oil distillate (Dean and Whitehead,

SEDIMENT/PETROLEUM	GEOLOGICAL AGE	CARBON RANGE NUMBER	REFERENCE
Various Marine sediments	Recent	19,20	Didyk et al., 1978
Puget Sound U.S.A.	Recent/marine	15-20	Barrick et al. 1980
Particulate traps, Baltic Sea	Recent/marine	15-20	Osterroht et al. 1983
Antrim Shale U.S.A.	Permian	16-21	Johns et al. 1966
Marine Shales	Cretaceous	9-14	Gohring et al. 1967
Shale, Africa	Cretaceous	15-25	Spyckerelle et al. 1972
Costa Rica oil seep		16,18-26,28,30	Haug and Curry, 1974
Rheingraben Dolomite	Tertiary	19,20,25	Waples et al. 1975
Spanish Crude oils	Various	9-45	Albaiges, 1980
California Crude oil	Miocene	18-36	Moldowan and Seifert,1980
Ottway Basin coastal bitumens Australia	Cretaceous	21-31	McKirdy et al., 1986

Table 1:1. Examples of 'Regular' (h-t) acyclic isopenoid hydrocarbons in the geosphere.

1961) and pristane in an East Texan petroleum (Bendoraitis et al., 1962) there have followed numerous reports of their occurrence in both Recent and Ancient sediments and in petroleum.

The biogenic source of pristane, phytane and other hydrocarbons of $<C_{20}$ has long been considered to be phytol ($\underline{7}$), the esterifying side chain alcohol of chlorophyll-$\underline{a}$ ($\underline{8}$) and probably the most abundant acyclic isoprenoid in the geosphere (Volkman and Maxwell, 1986) although in the light of recent reports, there exists several other probable sources (e.g. Holzer et al., 1979; Goossens et al., 1984). The origin of pristane, phytane and other $<C_{20}$ saturated hydrocarbons via diagenetic alteration of phytol from chlorphyll-$\underline{a}$ has never been proven but probably involves a complex series of reactions. A simpilified postulated scheme is illustrated in Fig. 1:3 (after Didyk et al, 1978). This scheme was compiled from various reports of phytol degradation involving incubation of radiolabelled phytol (i.e. Brooks and Maxwell, 1974; De Leeuw et al., 1974, 1977), stereochemical analyses (Brooks et al., 1978; Patience et al., 1978) and abiological experiments (Ikan et al., 1975). The scheme assumes that chlorophyll-$\underline{a}$ rapidly hydrolyses in sediments or the water column to liberate phytol or dihydrophytol ($\underline{9}$) which then undergoes reductive or oxidative degradation. Reduction would tend to preserve the carbon skeleton and may ultimately produce phytane. Oxidation, on the other hand, would lead to a greater degradation of the C_{20} skeleton perhaps leading to pristane $\underline{via}$ defunctionalisation of phytanic ($\underline{10}$) and/or pristanic acids ($\underline{11}$). Similar pathways of phytol degradation were invoked by Brooks et al. (1969) to explain the high pristane/phytane ratio of most coals and certain oils. It was argued that phytol in coaly material deposited in subereal marshy environments would be expected to

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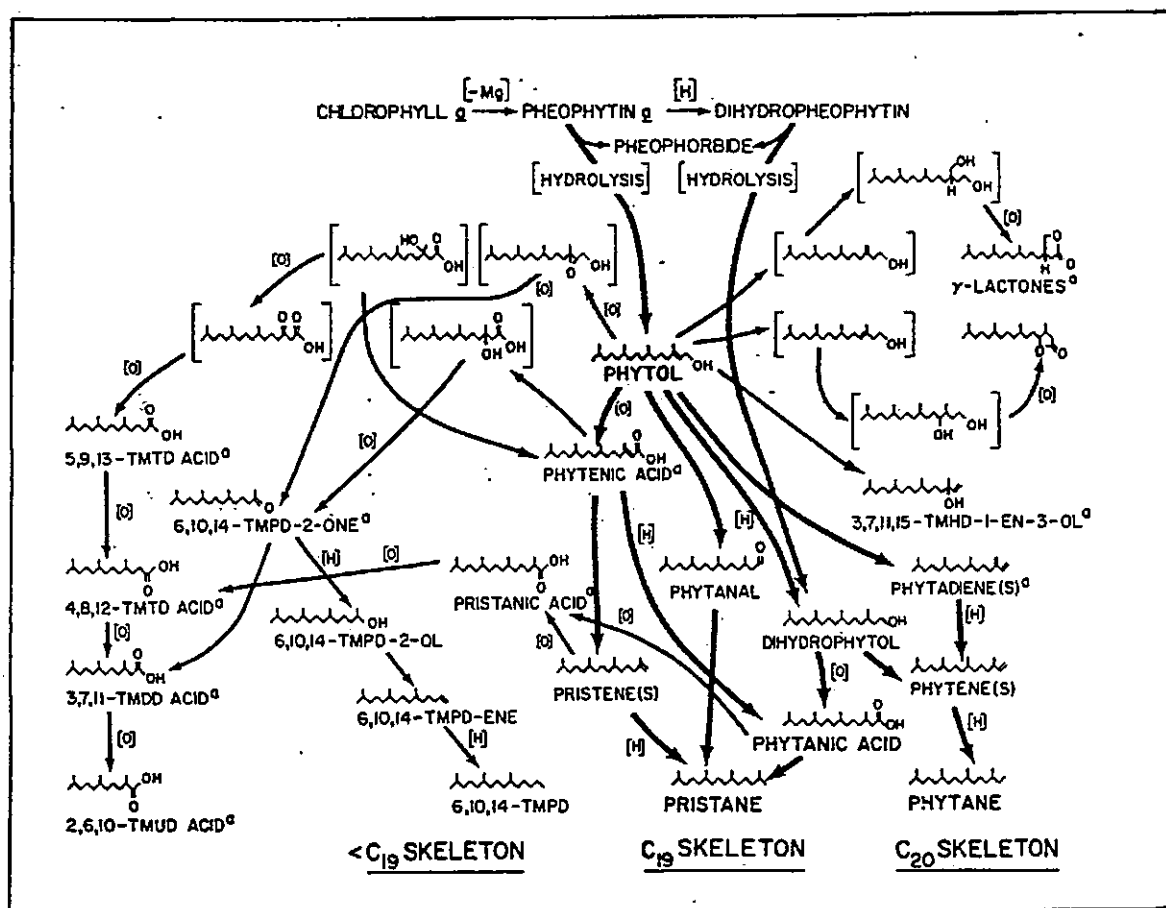
Fig.1:3   Postulated diagenetic pathways for the fate of the phytol side
chain of chlorophyll - a in aquatic environments. The isopren-
oids are named trivially, where possible, or by shorthand (for
example, 3,7,11,15-tetramethylhexadec-1-en-3-ol becomes
3,7,11,15-TMHD-1-en-3-ol). Initial reduction (shown by (H))
of phytol leads towards the right hand side of the diagram
preserving the C_{20} skeleton and hence favouring formation of
phytane, whereas initial oxidation (shown by (O)) tends to
the centre and left of the diagenetic scheme giving pristane,
with a C_{19} skeleton, and lower isoprenoids. The major pathways
to pristane and phytane formation are emphasised by the thicker
arrows. a = Compounds formed as radiolabelled products from
the incubation of $U-^{14}C$ phytol in sediments. The postulated
intermediates of these reactions are shown in square brackets.
(After Didyk et al. 1978).

undergo extensive oxidation leading to pristane. Later this led to the suggestion that ratios of pristane over phytane (pr/ph) in sediments may be correlated with the environmental conditions prevailing in the water column or sediment at time of deposition (i.e. Didyk et al., 1978). For example, a high pr/ph ratio (>2.0) was considered indicative of wholly oxic conditions and a low (<1.0) pr/ph ratio representative of anoxic conditions. A pr/ph ratio close to unity was thought to reflect environments with alternating periods of oxicity and anoxicity. The use of pr/ph ratios for determining the paleoredox conditions of sedimentary environments has been critically reviewed by Volkman and Maxwell (1986). It is reported that whilst such general-isation may be acceptable for assessing redox conditions in relatively Recent sediments, the use of the pr/ph ratio of crude oils as an indication of the paleoenvironment of deposition is not on firm ground because of various interfering factors. Pristane/phytane ratios are discussed further in section 4.4.1.

Lower molecular weight acyclic isoprenoid hydrocarbons are uncommon in unpolluted Recent sediments, but, in Ancient sediments are probably derived diagenetically by defunctionalisation of oxygenated phytol metabolites (see Fig. 1:3) and/or catagenesis of kerogen-bound phytol or metabolites. Degradation of chlorophyll-derived phytol by biota prior to sedimentary deposition provides an additional input of acyclic isoprenoids to Recent sediments. For example, in aquatic environments, certain calenoid zooplankton are known to produce pristane (Blumer et al., 1963), di/tri-unsaturated pristenes (12) (Blumer et al., 1969) and phytadienes (13) (Blumer and Thomas, 1965) from the chlorophyll—a in their diet. The occurrences of unsaturated pristanes and phytanes in

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Recent sediments is summarised by selected reference in Table 1:2.
Volkman and Maxwell (1986) propose that phytadienes are probably short
lived in sediments and rapidly react with each other, phytol or other
reactive species to form dimers or trimers,or become incorporated into
the accreting polymeric material.

Stereochemical studies have also provided evidence of a link between
'regular' acyclic isoprenoid hydrocarbons and phytol.   Naturally
occurring phytol exists as only one isomer (7(R) 11(R); 14) and
pristane isolated from zooplankton has been shown to have a
configuration identical at the relevant positions (meso-: 6(R) 10(S);
15; Patience et al., 1978), supporting earlier radiochemical evidence
that it was derived from phytol (perhaps via oxidation to phytanic acid
and subsequent decarboxylation; Brooks et al., 1969).   Pristane with
the meso- configuration was also identified as the sole isomer in
immature Messel oil shale (Eocene; Germany) suggesting it to originate
from chlorophyll-a - derived phytol during diagenesis (Patience et al.,
1978).   Isomerisation of the chiral centres of sedimentary meso-
pristane occurs with increasing thermal maturation.   For instance,
Green River shale (Eocene; Utah, U.S.A) which is considered to have
undergone a more severe thermal history than the Messel oil shale,
contains a mixture of 80% meso- pristane and 20% of [6(R) 10(R) 16 and
6(S) 10(S), 17] pristane. A mature crude oil, Djatibarang, was shown
to contain 50% meso- pristane and 25% of each of the 6(R) 10(R) and
6(S) 10(S) pristane isomers (Patience et al., 1978).   Convincing
evidence for progressive isomerisation at the chiral centres of
pristane with advancing thermal maturity was provided by MacKenzie et
al. (1980) in a detailed study of a sequence of the Toarcian shales
(Jurassic; Paris Basin, France) reported to have undergone progressive-

Table 1:2. Selected occurrences of phytenes and pristenes in Recent sediments.

Location	Type of Environment	Compounds present	Proposed source	Reference
Lake Greifensee Switzerland	Fresh water	'Phytenes'	Zooplankton	Giger and Schaffner,1977
Laguna Guerrero California, U.S.A.	Saline/algal mats	Phyt-1-ene, phyt-2-ene and 3 additional isomers		Philp et al., 1978
Puget Sound Washington State, U.S.A.	Land-locked marine	Phytadiene	Zooplankton or degradation of Phytol in sediments	Barrick et al., 1980
Japan Trench	Marine	Phytenes	Marine (non-bacterial)	Brassell et al., 1981
Upton Broad, U.K.	Freshwater	Neophytadiene 1,3(4)-phytadiene 2,4-phytadiene		Crawell,1982
Los Monegros, Spain	Hypersaline/ cyanobacterial mats	Phyt-1-ene Phyt-2-ene	Methanogenic bacteria	Albaiges et al., 1984a
Alfacs Bay, Spain	Marine	Pristene Isomers	Zooplankton?	Albaiges et al., 1984b

ly more heating with increasing depth of burial. The proportion of [6(R) 10(R) and 6(S) 10(S)] increased from 20% at shallow depth (700m) to 50% of the total pristane at 2000m.

Differencs in the configuration at the chiral centres of pristane between biogenic (i.e. meso-: 6(R) 10(S)) and petrogenic [i.e. 6(R) 10(R) and 6(S) 10(S)] sources has resulted in several investigators proposing their use as indicators of petroleum pollution. Quirk et al. (1980) demonstrated that pristane in a Recent lake sediment comprised a mixture of the meso- and [6(R) 10(R) + 6(S) 10(S)] isomers indicating inputs to the sediment from both pollutant and biogenic sources. One of the limits of this application is that it does not allow differention between an input of epimerised pristane from crude oil and an input of epimerised pristane from erosion of surrounding sedimentary rocks. Gasseman (1981) used isomer ratios in the C_{16} - C_{20} 'regular' isoprenoids to detect the presence of petrogenic hydrocarbons in seawater.

Whilst chlorphyll-derived phytol is potentially an important source of acyclic 'regular' isoprenoids ($<C_{20}$) in the geosphere there are several other possible biogenic precursors. For example, Goossens et al. (1984) suggested that the tocopherols may be additional major precursors of pristane in Ancient sediments and petroleums. Tocopherols are abundant in algae and cyanobacteria (Pennock, 1983). It was demonstrated that the major product upon flash pyrolysis of α-tocopherol (vitamin E; 18) was prist-1-ene (19) which has previously been shown to be a major product in pyrolsates of immature sediments and kerogens (Larter et al., 1979). Indeed, a detailed study of the Paris Basin indicated that with increasing maturity, the quantity of

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prist-1-ene liberated from the kerogen by pyrolysis decreased whilst the pristane content of the corresponding extractable bitumen increased (Van Graas et al., 1981). As the isoprenoid moiety in tocopherol is attached to the aromatic nucleus via a carbon-carbon bond, it is suggested that the tocopherols will exhibit greater sedimentary stability than the more labile phytol, but this remains an untested theory. Production of phytane is considered less likely via tocopherol diagensis since this would involve cleavage α-to the aromatic nucleus. Thus, the authors suggest pristane and phytane may wholly, or in part, originate from different precursors. In this respect it is interesting to note that from his studies of isoprenoids in crude oils, Illich (1983) was able to suggest that a proportion of the non-phytane isoprenoids in the oils had a different origin from that of phytane.

Whilst phytol (and tocopherol) may be considered as major precursors of 'regular' sedimentary acyclic isoprenoids $<C_{20}$, the isolation of 'regular' C_{21}, C_{23-25} isoprenoid alkanes in a Montana crude oil (Han and Calvin, 1969) suggested that isoprenoids with longer chains than phytol might also be a significant source of sedimentary isoprenoids. It was proposed that long chain unsaturated 'regular' acyclic isoprenoid alcohols (polyprenols; 20), containing up to one hundred carbon atoms could be sources of $>C_{20}$ hydrocarbons. The occurrence of polyprenols in nature has been reviewed by Morton (1968). They have been identified in many organisms including fungi (e.g. geranylnerolidol, C_{25}), higher plants (e.g. betulaprenols; C_{35} - C_{40} in Birchwood) and bacteria (e.g. C_{55} isoprenyl alcohol in Lactobacillus). Polyprenols were also postulated as the possible source of 'regular' C_{21}, C_{23} - C_{28} and C_{30} isoprenoid alkanes isolated from a Costa Rican seep oil (Haug and Curry, 1974). The

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absence of C_{17}, C_{22} and C_{27} 'regular' alkanes indicated that an origin from an 'irregular' (t-t) isoprenoid skeleton such as squalane (21) was unlikely.

Spyckerelle et al. (1972) identified C_{15} - C_{25} 'regular' isoprenoid alkanes in an African Cretaceous shale and proposed that the C_{21} - C_{25} alkanes were derived from diagenetic maturation and fragmentation either of polyprenols, or of naturally occurring acyclic sesterterpenoids. The occurrence of a 'regular' C_{25} isoprenoid hydrocarbon (2,6,10,14,18-pentamethyleicosane, 22) in a Tertiary sediment representing a lagoonal-type saline environment was reported by Waples et al. (1974). Whilst pristane (3) and phytane (4) were also present there was no report of 'regular' C_{16}, C_{18} or C_{21} - C_{24} alkanes. This unusual distribution suggested that thermal cracking of polyprenols/phytol was not a source and indicated that the C_{25} isoprenoid found in the saline sediments represented the actual carbon skeleton present in a living organism. It was further proposed that "once the precursor of the 'regular' C_{25} isoprenoid is identified, it's association with lagoonal-type, saline environments verified, the C_{25} isoprenoid may serve as a useful ecological marker". (This subsequently proved to be the case - see later).

Albaiges et al. (1978) identified, by synthesis of reference compounds, 'regular' C_{26} - C_{35} and C_{39} isoprenoid alkanes in a Spanish Miocene crude oil; C_{36}, C_{38} and C_{40} (23) members were also tentatively identified on the basis of mass spectral and gc retention data. 'Regular' C_{18} - C_{36} alkanes were reported in a Californian crude oil (Moldowan and Seifert, 1978) and Albaiges (1980) extended the carbon number range of reported 'regular' alkanes to C_{45}. Albaiges

et al. (1985) identified 'regular' C_{21} - C_{24} isoprenoids in a Western Mediterranean crude oil (Miocene) and proposed that they originated from catagenic degradation of a 'regular' C_{40} isoprenoid (Fig. 1:4, I) and an 'irregular' (t-t) C_{40} isoprenoid (Lycopane; Fig. 1:4, II) present in the oil.

The potential of polyprenols as precursors of 'regular' acyclic isoprenoid hydrocarbons is considered limited because of their restricted occurrence in time and space (Hahn, 1982) indicating that other sources must exist. Some evidence for a bacterial origin of 'regular' hydrocarbons was provided by the detection of pristane and phytane in bacteria (Han et al., 1968). Further evidence came with the identification of complex lipids containing the dihydrophytol 'regular' C_{20} moiety in halophilic bacteria (review by Kates, 1978). Halophilic bacteria are a distinct subgroup of the recently discovered Ur Kingdom Archaebacteria (Woese, 1981) which also encompasses methanogenic (methanogens) and thermoacidophilic bacteria (thermoacidophiles). The Archaebacteria are distinguished from other bacteria (i.e. Procaryotes) by their characteristic t-RNA composition, the lack of mumaric acid in their cell walls, the absence of fatty acid glyceryl ester lipids and the predominance of non-saponifiable lipids.

The lipid composition of the Archaebacteria consists of isoprenoid and hydroisoprenoid hydrocarbons and isopranylglycerol ether lipids (Langworthy et al., 1982). Isoprenoids occur in the form of alkanes, alkenes (the neutral non-polar lipids) and di- and tetra-ethers (the polar membrane lipids). The complex lipids of the Halobacteria mentioned above were shown to be diphytanylglyceroldiethers which consist of two C_{20}-phytanol chains ether linked to glycerol (24). De

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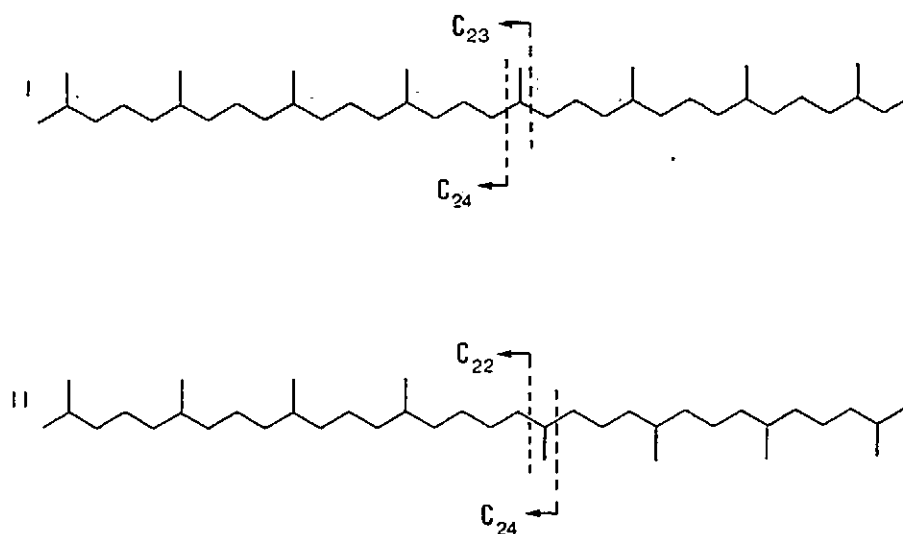
Fig.1:4   Catagenic degradation of C_{40} (h – t) and (t – t) isoprenoid alkanes
to produce C_{21} – C_{24} 'regular' (h – t) alkanes.
(After Albaiges et al.,1985).

Rosa et al. (1982) reported the identification of an asymmetrical diether lipid (25) comprising one phytanyl chain and a 'regular' C$_{25}$ 'sesterterpane' (2,6,10,14,18-pentamethyleicosane; 22) chain in alkaliphilic halophiles. Asymmetrical glycerol diether lipids containing phytanyl and 'regular' C$_{15}$ (farnesyl) or 'regular' C$_{25}$ chains have been observed in methanogenic bacteria (Mancuso et al., 1985).

The stereochemistry of the phytanyl groups in glycerol diether lipids of Halobacterium cutirubrum was shown to be 3(R) 7(R)11 (R) (26) (review by Kates, 1978). The same stereochemistry was observed for free dihydrophytol in sediments of the Dead sea, a highly saline environment where the halophile Halobacterium cutirubrum is abundant, thereby providing definitive evidence of a contribution of Archaebacterial lipids to sediments (Anderson et al., 1977). The later identification of free dihydrophytol in Halobacterium cutirubrum supports this initial conclusion (Kushwaha and Kates, 1978). In chlorophyll-a, phytol has the 7(R) 11(R) stereochemistry so non-stereospecific reduction of the double bond would be expected to yield both 3(R) 7(R) 11(R) and 3(S) 7(R) 11(R) isomers (Fig. 1:5). Thus, discrimination between a bacterial and algal origin for isoprenoids may be feasible (Volkman and Maxwell, 1986). The presence of mainly the 3(R) 7(R) 11(R) isomer of dihydrophytol in Messel oil shale (Eocene; Germany; Yon, 1982) was thought to indicate a significant input from Archaebacteria whereas the dihydrophytol isolated from Green River shale (Eocene; Utah, U.S.A) contained equal amounts of the 3(R) 7(R) 11(R) and 3(S) 7(R) 11(R) isomers and probably indicates an origin from phytol via nonstereospecific reduction (Yon, 1982).

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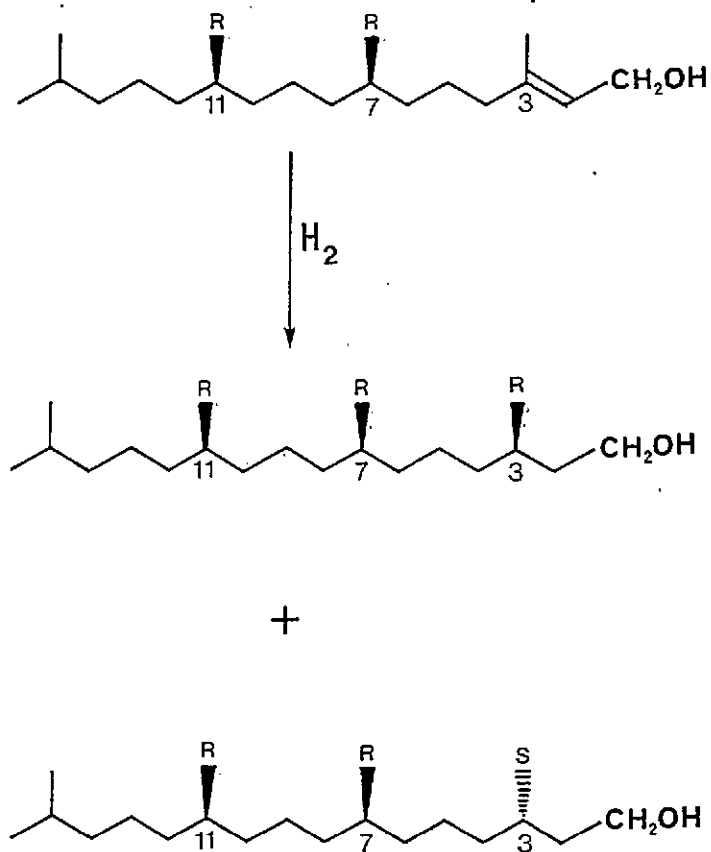
Fig. 1:5   Non-stereospecific hydrogenation of phytol.

Isoprenoids contained in the neutral, non-polar, lipid fraction of Archaebacteria are also considered as precursors of 'regular' acyclic isoprenoids in the geosphere (Hahn, 1982) though the number of reports of regular isoprenoids in Recent sediments (where such lipids might be expected to be observed) is low. Studies by Tornabene et al. (1979) and Holzer et al. (1979) on the neutral lipid composition of nine methanogens and three thermoacidophiles demonstrated the presence of 'regular' isoprenoids (alkanes and alkenes) ranging from C_{14} - C_{28} and C_{30} (Fig. 1:6; compounds labelled * are minor components). In addition to regular isoprenoids, (h-h) and (t-t) hydrocarbons were identified (discussed later). A summary of the isoprenoid and hydroisoprenoid derivatives identified in these studies is given in Tables 1:3 and 1:4. It can be seen that distribution patterns vary not only between the methanogens, thermoacidophiles and halophiles but also within these groups.

A more detailed study of the neutral lipids of Methanobacterium thermoautotrophicum was conducted by Risatti et al. (1984). The hydrocarbon distribution was shown to be dominated by 'regular' C_{20}, C_{25} and C_{30} isoprenoid alkenes and also contained 'regular' isoprenoid C_{15}, C_{16} and C_{18} - C_{20} alkanes. The seven C_{20} alkenes (three monoenes, four dienes) were shown by hydrogenation to possess the 'regular' phytanyl skeleton. Hydrogenation of the three C_{25} monoenes produced 'regular' 2,6,10,14,18-pentamethyleicosane (22). Analysis of the C_{25} alkane on a gc column (DEGS:PEGS) capable of separating diastereomers indicated the presence of a high degree of stereospecificity in the precursor alkenes. The occurrence of 2,6,10,14,18-pentamethyleicosane and related alkenes in a thermoacidophile (Holzer et al., 1979) and in certain sediments (e.g.
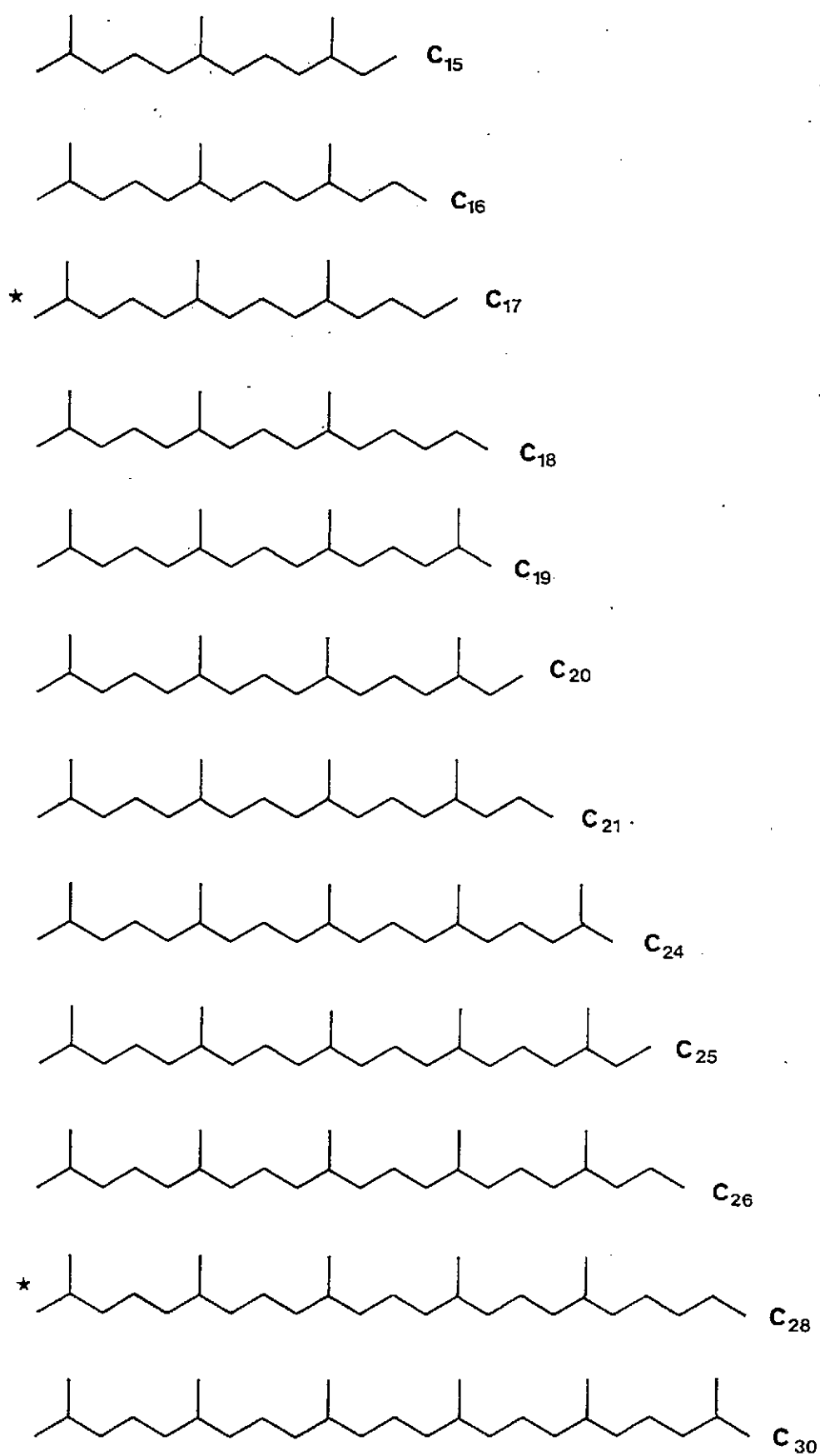
Fig. 1:6 C_{15} – C_{30} 'regular' isoprenoid (h – t) hydrocarbon
            skeletons identified in neutral lipids of Archaebacteria
            (After Tornabene et al., 1982).

Tables 1:3   Isoprenoid hydrocarbons from Archaebacteria.   Taken from Langworthy et al., 1982)

Microorganism	Type of Compound(s)	Isoprenoid Chain(s)	
		Carbon number	Linkage of isoprenoid elements
Methamococcus vannielii	alkanes	C_{18}, C_{19}	head-to-tail
Methanobacterium M.O.H.	alkanes, alkenes	C_{19}, C_{20}	head-to-tail
Methanobrevibacter PS and AZ	alkane	C_{20}	head-to-tail
Methanosareina barkeri	alkane, alkene	C_{25}	tail-to-tail
Methanobacterium thermoauto-trophieum	alkanes	C_{25}, C_{30}	tail-to-tail
Methanogens	alkene di- and tetraethers	C_{30} $C_{20}(2x)$, $C_{30}(2x)$	tail-to-tail head-to-tail and head-to-head
Thermoplasma	alkanes	$C_{15}-C_{20}$	head-to-tail
Sulfolobus	alkanes, alkenes alkanes, alkenes	C_{16}, $C_{18}-C_{20}$ C_{21}, C_{25}, C_{28}, C_{30}	head-to-tail head-to-tail
Sulfolobus spec.	alkanes	$C_{17}-C_{21}$	head-to-tail
Thermoacidophiles	tetraethers with 0-4 cyclopentylrings	$C_{10}(2x)$	head-to-tail and head-to-head
Halophiles	alkene diether	C_{30} $C_{20}(2x)$	tail-to-tail head-to-tail

Table 1:4   Isoprenoid hydrocarbons from Archaebacteria.   (Taken from Langworthy et al. 1982)

Organism	C_{30} Isoprenoid	Major Components* C_{28} Isoprenoid	C_{20}-C_{21} Isoprenoid	Minor Components C_{14}-C_{19} Isoprenoid
S. solfataricus	30:2; 30:1; 30:0	25:0	20:3; 20:2; 20:1	18:1; 18:0; 19:0
T. acidophilum	30:6	25:5	20:4; 20:0; 21:1	14:2; 14:1; 15:0; 16:2; 16:1; 17:1; 17:0; 18:1; 18:0; 19:0
M. thermoautotrophieum	30:6; 30:5; 30:4; 30:3; 30:2; 30:1; 30:0	25:3; 25:2; 25:1	20:2; 20:1	–
M. strain AZ	30:6		20:4; 20:1; 20:0	–
M. ruminantium PS	30:6; 30:5; 30:4; 30:3	25:5; 25:3	–	–
M. ruminantium M-1	30:6; 30:5; 30:4; 30:3 30:2; 30:1	–	–	–
M. strain M.o.H.	30:6; 30:5; 30:4	–	20:4; 20:3; 20:2; 20:1; 20:0	–
M. vannielii	30:6; 30:5; 30:4	25:5; 25:4; 25:3	20:4; 20:3; 20:2	15:0; 18:1; 19:0
M. strain P.S.	30:6; 30:5; 30:4; 30:3	25:3	–	15:0; 18:0; 19:0
M. hungatei	30:6; 30:5; 30:4	–	–	–
M. barkeri		25:3; 25:2; 25:1; 25:0	–	–
H. cutirubrum	30:8; 30:6; 30:5; 30:4	–	–	–

* First number indicates chain length, the second number indicates number of double bonds.

Spyckerelle et al., 1972; Waples et al., 1974) suggests that it has potential as a marker for Archaebacteria in sediments too immature to have generated petroleum. Indeed, ten Haven et al. (1985) reported the identification of 2,6,10,14,18- pentamethyleicosane in a bituminous marl/shale from a Messinian evaporitic basin (Miocene; Italy) and noted that the limited occurrence of the alkane in the hypersaline marl implied that the molecule may be a marker for certain organisms such as halophilic bacteria which only live in restricted salinity ranges.

The 'regular' C_{17} alkane (2,6,10-trimethyltetradecane; 27) isolated from Sulfolobus (Holzer et al., 1979) may be one of the sources of the 2,6,10-trimethyltetradecane identified in Antrim shale (Johns et al., 1966; McCarthy and Calvin, 1967). The origin of 2,6,10-trimethyltetradecane from phytol has been considered unlikely because cleavage of two C-C bonds is required (Gohring et al., 1967).

An additional series of quasi 'regular' isoprenoid alkanes with the methyl groups in the 3,7,11,..... positions were first identified by Haug and Curry (1974) who isolated 3,7,11-trimethyltetradecane (28) and 3,7,11-trimethylhexadecane (29) in a Costa Rican oil seep. An extended series (C_{21} - C_{39}) was identified in several Spanish crude oils on the basis of a comparison of measured gc retention indices with retention indices calculated using molecular additivity rules (Albaiges et al, 1978). Proposed origins for the quasi 'regular' isoprenoids include non-random cracking of 'regular' isoprenoids (Haug and Curry, 1974) and the thermocatalytic degradation of a parent C_{40} 'irregular' (h-h) hydrocarbon skeleton, 3,7,11,15,18,22,26,30 - octamethyldoctria-contane (30), a known component of certain crude oils (Albaiges, 1980). Albaiges et al. (1985) conclusively identified, by synthesis and mass

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spectral interpretation the C_{21} (<u>31</u>), C_{22} (<u>32</u>), C_{23} (<u>33</u>) and C_{24} (<u>34</u>) members of the quasi 'regular' isoprenoid alkanes in Western Mediterranean crude oils. Also occurring were 'regular' C_{21} - C_{24} isoprenoid alkanes and three isomeric C_{40} isoprenoid alkanes (Fig. 1:7) considered to be bacterial or Archaebacterial markers. The small relative abundance of the quasi 'regular' C_{23} alkane (<u>33</u>) compared to the quasi C_{21}, C_{22} and C_{24} alkanes suggested that these originated from categenic degradation of the 'regular' C_{40} isoprenoid hydrocarbon (Fig. 1:7; II) although their formation from the parent 'irregular' (h-h) isoprenoid (Fig. 1:7, I) could not be discounted.

In summary, 'regular' acyclic isoprenoid hydrocarbons have been identified in numerous biota, petroleums and sediments. Several origins for the sedimentary 'regular' isoprenoid hydrocarbons (<C_{20}) have been proposed including chlorophyll-derived phytol, α-tocopherol, polyprenols and Archaebacterial lipids. The exact contribution of each probably varies in any given situation. Proposed origins for the 'regular' isoprenoid hydrocarbons (>C_{20}) include polyprenols and Archaebacterial lipids. Since Archaebacteria are commonly found in 'extreme' environments (i.e. oxygen deficient) which were presumably commonplace in certain periods of the Archaen, it is perhaps not surprising that Archaebacterial lipids are proposed as a significant source of sedimentary 'regular' acyclic isoprenoids (Hahn, 1982; Volkman and Maxwell, 1986).
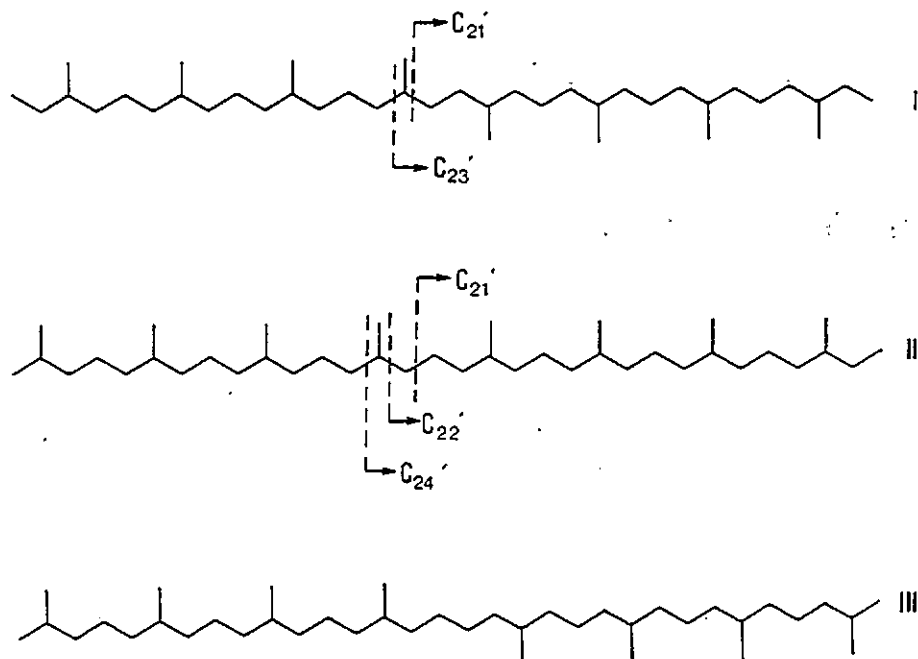
Fig. 1:7   Structures of C_{40} isoprenoid alkanes identified in Amposta crude oil (Miocene; Spain). The dashed lines indicate how catagenic degradation can produce the 'quasi - regular' isoprenoid alkanes identified concurrently in the oil (After Albaiges et al., 1985).

1.2.2 'Irregular' (t-t) and (h-h) acyclic isoprenoid hydrocarbons

A summary of the range of 'irregular' (t-t) and (h-h) linked acyclic isoprenoid hydrocarbons so far identified in the geosphere is summarised by selected reference in Table 1:5. The 'irregular' (t-t) acyclic isoprenoid hydrocarbon squalane (2,6,10,19,23,27- hexamethyl-tetracosane; 21) was first reported in a Nigerian petroleum (Gardner and Whitehead, 1972) where it was assumed to have originated via geochemical reduction of the unsaturated biogenic analogue, squalene (2,6,10,19,23,27-hexamethyltetracosa-2,6,10,14,18,22-hexene; 35). However, the identification of squalane and related squalenes in the neutral lipids of Archaebacteria (see Tables 1:3 and 1:4; Holzer et al., 1979; Tornabene et al., 1979; Risatti et al., 1984) has led to the proposal that squalane identified in non-polluted immature marine sediments (i.e. Dastillung and Corbet, 1980) is derived from a direct Archaebacterial input. Although squalane and related squalenes have been identified in various thermoacidophiles, halophiles and methanogens, only the latter are likely to have contributed significantly to deep sea sediments. This implies that the presence of squalane in marine sediments is an indicator of indigenous methanogenic activity. The co-occurrence in marine sediments of squalane with the 'irregular' (t-t) 2,6,10,15,19-pentamethyleicosane (36), another proposed marker of methanogenic activity, supports this implication (Brassell et al., 1981).

The tentative identification of 2,6,10,15,19-pentamethyleicosane in methanogens was first reported by Holzer et al. (1979) and later confirmed by synthesis (Rowland et al., 1982). Risatti et al. (1984) identified 2,6,10,15,19-pentamethyleicosane as the only hydrocarbon in Methanobacterium barkeri and showed by gc that the relative stereo-

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SEDIMENT/PETROLEUM	GEOLOGICAL AGE	CARBON RANGE NUMBER	TYPE	REFERENCE
Nigerian Petroleum		30	t-t	Gardner and Whitehead, 1972
Marine Sediments	Recent	25	t-t	Brassell et al., 1981
Marine Sediments	Recent	40	t-t	Dastilling and Corbet, 1981
Western Mediterranean	Various	40	t-t	Albaiges et al., 1985
Spanish Crude oils	Various	unclear	t-t	Albaiges et al., 1981
California Petroleum	Miocene	20-40	h-h	Moldowan and Seifert, 1979
Western Mediterranean	Various	27-40	h-h	Albaiges et al., 1980
Messel oil shale	Eocene	40	h-h	Chappe et al., 1979, 1980

Table 1:5. Examples of 'Irregular' (h-h, t-t) acyclic isopenoid hydrocarbons in the geosphere.

chemistry of the bacterial alkane was the same as found in three immature marine sediments (from Walvis Bay, Walvis Ridge and the Gulf of California). Combining this with the reported absence of 2,6,10,15,19-pentamethyleicosane from thermoacidophiles and halophiles, seems to be a powerful argument for the use of the alkane as a marker of sedimentary methanogenic activity.

The 'irregular' (t-t) acyclic isoprenoid lycopane (37) occurs in significant quantities in certain Recent sediments (Dastillung and Corbet, 1978; McEvoy et al., 1981) and in a Western Mediterranean crude oil (Albaiges et al., 1985) and could originate by diagenetic reduction of lycopene (38) which occurs widely in organisms. However, the occurrence of lycopane in sediments where methanogenic activity has been reported by Moldowan supports an origin from Archaebacteria although, as yet, no definite bacterial source has been found (Brassell et al., 1981; Volkman and Maxwell, 1986).

Two other 'irregular' (t-t) acyclic isoprenoid hydrocarbons identified in a crude oil are 2,6,10,15-tetramethylhexadecane (39) and 2,6,10,14,19-pentamethyleicosane (40) (Albaiges and Torradas, 1977). The alkanes may originate from the respective cracking of squalane (21) and lycopane (37) or through an, as yet, unreported source. The presence of a series of 'irregular' (t-t) isoprenoid alkanes in petroleums and sediments is reported by Albaiges et al. (1981).

The first identification of 'irregular' (h-h) linked acyclic isoprenoids in petroleum (Miocene; California, U.S.A) was reported Moldowan and Seifert (1979). In addition to a series of 'regular' (h-t) alkanes (C_{18} - C_{36}) they found a series of (h-h) alkanes, the

largest homologue being biphytane (i20-i20; <u>41</u>). The i19-i19 homologue
(<u>42</u>) was conclusively characterised by chemical synthesis of a
reference compound with the remaining (h-h) alkanes identified by mass
spectral interpretation. The same series of 'irregular' (h-h) alkanes
were detected in petroleums and source rocks from Canada, U.S.,
Venezuela, U.S.S.R. and also, in a separate study of a Western
Mediterranean crude oil (Albaiges, 1980). The proposed source of these
'irregular' (h-h) isoprenoid hydrocarbons were the biphytanyl
tetraethers identified contemporaneously in several species of
Archaebacteria (i.e. De'Rosa <u>et</u> <u>al</u>., 1977).

In addition to the diphytanylglycerol diethers (<u>24</u>) mentioned
previously, the membrane polar lipids of Archaebacteria can also
contain tetraethers which consist of two glycerol molecules bridged
through ether linkages by two identical pairs of C_{40}-biphytanyl
terminal diols (<u>43</u>) (Langworthy <u>et</u> <u>al</u>., 1982). The stereostructure of
the Archaebacterial tetraether has recently been established as (3R,
7R,11R,15S,18S,22R,26R,30R)-3,7,11,15,18,22,26,30-octamethyldotri-
contane-1,32-diol (<u>44</u> and <u>45</u>) (Heathcock <u>et</u> <u>al</u>., 1985). The
distribution between diethers and tetraethers within Archaebacterial
subgroups can vary considerably (Table 1:6) as does the actual
structure of the biphytanyl linkage (see Fig. 1:8). For instance, the
biphytanyl chain of <u>Thermoplasma</u> contains up to two cyclopentyl rings
whereas those of <u>Sulfolobus</u> two to four rings.

The (h-h) homologue alkanes lower than C_{40} identified in petroleum
are considered to be diagenetic debris from the biphytanyl moiety
(Moldowan and Seifert, 1979).

Archaebacterium	Diether (‰)	Tetraether (‰)
Sulfolobus solfataricus	5.0	95.0
Thermoplasma acidophilum	10.0	90.0
Methanospirilum strain AZ	37.5	62.4
Methanospirillum hungatei	40.5	59.5
Methanobacterium strain M.o.H.	43.5	56.5
Methanobacterium thermoautotrophicum	44.5	55.5
Methanobacterium ruminatium PS	44.7	55.3
Methanobacterium ruminatium M-1	71.8	28.2
Methanococcus vannielli	99.9	0.1
Methanococcus strain PS	100	0
Methanosarcina barkeri	100	0
Methanothrix söhngenii	100	0
Halobacterium cutirubrum	100	0
Halobacterium halobium	100	0
Halobacterium marismortui	100	0
Halobacterium saccharovorum	100	0
Halobacterium salinarium	100	0
Halobacterium volcanii	100	0
Halococcus morrhuae	100	0
Sarcina morrhuae	100	0
Sarcina literalis	100	0

Table 1:6   Diether and tetraether distribution in
              thermoacidophilic, halophilic and methanogenic
              Archaebacteria. (Taken from Langworthy _et al._, 1982).

METHANOGENS

Acyclic

Monocyclic

THERMOPLASMA

Bicyclic

SULFOLOBUS

Tricyclic
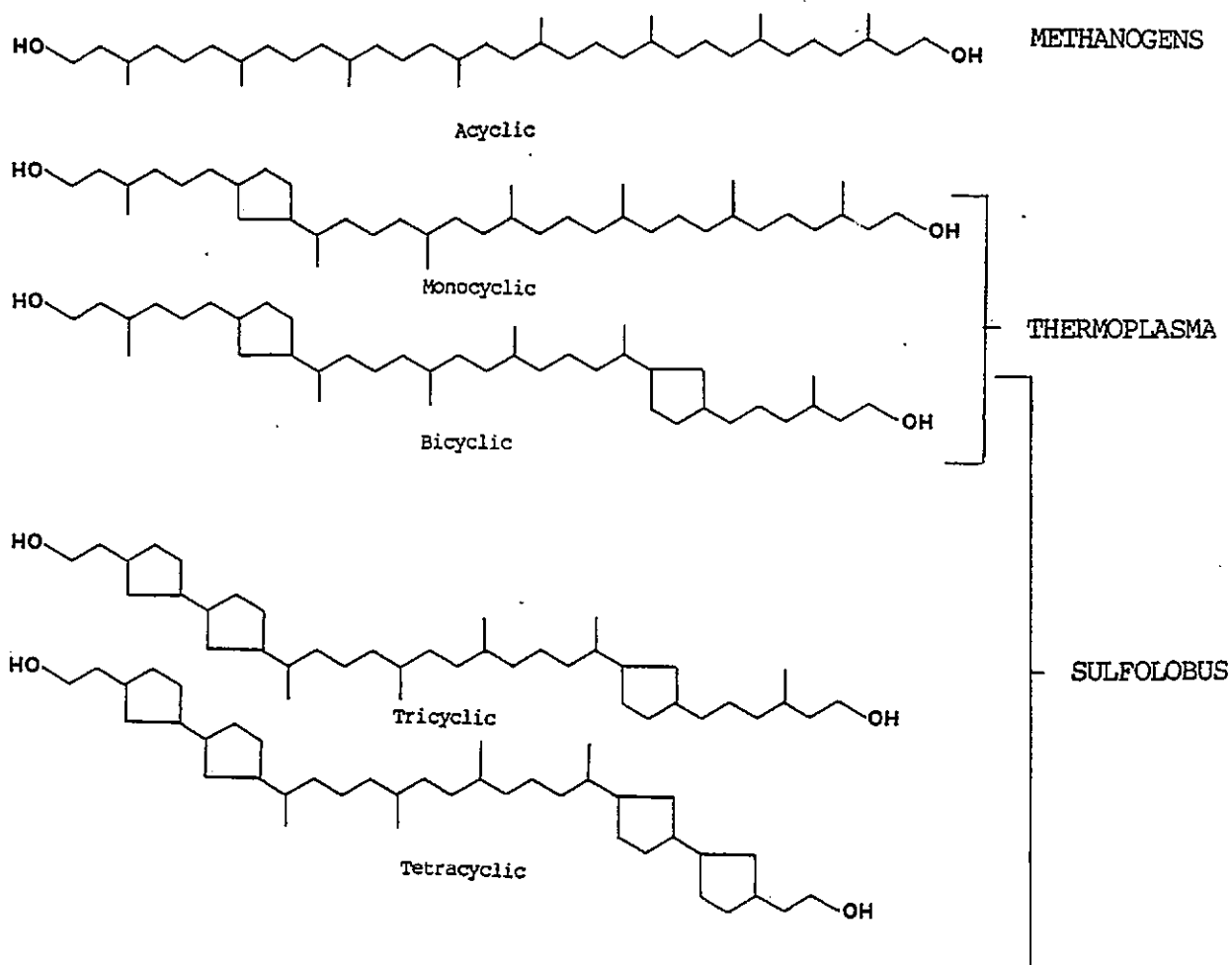
Tetracyclic

Fig.1:8   Structure and distribution of acyclic and cyclopentyl C_{40}-biphytanyl
          chains as the diols in the tetraethers of methanogenic and thermoacidophilic
          Archaebacteria (After Langworthy et al., 1982).

Evidence for the contribution of Archaebacterial lipids to kerogen has been provided by several studies (Michaelis and Albrecht, 1979; Chappe et al., 1979, 1980, 1982). Using boron tribromide to cleave ether linkages, Michaelis and Albrecht (1979) isolated phytane, C_{40} biphytane and a C_{40} (h-h) biphytanyl containing one cyclopentyl ring (46) from the Messel oil shale (Eocene; Germany) kerogen. A further study (Chappe et al., 1980) using LiAlD$_4$ in the reduction step, was able to demonstrate that phytane was bound to the kerogen by a single ether linkage and that biphytanyl was present in the form of a α, ω - ether. Identical products were obtained when the same reaction sequence was applied to Archaebacteria providing clear evidence of the incorporation of Archaebacterial lipids into the kerogen structure. The presence of phytane as a major component of the product mixture suggests, that in addition to chlorophyll-derived phytol, Archaebacterial lipids may be a significant source of sedimentary phytane. A study of the geological history of the Messel shale implicates methanogens as the most likely source of the Archaebacterial lipids. Subsequent work by Chappe et al. (1982) has demonstrated the presence of lipids typical of Archaebacteria in the polar lipids of several Recent and Ancient sediments and petroleums (e.g. Mahakam oil). The presence of the polar lipids in sediments at an advanced stage of maturation implies a high degree of stability in the ether linkage under geological conditions.

Polar lipids indicative of Archaebacteria have also been reported in Florida swamp sediment (Pauly and Van Vleet, 1986) and hot spring microbial mats (Ward et al., 1986), both known to contain methanogens. In nearly all the reported studies of sedimentary Archaebacterial lipids, various quantities of cyclopentyl biphytanes containing one to

three rings have been detected in addition to phytane and biphytane. The occurrence of these is puzzling in that they are not reported as constituents of the methanogens which are thought to have been the major Archaebacteria originally present in most of the sediments studied. The presence of cyclopentylbiphytanes may represent either inputs from some of the other Archaebacteria known to contain cyclopentylbiphytanes (such as Sulfolobus) or they may derive from an, as yet, unreported methanogenic source (Ward et al., 1986).

In summary, 'irregular' (t-t) and (h-h) acyclic isoprenoid hydrocarbons have been reported in numerous sediments and petroleums (Table 1:5). There is much evidence to indicate that the hydrocarbons represent geochemical fossils of Archaebacterial origin (Hahn, 1982). The major contribution is assumed to be derived from methanogens which thrive in anoxic sediments. Contributions from extreme halophiles and thermoacidophiles, particularly in Recent sediments, are thought to be less important in view of the extreme nature of the environments in which these species are abundant.

1.2.3 Unusual 'irregular' acyclic isoprenoid hydrocarbons in the geosphere: Botryococcanes

Botryococcanes represent one of the more unusual groups of 'irregular' acyclic isoprenoid hydrocarbons in the geosphere. The C_{34} botryococcane (10-ethyl-2,3,6,7,10,13,16,17,20,21-decamethyldocosane; 47) was first identified in petroleum by Moldowan and Seifert (1980) who reported a concentration of 1.4% in a Sumatran crude oil. The C_{34} botryococcane was identified from the mass spectrum which displays characteristic fragment ions at m/z 238, m/z 298 and m/z 448, generated by α-cleavages around the quaternary carbon atom. C_{34}

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botryococcane had an obvious biogenic precursor, a C_{34} botryococcene (48) which had been previously isolated from the fresh or brackish water green alga Botryococcus braunii (Maxwell et al., 1968). Structural assignment of the botryococcene was established through oxidative degradation and ^{13}C NMR analysis (Cox et al., 1973). The mass spectrum and retention index of the hydrogenated botryococcene was identical to that of the C_{34} botryococcane identified in the Sumatran petroleum. The presence of botryococcane in this petroleum was taken as evidence that the oil was generated primarily from organic matter in a brackish or fresh water depositional environment.

Recent studies (i.e. Casadevall et al., 1984) have both extended the carbon number range of reported botryococcenes, and established that there are two physiologically distinct varieties of Botryococcus braunii both able to biosynthesize large quantities of hydrocarbons. The first variety, Race A, contains up to 61% (by dry weight) of odd carbon numbered C_{25}-C_{31} n-alkadienes and trienes whereas the second variety Race B, contains 24-76% of polymethylated triterpenes termed botryococcenes (C_nH_{2n-10}, $30 \leqslant n \leqslant 37$). The term, botryococcene, is now generally used to describe the whole family of structurally similar isoprenoids identified in B. braunii. Work by several workers (Metzger and Casadevall, 1983; Galbraith et al., 1983; Metzger et al., 1985) has established that the current carbon number range of botryococcenes is C_{30} to C_{37}. The carbon number and structures are shown in Fig. 1:9. Cox et al. (1973) proposed a biosynthetic pathway for these which involved a tail to tail linkage of two C_{15} (h-t) units via a 1^1-3 condensation as had been previously suggested for isodigeranyl (Soucek et al., 1961).
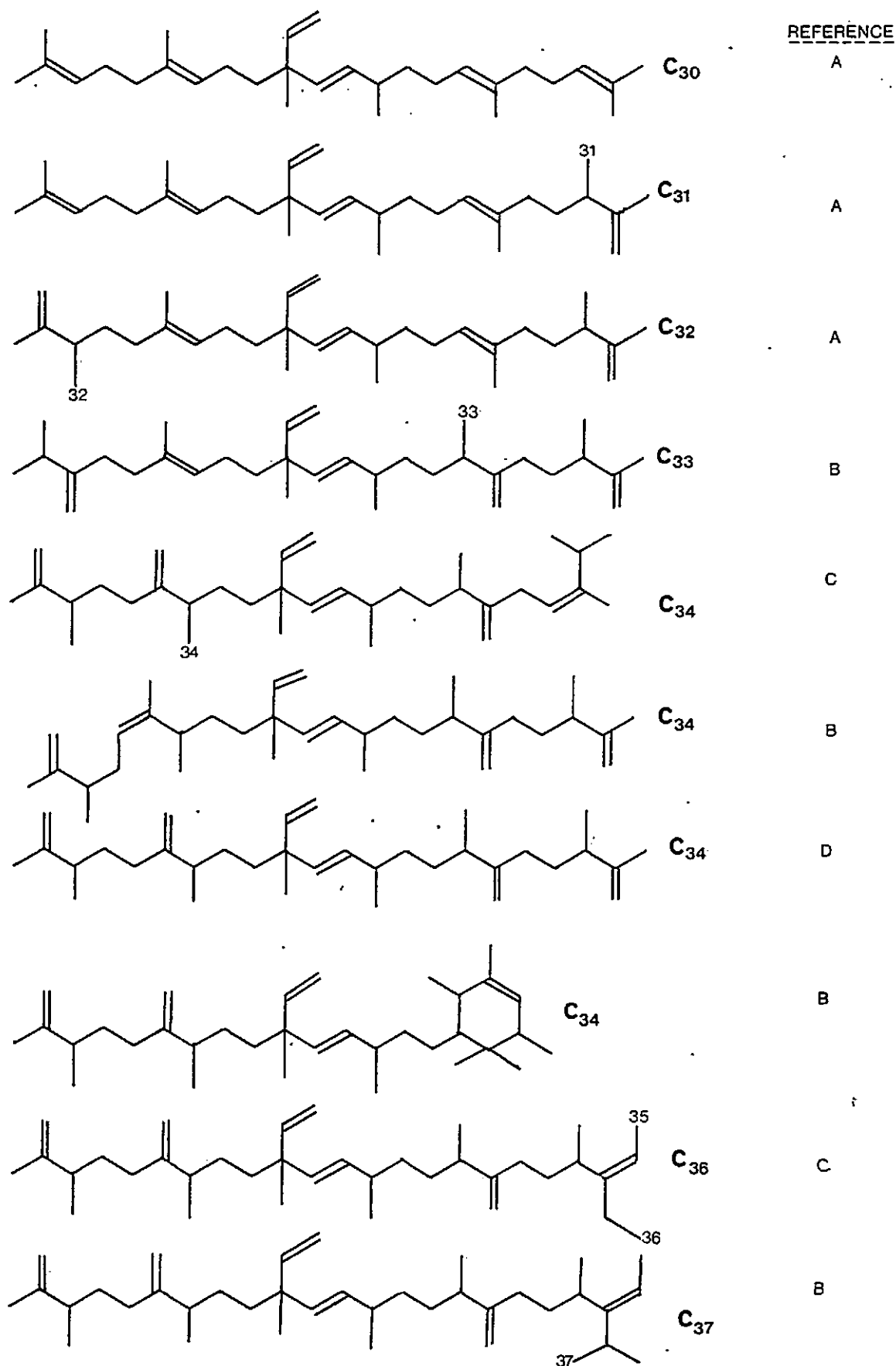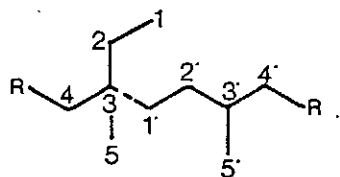
Fig.1:9   Structures of botryococcenes identified in Botryococcus braunii.

REFERENCES: A. Metzer and Casadevall, 1983
              B. Metzer et al.,1985
              C. Galbraith et al.,1983
              D. Cox et al., 1973.

Metzger et al. (1985) also suggested a terpenoid origin and proposed that the C_{30} botryococcene acts as a precursor, through progressive methylation, for the higher metabolites of the series.

The recent identification of C_{30}-C_{37} botryococcenes in B. braunii led Metzger et al. (1985) to state, that in addition to the C_{34} botryococcane identified in petroleum (Moldowan and Seifert, 1980), the identification of other botryococcanes C_{30}-C_{37} in petroleum is to be expected. Subsequently, Brassell et al. (1986a) tentatively identified C_{31} and C_{33} botryococcanes in the Maoming oil shale (Eocene; China). Identification was made by mass spectral interpretation. The reported mass spectra were different to those recorded upon hydrogenation of C_{31} and C_{33} botryococcenes of known structure isolated from B. braunii (Metzer et al., 1985) suggesting that a different alkylation pattern is present in the Maoming botryococcanes. Fig. 1:10 shows the mass spectra and proposed structure of the two Maoming oil shale botryococcanes. It is interesting to note that the ion abundances of the fragment ions generated by α-cleavage either side of the quaternary carbon are much lower than those observed in the mass spectrum of the C_{34} botryococcane (Moldowan and Seifert, 1980). The mass spectrum of the C_{33} botryococcane from the Maoming oil shale appears similar to that reported for an isoprenoid in a Sumatran (S.E. Balam) petroleum (Seifert and Moldowan, 1981). However, a direct comparison of the data
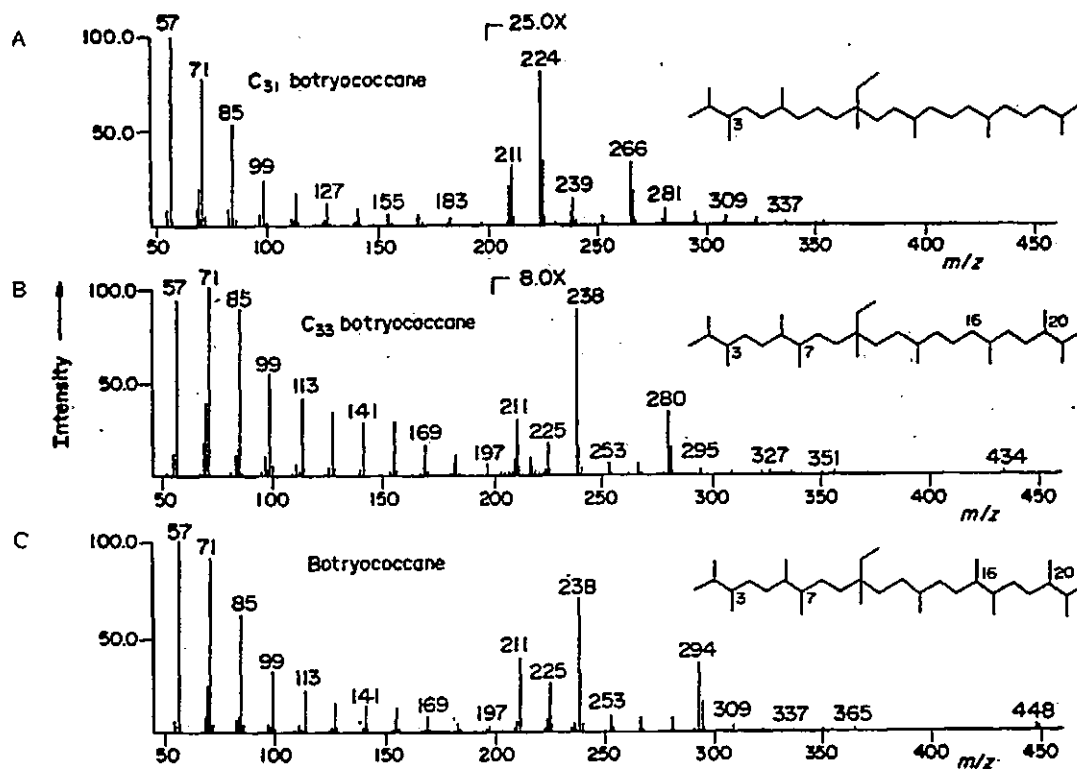
Fig. 1:10 Mass spectra as recorded by Brassell et al. (1986a) of two botryococcanes tentatively identified in Maoming oil shale (China). A. a C₃₁ botryococcane and B. A C₃₃ botryococcane. C. shows for comparative purposes the spectrum of authentic C₃₄ botryococcane (10-ethyl-2,3,6,7,10,13,16,17,20,21-decamethyldocosane).

is complicated as neither study provided gc retention data for the compounds. Also present in the Sumatran petroleum is an isoprenoid with a mass spectrum containing fragment ions at m/z 266/267, m/z 308/309 and m/z 434/435 indicative of a C_{37} botryococcane with the possible structure;

308/9

266/7
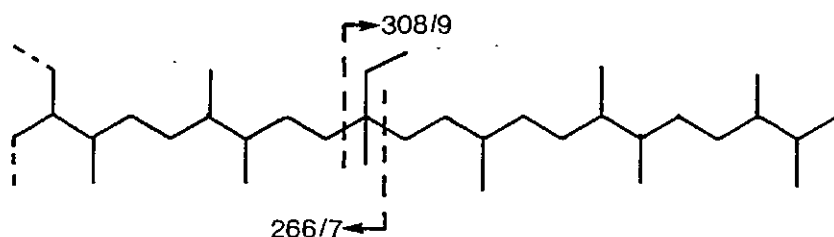
the solid lines representing the C_{34} botryococcane (Moldowan and Seifert, 1980) and the dashed lines the possible positions of the additional methyl groups.

Interestingly, the C_{34} botryococcane is absent in both the Maoming oil shale and the Sumatran petroleum, raising the possibility that the source organism B. braunii might be able to vary the carbon number range of the botryococcenes during biosynthesis. The presence of the botryococcanes in the Maoming oil shale suggests a fresh or brackish water (i.e. lacustrine) depositional environment which is consistent with the evidence provided by other biological markers such as the 4-methylsteroids which are indicative of fresh water dinoflagellates (Brassell et al., 1986a). C_{34} botryococcane has also been identified in a series of coastal bitumens from Southern Australia (McKirdy et al., 1986). Together with carbon isotope ratios, the presence of the botryococcanes provides evidence that the bitumens are members of a new class of non-marine Australian crude oils.

In summary, botryococcanes $(C_{31}-C_{34})$ have been identified (tentatively) in a number of Ancient sediments and petroleums. The only known biogenic precursors for the botryococcanes are the botryococcenes $(C_{31}-C_{37})$, which are found as major consituents of the fresh or brackish water green algae <u>Botryococcus</u> <u>braunii</u>. As a result, botryococcanes in sediments are excellent biological markers for lacustrine depositional environments.

1.3 BICYCLIC HYDROCARBONS IN THE GEOSPHERE

The geochemistry of bicyclic hydrocarbons has been comprehensively reviewed by Noble (1986) and it is not intended to duplicate that report here. Instead, attention is focused on reports of bicyclic hydrocarbons germane to the current investigation. There are very few reports of bicyclic hydrocarbons in Recent sediments; those that do exist concern themselves primarily with variations in the distribution and abundance of bicyclic hydrocarbons between different geographical locations. Generally, no purposeful attempt has been made at structural elucidation of the sedimentary hydrocarbons by mass spectral interpretation or synthesis of reference compounds. Such reports form the basis of section 1.4 and so will not be discussed further at this point. The majority of the literature on bicyclic hydrocarbons therefore focuses on their identification, occurrence and distribution in Ancient sediments and petroleums where they have been proposed as biological markers and indicators of the thermal maturity of sediments (Noble, 1986).

Kagramanova <u>et</u> <u>al</u>.(1976) used preparative gc to isolate two bicyclic alkanes occurring at high concentration (0.9% w/w) in a Russian crude oil. The compounds were assigned structures (<u>49</u>) and (<u>50</u>) using mass

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spectroscopy and ^{13}C NMR spectroscopy. With regard to the origin of these alkanes, they postulated a rearrangement of bicyclic alkanes such as (51) which could themselves be derived from the A/B ring of higher plant triterpenes. Another Russian worker, Vorob'eva et al. (1978) noted the occurrence of a group of seven C_{14}-C_{16} bicyclic alkanes (bicyclanes) in a petroleum from the Siva field (U.S.S.R.). Tentative structures were assigned to six of the bicyclanes on mass spectral interpretation; the seventh, a C_{16} bicyclane, was isolated by preparative gc and assigned by ^{13}C NMR spectroscopy (52). It was proposed that the bicyclanes were derived by degradation of pentacyclic triterpenes or from compounds formed by a variation in the biosynthetic cyclisation of squalene which terminated upon formation of the bicyclic ring structure. Dimmler et al. (1984) reported the identification of C_{15}-C_{24} bicyclic alkanes in the bitumen fraction of the Athabasca tar sands. The structures of the C_{18} (53) and C_{20} (54) hydrocarbons were assigned by comparison with synthesised reference standards. Microbial degradation of extended tricyclic terpanes was suggested as a possible source.

Alexander et al. (1983) reported the identification in petroleum of the bicyclic sesquiterpanes drimane (51) and eudesmane (55). Identifications were made by comparison of petroleum bicyclanes with synthesised alkanes of $4\beta(H)$-eudesmane (56) and $8\beta(H)$-drimane (57). $4\beta(H)$-eudesmane was reported as occurring only in those petroleum samples containing a significant contribution of terrigenous source material. Sesquiterpanes based on the eudesmane skeleton are widely distributed in higher plants and it was concluded that $4\beta(H)$-eudesmane is an unambiguous marker for higher plant precursors. On the other

hand, $8\beta(H)$-drimane was reported as occurring in sixteen crude oils and several bitumen extracts of sediments varying in age from Ordovician to Early Cretaceous. The occurrence of $8\beta(H)$-drimane in Cambrian-Ordovician samples is significant as this tends to eliminate derivation from higher plant precursors as a possible source. Indeed, the widespread geological and geographical occurrence of $8\beta(H)$-drimane indicated a microbial origin either from degradation of higher terpenoids such as the hopanes, or from direct bacterial synthesis of precursor compounds containing the bicyclic ring system. In a further study, Alexander et al. (1984) reported the identification in petroleum of $8\beta(H)$-drimane, $8\beta(H)$-homodrimane (58) and two other C_{15} bicyclanes (59 and 60) whose structures do not conform to the isoprene rule. The identification of the C_{15} bicyclanes was based on mass spectral interpretation and by comparison with the data of Kagramanova et al. (1976). $8\beta(H)$-drimane and $8\beta(H)$-homodrimane were identified by comparison with reference alkanes. The four compounds were shown to be present in 22 crude oils and 26 sediment extracts of varying geological age and geographical distribution. It was proposed that the C_{15} 'rearranged' bicyclanes (59 and 60) may be derived from a drimane based precursor by a rearrangement involving carbonium ion intermediates. A similar rearrangement in subsurface sedimentary environments is the clay catalysed conversion of sterenes (61) to diasterenes (62) (the so-called 'backbone rearrangement'). Eventually, reduction of these precursors results in the steranes and diasteranes which are present in varying proportions in petroleum. Using 34 crude oil and sediment extracts it was demonstrated that an increase in the ratio of C_{15} rearranged bicyclanes (59 and 60):drimane (51) was associated with a similar increase in the diasterane:sterane ratio. This indicated that the functions which determine the extent of rearrangement in

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sedimentary steroids may also affect the rearrangement of drimane precursors. With regard to origins, it was proposed that bicyclic hydrocarbons of the 'drimane type' are formed by biological alteration of hopanoid precursors during diagenesis. Further evidence of a link between hopanoids and drimanes was noted as the co-occurrence of A-ring methylated drimanes (63) and methylated hopanes (64) in a bitumen extract of a Western Australian sediment.

The work on the identification of bicyclic alkanes in Ancient sediments and petroleums was further broadened by Noble (1986) who reported the identification in sediments and petroleums of 13 bicyclic alkanes $C_{14}-C_{16}$ for which structures are shown in Fig. 1:11. Five compounds were assigned by comparison with synthetically prepared reference compounds, two by literature comparison and the remaining six bycyclanes were tentatively assigned structures from interpretation of mass spectra. The 'drimane-type' compounds (Fig. 1:12) were shown to be present in numerous sediments and petroleums irrespective of geological age or thermal maturity. This was again taken as evidence for a microbial source for the drimanes either through microbial degradation of bacteriohopanoids or from direct bacterial synthesis of compounds containing the bicyclic ring system. A scheme was proposed (Fig. 1:12) illustrating that 'drimane-type' compounds could derive through degradation of hopanoid precursors. The co-occurrence of A-ring methylated drimanes and methylated hopanes in ten crude oil samples provided further evidence of a common microbial source.

Other possible sources for the 'drimane-type' hydrocarbons included higher plant sesquiterpenoids and an origin via a variation in the
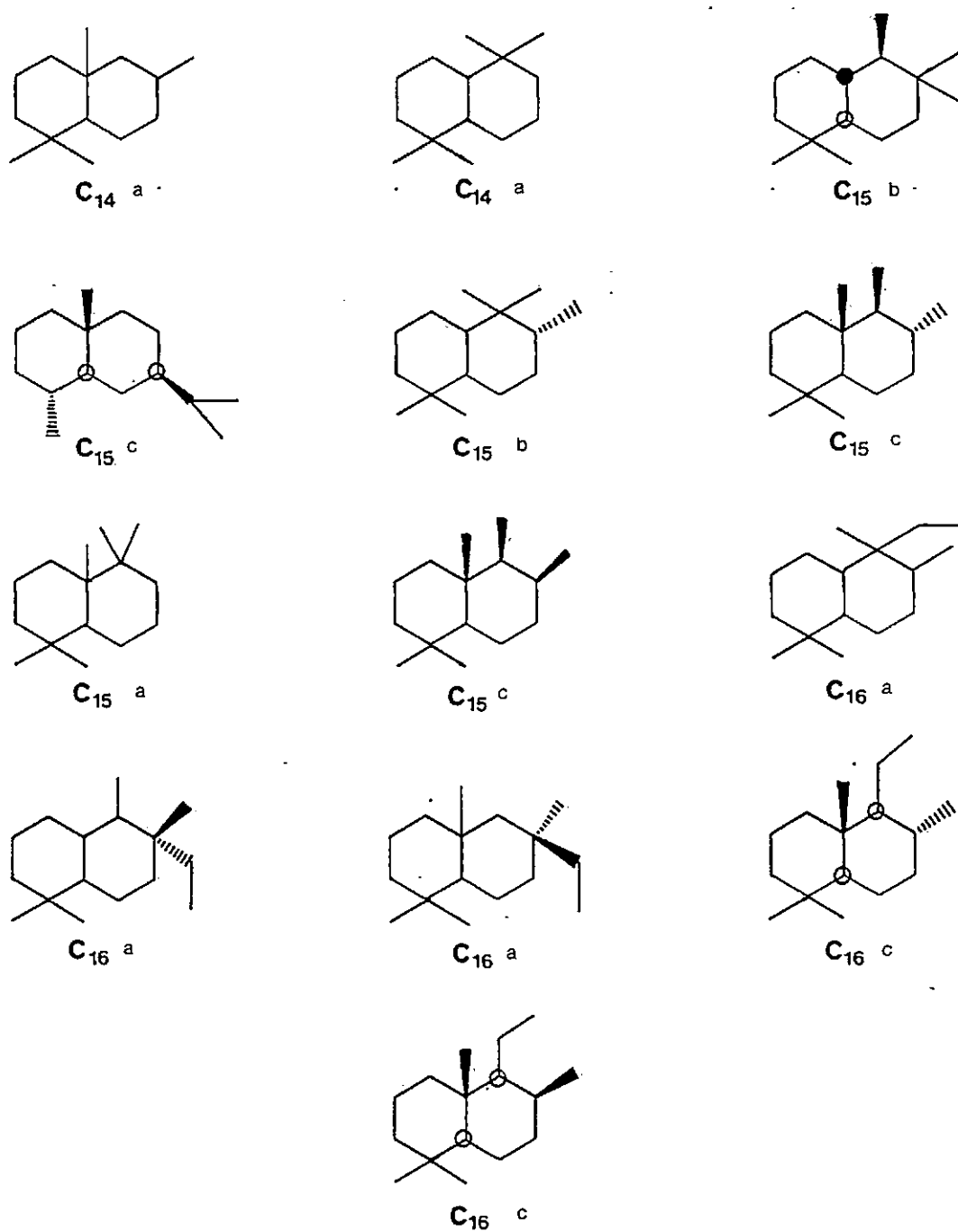
Fig.1:11 Structures of the bicyclic alkanes identified in
petroleums and Ancient sediments by Noble (1986).
Method of identification: a. mass spectral interpretation.
                          b. literature comparison
                          c. reference standards.

Fig.1:12   A proposed scheme for the degradation of $\Delta 11(12)$ -
           bacteriohopanetetrol to drimane-type precursors by
           microbial processes.   (After Noble, 1986).

MICROBIAL EPOXIDATION

REARRANGEMENT OF
EPOXIDE

+ KETONE ISOMER

EXCITATION OF KETONE BY PHOTOMIMETIC
MICROBIAL PROCESSES

H_2O,
β-CLEAVAGE

COOH

+ D/E Moiety   + D/E Moiety
+ C=C=O   + H_2
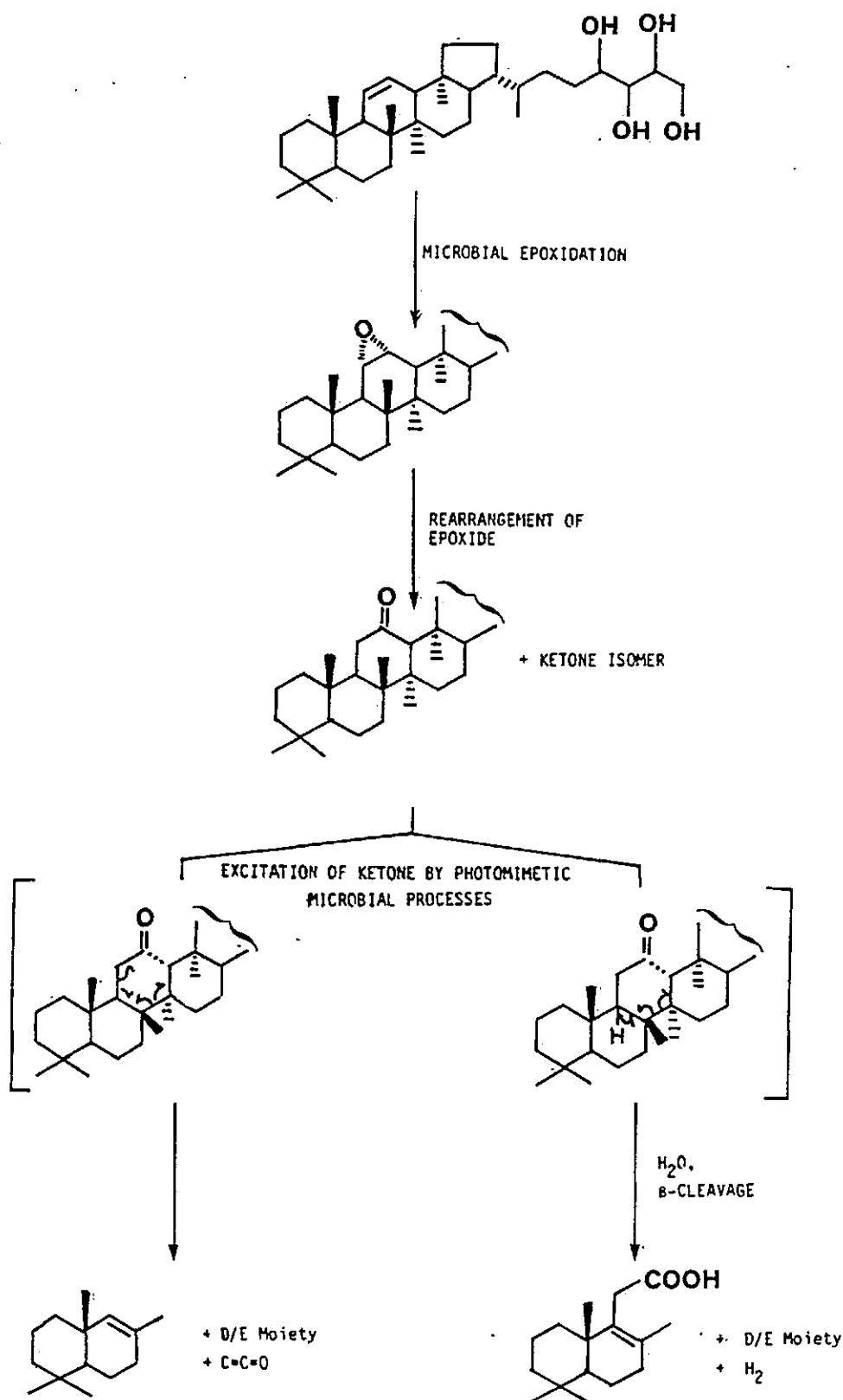
biosynthetic cyclisation of squalene with termination upon formation of the bicyclic ring structure. The latter pathway would give rise to a homologous series of bicyclic alkanes extending from C9 upwards which were not detected in any of the samples reported by Noble (1986). Indeed, the only reported occurrence of bicyclic alkanes $>C_{16}$ is that of Dimmler $\underline{et}$ $\underline{al}$. (1984) who observed bicyclanes $C_{15}-C_{24}$ in the Athabasca tar sands. This occurrence is probably a reflection of the extensive microbial alteration experienced by the oil sand and should, according to Noble (1986), be treated as an isolated case. Therefore, on the whole, there is little evidence to support the proposal that squalene is the biosynthetic precursor for the C_{14} - C_{16} compounds.

In summary, bicyclic alkanes have been detected in many Ancient sediments and petroleums irrespective of geological age, thermal maturity or geographical location. Their ubiquitous occurrence dictates a microbial origin, either through microbial degradation of bacteriohopanoids during diagenesis, or from a direct input from bacterial synthesis of bicyclic compounds.

1.4 UNUSUAL ACYCLIC AND CYCLIC C_{20}, C_{25} AND C_{30} HYDROCARBONS IN RECENT SEDIMENTS

Over the past ten years there have been numerous reports in the literature detailing the occurrence and distribution in (mostly Recent) sediments of a number of hydrocarbons containing 20, 25 or 30 carbon atoms (most recently reviewed by Rowland $\underline{et}$ $\underline{al}$., 1985). Few compounds in modern geochemical research can have been reported so frequently, yet remained unidentified for so long. Proposed structures for the compounds have included both acyclic and cyclic compounds either saturated or with varying degrees of unsaturation. Up to the present,

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the structure of only one of these unusual hydrocarbons has been established by spectroscopy and comparison with a synthetic alkane (Yon et al., 1982). Proposed structures for the other hydrocarbons have been based almost entirely on interpretation of mass spectra and remain unproven. The remainder of this introduction comprises a chronological review of the reported sedimentary occurrences of these unusual hydrocarbons. Table 1:7 summarises the sedimentary occurrences of the proposed acyclic hydrocarbons and Table 1:8 of the proposed cyclic hydrocarbons. In this review, individual hydrocarbons are referred to by the method of Barrick et al. (1980). For instance, an acyclic branched C_{25} diene is denoted as br25:2 and a bicyclic C_{30} triene as c30:3:2. In addition, in order to aid comparison of data from different sources, gc retention indices are also referred to (e.g. br25:2;$_{0V-1}$2082). Repeated references to Tables 1:7 and 1:8 are made throughout this review. In this way, it should become apparent, for example, that the C_{25} diene observed by Requego et al. (1984) (br25:2:2084) is the same as that reported by Vondrias and Smith (1986) (br25:2:2082) and so on. Examples of the sedimentary concentrations of these unusual acyclic and cyclic hydrocarbons are presented in Table 1:9.

A gas chromatogram of the aliphatic hydrocarbons extracted from surface sediments in the Gulf of Mexico revealed the presence of a group of compounds eluting between RI_{FFAP}2050-2150 (Gearing et al., 1976). The principal compound eluting at RI2075 was not adducted by urea but shifted upon hydrogenation to RI2015 indicating the presence of one or more degrees of unsaturation. Seven fractions of the RI2050-2150 mixture were collected by preparative gc and subjected to gcms. The molecular ion of highest intensity and occurrence was m/z 348

Investigation

Type of compound[a]	Gearing et al., 1976	Blanchard 1979	Prahl et al., 1980	Barrick et al., 1980	Venkatesan et al., 1980	Barrick & Hedges, 1981	Yon et al., 1982	Venkatesan & Kaplan, 1982	Osterroht et al., 1983	Requego & Quinn 1983a	Volkman et al., 1983	Albaiges et al., 1984a	Albaiges et al., 1984b	Requego et al., 1984	Requego & Quinn, 1985	Shaw et al., 1985	Dunlop & Jefferies 1985	Rowland et al., 1985	Voudrias et al., 1986
Column phase	FFAP	OV1	SP1000	SP2100	SP2100	SP2100	OV1	SP21	SP2100	SE30	SE30	SE30	OBS	SE30	SE30	OV101	OV1	OV1	OV1
br20:0	1640			1708			1709										1712	1705	
br20:1				1698															
br20:1				1702													1703	1700	
br25:0			2109	2109						2111			2105				2112	2112	
br25:1					2106														
br25:1																	2112		
br25:2										2070	2072		2068	2068					2067
br25:2		2080								2084		2082	2082	2084	2084			2082	2082
br25:2														2088	2088				
br25:3				2044						2044	2044				2044				
br25:3			2090	2090	2094				2089	2091	2092		2091	2091	2091	2092			2091
br25:3										2104					2104				
br25:3										2106	2107				2106				
br25:3													2119						
br25:4				2078				2055			2082								
br25:4				2124							2129								
br30:0			2524																
br30:5			2509			2509													

[a]: br denoted branched

Table 1:7 Sedimentary occurrances of the structurally related C20, C25 and C30 acyclic hydrocarbons
(figure stated is retention index)

Investigation

Type of compound[a]	Gearing et al., 1976	Farrington et al., 1977	Boehm & Quinn, 1978	Lytle & Lytle, 1979	Wakeham & Carpenter 1976	Crisp et al., 1979	Blanchard 1979	Prahl et al., 1980	Barrick & Hedges 1981	Osterroht et al., 1983	Requego & Quinn 1983a	Albaiges et al., 1984b	Requego et al., 1984
Column phase	FFAP	OV101	OV1	FFAP	FFAP	OV101	OVI	SP1000	SP2100	SP2100	SE30	DB5	SE30
c25:1:1[a]		2072	2025			2072					2079		et
c25:0:1(H)		2093	2125								2124		
c25:1:1							2133				2140		
c25:0:1(H)											2156	2139	
c25:4:1			2170									2127	
c25:0:1(H)			2110										
c25:2:2	2075	2102	2080	2074	2072	2073			2095		2097		
c25:0:2		2125	2145								2124		
c30:2:2											2499		2499
c30:0:2											2529/2550		
c30:2:2									2505				
c30:3:2								2558	2558	2548			
c30:4:2								2590	2590	2580			
c30:0:2								2550	2550				
c30:4:1								2563	2563				

[a]: denoted cyclic: number of double bonds: number of rings

Table 1:8 Sedimentary occurrences of cyclic C_{25} and C_{30} hydrocarbons
(figures stated are retention indices)

Table 1:9 Reported concentrations of selected cyclic or acyclic hydrocarbons in Recent sediments.

Location	Type of Environment	Major Hydrocabon present from cyclic or acyclic series	Surface sediment concentrations	Reference
Buzzards Bay U.S.A.	Recent/estuarine	$c25:2:2;_{FFAP}2075$	0.6^a (50% of total hydrocarbons)	Farrington et al., 1977
Rhode Island Sound, U.S.A.	Recent/estuarine	$c25:2:2;_{OV}2080$	1.55^a	Boehm and Quinn, 1978
Mid Narrangansett Bay, U.S.A.	Recent/estuarine	$c25:2:2$	1.32^a	Wade and Quinn, 1979
Narrangansett Bay, U.S.A.	Recent/estuarine	$c25:2:2$	4.2^b	Hurtt and Quinn, 1979
Southern Californian Bight, U.S.A.	Recent/estuarine	$C_{25}H_{48};_{OV1}2074$ (probably br25:2)	0.14^a	Venkatesan et al.,1980
Puget Sound , Washington State, U.S.A.	Recent/landlocked marine	$br25:3;_{SP2100}209$	$14.0;8.6^b$	Barrick et al., 1980
Narrangansett Bay, U.S.A.	Recent/estuarine	$c25:2:2;_{SE30}2097$	87.2^b	Requego and Quinn, 1983a
Peru continental shelf, Peru (Upwelling region)	Recent/marine	$br25:3;_{SE52}2092$	$10.1(surface)^a$ $0.41(16cm\ depth)$	Volkman et al., 1983
Peru continental shelf, Peru (Upwelling region)	Recent/marine	$br25:4$	40.0^a	Smith et al., 1983
Pettaquamscutt River, Rhode Island, U.S.A.	Recent/estuarine	$c30:2:2$	$1.9(surface)^a$ $0.1(30cm\ depth)$	Requego et al., 1984
Alfacs Bay, Spain	Recent/marine	$br25:1;_{OV1}2112$	15.0 ng/1/ particulate matter	Albaiges et al., 1984b
Washington coastal sediments, U.S.A.	Recent/marine	$br25:3$	27.0^b	Prahl and Carpenter, 1984
Shark Bay, Australia	Recent/marine/ hypersaline	$br25:1;_{OV1}2112$	0.161^a (18.4% of total hydrocarbons)	Dunlop and Jefferies, 1985
Round Swamp, Narrangansett Bay, U.S.A.	Recent/salt marsh	$br25:3;_{SE30}2091$	3.22^a	Requego and Quinn, 1985
Great Barrier Reef, Australia	Recent/marine	$C_{25}H_{48}$	0.0005^a	Coates et al., 1986

[a]: ppm dry sediment or

[b]: ppm organic carbon

(i.e. $C_{25}H_{48}$) followed by molecular ions at m/z 346 ($C_{25}H_{46}$), m/z 344 ($C_{25}H_{44}$) and m/z 350 ($C_{25}H_{50}$). The component with the molecular ion at m/z 344 ($C_{25}H_{44}$) exhibited an intense ion at m/z 247 suggesting a cyclic structure. Mass spectra of minor components suggested branched or cyclic structures. In sediments of the Florida coastline, this group of branched and/or cyclic unsaturated C_{25} isomers accounted for up to 50% of the aliphatic hydrocarbons. Sedimentary abundance of the C_{25} isomers was shown to reduce upon approaching the mouth of the River Mississippi suggesting that the hydrocarbons had a marine source. Another unidentified compound ($RI_{FFAP}1640$) displayed similar distributional characteristics to the C_{25} hydrocarbons. The compound was not adducted by urea or its retention index affected by hydrogenation. Gcms revealed the presence of a molecular ion at m/z 282 and fragment ions from possible branching points at m/z 169, m/z 197 and m/z 211. The hydrocarbon content of several species of benthic algae from the same sample points were examined but none were shown to contain either the C_{25} isomers or the compound RI1640. It was suggested that the sedimentary abundance of the C_{25} isomers in certain sediments (i.e. >50% of the aliphatic hydrocarbon content) made them worthwhile candidates for continued study in order that their exact structures and sources could be determined.

Wakeham and Carpenter (1976) reported the presence of an unusual hydrocarbon ($RI_{FFAP}2072$) which they proposed to be a branched alkane in surface sediments of Lake Washington, U.S.A.

Farrington et al. (1977) identified two C_{25} cycloalkenes ($RI_{OV101}2072$ and 2102) as the major hydrocarbons in North Western

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Atlantic coastal sediments. The mass spectra of the compound RI2072 and its hydrogenation product suggested a c_{25} monocyclic monoene (c25:1:1;2072). The mass spectrum of the second cycloalkene, RI2102, displayed a molecular ion at m/z 344 and intense fragments ions at m/z 231, m/z 247 and m/z 259. The presence of a molecular ion m/z 348 in the mass spectrum of the hydrogenation product indicated either a bicyclic structure or a monocyclic structure with a double bond which could not be readily hydrogenated. The former was favoured and RI2102 was proposed to be a bicyclic diene (i.e. c25:2:2;2102). In addition to the two major cycloalkenes, four minor alkenes were detected in the RI2000-2200 region. The concentration of these alkenes was shown to decrease with increasing depth in the sediment, presumably because of the labile nature of the double bonds. The alkenes were considered to represent diagenetic alteration products of some unknown organic precursor.

Brooks et al. (1977) noted the occurrence of an acyclic alkane whose mass spectrum was similar to that of 7- and 8-methylheptadecanes in sediments of three lacustrine lakes in Northern England. The alkane was tentatively assigned as $C_{18} H_{38}$ but this was subsequently shown to be incorrect (Yon, 1982; see later).

Two C_{25} cycloalkenes were identified as major hydrocarbons in surface sediments from Rhode Island Sound, U.S.A. (Boehm and Quinn, 1978). The two cycloalkenes (RI_{OV1}2080 and 2025) and their hydrogenation products were shown to have identical mass spectra to those hydrocarbons reported by Farrington et al. (1977) (i.e. the bicyclic diene, c25:2:2;$_{OV101}$2102 and the monocyclic monoene c25:1:1;$_{OV101}$2072 respectively). In addition, further spectroscopic analysis of the

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bicyclic alkene (c25:2:2;2080) by ^{1}H NMR and IR suggested a conjugated structure with a vinyl (^H_R>C=CH$_2$) and a trisubstituted double bond. The quantity of c25:2:2;2080 varied between sample locations but correlated strongly with the organic carbon (OC) content of the sediment. Although c25:2:2;2080 and c25:1:1;2025 were observed throughout Narrangansett Bay and Rhode Island Sound sediments, they were not detected in any of the phytoplankton, zooplankton or particulate matter analysed. However, the two C$_{25}$ cycloalkenes were observed in extracts of the ocean quahog Artica islandica in addition to another C$_{25}$ compound undetected in any of the sedimentary analysis. Mass spectral analysis of the latter and the hydrogenation product suggested a monocyclic structure with four double bonds (i.e. c25:4:1;2170). Whether the c25:4:1;2170 was synthesised directly by Artica or, by microorganisms within the bivalve's gut , was unknown but it was suggested that bivalves may have a prominent role in controlling the cycloalkene content of sediments. The concentration of c25:2:2;2080 and the other cycloalkenes decreased rapidly with increasing sediment depth whilst the OC content remained relatively constant indicating a rapid degradation of cycloalkene material (c.f. Farrington et al., 1977).

Keizer et al. (1978) reported the presence of a group of partially resolved components with RI2000-2100 in gas chromatograms of hydrocarbons from sediments of the Scotian Shelf, Canada. Their presence was affected by mild hydrogenation indicating unsaturated structures. The compounds were identified as C$_{25}$ cycloalkenes by comparison with previous studies (i.e. Farrington et al., 1977).

The identification of the bicyclic diene (c25:2:2) in sediments of Narrangansett Bay is reported by Hurtt and Quinn (1978). In contrast to the studies of Boehm and Quinn (1978), no correlation was found between c25:2:2 and the OC content of surface sediments. The ratio of c25:2:2 to OC content of sediments was shown to rise with increasing distance from the mouth of the River Providence and was presumed to be a result of the increased input of marine biogenic material. In agreement with other studies (i.e. Boehm and Quinn, 1978) a decrease in concentration of c25:2:2 was noted with increasing sediment depth.

The presence of two major peaks occurring at $RI_{FFAP}2074$ and 2154 in gas chromatograms of hydrocarbons from Texas coastal sediments was noted by Lytle and Lytle (1979). The compounds were suggested to be mixtures of c_{25} branched and cyclic hydrocarbons by reference to earlier studies (i.e. Farrington et al., 1977) although no particular structures were proposed. (A study of Table 1:8 suggests that the component with $RI_{FFAP}2074$ is may be the bicyclic diene c25:2:2).

The occurrence of c25:2:2 in sediments from the mid-Narrangansett Bay was also noted by Wade and Quinn (1979). The concentration of c25:2:2 (relative to OC content) was shown to decrease rapidly with increasing sediment depth; in contrast, the concentration of n-alkanes remained relatively constant. The decrease in c25:2:2 concentration was assigned to either a lower input in the past or to geochemical alteration (e.g. at double bond positions) with time in the sediments.

Blanchard (1979) noted the occurrence of three C_{25} polyenes ($RI_{OVI}2080$, 2133 and 2173) in kelp bed sediments of Loch Creran (U.K). Interpretation of the mass spectra suggested RI2080 and 2133

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were isomeric acyclic C_{25} dienes. The presence of a strong m/z 165 fragment ion in the mass spectrum of RI2173 indicated a cyclic C_{25} alkene (i.e. c25:1:1;2173). The probability of a marine origin for the C_{25} polyenes was indicated by a decrease in their sedimentary concentration upon approaching the mouth of the major river entering Loch Creran.

The hydrocarbon geochemistry of the Puget Sound region, U.S.A. has been comprehensively studied by Barrick et al. (1980) who reported the occurrence of a group of multibranched C_{20} and C_{25} hydrocarbons. The C_{20} compound (RI_{SP2100}1708) had an identical mass spectrum to the compound (RI_{FFAP}1640) reported previously in the Gulf of Mexico sediments (Gearing et al., 1976). The mass spectrum (Fig. 1:13a) contained a molecular ion at m/z 282 and an intense fragment ion at m/z 168 which, in combination with the low retention index (c.f. phytane ; 1808,., suggested a highly branched, but non-isoprenoid, structure (br20:0).

Also present in the sediment, but as minor components, were two previously unreported monounsaturated C_{20} compounds (RI_{SP2100}1698 and 1702) which were converted to br20:0 upon mild hydrogenation. The two alkenes exhibited similar mass spectra with prominent ions at m/z 210, m/z 196 and m/z 83. Surface concentrations of the three C_{20} hydrocarbons were shown to vary by a factor of 10 with no readily discernible pattern. The average concentrations of br20:0;1708 and br20:1;1698 were shown not to vary significantly with sediment depth suggesting the position of the double bond to be geochemically stable. The three C_{20} hydrocarbons were considered indigenous to the marine environment. Chromatograms of the aliphatic hydrocarbons from Puget
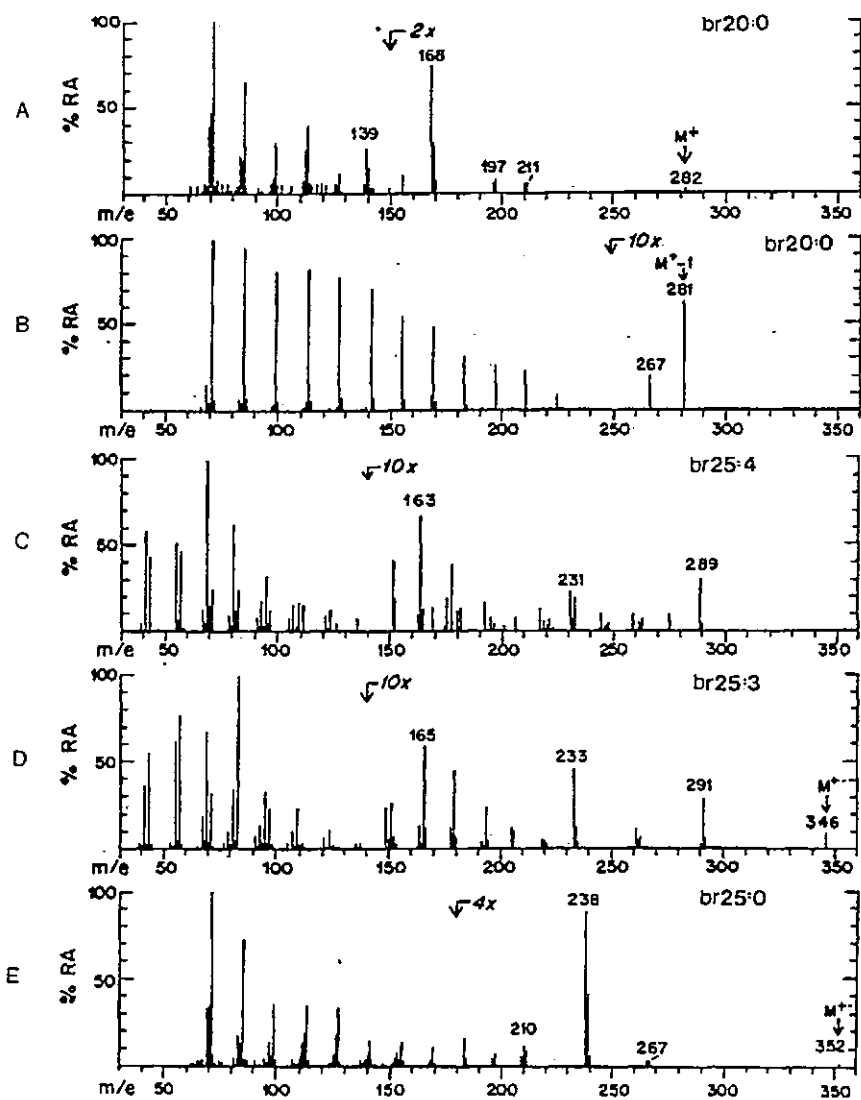
Fig.1:13   Mass spectra of sedimentary hydrocarbons from
            Puget Sound as recorded by Barrick et al. (1980).
            Electron impact (A) and CH_4 chemical ionisation
            (B) mass spectra of compound br20:0;   EI mass
            spectra of (C)br25:4, (D)br 25:3 and (E)br25:0.

Sound also showed four components eluting between RI2000-2150. These were converted by hydrogenation to a saturated acyclic C_{25} hydrocarbon (br25:0;2109) whose mass spectrum (Fig. 1:13e) displayed similar characteristics to the spectrum of br20:0 suggesting a structural relationship between the two alkanes. For example, the mass spectrum of the br25:0 contained ion pairs at m/z 238/239 and m/z 266/267 which are 70a.m.u. (i.e. C_5H_{10} the difference in molecular weight between br20:0 and br25:0) higher than those observed in br20:0. Further evidence of a structural relationship was the low retention times displayed by the two alkanes. The conversion upon hydrogenation of the four alkenes to the same br25:0 alkane dictated that they possessed the same carbon skeleton which differed only in the placement and geometry of the double bonds. Gcms indicated the presence of two C_{25} trienes (br25:3;2044 and br25:3;2090) and two C_{25} tetraenes (br25:4;2078 and br25:4;2124). Both the individual pairs of br25:3 and br25:4 hydrocarbons exhibited identical mass spectra (Fig. 1:13c and d). In addition, the difference in retention indices between the two trienes was identical to that between the tetraenes which was interpreted to indicate that the four molecules were structurally identical except for geometrical isomerisation (cis or trans) about one double bond and the presence of an additional double bond at a remote site in the br25:4 hydrocarbons. Sedimentary concentrations of the trienes were shown to exeed those of the tetraenes by a factor of 2-5. Surface sediment concentrations of br25 hydrocarbons were reported as correlating well with those of the br20 hydrocarbons suggesting a common origin. Unlike the br20 hydrocarbons, the br25 alkenes decreased exponentially with sediment depth from 70 μg/g OC in current day sediments to almost 0 μg/g OC in pre-1825 a.d. sediments. The decrease was assigned to in situ chemical degradation of the br25 hydrocarbons.

Prahl et al. (1980) reported significant concentrations of five unsaturated alkenes (RI$_{SP2100}$2090, 2509, 2558, 2563 and 2590) relative to other hydrocarbons in surface sediments of Dabob Bay, Washington, U.S.A. The mass spectrum of RI2090 and its hydrogenation product were reported to be identical to those of br25:3;2090 observed in Puget Sound (Barrick et al., 1980). Hydrogenation of the compounds with RI2509 and 2558 produced new chromatographic peaks at RI2524 and 2550 respectively. Mass spectra are illustrated in Fig. 1:14. The interpretation of spectra suggested that RI2509 was a monocyclic tetraene (c30:4:1;2509) and RI2558 a bicyclic triene (c30:3:2;2558). No attempts were made to postulate the structures of RI2563 and 2590. The alkenes (br25:3;2090), (c30:4:1;2509) and (c30:3:2;2558) were detected in two sediment trap samples (September-October, October-November)and the latter two alkenes were also detected at high concentration (i.e. 5% of the total aliphatic hydrocarbons) in a sample of mixed phytoplankton collected in November. The occurrence of c30:4:1;2509 and c30:3:2;2558 in only the November phytoplankton generally followed the temporal distribution of the two alkenes in trapped particulates and suggested a phytoplanktonic source. The authors were unable to assign a source to the remaining alkenes, br25:3;2090, RI2563 and 2590.

Venkatesan et al. (1980) reported the presence of a series of acyclic and cyclic C_{25} alkenes which eluted between n-C_{20} and n-C_{21} in gas chromatograms of the aliphatic hydrocarbons extracted from sediment cores taken from two basins in the Gulf of California. The concentrations of the three most prominent alkenes, $C_{25}H_{48}$;OV101 2074, $C_{25}H_{46}$;2094 and $C_{25}H_{50}$;2106 were shown to decrease with increasing depth in the sediment which was attributed to microbial

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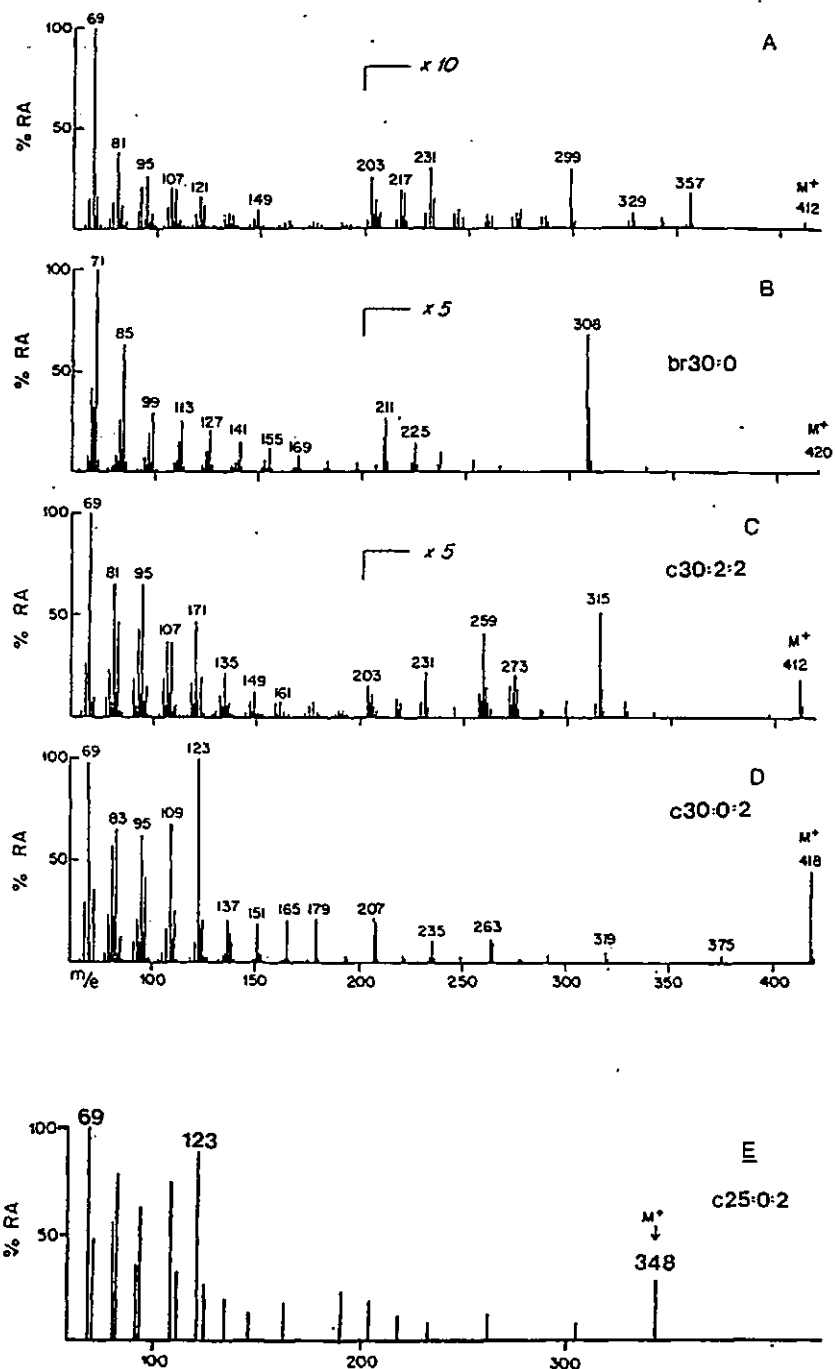
Fig.1:14   (A - D)   Mass spectra of sedimentary hydrocarbons
            from Dabob Bay as recorded by Prahl et al. (1980).
            Electron impact mass spectra of RI2509: (A) before
            hydrogenation and (B) after hydrogenation; and RI2558:
            (C) before hydrogenation and (D) after hydrogenation.
            E. A simplified mass spectrum showing the major ions
            recorded by Farrington et al. (1977) for a sedimentary
            hydrocarbon (RI2125) from Rhode Island Sound.

            Note the relative intensity of the m/e 123 fragment
            ion in spectra (D) and (E).

oxidation or polymerisation of the C_{25} alkenes. The compound $C_{25}H_{48};2074$ was noted as having an identical mass spectrum to the $c25:1:1;_{OV101}2072$ reported by Farrington et al. (1977).

In a further publication on the hydrocarbon geochemistry of the Puget Sound Region, U.S.A., Barrick and Hedges (1981) noted the presence of a bicyclic C_{25} diene ($c25:2:2;_{SP2100}2095$) and a group of cyclic and acyclic C_{30} alkenes. The $c25:2:2;2095$ possessed an identical mass spectrum to that of the $c252:2;_{OV1}2080$ published by Boehm and Quinn (1978). The mass spectrum of the hydrogenation product of $c25:2:2;2095$ (i.e. $c25:0:2$) was proposed as displaying a similar EI fragmentation pattern to that of the hydrogenation product ($c30:0:2;2550$) of two C_{30} bicyclic alkenes $c30:3:2;2558$ and $c30:4:2;2590$ also present in the sediment which suggested a structural relationship between the C_{25} and C_{30} alkenes. Two other C_{30} alkenes were present, designated $c30:4:1; 2563$ and HC412. The mass spectrum of the hydrogenated HC412 was reported to be analogous to those of the highly branched $br20:0$ and $br25:0$ alkanes noted in an earlier study (Barrick et al., 1980). The similarity observed in the electron impact (EI) fragmentation prompted the authors to suggest HC412 was a C_{30} structural homologue of $br20:0$ and $br25:0$. The only flaw in this argument was the apparent molecular ion m/z 420 in the hydrogenation product of HC412. This was interpreted to indicate the presence of a double bond in HC412 which was stable to hydrogen-ation suggesting HC412 was an acyclic C_{30} pentaene ($br30:5;2509$). The compound $br30:5$ was identical to that reported as a C_{30} monocyclic tetraene ($c30:4:1;2509$) by Prahl et al. (1980) in sediments of Dabob Bay. Further evidence of a structural relationship between the acyclic (i.e. $br20$; $br25$ and $br30$) compounds was provided by similarities in the gc

retention behaviour of the individual compounds. Concentrations of the C_{25} and C_{30} alkenes generally decreased with increasing sediment depth. Surface concentrations were shown to vary from 1 to 60 $\mu g/g$ OC with no apparent geographical trend within the bay. The mass spectrum of the br25:0 alkane was shown to be similar to that of hydrogenated moenocene (65), an alkane derived from hydrolysis of an antibiotic from a soil Actinomycetes. Although the compounds were not identical (e.g. different retention indices), the structural similarities were considered sufficiently striking to make the suggestion of a microbial source for the multibranched alkenes.

McEvoy et al. (1981) noted the occurrence of an unusual C_{25} alkane (i.e. br25:0) which eluted just after n-heneicosane (n-C_{21}) in hydrocarbon extracts of DSDP sediment cores from the California Continental borderland.

Anderlini et al. (1981) reported dominant peaks between RI2031-2188 in gas chromatograms of hydrocarbons extracted from an oyster, Pinctada margaratifera, collected along the coast of Kuwait. The peaks were attributed to various isomers of biogenic acyclic and cyclic C_{25} alkenes ranging in molecular weight from m/z 342 to 348.

The presence of an unidentified triplet eluting between n-C_{20} and n-C_{21} in hydrocarbon extracts of benthic invertebrates (i.e. Serolis cornuta) from Signy Island, Antarctica, was reported by Clarke and Law (1981). The triplet was not present in hydrocarbons from benthic invertebrates collected at South Georgia. Possible reasons for this difference were not discussed.

Venkatesan and Kaplan (1982) noted the occurrence of a $C_{25}H_{46}$ cyclic olefin (no RI given) and br25:4;$_{SP2100}$2055 in Alaskan Outer Continental Shelf sediments. The sedimentary abundance of the alkenes indicated an input from phytoplankton or zooplankton. Also present in the sediment were two $C_{30}H_{50}$ olefins (RI_{SP2100}2672 and 3027) suggested, from their mass spectra, to be bicyclic tetraenes with two fused rings at one end of the molecule.

Yon et al. (1982) reported the isolation and identification of a C_{20} highly branched alkane in Rozel point crude oil (age unknown; Utah, U.S.A) in which it was the second most abundant alkane (phytane was most abundant). The alkane, isolated by a combination of column chromatography, distillation and preparative gc, was subjected to spectroscopic analysis (i.e. ^1H NMR, ^{13}C NMR). Interpretation of the data implicated the structure (66) i.e. 2,6,10-trimethyl-7-(3-methylbutyl)dodecane. Confirmation of this assignment was provided by synthesis of the reference alkane which was shown to be spectroscopically indistinguishable from the geological alkane and to chromatograph with it on three different gc phases. The compound reported previously as a $C_{18}H_{38}$ alkane in surface sediments of Grasmere (U.K.; Brookes et al., 1977) was subsequently reidentified as 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (66). Indeed, 2,6,10-trimethyl-7-(3-methylbutyl)dodecane was reported as having an identical mass spectrum and retention index to that of the br:20:0 (Fig. 1:13a) observed by Barrick et al. (1980) and Gearing et al. (1976). The limited occurrence of 2,6,10-trimethyl-7-(3-methybutyl)dodecane (or br:20:0) in Ancient geological samples (e.g. Rozel Point crude oil) suggested to Yon et al. (1982) that it may have potential as a useful input biological marker.

Yon (1982) reported the co-occurrence of br20:0 with a br20:1 (no RI) and a previously unreported C_{25} monoene (no RI) is surface sediments from Grasmere (U.K.). The mass spectra of the two monoenes displayed strong similarities suggesting the C_{25} monoene to be a pseudohomologue of br20:1 (i.e. a br25:1). Hydrogenation of the C_{25} monoene produced an alkane with an identical mass spectrum to br25:0 thereby confirming the C_{25} monoene as a br25:1. The br20:0 alkane was also identified in Sabon Gida sediment (a lacustrine deposit; 90000 years old) and the Cariaco trench where it co-occurred with the br20:1 observed in Grasmere (U.K.). On the basis of mass spectral interpretation, Yon (1982) postulated that the structure of the br20:1 observed in Grasmere was either (67) or (68). Noting the similarity between the mass spectra of the now assigned br20:0 and the unknown br25:0, Yon (1982) also postulated on the structure of the br25:0 compound. Three structures were suggested (Fig. 1:15) with C giving the best fit to the observed mass spectrum.

A study of seasonal variations in hydrocarbons extracted from sedimentary particulates from Kiel Bight (F.R.G.) revealed the identification of br25:3;$_{SE5}$2089, c30:3:2;2548 and c30:4:2;2580 in particulates collected during September (Osterroht et al., 1983). The compounds were identified by comparison with the data of Prahl et al. (1980) and Barrick and Hedges (1981). However, in the paper by Osterroht et al. (1983) RI2563 should in fact read RI2590. The detection of the C_{25} and C_{30} alkenes in only the autumn sedimentary particulates agreed well with the results of Prahl et al. (1980). It was concluded that these compounds derived from plankton species appearing late in the yearly succession.
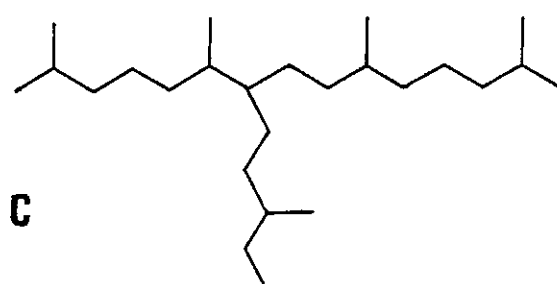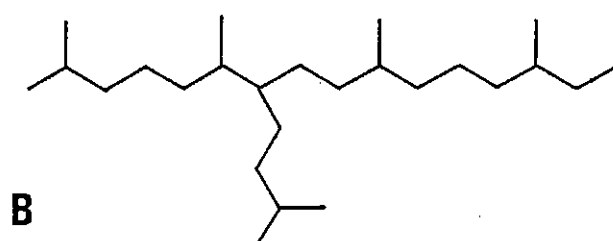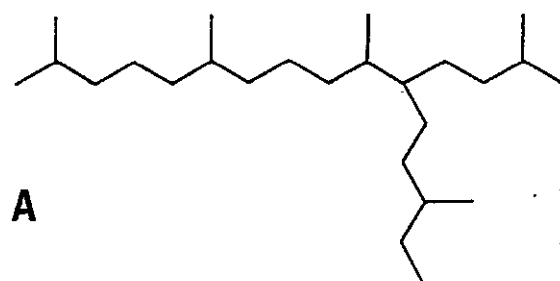
Fig.1:15   Proposed structures for br25:0 (from Yon, 1982).

Bayona et al. (1983) noted the presence of br20:0, two br25:3's and a C_{25} isocycloalkane in sedimentary particulate extracts from Alfaques Bay (Spain). The advantages of using two different chromatographic phases (i.e. SE52 and PEG20M) to aid identification of complex chromatographic mixtures was demonstrated.

The geochemistry of C_{25} and C_{30} biogenic alkenes of Narrangansett Bay estuary were discussed at length by Requego and Quinn (1983a). In addition to $c25:2:2_{SE30}2097$ and $c25:1:1;2079$ observed previously in Narrangansett Bay (Farrington et al. 1977) a further nine C_{25} and C_{30} alkenes were detected. Of these, five compounds (four C_{25} and one C_{30}) comprised 73-91% of the total alkenes in all the surface sediments analysed. Fig. 1:16 shows the mass spectra of the four C_{25} alkenes and Fig 1:17 the mass spectra of their hydrogenation products. The mass spectrum of the hydrogenation product of br25:2;2084 was almost identical to that of br25:0 (Barrick et al. 1980) indicating the hydrocarbon skeleton of br25:2;2084 to be identical to that of the br25:3 and br25:4 alkenes reported by Barrick et al. (1980). The compounds RI2079, which had an almost identical mass spectrum to br25:2;2084, was proposed to be a monocyclic monoene (c25:1:1;2079) because of the molecular ion m/z 350 observed in the spectrum of the hydrogenation product (c25:0:1;2104). On similar reasoning, the compound RI2140 was also proposed as a monocyclic monoene (c25:1:1a;2140). However, it was stressed that alternatively, both c25:1:1;2079 and c25:1:1a;2140 could contain a double bond resistant to hydrogenation, possess branch acyclic skeletons and be positional or geometrical isomers of br25:2;2084. The mass spectrum of c25:2:2;2097 was identical to that observed in previous studies (i.e. Boehm and Quinn, 1978; Barrick and Hedges, 1981). The mass spectrum of the major

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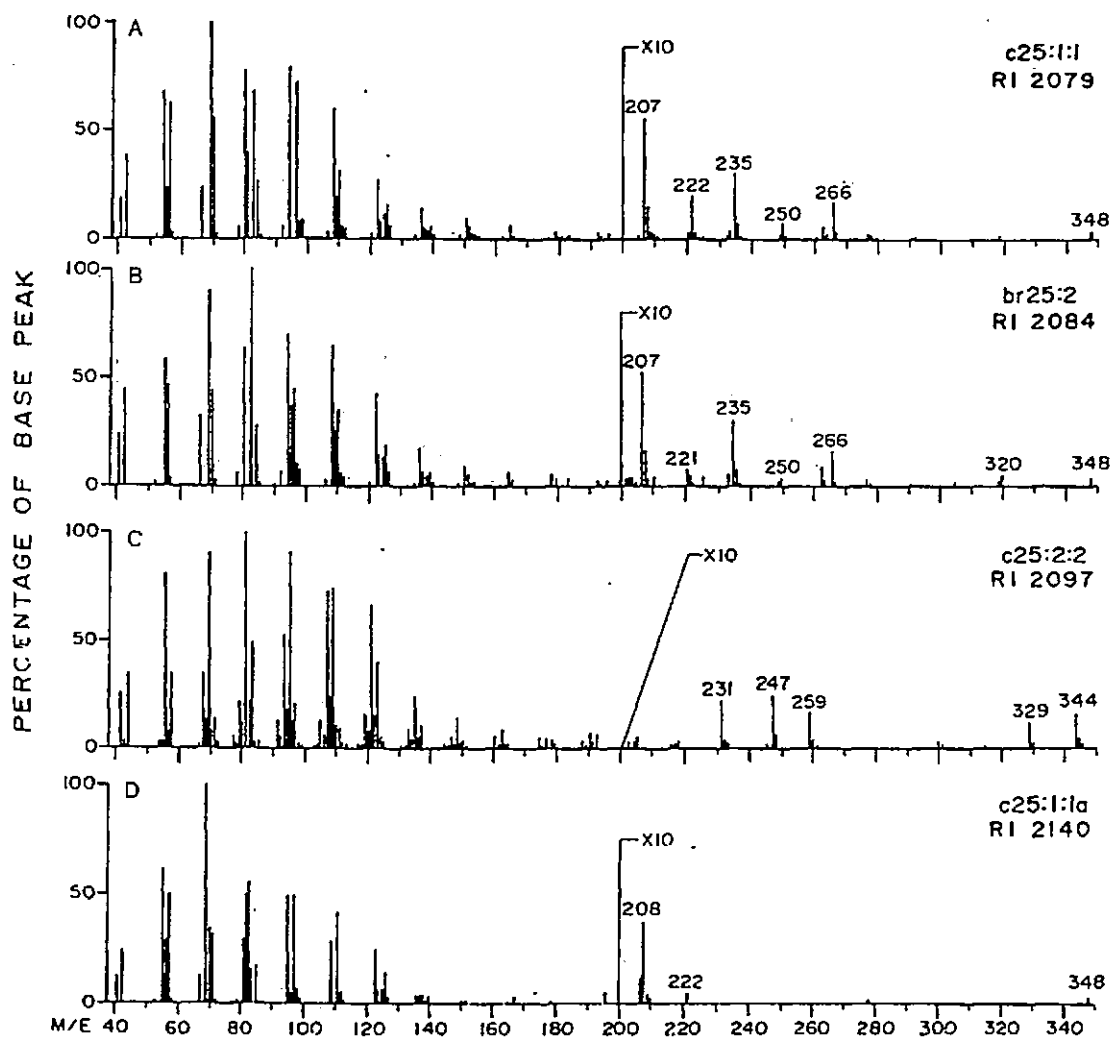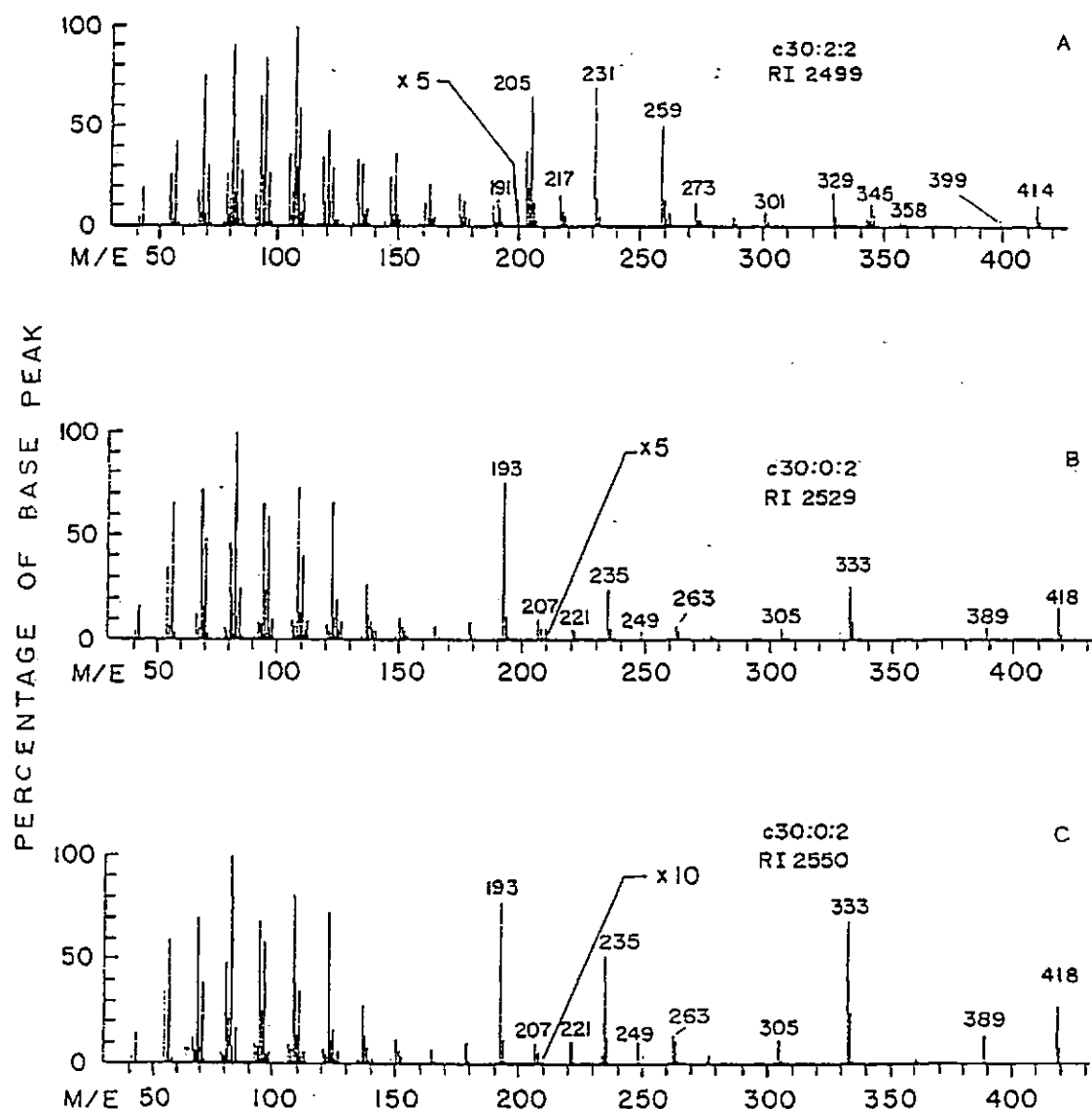
Fig. 1:16   Mass spectra as recorded by Requego and Quinn (1983a) of the major C_{25} alkenes detected in Narrangansett Bay sediments. Identifications as reported.
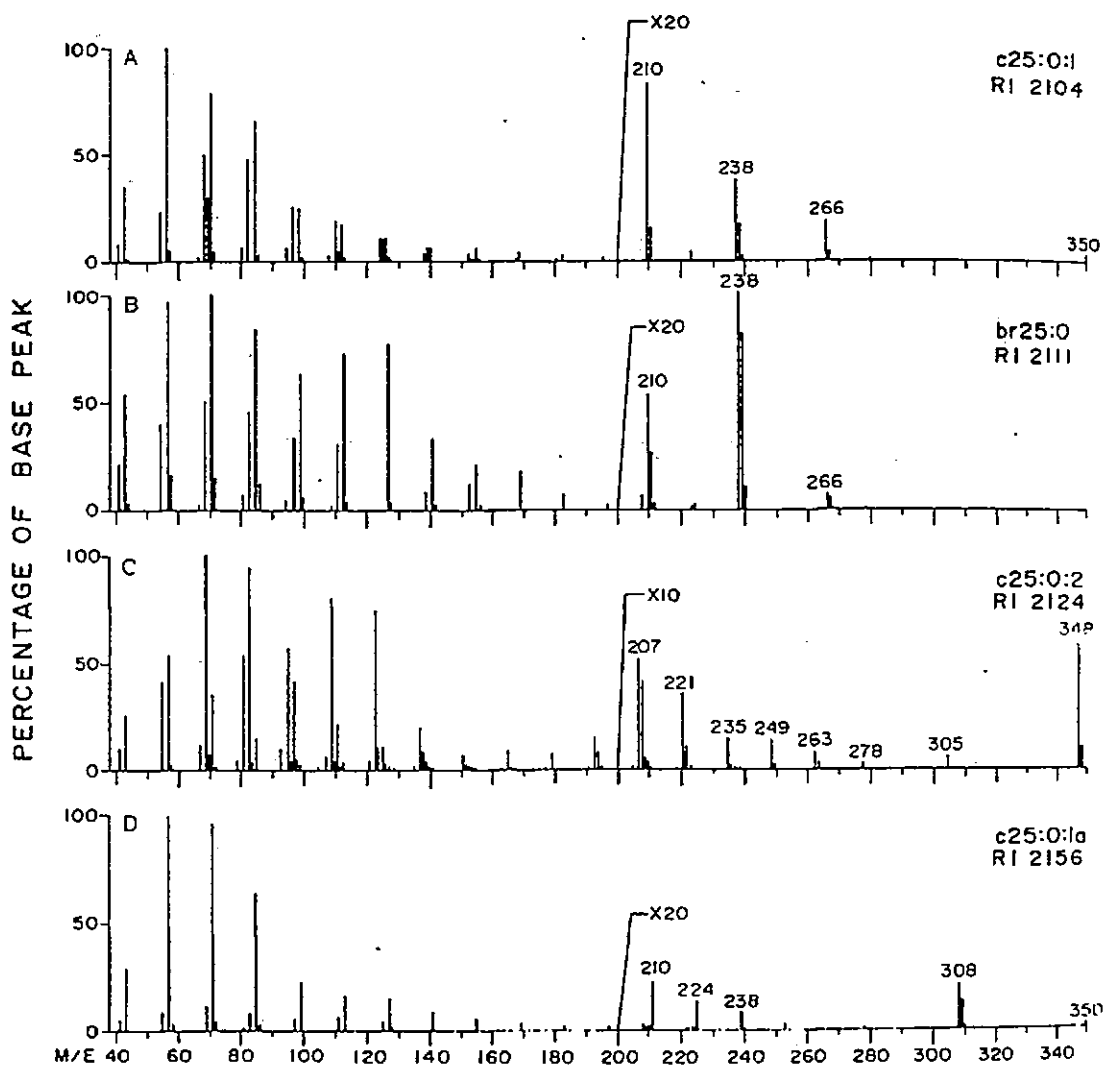
Fig. 1:17   Mass spectra as recorded by Requego and Quinn (1983a) of the respective hydrogenation products of the major C_{25} alkenes detected in Narrangansett Bay sediments. Identifications as reported.

C_{30} alkene and its hydrogenation product (Fig. 1:18) suggested a bicyclic diene (c30:2:2;2499) possessing structural similarities to c25:2:2;2097. However, structural differences were apparent as the c30:2:2;2499 yielded two diastereomers upon hydrogenation. The abundant m/z 193 ion in the spectra of both the diastereomers indicated a bicyclic methyl substituted ring system. c30:2:2;2499 was proposed as a structural analogue of the more highly unsaturated c30:3:2 and c30:4:2 reported by Barrick and Hedges (1981). The remaining alkenes identified in surface sediments of Narrangansett Bay were trace constituents and included four br25:3 compounds (RI2044,2091 and, previously unreported, 2104 and 2106), a positional or geometrical isomer of br25:2;2084 (i.e. br25:2;2070) and two unidentified C_{30} alkenes of molecular weight 414a.m.u. Significant geographic variations in the relative abundance of the five major compounds throughout the Bay were reported. The relative abundance of c25:2:2;2097 decreased near to the mouth of the Providence River whereas relative abundances of c30:2:2;2499, c25:1:1;2079 and c25:1:1a;2140 and br25:2;2084 increased. In addition, a comparison of alkene concentrations normalised to $\delta^{13}C$ values suggested that c25:1:1;2079 and c25:2:2;2097 were associated with input of marine organic matter. The concentration of c30:2:2;2499 was reported as independent of origin of organic matter as inferred from $\delta^{13}C$ values indicating the compound was of _in situ_ bacterial origin. The difference in source for c25:2:2;2097 and c30:2:2;2499 was thought unusual considering the proposed structural relationship between these two compounds. In addition, the occurrence of the presumed structural homologues of c30:2:2, c30:4:2 and c30:3:2, in mixed zooplankton samples (Prahl _et al_., 1980) supported the proposed _in situ_ bacterial

Fig.1:18   Mass spectrum as recorded by Requego and Quinn (1983a) of A. the major C_{30} alkene (c30:2:2) detected in Narrangansett Bay sediments.   B. and C. represent the mass spectra of the diastereoisomeric hydrogenation products of c30:0:2.

origin for c30:2:2. The explanation presented was that similar bacteria in both sediment and zooplankton guts were producing the alkenes through de novo synthesis or precursor modifying reactions. No conclusions were proposed regarding the origin of c25:1:1a;2140 or br25:2;2084. Depth profiles of the major alkenes demonstrated a slow decrease to constant levels within the upper 25cm of sediment; the decrease being attributed to in situ degradation and not a recently increased input. An exception was c25:2:2;2097 which showed a subsurface concentration increase at two locations in the estuary for which no explanation was advanced. The difference in alkane distributions between Narrangansett Bay and Puget Sound (Barrick et al., 1981) was proposed to reflect interregional differences in the agents determining the origin and fate of biogenic alkenes.

The occurrence of C_{25} trienes and tetraenes in coastal marine sediments from the Peru upwelling region has been reported by Smith et al. (1983) and Volkman et al.(1983). Both the trienes (br25:3;$_{SE52}$ 2044 and br25:3;2092) and the tetraenes (br25:4;2082 and br25:4;2129) were the same as those reported by Barrick et al.(1980) who suggested that they were structurally identical except for the occurrence of cis-trans isomers about one double bond. Volkman et al. (1983) reported that the difference in the gc retention indices of the two trienes was much greater than that found for cis-trans isomers of other unsaturated compounds such as 9-methyloctadecenoate. The distribution of the br25:3 and br25:4 compounds was similar to that observed in surface sediments of Puget Sound (Barrick et al., 1980) indicating a common biosynthesis, or transformations of the same precursor(s) and hence, the probability of a common origin from the same or related organisms. The detection of br25:3 and br25:4 in sediment traps located off Peru

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containing abundant zooplankton fecal material suggested that they may originate from an autochthonous source in the water column such as zooplankton or phytoplankton (Volkman et al., 1983). The absence of br25:3 and br25:4 in samples of mixed zooplankton was taken as evidence that the alkenes in the sediment trap material reflected an origin from zooplankton diet i.e. phytoplankton. The possibility of a phytoplankton source, especially from diatoms which predominated in the area, was supported by the observation that most of the other sedimentary lipids present in Peru sediments were directly related to a phytoplankton origin. The distribution and predominance (see Fig. 1:19) of the C_{25} trienes and tetraenes was similar in each surface sediment analysed but analysis of sediment cores indicated that the combined alkene concentration decreased by an order of magnitude between surface sediments and those at 4-5cm depth. This contrasted with the concentration of n-alkanes which actually increased over the same depth range. Reasons proposed for the observed rapid decrease in subsurface alkene concentrations were microbial degradation, considered important in view of the high bacterial biomass present, and the possibility of the alkenes being rapidly and irreversibly bound to accreting polymeric material via crosslinking involving the double bonds.

The C_{25} acyclic trienes (br25:3;2044 and br25:3;2091) and tetraenes (br25:4;2078 and br25:4;2124) have also been observed in coastal sediments off Washington (Prahl and Carpenter, 1984). The trienes were most abundant and occurred in a constant ratio to each other in all sediment samples analysed. Alkene concentrations were observed to be highest in shelf sediments and to decrease with increasing distance offshore. The absence of the C_{25} hydrocarbon series in suspended particulate matter or sediments of the Columbia River and, the apparent

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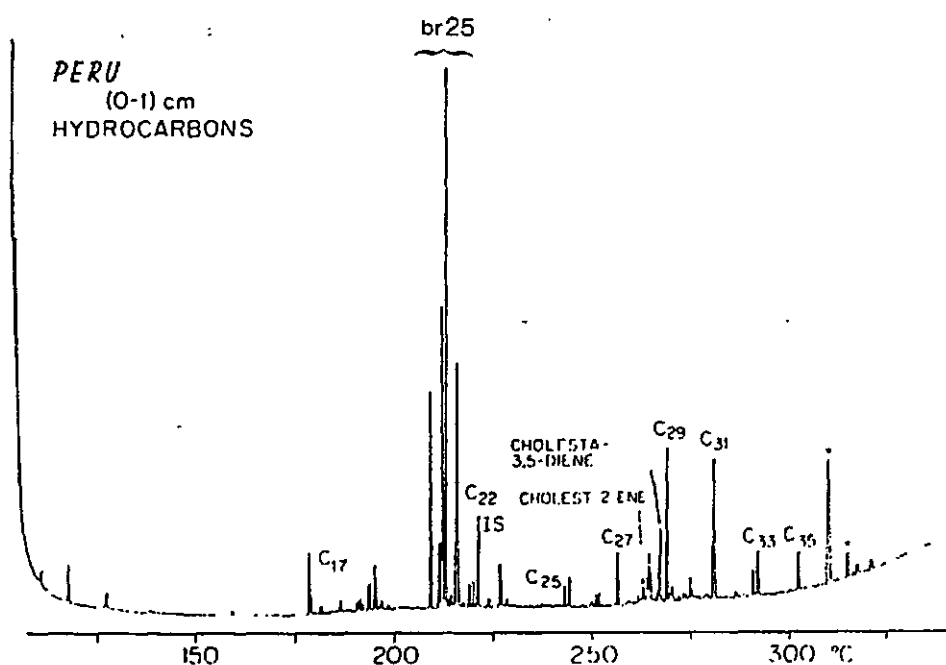
Fig.1:19   Gas chromatogram as recorded by Volkman et al.(1983)
of the aliphatic hydrocarbons isolated from the Peru
Upwelling region.   Note the abundance of the br25
hydrocarbons compared to normal alkanes (C_{17} etc) and
sterenes.   Peaks marked with a * represent long chain
methyl ketones (C_{37} and C_{38}).

confinement of the hydrocarbons to marine sedimentary environments, indicated a marine source. A rapid decrease in alkene concentration with sediment depth was observed, as documented in many previous studies (e.g. Barrick et al., 1980). Interestingly, the reasonably constant ratio between the C_{25} trienes observed in all surface sediment was maintained with depth in each sediment core analysed. This suggested that the remineralisation processes responsible for alkene degradation act non-selectively in the removal of individual compounds of the series.

Bates et al. (1984) reported the presence of multibranched acyclic C_{25} hydrocarbons in both suspended matter and sediments of the central main basin of Puget Sound, U.S.A. The presence of these compounds in upper sediment traps indicated a source other than in situ formation in deep bottom sediment. In contrast, a multibranched C_{20} compound eluting between $n-C_{17}$ and pristane was present only in lower sediment traps and bottom sediment suggesting in situ formation in bottom sediment.

Albaiges et al. (1984a) reported on the extractable and bound lipids of two lacustrine sediments; a eutrophic coastal lagoon (Ebro Delta) and a shallow hypersaline pond (Los Monegros) whose sediments consisted of cyanobacterial algal mats underlain by a layer of black sapropel. Hydrocarbon extracts of the lagoonal sediment showed the presence of a minor C_{25} diene (br25:2; $_{SE30}2084$) and a bicyclic C_{30} diene (c30:2:2;2500) which were suggested to be of bacterial origin. The occurrence in the lagoonal sediment of sclarene (69) and another bicyclic $C_{20}H_{32}$ diterpenoid (with base peak at m/z 137) suggested to have a perhydronapthalene structure was also noted. A dominant hydrocarbon in both the free and bound lipid extracts of the black

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sapropel (hypersaline pond) was shown to be a C_{25} triene (br25:3;2107) previously unreported in Recent sediments. Also present in the sapropel extracts were five isomeric phytenes and seven C_{30} iso-alkenes which condensed to squalane upon hydrogenation. The low level of diagenesis in the mud suggested the phytenes to be from a direct biological input rather than being diagenetic degradation products of phytol or Archaebacterial phospholipids. The presence of phytenes and hydrosqualenes in the neutral lipids of methanogenic bacteria (Langworthy et al., 1982) suggested a bacterial origin for these hydrocarbons in the sapropel sediment; the presence of methanogenic bacteria in the sediment being consistent with the observed production of methane. The observed association of br25:3;2107 with both the phytenes and hydrosqualenes in the bound fraction of the sapropel implied a common bacterial origin.

Albaiges et al. (1984b) reported the occurrence of C_{20} and C_{25} acyclic and cyclic hydrocarbons in extracts of particulate matter and surface sediments from the Alfacs and Fangar Bays of the Ebro Delta (Spain). The C_{20} alkane, br20:0;$_{SE30}$1705 (66) was reported to occur in significant concentrations in water particulates during summer only, thereby suggesting a marine planktonic origin. The range of acyclic and cyclic C_{25} hydrocarbons identified in particulates and sediment is shown in Table 1:10. Upon hydrogenation, the acyclic C_{25} alkenes condensed to br25:0;2105 and the monocyclic alkene (c25:1:1;2139) converted to an already existing monocyclic alkane (c25:0:1;2127). It was believed that this was the first report of c25:0:1;2127 in the marine environment where it was considered to represent a diagenetic or metabolic relation of c25:1:1;2139 which was observed in significant concentrations in spring particulates. In

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Table 1:10. Cyclic and acyclic C_{20} and C_{25} hydrocarbons identified in particulate matter from the Ebro delta. (Albaiges et al., 1984b)

Compound	Formula	Molecular weight	Retention Index DB5
br20:0	$C_{20}H_{42}$	282	1705
br25:3	$C_{25}H_{46}$	346	2091
br25:3	$C_{25}H_{46}$	346	2107
br25:3	$C_{25}H_{46}$	346	2119
br25:2	$C_{25}H_{46}$	348	2082
br25:2	$C_{25}H_{48}$	348	2068
c25:1:1	$C_{25}H_{48}$	348	2139
c25:0:1	$C_{25}H_{48}$	350	2127
br25:0	$C_{25}H_{50}$	352	2105
	$C_{25}H_{52}$		

Table 1:11. Cyclic and acyclic C_{25} and C_{30} hydrocarbons identified in the upper anoxic basin of the Pettaquamscutt River. (Requego et al., 1984)

			SE30
br25:3	$C_{25}H_{46}$	346	2091
br25:2	$C_{25}H_{46}$	348	2070
br25:2	$C_{25}H_{46}$	348	2084
br25:2	$C_{25}H_{46}$	348	2088
c30:2:2	$C_{25}H_{48}$	414	2499
	$C_{30}H_{54}$		

Table 1:12. Cyclic and acyclic C_{25} and C_{30} hydrocarbons identified in a New England Salt Marsh (Requego and Quinn, 1985).

			SE30
br25:3	$C_{25}H_{46}$	346	2044
br25:3	$C_{25}H_{46}$	346	2091
br25:3	$C_{25}H_{46}$	346	2104
br25:3	$C_{25}H_{46}$	346	2106
br25:2	$C_{25}H_{46}$	348	2084
br25:2	$C_{25}H_{48}$	348	2088
c30:2:2	$C_{25}H_{48}$	414	2499
	$C_{30}H_{48}$		

summer, the predominant components of the particulate hydrocarbons were two previously unreported acyclic alkenes br25:2;2068 and br25:3;2119. The remaining acyclic C_{25} alkenes existed as relatively minor components. Origins of the C_{25} hydrocarbons were uncertain. Surface sediments of Alfacs Bay contained a much simpler assemblage of C_{25} hydrocarbons than the particulates with br25:3;2107, br25:2;2082 and br25:0;2105 dominant. The occurrence of br25:0;2105 in sediments only was considered suggestive of a diagenetic origin from unsaturated analogues in the water column.

Biogenic C_{25} and C_{30} alkenes have also been identified in a sediment core from the upper anoxic basin of the Pettaquamscutt River (Rhode Island, U.S.A.; Requego et al.,1984). Several C_{25} and C_{30} hydrocarbons were identified (see Table 1:11) including a previously unreported acyclic C_{25} diene (br25:2;2088) whose mass spectrum was identical to that of br25:2;2084 suggesting them to be positional or geometric isomers. The concentrations of the C_{25} and C_{30} alkenes (normalised to OC content) in the sediment from the anoxic basin were compared to those in Bullock Creek, Upper Narrangansett Bay. The concentrations of br25:2;2084 and c30:2:2;2499 showed similar ranges between the two sites despite a 3% difference in the mean organic carbon $\delta^{13}C$ which indicated that the sources of organic matter from each location were different. Thus, it was considered that the occurrence of br25:2;2084 and c30:2:2;2499 was independant of source material input and instead reflected an origin from bacterial in situ production. The concentrations of all the five alkenes (see Table 1:9) detected in the upper anoxic basin exhibited subsurface maxima in direct contrast to the rapid decreases in alkene concentrations with sediment depth observed at other locations (i.e. Bullock Creek; Peru

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Upwelling region, Volkman et al., 1983). The increases in subsurface concentration were ascribed either to enhanced preservation of a past increase in alkene production by the extreme anoxic conditions or to a current bacterial in situ production at depth. Two cyclic C_{25} alkenes (c25:2:2 and c25:1:1) reported in sediments of Narrangansett Bay (Requego and Quinn, 1983a) where they demonstrated a correlation with the relative abundance of marine organic matter, were absent in the upper anoxic basin of the Pettaquamscutt River which was consistent with measured ^{13}C values for the organic matter in the upper basin which indicated the contribution from marine organic matter to be negligible.

Requego and Quinn (1985) examined the occurrence of C_{25} and C_{30} biogenic alkenes in sediments and detritus of Round Swamp (Narrangansett Bay, U.S.A.). In total, seven alkenes were detected (Table 1:12) including a previously unreported C_{25}diene (br25:2;$_{SE30}$2104). The general distribution of the alkenes in the anoxic sediments of the salt marsh were similar to those reported for other anoxic sediments (Requego et al., 1984) despite significant differences in the origin of the sedimentary organic matter at each location. This suggested the alkenes were sythesised in situ in association with anaerobic bacterial activity. It was reported that "similar" (though since the structures of these hydrocarbons were unknown it is difficult to justify the use of the expression) C_{20}, C_{25} and C_{30} hydrocarbons had been detected in several species of methanogenic bacteria (i.e. Holzer et al., 1979) highlighting the ability of anaerobic bacteria to synthesize unsaturated compounds. Size fractionation of the sediment and detritus indicated that significant quantities of the alkenes were associated with large

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fragments of decaying Spartina alternflora which implied that alkene synthesis may occur during microbial colonisation of the Spartina tissues. Attempts to induce alkene synthesis by anaerobically decomposing Spartina in the laboratory were shown to be unsuccessful (see also Requego and Quinn, 1983b). The absence of c25:2:2 and c25:1:1 in the swamp sediment was considered further evidence of a predominantly marine planktonic origin for these particular compounds and implied the existence of different origins for supposedly structurally related hydrocarbons (i.e. c25:2:2 and c30:2:2).

The first and so far, the only biological occurrence of these acyclic C_{20} and C_{25} hydrocarbons was reported by Rowland et al. (1985) who observed the presence of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane ($br20:0;_{OV1}1705$), a related C_{20} monoene (br20:1;1700) and a pseudohomologous C_{25} diene (br25:2;2082) in field-collected specimens of the green alga Entereomorpha prolifera. The mass spectrum of the br20:1;1700 was similar to that shown by Cranwell (1982) for a C_{20} monoene from Upton Broad (U.K.) and possessed similar fragment ions to the two br20:1 reported by Barrick et al. (1980). The mass spectrum of the C_{25} diene (br25:2;2082) was similar to that of the br25:2;2084 hydrocarbon reported in several studies (see Table 1:7). Similarities in the mass spectra of br20:0;1705 and the hydrogenation product of br25:2;2082 (i.e. br25:0;2112) led the authors to propose tentatively structure (70) for the br25:0 alkane. The occurrence of br20:0;1705, br20:1;1700 and br25:2;2082 in Entereomorpha, an alga that grows widely in both marine and aquatic environments suggested that it may be a potential source of the acyclic highly branched hydrocarbons in some sediments.

Dunlop and Jefferies (1985) observed differences in the distribution of sedimentary acyclic C_{20} and C_{25} hydrocarbons between oceanic and hypersaline waters of Shark Bay (W. Australia). The chromatogram of the hydrocarbon extract of the oceanic sediments was shown to be dominated by a complex hydrocarbon assemblage containing n-alkanes and a suite of branched acyclic compounds, whereas in contrast, the hydrocarbon extract of hypersaline sediments was dominated by a C_{25} monoene (br25:1;$_{0V1}$2112), a C_{20} monoene (br20:1;1703) and $C_{21}H_{44}$ (RI1803) and $C_{22}H_{46}$ (RI1902) alkanes. Interpretation of the mass spectra of the alkanes (Fig. 1:20) suggested they were structural homologues of br20:0, i.e. (71) and (72). Hydrogenation of br20:1;1703 and br25:1;2112 produced br20:0 and br25:0 respectively, which confirmed their identification as members of the 'br' series. Results from ozonolysis experiments on the two monoenes suggested the position of the double bond in the two structures to be (73) for the C_{20} monoene and (74) for the C_{25} monoene. The concentration of br25:1;2112 remained constant with sediment depth whereas the concentration of the alkanes ($C_{21}H_{44}$ and $C_{22}H_{46}$) increased. This was considered indicative of separate sources for alkenes and alkanes. The concentration of br25:1;2112 decreased significantly on passing from hypersaline to oceanic sediments (see Fig. 1:21) possibly reflecting changes in the biota present in the two different environments. However, the two distinct regions were reported as not aligning with the different macrobiotic seagrass communities present in Shark Bay. Indeed, a detailed study of the hydrocarbon chemistry of the seagrass sediments indicated that the oceanic and hypersaline alkenes derived from another source. Interestingly, it was noted that the hydrogenation product of the C_{25} alkenes present in the oceanic chemical assemblage was the same as that of the C_{25} monoene in the
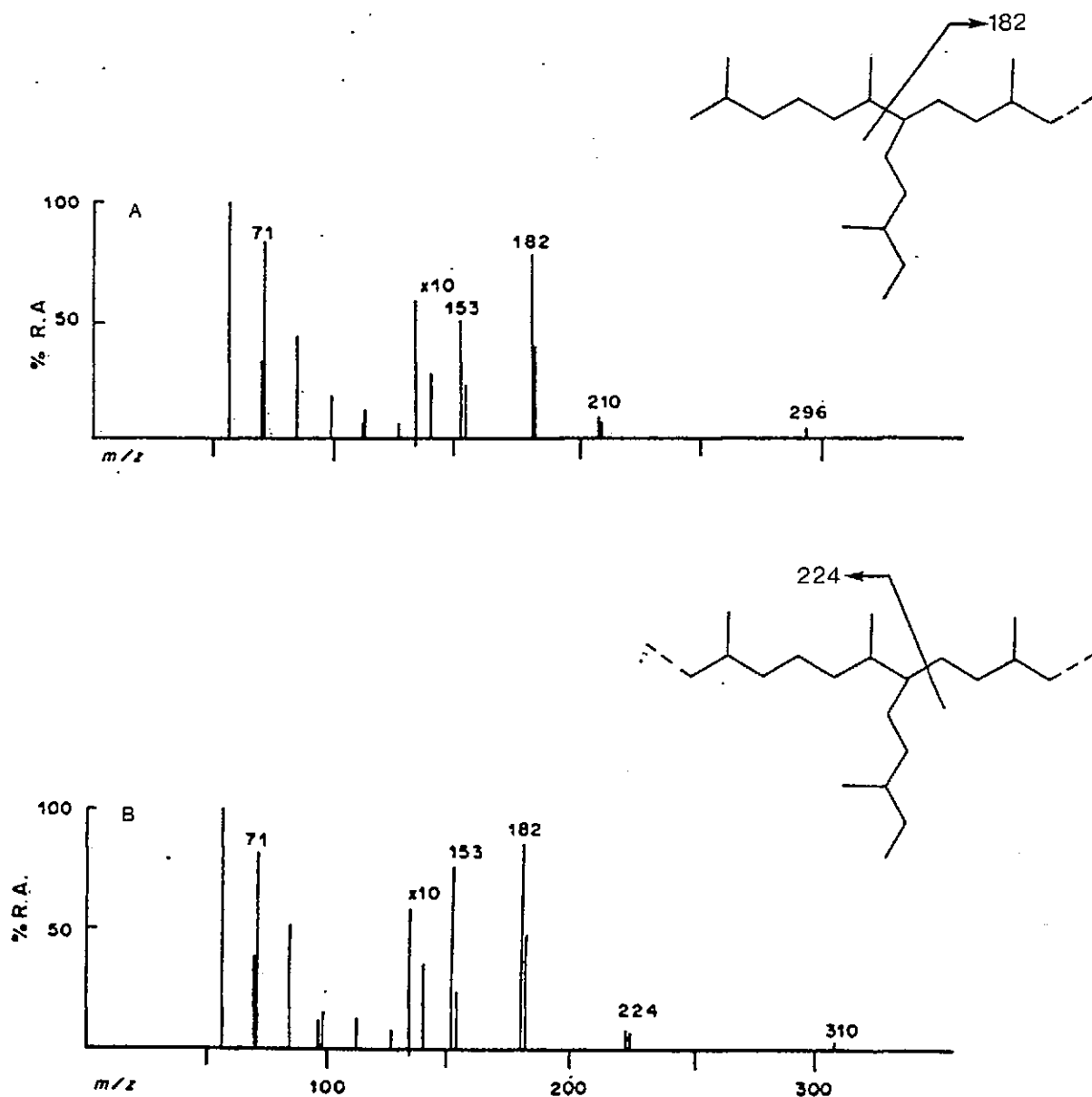
Fig.1:20   Mass spectra as recorded by Dunlop and Jefferies (1985) of two highly-branched C_{21} (A) and C_{22} (B) alkanes isolated from sediments of Shark Bay, Australia. Proposed structures as indicated.
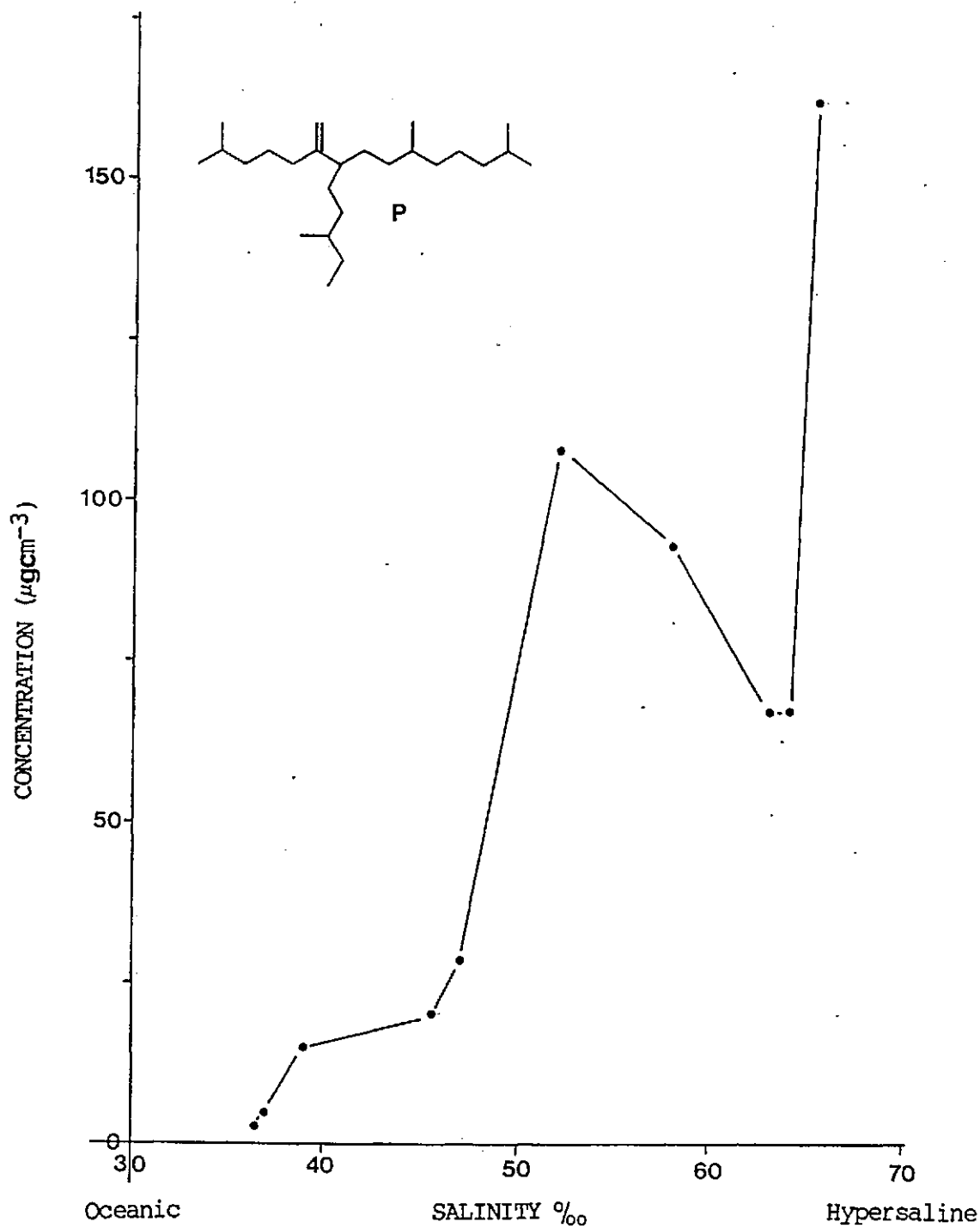
Fig.1:21   Graph of salinity _versus_ concentration for the major
C_{25} monoene of proposed structure (P) isolated from
sediments of Shark Bay, Australia: data from Dunlop
and Jefferies (1985). Note the increase in
concentration of P with increasing salinity.

hypersaline assemblage suggesting the possibility of a common origin.

Shaw et al. (1985) reported the occurrence of $br25:3;_{OV101}2092$ in sediments of Port Valdez (Alaska, U.S.A.) and Voudrias and Smith (1986) noted the occurrence of three compounds eluting between $n\text{-}C_{20}$ and $n\text{-}C_{21}$ in hydrocarbon extracts of three Eastern Virginia estuarine creeks (U.S.A.). The compounds, $C_{25}H_{48}$ ($RI_{SE52}2067$ and 2082) and $C_{25}H_{46}$ (2091) were incorrectly suggested to be cyclic by comparison with the data of Farrington et al. (1977). Coates et al. (1986) noted the occurrence of unidentified C_{25} hydrocarbons in Holothuria and sediments of the Great Barrier Reef (Australia). Sedimentary C_{25} hydrocarbons were dominated by a $C_{25}H_{50}$ monoene whereas those of Holothuria contained predominantly a $C_{25}H_{48}$ isoprenoid diene which was reported to be derived from plant material precursors in the diet.

Brassell et al. (1986a) reported the occurrence of two highly branched C_{30} alkanes with virtually identical mass spectra in hydrocarbon extracts of the Maoming oil shale (China). On the basis of mass spectral interpretation, the two C_{30} alkanes were proposed as C_{30} homologues of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (i.e. br20:0), namely 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecanes (i.e. br30:0; 75). Although Brassell et al. (1986a) did not provide retention index data for the two alkanes it is interesting to note both compounds eluted after n-hexacosane ($n\text{-}C_{26}$) (i.e. >RI2600) whereas the hydrocarbon suggested by Barrick and Hedges (1981) to be the C_{30} homologue of br20:0 eluted at RI2524.

In an overview of the Recent sediment hydrocarbon geochemistry of Atlantic and Gulf Coast Outer Continental Shelf environments, Boehm and

Requego (1986) noted that the series of C_{25} alkanes and C_{25} cycloalkanes observed in many Recent sediments (i.e. Barrick et al., 1980 etc.) are ubiquitous components of all Atlantic and Gulf of Mexico Outer Continental sediments. The compounds are proposed as being of a diagenetic and/or marine biogenic origin. Despite this being a review paper no mention was made of the synthesis of br20:0 by Yon et al. (1982) nor of the recent identification of br20:0 and a br25:2 in Entereomorpha (Rowland et al., 1985).

Sinninghe Damste et al. (1986) reported the identification of br20:0(66) and br25:0(70) in hydrocarbon extracts of a Messinian (Upper Miocene; Italy) marl layer deposited under hypersaline, euxinic conditions. Additionally identified was a C_{20} isoprenoid thiophene 5-(2,6-dimethyl-1-1(3-methylbutyl)-heptyl)-2,3-dimethylthiophene (76) which was suggested might arise by sulphur incorporation into br20 monoenes (br20:1) during early diagenesis.

In summary, a group comprising over twenty acyclic and cyclic C_{20}, C_{25} and C_{30} (and possibly other homologues) hydrocarbons have been reported to occur in high concentrations in Recent freshwater, estuarine, marine and hypersaline sediments from many different geographical locations. Apparent structural relations exist between members of the acyclic and cyclic hydrocarbon series but the only definite structural assignment made so far is for the acyclic highly branched C_{20} alkane (br20:0; 66). Analogous structures have been proposed for the br25:0 and br30:0 alkanes but, up to the present, these structures remain unproven. There has been little progress in determining the structures of the cyclic hydrocarbons except for suggestions that they contain a fused bicyclic ring structure.

There is much confusion in the literature concerning these compounds. For instance, certain hydrocarbons are referred to as cyclic when evidence suggests that the compounds are, in fact, acyclic. Although there is one reported biological occurrence of the acyclic hydrocarbons in a green alga, the ubiquitity of occurrence of both the acyclic and cyclic hydrocarbons in different sedimentary environments has led many workers to suggest they have a bacterial origin.

Most work has been concerned with monitoring distributional changes in hydrocarbon abundance between various sedimentary environments. Much is made of the potential of the acyclic and cyclic hydrocarbons to act as biological markers once the sources and definitive structures have been established. However, comparatively little has been done to conclusively identify the hydrocarbon structures. If the study of these unusual, abundant, acyclic and cyclic hydrocarbons is to progress, structural assignment of the major hydrocarbons is long overdue.

1.5 THE PRESENT STUDY

The present study describes the synthesis of the three acyclic highly branched alkanes reported to be br20:0, br25:0 and br30:0. Comparison of mass spectra and retention indices of the synthetic alkanes with the sedimentary alkanes demonstrated the structural assignments made herein to be correct. In addition, on the basis of mass spectral interpretation, structures for the bicyclic alkanes c25:0:2 and c30:0:2 are proposed and the reference alkanes synthesised from natural product precursors. A comparison of mass spectra and retention indices indicated the proposed structures for c25:0:2 and c30:0:2 to be similar but not identical to the sedimentary compounds. However, from the data

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presented more likely structures can be proposed.

Chapter 3 describes the syntheses of both the acyclic and bicyclic alkanes. Structural assignments based on full spectroscopic examination (i.e. ^{13}C NMR, ^{1}H NMR, HRMS) are discussed. Relationships between homologues of the acyclic and cyclic synthetic alkanes are examined.

Chapter 4 describes the use of the synthetic acyclic alkanes to assign structures to naturally occurring hydrocarbons in six sediments from different geographical locations. The assignments are then compared to those of previous investigations.

One of the reasons proposed for the high abundance of acyclic branched hydrocarbons in certain sediments is that they are resistant to biodegradation (Rowland et al., 1985). Biodegradation experiments using pure cultures of Pseudomonas aeruginosa and the synthetic acyclic alkanes and alkenes, designed to test this hypothesis, are described in Chapter 5. The results demonstrated the rapid biodegradation of n-hydrocarbons relative to the branched compounds.

Chapter 6 describes the synthesis of other 'irregular' (h-h) and (t-t) acyclic isoprenoid alkanes. The (h-h) alkane had been reported tentatively to occur in sediments of the MacArthur Basin (Australia) where it was suggested to be evidence for a contribution from organic biomass of Archaebacteria (Fowler, 1984).

CHAPTER TWO

EXPERIMENTAL DETAILS

EXPERIMENTAL DETAILS

2.1 GENERAL

Glassware was cleaned in chromic acid, rinsed in doubly distilled 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).

General purpose solvents (e.g. hexane, dichloromethane, methanol) were distilled in all-glass apparatus prior to use. Solvent purity was tested by evaporation (rotary evaporator) of $100cm^3$ of the solvent to approximately $50mm^3$ followed by analysis of a $0.5mm^3$ aliquot by gas chromatography. Diethyl ether (Et_2O) and tetrahydrofuran (THF) were purified by elution through basic alumina (BDH; 10g per $100cm^3$ solvent) and reflux with $LiAlH_4$ (30 min). Following distillation the ethers were stored over CaH_2 until use.

The silica gel (BDH; 60-120 mesh) and alumina (BDH; Grade I, neutral) used as adsorbents in column chromatography were soxhlet extracted with dichloromethane (48hr) before activation (120°C; 12hr). Deactivated alumina and silica gel were prepared by shaking (1hr) the adsorbent with the appropriate quantity of doubly distilled water. Thin layer chromatographic (tlc) plates were prepared on solvent-washed 20 x 20cm or 20 x 10cm glass plates with a coating of 0.25mm (analytical) or 0.5mm (preparative) silica gel (Merck Kiesel gel type 60G). Argentatious tlc plates were prepared from slurries of silica gel made up in an aqueous solution of 5% w/w $AgNO_3$. Following activation

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(110°C; 1hr) all plates were predeveloped in ethyl acetate and used without reactivation.

Concentrated hydrochloric acid, mercury, anhydrous sodium sulphate (anhyd. Na_2SO_4), aluminium foil and cotton wool were all extracted with dichloromethane before use.

2.2   EXTRACTION AND FRACTIONATION OF GEOCHEMICAL SAMPLES

2.2.1 Sediment collection and solvent extraction

Surface sediment samples G06, M73 and BAR10 were provided by Mr. S. Howells (Oil Pollution Research Unit) and sample GV1 by Dr. D. Jones (University of Newcastle upon Tyne). Both sets of samples were collected using a grab sampler and stored frozen in aluminium or glass cylinders. Surface sediments from the Tamar estuary were collected by spatula, transferred to clean glass jars and frozen immediately. The thawed samples were solvent extracted using the method of Douglas et al. (1981). Sediment (approximately 40g wet weight) was extracted with methanol (40cm^3) by ultrasonication (5min; Dawe model) with cooling (ice bath). The organic extract was separated by centrifugation (20min; 1800 rpm) and decanted. This procedure was repeated using dichloromethane/methanol (7:3), dichloromethane/methanol (4:1) and dichloromethane. The combined extracts were shaken (separating funnel) with doubly distilled water (30cm^3) and the lower organic layer collected, along with the dichloromethane washings (3 x 15cm^3) of the aqueous layer. Solvent was removed (Buchi rotary evaporator; 30°C) and the total organic extract transferred quantitatively to a vial and weighed.

2.2.2 Fractionation of total organic extract (toe).

The toe from the sediment was fractionated by tlc on silica gel (0.5mm) using hexane as mobile phase. The plate was visualised (UV light, 365μm; 0.5% Rhodamine 6G in methanol) and the "aliphatic hydrocarbon" band, corresponding to R_f 0.85 - 1.00, removed. The hydrocarbons were recovered from the silica gel by desorption with dichloromethane (20cm^3). Solvent was removed (Buchi; 30°C) and the "aliphatic hydrocarbons" transferred to a vial and weighed. Sulphur was removed from the "aliphatic hydrocarbons" by shaking with elemental mercury (1g) and hexane (1cm^3), allowing the sulphide precipitate to settle (>3hr) and removing the supernatant. The precipitate was washed with hexane (2 x 2cm^3) and the complete procedure was repeated with a further aliquot of mercury; the hexane layers were combined and the solvent removed (Buchi; 30°C). The "aliphatic hydrocarbons" were transferred to a vial, weighed and stored in dichloromethane (approximately 100mm^3) at 4°C.

2.2.3 Microscale hydrogenation of aliphatic hydrocarbons

Hydrogenation of an aliquot (10%) of the "aliphatic hydrocarbons" of each sediment extract was achieved by bubbling hydrogen gas (20cm^{3}min^{-1}) through the extract dissolved in hexane (10cm^3 20°C) containing $PtO_2.H_2O$ (0.1g).

The entire extraction and fractionation procedure was repeated for each sediment sample to test the reproducibility of the method. Results indicated that the concentration of any one compound can be determined to a precision of only approximately $\pm$ 14%. A blank analysis was performed by carrying out the entire extraction/fractionation procedure in the absence of sediment. Gas chromatography of the blank fractions

showed no interferents.

2.2.4 Solvent Extraction and Fractionation of Rozel Point Crude Oil

Rozel Point crude oil was provided by Dr. J. R. Maxwell (University
of Bristol) and extracted according to Yon (1982). Rozel Point crude
oil (10g) was poured into liquid N_2 in a mortar and was crushed with
a pestle. The resulting 'powder' was added to chilled, rapidly-stirred
hexane (250cm^3) and the mixture stirred overnight (12hr). The hexane
was decanted leaving an insoluble residue which was discarded. After
removal of solvent (Buchi; 30°C) the hexane soluble extract (3.71g) was
applied to three alumina columns (90g, Grade I, neutral). Elution with
hexane (200cm^3) afforded a hydrocarbon fraction (0.86g). Following
evaporation of solvent (Buchi; 30°C) the n-alkanes were removed from
the hydrocarbon fraction by molecular sieve. Molecular sieve (5A;
5.3g; activated at 450°C for 48hr) and the hydrocarbon fraction (296mg)
were refluxed (48hr) in 2,2,4-trimethylpentane (50cm^3). The solvent
was decanted and combined with washings (hot 2,2,4-trimethylpentane; 3
x 10cm^3) of the molecular sieve. Removal of the solvent (Buchi;
30°C) afforded the branched and cyclic fraction (267mg) which was
transferred to a vial and stored at 4°C.

2.2.5 Gas chromatography (gc)

Hydrocarbons were analysed on a Carlo Erba 4160 gas chromatograph
fitted with a 25m fused silica column (0.32mm i.d.) coated with OV1
(supplied by K. Hall, GC^2, Manchester) using flame ionisation
detection and on-column injection. The column oven was programmed from
40-80°C at 10°C min^{-1}, 80-290°C at 6°C min^{-1} and held at the final
temperature for 20 minutes. Hydrogen was the carrier gas at a flow
rate of 2cm^{3}min^{-1} (set at 250°C) supplied at a pressure of
0.6kgcm^{-2}.

Certain solvent extracts were also analysed on a Carlo Erba 5160 Mega gas chromatograph fitted with a 25m fused silica column (0.32mm i.d.) coated with CPWAX52 (Chrompack, Holland) using flame ionisation detection and on-column injection. The carrier gas was hydrogen $(2cm^3min^{-1})$ and the oven temperature programmed from 40-80°C at $10°Cmin^{-1}$, 80-240°C at $6°Cmin^{-1}$ and held at 240°C for 10 minutes.

Retention indices were calculated according to the standard method (Harris and Habgood, 1966). Standard alkane mixtures comprising: <u>a</u>. $n-C_{16}$, $n-C_{17}$, $n-C_{18}$; <u>b</u>. $n-C_{20}$, $n-C_{21}$, $n-C_{22}$; and <u>c</u>. $n-C_{24}$, $n-C_{25}$, $n-C_{26}$; were added to the hydrocarbon extracts where appropriate.

2.2.6 Quantitation

Quantitation of individual hydrocarbons was accomplished by measurements of gc peak areas using a Shimadzu CR3-A recording integrator. These were then compared to the responses from an alkane standard mixture of known concentration comprising: pristane, phytane, br25:0 and several n-alkanes of chain length C_{16} - C_{36}. This mixture was routinely injected into the gc prior to sample analysis. Gc reproducibility was ± 8% for multiple (x5) injections of the alkane standard and ± 11% for triplicate injections of complicated hydrocarbon extracts.

2.2.7 Gas chromatography mass spectrometry (gcms)

Analysis of selected hydrocarbon fractions was performed on a Carlo Erba 5160 Mega chromatograph coupled to a Finnigan 4000 quadrople mass spectrometer. A 25m fused silica column coated with OV1 (Hewlett-Packard Ultra series) 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 from 40-80°C at $10°Cmin^{-1}$, 80-290 at $4°Cmin^{-1}$ and held at 290° for 20 minutes. Mass spectrometer operating conditions were; ion source temperature 250°C, 40eV ionising energy and a filament emission current of 350 μA. Spectra (m/z 50-500) were collected every second using an Incos 2300 data system.

2.2.8 Compound identification

Individual hydrocarbons were identified by co-chromatography with authentic compounds on gc columns of differing polarity and by comparison of gas chromatographic retention indices with literature data. Additional identification 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.

2.3   BIODEGRATION EXPERIMENTS

2.3.1 Microbiological methods

Pseudomonas aeruginosa (National Centre for Industrial Bacteria) was used as the microorganism for the biodegradation experiments. Bacteria were grown in an OXOID nutrient broth (13g OXOID in $1000cm^3$ H_2O) for 24hr at 20°C.

2.3.2 Inoculation and incubation

All glassware involved in the degradation experiments was carefully sterilised by autoclave (120°C for 20min). Bacterial broth ($0.5cm^3$) was added to a conical flask ($25cm^3$) containing $10cm^3$ of a sterilised minimal salts solution (1000 cm^3 H_2O with 5% NH_4 Cl 1% NH_4NO_3; 2% Na_2SO_4; 3% K_2HPO_4; 1% KH_2PO_4 and

0.1% $MgSO_4.7H_2O$). The alkane or alkene mixture (see Tables 2:1 and 2:2 for compositional details) was dissolved in hexane ($100mm^3$) and added carefully to the flasks by syringe. Flasks were then stoppered with non-absorbent cotton wool and incubated aerobically in the light on an orbital shaker at 20°C. Control flasks, containing minimal salts solution and alkanes/alkenes but no bacterial inoculum were incubated under the same conditions to monitor abiological losses (e.g. evaporation).

2.3.3 Hydrocarbon Extraction

Hydrocarbons were extracted from the minimal salts solution with dichloromethane ($2 \times 10cm^3$). After drying (anhyd. Na_2SO_4; 30min) the solvent was transferred to a round bottomed flask, evaporated to near-dryness (Buchi; 30°C) and the extract transferred to a volumetric flask ($5cm^3$) and made up to volume with dichloromethane.

2.3.4 Gas chromatography

Extracted alkanes/alkenes from the biodegradation experiments were analysed under the same gas chromatographic conditions (i.e. OV1) used for geochemical analyses (section 2.2.5). During analysis of extracted alkenes it was observed that n-$C_{17:1}$ co-chromatographed with one of the br20:1 isomers rendering exact quantitation difficult. This problem was solved by reanalysing the alkene extract on a polar (CPWAX52) column where complete separation of n-$C_{17:1}$ and br20:1 alkenes occurred. Gas chromatographic conditions for the polar column were also detailed in section 2.2.5.

TABLE 2.1: QUANTITY OF ALKANES USED IN BIODEGRADATION EXPERIMENTS

ALKANE	ALKANE CONCENTRATION IN CONICAL FLASK AT DAY 0 ($\mu g\ cm^{-3}$)
Eicosane n-C_{20}	10.0
Pentacosane n-C_{25}	11.2
Triacontane n-C_{30}	10.2
2,6,10,14-tetramethyl hexadecane (Phytane) i20	10.3
2,6,10,14,18-pentamethyl eicosane i25	10.0
2,6,10,15,19,23-hexamethyl tetracosane i30	7.8
2,6,10-trimethyl-7-(3-methylbutyl)dodecane br20:0	10.4
2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane br25:0	11.6
2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane br 30:0	13.4

TABLE 2.2: QUANTITY OF ALKENES USED IN BIODEGRADATION EXPERIMENTS

ALKENE	ALKANE CONCENTRATION IN CONICAL FLASK AT DAY 0 (μg cm^{-3})
Heptadec-1-ene n-C$_{17:1}$	11.6
Eicos-1-ene n-C$_{20:1}$	11.6
2,6,10-trimethyl-7-(3-methylbutyl)dodecane br20:1	
RI[a]1679	10.5
RI1689	4.8
RI1690	4.7
RI1711	1.7
RI1714	1.5

TOTAL	23.2
2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane br25:1	
RI2078	8.3
RI2085	4.2
RI2092	4.3
RI2116	1.2
RI2125	1.1

TOTAL	19.1

[a]Retention indices: Gc conditions; OV1, 40-80°C at 10°C min^{-1}, 80-290°C at 6°C min^{-1}.

2.3.5 Quantitation

Quantitation of individual alkanes and alkenes was accomplished by measurement of gc peak areas using a Shimadzu CR3-A integrator. In the case of the alkanes, these areas were compared to the responses from an alkane mixture of known concentration containing all the components present in the biodegradation alkane mixture. Similarly alkenes were compared to an alkene mixture containing the suite of alkenes present in the biodegradation mixture. Repeat injections (x10) indicated gc reproducibility to be $\pm 11\%$ (alkanes) and $\pm 7\%$ (alkenes).

2.4   SYNTHESES

2.4.1   Instrumentation

2.4.1.1 Gas chromatography (gc)

Synthetic reaction mixtures were examined by use of a Carlo Erba 4160 gas chromatograph fitted with fused silica columns (25m x 0.25mm i.d.), Grob split vapourising injector (15:1 ratio) and a flame ionisation detector. The column phase and temperature programme employed for each analysis is indicated in the appropriate figure legend. The carrier gas was typically hydrogen at a flow rate of $2cm^3min^{-1}$ (set at 250°C) supplied at a pressure of approximately $0.6kgcm^{-2}$. Chromatograms were recorded using a Shimadzu CR3-A integrator.

2.4.1.2 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 double focussing magnetic sector mass spectrometer (gcms). The gas chromatograph was fitted with a 50m x 0.32mm i.d. fused silica column coated with OV1 (K Hall, GC^2) which led directly into the ion source of the mass spectrometer. On-column

injection and helium carrier gas were used throughout and the temperature of the column oven was typically programmed from 40-300°C at $6°Cmin^{-1}$. Spectra (m/z 40-532) were collected every 1.5 seconds using a DS90 data system. Typical mass spectrometer operating conditions were: ionisation energy 40eV; source temperature 250°C; filament emission current   A; trap current   A.

2.4.1.3 High Resolution Mass Spectrometry (HRMS)

High resolution electron impact mass spectra were recorded on a Kratos MS25 mass spectrometer. Samples were introduced by solid probe ($2\mu g$ in $1\mu l$ dichloromethane) and spectra collected every 2.5 seconds by the DS90 data system. Mass spectrometer operating conditions as for LRMS (section 2.4.1.2).

2.4.1.4 Infra Red Spectrometry (IR)

Infra Red spectra were recorded as either liquid films, KBr discs or solutions (in dichloromethane) on a Perkin Elmer Infra Red spectrometer. The $1603cm^{-1}$ peak in the spectra of polystyrene was used as a reference.

2.4.1.5 Nuclear Magnetic Resonance Spectrometry (NMR)

Proton or ^{1}H NMR spectra were recorded in deuterochloroform solution using a Perkin Elmer RB12 (60MHz) spectrometer.

The ^{1}H NMR of important intermediates and products were additionally recorded using a Bruker WH400 spectrometer (400MHz; S.E.R.C. WH400 Service, University of Warwick). 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

$\underline{d}$), triplet ($\underline{t}$), doublet of triplets ($\underline{d}$ of $\underline{t}$), quartet ($\underline{q}$) or multiplet ($\underline{m}$).

Carbon 13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded in deuterochloroform using a Bruker WH400 spectrometer (400MHz; S.E.R.C. WH400 Service, University of Warwick). TMS was the internal standard. Chemical shifts were obtained from broad band decoupled spectra and are reported on the δ scale.

2.4.2 Silylation of Alcohols

Simple alcohols were derivatised by silylation with bis (trimethylsilyl)trifluoroacetamide (BSTFA). BSTFA ($100mm^3$) was added to the dry alcohol ($\sim$1.0mg) and heated (60°C; 5min). Larger alcohols containing hindered hydroxyl groups were derivatised by silylation with N-trimethylsilylimidazole (TSIM) according to the method of Sakauchi and Horning (1971). TSIM ($200mm^3$) was added to the dry alcohol ($\sim$1.0mg) in a reacti-vial ($1cm^3$) and heated (120°C; 2hr).

2.4.3 Synthesis of

2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0)

Unless stated otherwise, all syntheses were performed under an inert atmosphere of dry, high purity argon.

2.4.3.1 Starting materials

The authenticity of all starting materials used in the synthesis of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane was confirmed by LRMS, IR and ^1H NMR spectroscopy.

6-methylhept-5-en-2-one

Gc purity : 94%

LRMS m/z : 126 ($M^{+\bullet}$,10%), 111 ($M^{+\bullet}$-CH_3,22%),

108 ($M^{+\bullet}$-H_2O,32%)   93 (14%), 69 (58%), 55 (62%), 43 (100%).

^1H NMR (60MHz)δ ppm:
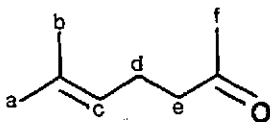
1.51(m;8H;a,b and d), 2.13 (s;3H;f), 2.34 (t;2H;J=7Hz;e), 5.03 (m;1H;c)

IR (liquid film): v(C=O) 1715cm^{-1}, ,v(C-CO-C) 1172cm^{-1},

v(C=C) 1625cm^{-1}, δ_s(CH_3) gemdimethyl 1380cm^{-1} 1370cm^{-1},

δ(C-H) oop 840cm^{-1} 810cm^{-1}.

3,7-dimethyloctdi-2,6-en-1-ol

Gc purity: 94%

LRMS m/z : 154 ($M^{+\bullet}$,0.2%), 136 ($M^{+\bullet}$-H_2O,4%), 121 (7%), 93 (27%),

69 (78%), 41 (100%).

Mass spectrum also showed a small ion at m/z 152 (0.1%) considered to be the $M^{+\bullet}$ from the small quantity (2%) of co-eluting 3,7-dimethyloctdi-2, 6-en-1-al (Citral) present.

LRMS (TMS ether) m/z : 226 ($M^{+\bullet}$,2%), 211 ($M^{+\bullet}$-CH_3,3%), 169 (8%),

143 (12%), 93 (38%), 75 (HO^+=Si(CH_3)$_2$,84%),

73[(CH_3)$_3^+$,100%].

^1H NMR (60MHz)δppm:
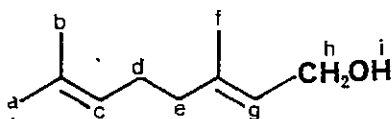
1.65($\underline{d}$;9H;a,b and f), 2.11 ($\underline{m}$;5H;d,e and$i$), 4.12 ($\underline{d}$;2H;J=7Hz;h),

5.3 ($\underline{m}$;2H;J=7Hz;c and g).

IR (liquid film): v(O-H) 3310cm^{-1} intermolecularly bonded,

v_{as}(CH$_3$) 2972cm^{-1}, v_{as}(CH$_2$) 2920cm^{-1},

v_s(CH$_3$) 2870cm^{-1}, v_s(CH$_2$) 2850cm^{-1}, v(C=C) 1660cm^{-1},

v(C-O) 1000cm^{-1}, primary unsaturated alchohol.

3-methylpentan-1-ol

Gc purity: 96%

LRMS (TMS ether) m/z : 159 (M$^{+\cdot}$-CH$_3$,11%),

103[CH$_2$=$\overset{+}{O}$Si(CH$_3$)$_3$,32%], 73[(CH$_3$)$_3$Si$^+$,100%].

^{1}H NMR (60MHz)δppm:
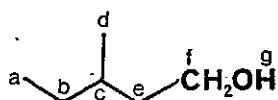
0.91($\underline{m}$;6H; J=6Hz; a and d), 1.38 ($\underline{m}$;5H;b,c and e), 2.42 ($\underline{s}$;1H; g),

3.64 ($\underline{t}$;2H;J=8Hz;f).

IR (liquid film): v(O-H) 3320cm^{-1} intermolecularly bonded,

δ_s(CH$_2$) 1460cm^{-1}, δ_s(CH$_3$) 1375cm^{-1}, v(C-O) 1055cm^{-1},

primary saturated.

2.4.3.2 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; 10.0g, 80mmol) was added to rapidly stirred hexane (150cm^3) containing preactivated (30min) PtO$_2$.H$_2$O (Adams catalyst; 0.15g). Hydrogen uptake was monitored and the reaction stopped upon uptake of the stoichimetric quantity (1770cm^3). Following filtration and drying (anhyd. Na$_2$SO$_4$;2g), solvent was removed (Buchi; 30°C) and the crude product purified by column chromatography on deactivated (4%) alumina (100g). Elution with hexane (200cm^3) and subsequent solvent removal (Buchi; 30°C) afforded pure 6-methylheptan-2-one (9.4g, 73mmol) which was assigned using LRMS, ^{1}H NMR and IR.

Gc purity: 95%

Yield   : 93%

LRMS m/z : 128 (M$^{+\cdot}$,3%), 110 (M$^{+\cdot}$-H$_2$O,12%), 95 (14%), 95 (14%), 71 (22%), 58 (100%).

^{1}H NMR (60MHz) δ ppm:
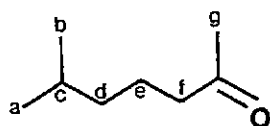
0.85 (d;6H; J=6Hz; a and b), 1.0 - 2.0 (m; 5H; c, d and e), 2.11 (s; 3H; g), 2.43 (t;2H;J=7Hz; f).

IR (liquid film): v(C=O) overtone 3420cm^{-1}, v(C=O) 1740cm^{-1}, δ_s(CH$_3$) gemdimethyl 1385cm^{-1} 1370cm^{-1}, v(C-CO-C) 1172cm^{-1}.

Repeat synthesis provided a further 4.3g (90% yield).

2.4.3.3 2,6-dimethylhept-1-ene

Two different synthetic methods were employed in the preparation of 2,6-dimethylhept-1-ene.

a.   Wittig synthesis of 2,6-dimethylhept-1-ene.

The method used was modified from Trippet (1960). To a rapidly stirred suspension of methyltriphenylphosphonium bromide (Aldrich; 5.58g) in THF ($20cm^3$) was added carefully NaH (BDH; 0.36g) in THF ($10cm^3$). The mixture was stirred (1hr) during which time the formation of methylenetriphenylphosphorane was noted by a change in colour from white to bright green. Dropwise addition of 6-methylheptan-2-one (2.0g, 15mmol) in THF ($8cm^3$) and subsequent heating (50°C; water bath; 4hr) of the reaction mixture was accompanied by a further colour change from green to creamy white. Following storage (4°C; 48hr), residual triphenylphosphine oxide $[(C_6H_5)_3PO]$ was removed by centrifugation (2200rpm; 10min). Attempts to remove $(C_6H_5)_3PO$ by partition into distilled water proved unsuccessful. The supernatant was decanted and combined with washings (3 x $10cm^3$ THF; 2 x $10cm^3$ hexane) of the residual $(C_6H_5)_3PO$. Solvent was removed (Buchi, 30°C) and the crude products applied to an activated (Grade 1) alumina (50g) column. Gc of the first hexane eluate (150 cm^3) revealed the presence of two 2,6-dimethylheptene isomers (0.16g, 1.2mmol) which were partially characterised by gcms. Attempts to separate the alkene isomers by argentatious tlc proved unsuccessful. The fraction was discarded and the synthesis repeated. After consideration of the poor yield (26%) and purity of 2,6-dimethylhept-1-ene obtained using the Wittig synthesis it was decided to prepare the alkene by a different synthetic method.

b.   Synthesis of 2,6-dimethylhept-1-ene by methylenation of 6-methylheptan-2-one using Zn - CH_2Br_2-$TiCl_4$.

The alkene was prepared in excellent yield using the method of Oshima et al. (1978).   To a suspension of zinc powder (Aldrich; 7.7g) in rapidly stirred THF (75cm^3) was added CH_2Br_2 (Aldrich; 10.3g).   Dropwise addition (1hr) of $TiCl_4$ (Aldrich 43cm^3 of a 1.0$\underline{M}$ dichloromethane solution) was accompanied by rapid evolution of heat and colour change from grey to dark brown; at this point, care was taken to ensure temperature in the reaction vessel did not exceed 35°C. $TiCl_4$ was transferred from reagent bottle to apparatus by passage through a double-ended syringe needle under the pressure of argon;   this avoided the excessive fuming (HCl) that occurred on contact of $TiCl_4$ with air.   6-methylheptan-2-one (5.17g, 40mmol) in THF (20cm^3) was added dropwise (45min) and the resulting mixture stirred at 25°C (16hr). Et_2O (20cm^3) and 1$\underline{M}$ HCl (50cm^3; dropwise) were added and the mixture transferred to a separating funnel (250cm^3).   The upper organic layer was removed and combined with washings (2 x 20cm^3 Et_2O; 2 x 20cm^3 dichloromethane) of the aqueous layer.   Following drying (anhyd. Na_2SO_4;3g) and filtration, the solvent was evaporated (Buchi; 30°C) and the crude reaction product purified by column chromatography on deactivated (4% H_2O) alumina (93g).   Elution with hexane (150cm^3) afforded pure 2,6-dimethylhept-1-ene (4.5g, 35mmol) which was assigned by LRMS, ^{1}H NMR and IR.

Gc purity : 95%

yield   : 91%

LRMS (Fig. 3:6b) m/z: 126(M$^+\cdot$,7%), 111 (M$^+\cdot$-CH_3,2%); 83(5%), 9(28%), 56(100%).

^{1}H NMR (60MHz) δ ppm:
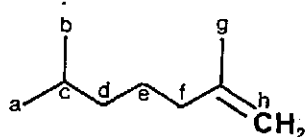
0.85 (d; 6H; J=6Hz; a and b), 1.33 (m; 5H; c, d and e), 1.72 (s; 3H;
g), 1.95 (t; 2H; f), 4.80 (s; 2H; h).

IR (liquid cell): vinyl ν(C-H) 3080cm^{-1}, vinyl ν(C=C) 1645cm^{-1},

δ_s(CH$_3$) gemdimethyl 1390cm^{-1} 1375cm^{-1}, vinyl (C-H) oop,

890cm^{-1}.

2.4.3.4 2,6-dimethylheptan-1-ol

Hydroboronation:oxidation was performed using the method of Brown
(1977). 2,6-dimethylhept-1-ene (4.52g, 36mmol) was added to rapidly
stirred THF (20cm^3) and cooled (0°C; ice bath). Borane:THF (BH$_3$:
THF) complex (Aldrich; 15cm^3 of a 1.0M THF solution; 12mmol plus 25%
excess) was added dropwise and the clear, colourless solution stirred
at 20°C for 30 minutes. The addition of distilled H$_2$O (10cm^3)
destroyed any excess hydride. 3M NaOH (30cm^3) was added and the
reaction mixture cooled (5°C; ice bath) whereupon H$_2$O$_2$ (BDH;
30cm^3 of a 30% solution) was added dropwise such that the temperature
in the reaction vessel did not exceed 40°C. After subsequent heating
(50°C; water bath; 2hr), the reaction mixture was transferred to a
separating funnel (250cm^3) and the upper organic layer removed and
combined with washings (2 x 15cm^3; Et$_2$O) of the lower aqueous
layer.

Following drying (anhyd. Na$_2$SO$_4$;2g) and filtration, solvent was
removed (Buchi; 30°C) and the crude alcohol purified by column

chromatography on deactivated (4%) alumina (92g). Elution with hexane (150cm^3) removed non-polar material and dichloromethane (150cm^3) afforded the alcohol. Solvent was evaporated (Buchi; 30°C) and 2,6-dimethylheptan-1-ol (4.8g, 33mmol) was assigned by LRMS (TMS ether; BSTFA), ^{1}H NMR and IR.

Gc purity : 92%

Yield   : 92%

LRMS (TMS ether) m/z: 201 (M$^{+\cdot}$-CH$_3$, 4%),

103 [CH$_2$=$\overset{+}{O}$Si(CH$_3$)$_3$, 64%], 75 [H$\overset{+}{O}$=Si(CH$_3$)$_2$,100%].

^{1}H NMR (60MHz) δ ppm:
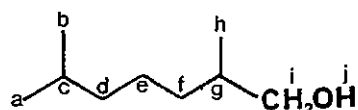
0.85 (d; 6H; J = 6Hz; a and b), 0.90 (d; 3H; J = 7Hz; h), 1.41 (m; 9H; c, d, e, f, g and j), 3.42 (d; 2H; J = 8Hz; i).

IR (liquid film): ν(O-H) 3320cm^{-1} intermolecularly bonded, ν(C-O) 1040cm^{-1} primary saturated.

Repeat synthesis yielded a further 3.3g.

2.4.3.5 2,6-dimethylheptan-1-al

2,6-dimethylheptan-1-al was prepared using the method of Omura and Swern (1978). This oxidation method was chosen after several unsuccessful attempts to prepare the aldehyde using pyridinium chlorochromate (Piancetilli et al., 1982). The Omura and Swern reaction was first performed on a pilot scale as follows:

Oxalic acid (0.97g; 1.1 equivalents) in dry dichloromethane (10cm^3) was cooled (-70°C; isopropanol/dry ice bath). Dimethylsulphoxide (DMSO; 1.3g; 2.4 equivalents; redistilled from CaH$_2$ onto 4A molecular sieve) in dichloromethane (3cm^3) was added dropwise over a period of

20 minutes and the resulting mixture stirred at -70°C (20 min). The dropwise addition of 2,6-dimethylheptan-1-ol (1.0g, 6.9mmol) in dichloromethane (5cm^3) over a period of 20 minutes resulted in the formation of a white precipitate. After stirring (-70°C; 20min) triethylamine (16.7g; 5 equivalents) was added dropwise over 10 minutes; after this time the cooling bath was removed, allowing the reaction vessel to reach ambient temperature. Upon the addition of H$_2$O (50cm^3) and stirring (15min) the precipitate disappeared and the reaction mixture was transferred to a separating funnel (250cm^3). The lower organic layer was removed and combined with washings (2 x 20cm^3; dichloromethane) of the aqueous layer. Following reduction of solvent volume to ca. 25cm^3 (Buchi; 30°C) the solution was washed successively with dilute HCl (25cm^3), 10% Na$_2$CO$_3$(25cm^3) and H$_2$O (25cm^3). After drying (anhyd. Na$_2$SO$_4$;2g) and filtration, solvent was removed (Buchi; 30°C) and the crude products purified by column chromatography on deactivated (4%) alumina (30g). Elution with hexane: Et$_2$O (95:5; 100cm^3) afforded a fraction containing the aldehyde and two significant contaminants. Following evaporation of solvent (Buchi; 30°C) the aldehyde was further purified by tlc (0.5mm silica gel; hexane mobile phase). Following visualisation (Rhodamine 6G; 365μm) the aldehyde band (R$_f$ = 0.41) was removed from the plate and the aldehyde recovered from the silica gel by desorption with dichloromethane (20cm^3). Evaporation of solvent (Buchi; 30°C) afforded the aldehyde (0.1g, 0.7mmol).

The "Swern" oxidation was repeated using the remaining quantity of 2,6-dimethylheptan-1-ol (6.0g, 42mmol). To minimise artifact formation, washing with acid etc. was avoided and the crude reaction product was purified by flash column chromatography on deactivated (8%) silica

(80g) according to the method of Still et al. (1978). Solvent was forced through the silica column under argon pressure (0.5kgcm^{-2}) at a flow rate of 30cm^{3}min^{-1}. Elution with hexane (100cm^3) removed non-polar material and hexane: Et$_2$0 (95:5; 200cm^3) removed the aldehyde. Solvent was evaporated (Buchi; 30°C) and 2,6-dimethylheptan-1-al (3.9g, 28mmol) characterised by LRMS, ^{1}H NMR and IR.

Gc purity : 91%

Yield   : 65%

LRMS (Fig. 3:7B)m/z: 142 (M$^{+\bullet}$,1%), 127 (M$^{+\bullet}$-CH$_3$,2%), 123 (4%), 71 (22%), 58 (100%).

^{1}H NMR (60MHz) (Fig. 3:8A) δ ppm:
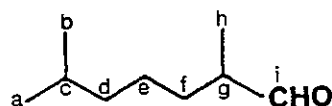
0.85 (d; 6H; J = 6Hz; a and b), 1.12 (d; 3H; J = 8Hz; h), 1.51 (m; 7H; c, d, e and f), 2.36 (m; 1H; g), 9.63 (d; 1H; J = 2Hz; i).

IR (liquid film) (Fig. 3:11B): aldehydic ν(C-H) 2710cm^{-1}, ν(C=O) 1730cm^{-1}, aldehydic δ(C-H) 1389cm^{-1}.

2.4.3.6 3,7-dimethyloctan-1-ol

3,7-dimethyloct-2,6-dien-1-ol (Geraniol; Aldrich; 6.0g,39mmol) was hydrogenated (hexane; 0.1g PtO$_2$.H$_2$0) to give a mixture of 3,7-dimethyloctan-1-ol, 3,7-dimethyloct-1-ene and 3,7-dimethyloctane. Solvent was evaporated (Buchi; 30°C) and the crude mixture purified by column chromatography on deactivated (4%) alumina (80g). Elution with hexane (150cm^3) removed the hydrocarbons and dichloromethane (200cm^3) afforded the alcohol. Removal of solvent (Buchi; 30°C)

afforded 3,7-dimethyloctan-1-ol (4.2g, 27mmol) which was assigned by LRMS (TMS ether, BSTFA), ^{1}H NMR and IR.

Gc purity : 99%

Yield   : 71%

LRMS (TMS ether) m/z: 215 ($M^+\cdot$-CH_3; 28%), 103 [CH_2 = $\overset{+}{O}Si$ $(CH_3)_3$, 40%], 89 (CH_2 = $\overset{+}{O}SiH(CH_3)_2$, 51%], 83 (100%), 73 (83%) .

^{1}H NMR (60MHz) δ ppm:
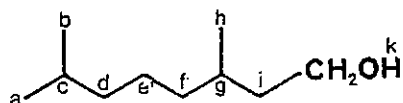
0.9 (d; 9H; J = 7Hz; a, b and h), 1.35 (m; 10H; c, d, e, f, g and i), 1.85 (s; 1H; k), 3.70 (t; 2H; J = 7Hz; j).

IR (liquid film): υ(O-H) 3320cm^{-1} intermolecularly bonded, δ_s(CH_3) gemdimethyl 1385cm^{-1} 1370cm^{-1}, υ(C-O) 1055cm^{-1} primary saturated.

2.4.3.7 1-bromo-3,7-dimethyloctane

This synthesis was performed using a modification of the method of Kamm and Marvel (1960). Concentrated H_2SO_4 (BDH Analar; 1.5g) was added carefully with stirring to 48% HBr (BDH Analar; 6.7g). Following the dropwise addition of 3,7-dimethyloctan-1-ol (4.2g, 27mmol) and a further aliquot of concentrated H_2SO_4 (1.0g), the reaction mixture was gently refluxed (24hr). When cool, hexane (15cm^3) and H_2O (15cm^3) were added and the mixture transferred to a separating funnel (100cm^3). The upper organic layer was removed and combined with washings (3x5cm^3; hexane) of the aqueous layer. Solvent was removed

(Buchi; 30°C) and the crude bromide purified by column chromatography on deactivated (4%) alumina (84g). Elution with hexane (200cm^3) and subsequent solvent evaporation (Buchi; 30°C) afforded 1-bromo-3,7-dimethyloctane (4.84g, 22mmol) which was characterised by LRMS, ^{1}H NMR and IR.

Gc purity : 98%

Yield : 82%

LRMS m/z: 220/222 (M$^{+\cdot}$,0.1%), 205/207 (M$^{+\cdot}$-CH$_3$,0.2%), 177/179 (9%), 149 (21%), 113 (37%), 71 (100%).

^{1}H NMR (60MHz) δ ppm:
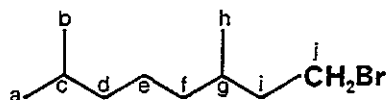
0.90 (d̲; 9H; J = 6Hz; a, b and h), 1.23 (m̲; 6H; d, e and f), 1.51 (m̲; 4H; c, g and i), 3.44 (t̲; 2H; J = 8Hz; j).

IR (liquid cell): δ_s(CH$_3$) gem dimethyl 1390 cm^{-1}, 1375cm^{-1}, ω(CH$_2$ - Br) 1260cm^{-1}.

2.4.2.8 1-bromo-3-methylpentane

1-bromo-3-methylpentane (9.4g, 57mmol after alumina column chromatography) was prepared from 3-methylpentan-1-ol (Sigma; 9.0g, 78mmol) using the modification of the method of Kamm and Marvel (1960) described in Section 2.4.3.7. The bromide was assigned by LRMS, ^{1}H NMR and IR.

Gc purity : 99%

Yield : 73%

LRMS m/z: 164/166 ($M^{+\cdot}$,1%), 135/137 (3%), 107/109 (5%), 85 (54%), 57 (100%).

^{1}H NMR (60Hz) δ ppm:
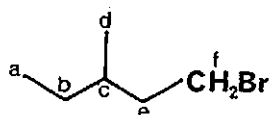
0.90 (<u>m</u>; 6H; a and d), 1.35 (<u>m</u>; 3H; b and c), 1.85 (<u>q</u>; 2H; e), 3.40 (<u>t</u>; 2H; J = 8Hz; f).

IR (liquid cell): δ_s(CH$_3$) 1380cm^{-1}, ω(CH$_2$Br) 1260cm^{-1}.

2.4.3.9 2,6,10,14-tetramethylpentadecan-7-ol

The Grignard synthesis was performed initially on a pilot scale using a standard procedure (Roberts <u>et</u> <u>al</u>., 1985). 1-bromo-3,7-dimethyloctane (323mg, 1.5mmol) in Et$_2$O (0.5cm^3, redistilled from LiAlH$_4$) was added to a RBF (10cm^3) containing Mg (41mg; freshly scraped from Mg ribbon) in Et$_2$O (2cm^2). Following the introduction of a crystal of iodine, the mixture was heated (30°C; water bath) and the formation of a cloudy precipitate observed. During subsequent reflux (1hr) the precipitate disappeared and the majority of the Mg scrapings were consumed. The reaction mixture was then cooled (0°C; ice bath), a solution of 2,6-dimethylheptan-1-al (200mg, 1.4mmol) in Et$_2$O (1cm^3) added and the mixture heated at reflux (4hr). When cool, ice water (2cm^3) and NH$_4$Cl (saturated solution; 2cm^3) were added and the mixture transferred to a separating funnel (100cm^3). The upper organic layer was removed and combined with washings (3x5cm^3; Et$_2$O) of the aqueous layer. Solvent was evaporated (Buchi; 30°C) and the crude products (240mg) purified by tlc (0.5mm silica gel; dichloromethane mobile phase). Following visualisation (Rhodamine 6G;

365μm) bands at R_f 0.89 - 1.00 (hydrocarbons; 24mg), R_f 0.80 - 0.88 (2,6,10,14-tetramethylpentadecan-7-one; 29mg) and R_f 0.58 - 0.65 (2,6,10,14-tetramethylpentadecan-7-ol; 60mg, 0.21mmol) were removed and the compounds recovered from the silica gel by desorption with dichloromethane (20cm^3). Solvent was evaporated (Buchi; 30°C) and the individual compounds transferred to vials to storage. 2,6,10,14-tetramethylpentadecan-7-ol from the pilot synthesis was assigned by LRMS of the TMS ether (TSIM; Sakauchi and Horning, 1971).

Gc purity : 84%

Yield   : 15%

LRMS : see below.

After consideration of the poor yield of the reaction above, the subsequent large scale preparation of 2,6,10,14-tetramethylpentadecan-7-ol) employed a different method (Kharasch and Reinmurth, 1954). Thus, 1-bromo-3,7-dimethyloctane (2.8g, 12mmol; 1.2 equivalent) in Et$_2$0 (12cm^3; 1:6 dilution) was added dropwise over the period of 1 hour to a RBF (100cm^3) containing Mg (305mg; 1.2 equivalents; freshly scraped from Mg ribbon) and iodine (3 crystals) in rapidly-stirred Et$_2$0 (30cm^3). During the course of the addition, a cloudy precipitate appeared and disappeared and the Mg scrapings were consumed. Following reflux (30min; water bath) the reaction mixture was cooled (0°C; ice bath) and 2,6-dimethylheptan-1-al (1.5g, 10mmol) in Et$_2$0 (10cm^3) added over 15 minutes. After a further period of reflux (3hr) the reaction mixture was cooled (0°C; ice bath) H$_2$0 (10cm^3) and NH$_4$Cl (saturated solution; 15cm^3) added and the mixture transferred to a separating funnel (250cm^3). The upper organic layer was removed and combined with washings (3x10cm^3,

Et$_2$O) of the aqueous layer. Solvent was evaporated (Buchi; 30°C) and the crude product purified by column chromatography on deactivated (4%) alumina (60g). Elution with hexane:Et$_2$O (150cm^2) removed non-polar material and dichloromethane (200cm^3) the alchohol. Solvent was evaporated and 2,6,10,14-tetramethylpentadecon-7-ol (1.6g,5.6mmol) assigned by IR and LRMS (TMS ether; TSIM).

Gc purity : 92%

Yield   : 57%

LRMS (TMS ether) (Fig. 3:12B) m/z: 341 (M$^{+\cdot}$-CH$_3$,2%),

243 (M$^{+\cdot}$-C$_8$H$_{17}$, 65%), 215 (M$^{+\cdot}$-C$_{10}$H$_{21}$, 36%), 129 (19%),

97 (69%), 83 (47%), 73 [(CH$_3$)$_3$Si$^+$;100%].

IR (liquid film): ν(O-H) 3605cm^{-1} free, ν(O-H) 3440cm^{-1} intermolecularly bonded, ν(C-O) 1140cm^{-1} saturated secondary, δ_s(CH$_3$)gemdimethyl 1390cm^{-1} 1375cm^{-1}.

2.4.3.10 2,6,10,14-tetramethylpentadecan-7-one

2,6,10,14-tetramethylpentadecan-7-one (1.23g, 4.3mmol after flash silica column chromatography) was prepared from 2,6,10,14-tetramethylpentadecan-7-ol (1.50g, 5.3mmol) using a modification of the method of Omura and Swern (1978) as described in section 2.4.3.5. The ketone was assigned by LRMS, ^{1}H NMR, ^{13}C NMR and IR.

Gc purity : 95%

Yield   : 83%

LRMS (Fig. 3.13B) m/z: 282 (M$^{+\cdot}$, 0.5%), 267 (M$^{+\cdot}$-CH$_3$, 0.3%),

198 (M$^{+\cdot}$-C$_6$H$_{12}$, 2%), 169 (11%), 156 (M$^{+\cdot}$-C$_9$H$_{18}$, 9%),

126 (23%), 72 [CH$_3$CH=C(CH$_3$)OH, 60%], 57 (81%), 43 (100%).

^{1}H NMR (400MHz) (Fig. 3.14A) δ ppm:
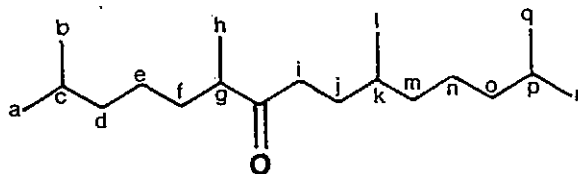
0.854 (d of d; 15H; J=6.5Hz; a,b,l,q and r), 1.041 (d; 3H; J=6.9Hz; h), 1.10-1.70 (m; 17H; c-f,j,k,m-p), 2.405 (t; 2H; J=9.0Hz;i), 2.515 (sextet; 1H; J=6.9Hz;g).

^{13}C NMR: see Table 3:4a.

IR (liquid film): ν(C=O) 1715cm^{-1}, δ_s(CH$_3$) gem dimethyl, 1385cm^{-1} 1370^{-1}, ν(C-CO-C) 1175cm^{-1}.

2.4.3.11 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol

The Grignard synthesis of 2,6,10,14-tetramethyl-7-(3-methylpentyl) pentadecan-7-ol was performed initially on a pilot scale using the method of Kharasch and Reinmuth (1954) (section 2.4.3.9). 1-bromo-3-methylpentane (100mg, 0.60mmol) in Et$_2$O (2cm^3; redistilled from LiAlH$_4$) was added dropwise to Mg scrapings (20mg; freshly prepared) and iodine (1 crystal) in rapidly stirred Et$_2$O (3cm^3). After reflux (30min) the reaction mixture was cooled and 2,6,10,14-tetramethylpentadecan-7-one (30mg, 0.11mmol) added. Following a further period of reflux (4hr), H$_2$O (1cm^3) and NH$_4$Cl (saturated solution; 1cm^3) were added and after stirring (16hr) the mixture transferred to a separating funnel (25cm^3). The upper organic layer was removed and combined with washings (3x5cm^3; Et$_2$O) of the aqueous layer. After drying (anhyd. Na$_2$SO$_4$;1g), solvent was evaporated (Buchi; 30°C) and the crude reaction products (53mg) purified by tlc [0.5mm silica gel; hexane: Et$_2$O (95.5) mobile phase].

Following visualisation (Rhodamine 6G; 365μm) bands at R_f 0.91 - 1.00 (hydrocarbons; 17mg), R_f 0.60 - 0.81 (unreacted 2,6,10,14-tetramethyl pentadecan-7-one; 18mg) and R_f 0.37 - 0.43 (2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol; 3.1mg, 0.006mmol) were removed and the products recovered from the silica gel by desorption with dichloromethane (20cm^3). The alcohol from the pilot synthesis was assigned by LRMS only.

Gc purity : 81%

Yield : 7%

LRMS: (see below)

The synthesis of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol was repeated using the "cerium chloride promoted Grignard addition" method of Imamoto et al. (1985). Initially performed on a pilot scale where a yield of 82% was recorded, this method was subsequently successfully employed in the large scale synthesis of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol as follows.

Part i: 1-bromo-3-methylpentane (0.80g, 4.8mmol; 1.4 equivalents) in THF (10cm^3; redistilled from LiAlH$_4$) was added dropwise over the period of 1 hour to Mg scrapings (0.13g; 1.5 equivalents; freshly prepared from Mg ribbon) and iodine (3 crystals) in rapidly stirred THF (15cm^3). During the course of the addition a cloudy white precipitate appeared and disappeared and the Mg scapings were consumed. Reflux (1hr) completed the preparation of 3-methylpentyl-1-magnesium bromide.

Part ii: CeCl$_3$7H$_2$O (1.30g; 1.3 equivalents) was quickly and finely powdered in a mortar and placed in a RBF (50cm^3). The water of crystallisation was removed by heating in vacuo (135-140°C; oil bath;

0.1mm Hg) for 1 hour, adding a magnetic stirrer and then stirring <u>in</u> <u>vacuo</u> at the same temperature for a further 1hr. Whilst still hot, Ar (dried over activated molecular sieve) was introduced, the flask cooled and THF ($20cm^3$; redistilled from $LiAlH_4$) added. Following stirring, the suspension was cooled (0°C; ice bath) and $25cm^3$ of the previously prepared (part <u>i</u>) THF solution of 3-methylpentyl-1-magnesium bromide carefully added. A further period of stirring (0°C; 1.5hr) was followed by the addition of 2,6,10,14-tetramethylpentadecan-7-one (0.90g, 3.9mmol) in THF ($5cm^3$). After stirring (0°C; 1hr) the mixture was transferred to a separating funnel ($100cm^3$) and treated with aqueous glacial acetic acid (4%; $50cm^3$). The organic layer was removed and the aqueous layer re-extracted with Et_2O ($2x10cm^3$). The combined organic extracts were washed successively with $NaHCO_3$ (saturated solution; $30cm^3$), brine ($30cm^3$) and H_2O ($20cm^3$). After drying (anhyd. Na_2SO_4;2g) solvent was removed (Buchi; 30°C) and the crude product purified by tlc [0.5mm silica gel; hexane: Et_2O (95:5) mobile phase]. Following visualisation (Rhodamine 6G; 365µm) bands at R_f 0.60-0.81 (unreacted 2,6,10,14-tetramethylpentadecan-7-one; 70mg) and R_f 0.37-0.50 (product alcohol) were removed and the compounds recovered from the silica gel by desorption with dichloromethane ($20cm^3$). Solvent was evaporated (Buchi; 30°C) and pure 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol (0.76g, 2.1mmol) assigned by LRMS, LRMS (TMS ether; TSIM), HRMS, ^{1}H NMR, ^{13}C NMR and IR.

Gc purity : 96%

Yield : 65%

LRMS (Fig. 3.19b) m/z: 350 ($M^{+\cdot}-H_2O$, 2%), 283 ($M^{+\cdot}-C_6H_{11}$, 6%),

255 ($M^{+\cdot}-C_8H_{17}$, 13%), 227 ($M^{+\cdot}-C_{10}H_{21}$, 11%),

140 (12%), 83 (52%), 57 (80%), 43 (100%).

LRMS (TMS ether) (Fig. 3.20b) m/z: 425 ($M^{+\cdot}-CH_3$, 0.4%),

355 ($M^{+\cdot}-C_6H_{13}$, 20%), 327 ($M^{+\cdot}-C_8H_{17}$, 61%),

299 ($M^{+\cdot}-C_{10}H_{21}$, 36%), 73 (100%).

HRMS: 255.2681 $C_{17}H_{35}O$ requires 255.2686

283.2989 $C_{19}H_{39}O$ requires 283.2999

^1H NMR (400MHz) δ ppm:
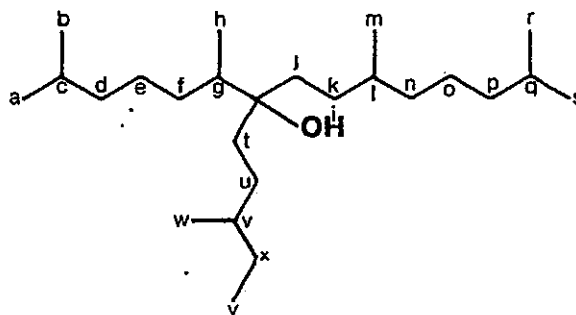
0.286 (m; 24H; J=6.5Hz; a,b,h,m,r,s,w and y), 1.00-1.50 (m; 28H; c-g, i-l, n-q, t-v and x).

^{13}C NMR: see Table 3.7A

IR (liquid film) (Fig. 3:21): ν(O-H) 3610cm^{-1} free, ν(O-H) 3430 intermolecularly bonded, ν(C-O) 1140cm^{-1} saturated, tertiary.

2.4.3.12 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecenes (br25:1)

2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol was dehydrated to a mixture of isomeric 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecenes by a modification of the method of Rinehart and Perkins (1963).

$POCl_3$ (BDH, 428mm^3) was added dropwise to a cooled (0°C; ice bath)

reacti-vial (5cm^3) containing 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol (300mg, 0.81mmol) and pyridine (Pearce silylation grade; 300mm^3). After stirring (24 hr; 25°C), the mixture was cooled and H$_2$0 (2cm^3) and hexane (2cm^3) added. The mixture was then transferred to a separating flask (25cm^3) where NaHCO$_3$ (saturated solution; 5cm^3) was added dropwise to destroy residual POCl$_3$. Extraction with hexane (3x10cm^3) and removal of solvent (Buchi; 30°C) afforded the crude products which were purified by tlc (3 x 0.5mm silica gel; hexane mobile phase). Following visualisation (Rhodamine 6G; 365μm), bands at R$_f$ 0.91 - 1.00 (alkenes) and R$_f$ 0.61-0.70 (unreacted 2,6,10,14-tetramethyl-7-(3-methylpentyl) pentadecan-7-ol; 126mg) were removed and the compounds recovered from the silica gel by desorption with dichloromethane (15cm^3). Solvent was evaporated (Buchi; 30°C) and the isomeric 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecenes (158mg, 0.45mmol) examined by gcms. The RI and mass spectrum of each of the alkene peaks recorded is listed in Table 3:10. Isomers were not assigned further.

2.4.3.13 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0)

The isomeric mixture of 2,6,10,14-tetramethyl-7-(3-methylpentyl) pentadecenes (100mg, 0.29mmol) were smoothly hydrogenated by bubbling hydrogen gas (20cm^{3}min^{-1}; 1hr) through a hexane solution (10cm^3; 20°C) containing PtO$_2$.H$_2$0 (20mg). Following filtration, solvent was evaporated (Buchi; 30°C) and the crude products purified by argentatious tlc [0.5mm silica gel (5% AgNO$_3$): hexane mobile phase]. Following visualisation (Rhodamine 6G; 365μm), the alkane band (R$_f$=0.93-1.00) was removed and the alkane recovered from the silica gel by desorption with dichloromethane (20cm^3). Solvent was

evaporated (Buchi; 30°C) and 2,6,10,14-tetramethyl-7-(3-methylpentyl)

pentadecane (97mg, 0.28mmol) assigned by LRMS, HRMS, ^{1}H NMR, ^{13}C NMR

and IR.

Gc purity : 96%

Yield   : 97%

LRMS (Fig. 3:26B)m/z: 267 ($M^{+\cdot}-C_6H_{13}$, 0.8%),

266 ($C_{19}H_{38}$,0.6%), 239 ($M^{+\cdot}-C_8H_{17}$, 4%), 238 ($C_{17}H_{34}$, 8%),

211 ($M^{+\cdot}-C_{10}H_{21}$, 2%), 210 ($C_{15}H_{30}$, 2.5%), 197 (2%), 183 (5%),

127 (10%), 113 (12%), 71 (65%), 57 (100%).

HRMS: 267.3066 $C_{19}H_{39}$ requires   267.3058;   238.2669 $C_{17}H_{34}$

requires 238.2666

^{1}H NMR (400MHz)(Fig. 3:28B) δ ppm:
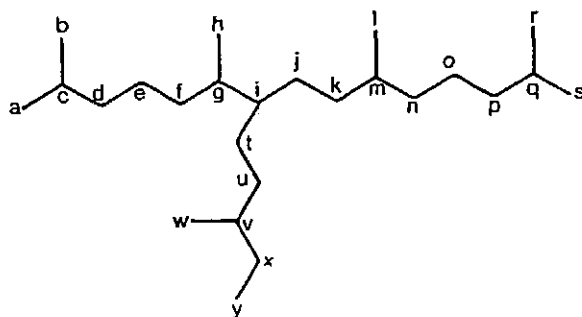
0.763 (<u>septet</u>; 3H; J=2.7Hz; one of a,b,h,l,r,s or w),

0.845 (<u>m</u>; 21H; J=6.6Hz; remainder of - CH_3),

1.00-1.40 (<u>m</u>; 25H; d-f,j,k,n-p,t,u,x and 3 - CH of c,m,q or v),

1.52 (<u>septet</u>; 3H; J=6.6Hz, g,i and one - CH from c,m,q or v).

^{13}C NMR: see Table 3:9A

IR (liquid film): $\nu_{as}(CH_3)$ 2962cm^{-1}, $\nu_{as}(CH_2)$ 2920cm^{-1},

$\nu_s(CH_2)$ 2875cm^{-1}, $\delta_s(CH_2)$, $\delta_{as}(CH_3)$ 1460cm^{-1},

$\delta_s(CH_3)$ gem dimethyl 1385cm^{-1}, 1370cm^{-1}.

2.4.4 Synthesis of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (br20:0)

2.4.4.1 Starting materials

The authenticity of commercial 1-bromo-3-methylbutane (Aldrich) used in the preparation of 2,6,10-trimethylundecan-7-ol was checked by LRMS, ^{1}H NMR and IR.

Gc. purity : 98%

LRMS m/z: 150/152 ($M^{+\cdot}$, 1%), 135/137 ($M^{+\cdot}$-CH_3, 3%),

107/109 ($M^{+\cdot}$-C_3H_7, 6%), 85 (41%), 57 (100%).

^{1}H NMR (60MHz) δppm:
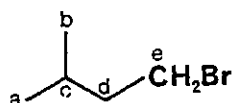
0.89 (<u>d</u>; 6H; J=6Hz; a and b), 1.50 (<u>m</u>; 3H; c and d), 3.32 (<u>t</u>; 2H; J=9Hz; e).

IR (liquid film):$\delta_s(CH_3)$ gem dimethyl, 1390cm^{-1} 1375cm^{-1},

$\omega(CH_2$-Br) 1265cm^{-1}.

2.4.4.2 2,6,10-trimethylundecan-7-ol

2,6,10-trimethylundecan-7-ol was prepared by Grignard synthesis (Kharasch and Reinmurh, 1954; described in section 2.4.3.9) from 1-bromo-3-methylbutane (Aldrich; 0.67g, 4.9mmol), Mg (0.11g, freshly scraped from Mg ribbon) and 2,6-dimethylheptan-1-al (0.50g, 3.5mmol) in Et_2O (redistilled from LiAlH$_4$). The crude products were purified by column chromatography on deactivated (4%) silica (42g). Elution with hexane:Et_2O (95.5; 100cm^3) removed non-polar material and dichloromethane (100cm^3) the alcohol. Solvent was removed (Buchi;

30°C) and 2,6,10-trimethylundecan-7-ol (0.43g, 1.5mmol) assigned by IR and LRMS (TMS ether; TSIM).

Gc purity : 89%

Yield : 58%

LRMS (TMS ether) (Fig. 3:11A)m/z: 285 ($M^{+\bullet}$-1, 0.25%), 271 ($M^{+\bullet}$-CH_3, 6%), 215 (18%), 173 (100%), 147 (26%), 83 (49%), 73 (96%).

IR (liquid film): ν (O-H) 3380cm^{-1} intermolecularly bonded, $\delta_{as}(CH_3)$, $\delta_s(CH_2)$ 1450cm^{-1}, δ_s (CH_3) gem dimethyl 1385cm^{-1} 1370cm^{-1}.

2.4.4.3 2,6,10-trimethylundecan-7-one

2,6,10-trimethylundecan-7-one (330mg; 1.2mmol after flash silica column chromatography) was prepared from 2,6,10-trimethylundecan-7-ol (400mg; 1.4mmol) using a modification of the method of Omura and Swern (1978) as described in section 2.4.3.5. The ketone was assigned by LRMS, ^{13}C NMR and IR.

Gc purity = 88%

Yield = 83%

LRMS (Fig. 3:13A)m/z: 212 ($M^{+\bullet}$, 2.2%), 197 ($M^{+\bullet}$-CH_3, 1.1%), 156 (5%), 141 (9%), 128 (26%), 99 (61%), 72 [CH_3CH=(CH_3)OH, 100%].

^{13}C NMR: see Table 3:3A.

IR (liquid film): ν (C=O) 1720cm^{-1}, $\delta_s(CH_3)$ gem dimethyl 1385cm^{-1} 1370cm^{-1}, ν(C-CO-C) 1170cm^{-1}.

2.4.4.4 2,6,10-trimethyl-7-(3-methylbutyl)dodecan-7-ol

2,6,10-trimethyl-7-(3-methylbutyl)dodecan-7-ol was prepared by "cerium chloride promoted Grignard" synthesis (Imamoto et al., 1985) from 1-bromo-3-methylpentane (337mg, 2.0mmol), Mg (47mg freshly scraped from Mg ribbon) $CeCl_3.7H_2O$ (780mg) and 2,6,10-trimethylundecan-7-one (300mg, 1.4mmol) in THF (redistilled from $LiAlH_4$) using the method described in section 2.4.3.11. The crude products were purified by column chromatography on deactivated (6%) silica (34g). Elution with hexane ($150cm^3$) removed the non-polar material and dichloromethane ($200cm^3$) afforded the alcohol. Following solvent evaporation (Buchi; 30°C), the residual 2,6,10-trimethyl-7-(3-methylbutyl)dodecan-7-ol (285 mg, 0.96mmol) was assigned by LRMS, LRMS (TMS ether; TSIM) HRMS, ^{1}H NMR, ^{13}C NMR and IR.

Gc purity = 91%

Yield   = 68%

LRMS (Fig. 3:19A)m/z: 280 ($M^{+\cdot}-H_2O$, 2%), 227 ($M^{+\cdot}-CH_5H_{11}$, 18%), 213 ($M^{+\cdot}-C_6H_{13}$, 28%), 185 ($M^{+\cdot}-C_8H_{17}$, 73%), 111 (17%), 83 (60%), 69 (94%), 43 (100%).

LRMS (TMS ether) (Fig. 3.20A)m/z: 369 ($M^{+\cdot}-1$, 0.1%), 355 ($M^{+\cdot}-CH_3$, 3%), 299 ($M^{+\cdot} - C_5H_{11}$, 31%), 285 ($M^{+\cdot}-C_6H_{13}$, 43%), 257 ($M^{+\cdot}-C_8H_{17}$, 100%), 73 (97%).

HRMS: 185.1906 $C_{12}H_{25}O$ requires 185.1904, 213.2213 $C_{14}H_{29}O$ requires 213.2218, 227.2376 $C_{15}H_{31}O$ requires 227.2373.

^{1}H NMR (400MHz) δ ppm:
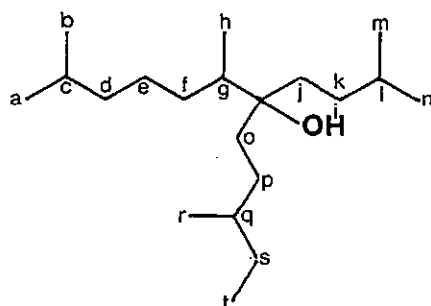
0.865 (d of d; 18H, J=7Hz; a,b,m,n,r and t), 0.890 (d; 3H; J=7Hz; h),
1.01-1.63 (m; 21H; c-g, i-l, o-q and s).

^{13}C NMR: see Table 3:6A.

IR (liquid film): ν(O-H) 3600cm^{-1} free, ν(O-H)3480cm^{-1}
intermolecularly bonded, δ_{as}(CH$_3$) δ_s(CH$_2$) 1450cm^{-1},
δ_s(CH$_3$) gem dimethyl 1385cm^{-1} 1370cm^{-1}.

2.4.4.5 2,6,10-trimethyl-7-(3-methylbutyl)dodecenes (br20:1)

2,6,10-trimethyl-7-(3-methylbutyl)dodecan-7-ol (100mg, 0.34mmol) was
dehydrated to a mixture of isomeric 2,6,10-trimethyl-7-(3-methylbutyl)
dodecenes (86.3mg, 0.30mmol after tlc) by the modified Rinehart and
Perkins (1963) method described in section 2.4.3.12. The RI and mass
spectrum of each of the alkene peaks recorded by gcms is listed in
Table 3:9. Isomers were not assigned further.

2.4.4.6 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (br20:0)

Hydrogenation (hexane; 0.05g PtO$_2$.H$_2$O) of the isomeric mixture of
2,6,10-trimethyl-7-(3-methylbutyl)dodecenes (50mg, 0.18mmol) by the
method described in section 2.4.3.13 afforded 2,6,10-trimethyl-7-(3-
methylbutyl)dodecane (49.6mg, 0.18mmol) which was assigned by LRMS,
HRMS, ^{1}H NMR, ^{13}C NMR and IR.

Gc purity : 97%

Yield   : 98%

LRMS (Fig. 3:26A)m/z: 211 ($M^{+\bullet}$-C_5H_{11}, 0.1%), 197

($M^{+\bullet}$-C_6H_{13}, 0.8%), 196 ($C_{14}H_{28}$, 0.6%), 169 ($M^{+\bullet}$-C_8H_{17};

8%), 168 ($C_{12}H_{24}$, 13%), 127 (10%), 113 (14%), 85 (36%), 71 (64%),

57 (100%).

HRMS: 211.2438 $C_{15}H_{31}$ requires   211.2434,   197.2277 $C_{14}H_{29}$

requires 197.2274, 168.1884 $C_{12}H_{24}$ requires 168.1884.

^{1}H NMR (400MHz) (Fig. 3:29A) δppm:
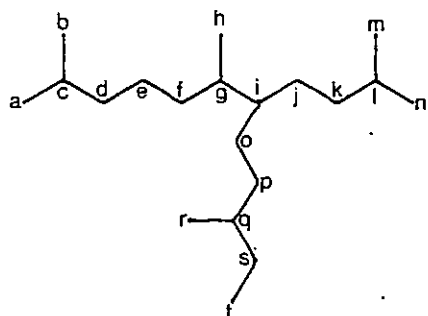
0.765 (<u>d</u> of <u>d</u>; 3H; h,r or t), 0.865 (<u>m</u>; 18H; J=6.6Hz; a,b,m,n, and 2-

CH_3 from h,r or t), 1.01-1.42 (<u>m</u>; 18H; d-f, j,k,o,p,s and 2-CH from

c,l or q), 1.51 (<u>m</u>; 3H; J=6.6Hz; g,i and one-CH from c,l or q).

IR (liquid film): $\nu_{as}(CH_3)$ 2960cm^{-1}, $\nu_{as}(CH_2)$ 2920cm^{-1},

$\nu_s(CH_2)$ 2875cm^{-1}, $\delta_s(CH_2)$, $\delta_{as}(CH_3)$ 1460cm^{-1},

$\delta_s(CH_3)$ gem dimethyl 1385cm^{-1}, 1370cm^{-1}.

2.4.5

Synthesis of 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane

2.4.5.1 Starting material

The authenticity of the 3,7,11-trimethyldodecan-2,6,10-trien-1-ol

(Aldrich; Farnesol) used in the preparation of 1-bromo-3,7,11-

trimethyldodecane was checked by LRMS (TMS ether; BSTFA), ^{1}H NMR and

IR.

Gc purity = 100%

LRMS (TMS ether)m/z: 294 ($M^{+\cdot}$, 0.7%), 279 ($M^{+\cdot}$-CH_3, 0.3%),

143 (11%), 107 (17%), 73 (88%), 69 (100%).

^1H NMR (60MHz) δ ppm:
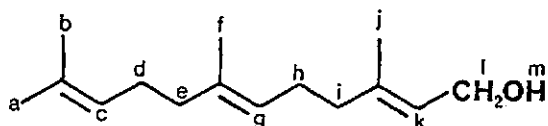
1.65 (s; 12H; a,b,f and j), 2.01 (m; 9H; d,e,h,i and m), 4.13 (d; 2H;

J=6Hz; 1), 5.32 (m; 3H; c,g and k).

IR (liquid film): ν (C-H) 3320cm^{-1} intermolecularly bonded, ν (C=C)

1660cm^{-1}, ν (C-O) 1010cm^{-1} primary, unsaturated alcohol.

2.4.5.2 3,7,11-trimethyldodecan-1-ol

3,7,11-trimethyldodecan-2,6,10-trien-1-ol (Aldrich: 2.5g, 11mmol) was

hydrogenated (hexane; 0.1g $PtO_2 \cdot H_2O$; see section 2.4.3.2) to give a

mixture of 3,7,11-trimethyldodecan-1-ol, 3,7,11-trimethyldodecane

(farnesane) and various C_{15} alkenes. The crude reaction products

were purified by column chromatography on deactivated (4%) alumina

(85g). Elution with hexane (150cm^3) removed the hydrocarbons and

dichloromethane (200cm^3) afforded 3,7,11-trimethyldodecan-1-ol (1.0g,

4.3mmol) which was assigned by LRMS (TMS ether; BSTFA), ^1H NMR and

IR.

Gc purity = 90%

Yield   = 39%

LRMS (TMS ether)m/z: 285 ($M^{+\cdot}$-CH_3, 34%), 125 (14%), 111 (31%),

103 [$CH_2=\overset{+}{O}Si(CH_3)_3$, 57%], 83 (71%), 57 (100%).

^{1}H NMR (60MHz) δ ppm:
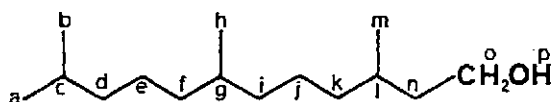
0.89 (<u>d</u>; 12H; J=6Hz; a,b,h and m), 1.35 (<u>m</u>; 18H; c-g,i-l,n and p), 3.70 (<u>t</u>; 2H; J=7Hz; o).

IR (liquid film): ν (O-H) 3320cm^{-1} intermolecularly bonded, ν(C-O) 1055cm^{-1} primary saturated, δ_s(CH$_3$) gem dimethyl 1385cm^{-1} 1370cm^{-1}.

2.4.5.3 1-bromo-3,7,11-trimethyldodecane

1-bromo-3,7,11-trimethyldodecane (0.67g, 2.3mmol after alumina column chromatography) was prepared from 3,7,11-trimethyldodecan-1-ol (1.0g, 4.3mmol) using the modification of the method of Kamm and Marvel (1963) described in section 2.4.2.6. The bromide was assigned by LRMS, ^{1}H NMR and IR.

Gc purity = 95%

Yield: = 47%

LRMS m/z: 290/292 (M$^{+\cdot}$, 0.3%), 275/277 (M$^{+\cdot}$-CH$_3$, 0.5%),

249/251 (M$^{+\cdot}$-C$_3$H$_7$, 10%), 219/221 (M$^{+\cdot}$-C$_5$H$_{11}$; 21%),

177/179 (M$^{+\cdot}$-C$_8$H$_{17}$, 36%), 113 (41%), 71 (100%), 57 (91%).

^{1}H NMR (60MHz) δ ppm:
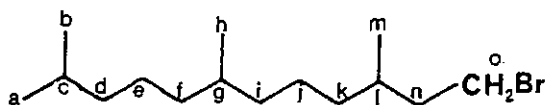
0.89 (<u>d</u>; 12H, J=7Hz; a,b,h and m), 1.25 (<u>m</u>; 12H; d,e,f,i,j and k), 1.50 (<u>m</u>; 5H; c,g,l and n), 3.40 (<u>t</u>; 2H; J=8Hz; o).

IR (liquid film): δ_s(CH$_3$) gem dimethyl 1385cm^{-1} 1370cm^{-1}, ω(CH$_2$-Br) 1255cm^{-1}.

2.4.5.4 2,6,10,14,18-pentamethylnonadecan-7-ol

2,6,10,14,18-pentamethylnonadecan-7-ol was prepared by Grignard synthesis (Kharasch and Reinmurth, 1954) from 1-bromo-3,7,11-trimethyldodecane (670mg, 2.3mmol), Mg (56mg, freshly scraped from Mg ribbon) and 2,6-dimethylheptan-1-al (270mg, 1.9mmol) in Et_2O (redistilled from $LiAlH_4$). The crude reaction products were purified by column chromatography on deactivated (5%) silica (41g). Elution with hexane:Et_2O (95.5; 100%) removed the non-polar material (hydrocarbons) and dichloromethane (150cm^3) the alcohol. Solvent was evaporated (Buchi; 30°C) and 2,6,10,14,18-pentamethylnonadecan-7-ol (433mg, 1.23mmol) assigned by LRMS (TMS ether; TSIM) and IR.

Gc purity : 87%

Yield   : 62%

LRMS (TMS ether) (Fig. 3:11C)m/z: 425 ($M^{+\cdot}$-1, 0.1%), 411 ($M^{+\cdot}$-CH_3, 1%), 313 (C_8H_{17}, 43%), 215 ($M^{+\cdot}$-$C_{15}H_{31}$, 32%), 129 (25%), 83 (33%), 73 (100%).

IR (liquid film): ν (O-H) 3380cm^{-1} intermolecularly bonded, $\delta_{as}(CH_3)$, $\delta_s(CH_2)$ 1455cm^{-1}, $\delta_s(CH_3)$ gem dimethyl 1390cm^{-1} 1375cm^{-1}, ν(C-O) 1060cm^{-1}, saturated secondary.

2.4.5.5 2,6,10,14,18-pentamethylnonadecan-7-one

2,6,10,14,18-pentamethylnonadecan-7-one (269mg, 0.84mmol after flash silica column chromatography) was prepared from 2,6,10,14,18-pentamethylnonadecan-7-ol (400mg, 1.1mmol) using a modification of the method of Omura and Swern (1978) as described in section 2.4.3.5. The ketone was assigned by LRMS, ^{13}C NMR and IR.

Gc purity = 91%

Yield   = 67%

LRMS (Fig. 3.13C) m/z: 352 ($M^{+\cdot}$, 2.2%), 337 ($M^{+\cdot}$ - CH_3, 2.1%),

268 (1.4%), 239 ($M^{+\cdot}$ - C_6H_{13}; 7%), 196 (16%), 156 (12%),

85 (32%), 43 (100%)

^{13}C NMR: see Table 3:5A.

IR (liquid film): ν(C=O) 1720cm^{-1}, δ_s(CH_2-CO) 1405cm^{-1},

δ_s(CH_3) gem dimethyl 1390cm^{-1} 1375cm^{-1}, ν(C-CO-C) 1165cm^{-1}.

2.4.5.6 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecan-7-ol

2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecan-7-ol was prepared by "cerium chloride promoted" Grignard synthesis (Imamoto et al., 1985) from 1-bromo-3-methylpentane (176mg, 1.1mmol), Mg (28mg, freshly scraped from Mg ribbon), $CeCl_3 \cdot 7H_2O$ (431mg) and 2,6,10,14,18-pentamethylnonadecan-7-one (250mg, 0.71mmol) in THF (redistilled from LiAlH$_4$) using the method described in section 2.4.3.11. The crude products were purified by column chromatography on deactivated (4%) silica (20g). Non-polar material was removed with hexane:Et$_2$O (95:5; 150cm^3) and the alcohol eluted with dichloromethane (150cm^3). Solvent was evaporated (Buchi; 30°C) and 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecan-7-ol (239mg, 0.54mmol) was assigned by LRMS, LRMS (TMS ether; TSIM), HRMS, ^{1}H NMR, ^{13}C NMR and IR.

Gc purity : 89%

Yield   : 76%

LRMS (Fig. 3:14C) m/z: 420 ($M^{+\cdot}$-H_2O), 353 ($M^{+\cdot}$-C_6H_{13}, 9%),

325 ($M^{+\cdot}$-C_8H_{17}, 16%), 227 ($M^{+\cdot}$-$C_{15}H_{31}$, 21%), 140 (15%), 83,

57 (100%).

LRMS (TMS ether) m/z: 509 ($M^{+\cdot}$-1, 0.1%), 495 ($M^{+\cdot}$-CH_3, 2%),

425 ($M^{+\cdot}$-C_6H_{13}, 29%), 397 ($M^{+\cdot}$-C_8H_{17}, 41%), 299

($M^{+\cdot}$-$C_{15}H_{31}$, 61%), 73 (100%).

HRMS: 227.2370 $C_{15}H_{31}O$ requires 227.2373, 325.3466 $C_{22}H_{45}O$ requires 325.3468, 353.3778 $C_{24}H_{49}O$ requires 353.3781.

^{1}H NMR (400MHz) δppm:
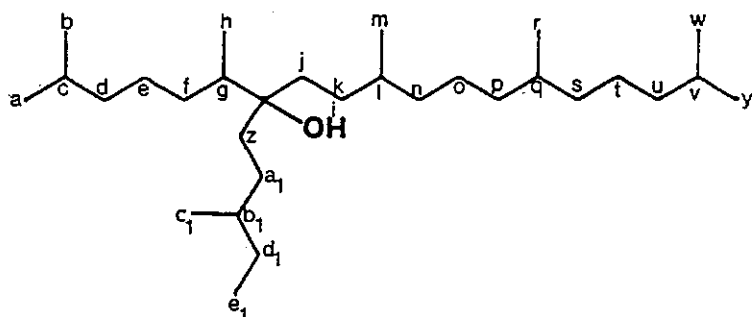
0.860 (<u>d</u> of <u>t</u>; 27H; J=6.6Hz; a,b,h,m,r,w,y,c_1 and e,), 1.00 - 1.60 (<u>m</u>, 35H; c-g, i-l, n-q, s-v, z, a_1,b_1 and d_1)

^{13}C NMR (400MHz): see Table 3:9A.

IR (liquid film): ν (O-H) 3620cm^{-1} free, ν(O-H) 3480^{-1} intermolecularly bonded, δ_{as}(CH$_3$), δ_s(CH$_2$) 1460cm^{-1}, ν (C-O) 1155cm^{-1} saturated tertiary.

2.4.5.7

2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecenes (br30:1)

2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecan-7-ol (100mg 0.23mmol) was dehydrated to a mixture of isomeric 2,6,10,14,18-penta-methyl-7-(3-methylpentyl)nonadecenes (57mg, 0.14mmol after tlc) by the modified Rinehart and Perkins (1963) method described in section 2.4.3.12. The RI and mass spectrum of each of the alkene peaks recorded by gcms is listed in Table 3:11. Isomers were not assigned further.

Gc purity = 93%

Yield = 59%

92

2.4.5.8

2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane (br30:0)

Hydrogenation (hexane; 0.05g $PtO_2.H_2O$) of the isomeric mixture of 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecenes (50mg, 0.12mmol) afforded 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane (33mg, 0.079mmol) which was assigned by LRMS, HRMS, ^{1}H NMR, ^{13}C NMR and IR.

Gc purity = 91%

Yield = 65%

LRMS (Fig. 3:26C)m/z: 337 ($M^{+\cdot}$-C_6H_{13}, 0.1%), 336 ($C_{24}H_{48}$, 0.1%), 309 ($M^{+\cdot}$-C_8H_{17} 2%), 308 ($C_{22}H_{44}$, 4%), 155 (7%), 127 (10%), 85 (36%), 57 (100%).

HRMS: 336.3758 $C_{24}H_{48}$ requires 336.3754, 308.3435 $C_{22}H_{44}$ requires 308.3441.

^{1}H NMR (400MHz) (Fig. 3:28C) δppm:
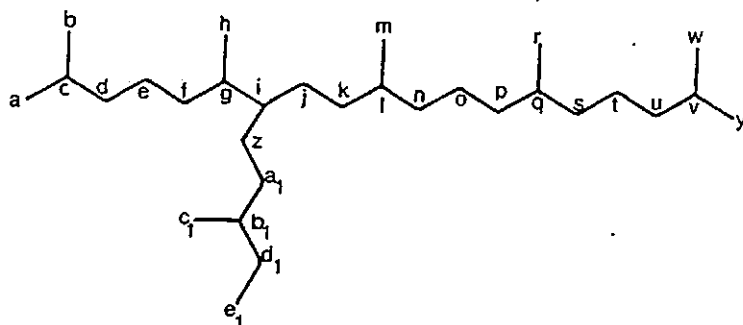
0.765 (quintet; 3H, one of a,b,h,m,r,w,y,c_1 or e_1), 0.850 (t; 24H; J=6.6Hz; remainder of -CH_3), 1.00-1.40 (m; 32H; d-f,j,k,n-p,s-u,z,a_1, d_1 and 4-CH from c,l,q,v or b_1), 1.40-1.60 (septet; 3H; J=6Hz, g,i and one other -CH from c,l,q,v or b_1).

^{13}C NMR (400MHz): see Table 3:15A.

IR (liquid film): $v_{as}(CH_3)$ 2960cm^{-1}, $v_{as}(CH_2)$ 2915cm^{-1}, $v_s(CH_2)$ 2875cm^{-1}, $\delta_s(CH_2)$ $\delta_{as}(CH_3)$ 1465cm^{-1}, $\delta_s(CH_3)$gem dimethyl 1385cm^{-1} 1370cm^{-1}.

2.4.6 Synthesis of 2,6,9,13-tetramethyltetradecane

2.4.6.1 2,6,9,13-tetramethyltetradecan-6-ol

2,6,9,13-tetramethyltetradecan-6-ol was prepared by Grignard synthesis (Kharasch and Reinmurth, 1954) from 1-bromo-3,7-dimethyloctane (611mg, 2.8mmol), Mg (66mg, freshly prepared from Mg ribbon) and 6-methylheptan-2-one (250mg, 1.98mmol) in Et_2O (redistilled from $LiAlH_4$). The crude products were purified by column chromatography on deactivated (10%) alumina (25g). Elution with hexane ($100cm^3$) removed non-polar material and hexane:Et_2O ($100cm^3$) the 2,6,9,13-tetramethyltetradecan-6-ol (333mg, 1.2mmol) which was assigned by LRMS, LRMS (TMS ether; TSIM), ^1H NMR, ^{13}C NMR and IR.

Gc purity : 88%

Yield : 62%

LRMS (Fig. 6:4A)m/z: 255 ($M^{+\cdot}-CH_3$, 1.2%), 252 ($M^{+\cdot}-H_2O$, 1.0%), 185 ($M^{+\cdot}-C_6H_{13}$, 18%), 129 ($M^{+\cdot}-C_{10}H_{21}$, 43%), 111 (28%), 83 (26%), 69 (100%).

LRMS (TMS ether) (Fig. 6:4B)m/z: 327 ($M^{+\cdot}-CH_3$, 8%), 257 ($M^{+\cdot}-C_6H_{13}$, 64%), 201 ($M^{+\cdot}-C_{10}H_{21}$, 100%), 143 (12%), 73 (100%).

^1H NMR (60Mz) δ ppm:
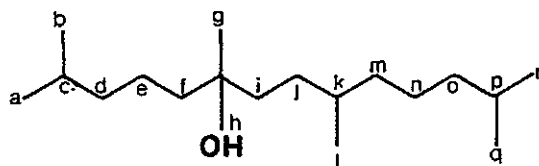
0.89 (d; 15H; J=7Hz; a,b,l,q and r), 1.18 (s; 3H; g), 1.19-1.80 (m; 19H; c-f,i-k, and m-p), 2.15 (s; 1H; h).

^{13}C NMR (400MHz): see Table 6:1A.

IR (liquid film): v(O-H) $3400cm^{-1}$ intermolecularly bonded, δ_s(CH_3) gem dimethyl $1390cm^{-1}$ $1375cm^{-1}$,v(C-O) $1170cm^{-1}$ satuated,tertiary.

2.4.6.2 2,6,9,13-tetramethyltetradecenes

2,6,9,13-tetramethyltetradecan-6-ol (150mg, 0.55mmol) was dehydrated to

a mixture of isomeric 2,6,9,13-tetramethyltetradecenes (99mg, 0.39mmol

after tlc) by the modified method of Rinehart and Perkins (1963). The

RI and low resolution mass spectra of each of the alkene peaks recorded

upon examination by gcms are listed in Table 6:2.

Gc purity = 100%   .

Yield   = 71%

2.4.6.3 2,6,9,13-tetramethyltetradecane

The isomeric mixture of 2,6,9,13-tetramethyltetradecenes (80mg,

0.32mmol) was hydrogenated by bubbling H_2 gas through a hexane

solution $(10cm^3;$ 20°C) containing $PtO_2.H_2O$ (2mg).   Following

filtration, solvent was evaporated (Buchi; 30°C) and the crude product

purified by argentatious tlc [0.5mm silica gel (5% $AgNO_3$); hexane

mobile phase]. Following visualisation (Rhodamine 6G; 365μm), bands at

R_f 0.93-1.00 (2,6,9,13-tetramethyltetradecane; 51mg, 0.20mmol) and

R_f 0.43-0.48 (2,6,9,13-tetramethyltetradecene; 2.5mg) were removed

and the hydrocarbons recovered from the silica gel by desorption with

dichloromethane $(20cm^3)$.   Following solvent evaporation (Buchi;

30°C), 2,6,9,13-tetramethyltetradecane was assigned by LRMS, ^{1}H NMR,

^{13}C NMR and IR. Gc of the band at 0.43-0.48 produced only one peak.

Attempts to assign the position of the double bond of this monoene by

^{1}H NMR (400MHz) and methoxy-mercuration (Blomquist et al., 1980) were

unsuccessful.

Alkane gc purity = 99%

Yield = 63%

*Pagination error
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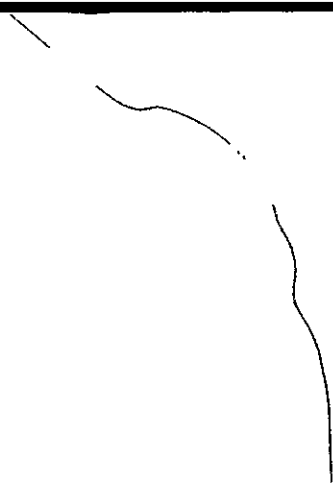
LRMS (Fig. 6:8B)m/z: 254 (M$^{+\bullet}$, 0.05%), 239 (M$^{+\bullet}$-15, 0.1%),

169 (M$^{+\bullet}$-C$_6$H$_{13}$, 6%), 127 (4%), 113 (12%), 85 (23%), 57 (100%).

^{1}H NMR (60MHz) δ ppm:
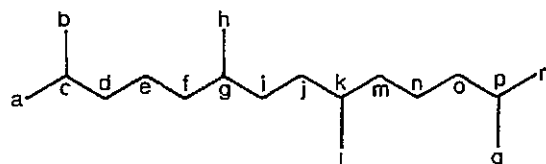
0.89 (<u>d</u>; 18H; J=7Hz; a,b,h,l,q and r), 1.00-1.55 (<u>m</u>; 20H; c-g, i-k and m-p).

^{13}C NMR (400MHz): see Table 6:3A.

IR (liquid film): ν_{as}(CH$_3$)2955cm^{-1}, ν_{as}(CH$_2$) 2925cm^{-1}, ν_s(CH$_3$) 2855cm^{-1}, δ_s(CH$_3$) gem dimethyl 1390cm^{-1} 1375cm^{-1}.

2.4.7 Synthesis of 2,6,11,15-tetramethylhexadecane

2,6,11,15-tetramethylhexadecane was a by-product in the Grignard syntheses of 2,6,10,14-tetramethylpentadecan-7-ol (section 2.4.4.9) and of 2,6,9,13-tetramethyltetradecan-6-ol (section 2.4.5.1). The alkane was separated from the respective alcohols by alumina column chromatography (hexane eluent) and further purified by argentatious tlc [0.5mm silica gel (5% AgNO$_3$); hexane mobile phase]. After visualisation (Rhodamine 6G; 365μm), the band at R$_f$ 0.93-1.00 was removed and the alkane recovered from the silica gel by desorption with dichloromethane (20cm^3). Solvent was evaporated (Buchi; 30°C) and 2,6,11,15-tetramethylhexadecane (23mg) assigned by LRMS, ^{1}H NMR, ^{13}C NMR and IR.

Gc purity = 99%

LRMSm/z: 267 (M$^{+\bullet}$-CH$_3$, 1%), 239 (M$^{+\bullet}$-C$_3$H$_7$, 1%),

197 (M$^{+\bullet}$-C$_6$H$_{13}$, 7%), 169 (M$^{+\bullet}$-C$_8$H$_{17}$, 8%), 127 (7%),

113 (20%), 57 (100%).

^{1}H NMR (60MHz) δ ppm:
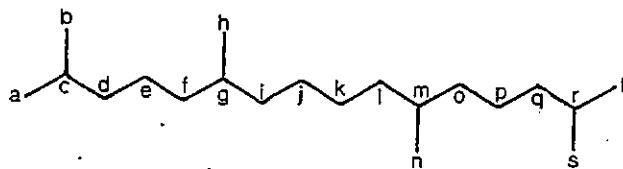
0.85 (<u>d</u>; 18H; J=7Hz; a,b,h,n,s and t), 1.0-1.55 (<u>m</u>; 24H, c-g, i-m and o-r).

^{13}C NMR (400MHz): see Table 6:4.

IR (liquid film): $\nu_{as}(CH_3)$ 2965cm^{-1}, $\nu_{as}(CH_2)$ 2860cm^{-1}, $\delta_s(CH_3)$ gem dimethyl 1390cm^{-1} 1375cm^{-1}.

2.4.8 Synthesis of 8α (H) $9\alpha\beta$ (H)11-tetrahydrogeranyldrimane

2.4.8.1 Starting materials

9α(H)-drimenol (Prof. C. Brooks,. Glasgow University) was assigned by LRMS and IR.

Gc purity = 99%

LRMSm/z: 222 (M$^{+\cdot}$, 6%), 207 (M$^{+\cdot}$-CH$_3$, 1%), 191 (7%), 124 (37%), 109 (100%), 81 (23%), 41 (41%).

IR (KBr disc): ν(O-H) 3380cm^{-1} intermolecularly bonded, ν(C=C) 1675cm^{-1}, $\delta_{as}(CH_3)$ 1460cm^{-1}, $\delta_s(CH_2)$ 1440cm^{-1}, ν(C-O) 1035cm^{-1} primary unsaturated.

2.4.8.2 $8\alpha\beta$(B) 9α(H)-drimanol

Hydrogenation (hexane; 2mg PtO$_2$.H$_2$O) of 9α(H)-drimenol (0.5g, 2.3mmol) afforded a isomeric mixture of 8α(H) 9α(H)-drimanol (85% by gc) and 8β(H) 9α(H)-drimanol (15% by gc) (0.45g,. 2.0mmol after column

chromatography on deactivated alumina). The alcohols were assigned by LRMS, ^{13}C NMR and IR.

Gc purity = 94%

Yield = 90%

LRMS 8α(H) 9α(H)m/z: 224 (M$^{+\bullet}$, 10%), 209 (M$^{+\bullet}$-CH$_3$, 19%),
191 (4%), 123 (92%), 109 (41%), 81 (70%), 55 (87%), 41 (100%).
8β(H) 9α(H)m/z: 224 (M$^{+\bullet}$, 12%), 209 (M$^{+\bullet}$-CH$_3$, 21%), 191 (5%),
123 (100%), 109 (41%), 81 (58%), 69 (73%).

^{13}C NMR: see Table 3:15.

IR (KBr disc): ν (O-H) 3350cm^{-1} intermolecularly bonded, ν (C-O) 1030cm^{-1} primary saturated.

2.4.8.3   8αβ(H) 9α(H)-drimanal

The isomeric mixture of 8αβ(H) 9α(H)-drimanol (400mg , 1.8mmol) was converted to 8α(H) 9α(H)-drimanal (85% by gc) and 8β(H) 9α(H)-drimanal (15% by gc) according to the method of Omura and Swern (1978) as described in section 2.4.3.5. The crude products were purified by flash column chromatography on deactivated (6%) silica (25g). The aldehydes (220mg, 0.98mmol) eluted in hexane:Et$_2$O (95:5; 100cm^3) and were assigned by LRMS and IR. The aldehyde was stored as a dilute solution in dichloromethane (5cm^3).

Gc purity = 88%

Yield   = 55%

LRMS 8α(H) 9 (H)m/z: 222 (M$^{+\bullet}$, 3.2%), 207 (M$^{+\bullet}$-CH$_3$, 4.5%),
189 (4%), 138 (14%), 123 (36%), 109 (29%), 41 (100%).

LRMS 8β(H) 9α(H) (Fig. 3:35A)m/z: 222 ($M^{+\bullet}$, 3%), 207 ($M^{+\bullet}$-CH_3,

8%), 189 (5%), 138 (12%), 123 (41%), 109 (38%), 41 (100%).

IR (KBr Disc): ν(C-H) 2695cm^{-1}, ν(C=O) 1720cm^{-1}, δ_s(CH_2)

1465cm^{-1}, aldehydic δ(C-H) 1390cm^{-1}.

Also present in IR: ν(O-H) 3400-2600cm^{-1} broad, ν(C-O) 1200cm^{-1};

indicative of carboxylic acid contamination.

2.4.8.4 $8\alpha\beta$ (H) 9α (H) $11\alpha\beta$(H)-tetrahydrogeranyldriman-11-ol

$8\alpha\beta$ (H) 9α (H) $11\alpha\beta$ (H)-tetrahydrogeranyldriman-11-ol was prepared by

Grignard synthesis (Kharasch and Reinmurth, 1954) from 1-bromo-3,7-

dimethyloctane (138mg, 0.62mmol), Mg (15mg, freshly scraped from Mg

ribbon) and 8 $\alpha\beta$ (H) 9α (H)-drimanal (100mg, 0.45mmol) in Et_2O

(redistilled from $LiAlH_4$). Benzene (0.5cm^3) was added to increase

the solubility of $8\alpha\beta$(H) 9α(H)-drimanal in Et_2O. The crude reaction

products were purified by tlc (0.5mm silica gel; hexane:Et_2O (95:5)

mobile phase). Following visualisation (Rhodamine 6G, 365μm), bands at

R_f 0.86-1.00 (hydrocarbons) and R_f 0.61-0.74 [$8\alpha\beta$ (H) 9α(H) $11\alpha\beta$ (H)-

tetrahydrogeranyldriman-11-ol (44% by gc) and $8\alpha\beta$ (H) 9α(H)-drimanal

(54% by gc)] were removed and the compounds recovered from the silica

gel by desorption with dichloromethane (20cm^3). Attempts to purify

the alcohol further using three different tlc systems (see Table 2:3)

succeeded in producing a fraction containing $8\alpha\beta$ (H) 9α(H) $11\alpha\beta$(H)-

tetrahydrogenanyldriman-11-ol (79% by gc) and $8\alpha\beta$(H) 9α(H)-drimanal (21%

by gc). The alcohol (2 gc peaks; 12.2mg, 0.03mmol) was assigned by

LRMS and IR. Attempts to derivitise the alcohol by silylation (TSIM;

Sakauchi and Horning, 1971) were unsuccessful.

Gc purity : 79%

Yield   : 7.4%

LRMS RI2558 (63% by gc) (Fig. 3:35C)m/z: 346 ($M^{+\cdot}-H_2O$, 1.2%),
331 (0.7%), 205 (6%), 191 (4%), 179 (17%), 123 (54%), 69 (100%).
RI2586 (16% by gc)m/z: 346 ($M^{+\cdot}-H_2O$, 1%), 331 (1.1%), 205 (6%),
191 (12%), 136 (11%), 123 (43%), 43 (100%).

IR (liquid cell; dichloromethane solution): $v(OH)$ 3380^{-1}
intermolecularly bonded, $v(C-H)$ aldehydic $2695cm^{-1}$,
$\delta_{as}(CH_3)$ 1455^{-1}, $\delta_s(CH_2)$ $1440cm^{-1}$, $\nu(C-O)$ $1035cm^{-1}$.

TABLE 2:3

tlc separation of crude reaction products from Grignard synthesis of
$8\alpha\beta$(H) 9α(H) $11\alpha\beta$(H)-tetrahydrogeranyldriman-11-ol

tlc system	Solid Phase	Mobile Phase	R_f band removed[a]	% by gc of alcohol:aldehyde
1	0.5mm SiO_2	hexane:Et_2O 95:5	0.61-0.74	44:54
2	0.5mm SiO_2	hexane	0.21-0.31	54:45
3	0.5mm SiO_2 + 5% $AgNO_3$	hexane	0.25-0.35	75:25
4	0.5mm SiO_2 + 5% $AgNO_3$	hexane:Et_2O 95:5	0.41-0.55	79:21

[a] transferred to origin of following plate

2.4.8.5 8αβ(H) 9 E,Z 11-tetrahydrogeranyldrimenes

8αβ (H) 9α(H) 11αβ (H)-tetrahydrogeranyldriman-11-ol (10mg, 0.03mmol) was dehydrated to 8αβ (H) 9 E,Z 11-tetrahydrogeranyldrimenes (2 gc peaks, 91% and 9%; 3.8mg, 0.01mol after tlc) by the modified Rinehart and Perkins (1963) method described in section 2.4.3.12. The RI and the mass spectrum of the major alkene peak recorded by gcms is listed below. The major alkene was also assigned using ^{1}H NMR.

Gc purity = 91%

Yield   = 39%

LRMS: RI 2359 (91% by gc) (Fig. 3:40B) m/z: 346 ($M^{+\cdot}$, 7%),
331 ($M^{+\cdot}$-CH_3, 8%), 205 (10%), 191 (51%), 136 (73%), 123 (43%),
109 (69%), 69 (100%).

^{1}H NMR (400MHz) δ ppm:
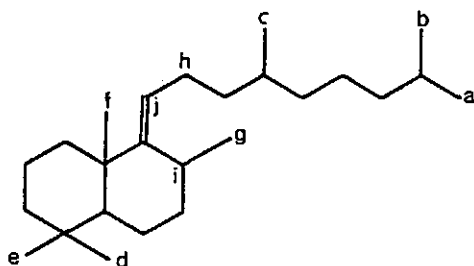
0.845 (m; 15H; a-e), 1.062 (s; 3H; f), 1.114 (d; 3H; g), 1.800-2.100
(m; 2H; h), 2.897 (m; 1H, i), 5.023 (t; 1H; j).

2.4.8.6 8α(H) 9αβ (H) 11-tetrahydrogeranyldrimane

Hydrogenation (hexane; 0.05g PtO_2.H_2O) of the isomeric mixture of 8α(H) 9 E,Z 11-tetrahydrogeranyldrimenes (0.5mg) afforded 8α(H) 9αβ(H) 11-tetrahydrogeranyldrimane (one gc peak, 0.4mg). The alkane was assigned by LRMS.

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Gc purity = 98%

Yield   = 81%

LRMS RI2381 (Fig. 3:42B) m/z: 348 ($M^{+\cdot}$, 9%), 333 ($M^{+\cdot}$-CH$_3$, 12%),

137 (10%), 123 (100%), 109 (35%), 69 (81%).

HRMS: 348.3742 C$_{25}$ H$_{48}$ requires 348.3753 123.1170 C$_9$ H$_{15}$

requires 123.1176.

2.4.9 Synthesis of 8$\alpha\beta$ (H) 9$\alpha\beta$ (H) 11-hexahydrofarnesyldrimane

2.4.9.1 8$\alpha\beta$(H) 9 α (H) 11$\alpha\beta$ -hexahydrofarnesyldriman-11-ol

8$\alpha\beta$ (H) 9 α(H) 11$\alpha\beta$ (H)-hexahydrofarnesyldriman-11-ol was prepared by

Grignard synthesis (Kharasch and Reinmurth, 1954) from 1-bromo-3,7,11-

trimethyldodecane (370mg, 1.2mmol), Mg (30mg, freshly scraped from Mg

ribbon) and 8$\alpha\beta$ (H) 9 α (H)-drimanal (200mg, 0.09mmol) in Et$_2$O

(redistilled from LiAlH$_4$). The crude reaction products were purified

by tlc (0.5mm silica gel; hexane mobile phase). Following visualis-

ation (Rhodamine 6G; 365μm), bands of R$_f$ 0.90-1.00 (hydrocarbons),

R$_f$ 0.20-0.31 [8$\alpha\beta$ (H) 9 $\prec$ (H) 11$\alpha\beta$ (H)-hexahydrofarnesyldriman-11-ol

(80% by gc) and 8$\prec\beta$ (H) 9$\prec$ (H) drimanal (20% by gc)] were removed and

the compounds recovered from the silica gel by desorption with

dichloromethane (20cm^3). Solvent was evaporated (Buchi; 30°C) and

8 $\prec\beta$ (H) 9 $\prec$ (H) 11$\prec\beta$ (H)-hexahydrofarnesyldriman-11-ol (3 gc peaks;

189mg, 0.4mmol) was assigned by LRMS, LRMS (TMS ether; TSIM), ^{13}C NMR

(partial) and IR.

Gc purity = 75%

Yield   = 33%

LRMS RI2882 (6% by gc) (Fig. 3:37B) m/z: 416 ($M^{+\cdot}$, H$_2$O, 0.1%),

205 (4%), 194 (18%), 179 (48%), 123 (56%), 69 (100%).

RI2905 (50% by gc) (Fig. 3:37C) m/z: 416 ($M^{+\cdot}$, H_2O, 4%), 401

(4%), 278 (5%), 205 (7%), 191 (26%), 179 (24%), 123 (65%), 69 (100%).

RI2934 (24% by gc) m/z: 416 ($M^{+\cdot}$, H_2O, 3%), 401 (3%), 278 (4%),

191 (26%), 179 (18%), 123 (61%), 69 (100%).

LRMS (TMS ether, 1 gc peak) (Fig. 3:38B) m/z: 401 (1%), 313 (65%),

295 (4%), 205 (31%), 129 (41%), 73 (100%).

^{13}C-NMR (400MHz): see Fig. 3:38A.

IR (liquid film): ν(O-H) 3380cm^{-1} intermolecularly bonded, ν(C-H)

aldehydic 2695cm^{-1}, δ_{as}(CH$_3$) 1455cm^{-1}, δ_s(CH$_2$) 1435cm^{-1},

ν(C-O) 1040cm^{-1}.

2.4.9.2 8αβ(H) 9 E,Z 11 E,Z-hexahydrofarnesyldrimenes

8αβ(H) 9≺(H) 11 ≺β (H)-hexahydrofarnesyldriman-11-ol (100mg, 0.23mmol)

was dehydrated to 8αβ (H) 9 E,Z 11 E,Z-hexahydrofarnesyldrimenes (3 gc

peaks; 81mg, 0.20mmol after tlc) by the modified Rinehart and Perkins

(1963) method described in section 2.4.3.12. The RI and mass spectrum

of the major alkene peak recorded by gcms is listed below.

Gc purity = 89%

Yield   = 84%

LRMS: RI2768 (80% by gc) (Fig. 3:40D) m/z: 416 ($M^{+\cdot}$, 3.5%), 401

($M^{+\cdot}$-CH$_3$, 3.5%), 291 (3.0%), 205 (9%), 191 (53%), 136 (64%), 123

(38%), 43 (100%).

2.4.9.3 8≺β (H) 9αβ (H) 11-hexahydrofarnesyldrimane

The isomeric mixture of 8≺β(H) 9 E,Z 11 E,Z -hexahydrofarnesyldrimenes

(50mg, 0.12mmol) was hydrogenated at 40°C and 500psi H_2 pressure in a

('bomb hydrogenator') using $PtO_2.H_2O$ (0.2g). After 6hr, the reaction products were filtered and solvent removed (Buchi; 30°C) prior to purification by argentatious tlc [0.5mm silica gel (5% $AgNO_3$); hexane mobile phase]. Following visualisation (Rhodamine 6G; 365μm), bands at R_f 0.91-1.00 (8αβ(H) 9αβ(H) 11-hexahydrofarnesyldrimane, 3 gc peaks, 90% by gc and 8 α (H) 9 E,Z -hexahydrofarnesyldrimene, 1 gc peak, 10% by gc) and R_f 0.35-0.49 (8α(H) 9 E,Z -hexahydrofarnesyl-drimene, 1 gc peak, 100% by gc) were removed and the hydrocarbons were recovered from the silica gel by desorption with dichloromethane (20cm^3). Solvent was evaporated (Buchi, 30°C) and the 8αβ (H) 9αβ(H) 11-hexahydrofarnesyldrimanes (15mg, 0.04mmol) characterised by LRMS, HRMS and by ^{13}C NMR. Gc of the band R_f 0.35-0.49 produced only one alkene peak (RI2768; 10.4mg) which was assigned by ^{1}H NMR and ^{13}C NMR.

Gc purity = 90%

Yield   = 33%

LRMS RI2784 (5% by gc) (Fig. 3:44B) m/z: 418 (M$^{+\cdot}$, 2.8%), 403 (M$^{+\cdot}$-CH$_3$, 4.1%), 191 (11%), 151 (21%), 123 (100%), 43 (81%).

RI2804 (4% by gc) m/z: 418 (M$^{+\cdot}$, 2.0%), 403 (M$^{+\cdot}$-CH$_3$, 2.1%), 191 (17%), 123 (61%), 43 (100%).

RI2836 (81% by gc) (Fig. 3:44C) m/z: 418 (M$^{+\cdot}$, 5.0%), 403 (M$^{+\cdot}$-CH$_3$, 5.3%), 137 (11%), 123 (100%), 43 (51%).

HRMS: 418.4557 $C_{30}H_{58}$ requires 418.4547, 403.4313 $C_{29}H_{55}$ requires 403.4312, 123.1171 C_9H_{15} requires 123.1176.

^{1}H NMR (400MHz) δ ppm:
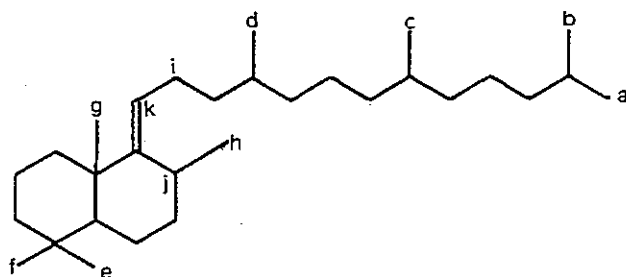
0.852 (m; 18H; a-f), 1.064 (s; 3H; g), 1.116 (d; 3H; h), 1.850-2.100 (m; 2H; i), 2.913 (m; 1H; j), 5.045 (t; 1H; k).

^{13}C NMR (400 MHz): δ 1. 153.1997ppm

Alkene·   2. 119.3069ppm
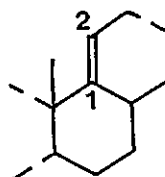
2.4.10 Synthesis of 2,6,10,14-tetramethylhexadecane (Phytane)

2,6,10,14-tetramethylhexadecane (Phytane: 0.5g, 1.8mmol) used in the biodegradation experiments (section 2:3) was prepared by hydrogenolysis (1% glacial acetic acid in hexane; 0.05g $PtO_2.H_2O$) of commercial E-2,6,10,14-tetramethylhexadec-2-en-1-ol   (Phytol,   Aldrich;   1.0g, 3.3mmol).   The   crude   reaction   products   were   purified   by   column chromatography   on   deactivated   (4%)   alumina   (25g).   2,6,10,14-tetramethylhexadecane eluted in hexane (100cm^3) and was characterised by LRMS.

Gc purity = 99%

Yield   = 55%

LRMSm/z: 282 (M$^{+\cdot}$, 0.3%), 267 (M$^{+\cdot}$-CH$_3$, 2%), 197 (4%), 183 (3%), 57 (100%).

CHAPTER THREE

SYNTHESES OF ACYCLIC AND CYCLIC
C_{20}, C_{25} AND C_{30} HYDROCARBONS

3.1 GENERAL POINTS

1. The numbering of the structures in Chapter 3 refer to the structures contained either in the text or at the end of the chapter. This numbering system is independent of that used in the other six chapters.

2. Correct IUPAC nomenclature is used throughout except when greater comparison between analogous compounds can be derived using different names. Cyclic compounds are called by trivial names based on the extended drimane (12) skeleton.

3.2 ACYCLIC ISOPRENOIDS

The syntheses of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (br20:0;1) 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0;2) and 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane (br30:0;3).

3.2.1 Structural assignment

The possibility of a structural homology between br20, br25 and br30 was first proposed by Barrick and Hedges (1981) who observed that the hydrocarbons had similar EI mass spectral fragmentation patterns and gave co-linear retention index plots. Yon et al. (1982) demonstrated that the C_{20} alkane (br20:0) in Rozel Point crude oil (Age unknown; Utah, U.S.A) and assorted Recent sediments (see Table 1:7) was 2,6,10-trimethyl-7-(3-methylbutyl)dodecane. The EI mass spectrum (Fig. 1:13A) of synthetic br20:0 prepared by Yon (1982) was characterised by ion doublets at m/z 168/169, m/z 196/197 and m/z 210/211 which were assigned to fragmentation about the C6-C7, C5-C6 (and C7-C8) and C7-C13 bonds respectively (Fig. 3:1A). The mass spectrum (Fig. 1:13C) of the

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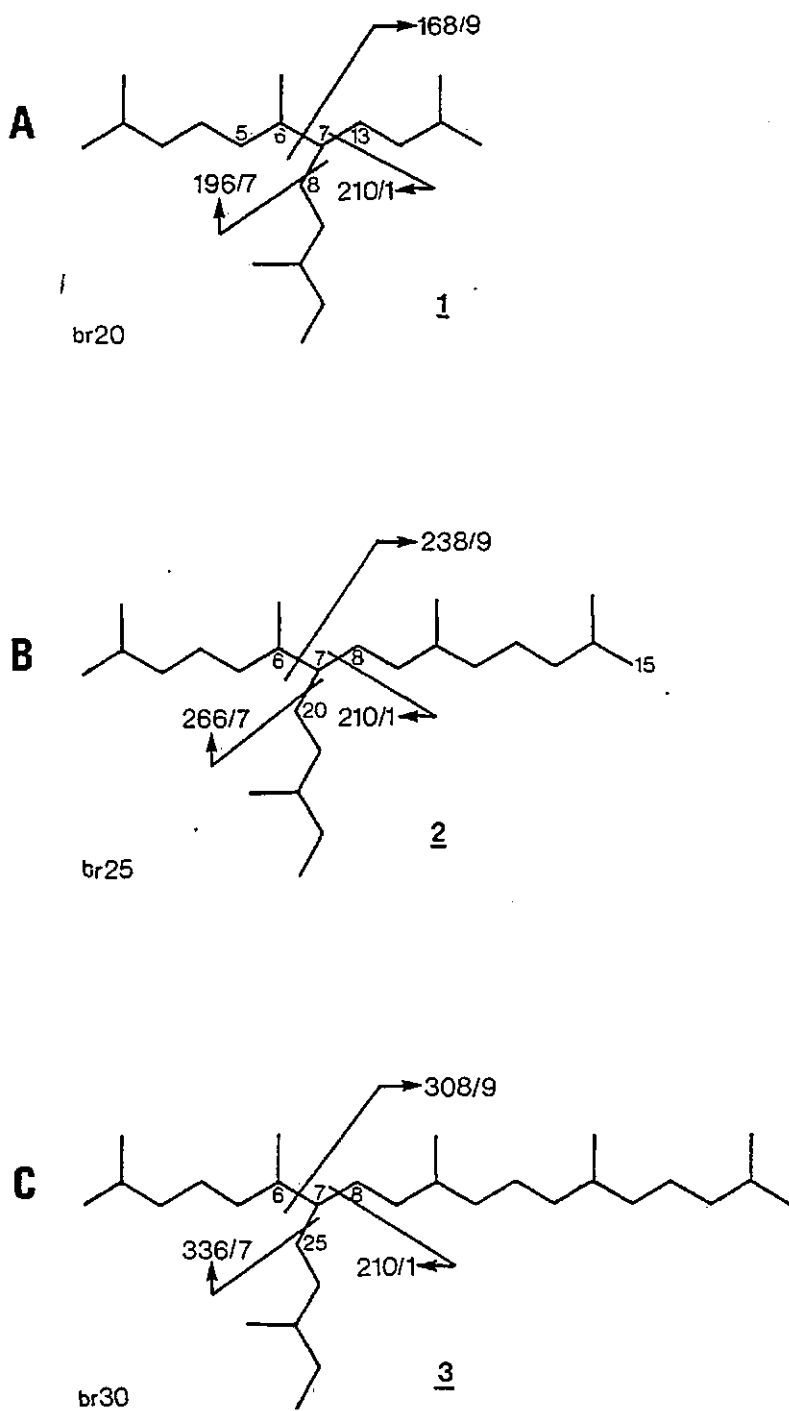
Fig.3:1   A. Observed structure and fragmentation pattern for
br20:0 (Yon,1982); B. and C. Proposed structures and
fragmentation patterns for br25:0 and br30:0
respectively. Structure numbers refer to text.

C_{25} alkane (br25:0) (obtained from hydrogenation of four C_{25} trienes and C_{25} tetraenes) proposed as structurally related to the br20:0 by Barrick et al. (1980) had a similar fragmentation pattern with ion pairs 70 a.m.u. higher at m/z 238/239, m/z 210/211 and m/z 266/267 (Fig. 3:1B). On this basis, Yon (1982) suggested three possible structures for the carbon skeleton of the alkane (Fig. 1:15A-C) and considered that structure C was most consistent with the mass spectrum. Subsequently, several investigators (Albaiges et al., 1984 a and b; Dunlop and Jefferies, 1985; Rowland et al., 1985) assigned structure C to the sedimentary hydrocarbons although the postulated assignment remains unconfirmed.

The mass spectrum of the C_{30} hydrocarbon obtained by hydrogenation of an acyclic C_{30} pentaene (Barrick et al., 1980; Prahl et al., 1980) is shown in Fig. 1:14B. The mass spectrum of the br30 alkane (br30:0) appears to demonstrate analogous fragmentation to br20:0 and br25:0 with ion pairs 70 a.m.u. higher (m/z 308/309 and m/z 336/337). By analogy with the structure proposed for the br25:0, the additional C_5 moiety in the br30:0 may be attached at C15 and thus, structure C (Fig. 3:1) is proposed for the carbon skeleton of the br30 hydrocarbons. The presence of the m/z 420 ion in the mass spectrum (Fig. 1:14B) of the br30:0 suggests the presence of a double bond located at such a position that it does not interfere with the major alkane fragmentation pattern.

To confirm the proposed structural assignments for the br25:0 and br30:0 it was decided to synthesise the hydrocarbons. The br20:0 was also prepared to aid interpretation of the spectra of the synthesised br25:0 and br30:0 and for use in subsequent biodegradation studies (Chapter 5).

3.2.2 Synthetic scheme

In designing a scheme for the synthesis of the reference hydrocarbons, it was obviously beneficial if a scheme which allowed the preparation of all three hydrocarbons with only one structural change in the starting materials could be employed. The scheme devised to fulfil this requirement is illustrated in Fig. 3:2 and uses only well-known synthetic reactions. By condensing the C_9 aldehyde with a C_5 (isopentyl R), C_{10} (geranyl, R^i) or C_{15} (farnesyl, R^{ii}) bromide it was possible to prepare C_{14}, C_{19} or C_{24} isoprenoid ketones which, on subsequent condensation with the C_6 (isohexyl) bromide and dehydration produced br20, br25 and br30 alkenes. The individual synthetic schemes for br20, br25 and br30 are shown in Figs. 3:3, 3:4 and 3:5 respectively. At each stage, products were routinely analysed by gc, gcms, IR and ^{1}H NMR (60MHz), with detailed NMR (400MHz; ^{1}H or ^{13}C) on the important intermediates as indicated in the text.

In the following discussion, the separate syntheses of br20, br25 and br30 are not discussed individually. Instead, the approach taken is to divide the three synthetic schemes into sections containing pseudohomologous compounds and then to discuss the three compounds in each section collectively. To aid understanding, Figs. 3:3, 3:4 and 3:5, which illustrate the synthetic schemes of br20, br25 and br30 respectively, are divided in this manner with section numbers corresponding to those in the text. It is thought that this approach allows for the greatest comparison of analogous data. The discussion first describes the synthesis of 2,6-dimethylheptan-1-al which is a synthon common to all three hydrocarbons.
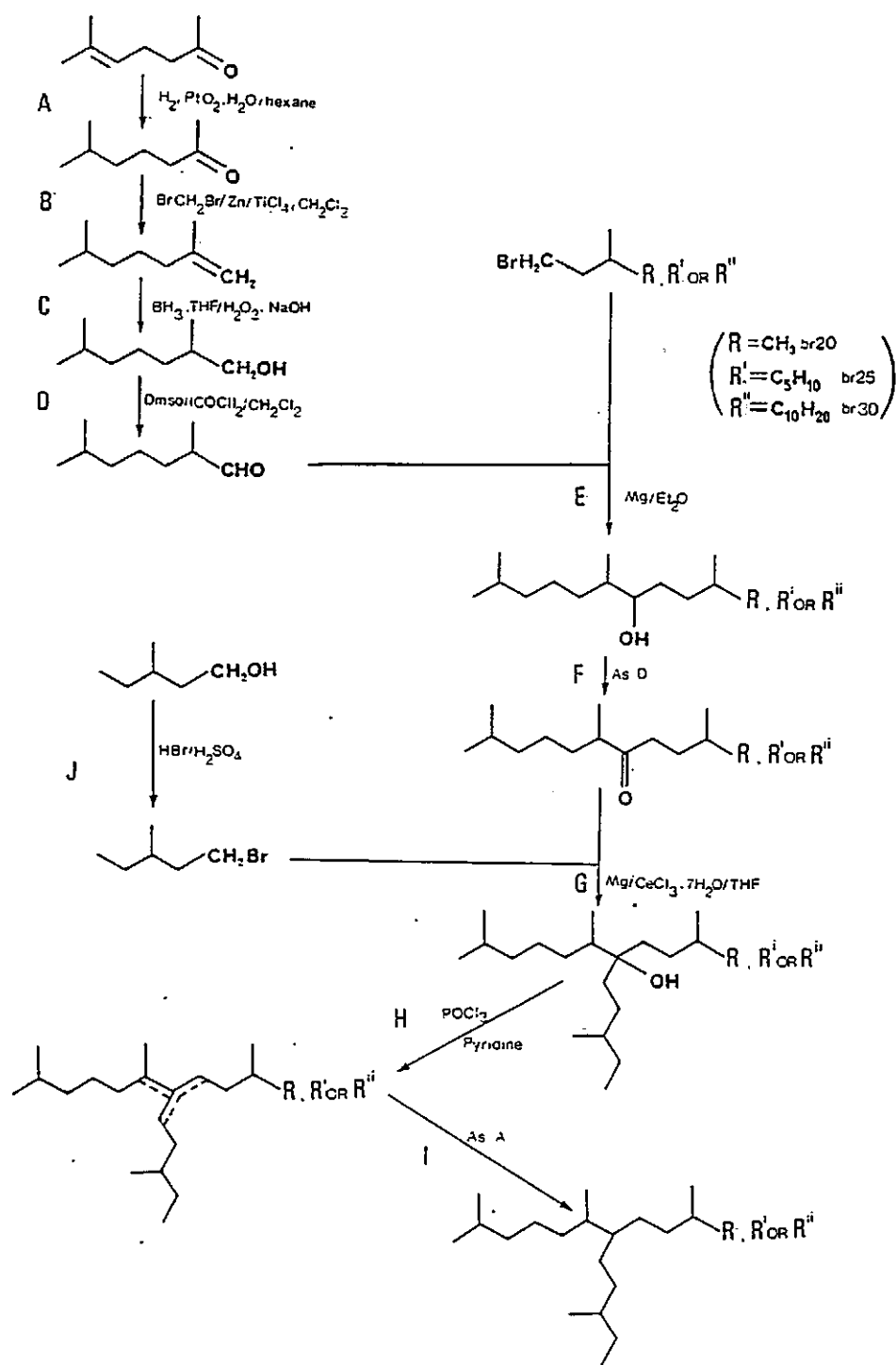
Fig.3:2   General synthetic scheme for the preparation of br20:0 (R), br25:0 (R^i) and br30:0 (R^{ii}).
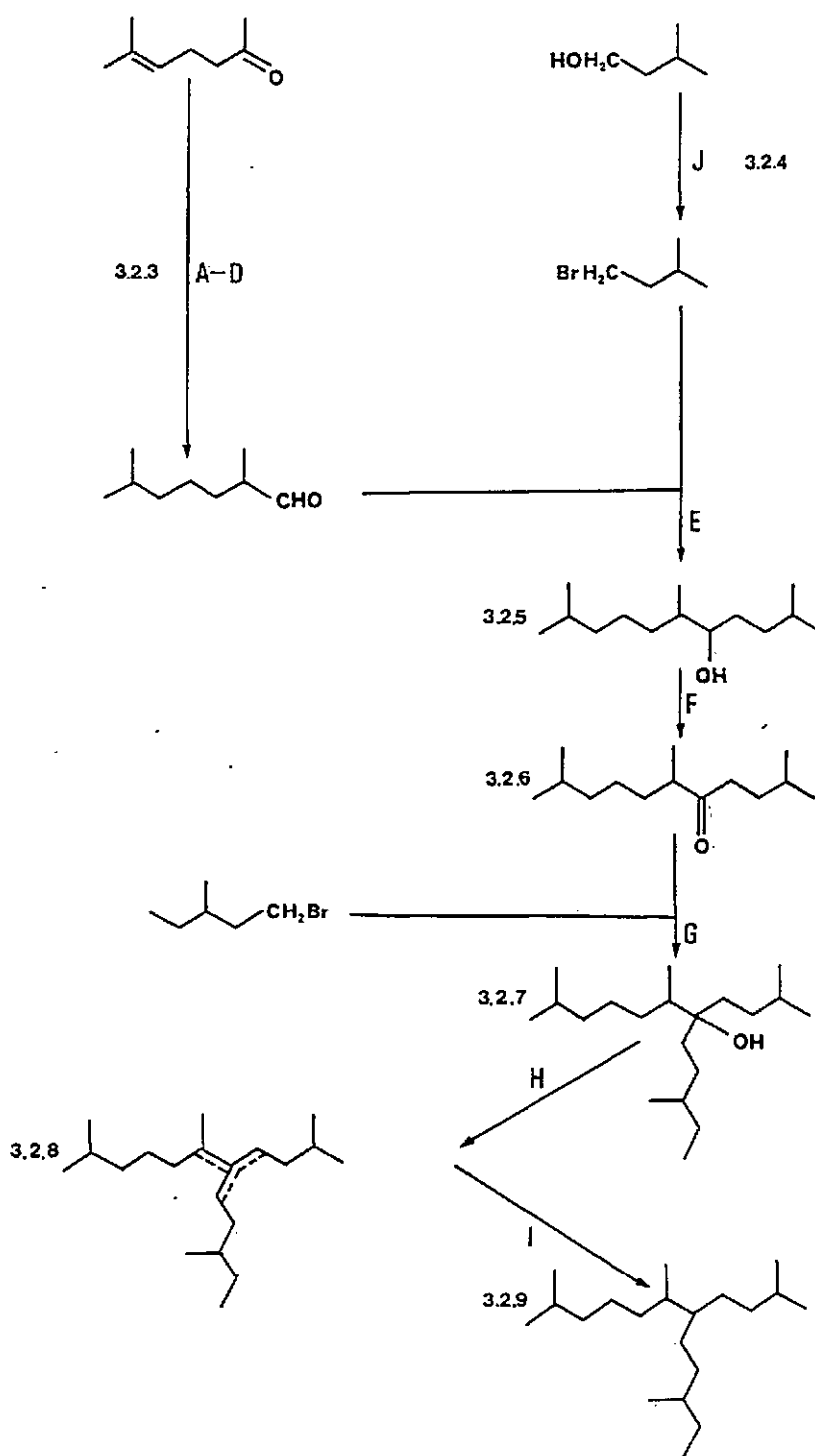
Fig.3:3   Synthesis of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane
(br20:0). Letters refer to the reagents in Fig.3:2
and numbers refer to the relevant sections in the text.
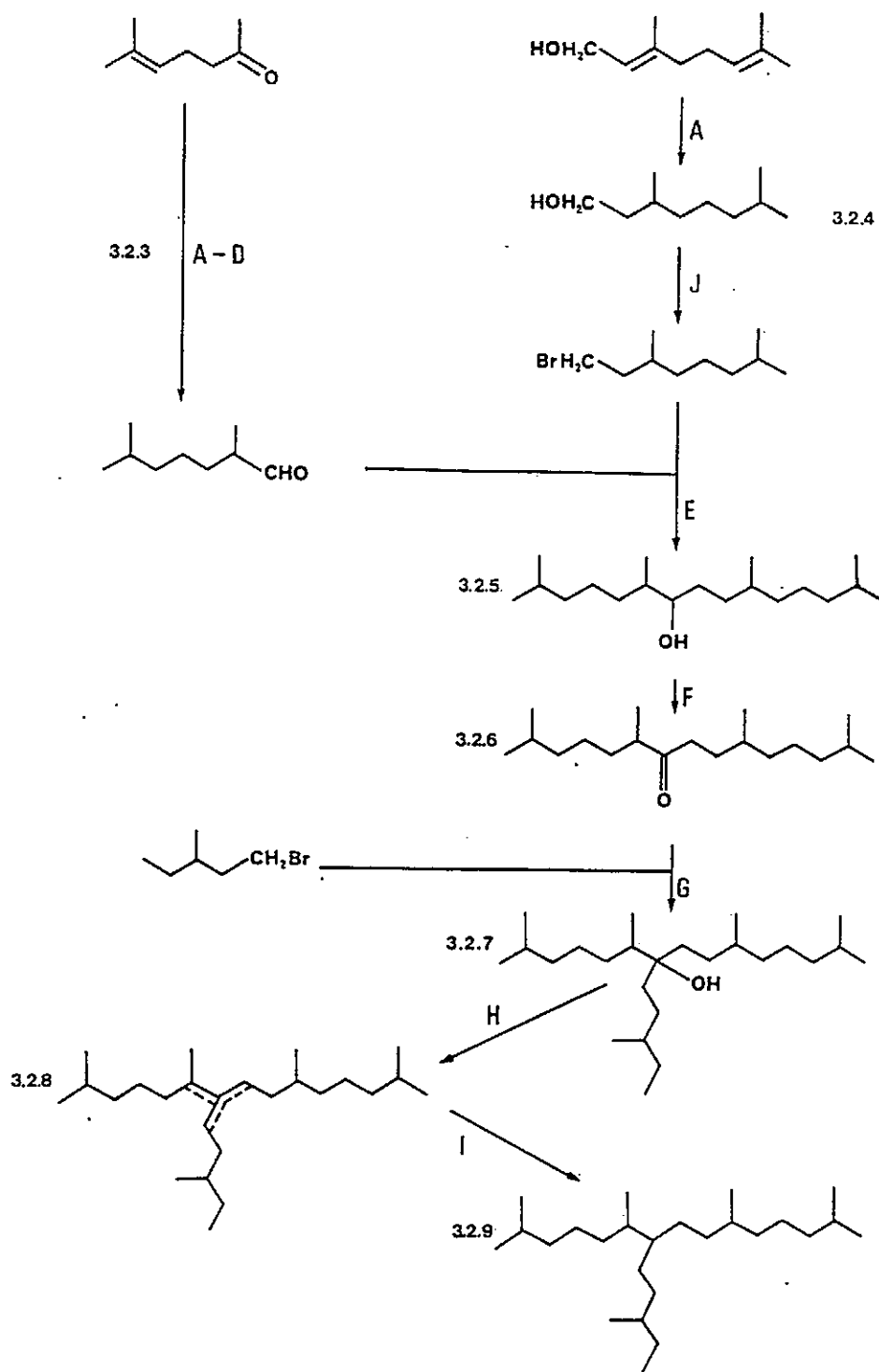
Fig.3:4   Synthesis of 2,6,10,14-tetramethyl-7-(3-methylpentyl)
          pentadecane (br25:0). Letters refer to the reagents
          in Fig.3:2 and numbers refer to the relevant sections
          in the text.
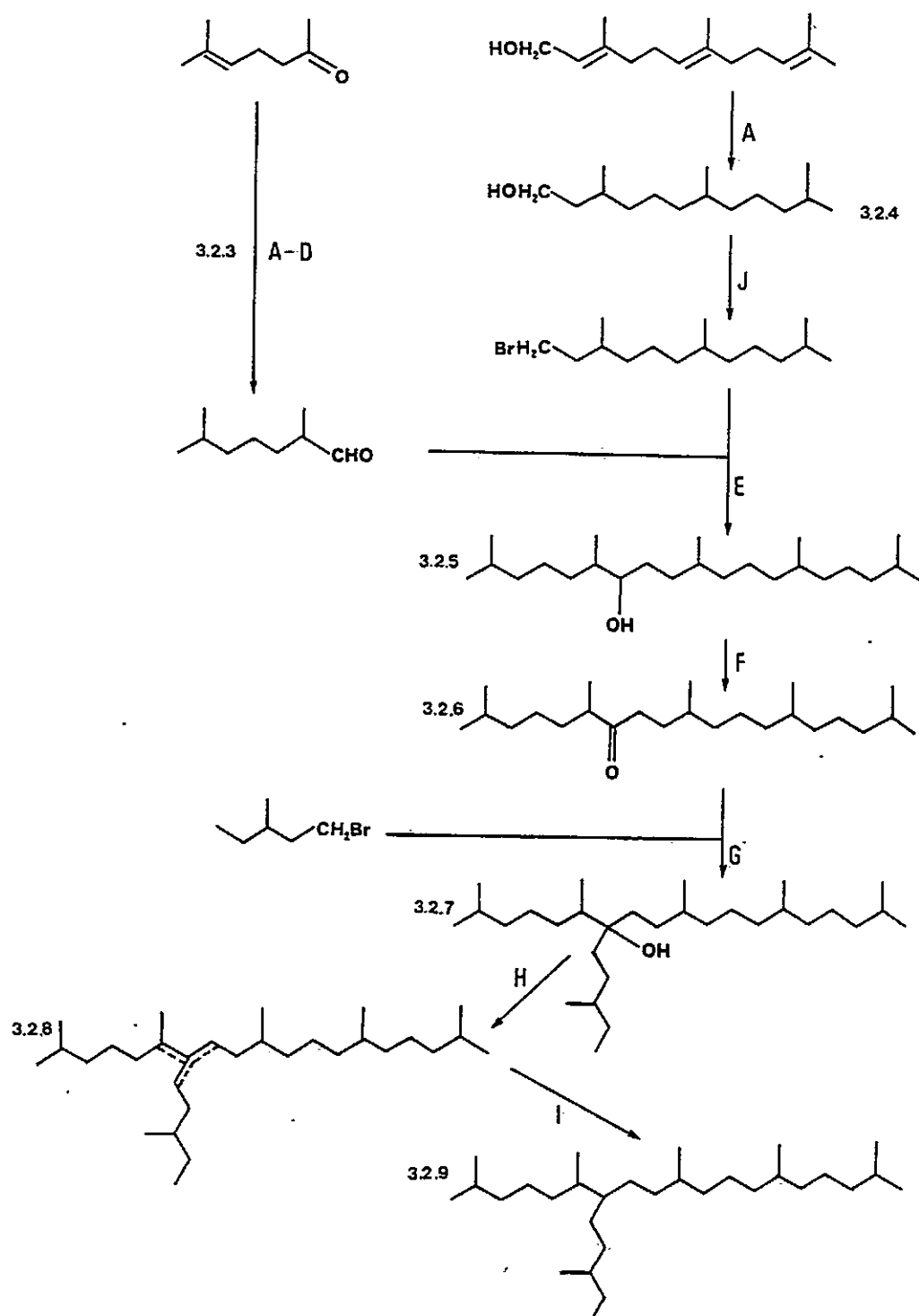
Fig.3:5   Synthesis of 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)
           nonadecane (br30:0). Letters refer to the reagents
           in Fig.3:2 and numbers refer to the relevant sections
           in the text.

3.2.3 Synthesis of 2,6-dimethylheptan-1-al
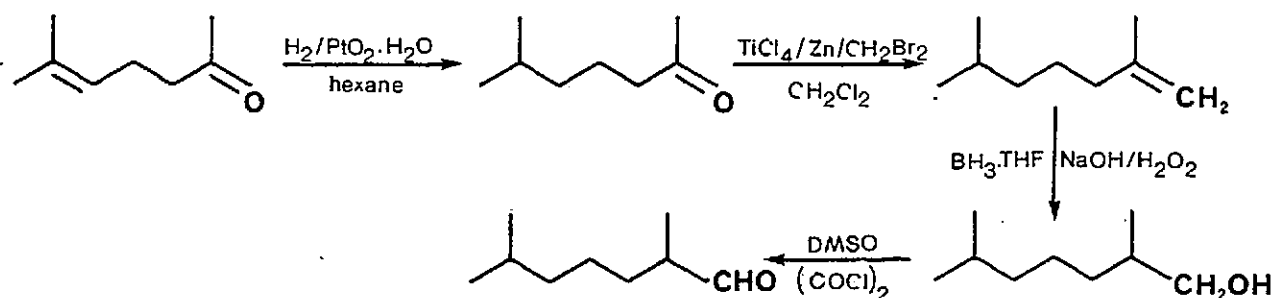
6-methylhept-5-en-2-one was smoothly hydrogenated over Adams catalyst ($PtO_2 \cdot H_2O$) to produce 6-methylheptan-2-one which was assigned by gcms, IR and ^1H NMR; the spectra being as expected. Interestingly, attempts to hydrogenate the unsaturated ketone using anhydrous catalyst (PtO_2) resulted in much lower yields (<30%) and also brought about some reduction of the ketone to 2,6-dimethylheptan-1-ol. 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:

2,6-dimethylhept-1-ene was prepared initially using the Wittig synthesis (Trippet, 1960) but poor yields (<26%) and problems in separation of the product alkene from triphenylphosphine oxide [$(C_6H_5)_3PO$], a by-product of the synthesis, prompted the search for a more effective method. The alkene was eventually prepared in excellent yield using the method of carbonyl methylenation involving $Zn/CH_2Br_2/TiCl_4$ described by Oshima et al. (1978) and Lombardo

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(1982). During early attempts to separate the product alkene from
unreacted ketone by passage through a chromatographic column of
activated (100%) alumina, it was observed that
2,6-dimethylhept-1-ene underwent partial isomerisation to produce a
second alkene (Fig. 3:6A). Figs. 3:6B and C respectively, show the
mass spectra of 2,6-dimethylhept-1-ene and its presumed isomer. It
is evident that both spectra contain the same ions but these differ
in relative distributions. Alkenes are known to undergo β-cleavage
without H transfer (allylic cleavage) and substituted alkenes
undergo γ-hydrogen rearrangement (Loudon and Maccoll, 1970;
McLafferty 1973). The base ion at even m/z 56 in the mass spectrum
(Fig. 3:6B) of 2,6-dimethylhept-1-ene is due to the γ-hydrogen
rearrangement; i.e

$$m/z\ 56$$
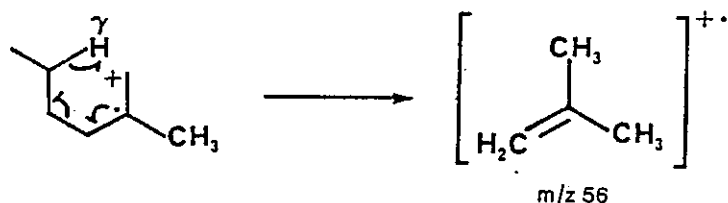
In contrast, the odd base ion m/z 69 in the mass spectrum (Fig.
3:6C) of the other isomer is from β-cleavage without hydrogen
transfer. Thus it would appear that migration of the double bond
occurred during column chromatography. The migration of double
bonds of alkenes on solid state catalysts such as alumina has been
widely investigated (reviewed by Mackenzie, 1970). The mechanism
of isomerisation is not fully understood but is believed to involve
hydride removal at the allylic position by the Lewis acid

A

100%=4734288   X   TIC

Retention time (mins) ——→

B

X

+H ——→ 56

C

Y

——→ 69

M/Z ——→
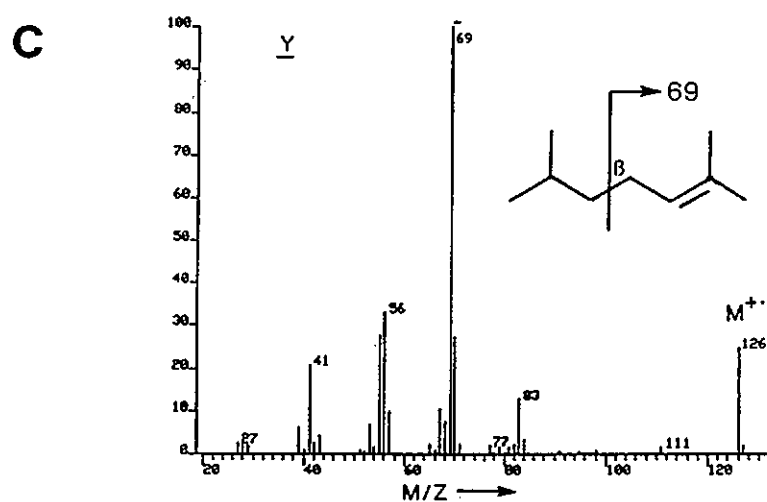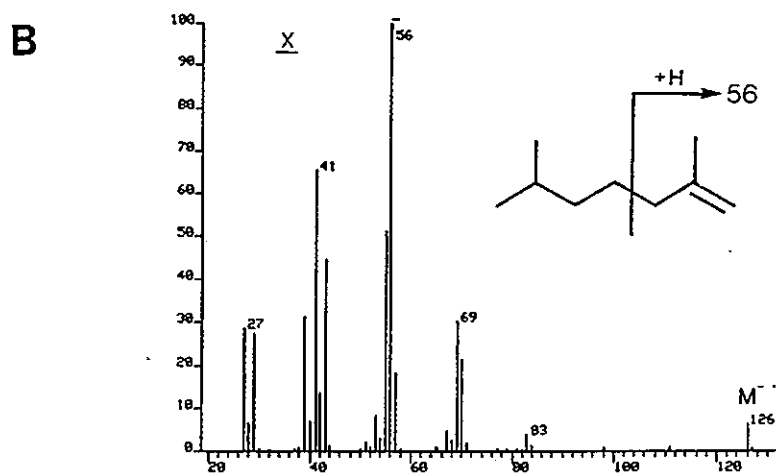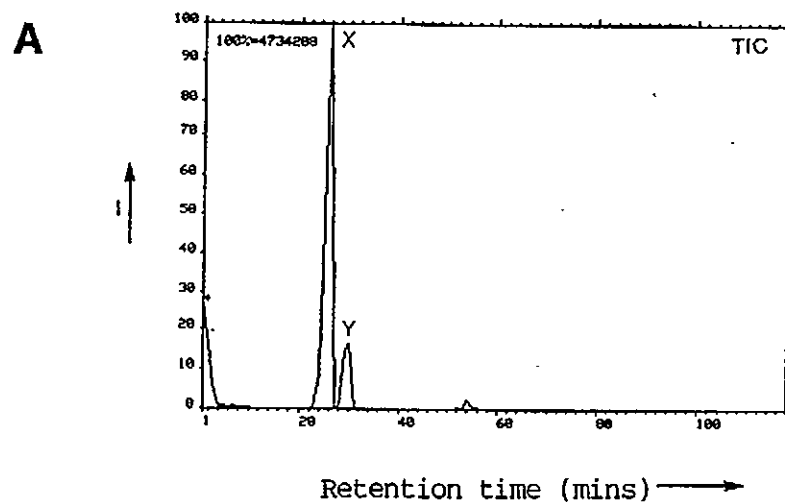
Fig.3:6   A. Total Ion Chromatogram (TIC) of the post-alumina
column products from the synthesis of 2,6-dimethylhept-
1-ene, B. mass spectrum of 2,6-dimethylhept-1-ene
(peak X) and C. mass spectrum of 2,6-dimethylhept-2-ene
(peak Y). Gc conditions: SE54, 40 – 80°C at 2°Cmin⁻¹.

sites on the catalyst, followed by addition of a hydride from an un-ionised substrate molecule. Isomerisation of 2,6-dimethylhept-1-ene was prevented in later purification steps by the use of 4% deactivated alumina (i.e. the addition of 4% water to activated alumina). The structural assignment of 2,6-dimethylhept-1-ene was confirmed by IR and ^{1}H NMR.

Hydroboronation-oxidation (Brown, 1977) of 2,6-dimethylhept-1-ene afforded 2,6-dimethylheptan-1-ol which was assigned by gcms, IR and ^{1}H NMR.

The method of Omura and Swern (1978) was chosen for the oxidation of 2,6-dimethylheptan-1-ol to 2,6-dimethylheptan-1-al after several unsuccessful attempts to prepare the aldehyde using pyridinium chlorochromate (Piancetilli et al., 1982). Purification of the crude reaction products from the pilot scale Omura and Swern (1978) oxidation of 2,6-dimethylheptan-1-ol was attempted by passage through a deactivated alumina column. Fig. 3:7A displays the gas chromatogram of the hexane:diethylether (95:5) eluate of that column. It shows, in addition to the aldehyde, that there are two other compounds present (Fig. 3:7A, I and II) which presumably formed from the aldehyde during passage through the alumina. The three components were separated by tlc (section 2.4.3.5) and compound I had mass and IR spectra consistent with 2,6-dimethylheptan-1-oic acid (4). The structure of compound II eluded spectroscopic determination but was thought to represent some form of alumina-catalysed condensation product of 2,6-dimethylheptan-1-al. The oxidation of aldehydes by atmospheric oxygen is well known (i.e. Verter, 1970; March, 1977). In addition, the possibility of aldehyde oxidation during the acid washing (HCl) stage of the method of
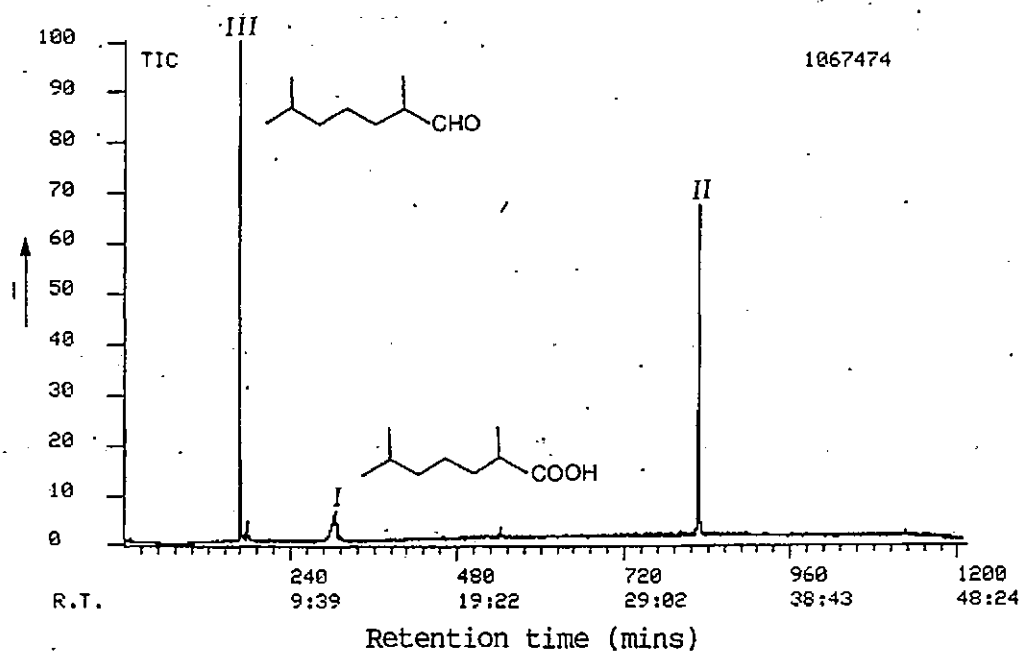
Fig.3:7A   Total ion chromatogram (TIC) of the post alumina column
products from the synthesis of 2,6-dimethylheptan-1-al.
Peak identifications: *III.* 2,6-dimethylheptan-1-al,
I. 2,6-dimethylheptanoic acid and *II.* ˉ uncharacterised
acid condensation product ?. Gc conditions: OV1,
40 - 290°C at 8°Cmin⁻¹.
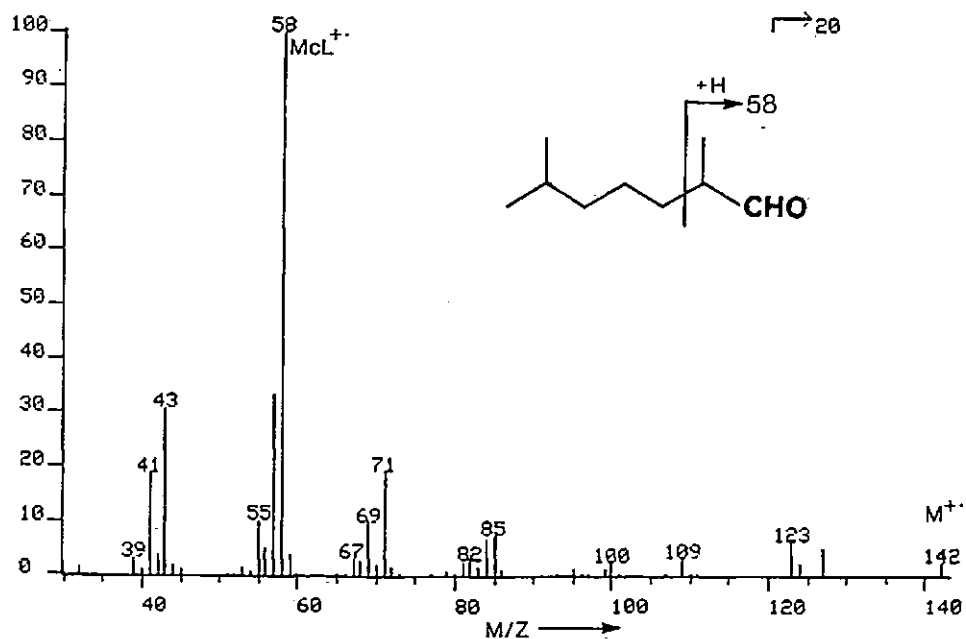
Fig.3:7B   Mass spectrum of 2,6-dimethylheptan-1-al.
McL⁺· denotes the McLafferty rearrangement ion.

Omura and Swern (1978) cannot be discounted. In subsequent sytheses of
2,6-dimethylheptan-1-al, formation of oxidation and condensation
products was kept to a minimum by eliminating acid washing, limiting
contact with the atmosphere and the use of 'flash' column
chromatography on deactivated silica (Still et al., 1978). The mass
spectrum of 2,6-dimethylheptan-1-al is given in Fig. 3:7B which shows
the molecular ion (M$^{+ \cdot}$) at m/z 142 and the expected McLafferty
rearrangement ion at m/z 58 (the base ion).
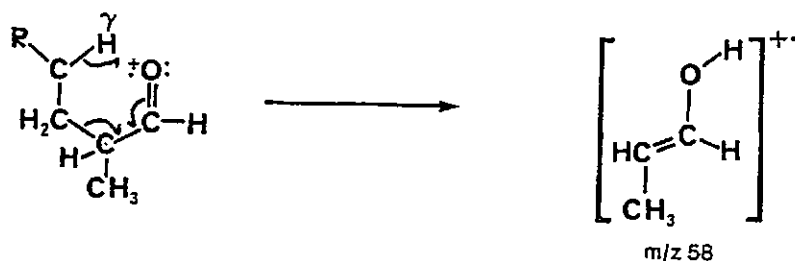
^{1}H NMR of 2,6-dimethylheptan-1-al revealed the aldehydic proton
(-CHO;i) as a doublet at δ9.6ppm (see Fig. 3:8A) and the methine proton
(CH$_2$-CH(CH$_3$)-CHO; g) as a multiplet at δ2.3ppm. The infra red
spectrum (Fig. 3:8B) was consistent with that of a saturated aldehyde
with strong carbonyl stretch at 1730cm^{-1} and aldehydic (C-H) stretch
at 2710cm^{-1}.
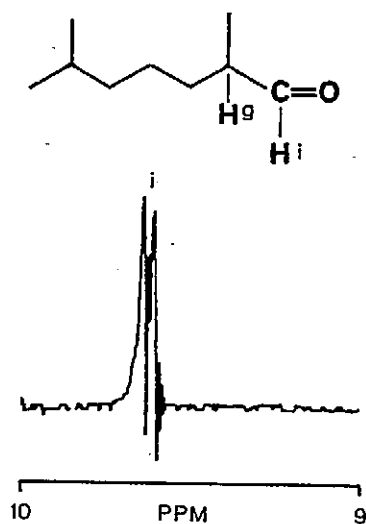
Fig.3:8A   Partial ^{1}H NMR (60MHz) spectrum of
            2,6-dimethylheptan-1-al.
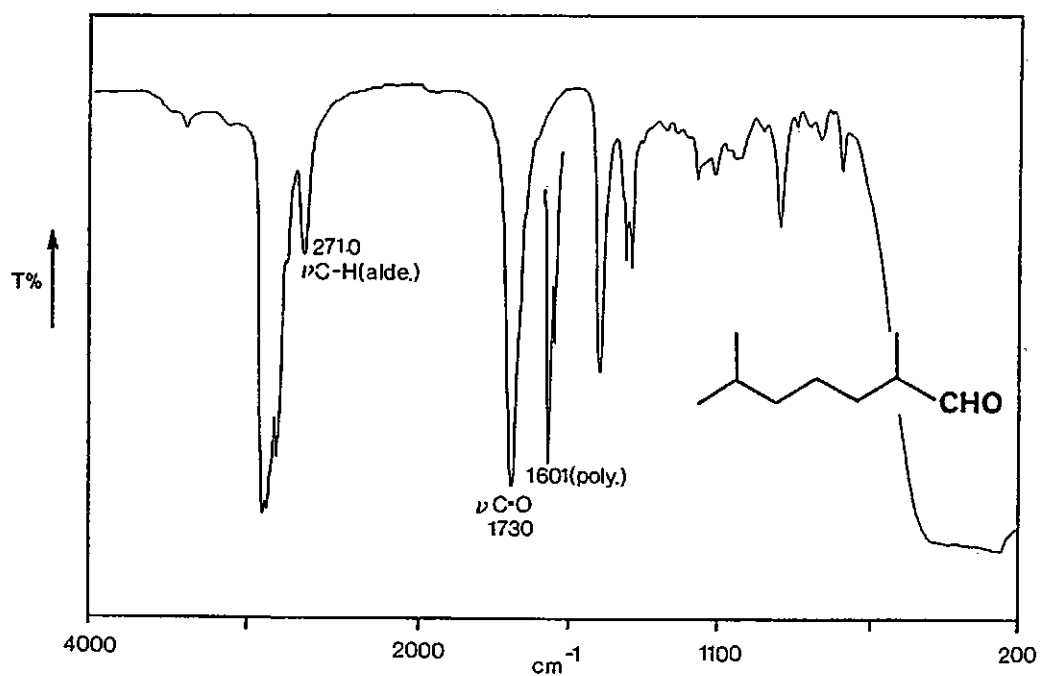
Fig.3:8B   IR spectrum of 2,6-dimethylheptan-1-al.

3.2.4 Synthesis of 1-bromo-3,7-dimethyloctane and 1-bromo-3,7,11-trimethyldodecane

1-bromo-3,7-dimethyloctane
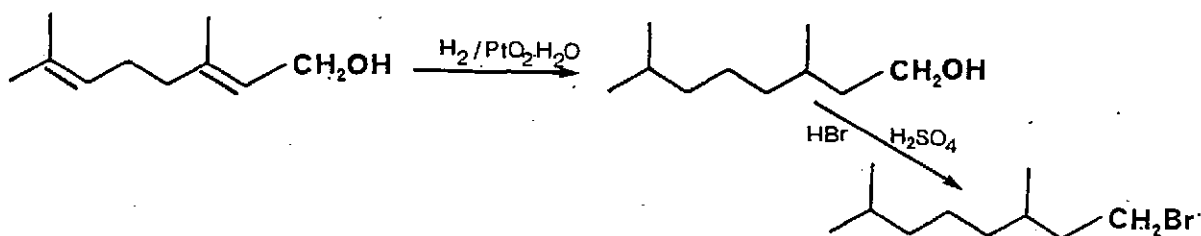
3,7-dimethyloct-2,6-dien-1-ol was smoothly hydrogenated to 3,7-dimethyloctan-1-ol and then converted to 1-bromo-3,7-dimethyloctane by the method of Kamm and Marvel (1960). Structural assignments were confirmed by spectroscopic data.

1-bromo-3,7,11-trimethyldodecane
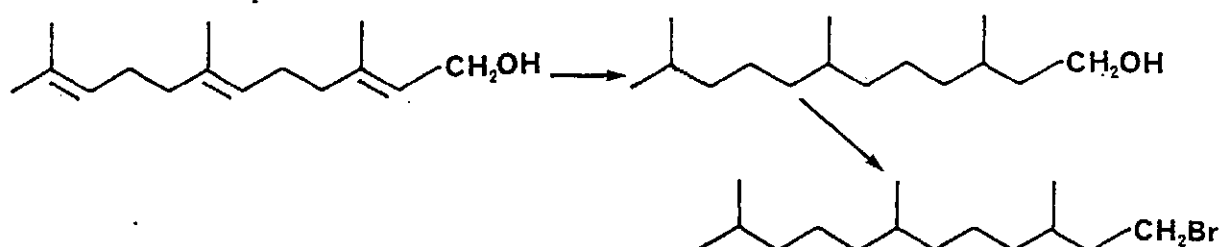
1-bromo-3,7,11-trimethyldodecane was prepared from 3,7,11-trimethyldodec-2,6,10-trien-1-ol (farnesol) in an analogous manner to the preparation of 1-bromo-3,7-dimethyloctane (see above). The ^{1}H NMR spectrum and mass spectrum of the C_{15} bromide were as expected.

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3.2.5   Synthesis of 2,6,10-trimethylundecan-7-ol, 2,6,10,14-tetramethylpentadecan-7-ol and 2,6,10,14,18-pentamethylnonadecan-7-ol.

Synthesis of 2,6,10,14-tetramethylpentadecan-7-ol was chosen for the pilot study and is therefore discussed first.

2,6,10,14-tetramethylpentadecan-7-ol (synthon for br25 carbon skeleton)
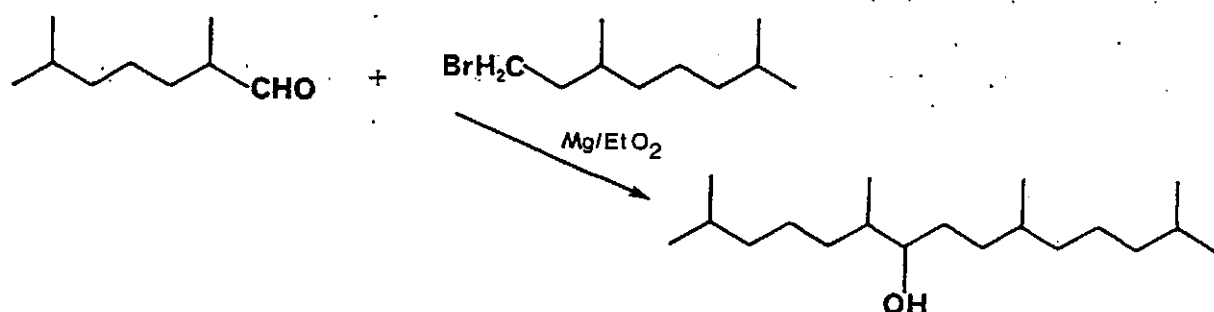
The gas chromatogram of the crude total reaction products of the initial Grignard coupling (method of Roberts et al., 1975) between 1-bromo-3,7-dimethyloctane and 2,6-dimethylheptan-1-al is shown in Fig. 3:9A. Peak numbers refer to Table 3:1. It is evident that several side reactions occurred alongside production of the desired Grignard

addition product, 2,6,10,14-tetramethylpentadecan-7-ol. There are several side reactions which can occur during Grignard reactions (March, 1977). The three most common are reduction, enolisation and Wurtz-type coupling. During reduction, which requires the Grignard reagent (RMgX) to have a β-hydrogen, the carbonyl compound is reduced to an alcohol by the Grignard reagent, which itself undergoes elimination to give an alkene. In enolisation, which requires that the carbonyl compound has a α-hydrogen, the Grignard reagent is converted to an alkane and the carbonyl compound to its elonate form which, on hydrolysis, is reconverted to the original aldehyde. During the third type of side reaction, 'Wurtz-type' coupling, a molecule of Grignard reagent (RMgX) combines with the alkyl halide (RX) to form a dimer (R2) and magnesium halide, i.e.

Fig.3:9   Gas chromatogram of the products of the Grignard
           addition of 1-bromo-3,7-dimethyloctane to
           2-6-dimethylheptan-1-al: A. method of Roberts
           et al (1985) and B. method of Kharasch and
           Reinmurth (1954).   Numbered peaks refer to Table 3:1.
           Gc conditions: SE54, 40 - 290°C at 8°Cmin^{-1}.

Table 3:1 Peak assignments for Fig. 3:9

Peak number	Assignment[a]	Structure
1	3,7-dimethyloct-1-ene	
2	3,7-dimethyloctane	
3	2,6-dimethylheptan-1-al	
4	2,6-dimethylheptan-1-ol	
5	2,6-dimethylheptanoic acid	
6	2,6,11,15-tetramethylhexdecane	
7	2,6,10,14-tetramethylpentadecan-7-ol	
8	2,6,10,14-tetramethylpentadecan-7-one	
9 & 10	2,6,10,14-tetramethylpentadecenes	

[a] Assignment by mass spectral interpretation and/or by co-chromatography with authentic compounds.

Table 3:2

Mass spectral fragmentation of 2,6,10-trimethylundecan-7-one (A); 2,6,10,14-tetramethypentadecan-7-one (B) and 2,6,10,14,18-pentamethylnonadecan-7-one (C). Numbers refer to the mass (a.m.u.) of the fragments observed in each mass spectrum. (Fig. 3:13 A-C).

MOLECULE	FRAGMENTS					
	$M^{+\cdot}$	$M^{+\cdot}$-15	McLafferty rearrangement ions		Double McLafferty	α-cleavage ion
			ii	iii		i
A	212	197	156	128	72	99
B	282	267	156	198	72	169
C	352	337	156	268	72	239

$$RMgX + RX \longrightarrow R_2 + MgX_2$$
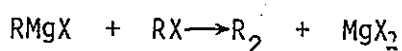
It is apparent from an examination of Fig. 3:10 that all three side reactions occurred with the products from each identified in Table 3:1. In addition, it appears that the product alcohol itself underwent both dehydration and oxidation reactions. Dehydration is commonly observed, especially in sterically hindered molecules (Kharasch and Reinmurth, 1954) upon hydrolysis but the cause of the oxidation is uncertain. According to Kharasch and Reinmurth (1954), the formation of side reaction products can be limited by use of a high initial dilution of the halide (RX) in the appropriate solvent, high quality, freshly prepared magnesium, a slow rate of addition of the halide to the magnesium, limited heating and rapid, continual agitation throughout the reaction. In contrast, the standard text on Grignard synthesis used herein and derived from more modern literature (i.e. Roberts et al., 1975) recommends almost the opposite with high concentrations of halide in solvent, relatively rapid addition rate of halide to magnesium etc. Fig. 3.9B shows the gas chromatogram of the crude reaction products from the repeated Grignard addition of 1-bromo-3,7-dimethyloctane to 2,6-dimethylheptan-1-al using the method of Kharasch and Reinmurth (1954) (section 2.4.5.10). Obviously the proportion of undesired products from side reactions has decreased relative to the desired secondary alcohol. The mass spectrum of 2,6,10,14-tetramethyl-pentadecan-7-ol is shown in Fig. 3:11B. $M^{+\cdot}$ (m/z 284) is absent but the spectrum exhibits ions at m/z 283 ($M^{+\cdot}$ - 1), m/z 282 ($M^{+\cdot}$ - 2) and m/z 266 ($M^{+\cdot}$ - H_2O). McLafferty (1973) reported that in mass spectrometry, alcohols often undergo thermal and catalytic reactions, especially in metal lined systems such as used herein, giving rise to such spurious peaks as ($M^{+\cdot}$ - 1) and ($M^{+\cdot}$ - 2). The ions at

116
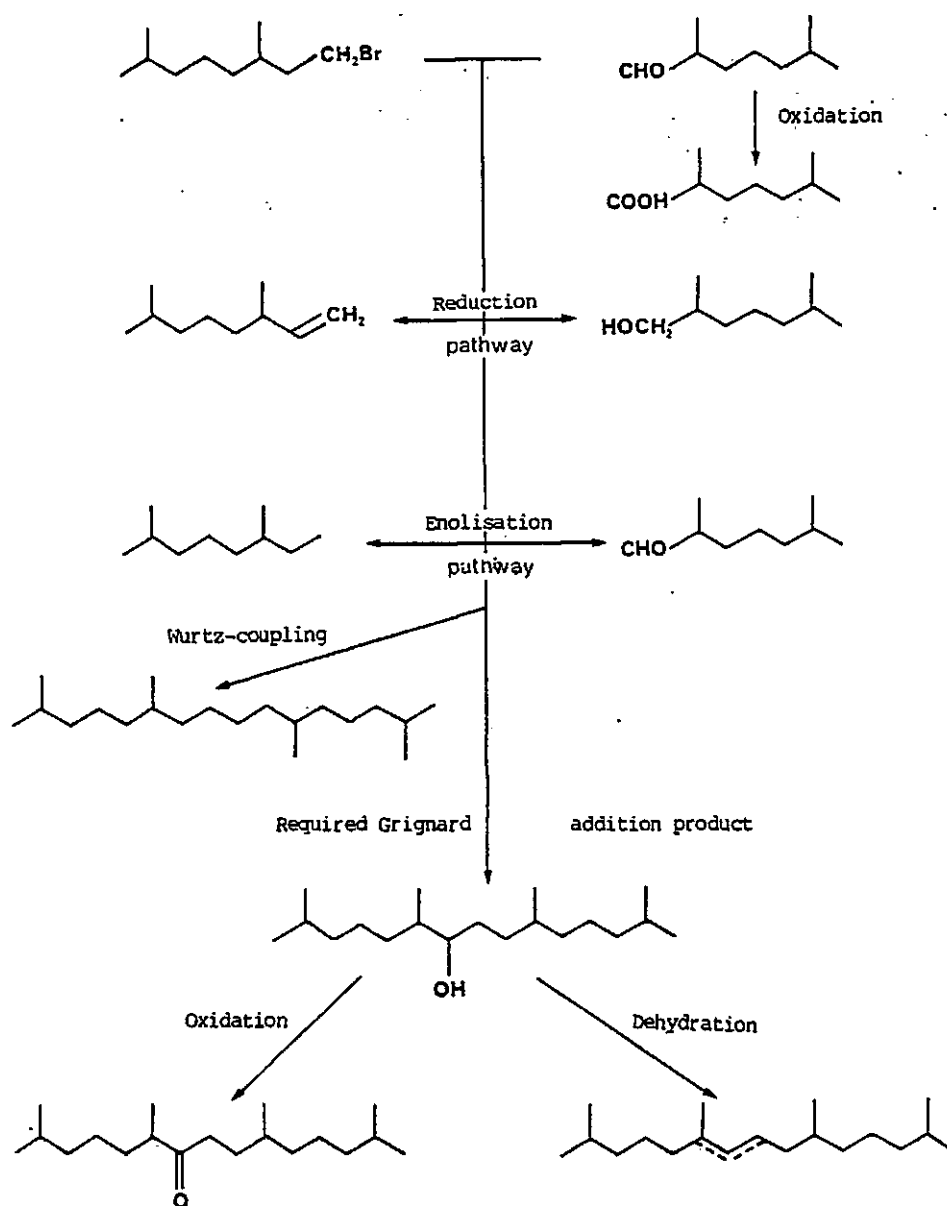
Fig. 3:10   The observed products in the Grignard addition of
             1-bromo-3,7-dimethyloctane to 2,6-dimethylheptan-1-al.
             Reaction classification as indicated.   For additional
             details see Table 3:1.
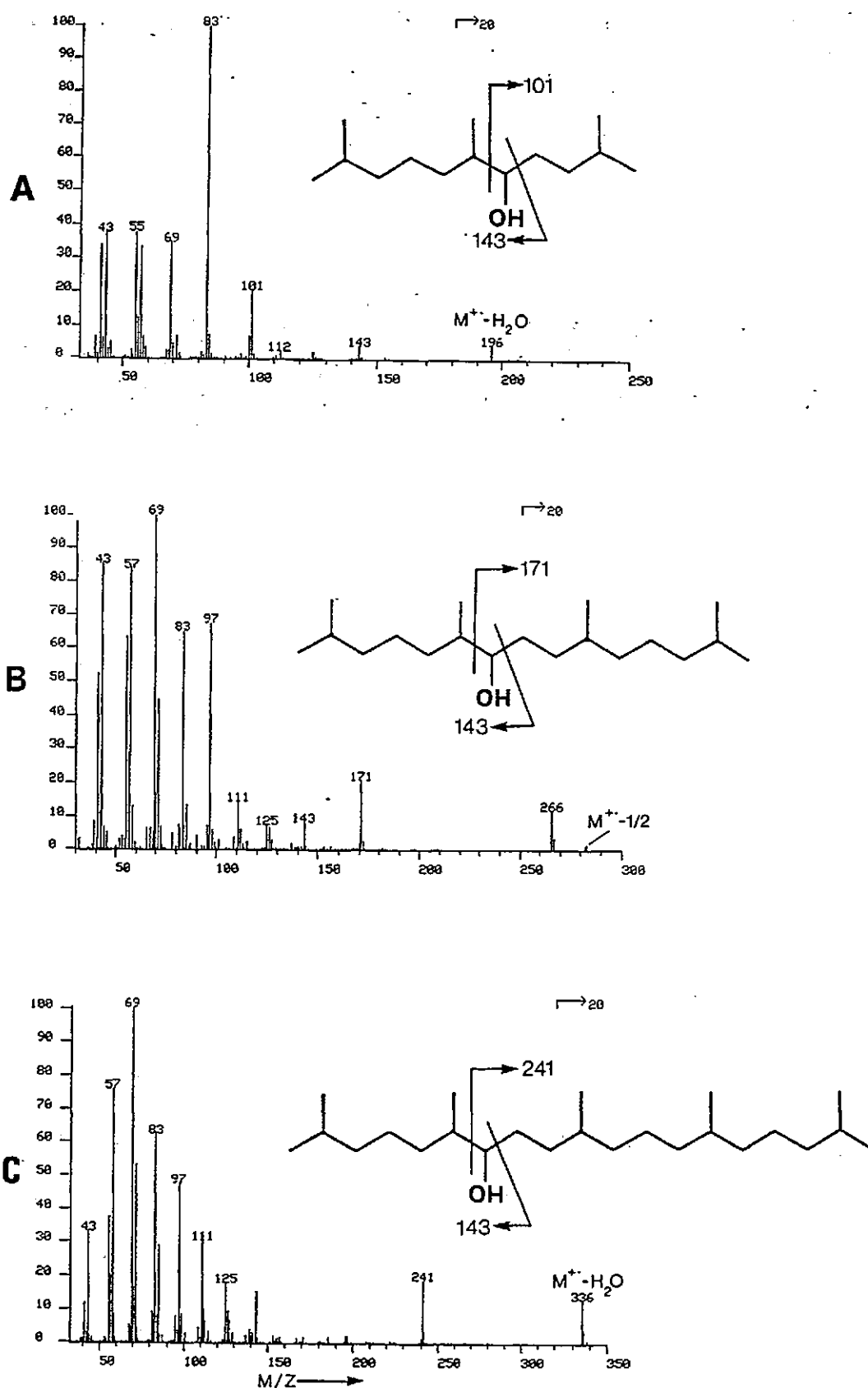
Fig.3:11   Mass spectra of A. 2,6,10-trimethylundecan-7-ol,.
            B. 2,6,10,14-tetramethylpentadecan-7-ol and
            C. 2,6,10,14,18-pentamethylnonadecan-7-ol.

m/z 143 and m/z 171 are generated by α-cleavage either side of the hydroxyl bearing carbon and the ions at m/z 69, m/z 97 and m/z 111 are fragments with one degree of unsaturation ($C_n H_{2n-1}^+$). The two intense α-cleavage ions in the mass spectrum of the TMS ether (Fig. 3:12B) confirm the position of the -OH group at C7 in the C_{19} skeleton.

2,6,10-trimethylundecan-7-ol
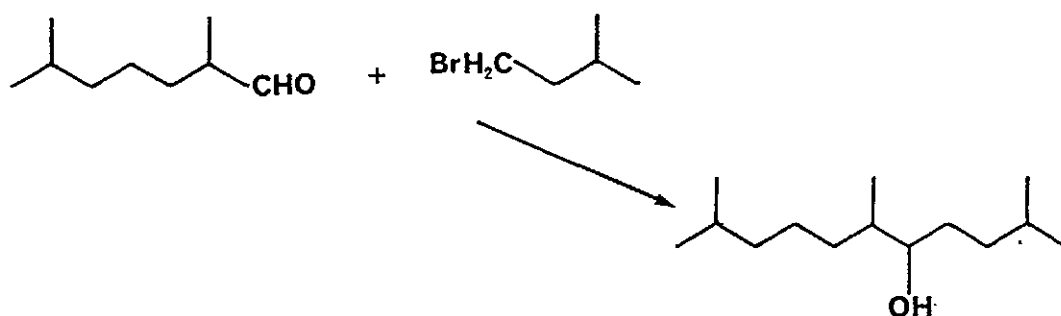
2,6,10-trimethylundecan-7-ol was prepared from the Grignard reaction of 1-bromo-3-methylbutane and 2,6-dimethylheptan-1-al using the method of Kharasch and Reinmurth (1954). The mass spectra of the secondary alcohol and the TMS ether are shown in Figs. 3:11A and 3:12A respectively. The position of the hydroxyl group at C7 in the C_{14} skeleton is confirmed by the two intense α-cleavage ions in the mass spectrum of the TMS ether of 2,6,10-trimethylundecan-7-ol (Fig. 3:12A).

2,6,10,14,18-pentamethylnonadecan-7-ol
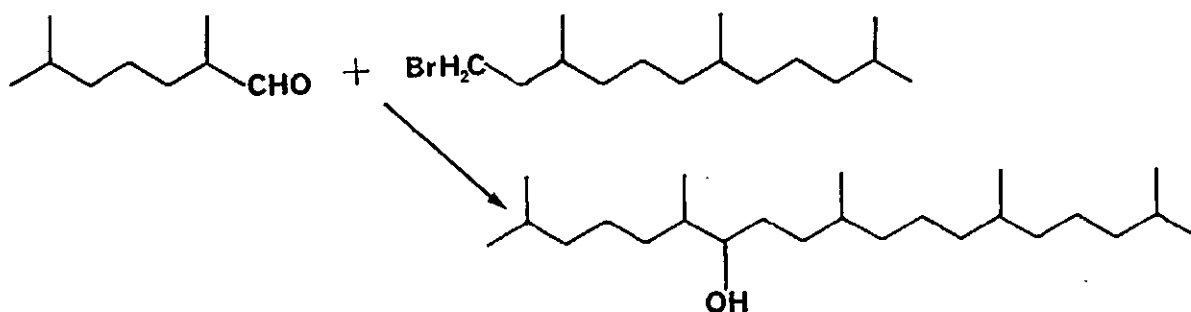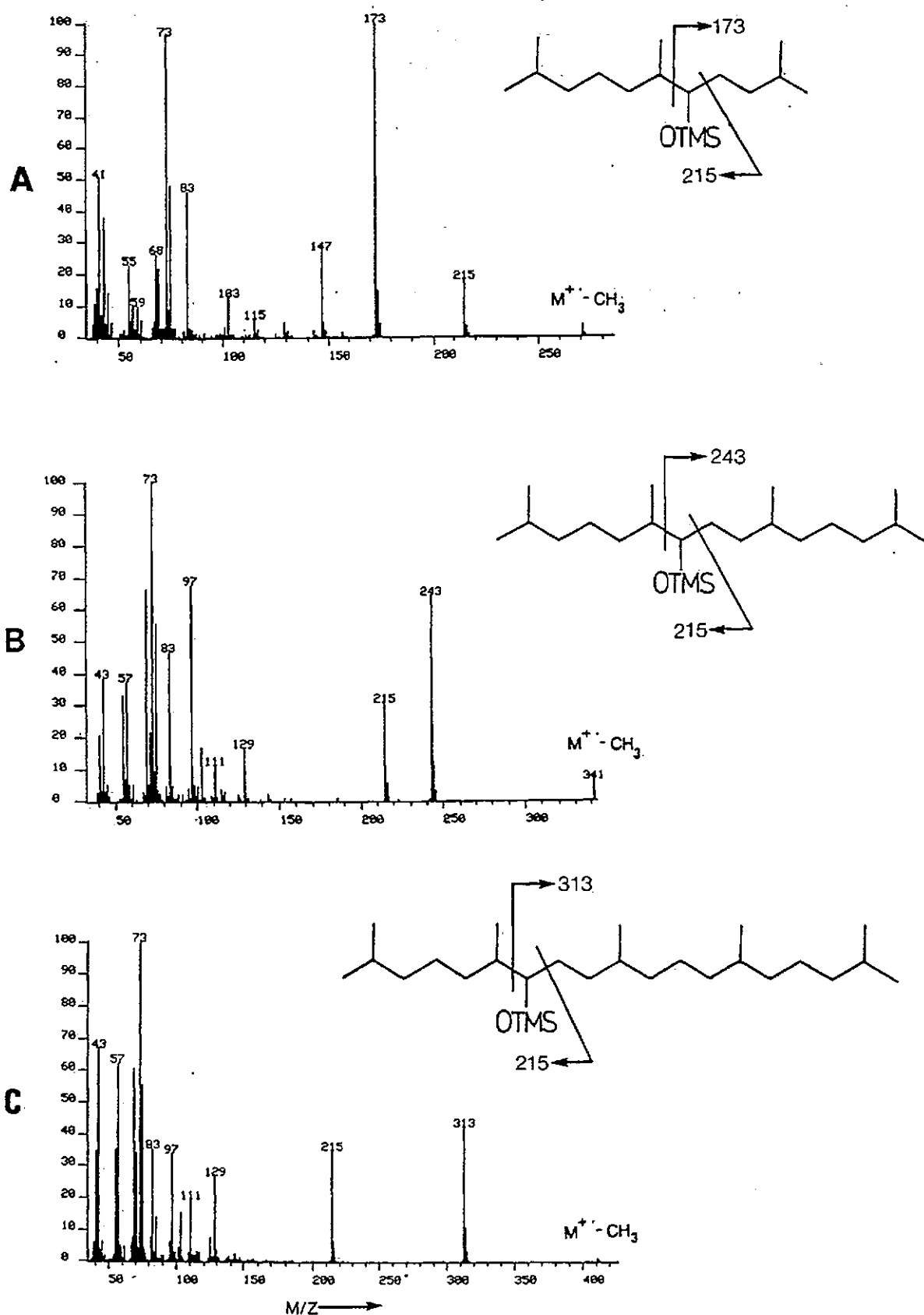
Fig.3:12   Mass spectra of the TMS ethers of A. 2,6,10-
            trimethylundecan-7-ol, B. 2,6,10,14-
            tetramethylpentadecan-7-ol and C. 2,6,10,14,18-
            pentamethylnonadecan-7-ol.

The C_{24} alcohol was prepared by Grignard reaction (Kharasch and Reinmurth, 1954) of 1-bromo-3,7,11-trimethyldodecane and 2,6-dimethylheptan-1-al. The mass spectra of the alcohol and the TMS ether are shown in Figs. 3:11C and 3:12C respectively. Again the presence of the two intense α-cleavage ions in the spectrum of the TMS ether of 2,6,10,14,18-pentamethylnonadecan-7-ol confirm the position of the -OH group at C7 on the C_{24} skeleton. The mass spectrum also displays a ($M^{+\cdot}$- CH_3) ion, often found in the spectra of trimethylsilyl ethers (Pierce, 1979). The yield of the C_{24} alcohol (62%) was higher than those of the corresponding C_{14} and C_{19} alcohols (58% and 57%) which is surprising as the formation of undesired products is reported to increase with increasing size of the Grignard reagent (March, 1977). In common with the synthesis of 2,6,10,14-tetramethylpentadecan-7-ol, a small quantity of the oxidation product of the C_{24} alcohol (i.e. a C_{24} ketone) was observed.

IR spectra of the C_{14}, C_{19} and C_{24} alcohols were consistent with those of saturated secondary alcohols. NMR spectra was not recorded.

3.2.6 Synthesis of 2,6,10-trimethylundecan-7-one; 2,6,10,14-tetramethylpentadecan-7-one and 2,6,10,14,18-pentamethylnonadecan-7-one

Mass spectra of the C_{14}, C_{19} and C_{24} ketones prepared by Omura and Swern (1978) oxidation of the respective C_{14}, C_{19} and C_{24} alcohols (section 3.2.5) are shown in Figs. 3:13A, B and C respectively. The mass spectrum of 2,6,10-trimethylundecan-7-one exhibits a $M^{+\cdot}$ at m/z 212, an ($M^{+\cdot}$ - 15) ion at m/z 197 and McLafferty rearrangement ions at m/z 156 and m/z 128:
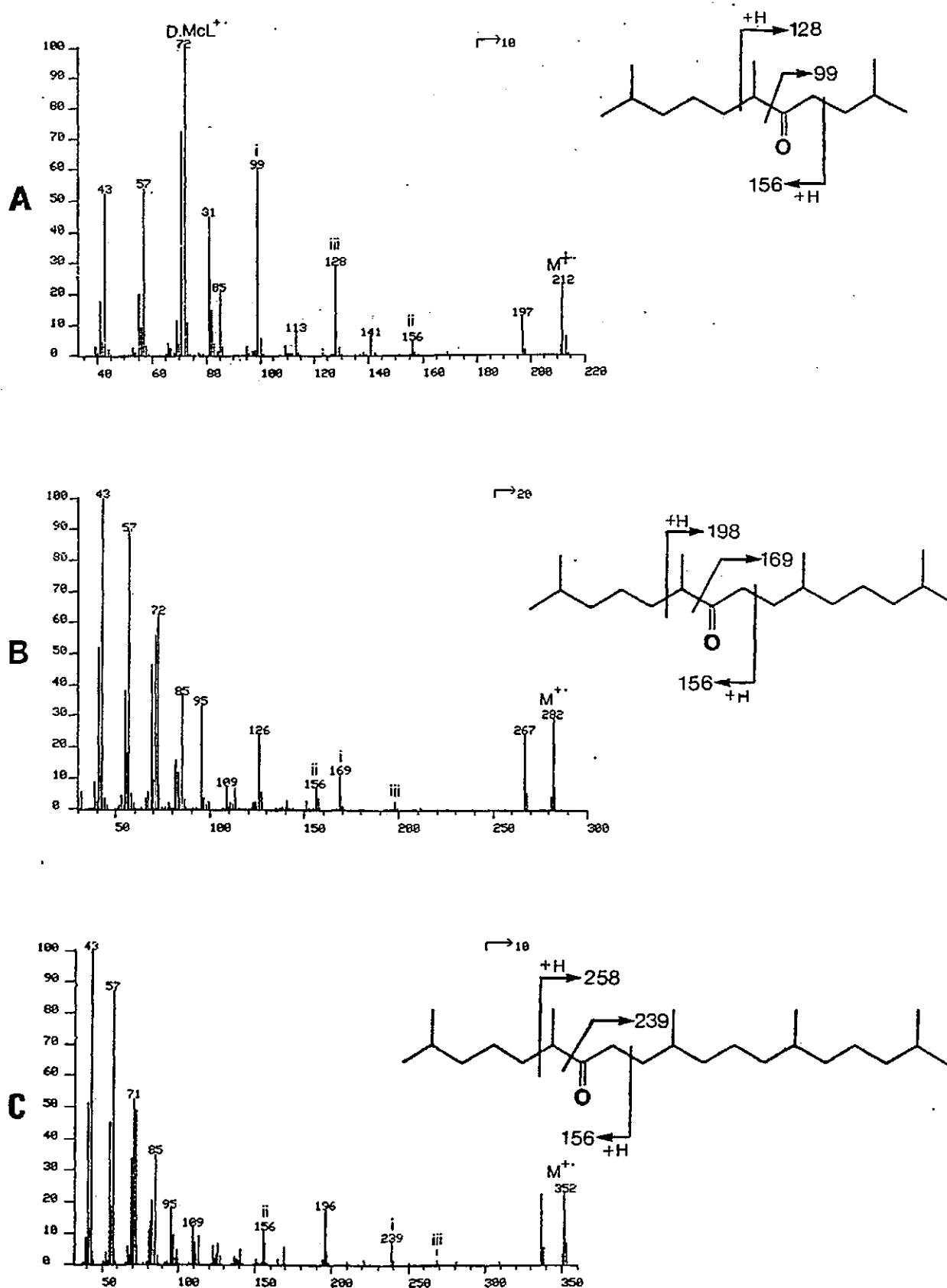
Fig.3:13   Mass spectra of A. 2,6,10-tetramethylundecan-7-one,
            B. 2,6,10,14-tetramethylpentadecan-7-one and
            C. 2,6,10,14,18-pentamethylnonadecan-7-one.
            Notations : D.McL⁺·= double McLafferty rearrangement ion;
            i = α-cleavage ion;   ii = McLafferty rearrangement ion 1 ;
            iii = McLafferty rearrangement ion 2 (see Table 3:2).

m/z 156
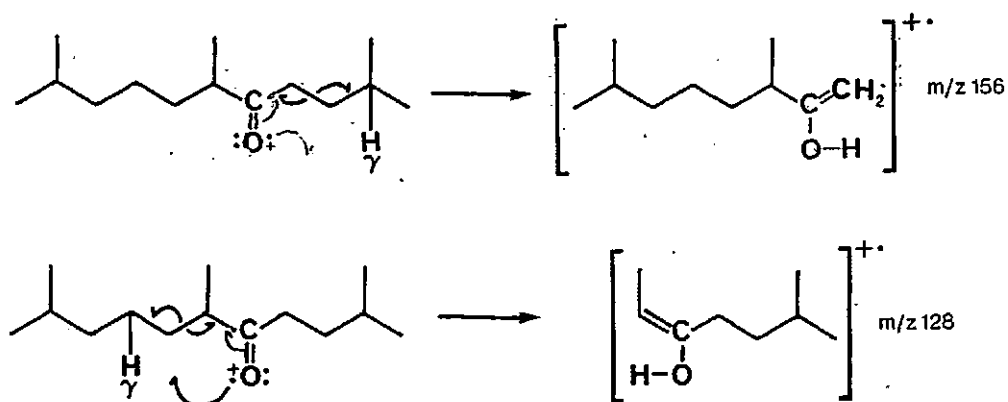
m/z 128

Additionally the presence of a double McLafferty rearrangement ion at m/z 72 is noted.

m/z 156

m/z 72
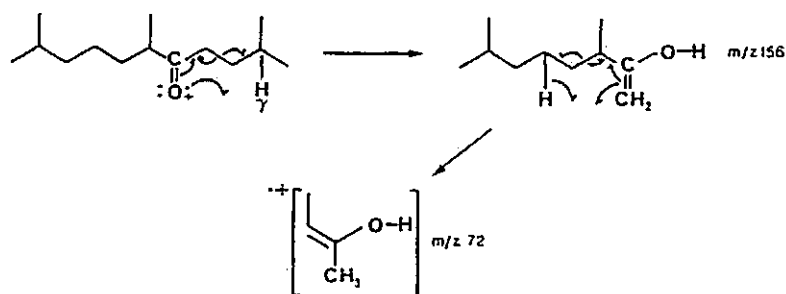
Obviously, a similar secondary rearrangement exists for the other primary McLafferty rearrangement ion (m/z 128). Also evident in the mass spectrum is the m/z 99 ion which arises from α-cleavage without H-transfer at the carbonyl carbon. Similar fragmentations occur in the mass spectra of 2,6,10,14-tetramethylpentadecan-7-one and 2,6,10,14,18-pentamethylnonadecan-7-one (Figs. 3:13B and C and summarised in Table 3:2). The partial ^{1}H NMR spectrum of 2,6,10,14-tetramethylpentadecan-7-one is displayed in Fig. 3.14A. The single proton α to the carbonyl carbon [CH_2-C(CH_3)$\underline{H}$-CO] is a sextet at $\delta 2.515$ppm and the methylene protons α to the carbonyl carbon [CO-C$\underline{H}_2$-CH_2] are seen as a triplet at $\delta 2.405$ppm. The triplet exhibited superimposed fine structure through coupling with other unknown protons. In addition, the methyl protons β to the carbonyl carbon (CH_2-C($C\underline{H}_3$)H-CO) appear as a downfield doublet at $\delta 1.041$ppm (not shown). ^{1}H NMR spectra for 2,6,10-trimethylundecan-7-one and 2,6,10,14,18-pentamethylnonadecan-7-one were not recorded. The IR spectra of the C_{14}, C_{19} and C_{24} ketones were all essentially

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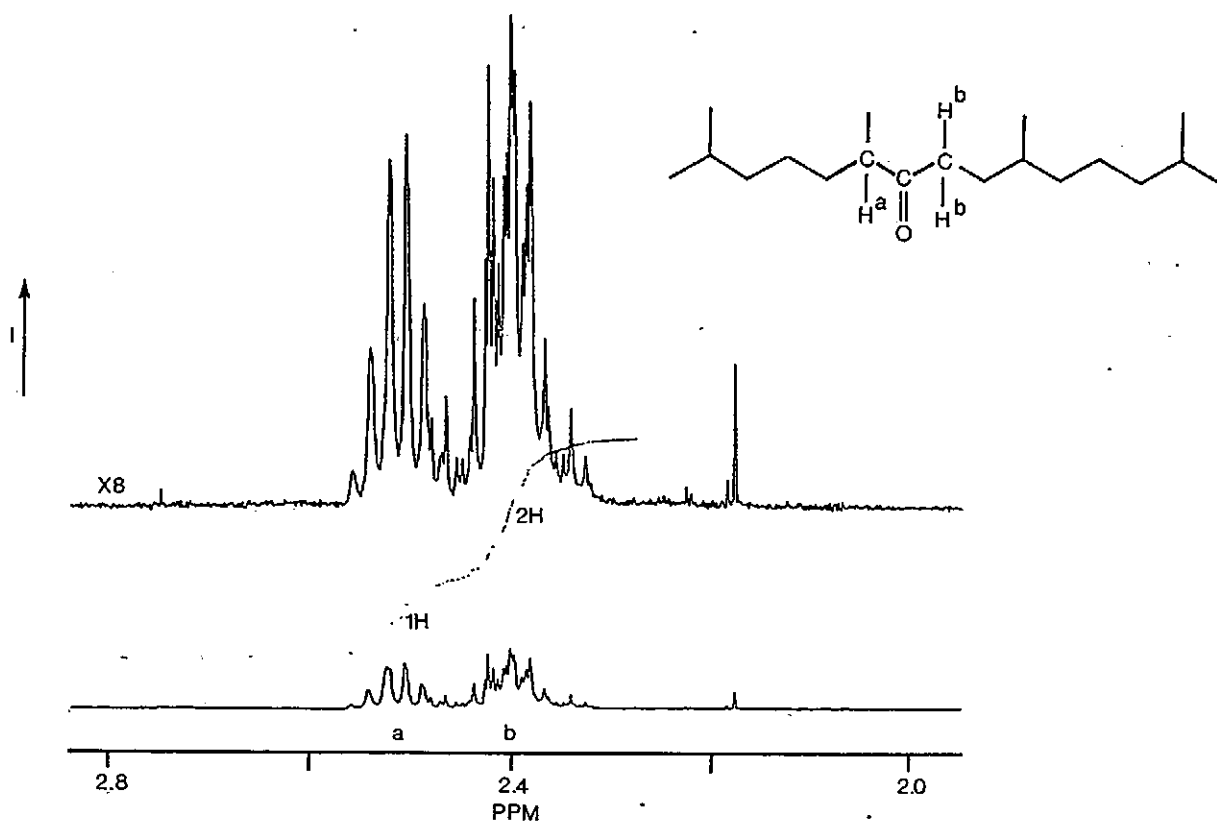
Fig.3:14A   Partial ^{1}H NMR (400MHz) spectrum of 2,6,10,14-tetramethylpentadecan-7-one.
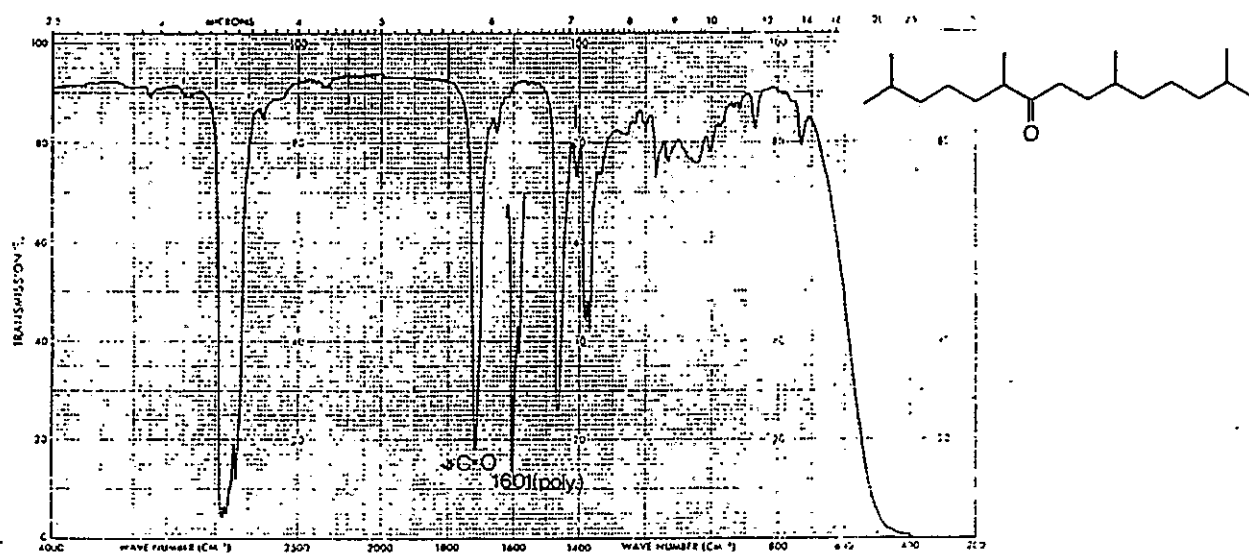
Fig.3:14B   IR spectrum of 2,6,10,14-tetramethylpentadecan-7-one. (poly.) = polystyrene.

similar, exhibiting strong carbonyl stretch at 1720cm^{-1}. The IR spectrum of the C_{19} ketone is shown as a example (Fig. 3.14B). The chemical shifts (δppm) and intensities, of the resonances observed in the ^{13}C NMR spectrum (Fig. 3.15) of 2,6,10-trimethylundecan-7-one are summarised in Table 3.3A. Also indicated is the type of carbon each peak represents as inferred by DEPT spectroscopy. In DEPT spectroscopy, methyl and methine carbon resonances produce positive peaks, methylene resonances give negative peaks and unprotonated carbons are not represented (Doddrell et al., 1982). The spectrum of 2,6,10-trimethylundecan-7-one was interpreted by comparison with data for other ketones presented by Breitmaier (1978) and Yon (1982). Breitmaier (1978) reported that the carbonyl carbon of alkanones appears between 203 and 217 ppm downfield of TMS, the magnitude of the downfield shift within that region being dependent on the number of α-protons. Yon (1982) noted, from data presented in Breitmaier et al. (1979) that the α- and β-carbons to the carbonyl carbon experience a shift change relative to the chemical shift of the corresponding carbon in the parent alkane. However, it appeared that the γ- and ϵ-carbon shift remained essentially unchanged. An n-alkanone was used as an example to illustrate this point.
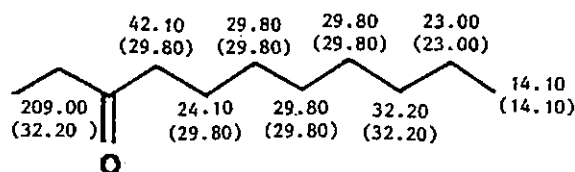
The figures in parentheses are those of the corresponding alkane. These observations were used by Yon (1982) in determining the carbon shift assignments for 2,6-dimethyldodecan-5-one as shown below.
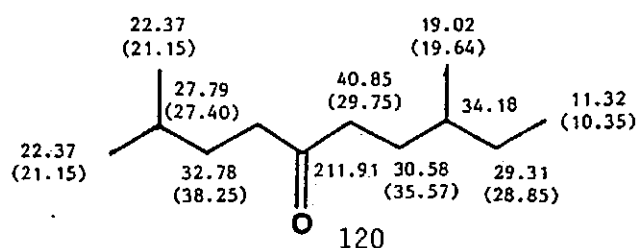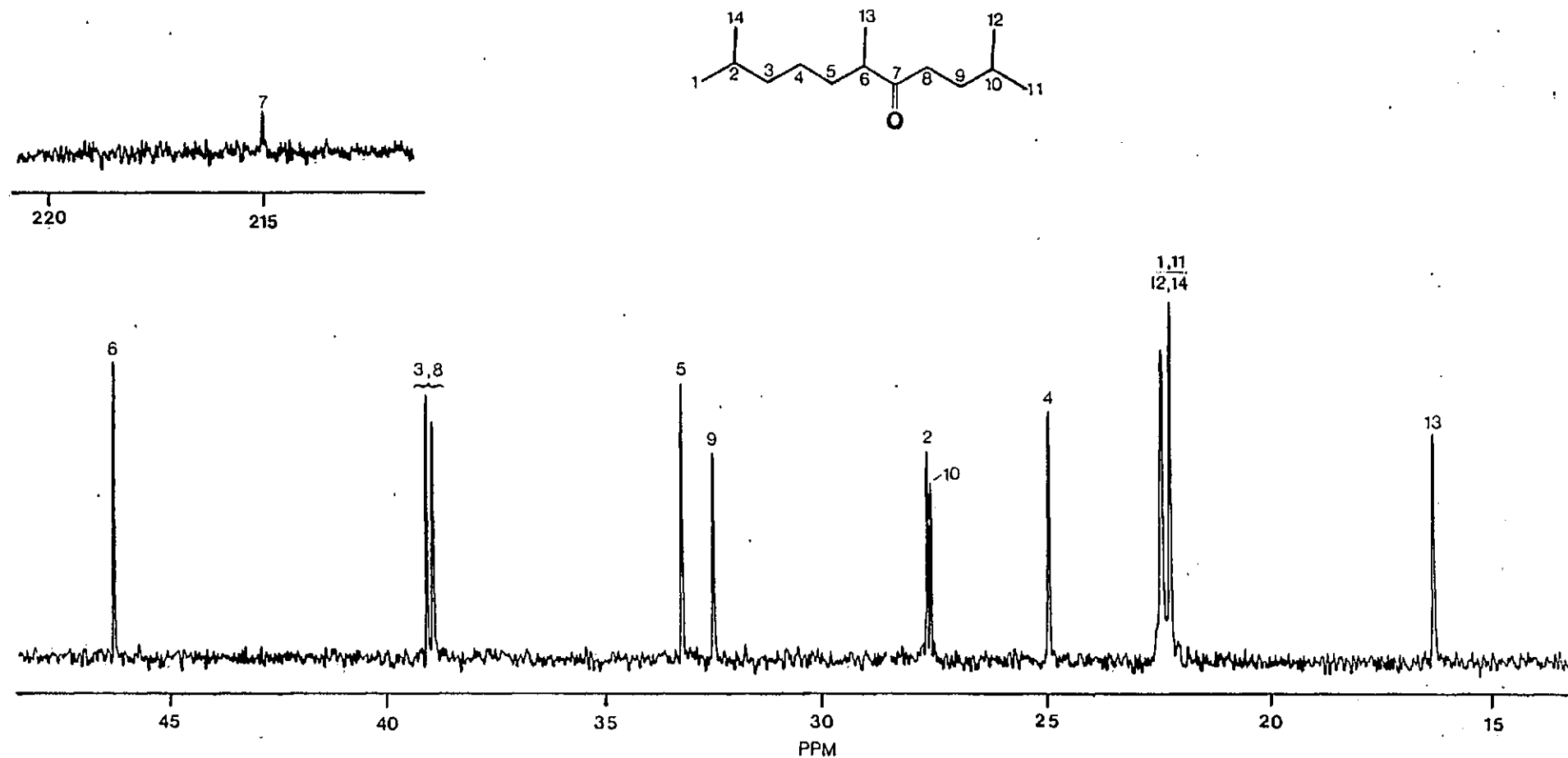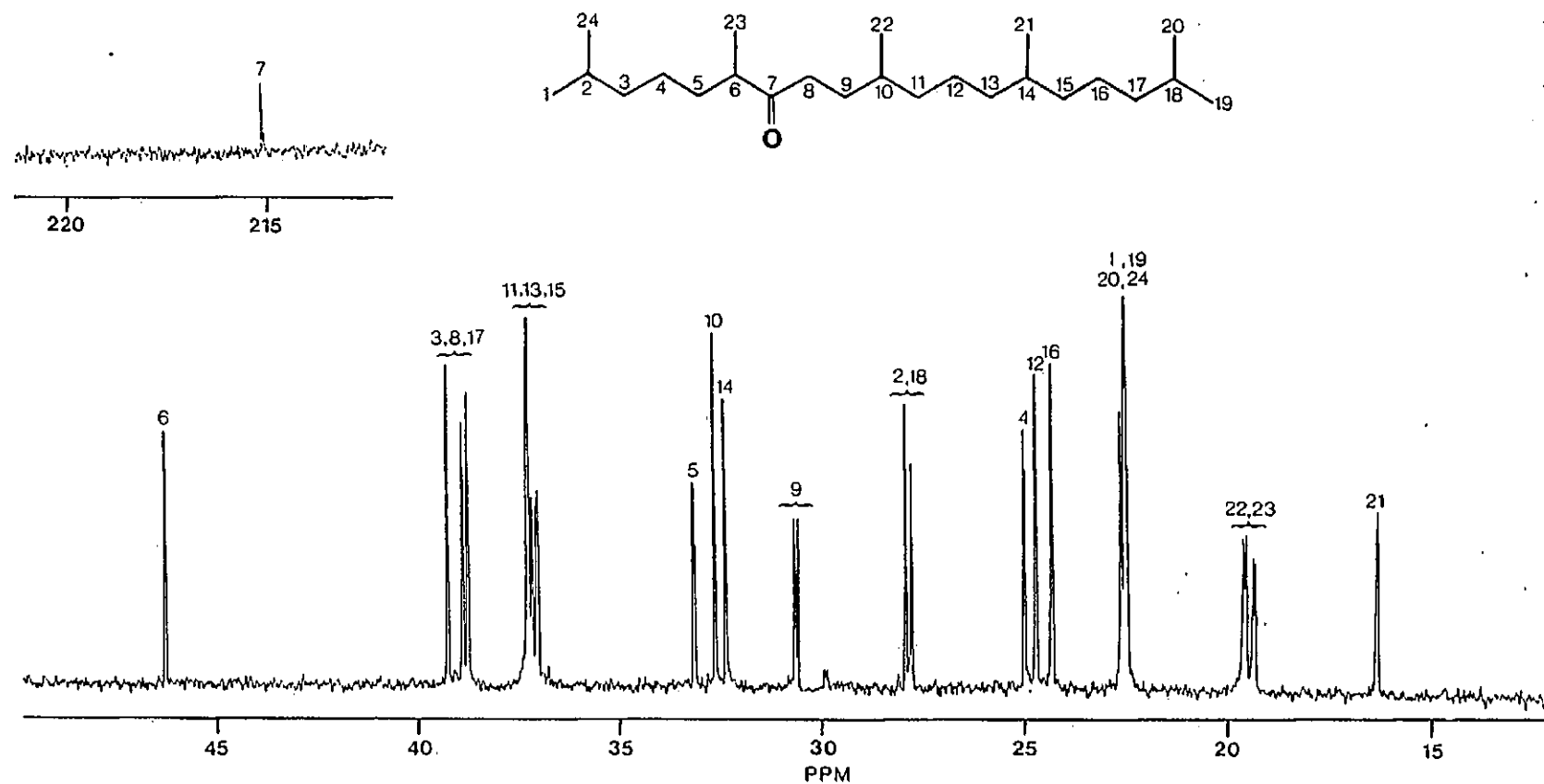
Fig.3:15 ^{13}C NMR (400 MHz) spectrum of 2,6,10-trimethylundecan-7-one. Proposed shift assignments (Table 3:3b) as shown. Numbers refer to the carbon number in the accompanying number.

As experimental carbon shift data for the alkane were not available the figures in parentheses represent shifts calculated using empirical molecular additivity coefficients available for alkanes (i.e. Grant and Paul, 1964; Carmen et al., 1973) which are discussed at greater length in section 3.2.9.

The application of these observations to the spectrum of 2,6,10-trimethylundecan-7-one enables a full interpretation (Table 3:3B).
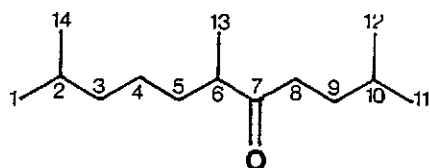
In the C_{14} ketone, the chemical shifts of C1-4, C10-12 and C14 would be expected to be the same as those in the corresponding alkane since only the shifts at the α- and β-carbons are affected. As chemical shift data for the alkane were not available it became necessary to use either calculated theoretical chemical shifts (Table 3:3B) or to compare the observed data with published spectra of analogous molecules such as pristane (Yon, 1982; see Table 3:4B). Thus, the peaks at 22.2682ppm and 22.4582ppm (Table 3:3A) were assigned to the methyl carbons C1, C11, C12 and C14 of the isopropyl groups. The observed difference in the chemical shifts is caused by magnetic non-equivalence of the methyl groups in each terminal isopropyl group (see section 3.2.9). Similarly, peaks at 27.7340 ppm and 27.6455 ppm were assigned to the methine carbons at C2 and C10 and peaks at 38.9020 ppm and 24.9817 ppm assigned to C3 and C4 respectively. The peak at 46.2762 ppm represents a methine carbon (CH; Table 3:3a) and was therefore assigned to C6. By analogy with the data of Breitmaier (1978), the peaks at 39.0431 ppm and 215.0283 ppm were assigned to C8 and C7 respectively. The peak at 16.3175 ppm must represent the remaining

Table 3:3A

Observed ^{13}C NMR chemical shift for 2,6,10-trimethylundecan-7-one

ppm	intensity	proposed number of carbons	Type of carbon[a]
16.3175	10.386	1	CH_3/CH
22.2683	16.358	2	CH_3/CH
22.4582	14.175	2	CH_3/CH
24.9817	11.376	1	CH_2
27.6455	8.069	1	CH_3/CH
27.7340	9.499	1	CH_3/CH
32.4856	9.528	1	CH_2
33.2038	12.526	1	CH_2
38.9020	10.929	1	CH_2
39.0431	12.068	1	CH_2
46.2762	13.495	1	CH_3/CH
215.0283	1.742	1	carbonyl
		14	

[a] From DEPT spectroscopy.

Table 3:3B

^{13}C-NMR shift assignments for 2,6,10-trimethylundecan-7-one
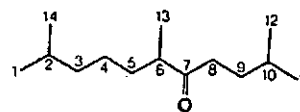
Carbon Number	ppm	Calculated chemical shift assignments[e] for parent C_{14} alkane ppm
13	16.3175[a]	20.44
1,11	22.2682[a]	22.50
12,14	22.4582[a]	22.50
4	24.981[b]	25.29
10	27.6455[b]	28.61
2	27.7340[b]	28.61
9	32.4856[c]	39.71
5	33.2038[c]	37.93
3	38.9020	39.99
8	34.0431	25.29
6	46.2762	33.02
7	215.0283	37.93

a, b, c: Chemical shift assignments with the same superscript may be interchanged.

d: skeleton numbered in this manner for comparative purposes.

e: calculated according to Carmen et al., 1973.

methyl carbon C13 which suggested that the two peaks at 33.2038 ppm and 32.4856 ppm corresponded to the β- carbons, C5 and C9. These assignments are in full agreement with the data obtained from DEPT spectroscopy.

The ^{13}C NMR spectrum of 2,6,10,14-tetramethylpentadecan-7-one is displayed in Fig. 3:16 and Table 3:4A lists the chemical shift and intensity data. The assignment of the spectrum was performed by comparison with the spectra of pristane and the preceding C_{14} ketone and is summarised in Table 3:4B.

Table 3:5a tabulates the ^{13}C NMR spectrum (Fig. 3:17) of 2,6,10, 14,18-pentamethylnonadecan-7-one. Also indicated is the type of peak (methyl, methylene, methine) inferred by DEPT spectroscopy. The assignment of the spectrum was performed by comparison with those of the preceding C_{14} and C_{19} ketones and with chemical shift data for the corresponding C_{24} parent alkane calculated using molecular additivity coefficients (Carmen et al., 1973). The assignment is summarised in Table 3:5B. In addition to the observed magnetic non-equivalence of the methyl groups of the isopropyl termini (i.e. pair of signals at 22.4946 ppm and 22.5973 ppm), the doublets for C22 (19.3390 ppm and 19.3961 ppm)* and for C23 (19.5630 ppm and 19.6284 ppm)* (Fig. 3:16) in the spectrum of the C_{24} ketone demonstrate the effect of magnetic non-equivalence resulting from differences in configuration (see later). The pair of signals for the C21 and C22 carbons are evidence for the presence of two diastereoisomeric pairs of enantiomers. The assignments in the spectrum of 2,6,10,14,18-

*Assignments may be interchanged.

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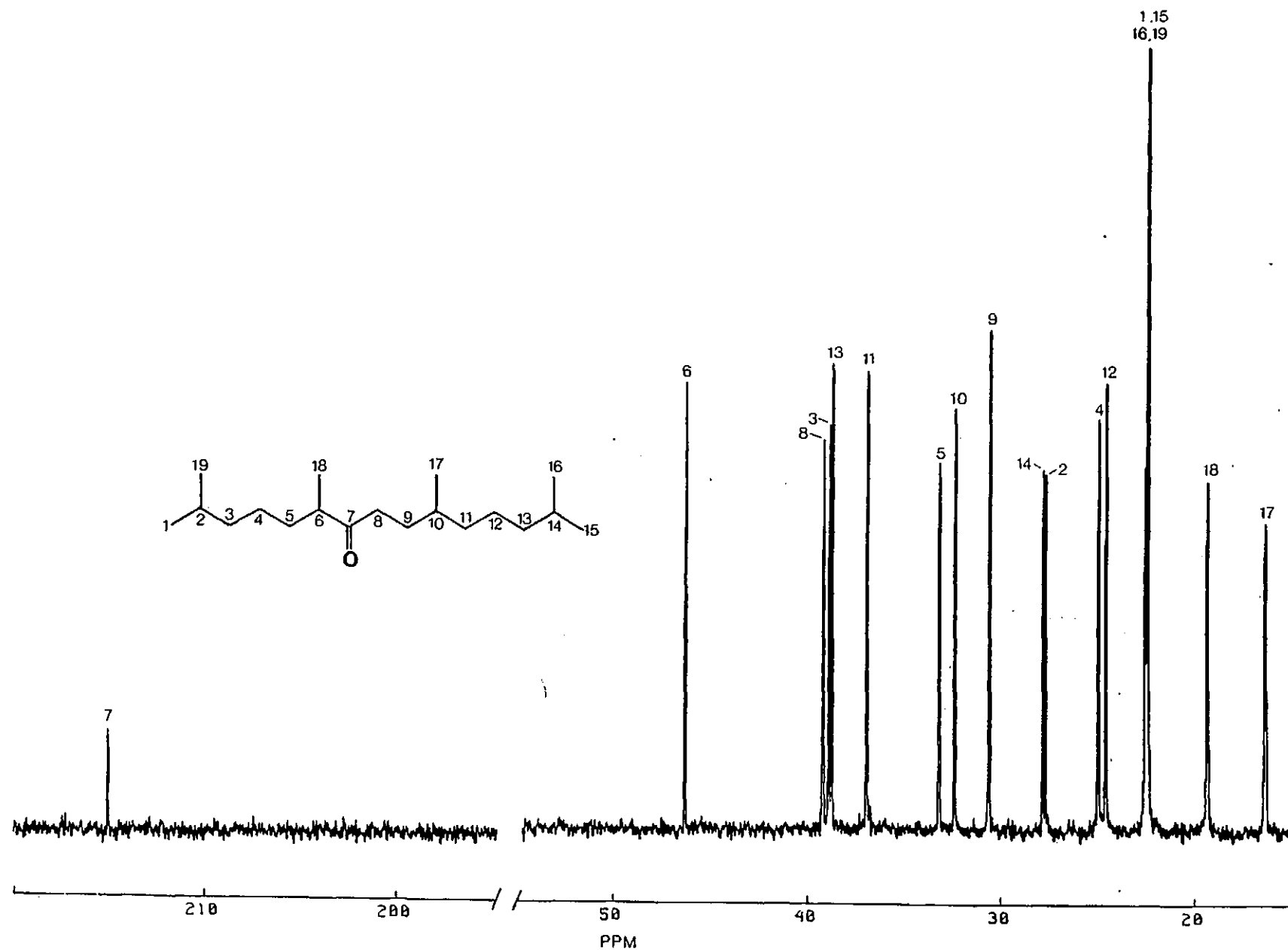
Fig.3:16 ^{13}C NMR (400 MHz) spectrum of 2,6,10,14-tetramethylpentadecan-7-one. Proposed shift assignments (Table 3:4b) as shown. Numbers refer to the carbon number in the accompanying structure.

Table 3:4A

Observed ^{13}C NMR chemical shifts for
2,6,10,14-tetramethylpentadecan-7-one

ppm	intensity	proposed number of carbons
16.3122	8.115	1
19.3557	9.359	1
22.4669	20.963	4
22.5678	9.540	
24.6007	11.790	1
24.9865	10.879	1
27.7318	9.427	1
27.8550	9.402	1
30.6385	11.551	1
32.4150	11.553	1
33.2159	9.629	1
36.9643	12.488	1
38.7660	12.337	1
38.9086	10.639	1
39.2068	10.484	1
46.2824	11.507	1
215.0609	2.500	1

Total: 19

Table 3:4B

^{13}C NMR shift assignments for 2,6,10,14-tetramethylpentadecan-7-one
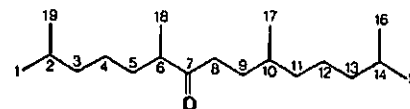
Carbon Number	ppm	Shift assignments for C_{19} alkane (pristane)[a] ppm
17	16.3122	19.79
18	19.3557	19.79
1,15	22.4669[b]	22.75
16,19	22.5678[b]	22.65
12	24.6007	24.85
4	24.9865	24.85
2	27.7318	28.02
14	27.8550	28.02
9	30.6385[c]	37.34
10	32.4150[c]	32.85
5	33.2159[c]	37.52
11	36.9643	37.52
13	38.7660	39.45
3	38.9086	39.45
8	39.2068	24.52
6	46.2824	32.85
7	215.0609	37.34

[a]: Yon,1982; Lamb,1982.

[b,c]: chemical shift assignments with the same superscript may be interchanged.

Table 3:5A

Observed ^{13}C NMR chemical shift for 2,6,10,14,18-pentametylnonadecan-7-one

ppm	intensity	proposed number of carbons	Type of carbon[a]
16.3217	8.813	1	CH$_3$/CH
19.3390	6.311	1	CH$_3$/CH
19.3961	6.484		
19.5630	7.573	1	CH$_3$/CH
19.6284	7.417		
22.4946	19.004	2	CH$_3$/CH
22.5973	13.543	2	CH$_3$/CH
24.3021	15.775	1	CH$_2$
24.6943	15.217	1	CH$_2$
24.9889	12.591	1	CH$_2$
27.7306	10.933	1	CH$_3$/CH
27.8758	14.049	1	CH$_3$/CH
30.5591	8.288	1	CH$_2$
30.6802	8.301		
32.4177	14.133	1	CH$_3$/CH
32.6808	17.091	1	CH$_3$/CH
33.2063	9.868	1	CH$_2$
37.0448	9.492	1	CH$_2$
37.1870	9.297	1	CH$_2$
37.2825	18.123	1	CH$_2$
38.7770	14.192	1	CH$_2$
38.9012	13.013	1	CH$_2$
39.2775	15.534	1	CH$_2$
46.2799	12.232	1	CH$_2$
215.1225	3.265	1	CH$_3$/CH
		Total: 24	

[a]: from DEPT spectroscopy

Table 3:5B

^{13}C-NMR shift assignments for 2,6,10,14,18-pentamethynonadecan-7-one
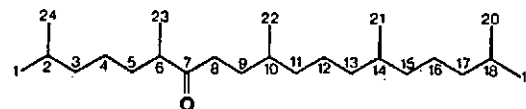
Carbon Number	ppm	Calculated chemical shift for C$_{24}$ alkane ppm
21	16.3275	20.44
22	19.3390[b]	20.44
	19.3961	
23	19.6284[b]	20.44
	19.5630	
1,19	22.4946[c]	22.50
20,24	22.5973[c]	22.50
16	24.3021	25.29
12	24.6943	25.57
4	24.9889	25.29
2	27.7306[d]	28.61
18	27.8758[d]	28.61
9	30.6802[e]	37.65
14	32.4177[f]	33.02
10	32.6808[f]	33.02
5	33.9868[e]	37.93
11	37.0448	37.94
15	37.1870[g]	37.93
13	37.6448[g]	39.08
17	38.7770	39.99
3	38.9012	39.99
8	39.2775	25.37
6	46.2799	33.02
7	215.1225	37.65

[a]: Calculated according to Carmen et al., 1973

b,c,d,e,f,g: chemical shift assignments with the same superscript may be interchanged.

Fig.3:17 ^{13}C NMR (400 MHz) spectrum of 2,6,10,14,18-pentamethylnonadecan-7-one. Proposed shift assignments (Table 3:5b) as shown. Numbers refer to the carbon number in the accompanying structure.

pentamethylnonadecan-7-one were in full agreement with the data obtained from DEPT spectroscopy.

3.2.7 Synthesis of 2,6,10-trimethyl-7-(3-methylbutyl)dodecan-7-ol, 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol and 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecan-7-ol.

The synthesis of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol was chosen for the pilot study.
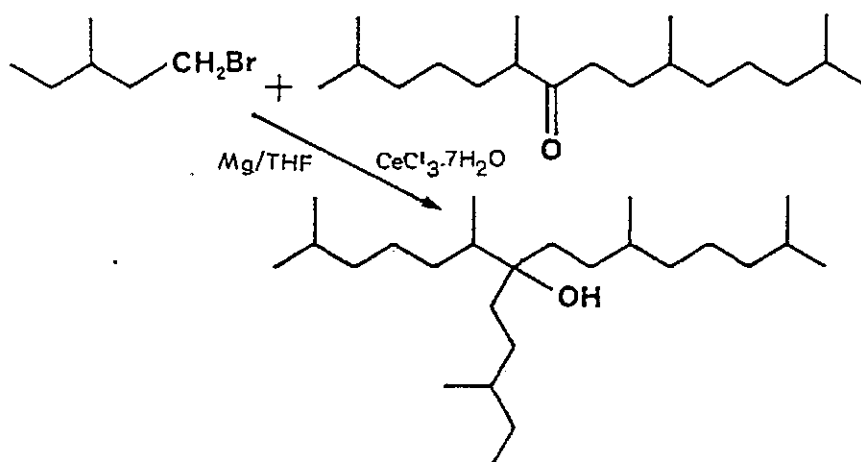
Fig. 3.18A displays the gas chromatogram of the crude reaction products from the Grignard addition (method of Kharasch and Reinmurth, 1954) of 1-bromo-3-methylpentane to 2,6,10,14-tetramethylpentadecan-7-one. It is evident that the chromatographic peak representing the desired product (labelled c) is a minor component compared with those representing the Wurtz-coupling product of 1-bromo-3-methylpentane (3,8-dimethyldecane, a) and residual unconsumed ketone (b). This distribution is reflected in the low yield (7%) of the desired product. March (1977) reported that highly hindered tertiary alcohols are typically produced in only extremely low yield by the standard addition of Grignard reagents to ketones because side reactions such as

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enolisation, reduction or Wurtz-coupling assume prominance. The non-polar tlc behaviour (i.e. R_f 0.4; SiO_2, hexane mobile phase) of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol and the inability to prepare the TMS ether of the alcohol using BSTFA indicate that the alcohol group is fairly hindered. The TMS ether of the tertiary alcohol was prepared eventually using TSIM (N-trimethyl-silylimidazole, 44) in a procedure designed specifically for the silylation of highly hindered hydroxyl groups in steroids (Sakauchi and Horning, 1971). The synthesis of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol was repeated using the recently developed "cerium chloride promoted Grignard addition" method of Imamoto et al. (1985). The method was designed for the synthesis of hindered tertiary alcohols and has been successfully used in the preparation of several alcohols considered difficult to produce (except in low yield) using the conventional Grignard addition. The method is thought to involve exchange of the Grignard reagent (RMgX) into the stronger nucleophile "$RCeCl_2$" which, exhibiting a pronounced affinity for carbonyl groups, undergoes almost exclusive nucleophilic addition to the carbonyl carbon. The pronounced affinity towards the carbonyl group is ascribed to the strong oxaphilicity of trivalent cerium (Imamoto et al., 1984). The gas chromatogram of the crude reaction products of the "cerium chloride promoted Grignard addition" of 1-bromo-3-methylpentane and 2,6,10,14-tetramethylpentadecan-7-one is shown in Fig. 3:18B. The increased production of 2,6,10,14-tetramethyl-7-(3-methylpentyl) pentadecan-7-ol is immediately obvious. The mass spectrum (Fig. 3:19B) of the product C_{25} tertiary alcohol exhibits a ($M^{+\bullet}$ - H_2O) ion at m/z 350 and three intense fragment ions at m/z 227, m/z 255 and m/z 283 formed by α-cleavage without hydrogen transfer at the hydroxyl bearing quaternary carbon. The presence of three intense α-cleavage ions in
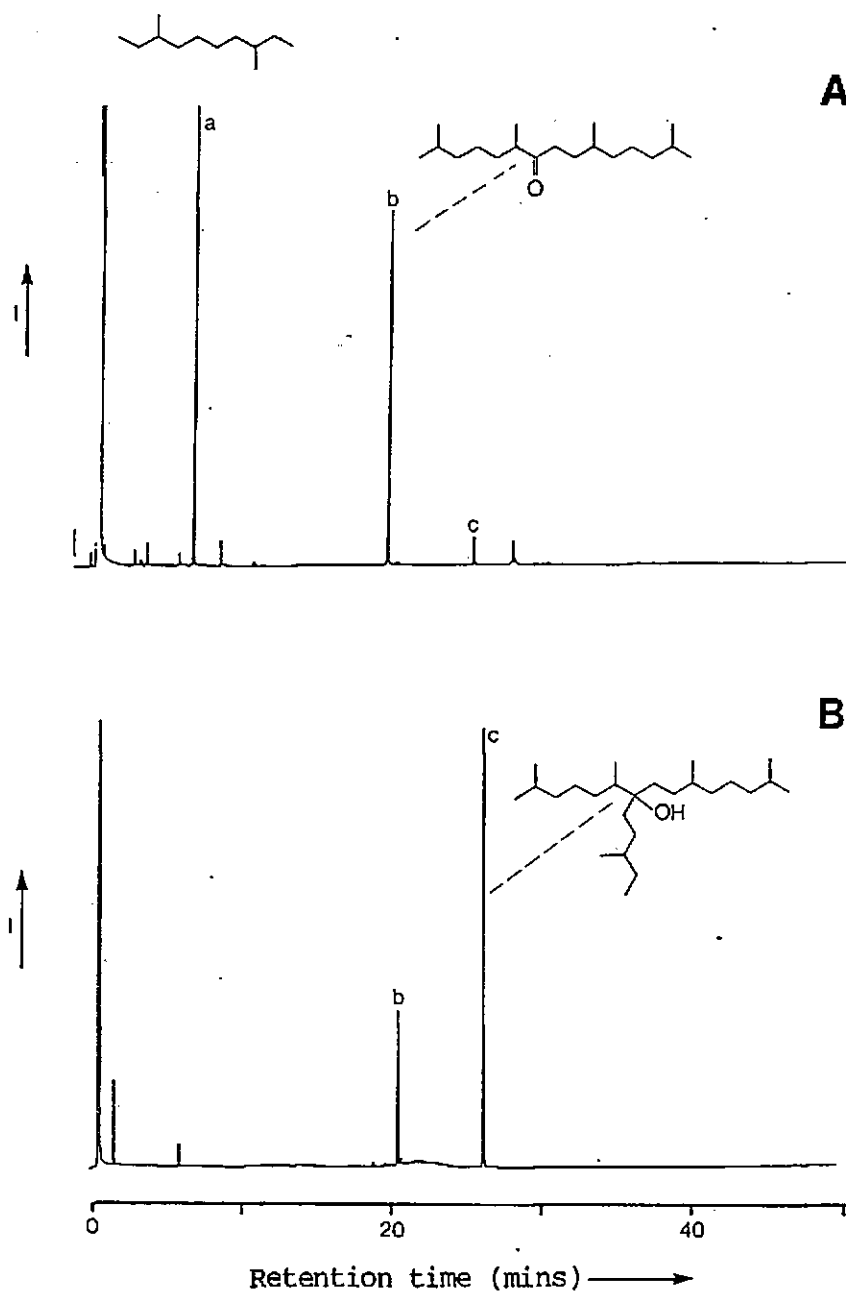
Fig.3:18   Gas chromatograms of the products of the Grignard addition of
1-bromo-3-methylpentane to 2,6,10,14-tetramethylpentadecan-7-one:
A. method of Kharasch and Reinmurth (1954) and B. method of
Imamoto et al. (1985). Peaks a, b and c represent
3,8-dimethyldecane, 2,6,10,14-tetramethylpentadecan-7-one and
2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol
respectively. Gc conditions: SE54, 40 - 290°C at 8°Cmin⁻¹.
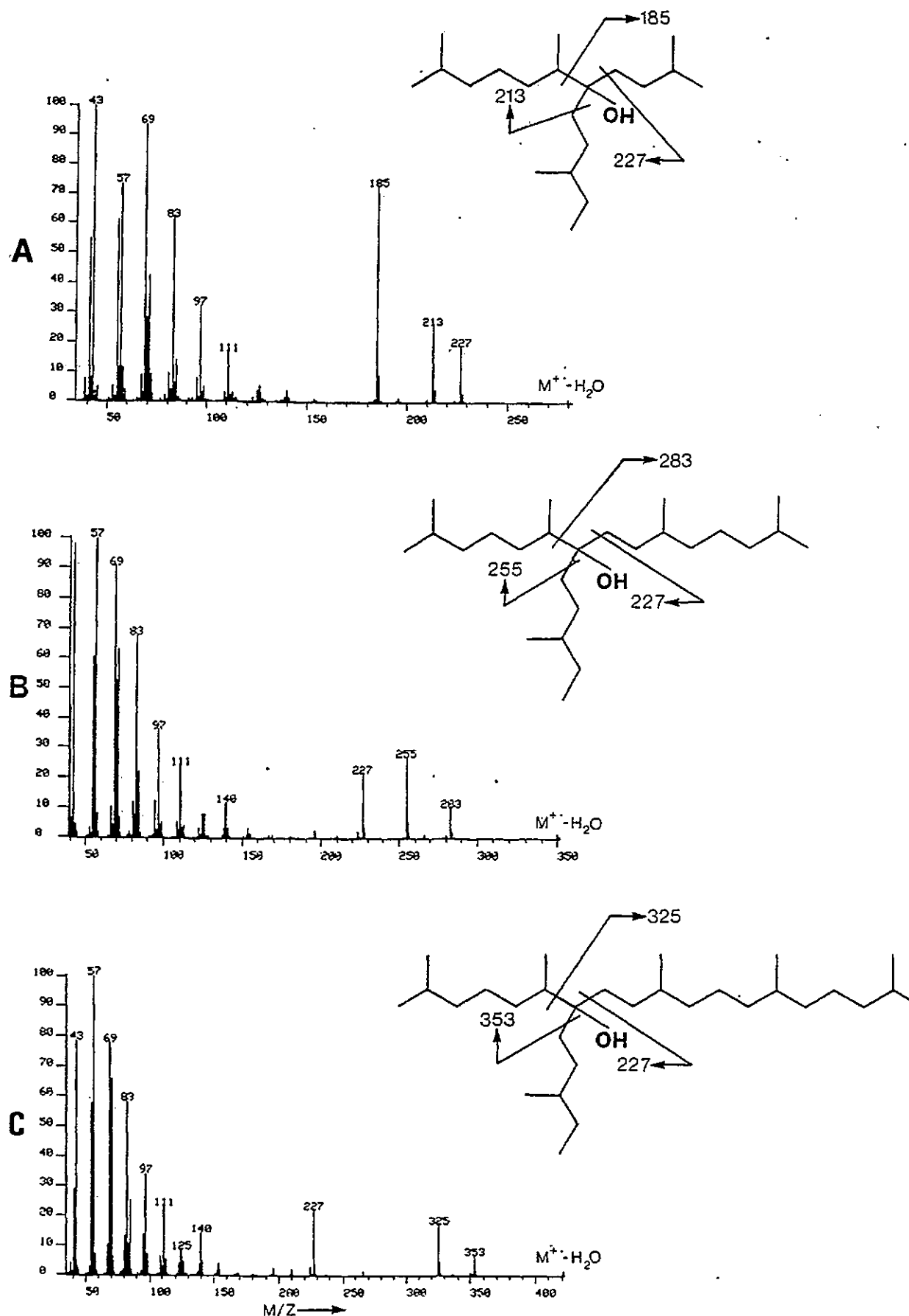
Fig.3:19   Mass spectra of A. 2,6,10-trimethyl-7-
           (3-methylbutyl)dodecan-7-ol, B. 2,6,10,14-
           tetramethyl-7-(3-methylpentyl)pentadecan-7-ol and
           C. 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecan-7-ol.

the mass spectrum (Fig. 3:20B) of the TMS ether of the tertiary C_{25}
alcohol confirms the assignment of the hydroxyl group to C7.
Interestingly, the most intense α-cleavage ion present in both spectra
is that formed by loss of the $C_8H_{17}^\bullet$ radical.

2,6,10-trimethyl-7-(3-methylbutyl)dodecan-7-ol.
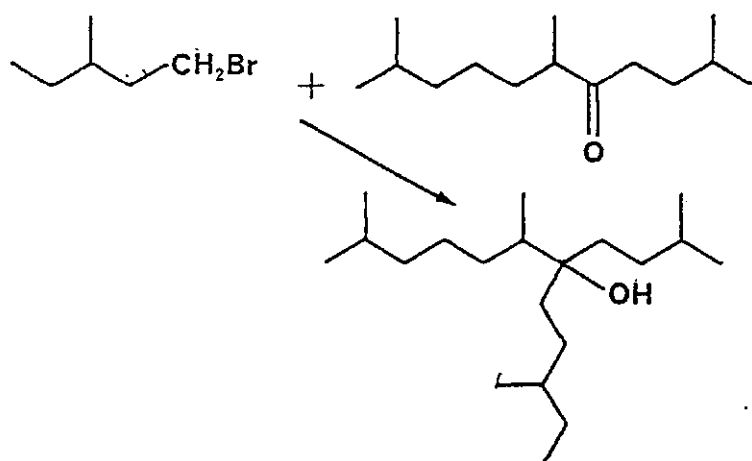
The mass spectrum of the C_{20} tertiary alcohol prepared by the "cerium
chloride promoted Grignard addition" of 1-bromo-3-methylpentane to
2,6,10-trimethylundecan-7-one is shown in Fig. 3:19A and the mass
spectrum of the TMS ether of the alcohol in Fig. 3:20A. The spectra
contain analogous α-cleavage ions around the quaternary carbon to
those observed in the spectrum of the C_{25} alcohol (see above).
Again, the most intense α-cleavage fragment ion present in both spectra
is that formed by loss of the $C_8H_{17}^\bullet$ radical. However in this
case, this also amounts to the loss of the largest alkyl group which is
considered the favoured α-cleavage in the mass spectra of tertiary
alcohols (Beynon et al., 1968; McLafferty, 1973).
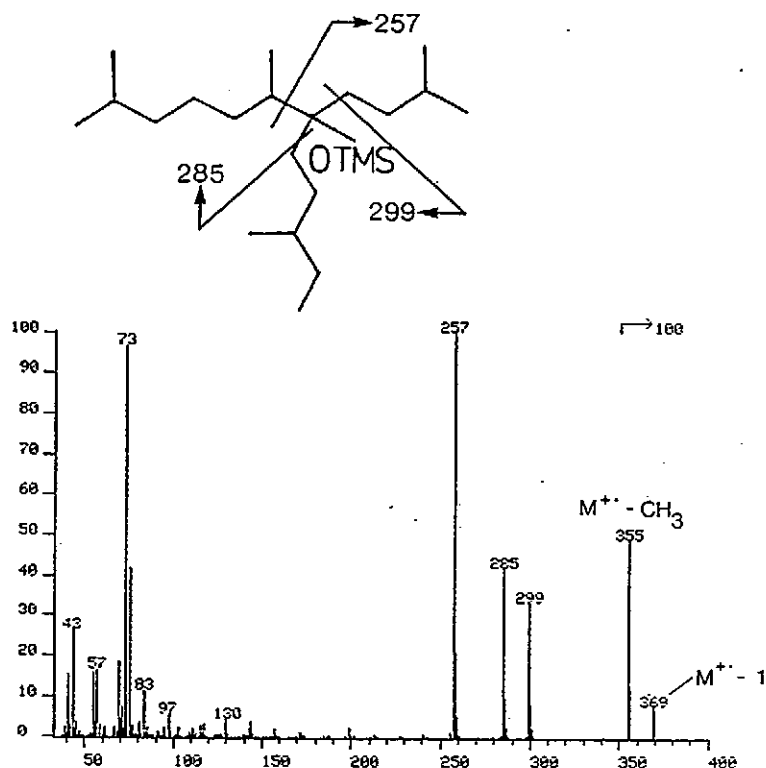
Fig.3:20A   Mass spectrum of the TMS ether of 2,6,10-trimethyl-
7-(3-methylbutyl)dodecan-7-ol.
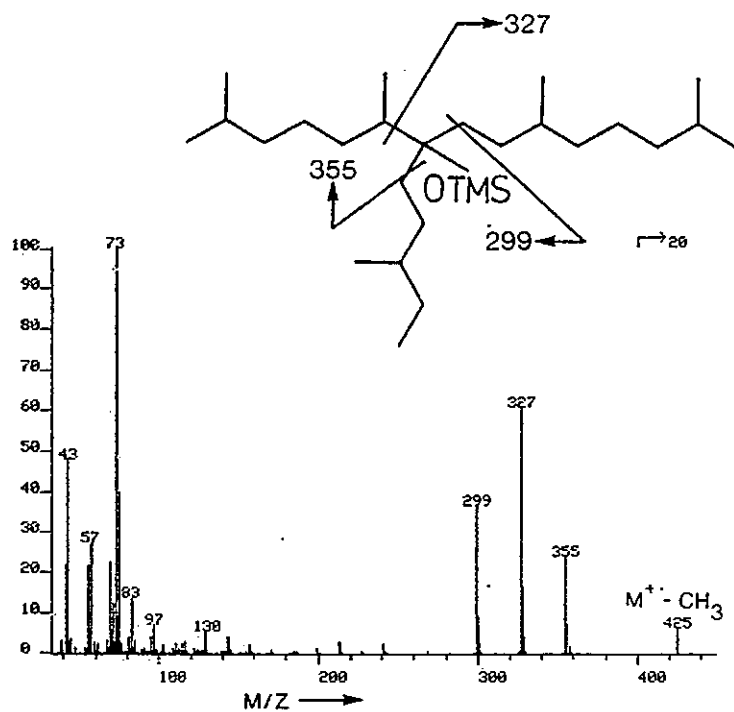
Fig.3:20B   Mass spectrum of the TMS ether of 2,6,10,14-tetra-
methyl-7-(3-methylpentyl)pentadecan-7-ol.

2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecan-7-ol
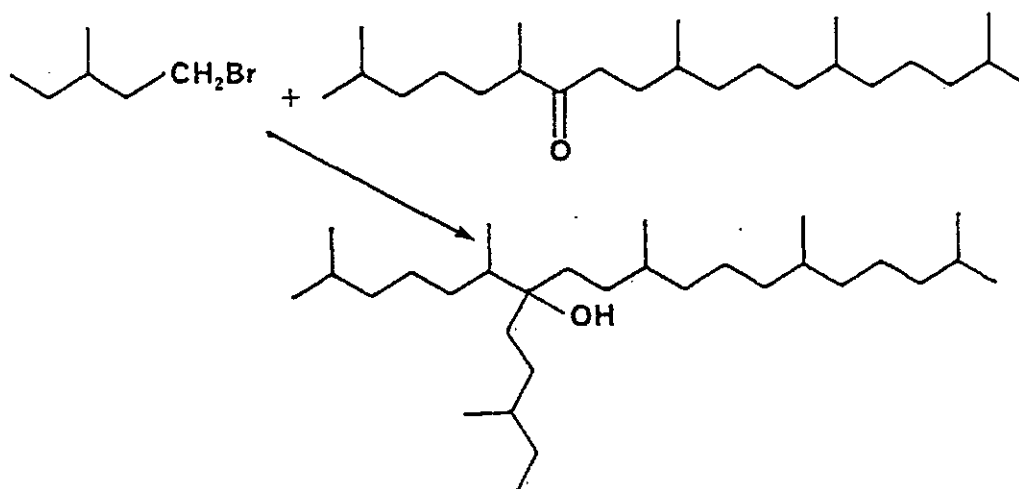
The "cerium chloride promoted Grignard addition" of 1-bromo-3-methylpentane to 2,6,10,14,18-pentamethylnonadecan-7-one produced the desired C_{30} alcohol in good yield (76%). The mass spectrum of the C_{30} tertiary alcohol is shown in Fig. 3:19C. In the spectrum, the prominant α-cleavage ion (m/z 227) is again that from the favoured loss of the largest alkyl radical $(C_{15}H_{31}\cdot)$ although the intensity is much reduced compared to that of the corresponding ion in the C_{20} alcohol (Fig. 3:19A). The reason for the reduced intensity of the α-cleavage ion in the spectrum of the C_{25} alcohol is uncertain.

High resolution mass spectra on the major α-cleavage ions present in the mass spectra of the C_{20}, C_{25} and C_{30} alcohols confirmed the above fragmentation assignment.

The IR spectra of the pseudohomologous tertiary C_{20}, C_{25} and C_{30} alcohols were essentially the same, each displaying sharp -OH absorptions at $3600cm^{-1}$ (free) and $3475cm^{-1}$ (intermolecularly bonded). The IR spectrum of 2,6,10,14-tetramethyl-7-(3-methylpentyl) pentadecan-7-ol is displayed as an example in Fig. 3:21.
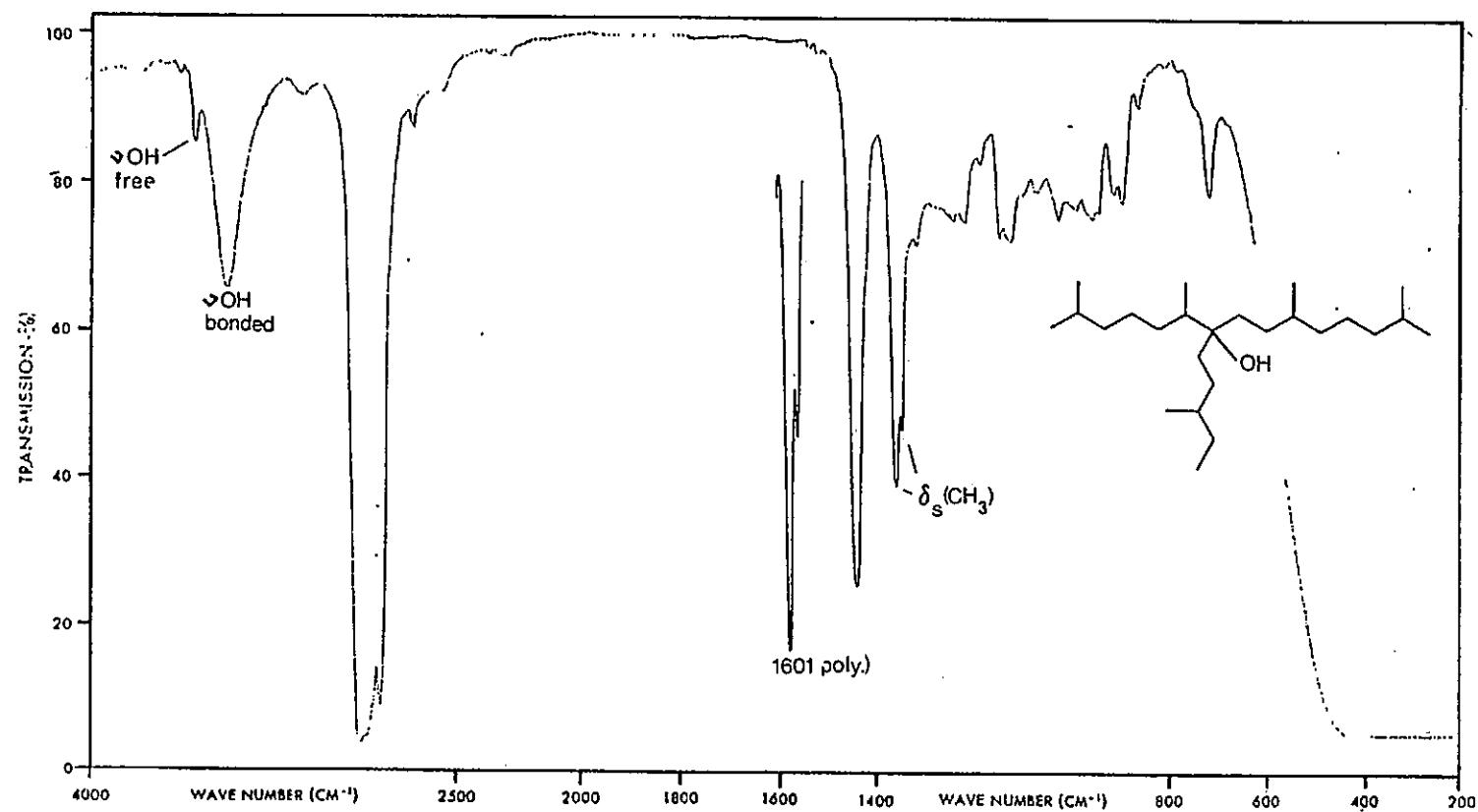
Fig.3:21   IR spectrum of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol.
            (poly.) = polystyrene.

The ^{1}H NMR spectra of the C_{20}, C_{25} and C_{30} alcohols integrated correctly into the appropriate number of $C\underline{H}_3$, $C\underline{H}_2$ and $C\underline{H}$ protons present in each case but displayed no distinguishing features worthy of prolonged discussion.

The ^{13}C NMR spectrum of 2,6,10-trimethy-7-(3-methylbutyl)dodecan-7-ol is shown in Fig. 3:22 and Table 3:6A. The interpretation of the spectrum is summarised in Table 3:6B and was performed by comparison with published ^{13}C NMR spectra of alcohols (Stothers, 1972; Breitmaier, 1978; Yon et al., 1982) and by comparison with the shift assignments for the parent C_{20} alkane 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (1; see section 3.2.9). Stothers (1972) noted through examination of the spectra of several tertiary alcohols, that carbons α, β and γ to the hydroxyl group experience an average change in chemical shift of +39.7, + 5.0 (2°) and -1.8 ppm respectively compared to the parent alkanes. For the δ and ϵ carbons, the effect of the hydroxyl moiety is noted as minimal with the chemical shifts essentially the same as in the corresponding alkane. By applying this rationale to the spectrum of 2,6,10-trimethyl-7-(3-methylbutyl) dodecan-7-ol (Fig. 3:22), it was possible to assign several carbons (i.e. C1-4,11,12,15-17,19,20) by direct comparison with the C_{20} alkane (Table 3:6b). The triplet observed for the methyl carbons of the isopropyl termini (i.e. C1,16,17,19) in the spectrum of the alkane (Fig. 3:29) appears as a quadruplet in the alcohol spectrum. This must be a consequence of the closer proximity of the hydroxyl group to one isopropyl terminus. Stothers (1972) noted that the hydroxyl-bearing carbon of several 2° and 3° alcohols is found between 71-76 ppm; therefore, the peak at 76.1853 ppm can be assigned to C7. The remainder of the spectrum is assigned by comparison with the shift
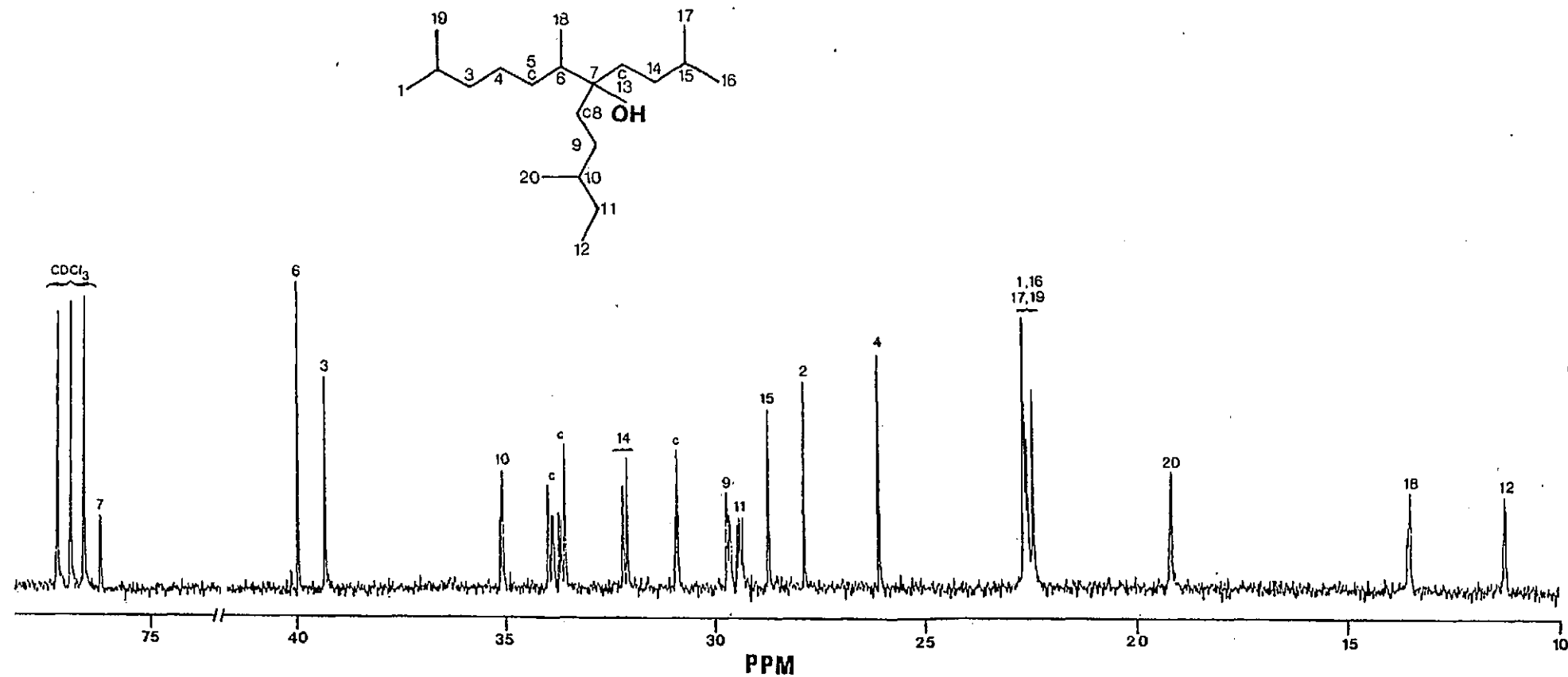
Fig.3:22 ^{13}C NMR (400 MHz) spectrum of 2,6,10-trimethyl-7-(3-methylbutyl)dodecan-7-ol. Proposed shift assignments (Table 3:6b) as shown. Numbers refer to the carbon number in the accompanying structure. c = assignment uncertain, one of C5, C8 or C13.
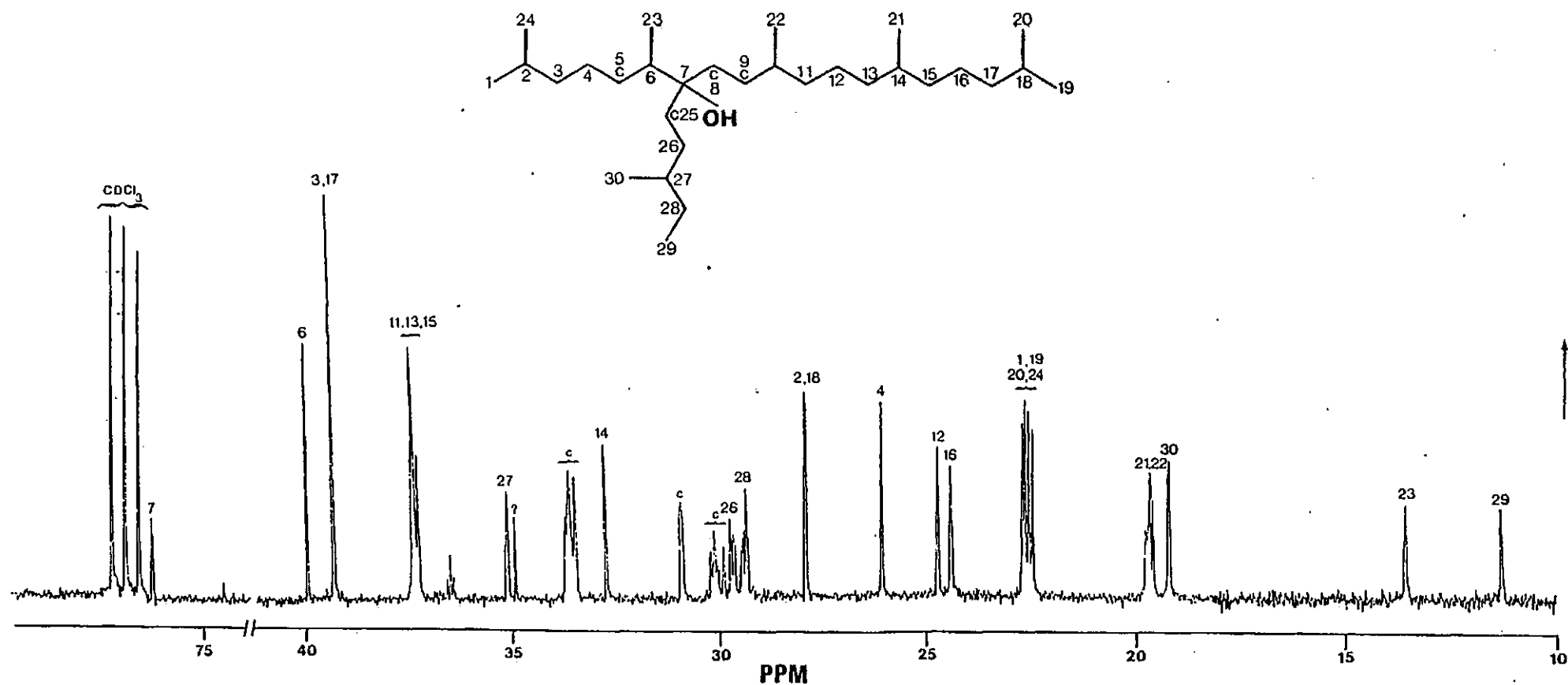
Table 3:6A

Observed ^{13}C NMR chemical shifts for
2,6,10-trimethyl-7(3-methylbutyl)dodecan-7-ol

| ppm	Intensity	Integral	Number of Carbons
76.1853	3.548	0.45	1
40.1232	.986		
39.9740	15.437	1.06	1
39.2790	10.505	1.22	1
35.1232	3.581	0.91	1
35.0672	5.6?4		
33.9695	4.972		
33.8819	3.705	1.22	1
33.8498	3.576		
33.7111	3.654		
33.6518	3.393	1.41	1
33.5684	6.968		
32.1725	4.960	1.22	1
32.0768	6.550		
30.9226	3.563	1.22	1
30.8791	6.821		
29.6842	4.614		
29.6235	3.525	1.22	1
29.5944	3.125		
29.4435	3.186		
29.4026	3.413	1.22	1
29.3664	3.301		
29.3155	3.525		
28.6842	8.570	0.91	1
27.8507	9.929	0.91	1
26.0618	11.304	1.07	1
22.6452	13.003		
22.5894	7.964	4.4	4
22.5484	7.087		
22.4216	9.390		
19.2054	5.454	1.1	1
13.5946	2.947	0.91	1
13.5423	4.588		
11.2873	4.483	0.76	1

Total: 20

Table 3:6B

^{13}C NMR shift assignments for 2,6,10-trimethyl-7-(3-methylbutyl)dodecan-7-ol.
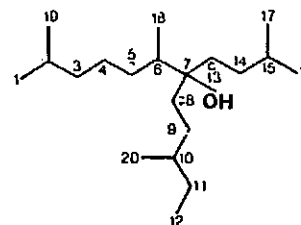
Carbon Number	ppm	Shift assignments for 2,6,10-trimethyl-7-(3-methylbutyl) dodecane ppm	Shift assignments for 2,6,10-trimethyl-7-(3-methylbutyl) dodec-2-en-7-ol (5)[a] ppm
12	11.2873	11.2793	11.44
18	13.5946 - 13.5423	15.6183 - 15.6352	13.56
20	19.205	19.1718 - 19.2874	19.33
1,16	22.4216 - 22.6452	22.5323 - 22.7202	22.71
4	26.0618	25.4464	N.C.C.[b]
2	27.8507	27.1460 - 27.5877	N.C.C.[b]
15	28.6842	27.8724	28.82
11	29.3155 - 29.4435	28.4777 - 28.8538	29.45
9	29.5944 - 29.6842[c] 30.8791 - 30.9226[c]	34.0789 - 34.3794	29.73
14	32.0768 - 32.1725[c] 33.5684 - 33.7111[c] 33.8498 - 33.9659[c]	34.8497 - 35.0253	32.20
10	35.0672 - 35.1232	37.1681 - 37.4874	34.07[b]
3	39.2790	39.3077	N.C.C.[b]
6	39.9740	34.6789 - 34.7847	40.70
7	76.1853	42.7643 - 42.9563	76.28

[a]: From Yon et.al., 1982.

[b]: No comparable carbon in this compounds

[c]: Assignment not possible. Either C5, C8 or C13.

assignments for an analogous C_{20} alcohol [2,6,10-trimethyl-7-(3-methylbutyl)dodec-2-en-7-ol; 5] prepared by Yon et al. (1982). Three peaks could not be assigned but must correspond to C_5 and the β-carbons C_8 and C_{13}. Interestingly, all three δ-carbons to the hydroxyl moiety experience a deshielding contribution of approximately 1 ppm. This is contrary to Stothers (1972) who noted only minimal substituent effects ($\simeq 0.1$ppm) at the δ-carbon. This difference probably reflects the highly branched nature of the current alcohol compared to those examined by Stothers (1972). The diastereoisomeric splitting apparent in certain peaks (i.e. 29.5944 - 29.6842 ppm) is due to the presence of four pairs of diastereoisomers. Comparison of the ^{13}C spectrum of the C_{20} alcohol (Fig. 3:22) and the C_{20} alkane (Fig. 3:29) demonstrates another interesting effect of the hydroxyl substituent; the increased separation of the peaks in the region 27.00 - 35.00 ppm. This effect is analogous to that of shift reagents (i.e. $Eu(dpm)_3$) often used in ^1H NMR. It is also evident that several of the doublets or quadruplets (produced by the magnetic non-equivalence of diastereoisomers) in the spectrum of the alkane (Fig. 3:29) condense to single peaks (or incompletely separated doublets) in the spectrum of the alcohol.

The interpretation of the ^{13}C NMR spectrum (Fig. 3:23) of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol is summarised in Table 3:7. Interpretation was possible by comparison with the shift assignments (Table 3:7) for the ^{13}C NMR spectrum (Fig. 3:30) of the parent C_{25} alkane (2,6,10,14-tetramethyl-7-(3-methylpentyl) pentadecane; 2) and those of the preceeding C_{20} alcohol. The absence of the signal at approximately 76 ppm (corresponding to the carbinol carbon) is most likely operator error, since all other data (IR etc.)

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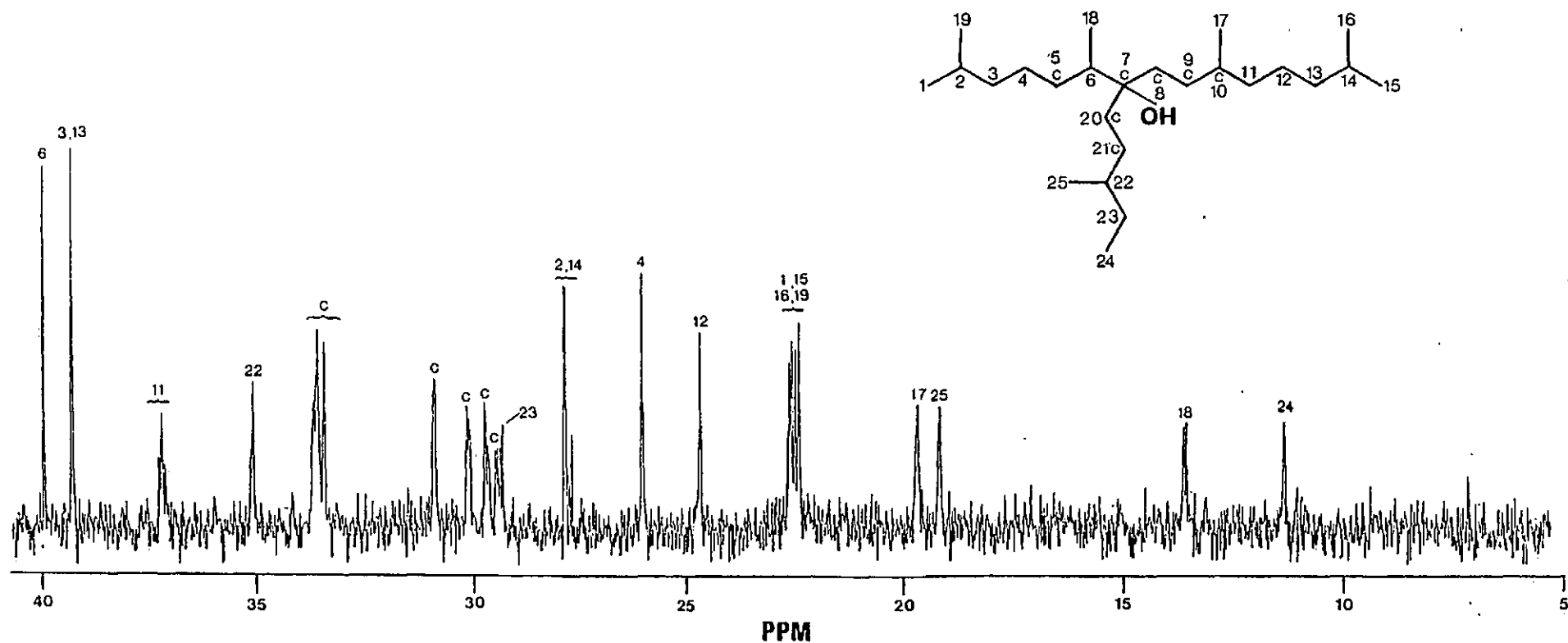
Fig.3:23 ^{13}C NMR (400 MHz) spectrum of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol. Proposed shift assignments (Table 3:7) as shown. Numbers refer to the carbon number in the accompanying structure. c = assignment uncertain, either C5, C8, C9, C20 or C21 .

Table 3:7

^{13}C NMR shift assignments for
2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecan-7-ol

19 18 17 16
5 7 9
1 2 3 4 c 6 c c c 11 12 13 14 15
c
10
8
20 c OH
21 c
25 22
23
24
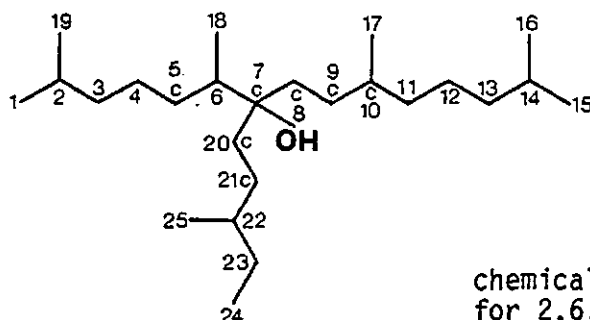
chemical shift assignments
for 2,6,10,14-tetramethyl-7-
(3-methylpentyl)pentadecane (2)

Carbon Number	ppm	ppm
24	11.2966	11.2786
18	13.5142 - 13.5738	15.4841 - 15.7649
25	19.2024	19.1815 - 19.2826
17	19.6908	19.6761 - 19.7961
1,15	22.4255 - 22.5080[a]	22.5282[a]
16,19	22.5930 - 22.6478[a]	22.6083[a]
12	24.7010	24.7024
4	26.0681	25.4479
2,14	27.7044 - 27.8864	27.2211 - 27.8815
23	29.3136	29.2930
	29.4194[b]	
	29.7192[b]	
	30.1430[b]	
	30.9000[b]	
	33.4284[b]	
	33.5839[b]	
22	35.0706	37.3330
11	37.1343 - 37.2091	37.0950
3,13	39.2764	39.3083
6	39.6454	34.7575
7	76?	

[a]: Chemical shifts with the same superscript in any one column may be interchanged.

[b]: assignment not possible but may be C5, C8, C9, C10, C20 or C21.

?: operator error?

showed that the compound was an alcohol.

The ^{13}C NMR spectrum of 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecan-7-ol is displayed in Fig. 3:24 and Table 3:8A. Again almost complete shift assignment was possible (summarised in Table 3:8B by comparison with the data for the parent C_{30} alkane (2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane; 3) and with that for the preceeding C_{20} and C_{25} alcohols.

The ^{13}C NMR spectra of both the C_{25} and C_{30} alcohols display several features which are also apparent in the spectra of the C_{20} alcohol: for instance, the quadruplet for the methyl carbons of the isopropyl termini and the increase in diastereoisomeric magnetic non-equivalence between the alcohols and their parent alkanes.

In summary, by a combination of gcms, HRMS, IR, ^1H and ^{13}C NMR, it has been possible to unambigously assign structures to the major alcohol product from each of the three "cerium chloride promoted Grignard additions".

3.2.8   Synthesis   of   2,6,10-trimethyl-7-(3-methylbutyl)dodecenes 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecenes and 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecenes.

2,6,10-trimethyl-7-(3-methylbutyl)dodecenes; br20:1

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Fig.3:24 ^{13}C NMR (400 MHz) spectrum of 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecan-7-ol. Proposed shift assignments (Table 3:8b) as shown.   Numbers refer to the carbon number in the accompanying structure. c = assignment uncertain, either C5, C8, C9, C10 or C25. ? = contaminant.
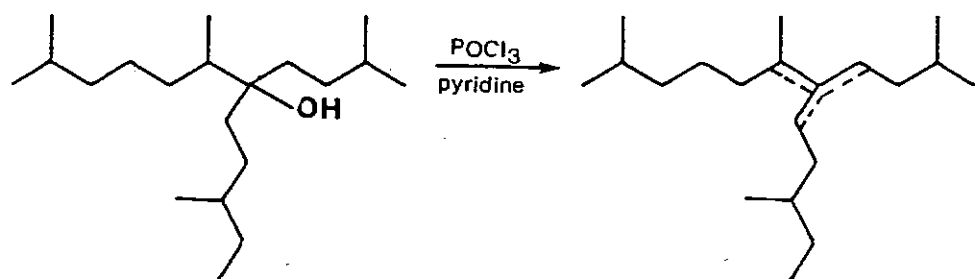
Table 3:8A

Observed ^{13}C NMR chemical shifts for 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecan-7-ol

ppm	intensity	integral	proposed number of carbons
76.2607	4.005	0.81	1
76.2349	3.201		
74.4733	0.814	IMPURITY	
53.2840	2.135		
39.9936	13.014	1.08	1
39.2907	19.325	1.89	2
37.3617	7.776		
37.3165	12.204	3.41	3
37.2146	6.939		
36.5266	1.079		
36.4538	2.184	IMPURITY	
36.3811	1.124		
35.0699	5.399	1.08	1
34.8935	3.975	IMPURITY	
33.6532	4.092		
33.5623	6.305	3.24	3
33.4328	6.060		
32.7133	7.497	1.08	1
30.8708	4.733		
30.1972	2.448	2.19	2
30.1053	3.384		
29.8932	2.626		
29.7181	4.070	1.08	1
29.6384	3.305		
29.4244	3.133		
29.3743	3.370	1.35	1
29.3268	5.356		
27.8849	9.960	1.65	2
27.8599	9.438		
26.0694	9.390	1.08	1
24.7096	7.621	1.08	1
24.3978	6.256	1.35	1
22.6566	8.368		
22.6100	9.429	3.78	4
22.5188	8.911		
22.4300	8.150		
19.6551	6.041	2.16	2
19.5897	4.706		
19.2144	6.588	1.21	1
13.5811	3.156		
		1.08	1
13.5218	3.497		
11.2866	4.978	1.08	1

Total 20

Table 3:8B

^{13}C shift assignments for 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecan-7-ol
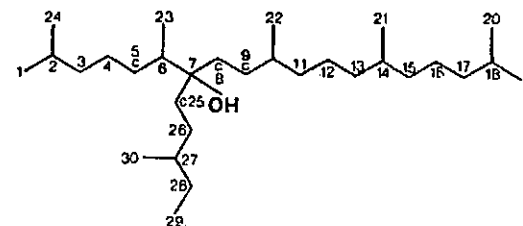
Carbon Number	ppm	shift assignments for 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane ppm
29	11.2866	11.3108
23	13.5218 - 13.5811	15.4833 - 15.7711
30	19.2144	19.1837
21,22	19.5897 - 19.6511	19.6647
1,19	22.4300 - 22.5188[a]	22.5327
20,24	22.6100 - 22.6566[a]	22.6110
16	24.3978	24.3826
12	24.7096	24.7161
4	25.0694	25.4497
2,18	27.8599 - 27.8849	27.1445 - 27.8836
28	29.3268 - 29.4244	29.3082
9	29.6384 - 29.7181	35.0741 - 35.4435
2.U.C[b,c]	29.8932 - 30.8708	
14	32.7133	32.7147
3.U.C[b,c]	33.4328 - 33.6532	
27	35.1699	37.2288 - 37.3929
11,13,15	37.2146 - 37.3617	37.2288 - 37.3929
3,17	39.2907	39.3078
6	39.9936	34.7590
7	76.2349 - 76.2607	42.6923 - 42.8870

[a]: chemical shift assignments with the same superscript may be exchanged.

[b]: 2/3 unassigned carbons

[c]: may be C5, C8, C10, C25 or C26

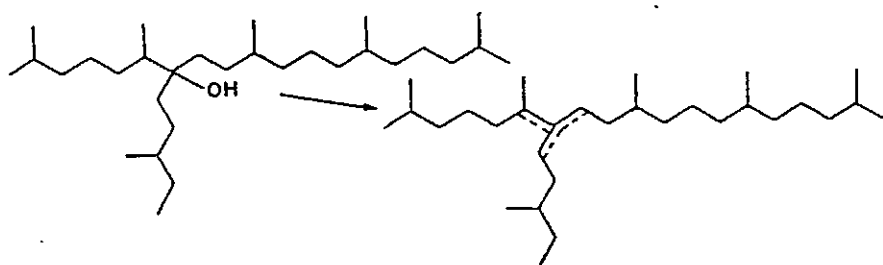Dehydration of 2,6,10-trimethyl-7-(3-methylbutyl)dodecan-7-ol produced a mixture of isomeric C_{20} monoenes represented by the five gas chromatographic peaks in Fig. 3.25A. Details of retention indices and mass spectra of the individual peaks are summarised in Table 3.9. The total number of positional and geometrical double bond isomers possible via dehydration of the C_{20} alcohol is six which suggests, assuming that all of the isomers were produced, that two of the monoenes co-chromatographed. McLafferty (1973) reported that cis and trans isomers have identical mass spectra: therefore, the spectral details in Table 3:9 indicate that monoenes RI1711 and 1714 are geometric isomers. The prominent fragmentation in the mass spectra of substituted alkenes is γ-hydrogen rearrangement (β-cleavage with H-transfer) with the most abundant ion resulting from the loss of the largest neutral fragment. Of the three possible positional double bond isomers, the following structure represents the monoene most likely to produce the fragmentation pattern present in RI1711 and 1714.
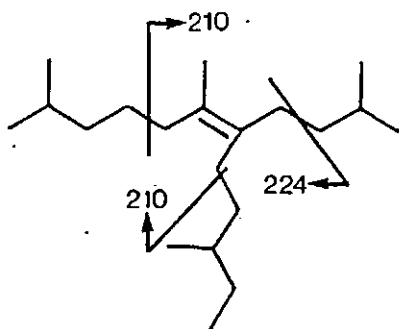
Differences in the mass spectra of the three remaining gas chromatographic peaks prevents further assignment. Non-similarity between the spectra of RI1679, 1686 and 1690 are not surprising as alkenes exhibit a strong tendency to isomerise through migration of the double bond in the source of the mass spectrometer. Indeed, McLafferty (1973) noted that the mass spectra of monoenes tend to be independent of the double bond position unless the double bond is highly
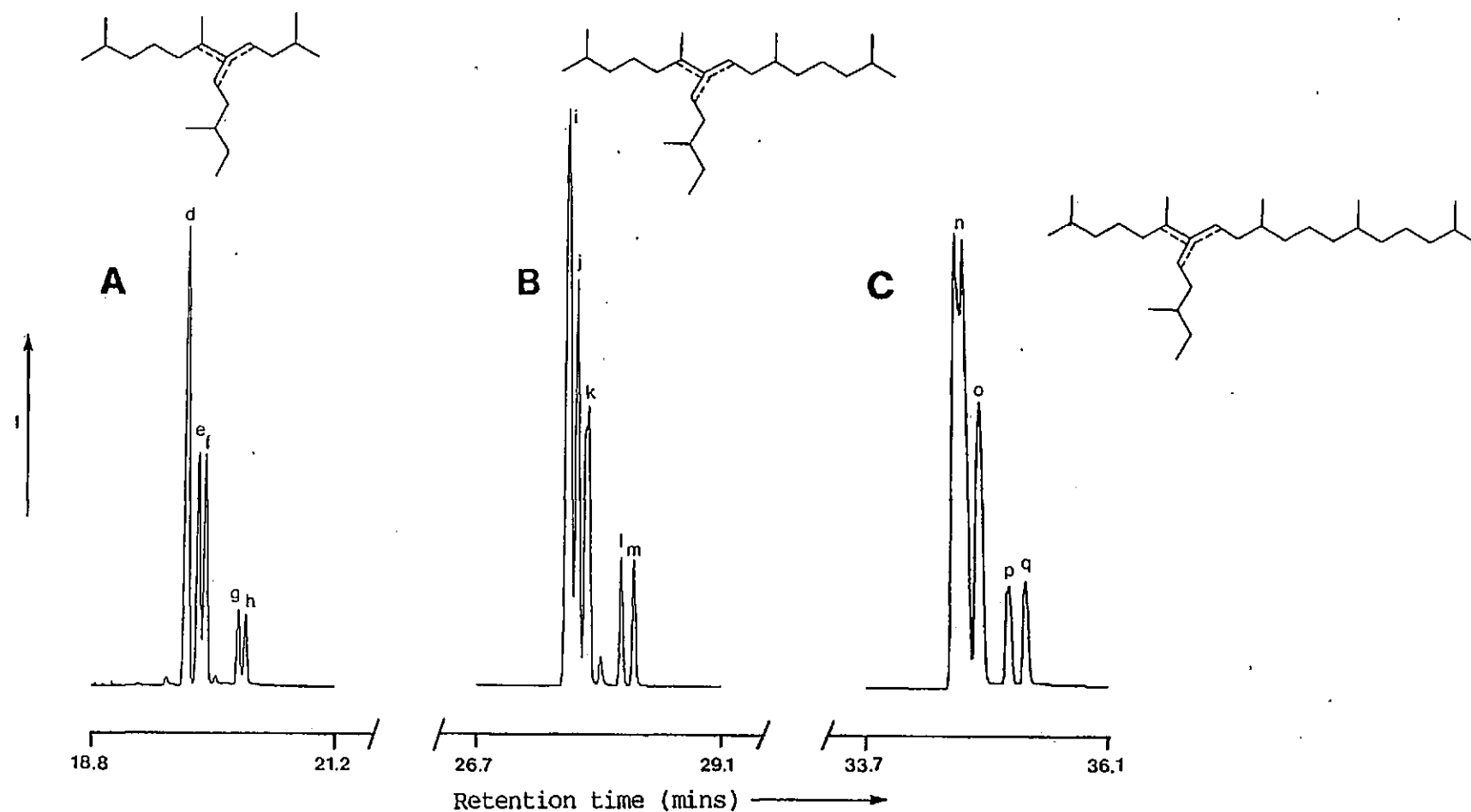
Fig.3:25　Gas chromatograms of A. the synthetic isomeric 2,6,10-trimethyl-7-
(3-methylbutyl)dodecenes(br20:1),　B. the synthetic isomeric
2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecenes (br25:1) and
C. the synthetic isomeric 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)
nonadecenes (br30:1).　Peaks d - h refer to Table 3:9; i - m to
3:10 and n - q to 3:11.　Gc conditions: OV1, 40 - 80°C at 10°Cmin^{-1}.
and 80 - 290°C at 6°Cmin^{-1}.

Table 3:9

LRMS of 2,6,10-trimethyl-7-(3-methylbutyl)dodecenes (br20:1)

Gc Retention Index[a]	Formula	$C_{20}H_{40}$	$C_{16}H_{32}$	LRMS $C_{15}H_{30}$	ION INTENSITY. % $C_{14}H_{28}$	$C_{11}H_{32}$	$C_{10}H_{20}$	C_9H_{18}	C_5H_4
RI	m/z	280	224	210	196	154	140	126	69
1679	d	5.1	1.9	3.0	3.9	4.0	13.5	20.0	100
1686	e	4.2	2.8	1.2	3.1	4.0	16.0	13.5	100
1690	f	3.6	0.3	3.5	2.8	3.0	8.0	26.0	100
1711	g	7.0	1.1	1.3	1.2	3.5	11.0	16.0	100
1714	h	5.2	0.6	1.1	0.9	3.0	10.0	14.0	100

[a]: OV1, 40-80°C at 10°Cmin^{-1}, 80-290°C at 6°Cmin^{-1}.

Table 3:10

LRMS of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecenes (br25:1)

Gc Retention Index[a]	Formula	$C_{25}H_{50}$	$C_{20}H_{40}$	$C_{19}H_{38}$	LRMS $C_{16}H_{32}$	ION INTENSITY. % $C_{15}H_{30}$	$C_{14}H_{28}$	$C_{10}H_{20}$	C_9H_{18}	83	(Base ion)
RI	m/z	350	280	266	224	210	196	140	126	83	
2078	i	2.4	2.0	1.5	1.7	0.1	5.0	19.0	12.0	68.0	100 (57)
2085	j	2.1	3.2	1.5	0.8	0.0	7.1	12.0	14.0	67.0	100 (43)
2092	k	2.8	0.6	0.0	6.0	0.0	2.0	28.0	8.0	67.0	100 (69)
2116	l	4.0	0.0	0.0	4.0	0.0	2.0	20.0	11.0	78.0	100 (69)
2125	m	3.8	0.0	0.0	1.6	0.0	5.0	21.0	10.0	74.0	100 (43)

[a]: OV1, 40-80°C at 10°Cmin^{-1}, 80-290°C at 6°Cmin^{-1}.

Table 3:11

LRMS of 2,6,10,14,18-pentamethyl-7-(3-methylpentylnonadecenes (br30:1)

Gc Retention Index[a]	Formula	$C_{30}H_{60}$	$C_{25}H_{50}$	$C_{24}H_{48}$	LRMS $C_{19}H_{38}$	ION INTENSITY. % $C_{16}H_{32}$	$C_{15}H_{30}$	$C_{14}H_{28}$	$C_{10}H_{20}$	(Base Ion)
RI	m/z	420	350	336	266	224	210	196	140	
2527	n	2.5	2.4	1.0	2.9	4.5	11.4	4.5	21.0	100 (57)
2543	o	2.5	0.5	0.8	1.1	10.4	1.1	4.4	3.0	100 (70)
2565	p	3.0	1.0	0.0	1.3	2.5	1.0	1.8	15.0	100 (43)
2579	q	3.0	1.1	0.0	1.4	2.5	0.9	1.8	18.8	100 (43)

[a]: OV1, 40-80°C at 10°Cmin^{-1}, 80-290°C at 2°Cmin^{-1}.

substituted. as in the case of the structure containing a tetra-substituted double bond proposed for RI1711 and 1714. The difference in retention indices ($\Delta RI = 3$) between these presumed geometrical isomers is similar to that observed between RI1690 and 1686 ($\Delta RI=4$) suggesting the latter peaks may also represent geometric isomers. Consequently, the peak at RI1679 may contain the remaining pair of isomers. Sojak et al. (1980) used a series of $C_{15} - C_{18}$ normal-alkenes to demonstrate that the difference in retention indices between geometric isomers depends strongly on the position of the double bond in the molecule. For example, cis - and trans-pentadec-4-enes co-chromatographed whereas the Δ^7 isomers differed by 4 RI units. It was also demonstrated that in certain cases, the retention difference between a pair of geometric isomers was greater than that between two different positional isomers of the same configuration (i.e. trans-Δ^7 and Δ^8).

The difficulties associated with ambiguous mass spectral identification of alkenes has led to the development of several methods for chemical derivatisation of the double bond. For example, Blomquist et al. (1980) suggested methoxy-mercuration whilst Francis and Veland (1981) noted that alkylthiolation provided derivatives stable to gc with easily recognisable fragments on electron-ionisation ms. However, both these methods proved unsuccessful for derivatisation of the present C_{20} monoenes. Presumably steric hindrance resulting from the highly branched nature of the C_{20} molecules prevented derivatisation. Another derivatisation method involves hydroxylation of the olefinic bond by oxidation with OsO_4 and subsequent reduction of the osmate with Na_2SO_3. Conversion of the resulting diols to their trimethylsilyl ethers renders them amenable to gcms analysis. This

method has been used previously by Rowland et al. (1982) to successfully determine the double bond positions in Δ^7 and Δ^8 2,6,11,15,19-pentamethyleicosenes (6). Application of this method to the isomeric 2,6,10-trimethyl-7-(3-methylbutyl)dodecenes again proved unsuccessful though it was apparent from mass spectral data that monohydroxy-mono TMS derivatives were produced. The large number of derivatives, coupled with evident rearrangements, made meaningful interpretation almost impossible. The production of a complex set of derivatives highlights the problems inherent in working with mixtures of isomers. Attempts to separate the isomeric 2,6,10-trimethyl-7-(3-methylbutyl)dodecenes by argentatious tlc proved unsuccessful.

2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecenes: br25:1
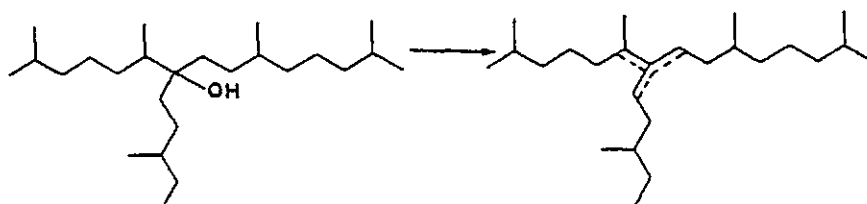
Dehydration of 2,6,10,14-tetramethyl-7-(3-methylbutyl)pentadecan-7-ol produced a mixture of isomeric C_{25} monoenes represented by the five gas chromatographic peaks in Fig. 3:25B. Details of retention indices and mass spectra of the individual peaks are summaried in Table 3:10. Two of the C_{25} monoenes, RI2078 and 2092, had identical mass spectra and retention indices to two C_{25} monoenes detected in sediments of the Tamar Estuary, U.K. (see section 4.3.1). The general distribution of the synthetic C_{25} isomers was similar to that of the C_{20} isomers (Fig. 3:25A) with a prominent early eluting peak (RI2078) and a symmetical doublet at RI2116 and 2125. By analogy with the

isomeric C_{20} monoenes RI2116 and 2125 might represent cis-trans isomers of the same structure; however, differences in mass spectra (Table 3:10) argue against this. Indeed, from mass spectral data, the two most likely geometic isomers are RI2092 and 2116 although the distribution of fragment ions gives no clues to the double bond positions. The difficulties already discussed in interpreting the mass spectral and chromatographic behaviour of alkenes makes further speculation unwise. Attempts to assign double bond positions using OsO_4, even under forcing conditions (6 hour reflux), were unsuccessful; as with the C_{20} monoenes, only monohydroxy-monoTMS derivatives were prepared (i.e. 7) with increased steric hindrance presumably preventing addition of the second TMS group.

2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecenes: br30:1

The gas chromatographic peaks representing the isomeric C_{30} alkenes produced on dehydration of 2,6,10,14,18-pentamethyl-7-(3-methylpentyl) nonadecan-7-ol are displayed in Fig. 3:25C. Details of retention indices and mass spectra are summarised in Table 3:11. The decrease in resolution of individual isomers through the C_{20}, C_{25} and C_{30} monoenes indicates that resolution decreases with increasing length of the alkyl chain. Interestingly, mass spectra (Table 3:11) of RI2565 and 2579 were identical, so they may represent geometric isomers.

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It is uncertain whether the later eluting symmetical doublet present in each of the C_{20}, C_{25} and C_{30} monoene isomers (Fig. 3:25A-C) represents in each case, <u>cis-trans</u> configurations of the same positional isomer. The similarity observed in the chromatographic behaviour of this doublet across the molecular weight range suggests this may well be the case. The C_{20}, C_{25} or C_{30} monoenes were not assigned further.

3.2.9

Synthesis of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (br20:0; <u>1</u>), 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; <u>2</u>) and 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane (br30:0; <u>3</u>).
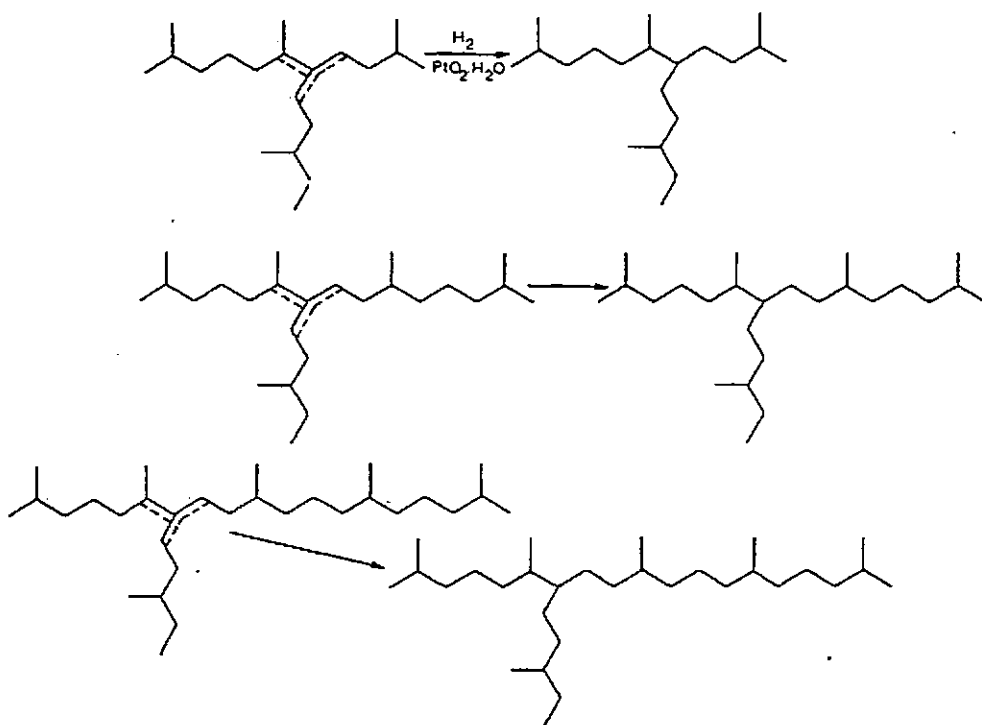
The mass spectra of the pseudohomologous C_{20}, C_{25} and C_{30} alkanes prepared from smooth hydrogenation of the respective isomeric C_{20}, C_{25} and C_{30} monoenes, are shown in Fig. 3:26A-C which also notes the retention indices. The mass spectrum (Fig. 3:26A) of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (br20:0; RI1708) is identical to that of the synthetic C_{20} alkane presented by Yon (1982) showing the characteristic ion doublets at m/z 168/169, m/z 196/197 and m/z 210/211 resulting from cleavage (with and without hydrogen transfer) about the C6-C7, C7-C8 and C7-C13 bonds respectively. The mass spectrum (Fig. 3:26B) of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; RI2109) exhibits an analogous fragmentation pattern with characteric ion doublets at m/z 238/239, m/z 266/267 and m/z 210/211 assigned (by HRMS) to cleavage about the C6-C7, C7-C20 and C7-C8 bonds respectively. The mass spectrum (Fig. 3:26C) of 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane (br30:0; RI2524) likewise demonstrates characteristic ion doublets 70 a.m.u. higher than in the pseudohomologous C_{25} alkane, at m/z 308/309 and m/z 336/337 assigned (by HRMS) to cleavage about the C6-C7 and C7-C25 bonds respectively.

The C_{20}, C_{25} and C_{30} alkanes plot co-linearly on a graph (Fig. 3:27) of retention index _versus_ carbon number as would be expected for structural homologues. Their relatively low retention indices compared to other acyclic isoprenoids of the same carbon number emphasises the highly branched nature of the structure.

The ^{1}H NMR spectrum of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane integrated correctly into the appropriate number of CH$_3$, CH$_2$ and CH protons and was identical to that of the synthetic C_{20} alkane presented by Yon (1982). The spectrum displays a set of four peaks
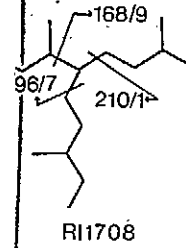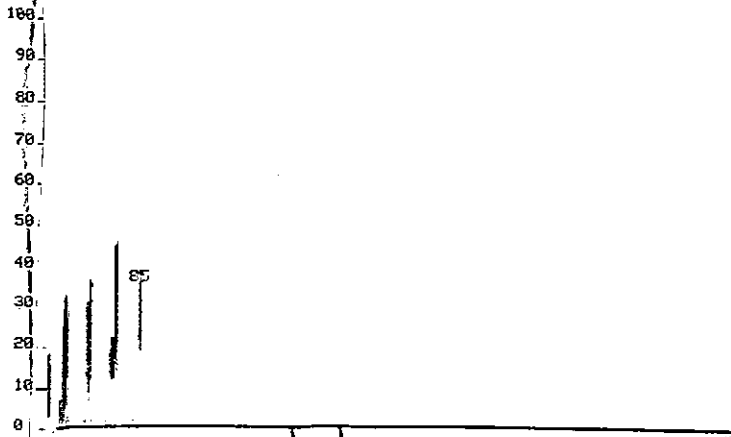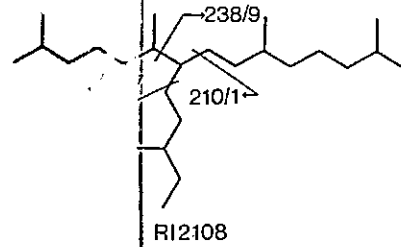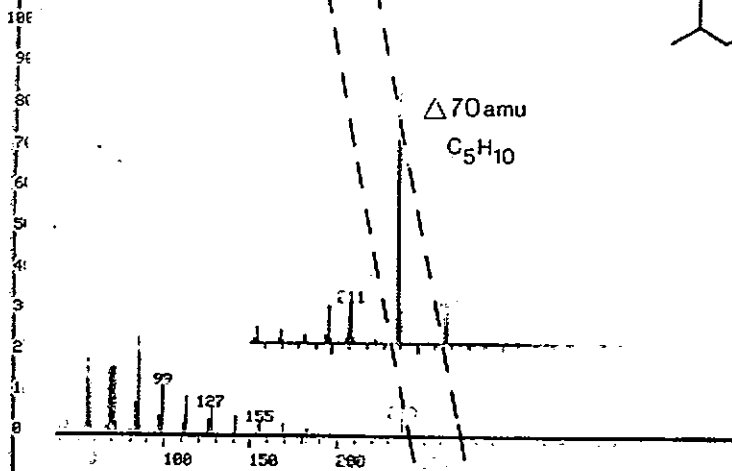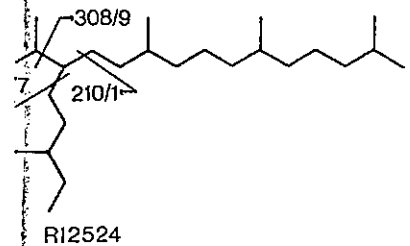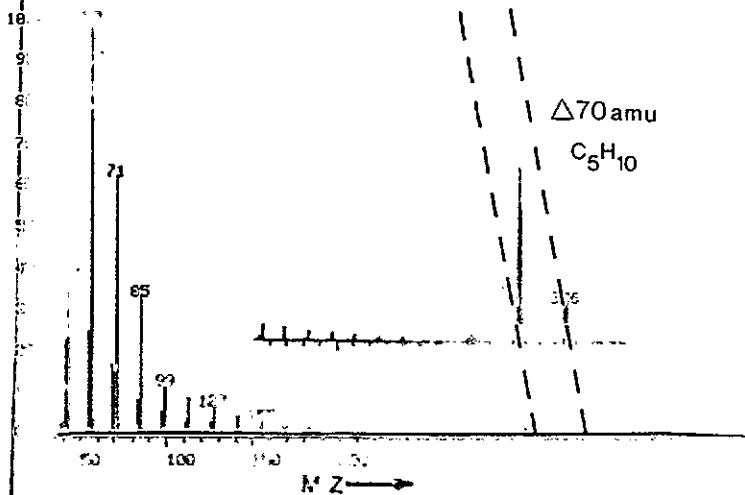
135

A

168/9
96/7
210/1
RI1708

B

△70 amu
C₅H₁₀

238/9
210/1
RI2108

99
127
155

100   150   200

C

△70 amu
C₅H₁₀

308/9
7
210/1
RI2524

71

85

99

50   100

M Z ⟶

Fig. :0   Mass spectra of A. . . .
(3-methylbutyl . . . . .
tetramethyl- . . .
and C. . . .
(3-methyl. . .
. . . . .
displayed by the three alkanes.

10,14-
(br25:0)

overlay
tion pattern
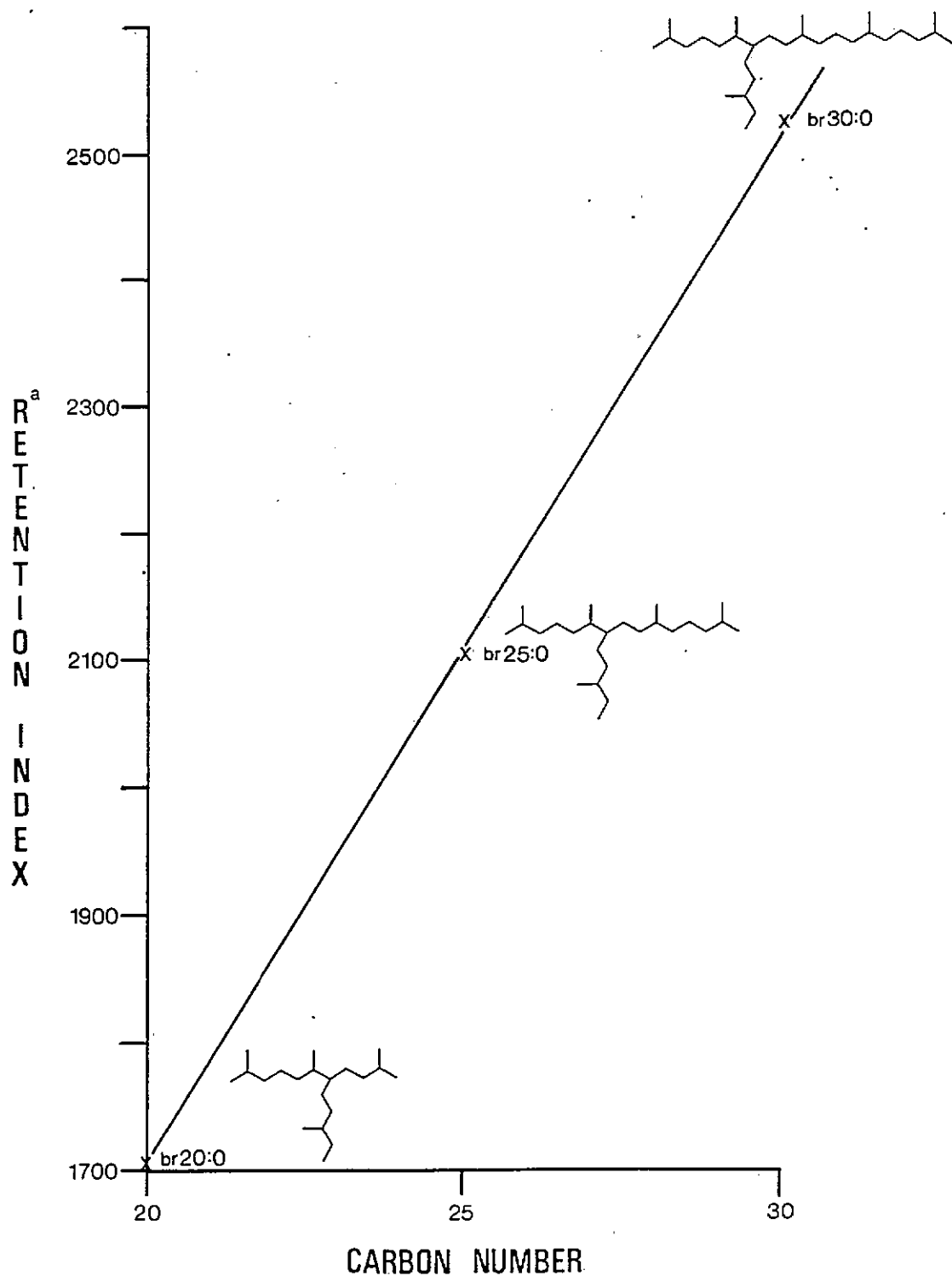
Fig.3:27 Graph of retention index versus carbon number for synthetic br20:0, br25:0 and br30:0. ^{a} = OV1,40 - 80°C at 10°Cmin^{-1}, 80 - 290°C at 6°Cmin^{-1}.

between 0.7476 - 0.7742 ppm (Fig. 3:28A) whose integration (3H) suggests they derive from a methyl group, possibly $\underline{C}$, $\underline{F}$ or $\underline{G}$. Similar peaks were observed as a doublet of doublets (0.764 ppm and 0.77 ppm) in the spectrum presented by Yon (1982) who gave no opinion as to their origin. The ^1H NMR spectra of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane and 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane were essentially the same as that for the C_{20} alkane and integrated correctly. The only discernible differences were in the 0.7 - 0.8 ppm region of the spectrum. In place of the four peaks present in the C_{20} alkane spectrum (Fig. 3:28A), both the C_{25} and C_{30} alkanes displayed complex multiplets (Fig. 3:28B and C respectively) whose integration again suggested a methyl group. The increase in splitting in this region is possibly caused by the increasing alkyl chain at C7 and the increased number of diastereoisomers present in the C_{25} (2^4 = 16) and C_{30} (2^5 = 32) alkanes. Unfortunately, it has not been possible to assign the methyl group responsible for these signals.

The ^{13}C NMR spectrum of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (br20:0; 1) is shown in Fig. 3:29 and listed in Table 3:12A. The spectrum was fully assigned by comparison with the ^{13}C NMR spectrum of the synthetic C_{20} alkane presented by Yon (1982), observed chemical shifts for other acyclic isoprenoids (i.e. pristane, phytane; Yon, 1982; Lamb, 1982) and with theoretical chemical shifts for br20:0 calculated according to Carmen et al. (1973). The assignment is summarised in Table 3:12B.

Grant and Paul (1964) first established that the chemical shift for any given carbon in an acyclic alkane could be predicted on an empirical
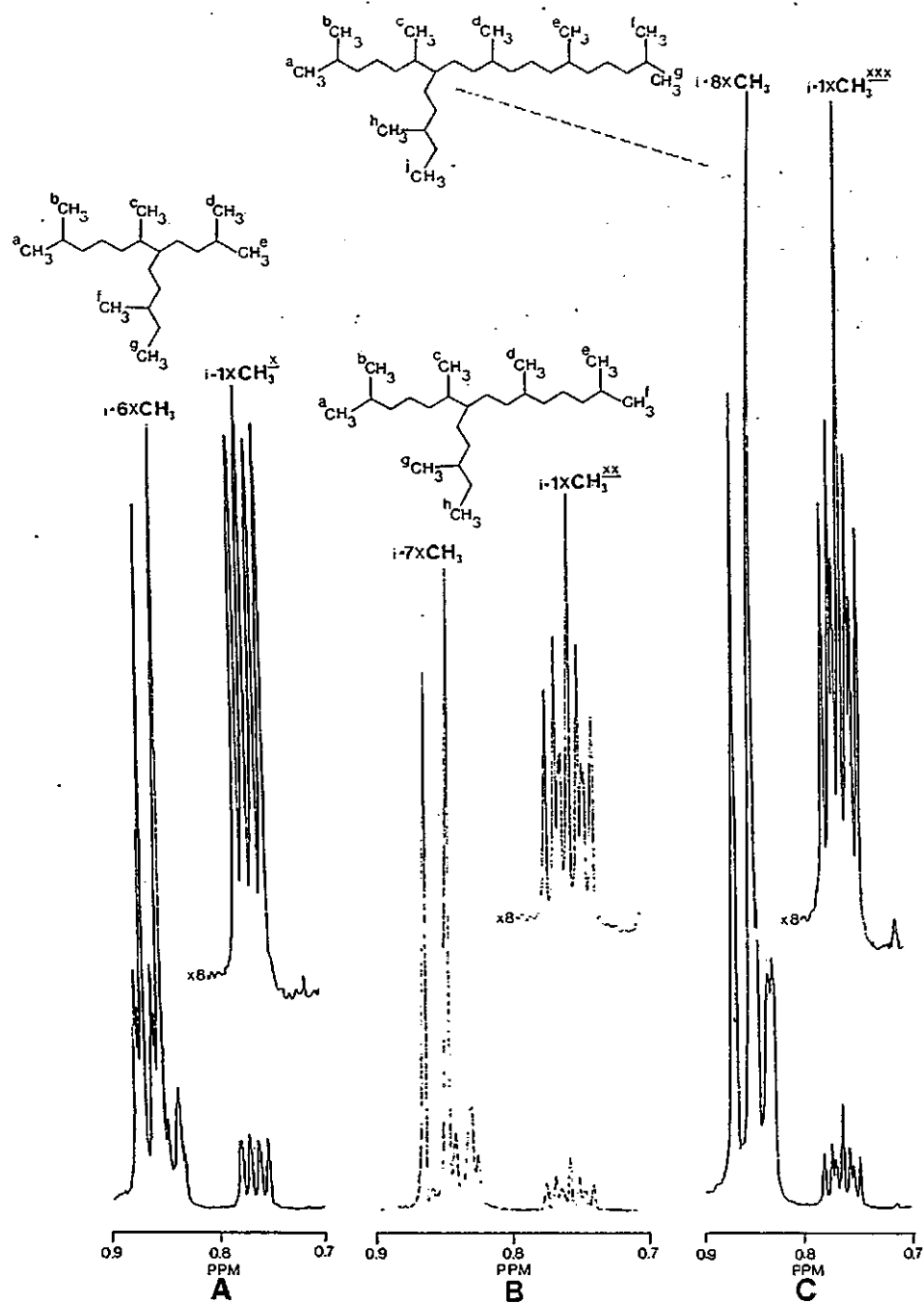
Fig.3:28 Partial ^{1}H NMR (400 MHz) spectra of A: 2,6,10-trimethyl-7-
(3-methylbutyl)dodecane (br20:0), B. 2,6,10,14-tetramethyl-7-
(3-methylpentyl)pentadecane (br25:0) and C. 2,6,10,14,18-
pentamethyl-7-(3-methylpentyl)nonadecane (br30:0).
i = integral; CH₃ = methyl group (or 3H).
X = c, f or g. xx = c, d, g or h. xxx = c, d, e, h or j.
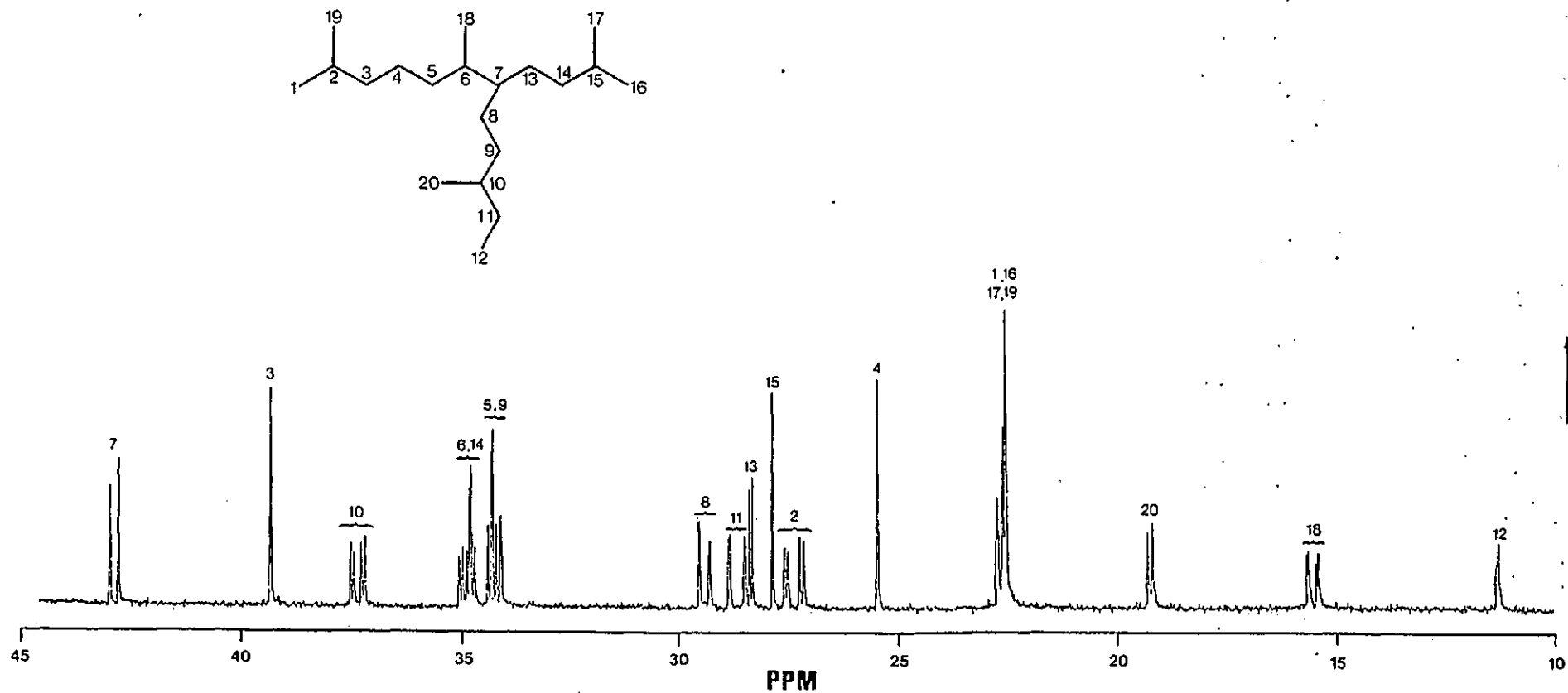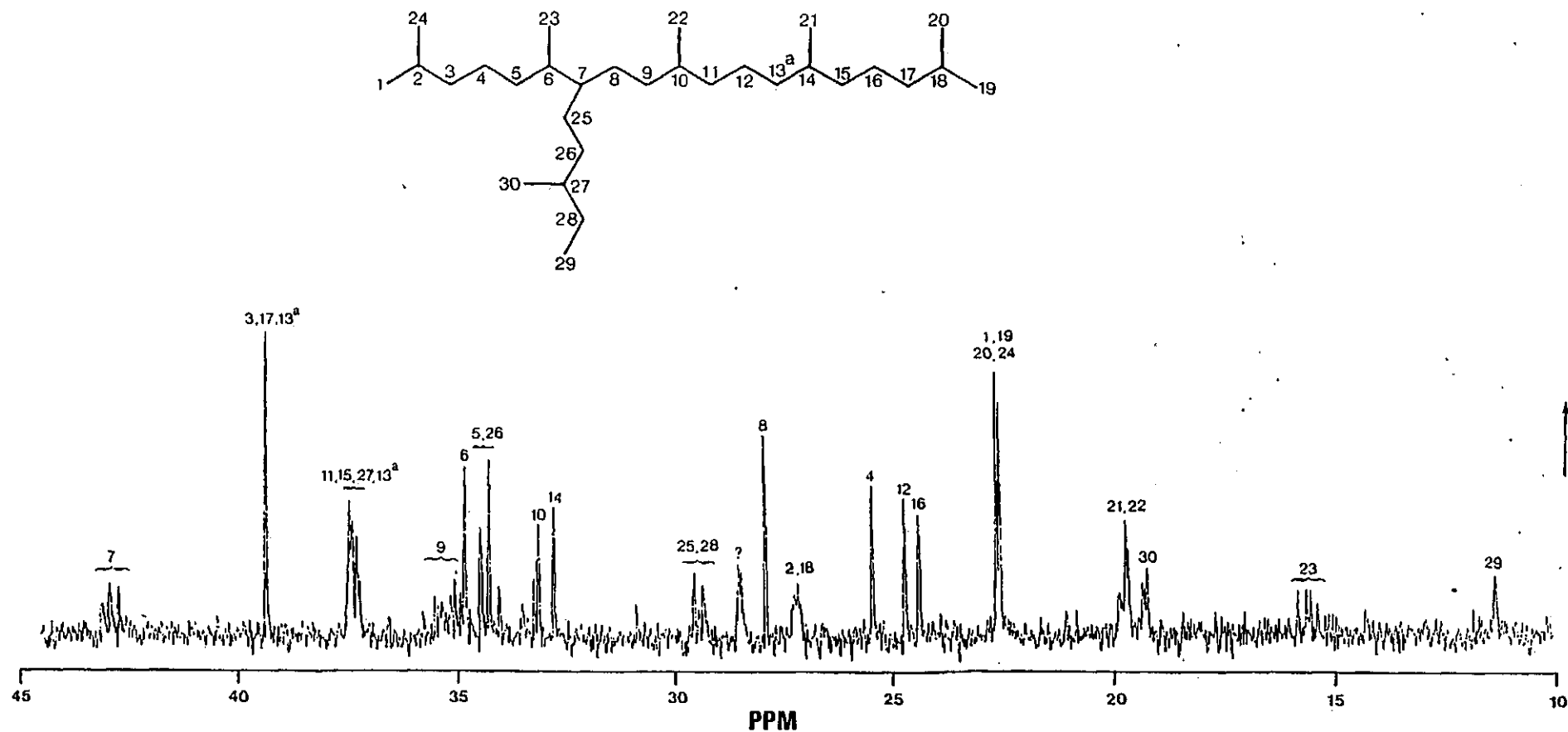
Fig.3:29　　^{13}C NMR (400 MHz) spectrum of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (br20:0). Proposed shift assignments (Table 3:12b) as shown.　Numbers refer to the carbon number in the accompanying structure.

Table 3:12A

Observed ^{13}C NMR chemical shifts for
2,6,10-trimethyl-7(3-methylbutyl)dodecane

ppm	intensity	integral	proposed number of carbons
42.9563	5.650	1.09	1
42.7643	6.576		
39.3077	9.911	0.96	1
37.4874	2.974	1.23	1
37.4177	2.624		
37.2427	2.924		
37.1681	3.202		
35.0253	2.390	1.91	2
34.9422	2.732		
34.8497	2.576		
34.7847	5.459		
34.7592	6.458		
34.6789	2.749		
34.3794	3.723	2.19	2
34.2898	8.107		
34.1832	3.806		
34.0789	4.183		
29.5241	3.961	1.08	1
29.2865	3.173		
28.8535	3.285	1.10	1
28.8324	3.507		
28.5071	3.328		
28.4777	3.263		
28.3872	5.396	1.3	1
28.3271	5.932		
27.8724	9.856	0.82	1
27.5877	2.891	1.23	1
27.5140	2.780		
27.2497	3.303		
27.1460	3.122		
25.4464	10.460	1.10	1
22.7202	5.101	3.97	4
22.5985	8.441		
22.5323	13.671		
19.2847	3.647	0.96	1
19.1713	3.996		
15.6352	2.503	1.10	1
15.6040	2.630		
15.4183	2.160		
15.3618	2.539		
11.2793	3.068	0.82	1
		Total	20

Table 3:12B

^{13}C NMR shift assignments for synthetic
2,6,10-trimethyl-7-(3-methylbutyl)dodecane
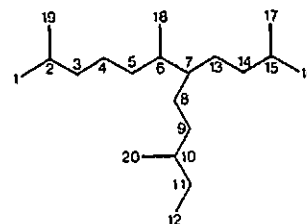
Carbon Number	ppm (this study)[a]	ppm (Yon, 1982)[b]	Calculated chemical shift[c] ppm
12*	11.2793	11.402 - 11.475	11.61
18*	15.4183 - 15.6352	15.426 - 15.718	18.38
20*	19.1718 - 19.2874	19.289 - 19.401	20.16
1,16 17,19	22.5323 - 22.7202	22.656 - 22.885	22.50
4	25.4464	25.560	25.57
2*	27.1460 - 27.5877	27.190 - 27.648	28.61
15	27.8724	27.978	27.90
13	28.3271 - 28.3872	28.436 - 28.494	28.75
11	28.4777 - 28.8538	28.557 - 28.913	30.20
8*	29.2865 - 29.5241	29.380 - 29.642	29.05
5*,9*	34.0789 - 34.3794	34.133 - 34.381	34.25,35.59
6*	34.6789 34.7847	34.751 - 34.873	34.46
14*	34.8497 - 35.0253	34.941 - 35.111	37.93
10*	37.1681 - 37.4874	37.242 - 37.573	35.36
3	39.3077	39.402	39.99
7*	42.7643 - 42.9563	42.822 - 43.017	41.49

[a]: 400 MHz NMR

[b]: 200 MHz NMR

[c]: calculated according to Carmen et al., 1973.

*: diastereoisomeric splitting.

basis using molecular additivity rules. Building up the carbon skeleton from methane, they were able to derive the following expression for predicting the chemical shift of any given carbon:

$$\delta_c(k) = B + \sum_K A_K N_{K,L}$$

In other words, the ^{13}C NMR chemical shift of the Kth carbon is equal to a constant plus the sum of the additive chemical shift coefficients multiplied by the number of carbons in the Lth position relative to K. The values for the chemical shift coefficients have been modified as a result of the greater statistical analysis of a wide survey of different compounds and correction factors have been proposed (Carmen et al., 1973) to compensate for extensively branched compounds such as acyclic isoprenoids. A representative calculation for the C7 methine carbon, common to the structures of the br20:0, br25:0 and br30:0 alkanes, is shown below. Figures in brackets are the chemical shift coefficients and the final two terms are the correction parameters to compensate for the two different branch points.
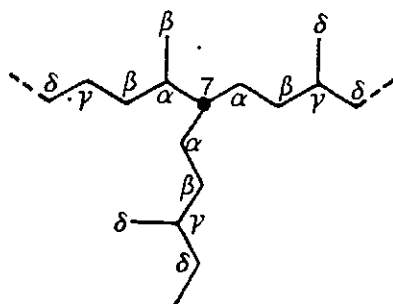
$\delta C7 = B + 3\alpha + 4\beta + 3\gamma + 5\delta + 2\ (3° - 2°) + 1\ (3'- 3')$

$= 2.35 \times 3(-8.85) + 4(-9.51) + 3(2.34) + 5(-0.28) + 2(3.04) + 9.05$

$\delta C7 = 41.49 \pm 1.27$ ppm downfield of TMS

The chemical shift assigned to C7 in the spectrum of the br20:0 alkane has a value of 42.7643 - 42.9563 ppm. Other theoretical systems for predicting chemical shifts (i.e. Grant and Paul, 1964; Lindeman and Adams; 1971) give values differing by up to 2 ppm. Whilst the theoretical chemical shifts rarely coincide exactly with the observed values, they are nonetheless useful.

The spectrum (Fig. 3:29) of the br20:0 alkane displays two interesting features reported previously in the spectra of other acyclic isoprenoids (i.e. pristane; Yon, 1982). The first is the observed magnetic non-equivalence of the individual methyl carbons of each isopropyl termini. This effect was first noted by Carmen et al. (1973), who demonstrated that the effect was independent of chirality by observing non-equivalence in achiral 2,4,6-trimethylheptane (8) and 2,5,8-trimethylnonane (9); the effect was attributed to conformational differences between the methyl carbons. The two signals at 22.65 ppm and 22.75 ppm in the ^{13}C NMR spectrum of meso-pristane were ascribed to magnetic non-equivalence of the individual methyl carbons of each isopropyl termini by Yon (1982). Similar effects have been observed in the spectra of 2,6,10,14,19-pentamethyleicosane (10) and 2,6,10,15,19-pentamethyleicosane (11) (Lamb, 1982). In the spectrum (Fig. 3:29) of br20:0, the three major signals in the 22.5325 - 22.7202 ppm region can be attributed to conformational differences in the methyl carbons of the isopropyl termini. The increased number of peaks relative to the same region in the spectrum of pristane probably reflects the proximity of one of the isopropyl groups to the C7 branch point.

The second interesting effect apparent in the spectrum (Fig. 3:29 of br20:0 is magnetic non-equivalence resulting from differences in

configuration (i.e. diastereoisomeric effects). Again, this was first demonstrated by Carmen et al. (1973) who noted that in the ^{13}C NMR spectrum of all isomer 2,5,8,11-tetramethyldodecane, the carbon atoms marked (*) produced clearly resolved doublets.
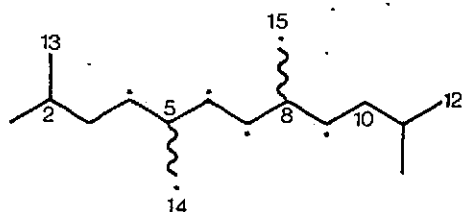
In the spectrum of br20:0, several of the carbon atoms appear to exhibit diastereoisomeric effects (see Table 3:12b; carbons with diastereoisomeric effects marked with an asterisk). The presence of four peaks in the chemical shifts assigned to C18 (15.4183 - 15.6352 ppm) and C10 (37.1681 - 37.4874 ppm) indicate that all four possible diastereoisomeric pairs are present in the synthetic br20:0. The spectrum of br20:0 exhibited herein is almost identical to that presented for synthetic br20:0 by Yon (1982) except that two signals (18.42 ppm and 39.86 ppm) which were attributed to contamination were absent in the present spectrum.

The ^{13}C NMR spectrum of 2,6,10,14-tetramethyl-7-(3-methylpentyl) pentadecane is shown in Fig. 3:30 and Table 3:13A. The interpretation of this spectrum is summarised in Table 3:13B and was made by comparison with the preceding br20:0 spectrum, published spectra (i.e. pristane; Yon, 1982) and calculated theoretical chemical shifts. Similarly the spectrum of br30:0 is shown in Fig. 3:31 and Table 3:14A and the assignments are summarised in Table 3:14B.

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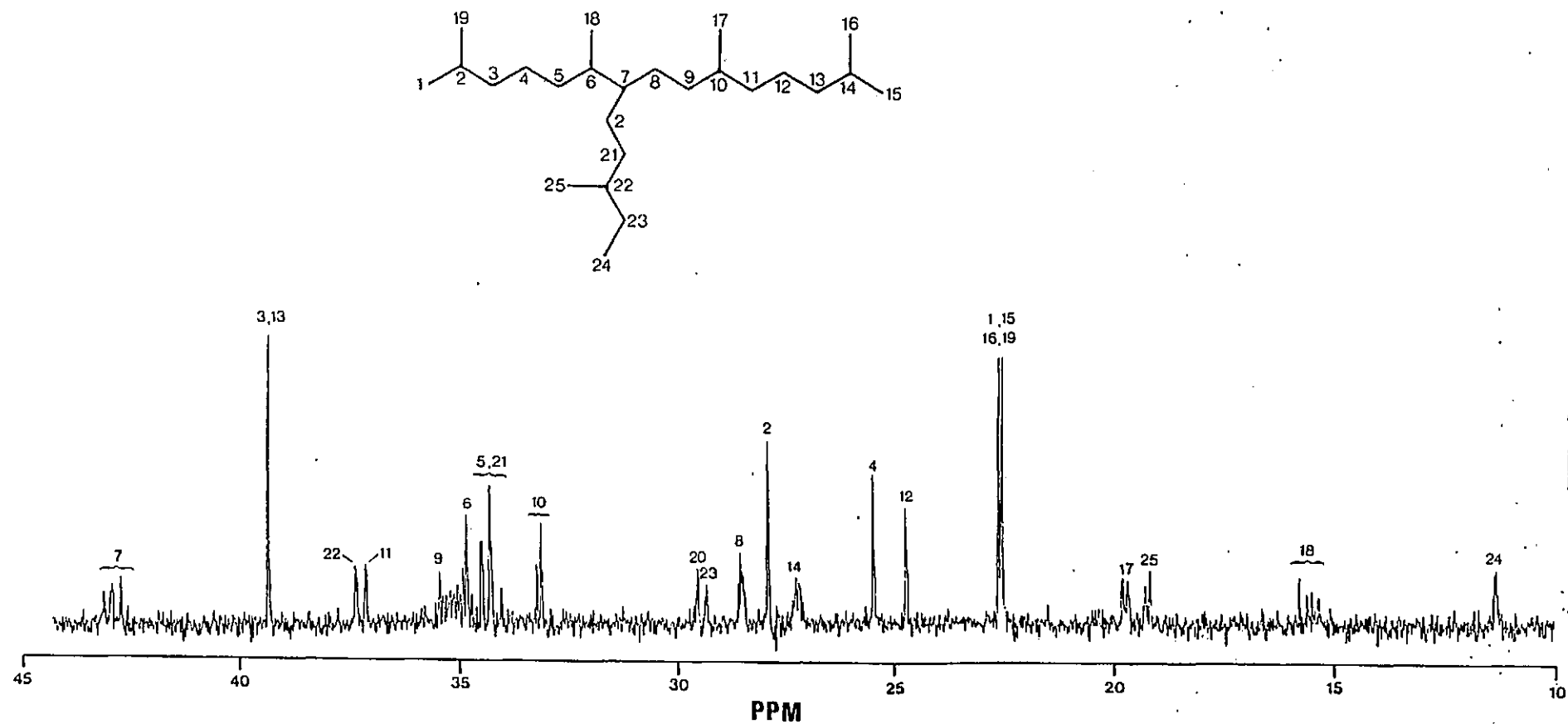
Fig.3:30 ^{13}C NMR (400 MHz) spectrum of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0). Proposed shift assignments (Table 3:13b) as shown. Numbers refer to the carbon number in the accompanying structure.

Table 3:13A

Observed ^{13}C NMR chemical shift for
2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane

ppm	intensity	proposed number of carbons	Type of carbon[a]
42.8954 42.6906	2.536 1.955	1	CH$_3$/CH
39.3082	13.481	2	CH$_2$
37.3330	3.150	1	CH$_2$
37.0950	3.022	1	CH$_2$
35.3798	1.991	1	CH$_2$
34.7575	6.385	1	CH$_3$/CH
34.4015	4.181	1	CH$_2$
34.2060	7.746	1	CH$_2$
33.9632	2.372	IMPURITY	
33.1650 33.0633	2.046 4.644	1	CH$_3$/CH
29.5351	2.117	1	CH$_2$
29.2930	1.851	1	CH$_2$
28.4920	3.630	1	CH$_2$
27.8815 27.2211	8.159 2.559	2	CH$_3$/CH
25.4479	7.417	1	CH$_2$
24.7024	5.770	1	CH$_2$
22.6063 22.5282	11.385 10.640	4	CH$_3$/CH
19.7961 19.6761	3.032 2.811	1	CH$_3$/CH
19.2826 19.1815	3.048 2.304	1	CH$_3$/CH
15.7649 15.4841	1.450 1.818	1	CH$_3$/CH
11.2786	2.413	1	CH$_3$/CH

[a]: as inferred from DEPT spectroscopy.

Table 3:13B

^{13}C NMR shift assignments for
2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane.
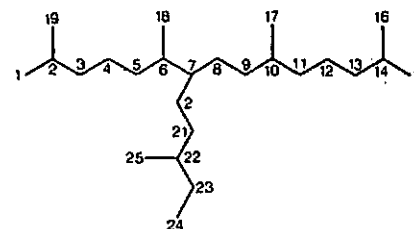
Carbon Number	ppm	Calculated chemical shift[b] ppm
24	11.2786	11.61
18	15.4841 - 15.7649	18.38
25	19.1815 - 19.2826	20.16
17	19.6761 - 19.7961	20.44
1,15	22.5282[a]	22.50
16,19	22.6063[a]	22.50
12	24.7024	25.29
4	25.4479	25.57
2,14	27.2211 - 27.8815	28.61
8	28.4920	28.75
23	29.2930	30.20
20	29.5351	29.05
10	33.0633 - 33.1650	33.30
5,21	34.2060 - 34.4015	34.25,35.59
6	34.7575	34.46
9	35.3798	35.87
11	37.0950	37.93
22	37.3330	35.36
3,13	39.3083	39.99
7	42.6906 - 42.8954	41.49

[a]: Chemical shift assignments with the same superscript may be interchanged.
[b]: Calculated according to Carmen et al., 1973.

Fig. 3:31 ^{13}C NMR (400 MHz) spectrum of 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane (br30:0). Proposed shift assignments (Table 3:14b) as shown. Numbers refer to the carbon number in the accompanying structure.   13^a = assignment uncertain.? = contaminant.

Table 3:14A

Observed ^{13}C NMR chemical shifts for
2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane

ppm	intensity	integral	number of carbons
42.8870 42.6923	4.695 4.408	1.22	1
39.3078	21.722	2.60	3
37.3929 37.2288	12.480 9.375	3.42	3
35.4435 35.0741	3.783 3.811	0.73	1
34.9847 34.7590 34.4046 34.2011	5.469 15.569 10.196 16.777	3.13	3
33.9682 33.4164 33.1686 33.0650 32.7147	4.679 3.332 5.579 10.943 12.174	2.31	2
29.5129	6.097	0.68	1
29.3082	5.005	0.63	1
28.4948	6.763	1.10	1
27.8836 27.1445	18.850 5.001	2.30	2
25.4497	14.017	1.07	1
24.7161	12.725	0.76	1
24.3826	11.224	1.07	1
22.6110 22.5327	24.131 21.323	3.71	4
19.6647 19.1837	10.456 6.004	3.36	3
15.7711 15.4833	3.938 4.084	0.91	1
11.3108	5.101	0.71	<u>1</u> 30

Table 3:14B

^{13}C NMR shift assignments for
2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane.
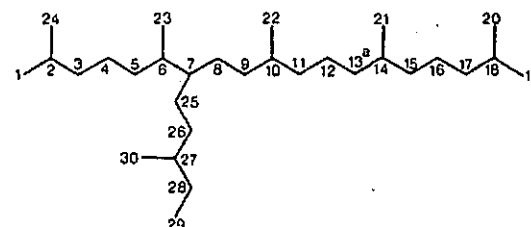
Carbon Number	ppm	Calculated chemical shifts[b] ppm
29	11.3108	11.61
23	15.4833 - 15.7711	18.38
30	19.1837	20.16
21,22	19.6647	20.44
1,19[c]	22.5327	22.50
20,24[c]	22.6110	22.50
16	24.3826	25.29
12	24.7161	25.57
4	25.4497	25.57
2,18	27.1445 - 27.8836	28.61
8	28.4948	28.75
28	29.3082	30.20
25	29.5129	29.05
14	32.7147 or 33.9682	33.02
10	33.0650 - 33.1686	33.30
5,26	34.2011 - 34.4046	34.25,35.59
6	34.7590	34.46
9	35.0741 - 35.4435	35.87
11,15, 27,13[a]	37.2288 - 37.3929	37.94,37.93,35.96
3,17,13[a]	39.3078	39.99,39.08,39.99
7	42.6923 - 42.8870	41.16

[a]: Assignment uncertain.

[b]: Carmen et al., 1973.

[c]: Chemical shifts with the same superscript may be interchanged.

There are some interesting comparisons to be made between the spectra. For instance, in each case the position of the terminal carbon of the ethyl group is seen as the most upfield resonance. Conversely, the position of the central tertiary carbon C7 is the most downfield signal in all three alkanes (Fig. 3:29-31). However, in the spectrum of br20:0 this resonance appears as a doublet (presumably due to diastereoisomeric coupling) whereas in both br25:0 and br30:0 this resonance is a quartet. This presumably reflects the increased number of isomers in br25:0 and br30:0. Evidence for the increasing alkyl chain (br20:0, br25:0, br30:0) is seen in the signals at 24 - 25 ppm and 40 ppm. In br20:0 only C4 occurs in the former area whereas C4 and C12 appear in the spectrum of br25:0 and C4, C12 and C16 in the spectrum of br30:0. At 40 ppm the resonance assigned to C3 in br20:0 increases to include C3 and C13 in br25:0 and C3, C13 and C17 in br30:0; again this shows the presence of the increasing alkyl chain.

The IR spectra of br20, br25, br30 were all consistent with saturated hydrocarbons.

Finally, the mass spectrum (Fig. 3:25B) and retention index of br25:0 were identical to those reported for an acyclic C_{25} alkane by Barrick et al. (1980). Indeed synthetic br25:0 co-chromatographed on two gc phases (OV1 Fig. 3:32 and CPWAX52) with the C_{25} hydrocarbon present in sediment from Milford Haven (section 4.3.3) which had an identical mass spectrum to that observed by Barrick et al. (1980). Similarly, the mass spectrum and retention index of br30:0 were identical to that of the acyclic C_{30} hydrocarbon reported by Prahl et al. (1980) and Barrick and Hedges (1981) and to that of the C_{30} alkane observed in Gluss Voe (section 4.3.2). Thus, by synthesis of reference alkanes it
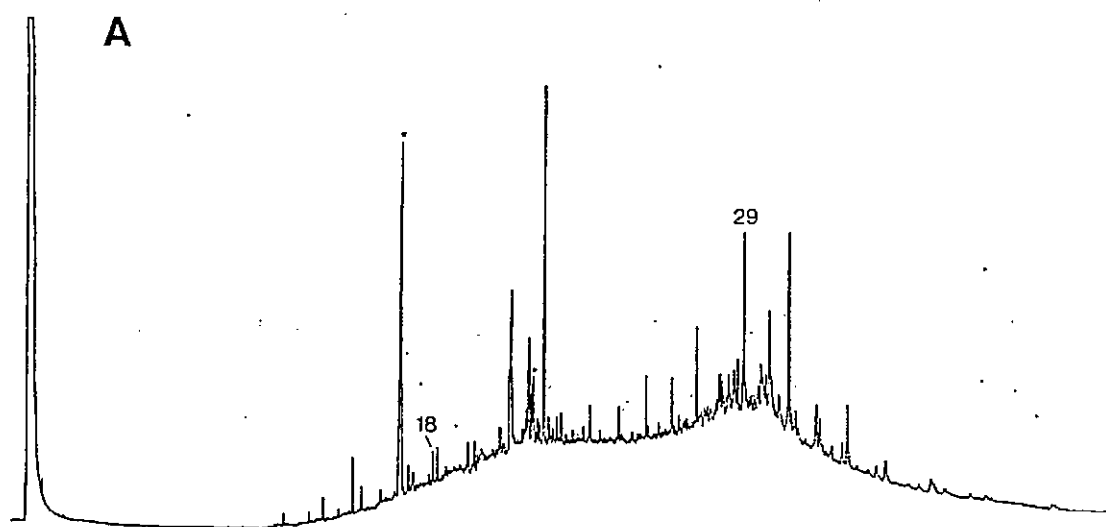
Fig.3:32   Gas chromatograms of A. the 'aliphatic hydrocarbons' from Milford
            Haven (section 4.3.3) and B. the same 'aliphatic hydrocarbons'
            co-injected with br20:0, br25:0 and br30:0.   Gc conditions as text.

Retention time (mins) ⟶
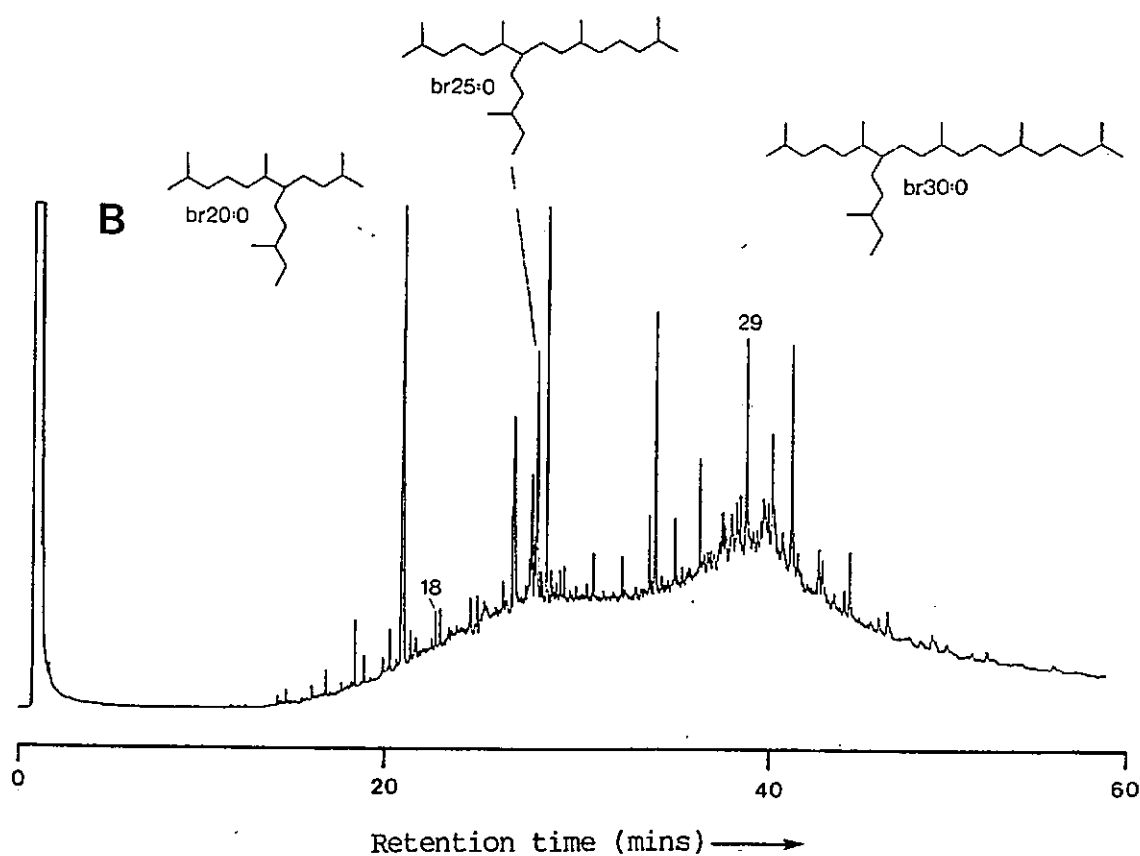
has been possible to assign the structures of br25:0 [i.e. 2,6,10,14,-tetramethyl-7-(3-methylpentyl)pentadecane] and br30:0 [i.e. 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane] to the sedimentary acyclic C_{25} and C_{30} hydrocarbons. The use of the synthetic br20:0, br25:0 and br30:0 hydrocarbons in the identification of sedimentary hydrocarbons is discussed at length in Chapter 4.

3.3 Bicyclic isoprenoids The synthesis of $8\alpha\beta$ (H) $9\alpha\beta$ (H) 11-tetrahydrogeranyldrimane (19) and $8\alpha\beta$ (H) $9\alpha\beta$ (H) 11-hexahydro-farnesyldrimane (20).

3.3.1 Structural assignment

The possibility of a structural relationship between certain sedimentary bicyclic C_{25} polyenes (a diene, c25:2:2) and a bicyclic C_{30} triene and tetraene (c30:3:2 and c30:4:2) was first suggested by Barrick and Hedges (1981) who observed similarities between gc retention behaviour and the EI fragmentation patterns of the bicyclic alkenes and their respective hydrogenation products (i.e. c25:0:2 and c30:0:2). This suggested that the bicyclic C_{30} alkenes had a fused bicyclic skeleton as had been proposed previously for the bicyclic C_{25} diene (Boehm and Quinn, 1978). An examination of the published mass spectra of the hydrogenation products (i.e. c25:0:2 and c30:0:2, Figs. 1:18E and D; Farrington et al., 1977 and Prahl et al., 1980 respectively) of the bicyclic C_{25} and C_{30} alkenes showed strong molecular ions and intense m/z 123 ions (base ion in c30:0:2). A base peak at m/z 123 is also exibited in the mass spectrum of the bicyclic sesquiterpane drimane (12) (a common bicyclic alkane present in petroleum), where the ion has been shown to be a fragmentation of the A-ring (Alexander et al., 1983, 1984):

Thus, the carbon skeleta of the bicyclic C_{25} and C_{30} hydrocarbons may contain the A-ring moiety of drimane (12) with the additional alkyl chain extending from one of the B-ring carbons (e.g. C6-C9). Alexander et al. (1983, 1984) also identified in numerous Ancient sediments and petroleums, a C_{16} homologue of drimane, homodrimane (13), in which the additional methyl carbon was attached to the existing methyl group at C9; the favoured fragmentation of the A-ring moiety (i.e. m/z 123) was not affected. Dimmler et al. (1984) reported the identification in Athabasca oil sand bitumen of a homologous series of C_{15} - C_{24} bicyclic alkanes containing the basic skeleton of drimane with the lengthening alkyl chain extending from C9. The structure of the C_{18} and C_{20} members
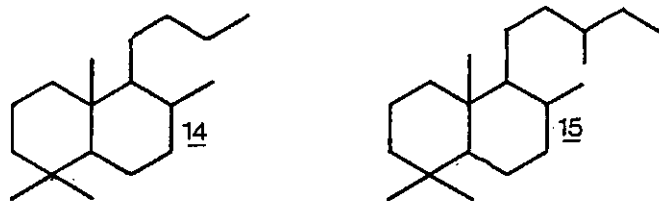
(14 and 15 respectively) were assigned by comparison with synthesized alkanes. The base ion at m/z 123 in the mass spectra of the C_{16}- C_{24} bicyclic alkanes indicated that the increase in carbon chain length at C9 does not effect A-ring cleavage. The proposed source of these hydrocarbons was via degradation of tricyclic terpanes. The possibility that the bicyclic C_{25} and C_{30} hydrocarbons currently of interest represented higher homologues (i.e. 17 and 18) of the series identified by Dimmler et al. (1984) was discounted because the

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retention index of the C_{24} member (16; $RI_{SE30}2272$) is greater than that of the reported retention index ($RI_{SE30}2125$) of the bicyclic C_{25} alkane (c25:0:2, Requego and Quinn, 1983). Indeed, the difference in retention indices between the C_{23} and C_{24} bicyclic alkanes is $\Delta RI = 72$ suggesting the approximate retention indices of the corresponding C_{25} alkane (17) would be RI2340 and the C_{30} alkane (18.) RI2690 (observed RI2590; Prahl et al. 1980).

Further possibilities for the carbon skeleta of the bicyclic C_{25} and C_{30} hydrocarbons (with the alkyl chain retained at C9) are (19) and (20).
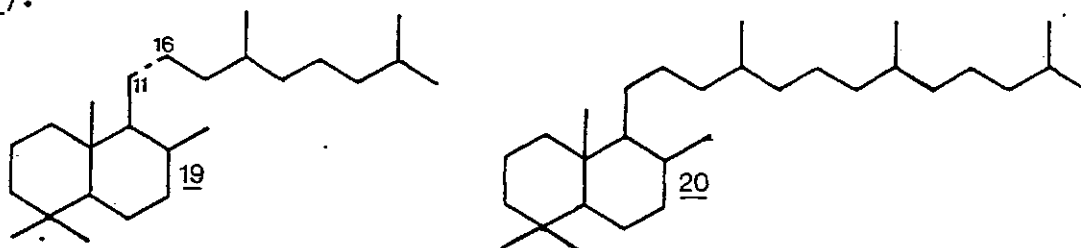
Such compounds could reasonably derive from limited cyclisation of the (t-t) acyclic isoprenoid, squalene. The postulated EI fragmentation of these proposed structures fitted well with the observed mass spectral fragmentation of c25:0:2 and c30:0:2 (Figs. 1:18E and D). In order to confirm if the sedimentary bicyclic C_{25} and C_{30} hydrocarbons had the postulated structures (19 and 20) the alkanes were synthesised.

3.3.2 Synthetic scheme

Examination of the proposed structures for the bicyclic C_{25} and C_{30} alkanes revealed a convenient disconnection at the C11-16 bond. Synthesis via the condensation of a bicyclic C_{15} aldehyde (21) with a 'regular' C_{10} isoprenoid moiety (i.e. 1-bromo- 3,7-dimethyloctane) would produce the C_{25} alkane (19), and the same bicyclic aldehyde when condensed with a 'regular' C_{15} isoprenoid moiety (i.e. 1-bromo-

3,7,11-trimethyldodecane) would produce the C_{30} alkane (<u>20</u>). The bicyclic C_{15} aldehyde was prepared from the naturally occurring sesquiterpenoid alcohol, 9α(H)-drimenol (<u>22</u>) which was kindly provided by Professors C. Brooks and H. Appel of Glasgow University, U.K. The full synthetic scheme for the bicyclic C_{25} and C_{30} alkanes is shown in Fig. 3:33.

3.3.3 $8\alpha\beta$(H) 9α(H)-drimanal (<u>25</u> and <u>26</u>) (see Fig. 3:34)

9α(H)-drimenol (<u>22</u>) was smoothly hydrogenated ($PtO_2.H_2O$; hexane) to afford a mixture of 8α(H) 9α(H)-drimanol (<u>23</u>) as the major product (85% by gc) and 8β(H) 9α(H)-drimanol (<u>24</u>) as the minor product (15% by gc). Assignments were made by reference to Appel <u>et al</u>. (1959). The ^{13}C NMR chemical shift assignments for the synthetic mixture of the C8-epimers of drimanol are presented in Table 3:15. These compared well with literature data for similar compounds (Kargramanov <u>et al</u>., 1976; Noble, 1986). The peaks at 60.5288 - 60.8131 ppm are readily assigned to the hydroxyl bearing carbon. Interestingly too, there are two signals in the spectrum for the methyl substituent (C12) at C8; a major signal at 16.9366 ppm and a minor signal at 21.8019 ppm which are assigned to the 8α(H) and 8β(H)-epimers respectively. The downfield shifts in signal betwen the epimers is attributed to the equatorial methyl substituent of the 8β(H)-epimer being sterically less crowded (i.e. greater deshielding) than the axial methyl substituent of the 8α(H)-epimer. A similar downfield shift is seen for C8 (Table 3.15). This effect is common in substituted cyclohexanes and is often described as the "steric compression shift" (Levy and Nelson, 1972). The larger signal for the 8α (H)- compared to the 8β (H)-epimer confirms the assignments made (cf Appel <u>et al</u>., 1959).
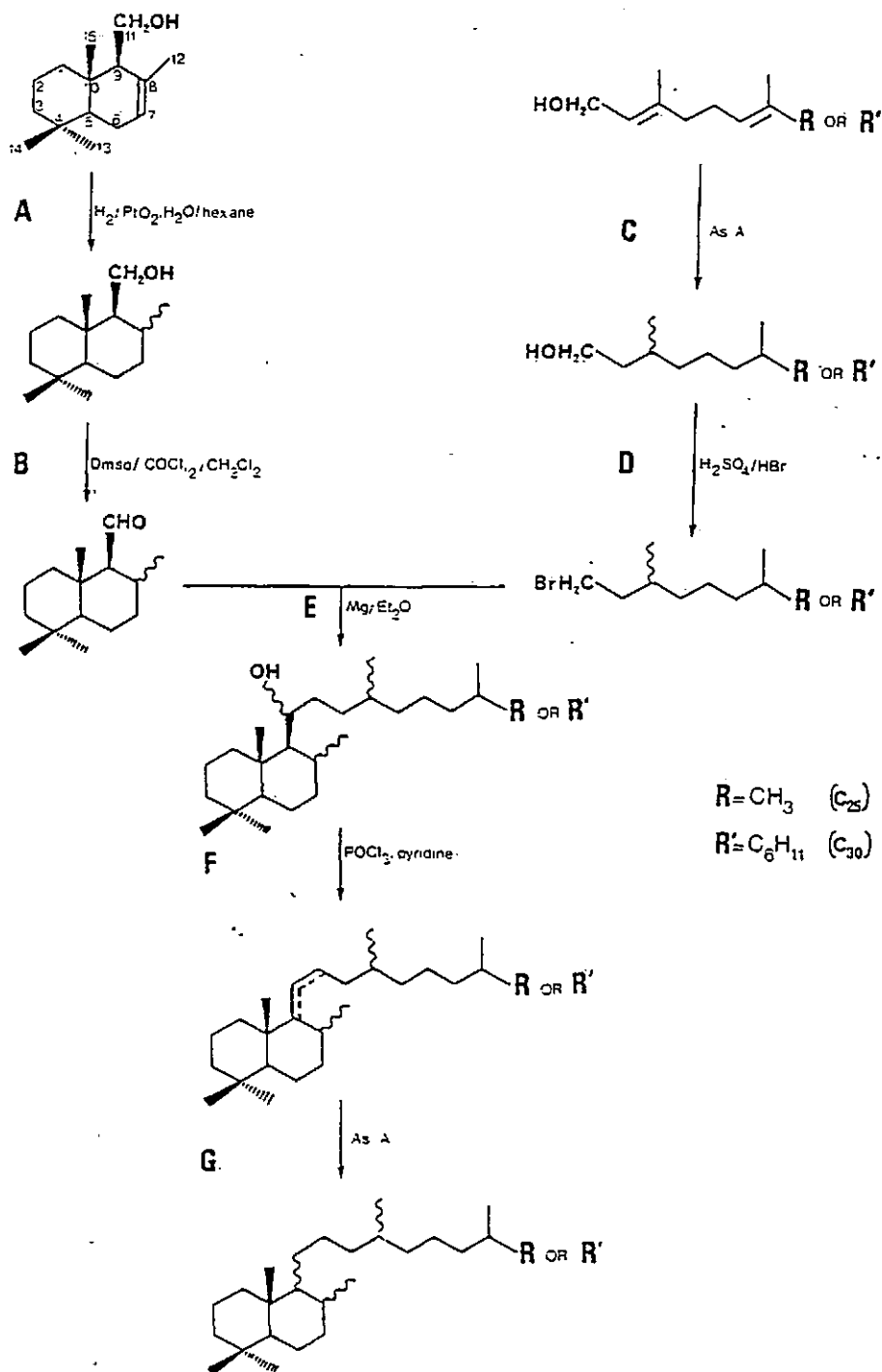
Fig.3:33   General synthetic scheme for the preparation of
            bicyclic alkanes, $8\alpha\beta(H)$ $9\alpha\beta(H)$ 11-tetrahydrogeranyl-
            drimane (R) and $8\alpha\beta(H)$ $9\alpha\beta(H)$ 11-hexahydrofarnesyl-
            drimane (R'). Note numbering system for carbon
            skeleton of $9\alpha(H)$ - drimenol.

Table 3:15

^{13}C NMR shift assignments for the synthetic mixture of
8α(H)-drimanol and 8β(H)-drimanol.
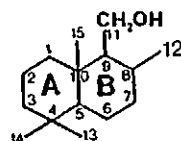
Carbon Number	Chemical shift ppm	Type of carbon[a] ppm
15	15.4788	CH$_3$/CH
12[bi]	16.9366	CH$_3$/CH
6	17.3933	CH$_2$
2	18.2799	CH$_2$
14	21.5008	CH$_3$/CH
12[bii]	21.8019	CH$_3$/CH
8[ci]	28.4359	CH$_3$/CH
4[d],13	33.1102 – 33.4420	CH$_3$/CH
7,8[cii]	34.3822	CH$_2$
1,10	37.4590 – 39.8087	CH$_3$/CH
3	41.8828	CH$_2$
9	55.6214	CH$_3$/CH
5	56.4186	CH$_3$/CH
11	60.5288 – 60.8131	CH$_2$

[a]: As inferred from DEPT spectroscopy

[bi]: 8α(H)   see text

[bii]: 8β(H)

[ci]: 8α(H)   see text

[cii]: 8β(H)

[d]: Quaternary carbons give no response in DEPT spectroscopy

Table 3:16

^{13}C NMR shift assignments for 8αβ(H) 9αβ(H) 11-
hexahydrofarnesyldrimane and related compounds.
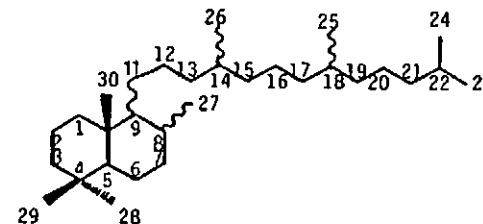
Carbon Number	Chemical shift ppm	Chemical shift 8αβ(H) 9α(H) 11αβ hexahydrofarnesyl driman-11-ol ppm	Chemical shift for 8αβ(H)-homodrimane[d] (13) ppm
1	39.56	39.71	39.7
2	18.45 – 18.75	18.5	
3	42.16 – 42.29[a]	41.45 – 41.95	42.3
4	33.02 – 34.29[a]		33.3
5	56.76		65.8
6	17.50		17.6
7	34.88		34.9
8	29.40/33.02–34.29[c]		28.7
9	53.28 – 54.85	54.91	55.5
10	37.51 – 38.28		38.5
11	25.33 – 25.90	72.24 – 72.83	
12	29.48		
13	36.48 – 36.80[a]		
14	33.02 – 34.29[a]		
15	37.10 – 37.33[b]		
16	24.71	24.69	
17	37.10 – 37.33[b]		
18	32.70		
19	37.10 – 37.33[b]		
20	24.34 – 24.40	24.32 – 24.69	
21	39.30	39.28	
22	27.89	27.85	
23	22.52	22.50	
24	22.61	22.59	
25	19.60 – 19.83	19.57	
26		19.63	
27	16.29/21.82[c]		16.4
28	21.55	21.90	21.6
29	33.02 – 34.29[a]		33.6
30	13.31 – 15.38		15.2

[a,b]: chemical shift unseparable

[c]: effect of α , β -configuration at C8(C27)

[d]: Noble (1986)

The Omura and Swern (1978) oxidation of 8α(H) - and 8β(H)-drimanol yielded two products, one major (85%) and one minor (15%), which were assigned as 8α(H)- and 8β(H)-drimanal (25 and 26) respectively by comparison of their gc retention behaviour with that of the C8-epimers of drimanol. The epimeric aldehydes displayed almost identical mass spectra. That of the major 8α(H)-epimer is displayed in Fig. 3:35A. The spectrum exhibits a molecular ion ($M^{+\cdot}$) at m/z 222 and a ($M^{+\cdot}$ - 15) ion at m/z 207 which, by analogy with the mass spectral behaviour of drimane (12) and homodrimane (13), results from the preferential cleavage of the methyl group at C10. The strong tendency of the aldehydes to oxidise in the presence of atmospheric oxygen to the corresponding acids (27 and 28) was prevented by storing the aldehydes under nitrogen as a dilute solution in dichloromethane.

3.3.4 $8\alpha\beta$(H) 9α(H) $11\alpha\beta$(H)-tetrahydrogeranyldriman-11-ol

(29; see Fig. 3:36) and $8\alpha\beta$(H) 9α(H) $11\alpha\beta$(H)-hexahydrofarnesyl -driman-11-ol (30; see Fig. 3:39)

$8\alpha\beta$(H) 9α(H) $11\alpha\beta$(H)-tetrahydrogeranyldriman-11-ol

The Grignard addition (method of Kharasch and Reinmurth, 1954) of 1-bromo-3,7-dimethyloctane and 8α(H)- and 8β(H)-drimanal produced in low yield (8%) the desired alcohol(s) which unfortunately could not be completely chromatographically separated (tlc) from the residual unconsumed $8\alpha\beta$(H)-drimanal (see Table 2:3). The product C_{25} alcohol gave two gc peaks (one major RI2558; 80%) and one minor (RI2586; 20%) (Fig. 3:35B) but it is uncertain if the obvious isomers represented configuration epimers at C8, C11 or a combination of both although the difference in retention indices (ΔRI = 28) is similar to that observed for 8α(H)- and 8β(H)-drimanal (ΔRI = 30). Interestingly, there is a change in the chromatographic behaviour of the major/minor products

CH₂OH

22

Hydrogenation

A (H₂/PtO₂.H₂0/hexane)

CH₂OH   CH₂OH

24   **23**

Minor 15%   Major 85%

B   Oxidation

(DMSO/(COCl)₂/CH₂Cl₂)

CHO   CHO

26   **25**

15%   85%

Oxidation

(atmospheric?)

COOH

27/28
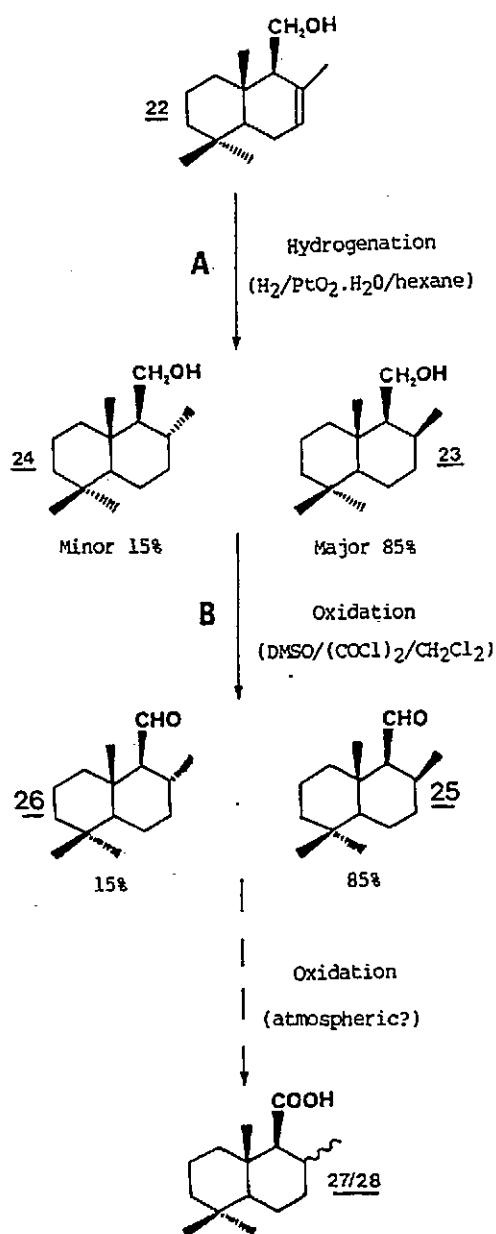
Fig.3:34   Synthesis of 8αβ(H) – drimanal.   Structure numbers
refer to text.   Letters refer to the related stages
in Fig.3:33.
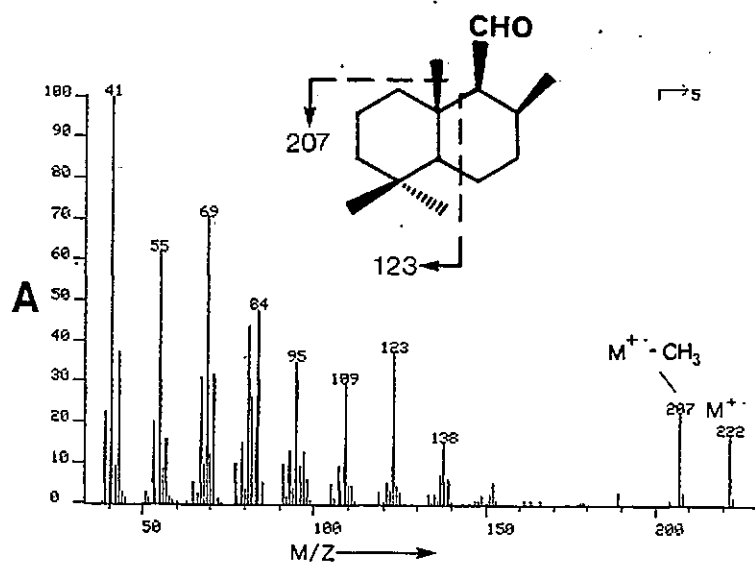
Fig.3:35A   Mass spectrum of 8α(H)-drimanal.
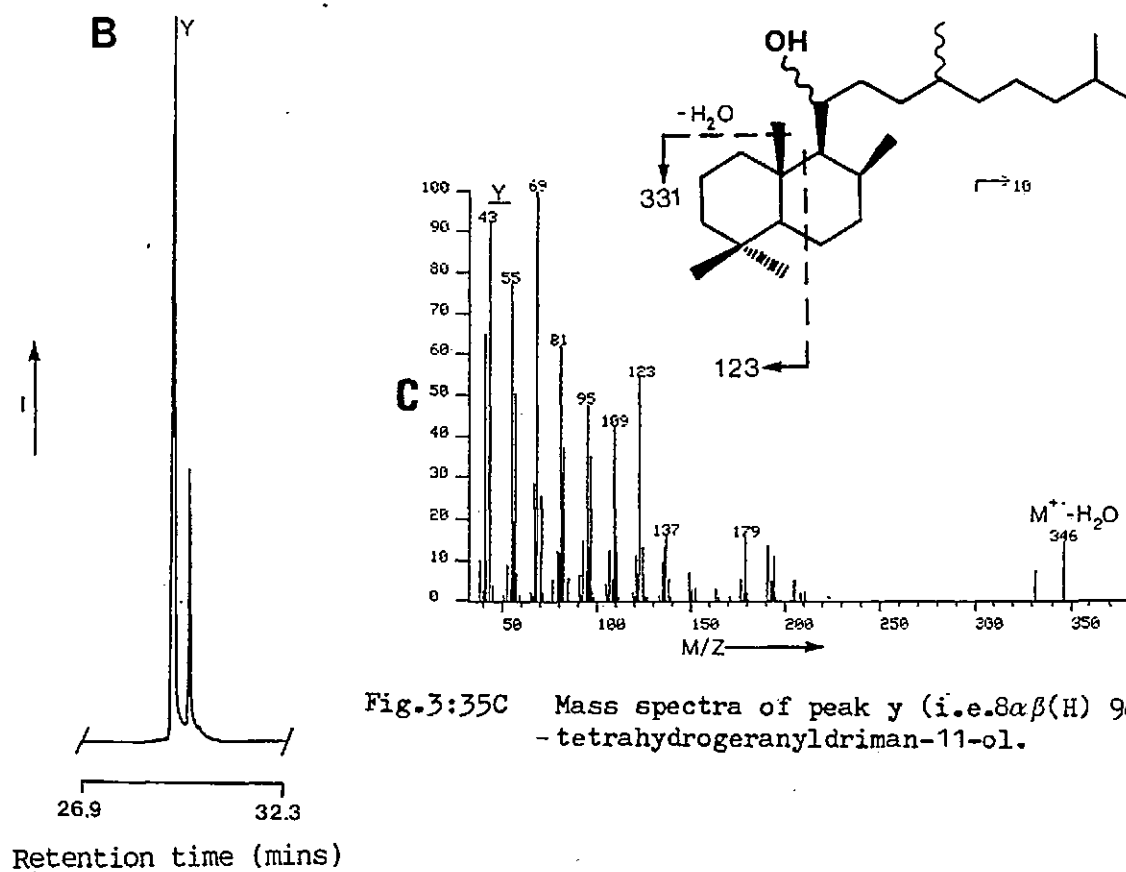
Fig.3:35C   Mass spectra of peak y (i.e.8αβ(H) 9α(H) 11αβ
             -tetrahydrogeranyldriman-11-ol.

Fig.3:35B   Gas chromatogram of the isomeric mixture of 8αβ(H) 9α(H)
             11αβ(H)-tetrahydrogeranyldriman-11-ols.
             Gc conditions: SE54, 40 - 290°C at 6°Cmin⁻¹.

between the aldehyde and the Grignard addition product. For instance, the major aldehyde (85%), assigned as 8 α (H)-drimanal, had a higher retention index than the minor product (15%), 8 β (H)-drimanal but the major isomer of 8 $\alpha\beta$ (H) 9 α (H) 11 $\alpha\beta$ (H)-tetrahydrogeranyldriman-11-ol had a lower retention index than the minor analogue. If the two gc peaks observed for the alcohol represent the C8 epimers then there are two possibilities to explain the observed shift in chromatographic behaviour: a), during the course of the Grignard addition, the configuration at C8 has changed from predominantely α to β or, b) the addition of a C_{10} alkyl group at C11 changes the elution order of the 8 α (H)- and 8 β (H)-epimers. ^{1}H NMR studies of the dehydration product of the bicyclic C_{25} alcohol indicated the latter to be the case (see section 3.3.5).

The mass spectrum of the major C_{25} alcohol isomer (RI2558) is displayed in Fig. 3:35C. The molecular ion ($M^{+\cdot}$) is absent but the spectrum exhibits an ($M^{+\cdot} - H_2O$) ion at m/z 346 and an ($M^{+\cdot} - H_2O - CH_3$) ion at m/z 331 which presumably derives from preferential cleavage of the methyl group at C10 (cf. drimane). Attempts to prepare the TMS ether of the alcohol using the powerful silylation reagent, N-trimethylsilylimidazole (TSIM) were unsuccessful. Apart from IR, further spectroscopic assignment (i.e. ^{1}H or ^{13}C NMR) of 8 $\alpha\beta$ (H) 9 α (H) 11 $\alpha\beta$ (H)-tetrahydrogeranyldriman-11-ol (29) was not performed.

8 $\alpha\beta$ (H) 9 α (H) 11 $\alpha\beta$ (H)-hexahydrofarnesyldriman-11-ol

The bicyclic C_{30} alcohol was prepared in moderate yield by the Grignard addition (method of Kharasch and Reinmurth, 1954) of 1-bromo-3,7,11-trimethyldodecane to 8 α (H)- and 8 β (H)-drimanal (25 and 26).
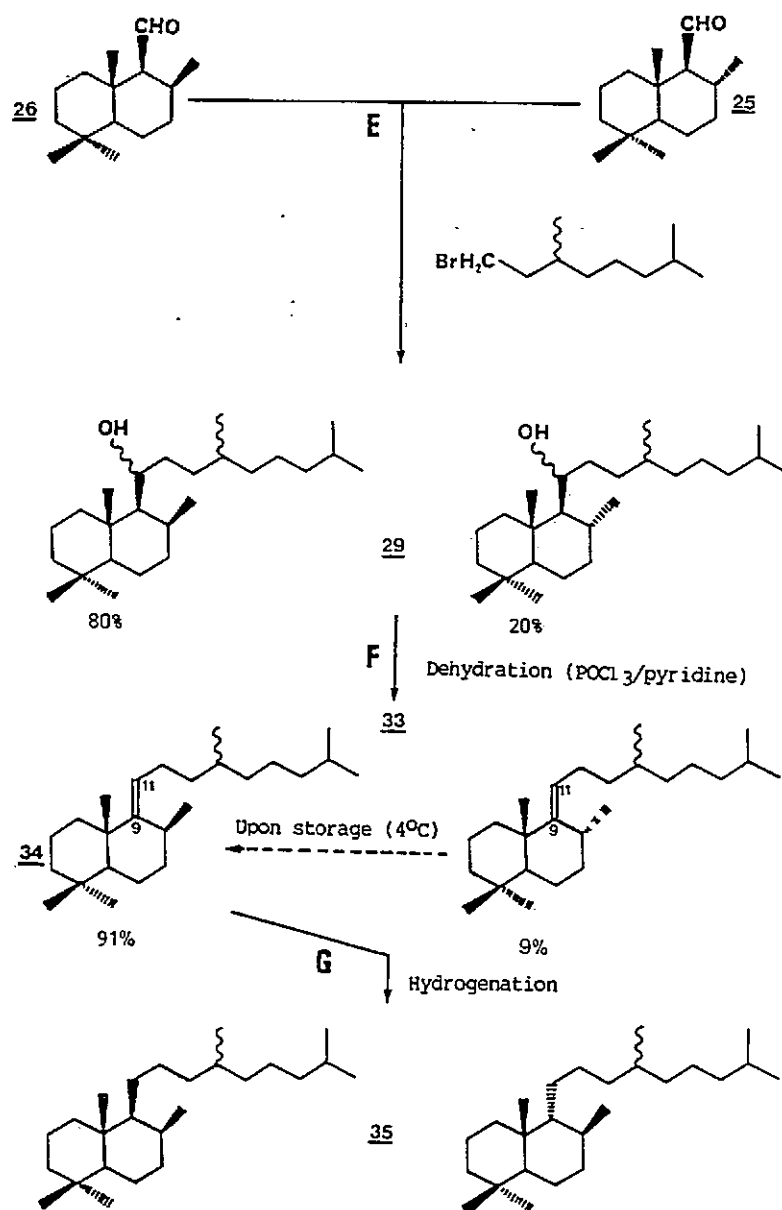
Fig.3:36   Synthesis of 8α(H) 9αβ(H)-tetrahydrogeranyldrimane.
Structure numbers refer to text. Letters refer to the
related stages in Fig.3:33

The alcohol gave three gc peaks (Fig. 3:37A). The early eluting peak (RI2882) had a different mass spectrum (Fig. 3:37B) to the others (Fig. 3.37C; only RI2905 shown). The additional isomer for the C_{30} alcohol compared to the bicyclic C_{25} alcohol (2 isomers), is probably the result of the longer C_{15} alkyl chain on the spatial configurations at C11.

The major peak (RI2905) probably represents the 8α (H)-epimer (31) and the peak of higher retention index (RI2934), the 8β (H)-epimer (32) although, if this is the case, it is interesting to note the increased abundance of the 8β (H)-epimer relative to that in $8\alpha\beta$ (H)-drimanal. The presence of four configurational isomers in the synthetic mixture of $8\alpha\beta$ (H) 9α (H) $11\alpha\beta$ (H)-hexahydrofarnesyldriman-11-ol is clearly demonstrated in the ^{13}C NMR spectrum in which the chemical shift assigned to C11 (which is the carbon α to the hydroxyl group) is split into four peaks (72.3991 - 72.8311 ppm; Fig. 3:38A). It is assumed that this represents the four molecules epimeric at C8 and C11 (Fig. 3:39). Full interpretation of the observed ^{13}C NMR spectrum of the bicyclic C_{30} alcohol is made complicated by the presence of several configurational isomers because it is difficult to know exactly the effect each particular configuration has on the shifts of the neighbouring carbons. Additionally, the spectrum is further complicated by the presence of 12% of $8\alpha\beta$ (H)-drimanal which could not be separated from the alcohol. However, it has been possible to assign certain chemical shifts which are listed in Table 3:16; assignments were made by comparison with chemical shifts for the corresponding bicyclic C_{30} alkane (section 3.2.6) and $8\alpha\beta$ (H)-drimanol (Table 3:15).
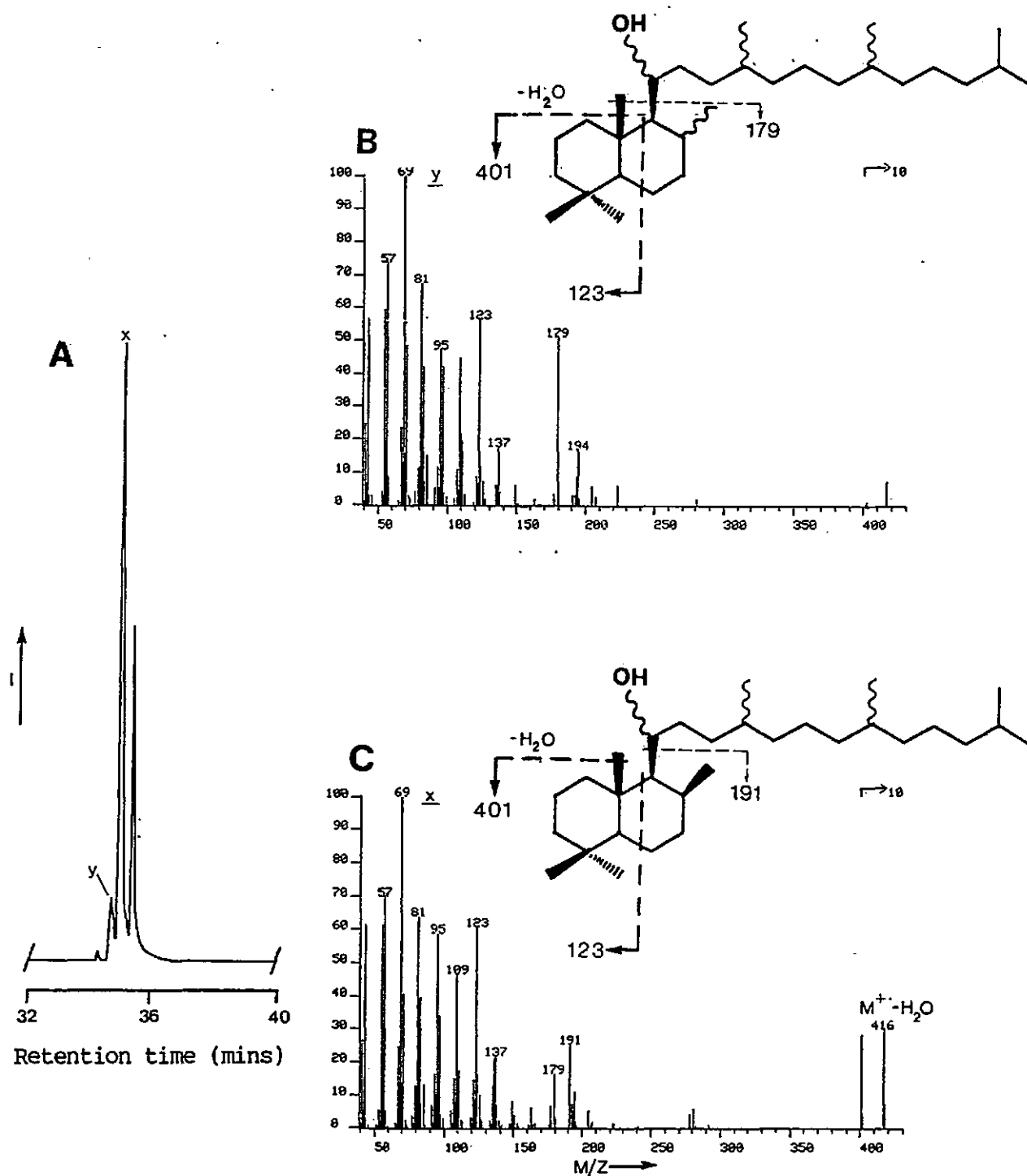
Fig.3:37   A. Gas chromatogram of the isomeric mixture of 8αβ(H) 9α(H)
11αβ(H)-hexahydrofarnesyldriman-11-ols. Gc conditions:
SE54, 40 - 290°C at 6°Cmin⁻¹. B. and C. mass spectra
of peaks y and x respectively.

Fig.3:38A   Partial ^{13}C NMR (400 MHZ) spectrum
of 8αβ(H) 9α(H) 11αβ(H)-hexahydro-
farnesyldriman-11-ol. Numbers
refer to the carbon number in the
accompanying structure.

PPM

M/Z→
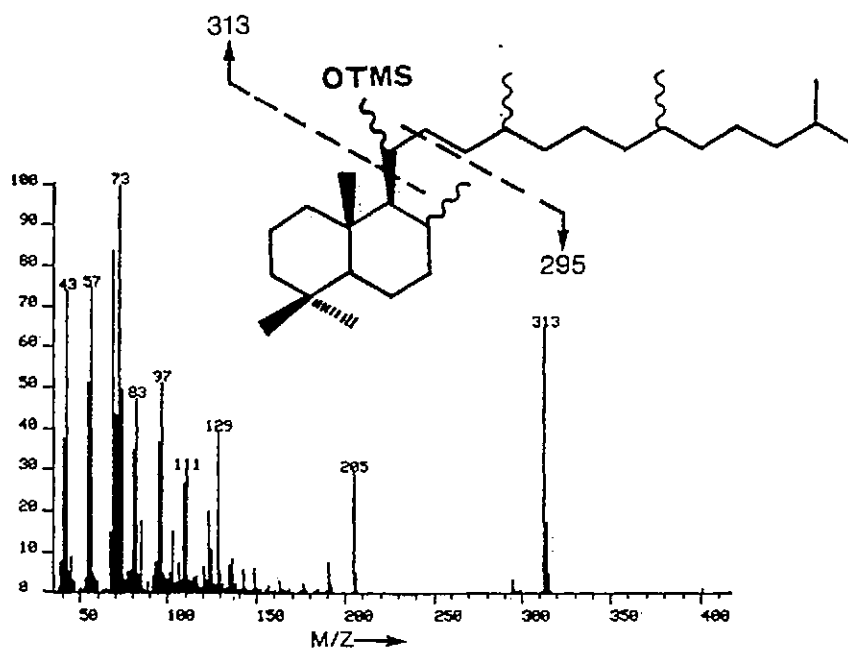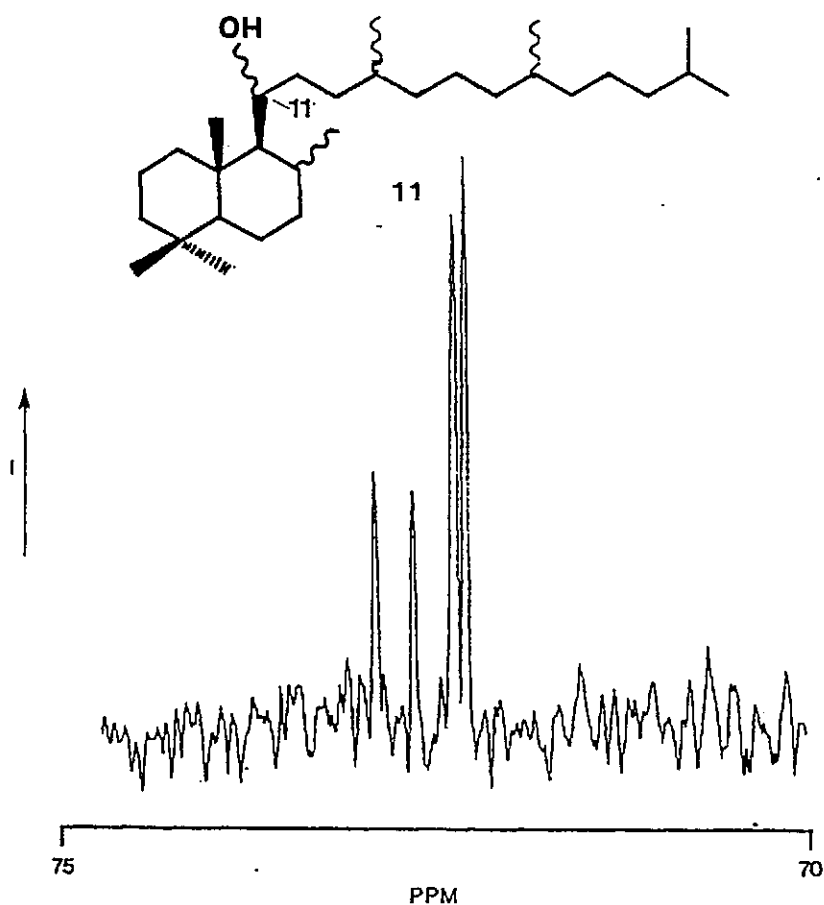
Fig.3:38B   Mass spectrum of the TMS ether of
8αβ(H) 9α(H) 11αβ(H)-
hexahydrofarnesyldriman-11-ol.
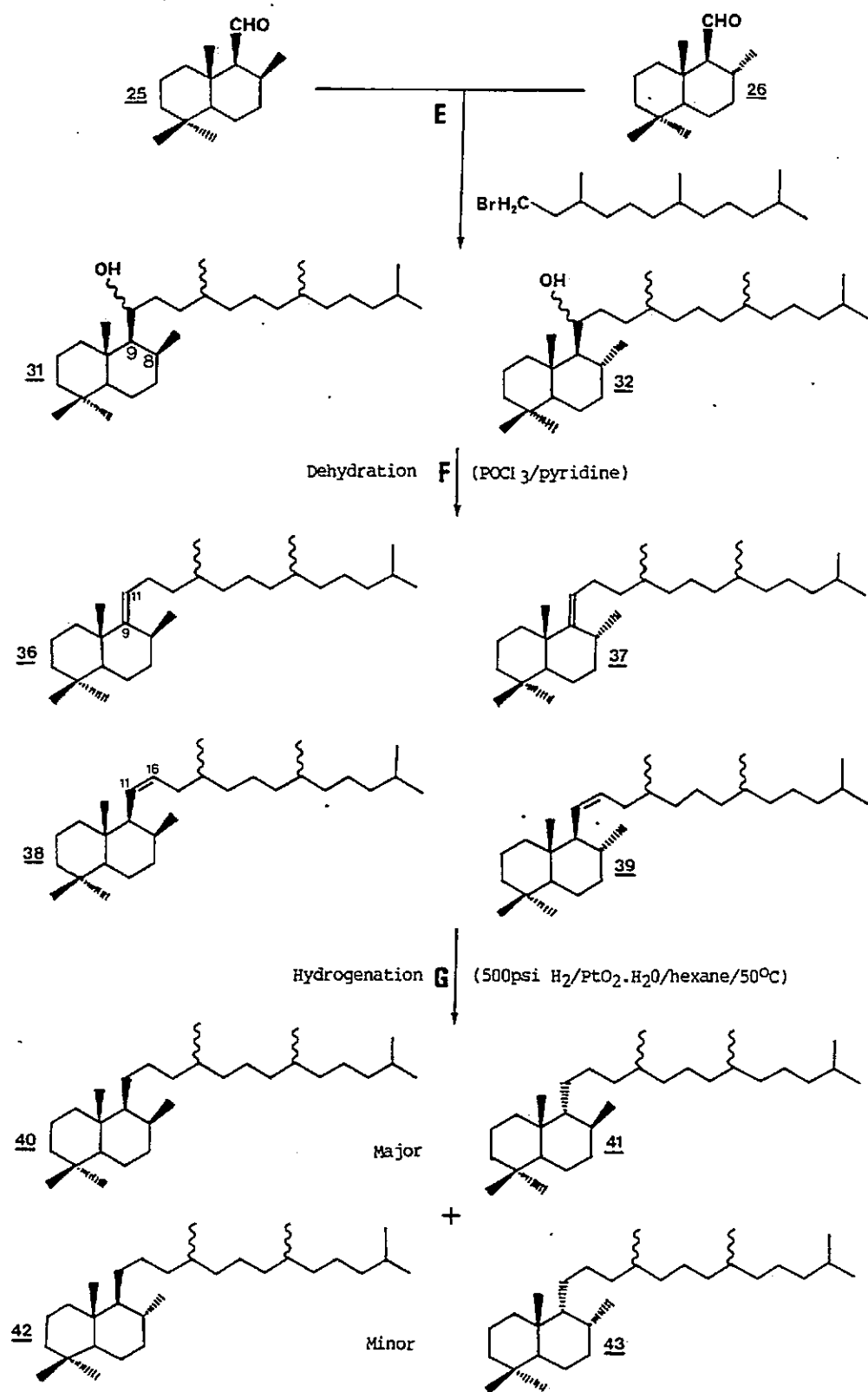
Fig.3:39 Synthesis of $8\alpha\beta(H)$ $9\alpha\beta(H)$-hexahydrofarnesyldrimane.
Structure numbers refer to text. Letters refer to
the synthetic stages in Fig.3:33.

The presence of α-cleavage ions in the mass spectrum (Fig. 3:38B) of the TMS ether of the bicyclic C_{30} alcohol confirms the position of the hydroxyl group at C11.

3.3.5   8 $\alpha\beta$ (H) 9E,Z-tetrahydrogeranyldrimene (33) and 8 α (H) 9$\alpha\beta$ (H) 11-tetrahydrogeranyldrimane (34) (Fig. 3:36).

Dehydration of 8 $\alpha\beta$ (H) 9 α (H) 11 $\alpha\beta$ (H)-tetrahydrogeranyldriman-11-ol produced a mixture of isomeric bicyclic C_{25} alkenes represented in the gas chromatogram shown in Fig. 3:40A. The chromatographic elution order of the major (91%) and minor (9%) peaks was the same as that observed for the precursor C_{25} alcohol (Fig. 3:35B) though the proportions were different. The mass spectra obtained for the two peaks were identical (e.g. Fig. 3:40B). The spectrum exhibits a molelcular ion at m/z 346 and an ($M^{+\cdot}$ - CH_3) ion at m/z 331 which is again attributed to the preferential cleavage of the methyl group at C10. The fragment ion at m/z 191 probably arises through migration of the double bond into the ring system followed by subsequent cleavage of the C9(C11) bond. Interestingly, upon storage (4°C; dichloromethane) of the alkene mixture, it was observed that the intensity of the minor product(s) decreased from 9% to zero suggesting a conversion to the more thermodynamically stable major product.

The position of the double bond in the major product was revealed by examination of the ^{1}H NMR spectrum (Fig. 3:41). The downfield triplet (δ5.043 ppm) with an integral of 1 H must represent a single vinyl proton (j) and thus indicates that the double bond is at the ring in the C9(C11) position. The multiplet at δ2.897 ppm (integral: 1H) is assigned to the methine proton (i) at C8 of the ring system and the multiplet at δ1.800 - 2.100 ppm (integral: 2H) represents the
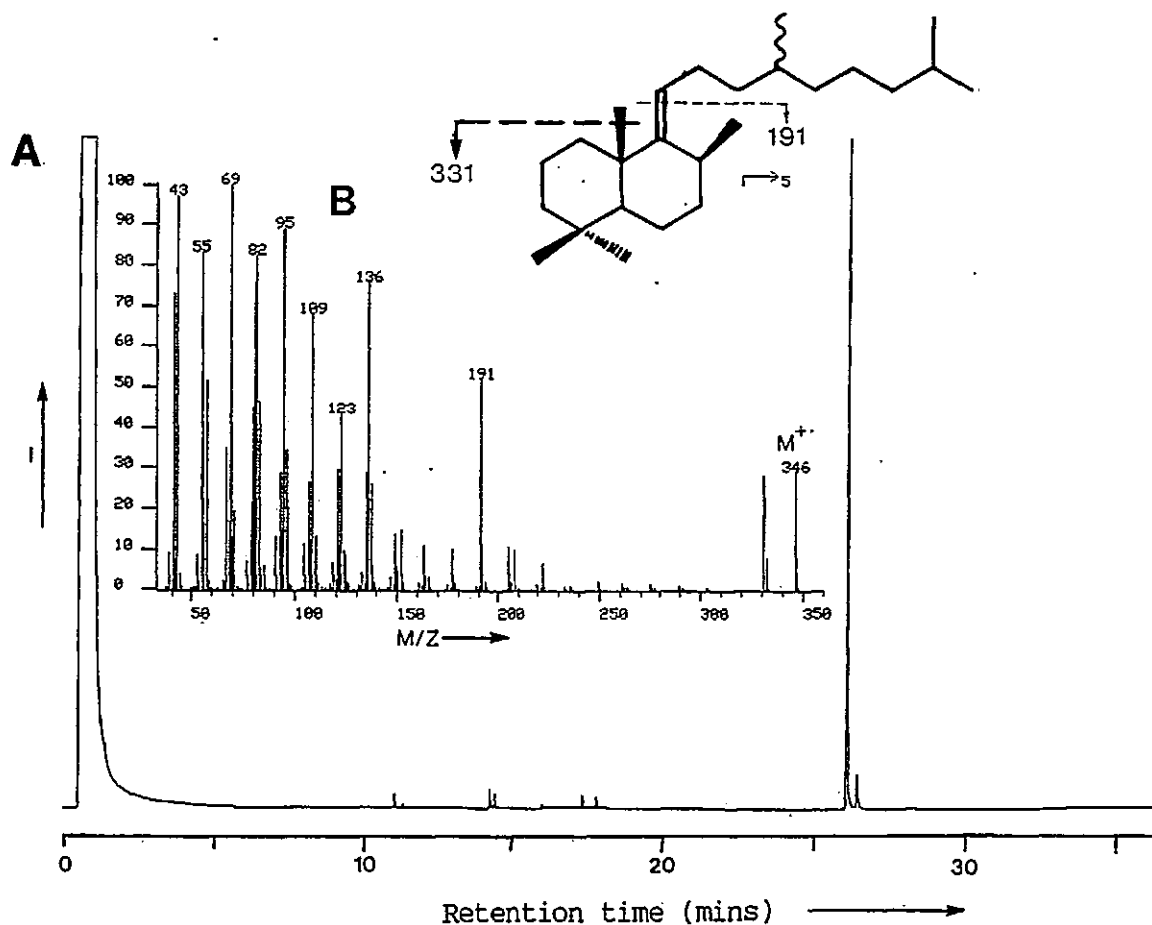
Fig. 3.40   A. Gas chromatogram and B. mass spectrum of
8α(H) 9E,Z 11-tetrahydrogeranyldrimene.
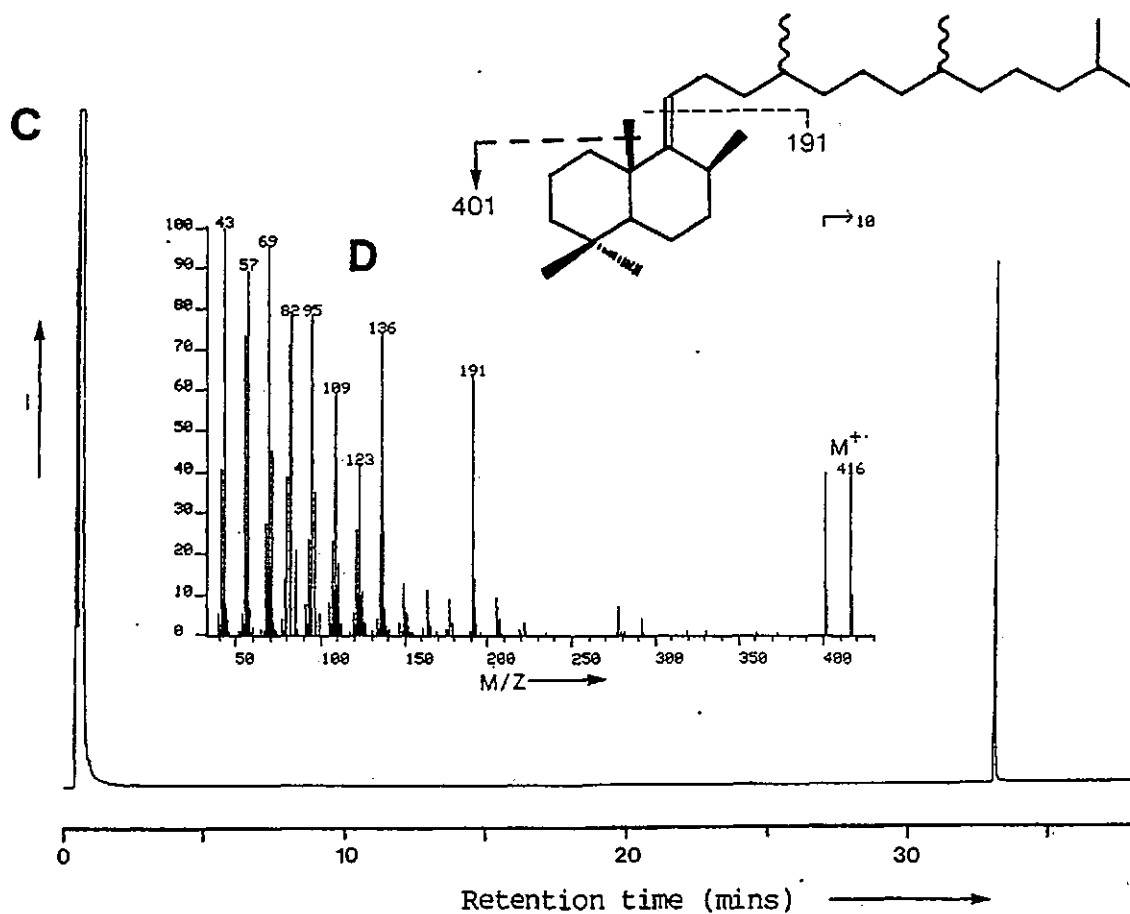
Fig.3:40   C. Gas chromatogram and D. mass spectrum of
8α(H) 9E,Z 11-hexahydrofarnesyldrimene.
Gc conditions: OV1, 40 - 290°C at 6°Cmin⁻¹.
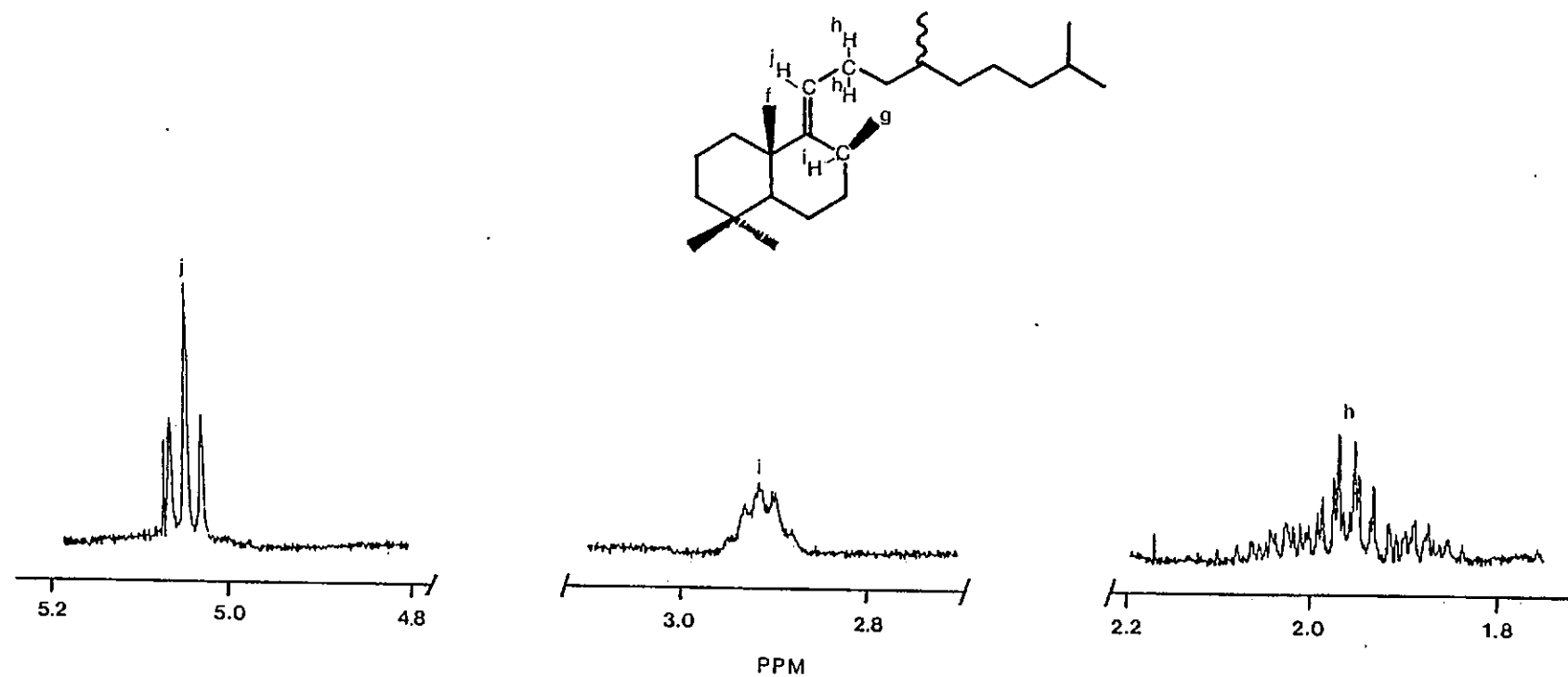
Fig.3:41   Partial ^{1}H NMR (400MHz) spectrum of 8α(H) 9E,Z 11-tetrahydrogeranyldrimene.

methylene protons (h) α to the double bond on the alkyl chain. Further notable features of the spectrum (not shown) include the doublet at $\delta 1.114$ ppm (integral: 3H) which was assigned to the methyl protons (g) and the singlet at $\delta 1.062$ ppm (integral: 3H) attributed to the bridgehead methyl protons (f). Further examination of the ^{1}H NMR spectrum using 2D correlation spectroscopy (COSY) confirmed the proposed structure (33) and indicated that both the methyl groups at C8 and C10 were in the axial configuration (i.e. 8α (H); Dr.O.W. Howarth - personal communication). Thus, it would appear that the predominant configuration at C8 is retained during the Grignard addition and dehydration steps. It is asumed that the major product of dehydration, 8 α (H) 9E,Z 11-tetrahydrogeranyldrimene (34), contains both geometric isomers.

Dehydration to give the C9(C11) double bond was favoured [rather than C11(C16)] presumably because of the increased stability associated with formation of a tetrasubstituted carbon at C9.

8 α (H) 9E,Z 11-tetrahydrogeranyldrimene was smoothly hydrogenated (PtO$_2$.H$_2$O) to a single product (Fig. 3:42A). Assuming the hydrogenation process does not alter the configuration at C8, the product can be assigned as 8 α (H) 11-tetrahydrogeranyldrimane (35). Although it is uncertain whether both or either of the C9 epimers [i.e. 9 α (H) or 9 β (H)] are present; the observed chromatographic separation of 8 α (H) and 8 β (H)-drimane (Alexander et al., 1983) and the presence of only one gc peak for the present compound suggests that just one C9 epimer is present.

Fig.3:42A Total ion chromatogram (TIC) of $8\alpha(H)$ $9\alpha\beta(H)$ 11-tetrahydrogeranyldrimane (peak x). Gc conditions: OV1, 40 - 290°C at 8°Cmin⁻¹.

Retention time (mins)
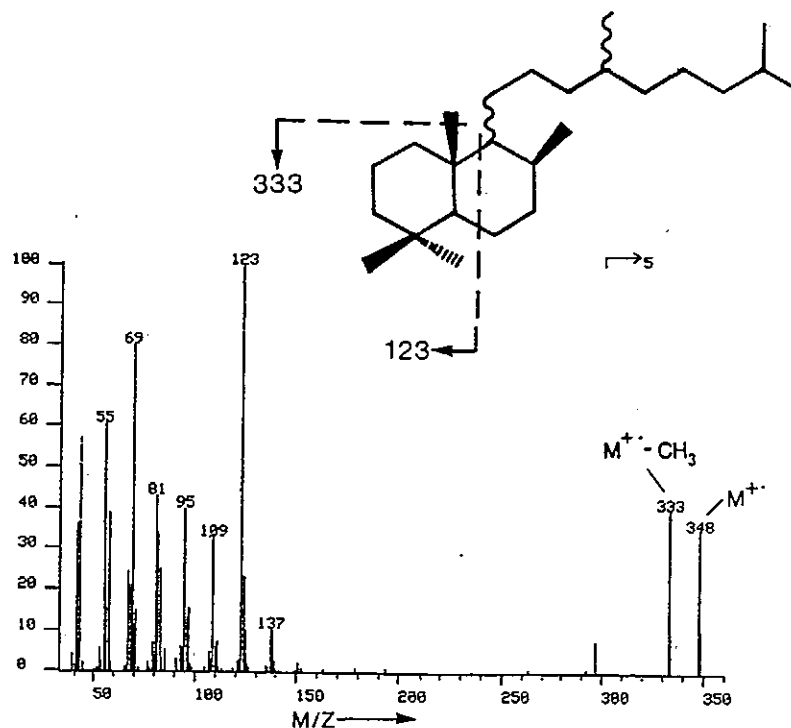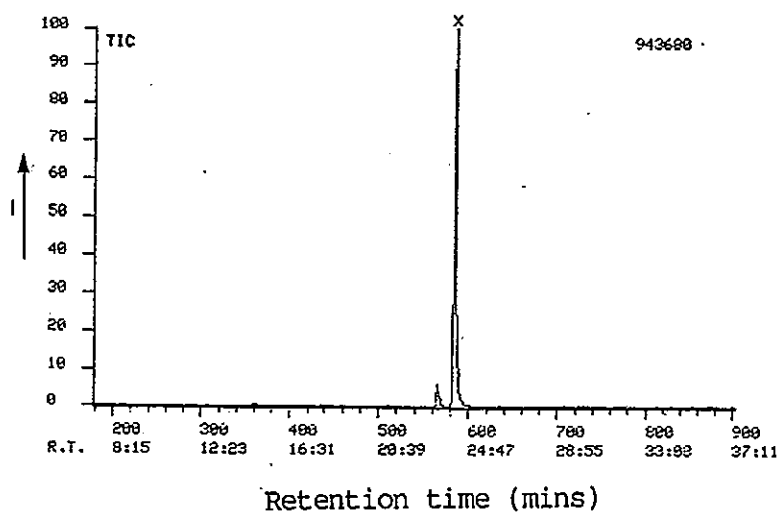
Fig.3:42B Mass spectrum of $8\alpha(H)$ $9\alpha\beta(H)$ 11-tetrahydrogeranyldrimane (peak x above).

The mass spectrum of the bicyclic C_{25} alkane is shown in Fig. 3:42B. An analogous fragmentation pattern to drimane (12) and homodrimane (13) is observed (Alexander et al., 1984). Thus, the spectrum of the C_{25} alkane exhibits a base ion at m/z 123 from A-ring fragmentation, a molecular ion (m/z 348) and an ($M^{+ \cdot}$ - CH_3) ion (m/z 333) which presumably arises from the preferential loss of the methyl substituent at C10 as observed for both drimane and homodrimane. Obviously the increased length of the alkyl chain at C9 does not interfere with the preferred cleavage of this methyl group. Unfortunately, the quantity (8mg) of pure bicyclic C_{25} alkane was insufficient for ^{13}C NMR.

Both the mass spectrum and retention index (RI2381) of 8 α (H) 11-tetrahydrogeranyldrimane (35) were different to the sedimentary bicyclic C_{25} alkane (c25:0:2) indicating the proposed carbon skeleton (section 3.3.1) for the sedimentary C_{25} hydrocarbons to be other than 35. The mass spectrum of the sedimentary alkane does not exhibit a strong ($M^{+ \cdot}$ - CH_3) ion suggesting the B-ring pattern of methyl substitution is different to that in 8 α (H) 11-tetrahydrogeranyldrimane (35). Further discussion on the implications of this discovery with reference to the proposal of other structures for the sedimentary bicyclic C_{25} hydrocarbons is included in Chapter 4.

3.3.6   8 $\alpha\beta$ (H) 9E,Z 11E,Z-hexahydrofarnesyldrimenes (36-39) and
8 $\alpha\beta$ (H) 9 $\alpha\beta$ (H) 11-hexahydrofarnesyldrimanes (40-43) (see Fig. 3:39)

Dehydration of 8 $\alpha\beta$ (H) 9 α (H) 11 $\alpha\beta$ (H)-hexahydrofarnesyldriman-11-ol (30) yielded a mixture of isomeric bicyclic C_{30} alkenes which produced three gc peaks (see Fig. 3:43A). The mass spectra of the three components were identical and that of the major product is shown in Fig. 3:40D. The presence of the mixture of isomers precluded
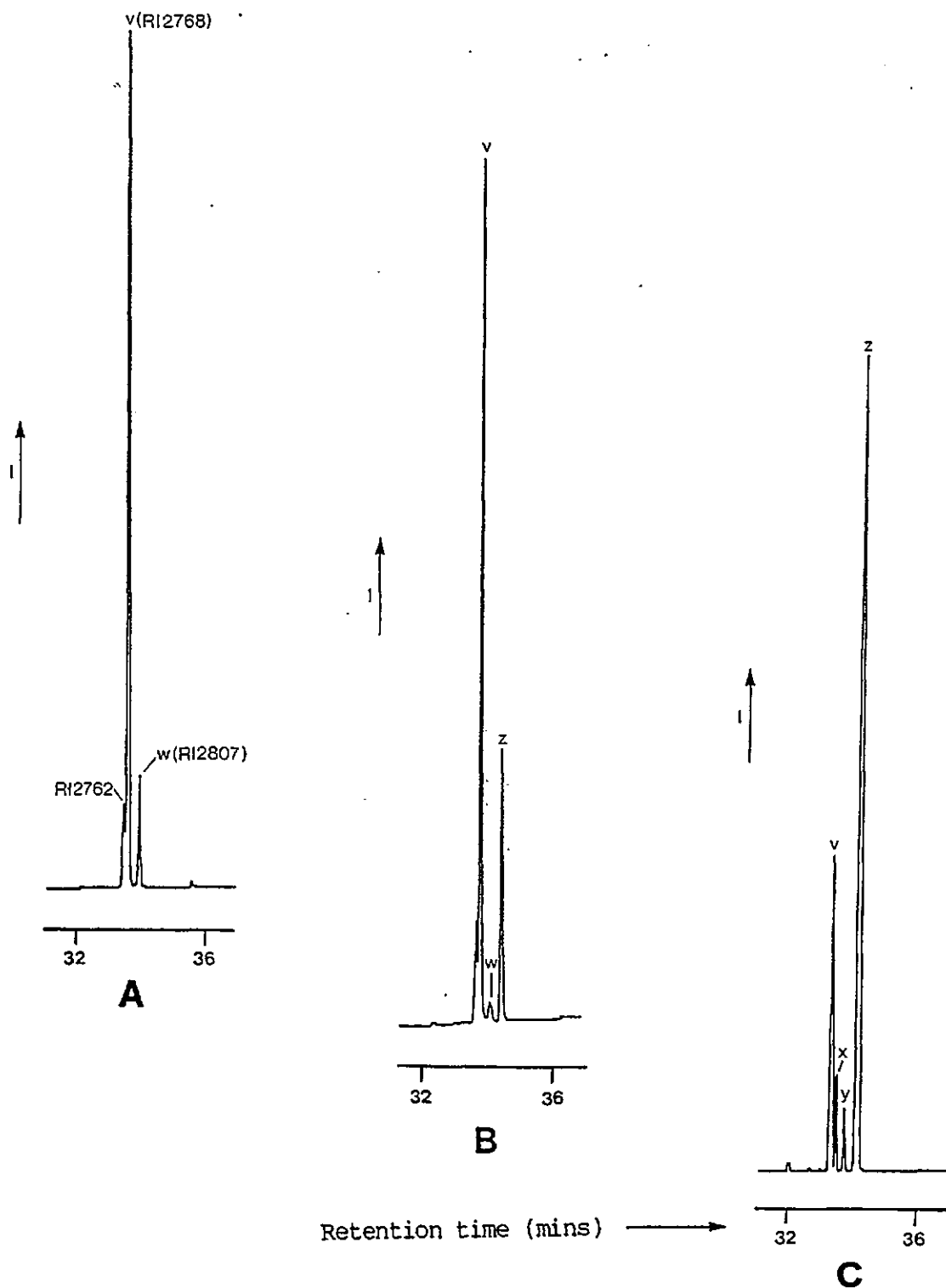
Fig.3:43   Gas chromatograms of A. the isomeric mixture of $8\alpha\beta$(H) 9,11E,Z-
hexahydrofarnesyldrimenes, B. the bicyclic C_{30} alkene/alkane
mixture after low pressure hydrogenation and C. the bicyclic
C_{30} alkene/alkane mixture after high pressure (500psi) hydrogenation.
Peak identifications as follows: v = 8α (H) 9E,Z 11-hexahydrofarnesyldrimene
(RI2768), w = an isomer of v (RI2807); z = 8α(H) 11-hexahydrofarnesyldrimane,
x and y = isomers of z.   GC conditions: OV1, 40 - 290°C at 6°Cmin⁻¹.

further spectroscopic examination (i.e. ^{1}H NMR or ^{13}C NMR). Fig. 3:43B shows the gas chromatogram of the products from the hydrogenation of the isomeric bicyclic C_{30} alkenes performed using the ambient pressure procedure (i.e. see section 2.4.3) utilised thus far throughout the synthetic studies. From a comparison with Fig. 3:43A (i.e. the precursor alkenes) it is apparent that whilst there has been a decrease in the intensity of the alkene RI2807 peak, the relative intensity of the major alkene(s) (RI2768) remains high indicating a degree of stability to normal hydrogenation. The gas chromatogram in Fig. 3:43C shows the products of high pressure hydrogenation (500 psi; 6hr) where it is obvious that the increased production of isomeric bicyclic C_{30} alkanes is associated with the decrease in the intensity of the C_{30} alkene(s) (RI2768) resistant to less forcing hydrogenation. Using argentatious tlc it proved possible to isolate the C_{30} alkene(s) remaining after hydrogenation in sufficient quantity and purity (Fig. 3:40C) to allow spectroscopic examination by ^{1}H NMR. The ^{1}H NMR spectrum of the bicyclic C_{30} alkene(s) (not shown) was very similar to that of the bicyclic C_{25} alkene (Fig. 3:41) indicating that the double bond was at the C9(C11) position. Additional examination by 2D-correlation spectroscopy (COSY) also demonstrated both C8 and C10 methyl substituents to be in the axial position (Dr. O.W. Howarth - personal communication). Thus, it would appear that the major alkene (RI2768) produced from dehydration of the bicyclic C_{30} alcohol is the C8 α (H)-epimer (36) which again illustrates the retention of the predominant configuration at C8 throughout the Grignard addition and dehydration steps. The increased resistance to hydrogenation of the 8 α (H)-epimer of the C_{30} alkene
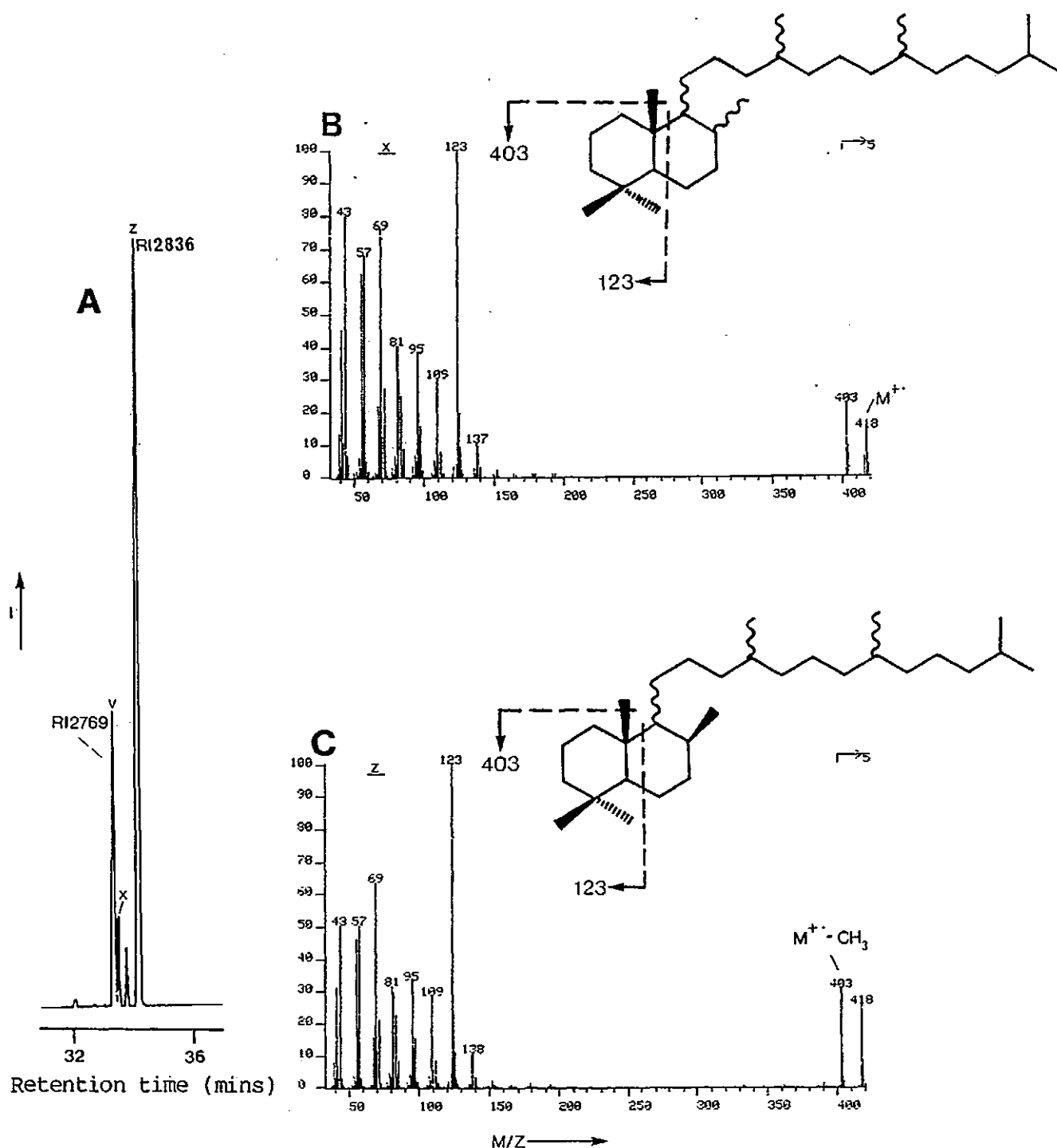
Fig.3:44   A. Gas chromatogram of the isomeric mixture of $8\alpha\beta(H)$ $9\alpha\beta(H)$
11-hexahydrofarnesyldrimanes (peak v = incompletely
separated $8\alpha(H)$ 9E,Z 11-hexahydrofarnesyldrimene), B. mass
spectrum of peak x (RI2784) and C. mass spectrum of peak z
($8\alpha(H)$ $9\alpha\beta(H)$ 11-hexahydrofarnesyldrimane; RI2836).
Gc conditions: OV1, 40 - 290°C at 6°Cmin-1.

compared to the 8 α (H)-epimer (34) of the C_{25} alkene may be the result of the elongated alkyl chain preventing easy access to the catalyst surface. The more easily hydrogenated isomers of the bicyclic C_{30} alkene (i.e. RI2762 and 2807) may represent the other positional double bond isomer [i.e. C11(C16) 38 and 39] or other configurational isomers (e.g. 8 β (H)-epimer) in which steric hindrance to hydrogenation is less significant. The position of the double bond at C9(C11) in the major 8 α (H)-epimer was confirmed from the ^{13}C NMR spectrum (not shown) which exhibited chemical shifts at 119.3069 ppm and 153.1997 ppm which can be assigned to C11 and C9 respectively (Stothers, 1972).

The gas chromatogram of the isomeric mixture of bicyclic C_{30} alkanes obtained following argentatious tlc is shown in Fig. 3:44A. The mass spectrum of the peak with RI2769 indicated that it was incompletely separated C_{30} alkene. The mass spectra of the two major (RI2784 and 2836) of the three peaks attributed to the 8 $\alpha\beta$ (H) 9 $\alpha\beta$ (H) 11-hexahydrofarnesyldrimanes (40-43) are shown in Figs. 3:44B and C. It is apparent that the spectra exhibit an analogous fragmentation pattern to that observed for the synthetic bicyclic C_{25} alkane (Fig. 3:42B), drimane and homodrimane (12 and 13; Alexander et al.; 1984). Again, as with the C_{25} alkane, the lengthened alkyl chain at C9 does not interfere with the preferential cleavage of the methyl substituent at C10. Assuming the configuration at C8 is preserved during hydrogenation then the major (81%) bicyclic C_{30} alkane can be assigned as 8 α (H) 11-hexahydrofarnesyldrimane (40 and 41): it is uncertain whether the major alkane represents one C9 epimer or both. The minor products may be attributed to the other configurational isomers at C8 and C9 (i.e. 42 and 43). The ^{13}C NMR spectrum of the isomeric mixture of bicyclic C_{30} alkanes is listed in Table 3:16.

Assignment was made by comparison with published spectra of other bicyclic $C_{14} - C_{16}$ alkanes (Voro'beva et al., 1976, Kargramanov et al., 1977; Noble, 1986) and the spectra of synthetic br30:0 (see Table 3:13B) which contains an identical C_{15} alkyl chain. Where it has not been possible to separate individual chemical shifts, these have been grouped together.

Both the mass spectra and the retention indices (RI2784 - 2836) of the $8\alpha\beta$ (H) $9\alpha\beta$ (H) 11-hexahydrofarnesyldrimanes are different to those exhibited by the sedimentary bicyclic C_{30} alkane (c30:0:2; Prahl et al., 1980). In common with the sedimentary bicyclic C_{25} alkane, the absence of an $(M^{+\cdot} - CH_3)$ ion in the mass spectrum of the c30:0:2 suggests that it has a different B-ring methyl substituent pattern to that present in $8\alpha\beta$ (H) $9\alpha\beta$ (H) 11-hexahydrofarnesyldrimane. Further discussion on the other possible carbon skeletons for the sedimentary bicyclic C_{30} alkane are included in Chapter 4.

Tetraenes related to $8\alpha\beta$ (H) $9\alpha\beta$ (H) 11-hexahydrofarnesyldrimane (20) have been reported to occur in Polypodioceous ferns (Shiojima et al)., 1983). The tetraenes (trivial name Polypodatetraenes, 45 and 46) were identified by comparison of their ^{1}H and ^{13}C NMR spectra with those of structurally similar cyclic hydrocarbons (47 and 48) of known structure. This identification was later confirmed by synthesis (Nishizawa et al., 1984). The presence of the tetraenes (or polypodatetraenes) in certain ferns may represent a possible input for hydrocarbons of structure (20) into sedimentary environments but their identification in sediments has not yet been reported. The chromatographic and spectral data presented herein for the bicyclic C_{30} alkane [8 $\alpha\beta$ (H) 9 $\alpha\beta$ (H) 11-hexahydrofarnesyldrimane or polypodane] can only help in future searches for hydrocarbons of structure (20) in sediments.

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$$(CH_3)_3SiNCH=NCH=CH$$

44

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46

47

48
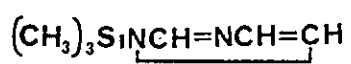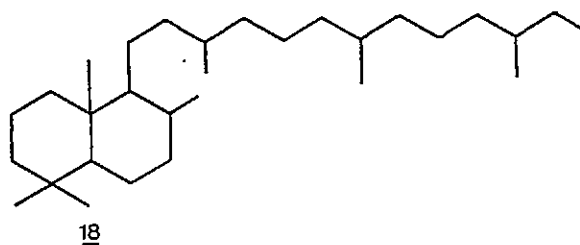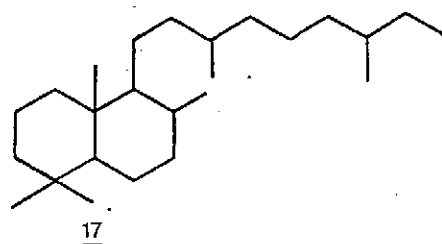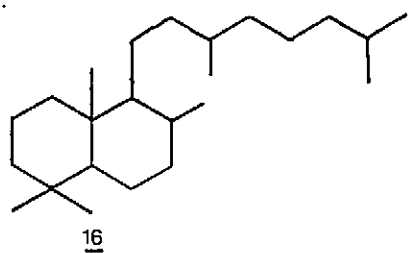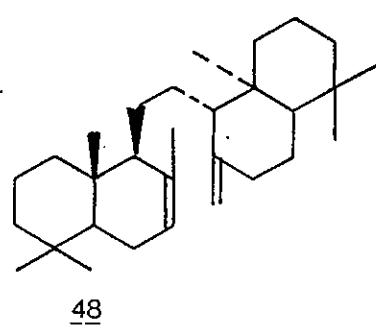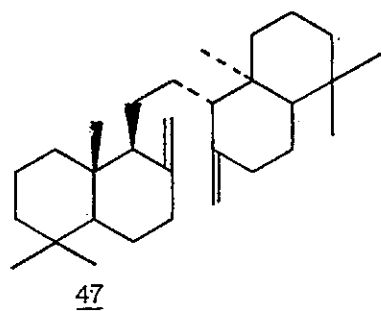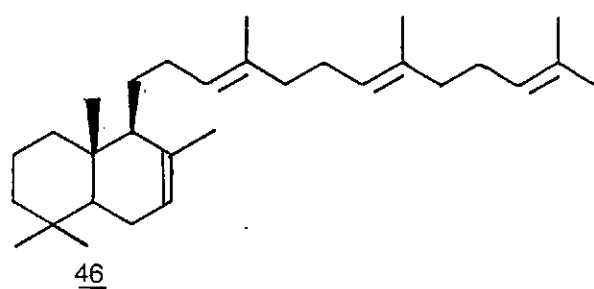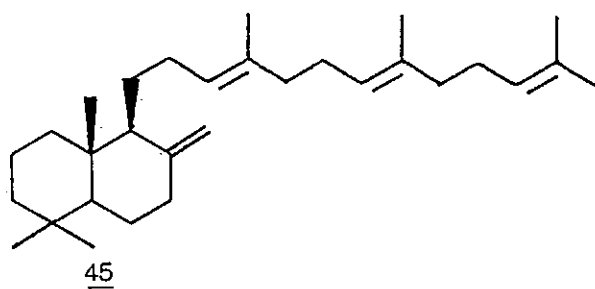
CHAPTER FOUR

INVESTIGATIONS INTO THE SEDIMENTARY

OCCURRENCES OF THE ACYCLIC AND

CYCLIC C_{20}, C_{25} AND C_{30} HYDROCARBONS

4.1 INTRODUCTION

The acyclic (br20, br25 and br30) and cyclic (C_{25} and C_{30}) hydrocarbons of interest in the present study are widely distributed (Table 4:1); this chapter details their occurrence in a further six sediments from diverse geographical locations (Fig. 4:1) and in which the compounds have not been reported previously. Use of the reference alkanes and alkenes synthesised in the present study (Chapter 3) has allowed the conclusive identification of several of the sedimentary hydrocarbons for the first time. This contrasts with most previous studies where, because of the lack of reference compounds, structures have either not been assigned or have been misassigned. Although it became apparent that the synthetic cyclic hydrocarbons (c25:0:2 and c30:0:2) prepared were not identical to the sedimentary compounds, similarities in the mass spectra have allowed further, reasoned speculation of likely structures.

4.2 SAMPLE LOCATION AND DESCRIPTION

The geographical locations of the six sediments are shown in Figs. 4:1. Sample descriptions and selected organic geochemical parameters(e.g. carbon preference index, concentration of 'aliphatic hydrocarbons' etc.) are shown in Table 4:2. The hydrocarbon assemblage of each individual sediment sample is discussed initially and followed by an overall discussion and review of the sedimentary occurrences.

Table 4:1. Occurrence of acyclic (br20, br25 and br30) and proposed cyclic (c25 and c30) hydrocarbons in sediments·

Locations	Age/Environment	Compounds					Reference
		br20	br25	br30	c25	c30	
Kiel Bight	Recent/marine		X			X	Osterroht et al., 1983
Scotian Shelf Nova Scotia	Recent/marine		X				Keizer et al., 1978
Shark Bay Western Australia	Recent/marine/ hypersaline	X	X				Dunlop and Jefferies, 1985
Alaskan Outer Continental shelf	Recent/marine		X			X	Venkatesan and Kaplan, 1982
Peru Continental shelf	Recent/marine		X				Volkman et al., 1983; Smith et al., 1983;
Alfacs Bay, Ebro River, Spain	Recent/marine	X	X		X		Bayona et al., 1983; Albaïges et al., 1984b
Dabob Bay, Washington State, U.S.A.	Recent/land-locked		X			X	Prahl et al., 1980
NE Gulf of Mexico	Recent/marine	X	X		X		Gearing et al., 1976
Southern California, U.S.A.	Recent/marine		X		X	X	Crisp et al., 1979
Rhode Island Sound, U.S.A.	Recent/estuarine		X		X		Boehm and Quinn, 1978
Buzzards Bay, Mass., U.S.A.	Recent/estuarine		X		X		Farrington et al., 1977
Narrangansett Bay, Rhode Is., U.S.A.	Recent/estuarine		X		X	X	Requego and Quinn, 1983a, 1985
Puget Sound, Washington State, U.S.A.	Recent/land-locked marine	X	X	X	X	X	Barrick et al., 1980; Barrick and Hedges, 1981
Sandyhaven, Dyfed, Wales	Recent/intertidal	X	X				Rowland et al., 1985
Grasmere, Cumbria, U.K.	Recent/freshwater lake	X	X				Yon, 1982
Camloch, Scotland	Recent/freshwater lake	X					Cranwell, 1982
Upton Broad, Norfolk, U.K.	Recent/freshwater lake	X					Cranwell, 1982
Rostherne Mere, Cheshire, U.K.	Recent/freshwater lake	X	X				Rowland et al., 1985
Cariaco Trench Venezuela	Pleistocene/marine	X					Yon, 1982
Sabon-Gida, Nigeria	Pleistocene/ lacustrine	X	X				Yon, 1982
Pettaquamscutt River, Rhode Is. U.S.A.	Recent/estuarine	X	X			X	Requego et al., 1984
Sullom Voe, Shetland Is., U.K.	Recent/intertidal	X	X				Jones, 1986
Los Monegros, Spain	Recent/hypersaline		X				Albaïges et al., 1984a
Maoming oil shale, China	Eocene/lacustrine			X			Brassell et al., 1986a
California Continental Borderland, U.S.A.	Recent/marine		X				McEvoy et al., 1981

(Adapted from Robson and Rowland, 1986)
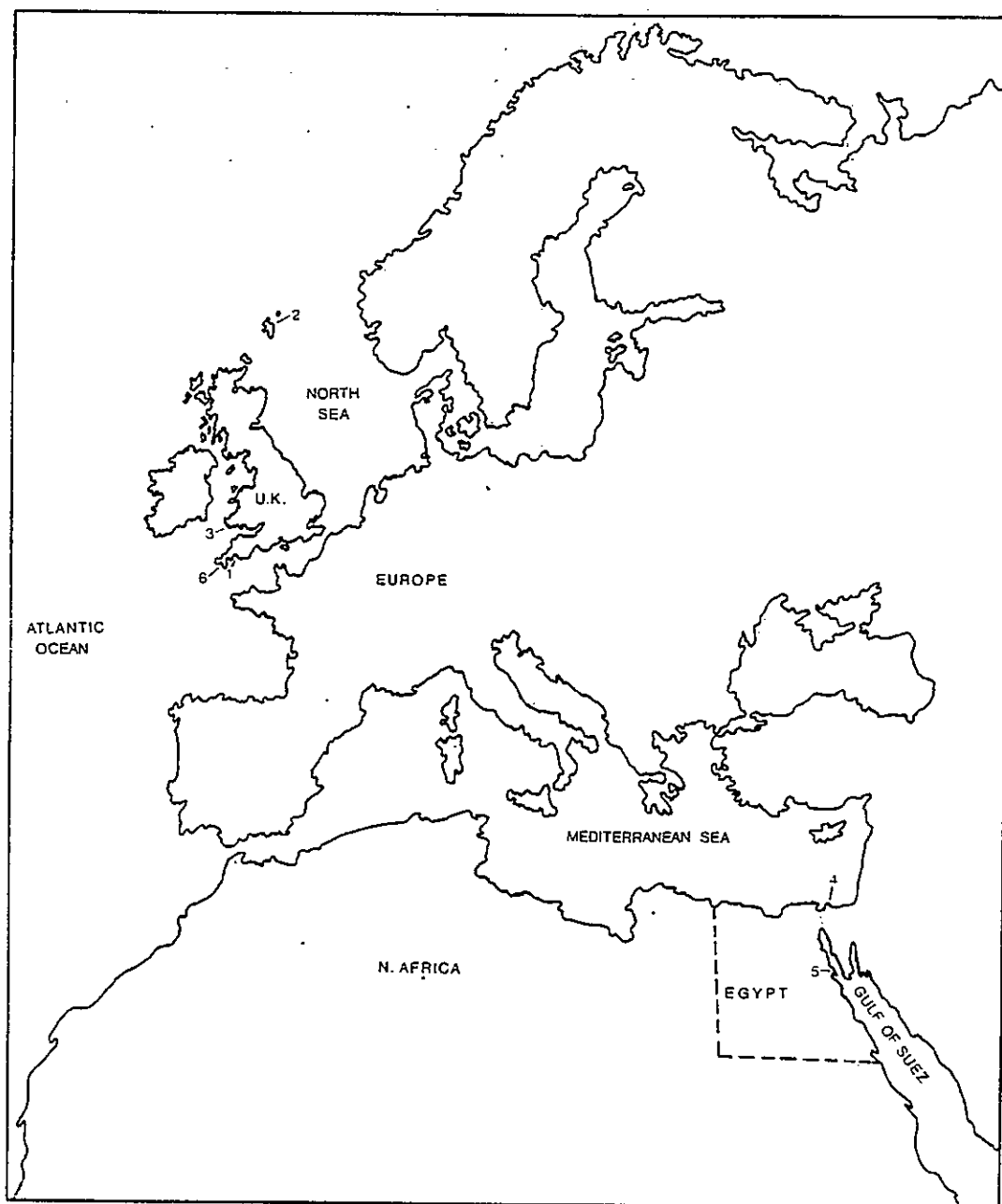
Fig.4:1 Map of Europe and Northern Africa illustrating the variation in the geographical location of the six sediments selected by this study for hydrocarbon analyses. Notations: 1 = Tamar Estuary, England; 2 = Gluss Voe, Shetland Isles; 3 = Milford Haven, Wales; 4 = Bardawil lagoon, Sinai; 5 = Gesium, Egypt; 6 = Loe Pool, England.

Table 4:2. Physical and chemical properties of the six sediment samples.

Sediment Sample Site	Environment (all Recent sediments)	Lithology	Total Organic Extract (μgg^{-1} dry sediment)	Total Aliphatic Hydrocarbons (μgg^{-1} dry sediment)	normal alkanes (% total aliphatic hydrocarbons[a])	acyclic and cyclic C_{20}, C_{25} and C_{30} compounds (% total aliphatic hydrocarbons[a])	Carbon preference Index (CPI)	
							C_{18-34}	C_{18-24}
Tamar (6A)	Intertidal Estuarine	Mud (organic rich)	2200	230	21.38	2.56	2.58	0.81
Loe Pool	Fresh water/ lacustrine	Mud (organic poor)	– –	– –	data not available		– –	– –
Gluss Voe	Intertidal	Mud/shells	2600	382	93.14	1.40	9.01	1.80
Bar 10	Lagoonally marine	Sand	166	13.3	61.70	12.18	1.18	1.46
G06	Marine	Sand/shells	756	58	15.13	3.02	1.08	1.01
M73	Intertidal	Sand/mud	767	55	22.50	10.61	1.49	1.29

[a]: calculated via gas chromatographic integration data.

4.3   SEDIMENT

4.3.1 Tamar Estuary U.K. (6AH)

The sample location is shown in Fig. 4:2A and Fig. 4:3 shows the gas chromatogram of the 'aliphatic hydrocarbon' extract. This is similar to that presented by Readman et al. (1986) for the hydrocarbon extract of an intertidal sediment from the Tamar Estuary. The chromatogram is dominated by high molecular weight n-alkanes (e.g. n-C_{27}, n-C_{29}, n-C_{31}) with a high odd-over-even carbon number preference (see Table 4:2) characteristic of a natural higher plant input (Eglinton and Hamilton, 1963; Thompson and Eglinton, 1978). The presence of a slight unresolved complex mixture (UCM) suggests contamination of the sediment by petroleum hydrocarbons (Brassell et al., 1978, Brassell and Eglinton, 1980). Further evidence for the presence of petroleum hydrocarbons is provided by examination of the m/z 191 mass fragmentogram. The m/z 191 ion is a major fragment in the mass spectra of hopanes (see Fig. 4:4). The hopanes present in unpolluted marine sediments are predominantly the 17β(H) 21β(H) hopanes and the C_{31} 17α (H) 21β(H) hopane with 22R stereo chemistry. In contrast, the hopanes of petroleum are principally the more thermodynamically stable 17α(H) 21β(H) hopanes with a 60:40 mixture of the 22S:22R diastereoisomers in compounds C_{31} and above (Mackenzie et al., 1980). The presence of extended C_{32} - C_{35} 17α(H) 21β(H) hopane (22S and 22R) doublets in Recent sediments is therefore considered indicative of petroleum contamination (Dastillung and Albrecht, 1976). The m/z 191 mass fragmentogram of the 6AH sediment extract (Fig. 4:5) is clearly dominated by the 17α(H) 21β(H) hopanes with C22S and C22R doublets in compounds > C_{31} and above.
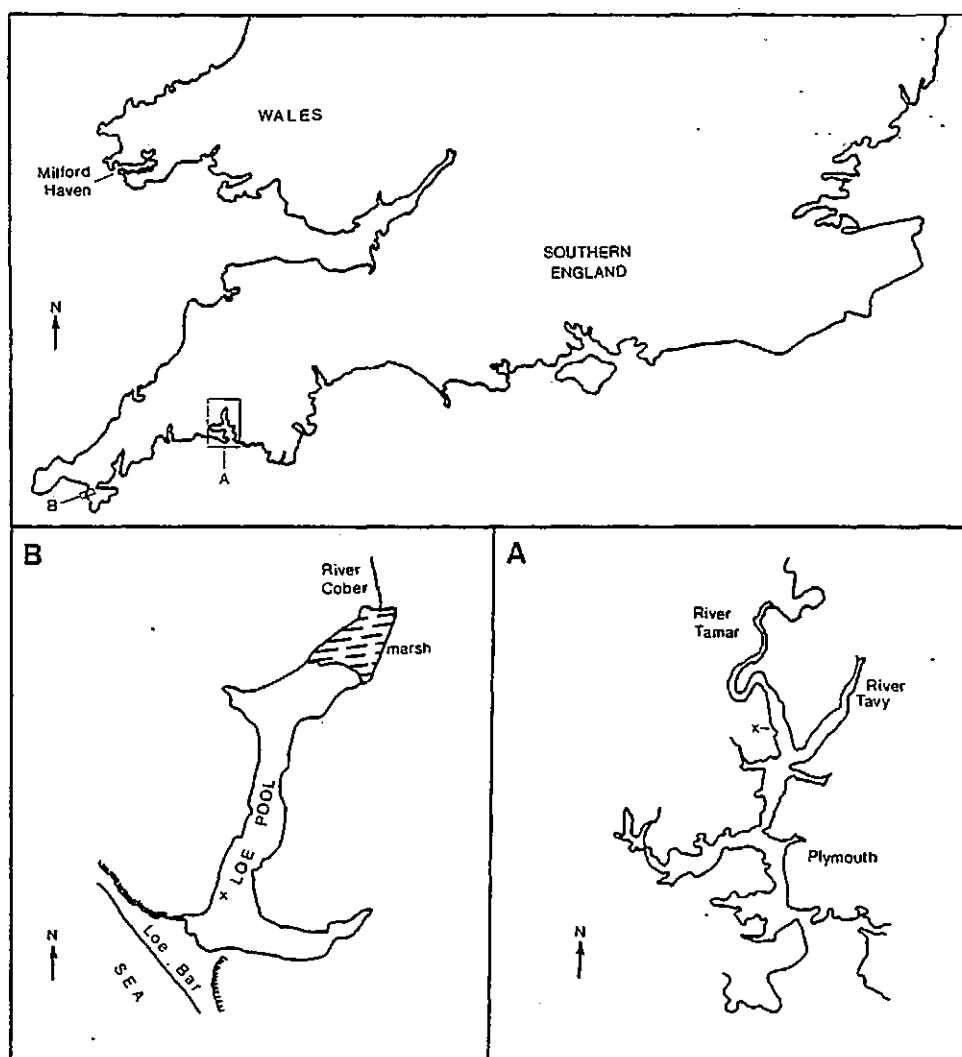
Fig.4:2 Map of Southern England and wales showing the relative locations of Milford Haven,
         Tamar Estuary and Loe Pool. Maps A (adapted from Readman et al.1982) and B
         (adapted from O'Sullivan et al. 1982) show in greater detail the sampling areas at
         the Tamar Estuary and Loe Pool respectively. The actual site of sediment collection
         is marked with a X.
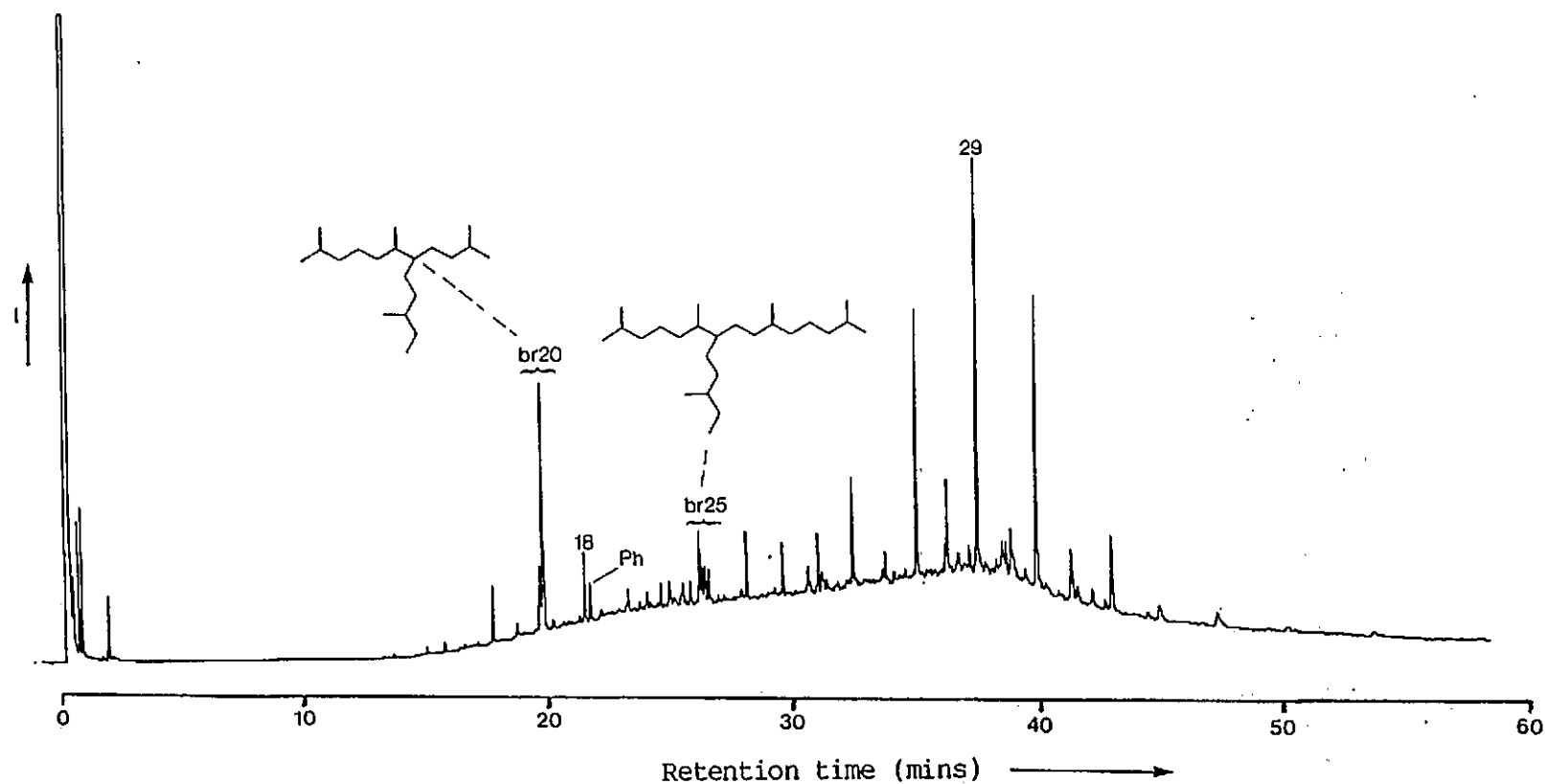
Fig.4:3   Gas chromatogram of the aliphatic hydrocarbons isolated from Tamar. Numbers refer to
the carbon chain length for the n-alkanes. Peaks labelled br20 and br25 represent
hydrocarbons with the carbon skeleton of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane
and 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane respectively. For
conditions see text.
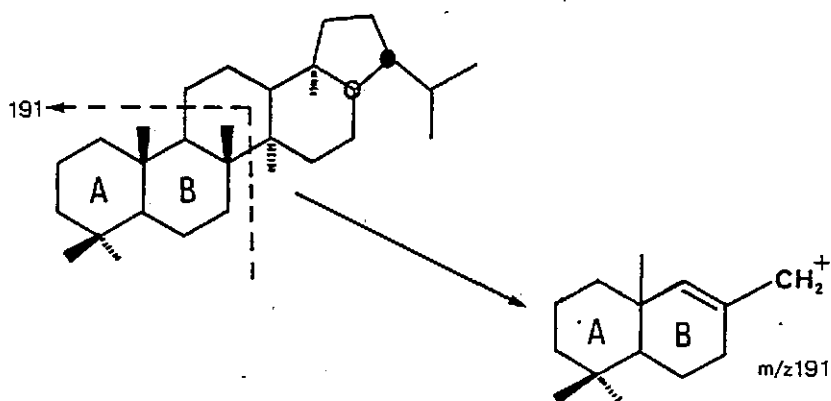
Fig.4:4   Mass spectral fragmentation of 17α(H) 21β(H)-hopane.
   (After Fowler, 1984).
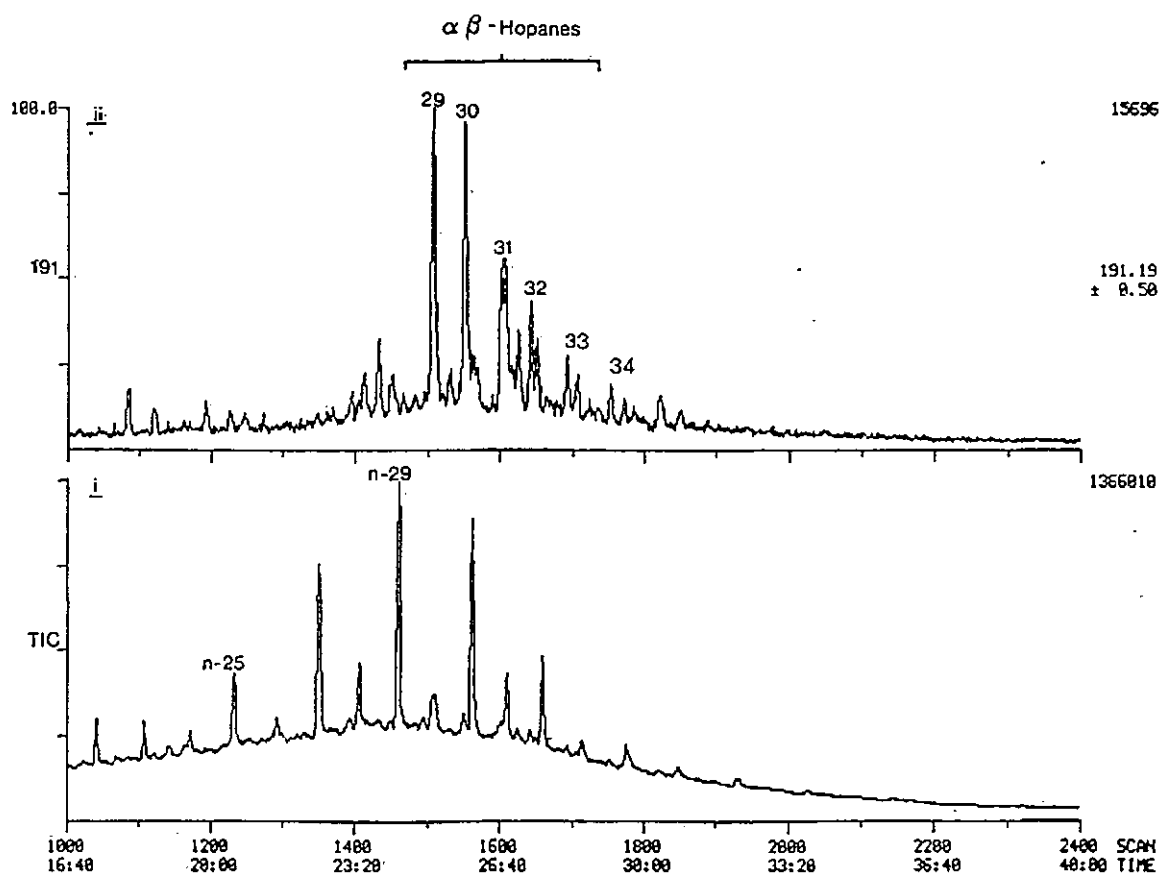
Fig.4:5   i Partial Total Ion Chromatogram (TIC) and ii m/z 191 mass
          fragmentogram of the aliphatic hydrocarbon extract of
          Tamar Estuary sediment.   n-29 = normal - C_{29} alkane.

The chromatographic peaks corresponding to the C_{20}, C_{25} and C_{30} hydrocarbons of significance to this study elute between RI1600 and 2600 (Fig. 4:6A) with the major peaks of interest at RI1695-1710 and RI 2000-2200. A gas chromatogram of the hydrogenated Tamar sediment hydrocarbons is shown in Fig. 4:6B. It is evident that the doublet at RI1700-1702 in Fig. 4:6A is removed on hydrogenation leaving only a singlet at RI1700 (Fig. 4:6B). The increase in the RI1707H peak suggests a conversion of RI1702 to 1707H. RI1707 and RI1707H have similar mass spectra (Fig. 4:7A) to synthetic 2,6,10-trimethyl-7-(3-methylbutyl) dodecane (br20:0:0; 66) (Fig. 4:7B) and an identical RI value on two different stationary phases (OV1 and CPWAX52). The mass spectrum of RI 1702 (Fig. 4:9A) contains a molecular ion at m/z 280 and characteristic fragment ions at m/z 126, 196 and 210 and is proposed to be a C_{20} monoene (i.e. a br20:1;1702). The retention index of br20:1;1702 is different to that of the available synthetic br20:1 (77) suggesting that the double bond is in a different position. A hydrocarbon with an identical mass spectrum and retention index to RI1702 has been reported in numerous sediments (see Table 1:7; Barrick et al., 1980; Dunlop and Jefferies, 1985; Rowland et al., 1985) and Dunlop and Jefferies (1985) used ozonolysis to suggest that the position of the double bond is as in structure (73). The shoulder peak on br20:0;1707 was shown to be pristane (RI1709) by co-chromatography.

Further examination of Fig. 4:6A and B indicates that the removal of peaks (RI2076, 2083 and 2091; Fig. 4:6A) by hydrogenation (Fig. 4:6B) is accompanied by an increase in the intensity of RI2107H suggesting that RI2076, 2083 and 2091 are hydrogenated to RI2107H. The mass spectrum of RI2107H (Fig. 4:8A) was very similar to that of synthetic 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane   (br25:0;   70)
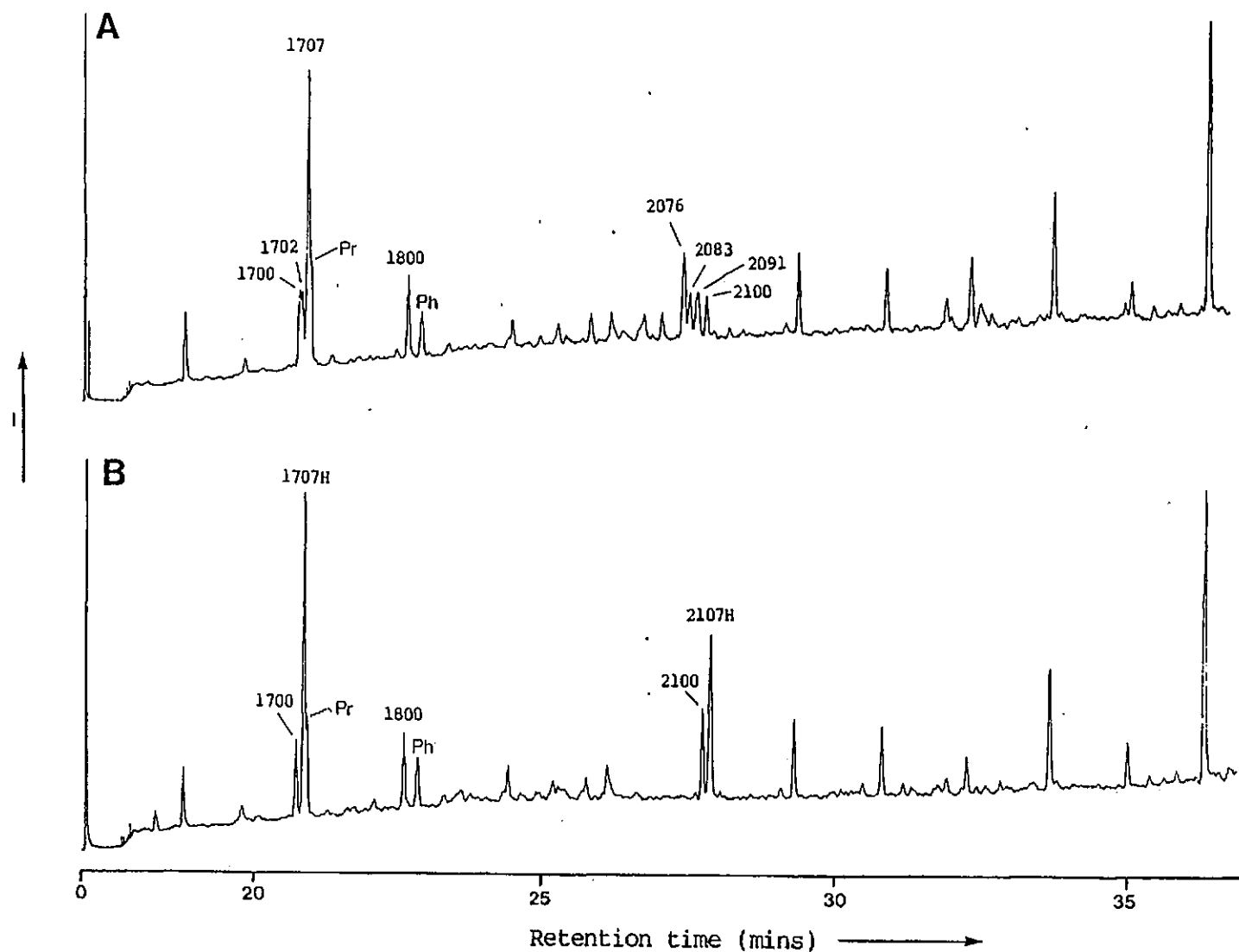
Fig.4:6 Partial gas chromatograms of A. the aliphatic hydrocarbons and B. the
hydrogenated aliphatic hydrocarbons from Tamar. Numbers refer to
Retention indices (Table 4:3). Pr = pristane and Ph = phytane.
For conditions see text
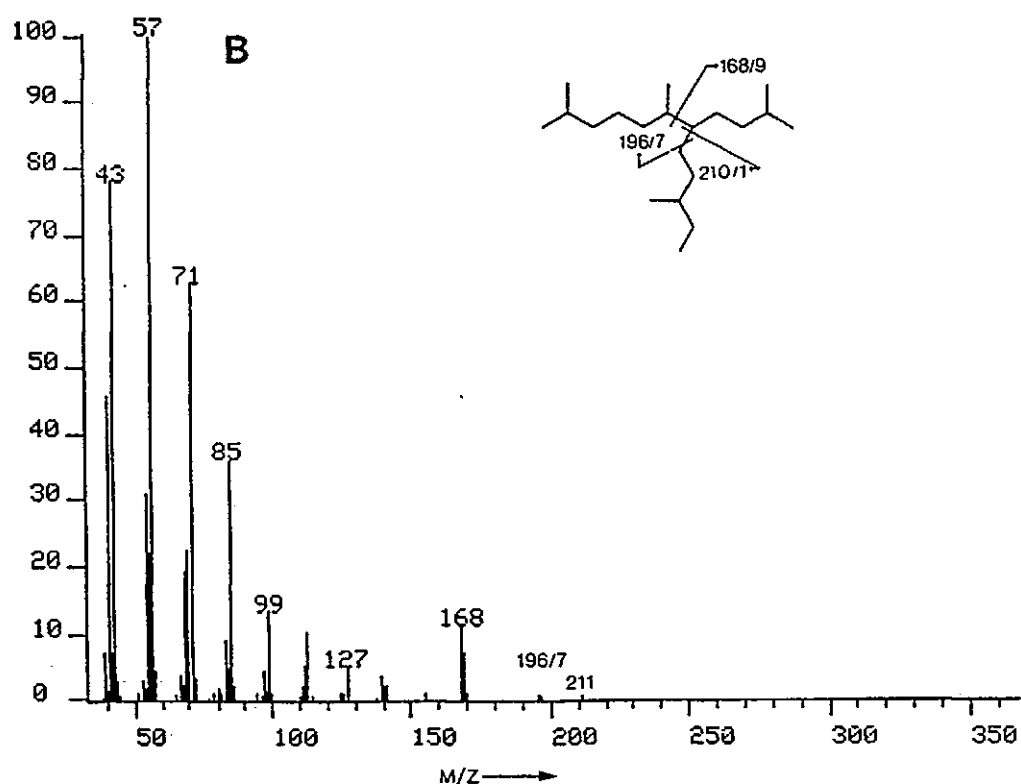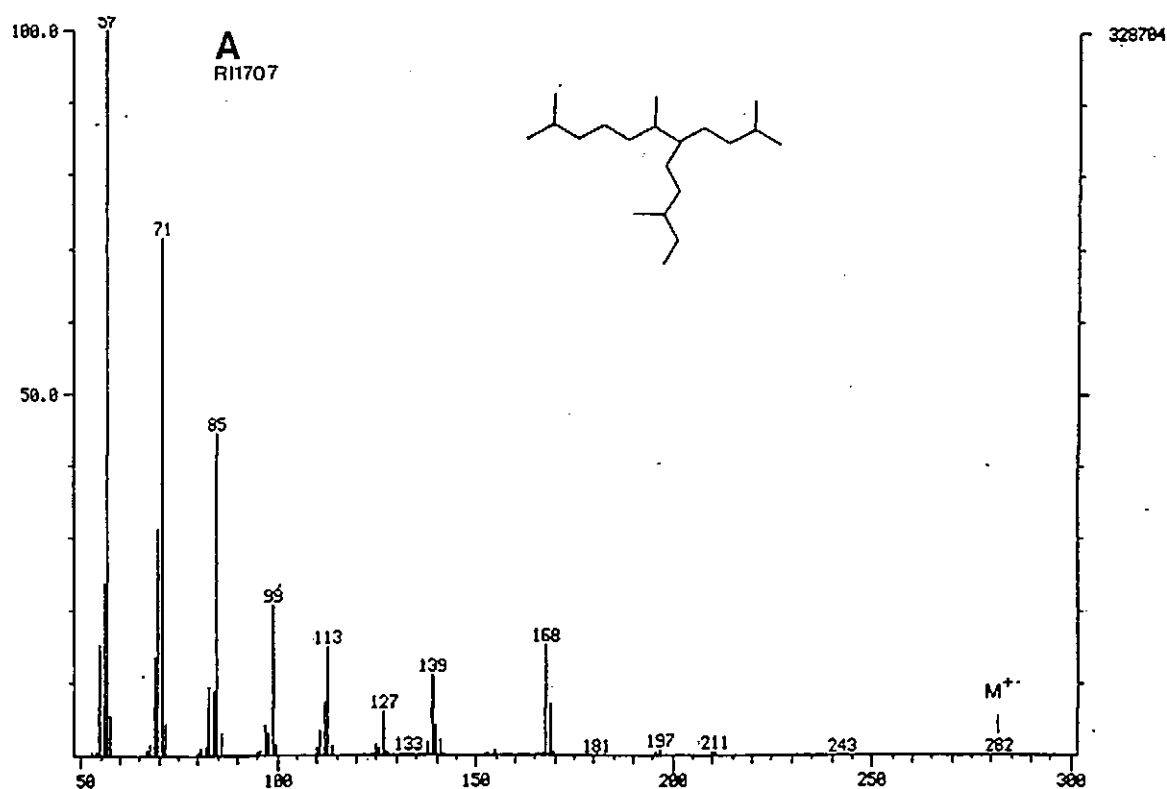
Fig.4:7   Mass spectra of A. RI1707 isolated from Tamar sediment and
B. synthetic 2,6,10-trimethyl-7-(3-methylbutyl)dodecane
(br20:0). Spectrum A recorded on a Finnigan 4000 and B. on
a Kratos MS25 (see Chapter 2).
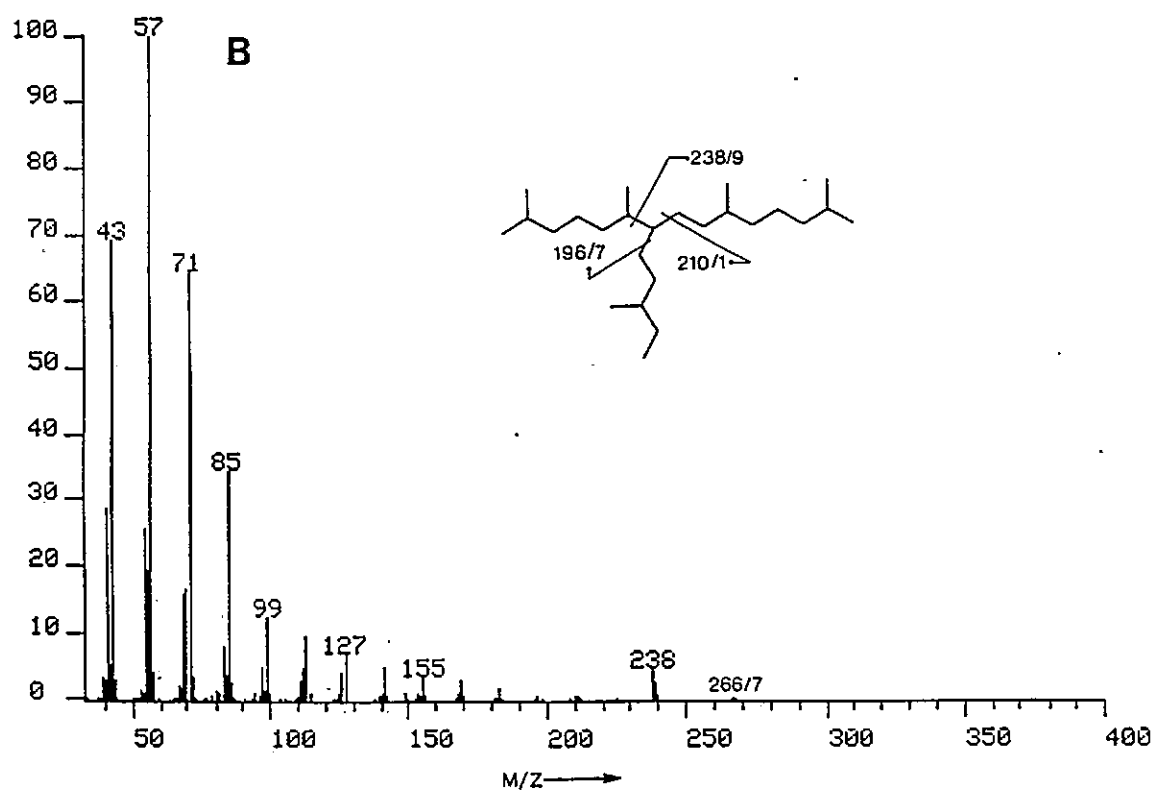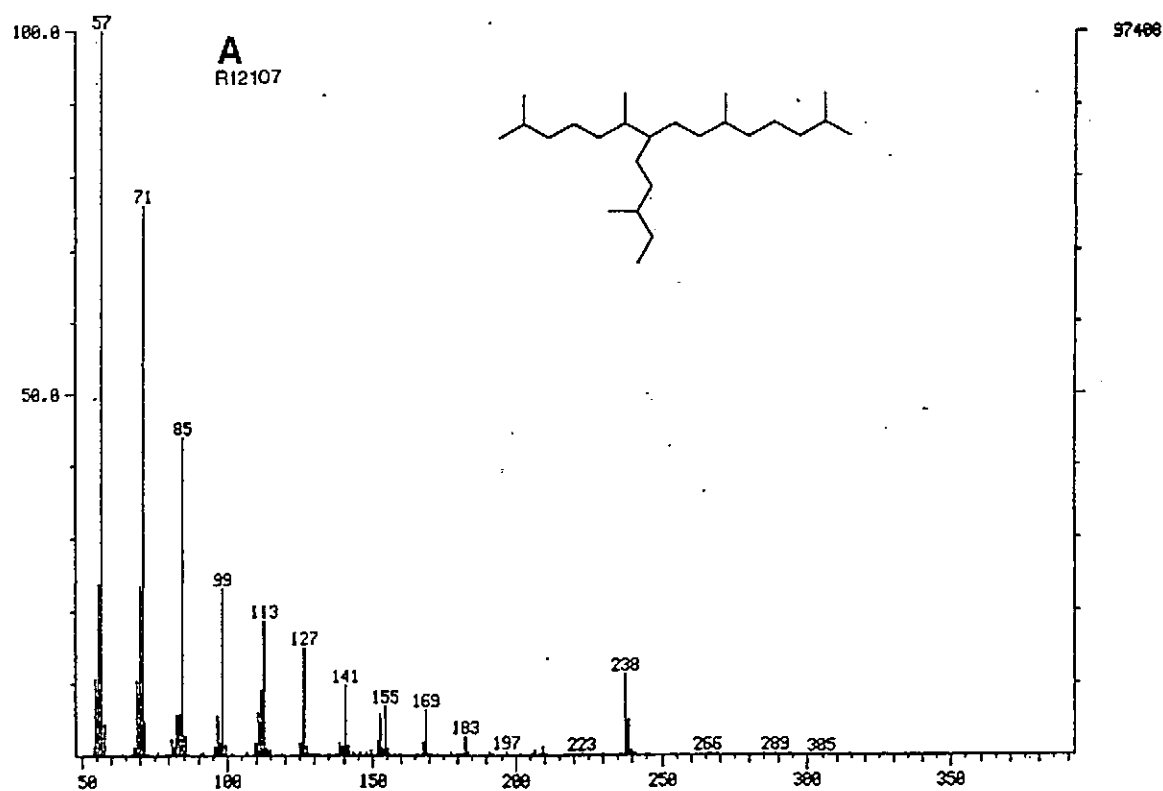
Fig.4:8   Mass spectra of A. RI2107 isolated from Tamar sediment and
          B. synthetic 2,6,10,14-tetramethyl-7-(3-methylpentyl)
          pentadecane (br25:0). Spectrum A recorded on a Finnigan 4000
          and B. on a Kratos MS25 (see Chapter 2).

(Fig. 4:8B) and co-chromatographed with it on two different gc phases (OV1 and CPWAX52). The mass spectrum of RI2083 (Fig. 4:9B) had a suspected molecular ion at m/z 348 suggesting that it was a C_{25} diene (br25:2;2083). A hydrocarbon of unknown structure with an almost identical mass spectrum and retention index was reported by Requego and Quinn (1983a) and by several other investigators (Table 1:7). Whilst the exact positions of the two double bonds are unknown, the hydrogenation data shows that the carbon skeleton of br25:2;2083 is that of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; 70). The mass spectra (Figs. 4:10A and 4:11A) and retention indices of RI2076 and 2091 were almost identical to those of two synthetic 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecenes (78; Figs. 4:10B and 4:11B) where the position of the double bond is in either the C6(C7), C7(C8) or C7(20) positions. Since the mass spectra of RI2076 and 2091 are different to each other the monoenes are clearly not cis-trans but positional isomers. Further examination of the mass spectrum of br25:1;2091 (Fig. 4:11A) revealed the presence of minor ions at m/z 231, 247 and 259. These ions are major fragments in the mass spectrum of a bicyclic C_{25} diene (c25:2:2) reported by Requego and Quinn (1983a) and other investigators (i.e. Farrington et al., 1977) suggesting that a trace of c25:2:2 was present in Tamar sediment. However, it is interesting to note that the c25:2:2 does not appear to produce a peak at RI2124 upon hydrogenation as reported by Requego and Quinn (1983a) (see later).

The identification and concentrations of various acyclic and cyclic C_{20} and C_{25} hydrocarbons in Tamar sediment is summarised in Table 4:3.
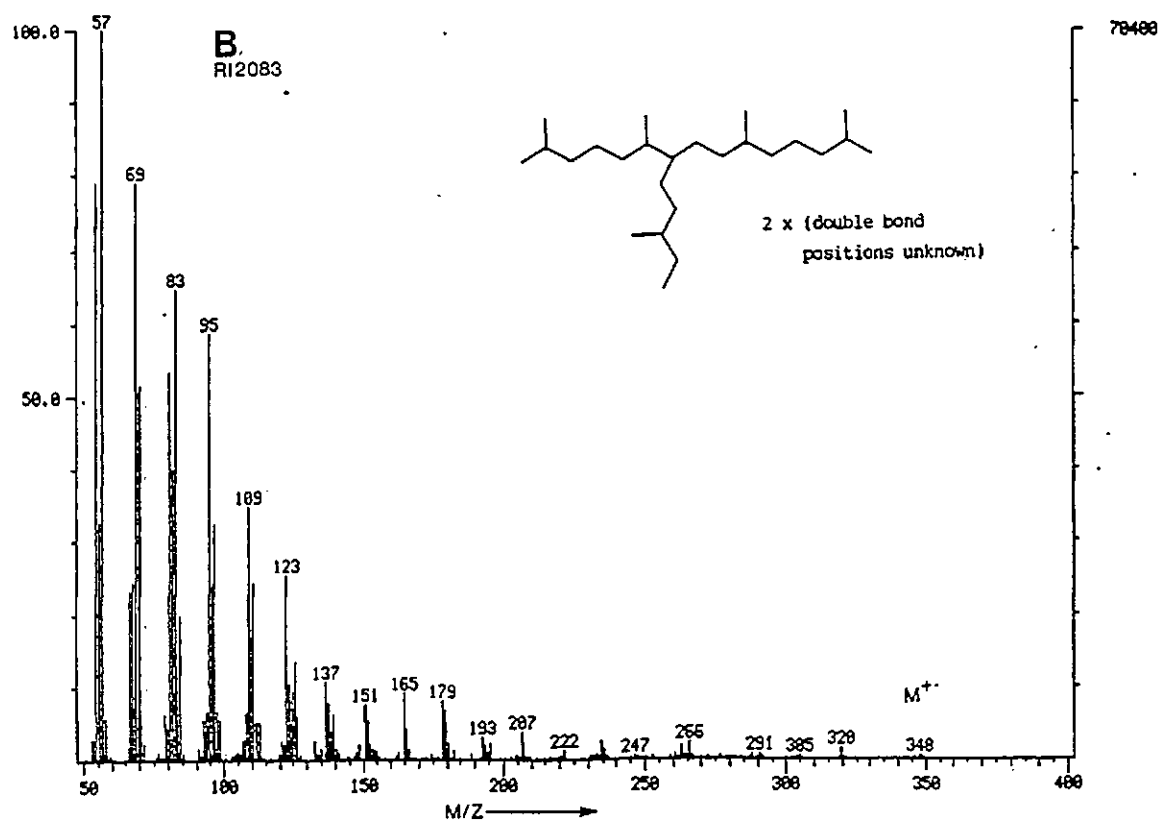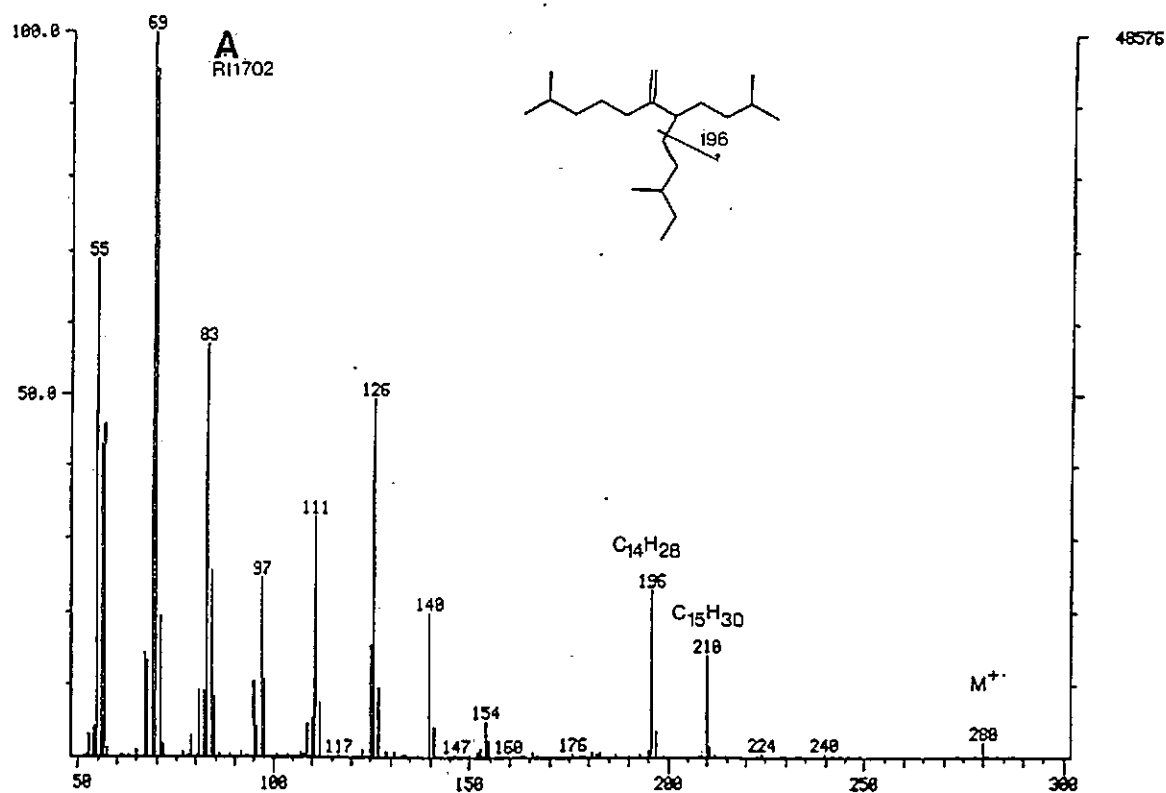
Fig.4:9   Mass spectra of A. RI1702 (br20:1) and B. RI2083 (br25:2)
           isolated from Tamar sediment. Proposed structures as
           indicated.
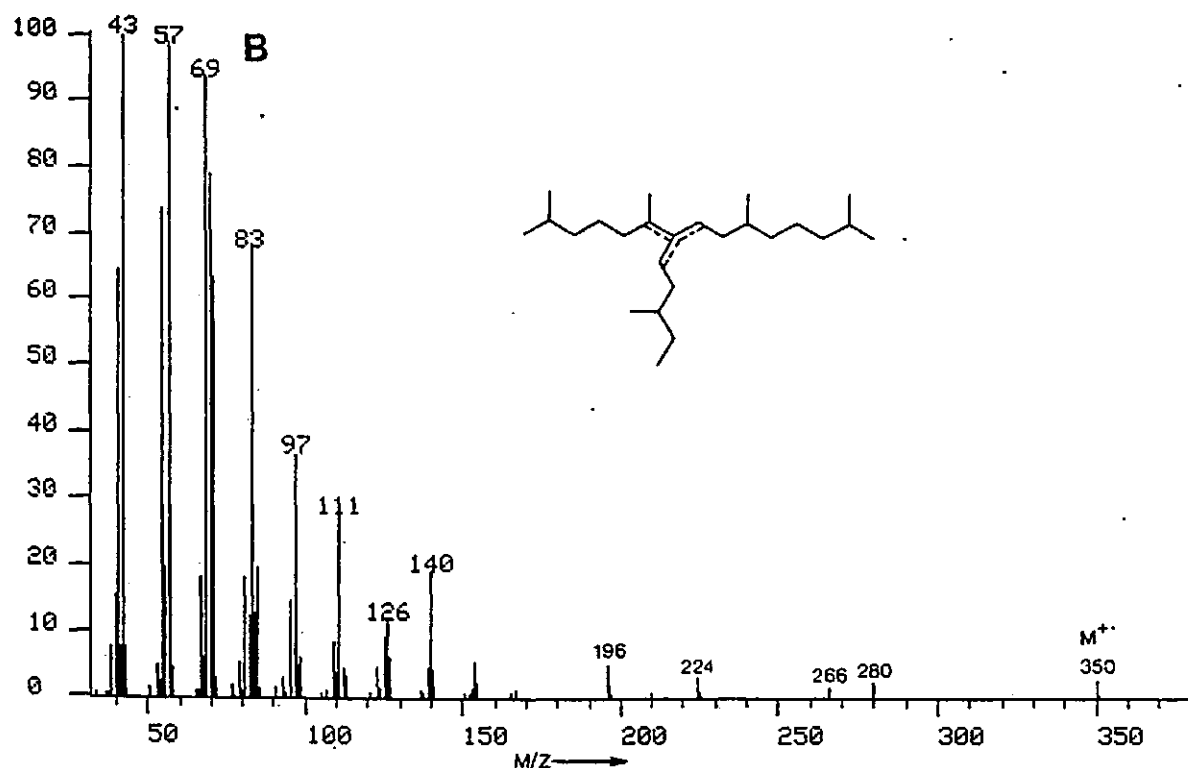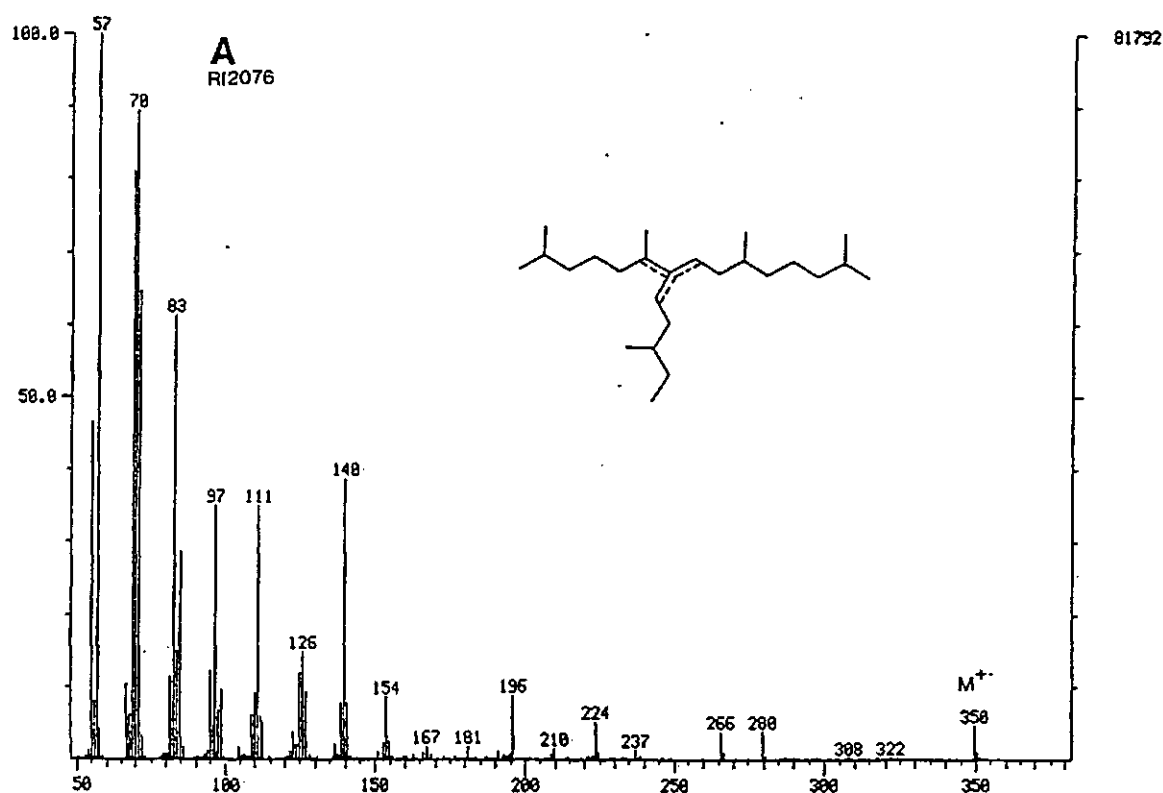
Fig.4:10   Mass spectra of A. RI2076 isolated from Tamar sediment and
           B. a synthetic 2,6,10,14-tetramethyl-7-(3-methylpentyl)
           pentadecene (br25:1; RI2078).   Spectrum A recorded on a
           Finnigan 4000 and B on a Kratos MS25 (see Chapter 2).
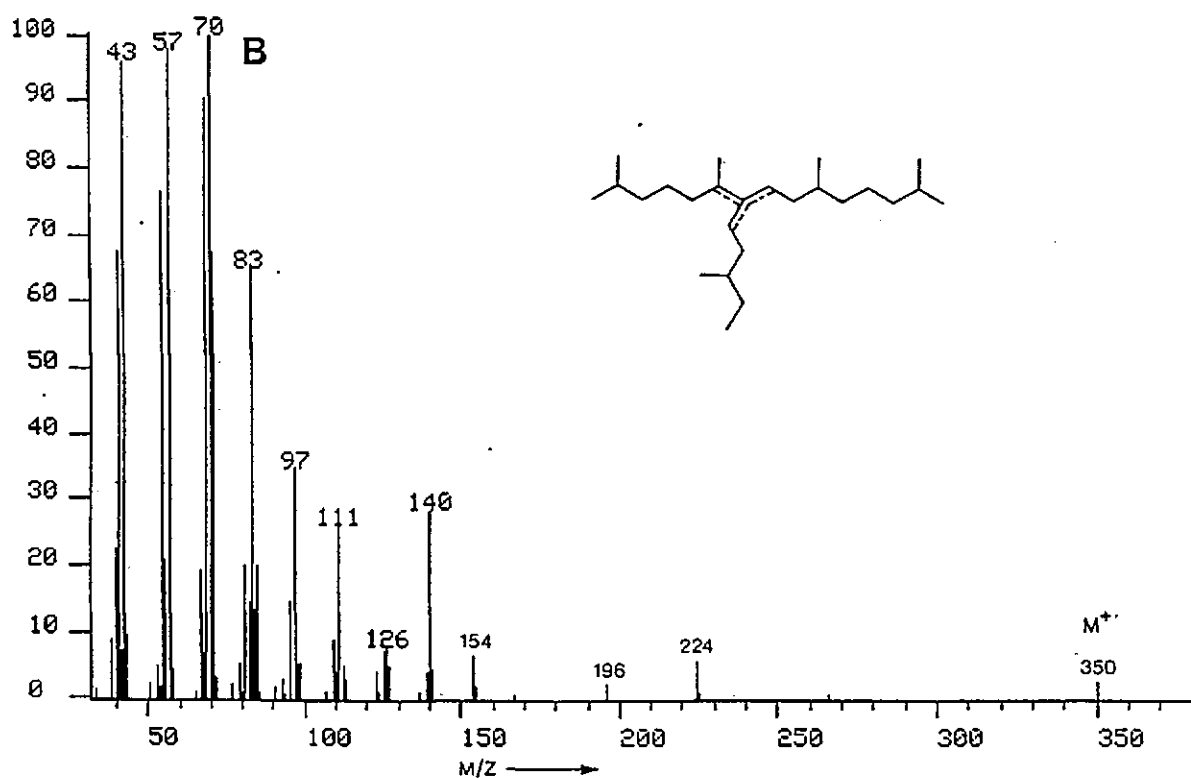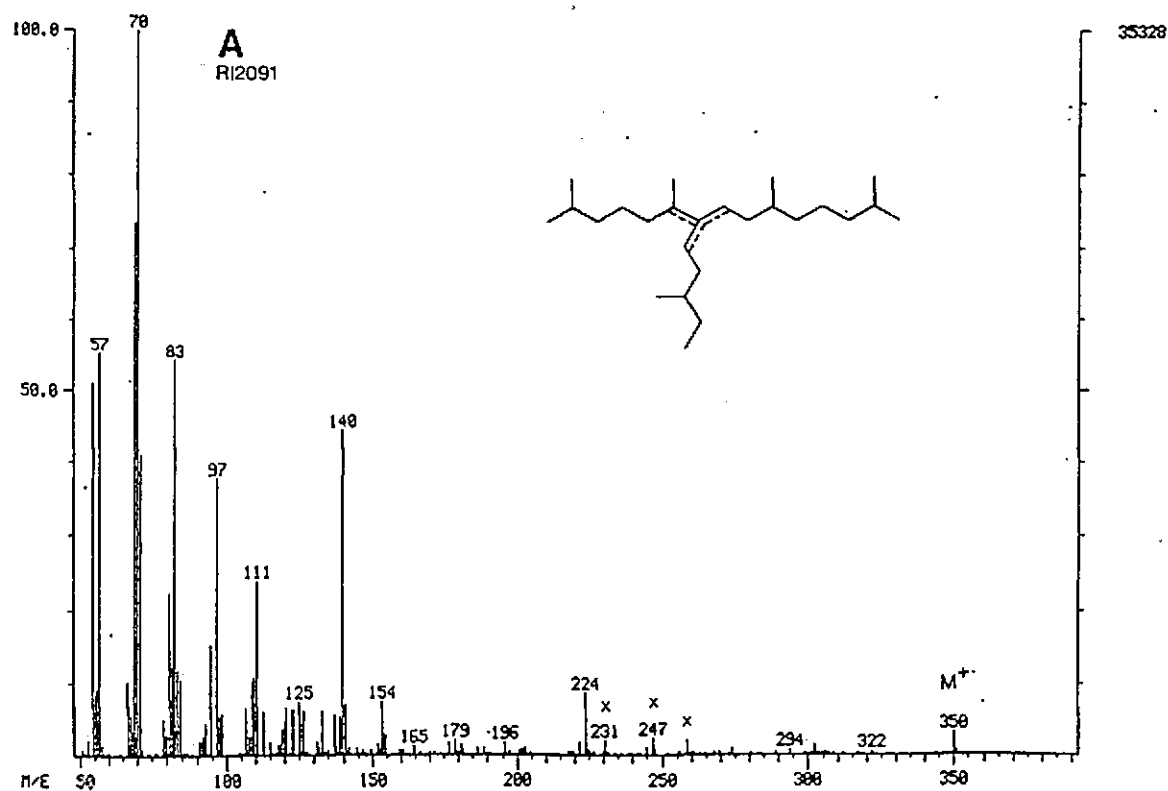
Fig.4:11   Mass spectra of A. RI2091 isolated from Tamar sediment and
            B. a synthetic 2,6,10,14-tetramethyl-7-(3-methylpentyl)
            pentadecene (br25:1; RI2092). x = minor contribution
            from co-chromatographing c25:2:2 (see text). Spectrum A
            recorded on a Finnigan 4000 and B on a Kratos MS25 (see
            Chapter 2).

Table 4:3. Summary of the identification of acyclic and cyclic C_{20}, C_{25} and C_{30} hydrocarbons in the six sediments examined in this study.

RETENTION INDEX (concentration in μgg^{-1} dry sediment)

Identification (Structure where known)	Tamar	Gluss Voe	Milford Haven	Bardawil	Gesium	Loe Pool
br20:0[a,b] (66)	1707 (2.23)	1708 (0.28)	1707 (1.08)	1708 (0.36)	1707 (0.22)	1707
br20:1[c]		1696 (0.11)		1696 (0.11)	1696 (0.10)	
br20:1[c,d] (73)	1702 (0.51)	1702 (0.23)	1702 (1.03)		1702 (0.22)	1702
br25:0[a,b] (70)			2107 (0.56)		2107 (0.39)[h]	2107
br25:1[a,b] (77)	2076 (1.26)					
br25:1[a,b] (77)	2091 (1.15)					
br25:1[c]			2100 (0.58)			
br25:1[c,d]		2108 (1.11)[h]			2106 (0.39)[h]	
br25:2[c,d]		2070 (0.80)				
br25:2[c,d]	2083 (0.73)				2084 (0.81)	2084
br25:3[c,d]		2044 (0.65)		2044 (0.61)	2044 (0.12)	
br25:3[c,d]		2091 (1.33)	2091 (0.82)	2091 (0.29)	2090 (0.08)	2091
br25:3[c,d]		2108 (1.11)[h]				
br25:4[c,d]		2078 (0.62)		2078 (0.14)	2078 (0. 2)	
br25:4[c,d,e]			2083 (0.57)			
br30:0[a,b] (75)		2523[g]	2523 (0.31)			
c30:2:2[c,d]		2499 (0.13)				
C_{25}Diene[c,d,f,]			2139 (1.67)			
C_{25}Alkane[c,d,f,g]			2154			

[a]: Gc co-injection with synthetic compound

[b]: Comparison of mass spectrum with synthetic compound

[c]: Interpretation of mass spectrum and hydrogenation to synthetic compound

[d]: Comparison of mass spectrum with literature data

[e]: Hydrocarbon displayed identical mass spectrum to compound designated as c25:2:2;2097 (Requego and Quinn, 1983a; see text)

[f]: see text

[g]: Hydrocarbon produced upon hydrogenation of aliphatic hydrocarbon extract

[h]: Summed concentration as individual hydrocarbons co-chromatographed

4.3.2 Gluss Voe U.K. (GV1)

The location of the sampling site in Gluss Voe (Shetland Isles) is shown in Fig. 4:12. Fig. 4:13 shows a gas chromatogram of the 'aliphatic hydrocarbon' extract which is dominated by higher-plant derived n-alkanes with a high odd/even predominance (see Table 4:2; Thompson and Eglinton, 1978). The absence of a pronounced UCM and $C_{32} - C_{35}$ 17 α (H) 21 β (H) hopane (22R + 22S) doublets in the m/z 191 mass fragmentogram suggests minimal contamination from petroleum hydrocarbons relative to biological hydrocarbons (Brassell et al., 1978). The distribution of n-alkanes and hopanes is similar to that reported for other unpolluted sediments in the Shetland Isles by Jones (1986).

The expanded RI1600 - 2700 chromatogram is shown in Fig. 4:14A whilst Fig. 4:14B displays that of the hydrogenated extract. The peaks at RI 1708H and 2108H in Fig. 4:14B had mass spectra and retention indices identical to those of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (br20:0; 66) and 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; 70) respectively. RI2523H had an identical mass spectrum to synthetic 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane (br30:0; 75) and co-chromatographed with it (OV1 and CPWAX52). Peaks at RI1690, 1696 and 1702 in Fig. 4:14A were reduced upon hydrogenation and exhibited mass spectra similar to n-$C_{17:1}$, br20:1;1696 and br20:1;1702 respectively. The mass spectrum (Fig. 4:15A) of br20:1; 1696 has not previously been presented in the literature but mention of a hydrocarbon with similar retention index was made by Barrick et al. (1980); br20:1;1702 was present in Tamar Estuary sediment and the shoulder peak to RI1708 (br20:0; 66) was pristane. The reduction in
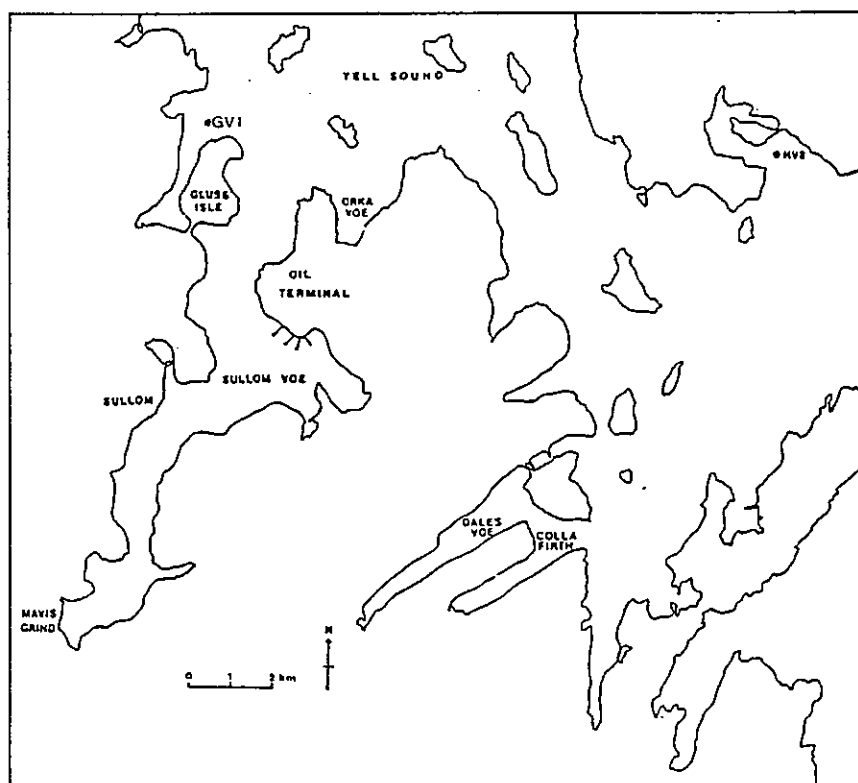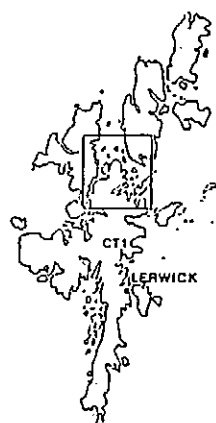
Fig.4:12   Map of Shetland Isles showing location of sampling
            site GV1.   (After Jones, 1986).
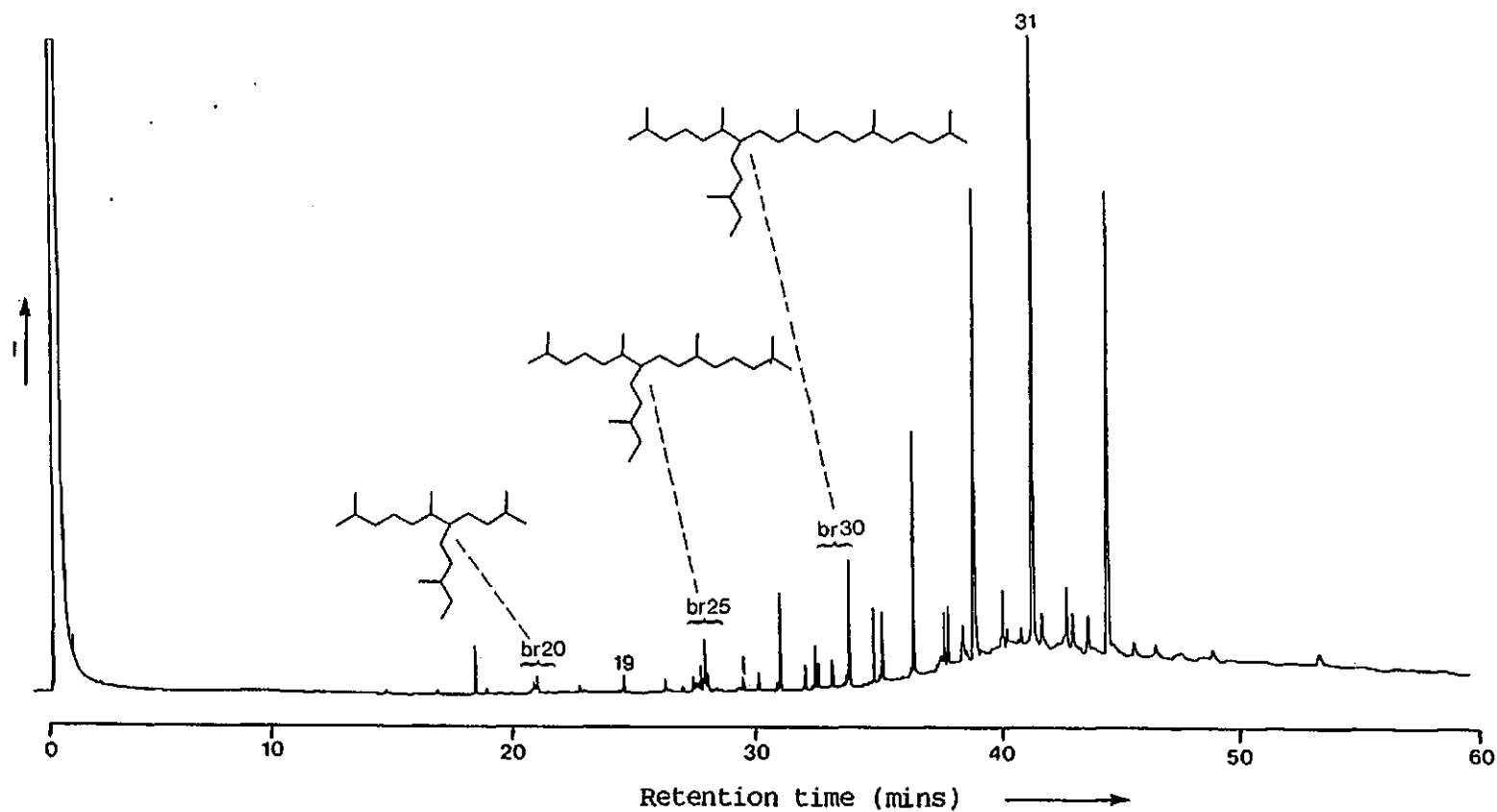
Fig.4:13   Gas chromatogram of the aliphatic hydrocarbons isolated from Gluss Voe. Numbers refer
to the carbon chain length for the n-alkanes. Peaks labelled br30 represent hydrocarbons
with the carbon skeleton of 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane.
For conditions see text.
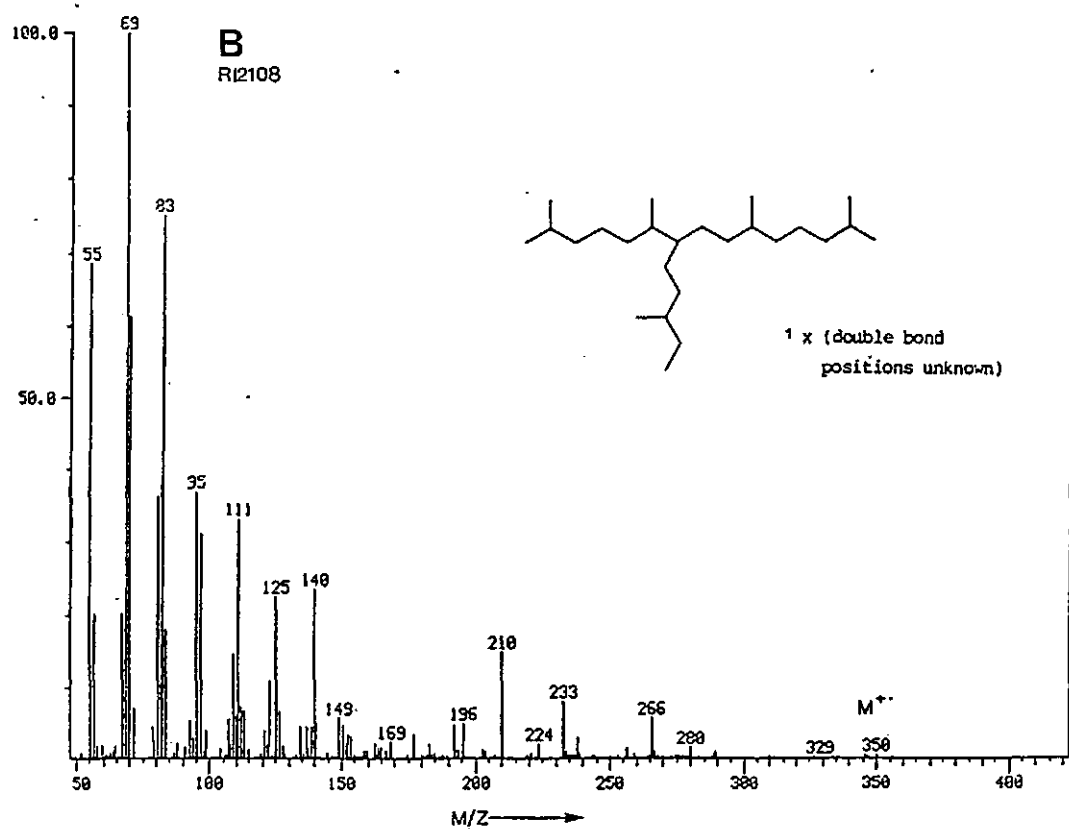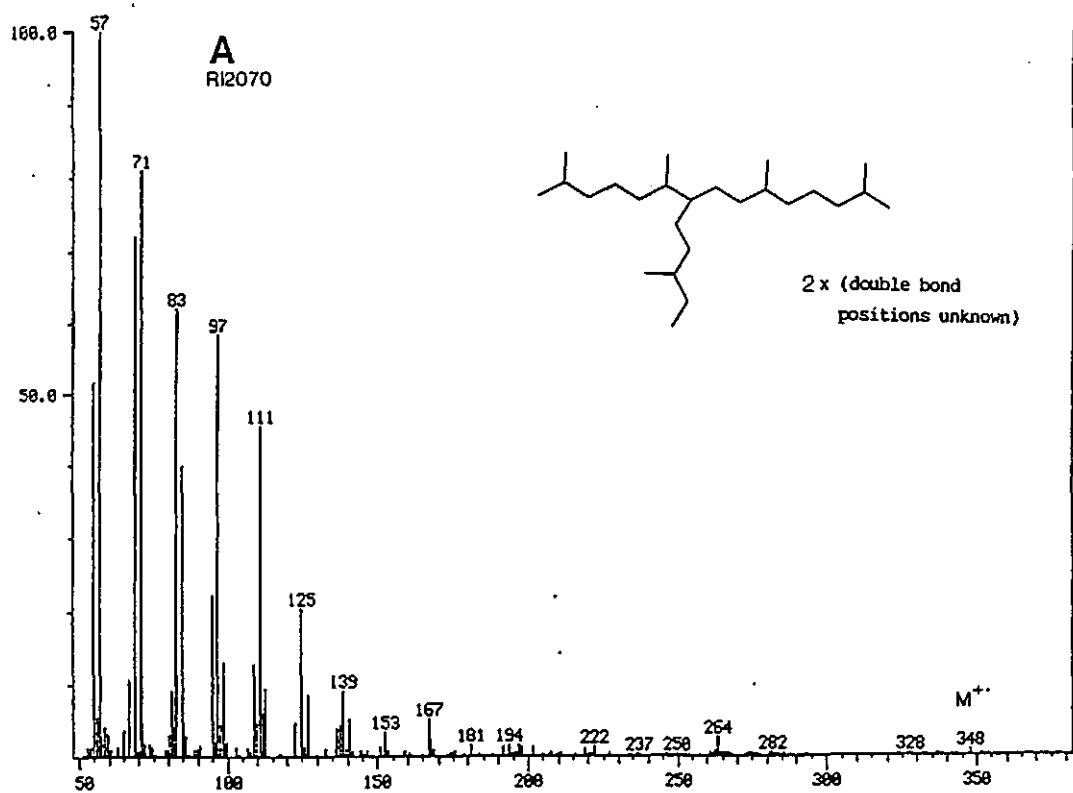
Fig.4:17   Mass spectra of A. RI2070 (br25:2) and B. RI2107 (br25:1)
            isolated from Gluss Voe sediment. Proposed structures as
            indicated.

the same region in the unhydrogenated chromatogram (Fig. 4:14A). This is unusual as hydrogenation generally simplifies chromatographic profiles (as observed in the RI2000 - 2100 region of the same two chromatograms). The compound at RI2523 was shown to be 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane (br30:0; 75) but it was not possible to determine which of the chromatographic peaks present in the GV1 extract was the unsaturated hydrocarbon precursor. Prahl et al. (1980) reported a C_{30} polyene with RI2509 (designated therein as c30:4:1) which converted upon hydrogenation to a C_{30} hydrocarbon (RI2524) whose mass spectrum suggested that it had one degree of unsaturation indicative of a cyclohexyl ring (i.e. $M^{+\cdot}$ m/z 420). The mass spectrum and retention index of that hydrocarbon are very similar to br30:0 (75) in all but the molecular ion indicating that the C_{30} hydrocarbons (RI2509 and 2524) reported by Prahl et al. (1980) as monocyclic alkenes are in fact acyclic (viz: a.pentaene and a monoene partial hydrogenation product) with the 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane (75) skeleton.

A second monocyclic (sic.) tetraene (of RI2563; also designated c30:4:1) was detected by Barrick and Hedges (1981) in Puget Sound, U.S.A. sediments. This compound hydrogenated to what was described as a monocyclic alkane since it contained one degree of unsaturation ($M^{+\cdot}$ m/z 420). However, the mass spectrum of the hydrogenation product (RI not given) is very similar to that of one of the synthetic br30:1 monoenes (79). Thus, it would appear that many of the acyclic compounds have been previously misidentified as cyclic because they have not been fully hydrogenated. This suggests that the occurrence of the 'br' compounds is therefore in fact even greater, than is indicated by Table 4:1.

The compound with RI 2499 in the chromatogram of GV1 exhibited a mass spectrum (Fig. 4:18A) and retention index identical to that of a compound designated as a bicyclic diene (c30:2:2) observed in Narrangansett Bay sediments (Requego and Quinn, 1983a). Upon hydrogenation, the compound reported by Requego and Quinn (1983a) converted to two diastereoisomeric bicyclic C_{30} alkanes (i.e. c30:0:2;2529 and c30:0:2;2550). Two peaks with similar retention indices (i.e. RI 2536H and 2549H) were present in the chromatogram of hydrogenated GV1 (Fig. 4:14B) although neither displayed a mass spectrum similar to those reported by Requego and Quinn (1983A). In addition, the mass spectra and retention indices were different to those of the synthetic C_{30} bicyclic alkanes (i.e. $8\alpha\beta$ (H) $9\alpha\beta$ (H) 11-hexahydrofarnesyldrimanes; 81) prepared as part of this study. Indeed, the mass spectra of the two compounds (RI 2536H and 2549H) present in hydrogenated GV1 bear no resemblance to any of the acyclic or cyclic hydrocarbons which form the basis of this study suggesting that they are produced via hydrogenation of, as yet, unidentified precursors. This highlights the difficulties that can be encountered during the interpretation of chromatographic profiles of hydrogenated and unhydrogenated extracts. The simple product-precursor relationships observed in the hydrogenation of single components (i.e. see synthetic work Chapter 3) are often obscured and thorough examination of both hydrogenated and unhydrogenated extracts is needed before relationships can be established.

The mass spectrum (Fig. 4:18B) of the compound with RI 2449 is similar to that of the c30:2:2;2499 (Fig. 4:18A), both spectra containing apparent molecular ions at m/z 414 and important fragment ions at m/z 231, 259, 301 and 345. RI 2449 may represent an isomer of c30:2:2;2499

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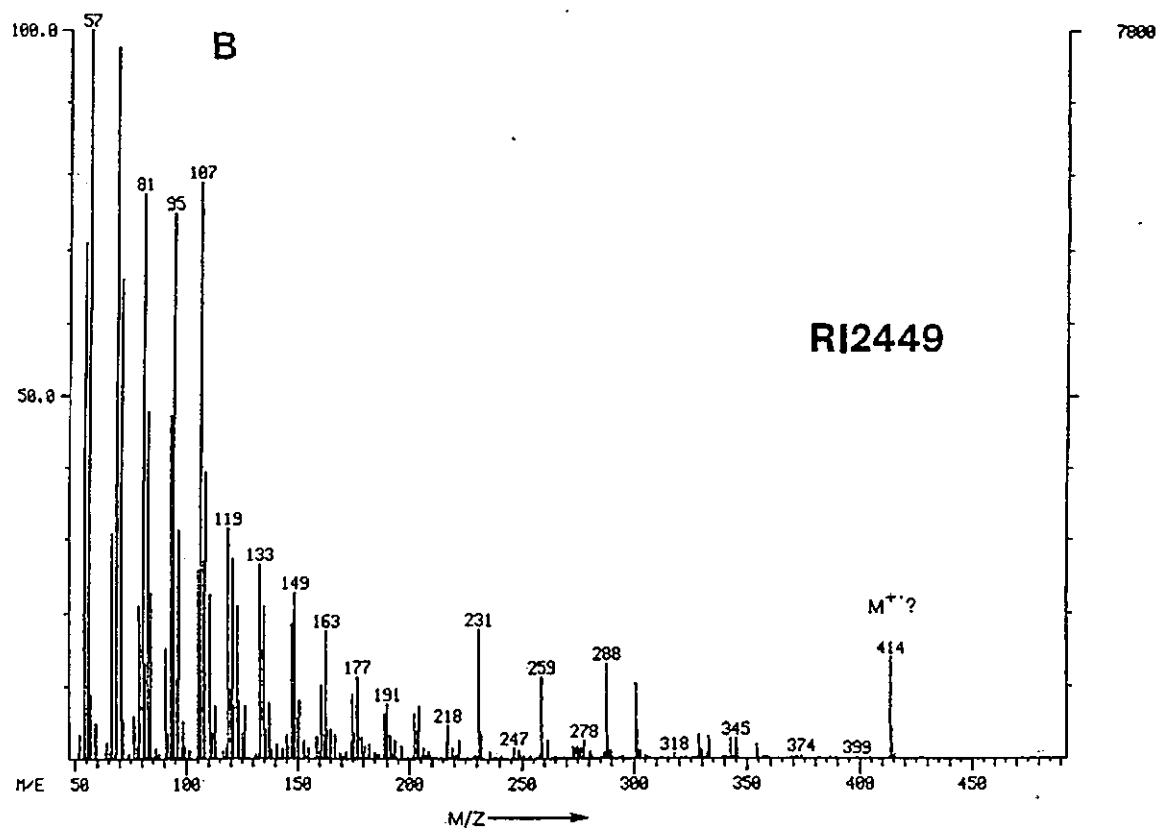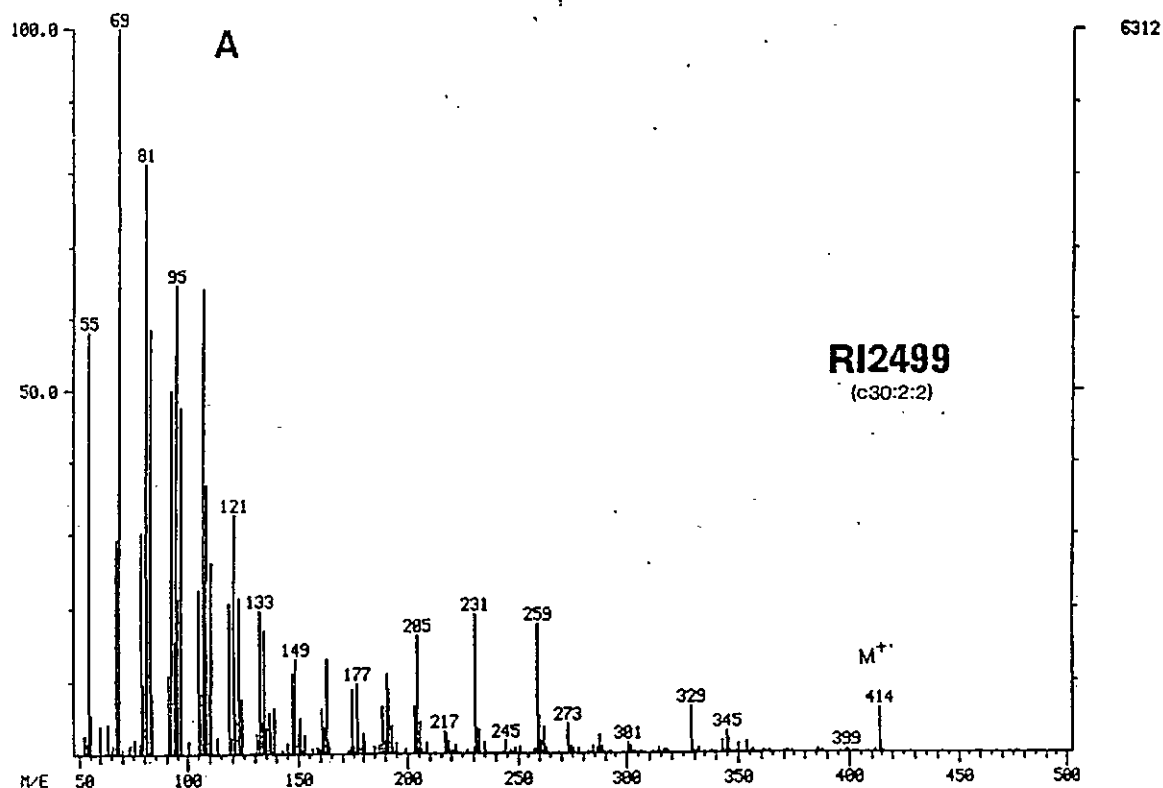
Fig.4:18   Mass spectra of A. RI2499 (c30:0:0) and B. RI2449 isolated
from Gluss Voe sediment.

or may be an acyclic hydrocarbon (i.e. br30:4) which is the precursor of the br30:0 (75) present in hydrogenated GV1.

Another interesting aspect of the hydrocarbon extract of GV1 is the compound with RI2083 which, from retention index alone might have been assumed to be a di-unsaturated analogue (i.e. br25:2) of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; 70). However, the mass spectrum (Fig. 4:19) of RI2083 differed considerably from that of br25:2;2083 (i.e. see Fig. 4:9B) and the apparent molecular ion at m/z 288 indicated a formula of $C_{21}H_{36}$. The intense ion at m/z 191 is a prominent ion in the mass spectra of 'extended' tricyclic terpanes (82) (Heissler et al., 1984) suggesting that RI2083 may be a tricyclic terpene with one degree of unsaturation (83). The retention index (RI2083) for the proposed C_{21} tricyclic terpene is not dissimilar to that ($RI_{OV101}2137$) quoted by Ekweozor and Strauss (1983) for a C_{21} tricyclic terpane (82) isolated from Athabasca Oil sands.

The identification of various acyclic and cyclic hydrocarbons in the 'aliphatic hydrocarbon' extract of GV1 is summarised in Table 4:3 together with concentration data.

4.3.3 Milford Haven, U.K. (M73)

The location of the sampling site at Milford Haven is shown in Fig. 4:2 and Fig. 4:20 shows the gas chromatogram of the 'aliphatic hydrocarbon' extract of M73. The presence of a low CPI (Table 4:2) and a considerable 'UCM' indicates contamination from petroleum hydrocarbons. This is supported by the m/z 191 mass fragmentographic data which is dominated by hopanes of a petrogenic origin. Figs. 4:21A and
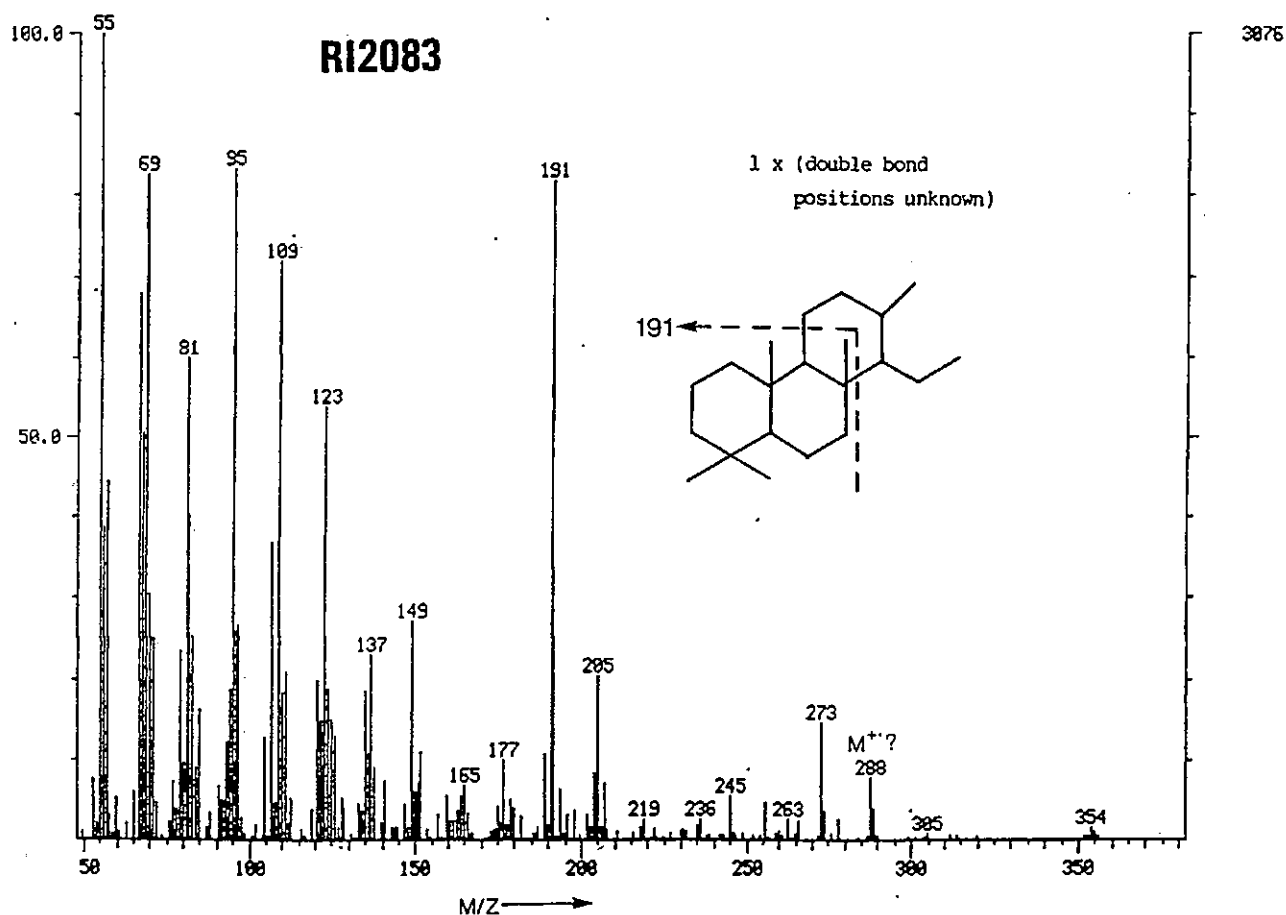
Fig.4:19   Mass spectrum of RI2083 isolated from Gluss Voe sediment.
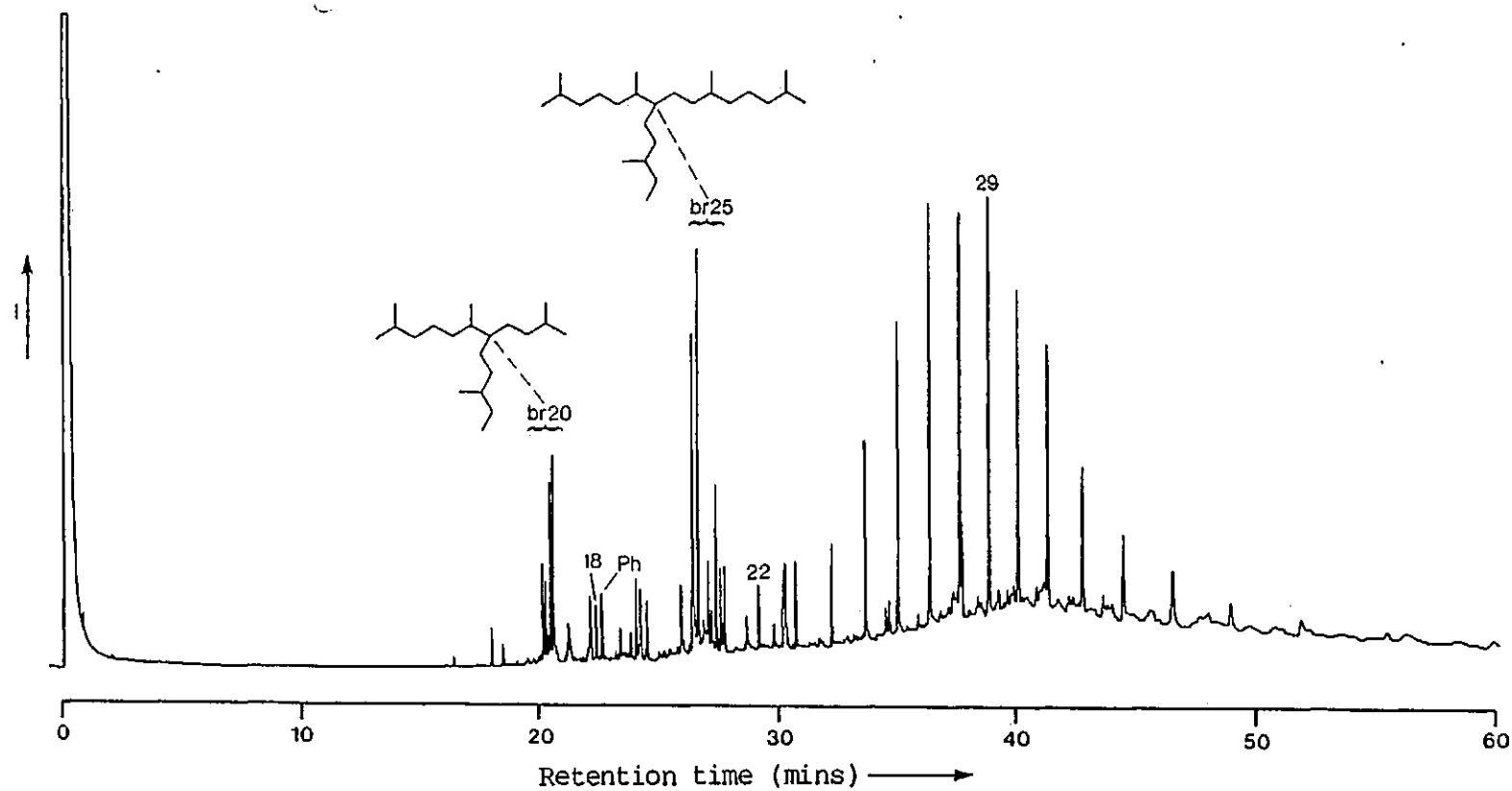Proposed structure as indicated.
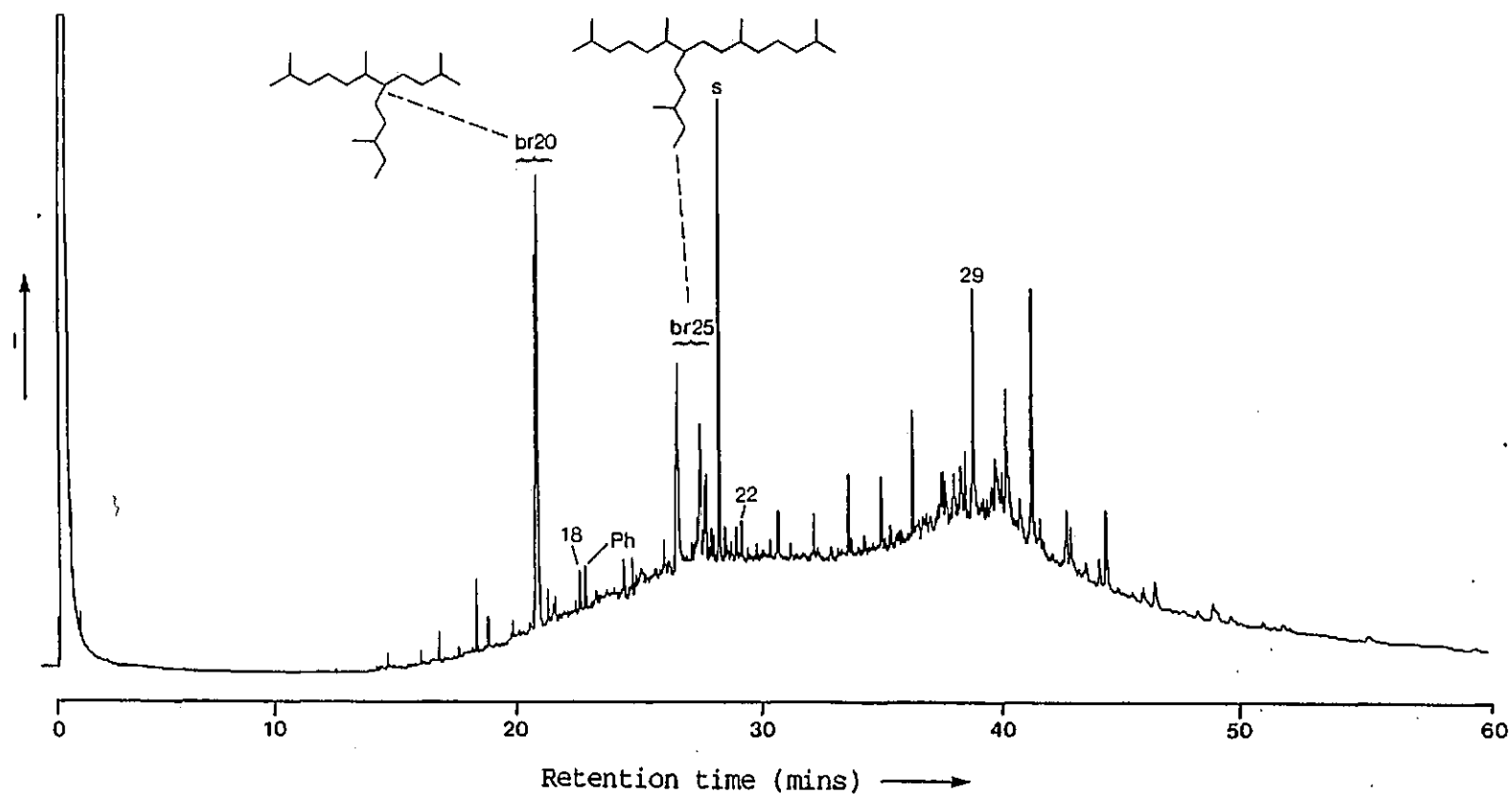
Fig. 4:20   Gas chromatogram of the aliphatic hydrocarbons isolated from Milford Haven.   Numbers refer
to the chain length for the n-alkanes.   's refers to compound RI2139.   For conditions see text.

B. show the expanded RI1600 - 2600 chromatograms of M73 and the hydrogenated hydrocarbon extract. It is evident that there has been a decrease in the relative intensity of RI1702 and an accompanying increase in RI1707H. RI1707 and 1707H have mass spectra identical to, and co-chromatograph with, synthetic 2,6,10-trimethyl-7-(3-methylbutyl) dodecane (br20:0; 66). The mass spectrum of RI1702 was identical to that of br20:1;1702 discussed previously (Tamar). The mass spectrum and retention index of the compound at RI2107 was identical to that of synthetic 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; 70). The large increase in intensity of RI2107H upon hydrogentation was associated with the disappearance of RI2083 and 2091. The mass spectrum of RI2091 indicated that it was a br25:3 identical to that found and discussed in Gluss Voe. Fig. 4:22A shows the mass spectrum of RI2083 which appears identical to that of a hydrocarbon proposed previously by several workers (i.e. Farrington et al., 1977; Boehm and Quinn, 1978; Requego and Quinn, 1983a) to be a bicyclic C_{25} diene although the retention index (RI2083) is different to that reported by Requego and Quinn (1983a) for a bicyclic C_{25} diene (c25:2:2;2097) in Narrangansett Bay sediments . The possibility arises that the hydrocarbon RI2083 present in M73 is a double bond isomer (positional and/or geometric) of c25:2:2;2097. According to Requego and Quinn (1983a), c25:2:2;2097 is converted by hydrogenation to a bicyclic alkane (c25:0:2;2124). If RI2083 is indeed an isomer of c25:2:2;2097 then it should also produce a hydrogenation product such as c25:0:2; 2124. However, an examination of the hydrogenated M73 chromatogram (Fig. 4:21B) showed no such product at ~RI2120. Indeed, the major change observed in the hydrocarbon extract of M73 upon hydrogenation, is the significant increase in the relative concentration of br25:0; 2107H. This suggests that the hydrocarbon RI2083 converts upon
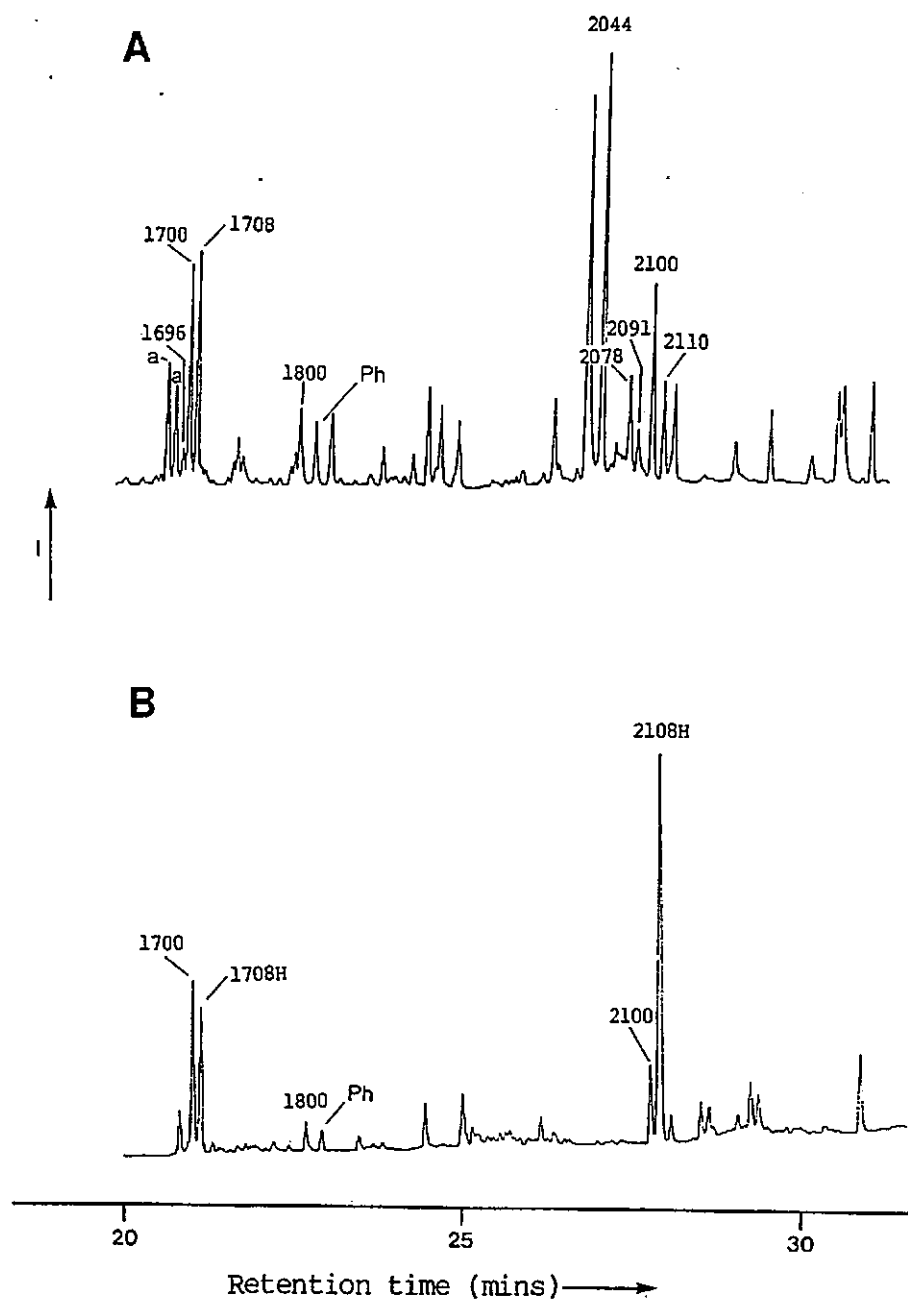
165
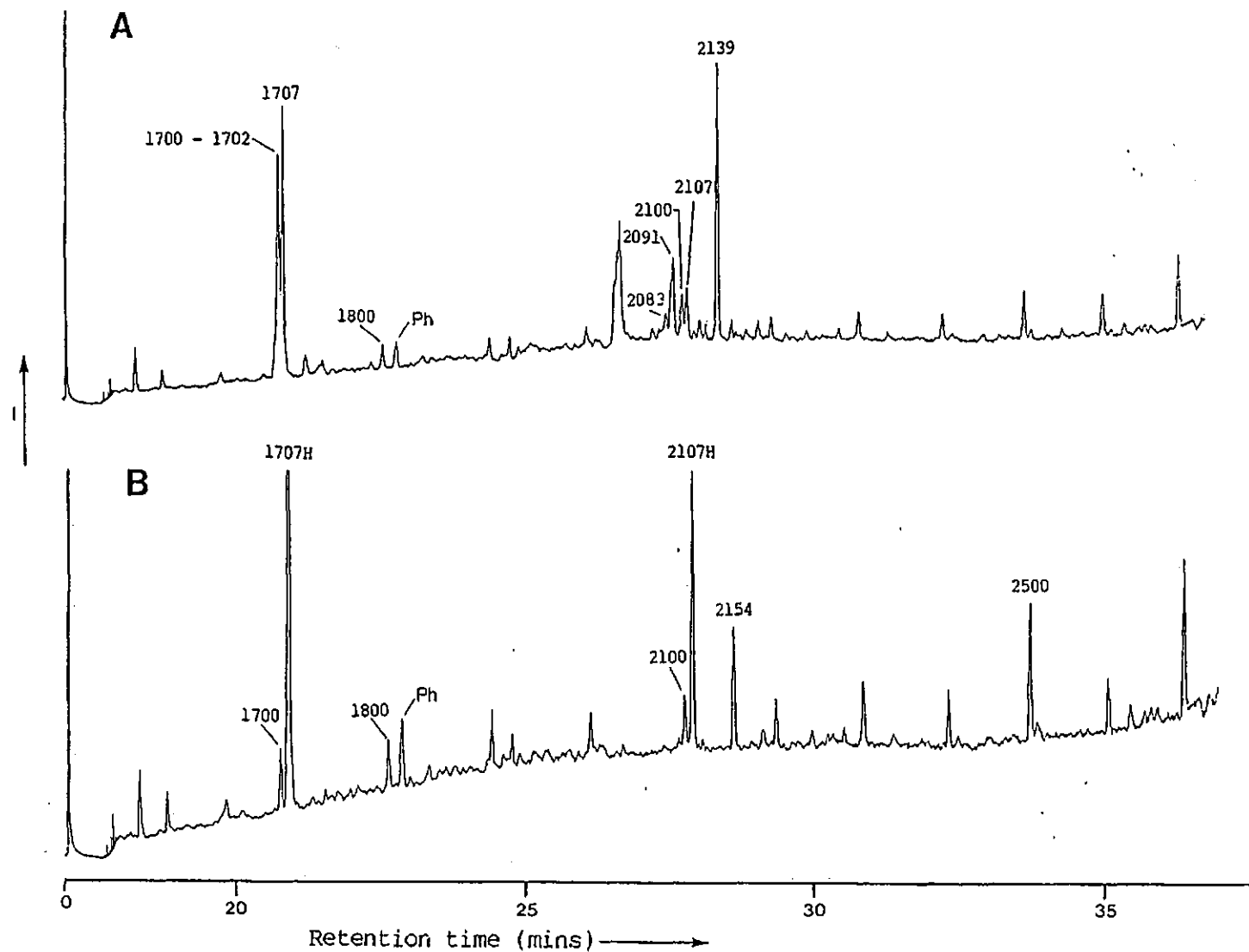
Fig.4:21   Partial gas chromatograms of A. the aliphatic hydrocarbons and B. the hydrogenated aliphatic hydrocarbons from Milford Haven.   Numbers refer to retention indices   (Table 4:3).   Pr = pristane and Ph = phytane. For conditions see text.
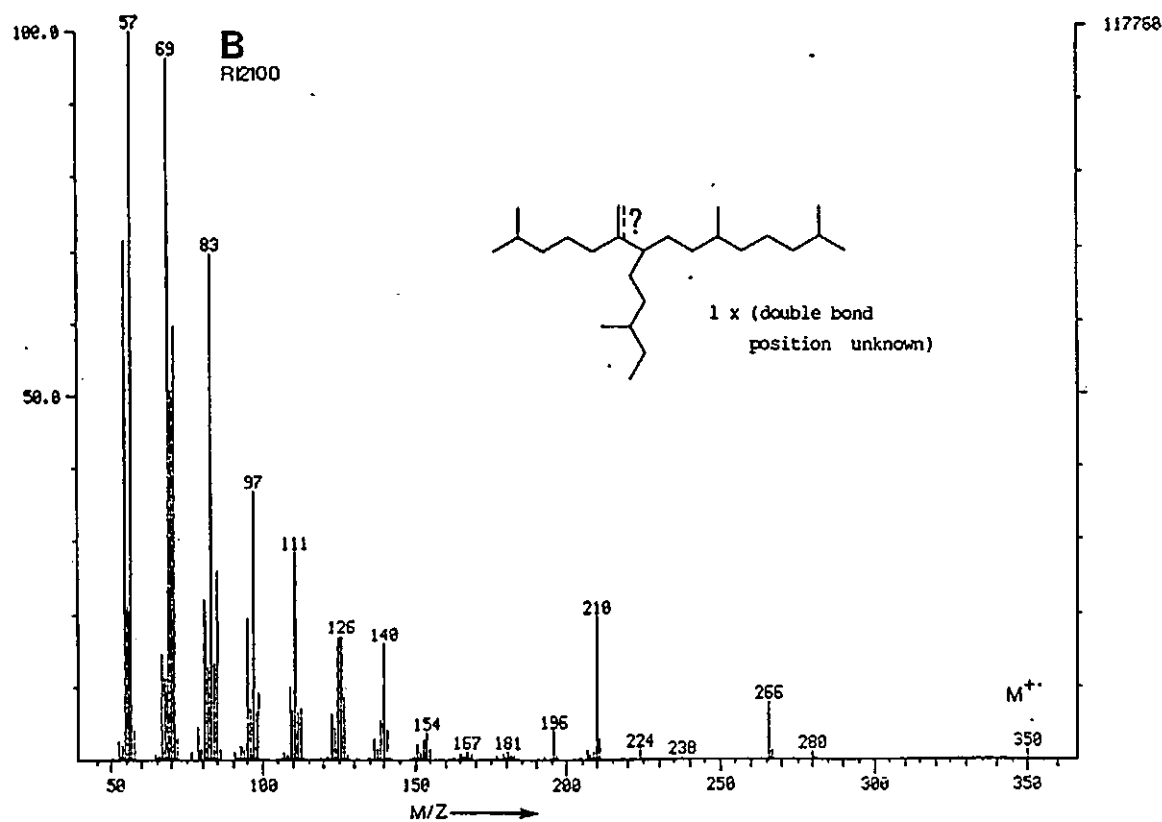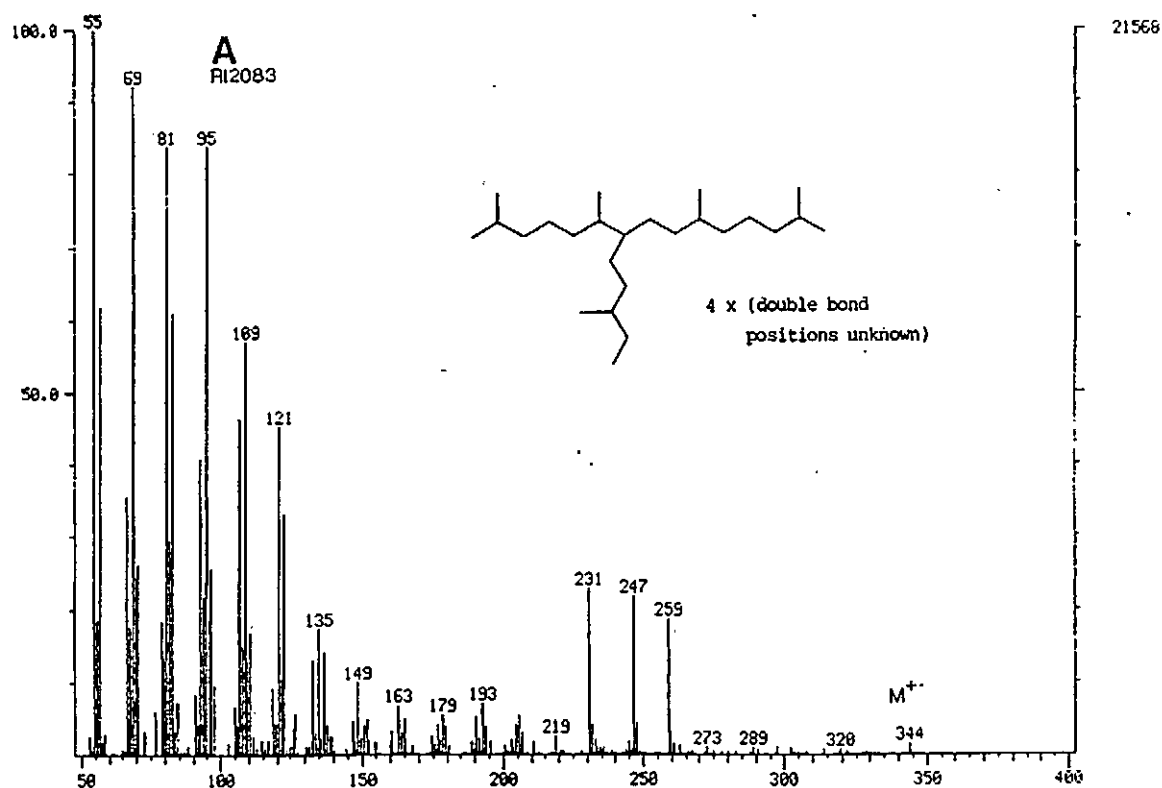
Fig.4:22   Mass spectra of A. RI2083 and B. RI2100 (br25:1) isolated
            from Milford Haven sediment. Proposed structure as indicated.

hydrogenation to br25:0;2107 indicating it to be acyclic and a C_{25} tetraene (i.e. br25:4) with the 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; 70) skeleton. If RI 2083 is indeed acyclic and a br25:4, then it raises the interesting question as to whether the hydrocarbon with an identical mass spectrum proposed by Requego and Quinn (1983a) as a bicyclic C_{25} diene (c25:2:2;2097) is also a tetraene (br25:4). The implications of this are discussed further in the concluding comments at the end of this chapter. It is interesting to note that the mass spectrum (Fig. 4:22A) of RI 2083 is different to that (Fig. 4:15B) of the tetra (br25:4;2078) -unsaturated analogue of br25:0 already identified suggesting that it is yet another isomer.

The hydrocarbon with RI 2100 displays a mass spectrum (Fig. 4:22B) indicative of a C_{25} monoene (br25:1) and is observed to be reduced by hydrogenation. An acyclic C_{25} monoene with a similar mass spectrum was detected in sediment of Shark Bay, Australia (Dunlop and Jefferies, 1985). That monoene converted upon hydrogenation to a C_{25} alkane whose mass spectrum and retention index were identical to that of br25:0. Although the difference in retention indices (RI 2100 vs RI 2112) between the C_{25} monoenes present in M73 and Shark Bay indicates they are not the same hydrocarbon, the similarities suggests that they possess the same carbon skeleton (74) and are geometrical isomers.

The mass spectrum (Fig. 4:23A) for the hydrocarbon at RI 2139 in the chromatogram of M73 is identical to that of a hydrocarbon proposed to be a monocyclic C_{25} monoene by Requego and Quinn (1983a) (c25:1:1; 2140) and Albaiges et al. (1984b) (c25:1:1;2139). Upon hydrogenation,
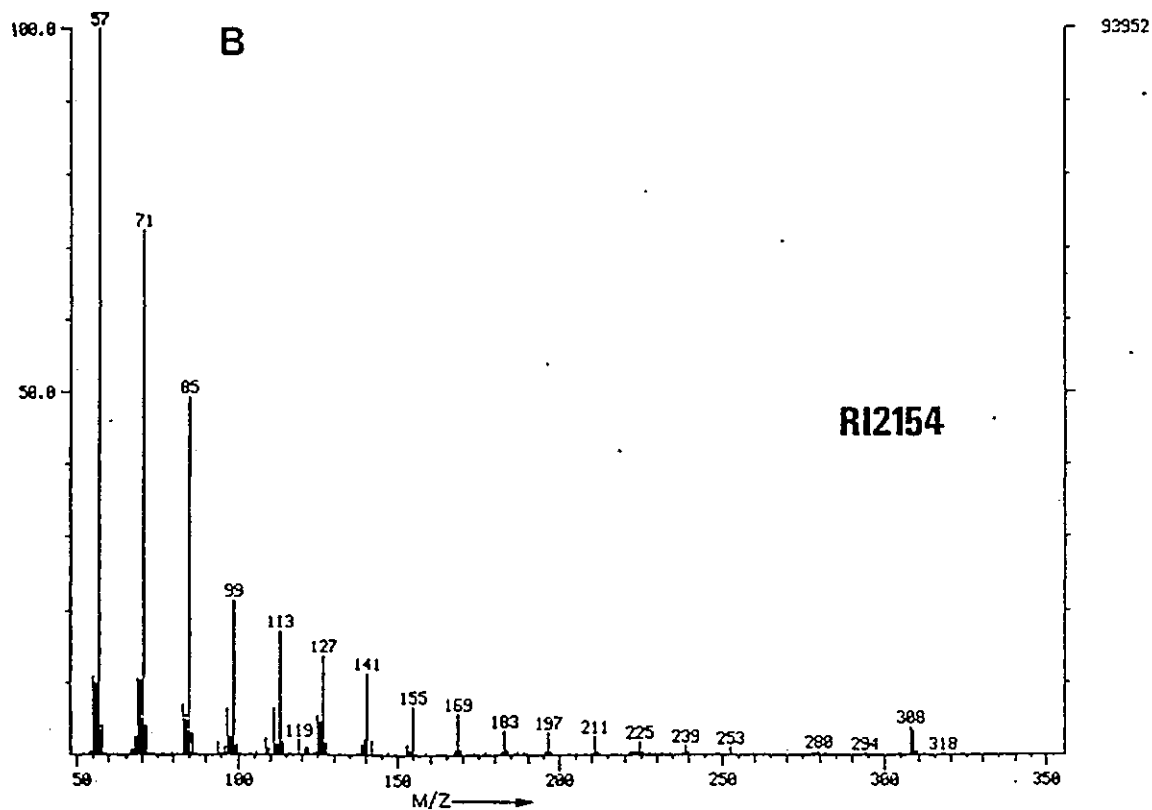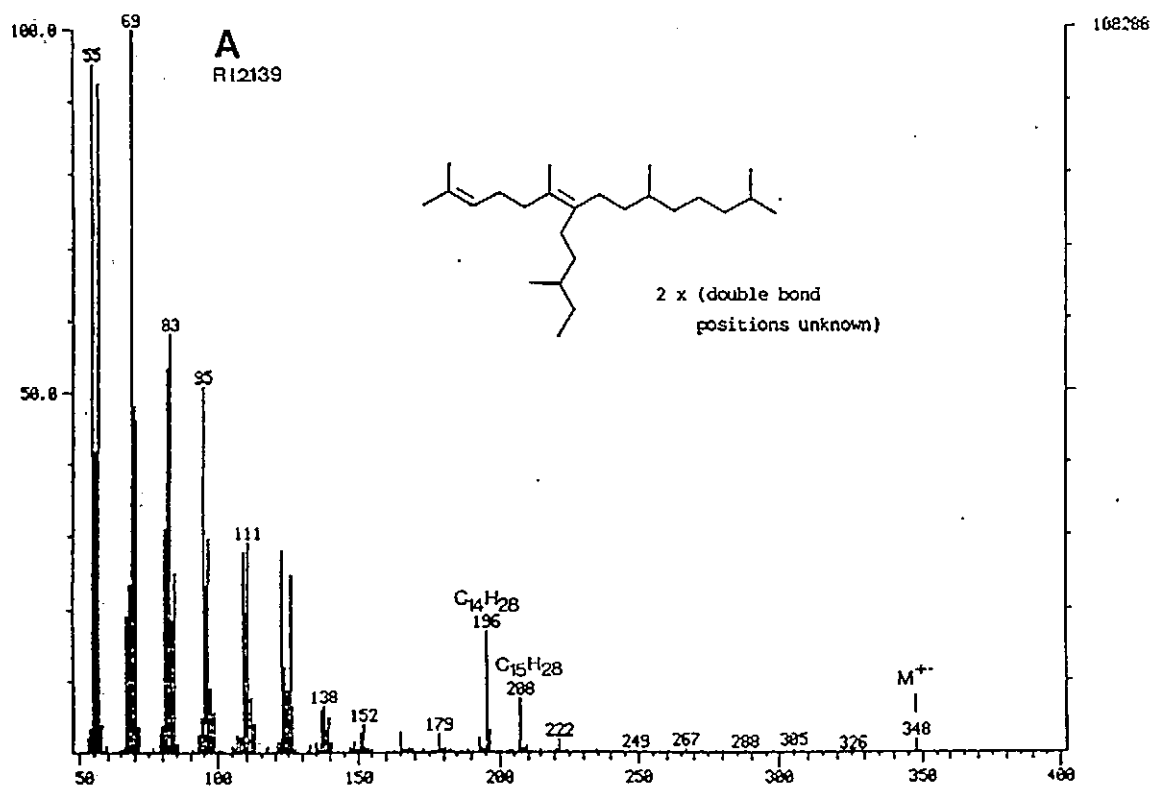
Fig.4:23   Mass spectra of A. RI2139 and B. RI2154 isolated from
           Milford Haven sediment. Proposed structures as indicated.

the hydrocarbon (c25:1:1;2140) reported by Requego and Quinn (1983a) was converted to a supposedly monocyclic C_{25} alkane (i.e. m/z 350; c25:0:1) with a retention index of RI2156. The mass spectrum of the hydrogenation product (c25:0:1;2156) noted by Requego and Quinn (1983a) is similar to that of a hydrocarbon (RI2154; Fig. 4:23B) present in the hydrogenated hydrocarbon extract of M73 (see Fig. 4:21B). Both spectra contain ions at m/z 308/309 but appear to differ in the absence of an ion at m/z 350 in the M73 hydrocarbon (RI2154). The fragmentation pattern in each is consistent with that of an acyclic alkane. It is unlikely that the hydrocarbon represents a monocyclic alkane (c25:0:1) or an acyclic alkene with a hindered double bond resistant to hydrogenation as proposed by Requego and Quinn (1983a). The mass spectra of monocyclic alkanes contain prominent fragment ions which result from cleavage of the bond joining the cyclohexyl ring to the alkyl chain (Fowler, 1984) and the mass spectra of monoenes, particularly those with the 2,6,10,14-tetramethyl-7-(3-methylpentyl) pentadecene (br25:1; 78) structure, contain prominent even mass fragment ions. Thus, it would appear that the hydrocarbon RI2154 (and RI2156; Requego and Quinn, 1983a) is an acyclic alkane although the carbon skeleton is evidently different to that of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; 70). If the hydrocarbon RI2154 does indeed represent the hydrogenation product of the RI2139 originally present in M73, then it implies that the latter also has an acyclic structure and is not a monocyclic monoene as proposed by Requego and Quinn (1983a). Interestingly, the predominant ions in the mass spectrum of RI2139 (i.e. m/z 196, 208 and 222) are also observed to be dominant in the mass spectra of several synthetic di (br20:2) -unsaturated analogues of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (84) presented by Yon (1982). Therefore by

analogy, RI2139 may represent a di (i.e. br25:2) -unsaturated analogue of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane and consequently be unrelated to RI2154 which must arise from hydrogenation of an unidentified precursor. The situation regarding the hydrocarbon proposed by Albaiges et al. (1984b) to be a monocyclic monoene (c25:1:1;2139) is confusing as the author quotes two different retention indices (RI2127 and 2139) for the same hydrogenation product! However, from similarities in mass spectra it seems likely that this hydrocarbon is also acyclic.

The identification of acyclic and cyclic hydrocarbons in the Milford Haven sediment is summarised in Table 4:3.

4.3.4 Bardawil, Egypt (Bar 10)

The exact location of the sampling site in the Bardawil lagoon is confidential so Fig. 4:24 illustrates the general location of Bardawil. Fig. 4:25 shows the gas chromatogram of the 'aliphatic hydrocarbons' isolated from BAR 10 sediment of which a notable feature is the high relative abundance of n-alkanes (C_{23} - C_{36}) which exhibit little odd-over-even carbon chain predominance (i.e. CPI = 1.18). This distribution is typical of fossil fuel products suggesting contamination by petrogenic hydrocarbons. This is further attested by the presence of minor quantities of petrogenic hopanes although surprisingly, the chromatogram does not display the pronounced 'UCM' typical of sediments contaminated by petroleum (Brassell et al., 1978). A similar distribution observed in coastal marine sediments from the Peru Upwelling region by Volkman et al. (1983) was attributed to a marine biogenic origin from phytoplankton and bacteria.

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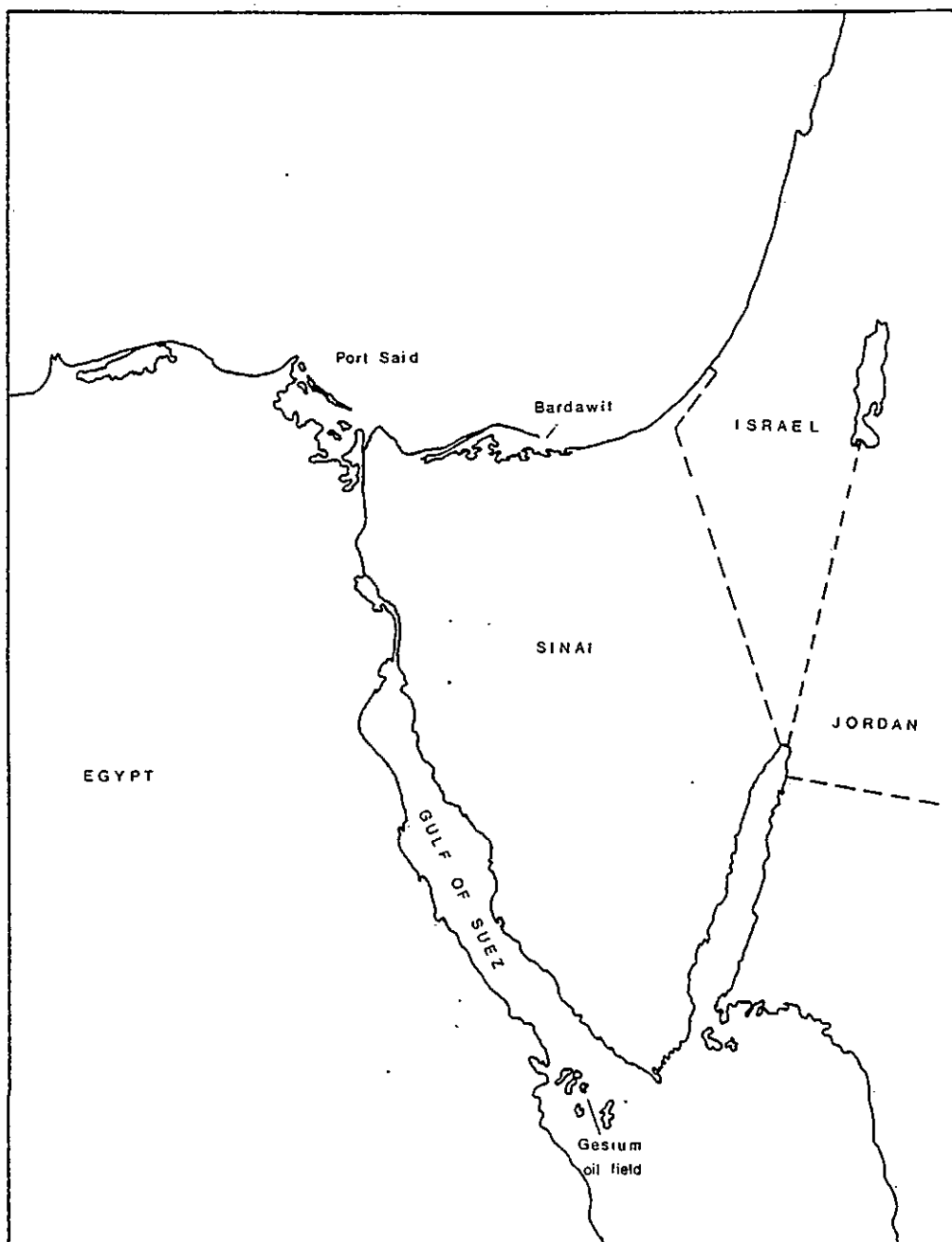
Fig.4:24   Map of the Gulf of Suez showing the relative locations of the sampling sites at
            Bardawil lagoon and Gesium.

Fig.4:25 Gas chromatogram of the aliphatic hydrocarbons isolated from Bardawil lagoon.
Numbers refer to the chain length for the n-alkanes. Ph = phytane.
For conditions see text.

The expanded RI1600 - 2300 chromatograms of Bar 10 and the hydrogenated hydrocarbon extract of Bar 10 are presented in Figs. 4:26A and B. Compound RI1708 was shown to be 2,6,10-trimethyl-7-(3-methylbutyl) dodecane (br20:0; 66). Peaks at RI1684 and 1690 were attributed to n-heptadecenes $(n-C_{17:1})$ whilst RI1696 had a mass spectrum and retention index identical to the br20:1;1696 observed in Gluss Voe. The decrease of RI2044, 2078 and 2091 upon hydrogenation was accompanied by a large increase in the intensity of RI2108H which was shown to be 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; 70). The mass spectra of RI2044, 2091 and 2078 showed that they were the tri (br25:3;2044 and br25:3;2091) and tetra (br25:4; 2078) -unsaturated analogues of br25:0 identified in Gluss Voe sediments. The mass spectrum (Fig. 4:27A) of RI2110 (unhydrogenated Bar 10) contained ions at m/z 196, 210, 266 and 350 indicative of a mono (br25:1) -unsaturated analogue of br25:0. Its mass spectrum and retention index were different to any of the synthetic 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecenes (78) (see Table 3:10) but identical to those of an acyclic C_{25} monoene (RI2112) observed in sediments of Shark Bay, Australia (Dunlop and Jefferies, 1985). The proposal based on ozonolysis that the acyclic C_{25} monoene (RI2112) from Shark Bay possessed the carbon skeleton of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; 70) is confirmed in the current investigation. Structure (74) is therefore assigned to RI2110.

The identification of acyclic C_{20} and C_{25} hydrocarbons in Bardawil lagoon sediment is summarised in Table 4:3 together with data on the sedimentary concentrations.

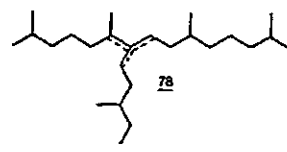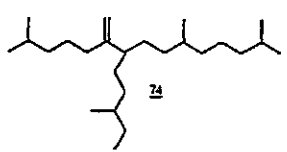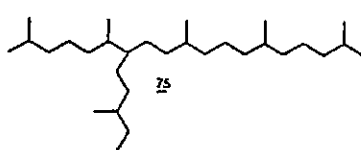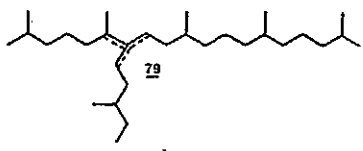Fig.4:26   Partial gas chromatograms of A. the aliphatic
           hydrocarbons and B. the hydrogenated aliphatic
           hydrocarbons from Bardawil lagoon. Numbers
           refer to retention indices (Table 4:3).
           Ph = phytane. Peaks labelled a represent
           n-heptadecenes. For conditions see text.
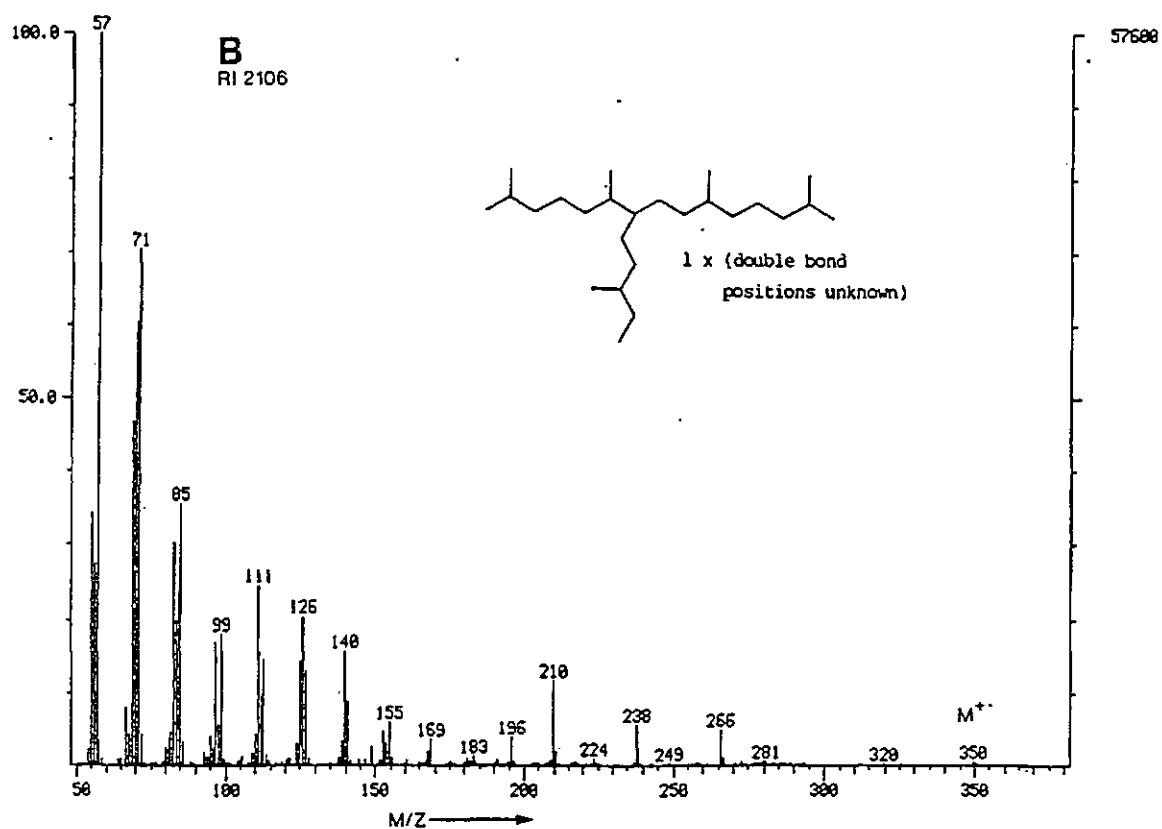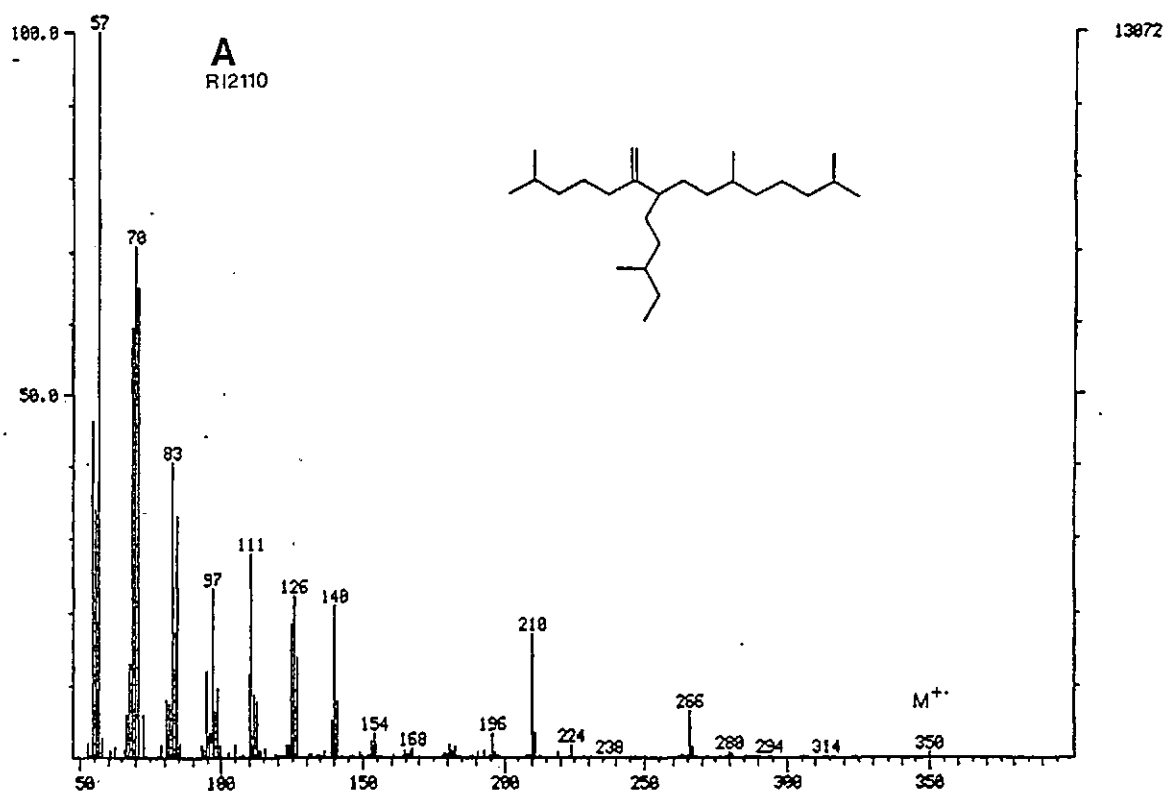
Fig.4:27 Mass spectra of A. RI2110 (br25:1) and B. RI2106
(br25:1) isolated from Bardawil and Gesium sediment
respectively.

4:3:5 Gesium, Egypt (GO6)

The exact location of the sediment sampling site is confidential but Fig. 4:24 shows the general location of Gesium in the Gulf of Suez. Fig. 4:28 shows the gas chromatogram of the 'aliphatic hydrocarbons' which is dominated by a large 'UCM' suggesting contamination of GO6 sediment by petrogenic hydrocarbons. Mass fragmentography (m/z 191) provided confirmation revealing the presence of petrogenic 17 α (H) 21 β (H) hopanes. The contamination of the GO6 sediment with petroleum hydrocarbons is not surprising considering the close proximity of the sampling site to Gesium oilfield (S. Howells - personal communication).

The expanded RI1600 - 2700 chromatograms of GO6 and the hydrogenated hydrocarbon extract of GO6 are presented in Figs. 4.29A and B respectively. The hydrocarbons RI1707 and RI2107 present in both chromatograms were shown to be 2,6,10-trimethyl-7-(3-methylbutyl) dodecane (br20:0; 66) and 2,6,10,14-tetramethyl-7-(3-methylpentyl) pentadecane (br25:0; 70) respectively by reference to the synthetic compounds. The slight shoulder on the chromatographic peak of RI1707 was due to pristane. The hydrocarbons RI1696 and 1702 were identified as the monounsaturated homologues of br20:0 (i.e. br20:1;1696 and br20:1;1702) and the hydrocarbons RI2084, 2044, 2090 and 2078 were identified as the di (br25:2;2084), tri (br25:3;2044 and br25:3;2090) and tetra (br25:4;2078) -unsaturated homologues of br25:0 discussed in earlier sections. The mass spectrum (Fig. 4:27B) of hydrocarbon RI2106 is identical to that of a hydrocarbon proposed by Requego and Quinn (1983a) to be the hydrogenation product (c25:0:1;2104) of a monocyclic monoene (c25:1:1;2079) present in Narrangansett Bay. However, an examination of Fig. 4:29B reveals that RI2106 decreases in intensity upon hydrogenation suggesting that it is a mono (br25:1;2106) -
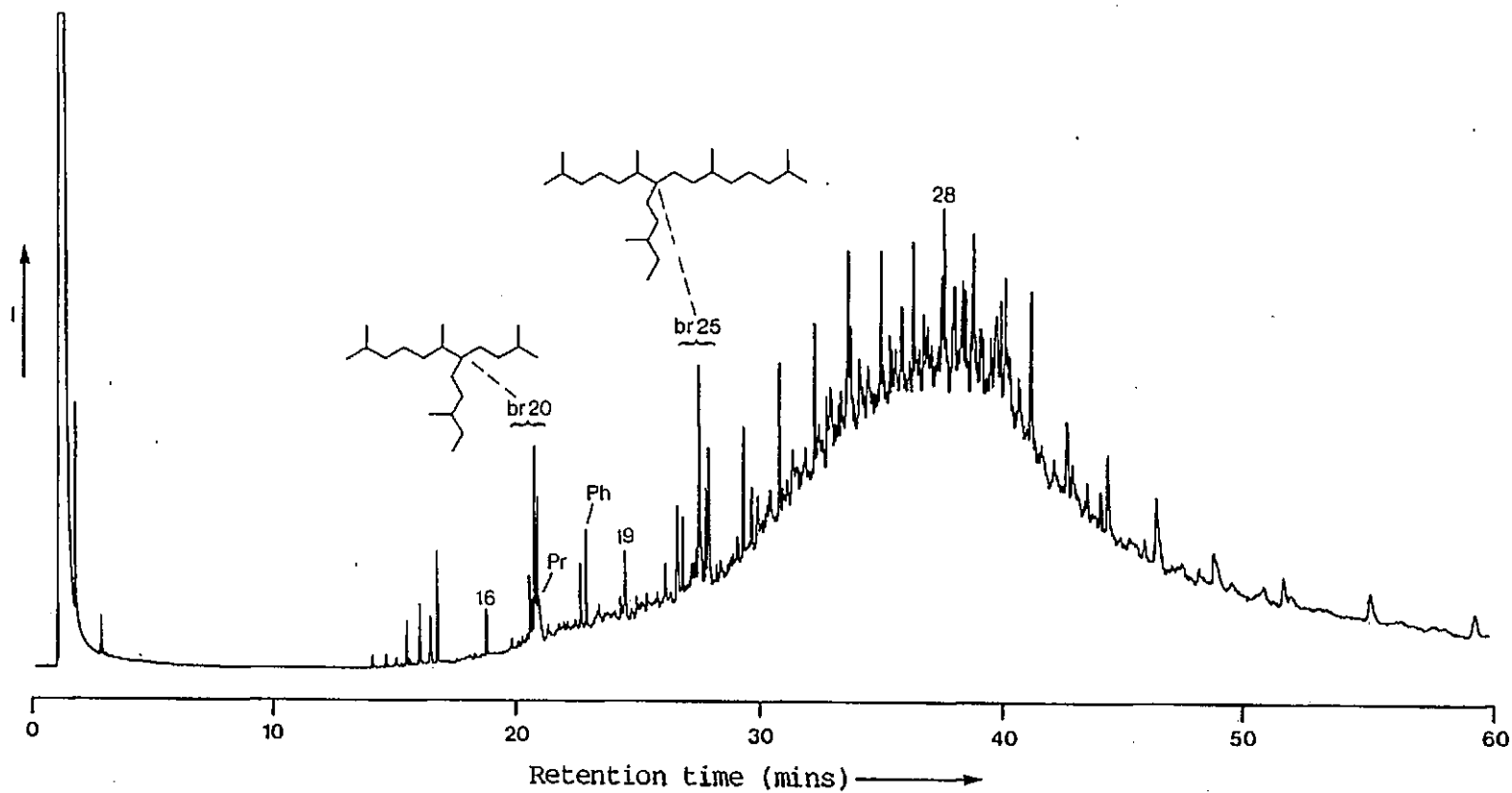
Fig.4:28 Gas chromatogram of the aliphatic hydrocarbons isolated from Gesium. Numbers refer to the chain length for the n-alkanes. Pr = pristane and Ph = phytane. For conditions see text.
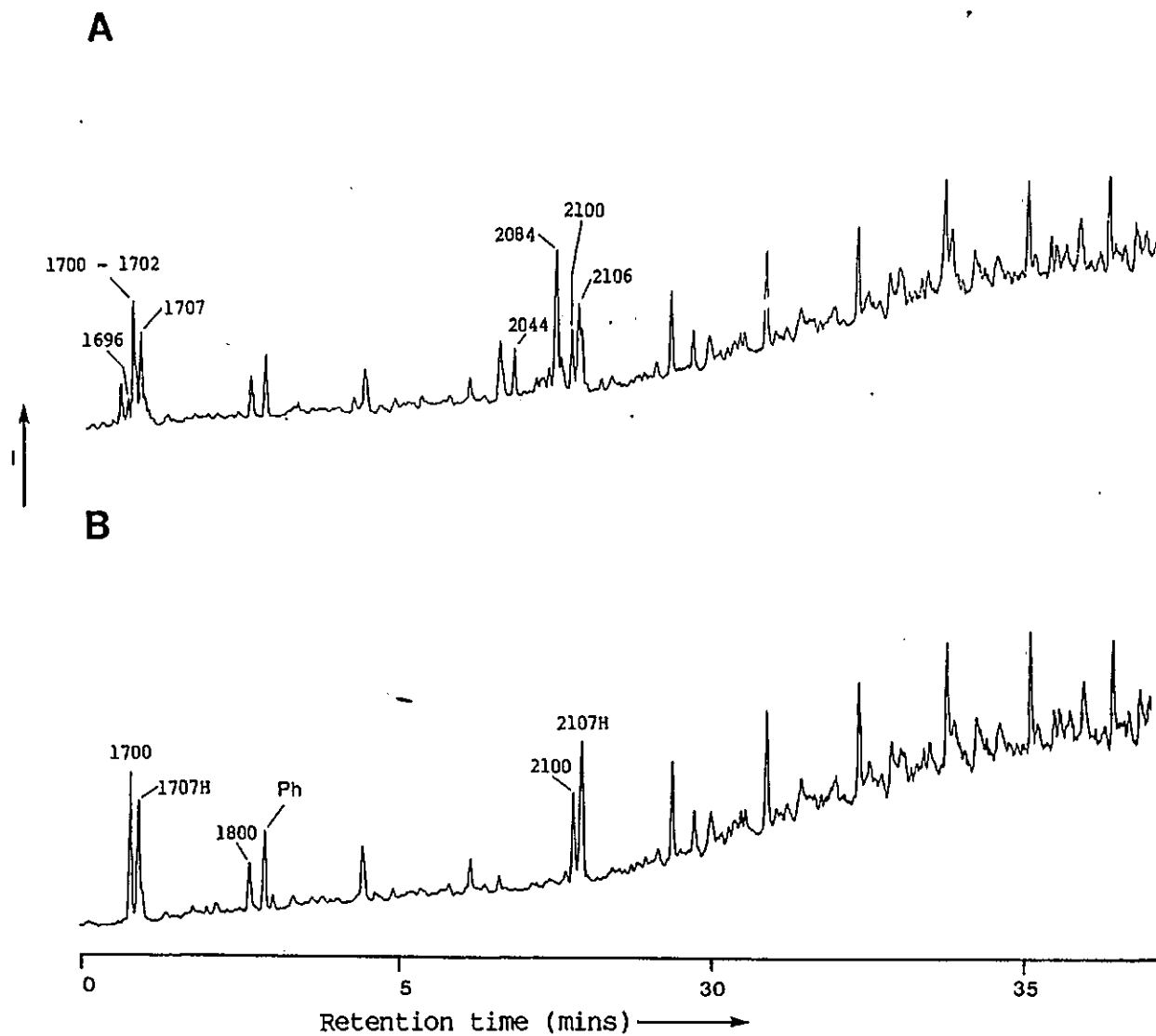
Fig.4:29 Partial gas chromatograms of A. the aliphatic hydrocarbons and B. the hydrogenated aliphatic hydrocarbons from Gesium. Numbers refer to retention indices. (Table 4:3)., Ph = phytane. For conditions see text.

unsaturated analogue of br25:0 and not a monocyclic alkane as proposed by Requego and Quinn (1983a). It appears that the conditions ("by passing H_2 35cm^{3}min^{-1} over PtO_2 in hexane at 25°C") for hydrogenation used by Requego and Quinn (1983a) were less forcing than those used in the current investigation (bubbling H_2 20cm^{3}min^{-1} through hexane containing $PtO_2 \cdot H_2O$ at ambient temp) and were therefore insufficient for hydrogenation of the herein designated br25:1;2104. The mass spectrum of the di-unsaturated hydrocarbon (c25:1:1;2079) proposed by Requego and Quinn (1983a) as the precursor of what has been shown herein to be a br25:1, is virtually identical to that of the di-unsaturated homologue of br25:0 (viz br25:2;2083, Fig. 4:9A) identified in Tamar sediments. Although the difference in retention indices (ΔRI = 5) indicates the two hydrocarbons are not the same compound, there is sufficient similarity to suggest that the hydrocarbon proposed previously as a monocyclic monoene (Requego and Quinn, 1983a) is now more correctly assigned the carbon skeleton of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (i.e. designated br25:2;2079). This brings the total number of isomers of di (br25:2) -unsaturated homologues of br25:0 identified in Recent sediments to five. The br25:1;2106 identified in GO6 sediment was also observed in Gluss Voe where it co-chromatographed with a br25:3;2108.

The identification of acyclic and cyclic C_{20} and C_{25} hydrocarbons in Gesium sediment is summarised in Table 4:3.

4.3.6 Loe Pool, U.K. (LP1)

Loe Pool is a eutrophic fresh water lagoon whose sediments are interesting as they exhibit annual laminations (O'Sullivan et al., 1982). The location of Loe Pool is shown in Fig. 4:2 . A previously

extracted 'aliphatic hydrocarbon' extract (urea non-adduct) from a winter lamination (1927 A.D) was provided by Mr D. A. Pickering. Pickering reports (PhD in preparation) that the 'aliphatic hydrocarbons' of LP1 (winter lamination) are dominated by high molecular weight n-alkanes (n-C_{25} - C_{31}) indicative of a higher plant input. The expanded RI1600 - 2700 chromatogram of the urea non-adduct of LP1 and hydrogenated LP1 are shown in Figs. 4:31A and B respectively. The hydrocarbons RI1707 and 2107 were shown to be 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (br20:0; 66) and 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; 70) respectively by reference to the synthetic compounds. The hydrocarbon RI1702 was identified as a mono-unsaturated homologue of br20:0 (br20:1;1702) and hydrocarbons RI2084 and 2091 as di (br25:2;2084) and tri (br25:3; 2091) -unsaturated homologues of br25:0. This represents the first identification of br25:0;2107 and br25:2;2084 in fresh water lacustrine sediments.

4.4 OVERALL DISCUSSION

Throughout the following discussion, constant reference should be made to Table 4:4 which summarises the identifications, past and present, of all the individual acyclic and cyclic hydrocarbons identified in sedimentary environments. Changes proposed to previous identifications in the light of the current investigation are included in this table.

4.4.1 Acyclic C_{20} hydrocarbons with the carbon skeleton of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (br20:0; 66)

It is now apparent that hydrocarbons designated as br20:0 and br20:1 in Tables 1:7 and 4:3 possess the carbon skeleton of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (br20:0; 66). The exact position of the
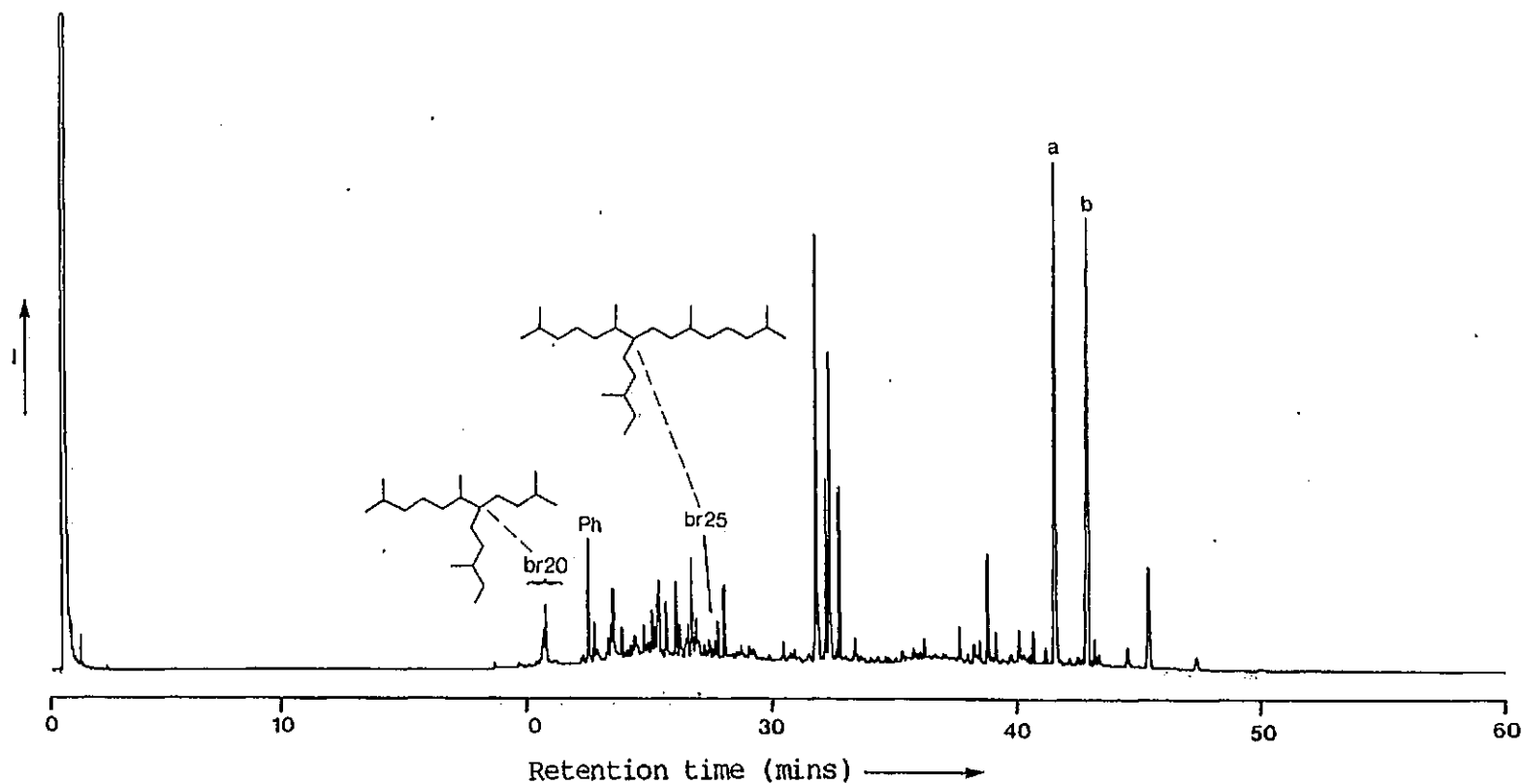
Fig.4:30   Gas chromatogram of the aliphatic hydrocarbons (urea non-adduct) isolated from Loe Pool (sample extract courtesy of D. A. Pickering). Ph = phytane, a = $C_{30}\beta\beta$ - hopene and b = $C_{31}\alpha\beta$ - hopane (22R). For conditions see text.
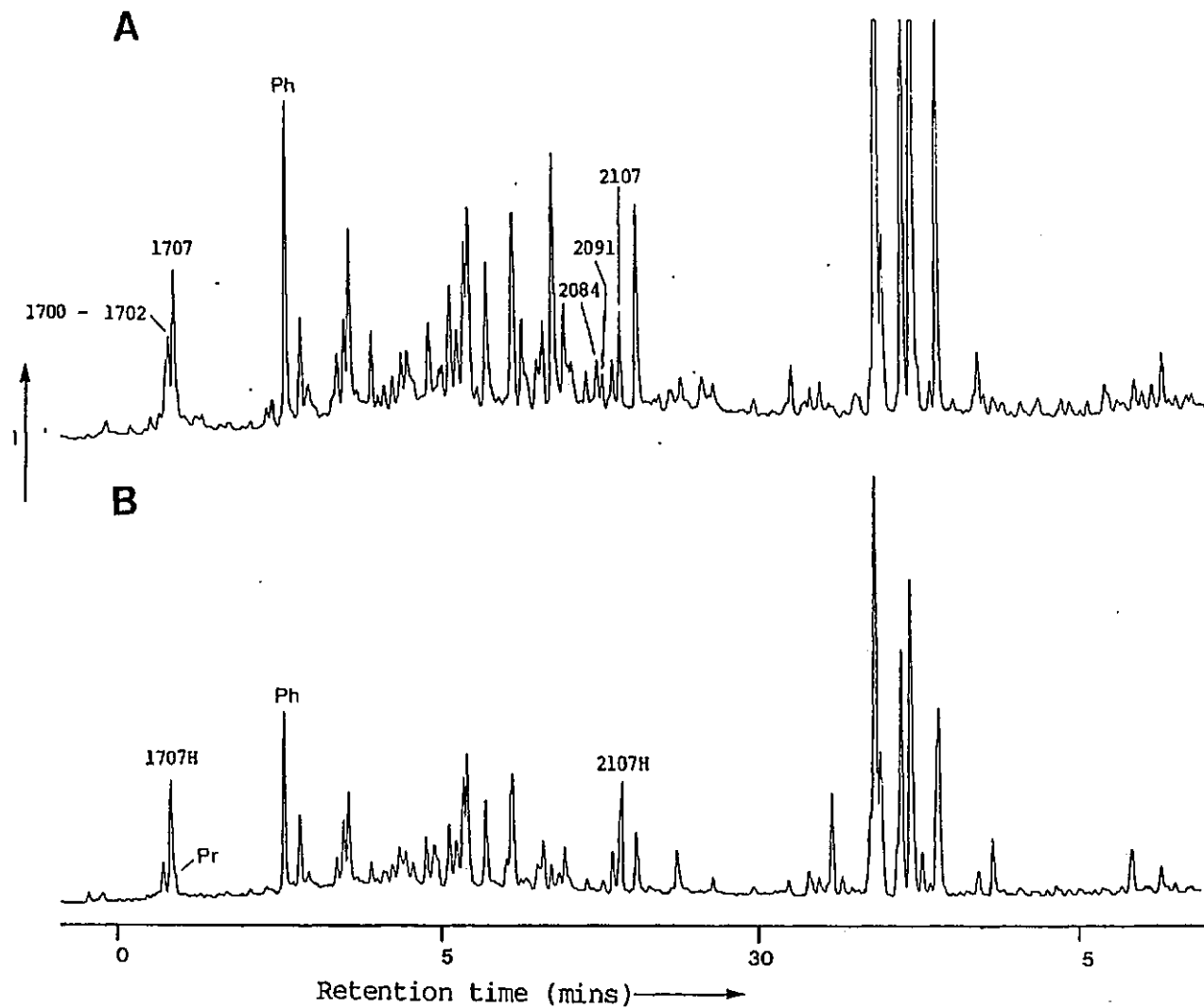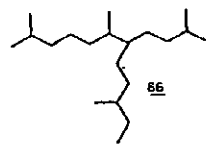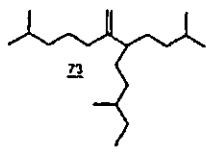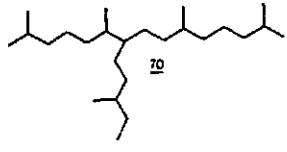
Fig.4:31   Partial gas chromatograms of A. the aliphatic hydrocarbons and B. the
            hydrogenated aliphatic hydrocarbons (urea non-adduct) from Loe Pool.
            Numbers refer to retention indices (Table 4:3). Pr = pristane and
            Ph = phytane. For conditions see text.

Table 4:4

Summary of the identification of acyclic and cyclic C_{20}, C_{25} and C_{30} hydrocarbons in Recent sediments.

COMPOUND RI OV1	ASSIGNMENT (PREVIOUS)	STRUCTURE	REFERENCE	
1696	br 20:1	66*	This Study	
1702	br 20:1	73	This Study	
1707	br 20:0	66	This Study	
2044	br 25:3	70*	This Study	
2070	br 25:2	70*	This Study	
2076	br 25:1	78	This Study	
2078	br 25:4	70*	This Study	
2079	br 25:2	70*	Requego and	
	(c25:1:1)		Quinn, 1983a	
2083	br 25:4	70*	This Study	
2084	br 25:2	70*	This Study	
2088	br 25:2	70*	Requego et al., 1984	
2090	br 25:3	70*	This Study	
2091	br 25:1	78	This Study	
2097	br 25:4	70*	Requego and Quinn, 1983a	
	(c25:2:2)		Farrington et al., 1977	
2100	br 25:1	70*	This Study	
2106	br 25:1	70*	This Study	
2107	br 25:0	70	This Study	
2110	br 25:1	74	This Study	
2509	br 30:5	75*	Barrick et al., 1980	
2523	br 30:0	75	This Study	
2563	br 30:1	79	Barrick	
	(c30:4:1)		et al., 1980	
2499	c30:2:2	94?	This Study	
2524	c30:3:2	?	Barrick and Hedges, 1981	STRUCTURALLY
2590	c30:4:2	?	Barrick and Hedges, 1981	RELATED

* Double bond position(s) unknown.

double bond in the monoenes remains equivocal although there is evidence to indicate that br20:1;1702 has structure (73) (Yon, 1982; Dunlop and Jefferies, 1985). Differences in mass spectra suggest that the remaining monoene must be a positional double bond isomer. One of the most important aspects of the current investigation is the identification of 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (br20:0; 66) as a major hydrocarbon in all of the sediments examined. Pristane (3), which has a similar retention index to br20:0 (i.e. 1711 vs 1708) was either absent or present only as a minor component as a shoulder peak on the side of the major chromatographic peak representing br20:0. The identification of both br20:0 and pristane was only made possible through the availability of synthetic alkane standards of br20:0 and pristane for co-chromato-graphy studies, the use of fused silica gas chromatographic columns of sufficient efficiency to separate br20:0 and pristane, and the availability of gcms. The use of gcms to facilitate identification of br20:0 and pristane is illustrated in Fig. 4:32 which shows the partial m/z 168 and 183 mass fragmentograms and the total ion chromatogram (T.I.C) of the 'aliphatic hydrocarbons' isolated from Gesium sediment (G06). The m/z 168 ion (characteristic fragment of br20:0) indicates the position of br20:0 and the m/z 183 ion the position of pristane. Without these advantages, the major peak at RI1708 would probably have been assigned as pristane. Thus, it is possible that br20:0 occurs more widely in sediments than currently thought. This has important ramifications for the use of pristane/phytane ratios as geochemical indicators of oxicity or for fingerprinting oil spills in marine sediments (e.g. Didyk et al., 1978; Erharhdt and Blumer, 1972). Other complicating factors in the identification of alkanes in Recent sediments may be the presence of br20:1;1696 and br20:1;1702 which nearly co-chromatograph with

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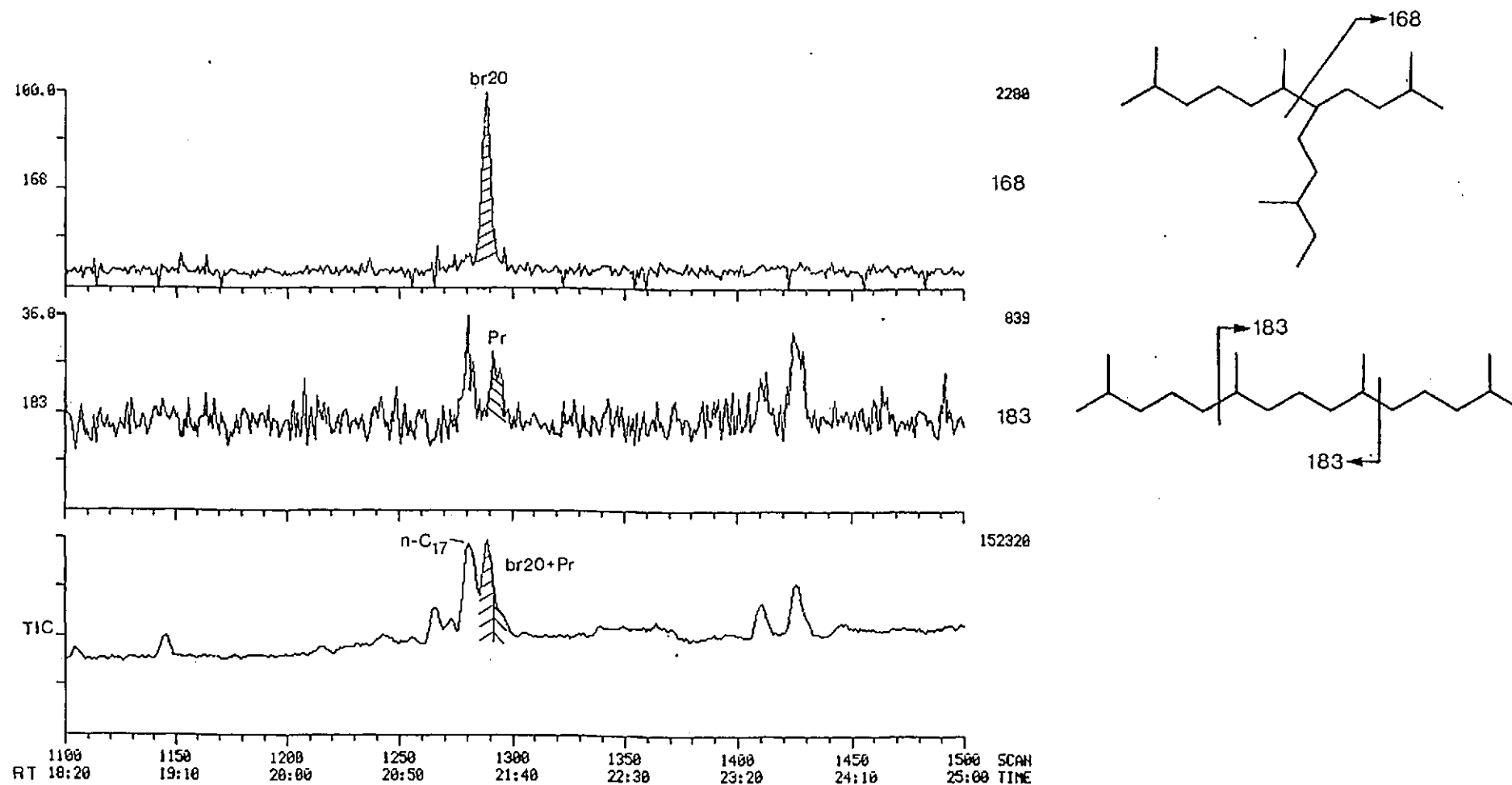
Fig.4:32   Partial Total ion chromatogram (TIC) and mass fragmentograms (m/z 168 and m/z 183) of the hydrogenated
aliphatic hydrocarbons isolated from Gesium. Note how mass fragmentography using the major fragment
ions of pristane (i.e. m/z 183) and br20 (2,6,10-trimethyl-7-(3-methylbutyl)dodecane; i.e. m/z 168)
can be used to distinguish the relative presence of the two co-chromatographing alkanes.

n-C_{17} (n-C_{17}/Pr ratios have also been used to fingerprint spilled oils).

Aspects of the stereochemistry of 2,6,10-trimethyl-7-(3-methylbutyl) dodecane (br20:0; 66) have been approached in four studies (Yon, 1982; Rowland, 1982; Yon et al., 1982 and Rowland et al., 1985). Yon (1982) used a high resolution gas chromatography column (100m DEGS/PEGS) to partially resolve synthetic all isomer br20:0 (synthesised by a different scheme) into two peaks of approximately equal area. The synthetic br20:0 was demonstrated to contain all four possible pairs of diastereoisomers (i.e. a total of eight isomers) suggesting that each of the peaks observed upon chromatographic analysis contained two pairs of diastereoisomers. The geological br20:0 isolated from Rozel Point crude oil was also partially resolved into two peaks of equal area showing that there was a minimum of one and a maximum of four isomers in each peak. However, ^{13}C NMR had previously indicated that all four possible diastereoisomers were present in the geological br20:0 suggesting that, like the synthetic br20:0, the two peaks partially resolved by chromatography each contained two pairs of diastereoisomers. The br20:0 present in sediments from the Cariaco Trench (off Venezuela) and Sandyhaven Bay (U.K) gave only one peak which co-chromatographed with the latter eluting peak of the synthetic br20:0 compound. This suggested that there was a minimum of one and a maximum of two pairs of diastereoisomers present and provided evidence that the br20:0 present in these sediments was biosynthesised in vivo with a specific configuration(s). The br20:0 alkane present in Grasmere sediments (U.K.) was resolved into two peaks with the latter eluting peak the major (i.e. 90%). It was proposed that non-specific hydrogenation of the monoene in Grasmere produced the two stereoisomers

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separable by gc. Rowland (1982) reported that the br20:0 alkane present in Rostherne Mere (U.K) produced only one chromatographic peak which was coincident with the second eluting peak from the synthetic (all isomer) alkane. The same result was observed for the br20:0 identified in field specimens of _Entereomorpha prolifera_ indicating the presence of only a limited number of isomers as was expected for a biosynthesised hydrocarbon (Rowland et al., 1985). Thus, it would appear that the br20:0 of limited configuration in the alga and assorted Recent sediments undergoes isomerisation at the chiral centres during diagenesis. This is in common with the other major branched isoprenoid hydrocarbons (pristane and phytane) present in sedimentary environments which have also been observed to undergo isomerisation with increasing maturation (Patience et al., 1978   ).

Attempts to assess the stereospecifity of the br20:0 hydrocarbons identified in the six sediments examined during this study proved unsuccessful. Unfortunately, no high resolution 100m DEGS/PEGS chromatographic column (cf Yon, 1982) was available so the stereoisomeric separations were attempted using 25m CPWAX52 (Chrompack, Holland) and 25m CARBOWAX 20M (Carlo Erba, U.K) chromatography columns which only proved capable of some minor separation of the diastereoisomers of a regular C_{25} isoprenoid [2,6,10,14,18-pentamethyleicosane (22)].

4.4.2 Acyclic C_{25} hydrocarbons with the carbon skeleton of 2,6,10, 14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; 70)

Within the current investigation it has been demonstrated that all the hydrocarbons designated br25 in Table 1:7 and in the six sediments studied herein (Table 4:3) possess the carbon skeleton of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; 70). From a comparison of these data with the literature for the acyclic C_{25} hydrocarbons it is also apparent that many of the compounds previously designated as cyclic also have the acyclic br25 skeleton. For example, the three hydrocarbons ($C_{25}H_{48}$;2072, $C_{25}H_{46}$;2044 and $C_{25}H_{44}$;2078) observed in coastal marine sediments off Southern California and proposed as cyclic by Crisp et al. (1979) can now be correctly assigned the acyclic carbon skeleton of br25:0. It has already been shown in section 4.3.5 that two hydrocarbons (RI2079 and the apparent hydrogenation product, RI2104) proposed by Requego and Quinn (1983a) as cyclic (c25:1:1;2079 and c25:0:1;2104) are acyclic hydrocarbons possessing the carbon skeleton of br25:0 (see Table 4:4). The mass spectra of the hydrocarbons RI2079 and 2104 presented by Requego and Quinn (1983a) are similar to those presented by Farrington et al. (1977) and Boehm and Quinn (1978) for a monocyclic monoene and hydrogenation product. The similarities are sufficiently significant to indicate that the two hydrocarbons proposed by Farrington et al. (1977) as cyclic are also acyclic (skeleton br25:0). If the br25:1 (i.e. RI2104) is the hydrogenation product of br25:2 (RI2079) then the supposition of Requego and Quinn (1983a) that both hydrocarbons contain a double bond hindered to hydrogenation seems to be correct. The retention indices of the two hydrocarbons provided by Farrington et al. (1977) were calculated using a packed gas chromatographic column (OV101) whereas those calculated by Requego and Quinn (1983a) used a

fused silica capillary column (SE30). This may account for the differences observed in the retention indices of the respective hydrocarbons in the two studies. Changes proposed to previous identifications of sedimentary acyclic and cyclic C_{25} hydrocarbons are included in Table 4:4. The following discussion on the various unsaturated analogues of 2,6,10,14-tetramethyl-7-(3-methylpentyl) pentadecane (br25:0; 70) takes into account the above observations.

Three previously unidentified mono (br25:1) -unsaturated analogues of br25:0 have been identified in the current investigation (i.e. br25:1; 2076, br25:1;2091 and br25:1;2100) bringing the total number of br25:1 monoenes identified in Recent sediments to five. Two of the br25:1 monoenes identified in Tamar sediment (i.e. br25:1;2078 and br25:1; 2091) had mass spectra and retention indices which were identical to two of the synthetic 2,6,10,14-tetramethyl-7-(3-methylpentyl) pentadecenes prepared during the course of this investigation (Chapter 3). Thus structure (78), in which the position of the double bond is restricted can be assigned to these two br25:1 monoenes although the exact position of the double bond remains unknown. Structure (74) was proposed for the br25:1;2110 (Dunlop and Jefferies, 1985) although at the time of their proposal the structure of br25:0 remained unproven. The position of the double bond in the remaining two sedimentary br25:1 compounds is uncertain although interpretation of mass spectra suggests that br25:1;2100 may be a geometric isomer of br25:1;2112 (74). The br25:1;2106 is the hydrocarbon proposed by Requego and Quinn (1983a) to have a double bond hindered to hydrogenation although no evidence for this was provided by current investigation in which br25:1;2106 was smoothly hydrogenated (section 4.3.5). The similarity in retention indices between br25:1;2106 and br25:0;2108 suggests that the intensity

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of the m/z 238 ion in the mass spectrum of br25:1;2106 (Fig. 4:27B) is due to co-elution of br25:0;2108: the relative strength of the m/z 85 to m/z 83 ion in this spectrum compared to the other br25:1 monoenes supports this assertion.

Hydrocarbons with identical mass spectra and retention indices to the two di (br25:2) -unsaturated analogues of br25:0 identified in the current investigation (i.e. br25:2;2070 and br25:2;2084) have been previously reported in the literature (see Table 1:7). Additional acyclic C_{25} hydrocarbons reported in the literature but now proposed to possess the carbon skeleton of br25:0 include br25:2;2088 (Requego et al., 1984) and the br25:2;2079 hydrocarbon suggested previously to be a monocyclic monoene (Farrington et al., 1977; Requego and Quinn, 1983a). The positions and geometry of the double bonds in the dienes remains uncertain.

Hydrocarbons with identical mass spectra and retention indices to the three tri (br25:3) -unsaturated analogues of br25:0 identified in the current investigation (i.e. br25:3;2044, br25:3;2091 and br25:3;2108) have been reported previously in the literature (Albaiges et al., 1984a; Barrick et al., 1980). Additional acyclic C_{25} trienes now proposed to have the br25:0 skeleton are br25:3;2104 (Requego and Quinn, 1983a and 1985) and br25:3;2119 (Albaiges et al., 1984b). As with the br25:2 dienes discussed above, the positions of the double bonds in the br25:3 trienes remains unknown. The identical mass spectra of br25:3;2044 and br25:3;2091 prompted Barrick et al. (1980) to propose that the two hydrocarbons were structurally identical except for geometric isomerisation (cis-trans) about one double bond. Volkman et al. (1983) noted that the difference in the retention indices of the
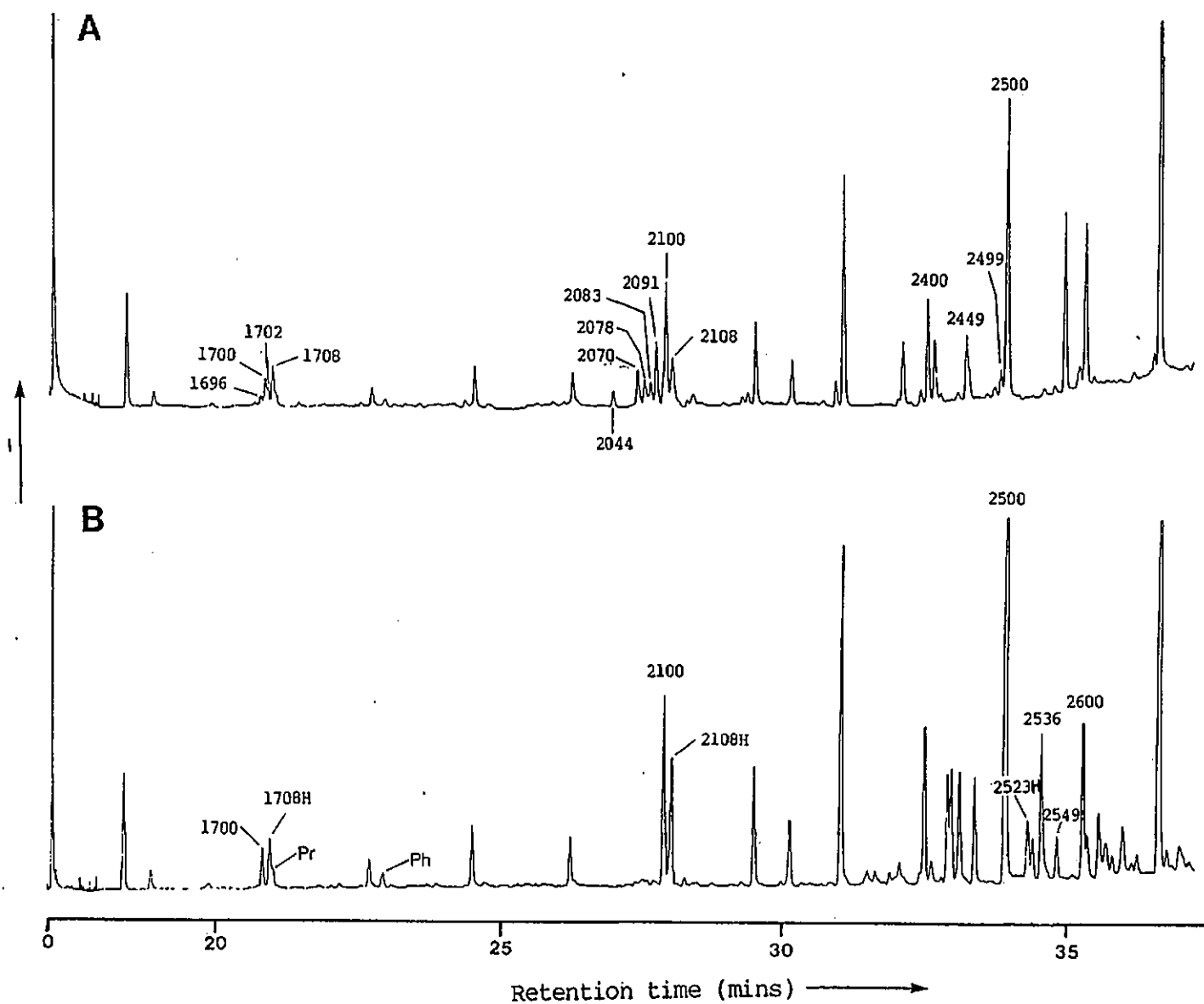
Fig.4:14   Partial gas chromatograms of A. the aliphatic hydrocarbons and B. the
hydrogenated aliphatic hydrocarbons from Class II

intensity of RI2044, 2070, 2078, 2083 and 2091 upon hydrogenation (cf. Fig. 4:14A and B) was accompanied by an increase in the intensity of RI2108H (br25:0; 70). The mass spectra (Fig. 4:16A and B) of RI2044 and 2091 displayed molecular ions at m/z 346 suggesting that they were C_{25} trienes (i.e. br25:3;2044 and br25:3;2091). Similar mass spectra and retention indices were presented for two isomeric acyclic C_{25} trienes by Barrick et al. (1980) and others (e.g. Volkman et al., 1983). In addition, the mass spectrum (Fig. 4:15B) and retention index of RI2078 was similar to that of one of a pair of acyclic C_{25} tetraenes also reported by Barrick et al. (1980) and others (see Table 1:7). The mass spectrum (Fig. 4:17A) and retention index of RI2070 was similar to that of a acyclic C_{25} diene reported by Requego et al. (1984). Examination of the mass spectrum (Fig. 4:17B) of RI2108 revealed a molecular ion at m/z 350, and fragment ions at m/z 196, 210, 224, 266 and 280 suggesting that it was a br25:1. Differences in the mass spectra and retention indices of RI2108 and the synthetic 2,6,10,14-pentamethyl-7-(3-methylpentyl)pentadecenes showed that the position of the double bond differed from that in structure (78). Minor fragment ions at m/z 191, 233, 261 and 346 are major fragments in the mass spectrum of an acyclic C_{25} triene of similar retention index (RI2107) reported by Albaiges et al. (1984b), suggesting the presence of br25:3;2107 in GV1 sediment. Although the position(s) of the double bond(s) in br25:1;2108, br25:3;2044, br25:3;2091, br25:3,2108 and br25:4;2078 are uncertain, hydrogenation showed that all possessed the 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane   (br25:0;   70) skeleton.

The RI2400 - 2600 region of the hydrogenated GV1 chromatogram (Fig. 4:14B) contained a more complex peak assemblage than that present in
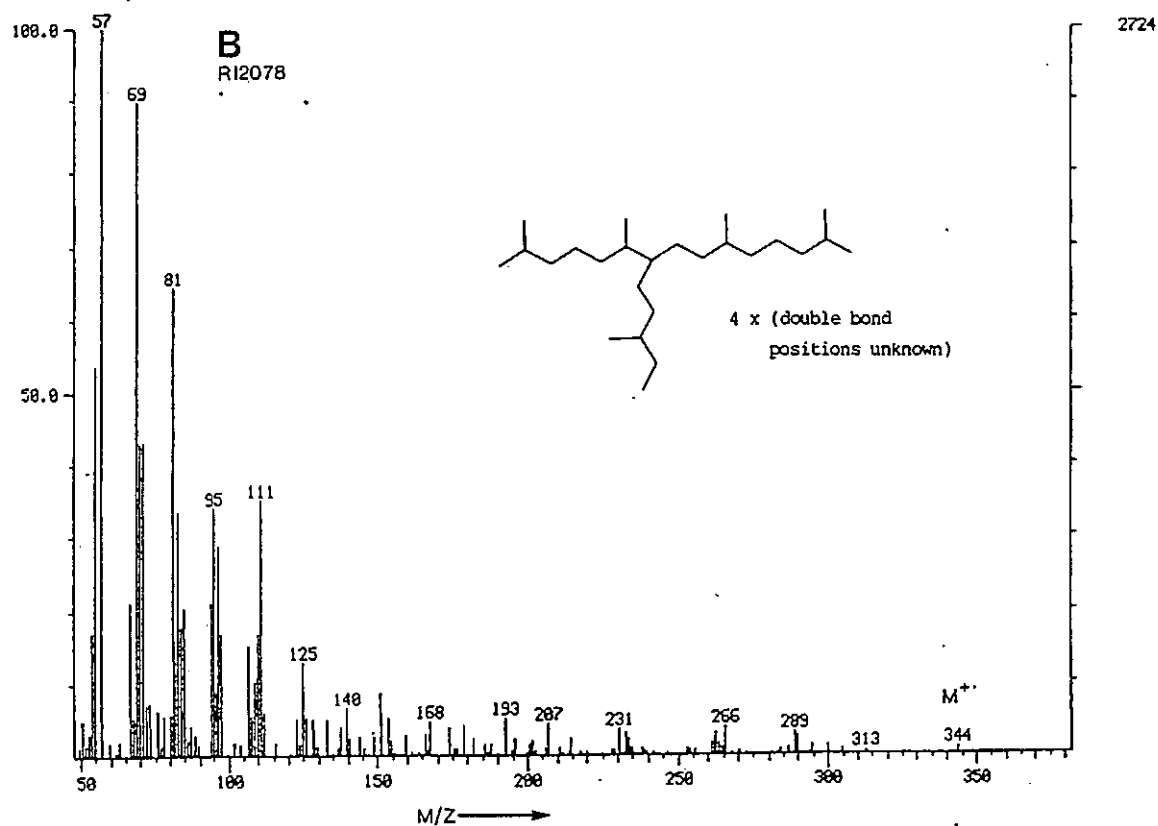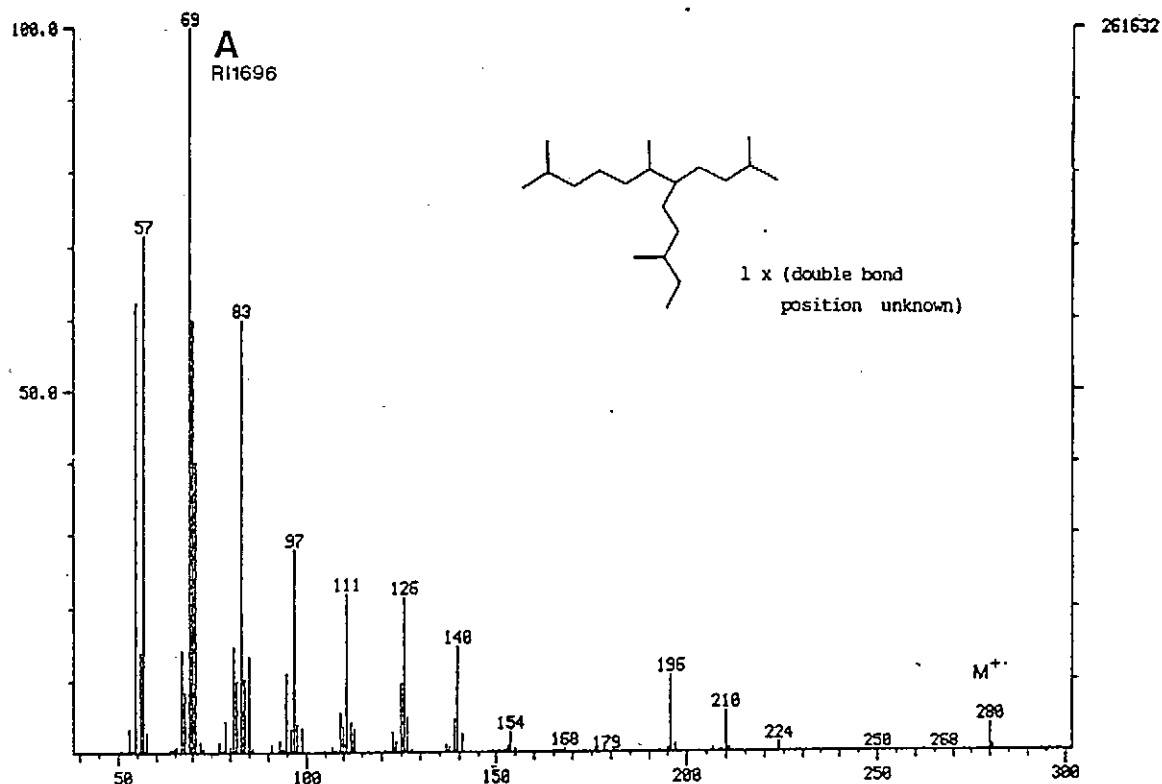
Fig.4:15 Mass spectra of A. RI1696 (br20:1) and B. RI2078 (br25:4) isolated from Gluss Voe sediment. Proposed structures as indicated.
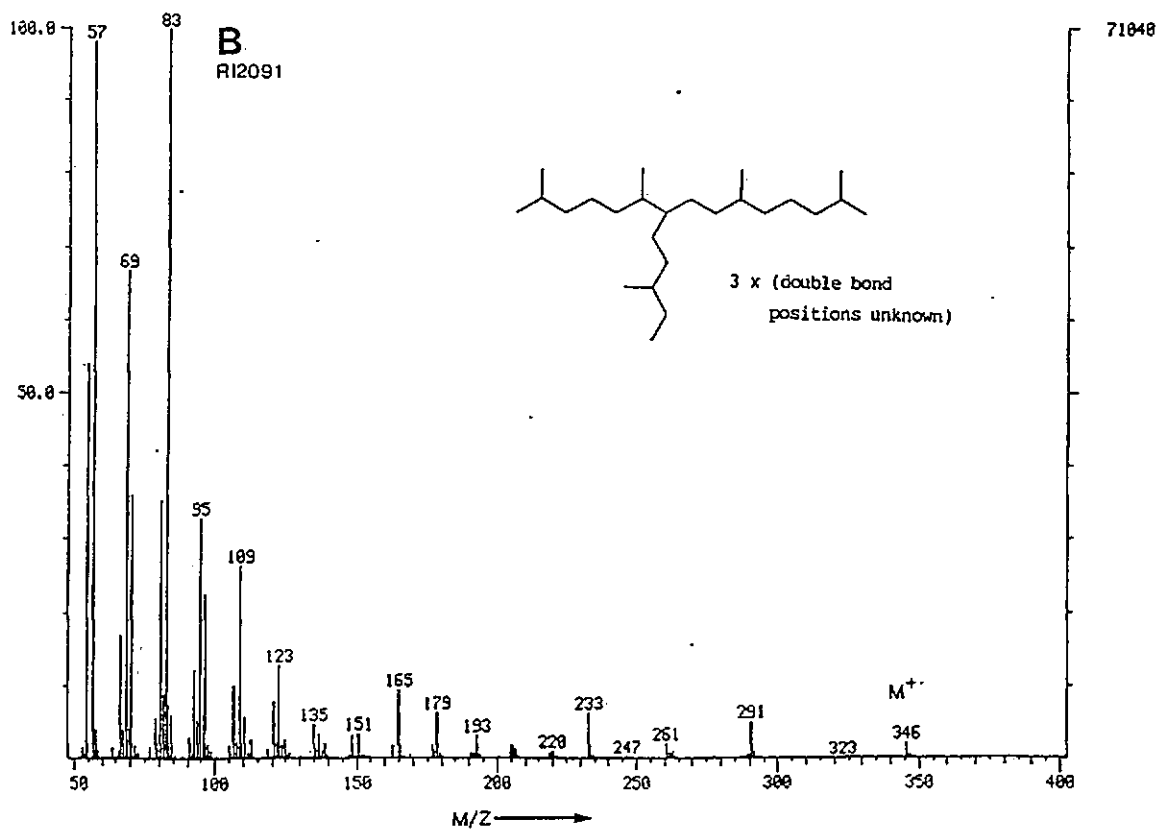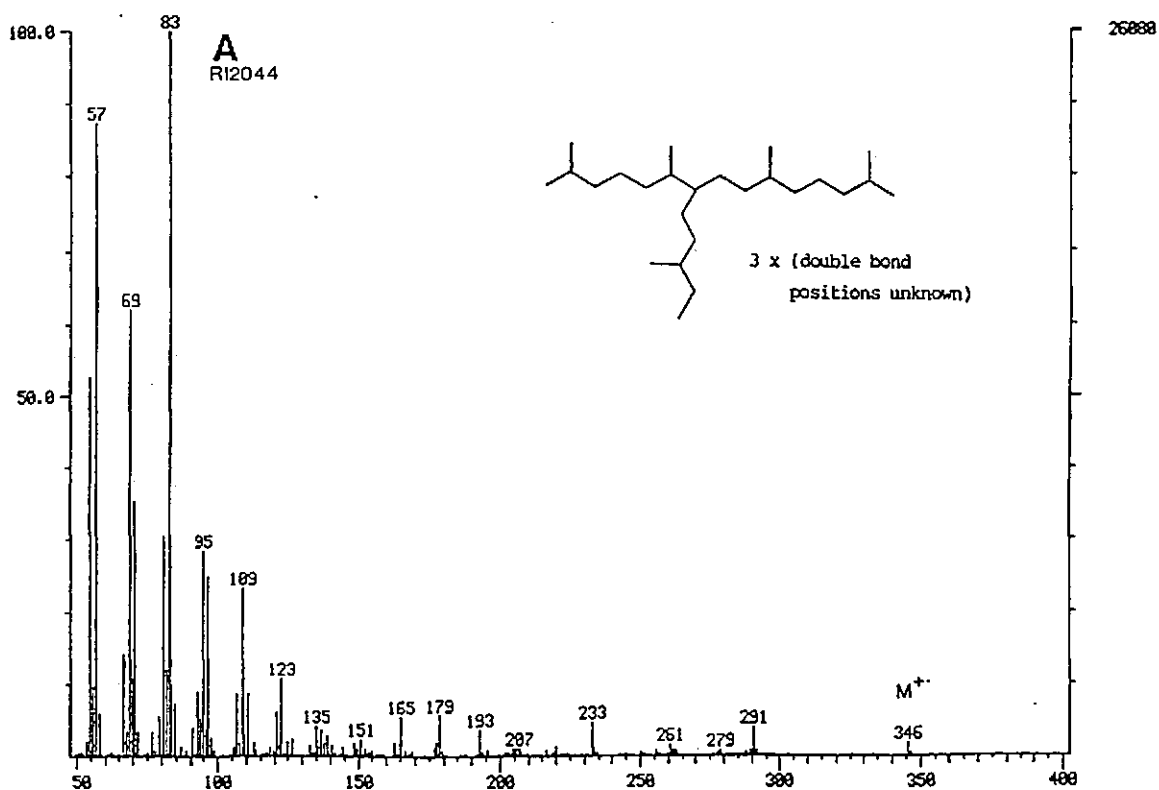
Fig.4:16   Mass spectra of A. RI2044 (br25:3) and B. RI2091 (br25:3)
            isolated from Gluss Voe sediment. Proposed structures as
            indicated.

two trienes was much greater than that found for cis - trans isomers of other unsaturated hydrocarbons and proposed that the trienes were geometric isomers about more than one double bond. An examination of Tables 1:7 and 4:3 indicates that the two br25:3 trienes (br25:3;2044 and br25:3;2091) are the most commonly reported of the family of alkenes with the br25:0 skeleton. Interestingly, there is only one report of br25:3;2044 in isolation to br25:3;2091 (Crisp et al., 1979); the remainder report the occurrence of either both br25:3;2044 and br25:3;2091 or br25:3;2091 alone. Three separate studies (Albaiges et al., 1984b; Osterroht et al., 1983; Prahl et al., 1980) have reported the identification of br25:3;2091 in sedimentary particulates from estuarine and marine sediments. Prahl et al. (1980) observed br25:3; 2091 in two sediment trap samples located in Dabob Bay (U.S.A) but failed to identify the triene in samples of mixed zooplankton collected at the same time. Osterroht et al. (1983) detected br25:3;2091 in autumn sediment trap samples and mixed plankton collected during November in Kiel Bight (F.R.G) and Albaiges et al. (1984b), noted the occurrence of br25:3;2091 in sedimentary particulates of the Erbo Delta (Spain). The absence of br25:3;2044 in sedimentary particulates, plus the general absence of isolated occurrences of br25:3;2044 in sediments suggests that it may represent a sedimentary diagenetic product of br25:3;2091.

Only one tetra (br25:4) -unsaturated analogue of br25:0 was identified in the currrent investigation (br25:4;2078). Previous reports of a hydrocarbon with identical mass spectra and retention index can be found in Table 1:7. The other sedimentary acyclic C_{25} tetraene now proposed to have the same skeleton is br25:4;2124 (Barrick et al., 1980). It is unclear if the C_{25} tetraene reported by Venkatesan and

Kaplan (1982) is the same as the br25:4;2078 or br25:4;2124 or whether it represents an additional br25:4 tetraene. The possibility that the hydrocarbons reported as bicyclic dienes (i.e. c25:2:2) may instead be tetra (br25:4) -unsaturated analogues of br25:4 is discussed in section 4.4.5.

The sedimentary concentrations of the br25 hydrocarbons in the six sediments examined in this study (Table 4:3) are similar to those reported previously for other sedimentary locations worldwide (see Table 1:9). However, care must be taken when directly comparing concentrations relative to dry sediment and those relative to organic carbon content. In none of the six sediments analysed herein do the br25 hydrocarbons display the predominance over other hydrocarbons observed in certain other sedimentary locations such as the Peru Upwelling region (Fig. 1:19; Volkman et al., 1983) and the Gulf of Mexico (Gearing et al., 1976).

Several workers have noted the rapid reduction in concentrations of br25 hydrocarbons with increasing sediment depth. For instance, Barrick et al. (1980) reported that the concentrations of four br25 hydrocarbons (br25:3;2044, br25:3;2091, br25:4;2078 and br25:4;2124) in sediment cores from Puget Sound (U.S.A) decreased exponentially from almost 15-70 ppm (organic carbon) in surface sediments to zero levels in sediment deposited 150 years before. The decrease in concentration was ascribed to in situ chemical degradation. Similar depth profiles for the four br25 hydrocarbons were observed in sediments of the Peru Upwelling regions (Volkman et al., 1983) where concentrations decreased by an order of magnitude between surface sediments and those at 4-5cm depth. Decreases in the sedimentary concentrations of the br25

hydrocarbons with sediment depth have also been observed in Narrangansett Bay, U.S.A. (Requego and Quinn, 1983a and 1985), Buzzards Bay, U.S.A. (Farrington et al., 1977) and Bullock Point, U.S.A. (Requego et al., 1984). The decrease observed by Volkman et al. (1983) in Peru sediments was ascribed to either microbial degradation or rapid and irreversible binding to accreting polymeric material via cross-linking reactions involving the double bonds.

The ability of complex polymers to incorporate functionalised lipids has been investigated by Larter and Douglas (1980). It was observed that synthetically prepared melanoidins (considered to have many of the properties of humic acids) were capable of binding functionalised acyclic isoprenoids (i.e. phytol). Upon examination by pyrolysis-gc, the thermally altered melanoidins released hydrocarbons related to the incorporated lipids (i.e. C_{14} - C_{20} isoprenoid alkenes and dienes). Results suggested that some of the alkyl fragments were bound to the melanoidin via hydrogen or covalent bonds. It was concluded that whilst the conditions of melanoidin synthesis used in the laboratory study were quite unlike those encountered in normal marine environments, the complex polymeric material such as melanoidins/humic acids produced in the water column (and/or sediments) have potential to act as important geochemical sinks for lipids abundant in marine environments. It has been reported (Volkman and Maxwell, 1986), that unsaturated isoprenoids such as phytadienes are probably short-lived in sediments and react rapidly with each other to form dimers, trimers or become incorporated into the accreting 'protokerogen'. The reports of Larter and Douglas (1980) and Volkman and Maxwell (1986) add credibility to the proposal of Volkman et al. (1983) that the rapid decrease in concentration of br25 hydrocarbons with increasing sediment depth is

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via their incorporation into accreting polymeric materials. If such material were sufficiently preserved during diagenesis to enter the catagenic zone, it would be subjected to the effects of increasing thermal maturation. Homolysis of single bonds in the br25 hydrocarbon structure could produce all the $C_5 - C_{15}$ (h-t) 'regular' acyclic isoprenoid hydrocarbons in addition to pristane (3) and the br20:0 alkane. Extensive crosslinking between the polymeric material and the double bonds in the br25 alkenes could explain the absence of br25:0 in geological materials since catagenesis would not presumably yield the intact structure. The br20:0 alkane, which occurs widely in Recent sediment, would not be expected to be incorporated into the kerogen in this way. It is interesting that this alkane is the only br hydrocarbon reported in crude oils (Yon et al., 1982).

Not all sedimentary environments display a pattern of decreasing br25 hydrocarbon concentration with sediment depth. Requego et al. (1984) observed that four br25 hydrocarbons (three br25:2 and one br25:3) in the upper anoxic basin of the Pettaquamscutt River exhibited subsurface concentration maxima. The increases in concentration were considered to result from either, enhanced preservation of a past increase in alkene production by the extreme anoxic conditions present in the sediment or, a current bacterial in situ production at depth. Dunlop and Jefferies (1985) reported that br25:1;2112 (74) present as the major hydrocarbon in sediments of the hypersaline basin of Shark Bay, (Australia) exhibited no significant change in concentration with depth in the sediment. It was also noted that the concentration of the related br20 hydrocarbons (br20:0; 66 and br20:1;1702, 73) displayed no apparent change with sediment depth. Although Barrick et al. (1980) reported an exponential decrease in the concentration of br25

hydrocarbons in Puget Sound (U.S.A) sediments no significant change was observed in the depth profiles of br20:0, br20:1;1696 or br20:1;1702 (73). Thus, it would appear that br20:1 and br25:1 monoenes are possibly more stable than br25 polyenes.

4.4.3 Acyclic C_{30} hydrocarbons with the carbon skeleton of 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane (br30:0; 75)

Within the current investigation it has been demonstrated that the hydrocarbons designated as br30:0 and br30:5 in Tables 1:7 and 4:3 have the carbon skeleton of 2,6,10,14,18-pentamethyl-7-(3-methylpentyl) nonadecane (br30:0; 75). From an examination of these Tables it can be seen that there are far fewer reports of br30 hydrocarbons than of the pseudohomologous br20 and br25 hydrocarbons. The br30:0 was observed in only two of the six sediments examined as part of this study (Gluss Voe and Milford Haven). In Gluss Voe, the br30:0 was produced by hydrogenation from an as yet, unidentified precursor. It is interesting to note whilst the retention index RI 2524 of the br30:0 reported by Prahl et al. (1980) (hydrogenation product of a sedimentary br30 pentaene) is exactly the same as that noted herein for the synthetic br30:0, its mass spectrum (Fig. 1:14B) contains an apparent molecular ion at m/z 420 ($C_{30}H_{60}$) and not at m/z 422 ($C_{30}H_{62}$) as expected. Unfortunately, the absence of a molecular ion in the spectrum (Fig. 3:26C) of the synthetic br30:0 prevents a direct comparison. However, obvious similarities in the remainder of the spectrum, together with the identical retention indices, dictate that the br30:0 reported by Prahl et al. (1980) has the structure (75). Barrick et al. (1980) explained the presence of the apparent molecular ion at m/z 420 by suggesting that the hydrocarbon contained a double bond which was stable to mild hydrogenation. However, the mass

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spectrum differs markedly from those of the synthetic 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecenes (br30:1; 79) (Table 3:11) prepared in this study. Therefore the double bond, if it exists, must be in a remote position which does not significantly alter either the retention index or the mass fragmentation pattern; it is difficult to see where that position might be. Alternatively, judging by the problems encountered in this study in obtaining a molecular ion for br30:0 (albeit on a different mass spectrometer) the ion at m/z 420 in the spectrum presented by Barrick et al. (1980) may represent a contaminant ion from a co-chromatographing compound.

4:4:4 Do homologues of br20:0, br25:0 and br30:0 exist?

Brassell et al. (1986a) reported the identification of two highly branched C_{30} alkanes with virtually identical mass spectra in the hydrocarbon extracts of the Maoming oil shale (Eocene; China). By comparison of the mass spectra of the two C_{30} alkanes with that of br20:0 (Rozel Point crude oil; Yon, 1982), it was proposed that the C_{30} hydrocarbons were the br30:0 (75) structural homologues of br20:0. It was not possible from gc retention times or mass spectra to determine whether the two C_{30} alkanes differed in structure (i.e. methylation pattern) or steric configuration. Comparison of the mass spectra of the C_{30} alkanes with that of the synthetic br30:0 reveals definite similarities; however, an examination of the total ion chromatogram of the hydrocarbon extract of Maoming oil shale indicates that both of the C_{30} alkanes chromatograph after n-hexacosane (n-C_{26}). In contrast, the retention index of synthetic 2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane (br30:0; 75) is 2524 indicating that the two C_{30} alkanes do not have the structure br30:0 proposed by Brassell et al. 1986a. It is possible that the alkanes may be higher molecular weight homologues (i.e. C_{31} and C_{32}) of br30:0.

Two hydrocarbons (RI1803 and RI1903) present in sediments of Shark Bay (Australia) were proposed to be C_{21} and C_{22} homologues of br20:0 (Dunlop and Jefferies, 1985). Mass spectral interpretation suggested structures 71 and 72 although this assignment was not confirmed by synthesis of the necessary reference compounds.

4.4.5 Sedimentary cyclic C_{25} and C_{30} hydrocarbons

Several investigators (see Table 1:8) have reported the occurrence in Recent sediments of a bicyclic C_{25} diene of unknown structure (c25:2:2). Barrick and Hedges (1981) noted similarities between the mass spectra of c25:2:2 and those of two bicyclic C_{30} polyenes (a triene, c30:3:2;2558 and a tetraene, c30:4:2;2590) which co-occurred with c25:2:2;2095 in sediments of Puget Sound (U.S.A). These similarities, and the corresponding relationship between the mass spectra of the respective hydrogenation products (c25:0:2 and c30:0:2) suggested to the authors that the C_{30} hydrocarbons were structurally homologous with the C_{25} compound. Similarities between the mass spectra of c25:0:2 and c30:0:2 include most notably the relatively high intensity of the m/z 123 ion. This point was ignored by previous workers (Barrick and Hedges, 1981) who drew more attention to high molecular weight minor fragment ions. The intensity of the m/z 123 ion suggested to the present author that the structures of the two hydrocarbons might be based upon the carbon skeleton of drimane (51) and homodrimane (52) whose mass spectra also demonstrate a base ion at m/z 123 (Alexander et al., 1984). However, after synthesis of (80) and (81), it became apparent (section 3.3) that the bicyclic C_{25} diene (c25:2:2) and the bicyclic C_{30} polyenes (c30:3:2 and c30:4:2) were not alkenes with these skeleta (though m/z 123 is the base peak ion in the synthetic compounds) since the retention indices of the synthesised

compounds were much greater than those of the sedimentary alkene hydrogenation products (c25:0:2 and c30:0:2). However, in the light of the data for the synthetic alkanes it is possible to speculate further on the structures of the sedimentary bicyclic hydrocarbons.

The presence of an intense m/z 123 ion in the spectra suggests that they do contain an A-ring moiety similar to drimane (51) but have a different structural arrangement of the B-ring. It has already been suggested (section 3.3) that structures 85 and 86 are unlikely as such compounds would have retention indices higher than those reported for the sedimentary c25:0:2 and c30:0:2 (cf Dimmler et al., 1984). Noble (1986) reported that a sedimentary bicyclic C_{15} alkane of proposed structure (87) had a mass spectrum with a strong m/z 123 ion. Substitution of an alkyl chain for one of the methyls on C9 suggests possible structures for c25:0:2 and c30:0:2 as follows:
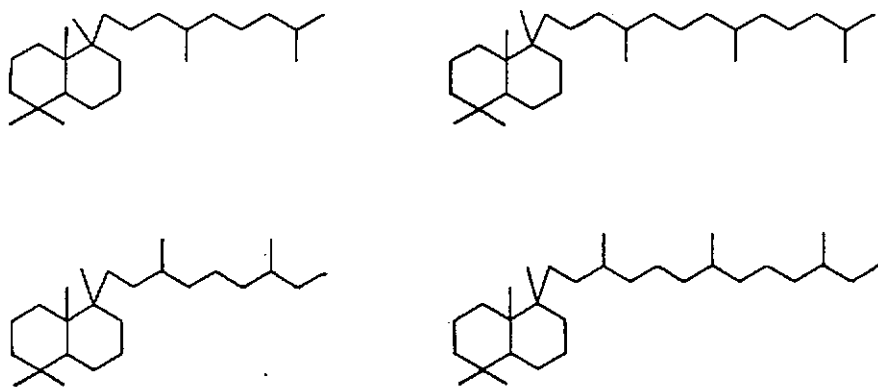
Neither of these compounds would be expected to give the observed mass spectral fragmentation of the sedimentary c25:0:2 and c30:0:2 although they ought to be similar. Other possible structures for c25:0:2 and c30:0:2 which retain an A-ring moiety similar to drimane are shown in Fig. 4:33. It is interesting to note that neither the mass spectra of c25:0:2 or c30:0:2 exhibit losses of $(M^{+\cdot}-CH_3)$ which is a relatively intense fragment ion in the mass spectra of drimane and 9-substituted drimanes. Alexander et al. (1984) used deuterium labelled drimane and homodrimane to demonstrate that the $(M^{+\cdot}-CH_3)$ ion was formed through loss of the methyl substituent at C10. Therefore, if the carbon skeletons of c25:0:2 and c30:0:2 have the A-ring moiety of drimane then the structural arrangement of the B-ring must prevent cleavage at C10. Cleavage of the C10 methyl group in the proposed structures for c25:0:2 and c30:0:2 (Fig. 4:33) may be prevented by the bulky isopranyl moiety at C9 increasing steric crowding.

An alternative proposition is that the c25:0:2 and c30:0:2 do not possess the A-ring moiety of drimane and that the m/z 123 ion arises from rearrangement of a different carbon skeleton. Noble (1986) assigned the structure (88) to a bicyclic C_{16} alkane occurring in an African crude oil. The base ion at m/z 123 was proposed to arise through cleavage at C6-C7 and C9-C10. Alkylation at C9 affords two further possible structures for c25:0:2 and c30:0:2:

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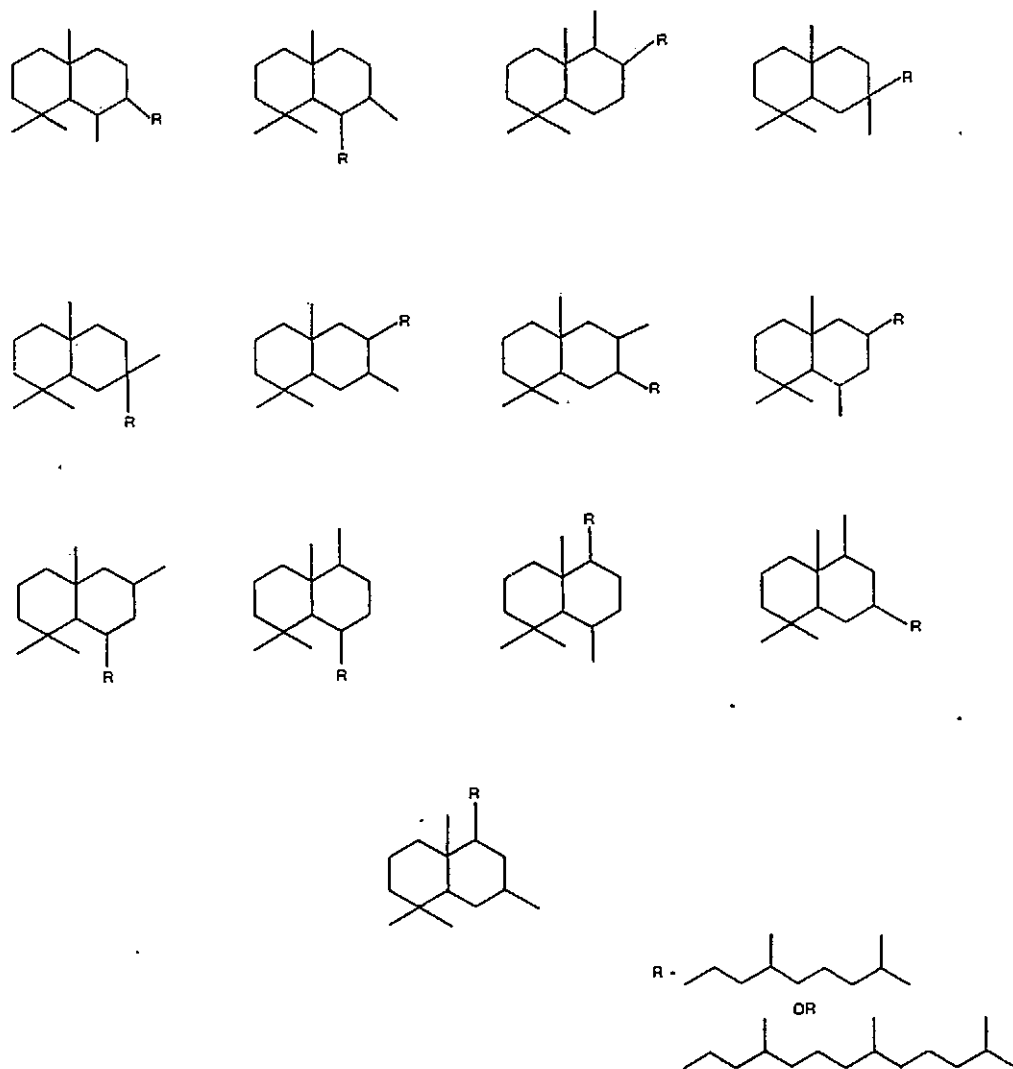
Fig. 4:33   Other possible carbon skeletons for the sedimentary bicyclic C_{25} and C_{30} hydrocarbons which retain the A-ring moiety of drimane.
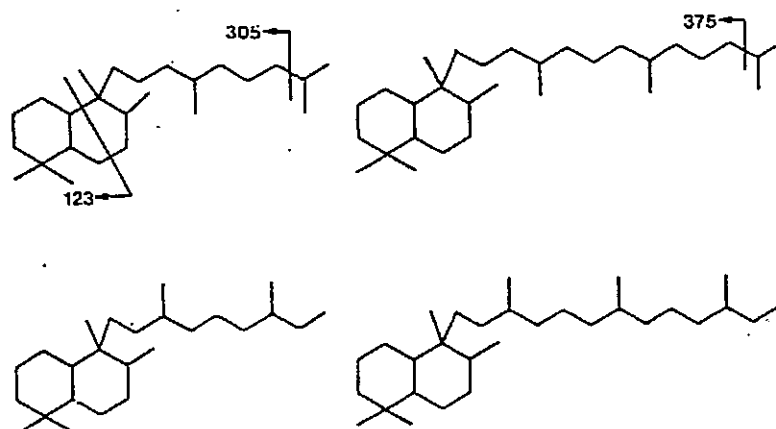
Again, neither predicted fragmentation fits exactly the mass spectra of c25:0:2 and c30:0:2. In addition, the mass spectrum of the bicyclic C_{16} alkane assigned the structure (88) by Noble (1986) contains an intense $(M^{+\bullet}-CH_3)$ ion through the loss of an unassigned methyl group; this is not observed in the mass spectra of c25:0:2 or c30:0:2.

In summary, it has not been possible to definitively assign structures to the hydrogenation products (c25:0:2;2124 and c30:0:2;2550) of the reported sedimentary bicyclic C_{25} diene (c25:2:2;2097) and the bicyclic C_{30} triene and tetraene (c30:3:2;2558 and c30:4:2;2590). They evidently do not possess the carbon skeletons of the 'extended' C_{25} and C_{30} drimanes (11-tetrahydrogeranyldrimane and 11-hexahydrofarnesyldrimane; 80 and 81) synthesised during the current investigation. However, this new data has allowed speculation of several other possible structures. The observed difference in retention behaviour between the synthetic bicyclic alkanes and the sedimentary hydrogenated bicyclic alkanes (see section 3.3) suggests that the latter have a greater degree of side chain branching.

The bicyclic hydrocarbons c25:2:2;2097, c30:3:2;2558 and c30:4:2;2590

were not observed in any of the sediments examined as part of this
study (Table 4:3) although a hydrocarbon exhibiting an identical mass
spectrum to that of c25:2:2;2097 (Requego and Quinn, 1983a; but a
different retention index, RI2083) was observed in the 'aliphatic
hydrocarbon' extract of Milford Haven. Since hydrogenation gave no
trace of a hydrocarbon displaying the mass spectrum or retention index
of the expected hydrogenation product (c25:0:2;2124) it was proposed
that the hydrocarbon RI2083 was converted to br25:0 and therefore
represented a tetra (br25:4) -unsaturated analogue of 2,6,10,14-
tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; 70). The mass
spectral similarities between RI2083 and c25:2:2;2097 suggests that the
latter is also a br25:4. If so, then the assumed hydrogenation product
(sic) c25:0:2;2124 must arise from some unidentified precursor. This
highlights the problems associated with the interpretation of gas
chromatograms obtained upon hydrogenation of complex hydrocarbon
assemblages.

Requego and Quinn (1983a) reported the identification in sediments of
Narrangansett Bay (U.S.A) of a bicyclic C_{30} diene (c30:2:2;2499)
whose mass spectrum was noted as similar to that of the co-occurring
c25:2:2, c30:3:2 and c30:4:2 reported previously. However,
hydrogenation yielded two diastereoisomeric bicyclic C_{30} alkanes
(c30:0:2;2529 and c30:0:2;2550) which had very different spectra to
that of the c30:0:2;2550 reported previously (Prahl et al., 1980).

Comparison of the mass spectra (Fig. 1:18C) of the diastereoisomeric
bicyclic C_{30} alkanes reported by Requego and Quinn (1983a) with those
of other geologically occurring bicyclic alkanes indicates to this
author that the hydrocarbons may have 'rearranged' bicyclic structures

(e.g. <u>89</u>) (Kargramanova <u>et al</u>., 1976; Vorob'eva <u>et al</u>., 1978; Noble, 1986) since spectra of such alkanes contain abundant m/z 193 ions due to favourable alkyl group cleavage at C8 (Noble, 1986). Thus, the following structure isomeric at C8 can be proposed for the bicyclic C_{30} alkanes (c30:0:2;2529 and c30:0:2;2550):
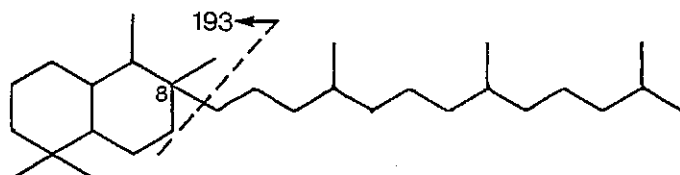
The fragmentation pattern expected for the proposed structures would fit that observed although it is difficult to rationalise the origin of the m/z 389 fragment ion (Fig. 1:18C). Unfortunately, it is not possible to compare the difference in retention indices observed for the two diastereoisomeric C_{30} bicyclic alkanes with those observed for structures <u>89</u> and <u>90</u> (also isomeric at C8) as the latter were not reported.. The position of the asymmetric carbon at C8 would place some constraints on the location of the double bonds in the precursor bicyclic C_{30} diene (c30:2:2) i.e.
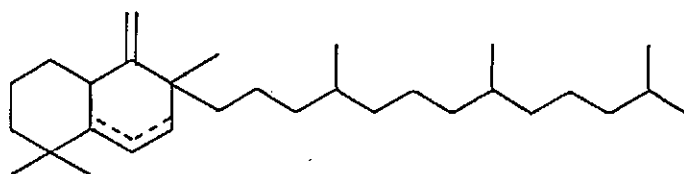
The proposed assignment must await confirmation by synthesis.

It is obvious that there is considerable confusion in the literature both over the exact relationship between alkenes and their presumed hydrogenation products and over the structures of these compounds. Much of this confusion has been caused by workers who have been too eager to assign structures in the absence of reference compounds. For example, Requego and Quinn (1983a) compared geographic variations in the sedimentary abundance of c25:2:2 and c30:2:2 with measurements of the $\delta^{13}C$ values of the sediment. The measurements inferred a marine planktonic origin for c25:2:2 and a bacterial origin for c30:2:2 which the authors found difficult to rationalise considering the presumed structural homology. Since it now appears that there may be no structural homology between c25:2:2 and c30:2:2 and that perhaps c25:2:2 is not even cyclic it is not surprising that the hydrocarbons were found to have different sources.

4.5 Possible origins for the sedimentary acyclic and cyclic C_{20}, C_{25} and C_{30} hydrocarbons

It is generally accepted that the occurrence of the acyclic and cyclic C_{20}, C_{25} and C_{30} hydrocarbons in Recent sediments can be attributed to a biogenic origin. The identification of these hydrocarbons in sediments from this study (i.e. Gluss Voe) which display no evidence of contamination from petroleum hydrocarbons agrees with this general prognosis. There have been no reports of the identification of any of the cyclic hydrocarbons in organisms and it is surprising, considering the almost ubiquitous occurrence of the acyclic hydrocarbons in Recent sediments, that there is only one report of their identification in organisms. Rowland et al. (1985) reported the identification of br20:0;1708 (66), br20:1;1702 (73) and br25:2;2084 in field specimens of the green alga Entereomorpha prolifera which occurs

widely in both freshwater and marine aquatic environments. It was
noted that the alga examined were not euxinic cultures and that the
hydrocarbons may not have been biosynthesised by the alga but by
associated epiphytes.

Requego and co-workers (1983a, 1984 and 1985) observed a good
correlation between the geographic sedimentary abundance of the cyclic
hydrocarbons c25:2:2;2097 and c25:1:1;2079 (the latter proposed herein
to be a br25:2;2079) and the distribution of marine organic matter (as
measured by δ^{13}C values) and proposed the hydrocarbons derived from a
marine planktonic source. In this regard it is interesting to note
that the mass spectra of the br25:2;2084 identified by Rowland et al.
(1985) in Entereomorpha prolifera, and the br25:2;2079 inferred by
Requego and Quinn (1983a) to have a marine planktonic origin, are
identical. The inferred planktonic source for c25:2:2;2097 agrees well
with the identification of the apparently structurally related (Barrick
and Hedges, 1981) c30:3:2;2558 and c30:4:2;2590 in particulate matter
and mixed zooplankton samples (November) collected from Kiel Bight
(F.R.G., Osterroht et al., 1983). It was suggested these compounds
were derived from plankton species appearing late in the yearly
succession, such as Dinophyceae and Bacillariophyceae. The results of
Osterroht et al. (1983) were in good temporal agreement with those of
Prahl et al. (1980) who observed c30:3:2;2558 in mixed plankton samples
(November) from Dabob Bay (U.S.A). The acyclic C_{25} hydrocarbons,
br25:3;2090, br25.3;2044, br25:4;2078 and br25:4;2124 have been
identified in sediment trap particulates collected from the Peru
Upwelling region above surface sediments in which the compounds are the
dominant hydrocarbons (Volkman et al., 1983). The acyclic C_{25}
trienes and tetraenes were not detected in any samples of mixed

zooplankton although their identification in zooplankton fecal matter suggested an origin from zooplankton food, i.e. phytoplankton. Thus there appears to be considerable, though disseminated evidence that these compounds have an algal source.

The ubiquitity of sedimentary occurrences of the acyclic hydrocarbons has caused several investigators to propose a bacterial source. Requego et al. (1984) observed that the distribution of br25:2;2084 in Pettaquamscutt River (U.S.A) sediments was independent of source (as inferred from $\delta^{13}C$ data) and proposed a bacterial origin. The sub-surface concentration maxima exhibited by the br25:2;2084 further suggested a bacterial in situ production. The occurrence of the acyclic C_{25} triene, br25:3;2107 in the bound hydrocarbon extract of black sapropel muds from the Los Monegros hypersaline lagoons (Spain) was associated with the identification of several hydrosqualanes indicating a common microbial origin, possibly from methanogenic bacteria in which hydrosqualenes are known constituents (Albaiges et al., 1984a). A bacterial origin has also been proposed for the bicyclic C_{30} diene (c30:2:2;2499) (Requego and co-workers, 1983a and b, 1984, 1985) proposed herein to have the carbon skeleton decribed above (94). For example, Requego et al. (1984) observed that the geographical distribution of c30:2:2;2499 was independent of sediment organic matter type indicating an origin from bacterial in situ production. A subsurface maximum in the sedimentary c30:2:2;2499 concentration profile further suggested a bacterial origin. If the c30:2:2;2499 does have structure (94) and a biogenic origin through bacterial synthesis this begs some interesting questions concerning the occurrences of 'rearranged' bicyclic alkanes in older geological materials.

Dunlop and Jefferies (1985) reported differences in the distribution of acyclic C_{20} and C_{25} hydrocarbons between oceanic and hypersaline waters of Shark Bay (Australia). In particular, the concentration of the br25:1;2112 (74) was shown to decrease from 161 ppb (dry sediment) in surface sediments below hypersaline (i.e. 65‰ S) waters to only 3 ppb (dry sediment) in the surface sediments underlying the oceanic waters. Associated with the decrease in the relative concentration of br25:1;2112 was an increase in the concentrations of related br25 dienes and trienes. It was reported that the chemically distinct regions were not aligned with the macrobiotic seagrass communities indicating that the acyclic C_{25} hydrocarbons were derived from another source, possibly Archaebacteria. The variation in hydrocarbon distribution between the hypersaline and oceanic waters could either reflect differences in the type of organisms present or more interestingly, the biosynthetic ability of similar source organisms in the two distinct environments to vary the degree of unsaturation of the br25 hydrocarbons according to the salinity apparent at each location. The increase in unsaturation with decreasing salinity may reflect changes in the bouyancy requirements of the organisms present. Interestingly, the hydrocarbon br25:1;2112 was only detected in one of the sediments examined during the present study (Bardawil lagoon, Egypt), a sample which also has a salinity greater than oceanic water (S. Howells - personal communtication).

The unsaturation of some organic molecules has been shown to reflect sea-surface temperatures but this does not seem to have been considered for the present compounds. Brassell et al. (1986b and d) reported that the unsaturation in long chain n-alkenones (C_{37} - C_{39}) of marine coccolithophorids decreased with increasing sea water temperature and

demonstrated that measurement of changes in unsaturation of sedimentary n-alkenones had potential as a molecular stratigraphic tool for paleoclimatic assessment. The possibility that the degree of unsaturation of the sedimentary acyclic br20, br25 and br30 hydrocarbons reflects water temperature cannot be discounted.

Variations in the degree of unsaturation of isoprenoids with oxygen content has also been reported. Tornabene (1978) observed that the degree of unsaturation in the C_{20}, C_{25} and C_{30} acyclic isoprenoid hydrocarbons (h-h and t-t) present in neutral lipids of Archaebacteria was controlled by the oxygen content of the growing media.

Requego et al. (1984) reported that the subsurface concentration maxima observed for acyclic C_{25} (br25:3;2091, br25:2;2070, br25:2;2084 and br25:2;2088) and cyclic C_{30} (c30:2:2;2499) hydrocarbons in sediments of the upper anoxic basin of the Pettaquamscutt River (U.S.A) correlated with an increase in pore-water sulphide suggesting that sulphate-reducing bacteria of the genus Desulphovibrio were a potential source of these hydrocarbons. Whilst it was noted that previous examinations of the lipid content of sulphate-reducing bacteria (i.e. Han and Calvin, 1969) had not revealed the presence of these compounds, it was suggested, as most of these reports were conducted 15 years previously, that re-examination of the bacterial lipids using high resolution gc and gcms might be worthwhile. The possibility of a relationship between the C_{20}, C_{25} and C_{30} hydrocarbons and sedimentary sulphur agrees well with the reported occurrences of br20:0 in Ancient sediments and petroleums (Yon, 1982) and with the identification of a sulphur analogue of br20:0 (i.e. 76) in a sulphur-rich evaporitic sediment (Sinninghe Damste et al., 1986).

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The sedimentary abundance of the br25:0 (70) and br30:0 (75) alkanes appears to be lower than that of the br20:0 (66) alkane. Of the three, only br20:0 has an identified source (Entereomorpha prolifera; Rowland et al., 1985). The br25:0 and br30:0 alkanes of sediments may represent sedimentary diagenetic products of their associated alkenes.

In summary, the only definite identification of the sedimentary acyclic hydrocarbons is in Entereomorpha proflifera from which three hydrocarbons with the carbon skeletons of br20:0 (66) and br25:0 (70) were isolated. Various other sources for the acyclic hydrocarbons have been proposed including marine planktonic (phytoplankton, zooplankton), bacteria and Archaebacteria. There appears to be good correlation between the occurrence of c25:2:2 (sic; br25:4), c30:3:2 and c30:4:2 with the sedimentary abundance of marine organic matter. The distribution of certain acyclic and cyclic hydrocarbons may reflect the sulphur contents of sedimentary environments and the extent of unsaturation in the br20, br25 and br30 hydrocarbons may be related to environmental parameters such as salinity, water temperature or oxygen content of water/sediments. All of these theories require further investigation.

CHAPTER FIVE

INVESTIGATIONS INTO THE BIODEGRADATION
OF NORMAL, ISOPRENOID AND HIGHLY BRANCHED
HYDROCARBONS

5:1 INTRODUCTION

It is evident from the preceding discussions that the acyclic br20, br25 and br30 hydrocarbons are not only geographically widespread (see Table 4:1) but also, at several locations, the abundance of the hydrocarbons exceeds that of co-occurring biogenic hydrocarbons. For instance, in coastal sediments from the Eastern Gulf of Mexico, Gearing et al. (1976) observed that alkenes with the carbon skeletons of br20:0 (66) and br25:0 (70) accounted for up to 50% of the resolvable aliphatic hydrocarbons. Similarly, in surface sediments from the Peru Upwelling region, alkenes with the carbon skeleton of br25:0 were far more abundant (i.e. 26.6 ppm dry sediment, 0-2 cm) than n-alkanes of higher plant origin (i.e. 6.9 ppm dry sediment, 0-2 cm), or isoprenoid hydrocarbons such as pristane and cholestenes thought to derive from planktonic inputs (Volkman et al., 1983). Few explanations for this have been forwarded.

It has previously been mentioned that the only reported biological source of the br20 and br25 hydrocarbons is the green macroalga Entereomorpha prolifera (Rowland et al., 1985). No quantitative information was available from that study but it was noted that the major hydrocarbons present in the alga were not the br20 and br25 hydrocarbons but n-alkenes (notably $n-C_{17:1}$, n-heptadecene). None of the sediments containing high concentrations of the acyclic br20 and br25 hydrocarbons (e.g. Peru; Volkman et al., 1983) exhibited similar high concentrations of n-heptadecene, although $n-C_{17:1}$ was present in small amounts in several of the sediments in which the branched hydrocarbons were found (e.g. Bayona et al., 1983 and Chapter 4, this study). Thus, there appears to be some discrepancy between the sedimentary abundance of the acyclic highly branched hydrocarbons and

their biological distributions; this may seem to cast doubt on whether E. prolifera is the source of br20 and br25 hydrocarbons in sediments. However, it has been suggested (Rowland et al., 1985) that a factor which may alter the E. prolifera distribution so that it is more like that found in sediments is preferential biodegradation of n-$C_{17:1}$ and other n-hydrocarbons. Whilst investigations into the biodegradation of petroleum-type alkanes are numerous (i.e. Atlas, 1984; Oudot, 1984; reviewed by Jones, 1986) there is comparatively little information available on the relative suceptibility of normal and branched alkenes to biodegradation. The present study was thus designed to provide experimental data to test the hypothesis that the relative abundance of acyclic br20 and br25 hydrocarbons is influenced by biodegradation.

The first experiment compares the biodegradation rates of normal alkanes, isoprenoid (h-t, t-t) alkanes and the synthetic highly branched br20, br25 and br30 alkanes. The second experiment compares the biodegradation rates of normal alkenes and the synthetic mixtures of isomeric br20:1 (77) and br25:1 (78) monoenes. Compositional details for the two experiments and data on the starting concentrations of the individual hydrocarbons are presented in Tables 2:1 (alkanes) and 2:2 (alkenes). Although the bacterial species chosen for this biodegradation study, Pseudomonas aeruginosa, is not a common bacterium in soils (Prof. L. Heath; personal communication), it has been used previously in several investigations of hydrocarbon degradation (reviewed by Atlas, 1984) and is a common bacterium in petroleum products and oil emulsions. The temperature chosen for the incubations (20°C) was a compromise between the optimum growth temperature (37°C) of P. aeruginosa and the much lower temperatures present in the average sedimentary environment.

Table 5:1

Daily concentrations (μgcm^{-3}) of individual hydrocarbons in alkane biodegradation experiments.

Day	n-C$_{20}$	n-C$_{25}$	n-C$_{30}$	i20	i25	i30	br20.0	br25.0	br30.0
0	10.0	11.2	10.2	10.3	10.0	7.9	10.4	11.6	13.4
1	10.5	11.5	10.4	10.5	9.6	8.1	10.2	11.3	13.4
2	7.4	10.3	10.6	10.0	9.6	7.8	9.6	11.1	13.4
3	3.9	6.8	7.6	9.4	9.1	7.8	9.6	10.5	13.4
4	3.0	4.2	5.4	10.0	9.3	7.7	9.2	11.1	13.4
5	2.1	3.6	4.7	9.7	9.2	7.8	8.7	11.1	13.4
6	1.3	2.5	4.1	9.2	9.6	7.9	8.2	11.2	13.4
7	1.3	1.3	3.3	9.4	9.6	7.8	8.7	11.5	13.4
8	1.0	2.0	1.8	9.3	9.5	7.6	8.5	11.3	13.4
9	--	- -	- -	Not available		- -	- -	- -	- -
10	0.6	1.2	1.2	8.8	9.4	7.9	8.1	11.1	13.4
11	0.0	1.0	0.9	8.5	9.4	7.8	7.4	11.0	13.4
12	0.0	1.0	1.1	9.1	9.5	7.9	8.2	11.1	13.4
13	0.0	1.2	1.6	8.9	9.5	7.7	6.9	11.1	13.4
14	0.0	1.1	1.2	8.9	9.5	7.9	7.3	10.9	13.4
21	0.0	0.8	1.3	8.4	9.6	7.7	6.2	11.2	13.4
28	0.0	0.0	1.0	8.0	9.5	7.7	5.0	11.1	13.4
14(S)*	9.6	11.2	10.1	8.9	9.6	8.0	7.3	11.2	13.4

*14(S) - Reference - No Bacterial Inoculum   Structure (Fig. 5:1)

n-C$_{20}$	n-eicosane	A
n-C$_{25}$	n-pentaeicosane	B
n-C$_{30}$	n-tricontane	C
i-20	2,6,10,14-tetramethylhexadecane	D
i-25	2,6,10,14,18-pentamethyleicosane	E
i-30	2,6,10,15,19,23-hexamethyltetracosane	F
br20:0	2,6,10-trimethyl-7-(3-methylbutyl)dodecane	G
br25:0	2,6,10,14-tetramethyl-7-(methylpentyl)pentadecane	H
br30:0	2,6,10,14,18-pentamethyl-7-(3-methylpentyl)nonadecane	I

Experimental error: ± 11%

Table 5:2

Daily concentrations (μgcm^{-3}) of individual hydrocarbons in alkene
biodegradation experiments.

(Figures in parentheses are expressed as a percentage of total br20:1 or br25:1 monoenes)

Day	n-C$_{17:1}$	br20:1					Total	n-C$_{20:1}$	br25:1					Total
		RI1679	RI1689	RI1690	RI1711	RI1714			RI2078	RI2085	RI2092	RI2116	RI2125	
0	11.6	10.5 (45.26)	4.8 (20.68)	4.7 (20.26)	1.7 (7.33)	1.5 (6.47)	23.2	11.6	8.3 (43.46)	4.2 (21.99)	4.3 (22.51)	1.2 (6.28)	1.1 (5.76)	19.1
1	10.8	7.7 (44.45)	3.6 (20.8)	3.5 (20.32)	1.2 (6.96)	1.3 (7.51)	17.3	9.9	7.5 (41.35)	4.2 (23.2)	4.2 (23.2)	1.1 (6.08)	1.1 (6.08)	18.1
2	8.2	7.6 (45.23)	3.5 (20.83)	3.4 (20.23)	1.2 (7.14)	1.1 (6.54)	16.8	6.7	7.4 (43.54)	3.7 (21.77)	3.9 (22.94)	1.0 (5.88)	1.0 (5.88)	17.0
3	5.3	7.5 (45.45)	3.4 (20.6)	3.2 (19.39)	1.2 (7.27)	1.2 (7.27)	16.5	4.3	7.1 (42.77)	3.8 (22.89)	3.7 (22.23)	1.0 (6.02)	1.0 (6.02)	16.6
4	3.0	6.0 (44.44)	2.8 (20.7)	2.7 (20)	1.0 (7.4)	1.0 (7.4)	13.5	3.1	7.4 (42.43)	3.8 (21.84)	4.1 (23.56)	1.1 (6.32)	1.0 (5.75)	17.4
5	1.6	5.3 (44.92)	2.5 (21.19)	2.4 (20.33)	0.8 (6.78)	0.8 (6.48)	11.8	1.3	6.6 (43.14)	3.4 (22.22)	3.5 (22.88)	0.9 (5.88)	0.9 (5.88)	15.3
10	1.0	1.9 (44.18)	0.9 (20.93)	1.0 (23.26)	0.3 (6.98)	0.2 (4.63)	4.3	1.2	5.6 (43)	2.9 (22.31)	3.0 (23.08)	0.7 (5.38)	0.8 (6.15)	13.0
10S	8.4	8.0 (43.24)	4.0 (21.62)	3.9 (21.08)	1.3 (7.02)	1.3 (7.02)	18.5	10.1	8.0 (43.24)	4.1 (22.16)	4.2 (22.7)	1.1 (5.95)	1.1 (5.95)	18.5

n-C$_{17:1}$ n-heptadec-1-ene

n-C$_{20:1}$ n-eicos-1-ene

br20:1   2,6,10-trimethyl-7-(3-methylbutyl)dodecenes

br25:1   2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecenes

5.2 RESULTS AND DISCUSSION

Biodegradation experiments were performed with a single bacterial species (Pseudomonas aeruginosa) and known concentrations of synthetic hydrocarbons in order that degradation rates could be measured. Normal, isoprenoid (h-t and t-t) and highly branched (i.e. br) C_{20}, C_{25} and C_{30} alkanes (e.g. Fig. 5:1; A, D and G) were used so that variations in degradation rates could be ascribed to either molecular structure (e.g. by comparing rates within the C_{20} alkanes) or to differences in molecular weight by comparing the degradation of C_{20} with C_{25} and C_{30} alkanes. The changes in concentration (μgcm^{-3}) of the individual hydrocarbons as a consequence of increasing biodegradation are shown in Tables 5:1 (alkanes) and 5:2 (alkenes). The graph in Fig. 5:2A compares the rate of degradation of the total (i.e. C_{20} + C_{25} + C_{30}) normal, isoprenoid and highly branched alkanes and Fig. 5:3 shows the gas chromatograms of the alkane mixture after 28 days (sterilised) and 28 days biodegradation. Clearly, under the conditions used, the n-alkanes were rapidly biodegraded and after 28 days only very low concentrations remained. Further examination of Fig. 5:2B indicates that the biodegradation rates of n-C_{20}, n-C_{25} and n-C_{30} are similar although it appears that the lag phase for n-C_{30} is longer than that of n-C_{20} and n-C_{25}. It is apparent from Fig. 5:2A that no effect attributable to biodegradation is observed in any of the isoprenoid or highly branched (br) isoprenoid hydrocarbons. The depletion in the concentrations of these hydrocarbons was due solely to abiological evaporation as shown by the similar depletion in the sterilised controls (see also Fig. 5:3). Thus, under these conditions the highly branched hydrocarbons are at least as resistant to biodegradation as the regular 'h-h' and 't-t' isoprenoids. The latter are well known to be more resistant to biodegradation than n-
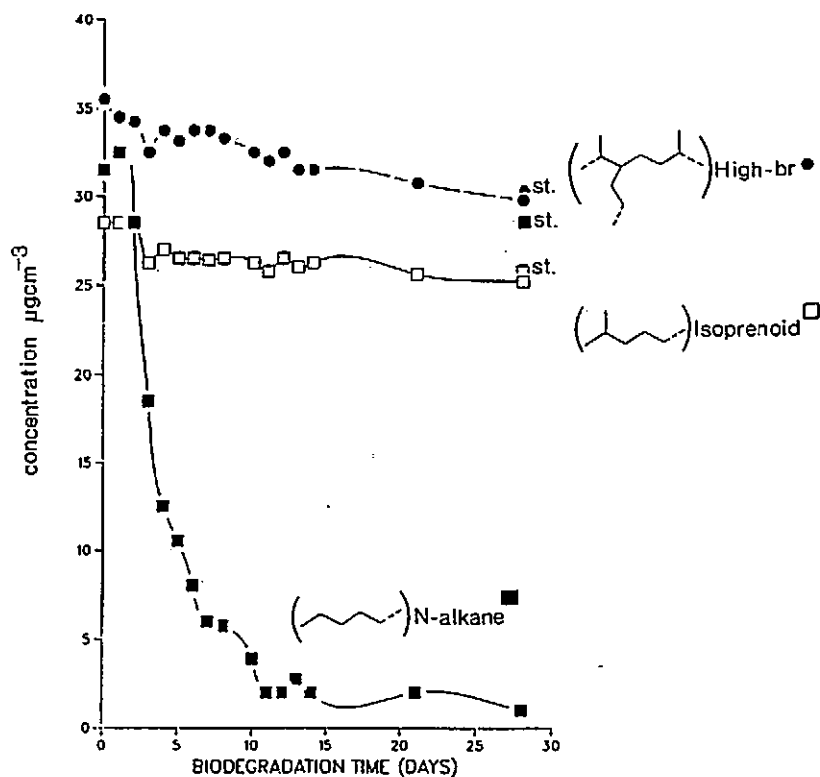
Fig.5:2A   Graph showing the relative biodegrada-
            tion rate of total n-alkanes compared
            to total isoprenoid and total high -
            br alkanes.   For details of individual
            components see Fig.5:1 and Table 5:1.
            st = sterilised.
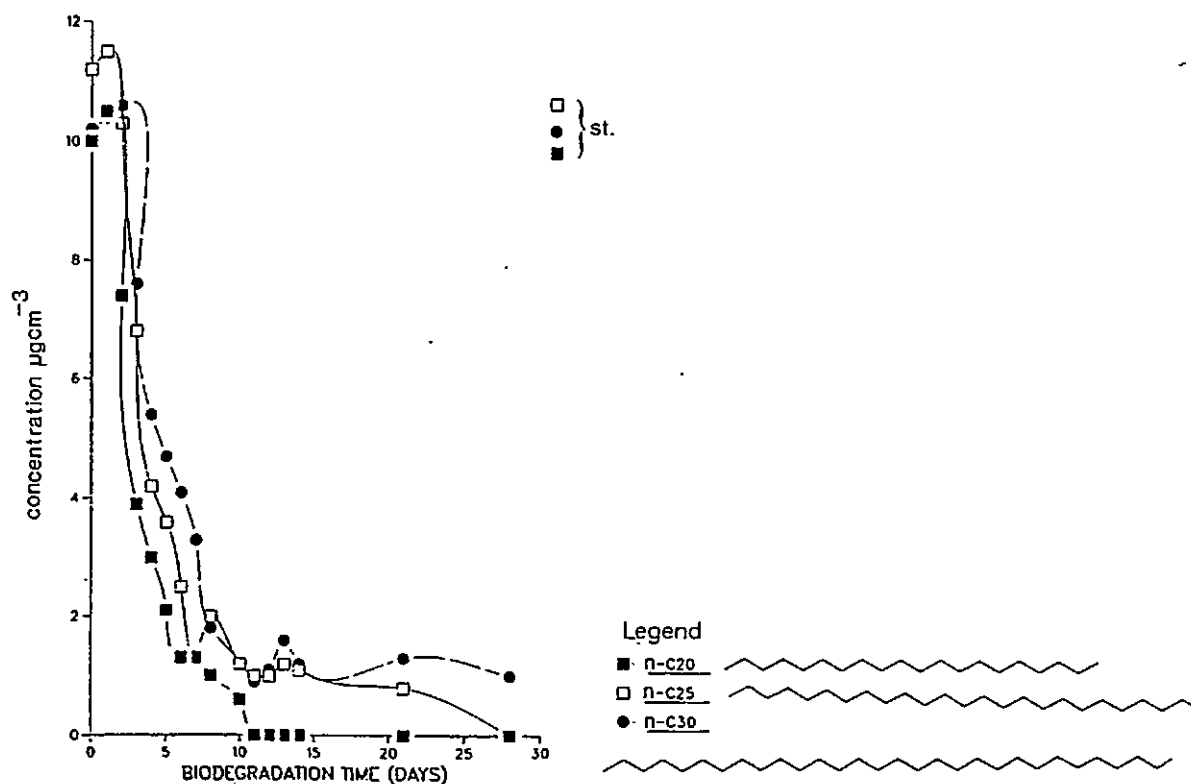
Fig.5:2B   Graph illustrating the relative bio-
            degradation of the individual n-alkanes
            used in the biodegradation studies.
            st = sterilised.

Fig. 5:1 Structures of the various alkanes used in the biodegradation experiments.
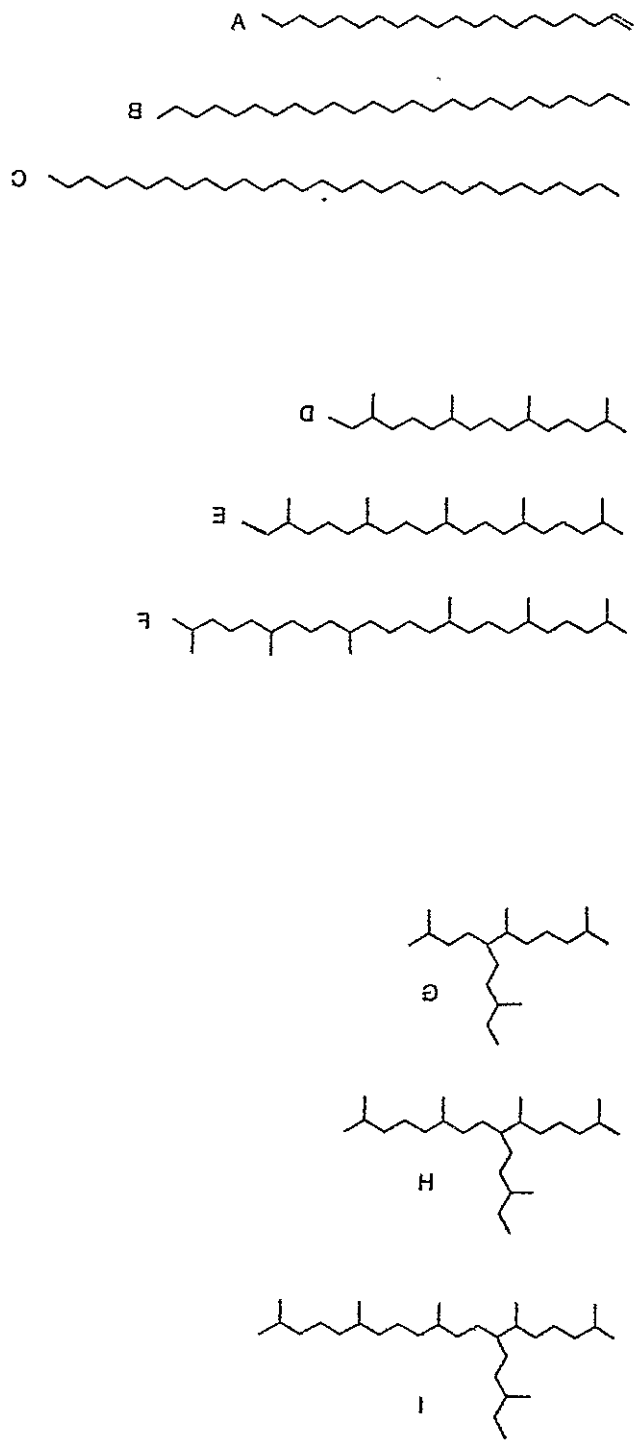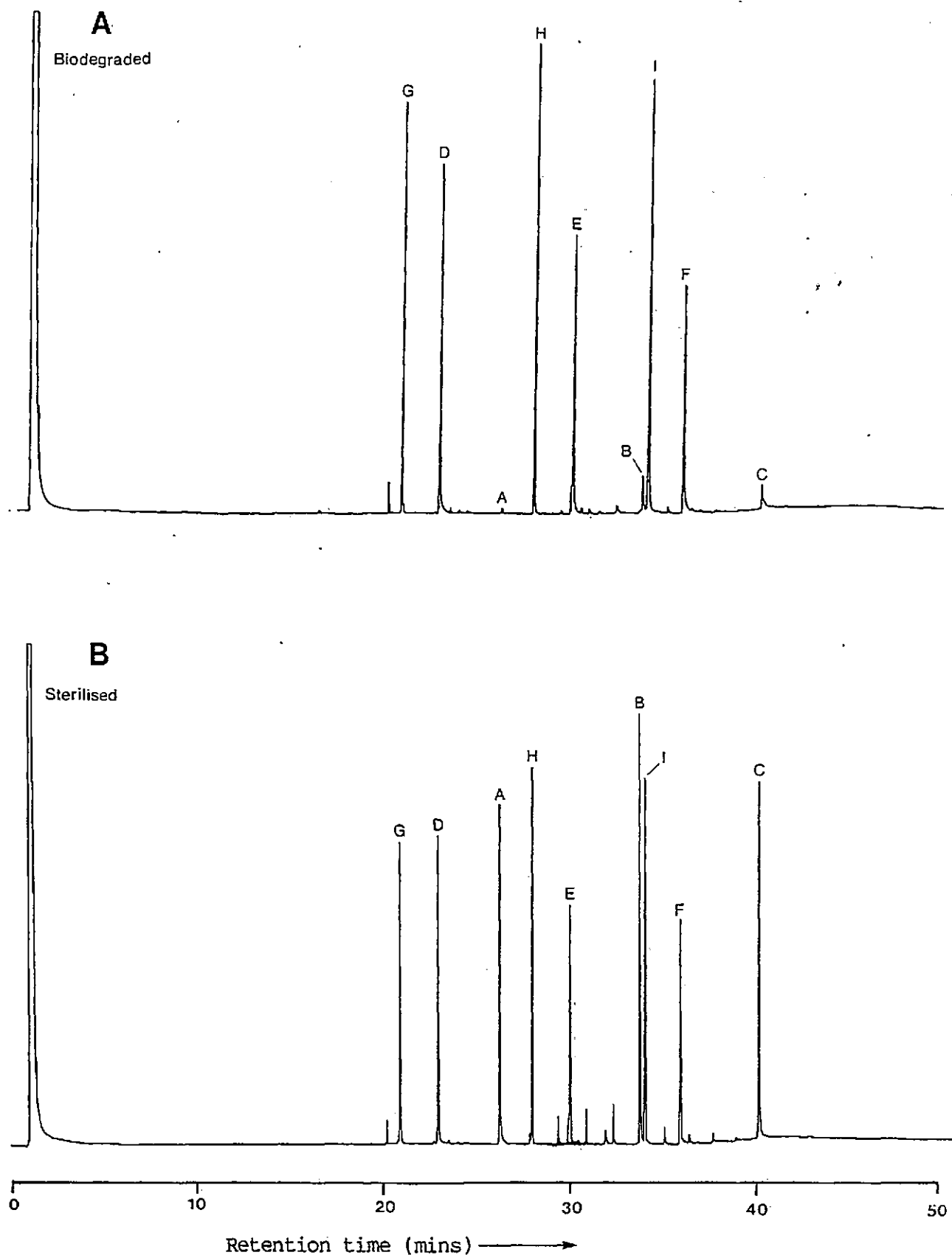
Fig.5:3   Gas chromatogram (A) of the alkane biodegradation mixture after 28
days biodegradation.   Chromatogram (B) represents the sterilised
control.   For identification of lettered peaks see Fig.5:1.
Gc conditions: OV1, 40 – 80°C at 10°Cmin^{-1} and 80 –290°C at 6°Cmin^{-1}.

alkanes (e.g. Oudot, 1984).

The recalcitrance of the highly branched alkanes may help to explain the abundance of, for example, br20:0 (66) in certain samples. Yon (1982) showed that br20:0 was the major hydrocarbon in lacustrine sediments from Grasmere, U.K. and it is the second most abundant alkane (phytane being major) in an unusually viscous, high-sulphur crude oil from Rozel Point, Utah, U.S.A. (Yon et al., 1982; see Fig. 5:8). Whilst it is likely that other factors, such as variable contributions of br20:0 or its precursors to the sediment at time of deposition, contribute to the high abundance of br20:0 in these and other sediments, the current data indicates that biodegradation could also be an influence. It was noted in the preceding Chapter that the br25:0 (70) and br30:0 (75) alkanes do not appear to be as abundant in sediments as br20:0. The absence of any report of a biological source for the br25 and br30 alkanes and the low concentrations of br25:0 in only a few sediments suggests they may not be biosynthesised in quantity and represent diagenetic products of unsaturated analogues (unlike br20:0 which is found in E. prolifera; Rowland et al., 1985).

Figure 5:4 shows gas chromatograms of the alkene mixture after 0 and 4 days biodegradation. To avoid problems of co-chromatography between normal alkenes and highly branched (i.e. br) alkene isomers it was necessary to use two stationary phases for analysis i.e. CPWAX52 (n-$C_{17:1}$ and br20:1) and OV1 (n-$C_{20:1}$ and br25:1). Fig. 5:5 compares the degradation rates of the normal, br20:0 and br25:0 alkanes with their unsaturated analogues. It appears, over the first 4 days of biodegradation, that the n-alkenes are degraded at a slower rate than the n-alkanes although it is interesting that the n-alkenes do not

4 Days (sterilised)

A

B

4 Days (biodegraded)

C

D

10   12

24   26

Retention time (mins) ⟶
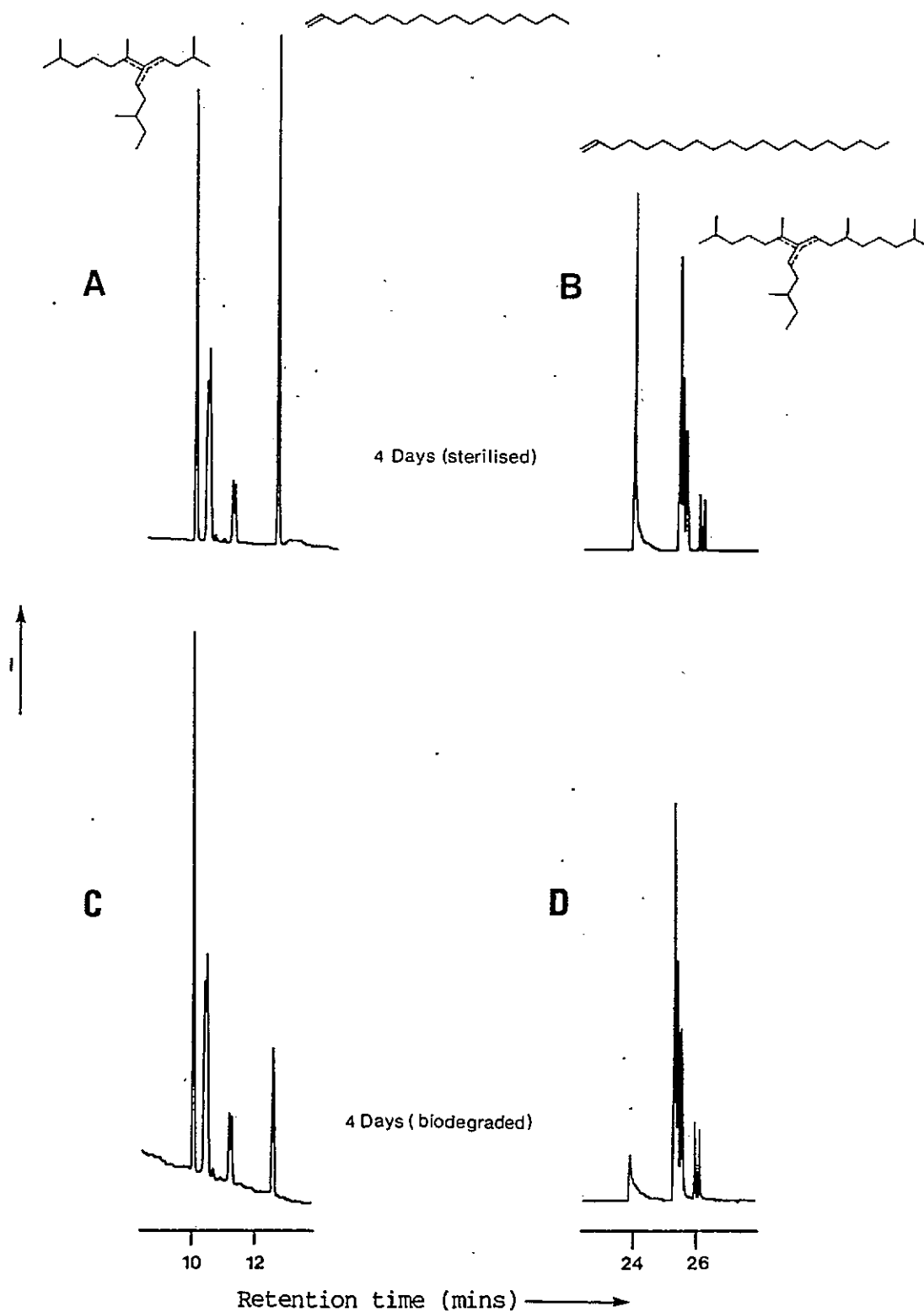
Fig.5:4   Partial gas chromatogram of the alkene
biodegradation mixture after 4 days biodegrada-
tion.   Chromatograms A and B represent the
sterilised control.   Peak identifications as
shown.   Gc conditions: A, C = CPWAX52, programme
as text; B, D = OV1, programme as text.
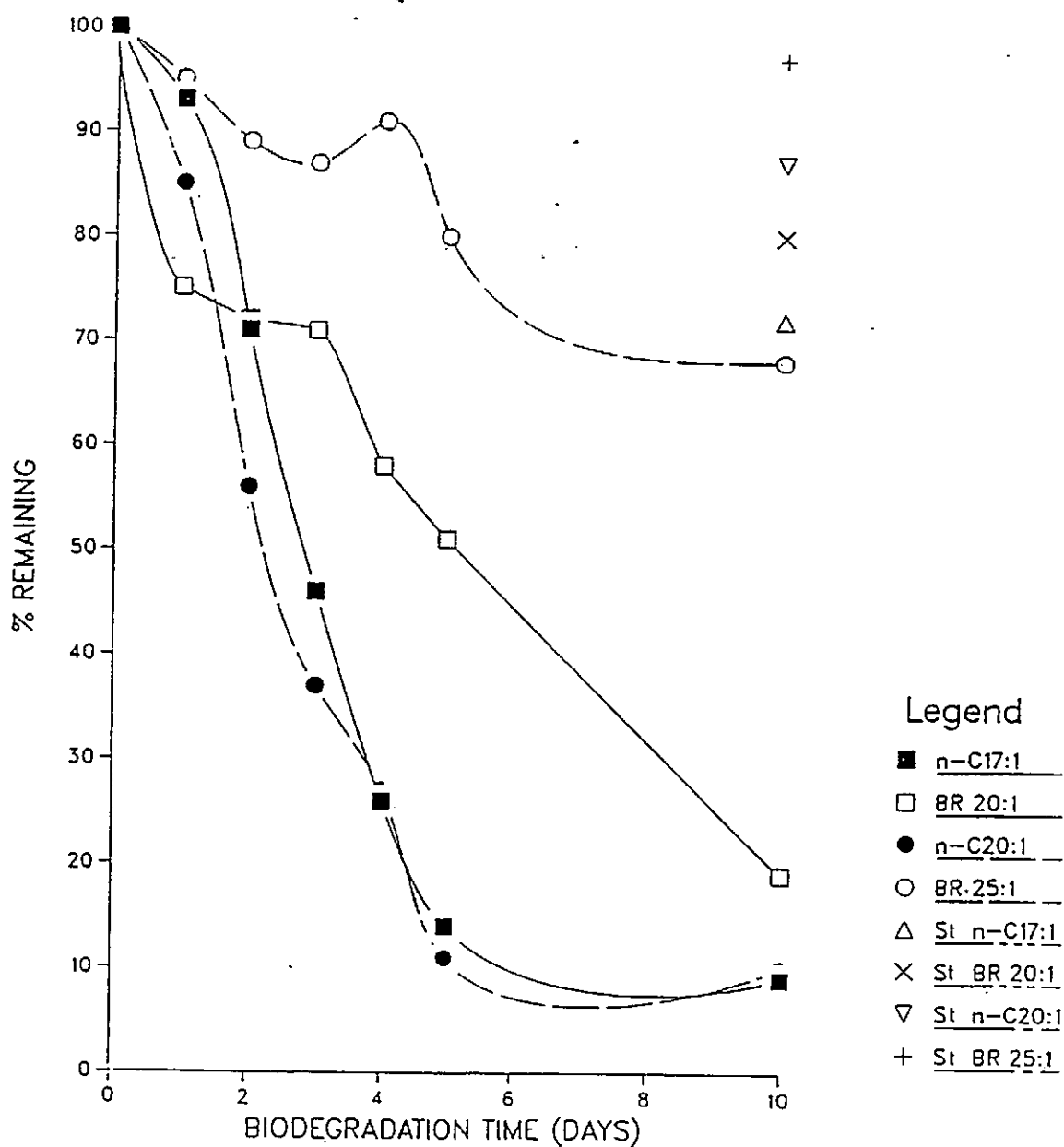
Fig. 5:5   Graph showing the relative biodegradation of
n-heptadecene ($n-C_{17:1}$), n-eicos-1-ene ($n-C_{20:1}$),
highly branched C_{20} alkenes (BR 20:1) and
C_{25} alkenes (BR 25:1). St = sterilised control.

demonstrate a lag phase like the n-alkanes. It is also apparent that under the same conditions of biodegradation, compared to the highly branched alkanes, the highly branched alkenes were considerably degraded within the study period. The depletion is not accounted for solely by abiological losses (e.g. evaporation) as shown by the sterilised control experiments. Although the highly branched alkenes are more recalcitrant than the n-alkenes or alkanes over the first four days of biodegradation (i.e. to almost half concentration) after that period the rate of degradation of all three hydrocarbon classes is similar. As with the n-alkenes, no lag phase is observed in the degradation of the highly branched alkenes. An examination of the data in Table 5:2 indicates that all the isomeric monoenes of br20:1 (77) and br25:1 (78) have the same rate of biodegradation with no preference for any one isomer detected within the limits of experimental error. The effect of molecular weight on biodegradation of the highly branched alkenes is shown by the more rapid removal (Fig. 5:6) of br20:1 compared to br25:1 hydrocarbons.

These results are also useful in explaining some of the sedimentary data. For example, br20 and br25 alkenes (including monoenes such as those used in the present experiments) are the major hydrocarbons of many coastal sediments (e.g. Volkman et al., 1983) and the present data suggests that this may be due in part to the more rapid, preferential biodegradation of co-occurring n-hydrocarbons. A previously unreported example is shown in Fig. 5:7 which displays a chromatogram of the 'aliphatic hydrocarbons' from North Sea surface sediments (for locational details see Johnson et al., 1978). In these sediments, the br25 alkenes (identified by retention position and mass spectra; Dr. S.J. Rowland, personal communication) were the major hydrocarbons
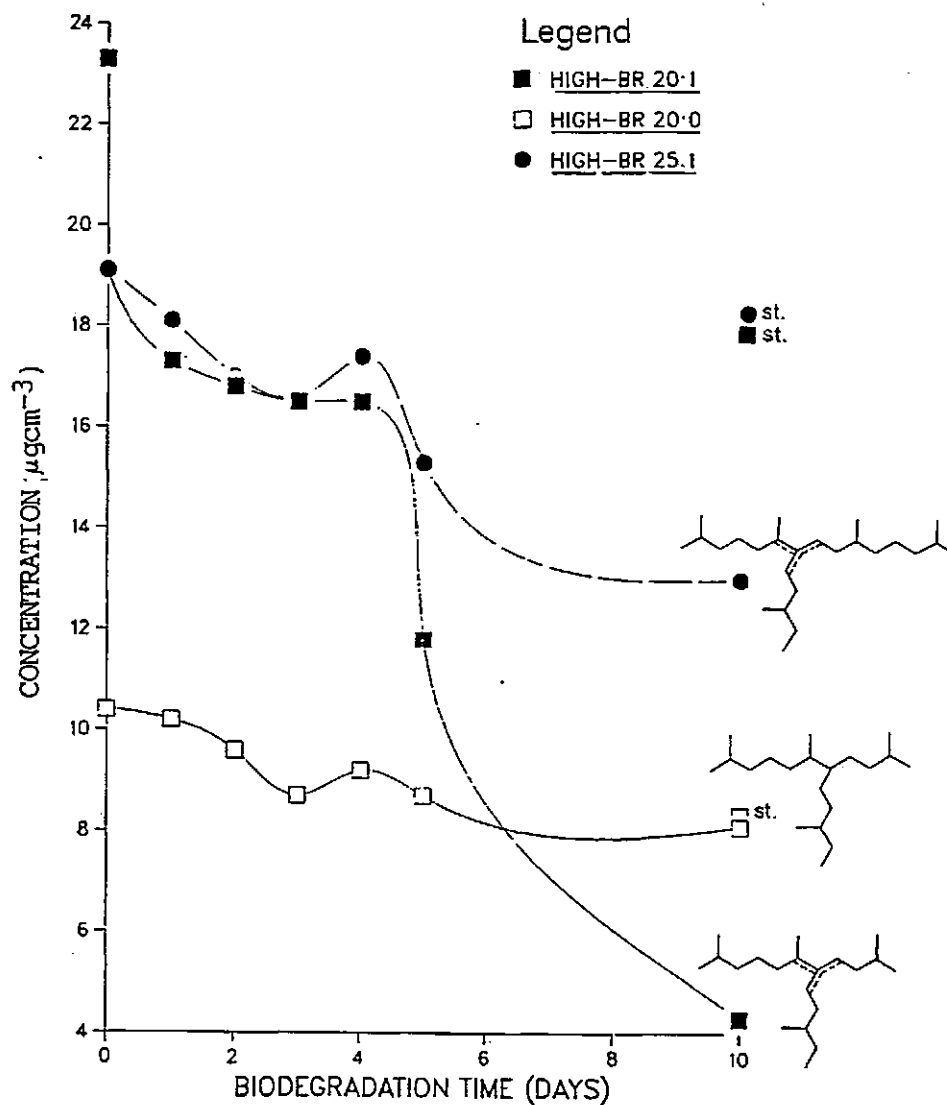
Fig.5:6   Graph illustrating the relative biodegradation
rates of high-br20:0   2,6,10-trimethyl-7-
(3-methylbutyl)dodecane , high br20:1
2,6,10-trimethyl-7-(3-methylbutyl)dodecenes
and high br25:1   2,6,10,14-tetramethyl-7-
(3-methylpentyl)pentadecenes .
st = sterilised.

Retention time (mins) ⟶
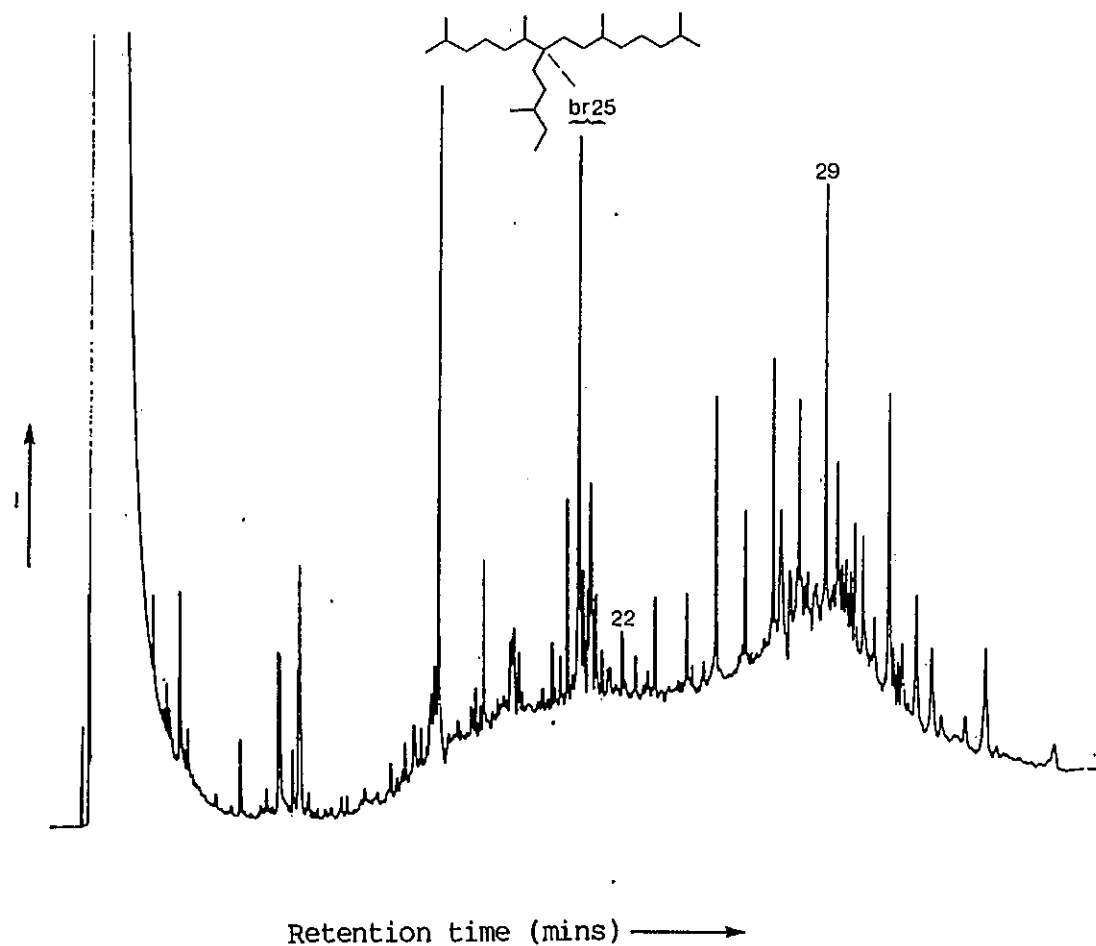
Fig.5:7   Gas chromatogram of the aliphatic hydrocarbons from a North sea sediment
(chromatogram courtesy of Dr. S. J.Rowland).   Numbers refer to the
chain length of the n-alkanes.   Peaks labelled br25 represent
hydrocarbons with the carbon skeleton of 2,6,10,14-tetramethyl-7-
(3-methylpentyl)pentadecane.   Gc conditions: OV1, 40 - 290°C at 4°Cmin^{-1}.
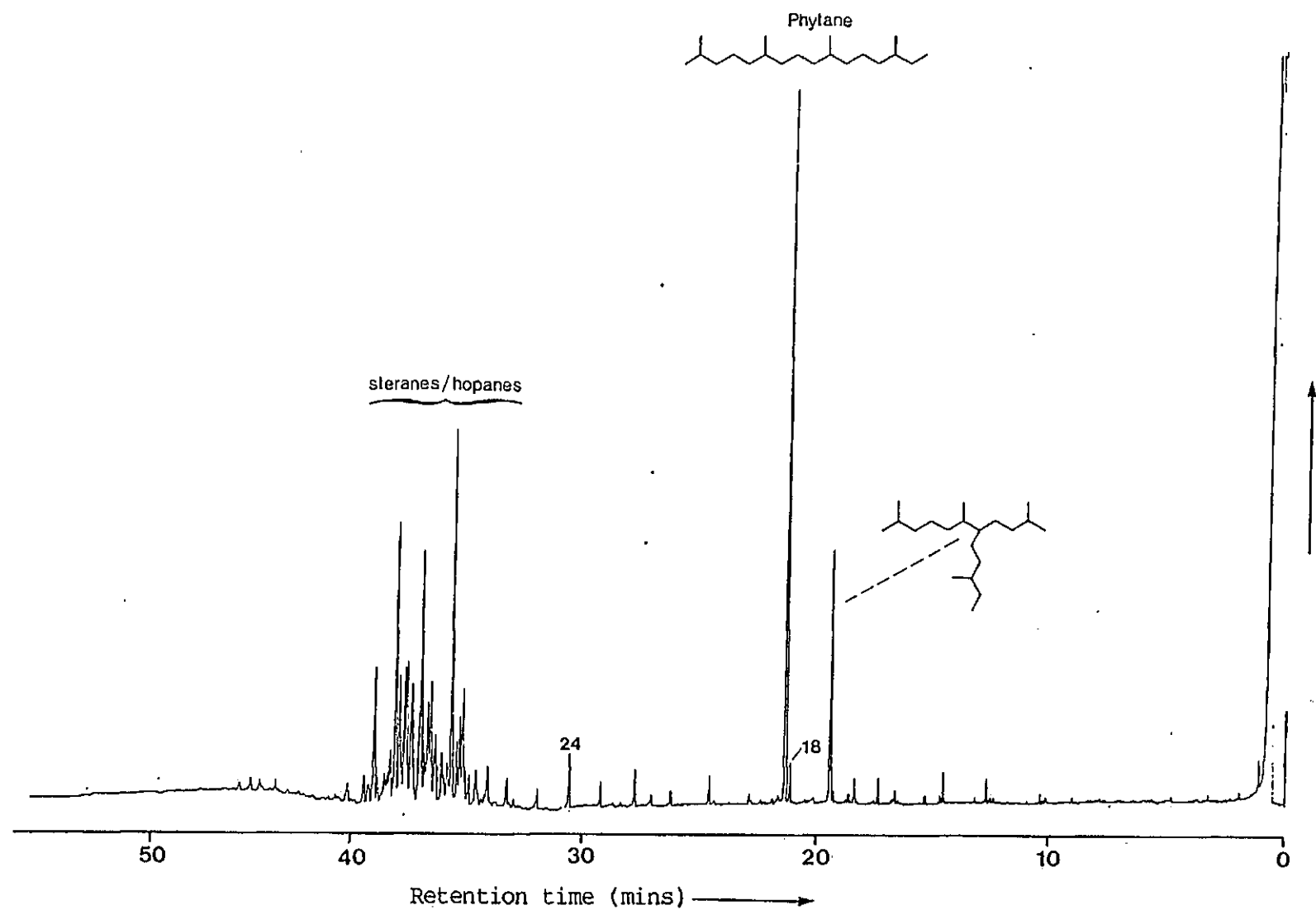
Fig.5:8   Gas chromatogram of the 'aliphatic hydrocarbons' from Rozel Point crude oil. Note
high abundance of br20:0 compared to n-alkanes (numbers refer to chain length).
Gc conditions as text.

present. Such hydrocarbons might have been expected to be less abundant than n-alkanes in these shelf sediments since the latter would probably have quite large fluxes from terrigenous higher plant material and from off-shore oil exploration activities. Clearly, this is not reflected in the relative abundances of the n-alkanes and the highly branched (br25) alkenes and it is suggested that this is in part due to the preferential biodegradation of n-alkanes as indicated in the present biodegradation experiments.

In Puget Sound sediments it was observed that the ratio of the br20:0 (66) alkane to related br25 alkenes (70; 3-4 double bonds) increased with increasing sediment depth (Barrick et al., 1980). This may be partly (or solely) due to preferential biodegradation, this time of the br25 alkenes relative to br20:0. Again this is supported by the data shown in Fig. 5:6 which compares the degradation of br20:0 to br25:1.

A final point of note in Fig. 5:5 is the relative degradation rates of n-heptadec-1-ene (n-$C_{17:1}$) and the highly branched hydrocarbons. A n-$C_{17:1}$ monoene (though not the 1-isomer) is the major hydrocarbon of Entereomorpha prolifera, the only reported biological source of the highly branched hydrocarbons, (Rowland et al., 1985). Yet, as mentioned previously, sediments containing abundant br20 and br25 hydrocarbons rarely contain analogously high concentrations of n-$C_{17:1}$. Again, the faster rate of biodegradation of the n-hydrocarbon (Fig. 5:5) offers an explanation for this disparity. Algal debris derived from Entereomorpha would likely suffer considerable degradation both before, during and after its incorporation into sediments.

5:3 SUMMARY

Laboratory biodegradation experiments involving incubation of synthetic alkanes and alkenes with cultures of the aerobe, Pseudomonas aeruginosa have shown that the different structural types of hydrocarbon suffer different degradation rates. Under the condition used (Pseudomonas aeruginosa, in air and light, 20°C, 1-28 days) the following degradation rates were observed for the C_{20}, C_{25} and C_{30} compounds.

n-alkanes $>$ (degraded before) n-alkenes $>$ highly branched alkenes (br) $>$ highly branched alkanes + 'normal' isoprenoids.

The results suggest that the relative resistance of the highly branched hydrocarbons to biodegradation is a factor in controlling the sedimentary distributions of these newly identified widespread, and often abundant, hydrocarbons.

CHAPTER SIX

MOLECULAR EVIDENCE FOR ARCHAEBACTERIA

BIOMASS IN ANCIENT SEDIMENTS?

6:1   SYNTHESIS OF 2,6,9,13-TETRAMETHYLTETRADECANE (5)

6.1.1 Introduction

It has been proposed, following the discovery of geochemical markers of Archaebacteria in several Ancient sediments (see Chapter 1; Moldowan and Seifert, 1979; Chappe et al., 1979, 1980) that most irregular (h-h) and some (t-t) isoprenoid hydrocarbons identified in Ancient sediments represent fossils of an Archaebacterial origin (Hahn, 1982). Recently, Fowler (1984) tentatively identified a group of C_{18} - C_{26} irregular isoprenoid hydrocarbons with a (h-h) linkage in sediments of the Corcoran formation of the McArthur Basin (PreCambrian, 1400 M.Y; Northern Territory, Australia) where it was suggested that the presence of these hydrocarbons indicated a contribution from Archaebacteria. Fig. 6:1 shows a gas chromatogram of the urea non-adduct of the hydrocarbons of one of the samples (LB67) from the Corcoran; peaks A,B,C,D,E and F were thought to be a series of related compounds in which the mass spectra demonstrated one major fragment in the higher molecular weight region which increased by 14 a.m.u. between members A to F. The mass spectral fragmentation pattern (Fig. 6:2) displayed by A suggested to Fowler (1984) structure (5), i.e. 2,6,9,13-tetramethyltetradecane. It was proposed that the major high molecular weight fragment ion at m/z 211 arose by cleavage (without H-transfer) adjacent to the tertiary carbon of one of the isopropyl termini. The carbon skeleton proposed for compound A could be derived like other (h-h) isoprenoid hydrocarbons identified in sediments and petroleums (Moldowan and Seifert, 1979) by thermocatalytic degradation of the polar dibiphytanyldiglycerol tetraethers (43) of Archaebacteria. If the proposed structure (5) for A were correct, then it could represent an important geochemical marker for Archaebacterial activity in Ancient sediments. In order to confirm the structural assignment proposed for
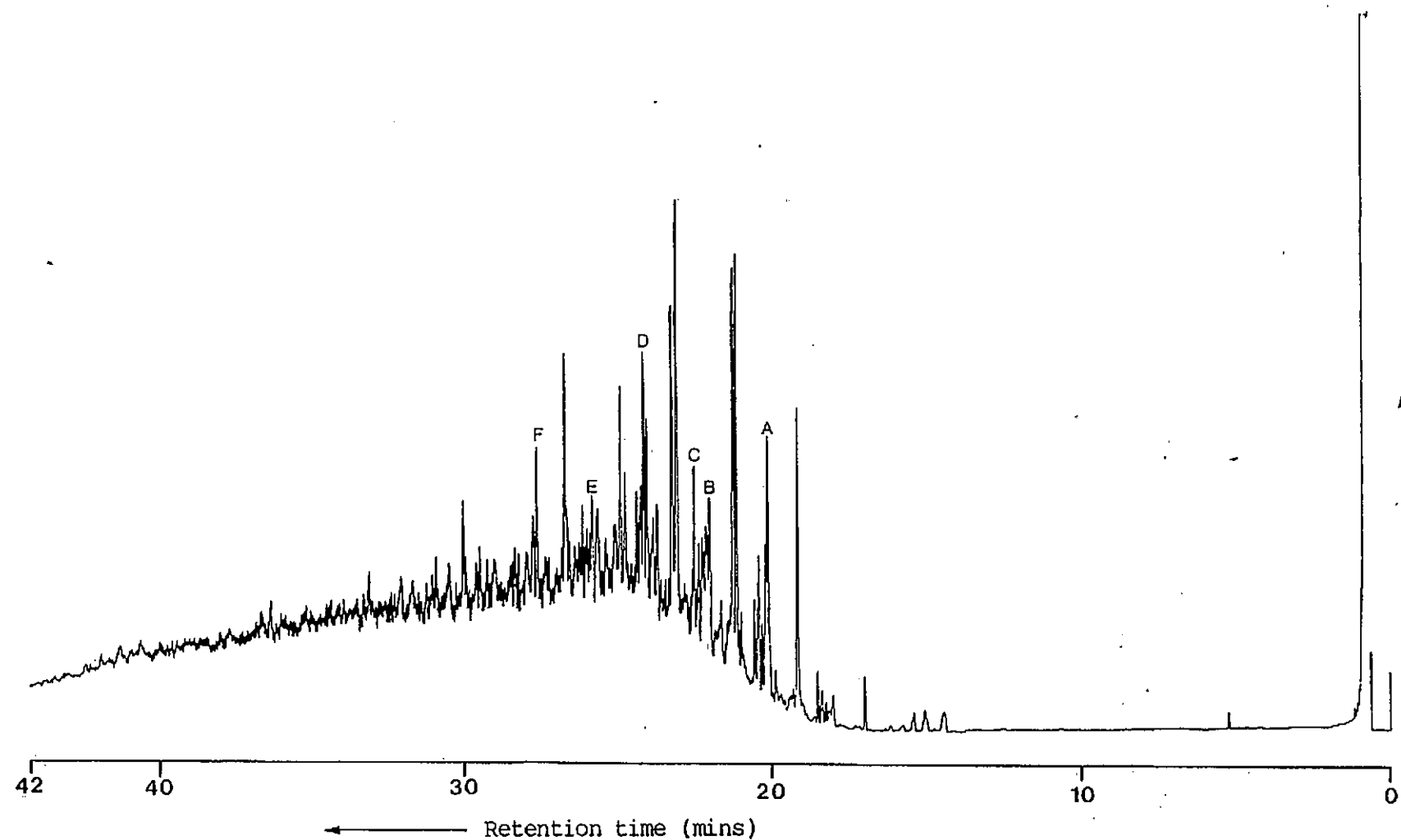
Fig.6:1   Gas chromatogram of the urea non-adduct of LB67 (sample extract courtesy of Dr. M. Fowler)
          Letters A - E refer to text.   Gc conditions as text.
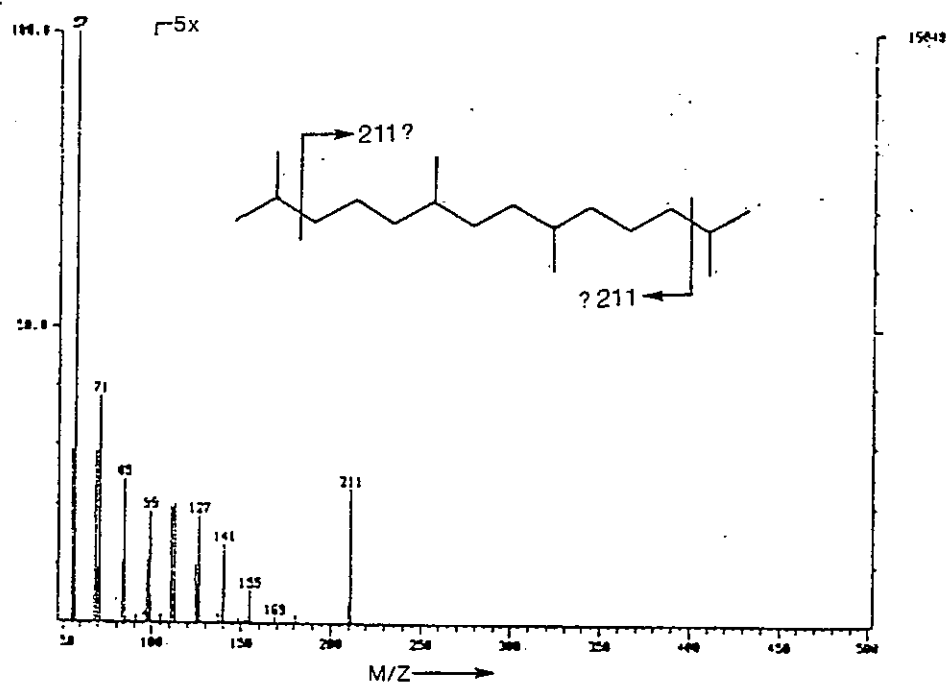
Fig.6:2   Mass spectrum of compound A proposed by
           Fowler (1984) to have structure
           (Taken from Fowler, 1984).

A by Fowler (1984) it was decided to synthesise the alkane.

6.1.2 Synthetic scheme

Examination of the structure for the C_{18} alkane revealed a convenient disconnection at the C6 - 7 bond. Synthesis <u>via</u> the condensation of a C_8 ketone (6-methylheptan-2-one) with a regular C_{10} isoprenoid
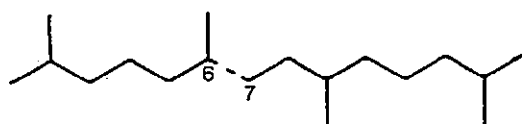
moiety (1-bromo-3,7-dimethyloctane) would produce a C_{18} tertiary alcohol which on subsequent dehydration and hydrogenation would yield the target alkane. The full synthetic scheme is shown in Fig. 6:3. Both the C_8 ketone and the C_{10} bromide were important synthons in the synthesis of 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane (br25:0; 70) and their preparation is discussed in Chapter 3.
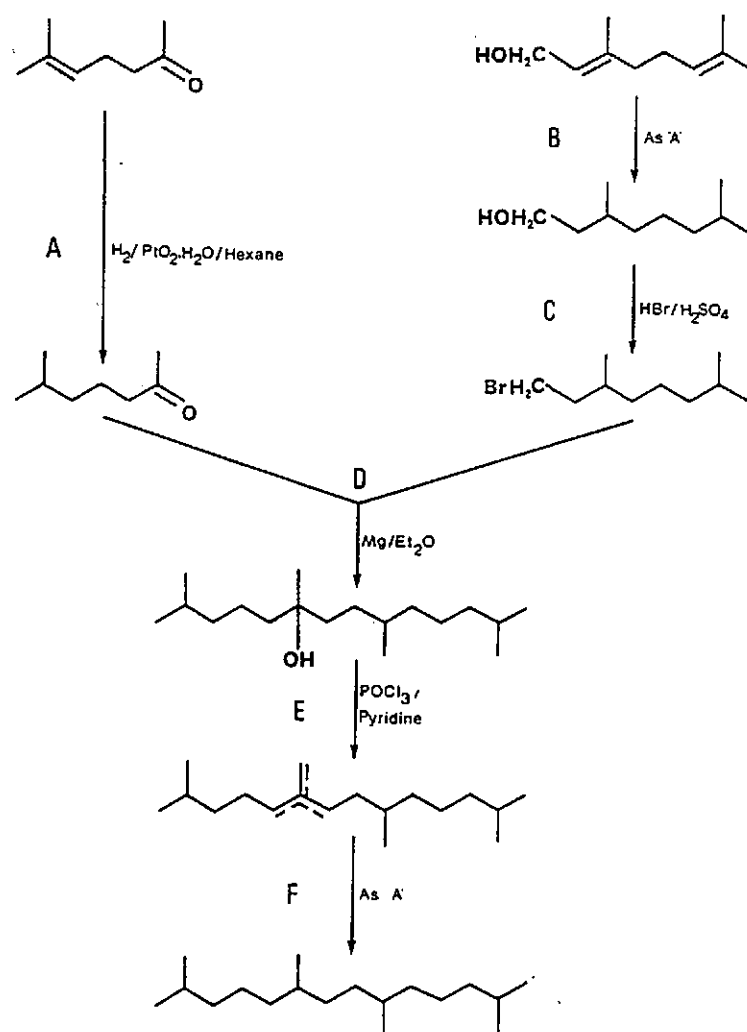
Fig.6:3   Synthesis of 2,6,9,13-tetramethyltetradecane.

6.1.3 Synthesis of 2,6,9,13-tetramethyltetradecan-6-ol
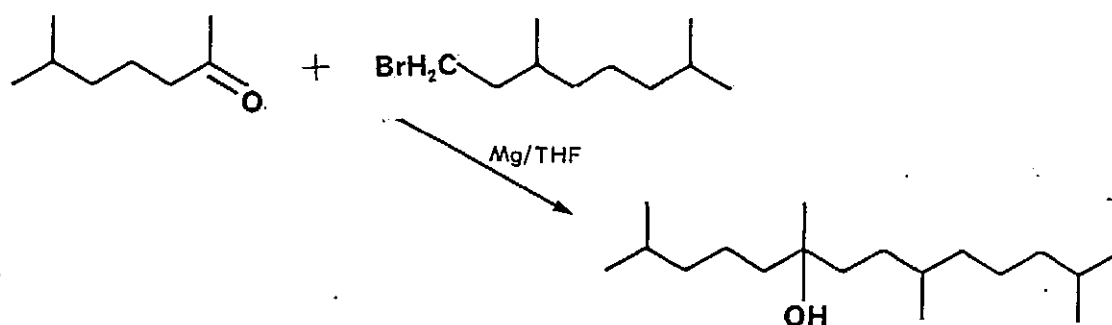
2,6,9,13-tetramethyltetradecan-6-ol was prepared in good yield (72%) by the Grignard addition (Kharasch and Reinmurth, 1954) of 1-bromo-3,7-dimethyloctane to 6-methylheptan-2-one. The mass spectrum (Fig. 6:4A) of the product C_{18} tertiary alcohol exhibited an $(M^{+\cdot} - CH_3)$ ion at m/z 255, an $(M^{+\cdot} - H_2O)$ ion at m/z 252 and two fairly intense fragment ions at m/z 129 and m/z 185 formed by α-cleavage without hydrogen transfer at the hydroxyl-bearing quaternary carbon. The most intense α- cleavage ion is that formed by the loss of the largest alkyl radical (i.e. $C_{10}H_{21}$) which is the favoured fragmentation in the mass spectra of tertiary alcohols (Beynon et al., 1968; McLafferty, 1973). The mass spectrum of the TMS ether of the alcohol (prepared using the powerful silylating reagent TSIM) is shown in Fig. 6:4B. The presence of two intense α- cleavage ions at m/z 201 and m/z 257 confirmed the position of the hydroxyl group at C6 on the C_{18} chain.

The ^{1}H NMR spectrum of the C_{18} alcohol integrated correctly into the assigned number of $C\underline{H}_3$, $C\underline{H}_2$ and $C\underline{H}$ protons but showed no distinguishing features worthy of prolonged discussion. The IR spectrum was consistent with that of a saturated tertiary alcohol.
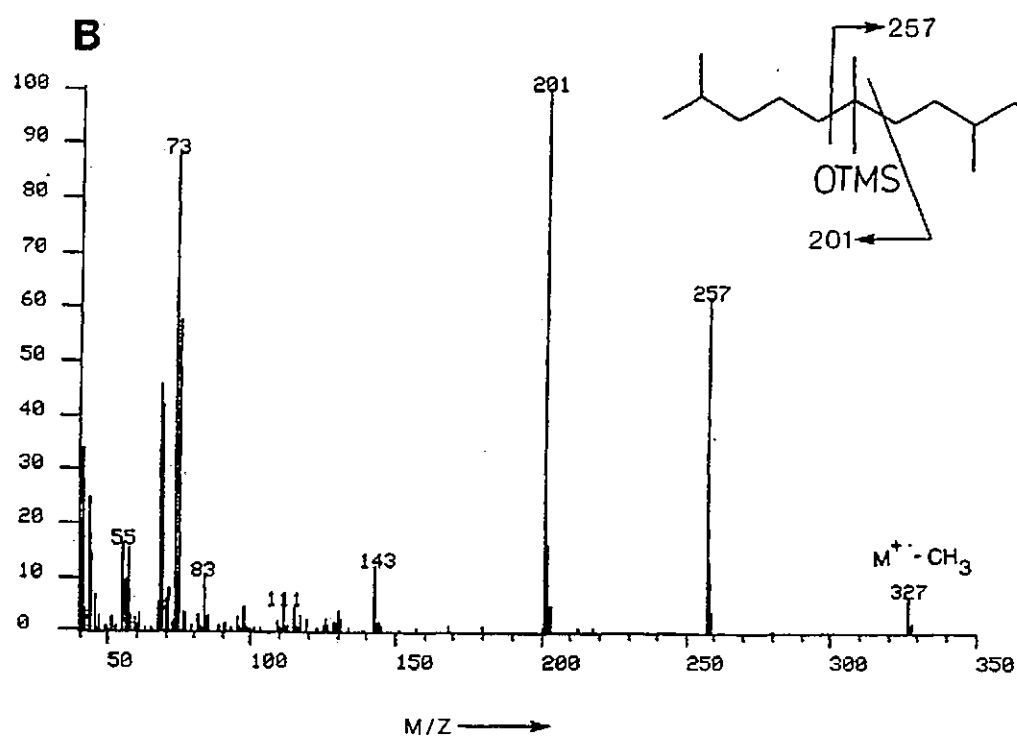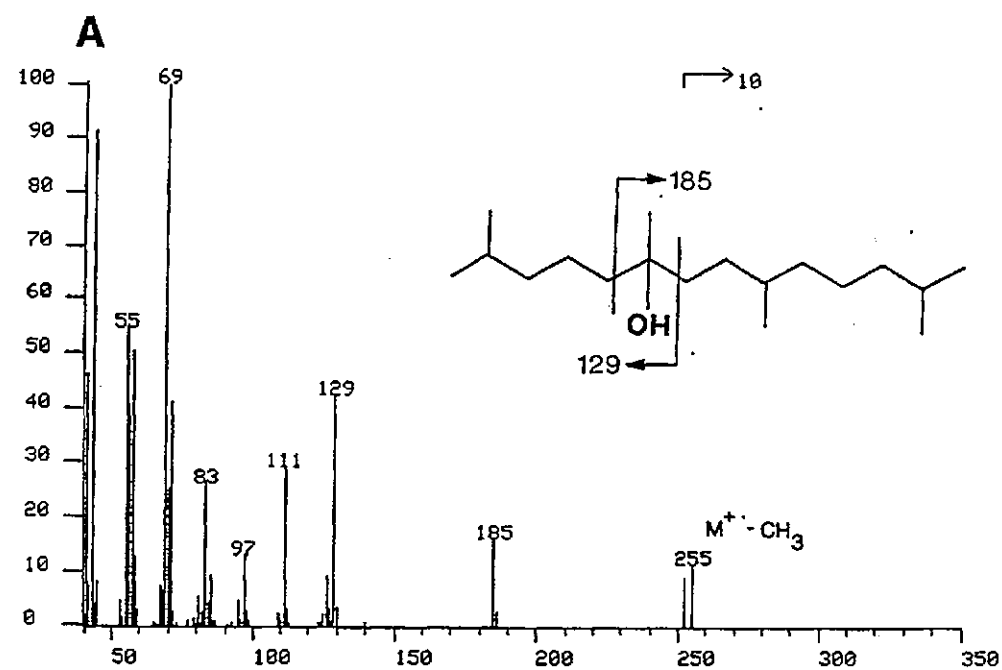
Fig.6:4   Mass spectra of A. 2,6,9,13-tetramethyltetradecan-6-ol
and B. its TMS ether.

The ^{13}C NMR spectrum of 2,6,9,13-tetramethyltetradecan-6-ol is shown in Fig. 6:5 and listed in Table 6:1A. The interpretation of the spectrum is summarised in Table 6:1B and was performed by comparison with published ^{13}C NMR spectra of alcohols (Stothers, 1972; Breitmaier 1978; Breitmaier et al., 1979), comparison with the spectra of the tertiary br20, br25 and br30 alcohols discussed in Chapter 3, and by comparison with the shift assignments for the parent C_{18} alkane; 2,6,9,13-tetramethyltetradecane (5; section 6.1.5). Interestingly, the chemical shift for the carbinol carbon is at lower field (~72 ppm) to that observed for the carbinol carbon in the tertiary br20 and br30 alcohols (i.e. ~76 ppm). The increase in shielding at the carbinol carbon in 2,6,9,13-tetramethyltetradecan-6-ol (compared to the br20 alcohol for example) is probably due to the decrease in the size of the alkyl substituents at the quaternary carbon in the C_{18} alcohol. The splitting apparent in certain peaks (i.e. 41.8703 - 41.9791 ppm) is due to the presence of the expected diastereoisomers although it is difficult to rationalise the observed three signals attributed to one carbon (possibly C17) at 26.7400 - 26.9788 ppm. However, the three signals of low intensity between 42.3998 - 51.3357 ppm are considered to reflect impurities in the 2,6,9,13-tetramethyltetradecan-6-ol so this may also account for the 'extra' peak at 26.7400 - 26.9788 ppm.
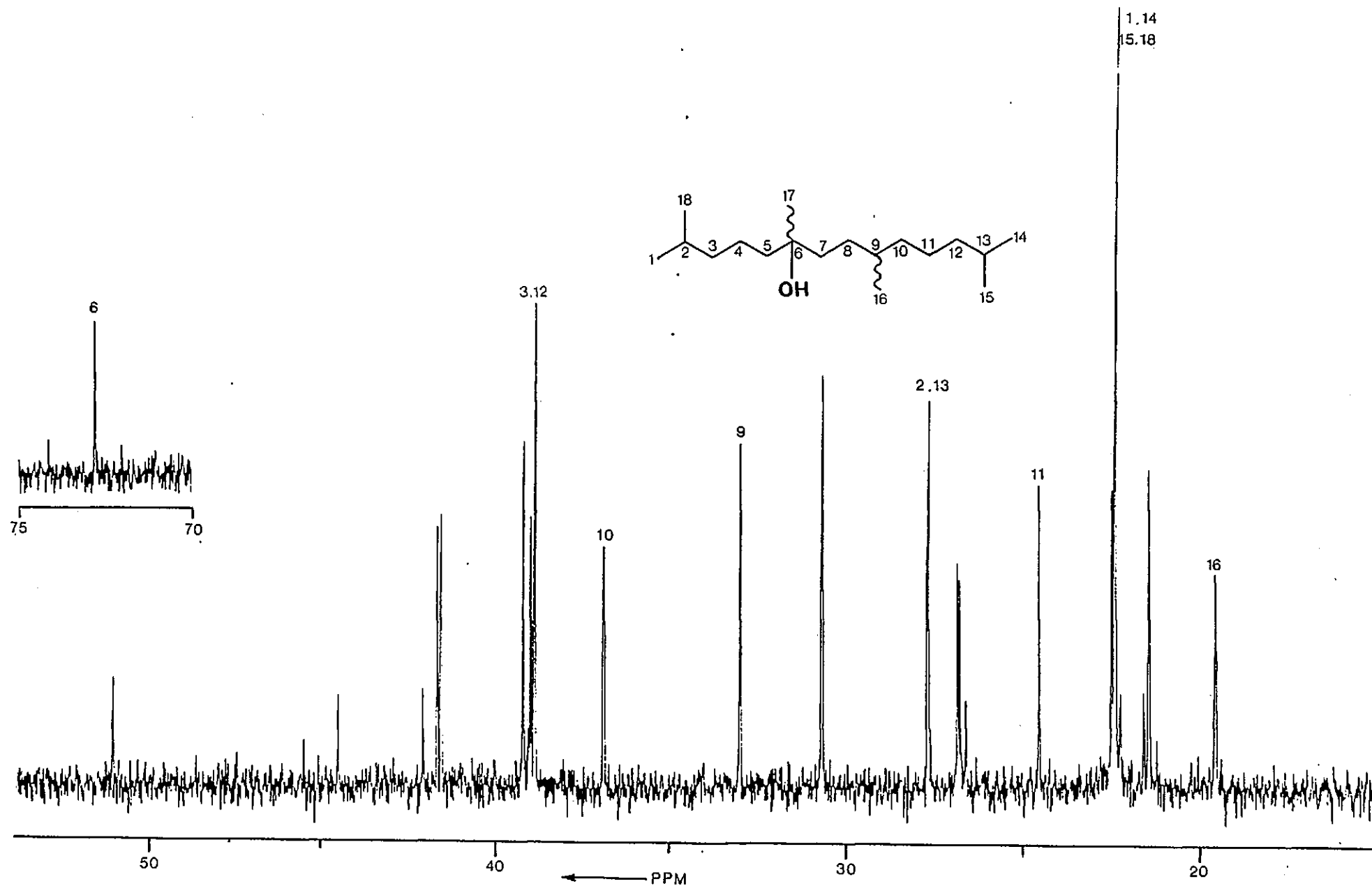
Fig.6:5 ^{13}C NMR (400 MHz) spectrum of 2,6,9,13-tetramethyltetradecan-6-ol. Proposed shift assignments (Table 6:1b) as shown. Numbers refer to the carbon number in the accompanying structure.

Table 6:1A

Observed ^{13}C NMR chemical shifts for
2,6,9,13-tetramethyltetradecan-6-ol.

ppm	intensity	proposed number of carbons
72.7562	4.605	1
51.3357	3.226	IMPURITY
44.8416	2.646	IMPURITY
42.3998	2.848	IMPURITY
41.9771	7.471	1
41.8703	7.777	
39.4924	10.147	
39.2521	8.252	3
39.1274	14.046	
37.1352	6.985	1
37.1104	6.959	
33.1917	10.058	1
30.8712	12.096	1
27.8680	7.283	
27.8279	11.130	2
27.7530	1.757	
26.9788	6.497	
26.9119	6.314	1
26.7400	2.748	
24.6651	9.524	1
22.5809	8.589	
22.5005	22.836	4
22.3295	3.041	
21.6573	2.849	1
21.5135	9.384	
19.6326	6.348	1
	Total	18

Table 6:1B

^{13}C-NMR shift assignments for 2,6,19,13-tetramethyltetradecan-6-ol.
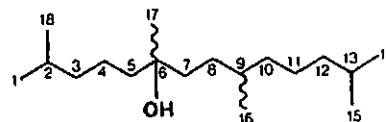
Carbon Number	ppm	shift assignment for 2,6,19,13-tetramethyltetradecane ppm
16	19.6326	19.6305 - 19.7814
4	21.5135 - 21.6573[a]	24.7118 - 24.7490
1;14 15,18	22.3295 - 22.5809	22.5366 - 22.6219
11	24.6651	24.7118 - 24.7490
17	26.7400 - 26.9788[a]	19.6305 - 19.7814
2,13	27.7530 - 27.8680 30.8712[b]	27.9150
9	33.1917	33.0137 - 33.0575
10	37.1104 - 37.1352[b]	37.1464 - 37.3947
3,12	39.1274 - 39.4924[b] 41.8703 - 41.9771[b]	39.3381
6	72.7562	33.0137 - 33.0575

[a]: Chemical shifts with the same superscript may be interchanged.

[b]: Chemical shift unassigned but either C5, C7 or C8.

6.1.4 Synthesis of 2,6,9,13-tetramethyltetradecenes (92)
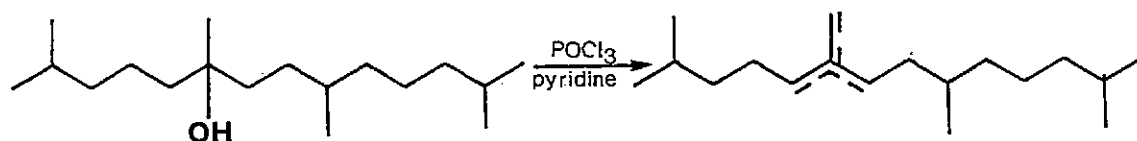
Dehydration of 2,6,9,13-tetramethyltetradecan-6-ol produced a mixture of isomeric C_{18} monoenes represented by the five gas chromatographic peaks in Fig. 6:6. Details of retention indices and mass spectra of individual peaks are summarised in Table 6:2. Examination of this Table suggests that RI 1633 and 1657 are geometric (cis-trans) isomers as they demonstrate almost identical mass spectra. The difficulties already discussed (Chapter 3; section 3.2.8) in interpreting the mass spectral and chromatographic behaviour of alkenes makes further speculation unwise. Attempts to assign double bond positions in the mixture of isomeric monoenes using methoxy-mercuration (Blomquist et al., 1980) and alkylthiolation (Francis and Veland, 1981) proved unsuccessful. The later eluting peak (RI 1717) proved separable from the other monoenes by argentatious tlc but eluded structural assignment by spectroscopy (see later).
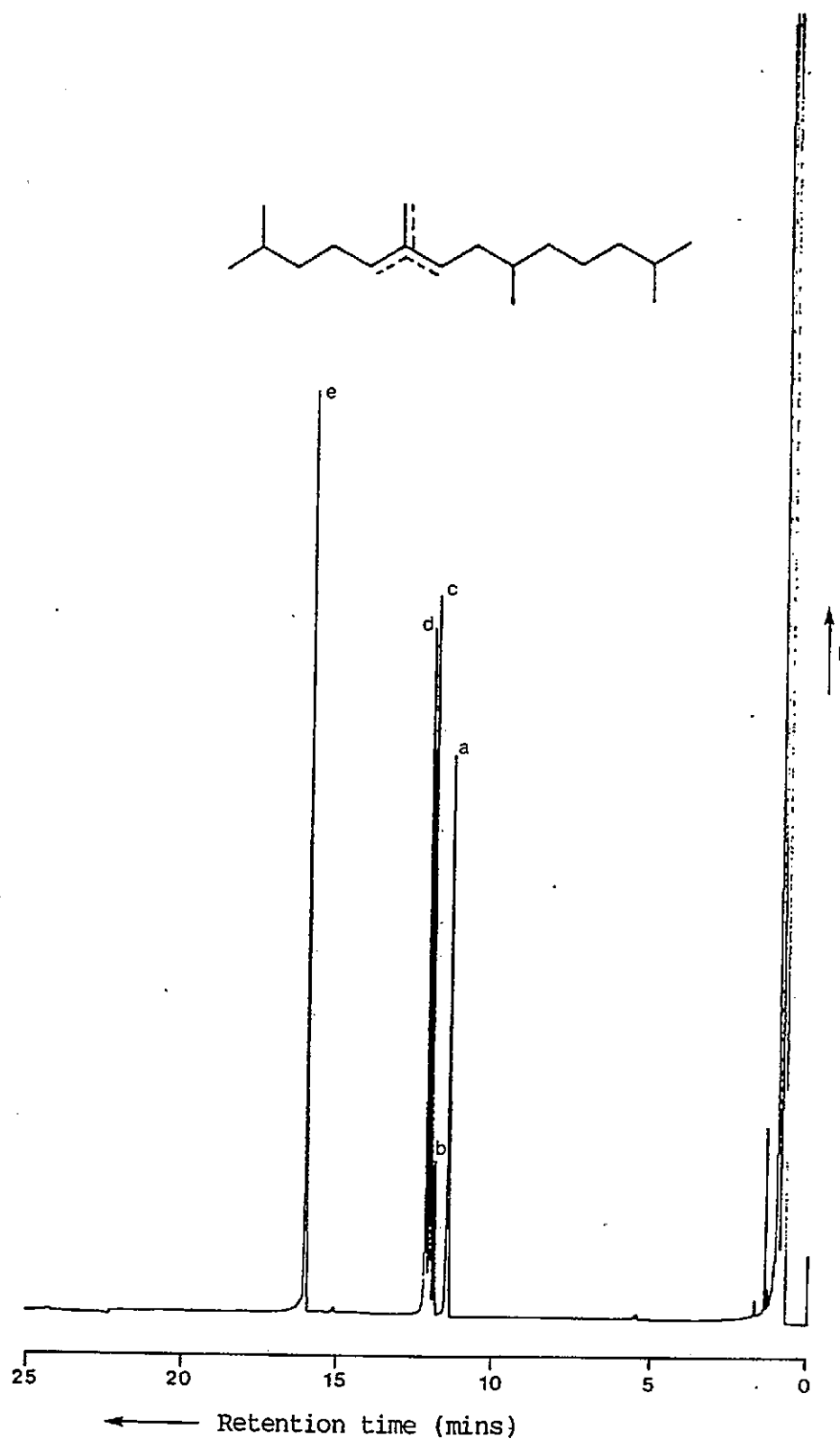
Fig 6:6   Gas chromatogram of the synthetic 2,6,9,13-tetramethyltetradecenes.
Letters a. - e refer to Table 6:3.   Gc conditions: OV1, 100°C - 200°C
at 4°Cmin⁻¹.

Table 6:2

LRMS of 2,6,9,13-tetramethyltetradecenes

Gc Retention Index[a] RI	Formula m/z	LRMS Ion Intensity %					
		$C_{18}H_{36}$ 252	$C_{13}H_{26}$ 182	$C_{10}H_{20}$ 140	C_9H_{18} 126	C_6H_{11} 83	(Base Peak Ion)
1633	a	5.0	1.5	3.5	24.0	68.0	100 (57)
1652	b	1.5	0.5	1.5	20.0	23.0	100 (56)
1657	c	5.0	1.5	4.0	23.0	78.0	100 (57)
1663	d	6.0	1.1	1.1	26.0	31.0	100 (56)
1817	e	4.0	2.0	3.1	30.5	42.5	100 (56)

[a]: OV1, 40-80°C at 10°Cmin^{-1}, 80-290°C at 6°Cmin^{-1}.

6.1.5 Synthesis of 2,6,9,13-tetramethyltetradecane
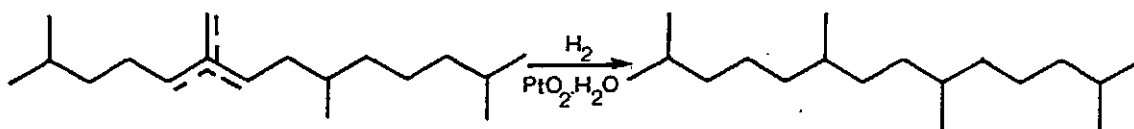
Fig. 6:7A shows the gas chromatogram of the products from the hydrogenation (ambient pressure; 25°C) of the isomeric C_{18} alkenes. From a comparison with Fig. 6:6 (i.e. the precursor alkenes) it is apparent that whilst the four early eluting alkene isomers (i.e. RI 1633 - 1663) hydrogenated smoothly to the parent C_{18} alkane (Fig. 6:7A; RI1605), the later eluting monoene isomer(s) (RI1717) were resistant to hydrogenation under the conditions used. Through argentatious tlc it was possible to isolate the C_{18} monoene in sufficient quantity and purity (Fig. 6:7B) to allow spectroscopic examination by ^{1}H NMR (400MHz). However, the observed spectrum did not allow unambiguous structural assignment. Attempts to assign the position of the double bond in the single monoene isomer using methoxy-mercuration (Blomquist et al., 1980) and alkylthiolation (Francies and Veland, 1981) proved unsuccessful.

Fig. 6:8A shows the gas chromatogram of the C_{18} alkane obtained after argentatious tlc of the crude hydrogenation products. It is interesting to note, that under the gas chromatographic conditions used (i.e. 25m x 0.32mm i.d. fused silica coated with OV1), the _meso_ - [i.e.
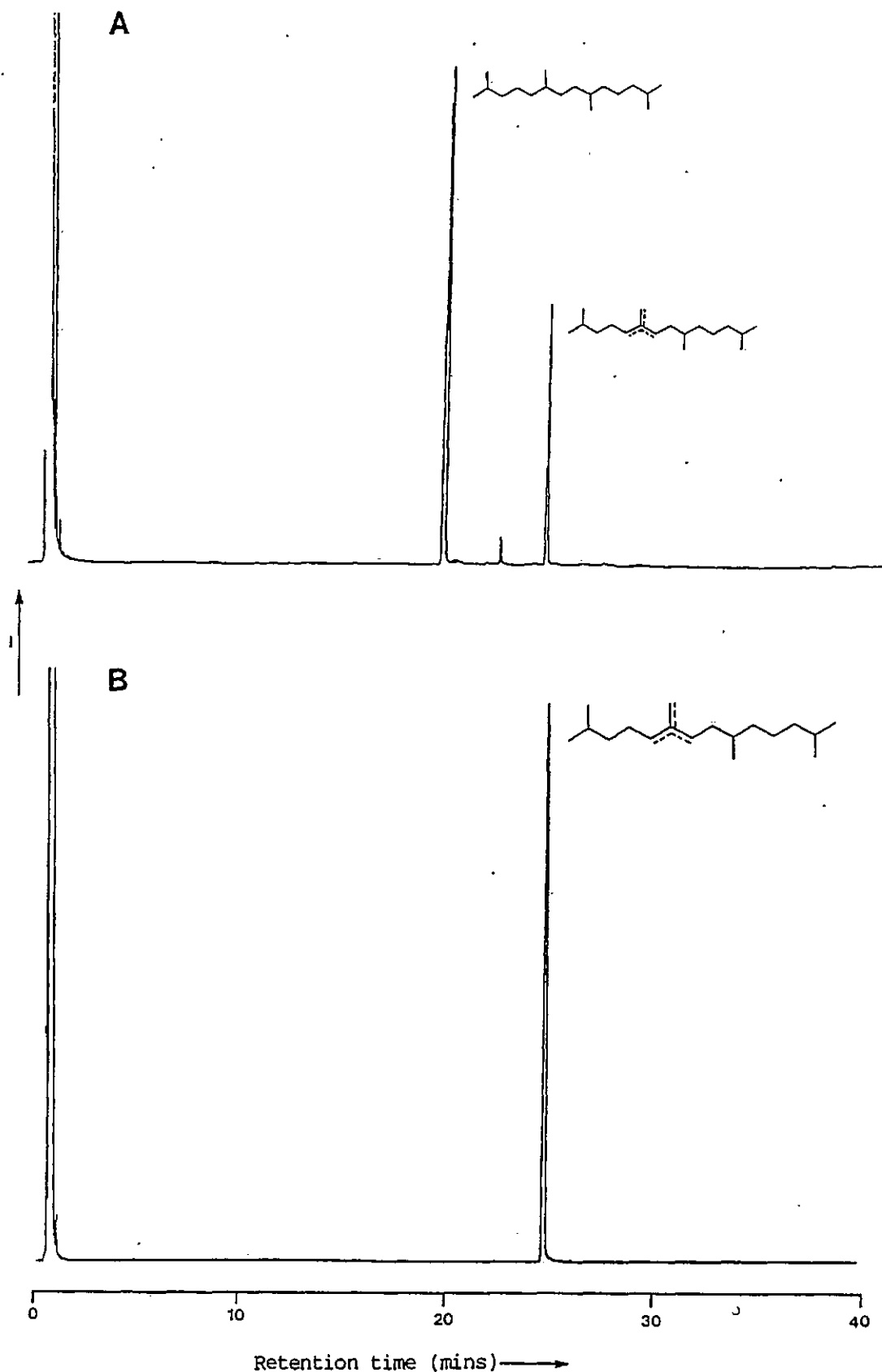
Fig.6:7   Gas chromatograms of A. the post-hydrogenation products of 2,6,9,13-
tetramethyltetradecene showing the presence of 2,6,9,13-tetramethyltetradecane
and the latter-eluting alkene isomer apparently stable to hydrogenation and
B. the latter-eluting alkene isomer after purification by argentatious tlc.
Gc conditions as text.
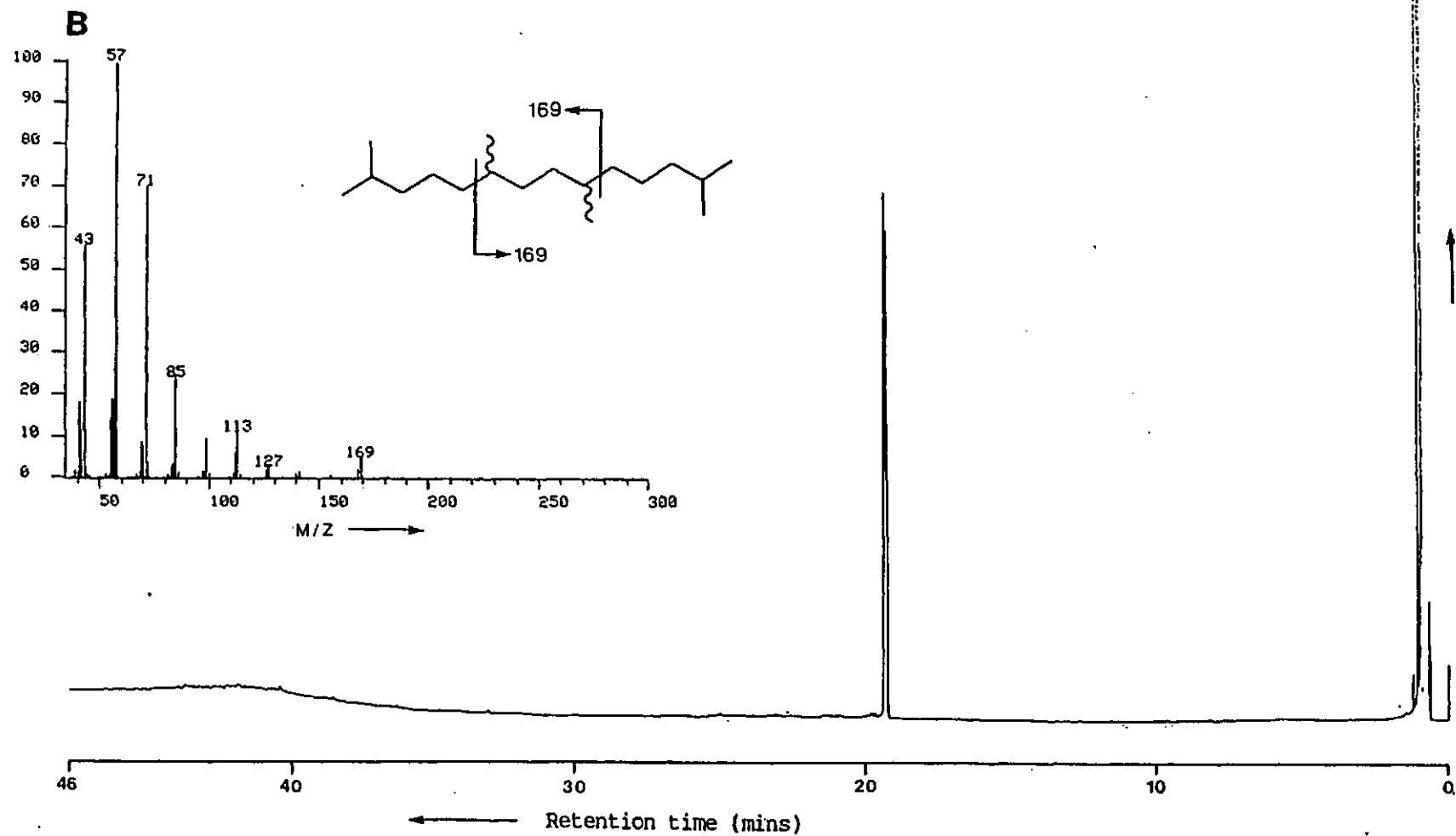
Fig.6:8   Gas chromatogram (A) and mass spectrum (B) of synthetic 2,6,9,13-tetramethyltetradecane.
Gc conditions as text.

6(R) 9(S) and 6(S) 9(R)] isomer of 2,6,9,13-tetramethyltetradecane was separated from the 6(R) 9(R) and 6(S) 9(S) isomers in an analogous situation to that observed for 2,6 (RS), 10 (RS),14-tetramethylpentadecane (pristane; $\underline{3}$). The different configurations of the C_{18} alkane are shown in Fig. 6:9.

The mass spectrum of the C_{18} alkane is shown in Fig. 6:8B. It is immediately obvious that the spectrum is different to that (Fig. 6:2) of the sedimentary C_{18} alkane presented by Fowler (1984). The high molecular weight region (i.e. > 150 a.m.u.) of the spectrum of the synthetic C_{18} alkane (Fig. 6:8B) exhibits a fragment ion at m/z 169, generated presumably by cleavage adjacent to the internal tertiary carbons, and does not demonstrate the intense m/z 211 ion observed in the mass spectrum of the sedimentary hydrocarbon (Fig. 6:2). The further difference in the retention indices displayed by the synthetic and sedimentary C_{18} alkanes (RI1605 vs 1654) indicates that the sedimentary alkane described by Fowler (1984) does not have the carbon skeleton of 2,6,9,13-tetramethyltetradecane ($\underline{5}$). This was additionally confirmed by co-injection of the synthetic C_{18} alkane with the urea non-adduct of sample LB67 (Figs. 6:10A and B).

The ^{1}H NMR spectrum of the C_{18} alkane integrated correctly into $C\underline{H}_{3}$, $C\underline{H}_{2}$ and $C\underline{H}$ protons but displayed few interesting features.

The ^{13}C NMR spectrum of 2,6,9,13-tetramethyltetradecane is shown in Fig. 6:11 and listed in Table 6:3A. The assignment of chemical shifts is summarised in Table 6:3B and was performed by comparison with published ^{13}C NMR spectra of acyclic isoprenoids (i.e. pristane, phytane; Yon, 1982; Lamb, 1982) and by comparison with theoretical

6 R 9 R

two enantomers : diastereoisomeric pair

6 S 9 S

6 R 9 S

meso –   : diastereoisomeric pair

6 S 9 R
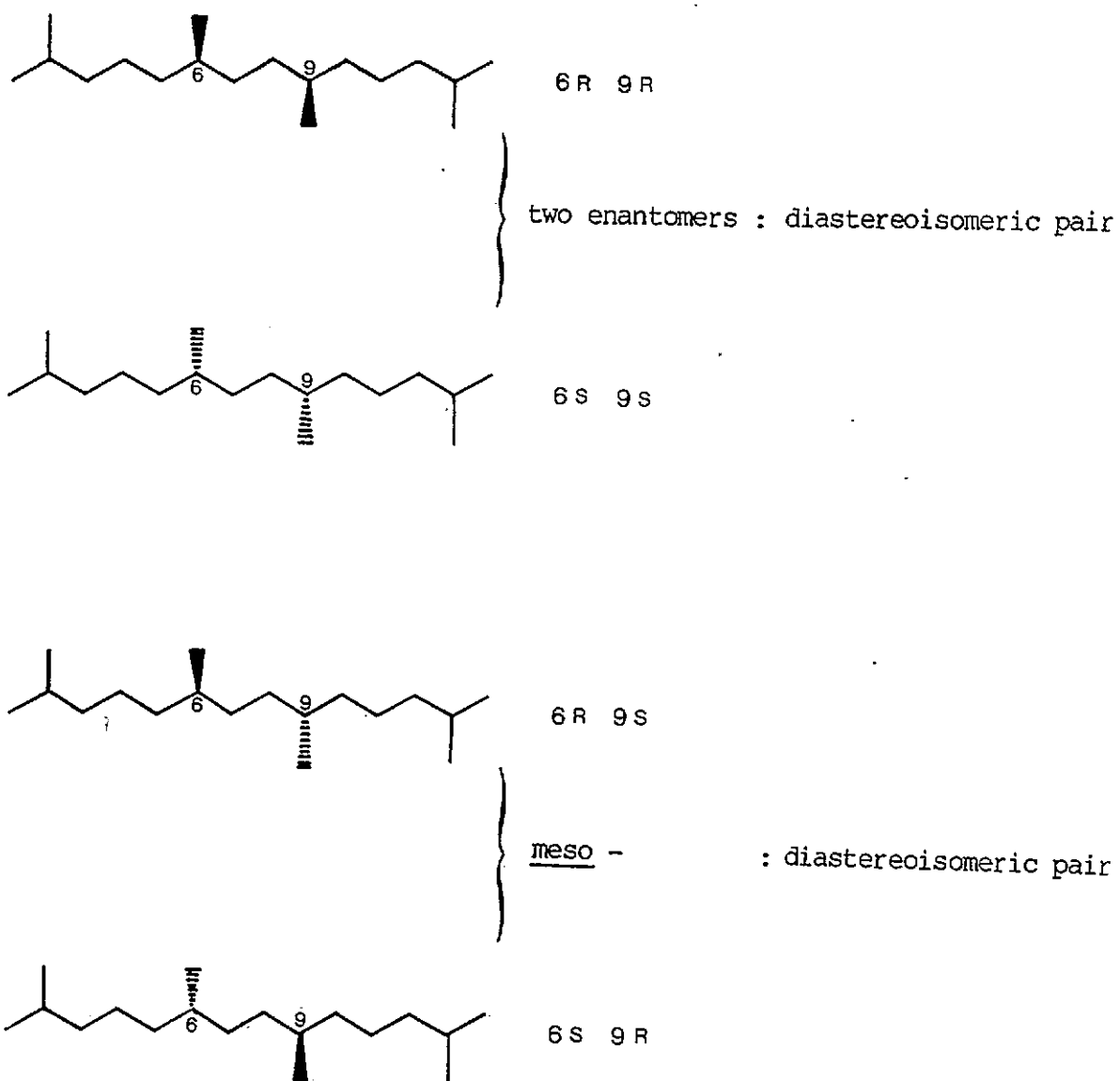
Fig.6:9   Stereochemistry of 2,6,9,13-tetramethyltetradecane.
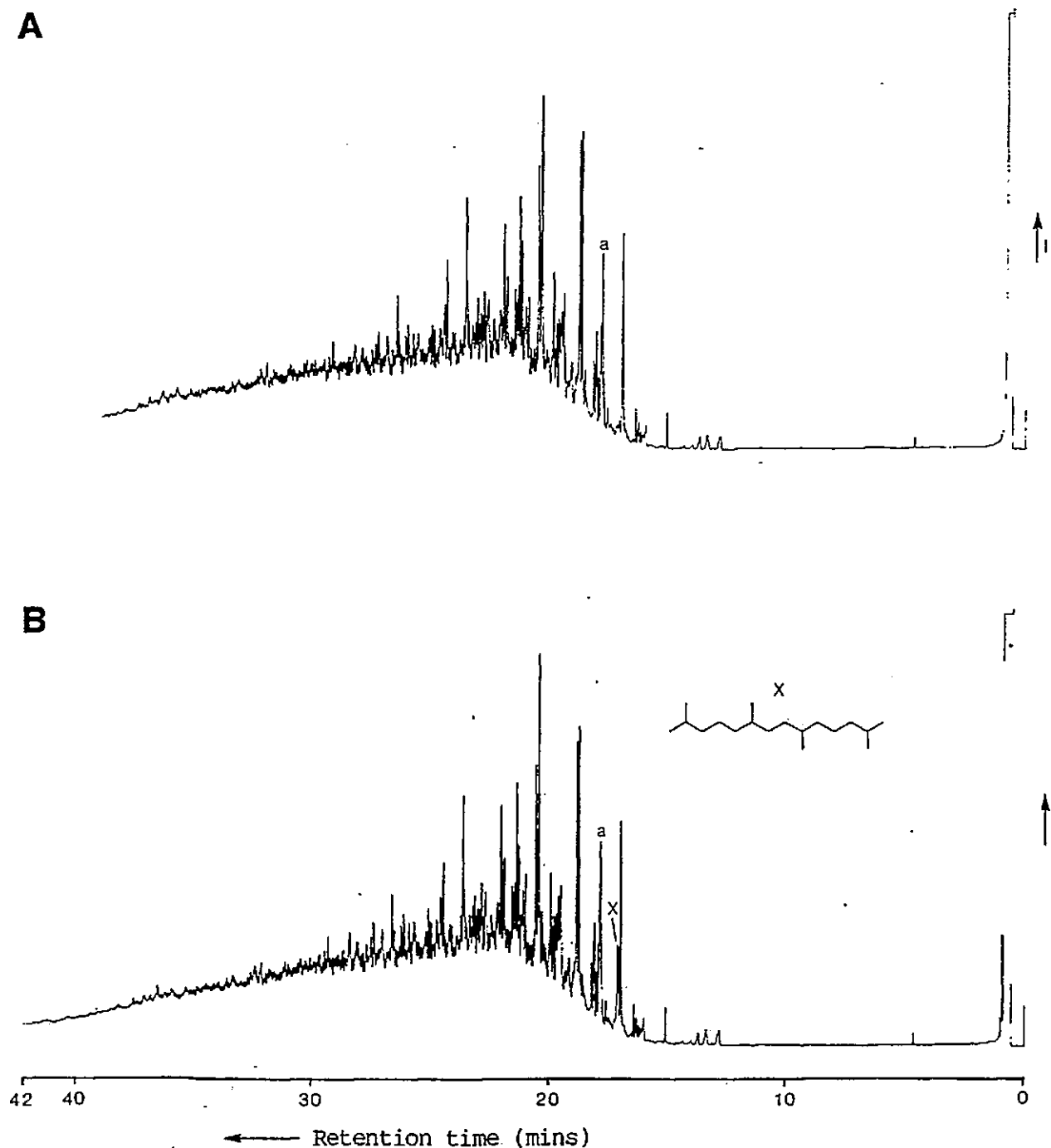
Fig.6:10   Gas chromatogram of the urea non-adduct of A. LB67 and
B. LB67 co-chromatographed with synthetic 2,6,9,13-
tetramethyltetradecane (peak X). Peak a. represents the
hydrocarbon suggested by Fowler (1984) to have the
structure of 2,6,9,13-tetramethyltetradecane.
Gc conditions as text.
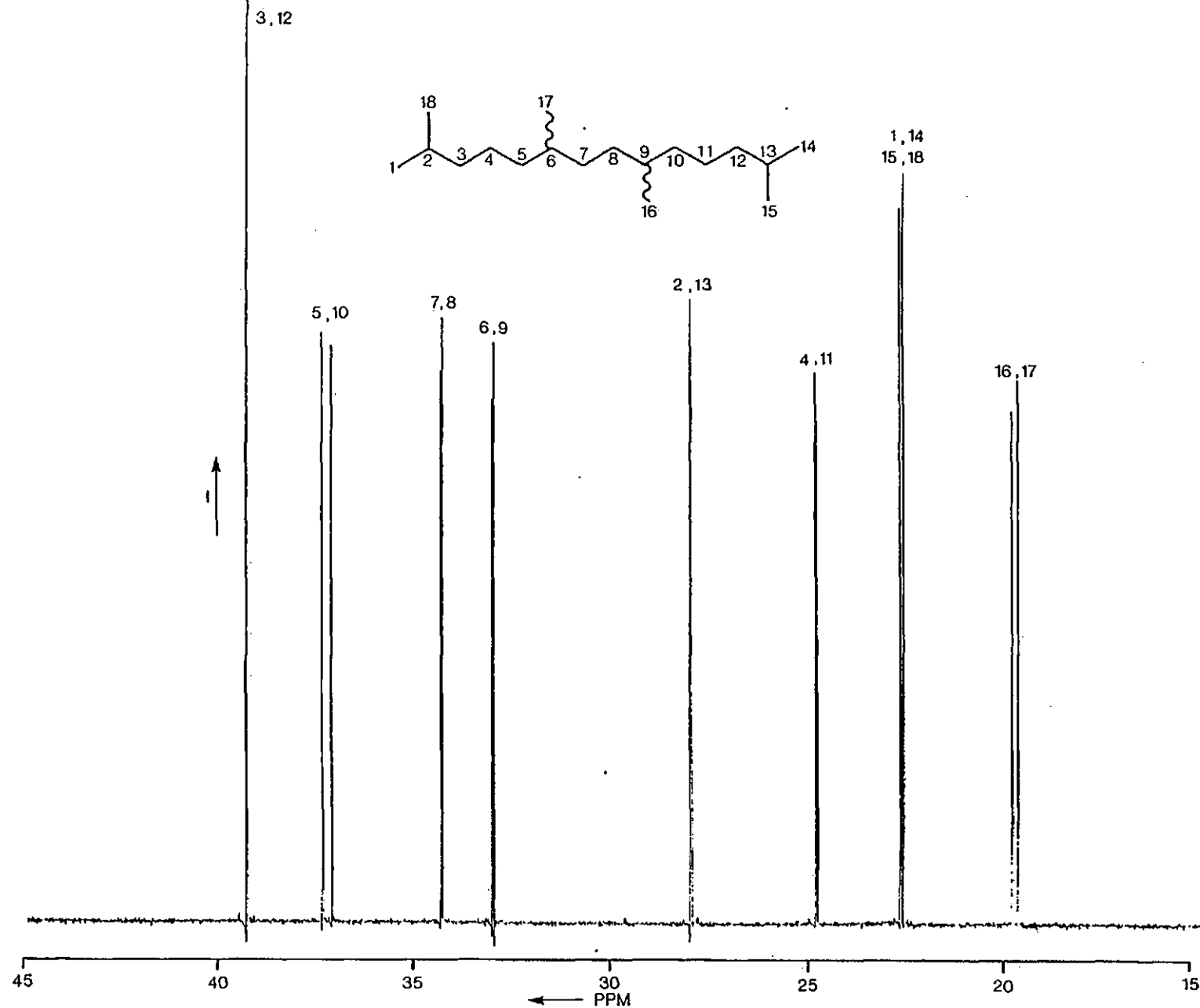
Fig.6:11 ^{13}C NMR (400 MHz) spectrum of 2,6,9,13-tetramethylhexadecane. Proposed shift assignments (Table 6:3b) as shown. Numbers refer to the carbon number in the accompanying structure.

Table 6:3A

Observed ^{13}C NMR chemical shifts for 2,6,9,13-tetramethyltetradecane

ppm	intensity	proposed number of carbons
39.3381	25.577	2
37.3947	15.098	
37.1464	14.691	2
34.3615	14.219	
34.3257	15.390	2
33.0575	13.465	
33.0137	14.729	2
27.9150	17.055	2
24.7490	14.011	
24.7118	12.966	2
22.6219	19.786	2
22.5366	19.867	2
19.7814	13.007	
19.6305	13.751	2
	Total	18

Table 6:3B

^{13}C NMR shift assignments for 2,6,9,13-tetramethyltetradecane.
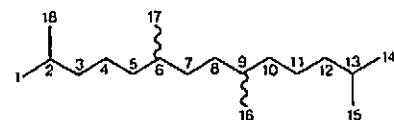
Carbon Number	ppm	Calculated shift assignments [a] ppm
16,17*	19.6305 19.7814	20.44
1,14	22.5366 [b]	22.50
15,18	22.6219 [b]	22.50
4,11*	24.7118 24.7490	25.29
2,13	27.9150	28.61
6,9*	33.0137 33.0575	33.30
7,8*	34.3257 34.3615	35.31
5,10*	37.1464 37.3947	37.93
3,12	39.3381	39.99

[a]: Calculated according to Carmen et al., 1973.

[b]: Chemical shift assignments with the same superscript may be interchanged.

* carbon atoms showing diastereoisomeric non-equivalence.

chemical shifts for the C_{18} alkane calculated according to Carmen et al. (1973). It is uncertain whether the signal doublets assigned to the carbons marked with an asterisk (Table 6:3B) represent magnetic non-equivalence due to conformational and/or configurational (i.e. diastereoisomeric) structure. The absence of doublets for carbons C4 - 13, C17 and C18 in the ^{13}C NMR spectrum of the single diastereo-

isomer meso-pristane (Lamb, 1982; Yon, 1982) suggests that the doublets observed in the spectrum of 2,6,9,13-tetramethyltetradecane result from diastereoisomeric differences in configuration at C6 and C9. However, it must be noted that the spectrum of pristane was run at lower field strength (i.e. Yon, 1982: 90MHz; Lamb, 1982: 200MHz) than the C_{18} alkane (400MHz). The almost equal symmetry of the doublets in the spectrum of the C_{18} alkane indicates that the relative abundances of the two diastereoisomers [i.e. meso- and 6(R) 9(R) + 6(S) 9(S)] are equal as would be expected from a non-stereospecific synthesis. Similar doublets for internal methyl and methylene carbons caused by magnetically non-equivalent configurations were recorded in the ^{13}C NMR spectrum of all-isomer 2,5,8,11-tetramethyldodecane (Carmen et al., 1973). Interestingly, the observed difference in the resonances for
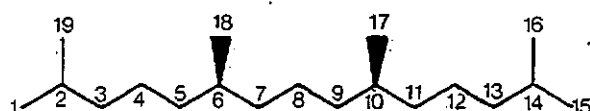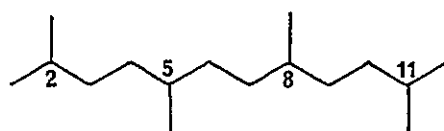
the internal methyl carbons ($\Delta^{13}C$ = 0.15 ppm) in that compound is identical to that observed for the C16 and C17 internal methyl carbons in the C_{18} alkane (see Table 6:2B). Unlike the current spectrum of the C_{18} alkane, no doublets were observed in the spectrum of

2,5,8,11-tetramethyldodecane for the internal methine carbons (C5 and C8) or the carbon atoms β to these carbons (i.e. C3 and C10) although this is probably a consequence of the lower field strength of the instrument used by Carmen et al. (1973). The doublet at 22.5366 - 22.6219 ppm is due to conformational magnetic non-equivalence of the methyl carbons of the isopropyl termini. Thus, it is apparent that the ^{13}C NMR spectrum of 2,6,9,13-tetramethyltetra-decane demonstrates magnetic non-equivalence due to both conformational and configurational structure.

6.2 SYNTHESIS OF 2,6,11,15-TETRAMETHYLHEXADECANE (6)

The 'irregular' C_{20} (t-t) isoprenoid alkane was prepared as a Wurtz coupling (i.e. RMgX + RX $\rightarrow$ RR + MgX; see section 3.2.5) by-product of the Grignard additions of 1-bromo-3,7-dimethyloctane to 6-methylheptan-2-one (section 6.1.3) and of 1-bromo-3,7-dimethyloctane to 2,6-dimethylheptan-1-al (3.2.5). The C_{20} alkane was isolated pure for spectroscopic analysis by argentatious tlc.

A gas chromatogram of the isolated alkane is shown in Fig. 6:12A and Fig. 6:12B displays the mass spectrum. The high molecular weight (>150 a.m.u.) region of the spectrum exhibits fragment ions at m/z 169 and m/z 197 generated by α-cleavage (without H-transfer) either side of the internal tertiary (i.e. C6 and C11) carbons. In common with the mass spectra of other acyclic isoprenoids (i.e. 2,6,9,13-tetramethyl-tetradecane; 5) the molecular ion is absent.

The ^1H NMR spectrum of the C_{20} alkane integrated into the correct number of CH_3, CH_2 and CH protons but displayed no distinguishing features worthy of prolonged discussion.
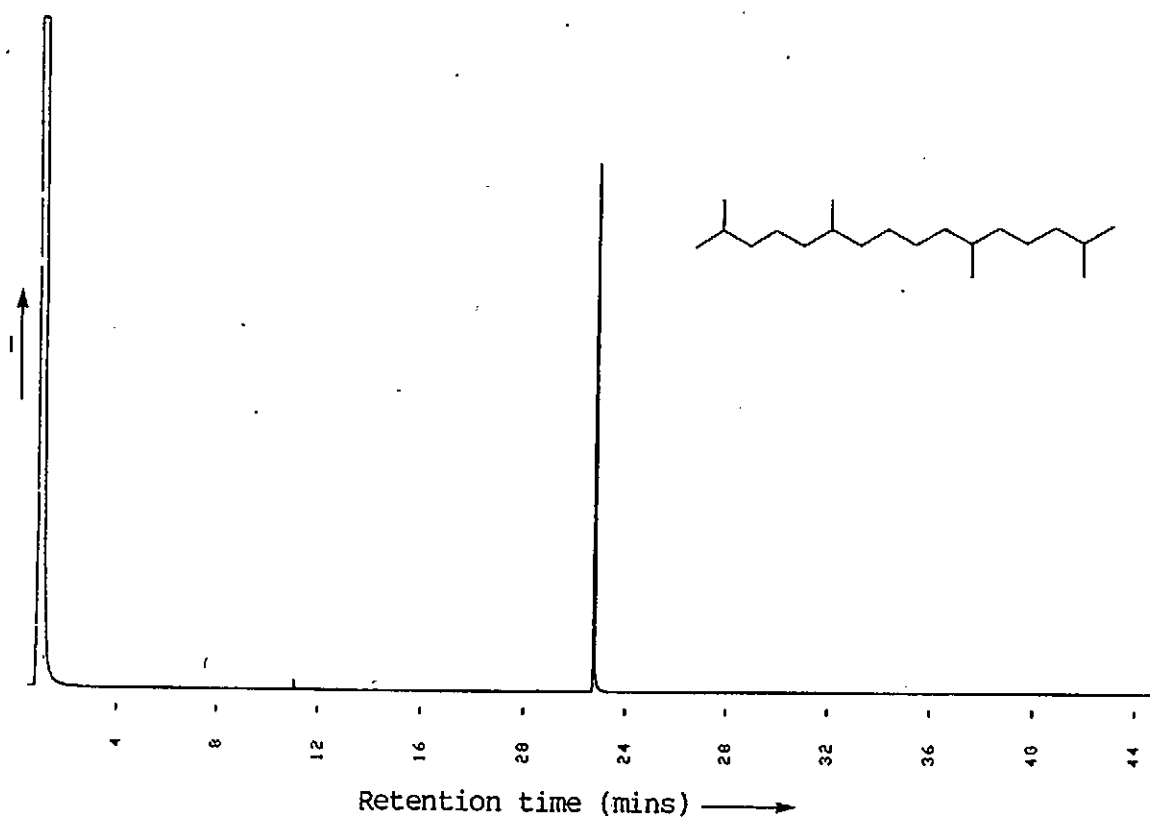
Fig.6:12A   Gas chromatogram of 2,6,11,15-tetramethylhexadecane.
             Gc conditions as text.
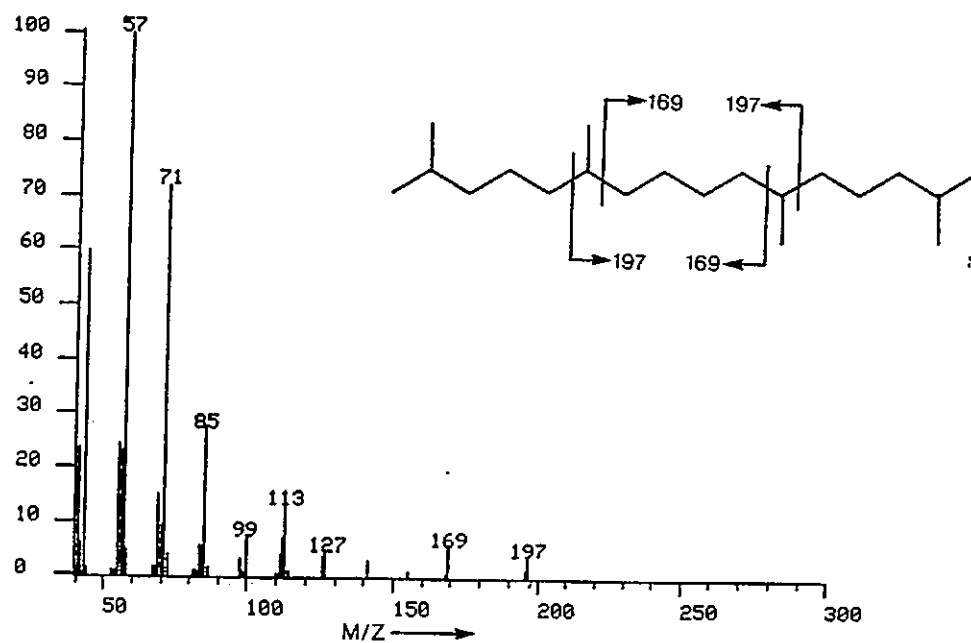
Fig.6:12B   Mass spectrum of 2,6,11,15-tetramethylhexadecane.

The ^{13}C NMR spectrum of 2,6,11,15-tetramethylhexadecane is displayed in Fig. 6:13 and listed in Table 6:4A. The assignment of chemical shifts is summarised in Table 6:4B and was performed by comparison with published spectra of acyclic isoprenoids (Breitmaier et al., 1979; Lamb, 1982; Yon, 1982; Yon et al., 1982) and by comparison with theoretical chemical shifts calculated according to Carmen et al. (1973). The symmetry of the molecule is obvious from the occurrence of only 11 resonance peaks for a C_{20} alkane. It is also interesting to note, that the spectrum of the C_{20} alkane does not contain the large number of signals due to magnetically non-equivalent carbons (resulting from configurational interactions) as seen in the ^{13}C NMR spectrum of 2,6,9,13-tetramethyltetradecane (Fig. 6:11). Indeed, strong configurational non-equivalence is only observed for the signals at 37.2796 - 37.4041 ppm (Fig. 6:14) which have been assigned to two of the methylene carbons (possibly C5 and C12 by analogy with the spectrum of 2,6,9,13-tetramethyltetradecane; Fig. 6:11) adjacent to the internal tertiary carbons (C6 and C11). Only slight magnetic non-equivalence is observed (Fig. 6:14) for the internal methyl carbons (C18 and C19). The reduction of configurational non-equivalence between the synthetic C_{18} and C_{20} isoprenoid alkanes probably results from the increased distance between the chiral centres in the C_{20} alkane. The doublet at 22.5248 - 22.6106 ppm is due to conformational magnetic non-equivalence of the methyl carbons of the isopropyl termini.

6.3 DISCUSSION

To the best of this author's knowledge, neither the 'irregular' C_{20} (t-t) or C_{18} (h-h) isoprenoid alkane synthesised herein have been shown to occur in sediments or petroleum. The tentative report of
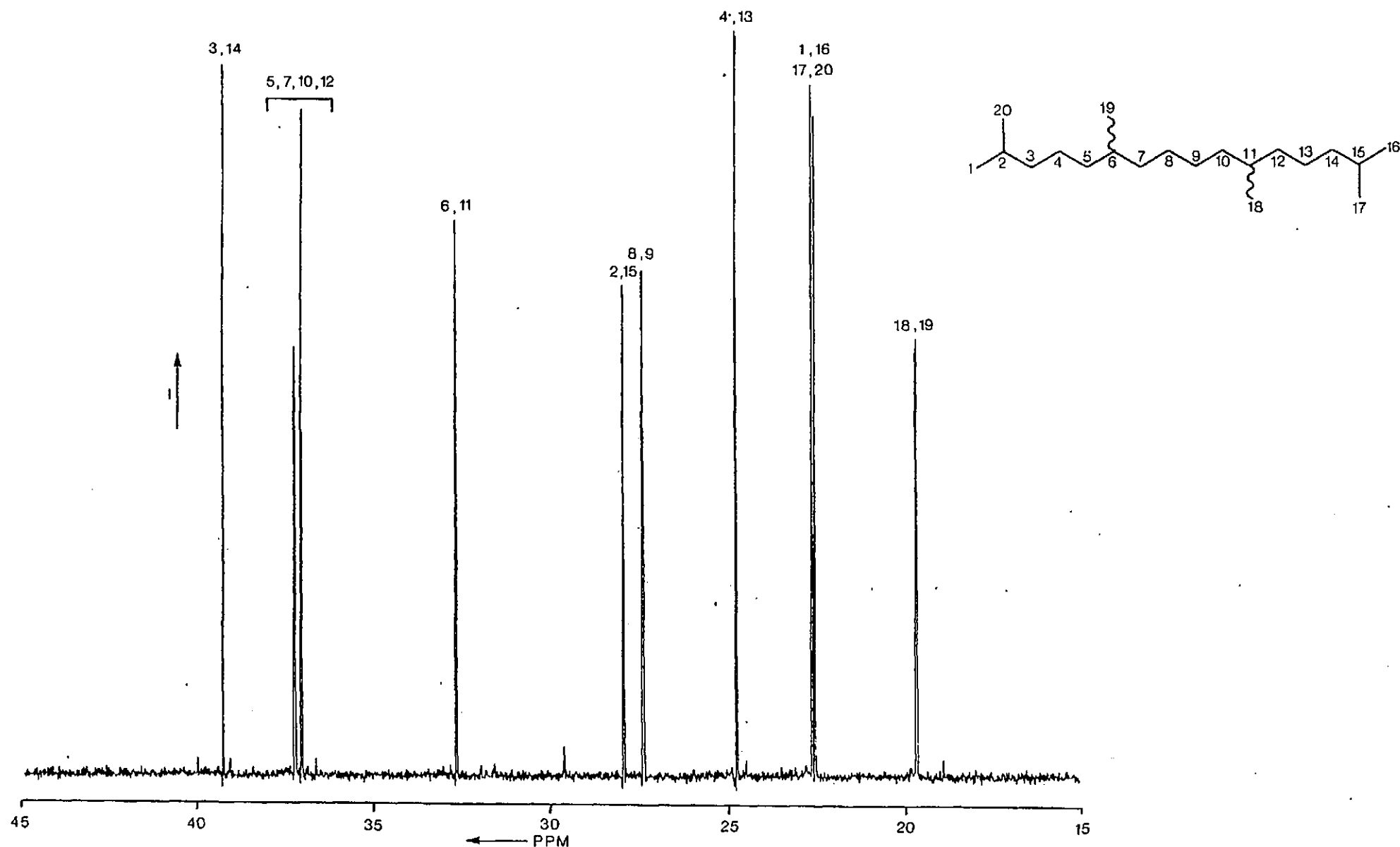
Fig.6:13 ^{13}C NMR (400 MHz) spectrum of 2,6,11,15-tetramethylhexadecane.   Proposed shift
assignments (Table 6:4b) as shown.   Numbers refer to the carbon number in the
accompanying structure.

Table 6:4A

Observed ^{13}C NMR chemical shifts for
2,6,11,15-tetramethyltetradecane

ppm	intensity	proposed number of carbons
39.3192	20.819	2
37.4041	12.478	
37.2796	12.278	4
37.0781	18.756	
32.7095	15.655	?
27.9006	14.283	2
27.3530	12.764	2
24.7156	21.792	2
22.6106	19.486	2
22.4811	19.5248	2
19.6403	12.508	2

Table 6:4B

^{13}C-NMR Chemical shift assignments for 2,6,11,15-tetramethylhexadecane.
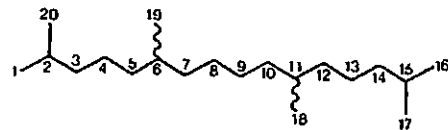
Carbon Number	ppm	Calculated shift assignments[a] ppm
18,19	19.6403	20.44
1,16	22.5248[b]	22.50
17,20	22.6016[b]	22.50
4,13	24.7156	25.29
8,9	27.3530	28.19
2,15	27.9006	28.61
6,11	32.7095	33.30
5,7,10,12	37.0781 37.2572 37.4041	37.65,37.93
3,14	39.3192	39.99

[a] : Calculated according to Carmen et al., (1973)

[b] : Chemical shift assignments in with the same superscript may be interchanged.
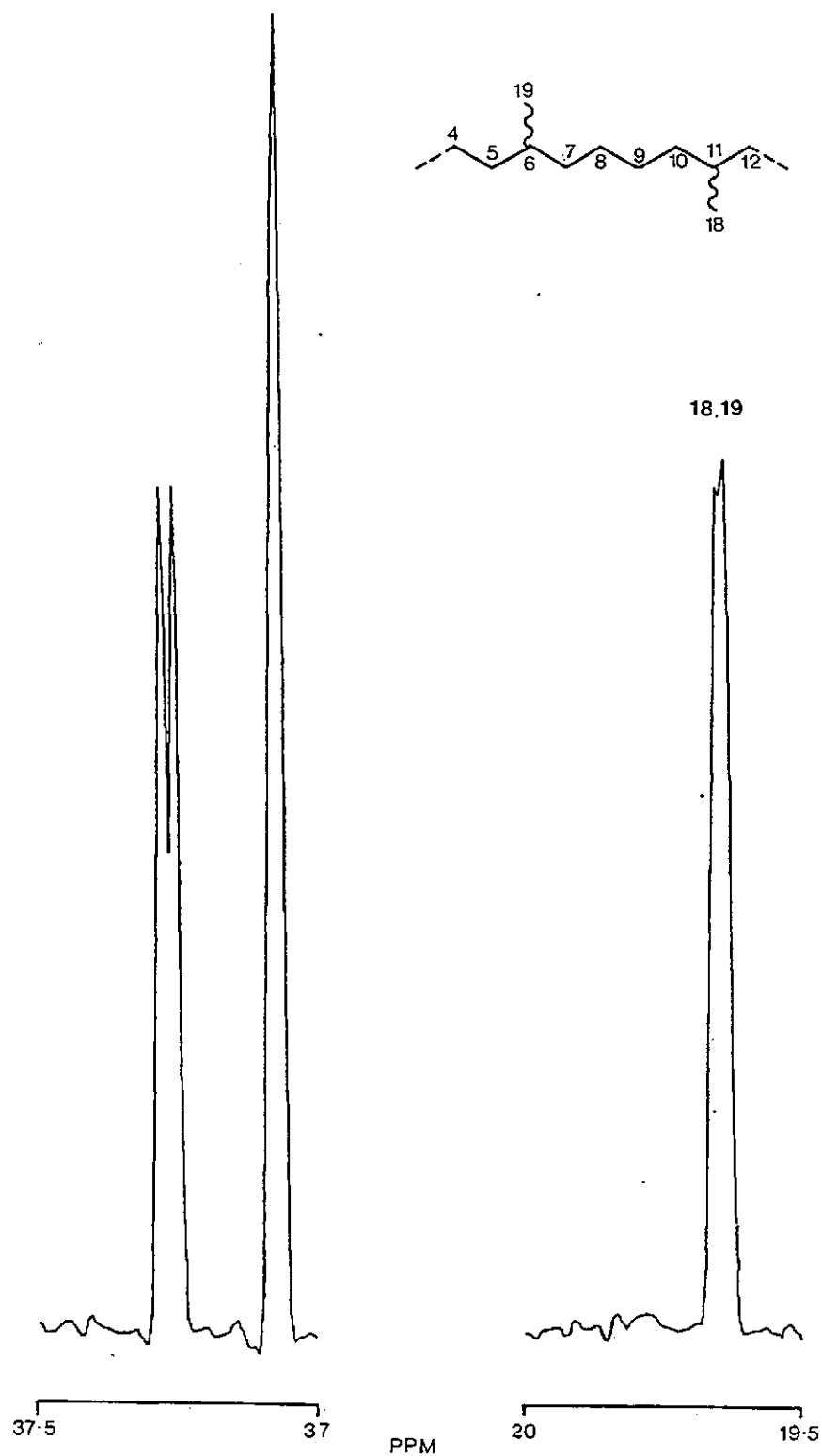
Fig.6:14   Partial expanded ¹³C NMR (400 MHz) spectrum
            of 2,6,11,15-tetramethylhexadecane.   Numbers
            refer to the carbon number in the accompany-
            ing structure.

Fowler (1984) is incorrect. However, it has been suggested that 2,6,9,13-tetramethyltetradecane ($\underline{5}$) could derive from thermocatalytic degradation of the C_{40} isoprenoid biphytane ($\underline{41}$) and C_{32} - C_{40} hydrocarbons pseudohomologous with the C_{18} alkane have been previously identified in petroleum (Moldowan and Seifert, 1979). Similarly, 2,6,11,15-tetramethylhexadecane ($\underline{6}$) could derive by degradation of squalane ($\underline{21}$) or lycopane ($\underline{37}$) or represent a direct biogenic input. C_{25} and C_{30} (t-t) isoprenoid hydrocarbons have already been identified in neutral lipids of Archaebacteria (Holzer $\underline{et}$ $\underline{al}$., 1979). The addition of the spectral data presented for the C_{18} and C_{20} isoprenoids to the existing library of spectral data on acyclic isoprenoid hydrocarbons can only aid future searches for these compounds in sedimentary environments.

The identity of the compounds noted by Fowler (1984) in Corcoran Formation PreCambrian sediments remains for the present unknown although, in the light of the new data, it is interesting to speculate on further structures. The higher retention index of the sedimentary alkane (RI1654) compared to the synthetic alkane (RI1605) suggests the structure is not as highly branched as 2,6,9,13-tetramethyltetradecane. By analogy with the mass spectra of other acyclic isoprenoid hydrocarbons, it is likely that the fragment ion at m/z 211 in the mass spectrum of the sedimentary alkane (Fig. 6:2) arises from cleavage adjacent to an internal methine carbon. Thus, structure ($\underline{93}$) can be proposed in which the m/z 211 fragment arises by cleavage
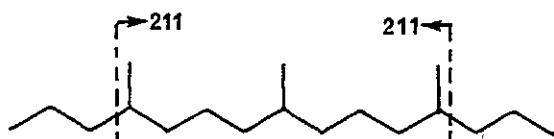
adjacent to C4 and C12. Structurally similar hydrocarbons (i.e. 31 and 32) have been reported in a Spanish crude oil (Miocene) by Albaiges et al. (1985) where they are thought to be derived from catagenic degradation of the 'regular' C_{40} (h-t) alkane (23). A similar origin can be envisaged for the structure (93) proposed above. This assignment of structure for the C_{18} alkane present in Corcoran Formation PreCambrian sediments must await confirmation by synthesis of the necessary reference alkane.

CHAPTER SEVEN

FUTURE WORK

FUTURE WORK

1. It was noted in Chapter 4 that two of the synthetic 2,6,10,14-
    tetramethyl-7-(3-methylpentyl)pent-adecenes (i.e. br25:1;2076 and
    br25:1;2091) were identified in the hydrocarbon extract of Tamar
    Estuary sediment.   Attempts to determine the position of the
    double bond in these and the other alkenes by chemical
    derivatisation (i.e. alkylthiolation, methoxy-mercuration and
    hydrolysis) proved unsuccessful.   A preparative hplc system
    should be developed to separate the individual synthetic isomeric
    br25 monoenes, this would allow unambiguous structural assignment
    of the individual hydrocarbons by ^{1}H and ^{13}C NMR.   Similar
    studies on the synthetic br20 and br30 monoenes would provide
    interesting data on the gas chromatographic behaviour of
    positional/geometric monoene isomers.   The same preparative hplc
    system could be used to isolate individual sedimentary acyclic
    br20, br25 and br30 alkenes in sufficient quantity and purity for
    spectroscopic analysis.   Additional information on the position
    of the double bond(s) within individual alkenes could be provided
    by chemical derivation using ozonolysis.   Dunlop and Jefferies
    (1985) used ozonolysis to successfully identify the position of
    the double bonds in two acyclic 'br' monoenes (br20:1;1702 and
    br25:1;2112).   Unfortunately, the apparatus required for
    ozonolysis was not available to the current investigator.

2. It was suggested in Chapter 4 that the abundance and degree of
    unsaturation of the acyclic br20, br25 and br30 hydrocarbons
    might be related to other environmental parameters   (e.g.
    salinity, sulphur, water/sediment temperature). A thorough study
    is required to investigate this. One possible location for such
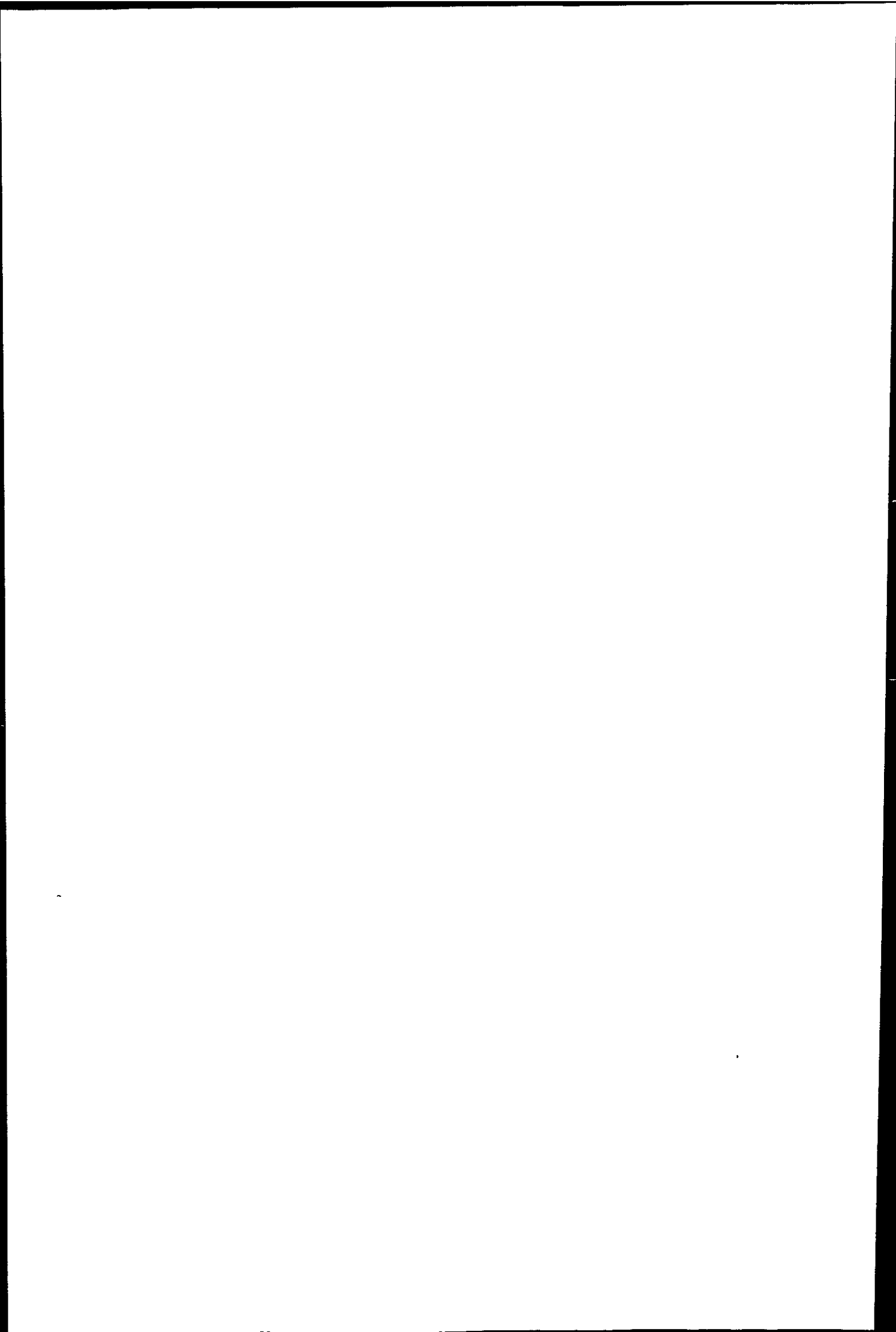
a study is the Tamar Estuary (U.K.) where attempts have been made previously (Readman et al., 1982) to relate the geographic distribution of polynuclear aromatic hydrocarbons (PAH) to salinity, water temperature, sulphate concentration etc. It has already been shown that the sediments of the Tamar Estuary contain several 'br' hydrocarbons for which definite structures are known, this makes it an ideal site for the type of investigation required.

3. There are several reports in the literature of the rapid decrease in concentration of br25 hydrocarbons with increasing depth in sediment cores (Barrick et al., 1980). This decrease has been attributed to in situ biological and chemical degradation and to the incorporation of the alkenes into accreting polymeric material. It is suggested that the preparation of the synthetic 'br' hydrocarbons be repeated but this time via a scheme which allows the incorporation of a radioactive carbon (^{14}C) into the molecule. Fig. 7:1 shows a synthetic scheme for the preparation of a radiolabelled br25 molecule with the radioactive carbon situated at the stable mid-point of the molecule (i.e. C7). The radiolabelled precursor synthon CH_2Br_2 could be prepared from radiolabelled bromoform ($CHBr_3$) which is commercially available from Amersham International. Incubation of the radiolabelled compounds insitu in a suitable sediment would provide data on the initial sedimentary fate of the 'br' hydrocarbons. Possible sites for the incubation experiments include the Tamar Estuary (possibly in tandem with the environmental survey) and Loe Pool (U.K) where sediments contain dated annual laminations which may prove helpful in relating the sedimentary concentration at depth
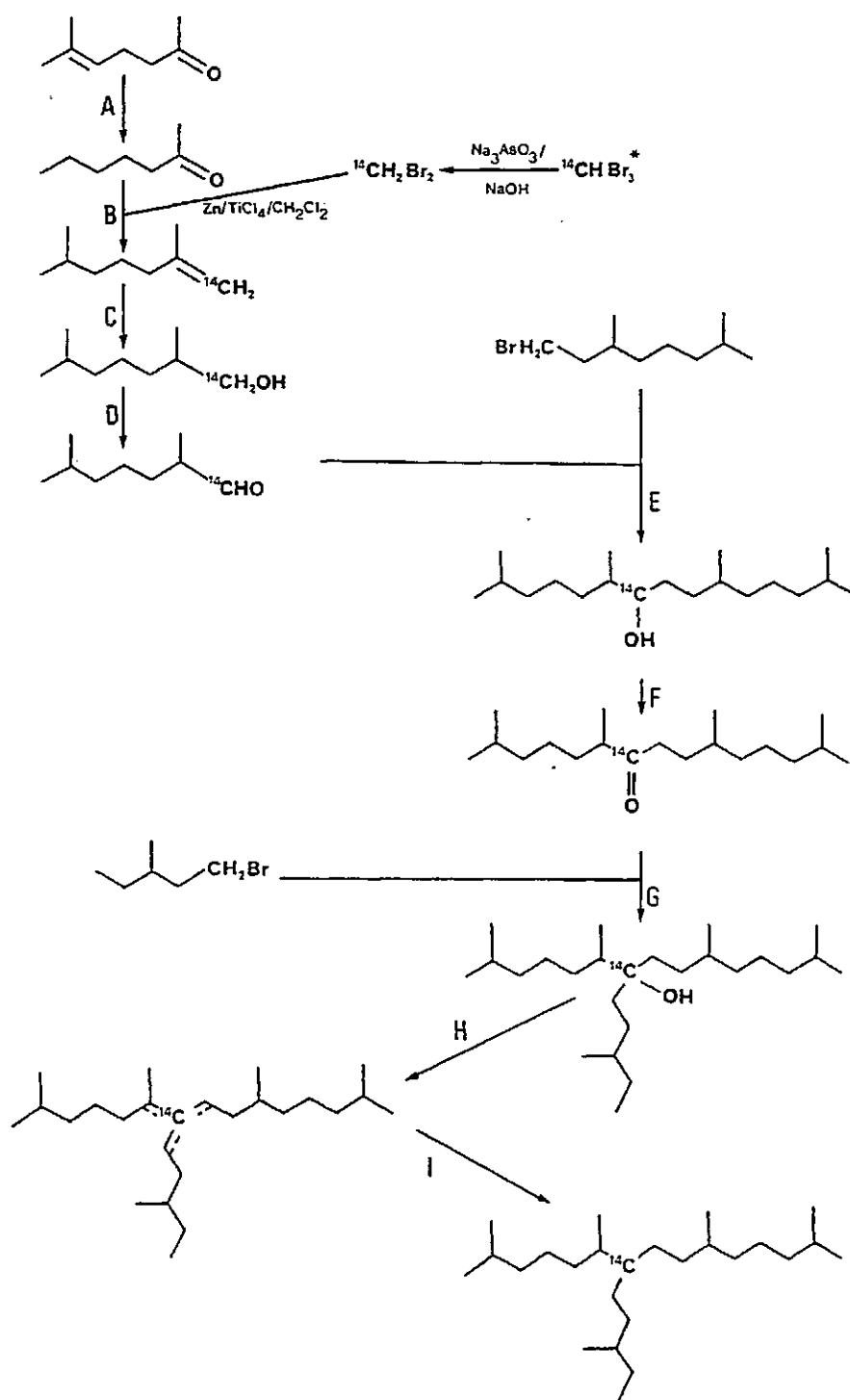
Fig.7:1   Proposed synthetic scheme for the preparation of ^{14}C - radiolabelled 2,6,10,14-tetramethyl-7-(3-methylpentyl) pentadecane (br25:0).   Letters refer to the related stages in Fig.3:2 where details of reagents can be found.   *^{14}C - radiolabelled bromoform is available from Amersham International.   Note the position of the labelled carbon at the tertiary carbon centre (i.e. C7) of the molecule.

with time in the sediments.

4. The suggestion that the rapid disappearance of br25 alkenes with depth in the sediment is related to their incorporation into rapidly accreting polymeric material (Volkman et al., 1983) should be subjected to further investigations. The polymeric material (i.e. 'protokerogen') isolated from a sediment core in which a rapid decrease in acyclic alkene concentration is evident (i.e. Peru Upwelling sediment), should be examined by degradative techniques such as pyrolysis-gc (py-gc) and pyrolysis-gcms (py-gcms) to determine if any molecular fragments associated with the alkenes can be found. Artificial maturation experiments (e.g. hydrous pyrolysis) involving isolated kerogens with added synthetic alkenes and/or isolated sedimentary alkene concentrates could provide interesting information on the type of mechanisms involved in the incorporation (i.e. crosslinking involving double bonds?).

5. In order to confirm the proposed carbon skeletons for the bicyclic C_{25} and C_{30} hydrocarbons it is necessary to synthesise reference compounds of relevant structure (see Chapter 3). Similarly, the structure (93) proposed for the C_{18} alkane in PreCambrian sediments requires confirmation by synthesis.

6. Finally, and probably most importantly, considerable further investigation is required into the biological sources of the acyclic and cyclic C_{20}, C_{25} and C_{30} hydrocarbons. The identification of br20 and br25 hydrocarbons in the green alga Entereomorpha prolifera requires further evaluation, with new

studies monitoring the hydrocarbon content of euxinic cultures and examining field speciments of the alga collected from different geographical locations. Such studies should help to confirm that the one reported identification of 'br' hydrocarbons in the alga E. prolifera (Rowland et al., 1985) is not an isolated example. In addition to E. prolifera, other species of alga should be examined to see if they also contain similar hydrocarbons. Many previous investigators have reported that the ubiquity of occurrence of the acyclic and cyclic hydrocarbons in Recent sediments (in some cases irrespective of organic source input) dictates an bacterial origin. It would be interesting, as Requego and Quinn (1985) suggest, to repeat some of the earlier analysis of bacterial hydrocarbons performed 10-15 years ago when chromatographic resolution and instrumentation was much less efficient than it is today. The possible association of the acyclic and cyclic hydrocarbons with sedimentary sulphur content suggests sulphate-reducing bacteria to be good candidates for initial examination.

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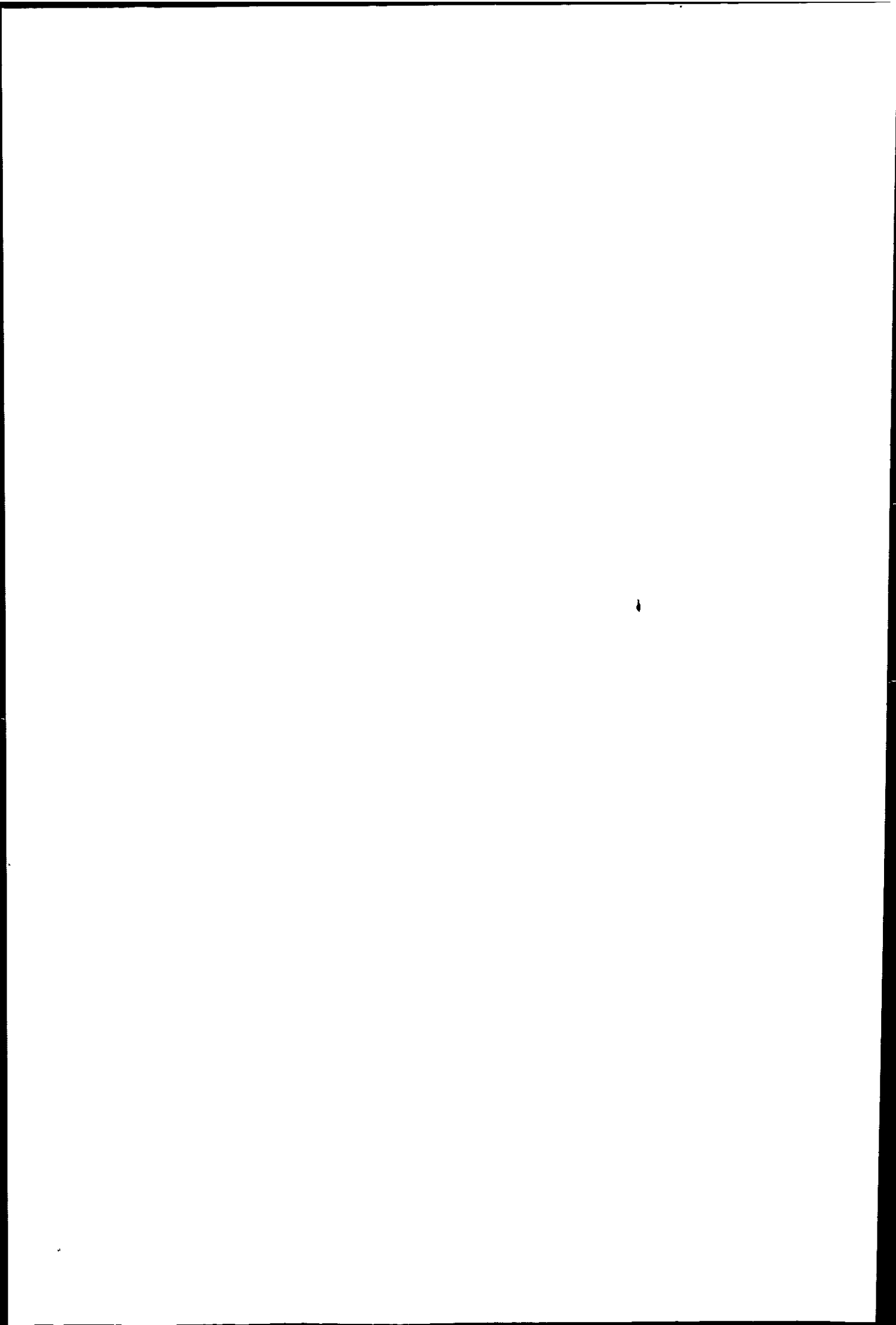
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STRUCTURES

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CH₂OH (structure 7)

8
PhytylO₂C H₃CO₃C

9
CH₂OH

10
COOH

11
COOH

12

13

14
CH₂OH

15

16

17

18
HO

19

20
H—(—CH₂—CH=CH—CH₂—)ₙ—OH
CH₃

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1 x (double bond
   position unknown)

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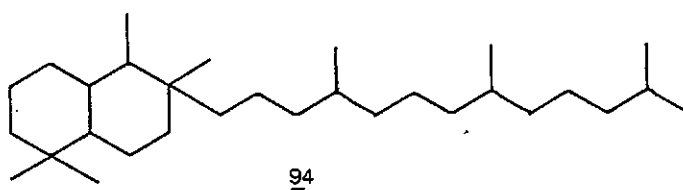
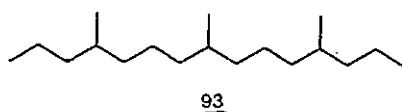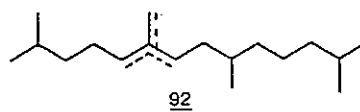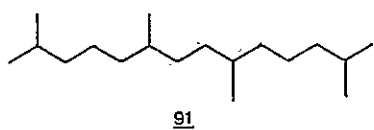86

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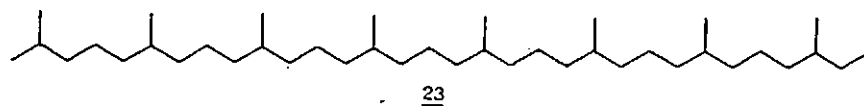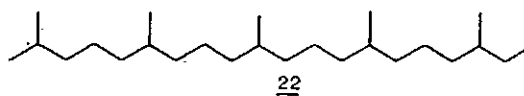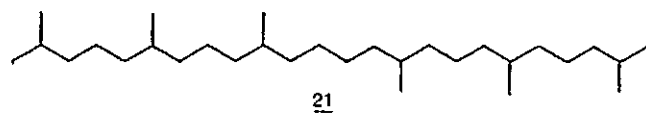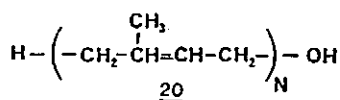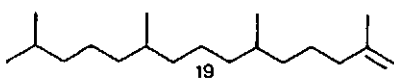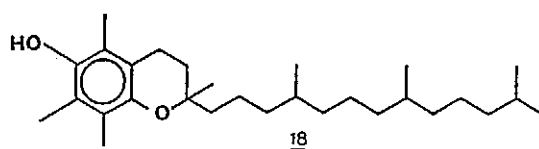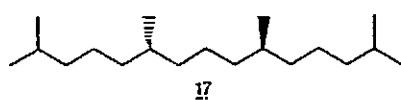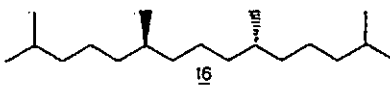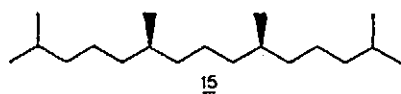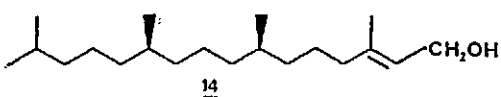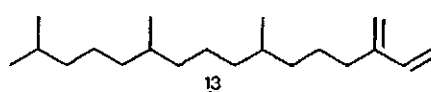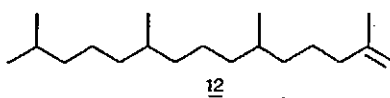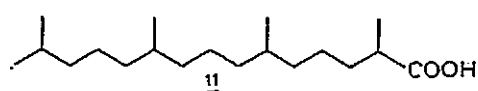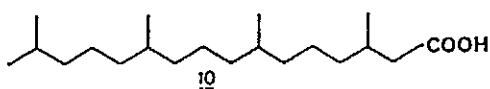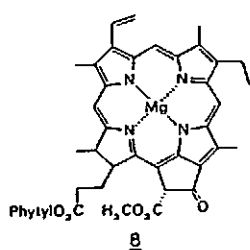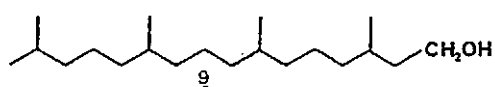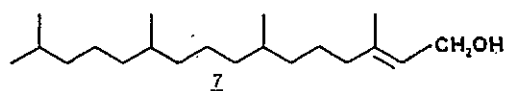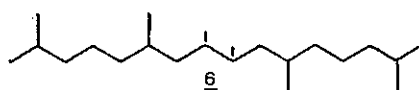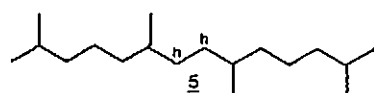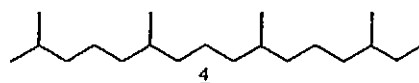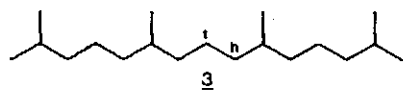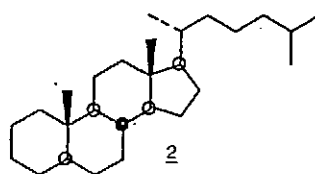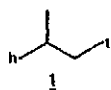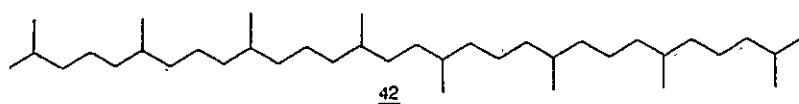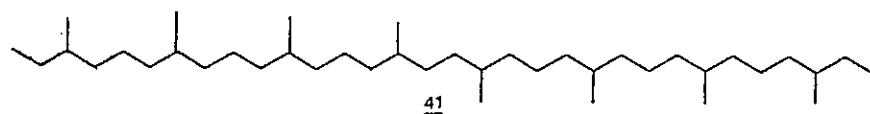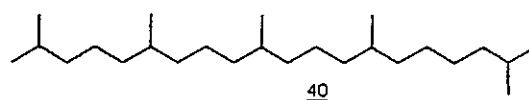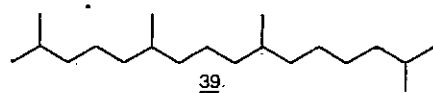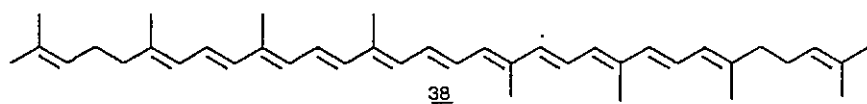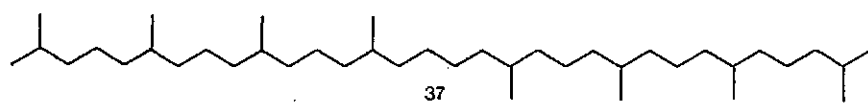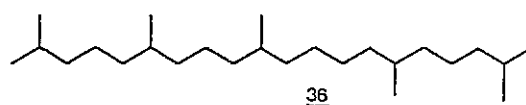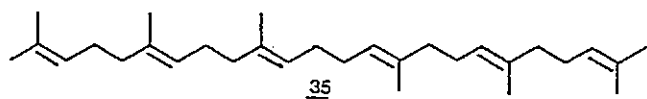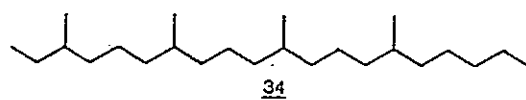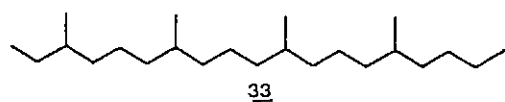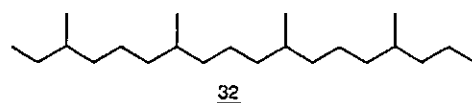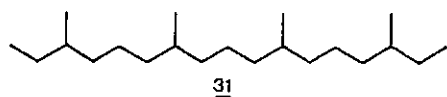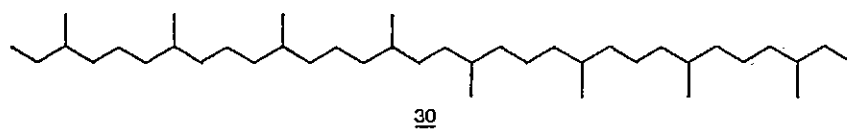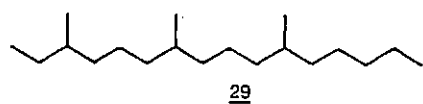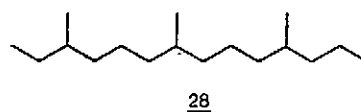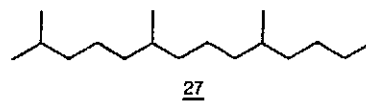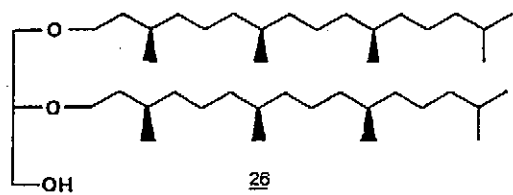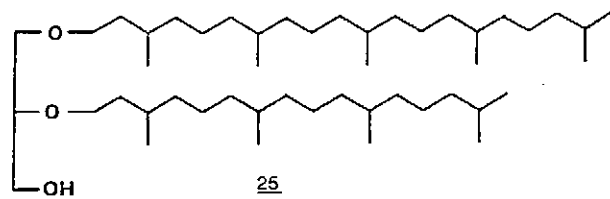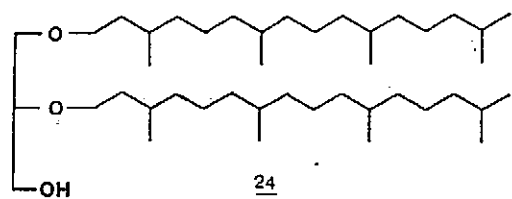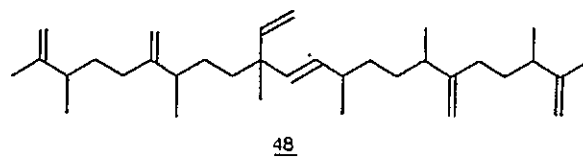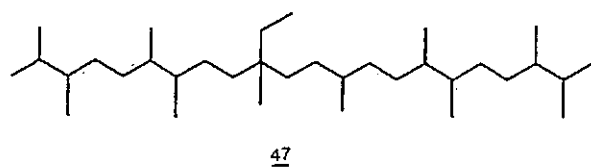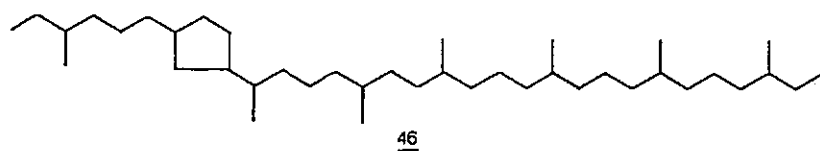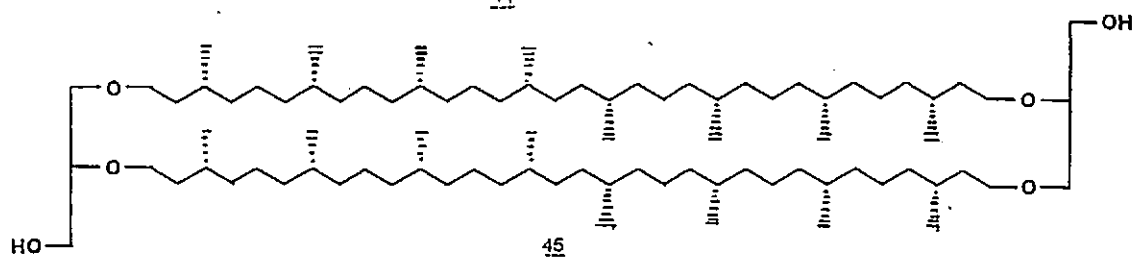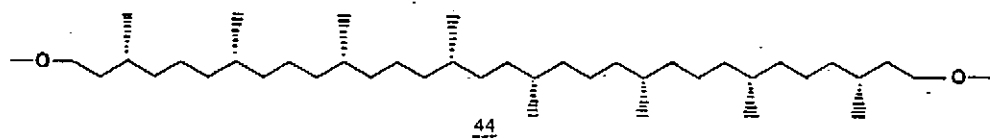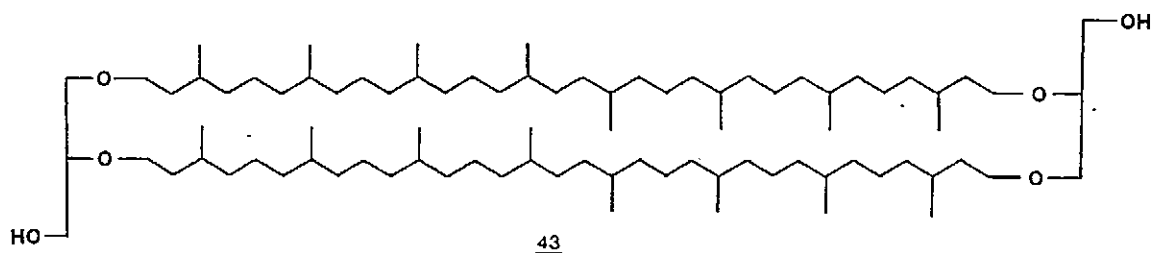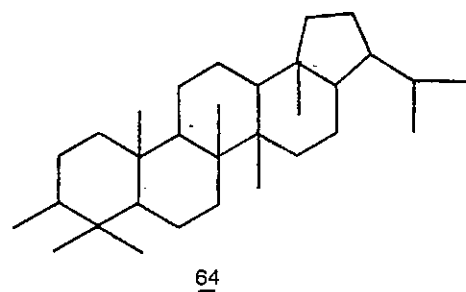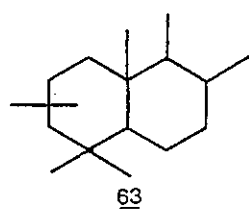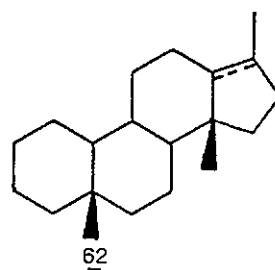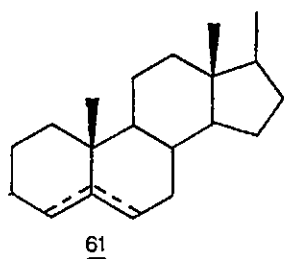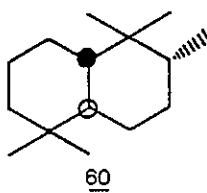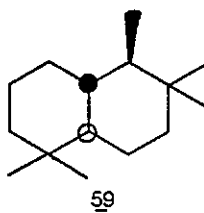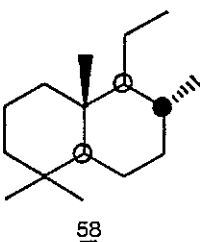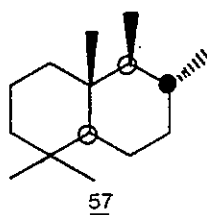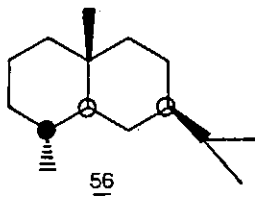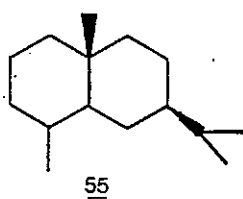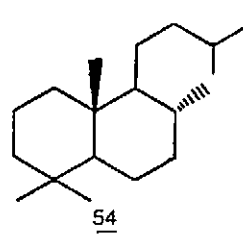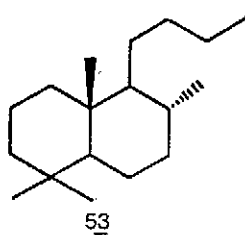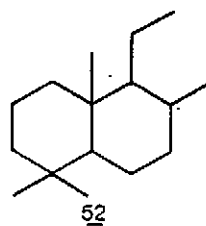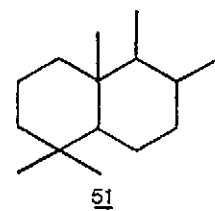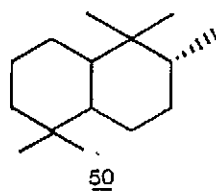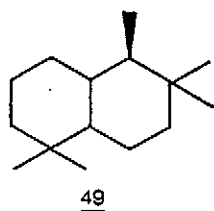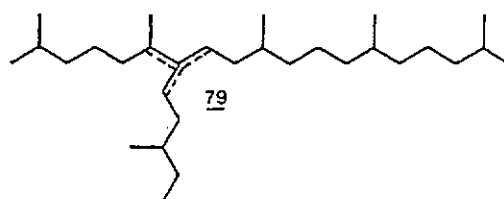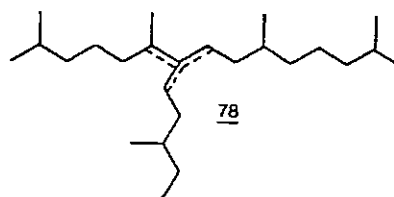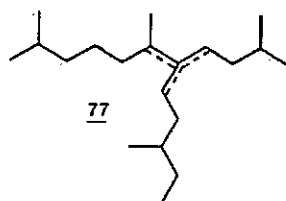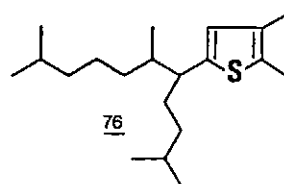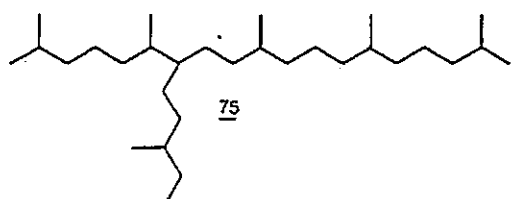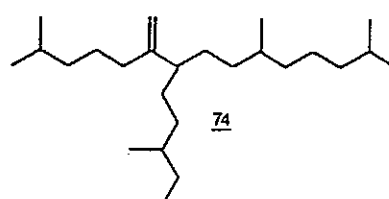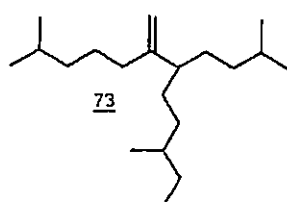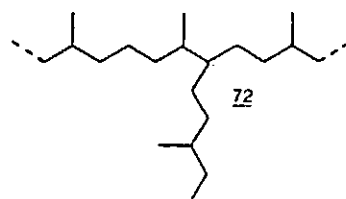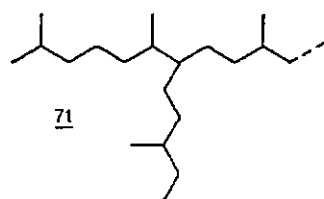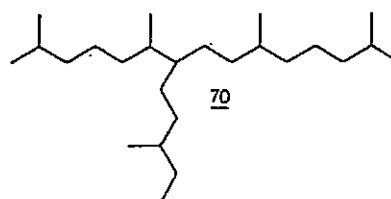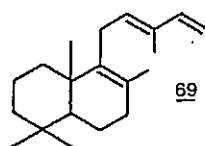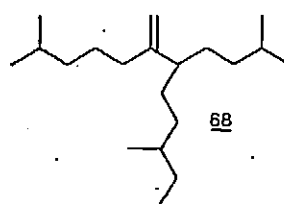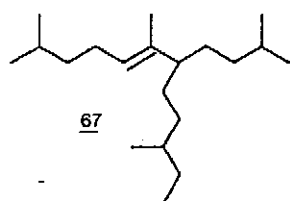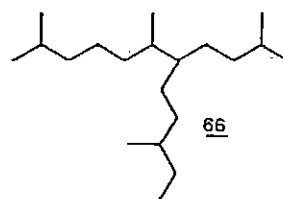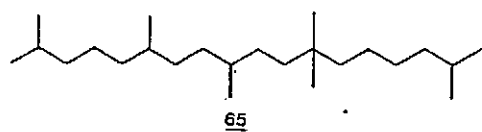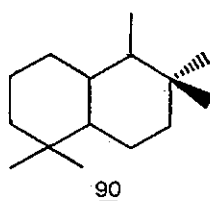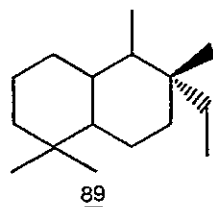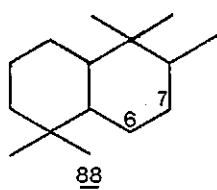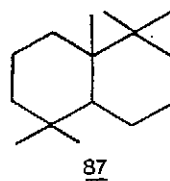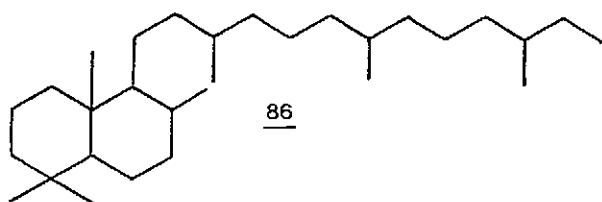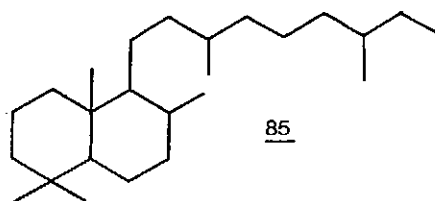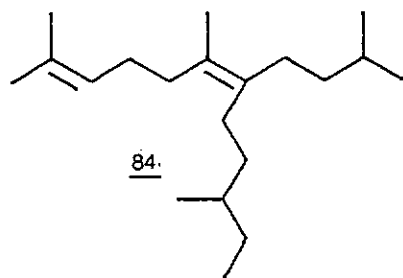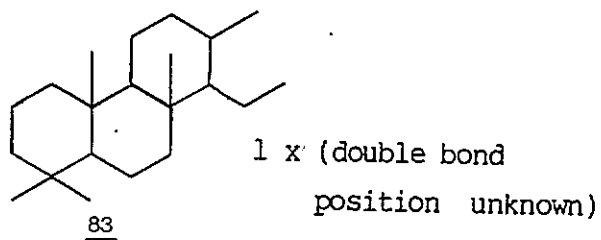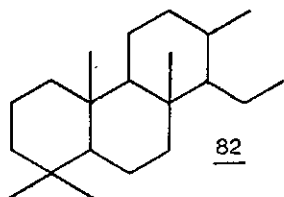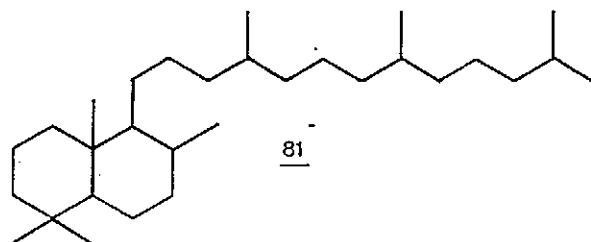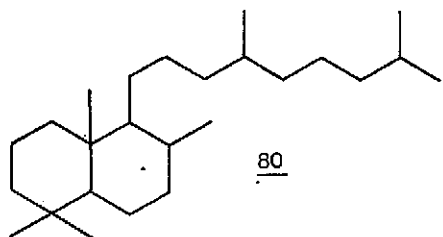
ASSOCIATED STUDIES

i.   Grob course in capillary gas chromatography. Manchester. June, 1984.

ii.   Gas chromatography-mass spectrometry workshop. Bristol Unversity. December, 1984.

iii. International conference: Advances in Organic Geochemistry 1985. Germany. September, 1985.

PRESENTATIONS AND PUBLICATIONS

As a result of the work reported in this thesis the following papers have been presented and published.

A.   Presentations

i.   Identification of C_{20}, C_{25} and C_{30} isoprenoid hydrocarbons in Recent marine sediments.
Paper presented as one of the Plymouth seminars in Marine Science. November, 1986.

B.   Publications

i.   Robson, J.N. and Rowland, S.J. (1986).
Identification of novel widely distributed sedimentary acyclic sesterterpenoids.
Nature, London 324, 561-563.
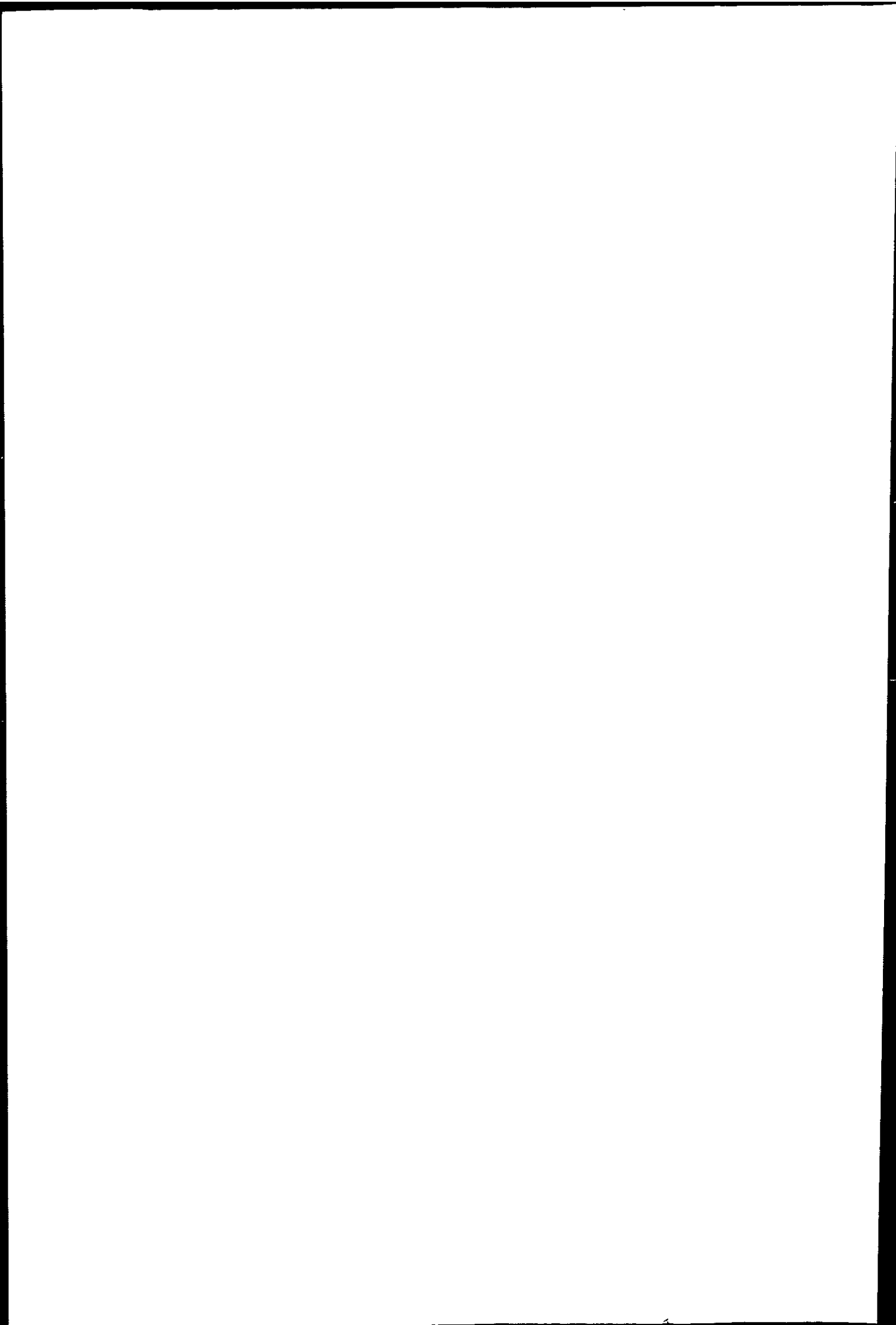
APPENDIX ONE

Identification of novel, widely distributed

sedimentary sesterterpenoids.

Robson, J.N. and Rowland, S.J. (1986)

Nature, London <u>324</u>, 561-563.

Identification of novel
widely distributed
sedimentary acyclic sesterterpenoids

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Isoprenoids, the general class of natural products biosynthesized
from isoprene units, are abundant in nature. Many isoprenoids
are also found in sediments, sedimentary rocks and crude oils
where they have proved useful as 'chemical fossils' of biological
activity and as indicators of geothermal stress[1]. However, acyclic
isoprenoids with 25 carbon atoms—so-called sesterterpenoids—
have been reported only rarely in the biosphere and the geosphere.
Indeed, the only acyclic sesterterpenoids of confirmed structure
reported in sediments are of pentamethyleicosane structural
isomers (Fig. 1, carbon skeleton I). For example, 2,6,10,14,18-
pentamethyleicosane has been proposed as a marker for halophilic
bacteria and the 2,6,10,15,19-isomer (I) as an indicator of
methanogens[2]. We have now identified a group of novel sesterter-
penoids that are major components of the hydrocarbons of recent
freshwater, marine and hypersaline sediments from many different
parts of the globe. Because they have unusual structures, these
sesterterpenes show promise as a new class of biological marker
compounds.

Over the past 10 years many workers (Table 1) have reported
the presence, in recent sediments, of a group of C_{25} hydrocarbons
of unknown structure. The compounds are abundant and are
very widely distributed; for example, they are the major hydro-
carbons present in sediments from a New England salt marsh,
Narragansett Bay (USA)[3]; North-east Gulf of Mexico[4]; the Peru
upwelling region[5]; Shark Bay, Western Australia[6] and the Ebro
River, Spain[7]. The same compounds have been detected in
aquatic filtering organisms and in sediment traps suspended in
the water column[7-10] and recently a C_{25} diene has been detected
in field specimens of the green alga, *Enteromorpha*[11]. Numerous
structures have been proposed for these C_{25} compounds (see
Fig. 1, skeletons II–V)[7,11,12] although no structure has been
confirmed. The electron impact mass spectrum and gas chomato-
graphic Kovats Index (KI) of the parent C_{25} alkane[12,13] and
ozonolysis of a C_{25} monoene[6] suggest structure II as the most
likely skeleton. This is supported by synthesis of a co-occurring

Table 1 Occurrence of alkanes and alkenes with skeletons II, VII and VIII in sediments*

Locations	Age/environment	II	VII	VIII	Refs
Kiel Bight, FRG	Recent/marine	√		√	17, 18
Scotian Shelf, Nova Scotia	Recent/marine	√			19
Shark Bay, Western Australia	Recent/marine/hypersaline	√	√		6
Alaskan Outer Continental Shelf	Recent/marine	√		√	20
Peru Continental Shelf	Recent/marine	√			5, 21
Alfacs Bay, Ebro River, Spain	Recent/marine	√	√		7, 22
Dabob Bay, Washington State, USA	Recent/land-locked marine	√		√	9
Northeast Gulf of Mexico	Recent/marine	√	√		4
Southern California, USA	Recent/marine	√			10
Rhode Island Sound, USA	Recent/estuarine	√			8
Buzzards Bay, Massachusetts, USA	Recent/estuarine	√			23
Narragansett Bay, Rhode Island, USA	Recent/estuarine	√		√	3, 15
Puget Sound, Washington State, USA	Recent/land-locked marine	√	√	√	12, 13
Sandyhaven, Dyfed, Wales	Recent/intertidal	√	√		11
Grasmere, Cumbria, UK	Recent/freshwater lake		√		14
Camloch, Scotland	Recent/freshwater lake		√		24
Loch Clair, Scotland	Recent/freshwater lake		√		24
Upton Broad, Norfolk, UK	Recent/freshwater lake		√		24
Rostherne Mere, Cheshire, UK	Recent/freshwater lake	√	√		11
Cariaco Trench, Venezuela	Pleistocene/marine		√		25
Sabon-Gida, Nigeria	Pleistocene/lacustrine	√	√		25
Loch Ewe, Scotland	Recent/marine loch		√		25
Esthwaite Water, Cumbria, UK	Recent/freshwater lake		√		26
Pettaquamscutt River, Rhode Island, USA	Recent/estuarine	√		√	27
Sullom Voe, Shetland Islands, UK	Recent/intertidal	√	√		28

* The same compounds have been found in marine filtering organisms[8], an alga[11] and certain crude oils[25].
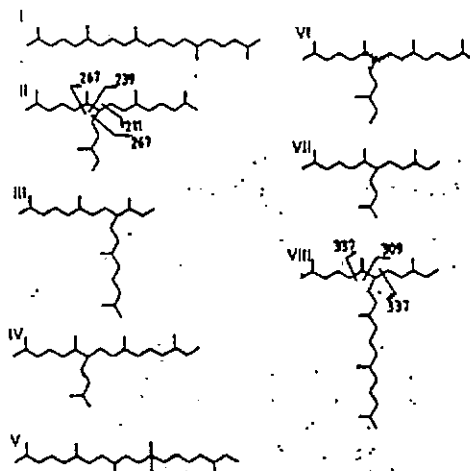

Fig. 1 Isoprenoids, carbon skeletons I-VIII.

C20 alkane (assumed to be pseudohomologous with the C25 alkane)[14]. Synthesis of V has shown this structure to be incorrect[12] and attempts to isolate the C25 alkenes by argentatious thin layer chromatography in quantities large enough for spectral characterization have been unsuccessful[15].

A mixture of five monoenes related to alkane II was synthesized in good yield from readily available starting materials by the scheme outlined in Fig. 2. Catalytic hydrogenation afforded the alkane. The electron impact mass spectrum of the synthetic alkane II is shown in Fig. 3. The spectrum, which is virtually identical to that of the sedimentary alkane[12] contains small but characteristic ion doublets at m/z 210,211; 238,239 and 266; 267 assigned to fragmentation about the C7-C8, C6-C7 and C5-C6 (and C7-C1') bonds (structure II). The KI value for both synthetic and sedimentary alkane is 2,109 (DB-1, 40-290 °C at 4 °C min⁻¹). These data confirm that the C25 alkenes and alkane identified in many previous studies (Table 1 and ref. 11) possess the 2,6,10,14-tetramethyl-7-(3-methylpentyl) pentadecane skeleton (structure II). Mass spectra of the synthetic monoenes (Fig. 1, structure VI) were characterized by even mass
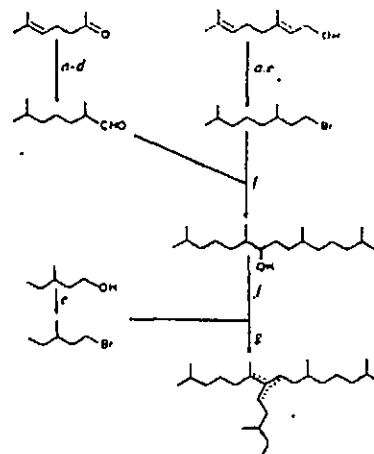

Fig. 2 Synthetic scheme for alkane II and alkenes VI. All products characterized by ¹H NMR mass and infrared spectroscopy. The second Grignard reaction (f) was repeated in improved yield (82%) by use of CeCl₃/Mg (ref. 29). a, H₂,PtO₂,hexane; b, BrCH₂Br,Mg; c, B₂H₆,THF,H₂O₂, OH d, (COCl)₂,DMSO; e, HBr/H₂SO₄; f, Mg,THF; g, POCl₃,Pyr.

ions at m/z 154,196,210 (weak), 224,266,280 and 350(M⁺) but none was identical to the spectra of any of the sedimentary monoenes. This is further indirect evidence that the sedimentary alkenes may indeed contain a methylene double bond as suggested previously from ozonolysis[6] and nuclear magnetic resonance (NMR)[8]. In sediments from Puget Sound[12] and Dabob Bay[9], Washington State (USA), the C25 hydrocarbons co-occurred with the C20 alkane (now known to be structure VII in Fig. 1[11]) and a C30 alkene of unknown structure but which from retention index measurements was considered to be pseudohomologous with both structures VII and II when hydrogenated. Given the structural relationship between VII and II demonstrated in the present study, it is reasonable to speculate that the C30 alkene is VIII (Fig. 1). This is consistent with the mass spectrum in that fragmentation about the C6-C7, C5-C6
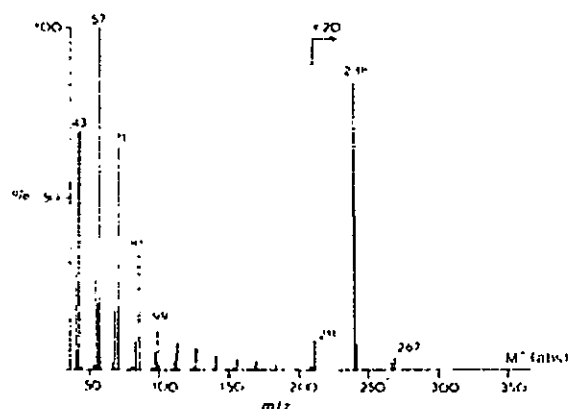
Fig. 3 Electron impact mass spectrum of synthetic II. Gas chromatography mass spectrometry conditions: Kratos MS25, 30 m × 0.3 mm CPSil 5 fused silica column, 40–300 °C at 4 °C min^{-1}. He carrier; 40 eV.

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and C7-C1 bonds would produce the ions observed at m/z 308,309; 336,337 (structure VIII). Thus, the abundant sesterterpenes appear to be the major and most commonly occurring components of a new class of terpenoids comprising $C_{20,25,30}$ and possibly other[6], hydrocarbons.

These highly branched hydrocarbons have already proved useful as environmental indicators. For instance, variations in their abundance in sediments have been successfully used to designate distinct chemogeographic regions. Dunlop and Jefferies[6] were able to classify oceanic and hypersaline sedimentary basins in Western Australia on this basis and Requego and Quinn[15] were able to show that the geographical variations of some of the alkenes reflected the distribution of marine organic matter in the estuary of Narragansett Bay, USA. Now that the structures of the C_{20} and C_{25} hydrocarbons have been established and one possible source organism proposed[11], future studies may be based on a firmer understanding. Further synthesis and incubation of synthetic ^{14}C-labelled analogues should help to elucidate the longer term sedimentary fate of these new biological markers.

Finally, the unusual structures of these molecules suggest that they may have a specific role in the lipid biochemistry of the source organisms. This has proved to be the case for other unusual isoprenoid lipids. For instance biphytanyl isoprenoid ethers act as rigidifiers in the cell membranes of archaebacteria[16] and are therefore of interest to biologists, biochemists and geochemists. Further study of the present multi-branched lipids may benefit from a similar multidisciplinary approach.

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