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Characterisation of unresolved complex mixtures of hydrocarbons

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CHARACTERISATION OF UNRESOLVED COMPLEX MIXTURES OF
HYDROCARBONS

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the degree of:

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FOR MY PARENTS

ABSTRACT

The hydrocarbons of Recent polluted sediments, in-reservoir and laboratory biodegraded crude oils, and certain petroleum products (e.g. lubricating oils) often display "humps" or Unresolved Complex Mixtures (UCMs) when analysed by gas chromatography (GC). Although widespread and often abundant, to date little is known of their detailed molecular composition.

Standard chromatographic methods of isolation of model aliphatic and aromatic hydrocarbon UCMs from lubricating oils followed by conventional methods of analysis provided little compositional detail. Thus GC and GC-electron impact mass spectrometry (GC-EIMS) was limited to an estimate of carbon number ranges and to the identification of certain series of "biological marker" compounds. However, these were well resolved and were estimated to account for <10% of the total detector response. Further analyses were performed by chemical ionisation-MS (CI-MS), probe distillation EI-MS, field ionisation-MS (FIMS), and elemental analysis; yet the information provided by each was limited to a few "average" molecular types.

In view of the limitations of conventional methods of analysis, alternative methods were adopted. These utilised novel chemical and pyrolytic degradations of the UCM hydrocarbons. Chemical oxidation with CrO_3 in glacial acetic acid produced reasonable yields of total recoverable material (40-80%). Furthermore, a high proportion were functionalised (>90%), and many resolved, which allowed their identification by EI and CI GC-MS.

Surprisingly, the most abundant products of oxidation of hydrocarbon UCMs were straight chain monocarboxylic acids. This appeared to contradict literature consensus on UCM composition, namely a predominance of highly branched and/or cyclic hydrocarbons. However, from literature reported CrO_3 oxidations of hydrocarbons, potential precursor compounds were proposed. These were monoalkyl substituted "T"-branched acyclic and monocyclic alkanes for the aliphatic UCM and alkyl "T"-branched monoaromatic hydrocarbons for the aromatic UCM.

Proposed precursor UCM hydrocarbons were confirmed by synthesis and chemical oxidation under the same conditions. Thus each of the synthetic candidate UCM hydrocarbons [7-*n*-hexylnonadecane, 9-(2-phenylethyl)-heptadecane, and 9-(2-cyclohexylethyl)-heptadecane] produced *n*-acids on oxidation with CrO_3 . Further correlations were found for products of other synthetic alkanes and less abundant UCM oxidation products. For example, *n*-alkan-2-ones, iso alkan-2-ones, and γ -methyl- γ -lactones could all be correlated with methyl substituted acyclic alkyl linkages on UCM hydrocarbons.

The application of chemical oxidation to aliphatic UCMs of varied origin showed the technique has great potential for "fingerprinting" such samples. GC-MS analysis of a selected series of resolved product compounds (alkyl ketones, γ -methyl- γ -lactones) showed good correlations for samples of the same origin, yet distinct differences for UCMs from different sources.

Biodegradation of the three candidate UCM hydrocarbons alongside acyclic isoprenoid alkanes and normal and monomethyl alkanes showed the UCM hydrocarbons were at least as resistant to microbial degradation as the isoprenoid alkanes. In this context it is therefore concluded that the candidate UCM compounds serve as good molecular models for hydrocarbon UCMs.

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ABBREVIATIONS USED IN THE TEXT

DCM	dichloromethane
anh. Na ₂ SO ₄	anhydrous sodium sulphate
TLC	thin layer chromatography
GC	gas chromatography
MS	mass spectrometry
CI	chemical ionisation
EI	electron impact
FIMS	field ionisation mass spectrometry
UNA	urea non adduct
UA	urea adduct
TUA	thiourea adduct
TUNA	thiourea non adduct
GPC	gel permeation chromatography
IR	infra red
NMR	nuclear magnetic resonance
MeOH	methanol
Ag ⁺ /TLC	argentatious TLC
UCM	unresolved complex mixture
HC	hydrocarbons
ECL	equivalent chain lengths
RRF	relative retention factor
F	fraction

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CHAPTER ONE

INTRODUCTION

INTRODUCTION

1.1 GENERAL

When examined by gas chromatography (GC), the hydrocarbons of most fresh crude oils consist of both resolved and unresolved compounds (Kissin, 1987). The former are usually most prominent, and typically comprise n-alkanes (n-C₁ -n-C₄₀₊), acyclic isoprenoids (usually C₁₄ - C₂₀), alkylated benzenes, naphthalenes and phenanthrenes (Adlard, 1972; Fig. 1.1). However, these distributions can be altered, for example by the combined natural processes known as "weathering", (i.e. water washing, evaporation, or microbial degradation); or by certain industrial refining processes. The net effect of both the natural and industrial alteration of crude oils is the removal of those components which are resolved by GC, and the enrichment of the unresolved components. The gas chromatograms of oils altered in this manner display a feature which is generally referred to as the Unresolved Complex Mixture or UCM (Fig. 1.1, e.g. Blumer et al., 1973). There are many reports of similar unresolved complex mixtures in the hydrocarbons of recent and ancient sediments, in refined petroleum products, biodegraded crude oils, air particulate extracts, and aquatic organisms. Some examples are presented in Fig. 1.1.

1.2 THE UCM IN BIODEGRADED CRUDE OILS

Fig. 1.2 (from Jones, 1986) shows the effects of microbial alteration on the aliphatic fraction of a crude oil biodegraded in the laboratory. The gas chromatogram of the fresh oil displays a typically well resolved distribution of normal alkanes (n-C₁₂ to n-C₃₅₊) and acyclic isoprenoids (C₁₉ + C₂₀) overlying a UCM. As biodegradation proceeds the most labile compounds, the n-alkanes, are

FIG. 1.1 GAS CHROMATOGRAMS OF A) TOTAL HYDROCARBONS FROM A NIGERIAN OIL (from Jones, 1986), B) AN ALIPHATIC UCM FROM A POLLUTED ESTUARINE SEDIMENT (from Thompson and Eglinton, 1978), AND C) AN AROMATIC UCM FROM AN IN-RESERVOIR BIODEGRADED CRUDE OIL (from Volkman et al, 1984)

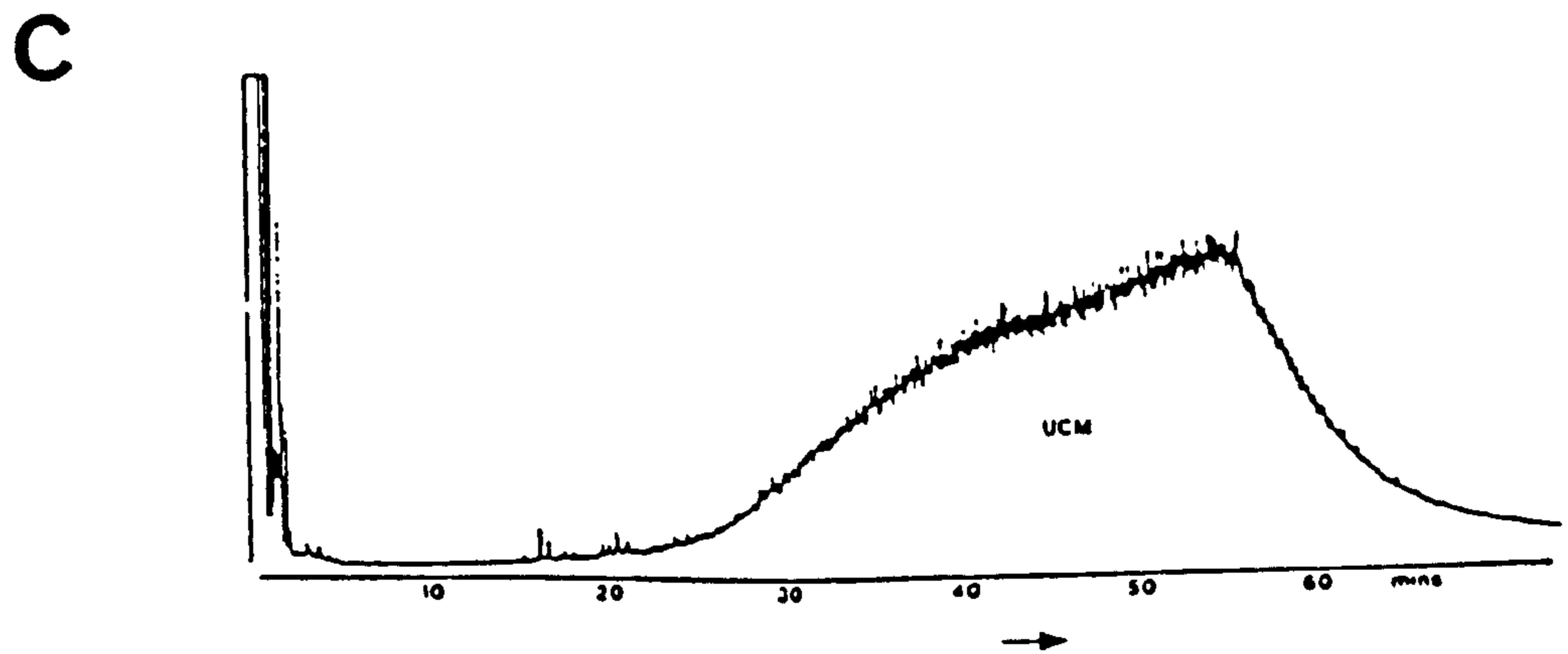
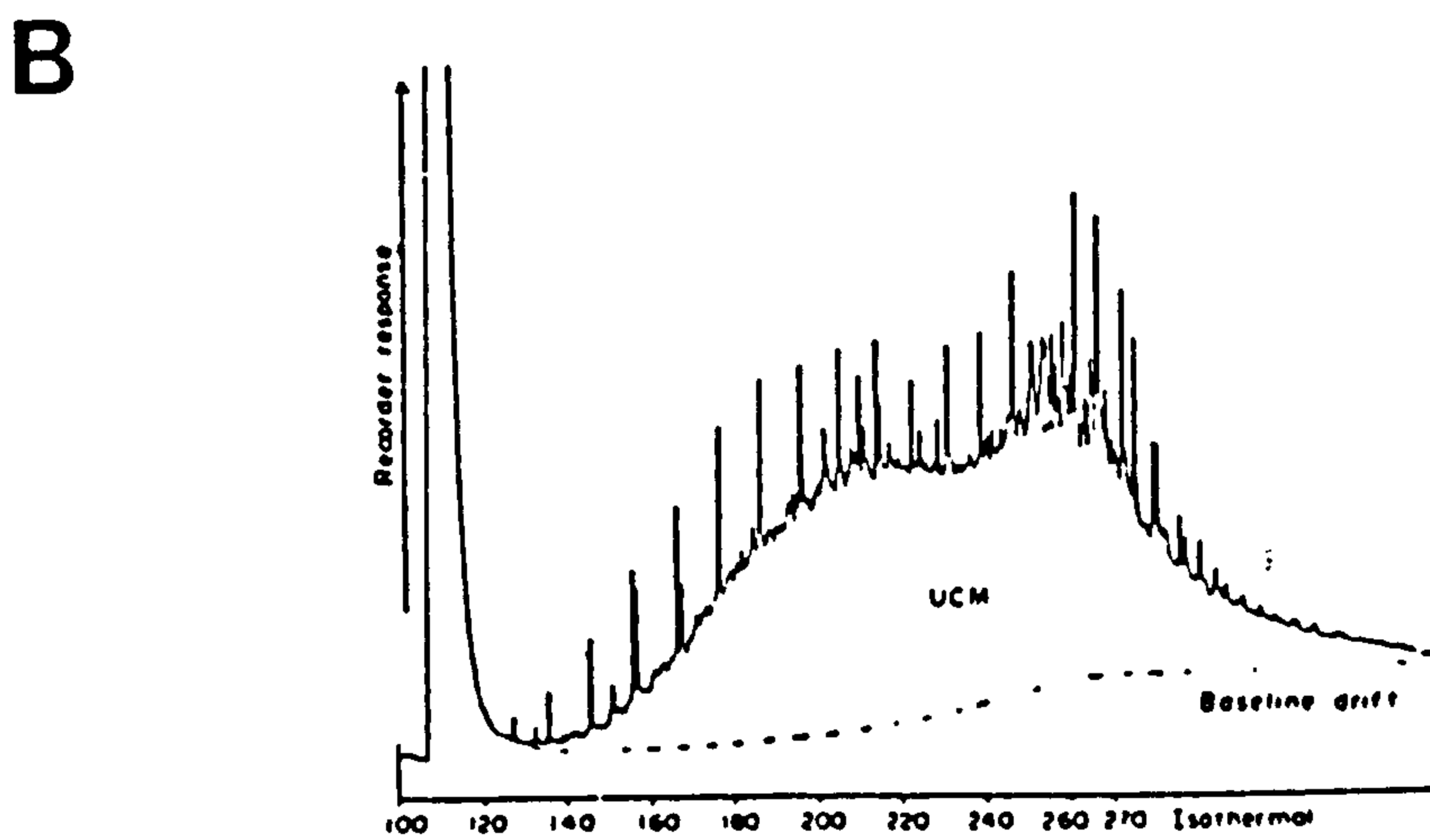
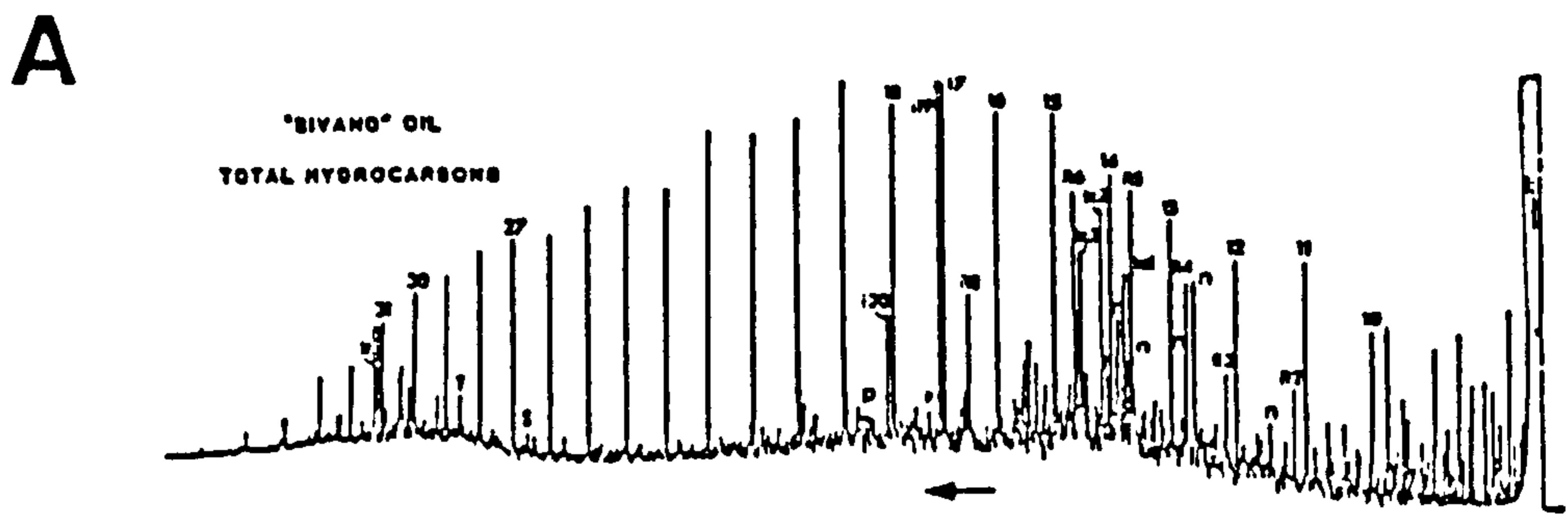
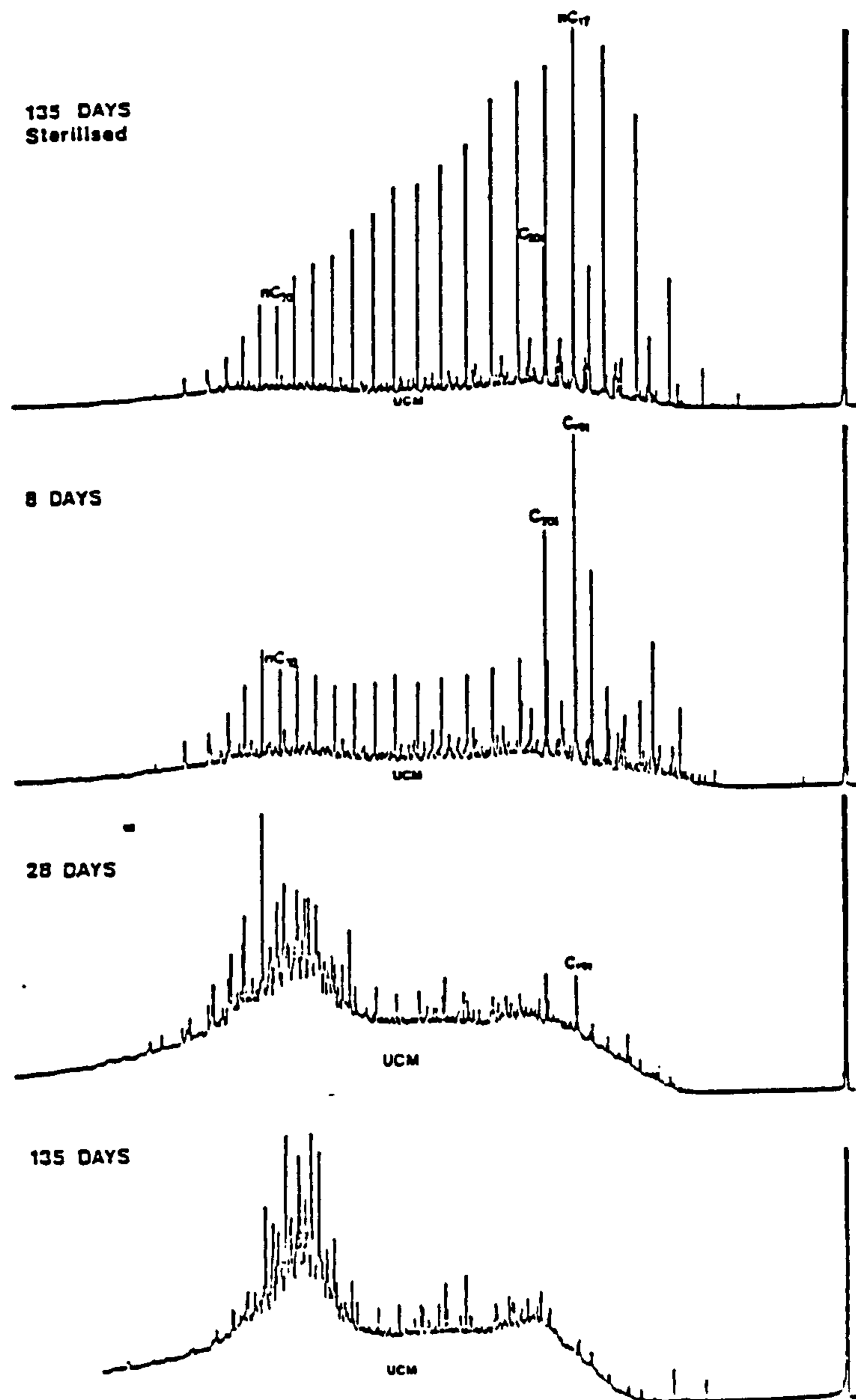


FIG. 1.2 GAS CHROMATOGRAMS SHOWING THE SEQUENTIAL LOSS OF RESOLVED ALIPHATIC HYDROCARBONS IN BRENT CRUDE OIL BIODEGRADED IN THE LABORATORY (from Jones, 1986)

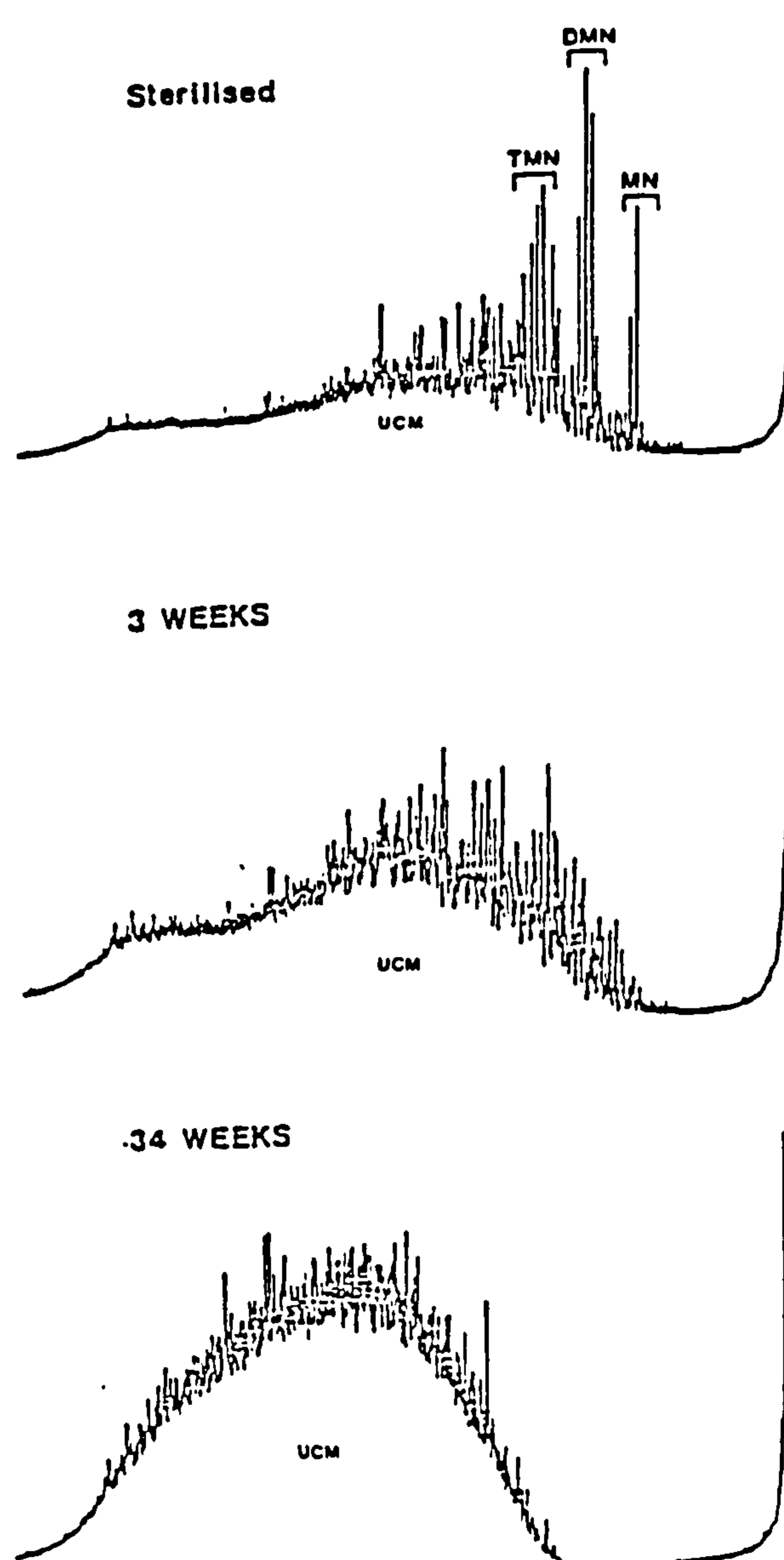
[GC: DB-5(J+W), 30m, 40-300°C @ 4°Cmin⁻¹]



nCn: n-alkanes
iCn: acyclic isoprenoid alkanes
UCM: unresolved complex mixture

FIG. 1.3 GAS CHROMATOGRAMS SHOWING THE SEQUENTIAL LOSS OF RESOLVED AROMATIC HYDROCARBONS IN A NIGERIAN CRUDE OIL BIODEGRADED IN THE LABORATORY (from Jones, 1986)

[GC: DB-5(J+W), 30m, 40-300°C @ 4°Cmin⁻¹]



mn:methyl naphthalenes
dmn:dimethylnaphthalenes
tmn:trimethylnaphthalenes
UCM:unresolved complex mixture

partially removed, resulting in a relative enrichment of the acyclic isoprenoids and the UCM. After a few weeks the n-alkanes are removed completely, the acyclic isoprenoids are reduced in abundance, and the UCM profile as a result of analytical amplification increases in magnitude. After 135 days only traces of the isoprenoids are detectable, and the chromatographic profile is dominated by a broad UCM with few resolved peaks. Many other studies have noted a similar loss of resolved components and an increase in the relative abundance of the UCM in the aliphatic fractions of crude oils spilled in the environment (e.g. Blumer and Sass, 1972; Blumer et al., 1973; Atlas et al., 1981, Berthou et al., 1981, Oudet et al., 1981, Jones et al., 1986); in laboratory biodegraded crude oils (e.g. Jobson et al., 1972, Bailey et al., 1973, Jones et al.; 1986) and in reservoir degraded crude oils (e.g. Deroo et al., 1974 ; Sassen, 1980; Volkman et al., 1983; Brooks et al., 1989^a).

The resolved components of the aromatic hydrocarbon fractions of crude oils have been shown to be degraded in a similar manner in preference to the unresolved components. The sequential loss of alkyl benzenes, naphthalenes and phenanthrenes with a concomitant increase in the relative abundance of the aromatic UCM profile is well documented for crude oils biodegraded in the laboratory (e.g. Jones et al., 1983); in Recent sediments affected by oil spills (e.g. Berthou et al., 1981; Atlas et al., 1981; Jones et al., 1986); and in crude oils biodegraded in the reservoir (e.g. Volkman et al., 1984; Connan, 1984). An example of the preferential removal of the resolved distributions of aromatic hydrocarbons in a Nigerian crude oil biodegraded in the laboratory is shown in Fig. 1.3.

Volkman et al., (1984), have summarised the effects of biodegradation on the composition of the resolved hydrocarbons of crude oils, and have proposed a scale to assess the extent of biodegradation based on the abundance of selected saturated and aromatic compounds. The initial appearance and extent of alteration of the UCM in this sequence is not accounted for. However, most published studies have shown that the aliphatic UCM becomes prominent at a stage when the majority of the n-alkanes have been removed. The UCM then typically persists with some alteration throughout the biodegradation sequence, and remains even when the "biological marker" compound distributions (e.g. acyclic isoprenoid, triterpenoidal, and steroidal alkanes) have been severely effected. Thus the UCM appears to consist of compounds which are relatively inert to microbial degradation, although the exact nature of these compounds is unclear.

1.3 THE UCM IN PETROLEUM PRODUCTS

Certain petroleum products which result from the distillation and refining of crude oils also contain hydrocarbon UCMs. Quantitatively the UCM is most important in kerosene (Kuchhal et al.; 1986) and fuel oil (Gearing et al., 1980) which are products of the middle distillate fractions (C10 -C20; 185°C - 345°C); and in high molecular weight vacuum distillate fractions (C18 - C45; 345°C - 540°C; Coleman et al., 1973). The UCM becomes particularly enriched when post distillation refining processes are used to further modify the composition of the straight-run distillate fractions. For

example, in the manufacture of lubricating base oils, solvent extraction with furfural or N-methyl-2-pyrrolidone removes the unsaturated and aromatic compounds, and further treatment by solvent or catalytic "dewaxing" removes normal alkanes (Klamann, 1984). The resulting oil is depleted in those compounds which are well resolved by GC, and rich in saturated paraffinic (branched) and naphthenic (cyclic) hydrocarbons (e.g. Dell'Acqua, 1975; Tanacredi, 1977; Lloyd, 1982). Other refined petroleum products which contain hydrocarbon UCMs include transmission fluids (Tanacredi, 1977; Dell'Acqua, 1975), hydraulic oils (Mackenzie and Hunter, 1979) and gear oils (Lloyd, 1982).

1.4 THE UCM IN RECENT SEDIMENTS

The UCM is a common feature in the hydrocarbon extracts of many aquatic sediments, and several authors have noted similarities between the UCMs of these samples and those of biodegraded crude oils and petroleum products. Table 1.1 summarises some of these reports. UCMs have been noted in many sedimentary depositional environments, including estuarine, riverine, coastal and marine. Qualitatively the UCM profiles have been shown to vary in carbon number range, appearance (e.g. unimodal/bimodal) and complexity (e.g. percentage resolved vs percentage unresolved). Quantitatively the UCM often accounts for the greatest proportion (>70% by weight) of the sedimentary hydrocarbon burden. A pollutant anthropogenic or natural (e.g. paleoseepage / weathered oil shale) fossil fuel origin has almost invariably been inferred for this material (Wakeham, 1976; Farrington and Quinn, 1973). In regions

TABLE 1.1 REPORTED OCCURRENCES OF HYDROCARBON UCHMS IN RECENT SEDIMENTS

REGION	SAMPLE TYPE	[UCH] (dry sediment)	UCH	FRACTION ANALYSED	CARBON RANGE (KI UNITS)	CARBON MAXIMUM	APPEARANCE	INFERRED ORIGIN	PROPOSED COMPOSITION	REFERENCE
PROVIDENCE RIVER, USA	Surface sediment	4500 $\mu\text{g/g}^{-1}$	85	TOTAL HC	1400-3400	2700	UNIMODAL	PETROLEUM	CYCLIC ALKANES, AROMATICS	VAN VLEET and QUINN, 1977.
BUZZARDS BAY, USA	Sediment Core (Surface)	105 $\mu\text{g/g}^{-1}$	96	AHC	1600-3400	2800	UNIMODAL	URBAN AIR	BRANCHED/CYCLIC HYDROCARBONS	FARRINGTON et al., 1977.
ROSTHERNE MERE, UK	bottom sediment (clay fraction)	64 $\mu\text{g/g}^{-1}$	63	AHC	1600-3300	2400	UNIMODAL	BIODEGRADED OIL	-----	THOMPSON and EGLINTON, 1978.
RHODE ISLAND SOUND USA	surface sediment	292 $\mu\text{g/g}^{-1}$	97	TOTAL HC	1500-1300+	2500	UNIMODAL	ANTHROPOGENIC	CYCLIC ALKANES, AROMATICS	BOEHM and QUINN, 1978.
NARRAGANSETT BAY, USA	sediment core	343 $\mu\text{g/g}^{-1}$	96	TOTAL HC	1400-3500+	2700	UNIMODAL	ANTHROPOGENIC	CYCLIC ALKANES, AROMATICS	HURTT and QUINN, 1979.
FUGET SOUND, USA,	surface sediment	3700 $\mu\text{g/g}^{-1}$	68	AHC	1600-3400	2600	UNIMODAL	FOSSIL FUELS-URBAN RUN OFF	BRANCHED/CYCLIC HYDROCARBONS	BARRICK et al., 1980.
HILLSBOROUGH RIVER, USA	surface sediment	196 $\mu\text{g/g}^{-1}$	93	AHC	1600-3200	2500	UNIMODAL	PETROLEUM-WASTE LUBE OILS	BRANCHED/CYCLIC COMPOUNDS	BROWN et al., 1985.
SARAH CREEK, USA	surface sediment	79 $\mu\text{g/g}^{-1}$	85	AHC	1600-3200	2650	UNIMODAL	LUBE OILS AND PYROLYSIS PRODUCTS	CYCLOALKANES	VOUDRIAS and SHITH, 1986.
STOCKHOLM ARCHIPELAGO,	suspended particles	1264 $\mu\text{g/g}^{-1}$	96	AHC	1200-3500+	2050, 2650	BIMODAL	PETROLEUM	-----	BROMAN et al., 1987.

close to urban and or industrial activity and unaffected by acute oil spillages or other sources of fossil hydrocarbons (e.g. oil seeps), the presence of a UCM in surface sediments has generally been attributed to chronic inputs of petroleum hydrocarbons introduced by Man. In particular, waste lubricating oils have often been implicated (Cooper et al., 1974; Wakeham and Carpenter, 1976; Tanacredi, 1977; Van Vleet and Quinn, 1977; Thompson and Eglinton, 1978; Hunter et al., 1979; Hoffman et al., 1983; Hamilton et al., 1984; Brown et al., 1985; Voudrias and Smith, 1986).

Direct evidence for a fossil origin for the UCM in recent sediments results from carbon isotope measurements. For example, more than 80% of the hydrocarbons in Narragansett Bay (mainly UCM) were ^{14}C dated at an age greater than 24,000 years old (Farrington and Quinn, 1973; Zafirion, 1973). Similarly, ^{14}C measurements made by Wakeham and Carpenter (1976) on UCMs isolated from Lake Washington surface sediments gave an age of 15000-20000 years before present; and by Farrington and Tripp (1977) on UCMs from New York Bight sediments indicated an age of 26,000 years before present. Such studies show the complex assemblage of hydrocarbons present in these surface sediments was not of recent biosynthetic origin.

Additional confirmatory evidence for a fossil origin of the UCM in surface sediments is the co-occurrence of UCMs with "mature" distributions of "biological marker" compounds (Eglinton and Calvin, 1967; Mackenzie et al., 1982; Volkman and Maxwell, 1984; Mackenzie, 1984). These are common and characteristic features of ancient sedimentary rocks and petroleums, and have been used extensively in the petroleum industry in oil/oil and oil/source rock correlations

(e.g. Seifert, 1977; Seifert and Moldowan, 1978; Mackenzie, 1984; Philp, 1985). In Recent sediments their presence is usually attributed to fossil hydrocarbon pollution, and many authors have correlated the presence of sedimentary hydrocarbon UCMs with mature distributions of acyclic isoprenoids, $17\alpha(H)$, $21\beta(H)$ - pentacyclic triterpanes, and normal and rearranged steranes (e.g. Dastillung and Albrecht, 1976; Albaiges and Albrecht, 1979; Barrick and Hedges, 1981; Rowland and Maxwell, 1984; Jones et al., 1986; Voudrias and Smith, 1986; Readman et al., 1986).

Only very few studies have attributed the occurrence of UCM in the aliphatic fractions of Recent sediments to other than a fossil fuel origin. For example, in Gulf of Alaska sediments, Venkatesan and Kaplan (1982) attributed the aliphatic UCM to autochthonous microbially altered algal detritus. A fossil origin was discounted since the triterpenoidal alkane distribution lacked an extended series of homologues, and the characteristic diastereomeric doublets ($22S/22R$) were either absent or not present in a ratio typical of mature petroleum. More recently, Grimalt et al. (1988) have attributed the UCM observed in the aliphatic fraction of Recent sediments stored at ambient temperature to the microbial reworking of autochthonous organic matter. Again the UCM was observed to elute over a narrow range ($n-C_{16}$ - $n-C_{22}$), and the hydrocarbon extracts were characterised by a low abundance of steranes and diasteranes, and hopanes typical of non petroleum polluted sediments i.e. $17\beta(H)$, $21\beta(H)$, $17\beta(H)$, $21\alpha(H)$. Clearly in these instances the UCM profiles and their associated molecular marker distributions are atypical of a fossil hydrocarbon origin, although these studies lack more direct

evidence of a biosynthetic origin (e.g. from carbon isotope measurements).

To summarise, the existence of a UCM in the gas chromatograms of biodegraded crude oils and petroleum products, coupled with carbon isotope measurements and biological marker distributions, provides good evidence for a fossil origin of the UCMs observed in Recent surface sediments. As a result the UCM has been adopted as a quantifiable measure of fossil hydrocarbons in these environments (e.g. Barrick and Hedges, 1981; Readman et al., 1986).

1.5 COMPOSITIONAL STUDIES OF HYDROCARBON UCMs

Of the distillate fractions of petroleum which are not resolved by GC, lubricating oils have been the most widely studied, probably because of their economic importance. Early studies relied on the determination of elemental compositions and physical properties, and estimates of the "average" molecular composition were made by a comparison with authentic hydrocarbons. Just such an approach was used in the analysis of a lubricant fraction derived from a representative crude oil (Ponca City) in a pioneering study of petroleum composition undertaken by the American Petroleum Institute (API). This data, summarised in Table 1.2, showed that the alkane fraction comprised mainly alkylcyclohexanes from ca. C25 to C40, and the aromatic fraction comprised mainly naphthoaromatic hydrocarbons from ca. C20 to C36. This research provided some of the earliest evidence for the molecular composition of lubricating oil hydrocarbons (Rossini et al., 1953).

TABLE 1.2

COMPOSITION OF THE LUBRICANT FRACTION OF PONCA CITY PETROLEUM
(ADAPTED FROM ROSSINI *et. al.*, 1953)

FRACTION	PERCENTAGE	MOLECULAR TYPE	PERCENTAGE OF THE FRACTION	PERCENTAGE OF TOTAL LUBRICANT FRACTION
"WATER-WHITE" ^a OIL"	35	1-RING } ^A	92	32
		2-RING }		
		3-RING }		
		NAPHTHENOENZENES } DINAPHTHENOENZENES }		
"EXTRACT" ^b OIL"	22	2-RING } ^A	8	2
		3-RING }		
		DI-AND } TRINAPHTHENOENZENES }		
		DINAPHTHENONAPHTHALENES } NAPHTHENOPHENANTHRENES }		

^a refers to residue of total lubricant fraction after dewaxing, solvent extraction (SO₂), and column chromatography (- "aliphatic" hydrocarbons).

^b refers to residue of total lubricant fraction after dewaxing, solvent (petroleum ether) extraction of the SO₂ solvent extract, combined with the residue "held back" by column chromatography (- "aromatic" hydrocarbons).

A: CYCLOALKANES

With the advent of more advanced, sensitive and structurally informative spectroscopic instrumentation, in particular electron impact mass spectrometry (EIMS), a more comprehensive view of lubricating oil composition was attained. Thus Clerc et al. (1955) produced a method based on fragment ion groups characteristic of compound types, and calibration matrices derived from the analysis of authentic compounds, and applied this to the analysis of a range of U.S. lubricating oil fractions (Table 1.3). The method provided a measure of the alkane/cycloalkane content of lubricating oils, as well as an indication of the types of compounds present in the monocyclic alkane fraction.

This approach was improved and extended to include aromatic hydrocarbons in a later study by Melpolder et al. (1956), on the composition of a thermally diffused lubricating oil saturate fraction. MS analysis showed the fraction to comprise predominantly isoparaffins (26.3%), monocyclo- and non-condensed cycloalkanes (36.9%), and bicyclic alkanes (19.8%), with low proportions of higher ring cycloalkanes (3-8 ring, 11.5%) and aromatic hydrocarbons (5.5%). At the same time and using a similar technique, Lumpkin (1956) provided additional mass spectroscopic evidence for the composition of the saturate fraction of a dewaxed lubricating oil. Thus MS analysis showed the fraction to contain mainly paraffins (41%) and monocyclo/noncondensed cycloalkanes (42%), with lesser amounts of 2-ring (12%), 3-ring (4%) and 4-ring (0.5%) cycloalkanes.

These early mass spectroscopic methods were reviewed and updated by Hood and O'Neal, 1959; and their method has been adopted by the petroleum industry as a standard procedure for the analysis of high

TABLE 1.3
COMPOSITION OF U.S. LUBRICATING OILS DETERMINED BY
EI-MS

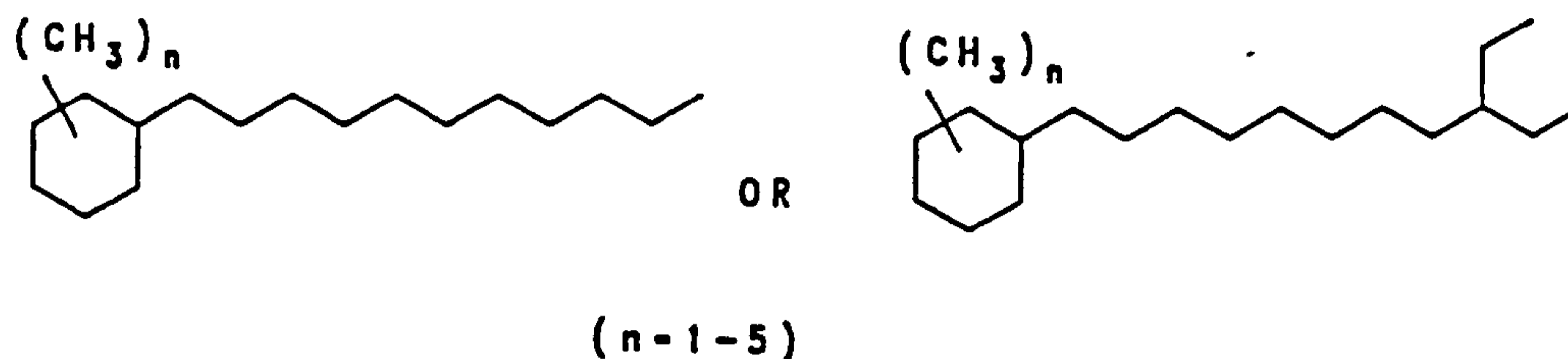
(ADAPTED FROM CLERC *et al.*, 1955)

LUBE OIL ORIGIN	COMPOUND TYPE (WEIGHT %)			CP/CH RING RATIO
	ALKANES	NONCONDENSED CYCLOALKANES	CONDENSED CYCLOALKANES	
Pensylvania	26	51	23	60/40
Mid- Continent	19	54	27	60/40
East Texas	15	54	31	60/40
Gulf Coast	9	53	38	65/35
California	3	53	44	70/30

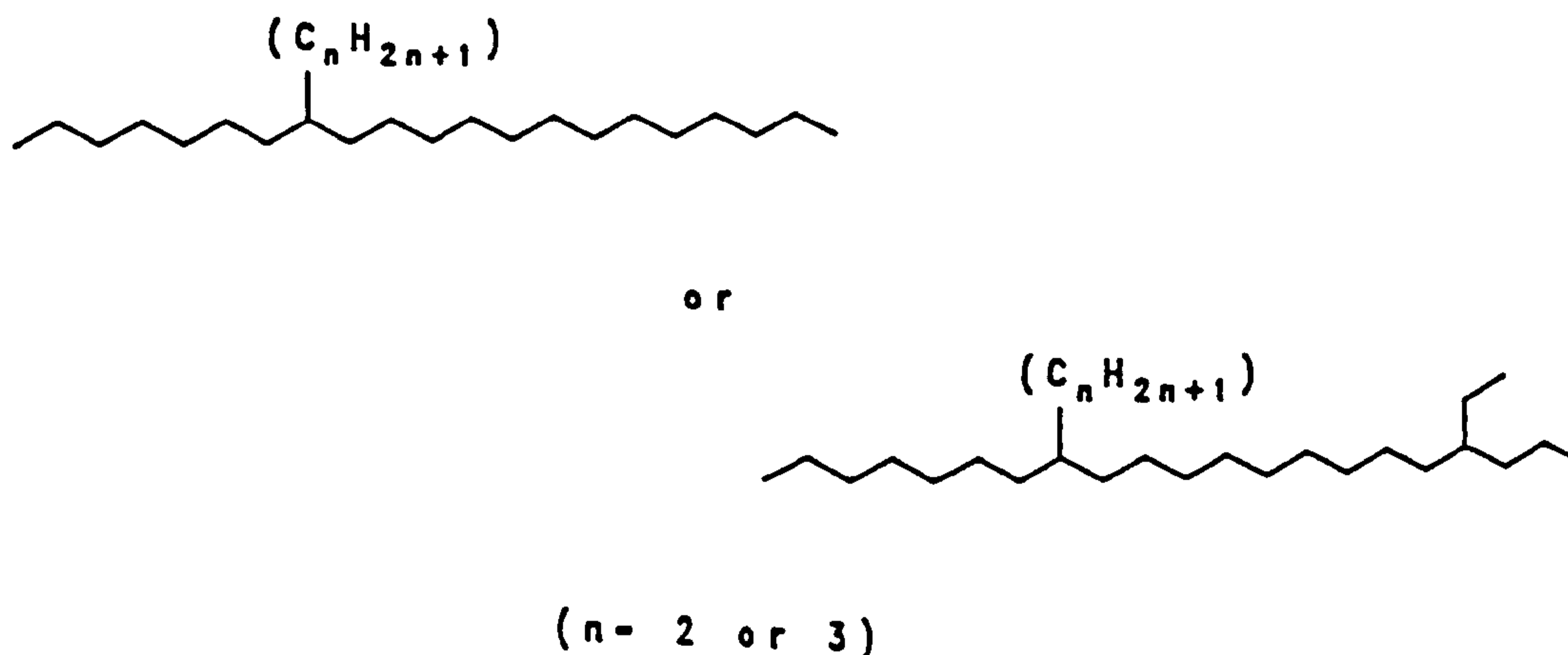
CP - CYCLOPENTYL
CH - CYCLOHEXYL

molecular weight petroleum saturate fractions (ASTM method D2786, 1971). As well as providing semi quantitative data on the proportions of acyclic through hexacyclic alkanes in a complex mixture, it also provides an indication of the molecular structure of lubricating oil alkanes. With supportive data provided by NMR and IR analyses, the types of molecules present in the saturate fraction of lubricating oils have been proposed (Hood et al., 1959). The monocycloalkanes in the lubricating oil saturate fractions are proposed to contain methyl branches on the ring, with one long alkyl chain, either unsubstituted, or slightly branched (e.g. monomethyl) at one end.

i.e.

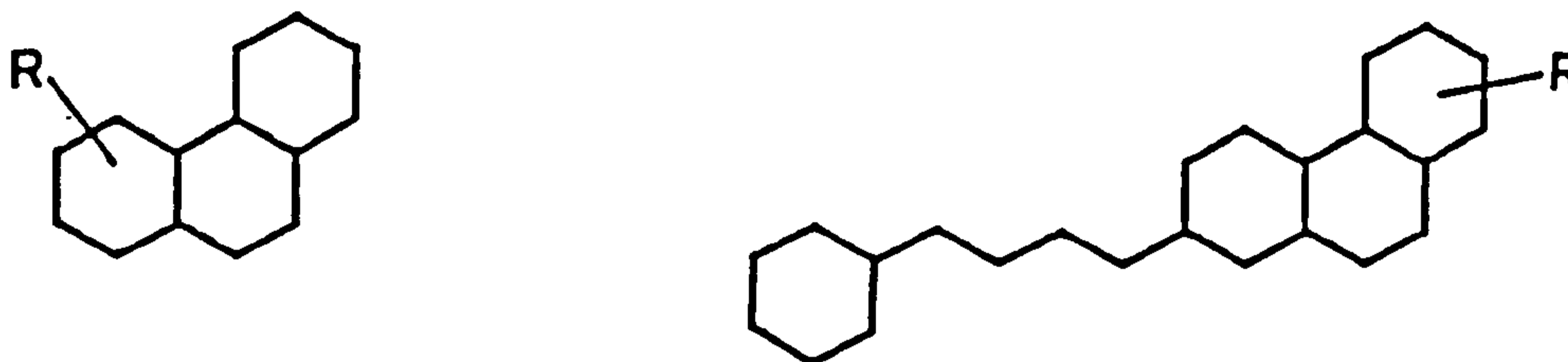


The acyclic alkanes were proposed to be predominantly simply branched, with the alkyl substituent only slightly larger than methyl (e.g. ethyl, propyl, butyl), i.e.



For the polycyclic alkanes, the general belief was of mainly

condensed ring nuclei, as opposed to non-condensed nuclei, i.e.



This method of ring analysis by mass spectrometry has been widely used in the petroleum industry for the characterisation of the saturate fractions of complex hydrocarbon mixtures (e.g. Tissot and Welte, 1978; Speight, 1984; Hala et al., 1981), and a selection of the available data is presented in Table 1.4. From this it can be seen that even in the absence of n- alkanes and acyclic isoprenoids, other acyclic alkanes are present. In fact the summed concentrations of acyclic and monocyclic alkanes account for the highest proportion of compound classes in the samples analysed.

The aromatic hydrocarbons of high molecular weight distillate fractions were also the subject of early studies by mass spectrometry, and similar procedures based on characteristic fragment ion abundances were developed (reviewed by Robinson, 1971; Kagler, 1973; Speight, 1984; Hala et al.; 1981). From these a generalised structure of the alkylbenzene fraction of lubricating oils was proposed. In comparison to the monocyclo- alkanes in the saturate fractions, the alkyl benzenes were thought to comprise one long substituted or simply (i.e. monomethyl) substituted alkyl chain, with up to 4 methyl substituents on the aromatic ring (Hood and O'Neal,

TABLE 1.4

APPLICATIONS OF THE EI-MS GROUP-TYPE ANALYSIS OF UCM ALKANES
BY THE METHOD OF HOOD AND O'NEAL, 1959

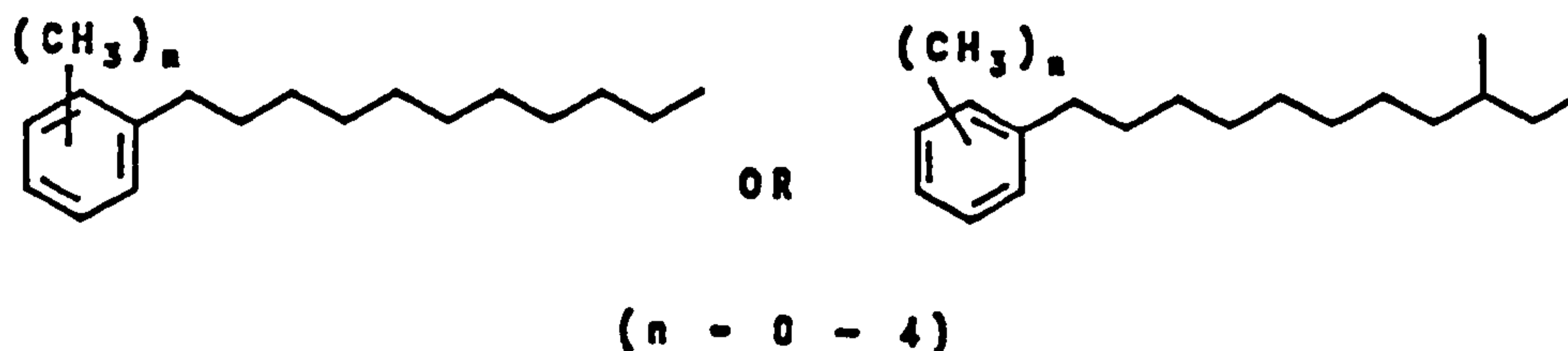
SAMPLE	CHROMATOGRAPHIC DATA		NO RINGS (WT %)								MA	
	UCM	RESOLVED ALKANES	0	1	2	3	4	5	6			
Prudhoe Bay distillate fraction	✓	n-alkanes- low	28	22	16	12	12	6	4	1	COLEMAN <u>et al.</u> 1973	
Pelican Oil ^a (Western Canada oil sand)	✓	n.d.	19	17	22	19	17	7	2	-	DEROO <u>et al.</u> 1977	
Emeraude Field ^a (West Africa)	✓	n.d.	11	13	21	22	18	11	4	-	CLARET <u>et al.</u> 1977	
Biodegraded Kuwait ✓ crude oil		n.d.	26	22	17	14	10	6	5	-	WALKER <u>et al.</u> 1976	

a : data estimated from histograms

n.d. : not observed in the gas chromatogram

MA : monoaromatic hydrocarbons

1959), i.e.



Additional components postulated included polyaromatic and naphthenoaromatic hydrocarbons of the type presented in Fig. 1.4. All these compound classes have been accounted for in a proposed method for the quantitative determination of aromatic hydrocarbons in petroleum distillates (Robinson and Cook, 1969; ATSM, D3239-73T). This method has been widely used and its applications include the characterisation of aromatic hydrocarbons in gas oils (Robinson and Cook, 1969); tar sand bitumens (Selucky et al., 1977, 1978), and biodegraded crude oils (Walker et al., 1975, 1976). Improvements to this method were suggested by Gallegos et al., (1967), by use of high resolution mass spectrometry, although Robinson (1971) provided similar data without the need of a high resolution instrument. This latter method, applied to a lubricating oil base stock, provided simultaneously a comprehensive analysis of the principal saturated and aromatic hydrocarbon group types, and this data is summarised in Table 1.5.

These early electron impact- mass spectroscopic studies therefore yield useful information concerning the "average" molecular structure of complex hydrocarbon mixtures, though the accuracy of the quantitative data which they provide is questionable. Since the

FIG. 1.4 PROPOSED TYPES OF AROMATIC HYDROCARBONS IN HIGH MOLECULAR WEIGHT FRACTIONS OF PETROLEUM (from Walker et al ,1976)

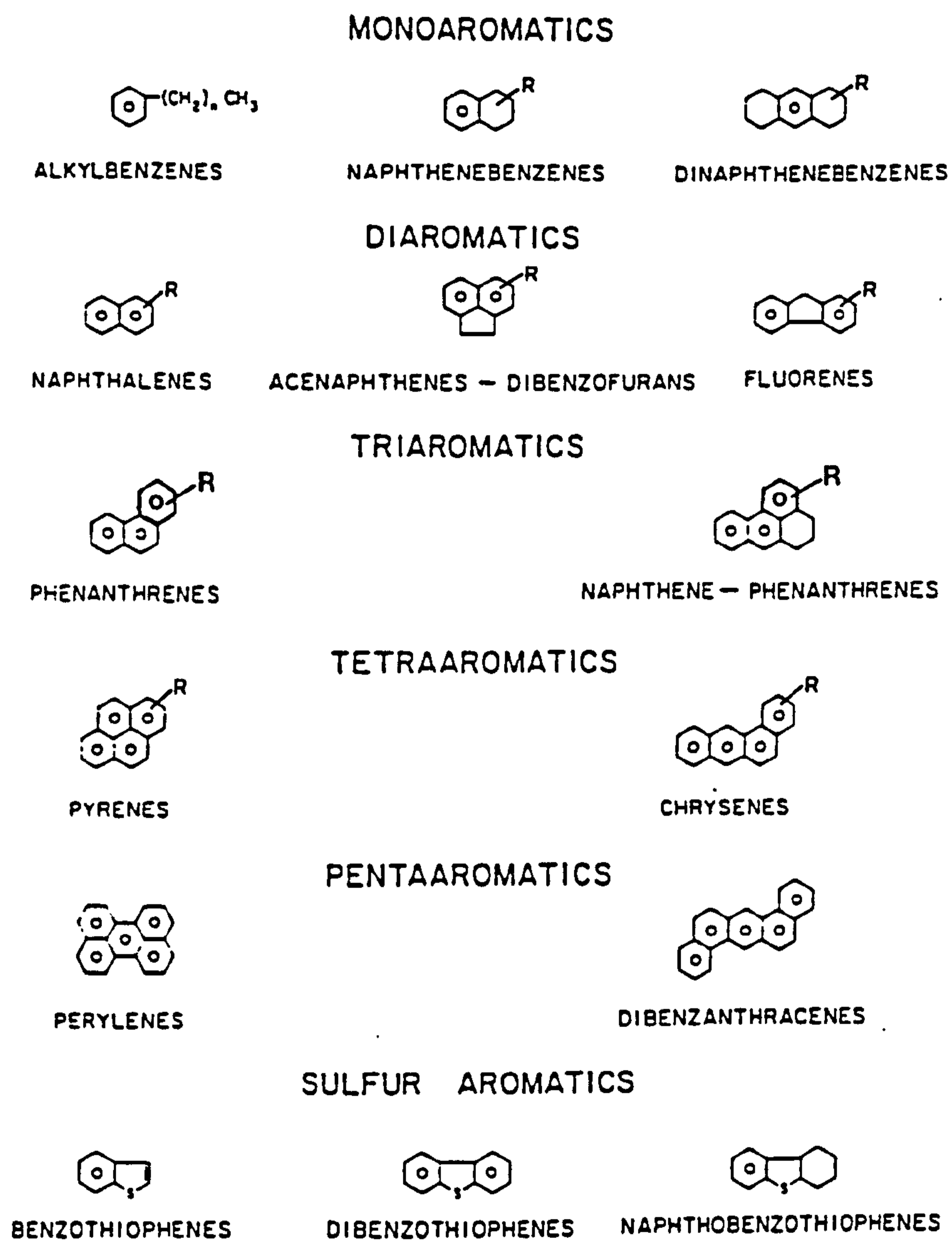


TABLE 1.5

COMPOSITION OF LOUISIANA LUBRICATING OIL BASE STOCK DETERMINED BY ELECTRON IMPACT-MASS SPECTROMETRY BY THE METHOD OF ROBINSON, (1971)

COMPOUND TYPE	LIQUID VOLUME(%)
Paraffins	15.5
Noncondensed cycloparaffins	36.7
2-Ring condensed cycloparaffins	17.6
3-Ring and condensed cycloparaffins	21.1
Total saturates	90.9
Benzenes	1.1
Naphthenobenzenes	0.0
Dinaphthenobenzenes	0.8
Naphthalenes	1.7
Acenaphthenes/dibenzofurans	1.0
Fluorenes	1.2
Phenanthrenes	0.0
Naphthenophenanthrenes	0.0
Pyrenes	0.0
Chryenes	0.1
Perylenes	0.2
Dibenzanthracenes	0.6
Benzothiophenes	0.2
Dibenzothiophenes	0.0
Naphthenobenzothiophenes	0.3
Unidentified	1.9
Total aromatics	9.1
Calculated Total ionisation (%)	97.0

technique relies on the use of calibration matrices derived from one laboratory, inconsistencies between laboratories and instruments (e.g. ionising conditions) can lead to erroneous results. A "Round-Robin" study by a number of mass spectrometry users has shown the validity of these methods is in doubt (Dr A. Herod, British Coal, personal communication). In addition, mass spectral group analysis of heavy aromatic hydrocarbon concentrates is often limited by the presence of heteroatom analogues, the masses of which can overlap the "characteristic" hydrocarbon mass fragment ion series (Speight, 1984).

Alternative mass spectral methods utilised in the analysis of complex petroleum derived hydrocarbon mixtures include those which result in relatively fragment free mass spectra which are useful for molecular weight determinations. As an example, Field Ionisation Mass Spectrometry (FIMS) has been used with success in the analysis of lubricating oils, petroleum distillates, and biodegraded crude oils (e.g. Schronk et al., 1982; Severin, 1976; Payzant et al., 1979, 1980, 1985^{a,b}; Lijmbach et al., 1981). Payzant et al. (1979, 1985^{a,b}) provided a comprehensive analysis of the gas chromatographically unresolved hydrocarbons of Western Canada oil sand bitumens by use of this technique. Thus the saturate fraction of an Athabasca oil sand bitumen was found to comprise mainly bi- and tricyclic alkanes, with lesser amounts of tetra-, penta-, mono-, hexa-, and acyclic alkanes, in that order (Table 1.6A, Payzant et al., 1985^a). Tetra- and pentacyclic alkanes were found to maximise at molecular weights corresponding to C29 and C30 compounds, respectively, whilst lower ring alkanes displayed several maxima at

TABLE 1.6

COMPOSITIONAL DATA FOR THE SATURATED AND MONOAROMATIC HYDROCARBON
FRACTION OF AN ATHABASCA OIL SAND BITUMEN OBTAINED BY FIMS
(ADAPTED FROM PAYZANT *et al.*, 1985^{a,b})

A) SATURATED HYDROCARBONS

COMPOUND TYPE	Z VALUE ^a	RELATIVE ABUNDANCE (%)	CARBON NO. MAXIMA
acyclic alkanes	+2	0.3	n.d.
monocyclic alkanes	0	11.1	C18
bicyclic alkanes	-2	28.3	C15, C19
tricyclic alkanes	-4	24.6	C19, C23, C29
tetracyclic alkanes	-6	21.4	C21, C27, C29
Pentacyclic alkanes	-8	12.7	C27, C30, C35
Hexacyclic alkanes	-10	1.5	n.d.

B AROMATIC HYDROCARBONS

COMPOUND TYPE	Z VALUE	ABUNDANCE (%)	C.NO MAXIMA
Alkylbenzenes	-6	13.7	C18
Alkyl naphthenobenzenes	-8	32.2	C18
Alkyldinaphthenobenzenes	-10	27.8	C19
Alkyl naphthalenes	-12	13.7	C22, C27, C29
Alkyl naphthenonaphthalenes	-14	7.0	C40
Alkyldinaphthenonaphthalenes	-16	3.4	n.d.
Alkylphenanthrenes	-18	1.2	n.d.

a : z derived from the expression C_nH_{2n+z}

n.d. : data not presented

lower carbon numbers (bicyclic: C15, C19; tricyclic: C19, C23, C29; monocyclic: C28). Aromatic hydrocarbon fractions were also analysed by FIMS, and the data for a monoaromatic hydrocarbon concentrate which accounted for ca. 8% of the total maltene fraction is summarised in Table 1.6 B. Thus alkylnaphthenobenzenes were quantitatively the most important fraction (ca. 33% of total monoaromatics), with lesser amounts of alkyldinaphthenobenzenes, alkyl benzenes and naphthalenes, alkylnaphthenonaphthalenes, alkyldinaphthenonaphthalenes, and alkylphenanthrenes; in that order (Payzant et al., 1985^b).

Additional spectroscopic techniques applicable to complex petroleum hydrocarbon mixture analysis include those based on infra red (IR) and nuclear magnetic resonance (¹H and ¹³C NMR) methods. Several significant reviews detail their use (e.g. Coggeshall, 1953; Kagler, 1973; Speight, 1984; Hala et al., 1981). In particular, NMR has been used extensively in the characterisation of petroleum and its distillate fractions (e.g. Clutler et al., 1972; Coleman et al., 1973; O'Donnell et al., 1980; Gerhards, 1980; Ali et al., 1985; Singh and Srivashava, 1985). For example, in a study of three refined lubricating oil base stocks, Singh and Srivashava (1985) provided a wealth of compositional information by a combination of ¹H and ¹³C NMR. This data is summarised in Tale 1.7. Thus for a solvent extracted and dewaxed lubricating oil base stock (oil 1), the sample was found to comprise 80.2% saturated C atoms and 19.8% aromatic C atoms. Of the saturates, 46% were associated with naphthenic nuclei, and 54% with paraffinic alkyl chains. Of the total aromatic C atoms, 24% were alkyl substituted (greater than methyl, type 1, Fig. 1.5); 7% methyl substituted (type 2, Fig. 1.5),

TABLE 1.7
 QUANTITATIVE DATA ON LUBRICATING OIL BASE STOCKS SUPPLIED BY
¹³C AND ¹H nmr MEASUREMENTS
 (ADAPTED FROM SINGH AND SRIVASHAVA, 1985)

CARBON ATOM TYPE	NO. ^a	WEIGHT(%)		
		OIL 1	OIL 2	OIL 3
Total saturates	7+8	80.2	86.6	87.4
Naphthenic	7	36.9	35.0	27.2
Paraffinic	8	43.3	51.6	60.2
Total aromatics	1-5	19.8	13.4	12.6
Alkylaromatics (>C1)	1	4.8	1.4	3.5
H-substituted Aromatics	3	4.5	5.4	2.1
CH ₃ -substituted Aromatics	2	1.4	1.4	1.9
2-, 3-, and Naphthenic junction Aromatics	4,5,6	9.1	5.2	5.1

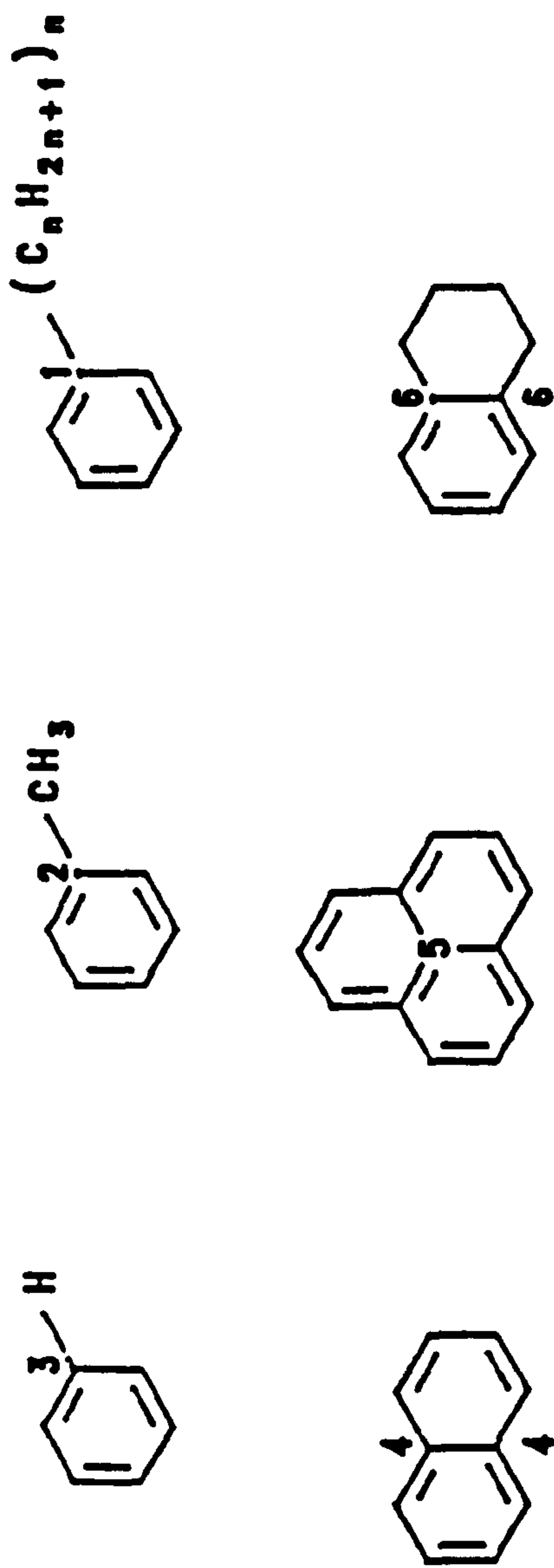
a : Carbon atom type number refers to Fig. 1.5

OIL 1: Assam crude oil; solvent extracted, dewaxed and clay finished

OIL 2: Assam crude oil; solvent extracted, dewaxed, acid treated, and clay finished

OIL 3: Darrius crude oil; solvent extracted, dewaxed and hydrofinished.

FIG. 1.5 CARBON ATOM ENVIRONMENTS QUANTIFIED BY ^{13}C NMR ANALYSES OF LUBRICATING BASE OIL STOCKS (from Singh and Srivishava, 1985)



46% associated with junctions to 1 or 2 aromatic or naphthenic rings (types 4- 6, Fig. 1.5), and 23% of type 3 (Fig. 1.5).

In summary, many spectroscopic techniques have been applied to the analysis of complex petroleum hydrocarbon mixtures known to be unresolved by GC. Most require only small amounts of sample and are rapid. However, the compositional information provided by these methods is limited to a few "average" molecular types. Furthermore, recent work suggests the validity of the most commonly used mass spectral methods is in doubt.

Some of the most specific molecular characterisations of high molecular weight petroleum hydrocarbons are those that are based on techniques of high separation power, for example GC. Even GC is limited however when samples are unresolved to providing a measure of the estimated carbon number range and the degree of complexity (e.g. % unresolved). When combined with electron impact mass spectrometry (EIMS) however, the techniques becomes particularly useful in the analysis of distributions of hydrocarbons which display characteristic and abundant ions in their mass spectra. Examples are the "biological marker" compounds which have been monitored in biodegraded crude oils and sediment extracts displaying prominent UCMs (e.g. Thompson and Eglinton, 1978; Rowland and Maxwell, 1984). It should be emphasised however that these compounds are usually relatively minor components, and are not representative of the UCM as a whole. As an example, a study of the distributions of 3- and 5-ring terpenoidal alkanes isolated from a biodegraded petroleum by zeolite adsorption showed these to be well resolved by GC and their summed concentrations represented only ca. 1.2% of the total UCM

(Dimmler and Strausz, 1983).

A number of studies have been performed on hydrocarbon UCMs isolated from environmental samples by GC-EIMS. Thus Cardoso (1976) used GC-EIMS to characterise the UCM hydrocarbons observed in Rostherne Mere surface sediments. The ion profiles m/z 85 (acyclic alkanes), m/z 83 (monocyclic alkanes), and m/z 81 (bicyclic alkanes) were shown to follow the total ion current (TIC) closely, though quantitatively the m/z 83 fragment ion series was more important. As a result a predominantly cyclic nature was inferred, though no definitive structural characterisation was made.

Brooks et al. (1977) examined the aliphatic UCM in Esthwaite Water surface sediments by GC-EIMS, and again the m/z 85 and m/z 83 alkane ion series were shown to resemble the TIC quite closely. Similar trends were noted for the ion distributions m/z 69, 97, 111, all characteristic of cyclic alkanes, and as a result a large contribution of cyclic alkanes to the UCM was proposed. Similarly Simoneit (1984) used the ion fragment series m/z 95 (C_nH_{2n-3}) characteristic of bicyclic alkanes to emphasise the distribution of the UCM in diesel exhaust particulate extracts. Again a predominantly cyclic nature of the UCM was inferred.

Thus the studies that have attempted an analysis of hydrocarbon UCMs by GC-EIMS are few, and reveal little compositional information at the molecular level. This is in part due to the inability of GC to resolve constituent compounds, but also, as in the case of alkanes; due to the high abundance of fragment ions induced by electron impact ionisation. Parent ions are often in low abundance, and as many

hydrocarbons display similar mass spectra, positive identification becomes difficult (Payzant et al., 1979). Some of these problems were overcome in a study of the mainly unresolved alkanes from a Western Canada tar sand bitumen by use of GC-field ionisation mass spectrometry (GC-FIMS). The total saturates fraction when treated with thiourea yielded 12% thiourea adducted alkanes and 88% non adducted alkanes. The former, when analysed by GC-FIMS was found to comprise overlapping homologous series of acyclic alkanes (e.g. pristane and phytane), and as major components a homologous series (C11 - C22+) of bicyclic alkanes, presumed to be alkyldecalins and alkylmethyldecalins. In addition, overlapping homologous series of tri- (presumed alkylperhydrophenanthrenes), tetra-, penta-, and hexacyclic alkanes were observed. Similar compound distributions, though more complex, were observed for the thiourea non adduct fraction. The major series were again alkyldecalins and alkylmethyldecalins, with a larger contribution of tri- and tetracyclic alkanes than the thiourea adduct. This technique, in particular when used in combination with EI-MS, therefore provided an excellent indication of the major hydrocarbon classes and their carbon number distributions in a complex, unresolved mixture (Payzant et al., 1980).

Although there are relatively few reports of the presence of aromatic hydrocarbon UCMs in Recent sediments and biodegraded crude oils, a number of significant studies have been published relating to their composition.

Killops and Readman (1985) used normal phase high performance liquid chromatography (HPLC) to fractionate the aromatic UCM obtained from a

Recent sediment according to the number of double bond equivalents. Though no compositional data was provided by GC-MS, the unresolved components which accounted for the majority of the total aromatic hydrocarbons were present almost exclusively in the monoaromatic (i.e. 3 double bond equivalents) fraction. This was confirmed in a later study in which the aromatic UCM derived from Bridgewater Bay sediment was fractionated using a similar procedure. Again the aromatic UCM was enriched in the monoaromatic fraction, which accounted for up to 30% of the total aromatics (Killops, 1986). Similar results were obtained by normal phase HPLC of the aromatic hydrocarbons observed in oysters following the Amoco Cadiz oil spill. The aromatic UCM was preferentially enriched in the 1-2 ring fractions (Berthou et al., 1981).

Perhaps the most thorough attempt at the characterisation of an aromatic UCM observed in a Recent sediment was that undertaken by Jones (1986). The aromatic UCM observed in sediment extracts from the Sullom Voe oil terminal, Shetland Islands, was fractionated by a combination of normal phase HPLC and gel permeation chromatography (GPC), and analysed further by GC and GC-MS. HPLC ring-class fractionation of the aromatic UCM again showed the majority of the unresolved compounds present in the 1-2 ring fractions. Furthermore, the majority of these were defined as being alkylated on the basis of their size exclusion behaviour on Sephadex LH-20. Individual compounds identified by GC-MS and GC- flame photometric detection (GC-FPD) included alkylbenzothiophenes and phenanthrenes; and alkyldibenzothiophenes were proposed to be major components with supporting evidence provided by low ionising voltage probe distillation mass spectra.

Degradative techniques (e.g. pyrolysis or chemical oxidation) have been applied with great success in the analysis of many varied and complicated organic matrices. For example pyrolysis ("Flash" or hydrous) in combination with GC or GC-MS has been used in the characterisation of fossil kerogens, crude oil asphaltenes, humic substances, and coals (Table 1.8). Chemical oxidation utilising a variety of oxidant/solvent systems has also proved of use in the characterisation of similar organic geochemical samples (Table 1.8). Despite these successes, little use has been made of pyrolytic or chemical degradations for the characterisation of complex hydrocarbon mixtures which are unresolved by GC. A notable exception however is the reported production of alkylated fluoren-9-ones by chemical oxidation of the gas chromatographically unresolved aromatic hydrocarbon fraction from an Athabasca oil sand bitumen. By comparison with the oxidation products of synthetic model hydrocarbons, specific structural assignments to precursor alkylated fluorenes could be made (Mojelsky and Strausz, 1986). This study represents the only reported characterisation of a hydrocarbon UCM at the molecular level.

TABLE 1.8
 CHEMICAL (OXIDATION) AND PYROLYTIC DEGRADATIONS APPLIED TO
 ORGANIC GEOCHEMICAL SAMPLE MATRICES

SAMPLE TYPE	DEGRADATION TECHNIQUE	AUTHOR (S)
COAL	Flashpyrolysis GC-MS	Chaffee <u>et al.</u> , 1984
	Chemical oxidation	Hayatsu <u>et al.</u> , 1982
KEROGEN	Flash pyrolysis GC-MS	Larter <u>et al.</u> , 1981
	Chemical oxidation	Ambles <u>et al.</u> , 1983 Machihara and Ishiwatari, 1987
ASPHALTENES	Flash pyrolysis GC	Behar <u>et al.</u> , 1984
	Hydrous pyrolysis/ GC, GC-MS	Jones <u>et al.</u> , 1987
	Chemical oxidation	Trifilieff, 1987
HUMIC ACIDS	Flash pyrolysis GC-MS	Wilson <u>et al.</u> , 1983
	Chemical oxidation	Schnitzer and Khan, 1972
LIGNIN	Flash pyrolysis GC-MS	Saiz-Jimenez and De Leeuw, 1984.
	Chemical oxidation	Hedges and Ertel, 1982
AROMATIC UCM HYDROCARBONS	Chemical oxidation	Mojelsky and Strausz, 1986

1.6 THE PRESENT STUDY

The present study has as a primary aim the isolation and structural characterisation of hydrocarbon UCMs of varied origin. Chapter 3 details developments in conventional methods of analysis applied to aliphatic and aromatic UCMs from lubricating base oils. These serve as ideal models as they are low in n-alkanes (through dewaxing), they are rich in hydrocarbons (through solvent extraction) and are readily available. Also described are more novel methods of UCM structural analyses which are based on the use of chemical or pyrolytic degradations. By a thorough characterisation of the products of these degradations and a knowledge of literature reported mechanisms potential UCM precursor compounds are proposed.

Chapter 4 details the methods and the results of the synthesis of these candidate UCM hydrocarbons. Also presented are the results of oxidation of synthetic compounds with CrO_3 under the same conditions as used for the hydrocarbon UCMs. Such an approach has been used with great success in the characterisation of many complex sample matrices in organic geochemistry (Table 1.8).

The potential of oxidation as a method for "fingerprinting" UCMs of varied origin is assessed in chapter 5. This describes oxidation of aliphatic UCM isolates from lubricating base oils (paraffinic and naphthenic) , from oil polluted Recent sediments, and from an in-resevoir biodegraded crude oil; and detailed analyses of the products of each. The chapter concludes with a comparison of a selected series of resolved oxidation product profiles for each of the UCM samples oxidised.

Chapter 6 investigates the behaviour of the synthetic hydrocarbons in

a microbial biodegradation study performed under controlled conditions. Also biodegraded were samples of aliphatic UCM material, and model alkanes not thought representative of the types of compounds present in hydrocarbon UCMs but which were used as reference controls.

EXPERIMENTAL DETAILS

2.1 GENERAL PROCEDURES

All glassware was precleaned in chromic acid or Decon 90 solution, rinsed thoroughly with tap water, distilled water, and oven dried (110°C) overnight. Glassware used in synthetic procedures was assembled whilst hot and placed immediately under an inert atmosphere (argon).

General purpose solvents were purified by distillation in an all-glass apparatus, and solvent purity routinely checked by rotary evaporation (Buchi, 40°C) of the solvent (ca. 100 cm³) to approximately 50 mm³, followed by analysis of an aliquot (0.5 mm³) by gas chromatography (GC). Diethyl ether (Et₂O) used in synthetic procedures was dried (CaCl₂ followed by Na wire), purified over alumina (basic grade, BDH), distilled over LiAlH₄, and stored over active molecular sieve (5A) prior to use.

Silica gel (BDH, 60-120 mesh) and aluminium oxide (BDH, grade 1, neutral) adsorbents used for chromatographic separations were Soxhlet extracted (DCM, 36 hr) and dried (40°C) prior to activation. Adsorbents with the required percentage water composition were prepared according to the procedures of Later et al. (1985); by drying (12 hr, SiO₂ @ 185°C; Al₂O₃ @ 450°C), cooling in a desiccator, addition of water (millipore grade) and homogenisation by mechanical shaking (3-5 hr).

Thin layer chromatography plates were prepared on solvent-washed glass plates (20 x 20 cm) spread with an aqueous slurry of pre-extracted (DCM, 36 hr) silica gel (Merke 60 G Kiesel gel).

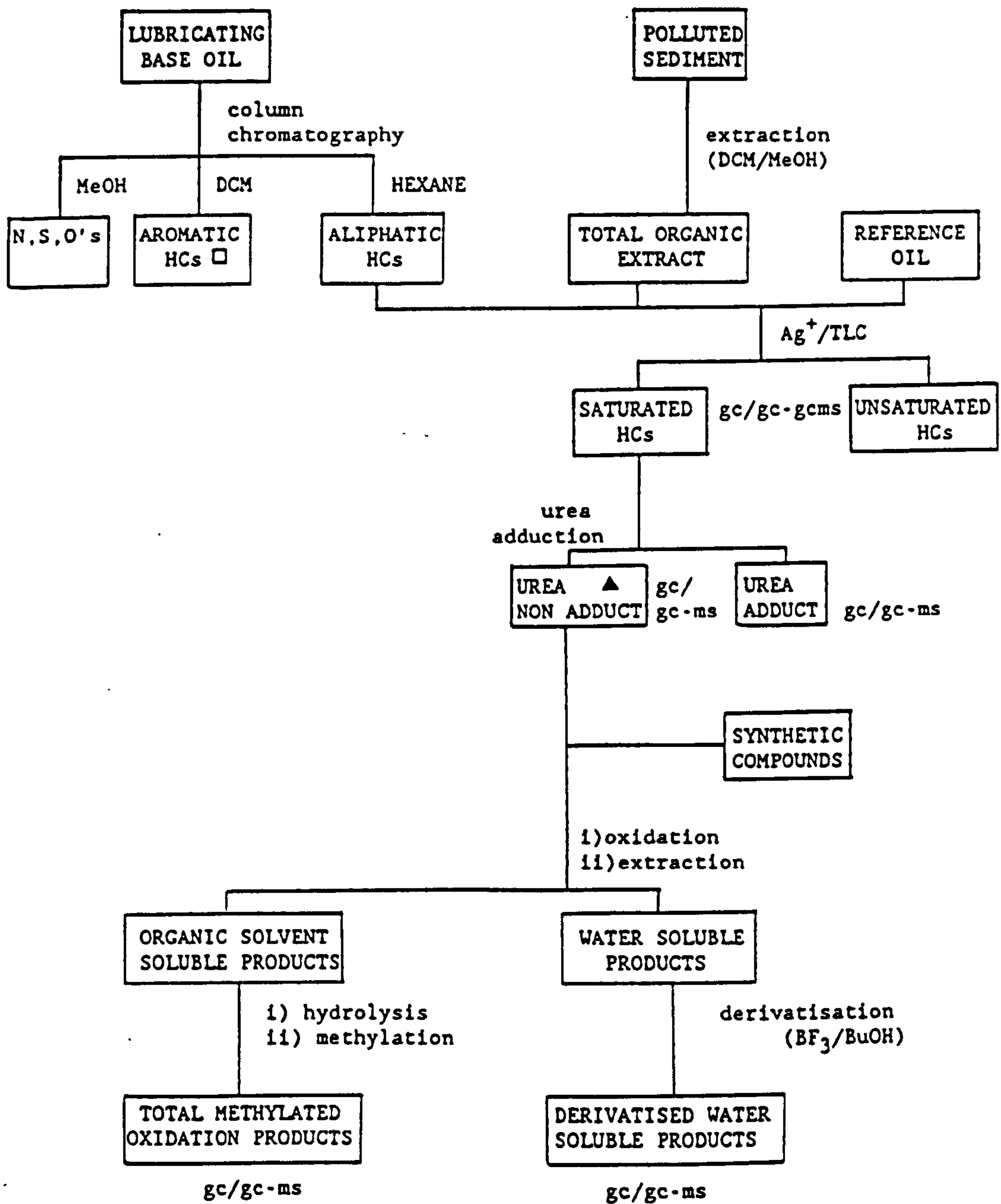
Argentatious TLC plates were prepared from slurries of silica gel made up in an aqueous solution of AgNO_3 (10% w/w). Freshly spread plates were dried (120°C , 1 hr); predeveloped (ethyl acetate, 2x) and reactivated (120°C , 30 mins) prior to use.

Concentrated hydrochloric acid (HCl), mercury, anhydrous sodium sulphate (anh. Na_2SO_4), cotton wool, and antibumping granules were all pre-extracted (DCM) before use.

2.2 ISOLATION OF ALIPHATIC AND AROMATIC UCM HYDROCARBONS

The lubricating oil used in this study as a source of hydrocarbon UCM material was a solvent refined Silkolene 150 (solvent neutral) lubricating mineral base oil provided by Mr Alan Walker, Silkolene Lubricants, UK. The precise origin or refining history of this oil is not known, though it is most likely to have been derived from a paraffinic crude oil of Middle Eastern or North Sea origin (Mr A. Walker, personal communication). The isolation of aliphatic and aromatic UCMs followed the scheme outlined in Fig 2.1. This involved fractionation of the base oil by open column liquid solid chromatography in glass columns (70 cm x 2 cm i.d.) packed with a solvent (hexane) slurry of silica gel (60-120 mesh; 5% $\text{H}_2\text{O}-\text{SiO}_2$; 40 g) under alumina (grade 1- neutral; 1.5% $\text{H}_2\text{O} - \text{Al}_2\text{O}_3$; 20 g). The oil (ca. 2g) was applied in hexane (2 cm^3) and the column eluted with hexane (178 cm^3), DCM (200 cm^3) and methanol (200 cm^3) to provide aliphatic, aromatic and polar fractions, respectively. Following solvent evaporation (Buchi, 40°C) and N_2 blow down, the isolated fractions were weighed.

FIG. 2.1
ISOLATION AND OXIDATION OF MODEL AND ENVIRONMENTAL UCM HYDROCARBONS



▲ ALIPHATIC UCM

□ AROMATIC UCM

The aliphatic fraction derived from the column chromatographic separation was purified further by argentatious TLC (10% AgNO₃ w/w) using hexane as the mobile phase. The plate was sprayed with Rhodamine 6G solution (0.5% in methanol) and examined under UV light (364 nm). The band corresponding to the alkanes (Rf: 0.85 - 1.0) was removed, transferred to a small column (20 cm x 1 cm i.d.), and the total alkanes recovered by desorption with DCM/hexane (1:1, 50 cm³). The solvent was removed to near dryness (Buchi, 40°C), the total alkanes transferred to a preweighed vial, and residual solvent blown down (N₂) to constant weight. The efficiency of the silver ion TLC separation was monitored routinely on selected plates using a standard hydrocarbon mixture composed of n-eicosane (n-C20:0), n-eicos-1-ene (n-C20:1), 1-phenyldecane, and anthracene (Rf values : 0.93-0.99; 0.62-0.71; 0.19-0.26; 0.06-0.26; respectively).

Normal and simply branched (i.e. monomethyl substituted) alkanes were removed from the total alkane fraction by urea adduction. This involved dissolution of alkanes (ca. 50 mg) in hexane/acetone (2:1, 10 cm³) followed by the dropwise addition of a saturated solution of urea in methanol (ca. 1 cm³). Solvent was removed (N₂ blow down) and the process repeated twice. Urea non adducts (UNA) were recovered by the addition of hexane (10 cm³) followed by ultrasonic agitation (Soniprep bath, 30 sec) and centrifugation (2,000 rpm, 10 min). The solvent supernatant was decanted and filtered (cotton wool plug) and the process repeated with fresh solvent (1 x 10 cm³, 1 x 5 cm³). Filtered solvent extracts were combined, evaporated (Buchi, 40°C) and the UNA alkanes transferred to a vial and weighed.

Urea adducts (UA) were recovered by the addition of water (10 cm³,

millipore grade), followed by heating (40°C, 5 min), and transfer to a separating funnel containing hexane (10 cm³). The aqueous layer was extracted (hexane, 2 x 10 cm³), the hexane extracts combined, dried (anh. Na₂SO₄), concentrated (Buchi, 40°C, N₂ blow down), and weighed after transfer to preweighed vials.

The urea non adduct thus provided and the aromatic fraction derived directly from the column chromatographic separation were used as the model aliphatic UCM and aromatic UCM respectively in the experiments described later in this section.

2.3 ISOLATION OF ENVIRONMENTAL UCM HYDROCARBONS

Oiled Humber Estuary surface sediment provided by Mr S. Howells, Oil Pollution Research Unit (OPRU), Field Studies Council (FSC); was collected by grab sampler and stored frozen in aluminium containers prior to extraction. The thawed samples were solvent extracted following the methods of Douglas et al. (1981). Sediment (ca. 50g wet weight) was extracted with methanol (40 cm³) by ultrasonic agitation (Soniprep 150- probe, 5 min) and the organic extract separated by centrifugation (2,000 rpm, 15 min) and decantation. This procedure was repeated using DCM/methanol (7:3), DCM/methanol (4:1) and finally DCM. The solvent extracts were combined and placed in a separating funnel, water (millipore grade, 30 cm³) was added, the mixture shaken, and the lower organic fraction collected. The aqueous layer was extracted further with DCM (3 x 15 cm³), the solvent extracts combined, dried (anh. Na₂SO₄), concentrated (Buchi, 40°C, N₂ blow down) and weighed.

Samples of "Sivand" tanker spill reference oil, Amoco Tank 207 oil,

and Newgale Beach oil were supplied by Mr S. Howells, OPRU, FSC. Samples of Athabasca oil sand bitumen were provided pre-extracted (in DCM) by Dr M. Fowler, Alberta Research Council, Canada. Samples of paraffinic (North Sea) and naphthenic (Venezualan) lubricating mineral base oils were provided by Mr M. J. Day, Shell Lubricants, UK. The total alkane fractions of each oil and the Humber sediment extract were isolated directly by silver ion TLC, and urea adduction provided urea non adducts which were used as environmental UCM samples.

2.4 UCM FRACTIONATIONS

2.4.1 THIOUREA ADDUCTION

Thiourea adductions were performed on samples of urea non adducted alkanes as described by Murphy et al., (1967). The UNA alkanes (ca. 10-20 mg) were dissolved in chloroform (5 cm³) to which was added a saturated solution of thiourea in methanol (0.5 - 1 cm³). The solution was warmed (water bath, 40°C) until complete dissolution of the crystals was observed. The mixture was then cooled at room temperature and stored overnight to complete adduct formation. Filtration of the crystals followed by solvent washing (2 x 1 cm³ CHCl₃) afforded thiourea non adducted alkanes (TUNA), whilst solubilisation of crystals in warm (40°C) water (millipore grade, 5 cm³) followed by hexane extraction (2 x 10 cm³) and drying (anh. Na₂SO₄ - column) provided the thiourea adducted alkanes (TUA).

2.4.2 GEL PERMEATION CHROMATOGRAPHY (GPC)

Aliphatic and aromatic hydrocarbon fractions derived from the dialysate fraction (Gough, 1987) of a commercially available fresh lubricating oil (20W40, Dulton) were isolated using column chromatography as described previously (section 2.2). Fractionation of these samples was achieved by use of gel permeation chromatography (GPC) at British Petroleum Research Centre (Geochemistry branch, Sunbury-on-Thames). Two XAD4 columns (divinylbenzene/styrene copolymer) were used in series with tetrahydrofuran as the mobile phase and combined UV (254 nm) and refractive index detection. Calibration was by use of authentic polystyrene polymer standards of known molecular weight ranges.

2.5 OXIDATION OF HYDROCARBON UCMS AND SYNTHETIC COMPOUNDS

2.5.1 CHEMICAL OXIDATIONS

Samples of model and environmental UCM material and model synthesised compounds were oxidised with chromium trioxide (CrO_3 , Aldrich) following the scheme outlined in Fig 2.2. The procedure used was a modification of that reported by Brooks et al. (1977), and involved addition of the sample (ca. 10-50 mg) to glacial acetic acid (May and Baker, AR grade, 10 cm^3) in a two-necked RBF (25 cm^3) equipped with a reflux condenser. The solution was heated ($70 \pm 2^\circ\text{C}$, water bath) with stirring (5 min) followed by the addition of the oxidant (CrO_3) at a 10:1 molar ratio of oxidant to substrate. A molar mass of 352 gmole^{-1} ($\text{C}_{25}\text{H}_{52}$) was assumed for the aliphatic UCM; 344 gmole^{-1} ($\text{C}_{25}\text{H}_{44}$) for the aromatic UCM. The solution was maintained at 70°C with stirring for 60 min, cooled (ice water bath), and transferred to a separating funnel with water (10 cm^3) and DCM (10 cm^3). The contents

were shaken, the lower DCM layer removed, and the aqueous layer re-extracted with additional DCM (2 x 10 cm³). The DCM extracts were combined and washed (water, 2 x 10 cm³), and the combined organic extracts and combined aqueous layer and water washings were prepared for analysis as detailed below.

2.5.2 DCM SOLUBLE PRODUCTS

The combined water washed DCM extracts were concentrated to near dryness (Buchi, 40°C) and hydrolysed using methanolic KOH (10% KOH: methanol w/v) under reflux (30 min). After cooling (room temperature) the hydrolysed material was acidified to pH ca. 1 (conc. HCL), water was added (millipore grade, 5 cm³), and the total hydrolysed organic material extracted into DCM (1 x 10 cm³, 2 x 5 cm³). The DCM extracts were combined, dried (anh. Na₂SO₄ column) and methylated using BF₃/methanol complex (BDH, 14%, 10 cm³) for 5 min. Total methylated oxidation products and residual unoxidised material were recovered by addition of water (10 cm³), extraction with DCM (1 x 10 cm³, 2 x 5 cm³), water washing of the combined DCM extracts (2 x 10 cm³), drying (anh. Na₂SO₄ column), and solvent removal (Buchi, N₂ blow down).

In certain cases, total oxidation products (as methyl esters) were separated from residual unoxidised material by column chromatography on deactivated silica gel (10% H₂O). Columns (20 cm x 1 cm i.d.) were slurry packed in hexane to a height of 18 cm, the sample (ca. 30 mg) applied in hexane (1-2 cm³), and eluted with hexane (15 cm³) to provide unoxidised material. A column "strip" using DCM (20 cm³)

followed by DCM:methanol (1:1, 20 cm³) provided the total methylated oxidation products.

2.5.3 WATER SOLUBLE PRODUCTS

The water:glacial acetic acid layer combined with the water washings of the DCM extracts was prepared for analysis by gas chromatography (GC) using a modification of the method of Eglinton et al. (1987).

An aliquot (10 cm³) was taken and the pH adjusted to ca. 8-9 using a concentrated solution of KOH in water (10M, ca. 7.5 cm³). At this stage a gelatinous blue-green precipitate was observed to form which was filtered (Whatman GF/C, precombusted 450°C), and the trapped solid was washed (2 x 5 cm³ H₂O). The filtrate plus washings was concentrated to near dryness by rotary evaporation and dried under a N₂ gas stream. BF₃/n-butanol complex was added (Chrompak, 5 cm³), and the flask heated in a steam bath (100°C) for 30 min. The flask was cooled, water added (10 cm³) and the derivatised products extracted into DCM (2 x 10 cm³). The DCM extracts were combined, washed (2 x 10 cm³ H₂O), dried (anh. Na₂SO₄ column) and concentrated (rotary evaporation, N₂ blow down) prior to analysis by GC.

2.6 BIODEGRADATION EXPERIMENTS

2.6.1 MICROBIOLOGICAL METHODS

The procedures used for the biodegradation experiments were based on

those reported by Robson and Rowland, 1989. *Pseudomonas fluorescens* (Dr. C. Gaylarde, City of London Polytechnic, personal communication) was grown in an oxid nutrient broth (13g oxid in 1000 cm³ H₂O) for 24 hr at 20°C. All glassware was Decon 90 washed and sterilised by autoclave (120°C, 30 min) prior to use.

2.6.2 INOCULATION AND INCUBATION

The synthetic hydrocarbon standard mixture (see Table 2.1 for compositional details) was added in hexane (100 cm³, Pederson pipette) to conical flasks (25 cm³) containing sterilised (autoclave, 120°C for 30 min) minimal salts solution (10 cm³, 5% NH₄Cl, 1% NH₄NO₃, 2% Na₂SO₄, 3% K₂HPO₄, 1% KH₂PO₄ and 0.1% MgSO₄.7H₂O) in 1000 cm³ H₂O). The hexane was allowed to evaporate, the bacterial broth added (0.5 cm³), and the flasks stoppered using non-absorbent cotton wool. Incubation was performed aerobically in the light on a shaking water bath (Tecam) temperature controlled at 20°C.

Samples of aliphatic UCM derived from the Silkolene 150 solvent neutral lubricating base oil (section 2.2) were biodegraded alongside model synthetic hydrocarbon standards using similar procedures. The UCM sample (83 mg) in hexane (200 mm³) was added to conical flasks (50 cm³) containing sterilised minimal salts solution (composition as above, 20 cm³). Bacterial broth (1 cm³) was added, the flasks stoppered (non-absorbent cotton wool), and the flasks and contents incubated as described previously.

2.6.3 EXTRACTION

Residual synthetic hydrocarbon standards and aliphatic UCM were extracted using DCM ($3 \times 10 \text{ cm}^3$), the DCM extracts combined and dried (anh. Na_2SO_4 column), the solvent removed to near dryness (Buchi, 40°C), transferred to vials (10 cm^3), and blown down to dryness (N_2). Samples were taken up in DCM (hydrocarbon standards: 2 cm^3 ; aliphatic UCM: 250 mm^3) prior to analysis by GC (section 2.7.2).

2.7 ANALYSES

2.7.1 ELEMENTAL ANALYSIS

Elemental analysis (C,H,N,S) of model aliphatic UCM material was performed at the microanalytical laboratories, School of Chemistry, University of Bristol, using a Carlo Erba elemental analyser.

2.7.2 GAS CHROMATOGRAPHY (GC)

Hydrocarbon UCM samples and oxidised material were examined on a Carlo Erba 5300 Mega Series gas chromatograph fitted with a 25m fused silica capillary column (0.32 mm i.d.) coated with DB-5 (J + W). Flame ionisation detection was used with on-column injection, and the column oven typically programmed from 50°C to 300°C at 5°C min^{-1} , and held at 300°C for 20 min. Hydrogen was used as a carrier at a flow rate of $2 \text{ cm}^3 \text{ min}^{-1}$ (set at 250°C) supplied at an inlet pressure of 0.55 KPa. Flame, air and hydrogen inlet pressures were set after "simplex" optimisation of detector responses relative to naphthalene (Trier, 1988) to values of 41 KPa (H_2) and 167 KPa (Air).

Mixtures of synthetic hydrocarbons used in the biodegradation experiments (section 2.6) were analysed by use of a Carlo Erba 5300 Mega Series gas chromatograph fitted with a 25 m fused silica capillary column (0.25 mm i.d.) coated with OV-1 (supplied by K Hall, GC2, Manchester). Hydrogen was the carrier at a flow rate of $1.5 \text{ cm}^3 \text{ min}^{-1}$ supplied with an inlet pressure of 75 KPa; detector air, hydrogen and N_2 make up gases were set at inlet pressures of 167, 41 and 70 KPa; respectively.

Intermediates in the synthesis of model hydrocarbons were examined by use of a Carlo Erba 4160 gas chromatograph fitted with a 25m fused silica capillary column (0.25 mm i.d.) coated with SE-54 (Carlo Erba). A Grob-type split vaporising injector (15:1 ratio, 280°C) and flame ionisation detection were used; and the carrier gas was nitrogen at a flow rate of $2 \text{ cm}^3 \text{ min}^{-1}$, supplied with an inlet pressure of 0.45 kg/cm². The oven temperature was typically programmed from 50°C to 300°C at $10^\circ\text{C}/\text{min}$, and held at 300°C for 10 min.

2.7.3 HIGH TEMPERATURE GAS CHROMATOGRAPHY (HTGC)

Model and environmental UCM samples were screened for high molecular weight components by high temperature capillary gas chromatography. This utilised an aluminium clad fused silica capillary column (25m x 0.25 mm i.d.) coated with methyl silicone "400" (0.1 μm film thickness, Quadrex Corp, USA) fitted into a conventional Carlo Erba 5300 Mega gas chromatograph. On-column injection was used with flame ionisation detection, however the flame tip was ceramic and the detector base set at 420°C . H_2 was the carrier at a flow rate of 1.5

cm³/min supplied with an inlet pressure of 90 KPa. The oven was temperature programmed from 100 - 400°C at 5°C/min, isothermal at 400°C for 10 min.

2.7.4 GAS CHROMATOGRAPHY - FLAME PHOTOMETRIC DETECTION (GC-FPD)

Samples of model aliphatic and aromatic UCM hydrocarbons were screened for organo-sulphur compounds by gas chromatography equipped with flame photometric detection (FPD) by Dr J. Robson, Organic Geochemistry Unit, School of Chemistry, University of Bristol.

2.7.5 QUANTITATION

All chromatograms and integration data were recorded using a Shimadzu CR3-A computing integrator. Retention indices were calculated according to standard methods (Harris and Habgood, 1966) using standard normal alkane mixtures containing n-C16, n-C17, n-C18, n-C20, n-C21, n-C22, n-C24, n-C25, n-C26, n-C28, n-C30, n-C32 and n-C36.

Biodegraded model synthetic hydrocarbon extracts (section 2.6) were quantified by measurement of GC peak areas relative to responses derived from a mixture of the same composition of known concentration (Appendix I). Replicate analyses (10x) indicated a GC reproducibility of ca. +6%.

Biodegraded model aliphatic UCM extracts were quantified relative to a calibration curve constructed from the GC responses of standards obtained by the serial dilution of a known quantity of UCM alkanes

(Appendix I). The calibration curve was linear over a concentration range of 2-8 mg cm⁻³ (ATTN. 5; 5 points), and replicate analyses (10x) indicated a GC precision of ±5.4%.

Estimates of percentage resolved components vs percentage unresolved components were obtained electronically using the Shimadzu CR3-A integrator in the "time slice area" measurement mode (Appendix I). Manual checks (chromatogram expansion and tracing on to mm² graph paper) were in good agreement (±1%) with those obtained electronically.

2.7.6 GAS CHROMATOGRAPHY - ELECTRON IMPACT MASS SPECTROMETRY (GC-EIMS)

Analysis of selected samples was performed on a Carlo Erba 5160 Mega gas chromatograph coupled to a Kratos MS25 double focussing magnetic sector mass spectrometer. A 30m fused silica capillary column coated with DB-5 (J + W) was used, with on-column injection and helium as the carrier gas. The column oven was typically temperature programmed from 50°C to 300°C at 5°C min⁻¹, and held at 300°C for 20 minutes. Electron impact mass spectrometer conditions were: ion source temperature: 250°C; ionising voltage: 40eV; filament emission current: 400 µA. Spectra (50 - 500 + Daltons) were collected each second using a Tektronix data system with DS90 software.

2.7.7 GAS CHROMATOGRAPHY - CHEMICAL IONISATION MASS SPECTROMETRY (GC - CIMS)

Selected aliphatic UCM hydrocarbon fractions and model hydrocarbon

standards were analysed by chemical ionisation (CI) GC - MS using the above described system. The reagent gas was isobutane fed into the ion source at a pressure of -0.67 bar, the ionising voltage was set at 58eV, and the filament emission current at 500 μ A. Ion counts due to background (impure isobutane) were minimised by data collection at >100 Daltons.

These conditions were optimised to achieve maximum abundance of the $[M-H]^+$ ion of synthetic 7 - n - hexylnonadecane (m/z 351; 100% relative intensity, 20% of total ion count). Samples were run at a temperature programme of 50°C to 180°C at 10°C min^{-1} , 180°C to 300°C at 5°C min^{-1} ; isothermal at 300°C for 20 mins.

2.7.8 PROBE ELECTRON IMPACT MASS SPECTROMETRY (EIMS)

Samples of model aliphatic UCM were analysed for ring class content by the methods of Hood and O'Neal, 1959, published as ASTM method D2786-71. Ion source conditions were standardised relative to n-hexadecane. Samples (ca. 5 μ g) were placed in quartz capillary tubes via syringe, the solvent evaporated (hot air stream) and the tubes placed in an air-cooled direct insertion probe. The probe and sample was fed directly into the ion source, and the probe heated to 50°C after 15 scans, 100°C after 50 scans, and finally to 150°C after 150 scans. The ionising voltage was 70 eV, the filament emission current 400 μ A, and the ion counts for each characteristic mass grouping (isoalkanes: m/z 71, 85, 99, 113; monocyclic alkanes: m/z 69, 83, 97, 111, 125, 139; 2-ring alkanes: m/z 109, 123, 137, 151, 165, 179, 193; 3-ring alkanes: m/z 149, 163, 177, 191, 205, 219, 233, 247; 4-ring alkanes: m/z 189, 203, 217, 231, 245, 259, 273, 287, 301; 5-ring

alkanes: m/z 229, 243, 257, 271, 285, 299, 313, 327, 341, 355) were collected using the DS-55 data system. Calculations of percentage composition as a function of ring class were performed by the use of published inverted matrices derived from the analysis of model hydrocarbon standards (Hood and O'Neal, 1959). The inverse matrix chosen was that derived for isoalkanes with an average carbon number composition of C28. A BBC microcomputer was programmed in BASIC to perform the lengthy calculations (Appendix II).

2.7.9 PYROLYSIS - GAS CHROMATOGRAPHY - MASS SPECTROMETRY (PY-GCMS)

Samples of model aliphatic and aromatic UCM hydrocarbons and selected model synthetic hydrocarbons were pyrolysed and analysed by gas chromatography - mass spectrometry using a CDS pyroprobe directly inserted into the modified heated (180°C) packed column injection port of a Carlo Erba 5160 Mega gas chromatograph coupled to a Kratos MS25 mass spectrometer. Samples (ca. 50 µg) in DCM were applied by syringe to quartz pyrolysis tubes packed with pre-extracted glass wool. Pyrolysis occurred for 20 sec at a maximum temperature of 800°C, and the column oven was temperature programmed from 40°C (for 5 min) to 300°C at 5°C min⁻¹, and held at 300°C for 15 min. The capillary column was fused silica (30m x 0.32 mm i.d.) coated with DB-1 (J + W). Electron impact mass spectra were recorded at an ionising voltage of 38 eV and an emission current of 400 µA over a mass range of 50 - 500 Daltons.

2.7.10 INFRA RED SPECTROSCOPY (IR)

Infra red spectra were recorded as either liquid films, KBr discs or solutions (in DCM) on a Perkin Elmer Infra Red Spectrometer. The 1601 cm^{-1} peak in the spectrum of polystyrene was used as a reference.

2.7.11 COMPOUND IDENTIFICATION

Individual hydrocarbons and functionalised compounds were identified by co-chromatography with authentic compounds on GC columns of differing polarity and by comparison of GC retention indices with literature reported values. Additional identifications were made by GCMS: components were identified by comparison with the spectra of authentic compounds, published spectra or by spectral interpretation. Mass fragmentography was used to characterise members of homologous or pseudohomologous series and to aid compound identification.

2.8 SYNTHESSES

2.8.1 OUTLINE OF SYNTHETIC PROCEDURES

Synthesis of model hydrocarbons followed the schemes outlined in Fig. 4.1 and was based on well known Grignard coupling reactions (Kharasch and Reinmuth, 1954). The main emphasis was on high purity, ready availability of pure starting materials, and low cost. The purity and authenticity of all starting materials was confirmed by capillary

gas chromatography, IR spectroscopy and/or low resolution mass spectrometry (LRMS).

Great care was taken to ensure that all glassware, solvents, reactants, magnesium and inert gases remained dry. Glassware was assembled whilst hot and placed immediately under an inert and dried (CaCl_2) argon stream. All solvents (section 2.1) were freshly distilled and stored over active molecular sieve prior to use. Magnesium ribbon was freshly scraped, washed in dry Et_2O and activated in the oven at 120°C for 30 min. All reactants (if liquid) were stored over freshly baked (450°C) anhydrous sodium sulphate, if solid were stored in desiccators under a dry N_2 atmosphere. Whilst in progress the reaction glassware was kept dry by the use of CaCl_2 tubes. In most cases activation of the scraped magnesium turnings was accomplished by the addition of 2-3 drops of 1,2-dibromoethane followed by slight heating (50°C , water bath).

2.8.2 SYNTHESIS OF MONOALKYL SUBSTITUTED ACYCLIC ALKANES (SCHEME A)

2.8.2.1 9-METHYLTETRACOSANE

Starting materials

Decan - 2 - one (2)

GC purity: 95%

LRMS m/z: 156 (M^+ , 5%), 58 (McL, 80%), 98 (M^+-MCL , 5%), 43 (C_3H_7 , 100%)

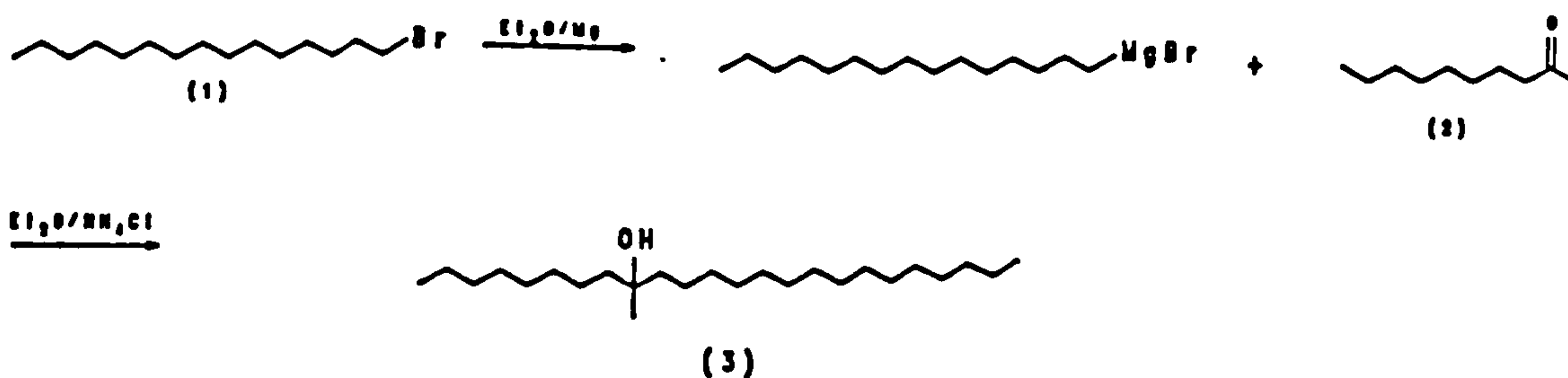
IR (liquid film): V(C=O) overtone 3440 cm^{-1} , V(C=O) 1720 cm^{-1} V(C-CO-C) 1172 cm^{-1}

1-Bromopentadecane (1)

GC purity: 96%

LRMS m/z: 290/292 (M^+ , 0.1%), 149/151 (M^+ - $C_{10}H_{21}$, 10%), 135/137 ($C_4H_8Br^+$, 42%)

9-Methyltetracosan-9-ol (3):



1-Bromopentadecane (1, 1.0036g, 3.5 mmol) in Et_2O (10 cm^3) was added dropwise to rapidly stirred activated (1,2-dibromoethane) magnesium scrapings (98 mg, 4 mmol) in Et_2O (10 cm^3). Gentle heat (water bath, 40°C) was applied intermittently during the addition stage, and a cloudy white precipitate was observed to form. The solution was then refluxed gently (1 hr) during which the precipitate disappeared and the majority of the Mg scrapings were consumed. The reaction mixture was then cooled (ice bath), a solution of decan-2-one (2, 450 mg, 2.9 mmol) in Et_2O (10 cm^3) added, and the mixture heated under gentle reflux for 4 hr. When cool, iced distilled water (5 cm^3) and NH_4Cl (saturated solution, 5 cm^3) were added, and after stirring (16 hr) the mixture was transferred to a separating funnel (100 cm^3). The

upper organic layer was removed, the aqueous layer re-extracted (Et_2O , $3 \times 10 \text{ cm}^3$) and the organic extracts combined and dried (anh. Na_2SO_4). After drying, the solvent was evaporated (Buchi, 30°C) and the crude products recovered and weighed (1.125g).

Crude reaction products (232 mg) were purified by open column chromatography on deactivated (4%) alumina (30g). Elution with hexane (100 cm^3) removed residual reactants, and dichloromethane (100 cm^3) provided the alcohol, 9-methyltetracosan-9-ol (3, 78 mg, 0.2 mmol). The purified alcohol was analysed by GC and assigned by LRMS.

GC purity : 95%

Yield : 36%

LRMS m/z : 350 ($\text{M}^+ - \text{H}_2\text{O}$, 1.8%), 255 ($\text{M}^+ - \text{C}_8\text{H}_{17}$, 10%),

157 ($\text{M}^+ - \text{C}_{15}\text{H}_{31}$, 26%)

9-Methyltetracosenes (4):



The synthetic alcohol was dehydrated according to the procedure of

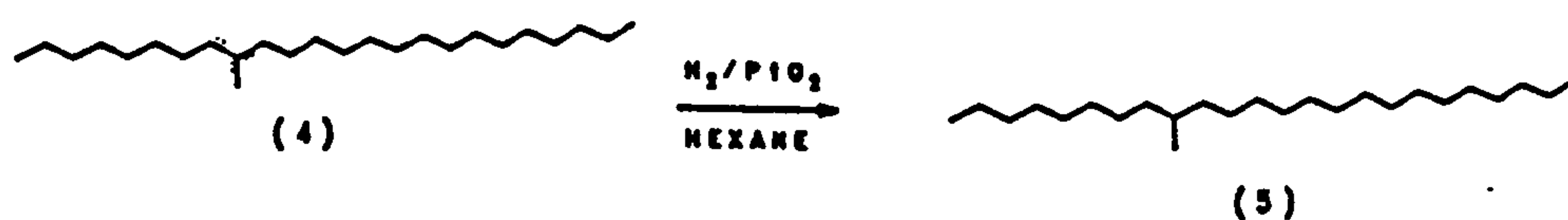
Rinehart and Perkins (1963). 9-Methyltetracosan-9-ol (3, 70 mg, 0.19 mmol) was added to pyridine (Pearce silylation grade, 500 mm³) in a "reacti vial". The solution was cooled (0°C, ice bath), POCl₃ (BDH, 200 mm³) was added dropwise and the solution stirred overnight (16 hr, 22°C). After stirring the mixture was cooled (0°C, ice bath) and chilled distilled water (0°C, 2 cm³) was added with care. The solution was transferred to a separating funnel with hexane (5 cm³) where NaHCO₃ (saturated solution, 5 cm³) was added to decompose residual POCl₃. Following hexane extraction (3 x 5 cm³) and solvent removal (Buchi, 40°C), the isomeric 9-methyltetracosenes (45 mg, 0.13 mmol) were examined by GC and GCMS.

GC purity (5 peaks) : 91%

Yield : 68%

LRMS m/z : 351 (M⁺ + H, 22%), 253 (2.1%), 155 (10%), 98 (40%),
55 (100%).

9-Methyltetracosane (5):



9-methyltetracosenes (4, 45 mg, 0.13 mmol) were hydrogenated by passing hydrogen gas (10 cm³ min⁻¹) through a well stirred hexane

solution (20 cm³, 20°C) containing Adam's catalyst (PtO₂.H₂O, 10 mg). The reaction progress was monitored by analysis of aliquots (10 mm³ in 1000 mm³) by GC. On completion, the solution was filtered (cotton wool plug), the solvent evaporated (Buchi, 40°C) and the crude products purified by argentatious TLC (0.5 mm SiO₂; 10% AgNO₃; hexane as mobile phase). Following visualisation (Rhodamine 6G, 365 nm), the alkane band (Rf: 0.92-1.00) was removed and the alkane recovered by desorption with hexane: dichloromethane (1:1, 25 cm³). Solvent removal provided the alkane 9-methyltetracosane (5, 22mg, 0.06 mmol) which was assigned by LRMS (CI and EI).

GC purity : 97%

KI (DB-5) : 2438

Yield : 46%

LRMS (EI) m/z : 352 (M⁺·, 0.3%), 337 (M⁺·-CH₃, 0.5%),

238 (C₁₇H₃₄, 7%), 239 (C₁₇H₃₅, 6%),

140 (C₁₀H₂₀, 21%), 141 (C₁₀H₂₁, 9%), 57 (100)

LRMS (CI-isobutane) m/z : 351 [(M-H)⁺, 100%]

2.8.2.2 7-n-Hexylnonadecane

Starting materials

Tridecan-7-one (7)

GC purity : 96%

LRMS m/z : 198 (M⁺·, 2%), 128 (McL, 22%), 113 (C₇H₁₃O, 100%),

58 (McL, 96%)

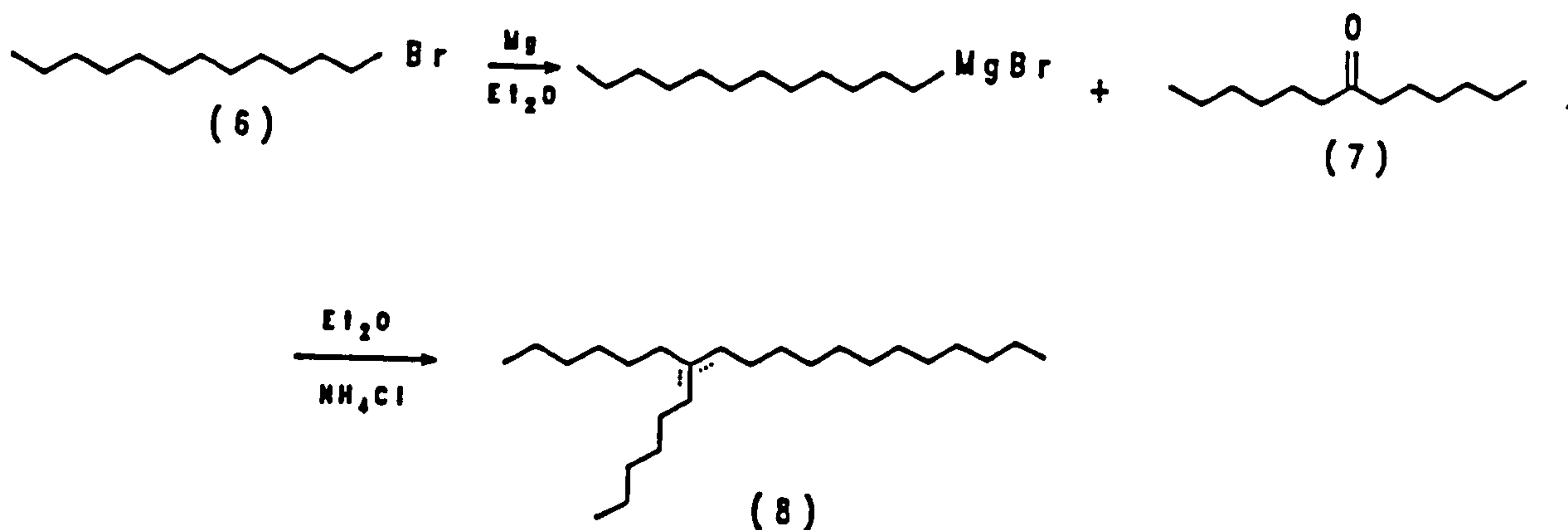
IR (liquid film) : V(C=O) overtone 3440 cm⁻¹, V(C=O) 1720 cm⁻¹, V(C-CO-C) 1140 cm⁻¹

1-Bromododecane (6)

GC purity : 97%

IR (liquid film) : $\psi(\text{CH}_2\text{-Br})$ 1270 cm^{-1}

7-n-Hexylnonadecenes (8):



1-Bromododecane (2g, 8 mmol) in Et₂O (10 cm³) was added dropwise to a rapidly stirred suspension of magnesium scrapings (227 mg, 9.33 mmol) in Et₂O (15 cm³). After formation of the Grignard reagent (section 2.8.2.1), tridecan-7-one (1.32g, 6.67 mmol) was added. After reflux (4 hr) and addition of water and NH₄Cl (sat. soln, H₂O); crude reaction products were extracted with Et₂O (3 x 15 cm³), the extracts combined and dried (anh. Na₂SO₄) and the total products weighed (2.47g). GC-MS of total products revealed the production of two main components, a mixture of isomeric alkenes and the Wurtz coupling product, n-tetracosane. Total products (200 mg) were urea adducted (section 2.2) and the urea non adduct recovered to yield the isomeric 7-n-hexylnonadecenes (8, 144 mg, 0.4 mmol). These were assigned by LRMS and IR.

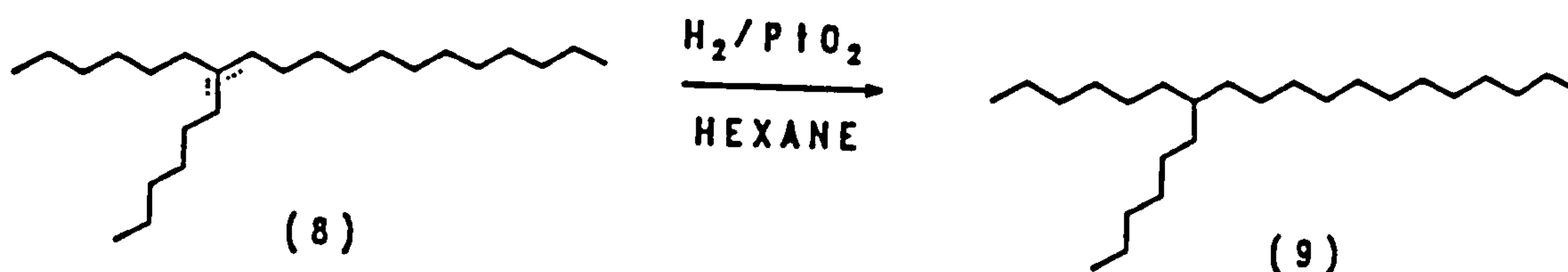
GC purity : 96%

Yield : 76%

LRMS m/z : 350 (M^+ , 0.5%), 280 ($C_{20}H_{40}$, 0.5%), 195/196 ($C_{14}H_{26}$, 2%), 69 (74%), 55 (100%)

IR (liquid film) : $\nu(C=C)$ 1670 cm^{-1}

7-n-Hexylnonadecane (9):



Isomeric 7-n-hexylnonadecenes (140 mg, 0.4 mmol) were smoothly hydrogenated to the alkane using Adam's catalyst (5 mg) in hexane (25 cm^3). Filtration and drying (anh. Na_2SO_4) provided the alkane, 7-n-hexylnonadecane (9, 73 mg, 0.21 mmol).

GC purity : 96%

KI(DB-5) : 2360

Yield : 53%

LRMS (EI) m/z : 352 (M^+ , 0.2%), 267 ($C_{19}H_{39}$, 38%),

266 ($C_{19}H_{38}$, 28%), 182 ($C_{13}H_{26}$, 30%), 57 (100%)

LRMS (CI-isobutane) m/z : 351 [$(M-H)^+$, 100%]

2.8.2.3 2-Methyltetracosane

Starting material

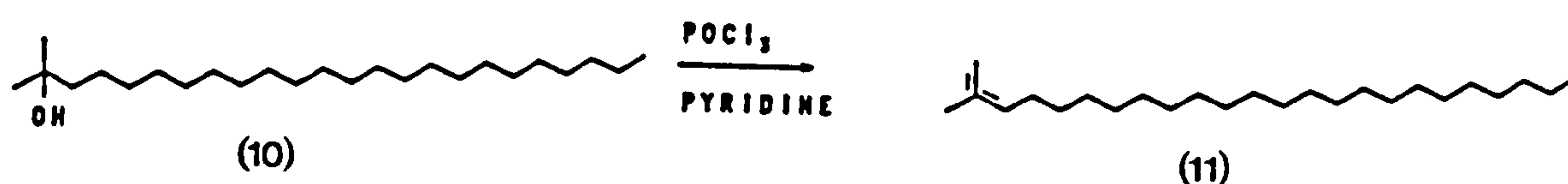
2-methyltetracosan-2-ol (10)

GC purity : 97%

LRMS m/z : 353 ($M^+ - CH_3$, 11%), 350 ($M^+ - H_2O$), 8%), 322 (2%),
360 ($C_3H_7O + H$, 100%)

IR (liquid film) : $\nu(OH)$ 3320 cm^{-1} ; δ_sCH_3 gem dimethyl 1365 cm^{-1} ,
1375 cm^{-1}

2-Methyltetracosenes (11):



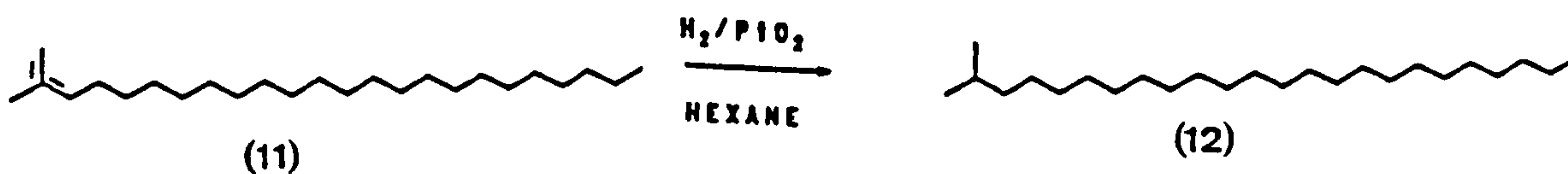
2-methyltetracosan-2-ol (10, 92 mg, 0.25 mmol) was dehydrated using $POCl_3$ (300 mm^3) in pyridine (500 mm^3). Hexane extraction (2 x 10 cm^3) provided the alkenes (11, 73mg, 0.2 mmol) which were examined by GC and LRMS.

GC purity : 95%

Yield : 80%

LRMS m/z : 351 ($M^+ + H$, 30%), 83 (66%), 69 (100%)

2-Methyltetracosane (12):



2-Methyltetracosenes (11, 70 mg, 0.2 mmol) were hydrogenated using Adam's catalyst (10 mg) in hexane (25 cm³). Total products were recovered (65 mg) and fractionated by argentatious TLC (0.55 mm SiO₂; 10% AgNO₃, hexane as developer). The band corresponding to the alkane (Rf: 0.85-0.99) was removed and desorped using DCM (30 cm³). The purified product, 2-methyltetracosane (12, 54 mg, 0.15 mmol) was examined by GC and LRMS.

GC purity : 97%

KI(DB-5) : 2463

Yield : 75%

LRMS m/z : 352 (M⁺·, 1.2%), 337 (M⁺·-CH₃, 0.6%), 309 (M⁺·-C₃H₇, 11%),
85 (50%), 57 (100%)

2.8.3 SYNTHESIS OF ALKYL MONOCYCLIC AND ALKYL AROMATIC HYDROCARBONS (SCHEME B)

2.8.3.1 9-(2-cyclohexylethyl)-heptadecane

Starting materials

3-cyclohexylpropanoic acid (13)

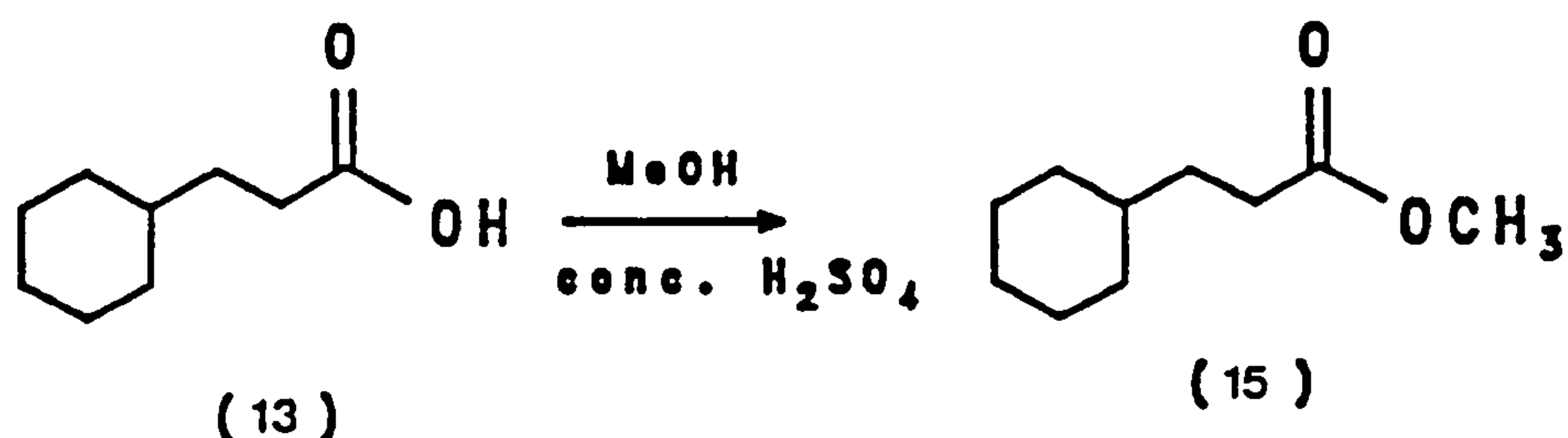
LRMS m/z : 156 (M⁺·, 1%), 138 (M⁺·-H₂O, 4%), 97 (C₇H₁₃, 100%),
83 (C₆H₁₁, 20%), 73 (C₃H₅O₂, 26%), 60 (McL; 13%)

1-Bromooctane (14)

LRMS m/z : 192/194 (M⁺·, 2%), 149/151 (M⁺· -C₃H₇, 10%),

135/137 (M^+ · -C₄H₉, 100%)

Methyl 3-cyclohexylpropanoate (15):



3-cyclohexylpropanoic acid was methylated according to Vogel (1978). The acid (13, 2.5g, 16 mmol) was added to methanol (5g, 160 mmol) in a RBF (50 cm³). Concentrated H₂SO₄ (0.42g, 4.3 mmol) was added cautiously, and the solution refluxed for 4 hr. After cooling and solvent removal to near dryness (Buchi, 40°C), distilled water (25 cm³) was added and the product extracted into DCM (15 cm³). The DCM extract was washed with water (10 cm³) and a saturated solution of NaHCO₃ (10 cm³), and dried over anh. Na₂SO₄. Filtration followed by solvent removal provided the ester, methyl 3-cyclohexylpropanoate (15, 2.0g, 11.8 mmol). This was examined by GC, LRMS and IR.

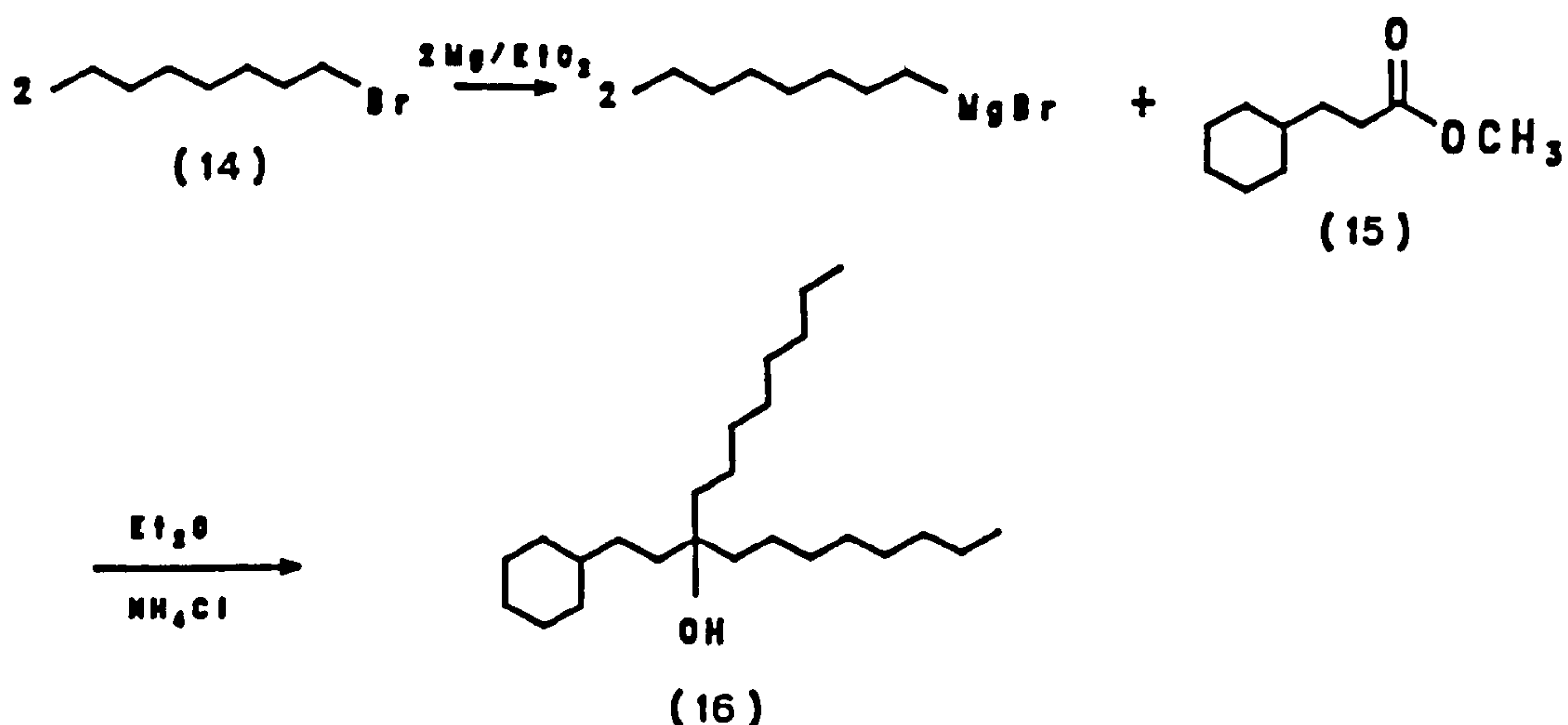
GC purity : 96%

Yield : 74%

LRMS m/z : 170 (M^+ ·, 2%), 139 (M^+ ·-OCH₃, 20%), 97 (C₇H₁₃, 100%),
87 (C₂H₄CO₂CH₃, 87%), 74 (McL, 52%)

IR (liquid film) : V(C=O) 1745 cm⁻¹, δ_{as} CH₃ (1440 cm⁻¹),
V(C-CO-O) 1180 cm⁻¹

9-(2-Cyclohexylethyl)-heptadecan-9-ol (16):



The C8 alkylbromide (14) was coupled to the methyl ester (15) following the procedures of Kharasch and Reinmuth (1954). 1-Bromooctane (14, 2.72g, 14 mmol) in Et₂O (15 cm³) was added to freshly scraped Mg (390 mg, 16 mmol) in Et₂O (10 cm³). On formation of the Grignard reagent the solution was cooled and the ester (15, 1g, 5.9 mmol) in Et₂O (10 cm³) added. After reflux (4 hr), the solution was cooled and water (5 cm³) and NH₄Cl (sat. soln, 5 cm³) added. After stirring (16 hr) the crude products were extracted with Et₂O (3 x 15 cm³), the extracts combined and dried (anh. Na₂SO₄), solvent was removed (Buchi, 40°C) and the total products weighed (2.8g).

Purification of the crude reaction products (1g) was achieved by column chromatography on Al₂O₃ (4% H₂O), 30g). Elution with hexane (80 cm³) removed unreacted bromide, and the DCM eluate (100 cm³) was collected (464 mg). When analysed by GC this was found to contain residual unreacted ester. Further purification of the fraction (111 mg) was achieved by TLC (0.5 mm, SiO₂; hexane: Et₂O (9:1) as mobile phase), the bands were visualised (Rhodamine 6G, 364 nm) and that

corresponding to the alcohol (Rf: 0.16-0.33) removed. Desorption with DCM (30 cm³) followed by solvent removal (Buchi, 40°C) provided the alcohol, 9-(2-cyclohexylethyl)-heptadecan-9-ol (16, 72mg, 0.2 mmol) which was examined by GC, LRMS, and IR.

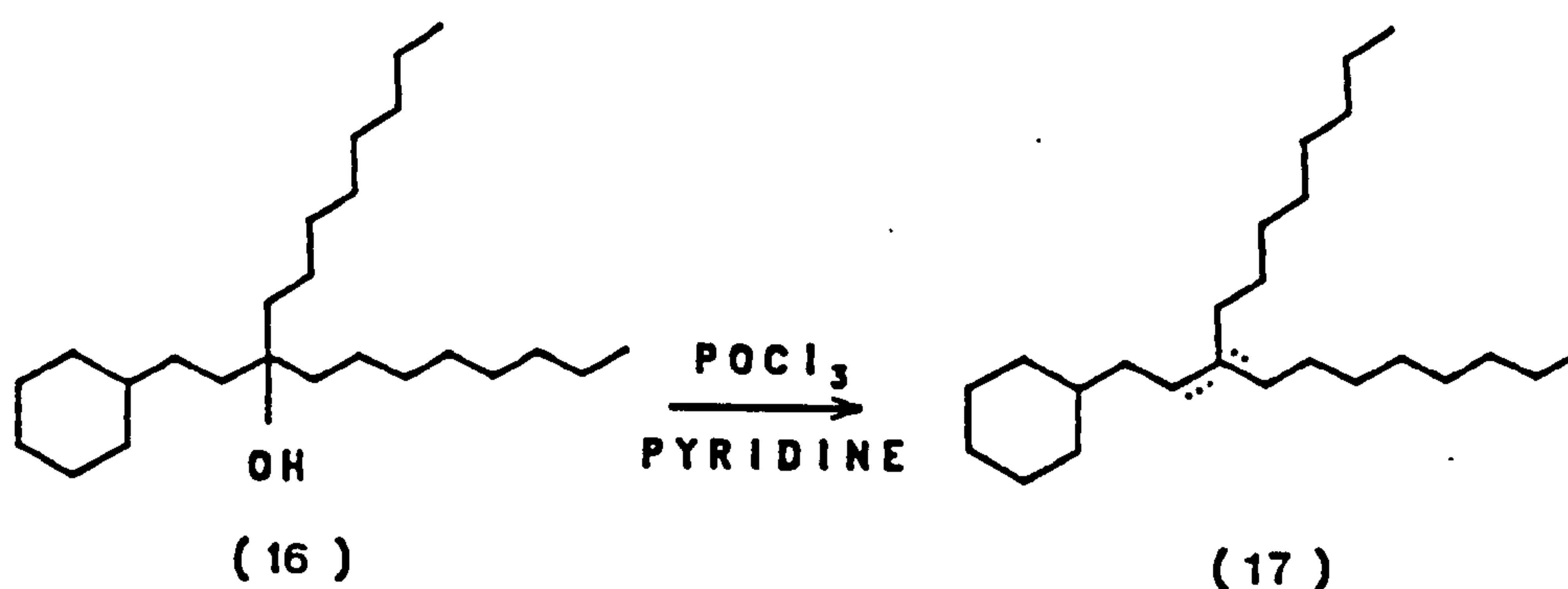
GC purity : 96%

Yield : 39%

LRMS m/z : 348 (M⁺·-H₂O, 16%), 253 (M⁺·-C₈H₁₇), 207 (42%),
154 (2 x McL, 67%), 96 (100%)

IR (Liquid film) : V(O-H) 3400 cm⁻¹ (H-bonded),
V (C-O) 1135 cm⁻¹, δ(CH₂) 1452 cm⁻¹

9-(2-Cyclohexylethyl)-heptadecenes (17):



The alcohol (16, 72 mg, 0.2 mmol) was dehydrated using POCl₃ (1.4 cm³) in pyridine (3 cm³). The crude product was extracted with hexane (2 x 10 cm³) to yield the alkenes, (17, 65 mg, 0.19 mmol) which were examined by GC, LRMS, and IR.

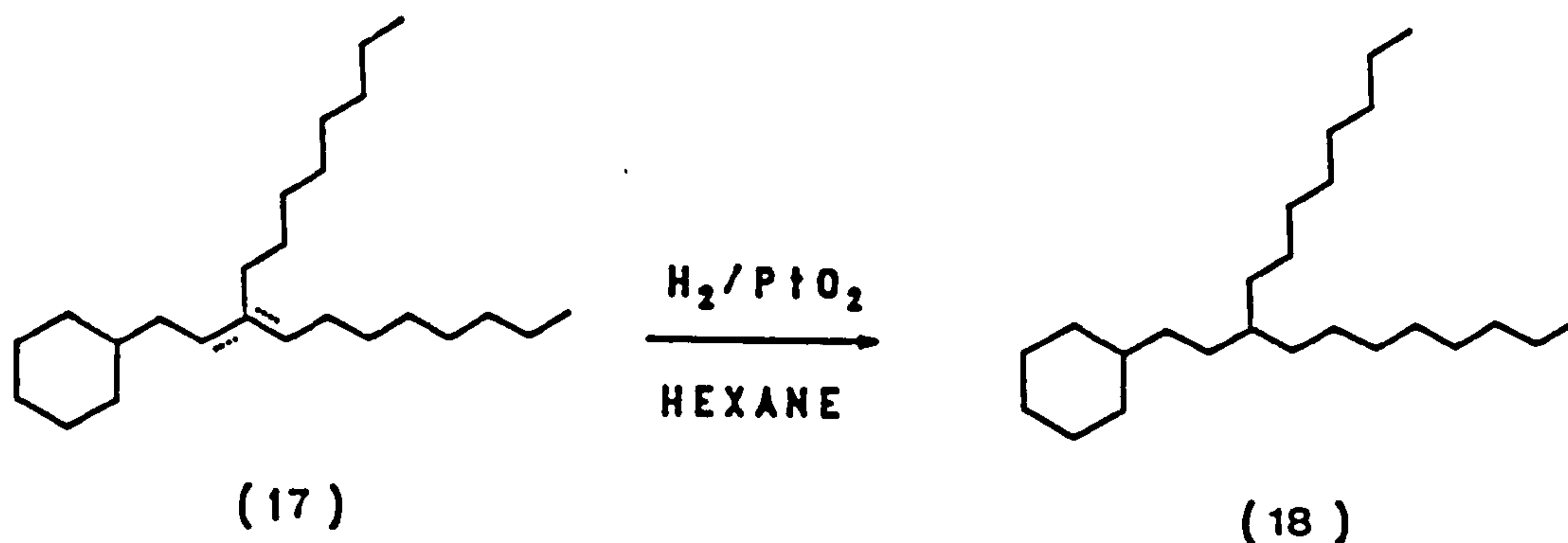
GC purity : 92%

Yield : 96%

LRMS m/z : 348 (M^+ , 0.5%), 253 ($M^+ - C_8H_{17}$, 12%), 154 (2 x McL, 47%), 139 (43%), 96 (90%), 84 (McL, 84%), 56 (100%)

IR (liquid film) : $\nu(C=C)$ 1645 cm^{-1} , oop(C-H bend) 750 cm^{-1}

9-(2-Cyclohexylethyl)-heptadecane (18):



The alkenes (17, 49 mg, 0.14 mmol) were hydrogenated to the alkane using Adam's catalyst (10 mg) in hexane (25 cm^3). The crude products (47 mg) were purified by argentatious TLC (0.5 mm SiO_2 , 10% $AgNO_3$, hexane as mobile phase) and after visualisation (Rhodamine 6G, 364 nm) the band corresponding to the alkane (R_f : 0.89-0.99) was removed. Desorption with hexane/DCM (1:1, 30 cm^3) provided a mixture (31 mg) which was purified further by thiourea adduction. The thiourea non adduct comprised the alkane 9-(2-cyclohexylethyl)-heptadecane (18, 22 mg, 0.06 mmol) which was examined by GC and LRMS.

GC purity : 97%

KI(DB-5) : 2420

Yield : 43%

LRMS m/z : 350 (M^+ , 0.4%), 238/239 ($M^+ - C_8H_{16}$, C_8H_{17} ; 33%),
237 ($M^+ - C_8H_{17}$, 65%), 236 ($M^+ - C_8H_{18}$, 47%),

97 (100%), 83 (98%)

2.8.3.2 9-(2-Phenylethyl)-heptadecane

Starting materials

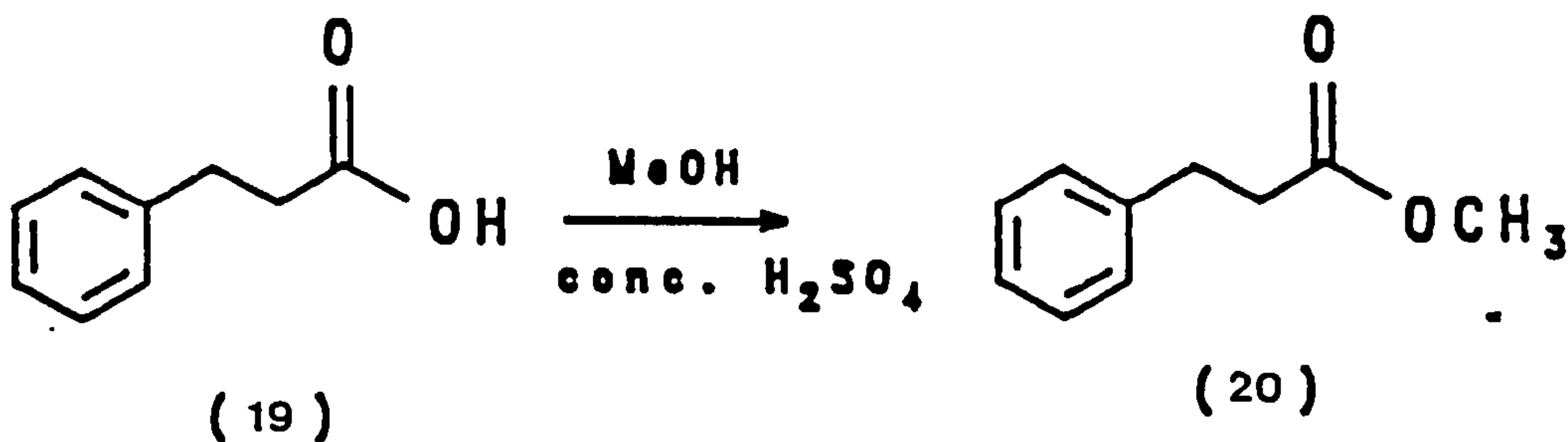
3-Phenylpropanoic acid (19)

LRMS m/z : 150 (M^+ , 65%), 104 (47%), 91 (100%), 77 (11%)

1-Bromooctane (14)

LRMS : (as above, 2.8.3.1)

Methyl 3-phenylpropanoate (20):



3-phenylpropanoic acid (19, 2.5g, 17 mmol) was added to methanol (5.44g, 0.17 mmol) and concentrated H₂SO₄ (0.42g, 4.3 mmol). The solution was refluxed for 4 hr, and after cooling solvent was removed to near dryness. Water was added (25 cm³) and the crude products extracted into DCM (15 cm³). The DCM extract was washed with water (10 cm³) and NaHCO₃ solution (sat., 10cm³), dried (anh. Na₂SO₄), and

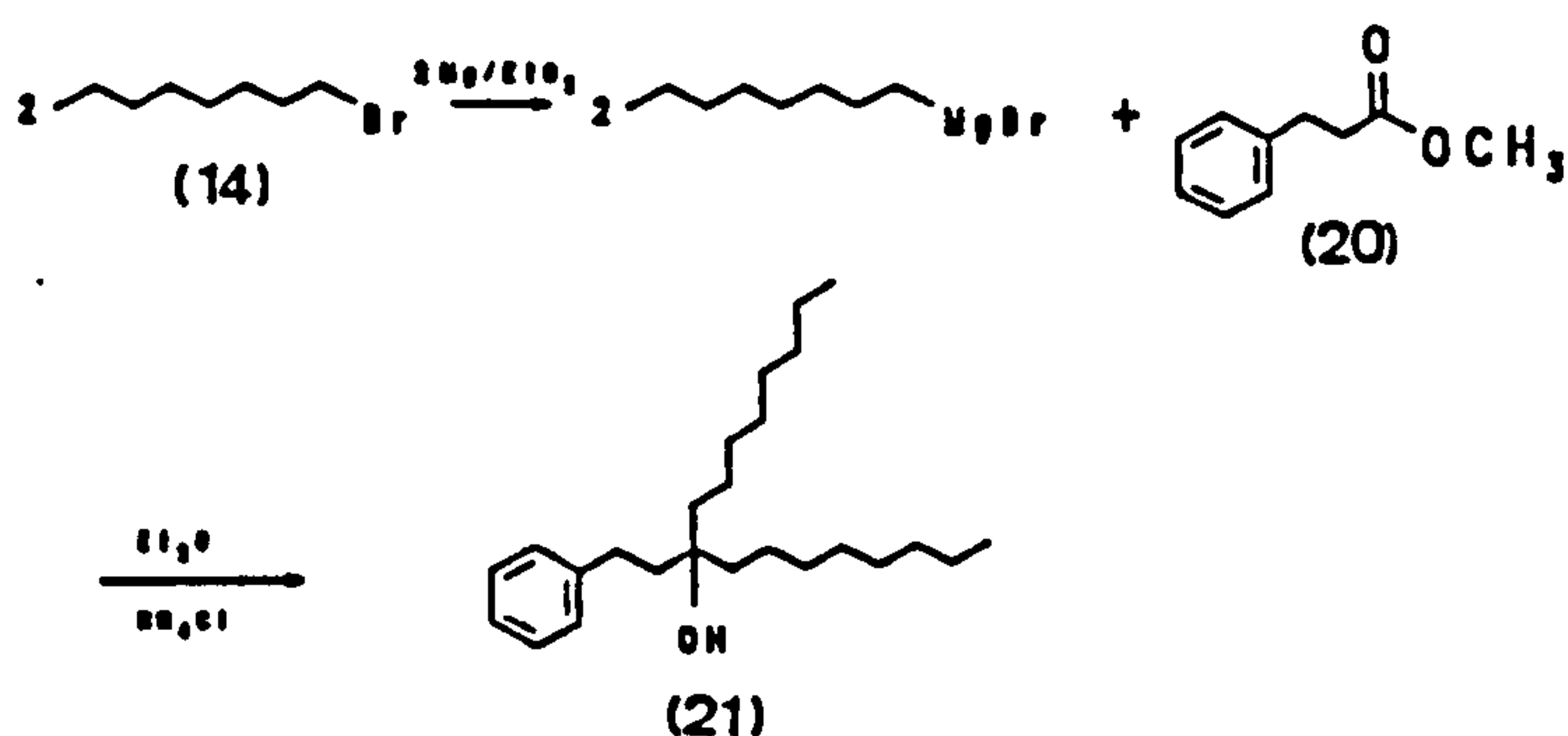
solvent removal (Buchi, 40°C) provided the ester, methyl 3-phenylpropanoate (20, 2.5g, 15mmol) which was examined by GC and LRMS.

GC purity : 97%

Yield : 75%

LRMS m/z : 164 (M⁺, 49%), 133 (M⁺-OCH₃, 17%), 104 (100%),
91 (52%), 77 (11%)

9-(2-Phenylethyl)-heptadecan-9-ol (21):



1-Bromooctane (14, 2.8g, 15 mmol) in Et₂O (15 cm³) was added to a stirred suspension of magnesium (freshly scraped, 430 mg, 18 mmol) in Et₂O (10 cm³). Following formation of the Grignard reagent the solution was cooled and the ester (20, 1g, 6 mmol) in Et₂O (10 cm³) added. After reflux (4 hr) and hydrolysis (H₂O, NH₄Cl, 16 hr), the crude products were extracted into Et₂O (3 x 15 cm³), the extracts were combined and dried (anh. Na₂SO₄) and the crude product (2.74 g)

recovered after solvent removal (Buchi, 40°C).

Reaction products (1.02g) were purified by column chromatography on alumina (4% H₂O, 30g). Elution with hexane (80 cm³) removed unreacted bromide and elution with DCM (100 cm³) provided the desired product plus unreacted methyl ester (550 mg). Further purification of the DCM fraction (330 mg) was achieved by hydrolysis with methanolic KOH (5% w/v, 10 cm³) for 30 min at room temperature, followed by addition of water (10 cm³) and extraction into DCM (2 x 10 cm³). After solvent removal (Buchi, 40°C) the hydrolysed product, 9-(2-phenylethyl)-heptadecan-9-ol (21, 200 mg, 0.6 mmol) was examined by GC, LRMS and IR.

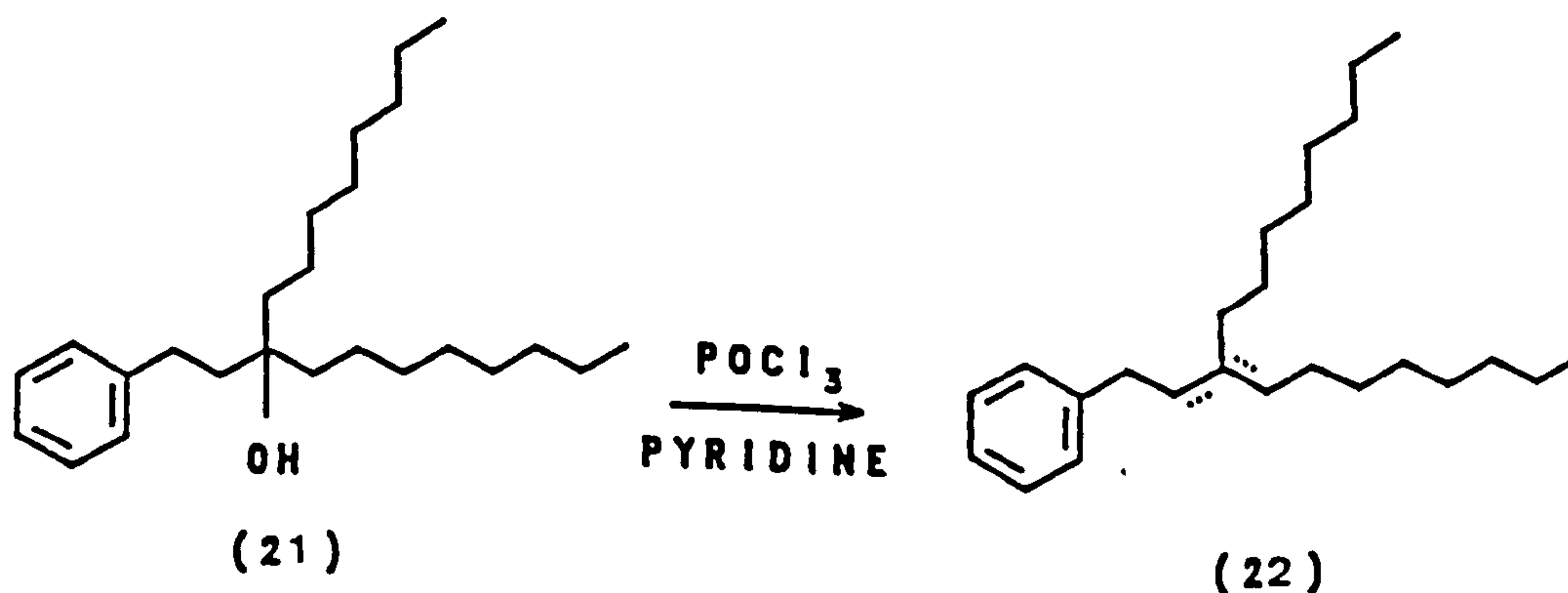
GC purity : 96%

Yield : 43%

LRMS m/z : 342 (M⁺·-H₂O, 26%), 247 (M⁺·-C₈H₁₇, 42%),
146 (2 x McL; 55%), 104 (78%), 91 (100%)

IR (liquid film) : V(O-H) 3400 cm⁻¹ (H-bonded), V(C-H arom)
3020 cm⁻¹, 3060 cm⁻¹, V(C-C arom) 1490 cm⁻¹,
-C-H oop bend 735 cm⁻¹, C-C ring bend 690 cm⁻¹

9-(2-Phenylethyl)-heptadecenes (22):



The purified alcohol (21, 200 mg, 0.6 mmol) was dehydrated using POCl_3 (4 cm^3) in pyridine (6 cm^3). The crude products were extracted with DCM ($2 \times 10 \text{ cm}^3$), the DCM extracts washed with dilute HCL (1%, 10 cm^3), and dried (anh. Na_2SO_4). Solvent removal (Buchi, 40°C) afforded the alkenes (22, 145 mg, 0.42 mmol) which were examined by GC, LRMS, and IR.

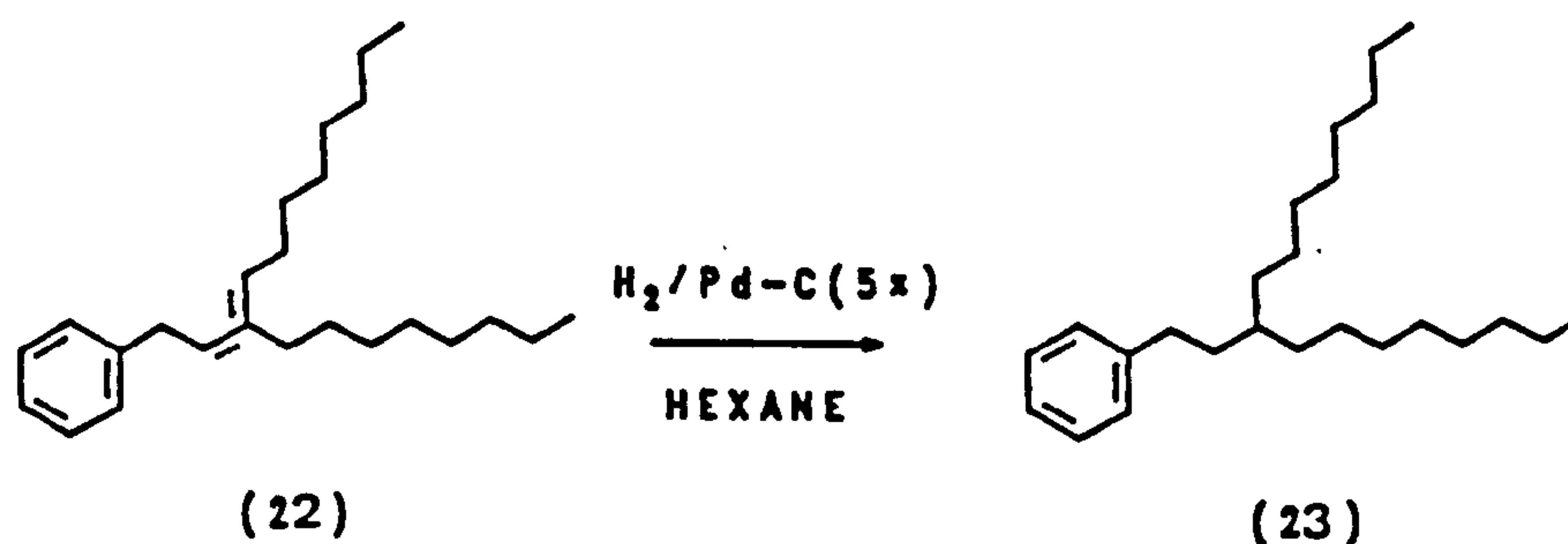
GC purity : 95%

Yield : 70%

LRMS m/z : 343 ($\text{M}^+ + \text{H}$, 23%), 244 (McL, 12%), 229 ($\text{M}^+ - \text{C}_8\text{H}_{17}$, 80%),
146 (52%), 104 (2 x McL, 86%), 91 (100%)

IR (liquid film) : $\nu(\text{C-H arom})$ 3020 cm^{-1} , 3060 cm^{-1} , $-\text{C-H oop}$
bend 740 cm^{-1} , $\nu(\text{C-C arom})$ 1490 cm^{-1}

9-(2-Phenylethyl)-heptadecane (23):



The isomeric alkenes (22, 110 mg, 0.32 mmol) were hydrogenated using palladium on carbon (5%, BDH, 20 mg) as the catalyst in hexane (30 cm^3). The crude products (94 mg) were purified by argentatious TLC

(0.5mm SiO₂, 10% AgNO₃ hexane as developer), the bands visualised (Rhodamine 6G, 364 nm), and the band corresponding to the alkyl aromatic hydrocarbon (Rf: 0.43-0.76) was removed. Desorption with DCM (30 cm³) provided 9-(2-phenylethyl)-heptadecane (23, 79 mg, 0.23 mmol) which was examined by GC and LRMS.

GC purity : 97%

KI(DB-5) : 2444

Yield : 72%

LRMS m/z : 344 (M⁺·, 2.3%), 253 (M⁺·-C₇H₇, 3.2%), 231 (M⁺·-C₈H₁₇, 0.2%), 105 (5%), 92 (100%)

2.8.4 2,6,10,14,18-Pentamethyleicosane (Scheme C):

Starting materials

Phytol (E-3,7R,11R,15-tetramethylhexadec-2-en-1-ol) (24)

GC purity : 95%

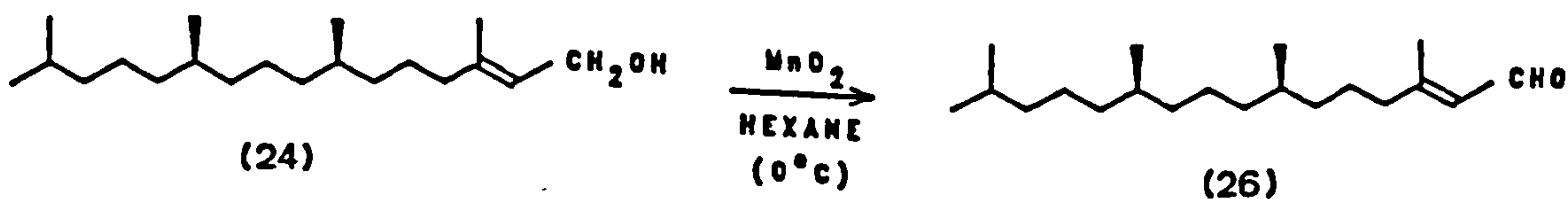
IR (liquid film) : V(O-H) 3310 cm⁻¹ (H-bonded), V(C=C) 1670 cm⁻¹, V(C-O) 1000 cm⁻¹, δs CH₃ gem dimethyl 1370 cm⁻¹, 1380 cm⁻¹

1-Bromo-2S-methylbutane (25)

GC purity : 96%

IR (liquid film) : V(C-Br) 620 cm⁻¹

E-3,7R,11R,15-Tetramethylhexadec-2-enal (phytenal) (26):



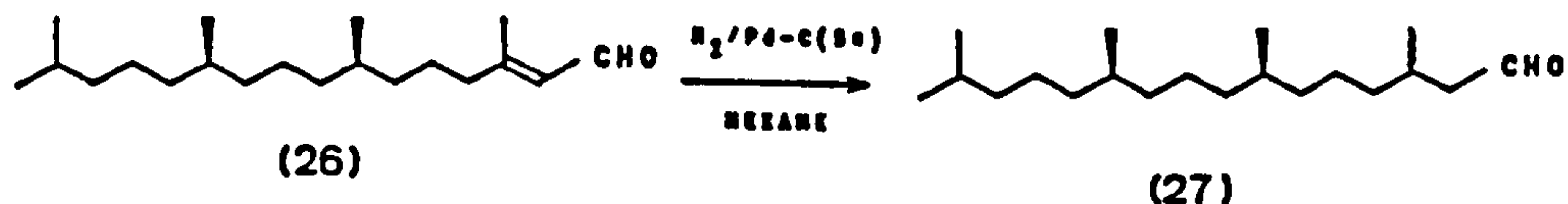
E-3, 7R, 11R, 15-tetramethylhexadec-2-en-1-ol (trans-phytol, Aldrich, 1.5g, 5.1 mmol) in hexane (150 cm³) with activated manganese dioxide (Attenburrow et al., 1952; 11.5g, 132 mmol) was stirred at 0°C (ice bath) for 30 min. The mixture was filtered (Whatman GF/F, precombusted 450°C), the solvent removed (Buchi, 40°C) and the product (1g, 3.4 mmol) examined by GC and IR.

GC purity : 95%

Yield : 67%

IR (liquid film) : $\nu(\text{C-H})$ aldehyde 2720 cm⁻¹, $\nu(\text{C-O})$ α, β - unsat. aldehyde 1680 cm⁻¹, $\delta_{\text{s}}\text{CH}_3$ gem dimethyl 1370 cm⁻¹, 1380 cm⁻¹

3RS, 7R, 11R, 15-Tetramethylhexadecanal (Phytanal) (27):



E-3,7R,11R,15-tetramethylhexadec-2-enal (26, 260 mg, 0.88 mmol) was hydrogenated using palladium on carbon (5%, BDH, 20 mg) as the catalyst in hexane (30 cm³) for 2 hr. Filtration followed by solvent

removal provided the saturated aldehyde 3RS,7R,11R,15-tetramethylhexadecanal (27, 194 mg, 0.66 mmol) which was examined by GC, LRMS and IR.

GC purity : 96%

Yield : 75%

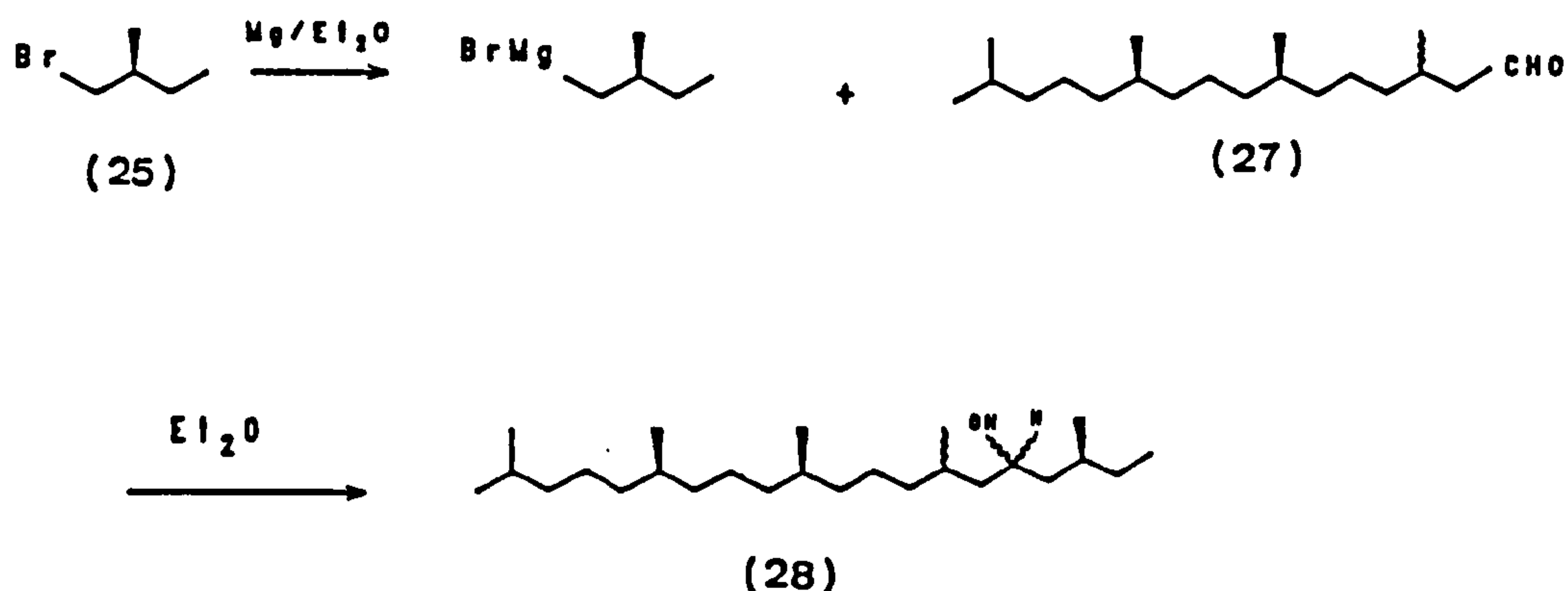
LRMS m/z : 296 ($M^{+\cdot}$, 1.3%), 278 ($M^{+\cdot}-H_2O$, 1.7%), 252 (McL, 1.1%)

IR (liquid film) : $\nu(C-H)$ aldehyde 2720 cm^{-1} , $\nu(C=O)$

1730 cm^{-1} , δ_sCH_3 gem dimethyl 1370 cm^{-1} ,

1380 cm^{-1}

3S, 7RS, 11R, 15R, 19-Pentamethyleicosan-5RS-ol (28):



The Grignard reagent formed from 1-bromo-2S-methylbutane (25, 119 mg, 0.79 mmol) and magnesium (22 mg, 0.9 mmol) was coupled with the aldehyde (27, 195 mg, 0.66 mmol) as described previously. The crude products (246 mg) were purified by TLC (0.5 mm, SiO₂, hexane:Et₂O 9.1), the band corresponding to the alcohol (Rf:0.23-0.42) was isolated and the pure compound (70 mg, 0.19 mmol) recovered by desorption with DCM (30 cm³) and solvent removal (Buchi, 40°C; N₂ blow down). This was assigned by GC, LRMS and IR.

GC purity : 95%

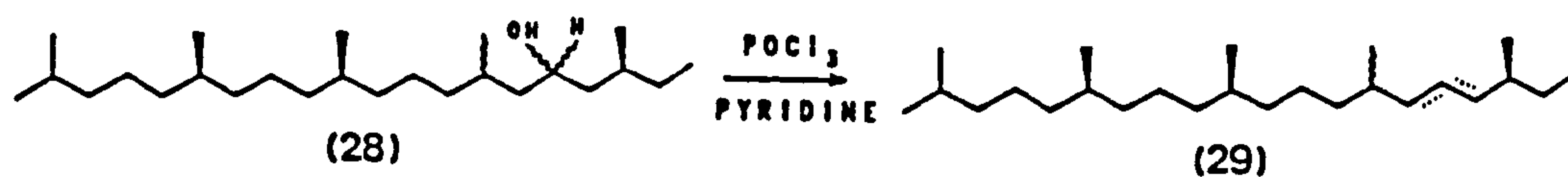
Yield : 29%

LRMS m/z : 369 ($M^+ + H$, 8%), 350 ($M^+ - H_2O$, 4%), 297 ($M^+ - C_5H_{11}$, 15%), 252 ($C_{18}H_{36}$, 10%), 173 (30%), 101 (42%), 83 (100%)

IR (liquid film) : $\nu(O-H)$ 3600 cm^{-1} (free), 3480 cm^{-1} (H-bonded)

δ_s CH_3 gem dimethyl 1380 cm^{-1} , 1370 cm^{-1}

2, 6R, 10R, 14RS, 18S-Pentamethyleicosenes (29):



The alcohol (28, 59 mg, 0.16 mmol) was dehydrated using $POCl_3$ (400 mm^3) in pyridine (800 mm^3). Extraction with hexane ($2 \times 10\text{ cm}^3$) provided the 2, 6R, 10R, 14RS, 18S-pentamethyleicosenes (29, 42 mg, 0.12 mmol) which were examined by GC and LRMS.

GC purity : 95%

Yield : 75%

LRMS m/z: 350 (M^+ , 12%), 280 ($M^+ - C_5H_{10}$, 1.6%), 266 ($M^+ - C_6H_{12}$, 3%), 210 (6%), 196 (6.4%), 140 (26%), 126 (35%), 57 (100%)

2, 6R, 10R, 14RS, 18S-Pentamethyleicosane (30):



Hydrogenation of the isomeric alkenes (29, 30 mg, 0.09 mmol) over Adam's catalyst (10 mg) in hexane (30 cm³) provided crude products (27 mg) which were purified by argentatious TLC (0.5 mm, SiO₂, 10% AgNO₃, hexane as developer). The band corresponding to the alkane (Rf: 0.87-0.99) was isolated and the purified product, 2, 6R, 10R, 14RS, 18S-pentamethyleicosane (30, 15 mg, 0.04 mmol) recovered by desorption with DCM (30 cm³) and solvent removal (Buchi, 40°C).

GC purity : 97%

KI(DB-5) : 2239

Yield : 44%

LRMS m/z : 352 (M⁺·, 0.4%), 338 (M⁺·-CH₂, 1.4%), 324 (1.5%),
 268 (48%), 253 (58%), 197 (12%), 183 (17%),
 113 (46%), 57 (100%)

CHAPTER THREE

STRUCTURAL CHARACTERISATION OF ALIPHATIC AND AROMATIC HYDROCARBON UCMS FROM LUBRICATING OILS

STRUCTURAL CHARACTERISATION OF ALIPHATIC AND AROMATIC HYDROCARBON UCMs FROM LUBRICATING OILS

3.1 THE ALIPHATIC UCM

3.1.1 GENERAL

The isolation of the aliphatic UCM from the Silkolene 150 solvent neutral lubricating oil followed the procedures of Douglas *et al.*, 1981, and Jones, 1986. Briefly this involved open column adsorption chromatography on deactivated silica and alumina, followed by argentatious TLC of the hexane eluate to remove residual monoaromatic hydrocarbons. The total alkanes were then adducted three times with urea to remove normal and simply branched (e.g. monomethyl) alkanes. The gravimetric data for these procedures (Table 3.1) showed the large contribution of the alkane fraction to the total oil, and this was confirmed by the GC profiles (Fig. 3.1) which were virtually indistinguishable. The high proportion of saturated hydrocarbons (greater than 80%), is consistent with the known refining history of the oil (solvent extraction to remove aromatic and polar compounds; IARC, 1984).

3.1.2. THE UREA ADDUCT

The low proportion of urea adducted compounds (<10%) is again consistent with the history of the refined lubricating base oil, where normal alkanes were removed by solvent dewaxing to improve the flow of oil under cold conditions (Klamann *et al.*, 1984). This is exemplified by the gas chromatogram of the urea adduct (Fig. 3.2) in which the normal alkanes (identified by coinjection and GC-MS) are

TABLE 3.1

GRAVIMETRIC DETERMINATIONS

A Column chromatography of Silkolene 150 solvent neutral lubricating oil

Sample, no.	mass oil(g)	Mass Recovered [g(%)]			
		aliphatics	aromatics	polars	recovery (%)
1	2.0259	1.823 (90)	0.186 (9)	0.016 (1)	100
2	2.0073	1.818 (91)	0.165 (8)	0.012 (1)	100
3	2.0094	1.761 (88)	0.190 (10)	0.017 (1)	99
4	2.0095	1.872 (93)	0.127 (6)	0.010 (1)	100
Mean (%)		91 _{±2}	8 _{±1}	1 _{±0}	100 _{±1}

B Fractionation of the total aliphatic hydrocarbons by argentatious TLC

Sample no.	mass applied(mg)	Mass recovered [mg(%)]		
		alkanes	monoaromatics	recovery(%)
1	27.3	21.4 (78)	2.0 (7)	86
2	27.5	20.9 (76)	1.5 (6)	82
3	28.2	23.1 (82)	1.5 (5)	87
4	29.4	23.6 (80)	1.4 (5)	85
Mean (%)		80 _{±3}	6 _{±1}	85 _{±2}

C Urea adduction of the total alkane fractions

Sample no.	mass adducted(mg)	mass adduct		recovery (%)
		[mg(%)]	mass non adduct [mg(%)]	
1	44.2	2.1 (5)	38.4 (87)	92
2	28.6	1.6 (6)	26.3 (92)	98
3	43.9	2.1 (5)	41.6 (95)	100
4	29.9	1.8 (6)	27.5 (92)	98
Mean (%)		5 _{±1}	91 _{±3}	97 _{±4}

FIG. 3.1 GAS CHROMATOGRAMS OF A) TOTAL OIL, B) TOTAL ALIPHATICS AND C) TOTAL ALKANES; SILKOLENE 150 LUBRICATING BASE OIL
[GC:DB-5(J+W), 50-180°C @ 10°C min⁻¹, 180-300°C @ 5°C min⁻¹, 300°C(20min)]

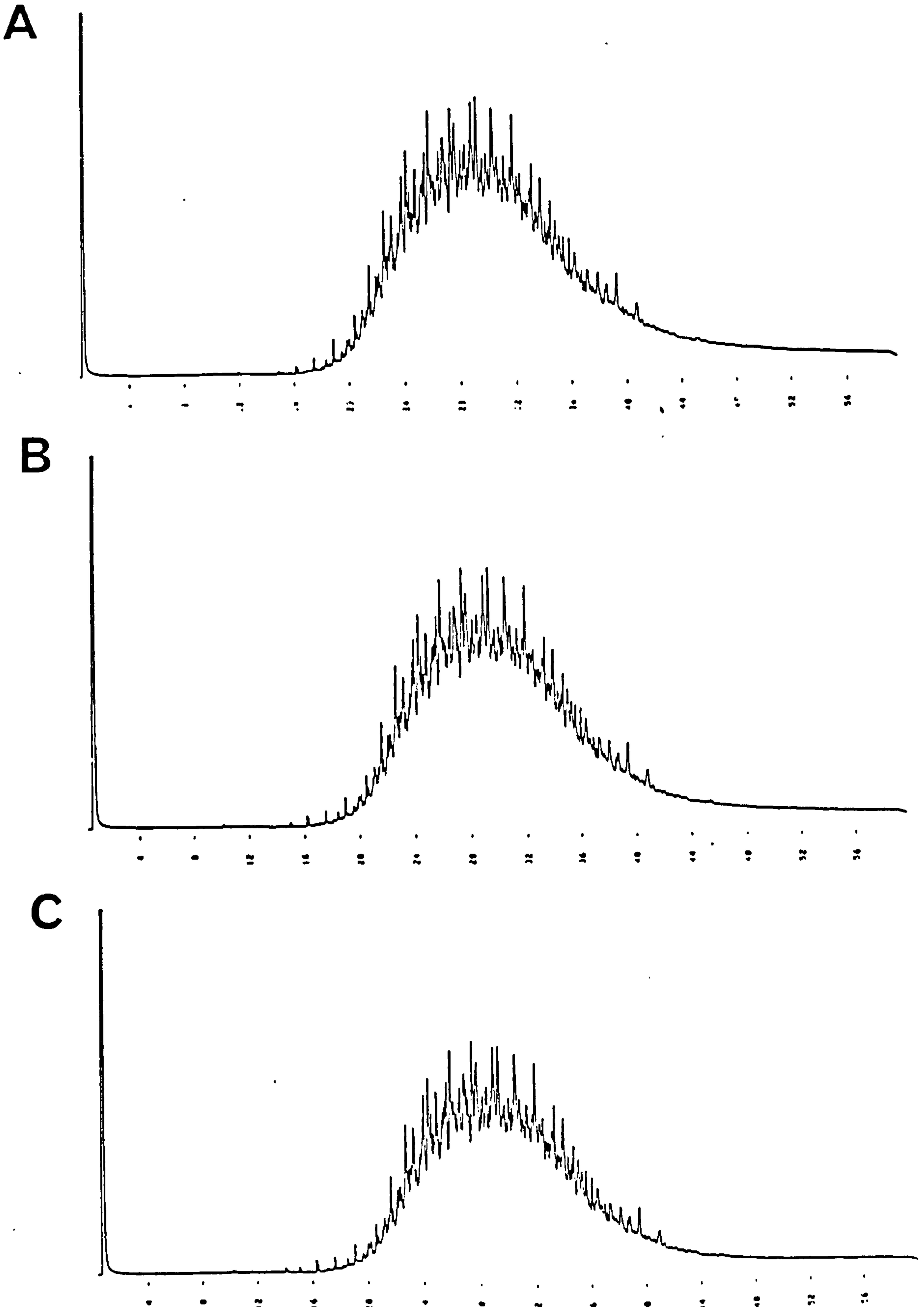
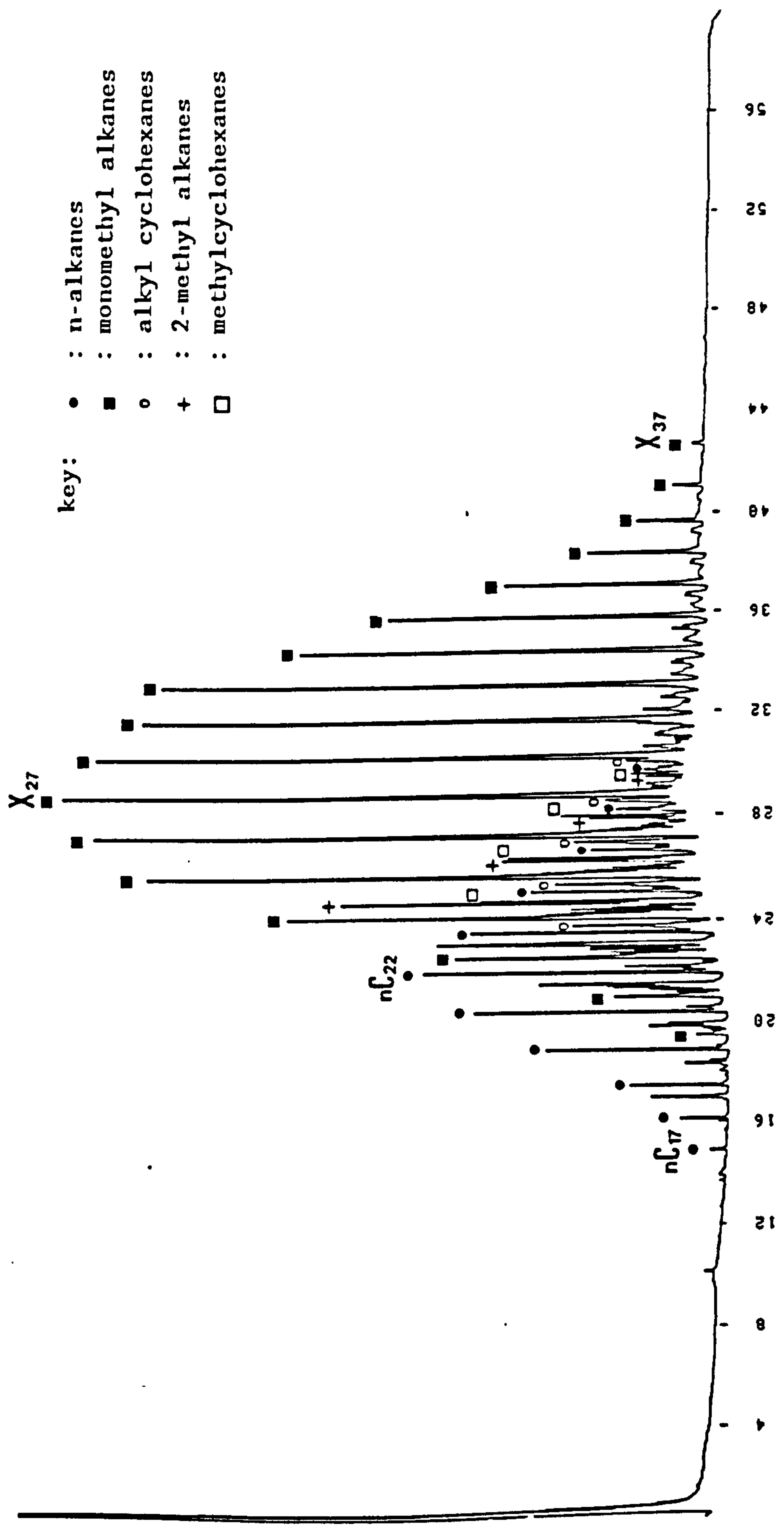


FIG. 3.2 GAS CHROMATOGRAM OF THE UREA ADDUCTED ALKANES,
 SILKOLENE 150 LUBRICATING OIL ALIPHATIC FRACTION
 [GC:DB-5(J+W), 50-180°C @ 10°C min⁻¹,
 180-300°C @ 5°C min⁻¹, 300°C(20min)]



minor components in the range n -C17 to n -C35, maximising at n -C22. A second homologous series of compounds of major abundance was identified and characterised by chemical ionisation (CI) and electron impact (EI) GC-MS, and by coinjection of a representative synthesised model compound (section 4.12). CI GC-MS (Fig. 3.3) revealed the carbon number distribution to be C21-C37, maximising at C27, and each compound in the homologous series was found to elute before the n -alkane of corresponding molecular weight, a feature which is characteristic of acyclic alkyl branched alkanes (Klomp, 1986). Molecular ions were absent in the EI mass spectra, a further indication of their branched nature. In addition each spectrum was found to comprise a pronounced series of even numbered fragment ions (C_nH_{2n}) in equal or greater abundance to the corresponding odd fragment ions (C_nH_{2n+1}). This feature is also typical of branched alkanes, and results from fragmentation of the molecule at the branch point (Han *et al.*, 1968; Klomp, 1986). The large number of even mass fragments in their spectra suggests that each peak contains a number of coeluting compounds, presumably isomeric monomethyl branched alkanes since these are known to form adducts with urea (Fowler, 1984; Klomp, 1986). For example, assuming that an even ion is produced from each branch point, the EI spectrum of the C25 compound would be consistent for a mixture of 9- to 12-methyltetracosanes (Fig. 3.4). Similarly, by analysis of the EI spectrum for the C26 compound, a mixture of 9- to 13-methylpentacosanes can be proposed (Fig. 3.5). The distribution of these isomers was clearly shown by mass fragmentography using these characteristic even mass ions (Fig. 3.4 and 3.5). The observed decrease in retention time as the methyl group is moved to the centre of the molecule is consistent with the

FIG. 3.3 CHEMICAL IONISATION(CH₄) MASS SPECTRA OF MONOMETHYL ALKANES IDENTIFIED IN THE UREA ADDUCT FRACTION, SILKOLENE 150 LUBRICATING OIL ALKANES: A) C₂₄, B) C₂₅, C) C₂₆.

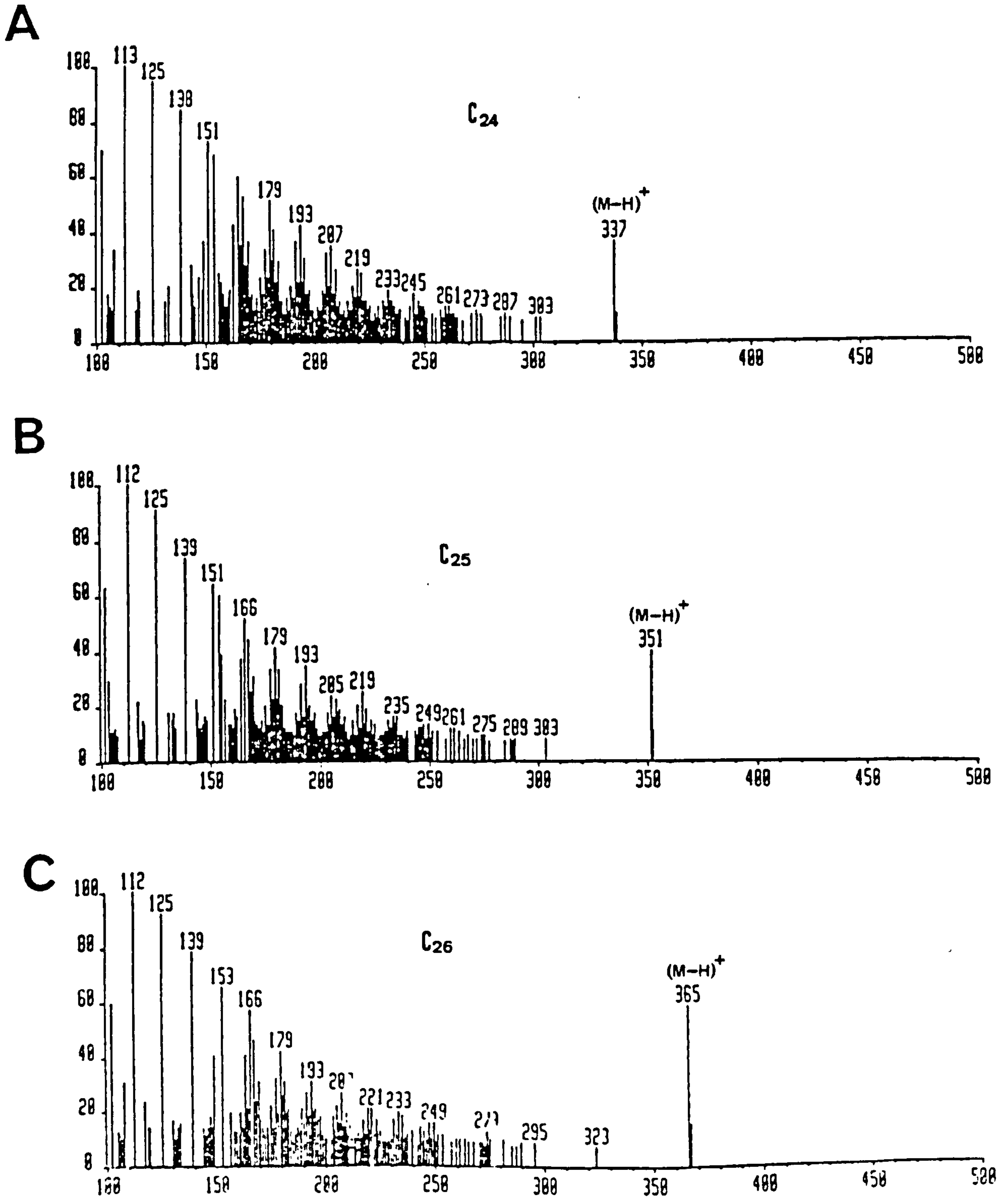


FIG. 3.4 EI MASS SPECTRUM OF A C25 MONOMETHYL ALKANE (X25) IDENTIFIED IN THE UREA ADDUCT FRACTION, SILKOLENE 150 LUBRICATING OIL ALKANES: A) TOTAL SPECTRUM, B) PARTIAL SPECTRUM (m/z 100-300), C) GC-MS MASS FRAGMENTOGRAM OF EVEN IONS

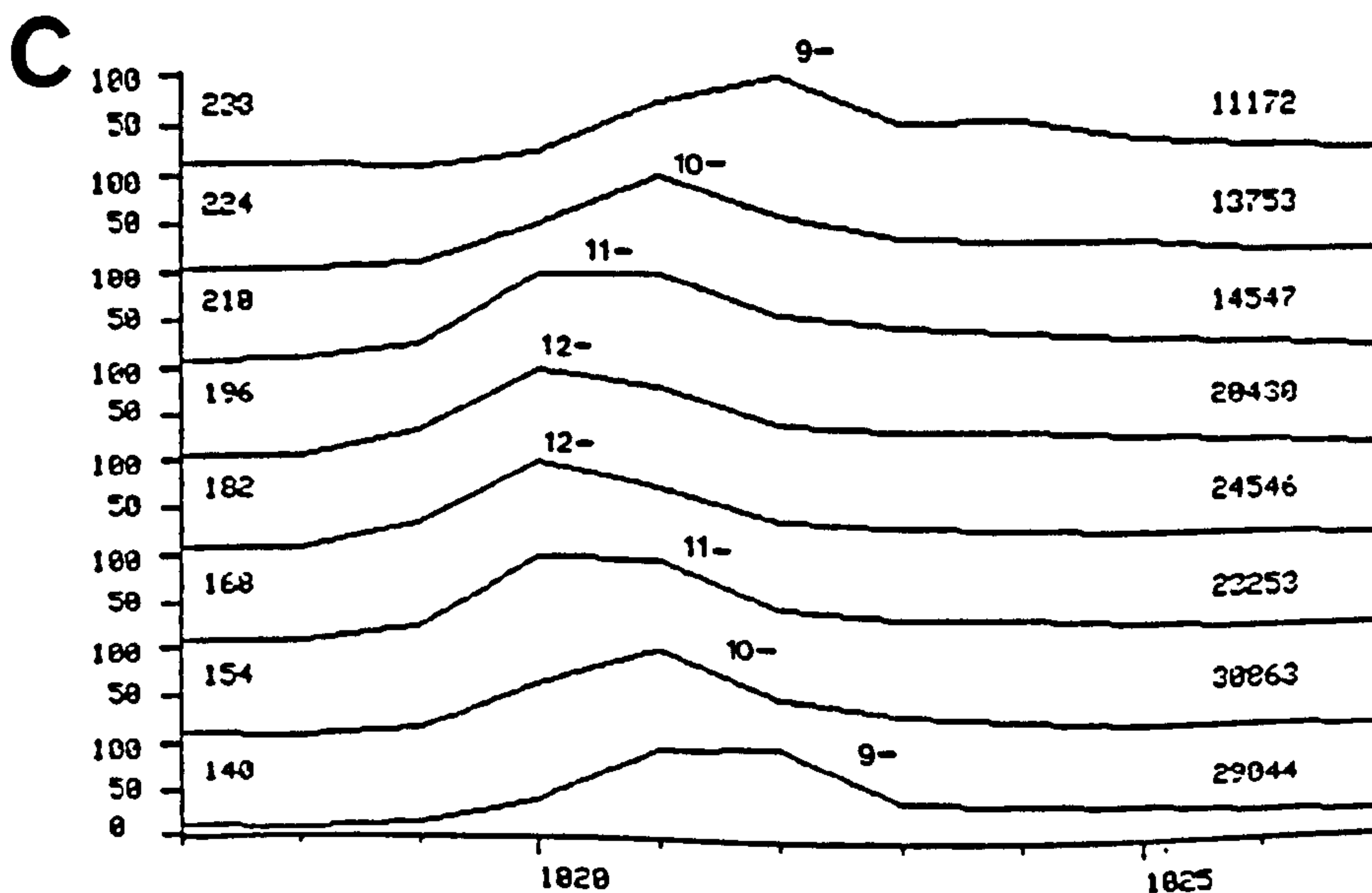
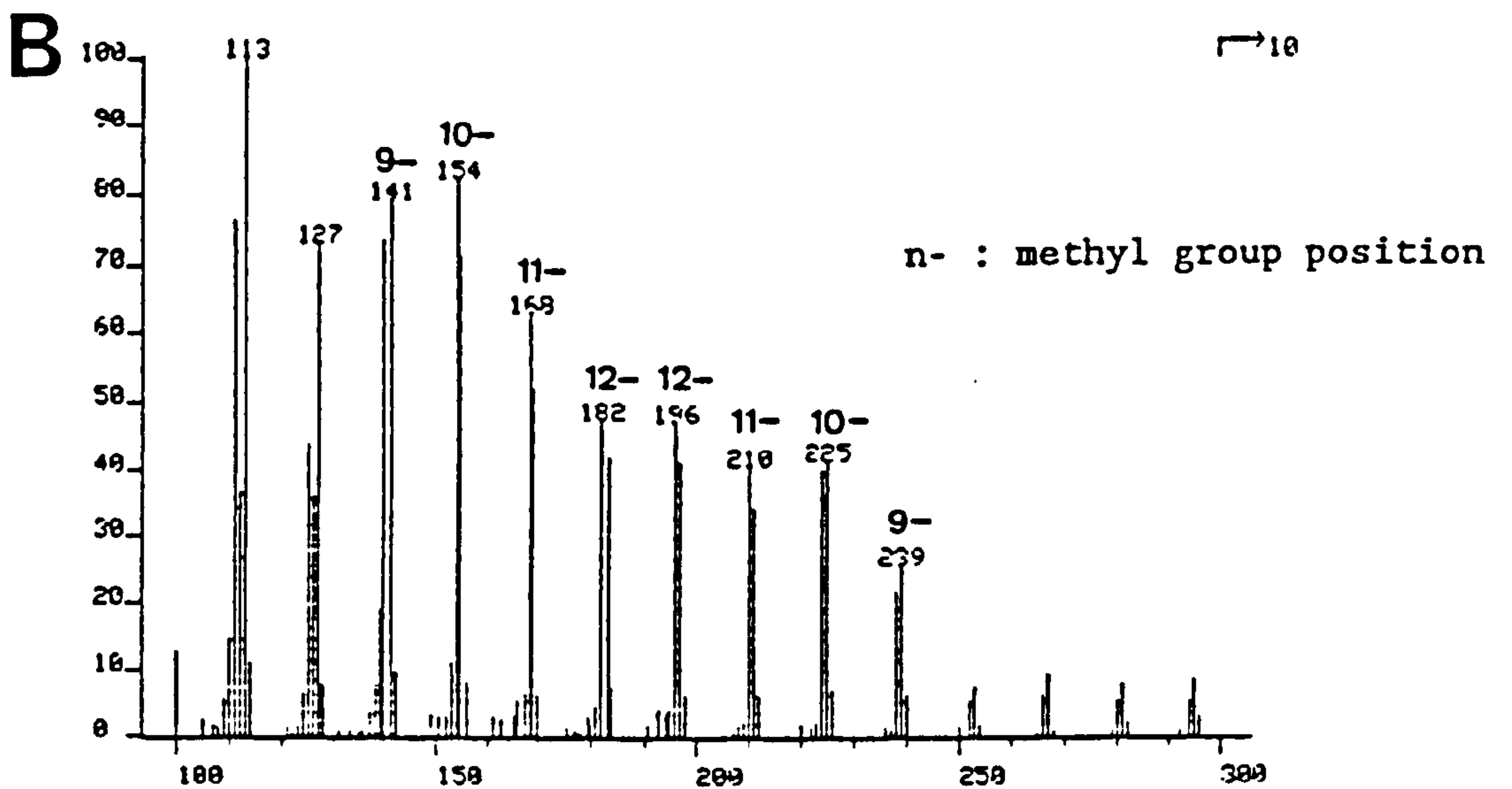
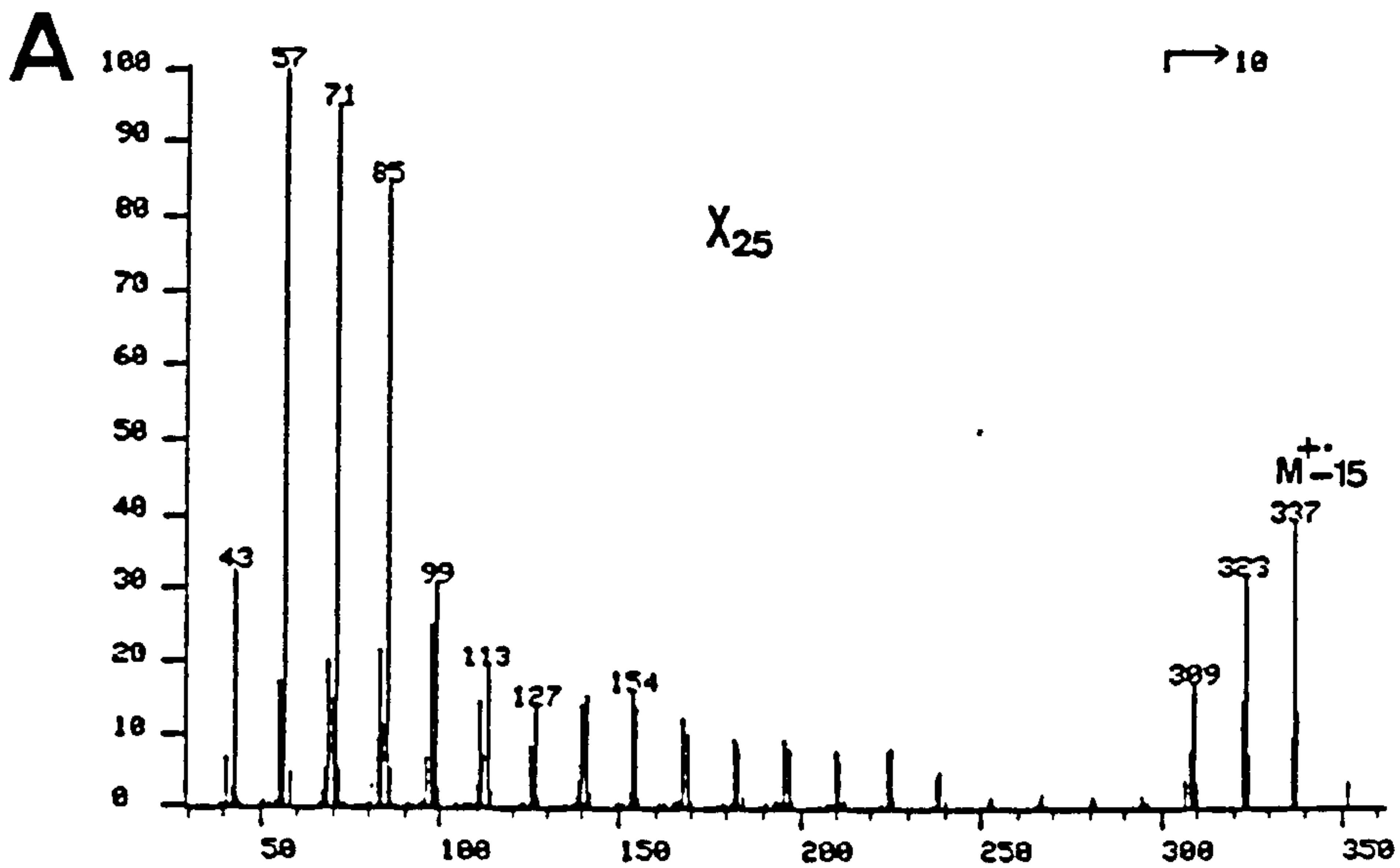
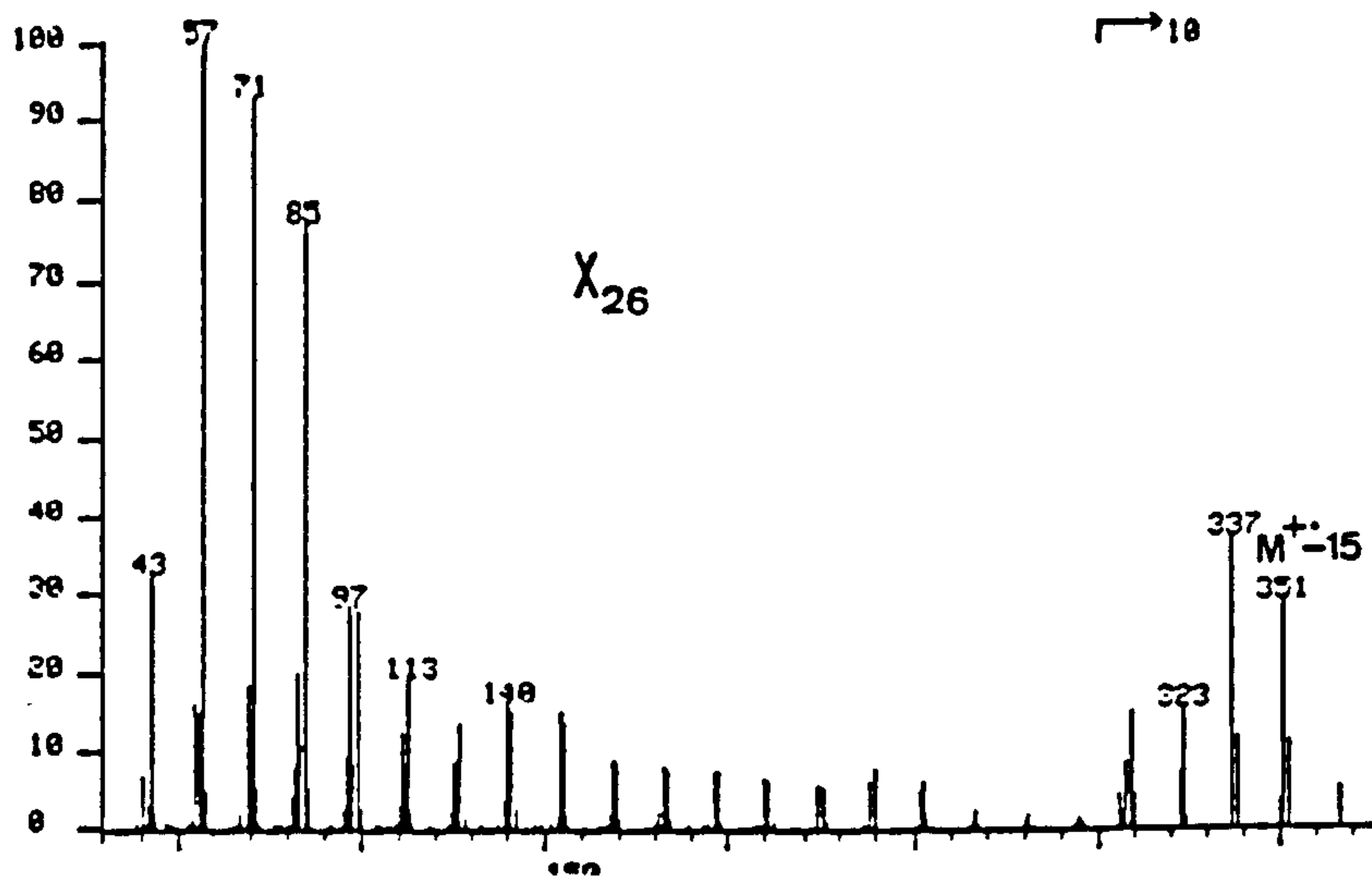
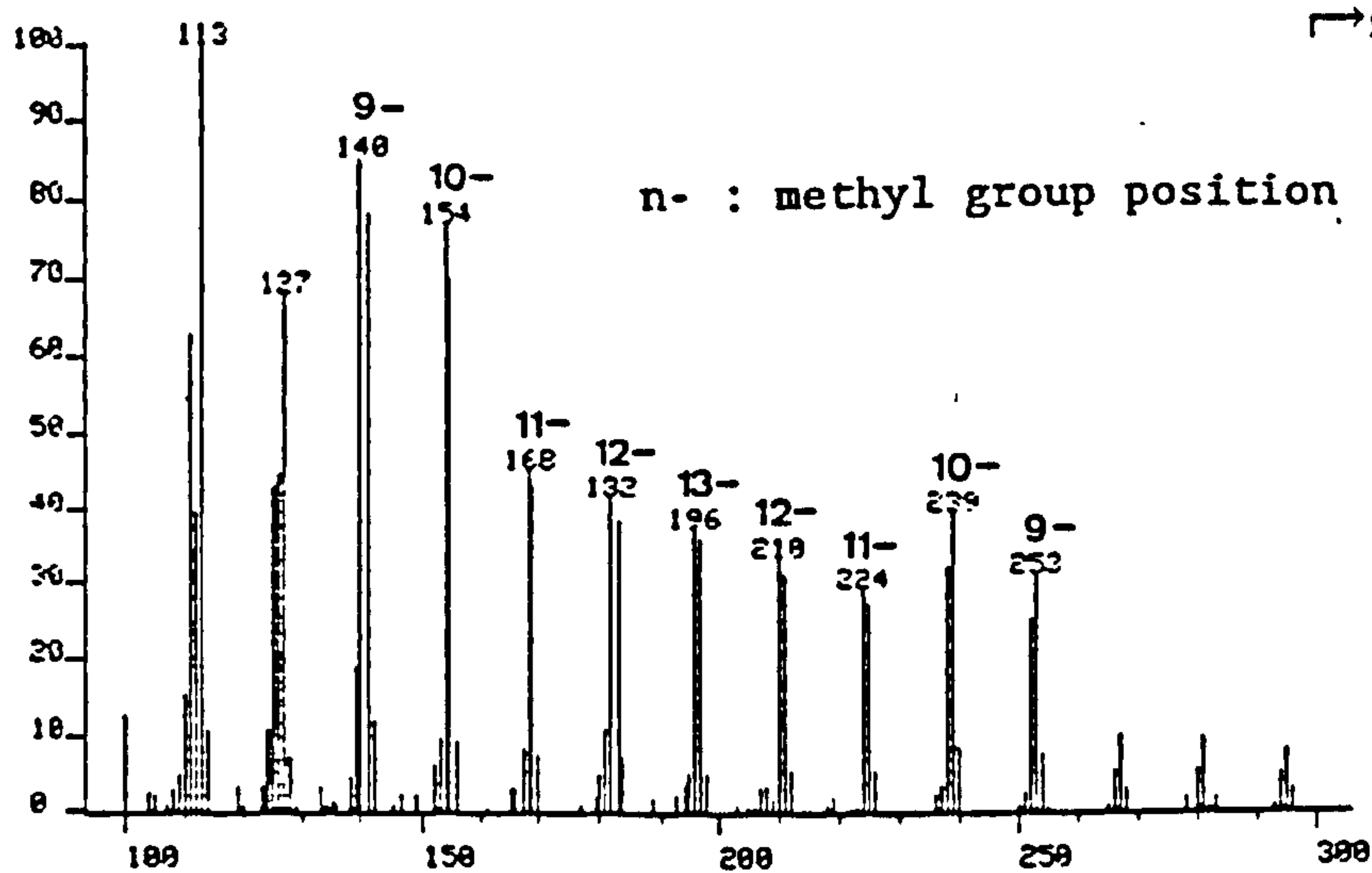


FIG. 3.5 EI MASS SPECTRUM OF A C₂₆ MONOMETHYL ALKANE (X₂₆) IDENTIFIED IN THE UREA ADDUCT FRACTION, SILKOLENE 150 LUBRICATING OIL ALKANES: A) TOTAL SPECTRUM, B) PARTIAL SPECTRUM (m/z 100-300), C) GC-MS MASS FRAGMENTOGRAM OF EVEN IONS

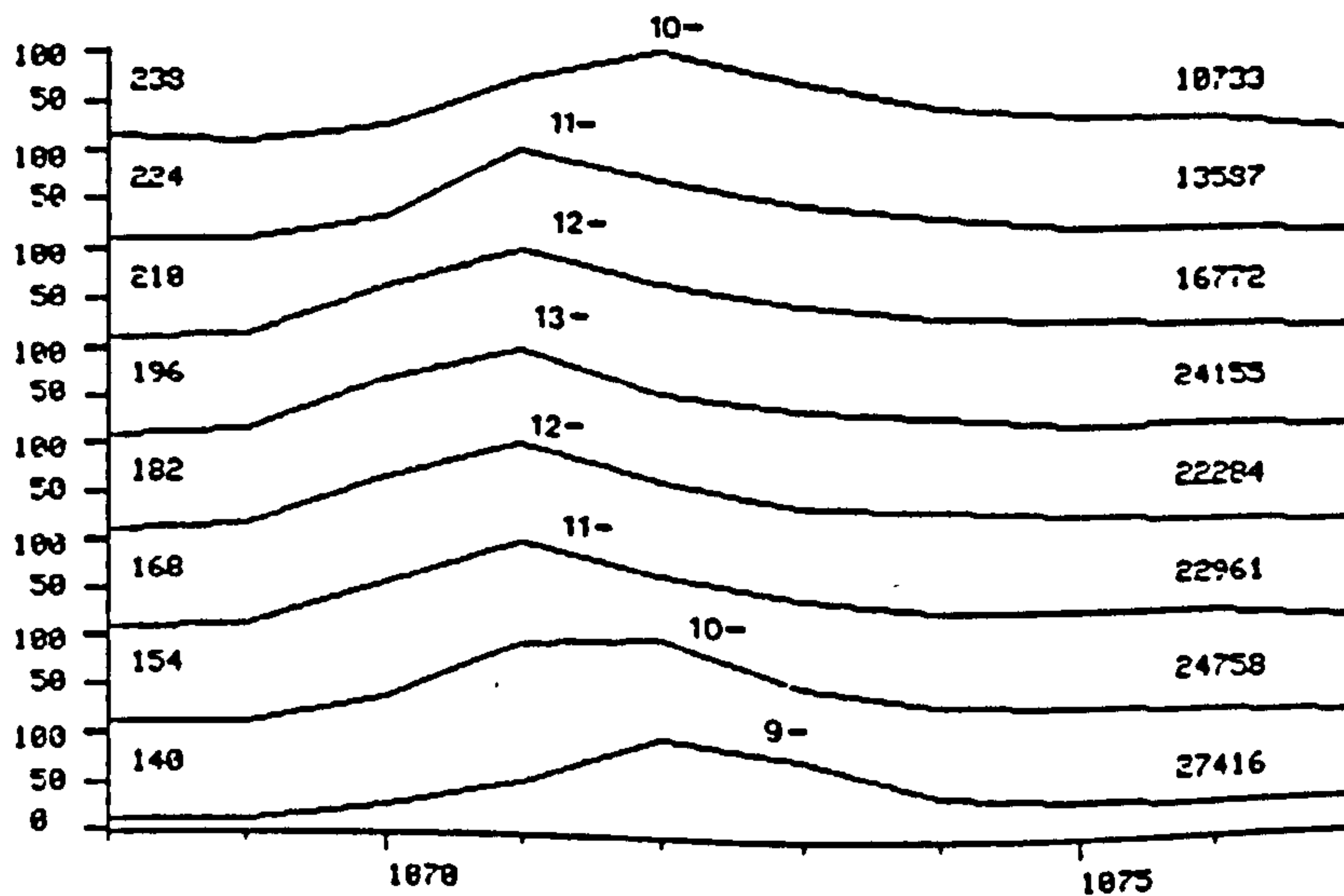
A



B



C



known elution order of monomethyl alkanes on apolar stationary phases (Hoering, 1981; Klomp, 1986; Kissin and Feulner, 1986). Further confirmation of the proposed nature of these compounds was provided by coinjection of authentic 9-methyltetracosane synthesised in this study (section 4.1.2.).

Similar coeluting mixtures of monomethyl branched alkanes have been observed in the urea adducts of Russian Precambrian oils (Fowler, 1984) and in South Oman crudes (Klomp, 1986). More recently Kissin and Feulner (1987), provided retention indices for these compounds derived from ethylene co-oligomerisation and hydrogenation reactions, and applied these to the identification of monomethyl alkanes in a number of crude oils (Kissin, 1987). The retention indices of the C23- C27 compounds identified in this study compare well with these published values (Table 3.2). Other components of the urea adduct tentatively identified by GC-MS include homologous series of 2-methyl alkanes, alkylcyclohexanes, and a series of unknown components which displayed abundant m/z 82 ions in their mass spectra (Fig. 3.6^b). These latter compounds appear to be alkylcyclohexanes (or possibly methylcyclopentanes) and await a more rigorous characterisation.

3.1.3. THE UREA NON ADDUCT (UNA) BY CONVENTIONAL TECHNIQUES

3.1.3.1. GAS CHROMATOGRAPHY / GAS CHROMATOGRAPHY - ELECTRON IMPACT MASS SPECTROMETRY (GC/GC-EIMS)

Urea did not adduct the bulk of the alkane fraction from the Silkolene 150 lubricating oil (>90%, Table 3.1), and when analysed by GC (Fig. 3.7) the UNA displayed a broad unresolved complex mixture

TABLE 3.2

A COMPARISON OF RETENTION INDICES FOR THE COELUTING SERIES OF MONOMETHYL ALKANES IDENTIFIED IN THE SILKOLENE 150 LUBRICATING OIL ALKANE UREA ADDUCT FRACTION

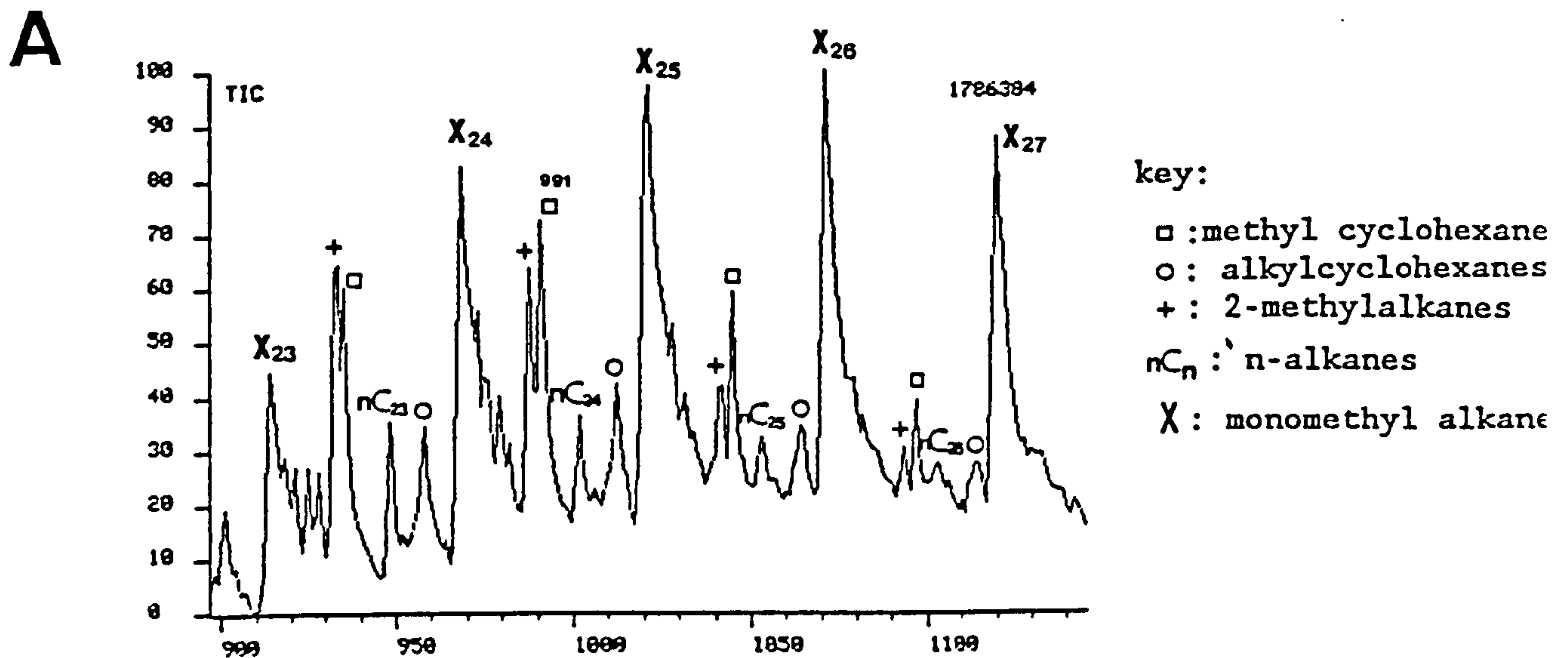
COMPOUND	THIS STUDY ¹	FOWLER, 1984 ²	KISSIN, 1986 ³
X23	2236	2245	2238
X24	2337	-	2337
X25	2437	-	2435
X26	2536	-	2534
X27	2637	-	2634

1 : DB-5, J+W, 30m, 50-300°C @ 5°C min⁻¹

2 : OV-101, 25m, isothermal (170°C)

3 : methylsilicone (OV-1), 50m, 40-300°C @ 5°C min⁻¹

FIG. 3.6 A) PARTIAL TOTAL ION CHROMATOGRAM OF THE UREA ADDUCTED ALKANES, SILKOLENE 150 LUBRICATING BASE OIL



B) EI MASS SPECTRUM OF AN UNIDENTIFIED C₂₃ ALKYL CYCLOHEXANE

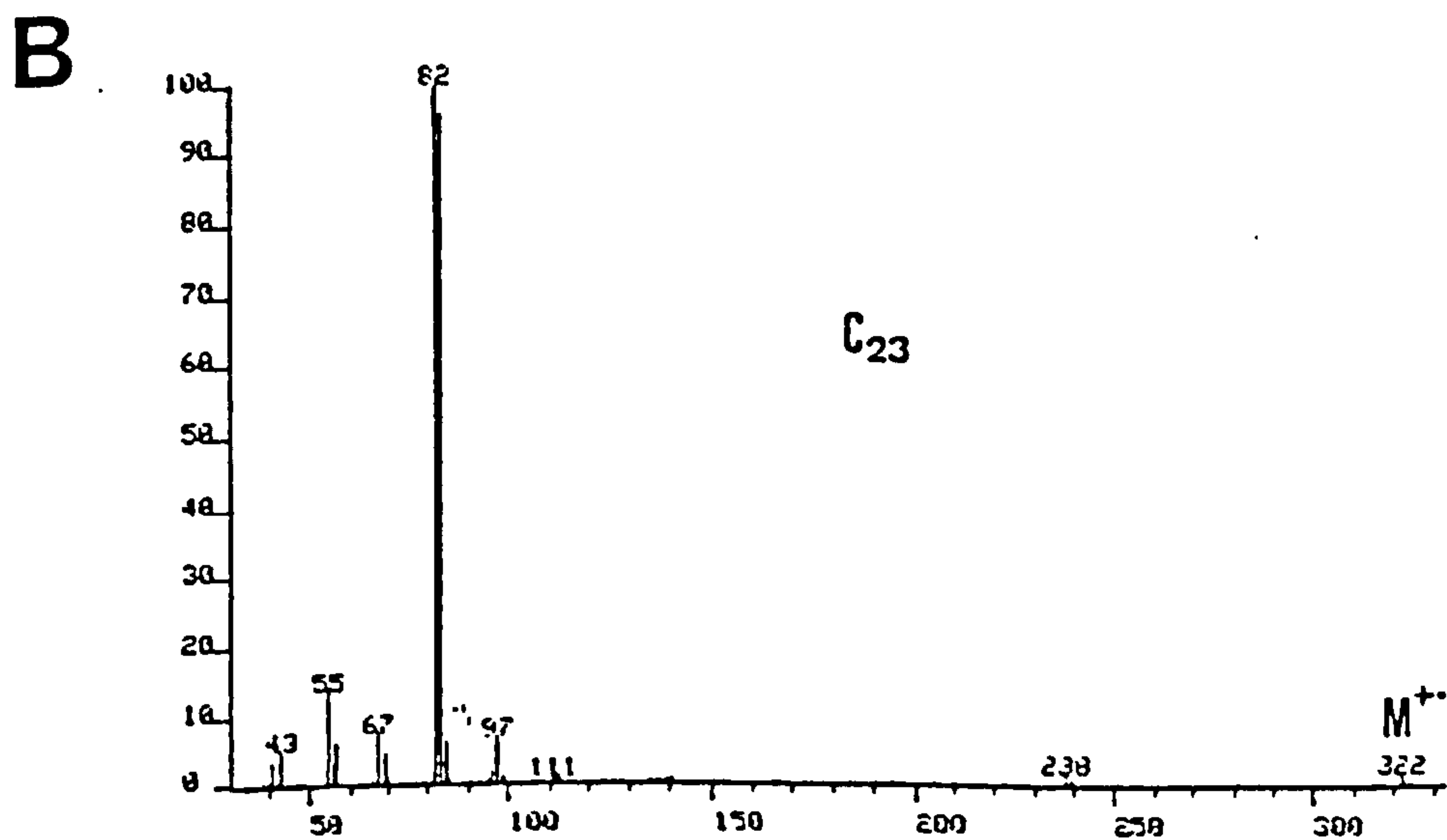
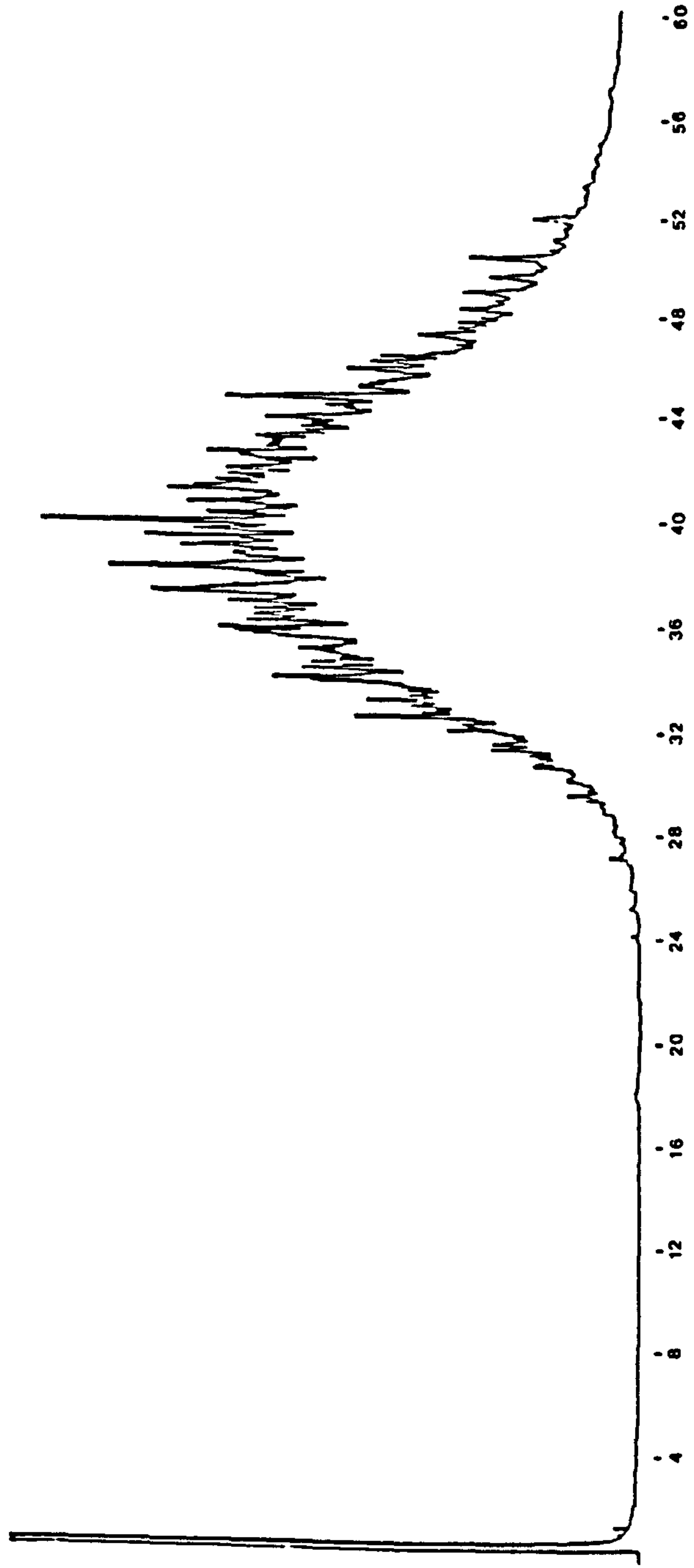


FIG. 3.7 GAS CHROMATOGRAM OF THE UREA NON ADDUCTED ALKANES (THE ALIPHATIC UCM), SIIKOLENE 150 LUBRICATING BASE OIL

[GC: DB-5(J+W), 50-300 °C @ 5 °Cmin⁻¹, 300 °C (10 MIN)]



(UCM) ranging from KI 1850 - 3600+, maximising at KI 2615. Superimposed on the UCM profile was a series of partially resolved components whose relative abundance (as monitored by the time slice area measurement, appendix I) represented less than 10% of the total FID response. The resolved monomethyl alkanes in the total alkane fraction (Fig. 3.1c) were reduced in the UNA, which is consistent with their observed enrichment in the urea adduct.

GC-MS mass fragmentography revealed the distributions of commonly occurring "biological marker" compounds (e.g. Philp, 1985). The m/z 183 fragment ion series (characteristic of acyclic isoprenoids) was found to coincide with the major resolved components superimposed on the UCM profile, and ranged from KI 2125 to >3365, maximising at 2659 (Fig. 3.8). The mass spectra of these components displayed prominent ion series characteristic of the branch positions of regular acyclic isoprenoids (i.e. $113 + 70N$; N = No. isoprenoid moieties; Albaiges *et al.*, 1981); and extrapolation of their scan numbers with previously determined KI values (GC-coinjection) allowed their identification with published KI values.

Thus the spectrum of compound 2 (Fig. 3.8) was found to contain ions m/z 113, 253, 323, 337 ($M^+ - 15$) characteristic of the C25 regular acyclic isoprenoid (Fig. 3.9a), and CI-MS (section 3.1.3.5., Fig. 3.9b) provided the expected quasi-molecular $(M-H)^+$ ion, m/z 351. The extrapolated KI value of 2232 provided additional evidence for a C25 regular isoprenoid since the synthetic alkane 2,6,10,14,18-pentamethyleicosane (section 4.17) was found to have a KI value of 2239. Having made this identification, the published retention index increments for regular acyclic isoprenoids were used to obtain their

FIG. 3.8 M/Z 183 MASS FRAGMENTOGRAM SHOWING THE SERIES OF
 REGULAR ACYCLIC ISOPRENOID ALKANES OBSERVED AS RESOLVED
 COMPONENTS OF THE UREA NON ADDUCT, SILKOLENE 150 LUBRICATING
 OIL ALKANES

(for peak identity refer to Table 3.3)

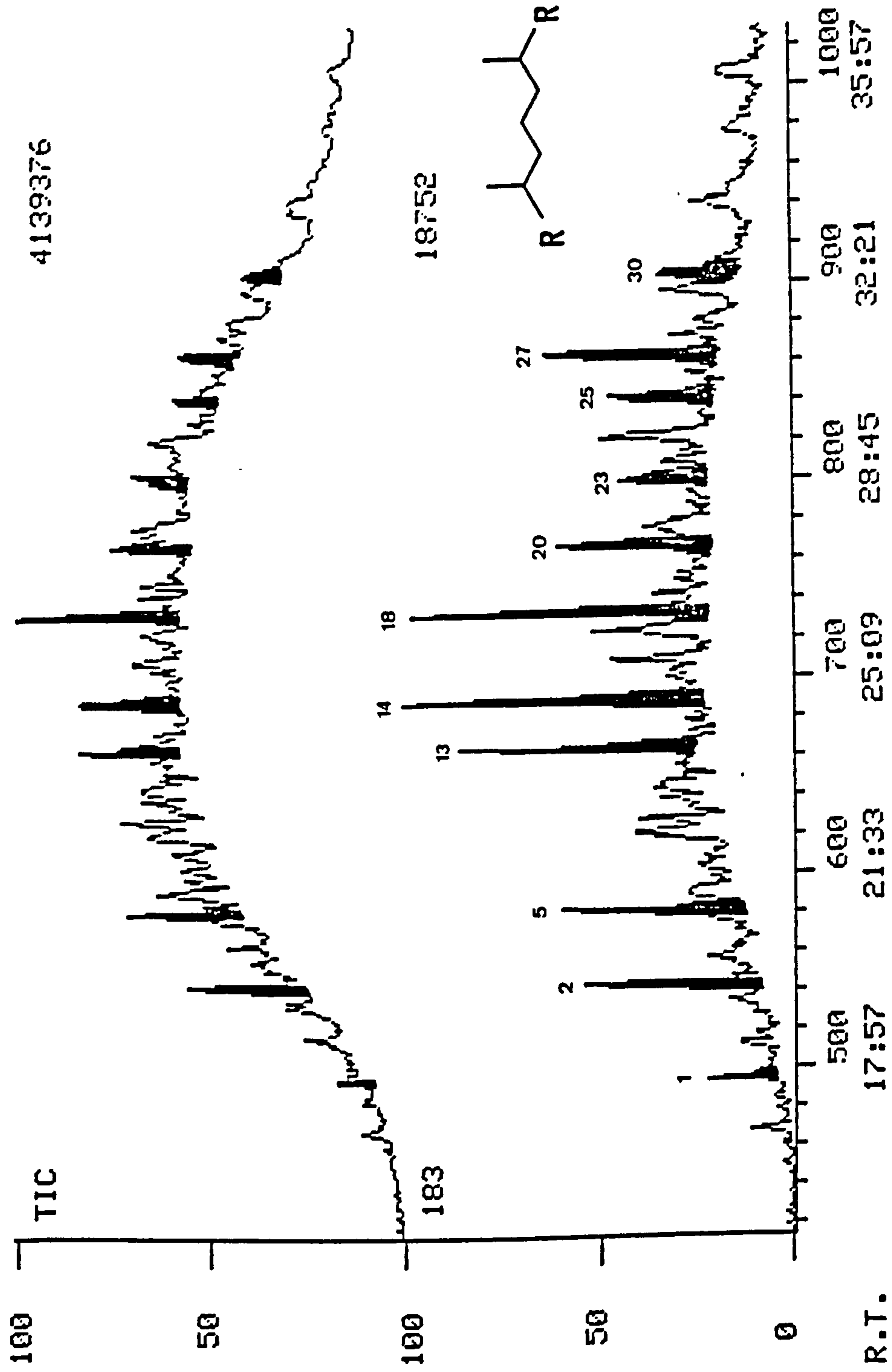
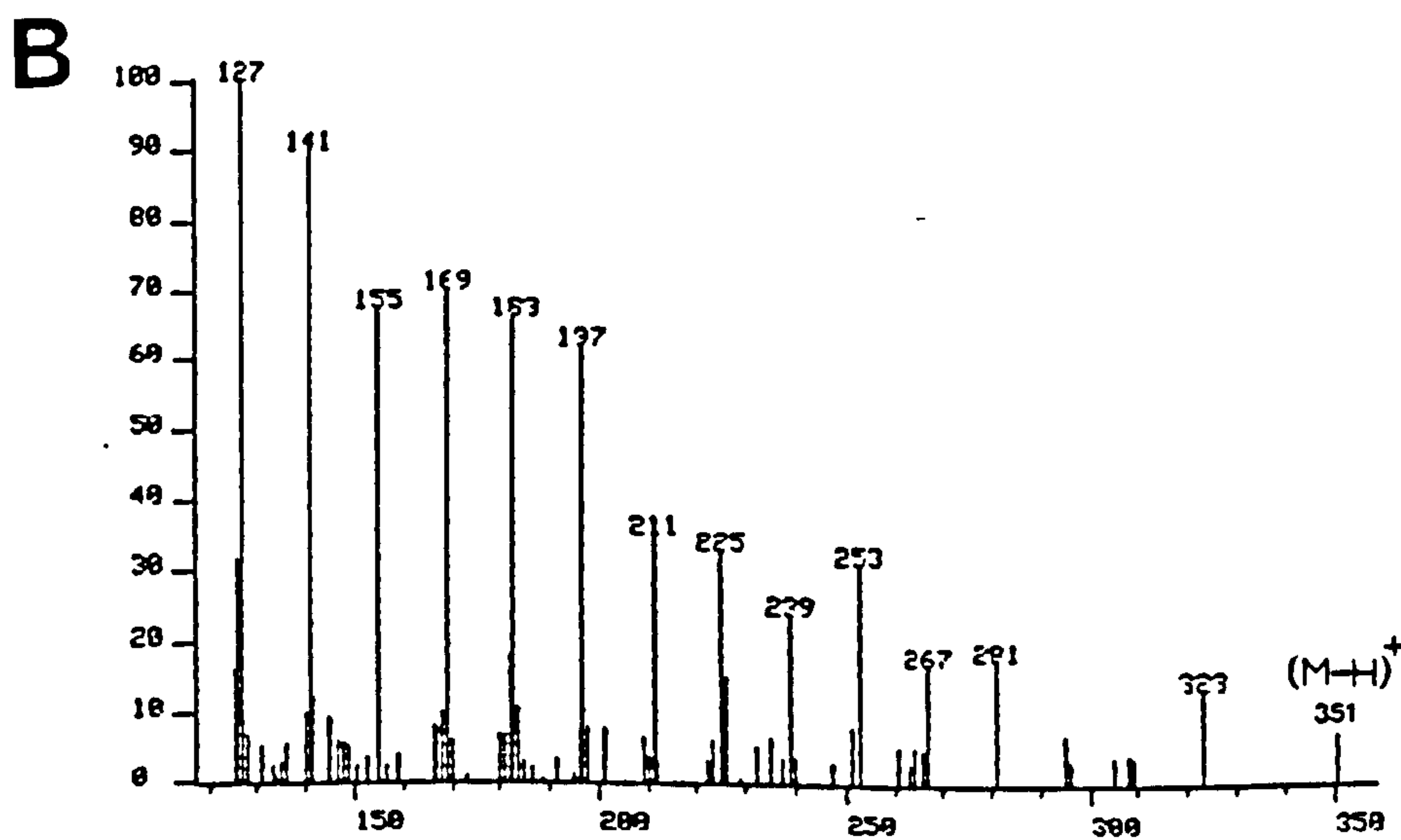
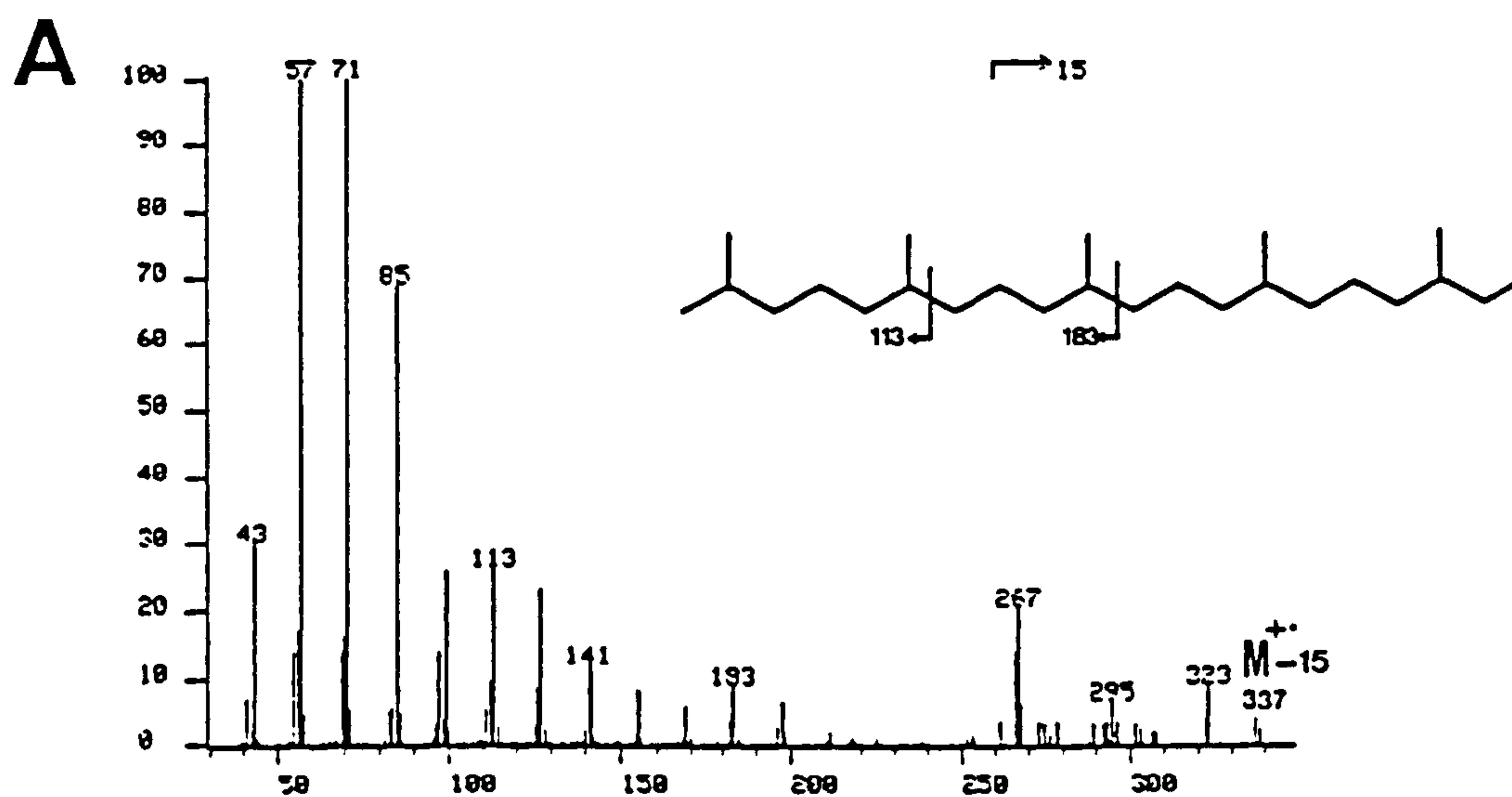


FIG. 3.9 A) EI AND B) CI (ISOBUTANE) MASS SPECTRA OF A C₂₅ REGULAR ACYCLIC ISOPRENOID ALKANE (COMPOUND 3, FIG. 3.8); UREA NON ADDUCT, SILKOLENE 150 LUBRICATING BASE OIL ALKANES.



predicted KI values, (Table 3.3; Albaiges *et al.*, 1981), and these were found to vary from those determined experimentally by only ca. ± 3 KI units. The mass spectrum of each component (e.g. Fig. 3.10) confirmed their proposed identities as a series of regular acyclic isoprenoids ranging from C24- C35.

Other fragment ion series monitored by GC-MS included those characteristic of acyclic alkanes (m/z 85), alkylcyclohexanes (m/z 83), alkylmethylcyclohexanes (m/z 97), alkyl decalins (m/z 137), pentacyclic triterpanes (m/z 191) and steranes (m/z 217). The m/z 85 mass fragmentogram characteristic of acyclic alkanes highlighted their distribution in the UNA, and the major series of resolved peaks identified coincided with the positions determined for the acyclic isoprenoids in the m/z 183 mass fragmentogram. A broad UCM was observed throughout the elution range of the UNA suggesting that acyclic alkanes (or possibly alkylbranched cyclic alkanes with the branch ≥ 6) contribute to the unresolved GC profile (Fig. 3.11a). The m/z 83 mass fragmentogram (Fig. 3.11b) also displayed a broad UCM underlying a repeating series of resolved peaks which were found to correspond to C19 - C26 alkylcyclohexanes. A closer examination showed that these coincided with a series of resolved compounds on top of the UCM, though in lower abundance to and only partially resolved from the series of acyclic isoprenoid alkanes. Likewise a homology of resolved compounds displaying prominent m/z 97 ions were identified in the UNA, and these presumably represent alkylmethylcyclohexanes (Fig. 3.11c). Their EI mass spectra however did not display molecular ions. Again these components were found to be superimposed on a broad UCM profile, and to contribute to the resolved peaks observed by GC analysis.

TABLE 3.3

EXPERIMENTAL AND PREDICTED KOVATS RETENTION INDICES (KI) FOR THE REGULAR ACYCLIC ISOPRENOIDS IDENTIFIED AS RESOLVED COMPONENTS OF THE UREA NON ADDUCT

COMPOUND NO.	KI(FOUND)	KI(PREDICTED)	δ KI ^a	CARBON NO.
1	2125	2129	+4	24
2	2232	-	-	25
5	2314	2317	+3	26
13	2500	2497	-3	28
14	2553	2550	-3	29
18	2659	2654	-5	30
20	2741	2738	-3	31
23	2822	2826	+4	32
25	2921	2918	-3	33
27	2974	2970	-4	34
30	3071	3073	+2	35

^a δ KI = KI(predicted) - KI(found)

FIG. 3.10 EI MASS SPECTRA OF A) A C29 REGULAR ACYCLIC ISOPRENOID ALKANE (COMPOUND 14, FIG. 3.8); AND B) A C30 REGULAR ACYCLIC ISOPRENOID ALKANE (COMPOUND 18, FIG.3.8).

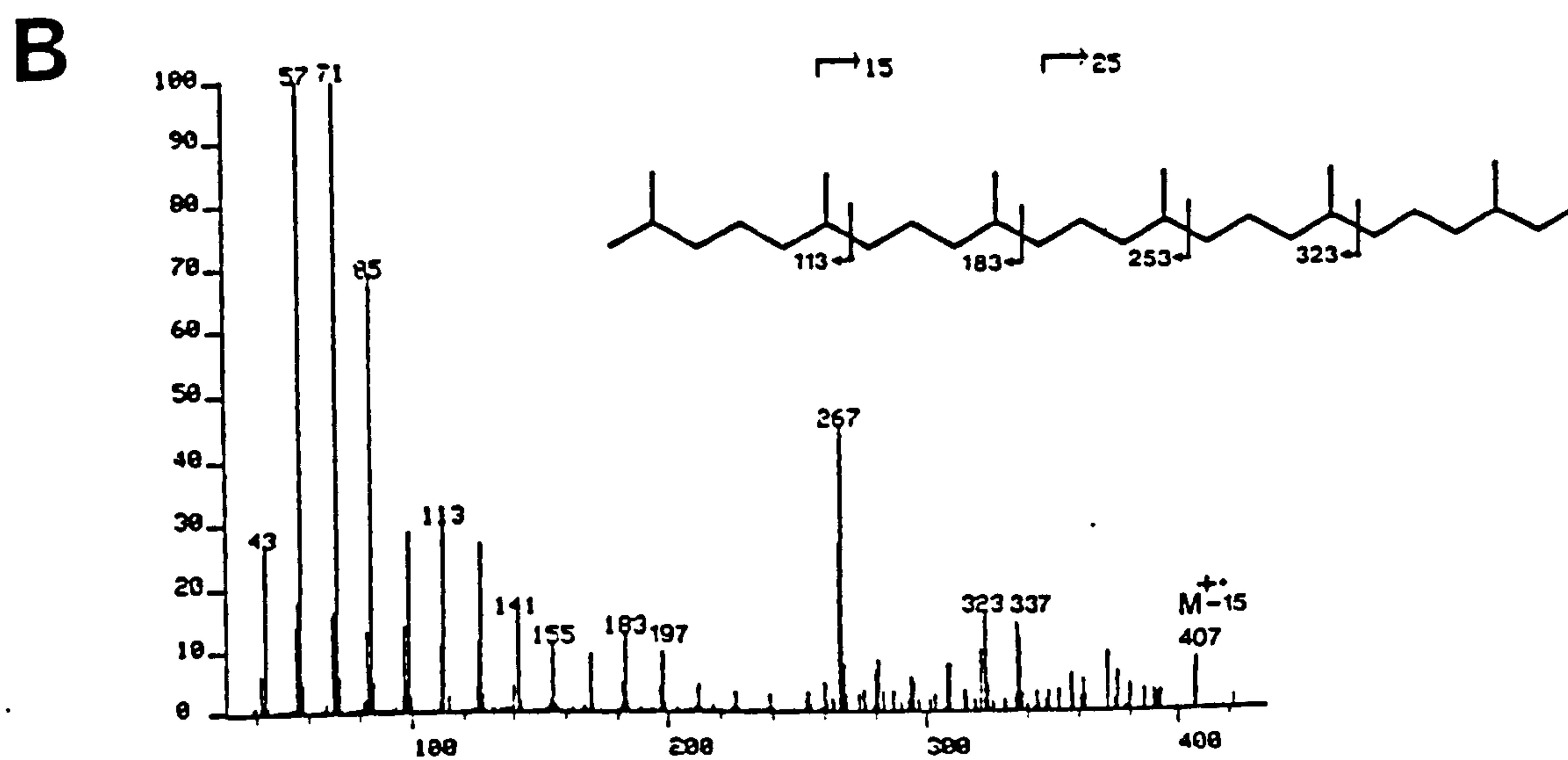
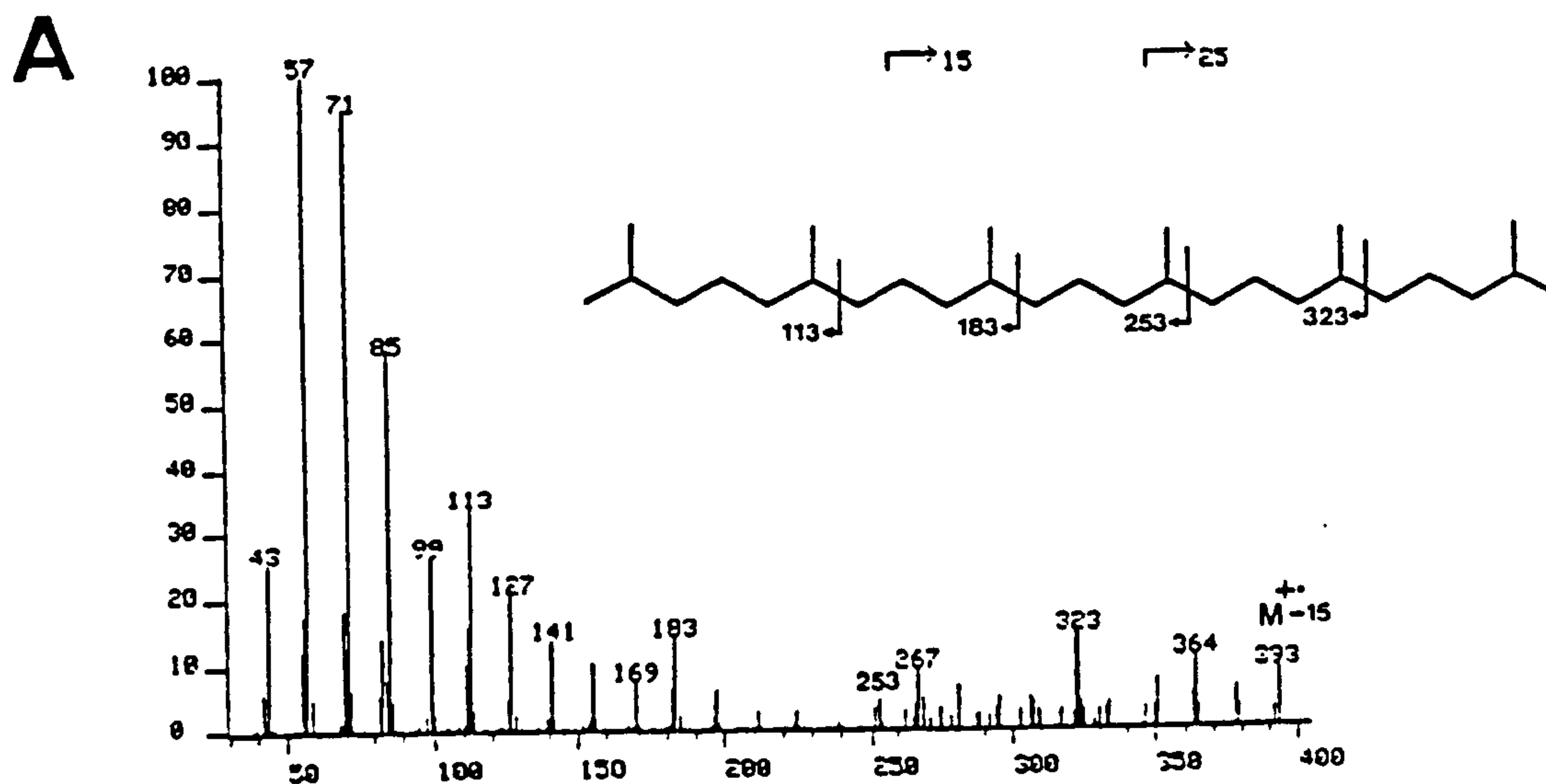
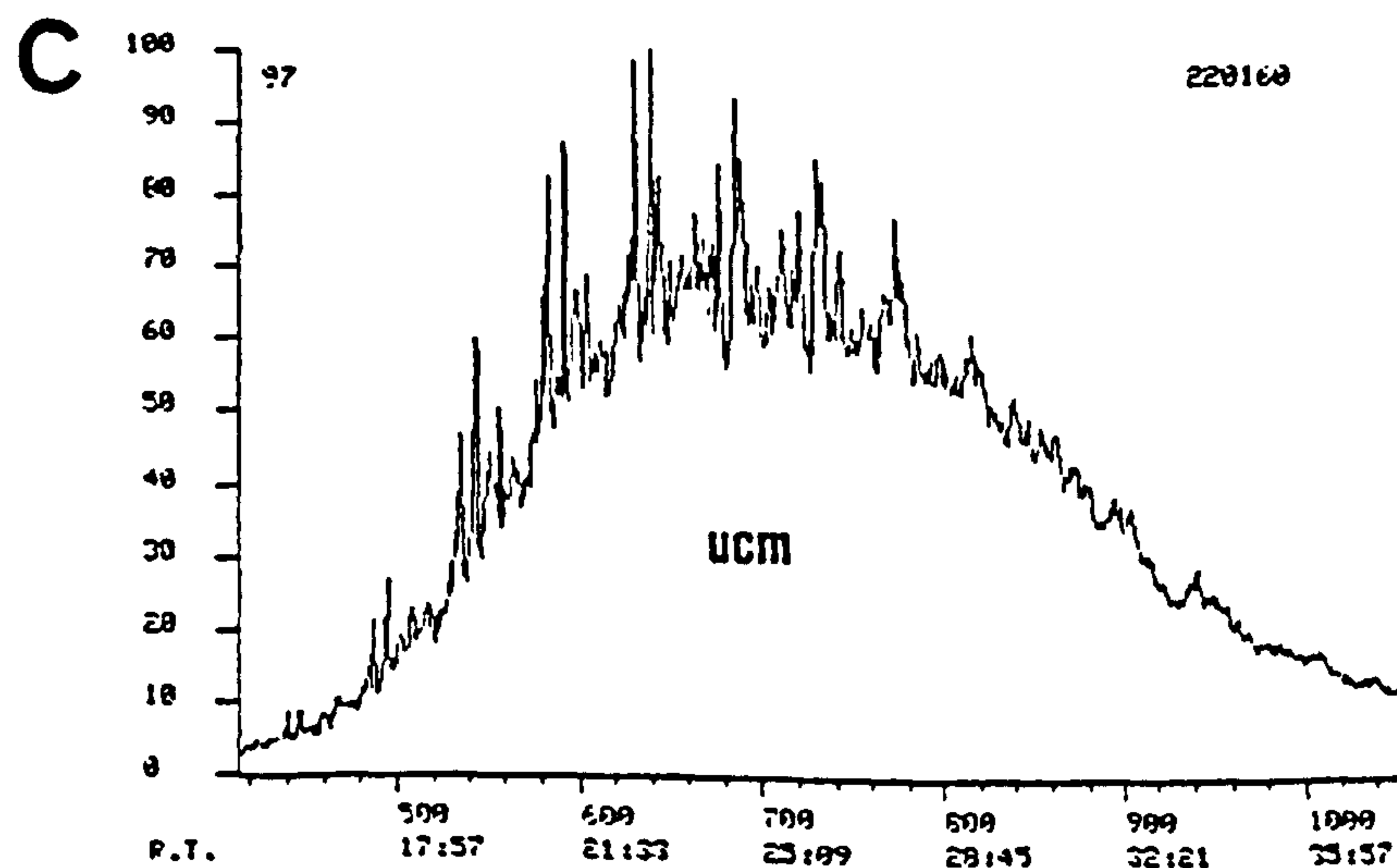
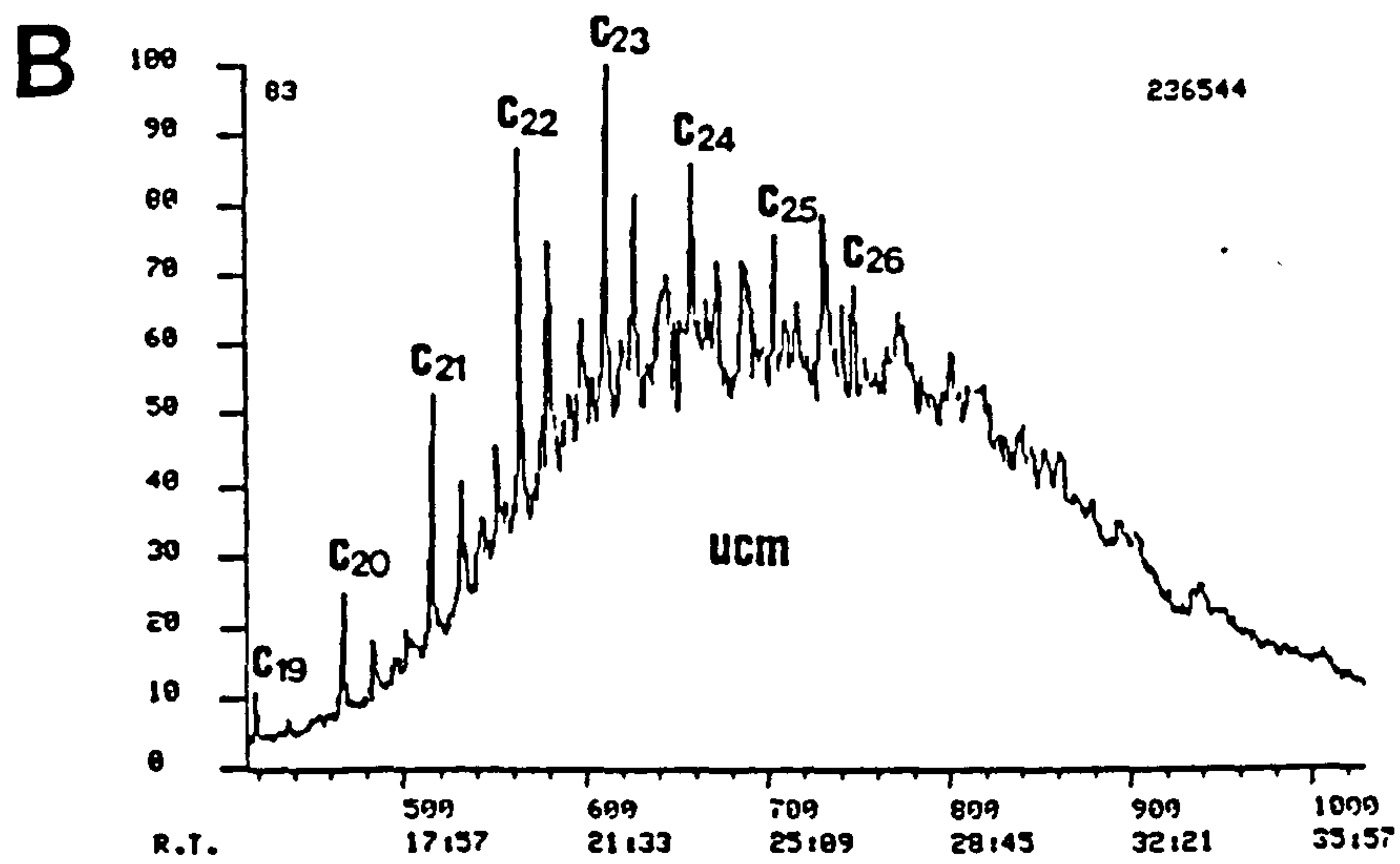
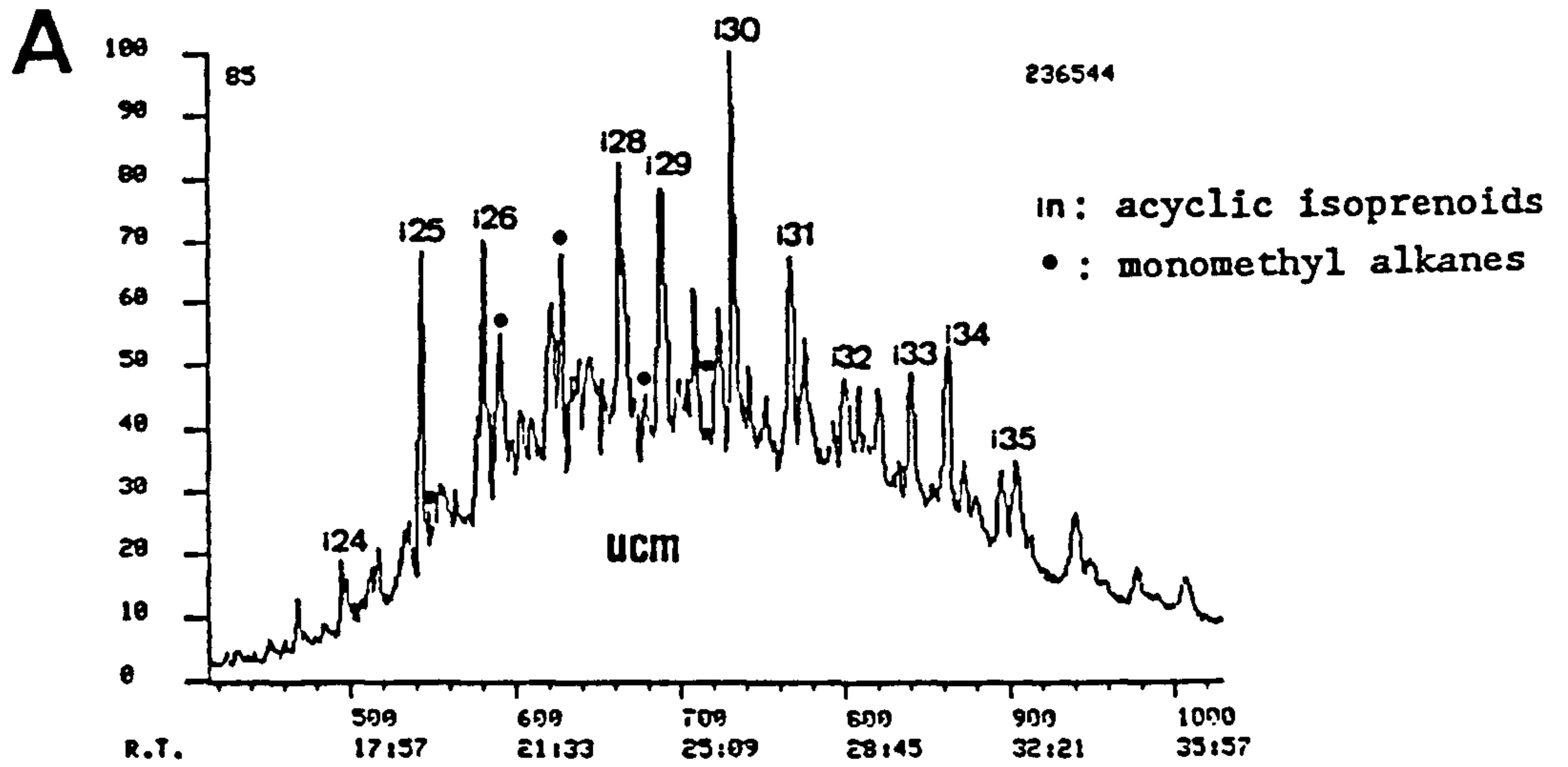


FIG. 3.11 GC-MS MASS FRAGMENTOGRAMS OF A) ACYCLIC ALKANES (m/z 85), B) ALKYL CYCLOHEXANES (m/z 83) AND C) ALKYMETHYL CYCLOHEXANES (m/z 97) OBSERVED IN THE UREA NON ADDUCT, SILKOLENE 150 LUBE OIL.

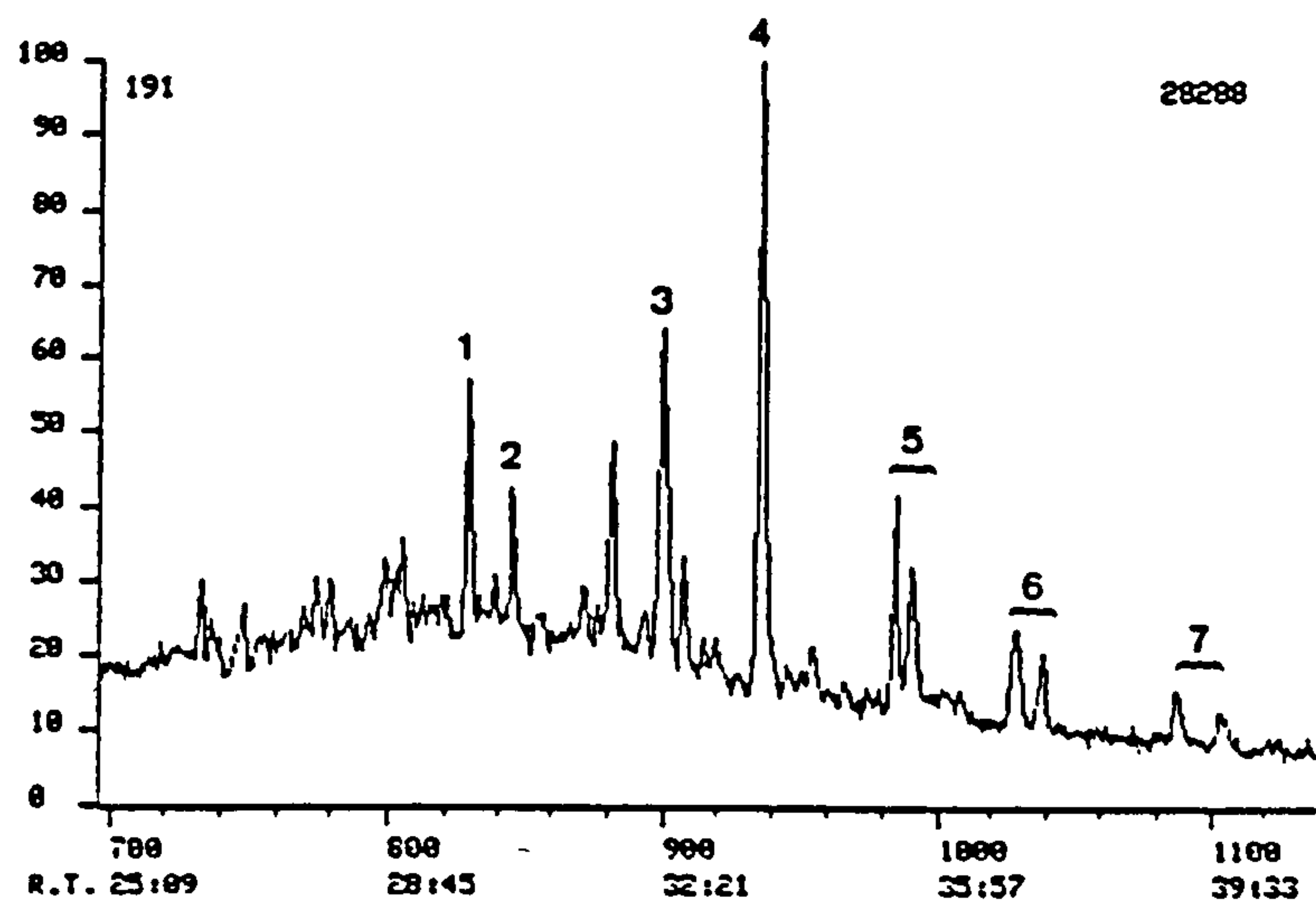


Pentacyclic triterpanes were observed in the UNA by monitoring the distribution of the m/z 191 ion characteristic of this series (Fig. 3.12). By an examination of their mass spectra and relative retention times these were characterised as $17\alpha(H)$, $21\beta(H)$ - hopanes typical of many mature petroleums (Table 3.4). These compounds were also evident amongst the resolved components of the UNA GC profile, though in low abundance compared to acyclic alkanes.

Steranes were identified in the UNA by the use of the m/z 217 mass fragment ion and again a typical distribution of compounds common to many petroleums was observed (Fig. 3.13). Though some components were unresolved, series of C27- C29 compounds possessing both a non-rearranged and a rearranged skeleton (as mixtures of stereoisomers) were tentatively identified by mass fragmentography of their molecular ions and their relative retention times. The m/z 218 fragment ion profile was used to accentuate the distribution of $14\beta(H)$, $17\beta(H)$ regular steranes as equilibrium mixtures of epimers at C20 (i.e. 20S/20R).

To summarise, GC and GC-MS of the urea non adducted alkanes provided useful information concerning the distributions of specific molecular marker compounds, and resolved series of acyclic isoprenoids, alkylcyclohexanes, steranes and triterpanes common to many petroleums were identified by mass fragmentography of their characteristic and abundant fragment ions. In each case the resolved series of compounds identified were also found to contribute to the resolved components overlying the UCM profile, though it should be emphasised

FIG. 3.12 PENTACYCLIC TRITERPANES IDENTIFIED IN THE UREA NON ADDUCT, SILKOLENE 150 LUBE OIL ALKANES, BY THEIR m/z 191 MASS FRAGMENTOGRAM.



for peak identity see Table 3.4

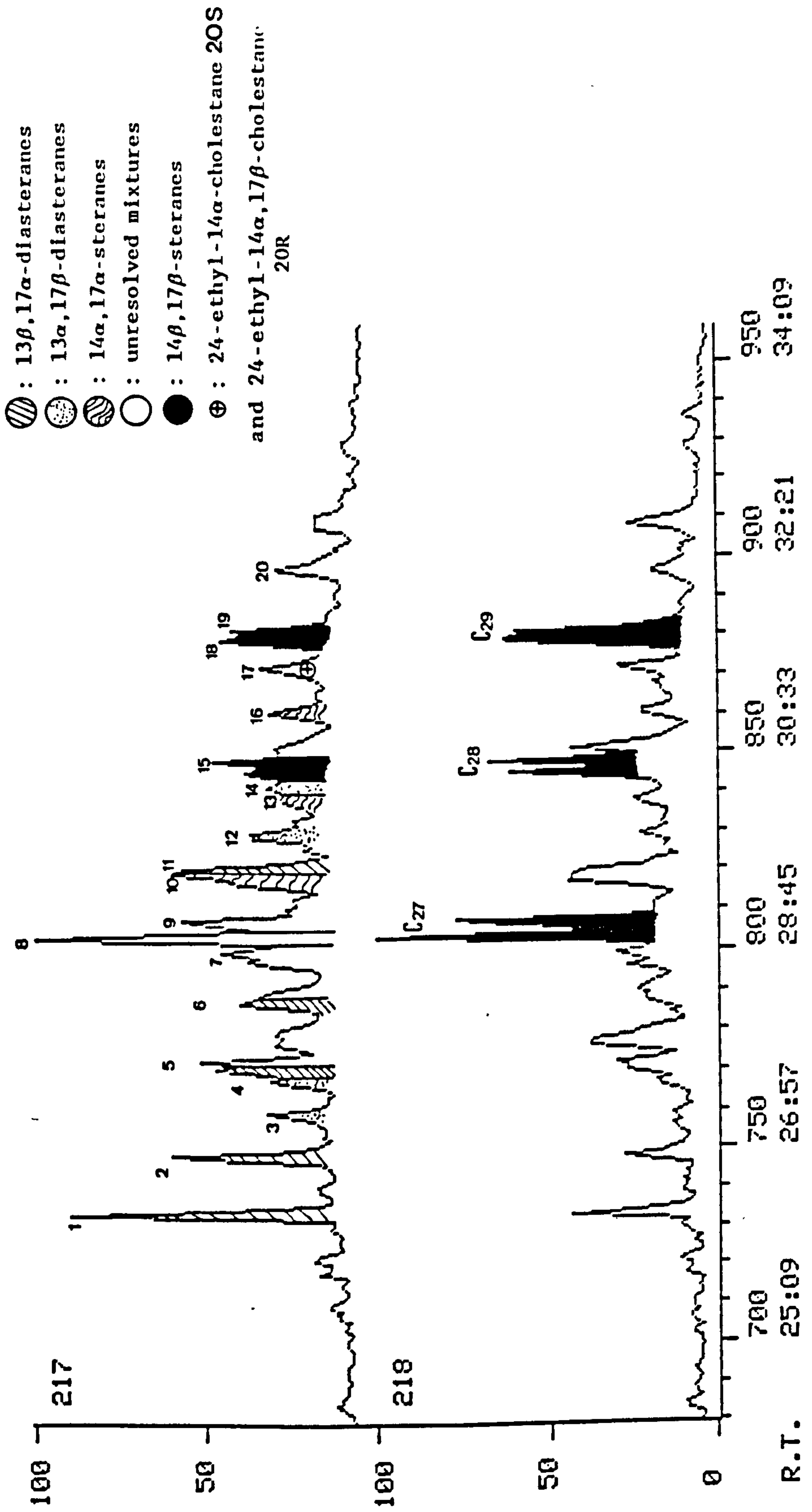
TABLE 3.4

PENTACYCLIC TRITERPANES IDENTIFIED IN THE SILKOLENE 150 LUBRICATING BASE OIL UREA NON ADDUCTED ALKANE FRACTION (GC-MS m/z 191 PROFILE, FIG. 3.12)

Peak no.	triterpane	Carbon no.
1	18 α (H)-22,29,30-trisnorneohopane	C27
2	17 α (H)-22,29,30-trisnorneohopane	C27
3	17 α (H), 21 β (H)-30-norhopane	C29
4	17 α (H), 21 β (H)-hopane	C30
5	17 α (H), 21 β (H)-homohopane (22S/22R)	C31
6	17 α (H), 21 β (H)-bishomohopane (22S/22R)	C32
7	17 α (H), 21 β (H)- trishomohopane (22S/22R)	C33

FIG. 3.13 STERANES AS MONITORED BY GC-MS MASS FRAGMENTOGRAPHY (m/z 217/218). UREA NON ADDUCTED ALKANES, SILKOLENE 150 LUBE OIL.

(for peak identity see Table 3.6)



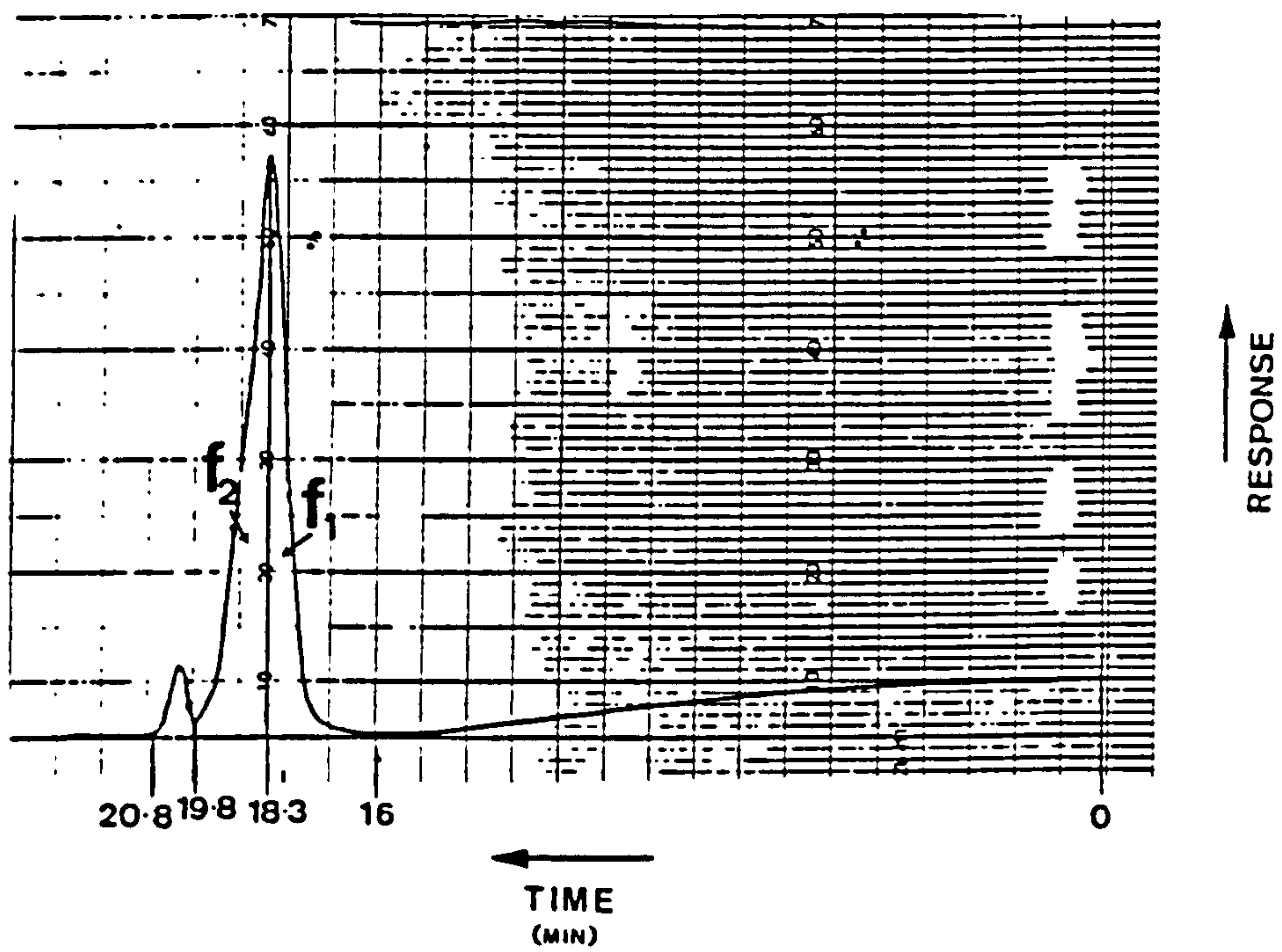
that the sum total of these were estimated to represent less than 10% of the total alkanes present. The ion series shown to follow the UCM profile closely were characteristic of acyclic alkanes (m/z 85) and alkylcyclohexanes (m/z 83, 97), which suggests that each contributes to the unresolved gas chromatographic profile. The absolute quantitative contribution of each series however could not be determined without internal standard methodologies, though estimates were made by use of the total ion count obtained at the maximum in each mass fragmentogram. Thus the m/z 85 and m/z 83 ion series maximised to a count of 236544; whilst the m/z 97 maximum provided a total count of 220160, this suggests the former are quantitatively more important. GC and GC-MS analysis of the UNA alkanes therefore provided little quantitative and only limited qualitative information concerning the molecular nature of the hydrocarbon composition.

Since GC and GC-MS alone provided little compositional information for the bulk hydrocarbon components of the aliphatic UCM, alternative methods were sought. Molecular clathration (e.g. thiourea) and gel permeation chromatography (GPC) were used to fractionate the UCM, and elemental analysis, probe MS, CI GC-MS, GC-FPD, and degradative techniques were used to gain molecular information. Each of these are discussed in greater detail in the following sections.

3.1.3.2. GEL PERMEATION CHROMATOGRAPHY (GPC)

GPC has been widely used in the petroleum industry for the separation and analysis of high molecular weight distillate fractions (Philip and Anthony, 1984; Oelert *et al.*, 1971; Altgelt, 1979; Lundanes and Greibrokk, 1985; Guieze and Williams, 1984). In the early part of this study it was used to separate the saturate fraction of a

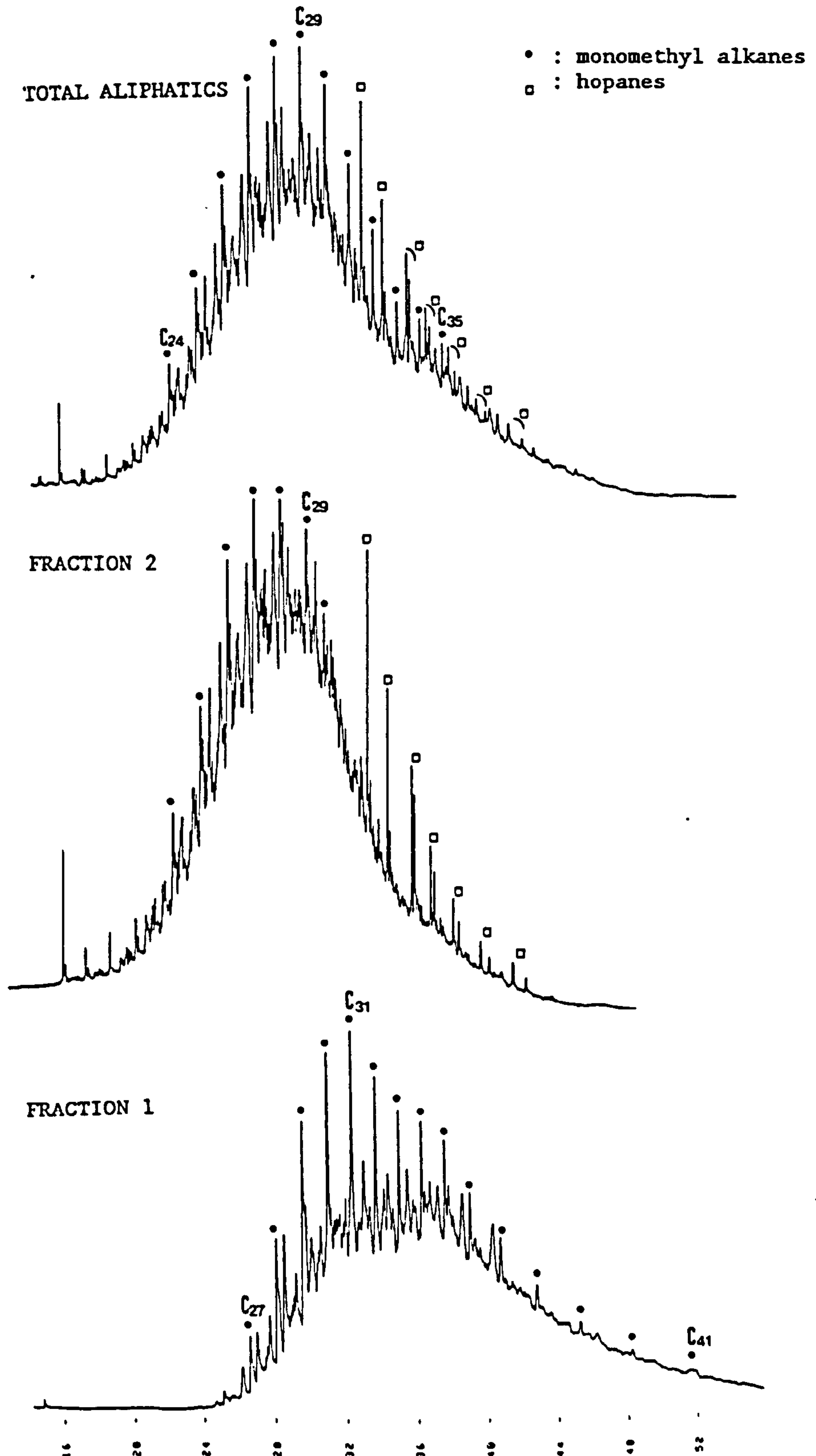
FIG. 3.14 GEL PERMEATION CHROMATOGRAM (REFRACTIVE INDEX DETECTION)
OF THE ALIPHATIC UCM, DULTON 20 W 40 LUBRICATING BASE OIL



commercially available lubricating oil that had been treated by dialysis to remove high molecular weight polymeric additives. Though no quantitative data was obtained a qualitative analysis by GC shows that a degree of separation was achieved. Thus the first fraction to elute from the GPC column (F1, 16.0 - 18.3min, Fig. 3.14) was found to comprise the higher molecular weight material in the approximate range C27 (380 gmole^{-1}) to C41 (576 gmole^{-1}), maximising at C33. This molecular weight distribution was determined by monitoring the positions of the monomethyl alkanes present as resolved components (Fig. 3.15). The expected elution order of the size exclusion technique, i.e. high molecular weight material first, was therefore confirmed by GC.

The second fraction to elute from the GPC column (F2, 18.3 - 19.8min, Fig. 3.14) comprised the bulk of the UCM hydrocarbons which when analysed by GC were found to range from ca. C18 (254 gmole^{-1}) to C28 (543 gmole^{-1}), maximising at C28 (394 gmole^{-1}). Some overlap was noted between the two fractions, since both contained C27 - C30 monomethyl alkanes, though in the second fraction the monomethyl alkanes $>C30$ were absent. The most notable effect of the GPC fractionation was the removal of UCM alkanes in the elution region of the pentacyclic triterpanes identified in this oil by GC-MS (Fig. 3.15). On the basis of molecular weight alone one would expect the members of this series heavier than the C31 monomethyl alkane, i.e. the C32 - C35 diastereomers, to elute in the earlier fraction, though these clearly remain in the lighter components less than C31. The reason for this apparent discrepancy is that GPC separations rely not only on differences in molecular weight but also on molecular

FIG. 3.15 PARTIAL GAS CHROMATOGRAMS OF THE FRACTIONS DERIVED FROM THE GEL PERMEATION CHROMATOGRAPHIC SEPARATION OF THE ALIPHATIC FRACTION, DULTON 20 W 40 LUBRICATING BASE OIL
 [GC:DB-5(J+W), 50-180°C @ 10°C min⁻¹,
 180-300°C @ 5°C min⁻¹, 300°C(20min)]



dimensions. In particular the effective linear molecular size is known to be an important criterion for explaining the mechanisms involved in size exclusion chromatography (e.g. Philip and Anthony, 1986). Thus a fused ring pentacyclic structure with a maximum side chain length of seven carbon atoms has an effective linear molecular size of 17-18, i.e. well below that of the last eluting member of the monomethyl alkane series in the second fraction. This effect may prove particularly useful for the characterisation of the hopane series, as their enrichment relative to the UCM components allows their identification and possible quantitation by GC alone.

To summarise the use of GPC in the characterisation of the aliphatic UCM was limited in the amount of compositional information it provided, although it did appear promising for the preparative fractionation of complex hydrocarbon mixtures prior to more detailed chemical analysis.

3.1.3.3. THIOUREA ADDUCTION

Thiourea adduction as a form of molecular clathration has been quite widely used in petroleum and organic geochemistry for the enrichment of specific compound classes from complex alkane mixtures. In general linear methyl-substituted acyclic alkanes (including regular acyclic isoprenoids) readily form adducts, though a more highly branched acyclic isoprenoid 2,6,10-trimethyl-7-(3-methylbutyl)-dodecane does not (Yon, 1981). Because of the selectivity of thiourea towards regular acyclic isoprenoids the procedure is used for their isolation (e.g. Albaiges, 1980). Other compounds known to

form adducts include alkyl and alkylmethylcyclohexanes, decalin and alkyl decalins, and possibly perhydrophenathrenes (Payzant et al., 1980; Baron, 1961). In contrast polycyclic alkanes such as $17\alpha(H)$, $21\beta(H)$ - hopanes and rearranged steranes do not form adducts with thiourea (Rubinstein et al., 1977). Steranes possessing the non-rearranged skeleton however, can form adducts depending on the presence and length of the alkyl side chain at C-17. Thus the unsubstituted regular C19 sterane androstane was not observed to form an adduct with thiourea, whereas cholestane (C27), 24-methylcholestane (C28) and 24-ethylcholestane (C29) did (Murphy et al., 1967). Other workers have noted the apparent selectivity of thiourea towards this class of alkanes (Mulheirn and Ryback, 1974; Cardoso, 1976; Rubinstein et al., 1977; Payzant et al., 1980). The results of the adduction of the aliphatic UCM with thiourea are shown in Fig. 3.16. The gravimetric data (Table 3.5) shows the majority of the UNA alkanes were excluded by the sieve (>58%). GC showed an enrichment of resolved components within the adduct, whereas the non adduct consisted almost entirely of a UCM. This is consistent with previous studies that have used thiourea to fractionate alkane UCMs from Recent sediments and from Athabasca oil sand bitumens (Cardoso, 1976; Payzant et al., 1980).

GC-MS of the thiourea adduct confirmed the major series of resolved acyclic and monocyclic alkanes were concentrated in this fraction (Fig. 3.17), and their mass fragmentograms showed proportionately less of a UCM profile than that observed in the urea non adduct. The same ion profiles for the thiourea non adduct in each case showed a greater contribution of the unresolved envelope (Fig. 3.18), though a small number of resolved components did remain. Estimates of the

FIG. 3.16 GAS CHROMATOGRAMS OF THE A) UREA NON ADDUCT, B) THIOUREA ADDUCT AND C) THIOUREA NON ADDUCT, SILKOLENE 150 LUBRICATING OIL ALKANE FRACTION
[GC:DB-5(J+W), 50-180°C @ 10°C min⁻¹, 180-300°C @ 5°C min⁻¹, 300°C(20min)]

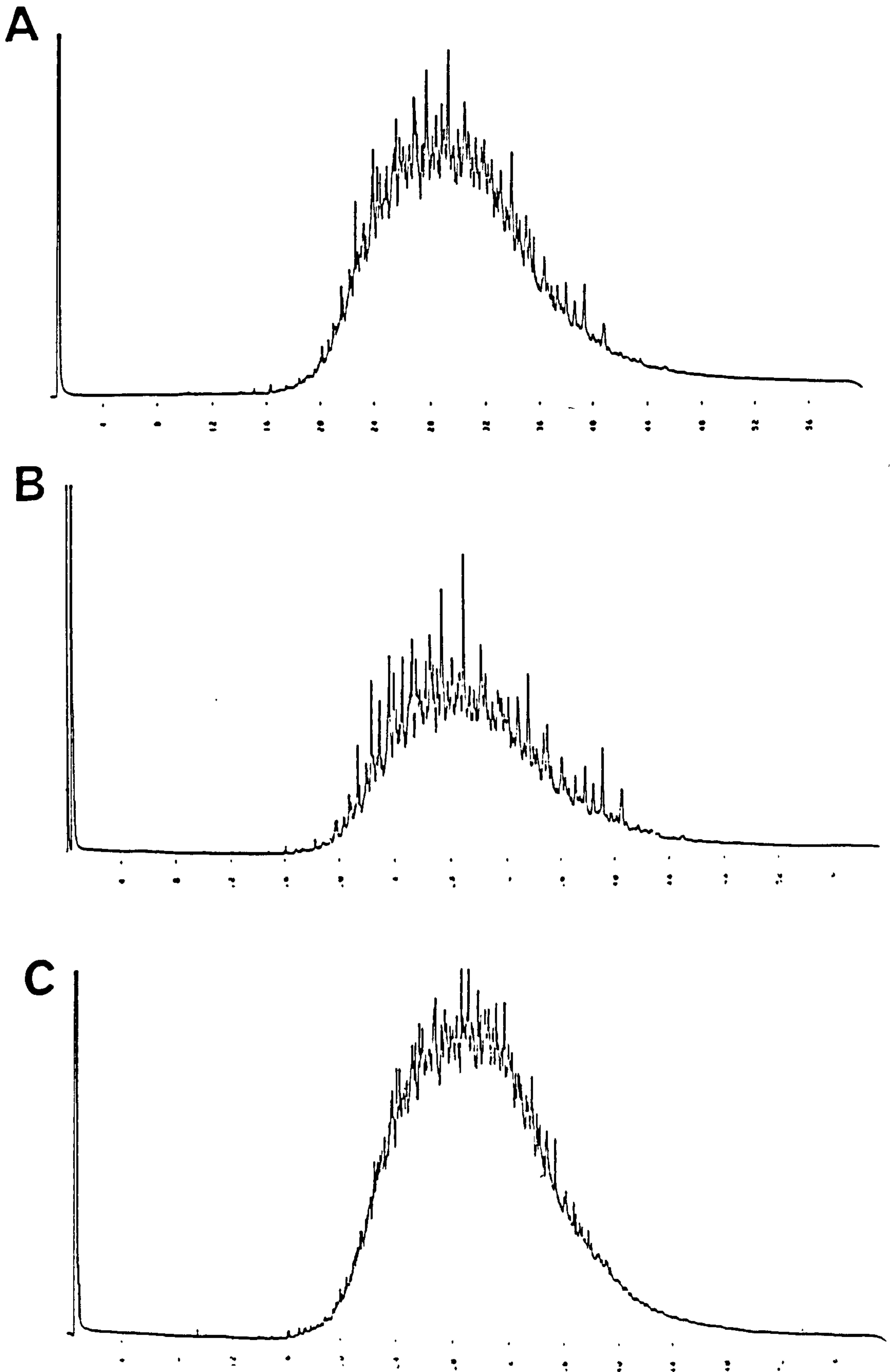


TABLE 3.5

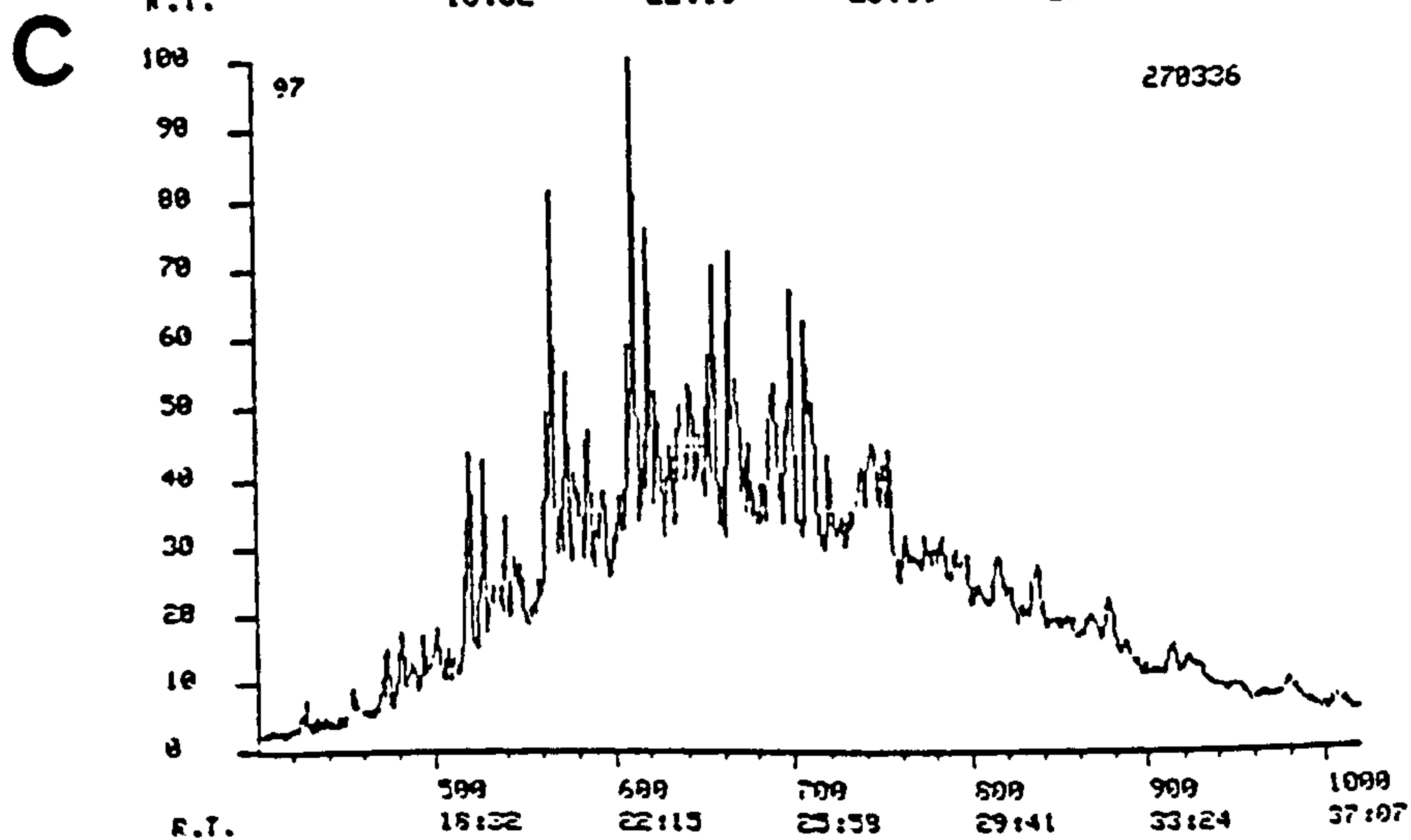
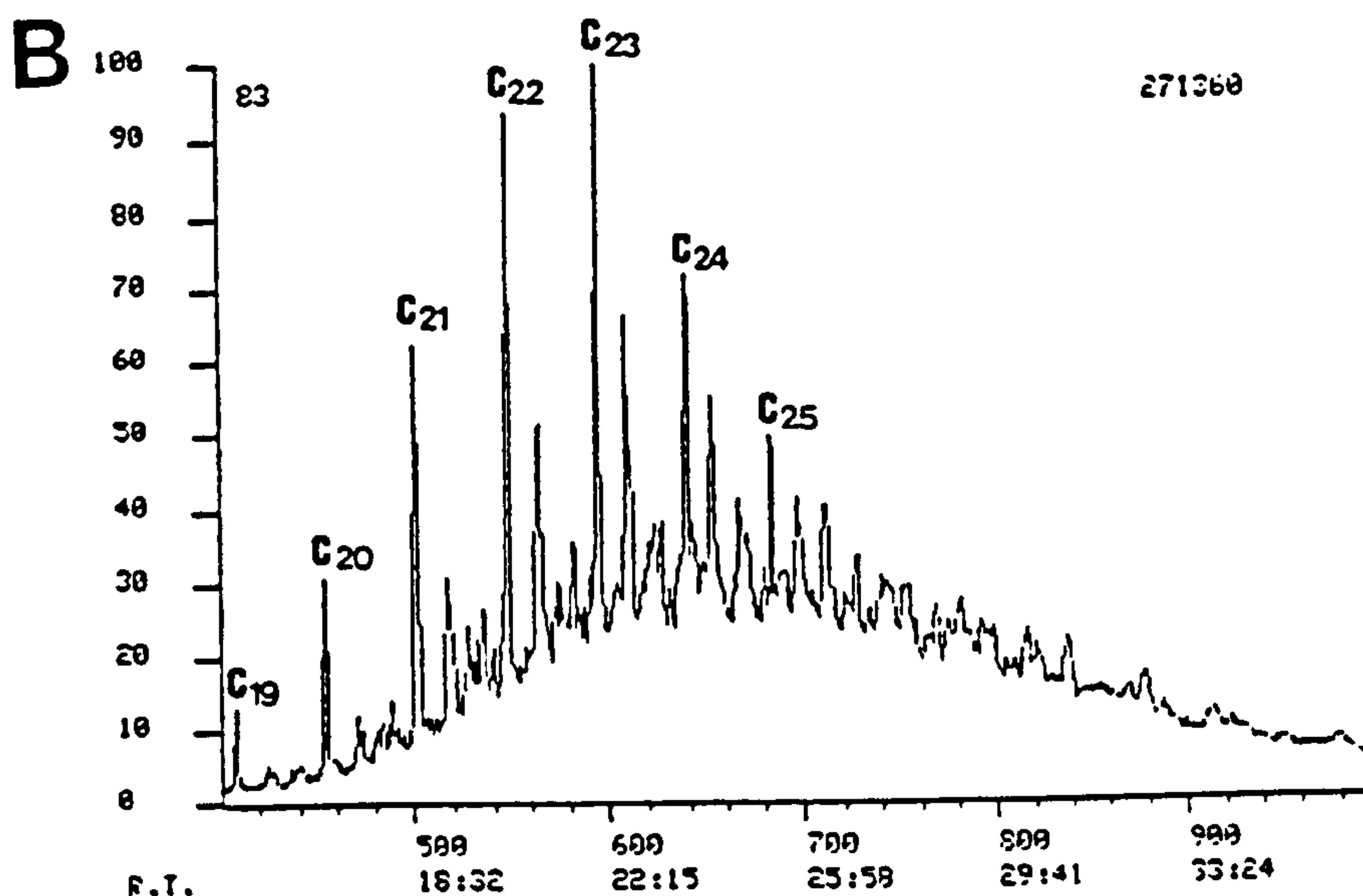
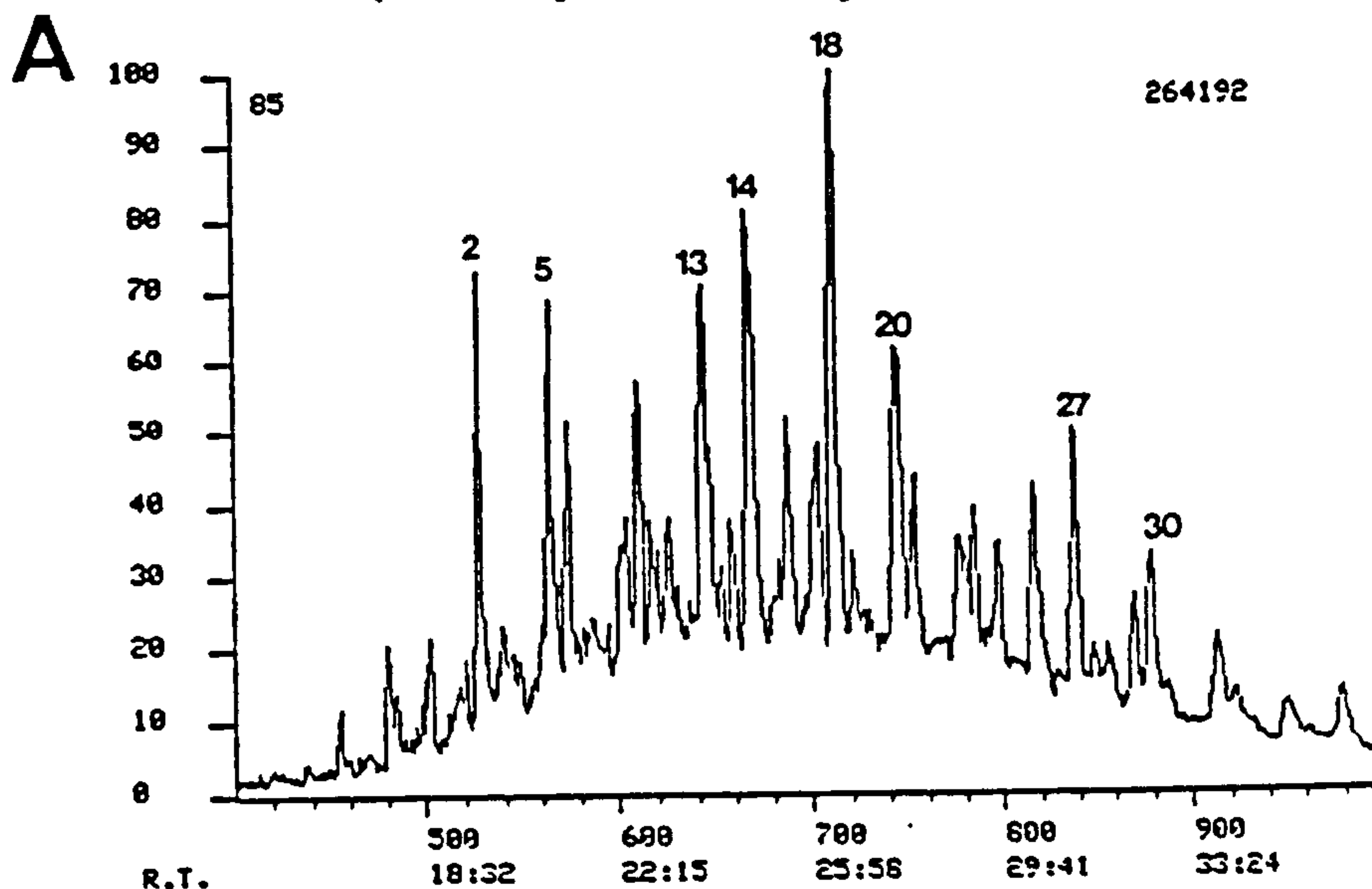
GRAVIMETRIC DATA FOR THE THIOUREA ADDUCTION PROCEDURE

MASS SAMPLE (mg)	MASS ADDUCT ^a [mg (%)]	MASS NON ADDUCT ^a [mg (%)]	RECOVERY (%)
25.5	8.5 (39)	13.4 (61)	86
25.5	8.7 (41)	12.5 (59)	83

a: % yields based on total mass products recovered

FIG.3.17 GC-MS MASS FRAGMENTOGRAMS OF A) ACYCLIC ALKANES (m/z 85), B) ALKYL CYCLOHEXANES (m/z 83) AND C) ALKYL METHYL CYCLOHEXANES (m/z 97) IN THE THIUREA ADDUCT, SILKOLENE 150 LUBE OIL ALKANES

(A: for peak identity see Table 3.3)



quantitative contribution of each ion series based on total ions counts at the profile maximum are alkylcyclohexanes >n-alkylmethylcyclohexanes> acyclic alkanes for the thiourea adduct, and alkylmethyl cyclohexanes > alkylcyclohexanes > acyclic alkanes for the thiourea non adduct.

The fragmentogram characteristic of alkyl decalins (m/z 137) was also plotted for each fraction (Fig. 3.19). The UNA comprised a broad UCM with a series of resolved doublet peaks, in contrast the thiourea adduct was enriched in the lower eluting compound of each doublet. The mass spectra of these latter compounds were characterised by m/z 137 as the base peak with abundant molecular ions (ca. 15%), and were tentatively identified as C19 - C28 alkyldecalins.

The hopanes identified in the UNA were present almost exclusively in the TNA, consistent with their known adduction behaviour (e.g. Payzant et al., 1980). The steranes were found to differ from those observed in the UNA suggesting selective inclusion of some members in the adduct. Mass fragmentography of molecular ions showed that rearranged and C29 regular steranes were absent in the adduct, whereas the regular C27 14 α , 17 α epimers and the C28 14 α , 17 α epimers were present. Also the C27 14 β ,17 β - cholestane 20R isomer was observed though not the 20S (Fig. 3.20 A + B; Table 3.6). As expected these thiourea adducted compounds were not identified in the TNA (Table 3.6, Fig. 3.20 B).

3.1.3.4 ELEMENTAL ANALYSIS AND GAS CHROMATOGRAPHY - FLAME PHOTOMETRIC DETECTION

Elemental analysis provides a simple method for estimating the degree

FIG.3.18 GC-MS MASS FRAGMENTOGRAMS OF A) ACYCLIC ALKANES (m/z 85), B) ALKYL CYCLOHEXANES (m/z 83) AND C) ALKYL METHYL CYCLOHEXANES (m/z 97) OBSERVED IN THE THIOUREA NON ADDUCT, SILKOLENE 150 LUBE OIL ALKANES

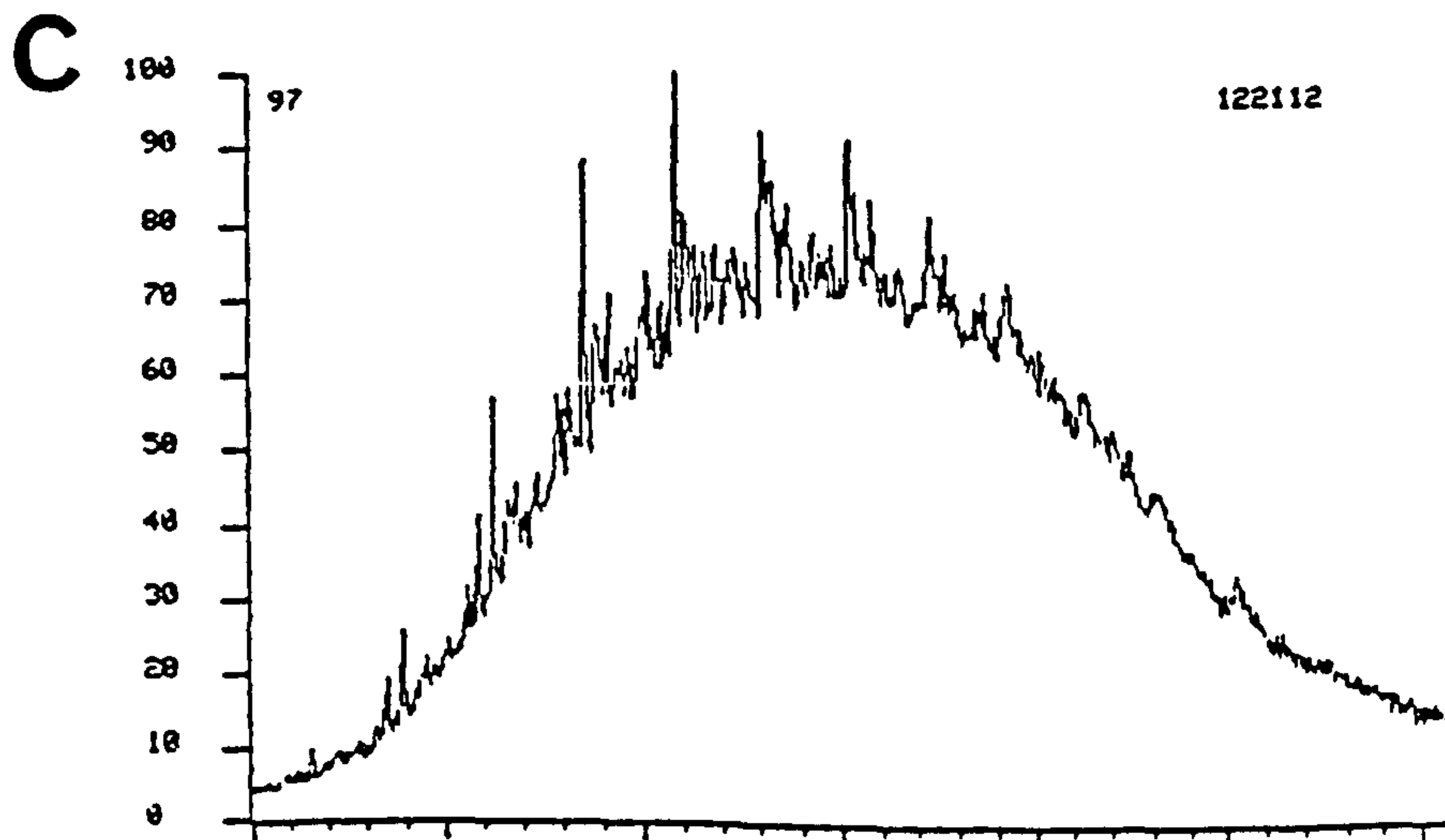
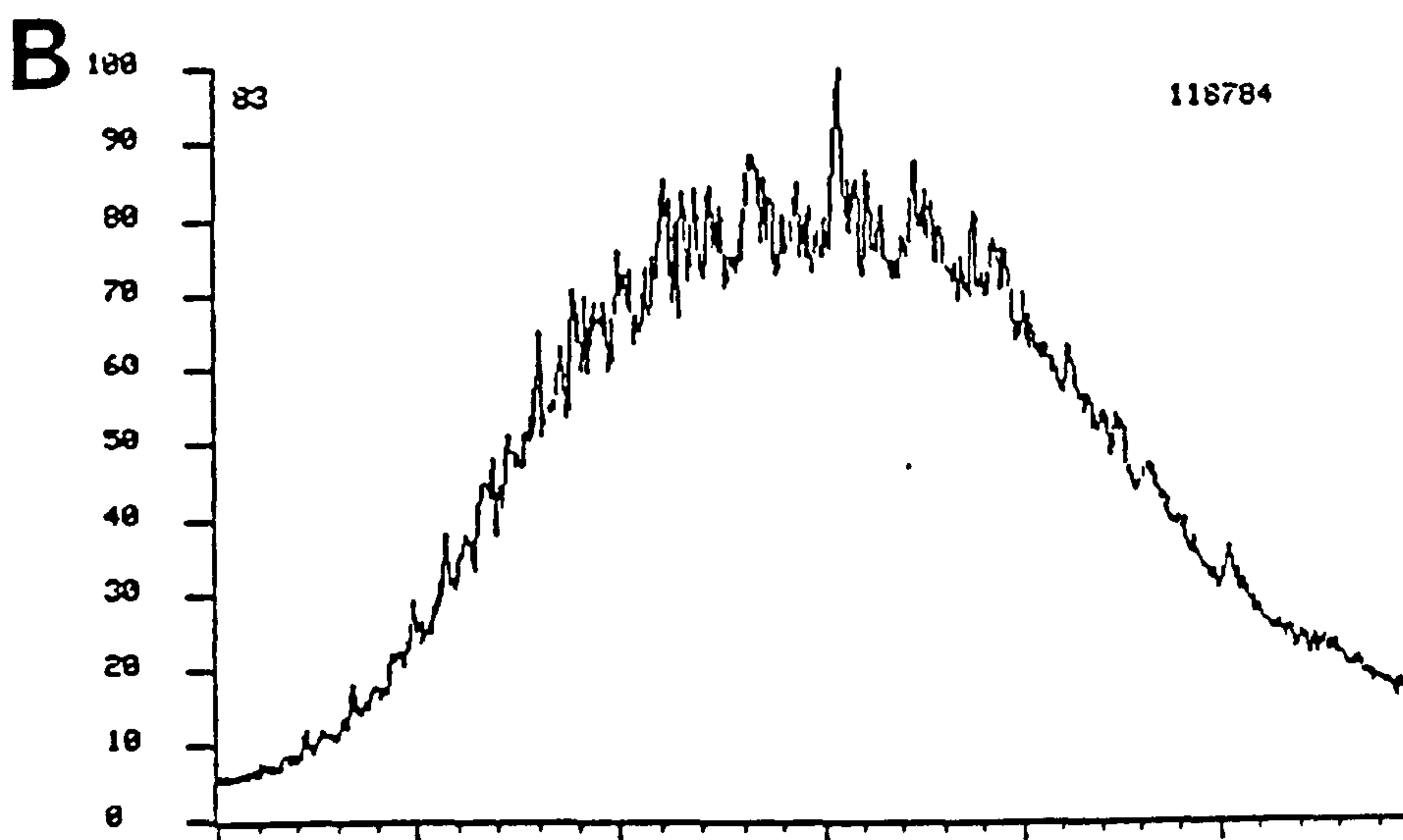
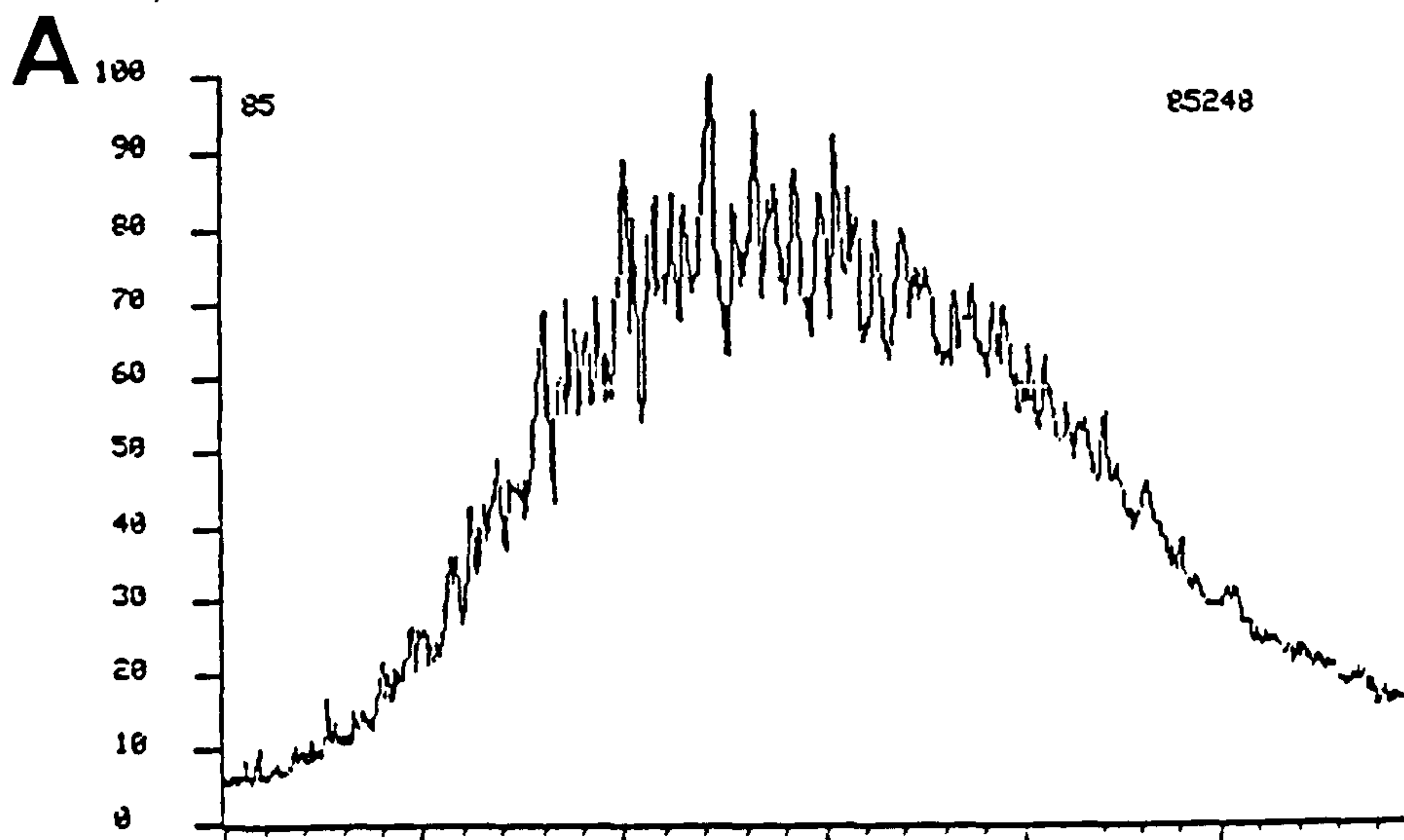


FIG. 3.19 GC-MS MASS FRAGMENTOGRAMS OF ALKYL DECALINS (m/z 137) OBSERVED IN THE A) UREA NON ADDUCT, B) THIOUREA ADDUCT AND C) THIOUREA NON ADDUCT FRACTIONS, SILKOLENE 150 LUBE OIL ALKANES

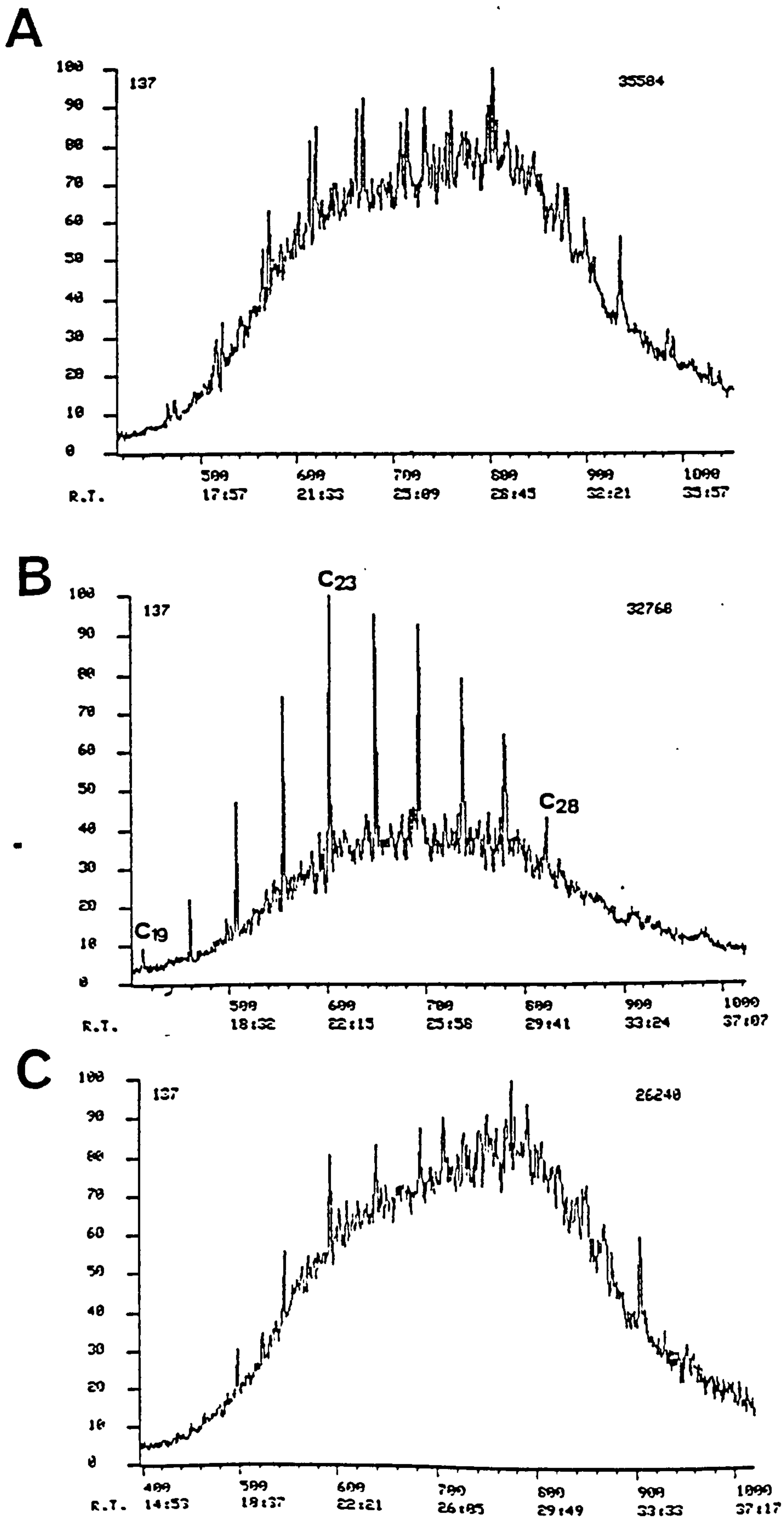
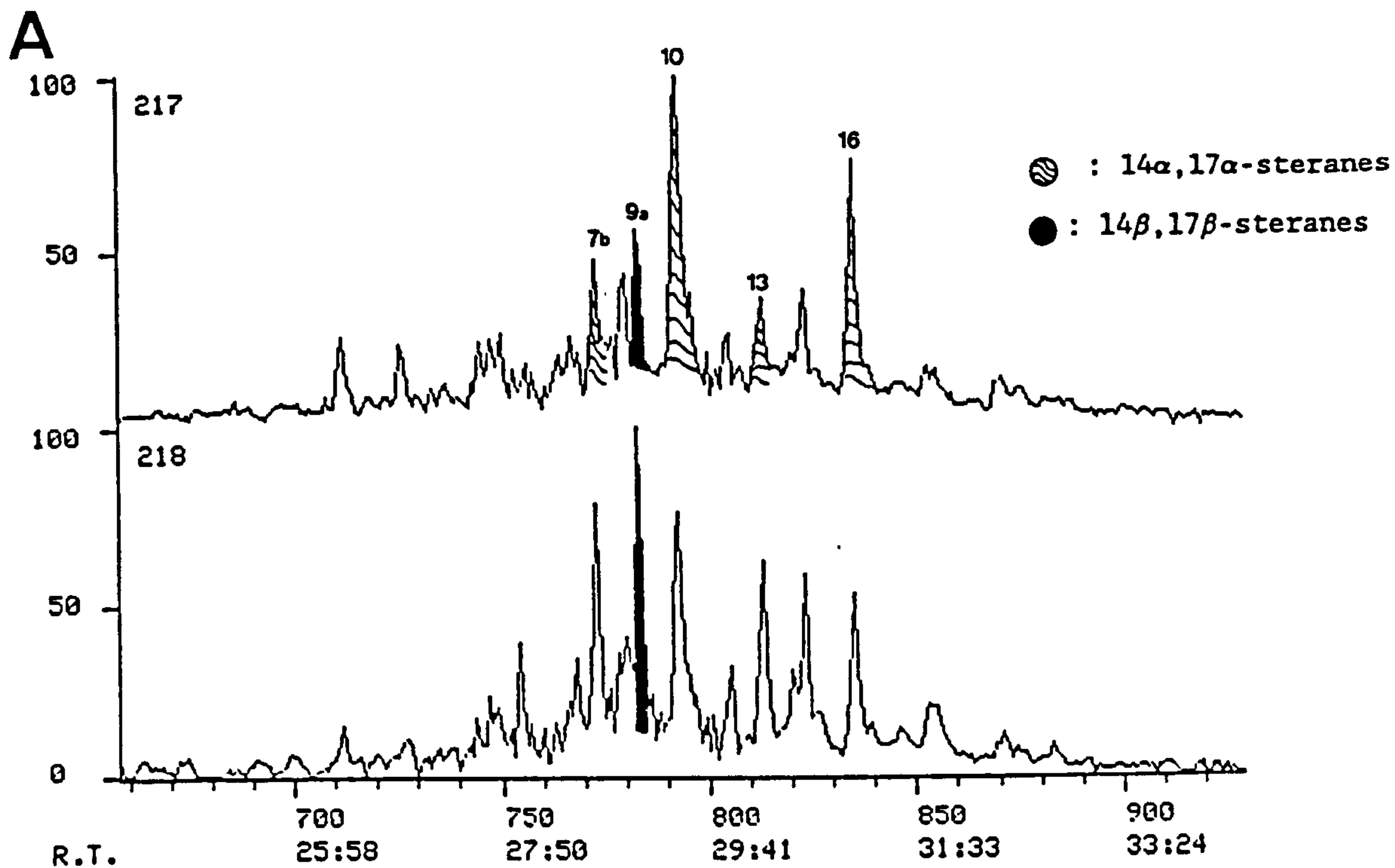


FIG. 3.20 STERANES AS MONITORED BY GC-MS MASS FRAGMENTOGRAPHY (m/z 217/218): A) THIOUREA ADDUCT AND B) THIOUREA NON ADDUCT, SILKOLENE 150 LUBE OIL ALKANES.



(for peak identity see Table 3.6)

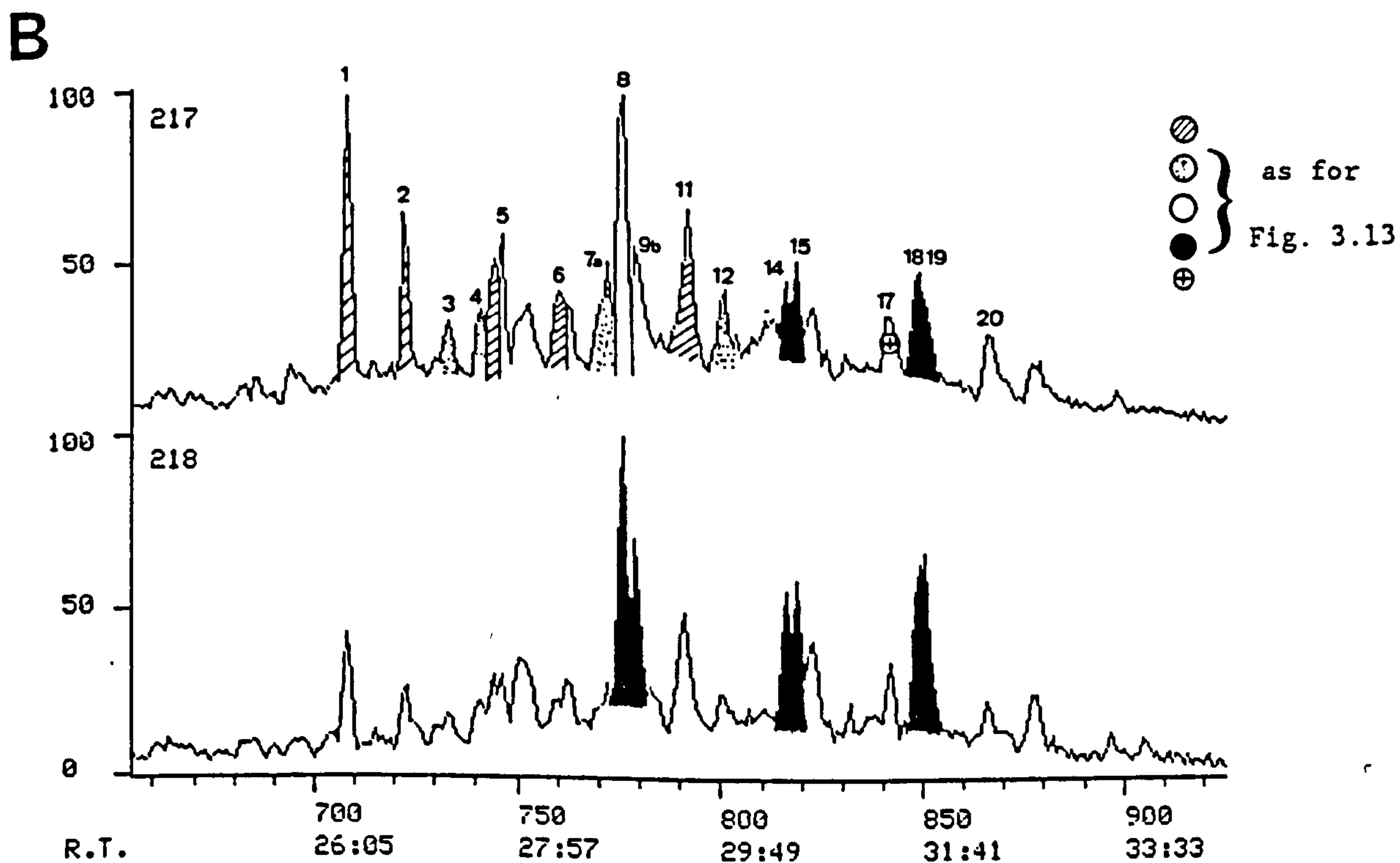


TABLE 3.6

TENTATIVE ^a IDENTIFICATIONS OF STERANES AND DIASTERANES IN THE UNA, TNA AND TA OF THE SILKOLENE 150 LUBRICATING OIL- ALKANE FRACTION

COMPOUND NO.	TYPE	C20	C.NO.	UNA	TNA	TA
1	13 β ,17 α -diacholestane	20S	C27	/	/	x
2	13 β ,17 α -diacholestane	20R	C27	/	/	x
3	13 α ,17 β -diacholestane	20S	C27	/	/	x
4	13 α ,17 β -diacholestane	20R	C27	/	/	x
5	24-methyl-13 β ,17 α -diacholestane	20S	C28	/	/	x
6	24-methyl-13 β ,17 α -diacholestane	20R	C28	/	/	x
7a	24-methyl-13 α ,17 β -diacholestane	20S	C28	/	/	x
7b	14 α ,17 α -cholestane	20S	C27	/	x	/
8a	24-ethyl-13 β ,17 β -diacholestane	20S	C29	/	/	x
8b	14 β ,17 β -cholestane	20S	C27	/	/	x
9a	14 β ,17 β -cholestane	20R	C27	/	?	/
9b	24-methyl-13 α ,17 β -diacholestane	20R	C28	/	?	?
10	14 α ,17 α -cholestane	20R	C27	/	x	/
11	24-ethyl-13 β ,17 α -diacholestane	20R	C29	/	/	x
12	24-ethyl-13 α ,17 β -diacholestane	20S	C29	/	/	x
13	24-methyl-14 α ,17 α -cholestane	20S	C28	/	x	/
14a	24-ethyl-13 α ,17 β -diacholestane	20R	C29	/	/	x
14b	24-methyl-14 β ,17 β -cholestane	20R	C28	/	/	x
15	24-methyl-14 β ,17 β -cholestane	20S	C28	/	/	x
16	24-methyl-14 α ,17 α -cholestane	20R	C28	/	x	/
17	24-ethyl-14 α -cholestane	20S	C29	/	/	x
18	24-ethyl-14 β ,17 β -cholestane	20R	C29	/	/	x
19	24-ethyl-14 β ,17 β -cholestane	20S	C29	/	/	x
20	24-ethyl-14 α ,17 β -cholestane	20R	C29	/	/	x

a: based on mass spectral data, molecular ion mass fragmentograms, and relative retention times (e.g. Philp, 1985)

b: / : present x : absent

of unsaturation of hydrocarbon mixtures, and the technique in combination with catalytic hydrogenation formed the basis of early structural group determinations developed for petroleum analysis (Van Nes and Van Westen, 1951; Rossini, 1953). The results of the method applied to the aliphatic UCM and the products of thiourea adduction are presented in Table 3.7. From this data average molecular formulae were calculated and compared with those predicted for pure hydrocarbon classes.

For the urea non adduct (UNA), the experimentally determined general molecular formula of $C_nH_{2n+0.26}$ falls between the expected values for monocyclic (C_nH_{2n+0}) and acyclic alkanes (C_nH_{2n+2}). From this it would appear that the proportion of molecules containing ring systems larger than one is small, and that the UNA is comprised in the main of monocyclic and acyclic alkanes with the former predominating.

The products of the thiourea adduction process were also monitored by elemental analysis, and the determined values agree with the known behaviour of alkanes by this form of molecular clathration. Thus the elemental formula for the thiourea adduct (TUA) of $C_nH_{2n+0.44}$ indicates a higher proportion of acyclic alkanes, consistent with the known behaviour of such compounds (e.g. acyclic isoprenoids, Payzant et al., 1980). In contrast, the elemental formula for the thiourea non adduct ($C_nH_{2n-0.01}$) suggests a lower proportion of acyclic alkanes, which is again consistent with the known enrichment of cyclic alkanes in this fraction (e.g. Rubinstein et al., 1977). The z value determined however is only slightly less than that expected for a mixture of pure monocyclic alkanes, it appears likely therefore that these components predominate.

TABLE 3.7

ELEMENTAL ANALYSIS OF THE UREA NON ADDUCT (UNA), THIOUREA ADDUCT (TA)
AND THIOUREA NON ADDUCT (TNA), SIKLOLENE 150 LUBRICATING OIL
ALKANE FRACTION

Sample	Weight (%)			H/C RATIO	TOTAL(%)
	C	H	N		
UNA	86.78	14.72	-0.04	2.04	101.46
TA	84.16	14.47	0.00	2.06	98.63
TNA	85.82	14.29	0.01	2.00	100.12

COMPARISON OF ELEMENTAL FORMULAE

Alkane type	z value ^a	H/C ratio ^b	General formula
acyclic	+2	2.08	C_nH_{2n+2}
monocyclic	0	2.00	C_nH_{2n+0}
bicyclic	-2	1.92	C_nH_{2n-2}
tricyclic	-4	1.84	C_nH_{2n-4}
tetracyclic	-6	1.76	C_nH_{2n-6}
pentacyclic	-8	1.68	C_nH_{2n-8}
hexacyclic	-10	1.60	C_nH_{2n-10}
UNA	0.26	2.04	$C_nH_{2n+0.26}$
TA	0.44	2.06	$C_nH_{2n+0.44}$
TNA	-0.01	2.00	$C_nH_{2n-0.01}$

a : z is derived from the expression C_nH_{2n+z} , where $z = \%H - (\%C/12 \times 2)$

b : H/C ratios determined for a C25 alkane

A small proportion of sulphur was determined in the urea non adduct (ca. 0.5%), this result was surprising since the oil was solvent extracted to remove heterocyclic compounds during refining. The results of the sulphur determinations also showed large variations (+50%), and GC-FPD showed no significant response above the background signal. The values of sulphur determined by elemental analysis may therefore be in error.

3.1.3.5. CHEMICAL IONISATION (CI) MASS SPECTROMETRY

"Soft" MS ionisation techniques play an important role in the characterisation of hydrocarbons and derivatives in petroleum and organic geochemistry. Of these, chemical ionisation (CI) is perhaps the most widely used in view of its relative simplicity and compatibility with conventional EI GC-MS systems (Chapman, 1985). The main advantage of CI over conventional EI ionisation is the production of virtually fragment free mass spectra, a feature which is particularly beneficial for molecular weight determination when EI fails to produce a molecular ion (Harrison, 1983). Thus CI GC-MS has been used for the characterisation of a wide range of hydrocarbons including acyclic isoprenoids (Bayer et al., 1981), monomethylalkanes (Klomp, 1986), alkylbenzenes (Hawthorne and Miller, 1985), steranes (Goodwin et al., 1983) and monoaromatised steroids (Mackenzie et al., 1981). In this study CI GC-MS with isobutane as reagent gas was used to characterise the urea non adduct, thiourea adduct, and thiourea non adduct derived from the Silkolene 150 lubricating base oil alkane fraction.

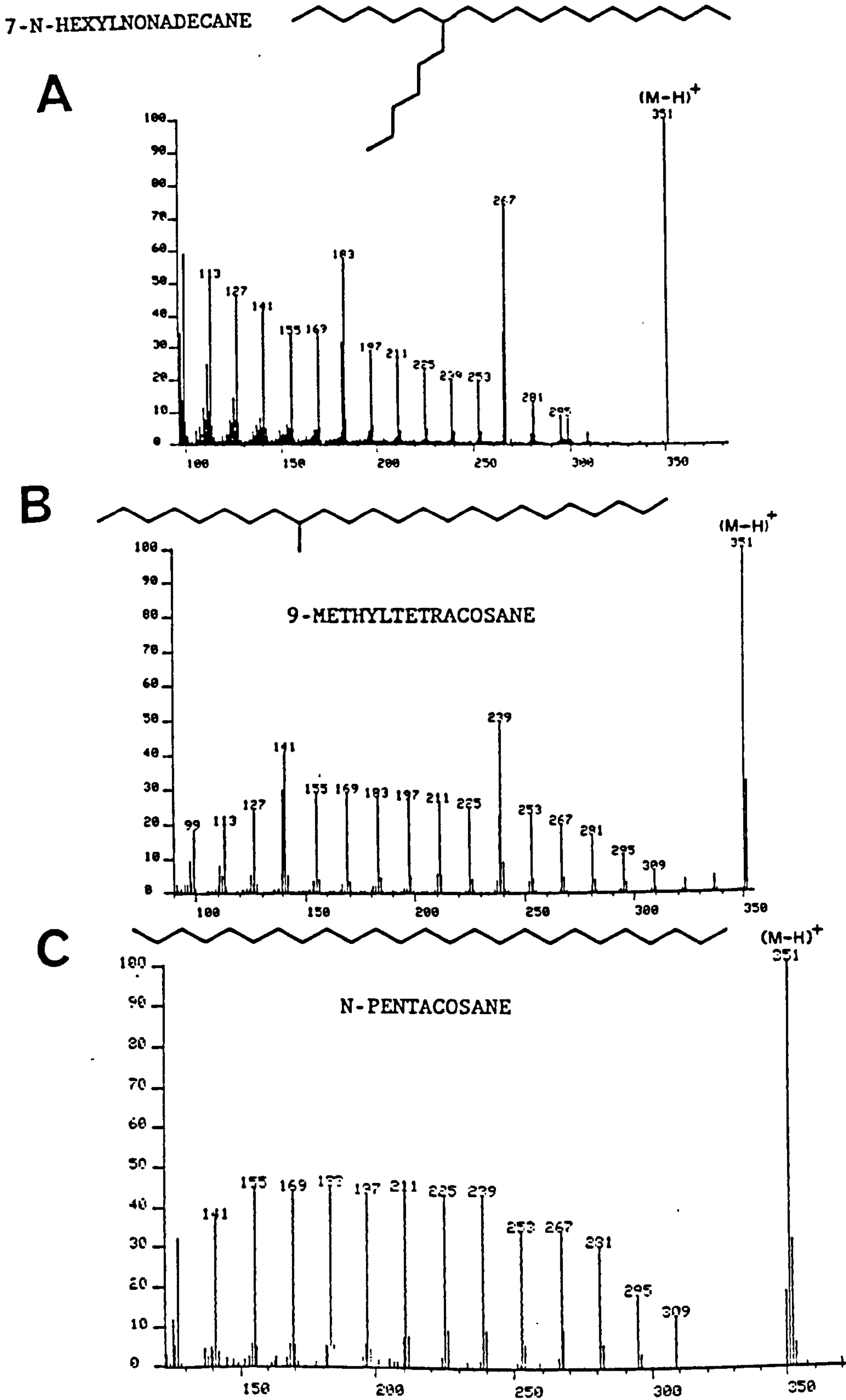
Instrumental ionising conditions were optimised to achieve the

maximum abundance of the quasi-molecular ion $[(M-H)^+]$ of the synthetic model alkane 7-n-hexylnonadecane (section 4.1.3). Under these conditions (section 2.7.7), the mass spectrum (Fig. 3.21A) of the alkane showed the $(M-H)^+$ ion as the base peak, with a high abundance of fragment ions caused by cleavage of the molecule at the branch position ($C_{13}H_{27}$: 59%; $C_{19}H_{39}$: 74%). A number of synthetic C25 alkanes were analysed by CI-MS to determine the effect of alkyl branching on the $(M-H)^+$ ion abundance, and the extent of fragmentation. For synthetic 9-methyltetracosane (section 4.1.2) the $(M-H)^+$ ion was the base peak and the abundance of $C_{10}H_{21}$ (41%) and $C_{17}H_{35}$ (50%) ions was high (Fig. 3.21B). In addition, the $M^{+}-15$ ion was apparent, which in CI (and EI) mass spectra is usually attributed to methyl branching (Arsenault, 1972; Klomp, 1985). This ion was absent in the CI mass spectrum of 7-n-hexylnonadecane.

Authentic n-pentacosane (Aldrich Chem. Co.) was also examined by CI-MS and a high relative abundance of the m/z 351 ion was again observed in the spectrum (100, Fig. 3.21C). However, no significant enhanced abundance was noted for any fragment ions, and the $M^{+}-15$ ion was absent. This is consistent with the known fragmentation behaviour of n-alkanes under both electron impact and chemical ionisation conditions (Field et al., 1966; McLafferty, 1978).

Other C25 alkanes examined by CI-MS were synthetic 2,6,10,14,18-pentamethyleicosane (30, section 4.1.7) and 2,6,10,14-tetramethyl-7-(3-methylpentyl)-pentadecane [the latter synthesised in a previous study (Robson and Rowland, 1986)]. In both cases, the quasi-molecular ion $(M-H)^+$ though present was found in much reduced abundance in comparison to the monoalkyl and unsubstituted C25

FIG. 3.21 CHEMICAL IONISATION (ISOBUTANE) MASS SPECTRA OF AUTHENTIC C₂₅ ALKANES



alkanes (Fig. 3.22 A+B). This is typical of multiply branched alkanes; the intensity of the $(M-H)^+$ ion formed under CI conditions has been shown to decrease with increased branching (Harrison, 1983). The abundances of the $(M-H)^+$ ions of the acyclic isoprenoids were nonetheless much greater than their molecular ions obtained under conventional EI conditions, thereby providing additional diagnostic information for their structures (Table 3.8). The effects of branching were also evident from the fragment ions, though these were not as marked as those observed for the monoalkyl substituted isomers. Thus the regular isoprenoid was found to show an enhanced m/z 183 ion analogous with the EI mass spectrum (section 4.1.7), and the highly branched isoprenoid displayed an abundant m/z 238/239 doublet, again a feature of the EI mass spectrum (Robson and Rowland, 1986).

These optimised CI conditions appeared promising for the selective determination of monoalkyl substituted alkanes in a complex mixture, and to this end the urea non adduct was monitored by CI GC-MS using isobutane as the reagent gas. The contribution of impurities present in the gas to the total ion current profile was minimised by the exclusion of all masses below m/z 100 during data acquisition.

The total ion current of the CI GC-MS run compared well with the profile obtained by GC-FID analysis, and the positions of the quasi-molecular ions characteristic of acyclic alkanes $(C_nH_{2n+2} - H)^+$ were monitored by mass fragmentography (Fig. 3.23). This showed a series of compounds at each carbon number which were found to range from C22 (m/z 309) to >C32 (m/z 449), with the maximum normalised total ion count occurring in the fragment ion series belonging to a C24 acyclic

FIG. 3.22 CHEMICAL IONISATION (ISOBUTANE) MASS SPECTRA OF AUTHENTIC C₂₅ ALKANES

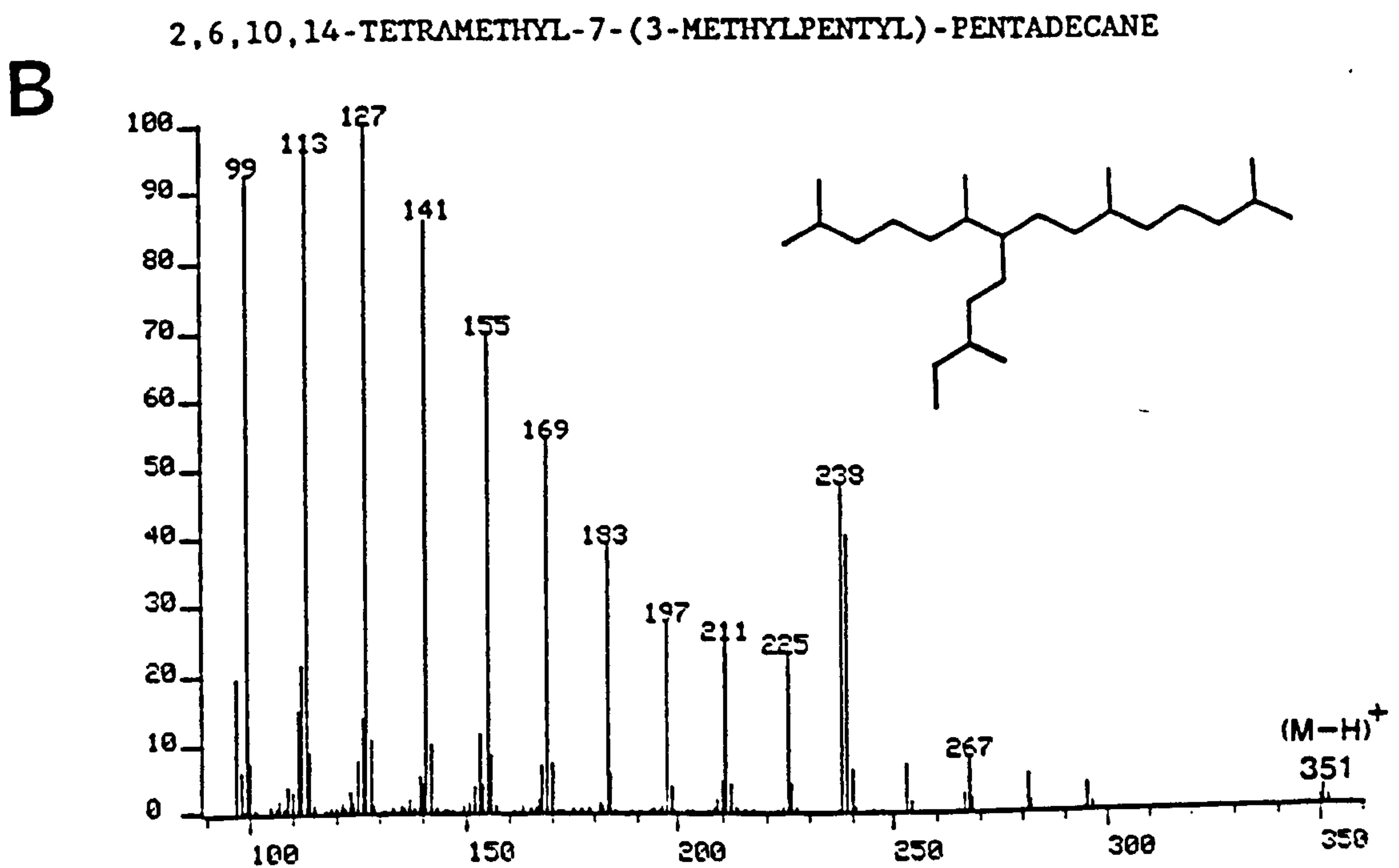
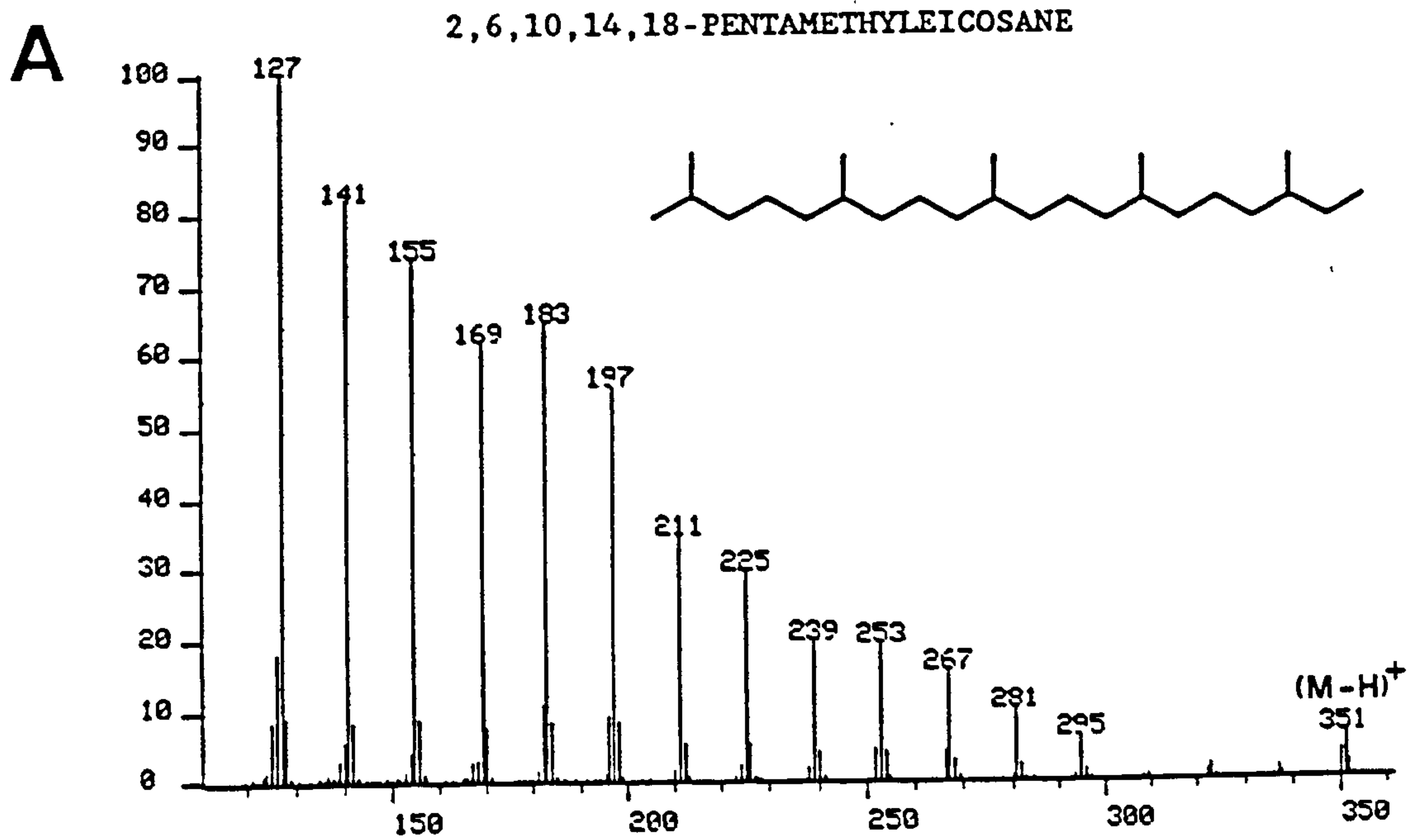


TABLE 3.8

A COMPARISON OF MOLECULAR AND QUASI-MOLECULAR ION ABUNDANCES
FOR ISOMERIC C-25 ALKANES DETERMINED BY EI AND CI MASS
SPECTROMETRY.

Compound	Relative % intensity	
	EI (M ⁺) ^a	CI(M-H) ^{+b}
<u>n</u> -pentacosane	5	100
9-methyltetracosane	0.3	100
7- <u>n</u> -hexylnonadecane	0.2	100
2,6,10,14,18-pentamethyleicosane	0.4	8
2,6,10,14-tetramethyl-7- ^c (3-methylpentyl)-pentadecane	0.0	1.5

a : obtained under standard electron impact conditions (ionising voltage : 40eV, source temperature : 230°C)

b : obtained under optimised CI ionising conditions (ionising voltage : 58eV, source temperature : 230°C, CI inlet gas pressure: - 0.67 bar)

c : Robson and Rowland, 1986

alkane. Extrapolation of the scan numbers of the major resolved peaks in the urea non adduct with their KI values provided approximate retention index ranges. Thus the compounds were found to elute in the approximate relative retention factor (RRF) range of -1.50 to -0.30 (Table 3.9; Kissin and Feulner, 1986). From published data and assuming that each compound is monoalkyl substituted, this could represent internally branched iso-alkanes with the length of the branch varying from C1 (i.e. methyl) to C4- C5 (i.e. butyl or pentyl) (Kissin and Feulmer, 1986). The presence of more highly substituted alkanes cannot be discounted however, since di- and trimethylalkanes are known to elute in the same approximate region (Kissin et al., 1986), and their behaviour under these CI conditions is not known.

Major peaks in each series of acyclic alkanes were found to comprise a homologous series whose estimated KI values varied by ± 4 units from those determined for the monomethyl alkanes identified in the urea adduct (section 3.2.1). A closer analysis by coinjection confirmed that these compounds are the same, and that they constitute a proportion of the resolved components of both fractions. Their abundance in the UNA is however greatly reduced.

Mass spectra showed a high abundance of quasi-molecular ions (as expected from the analysis of the model monomethylalkanes) though evidence of further branching and "background" was also present (Fig. 3.24).

Similar series of quasi-molecular ions corresponding to acyclic alkanes were noted for both the thiourea adduct and thiourea non

FIG. 3.23 CHEMICAL IONISATION (ISOBUTANE) GC-MS OF THE UREA
NON ADDUCT, SILKOLENE 150 LUBE OIL , ACYCLIC ALKANES.
(shaded peaks -monomethyl alkanes)

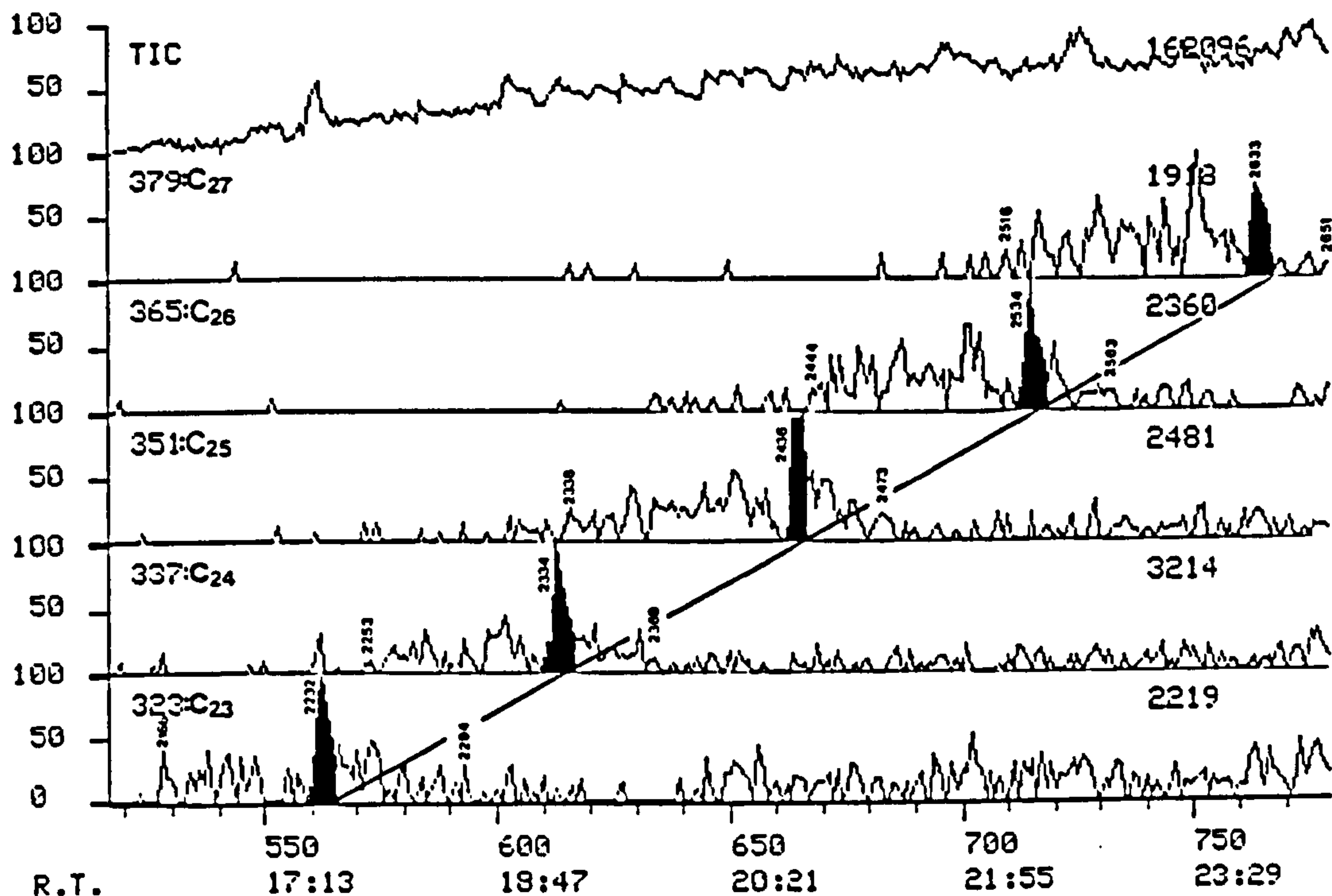


FIG. 3.25 CHEMICAL IONISATION (ISOBUTANE) GC-MS OF THE THIOUREA
ADDUCT, SILKOLENE 150 LUBE OIL , ACYCLIC ALKANES.
(shaded peaks -monomethyl alkanes)

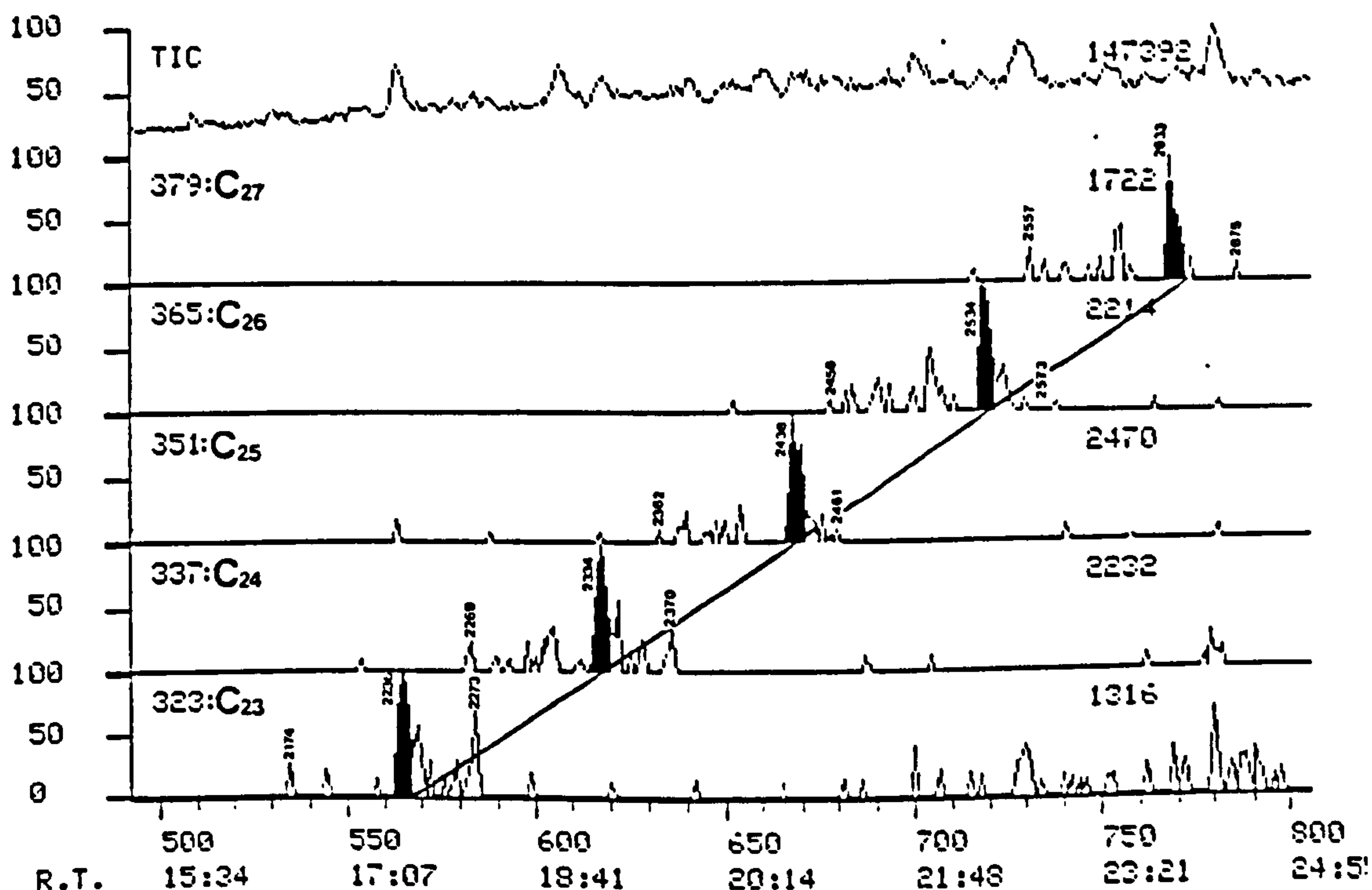
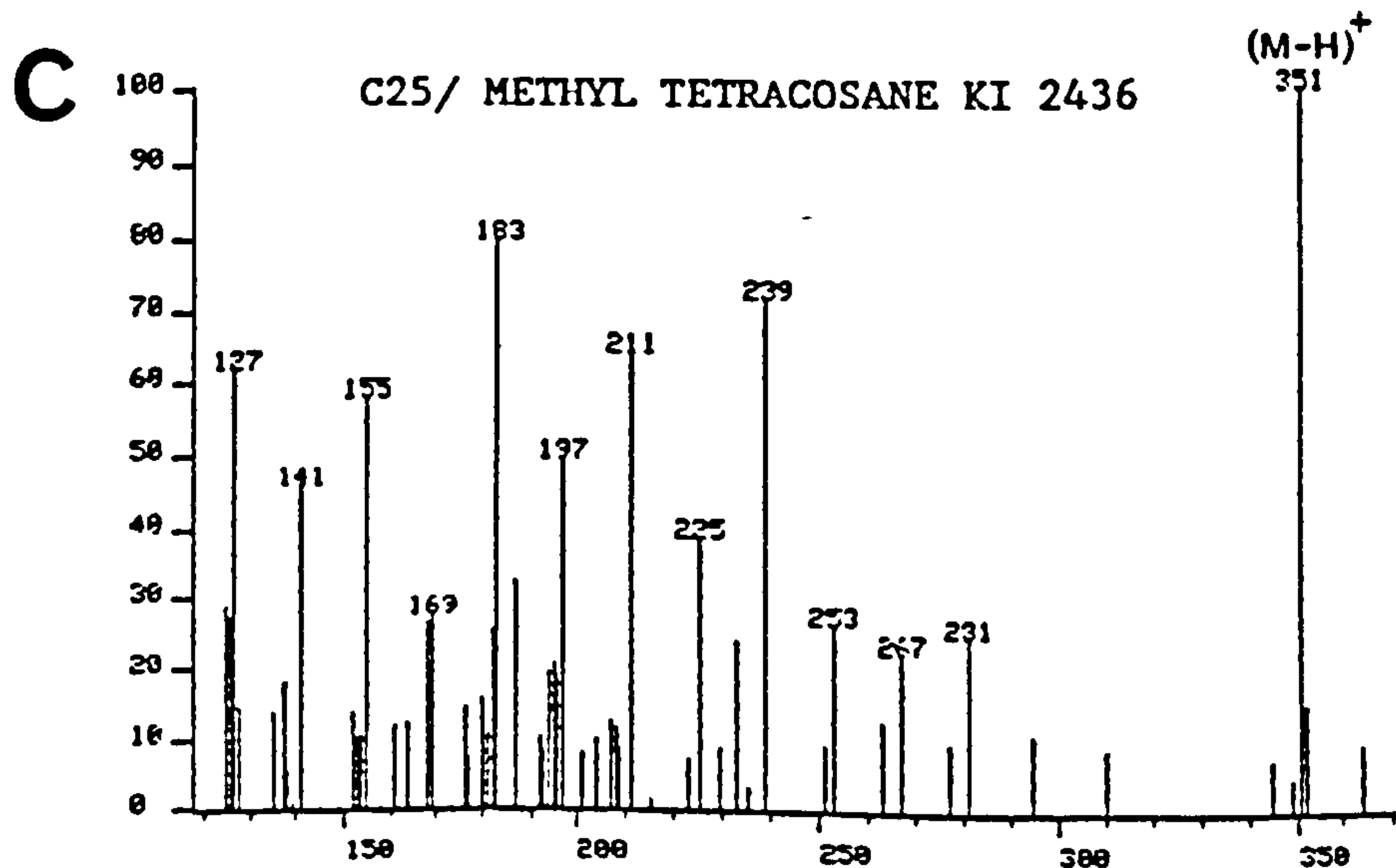
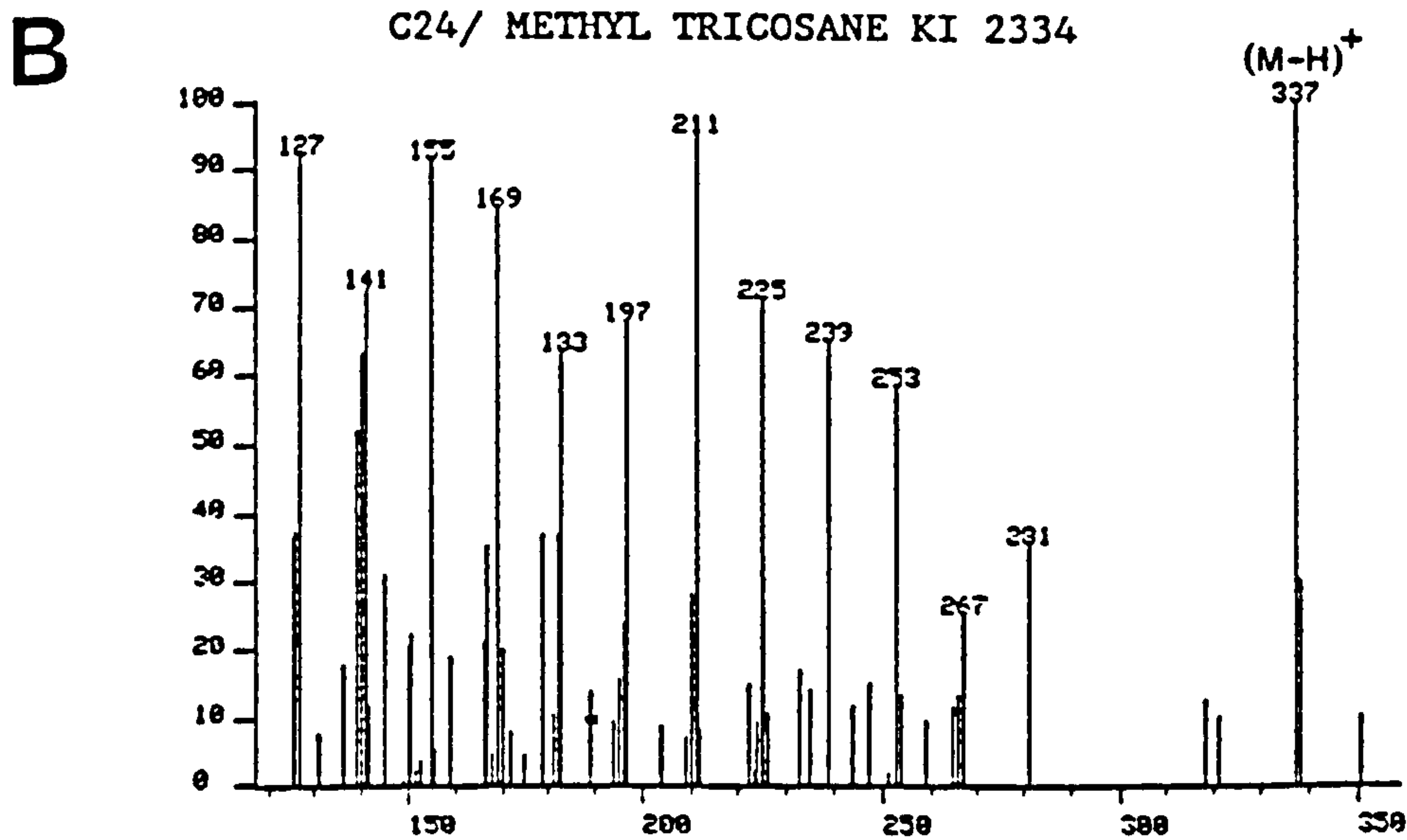
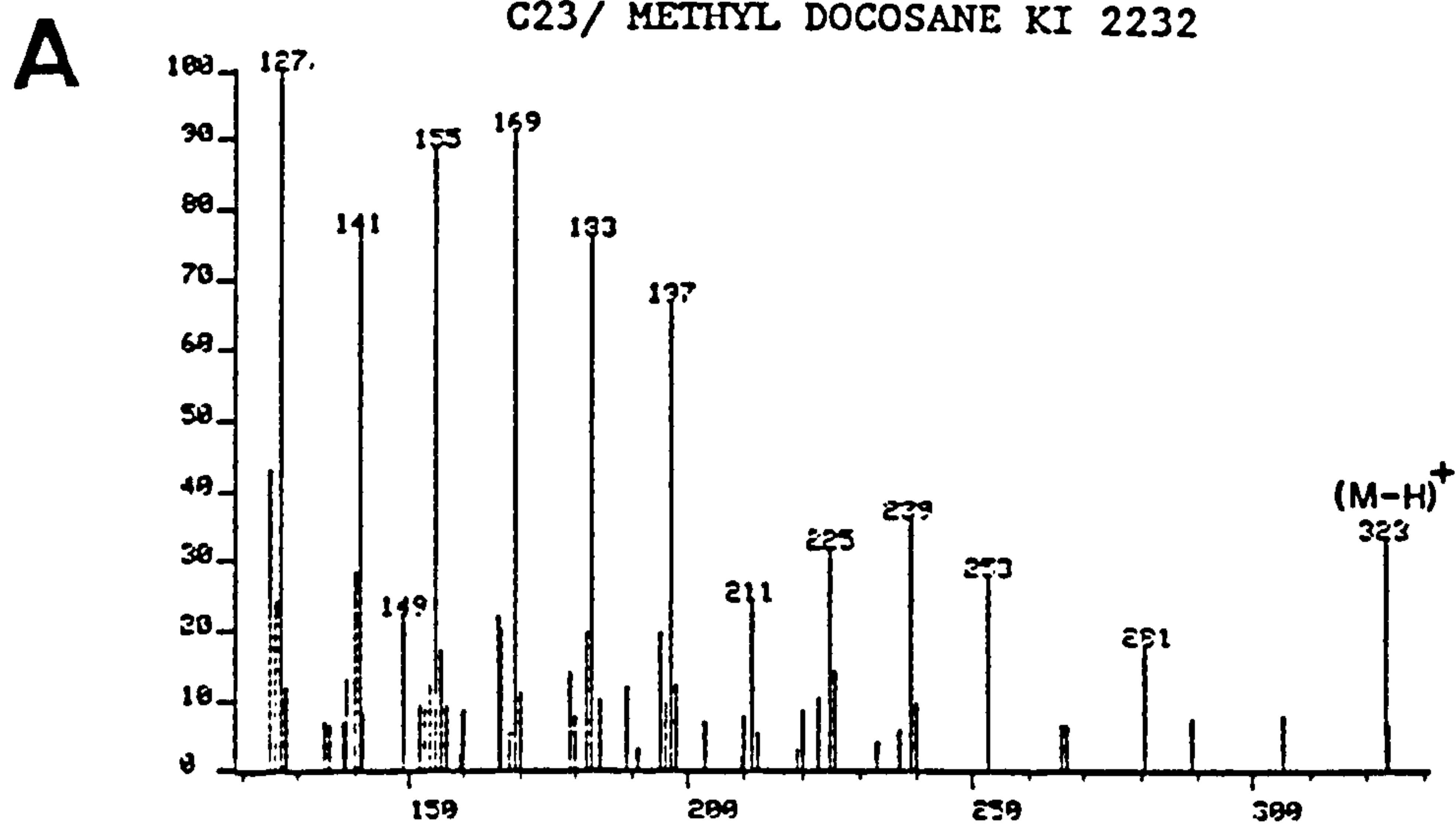


FIG. 3.24 CHEMICAL IONISATION (ISOBUTANE) MASS SPECTRA OF C23-C25 ACYCLIC ALKANES IDENTIFIED BY MASS FRAGMENTOGRAPHY OF THEIR QUASI-MOLECULAR (M-H)⁺ IONS



adduct subfractions of the urea non adduct. In the former a narrower series of isomeric compounds was noted at each carbon number (Fig. 3.25), though the same homology of monomethyl alkanes was identified. These appeared enriched in the thiourea adduct, and this was confirmed by GC. Monomethyl alkanes were also evident in the thiourea non adduct (Fig. 3.26), though in reduced abundance compared with lower eluting compounds. Since gas chromatographic retention decreases with the length of a monoalkyl side chain, the enrichment of lower eluting isomers in the TNA possibly represents the exclusion of longer alkyl substituents.

Similar series of compounds at each carbon number were observed when the quasi-molecular ions characteristic of monocyclic alkanes [i.e. $(C_nH_{2n-H})^+$] were monitored in each alkane fraction. These components were found in general to elute at higher retention index values than the corresponding acyclic alkanes, consistent with their observed elution order in the urea adduct (section 3.1.2). In the urea non adduct (Fig. 3.27) the monocyclic alkanes were found to elute in the approximate relative retention factor (RRF) range of -1.2 to +0.90, and though each profile was complex, a number of homologous series were identified by a comparison of extrapolated KI values. Thus homologies 1 and 3 (Fig. 3.27) coincided with series of alkylcyclohexanes identified by EI GC-MS of their m/z 83 ions, and by the same approach homologies 2 and 4 corresponded with the series of alkylmethylcyclohexanes identified from the m/z 97 mass fragmentogram. Again an enrichment of later eluting isomers was found in the thiourea adduct (Fig. 3.28) whilst lower eluting components (homology 4) were preferentially found in the thiourea non adduct (Fig. 3.29). This was confirmed by EI GC-MS (section

FIG. 3.26 CHEMICAL IONISATION (ISOBUTANE) GC-MS OF THE THIOUREA
NON ADDUCT, SILKOLENE 150 LUBE OIL , ACYCLIC ALKANES.
(shaded peaks =monomethyl alkanes)

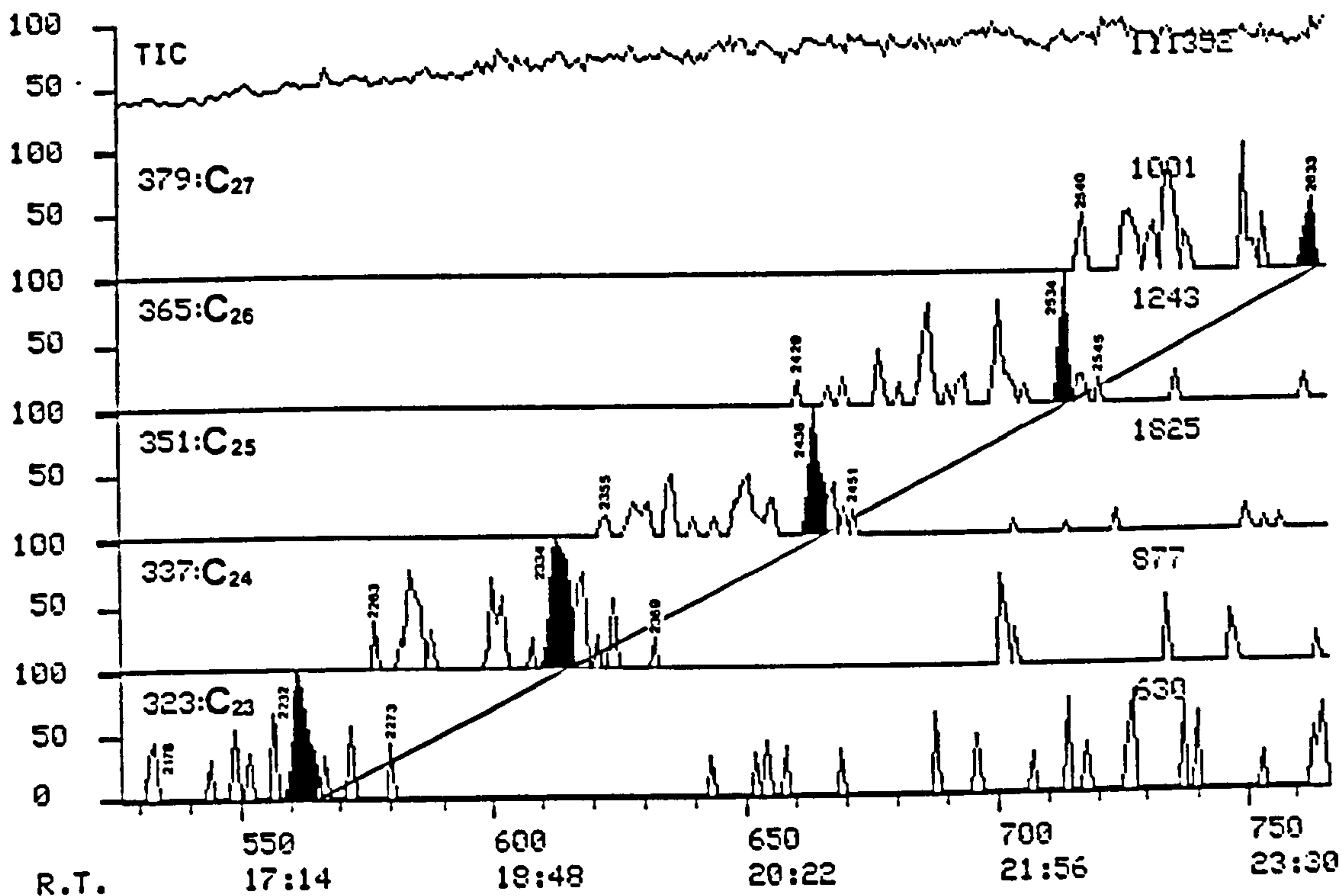
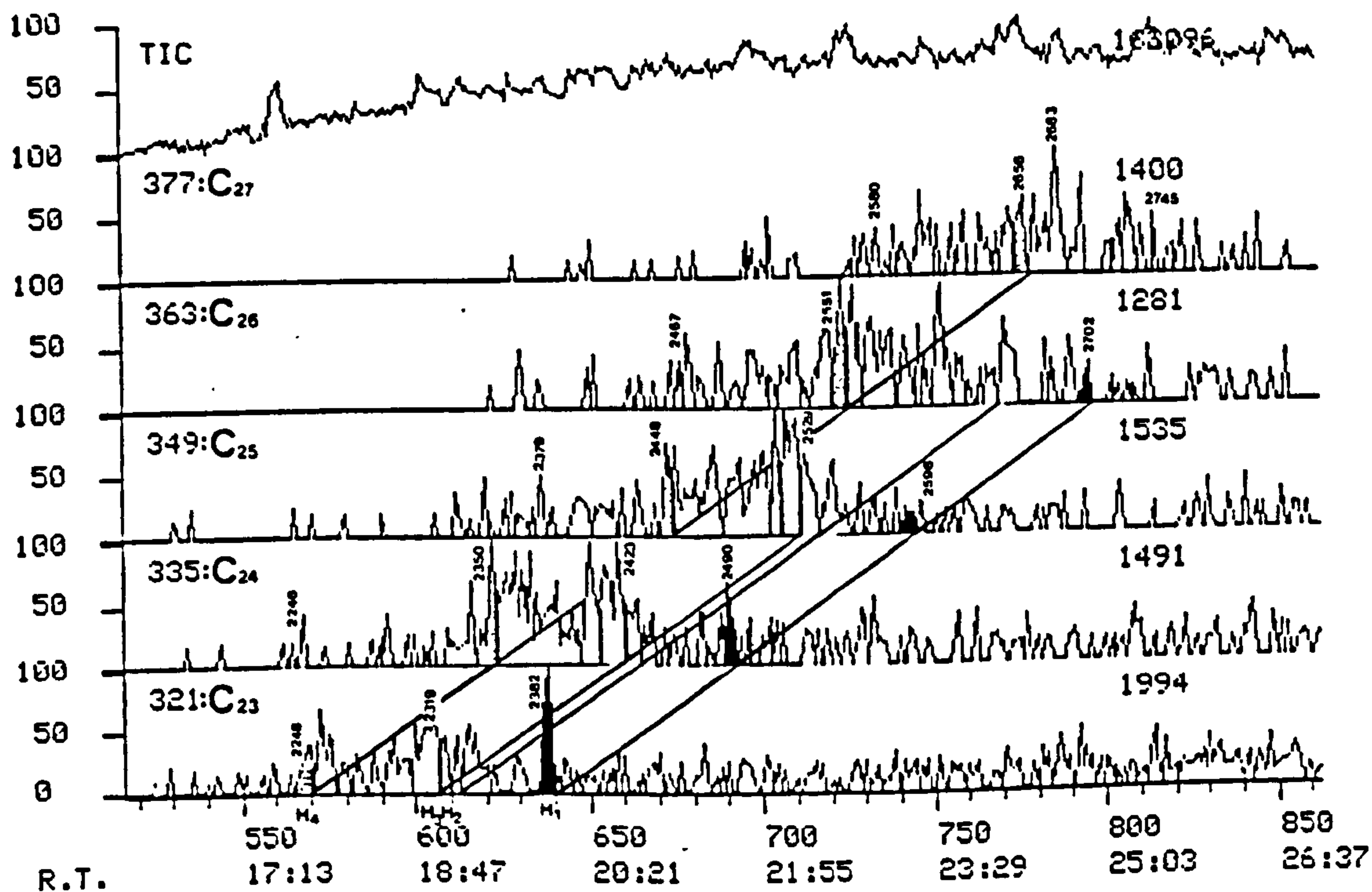
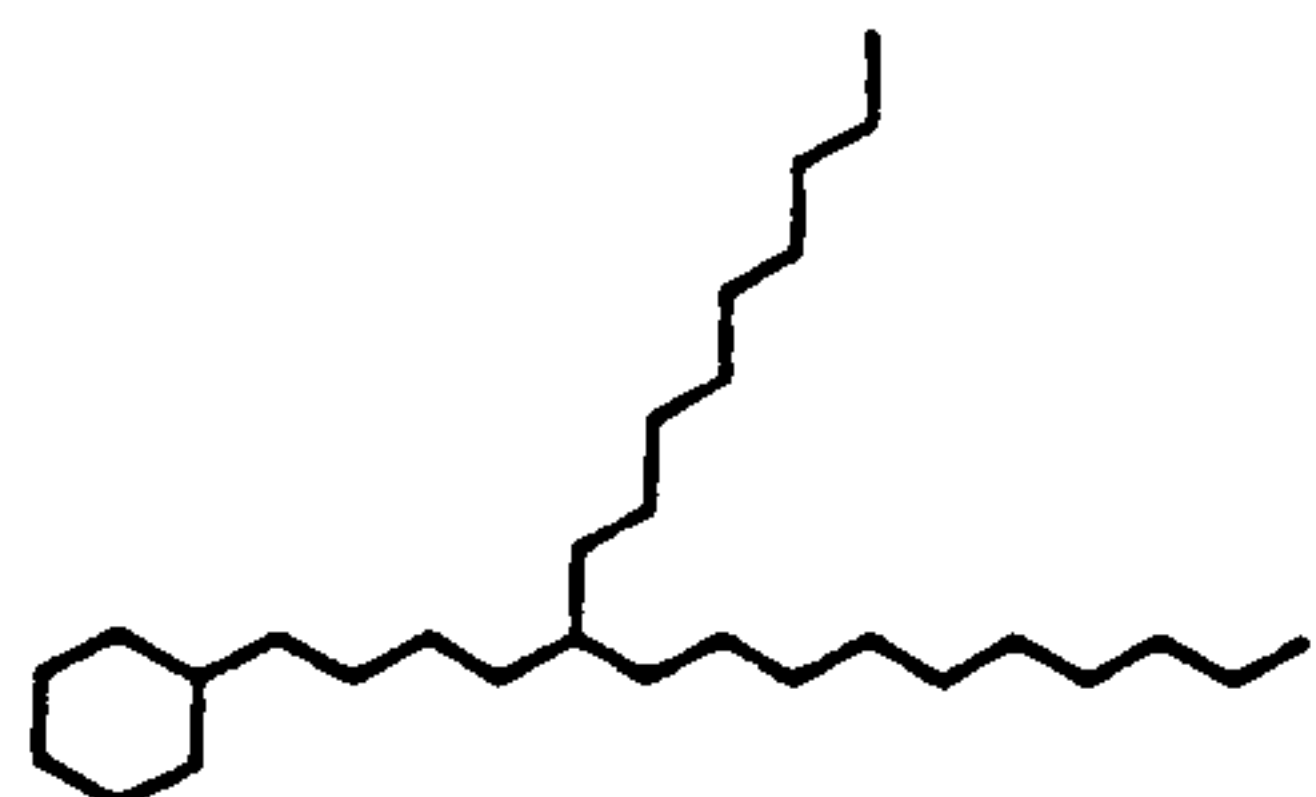


FIG. 3.27 CHEMICAL IONISATION (ISOBUTANE) GC-MS OF THE UREA
NON ADDUCT, SILKOLENE 150 LUBE OIL , MONOCYCLIC ALKANES.

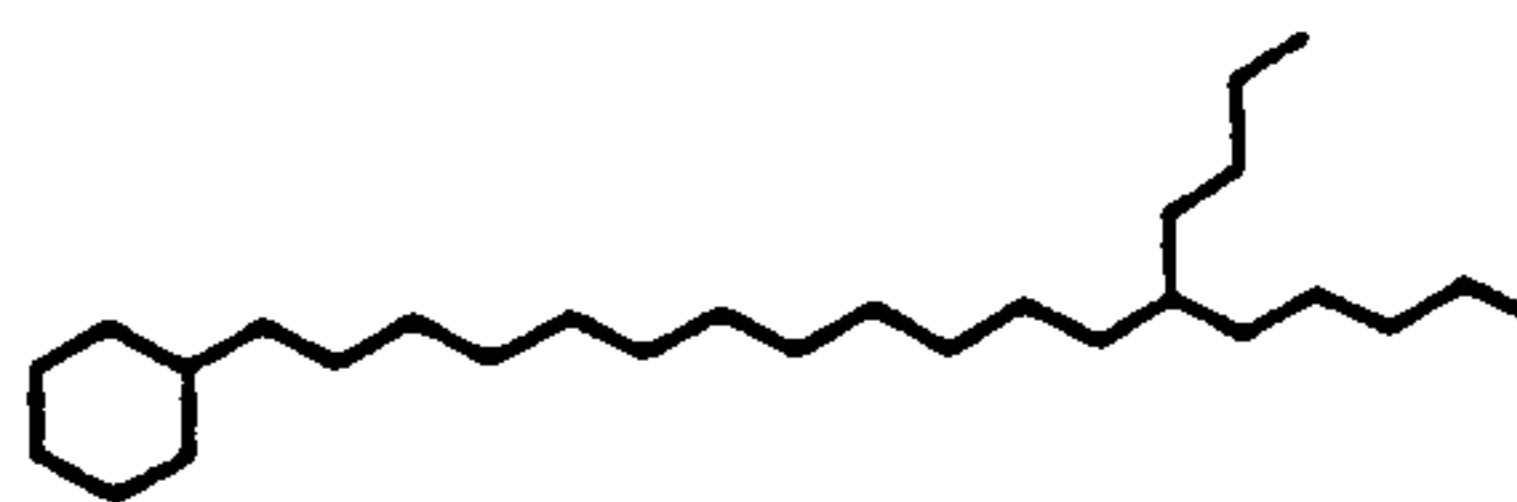


for peak identity see text (section 3.1.3.5)

3.1.3.1). By analogy with the observed distribution of acyclic alkanes monitored by CI GC-MS, this may represent exclusion of longer alkyl branched alkyl chains i.e.



LOWER ELUTING
(THIOUREA NON ADDUCT)



HIGHER ELUTING
(THIOUREA ADDUCT)

Indeed the synthetic monocyclic alkane 9-(2-cyclohexylethyl)-heptadecane with a C8 alkyl branch was not adducted by thiourea (section 4.1.5).

To summarise, the CI instrumental conditions optimised for 7-n-hexylnonadecane were found to produce abundant quasi-molecular $(M-H)^+$ ions for analogous simply branched or unbranched standard alkanes. Synthetic acyclic isoprenoid alkanes under the same ionising conditions did not show abundant quasi-molecular ions. When applied to the analysis of a complex alkane mixture, certain homologous series of compounds were identified by mass fragmentography of these ions. CI GC-MS therefore provided complementary and often confirmatory information for resolved series of monomethylalkanes, alkylcyclohexanes and alkylmethyl cyclohexanes; though in the main these appeared as resolved components of the samples analysed. CI GC-MS did provide however tentative evidence for the exclusion of long alkyl branched acyclic and monocyclic alkanes by clathration with thiourea.

3.1.3.6 ELECTRON IMPACT (EI) PROBE MASS SPECTROMETRY

High ionising voltage electron impact mass spectrometry as a method

FIG. 3.28 CHEMICAL IONISATION (ISOBUTANE) GC-MS OF THE THIOUREA ADDUCT, SILKOLENE 150 LUBE OIL , MONOCYCLIC ALKANES.

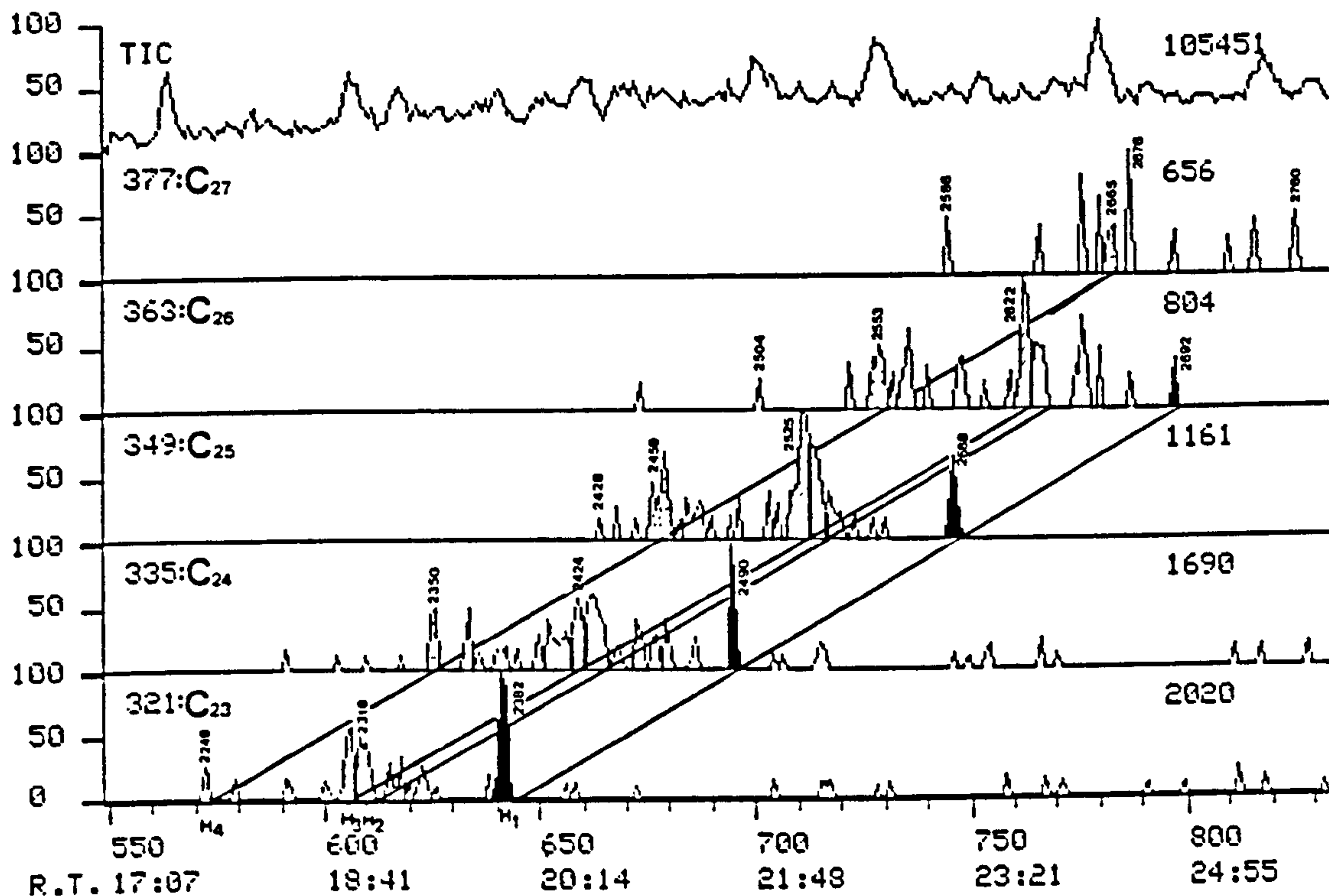
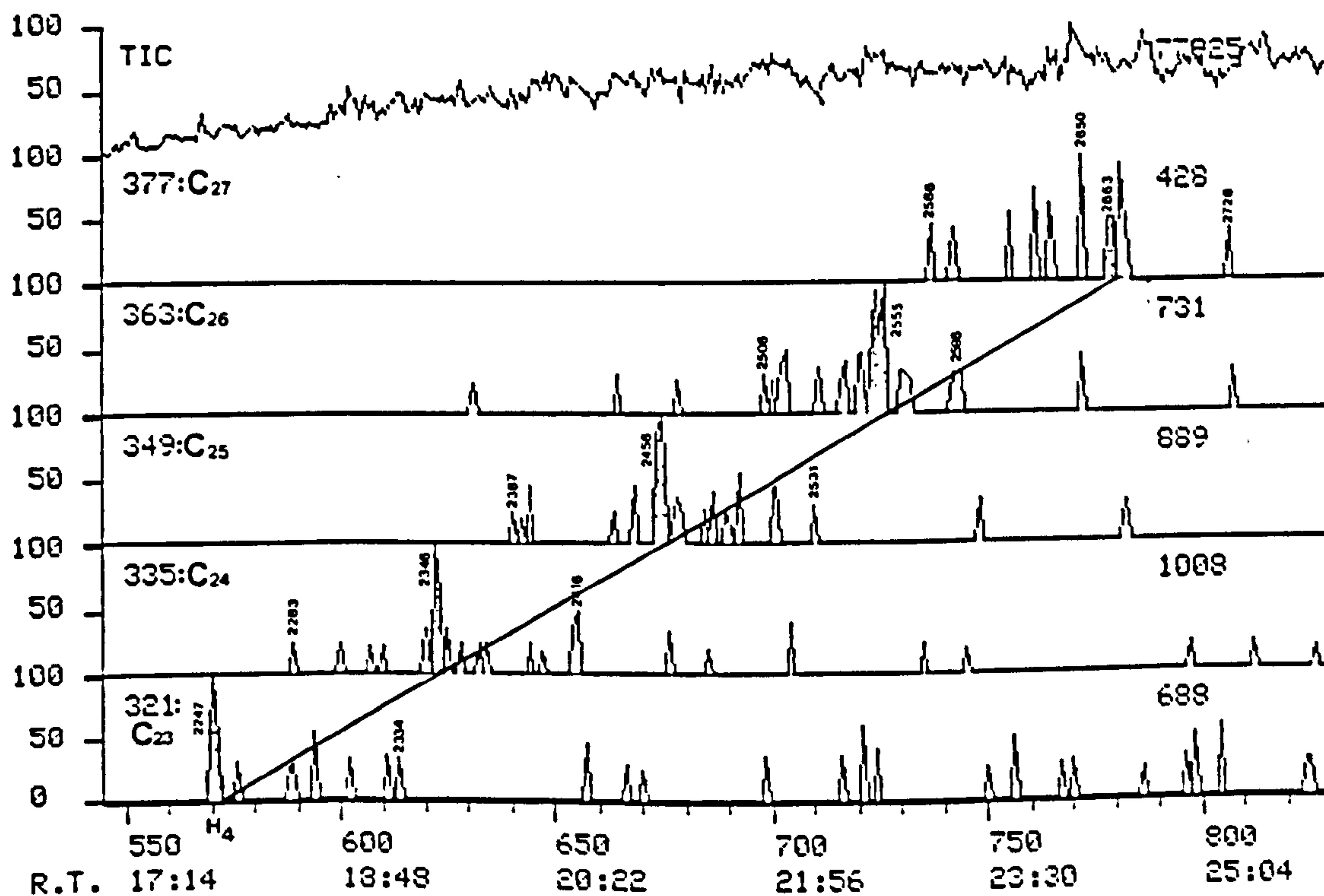


FIG. 3.29 CHEMICAL IONISATION (ISOBUTANE) GC-MS OF THE THIOUREA NON ADDUCT, SILKOLENE 150 LUBE OIL , MONOCYCLIC ALKANES.



for peak identity see text (section 3.1.3.5)

TABLE 3.9

APPROXIMATE RETENTION INDEX DATA DETERMINED FOR ACYCLIC ALKANES
IN THE UREA NON ADDUCT BY CI GC-MS

Carbon no.	approx elution ^a range (KI)	RRF range ^b	proposed length of monoalkyl ^c substituent
23	2166 - 2294	-1.34 to -0.06	C4 - C1
24	2253 - 2367	-1.47 to -0.33	C5 - C1
25	2336 - 2473	-1.64 to -0.27	>C5 - C1
26	2444 - 2563	-1.56 to -0.37	>C5 - C1
27	2516 - 2651	-1.84 to -0.49	>C5 - C1

a : estimated by coinjection of n-alkanes and interpolation of scan number.

b : $RRF = (KI \text{ iso alkane} - KI \text{ n- alkane})/100$; Kissin and Feulmer, 1986.

c : Cn values relate to possible length of monoalkyl substituent and are estimated from the graphical data provided by Kissin and Feulmer, 1986 (C1 - methyl, C2 -ethyl, C3- propyl etc)

of structural group analysis had been used for a wide range of complex hydrocarbon mixtures over many years (e.g. Coleman et al., 1973; Deroo et al., 1977; Claret et al., 1977). The method that is usually chosen for such analysis of saturated alkane fractions from crude petroleums and lubricating oils was originally developed by Hood and O'Neal (1959) and later adopted by the American Society for Testing and Materials (ASTM) as a standardised method (ASTM : D2786-81). The basis of the ASTM method (so-called "fragment-peak" method) is summation of the ion intensities of those mass fragments which are characteristic of acyclic and monocyclic though hexacyclic alkanes. This summation is then corrected for hydrocarbon type response by the use of inverted matrices painstakingly derived by Hood and O'Neal from the analysis of a wide range of authentic alkanes. By the use of these matrices only one standard alkane calibrant (n-hexadecane) is needed to calibrate the mass spectrometer prior to each analysis (Hood and O'Neal, 1959; ASTM: D2786-81).

Although this ASTM method has been used extensively in the petroleum industry, a recent "round-robin" interlaboratory study has shown certain deficiencies. From this it seems that modern mass spectrometers differ so much from those used by Hood and O'Neal (1959) to obtain the inverse matrices, that individual modern mass spectrometers may require extensive recalibration to give reliable quantitative data (Dr A. Herod, British Coal, personal communication).

Nonetheless since ASTM D2786-81 is one of the few mass spectral methods by which alkane ring class information can be obtained, it

was used herein for the examination of several alkane fractions obtained by molecular clathration of the Silkolene 150 lubricating oil total alkanes. The Kratos MS25 mass spectrometer was calibrated with n-hexadecane as recommended (ASTM: 02786-81) and after optimisation the summed peak intensities for ion series characteristic of acyclic alkanes (m/z 71,85,99 and 113) and monocyclic alkanes (m/z 69,83,97,111,125 and 139) were found to lie just outside the "acceptable range" (determined ratio: 0.17, acceptable range: 0.18 - 0.22). However, the recent "round-robin" study with modern mass spectrometers suggests the range may be extended to include this value (Dr A. Herod, personal communication).

Analysis of urea adduct, urea non adduct, thiourea adduct, and thiourea non adduct alkane fractions of the Silkolene 150 oil produced Gaussian total ion current (TIC) profiles. Mass spectra were taken at the maxima (Figs. 3.30 - 3.33). Summed partial ion intensities for each alkane class are presented in Table 3.10a, and these were used in published inverse matrix calculations to yield corrected partial ion intensities (Table 3.10b); assuming an average carbon number of 30. These calculations were performed by computer using a specially written BASIC program (Appendix II, B. Fairman, personal communication). Corrected partial ion intensities were then normalised and expressed as a weight percent of the total alkanes (Table 3.10c).

Values obtained for the urea adduct (73% acyclic alkanes, 19% monocyclic alkanes) appear reasonable given the abundance and distribution of methyl-alkanes observed by GC and GC-MS (ca. 83% acyclic alkanes), and the elemental analysis data which suggested a

FIG. 3.30 ELECTRON IMPACT PROBE MS OF THE UREA ADDUCT, SILKOLENE 150 LUBE OIL ALKANE FRACTION

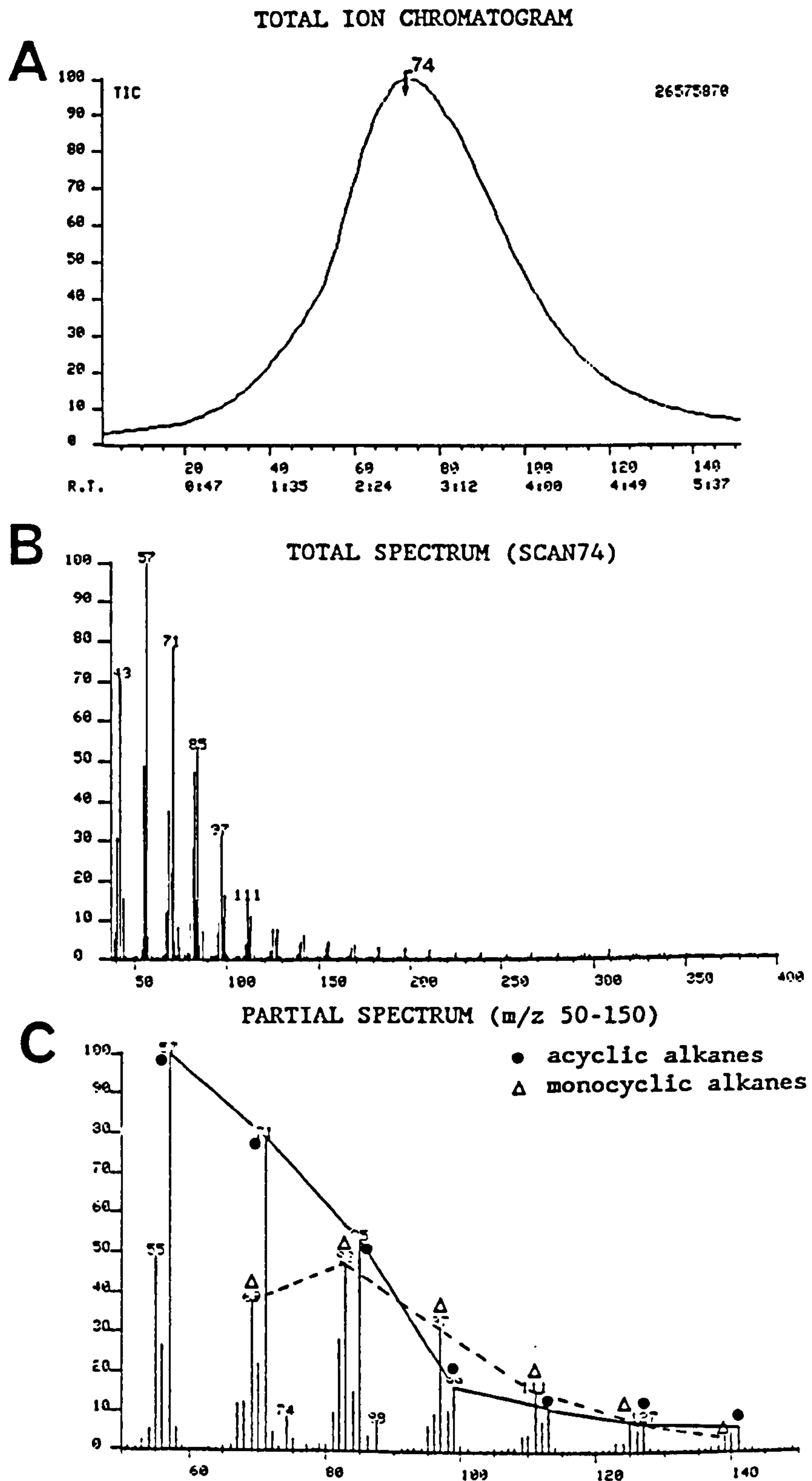


FIG. 3.31 ELECTRON IMPACT PROBE MS OF THE UREA NON ADDUCT,
SILKOLENE 150 LUBE OIL ALKANE FRACTION

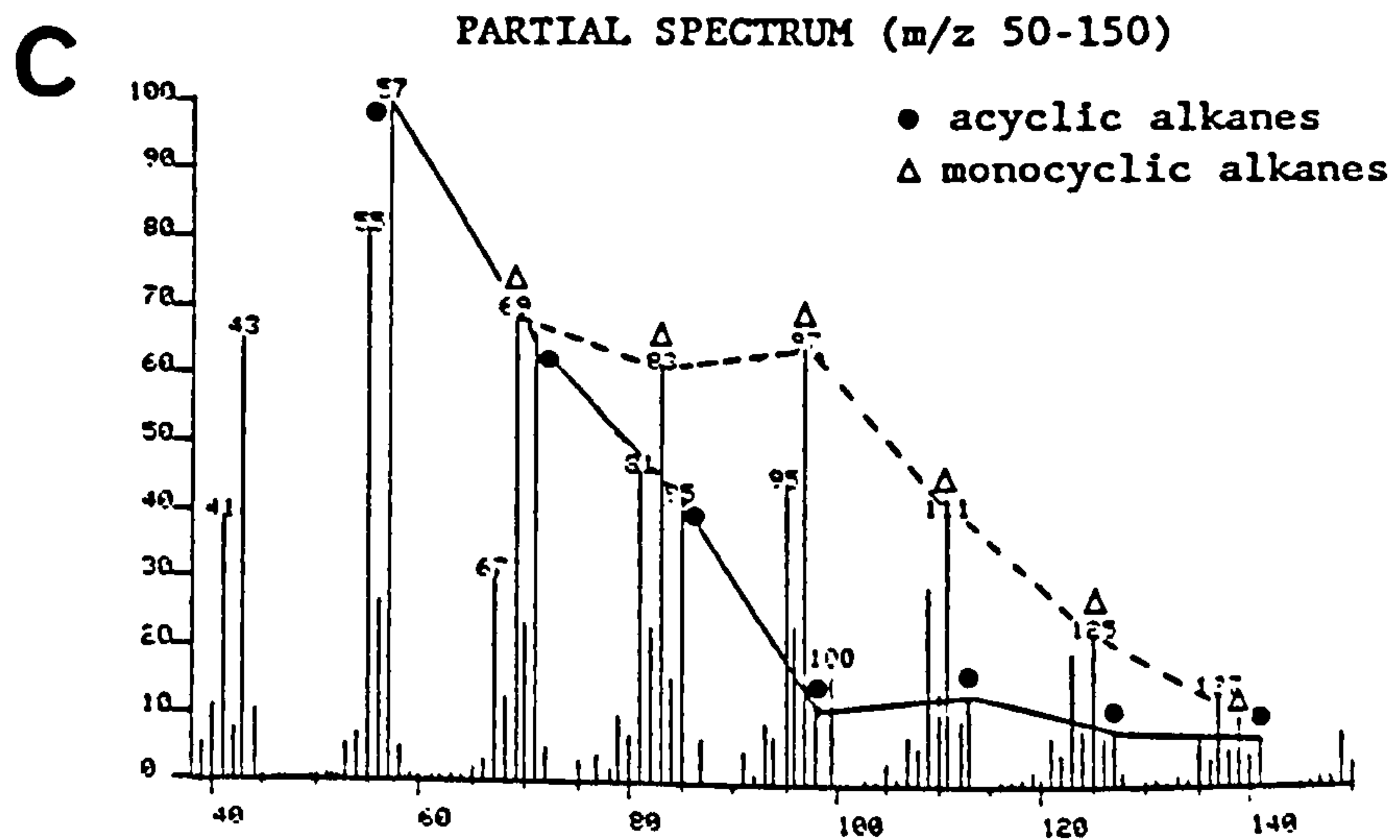
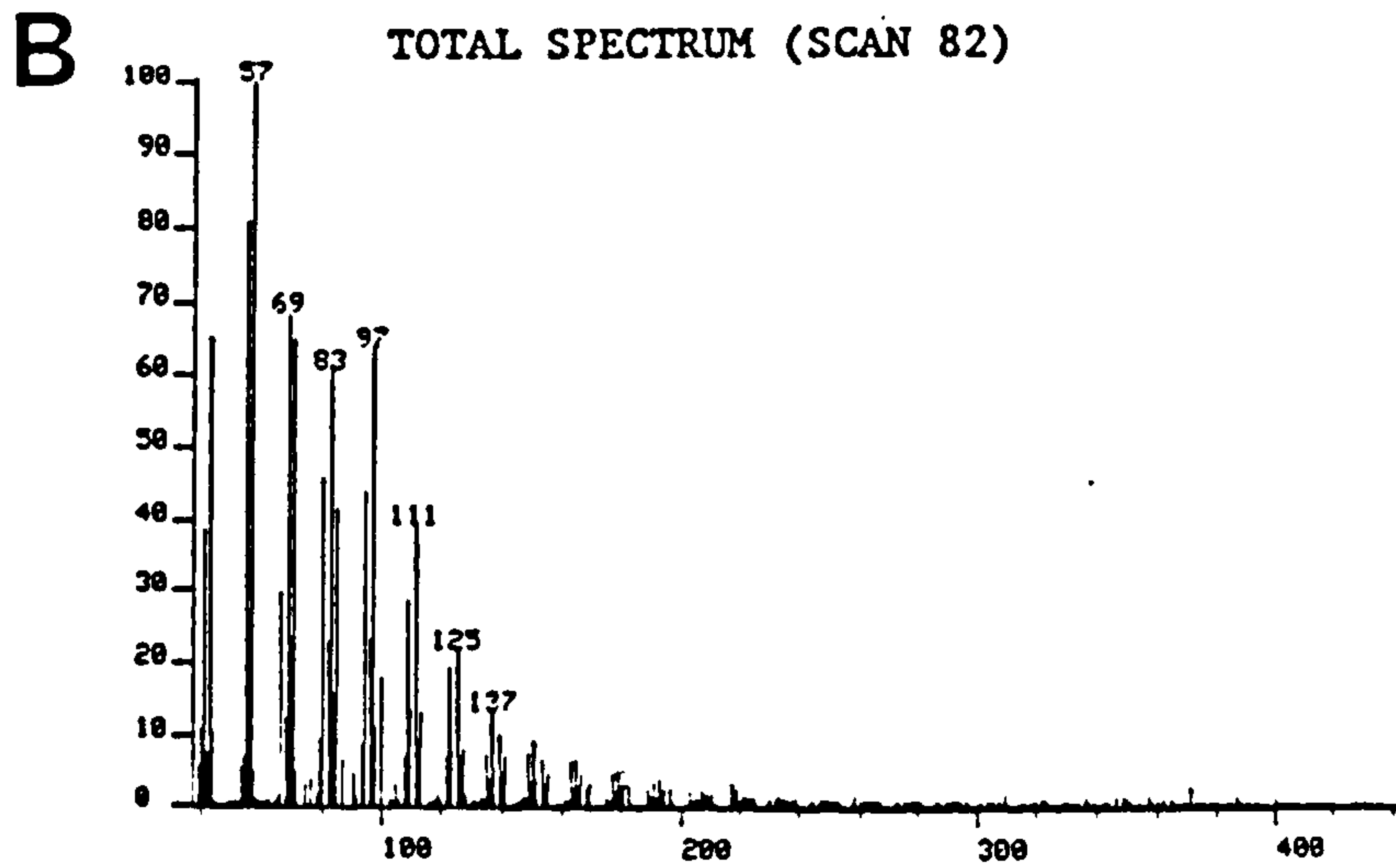
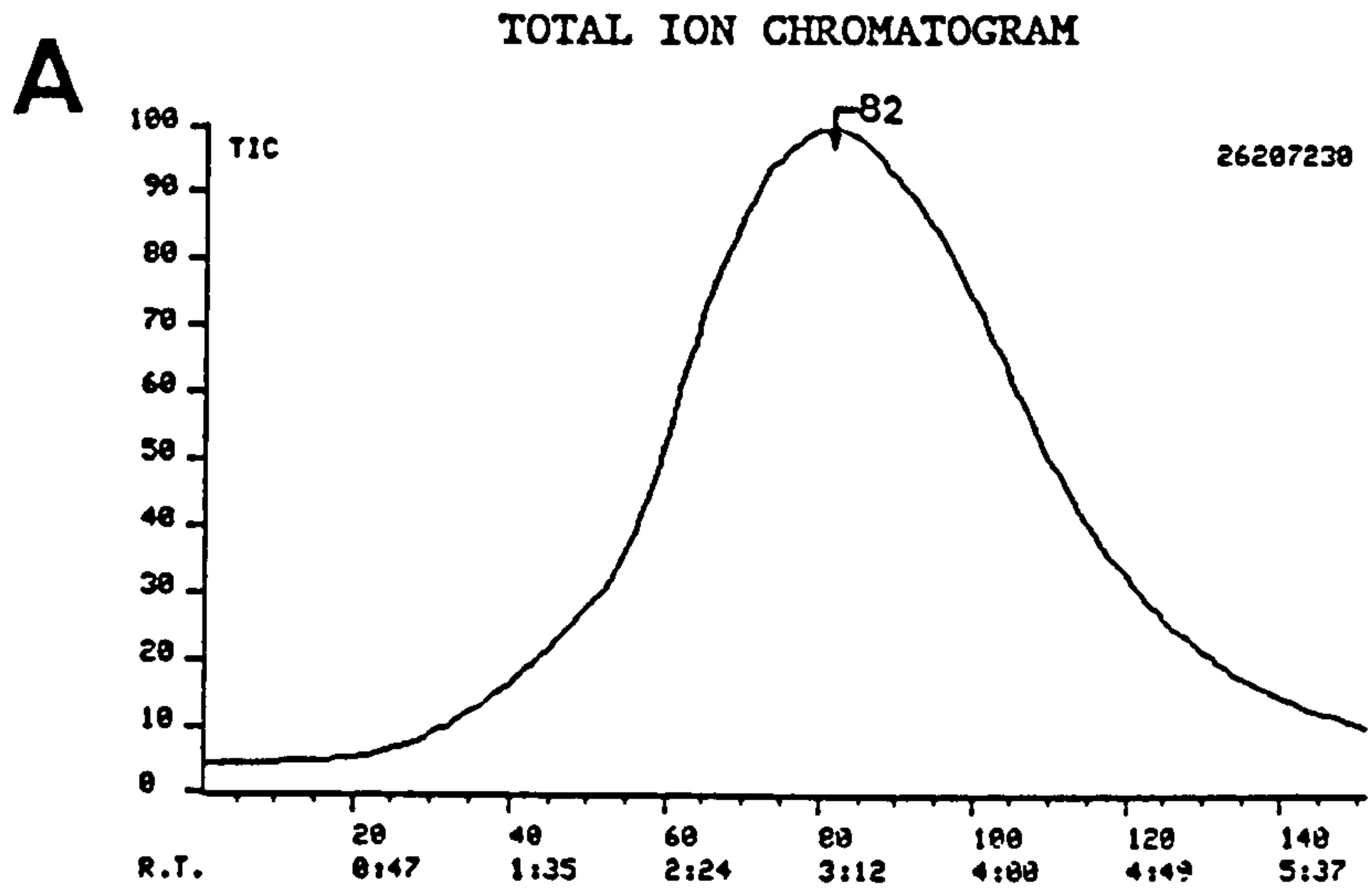
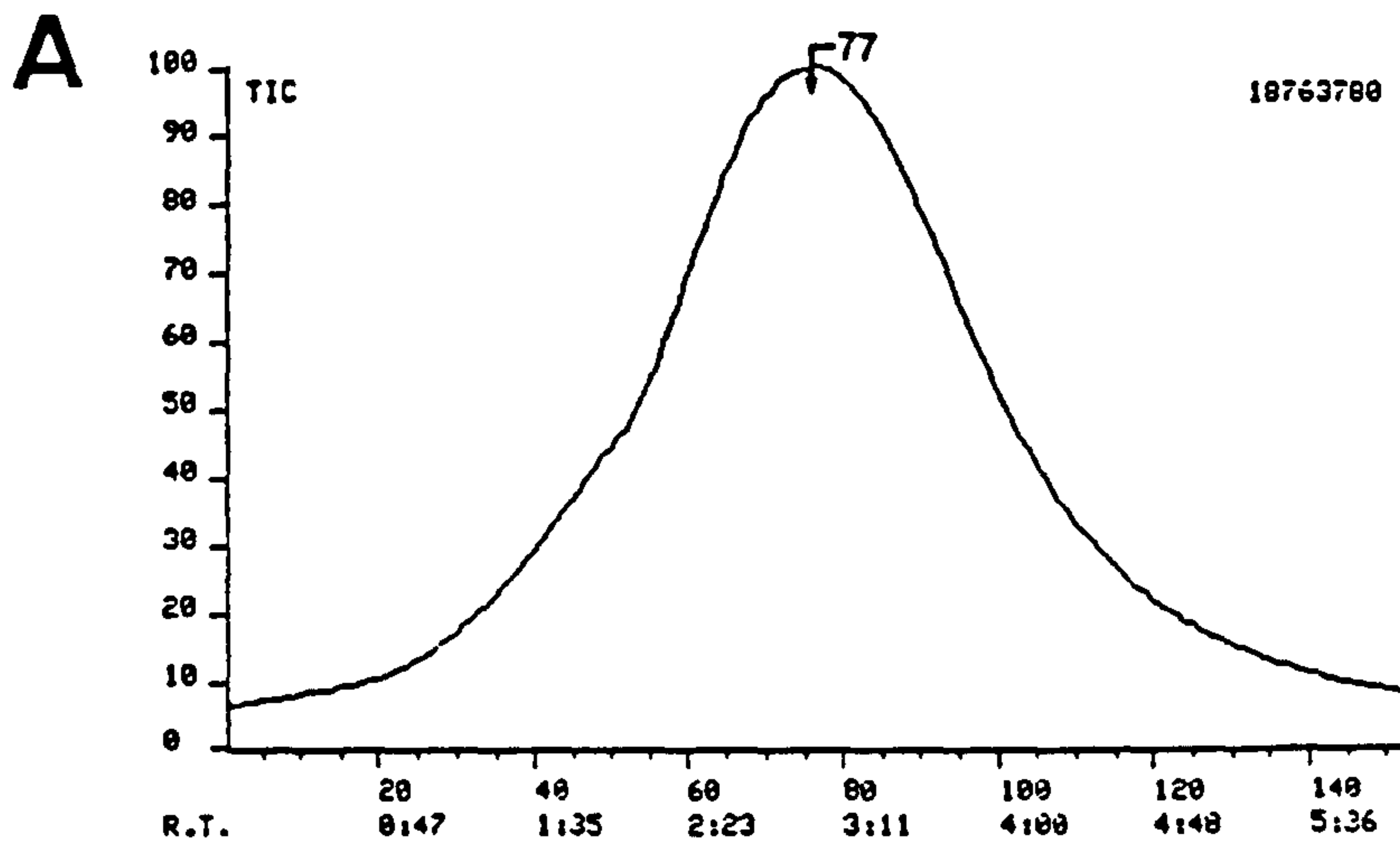
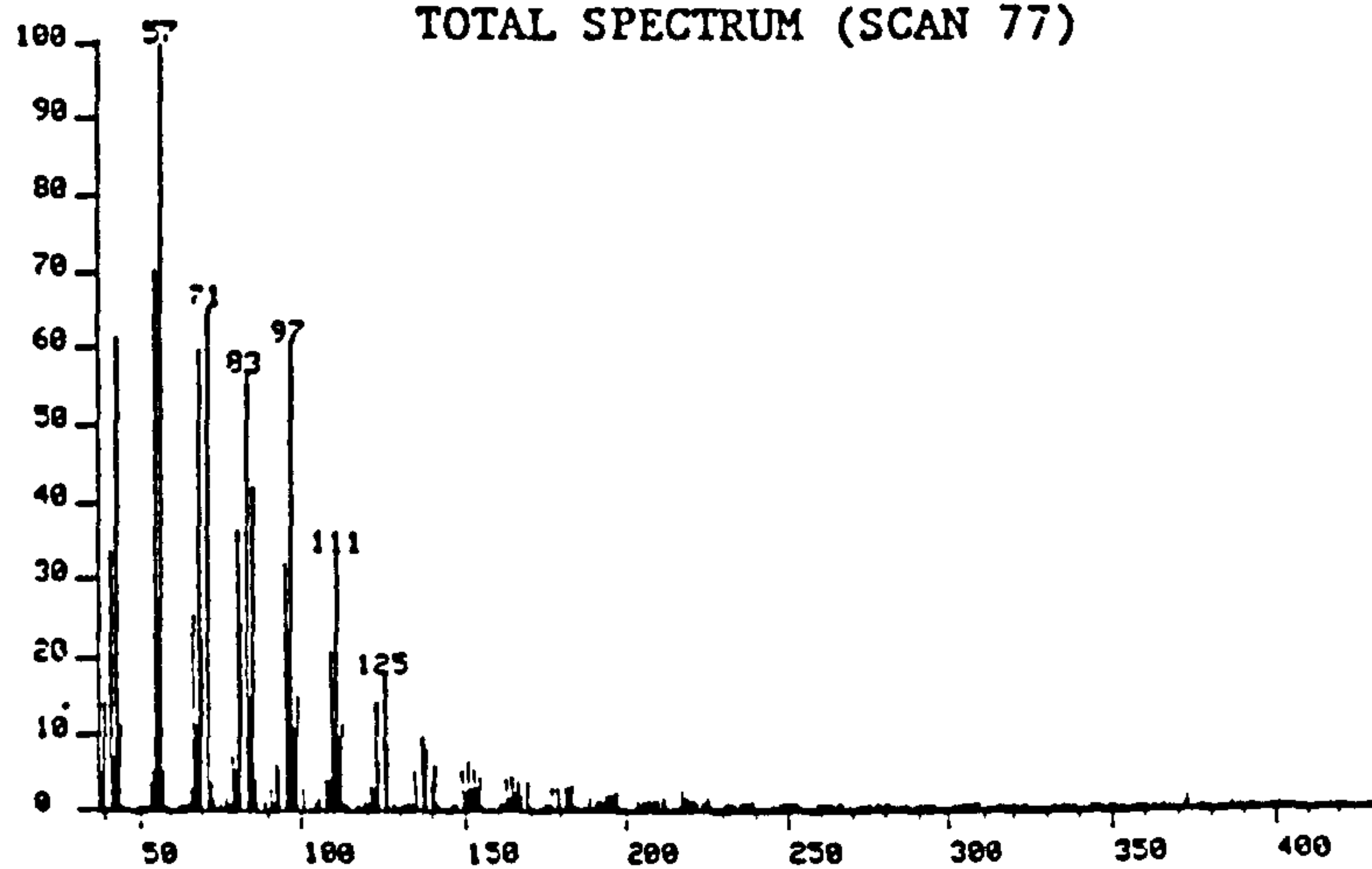


FIG. 3.32 ELECTRON IMPACT PROBE MS OF THE THIOUREA ADDUCT,
SILKOLENE 150 LUBE OIL ALKANE FRACTION

TOTAL ION CHROMATOGRAM



B TOTAL SPECTRUM (SCAN 77)



C PARTIAL SPECTRUM (m/z 50-150)

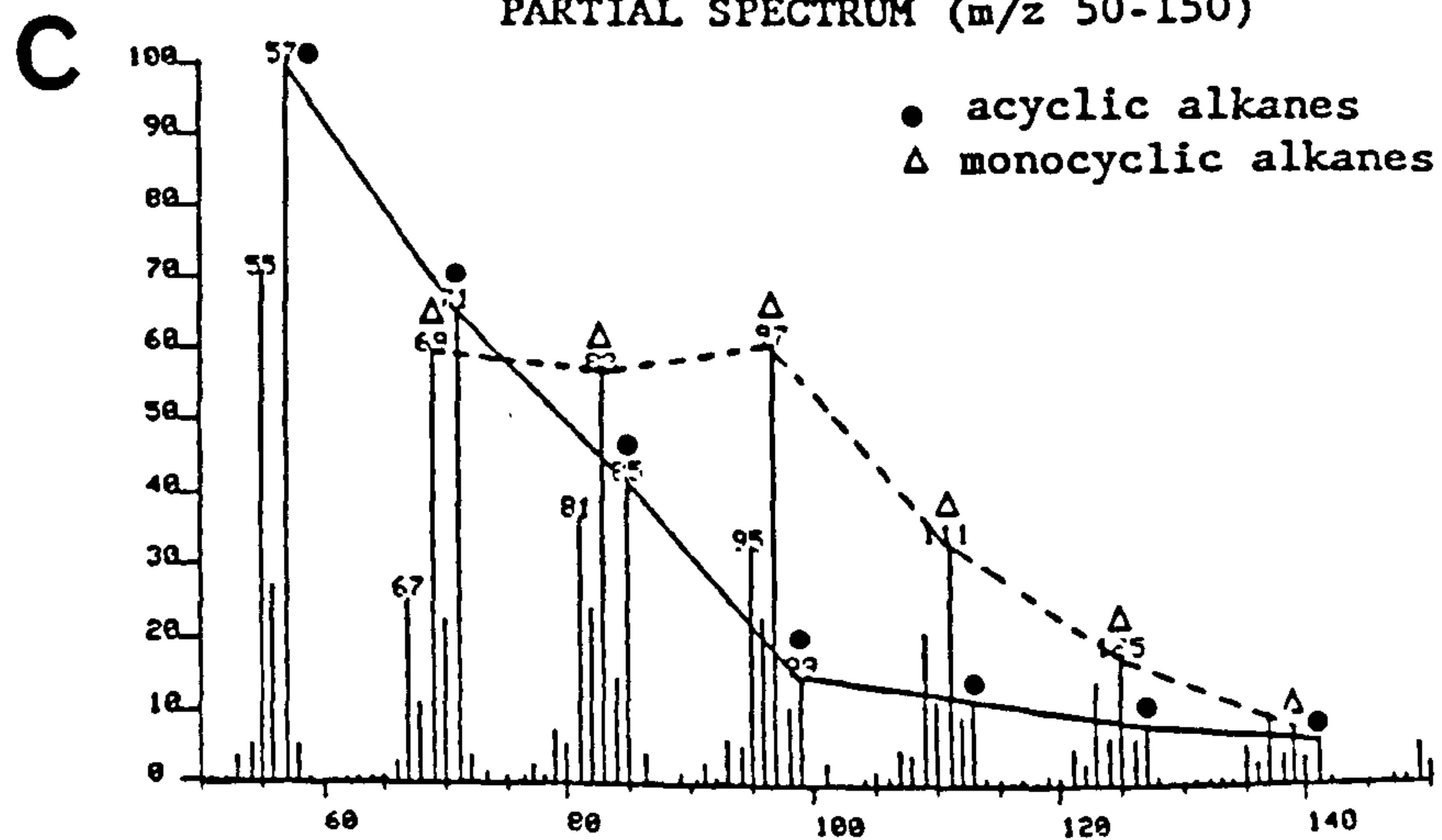
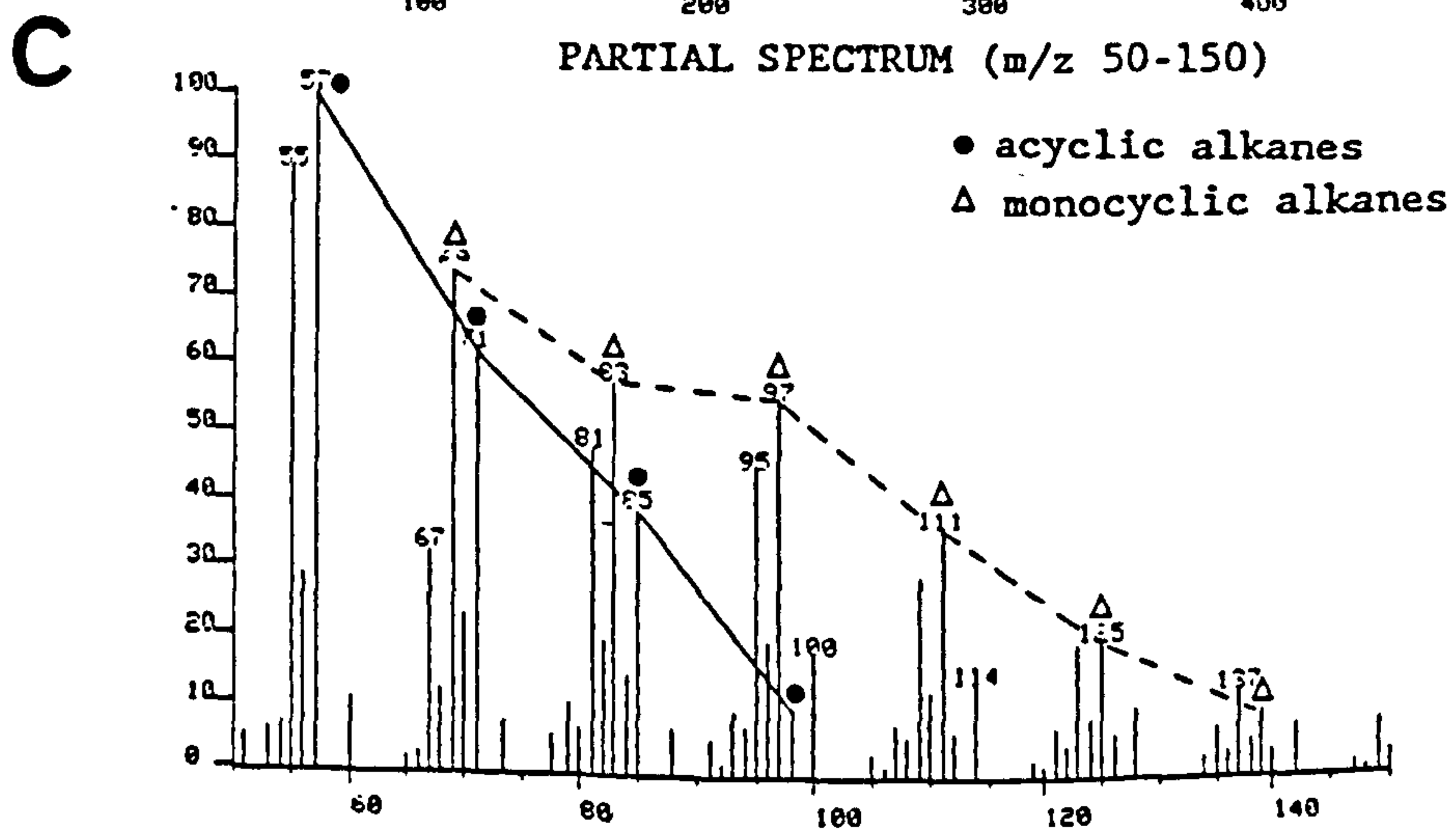
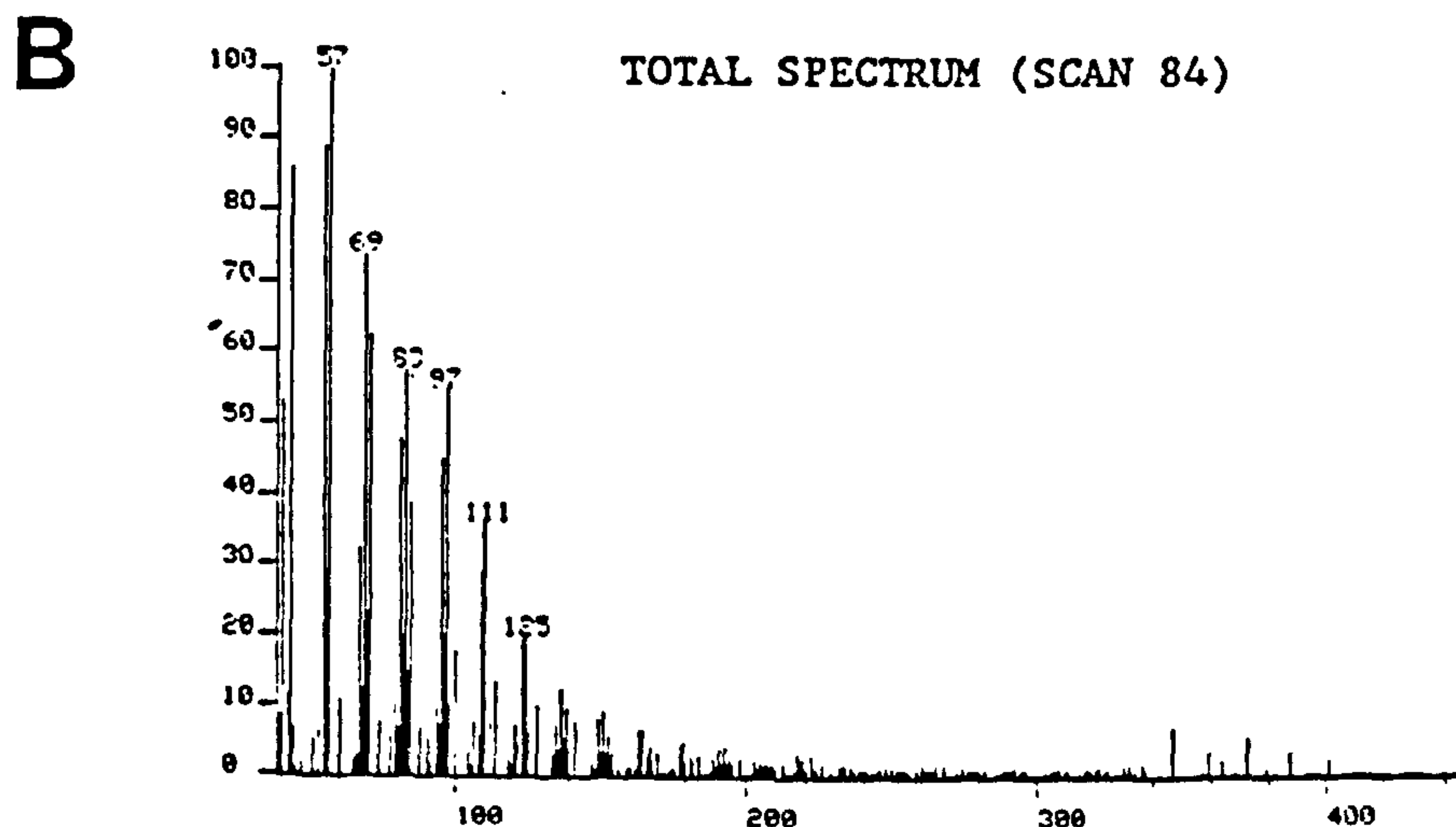
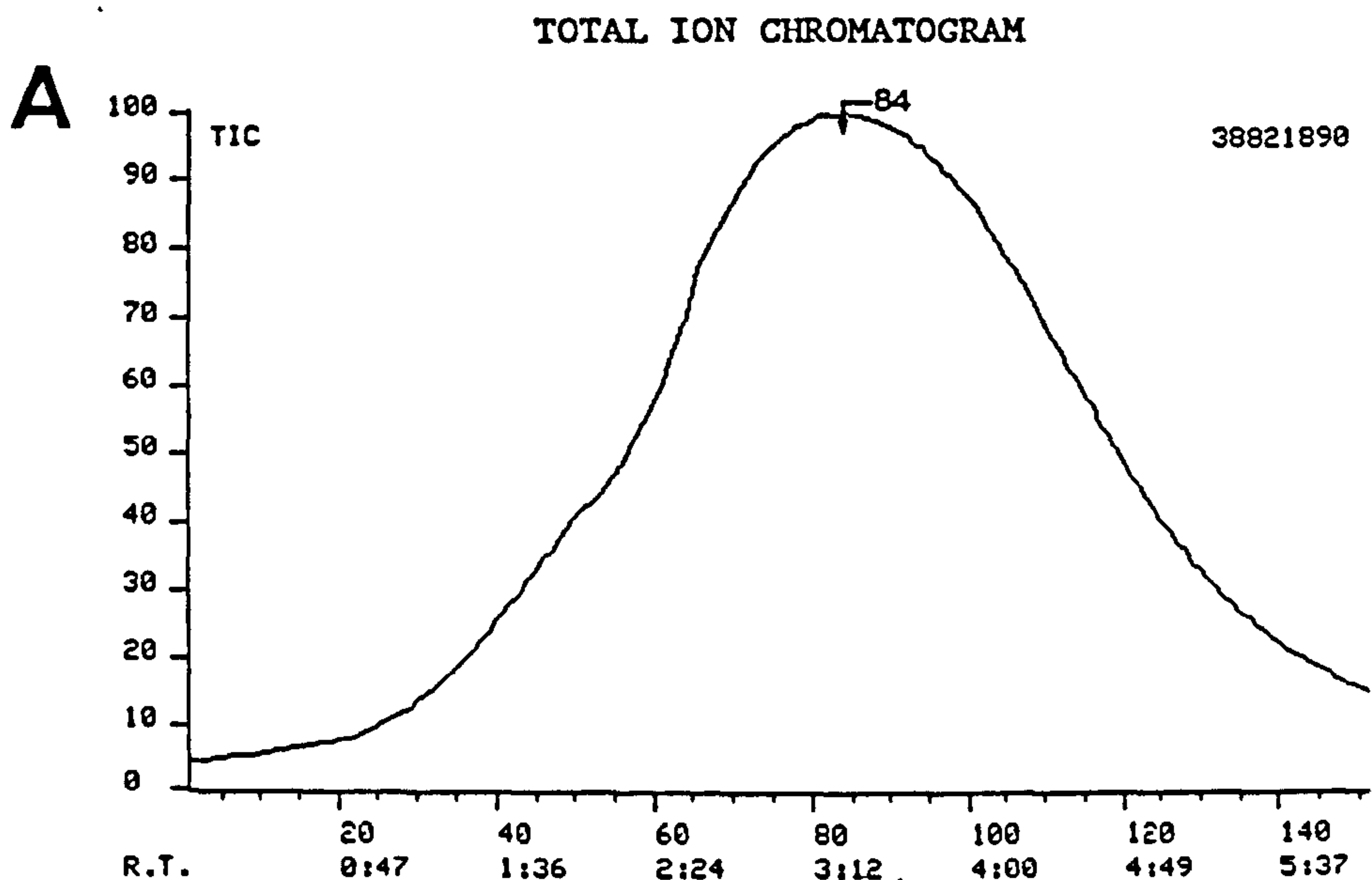


FIG. 3.33 ELECTRON IMPACT PROBE MS OF THE THIOUREA NON ADDUCT, SILKOLENE 150 LUBE OIL ALKANE FRACTION



high proportion of these alkane types (section 3.1.3.4).

The large contribution of acyclic and monocyclic alkanes to the urea nonadduct (83%) is also in agreement with the elemental analysis data which provided an average molecular formula of $C_nH_{2n+0.26}$ (c.f. acyclic alkane: C_nH_{2n+2} ; monocyclic alkane: C_nH_{2n+0}). Both methods provided evidence for the predominance of monocyclic alkanes.

The thiourea adduct fraction showed an enrichment in the proportions of 2-5 ring alkanes compared to the urea adduct and urea non adduct which is inconsistent with the data provided by elemental analysis ($C_nH_{2n+0.44}$). The reasons for this discrepancy are not known. The adduction behaviour of alkanes by thiourea is not fully understood (Mulheirn and Ryback, 1974), though the depletion of acyclic and monocyclic alkanes in the thiourea non adduct is as expected, with monocyclic alkanes again predominating over acyclic alkanes (Table 3.10c). These results are again supported by the elemental analysis data which showed evidence for a higher proportion of cyclic alkanes in this fraction ($C_nH_{2n-0.01}$).

In summary, probe EI mass spectrometry has shown that the Silkolene 150 lubricating oil urea non adduct fraction is comprised mainly of acyclic and monocyclic alkanes with lower proportions of higher ring alkanes. The ratio of acyclic vs. monocyclic alkanes is near unity but given the limitations of the ASTM method this quantitative result should be viewed as containing a wide margin of error. It is apparent that detailed information of alkane structural groups is beyond the scope of existing mass spectral methods.

TABLE 3.10

A) SUMMED ION INTENSITIES FOR EACH CHARACTERISTIC MASS GROUPING OBTAINED FROM THE 70eV IONISING VOLTAGE MASS SPECTRAL ANALYSIS OF ALKANE FRACTIONS

Sample	Summed ion intensities ($\times 10^3$)						
	(71	(69	(109	(149	(189	(229	(269
UA	4389	3941	275.946	78.432	37.619	15.105	13.022
UNA	2382	4596	1494.184	509.593	257.162	55.547	25.534
TUA	1906	3371	871.044	275.149	144.103	26.763	13.046
TUNA	568	6408	2159	763.590	283.687	33.913	10.968

B) CORRECTED PARTIAL ION INTENSITIES ($\times 10^3$)

Sample	(71	(69	(109	(149	(189	(229	(269	TOTAL
UA	2277.252	586.369	60.727	44.890	49.294	43.802	52.731	3115.065
UNA	944.077	1122.648	161.067	102.814	62.410	12.501	82.965	2488.542
TUA	786.965	679.495	352.409	169.033	232.529	75.713	60.209	2356.353
TUNA	825.385	1356.060	896.872	601.302	471.442	116.489	82.350	4349.900

C NORMALISED PARTIAL ION INTENSITIES EXPRESSED AS WEIGHT PERCENT

Sample	0-ring (71)	1-ring (69)	2-ring (109)	3-ring (149)	4-ring (189)	5-ring (229)	6-ring (269)
UA	73.1	18.8	2.0	1.4	1.6	1.4	1.7
UNA	37.9	45.1	6.5	4.1	2.5	0.5	3.3
TUA	33.4	28.8	15.0	7.2	9.9	3.2	2.6
TUNA	19.0	31.2	20.6	13.8	10.8	2.7	1.9

3.1.4. THE UREA NON ADDUCT BY CHEMICAL OXIDATION

Since conventional techniques for characterisation of the aliphatic UCM provided only limited information, alternative methods were sought. Chemical degradation was attractive, in view of its success in the characterisation of many varied and complex organic sample matrices, yet the choice of method was limited due to the relative stability of C-H and C-C single bonds, and hence lack of reactivity that characterises alkanes (March, 1984). Indeed, the name paraffin - derived from the latin *parum affinus* meaning "little affinity" - is used to describe this inert class of hydrocarbons (Finar, 1981). Of the few reactions known, oxidation is perhaps the most widely studied in view of its importance in synthetic organic chemistry, yet relatively few oxidants are available that are : i) powerful enough to break fully saturated C-H and C-C single bonds and ii) capable of selective functionalisation of alkanes (Gretz et al., 1987). Amongst these, derivatives of hexavalent chromium (CrVI) and heptavalent manganese (Mn VII) are most common (e.g. see reviews by Wiberg, 1965; Lee, 1980; Cainelli and Cardillo, 1984; Freeman, 1986). In particular, chromium species have been used with success in the classical chemical structural characterisation of methyl groups in aliphatic and aromatic hydrocarbons (Kuhn and Roth, 1933; Brandenberger et al., 1961; Cason et al., 1959) and in the determination of steroid side chains (Barbier and Locquin, 1913). Chromium has also been used in organic geochemistry for the characterisation of kerogen (Simoneit and Burlingame, 1974); and in a series of studies aimed at determining the absolute stereochemistries of acyclic isoprenoids from a variety of geological environments (e.g. Cox et al., 1972; Maxwell et al.,

1972; Brooks et al., 1977; Patience et al., 1978, 1979; Rowland and Maxwell, 1983). Given these successes this method was chosen for the oxidation of the aliphatic UCM. It was hoped that oxidation of the UCM alkanes would yield functionalised (i.e. O-containing) products which could be separated from unoxidised alkanes by conventional preparative chromatography. By GC and GC-MS analysis of the compounds produced, the molecular nature of precursor UCM alkanes could then be postulated.

In the initial phases of this research the method of Brooks et al., (1977) was applied to samples of aliphatic UCM obtained by dearomatisation and urea adduction of the alkane fraction of a commercially available lubricating oil (20 W 40, Dulton). Approximately 50mg of sample was oxidised in a mixture of CrO₃ (250mg) in glacial acetic acid (10cm³) for 5, 30 or 60 minutes at 70°C. The products and residual alkanes were extracted with hexane, hydrolysed, and methylated prior to analysis by GC. With increasing time of reaction the yield of total recovered material was found to decrease (Table 3.11) though a gradual increase in the formation of a series of resolved compounds eluting prior to residual UCM alkanes was noted (Fig. 3.34). These were identified by GC-MS (after preparative TLC) as C13- C24 (max. C17) n-alkanoic acids (methyl esters, Fig. 3.35). This was surprising in view of the proposed highly branched and/or cyclic nature of the UCM alkanes (Farrington et al., 1977). The products obtained however were not thought to be representative of the UCM as a whole in view of the low yields which were nonetheless typical for the procedure (e.g. Patience et al., 1979).

TABLE 3.11

PRELIMINARY OXIDATION^a EXPERIMENTS OF THE ALIPHATIC UCM, DULTON 20 W 40
LUBRICATING OIL, CrO₃/GLACIAL ACETIC ACID - HEXANE EXTRACTANT

Sample No.	mass(mg)	mass CrO ₃ (mg)	ratio oxidant: substrate	time (min)	yield [mg(%)]
1	52.2	250	17:1	5	23.8(45)
2	63.9	250	13.8	30	26.5 (42)
3	52.5	250	16.8	60	15.7 (28)

a: all oxidations in glacial acetic acid (10cm³) at 70±2°C

TABLE 3.12

MODIFIED OXIDATION PROCEDURE APPLIED TO THE ALIPHATIC UCM FROM THE SILKOLENE 150
LUBRICATING OIL, CrO₃/GLACIAL ACETIC ACID - DCM EXTRACTANT

Sample no.	mass(mg)	mass CrO ₃ (mg)	ratio oxidant: substrate	yield[mg(%)]
1	42.5	153	9.8:1	18.4 (43)
2	44.2	127	10.1:1	34.2 (77)
3	42.3	120	10.0:1	35.2 (83)

a :all oxidations in glacial acetic acid (10cm³) at 70±2°C

FIG. 3.34 GAS CHROMATOGRAMS OF TOTAL RECOVERED MATERIAL (METHYLATED) - DULTON 20 W 40 LUBE OIL ALIPHATIC FRACTION OXIDATION-(CrO₃ in glacial acetic acid, hexane as extractant)

[GC: OV-1 (GC²), 25m, 50-300°C @ 5°C min⁻¹, 300°C for 20min]

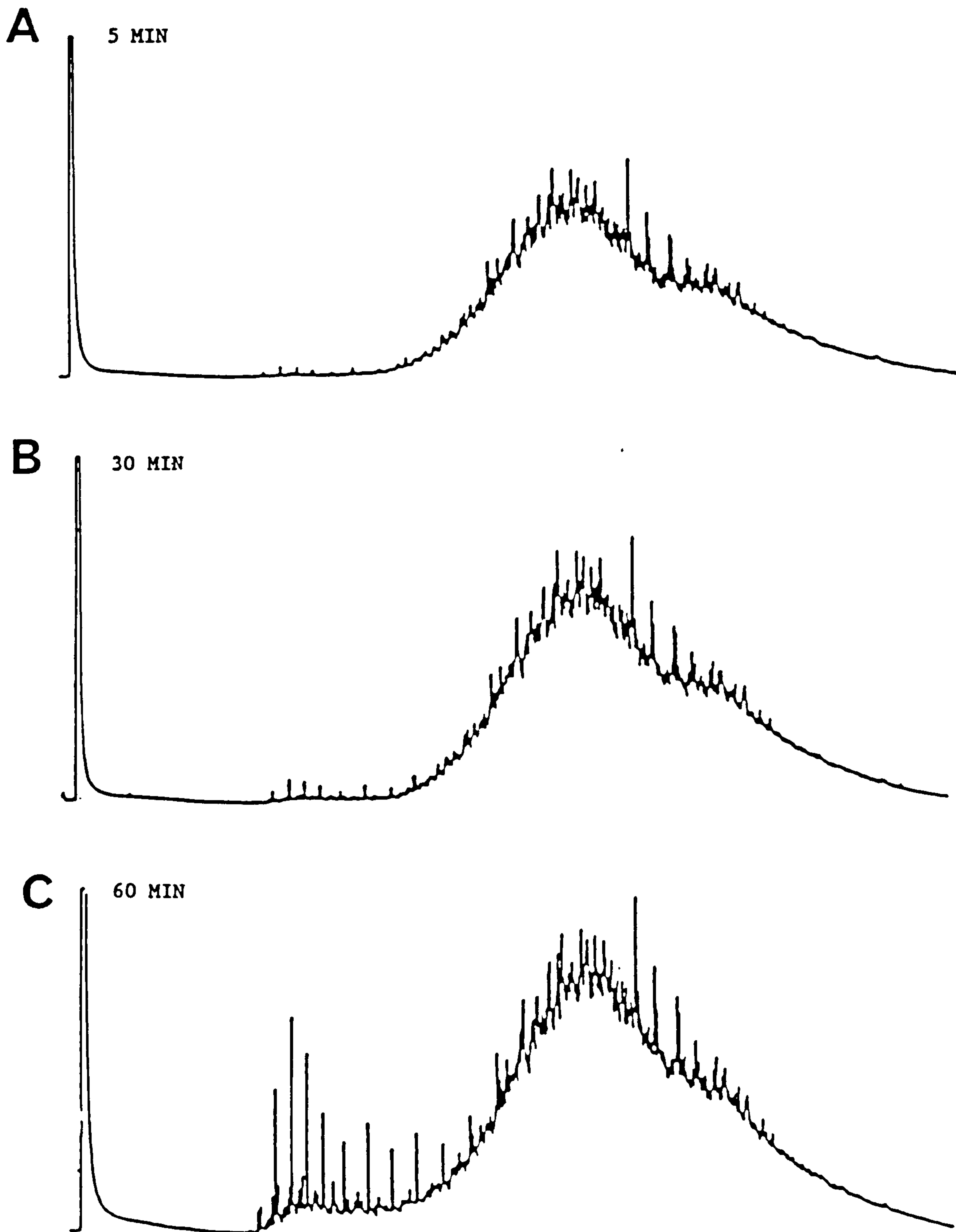
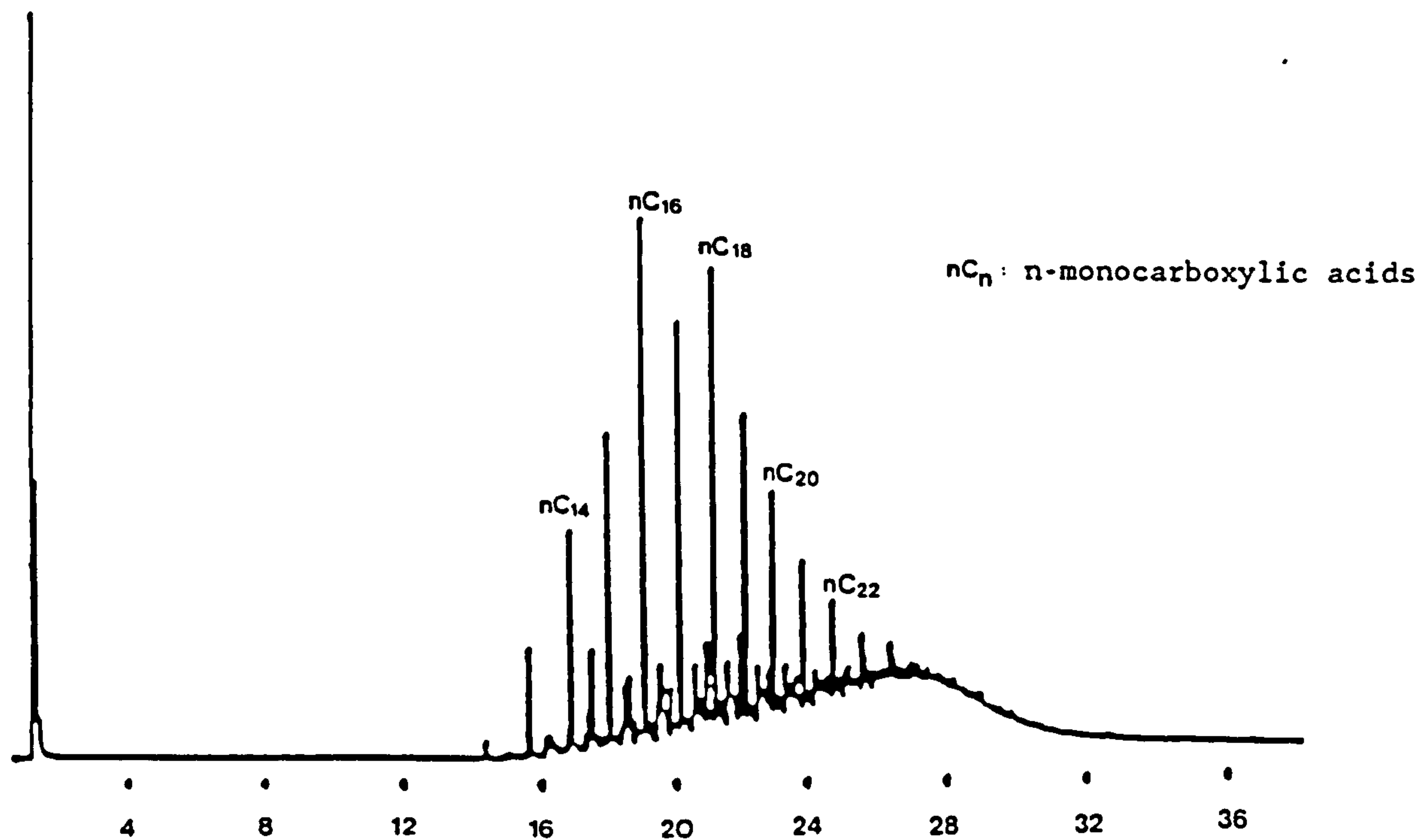


FIG. 3.35 GAS CHROMATOGRAM OF ISOLATED OXIDATION PRODUCTS (METHYL ESTER BAND) - DULTON 20 W 40 ALIPHATIC FRACTION OXIDATION-(CrO₃ in glacial acetic acid, hexane as extractant, 60 min)

[GC: OV-1 (GC²), 25m, 50-300°C @ 5°C min⁻¹, 300°C for 20min]



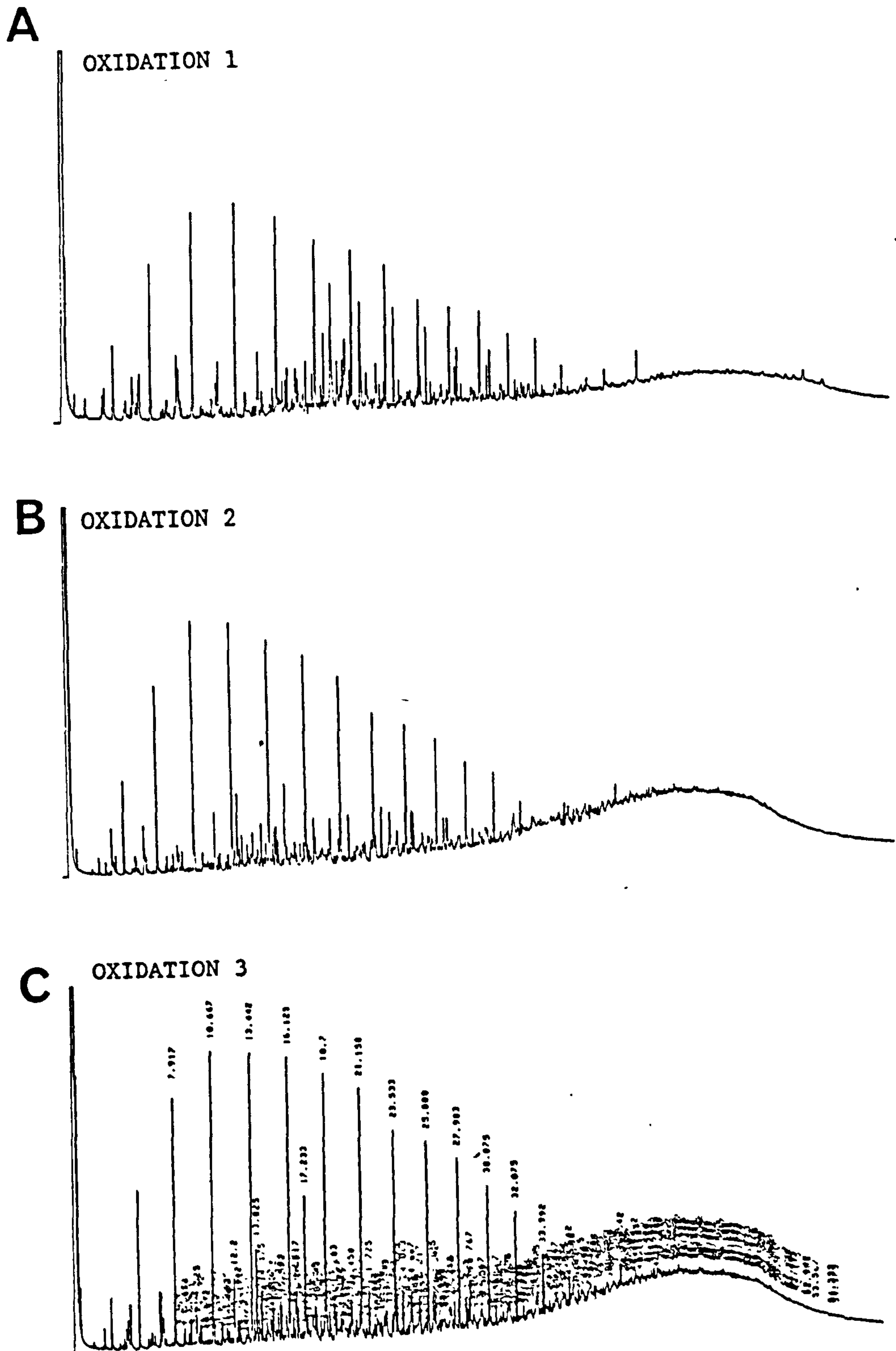
During the course of these initial oxidations a degree of colouration remained in the post hydrolysis/hexane extracted aqueous layers. This suggested the presence of more polar material not fully extractable into hexane. Reextraction with DCM was found to isolate quantitatively more material than hexane, and the method was therefore modified by the replacement of hexane with DCM as the extractant at each stage. The time of oxidation chosen was that which produced optimum quantities of resolved products (i.e. 1 hour), and the oxidant/substrate molar ratio reduced from ca. 18:1 to 10:1 (assuming an average molecular weight of UCM alkanes as 352 gmole^{-1}) in order to reduce the extent of over oxidation. The resulting method applied to the aliphatic UCM derived from the Silkolene 150 lubricating base oil resulted in a higher yield of total recovered material (40%, Table 3.12), with a much larger contribution of resolved compounds to the gas chromatogram (Fig. 3.36). Additional oxidations were performed and with greater care taken in the evaporative procedures (rotary evaporation and N_2 blow down) a much larger and more consistent yield of total products was obtained (77% and 83%).

A comprehensive characterisation of the products of oxidation of the aliphatic UCM was achieved by GC, EI GC-MS, and coinjection of authentic standards (where available). Identifications made by GC-MS relied on library or published spectra- in cases where no match was obtained tentative assignments were made by spectral interpretation. In certain cases further confirmation was made by chromatographic isolation (Table 3.13) and CI GC-MS (ammonia as reagent gas), or by a comparison with model compound oxidation products (chapter 4).

FIG. 3.36 GAS CHROMATOGRAMS OF THE TOTAL RECOVERED MATERIAL (METHYLATED) FROM REPLICATE OXIDATIONS OF THE ALIPHATIC UCM, SILKOLENE 150 LUBRICATING BASE OIL

(CrO₃/GLACIAL ACETIC ACID, 60 MINS, DCM EXTRACTANT)

[GC: DB-5(J+W), 50-300 °C @ 5 °Cmin-1, 300 °C (20 MIN)]



Preliminary GC-MS analyses of unfractionated total recovered material identified the major products from each UCM oxidation as C6-C20 (max. C9) n-alkanoic acids (Fig. 3.37), with no carbon predominance. This confirmed preliminary results obtained by hexane extraction, though with DCM as the extractant the carbon number range was extended to lower homologues. Furthermore, in view of the much higher yields of total recovered material (up to 80%), most of which was functionalised compounds (Table 3.13), these appeared to be representative of the UCM as a whole. This was confirmed by an examination of the blank (Fig. 3.38) which did not reveal any significant contribution of impurities to the resolved compound distribution.

Column chromatographic fractionation of the total recovered products of UCM oxidation provided gravimetric data for the proportion of oxidised compounds vs. unoxidised alkanes (Table 3.13). Thus fraction 1 containing unoxidised alkanes represented < 12% of the total recovered material, whereas the DCM: methanol fraction (F4) accounted for more than 70% of the total material. In total ca. 89% of the material recovered was found to comprise functionalised compounds.

The quantitatively most important fraction (F4) was analysed by EI and CI GC-MS, and the major compounds (Fig. 3.39) identified as a homologous series of C6-C14 (C9 max.) α,ω -n-dicarboxylic acids. Mass spectra were comparable with library and published spectra (Fig. 3.40, Ryhage and Stenhagen, 1959), the notable features of which were the absence of molecular ions, abundant $M^+ - 31$ ($-\text{OCH}_3$) and $M^+ - 64$ ($-2\text{CH}_3\text{OH}$) ions, and significant $M^+ - 91$ ($-\text{CO}_2\text{CH}_3$ and CH_3OH), $M^+ - 105$

FIG. 3.37 GC-MS MASS FRAGMENTOGRAM (m/z 74) SHOWING THE DISTRIBUTION OF N-ACIDS OBSERVED AS OXIDATION PRODUCTS OF THE ALIPHATIC UCM

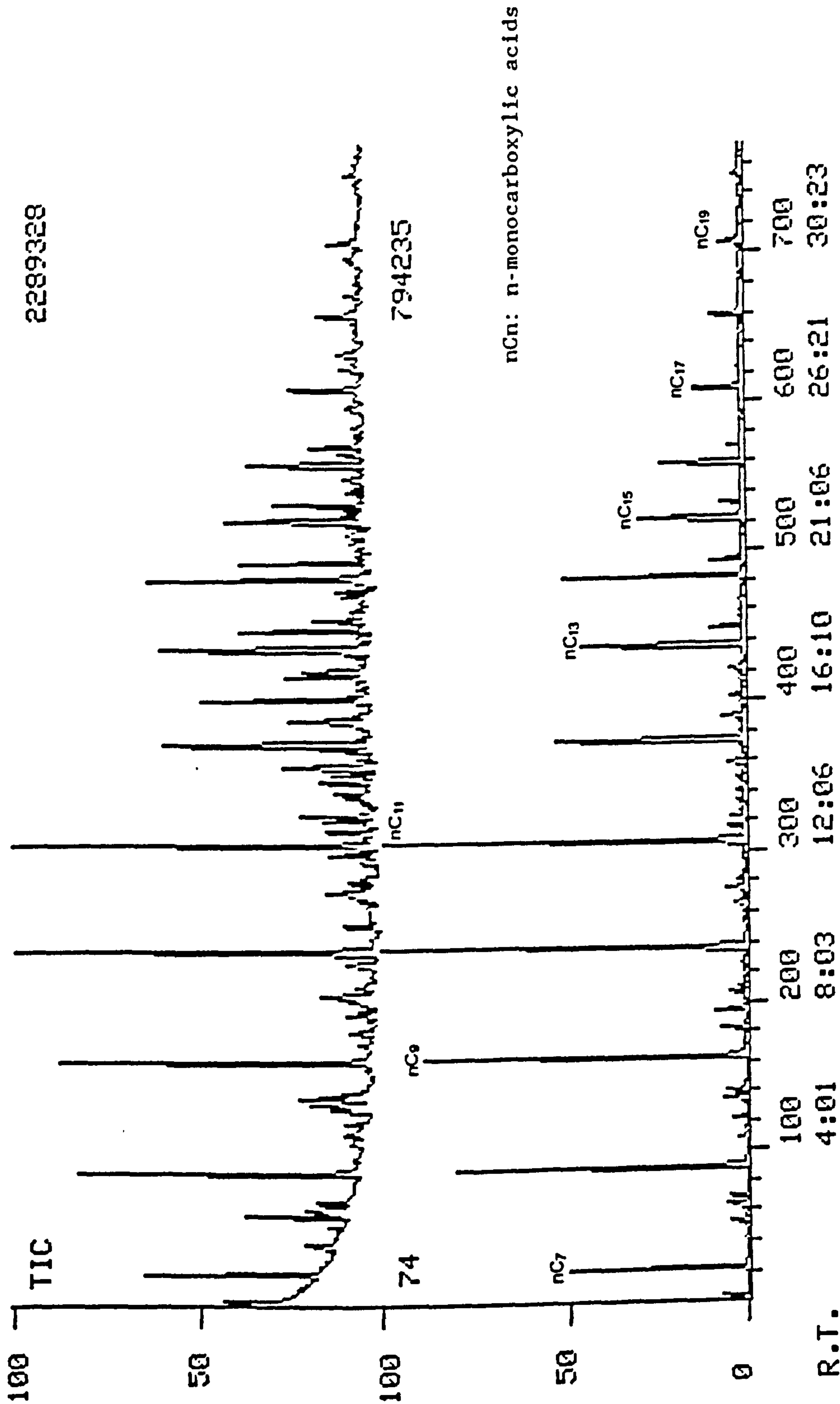


TABLE 3.13

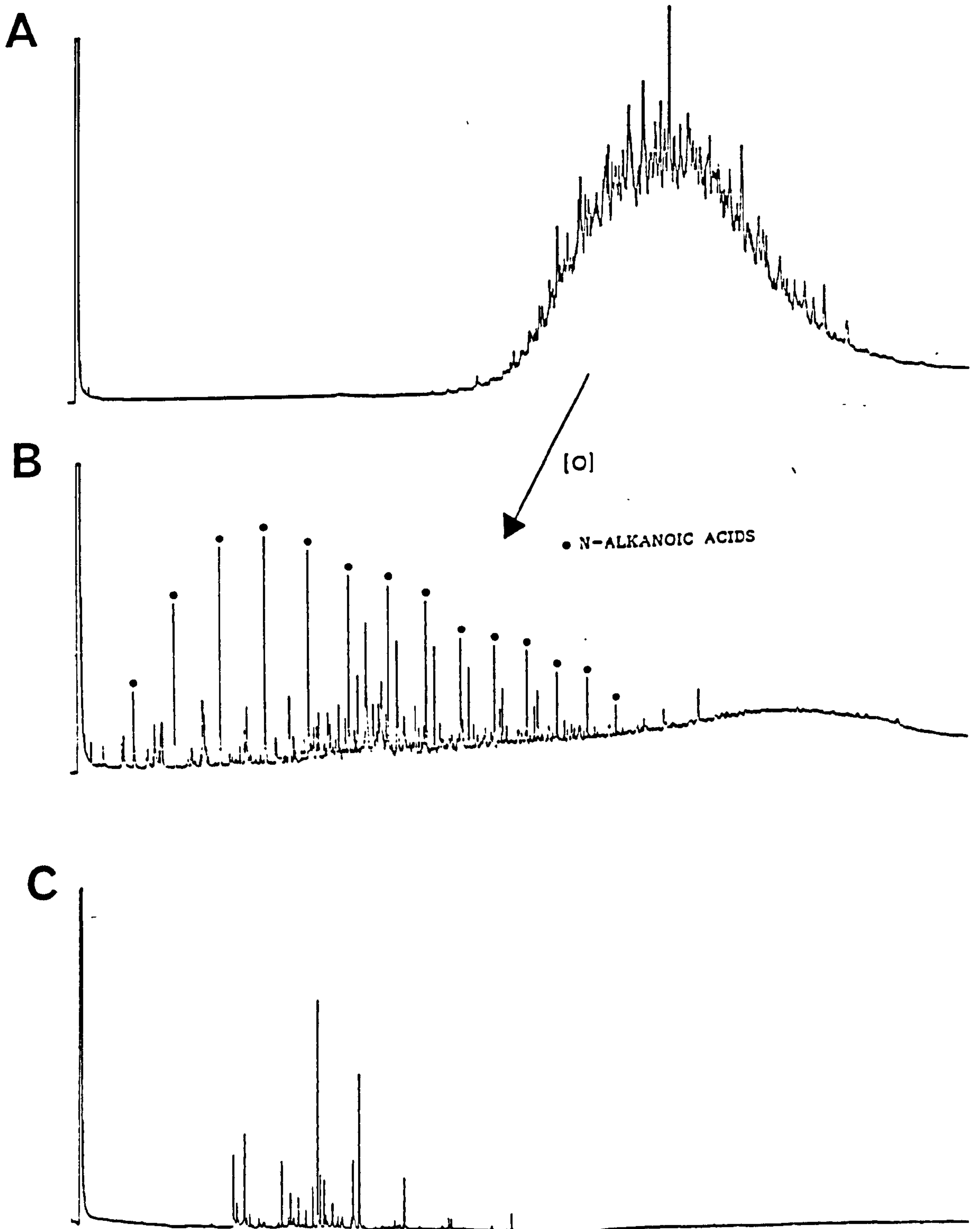
CHROMATOGRAPHIC ISOLATION OF THE TOTAL METHYLATED OXIDATION PRODUCTS OF UCM
OXIDATION 1

mass applied	mass F1(mg)	mass F2 (mg)	mass F3 (mg)	mass F4 (mg)	recovery(%)
17.7	1.3	1.0	0.9	8.2	71

fraction	solvent	%total recovered
F1	hexane	11.4
F2	hexane/DCM (1:1)	8.8
F3	DCM	7.9
F4	DCM/MeOH (1:1)	71.9

FIG.3.38 GAS CHROMATOGRAMS OF A) THE ALIPHATIC UCM BEFORE OXIDATION, B) THE ALIPHATIC UCM AFTER OXIDATION AND C) THE PROCEDURAL BLANK

[GC: DB-5(J+W), 50-300 °C @ 5 °Cmin-1, 300 °C (20 MIN)]



($-\text{CH}_2\text{CO}_2\text{CH}_3$ and CH_3OH), and m/z 74 (McLafferty rearrangement) ions. These fragments are characteristic of the methyl esters of n-diacids (Ryhage and Stenhagen, 1959; Hermann et al., 1986), and CI GC-MS provided the expected quasi-molecular ions (e.g. Fig. 3.40) for this series. Further confirmation was achieved by the use of relative retention times expressed as equivalent chain length (ECL) values (Douglas et al., 1977; Table 3.14) which compared well with published values (Rostad and Perieva, 1986).

A major compound was noted in each of the total products (labelled "O", Fig. 3.39). EIMS (Fig. 3.41A) did not produce a molecular ion but CI-MS produced a quasi-molecular ion m/z 201 ($M + H$)⁺ and an adduct ion m/z 218 ($M + \text{NH}_4$)⁺ indicative of a compound with a molecular weight of 200 (Fig. 3.41C). Use of this mass provided a satisfactory library match with the entry for the C10 keto acid 9-oxo-decanoic acid (methyl ester) (Fig. 3.41B). However, the oxidation products of authentic 2,6,10,14-tetramethylpentadecane (pristane, section 4.2.7) comprised a compound which displayed a near identical mass spectrum, which was characterised as the methylbranched C10 keto acid 8-oxo-4-methylnonanoic acid. A comparison of ECL values of this latter compound with that observed as a major product of the UCM showed them to be the same (ECL = 11.44). Other keto acids were identified as products of the aliphatic UCM (C8 and C11). Alkyl ketones were also observed by mass fragmentography (m/z 58 derived from γ -H-rearrangement; McLafferty, 1978); though their abundance was low (Fig. 3.42). The major compounds were identified by a comparison of published spectra and retention indices (Table 3.14) as a homologous series of C8-C15 (C12

FIG. 3.39 GC-MS MASS FRAGMENTOGRAMS OF γ -LACTONES (m/z 99) AND N- α,ω -DIACIDS (m/z 74/87) OBSERVED AS OXIDATION PRODUCTS OF THE ALIPHATIC UCM (FRACTION 4, POLAR MATERIAL)

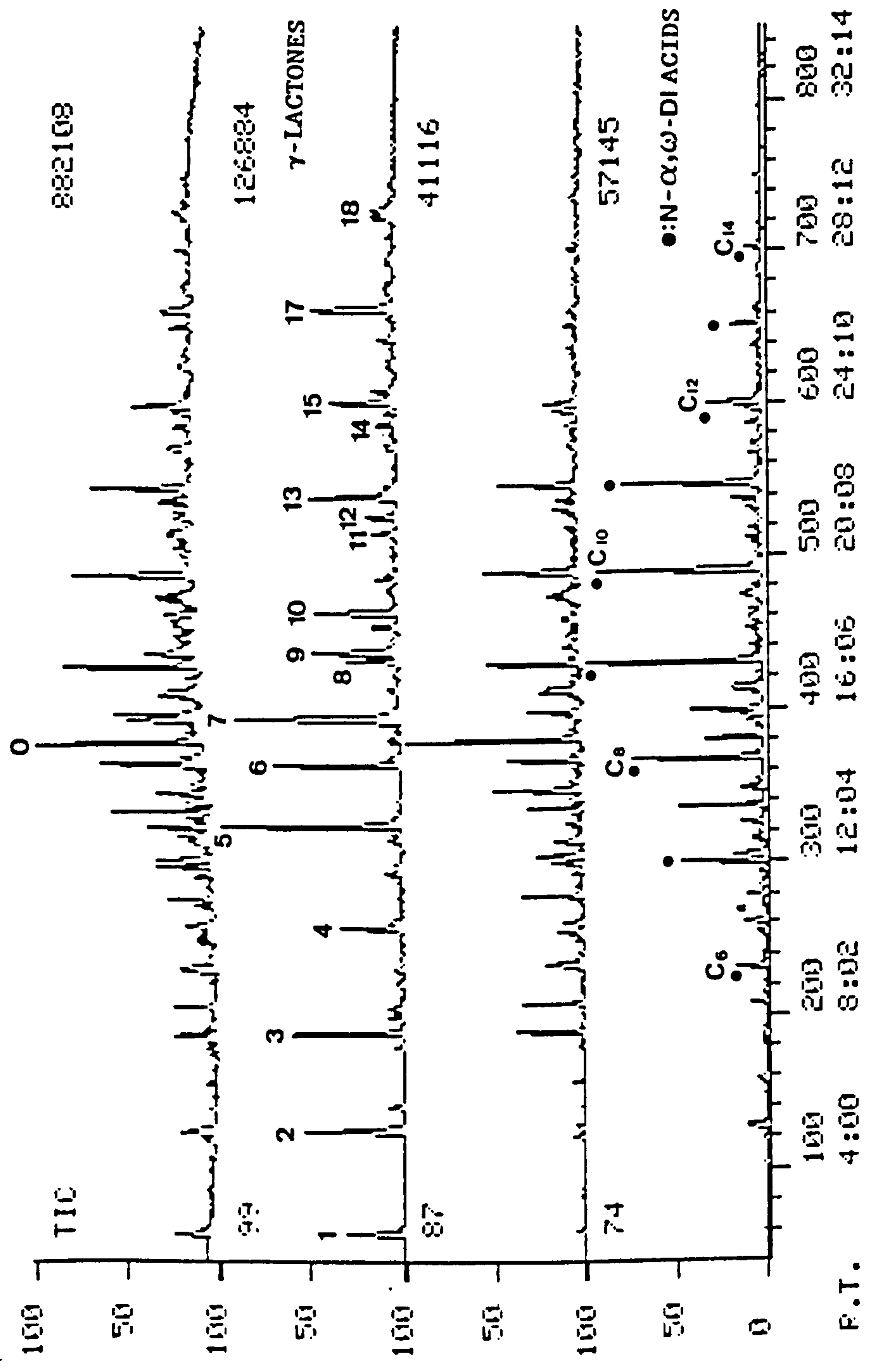
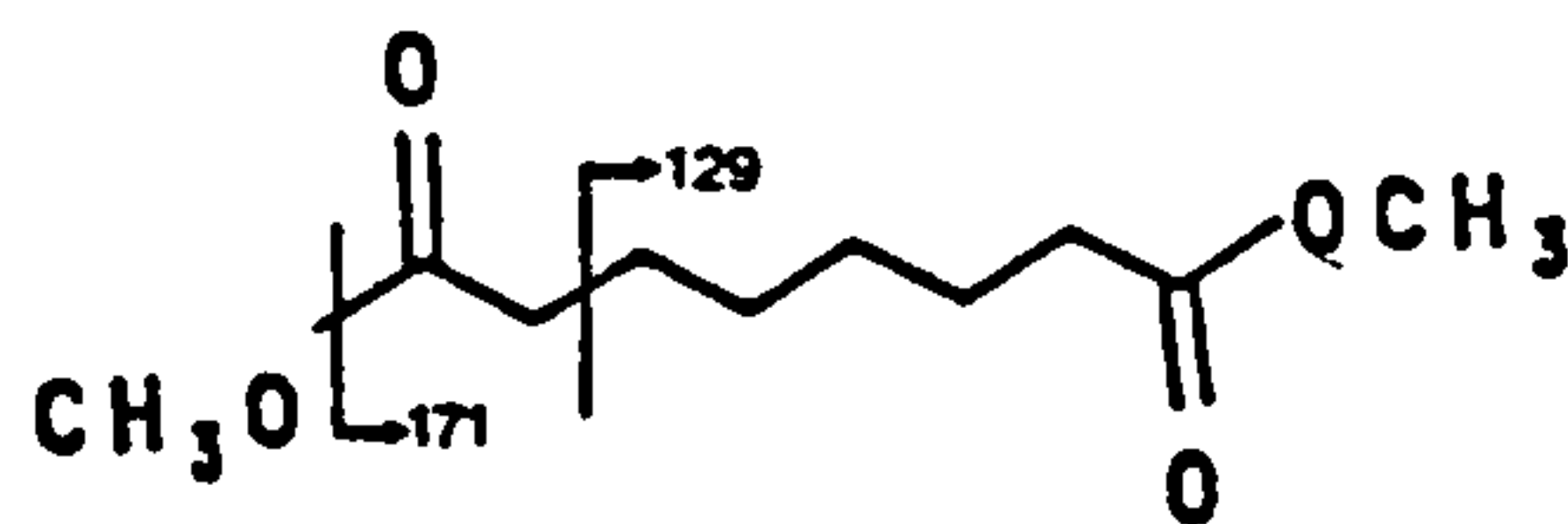
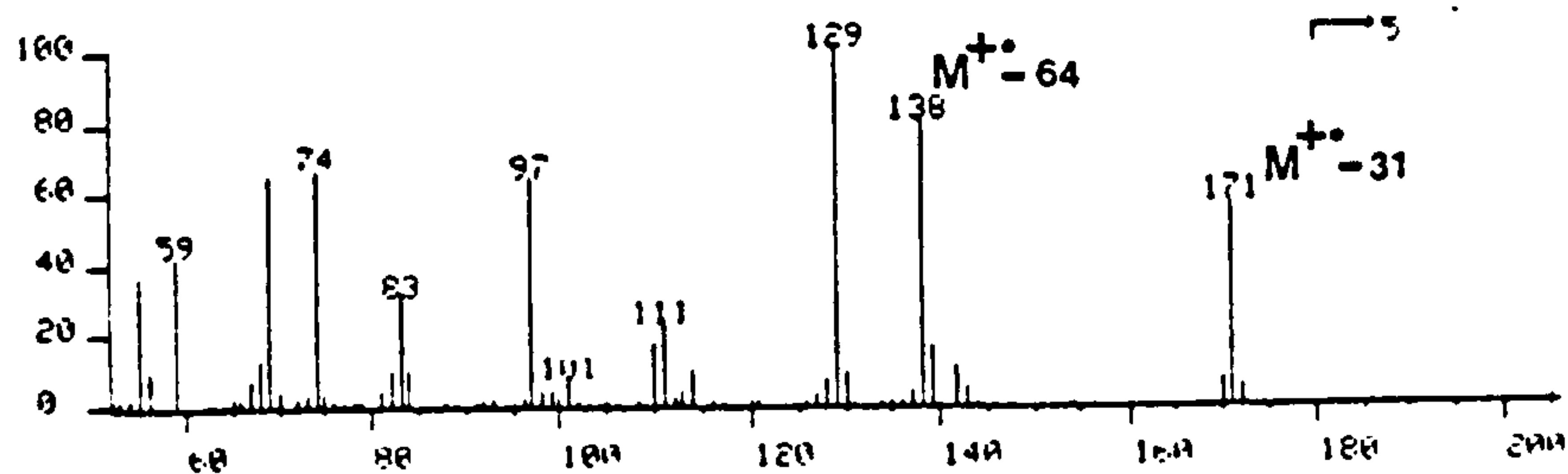


FIG. 3.40 EI AND CI(NH₃) MASS SPECTRA OF A C₈- α,ω DICARBOXYLIC ACID (DIMETHYL ESTER) IDENTIFIED AS AN ALIPHATIC UCM OXIDATION PRODUCT

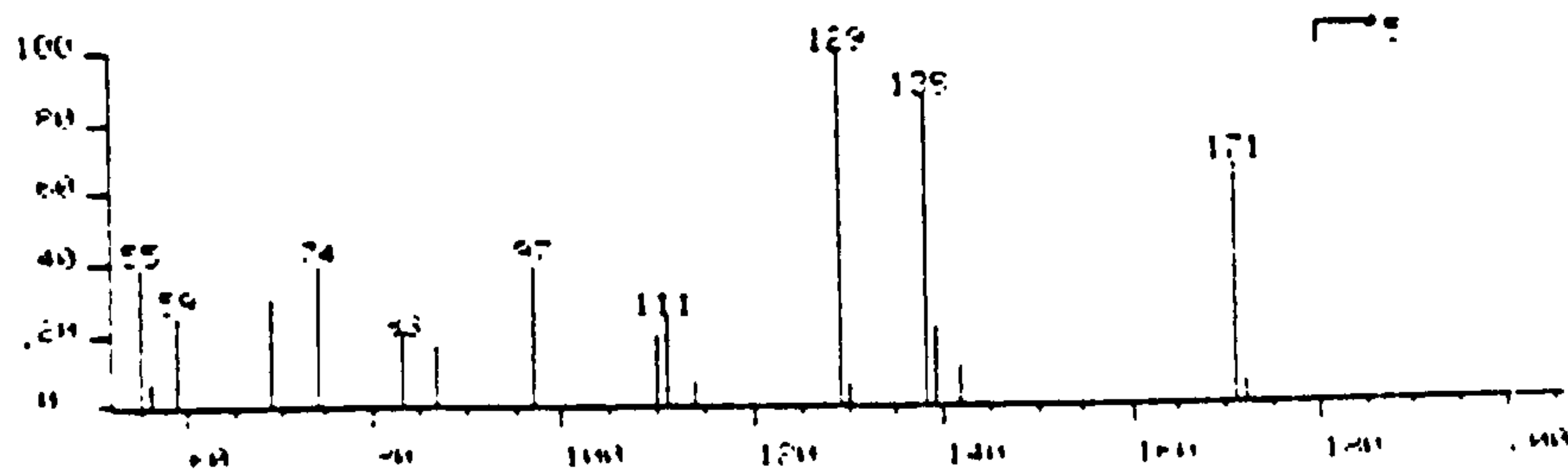


A



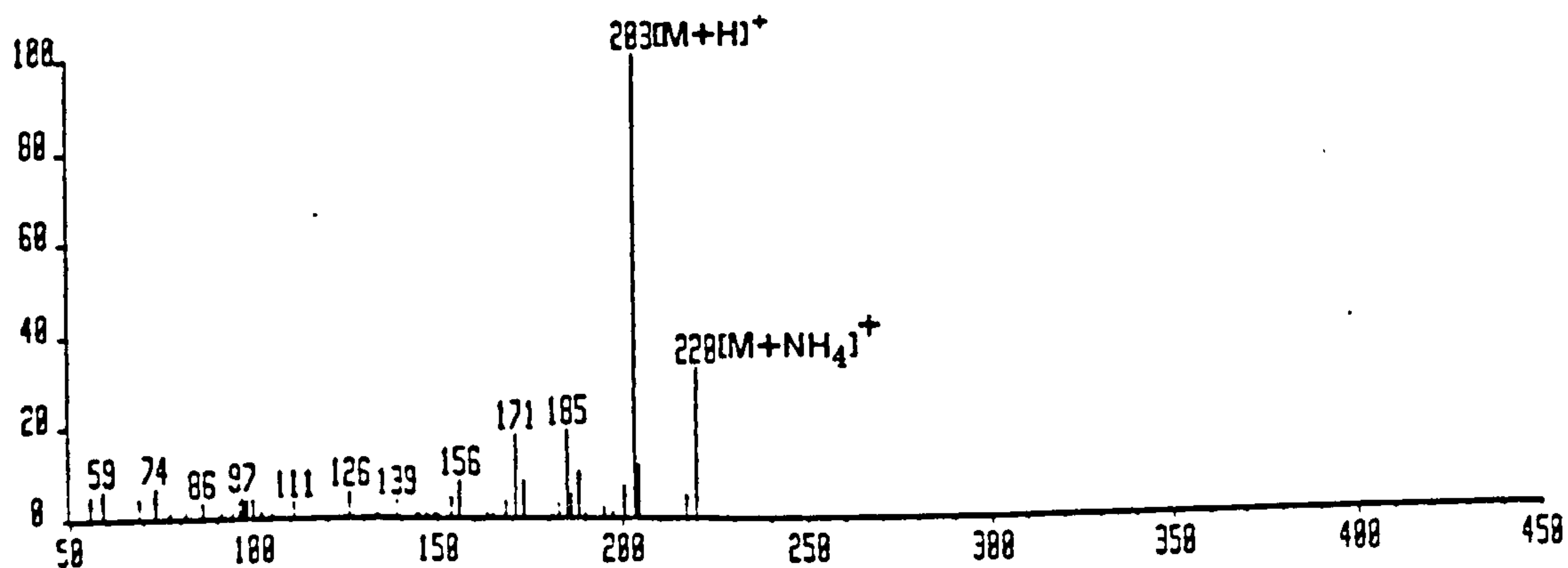
EI MASS SPECTRUM

B



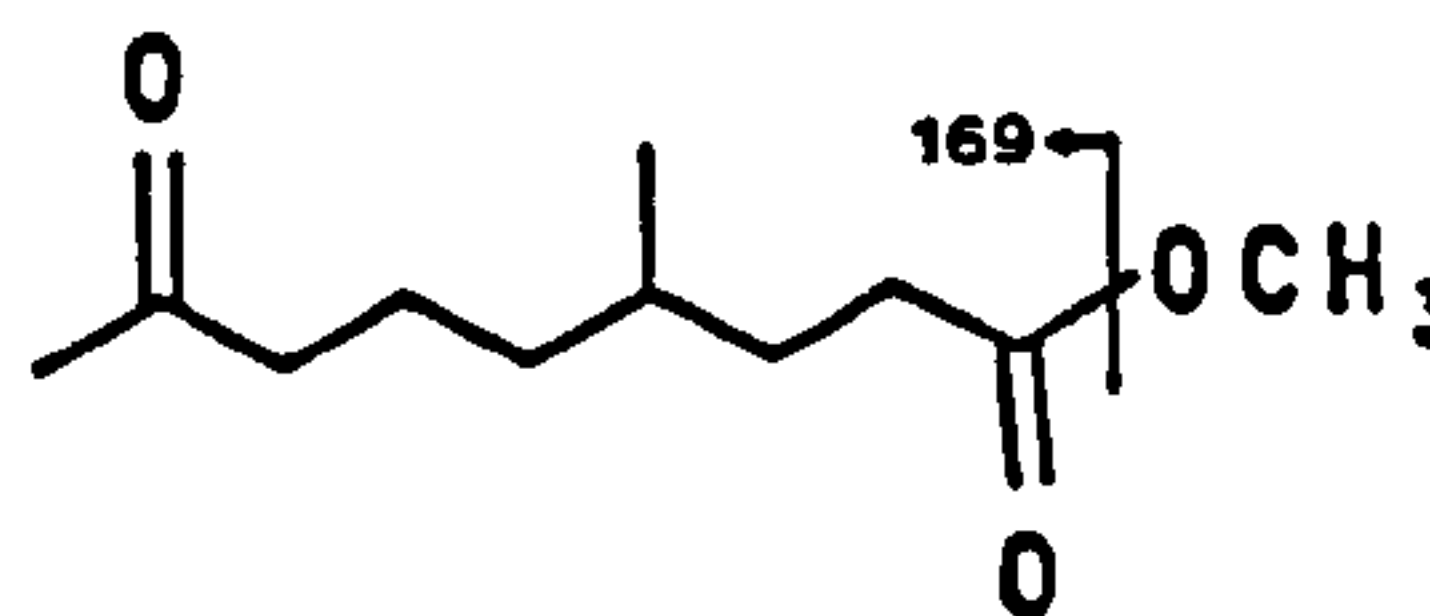
LIBRARY MATCH

C

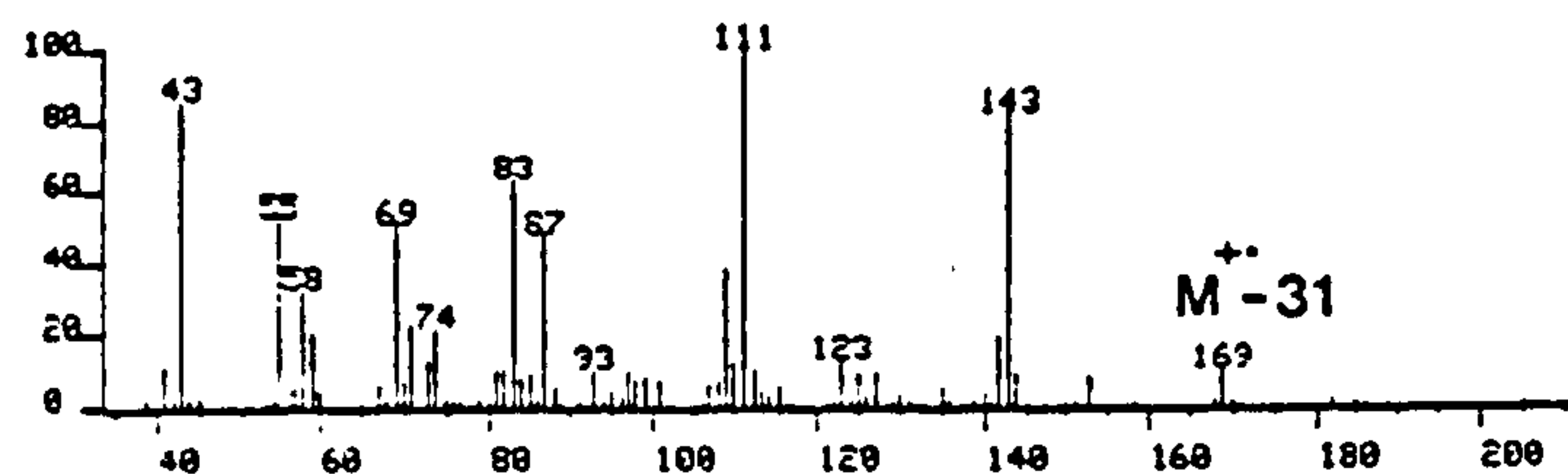


CI(NH₃) MASS SPECTRUM

FIG. 3.41 EI AND CI(NH₃) MASS SPECTRA OF A C₁₀ KETO ACID IDENTIFIED AS AN OXIDATION PRODUCT OF THE ALIPHATIC UCM



A



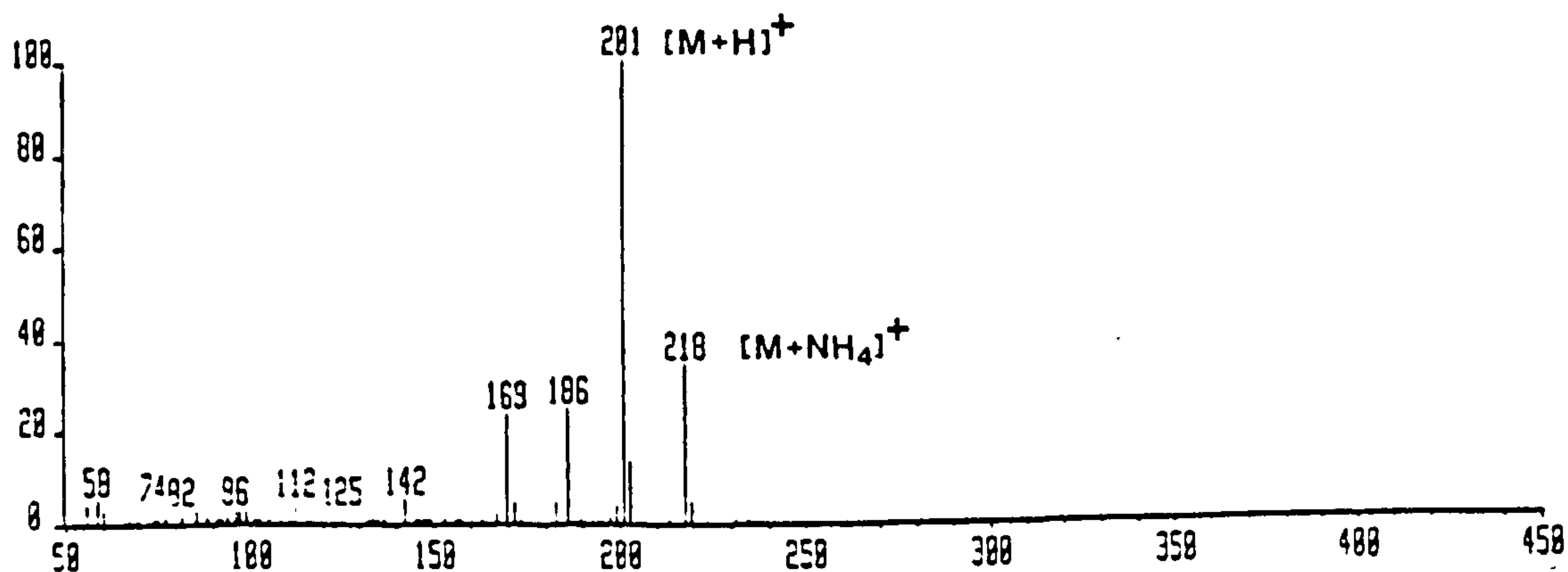
A) EI MASS SPECTRUM

B



B) PROPOSED LIBRARY ENTRY

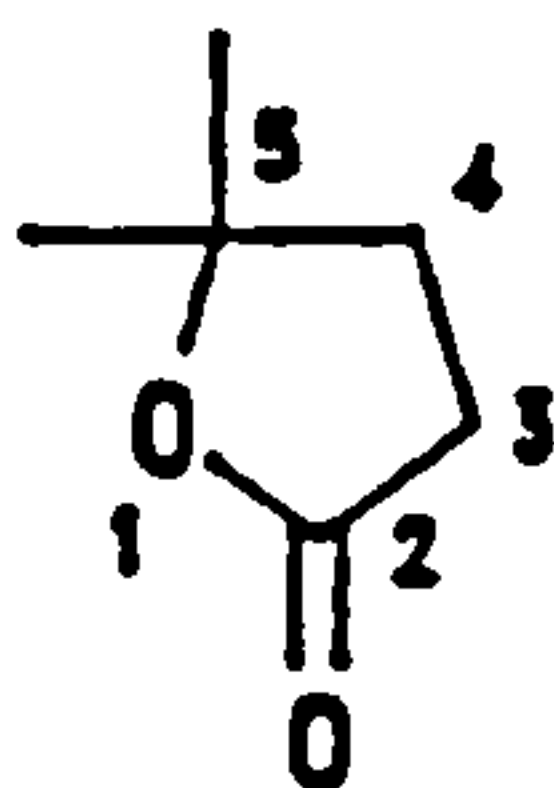
C



C) CI(NH₃) MASS SPECTRUM

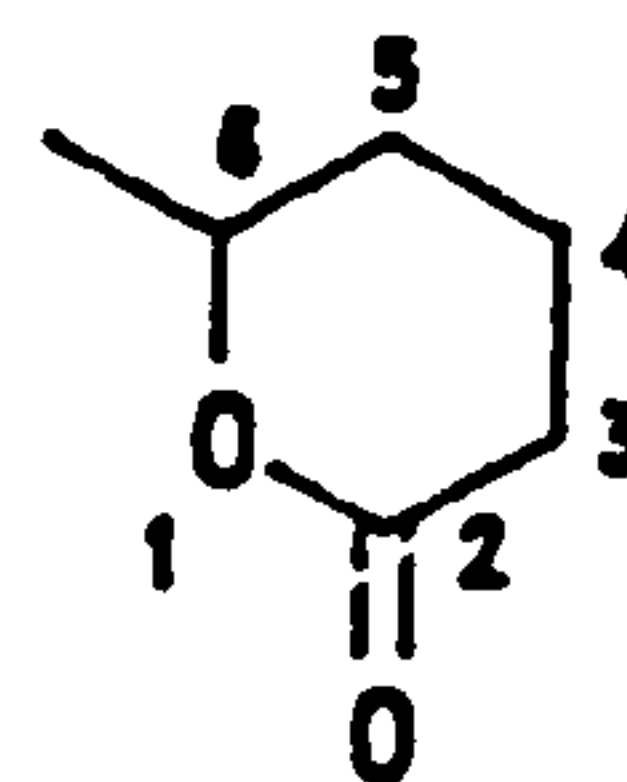
max.) n-alkan-2-ones. A second series was found to elute at lower relative retention times by a difference of approximately 0.4 ECL units, presumably as a result of methyl branching. Mass spectral correlations and a comparison of published ELC values (Rostad and Pereira, 1986) showed these to be "iso"-methylbranched alkan-2-ones (C8-C12; max.C8).

A quantitatively important series of compounds were found to be enriched in the polar fraction of the total oxidation products (Fig. 3.43). EI mass spectra contained prominent m/z 99 ions and from retention time data, EI and CI mass spectra, published and library spectra, and a comparison of the oxidation products of the authentic alkanes 9-methyltetracosane and 2,6,10,14-tetramethylpentadecane (chapter 4); these were tentatively identified as varied series of dialkyl substituted γ -lactones. Thus compound 1 (Fig. 3.43) exhibited a molecular ion in it's EI mass spectrum (m/z 114) confirmed by CI GC-MS [$(M+H)^+$: 115; $(M+NH_4)^+$: 132; Fig. 3.44]. The EI mass spectrum matched well with a library entry for C6- γ -methyl- γ -lactone (Fig. 3.44). The intense m/z 99 ion is due to α -cleavage at C-5, a distinguishing feature of dialkyl substituted γ -lactones at this position (Porter and Baldas, 1977). δ -lactones could be discounted as the C6 member has a low abundance of m/z 99 (Honkanen *et al.*, 1965).



$C_6H_{10}O_2$ /114
(m/z 99, 100%)

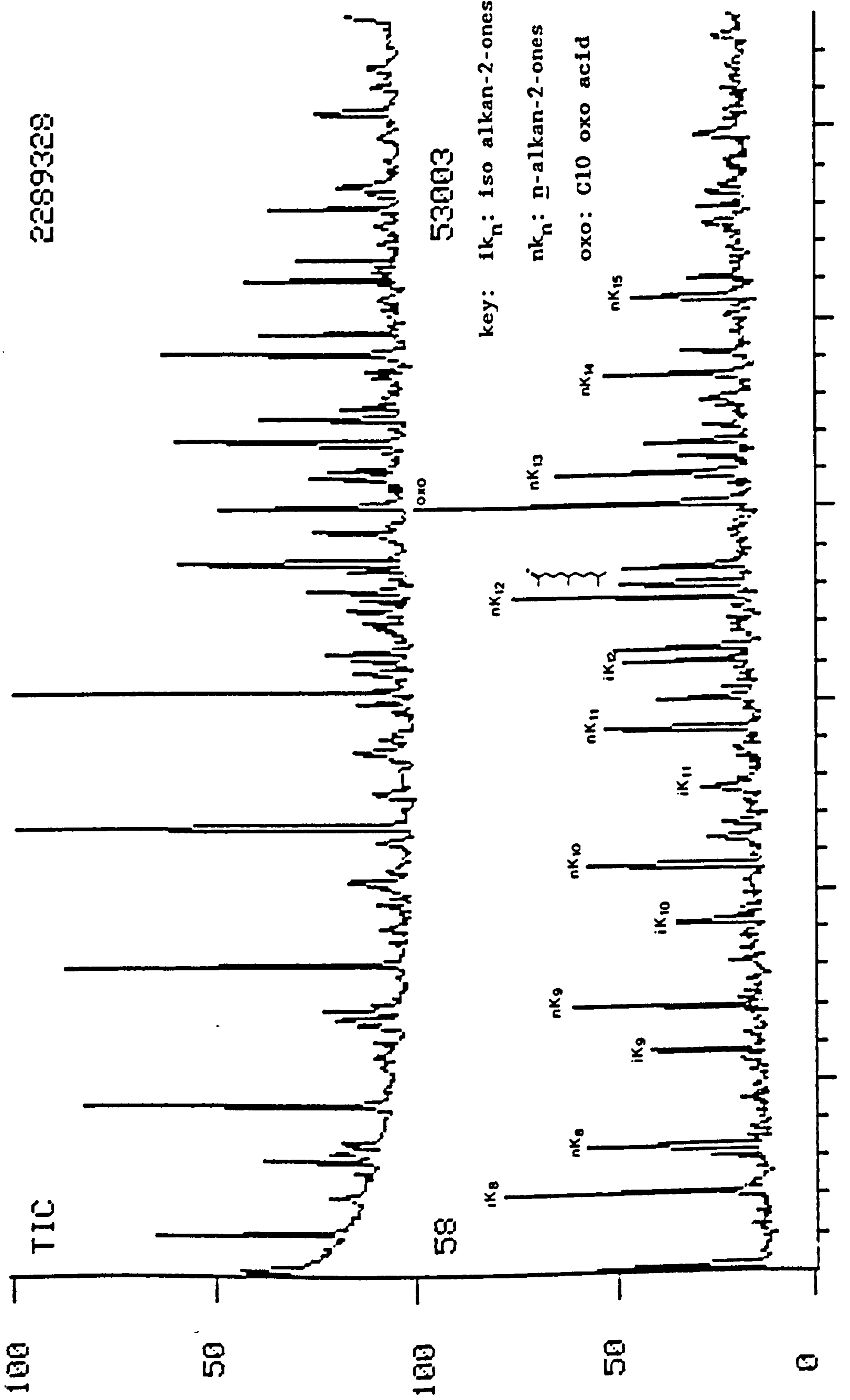
C-5 alkyl substituted
 γ -methyl- γ -lactone



$C_6H_{10}O_2$ / 114
(m/z 99- low)

6-methyl- δ -lactone

FIG. 3.42 PARTIAL GC-MS m/z 58 MASS FRAGMENTOGRAM OF ALKYL
KETONES IDENTIFIED AS ALIPHATIC UCM OXIDATION PRODUCTS



Compounds 2-5 (Fig. 3.43) also had m/z 99 as the base peak in their EI mass spectra though molecular ions were absent. However, CIMS provided the carbon number range C7-C10, and the ion of highest mass in each spectrum was found to represent loss of a methyl group from the molecular ion (i.e. $M^+ - 15$, Fig. 3.45). On this basis these were characterised as homologues of 1, and this was confirmed by a plot of their extrapolated ECL values against their carbon numbers (Fig. 3.46).

Compounds 6 and 8 (Fig. 3.43) showed comparable ECL values (11.25 and 12.29, respectively) and their EI mass spectra appeared pure and indicative of C11 and C12 γ -methyl- γ -lactones (e.g. Fig. 3.47). CI confirmed their proposed molecular masses (Fig. 3.47), though their GC elution positions were reduced by ca. 0.45 units compared with the corresponding series described above. Methyl branching of the alkyl side chain could account for this reduction in retention, and further evidence of this was found by oxidation of a weathered crude oil residue (chapter 5).

Compounds 7,10,12 and 14 (Fig. 3.43) initially appeared as higher homologues of compounds 1-5 by retention data alone, though their mass spectra did not exhibit similar ions. Instead ions indicative of a methyl ester functionality (i.e. m/z 74; $M^+ - 31$ and $M^+ - 15$) characterised their EI mass spectra, and CI provided relatively pure

FIG. 3.43 PARTIAL GC-MS m/z 99 MASS FRAGMENTOGRAM SHOWING
 THE DISTRIBUTION OF γ -LACTONES IDENTIFIED AS OXIDATION
 PRODUCTS OF THE ALIPHATIC UCM

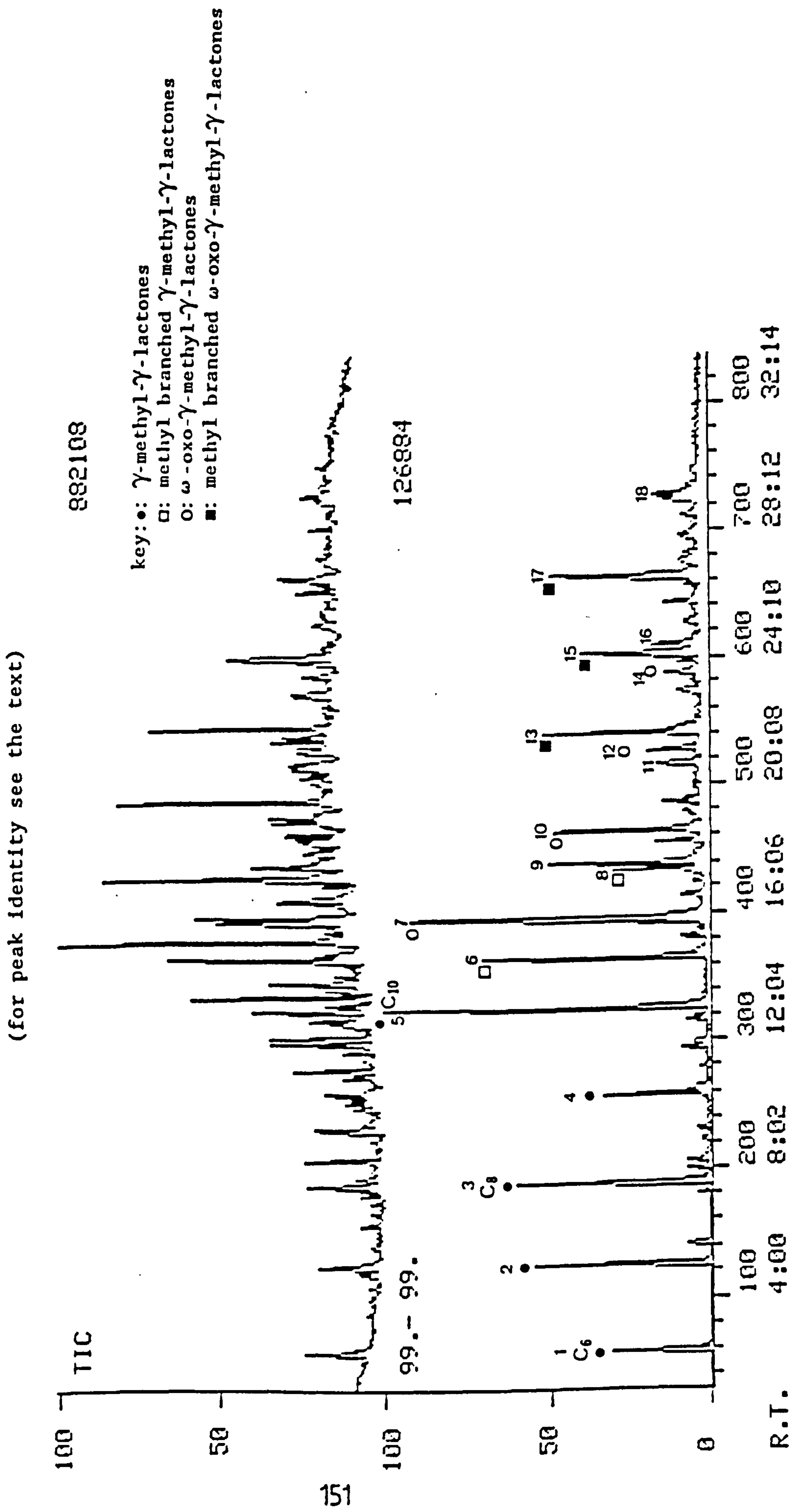
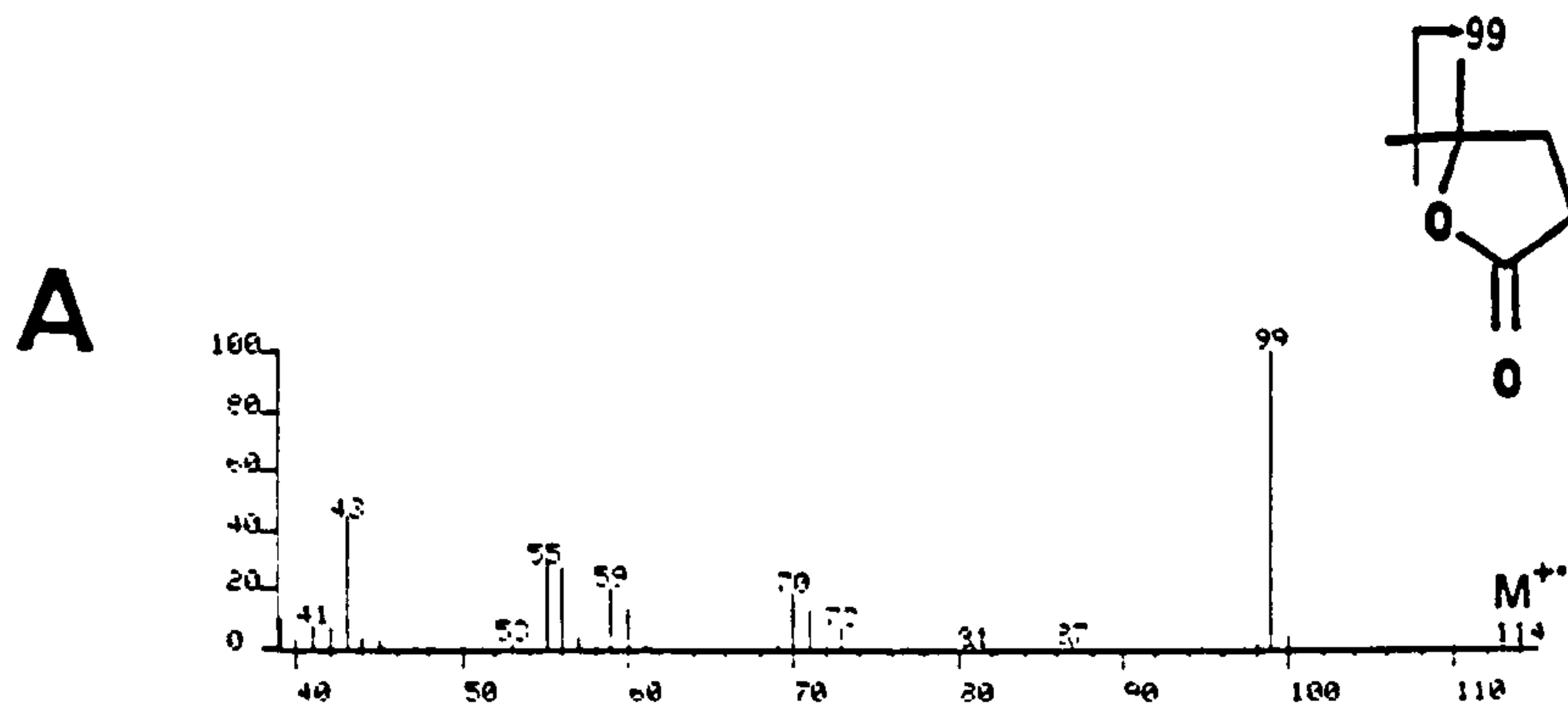
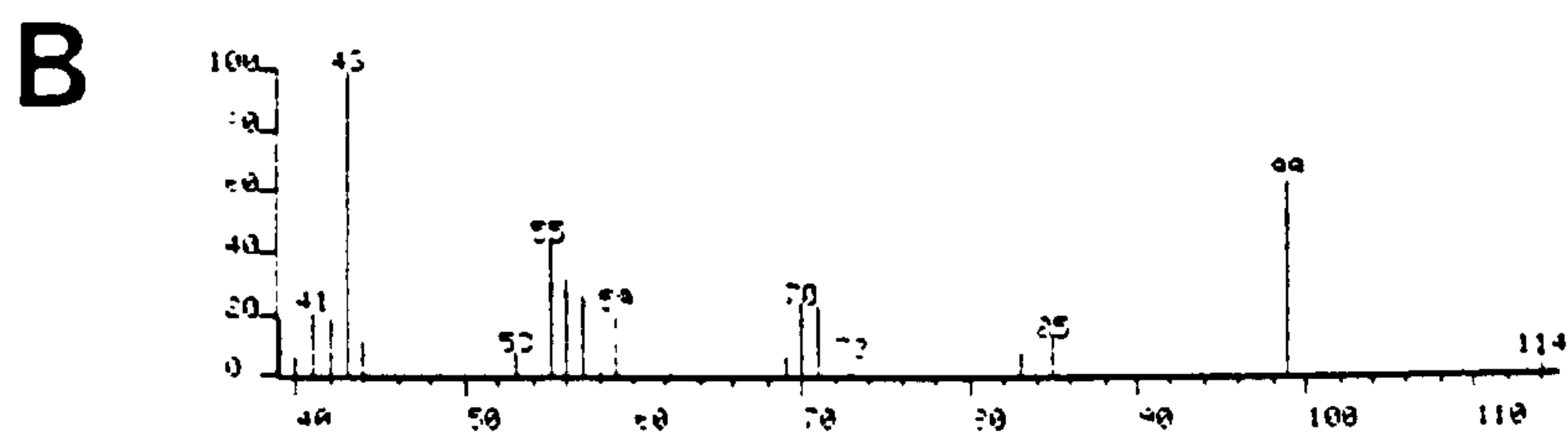


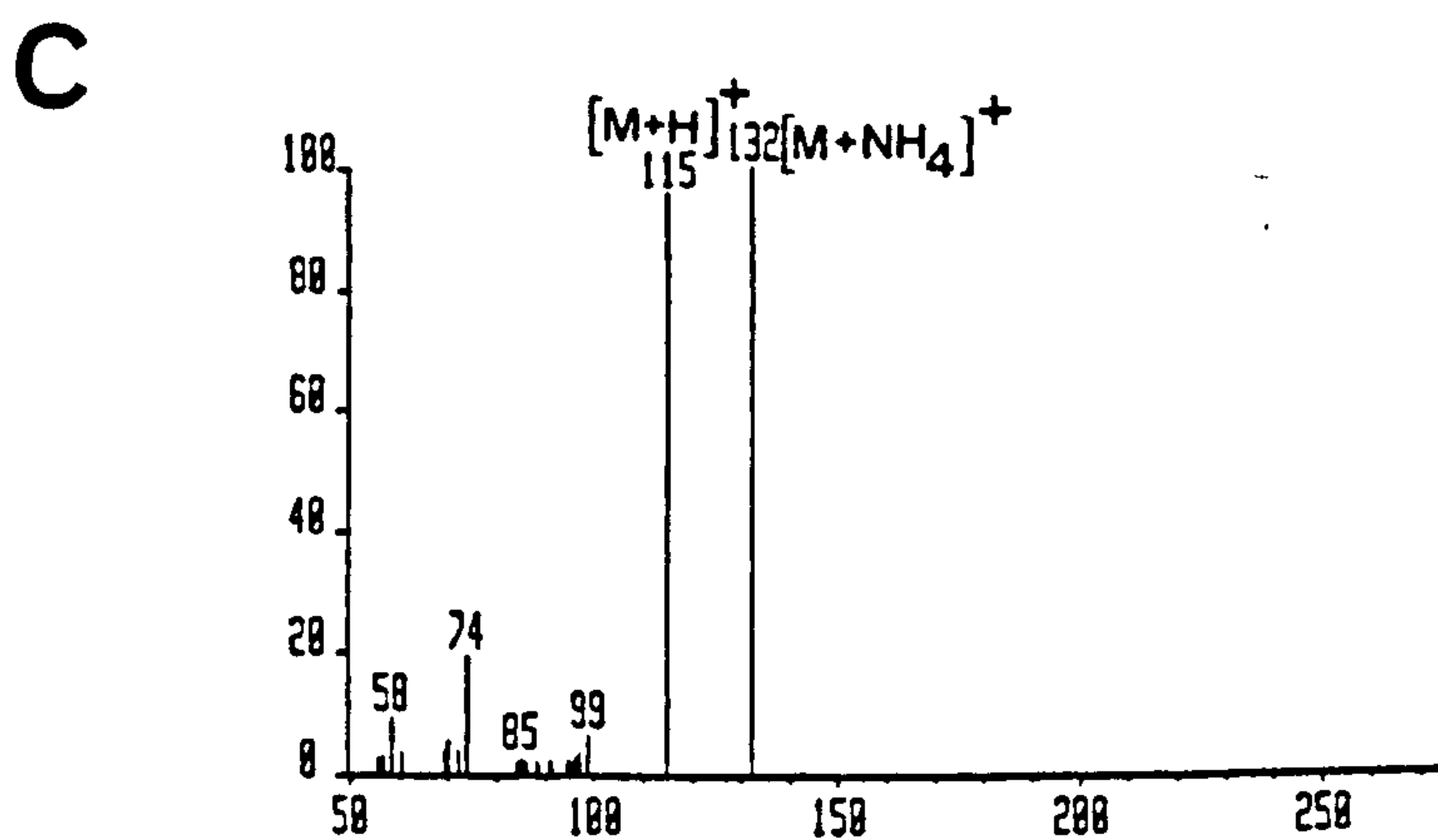
FIG: 3.44 EI AND CI(NH₃) MASS SPECTRA OF A C₆- γ -METHYL- γ -LACTONE IDENTIFIED AS AN OXIDATION PRODUCT OF THE ALIPHATIC UCM



A) EI MASS SPECTRUM

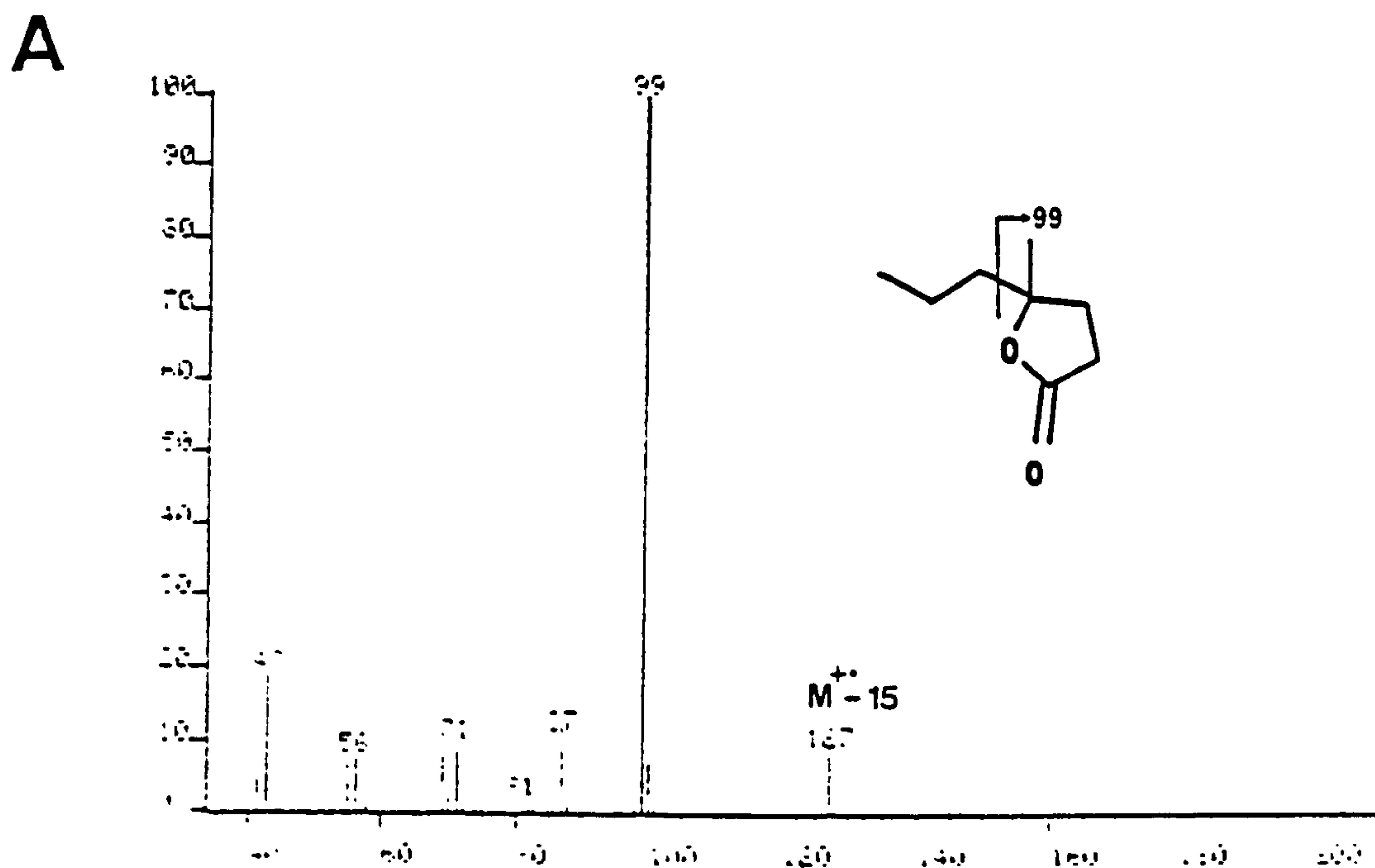


B) LIBRARY MATCH

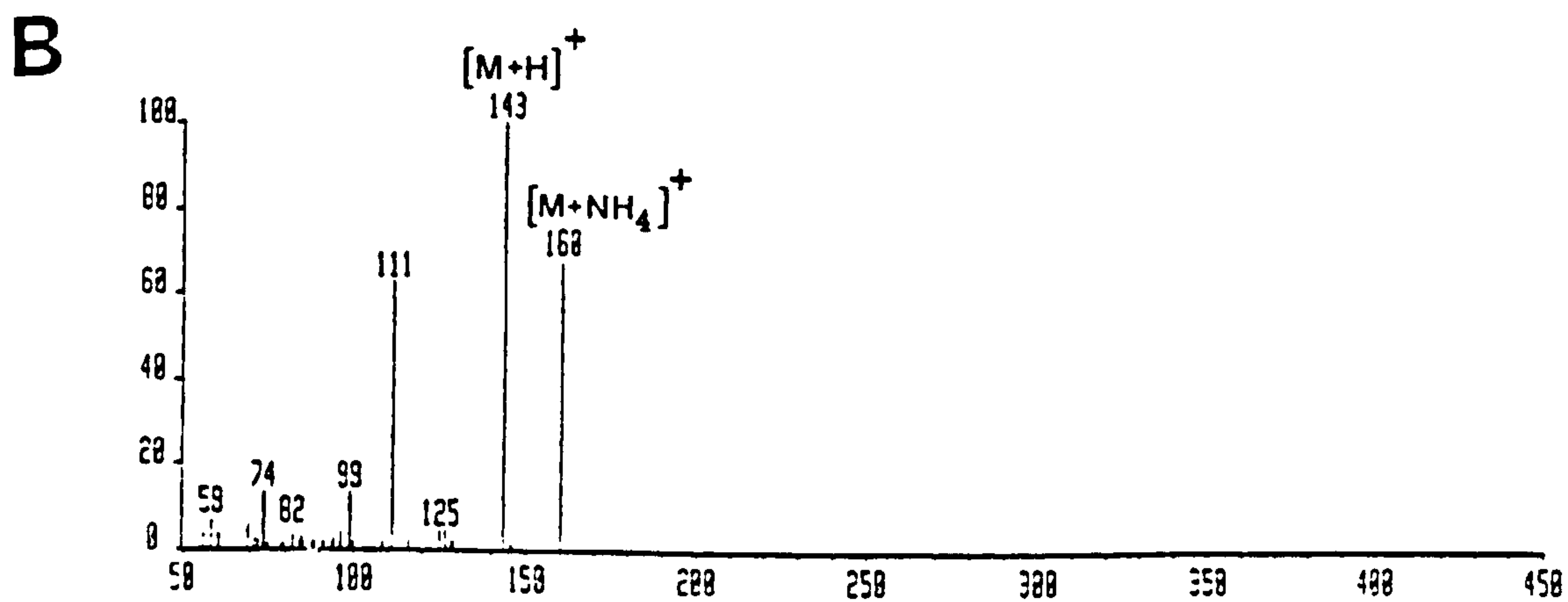


C) CI(NH₃) MASS SPECTRUM

FIG.3.45 EI AND CI (NH₃) MASS SPECTRA OF A C₈- γ -METHYL- γ -LACTONE IDENTIFIED AS AN OXIDATION PRODUCT OF THE ALIPHATIC UCM



A) EI MASS SPECTRUM



B) CI (NH₃) MASS SPECTRUM

spectra consistent for a series of C8-C10 carboxy lactones (e.g. Fig. 3.48). Furthermore this same series of compounds was identified as products of the oxidation of synthetic 9-methyltetracosane (section 4.2.1) and the relative retention data (as ECL units) correlated exactly. These considerations therefore led to their tentative identification as a homologous series of γ -methyl- ω -carboxy- γ -lactones ranging from C8-C10.

Compounds 13, 15 and 17 observed in the m/z 99 mass fragmentogram provided CI and EI mass spectra indicative of C11 - C13 ω -carboxy- γ -methyl- γ -lactones, though at a lower relative GC retention than the previously described series. This perhaps represents methyl branching of the alkyl side chain, and indeed the first member (compound 13) exhibited the m/z 88 ion characteristic of a McLafferty rearrangement involving a α -methyl substituent (Fig. 3.49). This same compound was identified as an oxidation product of authentic 2,6,10,14-tetramethylpentadecane (pristane, section 4.2.7). This series was therefore tentatively identified as methylbranched ω -carboxy- γ -methyl- γ -lactones in the range C11-C13.

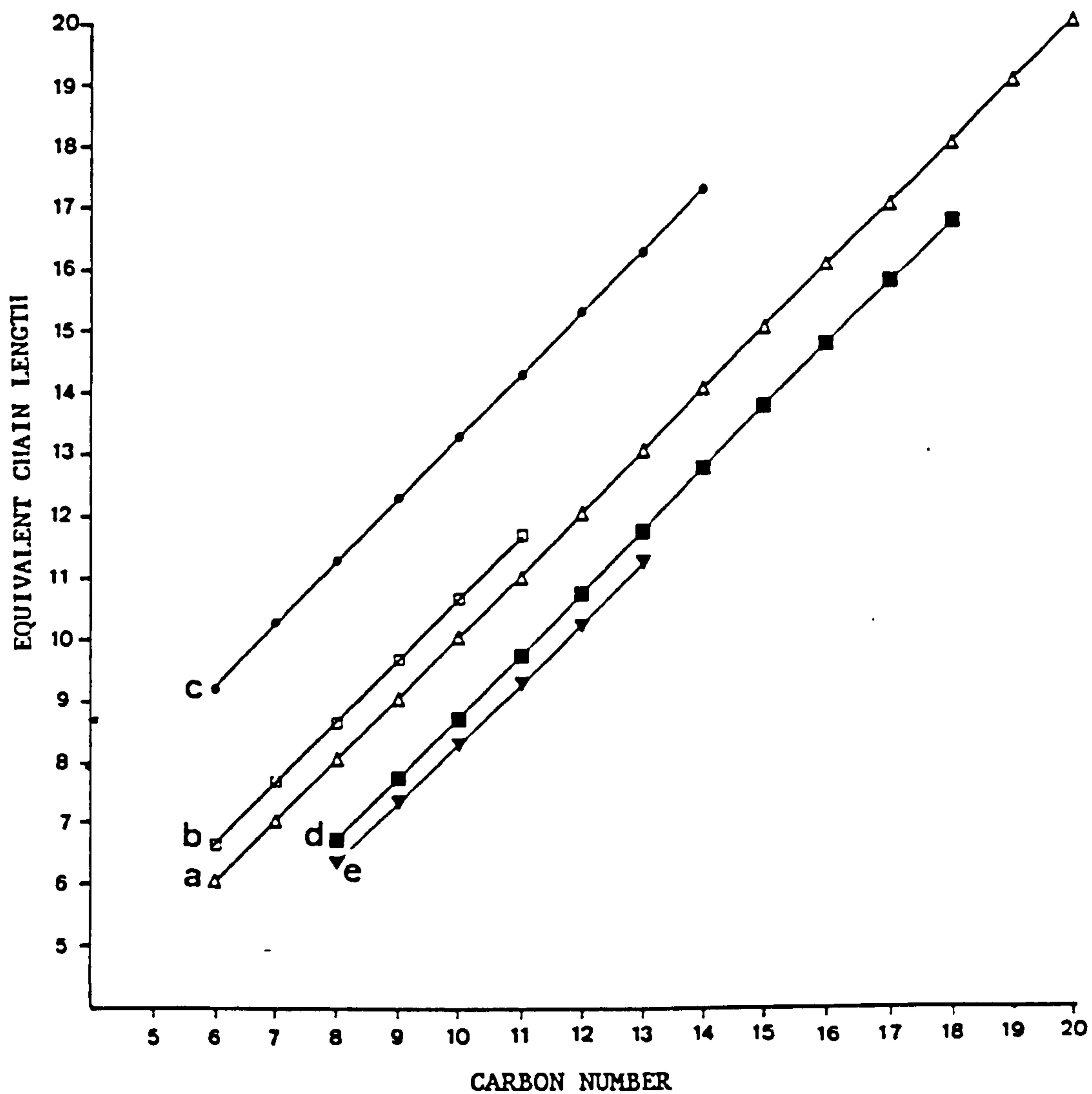
Monomethyl branched monocarboxylic acids were also products of the oxidation of the aliphatic UCM, though in much reduced abundance in comparison to normal monocarboxylic and normal α,ω - dicarboxylic acids. Tentative identifications were made by a comparison of retention indices with published values and by spectral interpretation (Table 3.15). C7-C11 compounds were identified and of these, iso (C_{n-1} methyl- C_n) and anteiso (C_{n-2} methyl- C_n) monocarboxylic acids predominated.

TABLE 3.14

EQUIVALENT CHAIN LENGTH (ECL) VALUES FOR THE PRINCIPAL HOMOLOGOUS SERIES OF RESOLVED HYDROCARBON UCM OXIDATION PRODUCTS

C. No.	N-ACIDS		N- α,ω -DIACIDS		N-ALKAN-2-ONES		ISO ALKAN-2-ONES		γ -METHYL- γ -LACTONES		ω -CARBOXY- γ -METHYL γ -LACTONES		
	M.Wt.	ECL	M.Wt.	ECL ^a	ECL ^b	M.Wt.	ECL ^a	M.Wt.	ECL ^a	M.Wt.	ECL ^a	M.Wt.	ECL ^a
6	130	6.00	174	9.17	-	-	-	114	6.61	-	-	-	-
7	144	7.00	188	10.24	-	-	-	128	7.67	-	-	-	-
8	158	8.00	202	11.26	11.22	128	6.68	128	6.36	142	8.61	186	11.70
9	172	9.00	216	12.25	12.23	142	7.65	142	7.36	156	9.62	200	12.85
10	186	10.00	230	13.25	13.24	156	8.69	156	8.27	170	10.64	214	13.97
11	200	11.00	244	14.26	-	170	9.70	170	9.27	184	11.67	228	14.97
12	214	12.00	258	15.25	15.26	184	10.71	184	10.21	-	-	-	-
13	228	13.00	272	16.26	-	198	11.71	198	11.21	-	-	-	-
14	242	14.00	286	17.26	-	212	12.75	-	-	-	-	-	-
15	256	15.00	-	-	-	226	13.74	-	-	ECL ^a : ECL found			
16	270	16.00	-	-	-	240	14.75	-	-	ECL ^b : literature reported ECL value			
17	284	17.00	-	-	-	254	15.75	-	-	(Rostad and Pereira, 1986)			
18	298	18.00	-	-	-	268	16.77	-	-	-	-	-	-

FIG.3.46 PLOTS OF CARBON NUMBER vs EQUIVALENT CHAIN LENGTH (ECL) VALUES FOR THE PRINCIPAL HOMOLOGOUS SERIES OF UCM OXIDATION PRODUCTS



- a : N-MONOCARBOXYLIC ACIDS
- b : γ -METHYL- γ -LACTONES
- c : N- α,ω -DICARBOXYLIC ACIDS
- d : N-ALKAN-2-ONES
- e : ISOALKAN-2-ONES

FIG. 3.47 EI AND CI (NH₃) MASS SPECTRA OF A C11-METHYL BRANCHED γ -METHYL- γ -LACTONE IDENTIFIED AS AN OXIDATION PRODUCT OF THE ALIPHATIC UCM

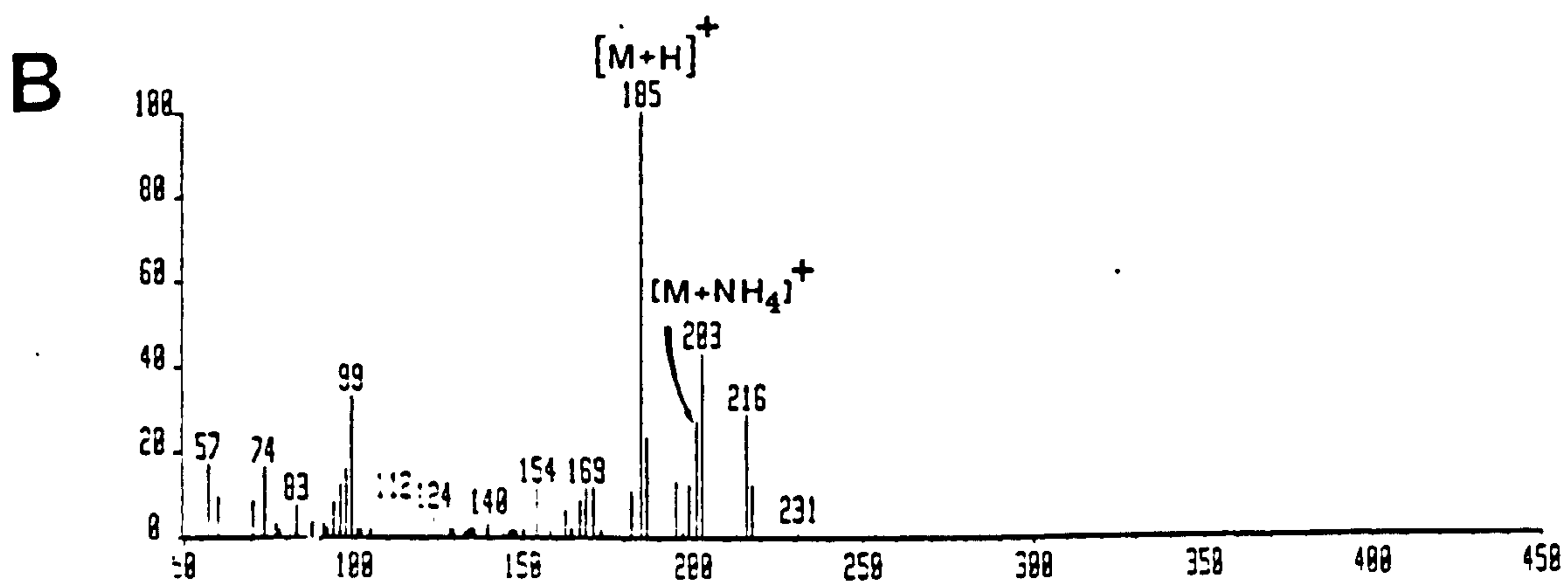
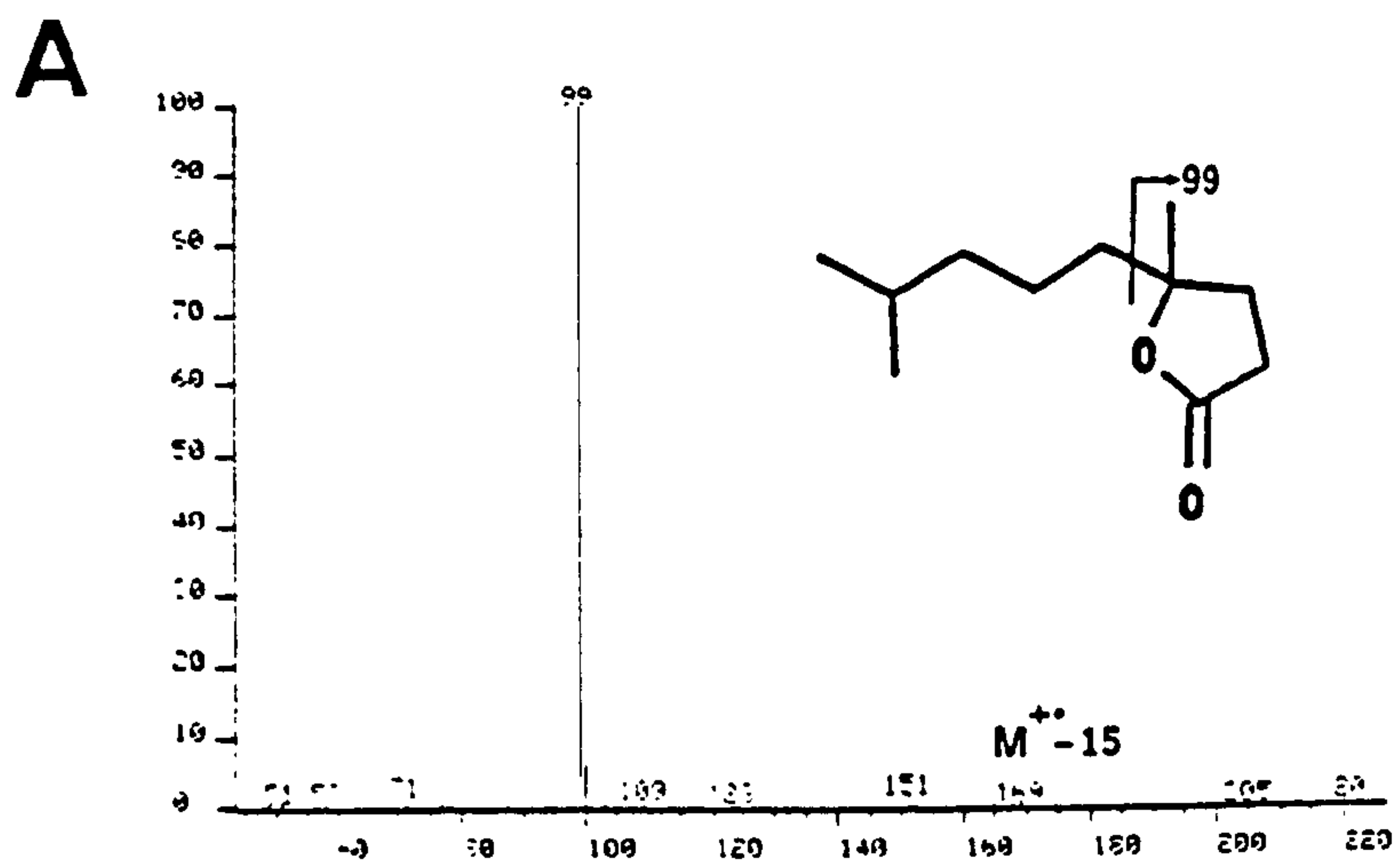
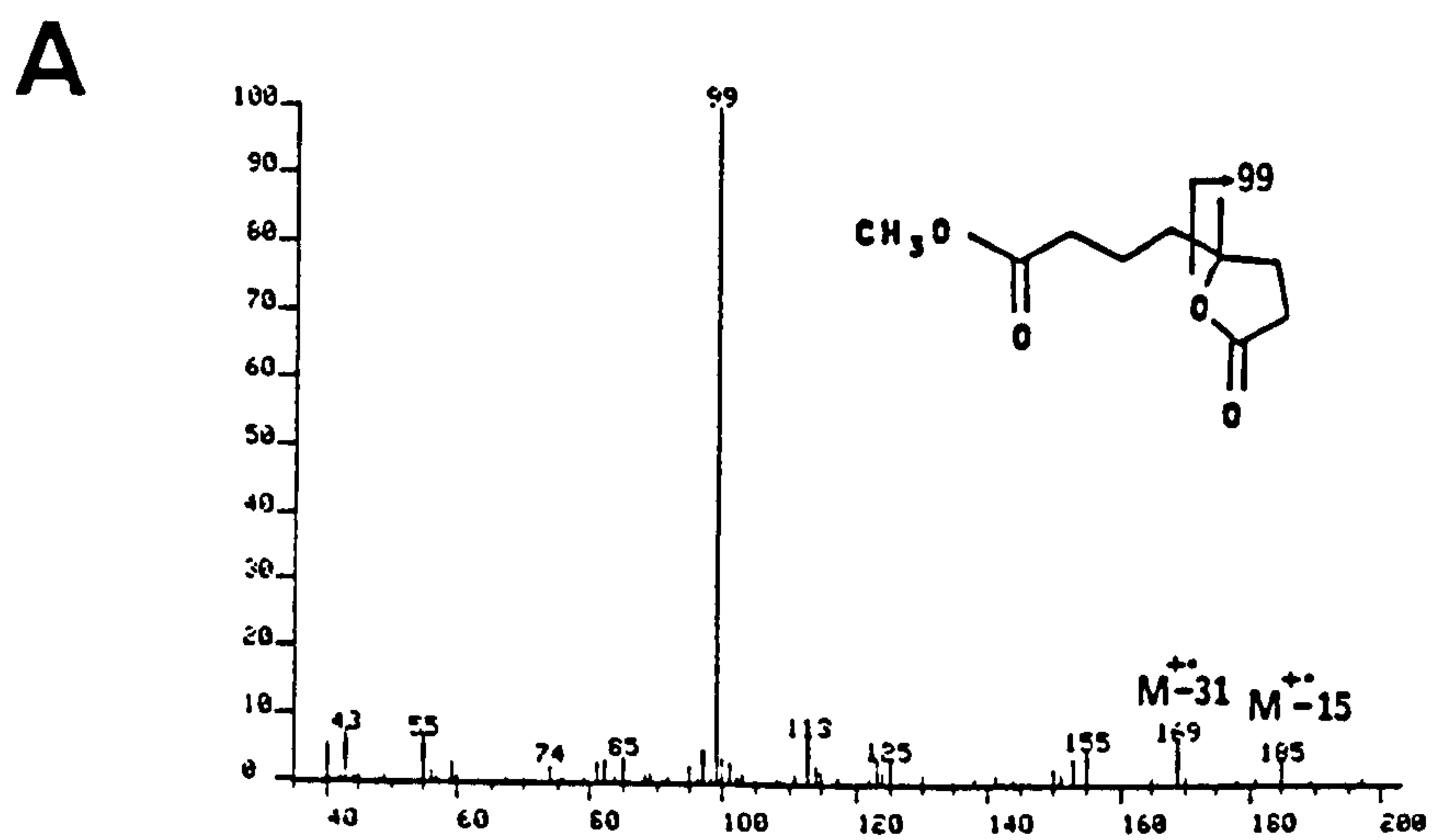
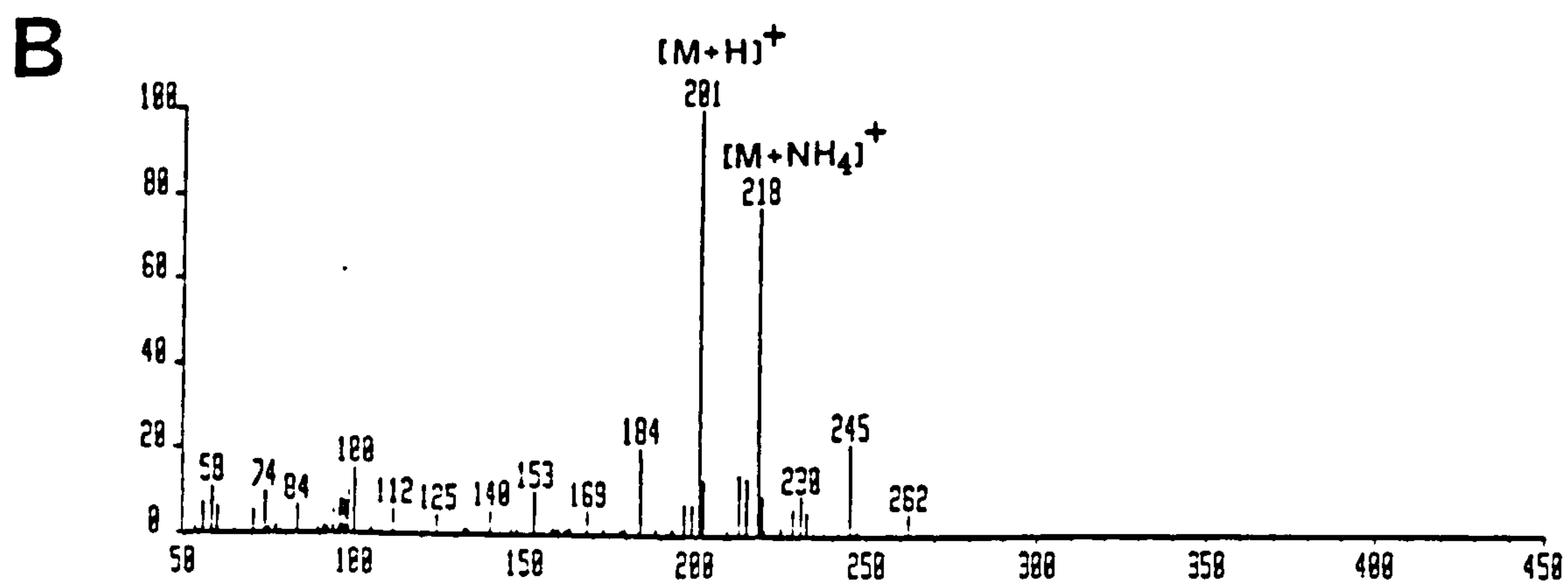


FIG. 3.48 EI AND CI (NH₃) MASS SPECTRA OF A C₉- ω -CARBOXY- γ -METHYL- γ -LACTONE IDENTIFIED AS AN OXIDATION PRODUCT OF THE ALIPHATIC UCM

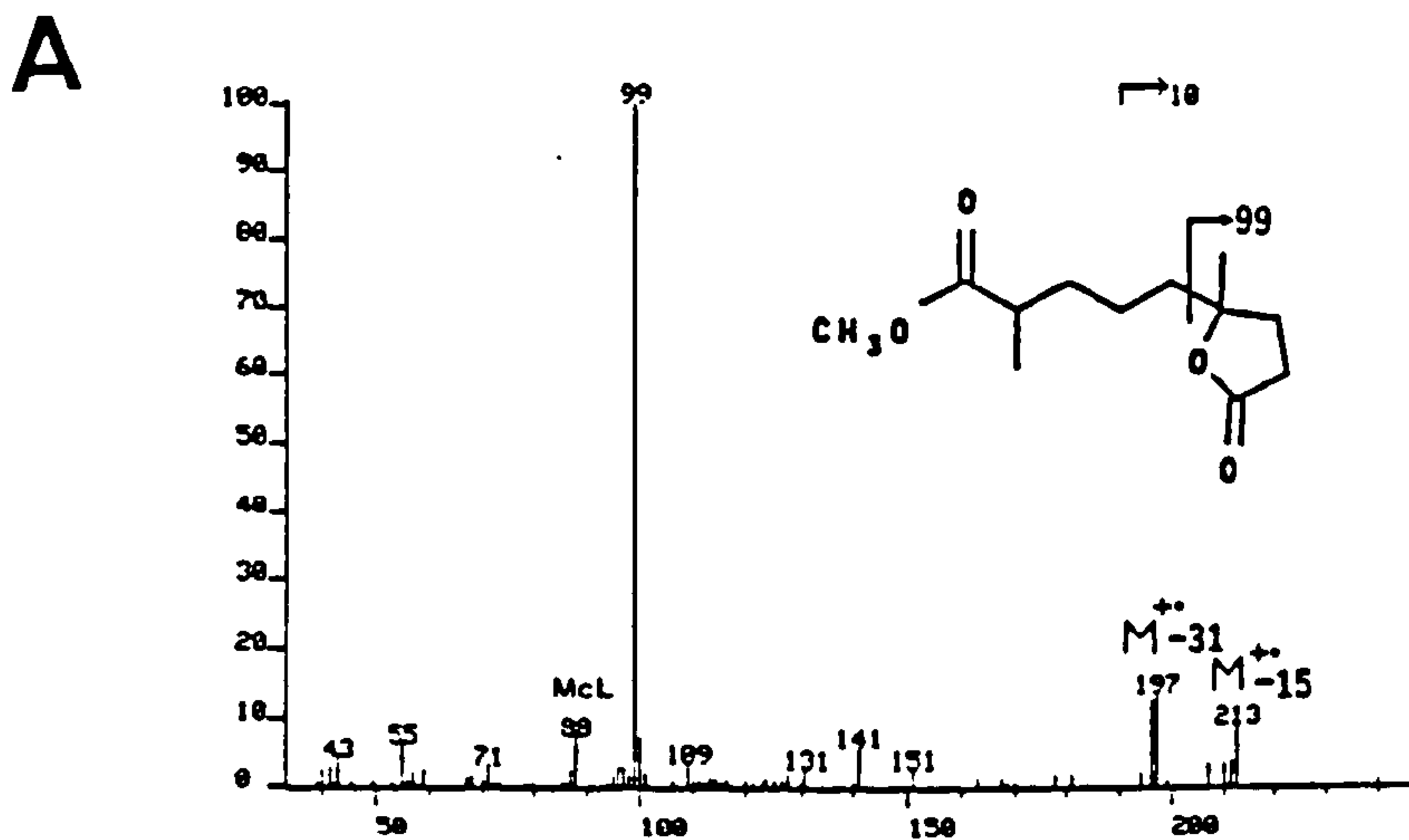


A) EI MASS SPECTRUM

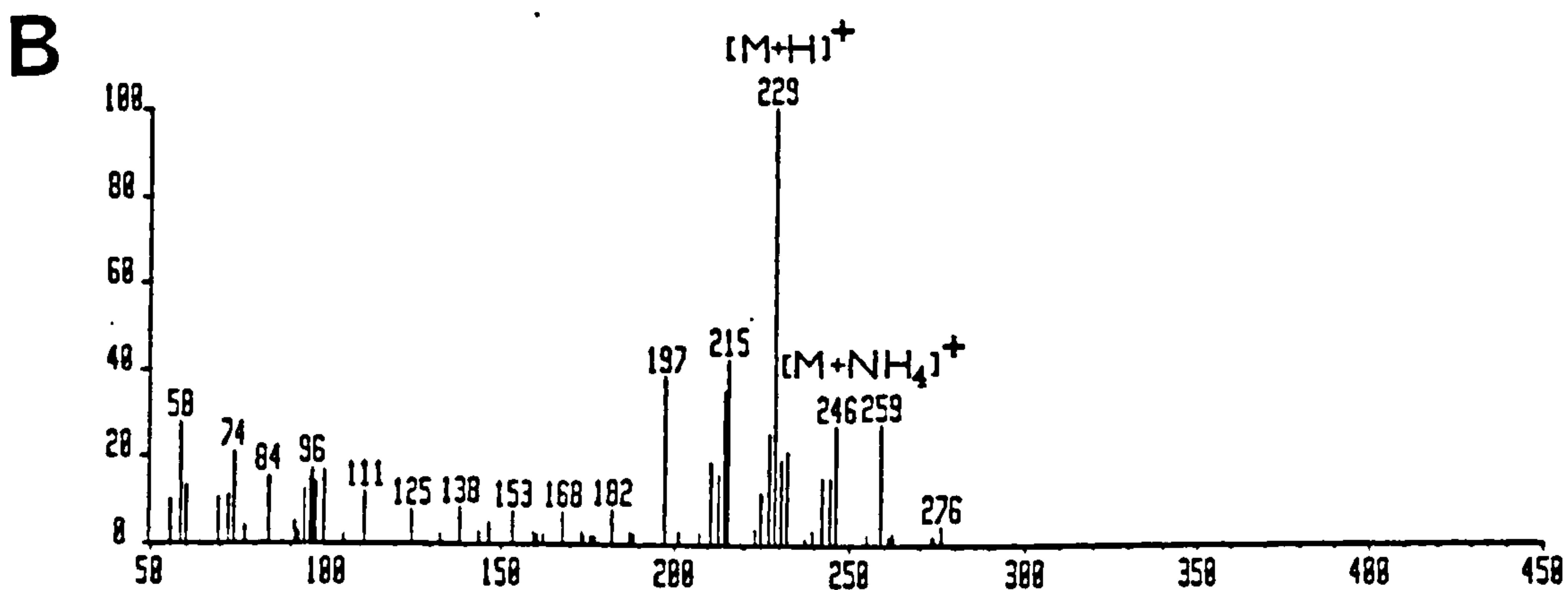


B) CI (NH₃) MASS SPECTRUM

FIG. 3.49 EI AND CI (NH₃) MASS SPECTRA OF A C11 METHYL BRANCHED ω-CARBOXY-γ-METHYL-γ-LACTONE IDENTIFIED AS AN OXIDATION PRODUCT OF THE ALIPHATIC UCM



A) EI MASS SPECTRUM



B) CI (NH₃) MASS SPECTRUM

TABLE 3.15

MONOMETHYL BRANCHED MONOCARBOXYLIC ACIDS (METHYL ESTERS)
IDENTIFIED AS ALIPHATIC UCM OXIDATION PRODUCTS

Scan	Diagnostic Ions	MOL.WT	Proposed compound ^a	ECL (found)	ECL ^b (lit)
64	74(100), 87(20), 101(22), 113(5)	144	5-MC6ma(Iso)	6.67	
70	74(100), 87(88), 113(22), 115(20)	144	4-MC6ma(anteIso)	6.75	6.71
122	74(100), 87(15), 101(27), 129(26)	158	3-MC7ma	7.44	
135	74(100), 87(59), 115(25), 127(12), 129(4)	158	6-MC7ma(Iso)	7.61	7.63
140	74(100), 87(20), 101(10), 127(5), 129(8)	158	5-MC7ma(anteIso)	7.68	
194	74(100), 86(4), 101(26), 141(5)	172	4-MC8ma	8.42	
206	74(100), 87(2), 101(10), 129(12), 141(5)	172	7-MC8ma(Iso)	8.59	
210	74(100), 87(55), 101(8), 129(24), 141(4), 143(3)	172	6-MC8ma(anteIso)	8.65	8.69
265	74(100), 101(48), 85(13), 129(5), 155(10)	186	5-MC9ma	9.43	
275	74(94), 87(16), 113(25), 129(10), 155(10)	186	4-MC9ma	9.56	9.61
281	74(100), 87(78), 143(23)	186	8-MC9ma(Iso)	9.65	9.65
316	74(54), 87(100), 127(15), 169(8)	200	4-MC10ma	10.15	
333	74(100), 87(10), 101(65), 169(10)	200	5-MC10ma	10.41	

a: Code : 5-MC6ma = 5-methylhexanoic acid (methyl ester)

b: ECL values obtained from Body, D.R., 1984 (10% OV-101 on Gas-chrom Q)

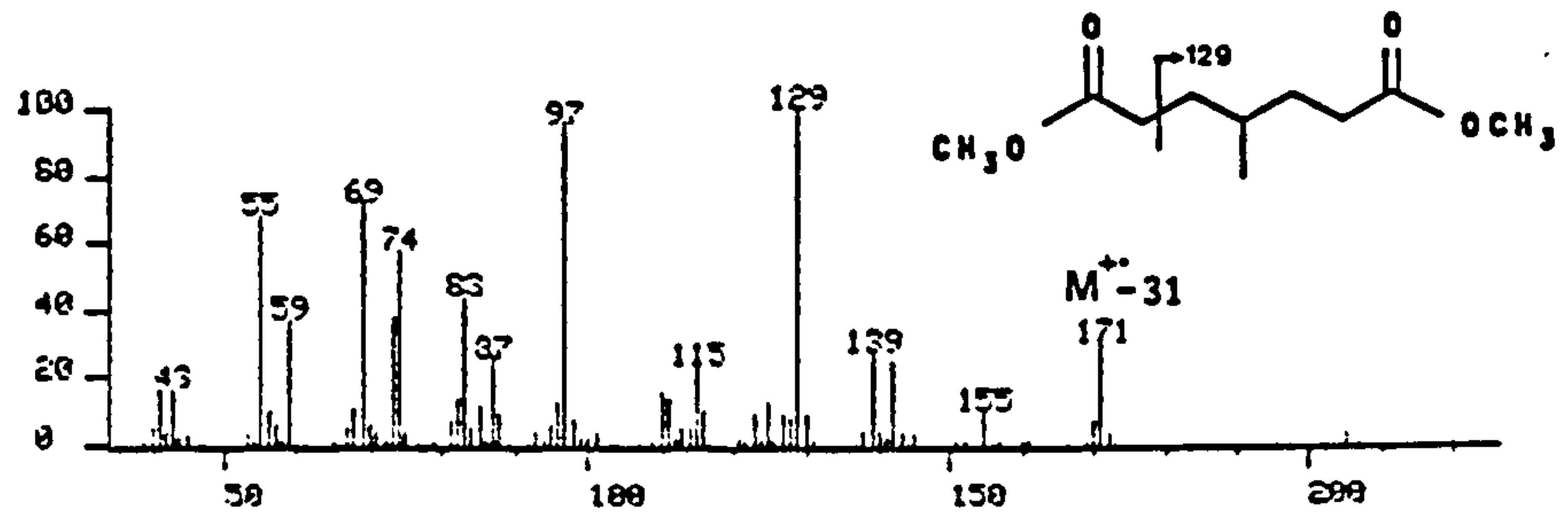
Methyl branched α,ω -dicarboxylic acids were identified as aliphatic UCM oxidation products by a combination of EI and CI GC-MS, and representative spectra are shown in Fig. 3.50. Again their abundance was reduced in comparison to n - α,ω -dicarboxylic acids, and monomethyl substituted compounds were found to predominate. Certain monomethyl substituted ω -oxo-carboxylic acids were also identified (Fig. 3.51). Cyclohexyl carboxylic acids were generally absent with the important exception of 2-cyclohexylethanoic acid (Fig. 3.52) which was identified by a comparison with the oxidation products of the model cycloalkane 9-(2-cyclohexylethyl)-heptadecane (section 4.2.5).

Additional compounds were identified which were consistent with an acyclic isoprenoid type origin although in low abundance in comparison with n -monocarboxylic acids and n - α,ω -dicarboxylic acids. These were the isoprenoid derived monocarboxylic acids 4,8-dimethylnonanoic acid and 4,8,12-trimethyltridecanoic acid; and the isoprenoid derived ketone 6,10-dimethylundecan-2-one (Fig. 3.42). Their identification was aided by a comparison of extrapolated ECL values from the products of oxidation of authentic pristane (section 4.2.7).

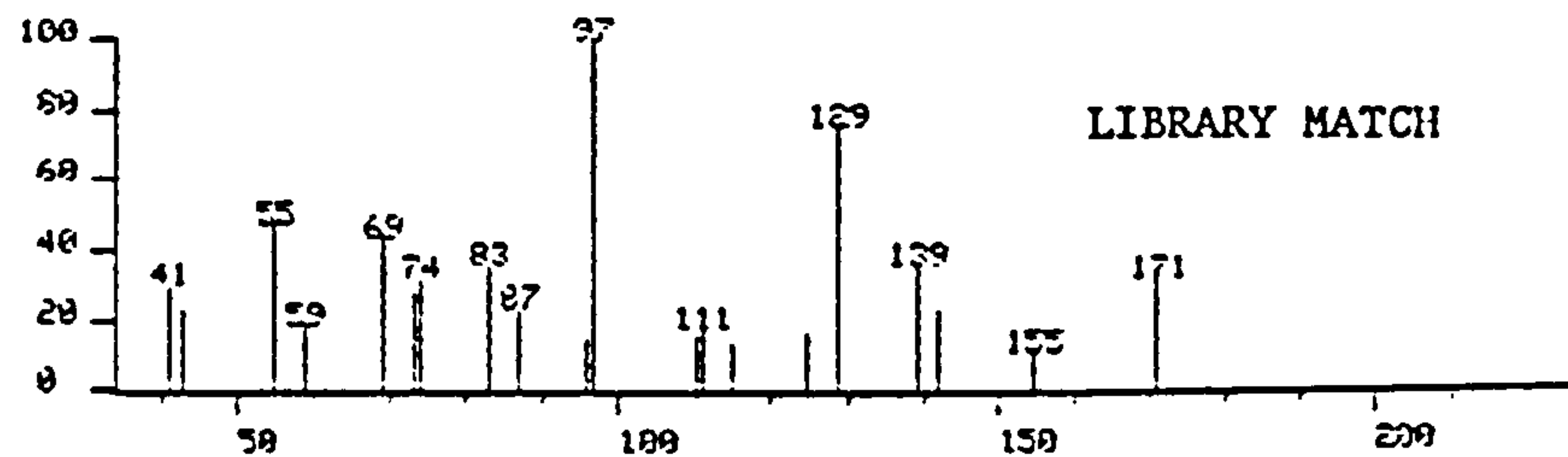
In summary, the CrO_3 /glacial acetic acid oxidation of the aliphatic UCM produced series of functionalised compounds in good yield and which were well resolved by GC. Many of these were identified by EI and CI GC-MS, comparisons with published spectra and relative retention indices, and by analysis of the oxidation products of synthetic model hydrocarbons (section 4.2). The compounds identified are summarised in Table 3.16. Perhaps the most surprising result of the oxidation of what has been presumed by most workers to be a

FIG. 3.50 EI AND CI (NH₃) MASS SPECTRA OF METHYL BRANCHED α,ω -DICARBOXYLIC ACIDS IDENTIFIED AS OXIDATION PRODUCTS OF THE ALIPHATIC UCM

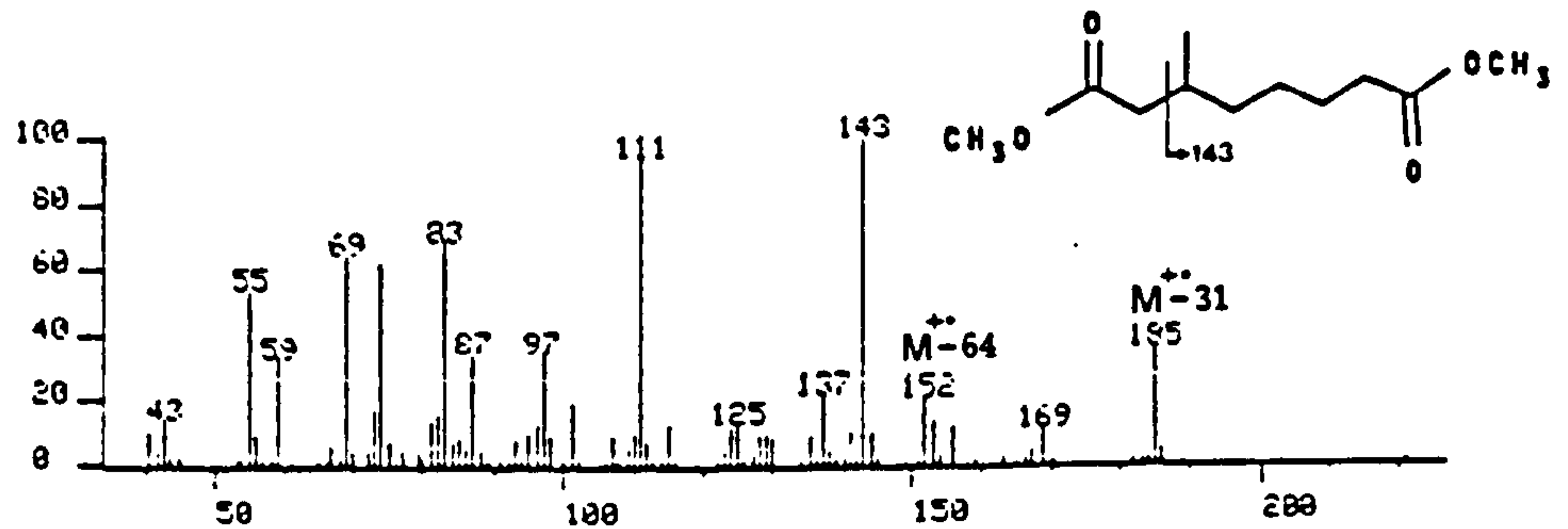
A



A) 4-METHYLHEPTANDIOIC ACID (EI)



B



B) 3-METHYLOCTANDIOIC ACID (EI + CI)

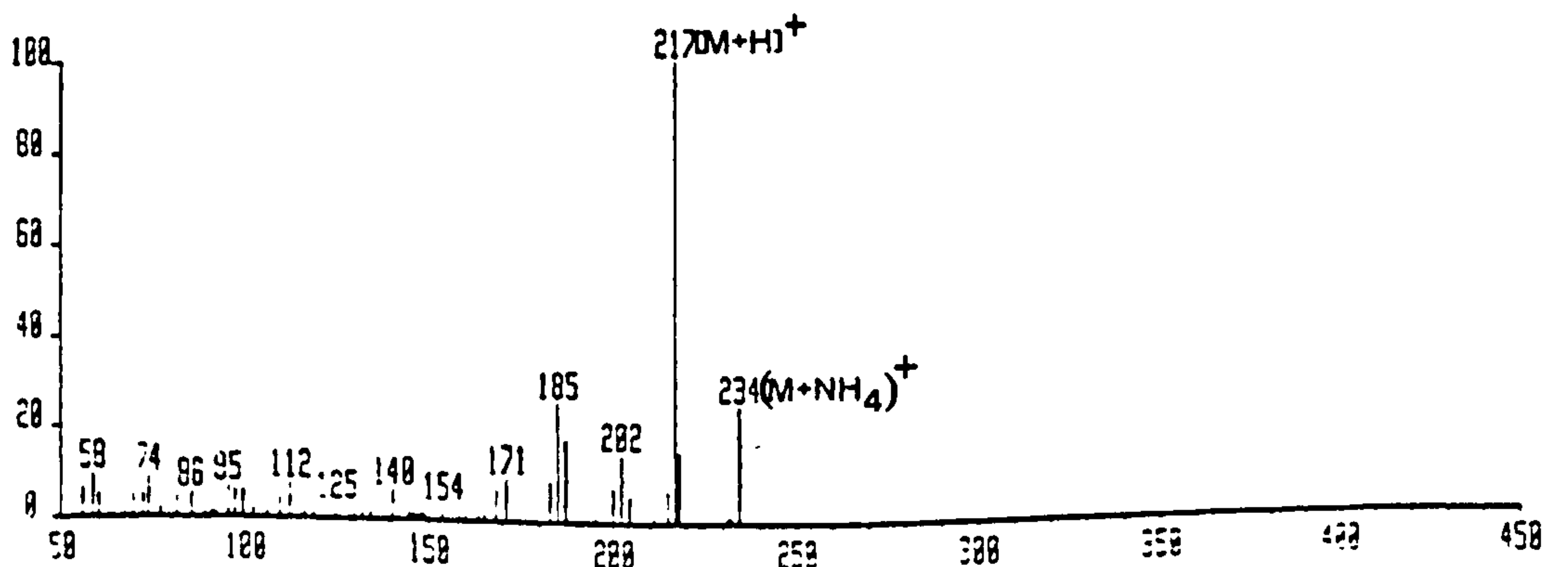
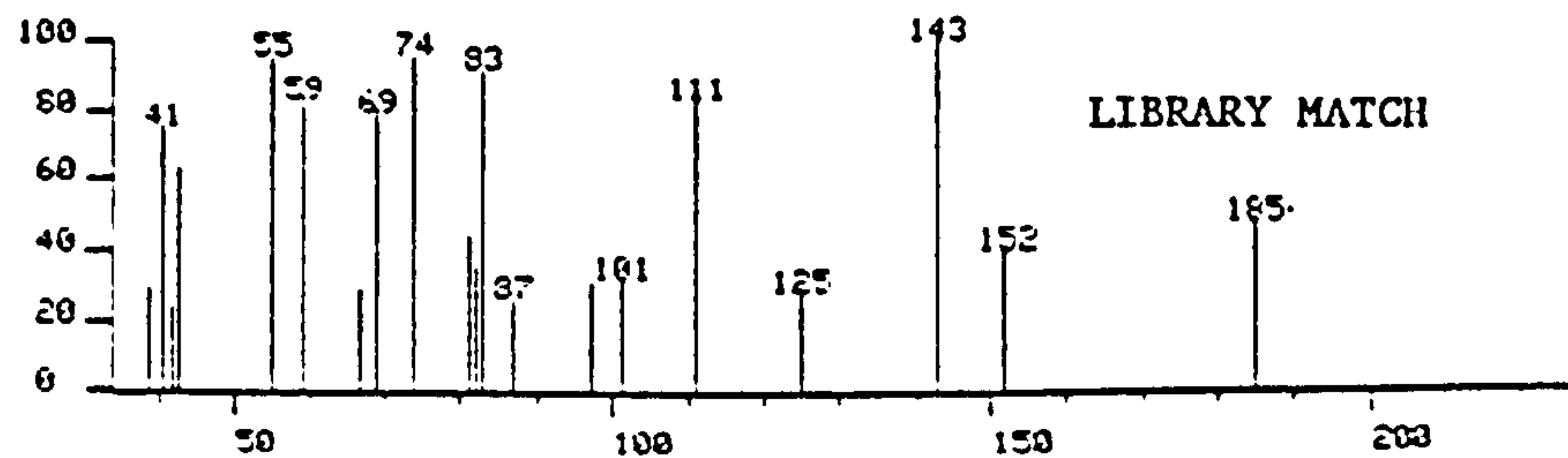


FIG 3.51 EI MASS SPECTRUM OF A C8 METHYL BRANCHED KETO ACID IDENTIFIED AS AN OXIDATION PRODUCT OF THE ALIPHATIC UCM

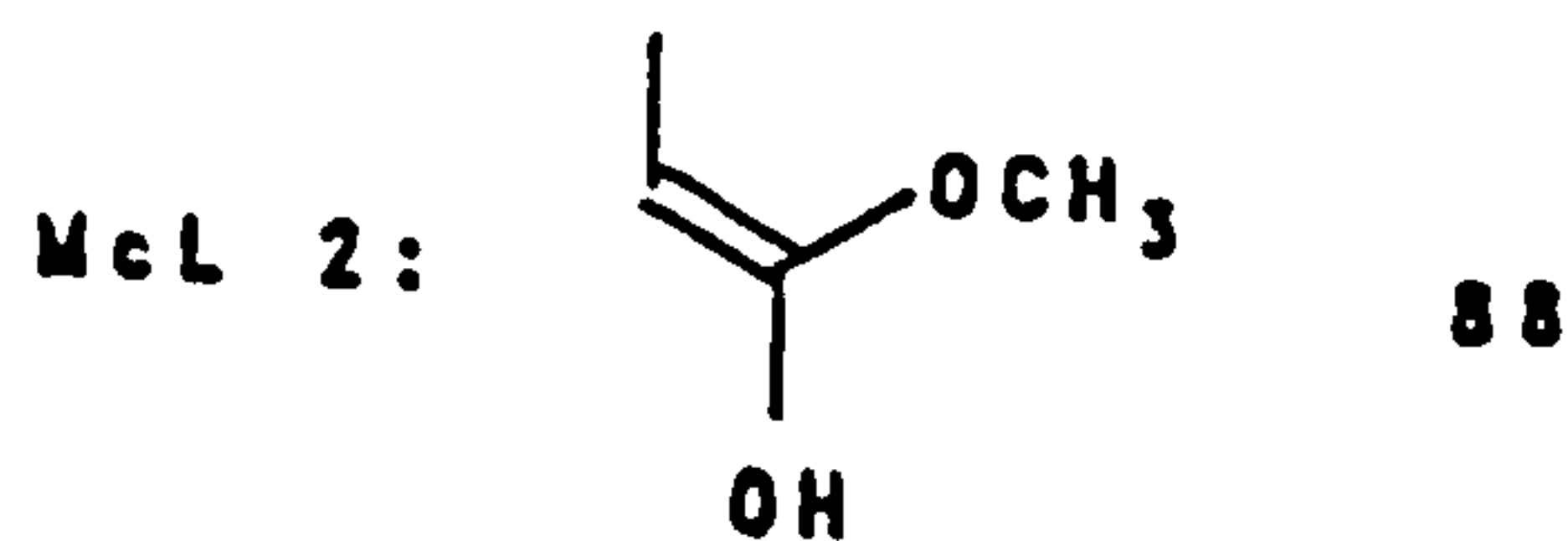
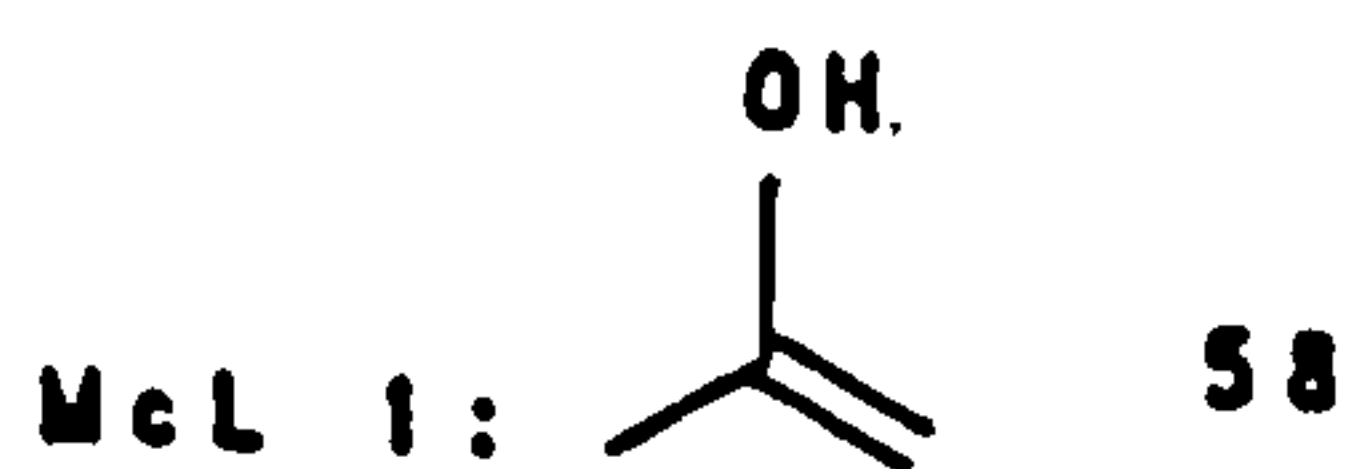
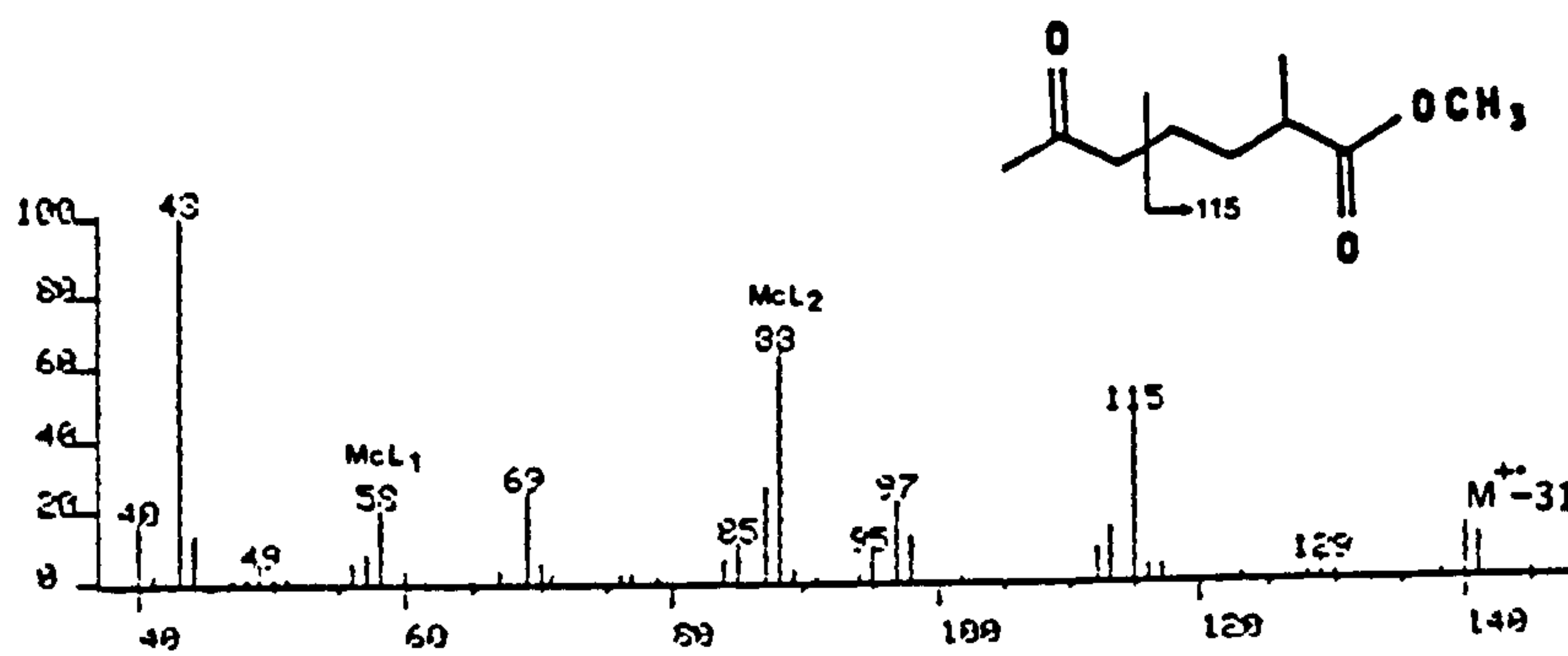


FIG 3.52 EI MASS SPECTRUM OF 2-CYCLOHEXYLETHANOIC ACID /METHYL ESTER IDENTIFIED AS A) AN ALIPHATIC UCM OXIDATION PRODUCT AND B) A PRODUCT OF OXIDATION OF 9-(2-CYCLOHEXYLETHYL)-HEPTADECANE

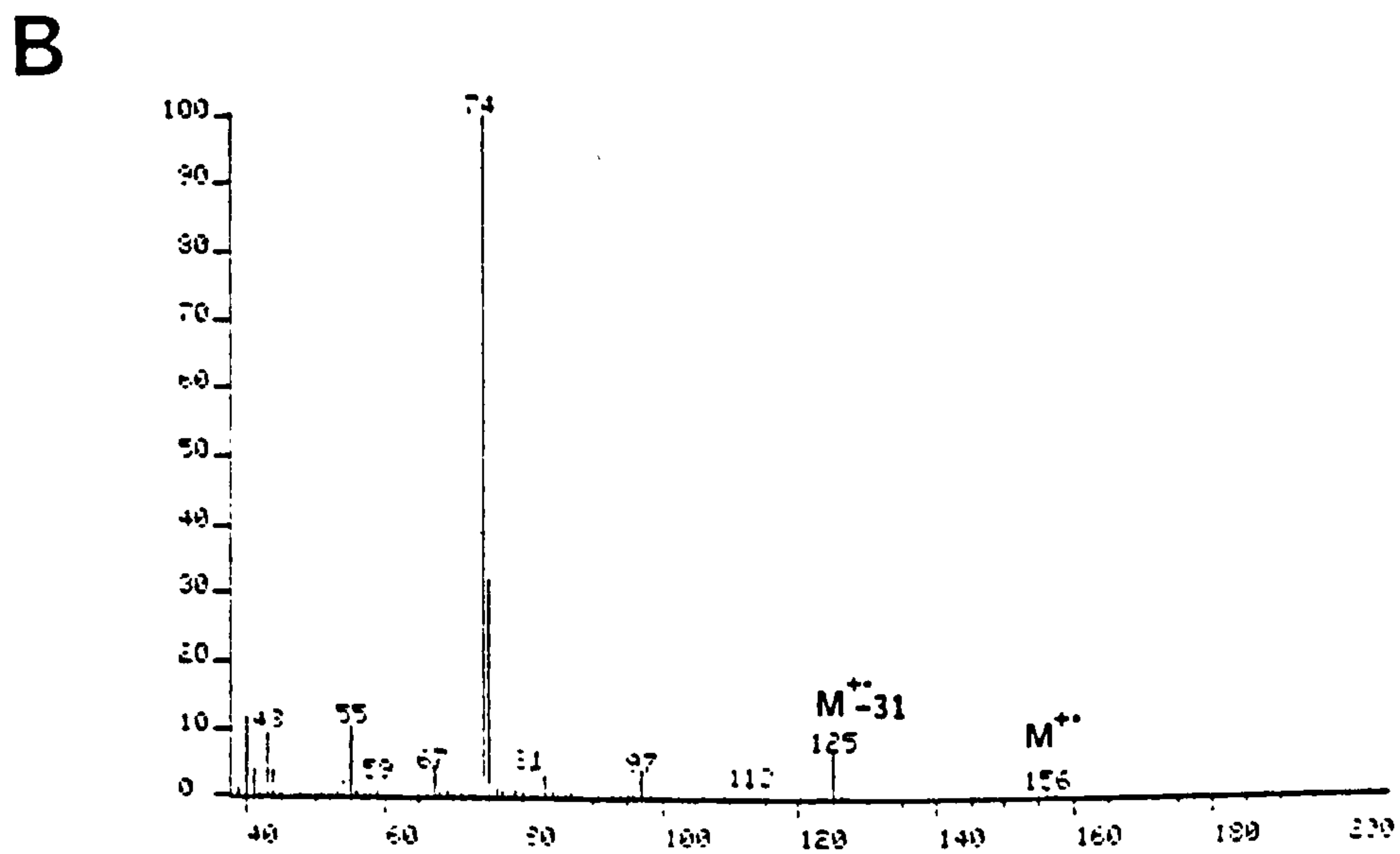
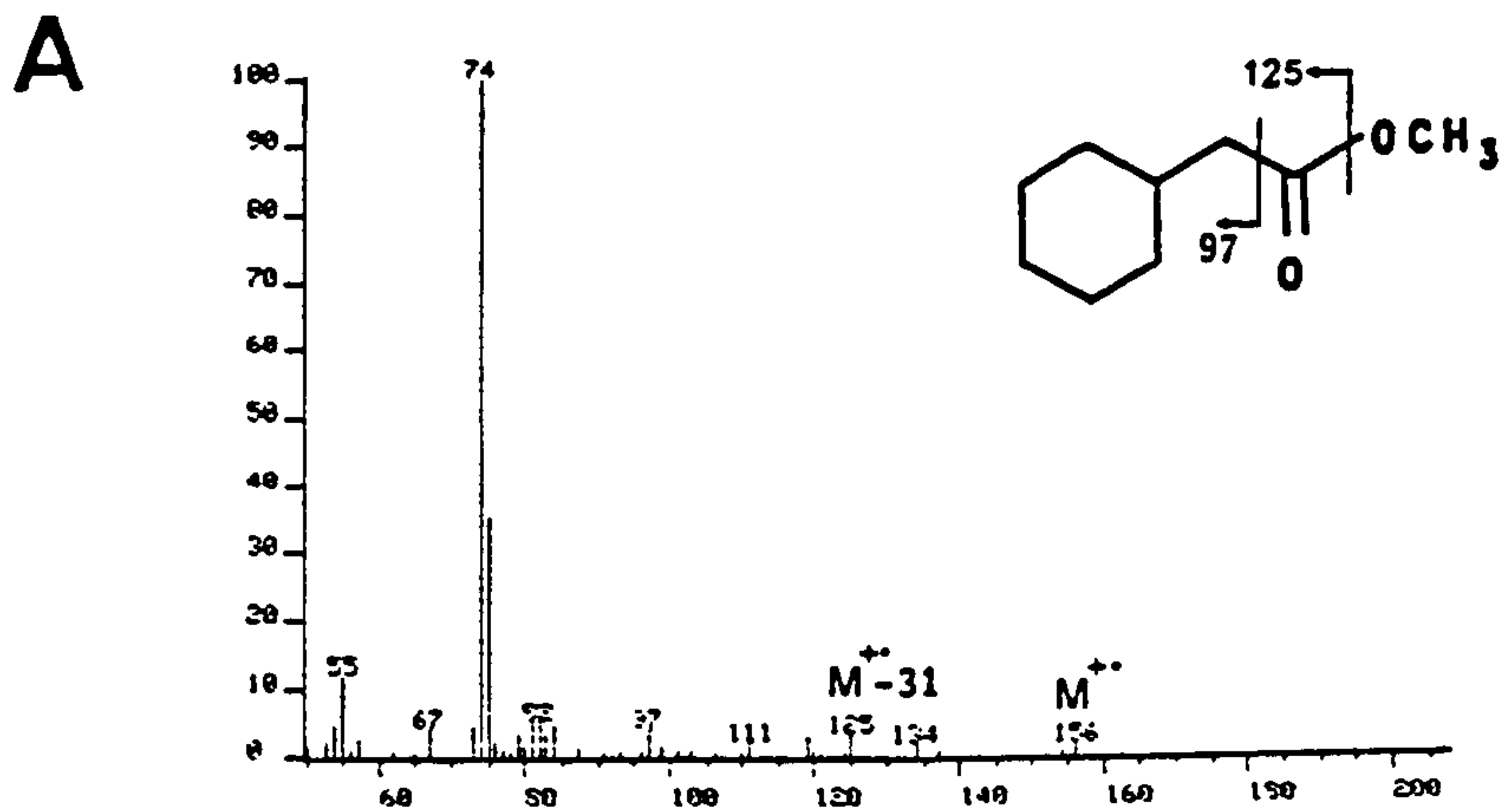


TABLE 3.16

A SUMMARY OF IDENTIFIED OXIDATION PRODUCTS OF THE ALIPHATIC UCM

Compound	Carbon no. range	maximum
<u>n</u> -monocarboxylic acids	C6 - C20	C9
α,ω - <u>n</u> -diacarboxylic acids	C6 - C14	C9
Keto acids	C8, C10, C11	C10
<u>n</u> -alkan-2-ones	C8 - C15	C12
"iso"-methylbranched alkan-2-ones	C8 - C12	C8
γ -methyl- γ -lactones	C6 - C10	C8
methylbranched γ -methyl γ -lactones ^a	C11 - C12	C11
ω -carboxy- γ -methyl γ -lactones	C8 - C11	C8
methylbranched- ω -carboxy- γ - ^a methyl- γ -lactones	C11 - C13	C11
methylbranched monocarboxylic acids ^b	C6 - C10	-
methylbranched α,ω -diacids ^b	C8, C9, C11	-
cyclohexyl carboxylic acids	C8	C8 ^c
iosprenoid acids	C11 - C16	-
isoprenoid ketones	C13	C13 ^c

Code : ^a in-chain methyl group position not definitely known

^b varied positions for methyl group

^c one compound identified only

mixture of "highly branched and/or cyclic" hydrocarbons (e.g. Farrington et al., 1977) was the relatively simple structure of the compounds produced. Thus normal mono- and di-carboxylic acids were quantitatively the most important components of the resolved oxidation products, whereas polymethyl and cyclic carboxylic acids were virtually absent.

To account for this relatively simple distribution of observed oxidation products a consideration of the proposed mechanism of CrO_3 /glacial acetic acid oxidation of alkanes was made. Though a wide range of alkanes have been oxidised by this reagent the precise mechanism of oxidation remains unclear, and the intermediates and ultimate products often vary depending on reaction conditions (reviewed by Wiberg, 1965; Cainelli and Cardillo, 1984; Freeman, 1986). The major findings are:

i) the oxidation is acid catalysed and the rate is first order with respect to both the oxidant and the alkane,

i.e, $r = k[\text{alkanes}][\text{CrO}_3]h_0$; where h_0 is the acidity function.

ii) the relative rates of oxidation of primary, secondary and tertiary C-H bonds are 1: 110 : 7000.

iii) A kinetic isotope effect is observed for C-H bond activation ($K_H/K_D=2.5$).

iv) steric acceleration is generally observed, i.e. by increasing the bulky nature of alkyl substituents around a tertiary centre an increase in oxidation rate is observed.

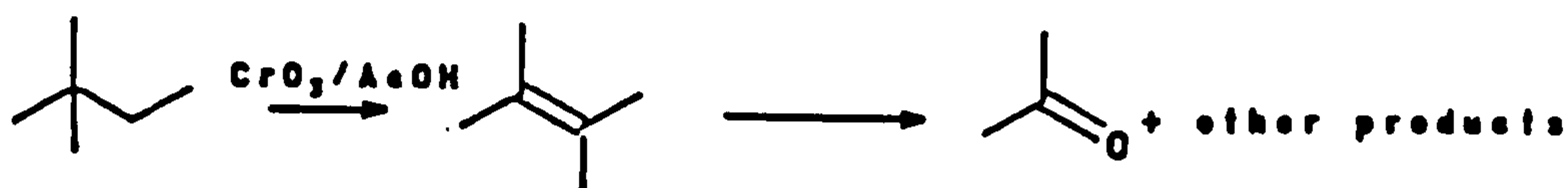
v) the initial product of the oxidation at the tertiary centre is the corresponding tertiary alcohol.

vi) oxidation of asymmetric carbon centres generally leads to the tertiary alcohol with retention of stereochemical configuration.

7) in certain cases the addition of azide ion (N_3^-) to the reaction mixture produces the corresponding alkyl azide.

8) in the CrO_3 glacial acetic acid oxidation of 2,3-dimethylbutane (neohexane), a major product is acetone, formed presumably via methyl migration and formation of the intermediate alkene 2,3-dimethylbut-2-ene.

i.e.



To account for these (often conflicting) observations an elaborate mechanism has been proposed (Wiberg, 1965). In aqueous solutions the reacting Cr(VI) species is believed to be the acid chromate ion (HCrO_4^-); though in acetic acid under strictly anhydrous conditions it is not known whether the oxidant exists as monomeric CrO_3 , as acid chromate (H_2CrO_4) or as the acetochromate ion ($\text{CH}_3\text{COCrO}_2^-$, Wiberg, 1965). For the purpose of comparison with literature oxidations, the reacting species will be assumed to be acid chromate (H_2CrO_4).

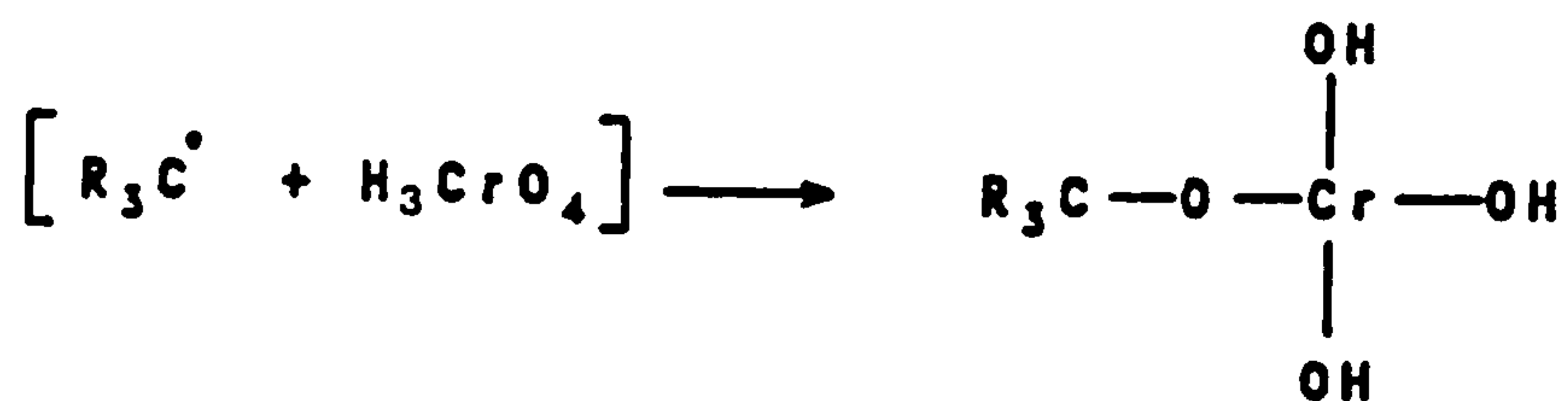
The observation of a large kinetic isotope effect suggests the rate controlling step is C-H bond cleavage, presumably via homolytic

cleavage to form a free radical.

i.e.

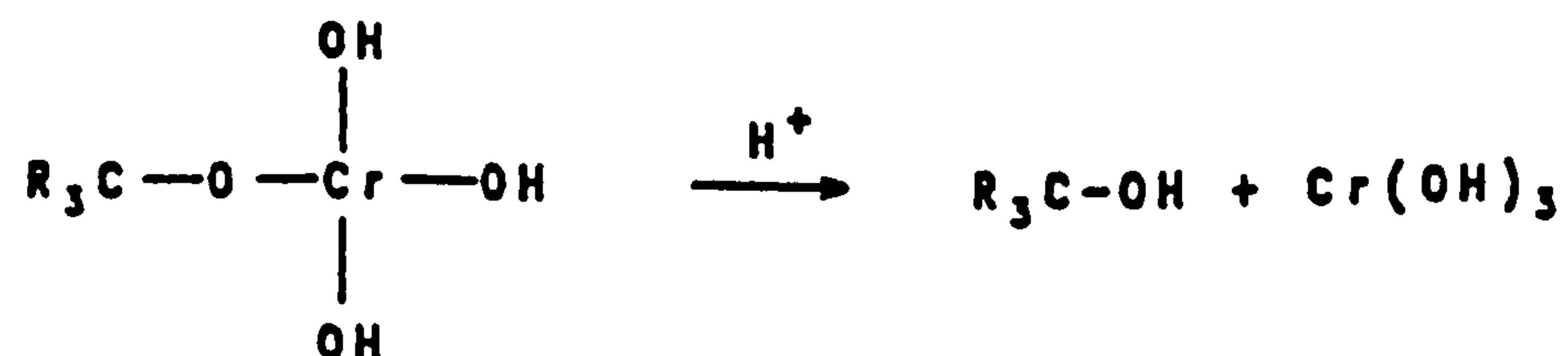


To account for the general lack of rearrangement the radical species is probably not free, but remains trapped within a solvent cage. It may then undergo recombination with the Cr(V) species to produce a chromate ester:-



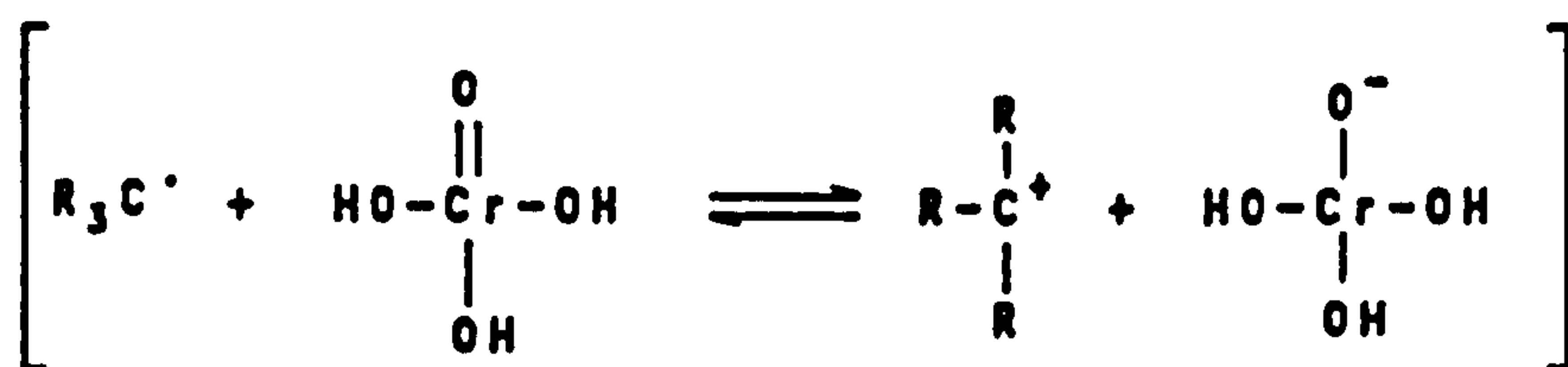
Acid catalysed hydrolysis with Cr-O bond cleavage provides a tertiary alcohol with retention of configuration.

i.e.



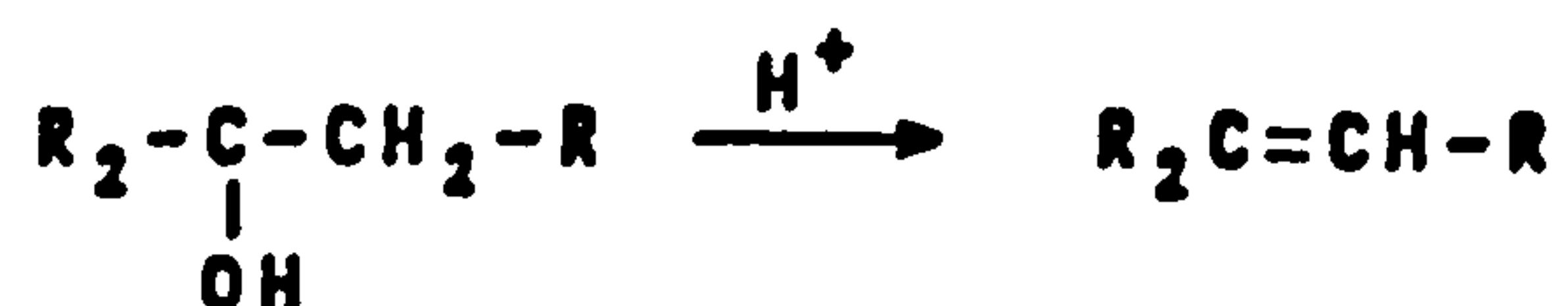
To account for the small amount of rearrangement that does occur and the formation of alkyl azides, a degree of cationic character is required. This was explained by assuming the radical is present as a resonance hybrid within the solvent cage.

i.e.



From this the radical species may recombine to form the chromate ester with retention of configuration, or diffuse apart and be oxidised by further chromic acid to produce a carbocation. Alternatively, a carbocation could be formed by resonance within the solvent cage, and by diffusion apart and recombination with chromate anion followed by hydrolytic cleavage a racemic mixture of tertiary alcohols would be produced.

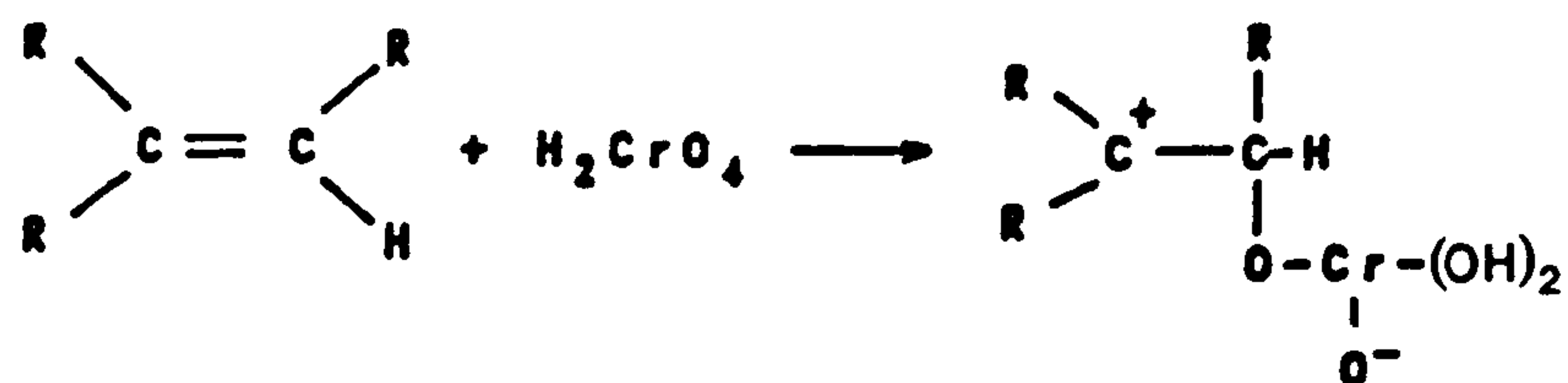
Although the tertiary alcohol (initial oxidation product) is observed in certain cases (Cainelli and Cardillo, 1984), the product is often unstable under acidic conditions, and undergoes acid-induced dehydration to form an alkene (providing α -hydrogens are present).



The resulting alkene is subject to further oxidation by additional chromic acid, and a number of products may result. Most normally these are acids and ketones, though other products (e.g. epoxides, glycols, diketones) have been isolated. Again the precise intermediates are unknown, though they may result from

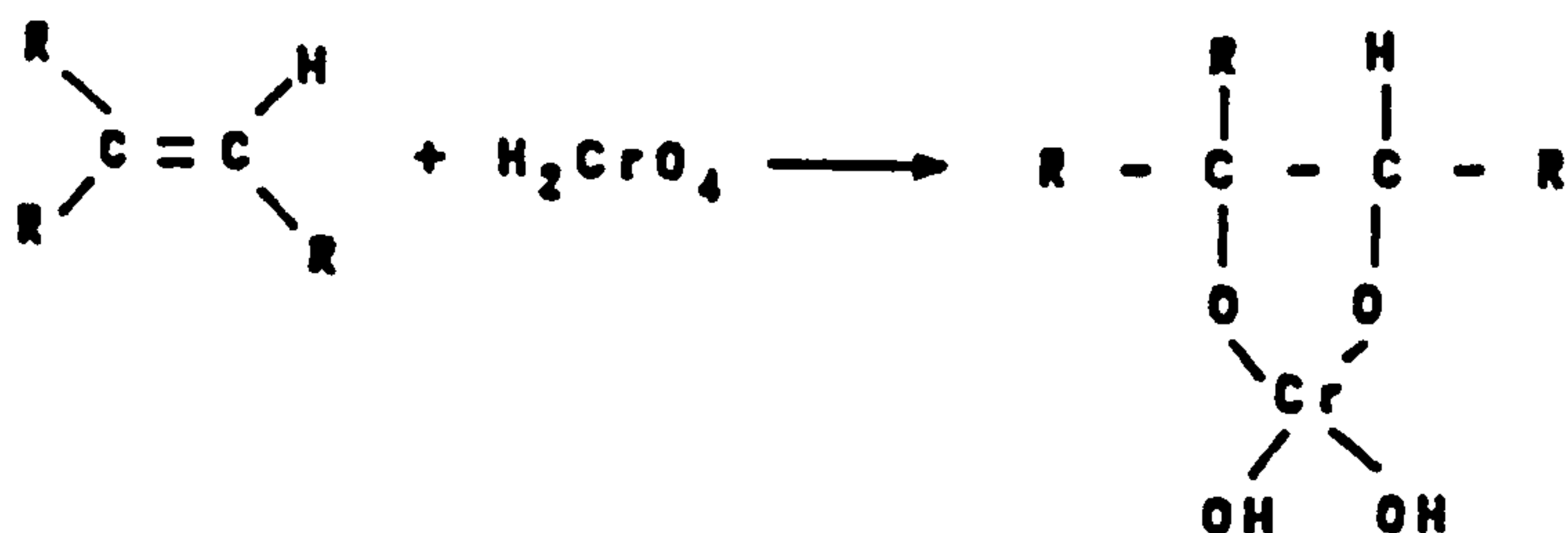
i) donation of an electron from the double bond :

i.e.



or ii) simultaneous addition across the double bond to form a cyclic chromate ester;

i.e.



Both could account for the formation of epoxides or 1,2-diols, though these are also known to undergo oxidative cleavage of the C-C bond to

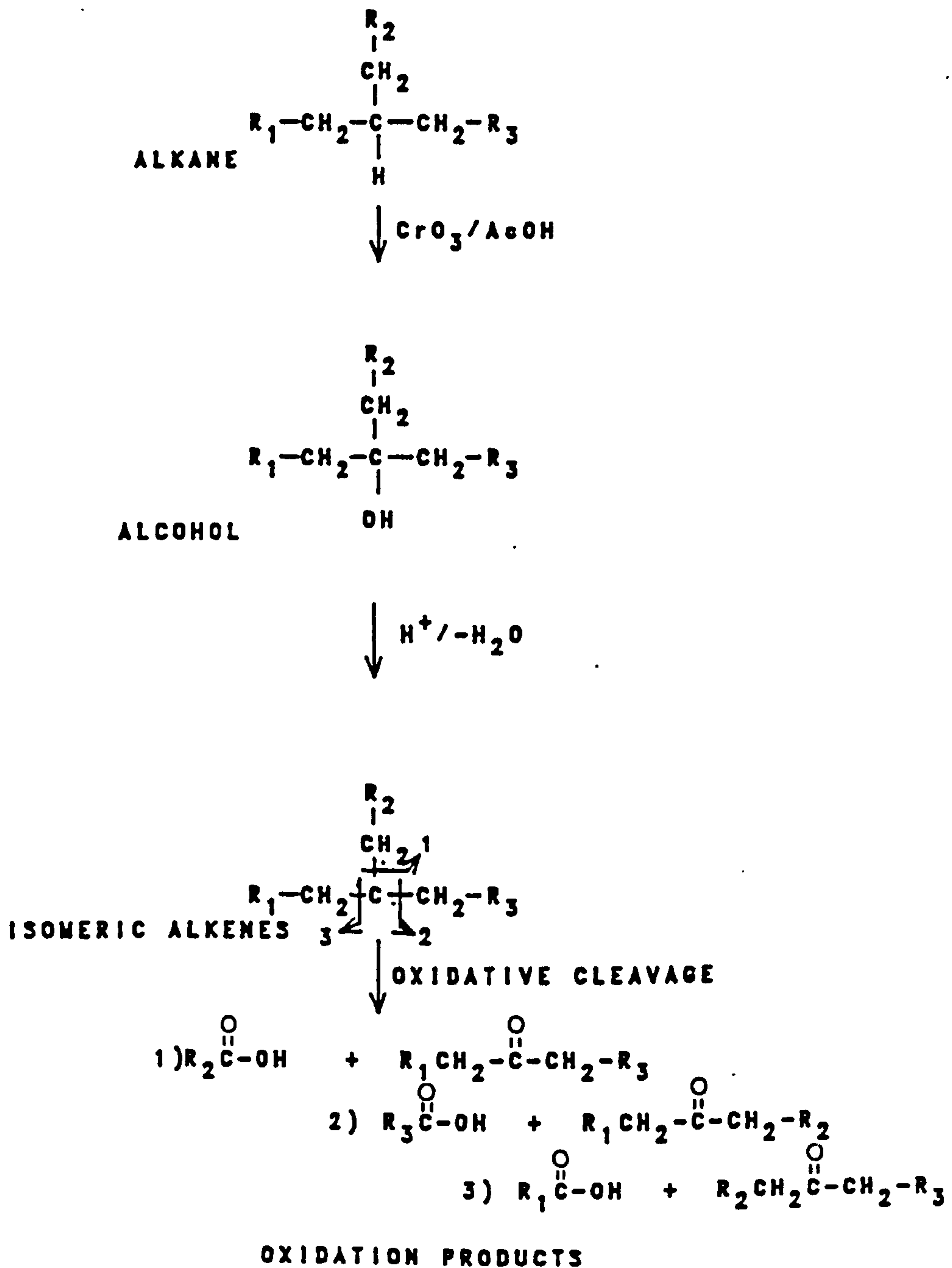
form mixtures of ketones and aldehydes or acids (March, 1985). It is clear however that the majority of alkanes oxidised produce mixtures of ketones and acids (dependent on the degree of alkyl substitution), and that these results from oxidative cleavage of the intermediate double bond. This proposed reaction scheme is summarised in Fig. 3.53.

On the assumption that the above mechanism operates in the CrO_3 / glacial acetic acid oxidation of the aliphatic UCM, the observed distribution of oxidation products can be ascribed to cleavage at specific linkages in precursor alkanes. The variety of products identified presumably result from cleavage at different alkyl linkages and each of these will be discussed in turn.

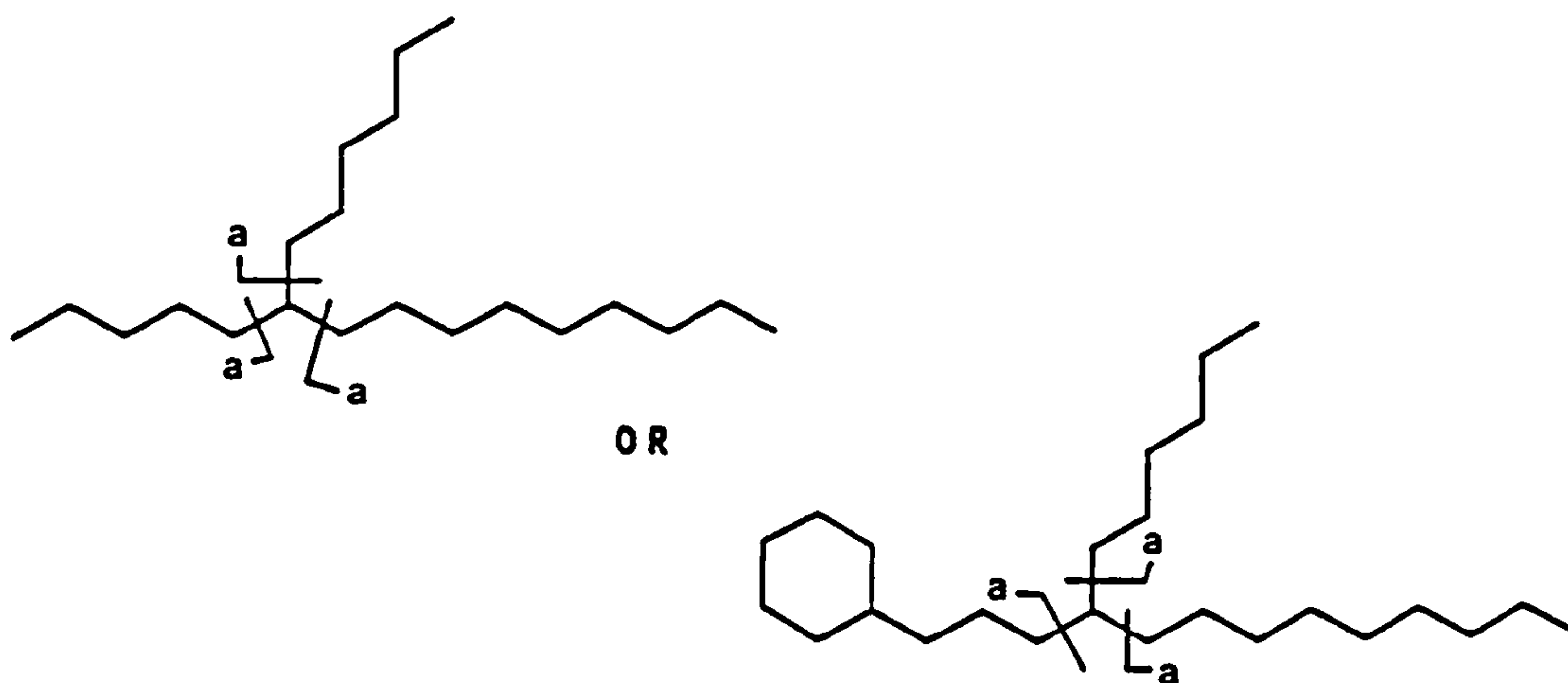
n-monocarboxylic acids :

The high proportion of straight chain monocarboxylic acids observed as aliphatic UCM products could conceivably be produced from terminal oxidation of n-alkanes. This is unlikely however, since i) lubricating oils are dewaxed during refining (Klamann, 1984) and ii) multiple urea adductions were performed prior to oxidation to remove traces of n-alkanes which survived dewaxing (observed in the urea adduct fraction and found to be low in comparison to monomethyl alkanes, section 3.12). A more likely explanation is that n-monocarboxylic acids originate from i) residual monomethyl alkanes not fully adducted by the sieve, and/ or ii) monoalkyl substituted alkanes with a sufficiently long alkyl substituent to prevent adduction by urea. Though some monomethyl alkanes did remain in the urea non adduct, their abundance as resolved compounds was much too

FIG. 3.53 SUMMARY OF THE MECHANISM OF CrO_3 /GLACIAL ACETIC ACID
OXIDATION OF BRANCHED ALKANES



low to produce the high proportion of acids observed. The second alternative is more likely, i.e. n-acids result from oxidation of monoalkyl branched acyclic (or possibly monocyclic) alkanes, with the length of the monoalkyl substituent sufficiently long to prevent their adduction into urea. Possible precursor alkanes for n-acids could therefore include structures of the following type :

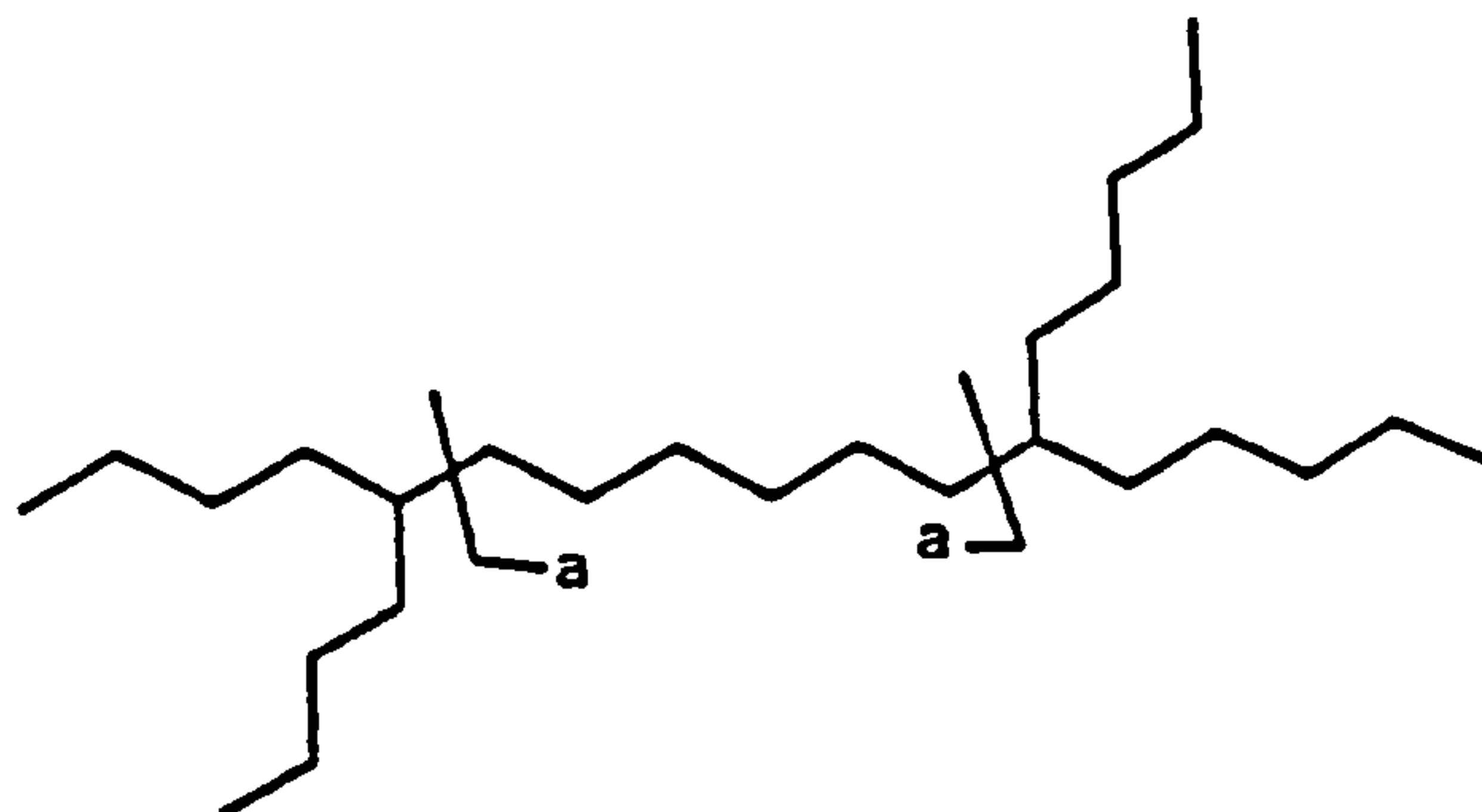


(a-acid)

To account for the observed carbon number range of n- monocarboxylic acids, the length of the alkyl chain must vary from C6 to C20.

n- α,ω -dicarboxylic acids :

From the above reasoning, α,ω -n-diacids could result from dialkyl substituted n-alkyl chains, i.e.

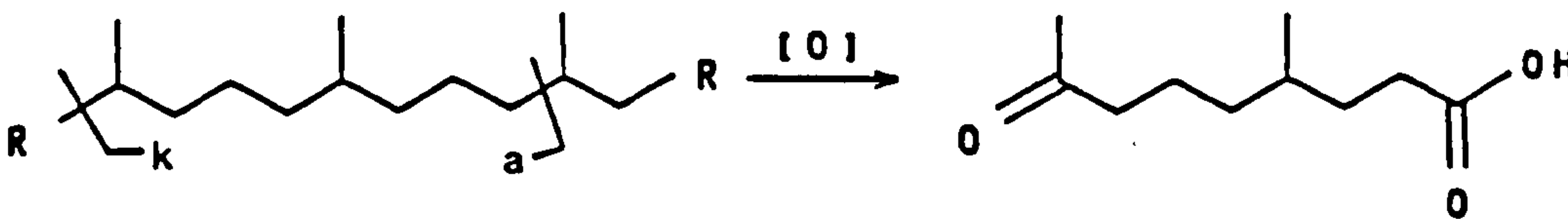


The range of compounds observed (C6-C13) may reflect the length of the alkyl chain lying between the branch positions. Alternatively straight chain diacids could arise as secondary oxidation products of n-monocarboxylic acids (e.g. see oxidation of n-pentacosane, section 4.2.3).

Ketoacids :

The observation of a series of these compounds as UCM oxidation products can be related to possible precursor methyl-branched alkyl linkages. Their derivation appears to proceed via simultaneous cleavage of a (at least) dialkyl substituted alkyl chain, producing both a keto and acid functionality in the same molecule. For instance, the major compound identified most likely originates from an acyclic isoprenoid-type moiety,

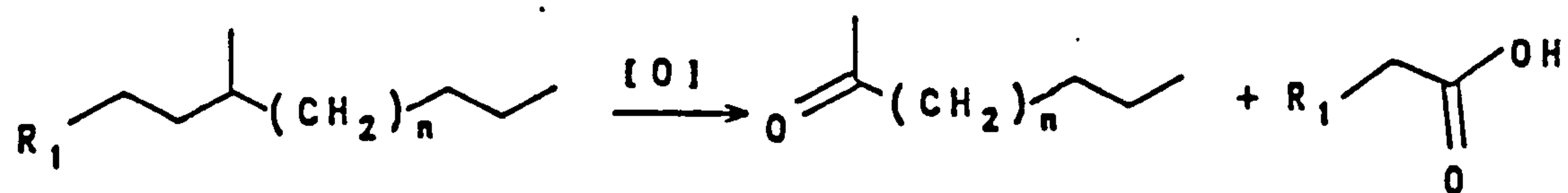
viz :



This was confirmed by oxidation of authentic pristane (section 4.2.7).

n-alkan -2-ones :

Like wise a homology of n-alkan-2-ones could result from oxidative cleavage of a methyl substituted alkyl chain linkage, viz:

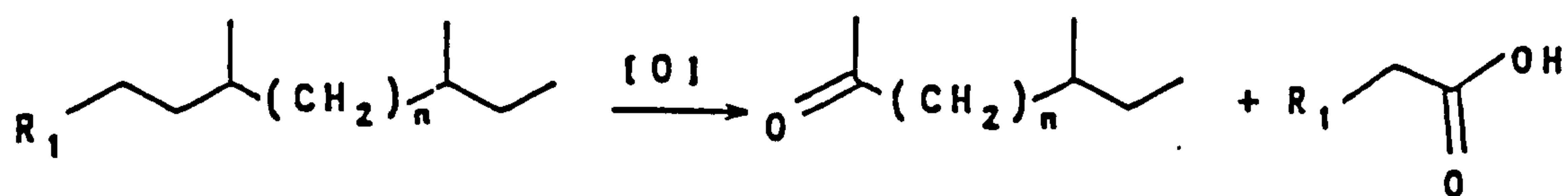


The carbon number range of the components identified (C8-C15) would require the presence of a methyl group at the C-7 to C-14 positions along a n-alkyl chain.

methylbranched alkan-2-ones :

By a similar mechanism, methylbranched alkan-2-ones could be produced from precursor alkanes containing two methyl group substituents :

i.e.

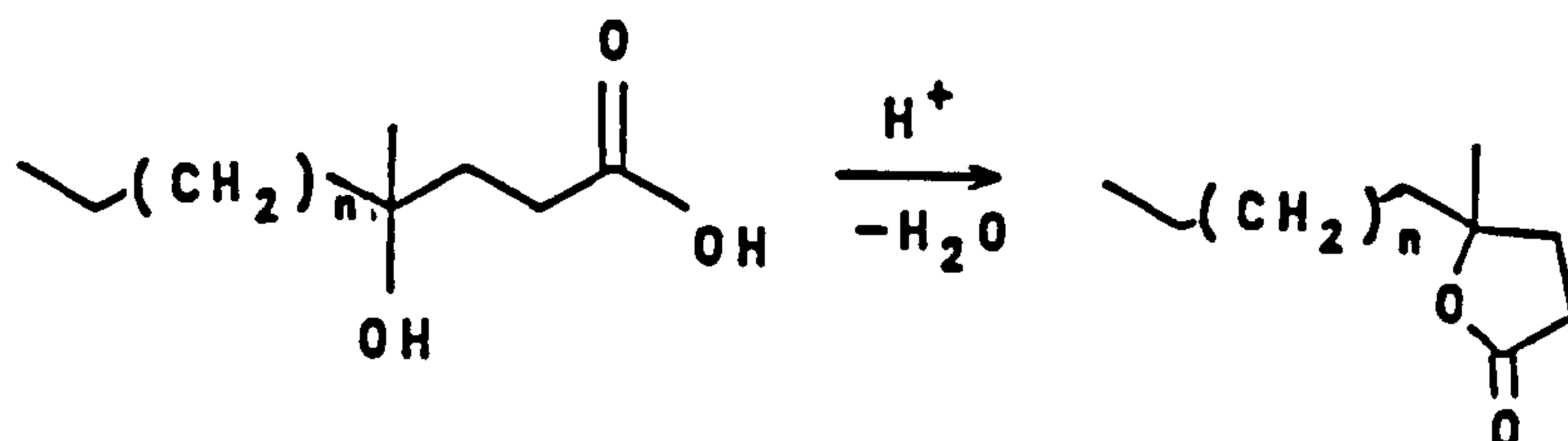


The major series of methyl branched alkyl ketones identified were "iso", thus requiring at least one methyl group in the 2- position of an alkyl chain.

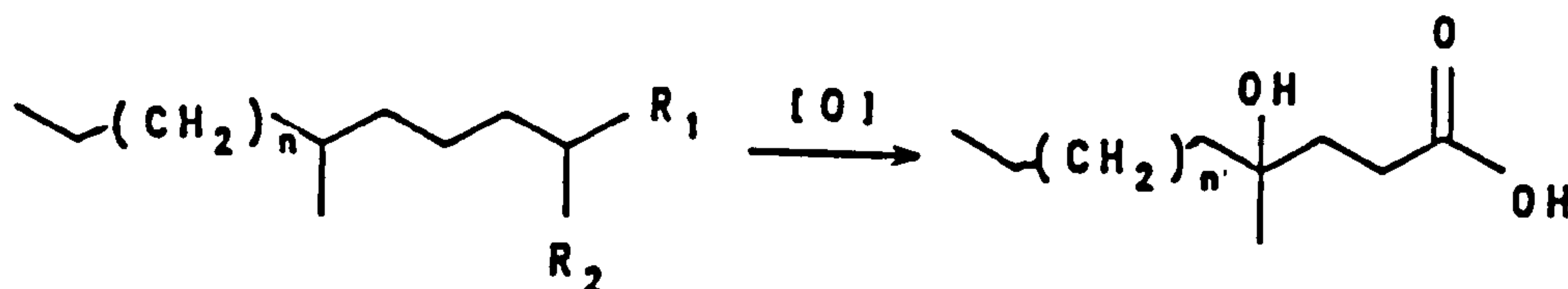
γ -methyl- γ -lactones :

In an acidic medium, γ -methyl- γ -lactones would result from the intramolecular esterification of a 4-hydroxy-4-methyl carboxylic acid

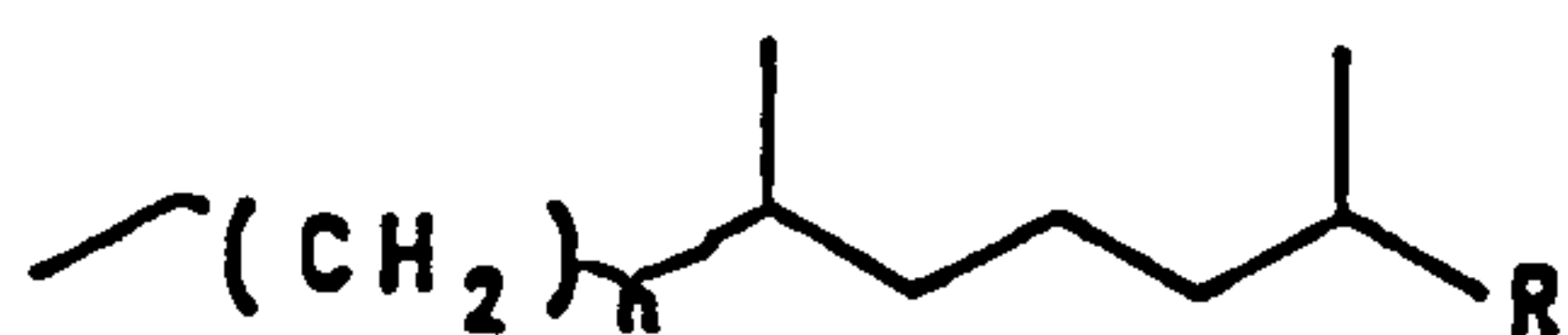
viz:-



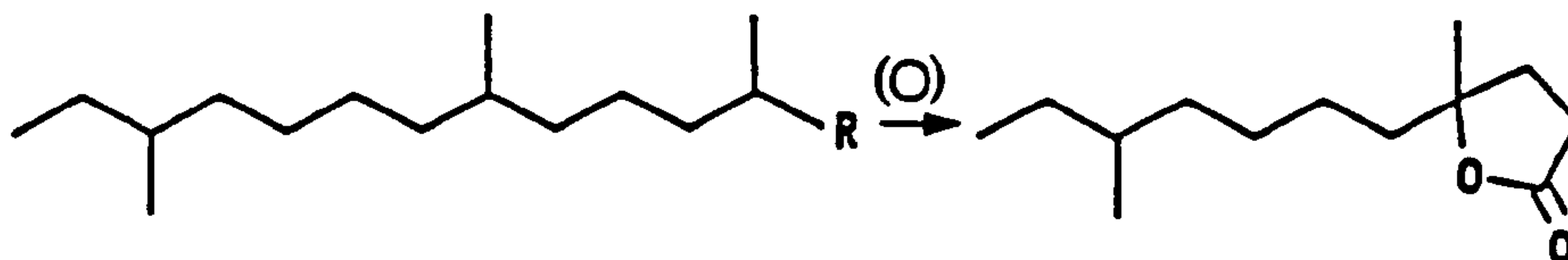
Possible precursor alkanes could therefore comprise dialkyl substituted alkyl chains i.e.



The identification of γ -methyl- γ -lactones in the carbon number range C6-C10 would therefore require the methyl group to be positioned at C-2 (i.e. iso), C-3 (i.e. anteiso) or C-4 to C-6. If R_2 (or R_1) equals methyl, then this possibly represents an isoprenoidal moiety in the precursor alkane, i.e.



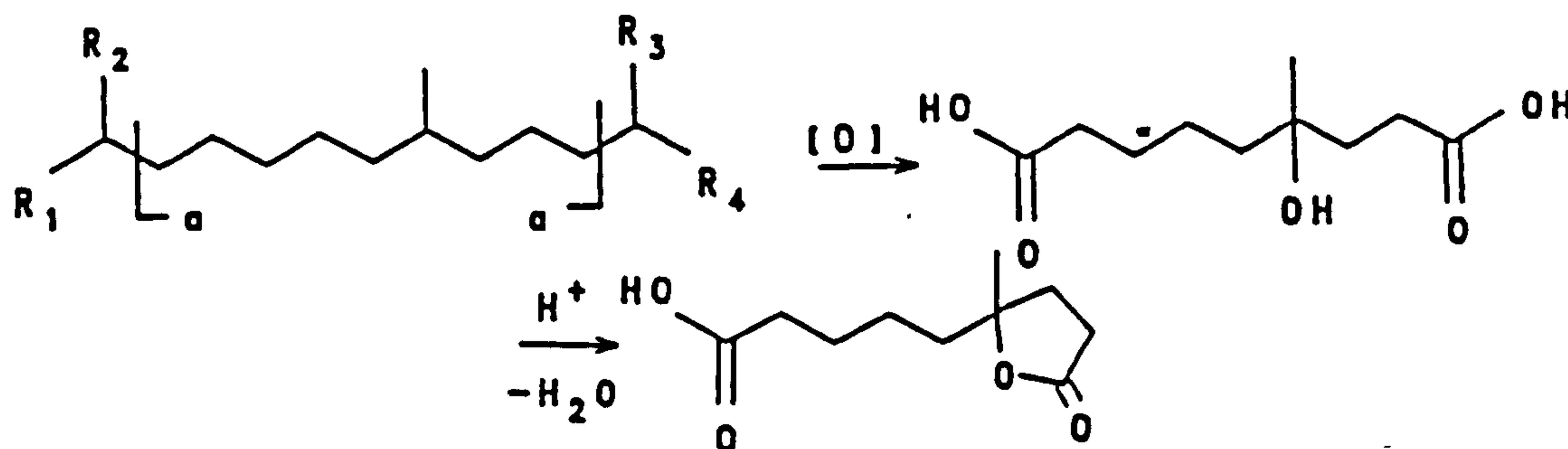
Likewise methyl branched γ -methyl- γ -lactones would originate from a further methyl substituent in the alkyl chain, e.g.



The position of the methyl substituent could not however be determined from mass spectral data alone.

ω -carboxy- γ -methyl- γ -lactones :

By the above reasoning, ω -carboxy- γ -methyl- γ -lactones would originate from the internal esterification of an ω -carboxy-4-hydroxy-4-methyl carboxylic acid. This itself could result from a precursor alkane containing at least three alkyl substituents, i.e.

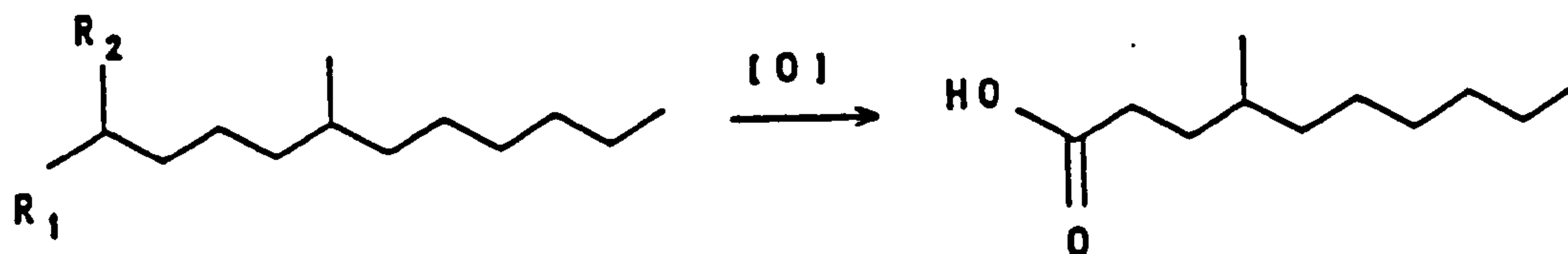


The observed distribution of oxidation products (C8-C11) reflects the length of the alkyl chain, i.e. $n = 2$ to 5 . Methyl branched ω -carboxy- γ -methyl- γ -lactones would be produced by the same mechanism, provided an additional methyl group is situated on the alkyl chain.

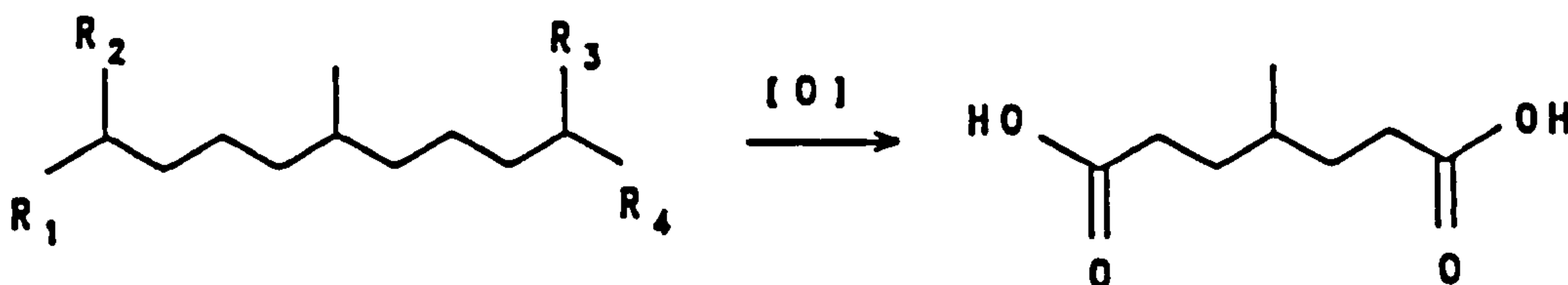
Methylbranched mono-and dicarboxylic acids:

The majority of these compounds were found to be monomethyl substituted and therefore likely to originate from simply branched alkyl linkages on precursor alkanes. Monomethyl monocarboxylic acids could possibly originate from a methyl substituted alkyl chain,

viz :-



and likewise methylbranched α,ω -dicarboxylic acids may originate from more highly substituted alkyl chains, e.g.



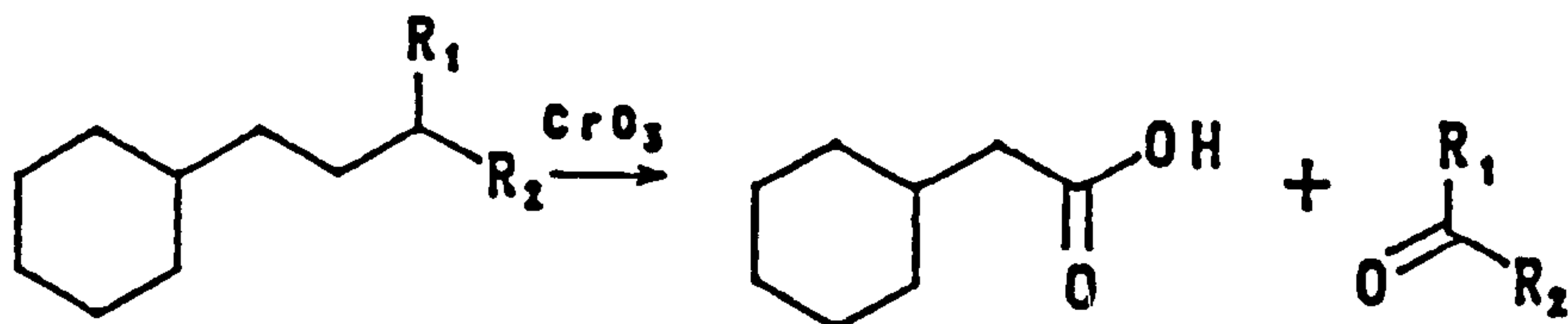
Again, if R₂ = R₃ = CH₃, this is representative of an isoprenoidal moiety.

Cyclohexylcarboxylic acids :

Only one example of this type of functionalised compound was observed as an oxidation product of the aliphatic UCM, which from the proposed mechanism presumably originates via oxidative cleavage about a

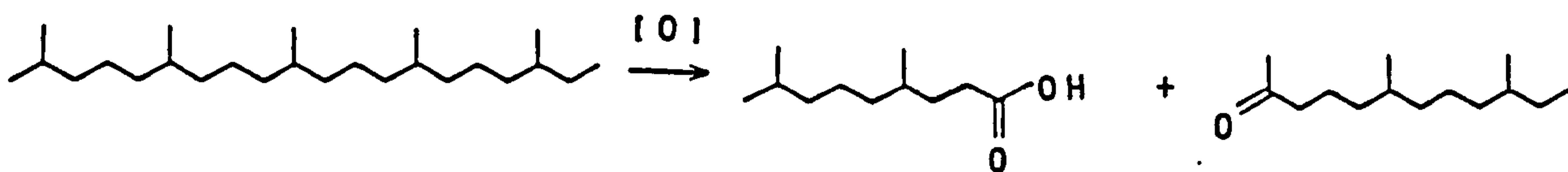
tertiary position. The precursor alkane must comprise a branched alkyl substituted monocyclohexyl moiety,

viz :

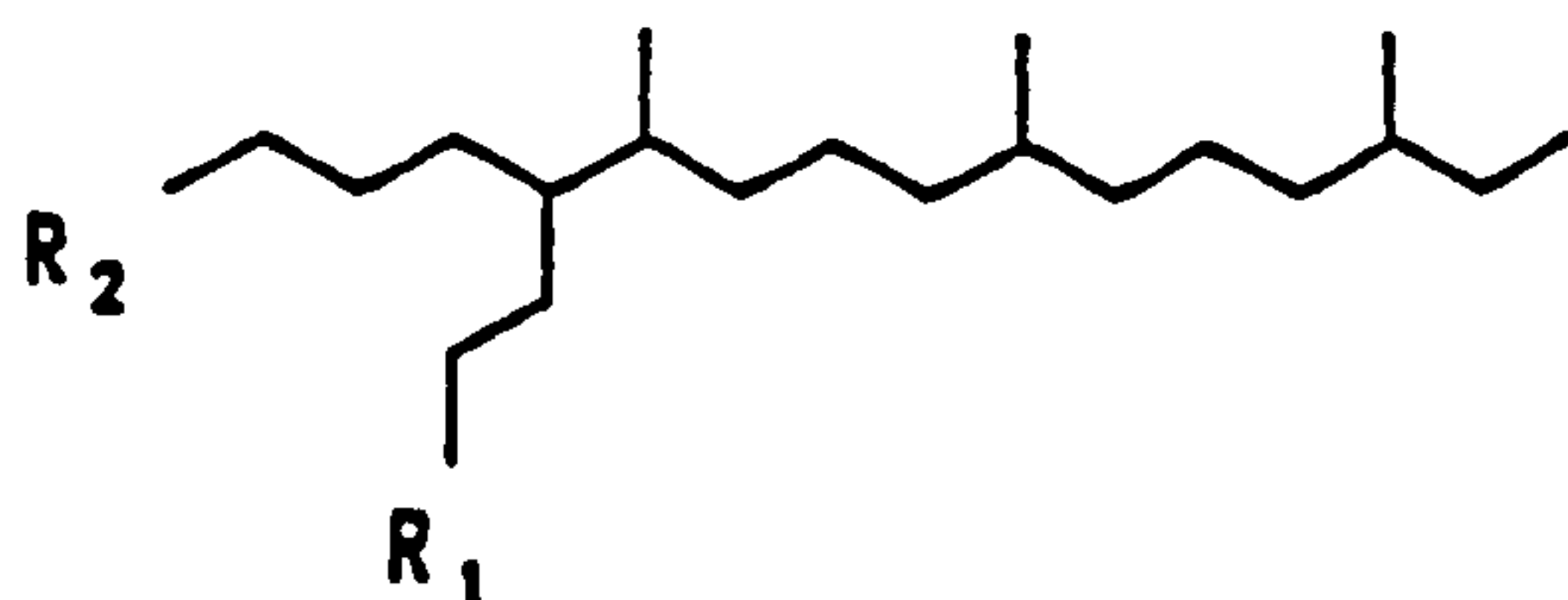


isoprenoid-derived acids and ketones :

These could be correlated specifically with the oxidation products of pristane (section 4.2.7). Thus cleavage of a regular acyclic isoprenoid (resolved compound) could produce both a ketone and an acid :-



Alternatively, these products may be derived from isoprenoid-type alkyl linkages within the UCM (Chapter 5); e.g.



3.2 THE AROMATIC UCM

In contrast to the high proportion of aliphatic hydrocarbons obtained by column chromatography of the Silkolene 150 lubricating base oil (ca. 90%), the aromatic hydrocarbon fraction was found to account for only ca. 8% of the total oil (Table 3.1). This was perhaps to be expected as lubricating oils are usually dearomatised by furfural treatment during processing (Klamann, 1984).

3.2.1 ANALYSIS BY CONVENTIONAL TECHNIQUES

3.2.1.1 GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY - MASS SPECTROMETRY (GC/GC-MS)

When analysed by GC (Fig 3.54a) the aromatic fraction showed a broad UCM ranging from KI 1950 to KI 3600 +, maximising at KI 2700. In contrast to the aliphatic UCM (section 3.1), the proportion of resolved components was small (ca. 3%, as estimated by the time slice area measurement, appendix I.)

GC-MS analysis showed ion series indicative of phenyl alkanes (m/z 91/92), methylphenyl alkanes (m/z 105/106), dimethylphenyl alkanes (m/z 119/120) and trimethylphenyl alkanes (m/z 133/134). Each ion profile exhibited a UCM, and though EI GC-MS of individual resolved compounds did provide carbon number distributions in certain cases (e.g. C19-C28 1-phenylalkanes), exact positional isomers and the extent of chain branching could not be determined (Fig 3.55).

An attempt was made to characterise the UCM components by obtaining EI mass spectra at selected intervals through the UCM. Four scans

FIG. 3.54 GAS CHROMATOGRAMS OF THE AROMATIC UCM (SILKOLENE 150 LUBE OIL) A) BEFORE OXIDATION AND B) AFTER OXIDATION WITH CrO_3 IN GLACIAL ACETIC ACID

[GC: DB-5 (J+W), 25m, 50-300°C @ 5°C min⁻¹, 300°C for 20min]

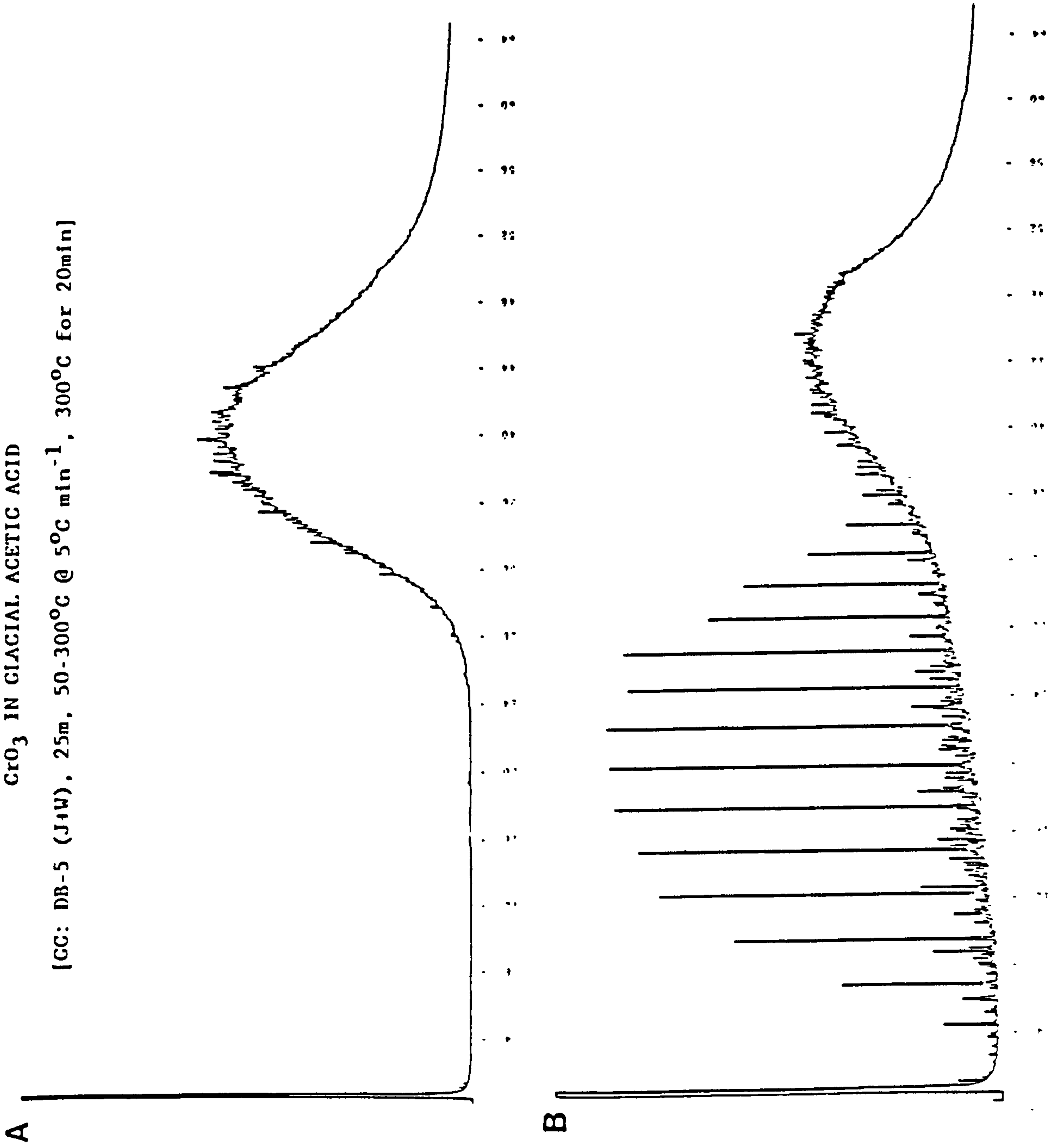
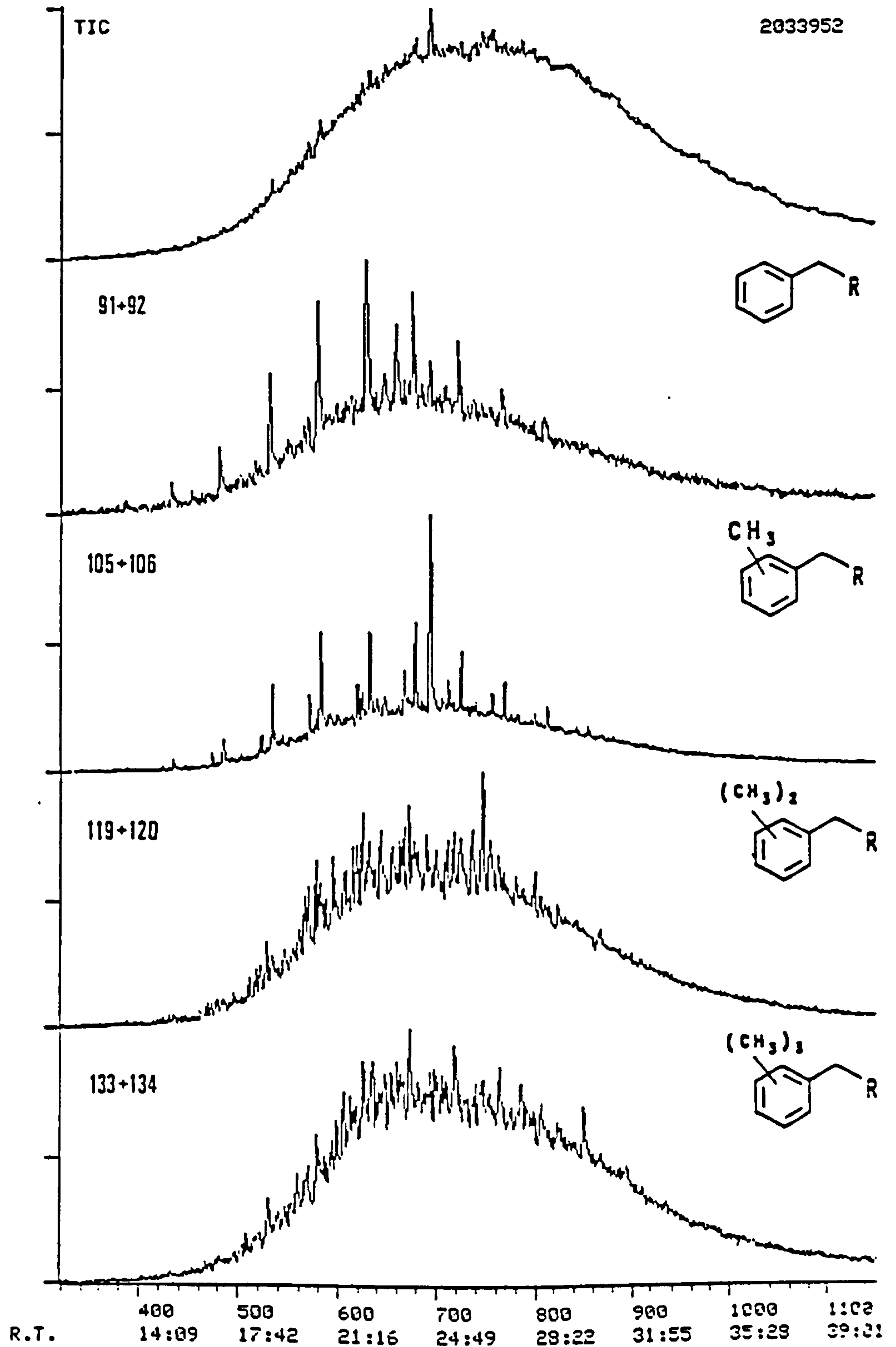


FIG. 3.55 GC-MS MASS FRAGMENTOGRAMS OF 1-PHENYL ALKANES (m/z 91/92), METHYLPHENYL ALKANES (m/z 105/106), DIMETHYLPHENYL ALKANES (m/z 119/120), AND TRIMETHYLPHENYL ALKANES (m/z 133/134) OBSERVED FOR THE AROMATIC UCM, SILKOLENE 150 LUBE OIL.



were taken, and within each spectrum (m/z 45-200 region) the relative abundances of fragment ions characteristic of acyclic and monocyclic alkanes (C_nH_{2n+1} , C_nH_{2n-1} , respectively), and alkylmonoaromatic (C_nH_{2n-7}), alkyl-naphthenobenzene (C_nH_{2n-9}) and alkyl-naphthalene (C_nH_{2n-11}) hydrocarbons were compared (Fig. 3.56). These fragment ion distributions were essentially similar throughout the UCM profile, and showed, interestingly, a predominance of mass fragments characteristic of acyclic and monocyclic alkanes, and monoaromatic hydrocarbons. This is perhaps indicative of a mixture of alkylated monoaromatic hydrocarbons. This is in accordance with the HPLC ring-class fractionation behaviour of aromatic UCM hydrocarbons observed in previous studies (Killops and Readman, 1985; Jones, 1986).

3.2.1.2 ELEMENTAL ANALYSIS AND GAS CHROMATOGRAPHY- FLAME PHOTOMETRIC DETECTION (GC-FPD)

Elemental analysis provided an "average" H/C ratio of 1.62, which approximates that expected for a C25 alkyl dinaphthenobenzene (1.60). Although proportionately greater amounts of sulphur were determined by elemental analysis in comparison to the aliphatic UCM (ca. 1.5%), no evidence of sulphur containing compounds was observed by GC-FPD.

3.2.1.3 GEL PERMEATION CHROMATOGRAPHY (GPC)

The GPC system described for the aliphatic UCM (section 3.1.3.2) was used to fractionate the aromatic UCM. Detection was by U.V. (254nm), and the chromatogram (Fig. 3.57) shows the positions of preparative fractionation. Subsequent GC analyses (Fig. 3.58) showed that the fractionation was successful, and that the expected GPC elution order

FIG. 3.56 EI MASS SPECTRA (m/z 45-200) TAKEN AT SELECTED INTERVALS THROUGH THE AROMATIC UCM GC-MS PROFILE.

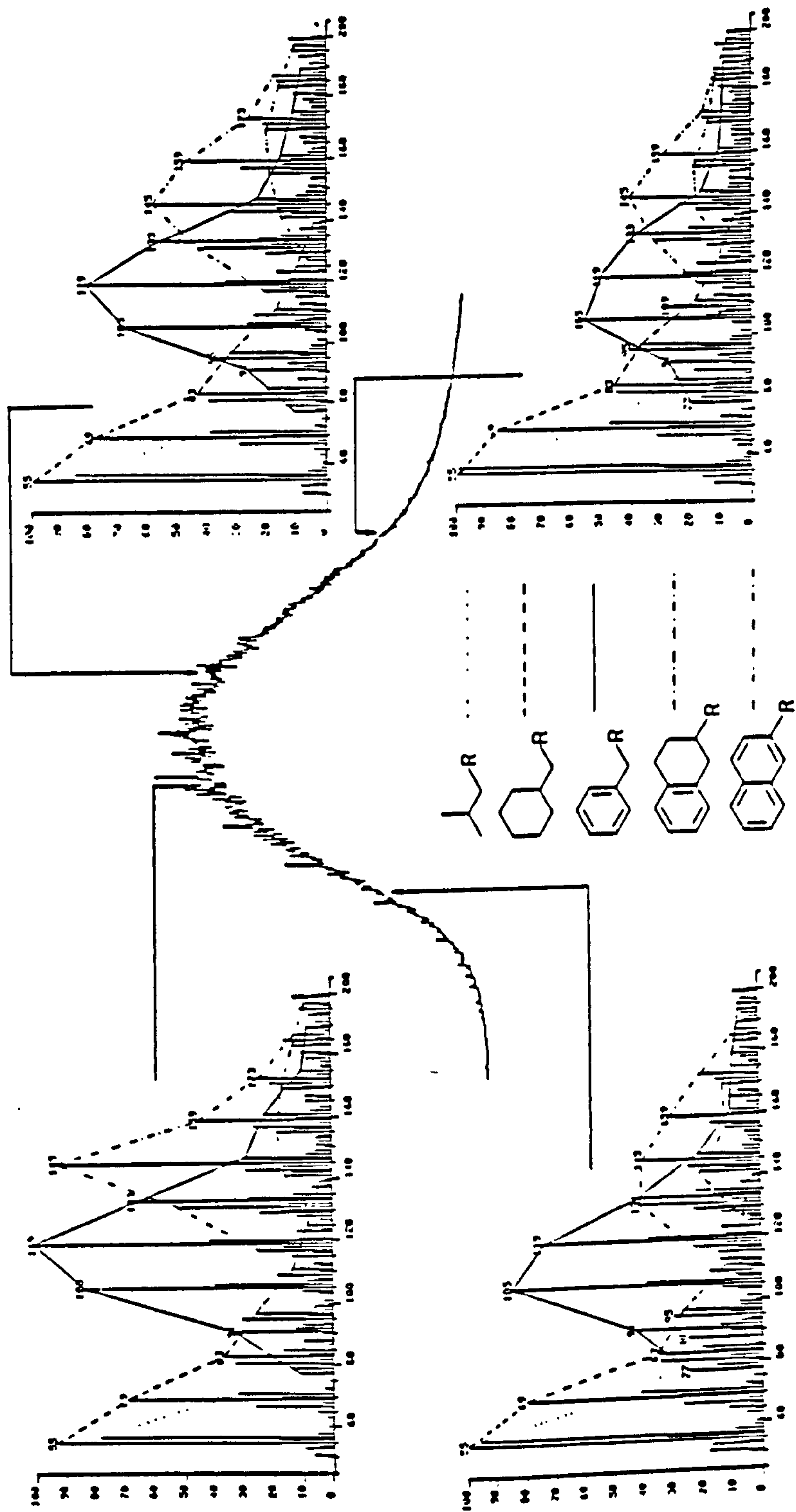


TABLE 3.17

ELEMENTAL ANALYSIS OF THE AROMATIC UCM, SILKOLENE 150 LUBRICATING OIL

RUN	WEIGHT (%)			H/C RATIO	TOTAL (%)
	C	H	N		
1	87.50	12.20	0.10	1.67	99.80
2	87.96	11.52	0.07	1.57	99.55
3	87.99	11.77	0.11	1.62	99.87
Mean	87.82	11.83	0.09	1.62	99.74

COMPARISON OF ELEMENTAL FORMULAE

AROMATIC HYDROCARBON TYPE	Z VALUE	H/C RATIO ^a	GENERAL FORMULA
alkylbenzene	-6	1.76	C_nH_{2n-6}
alkylnaphthenobenzene	-8	1.68	C_nH_{2n-8}
alkyldinaphthenobenzene	-10	1.60	C_nH_{2n-10}
alkylnaphthalene	-12	1.52	C_nH_{2n-12}
alkylnaphthenonaphthalene	-14	1.44	C_nH_{2n-14}
alkyldinaphthenonaphthalene	-16	1.36	C_nH_{2n-16}
alkylphenanthrene	-18	1.28	C_nH_{2n-18}
aromatic UCM		1.62	

a: for a C25 aromatic hydrocarbon

FIG. 3.57 GPC CHROMATOGRAM (UV 254 nm) OF THE AROMATIC UCM

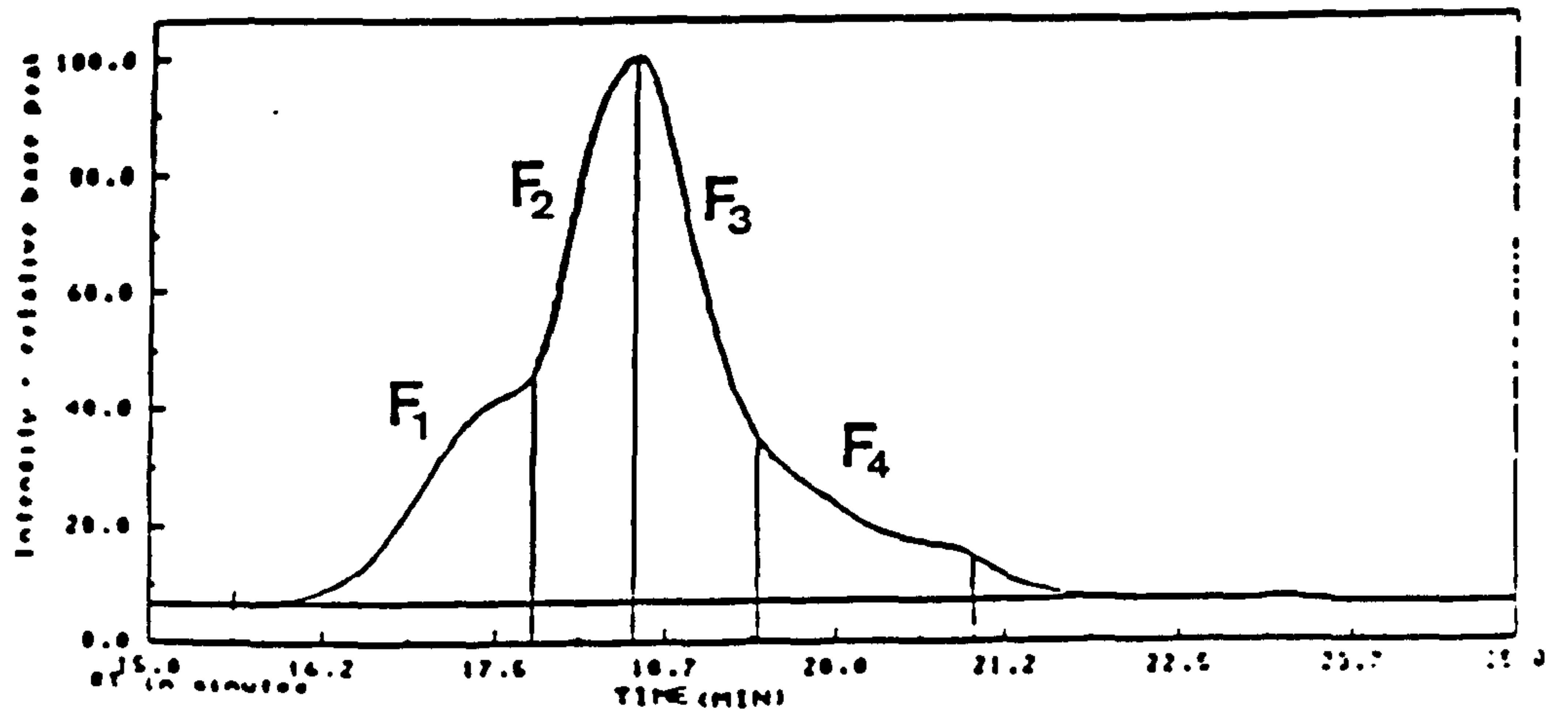
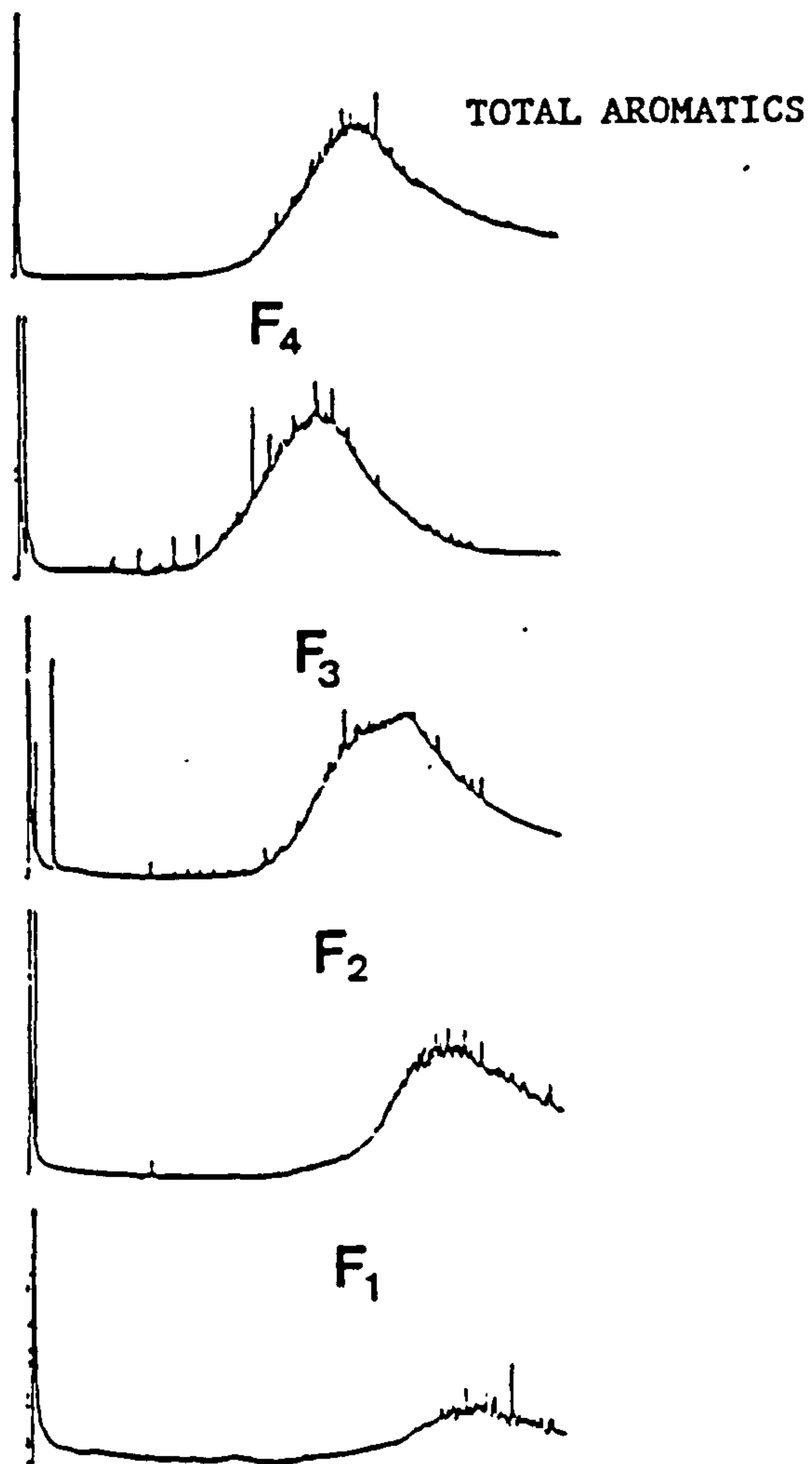


FIG. 3.58 GAS CHROMATOGRAMS OF GPC FRACTIONATED AROMATIC UCM MATERIAL



[GC:OV-1(GC²), 50-180°C @ 10°C min⁻¹, 180-300°C @ 5°C min⁻¹, 300°C(20min)]

of higher molecular weight material first was in fact observed. As for the aliphatic UCM, GPC appears promising for the preparative fractionation of aromatic hydrocarbon UCMs prior to more detailed analyses; these were not conducted in the time available.

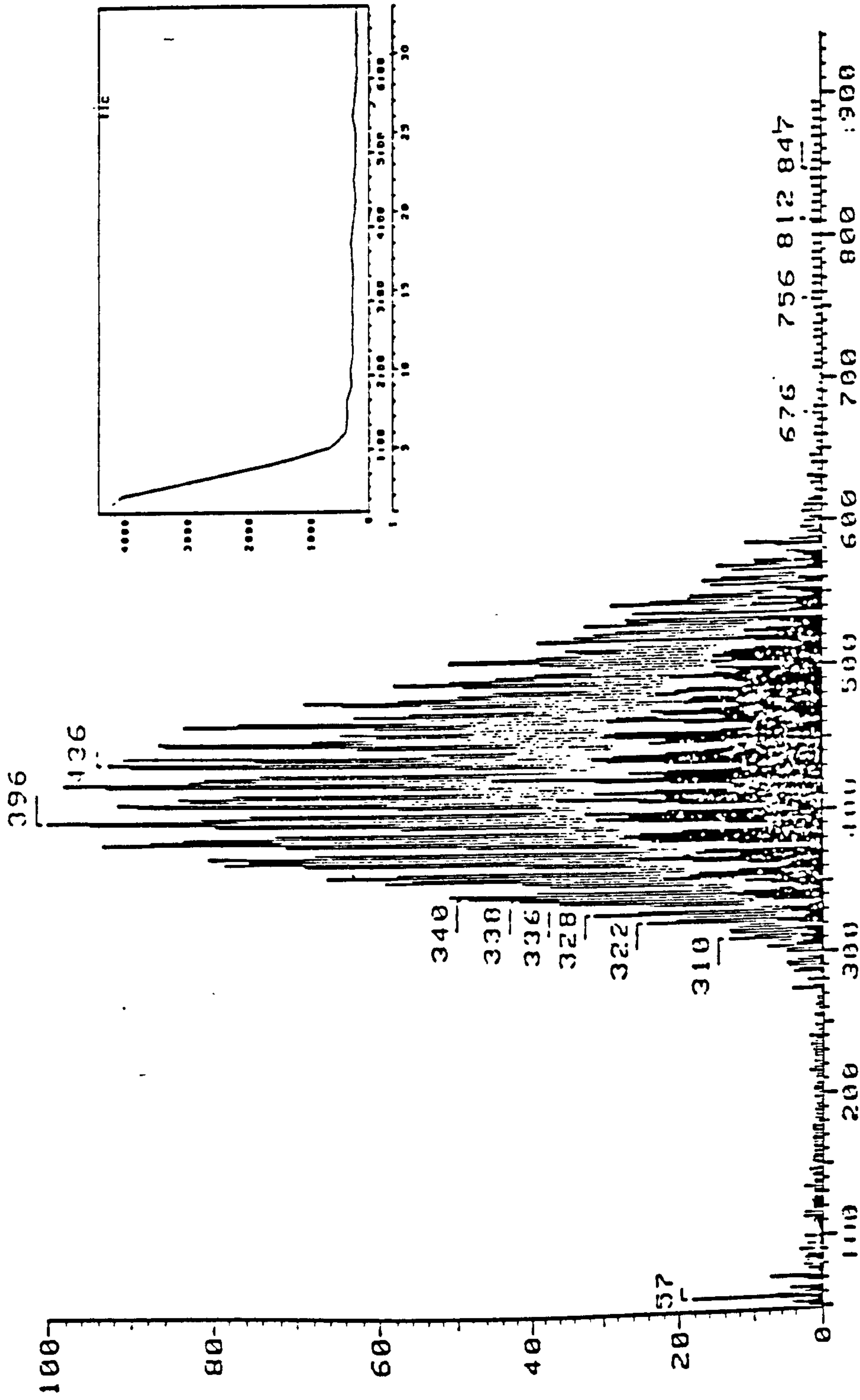
3.2.1.4 FIELD IONISATION MASS SPECTROMETRY (FIMS)

Field ionisation mass spectrometry invariably results in virtually fragment free mass spectra which are useful for molecular weight determination (Becky, 1977) and is particularly suited to less polar compounds. As a result it has been widely used in the petroleum industry for the characterisation of hydrocarbons and residuals (e.g. McKay and Latham, 1981; Wanless, 1970; Kuras et al., 1976; Allen et al., 1985). Although typically used with direct insertion (e.g. probe distillation) methods, FIMS has also been used in combination with GC (Payzant et al., 1980; Schulten and Halket, 1986) and with pyrolysis (Schulten et al., 1987; Schulten and Halket, 1986).

FIMS has been extensively used in the characterisation of the mainly chromatographically unresolved hydrocarbon fractions of Alberta oil sand bitumens (Payzant et al., 1979, 1980, 1985^{a,b}). For the saturate fraction, the major components observed were bicyclic alkanes, with lesser amounts of tri-, tetra-, penta-, mono-, hexa-, and acyclic alkanes; in that order. The aromatic hydrocarbon fraction was found to comprise a complex mixture of alkylbenzenes, tetralins, naphthalenes, phenanthrenes, and thiophenes, with members of the class $z-14$ (where $z = C_nH_{2n-z}$) equivalent to naphthenonaphthalenes or monoaromatised pentacyclanes predominating.

In the present study an attempt was made to characterise the aromatic

FIG. 3.59 FIELD IONISATION (FI) MASS SPECTRUM OF THE AROMATIC UCM (insert: total ion chromatogram)



UCM derived from the Silkolene 150 lubricating oil by FIMS. This was kindly performed by Prof. H. Schulten, Fachhochschule Fresenius, Wiesbaden, FRG. For this the sample was thermally desorbed but under non optimum conditions (Schulten et al., 1987) into the modified FI ion source of a Finnigan Mat 731 double focussing mass spectrometer.

From the total ion chromatogram profile (see insert, Fig. 3.59), it is evident that the sample was rapidly desorbed into the ion source within the first five scans. The FI spectrum was recorded at the maximum (scan 1) and is shown in Fig. 3.59. Although it is not known whether this position of the TIC is representative of the sample as a whole, the mass spectrum showed a near Gaussian profile of molecular masses in the range 300-600 Daltons. From this data, and assuming no overlap between compounds of the same z number, five principal series of compounds were identified (Table 3.18) between m/z 300 and m/z 530. The z values ranged from -6 (alkylbenzenes) through to -14 (alkyl naphthenonaphthalenes), with the most abundant peak in the mass spectrum corresponding to a C₂₉ dinaphthenobenzene (m/z 396, z=-10). Peak heights were recorded and by normalisation to the height of the most abundant peak (m/z 396), a measure of the relative percentage intensity for each series was obtained. By plotting these against carbon number an alkyl homologue distribution (AHD, e.g. Blumer, 1976 ; Jones, 1986) could be constructed (Fig. 3.60). Each series maximised at ca. C₂₈-C₃₂, and the UCM was comprised in the main of alkyl dinaphthenobenzenes > alkyl naphthalenes > alkyl naphthenonaphthalenes > alkyl tetralins > alkyl benzenes.

Under non optimised conditions therefore, FIMS provided a crude and only semi-quantitative analysis of the principal components of the

FIG. 3.60 ALKYL HOMOLOGUE DISTRIBUTION (AHD) CONSTRUCTED FROM THE FIMS SPECTRUM OF THE AROMATIC UCM

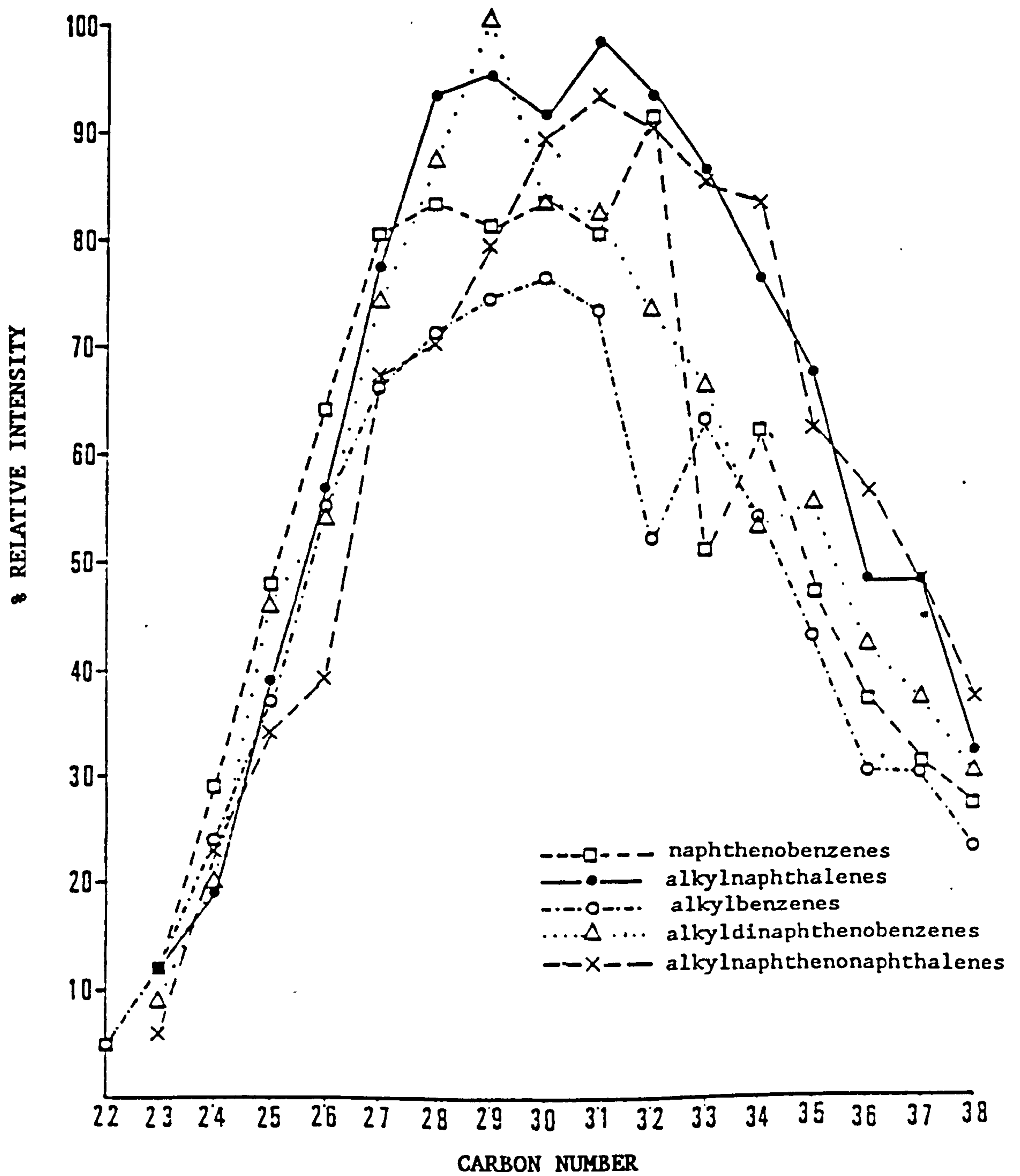


TABLE 3.18

PRINCIPAL SERIES OF AROMATIC HYDROCARBONS IDENTIFIED IN THE
AROMATIC UCM BY FIELD IONISATION MASS SPECTROMETRY (FIMS)

z VALUE ^a	MOLECULAR WT. RANGE	CARBON NO. RANGE	PROPOSED COMPOUND	SYMBOL ^b
-6	302-526	C22-C38	alkylbenzenes	- · - · - ○ - · - · -
-8	300-524	C22-C38	naphthenobenzenes	- - - □ - - -
-10	312-522	C23-C38	dinaphthenobenzenes	· · · · · Δ · · · · ·
-12	310-520	C23-C38	naphthalenes	————●————
-14	308-518	C23-C38	naphthenonaphthalenes/ monoaromatic pentacyclanes	- - - x - - -

a: from the expression C_nH_{2n+z}

b: symbol refers to Fig. 3.60

aromatic UCM. For this a number of assumptions had to be made; namely i) an absence of sulphur-containing aromatic compounds, e.g. benzothiophenes (z--10) and naphthenobenzothiophenes (z--12); and ii) an absence of higher ring aromatic hydrocarbons, i.e. naphthenophenanthrenes and above. For example a C21 naphthenophenanthrene has the same molecular weight as a C20 alkylbenzene (274 gmole^{-1}), and these may interfere in the determination of alkyl benzenes.

3.2.2 THE AROMATIC UCM BY CHEMICAL OXIDATION

Three CrO_3 /glacial acetic acid oxidations of the aromatic UCM were performed using the procedure described for the aliphatic UCM (section 3.1). The gravimetric data is summarised in Table 3.19. Each UCM oxidation produced a good yield of total recovered material (78-87%), and GC analyses (Fig 3.54 B and 3.61) showed the production of a major series of resolved compounds. GC-MS confirmed these as n-alkanoic acids in the range C6-C20, maximising at C13, C14 (Fig. 3.62). In comparison to the oxidation products of the aliphatic UCM, the proportion of residual and/or functionalised UCM in the gas chromatograms was greater, yet the proportion of additional resolved products relative to n-acids was less. However, GC-MS showed that many of these products were in fact the same, i.e. series of aliphatic acids, ketones and lactones. These included series of n-alkan-2-ones (C18-C19, max. C12) and iso-methyl branched alkan-2-ones (C8-C10, C12 max. C8) (Fig. 3.62). The major component of the m/z 58 mass fragmentogram (Fig. 3.62) was however identified as the isoprenoid ketone 6,10-dimethylundecan-2-one. Also identified was the C18 isoprenoid ketone 6,10,14-trimethylpentadecan-2-one. Series

FIG. 3.61 GAS CHROMATOGRAMS OF REPLICATE OXIDATIONS OF THE AROMATIC UCM (TOTAL RECOVERED MATERIAL)

(CrO₃ in glacial acetic acid, 60 mins, DCM extractant)

[GC: DB-5(J+W), 50-300 °C @ 5 °Cmin⁻¹]

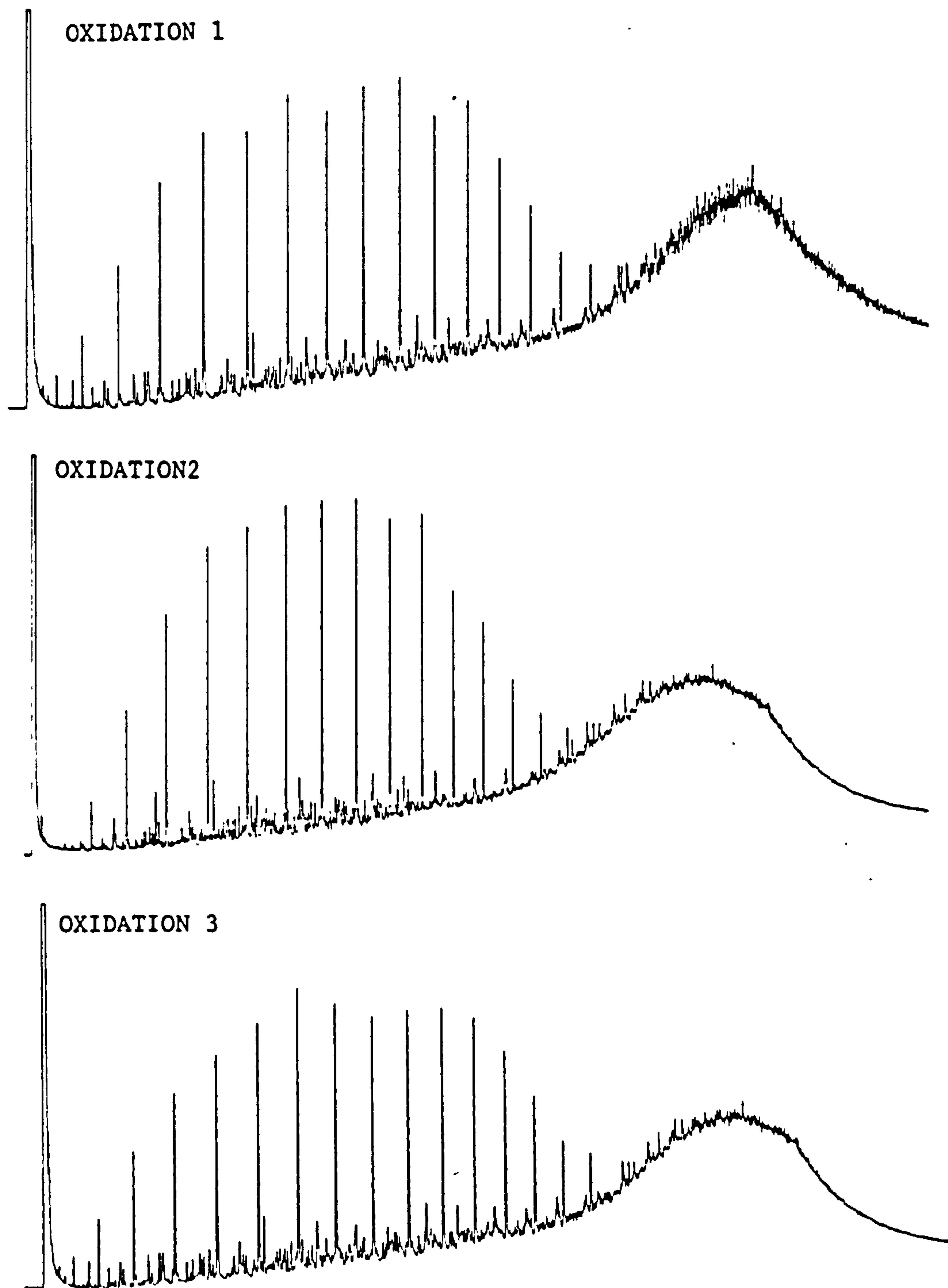


FIG. 3.62 GC-MS MASS FRAGMENTOGRAMS OF THE RESOLVED PRODUCTS OF OXIDATION OF THE AROMATIC UCM (m/z 74: n-acids; m/z 58:alkyl ketones; m/z 99: γ -lactones)

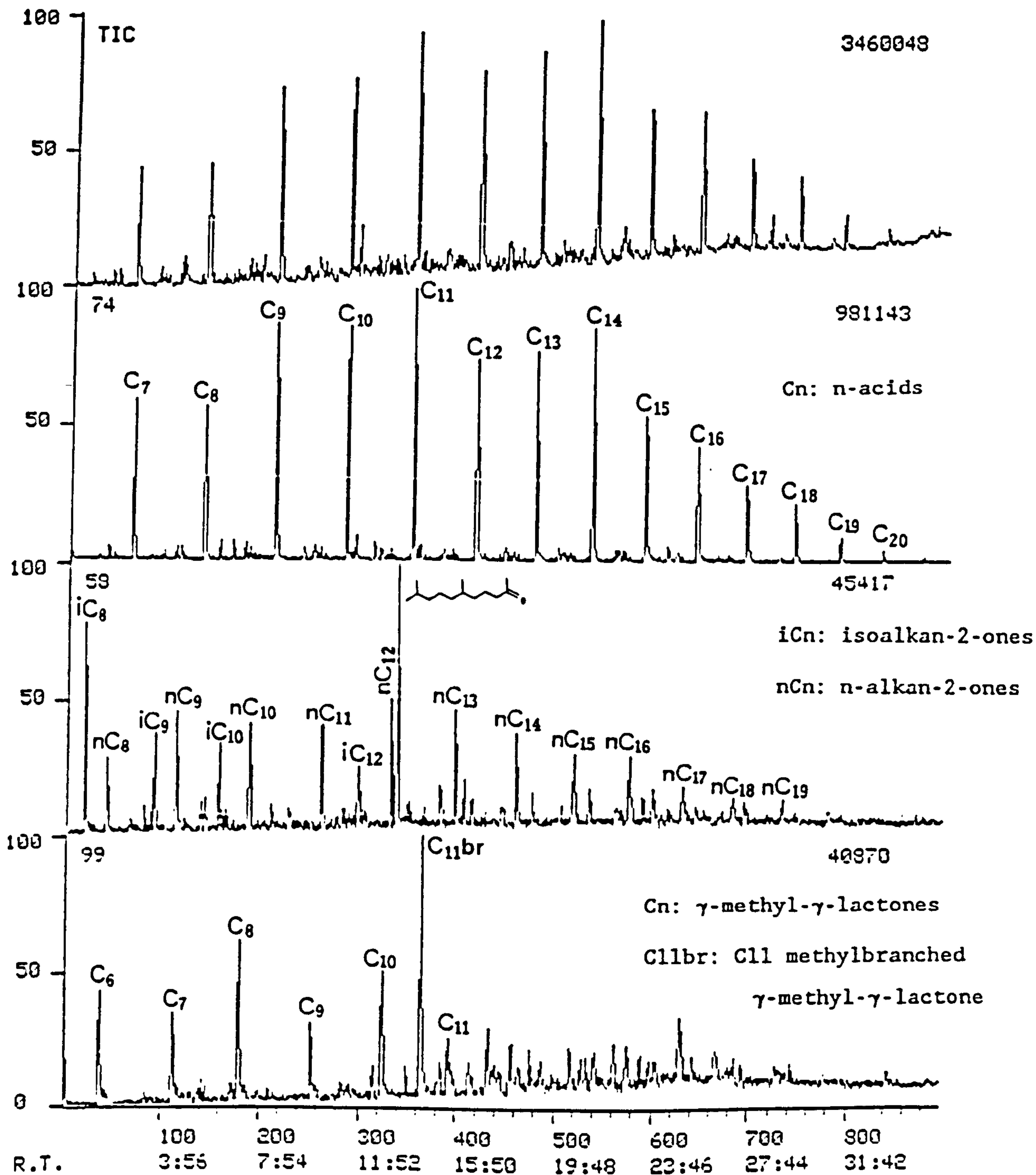


TABLE 3.19

GRAVIMETRIC DATA FOR THE CrO_3 /GLACIAL ACETIC ACID OXIDATION OF
THE AROMATIC UCM - SILKOLENE 150 LUBRICATING BASE OIL

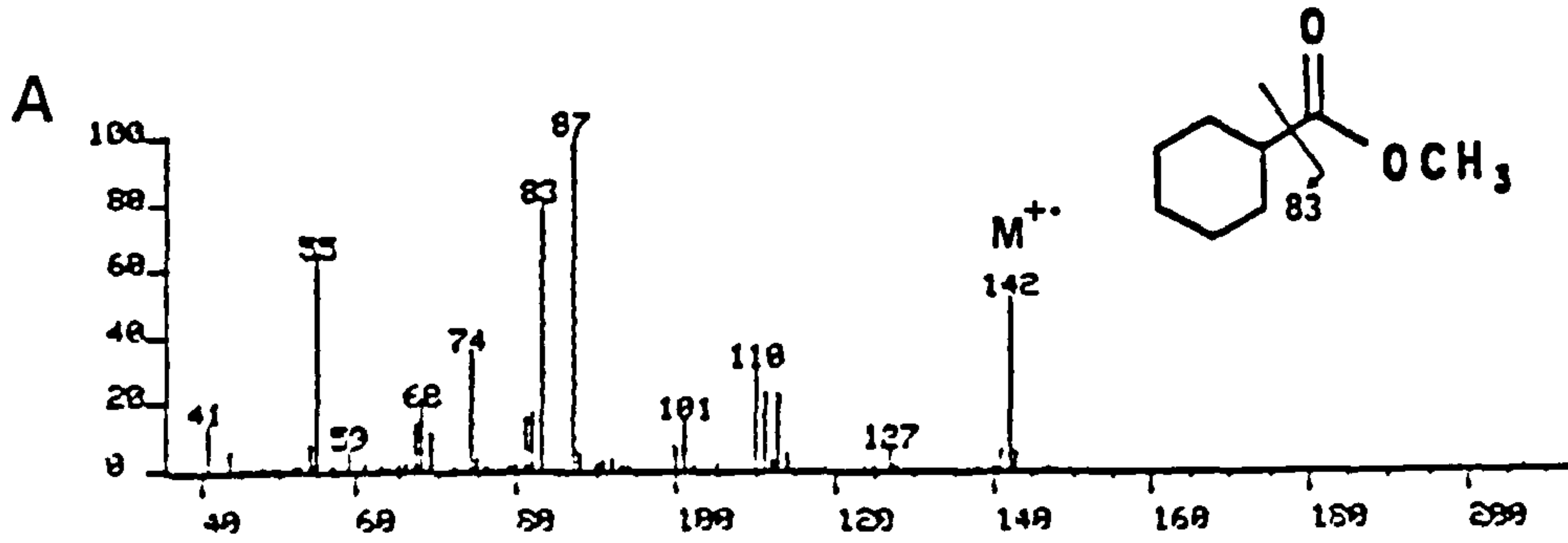
SAMPLE NO.	MASS OXIDISED(mg)	MASS CrO_3 (mg)	RATIO OXIDANT: SUBSTRATE	YIELD (mg (%))
1	42.6	121.9	9.8:1	36.9 (87)
2	59.6	170.6	9.9:1	46.4 (78)
3	50.3	146.3	10.0:1	42.1 (84)

of γ -methyl- γ -lactones similar to those observed as oxidation products of the aliphatic UCM were also identified as aromatic UCM oxidation products (namely C6-C11, max. C8, Fig. 3.62). However the major component of the m/z 99 mass fragmentogram (Fig. 3.62) was characterised by mass spectral and retention index correlations as a C11 methylbranched γ -methyl- γ -lactone. This was tentatively assigned an isoprenoid - type origin on the basis of oxidation of a weathered crude oil residue which contained a high proportion of acyclic isoprenoids (i.e. pristane and phytane, "Sivand" oil spill, section 5.4).

Isoprenoid acids were identified as aromatic UCM oxidation products, and their proportions exceeded those observed as products of the aliphatic UCM. These included 4-methylhexanoic acid, 4,8-dimethylnonanoic acid, and 4,8,12-trimethyltridecanoic acid.

In addition to series of functionalised aliphatic compounds described above, certain cyclic and aromatic acids and ketones were observed as products of the aromatic UCM. Their identification was assisted by a knowledge of the products of oxidation of model compounds (9-(2-cyclohexylethyl)-heptadecane, section 4.2.5; and 9-(2-phenylethyl)-heptadecane, section 4.2.6); and by library spectral matching. These included cyclohexylcarboxylic acid (ELC:7.32), a monomethyl substituted cyclohexyl carboxylic acid (ELC:7.90), 2-cyclohexylethanoic acid (ELC:8.24), and 3-cyclohexylpropanoic acid (ELC:9.38). Also identified were the aromatic acids, benzoic acid (ELC:7.66), mono- and dimethyl substituted benzoic acids (ELC:8.78 and 10.19, respectively), and a methyl substituted aromatic ketone (ELC:10.32). Examples of their mass spectra are shown in Figs. 3.63

FIG. 3.63 EI MASS SPECTRA OF CYCLOHEXYL CARBOXYLIC ACIDS IDENTIFIED AS AROMATIC UCM OXIDATION PRODUCTS



A) CYCLOHEXYL CARBOXYLIC ACID



B) METHYLCYCLOHEXYL CARBOXYLIC ACID

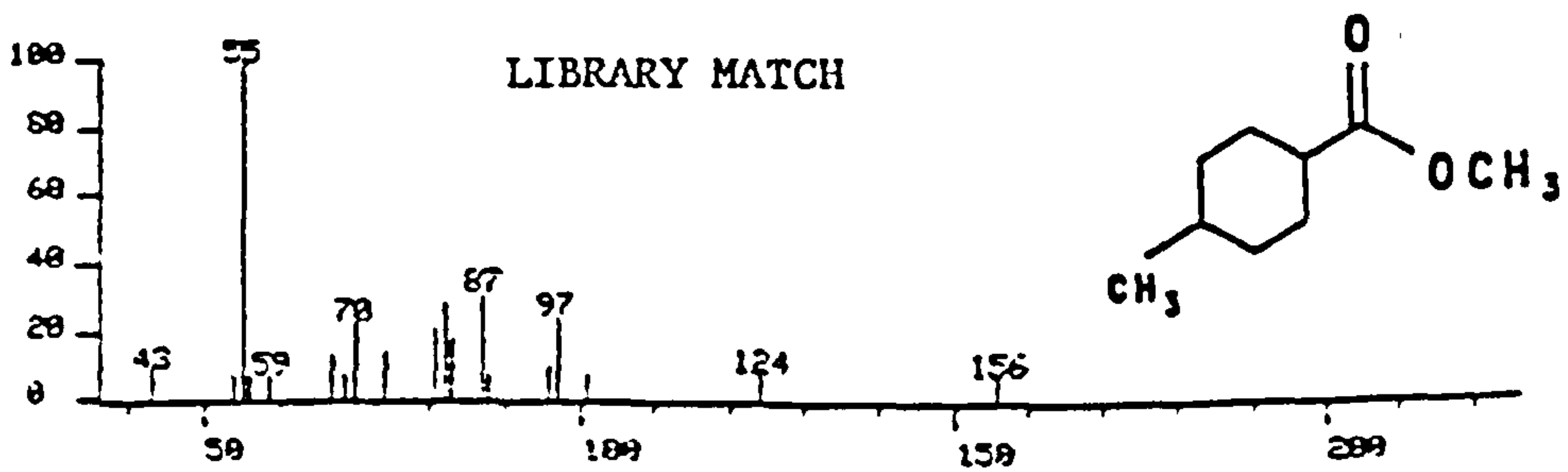
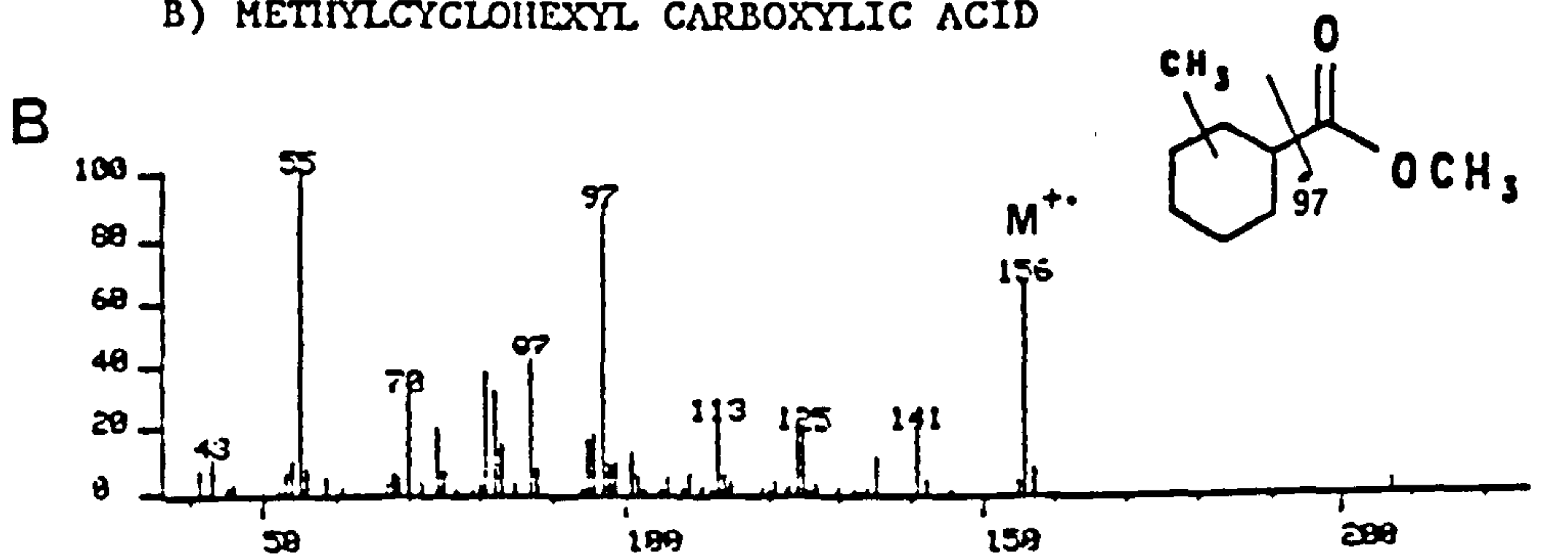
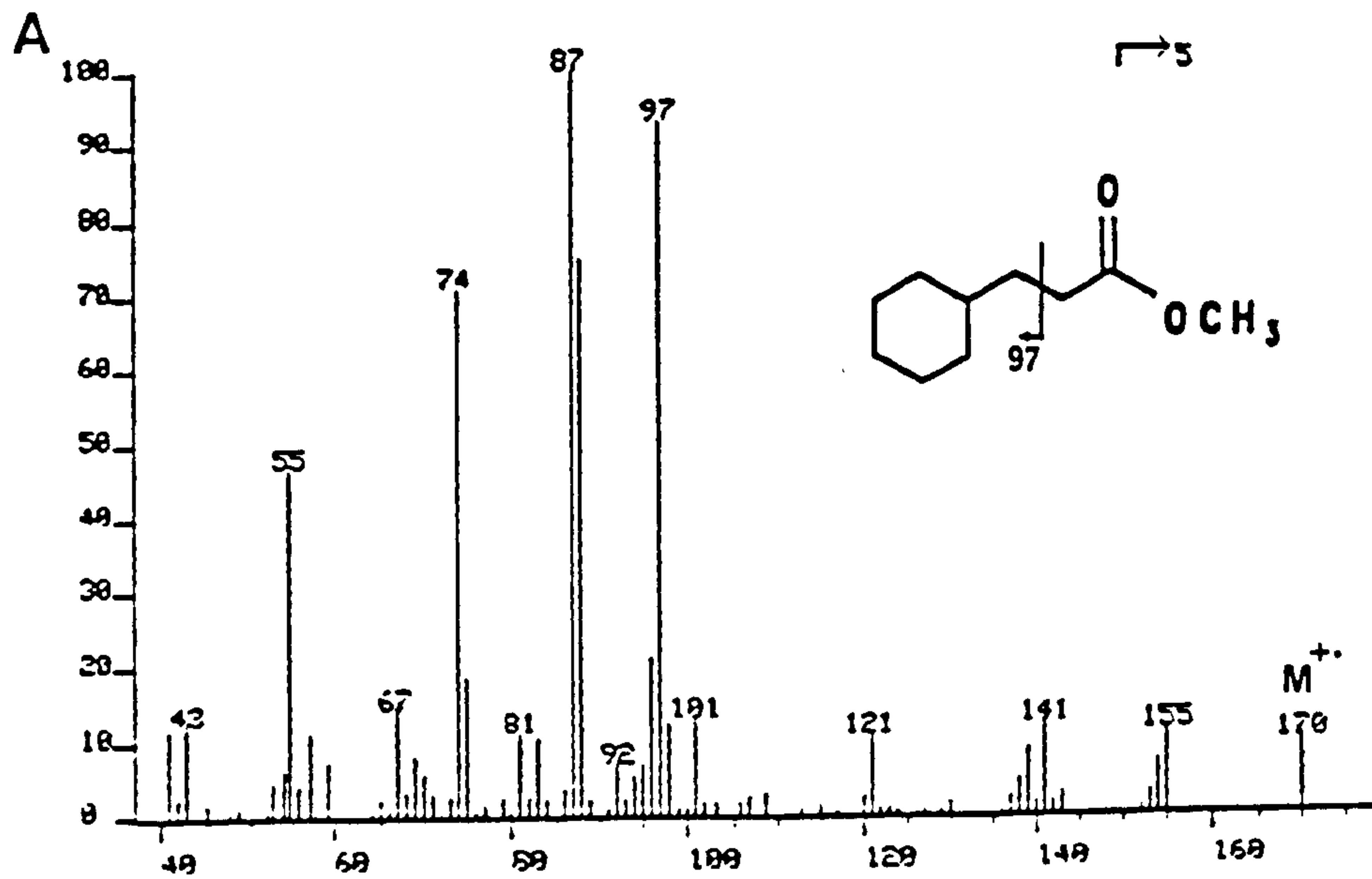


FIG. 3.64 EI MASS SPECTRA OF CYCLOHEXYL AND AROMATIC ACIDS IDENTIFIED AS AROMATIC UCM OXIDATION PRODUCTS



A) 1-CYCLOHEXYLPROPANOIC ACID

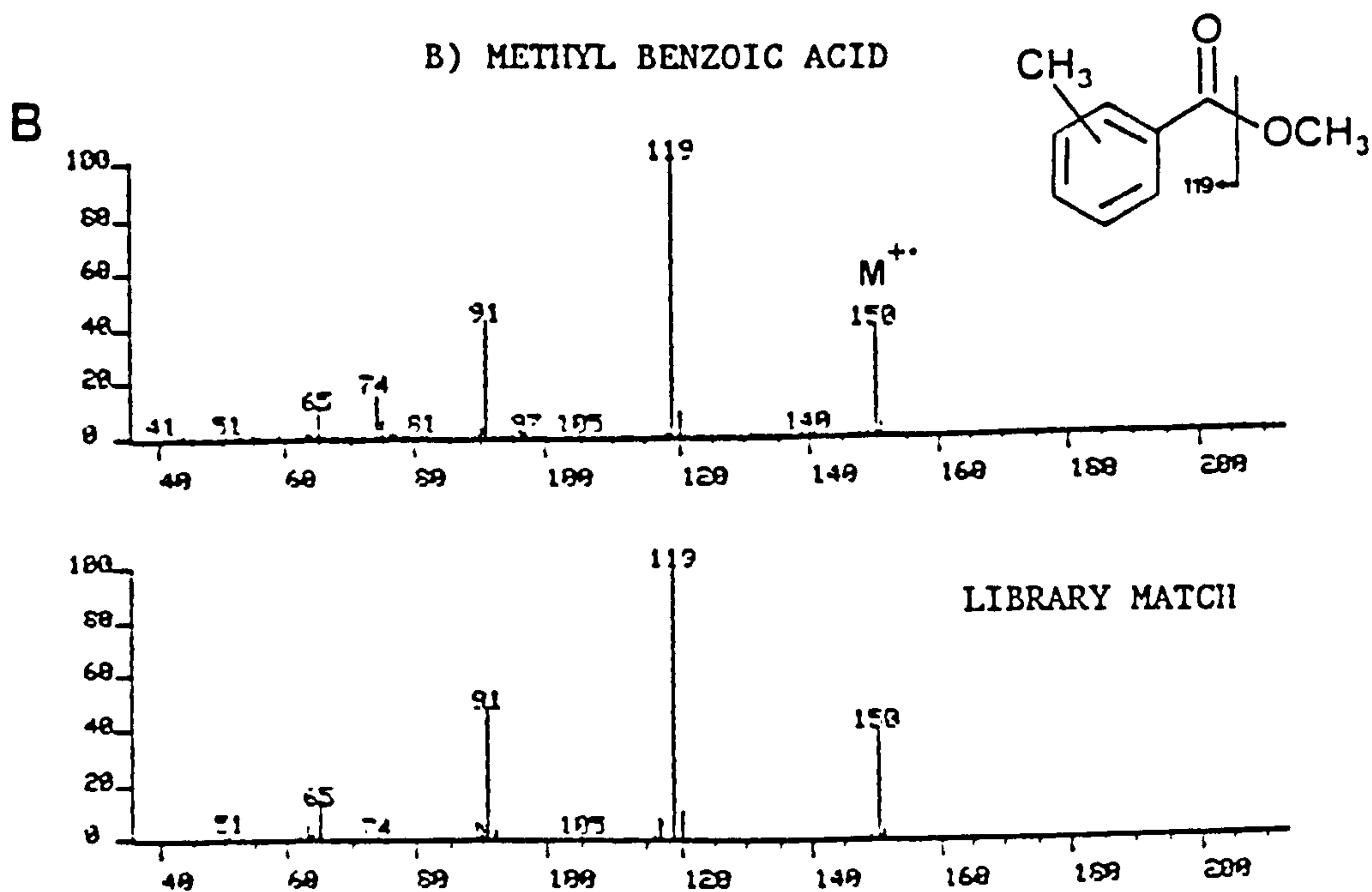
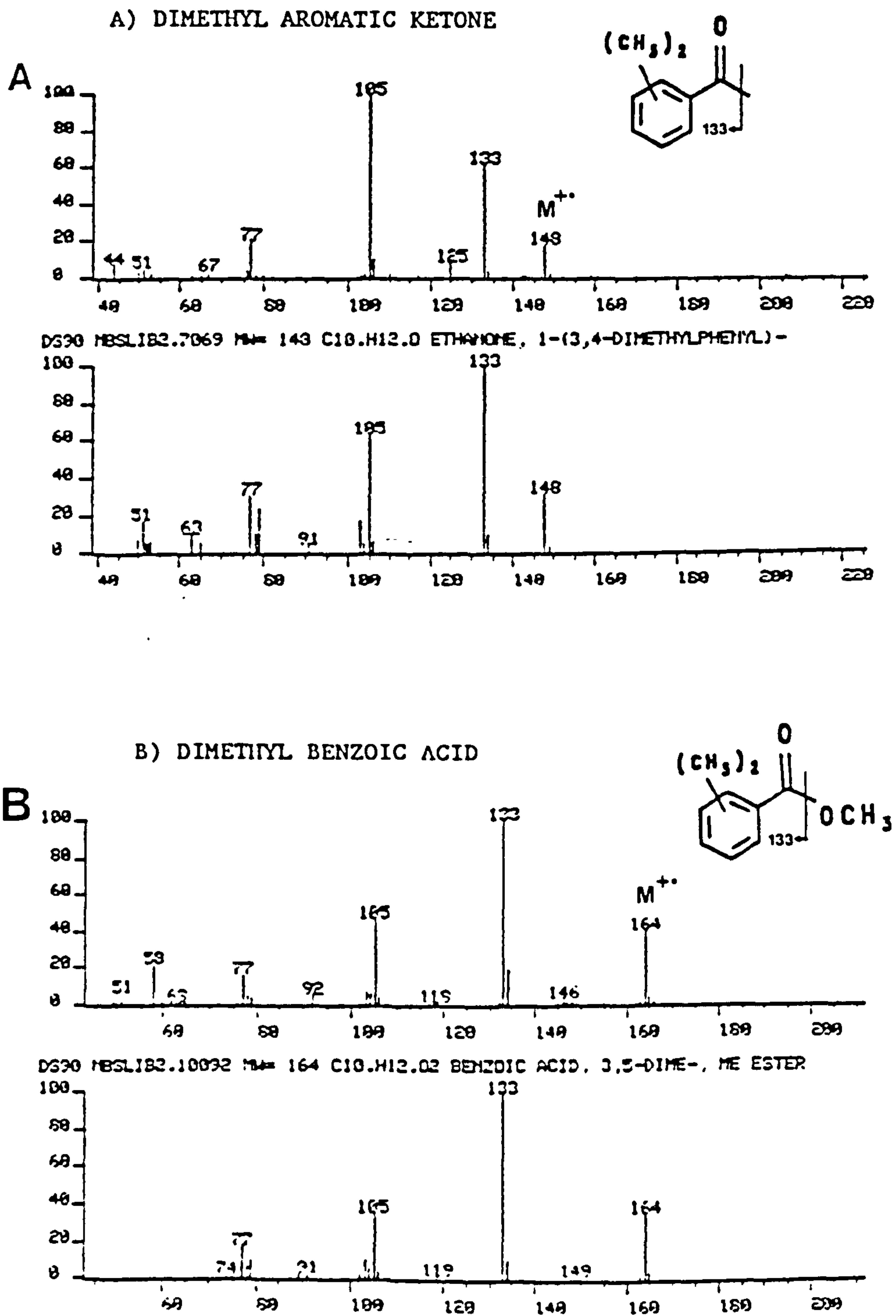
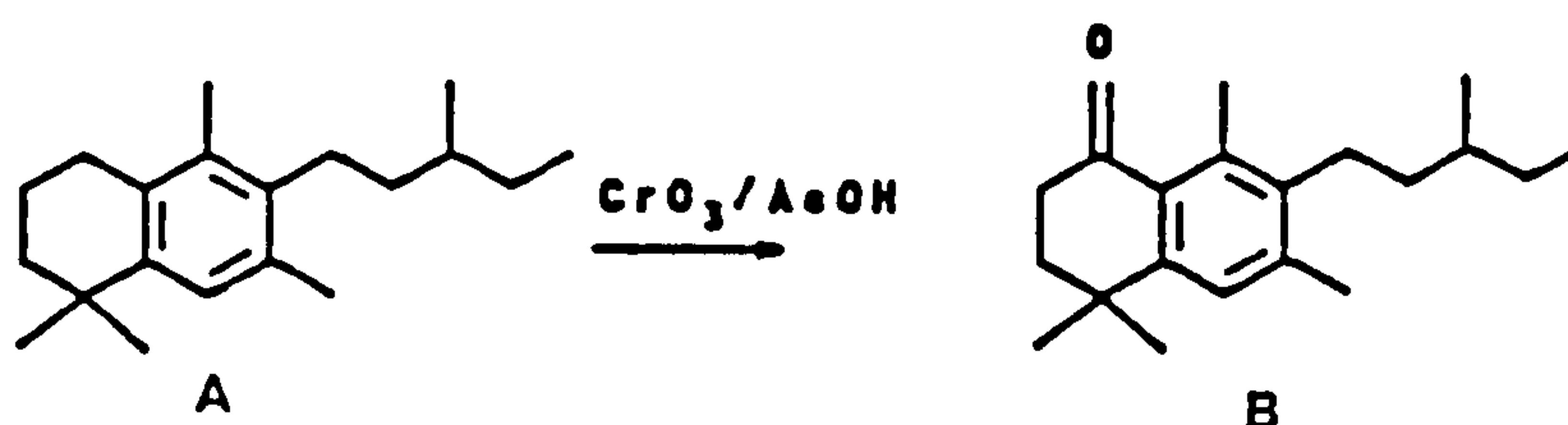


FIG. 3.65 EI MASS SPECTRA OF AROMATIC UCM OXIDATION PRODUCTS

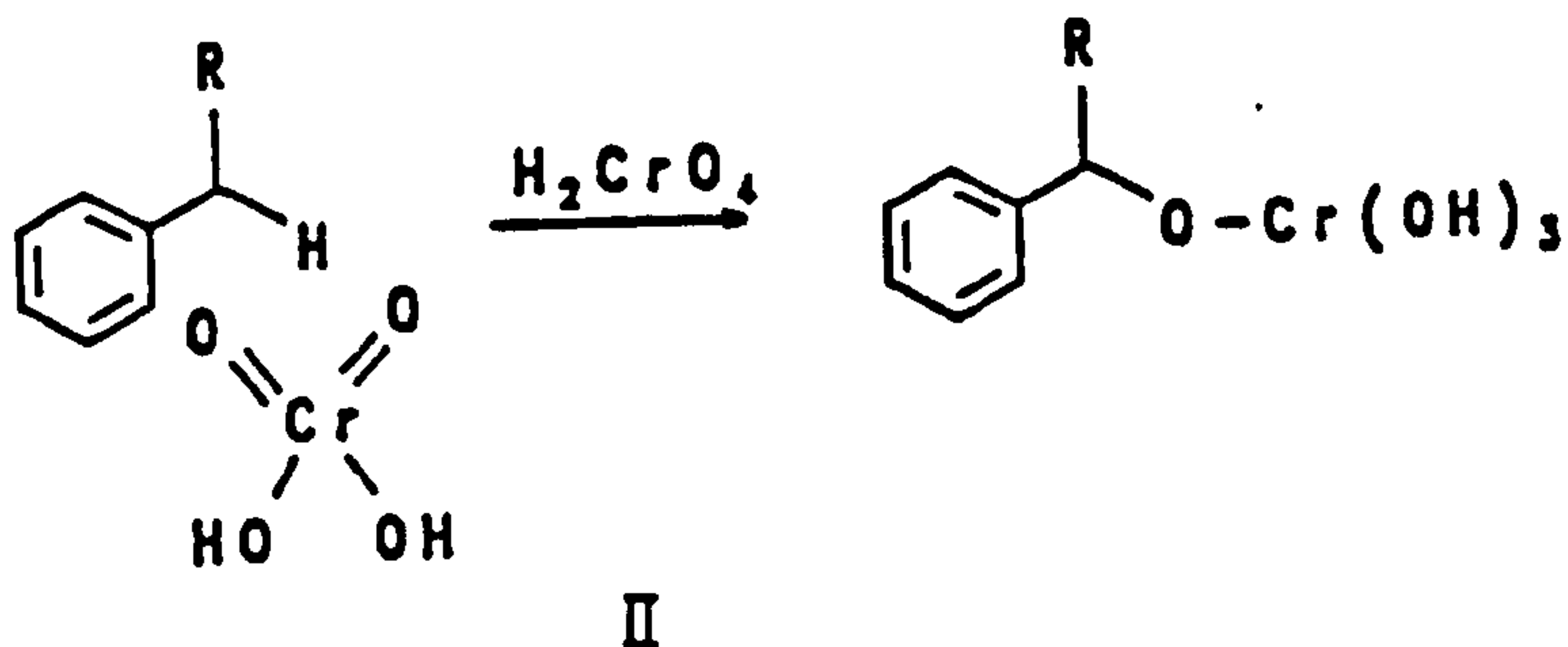
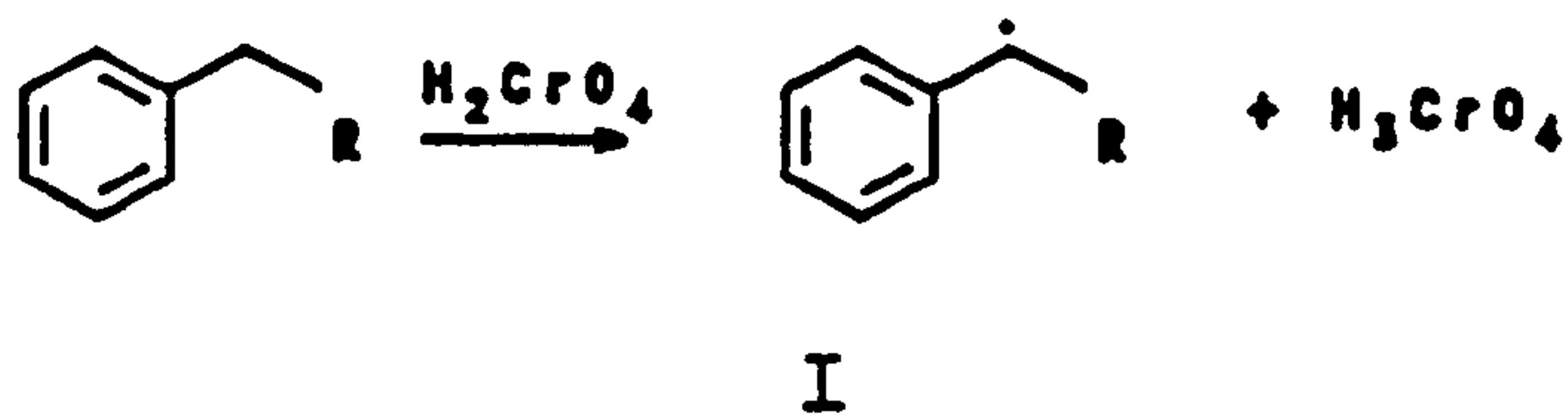


The mechanism of oxidation of alkyl aromatic hydrocarbons by CrO_3 in glacial acetic acid has been reviewed previously (Wiberg, 1965; Cainelli and Cardillo, 1984; Freeman, 1986). In contrast to the oxidation of branched alkanes, which takes place preferentially at tertiary C-H bonds (section 3.3.14) the oxidation of alkylaromatics occurs almost exclusively at the benzylic position. For example, the oxidation of ethyl benzene affords benzoic acid and acetophenone as major products (Cainelli + Cardillo, 1984). In a similar way, oxidation of the alkyl tetralin (A) produces the corresponding alkyl tetralone (B) via benzylic oxidation. This occurs in preference to oxidation at the tertiary position of the alkyl substituent (Cainelli and Cardillo, 1984)

i.e.

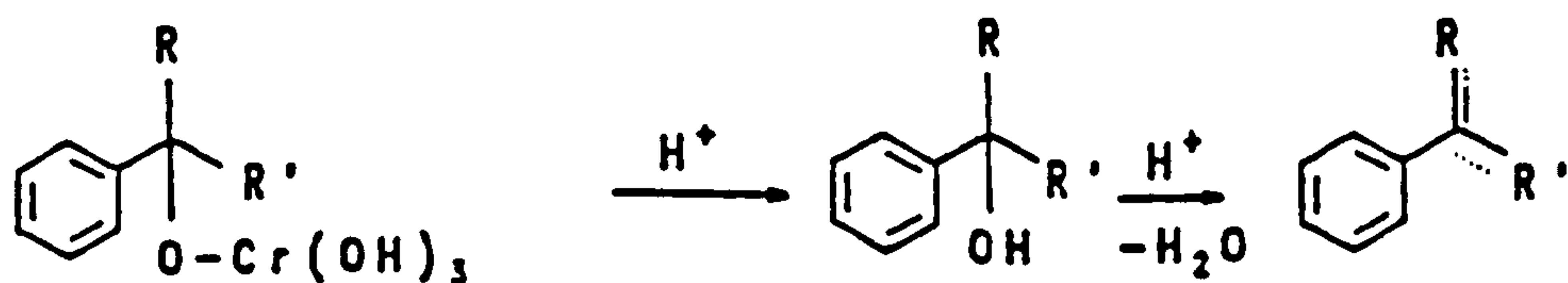


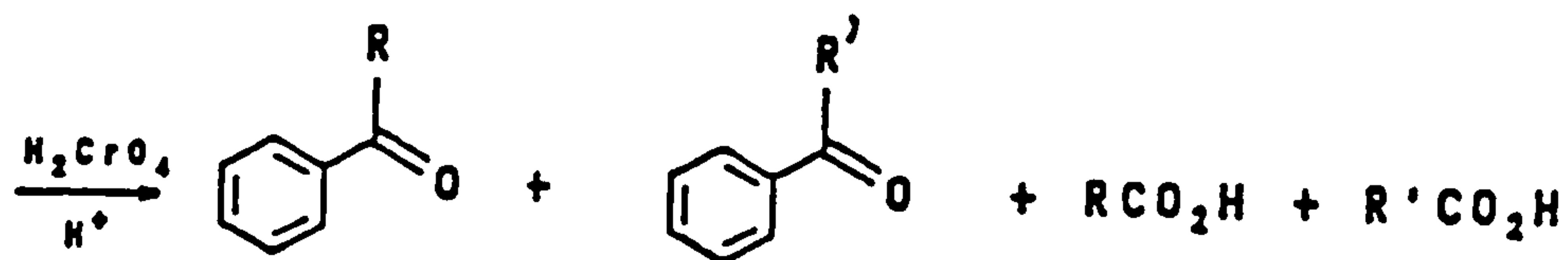
The initial rate - determining step appears to be abstraction of a benzylic hydrogen atom producing a free radical (I, c.f. the oxidation of branched alkanes, section 3.1.4) Alternatively, a concerted mechanism leading to a chromate ester may occur (II), i.e.



The intermediate radical species formed via mechanism I may then be further oxidised to a carbocation, or alternatively recombine with additional chromic acid to form a chromate ester. Decomposition of the chromate esters would then yield a carbonyl compound (providing the benzylic position is primary or secondary) This could then undergo further oxidation with additional oxidant to form the acid (if primary), or a mixture of aromatic and aliphatic acids (if secondary).

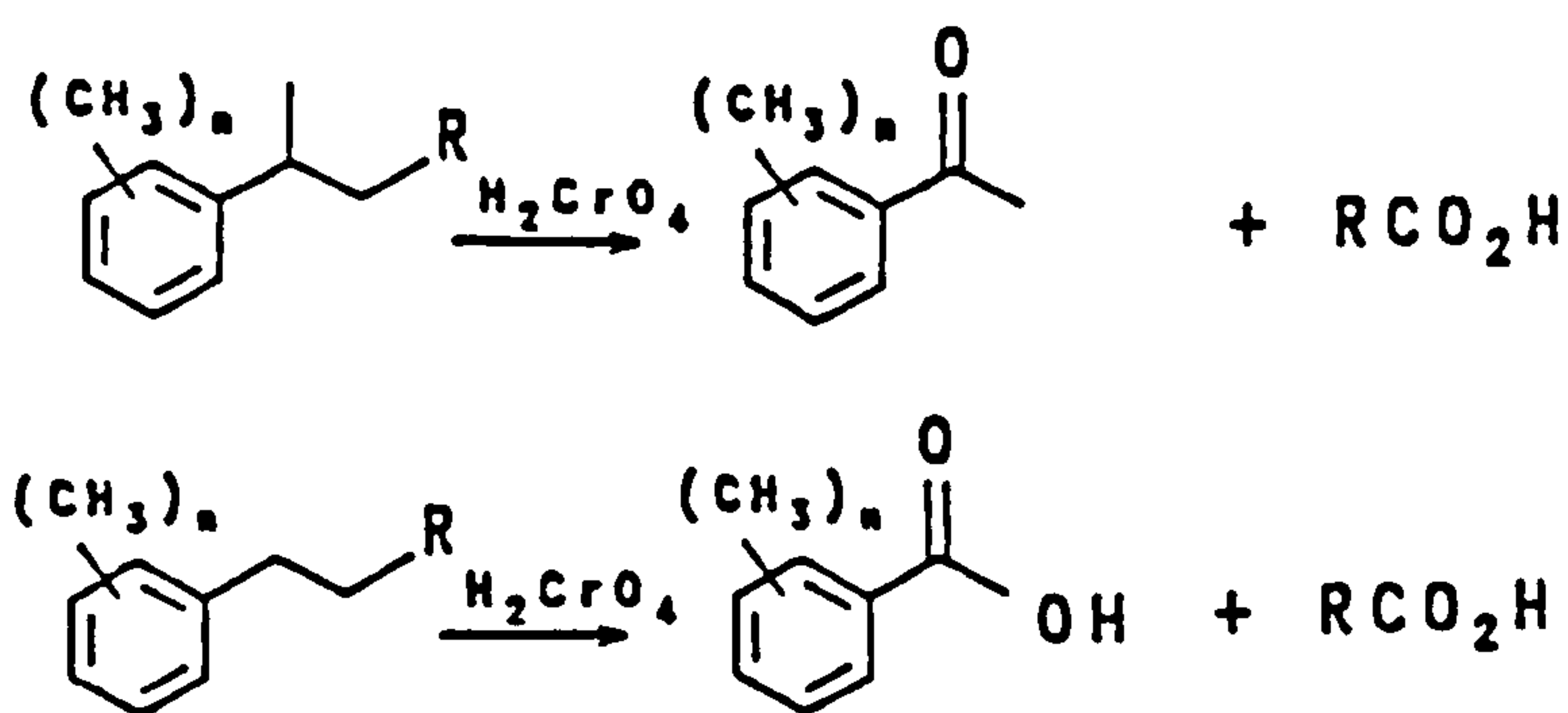
Tertiary chromate esters would undergo hydrolytic cleavage to produce the corresponding tertiary alcohol, which providing α -hydrogens are present, would undergo acid-induced dehydration to an olefin followed by oxidative cleavage of the double bond to form an acid and a ketone (c.f. the oxidation of alkanes, section 3.1.4).





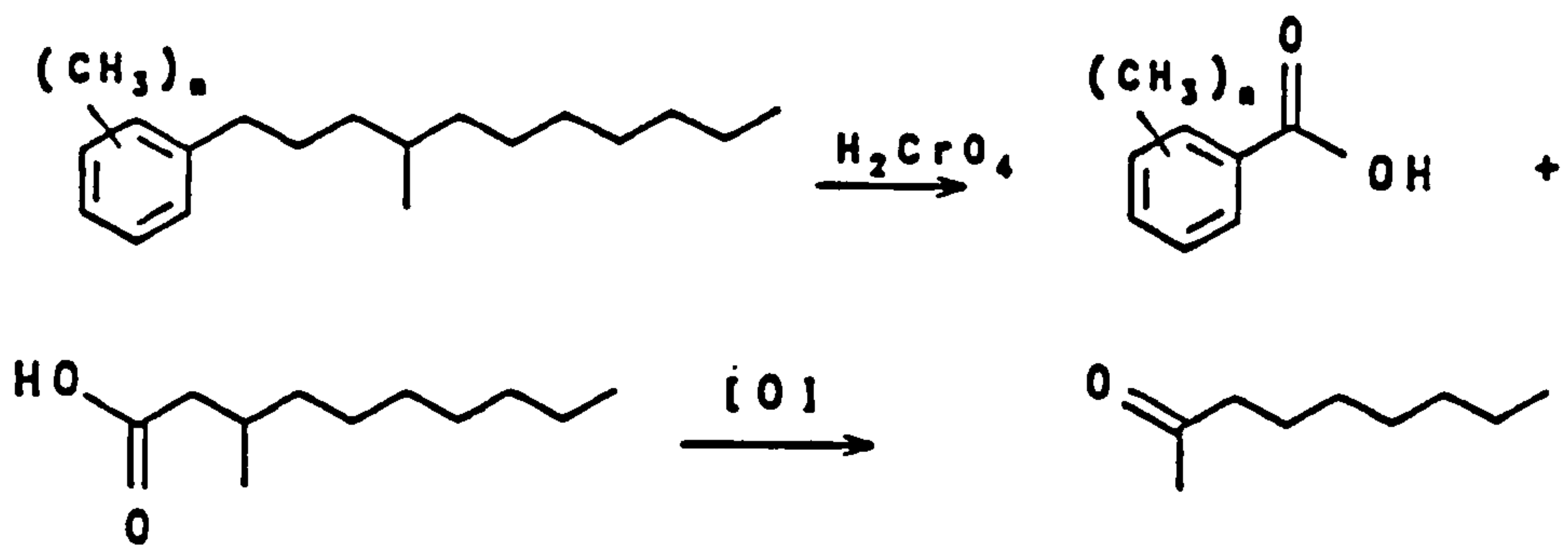
On the basis of the above mechanism, the observed oxidation products of the aromatic UCM can be correlated with potential precursor alkyl aromatic hydrocarbons.

The aromatic oxidation products identified were essentially unsubstituted or methyl substituted benzoic acids, or monoaromatic ketones. This is perhaps indicative of a predominance of alkyl substituted monoaromatic hydrocarbons. The major resolved aliphatic compounds identified were n-monocarboxylic acids, these therefore most likely originate directly from oxidation of linear or simply branched alkyl chains, providing the branch is methyl and α to the ring:



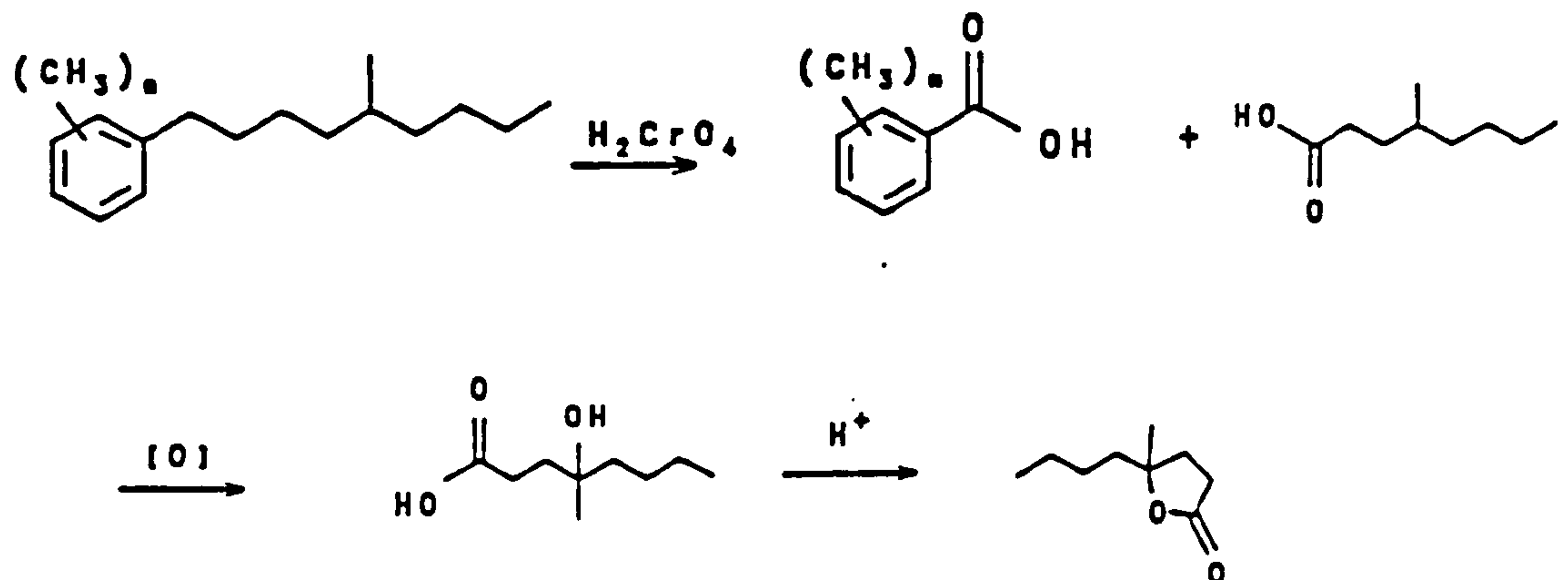
On the assumption that benzylic positions are oxidised first, series of n- and "iso"-methylbranched alkan-2-ones must arise through secondary oxidation of methyl substituted alkyl side chains, (c.f.

the oxidation of alkanes, section 3.14).

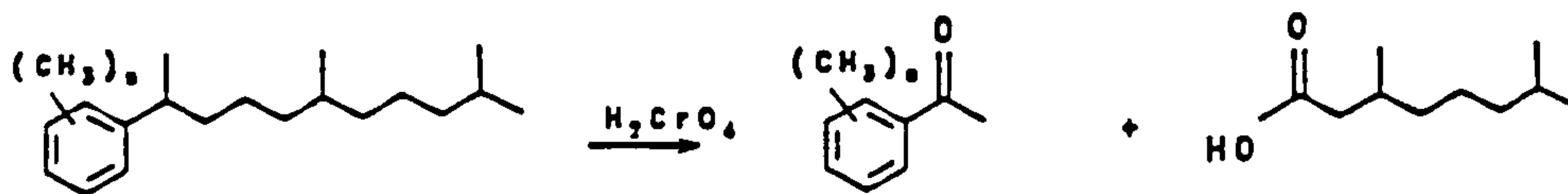


Likewise, γ -methyl- γ -lactones must originate from secondary oxidation of methyl branched alkyl side chains,

i.e.

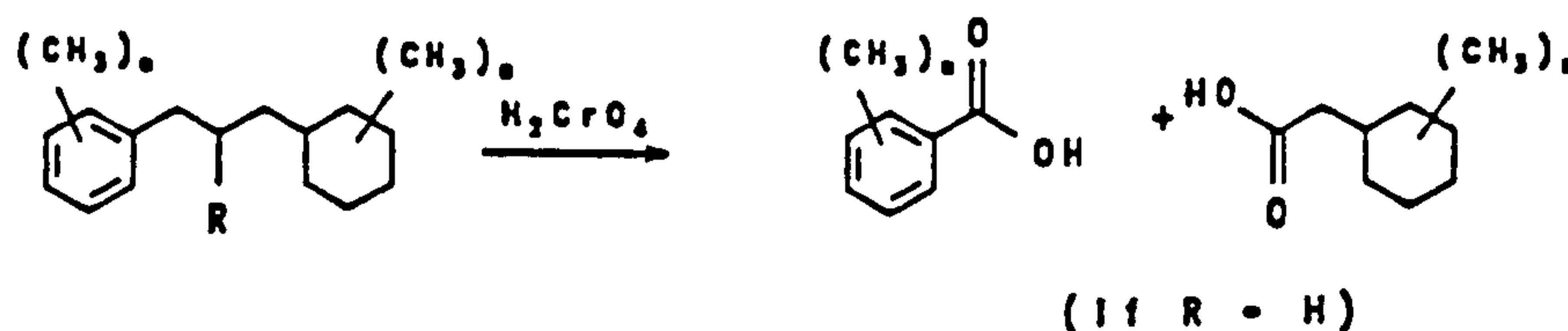


A high proportion of acyclic isoprenoid acids and ketones were identified as aromatic UCM products. These therefore most probably arise through primary and secondary oxidation of monoaromatic hydrocarbons bearing acyclic "isoprenoid" alkyl chains:



Similar "isoprenoid" alkyl substituted aromatic hydrocarbons have recently been identified in sediments and petroleums (Sinninghe Damste et al., 1988).

Of particular interest was the identification herein of a number of cyclohexyl carboxylic acids. These oxidation products suggest the presence of non condensed naphthenic/aromatic hydrocarbons of the type:-

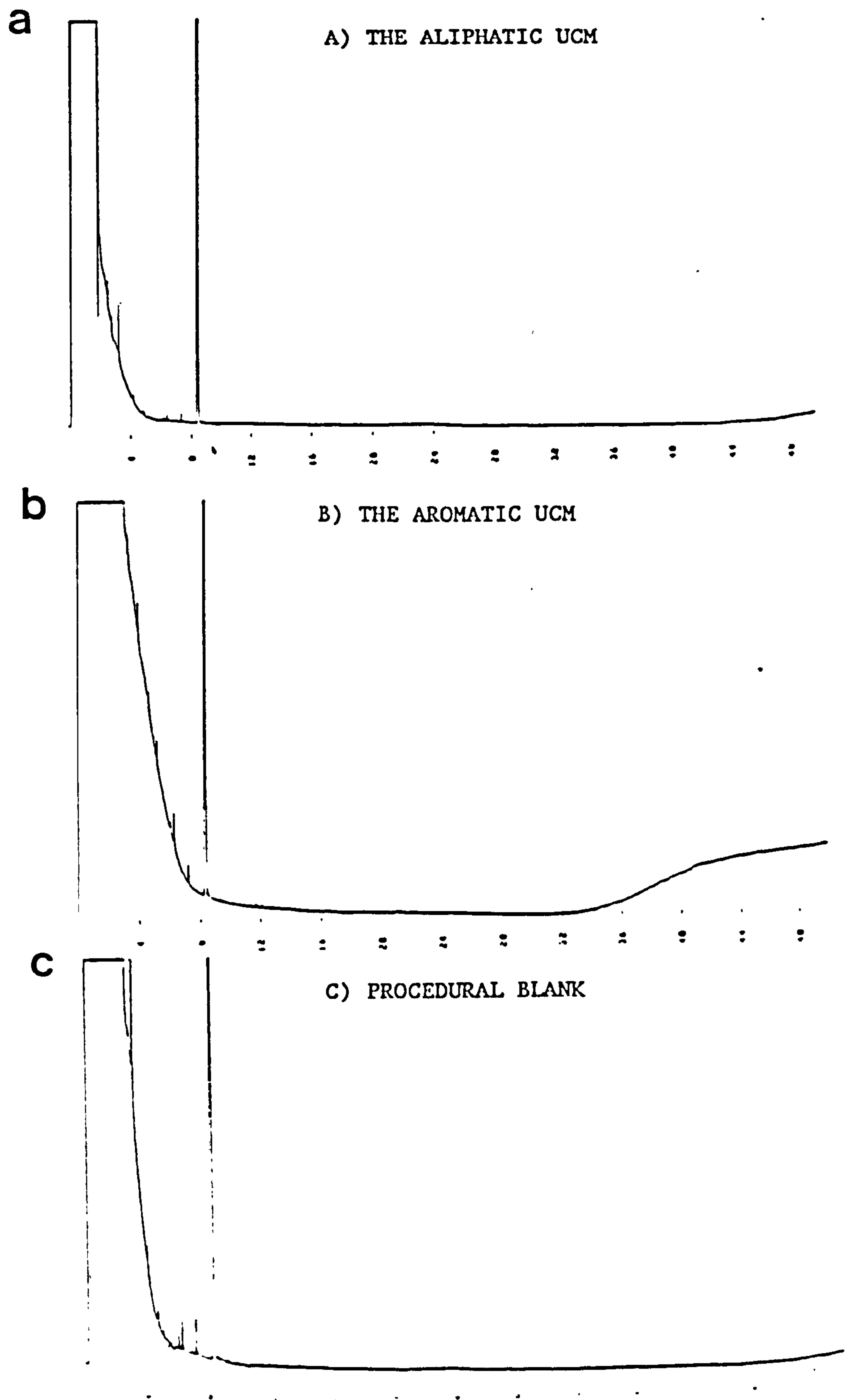


Evidence of non-condensed naphthenic/aromatic nuclei was provided by GC- EIMS analysis of the aromatic UCM (section 3.2.1.1).

3.3 AN EXAMINATION OF OXIDATION PRODUCTS ASSOCIATED WITH THE AQUEOUS PHASE

Yields of DCM-Soluble aromatic and aliphatic UCM oxidation products though generally high were not 100%. A possible explanation is that DCM did not recover all the oxidation products, in particular low

FIG. 3.66 GAS CHROMATOGRAMS OF THE AQUEOUS PHASE DERIVATISED (BF₃/n-BUTANOL) UCM OXIDATION PRODUCTS



molecular weight and/or more polar organic acids which are more soluble in water. An attempt was therefore made to characterise organic acids associated with the aqueous phase by an adaptation of the method of Eglinton *et al.*, 1987. This involved adjustment of the pH of an aliquot (10cm³) of post-UCM oxidation, DCM extracted, acetic acid/water mixtures by concentrated KOH solution (15M, ca. pH 8-9). At this stage a gelatinous blue-green precipitate was observed to form (presumed to be Cr(OH)₃), which was removed by filtration (Whatman GF/F, precombusted, 450°C - 12hr). The filtrate was concentrated to near dryness by rotary evaporation (Buchi, 70°C), and dried under a stream of N₂. BF₃/n-butanol (5cm³, 14% v/v, chrompack) was added, and after ultrasonication (2min), the mixture was heated in a water bath (100°C) for 30min. The solution was cooled, water added, (15cm³), and products extracted into DCM (3x10cm³), dried (Na₂SO₄), and the solvent removed.

A considerable volume of solution remained after DCM removal (presumed butyl acetate/residual n-butanol), which was not concentrated further. GC analysis of the derivatised aqueous - phase revealed a broad solvent front, with few additional peaks. GC of the procedural blank showed an almost identical profile (Fig. 3.66 c).

It was therefore concluded that although low molecular weight organic acids are likely to have been produced on oxidation of hydrocarbon UCMs, the use of acetic acid as a solvent medium inhibits their detection by use of the above described procedure.

3.4 PYROLYSIS GAS CHROMATOGRAPHY - MASS SPECTROMETRY (PY-GCMS) OF ALIPHATIC AND AROMATIC UCMs, AND MODEL COMPOUNDS.

3.4.1 GENERAL :

Pyrolysis gas chromatography (Py-GC) and pyrolysis gas chromatography-mass spectrometry (Py GC-MS) have been used extensively in petroleum and environmental organic geochemistry for the characterisation of a wide range of complex, organic sample matrices (reviewed by Larter and Douglas, 1982; Philp, 1982). These include kerogen (Eglinton et al., 1988), crude oil asphaltenes (Behar et al., 1984), coal (Chaffee et al., 1984) and lignin (Saiz-Jimenez and de Leeuw, 1984). In the present study use was made of pyrolysis GC-MS as a rapid method for the preliminary characterisation of aliphatic and aromatic hydrocarbon UCMs from lubricating oils.

The system used was that described recently (Patience et al., 1989), and the methods involved application of samples (ca. 50 g) via syringe to quartz pyrolysis tubes packed with preextracted (DCM, 48hr) glass wool. Pyrolysis was performed at a maximum temperature of 800°C for 20 secs, and the volatile pyrolysates cryogenically focussed at the column head (liquid N₂, 2min), prior to temperature programmed GC-MS. This temperature of pyrolysis was chosen for optimum formation of degradation products relative to residual unaltered UCM.

3.4.2 RESULTS

Although relative yields of pyrolysis products were low, pyrolysis of the aliphatic UCM produced a resolved series of compounds (Fig. 3.67 A), the major components of which were identified as n-alkenes with one degree of unsaturation in the range C₉ to C₂₀ (nC₉:1 to nC₂₀:1, Fig. 3.67 B). This is in accordance with the results of the CrO₃

FIG. 3.67 PYROLYSIS GC-MS OF THE ALIPHATIC UCM

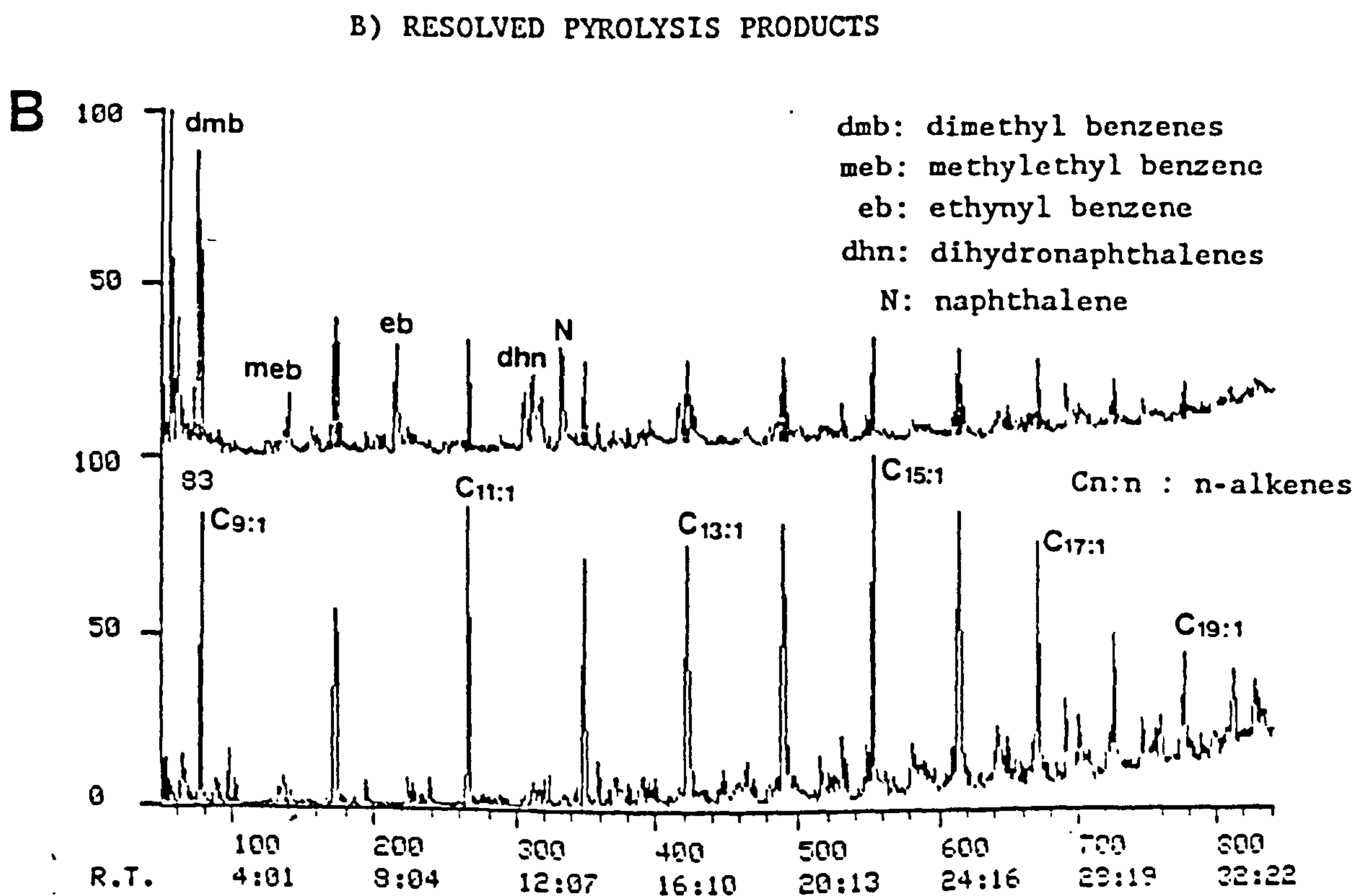
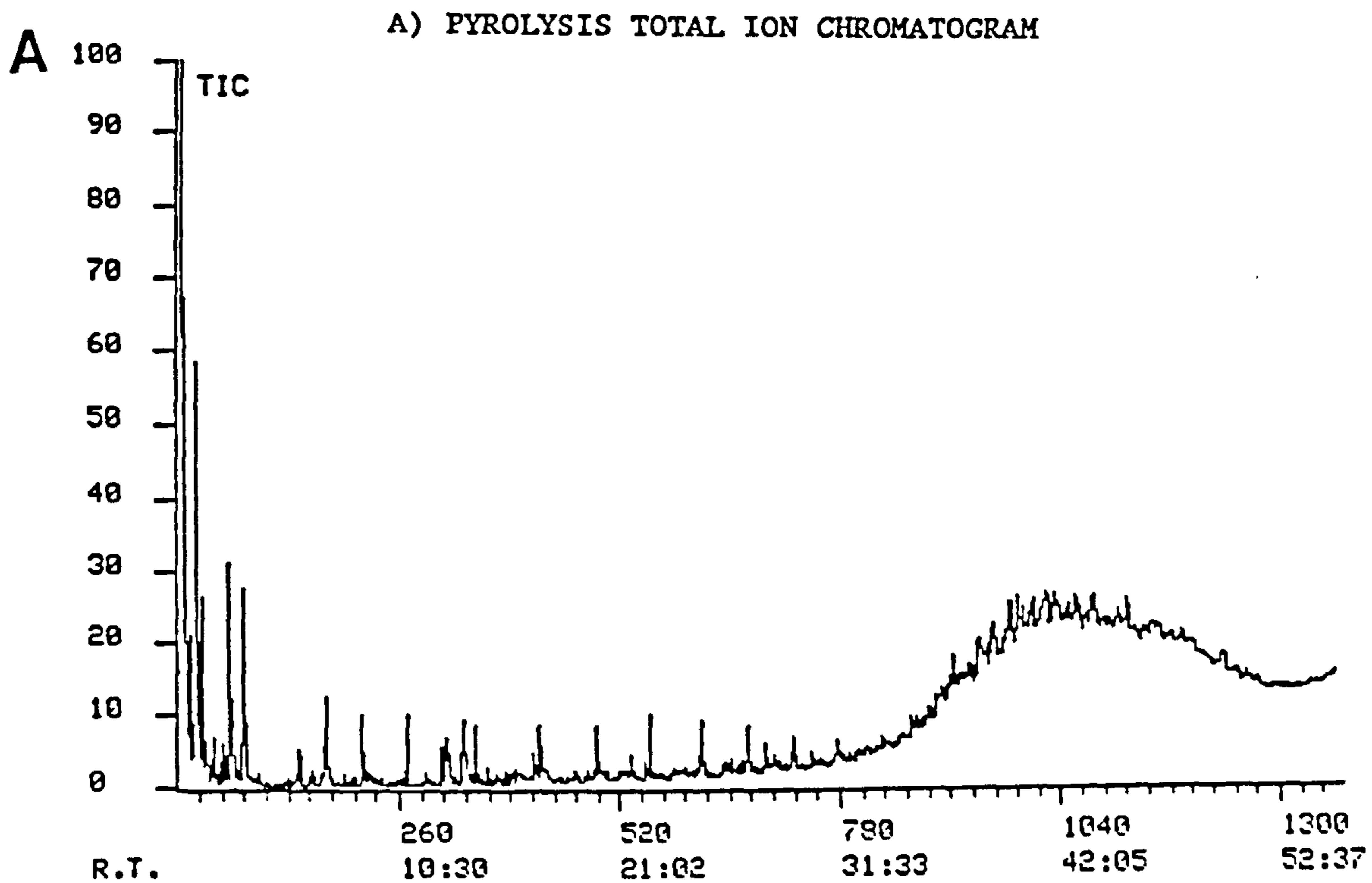
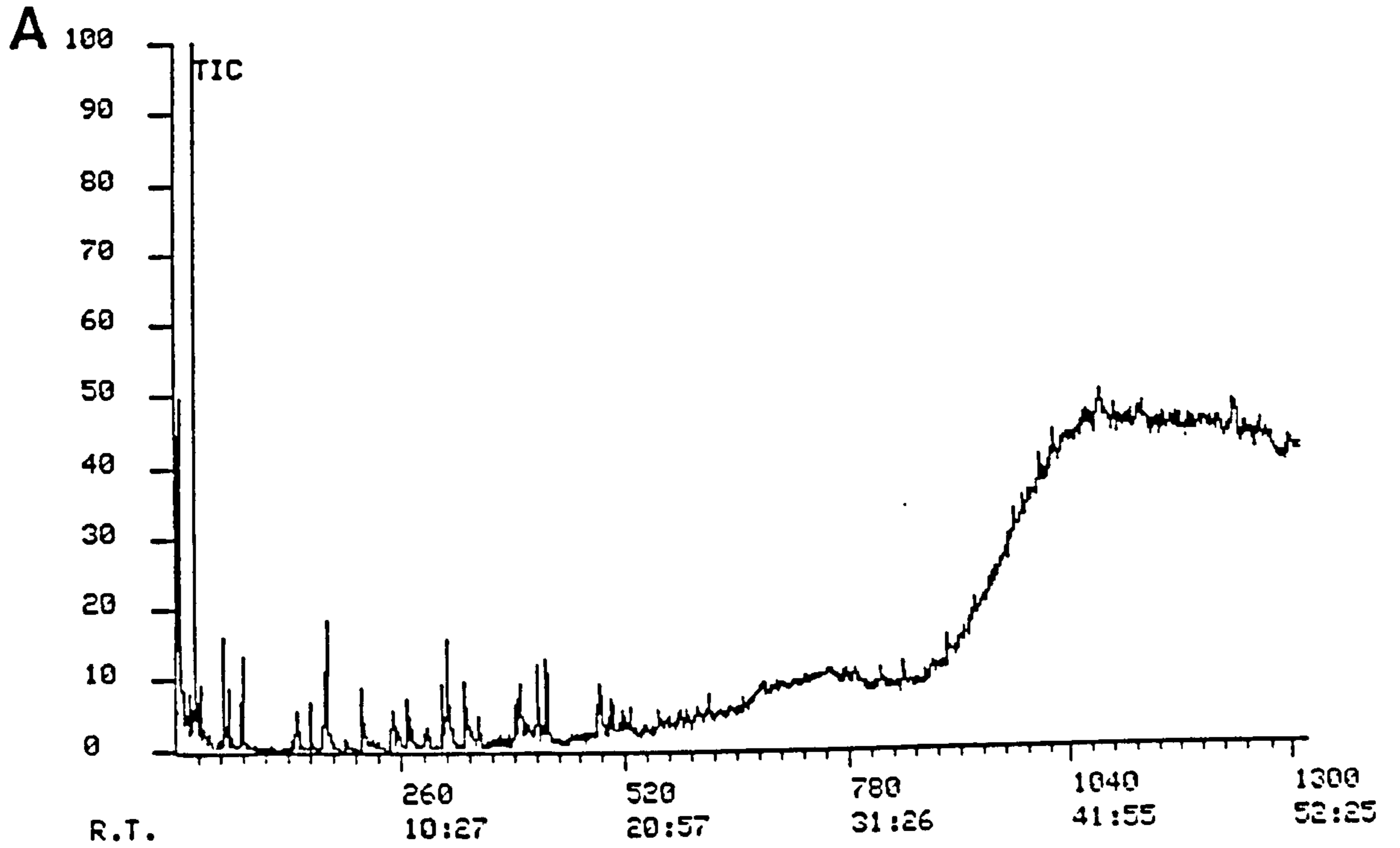
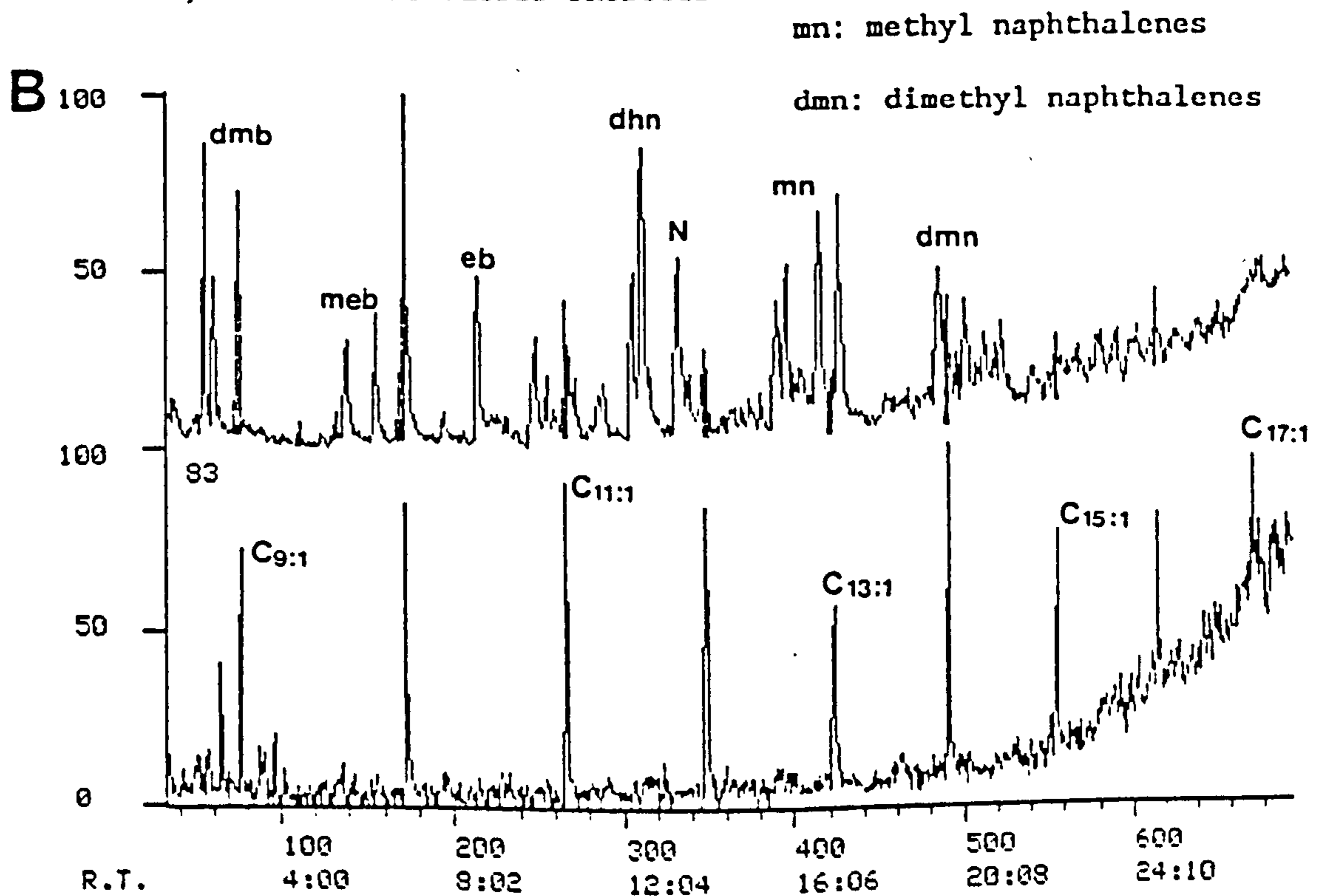


FIG. 3.68 PYROLYSIS GC-MS OF THE AROMATIC UCM

A) PYROLYSIS TOTAL ION CHROMATOGRAM



B) RESOLVED PYROLYSIS PRODUCTS



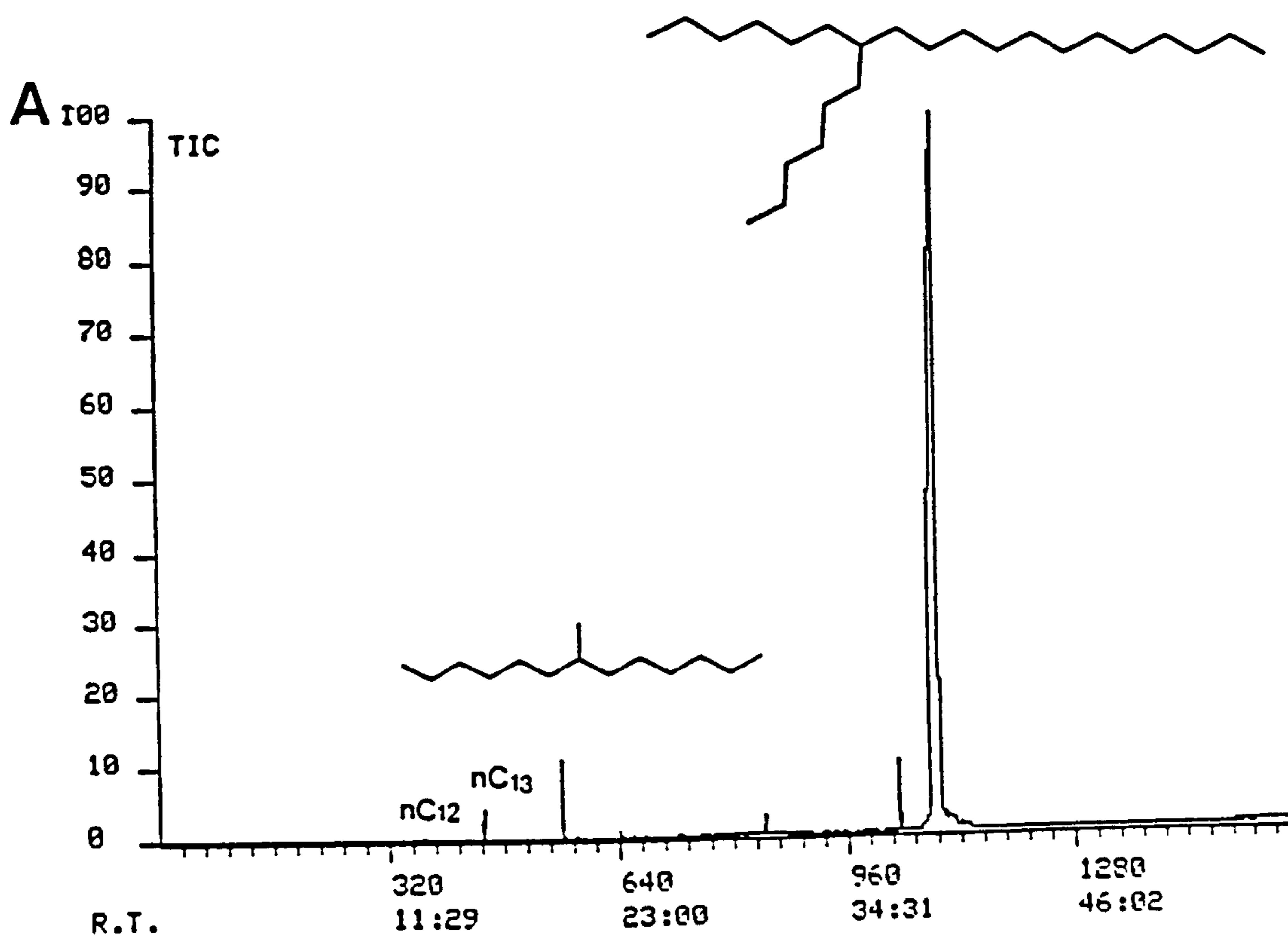
oxidation of the aliphatic UCM which produced as major compounds, n-alkanoic acids. The n-alkenes are presumably produced through pyrolytic cleavage of monoalkyl substituted acyclic or monocyclic alkanes at the branch position. Tertiary C-H bonds are known to be the most active in radical hydrogen abstraction reactions (Kissin, 1986). Also identified as aliphatic UCM pyrolysis products were alkyl aromatic hydrocarbons. These included dimethylbenzenes, methylethylbenzene, ethynylbenzene, dihydronaphthalenes, and naphthalene (Fig. 3.67 B). These are however unlikely to originate from alkylated aromatic hydrocarbons within the UCM, but are presumably formed via radical induced aromatisation reactions which are known to occur at elevated temperatures (Badger et al., 1958).

Pyrolysis of the aromatic UCM also produced a series of resolved products, these were surprisingly similar to those observed on pyrolysis of the aliphatic UCM. Thus a homologous series of n-alkenes were identified in the range C9 to C17 (C9:1 to C17:1, Fig. 3.68 A+B). Also observed were similar series of aromatic hydrocarbons, namely dimethylbenzenes, methylethylbenzenes, ethyl benzene, dihydronaphthalenes and naphthalene. Quantitatively a much greater proportion of aromatic hydrocarbons were noted as products of the aromatic UCM. Also observed were isomeric methyl and dimethylnaphthalenes, these were not observed as aliphatic UCM pyrolysis products.

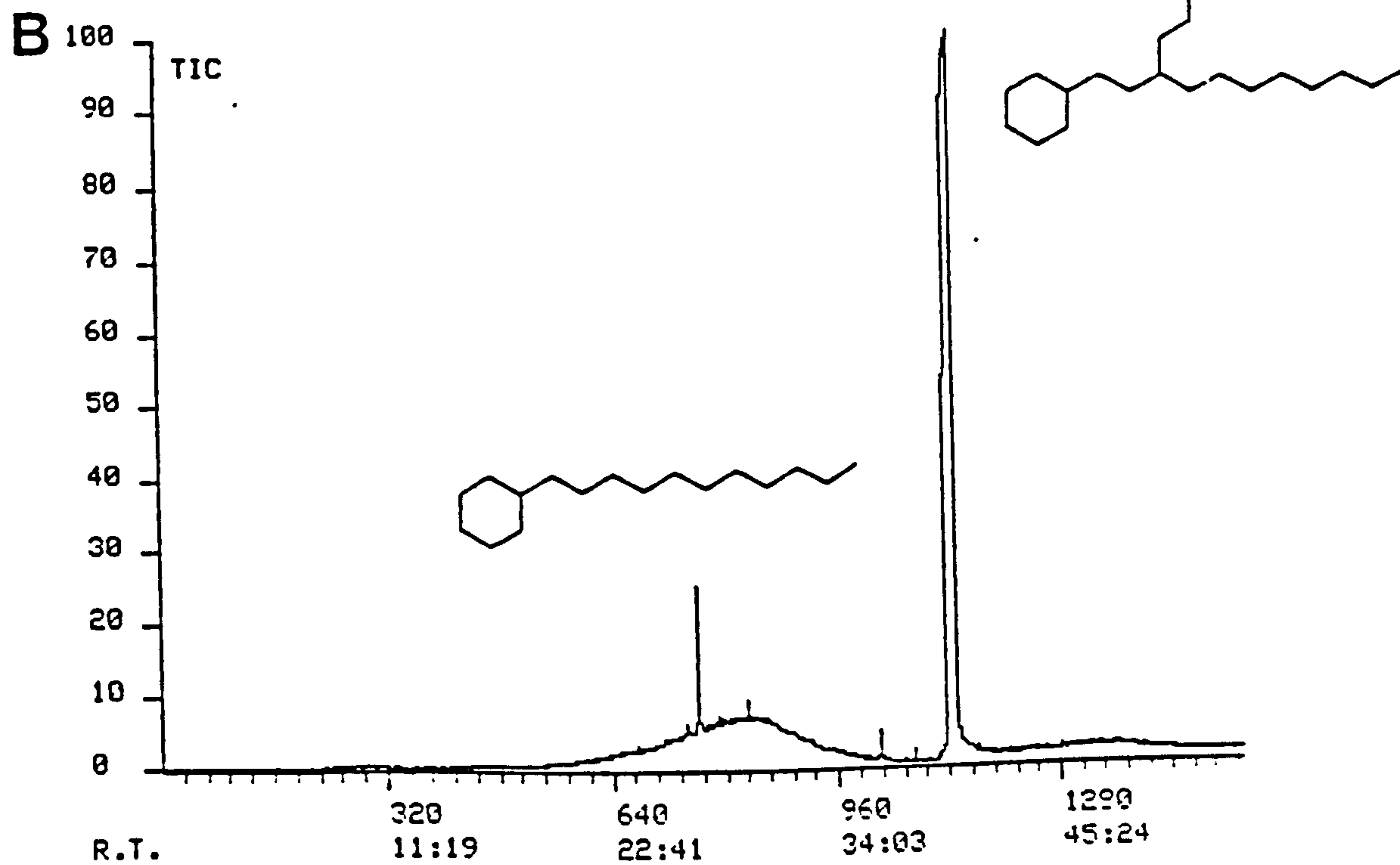
Yields of pyrolysis products of synthetic model UCM hydrocarbons (7-n-hexylnonadecane, 9-(2-cyclohexylethyl)-heptadecane and 9-(2-phenylethyl)-heptadecane, chapter 4) relative to residual hydrocarbon

FIG. 3.69 PYROLYSIS GC-MS OF MODEL HYDROCARBONS

A) 7-N-HEXYLNONADECANE



B) 9-(2-CYCLOHEXYLETHYL)-HEPTADECANE



were low. Pyrolysis of 7-n-hexylnonadecane (Fig. 3.69 A) produced three main products, these were characterised as n-C12 and n-C13 alkanes, and a branched C14 alkane (M^+ 198, m/z 113 as base peak), presumed to be 7-methyltridecane. Pyrolysis therefore appeared to occur preferentially at or adjacent to the branch position.

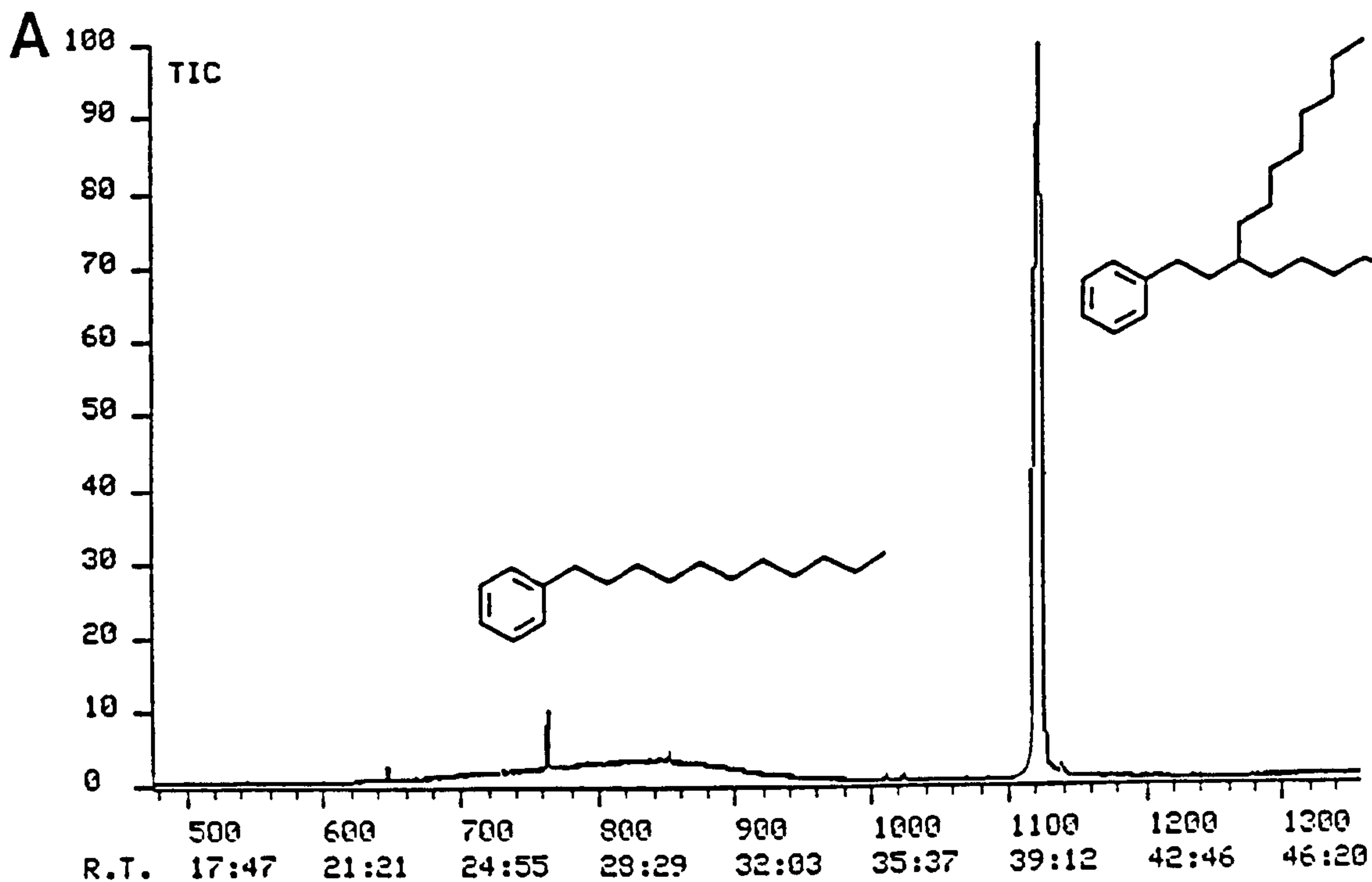
Pyrolysis of 9-(2-cyclohexylethyl)-heptadecane produced a single product, the C17 monocyclic alkane 1-cyclohexylundecane (Fig. 3.69 B and Fig. 3.70 B). Again pyrolysis appeared favoured at the branch position of the alkyl chain. Pyrolysis of the alkyl aromatic hydrocarbon 9-(2-phenylethyl)-heptadecane was also observed to produce a single compound, 1-phenylundecane (Fig. 3.70A and B). Pyrolysis therefore occurred at the tertiary position of the alkyl side chain in preference to pyrolysis at the benzylic position.

3.4.3 SUMMARY

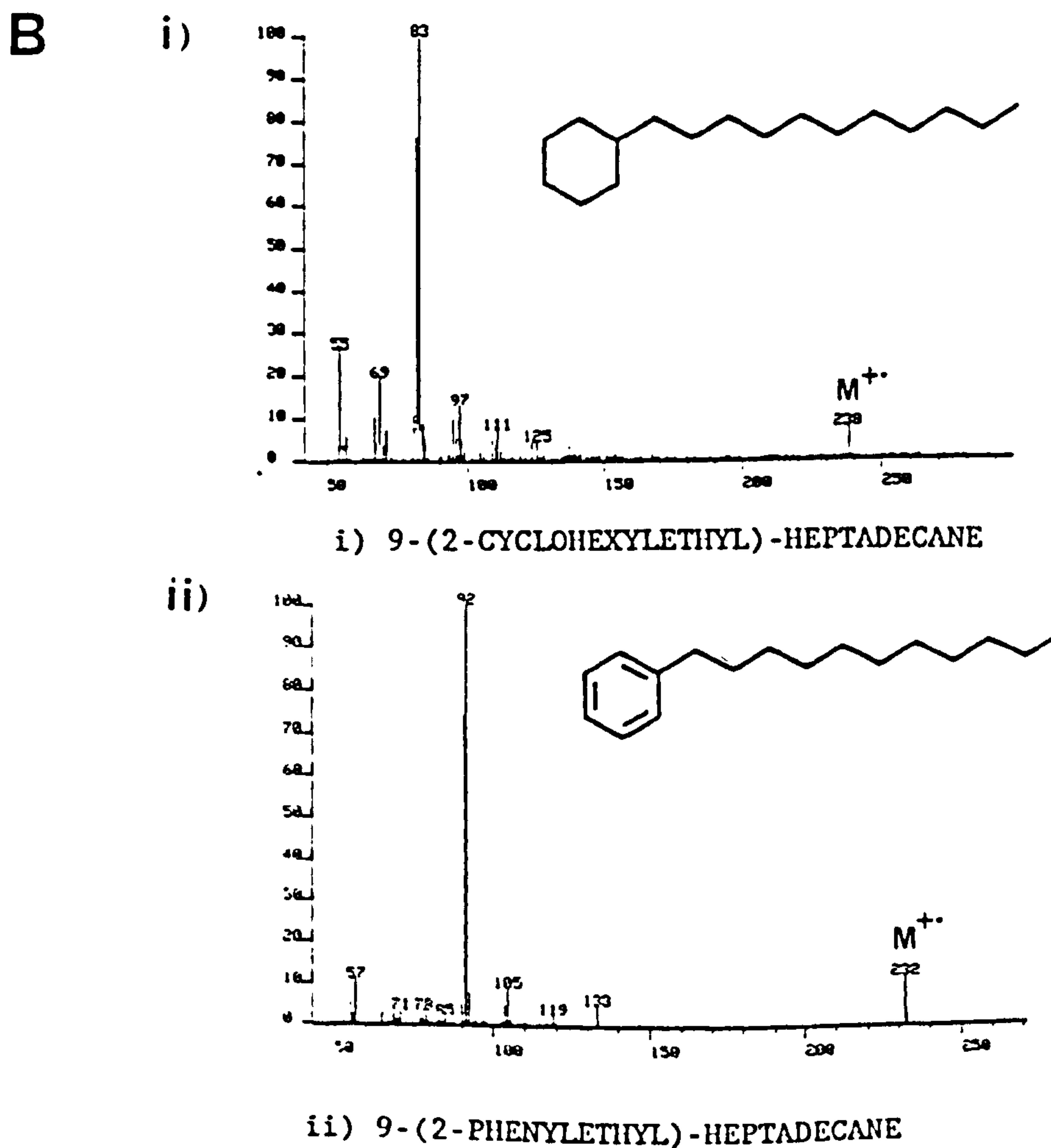
Preliminary pyrolysis GC-MS of aliphatic and aromatic UCM hydrocarbons appeared promising for the characterisation and hence possible rapid "fingerprinting" of such samples. Major products identified in each case were homologues series of n-alkenes, and alkyl aromatic hydrocarbons. The former appear to provide confirmatory evidence for a high proportion of monoalkyl substituted acyclic, monocyclic or alkylaromatic hydrocarbons in the samples under study. The identification of certain of the latter as aliphatic UCM pyrolysis products suggests these may arise as artefacts of the high temperature procedure. However, methyl and dimethylnaphthalenes were only identified as aromatic UCM pyrolysis

FIG. 3.70 PYROLYSIS GC-MS OF MODEL HYDROCARBONS

A) 9-(2-PHENYLETHYL)-HEPTADECANE



B) EI MASS SPECTRA OF PRODUCTS OF PYROLYSIS OF



products.

Pyrolysis of model compounds did not produce series of n-alkenes, instead n-alkanes were observed as products of the acyclic alkane 7-n-hexylnonadecane. For the monocyclic and monoaromatic hydrocarbon standards, pyrolysis occurred preferentially at the tertiary position of the alkyl side chain, with the loss in each case of a C8 alkyl fragment. These preliminary pyrolysis experiments therefore show promise for the rapid characterisation of complex hydrocarbon mixtures. Further work is required for the optimisation of the technique with particular reference to the production of sufficient yields of degradation products, whilst at the same time minimising the production of artefacts.

CHAPTER FOUR

SYNTHESIS AND OXIDATION OF MODEL HYDROCARBONS

4.1 SYNTHESIS OF MODEL HYDROCARBONS

4.1.1 OUTLINE

From the products of oxidation of aliphatic and aromatic UCMs and the reported mechanism of CrO_3 /glacial acetic acid oxidation of hydrocarbons, potential precursor compounds were proposed (Chapter 3). To confirm these proposals it was decided to synthesise certain hydrocarbons and to oxidise these under identical conditions to the UCM. Such an approach has been used with success in similar degradation studies of unresolved aromatic hydrocarbons in Athabasca oil sands (Mojelsky and Strausz, 1986); of kerogen (Machihara and Ishiwatari, 1987); of crude oil asphaltenes (Trifilieff, 1987), and of coal (Hayatsu *et al.*, 1982).

The compounds chosen for synthesis are shown in Table 4.1. Twenty-five was chosen as the carbon number since this approximates the middle of the elution range of typical hydrocarbon UCMs observed in biodegraded crude oils and in Recent sediments (e.g. Jones, 1986; Thompson and Eglinton, 1976).

Normal and monomethylalkanes, although prevalent in many mature petroleums (Kissin, 1987), are not thought to be representative of the UCM since they are mainly removed by urea adduction (Section 3.1.2). However, they were used as reference alkanes for both oxidation and biodegradation studies (Chapter 6). Hydrocarbons thought to be more typical of the aliphatic UCM were monoalkyl

TABLE 4.1
MODEL C25 HYDROCARBONS

NAME	COMPOUND	ORIGIN
<u>n</u> -pentacosane	<u>36</u>	Aldrich Chemical Co
2-methyltetracosane	<u>12</u>	Synthetic/this study
9-methyltetracosane	<u>5</u>	Synthetic/this study
7- <u>n</u> -hexylnonadecane	<u>9</u>	Synthetic/this study
9-(2-cyclohexylethyl)-heptadecane	<u>18</u>	Synthetic/this study
9-(2-phenylethyl)-heptadecane	<u>23</u>	Synthetic/this study
2,6,10,14,18-pentamethyleicosane	<u>30</u>	Synthetic/this study

substituted acyclic and monocyclic alkanes. Thus 7-n-hexylnonadecane and 9-(2-cyclohexylethyl)-heptadecane were synthesised. By analogy, the monoaromatic hydrocarbon 9-(2-phenylethyl)-heptadecane was synthesised as a potential aromatic UCM component. The regular isoprenoid 2,6,10,14,18-pentamethyleicosane was not considered representative of the types of compounds present in the aliphatic UCM, since oxidation of the analogous C19 regular isoprenoid 2,6,10,14-tetramethylpentadecane (Pristane) with chromic acid is known to produce polymethyl substituted carboxylic acids (Brooks et al., 1977; Patience et al., 1979; Rowland and Maxwell, 1983). These compounds were virtually absent as resolved aliphatic UCM oxidation products; however, the C25 isoprenoid was synthesised as a reference for the biodegradation study (Chapter 6).

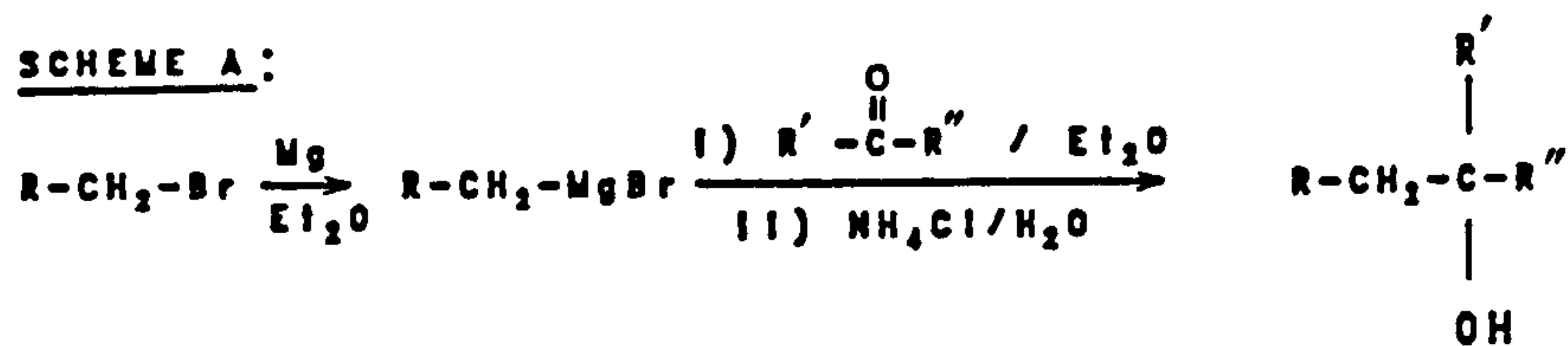
The choice of synthetic method was dependent on the availability of pure and inexpensive starting materials and on a relatively simple sequence of reactions which would produce the desired hydrocarbon in good yield. For this reason the coupling of alkylhalides with a carbonyl compound via a Grignard reagent was considered suitable, in view of its versatility and widely documented use in the synthesis of petroleum-type hydrocarbons (e.g. McLaughlin and Schiessler, 1954; Cosby and Sutherland, 1941; Schiessler et al., 1942). The generalised schemes chosen are summarised in Fig 4.1, and the synthesis of each individual model hydrocarbon is discussed below.

4.1.2 9-METHYLTETRACOSANE

Initial attempts to couple the alkyl halide (1-bromopentadecane, 1) via a Grignard reagent to the methylketone (decan-2-one, 2) in

FIG. 4.1 GENERAL GRIGNARD COUPLING SCHEMES FOR THE SYNTHESIS OF MODEL COMPOUNDS

SCHEME A:



9-METHYLTETRACOSANE

7-N-HEXYLNONADECANE

$R = C_{14}H_{29}$

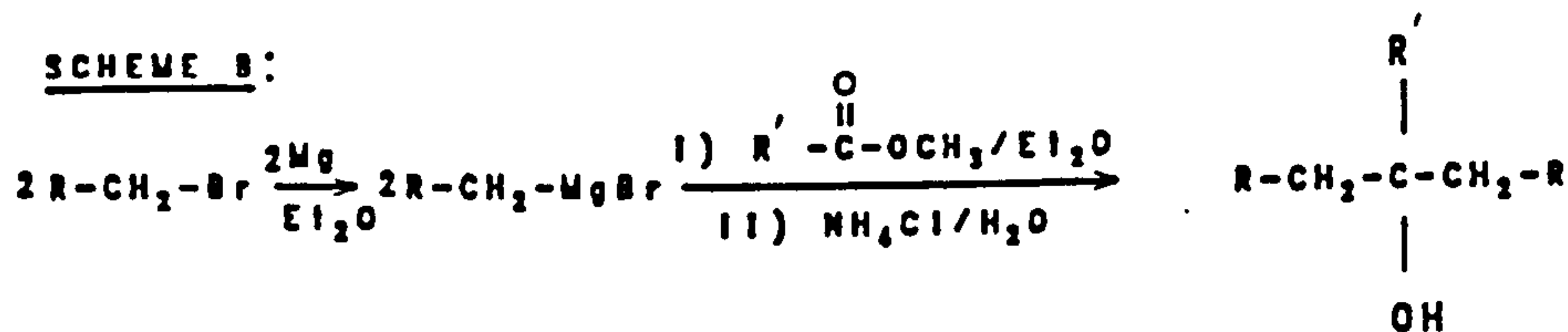
$R = C_{11}H_{23}$

$R' = C_9H_{19}$

$R' = R'' = C_8H_{17}$

$R'' = CH_3$

SCHEME B:

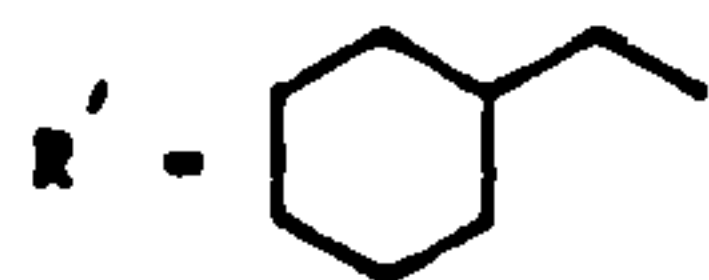


9-(2-CYCLOHEXYLETHYL)-HEPTADECANE

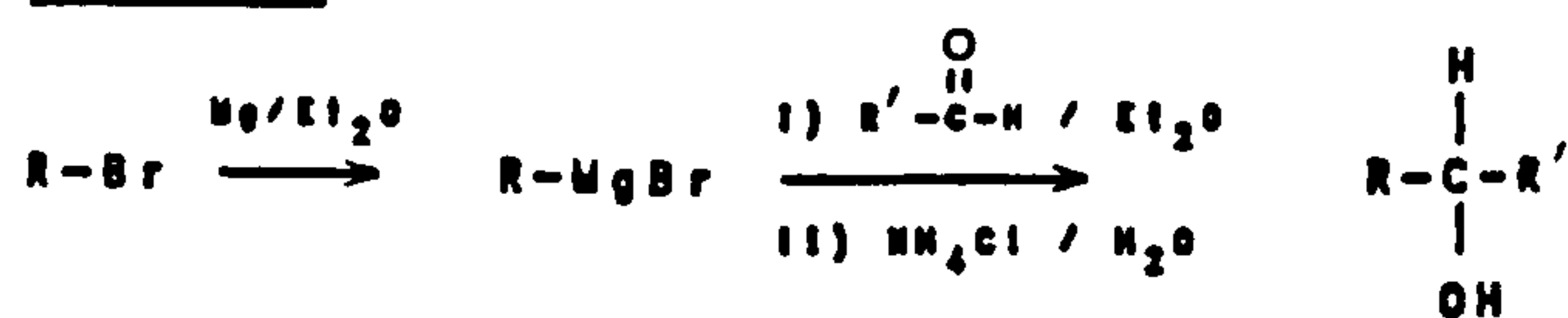
9-(2-PHENYLETHYL)-HEPTADECANE

$R = C_7H_{15}$

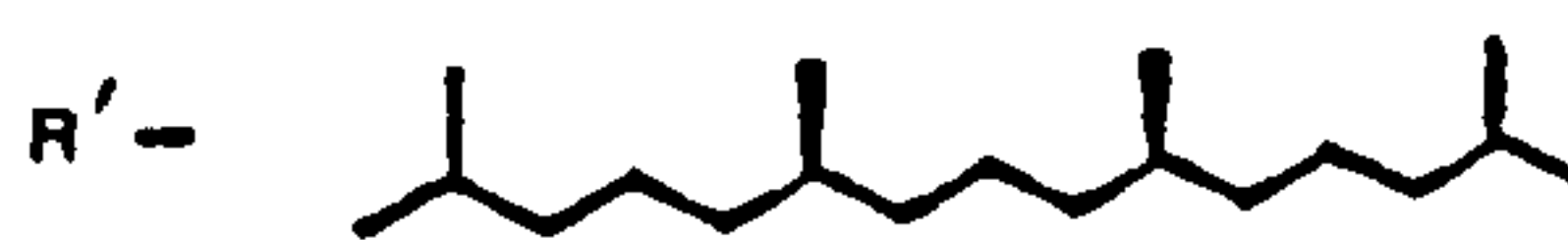
$R = C_7H_{15}$



SCHEME C:



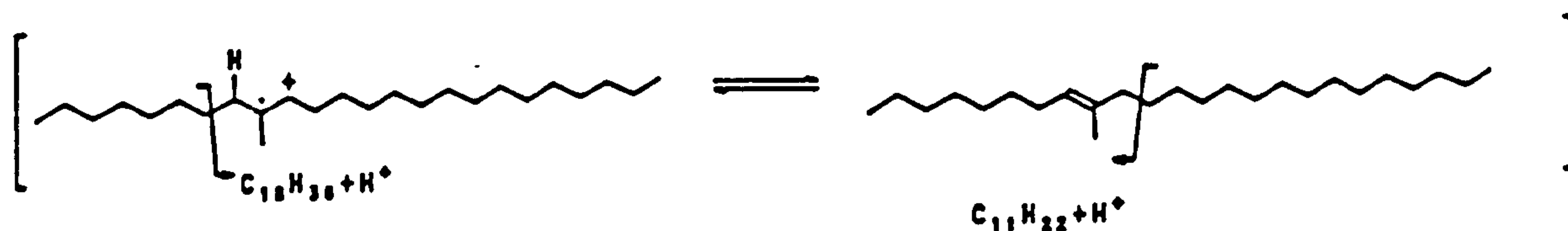
2.5.10.14.18-PENTAMETHYLEICOSANE



sodium-dried Et₂O in a nitrogen atmosphere (Scheme A) proved unsuccessful. This was presumably due to the presence of trace quantities of water which prevented formation of the Grignard reagent. As a result all subsequent Grignard coupling reactions were modified by the use of scrupulously dried Et₂O (Na; active alumina, redistilled over LiAlH₄) and a dried (CaCl₂) argon stream. Use of these procedures produced the desired alcohol, 9-methyltetracosan-9-ol (3), in moderate yield (36%). LRMS of the tertiary alcohol (Fig. 4.2) showed the M⁺·-H₂O ion (m/z 350) typical of aliphatic alcohols (McLafferty, 1978), and intense ions at m/z 353, m/z 255, and m/z 157 indicative of α-cleavage about the tertiary centre. These confirmed the position of the hydroxyl group at C-9.

The alcohol (3) was dehydrated using POCl₃ in pyridine (Rinehart and Perkins, 1963), and the resulting product was found to comprise the expected mixture of five isomeric 9-methyltetracosenes (4) when examined by GC (Fig. 4.3) and GC-MS. Due to virtually indistinguishable EI mass spectra however, positional and geometrical isomers could not be differentiated by MS alone. In addition, each spectrum showed evidence of double bond migration, as exhibited by the most abundant isomer (Fig. 4.4). Thus the intense m/z 253 (C₁₈H₃₇) and m/z 155 (C₁₁H₂₃) ions are presumably derived from allylic cleavage (with H-transfer) of a resonance intermediate resulting from 1,3 H-transfer and migration of the double bond (McLafferty, 1978; Loudon and Maccoll, 1970).

i.e.



Hydrogenation of the alkene intermediates (4, PtO_2 ; hexane) proceeded smoothly with the formation of the alkane, 9-methyltetracosane (5) plus residual unreacted alkenes. These were subsequently removed by argentatious TLC, and the purified alkane was examined by GC and GC-MS. The EI mass spectrum (Fig. 4.5) showed ions characteristic of $M^+ - CH_3$ (m/z 337), and $C_{10}H_{20}/C_{10}H_{21}$ (m/z 140/141), $C_{15}H_{30}/C_{15}H_{31}$ (m/z 210/211) and $C_{17}H_{34}/C_{17}H_{35}$ (m/z 238/239) ions derived from α -cleavage about the branch position. This confirms the methyl group position at C-9. The doublet even:odd ions are typical of branched alkanes and are thought to arise from secondary H-transfer involving a cyclic intermediate (McCarthy *et al.*, 1968). Further characterisation of the alkane was made by CI-MS (isobutane), and the mass spectrum (Fig. 4.6) showed the expected quasi-molecular $(M-H)^+$ ion (m/z 351), and abundant fragment ions derived from α -cleavage at the branch position.

Coinjection of the synthetic alkane (5) with the urea adduct of the Silkolene 150 lubricating oil alkanes (Section 3.1.2) confirmed the proposed nature of the major components as a homologous series of internally branched monomethyl alkanes (Fig. 4.7).

FIG. 4.2 EI MASS SPECTRUM OF SYNTHETIC 9-METHYLTETRACOSAN-9-OL

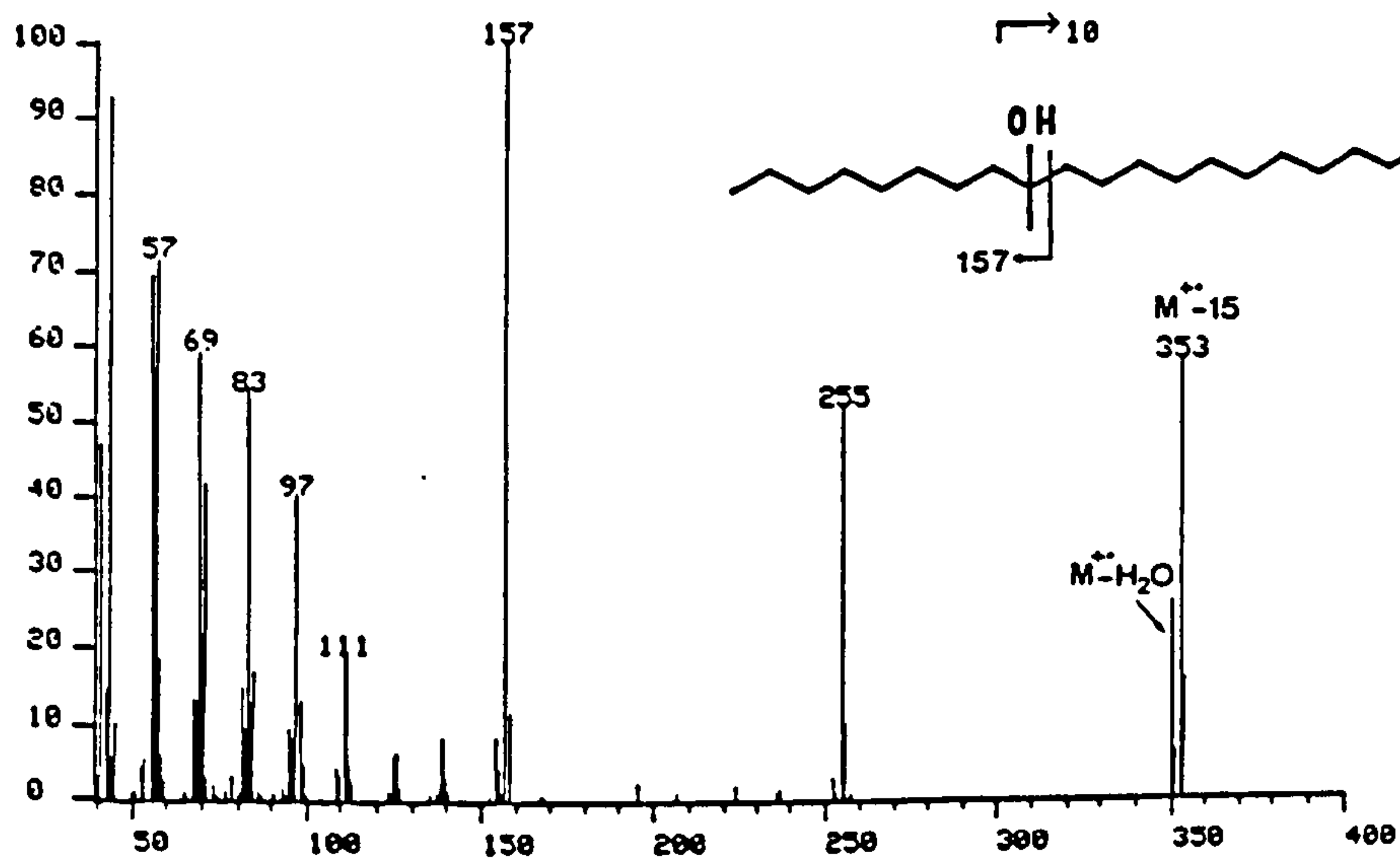
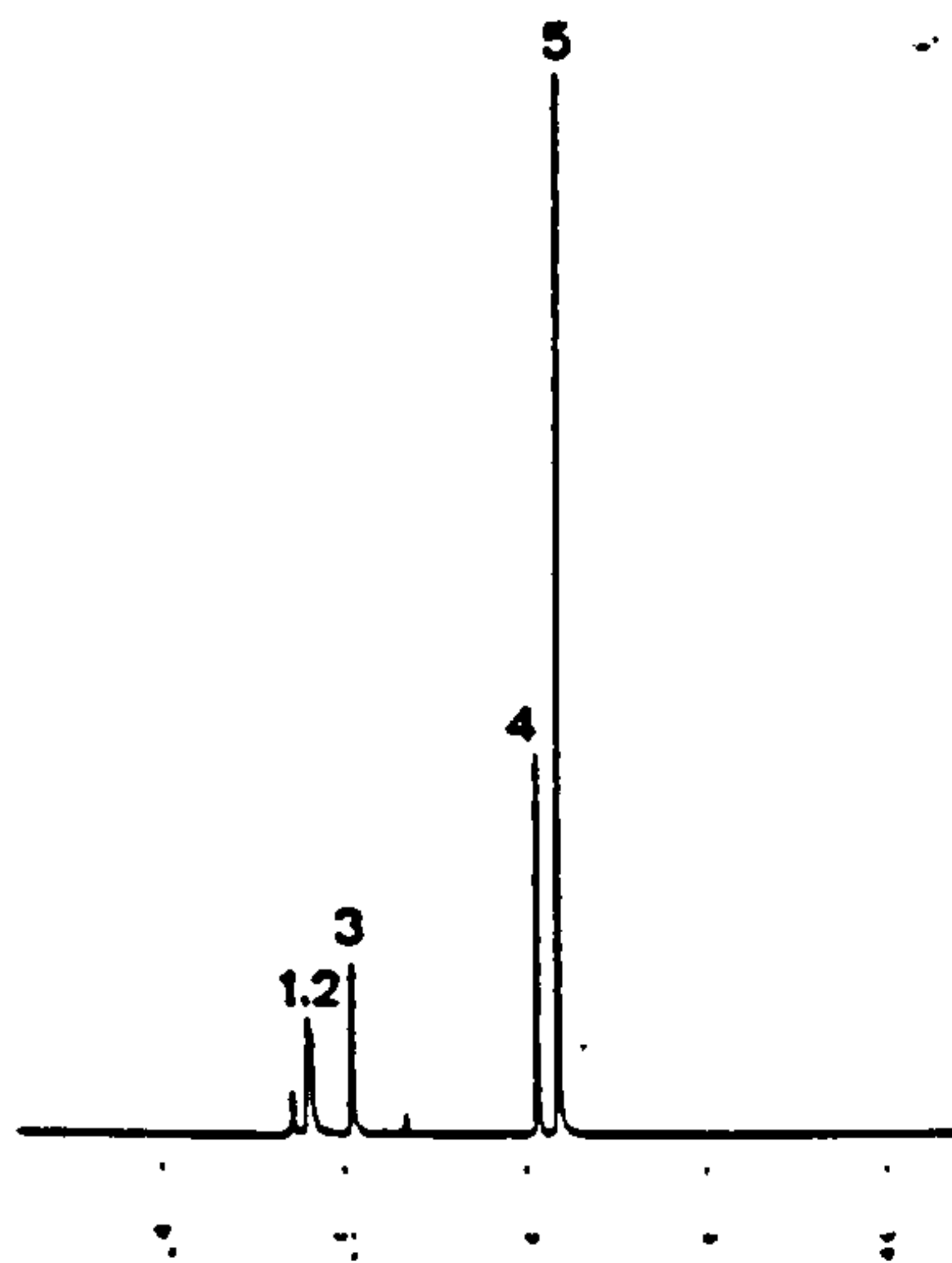


FIG. 4.3 PARTIAL GAS CHROMATOGRAM OF DEHYDRATION (POCl₃) PRODUCTS OF 9-METHYLTETRACOSAN-9-OL

[GC: SE-54(CARLO ERBA), 25m, 50-300 °C @ 10 °C min⁻¹]



(isomeric 9-methyltetracosenes)

FIG. 4.4 EI MASS SPECTRUM OF SYNTHETIC 9-METHYLTETRACOSENES

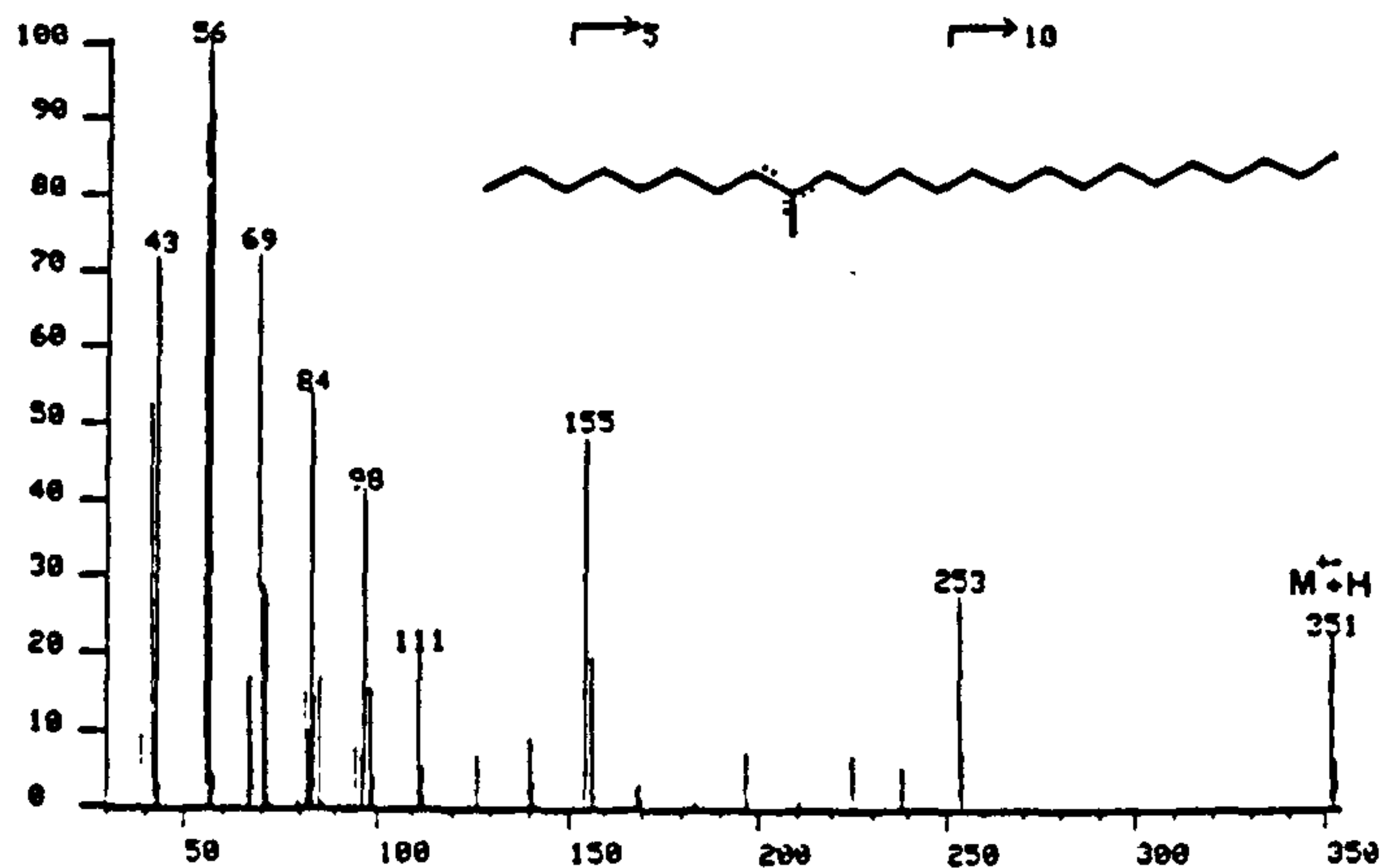


FIG. 4.5 EI MASS SPECTRUM OF SYNTHETIC 9-METHYLTETRACOSANE

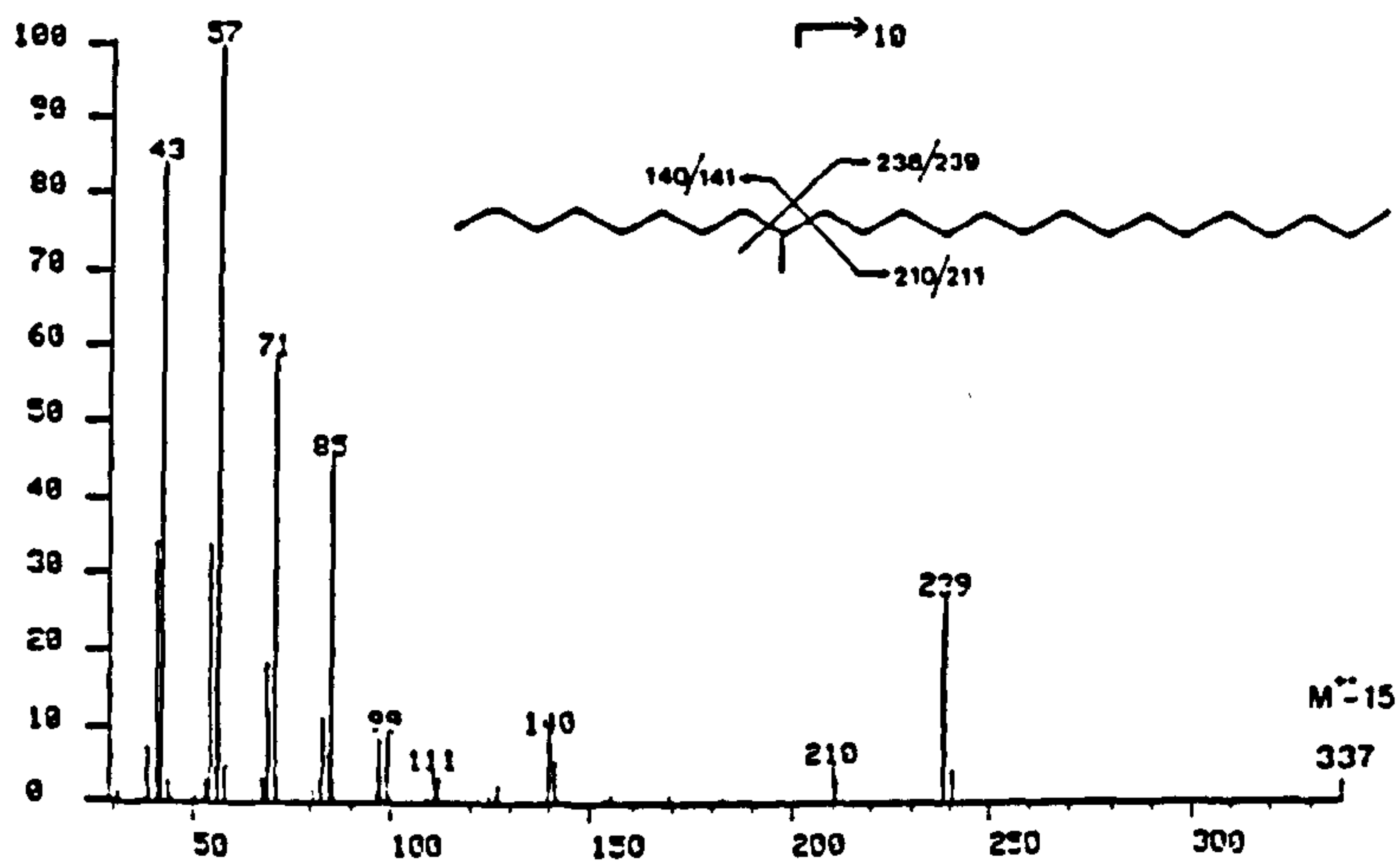


FIG. 4.6 CI (ISOBUTANE) MASS SPECTRUM OF SYNTHETIC 9-METHYLTETRACOSANE

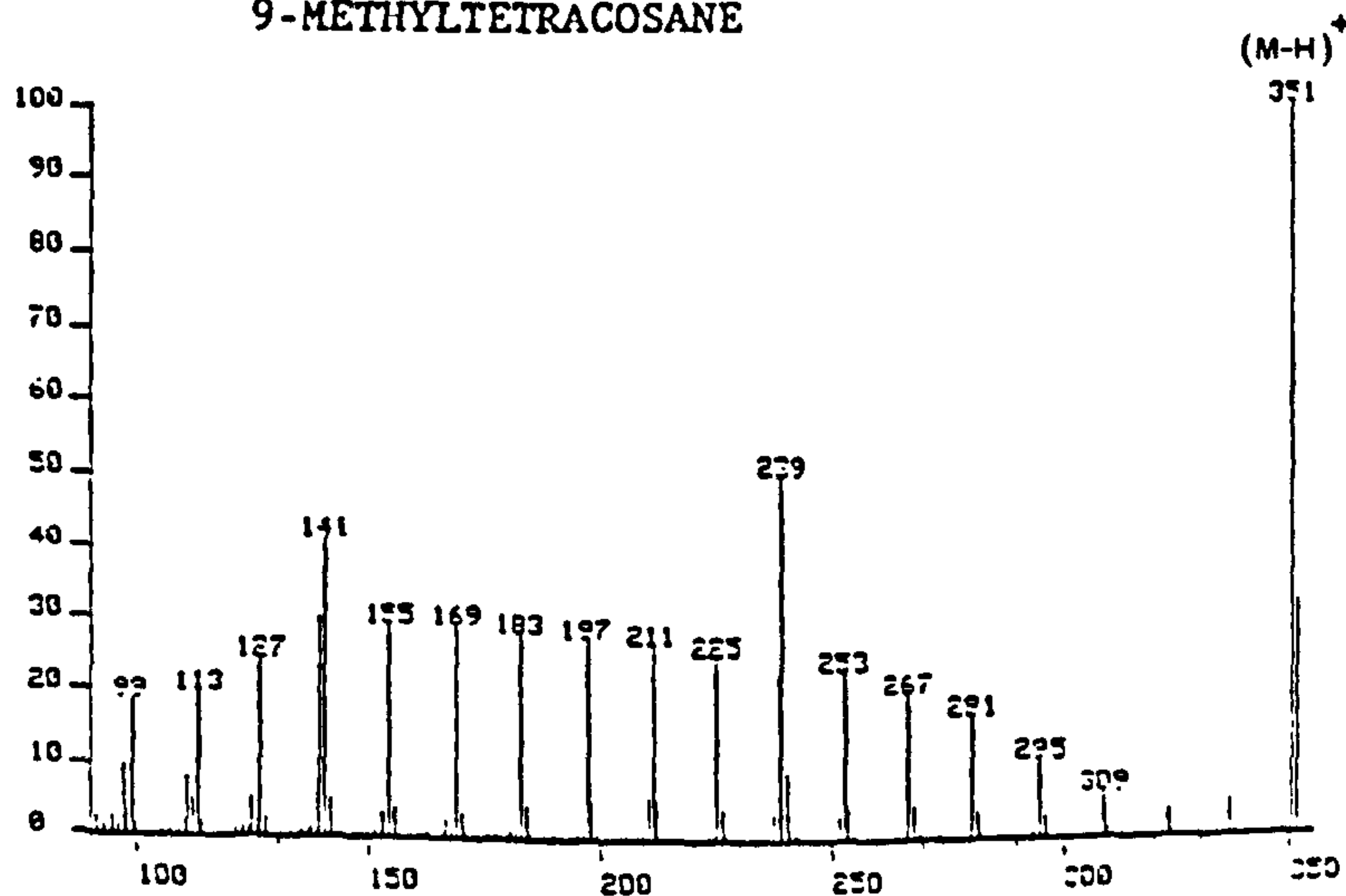
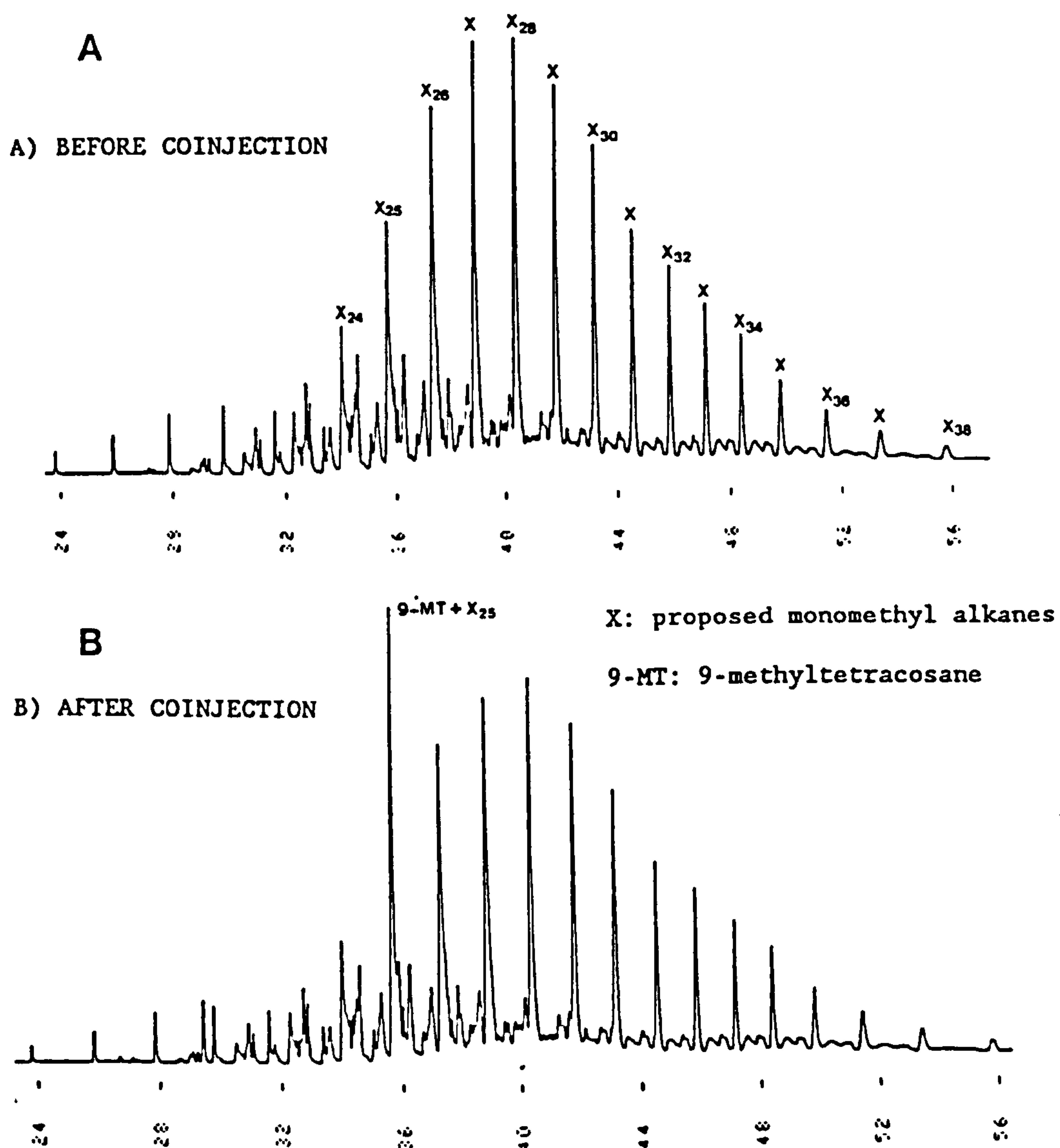


FIG. 4.7 COINJECTION OF 9-METHYLTETRACOSANE WITH THE UREA
ADDUCT-SILKOLENE 150 LUBE OIL, ALIPHATIC FRACTION.

[GC: OV-1(GC²), 25m, 50-300 °C @ 5 °C min⁻¹, 300°C (20 min)]



(N.B. The broad nature of these peaks is due to the coelution
of positional isomers)

4.1.3 7-N-HEXYLNONADECANE

The crude reaction products formed from the Grignard coupling of 1-bromododecane (6) and tridecan-7-one (7) were analysed by GC and GC-MS (Fig. 4.8) and found to comprise a poorly resolved triplet of isomeric alkenes and the "Wurtz" coupling product n-tetracosane. The latter is a common by-product of Grignard reactions and is formed by reaction between the Grignard reagent and residual alkylhalide (Kharasch and Reinmuth, 1954), i.e. $\text{RMgX} + \text{RX} \longrightarrow \text{R}_2 + \text{MgX}$.

Attempts to silylate (BSTFA) the total reaction product mixture proved unsuccessful and IR of the total products did not reveal any absorbance indicative of a hydroxyl group. Instead, a peak was observed at 1670 cm^{-1} typical of an alkene [$\nu(\text{C}=\text{C})$], which suggested the product alcohol had undergone dehydration, presumably during hydrolysis. Again, this is a commonly observed side reaction in Grignard additions (Kharasch and Reinmuth, 1954). LRMS of the major products confirmed their identity as isomeric 7-n-hexylnonadecenes (8, Fig. 4.9).

The alkenes (8) were purified from the Wurtz product (n-C₂₄) by urea adduction, then smoothly hydrogenated ($\text{PtO}_2 \cdot \text{H}_2\text{O}$, hexane) to the alkane, 7-n-hexylnonadecane (9) in moderate yield (53%). The EI mass spectrum showed the expected molecular ion (m/z 352), and fragment ions m/z 182/183 ($\text{C}_{13}\text{H}_{26}/\text{C}_{13}\text{H}_{27}$) and m/z 226/267 ($\text{C}_{19}\text{H}_{38}/\text{C}_{19}\text{H}_{39}$) derived from α -cleavage at the branch position (Fig. 4.10). CI-MS confirmed the EI data, with the quasi-molecular ion as the base peak (m/z 351) and abundant fragment ions generated from cleavage at the

FIG. 4.8 PARTIAL RECONSTRUCTED ION CHROMATOGRAM OF THE PRODUCTS OF THE GRIGNARD COUPLING OF 1-BROMODODECANE AND TRIDECAN-7-ONE

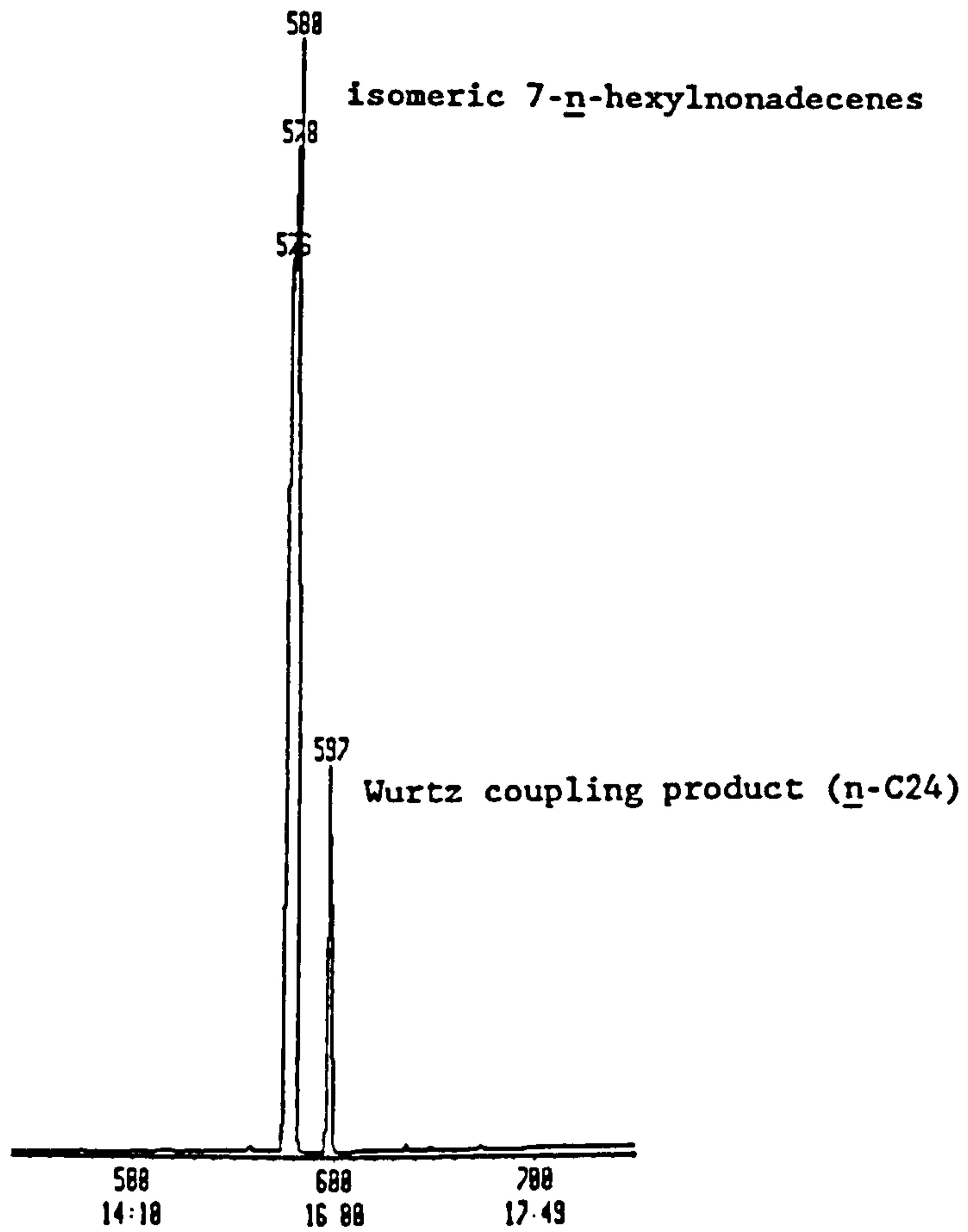


FIG. 4.9 EI MASS SPECTRUM OF SYNTHETIC 7-N-HEXYLNONADECENES

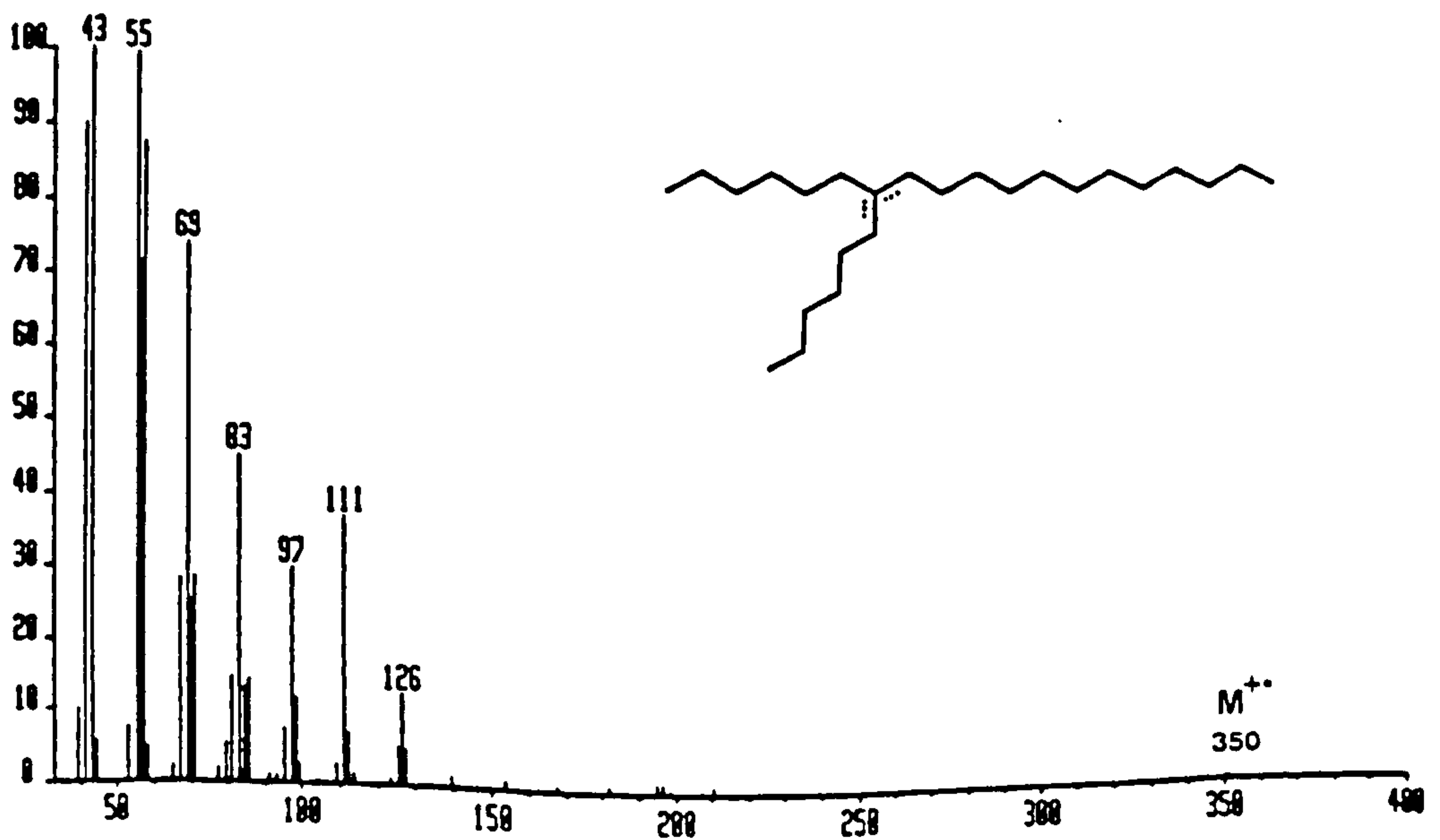


FIG. 4.10 EI MASS SPECTRUM OF SYNTHETIC 7-N-HEXYLNONADECANE

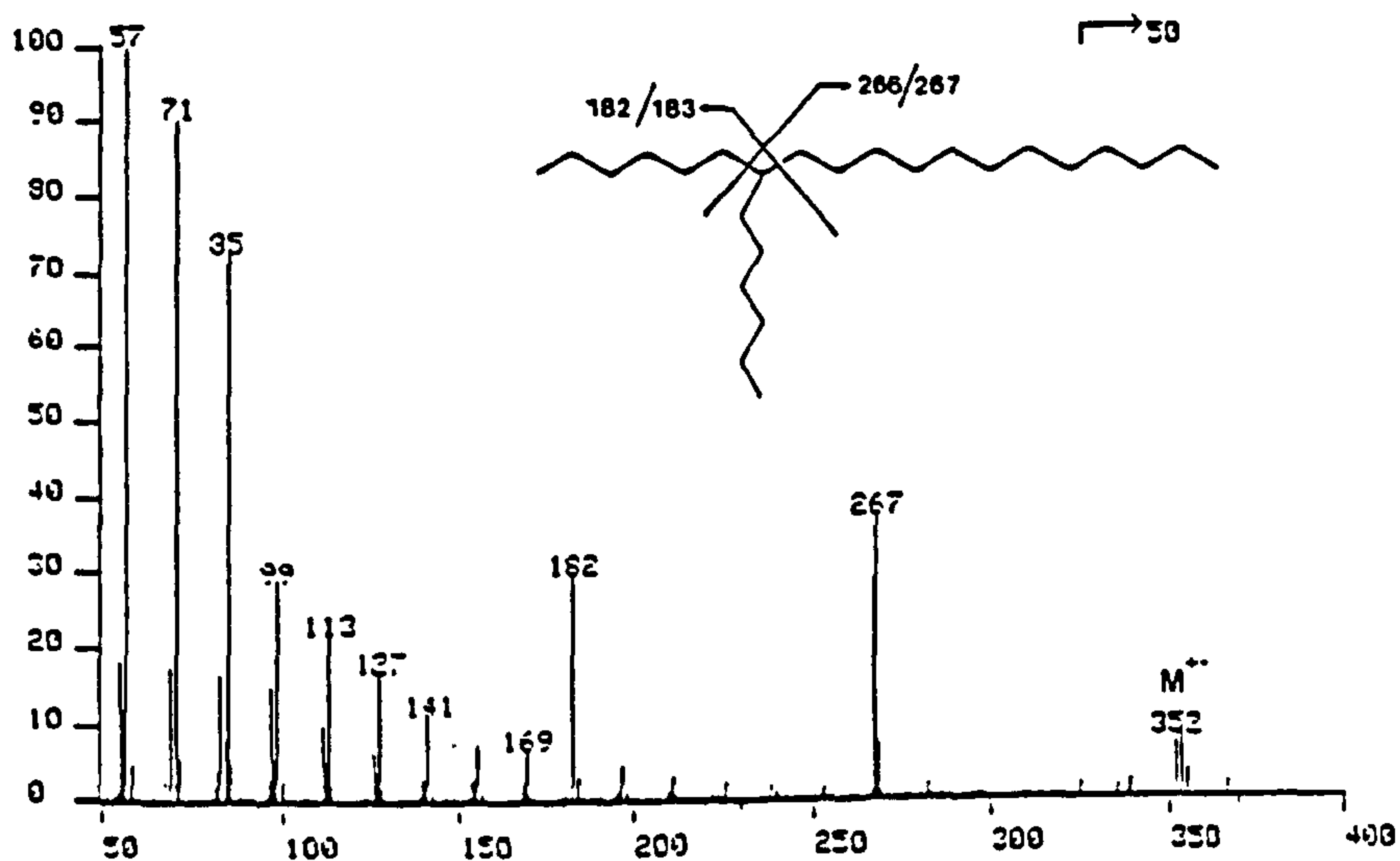
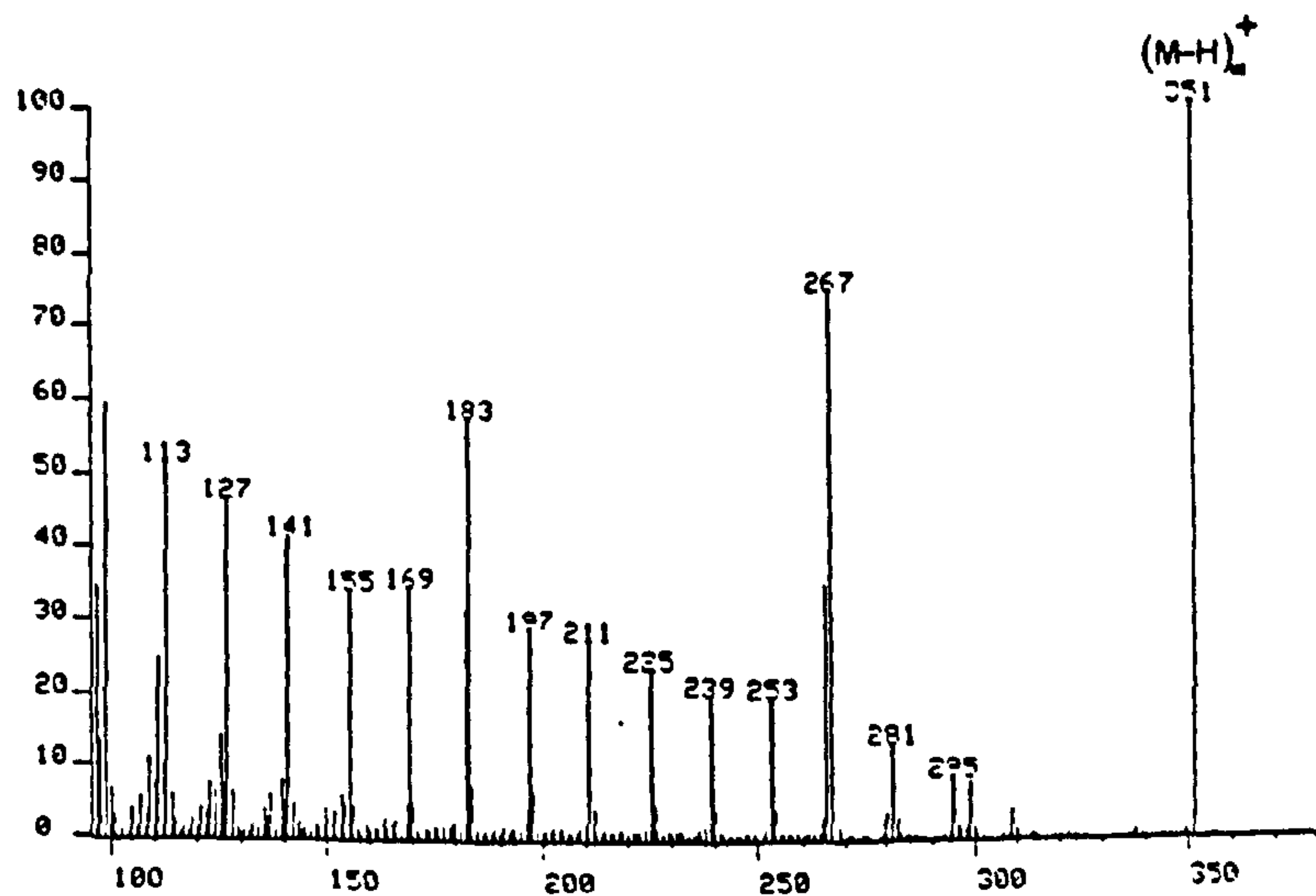


FIG. 4.11 CI (ISOBUTANE) MASS SPECTRUM OF SYNTHETIC 7-N-HEXYLNONADECANE



tertiary centre (Fig. 4.11).

4.1.4 2-METHYLTETRACOSANE

The intermediate alcohol 2-methyltetracosan-2-ol (10) was provided from a separate study (A. Tonkin, personal communication), which involved coupling acetone with 1-bromodocosane in a Grignard reaction. Dehydration ($\text{POCl}_3/\text{pyridine}$) herein followed by hydrogenation ($\text{PtO}_2 \cdot \text{H}_2\text{O}/\text{hexane}$) and purification by argentatious TLC (R_f : 0.87-0.99) provided the alkane, 2-methyltetracosane (12), the EI mass spectrum of which (Fig. 4.12) was consistent with the proposed structure [m/z 352 (M^+), m/z 337 ($\text{M}^+ - \text{CH}_3$), m/z 309 ($\text{M}^+ - \text{C}_3\text{H}_7$)].

4.1.5 9-(2-CYCLOHEXYLETHYL)-HEPTADECANE

The synthesis of 9-(2-cyclohexylethyl)-heptadecane (18) involved coupling two molar equivalents of the alkylhalide 1-bromooctane (14) with one molar equivalent of the methyl ester of cyclohexylpropanoic acid (13) via reaction scheme B (Fig. 4.1). The ester (15) was formed in good yield (74%) from the carboxylic acid (13) by mineral acid catalysed esterification in methanol (Vogel, 1978). The EI mass spectrum (Fig. 4.13) showed the molecular ion m/z 170, m/z 97 as the base peak (derived from α -cleavage) and ions indicative of a methyl ester functionality [m/z 87, m/z 74, m/z 139 ($\text{M}^+ - \text{OCH}_3$)].

The Grignard coupling of methylcyclohexylpropanoate (15) to two molar equivalents of 1-bromooctane (14) proceeded smoothly and on hydrolysis the desired alcohol 9-(2-cyclohexylethyl)-heptadecan-9-ol (16) was produced in moderate yield (39%). IR indicated the presence

FIG. 4.12 EI MASS SPECTRUM OF SYNTHETIC 2-METHYLTETRACOSANE

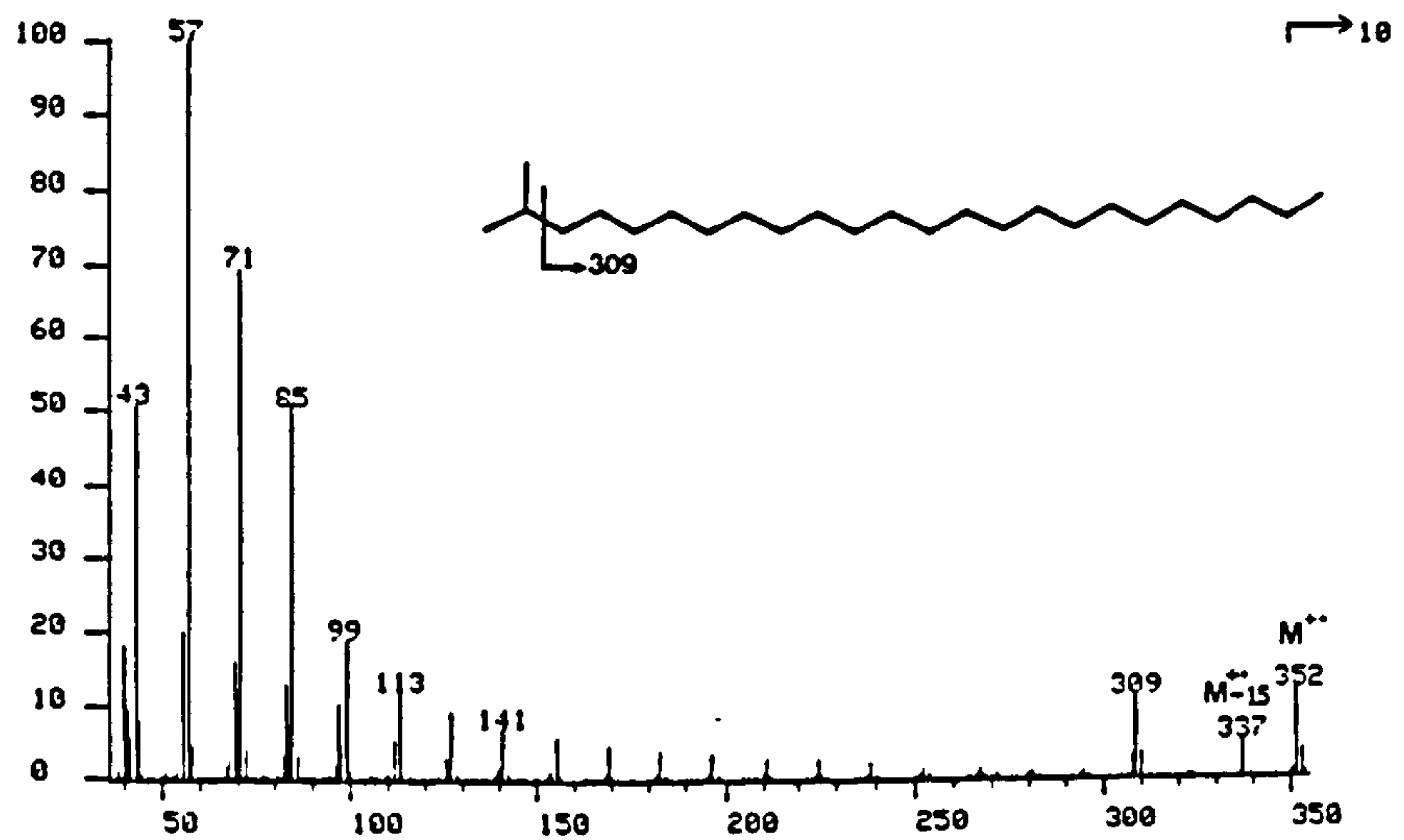
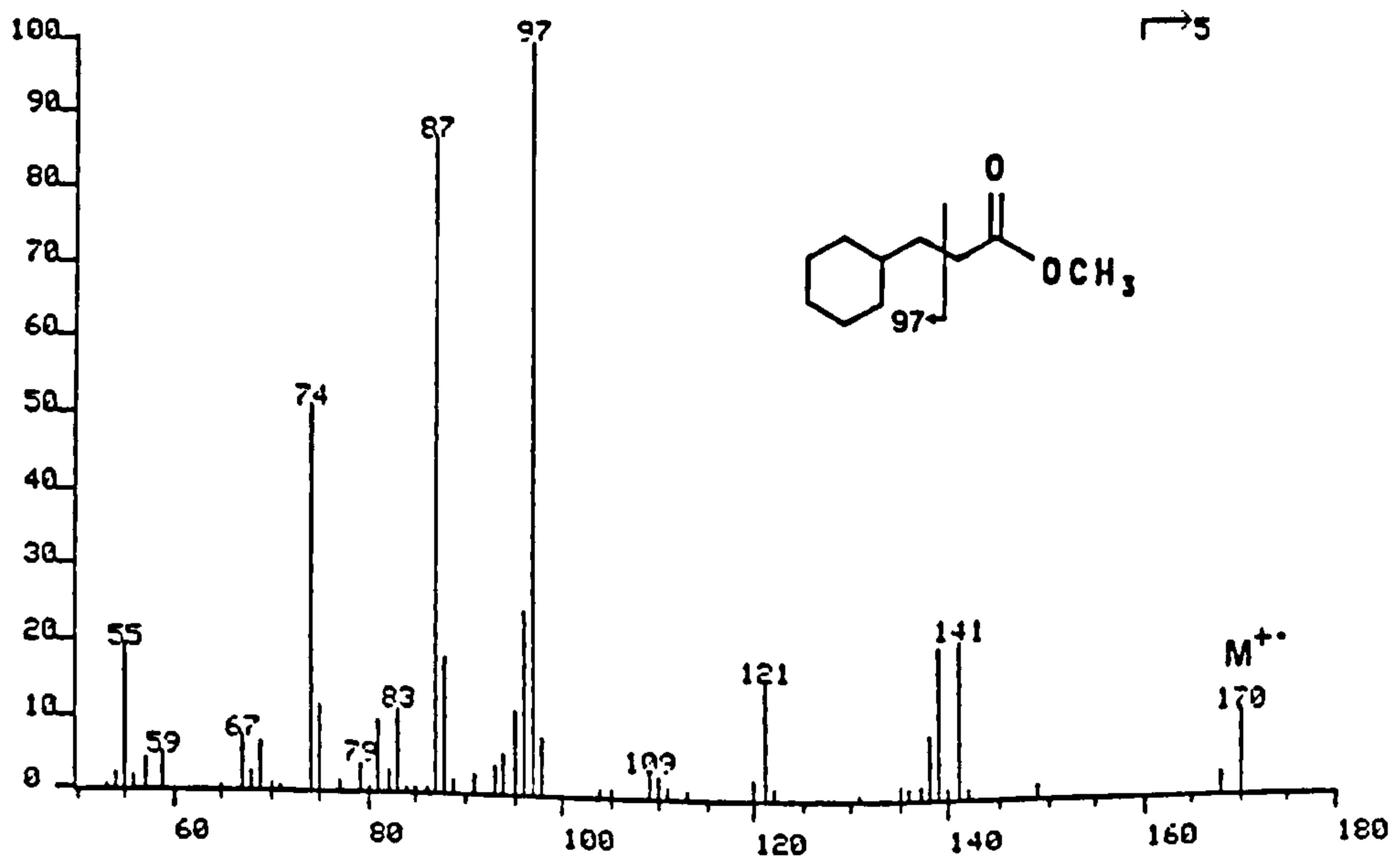
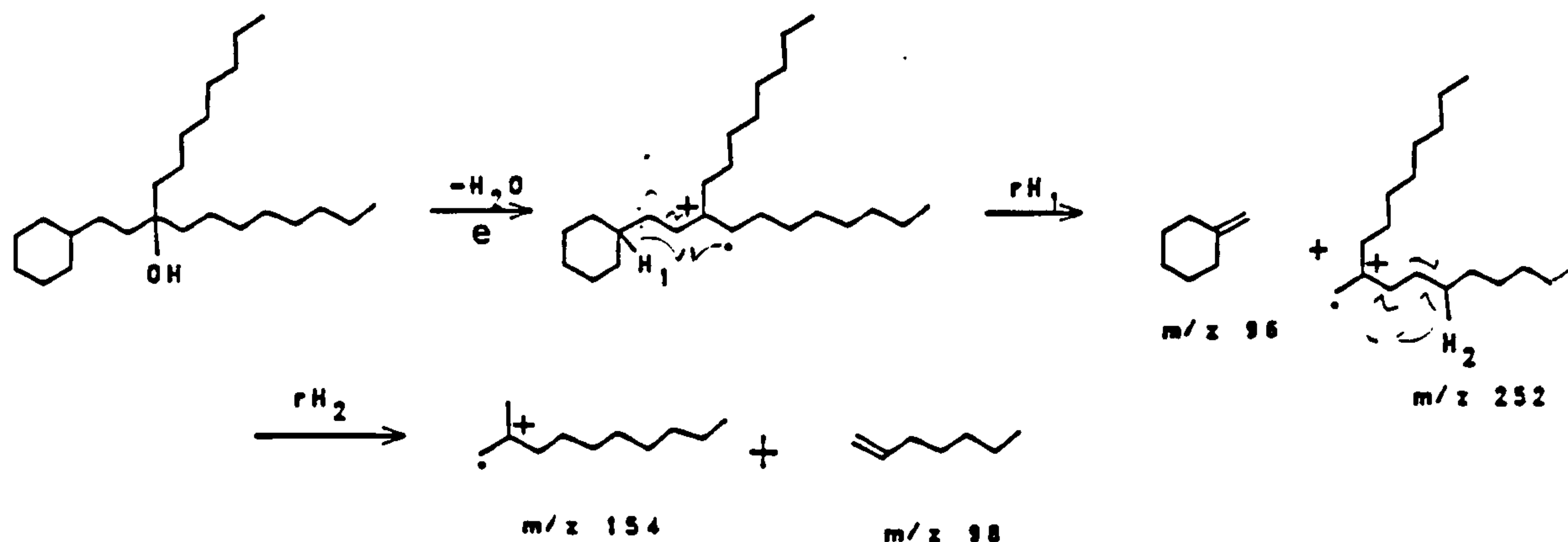


FIG. 4.13 EI MASS SPECTRUM OF SYNTHETIC METHYLCYCLOHEXYL PROPANOATE



of a H-bonded hydroxyl group ($\nu(\text{O-H})$: 3400cm^{-1}) and LRMS (Fig. 4.14) showed ions typical of an aliphatic alcohol. Thus m/z 348 is derived from loss of H_2O ($M^+ - 18$) and m/z 253 is derived from α -cleavage at the position of the hydroxyl group. The ions m/z 96 and m/z 154 presumably originate from a double McLafferty rearrangement of dehydrated molecular ion, i.e.



The dehydration ($\text{POCl}_3/\text{pyridine}$) products of the tertiary alcohol (16) were found to comprise a mixture of three compounds when analysed by GC (Fig. 4.15A). GC-MS confirmed the structure of the major compound (Y, Fig. 4.15A) as a (possible) mixture of 9-(2-cyclohexylethyl)-heptadecenes (17). Thus the EI mass spectrum (Fig. 4.16) showed comparable ions to those observed for the precursor alcohol (Fig. 4.14), with m/z 96, 252 and 154 being generated by analogous McLafferty-type H atom rearrangements, i.e. as above.

Compound W (Fig. 4.15A) showed a near-identical EI mass spectrum to compound X (Fig. 4.17A and B) with a molecular ion (m/z 236) indicative of a C_{17} monocyclic alkene with one degree of unsaturation. A possible structure is 1-cyclohexylundec-3-ene, which via a McLafferty γ -H rearrangement would produce the observed m/z 96 ion, i.e.

FIG. 4.14 EI MASS SPECTRUM OF SYNTHETIC 9-(2-CYCLOHEXYLETHYL)-HEPTADECAN-9-OL

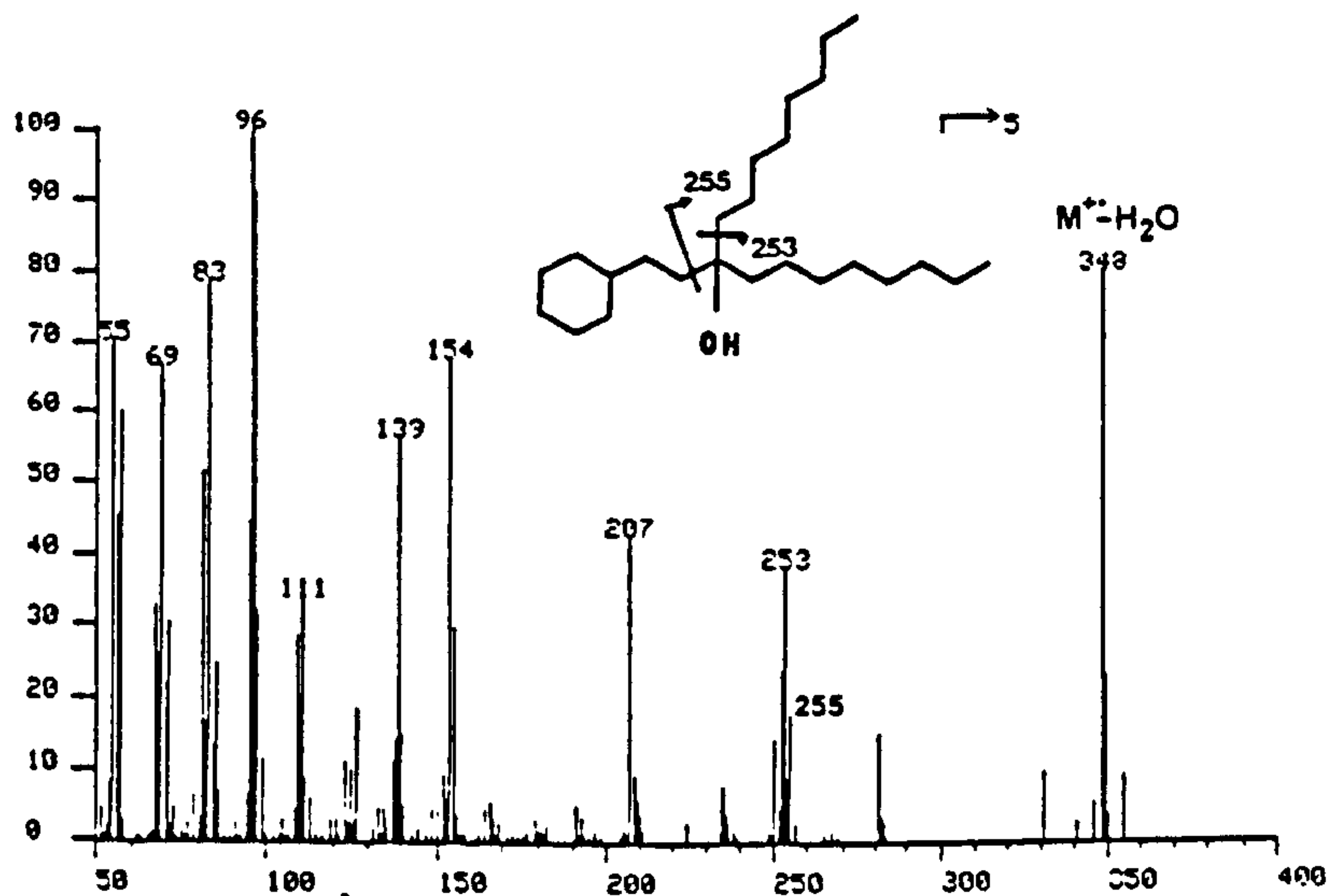
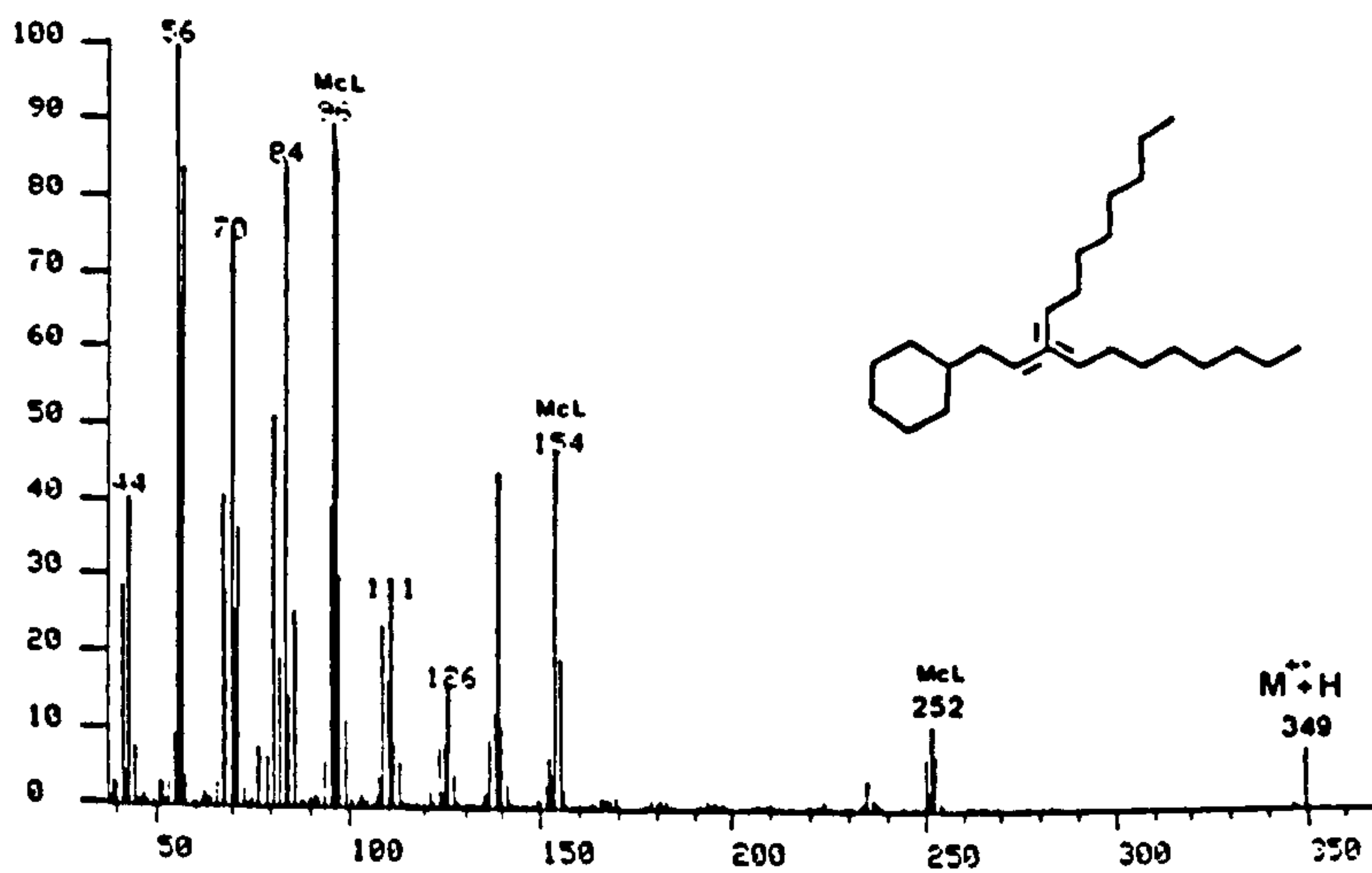


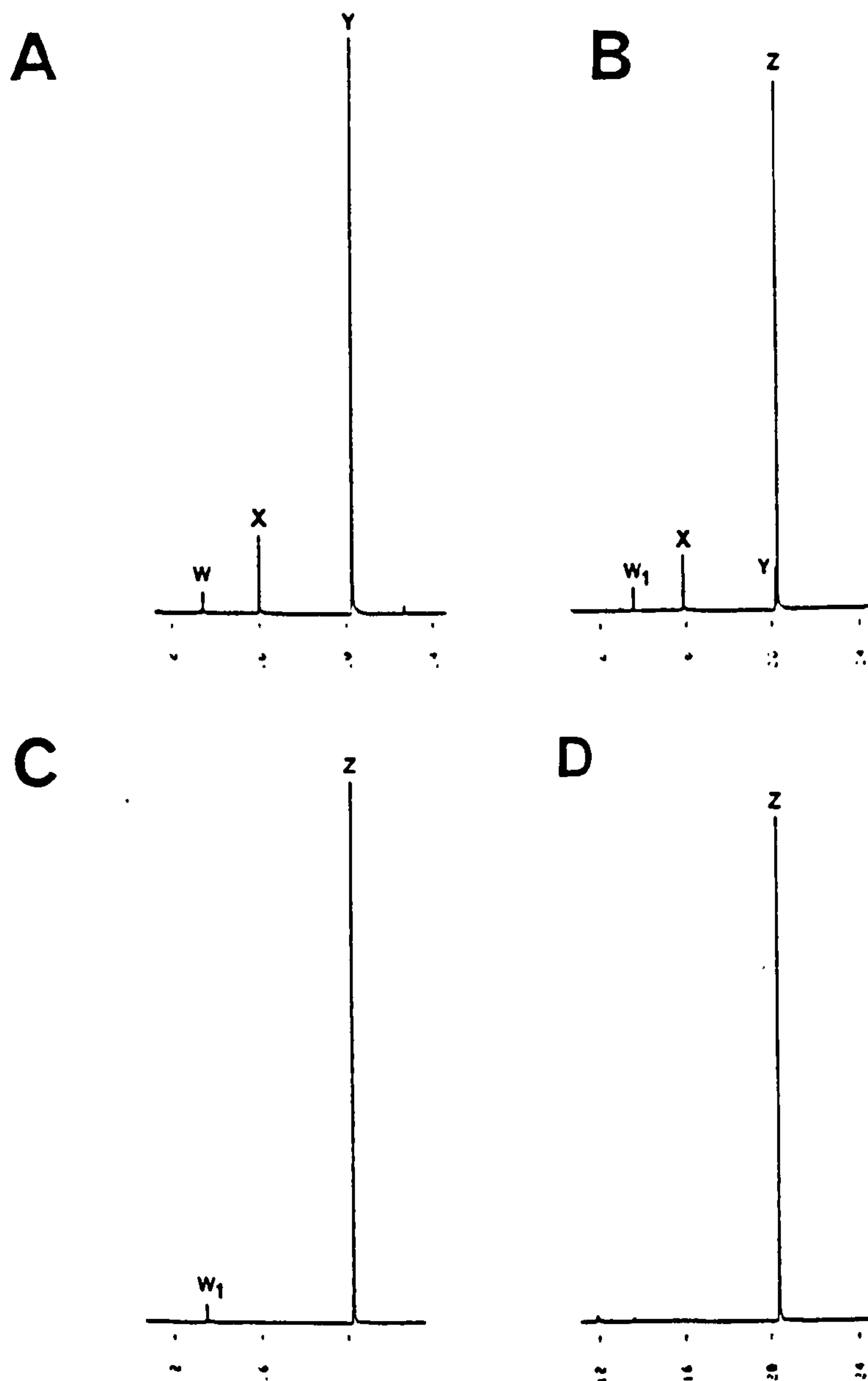
FIG. 4.16 EI MASS SPECTRUM OF SYNTHETIC 9-(2-CYCLOHEXYLETHYL)-HEPTADECENES



(McL: denotes McLafferty rearrangement ions)

FIG.4.15 PARTIAL GAS CHROMATOGRAMS FOR THE DEHYDRATION ,
HYDROGENATION, AND PURIFICATION OF 9-(2-CYCLOHEXYLETHYL)-
HEPTADECANE

[GC: SE-54(CARLO ERBA), 25m, 50-300 °C @ 10 °C min⁻¹]

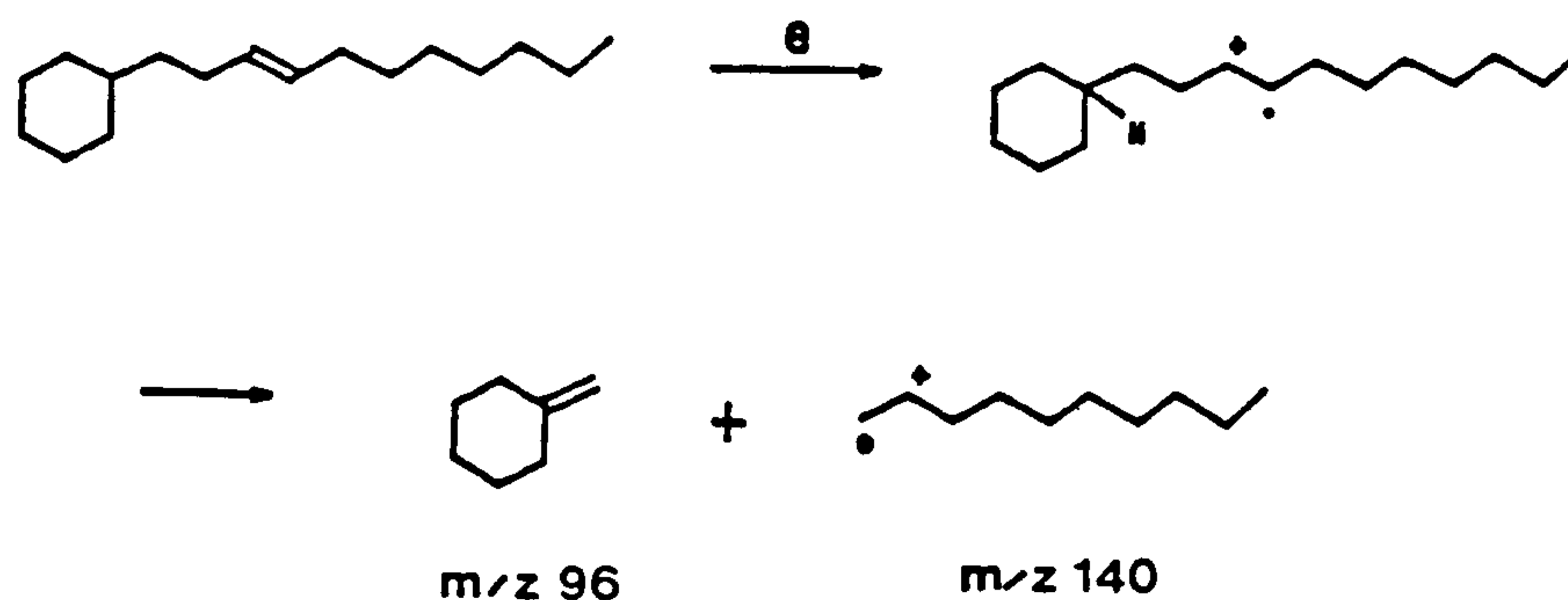


A) DEHYDRATION PRODUCTS OF 9-(2-CYCLOHEXYLETHYL)-HEPTADECAN-
9-OL

B) HYDROGENATED (PtO₂/hexane) ALKENES

C) PURIFIED (Ag⁺ TLC) HYDROGENATION PRODUCTS

D) PURIFIED (THIOUREA NON ADDUCT) 9-(2-CYCLOHEXYLETHYL)-
HEPTADECANE



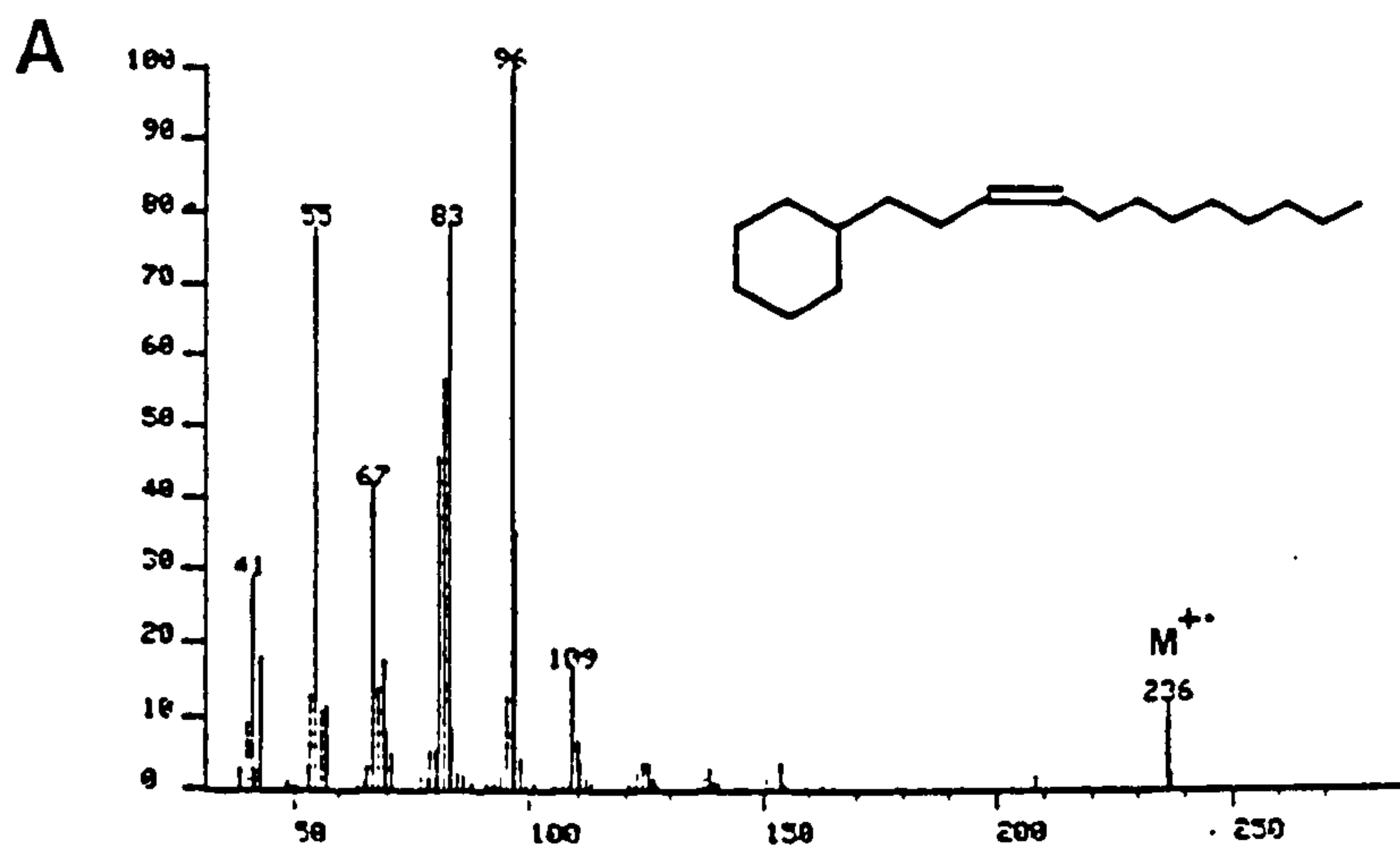
Compounds W and X therefore most likely represent geometric (i.e. cis and trans) isomers, though their derivation is unclear. They may originate as by-products of the dehydration procedure of the tertiary alcohol, or alternatively may have been present as an impurity (in some form) prior to dehydration. Capillary GC of the purified alcohol however did not reveal the presence of any additional component.

Upon hydrogenation ($\text{PtO}_2 \cdot \text{H}_2\text{O}$, hexane), 9-(2-cyclohexylethyl)-heptadecenes were reduced and a new compound was observed (compound Z, Fig. 4.15B). EI GC-MS confirmed this as the desired alkane, 9-(2-cyclohexylethyl)-heptadecane (18), the mass spectrum of which (Fig. 4.18) showed the expected molecular ion $m/z\ 350$ and ions characteristic of alkylmonocyclohexyl alkanes ($\text{C}_n\text{H}_{2n-1}$; e.g. $m/z\ 83, 97, 111$). The cluster of ions in the $m/z\ 237$ region (see insert, Fig. 4.18) are derived from cleavage about the branch position, the even ions being produced from H-transfer (McCarthy *et al.*, 1968).

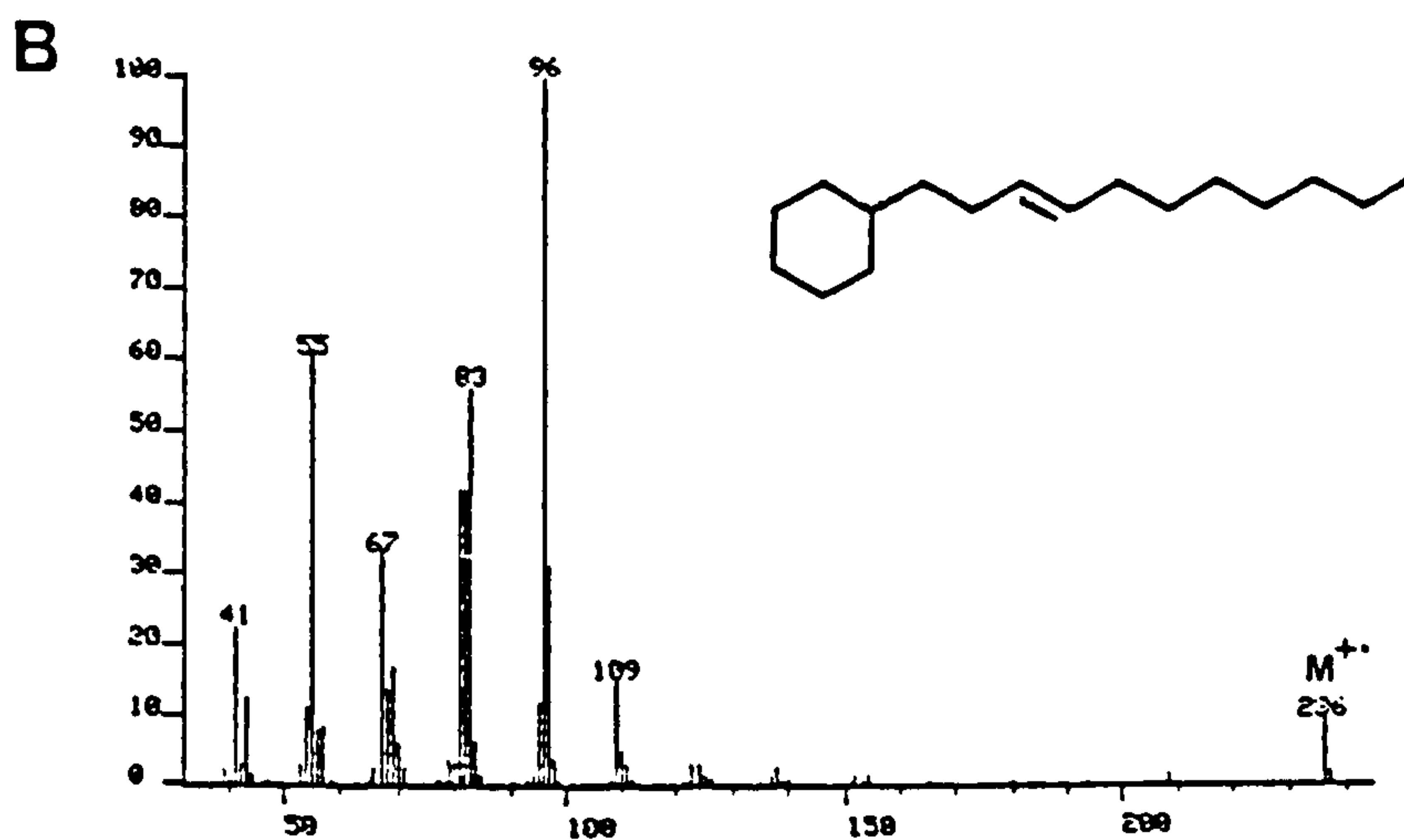
GC-MS also showed that compound W was hydrogenated to the fully saturated 1-cyclohexylundecane, whereas X remained unchanged. W

FIG. 4.17 EI MASS SPECTRA OF ISOMERIC DEHYDRATION PRODUCTS OF 9-(2-CYCLOHEXYLETHYL)-HEPTADECAN-9-OL

A) COMPOUND W: possible cis alkenyl cyclohexane



B) COMPOUND X: possible trans alkenyl cyclohexane



possibly represents therefore the cis isomer, since hydrogenation of the least sterically-hindered cis geometry is usually favoured (March 1987; Friefelder, 1971). Alternatively, X may represent a positional isomer of W, produced by double bond migration into the ring. Endocyclic double bonds are known to require more forcing conditions to hydrogenate than exocyclic double bonds (Friefelder, 1971). If this were the case however, their mass spectra would be expected to differ.

The alkane was purified from residual alkenes by silver ion TLC, the products of which when analysed by GC (Fig. 4.15C) were found to comprise (as expected) the two alkylcycloalkanes W and 18. Attempts to purify the branched cycloalkane by urea adduction were not successful, although thiourea adduction did result in a satisfactory reduction of the n-alkylcyclohexane. The resultant "T"-branched cyclohexane 9-(2-cyclohexylethyl)-heptadecane (20) was 97% pure by capillary GC (Fig. 4.15D).

4.1.6 9-(2-PHENYLETHYL)-HEPTADECANE

The synthesis of the monoaromatic hydrocarbon 9-(2-phenylethyl)-heptadecane (23) used the same approach as that described for the corresponding alkane (18) which is summarised in Fig. 4.1. The intermediate ester methyl 3-phenylpropanoate (20) was formed in good yield (75%) by mineral acid catalysed esterification of 3-phenylpropanoic acid (19) in methanol. The EI mass spectrum (Fig. 4.19) showed the required molecular ion (m/z 164), ions indicative of

FIG. 4.18 EI MASS SPECTRUM OF SYNTHETIC 9-(2-CYCLOHEXYLETHYL)-HEPTADECANE

(insert: expanded portion, m/z 235-240)

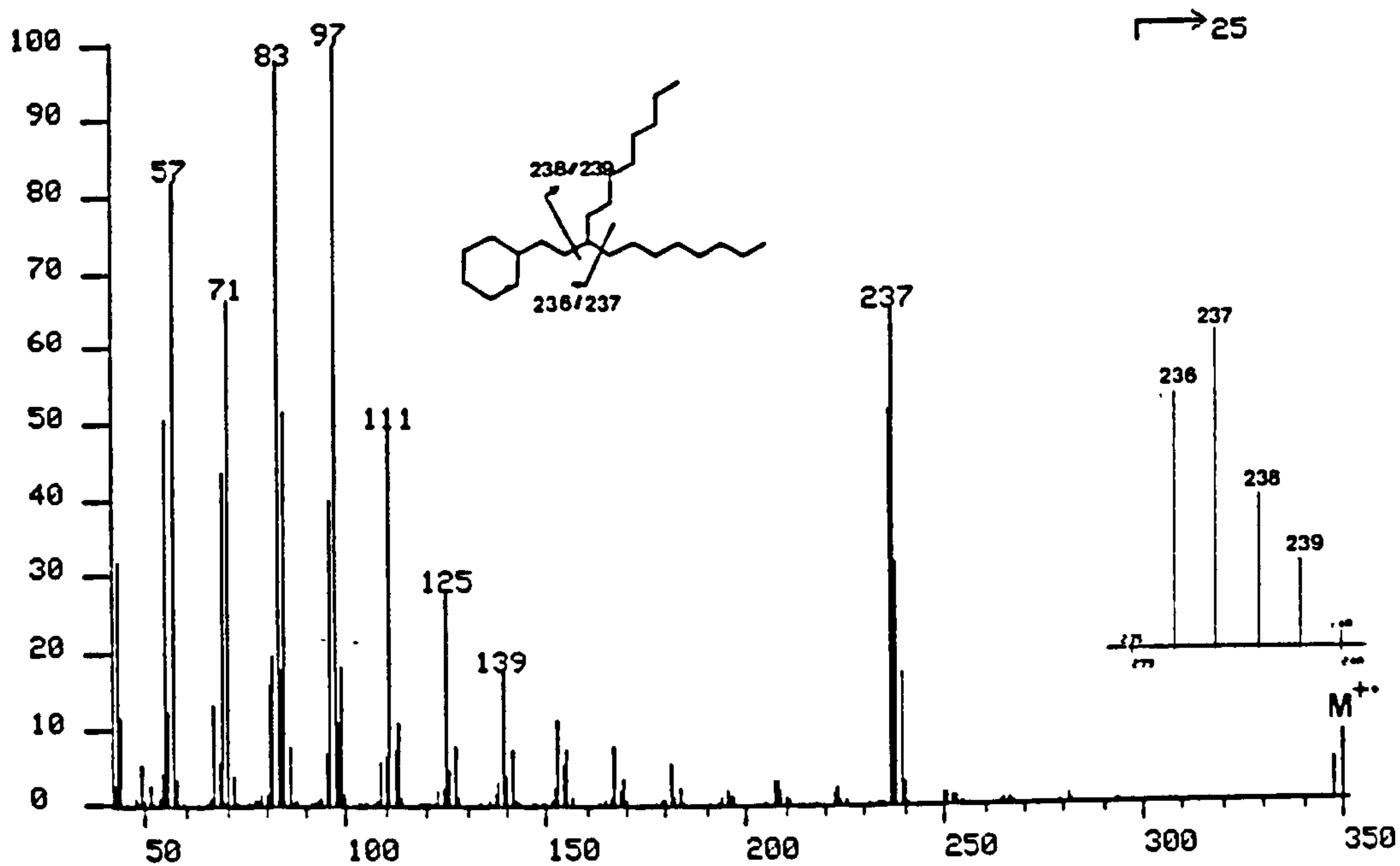
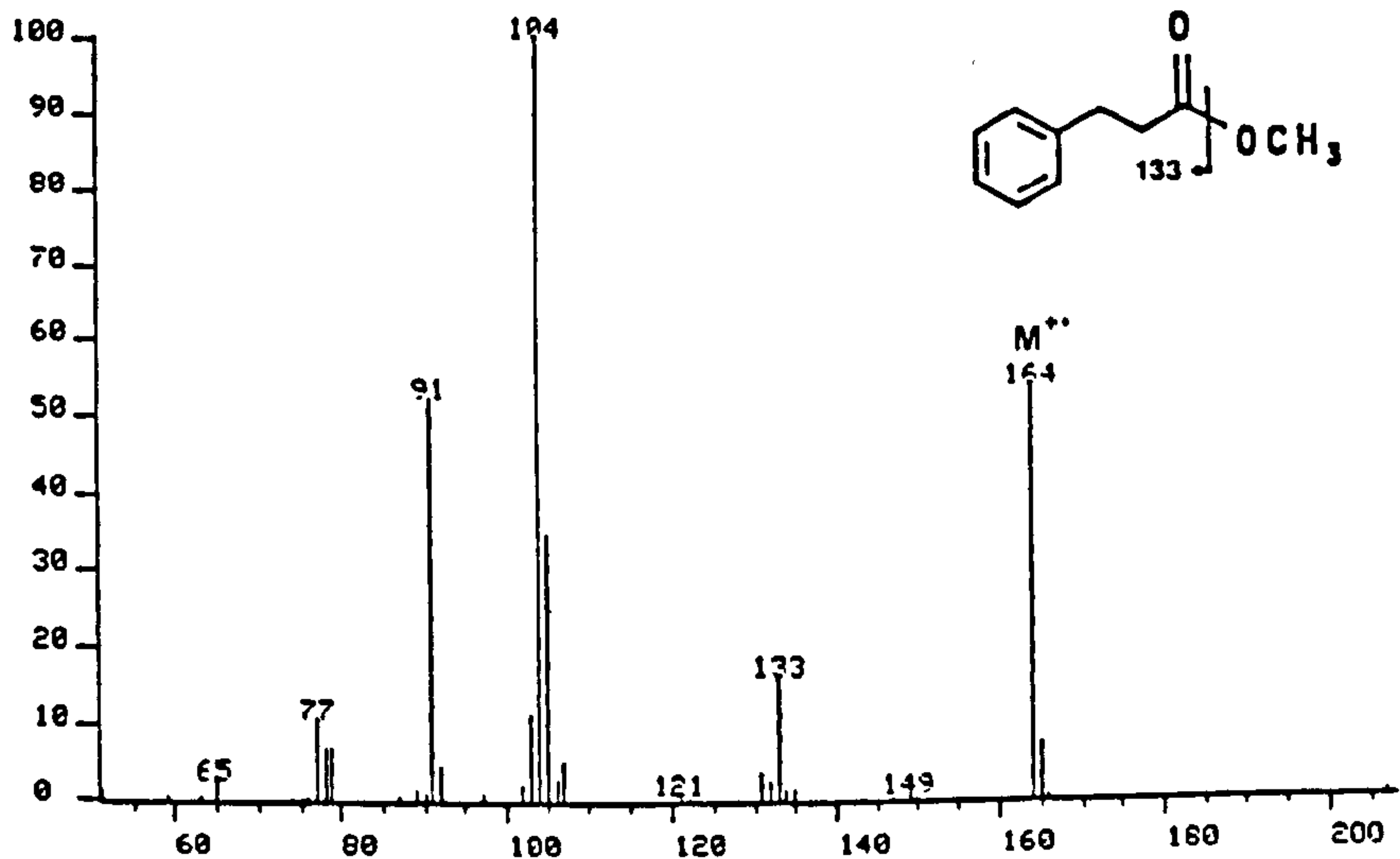


FIG. 4.19 EI MASS SPECTRUM OF SYNTHETIC METHYL 3-PHENYLPROPANOATE



a substituted benzene (m/z 77, 91) and $M^{\cdot+} \cdot \text{-CO}_2\text{CH}_3 + \text{H}$ (m/z 104) as the base peak.

The crude product of the Grignard reaction between methyl 3-phenylpropanoate (20) and two molar equivalents of 1-bromooctane (14) followed by hydrolysis ($\text{NH}_4\text{Cl}/\text{H}_2\text{O}$) was found to comprise four components when analysed by GC. GC-MS showed these to be residual alkylbromide and methyl ester, the Wurtz coupling product n-hexadecane and the desired alcohol 9-(2-phenylethyl)-heptadecan-9-ol (21). Initial attempts to purify the alcohol from residual methyl ester using a combination of column and thin layer chromatography were unsuccessful. Instead, hydrolysis of the mixture with methanolic KOH (5% w/v, 10cm^3) at room temperature followed by addition of water and extraction with DCM was found to remove all traces of residual ester as the acid salt. The resulting purified alcohol was assigned by IR and LRMS. IR confirmed the presence of a hydroxyl group ($\nu(\text{O-H}): 3400\text{ cm}^{-1}$) and the EI mass spectrum (Fig. 4.20) showed ions indicative of loss of water ($M^{\cdot+} \cdot -18$, m/z 342) and the aromatic ring (m/z 91, tropylium ion). Further ions were indicative of α -cleavage (m/z 255, $M^{\cdot+} \cdot \text{-C}_8\text{H}_9$; m/z 247, $M^{\cdot+} \cdot \text{-C}_8\text{H}_{17}$) which confirmed the position of the hydroxyl group at C-9. Other ions present in the EI mass spectrum could be attributed to loss of water followed by McLafferty type rearrangements of the resulting double bond (e.g. m/z 146; double McLafferty rearrangement). Evidence of double bond migration into conjugation with the aromatic ring was indicated by the presence of the m/z 229 ion, presumably derived from allylic cleavage without H-transfer, i.e.

FIG. 4.20 EI MASS SPECTRUM OF SYNTHETIC 9-(2-PHENYLETHYL)-HEPTADECAN-9-OL

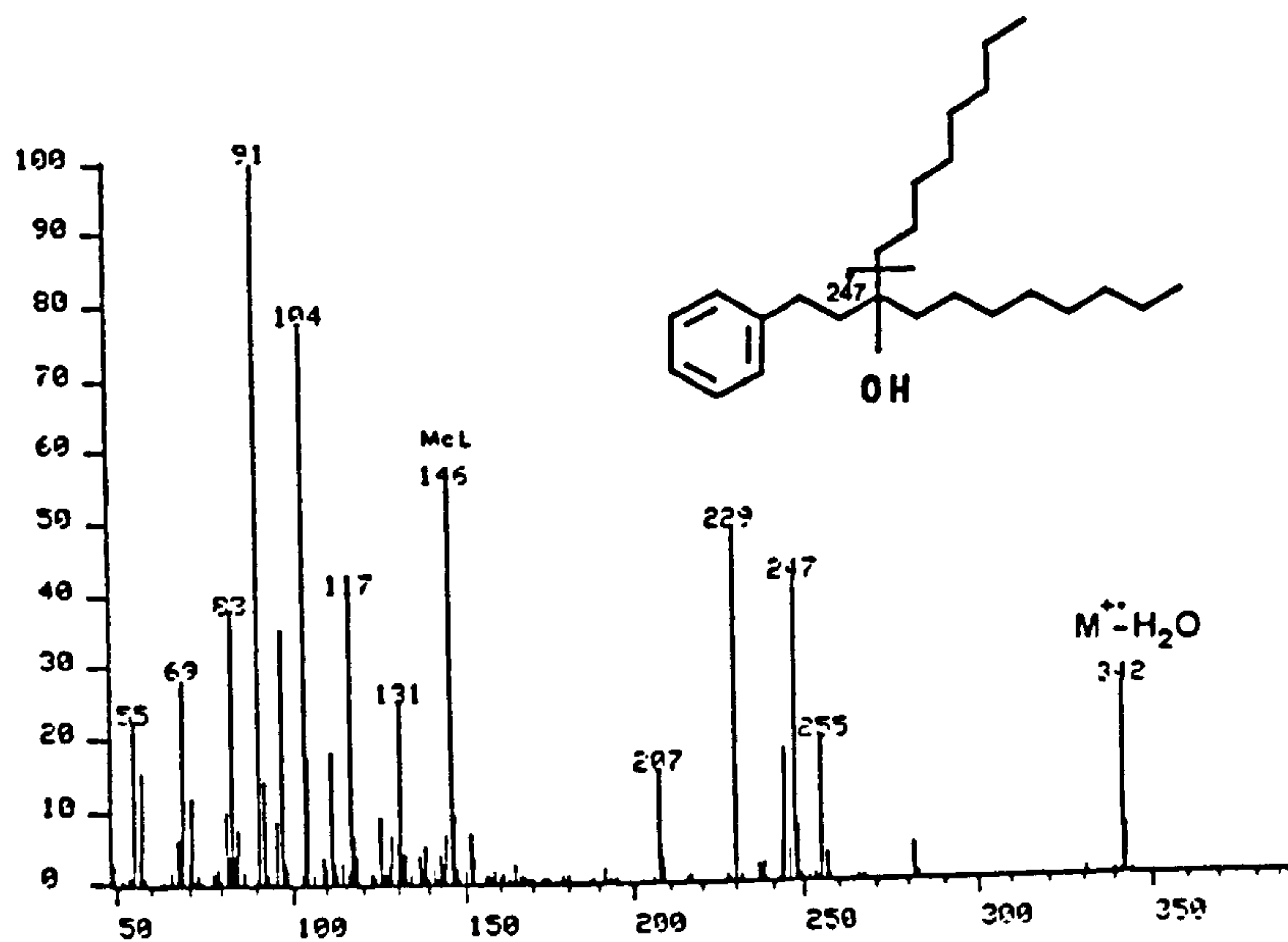
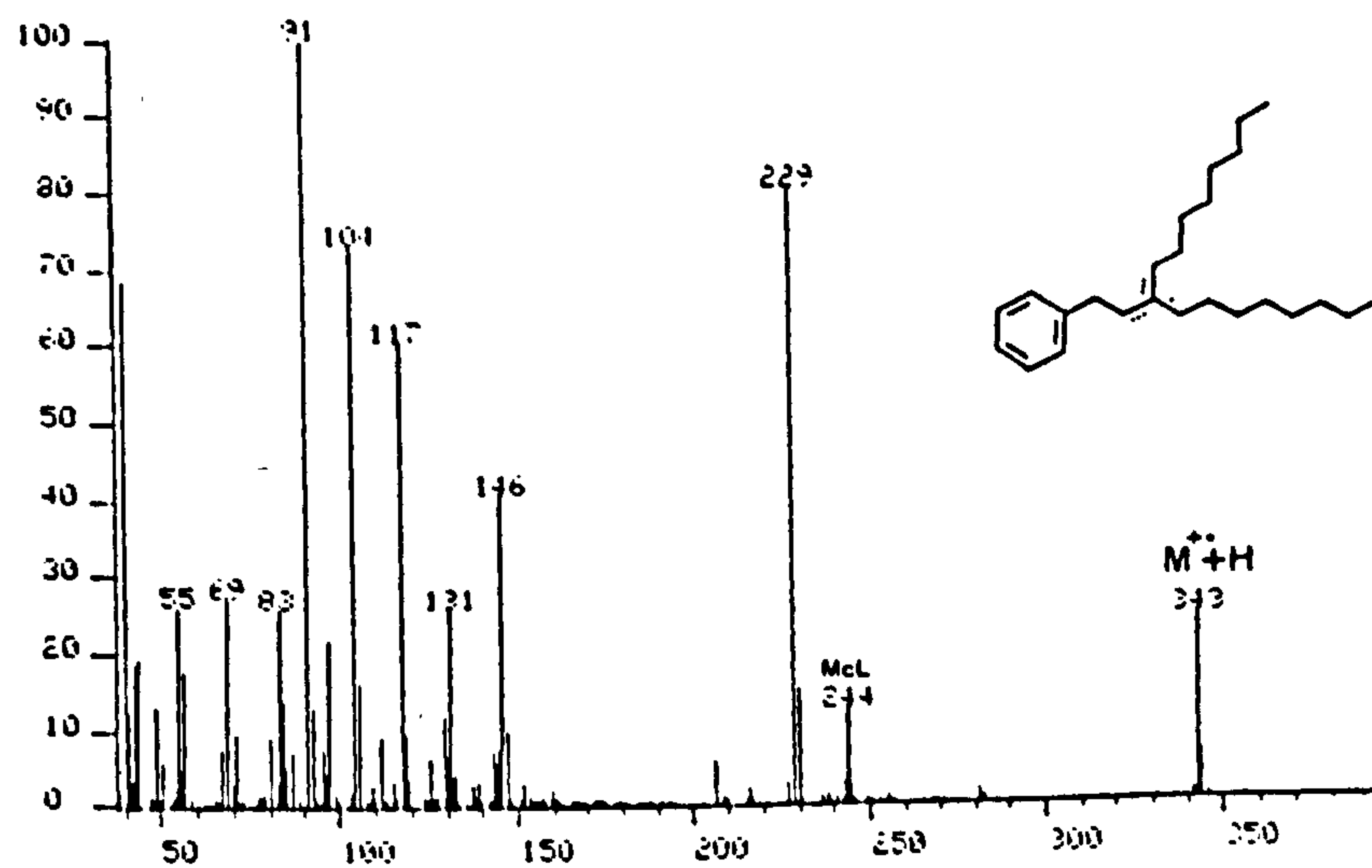
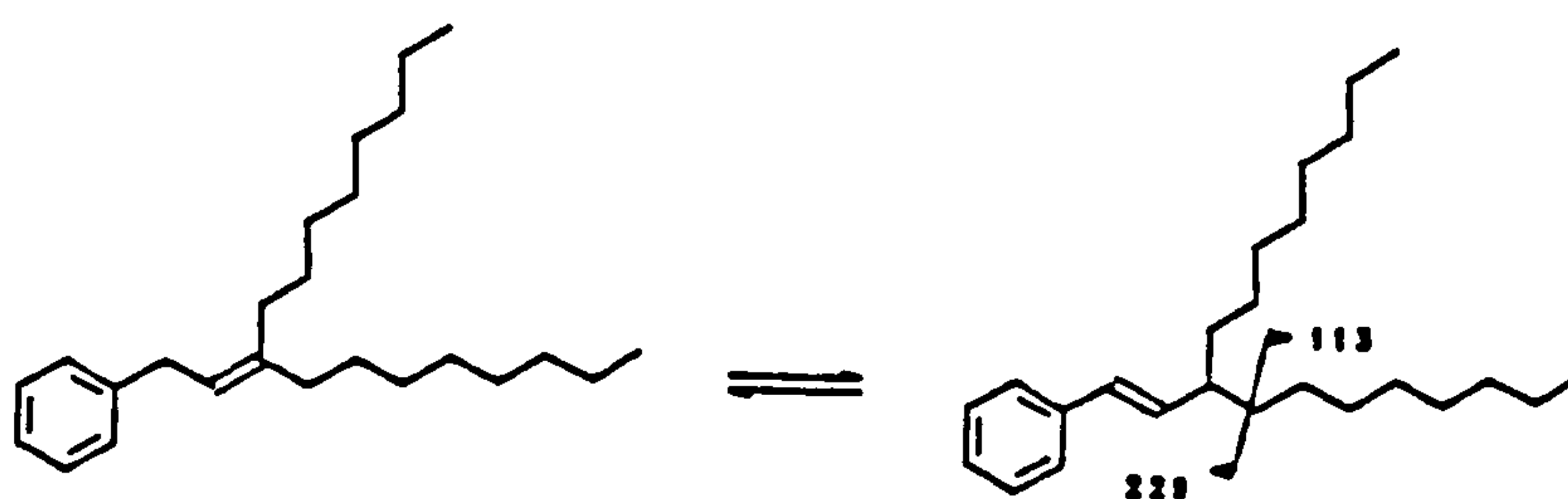


FIG. 4.21 EI MASS SPECTRUM OF SYNTHETIC 9-(2-PHENYLETHYL)-HEPTADECENES





The dehydration ($\text{POCl}_3/\text{pyridine}$) products of the tertiary alcohol (21) were found to comprise two compounds when analysed by GC and GC-MS. The EI mass spectrum of each compound was virtually indistinguishable and each showed ions comparable to those observed for the precursor alcohol (Fig. 4.21). Thus the m/z 146 ion is derived via an analogous double McLafferty-type rearrangement. Evidence of double bond migration was again observed with ions m/z 230/229, indicative of allylic cleavage with and without H-transfer, respectively. The m/z 244 ion presumably originates from a γ -H rearrangement of a predicted product alkene. The $\text{M}^+ + \text{H}$ ion (m/z 343) provided further confirmation of the desired alkenes 9-(2-phenylethyl)-heptadecenes (22).

Initial attempts to hydrogenate the isomeric alkenes (22) using $\text{PtO}_2 \cdot \text{H}_2\text{O}$ (Adam's catalyst) in hexane at room temperature and atmospheric pressure were found to result in a virtually quantitative simultaneous reduction of both the ring and the double bond to the cycloalkane 9-(2-cyclohexylethyl)-heptadecane (18). This result was surprising since higher temperatures and pressures are usually

required to catalytically hydrogenate aromatic rings (March, 1985). In addition, aryl substitution of olefins is known to retard the hydrogenation of the double bond when platinum is used as a catalyst (Friefelder, 1971). Alternative use of palladium on carbon (5%) was found to selectively hydrogenate the double bond whilst the aromatic ring remained unchanged. A possible explanation for the observed variations in selectivity of the two catalysts is that aromatic rings are thought to be adsorbed less strongly to palladium surfaces than platinum (Friefelder, 1971). This would allow a more rapid transfer of the (presumably) most labile olefinic double bond and its reduction product, prior to hydrogenation of the ring. The product alkylaromatic hydrocarbon was purified from residual unreacted alkene by argentatious TLC and assigned by LRMS. The EI mass spectrum (Fig. 4.22) showed the required molecular ion (m/z 344) and ions derived from α -cleavage at the branch position (m/z 231, $M^+ - C_8H_{17}$) and benzylic to the ring (m/z 253, $M^+ - C_7H_7$). The base peak (m/z 92) is derived from a McLafferty γ -H rearrangement which is a characteristic feature of alkyl substituted benzenes, provided the alkyl substituent is propyl or larger (McLafferty, 1978) i.e.

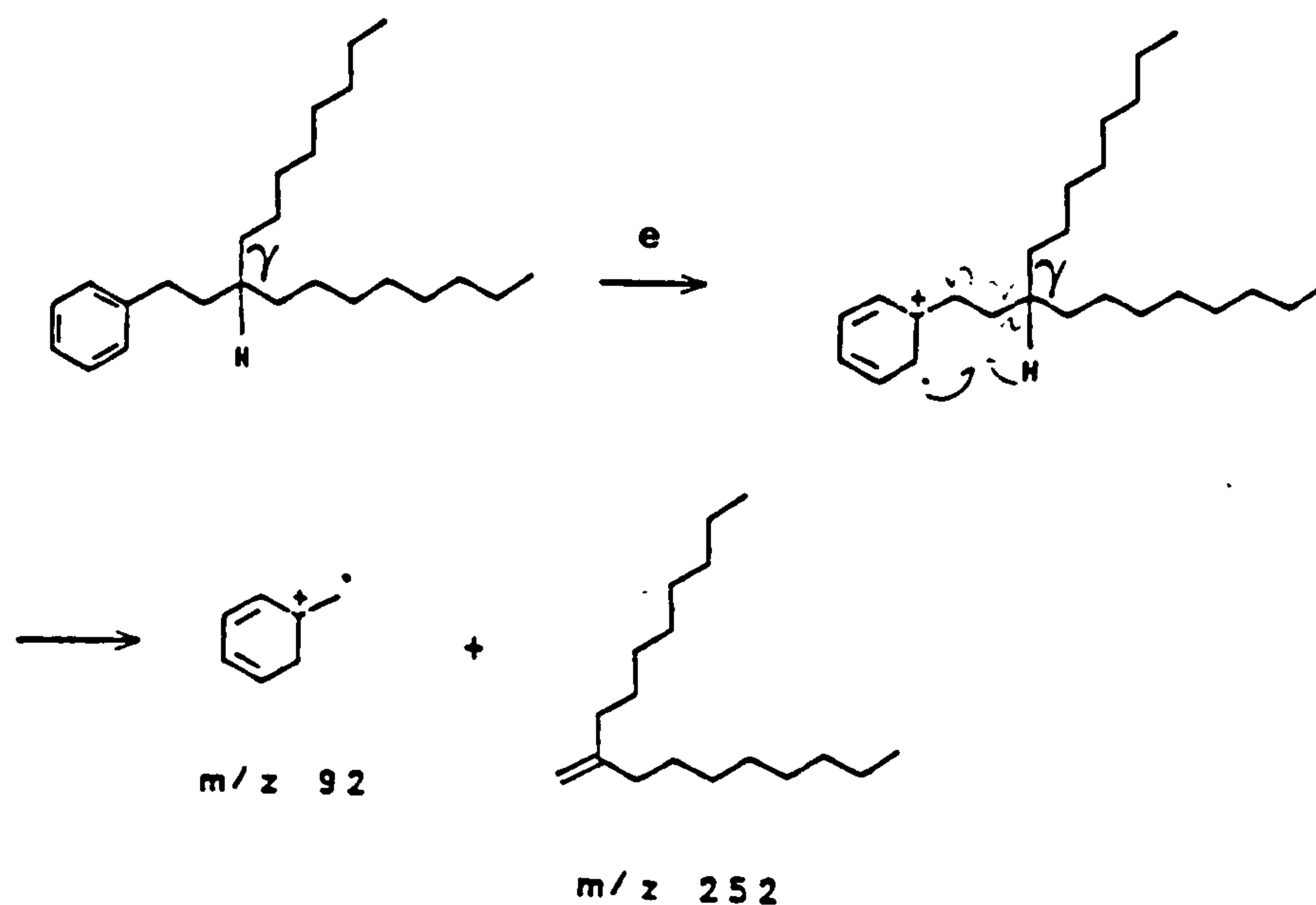
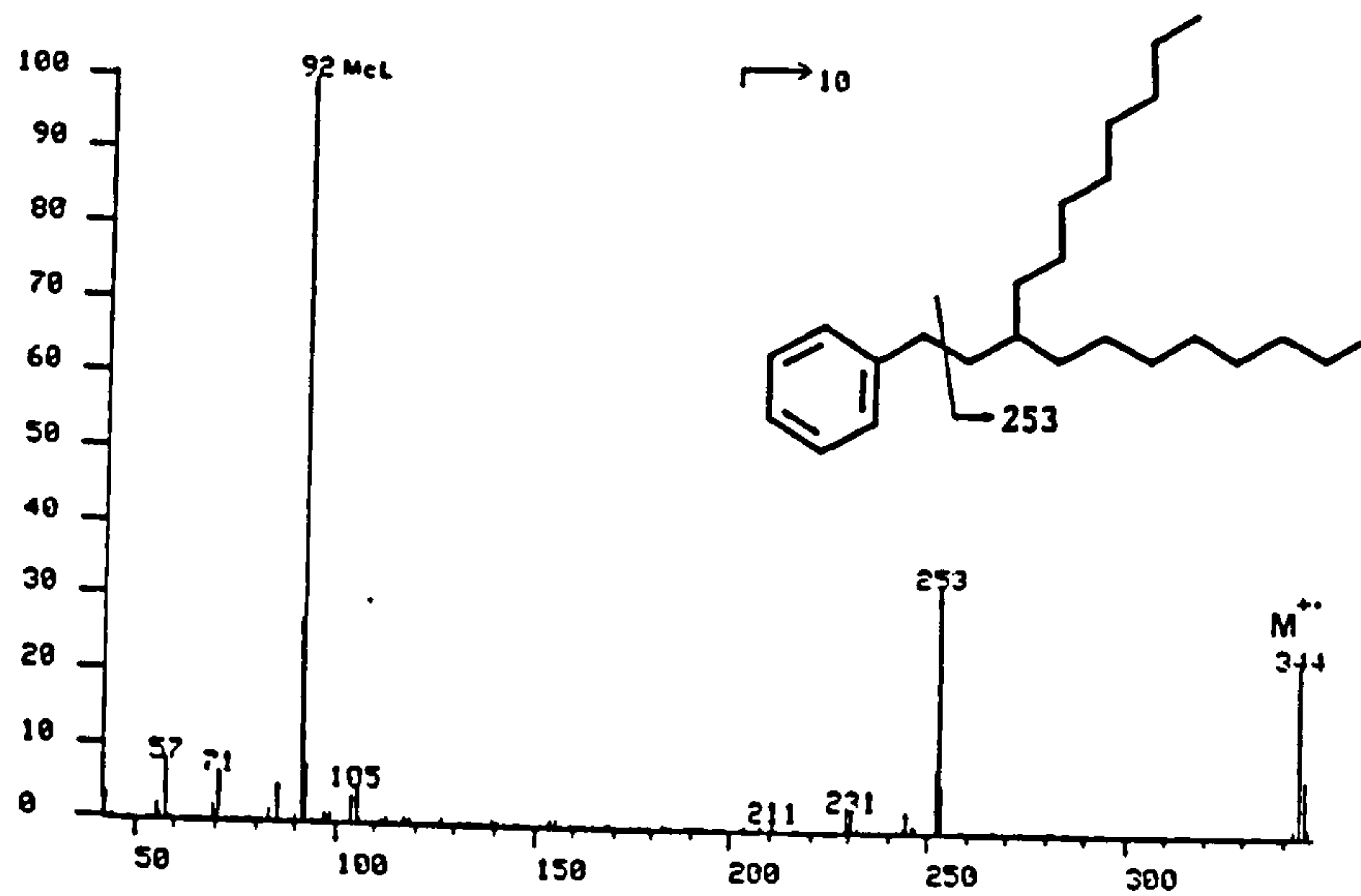


FIG. 4.22 EI MASS SPECTRUM OF SYNTHETIC 9-(2-PHENYLETHYL)-HEPTADECANE



4.1.7 2,6R,10R,14RS,18S-PENTAMETHYLEICOSANE

The synthetic route chosen for the regular C₂₅ acyclic isoprenoid 2,6R,10R,14RS,18S-pentamethyleicosane (30) was similar to that used by Rowland *et al.* (1982) and is summarised as Scheme C, Fig. 4.1. The route to the intermediate aldehyde 3RS,7R,11R,15-tetramethylhexadecanal (27) involved oxidation of trans-phytol [E-3,7R,11R,15-tetramethylhexadec-2-en-1-ol, (24)] with active manganese dioxide (Attenburrow *et al.*, 1952) to form the α,β -unsaturated aldehyde 3,7R,11R,15-tetramethylhexadec-2-enal (26). IR of the product (Fig. 4.23B) revealed the presence of an aldehydic proton [$\nu(\text{C-H}):2720\text{cm}^{-1}$] and a carbonyl absorption [$\nu(\text{C=O}):1680\text{cm}^{-1}$] typical of α,β -unsaturated aldehydes (Silverstein *et al.*, 1981). Hydrogenation of 26 using Pd on carbon (5%) provided the saturated aldehyde 3RS,7R,11R,15-tetramethylhexadecanal (phytanal, 27) in good yield (75%). IR showed a shift towards higher frequency for the carbonyl absorption [$\nu(\text{C=O}):1730\text{cm}^{-1}$] indicative of loss of the α,β -double bond; whilst the position of the aldehydic C-H stretch [$\nu(\text{C-H}):2720\text{cm}^{-1}$] remained unchanged (Fig. 4.23C). LRMS (Fig. 4.24) showed the expected molecular ion m/z 296 along with m/z 278, indicative of loss of water ($\text{M}^+ - 18$). Most of the even ions in the high mass region of the spectrum could be attributed to recurrent γ -H rearrangements [e.g. m/z 252 (single), m/z 210 and m/z 196 (double)]. The saturated aldehyde (27) was coupled to the Grignard reagent derived from 1-bromo-2S-methylbutane (25) using the above procedures. Hydrolysis followed by TLC purification (SiO_2 , hexane: Et_2O , 9:1; Rf: 0.23-0.42) provided the secondary alcohol 3S,7RS,11R,15R,19-pentamethyleicosan-5RS-ol (28) which was assigned by IR and LRMS. IR

FIG. 4.23 INFRA RED (IR) SPECTRA OF A) PHYTOL, B) PHYTENAL AND C) PHYTANAL

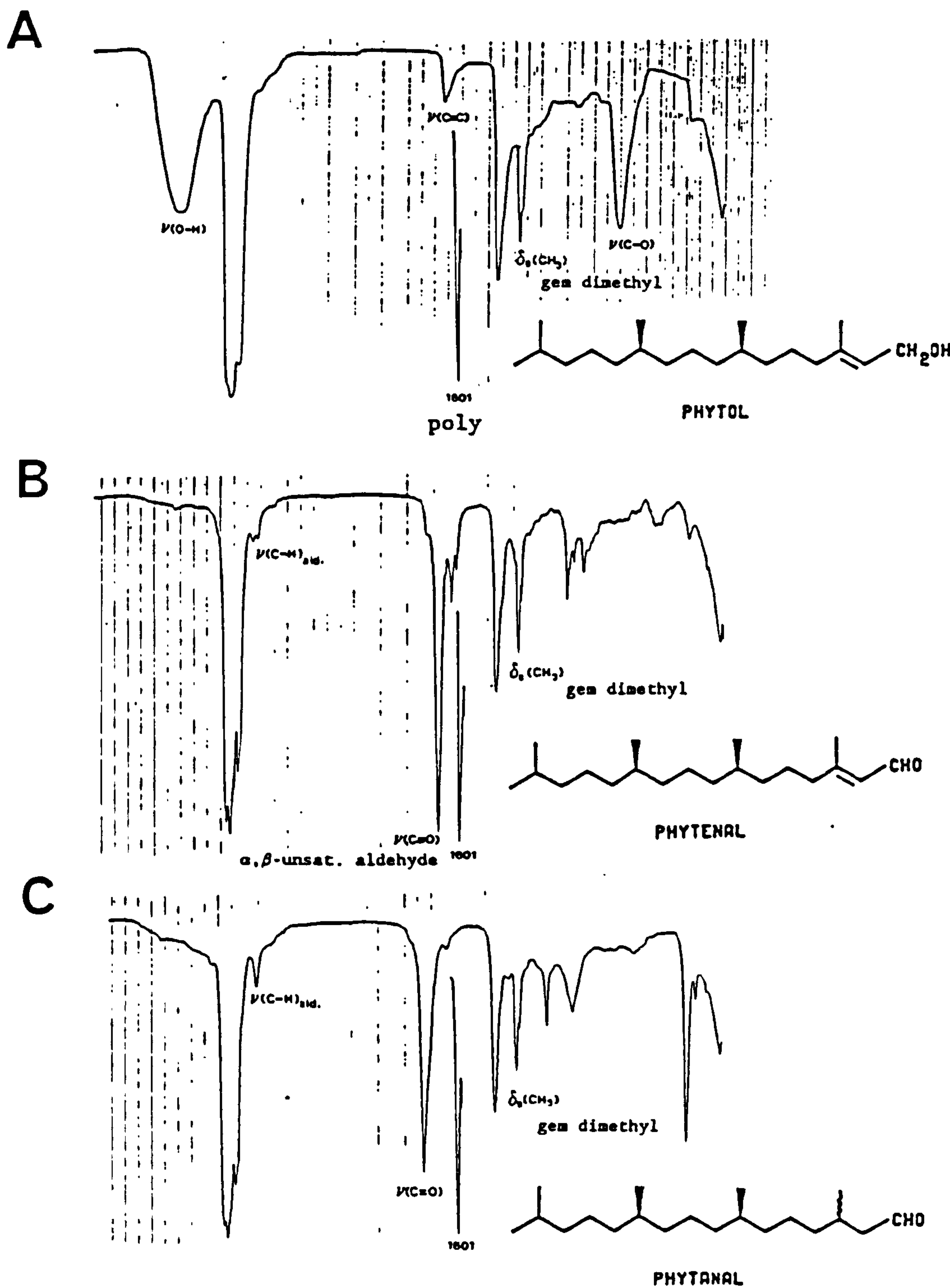


FIG. 4.24 EI MASS SPECTRUM OF SYNTHETIC PHYTANAL
 (• denotes McLafferty rearrangement ions)

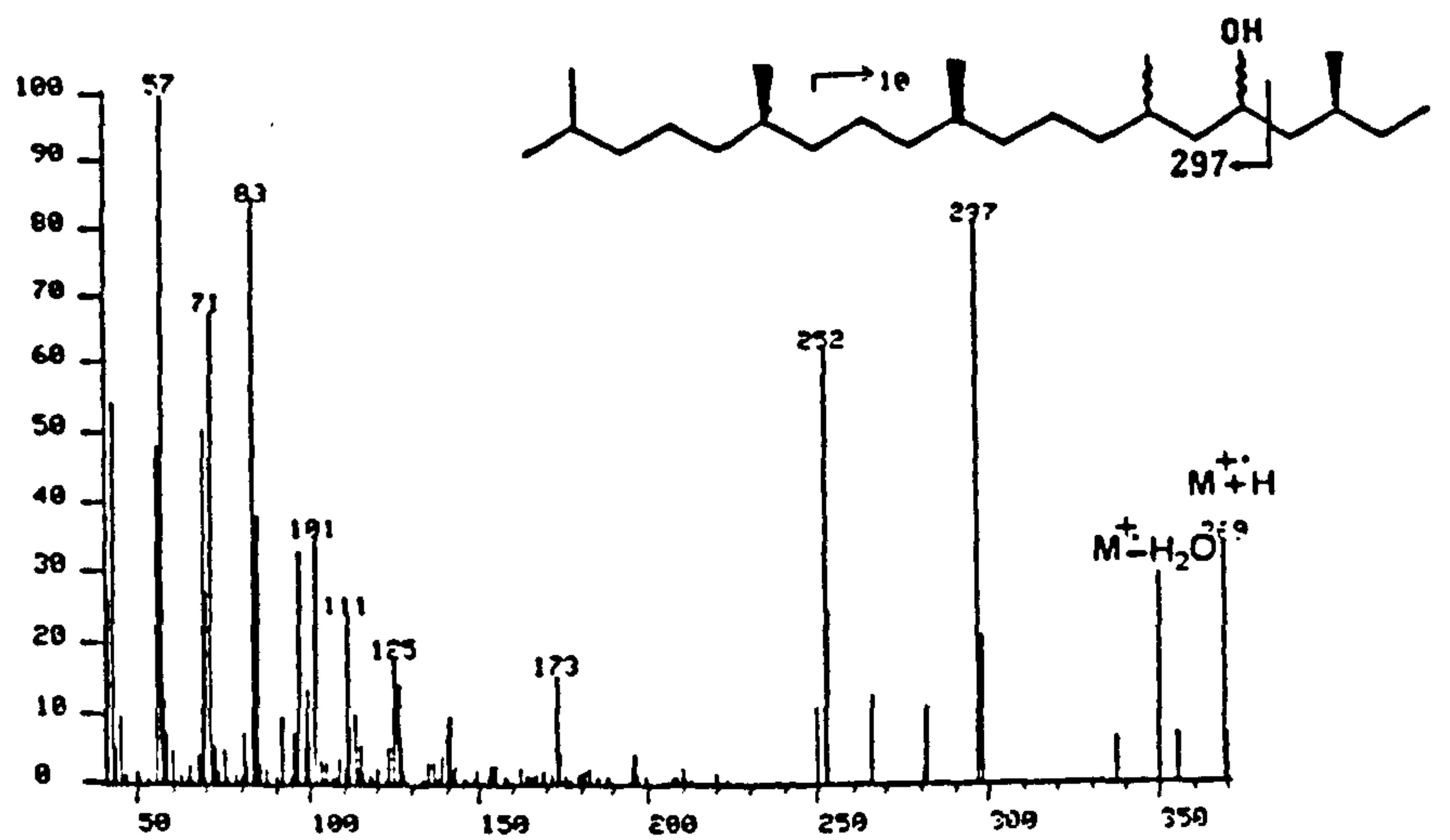
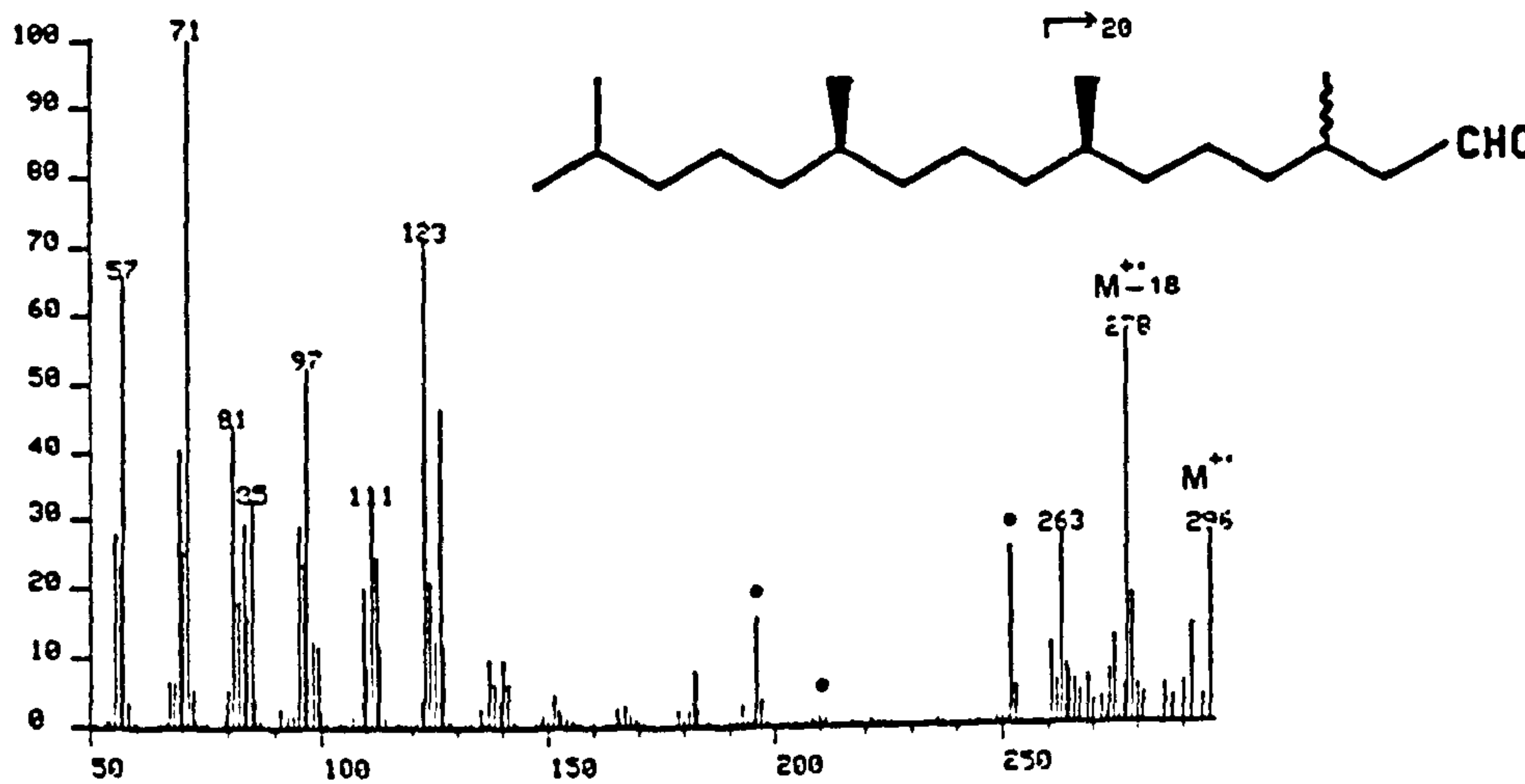


FIG 4.25 EI MASS SPECTRUM OF SYNTHETIC 3S,7RS,11R,15R,19-PENTAMETHYLEICOSAN-5RS-OL

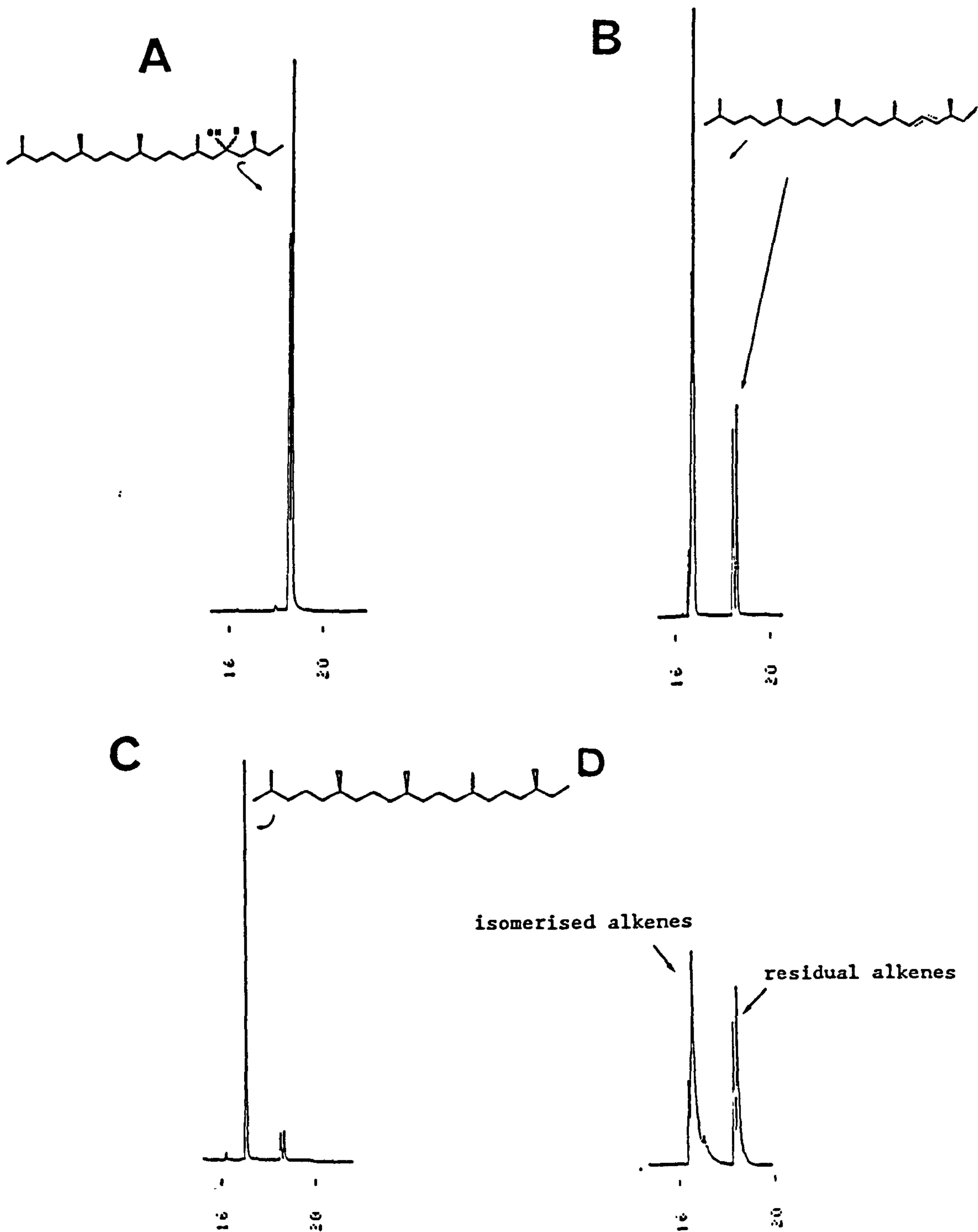
showed evidence of a H-bonded hydroxyl group [$\nu(\text{O-H}):3480\text{cm}^{-1}$] and capillary GC (Fig. 4.26A) showed a partially resolved doublet presumably arising from diastereomeric separation (Lamb, 1982). The EI mass spectra were identical and showed ions diagnostic of a C25 aliphatic alcohol (Fig. 4.25), namely m/z 369 ($\text{M}^{\cdot+} + \text{H}$) and m/z 350 ($\text{M}^{\cdot+} - \text{H}_2\text{O}$); (McLafferty, 1978). The ions m/z 71, 101, 297 and 267 are all derived from α -cleavage adjacent to the hydroxyl group and confirm the position as C-5. The m/z 252 ion presumably originates from a McLafferty-type γ -H rearrangement involving the molecular ion.

Dehydration of the secondary alcohol (28, $\text{POCl}_3/\text{pyridine}$) produced a mixture of five C25 monoenes when analysed by GC and GCMS (Fig. 4.26B, Fig. 4.27). EI mass spectra of the isomers were virtually indistinguishable. For example the EI mass spectrum (Fig. 4.27) of the major compound (compound C, Fig. 4.26B) showed ions similar to those observed for the intermediate alcohol (28), which are presumably derived from analogous consecutive γ -H rearrangements.

Hydrogenation ($\text{PtO}_2 \cdot \text{H}_2\text{O}/\text{hexane}$) of the isomeric alkenes (29) converted the earlier eluting isomers to a single peak (Fig. 4.26C) but the later eluting isomers appeared to remain unchanged. Purification by argentatious TLC provided 2,6R,10R,14RS,18S-pentamethyleicosane (30, R_f : 0.87-0.99) and the mass spectrum (Fig. 4.28) compared well with that reported previously (Holzer *et al.*, 1979; Lamb, 1982). An examination of the residual alkene band (R_f : 0.00-0.05, Fig. 4.26D) showed that the later eluting monoenes had undergone some isomerisation, possibly mediated by the silica surface, to the preceding isomers. The same behaviour has been noted by Lamb (1982) for analogous 2,6RS,11RS,15RS,19-pentamethyleicosenes

FIG. 4.26 PARTIAL GAS CHROMATOGRAMS OF:

[GC: SE-54(CARLO ERBA), 25m, 50-300 °C @ 10 °C min⁻¹]

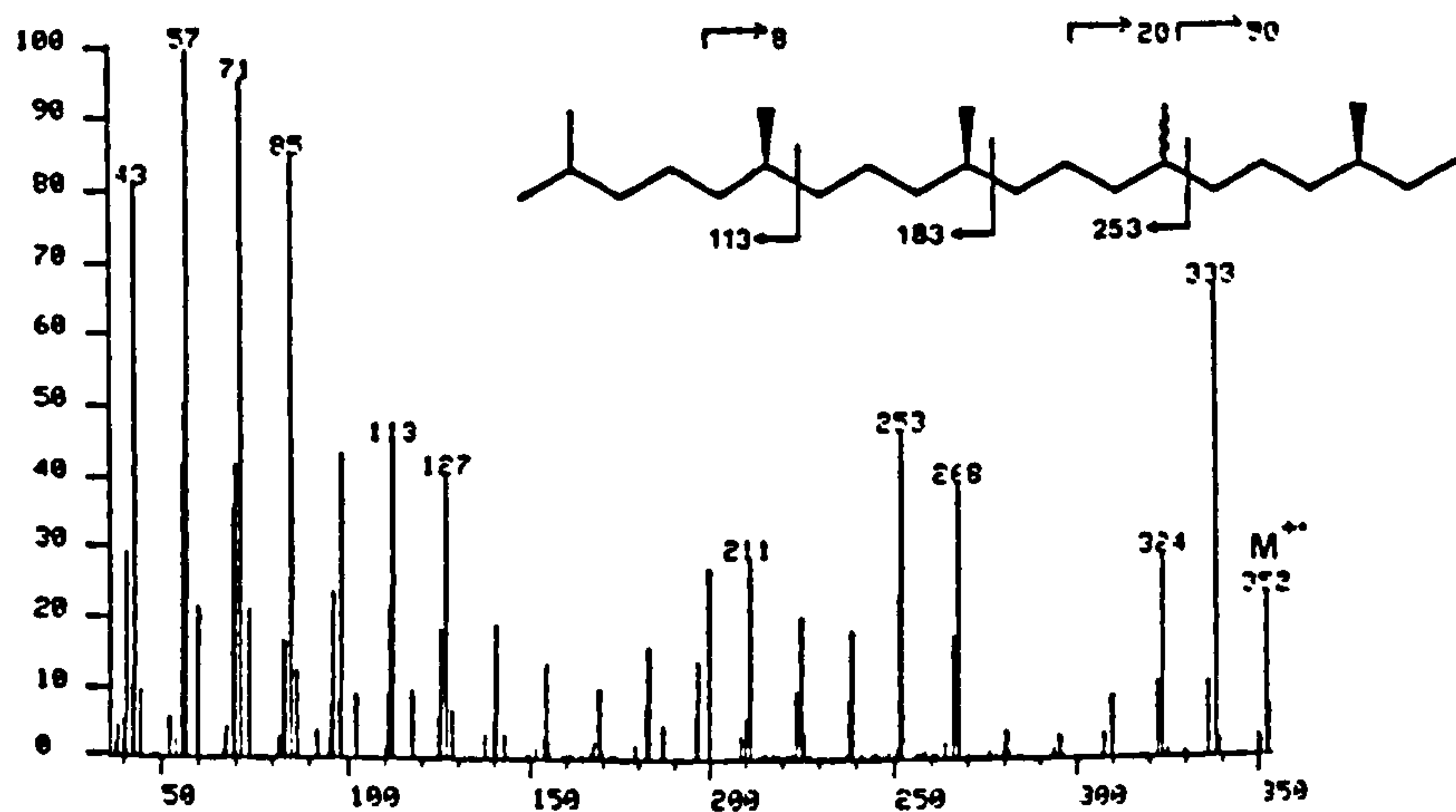


- A) PURIFIED 3S,7RS,11R,15R,19-PENTAMETHYLEICOSAN-5RS-OL
- B) DEHYDRATION PRODUCTS
- C) HYDROGENATED PRODUCTS
- D) RESIDUAL ALKENE BAND

FIG. 4.27 EI MASS SPECTRUM OF SYNTHETIC 3,7,11,15,19-PENTAMETHYLEICOSENES



FIG. 4.28 EI MASS SPECTRUM OF SYNTHETIC 2,6R,10R,14RS,18S-PENTAMETHYLEICOSANE



(31) derived from dehydration of synthetic 2,6RS,11RS,15RS,19-pentamethyleicosan-8RS-ol (32). As observed previously (Lamb, 1982), the later eluting isomeric monoenes were inert to hydrogenation, yet were found to readily isomerise to more easily hydrogenated monoenes on a Ag^+/SiO_2 surface. This has been attributed to stereospecific dehydration of the secondary alcohol to form trans-monoenes (inert to hydrogenation), which then underwent trans-cis isomerisation on the solid surface (Lamb, 1982). A similar mechanism may therefore account for the behaviour of 2,6,10,14,18-pentamethyleicosenes observed in this study.

4.2 OXIDATION OF MODEL HYDROCARBONS

4.2.1 OXIDATION OF 9-METHYLTETRACOSANE

Two CrO_3 /glacial acetic acid oxidations of 9-methyltetracosane (5) were performed, the first for ten minutes using hexane as the extractant (c.f. Brooks *et al.*, 1977) and the second for sixty minutes with DCM as the extractant. The gravimetric data for these procedures is summarised in Table 4.2. The oxidation using hexane as the extractant provided total methylated oxidation products* (10%), which when analysed by GC (Fig. 4.29) were found to comprise three major components. GC-MS (Table 4.3) showed these to be residual alkane (compound C) and some predicted products of oxidation at the tertiary centre, *n*-pentadecanoic acid (A) and heptadecan-2-one (B, Fig. 4.29 and Table 4.3).

*NB The term "product" is used loosely herein to describe both residual unoxidised alkane and functionalised oxidation products.

TABLE 4.2

THE CrO₃/GLACIAL ACETIC ACID OXIDATION OF MODEL HYDROCARBONS

COMPOUND	MASS OXIDISED (mg)	MOLAR RATIO OXIDANT/ HYDROCARBON	TIME (MIN)	EXTRACTION SOLVENT	MASS PRODS (mg)	YIELD (%)
9-methyltetracosane	5	10.6	10	Hexane	0.5	10
9-methyltetracosane	6.4	11.0	60	DCM	0.8	13
7- <u>n</u> -hexylnonadecane	41.2	10.4	60	DCM	22.3	54
9-(2-cyclohexylethyl)- heptadecane	19.6	10.2	60	DCM	13.0	66
9-(2-phenylethyl)- heptadecane	11.3	10.1	60	DCM	7.6	68
2-methyltetracosane	20.6	10.2	60	DCM	3.9	19
<u>n</u> - pentacosane	51.6	10.0	60	DCM	26.5	51
2,6,10,14-tetramethyl pentadecane (Pristane)	52.0	13.4	60	Hexane	2.4	5
2,6,10,14-tetramethyl pentadecane	42.6	9.8	60	DCM	35.9	84
Mixture	9.7	10.0	60	DCM	3.2	33

FIG. 4.29 PARTIAL GAS CHROMATOGRAM OF TOTAL OXIDATION PRODUCTS (METHYLATED) OF 9-METHYLTETRACOSANE

(CrO₃/glacial acetic acid, 10 min, hexane extractant)

[GC: DB-5(J+W), 25m, 50-300 °C @ 10 °C min⁻¹]

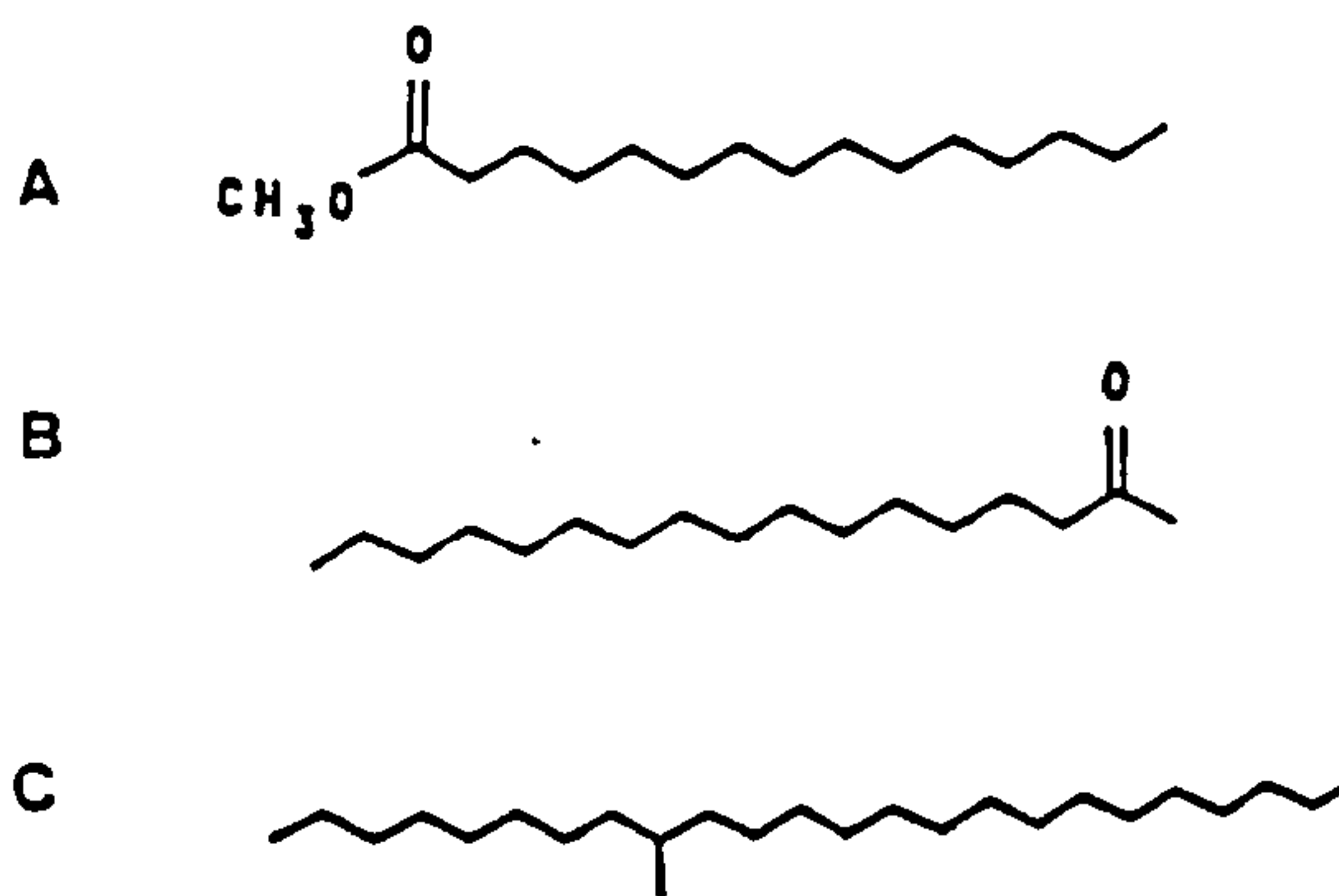
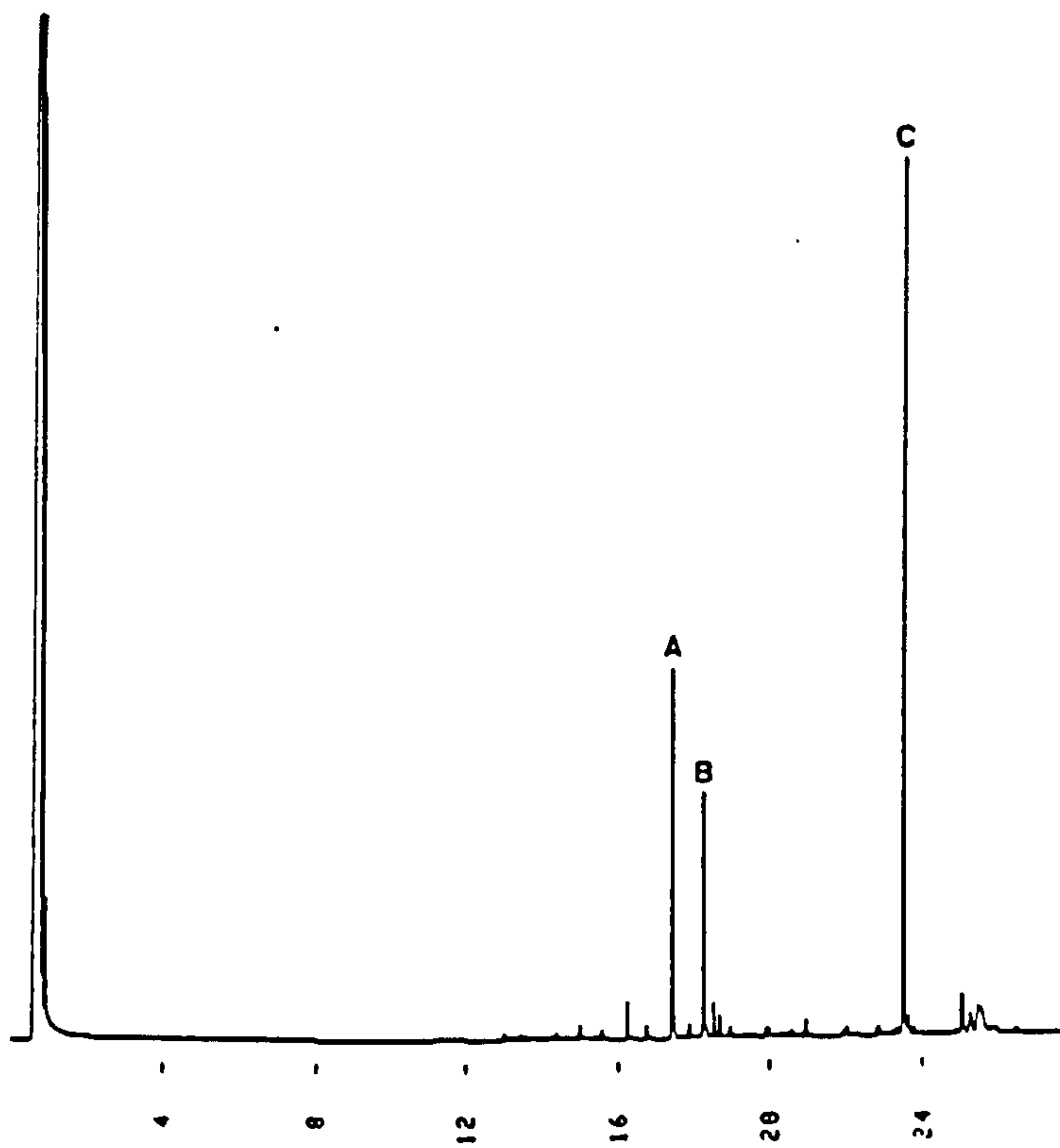


TABLE 4.3

MASS SPECTRAL DATA FOR THE CrO_3 /GLACIAL ACETIC ACID OXIDATION OF
9-METHYLTETRACOSANE (10 MIN/HEXANE EXTRACTANT)

COMPOUND	NAME	MAJOR IONS(% ABUNDANCE)
A	<u>n</u> -pentadecanoic acid ^a	74(100), 87(58), 143(10), 256(8)
B	<u>n</u> -heptadecan-2-one	58(100), 71(43), 196(3), 254(5)
C	9-methyltetracosane	57(100), 140/141 (10/5), 210/211(0.5/0.3) 238/239(0.2/0.25), 337 (0.4)
a:	as methyl ester	

The low yield is typical for the procedure using hexane as the extractant (e.g. Patience et al., 1979), presumably due to the inefficiency of the low polarity solvent in the extraction of more polar products - neither predicted product compounds octanoic acid and decan-2-one were recovered.

The second oxidation of 9-methyltetracosane (5) was a modification of the initial procedure of Brooks et al. (1977), and utilised DCM as the extractant instead of hexane. The time of the oxidation was extended to sixty minutes for a more direct comparison with the oxidation of the aliphatic UCM (Section 3.1.4).

GC and GC-MS analyses (Fig. 4.30 and Fig. 4.31) of the total recovered material revealed a complex mixture of compounds in which the residual alkane (X, Fig. 4.30) was identified as a minor component. The major compounds identified were found to comprise a homologous series of n- α,ω -diacids (C6-C11) as well as a homologous series of compounds which showed abundant m/z 99 ions in their EI mass spectra (compounds a-e, Fig. 4.31). CI GC-MS using NH₃ as the reagent gas provided molecular weight data consistent for a series of ω -carboxy- γ -methyl- γ -lactones comparable to those observed as oxidation products of the aliphatic UCM (Section 3.1.4). Thus the EI mass spectrum of component b matched well with that identified as an aliphatic UCM oxidation product (Fig. 4.32, A + B), and showed ions consistent with a C9 carboxy lactone-type structure. CI-MS confirmed the proposed molecular weight (Fig. 4.32C).

The precise origin of these two series of major oxidation products of 9-methyltetracosane is unclear, since neither are primary oxidation

FIG. 4.30 PARTIAL GAS CHROMATOGRAM TOTAL OXIDATION PRODUCTS (METHYLATED) OF 9-METHYLTETRACOSANE

(CrO₃/glacial acetic acid, 60 min, dcm extractant)

[GC: DB-5(J+W), 25m, 50-300 °C @ 5 °C min⁻¹]

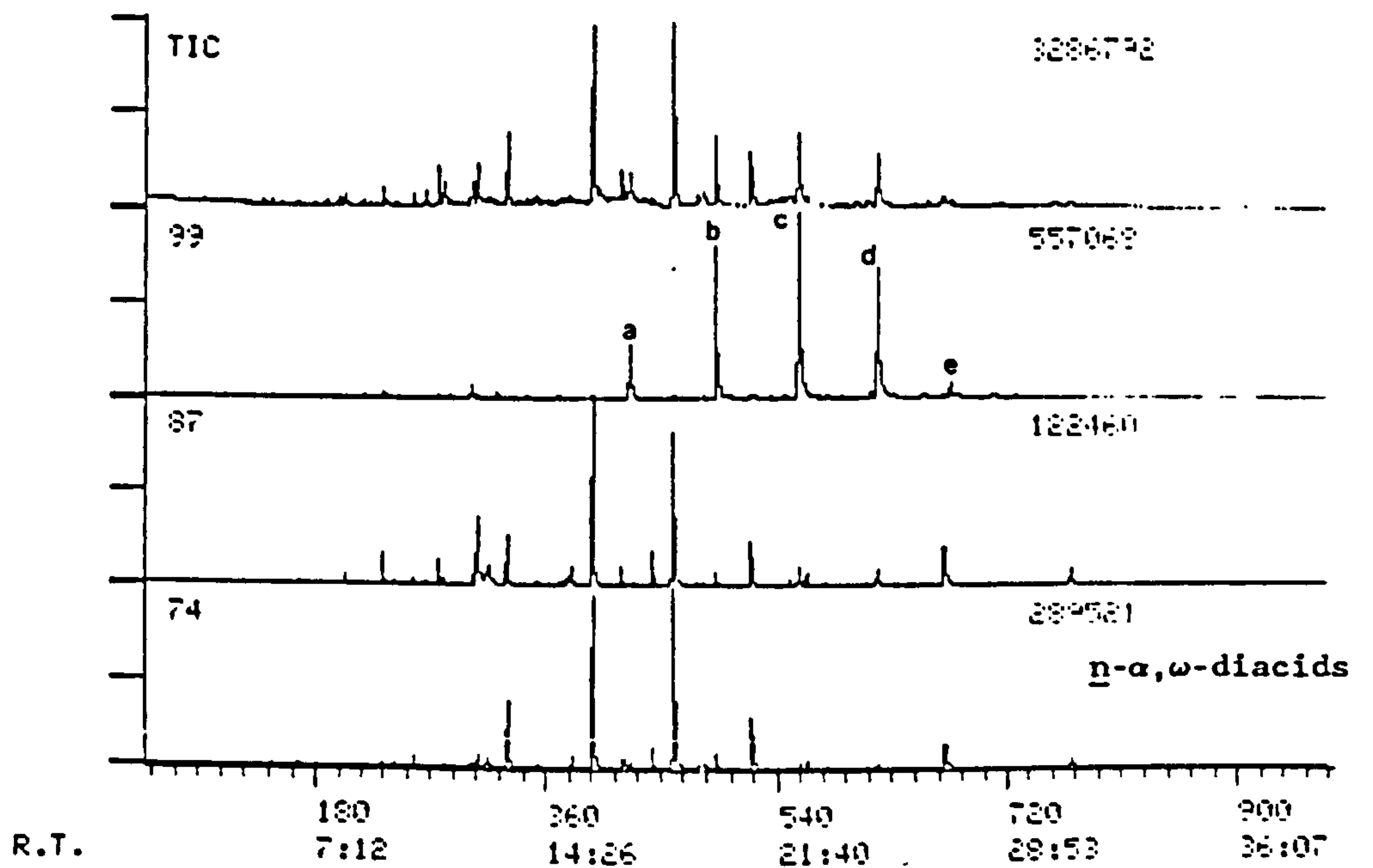
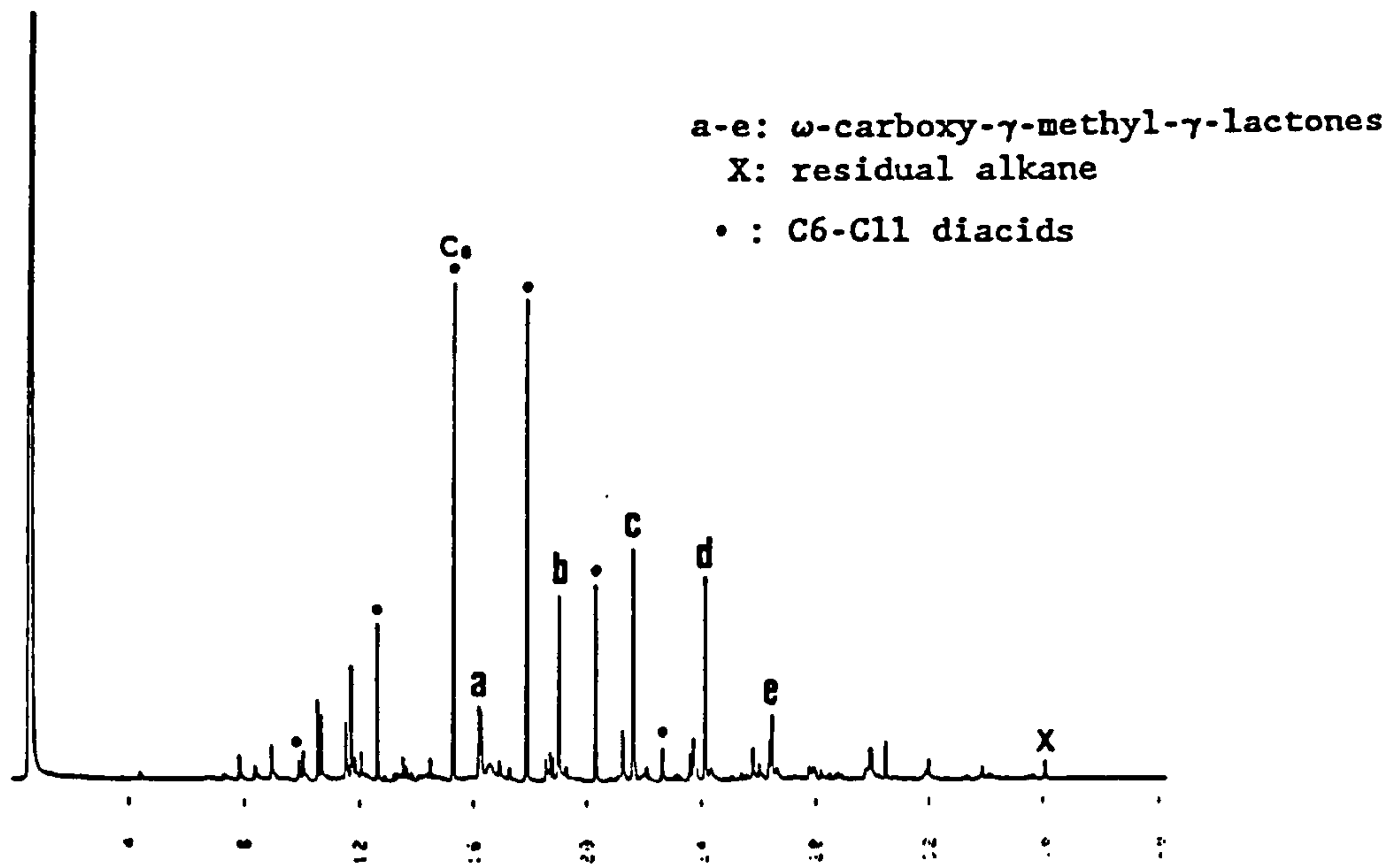
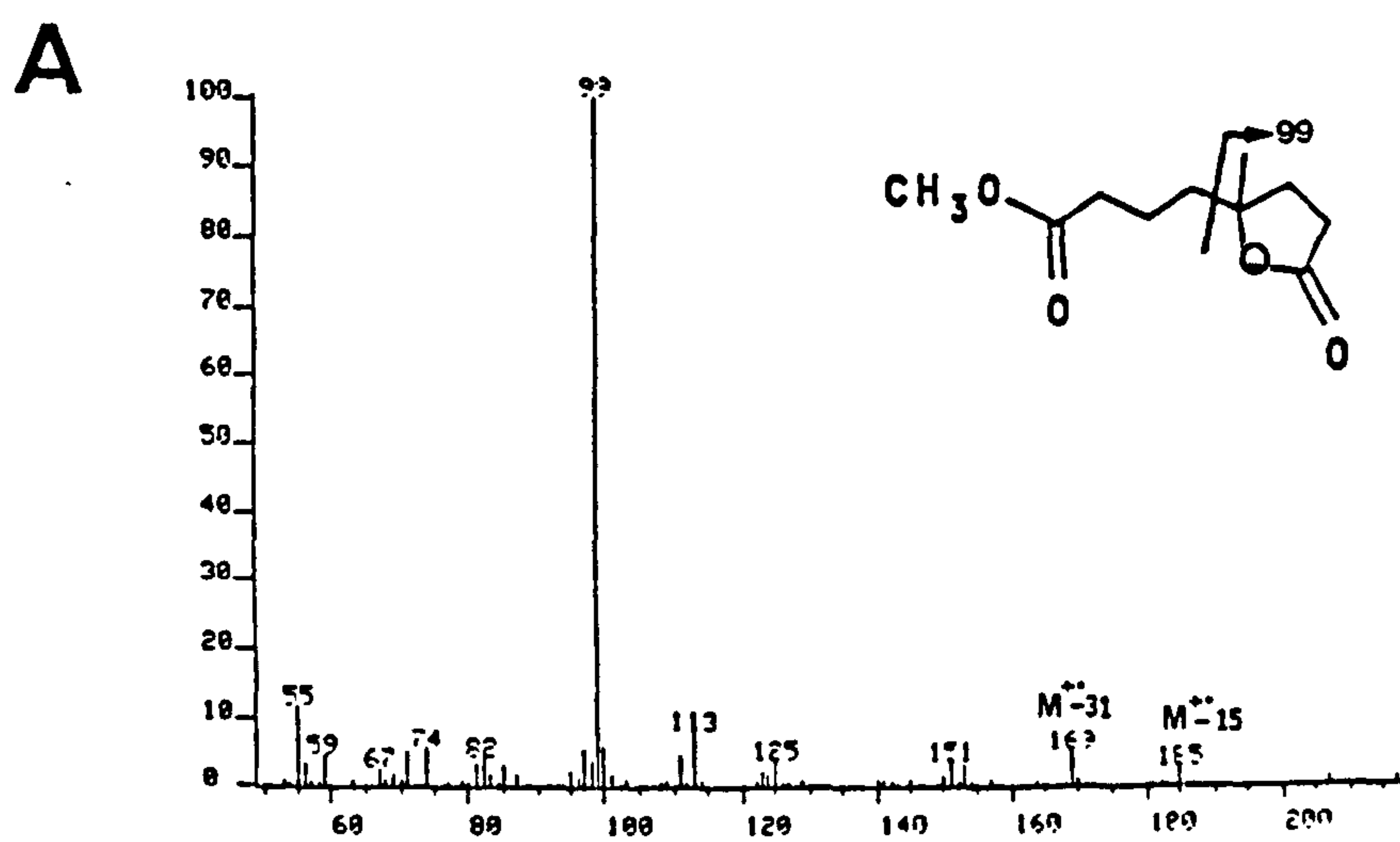


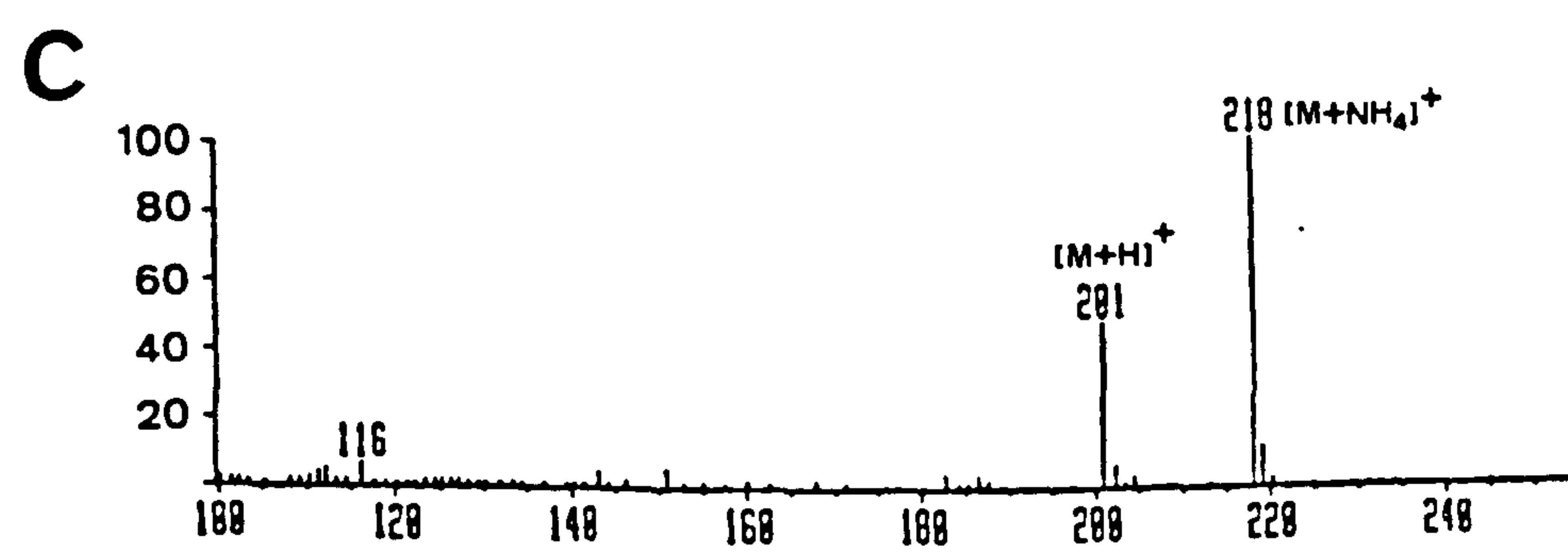
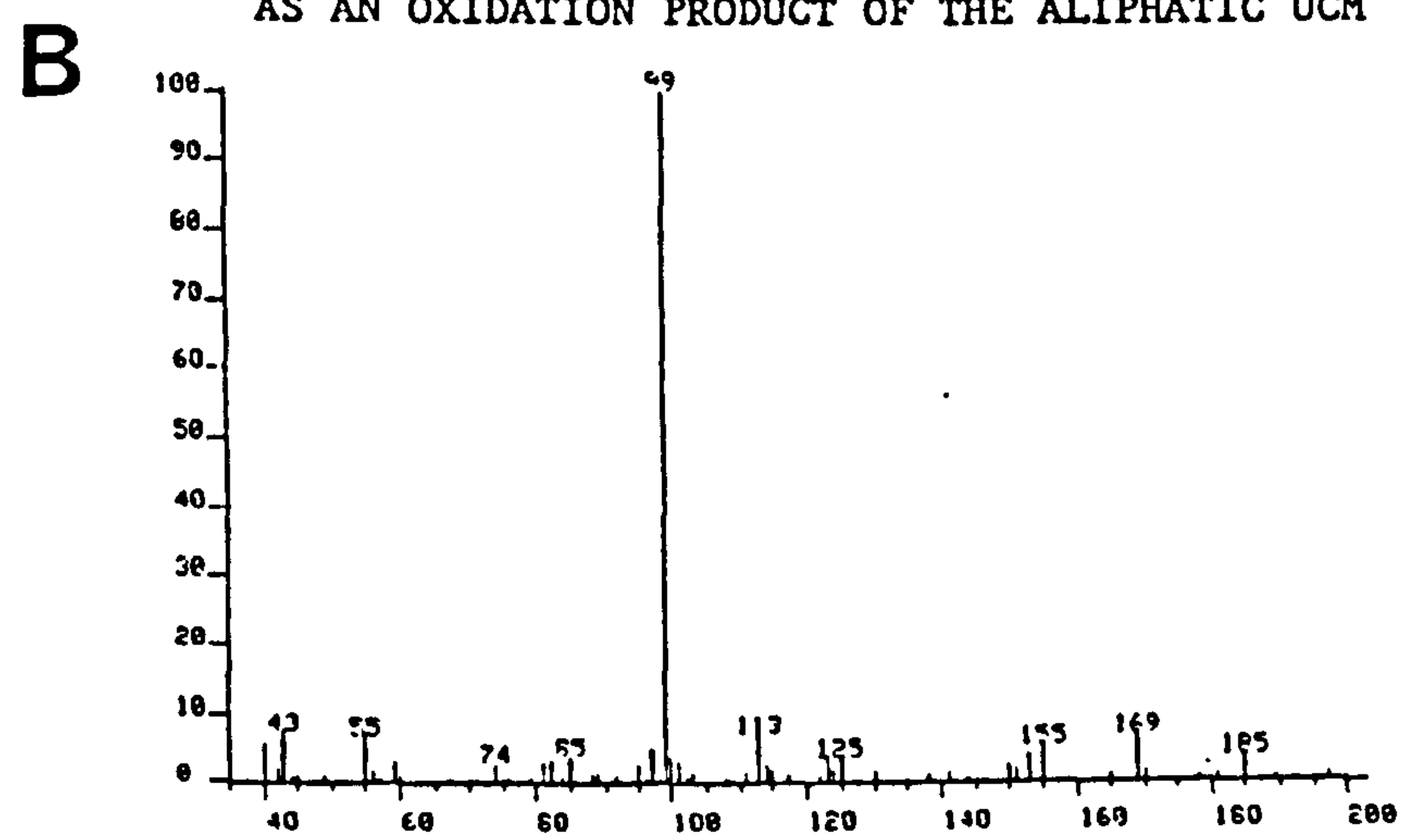
FIG. 4.31 GC-MS MASS FRAGMENTOGRAM OF 9-METHYLTETRACOSANE OXIDATION PRODUCTS

(CrO₃/glacial acetic acid, 60 min, DCM extractant)

FIG. 4.32 A) EI MASS SPECTRUM OF COMPONENT b (FIG. 4.31), AN OXIDATION PRODUCT OF 9-METHYLTETRACOSANE



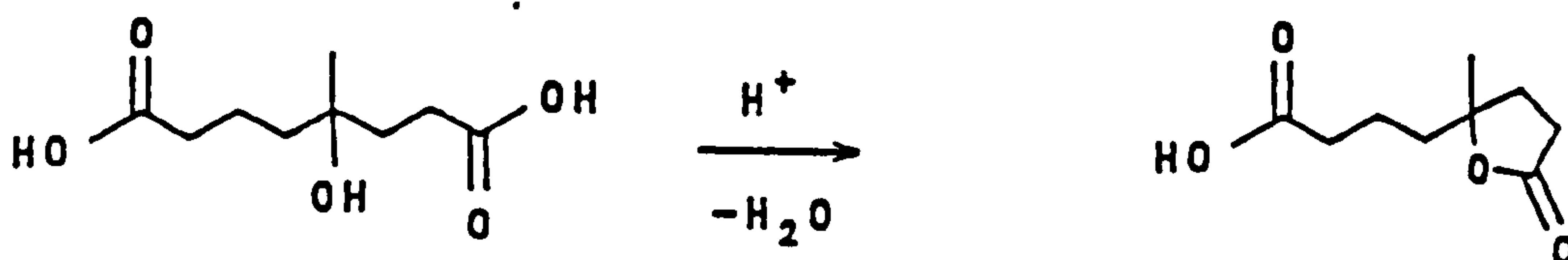
B) EI MASS SPECTRUM OF A C9-CARBOXY LACTONE IDENTIFIED AS AN OXIDATION PRODUCT OF THE ALIPHATIC UCM



C) CI(NH₃) MASS SPECTRUM OF COMPONENT b

products (see ten minute oxidation data). The series of \underline{n} - α,ω -diacids must originate from secondary oxidation of either the initial methyl ketones or the monocarboxylic acids. Secondary oxidation is commonly observed in chromic acid oxidations of saturated hydrocarbons and both ketones and acids are susceptible to degradation by the oxidant (Lee, 1980). Ketones in particular appear to be degraded quite rapidly to carboxylic acids via C-C bond cleavage adjacent to the carbonyl and as a result are rarely observed as major products in CrO_3 oxidations (Wiberg, 1965). In contrast, the presence of a carboxylic acid functionality in a linear alkyl chain appears to exert a stabilising effect at the C-1 to C-5 positions - oxidation preferentially takes place at positions C-6 and above (Cason et al., 1959). For the observed pentadecanoic acid this would result in the production of di-acids in the range C6-C15, close to that observed in the oxidation of 9-methyltetracosane (C6-C11).

The origin of a homologous series of carboxy lactones is more difficult to explain, as they cannot be related to any of the primary or indeed secondary oxidation products observed. Their derivation as lactones would require the intramolecular esterification of a precursor γ -hydroxy- γ -methyl substituted diacid, i.e.



This itself must originate from a primary intermediate in the oxidation sequence, i.e. the tertiary alcohol 9-methyltetracosan-9-ol. However, under the acidic conditions of the experiment the labile alcohol must in some way be protected against dehydration, possibly as a chromate ester, the rate of hydrolysis of which is sufficiently low to allow oxidation of the adjacent alkyl side chains. Subsequent hydrolysis (KOH/MeOH) would yield the hydroxy diacid which under the conditions of recovery (i.e. acidification with HCl) may undergo intramolecular cyclisation to form the lactone. It appears that the oxidation of 9-methyltetracosane proceeds via a complex series of competitive reactions involving oxidation of substrate, intermediates and products. The ultimate nature of the end product is governed by the lability of each molecular type involved. Whatever the precise mechanism, it is of interest that the series of carboxy lactones observed as oxidation products of the aliphatic UCM (Section 3.1.4) were also produced as oxidation products of a proposed precursor alkane containing internal methyl branching.

4.2.2 OXIDATION OF 7-N-HEXYLNONADECANE

The CrO₃ /glacial acetic acid oxidation of 7-n-hexylnonadecane (9) produced a mixture of compounds in moderate yield (54%). GC (Fig. 4.33) and GC-MS (Table 4.4) revealed the major component to be residual unoxidised alkane (X, Fig. 4.33). Compounds identified as oxidation products included dodecanoic acid, nonadecan-7-one, and tridecan-7-one, each of which represents a predicted product derived

FIG. 4.33 PARTIAL GAS CHROMATOGRAM OF TOTAL OXIDATION PRODUCTS (METHYLATED) OF 7-n-HEXYLNONADECANE

(CrO₃/glacial acetic acid; 60 min, DCM extractant)

[GC: DB-5(J+W), 25m, 50-300 °C @ 5 °C min⁻¹]

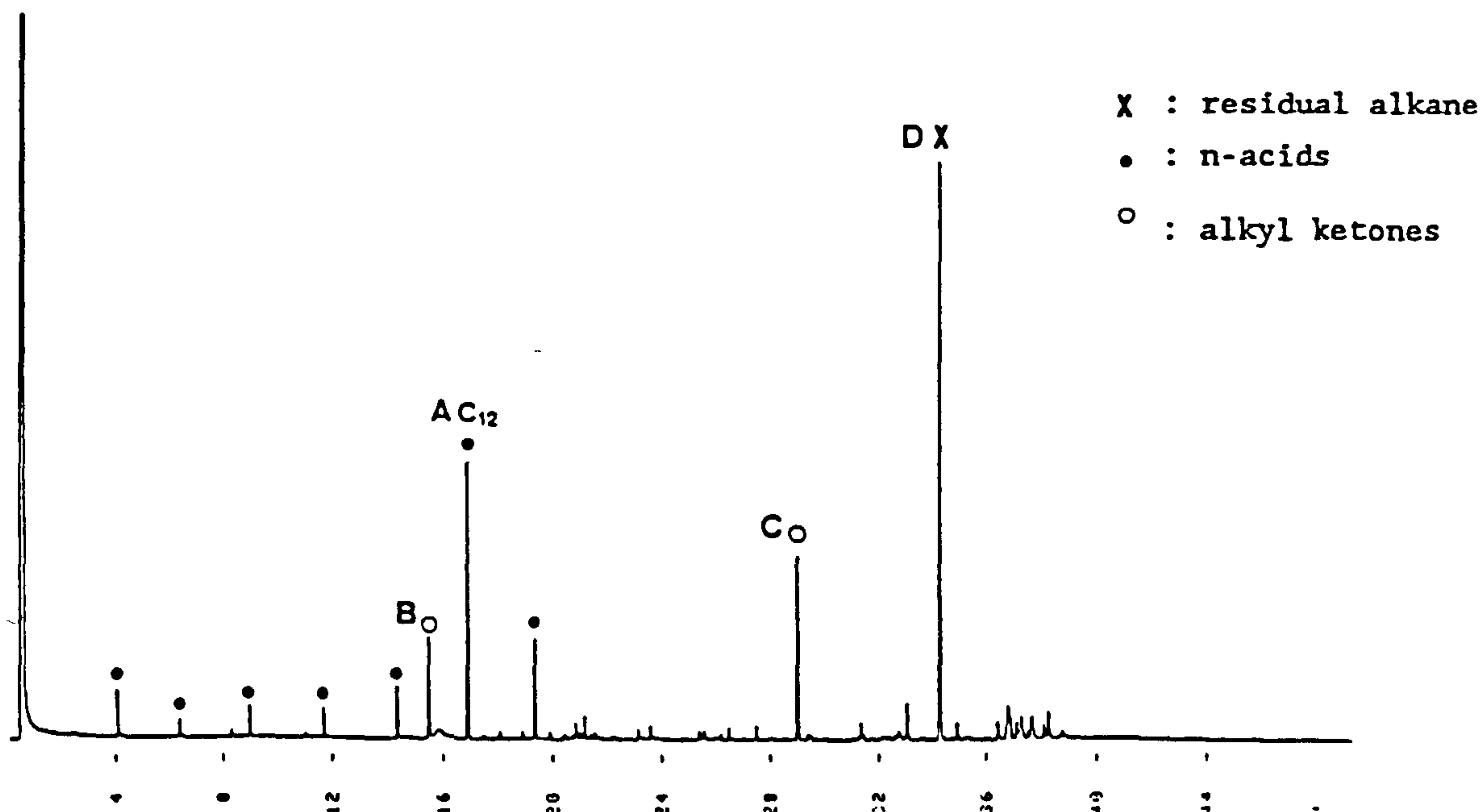


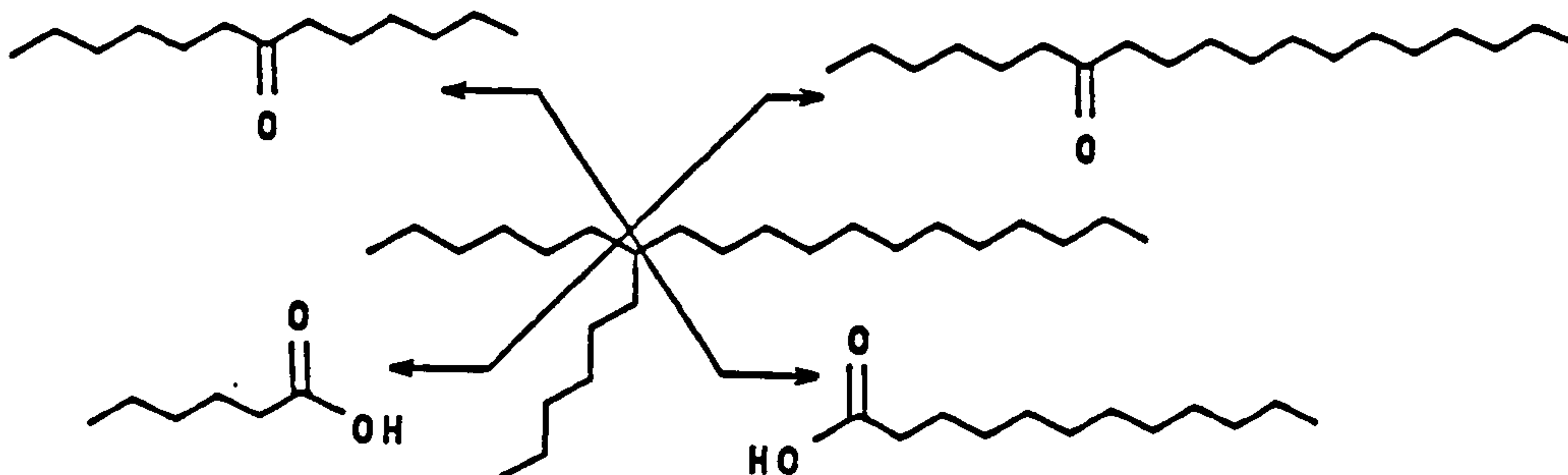
TABLE 4.4

MASS SPECTRAL DATA FOR THE CrO₃/GLACIAL ACETIC ACID OXIDATION OF 7-N-HEXYLNONADECANE (60MIN/DCM EXTRACTANT)

COMPOUND	NAME	MAJOR IONS (% ABUNDANCE)
A	<u>n</u> -dodecanoic acid ^a	74(100), 87(48), 183(10), 214(8)
B	<u>n</u> -tridecan-7-one	58(95), 71(55), 85(6), 113(100), 128(26), 198(5)
C	<u>n</u> -nonadecan-7-one	58(100), 71(89), 113(74), 128(30), 197(28), 212(8), 282(2)
D	7- <u>n</u> -hexylnonadecane	57(100), 182(30), 266(28), 267(38), 252(0.2)

a: as methyl ester

from oxidative cleavage adjacent to the tertiary C atom, viz:

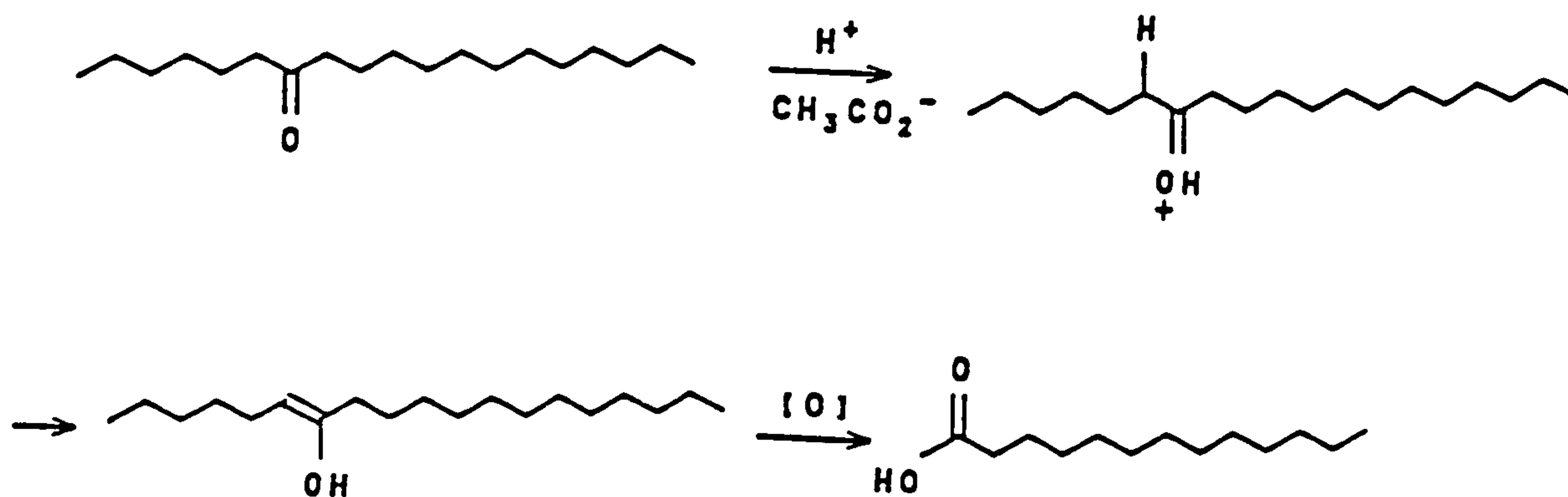


The predicted C₆ monocarboxylic acid was not recovered, presumably due to its volatility or preferential solubility in the aqueous layer.

It is interesting to note the abundance of the C₁₂ monocarboxylic acid relative to the alkyl ketone derived from cleavage of the same C-C bond. On a molar basis and assuming comparable GC response factors a 1:1 mixture would be expected. The proportion of the acid is however almost twice that of the ketone (as estimated from integrated peak areas). This possibly represents the greater lability of the ketone to additional oxidant in the reaction mixture, a feature which is commonly observed in the CrO₃ oxidation of saturated hydrocarbons (Wiberg, 1965).

Other components observed as minor products of the oxidation of 7-n-hexylnonadecane were a homologous series of n-monocarboxylic acids in the range C₇-C₁₃. From previous considerations (Section 4.2.1), these must be produced from secondary oxidation of the predicted

alkyl ketone(s). The C13 acid is not a product predicted from theory, although its origin can be explained by assuming acid catalysed enolisation of the higher ketone followed by oxidative cleavage of the double bond, i.e.:



Such a reaction sequence has been proposed for the CrO_3 oxidation of aliphatic ketones in acidic media (Wiberg, 1965).

4.2.3 OXIDATION OF N-PENTACOSANE

The CrO_3 /glacial acetic acid oxidation of n-pentacosane (Aldrich) produced a mixture of compounds in moderate yield (51%, Table 4.2). GC (Fig. 4.34) and GC-MS analyses showed these to comprise, as major components, a homologous series of n-alkanoic acids (C6-C24, max. C8). Also identified though in lesser abundance was a homologous series of n- α,ω -diacids (C7-C20, max. C10). A much higher abundance of residual alkane was noted in comparison to the oxidation of branched alkanes, consistent with the reported lower reactivity of primary and secondary C-H bonds (Cainelli and Cardillo, 1984). The EI mass spectrum (Fig. 4.35) of an additional component observed in

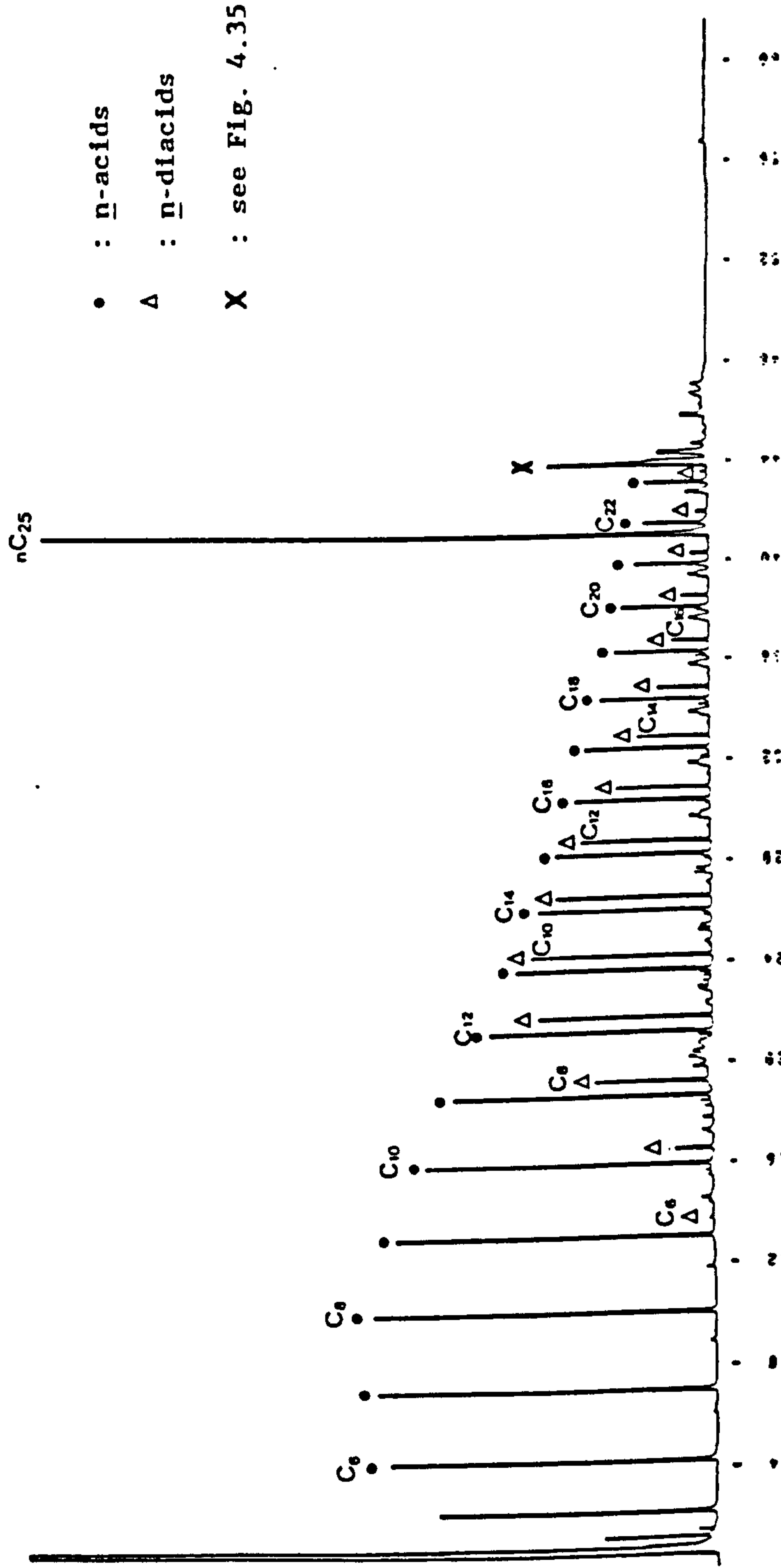


FIG. 4.34 GAS CHROMATOGRAM OF TOTAL OXIDATION PRODUCTS
(METHYLATED) OF N-PENTACOSANE

(CrO₃/glacial acetic acid; 60 min, DCH extractant)

[GC: DB-5(J+W), 25m, 50-300 °C @ 5 °C min⁻¹]

the gas chromatogram (X, Fig. 4.34) showed an ion at m/z 366 which possibly represents the molecular ion of a C₂₅ aldehyde or ketone (C₂₅H₅₀). However, no γ -H rearrangement ions (e.g. m/z 44, m/z 322; m/z 58, m/z 308; respectively) were noted to confirm these structures. A possible alternative is that the m/z 366 ion originates by loss of water ($M^+ - 18$) from a C₂₅-diol, though the exact positions of the hydroxyl groups are unclear.

The formation of a homologous series of n-alkanoic acids is consistent with previous reports that all methylene groups in a linear alkyl chain are equivalent and oxidised at the same rate (Roceck and Mares, 1959). The series of α, ω -n-diacids presumably originate from secondary oxidation of the initial n-monocarboxylic acids.

4.2.4 OXIDATION OF 2-METHYLTETRACOSANE

In contrast to the oxidation of n-pentacosane (Section 4.2.3), the major products of the CrO₃/glacial acetic acid oxidation of 2-methyltetracosane (12) were identified by GC (Fig. 4.36) and GC-MS as a homologous series of n- α, ω -diacids (C₆-C₁₈, max. C₉). This is consistent with a relatively rapid formation of the predicted n-C₂₂ acid (derived from oxidative cleavage of the C-C bond adjacent to the branch position) followed by oxidation of the alkyl chain. The low abundance of the residual alkane (Fig. 4.36) is also consistent with the known greater reactivity of tertiary C-H bonds in comparison to secondary and primary C-H bonds. The oxidation of n-monocarboxylic acids is known to preferentially take place at positions C-6 and above (Cason et al., 1959), and this may account for the absence of

FIG 4.35 EI MASS SPECTRUM OF COMPOUND "X" OBSERVED AS AN OXIDATION PRODUCT OF N-PENTACOSANE

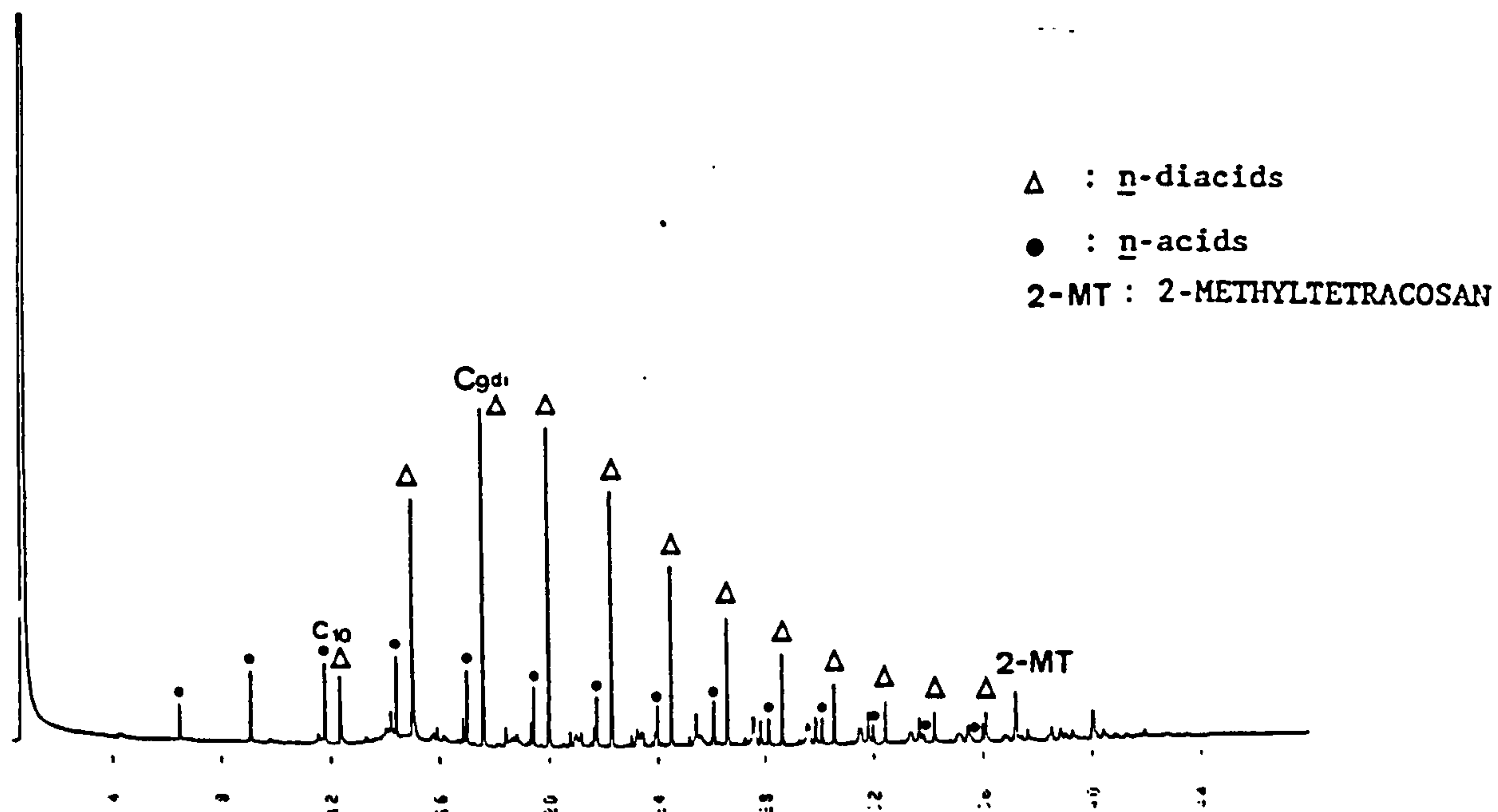
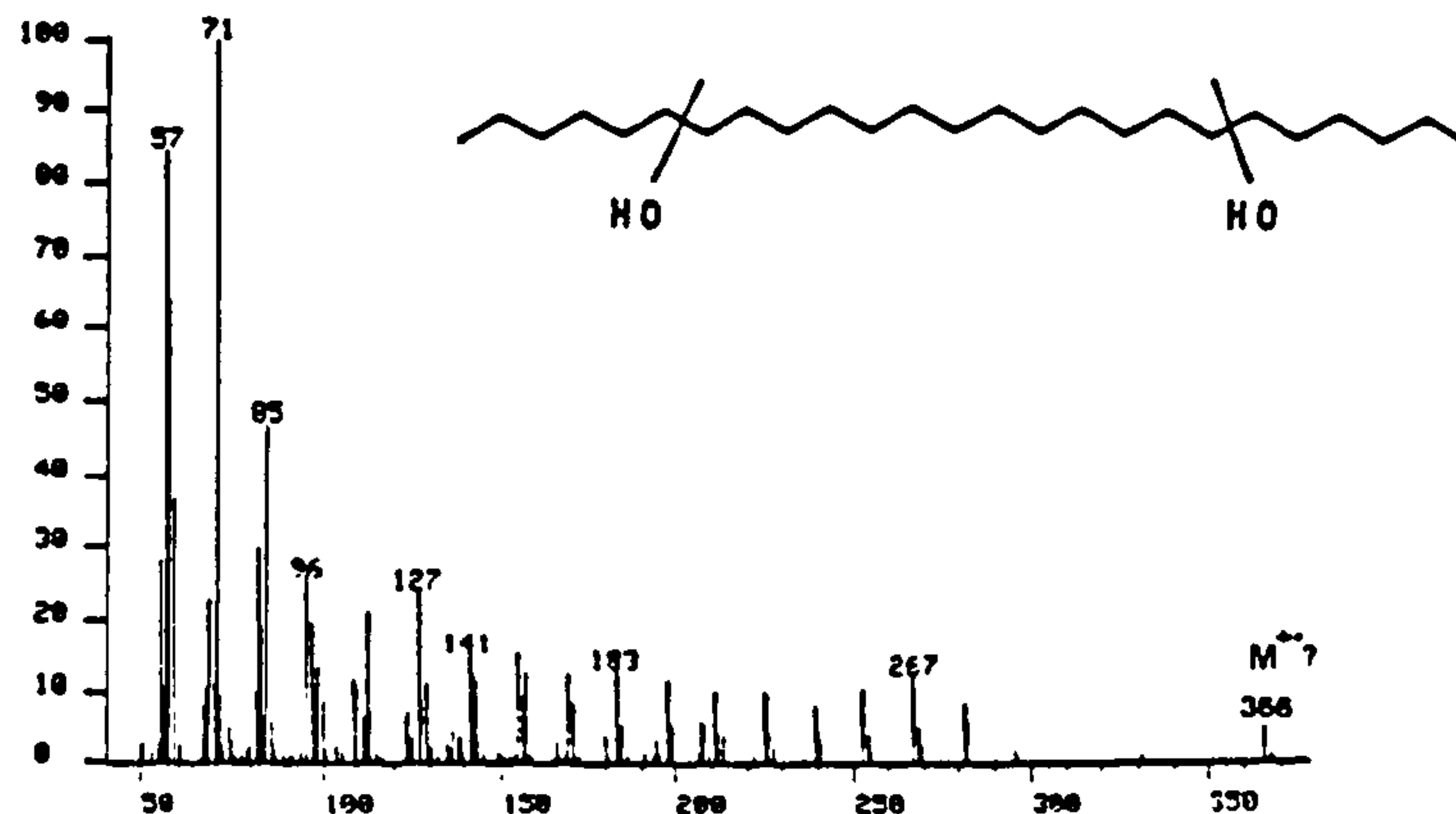


FIG. 4.36 GAS CHROMATOGRAM OF TOTAL OXIDATION PRODUCTS (METHYLATED) OF 2-METHYLTETRACOSANE

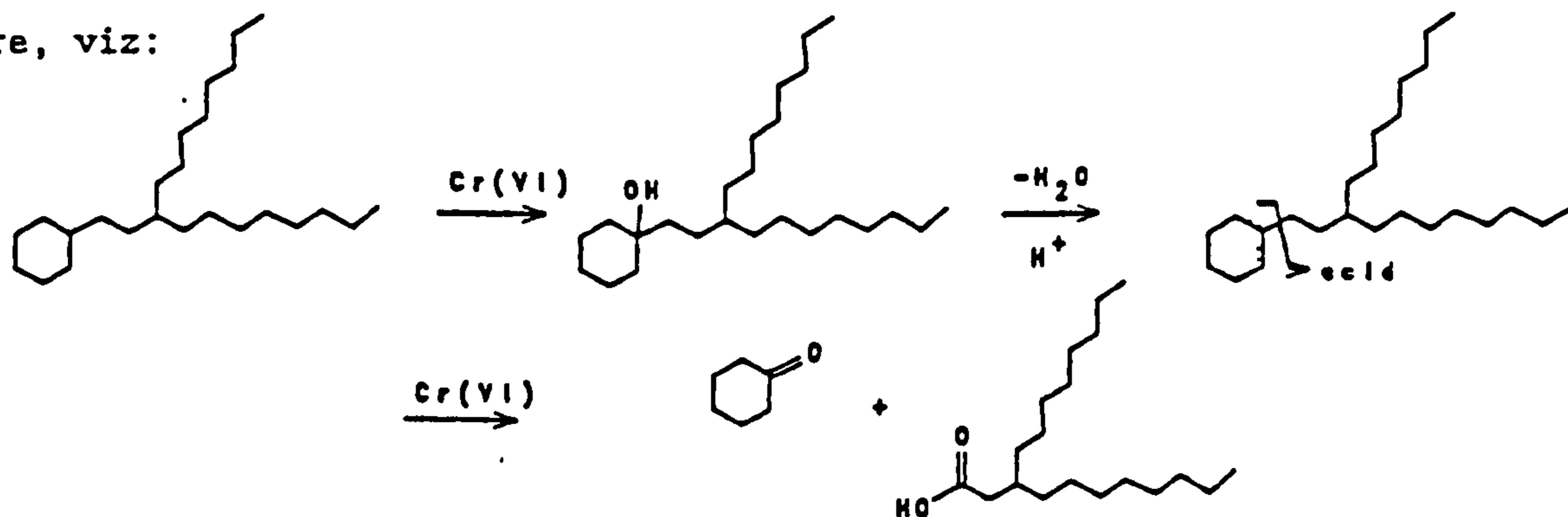
(CrO₃/glacial acetic acid; 60 min, DCM extractant)

[GC: DB-5(J+W), 25m, 50-300 °C @ 5 °C min⁻¹]

diacids below C6.

4.2.5 OXIDATION OF 9-(2-CYCLOHEXYLETHYL)-HEPTADECANE

The CrO_3 /glacial acetic acid oxidation of 9-(2-cyclohexylethyl)-heptadecane (18) produced a series of compounds in moderate yield (66%), the majority of which could be assigned by GC (Fig. 4.37) and GC-MS (EI and CI, Table 4.5). The major product was *n*-octanoic acid, i.e. the predicted product of oxidation at the tertiary position of the alkyl side chain. Other *n*-acids identified were heptanoic acid and nonanoic acid. These presumably originate from secondary oxidation of the predicted alkyl ketone, heptadecan-9-one (compound 7, Fig. 4.37). The monocyclic acid 2-cyclohexylethanoic acid (compound 3, Fig. 4.37) is also a predicted product from oxidation at the tertiary position of the alkyl side chain, whereas 3-cyclohexylpropanoic acid (compound 5, Fig. 4.37) could be derived from secondary oxidation of the predicted monocyclic alkyl ketone, 1-cyclohexylundecan-3-one (compound 8, Fig. 4.37). Compound 9 was tentatively identified as 3-octylundecanoic, possibly arising from oxidation at the ring-alkyl junction, which is also a tertiary centre, viz:



Alkyl substituted cyclohexanes are known to produce alkyl monocarboxylic acids derived from oxidation at this position (Wiberg,

FIG. 4.37 GAS CHROMATOGRAM OF TOTAL OXIDATION PRODUCTS
(METHYLATED) OF 9-(2-CYCLOHEXYLETHYL)-HEPTADECANE

(CrO₃/glacial acetic acid; 60 min, DCM extractant)

[GC: DB-5(J+W), 25m, 50-300 °C @ 5 °C min⁻¹]

(for peak identities refer to TABLE 4.5)

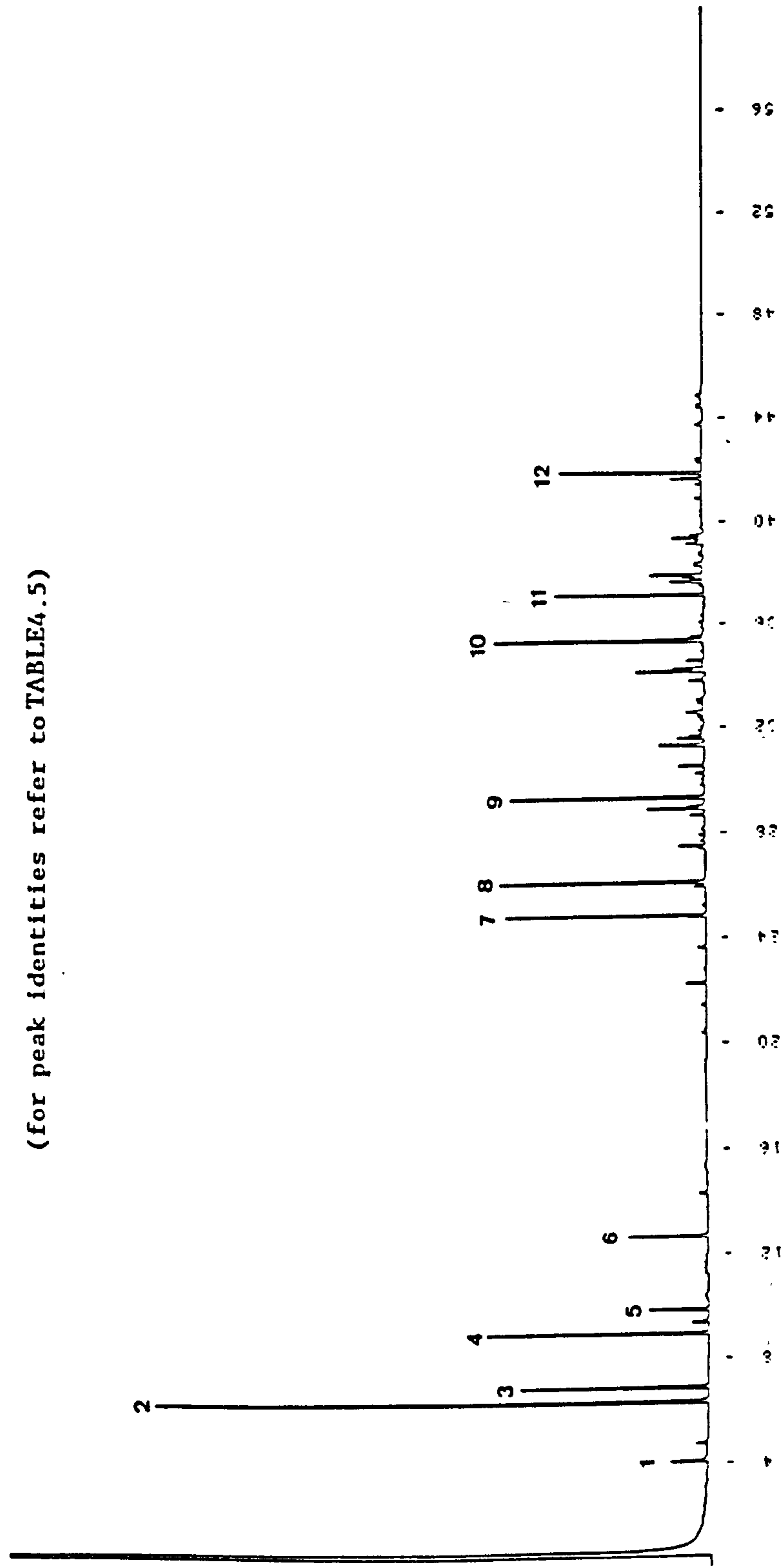


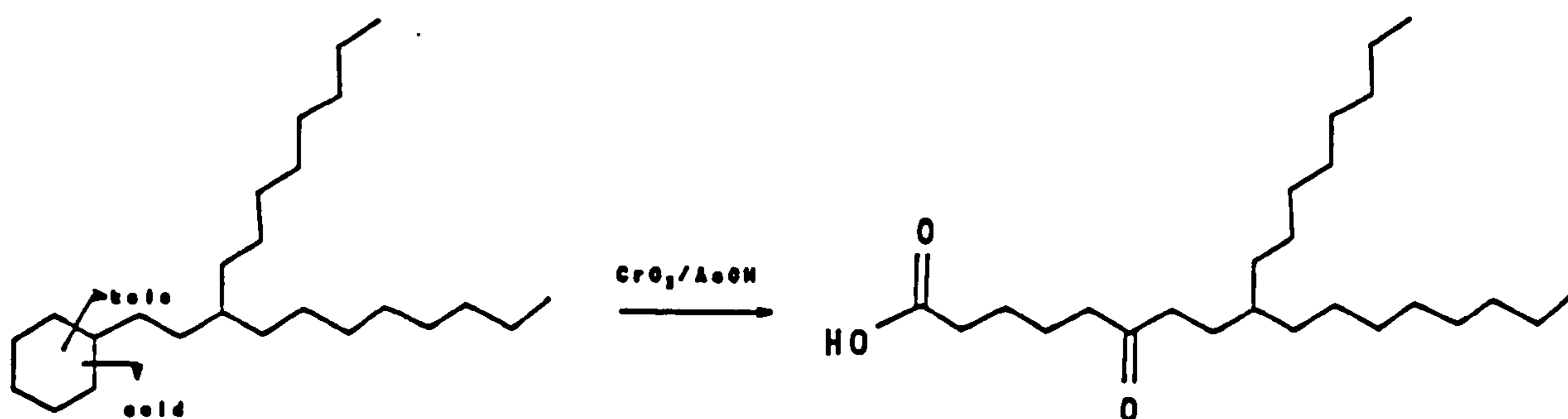
TABLE 4.5

MASS SPECTRAL DATA FOR THE CrO_3 /GLACIAL ACETIC ACID OXIDATION OF 9-(2-CYCLOHEXYLETHYL)-HEPTADECANE

COMPOUND NO	SCAN	DIAGNOSTIC IONS (M/Z) EI	CI	COMPOUND	ECL
1	86	74(100), 87(30), 113(10), 144(3)		heptanoic acid	7.00
2	160	74(100), 87(48), 127(17), 158(4)		octanoic acid	8.00
3	178	74(100), 97(4), 113(4), 125(7), 156(2)		2-cyclohexylethanoic acid	8.25
4	232	74(100), 87(42), 141(8), 172(1)		nonanoic acid	9.00
5	260	87(100), 97(96), 74(86), 139(13), 170(1.2)		3-cyclohexylpropanoic acid	9.39
6	296	56(100), 69(43), 84(84), 99(67), 117(95), 157(10)		unknown	10.17
7	629	71(100), 58(60), 141(94), 156(4), 169(20)	(255/272)	heptadecan-9-one	16.26
8	663	96(100), 121(55), 141(28), 157(41), 169(12), 252(4)	(253/270)	1-cyclohexyl- undecan-3-one	16.88
9	736	74(100), 199(28), 238(6), 255(0.8), 281(1.1), 312(1.4)	(313/331)	3-octylundecanoic acid	18.21
10	883	83(98), 97(100), 236/237(60), 238/239(30)	-	9-(2-cyclohexylethyl)- heptadecane	20.90
11	930		(386/403)	unknown	21.76
12	1106		(412/429)	unknown	24.98

1965).

Other compounds identified included the residual alkane (compound 10, Fig. 4.37) and two additional compounds of higher molecular weight (compounds 11 and 12, Fig. 4.37). Electron impact mass spectra for these latter two components were weak and did not provide significant diagnostic ions. However, CI provided possible molecular masses of 384 and 410 respectively. Thus compound 12 possibly represents a C25 keto acid which could conceivably be produced from oxidation at the second tertiary centre, i.e.



The corresponding C7 keto acid 6-oxo-heptanoic acid is reported to be a major product in the CrO_3 oxidation of methylcyclohexane (Wiberg, 1965).

4.2.6 OXIDATION OF 9-(2-PHENYLETHYL)-HEPTADECANE

The CrO_3 /glacial acetic acid oxidation of the alkyl aromatic

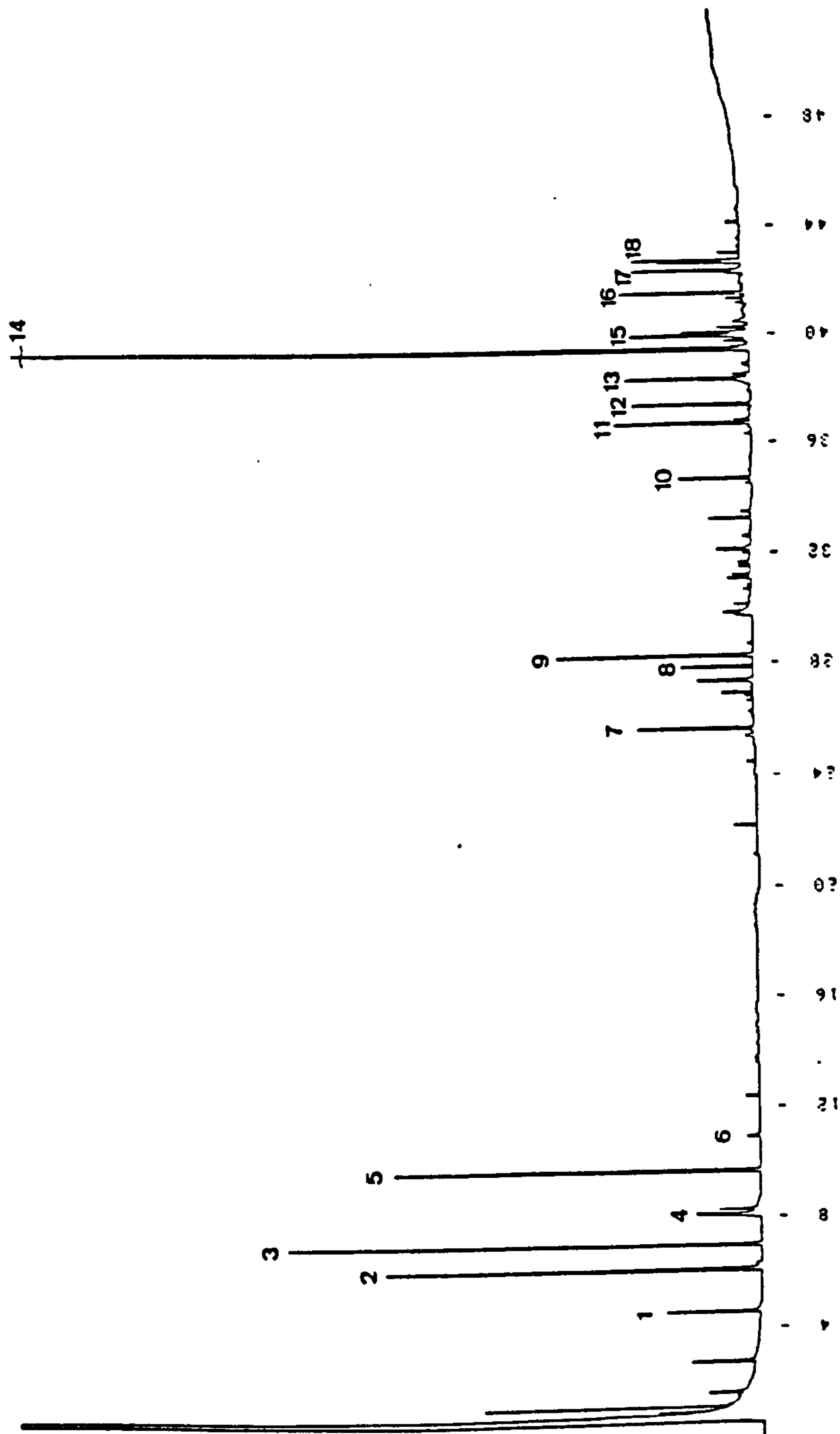
hydrocarbon 9-(2-phenylethyl)-heptadecane (23) produced a mixture of compounds in moderate yield (68%, Table 4.2). GC (Fig. 4.38) and GC-MS (Table 4.6) revealed a series of products of low molecular weight which were assigned as alkyl and alkylaromatic acids (as methyl esters). Octanoic and 2-phenylethanoic acids are predicted products of the CrO_3 oxidation at the tertiary position of the alkyl substituent (c.f. the oxidation of 9-(2-cyclohexylethyl)-heptadecane, section 4.2.5). The C7 and C9 aliphatic acids presumably arise through secondary oxidation of the predicted ketone, heptadecan-9-one (compound 7, Fig. 4.38). By analogy, benzoic acid and 3-phenylpropanoic acid possibly originate from secondary oxidation of the alkylaromatic ketone 1-phenylundecan-3-one (compound 8, Fig. 4.38). 2-octyldecanoic acid (compound 9, Fig. 4.38) appears to have been formed via oxidative cleavage of the isomerised and resonance stabilised intermediate alkene 1-phenyl-3-octylundec-1-ene (compound 12, Fig. 4.38).

The most abundant product identified (compound 14, Fig. 4.38) exhibited a mass spectrum (Fig. 4.39) indicative of a C25 alkyl aromatic ketone derived from oxidation at the benzylic position. Benzylic oxidation is a common feature in the CrO_3 oxidation of aryl alkanes and alkylaryl ketones are often observed as major products (Cainelli and Cardillo, 1984). Attack at this position is presumably favoured as a result of the high degree of stability of the proposed intermediate radical species formed by a hydrogen abstraction mechanism (i.e. similar to that described for the oxidation of alkanes and the aromatic UCM; Chapter 3).

Other minor products were also found to exhibit m/z 120 as the base

FIG. 4.38 GAS CHROMATOGRAM OF TOTAL OXIDATION PRODUCTS
(METHYLATED) OF 9-(2-PHENYLETHYL)-HEPTADECANE

(CrO₃/glacial acetic acid; 60 min, DCM extractant)



[GC: DB-5(J+W), 25m, 50-300 °C @ 5 °C min⁻¹]

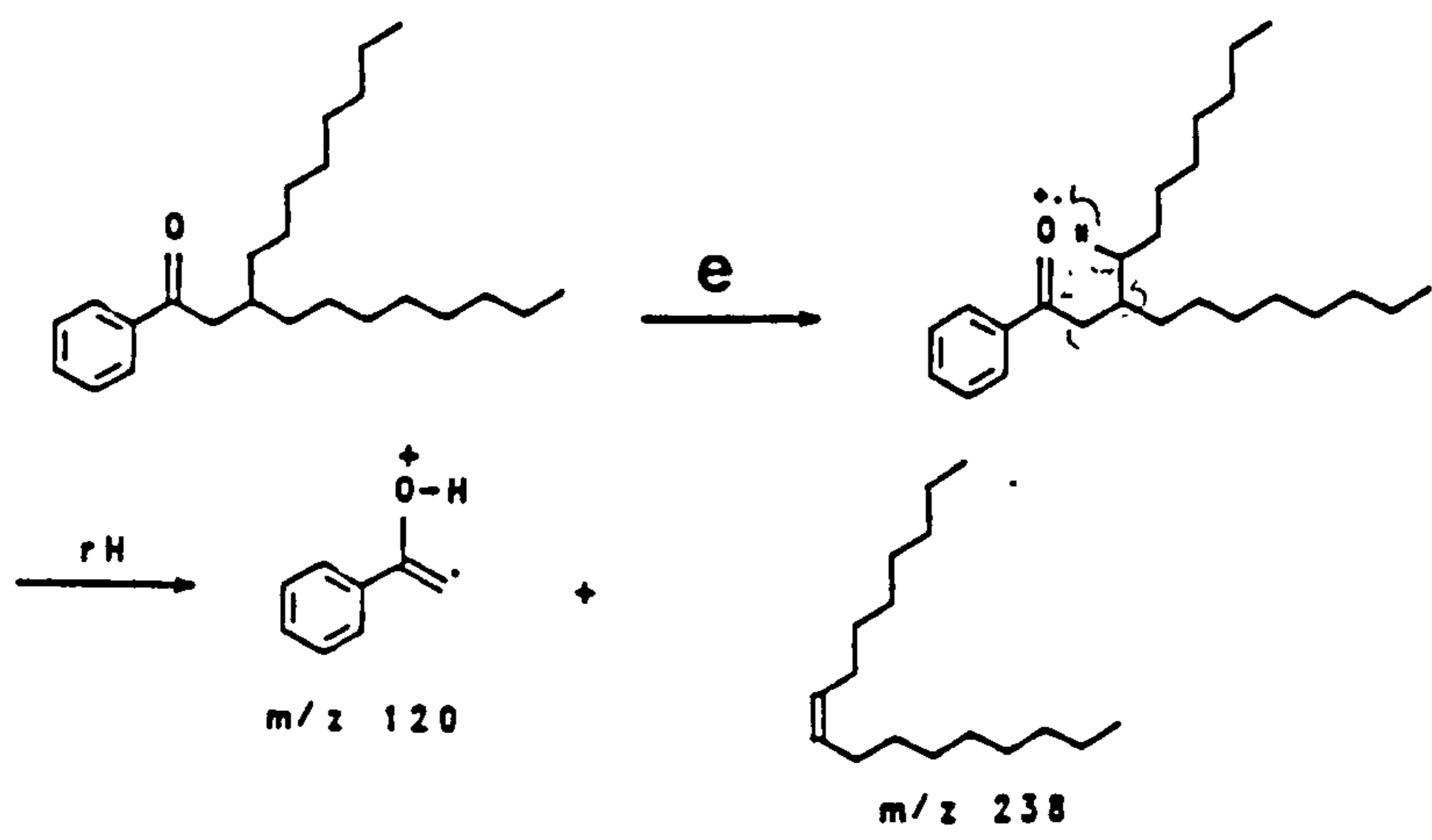
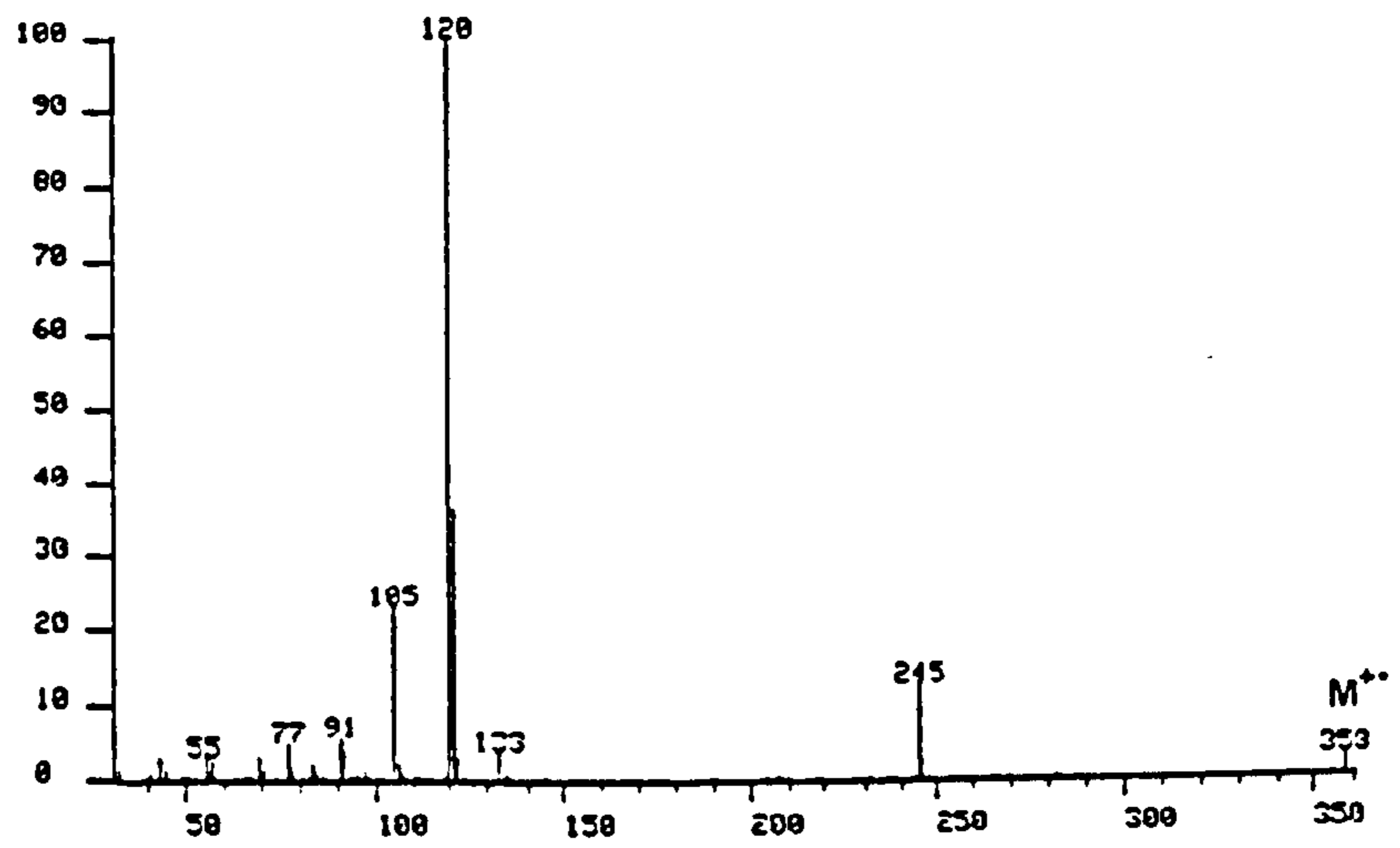
(for peak identities refer to TABLE 4.6)

TABLE 4.6

MASS SPECTRAL DATA FOR THE CrO_3 /GLACIAL ACETIC ACID OXIDATION OF 9-(2-PHENYLETHYL)-HEPTADECANE

COMPOUND NO.	SCAN	MAJOR IONS [M/Z (%ABUNDANCE)]	COMPOUND	ECL
1	34	74(100), 87(38), 101, (15), 113(16), 144(1)	heptanoic acid	7.00
2	75	105(100), 77(40), 136(40), 136(40)	benzoic acid	7.64
3	98	74(100), 87(42), 127(12), 158(2)	octanoic acid	8.00
4	129	92(100), 119(2), 135(2), 150(34)	2-phenylethanoic acid	8.49
5	161	74(100), 87(50), 141(13), 172(4)	nonanoic acid	9.00
6	193	104(100), 91(50), 77(10), 133(10), 164(30)	3-phenylpropanoic acid	9.61
7	541	141(100), 71(72), 58(48), 169(29), 254(5)	heptadecan-9-one	16.26
8	595	120(100), 105(72), 77(17), 133(10), 246(6)	1-phenylundecan-3-one	17.59
9	601	186(100), 87(79), 74(15), 200(4), 267(1), 298(6)	2-octyldecanoic acid	17.71
10	757	120(100), 105(68), 77(9), 198(7), 245(0.3), 287(2)	unknown	20.80
11	803	120(100), 105(100), 77(12), 259(1.4), 281(0.2)	unknown	21.70
12	817	229(100), 117(85), 91(68), 104(50), 342(20)	1-phenyl-3-octyl undec-1-ene	21.98
13	842	120(100), 105(50), 77(10), 245(10)	unknown	22.48
14	866	120(100), 105(15), 77(5), 91(7), 245(15), 358(4)	1-phenyl-3-octyl undecan-1-one	22.95

FIG. 4.39 EI MASS SPECTRUM OF COMOUND 14: THE MAJOR PRODUCT OF THE CrO_3 /GLACIAL ACETIC ACID OXIDATION OF 9-(2-PHENYLETHYL)-HEPTADECANE



peak in their EI mass spectra, though molecular ions were absent. These possibly represent products oxidised at both the benzylic position and the tertiary centre; their exact nature however remains unclear.

4.2.7 OXIDATION OF 2,6,10,14-TETRAMETHYLPENTADECANE (PRISTANE)

Two CrO_3 /glacial acetic acid oxidations of pristane (Pfalz and Bauer Inc.) were performed, the first for 60 minutes with hexane as the extractant (i.e. using the methods of Brooks et al., 1977) and the second using a modified procedure with DCM as the extractant. With hexane a typically low yield of total methylated oxidation products was recovered (5%, e.g. Patience et al., 1979) and GC-MS (Fig. 4.39) revealed a series of isoprenoid acids and ketones similar to those reported previously for the chromic acid oxidation of pristane (e.g. Patience et al., 1979; Rowland, 1977).

With DCM as the extractant a much higher yield of products was obtained (84%) and GC (Fig. 4.40) showed an additional series of compounds of lower molecular weights. GC-MS (Table 4.7) showed the most abundant compounds were the expected series of isoprenoid acids and ketones derived from oxidative cleavage at the methyl group positions. Thus compounds 1 (4-methylpentanoic acid), 2 (6-methylheptan-2-one), 6 (4,8-dimethylnonanoic acid), 7 (6,10-dimethylundecan-2-one), 12 (4,8,12-trimethyltridecanoic acid) and 13 (6,10,14-trimethylpentadecan-2-one) are all primary oxidation products predicted from theory (Chapter 3). An isoprenoid keto acid 8-oxo-4-methylnonanoic acid (compound 9, Fig. 4.40) was also identified from the EI mass spectrum (Fig. 4.41); this presumably

FIG. 4.40 PARTIAL TOTAL ION CHROMATOGRAM OF THE TOTAL OXIDATION PRODUCTS (METHYLATED) OF PRISTANE

(CrO₃/glacial acetic acid; 60 min, hexane extractant)

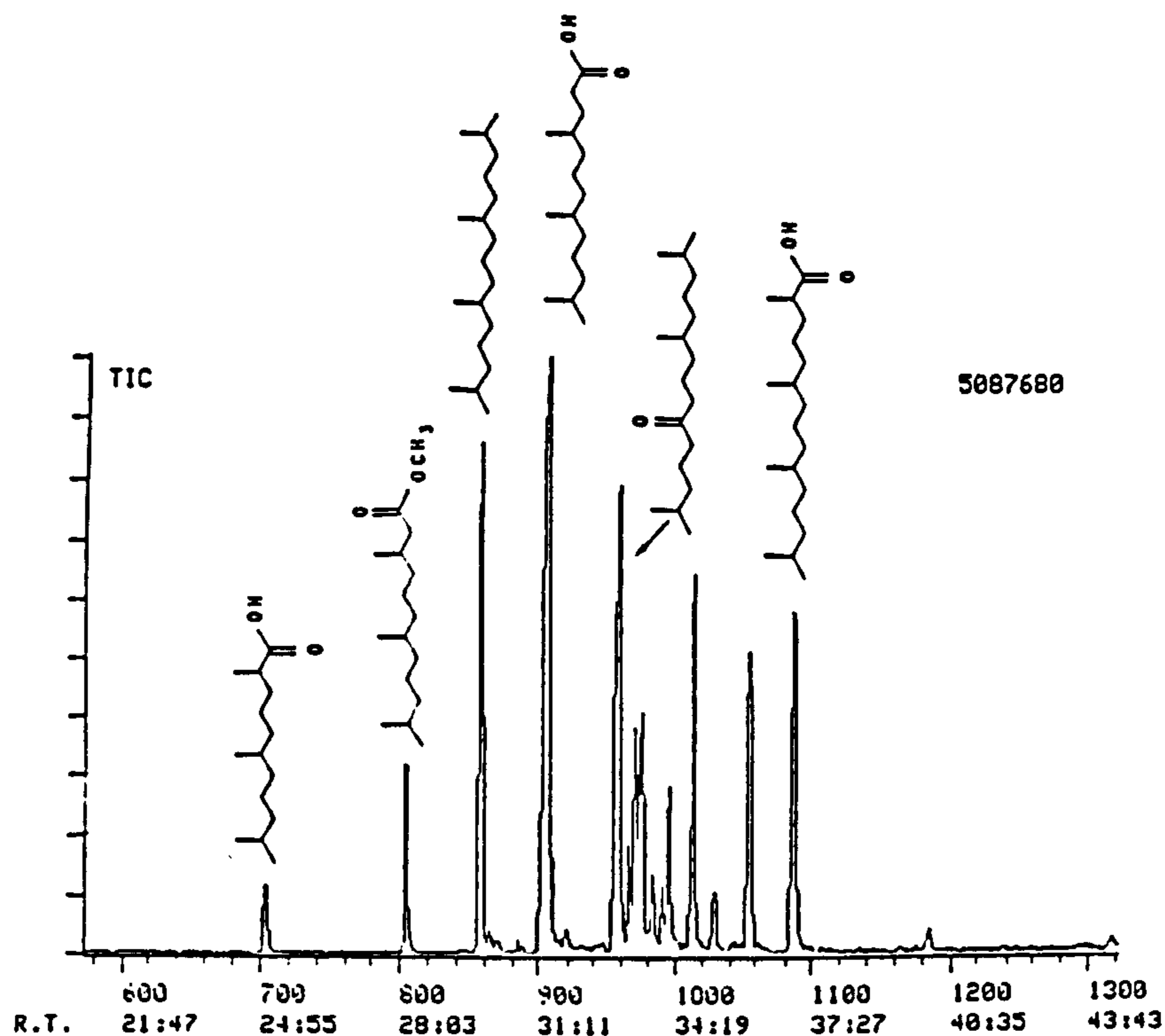
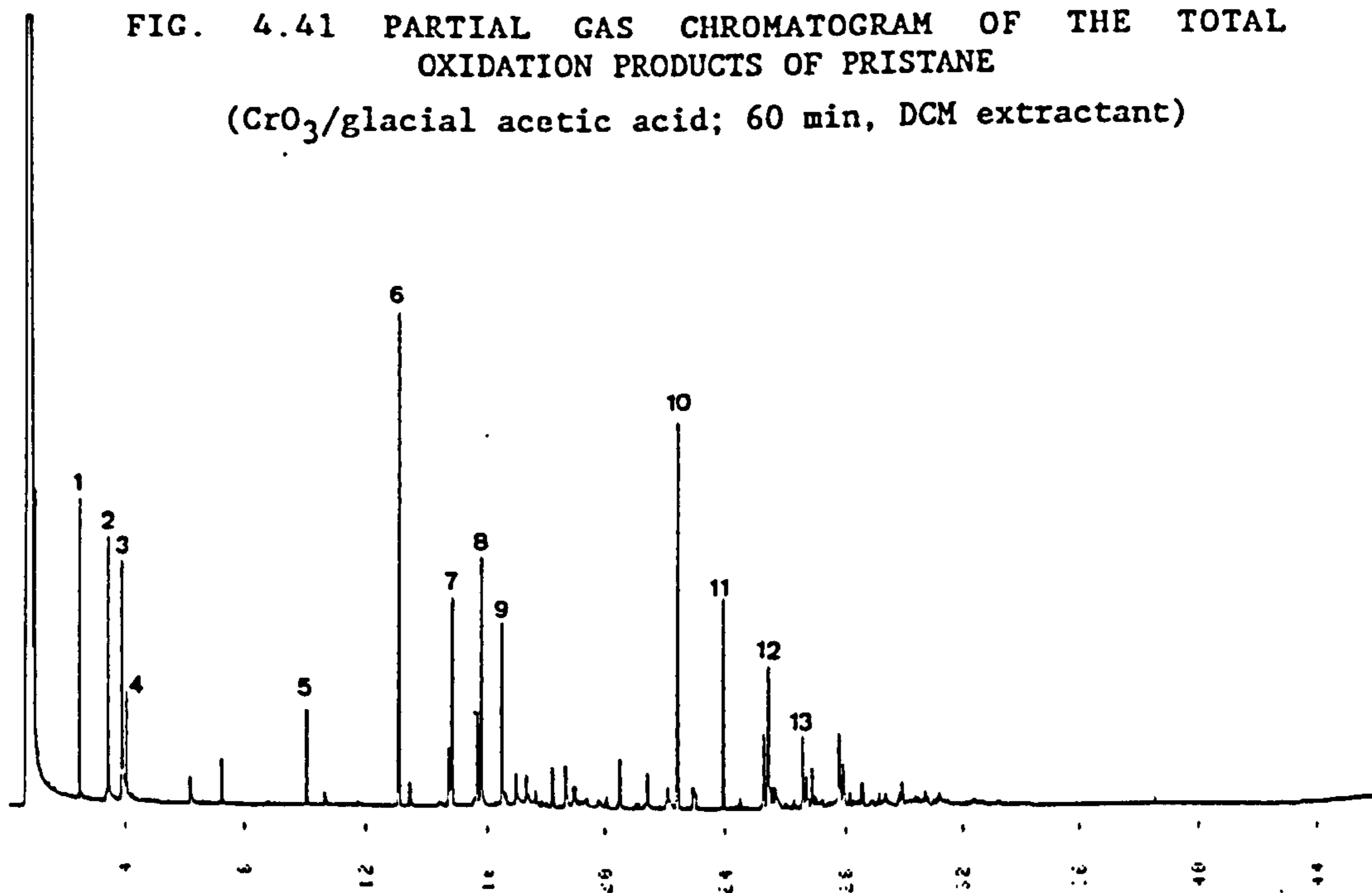


FIG. 4.41 PARTIAL GAS CHROMATOGRAM OF THE TOTAL OXIDATION PRODUCTS OF PRISTANE

(CrO₃/glacial acetic acid; 60 min, DCM extractant)



[GC: DB-5(J+W), 25m, 50-300 °C @ 5 °C min⁻¹]

(for peak identities refer to Table 4.7)

TABLE 4.7

MASS SPECTRAL DATA FOR THE CrO₃/GLACIAL ACETIC ACID OXIDATION
OF PRISTANE (60 MIN/DCM EXTRACTANT)

COMPOUND	SCAN	MAJOR IONS [m/z (% ABUNDANCE)]	COMPOUND *	PREDICTED PRODUCT	APPROX ECLVAL	ECL ^{a,b} VALUES
1	89	74(100), 87(60), 99(32), 115(8)	4-methyl- pentanoic acid	✓	5.98	-
2	130	58(100), 71(24), 95(15), 110(14), 113(3), 128(4)	6-methylheptan- -2-one	✓	6.37	6.36 ^b
3	150	99(100), 114(3)	C6 γ-methyl -γ-lactone		6.58	6.61 ^a
4	154	74(100), 87(22), 101(20) 113(6), 129(1)	5-methylhexanoic acid		6.64	6.67 ^a
5	345	74(100), 87(5), 101(60), 143(4), 155(5), 171(1.5) 186(0.6)	3,7-dimethyl- octanoic acid		9.03	-
6	434	87(100), 74(59), 127(12)	4,8-dimethyl- nonanoic acid	✓	10.16	-
7	485	58(100), 71(15), 109(17), 140(10), 180(14)	6,10-dimethyl undecan-2-one	✓	10.81	
8	515	99(100), 114(4), 129(2), 151(2)	γ-methyl-γ- lactone(?)		11.17	-
9	531	55(74), 58(45), 74(25), 87(58), 111(100), 143(72), 169(11), 185(1)	8-oxo-4-methyl nonanoic acid		11.44	11.44 ^b
10	694	57(100), 113(25), 183(10), 197(1), 253(1)	2,6,10,14-tetra- methyl pentadecane (pristane)		13.82	-
11	714	99(100), 88(10), 113(2), 169(2), 197(2)	4-hydroxy-4,8- dimethyl nonanoic acid-γ-lactone		14.06	14.19 ^a
12	737	74(36), 87(100), 157(8), 255(0.2), 270(0.6)	4,8,12-trimethyl tridecanoic acid	✓	14.50	-
13	780	58(100), 71(68), 112(18), 149(10), 199(14), 210(3), 250(4)	6,10,14-trimethyl pentadecan-2-one	✓	15.16	-

* acids identified as methyl esters

a observed as a product of the aliphatic UCM

b observed as a product of the aromatic UCM

FIG. 4.42 EI MASS SPECTRUM OF A C10 KETO ACID OBSERVED AS AN OXIDATION PRODUCT OF PRISTANE (compound 9, Fig.4.41)

(McL: denotes McLafferty rearrangement ions)

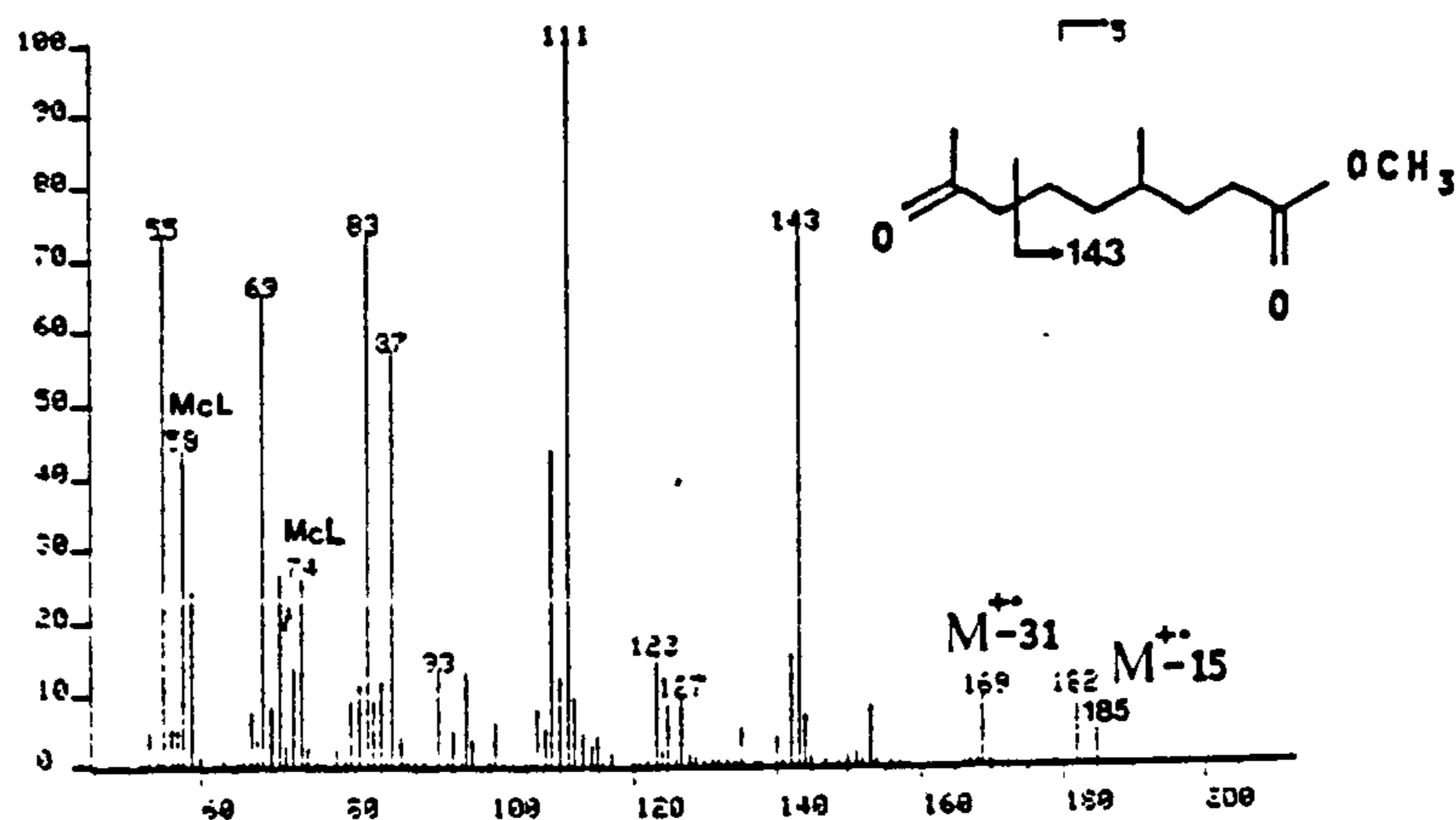
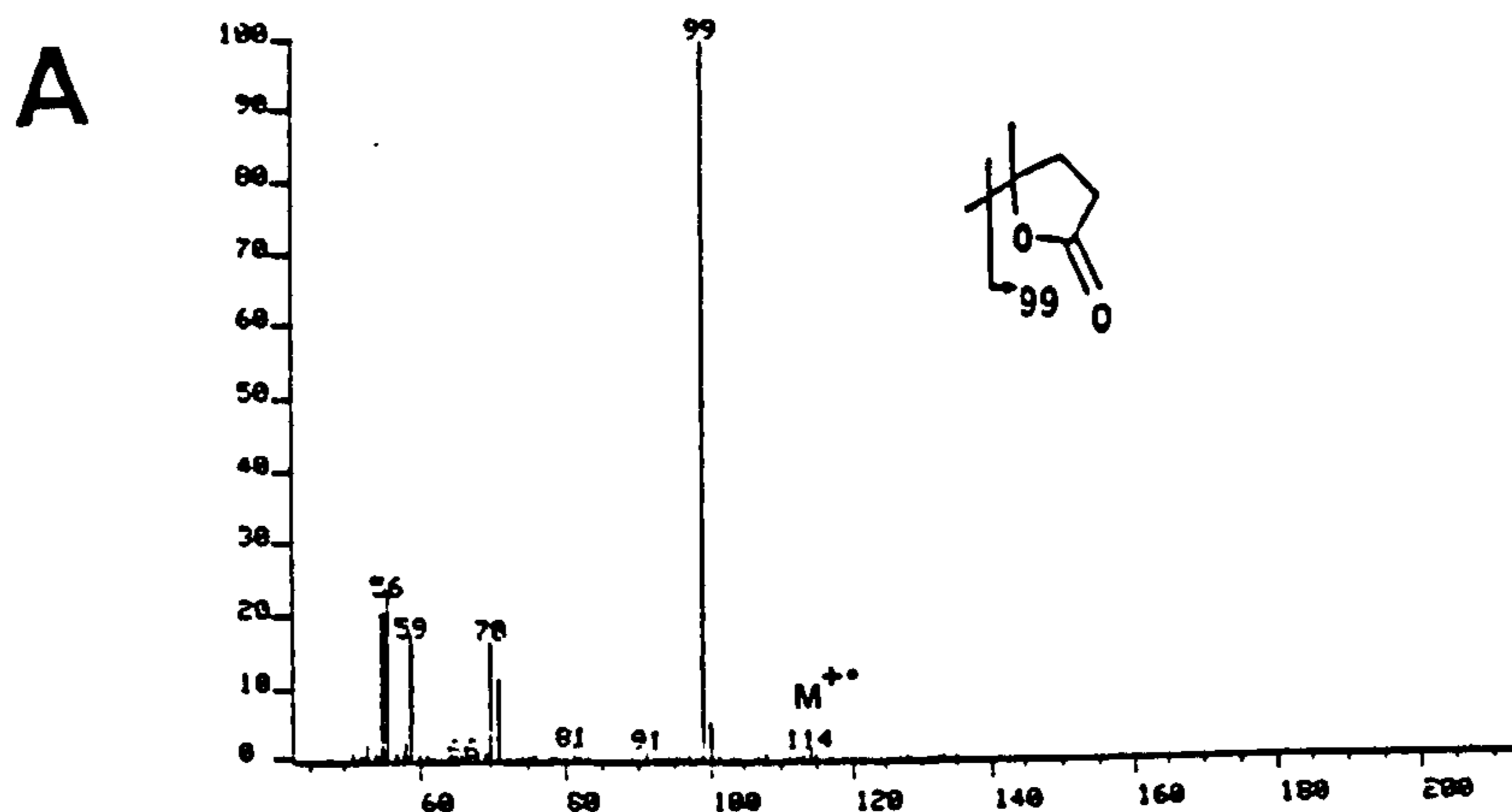
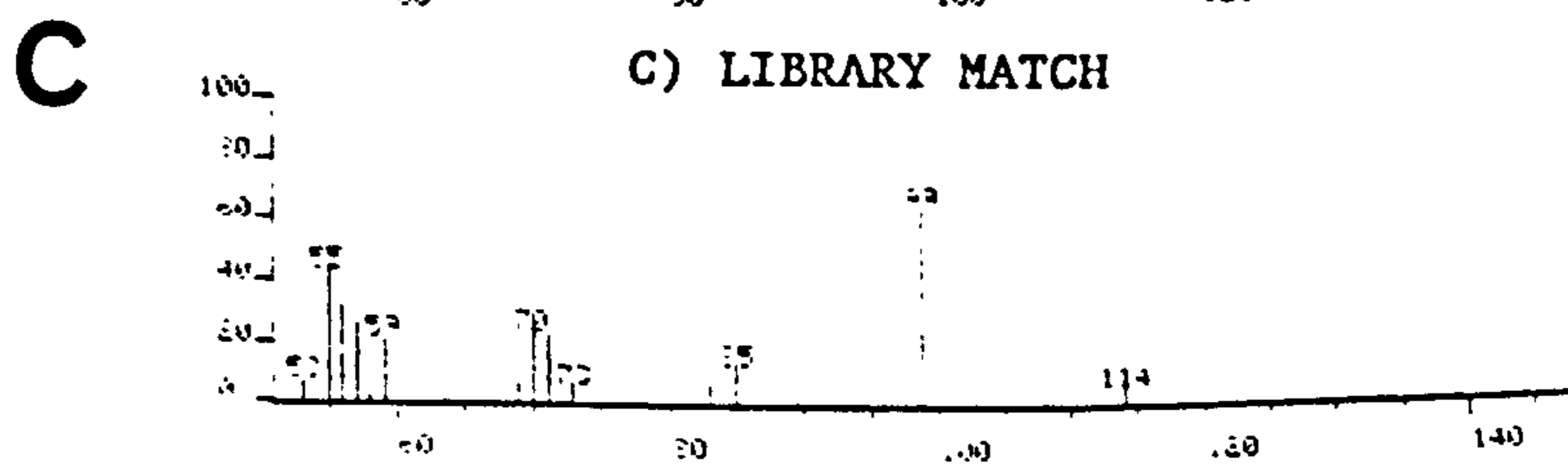
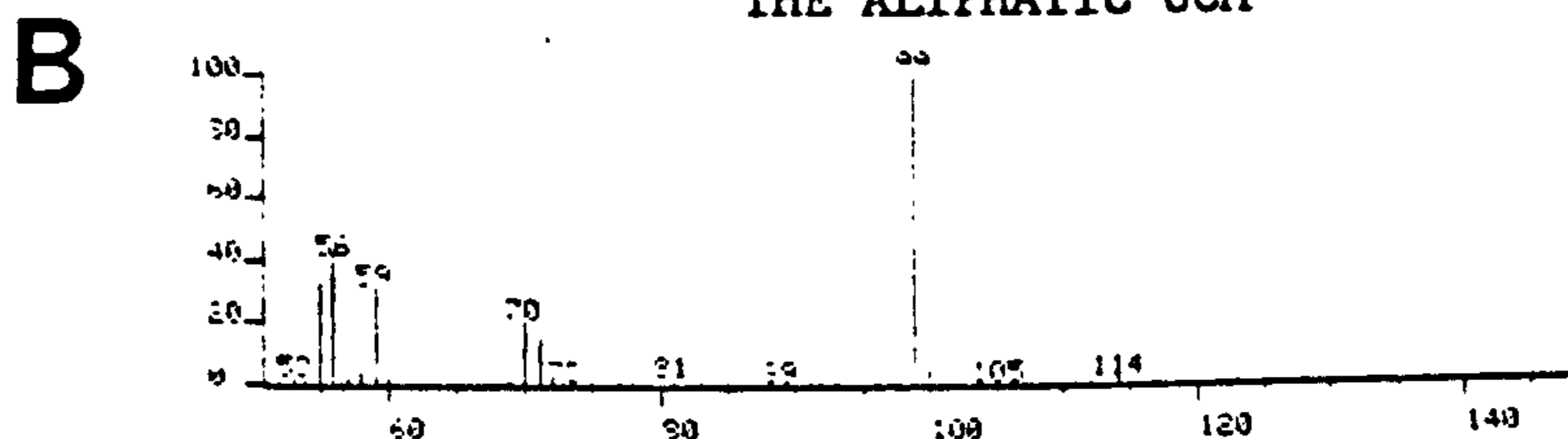


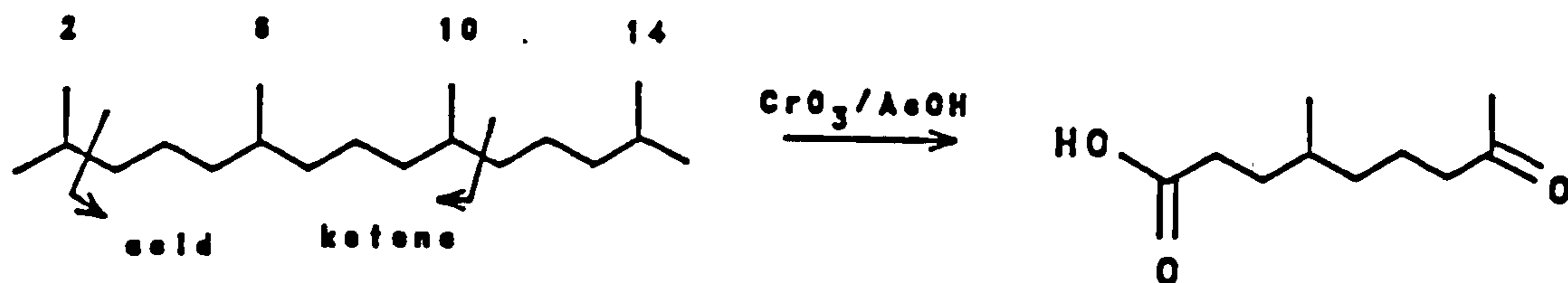
FIG. 4.43 EI MASS SPECTRUM OF A) C6- γ -METHYL- γ -LACTONE OBSERVED AS AN OXIDATION PRODUCT OF PRISTANE (compound 3, Fig. 4.41)



B) THE SAME COMPOUND OBSERVED AS AN OXIDATION PRODUCT OF THE ALIPHATIC UCM



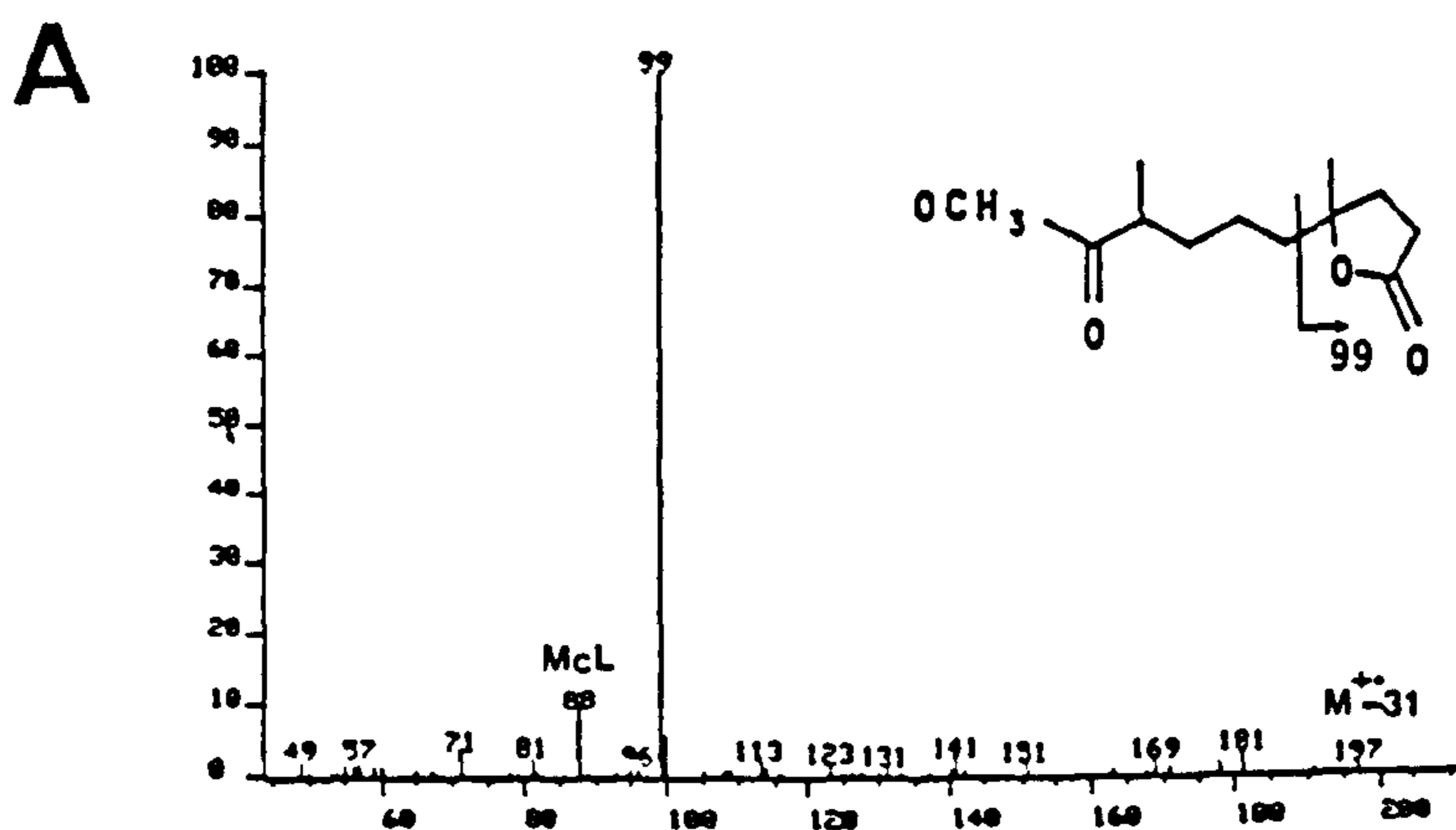
originates from oxidative cleavage at the C-2 and C-10 methyl group positions, viz:



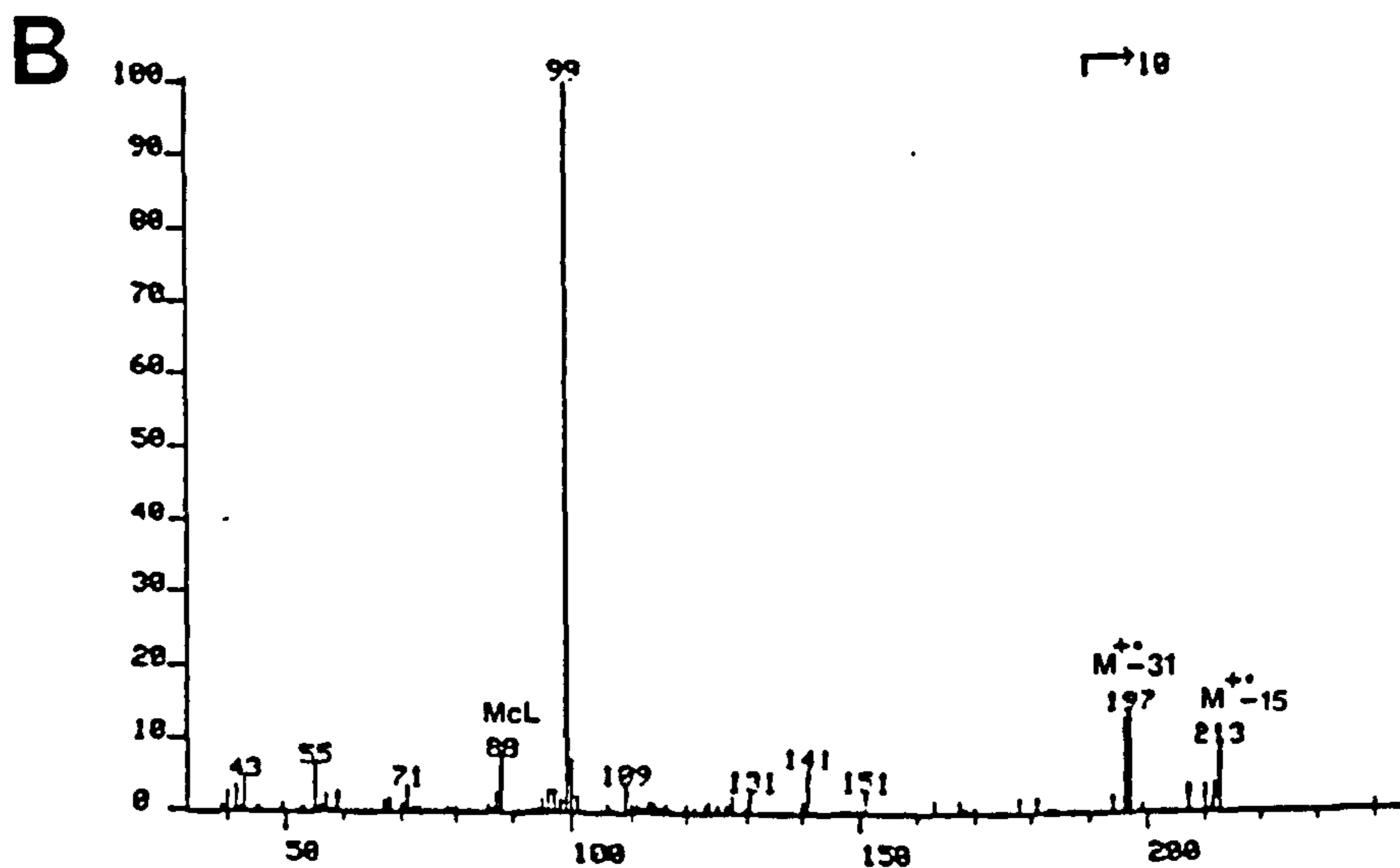
The library spectrum of the corresponding linear C10 keto acid (9-oxo-decanoic acid) was virtually indistinguishable from that of the isoprenoid keto acid and on this basis the C10 keto acid identified as an oxidation product of the aliphatic UCM (Chapter 3) was assigned a linear structure. However, a closer examination of extrapolated ECL values (Table 4.7) shows an exact match between the keto acid derived from pristane and that observed as a product of the aliphatic UCM.

Other products of pristane oxidation included a series of γ -methyl- γ -lactones similar to those observed as oxidation products of the aliphatic UCM and the model alkane 9-methyltetracosane (Sections 3.1.4 and 4.2.1 respectively). Thus compound 3 (Fig. 4.40) was identified from the mass spectrum (Fig. 4.42A) as a C6 γ -methyl- γ -lactone derived from intramolecular esterification of 4-hydroxy-4-methylpentanoic acid. The spectrum matched well with a C6 γ -methyl- γ -lactone observed as an oxidation product of the aliphatic UCM and present as a library entry (Fig. 4.42B+C). The identification of compound 11 as a methyl branched γ -methyl- ω -carboxy- γ -lactone is significant since a similar series of compounds were tentatively

FIG. 4.44 EI MASS SPECTRUM OF A) C11 METHYLBRANCHED ω -CARBOXY- γ -METHYL- γ -LACTONE OBSERVED AS AN OXIDATION PRODUCT OF PRISTANE (compound 11, Fig. 4.41)



B) THE SAME COMPOUND OBSERVED AS AN OXIDATION PRODUCT OF THE ALIPHATIC UCM



identified as oxidation products of the aliphatic UCM (Section 3.1.4). . Indeed, a comparison of ECL values (Table 4.7) and EI mass spectra (Fig. 4.43) confirm the assignment for the C11 member.

4.2.8 OXIDATION OF SYNTHETIC HYDROCARBON MIXTURE

In the oxidation of model compounds the yields of oxidation products relative to unreacted hydrocarbon and the yields of total recovered material were observed to vary. Although great care was taken in each oxidation to reproduce conditions exactly, the complexity of the procedure meant a degree of variability was inevitable in the handling of oxidation products, particularly during the evaporative procedures. Furthermore, secondary and possibly tertiary oxidation products were observed to form depending on the reactivity of the primary product relative to the residual hydrocarbon. This is a reported disadvantage in chromium oxidations which limits its use as a synthetic reagent (e.g. Cainelli and Cardillo, 1984). In an attempt to compare the relative susceptibility of the different model C25 hydrocarbons to these effects a mixture of all eight was oxidised (see Table 4.8 for details).

Oxidation of the mixture produced a series of compounds in moderate yield (33%, Table 4.2). GC (Fig. 4.44) and GC-MS (Table 4.9) showed the expected functionalised products, many similar to those observed for the individual model hydrocarbon and aliphatic UCM oxidations. The principal products were homologous series of n-monocarboxylic acids in the range C6-C22, with elevated abundances observed for

those acids expected from the primary oxidation of tertiary C atoms (C6-compound 3; C8-compounds 4, 5 and 6; C12-compound 3; C15-compound 5; C22-compound 7). The C7 and C9 n-monocarboxylic acids were also observed in greater abundance relative to other n-acids, these are proposed secondary oxidation products of compounds 4 (Section 4.2.5) and 6 (Section 4.2.6). Other compounds identified included n-alkylketones (C10, C13, C17 and C19); and isoprenoid-derived acids (C6, C7, C10 and C21), a ketone (C8), an oxoacid (C10) and various γ -methyl- γ -lactones. Dicarboxylic acids (with the exception of C9 and C10) were generally absent as too were ω -oxo- γ -methyl- γ -lactones.

The abundances of residual unoxidised hydrocarbons were found to vary depending on their structure. An attempt was made to semi-quantitatively determine their relative reactivity on an individual compound basis by normalising integrated peak areas to that of n-pentacosane. By this approach the synthetic alkanes showed an order of reactivity which broadly correlated with the number of tertiary C atoms available for oxidation (Table 4.8). Thus the acyclic isoprenoids 2,6,10,14-tetramethyl-7-(3-methylpentyl)-pentadecane (compound 1, 6 tertiary C atoms) and 2,6,10,14,18-pentamethyleicosane (compound 2, 5 tertiary C atoms) were reduced in abundance relative to n-C25 by 91% and 93% respectively. The slightly greater reactivity observed for the linear isoprenoid may be as a result of the geometry of the more highly branched isoprenoid in three dimensions - the central position is known to be hindered to e.g. epoxidation, from previous studies (Robson, 1987). The cycloalkane (compound 4, 2 tertiary C atoms) was next in order of reactivity followed by the singly branched alkanes containing 1 tertiary C atom.

FIG. 4.45 GAS CHROMATOGRAM OF THE TOTAL OXIDATION PRODUCTS
(METHYLATED) OF THE SYNTHETIC HYDROCARBON MIXTURE

(CrO₃/glacial acetic acid; 60 min, DCM extractant)

[GC: DB-5(J+W), 25m, 50-300 °C @ 5 °C min⁻¹]

(for peak identities refer to Table 4.9)

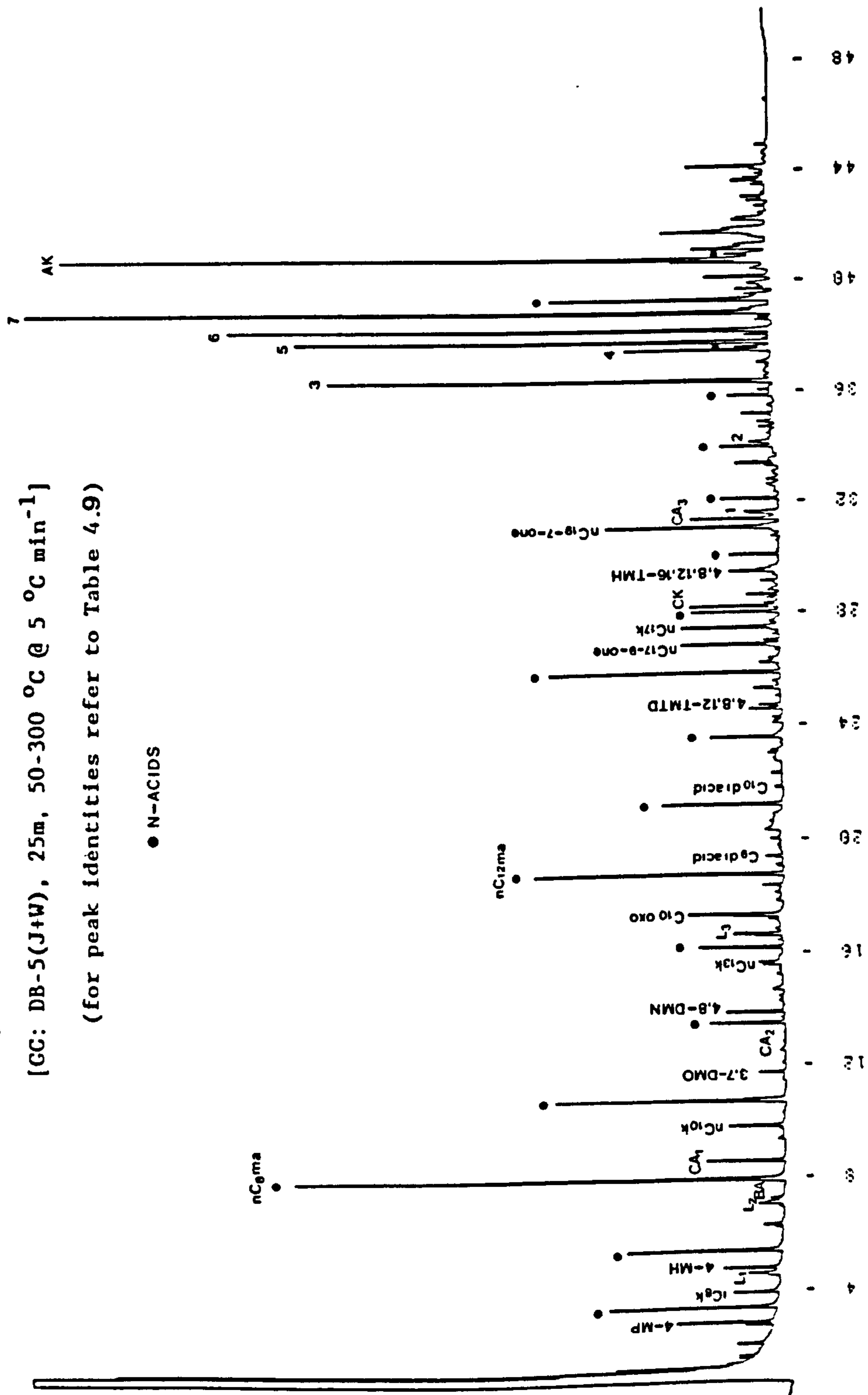


TABLE 4.8
COMPOSITION, ELUTION ORDER, AND RELATIVE REACTIVITIES OF
THE SYNTHETIC HYDROCARBON MIXTURE OXIDISED WITH CrO₃ IN GLACIAL ACETIC ACID

COMPOUND	ELUTION ORDER	No. 3° C ATOMS	NORMALISED RATIOS BEFORE[O]	AFTER[O]	% REMAINING	ORDER OF REACT- IVITY
2,6,10,14- tetramethyl -7-(3-methylpentyl) pentadecane	1	6	104	9	9	3
2,6,10,14,18- pentamethyl eicosane	2	4	98	7	7	2
7-n-hexylnonadecane	3	1	103	50	49	5
9-(2-cyclohexylethyl)- heptadecane	4	2	99	18	18	4
9-methyltetracosane	5	1	97	51	53	6
9-(2-phenylethyl)- heptadecane	6	2	106	2	2	1
2-methyltetracosane	7	1	104	62	60	7
n- pentacosane	8	0	100	100	100	8

TABLE 4.9

GC DATA FOR THE PRODUCTS OF OXIDATION OF THE SYNTHETIC HYDROCARBON MIXTURE

CODE	COMPOUND	ORIGIN ^a	ECL VALUE
4-MP	4-methylpentanoic acid	1,2	-
1C8k	6-methylheptan-2-one	1,2	6.36
L1	C6 γ -methyl- γ -lactone	1,2	6.61
4-MH	4-methylhexanoic acid	2	6.63
L2	C7 γ -methyl- γ -lactone	2	7.67
BA	benzoic acid	6	7.70
CA ₁	1-cyclohexylethanoic acid	4	8.27
nC10k	<u>n</u> -decan-1-one	5	8.69
3,7-DMO	3,7-dimethyloctanoic acid	1	9.04
CA ₂	1-cyclohexylpropanoic acid	4	9.43
4,8-DMN	4-8-dimethylnonanoic acid	2	10.15
nC13k	<u>n</u> -tridecan-7-one	3	10.81
L3	C11 branched γ -methyl γ -lactone	2	11.23
C10 oxo acid	4-methyl-8-oxo-nonanoic acid	1,2	11.44
C9 diacid	nonandioic acid	3,8	12.25
C10 diacid	decandioic acid	3,8	13.25
4,8,12-TMTD	4,8,12-trimethyltridecanoic acid	2	14.50
<u>n</u> C17-9-one	<u>n</u> -heptadecan-9-one	4,6	15.50
<u>n</u> -C17k	<u>n</u> -heptadecan-2-one	5	15.78
Ck	1-cyclohexylundecan-3-one	4	16.19
4,8,12,16-TMH	4,8,12,16-tetramethyl heptadecanoic acid	2	16.69
<u>n</u> -C19-7-one	<u>n</u> -nonadecan-7-one	3	17.51
CA ₃	3-octylundecanoic acid	4	17.63
AK	1-phenyl-3-octyl undecan-1-one	6	22.95

a: see Table 4.8

TABLE 4.10

OXIDATION OF SYNTHETIC HYDROCARBON MIXTURE -
ESTIMATED PERCENTAGE CONTRIBUTION OF ALKANES BEFORE OXIDATION AND ALKANES PLUS
IDENTIFIED PRODUCTS AFTER OXIDATION TO THE TOTAL INTEGRATED AREA RESPONSE

ALKANE TYPE*	% ALKANE BEFORE ^A [0]	%ALKANE + RELATED ^B PRODUCT AFTER [0]	CHANGE ^C
isoprenoids (1+2)	29	13	-16
7- <u>n</u> -hexylnonadecane (3)	15	15	0
9-(2-cyclohexylethyl)- (4) heptadecane	14	15	+1
9-methyltetracosane (5)	14	13	-1

a: expressed as a percentage of the total integrated GC-FID areas.

b: the summed integrated GC-FID areas of residual alkanes plus identified
alkane derived products expressed as a percentage of the total
integrated GI-FID area.

c: % alkane before [0] - % alkane + alkane-derived products after [0]

[0]- oxidation

* compound numbers refer to elution orders (e.g. Table 4.8)

The most reactive hydrocarbon under the conditions of oxidation was found to be the alkyl aromatic (compound 6) which was almost quantitatively converted by benzylic oxidation to the alkyl aromatic ketone 1-phenyl-3-octylundecan-1-one (Fig. 4.38, Table 4.9).

The observation of a greater degree of reactivity for the acyclic isoprenoids has important implications in the structural characterisation of UCMs by chemical oxidation. Of particular concern is the lability of initially formed oxidation products to further oxidation. If this were the case then the proportion of multiply branched components may be underestimated by the procedure. To determine if this were so, a semi-quantative mass balance calculation was performed by summing the integrated area responses for each component identified. Products derived from the aromatic hydrocarbon were discounted from the calculation. Prior to oxidation the acyclic isoprenoids accounted for 29% of the total integrated area response. After oxidation products identified as being derived from the isoprenoids and including the residual alkanes comprised 13% of the total integrated area response. Assuming all FID responses were equal and a quantitative transfer of alkane to identified product, then ca. 16% of the expected isoprenoid-derived products were unaccounted for. This approach was extended to other alkanes in the mixture (Table 4.10) and for these the estimated percentage contribution to the total integrated area varied by only $\pm 1\%$ before and after oxidation. On the basis of this crude calculation it would therefore appear that the oxidation procedure does underestimate the proportion of multiply branched structures present in a complex mixture, presumably through oxidation to volatile (e.g. CO₂) or more water soluble shorter chain acids. Though attempts were made to

identify water soluble products of the aliphatic and aromatic UCM oxidations (Chapter 3), none were determined in any significant abundance relative to the blank.

CHAPTER FIVE

POTENTIAL APPLICATIONS OF CHEMICAL OXIDATION

FOR "FINGERPRINTING" UCMs OF VARIED ORIGIN

5.1 INTRODUCTION

Many methods have been used for the source recognition or "fingerprinting" of crude oils and crude oil residues, both in an environmental (e.g. oil spill) context and in petroleum organic geochemistry (e.g. reviewed by Adlard 1972; Clark and Brown 1977; Seifert and Moldowan, 1978; Albaiges and Albrecht, 1979; Petrakis et al., 1980; Douglas et al., 1981; NAS, 1985; Philp, 1985; and Jones, 1986). Although it is generally accepted that a multi-technique approach is best suited for oil - oil correlations (e.g. Shen, 1984; Urdal et al., 1986), probably the single most important analytical technique has been the use of GC-MS analysis of selected series of "biological marker" compounds. In particular the distributions of acyclic isoprenoids, pentacyclic triterpanes and steranes have been shown to vary with source, maturity, and degree of in-reservoir alteration (e.g. migration/biodegradation); and as such reflect the depositional and post depositional history of the oil. These highly specific "biological marker" profiles act as useful tools in oil-oil and oil-source rock correlations (e.g. see reviews by Hunt, 1979; Mackenzie et al., 1982; Mackenzie, 1984; Tissot and Welte, 1980; Philp, 1985; Volkman and Maxwell, 1986; Philp and Oung, 1988).

Although the "biological marker" approach has been used with great success in petroleum and environmental organic geochemistry, it does suffer from certain disadvantages. Perhaps the most obvious is the often low abundance of the compounds relative to the bulk oil components (e.g. Dimmler and Strausz, 1983), which dictates the use

of sophisticated and thereby costly instrumentation (i.e. GC-MS). A second disadvantage is the alteration of "biological marker" compound distributions by microbial degradation which sometimes occurs. In particular, acyclic isoprenoids are often rapidly degraded (e.g. Bailey et al., 1973; Seifert and Moldowan, 1979; Volkman et al., 1983, 1984; Jones, 1986; Brooks et al., 1989^a) which render them less effective as source indicators. In extreme cases, the microbially more resistant polycyclic alkanes (e.g. steranes and triterpanes) may also be affected by biodegradation. This has been demonstrated both in the laboratory (e.g. Rubinstein et al., 1977; Connan, 1980; Goodwin et al., 1983; Jones, 1986) and by field observations (e.g. Reed, 1977; Seifert and Moldowan, 1979, Rullkotter and Wendisch, 1982; Mckirdy et al., 1983; Alexander, 1984). In such situations it becomes difficult to conclusively assign an origin to extremely biodegraded crude oils based on "biological marker" fingerprints alone (e.g. Seifert et al., 1984; Volkman et al., 1984).

An important, but until now largely ignored, feature of most biodegraded crude oils is the often high abundance of the unresolved complex mixture (UCM) in the gas chromatograms of both aliphatic and aromatic hydrocarbon fractions. The UCM, though present in most, perhaps all oils, becomes more evident in the early stages of microbial alteration when the majority of labile n-alkanes have been removed (e.g. Connan, 1984; Jones et al., 1983; 1986). The UCM then persists (though with some modification) throughout the biodegradation sequence, and remains even when the "biological marker" distributions have been severely affected (Volkman, 1983, 1984; Brooks et al., 1989^b). Therefore any method which enables the UCM of a biodegraded crude oil to be characterised at the molecular

level has potential as a method for "fingerprinting" that oil.

Oxidation using CrO_3 in glacial acetic acid has been shown herein to provide important information concerning the molecular structure of hydrocarbon UCMs from a paraffinic lubricating base oil (chapter 3). This approach was extended to a series of aliphatic UCMs of varied origin in the hope that oxidation might liberate specific series of resolved compounds unique to each. A rapid method of UCM enrichment was used for each of the oils studied, involving chromatographic fractionation of whole oils by argentatious TLC followed by urea adduction of the alkane fractions (R_f : 0.85 - 0.98). The efficiency of each fractionation was monitored by the use of a mixture of authentic hydrocarbons containing n -eicosane, n -eicos-1-ene, 1-phenyl decane, and anthracene. Under the conditions used [10% AgNO_3 : SiO_2 (w : w); hexane] each component was well resolved from each other. The efficiency of the urea adduction procedure was monitored by GC, and all UCMs were screened for high molecular weight material prior to oxidation by high temperature GC (section 2.7.3). Each of the resultant urea non adducts was oxidised using the procedure described previously (chapter 3). Total methylated oxidation products were then screened by GC and GC-MS, and where time allowed fractionated by open column chromatography to provide a quantitative measure of oxidised vs unoxidised material. The gravimetric data for these procedures is summarised in Table 5.1 and Table 5.3.

5.2 SHELL PARAFFINIC LUBRICATING BASE OIL

The paraffinic lubricating base oil originated from the refining of

TABLE 5.1
GRAVIMETRIC DATA FOR THE ISOLATION AND CrO₃/GLACIAL ACETIC ACID
OXIDATION OF UCMs OF VARIED ORIGIN

SAMPLE	%ALKANES ^a	%UCM ^b	MASS UCM OXIDISED (mg)	MASS TOTAL RECOVERED MATERIAL (mg)	YIELD (%)
Paraffinic lubricating oil	69	83	29.9	22.4	75
Naphthenic lubricating oil	66	94	30.6	22.2	73
"Sivand" oil spill - ref. oil	55	76	39.9	33.1	83
"Sivand" oil spill- sediment extract	43	87	44.6	37	83
Amoco tank spill- ref. oil	16	71	40.9	35.5	87
Amoco tank spill- Newgate Beach oil	16	68	21.1	15.8	75
Athabasca oil sand bitumen	8	94	46.1	42.7	93

a: obtained by TLC fractionation

b: obtained by urea adduction, urea non adduct = UCM

several North Sea Crudes (including Brent, Murcheson, Dunlin and Cormorant) and was supplied by Shell Lubricants U.K. The oil was produced by vacuum distillation of the atmospheric residuum, solvent extraction (furfural) and solvent dewaxing to remove n-alkanes. The total polar content was 17% (maximum, obtained by HPLC analysis), and carbon type analysis (see note 1) provided 2% aromatic C atoms, 31% naphthenic C atoms, and 67% paraffinic C atoms (Mr M. Day, Shell Lubricants U.K.; personal communication). The gravimetric data for the UCM isolation (Table 5.1) showed a high proportion of alkanes in the total oil (69%, as expected for a solvent-extracted vacuum distillate (Klamann, 1984). The high proportion of urea non adducted alkanes (83%) is again typical of an oil which has undergone solvent dewaxing to remove n-alkanes in its refining history.

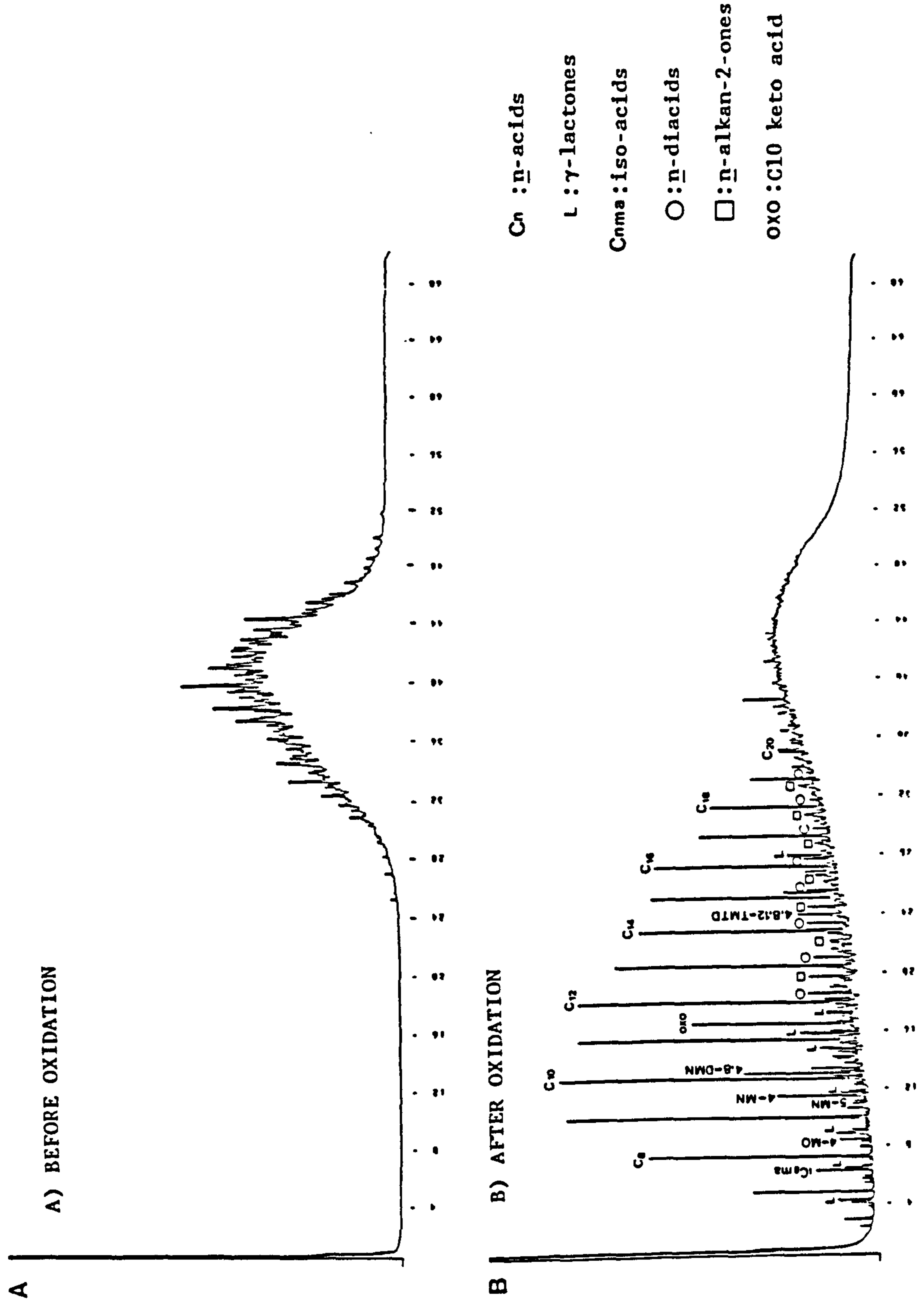
GC of the urea non adduct fraction showed a broad UCM ranging from KI 1900 - 3300, maximising at KI 2540 (Fig. 5.1A). Superimposed on the UCM profile was a series of partially resolved compounds (less than 7% of the total FID response, as estimated by the time slice area measurement - Appendix 1).

GC-MS analysis was used to emphasise the distribution of "biological marker" compounds proposed as useful indicators of crude oil origin and maturity (e.g. Ensminger et al., 1975; Seifert, 1977; Seifert and Moldowan, 1978; Albaiges and Albrecht, 1979; Jones et al., 1986).

(note: obtained by ¹³C nmr analysis, Mr M. Day, Shell Lubricants personal communication)

FIG. 5.1 GAS CHROMATOGRAMS OF THE PARAFFINIC LUBE OIL ALIPHATIC UCM

(CrO₃/GLACIAL ACETIC ACID, 60 MIN, DCM EXTRACTANT)

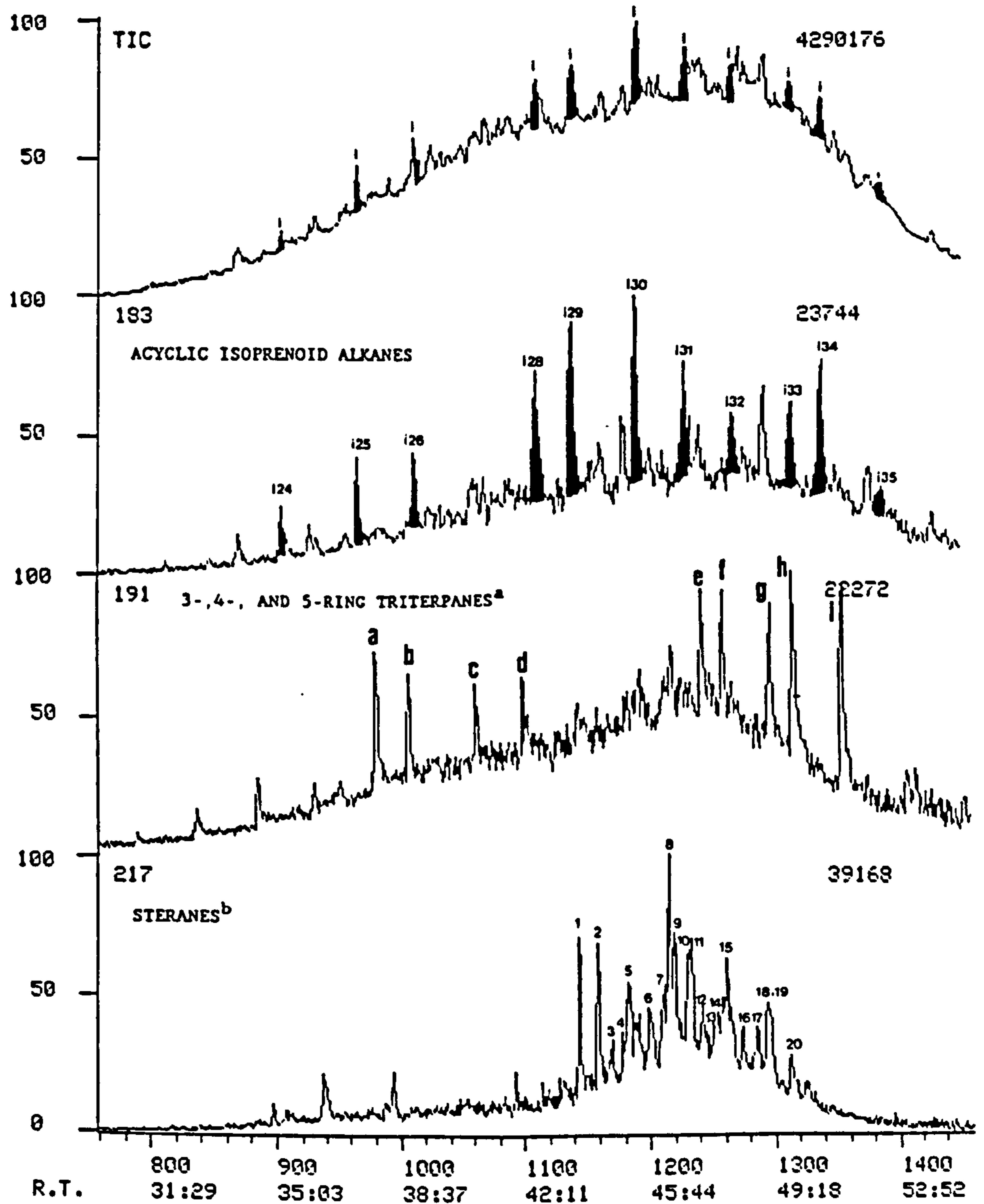


Thus the m/z 183 mass fragment ion series, chosen to illustrate the regular acyclic isoprenoids, revealed the distribution of these compounds throughout the UCM profile (Fig 5.2). By a comparison of retention indices and mass spectra the major components were identified as a C24 - C35 series. These matched almost exactly, both in carbon number range and relative abundance, the distribution of acyclic isoprenoids identified in the Silkolene 150 lubricating base oil UCM (section 3.1). Again the isoprenoids were found to contribute significantly to the resolved series of components superimposed on the UCM profile.

The m/z 191 mass fragmentogram was used to highlight the distribution of tri- and pentacyclic terpanes within the UCM (Fig. 5.2) and certain components were tentatively identified by their mass spectra (e.g. Philp, 1985) and relative retention times (Table 5.2). The hopanes identified were confined to C28-C30 compounds; the extended C31-C35 hopanes as observed for the aliphatic UCM of the Silkolene 150 lubricating oil, were absent. In contrast to the m/z 191 mass fragmentogram, the m/z 217 sterane profile (Fig. 5.2) was near identical to that observed earlier for the Silkolene oil aliphatic UCM (section 3.1).

Oxidation of the aliphatic UCM produced a well-resolved series of compounds (Fig. 5.1B) in good yield (75%, Table 5.1). GC-MS using fragment ion series characteristic of carboxylic acids-methyl esters (m/z 74), alkyl ketones (m/z 58) and lactones (m/z 99) revealed series of functionalised compounds similar to these observed as oxidation products of the Silkolene 150 lubricating oil aliphatic UCM

FIG. 5.2 GC-MS ANALYSIS OF SELECTED "BIOLOGICAL MARKER" COMPOUNDS, PARAFFINIC LUBE OIL ALIPHATIC UCM



a: for peak identity see Table 5.2
 b: for peak identity see Table 3.6

TABLE 5.2

A SUMMARY OF MASS SPECTRAL DATA FOR THE RESOLVED COMPONENTS OF
THE m/z 191 MASS FRAGMENTOGRAM- SHELL PARAFFINIC LUBE OIL, UREA
NON ADDUCT.

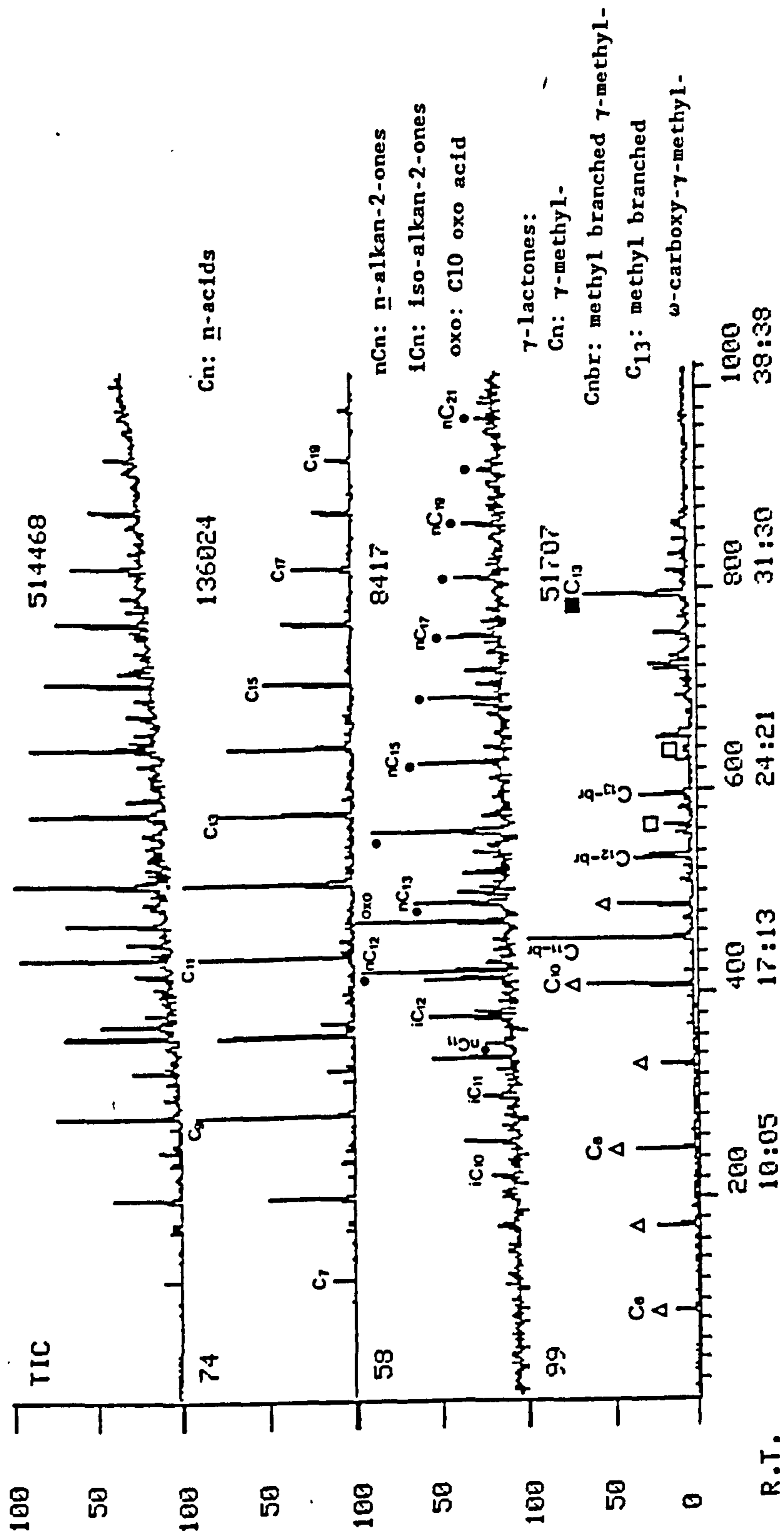
COMPOUND	DIAGNOSTIC IONS [m/z (% ABUNDANCE)]	TENTATIVE ASSIGNMENT	FORMULA
A	57(100), 191(30), 303(2), 318(3)	C23 tricyclic terpane	C23H42
B	123(30), 191(100), 219(15), 317(15) 322(6)	C24 tricyclic terpane	C24H44
C	83(100), 191(47), 323(15), 331(6), 346(3)	C25 tricyclic	C25H46
D	123(50), 177(15), 191(100), 315(9) 330(12)	C24 tetracyclic	C24H42
E	135(55), 191(100), 371(17), 386(16)	C28 tetracyclic	C28H50
F	123(83), 191(100), 218(82), 317(16), 386(29)	C28 tetracyclic	C28H50
G	191(100), 369(1), 384(18)	C28 hopane	C28H48
H	123(50), 177(42), 191(100), 383(6), 398(7)	C29 hopane	C29H50
I	191(100), 397(8), 412(10)	C30 hopane	C30H52

(Fig. 5.3). The major resolved components were again identified as a homologous series of n-monocarboxylic acids (C7-C20; max. C10); with additional series of n- α,ω -dicarboxylic acids (C9-C16), n-alkan-2-ones (C10-C21), iso-methylbranched alkan-2-ones (C10-C12), γ -methyl- γ -lactones (C6-C11), ω -carboxy- γ -methyl- γ -lactones (C9,C10), and methylbranched γ -methyl- γ -lactones (C11-C13) and ω -carboxy- γ -methyl- γ -lactones (C13). Products consistent with an acyclic isoprenoid origin included the isoprenoid C10 ω -oxo-carboxylic acid (marked "oxo", Fig. 5.1B and Fig. 5.3), and the C11 and C16 isoprenoid acids 4,8-dimethylnonanoic acid (marked 4,8-DMN, Fig. 5.1B) and 4,8,12-trimethyltridecanoic acid (marked 4,8,12-TMTD). Other monomethyl branched monocarboxylic acids were identified, these included iso and anteiso-methylbranched acids (C8 -C10), and certain internally - branched acids, e.g. 4-methyloctanoic acid (4-MO, Fig. 5.1B) and 4- and 5-methylnonanoic acids (4-MN and 5-MN, respectively, Fig 5.1B).

Many of the products identified could therefore be correlated with oxidation products previously observed for the Silkolene 150 lubricating oil aliphatic UCM. In fact it was difficult to distinguish the two oxidation product profiles by GC alone. However, a close comparison of the m/z 99 mass fragmentogram showed certain differences which appear to have potential uses as "fingerprints" specific to the oil in question. This was investigated further by an analysis of the oxidation products of several other aliphatic UCMs isolated from oils of varied origin (section 5.6).

The column chromatographic fractionation of the total products recovered from the paraffinic lube oil UCM oxidation showed the bulk

FIG. 5.3 GC-MS MASS FRAGMENTOGRAMS SHOWING THE DISTRIBUTION OF CARBOXYLIC ACIDS/METHYL ESTERS (m/z 74), ALKYL KETONES (m/z 58), AND γ -LACTONES (m/z 99) AS OXIDATION PRODUCTS OF THE PARAFFINIC LUBE OIL ALIPHATIC UCM



of the material (79%) to be comprised of functionalised compounds (Table 5.3). This is compared to greater than 88% of functionalised compounds observed for the Silkolene 150 lubricating oil aliphatic UCM (section 3.1). GC analysis of this fraction (Fig 5.4B) showed some loss of low molecular weight material (during rotary evaporation and N₂ blow down) though the remaining profile resembled closely that observed for the unfractionated products. A major UCM was also noted in the gas chromatogram which presumably represents unresolved functionalised compounds. The exact nature of these is unclear and requires further study. The residual hydrocarbon fraction comprised only ca. 9% of the total material recovered, and the gas chromatogram (Fig. 5.4A) showed that changes had occurred (Fig. 5.1A), most notably a greater proportion of resolved components superimposed on the UCM. GC-MS analysis was used to monitor changes which had occurred in the "biological marker" distributions caused by oxidation.

The m/z 183 mass fragmentogram (Fig. 5.5) indicated that many of the additional resolved components superimposed on the UCM were isoprenoids, though these were not characterised further. The m/z 191 mass fragmentogram also showed major changes in comparison to the initial m/z 191 profile. Thus a general enrichment of these components was noted relative to the UCM, although certain individual compounds appeared to have been reduced in abundance (Fig. 5.5). These included compounds f, g, h, and i; tentatively identified as a C₂₈ tetracyclic terpane, and C₂₈-C₃₀ hopanes (Table 5.2). In contrast the lower molecular weight tri - and tetracyclic terpenoidal alkanes appeared to be only slightly affected by the oxidation. The reasons for their contrasting reactivities towards the oxidant were

TABLE 5.3

GRAVIMETRIC DATA FOR THE COLUMN CHROMATOGRAPHIC FRACTIONATION OF
TOTAL OXIDATION PRODUCTS

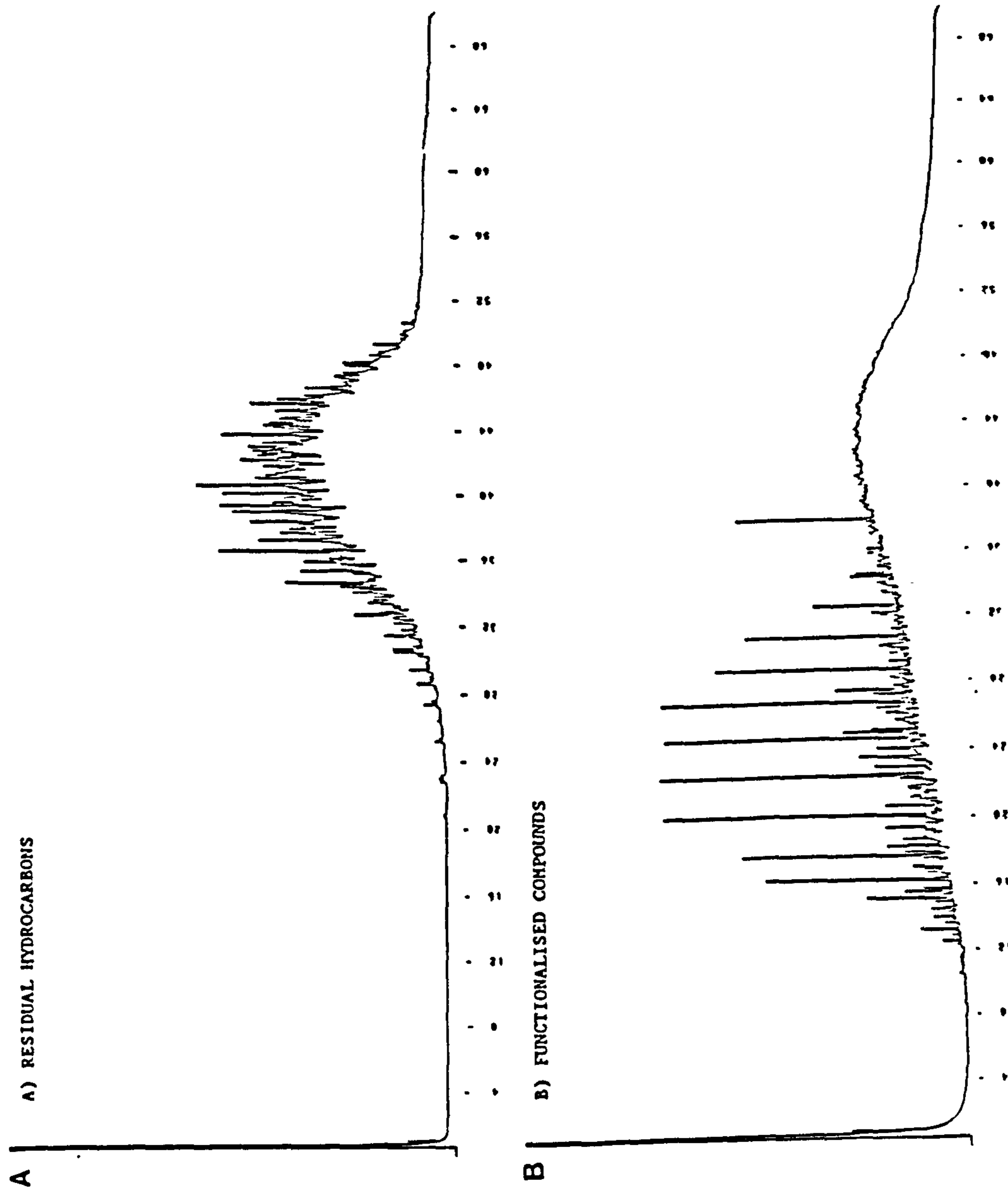
OIL OXIDISED	MASS APPLIED (mg)	MASS FRACTION 1 ^a [mg(%)]	MASS FRACTION 2 ^b [mg(%)]
SHELL PARAFFINIC LUBE OIL - UCM	11.2	0.8 (8.9)	8.2 (91.1)
SHELL NAPHTHENIC LUBE OIL - UCM	11.1	0.6 (7.9)	7.0 (92.1)

^a Fraction 1: Hexane as elution solvent

^b Fraction 2: DCM, DCM: MeOH (1:1) as elution solvent

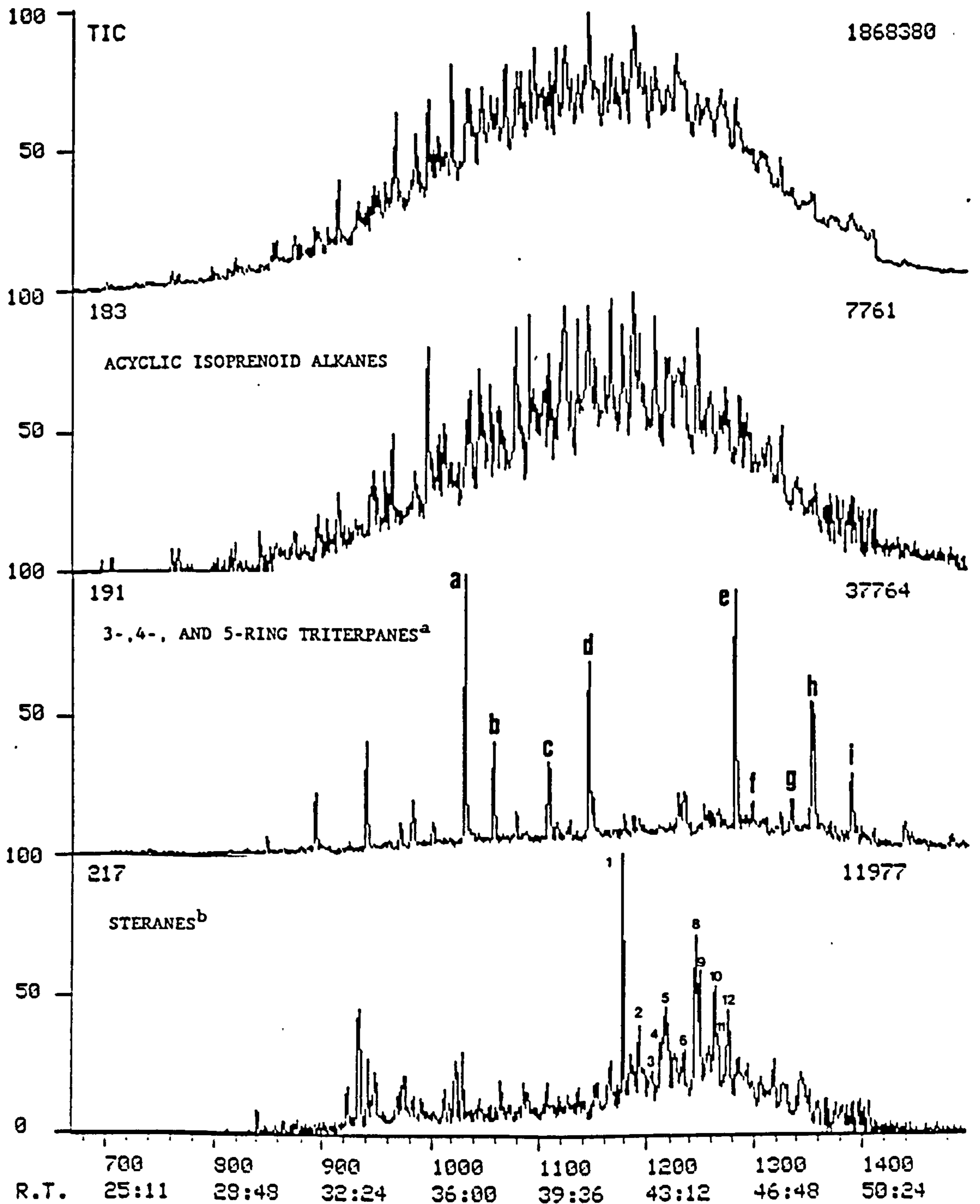
(recoveries are expressed as a percentage of the total mass of
material eluted from the column)

FIG. 5.4 GAS CHROMATOGRAMS OF THE TOTAL, PARAFFINIC LUBE OIL ALIPHATIC UCM OXIDATION PRODUCTS AFTER CHROMATOGRAPHIC FRACTIONATION



[GC: DB-5(J+W), 25m, 50-300°C @ 5°C min⁻¹, 300°C(20min)]

FIG. 5.5 GC-MS ANALYSIS OF "BIOLOGICAL MARKER" COMPOUNDS PRESENT IN THE RESIDUAL HYDROCARBON FRACTION OF THE PARAFFINIC LUBE OIL UCM AFTER OXIDATION



a: for peak identity see Table 5.2
 b: for peak identity see Table 3.6

not investigated further.

The sterane profile (m/z 217, Fig. 5.5) was also markedly affected by the CrO₃ glacial acetic acid oxidation. Most notably the enrichment of compounds 1 [presumed to be a C₂₇ 13 β ,17 α -diasterane (20S)] relative to its epimer and higher steranes and diasteranes was observed. Again the reasons for this apparent stereochemical selectivity towards the oxidant require further study.

5.3 SHELL NAPHTHENIC LUBRICATING BASE OIL

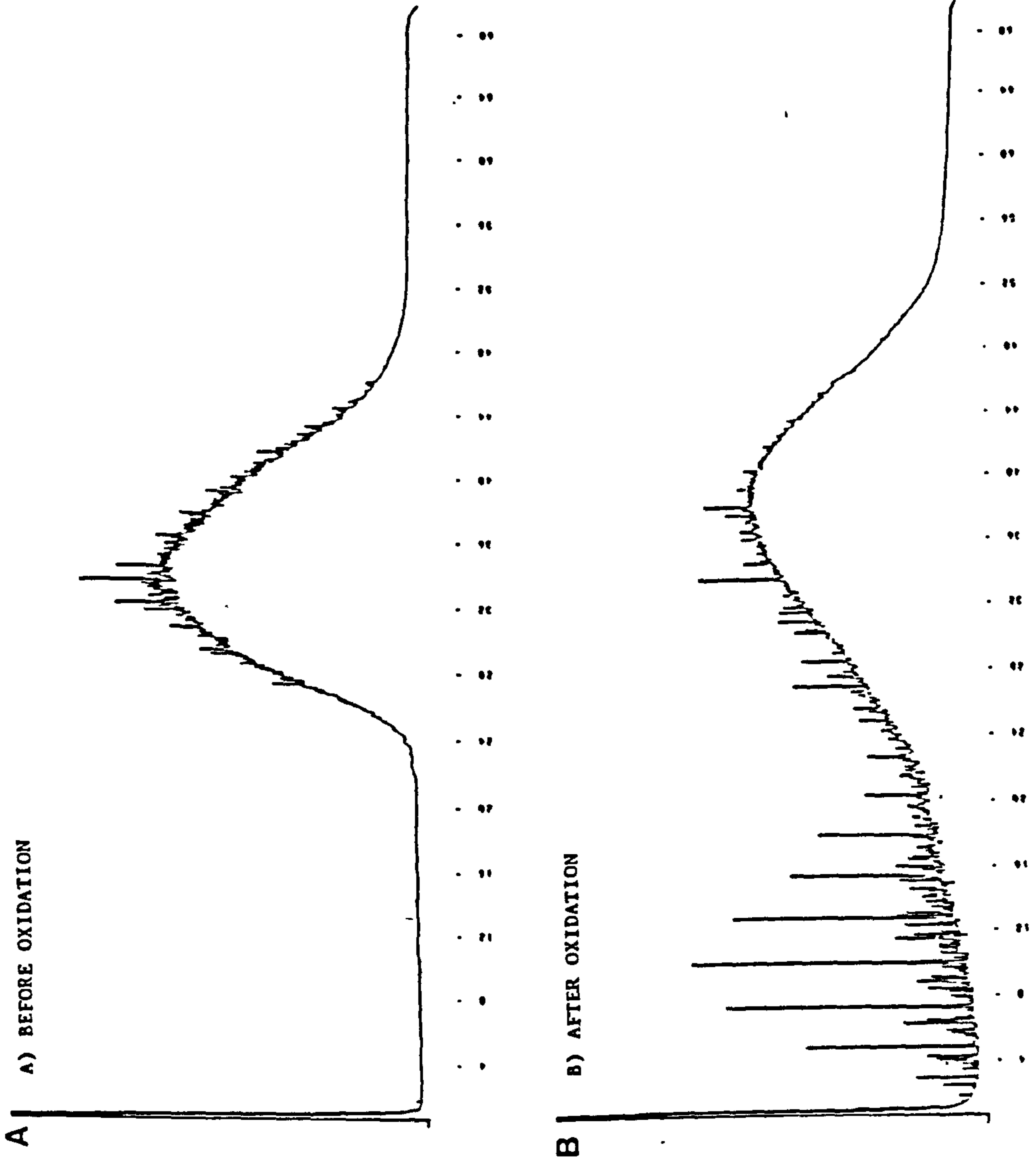
The naphthenic lubricating base oil was of heavy Venezuelan Crude oil origin from Tia Juana Pesado and was supplied by Shell Lubricants U.K.. Like the paraffinic lubricating base oil it was produced by vacuum distillation of the atmospheric residuum and by solvent extraction with furfural. The oil was not dewaxed since heavy Venezuelan Crudes are characterised by low n-alkane contents consistent with a microbially altered origin (Demaison, 1977). The total polar content was 19-22% (as measured by HPLC), and the carbon type analysis (section 5.1) provided 1% aromatic C atoms, 46% naphthenic C atoms, and 53% paraffinic C atoms. (Mr M. Day, Shell Lubricants U.K., personal communication).

The gravimetric data for the UCM isolation (Table 5.1) showed a high proportion of alkanes (66%) and urea non adducted alkanes (94%), as expected for a solvent extracted distillate fraction low in n-alkanes.

GC analysis of the urea non adduct showed a broad UCM in the range KI

FIG. 5.6 GAS CHROMATOGRAMS OF THE NAPHTHENIC LUBE OIL
ALIPHATIC UCM

(CrO₃/GLACIAL ACETIC ACID, 60 MIN, DCM EXTRACTANT)



[GC: DB-5(J+W), 25m, 50-300°C @ 5°C min⁻¹, 300°C(20min)]

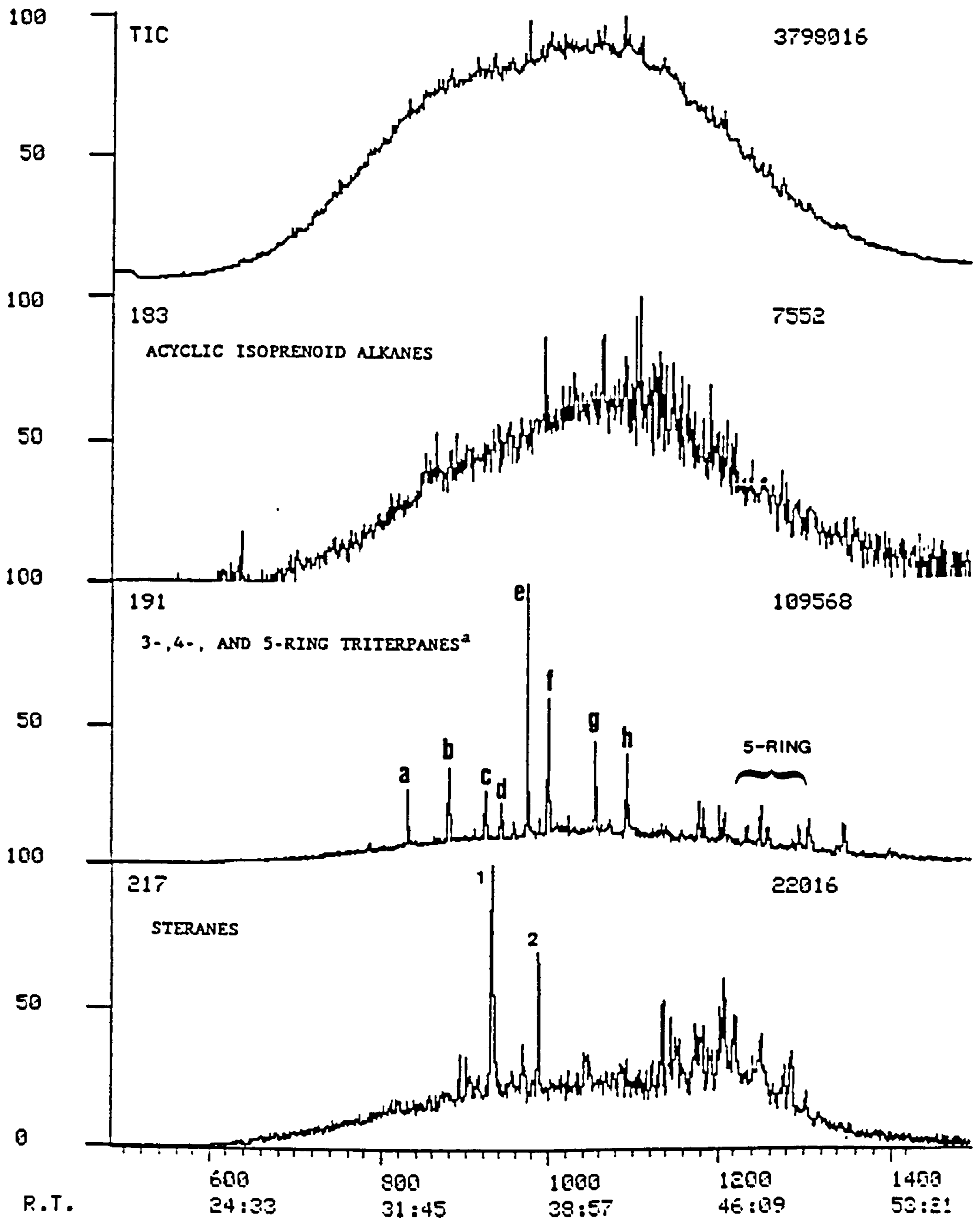
1600 - 3400, maximising at KI 2300 (Fig. 5.6A). In contrast to the paraffinic lube oil UCM few resolved peaks were observed, these were found to contribute less than 4% to the total FID response (as estimated by the time slice area measurement, appendix 1).

GC-MS analysis (Fig. 5.7) showed few resolved peaks in the m/z 183 mass fragmentogram characteristic of acyclic isoprenoid alkanes. The m/z 191 mass fragmentogram however was dominated by an intense ion series observed to elute in the tricyclic terpane region. By mass spectral correlations and relative times (e.g. Philp, 1985) these were identified (Table 5.4) as a series of C20 - C26 tricyclic terpanes common to many petroleums and which have been reported to be relatively resistant to biodegradation (e.g. Aquino Neto *et al.*, 1983; Ekweozor and Strausz, 1982, 1983). In contrast to the tricyclic terpanes, the pentacyclic triterpanes showed an atypical profile which appeared to have been effected by biodegradation.

The sterane profile (m/z 217, Fig. 5.70) was also atypical of most mature petroleums and again appeared effected by biodegradation. The two major components (1 and 2, Fig. 5.7) had mass spectra (Fig. 5.8) characteristic of C21 and C22 regular steranes (Philp, 1985).

Oxidation of the naphthenic lube oil UCM with CrO_3 /glacial acetic acid produced some resolved compounds (Fig. 5.6B) with a good overall recovery (73%, Table 5.1). GC-MS (Fig. 5.9) showed that the resolved compounds were similar to those observed for both the Silkolene 150 and paraffinic lubricating oil alkane UCM oxidations, but their proportion was small. Again the major resolved components were identified as n-monocarboxylic acids (as methyl esters) in the range C6 - C20, maximising at C9. Other compounds identified included n-

FIG. 5.7 GC-MS ANALYSIS OF SELECTED "BIOLOGICAL MARKER" COMPOUNDS, NAPHTHENIC LUBE OIL ALIPHATIC UCM



a: for peak identity see Table 5.4 (terpanes)

FIG. 5.8 EI MASS SPECTRA OF A) COMPONENT 1 AND B) COMPONENT 2 OBSERVED IN THE STERANE (m/z 217) GC-MS PROFILE OF THE NAPHTHENIC LUBE OIL UCM

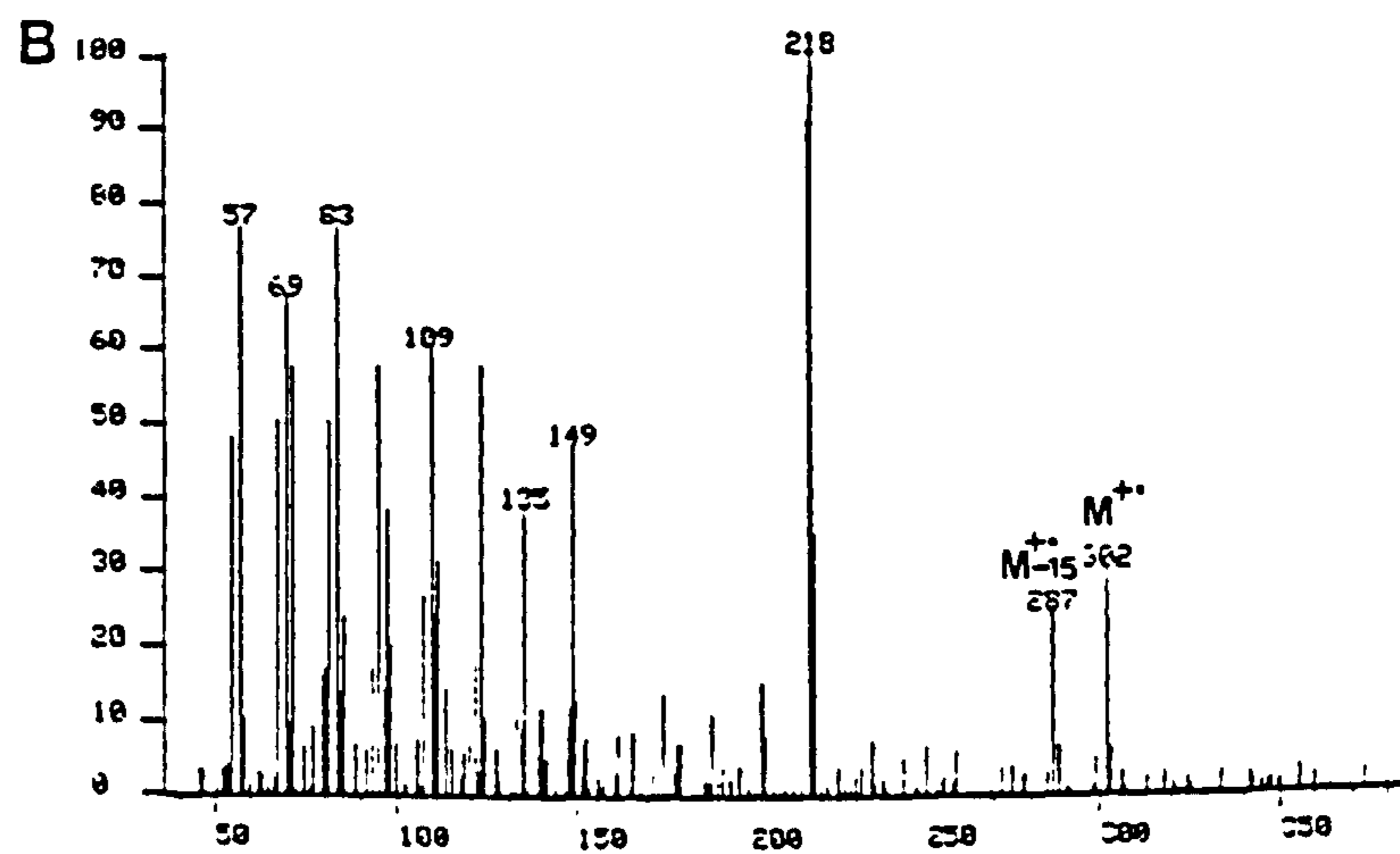
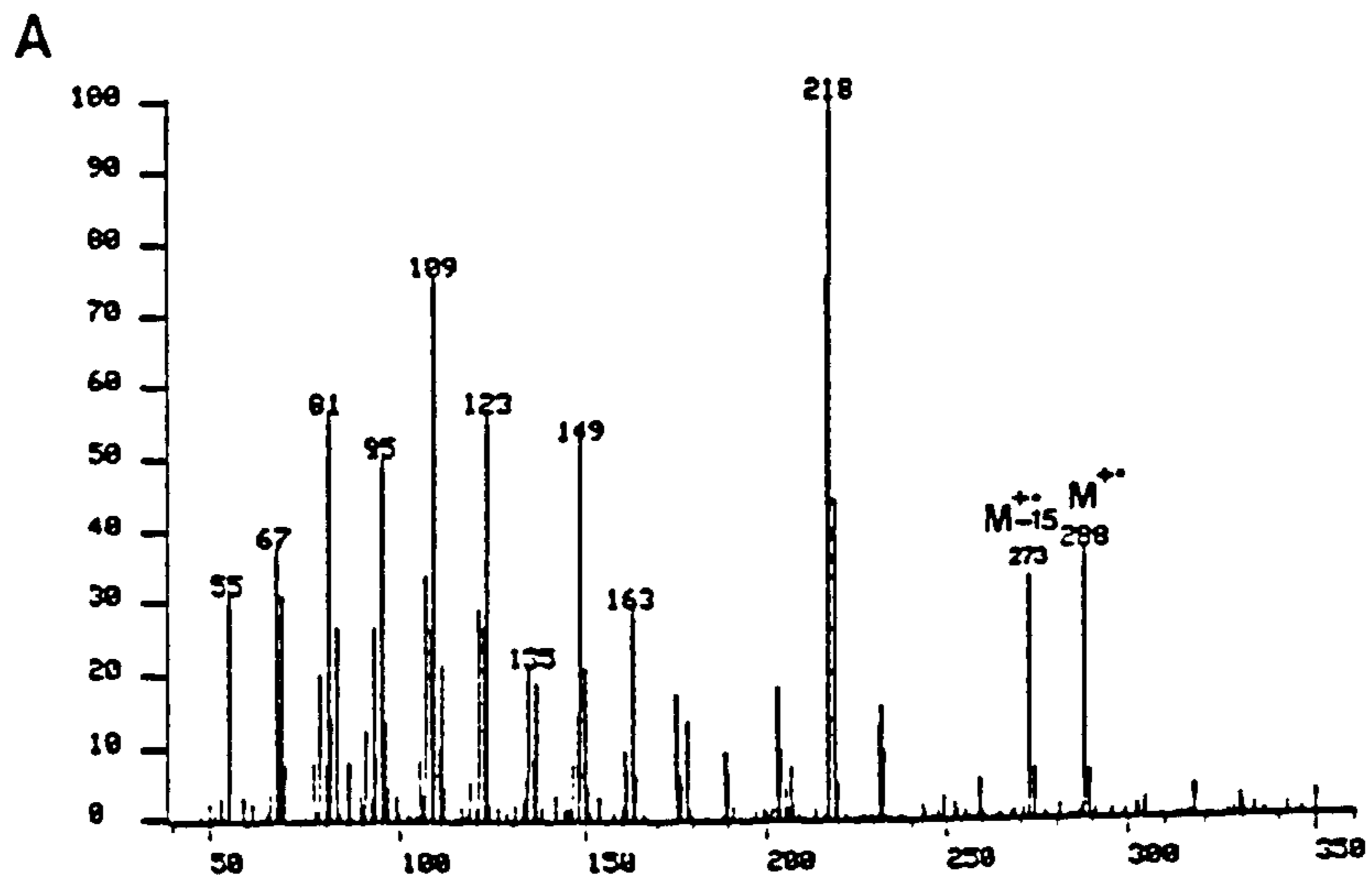


TABLE 5.4

SUMMARY OF MASS SPECTRAL DATA FOR THE RESOLVED COMPONENTS OF THE
 m/z 191 MASS FRAGMENTOGRAM -SHELL NAPHTHENIC LUBRICATING OIL
 (UREA NON ADDUCT)

COMPOUND	SCAN	MAJOR IONS [m/z (% ABUNDANCE)]	ASSIGNMENT
A	830	69(92),123(58),163(31), 191(100),261(20),276(18)	C20 tricyclic terpane
B	878	69(29),81(30),123(22),191(100), 275(15), 290 (14)	C21 tricyclic terpane
C	922	191(100), 289(12), 304(14)	C22 tricyclic terpane
D	941	95(45),177(28),191(100),303(19), 318 (16)	C23 tricyclic terpane
E	972	69(31),123(21),137(76),191(100), 303(14), 318(12)	C23 tricyclic terpane
F	998	95(32),123(79),191(100),317(10), 332(8)	C24 tricyclic terpane
G	1052	95(38),123(15),137(21),191(100), 331(12) , 346(8)	C25 tricyclic terpane
H	1090	69(26),95(3),191(100), 345(3), 360 (3)	C26 tricyclic terpane

alkan-2-ones (C8-C16), iso-methylbranched alkan-2-ones (C8-C13), γ -methyl- γ -lactones (C16-C11) and a methyl branched γ -methyl- γ -lactone (C11). Products consistent with an acyclic isoprenoid origin included the C13 isoprenoid ketone 6,10-dimethylundecan-2-one and the C10 isoprenoid oxo-acid (Fig 5.10). Methyl branched carboxylic acids were also identified (mainly iso- and anteiso-). Generally absent or in low abundance were series of n - α,ω -dicarboxylic acids and ω -oxo- γ -methyl- γ -lactones.

A dominant feature of the gas chromatogram of the total oxidation products (Fig. 5.6B) was a broad UCM initially thought to represent residual unoxidised alkanes. Chromatographic fractionation (Table 5.3) and GC analysis (Fig. 5.10B) however showed that in excess of 90% of the total recovered products were functionalised. GC-MS mass fragmentography of the UCM region using the ions characteristic of carboxylic acids - methyl esters (m/z 74), alkyl ketones (m/z 58), and γ -methyl- γ -lactones (m/z 99) did not produce a strong signal. Instead a spectrum was taken from a position near to the maximum of the UCM and this compared with a scan taken at a similar position in the UCM before oxidation and in the UCM of the residual hydrocarbon fraction (Fig.5.11). The UCM before oxidation showed ions indicative of monocyclic alkanes, bicyclic alkanes and acyclic alkanes; with the former predominating. After oxidation (but before chromatographic fractionation) the UCM showed similar ions though the bicyclic series was found to predominate. The scan taken of the residual hydrocarbon fraction UCM was similar to that observed for the UCM before oxidation. Though some change has occurred in the alkane ion series after oxidation, this approach did not provide any

FIG. 5.9 GC-MS OF THE RESOLVED PRODUCTS OF OXIDATION OF THE NAPHTHENIC LUBE OIL ALIPHATIC UCM : (m/z 74- MONOCARBOXYLIC ACIDS, m/z 58- ALKYL KETONES, AND m/z 99- γ -LACTONES)

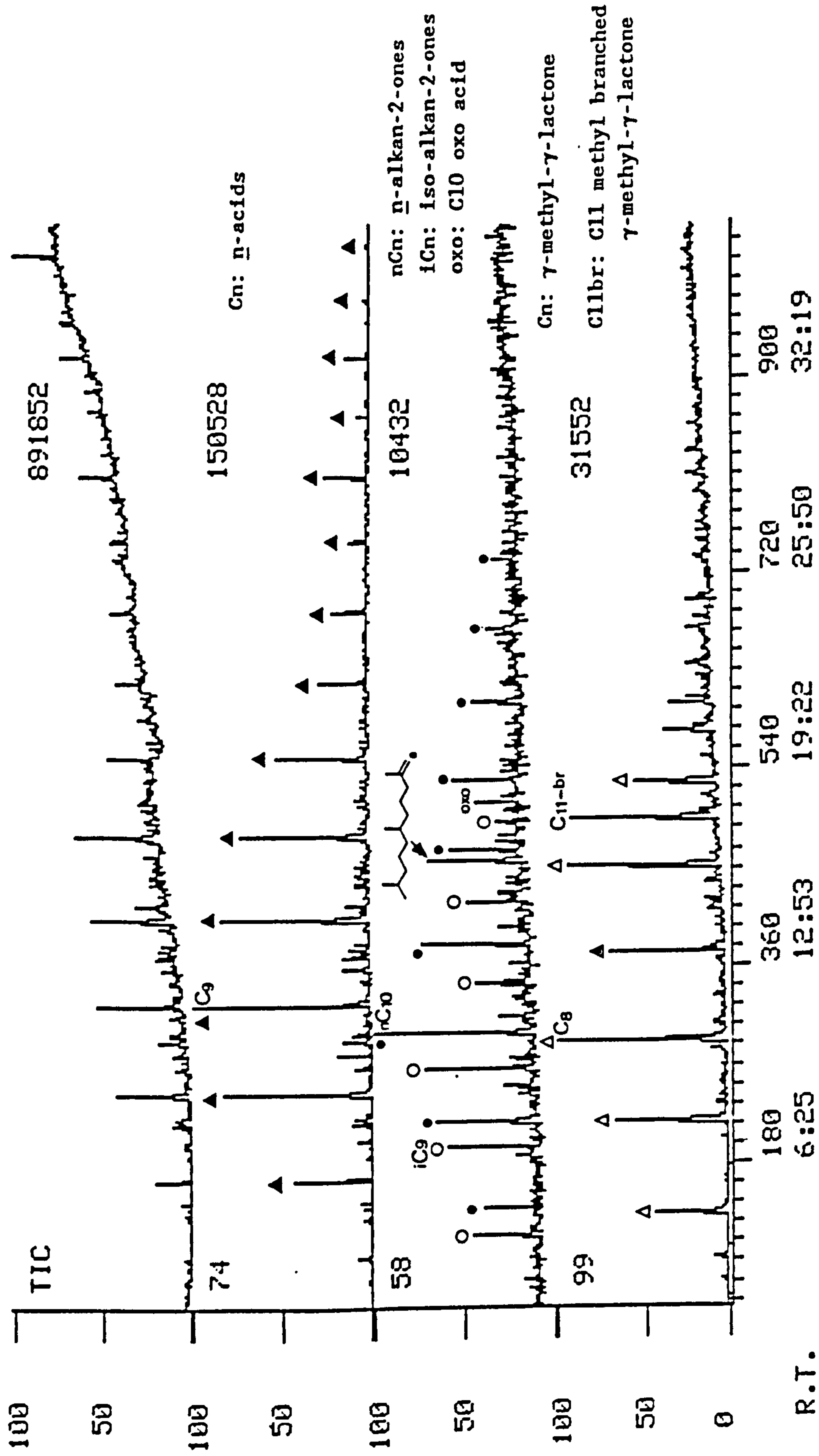
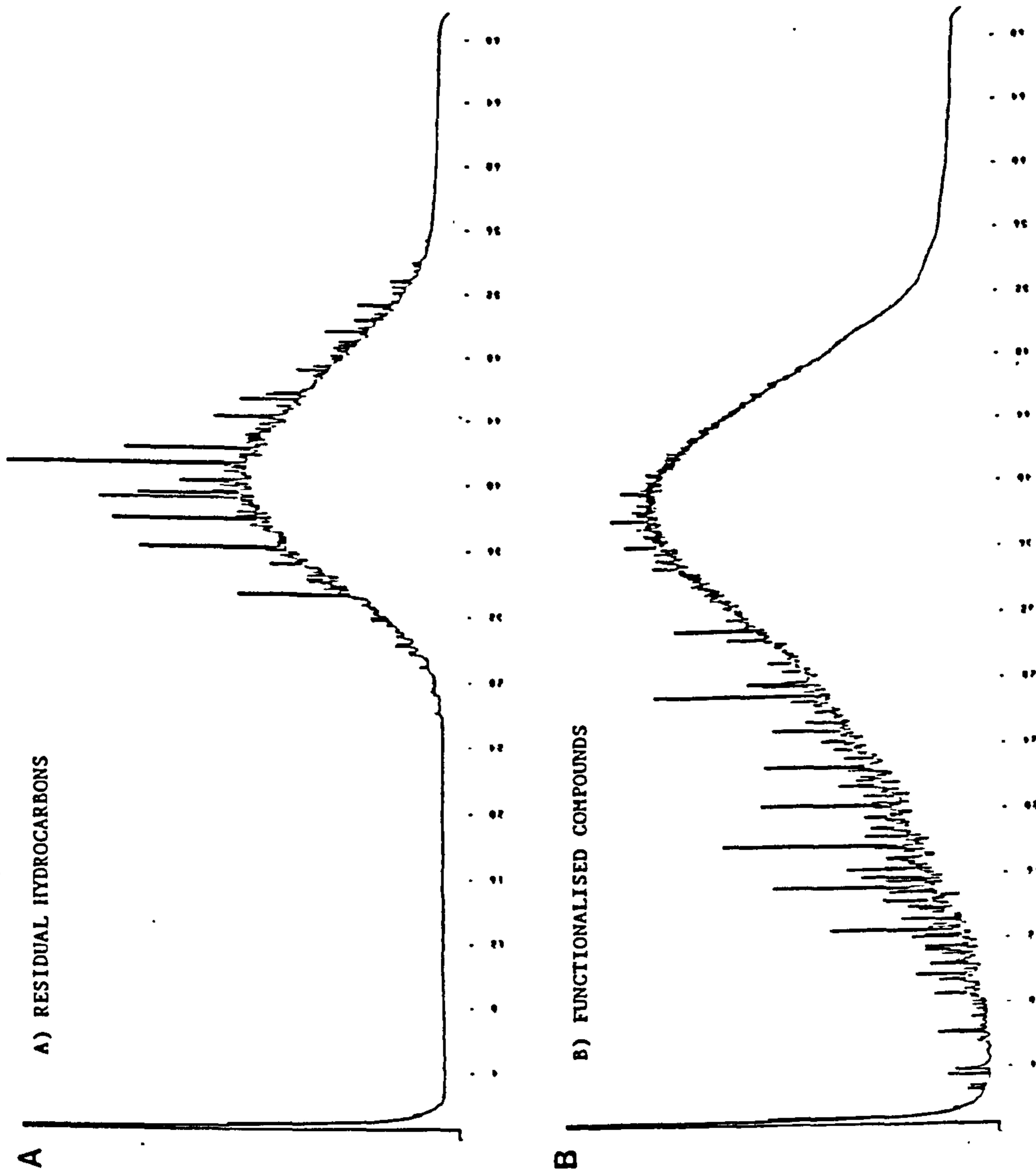


FIG. 5.10 GAS CHROMATOGRAMS OF THE TOTAL NAPHTHENIC LUBE OIL ALIPHATIC UCM OXIDATION PRODUCTS AFTER CHROMATOGRAPHIC FRACTIONATION



[GC: DB-5(J+W), 25m, 50-300°C @ 5°C min⁻¹, 300°C(20min)]

information as to the type and extent of "functionalisation" of the UCM. This would require further study (chapter 7).

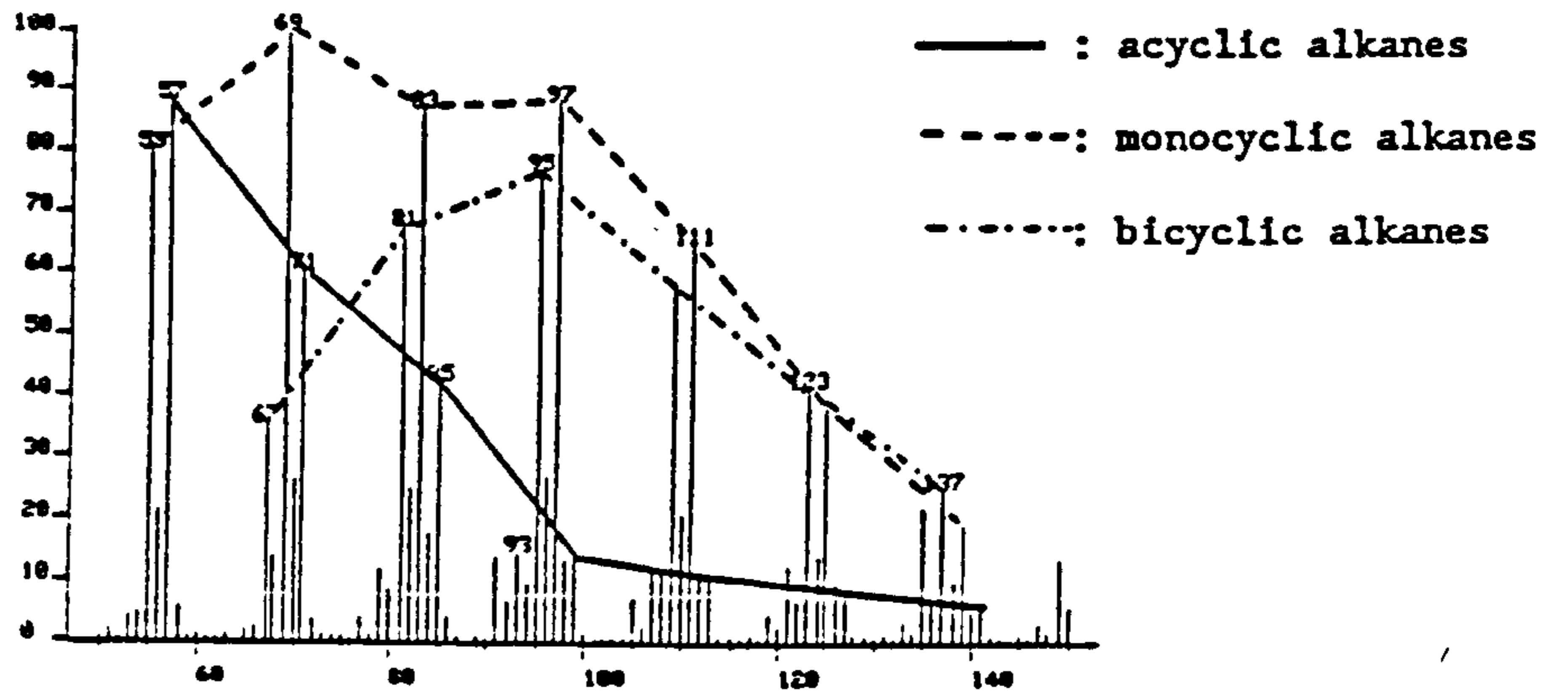
GC of the residual hydrocarbon fraction isolated by column chromatography of the total UCM oxidation products showed an enrichment of a series of compounds which appeared unaffected by oxidation (Fig. 5.10A). GC-MS (Fig. 5.12) confirmed these to comprise the series of tricyclic terpanes identified in the UCM before oxidation (Fig. 5.7, Table 5.4). It appears that these compounds are not only resistant to biodegradation (e.g. Aquino Neto *et al.*, 1983) but also relatively resistant to chemical oxidation by CrO_3 . It is unclear as to why this may be. Compounds above C23 which possess a tertiary and therefore labile C atom on the side chain (compounds f, g, and h, Fig. 5.12) were however noticeably reduced in abundance relative to the C23 member as estimated by peak height ratios, Table 5.5). This is consistent with the proposed mechanism of CrO_3 oxidation (chapter 3).

5.4 THE "SIVAND" OIL SPILL

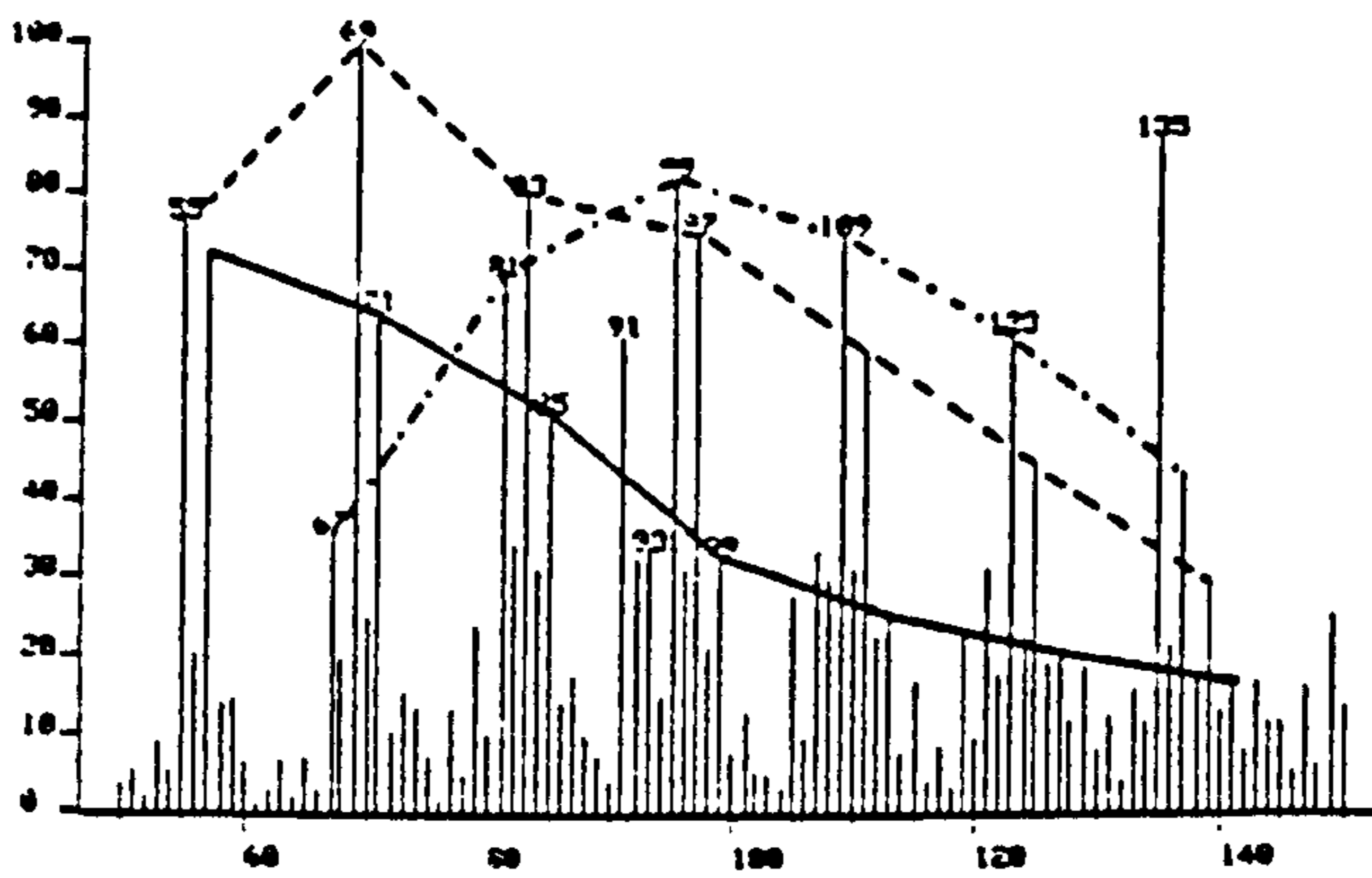
In September 1983, 6,000-7,000 tonnes of Nigerian crude oils were accidentally spilled from the "Sivand" oil tanker into the Humber Estuary (Fig. 5.13A). Due to tidal action this oil became distributed over the entire 50 miles of the estuary, and rapidly became associated with surficial sediments the lithology of which ranged from fine grained muds (ca. 90% clay) to coarse sands (ca. 9% clay). The fate of this oil in the estuarine environment was the

FIG 5.11 EI MASS SPECTRA TAKEN AT A MAXIMUM OF THE GC-MS UCM PROFILE FOR A) THE NAPHTHENIC OIL UCM BEFORE OXIDATION, B) THE UCM AFTER OXIDATION (BEFORE FRACTIONATION) AND C) THE RESIDUAL HYDROCARBON UCM

A



B



C

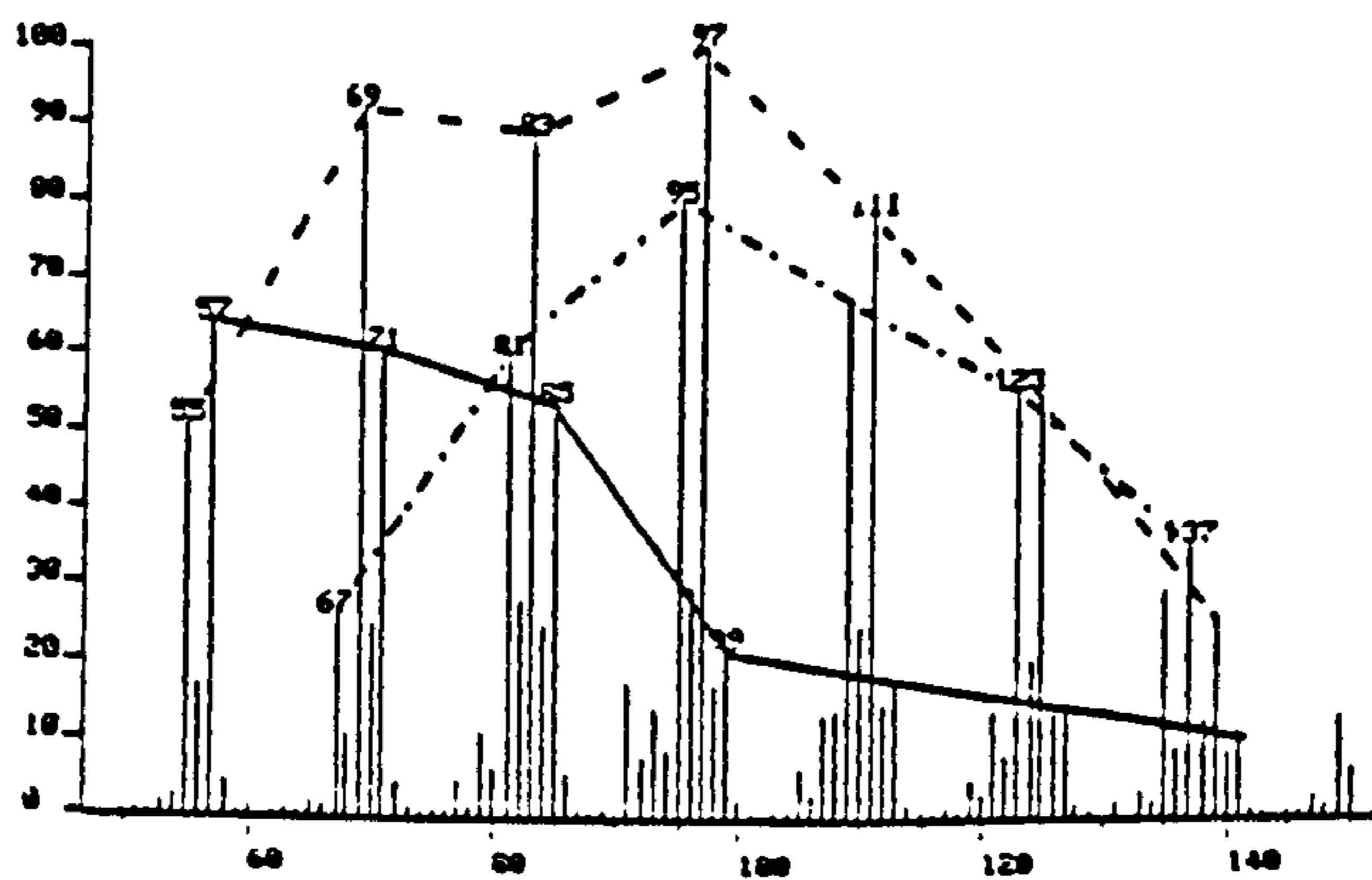
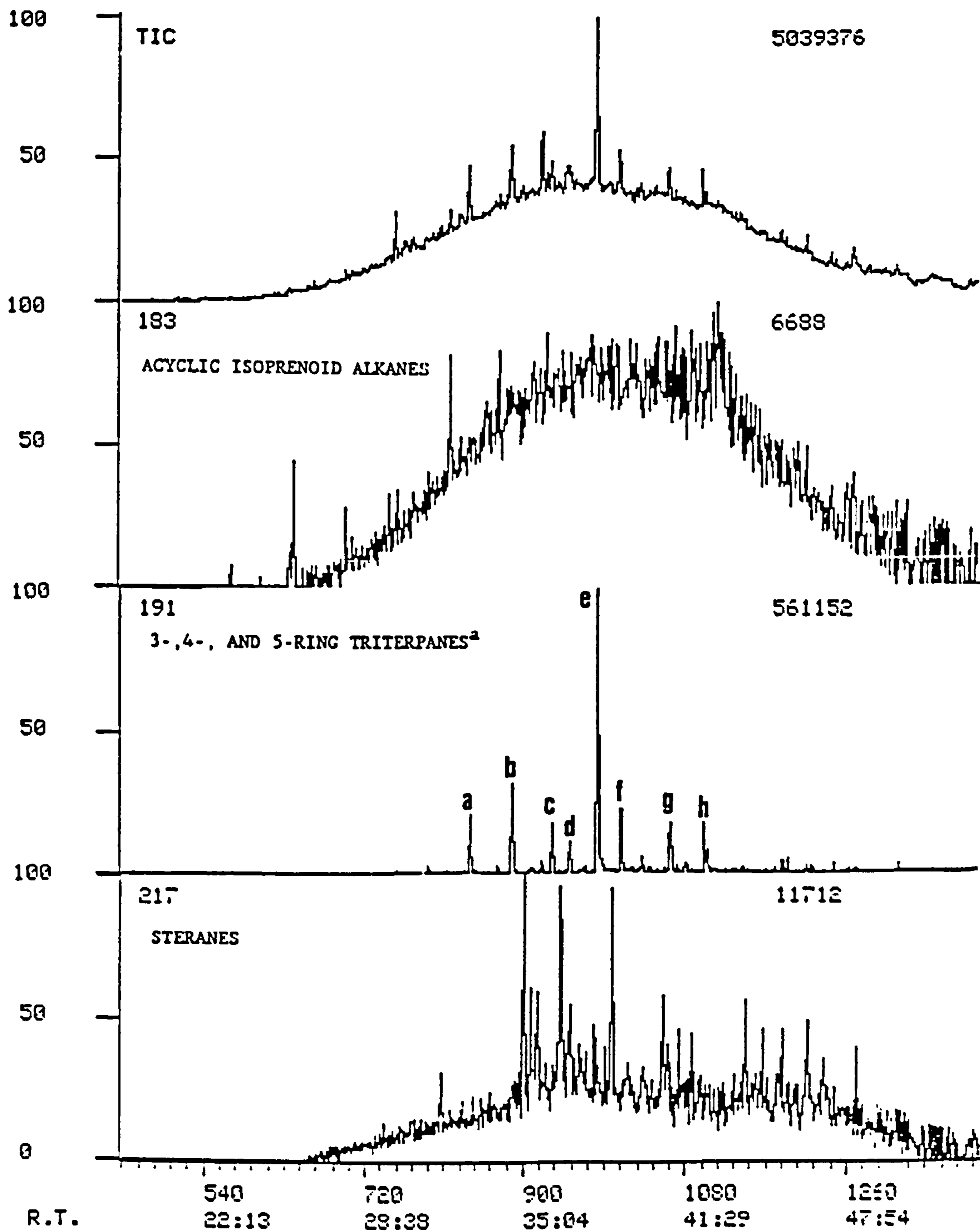


FIG. 5.12 GC-MS ANALYSIS OF "BIOLOGICAL MARKER" COMPOUNDS
PRESENT IN THE RESIDUAL HYDROCARBON FRACTION OF THE
NAPHTHENIC LUBE OIL UCM AFTER OXIDATION



a: for peak identity see Table 5.4

TABLE 5.5

PEAK HEIGHT RATIOS (RELATIVE TO THE C23 MEMBER) OF THE TRICYCLIC TERPANE SERIES BEFORE AND AFTER OXIDATION WITH CrO_3

COMPOUND	CARBON NUMBER	RATIO BEFORE [O]	RATIO AFTER [O]
A	C20	0.2	0.2
B	C21	0.3	0.2
C	C22	0.2	0.2
D	C23	0.1	0.1
E	C23	1.00	1.00
F	C24	0.5	0.2
G	C25	0.4	0.2
H	C26	0.3	0.2

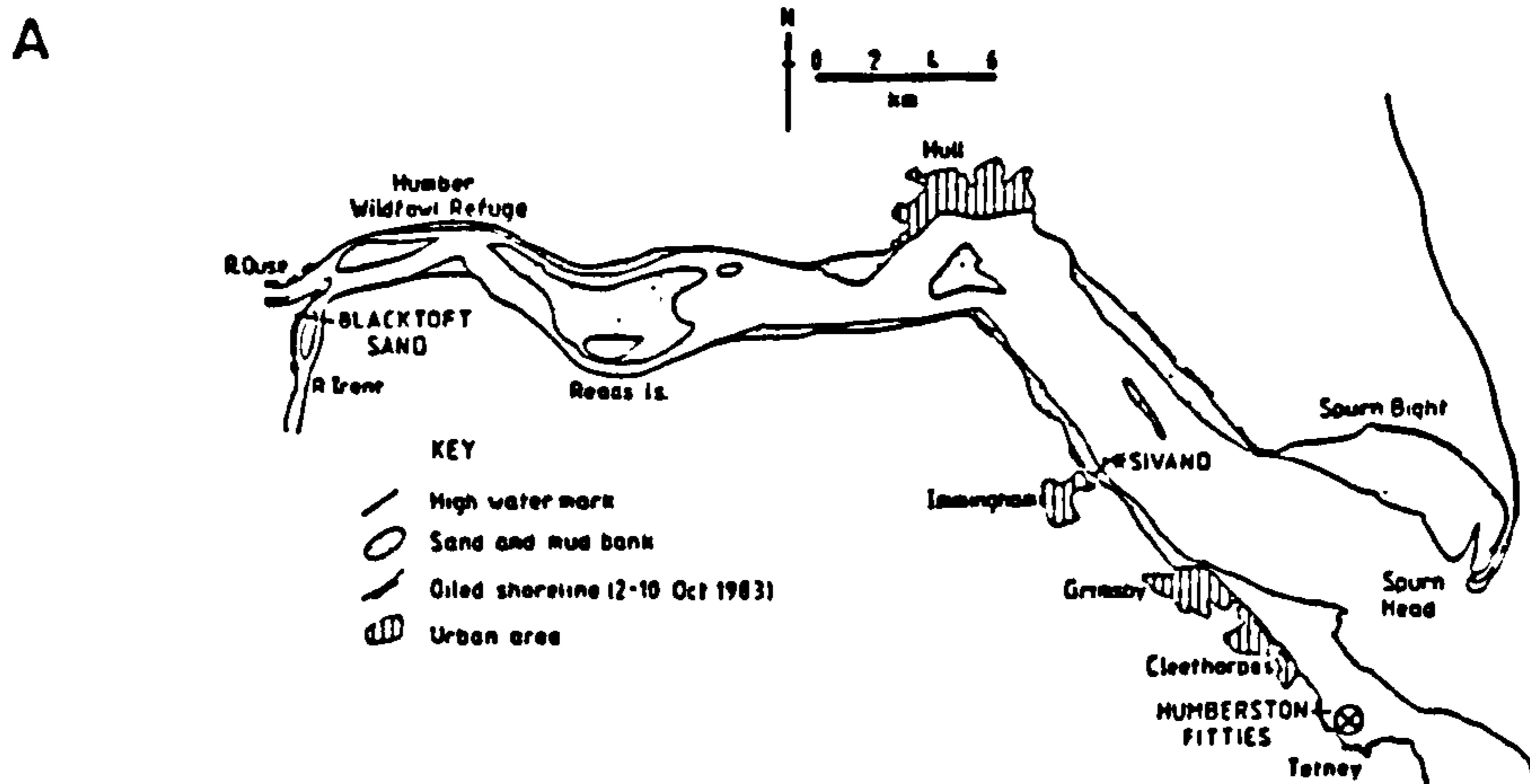
subject of an intensive study over the twelve months following the spill (Jones et al., 1986; Mr S. Howells, Oil Pollution Research Unit (OPRU), Field Studies Council, personal communication). Amongst the findings was a decrease in the proportion of n-alkanes in the aliphatic hydrocarbon fractions with a concomitant increase in the abundance of the aliphatic UCM. This is a commonly observed feature in oil spill studies and is attributed to the preferential microbial oxidation of n-alkanes (Blumer et al., 1973; Atlas et al., 1981; Oudet et al. 1981; Payne and McNabb, 1984; Kennicutt, 1988).

The enrichment of the UCM and the availability of oil-polluted sediment and a sample of the spilled oils made this a good case for study. It was hoped that oxidation of the aliphatic UCM isolated from a weathered crude would yield products comparable both in type and abundance to those obtained from the reference oil UCM. Furthermore, the high abundance of distinctive "biological marker" compounds reported for this oil would allow an examination of their fates under the conditions of oxidation. (Jones et al., 1986).

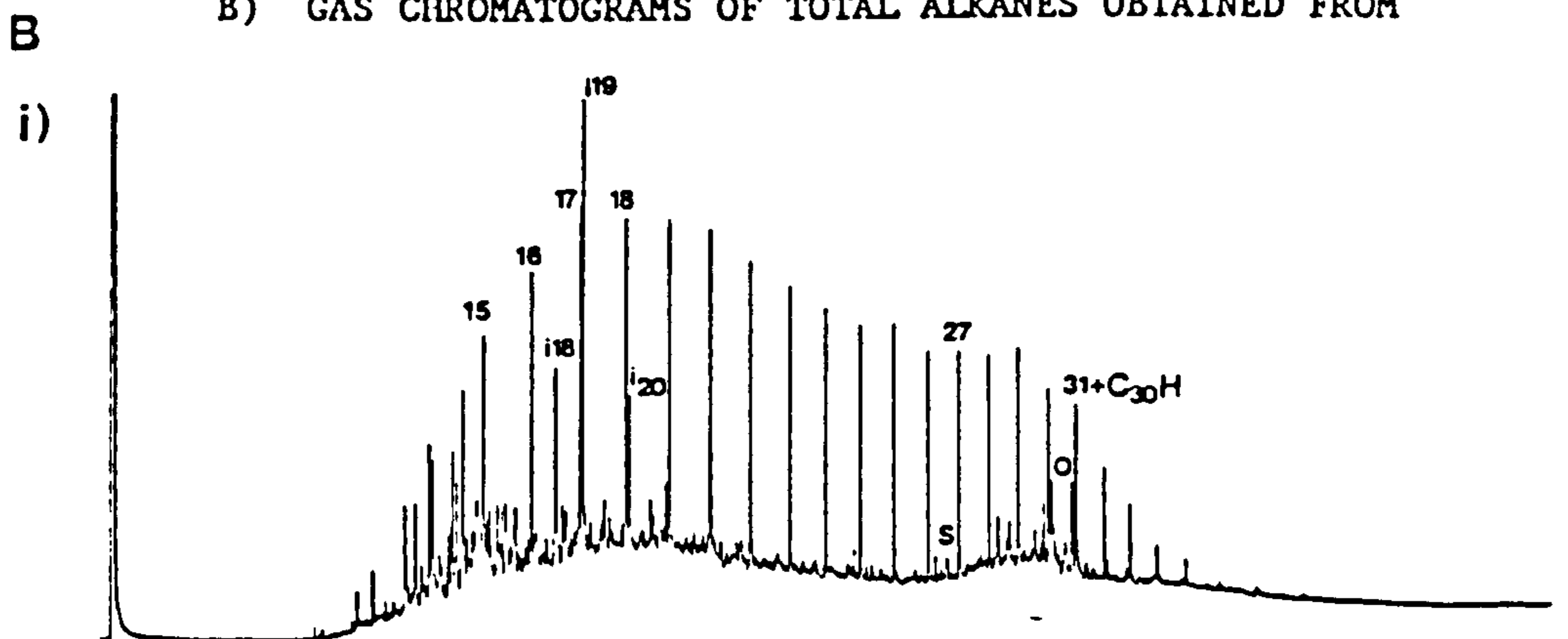
The sediment chosen for study was sampled from a sandy site at Humberston Fitties (Fig. 5.13 A) twelve months after the spill. The reference oil was obtained from the Hessel Strandline (Fig. 5.13A) four days after the spill. The isolation of the sedimentary hydrocarbon UCM involved ultrasonic extraction of freeze dried sediment with DCM/methanol according to published procedures (Douglas et al., 1981; Jones et al., 1986). This provided $2101\mu\text{gg}^{-1}$ dry sediment of total organic extract (TOE) Fractionation of the TOE by silver ion TLC provided 43% alkanes of which 87% were urea non adducted alkanes (Table 5.1). The reference oil was solvent

FIG. 5.13

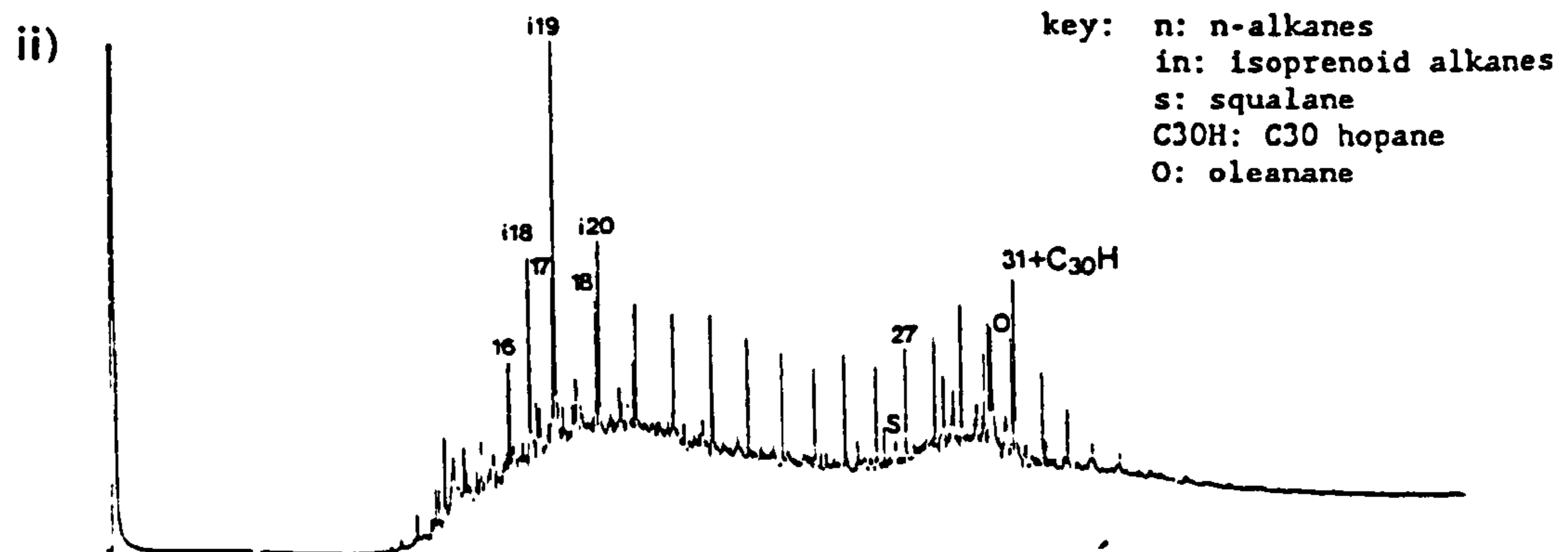
A) MAP SHOWING LOCATION OF "SIVAND" OIL SPILL SITE AND SAMPLE LOCATION (⊗)



B) GAS CHROMATOGRAMS OF TOTAL ALKANES OBTAINED FROM



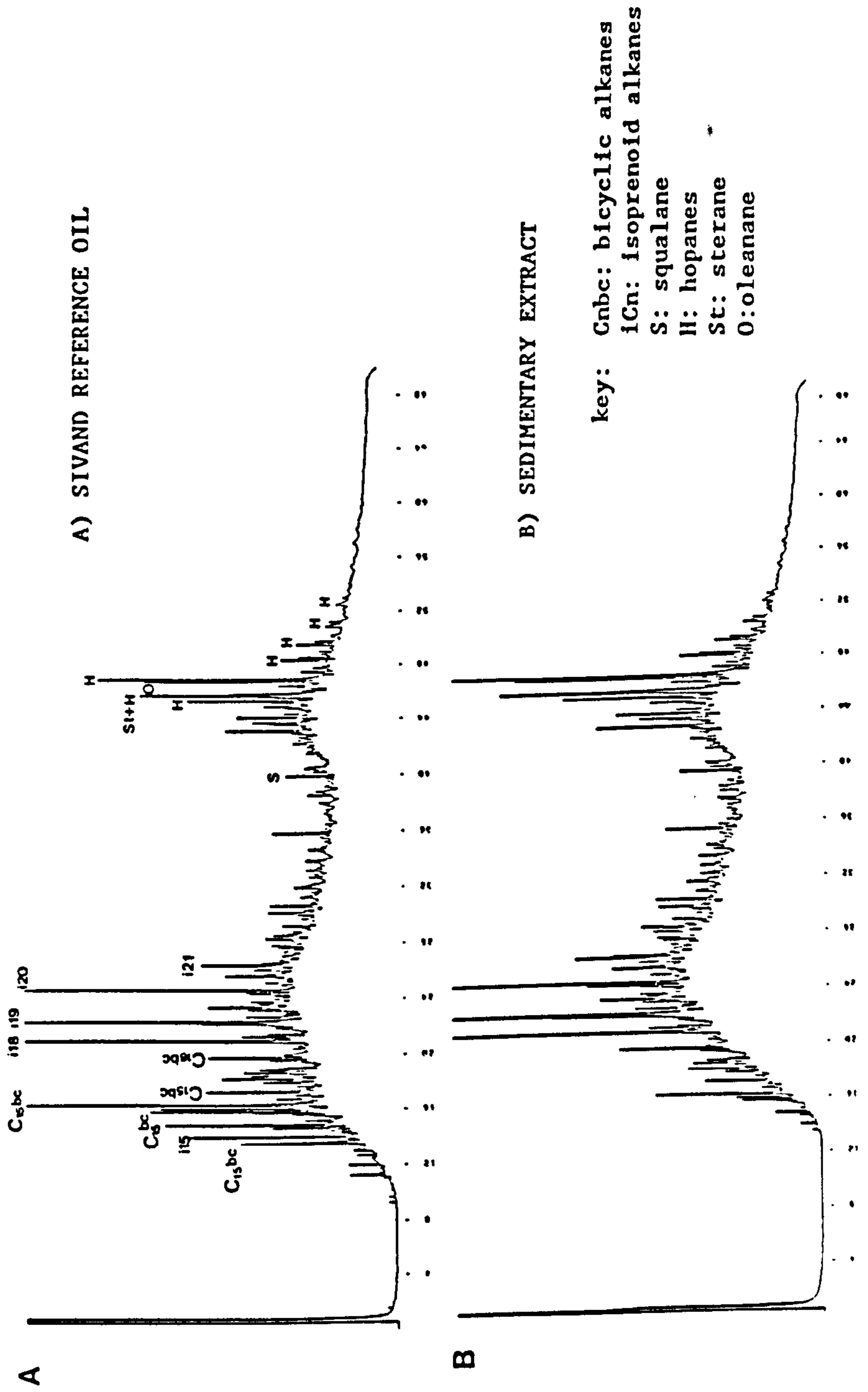
i) THE SIVAND REFERENCE OIL (4 DAYS POST SPILL)



ii) HUMBERSTON FITTIES (1 YEAR POST SPILL)

[GC: DB-5(J+W), 25m, 50-300°C @ 5°C min⁻¹, 300°C(20min)]

FIG. 5.14 GAS CHROMATOGRAMS OF UREA NON ADDUCTED ALKANES FOR



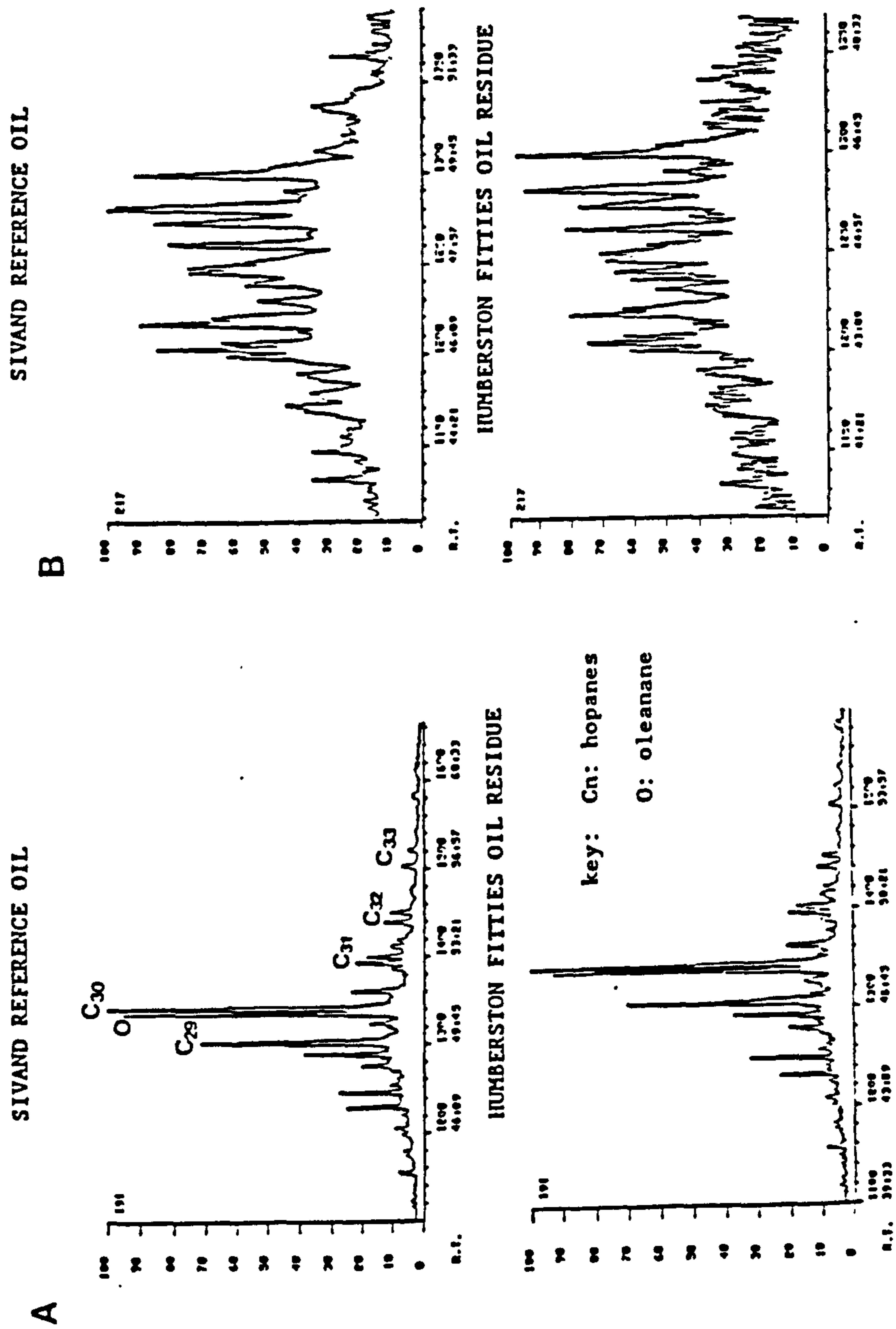
extracted to remove residual water, the DCM extracts dried (anh. Na_2SO_4) and following solvent removal the organic extract was subjected to the same chromatographic and urea adduction procedures. This provided 55% total alkanes of which 76% were urea non adducted alkanes (Table 5.1).

Gas chromatograms of the total alkanes recovered from each site (Fig. 5.13B) showed a partial reduction in the proportion of n-alkanes relative to branched alkanes (e.g. acyclic isoprenoids) and the UCM over the twelve month period. The gas chromatograms of the urea non adducted alkanes were virtually indistinguishable however except for a reduction in certain low molecular weight components (Fig. 5.14). It appears microbial alteration of the urea non adducted alkanes was minimal. GC-MS confirmed the major resolved components of both alkane fractions as acyclic isoprenoids (C15 - C21, squalane - C30) bicyclic alkanes (C15-C16), and triterpenoidal alkanes (e.g. C29 - C33 $17\alpha(\text{H})$, $21\beta(\text{H})$ - hopanes). Also present was an isomer of oleanane, the latter a characteristic feature of many Nigerian crude oils (e.g. Whitehead, 1974; Ekweozor et al., 1979; Jones et al., 1986) (Fig. 5.13).

GC-MS mass fragmentography using the ions m/z 191 (pentacyclic triterpanes) and m/z 217 (steranes) showed similar profiles for both isolated UCMs which along with the GC data provides good evidence for a common origin (Fig. 5.15).

Oxidation using CrO_3 /glacial acetic acid provided a yield of 83% total recoverable material for both the reference oil UCM and the UCM derived from the partially weathered oil residue (Table 5.1). GC of

FIG. 5.15 PARTIAL GC-MS MASS FRAGMENTOGRAMS OF A) PENTACYCLIC TRITERPANES (m/z 191) AND B) STERANES (m/z 217) OBSERVED FOR THE "SIVAND" REFERENCE OIL AND THE HUMBERSTON FIFTIES OIL RESIDUE ALKANES



both showed an almost identical distribution of resolved oxidation products (Fig. 5.16). Again the most abundant components observed were a series of n-monocarboxylic acids in the range C6 - C21, maximum C7, C8. Other products identified compared well with those observed in previous UCM oxidations. These included n-alkan-2-ones (C8 - C16), iso alkan-2-ones (C8-C12), γ -methyl- γ -lactones (C16-C11), iso- and anteiso-monocarboxylic acids (C15-C13), and certain monomethyl branched monocarboxylic acids, particularly 3-methyl substituted (C18-C11). Elevated abundances were noted for products consistent with an acyclic isoprenoid origin. These included the isoprenoid acids 4-methylpentanoic acid, 4-methylhexanoic acid, 3,7-dimethyloctanoic acid, 4,8-dimethylnonanoic acid, 4,8,12-trimethyltridecanoic acid, the isoprenoid-derived alkyl ketones 6,10-dimethylundecan-2-one and 6,10,14-trimethylpentadecan-2-one, and the isoprenoid-derived keto acid 8-oxo-4-methylnonanoic acid (Figs. 5.16/5.17) Table 5.6). The high proportion of these components is not surprising in view of the high abundance of pristane and other acyclic isoprenoids in the urea non adducts prior to oxidation. Most of the pristane, though not all, was consumed during the oxidation (Fig. 5.16). Also noted in higher abundance relative to other members of the series was a C11 branched γ -methyl- γ -lactone (Fig. 5A). This compound has been observed in previous UCM oxidations though generally in lesser abundance. There is indirect evidence therefore of an acyclic isoprenoid-type origin for this component, as such it's molecular structure may be:-

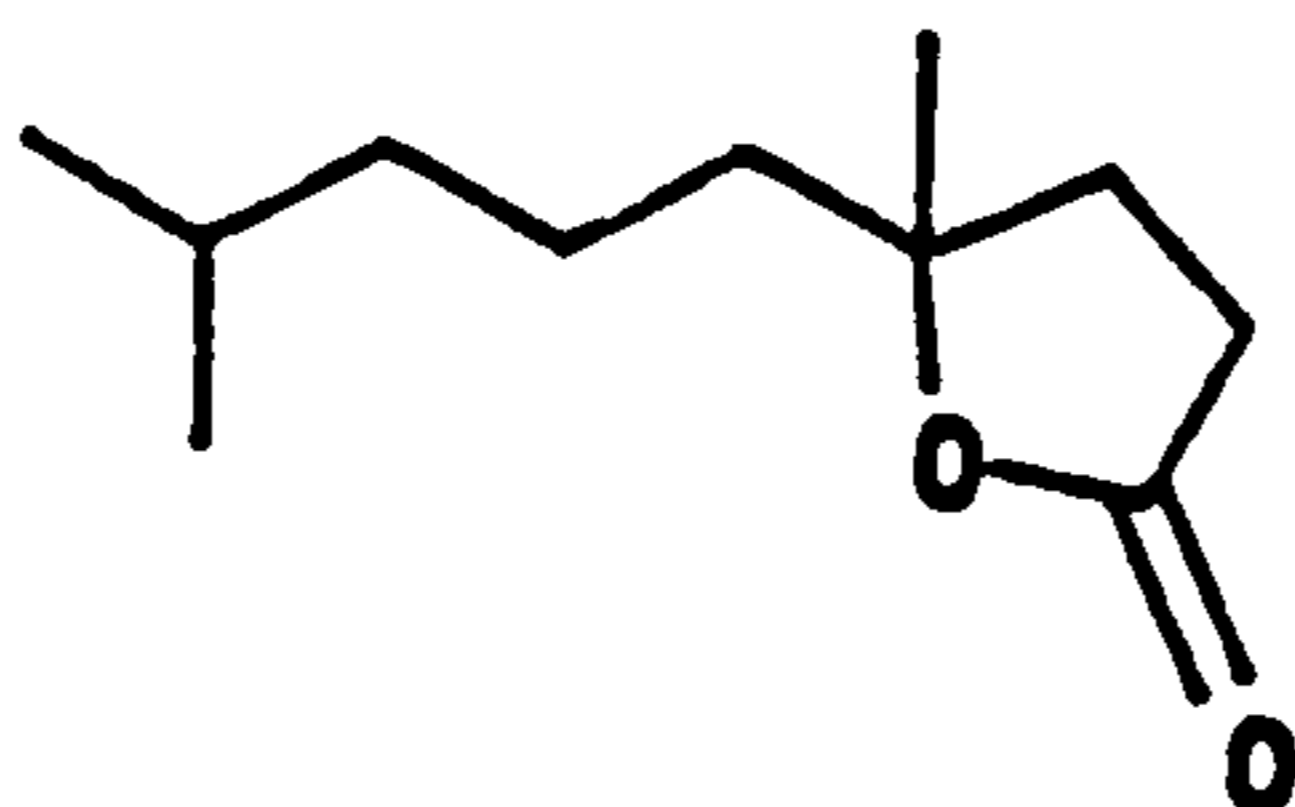


FIG. 5.16 GAS CHROMATOGRAMS OF THE TOTAL OXIDATION PRODUCTS OF A) THE SIVAND REFERENCE OIL ALKANE UCM AND B) THE HUMBERSTON FITTIES OIL RESIDUE ALKANE UCM

[GC: DB-5(J+W), 25m, 50-300°C @ 5°C min⁻¹, 300°C(20min)]

(for peak identity see Table 5.6)

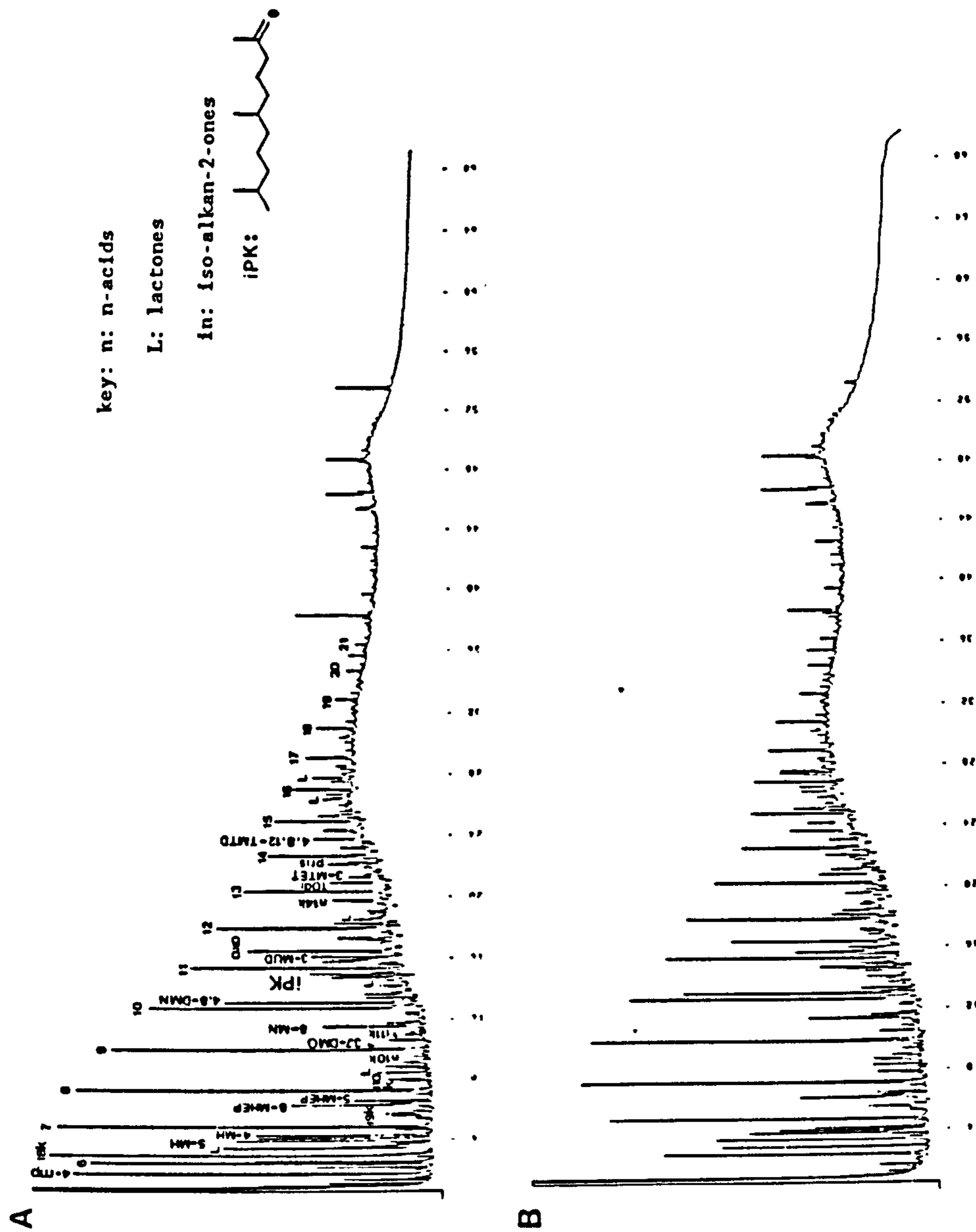
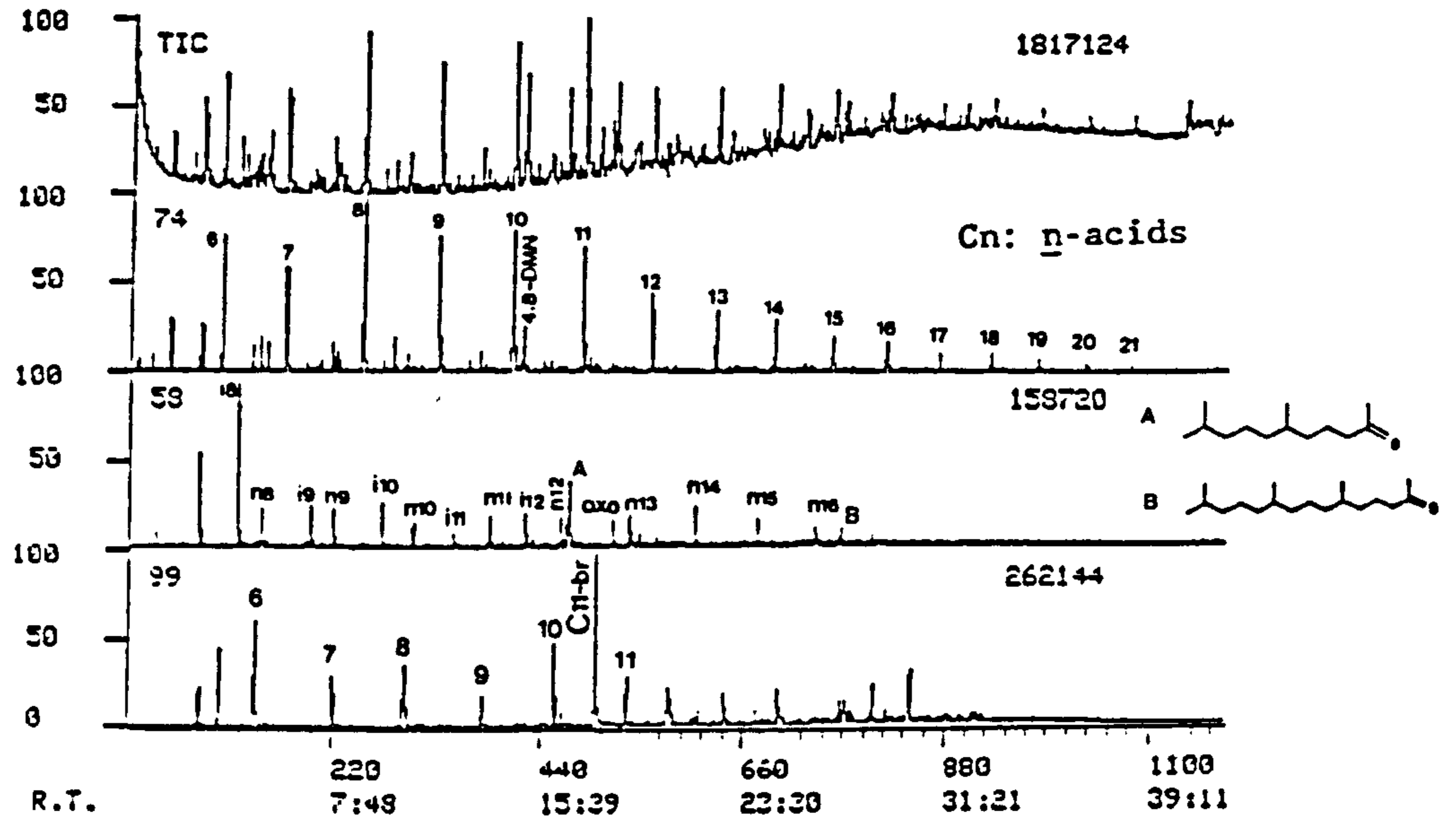


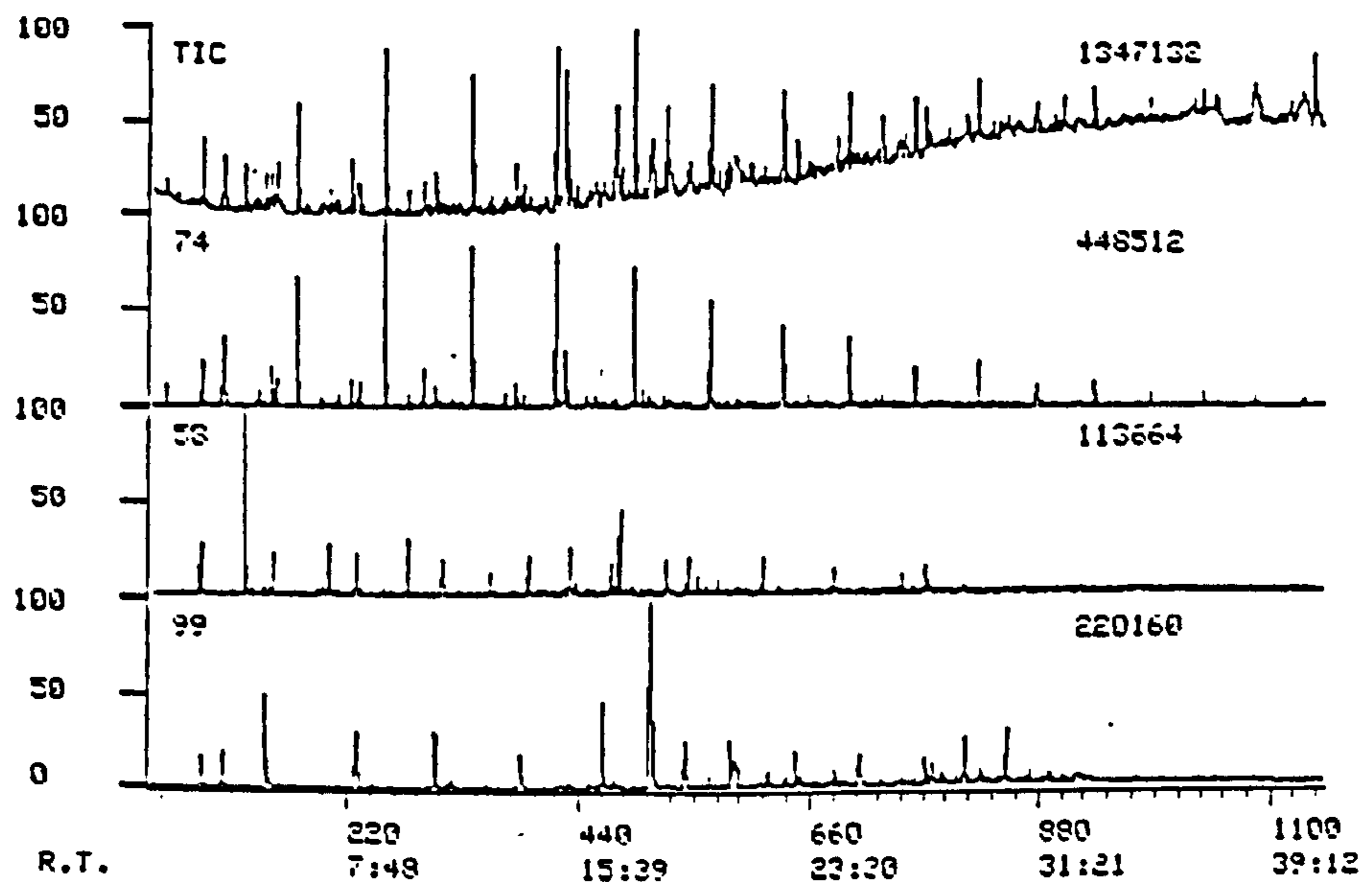
FIG. 5.17 COMPARATIVE GC-MS MASS FRAGMENTOGRAMS OF THE RESOLVED PRODUCTS OF OXIDATION OF A) THE SIVAND REFERENCE OIL UCM AND B) THE HUMBERSTON FITTIES OIL RESIDUE UCM

(m/z 74: N-ACIDS, m/z 58: ALKYL KETONES, m/z 99: γ -LACTONES)

A



B



nCn: n-alkan-2-ones

Cn: γ -methyl- γ -lactone

iCn: iso-alkan-2-ones

C11br: C11 methyl branched γ -methyl- γ -lactone

oxo: C10 oxo acid

TABLE 5.6

COMPONENTS IDENTIFIED BY GC-MS (MASS SPECTRA AND EQUIVALENT CHAIN LENGTHS)
AS OXIDATION PRODUCTS OF THE SIVAND OIL SPILL WEATHERED OIL UCM AND
REFERENCE OIL UCM (EXCLUDING n- MONOCARBOXYLIC ACIDS).

COMPOUND ¹ CODE	NAME ²	ECL VALUE	POSSIBLE ORIGIN
4-MP	4-methylpentanoic acid	5.75	isoprenoid
iC ₈ K	6-methylheptan-2-one	6.28	isoprenoid
5-MH	5-methylhexanoic acid	6.62	ucm
4-MH	4-methylhexanoic acid	6.72	ucm/isoprenoid
iC ₉ K	7-methyloctan-2-one	7.36	ucm
6-MHEP	6-methylheptanoic acid	7.62	ucm
5-MHEP	5-methylheptanoic acid	7.69	ucm
iC ₁₀ K	8-methylnonan-2-one	8.27	ucm
3-MN	3-methylnonanoic acid	8.43	ucm
n-C ₁₀ K	<u>n</u> -decan-2-one	8.69	ucm
3,7-DMO	3,7-dimethyloctanoic acid	9.04	isoprenoid
iC ₁₁ K	9-methyldecan-2-one	9.23	ucm
8-MN	8-methylnonanoic acid	9.55	ucm
4,8-DMN	4,8-dimethylnonanoic acid	10.15	isoprenoid
3-MUD	3-methylundecanoic acid	11.40	ucm
C ₁₀ oxo	8-oxo-4-methylnonanoic acid	11.44	isoprenoid
n-C ₁₄ K	<u>n</u> -tetradecan-2-one	12.75	ucm
C ₁₀ diacid	<u>n</u> -decandioic acid	13.24	ucm
3-MTET	3-methyltetradecanoic acid	13.39	ucm
pris	pristane	13.82	isoprenoid
4,8,12-TMTD	4,8,12-trimethyltridecanoic acid	14.50	isoprenoid

1: see Fig. 5.15 for elution order

2: identified as methyl esters

L: corresponds to varied lactones

6-12: corresponds to n-monocarboxylic acids with that number of C atoms

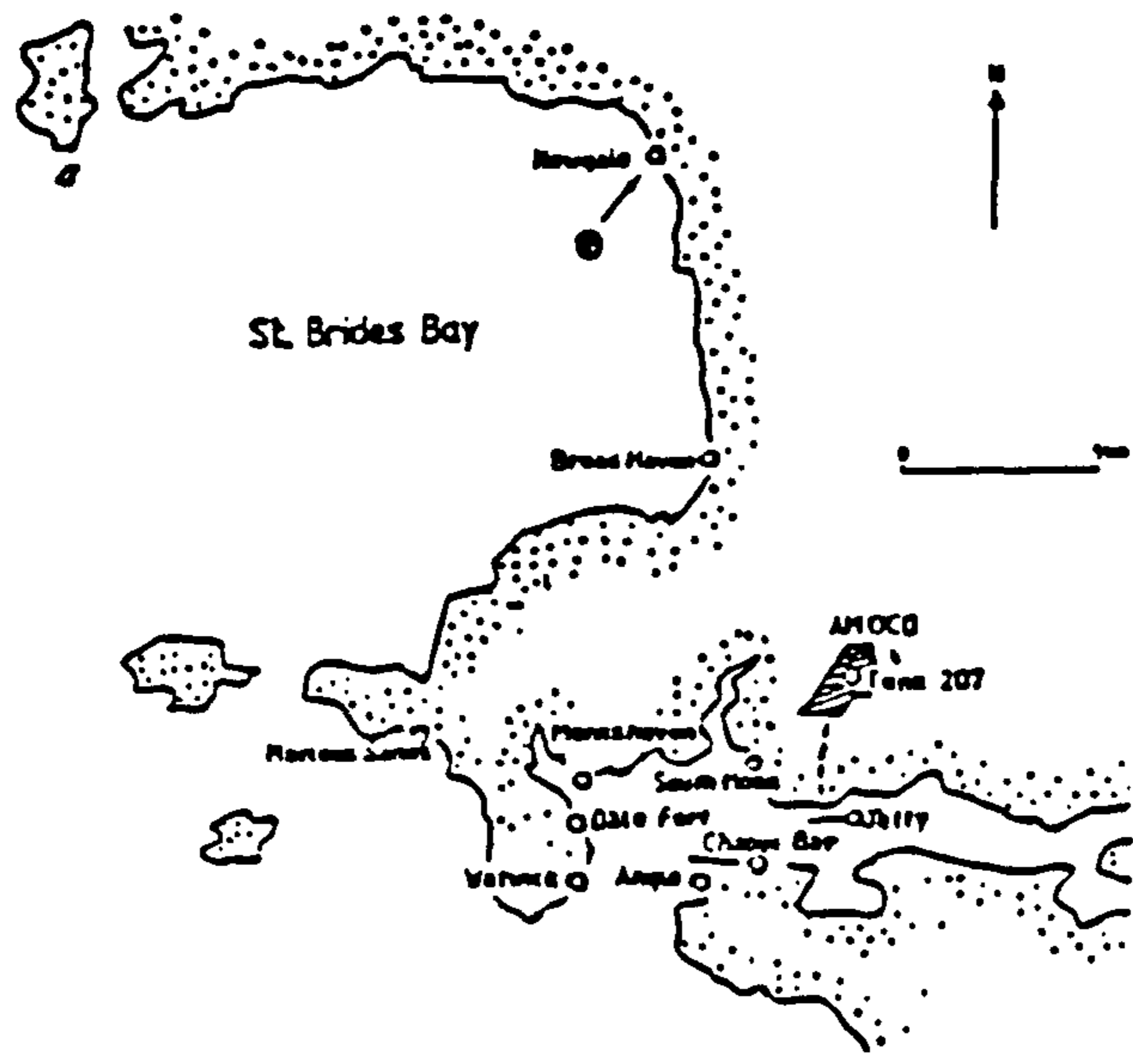
In summary there was an excellent correlation between the two oils and their products of oxidation, with almost identical distributions of resolved functionalised compounds observed both by GC (Fig. 5.16) and GC-MS analysis (Fig. 5.17). In this respect the technique would appear to have excellent potential as a means of distinguishing UCMs from various sources. This potential was examined further by oxidation of a spilled oil UCM with a candidate (though not proven) reference oil UCM (section 5.5).

5.5 THE AMOCO TANK OIL SPILL

On the 27 February 1986, approximately 4 tonnes of heavy fuel oil was released into Milford Haven from a fractured pipeline on the jetty of the Amoco Milford Refinery (Fig 5.18A). Though a clean up operation was organised by Milford Haven Conservancy Board, the bulk of the oil dispersed out of the estuary under the influence of surface currents and a strong easterly wind. Ten days later, oil of unknown origin was observed coming ashore on the Marloes Peninsula and on the beaches of St. Brides Bay (Fig. 5.18A). The oil company denied responsibility for this beached oil citing the higher sulphur content of the beached oil compared to the spilled oil, as evidence that the oils were not the same. No reports of any other spillage of oil in or around Milford Haven were made at the time. (Mr S. Howells, OPRU, Field Studies Council, personal communication).

Samples of stranded oil from Newgale Beach and a candidate reference oil from the Amoco Refinery Tank No. 207 (Fig. 5.18A) were supplied by Mr S. Howells, OPRU, Field Studies Council. These were fractionated by silver ion TLC followed by urea adduction which

FIG. 5.18 A) MAP SHOWING LOCATION OF AMOCO MILFORD REFINERY AND SITE OF COLLECTION OF BEACHED OIL (⊙)

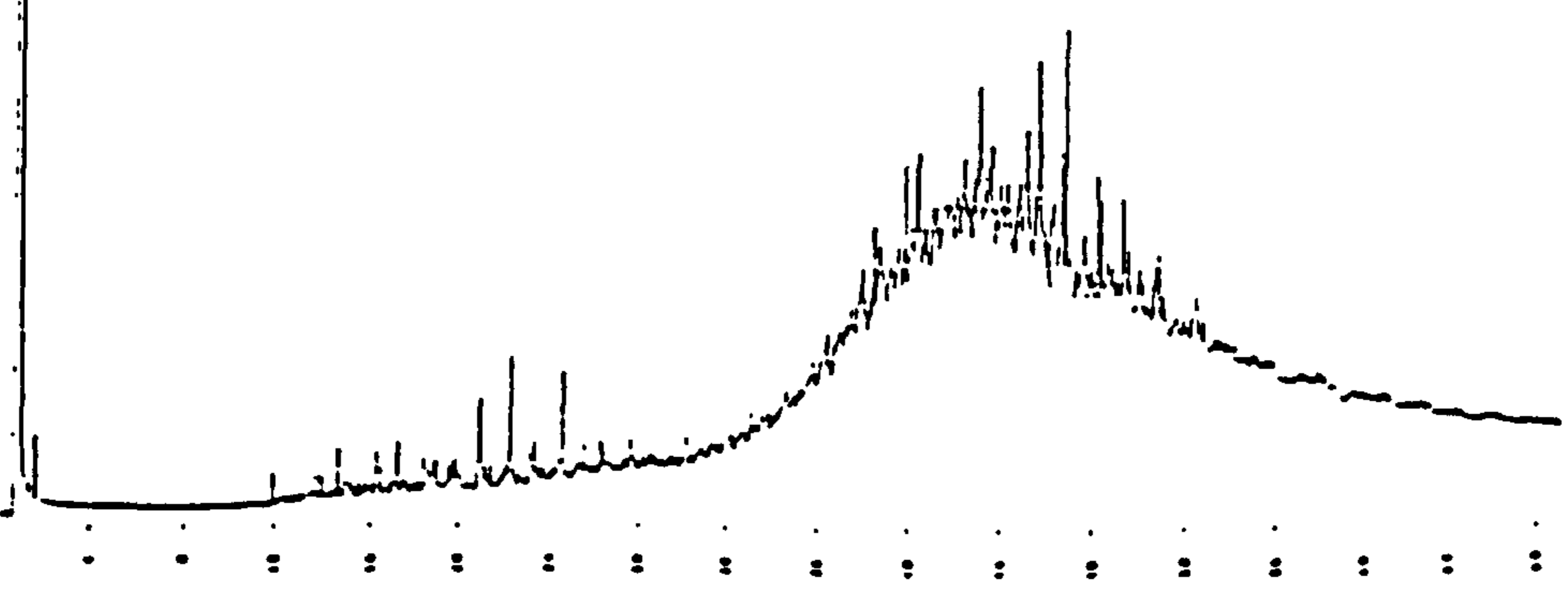


B) GAS CHROMATOGRAMS OF TOTAL UREA NON ADDUCTED ALKANES OBTAINED FROM AMOCO TANK 207 AND NEWGALE BEACH

AMOCO TANK 207

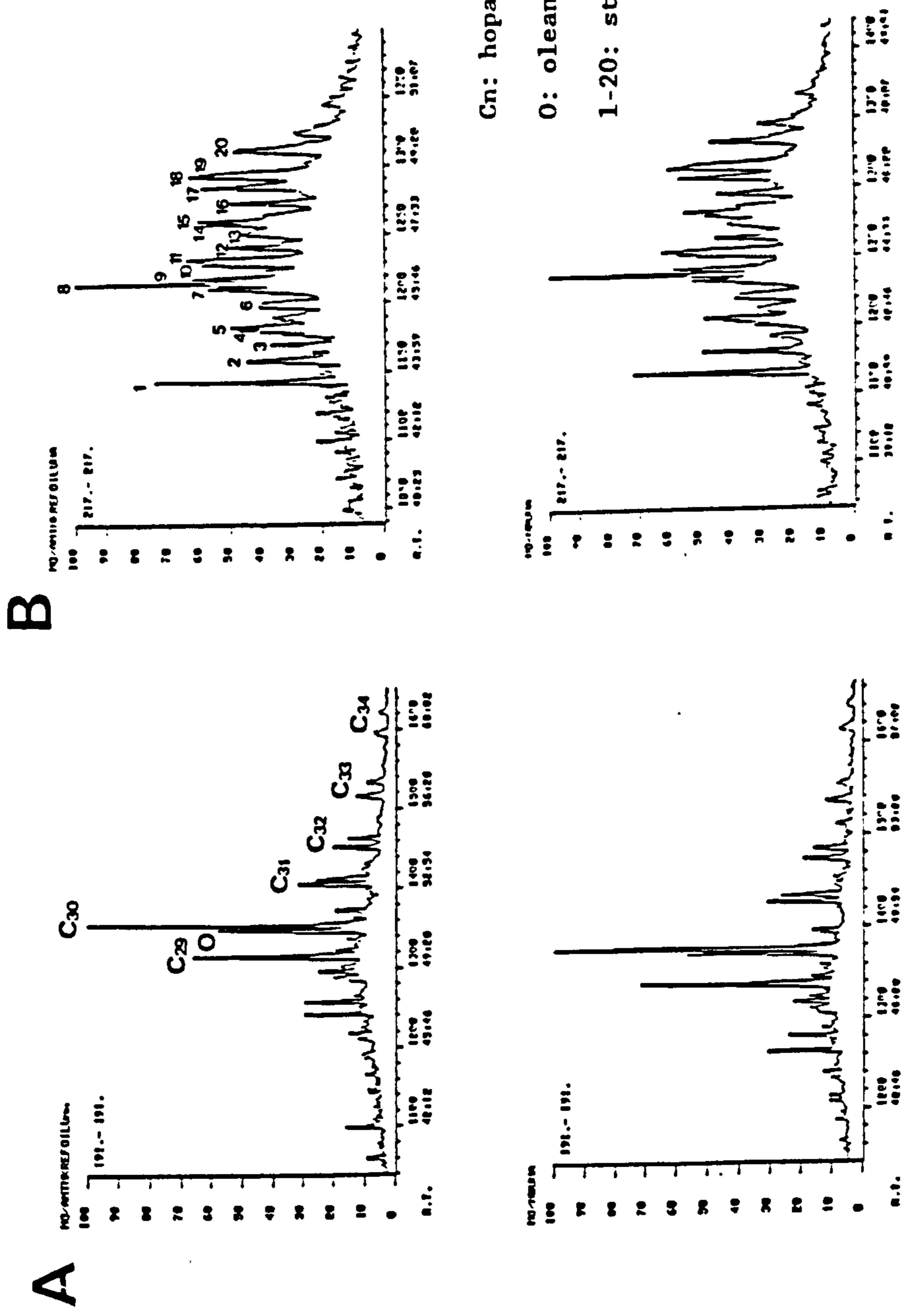


NEWGALE BEACH



[GC: DB-5(J+W), 25m, 50-300°C @ 5°C min⁻¹, 300°C(20min)]

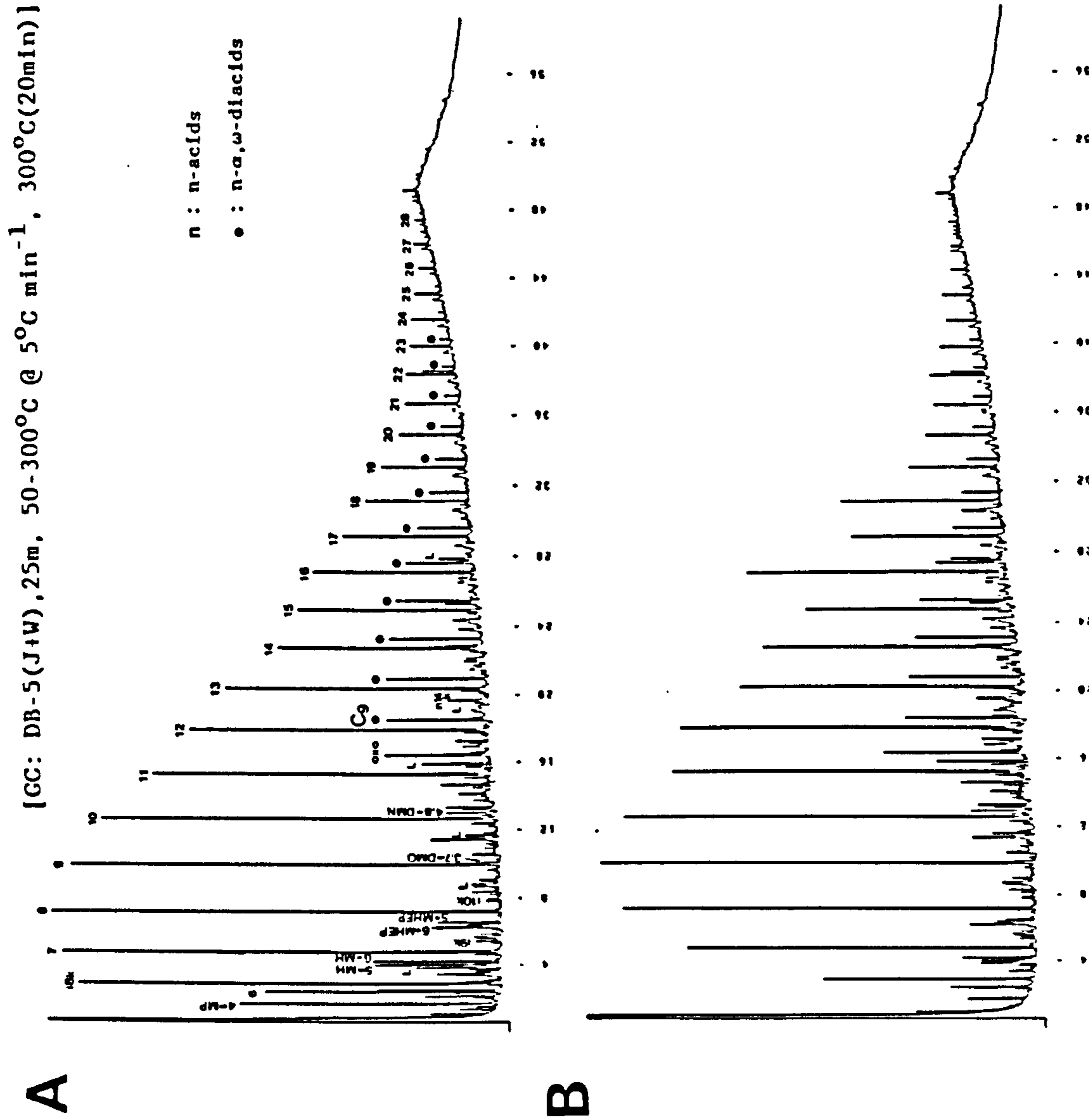
FIG 5.19 PARTIAL GC-MS MASS FRAGMENTOGRAMS OF A)
 PENTACYCLIC TRITERPANES (m/z 191) AND B) STERANES (m/z 217)
 OBSERVED FOR THE AMOCO TANK REFERENCE OIL AND THE STRANDED
 OIL FROM NEWGALE BEACH



provided 10% total alkanes for each sample, of which 71% comprised urea non adducted alkanes for the reference oil, 61% for the beached oil (Table 5.1). The gas chromatograms of the urea non adducted alkanes (Fig. 5.18B) were virtually indistinguishable, and GC-MS confirmed the major resolved components as regular acyclic isoprenoids (C18-C20), steranes and triterpanes. GC-MS mass fragmentography using the ions m/z 191 (pentacyclic triterpanes) and m/z 217 (steranes) also showed near identical profiles for both samples (Fig. 5.19). In particular, an isomer of oleanane was identified (marked O, Fig. 5.18B and Fig. 5.19), this is thought to be of higher plant origin and as such is a characteristic feature of terrestrially-derived or deltaic crude oils (Whitehead, 1974; Ekweozor et al., 1979; Grantham et al., 1983; Hoffman et al., 1984; Philp and Gilbert, 1986).

Oxidation of the urea non adduct with CrO_3 in glacial acetic acid produced a yield of total recovered material of 87% for the reference oil and 75% for the beached oil (Table 5.1). GC of the products of oxidation (Fig. 5.20) showed an almost identical distribution of components for both samples. These were confirmed by GC-MS as n-monocarboxylic acids (C16-C28; max. C8); n- α,ω -diacids (C9-C23, max. C9); n-alkan-2-ones (C7-C11, max. C7); iso-methyl branched alkan-2-ones (C8-C11); γ -methyl- γ -lactones (C6-C11) and a methyl branched γ -methyl- γ -lactone [C11; (Fig. 5.21)]. Other products identified included monomethyl branched acids (particularly iso, anteiso, and 3-methyl), and products consistent with an acyclic isoprenoid-type origin; e.g. the isoprenoid acids 3,7-trimethyltridecanoic acid, the isoprenoid ketones 6,10-dimethylundecan-2-one and 6,10,14 -

FIG. 5.20 GAS CHROMATOGRAMS OF THE TOTAL OXIDATION PRODUCTS OF A) THE AMOCO TANK REFERENCE OIL ALKANE UCM AND B) THE NEWGALE BEACH OIL RESIDUE ALKANE UCM

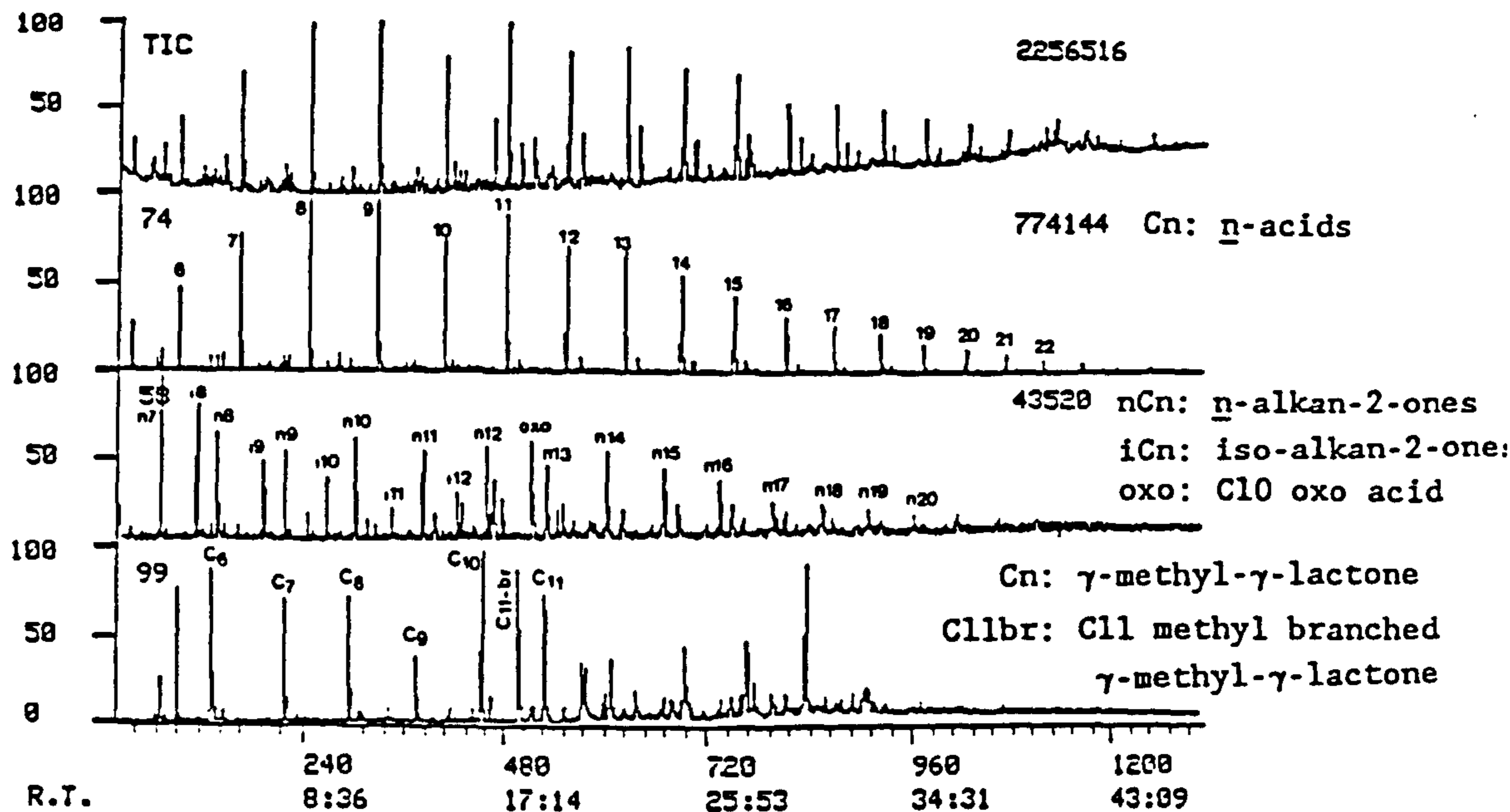


(for peak identity see Table 5.6)

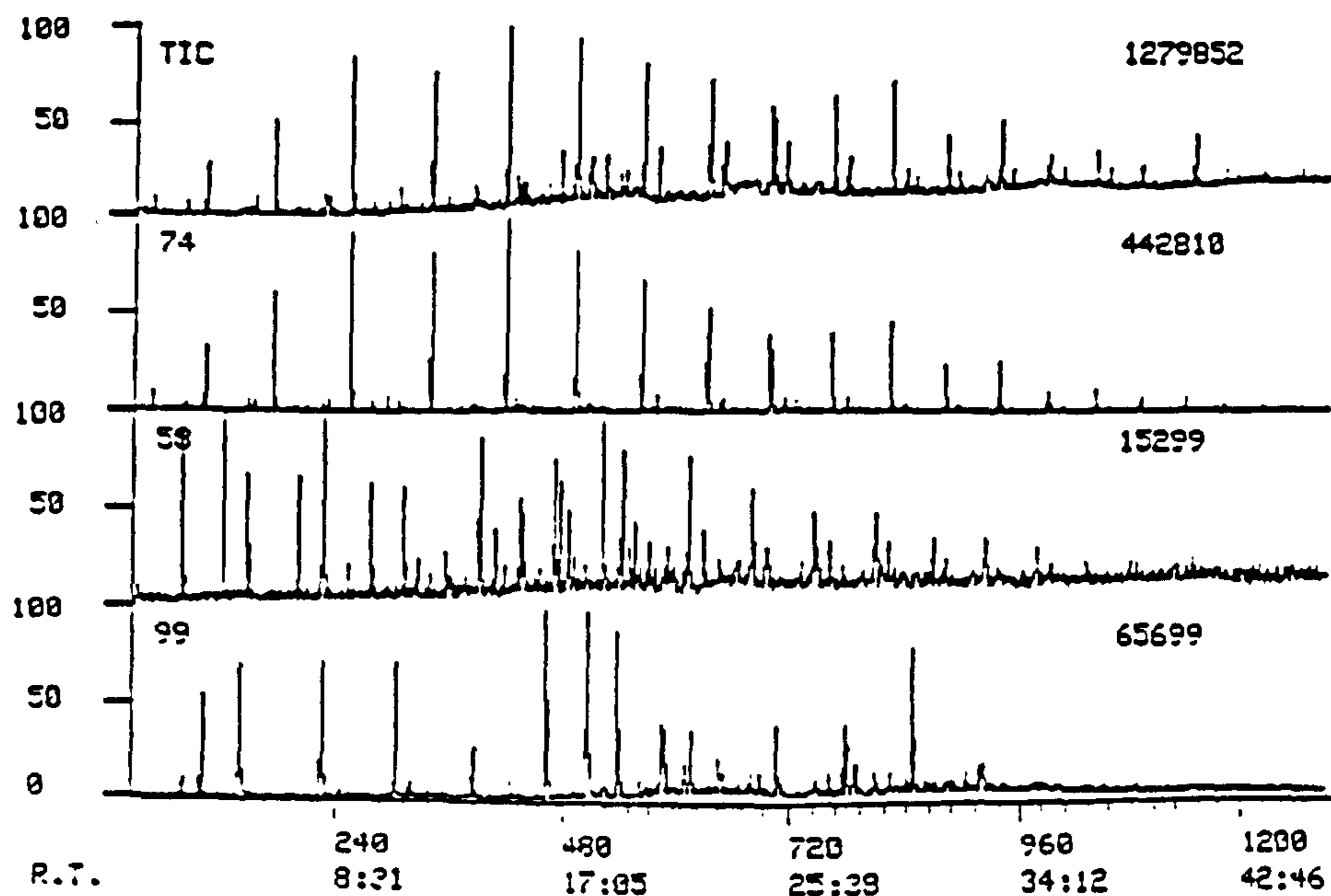
FIG. 5.21 COMPARATIVE GC-MS MASS FRAGMENTOGRAMS OF THE RESOLVED PRODUCTS OF OXIDATION OF A) THE AMOCO TANK REFERENCE OIL UCM AND B) THE HUMBERSTON FITTIES OIL RESIDUE UCM

(m/z 74: N-ACIDS, m/z 58: ALKYL KETONES, m/z 99: γ -LACTONES)

A



B



trimethylpentadecan-2-one; and the isoprenoid-derived keto acid 8-oxo-4-methylnonanoic acid (Fig. 5.20 and Fig. 5.21). These latter components may originate as products of oxidation of the regular acyclic isoprenoids identified as resolved components of the urea non adducted alkanes (Fig. 5.18B). However, in view of their abundance relative to other resolved oxidation products, the low abundance of identified acyclic isoprenoids prior to oxidation, and the susceptibility of these compounds to further oxidation (e.g. oxidation of pristane, chapter 4), it would appear that these may also arise as products of oxidation of isoprenoid - type alkyl linkages within the UCM.

To summarise, classical GC and GC-MS techniques of "fingerprinting" crude oils provided good correlations between the profiles observed for the beached oil and the candidate reference oil obtained from the Amoco Refinery. Further evidence of a common origin was provided by oxidation of the urea non adducted alkanes - the GC and GC-MS profiles of the products of oxidation were virtually indistinguishable. In particular the extended range of n-monocarboxylic acids (up to C28), and the relative abundance and range of n- α,ω -diacids (C9-C23) appeared unique to this oil. Further correlations were sought and found in the m/z 99 (γ -methyl- γ -lactone) and m/z 58 (alkyl ketone) mass fragmentograms. These will be discussed with reference to other UCM oxidations in section 5.7.

5.6 ATHABASCA OIL SAND

The Alberta oil sands of Western Canada represent one of the World's largest accumulations of non conventional crude oil. In this region

an estimated 892 billion barrels (142 billion m³) of heavy oil are in place. This accounts for ca. 42% of the total known World's reserves of heavy oil (Demaison, 1977). In view of the economic importance of such vast deposits of potentially recoverable oil a considerable volume of research has focused on their distribution (Govier, 1984; Jardine, 1974; Demaison, 1977); origin (Evans et al., 1971; Deroo et al., 1974; Montgomery et al., 1974; Rubinstein et al., 1977); and composition (e.g. see Deroo et al., 1974, 1977; Selucky et al., 1977; and Brooks et al., 1989^b for general reviews). Although previously a matter of some debate (e.g. Deroo et al., 1974; Montgomery et al., 1974) it is now generally accepted that the Alberta oil sand bitumens have arisen from the water washing and biodegradation of conventional crude oils (Rubinstein et al., 1977; Brooks et al., 1989^b). As such, the gas chromatograms of hydrocarbon fractions isolated from these bitumens are typical of in-reservoir biodegraded oils, and are characterised by an absence of n-alkanes, few resolved compounds, and a dominant UCM. (Deroo et al., 1977; Mojelsky and Strausz, 1986; Brooks et al., 1989^a).

Several reports detail attempts at the chemical characterisation of these complex hydrocarbon mixtures by a number of analytical techniques. These include "biological marker" distributions by GC-EI MS (e.g. Selucky et al., 1977; Rubinstein et al., 1977; Ekweozor and Strausz, 1983; Hoffman and Strausz, 1986; Brooks et al., 1989^{a,b,c}); probe distillation field ionisation mass spectrometry [FIMS saturates: Payzant et al., 1979, 1984; aromatics: Payzant et al., 1979, 1985) , and GC-FIMS (Payzant et al. 1980) . Of particular interest to the current study is the reported production of alkylated

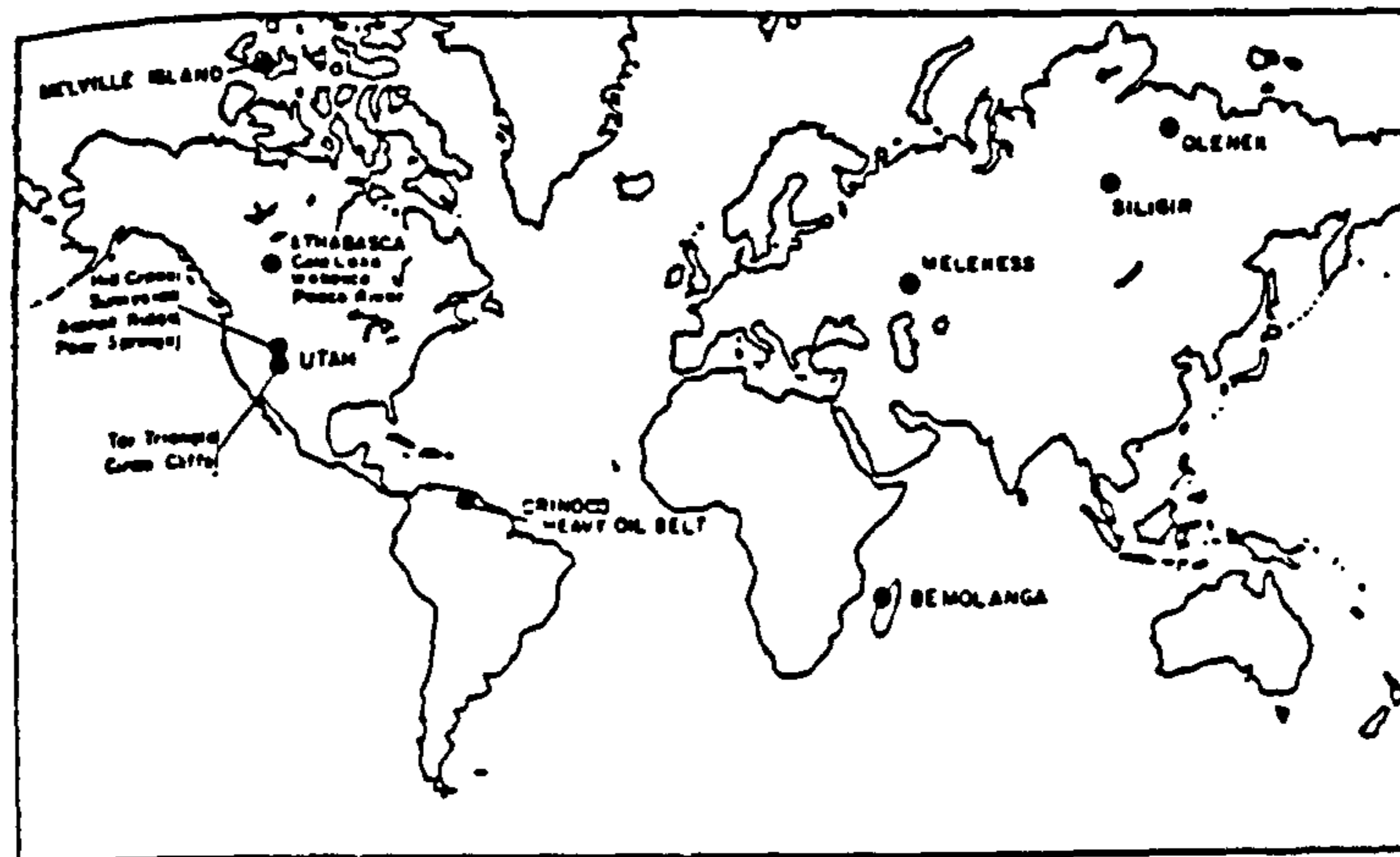
fluoren-9-ones by chemical oxidation of the aromatic hydrocarbon fraction isolated from an Athabasca oil sand bitumen (Mojelsky and Strausz, 1986). This is one of the few attempts at structural characterisation of a hydrocarbon UCM by chemical oxidation. Apparently no attempt has yet been made to characterise the aliphatic UCM derived from a similar oil sand bitumen by chemical oxidation.

The sample chosen for study was from the Devonian Grosmont formation - part of the so-called "Carbonate Triangle" (Fig. 5.22), and kindly supplied by Dr Martin Fowler, Alberta Research Council, Canada. The bitumen was obtained from a core at 292.3 - 292.9m depth, and extract dissolved in DCM was provided. Argentatious TLC and urea adduction provided 8% total alkanes of which 94% comprised urea non adducted alkanes (Table 5.1). The low proportion of saturated hydrocarbons is typical of Western Canada oil sand bitumens. In particular the present sample has a reported composition of 8% saturates, 17% aromatics, 9.5% resins and 57% asphaltenes (Brooks et al., 1989^b).

GC of the total urea non adducted alkanes (Fig. 5.23A) showed a broad UCM with few resolved components. Normal and acyclic isoprenoid alkanes were absent in agreement with the data of Brooks et al., (1989^b). GC-MS mass fragmentography using ions characteristic of 3- and 5- ring terpenoidal alkanes (m/z 191) and steranes (m/z 217) revealed the distribution of these compounds in the urea non adduct (Fig. 5.24, Table 5.7). Their distribution compared well with those reported by Brooks et al., (1989^b).

Oxidation of the urea non adducted alkanes produced a series of resolved peaks (Fig. 5.23B) in an overall yield of 93% total recovered material. GC-MS (Fig. 5.25) showed these to comprise n-

FIG. 5.22 A) MAP SHOWING GLOBAL OCCURENCES OF HEAVY OIL DEPOSITS (AFTER DEMAISON. 1977)



B) MAP SHOWING LOCATION OF ATHABASCA OIL SAND DEPOSITS AND SAMPLE SITE (circled) (after Brooks et al., 1989^a)

FIG 5.23 GAS CHROMATOGRAMS OF ATHABASCA OIL SAND ALKANE UCM
 A) BEFORE OXIDATION AND B) AFTER OXIDATION

[GC: DB-5(J+W), 25m, 50-300°C @ 5°C min⁻¹, 300°C(20min)]

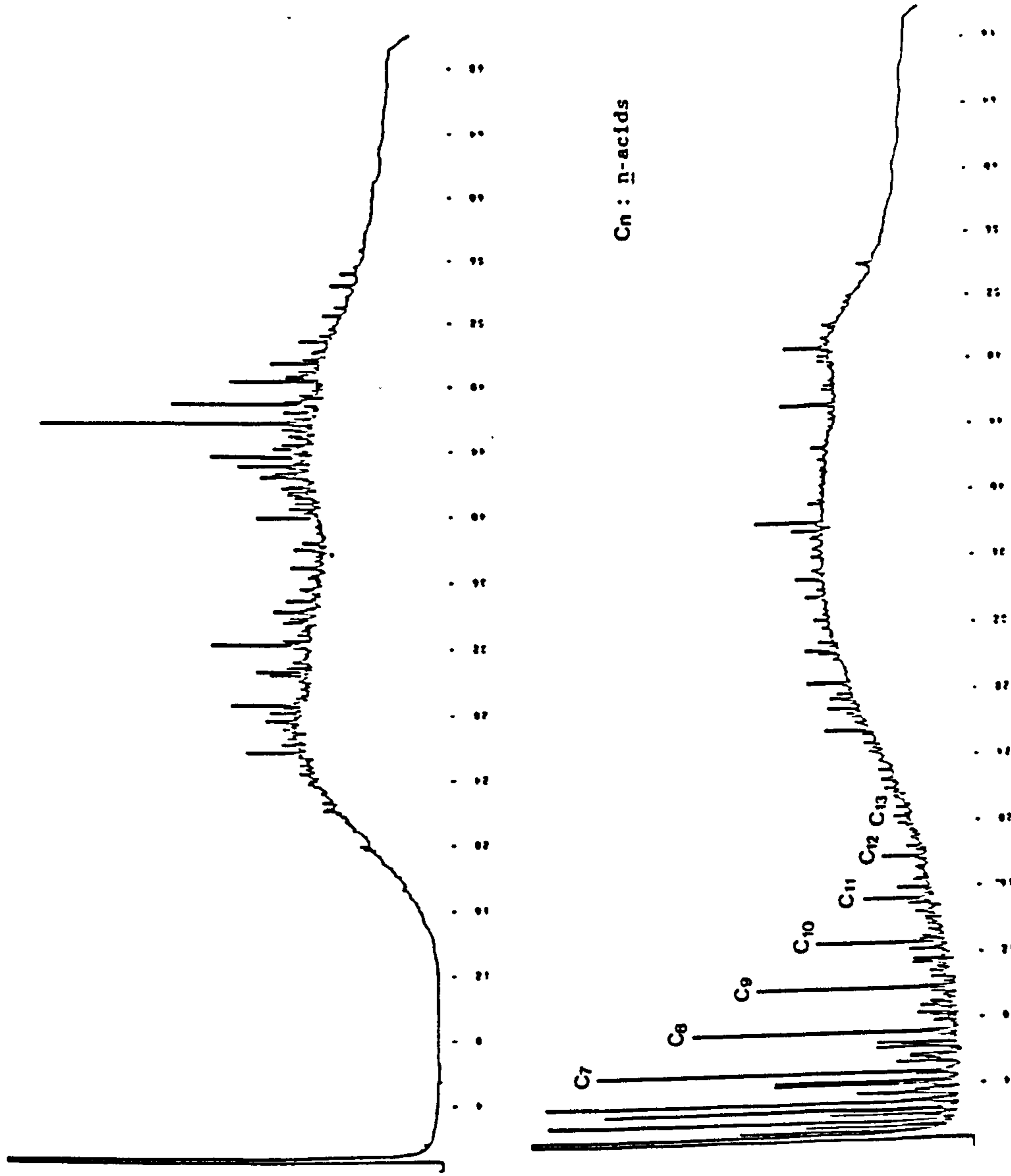
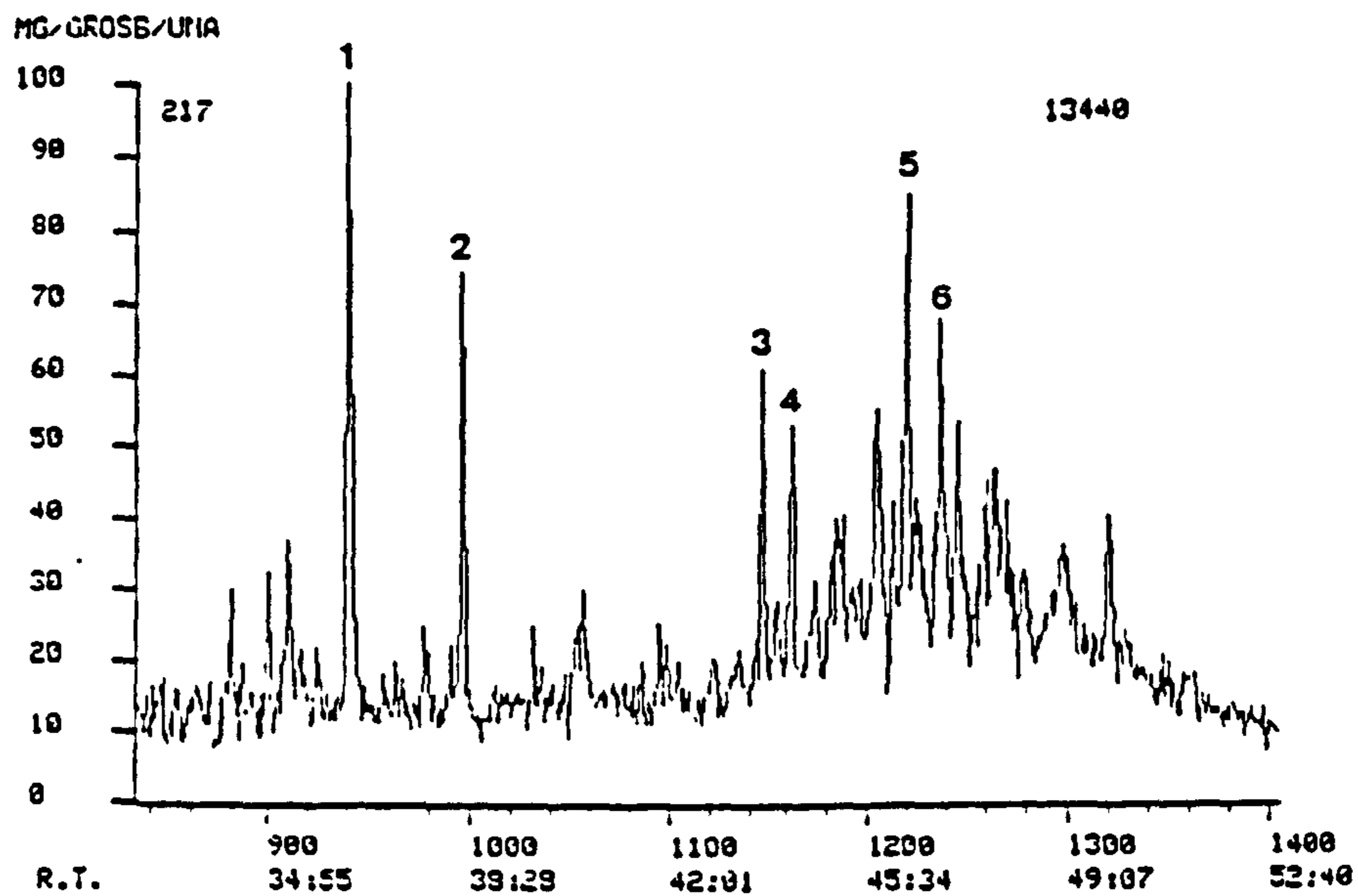
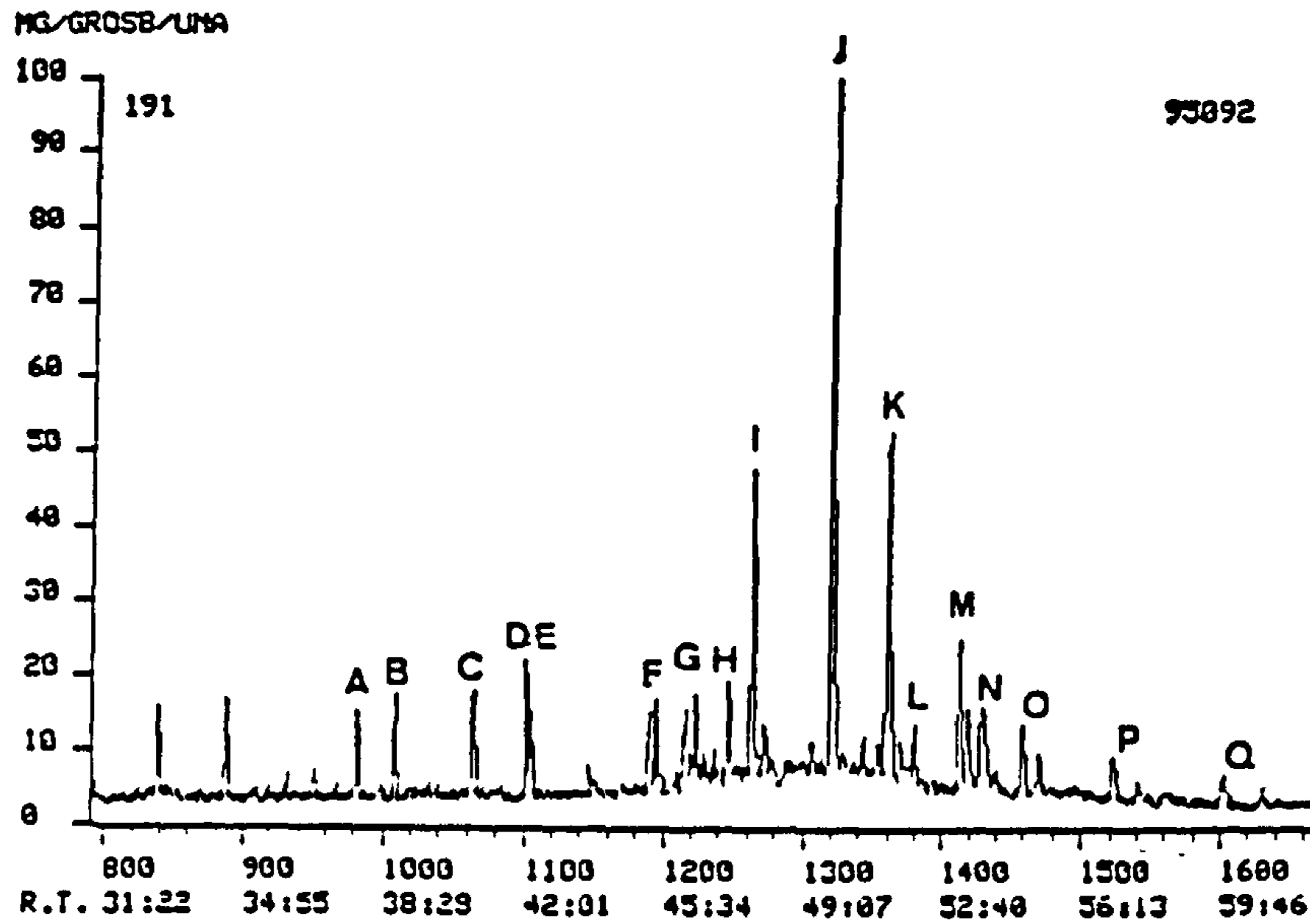


FIG. 5.24 GC-MS MASS FRAGMENTOGRAMS OF A) TERPENOIDAL ALKANES (m/z 191) AND B) STERANES (m/z 217) IDENTIFIED IN THE ATHABASCA OIL SAND BITUMEN ALKANE UCM



(for peak identity see Table 5.7)

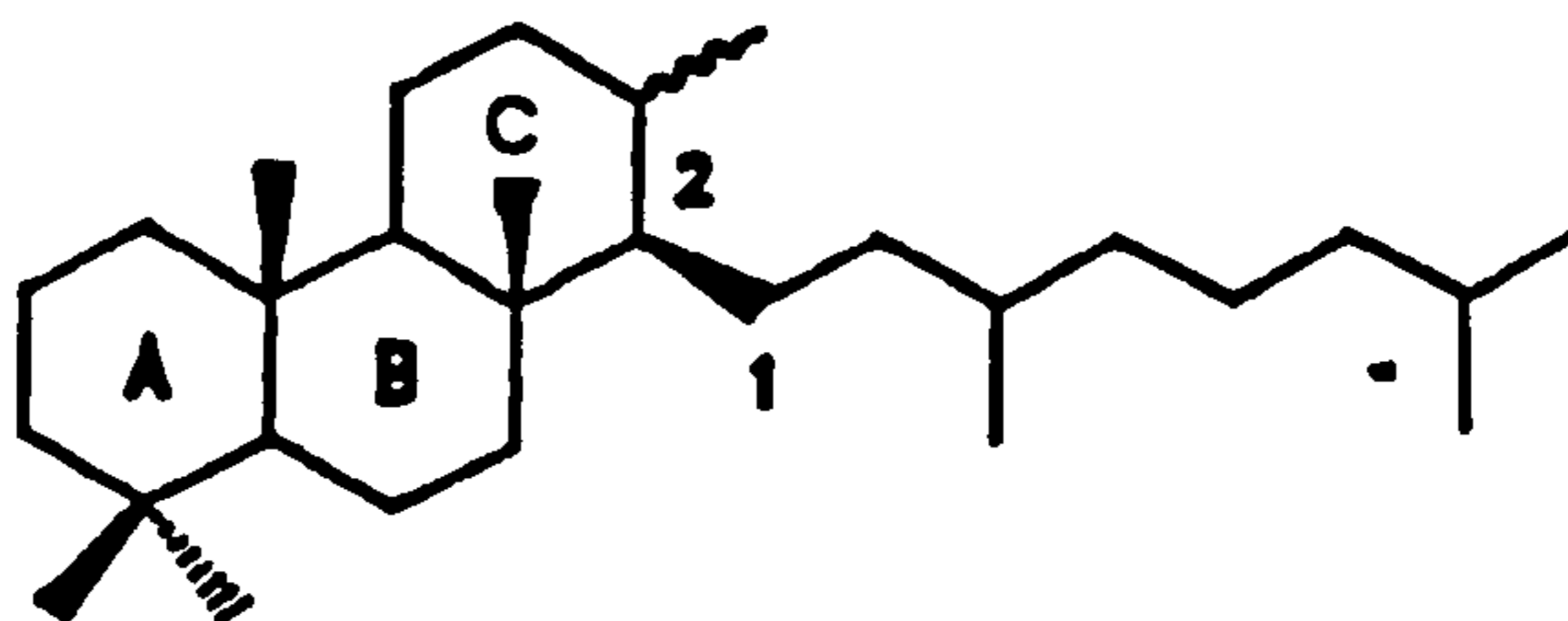
TABLE 5.7

SUMMARY OF GC-MS IDENTIFICATIONS OF THE RESOLVED COMPONENTS OF THE m/z 191 (3- + 5- RING TERPENOIDAL ALKANES) and m/z 217 (STERANES) MASS FRAGMENTOGRAMS- ATHABASCA OIL SAND BITUMEN UREA NON ADDUCTED ALKANE FRACTION

PEAK	m/z 191 COMPOUND	m/z 217
A	C23 tricyclic terpane	1 C21 sterane
B	C24 tricyclic terpane	2 C22 sterane
C	C25 tricyclic terpane	3 13 β (H),17 α (H)- diacholestane (20S)
D	C24 tetracyclic terpane	4 13 β (H),17 α (H)- diacholestane (20R)
E	C26 tricyclic terpane - isomers	5 5 α (H),14 β (H),17 β (H)- cholestane (20R)
F	C28 tricyclic terpane - isomers	6 24-ethyl-13 β (H),17 α (H)- cholestane
G	C29 tricyclic terpane - isomers	
H	18 α (H)- trisnorhopane	
I	17 α (H)- trisnorhopane	
J	17 α (H),21 β (H)-norhopane	
K	17 α (H), 21 β (H)- hopane	
L	17 β (H),21 α (H)- moretane	
M	17 α (H), 21 β (H)- homohopanes	
N	gammacerane	
O	17 α (H), 21 β (H)- bishomohopanes	
P	17 α (H), 21 β (H)- trishomohopanes	
Q	17 α (H), 21 β (H)- tetrakishomohopanes	

(identification based on comparative retention times; Brooks et al,1989^a)

monocarboxylic acids (C5-C12; max. C7); n-alkan-2-ones (C7-C13); iso-methyl branched alkan-2-ones (C7-C12); γ -methyl- γ -lactones (C6-C11), and a methyl branched γ -methyl- γ -lactone (C11). Mono methyl branched carboxylic acids were also identified, as too were compounds consistent with an acyclic isoprenoid-type origin. These included the isoprenoid acids 3,7-dimethyloctanoic acid, 4,8-dimethylnonanoic acid, the isoprenoid-derived ketone 6,10-dimethylundecan-2-one; and the isoprenoid keto acid 8-oxo-4-methylnonanoic acid. The identification of these components is surprising as resolved acyclic isoprenoid alkanes were not detected in the UCM prior to oxidation. However, the resolved cyclic isoprenoids, in particular the tricyclanes do have isoprenoid-type alkyl side chains which could potentially contribute to the isoprenoid-derived functionalised compounds. The longest isoprenoid alkyl chain would occur for the C29 tricyclic terpane isomers (G; Fig. 5.24) and would have ten carbon atoms viz:-

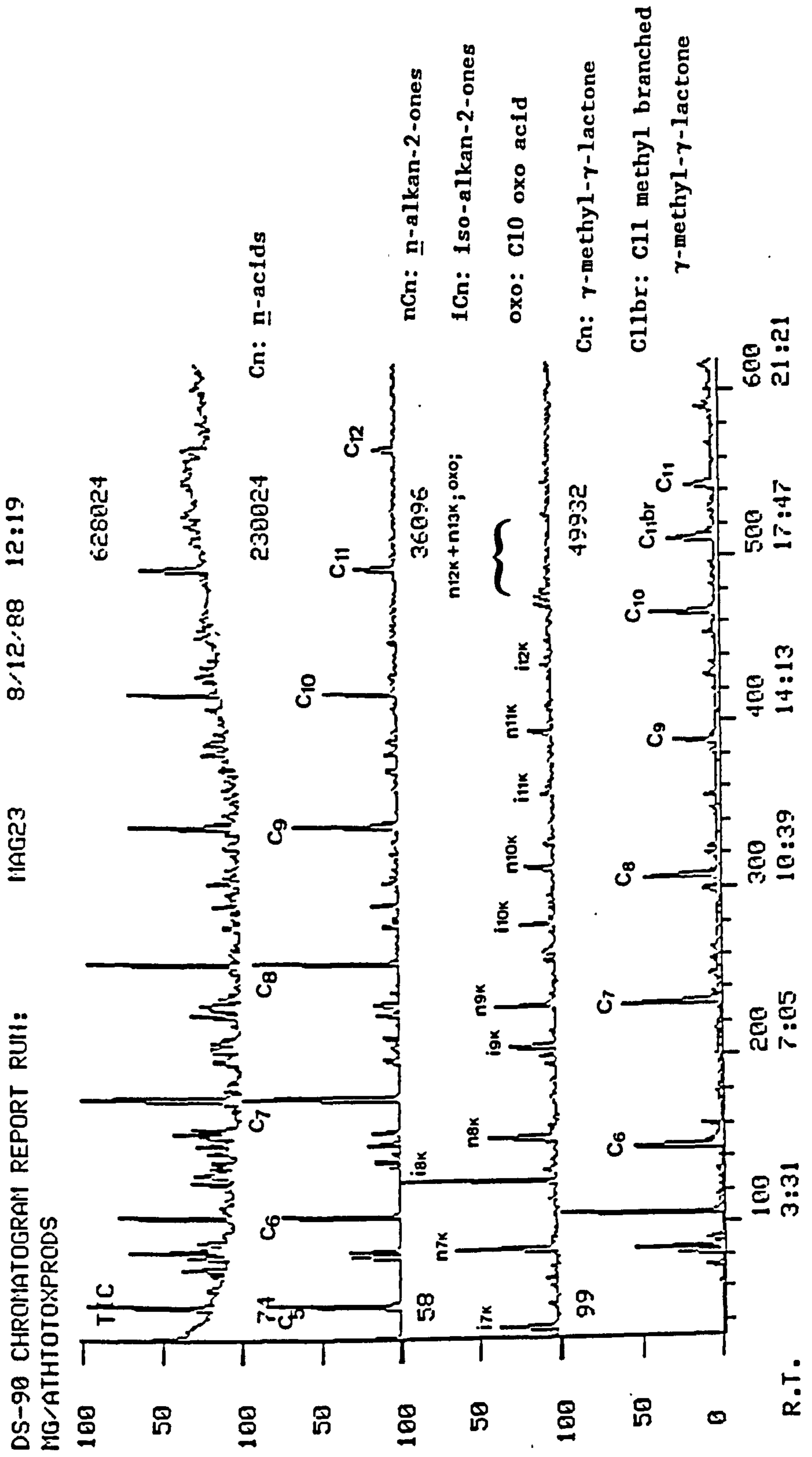


C29 tricyclic terpane (compound G, Fig. 5.24)

Oxidation at the position of alkyl chain substitution (2) with cleavage of the 1-2 bond could conceivably produce 3,7-dimethyl octanoic acid. However, a mechanism whereby C-ring C-C bonds adjacent to position 2 cleave to produce either the C11 acid or the C10 oxo acid would be difficult to invoke. On this basis it appears

FIG. 5.25 GC-MS MASS FRAGMENTOGRAMS OF THE RESOLVED PRODUCTS OF OXIDATION OF THE ATHABASCA OIL SAND BITUMEN ALKANE UCM

(m/z 74: N-ACIDS, m/z 58: ALKYL KETONES, m/z 99: γ -LACTONES)



that isoprenoid-type functionalised compounds can be produced by oxidation of (presumably isoprenoid-type) alkyl linkages within the UCM.

Of all the UCMs oxidised the Athabasca oil sand bitumen produced the lowest carbon number range of resolved functionalised products. The reasons for this are at present unclear though it may reflect the extent of biodegradation of the oil.

5.7 SUMMARY OF UCM OXIDATIONS AND POTENTIAL USE OF THE TECHNIQUE IN OIL - OIL CORRELATIONS

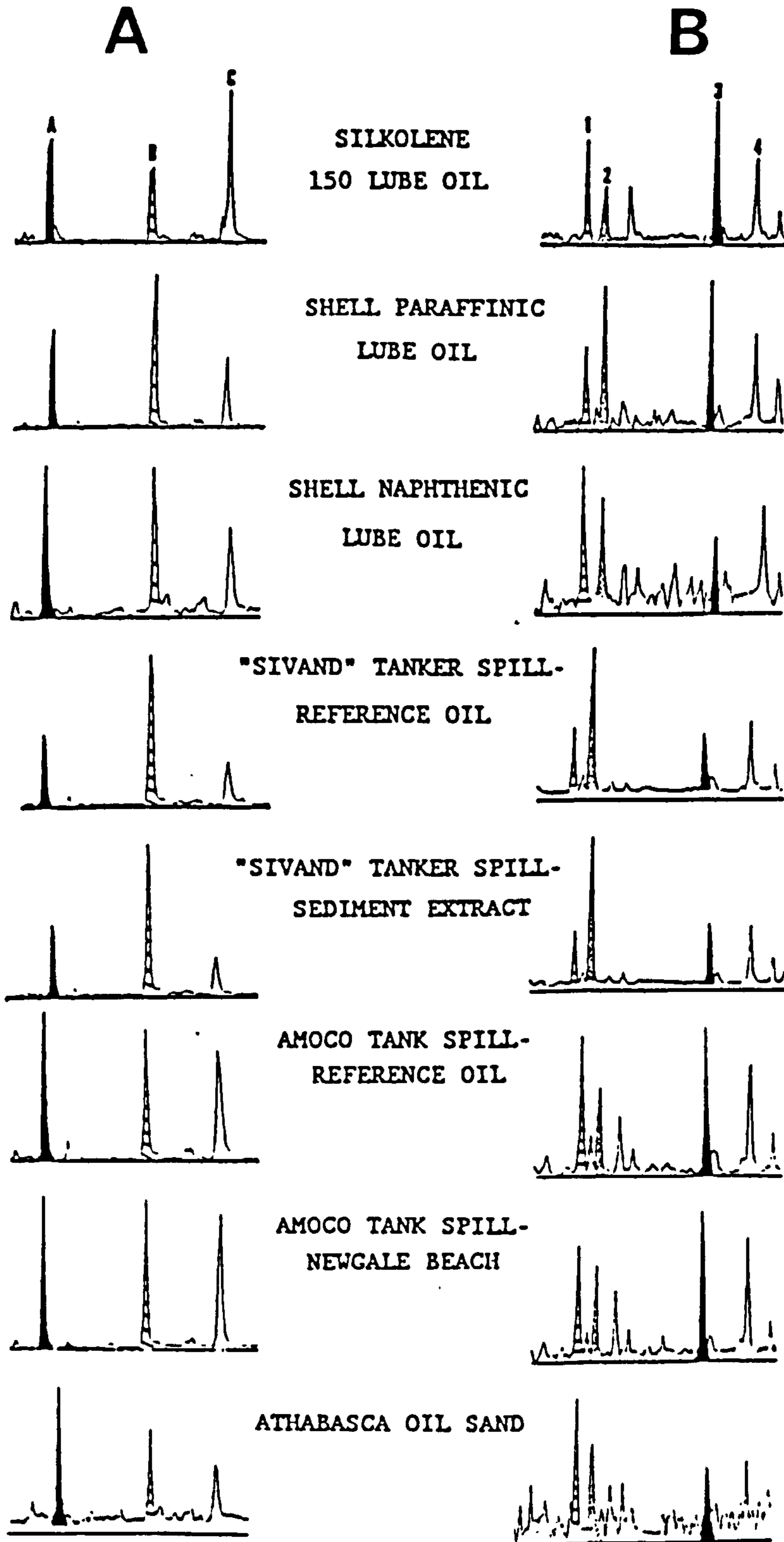
A total of nine aliphatic UCMs were isolated from mineral oil samples of various origin and oxidised using CrO_3 in glacial acetic acid. In each case a series of resolved functionalised products was obtained of which n-monocarboxylic acids were found to predominate. Although many of the products identified from each UCM were in fact similar, their proportions relative both to each other and to residual and/or functionalised UCM varied. In particular an inverse correlation was noted between the extent of biodegradation of an oil and the relative abundance and carbon number range of resolved UCM oxidation products (e.g. Athabasca oil sand and Shell naphthenic lube oil UCMs). Also noted were high relative abundances of resolved isoprenoid-derived acids, ketones and keto acids for those samples which contained a high proportion of acyclic isoprenoids prior to oxidation (i.e. "Sivand" oil spill UCMs). However, these same products were also observed from UCMs in which resolved acyclic isoprenoids were either absent or low in abundance (i.e. Athabasca and Shell naphthenic

oils). This suggests a dual origin for these compounds; they may originate as oxidation products of resolved compounds or as products of isoprenoid-type linkages within the UCM.

The compound type, carbon number range, and relative abundance of resolved oxidation products was reproducible for a given oil UCM (chapter 3); a feature which is important if the technique is to be considered for "fingerprinting" UCMs. In fact an excellent correlation was obtained between the products of oxidation of a partially weathered oil UCM and its identified reference oil UCM ("Sivand" oil spill, section 5.4). On this basis, a common origin was inferred for the sample of stranded oil obtained from Newgale Beach and a candidate (though not proven) reference oil from the Amoco Refinery (section 5.5).

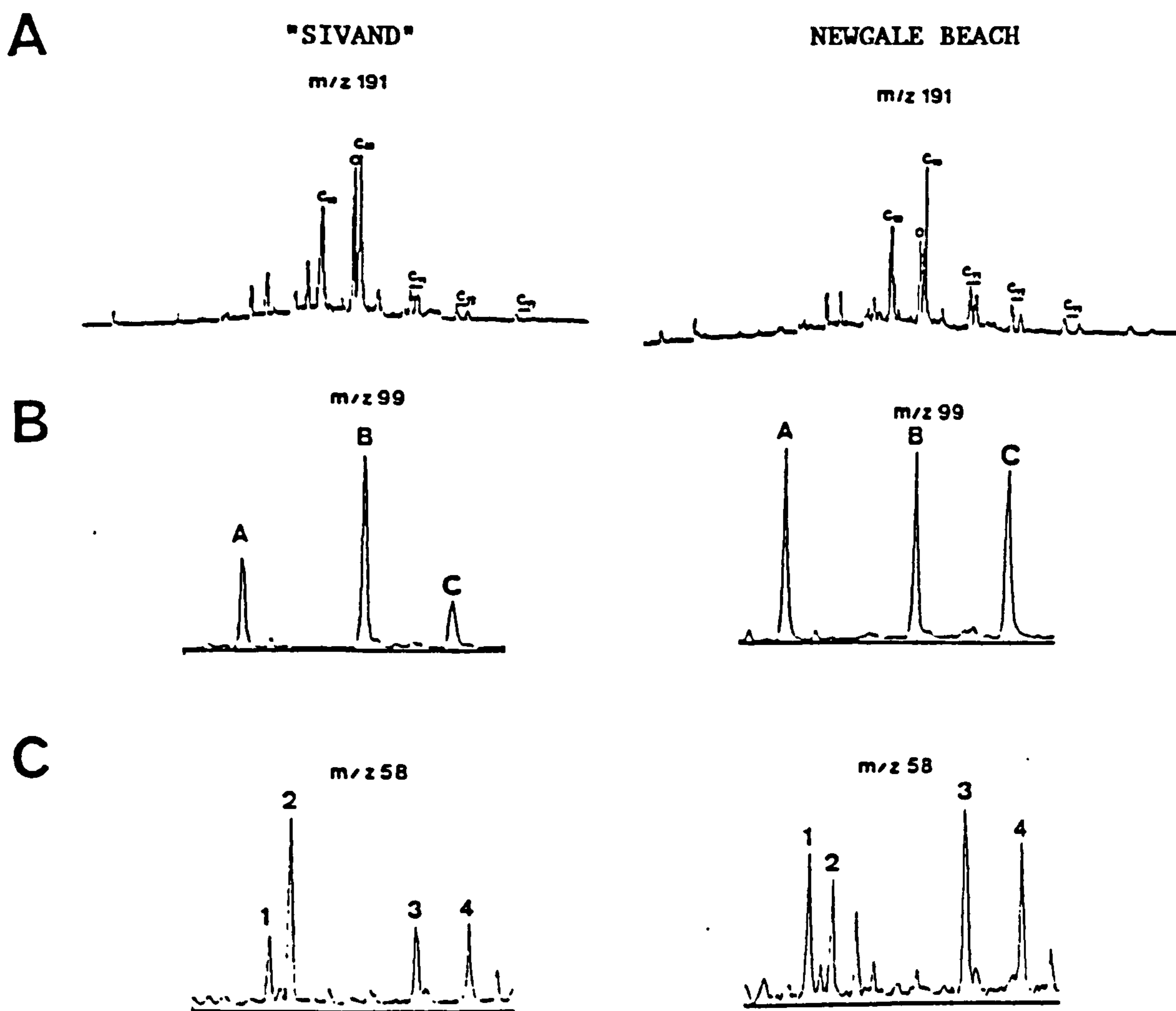
More detailed comparisons of the oxidation product profiles of each UCM were made by examination of the relative proportions of a selected series of γ -methyl- γ -lactones and alkyl ketones as observed by GC-MS mass fragmentography (Fig. 5.27). Compounds A, B and C in the partial GC-MS m/z 99 profile elute in the ECL 10.00 - 12.00 region and were characterised previously as C10-, C11-methylbranched-, and C11- γ -methyl- γ -lactones, respectively. Compounds A and C appear to be derived from simply branched monomethyl linkages within the UCM whereas compound B is most likely of acyclic isoprenoidal origin (resolved and/or unresolved). All of the UCMs oxidised produced these three compounds in variable proportions relative to other resolved oxidation products. Furthermore the abundance of these compounds relative to each other varied depending on the UCM origin, yet were similar for those UCMs derived from a common source.

FIG. 5.26 COMPARATIVE PARTIAL GC-MS MASS FRAGMENTOGRAMS OF
 A) γ -LACTONES (m/z 99) AND B) ALKYL KETONES (m/z 58) OBSERVED
 FOR EACH OF THE PRODUCTS OF OXIDATION OF ALIPHATIC UCMs



(for peak identity see the text)

FIG. 5.27 COMPARATIVE GC-MS PARTIAL MASS FRAGMENTOGRAMS SHOWING DISTRIBUTIONS OF A) PENTACYCLIC TRITERPANES (BEFORE OXIDATION) AND B) γ -LACTONES AND C) ALKYL KETONES (AFTER OXIDATION) FOR THE "SIVAND" SEDIMENTARY UCM ALKANES AND THE NEWGALE BEACH UCM ALKANES



(for peak identity see the text)

In this context therefore they may act as useful "fingerprints" for oil-oil (and possibly oil-source rock) correlations. No quantitative data was obtained for these selected ion profiles.

Also identified in the same elution range as the above described lactones (ECL: 10.00 - 12.00) was a series of compounds which exhibited m/z 58 ions in their mass spectra (compounds 1-4; Fig. 5.27). Compounds 1 (n-dodecan-2-one) and 4 (n-tridecan-2-one) are derived from simply branched (i.e. monomethyl) linkages within the UCM whereas compounds 2 (6,10-dimethylundecan-2-one) and 3 (8-oxo-4-methylnonanoic acid) are derived from acyclic isoprenoid type alkanes (resolved and/or unresolved). Again the distribution of these compounds (as monitored by GC-MS mass fragmentography of their m/z 58 ions) was shown to vary depending on the UCM origin, yet was similar for those oils derived from a common source. These compounds may also therefore act as specific "fingerprints" which have potential for oil-oil and oil-source rock correlations. Of particular interest was the distinction between the "Sivand" oil spill UCMs and the Amoco Tank oil spill UCMs provided by these two profiles. The conventional "biological marker" m/z 191 pentacyclic triterpane profiles for these two oils showed strong similarities, in particular since both showed an unusual occurrence of an isomer of oleanane (Fig. 5.28). On the basis of this conventional "biological marker fingerprint" these two oils would be difficult to differentiate. The UCM oxidation product m/z 99 and m/z 58 GC-MS profiles however allowed a clear distinction to be made.

6.1 INTRODUCTION

The degradation of petroleum by bacteria often results in the progressive depletion of chromatographically resolved hydrocarbons (e.g. *n*-alkanes, acyclic isoprenoid alkanes; alkyl benzenes, naphthalenes and phenanthrenes) relative to unresolved hydrocarbons (i.e. the UCM). This enrichment of the UCM through microbial degradation has been noted in recent sediments affected by oil spills (e.g. Blumer et al., 1973; Atlas et al., 1981; Oudet et al., 1981); in laboratory degraded crude oils (e.g. Bailey et al., 1973; Rubinstein et al., 1977; Jones et al., 1986) and in crude oils biodegraded in the reservoir (e.g. Deroo et al., 1974; Volkman et al., 1983, 1984; Connan, 1984; Brooks et al., 1989^a). Hence, the UCM is thought to comprise compounds which are relatively inert to microbial degradation. The general consensus is that the UCM is a mixture of many structurally complex isomers and homologues of branched and cyclic hydrocarbons (e.g. Farrington et al., 1973; Eglinton et al., 1975; Alexander et al., 1982; Sanders and Tibbetts, 1987). Oxidative studies herein (Chapter 3-5) however, indicated that some UCMs comprise mixtures of structurally simple compounds such as isomeric monoalkyl substituted "T"-branched alkanes [e.g. 7-*n*-hexylnonadecane (9)]. If this supposition is correct, the resistance of the UCM to microbial degradation is perhaps somewhat surprising since

many structurally simple hydrocarbons are relatively easily metabolised by microbes. For example, there are many reports of the preferential microbial oxidation of n-alkanes relative to branched alkanes, both in laboratory studies using pure bacterial strains (e.g. McKenna and Kallio, 1964; Pirnik et al., 1974; Robson and Rowland, 1987) and in environmental oil spills (e.g. Atlas et al., 1981; Jones et al., 1986). Simply branched (e.g. monomethyl) alkanes are also susceptible to attack by microbes, as observed with individual hydrocarbons (e.g. Thijsse and Van der Linden, 1961; McKenna and Kallio, 1964; Pirnik et al., 1974) and in crude oil biodegradation studies (Connan et al., 1980; Connan, 1984). It was therefore considered necessary to assess the susceptibility of the proposed UCM components (i.e. 7-n-hexylnonadecane, 9-(2-cyclohexylethyl)-heptadecane and 9-(2-phenylethyl)-heptadecane) to microbial degradation.

An experiment was devised to measure the biodegradation rates of these hydrocarbons. Included in the test mixture (see table 6.1 for compositional details) were compounds not thought to be representative of the aliphatic UCM, but which were used for comparisons (n-pentacosane, 2-methyltetracosane, 9-methyltetracosane, 2,6,10,14,18-pentamethyleicosane, and 2,6,10,14-tetramethyl-7-(3-methylpentyl)-pentadecane). The organism chosen for the study was a pure strain of *Pseudomonas fluorescens*, obtained by enrichment from a used metal working fluid (Dr. D. Gaylarde, City of London Polytechnic; personal communication). *Pseudomonas* species are well known

hydrocarbon degraders, and are wide spread and often the most dominant of the hydrocarbon utilising microbes in the marine environment (Karrick, 1977). A pure strain was used so that microbial degradation rates could be measured, and all hydrocarbons were C25 so that any variations could be ascribed solely to molecular structure.

The procedures used were based on those reported by Robson and Rowland, (1989), and involved addition of a known amount of hydrocarbon mixture to flasks containing sterilised minimal salts medium and bacterial broth. The aliphatic UCM was treated in an identical manner in separate flasks at the same sample loading (i.e. ca. 80 μgcm^{-3}). The flasks and hydrocarbon contents were incubated for varying periods of time in the dark on a shaking water bath at 20°C. Residual hydrocarbons were recovered by extraction with DCM, the extracts were dried (anh. Na_2SO_4), the solvent removed, and made up to volume with DCM prior to analysis by GC.

The synthetic hydrocarbon mixture was quantified by reference to an external standard composed of all eight hydrocarbons at a known concentration. Replicate analysis (x10) indicated a GC reproducibility of ca. 6% per compound. Variation in detector response was assessed by monitoring integrated peak areas of each component daily. Throughout the course of the experiment these did not fall outside the limits imposed by experimental error.

The aliphatic UCM was quantified by reference to a calibration curve constructed from the FID responses of known concentrations of UCM. For this an automated time slice area integral measurement was used which at the same time provided a measure of the percentage resolved alkanes vs. percentage unresolved alkanes (Appendix I). Replicate analyses (x10) indicated a GC precision of 5.4%. Daily monitoring of detector response variations again showed that during the experiment integrated standard UCM areas did not fall outside the experimentally determined error limits.

6.2 RESULTS AND DISCUSSION

The daily concentration of individual synthetic hydrocarbons are shown in Table 6.2, and expressed as a percentage of the starting concentration in Table 6.4. Changes in concentration of the aliphatic UCM monitored over the same period are shown in Table 6.3. Also presented are daily variations in the percentage resolved alkanes vs. percentage unresolved alkanes obtained by the time slice area measurement (Table 6.3).

Fig. 6.2 depicts the changes observed in the concentration of n- and monomethyl branched C25 alkanes during the course of the study. Fig. 6.1 shows the gas chromatograms of the hydrocarbon mixture after 5, 14 and 25 days and the day 25 sterilised control. It is evident that under the conditions used, the normal and monomethyl substituted alkanes were

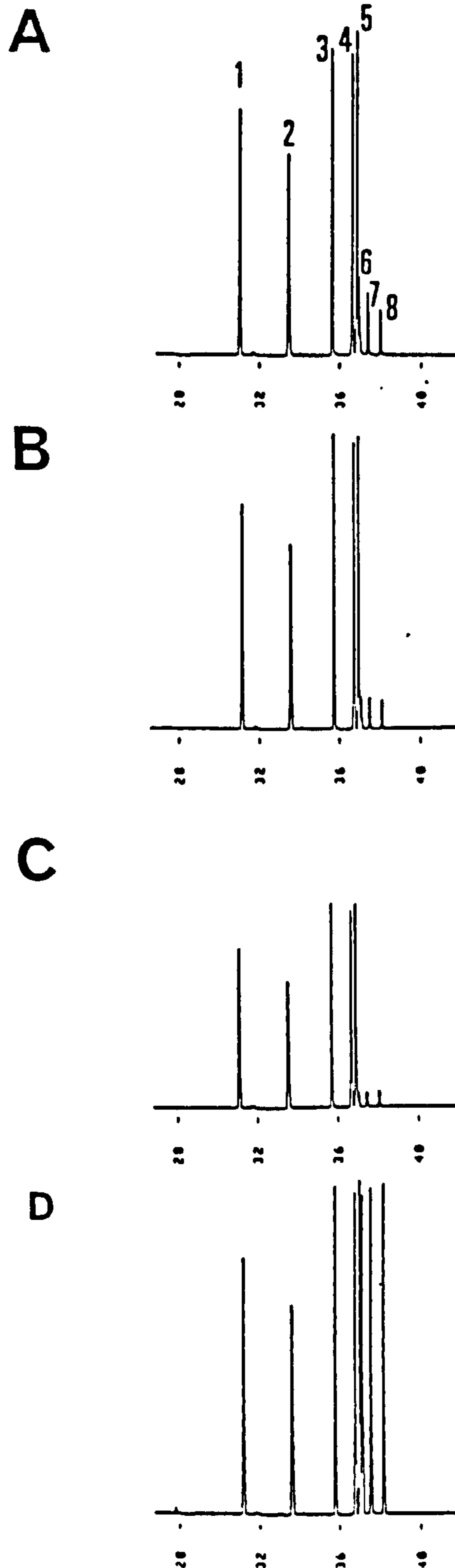
TABLE 6.1

COMPOSITION AND CONCENTRATION AT DAY 0 OF THE SYNTHETIC
HYDROCARBON BIODEGRADATION MIXTURE.

COMPOUND NO.	COMPOUND	CONCENTRATION (μgcm^{-3})	AT DAY 0
1	2,6,10,14-tetramethyl-7-(3-methylpentyl)-pentadecane	10.4	
2	2,6,10,14,18-pentamethyleicosane	10.6	
3	7- <u>n</u> -hexylnonadecane	10.8	
4	9-(2-cyclohexylethyl)-heptadecane	10.0	
5	9-(2-phenylethyl)-heptadecane	10.4	
6	9-methyltetracosane	10.0	
7	2-methyltetracosane	10.8	
8	<u>n</u> -pentacosane	9.8	

FIG. 6.1 PARTIAL GAS CHROMATOGRAMS OF THE SYNTHETIC BIODEGRADATION MIXTURE AT A) DAY 5, B) DAY 14, C) DAY 25 AND D) DAY 25 STERILISED CONTROL

(for peak identity see Table 6.1)



rapidly degraded within the first ten days in the approximate order n -C25 > 2-methyltetracosane > 9-methyltetracosane. These changes could be attributed almost solely to microbial alteration as the sterilised control (Fig. 6.1) revealed little reduction in the concentrations of alkanes caused by abiotic factors (e.g. evaporation). After approximately 14 days the rates of degradation of these compounds began to level off, though they were observed to degrade further (Fig. 6.2).

In contrast to the behaviour observed for n - and monomethyl C25 alkanes, the remaining hydrocarbons in the mixture were observed to degrade at a relatively slow rate up to day 14. Thus Fig. 6.3 depicts the observed changes in daily concentrations for the proposed UCM hydrocarbons (7- n -hexylnonadecane, 9-(2-cyclohexylethyl)-heptadecane, and 9-(2-phenylethyl)-heptadecane) compared to the changes observed for the aliphatic UCM. The behaviour of the three candidate hydrocarbons was similar throughout the 25 day period. This was exemplified by coplotting the day 0 to day 25 data sets for 7- n -hexylnonadecane and 9-(2-cyclohexylethyl)-heptadecane (Fig. 6.7a). This plot was near linear with a correlation coefficient $r = 0.997$. Up to day 14 a relatively slow rate of decrease was observed, this was approximated as linear and regression of the 7- n -hexylnonadecane data points (day 0 to day 14) provided a measure of the rate of decrease as -0.74% day^{-1} ($r = 0.619$). After 14 days the rate of decrease of the candidate UCM hydrocarbons was more rapid, and linear

FIG. 6.2 GRAPH ILLUSTRATING THE RELATIVE BIODEGRADATION RATES OF ○: N-PENTACOSANE, □: 2-METHYLTETRACOSANE, △: 9-METHYLTETRACOSANE.

(SC: sterilised control)

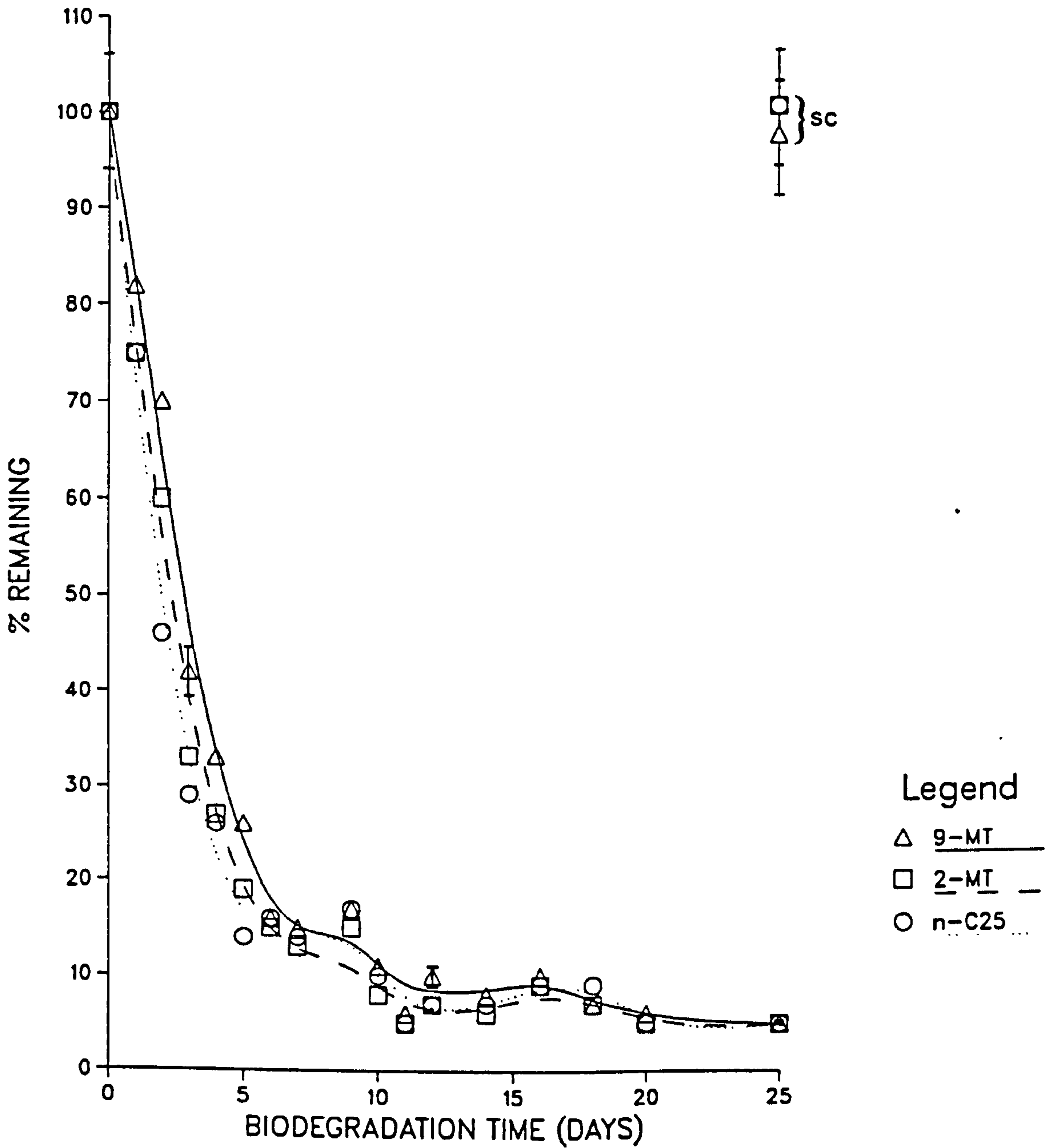


TABLE 6.2

DAILY CONCENTRATION (μgcm^{-3}) OF INDIVIDUAL COMPOUNDS IN THE HYDROCARBON MIXTURE BIODEGRADATION STUDY

DAY	COMPOUND NO. ^a							
	1	2	3	4	5	6	7	8
0	10.4	10.6	10.8	10.0	10.0	10.4	10.8	9.8
1	10.3	10.6	10.8	10.1	10.0	8.5	8.1	7.3
2	9.7	10.0	10.1	9.6	9.6	7.3	6.5	4.5
3	10.0	10.2	10.5	9.8	9.9	4.4	3.6	2.8
4	10.4	10.7	10.9	10.1	10.1	3.4	2.9	2.5
5	9.9	10.2	10.5	9.7	9.7	2.7	2.1	1.4
6	9.2	9.4	9.6	9.0	9.0	1.7	1.6	1.6
7	9.2	9.4	9.5	8.8	8.8	1.6	1.4	1.4
8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
9	9.7	9.8	10.1	9.3	9.3	1.8	1.6	1.7
10	9.4	9.5	9.7	9.1	9.0	1.1	0.9	1.0
11	8.6	8.7	9.0	8.3	8.3	0.6	0.5	0.5
12	9.7	9.8	10.2	9.5	9.5	1.0	0.8	0.7
14	9.4	9.6	10.0	9.3	9.2	0.8	0.6	0.6
16	8.8	9.0	9.4	8.9	8.6	1.0	1.0	0.9
18	7.8	7.8	8.3	7.8	7.7	0.7	0.7	0.9
20	7.2	7.3	7.8	7.3	7.2	0.6	0.5	0.5
25	6.2	6.3	6.7	6.2	6.1	0.5	0.5	0.6
25SC ^b	10.2	10.5	10.8	10.2	10.1	10.2	10.8	9.9

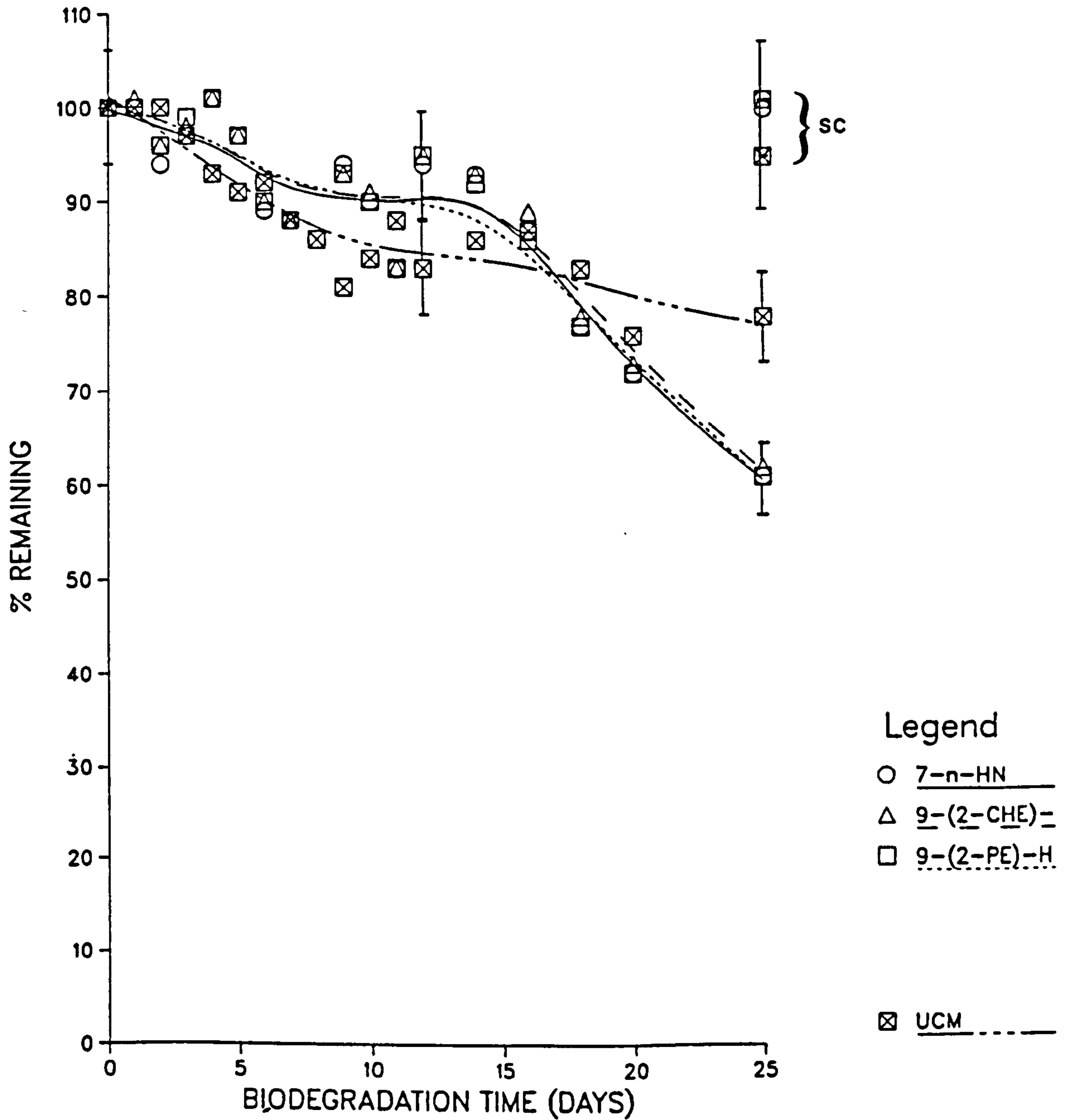
a: for compound identity refer to Table 6.1

b: SC corresponds to sterile control 1

n.d.: not determined

FIG. 6.3 GRAPH ILLUSTRATING THE RELATIVE BIODEGRADATION RATES OF CANDIDATE UCM HYDROCARBONS (\circ :7-N-HEXYLNONADECANE, Δ :9-(2-CYCLOHEXYLETHYL)-HEPTADECANE, \square :9-(2-PHENYLETHYL)-HEPTADECANE) COMPARED TO THE ALIPHATIC UCM (\boxtimes)

(SC: sterilised control)



regression of the 7-n- hexylnonadecane data points (days 14, 16, 18, 20 and 25) provided a daily rate decrease of -2.94% day^{-1} ($r= 0.985$). It is interesting to note that after day 14 the rate of decrease of the n- and monomethyl alkanes slowed and became near linear ($r= 0.652$) with a rate decrease of -0.3% day^{-1} . It appears that at this stage in the biodegradation sequence the bacteria began to utilise the UCM candidate hydrocarbons in preference to the small remaining amounts of the initially more labile n- and monomethyl C25 alkanes.

Over the first 14 days the aliphatic UCM was also observed to decrease in concentration (Fig. 6.3, Fig. 6.4), and linear regression provided a rate decrease of -1.36% day^{-1} ($r= 0.890$), i.e. slightly greater than that observed for the candidate UCM hydrocarbons. Thereafter however the rate of decrease did not parallel that observed for the model UCM hydrocarbons, and remained fairly constant (-0.93% day^{-1} , $r= 0.807$). An examination of the values of percentage resolved alkanes vs. percentage unresolved alkanes provided by the time slice area measurement (Table 6.3, Fig. 6.5) did not show any increase in the proportion of unresolved alkanes within the limits of experimental error ($\pm 8\%$). It appears therefore that the UCM was degraded as a whole and that individual resolved alkane components were degraded at the same rate as unresolved components.

Of particular interest to this study was the behaviour of the two candidate aliphatic UCM alkanes relative to that observed

FIG. 6.4 GAS CHROMATOGRAMS OF BIODEGRADED ALIPHATIC UCM RESIDUES AT
A) DAY 5, B) DAY 14, C) DAY 25 AND D) DAY 25 STERILE CONTROL.

[GC: DB-5(J+W), 30m, 50-300°C @ 5°Cmin-1]

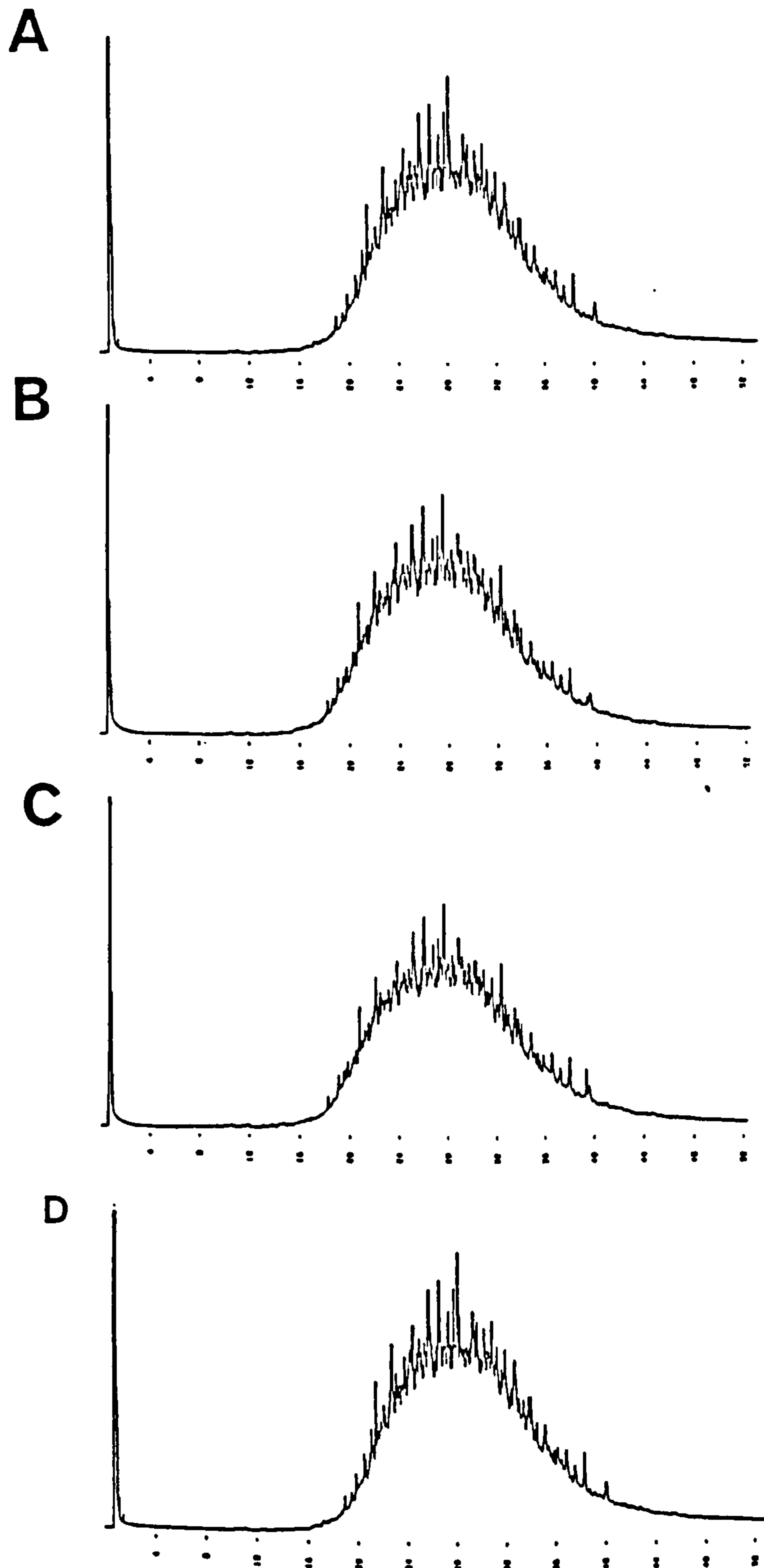
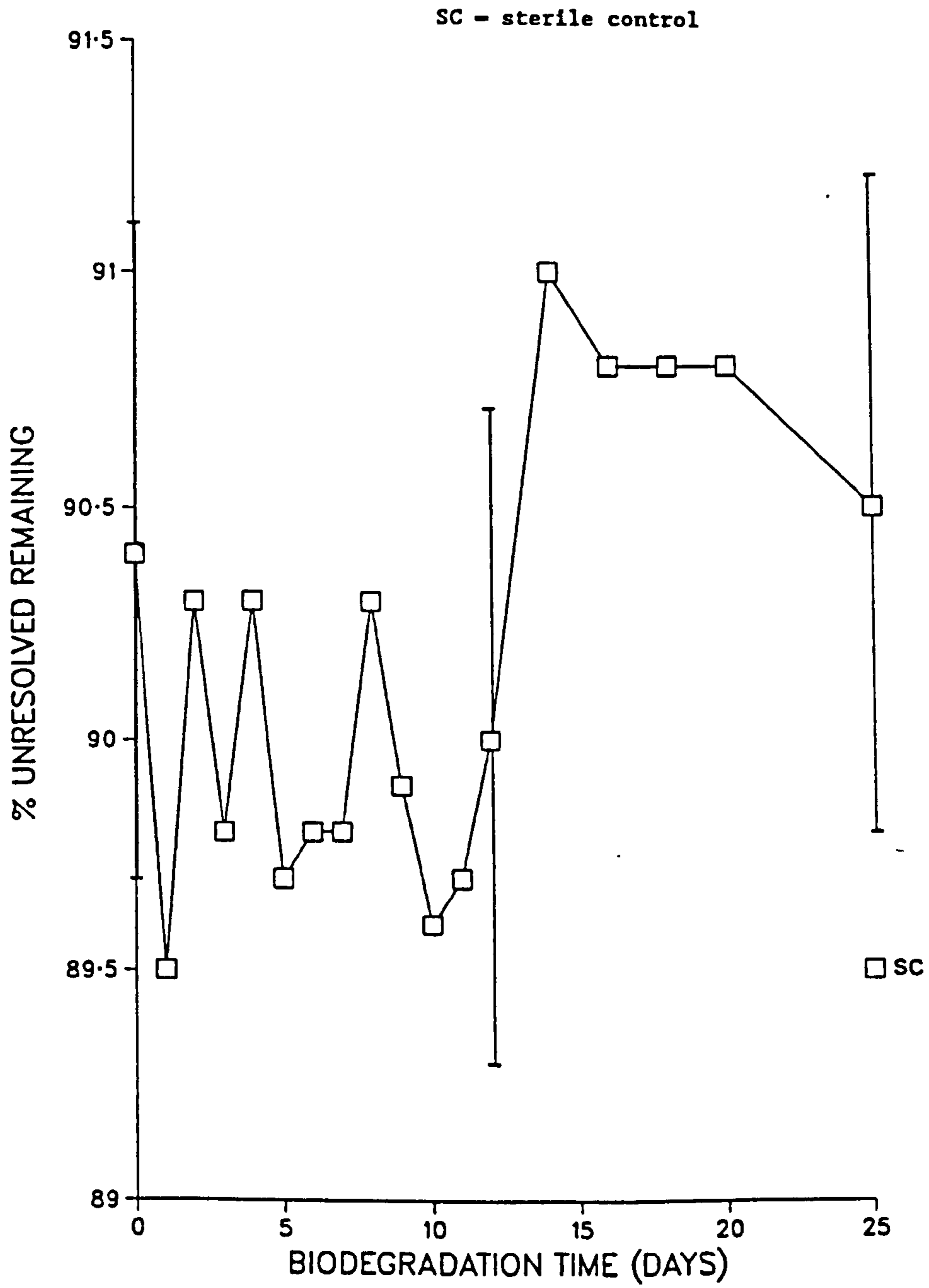


FIG. 6.5 GRAPH ILLUSTRATING VARIATIONS OBSERVED IN THE PERCENTAGE UNRESOLVED ALKANES OF THE ALIPHATIC UCM IN THE BIODEGRADATION STUDY



for the acyclic isoprenoid alkanes included in the hydrocarbon mixture. Though it is generally recognised that acyclic isoprenoid alkanes are relatively resistant to microbial degradation compared to n- and monomethyl alkanes, certain isoprenoids have been shown to be degraded by both pure and mixed cultures (e.g. McKenna, 1971; Cox et al., 1974; Pirnik et al., 1974; Rontani et al., 1986). In particular the regular acyclic isoprenoids pristane (C19) and phytane (C20) have been observed to degrade before any observable change in the aliphatic UCM profile (Deroo et al., 1974, 1977; Brooks et al., 1989^a). If the monoalkyl substituted acyclic and monocyclic alkanes are good models for the alkane components of the UCM, then these should be at least, possibly more; resistant to microbial degradation than the regular acyclic isoprenoid alkane.

Fig. 6.6 depicts the changes observed for the monoalkyl substituted acyclic alkane 7-n-hexylnonadecane vs. the acyclic isoprenoid alkanes 2,6,10,14,18-pentamethyleicosane and 2,6,10,14-tetramethyl-7-(3-methylpentyl)-pentadecane during the biodegradation study. Also plotted for comparison is the data for n-pentacosane. All three branched alkanes were observed to degrade at a relatively slow rate up to day 14 followed by a more rapid decrease up to day 25. Though a slightly greater resistance was noted for the monoalkyl 'T'-branched alkane, the difference did not exceed the limits imposed by experimental error. In fact, the data sets for the regular acyclic isoprenoid alkane and the "T"- branched

TABLE 6.3

DAILY CONCENTRATIONS (μgcm^{-3}) OF ALIPHATIC UCM AND PERCENTAGE RESOLVED VS. PERCENTAGE UNRESOLVED ALKANES IN THE ALIPHATIC UCM BIODEGRADATION STUDY.

DAY	CONCENTRATION (μgcm^{-3})	RESOLVED (%)	UNRESOLVED
0	82.5	9.6	90.4
1	82.5	10.5	89.5
2	82.7	9.7	90.3
3	80.3	10.2	89.8
4	76.9	9.7	90.3
5	75.2	10.3	89.7
6	75.5	10.2	89.8
7	72.5	10.2	89.8
8	71.2	9.7	90.3
9	67.2	10.1	89.9
10	69.2	10.4	89.6
11	72.9	10.3	89.7
12	68.8	10.0	90.0
14	71.0	9.0	91.0
16	71.7	9.2	90.8
18	68.2	9.2	90.8
20	63.0	9.2	90.8
25	64.0	9.5	90.5
25SC	78.0	10.5	89.5

TABLE 6.4

DAILY AMOUNTS OF INDIVIDUAL HYDROCARBONS AND ALIPHATIC UCM EXPRESSED AS A PERCENTAGE OF THEIR CONCENTRATIONS AT DAY 0

DAY	COMPOUND NO ^a								UCM
	1	2	3	4	5	6	7	8	
0	100	100	100	100	100	100	100	100	100
1	99	100	100	101	100	82	75	75	100
2	93	94	94	96	96	70	60	46	100
3	96	96	97	98	99	42	33	29	97
4	100	101	101	101	101	33	27	26	93
5	95	96	97	97	97	26	19	14	91
6	89	89	89	90	90	16	15	16	92
7	89	89	88	88	88	15	13	14	88
8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	86
9	93	93	94	93	93	17	15	17	81
10	90	90	90	91	90	11	8	10	84
11	83	82	83	83	83	6	5	5	88
12	93	92	94	95	95	10	7	7	83
14	90	91	93	93	92	8	6	7	86
16	85	85	87	89	86	10	9	9	87
18	75	74	77	78	77	7	7	9	83
20	69	69	72	73	72	6	5	5	76
25	60	59	61	62	61	5	5	5	78
25sc ^b	98	99	100	101	101	98	100	101	95

a: for peak identity refer to Table 6.1

b: SC - sterile control

n.d.: not determined

FIG. 6.6 GRAPH ILLUSTRATING THE RELATIVE BIODEGRADATION RATES OBSERVED FOR THE ACYCLIC ISOPRENOID ALKANES [Δ : 2,6,10,14-TETRAMETHYL-7-(3-METHYLPENTYL)-PENTADECANE, \square : 2,6,10,14,18-PENTAMETHYLEICOSANE], THE "T"-BRANCHED MONOALKYL SUBSTITUTED ACYCLIC ALKANE (\boxtimes : 7-N-HEXYLNONADECANE), AND N-PENTACOSANE (\circ).

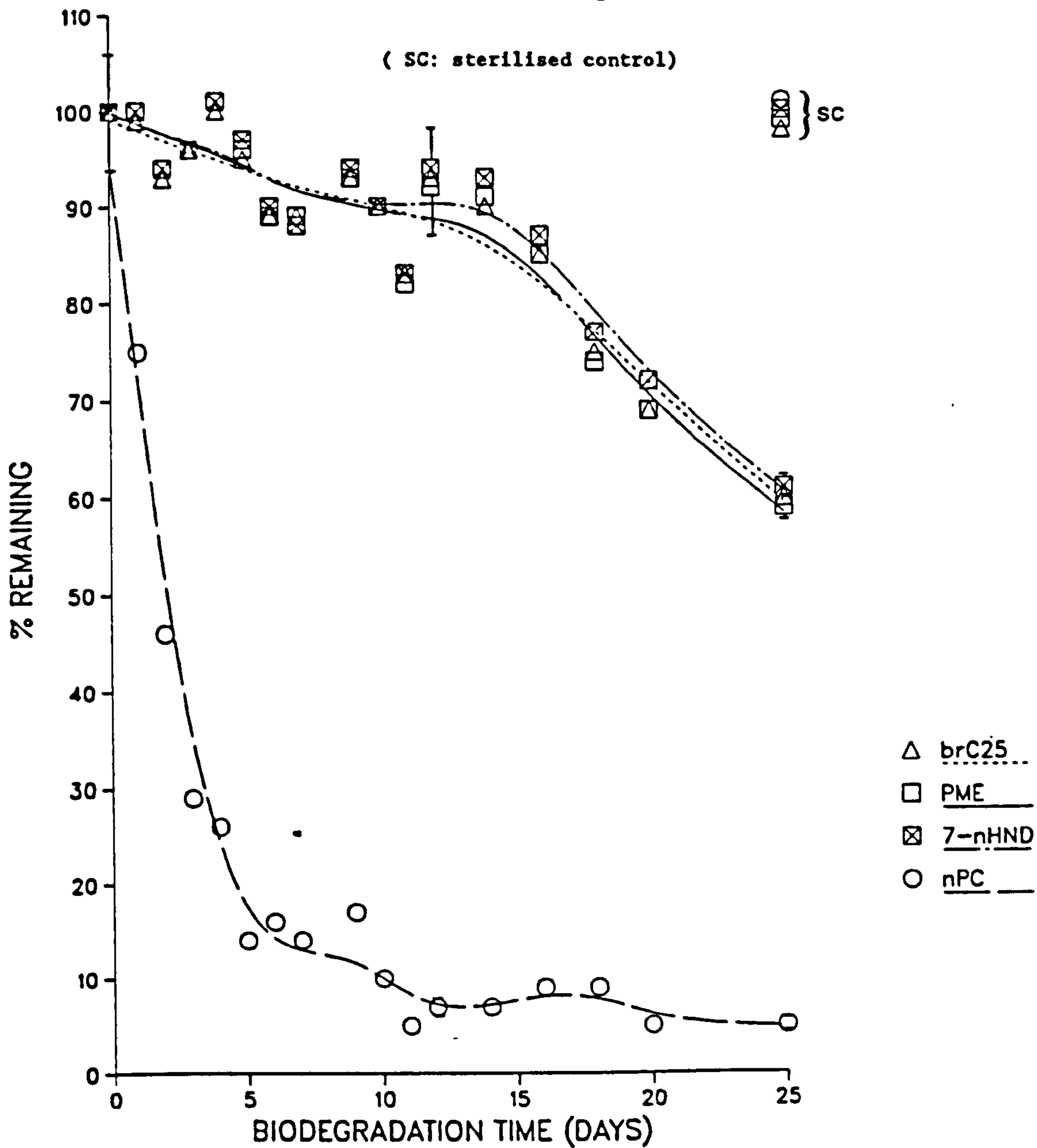
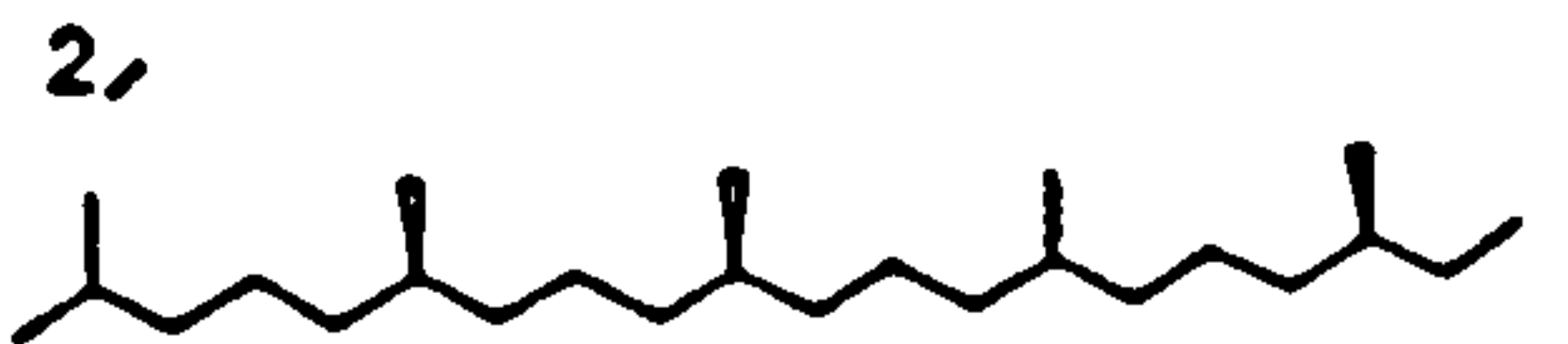
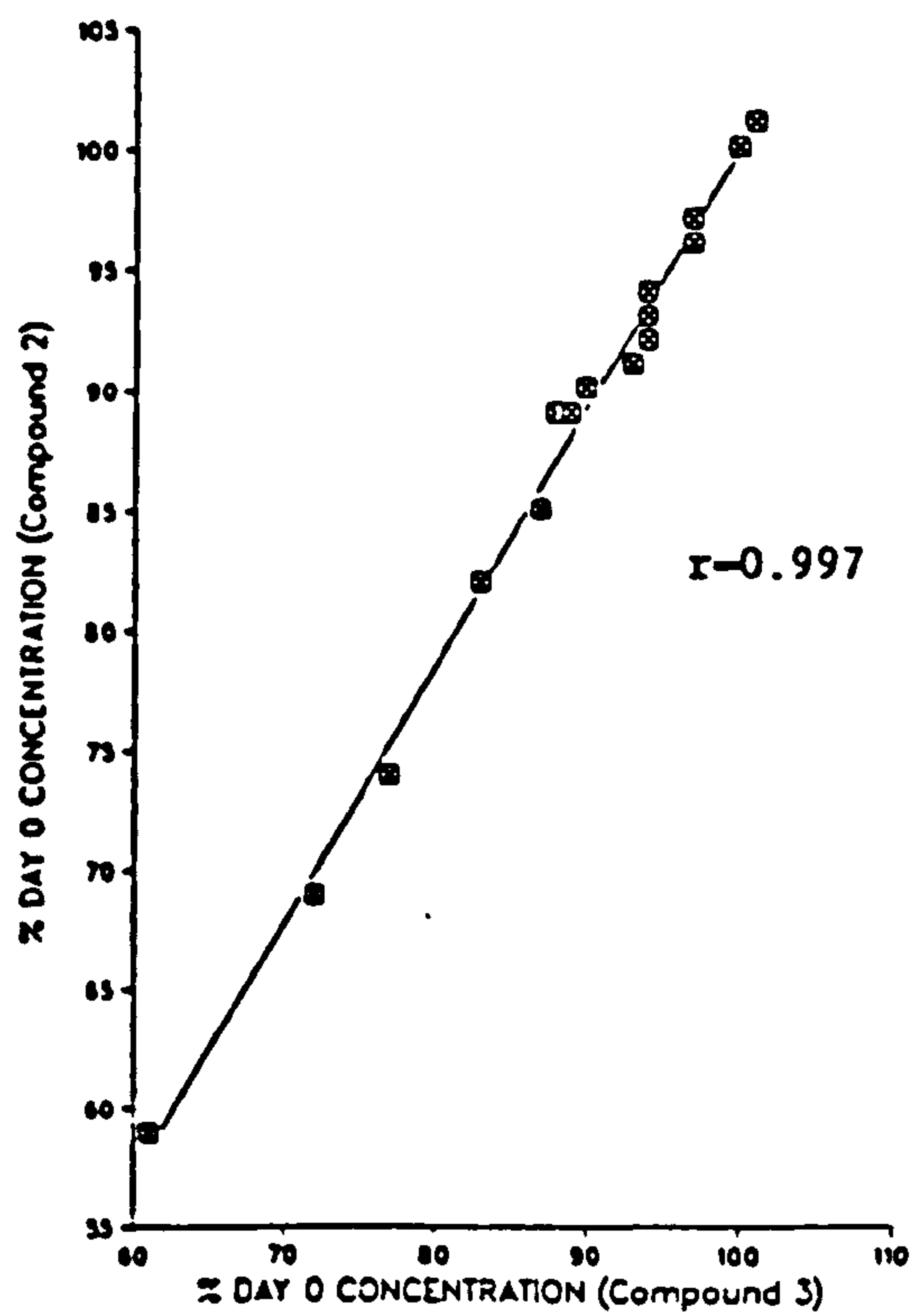
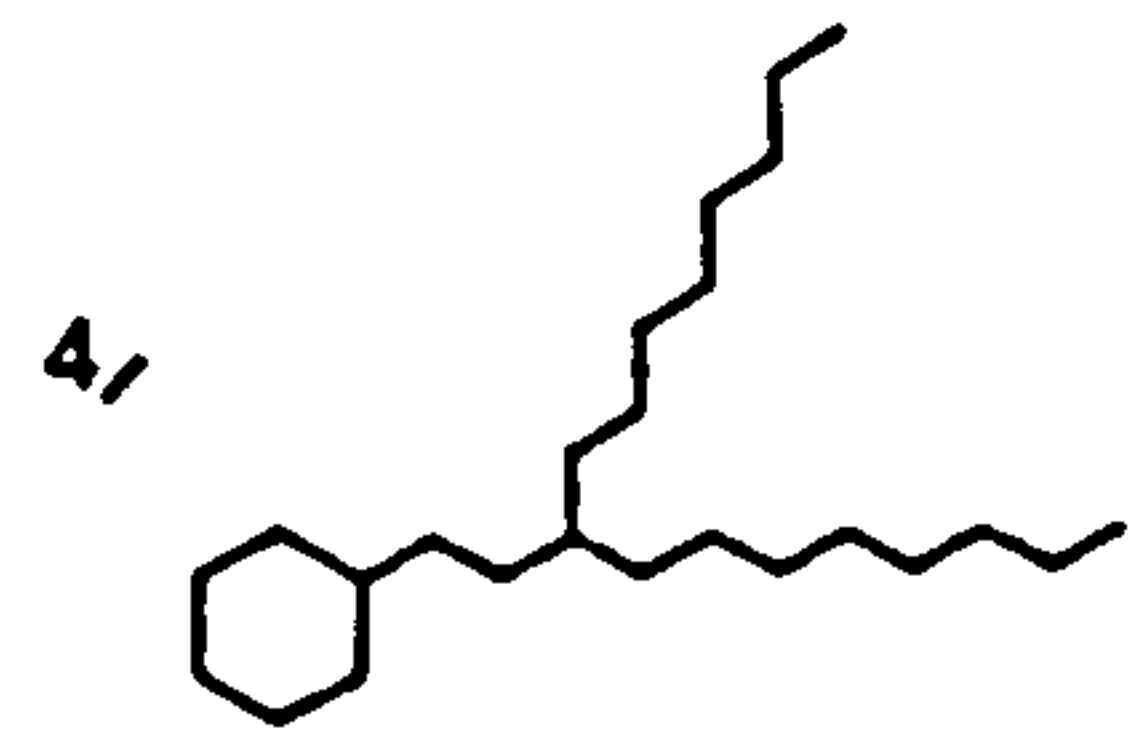
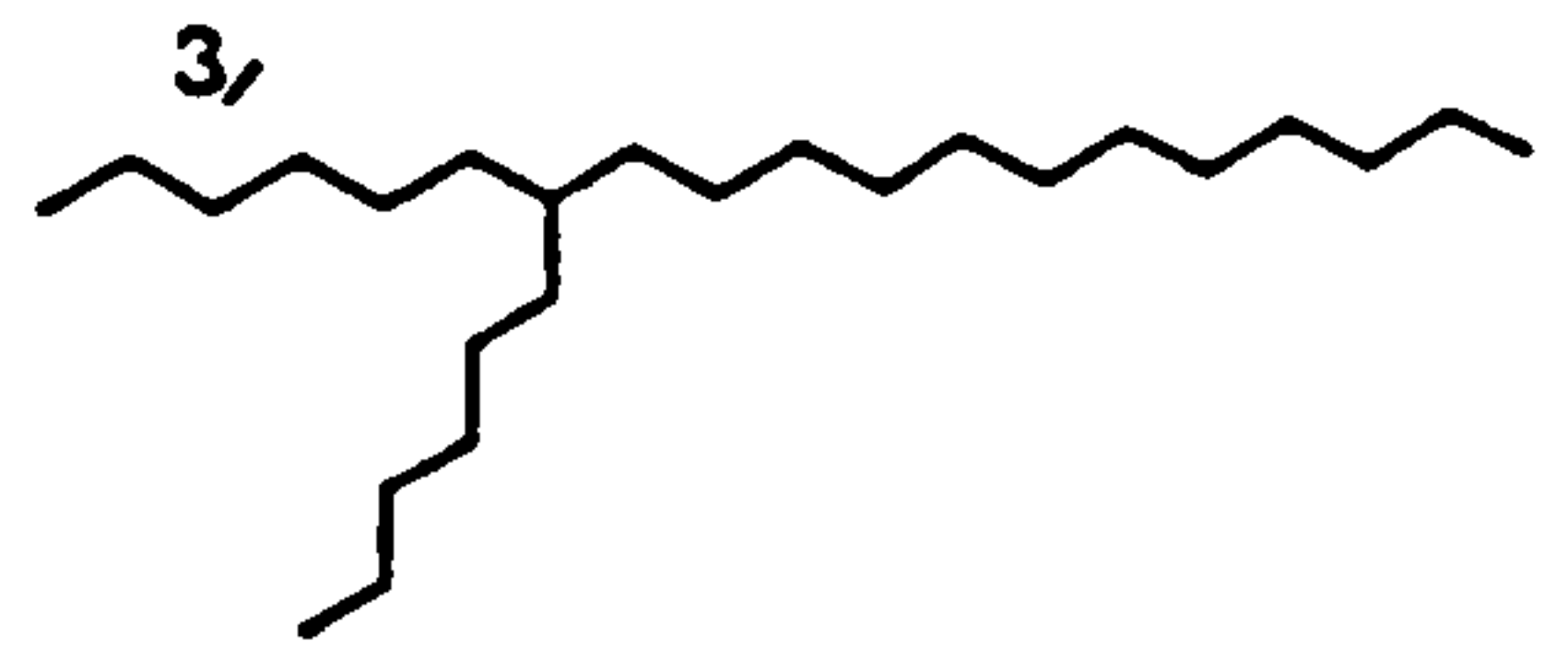
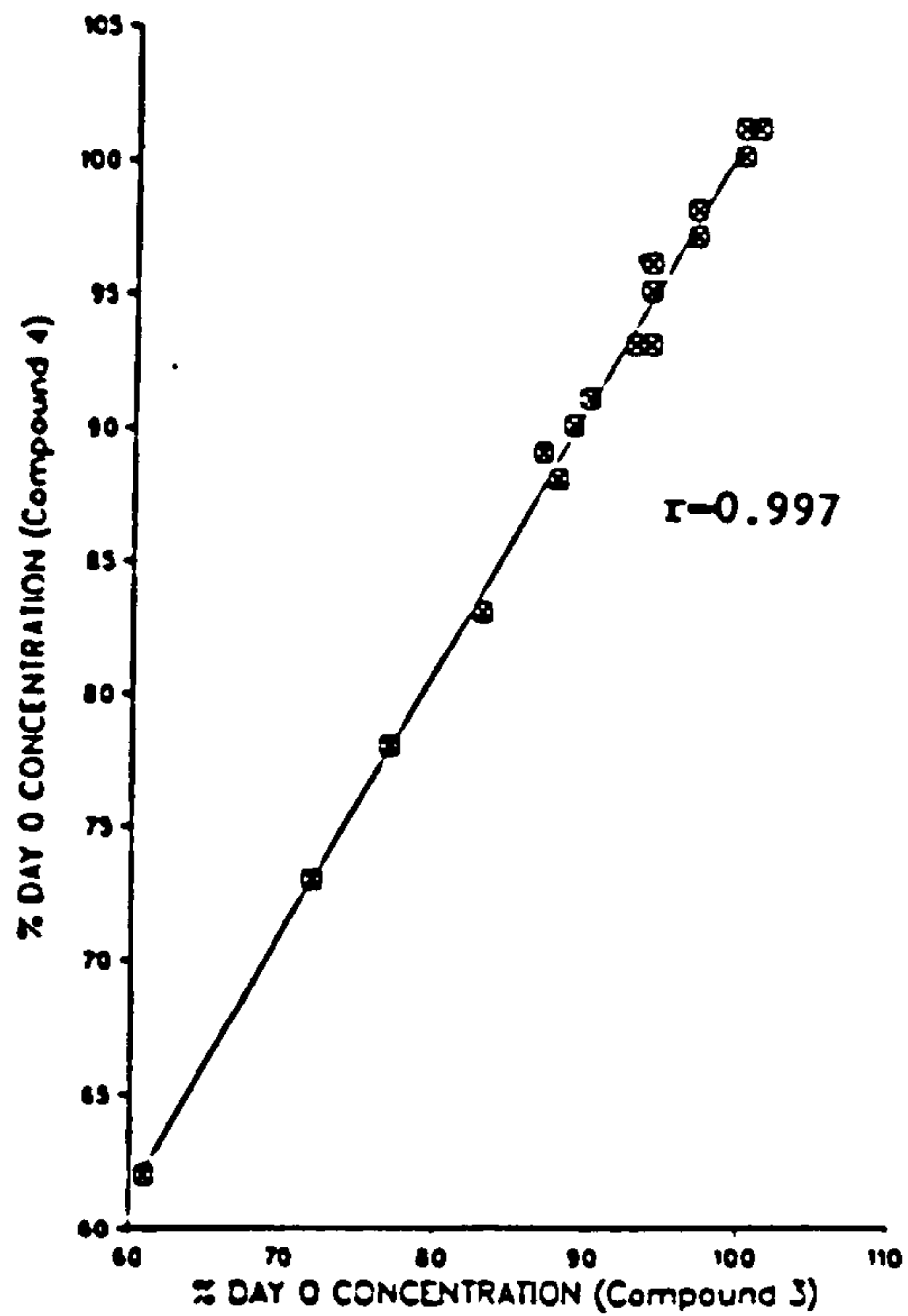


FIG 6.7 PLOTS OF PERCENT REMAINING FOR A) 9-(2-CYCLOHEXYLETHYL)-HEPTADECANE vs 7-N-HEXYLNONADECANE, AND B) 2,6,10,14,18-PENTAMETHYLEICOSANE vs 7-N-HEXYLNONADECANE OBSERVED IN THE MODEL COMPOUND BIODEGRADATION STUDY



acyclic alkane covaried with a regression coefficient of $r=0.997$ (Fig. 6.7b). No appreciable difference in resistance was therefore noted for these two alkane types.

6.3 SUMMARY AND CONCLUSIONS

Laboratory biodegradation of a mixture of eight isomeric C₂₅ hydrocarbons with the aerobe *Pseudomonas fluorescens* showed that the rate and extent of degradation was influenced by molecular structure. Thus the normal and monomethylalkanes were rapidly degraded within the first 10 days of the experiment in the approximate order n-pentacosane > 2-methyltetracosane > 9-methyltetracosane. This is in accordance with previous studies of alkane biodegradation, both in the laboratory and from field observations (e.g. Thijsse and Van der Linden, 1961; McKenna and Kallio, 1964; Pirnik et al., 1974; Connan et al., 1980).

The remaining synthetic hydrocarbons were also observed to degrade though to a limited extent in comparison to normal and monomethyl alkanes. A relatively slow decrease was noted up to day 14 (ca. -0.7% day⁻¹), followed by a more rapid decrease in concentration (ca. -3% day⁻¹) to a value of ca. 60% of the starting material. Of particular interest was the observation that the monoalkyl "T" branched alkane 7-n-hexylnonadecane was at least as resistant to microbial alteration as the isomeric acyclic isoprenoid alkane 2,6,10,14,18-pentamethyleicosane. Similar results were obtained by McKenna and Kallio (1964) when screening 14

isomers of hexadecane for growth with *Micrococcus* and *Nocardia* species. Both microbes were able to utilise n-hexadecane, methylpentadecanes (C-2 to C-8), and 3-ethyltetradecane, though only the *Nocardia* species was able to utilise longer alkyl branches (4-, 6-, and 7-propyltridecane, 5-butyl-dodecane, and 6-pentylundecane). A strain of *Micrococcus* *cerificans* was however observed to grow on pristane (2,6,10,14-tetramethylpentadecane). A comparable selectivity was observed for *Pseudomonas* species. Again these were observed to grow on monomethyl substituted pentadecanes (C-4, C-5 and C-6) though not on the isomeric "T"-branched alkane 6-pentylundecane. The reason for the partial recalcitrance of these alkanes is not known, though as microbial utilisation of alkanes is mediated by bacterial enzymes, then the geometry of the compound in three dimensions may be of importance.

The aliphatic UCM was also observed to partially degrade in the biodegradation experiment, initially at a comparable rate to that observed for the candidate UCM alkanes (ca. 1% day⁻¹, up to day 14). Thereafter however the rate of decrease remained essentially constant, whereas the candidate UCM alkanes were observed to degrade more rapidly (ca. 3% day⁻¹). Of particular interest was the observation that the UCM appeared degraded "as a whole", i.e. no significant reduction in the proportion of resolved alkanes vs. unresolved alkanes was noted. A significant proportion of these resolved alkanes were identified previously (Chapter 3) as regular

CHAPTER SEVEN

FINAL CONCLUSIONS AND FUTURE RESEARCH

FINAL CONCLUSIONS AND FUTURE RESEARCH

7.1 FINAL CONCLUSIONS

Since the application of gas chromatography (GC) to petroleum and environmental organic geochemistry, unresolved complex mixtures (UCMs) or "humps" of hydrocarbons have been observed in the solvent extracts of oil polluted sediments, laboratory and in-reservoir biodegraded crude oils, and in certain petroleum products (e.g. lubricating oils). In polluted Recent sediments, the UCM has been adopted as a quantifiable measure of chronic oil pollution, and measurements have shown that the UCM often accounts for the greatest proportion of the pollutant hydrocarbon burden.

Despite the abundance and ubiquity of the UCM little is known of the detailed composition. Literature consensus is of a mixture of many structurally complex isomers and homologues of branched and cyclic alkanes. The molecular type, degree of complexity and number of isomers present is however not a present known.

The main aim of this study was to isolate hydrocarbon UCMs from a variety of sources, and to undertake structural analyses using both conventional and non-conventional methods. For this purpose mineral lubricating base oils were chosen as a source of hydrocarbon UCMs. These serve as ideal models as they are readily available, their n-alkane content is low (through dewaxing), and they are rich in hydrocarbons (through solvent extraction). Furthermore, many reports of the presence of hydrocarbon UCMs in Recent polluted sediments have implicated waste automobile lubricating oils as a likely source.

The isolation of hydrocarbon UCMs from the Silkolene 150 lubricating oil involved standard column and thin layer chromatographic separations, and urea adduction to remove n- and simply branched (e.g. monomethyl alkanes). By this approach the total oil was found to comprise 66% urea non adducted alkanes (the aliphatic UCM) and 8% aromatic hydrocarbons (the aromatic UCM). GC of the aliphatic and aromatic UCMs provided little compositional information, this was limited to an estimate of the carbon number range (aliphatic UCM: KI 1850 - 3600 +, max. 2615; aromatic UCM: KI 1950 - 3600 +, max. 2700); and the degree of complexity (i.e. percentage unresolved, aliphatic UCM: > 90%; aromatic UCM: > 95%). GC-MS analyses of the aliphatic UCM were of use in the detection of commonly occurring "biological marker" compounds (e.g. acyclic isoprenoid alkanes, pentacyclic triterpanes, steranes), however these accounted for less than 10% of the total integrated FID response. GC-MS mass fragmentography of ions characteristic of acyclic, monocyclic and bicyclic alkanes revealed series of these compounds within the aliphatic UCM. However the exact molecular nature and absolute quantitative contribution of each alkane type to the UCM profile could not be determined by GC-MS alone.

Conventional GC and GC-MS methods of analyses applied to hydrocarbon UCMs in this study therefore provided only limited molecular detail. As a result, alternative methods were sought. These included fractionation of the UCM hydrocarbons by thiourea adduction and gel permeation chromatography (GPC), and UCM analyses by CI GC-MS, probe EI-MS, probe FI-MS and elemental analysis. Thiourea was shown to remove a major proportion of the resolved compounds overlying the

aliphatic UCM (mainly regular acyclic isoprenoids). GPC was also shown to be a promising technique for the preparative fractionation of UCM hydrocarbons.

CI GC-MS of the aliphatic UCM was limited to the provision of molecular weight data for a narrow range of alkane types, in the main these were identified as resolved alkanes. CI GC-MS did however provide tentative evidence for the existence of certain alkyl branch acyclic and monocyclic alkanes, with the branch length varying from CI (i.e. methyl) to C4, C5 (i.e. butyl, pentyl)

A published method of probe EIMS used routinely in the petroleum industry provided evidence for the predominance of acyclic and monocyclic alkanes in the aliphatic UCM, in a 1:1.2 ratio. This appeared consistent with the data provided by elemental analysis for the same sample, which also indicated a higher proportion of monocyclic alkanes. However, use of this particular method of probe-MS analysis of complex petroleum fractions has recently been questioned following a "round-robin" study by users of the technique in the U.K.. The quantitative data which it provides may therefore be in error.

Probe FIMS appeared promising for the analysis of complex hydrocarbon mixtures, in particular as a method of obtaining reliable molecular weight data. Thus FIMS of the aromatic UCM provided evidence for the presence of varied homologous series of alkylated benzenes, naphthenobenzenes, naphthalenes and naphthenonaphthalenes.

Each of the above described methods provided only limited structural information at the molecular level. As a result, an alternative

approach was adopted which utilised chemical and pyrolytic degradations of the UCM hydrocarbons. Chemical oxidation with CrO_3 in glacial acetic acid produced reasonable yields of total recoverable material (40-80%). Furthermore, a high proportion (>90%) were functionalised, and many resolved which allowed their identification by conventional EI and CI GC-MS. These products are summarised in Table 7.1.

The most surprising result of the oxidation of both aliphatic and aromatic UCMs was the high proportion of straight chain monocarboxylic acids produced. This appeared to contradict the literature reports on the composition of hydrocarbon UCMs, namely a predominance of highly branched and/or cyclic compounds, but from a knowledge of the mechanism of CrO_3 oxidation of hydrocarbons, potential precursor UCM compounds could be proposed. N-monocarboxylic acids were proposed to originate from oxidative cleavage of monoalkyl substituted "T"- branched acyclic and monocyclic alkanes for the aliphatic UCM, and monoalkyl "T"- branched monoaromatic hydrocarbons for the aromatic UCM.

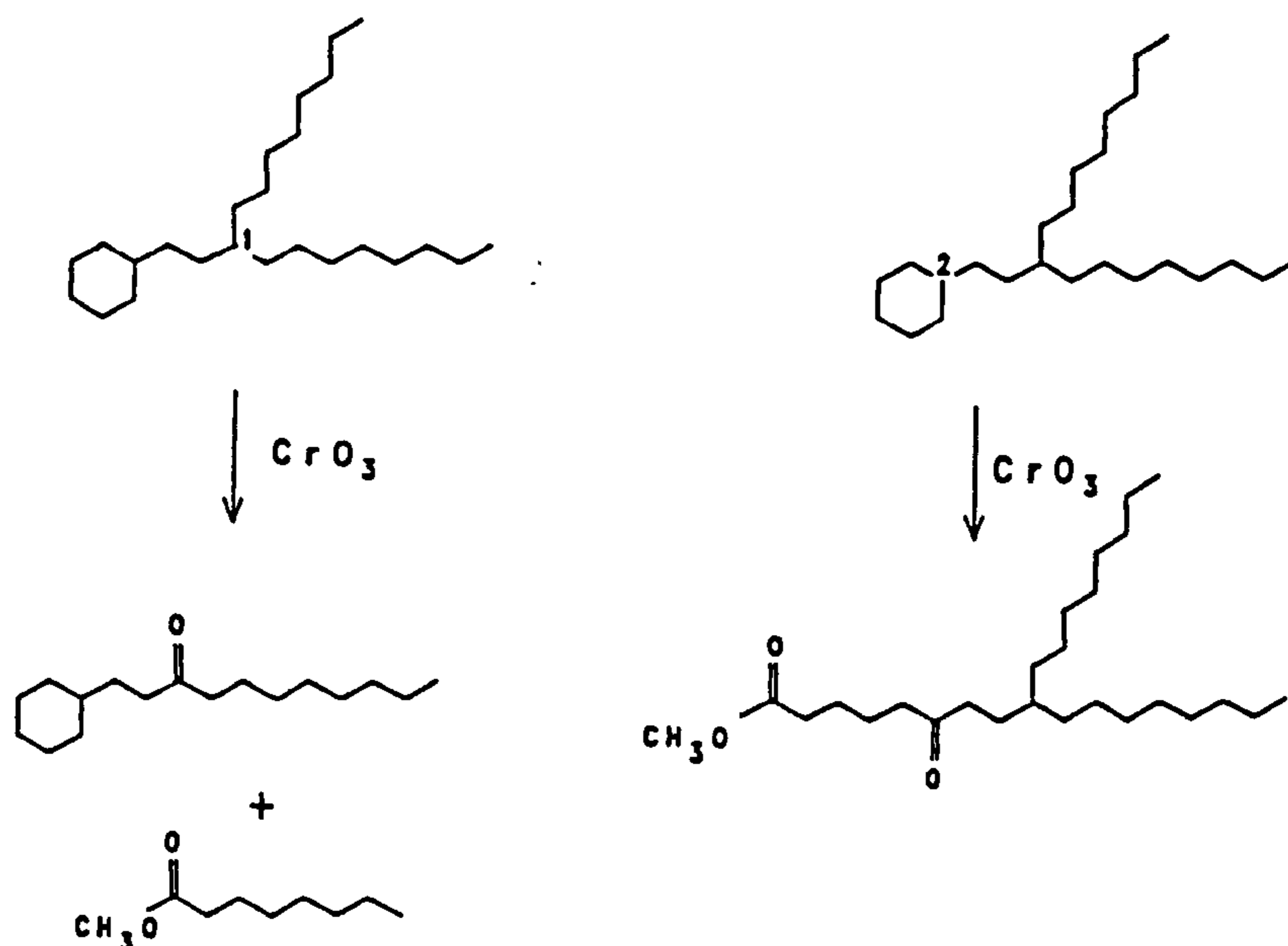
Many other types of functionalised compounds were identified as hydrocarbon UCM oxidation products (summarised in Table 7.1), most of which could be correlated with specific types of alkyl linkages.

Thus n-alkan-2-ones would originate from methyl substituted acyclic linkages, whereas iso-alkan-2-ones would originate from 2 -methyl substituted alkyl chains with a second mid- chain methyl group. γ -methyl- γ -lactones (C6 to C11) were presumed to originate from 2- to 7- methyl substituted alkyl chains with a second alkyl substituent at

C-6 to C-11. Analogous methyl branched ω -oxo- γ -methyl- γ -lactones were all identified as UCM oxidation products.

Isoprenoid acids and ketones were identified as products of both aliphatic and aromatic hydrocarbon UCMs, although quantitatively more important as products of the latter. As aliphatic UCM oxidation products, these may arise through oxidation of resolved regular acyclic isoprenoids. As products of the aromatic UCM, these may arise through oxidation of alkyl isoprenoid benzenes of the type identified recently in certain crude oils. (Sinninghe Damste *et al.*, 1988). The isoprenoid-derived C10 keto acid 8-oxo-4-methylnonanoic acid was identified as a major product of the aliphatic UCM only.

Although GC-MS, probe EI-MS and elemental analysis all provided evidence for a substantial proportion of monocyclic alkanes in the aliphatic UCM, only one cyclohexyl carboxylic acid was identified as an oxidation product. A possible cause is oxidation of the ring-alkyl chain junction (a tertiary position) which would cause the ring to open with the production of an alkylbranched keto acid, via:



This mechanism may therefore account for the low proportion of monocyclic acids identified as UCM oxidation products. Some evidence of cleavage at this position was provided by oxidation of the synthetic monocyclic alkane (chapter 4).

Alternatively the abundance of monocyclic alkanes may be over estimated by conventional methods of analysis, namely probe EI-MS and/or GC EI-MS. In the analysis of the authentic monocyclic alkane 9-(2-cyclohexylethyl)-heptadecane by EI-MS (chapter 4), a substantial proportion of the total ion current (76%) was attributed to fragments retaining the cyclohexyl ring moiety (estimated by summation of peak heights). However, the ring itself only accounts for 6/25 = 24% of the total carbon atoms present. This highlights a major problem in quantitative mass spectrometry, namely the determination of molecular amount based on a few nonrepresentative fragment ions.

Somewhat surprisingly, cyclohexyl and methyl cyclohexyl carboxylic acids were more abundant as products of the aromatic UCM. Monoaromatic acids and ketones were also identified. This is perhaps indicative of a high proportion of noncondensed naphthenic/aromatic nuclei on precursor aromatic UCM hydrocarbons.

Many of the proposed precursor UCM hydrocarbons were confirmed by synthesis and oxidation of model compounds. The synthetic procedures chosen involved the coupling of alkyl halides with carbonyl compounds (ketones, aldehydes or esters) via well known Grignard addition reactions. Dehydration of the resultant secondary or tertiary alcohols followed by catalytic hydrogenation produced the hydrocarbons, which were purified by preparative chromatography.

Oxidation of authentic hydrocarbons utilised the same conditions as used for the aliphatic and aromatic UCM, and the products were identified by GC and GC-MS. These are summarised in Table 1.7. In accordance with the reported mechanism of hydrocarbon oxidation by chromic acid, attack occurred preferentially at tertiary carbon centers in the case of aliphatic hydrocarbons, and at the benzylic position in the case of the aromatic hydrocarbon. Thus many of the identified compounds were the predicted oxidation products according to theory.

Most significantly, n-monocarboxylic acids were identified as products of each of the candidate UCM hydrocarbons, 7-n-hexylnonadecane, 9-(2-cyclohexylethyl)-heptadecane and 9-(2-phenylethyl)-heptadecane. Although n-acids were observed as products of the n- and monomethyl alkanes, these were removed prior to oxidation by adduction with urea. The most likely origin of n-acids observed as UCM oxidation products is therefore through oxidative cleavage of monoalkyl substituted "T"-branched acyclic, monocyclic or monoaromatic hydrocarbons.

The oxidation of model compounds was of particular use in the identification of certain unknown products of oxidation of both aromatic and aliphatic UCMs. Thus the series of ω -carboxy- γ -methyl- γ -lactones identified as aliphatic UCM oxidation products could be correlated specifically with the products of oxidation of 9-methyltetracosane. The C11 methylbranched γ -methyl- γ -lactone, assigned an "isoprenoid-type" origin on the basis of its GC retention behaviour, was also observed as a product of the synthetic

hydrocarbon mixture. This contained two acyclic isoprenoid alkanes, which strongly suggests such an origin for this compound. A methyl branched ω -carboxy- γ -methyl- γ -lactone, also assigned an acyclic isoprenoid origin, was identified as an oxidation product of both the aliphatic UCM and pristane. Further correlations between the products of oxidation of hydrocarbon UCMs and model compounds are summarised in Table 7.1.

Although observed as products of oxidation of "T"-branched hydrocarbons, mid chain alkyl ketones were not identified as aliphatic or aromatic UCM oxidation products. The reason for this discrepancy is not at present clear. A possible cause is their greater reactivity towards residual oxidant, a feature which was noted in the oxidation of individual authentic "T" branched compounds. In each case the corresponding acid derived from cleavage of the same C-C bond was significantly more abundant than the alkyl ketone. Alternatively, mid chain (not identified) and methyl (identified) ketones may display differing reactivities towards the oxidant. This needs to be assessed by the oxidation of authentic ketone isomers.

The currently available evidence based on the oxidation of both hydrocarbon UCMs and model compounds therefore suggests a high proportion of UCM compounds comprise relatively simple monoalkyl substituted acyclic alkyl linkages. In view of the apparent simplicity of such a mixture it was decided to calculate the number of theoretically possible acyclic alkane isomers within a given carbon number range. This was performed by computer with the assistance of Mr A. Aldrige, (Database). Within the range C20 to

TABLE 7.1

SUMMARY OF UCH OXIDATION PRODUCTS AND CORRELATIONS WITH THE PRODUCTS OF OXIDATION OF MODEL COMPOUNDS

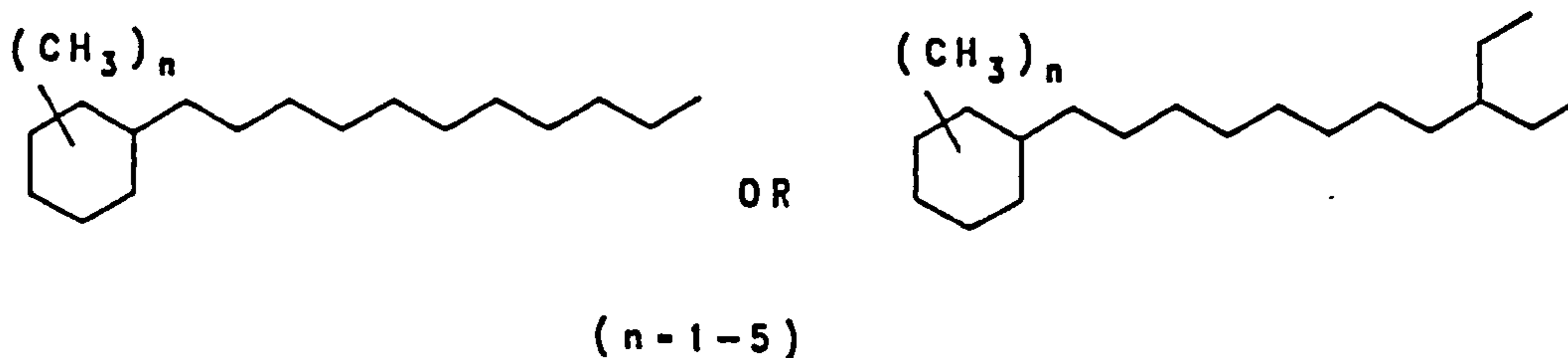
COMPOUND TYPE	ALIPHATIC UCH	AROMATIC UCH	<u>N</u> -C25	2-MT	9-MT	7- <u>N</u> -HN	9-(2-CHE)-H	9-(2-PE)-H	PRIS	MIX
<u>n</u> -monocarboxylic acids	C6-C20 (C9)	C6-C20 (C14)	C6-C24 (C14)	C6-C19 (C9)	C15 ^a	C7-C13 (C12)	C7-C9 (C12)	C7-C9 (C18)	-	C6-C23 (C8)
<u>n</u> - α,ω -di carboxylic acids	C6-C14 (C9)	-	C7-C20 (C10)	C6-C18 (C9)	C6-C11 ^b (C18)	-	-	-	-	C9,C10
<u>n</u> -alkan-2-ones	C8-C15 (C12)	C8-C19 (C12)	-	-	C17 ^a	-	-	-	-	C10-C17
isoalkan-2-ones	C8-C12 (C8)	C8-C10,C12 (C8)	-	-	-	-	-	-	C8	C8
γ -methyl- γ lactones	C6-C11 (C10)	C6-C11 (C8)	-	-	-	-	-	-	C6	C6,C7
methylbranched γ -methyl- γ -lactones	C11,C12 (C11)	C11	-	-	-	-	-	-	-	C11
ω -oxo- γ -methyl- γ -lactones	C8,C10 (C8)	-	-	-	C8-C12 ^b (C10)	-	-	-	-	-
methylbranched ω -oxo- γ -methyl- γ -lactones	C11-C13 (C11)	-	-	-	-	-	-	-	C11	-
isoprenoid acids	C11,C16	C7,C11, C16	-	-	-	-	-	-	C6,C7, C10,C11, C16	C6,C7, C10,C11, C16,C21
isoprenoid ketones	C13	C13,C18	-	-	-	-	-	-	C8,C13, C18	C8
methylbranched acids	C7-C11 (C8)	-	-	-	-	-	-	-	C6,C7	C6,C7
keto acids	C10	-	-	-	-	-	-	-	C10	C10
cyclohexyl carboxylic acids	C8	C7-C9	-	-	-	-	C8,C9	-	-	C8,C9
aromatic acids	-	C7-C9	-	-	-	-	-	C7,C9	-	C7
aromatic ketones	-	C9	-	-	-	-	-	C17,C25	-	C25
mid chain alkyl ketones	-	-	-	-	-	C13,C19	C17	C17, C19	-	C13,C17

a: 10 min. oxidation
b: 60 min. oxidation

C30 a total of 536 individual monoalkyl substituted acyclic alkanes exist, with the branch length varying from C1 (i.e. methylnonadecanes to methylnonacosanes) to C10 (i.e. 10-n-decyleicosane). Under standard chromatographic conditions [DB-5 (J+W), 25m, 50-300°C @ 5°Cmin⁻¹], C20 to C30 n-alkane standards require 16.7 minutes for complete elution (1003 seconds). Each monoalkyl substituted acyclic alkane would then require 1003/536=1.9seconds to elute and be baseline resolved for complete resolution. However, the elution time of authentic n-alkanes in this region was estimated at 11.7 seconds/component. The theoretical number of monoalkyl substituted acyclic alkane isomers under these conditions would thereby form an unresolved complex mixture when analysed by GC.

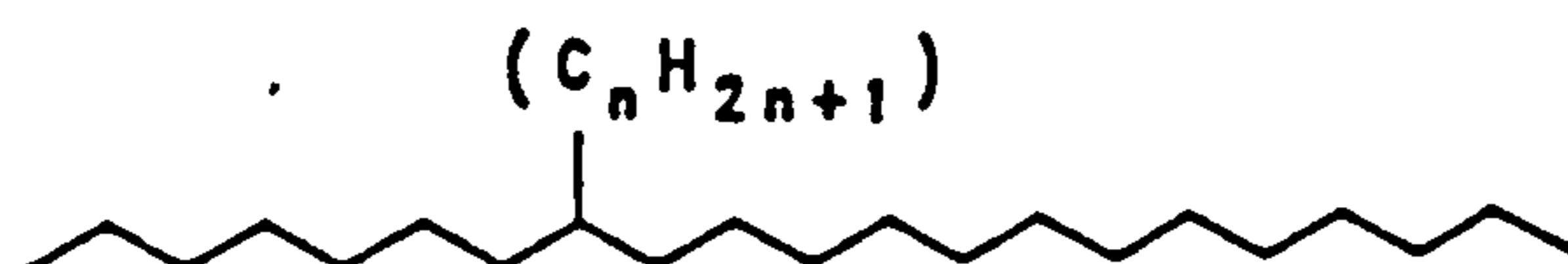
Theoretical and experimental considerations presented in this study therefore point to a surprisingly simple mixture of components for the hydrocarbon UCMs analysed. This is in accordance with the conclusions of Hood et al. (1959) following many years of research into the composition of the distillate fraction of petroleum (API Research Project 6). They proposed the monocyclic alkanes of the lubricant saturate fraction of petroleum to comprise a single long alkyl branch which is unsubstituted or simply substituted (e.g. methyl) at one end, with 0 to 5 methyl groups on the ring

i.e.

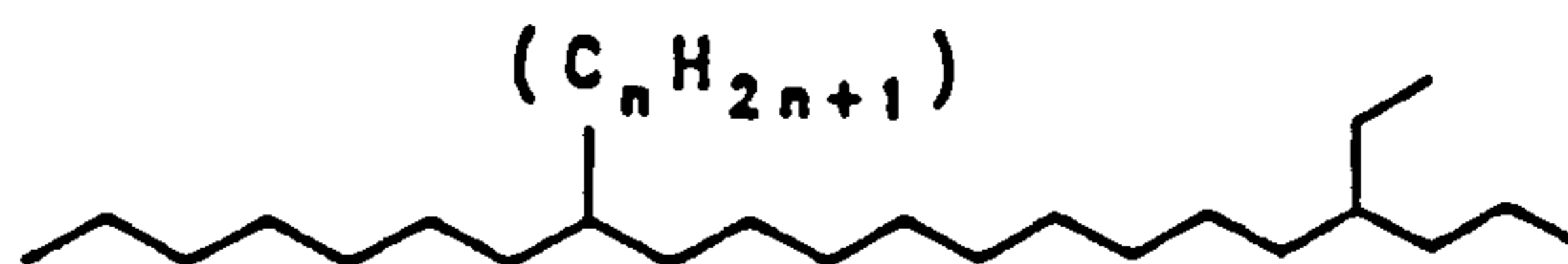


The acyclic alkanes in the lubricant saturate fractions were proposed to comprise mainly monoalkyl substituted acyclic alkanes, with possibly a second "short" alkyl substituent at the opposite end of the chain

i.e.



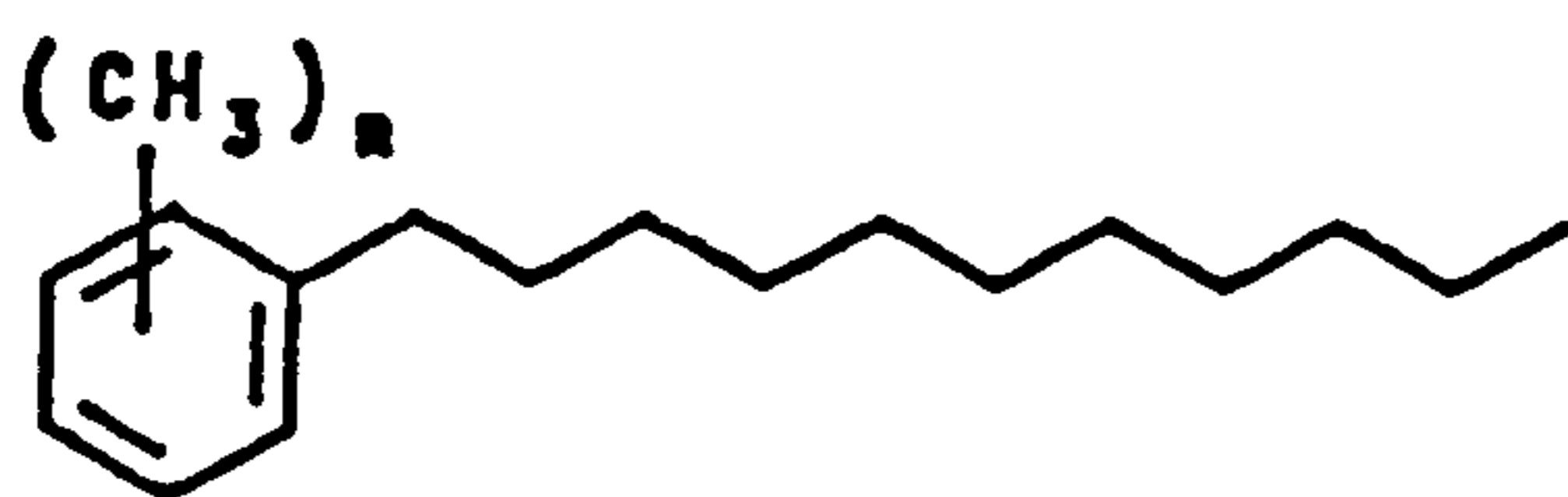
or



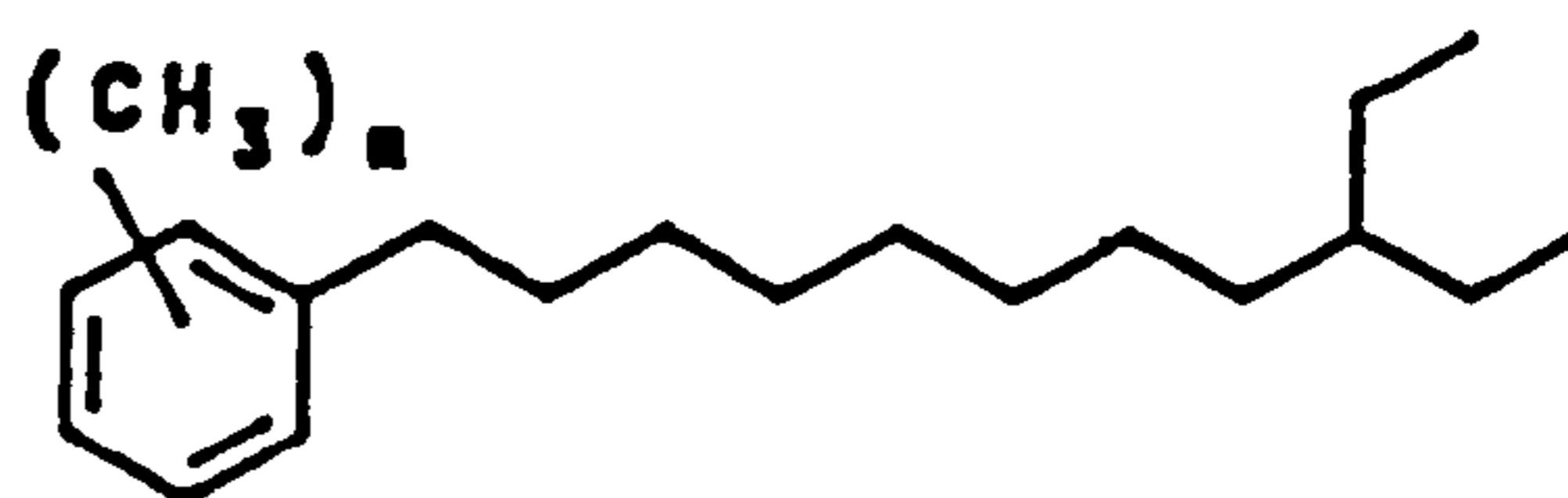
(n = 2 or 3)

Likewise, the alkyl benzene fractions of lubricating oils were thought to comprise a single long alkyl chain, simply branched at one end, and with 0-4 methyl substituents on the ring.

i.e.



OR



(n = 0 - 4)

Some thirty years after this work was first published, oxidation of the hydrocarbon fractions of lubricating oils undertaken in this study appears to confirm their findings.

The correlation of biodegraded crude oils with their source rocks or unaltered oils is a recognised problem in organic geochemistry, the parameters most often used to achieve such correlations, namely "biological marker" fingerprints, are often altered beyond recognition by extensive microbial degradation. However, one feature which retains prominence in most biodegraded crude oils is the UCM, yet until now this has largely been ignored as a source of compositional information. Oxidative studies herein showed promise as a means for "fingerprinting" hydrocarbon UCMs on the basis of different products of oxidation. This potential was assessed by oxidation of a total of nine aliphatic UCMs of varied origin. Although n-monocarboxylic acids were major resolved oxidation products in each case, their abundance relative to both residual and/or functionalised UCM and other products was shown to vary depending on the UCM origin. A closer examination of certain resolved oxidation product profiles by GC-MS (γ -lactones, m/z 99; alkyl ketones, m/z 58) showed that these varied depending on the UCM origin, yet were almost identical for UCMs from a common source. In this context these may therefore act as specific "fingerprints" which have excellent potential as a method for oil-oil and oil-source rock correlations.

In view of the relative simplicity of the proposed UCM hydrocarbons (7-n-hexylnonadecane, 9-(2-cyclohexylethyl)-heptadecane, and 9-(2-

phenylethyl)- heptadecane), an experiment was devised to assess their resistance to microbial degradation. The organism chosen was a pure strain of *Pseudomonas fluorescens*, a common hydrocarbon degrader in many sedimentary environments.

Under the conditions of the experiment, normal and monomethyl alkanes were rapidly degraded within the first ten days, in the approximate order *n*-pentacosane > 2-methyltetracosane > 9-methyltetracosane. This was in accordance with previous studies of alkane biodegradation.

The remaining synthetic hydrocarbons were also shown to degrade although to the limited extent. Of particular interest was the observation of comparable rates of degradation for the candidate UCM hydrocarbons and the regular acyclic isoprenoid alkane 2,6,10,14,18-pentamethyleicosane. Their concentrations covaried throughout the study with a high degree of linearity ($r = 0.997$, day 0 to day 25). In this context therefore, monoalkyl substituted "T"-branched hydrocarbons serve as good models for the hydrocarbon UCMs studied.

7.2 PROPOSALS FOR FUTURE RESEARCH

Proposals for future research are summarised below:

1) Further methods of preparative fractionation of hydrocarbon UCMs should be sought. Although thiourea adduction and GPC fractionations were of some success in the present study, no additional analyses were undertaken. Alternative methods may include those based on the selective inclusion of UCM hydrocarbons by zeolites (e.g. Dimmler and Strausz, 1983) for which a wide variety of pore sizes are available. These would be of particular use in the removal of resolved acyclic isoprenoid alkanes, evidence presented herein suggests these contribute in part to the resolved acyclic-isoprenoid-type acids and ketones produced by oxidation.

Alternatives to molecular sieving include preparative GPC, GC, or HPLC; the latter would be of particular use for the preparative fractionation of the aromatic UCM and should confirm the supposition of a predominance of alkyl substituted monoaromatic UCM hydrocarbons (e.g. Killops and Readman, 1985).

2) Methods which are of particular promise for future UCM analysis are FIMS and ^{13}C nmr. The former is perhaps the most reliable method for obtaining accurate compound type (i.e. z number) and carbon number ranges. The latter was not investigated in this study, although literature reports show the technique can provide a wealth of compositional information (e.g. Singh and Srivashava, 1985).

3) The use of alternative oxidants should be investigated for both aliphatic and aromatic UCMs. For the former relatively few oxidants are available that are capable of cleaving fully saturated C-C and C-H bonds. One example however is chromyl chloride, this has the advantage of being soluble in aprotic solvents (e.g. DCM, Cainelli and Cardillo, 1984). For the aromatic UCM, the reported and observed greater reactivity of the benzylic position (e.g. March, 1987) allows the use of many more oxidants suitable for the selective functionalisation of aromatic UCM hydrocarbons. One example is ruthenium tetroxide, this is reported to selectively cleave alkyl aromatic hydrocarbons at this position and in good yield (Trifilieff, 1987). An added advantage is the solubility of the reagent in CCl_4 .

4) Methods of synthesis of model hydrocarbon UCMs should be sought. A possible means is via hydrous pyrolysis of synthetic polymers of varying degrees of cross chain branching. Alternatively, the "methylene insertion" reaction is known to produce random methylation of a hydrocarbon chain with the formation of all possible methyl-substituted isomers (Hala et al., 1981). The reported reaction appears simple, and by use of a mixture of high molecular weight n-alkane standards ($>C_{20}$), a large number of methyl substituted alkanes would be formed. These may reveal a UCM when analysed by GC.

Synthetic UCMs could then be oxidised and compared with those produced naturally by the maturation of organic matter. This in itself has not been addressed in this study. In fact little is known of the origin and transformations of hydrocarbon UCMs under geological conditions. This could be investigated by an examination

of isolated UCM profiles from well defined maturity sequences such as those used to study "biological marker" compounds (e.g. Toarcian shales, Mackenzie et al., 1981).

5) The production of a quantitatively important functionalised UCM was noted in the oxidation of certain aliphatic UCMs in this study. The composition of this mixture is at present not known. However, isolation of the functionalised UCM can be readily achieved by standard chromatography followed by urea adduction to remove resolved n-acids. The UCM could then be analysed directly by alternative methods (e.g. FAB-MS), or fractionated by preparative chromatography (e.g. HPLC).

6) The biodegradation experiments performed in this study should be modified and extended to examine the effects of microbially induced compositional changes on oxidation product profiles. For example, the most heavily biodegraded UCMs examined produced the least products. Ideal biodegradation sequences exist in the Alberta oil sand bitumens of Western Canada (e.g. Deroo et al., 1974; Brooks et al., 1989^a).

The resistance of the "T"-branched isoalkane to microbial alteration observed in this study should be confirmed. By use of a mixture comprising only the monoalkyl hydrocarbon and regular and highly branched isoprenoids, a more accurate assessment of their relative resistance could be made.

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APPENDIX ONE

A) THE ALIPHATIC UCM:

The aliphatic UCM was quantified electronically using the Shimadzu CR3-A integrator. This involved use of the "time slice area measurement" function, and programming the integrator in BASIC to sum integral areas for each "time slice".

i) TOTAL (UCM + resolved peaks) INTEGRATED SIGNAL

METHOD: width: 50
drift: 0
attenuation: 5
method: 4840
slope:automatic
minimum area: 1000

BASIC PROGRAM:

```
10 PRINT MAXSL
20 S=0
30 FOR I=1 TO MAXSL
40 S=S+SLAR(I)
50 NEXT I
60 PRINT S
70 END
```

ii) INTEGRATED RESOLVED PEAK SIGNAL:

METHOD: width: 5
drift: 1000
attenuation: 5
method: 0840
slope:automatic
minimum area: 40000

iii) RESULTS

RUN	AREA _R	AREA _{UR}	%R	%UR
1	0.08	0.98	8.5	91.5
2	0.09	1.01	9.0	91.0
3	0.10	1.06	9.6	90.4
4	0.12	1.20	10.1	89.9
5	0.89	1.06	8.4	91.6
6	0.11	1.14	10.0	90.0
7	0.11	1.08	9.9	90.1
8	0.11	1.08	10.4	89.6
9	0.10	1.06	9.5	90.5
10	0.11	1.08	10.2	89.8

R=RESOLVED

UR=UNRESOLVED

MEAN AREA_{UR} = 1.07 ± 0.06 (5.7%)

MEAN %UR: 90.5

these values were found to differ from those determined manually (chromatogram expansion and tracing onto graph paper) by ± 2%.

iv) CALIBRATING THE TOTAL AREA SIGNAL:

RUN	CONC (mgml ⁻¹)	TOTAL AREA (x 10 ⁶)	%R	%UR
1	2.12	0.35	9.4	90.6
2	4.24	0.89	8.4	91.6
3	5.00	1.07	9.3	90.7
4	6.36	1.41	9.4	90.6
5	7.95	1.80	9.2	90.8

Linear in the range 2-8 mgml⁻¹; r=0.99999, intercept = 0.007 mgml⁻¹

B) THE SYNTHETIC HYDROCARBON MIXTURE

REPLICATE ANALYSES

COMPOUND	RUN NO. (AREA x 10 ⁵)									
	1	2	3	4	5	6	7	8	9	10
1	1.51	1.28	1.43	1.51	1.55	1.39	1.33	1.56	1.45	1.53
2	1.44	1.22	1.35	1.44	1.47	1.32	1.32	1.48	1.37	1.45
3	1.51	1.28	1.42	1.51	1.55	1.38	1.35	1.56	1.44	1.51
4	1.46	1.24	1.37	1.46	1.50	1.33	1.34	1.51	1.39	1.47
5	1.53	1.30	1.44	1.53	1.55	1.39	1.34	1.58	1.46	1.53
6	1.44	1.22	1.35	1.44	1.49	1.31	1.26	1.49	1.37	1.45
7	1.52	1.29	1.43	1.52	1.56	1.39	1.32	1.57	1.45	1.54
8	1.46	1.24	1.37	1.45	1.49	1.33	1.27	1.51	1.39	1.47

(see text, Chapter 6 (Table 6.1), for peak identity)

MEANS AND STANDARD DEVIATIONS

COMPOUND NO.	MEAN AREA (x 10 ⁶)	$\delta n-1$	% variation
1	1.45	0.09	6.3
2	1.39	0.08	5.9
3	1.45	0.09	6.2
4	1.41	0.08	5.9
5	1.47	0.09	6.2
6	1.38	0.09	6.6
7	1.46	0.10	6.5
8	1.40	0.09	6.4

APPENDIX TWO

BBC "BASIC" PROGRAM FOR THE QUANTITATIVE
ANALYSIS OF ALKANE TYPES IN PETROLEUM SAMPLES BY PROBE EI-MS

LIST

```

10 DIM A(10,10), B(10), R(10), SI(10)
12 REM READING SIGMA DATA
15 FOR I=1 TO 7:READ SI:SI(I)=SI:SI=0:NEXT I
20 CLS
30 PRINT " ©COPYRIGHT 1988 B.FAIRMAN AND M.GOUGH "
40 PRINT "-----"
50 PRINT:PRINT
60 PRINT " MATRIX CALCULATIONS FOR THE RING-TYPE ANALYSIS OF PETROLEUM SATUR
ATE FRACTIONS"
70 PRINT TAB(5,20)"PRESS [SPACEBAR] TO CONTINUE"
80 VDU 23,1,0;0;0;0;
90 B$=GET$
85 IF B$<>" " GOTO 70
100 *FX5,1
110 *FX8,1
130 REM INPUT DATA
140 VDU 23,1,1;0;0;0;
150 CLS
160 INPUT " ENTER NAME OF RUN";FIL$:PRINT:PRINT
170 INPUT " ENTER THE NUMBER OF ALKANE RINGS THAT HAVE BEEN DETERMINED (1-8)
";R
175 IF R<1 OR R>8 GOTO 170 ELSE R=R+1
180 PRINT
190 INPUT " ENTER NUMBER OF MASS GROUPS TO BE USE IN THE CALCULATIONS (1-7)"
;G
185 IF G<1 OR G>7 GOTO 190
200 CLS
210 FOR I=1 TO G
220 FOR J=1 TO R
230 PRINT TAB(7,0)"ENTER DATA FOR MASS GROUP ";I
240 PRINT TAB(8,1)"-----"

```

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250 PRINT TAB(0,3)"ENTER INVERSE MATRIX COEFFICIENTS"
260 PRINT TAB(0,5+J)"VALUE FOR RING-";J-1;"=
270 INPUT TAB(18,5+J);A(I,J)
280 NEXT J
290 PRINT TAB(0,10+R)"ENTER SUMMED INTENSITY FOR MASS GROUP ";I
310 PRINT "(SIGMA ";SI(I);")"
320 INPUT TAB(12,11+R);B(I)
330 FOR JJ=1 TO R
340 PRINT TAB(17,5+JJ)"
350 NEXT JJ
360 PRINT TAB(0,10+R)"
370 PRINT TAB(0,11+R)"
380 NEXT I
390 REM CALCULATING PARTIAL ION INTENSITIES
400 FOR X=1 TO G
410 FOR I=1 TO G
420 S=B(I)*A(I,X)
430 H=H+S:S=0:NEXT I
440 R(X)=H:H=0
450 NEXT X
460 REM RESULTS
470 CLS
480 INPUT "          DO YOU WANT A HARD-COPY OF THE RESULTS  Y/N";T$
485 CLS
490 IF T$="Y" THEN VDU2
510 PRINT:PRINT
520 PRINT "          RESULTS FOR RUN ";FIL$
530 PRINT:PRINT
540 PRINT "    RING NUMBER";"    PARTIAL ION INTENSITY"
550 FOR I=1 TO R

```

```
555 PRINT
560 PRINT"          ";I-1;"          ";R(I)
570 NEXT I
580 IF T$="Y" GOTO 590 ELSE 600
590 VDU3
600 INPUT TAB(10,20)"ANOTHER  Y/N";Y$
610 IF Y$="Y" GOTO 20
620 END
630 DATA 71,69,108,149,189,229,269
```

>