

**CHARACTERISATION OF UNRESOLVED COMPLEX MIXTURES OF**  
**HYDROCARBONS BY DEGRADATIVE METHODS**

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**TO MY PARENTS**

# CHARACTERISATION OF UNRESOLVED COMPLEX MIXTURES OF HYDROCARBONS BY DEGRADATIVE METHODS

by  
Andrew Thomas Revill

## ABSTRACT

*Unresolved Complex Mixtures* (UCMs) of hydrocarbons are found in crude and refined oils and in water, sediments and biota polluted with oils. The concentrations of UCMs in oils are significant (e.g. >65% of the aliphatic hydrocarbons in fresh Kuwait crude) and it is perhaps surprising that virtually nothing is known about UCM composition. The present study sought to redress this paucity of information in three main ways:

First, following two recent studies of aliphatic UCMs, an investigation of the composition of the "aromatic" UCM of Venezuelan Tia Juana Pesado crude oil was made by spectroscopic (IR, NMR, MS) and oxidative (CrO<sub>3</sub>, RuO<sub>4</sub>) methods. These showed that the UCM was, in fact, highly aliphatic. The major compounds identified were alkyl substituted naphthoaromatics with one and two aromatic rings. Chemical oxidation indicated that the alkyl branched side chains extended to at least twenty three carbon atoms.

Second, an investigation into the origins of UCMs was made. The products of hydrous pyrolysis of man-made (polythene) and biogenic (cutan) polymers under conditions proposed previously to simulate catagenesis, included, in the hydrocarbons, high proportions of UCMs (50% - >70%). Hydrous pyrolysis of polythene produced a mixture of saturated (56%) and olefinic (44%) hydrocarbons, whilst pyrolysis of cutan produced hydrocarbon (aliphatic and aromatic; 30-75%) and non-hydrocarbon (70-25%) fractions, both with >60% unresolved components. Oxidative characterisation of these UCMs produced mainly *n*-acids with somewhat similar results to those found when oil UCMs were oxidised. However, the laboratory generated UCMs are not perfect oil UCM models since some oil UCM oxidation products were not observed in the laboratory models.

Finally, an attempt was made to release the geochemical information contained within UCMs. Replicate oxidations of milligram quantities of oil UCMs followed by quantitative GCMS characterisation and multivariate statistical analysis of the resolved oxidation products gave reproducible distributions with >80% similarity. Application of this method to two oil spill incidents where the source oil was known (Milford Haven and the Humber Estuary) gave good correlations between sediment and source. In contrast analysis of Mersey Estuary sediments contaminated with heavy asphaltic oil and of Sullom Voe sediments contaminated with UCMs failed to show any correlation between the sediments and the source oils. However, subsequent re-analysis of the data excluding the major UCM oxidation products (*n*-carboxylic acids) produced better correlations which indicated that the greatest correlation potential for these UCMs was contained within the minor oxidation products. A similar study of UCMs from two oil seeps from the Siljan Ring region of Sweden failed to show any correlation with potential source rocks, in agreement with biomarker data.

This study has extended present knowledge of UCM composition and suggested a mechanism for UCM formation. Furthermore, quantitative and statistical analysis of UCM oxidation products has been shown to be useful for oil identification. There is still much to be learned about UCMs and the subject should provide a fruitful area for further research. Some possible approaches are suggested.

Parts of this work have been published [Revill et al. (1991), *Organic Geochemistry: Advances and Applications in Energy and the Natural Environment*, Manchester University Press (Abstract), 649-651; Revill et al. (1992), *J. Chromatogr.*, 589, 281-286].



## ACKNOWLEDGEMENTS

I was told recently, by someone with more experience than myself in these matters, that the acknowledgements are the most read part of any thesis. With this in mind I should like to indulge a little.

It is customary to thank ones supervisor first and I have every intention of doing the same. However, I should like to say that these thanks are not given lightly but with deepest sincerity. I am genuinely indebted to Dr. Steve Rowland for initially rescuing me from industrial oblivion and then his continued enthusiasm and encouragement throughout the course of the project. I feel no one could have had a better supervisor.

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No words can express my feelings of gratitude towards my parents, sister and brother-in-law who have supported me through the academic disasters and financial frugality.

Finally, I have reserved the best till last. A very special thanks to Hilary for being there.

## ABBREVIATIONS USED IN THE TEXT

DCM	dichloromethane
anh. Na <sub>2</sub> SO <sub>4</sub>	anhydrous sodium sulphate
TLC	thin layer chromatography
GC	gas chromatography
MS	mass spectrometry
EI	electron impact
FDMS	field desorption mass spectrometry
UNA	urea non adduct
UA	urea adduct
TUA	thiourea adduct
TUNA	thiourea non adduct
GPC	gel permeation chromatography
IR	infra red
UV	ultra violet
UVF	ultra violet fluorescence
NMR	nuclear magnetic resonance
UCM	unresolved complex mixture
KI	kovats index



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## CHAPTER ONE

### INTRODUCTION

Unresolved complex mixtures are a common feature in gas chromatograms involving hydrocarbon fractions, yet their composition and origin remain little studied. This chapter reviews the occurrence of UCMs and current knowledge of their composition. Possible precursor compounds which may form UCMs during maturation are discussed and how this might relate to petroleum formation in general. Finally, the potential application of UCM composition in correlation studies is considered.

*The journey of a thousand miles begins with a single step.*

**Lao Tze**



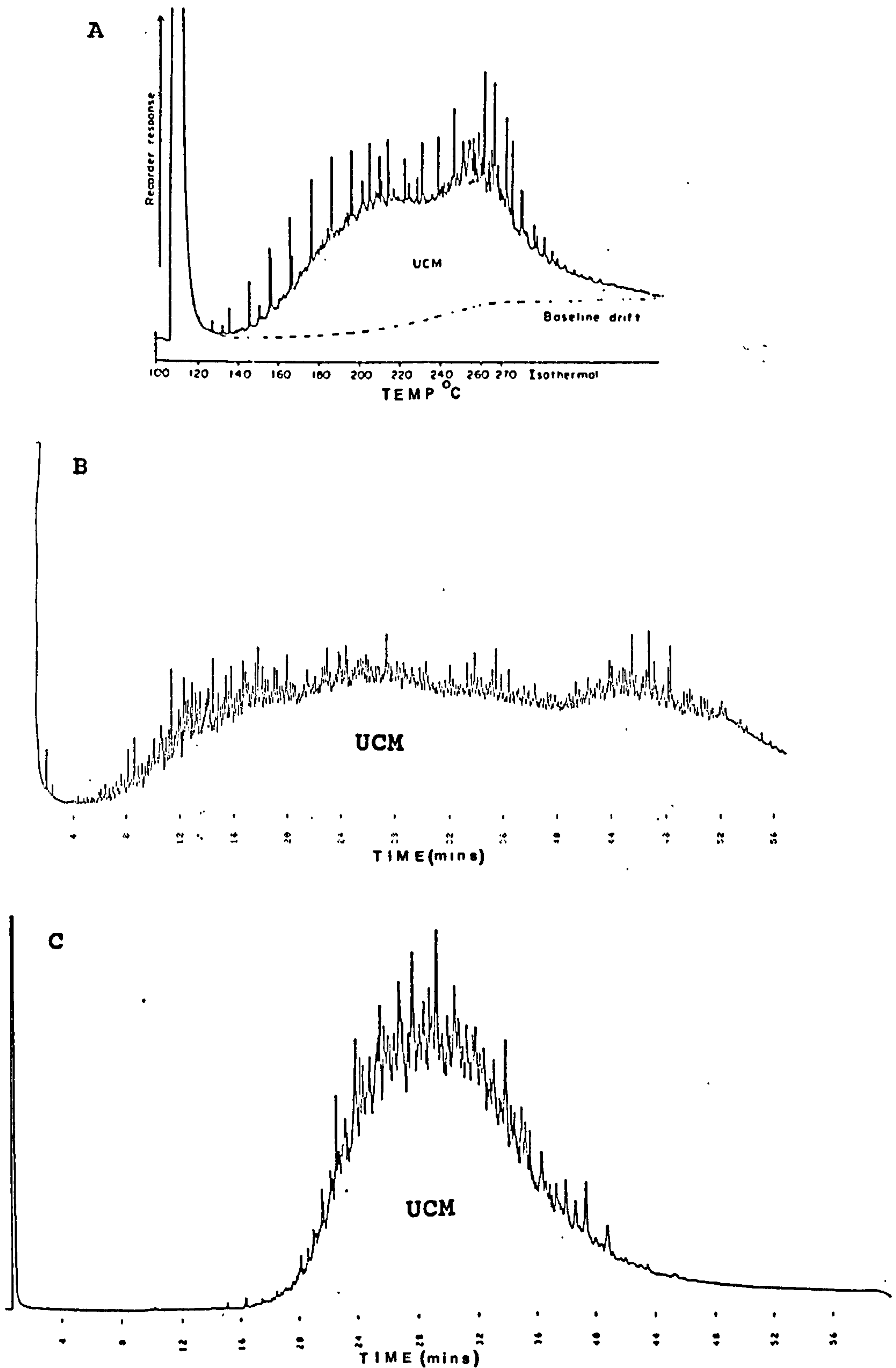
## INTRODUCTION

### 1.1 GENERAL

Gas chromatograms of hydrocarbons isolated from oil-polluted sediments, biodegraded crude oils and some refined petroleum products, often contain a feature which is commonly referred to as the Unresolved Complex Mixture or UCM (Figures 1.1 and 1.2). This is thought to result from the poor resolution of complex mixtures of hydrocarbons with similar physical and chemical properties (reviewed by Gough, 1989).

UCMs often account for more than 70% of the hydrocarbon burden of contaminated sediments, and UCM concentrations of 64-4500  $\mu\text{g g}^{-1}$  dry weight of sediment have been reported. Recently, UCMs have been shown to occur as mineral oil contamination in some food products (Grob et al., 1991) in riverine suspended matter (Qui et al., 1991) and other environmental samples (Table 1.1). The use of  $^{14}\text{C}$ -dating has shown that the UCM in sediments is almost invariably petrogenic in origin (Farrington and Quinn, 1973; Zofiriou, 1973; Wakeham and Carpenter, 1976; Farrington and Tripp, 1977).

Despite the widespread occurrence of UCMs, relatively little is known about UCM composition or origin. Hence, the geochemical information contained within these complex



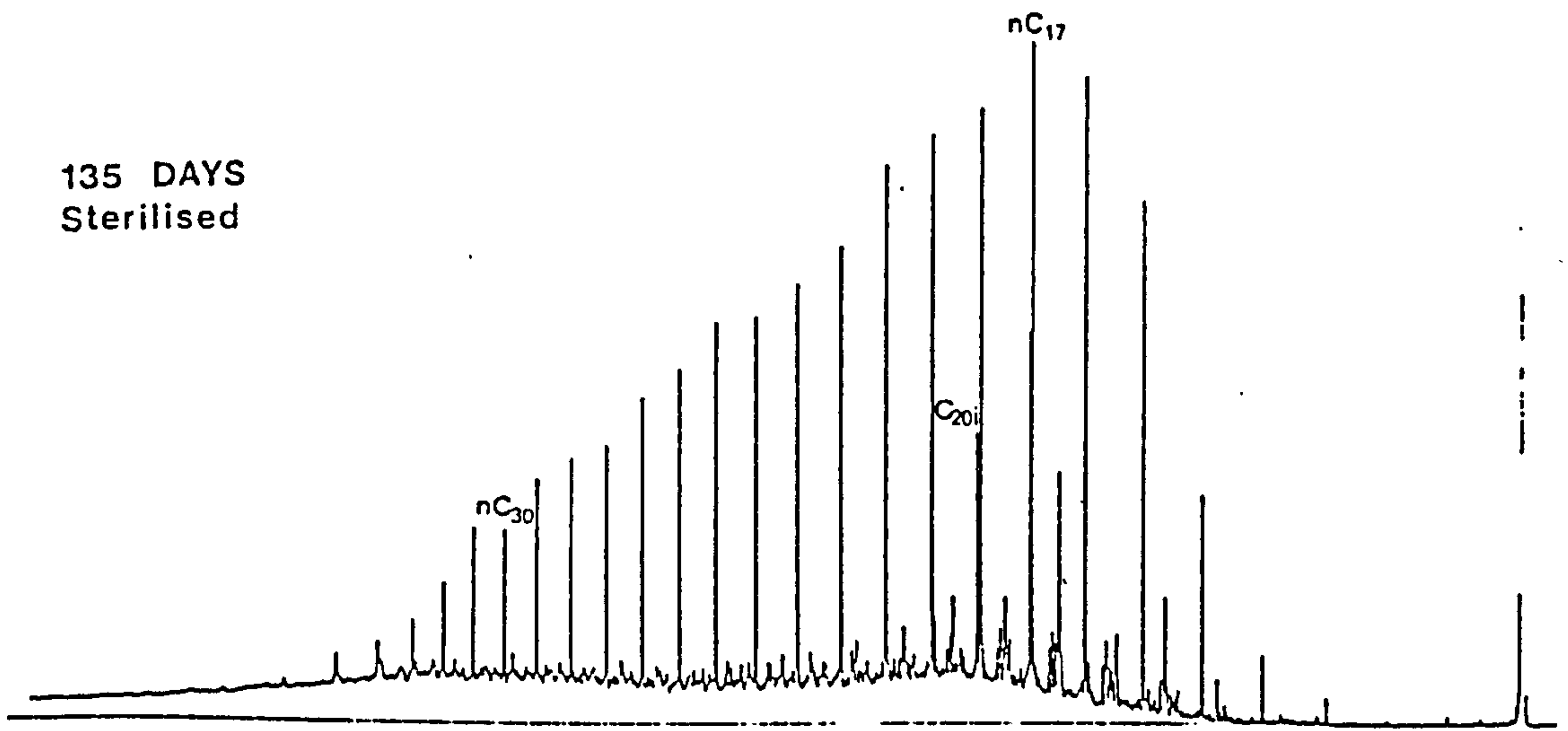
**FIGURE 1.1. EXAMPLES OF GAS CHROMATOGRAMS EXHIBITING UCMs**

(A) Polluted sediment extract (Thompson and Eglinton, 1978)

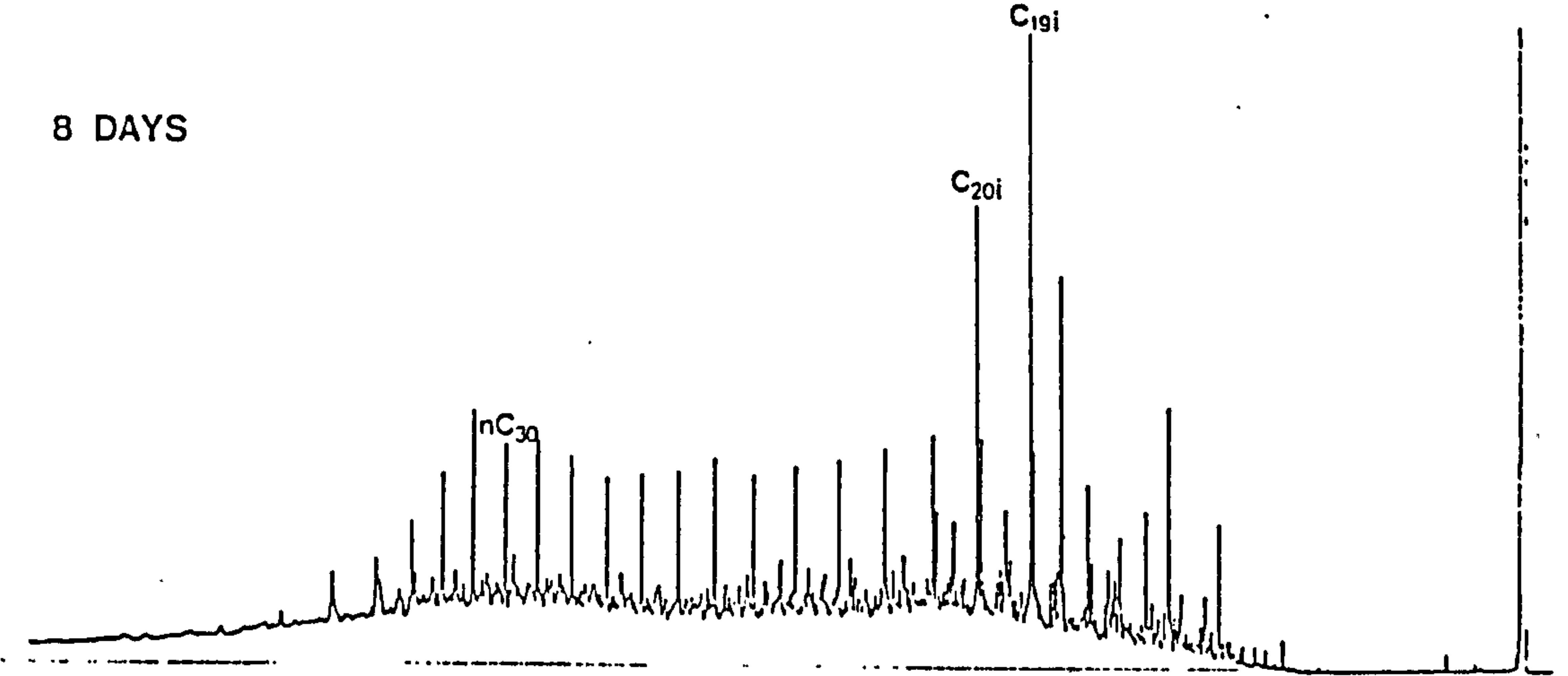
(B) In reservoir degraded crude oil (Venezuela)

(C) Lubricating oil

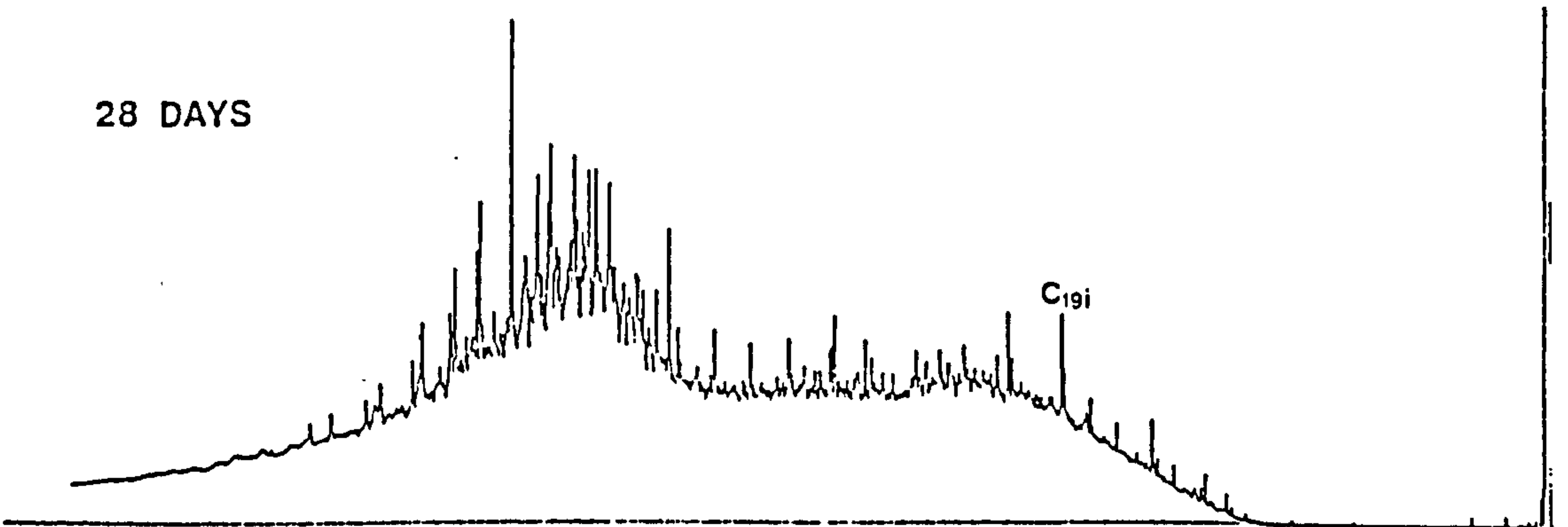
135 DAYS  
Sterilised



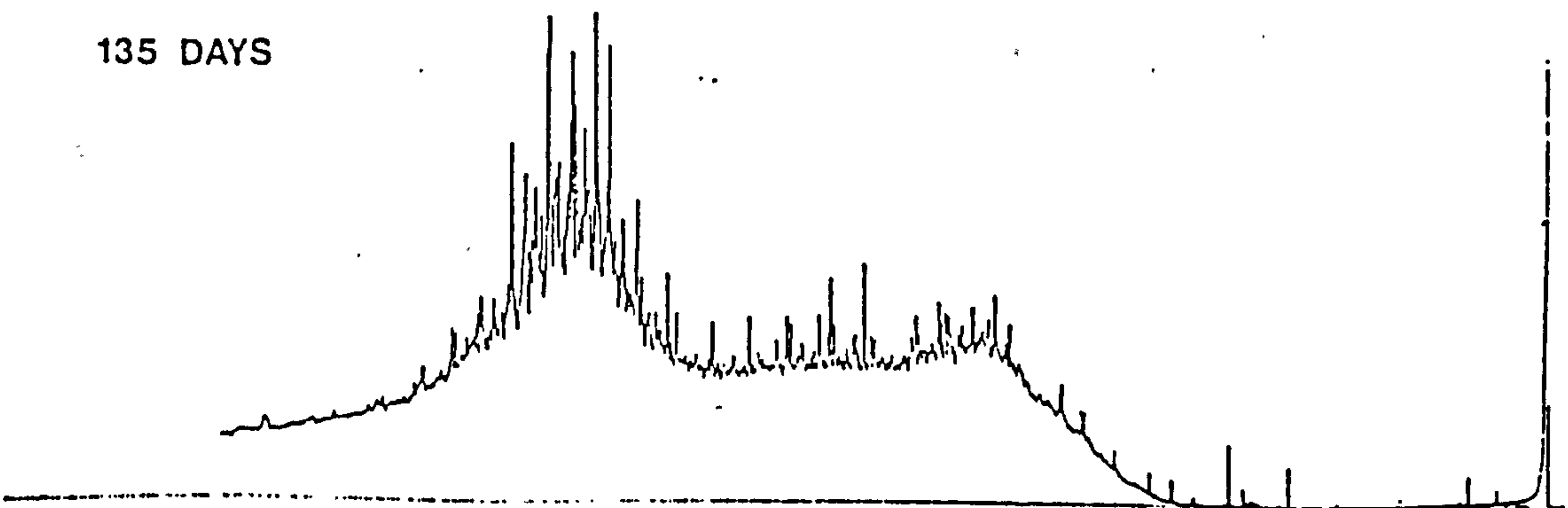
8 DAYS



28 DAYS



135 DAYS



← TIME

FIGURE 1.2. CRUDE OIL BIODEGRADATION SEQUENCE (Jones, 1986)



TABLE 1.1 REPORTED OCCURRENCES OF HYDROCARBON UCMs IN RECENT SEDIMENTS (Adapted from Gough, 1989)

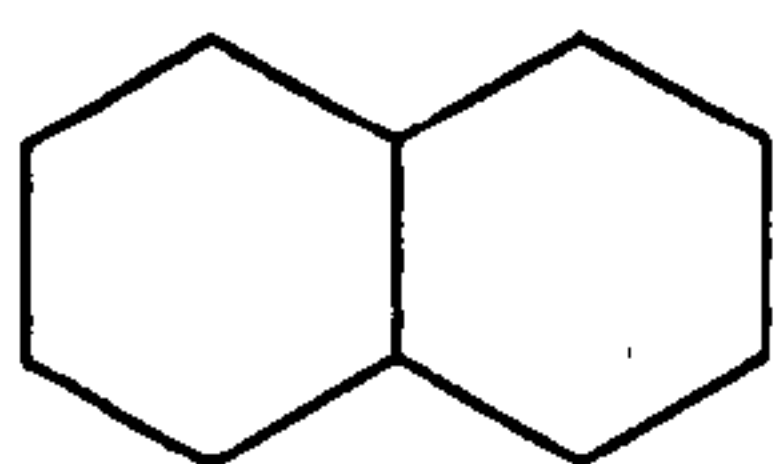
REGION	SAMPLE TYPE	[UCM] #g <sup>-1</sup>	% UCM	FRACTION ANALYSED	CARBON RANGE (KI UNITS)	CARBON MAXIMUM (KI UNITS)	APPEARANCE	INFERRED ORIGIN	PROPOSED COMPOSITION	REFERENCE
NARRAGANSETT BAY, USA	Sediment Core	343 #g <sup>-1</sup>	96	Total HC	1400-3500+	2700	Unimodal	Anthropogenic	Cyclic Alkanes, Aromatics	Hurtt and Quinn, 1979
PUGET SOUND, USA	Surface Sediment	3700 #g <sup>-1</sup>	68	AHC	1600-3400	2600	Unimodal	Fossil Fuels - Urban Run Off	Branched/Cyclic Hydrocarbons	Barrick et al., 1980
PORT VALDEZ, Alaska	Surface Sediment	1-210 #g <sup>-1</sup>	84	Total HC	1200-3200	2300	Unimodal	Petroleum		Shaw et al., 1985
SARAH CREEK, USA	Surface	79 #g <sup>-1</sup>	85	AHC	1600-3200	2650	Unimodal	Lube Oils and Pyrolysis Products	Cycloalkanes	Voudrias and Smith, 1986
STOCKHOLM ARCHIPELAGO	Suspended particles	1264 #g <sup>-1</sup>	96	AHC	1200-3500+	2050, 2650	Bimodal	Petroleum		Broman et al., 1987
RICHARDS BAY, S. AFRICA	Surface micro-layer	1350 #g <sup>-1</sup>	84	AHC	1500-3200	2800	Unimodal	Petroleum	Branched/Cyclic	Butler and Sibbald, 1987
BALTIC SEA	Sediment Core	90 #g <sup>-1</sup>	70	AHC	1400-3400	2300	Unimodal	Petroleum		Pihlaja et al., 1990
YANGTSE RIVER	Suspended Sediment	3 #g <sup>-1</sup>	71	AHC	1700-3500+	2200+3000	Bimodal	Anthropogenic		Qui et al., 1991
KUWAIT	Surface Sediment	30 #g <sup>-1</sup>	91	AHC	1300-3500+	2200+3000	Bimodal	Petroleum		Morel et al., 1991

AHC = ALIPHATIC HYDROCARBONS

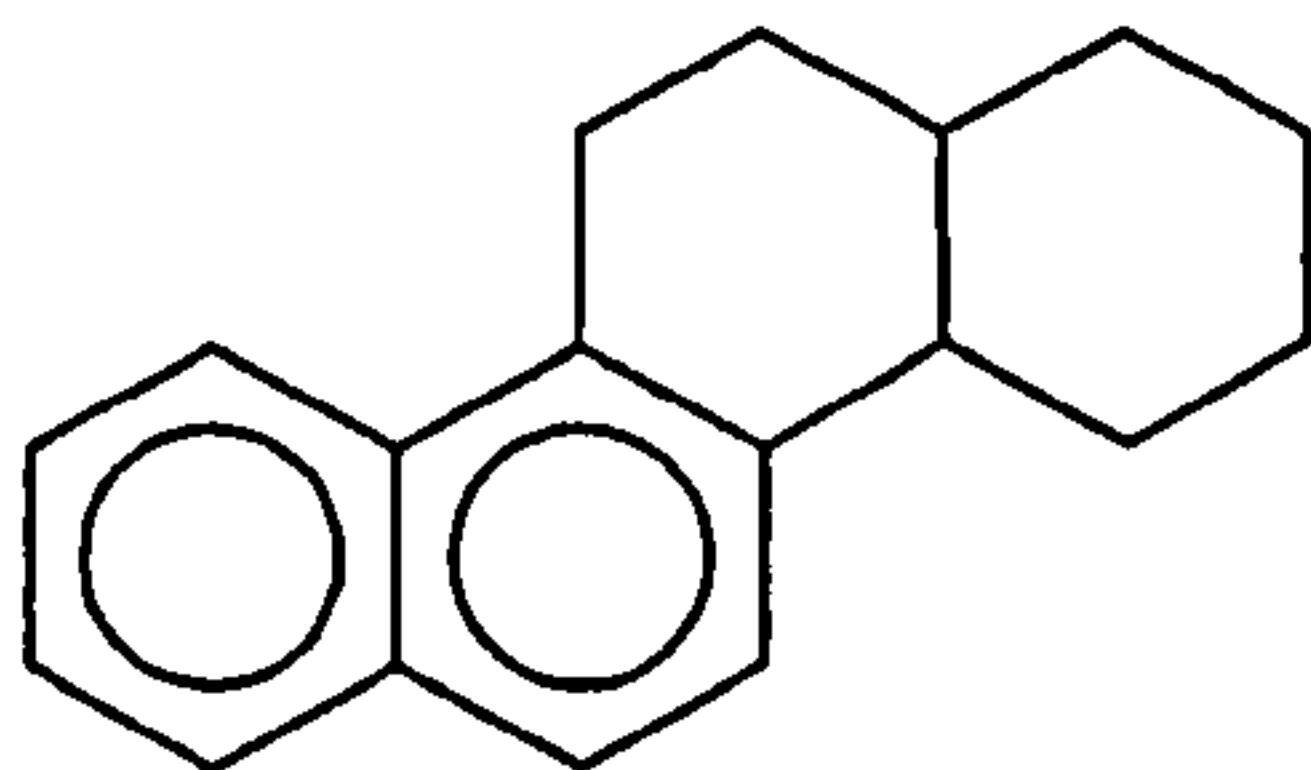
mixtures has scarcely been utilised. It is the purpose of this introduction to summarise our knowledge of UCMs. The subject area has been extensively reviewed by Gough (1989), so the present account merely emphasises reports made since 1989.

## 1.2 UCM COMPOSITION

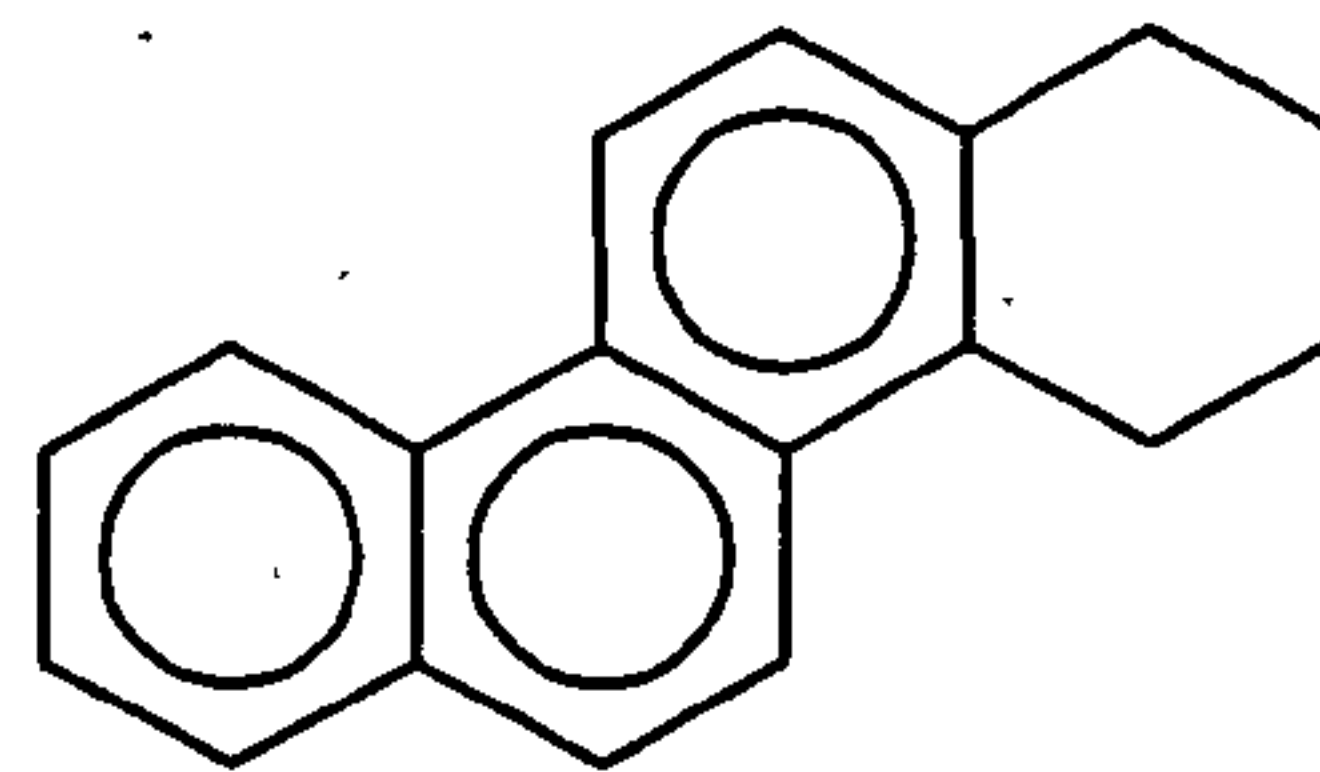
Until relatively recently, little was known about UCM composition. Early research (eg. Rossini *et al.*, 1953) established typical elemental compositions and physical properties for UCMs from lubricating oils and suggested that they mainly comprised one to three ring cycloalkanes (e.g. I), dinaphthenonaphthalenes (II) and naphthenophenanthrenes (III).



I



II



III

Later, EI-MS studies (eg. Clerc *et al.*, 1955; Hood and O'Neal, 1959) confirmed these average structures. In most later studies UCM composition has not been assigned but simply referred to as a mixture of branched/cyclic compounds (Table 1.1).

Recently, the early studies have been complemented by a series of reports which have utilised chromic acid

oxidation of the unresolved hydrocarbons to yield resolved, functionalised compounds (Gough, 1989; Gough and Rowland, 1990; Killops and Al-Juboori, 1990; Gough and Rowland, 1991). Although UCMs occur in both aliphatic and aromatic hydrocarbon fractions, most of the present research has concentrated on aliphatic UCM composition.

### 1.2.1 THE ALIPHATIC UCM

Early mass spectrometric analyses suggested that aliphatic UCMs consist of branched alkanes and alkylcyclohexanes, with as many as three condensed cyclohexyl rings (reviewed by Gough, 1989; Table 1.2). Somewhat surprisingly, therefore, chromic acid oxidation of an aliphatic UCM from a lubricating oil examined by Gough (1989), yielded resolved components which were predominantly *n*-carboxylic acids and relatively few cyclic products. The absence of cyclics may have been due to ring opening during oxidation (Gough and Rowland, 1990) or the cyclic products may still have been unresolved by GC (Gough and Rowland, 1991). Killops and Al-Juboori (1990) subjected a heavily biodegraded Alaskan crude oil (total hydrocarbons), to a variety of chemical, spectroscopic and spectrometric techniques. Elemental and CI-MS analyses suggested a predominance of aliphatic hydrocarbons, of which acyclic (30%), mono- (40%) and di- (15%) cyclic compounds were abundant. Chromic acid oxidation of this sample produced low yields (contrasting with those obtained by Gough and

**TABLE 1.2: RESULTS OF SEVERAL GROUP TYPE ANALYSES OF UCM ALIPHATIC COMPOUNDS (ADOPTED FROM GOUGH, 1989; SEE SOURCE FOR REFERENCES)**

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
Pelican Oil* (Western Canada oil sand)	/	n.d.	19	17	22	19	17	7	2	-
Emeraude Field* (West Africa)	/	n.d.	11	13	21	22	18	11	4	-
Biodegraded Kuwait crude oil	/	n.d.	26	22	17	14	10	6	5	-

\* : data estimated from histograms

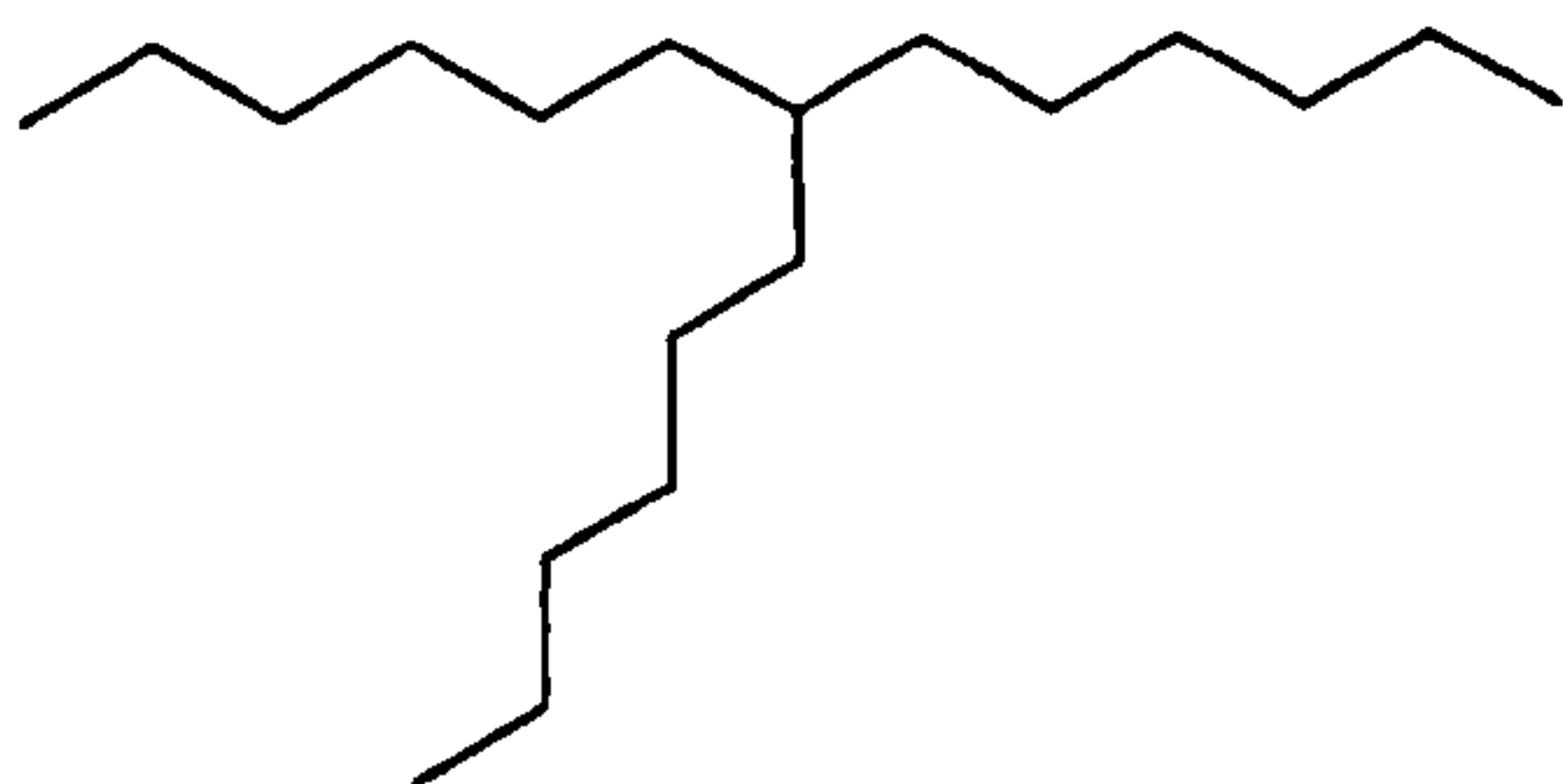
n.d.: not observed in the gas chromatogram

MA : monoaromatic hydrocarbons

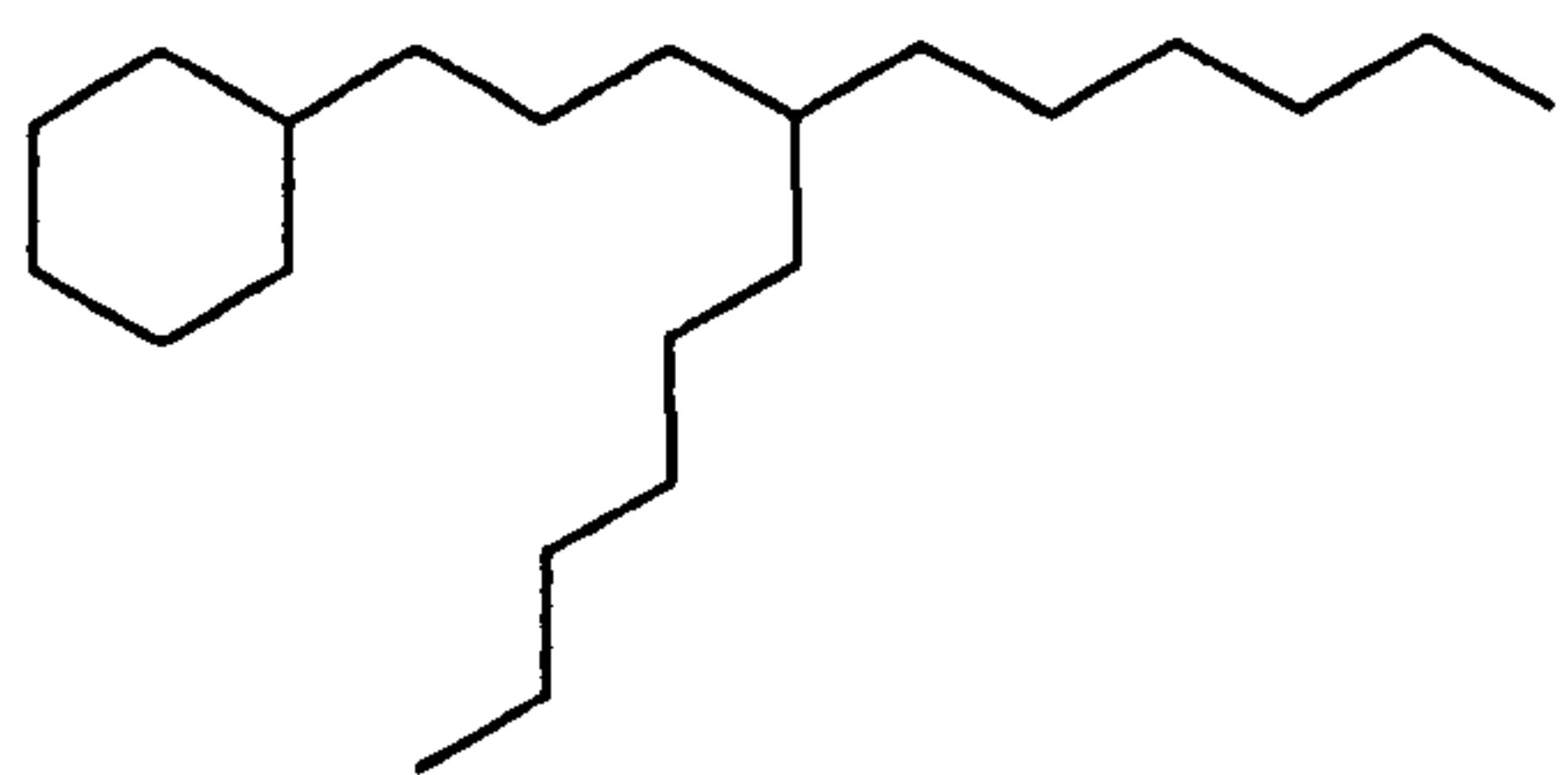


Rowland, 1990), but the qualitative data was comparable to the earlier studies (Gough, 1989; Gough and Rowland, 1990), with *n*-carboxylic acids forming the major products and  $\alpha,\omega$ -dicarboxylic acids and monocycloalkanoic acids as minor products.

Killops and Al-Juboori (1990) suggested that the UCM contained an average aliphatic component with ca. 20 carbons and 2.5 rings. They also suggested that alkyl chains up to C<sub>19</sub> are present. In agreement with this, Gough (1989) and Gough and Rowland (1991) suggested that such *n*-carboxylic acids are derived from simple branched alkanes (e.g. IV) which may have cyclohexyl rings attached (e.g. V).



IV



V

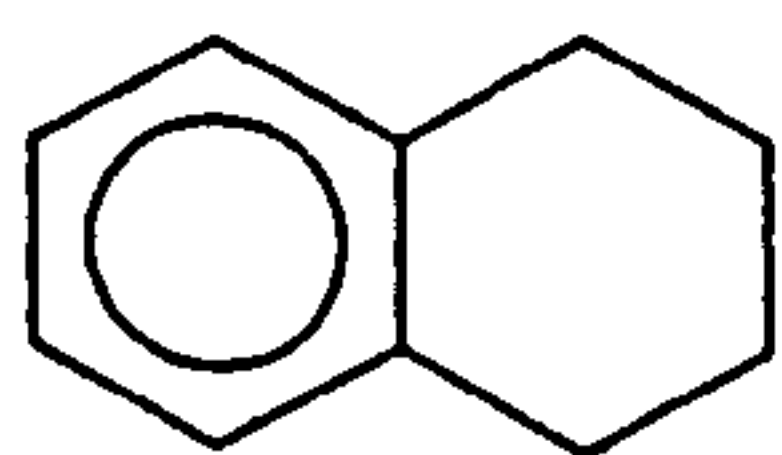
For C<sub>20-30</sub> compounds of type IV, Gough and Rowland (1990) point out that 536 isomeric structures are possible (allowing for alkyl chain lengths from C<sub>1</sub> to C<sub>6</sub>), a mixture which would probably be unresolved by GC.

Further evidence for these types of compounds was given by biodegradation studies (Gough et al., 1991). Using a

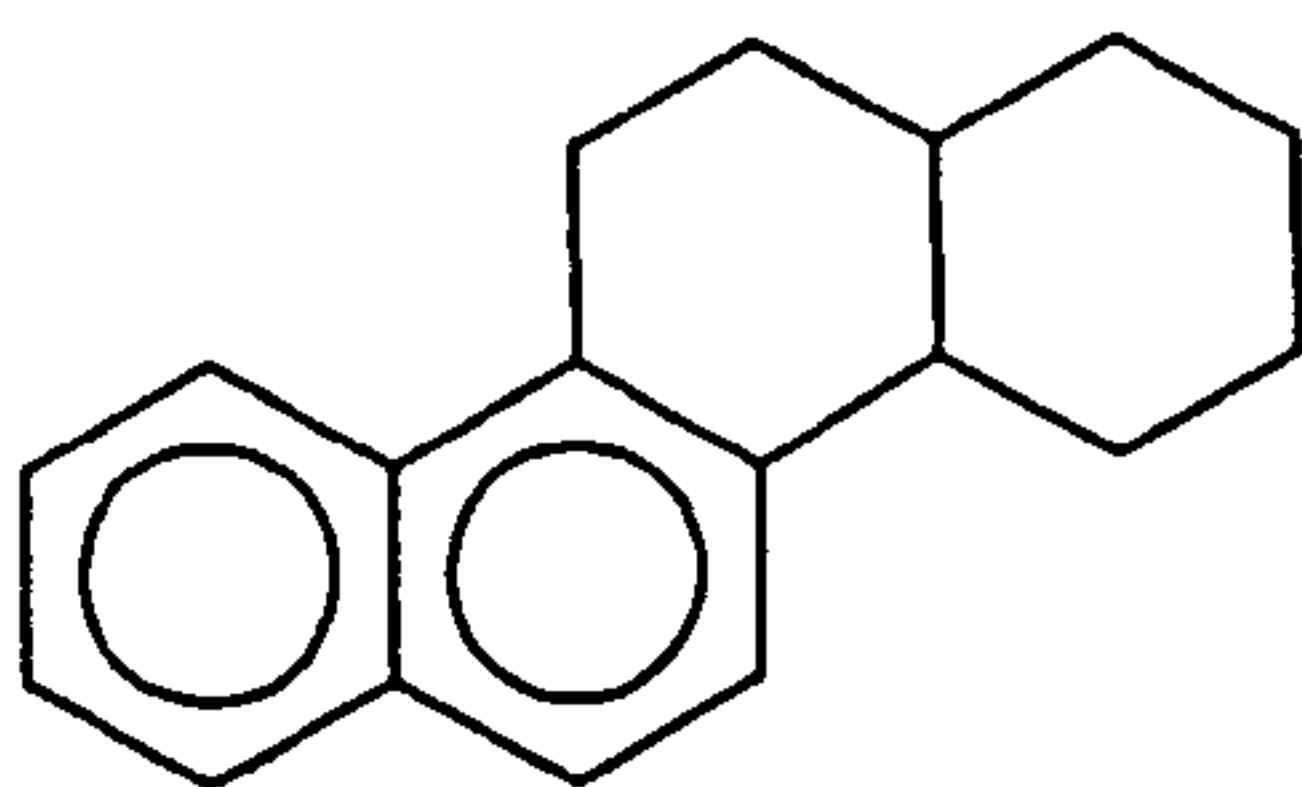
mono-culture of *Pseudomonas fluorescens* (a known hydrocarbon degrader), the authors showed that compounds of types IV and V exhibited a degree of resistance analogous to that of the UCM (Figure 1.3a). The UCM degradation profile was very similar to that seen in a tropical ecosystem when spilled oil was degraded (Oudot and Dutrieux, 1989; Figure 1.3b). This suggests that these structures proposed by Gough and Rowland (1991) are a reasonable model for some aliphatic UCM components.

### 1.2.2 THE AROMATIC UCM

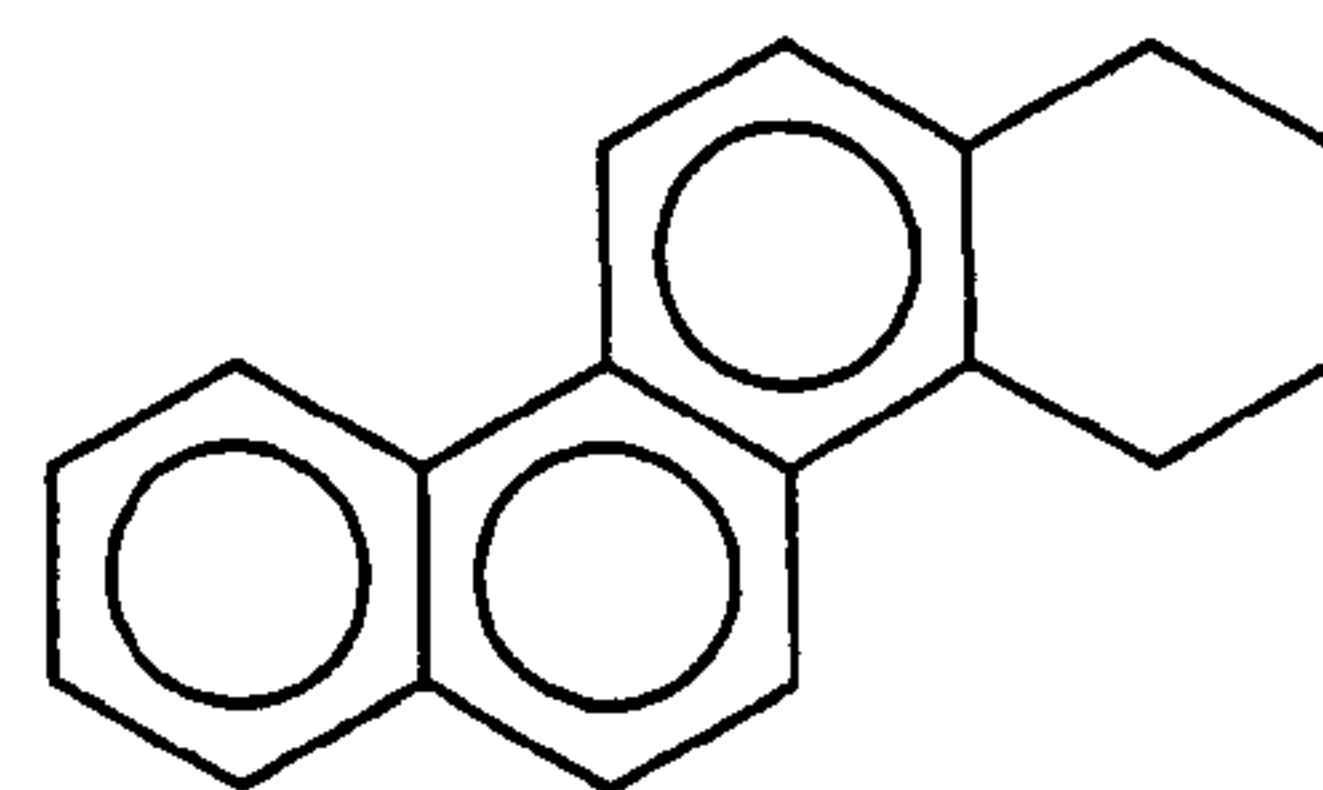
Early studies of the hydrocarbons in the lubricant fraction of crude oil (Rossini *et al.*, 1953) suggested that the major aromatic compounds present were mono nuclear aromatics with one to three cycloparaffin rings (e.g. VI) (37% of aromatics), dinuclear aromatics condensed with one to two cycloparaffin rings (e.g. VII) (34%) and trinuclear aromatics condensed with one to two aliphatic rings (e.g. VIII) (29%).



VI



VII

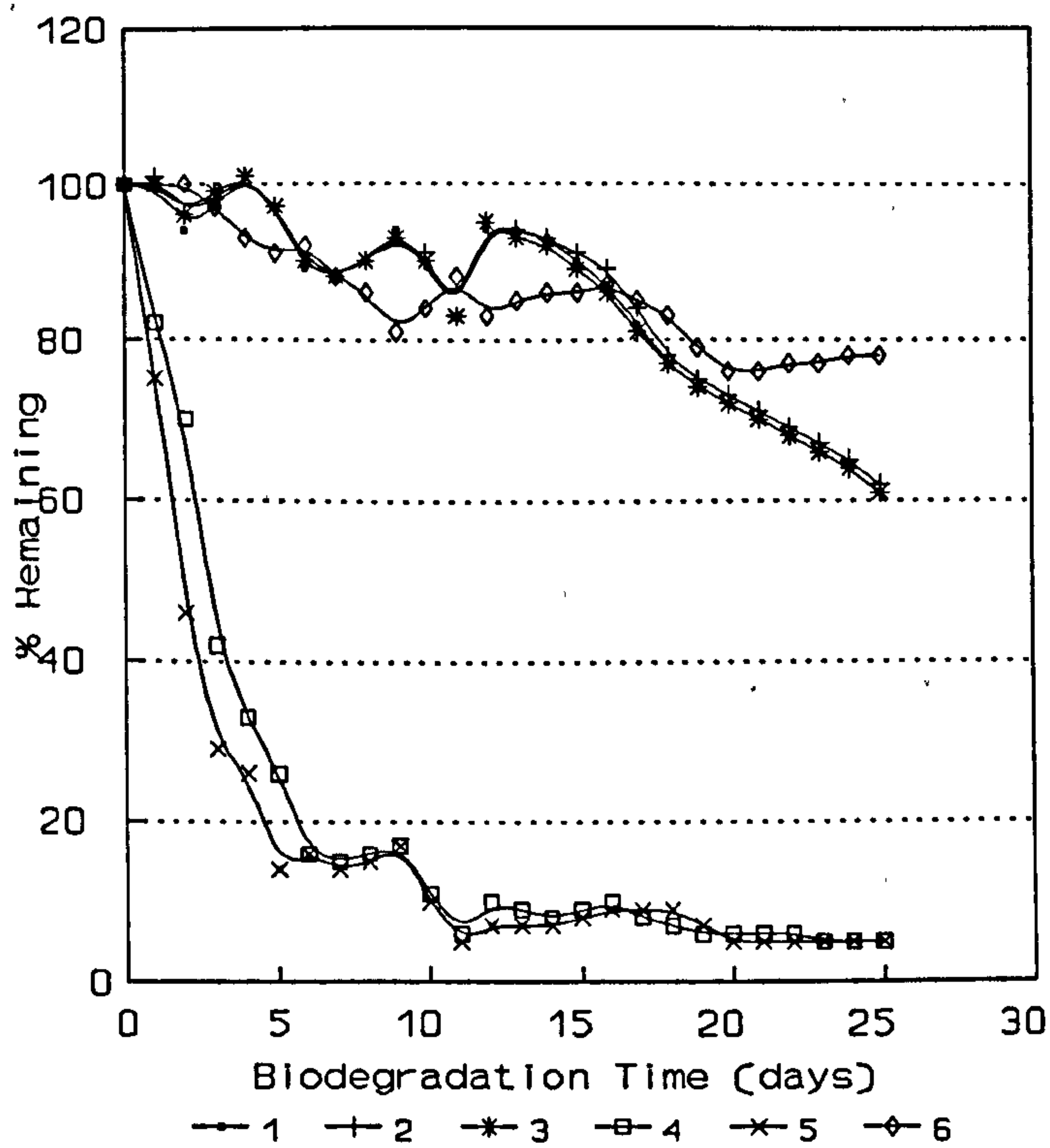


VIII

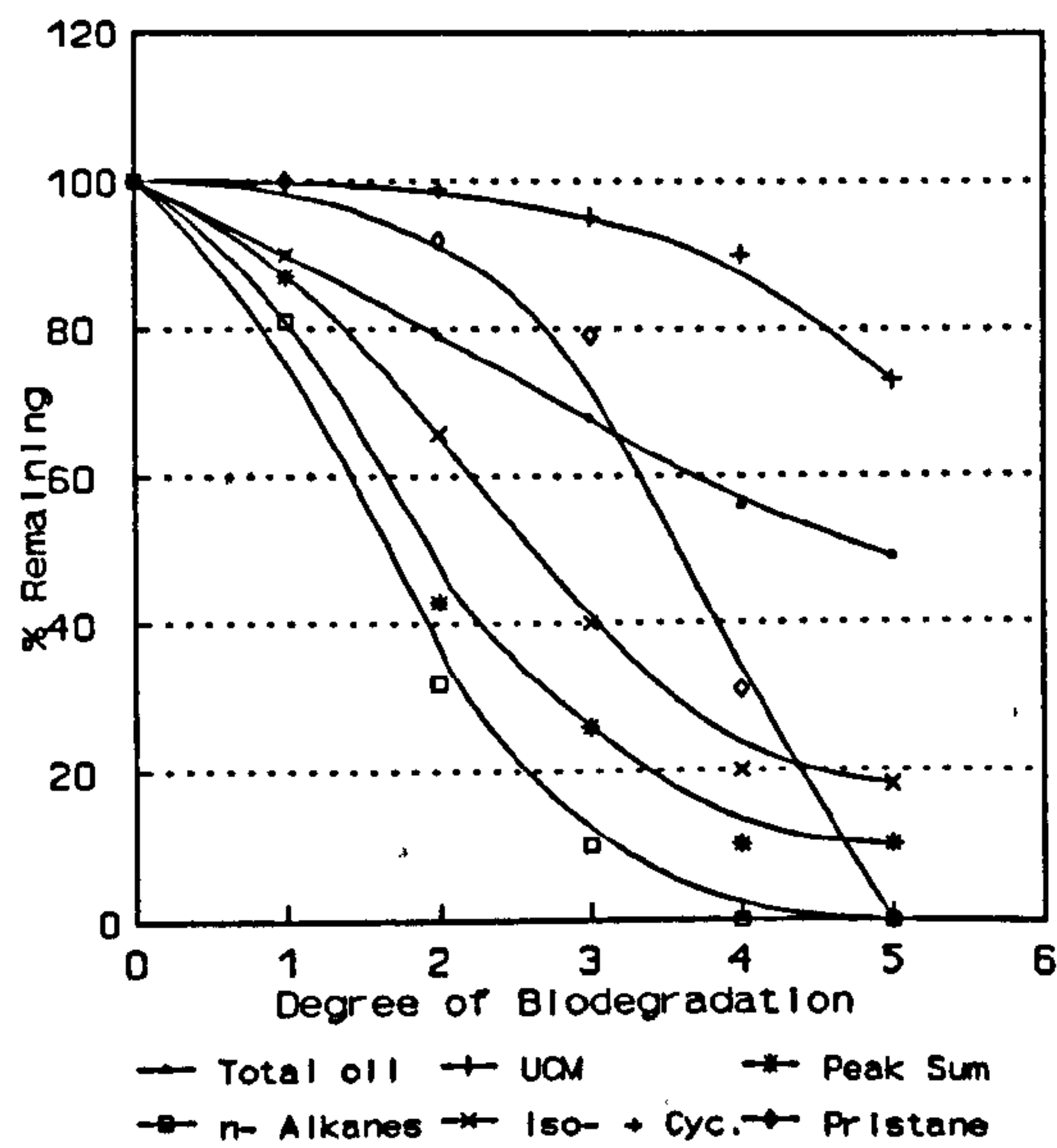
Farcasiu and Rubin (1987) examined the "heavy end" of petroleum using spectroscopic and dehydrogenation methods

KEY TO COMPOUNDS IN FIGURE 1.3a

Symbol number	Compound
1	7- <i>n</i> -hexylnonadecane
2	9-(2cyclohexylethyl)heptadecane
3	9-(2-phenylethyl)heptadecane
4	9-methyltetracosane
5	<i>n</i> -pentacosane
6	UCM



**FIGURE 1.3a. BIODEGRADATION OF SELECTED COMPOUNDS AND THE UCM. (Adapted from Gough et al. 1991)**  
 (For key to compounds see opposite)



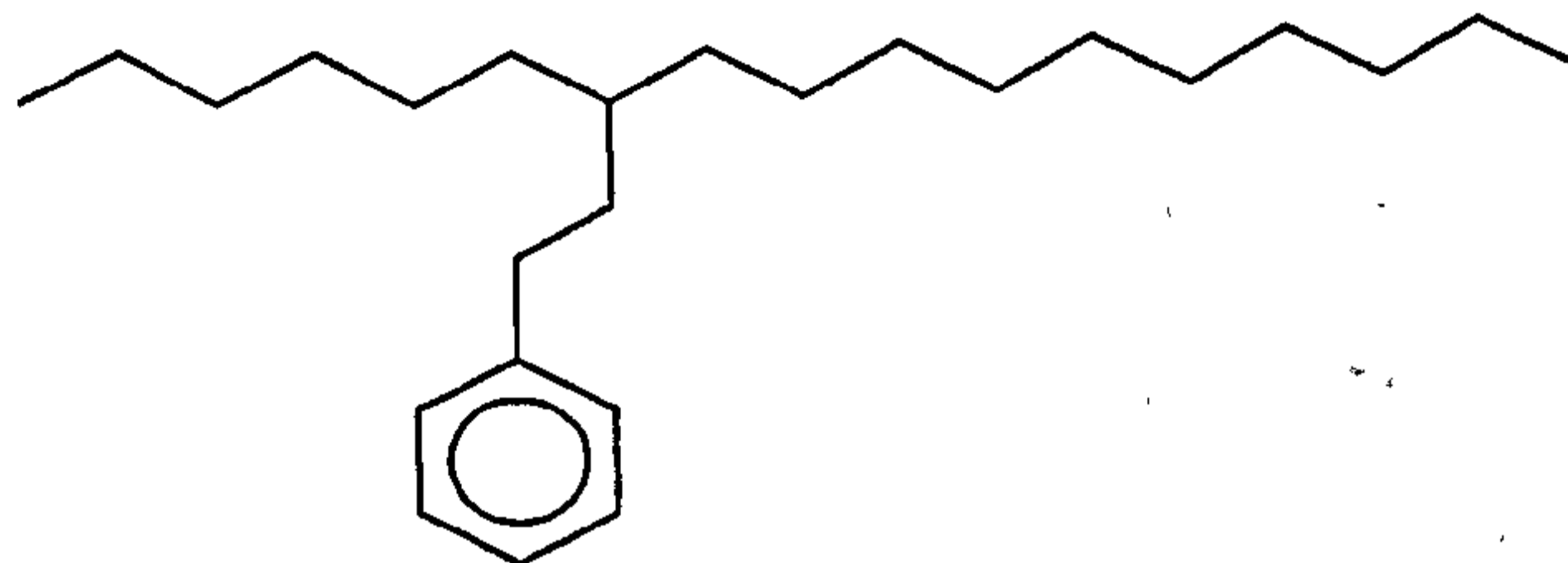
**FIGURE 1.3b. LEVELS REMAINING AT VARIOUS STAGES OF BIODEGRADATION IN A TROPICAL ECOSYSTEM. (Adapted from Oudot and Dutrieux, 1989)**



in an attempt to characterise the aromatic hydrocarbons. An average structure of a monoaromatic with an aliphatic side chain consisting of two to four saturated rings was suggested.

Gough (1989) used EIMS, GPC and FIMS to analyse the aromatic fraction of a lubricating oil, which indicated that the major compound classes were alkylbenzenes, alkyl-naphthalenes, alkyl-tetralins and other combinations of aromatic and saturated ring systems. Killops and Al-Juboori (1990), again working on a biodegraded Alaskan crude oil (total hydrocarbons), also utilised a variety of techniques to examine the aromatic UCM compounds. However, the only structural inferences made were that aliphatic cyclic moieties were important in the aromatic fraction and that there may have been 0 to 3.5 aliphatic rings associated with each aromatic structure. The lack of specific information may be partly due to the use of a sample with only ca. 10% aromatic content. Similarly Gough (1989) analysed the aromatic UCM of lubricating oils which are anomalously low in aromatic compounds due to refining. The only obvious aromatic products from these studies were alkylbenzoic acids indicating an average of two to three alkyl substituents per ring. The chromic acid oxidation performed by Gough (1989) produced similar products to those of the aliphatic UCM (namely *n*-carboxylic acids with  $\alpha,\omega$ -dicarboxylic acids,  $\gamma$ -lactones and ketones as minor products). These results, in conjunction with the

instrumental analyses, led Gough (1989) to propose an average structure of a monoaromatic compound, substituted with an aliphatic component similar to those proposed for the aliphatic UCM (e.g. IX).



IX

This type of structure could, as shown for the aliphatic UCM, generate sufficient numbers of compounds to produce an unresolvable mixture by GC. This model was supported by biodegradation studies (Gough et al., 1991) utilising cultures of *Pseudomonas fluorescens*. Compounds similar to IX (e.g. 9-(2-phenylethyl)heptadecane) exhibited a degree of resistance comparable to the UCM (Figure 1.3a; compound 3) suggesting that this was a viable model for some aromatic UCM compounds. However, this compound is lacking in the saturated cyclic moiety suggested by previous research (e.g. Rossini et al., 1953; Farcasiu and Rubin, 1987), which suggests that while it is a potential model compound, it is not entirely correct.

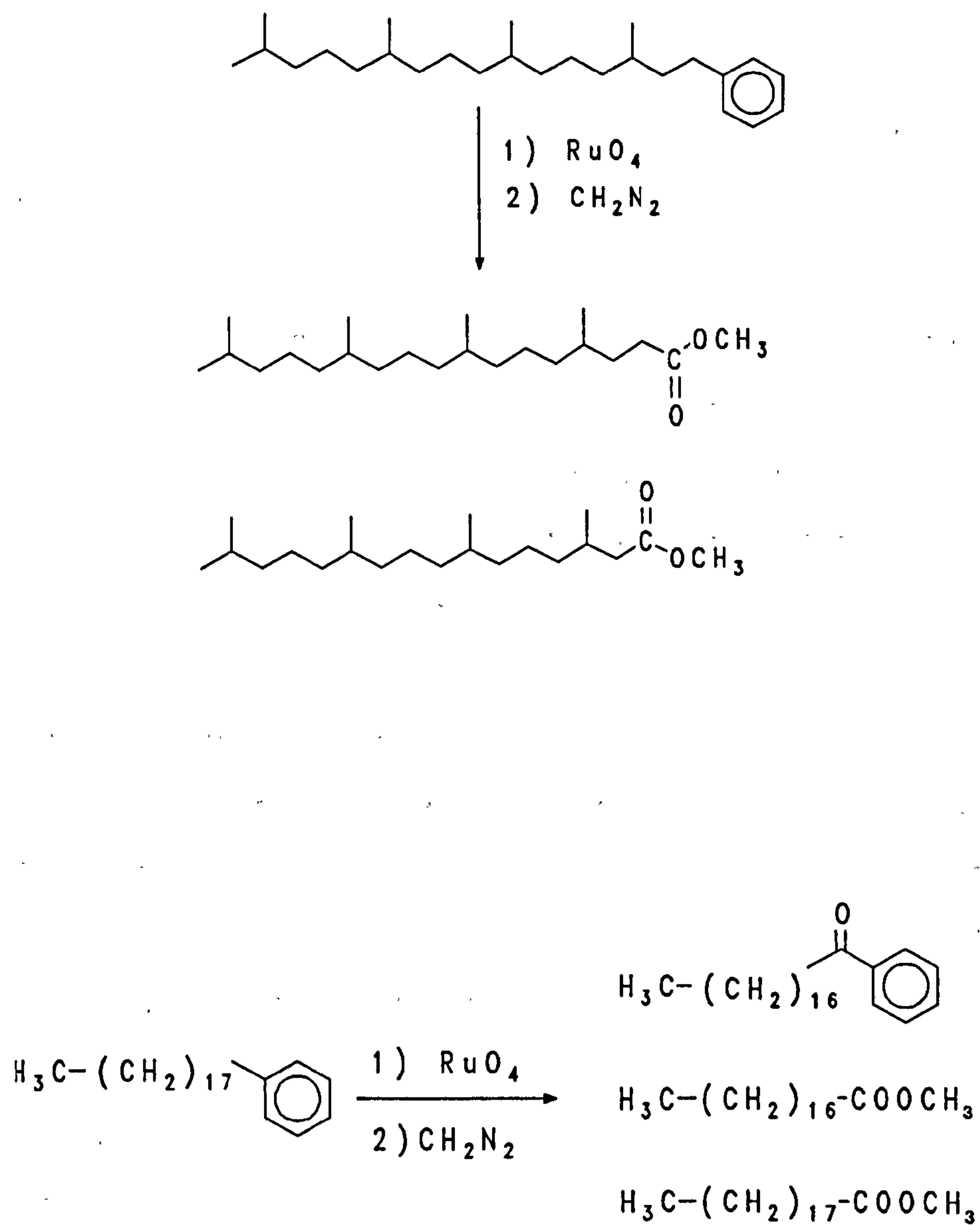
Gough (1989) suggested the use of more selective oxidants to provide further information, since chromic acid, while preferentially oxidising benzylic carbons, also oxidises tertiary carbons in the side chain, thus destroying the

most important structural feature of the compound. Ruthenium tetroxide ( $\text{RuO}_4$ ), which was first used as an oxidant by Djerassi and Engle (1953) to produce sulfoxides from sulfides, has been finding increasing popularity in a variety of oxidative applications. The methodology, particularly in the use of solvents and co-oxidants, has undergone several modifications from the systems used initially (e.g. Oberender and Dixon, 1959; Nakata, 1963 and Sharpless *et al.*, 1981) to those used by Stock and Tse (1983), Trifilieff (1987) and Boucher *et al.*, (1990). The advantages of this oxidant are the low quantities required (due to the use of a co-oxidant) and the specificity for carbon-carbon double bonds and aromatic rings, leaving the aliphatic and alicyclic moieties intact (Figure 1.4). Ideally, one of the advantages of more detailed structural analysis of the UCM hydrocarbons (both aliphatic and aromatic) would be a "retro-analysis" to determine possible UCM precursors.

### 1.3 ORIGINS OF UCMS

$^{14}\text{C}$ -dating of UCMS isolated from sediments (reviewed by Gough, 1989) gave values of 15000-26000 years B.P., effectively discounting, for these cases, recent biosynthetic sources. This is also consistent with the co-occurrence of UCMS and "mature" biomarker profiles (Eglinton and Calvin, 1967; Volkman and Maxwell, 1986). In a recent study of hydrocarbons in Baltic sediments,



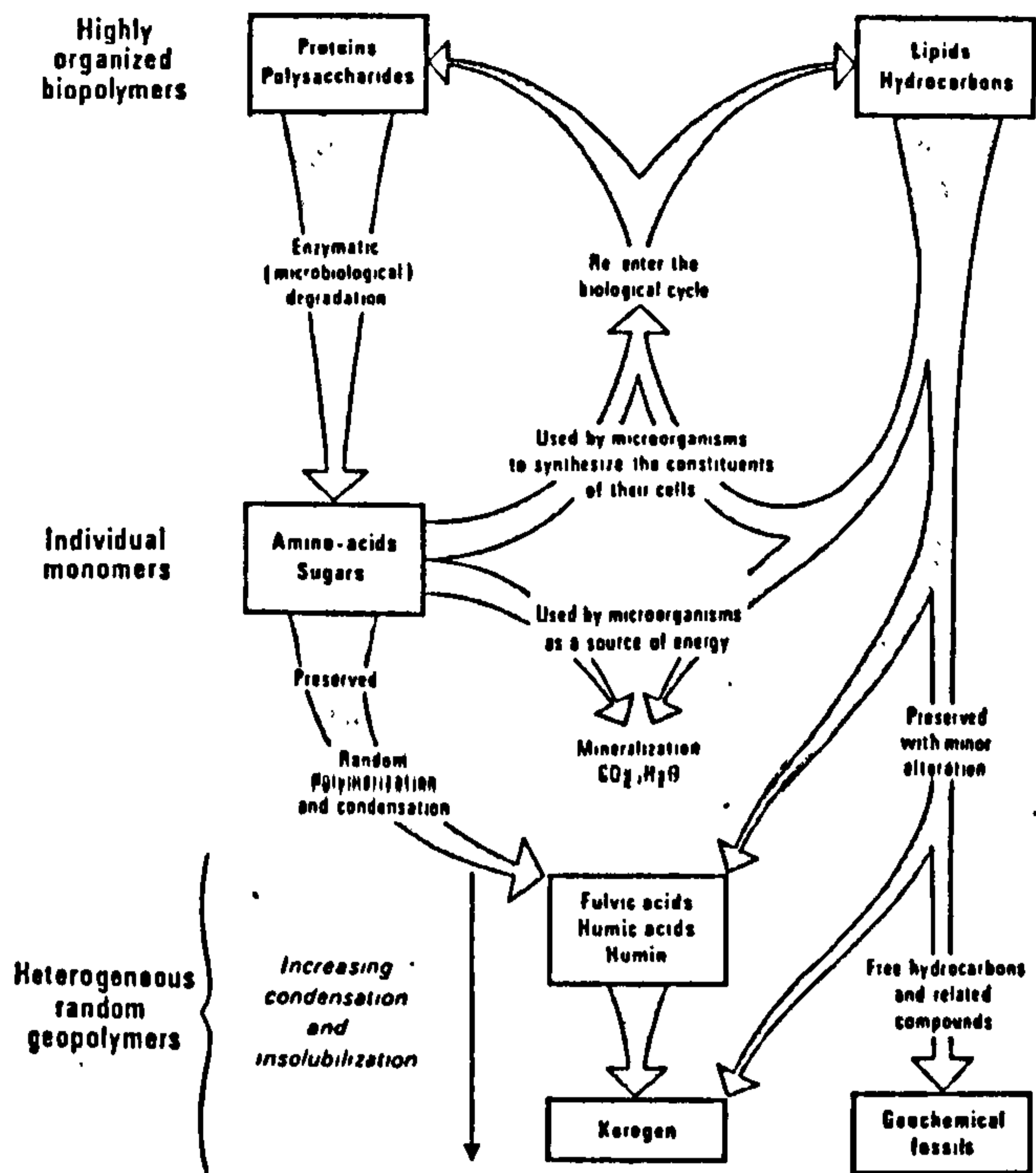


**FIGURE 1.4. OXIDATION OF AROMATIC COMPOUNDS BY RUTHENIUM TETROXIDE. (Adapted from Stock and Tse, 1983 and Trifilief, 1987)**

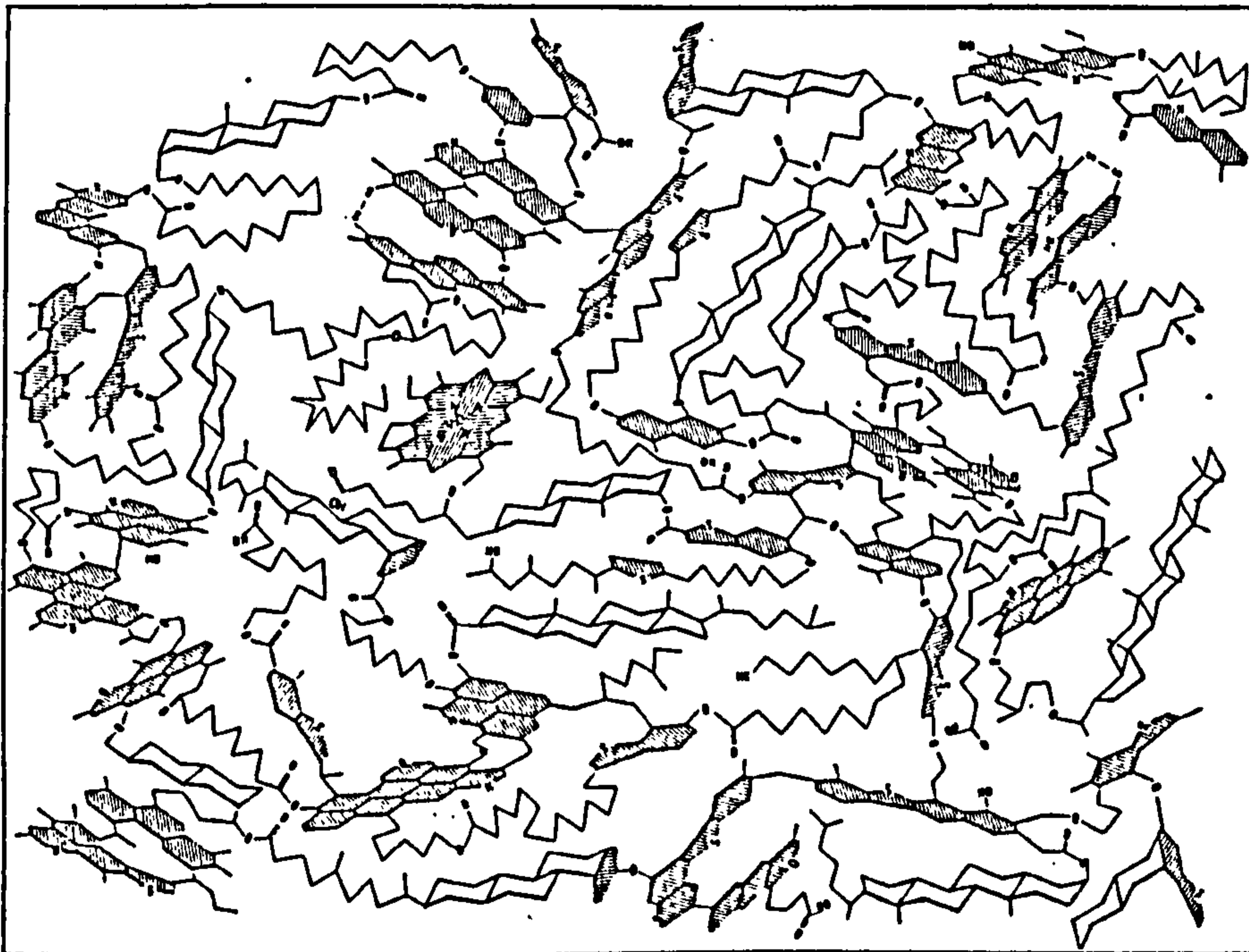


variations in UCM concentration closely paralleled those of the petrogenic alkanes (Pihlaja et al., 1990) providing further evidence of a petrogenic origin. In only a few cases have non-petrogenic UCMs been reported (Gough, 1989). These have very narrow carbon number ranges (eg. (C<sub>16</sub> - C<sub>22</sub>) compared with typical petrogenic UCMs (C<sub>14</sub> - C<sub>34</sub>) and have generally been attributed to microbial re-working of organic matter (Venkatesan and Kaplan, 1982, Grimalt et al., 1988). Since UCMs are generally accepted to be petrogenic in origin, it is reasonable to assume that the UCM precursor is kerogen. There are essentially two current theories of kerogen formation:

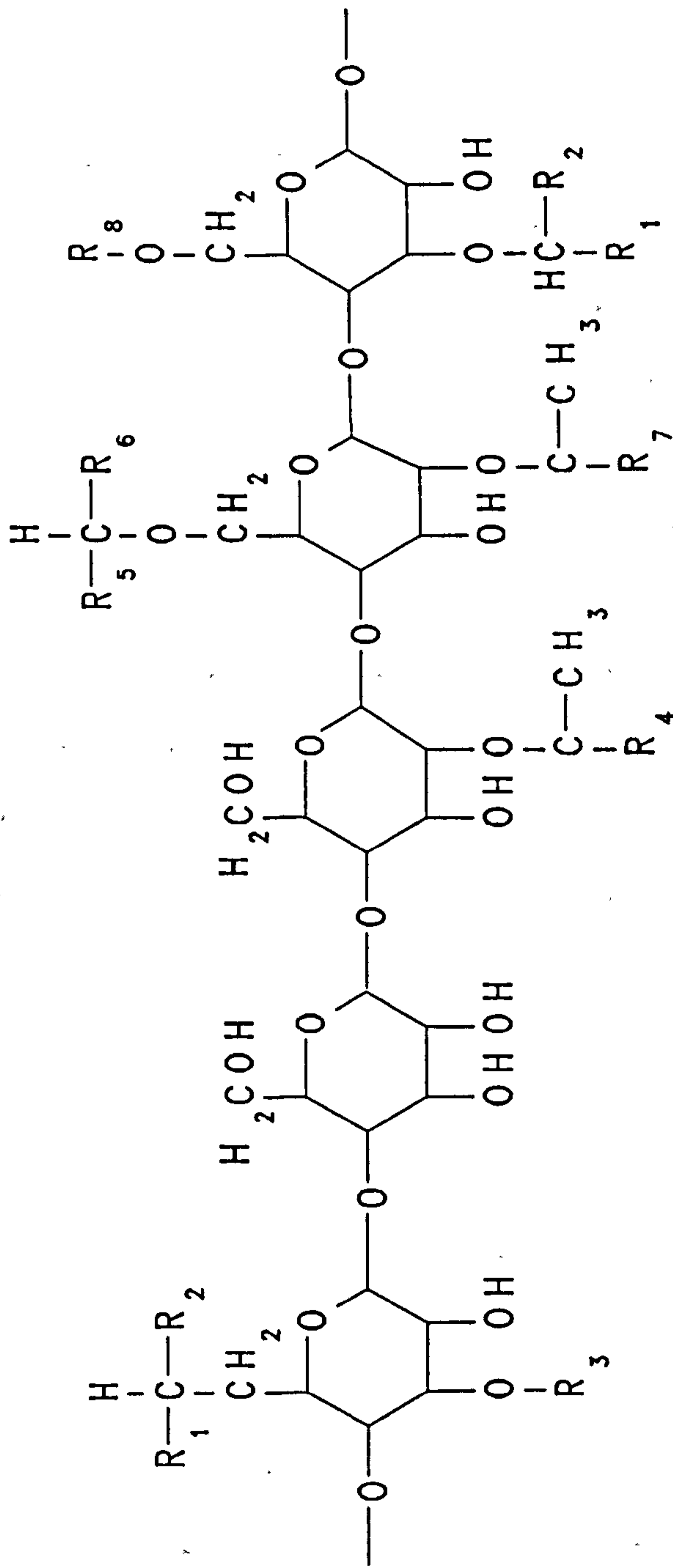
- (1) Kerogen results from the degradation of biochemical macromolecules into individual sub-units, followed by polycondensation and insolubilisation (Tissot and Welte, 1984; Figure 1.5a). The resulting structure is often represented as having aliphatic and aromatic rings with aliphatic side chains, cross linked by functional groups (Tissot and Welte, 1984; Behar and Vandembroucke, 1987; Figure 1.5b).
- (2) Philp and Calvin (1976) suggested that kerogen may be formed from insoluble, selectively preserved residues from algae and bacteria. This alternative view of kerogen formation has been recently re-investigated by several workers. Nip et al. (1986a, 1986b) identified the presence of a highly aliphatic



**FIGURE 1.5a. "CLASSICAL" PATHWAY TO KEROGEN FORMATION (Tissot and Welte, 1984)**



**FIGURE 1.5b. TRADITIONAL VIEW OF KEROGEN STRUCTURE (Behar and Vandenbroucke, 1987)**



**FIGURE 1.6. PROPOSED STRUCTURE OF THE HIGHLY ALIPHATIC BIOPOLYMER FROM AGAVE AMERICANA (Tegelaar et al. 1989a)**

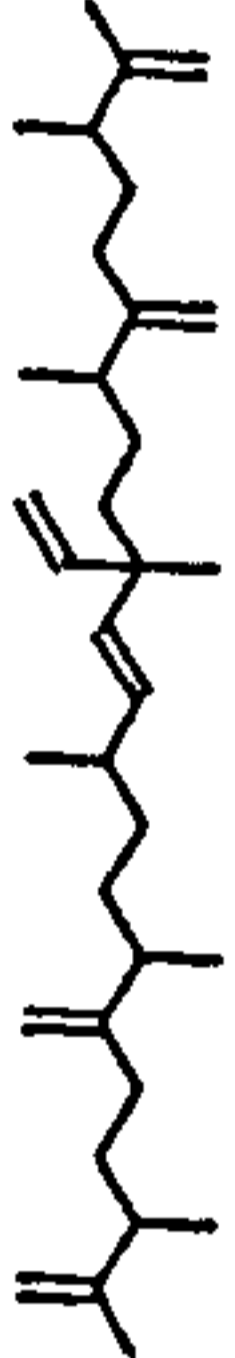



biopolymer in extant and fossil plant cuticles and suggested that this new biopolymer exhibited a greater degree of preservation over geological time than cutin and cuticle waxes. Further studies (Nip *et al.*, 1987; Nip *et al.*, 1988; Derenne *et al.*, 1988; Goth *et al.*, 1988; Nip *et al.*, 1989; Derenne *et al.*, 1991) have identified resistant biopolymers in a variety of plant cuticles, coals, torbanites and oil shales. Tegelaar *et al.* (1989a) proposed a tentative structure (Figure 1.6) for the biopolymer from the cuticle of *Agave americana*, though this is currently under review (De Leeuw, Personal Communication 1991). A variety of biopolymers in extant organisms have been identified (Tegelaar *et al.*, 1989b; Table 1.3) and this has led to a new mechanism of kerogen formation being proposed (Tegelaar *et al.*, 1989c; Figure 1.7) based on the preservation potentials of many biomacromolecules (Table 1.4).

If such kerogen models are to be accepted, the effects of diagenesis and catagenesis on these macromolecules have to result in the formation of crude oils with an average composition of 57% saturated hydrocarbons and 29% aromatic hydrocarbons (based on 500 oils, Tissot and Welte, 1984). Both the saturated and aromatic components include UCMs, yet the presence of the UCM in the theories of kerogen formation appears to have been ignored. For oils which contain saturated and aromatic UCMs in significant proportions, precursor kerogens must contain suitable



TABLE 1.3. THE OCCURRENCE OF INSOLUBLE, ALIPHATIC BIOPOLYMERS IN EXTANT ORGANISMS  
(Tegelaar et al. 1989b)

ORGANISM	MAJOR LIPIDS	CELL WALL BIOPOLYMER
<i>Botryococcus braunii</i> (Race A)	$\text{CH}_3-(\text{CH}_2)_7-\text{CH}=\text{CH}-(\text{CH}_2)_n-\text{CH}=\text{CH}_2$ $\text{CH}_3-(\text{CH}_2)_5(-\text{CH}=\text{CH})_2-(\text{CH}_2)_n-\text{CH}=\text{CH}_2$ $n=13, 15, 17, 19 / \text{C}_{25}-\text{C}_{31}$	"PRB A" $-\text{O}-(\text{CH}_2)_n-\text{O}-(\text{CH}_2)_m-\text{O}-$ (cycloalkyl units)
<i>B. braunii</i> (Race B)	 <p>e.g. "Botryococcenes"</p>	"PRB B" Identical to "PRB A"
<i>B. braunii</i> (Race L)	 <p>"lycopadiene"</p>	"PRB L" "polylycopadiene"
<i>Tetraedron minimum</i>	-	$-\text{O}-(\text{CH}_2)_n-\text{O}-(\text{CH}_2)_m-\text{O}-$ (no cycloalkyl units)
<i>Scenedesmus obliquus</i>	-	$-(\text{CH}_2)_n-$ moieties
<i>Chlorella pyrenoidosa</i>	-	$-(\text{CH}_2)_n-$ moieties
<i>Dunaliella tertiolecta</i>	-	$-(\text{CH}_2)_n-$ moieties
Plant Cuticles	Waxes	$-(\text{CH}_2)_n-$ polysacch. moieties / Cutins
Plant Periderms	Waxes	$-(\text{CH}_2)_n-$ moieties/ Suberins

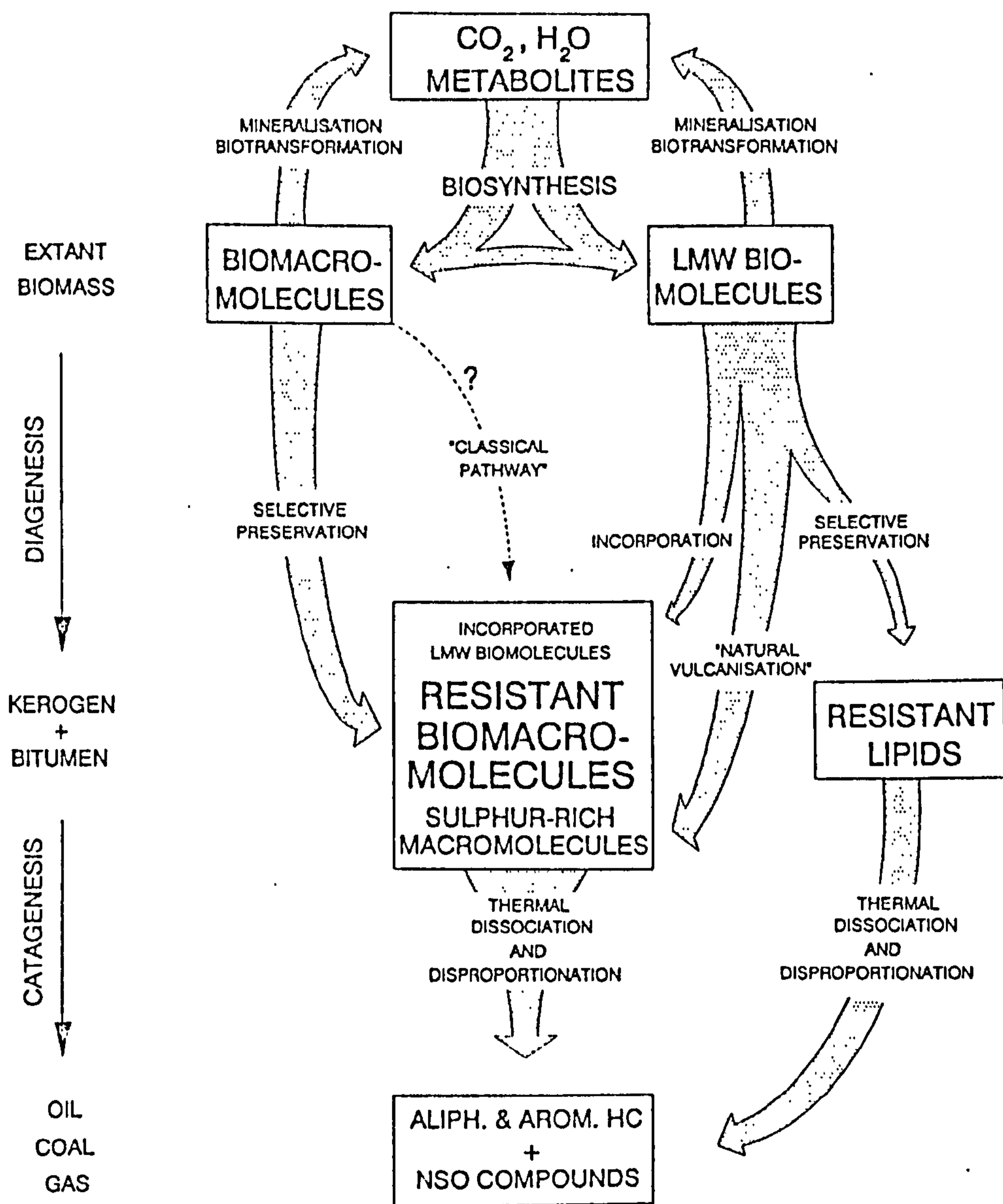


FIGURE 1.7. PROPOSED REVISED PATHWAY TO KEROGEN FORMATION (Tegelaar et al., 1989c)

**TABLE 1.4: KNOWN BIOMACROMOLECULES, THEIR OCCURRENCE, PRESERVATION POTENTIAL AND LIKELY CATAGENIC PRODUCTS.**  
(Adapted from Tegelaar et al., 1989)

Biomacromolecule	Occurrence	Preservation potential <sup>a</sup>	Macerals	Expected major catagenic products
Starch	Vascular plants; some algae; bacteria	-		
Glycogen	Animals	-		
Fructans	Vascular plants; algae; bacteria	-		
Laminarans	Mainly brown algae; some other algae and fungi	-		
Poly β-hydroxyalkanoates	Eubacteria	-		
Cellulose	Vascular plants; some fungi	-/+		
Xylans	Vascular plants; some algae	-/+		
Pectins	Vascular plants	-/+		
Mannans	Vascular plants; fungi; algae	-/+		
Galactans	Vascular plants; algae	-/+		
Mucilages	Vascular plants (seeds)	+		
Gums	Vascular plants	+		
Alginic Acids	Brown algae	-/+		
Fungal Glucans	Fungi	+		
Dextrans	Eubacteria; fungi	+		
Xanthans	Eubacteria	+		
Chitin	Arthropods; copepods; crustaceae; fungi; algae	+		
Acid Mucopolysacch.	Mammals; some fish; eubacteria	-/+		
Proteins	All organisms	-/+		
Extensin	Vascular plants; algae	-/+		
Murein	Eubacteria	+		
Teichoic Acids	Grampositive eubacteria	+		
Teichuronic Acids	Grampositive eubacteria	+		
Lipoteichoic Acids	Grampositive eubacteria	+		
Lipopolysacch. (LPS)	Grampositive eubacteria	++		
DNA, RNA	All organisms	-		
Glycolipids	Plants; algae; eubacteria	+ / ++		
Rubber, Gutta, Dolichols	Vascular plants; animals	+		
Resins, Ambers	Vascular plants	+ / ++		
Cutins, Suberins	Vascular plants	+ / ++		
Lignins	Vascular plants	+++		
Tannins	Vascular plants; algae	+++		
Sporopollenin	Vascular plants	+++	Vitrimite	condensed aromatics
Algacnan <sup>b</sup>	algae	+++	Vitrimite	condensed aromatics
Cutan <sup>c</sup>	Vascular plants	+++	Sporinites	n-alkanes/(condensed) aromatics
Suberan <sup>d</sup>	Vascular plants	+++	Alginites	n-alkanes/some aromatics
			Cutinite	n-alkanes
			Suberinite	n-alkanes and aromatics

a) Preservation potential ranges from - (extensive degradation) to +++ (no degradation) for any depositional environment.

b) Algacnan is the collective term for the resistant non-hydrolyzable macromolecule in cell walls of (micro)algae.

c) Cutan is the collective term for the non-hydrolyzable macromolecule in many higher plant cuticles.

d) Suberan is the collective term for the non-hydrolyzable macromolecule in many higher plant periderms.

UCM-type aliphatic and aromatic structures. If the hypothesis that kerogen contains UCM precursors is accepted, then the UCM composition of oils might reasonably be expected to contain palaeoenvironmental information. Thus, different UCMS may be expected to show variations in composition which would reflect the type of organic matter at the time of deposition and subsequent geochemical fate (Gough, 1989; Gough and Rowland, 1990). If this information can be released, the source of oils may be distinguishable on the basis of the differences in UCM composition.

#### **1.4 FINGERPRINTING OF UCMS**

Part of the evidence for a petrogenic source for UCMS (Section 1.3) is the co-occurrence of UCMS with mature "biological marker" profiles. The information contained within the distribution and stereochemistry of these biomarkers has led to their widespread use as fingerprinting compounds in oil/oil and oil/source rock correlations (Pym *et al.*, 1975; Seifert and Moldowan, 1978; Seifert *et al.*, 1979; Philp, 1983; Jones *et al.*, 1986 and Peters *et al.*, 1989). These compounds (especially steranes and pentacyclic triterpanes) have been shown to be susceptible to biodegradation under certain conditions (Goodwin *et al.*, 1983; Connan, 1984; Philp and Lewis, 1987; Chosson *et al.*, 1991) and the relative rates and order of degradation to vary, depending upon the environment.



Therefore in any correlation study where degradation has occurred, the use of these compounds is prone to error. Indeed, a requirement in this type of situation would be to know which compounds have been degraded and by how much (Poynter and Eglinton, 1991). In addition, some oils contain a wide range of biomarker concentrations, which vary by as much as three orders of magnitude (though rarely accounting for greater than 1% of the C<sub>15+</sub> hydrocarbons, Rullkötter et al., 1984). Thus an input of two oils, with widely differing biomarker concentrations could result in misleading biomarker profiles. The pentacyclic triterpane distribution can be further confused by inputs of naturally occurring compounds (e.g. from peat deposits; Quirk et al., 1980).

The use of UCM oxidation profiles to provide a complementary fingerprint was demonstrated for two, well documented, acute oil spills by Gough (1989) and Gough and Rowland (1990) (Figure 1.8). These studies utilised variations in seven oxidation products (three lactones and four ketones) to compare the oils and polluted sediments. However, these oxidation products were also only minor products (<1%) and presumably represented correspondingly minor UCM components. Thus, use of these compounds for fingerprinting suffers from the same disadvantages as that of traditional biomarkers. A more acceptable method might be to consider the oxidation products as a whole but any attempt to do this would require the comparison of complex

LACTONES  
(m/z 99)

KETONES  
(m/z 58)

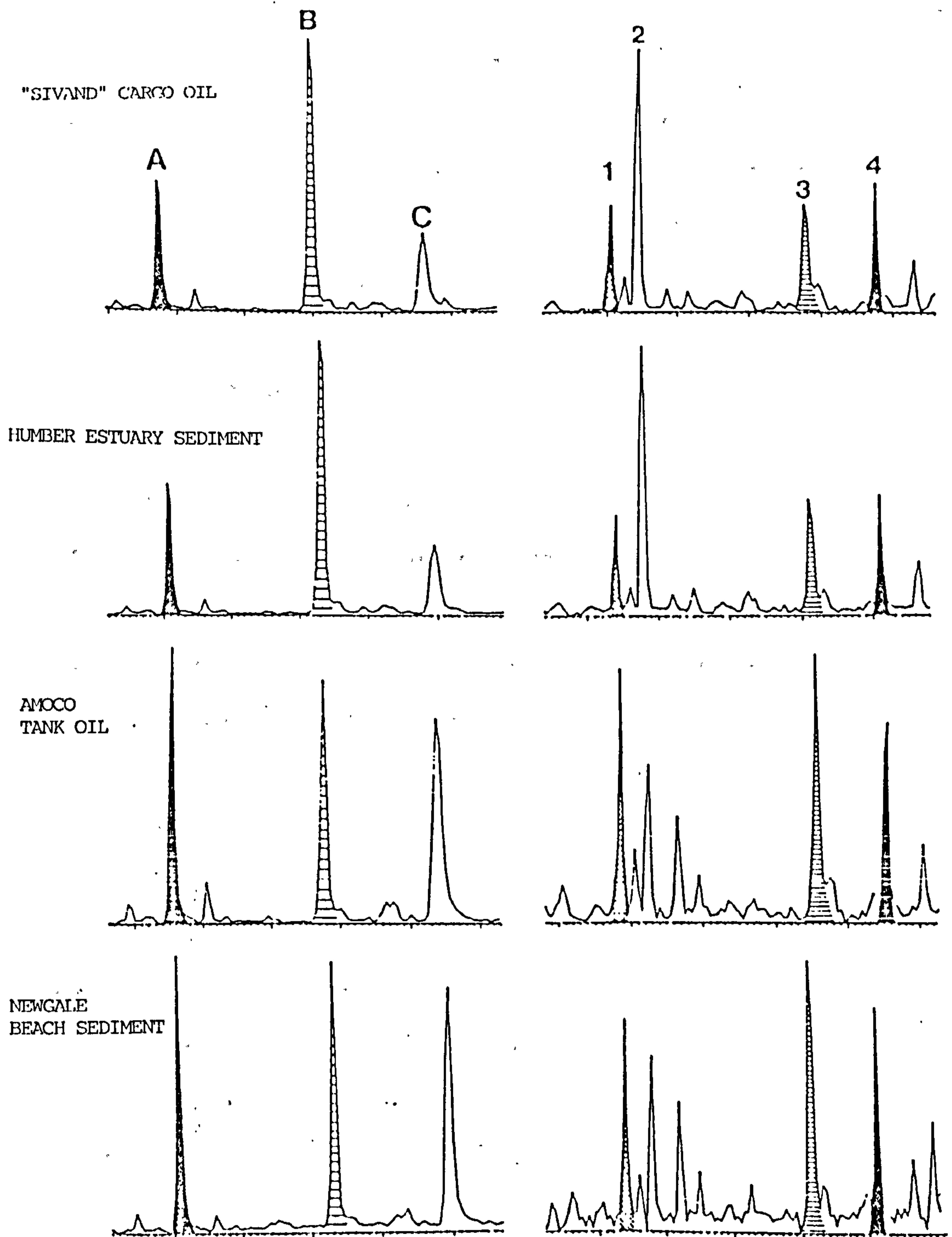


FIGURE 1.8. THE USE OF UCM OXIDATION PRODUCTS TO FINGERPRINT TWO ACUTE OIL SPILLS (Gough and Rowland, 1990)

chromatograms (for examples see Gough and Rowland, 1990; Gough and Rowland, 1991), which would be difficult and subjective if carried out manually. This problem undoubtedly lends itself to multivariate statistical techniques, similar to those which have become routinely used in exploration geochemistry (e.g. Birks, 1987; Barth, 1987; Esbensen and Martens, 1987; Esbensen et al., 1987).

### **1.5 THE PRESENT STUDY**

The present study is intended to advance current knowledge of UCMs in three interconnected areas.

Chapter 1 has reviewed the current state of knowledge of UCM composition, how this may help in elucidating the origins of the UCM and the possible geochemical information contained within this complex mixture.

Chapter 2 details the use of instrumental and chemical techniques to characterise an aromatic UCM. In this investigation the UCM was degraded to a series of resolved components using a specific oxidant and the products compared with those formed by the oxidation of known aromatic hydrocarbons.

Chapter 3 combines the structural information from Chapter 2 with that of previous studies, to try to formulate ideas on the origins of UCMs. Laboratory maturation of possible

kerogen precursors has yielded UCMs, the oxidation products of which were compared to those of UCMs from oils. The results have implications for both UCM origins and theories of kerogen structure.

Chapter 4 reports the fingerprinting of UCMs by analysis of the oxidation products from a series of different aliphatic UCMs. This work advances the results of previous studies by the use of purpose written computer programs to compare chromatograms and multivariate statistical techniques to produce objective UCM correlations.



"Do you know what this is?"  
"No" said Piglet  
"It's an A"  
"Oh", said Piglet  
"Not O---A" said Eeyore severely

A.A. Milne (The House at Pooh Corner)

## STRUCTURAL CHARACTERISATION OF THE AROMATIC UCM

### 2.1 GENERAL

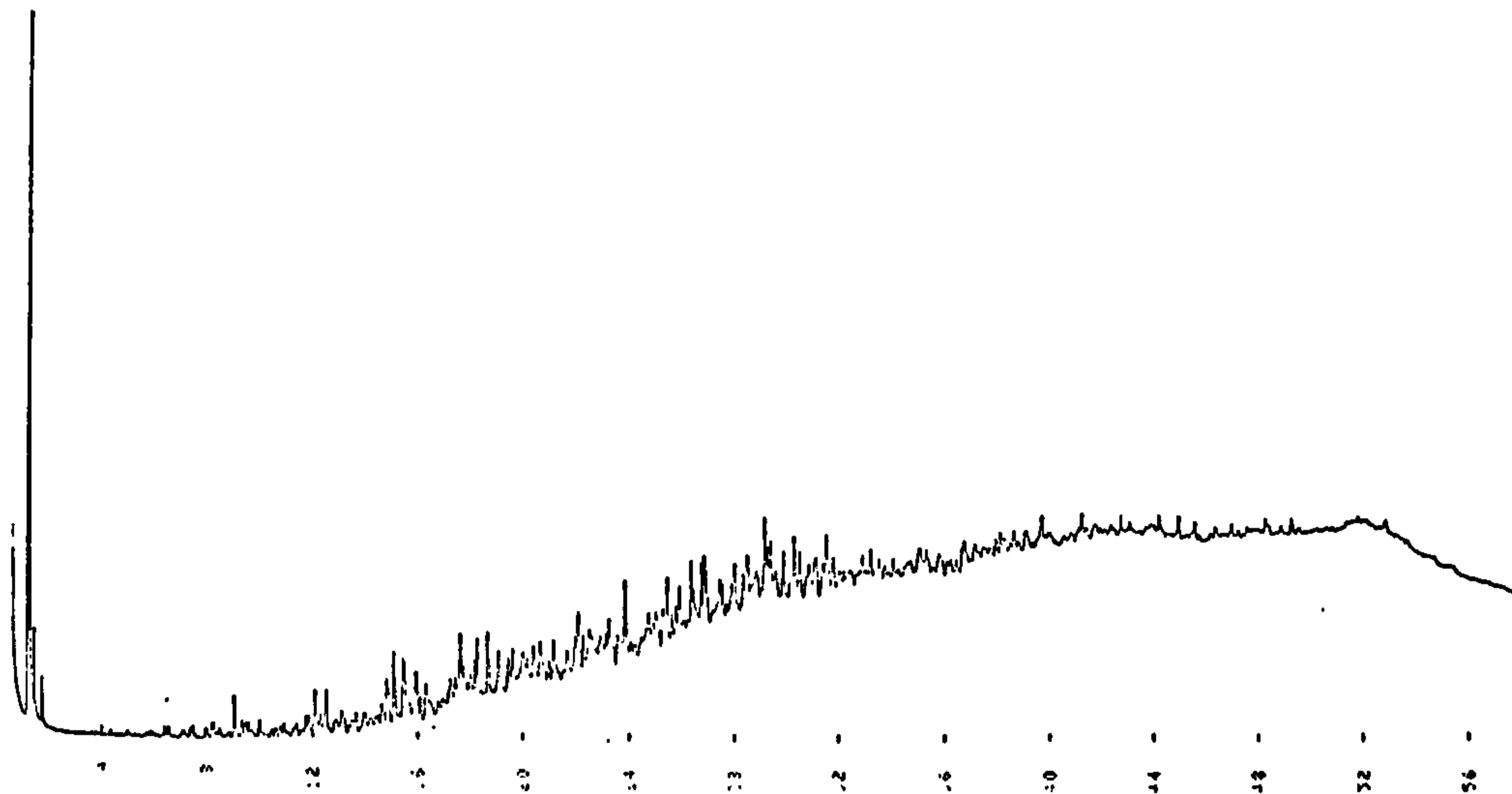
If relatively little is known about aliphatic UCM composition, even less is known about the aromatic UCM. Although Killops and Al-Juboori (1990) studied the UCM of a whole degraded oil, including the aromatic hydrocarbons (see Section 1.2) and Gough (1989) used Cr(IV) oxidation to study the aromatic UCM from lubricating oil and both concluded that the UCMs were comprised of monoaromatic compounds with branched alkyl side chains, it is not known how generally applicable their conclusions are.

A major problem in the study of aromatic UCMs, is the initial isolation of the unresolved components from the resolved components. Killops and Readman (1985) used HPLC to separate the aromatic fraction according to the number of aromatic double bonds. The majority of the UCM was monoaromatic, but this fraction still contained a high proportion of resolved components. Recently, Grimalt et al. (1991) achieved a similar result using column chromatography to fractionate Amposta oil (Spain).

At present there appears to be no satisfactory chemical method for the removal of the resolved components. For the present study this problem was overcome by using the aromatic UCM of a biodegraded oil (Tia Juana Pesado Crude [TJP], Venezuela) from which the resolved components had

already been removed by natural bacterial action. Although a biodegraded oil is not an ideal substrate from which to draw general conclusions about UCM structure, such oils nonetheless represent a massive amount of oil accumulated (2,800,000 MMBbl; Roadifer, 1987), including the world's three largest oil accumulations (the Orinoco heavy oil belt, Venezuela [1,200,000 MMBbl], Athabasca tar sand, Canada [868,600 MMBbl] and Cold Lake tar sand, Canada [270,000 MMBbl] (Roadifer, 1987)) and such oils are themselves the subject of considerable geochemical interest (reviewed by Hunt, 1979; Cassani and Eglinton, 1986; Roadifer, 1987; Cassani and Eglinton, 1991). Furthermore since the limited evidence obtained so far has suggested that the UCM is relatively inert to biodegradation (Gough *et al.*, 1991), the UCM structure of a biodegraded oil might be expected to be similar to other, less degraded, oils.

Isolation of the aromatic fraction from TJP crude followed the method of Davies and Wolff (1990). Initially the high percentage of aromatic compounds (Table 2.1) modified the apolar solvent used as an eluent for column chromatography and severely reduced chromatographic separation so that an unusually high ratio of adsorbent to sample was required in the eventual separation of the aromatic UCM from the total oil (see experimental).



**FIGURE 2.1. GC-FID OF TJP AROMATICS**

(J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub>carrier)

**TABLE 2.1. YIELD FOR FOUR DIFFERENT FRACTIONATIONS OF TJP  
CRUDE OIL FRACTIONS FROM COLUMN CHROMATOGRAPHY.**

Total Oil (mg)	Aliphatics (mg(%))	Aromatics (mg(%))
236	67.5 (28.6)	91.8 (38.9)
190	52.1 (27.4)	73.8 (38.8)
130	37.7 (29.0)	57.5 (44.2)
430	120.3 (27.9)	168.2 (39.1)
RSD (%)	2.2	5.7



## 2.2 BULK CHARACTERISTICS

GC of the aromatic hydrocarbons revealed a broad UCM ranging from KI 1300-3600 (2700 max.) with only ca. 5% resolved components (Figure 2.1), as estimated by the time slice area measurement (Appendix I). Elemental analysis gave an average composition of:-

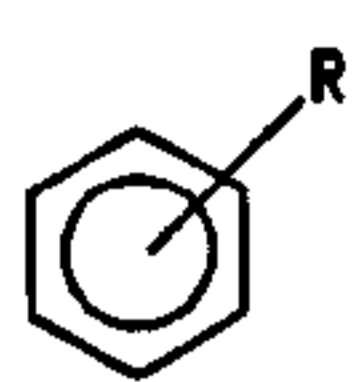
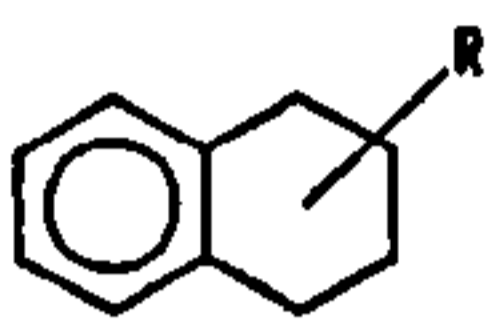
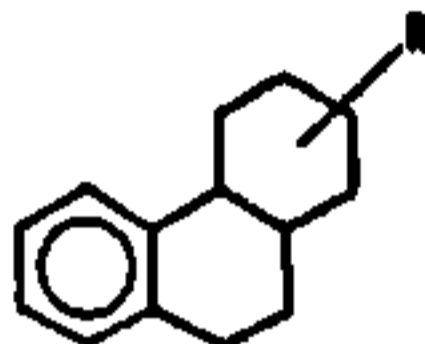
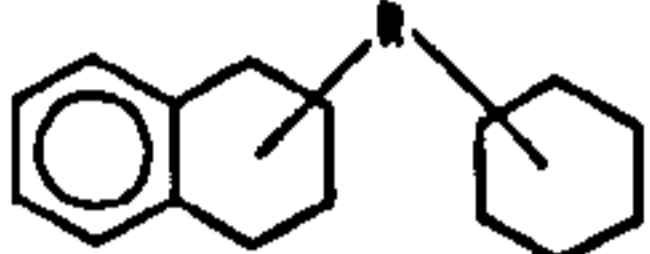


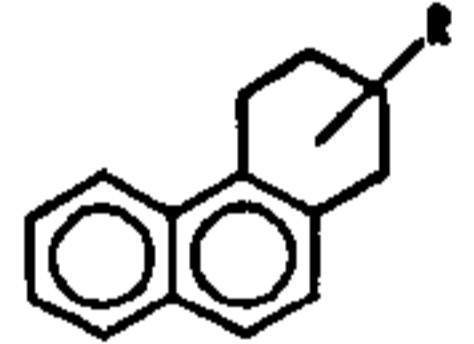
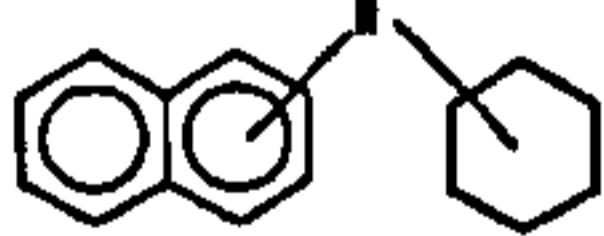
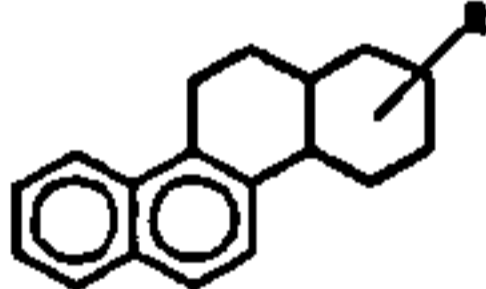

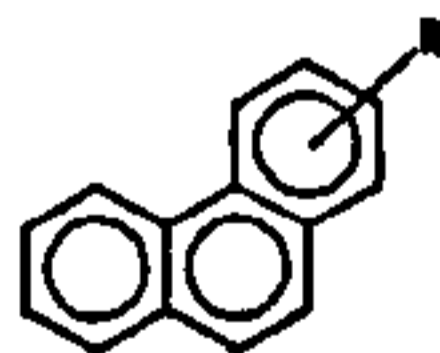
C%	H%	N%	S%	O%*	H/C
82.33	10.10	0.0	4.04	3.53	
83.92	10.00	0.0	4.08	2.00	
<hr/>					
83.13	10.05		4.06	2.77	1.45

\* Determined by difference

The H/C ratio suggests the presence of naphthenonaphthalenes (assuming an average composition of a  $C_{25}$  hydrocarbon,  $Z = -14$ , Table 2.2). However, this value cannot be taken as a true indication of the average compound class, since the relatively high sulphur content decreases the H/C ratio. For example, dibenzothiophene ( $C_{12}H_8S$ ) contains 17% sulphur (wt/wt) and has a H/C ratio of 0.67 whereas biphenyl ( $C_{12}H_{10}$ ) has a H/C ratio of 0.8. This effect is shown graphically for a variety of sulphur compound classes in Figure 2.2. This figure highlights the fact that the UCM is unlikely to consist entirely of

TABLE 2.2 ELEMENTAL ANALYSIS

Aromatic Hydrocarbon                      Z<sup>a</sup> Value                      H/C Ratio<sup>b</sup>                      General Formula

	-6	1.76	C <sub>n</sub> H <sub>2n-6</sub>
	-8	1.68	C <sub>n</sub> H <sub>2n-8</sub>
	-10	1.60	C <sub>n</sub> H <sub>2n-10</sub>
	-12	1.52	C <sub>n</sub> H <sub>2n-12</sub>
	-14	1.44	C <sub>n</sub> H <sub>2n-14</sub>
	-16	1.36	C <sub>n</sub> H <sub>2n-16</sub>
	-18	1.28	C <sub>n</sub> H <sub>2n-18</sub>
			
			
			
			

Aromatic UCM (Gough, 1989)  
THIS STUDY

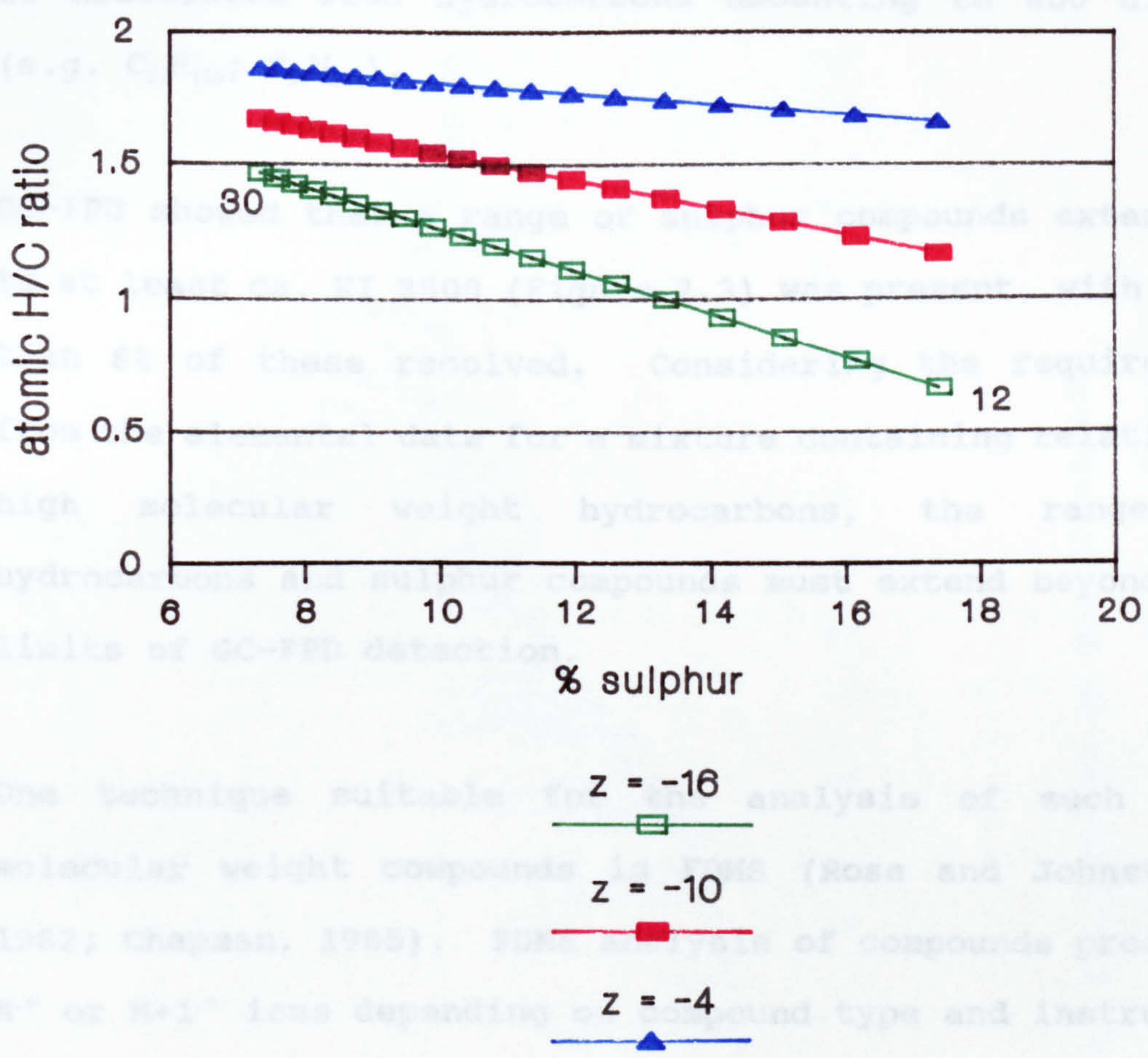
1.62  
1.45

<sup>a</sup> Z value = C<sub>n</sub>H<sub>2n+z</sub>

<sup>b</sup> for a C<sub>25</sub> aromatic hydrocarbon



aromatic sulphur compounds. The 4% S content of the UCM must be due to dilution of the sulphur compounds by hydrocarbons with a H/C ratio greater than 1.45. Indeed, to obtain a sulphur content of 4%, each sulphur atom must be associated with hydrocarbons amounting to 800 a.m.u. (e.g. C<sub>12</sub>H<sub>22</sub>S).



**FIGURE 2.2. ATOMIC H/C RATIOS vs. %S FOR DIFFERENT Z VALUES C<sub>12</sub> - C<sub>30</sub>**

One technique suitable for the analysis of such high molecular weight compounds is FIMS (Rose and Johnston, 1967; Chapman, 1965). FIMS analysis of compounds produces M<sup>+</sup> or M+1<sup>+</sup> ions depending on compound type and instrument conditions (H. Major, W. Analytical Ltd., Personal Communication, 1991). The aromatic UCM and three standards, 7-hexylundecane, 5-(1-cyclohexylethyl)heptadecane and 9-(2-phenylethyl)undecane are analysed by FIMS. The authentic compounds all produced strong M+1<sup>+</sup> ions (Figure 2.4) while FIMS of the UCM produced both M<sup>+</sup> and M+1<sup>+</sup> ions (Figure 2.5) and a molecular weight range of 200-1200 Daltons, from which the distributions of the

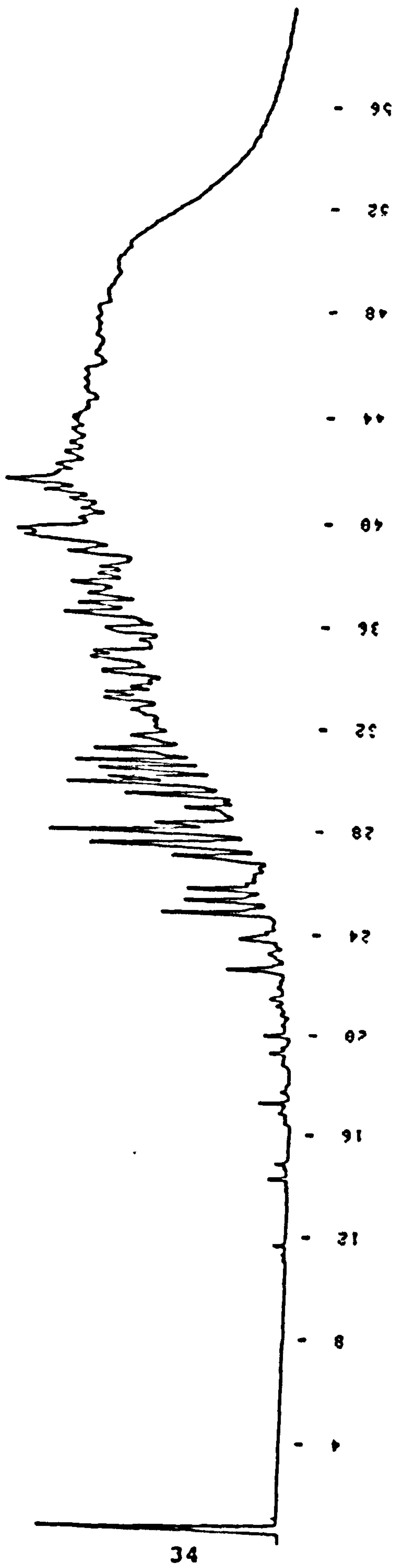


aromatic sulphur compounds. The 4% S content of the UCM must be due to dilution of the sulphur compounds by hydrocarbons with a H/C ratio greater than 1.45. Indeed, to obtain a sulphur content of 4%, each sulphur atom must be associated with hydrocarbons amounting to 800 a.m.u. (e.g.  $C_{57}H_{116}$ ;  $C_{53}H_{104}$ ).

GC-FPD showed that a range of sulphur compounds extending to at least ca. KI 3500 (Figure 2.3) was present, with less than 8% of these resolved. Considering the requirement from the elemental data for a mixture containing relatively high molecular weight hydrocarbons, the range of hydrocarbons and sulphur compounds must extend beyond the limits of GC-FPD detection.

One technique suitable for the analysis of such high molecular weight compounds is FDMS (Rose and Johnstone, 1982; Chapman, 1985). FDMS analysis of compounds produces  $M^+$  or  $M+1^+$  ions depending on compound type and instrument conditions (H. Major, VG Analytical Ltd., Personal Communication, 1991). The aromatic UCM and three standards, 7-hexylnonadecane, 9-(2-cyclohexylethyl)heptadecane and 9-(2-phenylethyl)heptadecane) were analysed by FDMS. The authentic compounds all produced strong  $M+1^+$  ions (Figure 2.4) while FDMS of the UCM produced both  $M^+$  and  $M+1^+$  ions (Figure 2.5) and a molecular weight range of 200-1200 Daltons, from which the distributions of the





**FIGURE 2.3. GC-FPD OF TJP AROMATICS**

**(J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier)**

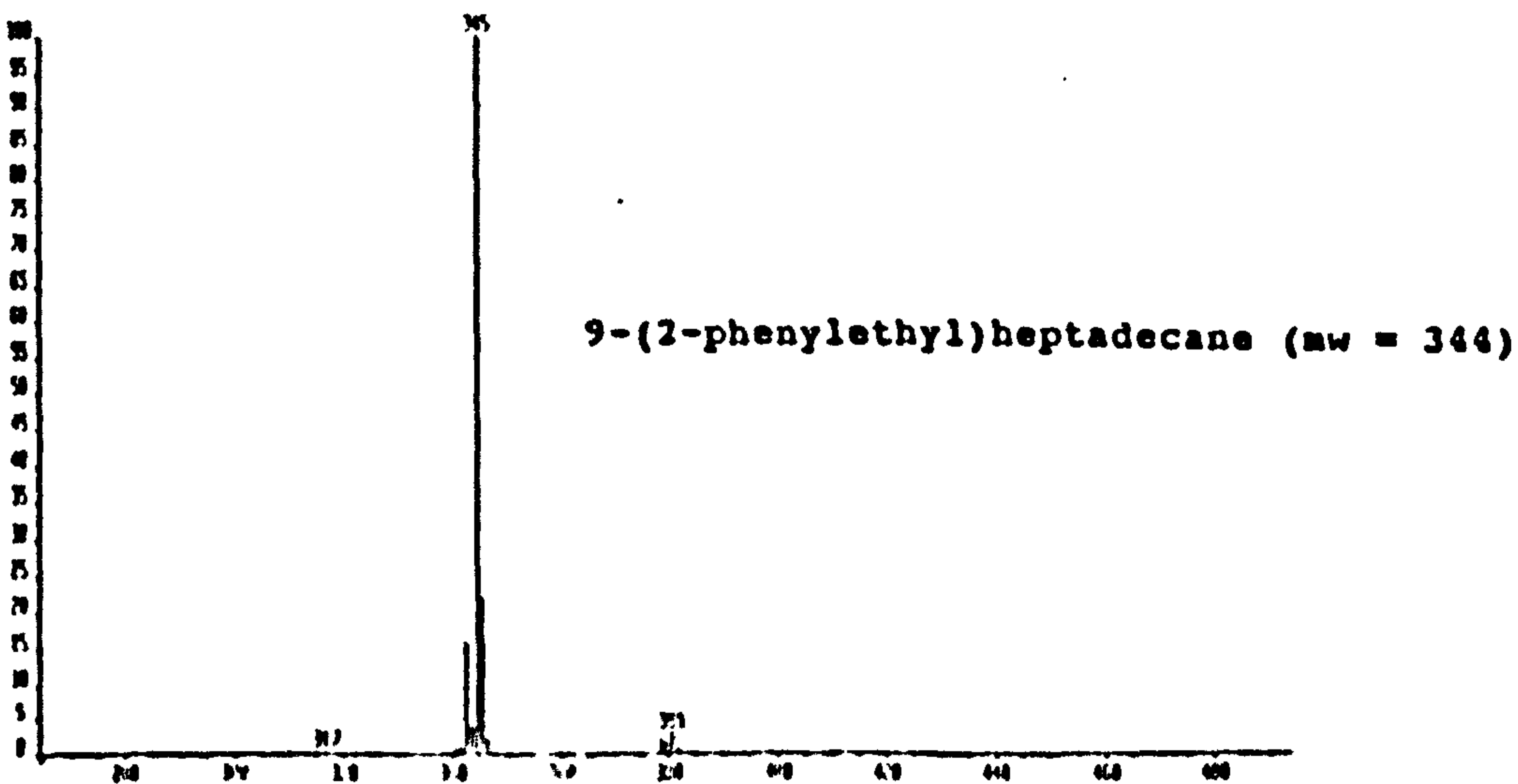
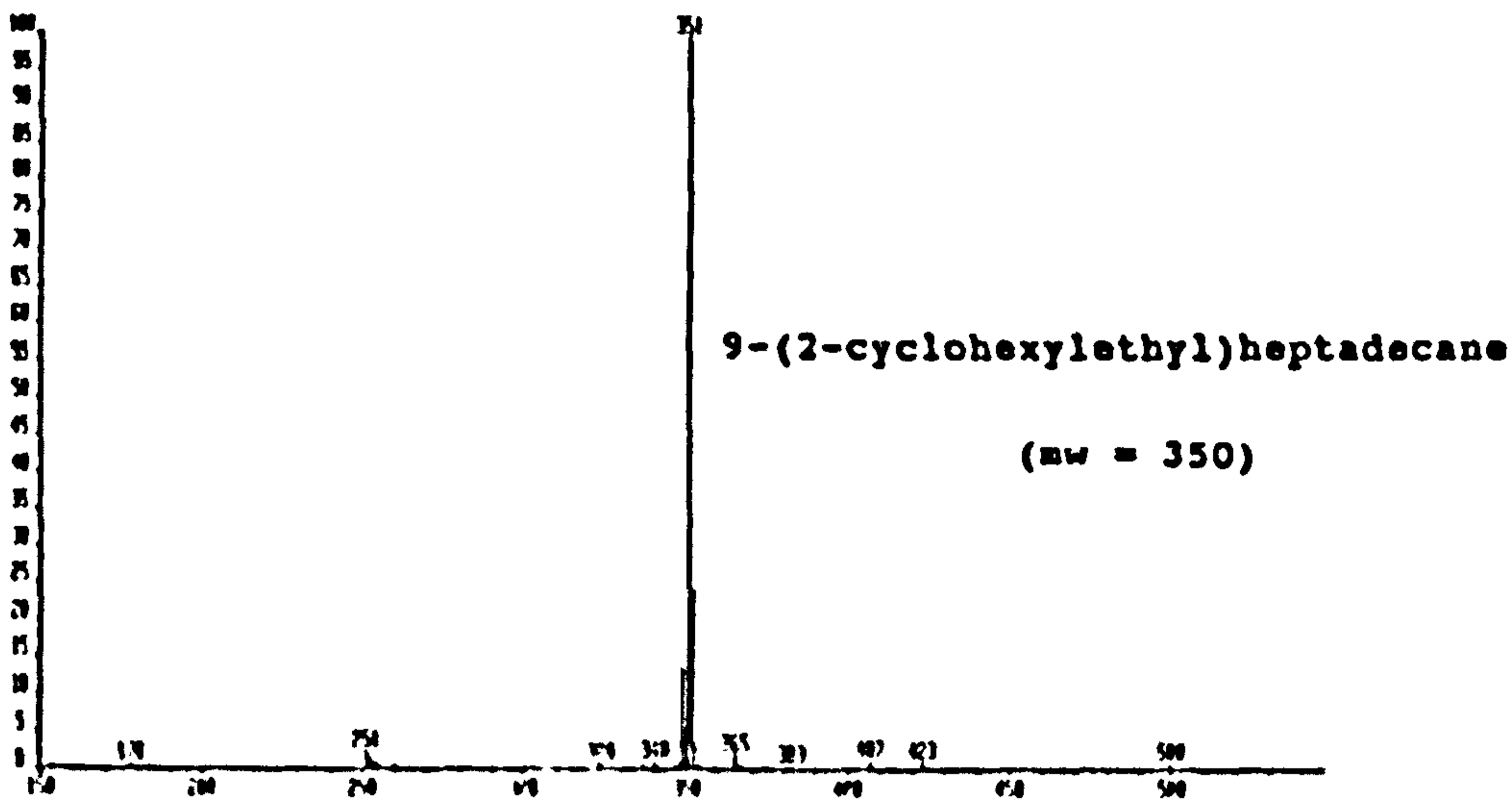
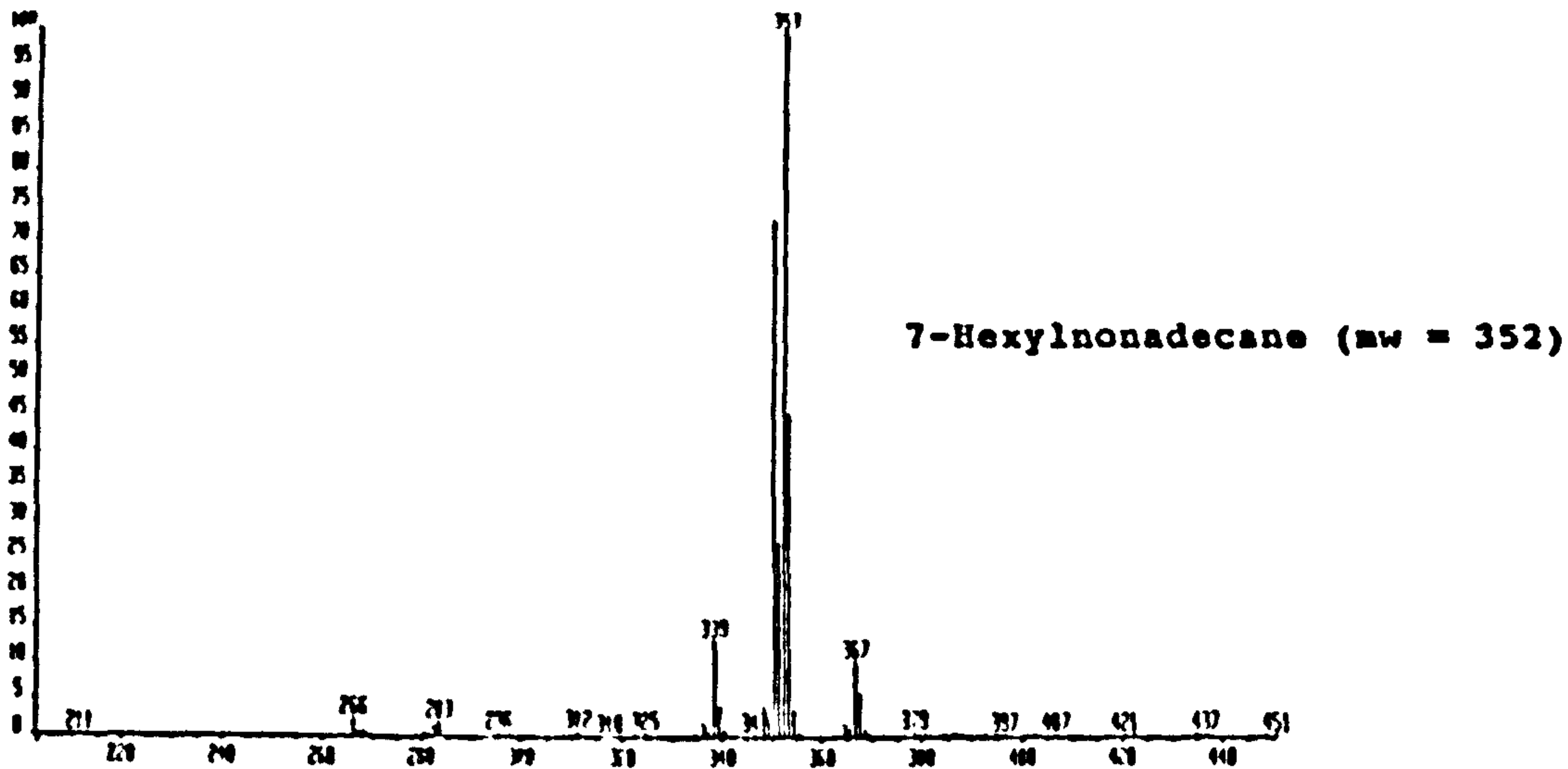


FIGURE 2.4. FDMS OF AUTHENTIC COMPOUNDS

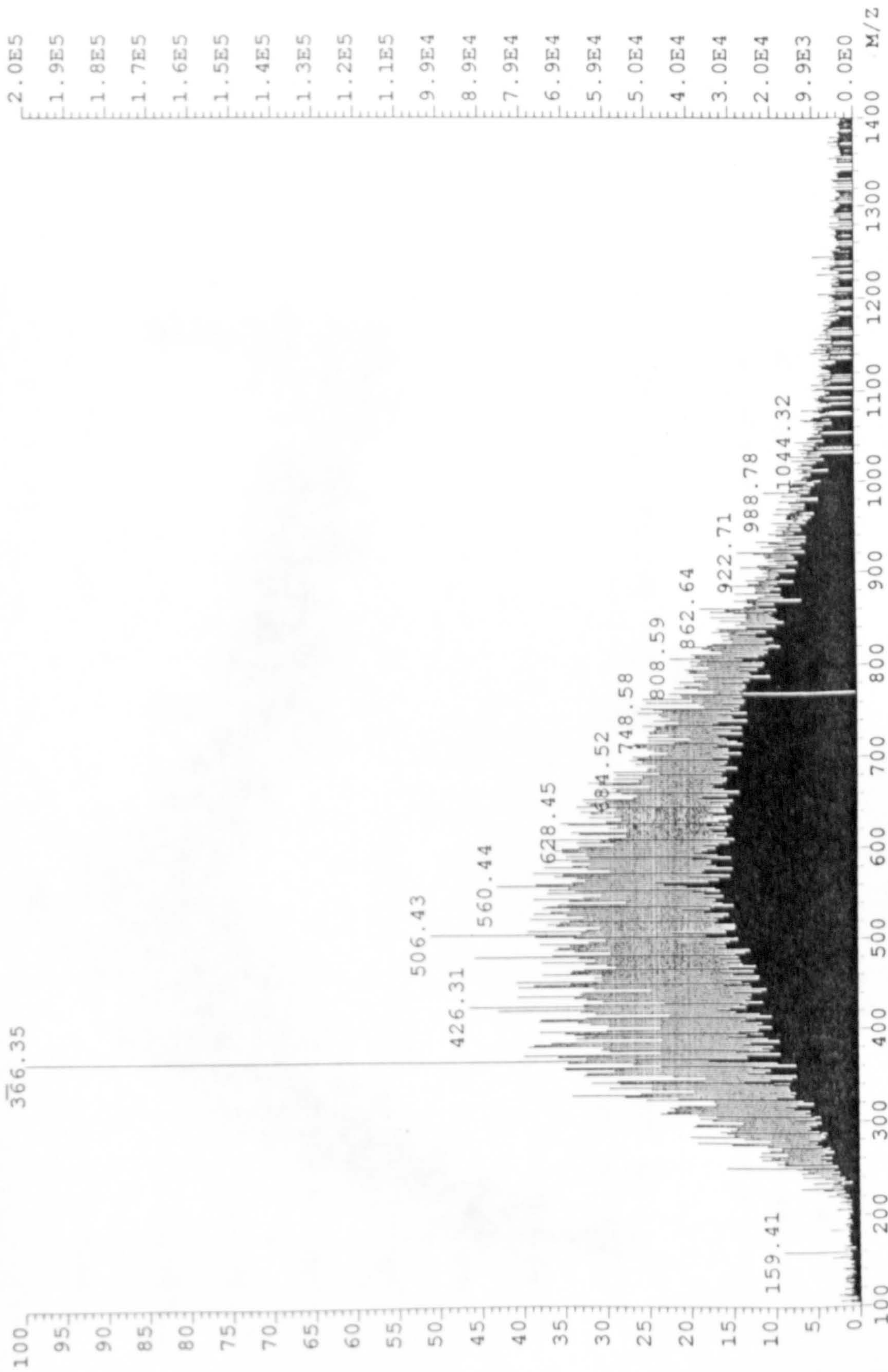


FIGURE 2.5. FDMS OF TJP AROMATICS



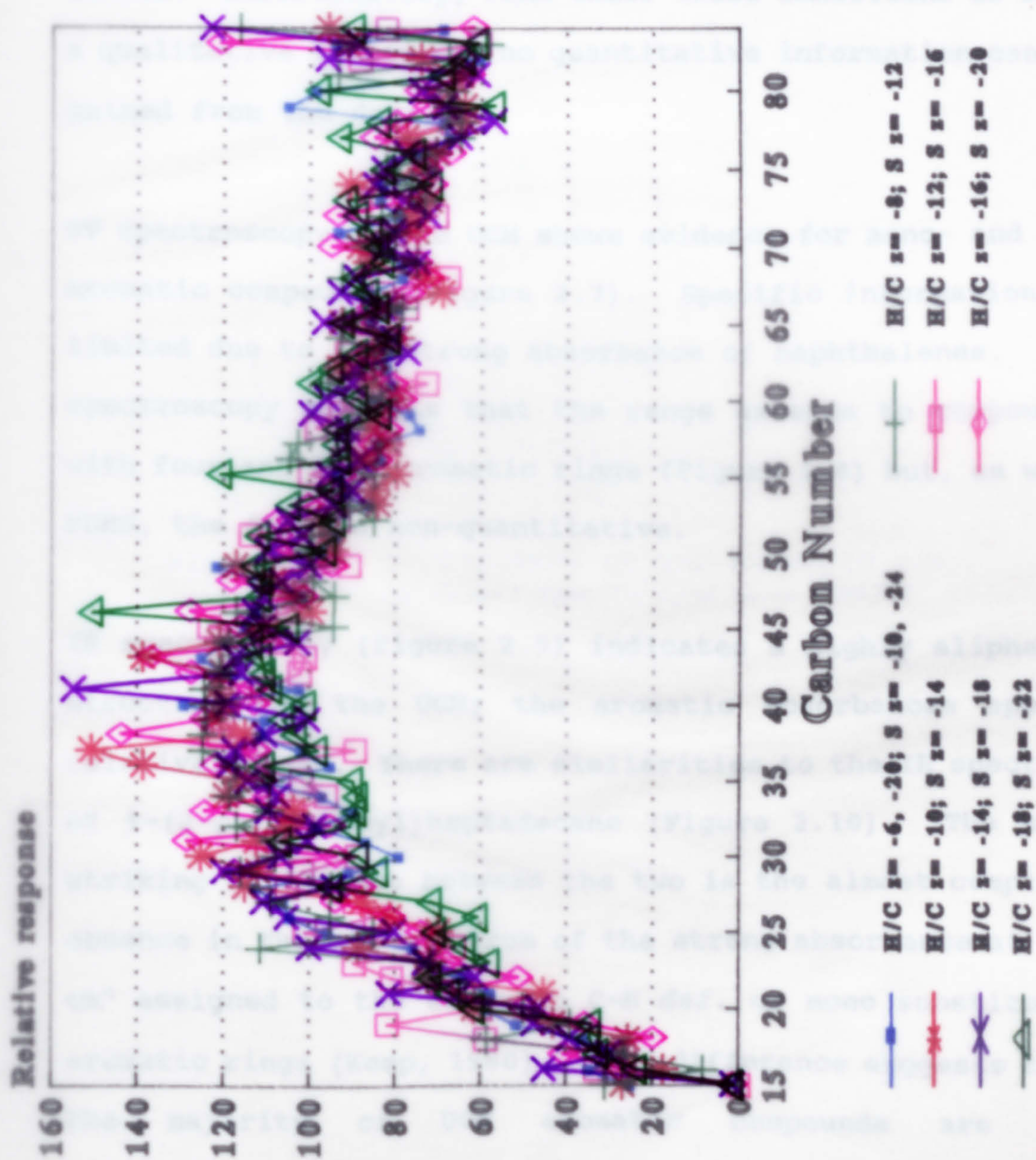


FIGURE 2.6. FDMS ANALYSIS OF THE AROMATIC UCM



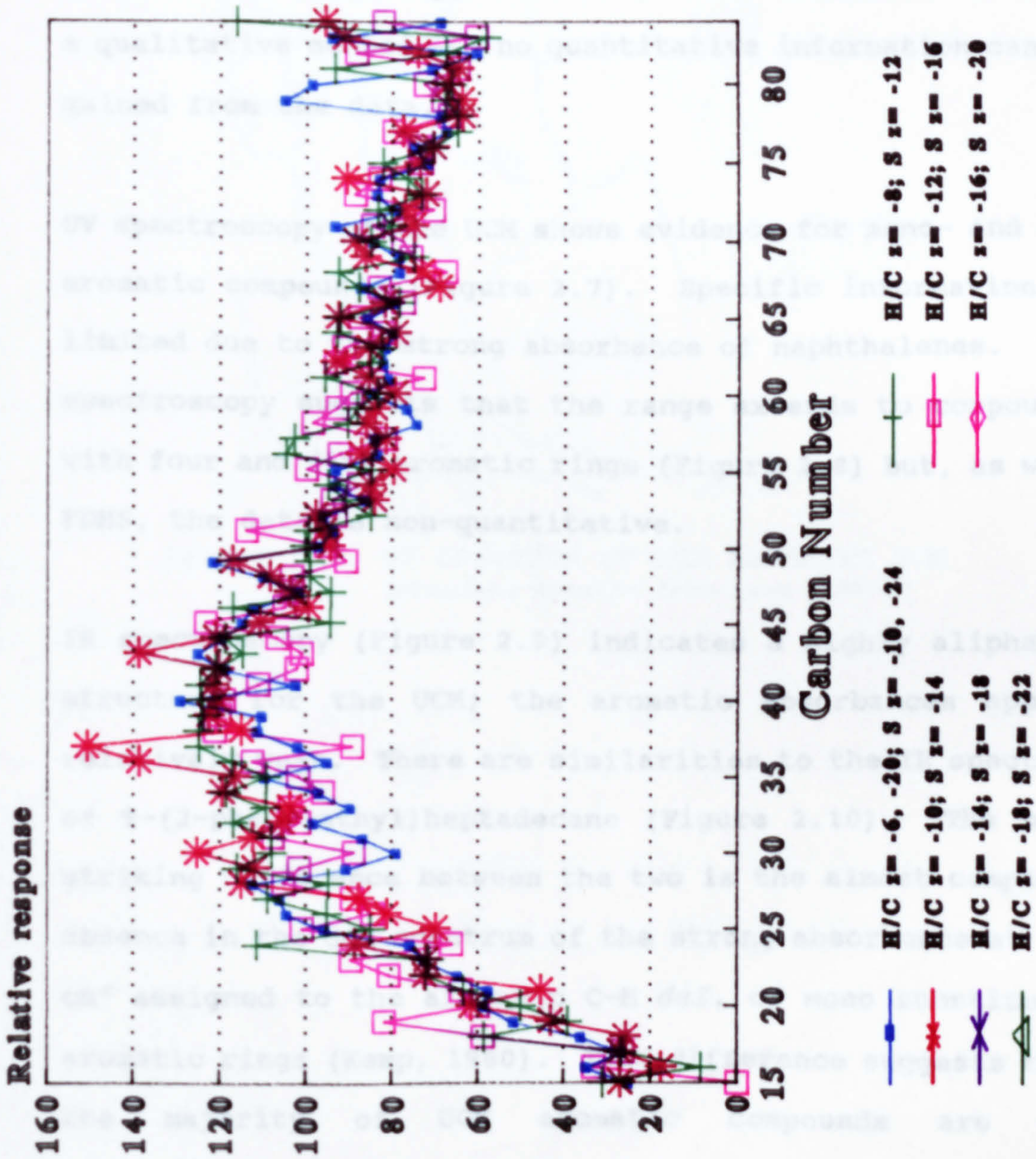


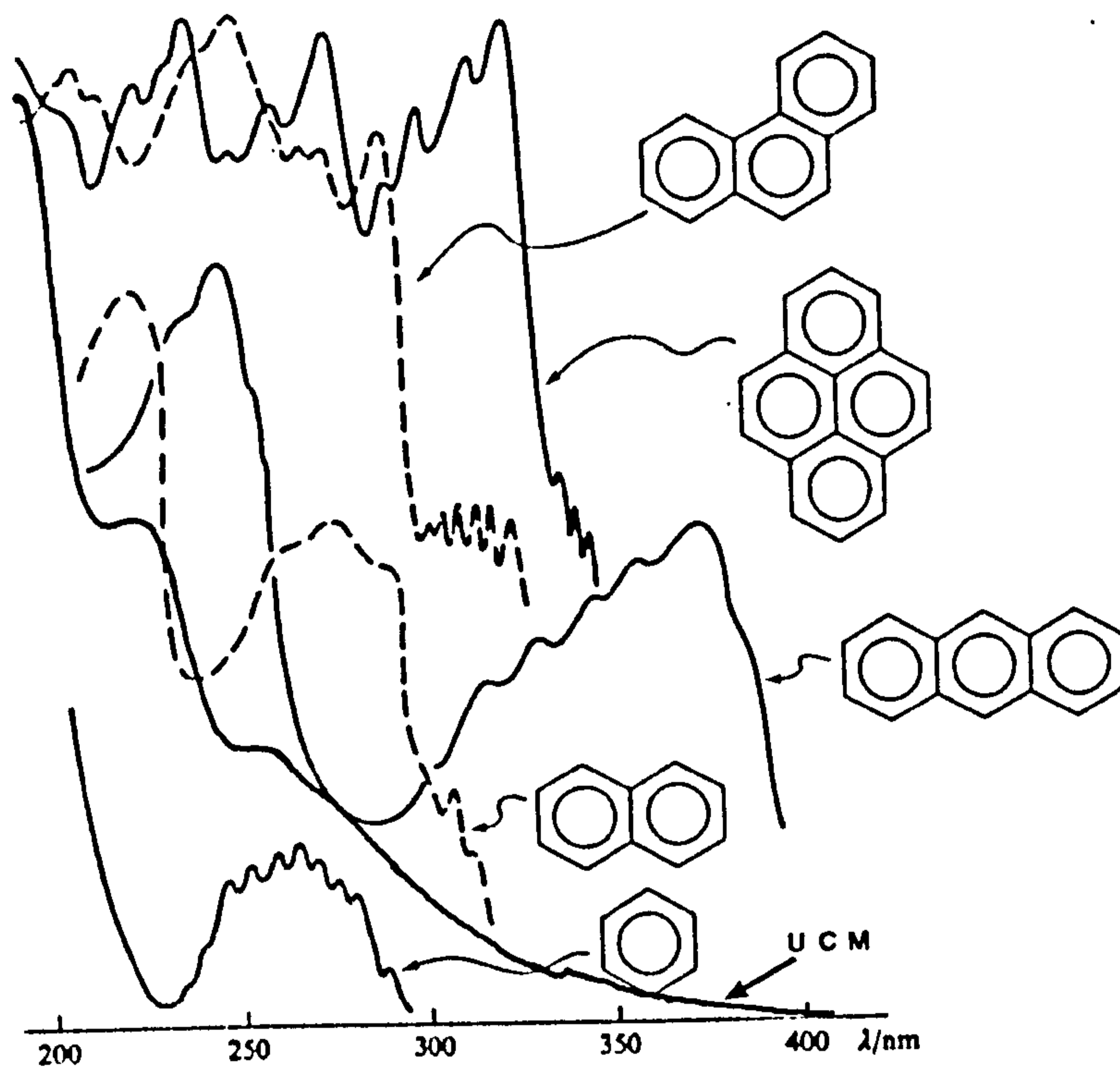
FIGURE 2.6. FDMS ANALYSIS OF THE AROMATIC UCM



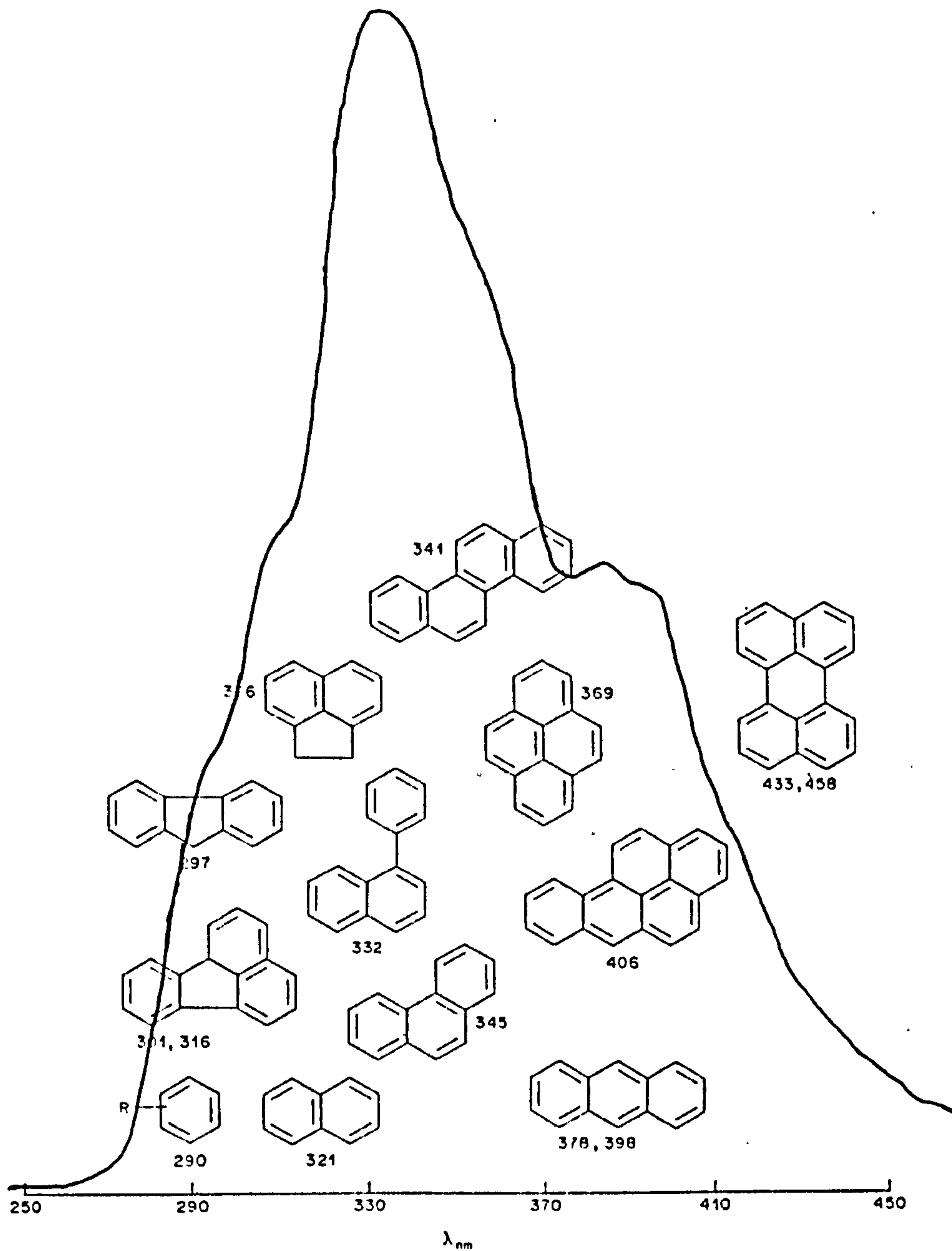
common hydrocarbon and sulphur containing aromatics were plotted (Figure 2.6). The data show that there is little difference between the relative distributions of the various compound classes and that there is a proportion of the sample which is indeed outside the normal limits of GC-FID. Unfortunately, FDMS under these conditions is only a qualitative method and no quantitative information can be gained from the data.

UV spectroscopy of the UCM shows evidence for mono- and di-aromatic compounds (Figure 2.7). Specific information is limited due to the strong absorbance of naphthalenes. UVF spectroscopy suggests that the range extends to compounds with four and five aromatic rings (Figure 2.8) but, as with FDMS, the data is non-quantitative.

IR spectroscopy (Figure 2.9) indicates a highly aliphatic structure for the UCM; the aromatic absorbances appear relatively weak. There are similarities to the IR spectrum of 9-(2-phenylethyl)heptadecane (Figure 2.10). The most striking difference between the two is the almost complete absence in the UCM spectrum of the strong absorbance at  $700\text{ cm}^{-1}$  assigned to the aromatic C-H def. of mono substituted aromatic rings (Kemp, 1980). This difference suggests that the majority of UCM aromatic compounds are not mono-alkylbenzenes but rather *ortho*-disubstituted benzenes ( $730\text{ cm}^{-1}$ ), *para*-disubstituted benzenes or substituted naphthalenes ( $810\text{ cm}^{-1}$ ,  $870\text{ cm}^{-1}$ ) with the strongest

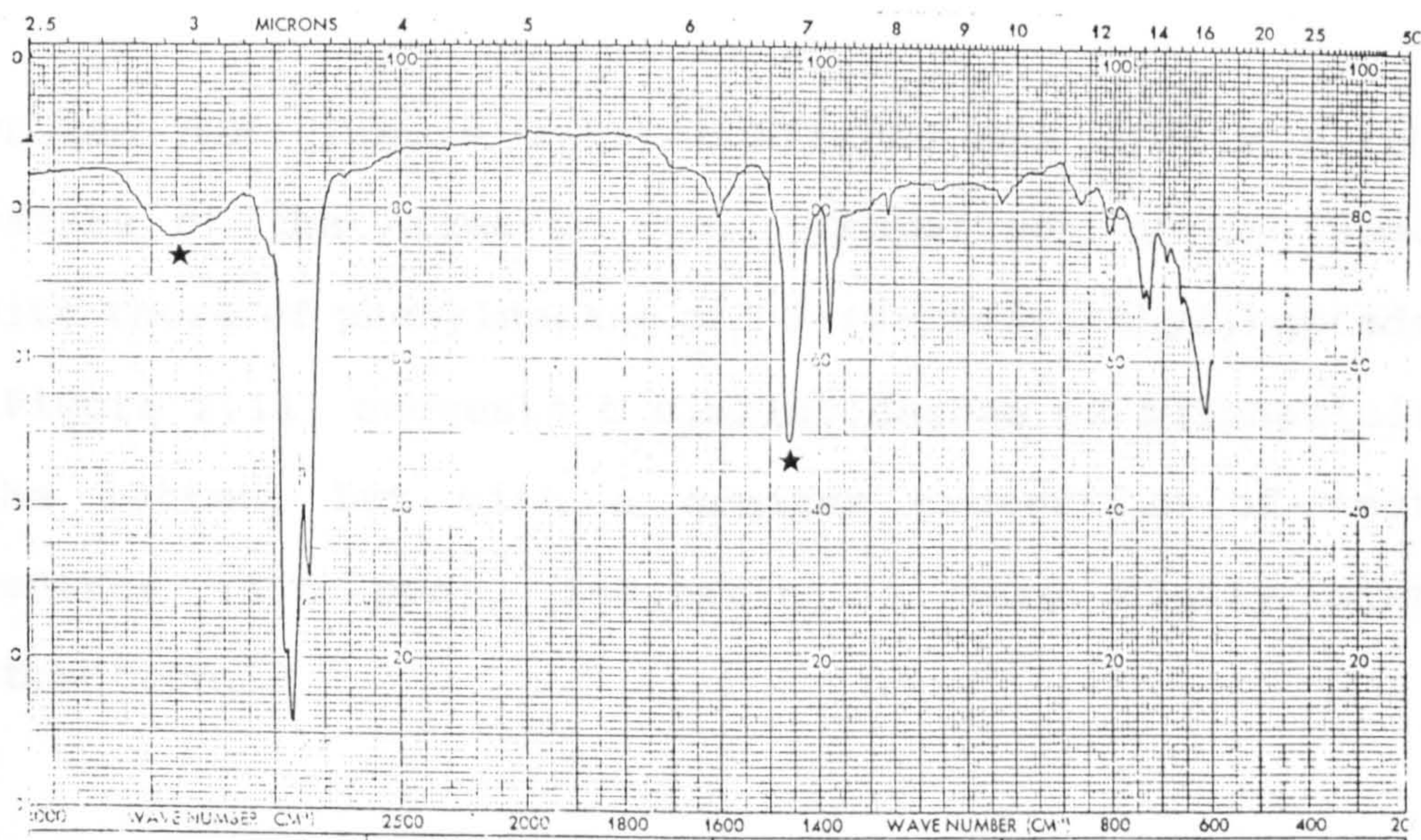


**FIGURE 2.7. UV SPECTRUM OF THE AROMATIC UCM**  
 [Standard spectra from Kemp (1987)]

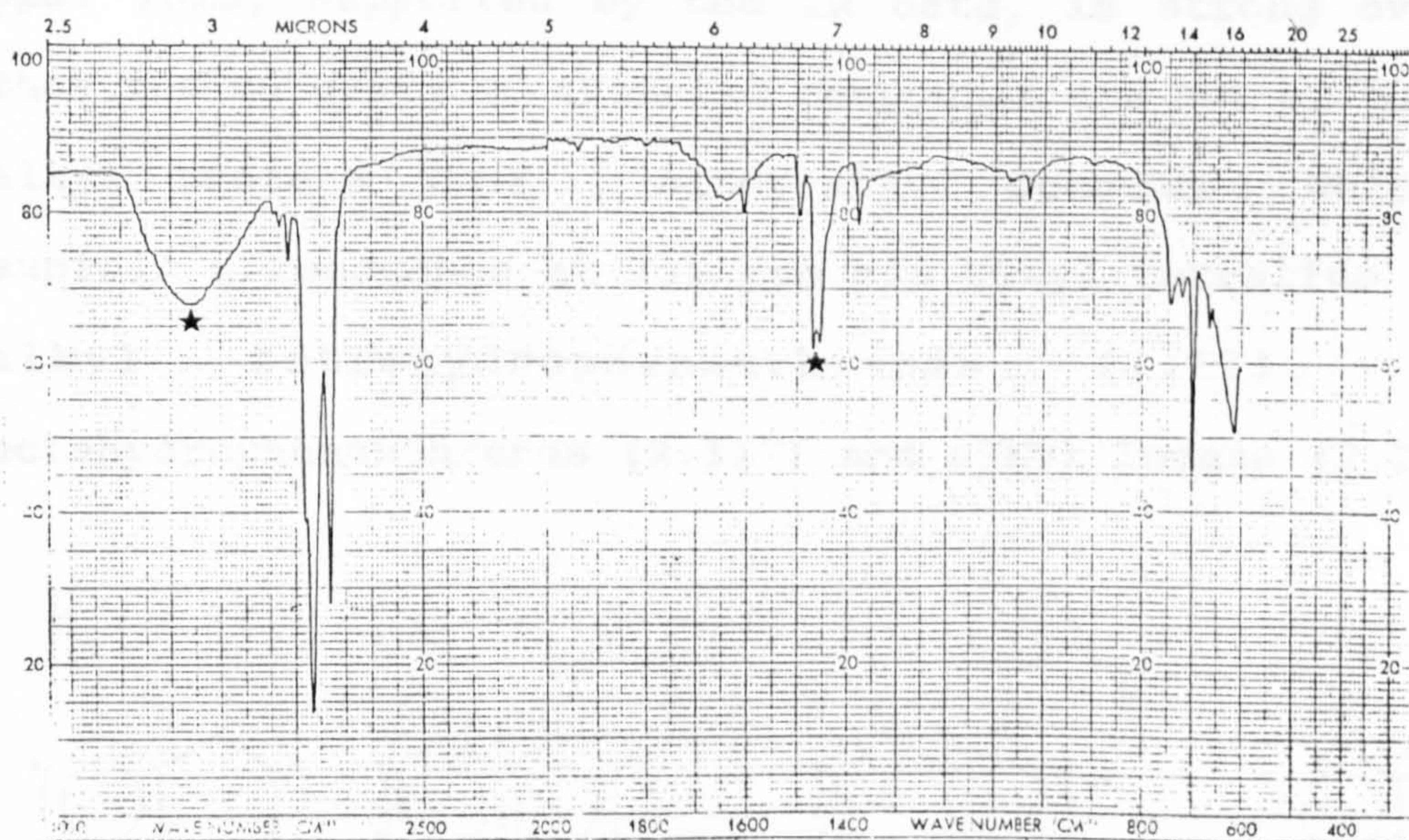


**FIGURE 2.8. UVF SYNCHRONOUS SCANNING SPECTRUM OF THE AROMATIC UCM**  
 (Relative positions of aromatic compounds by reference to Mille et al., 1988)





**FIGURE 2.9. IR SPECTRUM OF THE AROMATIC UCM**  
 (Sample analysed as a thin film on NaCl discs. \* indicates absorbtions due to the slightly hydrosopic nature of the discs)



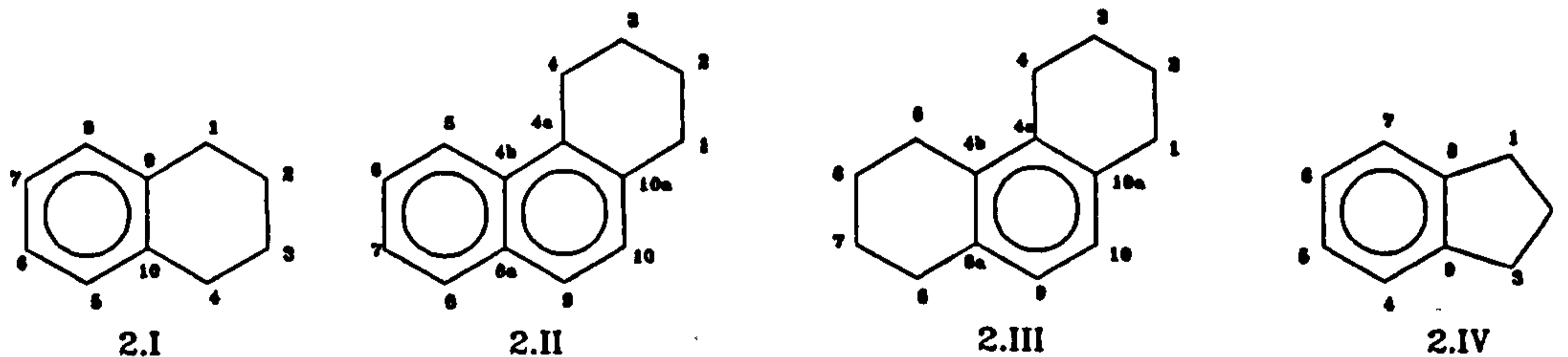
**FIGURE 2.10. IR SPECTRUM OF 9-(2-PHENYLETHYL)HEPTADECANE**  
 (Sample analysed as a thin film on NaCl discs. \* indicates absorbtions due to the slightly hydrosopic nature of the discs)



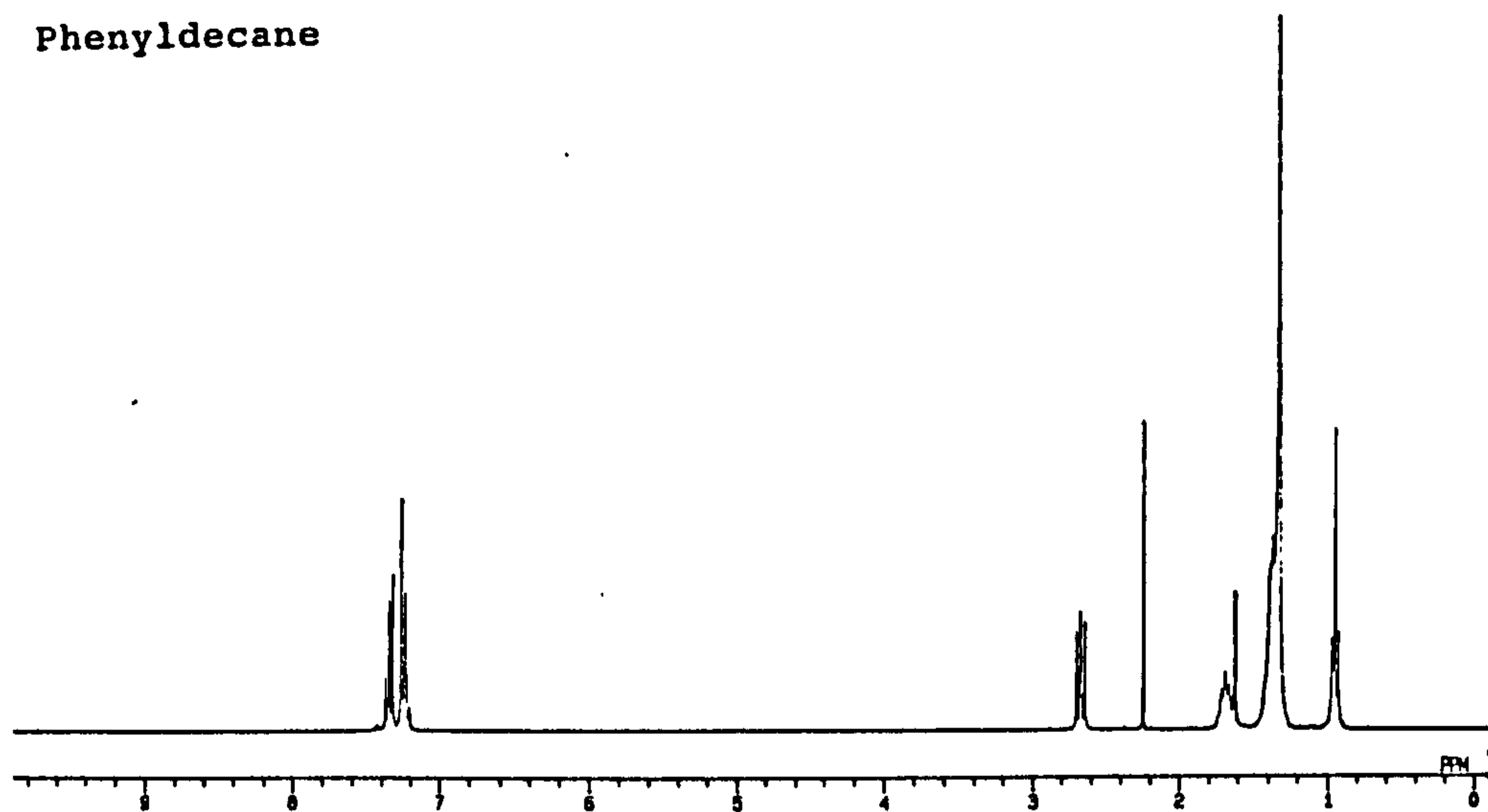
absorbption at 730  $\text{cm}^{-1}$ .

Proton NMR (Figure 2.11) confirmed the highly aliphatic nature of the aromatic UCM. Comparison of the spectrum with those of phenyldecane and 9-(2-phenylethyl)heptadecane (Figure 2.11) suggests a similar degree of aliphaticity to the latter, but with a greater proportion of tertiary centres (1.6 ppm), indicating a more highly branched structure.

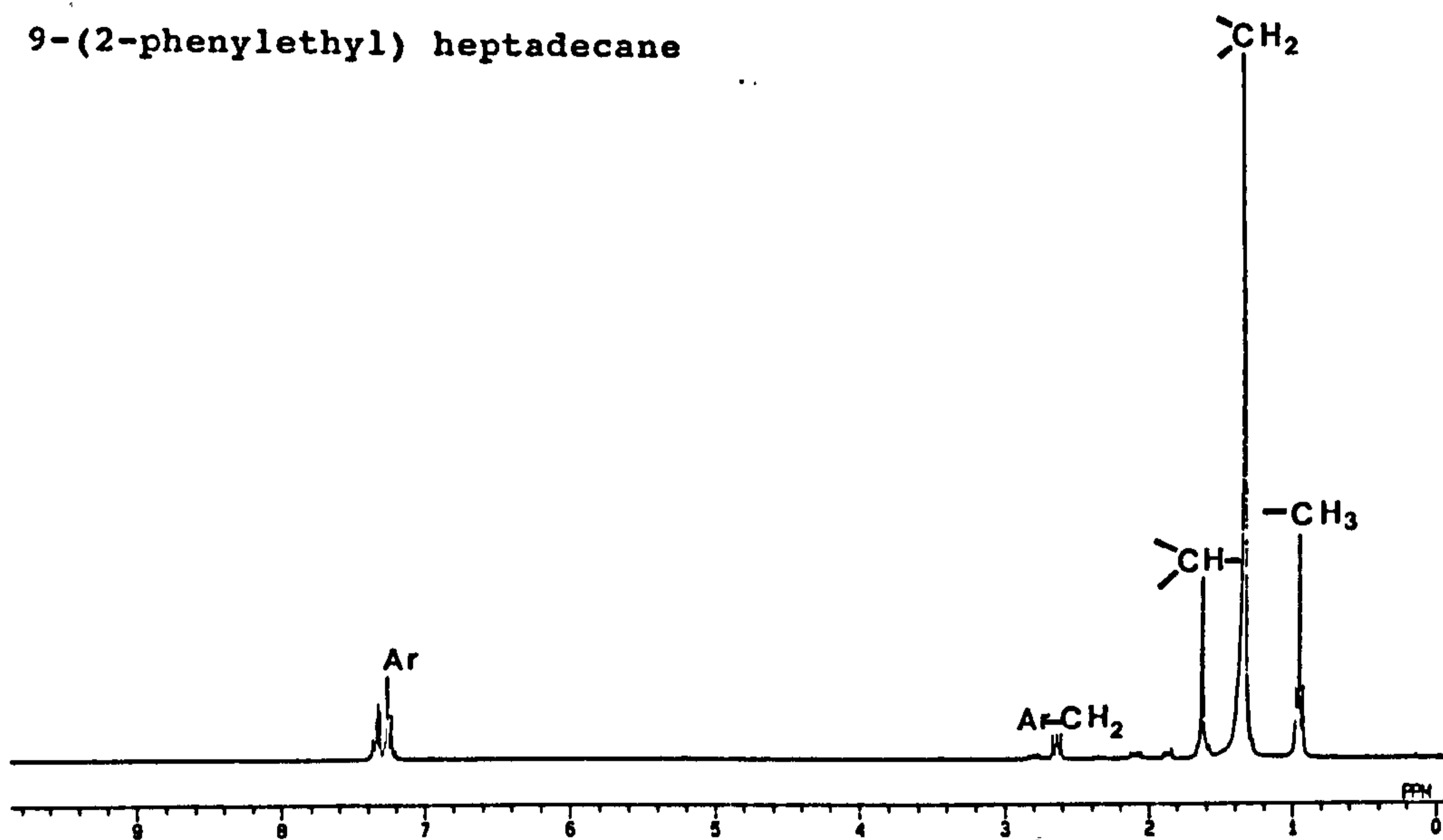
$^{13}\text{C}$ -NMR spectra of the two authentic compounds each exhibited a resonance at 143 ppm assigned to the ipso carbon (benzene carbon, 128 ppm + substitution effect, 15 ppm: Kemp, 1980). This shift is absent in the UCM spectrum (Figure 2.12), which instead exhibits a resonance at 138 ppm. This, supported by the IR data, is strong evidence that the majority of aromatic compounds are not of the mono alkyl benzene type. Alternative compounds which may explain a resonance at 138 ppm are alkyl tetralins (2.I), alkyl tetrahydrophenanthrenes (2.II), alkyl octahydrophenanthrenes (2.III) and alkyl indans (2.IV).



Phenyldecane



9-(2-phenylethyl) heptadecane



Aromatic UCM

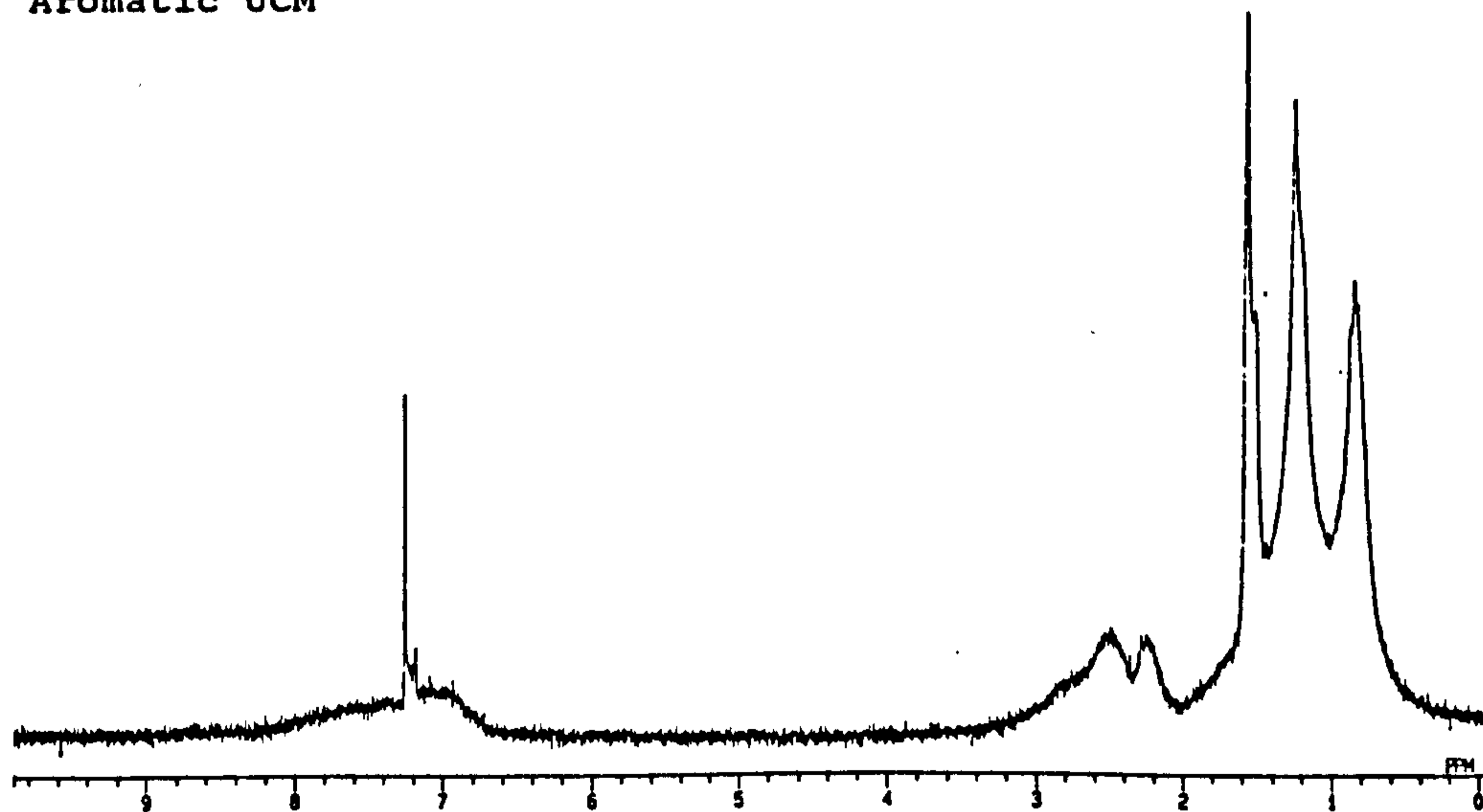
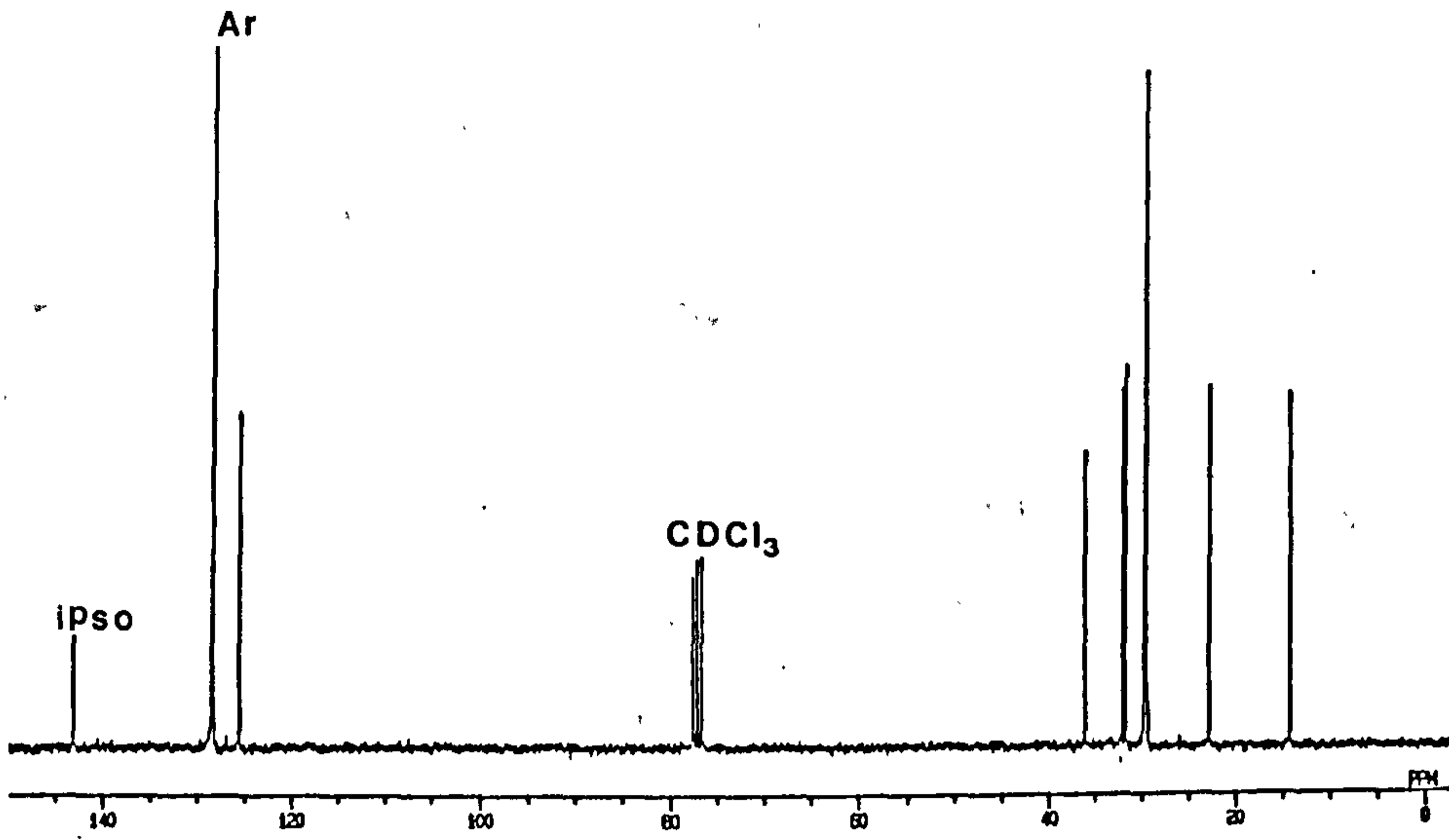
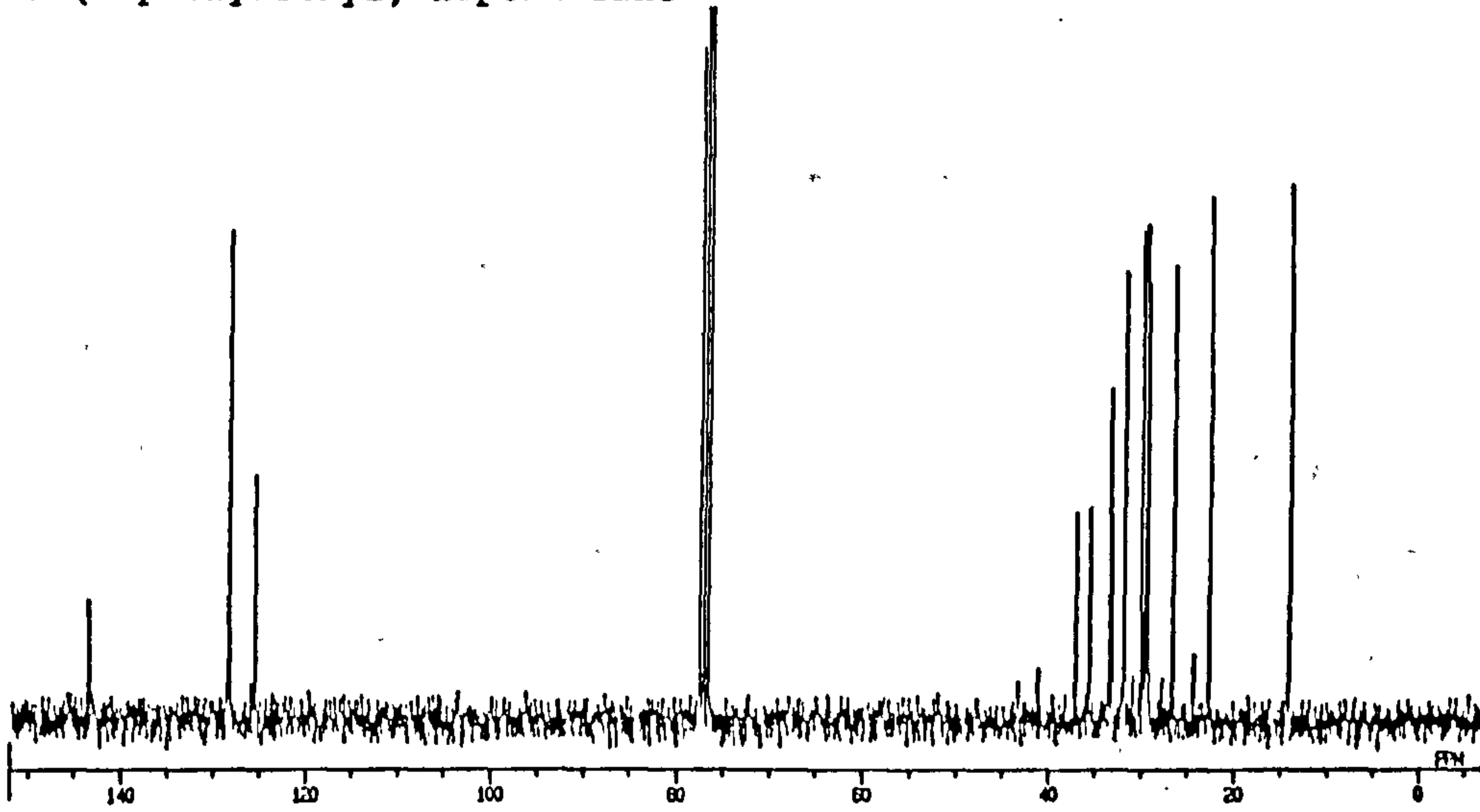


FIGURE 2.11. <sup>1</sup>H-NMR SPECTRA  
(Jeol 270MHz; Sample in CDCl<sub>3</sub>)

Phenyldecane



9-(2-phenylethyl) heptadecane



Aromatic UCM

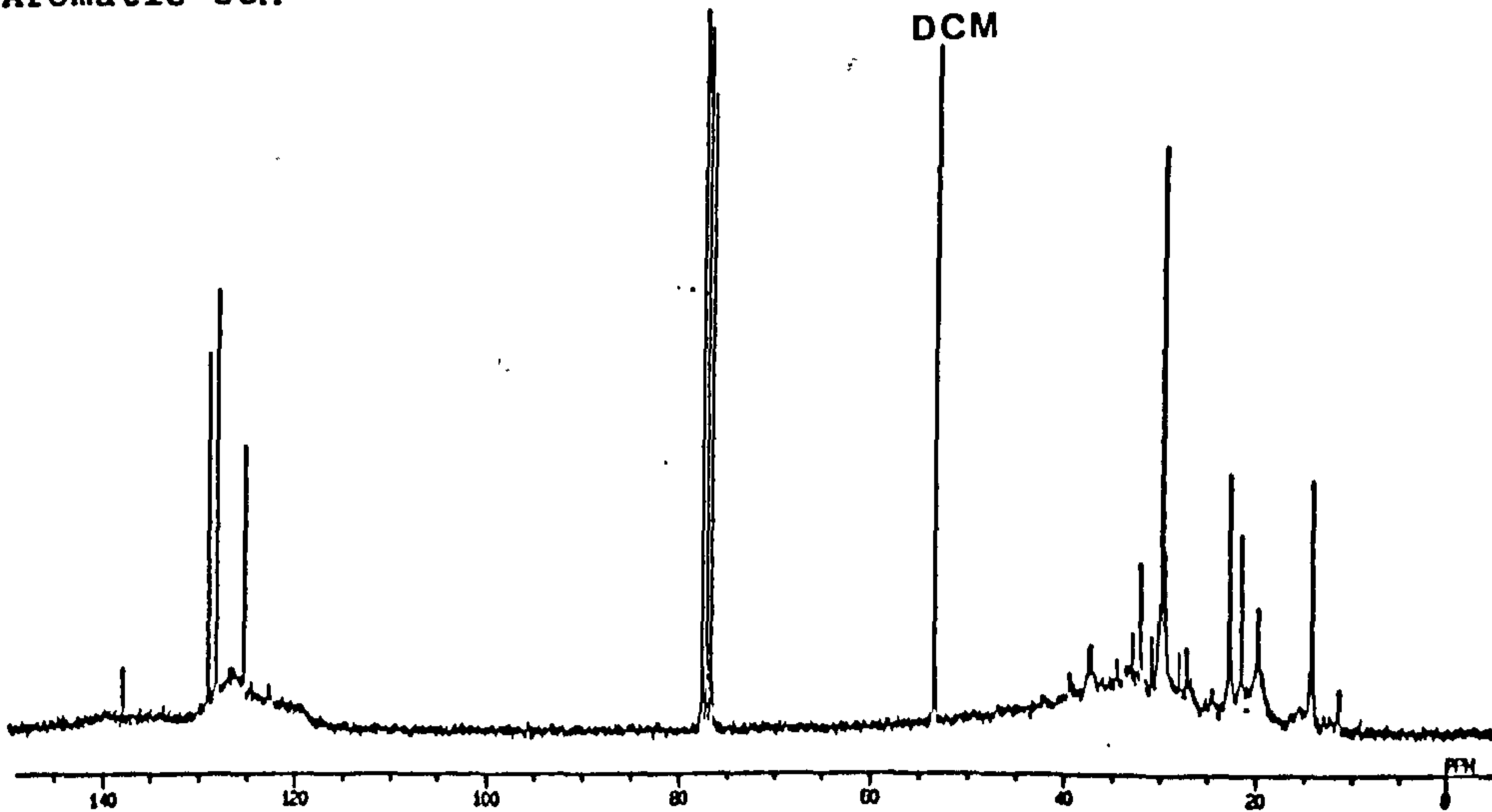


FIGURE 2.12.  $^{13}C$ -NMR SPECTRA  
(Jeol 270MHz; Sample in  $CDCl_3$ )



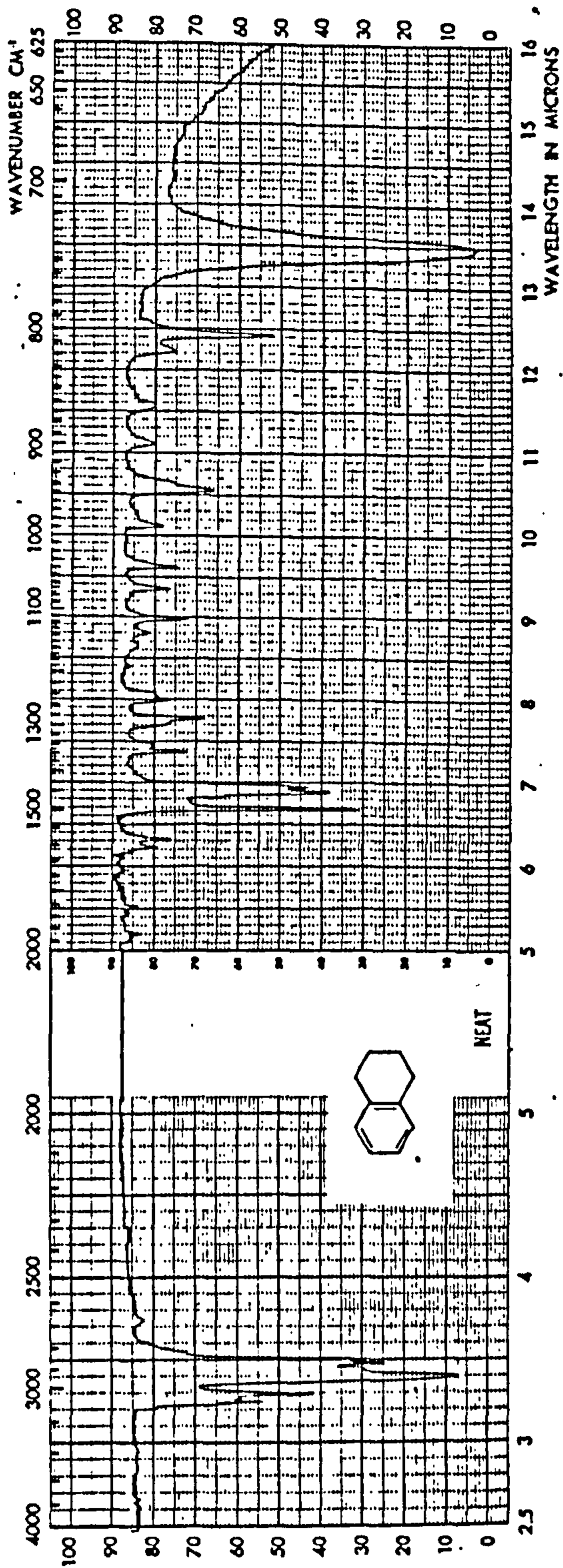
<sup>13</sup>C-NMR data for the alkylated homologues of three of these compound classes (2.I, 2.II and 2.IV) and for the parent compound of the fourth (Table 2.3), show that the closest correlation is between the UCM and alkyltetralins, with substitution on the carbons  $\beta$  to the aromatic ring (carbon 2). Substitution at any other position produces chemical shifts at 140 ppm or greater. Although no data were available for alkylated homologues of octahydrophenanthrene or alkyl substituents greater than methyl for the tetrahydrophenanthrenes, comparison of the resonance values for the parent hydrocarbons with the effects of alkyl substitution in the other compound classes suggests that significant differences would occur, especially at carbons 4a, 4b, 8a and 10a (Structures 2.II and 2.III) which would give rise to resonances at 132-134 ppm. The UCM exhibited no resonances in this region. Furthermore the differences between chemical shifts reported for alkyltetralins and those recorded for the aromatic UCM are  $\leq 0.8$  ppm, with most  $\pm 0.3$  ppm. Differences of this magnitude are probably insignificant (Dr. N Turner, Exeter University, Personal Communication, 1991). It is also noteworthy that the IR spectrum of tetralin (Figure 2.13) exhibits a strong absorbance at 730  $\text{cm}^{-1}$  (out of plane C-H def.) which was the strongest of the aromatic absorbances in the UCM spectrum (Figure 2.9).

EI mass spectra of the UCM (Figure 2.14) are characterised by a wide range of ions, some diagnostic of particular

TABLE 2.3 <sup>13</sup>C-NMR DATA FOR ALKYL SUBSTITUTED TETRALINS, INDANS, TETRA- AND OCTA-HYDRO PHENANTHRENES  
(Adapted from Adamczyk *et al.*, 1984; Morim *et al.*, 1985 and Seshadri *et al.*, 1978). Figures in brackets indicate closest fit from UCM. Differences >1 ppm are highlighted.

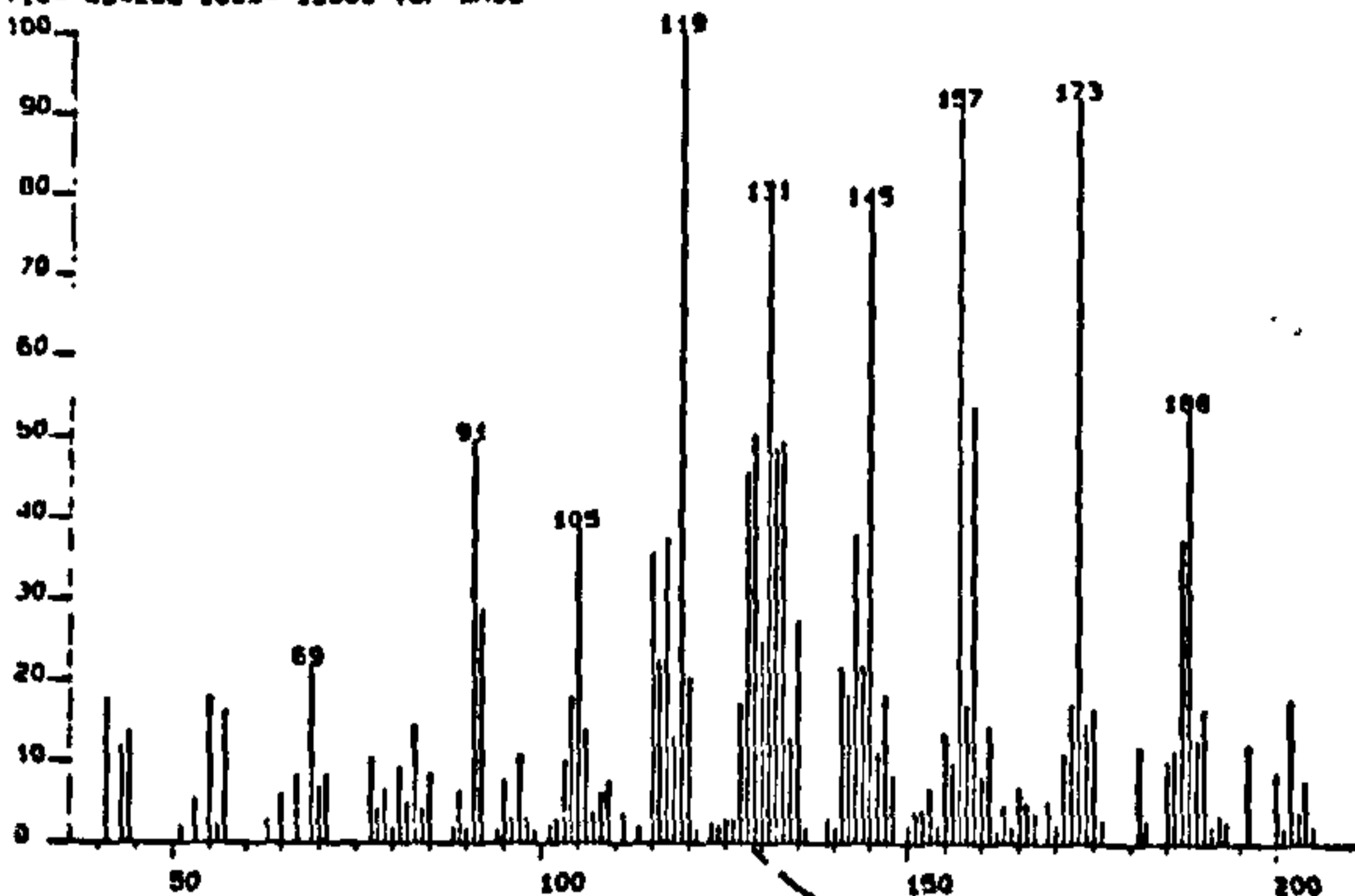
COMPOUNDS	CARBON ATOM													
	1	2	3	4	4a	4b	5	6	7	8	8a	9	10	10a
Tetralin	29.6	23.6	23.6	29.6			129.4	125.8	125.8	129.4		137.1	137.1	
1-butyltetralin	37.6 (37.5)	27.5 (29.3)	19.8 (19.9)	29.8 (29.7)			129.0 (128.9)	125.4 (125.2)	125.3 (125.2)	128.5 (128.2)		141.6 (137.8)	136.9 (137.7)	
2-butyltetralin	36.3 (37.0)	34.2 (35.0)	29.6 (29.7)	29.2 29.3			129.1 (128.9)	125.3 (125.2)	125.4 (125.2)	128.8 (128.9)		136.9 (137.7)	137.0 (137.7)	
6-butyltetralin	29.4 (29.4)	23.4 (22.7)	23.3 (22.7)	29.0 (29.3)			129.0 (128.9)	139.9 (137.7)	125.6 (125.2)	129.0 (128.9)		134.2 (137.7)	136.8 (137.7)	
Indan	33.0	25.4	33.0	124.4			126.2	126.3	124.0	144.0		144.0		
1-butylindan	44.8 (37.5)	32.2 (31.9)	31.7 (31.9)	124.3 (125.2)			125.9 (125.2)	126.1 (126.2)	123.5 (125.2)	147.8 (137.7)		144.0 (137.7)		
2-butylindan	39.4 (37.5)	40.2 (37.5)	39.4 (37.5)	124.3 (125.2)			125.9 (126.2)	125.9 (126.2)	124.3 (125.2)	143.7 (137.7)		143.7 (137.7)		
5-butylindan	32.8 (32.8)	25.6 (22.7)	32.5 (32.8)	124.4 (125.2)			140.8 (137.7)	126.2 (126.2)	124.0 (125.2)	141.3 (137.7)		144.2 (137.7)		
Tetrahydrophenanth.	30.4	23.2	23.0	25.5	132.5	131.5	123.0	125.0	124.7	128.6	132.9	125.9	128.3	134.2
1-methyl-	33.2	30.8	19.7	26.1	132.3	131.4	123.3	126.0	125.0	128.6	132.8	126.1	127.5	139.4
3-methyl-	30.4	31.1	29.2	34.3	132.4	131.4	123.0	125.8	124.8	128.6	132.7	125.8	128.1	133.9
Octahydrophenanth.	30.2	23.8	23.2	26.3	134.0	134.0	26.3	23.2	23.8	30.2	134.8	126.5	126.5	134.8



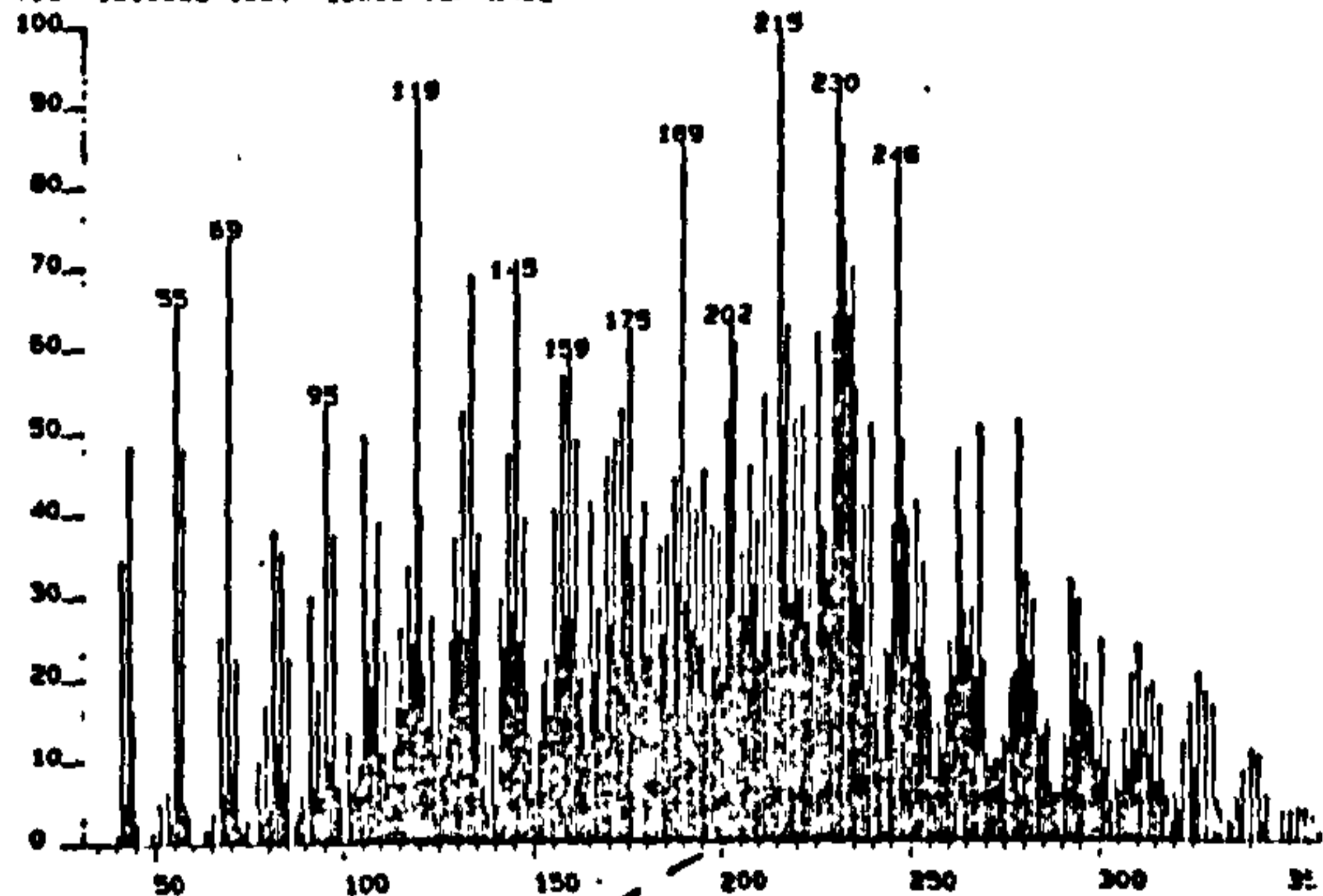


**FIGURE 2.13. IR SPECTRUM OF 1,2,3,4-TETRAHYDRONAPHTHALENE  
(TETRALIN; Taken from Pouchert, 1975)**

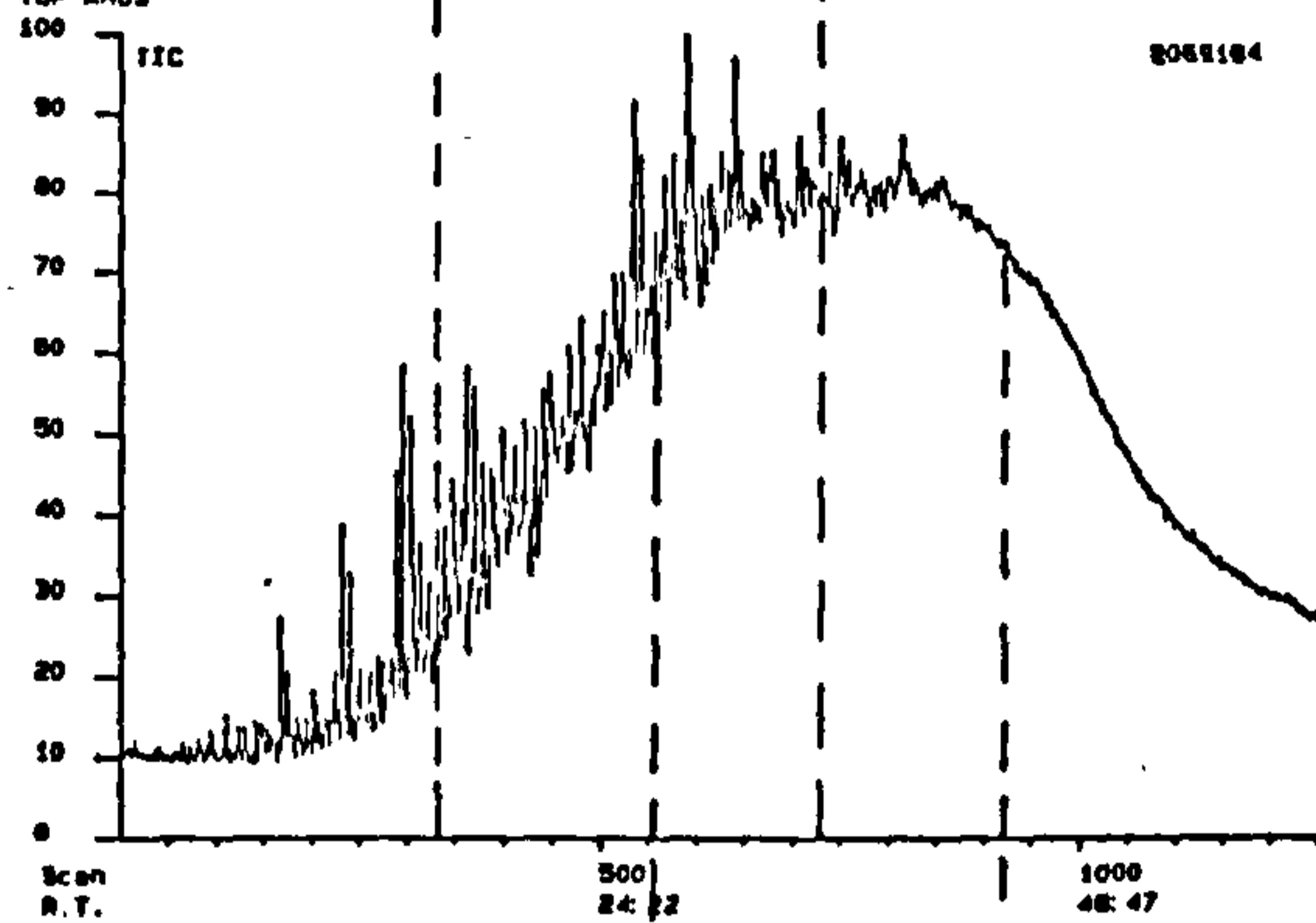
Exact Nominal Multiplet Ref / Lock Exc / Half Significant Saturated  
DS90 REVILLO080 329 RT= 18: 01 +EI SLAP 14-Oct-81 14: 41  
TIC= 454288 100%- 15360 T.J.P AROS



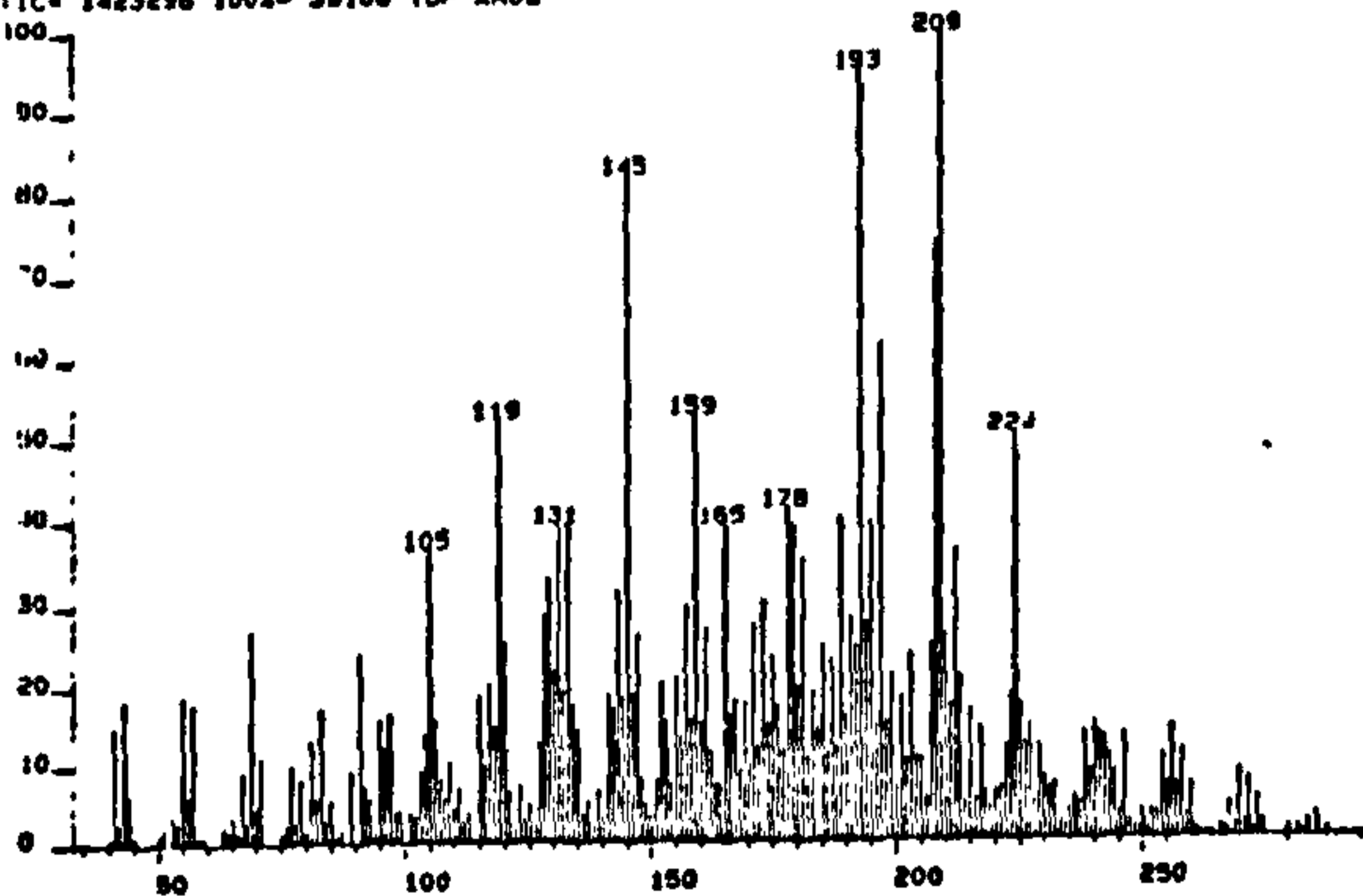
Exact Nominal Multiplet Ref / Lock Exc / Half Significant Saturated  
DS90 REVILLO080 729 RT= 33: 33 +EI SLAP 14-Oct-81 14: 41  
TIC= 1619328 100%- 19328 T.J.P AROS



DS90 Chromatogram Report Run: REVILLO080, 14-Oct-81 14: 41



Exact Nominal Multiplet Ref / Lock Exc / Half Significant Saturated  
DS90 REVILLO080 552 RT= 28: 54 +EI SLAP 14-Oct-81 14: 41  
TIC= 1423296 100%- 39168 T.J.P AROS



Exact Nominal Multiplet Ref / Lock Exc / Half Significant Saturated  
DS90 REVILLO080 919 RT= 44: 50 +EI SLAP 14-Oct-81 14: 41  
TIC= 1529728 100%- 15936 T.J.P AROS

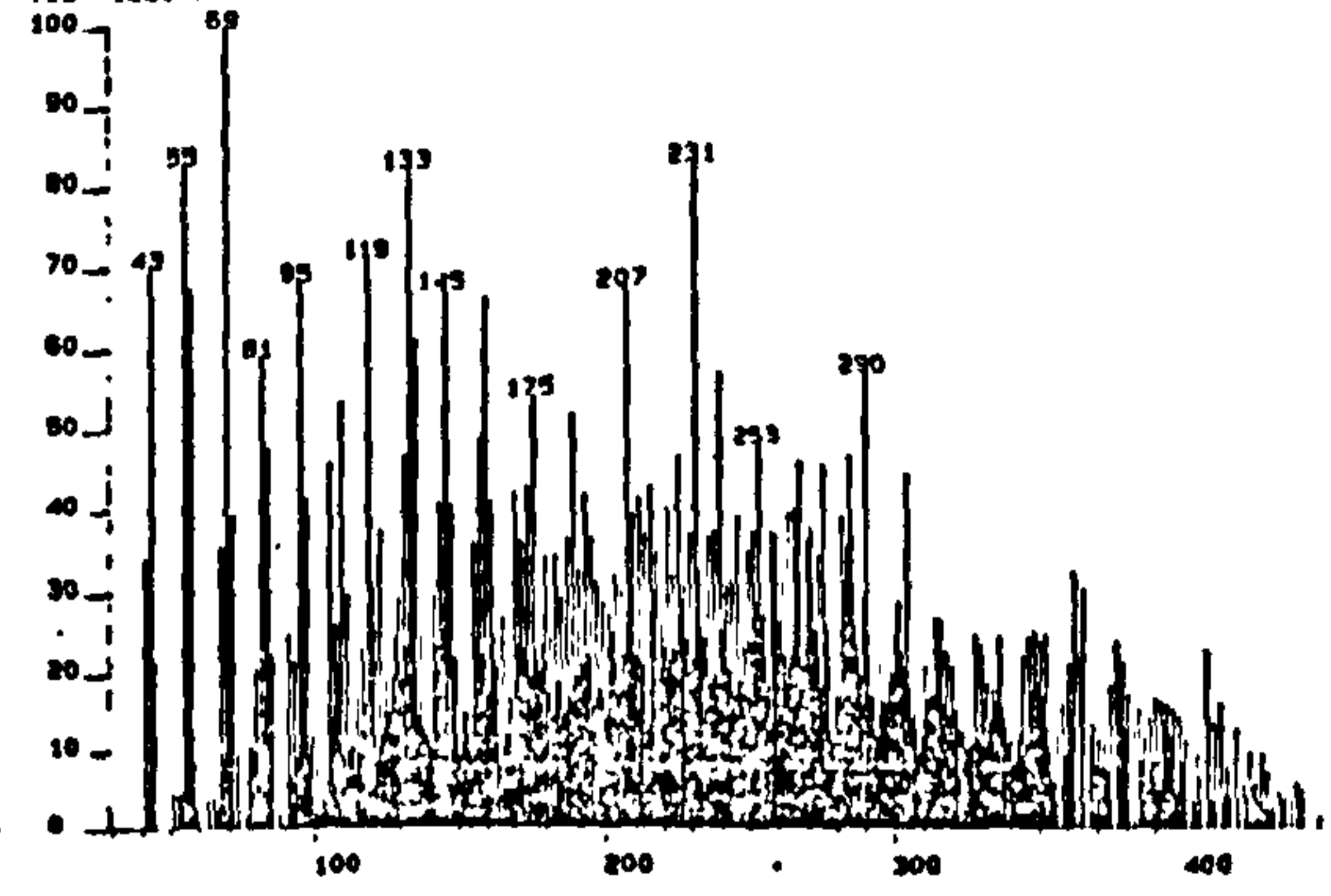


FIGURE 2.14. EI-GCMS OF THE AROMATIC UCM  
(J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier)



compound classes. One series is associated with alkylbenzenes ( $m/z$  91, 105, 119) and a second ( $m/z$  131, 145, 159, 173) could be assigned to naphthenobenzenes (e.g. tetralins), but in general the spectra are too complex to draw many detailed conclusions.

In summary elemental, IR, UV, UVF, NMR and MS of the aromatic UCM suggest that it is characterised by naphthenobenzene components, with aliphatic substitution on the saturated ring. Interestingly, such compounds have been shown to represent a relatively large proportion of the aromatic fraction of some ancient sediments (e.g. Green River shale; Anders *et al.* 1973; Gallegos, 1973; Anders *et al.*, 1975).

## **2.3 CHEMICAL OXIDATION OF THE AROMATIC UCM**

### **2.3.1 GENERAL**

Gough (1989) used chromium (VI) ( $\text{CrO}_3/\text{acetic acid}$ ) to oxidise the aliphatic UCMs of both crude and lubricating oils in an attempt to obtain structural information. He also used the same reagent to oxidise the aromatic fraction of a lubricating oil. This produced similar products to those of the aliphatic UCM i.e. *n*-carboxylic acids,  $\gamma$ -lactones and ketones. However, since the feedstock oils from which lubricating oils are made are de-aromatised during refining, these results are possibly of only limited

value and not relevant to the composition of the aromatic UCMs of crude oils in general. Few, if any, other studies of aromatic UCMs have been made and no other oxidants appear to have been used to characterise aromatic UCMs, even though a variety of reagents are available. Use of reagents such as ruthenium tetroxide ( $\text{RuO}_4$ ) which are known to specifically oxidise aromatic rings, might be expected to yield additional compositional information.

Therefore, in the present study both Chromium (VI) and  $\text{RuO}_4$  were used to oxidise the aromatic UCM of biodegraded TJP oil with the aim of obtaining more generally applicable compositional information.

### 2.3.2 CHROMIUM TRIOXIDE OXIDATION

Oxidation of the aromatic UCM was carried out according to the method of Gough (1989). The recovery of oxidation products (>80%) was comparable to Gough (1989) (Table 2.4). These resolved oxidation products accounted for ca. 25% of the recovered material by GC (estimated by the time slice area measurement, Appendix I). The major resolved oxidation products were *n*-monocarboxylic acids (Figure 2.15) (*n*- $\text{C}_{5-23}$ ) with a strong odd over even predominance between *n*- $\text{C}_9$  and *n*- $\text{C}_{16}$ . This is comparable to the results of Gough (1989) for a lubricating oil (*n*- $\text{C}_{7-20}$ ). Other, relatively minor oxidation products were alkylketones ( $\text{C}_7$ - $\text{C}_{20}$ ; max  $\text{C}_{11}$ ), including a secondary series of iso-methyl

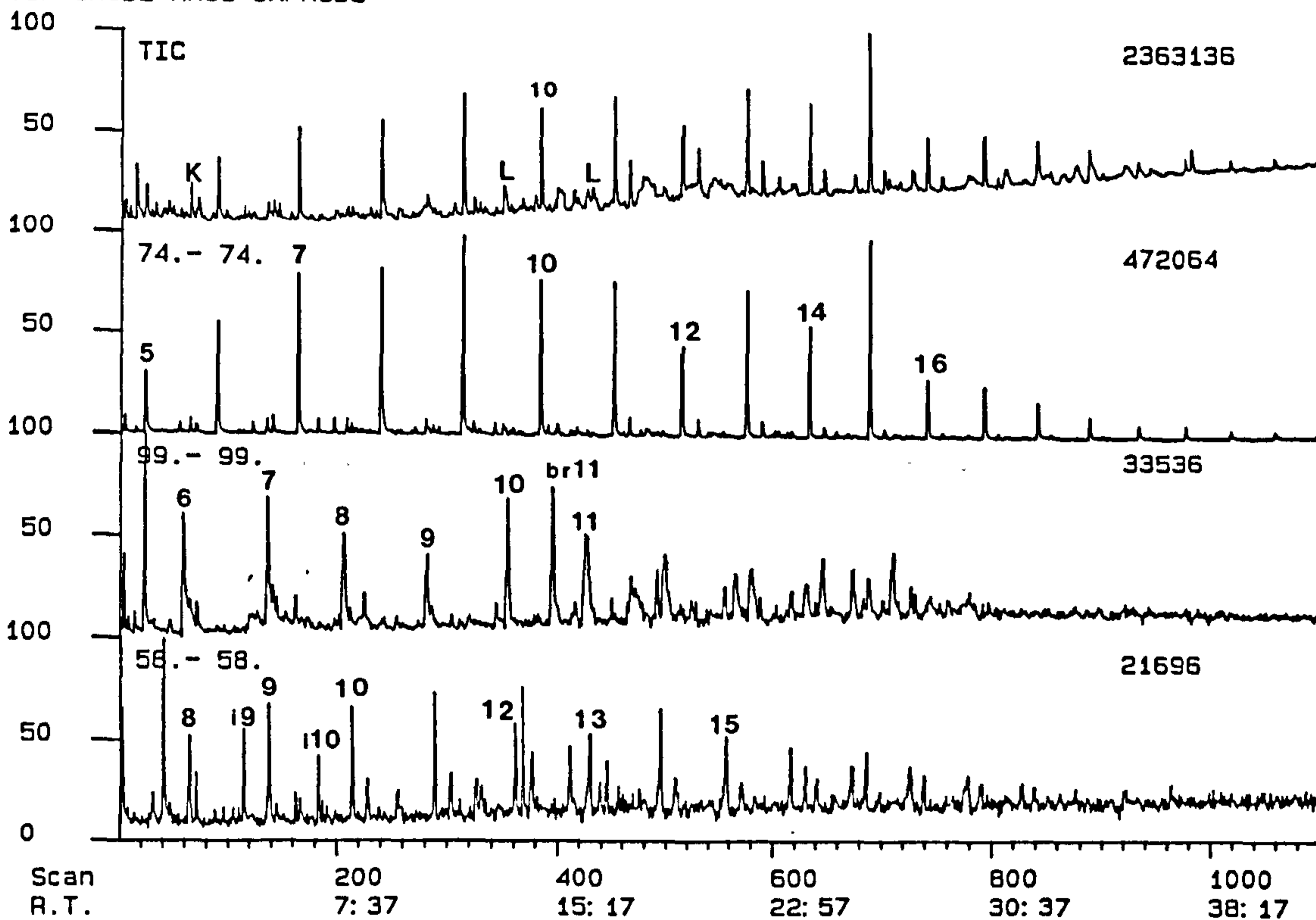
**TABLE 2.4 TJP Crude Oxidations**

Sample Weight (mg)	Total Recovered Material (mg)	Recovery (%) (By weight)
<i>chromium trioxide</i>		
<i>this study</i> 10	8.1	81
<i>Gough (1989)</i> 50.8	42.2	83*
<i>ruthenium tetroxide</i>		
90	89	98
66.4	57.8	87
55.0	26.5	48

\* Results from Gough (1989) are from oxidation of a lubricating oil.



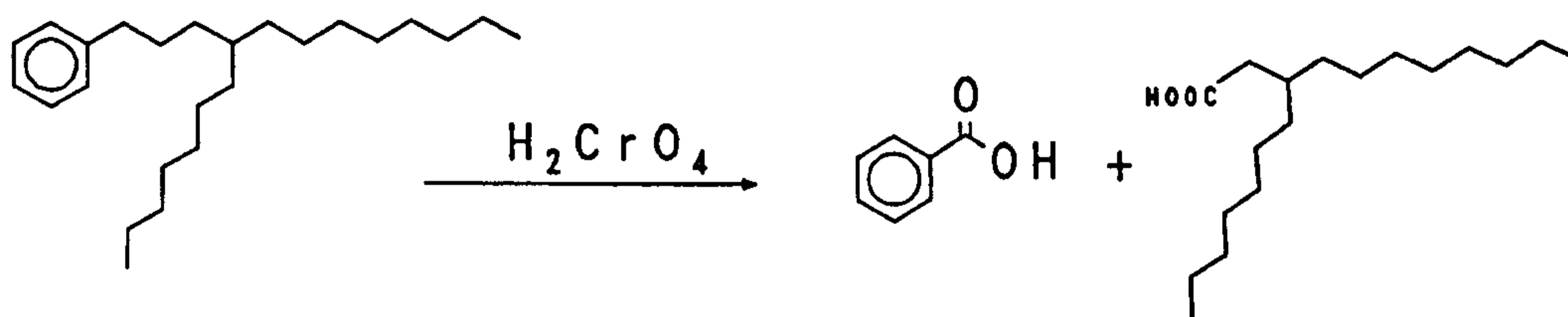
DS90 Chromatogram report Run: REVILL0024, 15-Oct-90 14:40  
 TJP CRUDE AROS OXPRODS



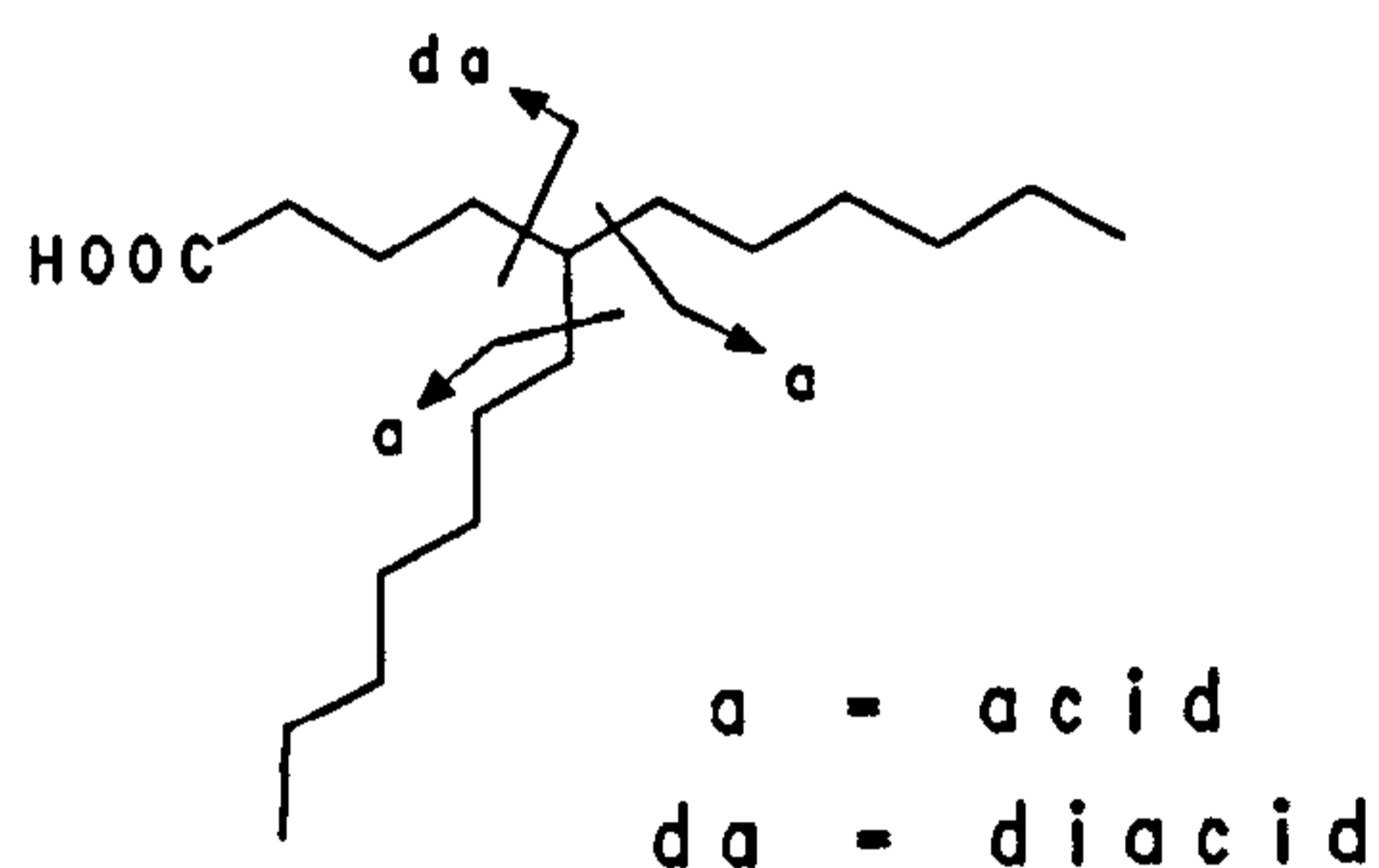
**FIGURE 2.15. CHROMATOGRAMS FOR THE AROMATIC UCM  $\text{CrO}_3$  OXIDATION PRODUCTS**

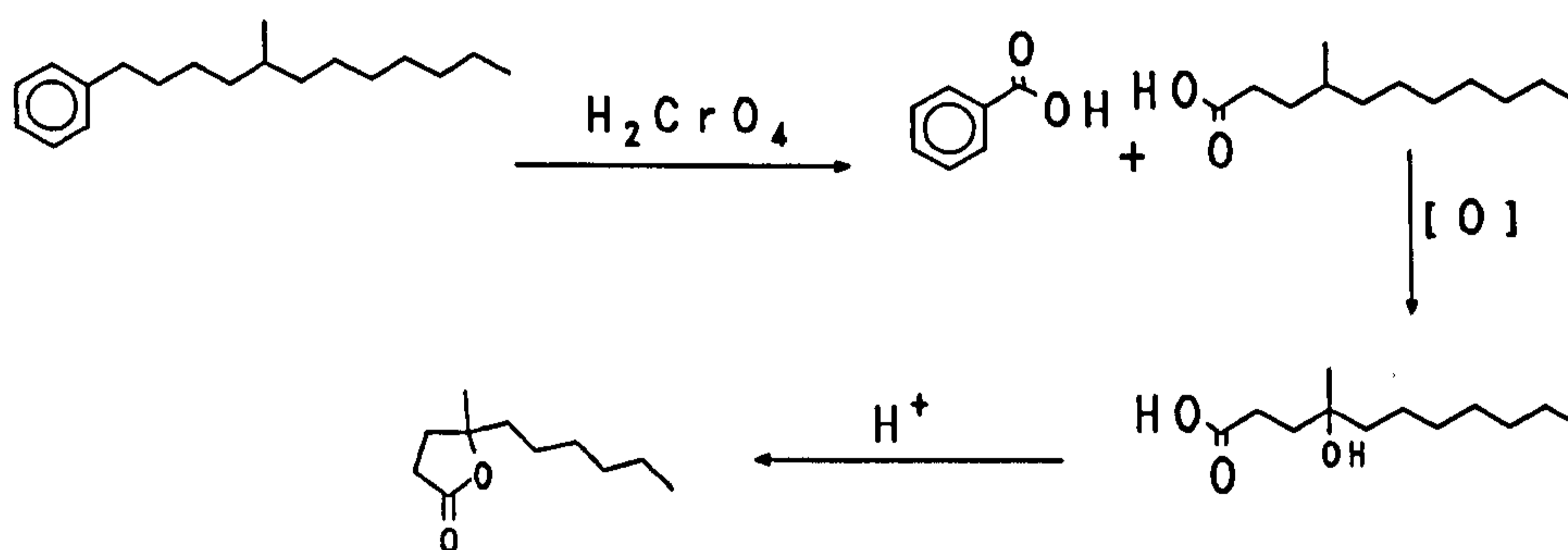
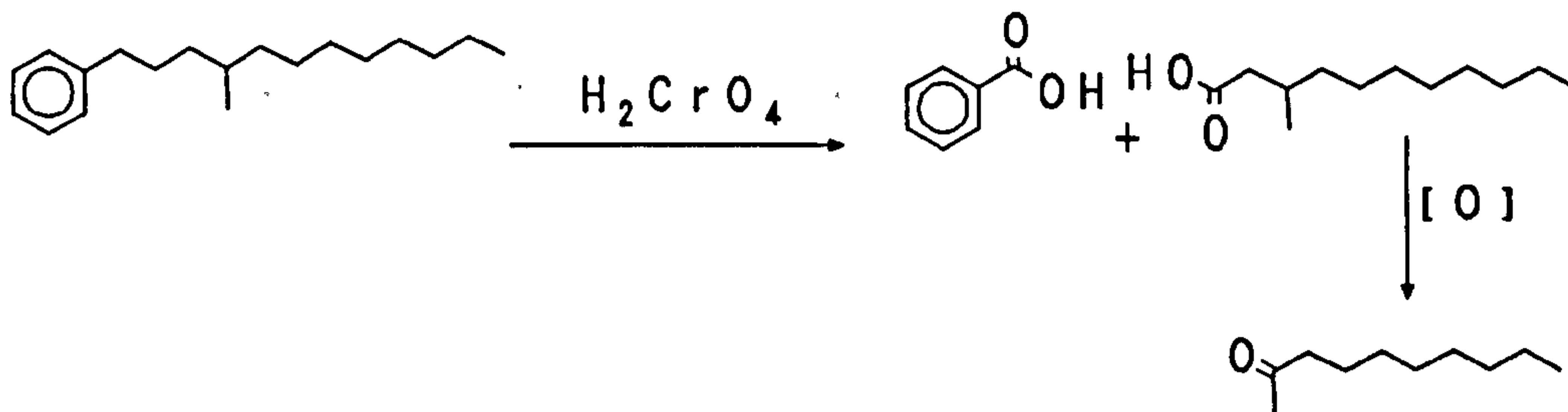
( $m/z$  74 = *n*-carboxylic acid methylesters;  $m/z$  99 =  $\gamma$ -lactones;  
 $m/z$  58 = ketones; numbers indicate carbon number, br = branched,  
 i = iso, K = ketone, L = Lactone)

branched ketones, and a series of  $\gamma$ -methyl- $\gamma$ -lactones ( $C_5$ - $C_{11+}$ ; max  $C_{10}$ ). The dominant ketone was identified, by comparison of retention time and MS with Gough (1989), as a  $C_{13}$  isoprenoid. Gough (1989) attributed similar oxidation products to oxidation of the alkyl side chain of alkylbenzenes due to preference for attack at the benzylic carbon,

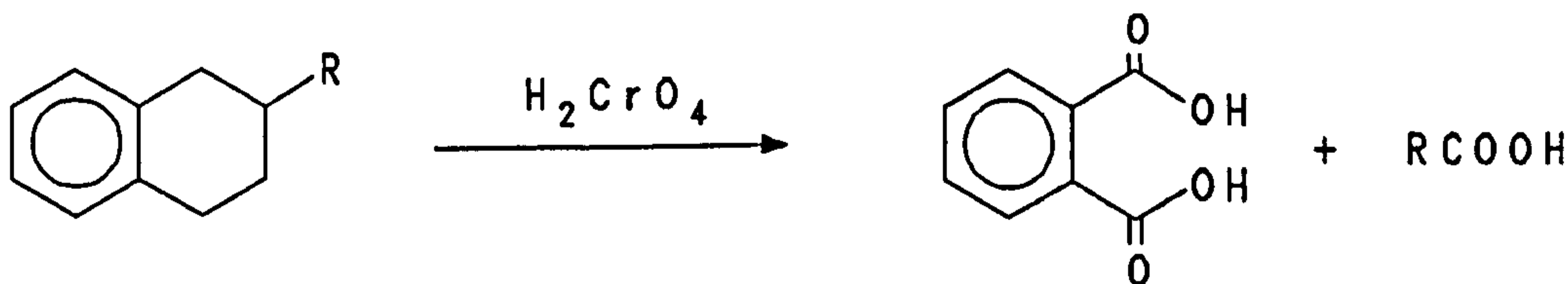


followed by secondary oxidation of the alkyl chain to yield other acids, ketones or lactones; viz.,



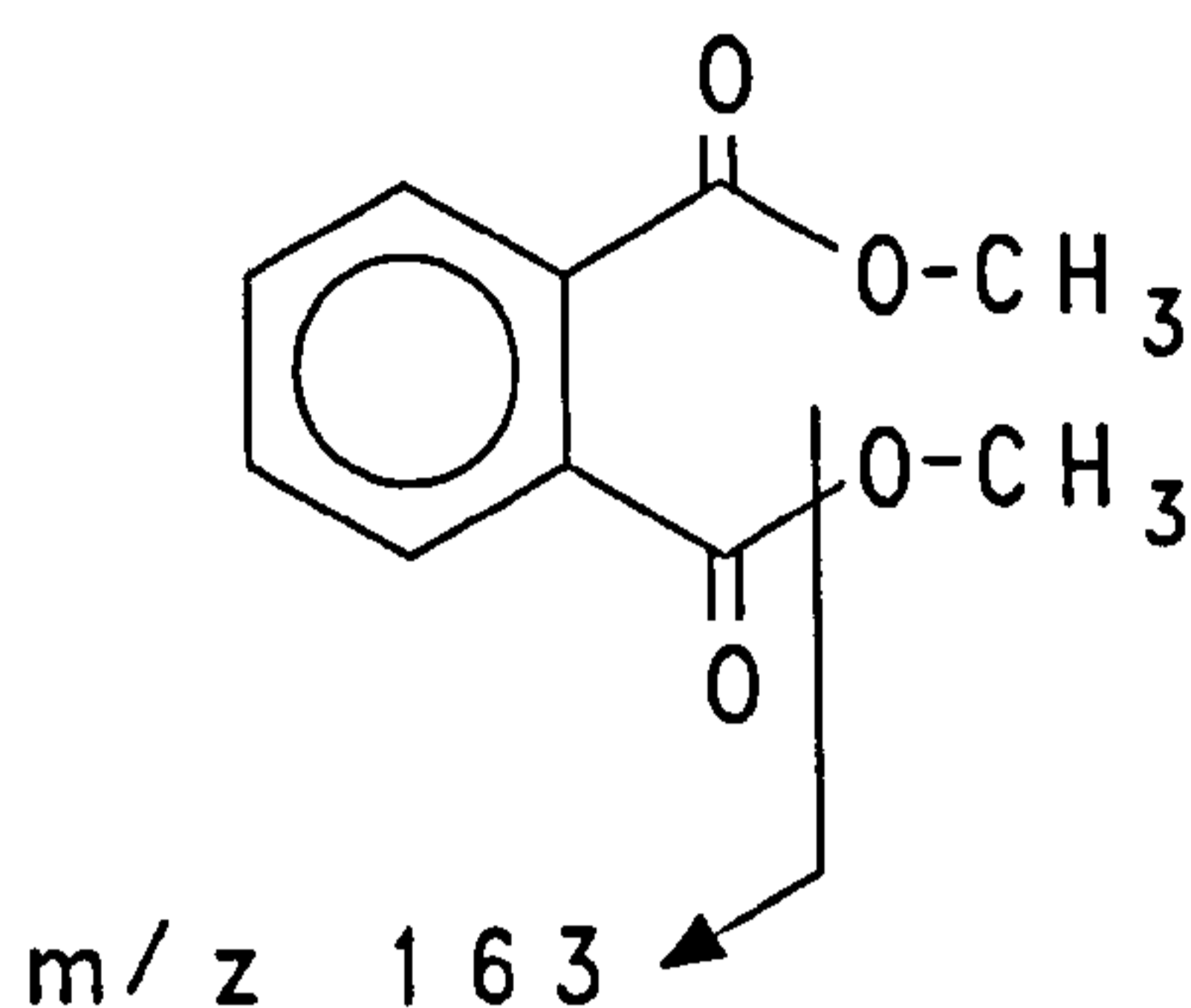


However, the IR and NMR data of the TJP aromatic UCM (Section 2.2) indicated that the alkyl groups were not on the aromatic rings as suggested by Gough (1989) for the lubricating oil but rather that naphthenoaromatic, tetralin-type compounds were present. If this is the case, other oxidation products, notably *o*-phthalic acid might be expected.

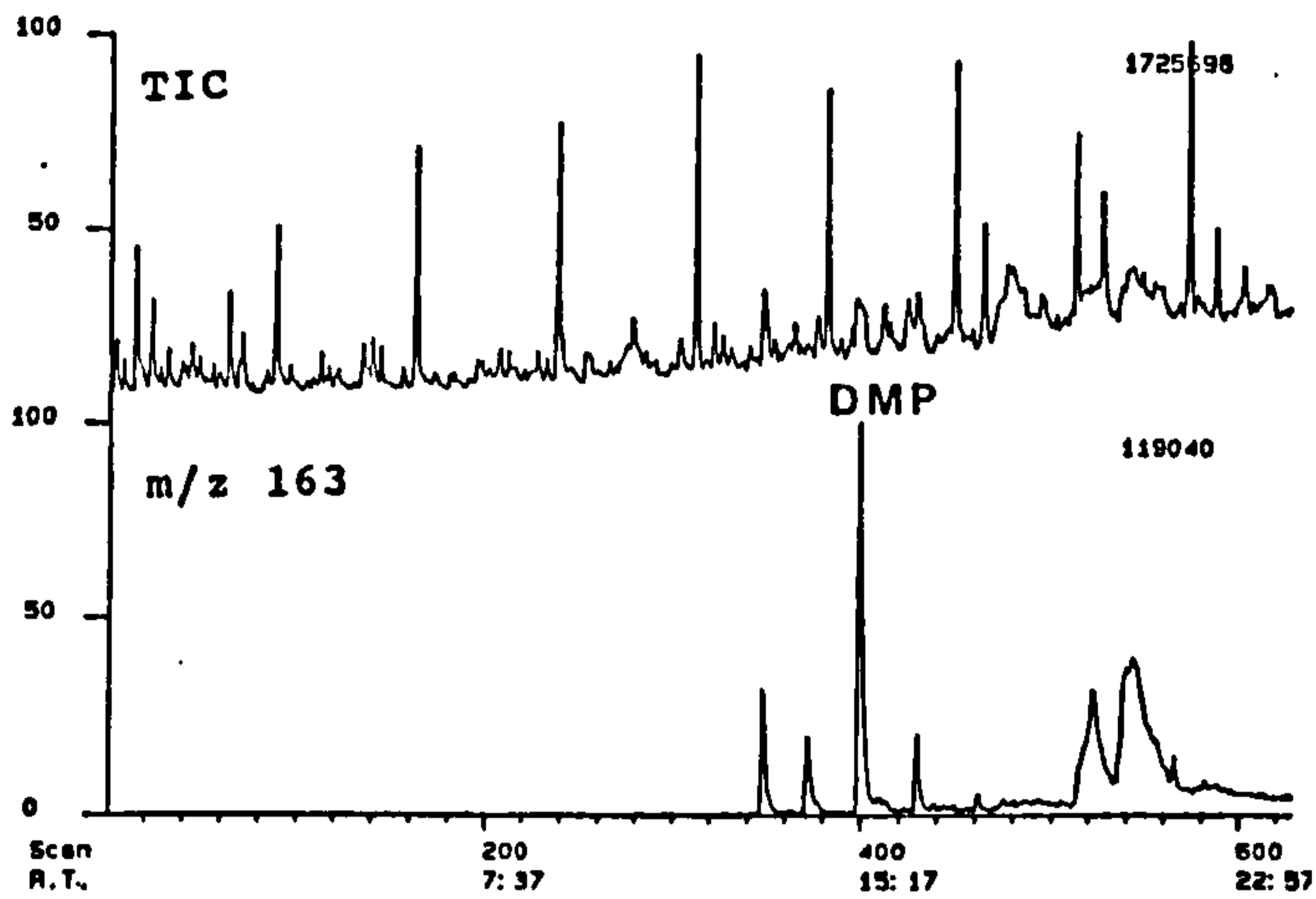




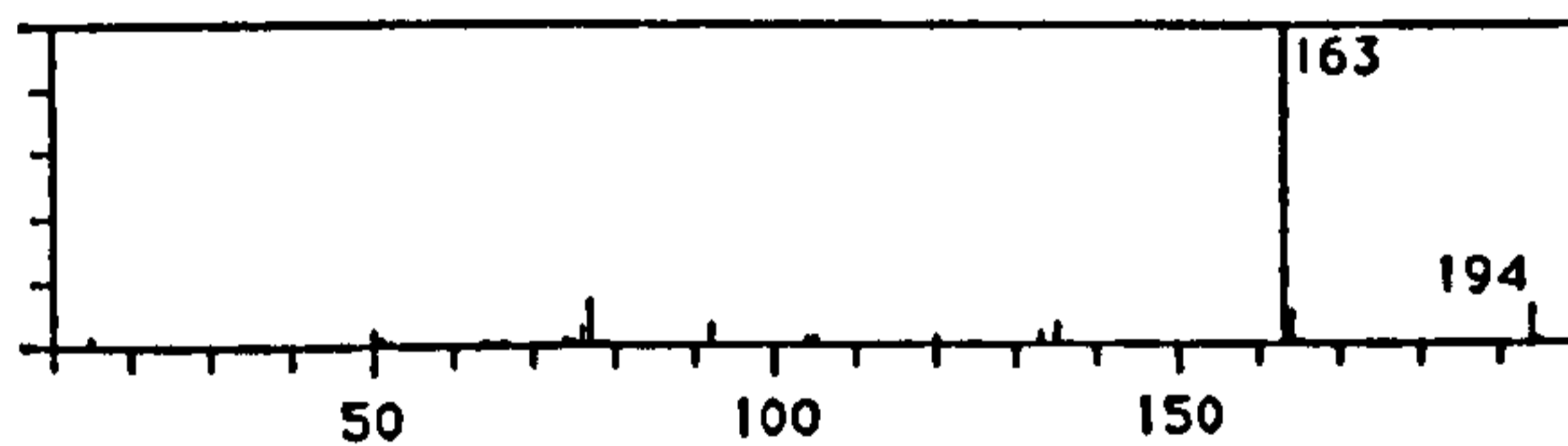
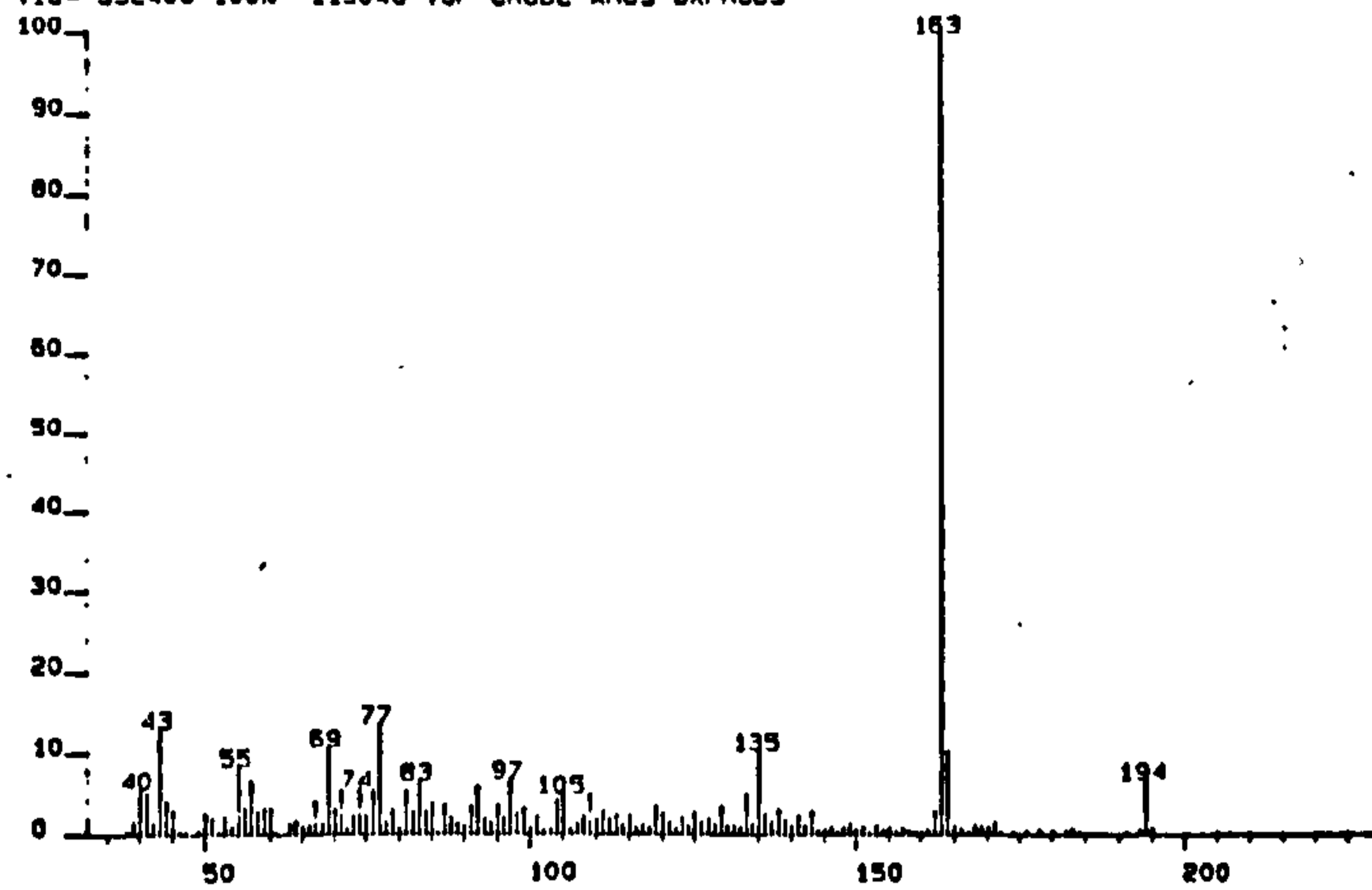
After methylation during work-up phthalic acid would be present as the dimethyl ester, which unlike other phthalate esters, has a base peak at  $m/z$  163 (cf.  $m/z$  149) (Middleditch, 1989).



Dimethylphthalate was identified as a minor component (ca. 5% of TIC) of the UCM oxidation products by GCMS. The spectrum showed strong similarities to that reported for dimethylphthalate (Figure 2.16; Middleditch, 1989). (Although dimethylphthalate has been reported as an analytical artefact (plasticiser) in some solvents and in water which has passed through plastic tubing (Middleditch, 1989), there is no evidence for this compound in the procedural blank (Figure 2.17). Thus the presence of dimethylphthalate is strong evidence for the presence of naphthenobenzenes in the UCM). No evidence for esters of methyl phthalic acid or tetrahydrophenanthrene-type structures could be found, in agreement with the results from <sup>13</sup>C-NMR spectroscopy that such structures were absent.



Exact Nominal Multiplet Ref / Lock Exc / Half Significant Saturated  
 DS90 REVILL0024.400 RT= 15:17 +EI SLAP 15-Oct-90 14:40  
 TIC= 532400 100%- 119040 TJP CRUDE AROS OXPRODS



Library spectrum  
 Middleditch (1989)

FIGURE 2.16. EXPANDED CHROMATOGRAM AND MASS SPECTRUM TO SHOW THE PRESENCE OF DIMETHYLPHTHALATE IN THE  $\text{CrO}_3$  OXIDATION PRODUCTS

DS90 Chromatogram report Run: REVILL0081. 14-Oct-91 16:10  
 CR03 OXID BLANK

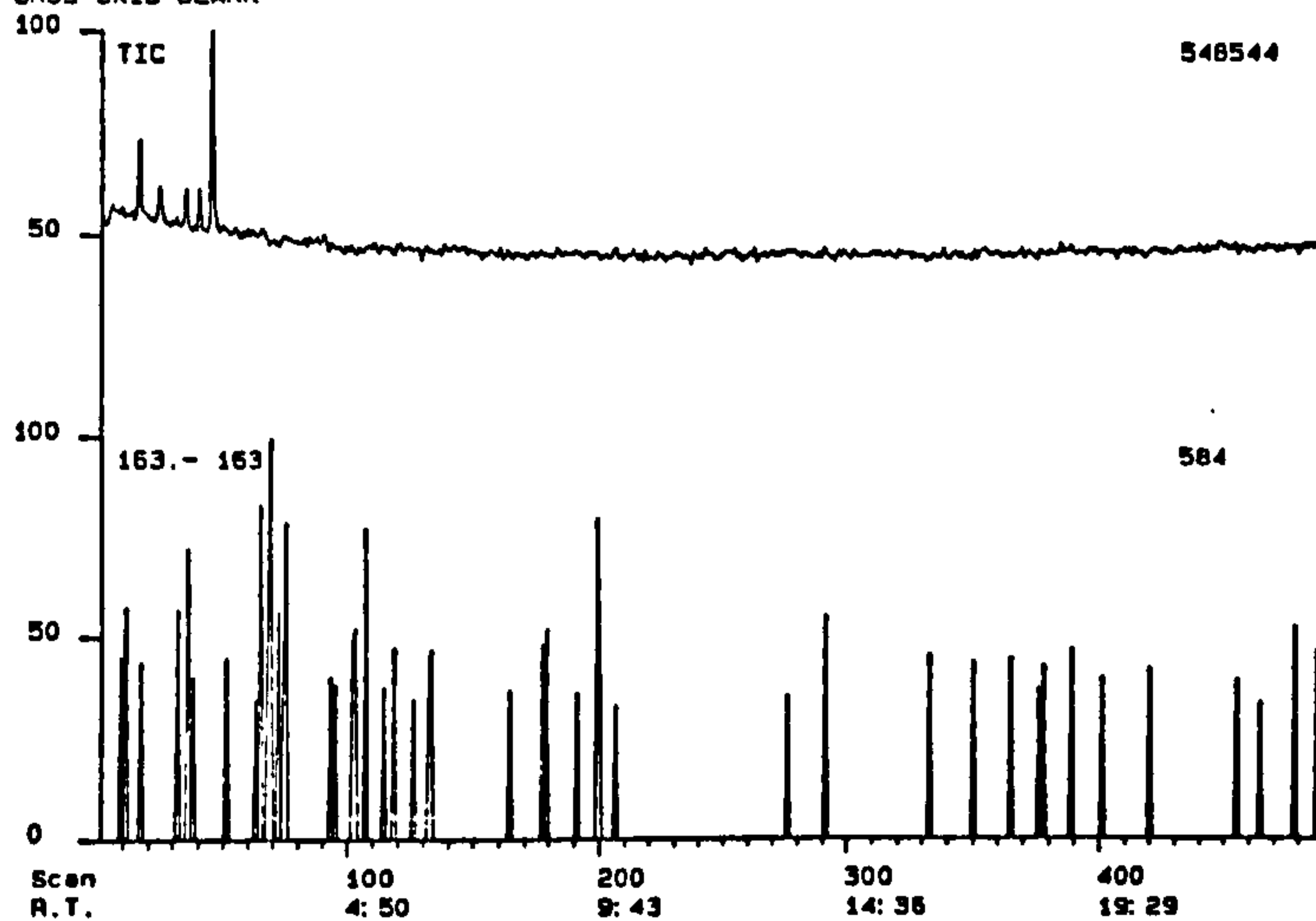
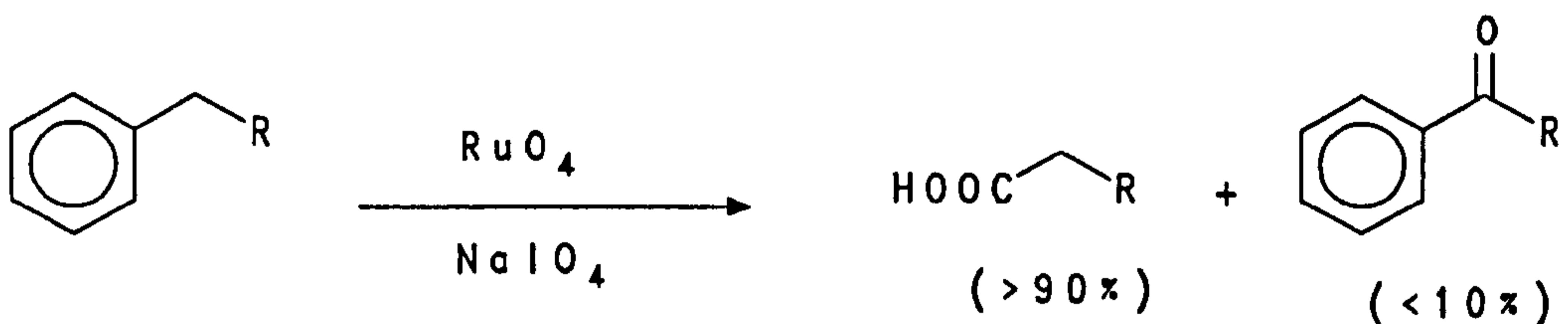


FIGURE 2.17. M/Z 163 CHROMATOGRAM OF THE OXIDATION BLANK

### 2.3.3 RUTHENIUM TETROXIDE OXIDATION

The relatively non-specific mechanism of the Cr(VI) oxidation (March, 1985) was followed by investigating a more specific reagent for the oxidation of aromatic hydrocarbons (*viz.* RuO<sub>4</sub>). The oxidation conditions were modified from those of Stock and Tse (1983) and Boucher *et al.* (1990), and were validated by a series of trials utilising phenyldecane as substrate (Table 2.5). Recoveries of *ca.* 60% were achieved, with undecanoic acid (analysed as a methyl ester) accounting for up to 80% of the products. Interestingly, there was no evidence for the benzylic ketone previously reported as a by-product (Stock and Tse, 1983; Trifilieff, 1987);



Oxidation of the aromatic UCM generally gave good recoveries (48-99%; Table 2.4) and produced a series of resolved (5% by GC *cf.* 25% by CrO<sub>3</sub>/acetic acid) components superimposed on a UCM (Figure 2.18). The former were mostly *n*-carboxylic acids. However, the *m/z* 74 chromatogram, typical of carboxylic acid methyl esters, also exhibited a significant UCM, suggesting the presence



TABLE 2.5 RUTHENIUM TETROXIDE OXIDATIONS OF PHENYLDECANE

Substrate (mg)	RuCl <sub>3</sub> mg(molar ratio)	NaIO <sub>4</sub> mg(molar ratio)	Solvent	Mixing	T°C	Products (mg)	% Recovery
205	6.0(0.03)	2.0(10)	CCl <sub>4</sub>	static	Amb.	120	59
200	50 (0.26)	2.0(10)	CCl <sub>4</sub>	static	Amb.	124	62
200	100 (0.53)	1.0(5.2)	CCl <sub>4</sub>	shaken	Amb.	138	69
218	2.6(0.01)	3.4(17.3)	CCl <sub>4</sub>	static	Amb.	189	86
200	2.6(0.01)	3.4(17.3)	CCl <sub>4</sub>	m/s	35 <sup>a</sup>	128	64
200	2.6(0.01)	3.4(17.3)	CCl <sub>4</sub>	mechanical	35 <sup>b</sup>	130	65
200	2.6(0.01)	3.4(17.3)	chloroform	mechanical	35 <sup>b</sup>	126	63

<sup>a</sup> = non-thermostated

<sup>b</sup> = thermostated

Amb. = ambient temperature

m/s = magnetic stirrer

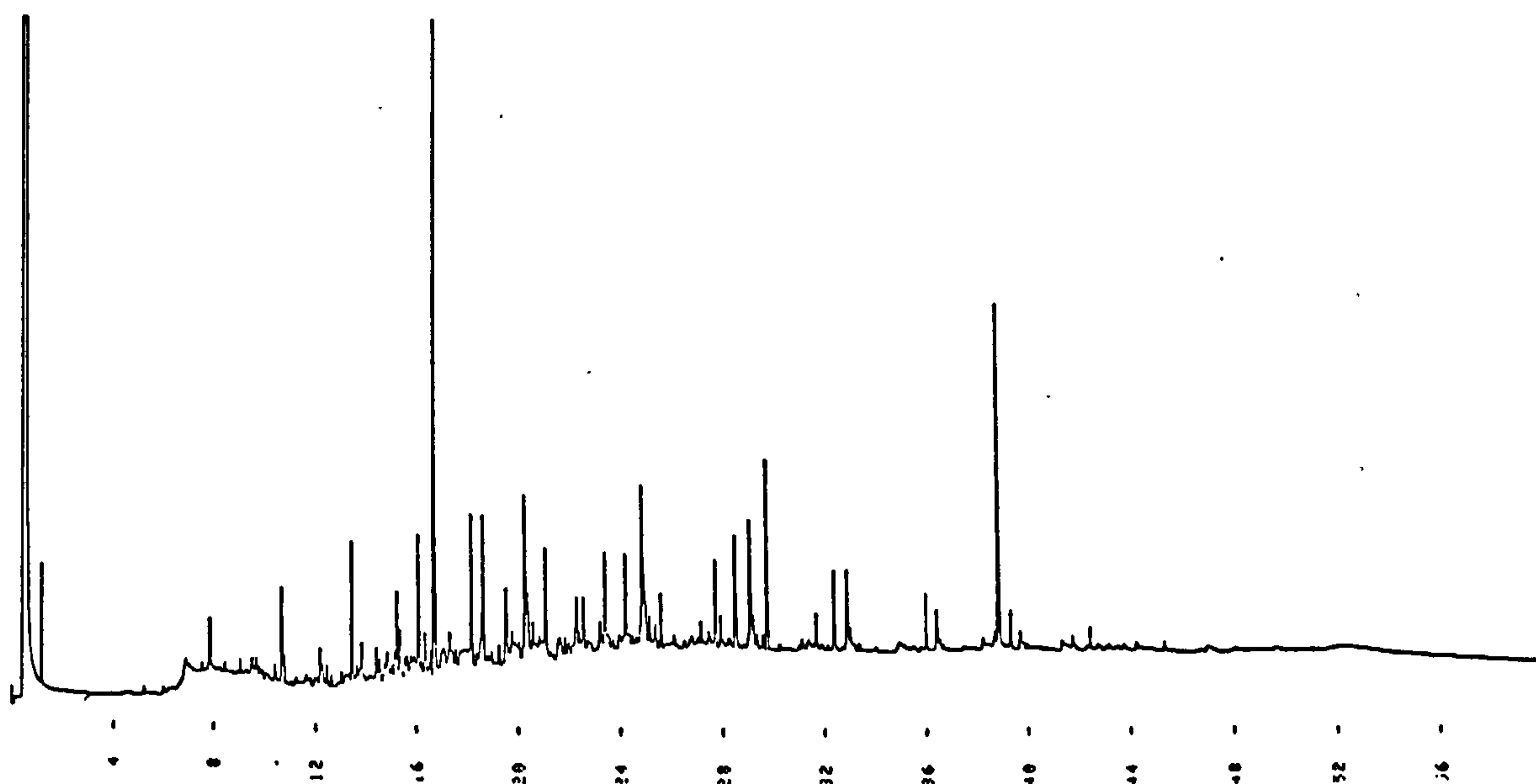


FIGURE 2.18. GC-FID OF THE AROMATIC UCM RuO<sub>4</sub> OXIDATION PRODUCTS  
(J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier)

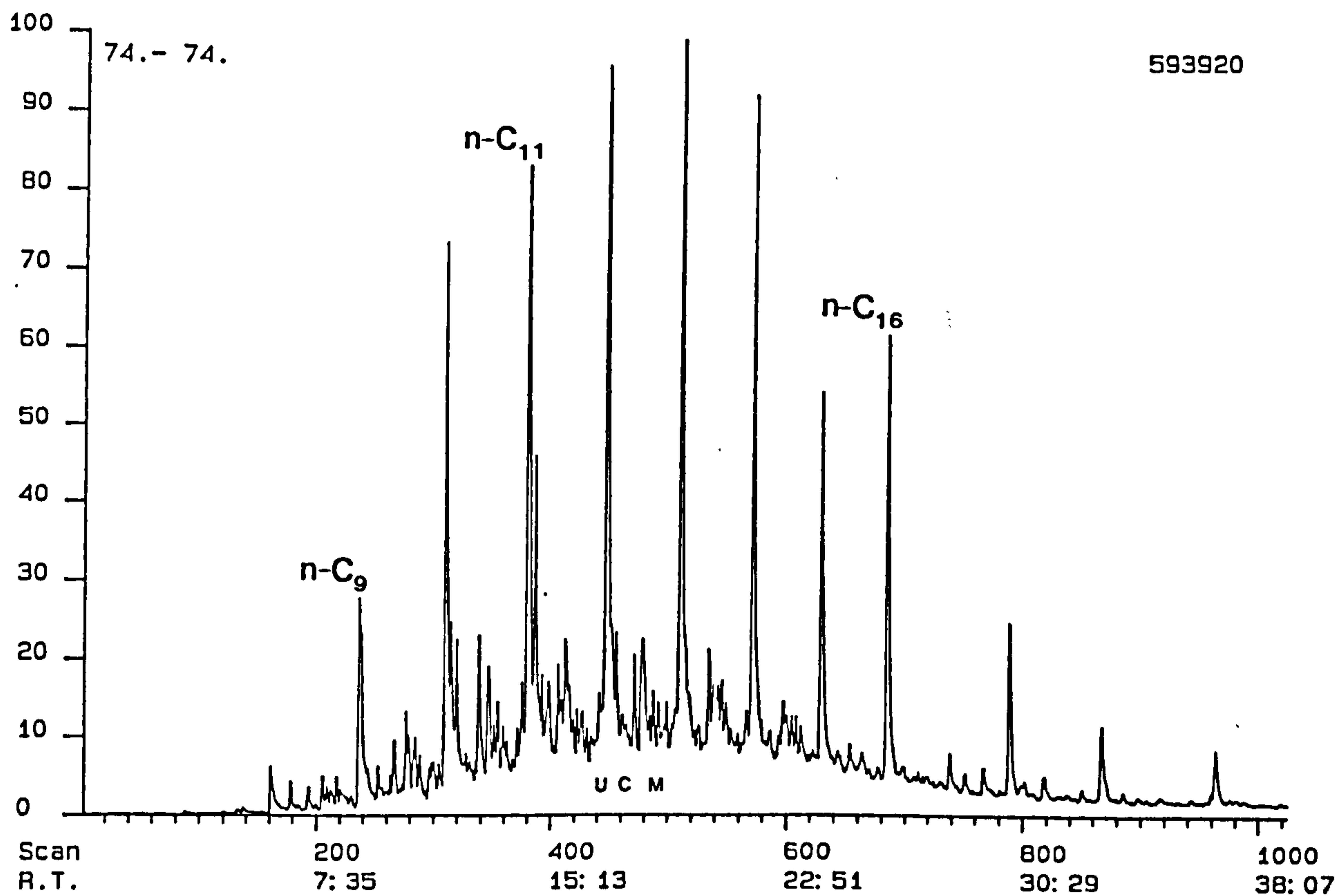


FIGURE 2.19. M/Z 74 CHROMATOGRAM OF THE AROMATIC UCM RuO<sub>4</sub> OXIDATION PRODUCTS  
(J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier)

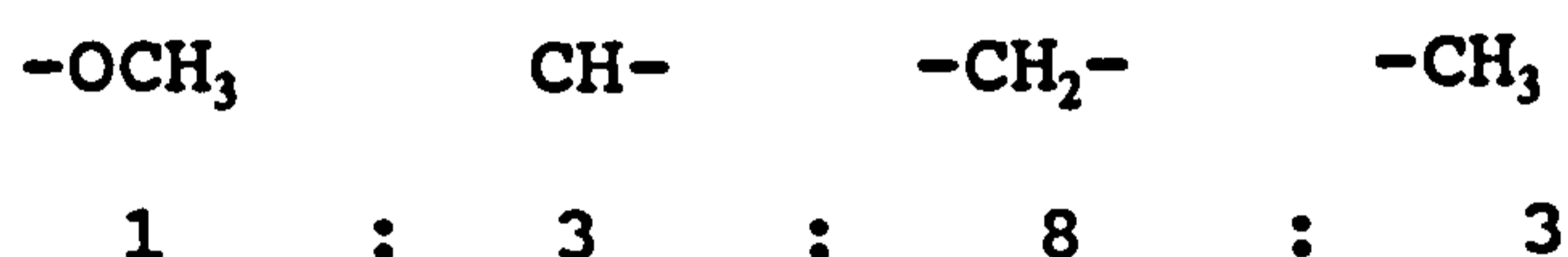
of acidic unresolved products (Figure 2.19). The *n*-monocarboxylic acid profile from this oxidation was also different to that of the Cr(VI) oxidation. The distribution shows no even/odd predominance up to C<sub>14</sub> (equivalent to C<sub>13</sub> in the Cr(VI) oxidation) and thereafter a strong even/odd predominance up to C<sub>22</sub> (RuO<sub>4</sub> oxidation abstracts one carbon from the aromatic ring, so this is equivalent to an odd/even predominance from the Cr(VI) oxidation).

Assuming that these *n*-carboxylic acids are liberated from alkyl chains attached directly to an aromatic ring, the chain lengths (C<sub>8</sub>-C<sub>22</sub>) represent the alkyl moieties present in the sample (actual chain lengths C<sub>n-1</sub>). These results suggest that chain lengths of 16, 18 and 20 carbons are minor or even absent. Additionally there is little evidence for chain lengths greater than C<sub>21</sub>. The differences between the oxidation product profiles of the RuO<sub>4</sub> and CrO<sub>3</sub> oxidations must be due to secondary oxidation of side chains in the latter, presumably at tertiary centres, to yield further *n*-carboxylic acids. This is further supported by the presence of a UCM in the *m/z* 74 chromatogram of the RuO<sub>4</sub> oxidation (which presumably contains branched compounds) and the absence of a strong UCM in the Cr(VI) oxidation *m/z* 74 chromatogram. Fractionation of the material recovered from the RuO<sub>4</sub> oxidation, into acidic and non-acidic (*i.e.* reacted and unreacted) portions, yielded 50% acids, of which 16% were resolved by GC (*cf.* 5% for the



total recovered products). This fraction was examined by several techniques in an attempt to arrive at an average structure for the unresolved acids.

<sup>1</sup>H-NMR (Figure 2.20) gave ratios for the various proton types as,



These figures, assuming direct attachment to a mono aromatic ring would indicate a C<sub>19</sub> alkylbenzene or if diaromatic, a C<sub>23</sub> alkyl naphthalene precursor. Increasing these figures however, to allow for the formation of diacids, the precursor becomes a C<sub>38</sub> diaromatic (assuming the alkyl chain is attached to an aromatic ring at two separate points). These two hypothetical structures are not ideal in terms of the observed KI range (max. KI 2700) of the UCM and evidence from IR, NMR and the chromic acid oxidation for the predominance of naphthenobenzene type structures. Stock and Tse (1983) oxidised tetralin with RuO<sub>4</sub> to yield predominantly adipic acid, HOOC(CH<sub>2</sub>)<sub>4</sub>COOH and some glutaric acid, HOOC(CH<sub>2</sub>)<sub>3</sub>COOH, and therefore the presence of alkylated naphthenobenzenes should lead to dicarboxylic acid oxidation products, which if the alkyl portion is significantly branched, may remain unresolved by GC.

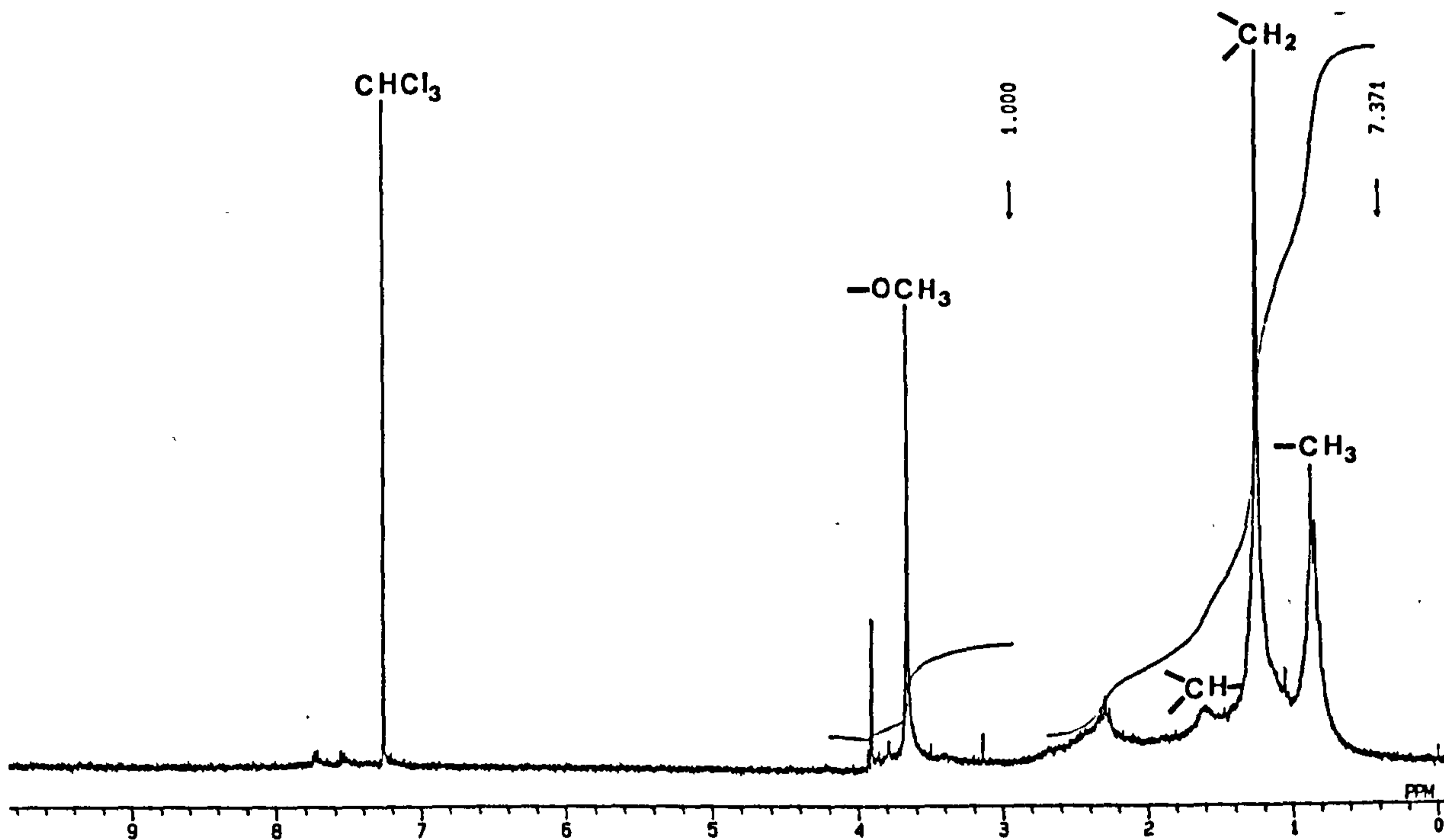
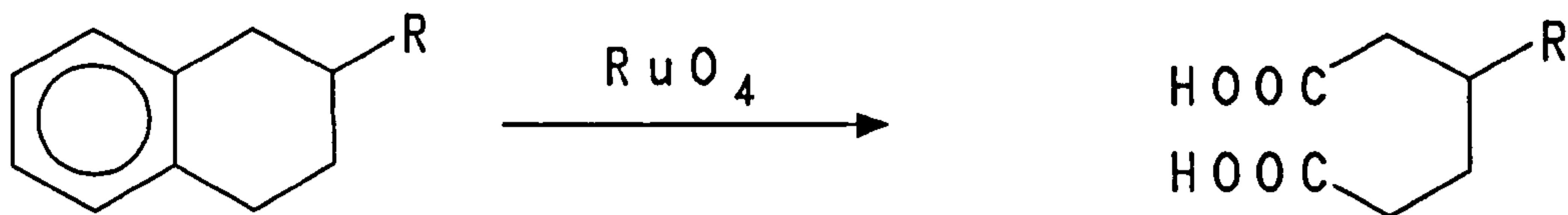
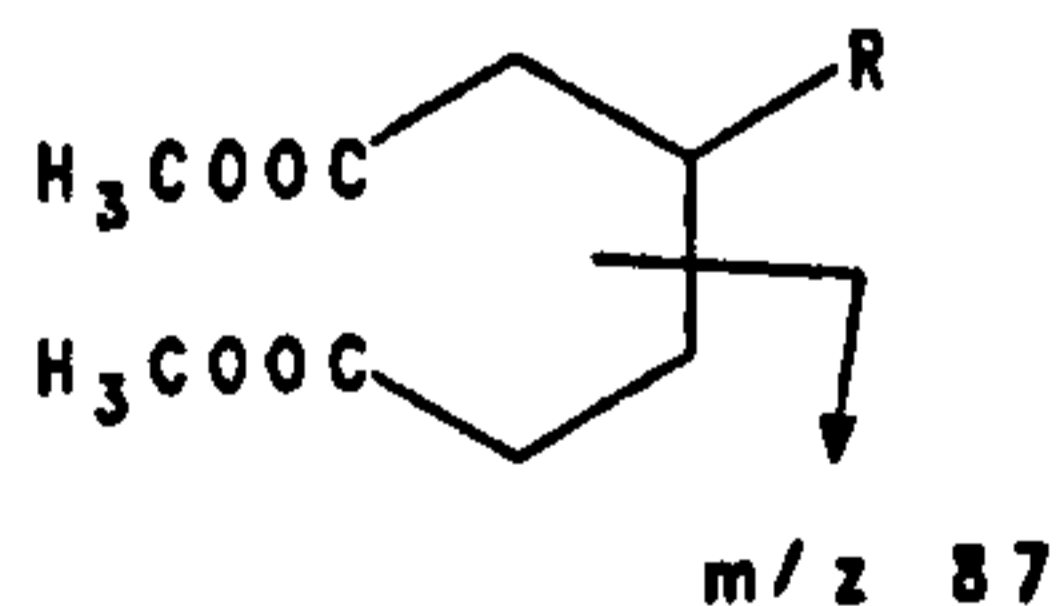


FIGURE 2.20.  $^1\text{H-NMR}$  SPECTRUM OF THE ACIDIC FRACTION PRODUCED BY  $\text{RuO}_4$  OXIDATION OF THE TJP AROMATIC UCM



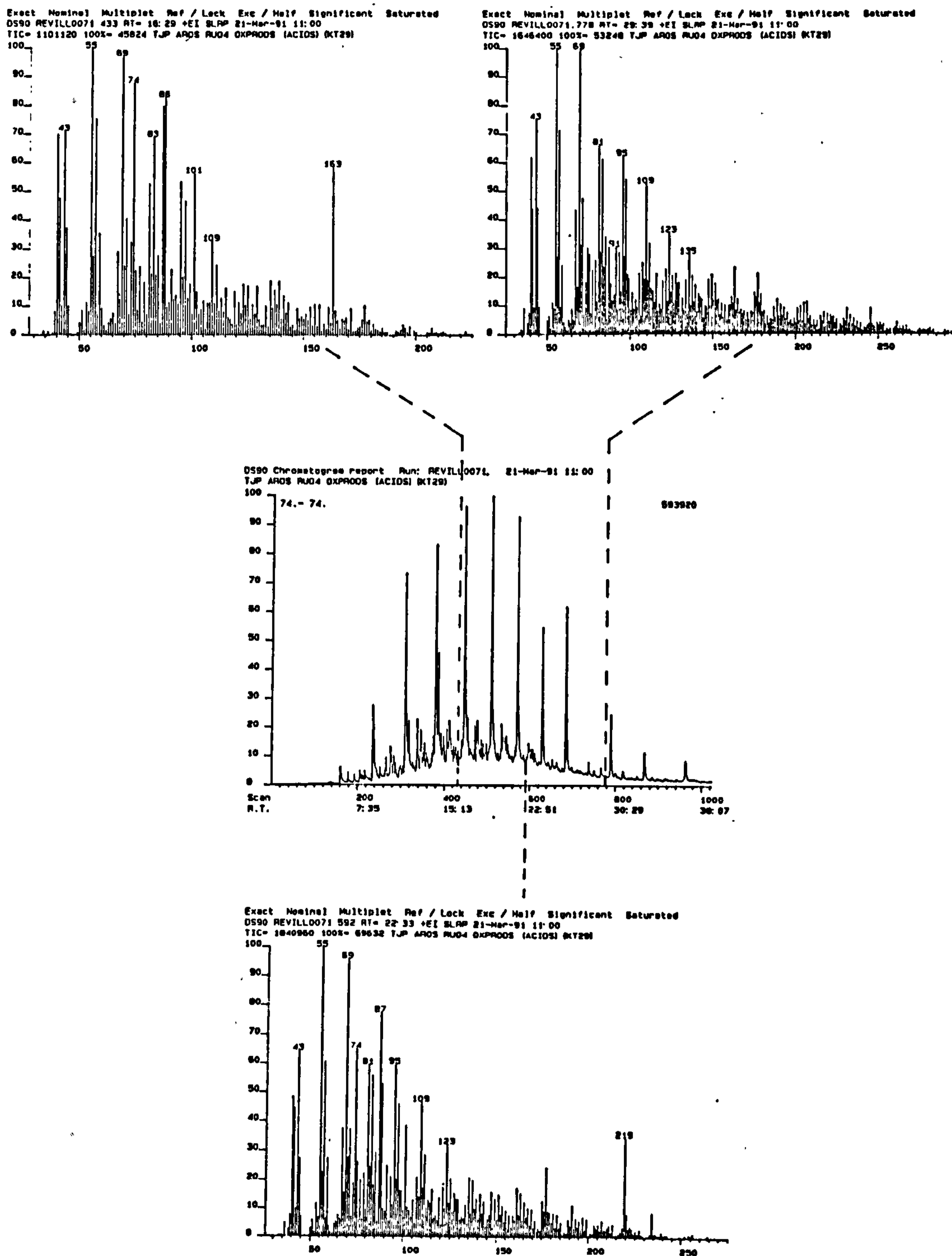
If this were the case, then the dicarboxylic acid products suggested by NMR would require a C<sub>32</sub> monoaromatic precursor, much closer to the GC maximum of KI 2700.

Mass spectra taken through the unresolved portion of the acidic fraction (Figure 2.21) confirm the presence of carboxylic acids (as methyl esters,  $m/z$  74, Mclafferty rearrangement) but also exhibit other important ions. All three spectra possess a relatively strong  $m/z$  87 ion in relation to the  $m/z$  74. In *n*-carboxylic acid methyl esters, the  $m/z$  74 is the base peak, with  $m/z$  87 present at 50-60%. The relatively enhanced  $m/z$  87 ions in these spectra compared with spectra of *n*-carboxylic acid methylesters could be due to enhanced fragmentation  $\gamma$  to the acid group:



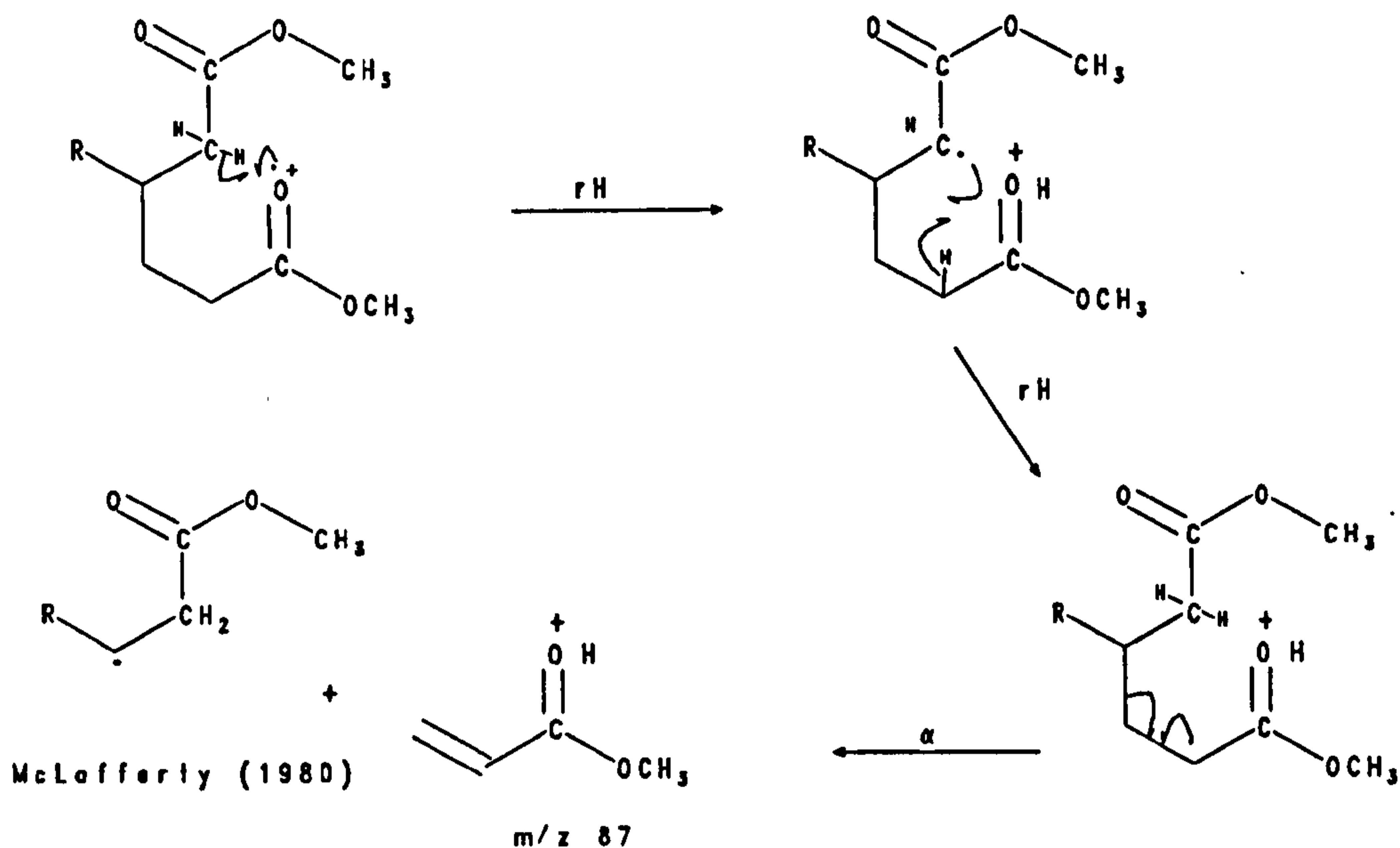
A more likely alternative is a  $\delta$ H rearrangement (Mclafferty, 1980). *n*-Carboxylic acid methyl esters generally rearrange  $\gamma$  to the carbonyl carbon, yielding the





**FIGURE 2.21. EI-GCMS OF THE UNRESOLVED ACIDS PRODUCED BY THE RuO<sub>4</sub> OXIDATION OF THE TJP AROMATIC UCM (J&W DB-5, 25m, 40-300°C @ 5°C/min. He carrier)**

$m/z$  74 ion. However, the presence of a suitable group may cause the  $\delta H$  to become sterically more accessible, allowing for the formation of the  $m/z$  87 ion; the second acid group may fulfill this role.

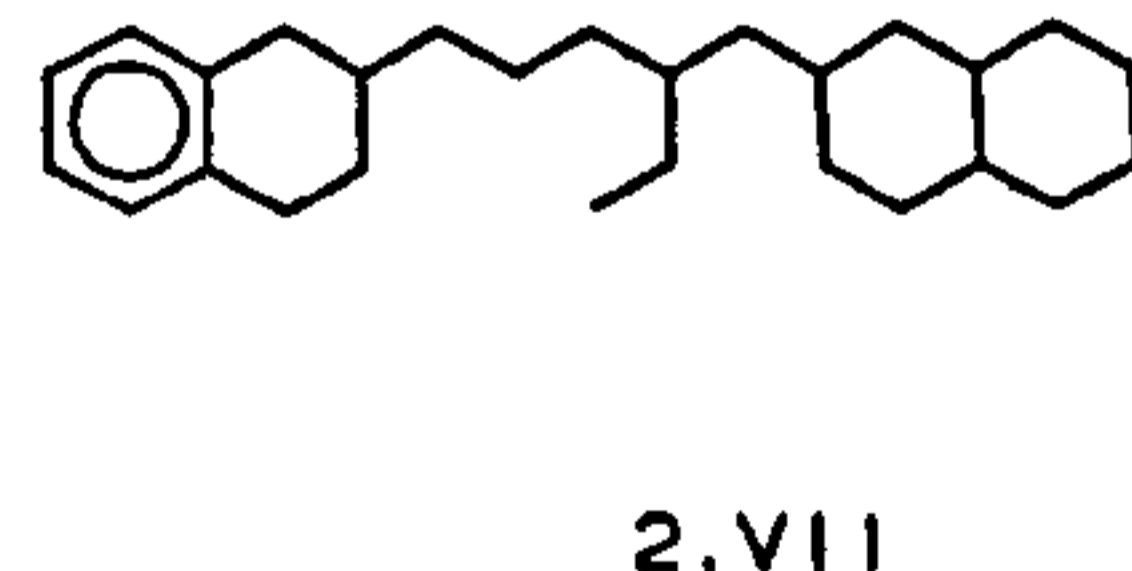
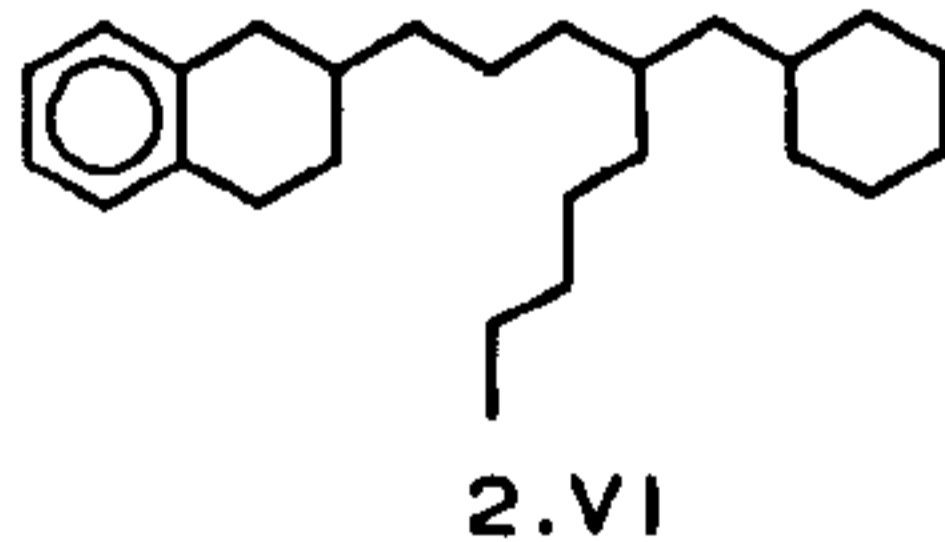
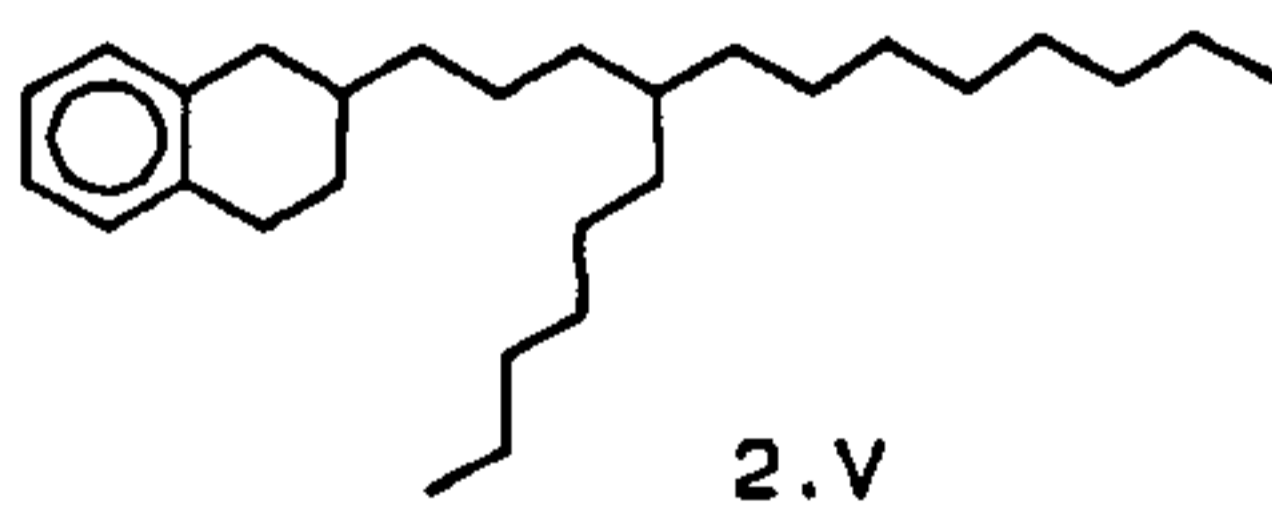


The spectra also exhibit two very distinct ion series at  $m/z$  55, 69, 83, 97 and  $m/z$  95, 109, 123, 137 which can be assigned to mono- and dienes or mono- and bicyclic compounds respectively. There is no evidence from  $^1H$ -NMR for the presence of alkenes (4-6 ppm), and none would be expected since double bonds are attacked by  $RuO_4$  and are not produced. This leaves, therefore, the presence of mono- and bicyclic alkanes in the unresolved oxidation products, suggesting these comprise part of the alkyl chain and presumably are not condensed to the naphthenobenzene structure. Corroborating evidence is provided by Farcasiu

and Rubin (1987) who analysed petroleum residues by dehydrogenation and suggested that the aliphatic portion of aromatic compounds should consist of two to four saturated rings per molecule.

#### 2.4 SUMMARY

Bulk UCM analyses (elemental, UV, UVF, IR,  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR) produced strong evidence that the majority of compounds in the aromatic UCM are highly aliphatic, with only one or two aromatic rings. In general the evidence suggested the presence of naphthenoaromatic compounds rather than monoalkylbenzenes and  $^{13}\text{C}$ -NMR corroborated this conclusion. The presence of dimethylphthalate in the chromic acid oxidation products (and absence in the procedural blank), plus evidence for branched dicarboxylic acids from  $\text{RuO}_4$  oxidation are also consistent with this. The data strongly suggest that the aliphatic side chain could include saturated rings and structures 2.V to 2.VII are proposed as possible candidates.

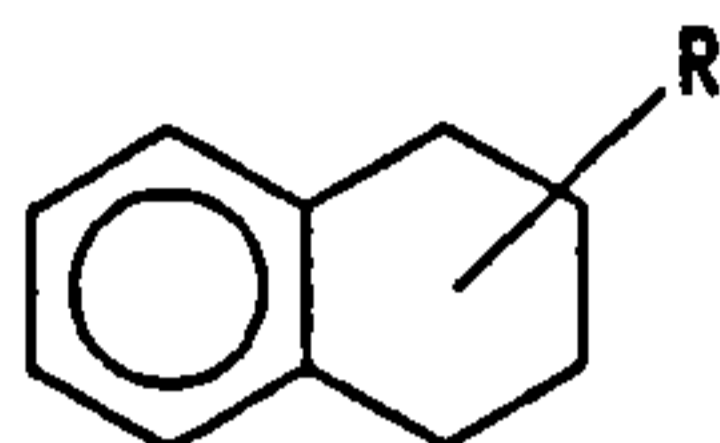


Gough and Rowland (1990) showed that for a  $\text{C}_{20}$ - $\text{C}_{30}$  "T-branch"



alkane, there were 536 possible isomers. The number of isomers (excluding stereoisomers) for the three types of aromatic compounds suggested (2.V-2.VII), between C<sub>20</sub> and C<sub>30</sub> was calculated using a purpose written computer program (Appendix II) (Tables 2.6-2.8) and found to be 1,213, not including multiple branching on the chain or methyl branching on the saturated rings. For the purpose of this calculation it was assumed that for a particular carbon number, the number of carbon atoms in the alkyl chain (n) was equal to the total less those in the ring system(s). Within this side chain, the monoalkyl moiety was allowed to have from zero to n-1 carbon atoms (i.e. there must always be at least one carbon atom between any two ring systems). At an oven temperature ramp of 5°C per minute C<sub>20</sub> and C<sub>30</sub> are separated by 17min. (1020s; J&W DB-5) with a baseline peak width of ca. 10 seconds, thus GC could in theory resolve only 102 compounds in this time. Clearly, naphthenoaromatic compounds with side chains containing saturated rings could easily account for the unresolved nature and other chemical characteristics of the aromatic UCM.

**TABLE 2.6. RESULTS FROM THE "T-BRANCH" PROGRAM FOR A MONO-AROMATIC NAPHTHENO COMPOUND (Z = -8) BETWEEN C<sub>20</sub> AND C<sub>30</sub>**



**NUMBER OF T-BRANCH ALKANES, EXCLUDING STEREOISOMERS MOLECULE ASYMMETRIC AT ONE END ONLY**

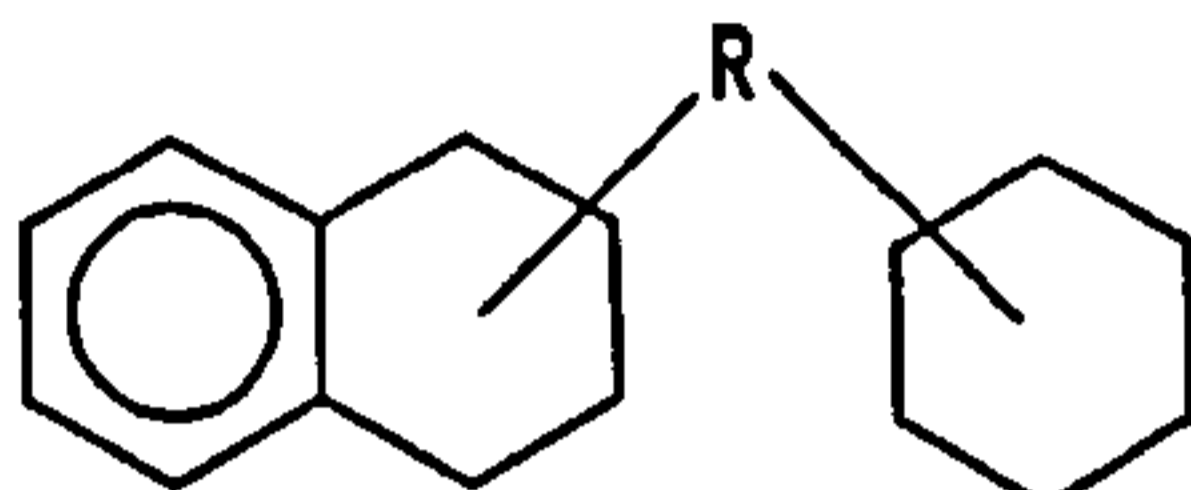
Total <sup>a</sup> Carbons	Branch Carbons <sup>b</sup>									Total
	1	2	3	4	5	6	7	8	9	
10	8	6	4	2						20
11	9	7	5	3	1					25
12	10	8	6	4	2					30
13	11	9	7	5	3	1				36
14	12	10	8	6	4	2				42
15	13	11	9	7	5	3	1			49
16	14	12	10	8	6	4	2			56
17	15	13	11	9	7	5	3	1		64
18	16	14	12	10	8	6	4	2		72
19	17	15	13	11	9	7	5	3	1	81
20	18	16	14	12	10	8	6	4	2	90
										=====
										565

Total isomers including unbranched parent = 576

<sup>a</sup> Refers to the number of side chain carbons *i.e.* the C<sub>20</sub> compound has 10 carbons in the parent and therefore 10 carbons in the chain.

<sup>b</sup> Refers to the number of carbons in the branch of the side chain *i.e.* in a side chain of 10 carbons there can only be 4 carbons in the branch (6 in the main chain) before isomers start to be repeated.

**TABLE 2.7. RESULTS FROM THE "T-BRANCH" PROGRAM FOR A MONO-AROMATIC NAPHTHENO COMPOUND WITH ONE CYCLOHEXYL RING IN THE SIDE CHAIN BETWEEN C<sub>20</sub> AND C<sub>30</sub> (Z = -10)**



**NUMBER OF T-BRANCH ALKANES, EXCLUDING STEREOISOMERS  
MOLECULE ASYMMETRIC AT BOTH ENDS**

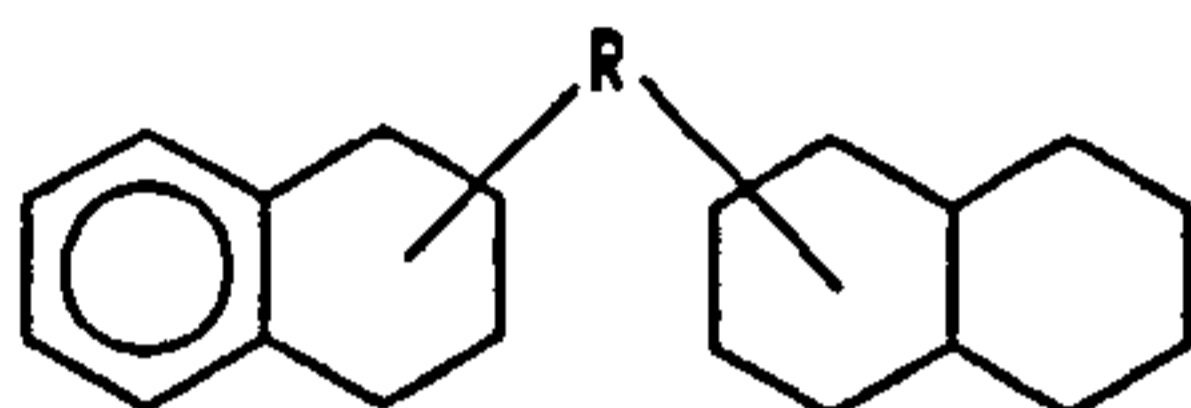
Total <sup>a</sup> Carbons	Branch Carbons <sup>b</sup>													Total	
	1	2	3	4	5	6	7	8	9	10	11	12	13		
4	3	2	1												6
5	4	3	2	1											10
6	5	4	3	2	1										15
7	6	5	4	3	2	1									21
8	7	6	5	4	3	2	1								28
9	8	7	6	5	4	3	2	1							36
10	9	8	7	6	5	4	3	2	1						45
11	10	9	8	7	6	5	4	3	2	1					55
12	11	10	9	8	7	6	5	4	3	2	1				66
13	12	11	10	9	8	7	6	5	4	3	2	1			78
14	13	12	11	10	9	8	7	6	5	4	3	2	1	1	91
															<u>451</u>

Total isomers including unbranched parent = 462

<sup>a, b</sup> Notes as per table 2.6



**TABLE 2.8. RESULTS FROM THE "T-BRANCH" PROGRAM FOR A MONO-AROMATIC NAPHTHENO COMPOUND WITH TWO FUSED CYCLOHEXYL RINGS IN THE SIDE CHAIN BETWEEN C<sub>20</sub> AND C<sub>30</sub> (Z = -12)**



NUMBER OF T-BRANCH ALKANES, EXCLUDING STEREOISOMERS  
MOLECULE ASYMMETRIC AT BOTH ENDS

Total <sup>a</sup> Carbons	Branch Carbons <sup>b</sup>									Total	
	1	2	3	4	5	6	7	8	9		
1											0
2	1										1
3	2	1									3
4	3	2	1								6
5	4	3	2	1							10
6	5	4	3	2	1						15
7	6	5	4	3	2	1					21
8	7	6	5	4	3	2	1				28
9	8	7	6	5	4	3	2	1			36
10	9	8	7	6	5	4	3	2	1		45
											=====
											165

Total isomers including unbranched parent = 175

<sup>a, b</sup> Notes as per table 2.6

## CHAPTER THREE

### ORIGINS OF THE UCM

*This chapter describes investigations into the origins of UCMs. The products of hydrous pyrolysis of man-made (polythene) and biogenic (cutan) polymers under conditions proposed previously to simulate catagenesis, included, in the hydrocarbons, high proportions of UCMs (50% - >70%). Hydrous pyrolysis of polythene produced a mixture of saturated (56%) and olefinic (44%) hydrocarbons, whilst pyrolysis of cutan produced hydrocarbon (aliphatic and aromatic; 30-75%) and non-hydrocarbon (70-25%) fractions, both with >60% unresolved components. Oxidative characterisation of these UCMs produced mainly n-acids with somewhat similar results to those found when oil UCMs were oxidised. However, the laboratory generated UCMs are not perfect oil UCM models since some oil UCM oxidation products were not observed in the laboratory models.*

*Estragon: Let's pass on now to something else, do you mind?*  
*Vladimir: I was just going to suggest that*  
*Estragon: But to what?*  
*Vladimir: Ah!*  
*Silence*

**Beckett (Waiting for Godot)**



## ORIGINS OF THE UCM

### 3.1 THEORIES OF PETROLEUM FORMATION

It is widely accepted that petroleum formation follows the general pathway:



(Tissot and Welte, 1984)

At present there are essentially three theories of kerogen and petroleum formation. One is the classical view that macromolecules are broken down to smaller units, followed by re-condensation to produce kerogen and catagenesis to form petroleum (Tissot and Welte, 1984). The second is that of selective preservation of biomacromolecules in conjunction with polycondensation to form the kerogen structure (Philp and Calvin, 1976; Tegelaar *et al.*, 1989c) followed by catagenesis. Both these processes may play important roles in petroleum formation.

A third, alternative, but less widely accepted view of petroleum formation, is that at least part of the petroleum mixture has an abiogenic origin (Robinson, 1963; Robinson, 1966; Gold, 1979; Gold, 1986; Gold *et al.*, 1986). Such theories propose that an input of meteoritic hydrocarbons,

often referred to as kerogen-like (Pering and Ponnampereuma, 1971; Cronin *et al.*, 1987; Kerridge *et al.*, 1987; Morgan *et al.*, 1991) occurred during the formation of the Earth which, under the prevailing conditions produced a mixture of (primarily) methane and some liquid hydrocarbons (Gold *et al.*, 1986). It is suggested that the high pressures present had a stabilising effect, preventing dissociation of the compounds and producing a good solvent in the form of liquid methane. This mixture could then migrate out from the deeper regions of the Earth, undergoing a series of important reactions, such as those demonstrated by Gold *et al.* (1986) in the laboratory. These workers showed that methane, under geological pressures (1000 atm) and in the presence of a clay catalyst (e.g. montmorillonite), formed C<sub>2</sub> units which condensed to form benzenes, alkylated aromatic rings and a polyethylene-type polymer on the catalyst surface. Gold (1986) suggests that these reactions could account for the major components in crude oils and that larger polymeric material can be accumulated either by condensing out from the solvent (methane) at reduced pressures, or as a result of polymerising reactions involving C<sub>2</sub> units. Efforts are currently underway in the Siljan Ring region of Sweden (Haggin, 1986; Osborne, 1986; Anon, 1987; Shirley, 1987; Aldhous, 1991) to provide geological evidence for this hypothesis.

If any of the above theories of petroleum formation are correct, they must also account for the production of

quantitatively significant hydrocarbon UCMs by catagenesis. Indeed, if the results of recent studies of UCM composition are correct (Gough and Rowland, 1990, 1991; Killops and Al-Juboori, 1990) the UCM produced should comprise significant proportions of simply branched (so-called "T-branched") compounds. <sup>14</sup>C-dates of 15000-26000 years and co-occurrence with mature biomarker profiles are regarded as strong evidence that UCMs are petrogenic (reviewed by Gough, 1989; see section 1.3) and analyses in this study show that UCMs are abundant in petroleum and can account for up to 30% of fresh crude oils (Table 3.1); a fact often overlooked when oils are examined by GC alone due to normalisation on the resolved compounds. Previous investigations into petroleum formation have not accounted for the presence of this abundant unresolved portion.

### **3.1.1 THE PRESENT STUDY**

Each of the above theories of petroleum formation considers that at least part of crude petroleum is derived from catagenesis of macromolecular organic matter, whether this is biogenic, as in the case of kerogen, or abiogenic, as in the case of the polyethylene-type material observed by Gold and co-workers (e.g. Gold et al., 1986) and the materials detected in meteorites (e.g. Morgan et al., 1991). Therefore in the present study a series of laboratory simulated catagenesis experiments on both biogenic (biopolymeric) and abiogenic (polythene) materials were



TABLE 3.1: GRAVIMETRIC COMPOSITION OF SIX CRUDE OILS FROM  
VARIOUS DEPOSITIONAL ENVIRONMENTS

OIL	% ALIPHATICS	% AROMATICS	% ASPHALTENES AND POLARS*	ALIPHATIC		ALIPHATIC		UREA NON ADDUCT* % OF OIL
				UREA ADDUCT % OF ALIPHATICS	UREA NON ADDUCT* % OF ALIPHATICS	UREA ADDUCT % OF ALIPHATICS	UREA NON ADDUCT* % OF OIL	
Nigerian Light <sup>1</sup>	52	17	31	37	63	37	63	33
Kuwait <sup>2</sup>	33	24	43	34	66	34	66	22
Forties <sup>3</sup>	42	18	40	38	62	38	62	26
Libyan <sup>2</sup>	50	16	34	44	56	44	56	28
Iranian Light <sup>2</sup>	40	19	41	37	63	37	63	25
Tia Juana Pesado <sup>3</sup> (Venezuela) (Biodegraded)	23	35	42	10	90	10	90	21

\* By Difference  
+ Taken to represent UCM

- 1 Deltaic sourced
- 2 Carbonate sourced
- 3 Marine sourced

carried out in order to investigate (a) whether UCM hydrocarbons were produced, (b) if so, in what quantities and (c) how the composition of these UCMS compared with the UCMS found in natural petroleum.

## **3.2 LABORATORY CATAGENESIS**

### **3.2.1 GENERAL**

The best available and most widely accepted method for the laboratory simulation of catagenesis is hydrous pyrolysis (reviewed by Eglinton, 1988; Siskin and Katritzky, 1991). First used by Lewan *et al.* (1979), hydrous pyrolysis involves subjecting the sample, most commonly kerogen or rock chips, to heat (300-360°C) in the presence of water and an inert atmosphere (helium or nitrogen) in a sealed pressure vessel. At these temperatures, the vapour pressure of water increases the pressure inside the vessel to > 120 atm. Samples are commonly heated for 1-4 days depending on the temperature. Artificial maturation of kerogens isolated from petroleum source rocks often yields an organic-soluble fraction in which the non-aromatic hydrocarbons are dominated by a series of *n*-alkanes and (though often ignored) a UCM (e.g. Huizinga *et al.*, 1987; Eglinton *et al.*, 1988; Eglinton, 1988). Type I and II kerogen structures (*i.e.* the most important oil-prone kerogens) are traditionally viewed as comprising a series of polymethylenic chains, cross linked by functional groups

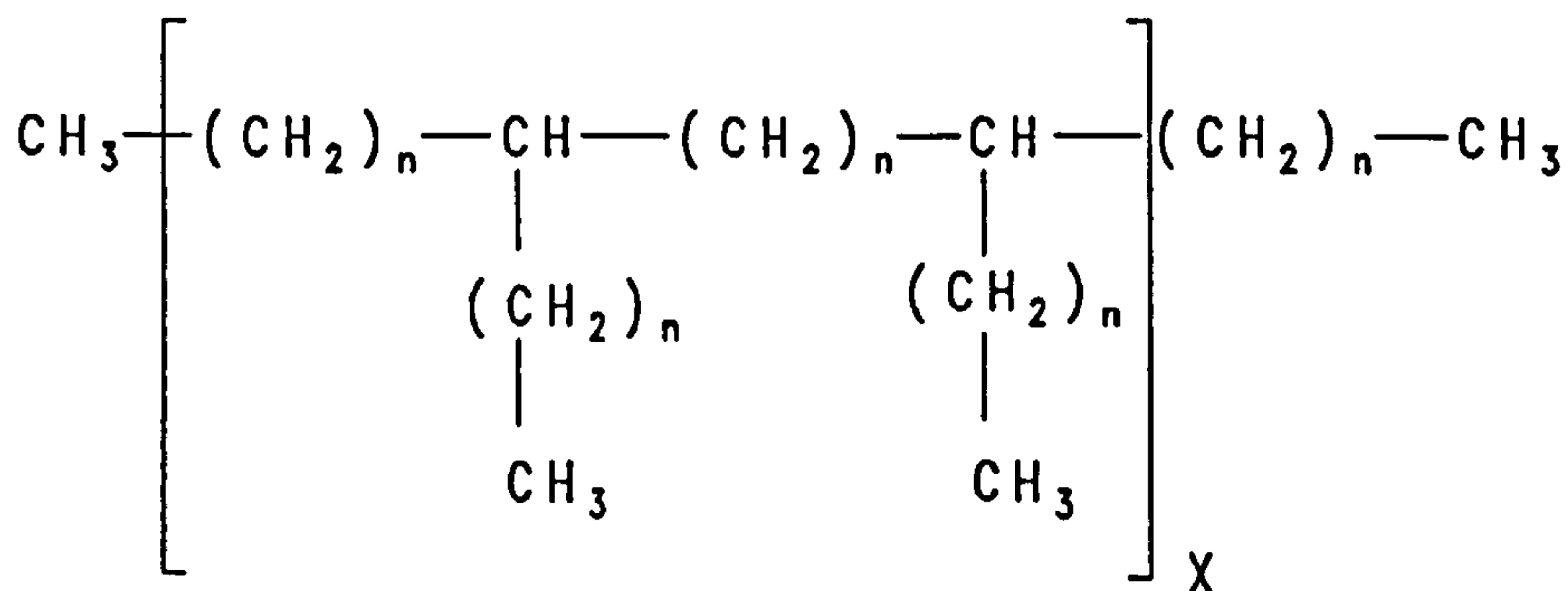
(Tissot and Welte, 1984; Behar and Vandembroucke, 1987). Such polymethylene chains would appear to fulfil the structural pre-requisite for the simply branched T-shaped alkanes by proposed Gough and Rowland (1990; 1991) to form part of the aliphatic UCM.

Therefore in the present study, hydrous pyrolysis of two macromolecules, one man-made, the other a biomolecule, was carried out in an attempt to discover which types of "kerogen-like" structures might yield a UCM under catagenic conditions.

### 3.2.2 HYDROUS PYROLYSIS OF ALKATHENE

#### 3.2.2.1 DISCUSSION OF RESULTS

In order to attempt to produce a UCM under thermal stress in the laboratory, "Alkathene", (the trade name for a man-made, low density polythene, with a general formula of:-





with an average value of  $n = 30$  and approximately 10 methyl groups/1000 carbons) was subjected to hydrous pyrolysis at 350°C for 24 hours and 48 hours in a stainless steel bomblet.

The different pyrolysis times appear to have a significant effect on the quantity of material recovered (Table 3.2) and on the appearance of the gas chromatograms of the extractable fractions (Figure 3.1). The longer pyrolysis time (experiment 2) produced much less organic-soluble material and a much smoother distribution of *n*-alkanes ( $n-C_{13-32}$ ; max  $n-C_{17}$ ) with a slight odd over even predominance. Neither GC profile showed an obvious UCM. However, urea adduction of the organic extracts from both experiments and GC of the UNA, revealed the presence of quite significant proportions of UCMs (Table 3.2, 43% and 58%; Figure 3.2). The extended pyrolysis time appears to have reduced the amount of GC resolvable material in the UNA fraction (35% in experiment 1; 17% in experiment 2) and altered the UCM profile from bimodal (experiment 1) to a much smoother distribution<sup>1</sup>

Argentatious TLC of the UNA of experiment 1 produced a

---

<sup>1</sup>FOOTNOTE: It has often been proposed that bimodal UCMs in sediments represent an input of two different oils (Thompson and Eglinton, 1978) or that the oil maybe sourced from two different kerogens (Killops and Al-Juboori, 1990). Biodegradation of UCMs can also alter the overall distribution of the aliphatic UCM, even producing a single maximum UCM from a bimodal distribution (Jones et al., 1986). However, the present results show that a UCM produced from one source can also be bimodal.

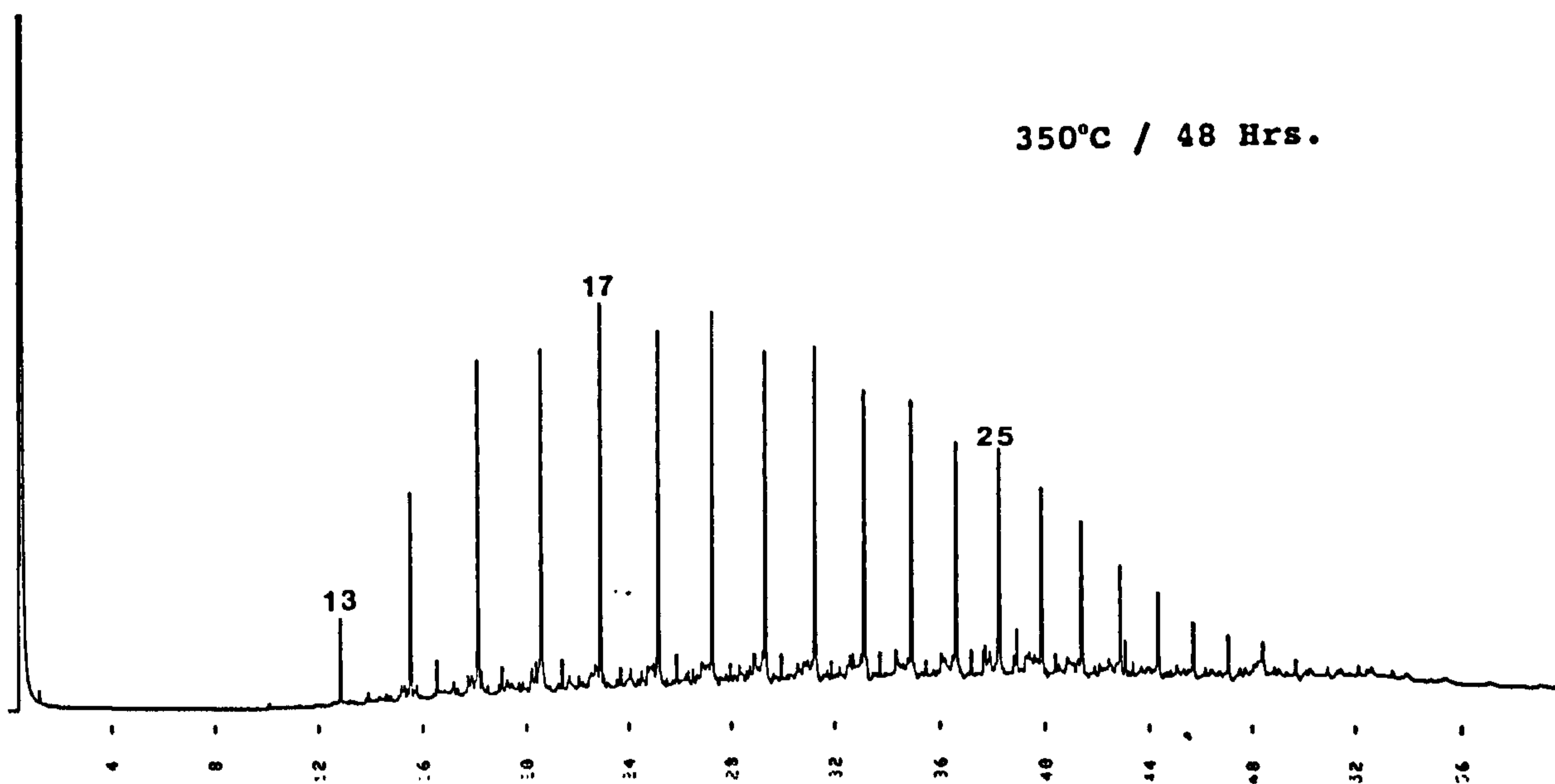
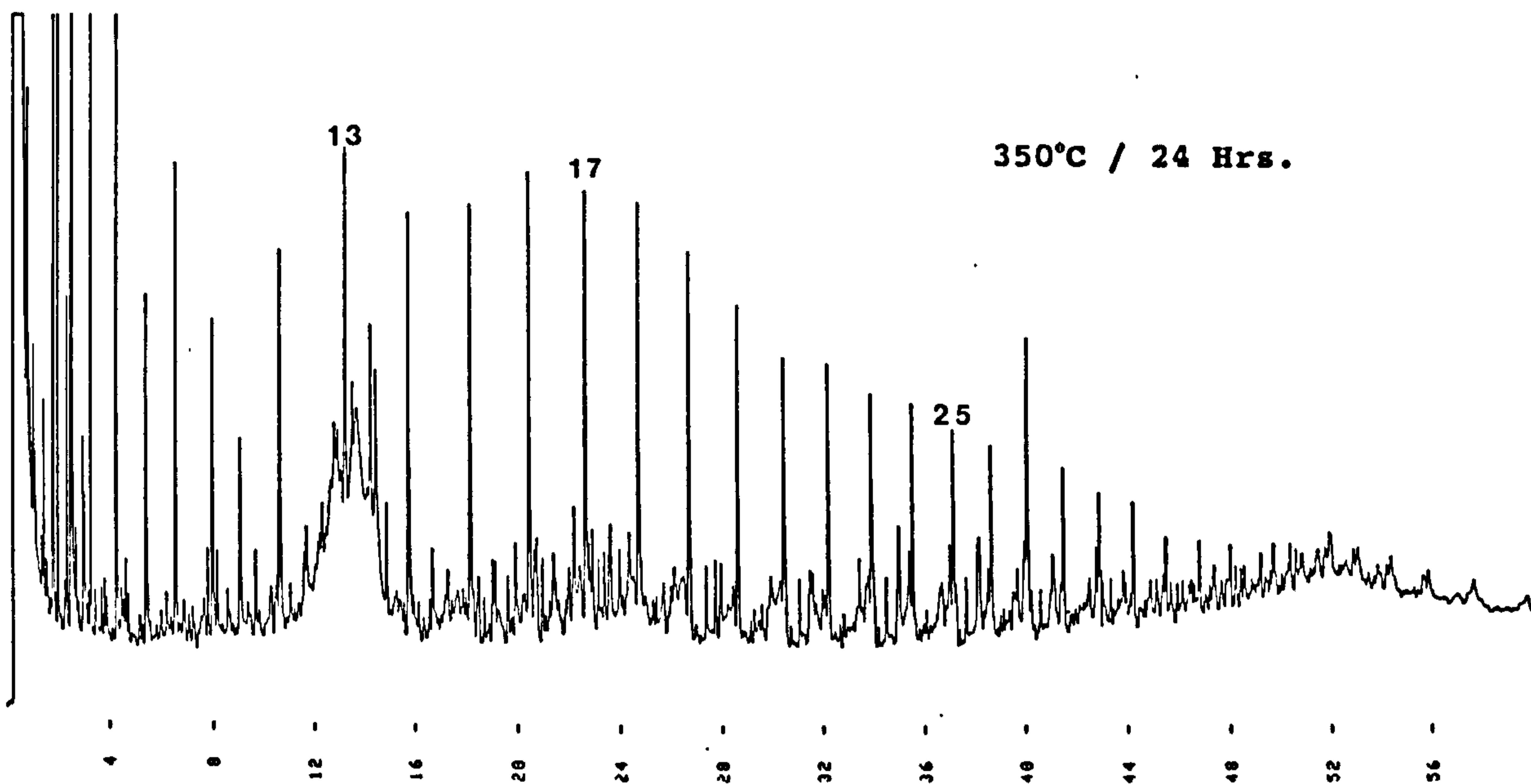
**TABLE 3.2a. QUANTITATIVE DATA FOR THE HYDROUS PYROLYSIS OF ALKATHENE AND CUTAN**

Amount of Material	T <sub>c</sub>	Time	Total Yield (mg)	Amount of Total products GC Resolved (%)	UNA (mg)	UA (mg)	UNA Resolved (%)
<b>Alkathene</b>							
1.2g	350	24hrs	93.4	50	19.7 (43%)	25.8	36
1.0g	350	48hrs	5.6	25	2.8 (58%)	2.0	17
<b>Agave americana</b>							
11.4mg	350	48hrs	2.4	29	0.5 (25%)	1.5	25
N.A.	365	72hrs	38.1	N.A.	8.9 (28%)	22.7	25

N.A. = Data not available

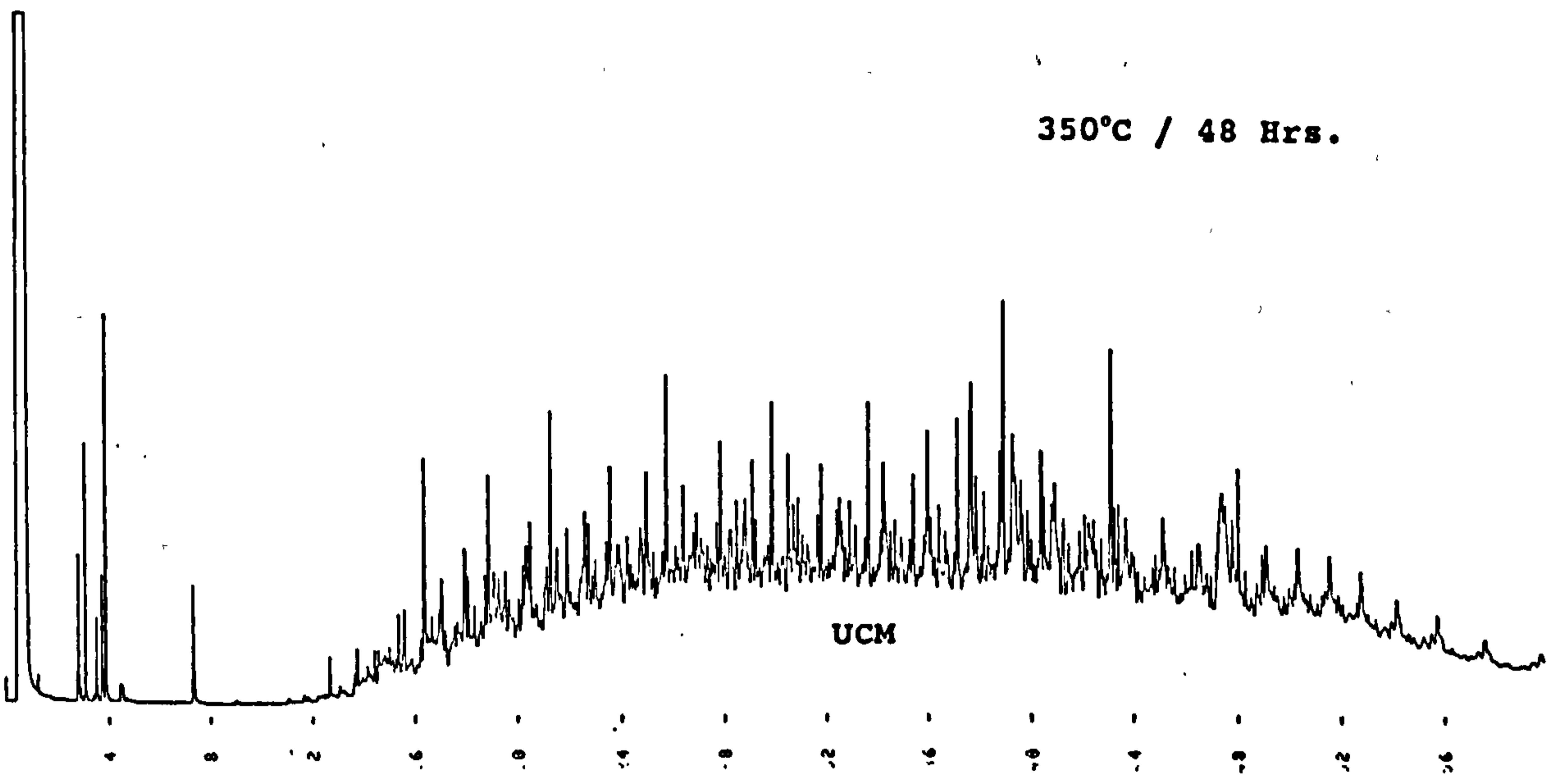
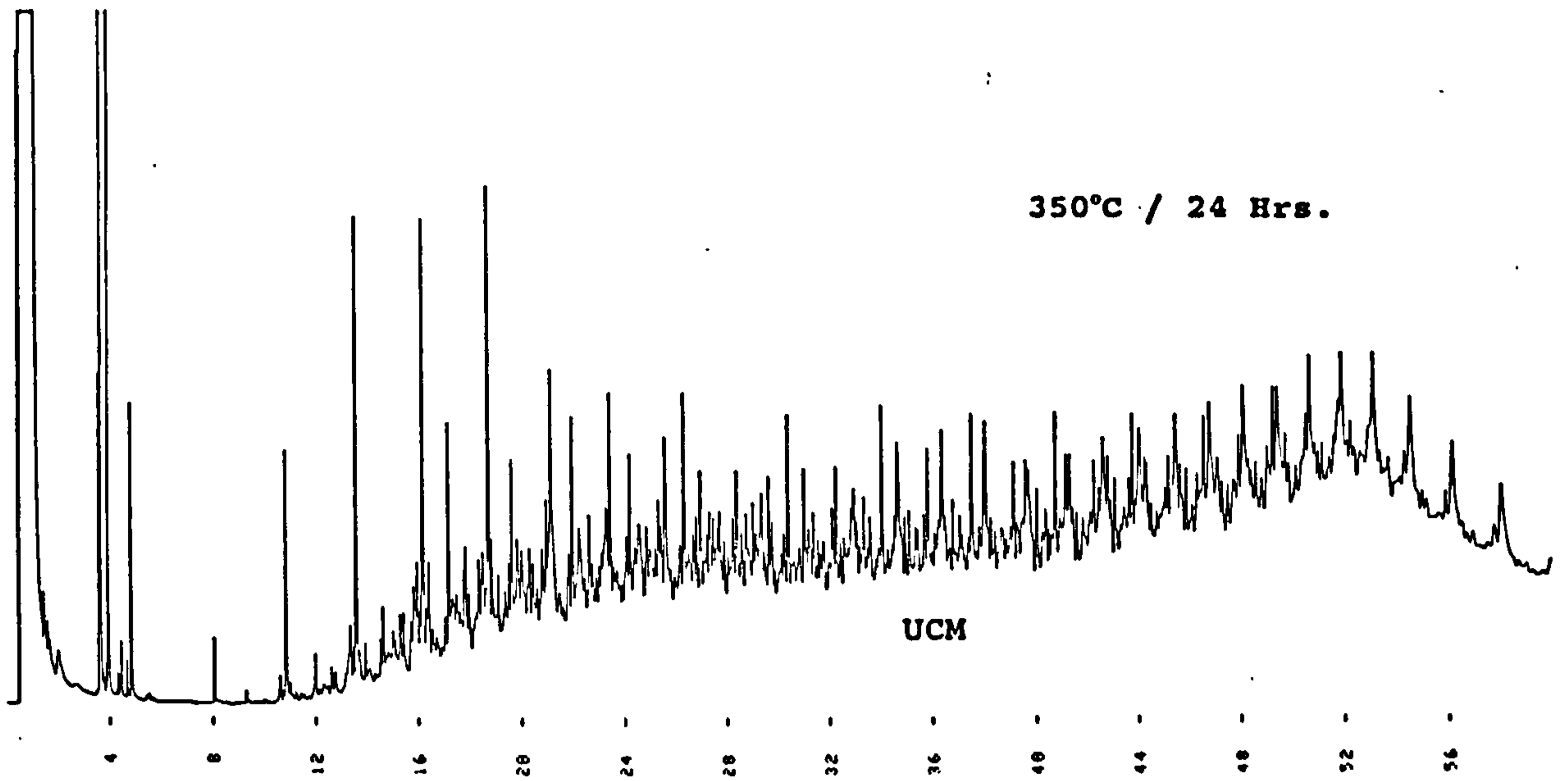
**FIGURE 3.2b. QUANTITATIVE DATA FOR THE OXIDATION OF ARTIFICIALLY PRODUCED UCMS**

Material	Amount of UNA Oxidised (mg)	Total recovered Material (mg(%))	Amount of recovered Material resolved By GC (%)
Alkathene	3.5	2.4 (69)	72
Cutan	3.1	2.8 (90)	70



**FIGURE 3.1. ALKATHENE HYDROUS PYROLYSIS PRODUCTS**  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)  
 (Total organic solubles, numbers indicate *n*-alkane chain length)

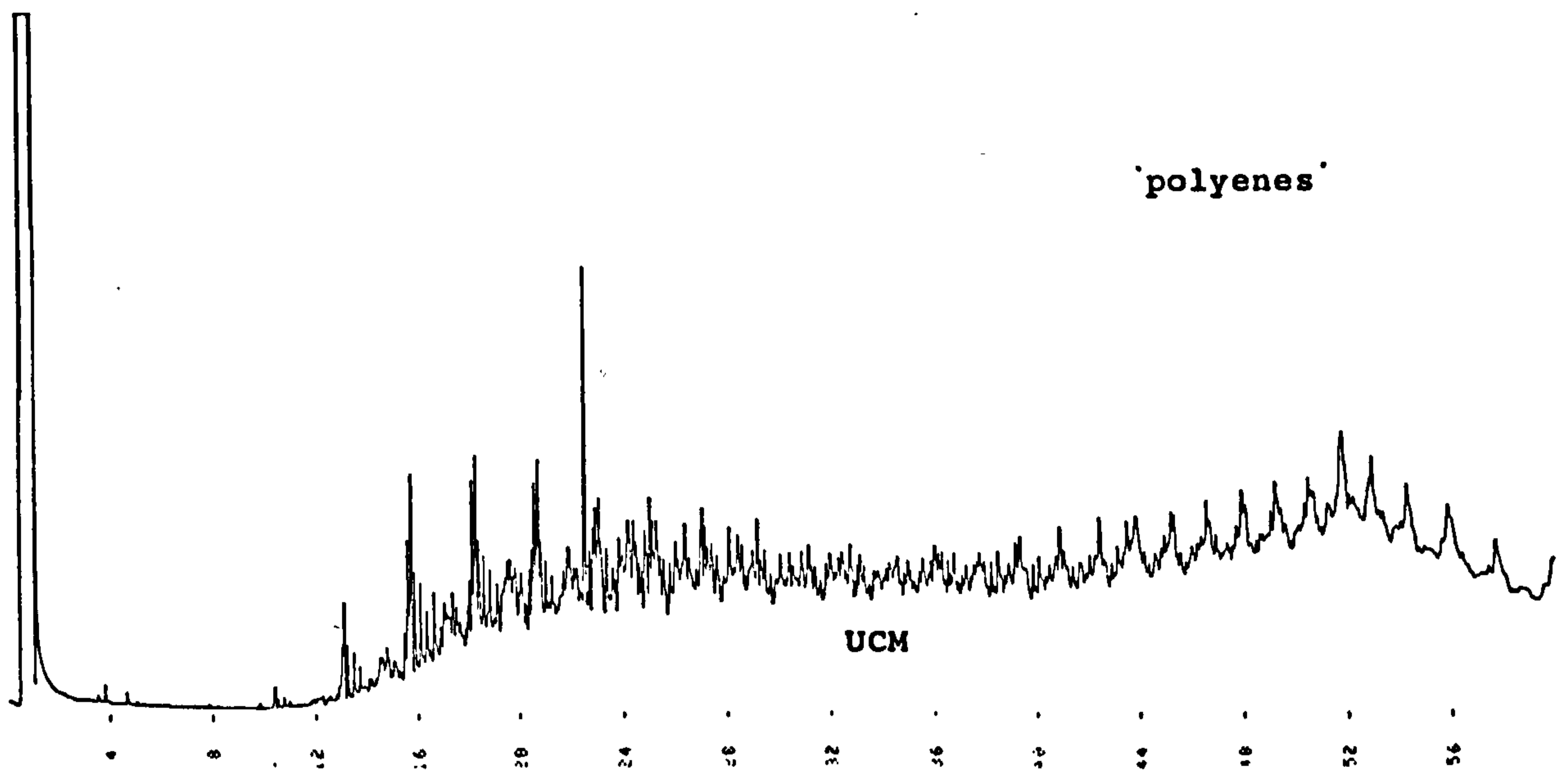
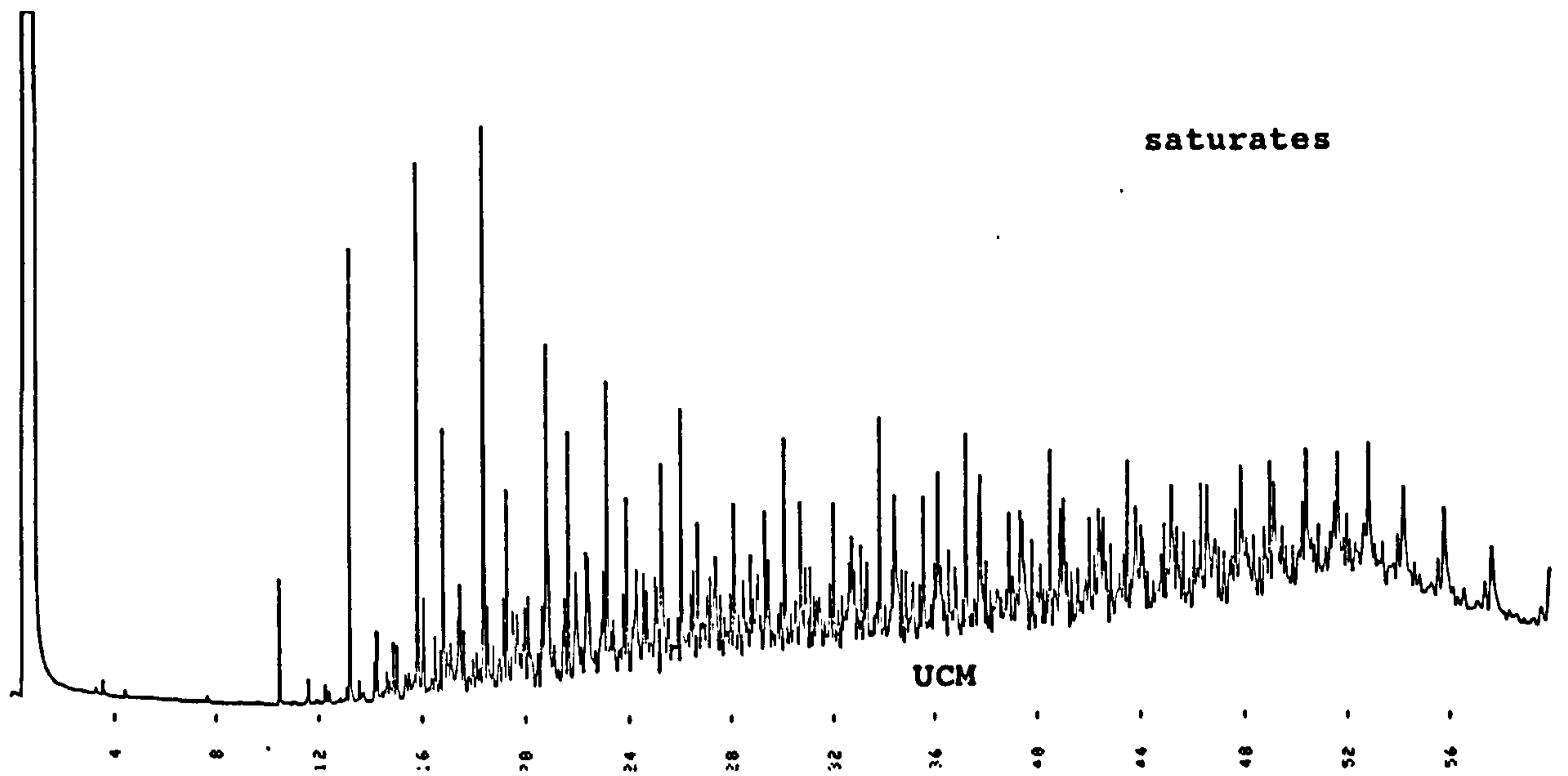




**FIGURE 3.2. UREA NON ADDUCT FRACTIONS OF ALKATHENE HYDROUS PYROLYSIS TOTAL PRODUCTS**  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)

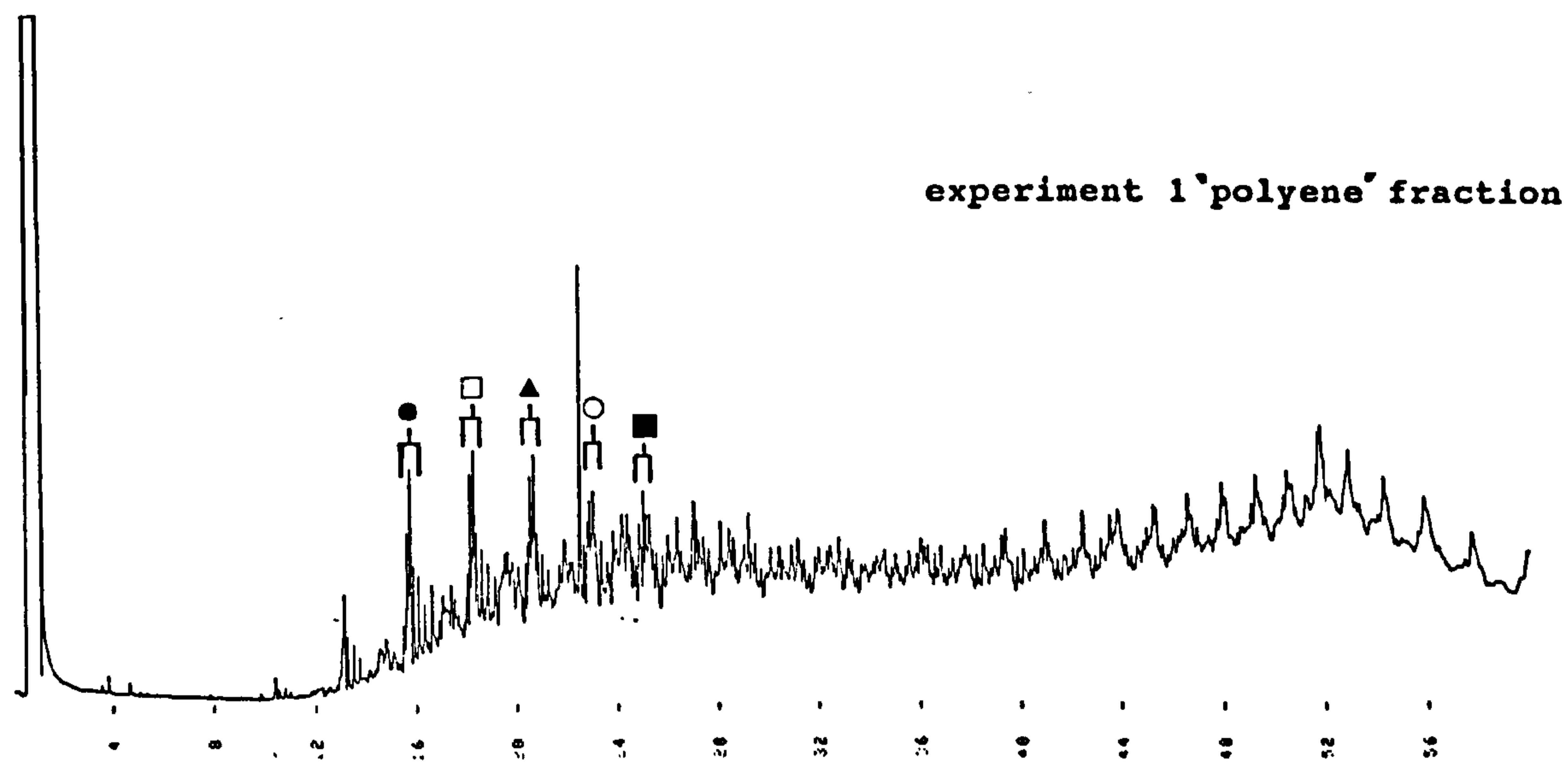
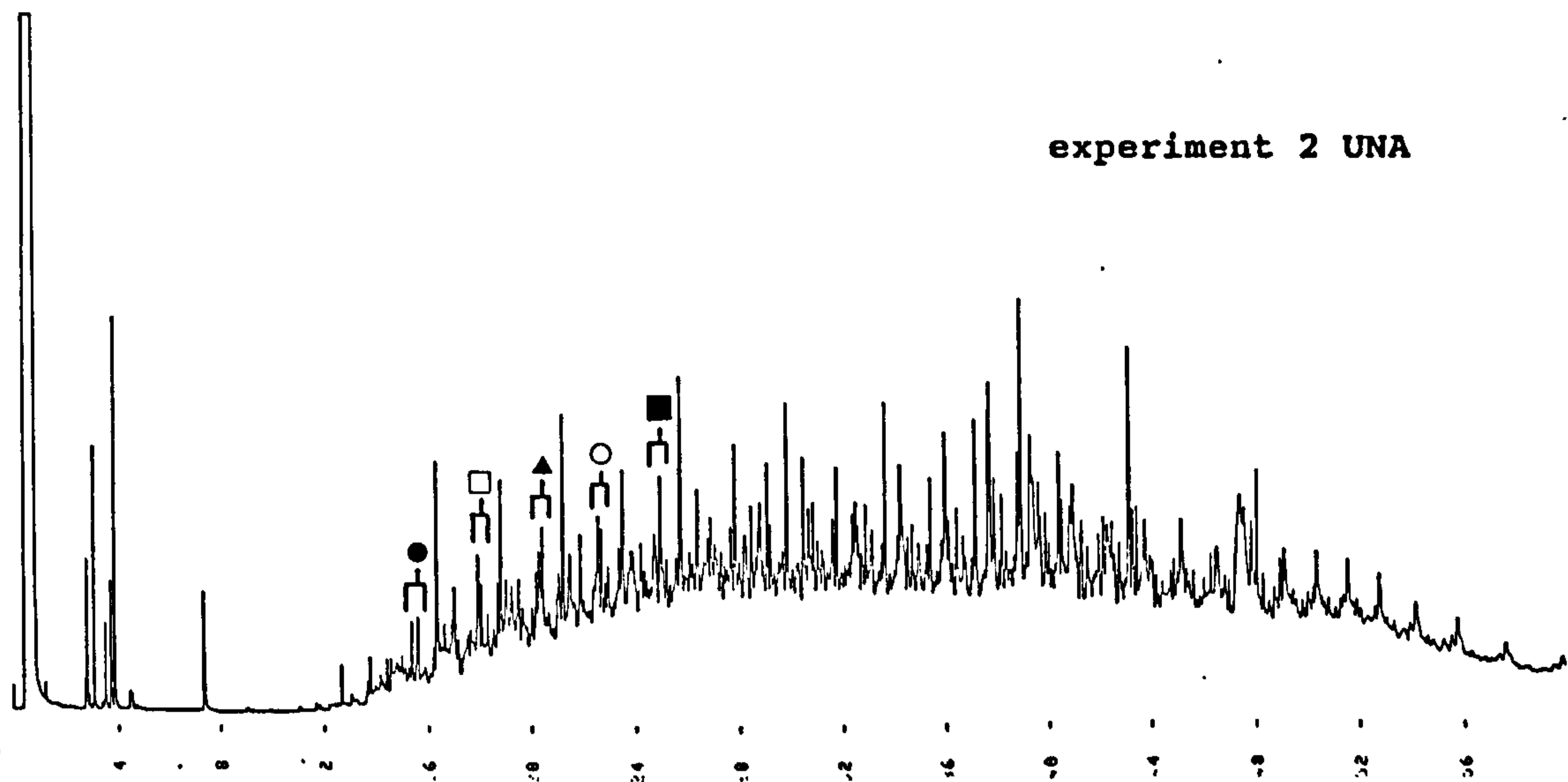
saturates fraction ( $R_f$  0.6-0.96; 56%) and a polyene portion ( $R_f$  0.03-0.5; 44%) with the latter exhibiting the more pronounced UCM by GC (86% unresolved *cf.* 64% unresolved in the saturates fraction; Figure 3.3). Unfortunately, insufficient material was available from experiment 2 to allow a similar fractionation, but comparison of the GC results for the UNA from experiment 2 and the polyene fraction of experiment 1 (Figure 3.4) suggests that the experiment 2 UNA contains similar saturated and polyene components.

GCMS spectra of the saturated UCM showed significant  $m/z$  83, 97, 111 fragments (Figure 3.5). These indicate the presence of either abundant cyclic hydrocarbons or alkenes. Several pieces of evidence confirm that the compounds are not alkenes: TLC resolution was monitored by authentic reference compounds (eicosane and eicosene) and alkenes would not be expected in this TLC fraction;  $^1\text{H-NMR}$  indicated a high degree of branching ( $3^\circ$ carbon, 1.6 ppm) and a lack of olefinic protons (4-6 ppm; Figure 3.6). The mainly unresolved nature of this fraction in the GCMS analysis (Figure 3.5) suggests that the cyclic compounds possess alkyl side chains which presumably contain some branching, possibly of the simple "T-branch" type. If this is the case, the UCM in this fraction would have a composition similar to that proposed for petrogenic UCMs by Gough and Rowland (1990, 1991).

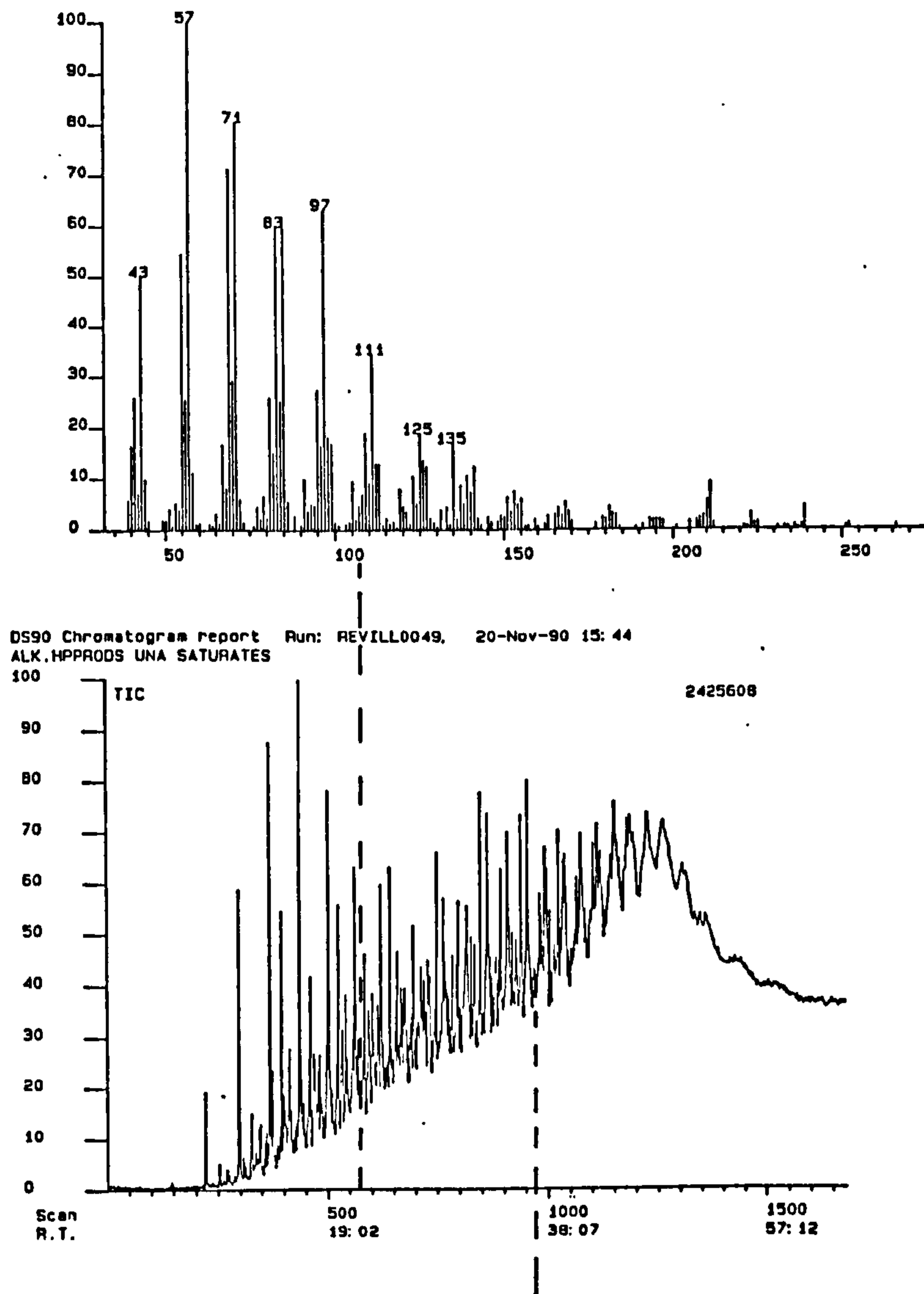


**FIGURE 3.3. SATURATED AND "POLYENE" FRACTIONS FROM ALKATHENE EXPERIMENT 1 UNA FRACTION (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas; hydrous pyrolysis at 350°C for 24 hours)**





**FIGURE 3.4. COMPARISON OF ALKATHENE EXPERIMENT 2 UNA FRACTION AND EXPERIMENT 1 'POLYENE' FRACTION (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas) (Matching symbols indicate some of the peaks which correspond by retention time)**



**FIGURE 3.5. GCMS ANALYSIS OF THE ALKATHENE UNA SATURATED FRACTION**  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas  
 hydrous pyrolysis at 350°C for 24 hours)

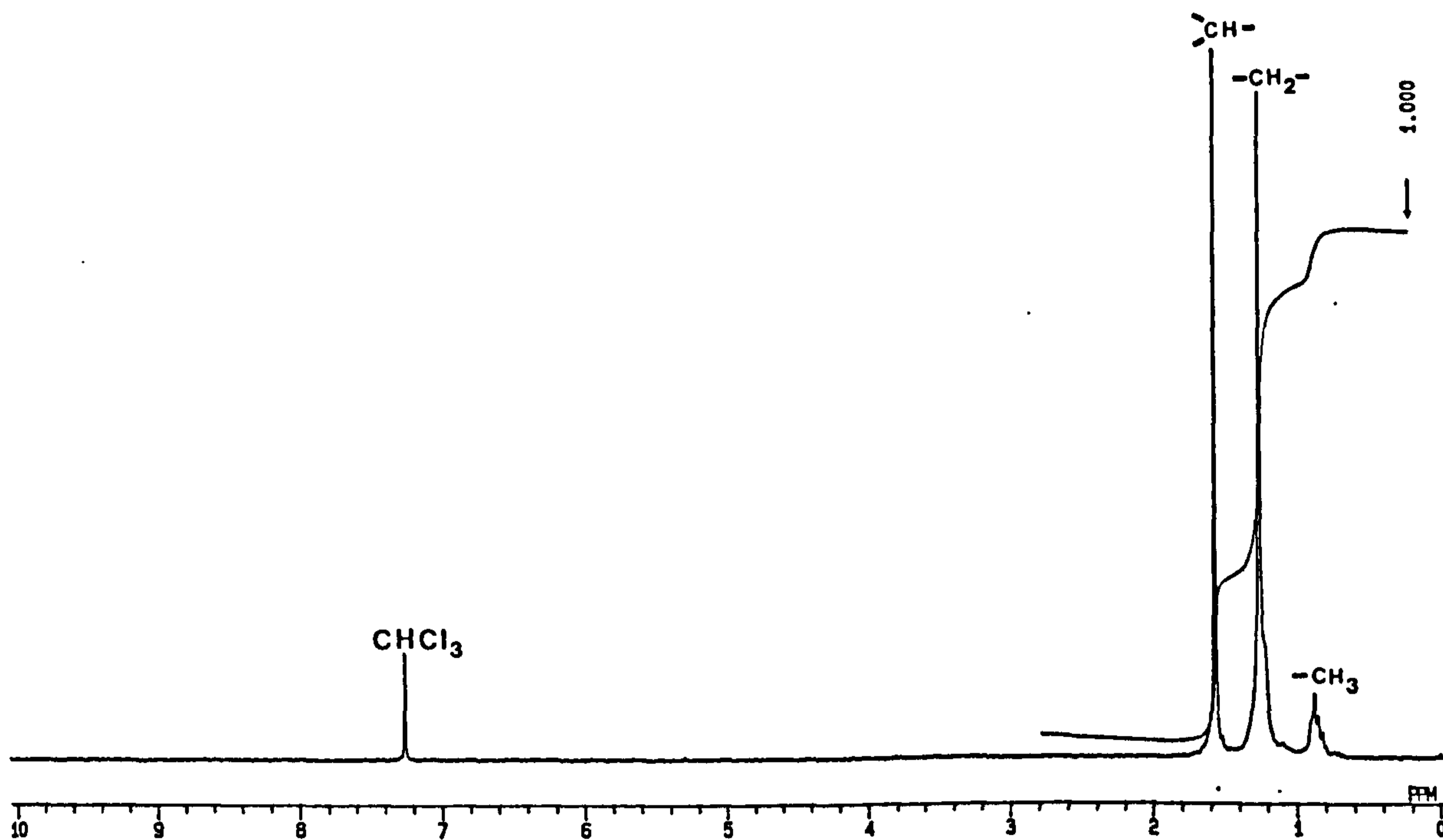


FIGURE 3.6.  $^1\text{H-NMR}$  SPECTRUM OF THE ALKATHENE UNA SATURATED FRACTION



In contrast, GCMS analysis of the polyene fraction from TLC (Figure 3.7) did indicate the presence of unresolved alkenes ( $m/z$  69) with little evidence for unresolved aromatic compounds, though these do appear to be present as resolved compounds (Figure 3.8).  $^1\text{H-NMR}$  of this fraction confirmed the presence of alkenes and the absence of significant amounts of aromatic compounds (Figure 3.9). Interestingly, there was only a very weak NMR resonance attributable to tertiary carbons, suggesting a reduced degree of branching compared with the saturated fraction.

In order to characterise the saturated UCM further and to allow comparison with petrogenic UCMs, the UNA saturated fraction (experiment 1) was subjected to chromic acid oxidation using the method of Gough (1989).

Oxidation produced 69% of recovered material, which is comparable to that from petrogenic UCMs (Gough and Rowland, 1990). However, only about 28% was unresolvable by GC (Figure 3.10). The latter figure is considerably lower than that produced from petrogenic UCMs, which typically have shown about 70% of unresolved oxidised material (Gough and Rowland, 1990). This could indicate either that the oxidant to substrate ratio is too great, leading to over oxidation, or that the Alkathene UCM contains a greater proportion of simple, more readily oxidised alkanes, than petrogenic UCMs, *i.e.* petrogenic UCMs are much more complex in their molecular structure than the Alkathene UCM.

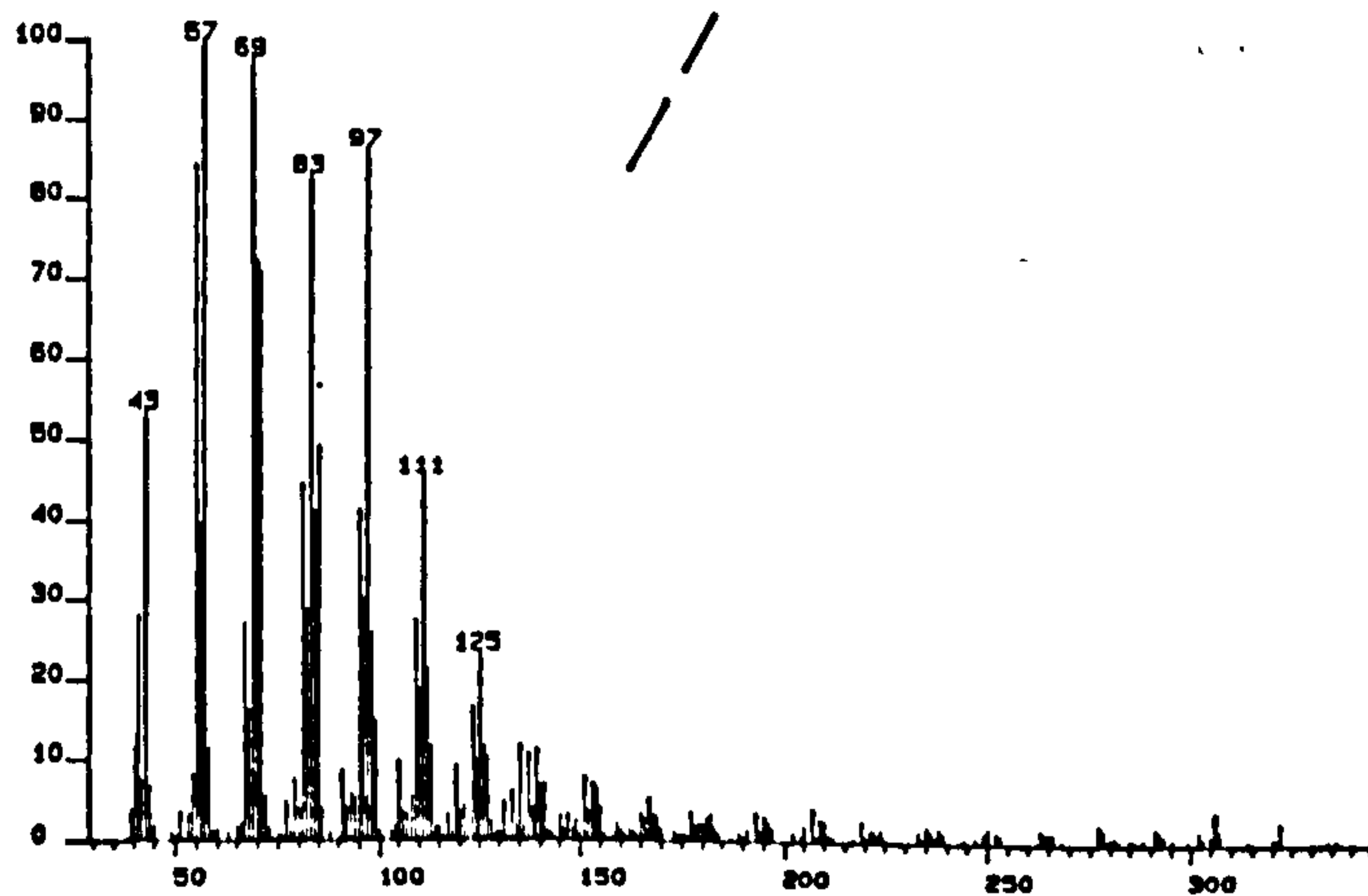
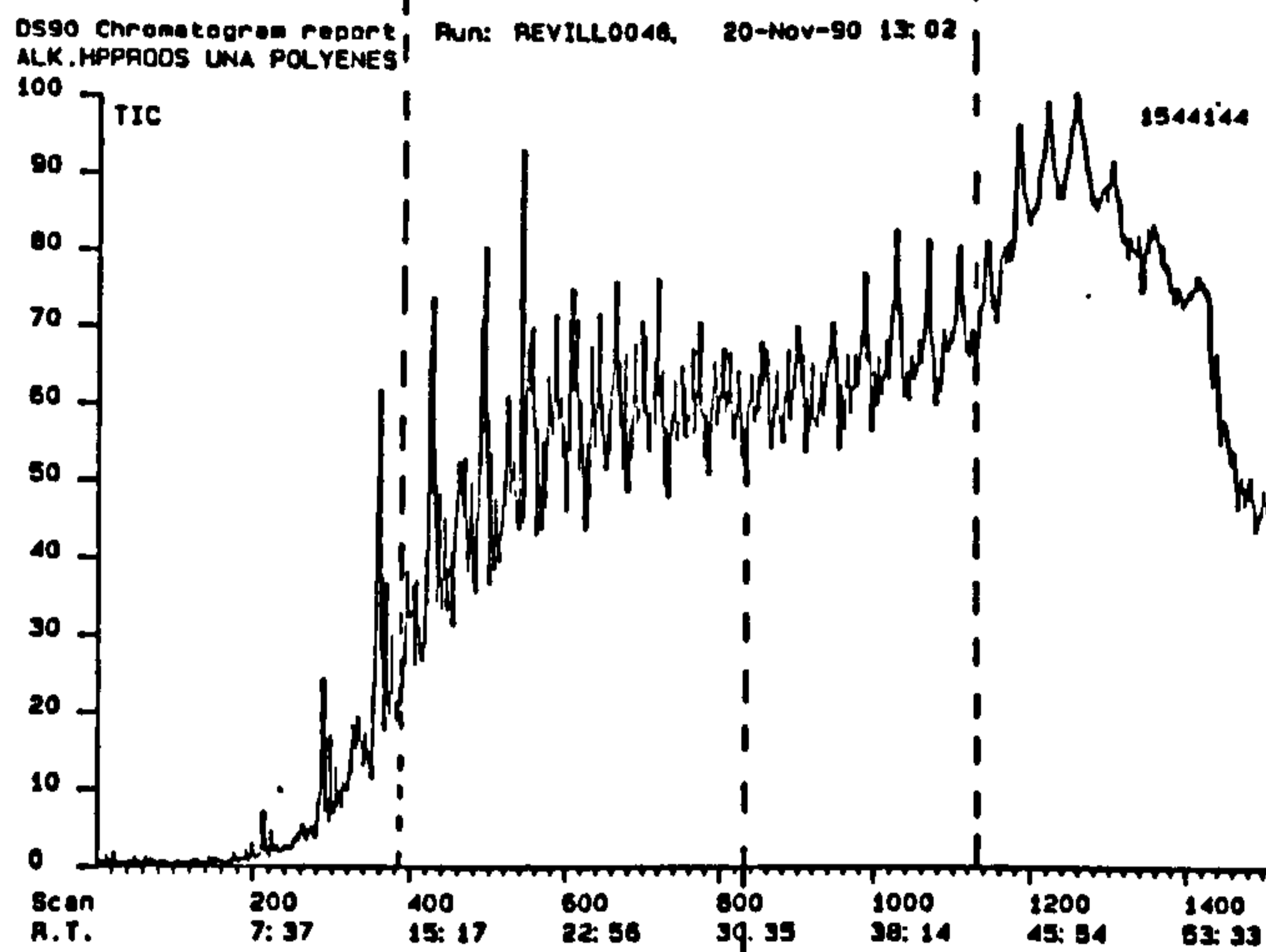
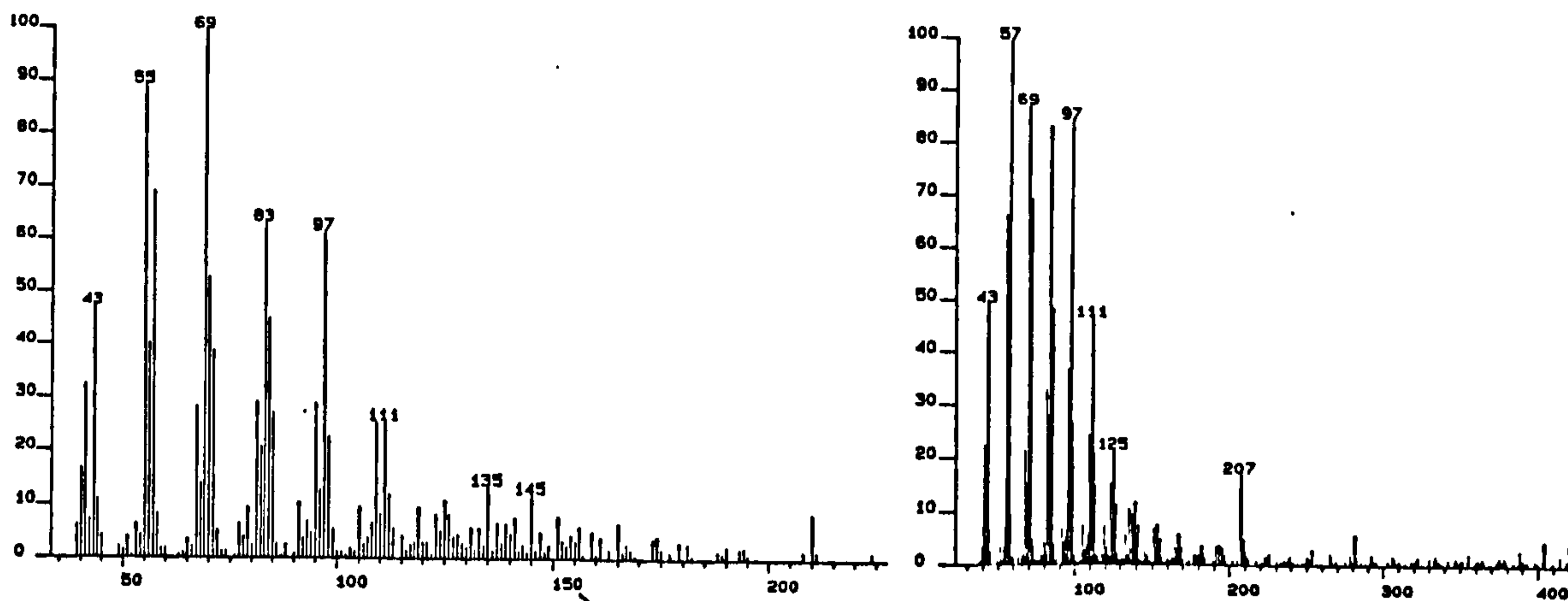
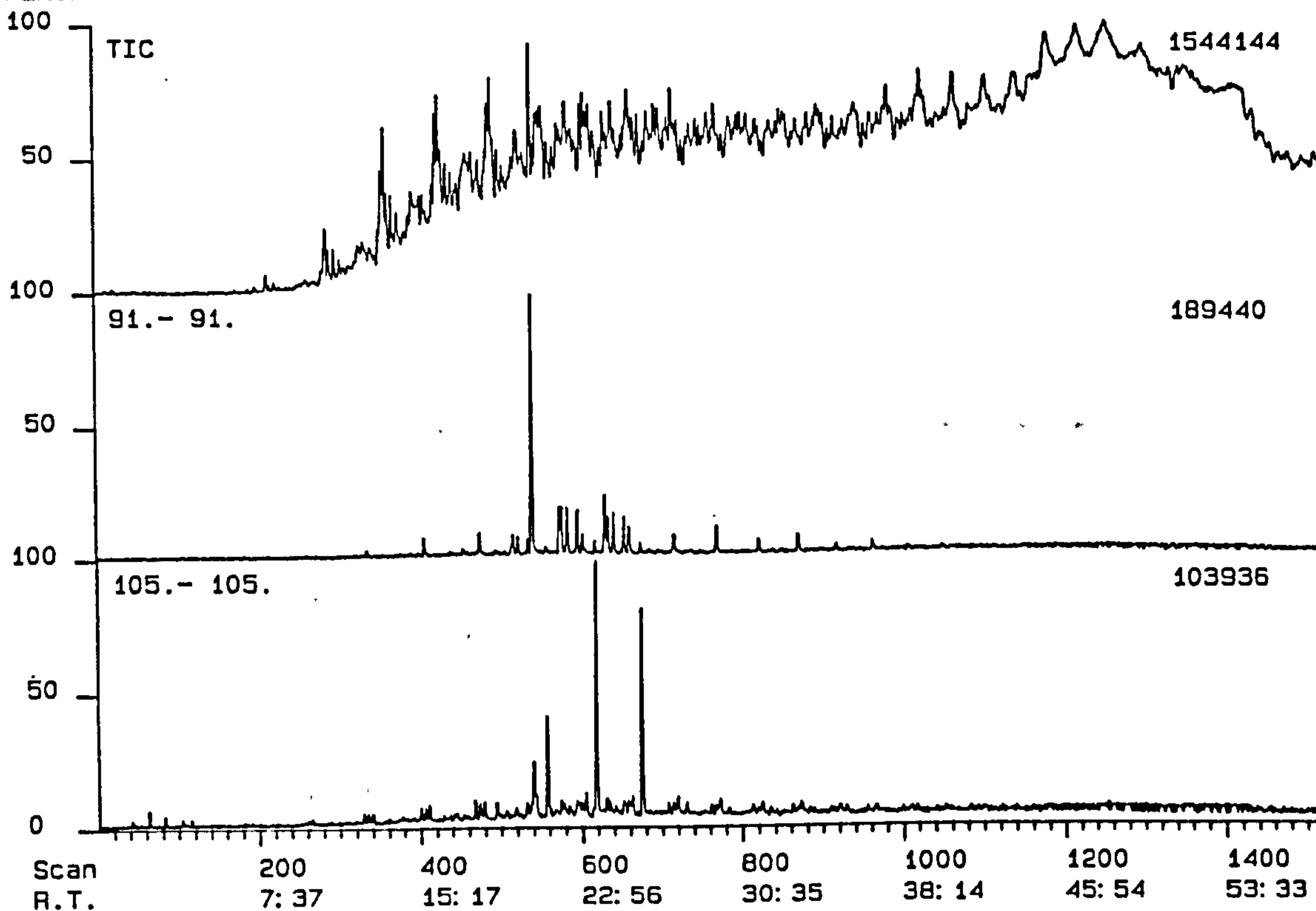


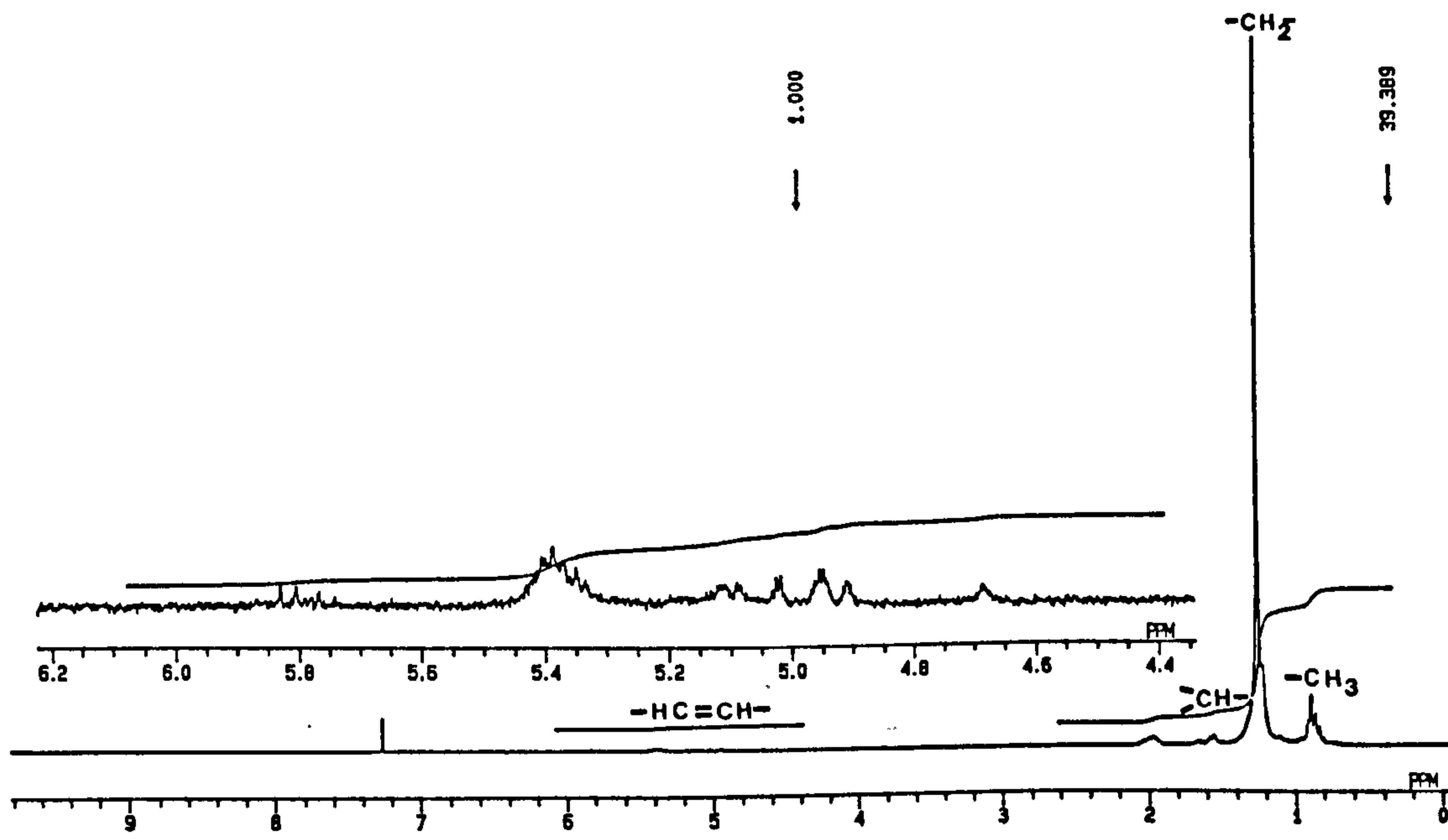
FIGURE 3.7. GCMS ANALYSIS OF THE ALKATHENE UNA 'POLYENE' FRACTION (J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas)

DS90 Chromatogram report Run: REVILL0048, 20-Nov-90 13:02  
ALK.HPPRODS UNA POLYENES

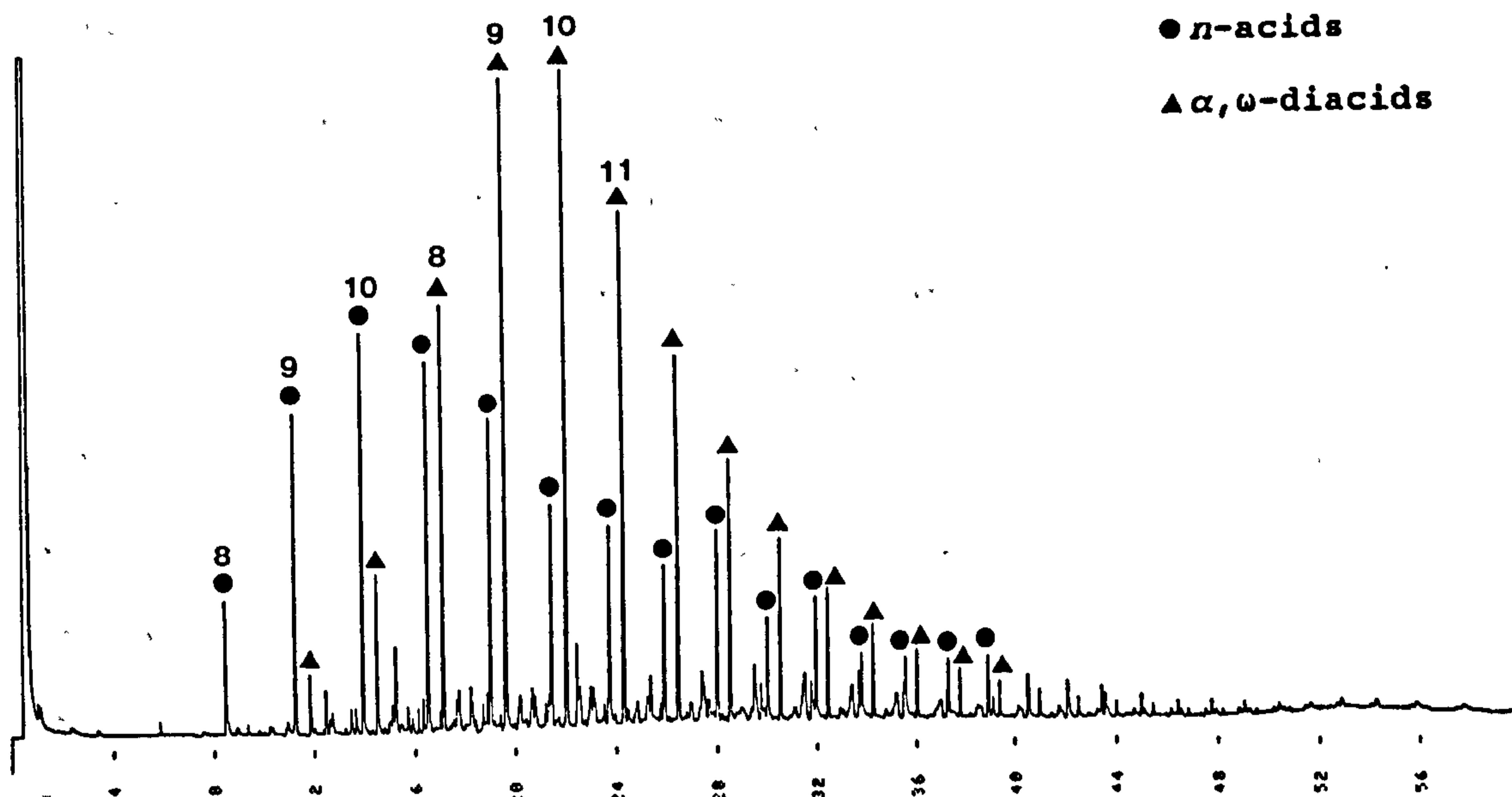


**FIGURE 3.8. MASS CHROMATOGRAMS TO SHOW THE PRESENCE OF AROMATIC COMPOUNDS IN THE ALKATHENE UNA 'POLYENE' FRACTION**  
(J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas)



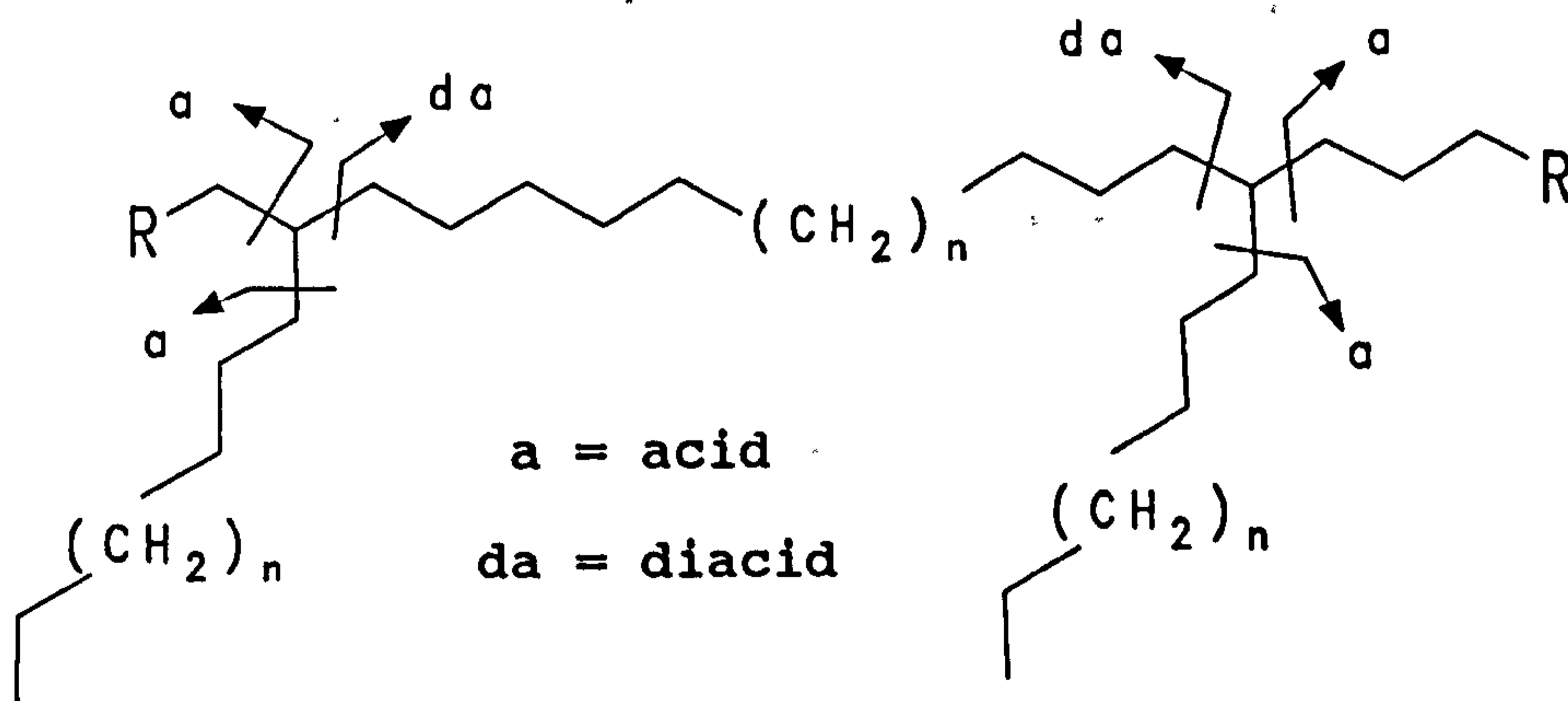


**FIGURE 3.9. <sup>1</sup>H-NMR SPECTRUM OF THE HYDROUS PYROLYSIS PRODUCTS OF ALKATHENE UNA "POLYENE" FRACTION**



**FIGURE 3.10. GAS CHROMATOGRAM OF THE  $\text{CrO}_3$  OXIDATION PRODUCTS FROM THE SATURATED HYDROCARBON UNA FRACTION ISOLATED FROM THE HYDROUS PYROLYSIS PRODUCTS OF ALKATHENE**  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min.,  $\text{H}_2$  carrier gas)  
 (numbers indicate chain length)

The resolved compounds in the oxidised Alkathene UCM were found to be *n*-monocarboxylic acids ( $n-C_{8-25}$ ; max.  $C_{10}$ ) and  $\alpha,\omega$ -dicarboxylic acids ( $C_{6-20}$ ; max.  $C_{9,10}$ ). Production of predominantly straight chain components from an artificially-produced UCM is a striking similarity to the result of oxidation of petrogenic UCMs. However, the relative dominance of *n*-dicarboxylic acids over *n*-monocarboxylic acids in the GC profile is in contrast to the oxidation product profiles seen from oxidation of petrogenic UCMs (Gough, 1989, Killips and Al-Juboori, 1990; Gough and Rowland, 1990, 1991; this study, Chapter 4). There are at least two possible explanations for the latter observation. One is that Alkathene, which has a branch point approximately every 30 carbons, fragments during hydrous pyrolysis at alternate inter-branch positions, leaving compounds with two branch points, which would then yield diacids upon oxidation:-



However, if this were the case, a predominance of diacids



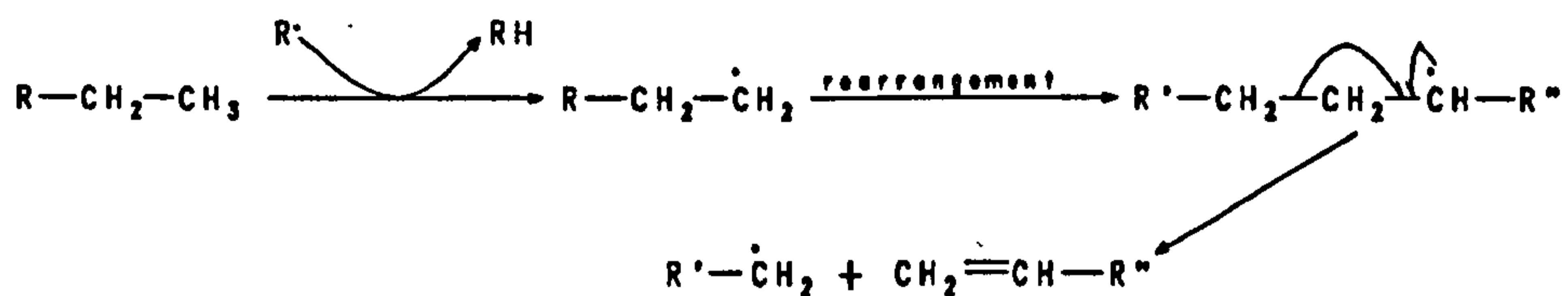
with around 30 carbons would be expected since this is the number of carbons between branch points. In addition, GCMS of the saturated UCM suggested a high proportion of compounds with a cyclic moiety, which would appear to refute the above mechanism, and in oxidations of authentic reference compounds performed by Gough (1989), 9-(2-cyclohexylethyl)heptadecane did not yield any diacids. Alternatively, the predominance of diacids could be due to secondary oxidation. In this study the oxidation method was the same as that used for petrogenic UCMs and the substrate was assumed to have a molecular weight of 352. Should this have been an overestimation, an excess of the oxidant might have produced over oxidation. Since Roceck and Mares (1959) proposed that all methylene groups in a linear alkyl chain are equivalent and should be oxidised at the same rate, and Gough (1989) found that a series of  $\alpha,\omega$ -dicarboxylic acids were formed in the oxidation of *n*-pentacosane, presumably from secondary oxidation, this appears to be the more reasonable explanation for the present results. Interestingly, the *n*-mono acids produced in the oxidation exhibit a strong even over odd predominance between C<sub>14</sub> and C<sub>20</sub>, while the diacids exhibit no apparent carbon number predominance. Further investigations are required to explain this result.

No evidence could be found for the presence of ketones and  $\gamma$ -lactones in the oxidation products, although these have been found in all of the petrogenic oxidation products to

date. Such compounds originate from methyl branched and isoprenoidal compounds in oils as shown by oxidation of authentic compounds (Gough and Rowland, 1991). So it is perhaps unsurprising that they were absent from the Alkathene UCM oxidation products.

### 3.2.2.2 POSSIBLE MECHANISMS FOR THE FORMATION OF A UCM BY LABORATORY CATAGENESIS OF ALKATHENE

Hoering (1984) concluded from hydrous pyrolysis experiments utilising deuterium labelled water that the reaction mechanism occurring in hydrous pyrolysis (and by inference natural catagenesis) were consistent with a free radical process. Abbott et al. (1990), studying biological marker release from kerogen during hydrous pyrolysis, also stated that the resulting kinetic calculations supported a free radical mechanism. Such a mechanism would yield predominantly alk-1-enes from alkyl chains, viz;



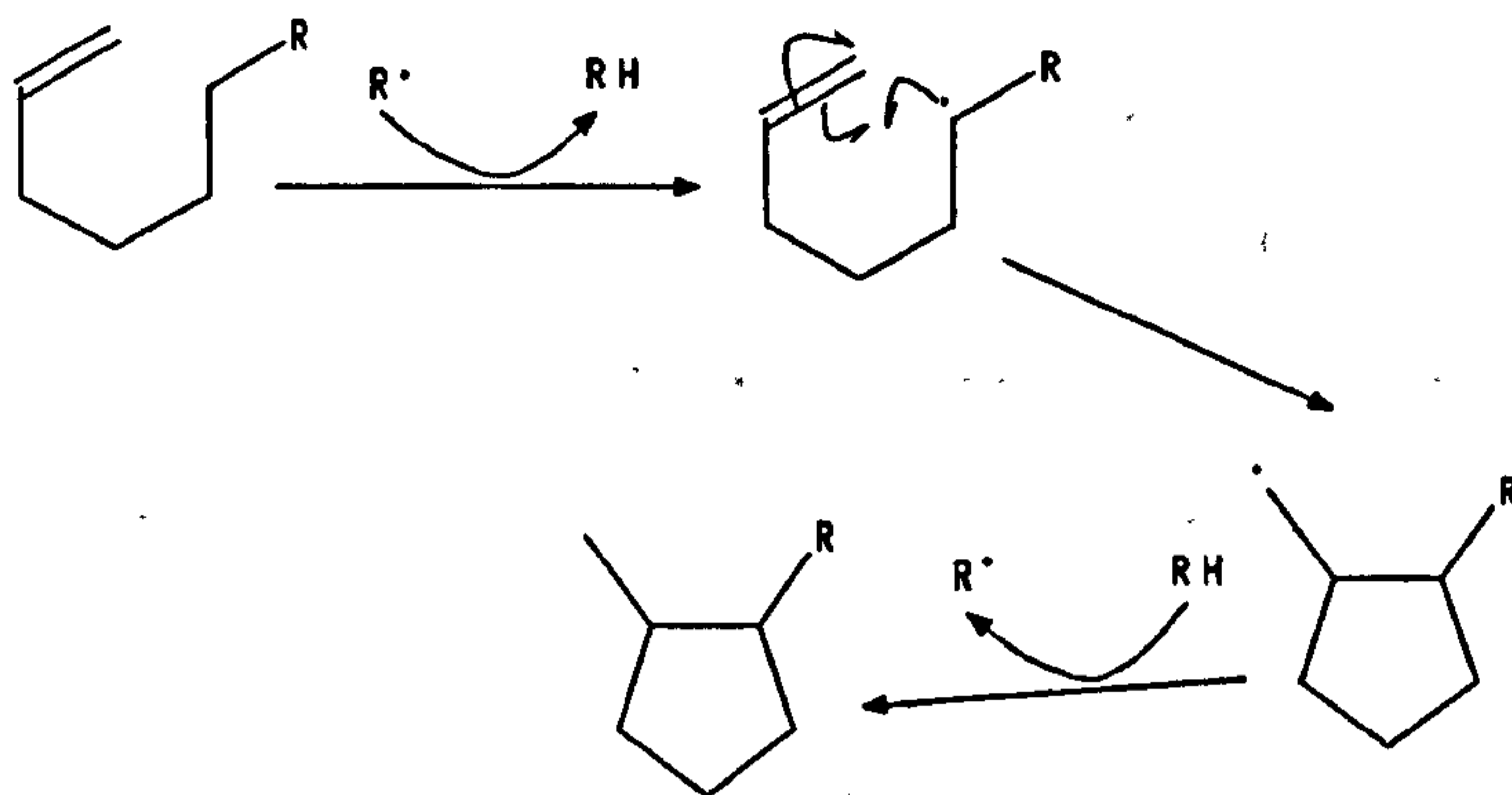
(after Henderson et al., 1968 and de Leeuw, Personal Communication, 1991)

Terminal alkenes however could reasonably be expected to

become hydrogenated within the hydrous pyrolysis free radical system by the scavenging of hydrogen (Hoering, 1984). Rowland (1990) reported the production of branched alkenes (e.g. phyt-1-ene) in the hydrous pyrolysis of methanogenic bacteria and it is well known that alkyl substituted alkenes have increased stability, due to steric hindrance of the hydrogenation step (Rylander, 1979). Thus, it is unexpected that terminal alkenes would survive under the conditions which prevail in hydrous pyrolysis and it seems likely that the unsaturation is associated with a more hindered carbon (e.g. a tertiary carbon), possibly due to migration away from the terminal position. This could explain the predominantly unresolved nature of the polyene fraction, since some form of branching is presumed to be required to produce the unresolved mixture, e.g. the "T-branch" type structures proposed for the aliphatic UCM (Gough and Rowland, 1990).

Comparing the UCM produced in this study with those in oils, it is perhaps the saturated fraction which is of most interest. GCMS and <sup>1</sup>H-NMR suggest a high degree of branching and cyclicity within the unresolved portion of this fraction. Bearing in mind the initial formation of terminal alkenes as previously discussed, it is conceivable that free radical cyclisation (Ege, 1989) could occur, producing alkylcyclopentanes, viz;





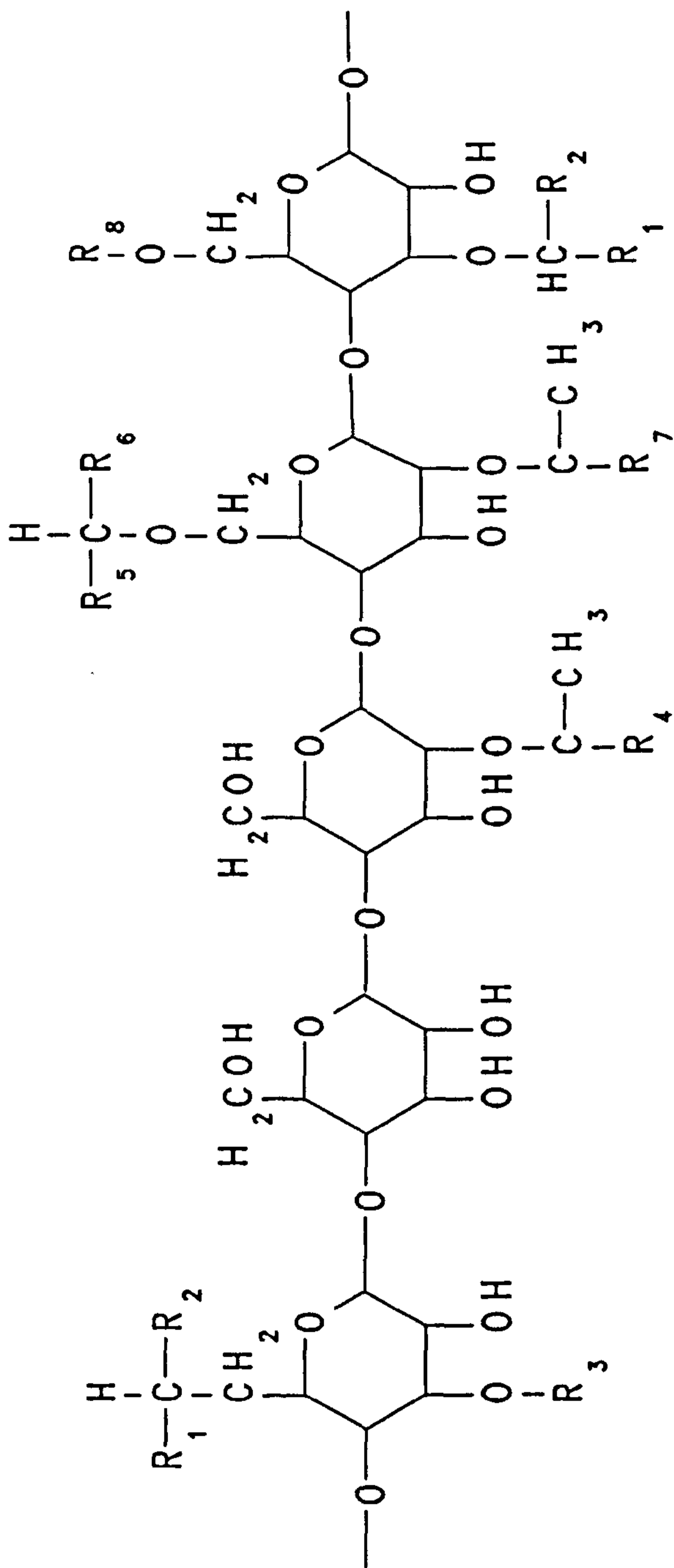
However, it should be possible for the corresponding six membered ring to form, since these are slightly more stable (March, 1985). Mango (1990), in studying possible origins for the light cycloalkanes in petroleum, used heating experiments to show that an *n*-alkane (octadecane) heated in sealed tubes, with cholestane and adamantane, without water at 330°C for 4 weeks yields alkylated homologues with both five and six membered rings, which were not produced from degradation of the cholestane or adamantane (determined by mass). This would suggest that polymethylenic chains will undergo reactions which can produce cyclic compounds, possibly by mechanisms similar to those described.

### 3.2.3 HYDROUS PYROLYSIS OF AN ALIPHATIC BIOPOLYMER

Recently, there has been renewed interest in the role that selective preservation of biomacromolecules plays in the formation of kerogen (e.g. Tegelaar et al., 1989b; 1989c). This has led to the identification of a wide range of

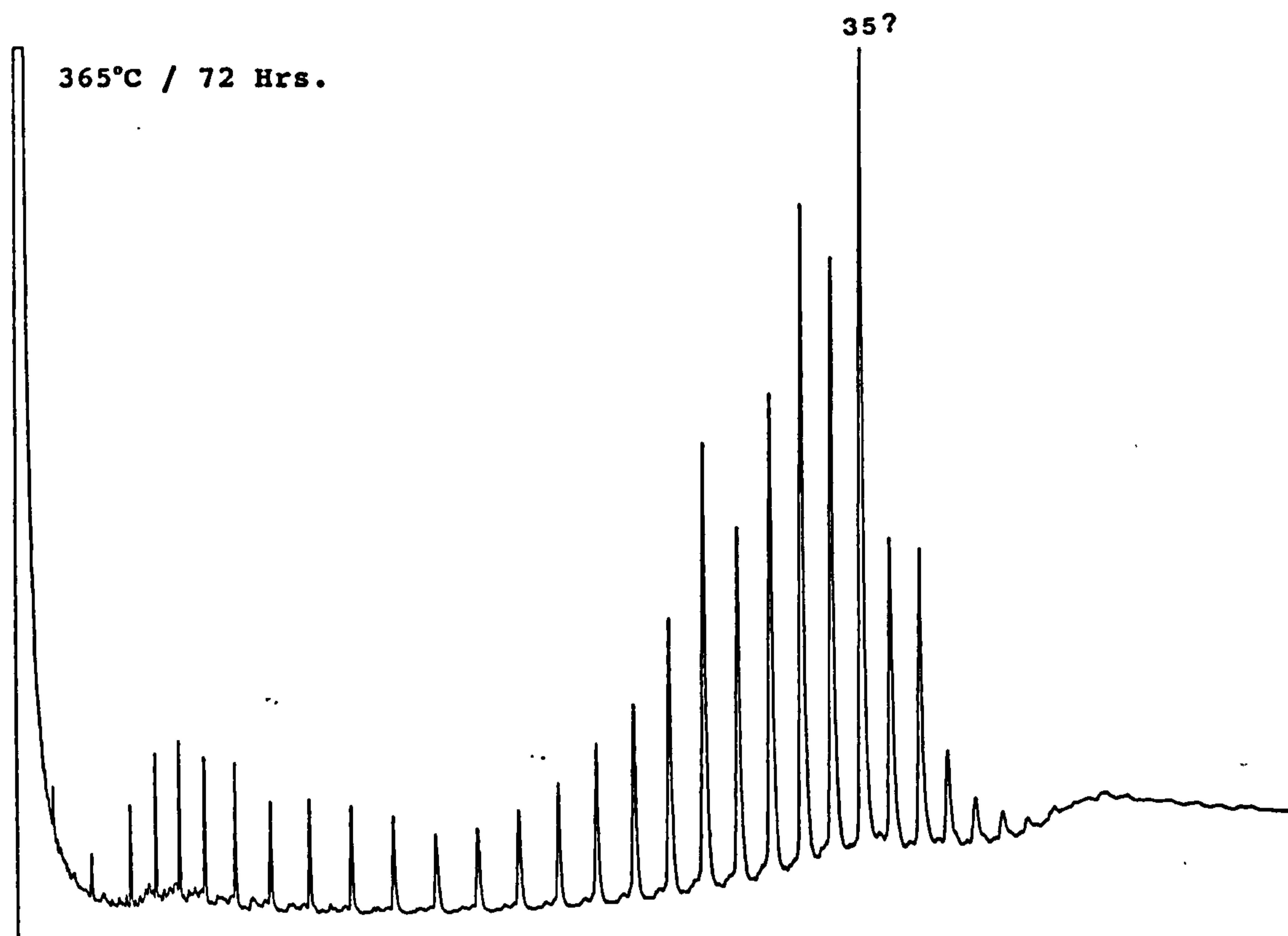
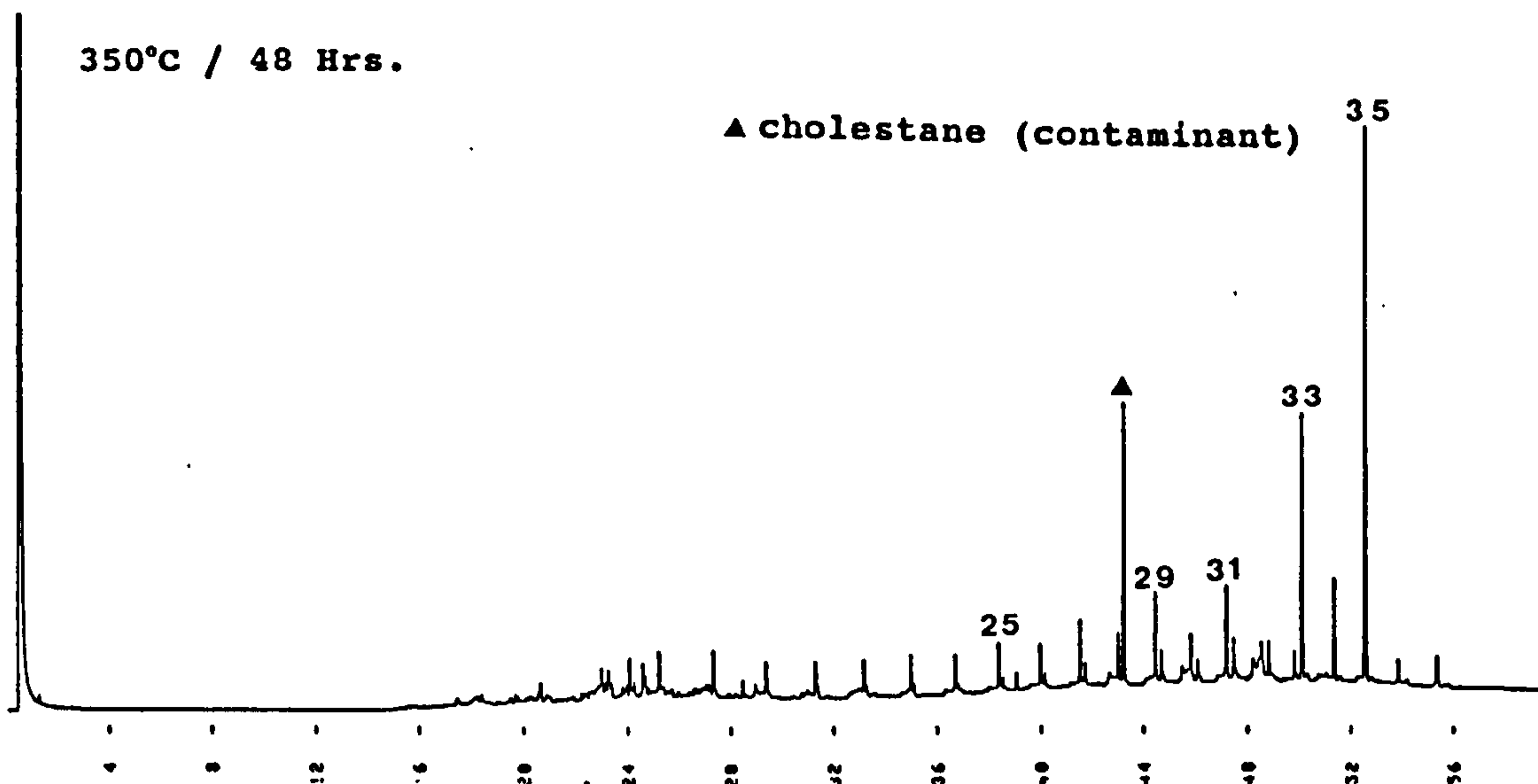
resistant biopolymers (e.g. Largeau et al., 1984; Derenne et al., 1988; Goth et al., 1988; Tegelaar et al., 1989c; Derenne et al., 1991) and one such, is the biopolymer of *Agave americana* (L). This biopolymer, referred to as cutan, is proposed to consist of a carbohydrate backbone linked to aliphatic moieties via ether bonds (Figure 3.11). Cutan has also been used in the present study as a substrate for laboratory formation of a UCM.

The sample of cutan was subjected to hydrous pyrolysis at 350°C for 48 hours and, in a second experiment, at 365°C for 72 hours. These times and temperatures were adventitious since the samples were provided by other workers (see acknowledgements). Quantitative data, where available, are given in Table 3.2 (page 78). The GC profiles appear to contain several similarities (Figure 3.12). Both exhibit a range of *n*-alkanes (*n*-C<sub>14-40</sub>; max. *n*-C<sub>35</sub>) with a strong odd over even predominance between C<sub>29</sub> and C<sub>35</sub>. The profile from experiment 1 is 29% resolved by GC (Table 3.2; no data were available for experiment 2). The UNA fractions exhibited broad UCMs (that from experiment 2 was bimodal; Figure 3.13). Both UNA fractions were 75% unresolved by GC (Table 3.2). Fractionation of the UNA from both experiments into hydrocarbons and non-hydrocarbons (Table 3.3; Figures 3.14 and 3.15) indicated that the relative proportions of hydrocarbons increased with an increase in pyrolysis temperature/time. In addition, the UCM decreased from 85% to 60% (Table 3.3).

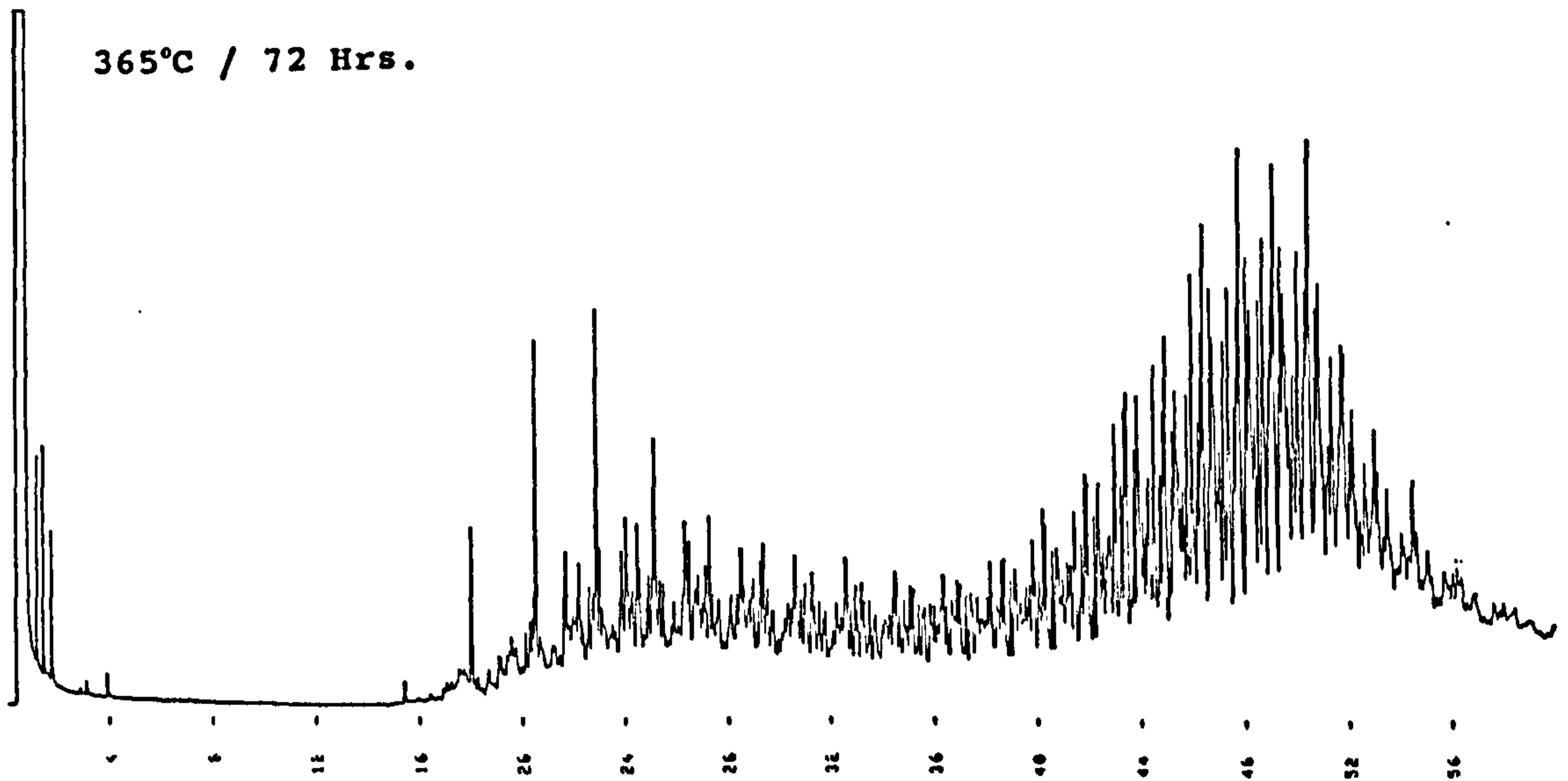
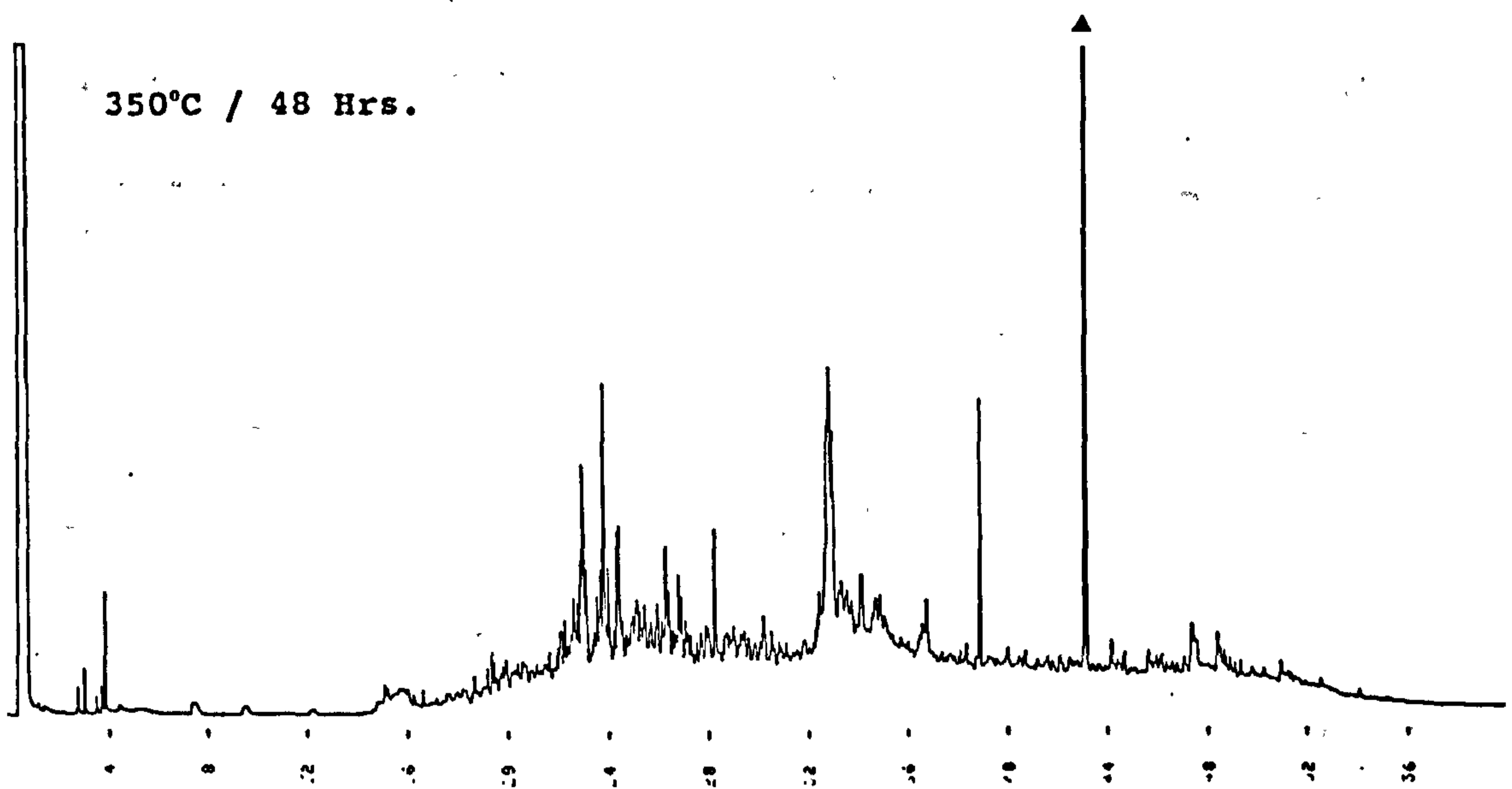


**FIGURE 3.11. PROPOSED STRUCTURE OF THE HIGHLY ALIPHATIC BIOPOLYMER FROM AGAVE AMERICANA (Tegelaar et al. 1989a)**





**FIGURE 3.12. HYDROUS PYROLYSIS PRODUCTS OF THE ALIPHATIC BIOPOLYMER ISOLATED FROM AGAVE AMERICANA, TOTAL ORGANIC SOLUBLES**  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)  
 (numbers indicate *n*-alkane chain length; assignment in experiment 2 is by comparison with experiment 1 results)



**FIGURE 3.13. CUTAN HYDROUS PYROLYSIS PRODUCTS UNA FRACTIONS**  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)  
 [ ▲ cholestane (contaminant) ]

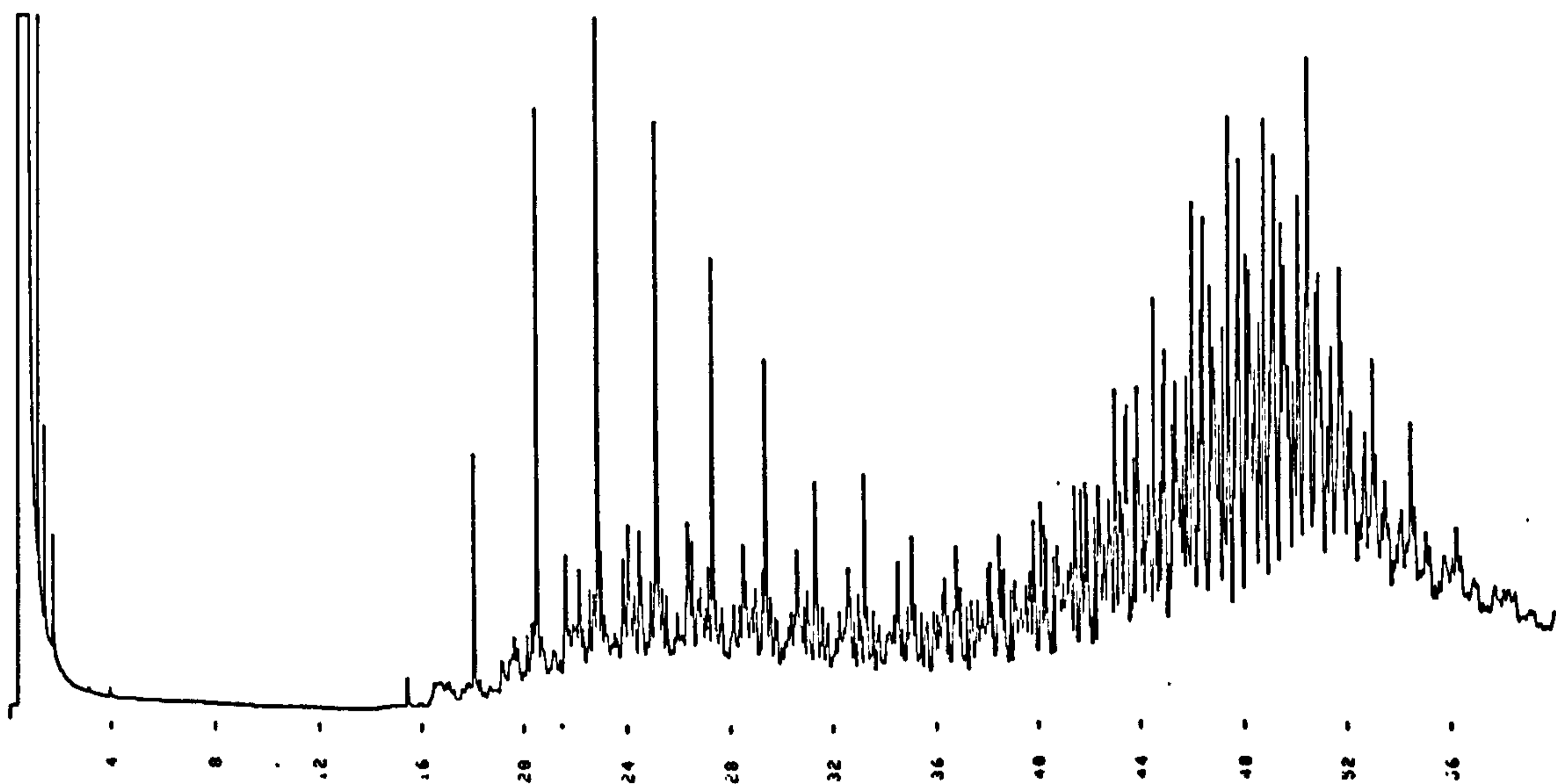
**TABLE 3.3. QUANTITATIVE DATA FOR THE FRACTIONATION OF CUTAN PYROLYSATE**

Pyrolysis Temp/Time	Total UNA (mg)	% Resolved	H/C		NON H/C	
			mg	% res.	mg	% res.
1. 350°C/48hrs	0.5	25	0.15 (30%)	15	0.36 (70%)	15
2. 365°C/72hrs	8.9	25	6.7 (75%)	40*	2.2 (25%)	11

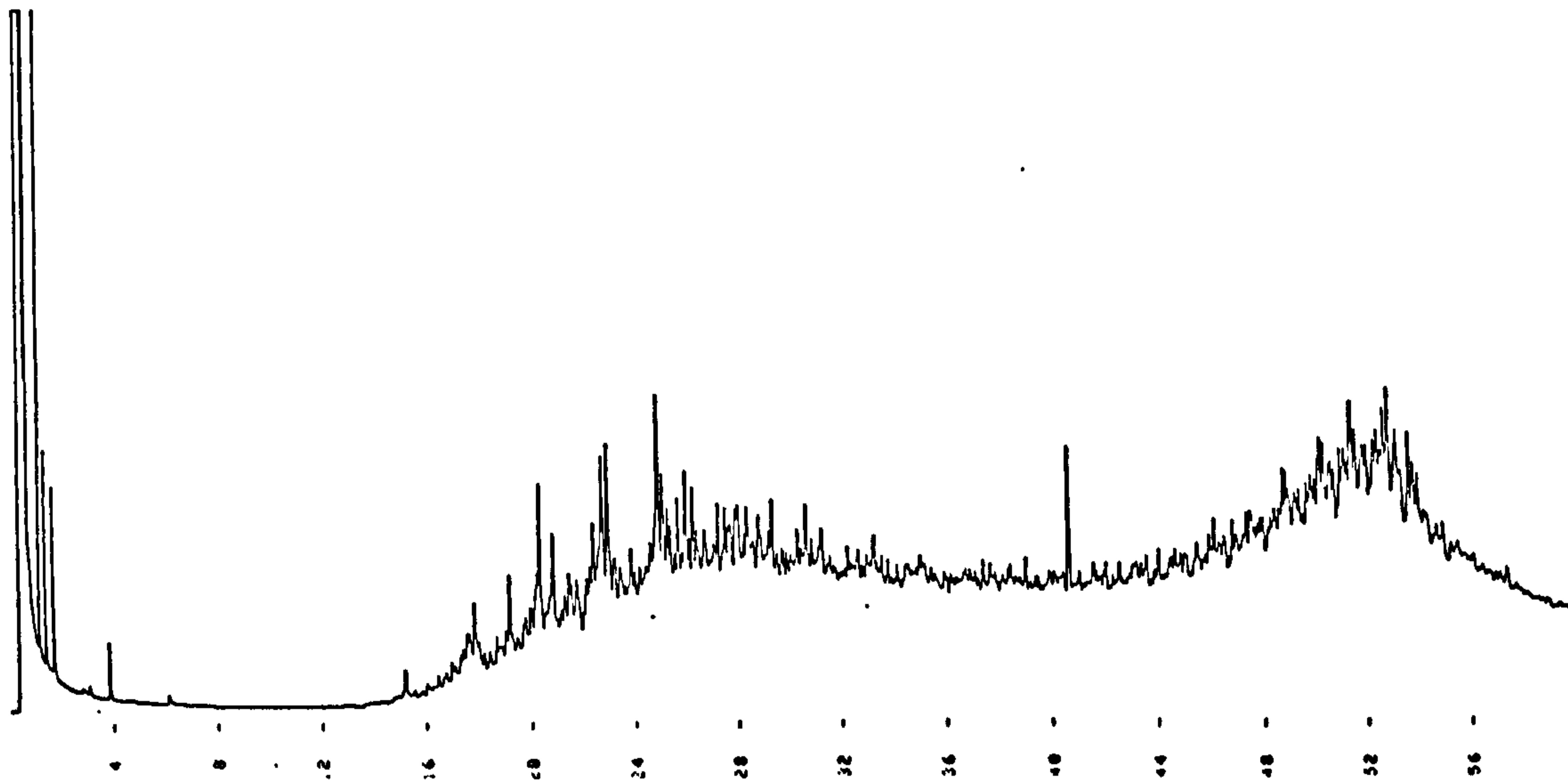
\* This value becomes 35% if the residual alkanes are removed from the calculation



Hydrocarbon fraction



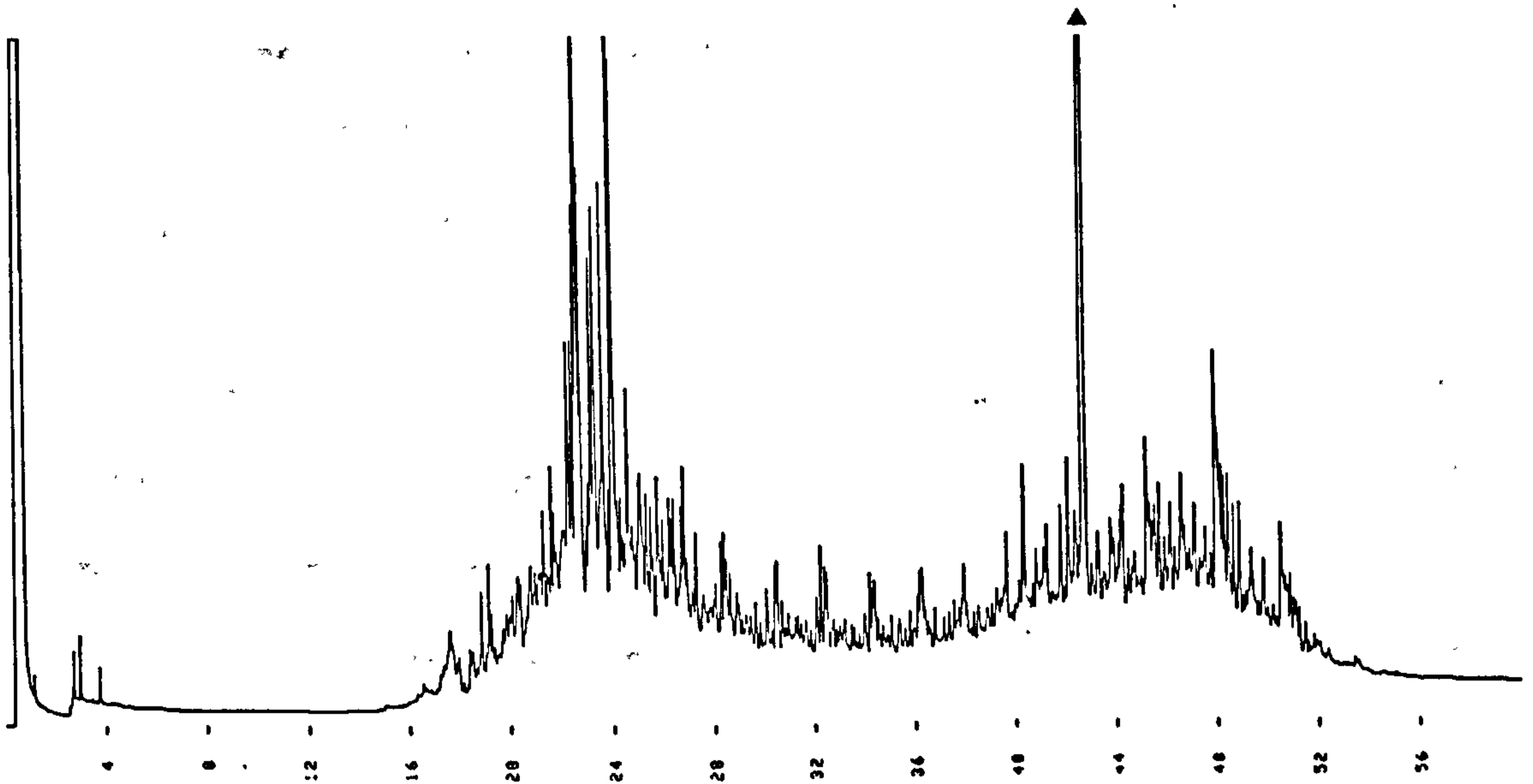
non-hydrocarbon fraction



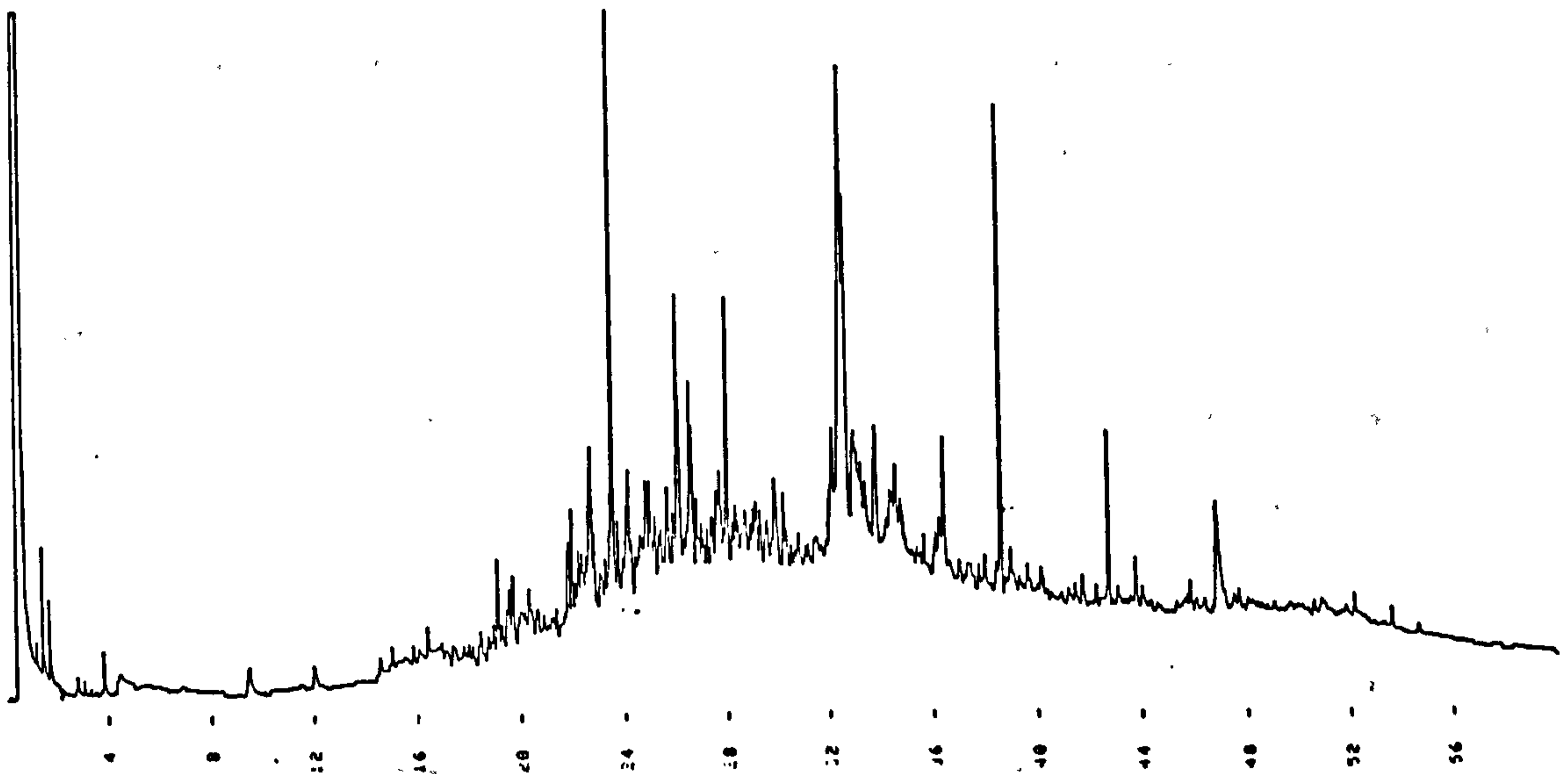
**FIGURE 3.14. CUTAN HYDROUS PYROLYSIS EXPERIMENT 2 UNA  
HYDROCARBON AND NON-HYDROCARBON FRACTIONS  
(J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)**

Hydrocarbon fraction

[▲cholestane (contaminant)]



non-hydrocarbon fraction



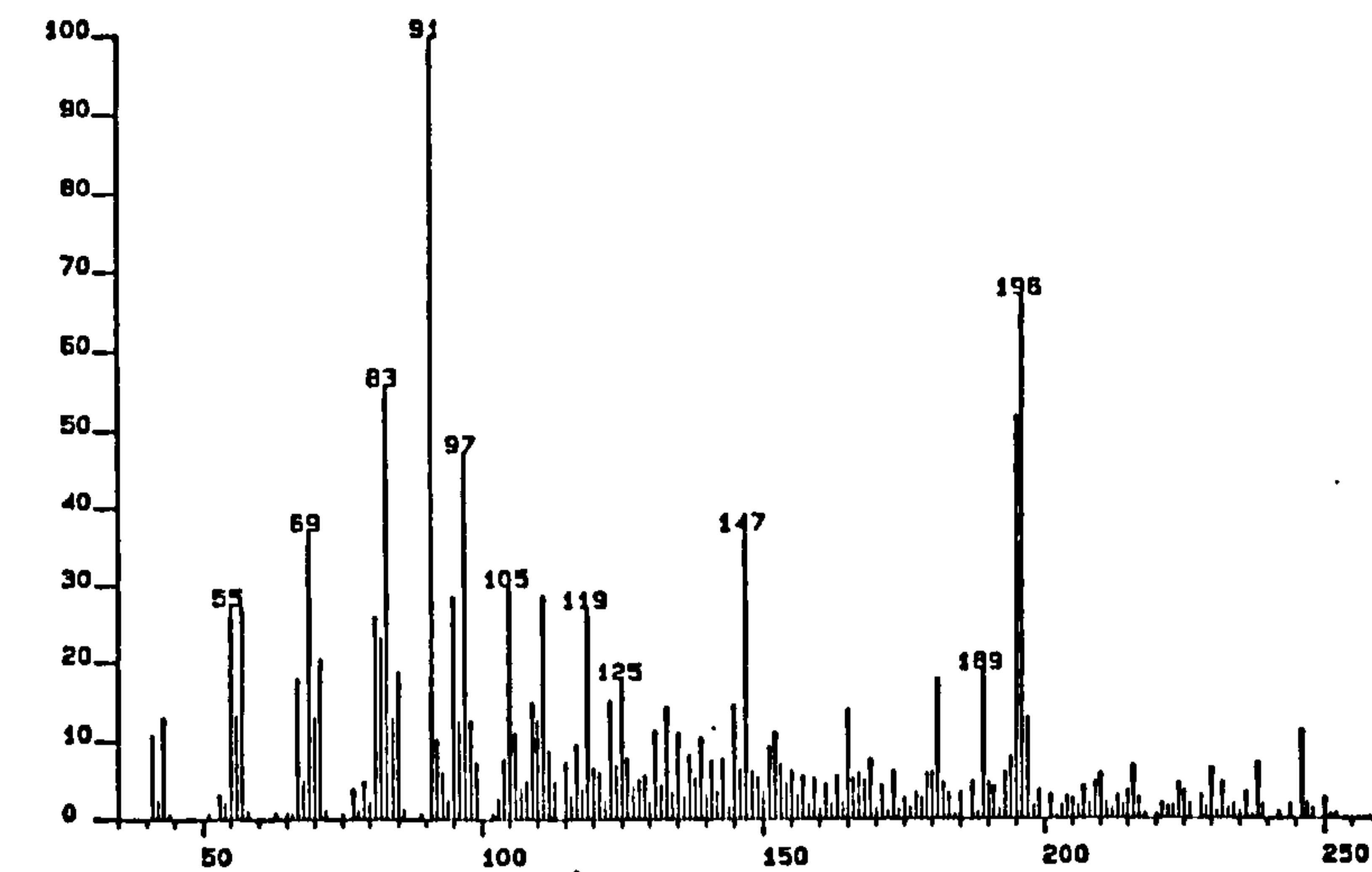
**FIGURE 3.15. CUTAN HYDROUS PYROLYSIS EXPERIMENT 1 UNA HYDROCARBON AND NON-HYDROCARBON FRACTIONS (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)**

Unfortunately, the relatively small amount of material isolated from experiment 1 precluded any subsequent analysis. GCMS and  $^1\text{H-NMR}$  of the UCM hydrocarbon fraction from experiment 2 indicated the presence of cyclic ( $m/z$  69, 83, 97), branched (1.5 ppm) and some aromatic ( $m/z$  91; 7 ppm) compounds (Figures 3.16 and 3.17).

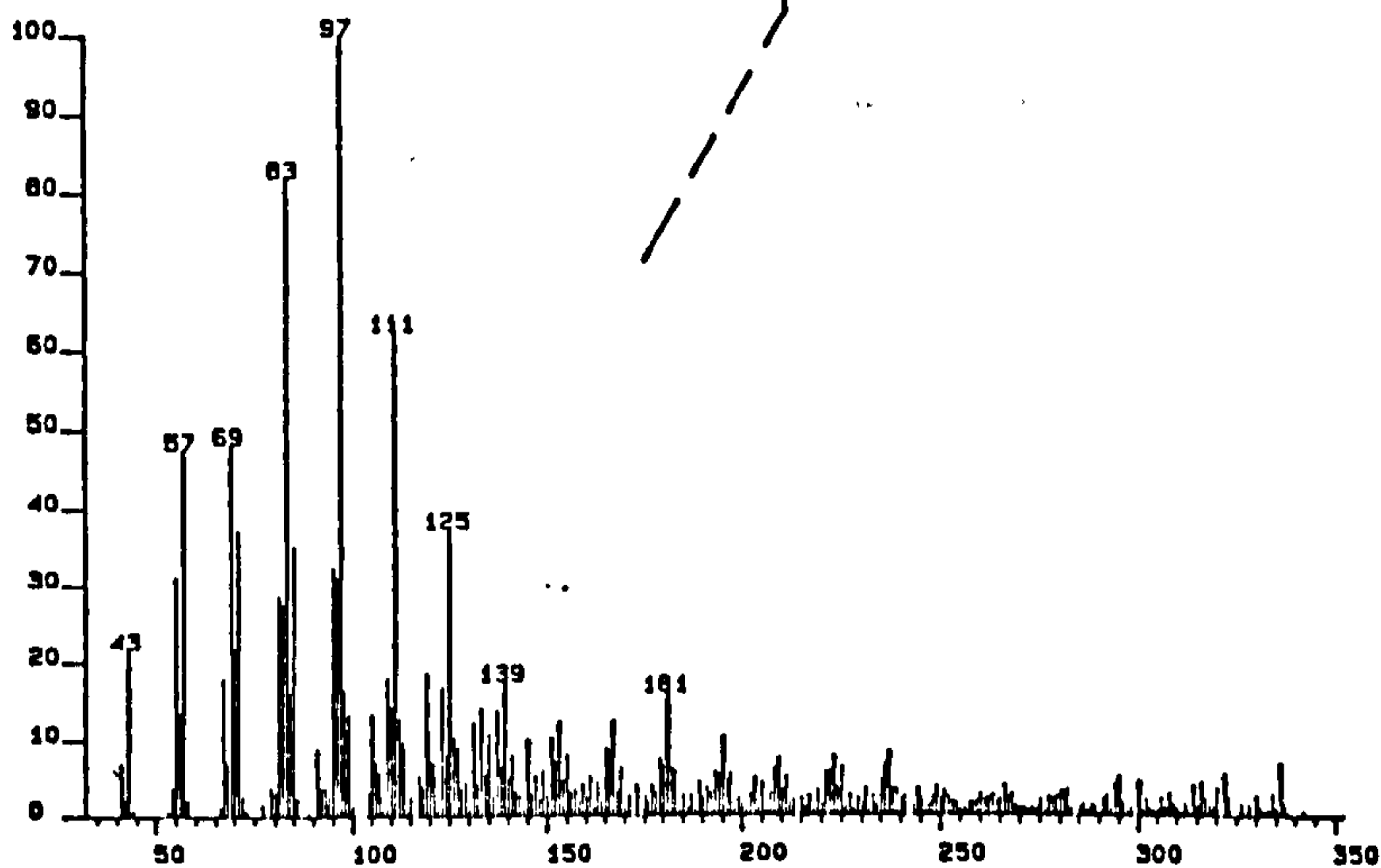
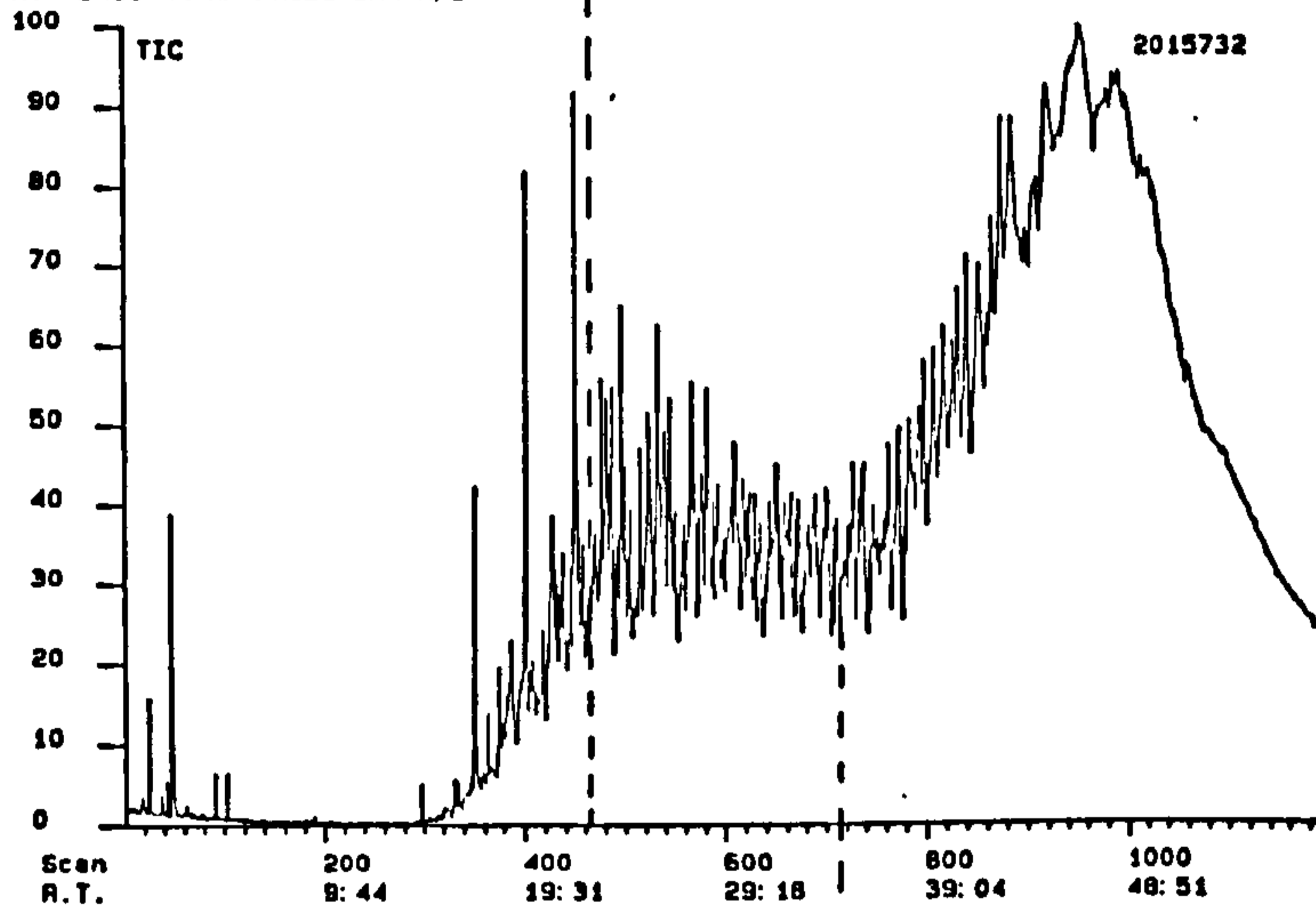
The corresponding non-hydrocarbons fraction was 89% unresolved by GC (Figure 3.14) and from GCMS, IR and  $^1\text{H-NMR}$  shown to contain highly aliphatic (e.g.  $2930\text{ cm}^{-1}$ , C-H str.; 1.3 ppm) functionalised (e.g.  $1700\text{ cm}^{-1}$ , C=O str.) compounds (Figures 3.18-3.21). This possibly suggests that ether linkages in the original biopolymer were incorporated into aliphatic structures e.g. as ketones or aldehydes.

Interestingly,  $^1\text{H-NMR}$  showed that neither fraction contained any alkenes, which were abundant in the hydrous pyrolysate of Alkathene. Although the reason for this is not known it is not surprising that there is such a difference since the two substrates are very different. The UNA hydrocarbon fraction from experiment 2 was also subjected to oxidation with chromic acid. Recovery of material exceeded 90% of which 70% was resolved by GC (Figure 3.22). The major oxidation products were again straight chain compounds viz,  $n$ -monocarboxylic acids ( $\text{C}_6\text{-C}_{32}$ ; max.  $\text{C}_{11,12}$ ) and  $\alpha,\omega$ -dicarboxylic acids ( $\text{C}_8\text{-C}_{21}$ ; max.  $\text{C}_{11}$ ). Thus, this artificially-produced UCM is also, to some extent, similar to the UCM found in oils. The  $n$ -diacids were less abundant

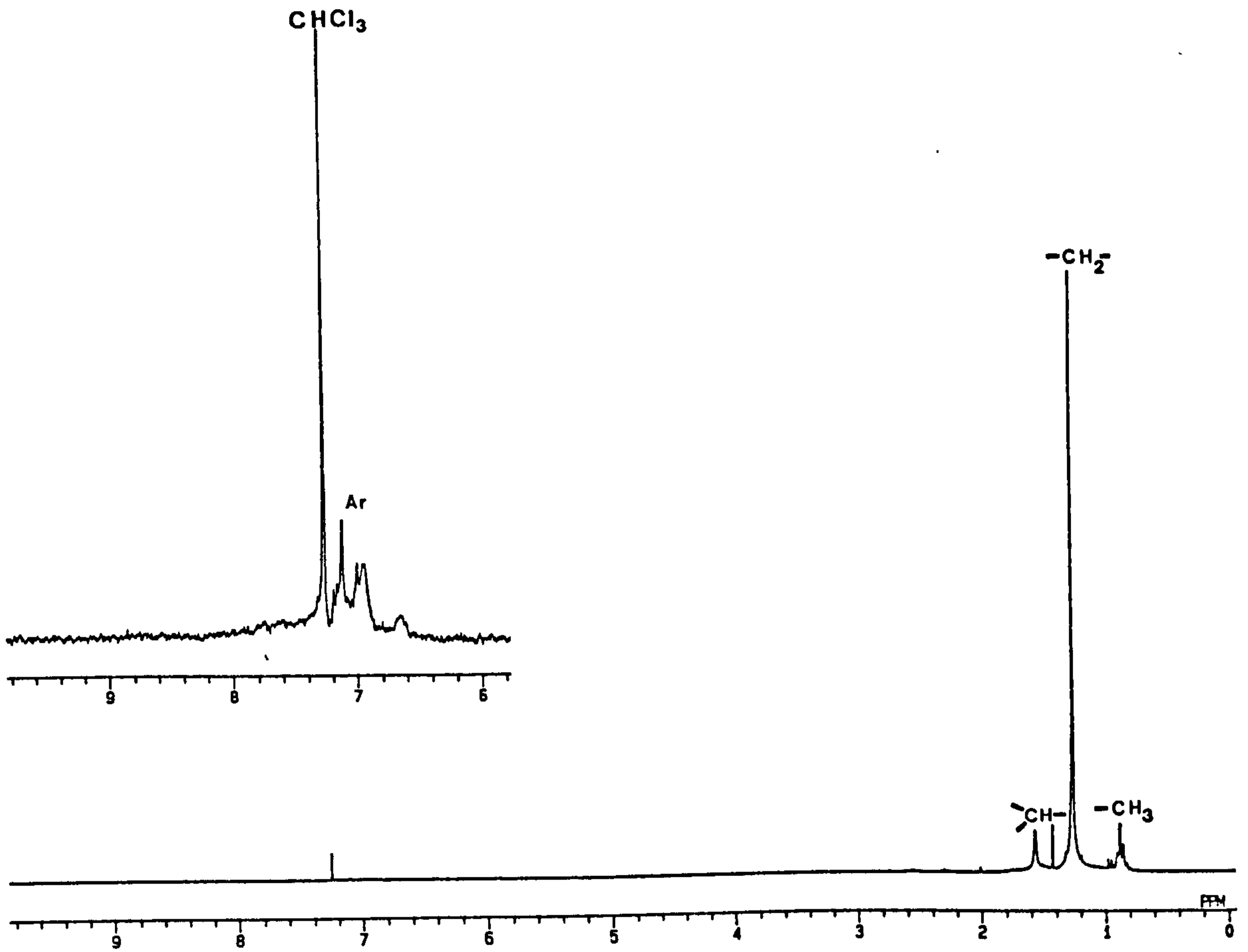




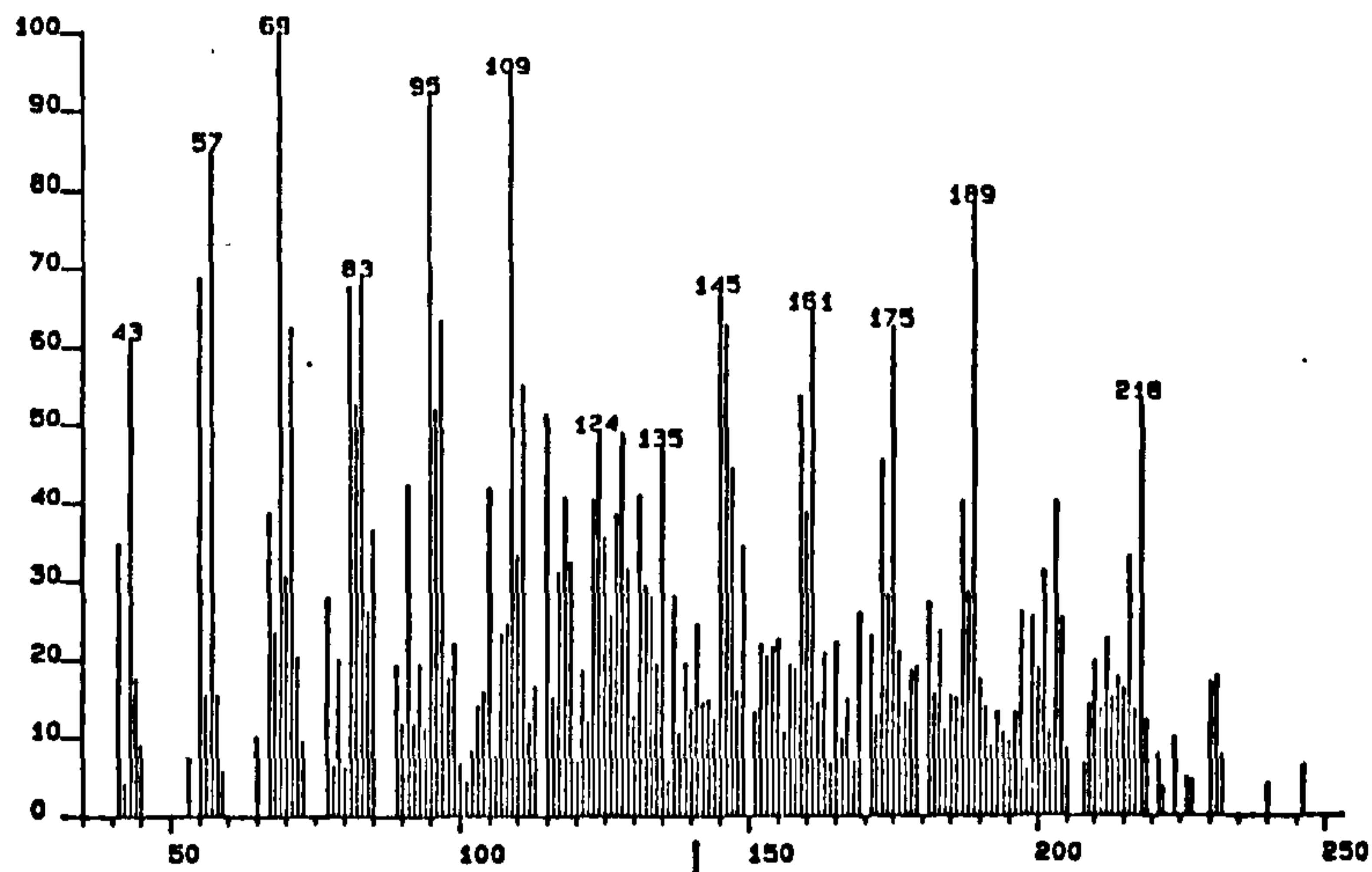
DS90 Chromatogram report Run: REVILL0083, 1-Nov-91 12:57  
 A.AMERICANA HP PRODS UNA H/C



**FIGURE 3.16. GCMS ANALYSIS OF CUTAN HYDROUS PYROLYSIS  
 EXPERIMENT 2 UNA HYDROCARBON FRACTION  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas)**



**FIGURE 3.17. <sup>1</sup>H-NMR SPECTRUM OF CUTAN EXPERIMENT 2  
UNA HYDROCARBON FRACTION  
(Jeol 270MHz; sample in CDCl<sub>3</sub>)**



DS90 Chromatogram report Run: REVILL0079, 14-Oct-91 13:10  
 A.AMERICANA HP PRODS UNA NON/HC

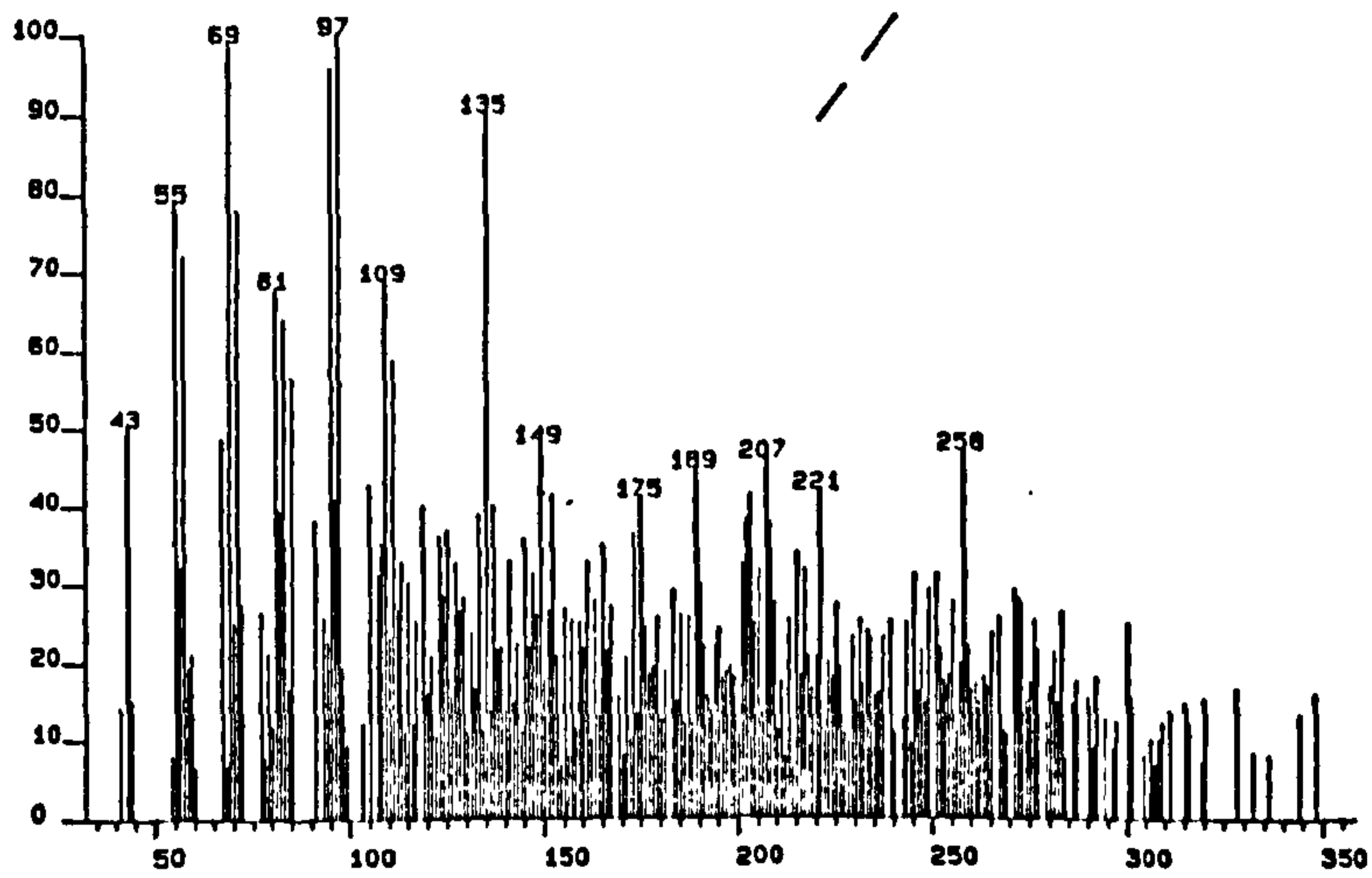
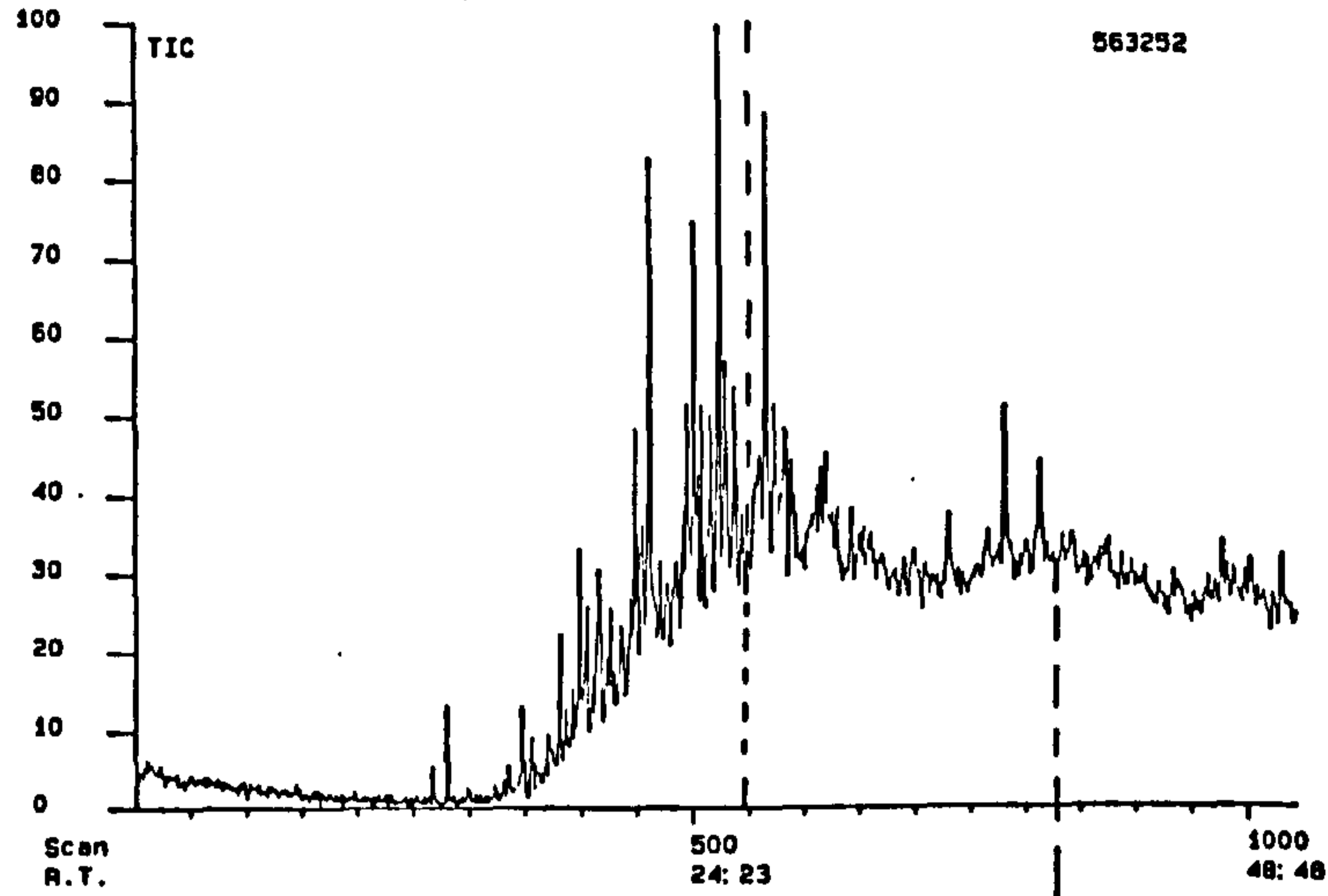
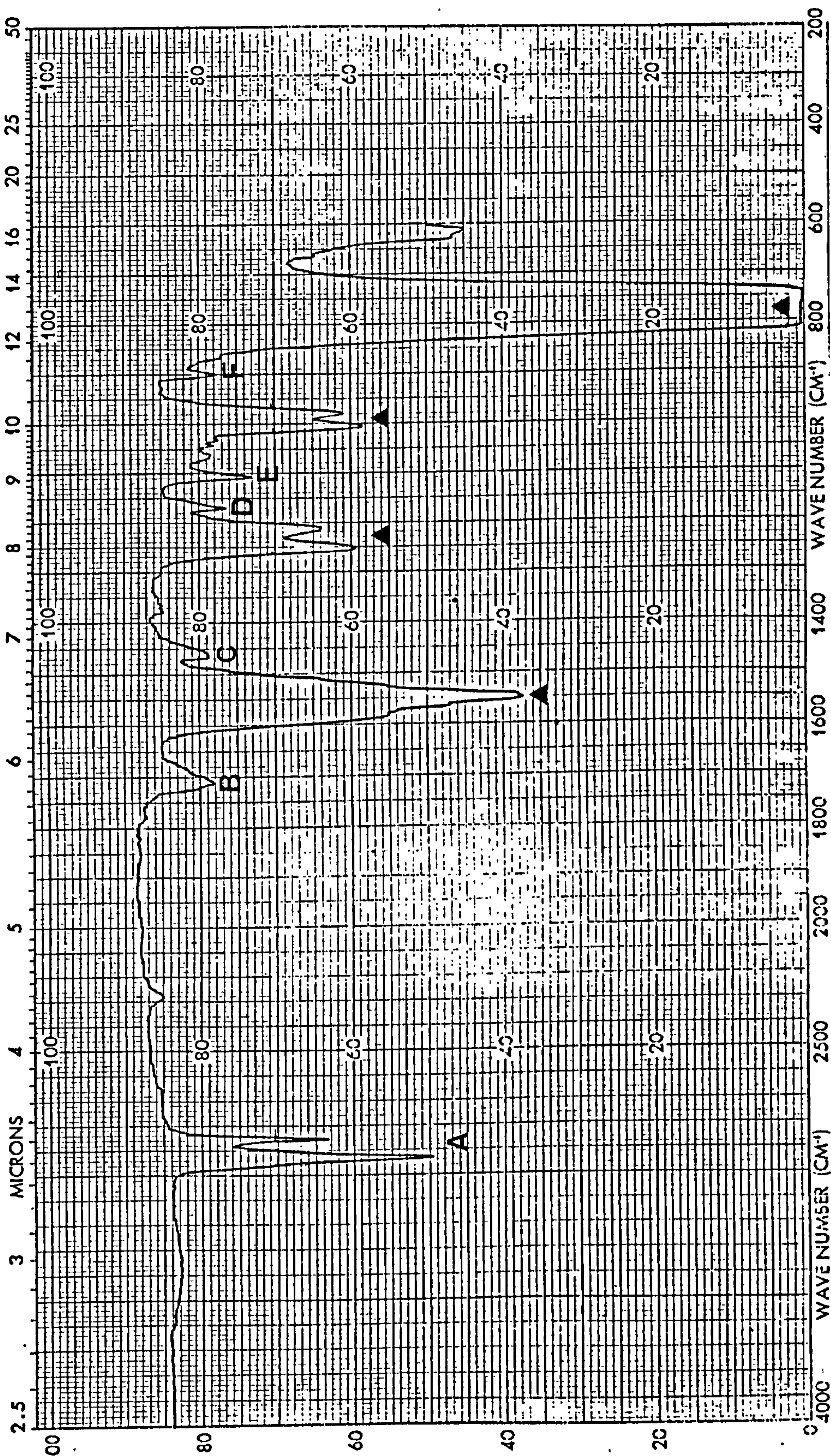


FIGURE 3.18. GCMS ANALYSIS OF CUTAN EXPERIMENT 2 UNA  
 NON-HYDROCARBON FRACTION  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas)





**FIGURE 3.19. IR SPECTRUM OF CUTAN UNA EXPERIMENT 2  
NON-HYDROCARBON FRACTION**

(A = C-H str., B = C=O str., C = C-H def., D = C-O str., E = C-O str.,  
F = C-H def.; A indicates absorbance bands due to solvent CCl<sub>4</sub>)

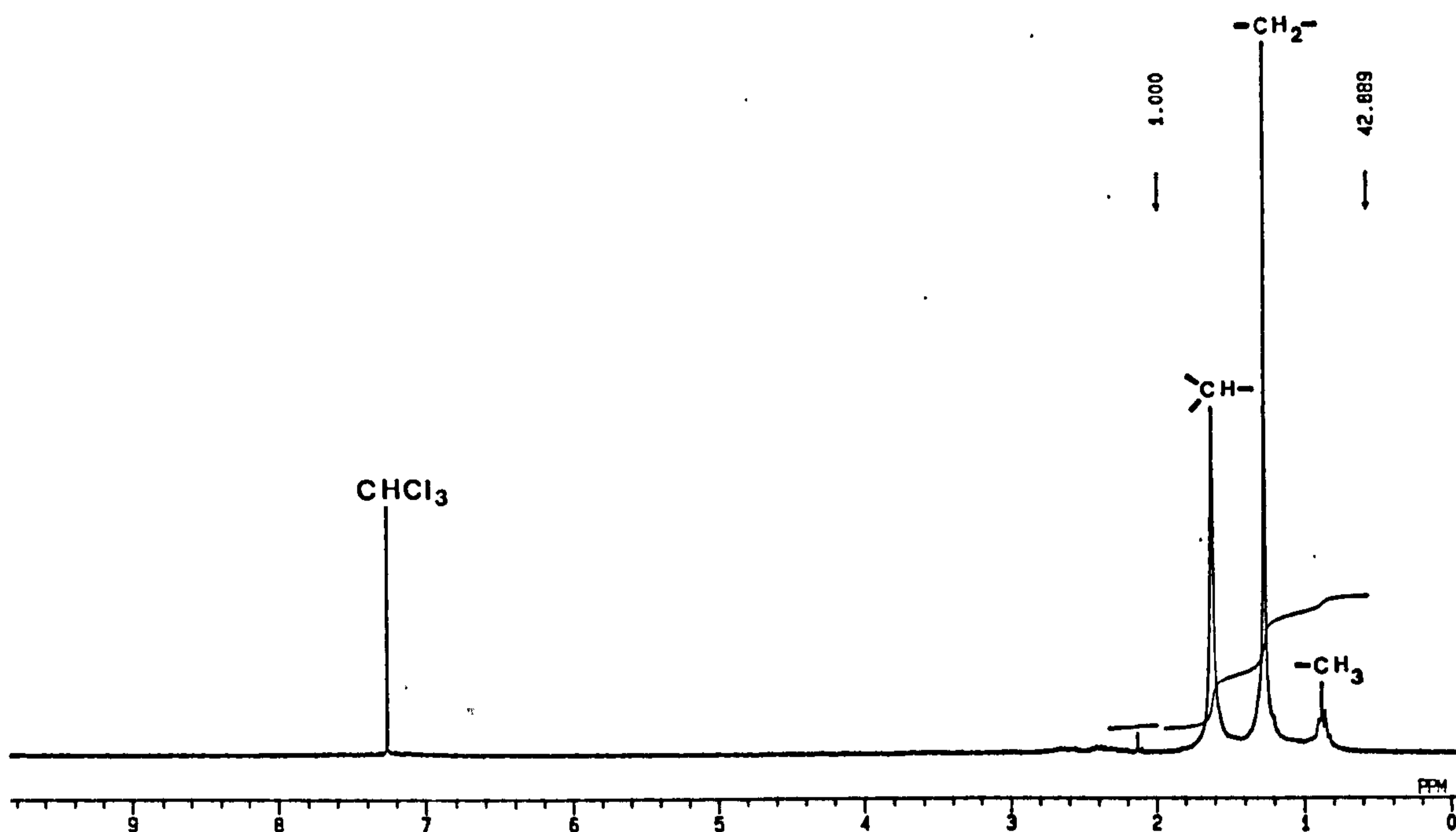


FIGURE 3.20.  $^1\text{H}$ -NMR SPECTRUM OF CUTAN HYDROUS PYROLYSIS EXPERIMENT 2 UNA NON-HYDROCARBON FRACTION (Jeol 270MHz; sample in  $\text{CDCl}_3$ )

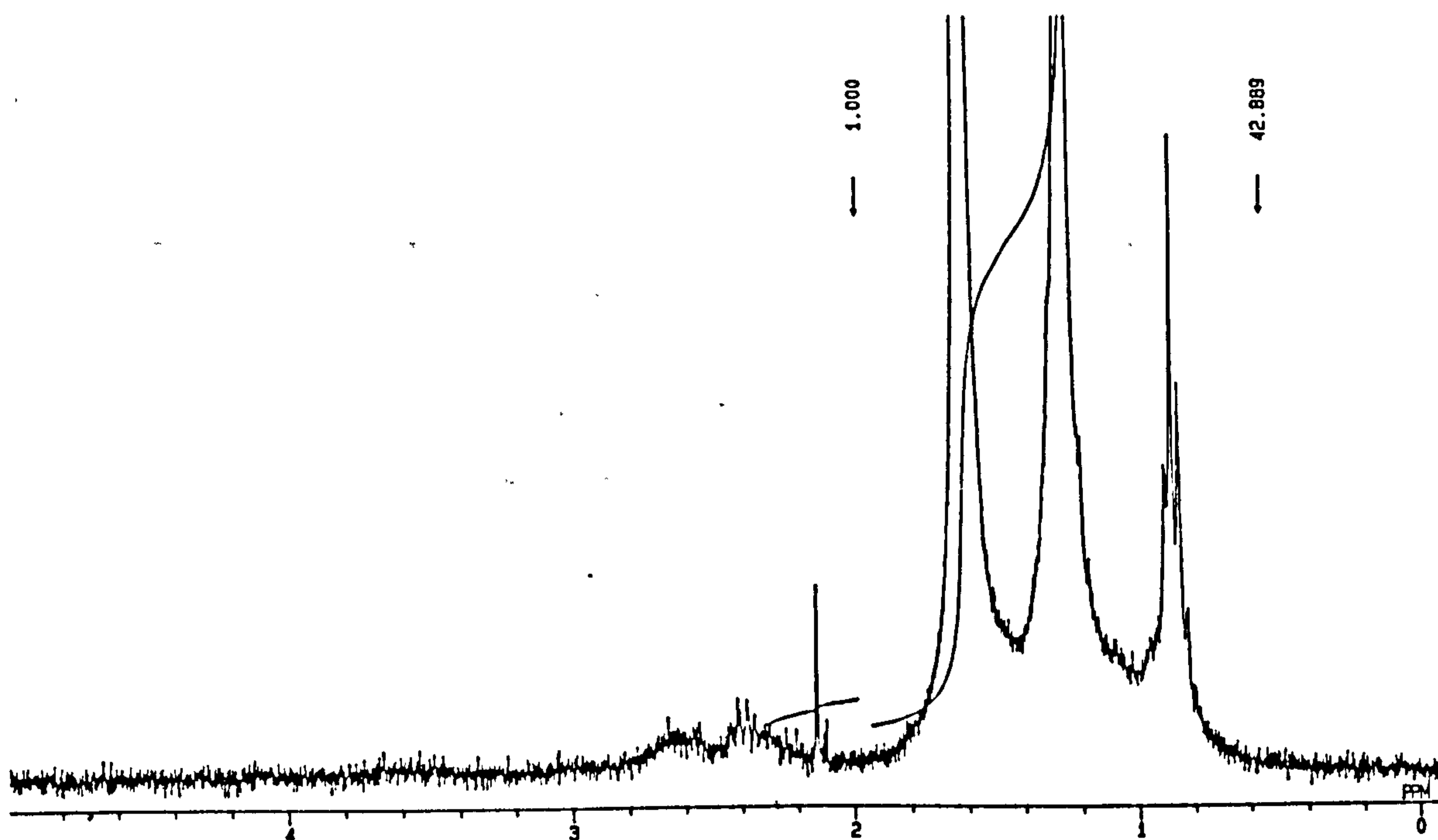
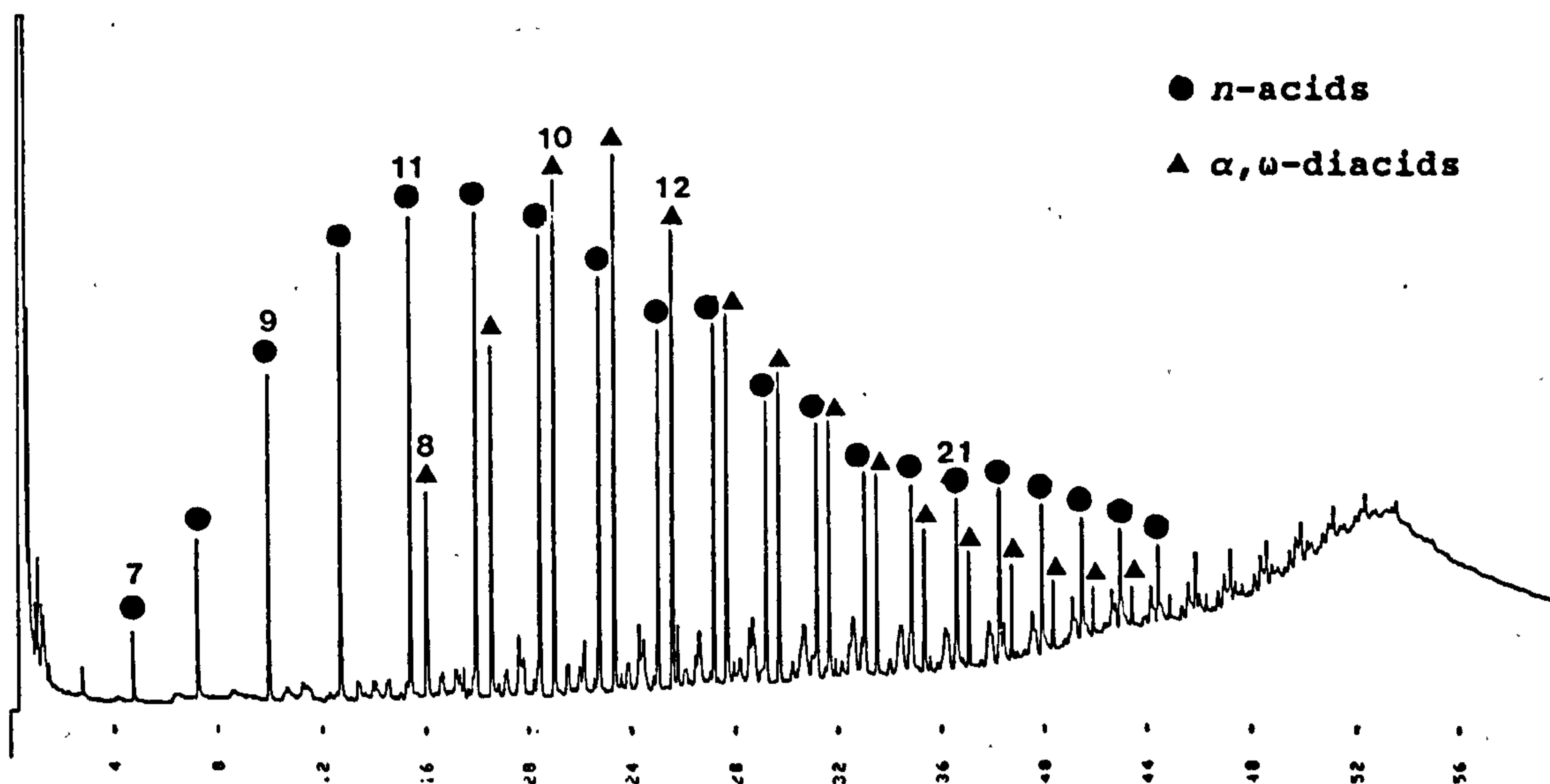


FIGURE 3.21. EXPANDED  $^1\text{H}$ -NMR SPECTRUM OF CUTAN HYDROUS PYROLYSIS EXPERIMENT 2 NON-HYDROCARBON FRACTION (Jeol 270MHz; sample in  $\text{CDCl}_3$ )





**FIGURE 3.22. GAS CHROMATOGRAM OF THE  $\text{CrO}_3$  OXIDATION PRODUCTS FROM THE SATURATED HYDROCARBON UNA FRACTION PRODUCED BY CUTAN HYDROUS PYROLYSIS EXPERIMENT 2**  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min.,  $\text{H}_2$  carrier gas)  
 (numbers refer to chain length)



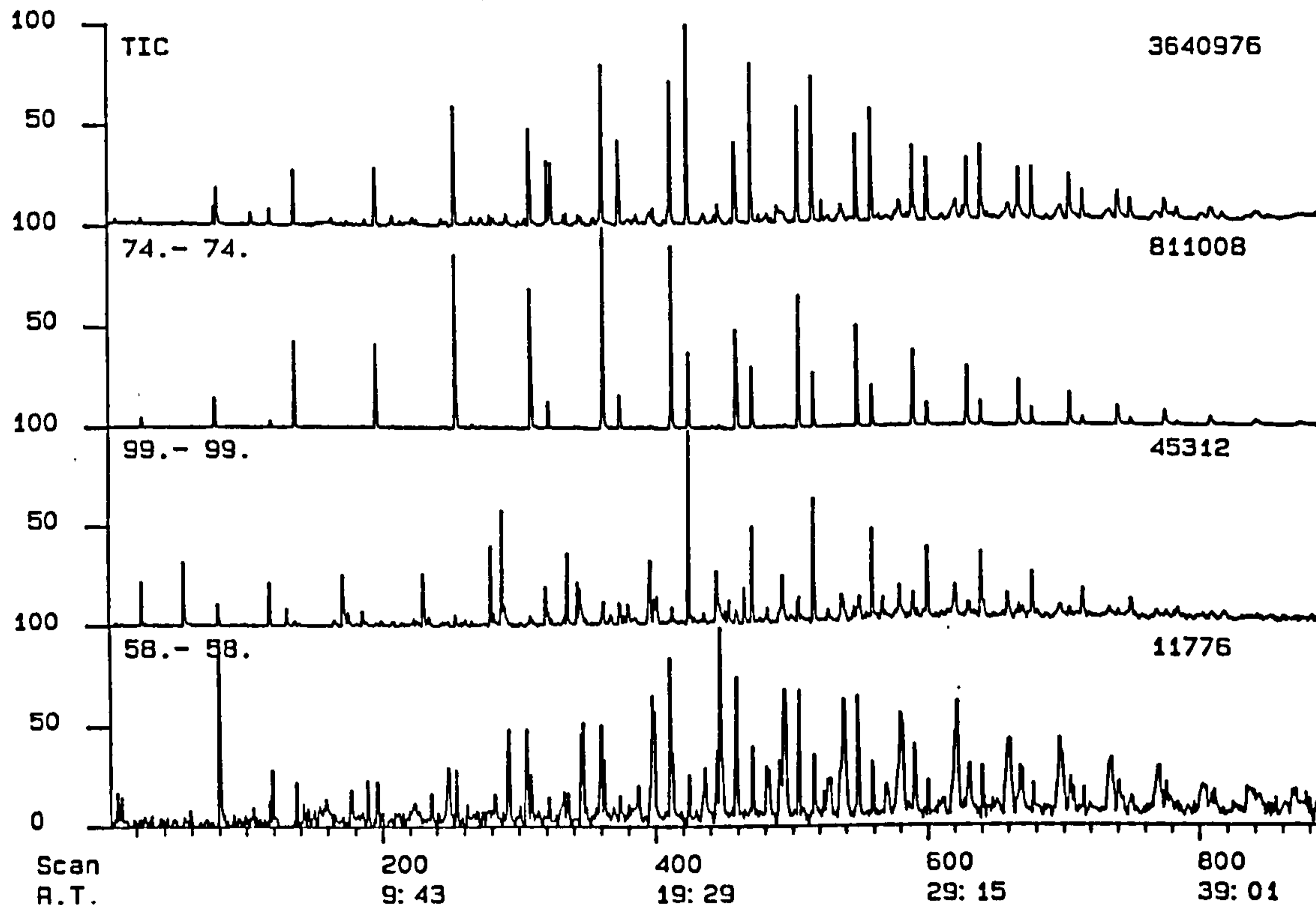
than in the case of Alkathene, which again is similar to the oxidation products of most crude oils. The monocarboxylic acids exhibited a slight even/odd predominance between C<sub>15</sub> and C<sub>22</sub>, but no carbon number preference was exhibited by the diacids. There was some evidence, from GCMS, for the presence of  $\gamma$ -lactones and ketones within the oxidation products (Figure 3.23) which is a further similarity to natural UCMs.

The formation of ketones and lactones by oxidation indicates that a proportion of the branching in the UCM is due to mono- and dialkyl substituted alkyl chains, as shown by oxidation of authentic compounds (Gough, 1989). However, these alkanes were relatively minor in the *A. americana* UCM since the <sup>1</sup>H-NMR resonance attributable to 3° carbons (1.5 ppm) in the hydrocarbon UCM was relatively weak.

### 3.3 SUMMARY

The above experiments have shown that hydrous pyrolysis of polymers containing polymethylenic chains produces mixtures of compounds which include substantial proportions of unresolvable compounds (25-55% UNA). This applies to both biogenic and abiogenic polymers. These results indicate that catagenesis of polymethylenic type I and II kerogens (Behar and Vandenbroucke, 1987) would probably produce a proportion of the UCMs found in oils. When subjected to

DS90 Chromatogram report Run: REVILL0082, 29-Oct-91 12:39  
AGAVE AMERICANA HP PRODS II H/C OXPRODS



**FIGURE 3.23. GCMS ANALYSIS OF THE CrO<sub>3</sub> OXIDATION PRODUCTS OF THE CUTAN SATURATED HYDROCARBON UNA FRACTION PRODUCED BY CUTAN HYDROUS PYROLYSIS EXPERIMENT 2**

(J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)


(*m/z* 74 = *n*-carboxylic acid methylesters,

*m/z* 99 =  $\gamma$ -lactones, *m/z* 58 = ketones)

CrO<sub>3</sub> oxidation, both laboratory generated UCMs yielded a series of resolved compounds, dominated by *n*-mono- and diacids. The relative proportion of the latter was greatest in the case of the UCM from the Alkathene pyrolysis, which was attributed to secondary oxidation of mono-acids formed initially. Significantly, the fraction of the UCM that was oxidised to resolved acids was about 70% in both cases, suggesting that simple "T-branched" alkane and/or cyclic structures make-up a much greater proportion of these laboratory UCMs than of those in oils (15-20% resolved). This is consistent with the highly aliphatic nature of the individual polymers used in these experiments. These results indicate the need for better models of UCM precursors to be investigated, such that the oxidation products from the UCMs formed produce components similar to those from natural UCMs.



the adventures of  
FAT FREDDY'S  
**CAT**




COPYRIGHT © 1978 BY GILBERT SHELTON

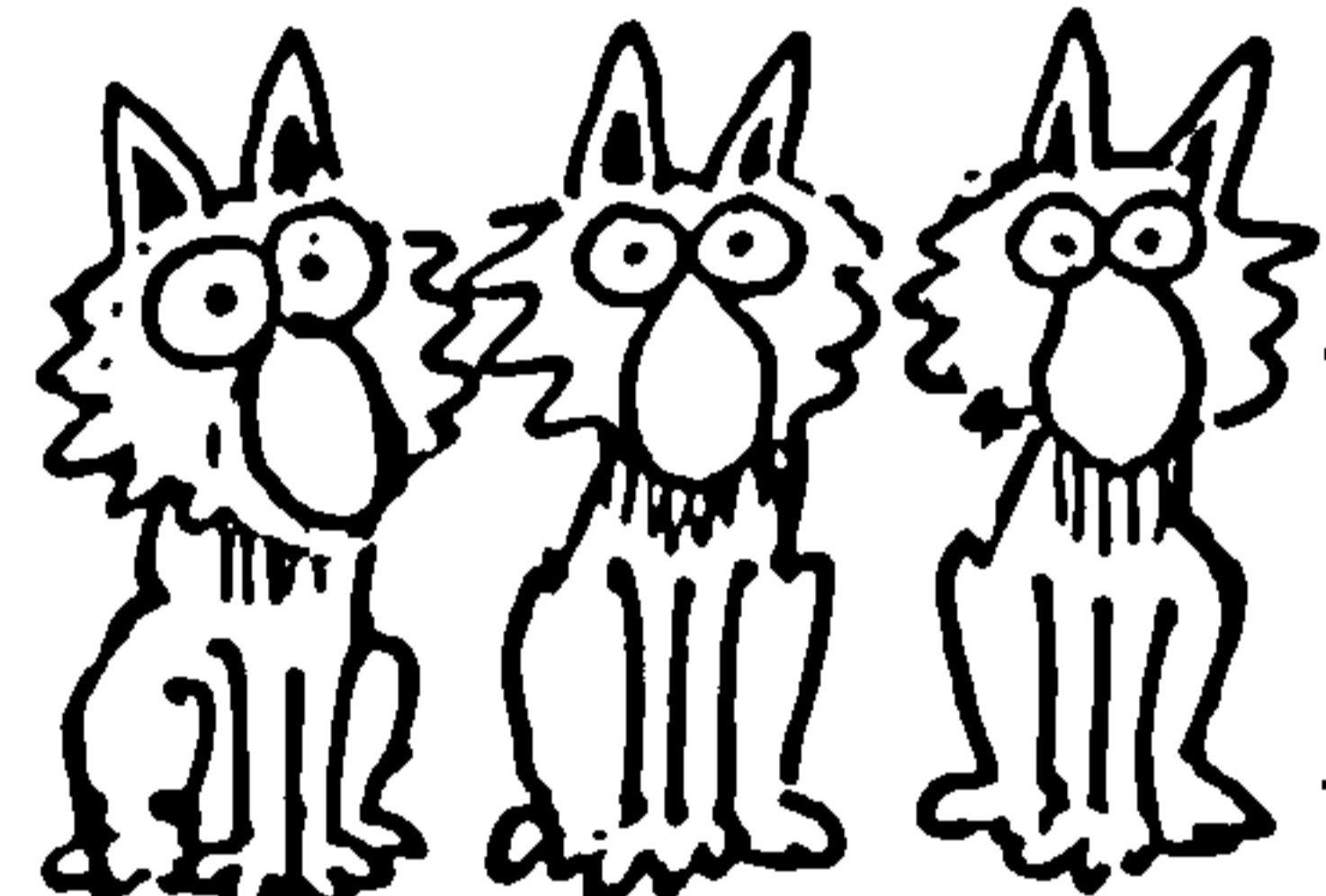
MAY WE GO PLAY IN  
THE STREET, UNCLE F.?



THAT'S VERY RISKY! YOU  
MIGHT RUN INTO A  
DIMENSIONAL  
TRANSMOGRIFIER!




WHAT'S THAT?



SOME PEOPLE CALL THEM  
"CARS" AND "TRUCKS"; I CALL  
THEM DIMENSIONAL TRANSMOGRIFIERS...



...BECAUSE THEY CHANGE  
THREE-DIMENSIONAL CATS  
INTO TWO-DIMENSIONAL ONES!



## FINGERPRINTING OF UCMs

### 4.1 INTRODUCTION

The limitations associated with the use of biomarkers for comparing degraded oils with non-degraded oils or their source rocks have already been discussed in Section 1.4. As a result of these limitations, Gough and Rowland (1990) suggested that, in addition to the biomarker profiles, UCM oxidation product profiles could be used to fingerprint degraded oils. The technique was applied successfully to two acute oil spills where partly degraded beached oils were correlated with oils from a tanker and ruptured pipeline (Figure 4.1). However, the profiles used in that study (shown in Figure 4.1) represented only a few, randomly chosen, components of the total oxidation products and furthermore only two spills were investigated.

The aims of the present study are (i) to use the oxidation product profiles as a whole to "fingerprint" degraded oils, (ii) to use non-subjective, computer based statistical techniques to compare these profiles (*i.e.* multivariate analysis) and (iii) to extend the study to include further case histories of oil/oil and oil/source rock correlations.

LACTONES  
(m/z 99)

KETONES  
(m/z 58)

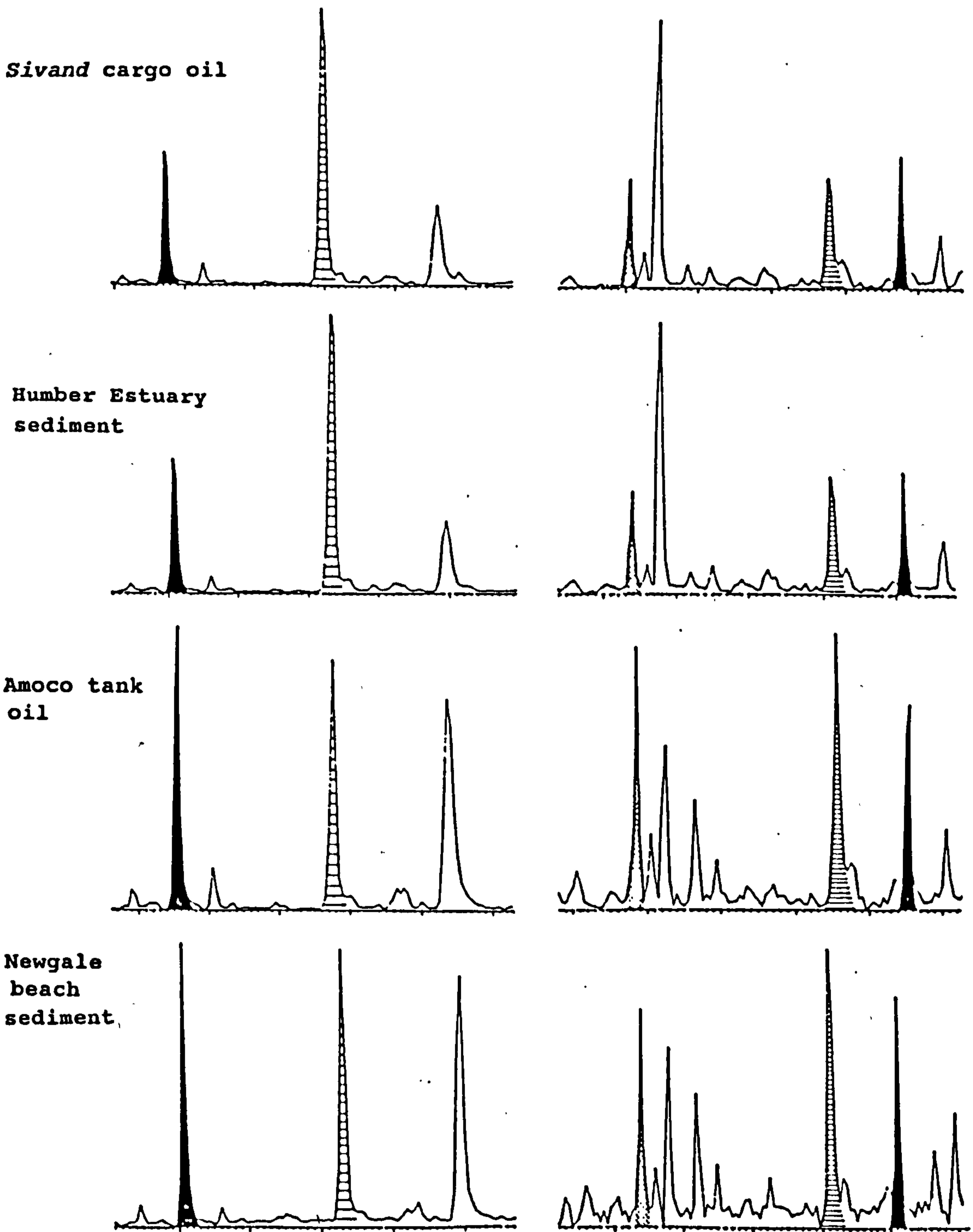


FIGURE 4.1. THE USE OF SELECTED UCM OXIDATION PRODUCTS TO FINGERPRINT TWO ACUTE OIL SPILLS (Gough, 1989) (1983 spill of Nigerian crude oil from the *Sivand* into the Humber Estuary and the 1986 fuel oil spill from the Amoco Milford Refinery into Milford Haven, sample collected from Newgale beach)



## 4.2 INTEGRATION OF CHROMATOGRAPHIC DATA

UCMs isolated from crude oils, source rocks and sediments were oxidised and the oxidation products examined and quantified according to the methods outlined in Chapter 6.

Gough (1989) used a time slice area measurement program written for a Shimadzu integrator (C-R3A; Appendix I) in order to estimate the percentage resolved components in isolated UCMs by GC. This method has been re-evaluated with results which suggest that in certain cases the program may have overestimated the amount of resolved material in the chromatograms. An important integration parameter for Shimadzu integrators is the "drift value", which is normally set to a value of ca. 10000 to allow for valley to valley peak integration (Figure 4.2). Gough (1989) used a value of zero, assuming this would give a near flat baseline, however this value causes integration between major valleys in the chromatogram (Figure 4.2). In the present study it was found that a method of manual peak detection, followed by the time slice area measurement, gave more reliable results. This involved consideration of the whole UCM as one "peak" as illustrated in Figure 4.2. The differences shown in Figure 4.2 can be expressed numerically if each method is used to integrate the same chromatogram. The chromatogram used exhibited a UCM which eluted over 52 minutes, which is sufficient time for 624 slice areas to be measured (Shimadzu manual). The

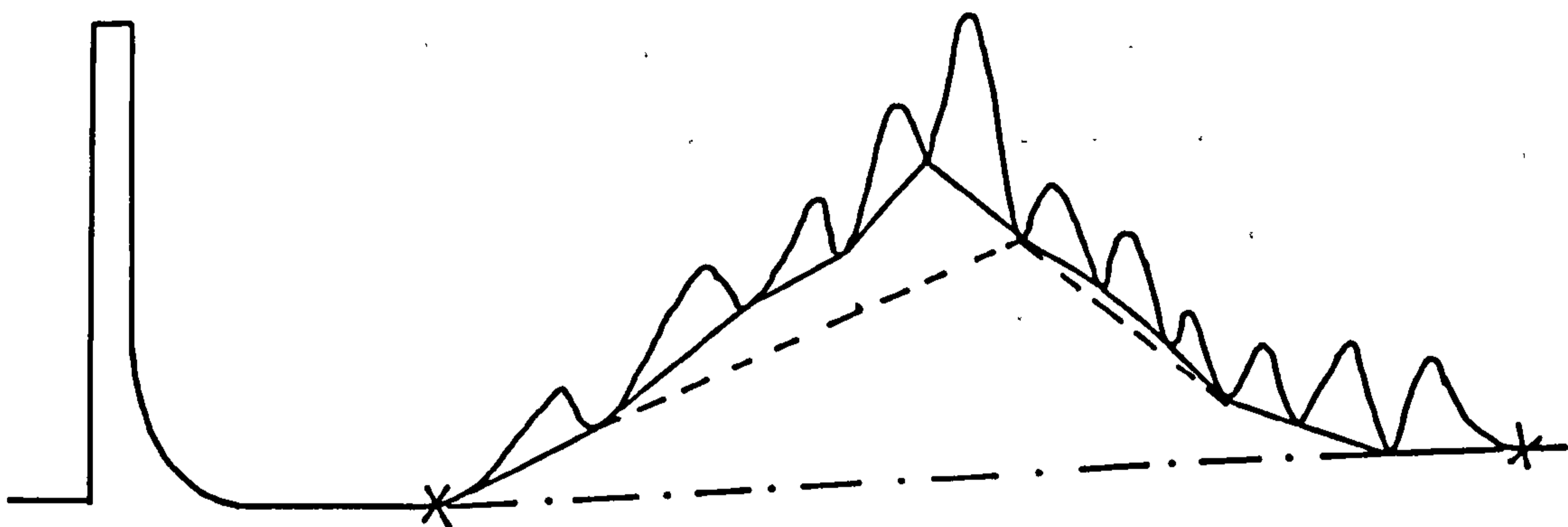


FIGURE 4.2. EXAGGERATED DIFFERENCES BETWEEN GC INTEGRATION METHODS FOR THE UCM (Shimadzu integrator)

- Normal peak integration (DRIFT = 10 000)
- "Auto" UCM integration (Gough, 1989; DRIFT = 0)
- .- UCM integration by manual peak detection
- X Manual peak limits

The relative proportions of UCM and resolved compounds are assessed by integration of the total chromatogram (resolved + UCM) followed by integration of the resolved peaks only. Thus a drift value of 0 could underestimate the total area and therefore overestimate the proportion of resolved compounds.

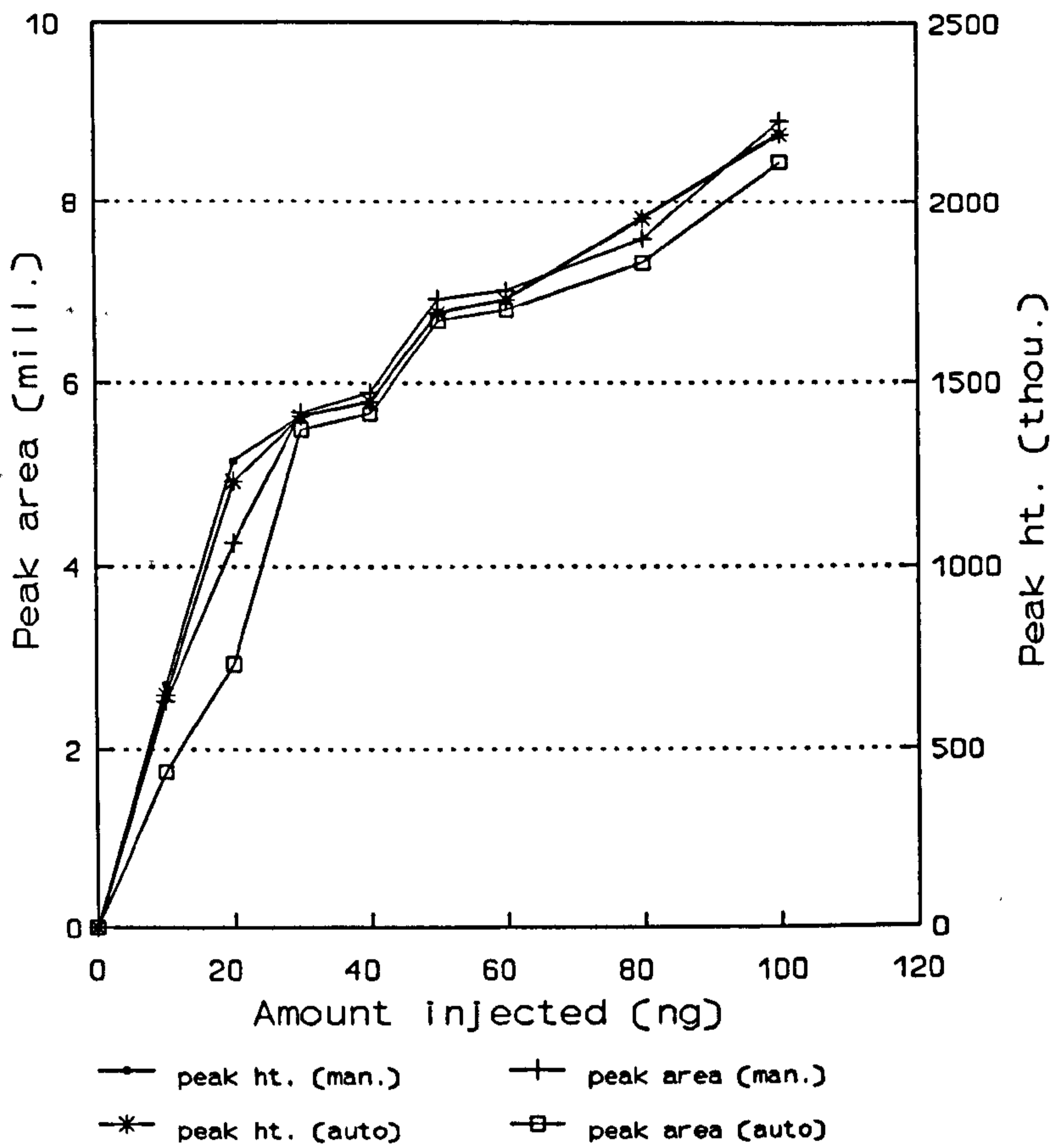
following results were obtained:

Drift Setting	No. of Slices Reported as Measured	% Resolved Components Calculated
0	353	45
1	494	30
Manual Peak Detection	622	15

Thus only the manual peak detection method in this example reported a number of slices (622) close to the theoretical number (624). Not all examples measured show such a large difference between methods and some produced near concurrent data; however it is apparent that the automatic drift setting underestimates the UCM area. The manual method is more reliable but may still introduce errors if the chromatographic baseline does not return to the level which existed at the start of the GC analysis, as is the case with column bleed.

There were fewer problems encountered with integration using the Kratos DS90 (rev.5) GCMS software. Since the data system displays the chromatogram and the integrated baseline, one is better able to judge the appropriateness of the integration parameters. However it was important to check the baseline for each integration and, where necessary, alter the various parameters until a reasonable baseline was achieved. Interestingly, little difference was found between using peak height or peak area for



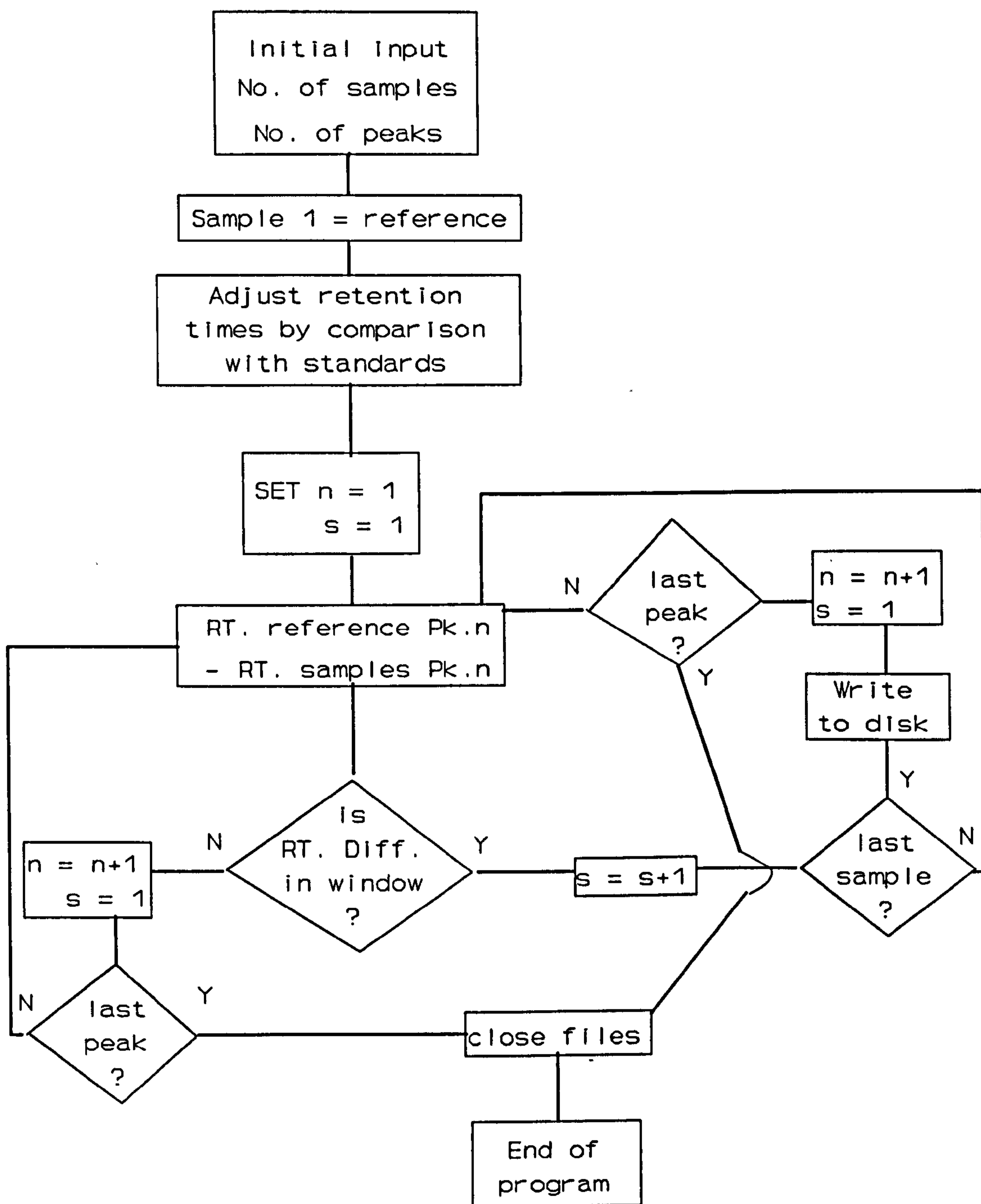


**FIGURE 4.3. COMPARISON OF GCMS INTEGRATION METHODS AND CALIBRATION OF PEAK HEIGHT AND PEAK AREA MEASUREMENTS FOR D<sub>8</sub>-NAPHTHALENE.**  
 (man. = manual integration, auto = software integration)

quantification (Figure 4.3).

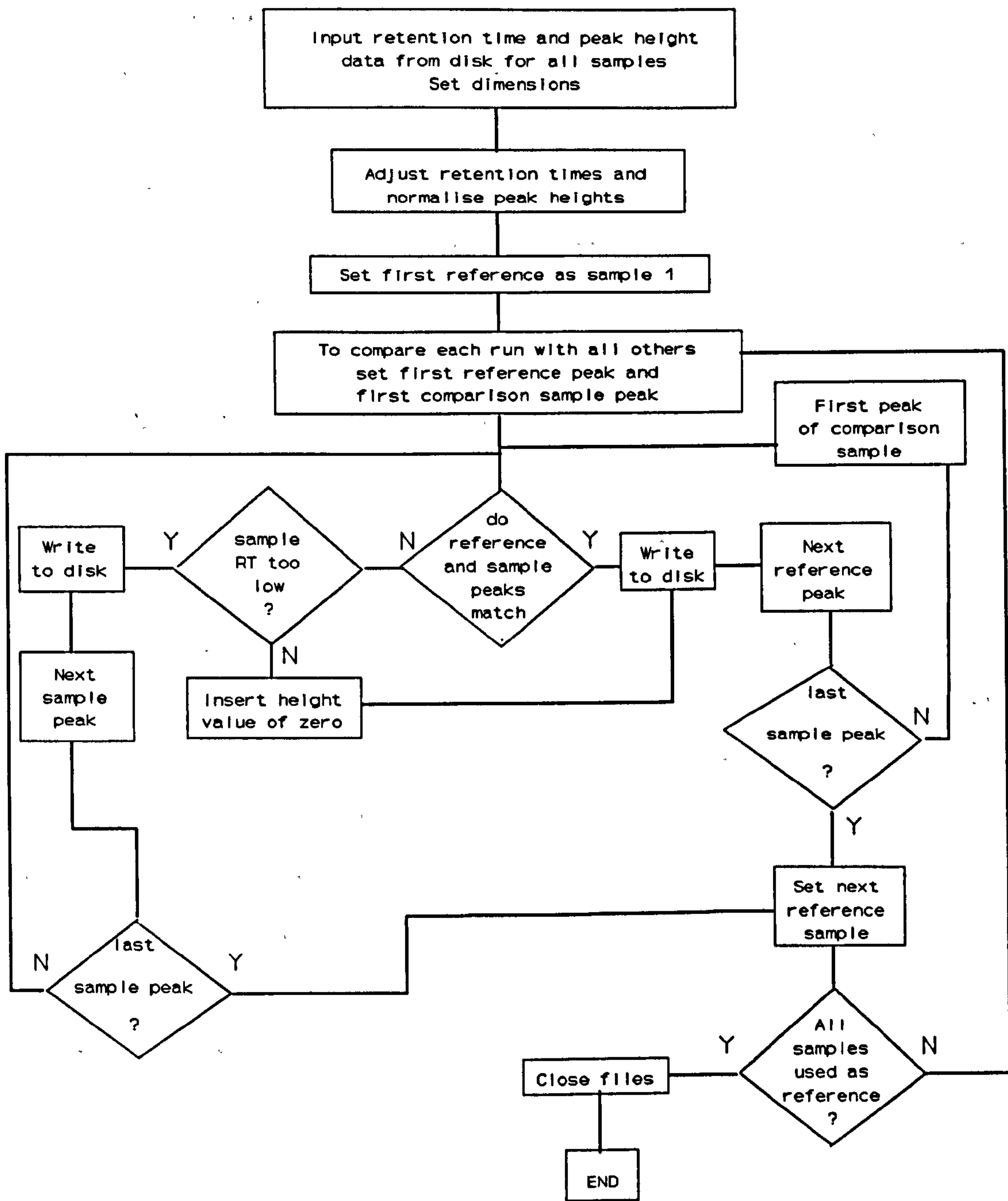
In addition to the commercial integration software for GC and GCMS, two BASIC programs were written (Appendix III). One allowed comparison of the chromatographic integration data for each sample and found peaks which were present in all samples (PEAK-MATCH). The second, more complex program, compared each data set in turn with all others and where peaks were absent, inserted an integration value of zero (DB-MATCH). These programs are shown schematically in Figure 4.4 and 4.5. Since peaks were matched by retention time (scan number) it was found to be necessary to include two internal standards, one towards the beginning (*d*<sub>8</sub>-naphthalene) and one towards the end (perylene) of the chromatographic analysis. Thus retention time variations between runs could be monitored. For example, the run to run variation in GCMS scan number (retention time) is often 5 scans at the start of an analysis, but increases to 10 scans or more by the end of the analysis. A chromatographic correction parameter was built into the program to compensate for this phenomenon. Once the chromatographic data had been produced and integrated and pre-processed by the aforementioned programs, the data could be analysed by computer based statistical methods.

Brereton (1987) emphasises the importance of understanding all steps in the acquisition and processing of data, and nowhere is this more apparent than with the problems



**FIGURE 4.4. OUTLINE OF THE PURPOSE WRITTEN PEAK MATCH PROGRAM**  
(Y = Yes, N = No)





**FIGURE 4.5. OUTLINE OF THE PURPOSE WRITTEN DBMATCH PROGRAM**  
 (Y = Yes, N = No)

highlighted here with respect to peak integration. Whilst computer based integration is essential with the increased sample through-put made possible by modern instrumentation, it is important that the operator does not treat this as a "black box" but attempts to understand the processes involved and to validate the results obtained.

#### 4.3 MULTIVARIATE STATISTICAL TECHNIQUES

Multivariate analysis is a term used to describe a collection of statistical techniques which allow a number of variables ( $p$ ) measured for a number of samples ( $n$ ) from a data matrix ( $n \times p$ ) to be tested for intersample correlations. The use of these techniques in chemistry is often called chemometrics (Massart *et al.*, 1989). The data matrix is often very large (e.g.  $100 \times 20$ ) so chemometric analysis is invariably computer based, with the underlying aim of reducing the data in both size and complexity to allow easier understanding. The range of multivariate statistical techniques is wide (for an introduction see Chatfield and Collins, 1980) and it is important that the most appropriate technique is chosen for the task in hand. Table 4.1 gives an indication of the most appropriate type of analysis for each different type of data. Mellinger (1987) suggests a useful preliminary categorisation of multivariate techniques into *factor analysis methods* and *classification methods*.

**TABLE 4.1: CLASSIFICATION OF MULTIVARIATE METHODS ACCORDING TO DATA TYPE AND AIMS OF THE ANALYSIS (Adapted from Feinberg and Ducause, 1991)**

CONT	data type			aims of analysis				Method
	ORDR	UNOR	BINA	PRED	DESC	GROU	CLAS	
+	+	-	-	✓				Multiple Linear Regression MLR
+	-	-	-	✓	X			Canonical Correlation CC
+	+	-	-	✓	X			Partial Least Squares PLS
-	+	+	+		✓	X		Corresponding Factor Analysis CFA
+	-	-	-	X	✓	X		Principal Components Analysis PCA
+	+	-	-		✓	X		Factor Analysis FA
+	+	-	-		X		✓	Factorial Discriminant Analysis FDA
+	-	-	-				✓	Soft Independent Modelling by Class Analogy SIMCA
+	-	-	-			✓		Partitioning Classification PC
+	-	-	-			✓		Hierarchical Classification HC

CONT, Continuous; ORDR, Ordered Categorical; UNOR, Unordered Categorical; BINA, Binary; PRED, Prediction;  
DESC, Description; GROU, Clustering; CLA, Classification

+ Possible  
- Impossible  
✓ Suitable  
X Possible, not ideal



#### 4.3.1 FACTOR ANALYSIS METHODS

Factor analysis techniques (most commonly principal components analysis [PCA] and factor analysis [FA]) are methods by which multidimensional data can be represented in two (or less commonly three) dimensions (Massart *et al.*, 1989). The most commonly used of these techniques is PCA (Smith, 1991), which has found wide use in organic geochemistry (e.g. Kvalheim and Telnaes, 1986a; Telnaes *et al.*, 1987; Mello *et al.*, 1988; Grahl-Nielsen and Lygre, 1990; Yunker *et al.*, 1991). PCA produces new axes (Principal components) for the original data, which are orthogonal and represent the greatest variance within the data (Smith, 1991) with each successive principal component representing a decreasing amount of variance. Thus, the common 2-D plot of PC1 v. PC2 displays the greatest variance. Therefore, principal components are composite variables and as such rarely produce straightforward answers (Kvalheim and Telnaes, 1986b) because one factor may have an overwhelming influence on the data (e.g. biodegradation, maturity). A further drawback of PCA is that in general at least five samples are required for each variable (Tabachnick and Fidell, 1989). Whilst some statisticians (M. Carr, Personal communication, 1991) feel that this ratio may be excessive, certainly a greater number of samples than variables is required because PCA (and FA) are sensitive to the size of correlations.

The resulting 2-D plots of PC1 v. PC2 etc. can be used to identify groupings of samples or correlations between variables. However these groupings are separated only in two dimensions, and individuals within each group may well be separated in other dimensions (Brill et al., 1985). To enhance the results from PCA, the new, reduced, number of components describing the data can be subsequently subjected to cluster analysis (Adams, 1991) which allows intra-group correlations to be assessed. Thus a combination of the two techniques will allow groups of samples to be identified and the relationships of individuals to each other within a group to be studied.

#### **4.3.2 CLASSIFICATION METHODS**

The most common form of classification is cluster analysis (Mellinger, 1987) which has the aims of searching for unbiased groupings in the data (Meglen, 1988). The overriding concept in cluster analysis (and to some extent all multivariate data analysis) is one of similarity, dissimilarity or distance (for an introduction see Chatfield and Collins, 1980 and Everitt, 1980; Birks, 1987).

The most widely used distance measure is Euclidean distance, given by equation (1)

$$d_{ij} = \left\{ \sum_{k=1}^R (X_{ik} - X_{jk})^2 \right\}^{1/2} \dots \dots \dots (1)$$

$x_{ik}$  = value of kth variable for sample i

$x_{jk}$  = value of kth variable for sample j

$d_{ij}$  = distance between samples i and j

Other distance measures are the city block (2) and Mahalanobis  $D^2$  values (3)

$$d_{ij} = \sum_{k=1}^R |X_{ik} - X_{jk}| \dots \dots \dots (2)$$

$$d_{ij} = (X_i - X_j)' \Sigma^{-1} (X_i - X_j) \dots \dots \dots (3)$$

$\Sigma^{-1}$  = pooled within groups variance co-variance matrix

$x_i, x_j$  = vectors of scores for entities i and j.

Each distance measure has advantages and disadvantages (see Everitt, 1980) but as a rule, Euclidean type distances are used for individuals whilst Mahalanobis  $D^2$  distances tend to be used for inter-group distances. The difference between some distance measures is shown graphically in Figure 4.6. The net result of distance measurement is to produce a matrix of distances between all samples in multidimensional space. If the number of samples is given by n, there will be n-1 dimensions. For example, the distances between three samples can be represented in two dimensions (a triangle). Once the multidimensional distances have been calculated, the data can then be used in a cluster analysis



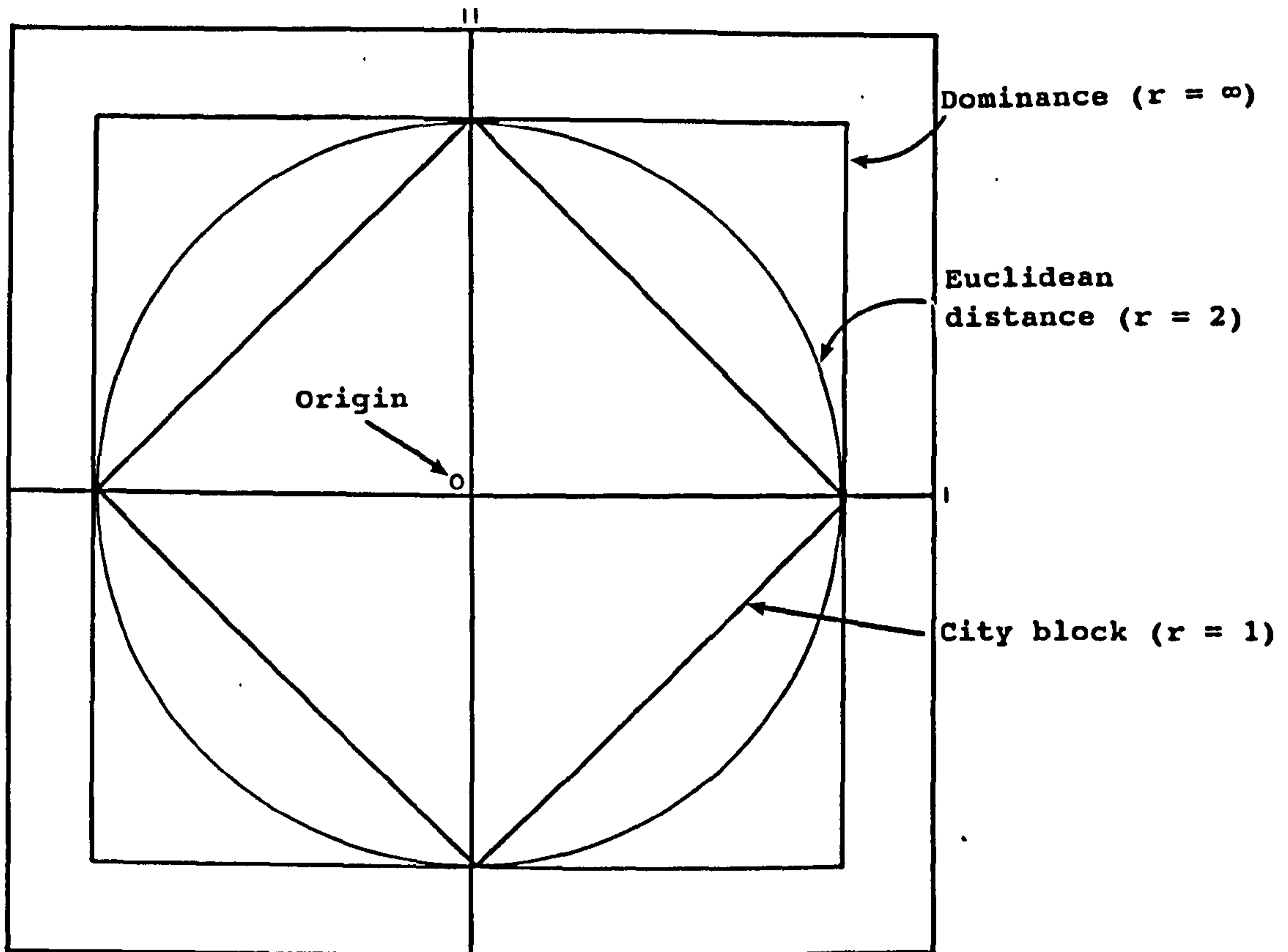


FIGURE 4.6. EQUAL DISTANCE CONTOURS FOR THREE METRIC

DISTANCES OF THE FORM  $d_{jk}^r = \sqrt[r]{\sum_k |X_{ik} - X_{jk}|^r}$   
 (Coxon, 1982)

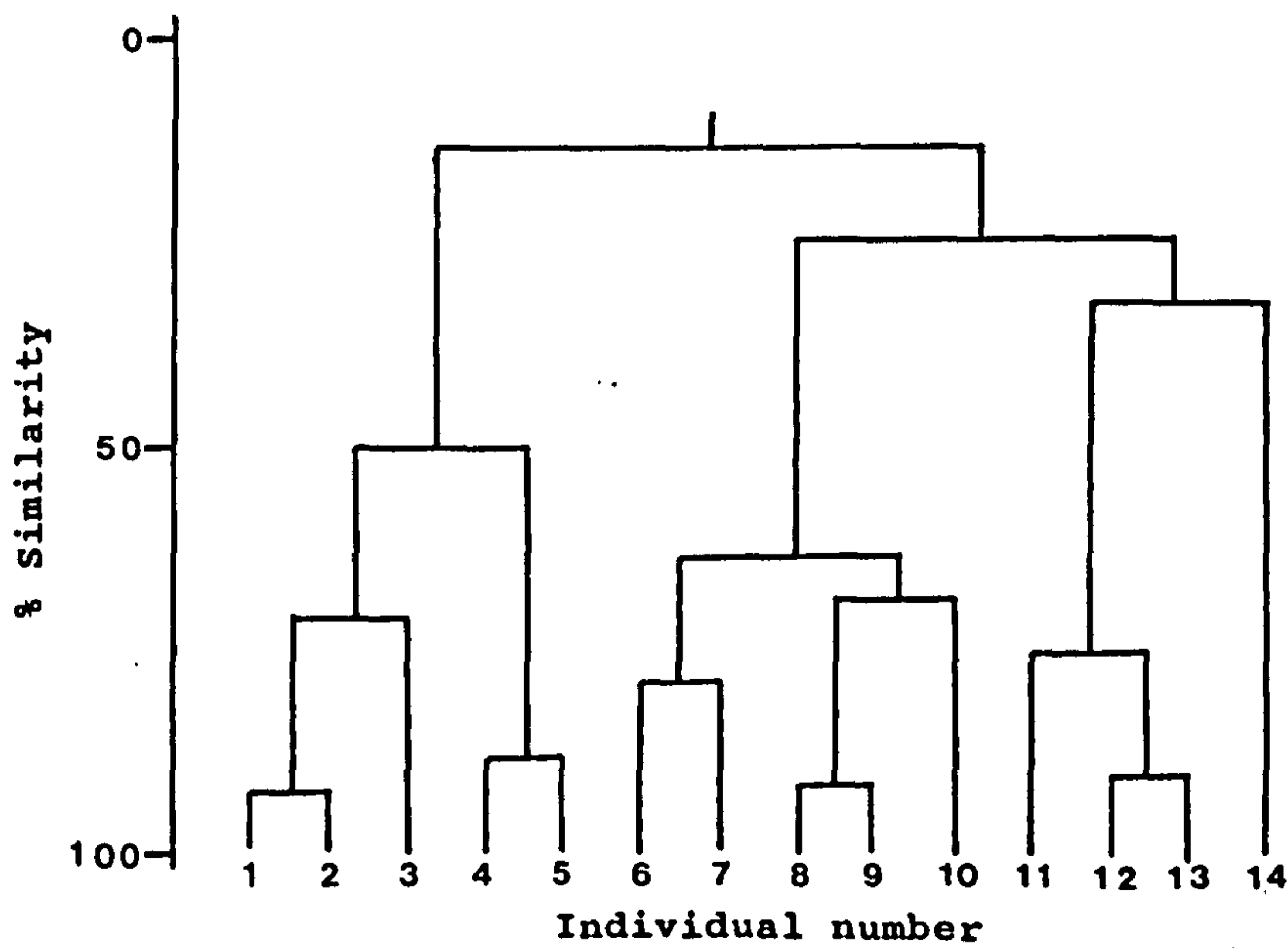


FIGURE 4.7. EXAMPLE OF A HIERARCHICAL DENDROGRAM

program, most of which produce hierarchical dendrograms (Figure 4.7), although there are alternative pictorial representations possible (see Everitt, 1980; Chatfield and Collins, 1980; Tabachnick and Fidell, 1989). Hierarchical dendrograms relate samples to each other (by horizontal lines; Figure 4.7) as well as the degree of their similarity (by vertical lines, Figure 4.7). On its own, this technique suffers from the opposite problems to PCA in that whilst it gives detailed groupings, it does not show spatial distributions. However, as stated earlier, cluster analysis can be used in conjunction with PCA to provide complementary information. An alternative treatment of distance measures is multi-dimensional scaling (MDS, Kruskal and Wish, 1978). MDS takes inter sample distances in multidimensional space and projects the samples onto two dimensions whilst attempting to measure the relative positions of samples as closely as possible and minimise the so called "stress function" (4) (Mantoura, et al., 1982).

$$S = [ \sum_{ij} (d_{ij} - \hat{d}_{ij})^2 / \sum_{ij} d_{ij}^2 ]^{1/2} \dots \dots \dots (4)$$

$\hat{d}_{ij}$  = Fitted distance from the regression corresponding to the dissimilarity function

S = stress on the data

Essentially, MDS ranks the inter-sample distances (dissimilarities) and uses these to determine the relative

positions in two dimensions. Thus the orientation and scale of an MDS plot are arbitrary (Mantoura et al., 1982). One example of a distance matrix is the common between towns road distance chart found in the front of most road atlases, and if this is used as input to the MDS program, the result looks very much like a map of mainland U.K. (Figure 4.8) except that the image is rotated through 180° and some towns are slightly out of place because road distances are not true Euclidean distances. The advantage of MDS is in its flexibility, compared with other techniques (e.g. PCA) in dealing with situations where the number of samples is small compared to the number of variables (cf. PCA) (Mantoura et al., 1982). This is because MDS utilises distance rather than variance data. For example, five samples can all have a distance from each other where as the variance of these (few) samples would probably cause most variables to be grouped into one principal component, thereby producing a loss of information.

In conclusion, where whole chromatograms of a small number of samples are to be compared, MDS and cluster analysis are more appropriate than PCA and these methods were therefore adopted for the analysis of the chromatographic data resulting from oxidation of UCMs in the following case studies.



1	Aberdeen	1000
2	Aberdeen	1000
3	Barnsley	1000
4	Barnsley	1000
5	Birmingham	1000
6	Birmingham	1000
7	Bradford	1000
8	Bradford	1000
9	Cambridge	1000
10	Cardiff	1000
11	Cardiff	1000
12	Cardiff	1000
13	Cardiff	1000
14	Cardiff	1000
15	Cardiff	1000
16	Cardiff	1000
17	Cardiff	1000
18	Cardiff	1000
19	Cardiff	1000
20	Cardiff	1000
21	Cardiff	1000
22	Cardiff	1000
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33	Cardiff	1000
34	Cardiff	1000
35	Cardiff	1000
36	Cardiff	1000
37	Cardiff	1000
38	Cardiff	1000
39	Cardiff	1000
40	Cardiff	1000
41	Cardiff	1000
42	Cardiff	1000

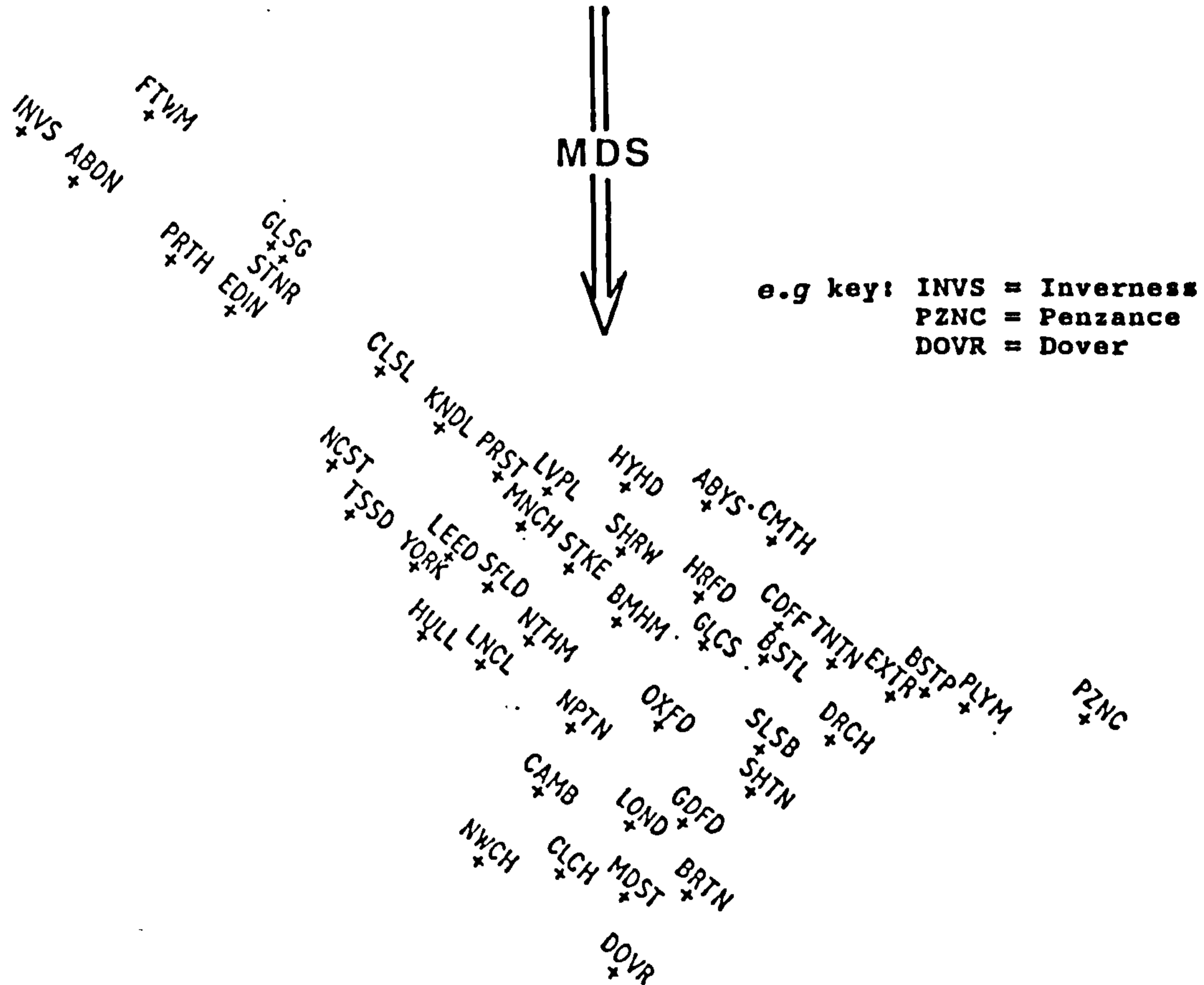


FIGURE 4.8. THE RESULT OF PERFORMING MDS ANALYSIS ON A ROAD DISTANCE MATRIX (After Chatfield and Collins, 1980)

## 4.4 CASE STUDIES

### 4.4.1 SULLOM VOE OIL TERMINAL

#### 4.4.1.1 GENERAL

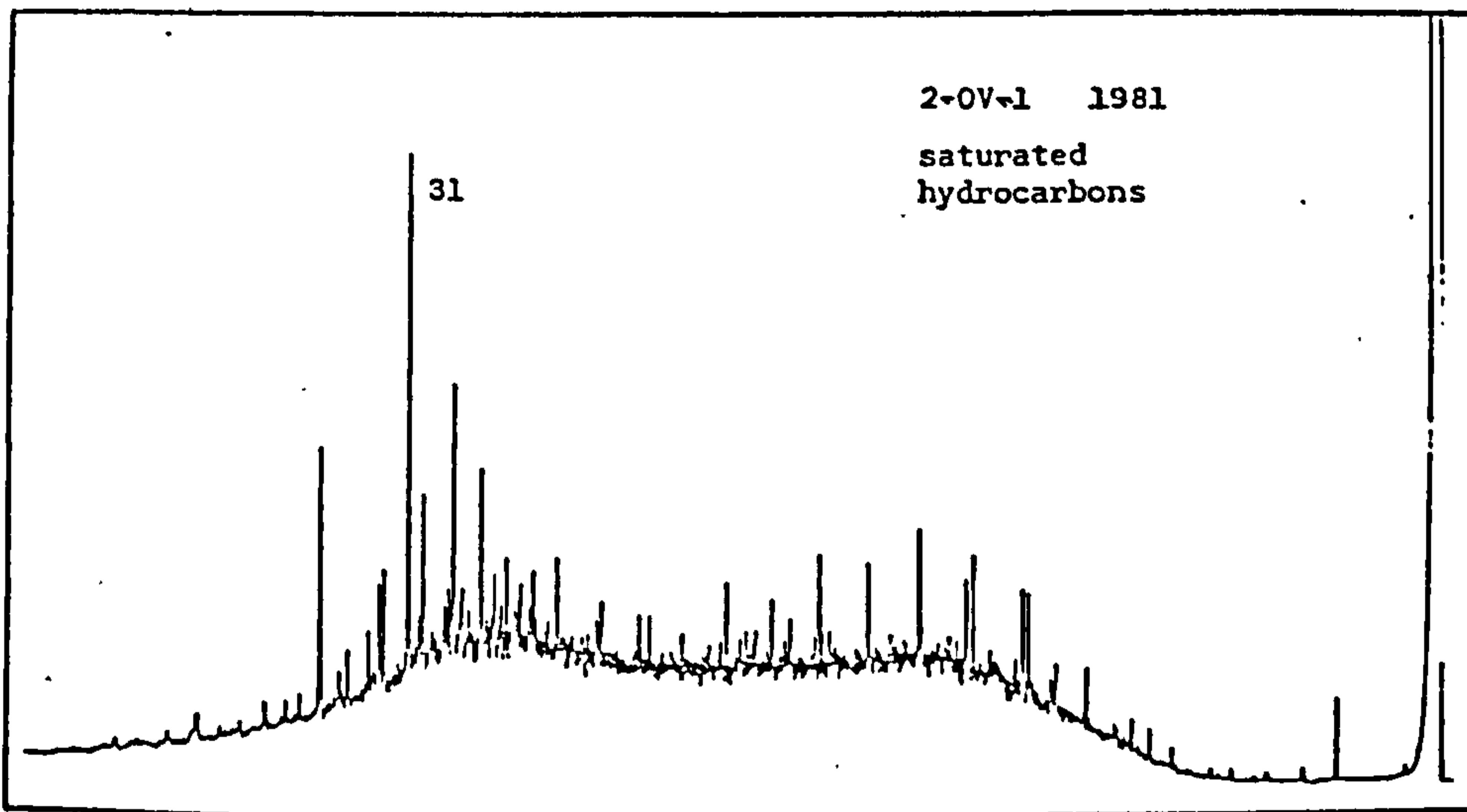
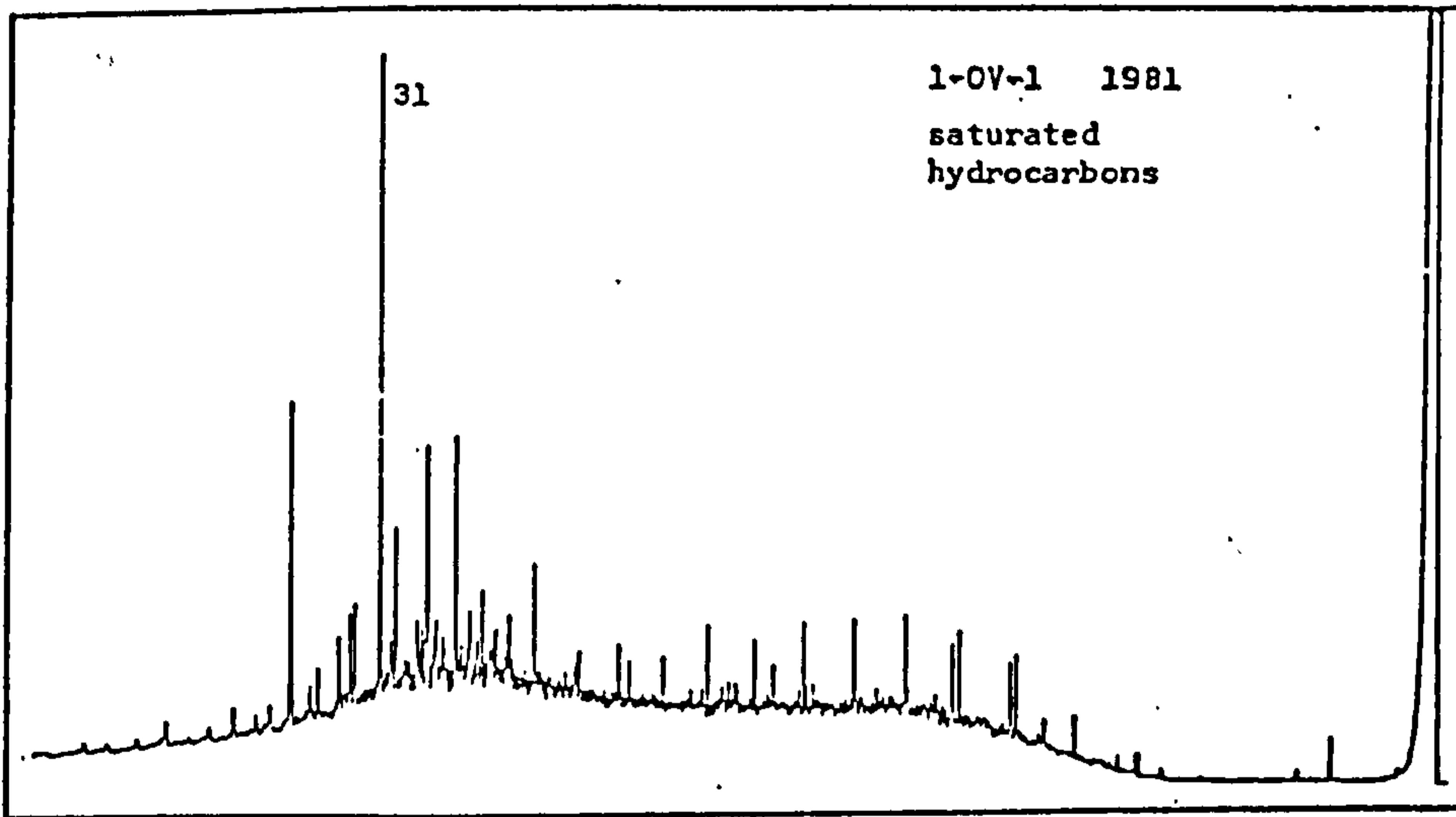
Sullom Voe oil terminal (Shetland, U.K.; Figure 4.9) receives oil from the North Sea Ninian Field where the oil is stabilised and stored for further transport. Permission to build the terminal stipulated that an annual survey of the (pollutant) hydrocarbons of benthic sediments should be performed to monitor the impact of terminal operations on this environmentally sensitive area.

These surveys (reviewed by Dodd and Howells, 1985) have repeatedly shown that concentrations of aliphatic hydrocarbons in sublittoral sediments around the oil terminal rarely exceed an average of  $100 \mu\text{g g}^{-1}$  dry sediment. However, in some sediments concentrations were higher than this average and the gas chromatographic profiles included significant UCMS (Figure 4.10). Some concern was expressed over this and the Shetland Oil Terminal Environmental Advisory Group (SOTEAG) sponsored an investigation into the likely sources of these UCMS, with four possibilities having been previously suggested. These were (a) a general background of "chronic" hydrocarbon accumulation from the many activities in the area. (Dodd and Howells, 1985), (b) biodegradation of Ninian crude oil, known to be legally



**FIGURE 4.9. MAP SHOWING THE LOCATION OF THE SHETLAND ISLANDS**





← TIME

**FIGURE 4.10. EXAMPLES OF UCMS IN SAMPLES FROM THE 1981 BENTHIC SEDIMENT SURVEY AROUND THE SULLOM VOE OIL TERMINAL (numbers indicate  $n$ -alkane chain length)**

discharged to the area from the Calbeck Ness diffuser, (c) fuel oil, originally spilled from the Esso *Bernicia* in 1978, remnants of which may have survived in the area (cf. Grigson, 1981) or (d) a contribution of hydrocarbons from nearby peat deposits (S. Howells, Personal Communication, 1989).

#### 4.4.1.2 GRAVIMETRY AND GAS CHROMATOGRAPHY

Gravimetric data for samples from various locations in the Sullom Voe area (Figure 4.11) are shown in Table 4.2. Each sediment sample contained similar concentrations of aliphatic hydrocarbons (Table 4.2) which were dominated by *n*-alkanes with a distribution characteristic of higher plant waxes (Figure 4.12). Removal of these by urea adduction yielded a small amount of UCM (KI 1500 - KI 4000+; max. KI 2900, KI 3700) superimposed upon which were a series of resolved compounds attributable to acyclic isoprenoids, steranes and triterpanes (Figure 4.12).

Similar analysis of the peat sample (Figure 4.13) produced a UNA fraction of <1mg from the 100g extracted, comprising a series of resolved compounds identified as predominantly triterpanes. For example, the major component was identified by relative retention time as the  $17\alpha(H), 21\beta(H)C_{31}$  homohopane (22*R*) of presumed biogenic origin (cf. Quirk et al., 1980). No measurable UCM was present in the UNA of the peat.

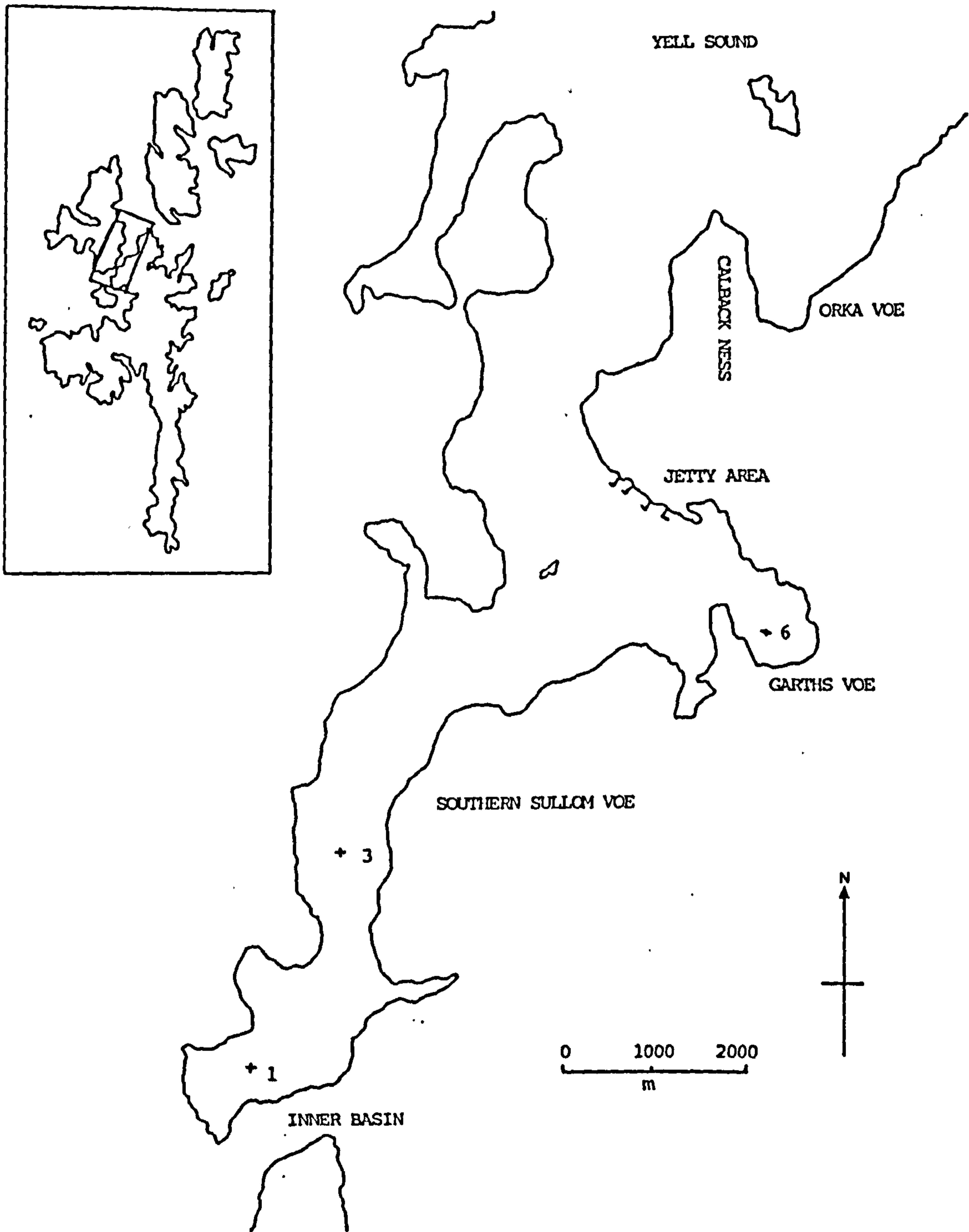


FIGURE 4.11. SAMPLE SITE LOCATIONS FOR SULLOM VOE SAMPLES

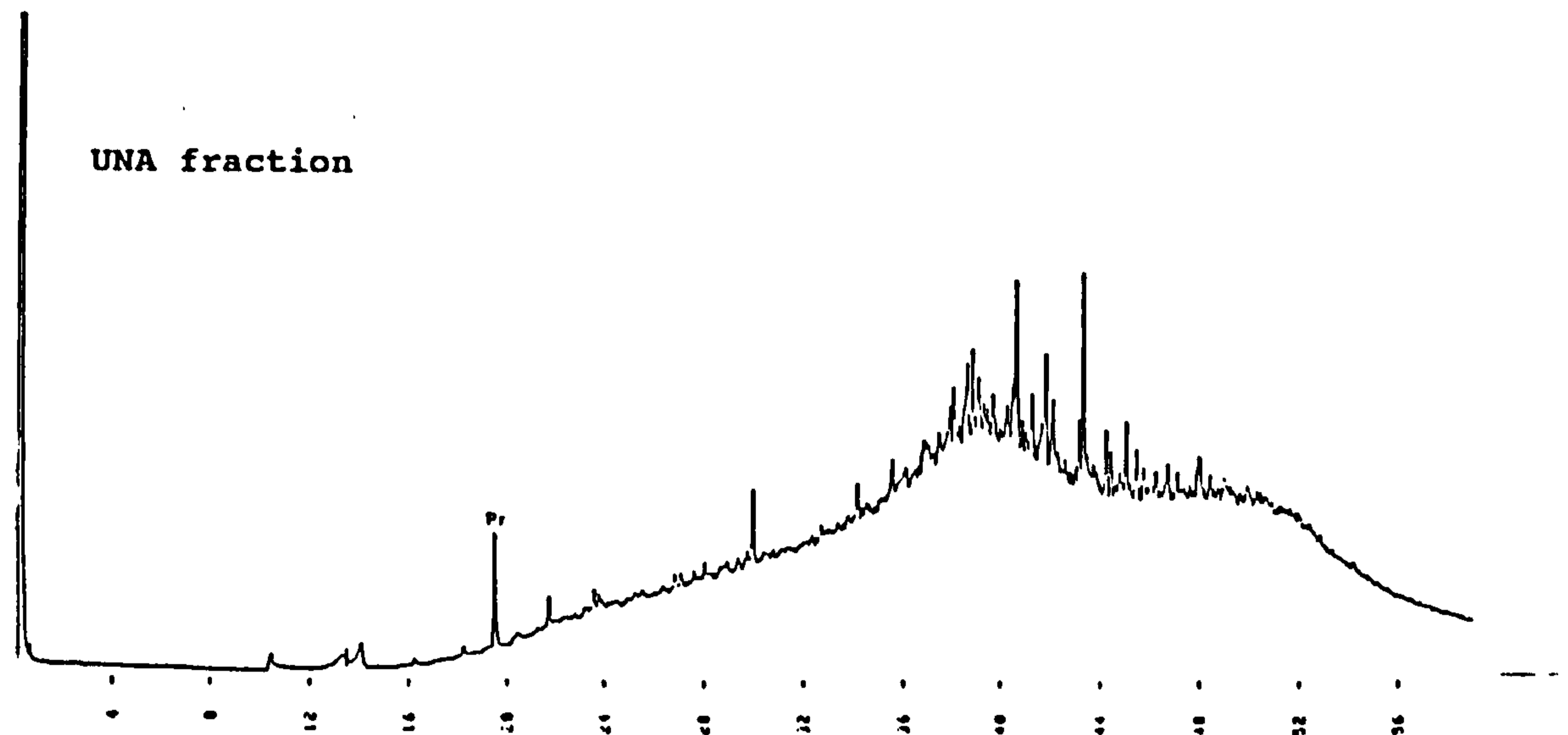
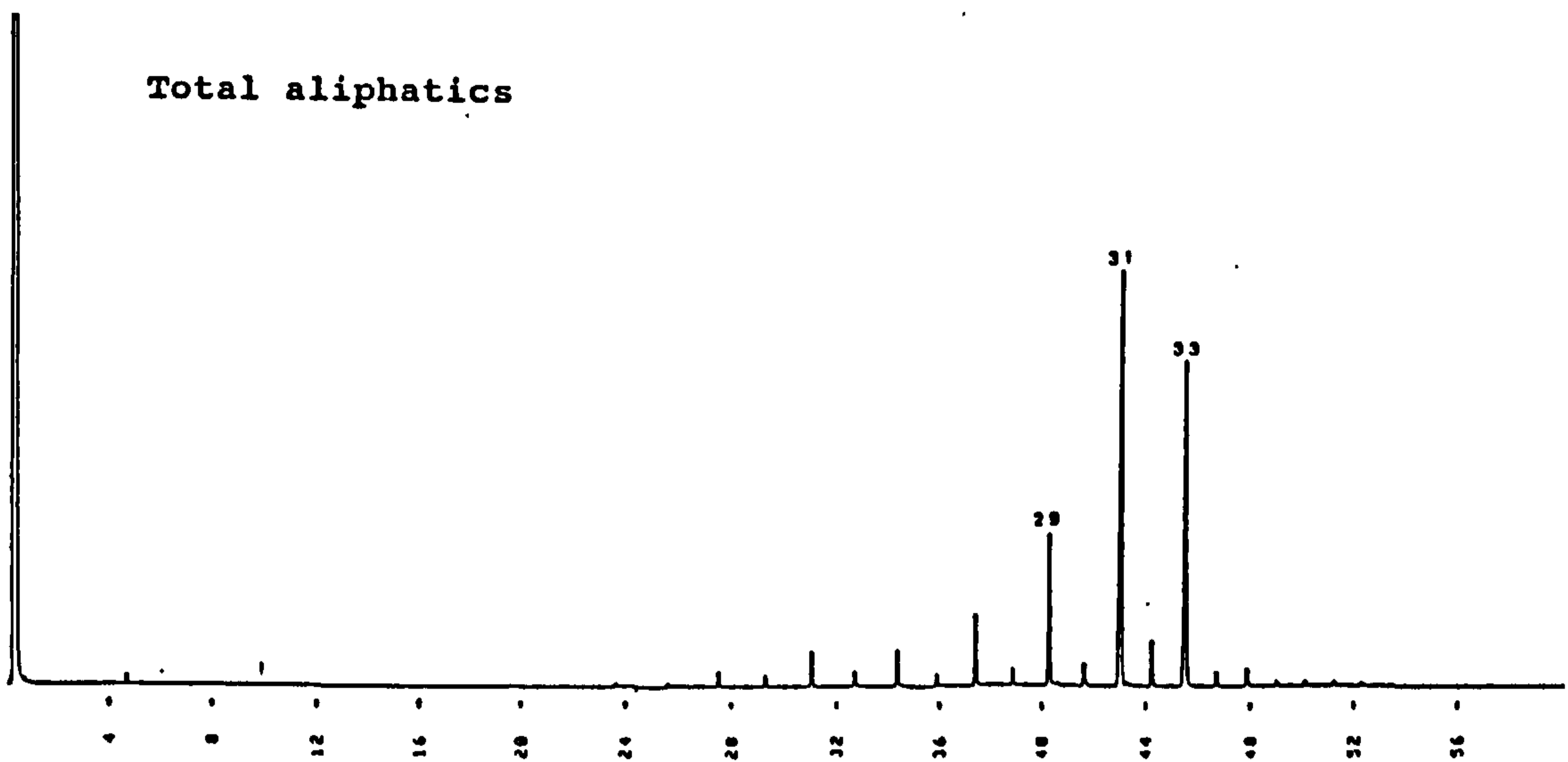


**TABLE 4.2: CONCENTRATION OF FRACTIONS IN SULLOM VOE SEDIMENTS ( $\mu\text{g g}^{-1}$  DRY WEIGHT)**

FRACTION	SEDIMENT			SCATSA NESS PEAT
	1	3	6	
TOE*	4300	1300	6000	40,000
Total Aliphatics	300	200	360	97
UNA Aliphatics	33	25	32	nd
Total Aromatics	680	460	1900	1200

nd = not detected

\* = Total Organic Extract



**FIGURE 4.12. GAS CHROMATOGRAMS OF SULLOM VOE STATION 3  
 TOTAL ALIPHATICS AND UNA FRACTIONS  
 (numbers indicate n -alkane chain length, Pr = pristane)  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)**

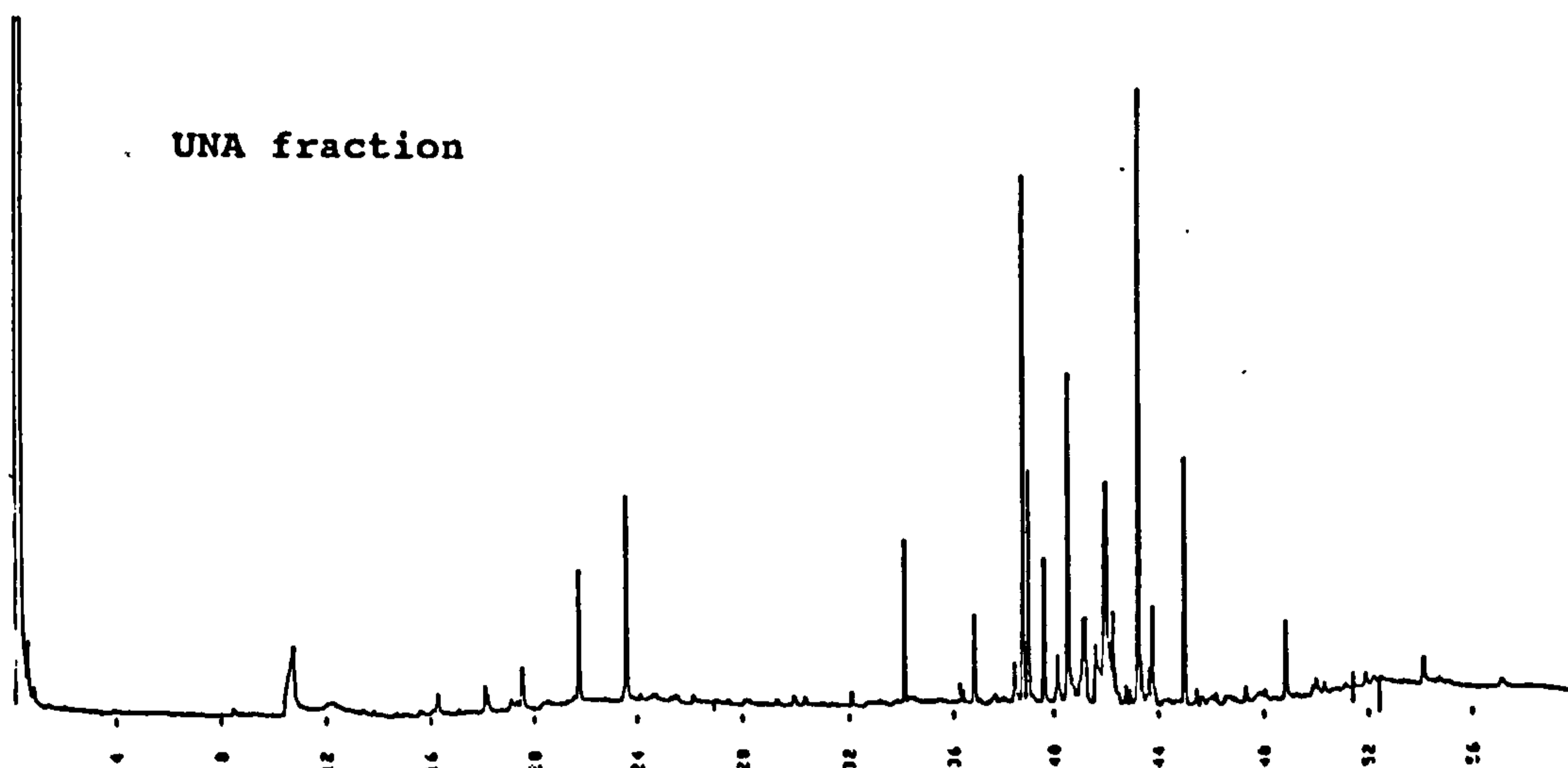
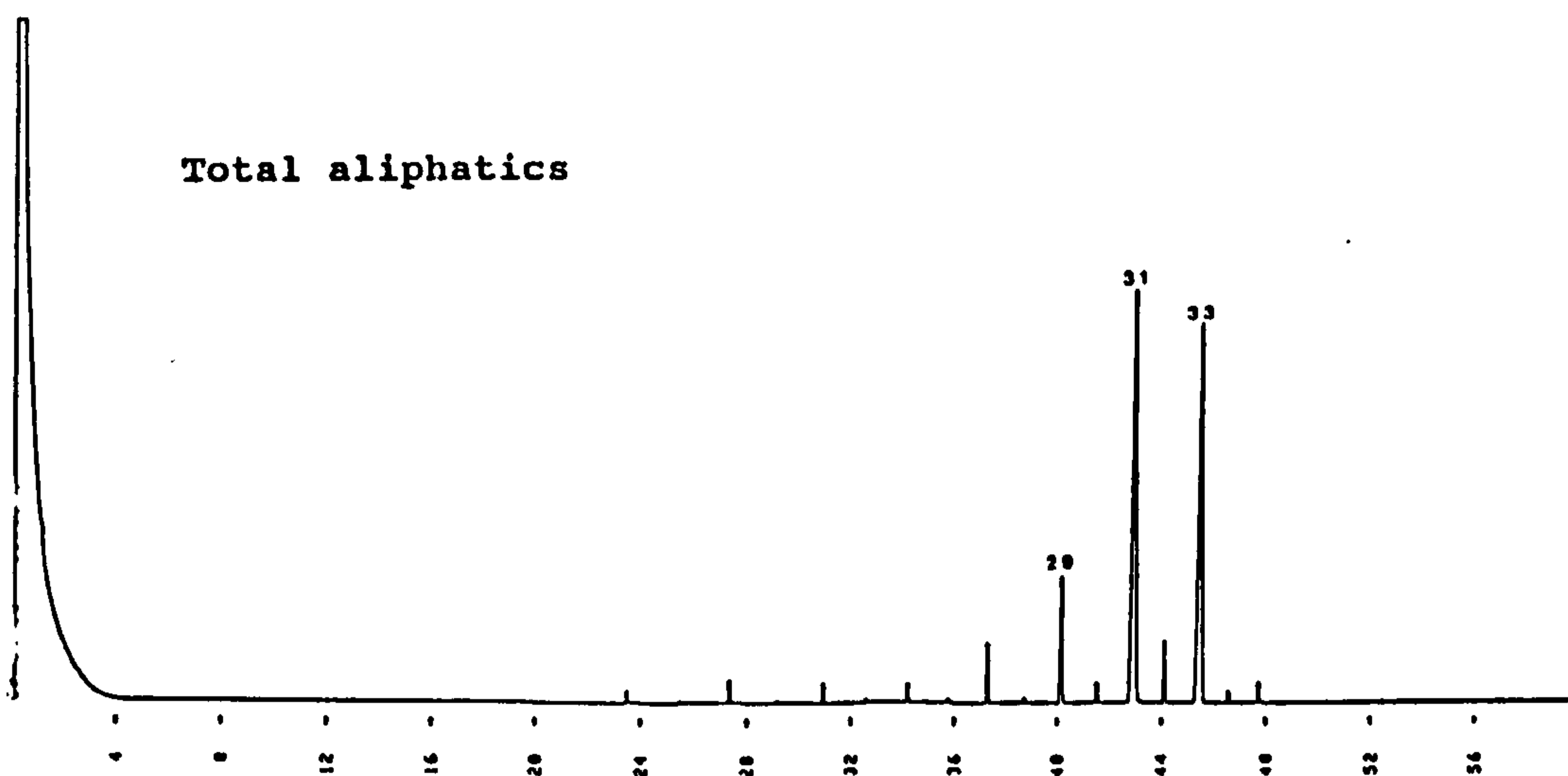


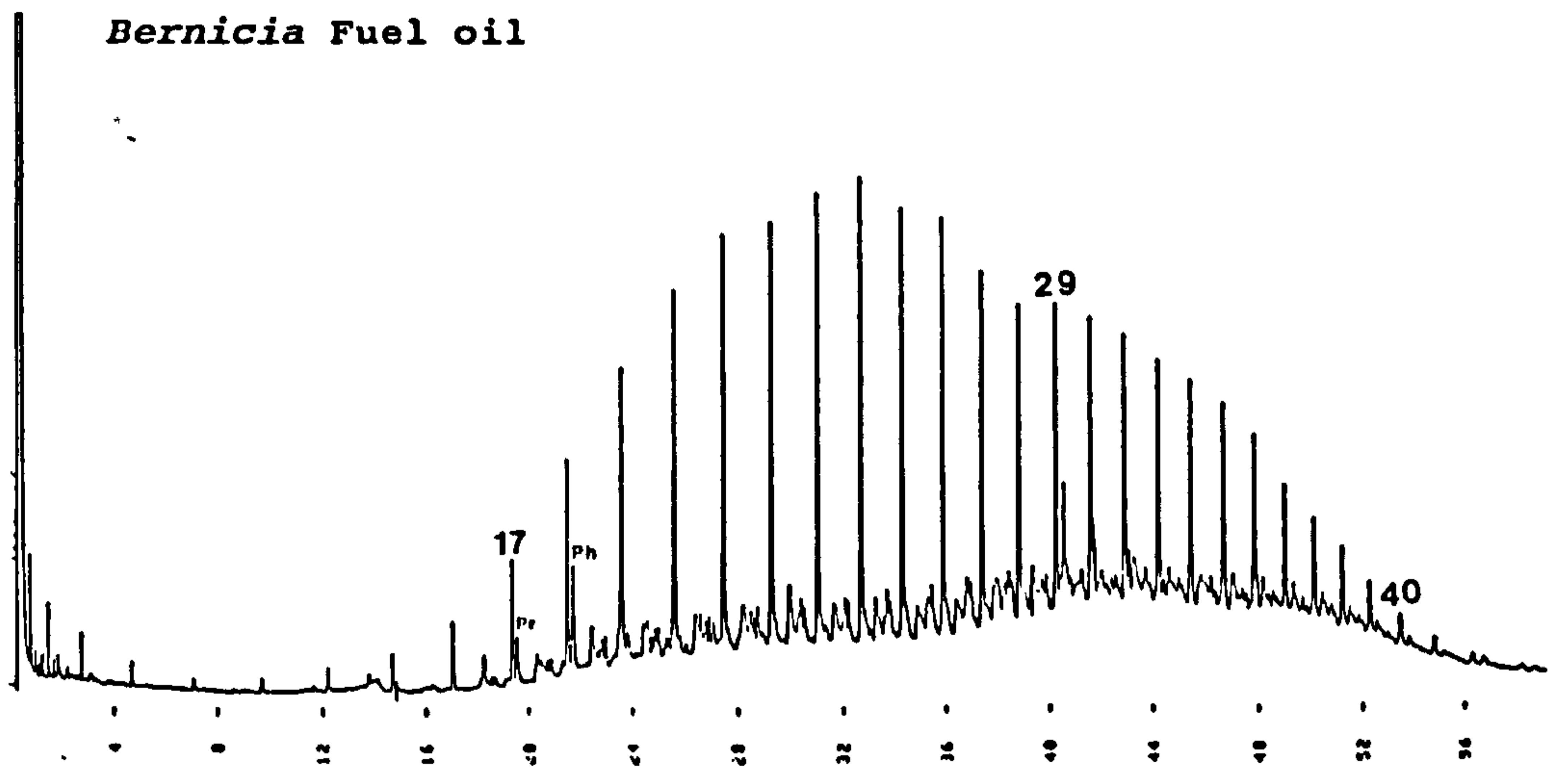
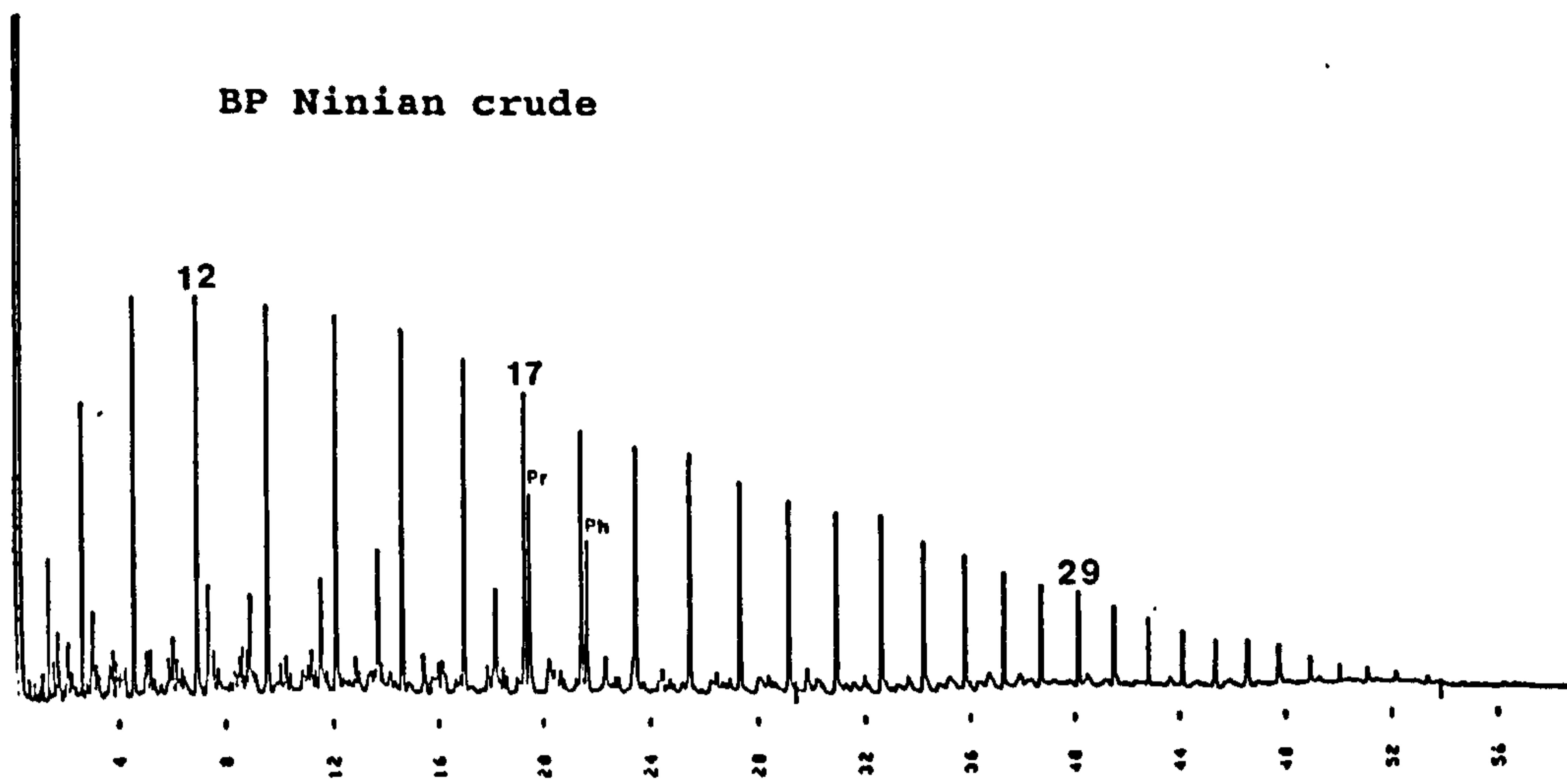
FIGURE 4.13. GAS CHROMATOGRAMS OF SCATSA NESS PEAT TOTAL ALIPHATICS AND UNA FRACTIONS (numbers indicate  $n$ -alkane chain length, Pr = pristane) (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)



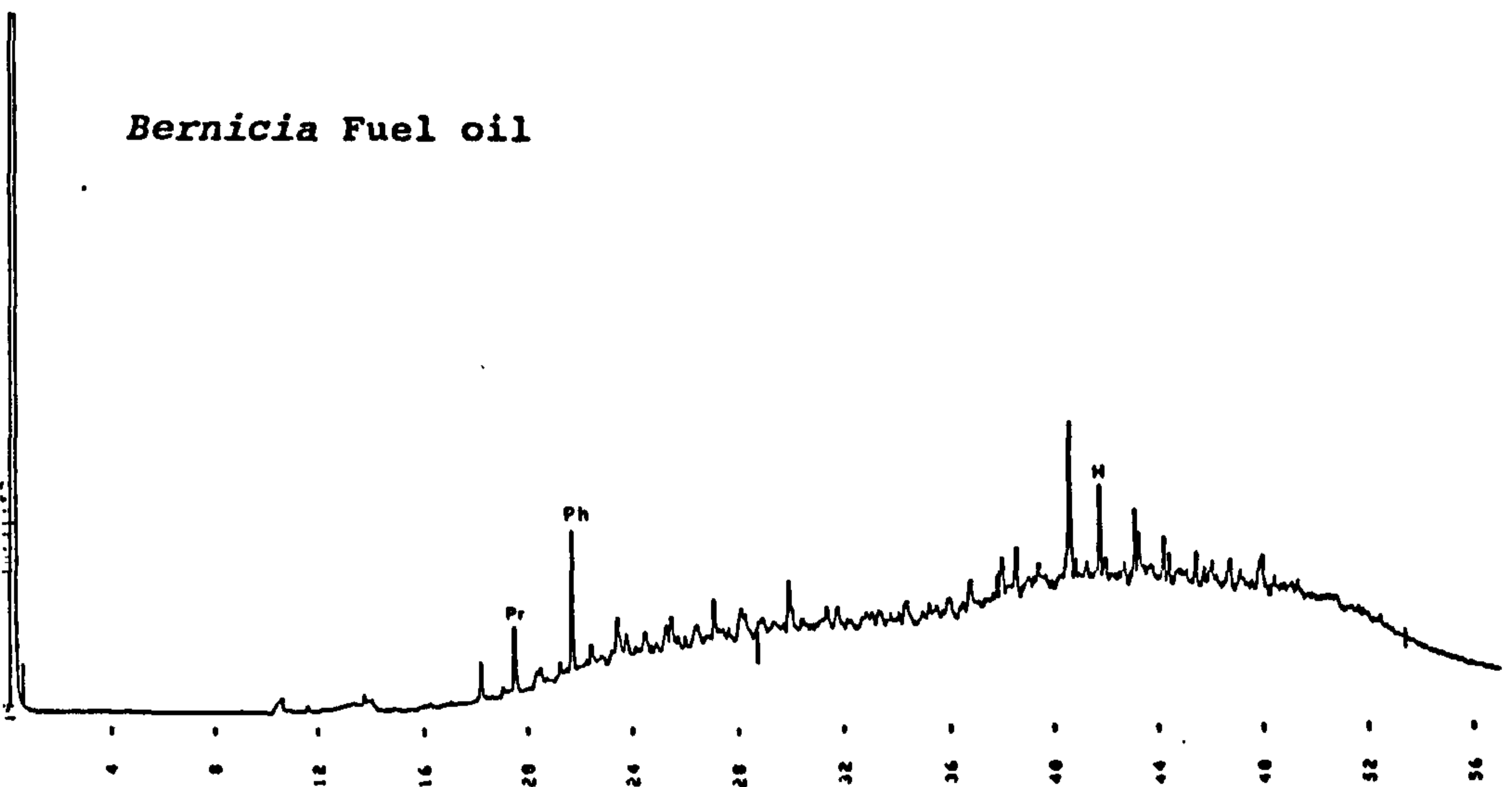
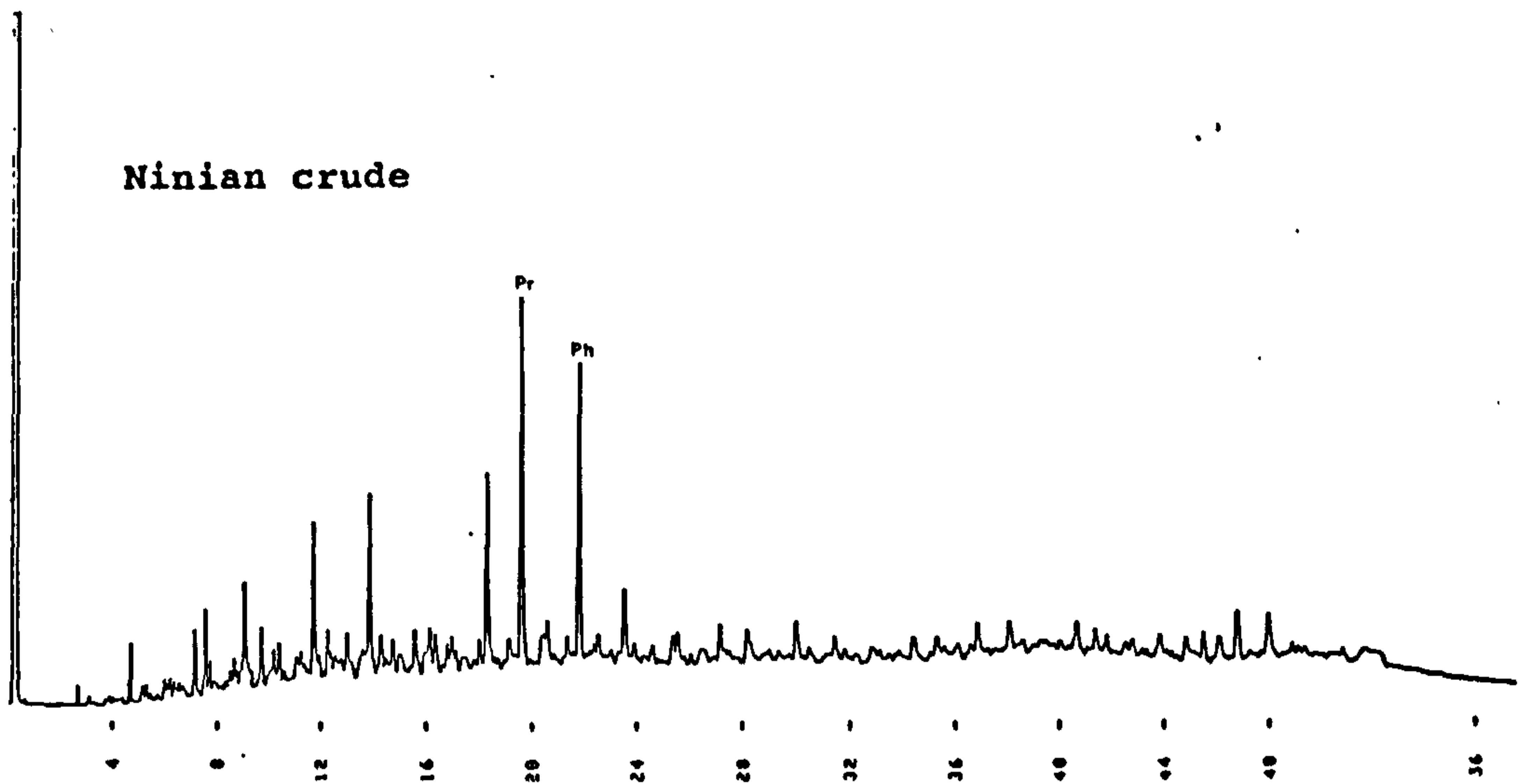
Gas chromatograms of the BP Ninian Crude and Esso Bernicia fuel oil are shown in Figure 4.14. The UNA fractions were dominated by a UCM with resolved peaks (mainly pristane, phytane and triterpanes) superimposed (Figure 4.15). Since Ninian crude contains relatively high proportions of acyclic isoprenoids the UNA was further subjected to thiourea adduction. The final, isolated UCMS of the two oils were somewhat similar, ranging from ca. KI 1200 to KI 4000 with bimodal distributions (Figure 4.16).

#### 4.4.1.3 GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Figure 4.17 shows partial  $m/z$  191 mass chromatograms, illustrating the distributions of pentacyclic triterpanes in the sediments, peat, Bernicia fuel oil and Ninian crude. The peak assignments are given in Table 4.3 (facing page 143). The sediment samples contained both petrogenic (e.g. norhopane, hopane) and biogenic (e.g. 22R homohopane in relatively high amounts) triterpanes. The peat sample comprised triterpanes which are usually regarded as biogenic in origin. Indeed, a similar distribution is observed in Sullom Voe sediments, suggesting that the peat is the major source of biogenic triterpenoid hydrocarbons in the sediments. The distribution of petrogenic triterpanes in the sediments is similar to that of the Bernicia fuel oil (i.e. norhopane/hopane >1). Whilst this suggests that the major source of sediment petrogenic triterpanes is the Bernicia fuel oil, this is by no means

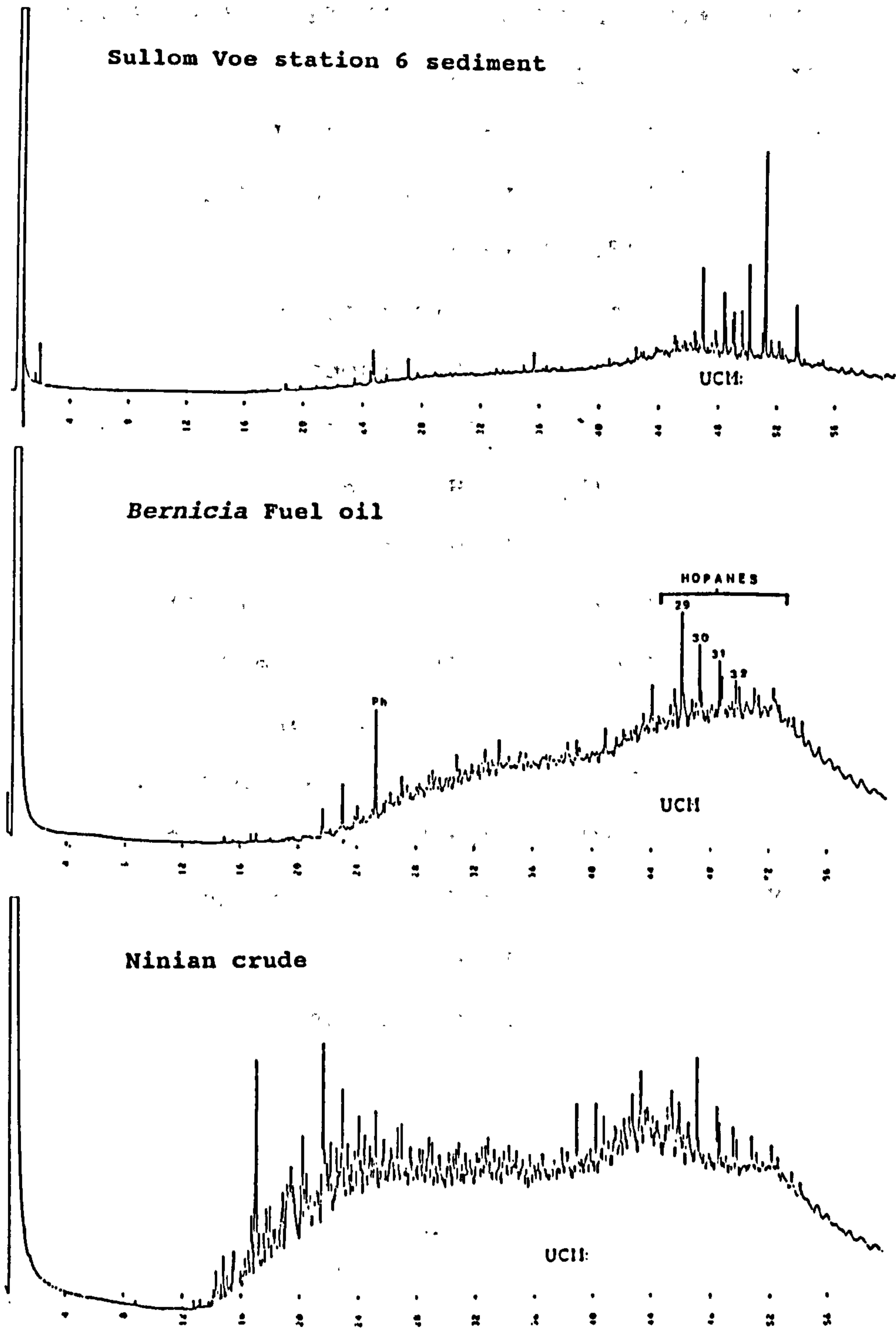


**FIGURE 4.14. GAS CHROMATOGRAMS OF NINIAN CRUDE AND BERNICIA FUEL OILS TOTAL ALIPHATIC FRACTIONS**  
 (numbers indicate *n*-alkane chain length, Pr = pristane)  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)

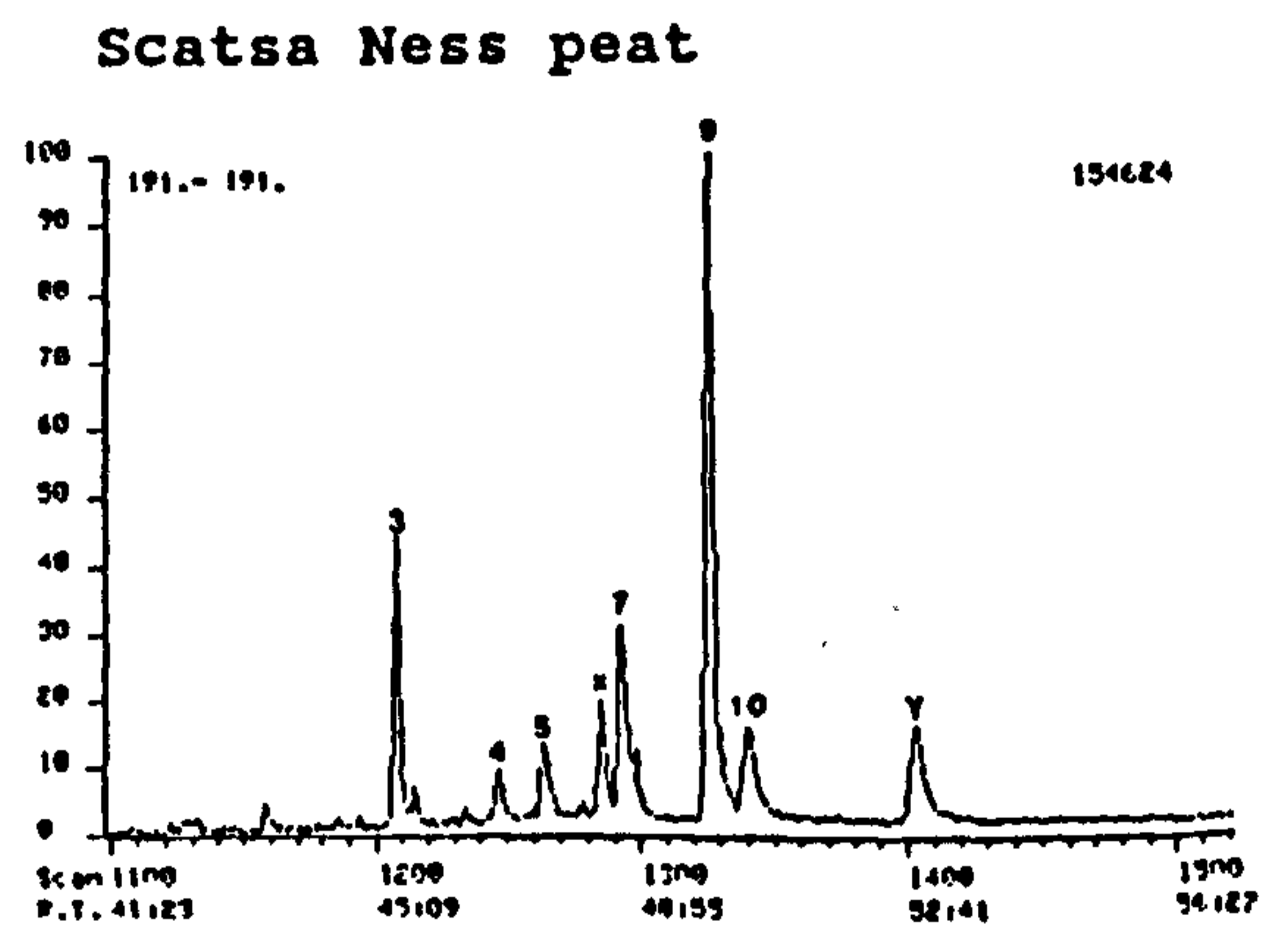
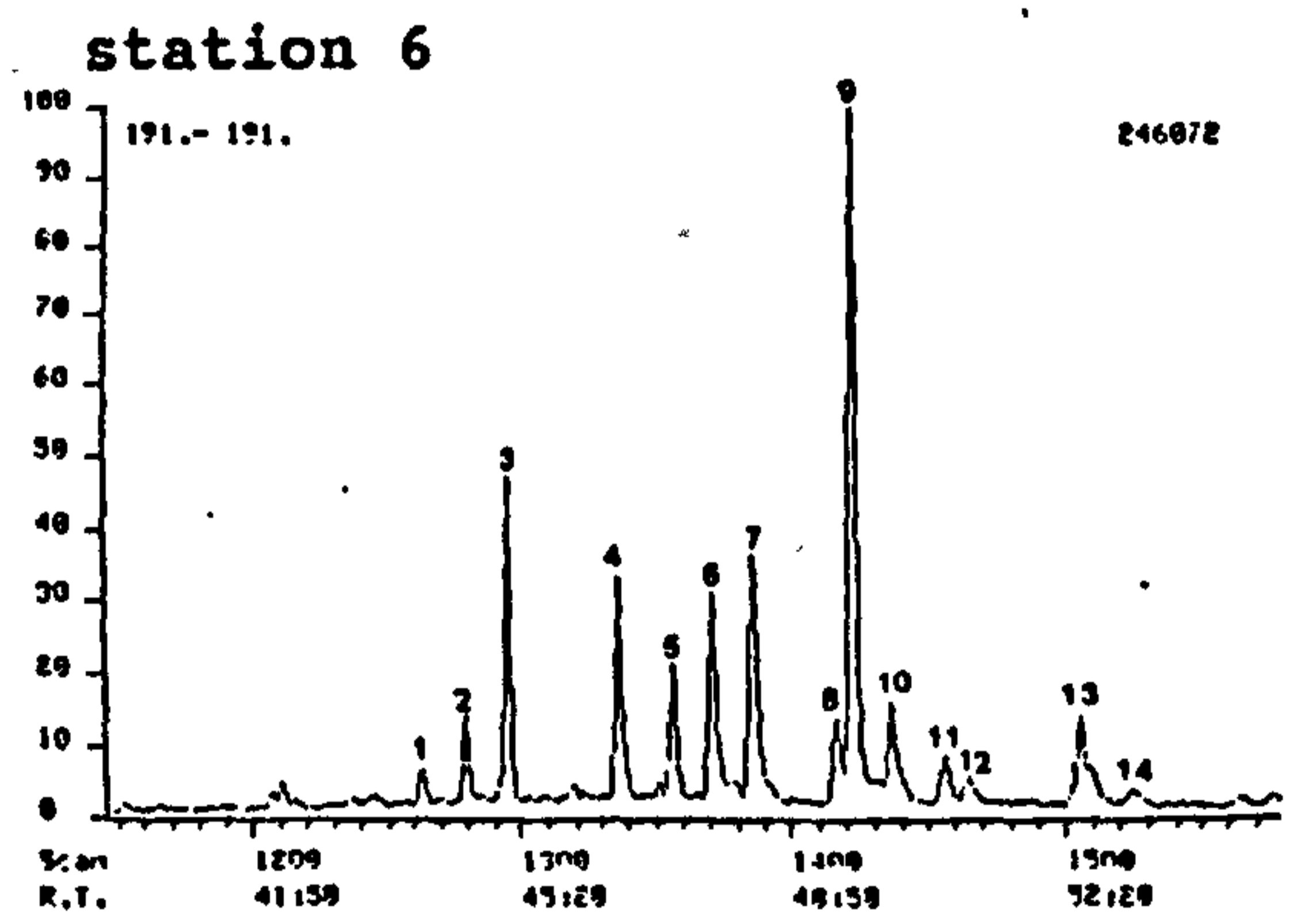
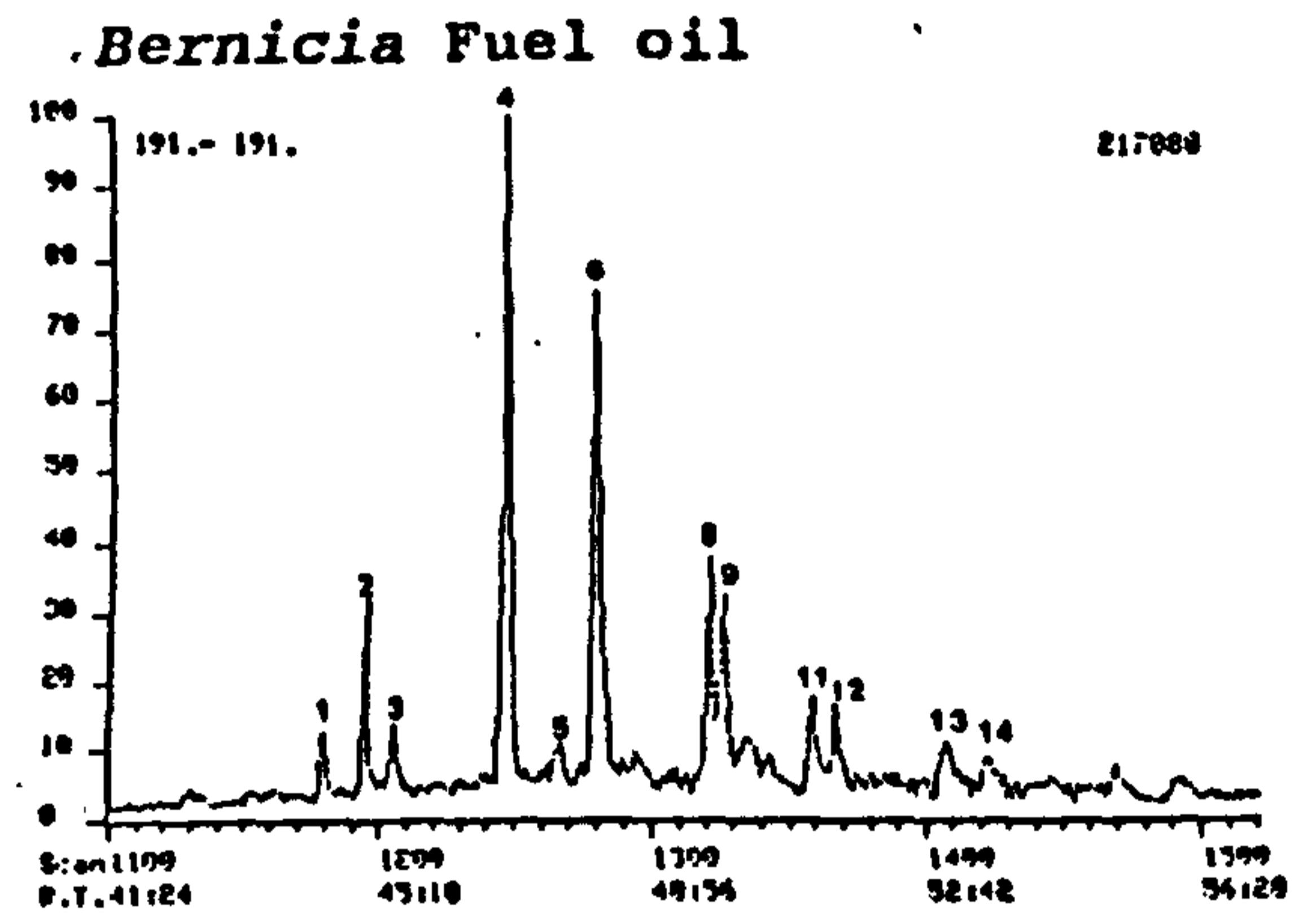
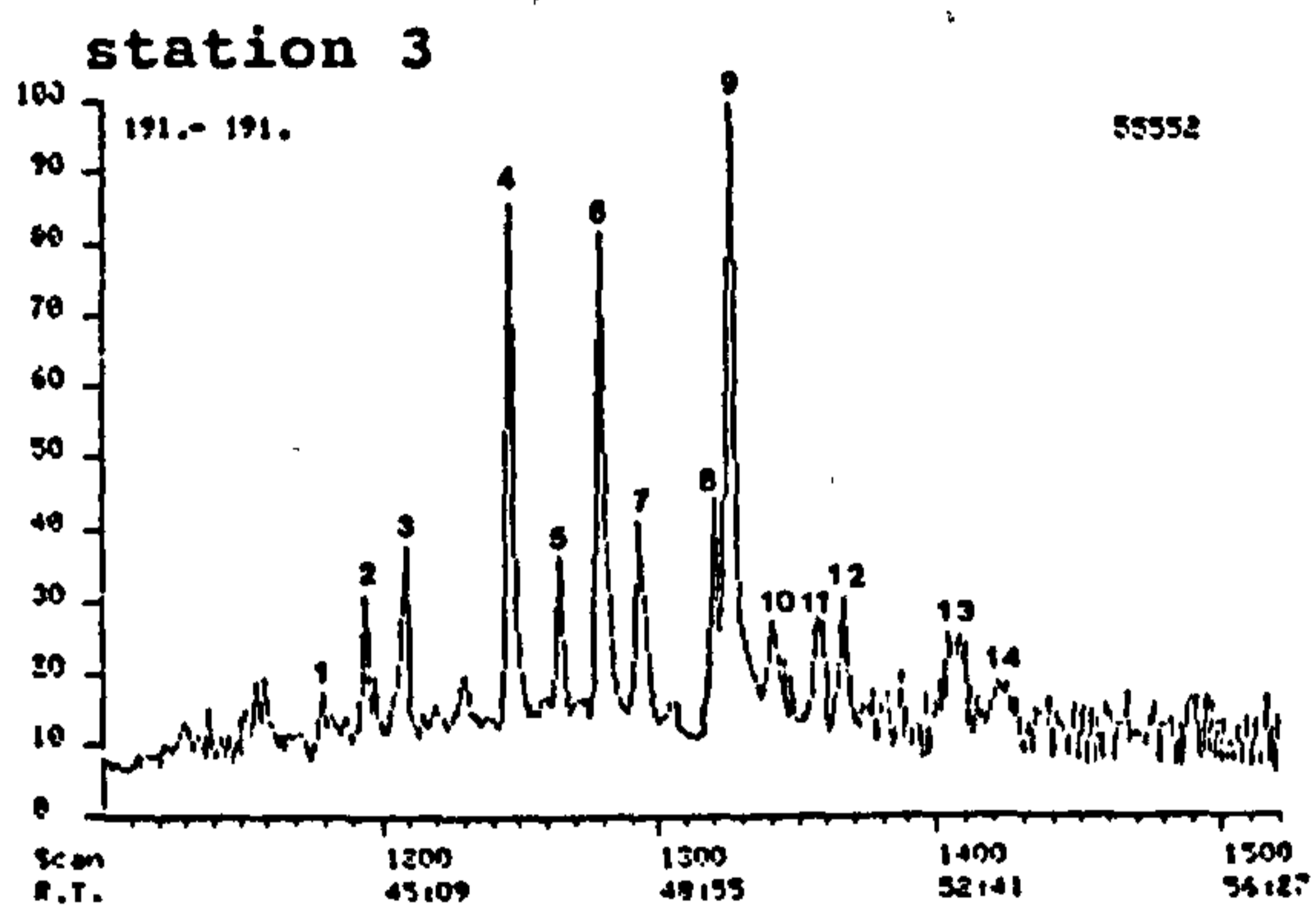
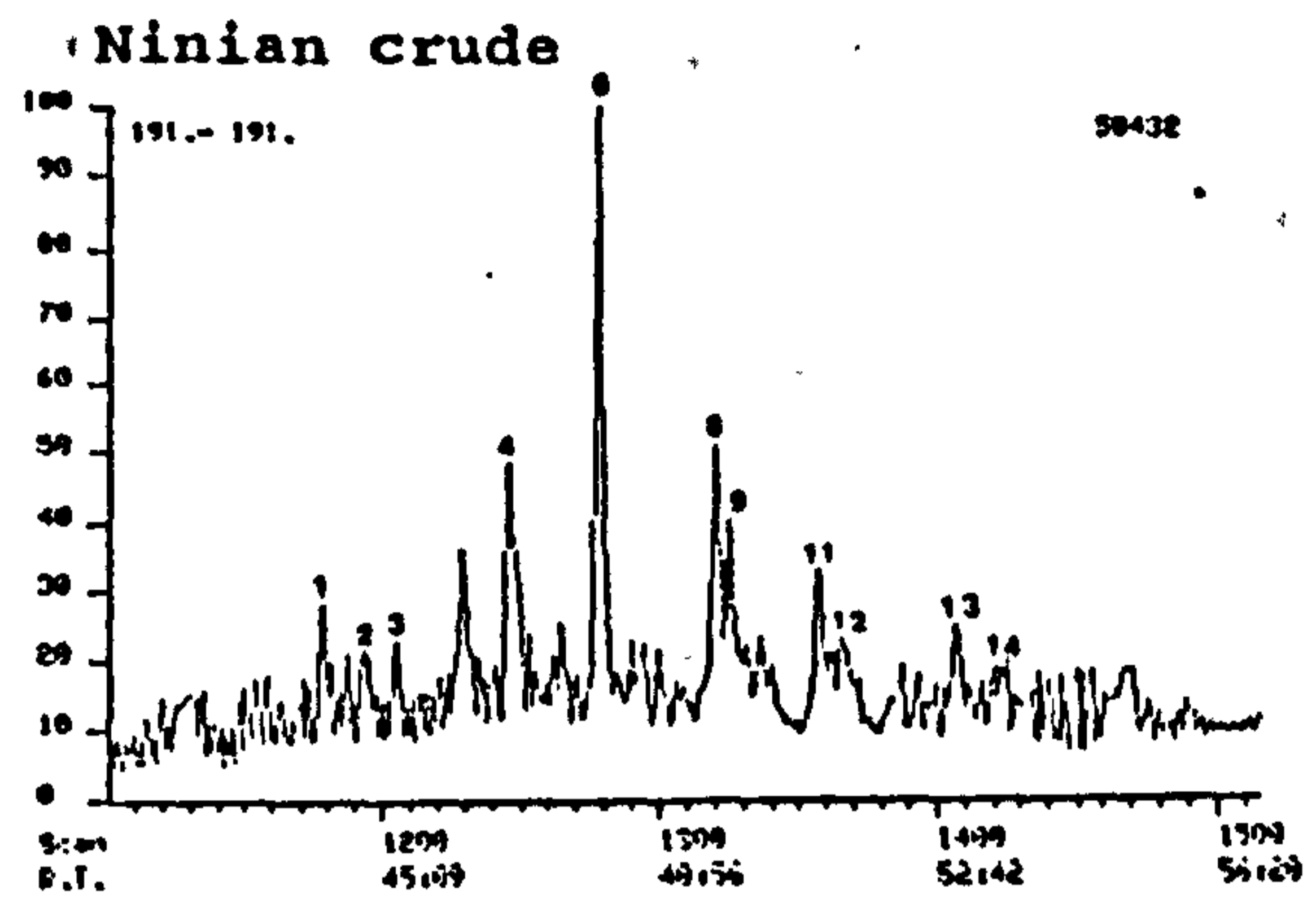
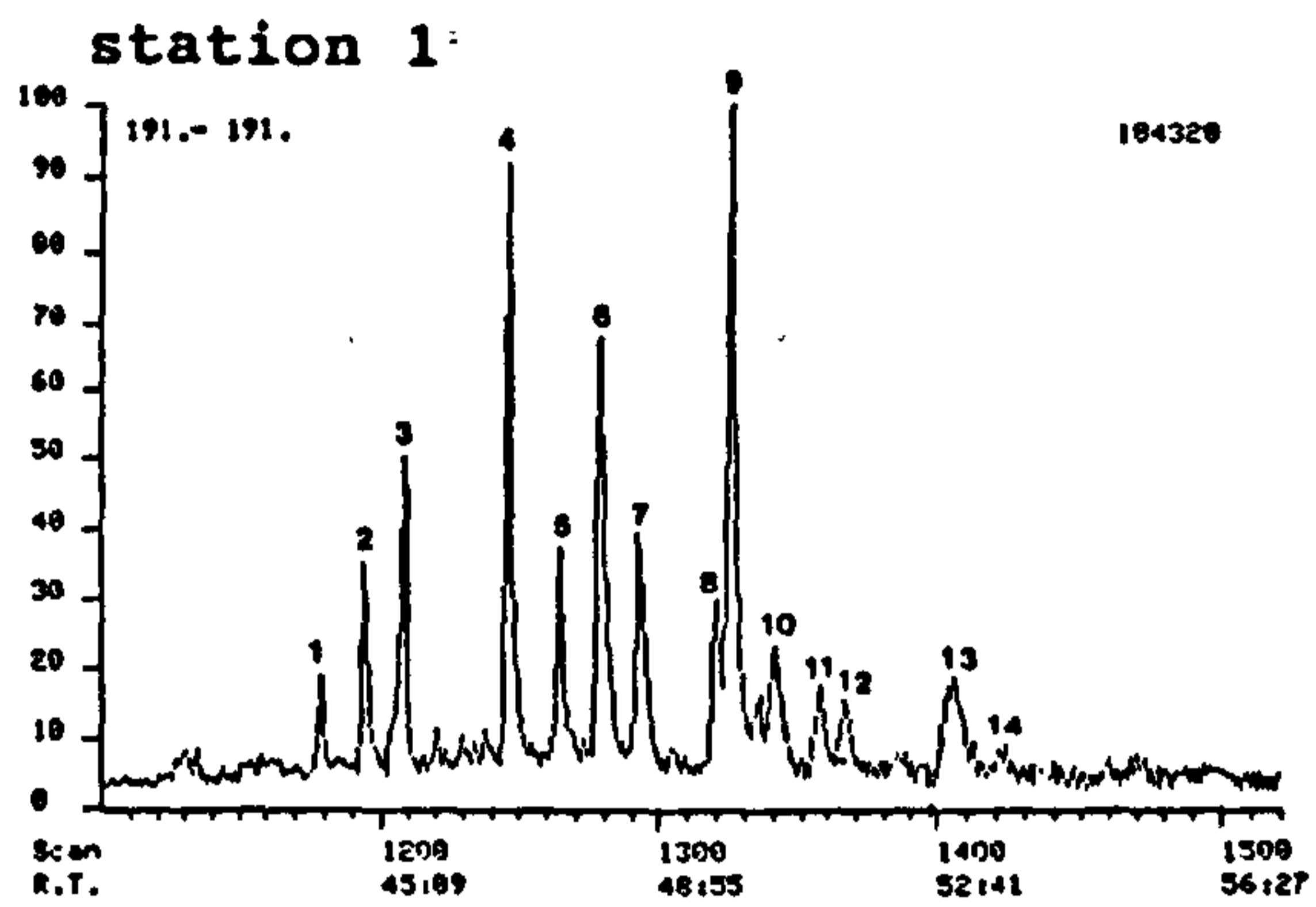


**FIGURE 4.15. GAS CHROMATOGRAMS OF REFERENCE OILS UNA FRACTIONS**  
 (numbers indicate *n*-alkane chain length, Pr = pristane  
 Ph = phytane, H = C<sub>30</sub> hopane)  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)





**FIGURE 4.16. UCMS ISOLATED FROM SULLOM VOE SAMPLES**  
 (numbers indicate carbon number, Ph = phytane)  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)



**FIGURE 4.17. PARTIAL M/Z 191 CHROMATOGRAMS OF UNA FRACTIONS FROM SULLOM VOE SAMPLES AND REFERENCE OILS (J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas) (for peak identification see table opposite)**

conclusive evidence since a norhopane/hopane ratio >1 has been reported in several oils, notably those of Middle Eastern origins (e.g. Tibbetts et al., 1983) and such a distribution can also be the product of selective biodegradation of the C<sub>30</sub> hopane (Seifert and Moldowan, 1979; Goodwin et al., 1983). In addition, the concentrations of hopanes in the fuel oil and Ninian crude (Table 4.4) were quite different (2.5:1) and a small amount of residual fuel oil could have a relatively large effect upon the biomarker distributions of a UCM residue derived largely from Ninian Crude. The biomarker evidence was therefore inconclusive and an attempt to fingerprint the quantitatively more important UCM was made, utilising oxidative degradation, GCMS analysis and multivariate statistical techniques (MDS and cluster analysis).

#### 4.4.1.4 OXIDATIVE DEGRADATION (CrO<sub>3</sub>)

Although the aliphatic hydrocarbons in Sullom Voe sediments exhibited a strong UCM, their relatively low concentrations meant that only ca. 2-3 mg of UCM could be isolated from the sediment collected at Station 6 and less than 1 mg from stations 1 and 3.

Previous oxidations (Gough, 1989; Gough and Rowland, 1990) had utilised 50 mg of sample. Therefore, a series of validation experiments using Silkolene lubricating oil feedstock were carried out to test the feasibility and



**TABLE 4.4: CONCENTRATIONS OF BIOMARKERS IN OILS ( $\mu\text{g g}^{-1}$ )**

OIL	TOTAL HOPANES	C <sub>29</sub>	C <sub>30</sub>	TOTAL STERANES
Ninian crude	1100	100	380	1100
Bernicia fuel oil	2500	550	900	1400

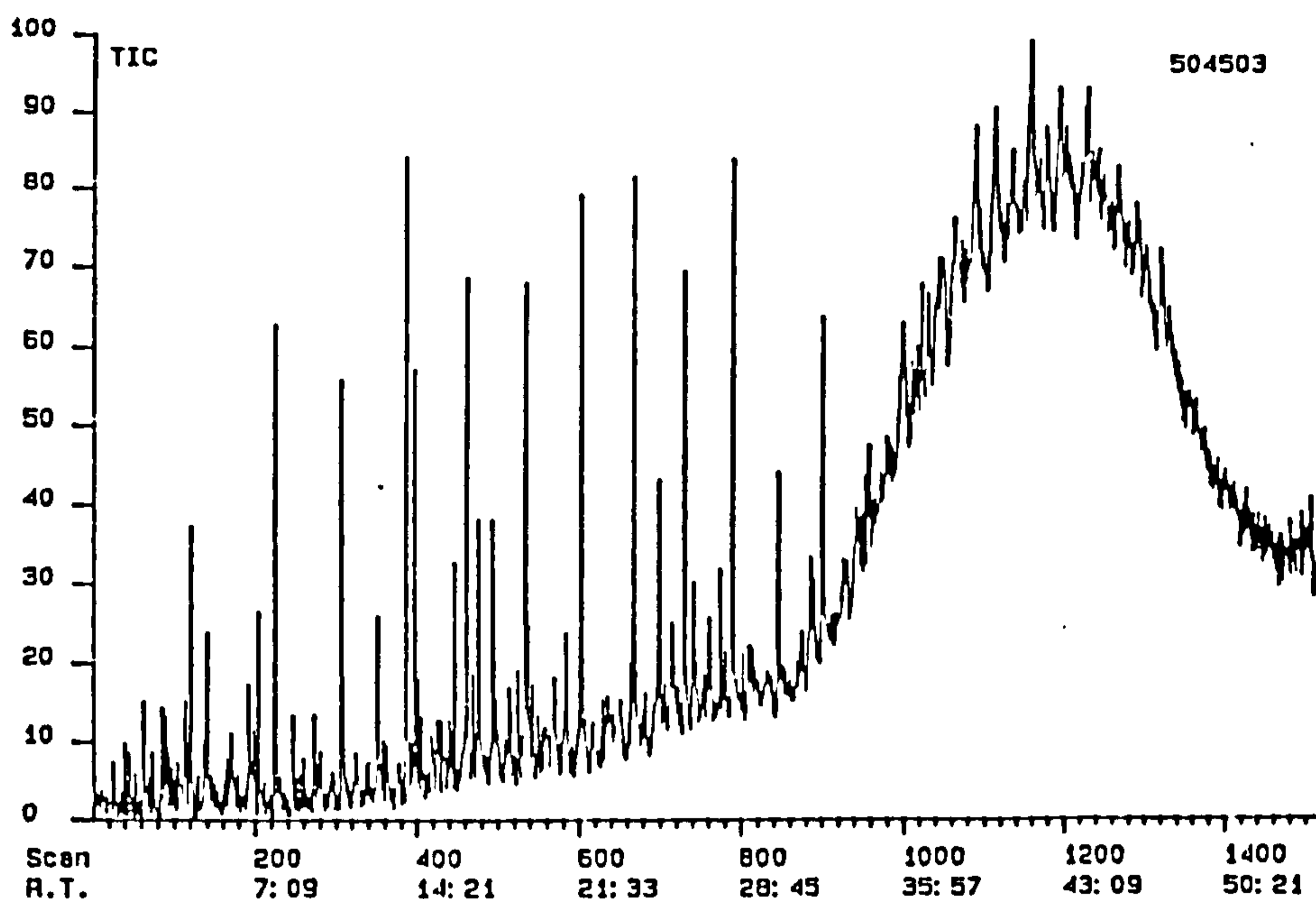
**TABLE 4.5. RESULTS OF OXIDATION OF SULLOM VOE SAMPLES**

Sample	% Alkanes	% UCM	Total Yield from Oxidation (%)	Isolated oxidised products (%)
Sullom Voe sediments	66	9	95	70
Ninian crude	52	31	89	55
Bernicia fuel oil	28	62	84	69

reproducibility of oxidation of 2 mg samples (see experimental for details). These gave a high recovery of material (80-90%) with good repeatability when the oxidation product profiles were examined visually (Figure 4.18).

Application of this refined method to the isolated UCMs (Table 4.5) produced a series of GC resolved components, superimposed on a reduced UCM. Mass chromatography of the major oxidation products (Carboxylic acids as methyl esters,  $m/z$  74; ketones,  $m/z$  58 and  $\gamma$ -methyl- $\gamma$ -lactones,  $m/z$  99) highlighted some similarities and some differences between the oxidation product profiles (Figures 4.19-4.21). The most noticeable difference was the relatively enhanced level of low molecular weight compounds in the sediment oxidation products. This may be a function of sample work-up although all samples were treated similarly. However, it is clear that the final chromatograms are complex and that a thorough comparison of even only three samples by eye is extremely difficult and that statistical treatment of the data is needed. Three samples are too few for a valid statistical analysis, so the results from these analyses were combined with data from further samples in a second case study from the River Mersey (Section 4.4.2), from a reproducibility study and from two previous case studies involving acute oil spills (Gough and Rowland, 1990).

Oxidation 1



Oxidation 2

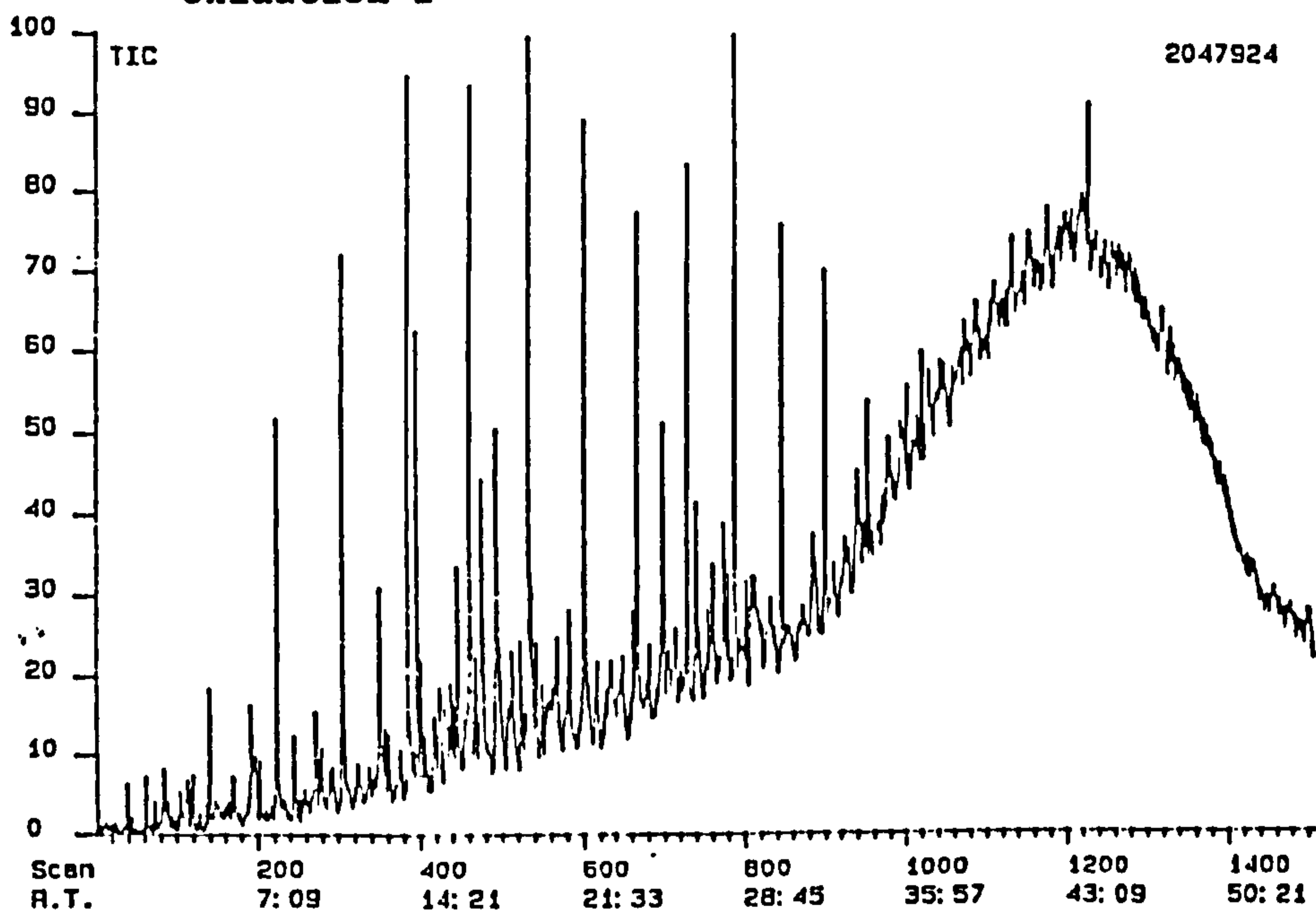
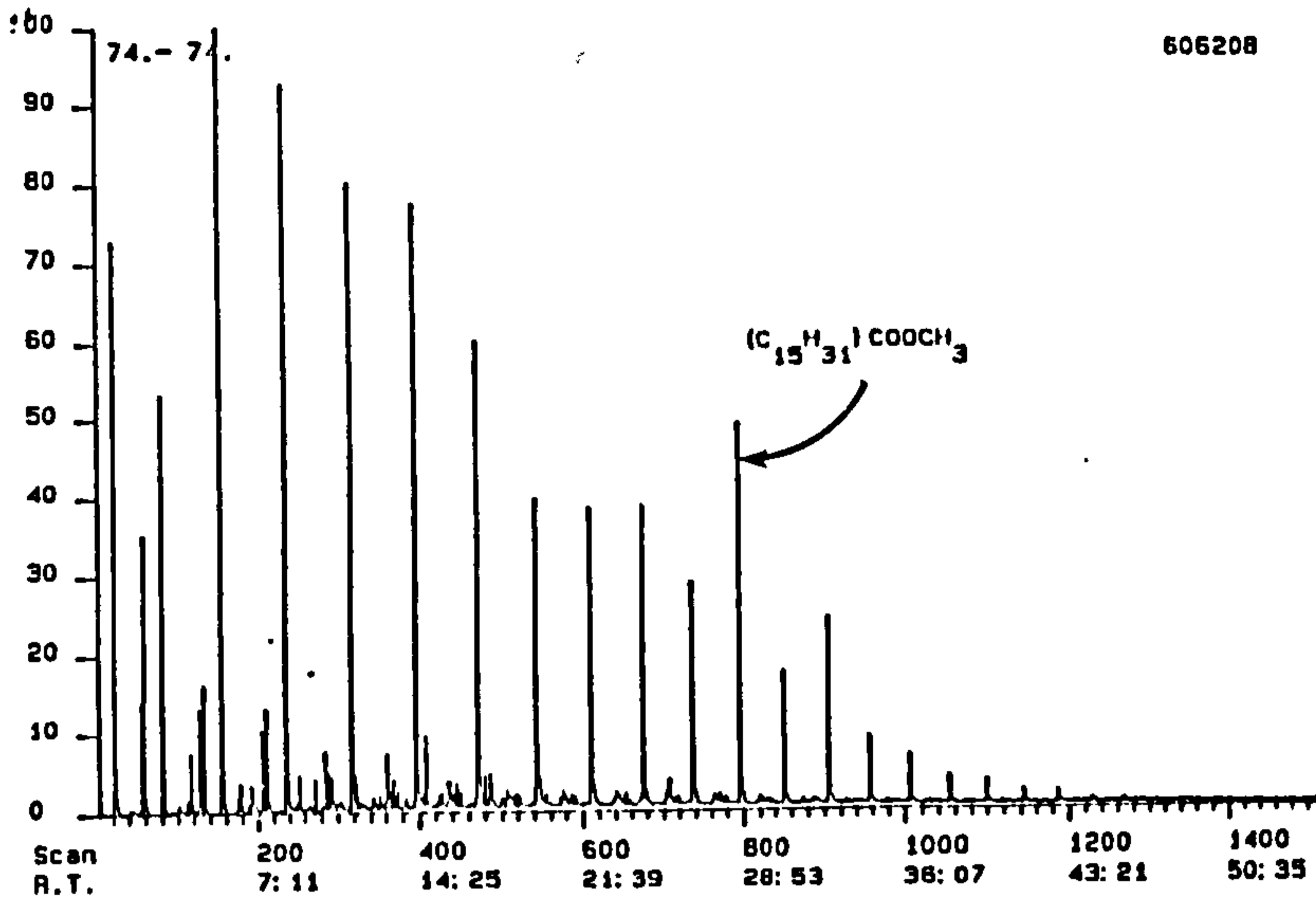


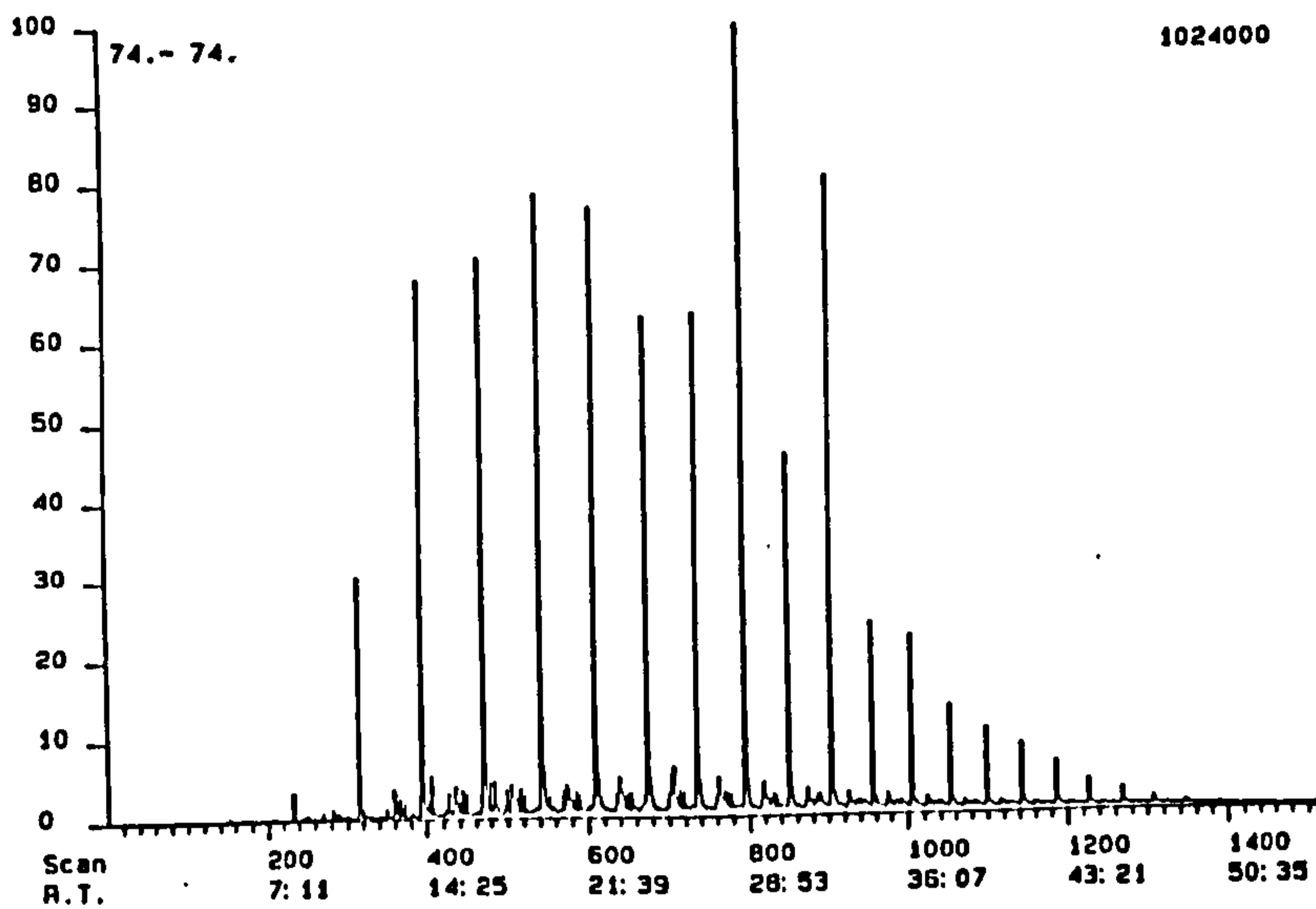
FIGURE 4.18. REPEAT OXIDATIONS OF 2MG SAMPLES OF SILKOLENE LUBRICATING OIL: TOTAL OXIDATION PRODUCTS (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)



Sullom Voe station 6



Bernicia Fuel oil



Ninian crude

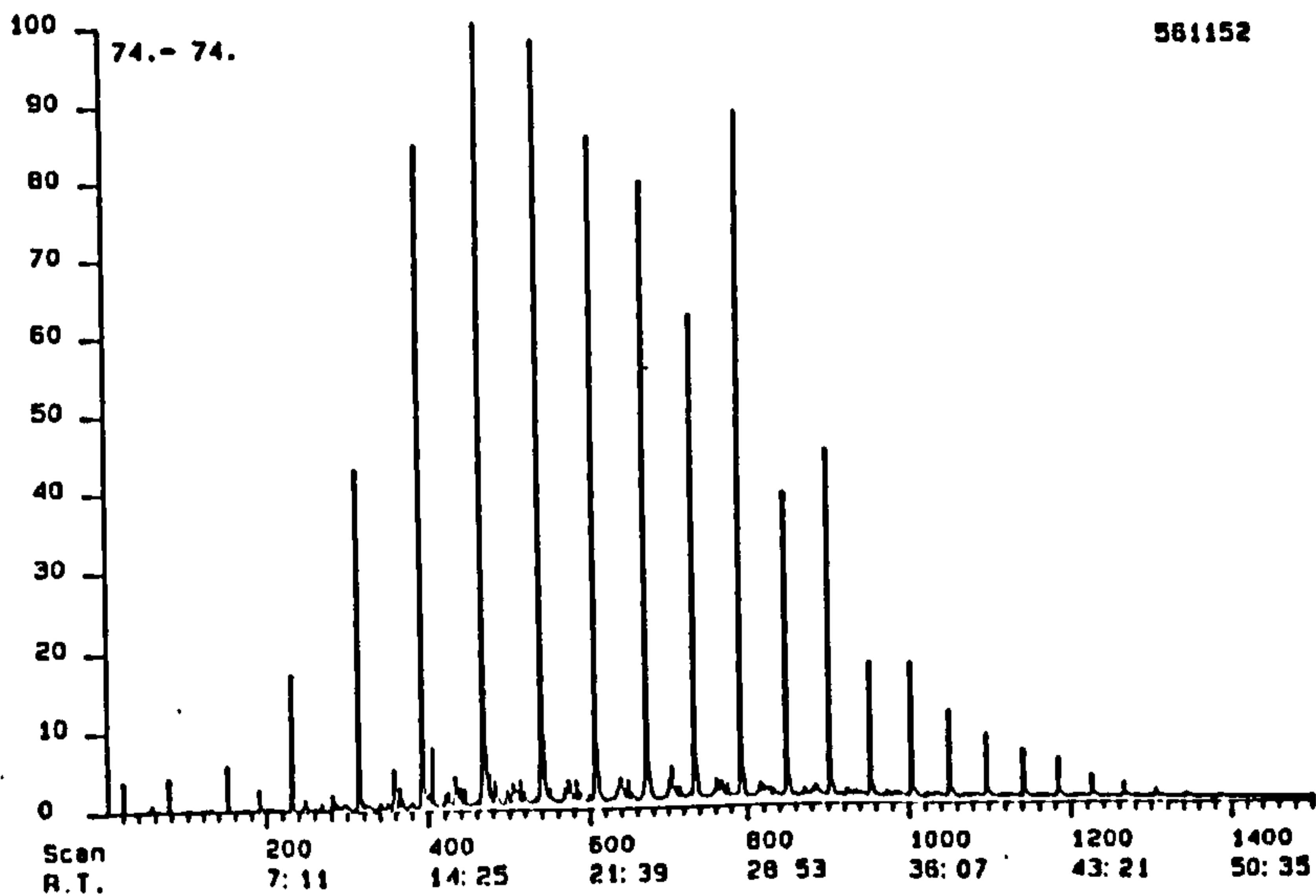
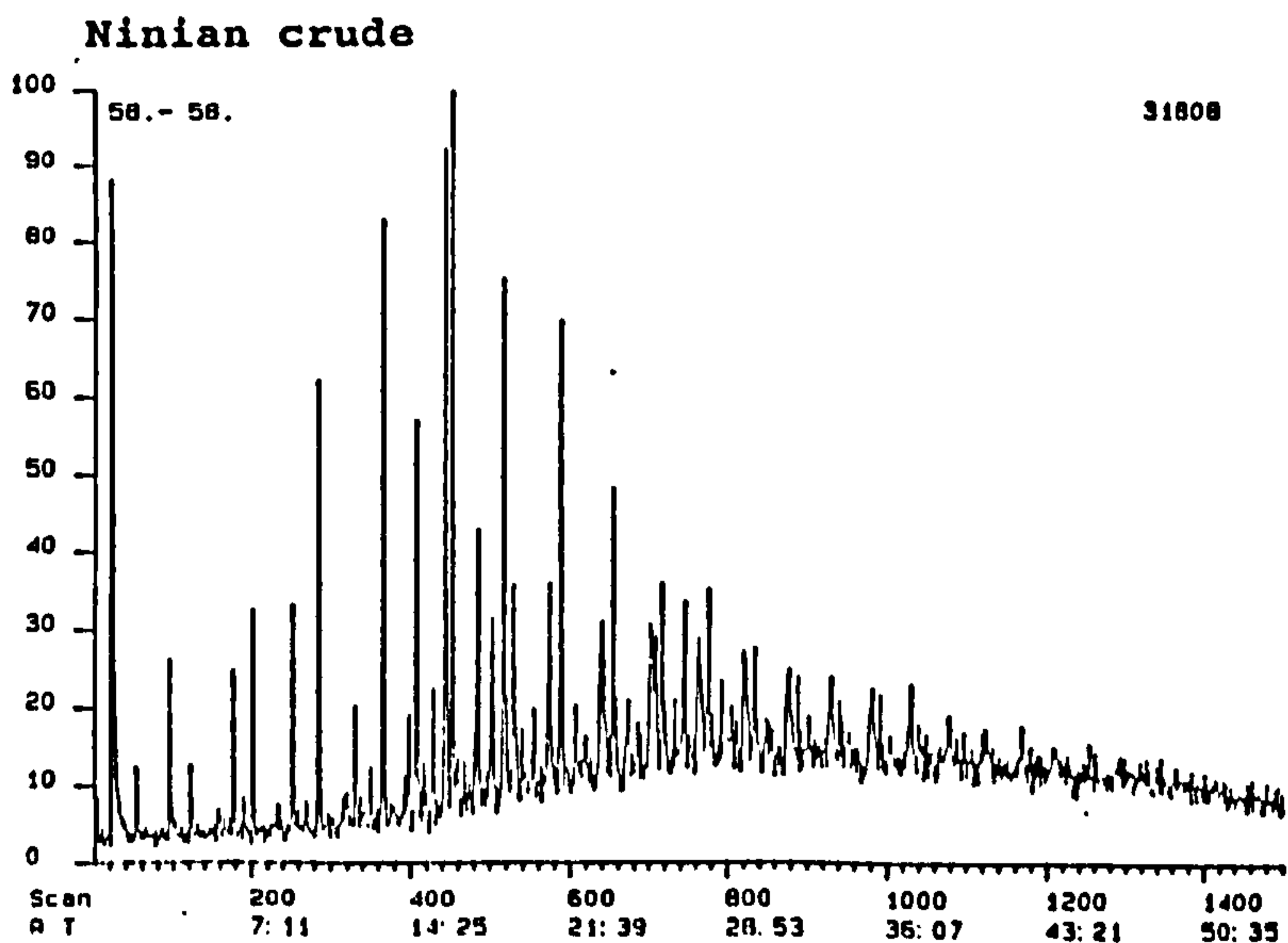
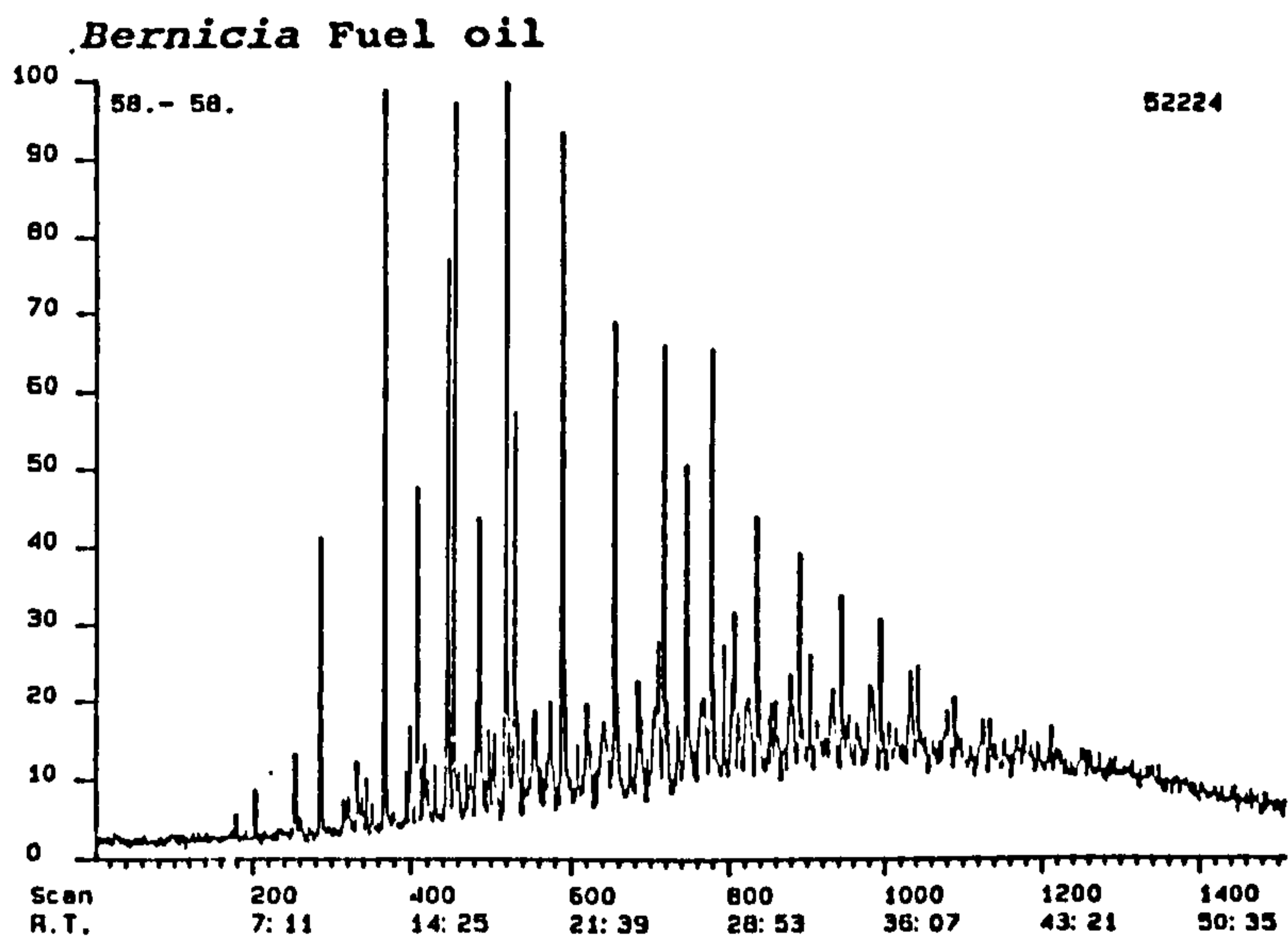
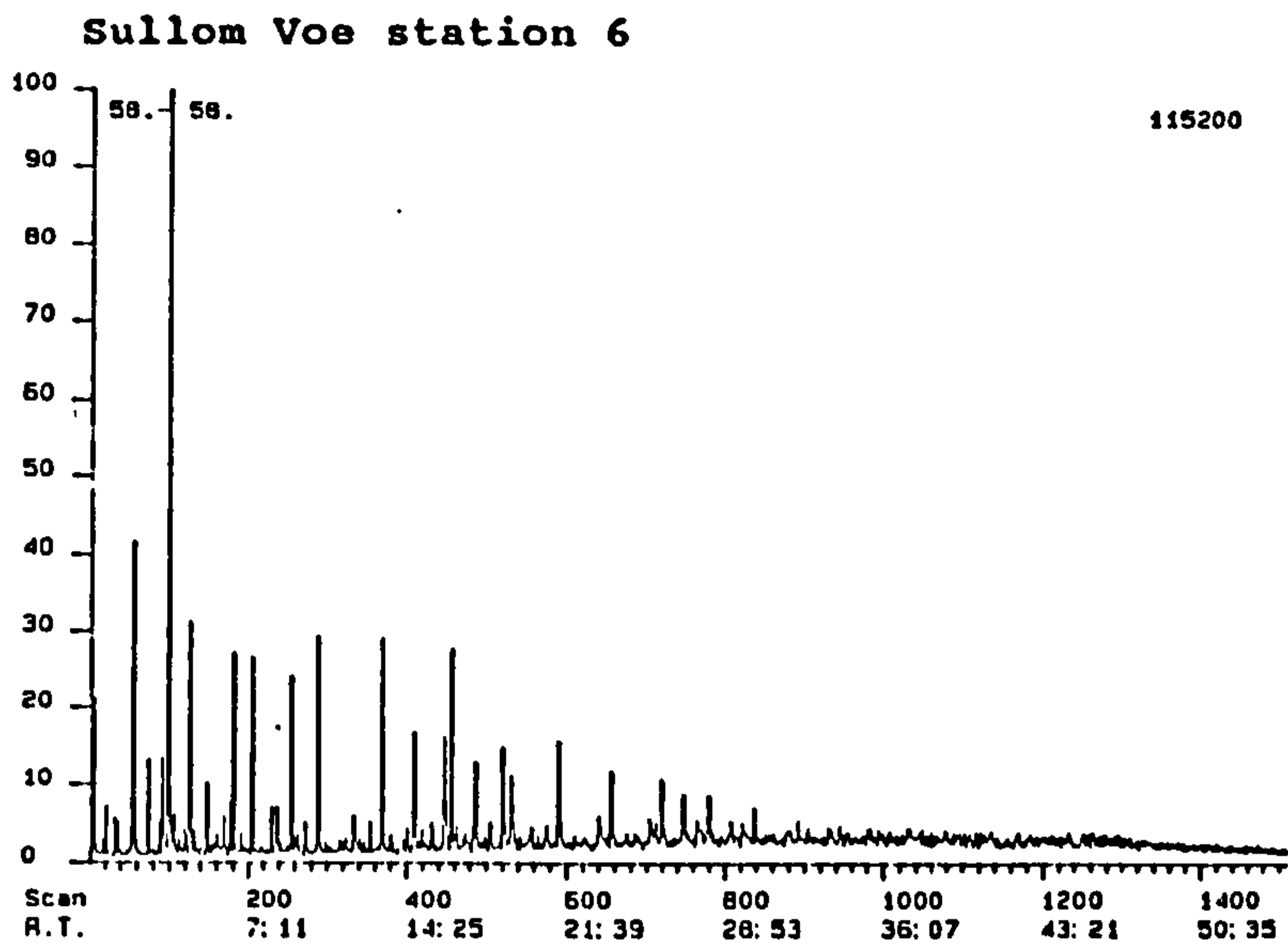
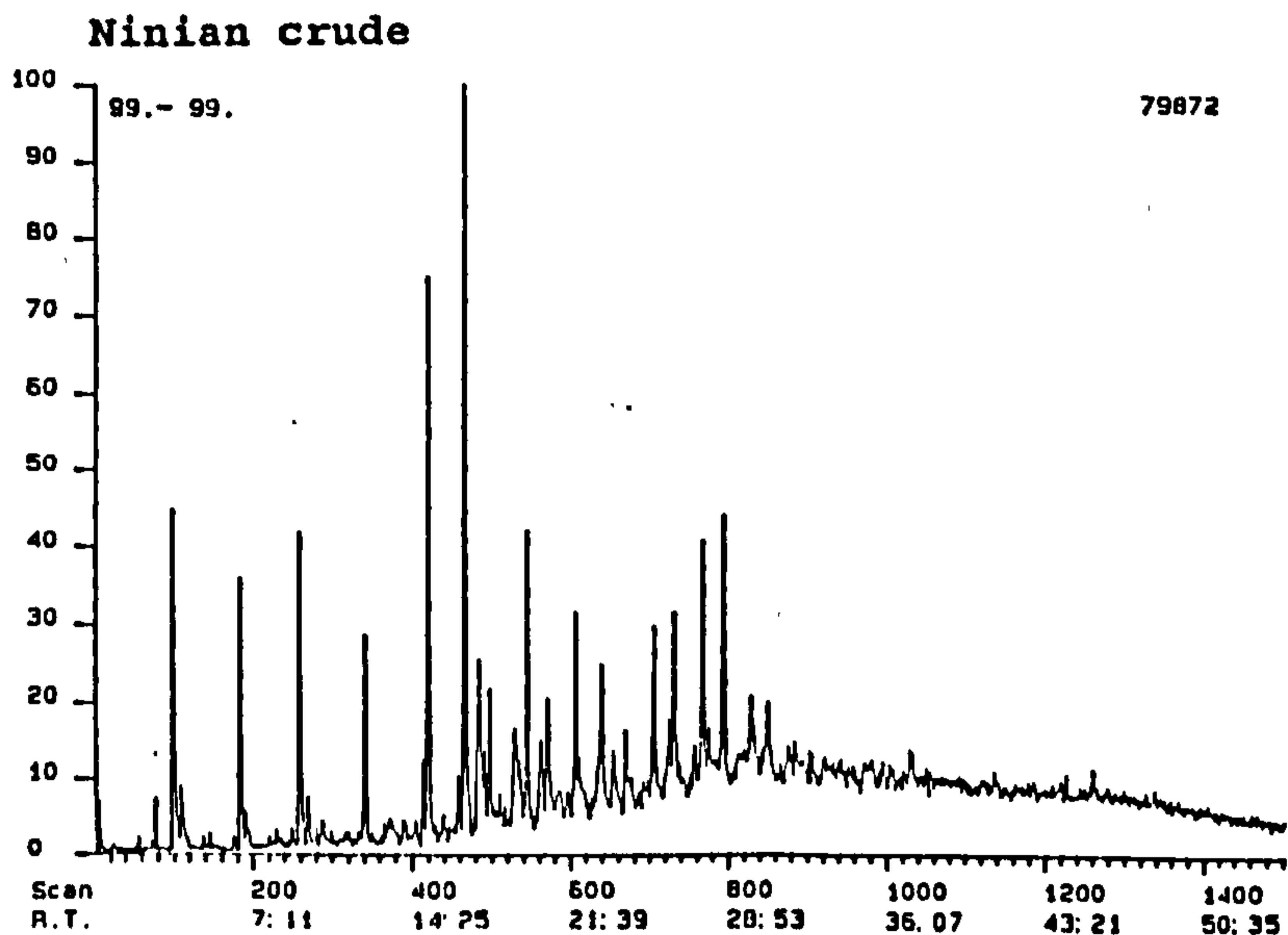
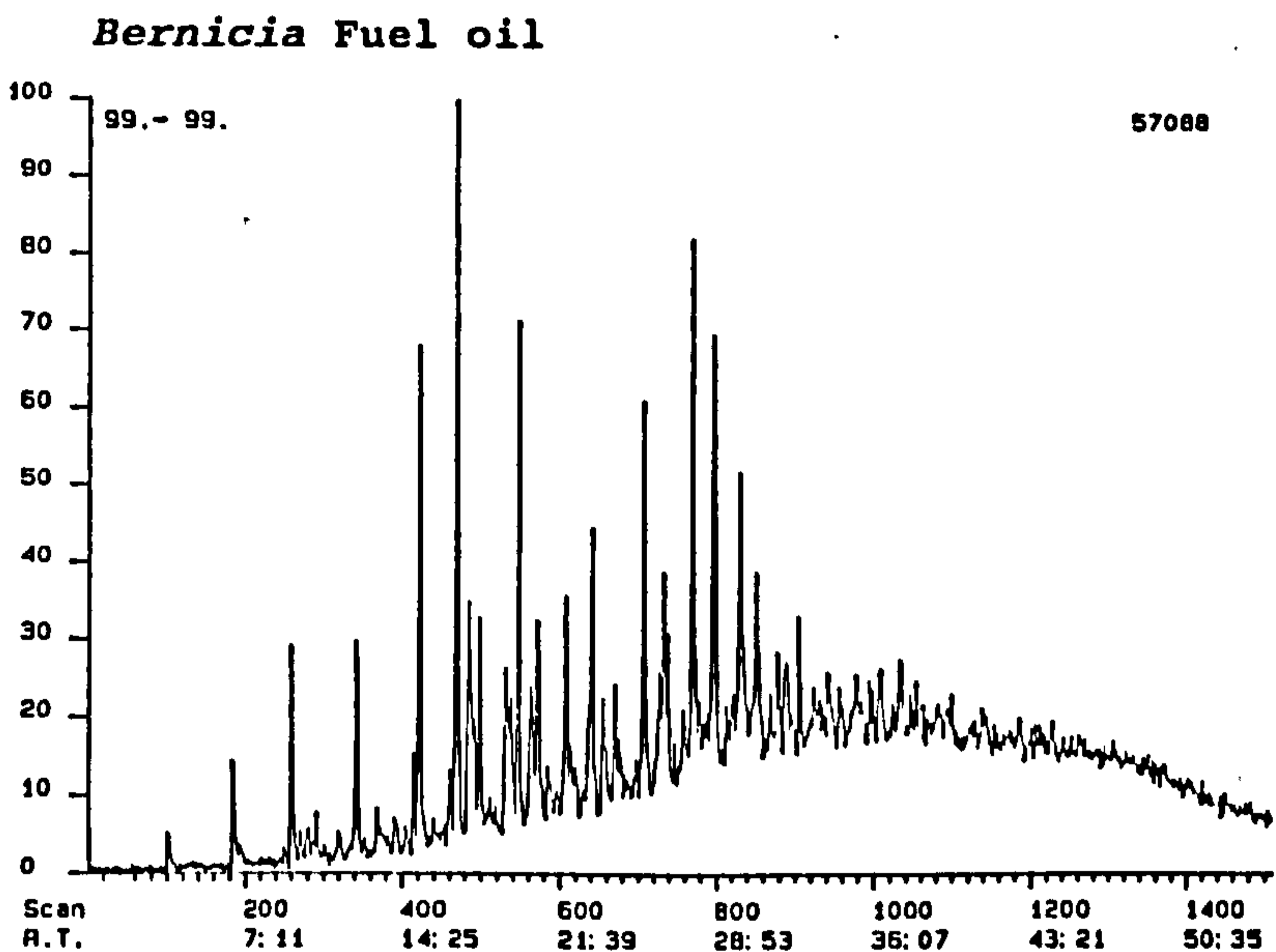
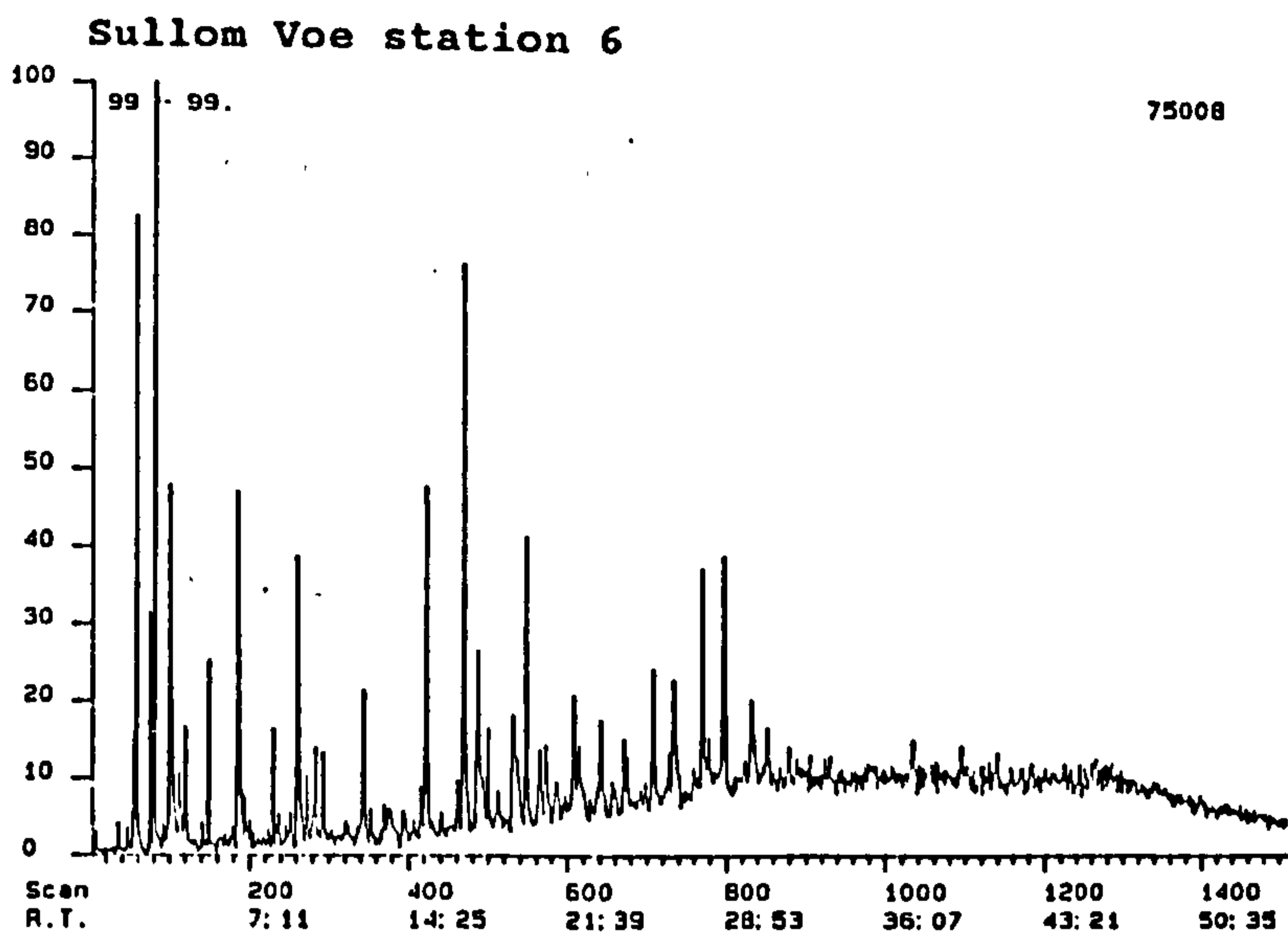


FIGURE 4.19. M/Z 74 CHROMATOGRAM FOR SULLOM VOE UCM CrO<sub>3</sub> OXIDATION PRODUCTS (J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas)



**FIGURE 4.20. M/Z 58 CHROMATOGRAM FOR SULLOM VOE UCM  
CrO<sub>3</sub> OXIDATION PRODUCTS  
(J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas)**



**FIGURE 4.21. M/Z 99 CHROMATOGRAM FOR SULLOM VOE UCM  
CrO<sub>3</sub> OXIDATION PRODUCTS  
(J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas)**



## 4.4.2 MERSEY OIL SPILL

### 4.4.2.1 INTRODUCTION

On the 19 August 1989, there was an accidental spillage of 150 tonnes of Tia Juana Pesado (TJP; Venezuela) heavy asphaltic crude oil from the Shell refinery at Stanlow, Elsmere Port into the Mersey Estuary (Davies and Wolff, 1990). Since TJP crude is a UCM-rich degraded oil, this spill provided a good test of the use of the UCM "fingerprinting" method.

### 4.4.2.2 GRAVIMETRY AND GAS CHROMATOGRAPHY

Gravimetric data for the oil and sediment (Dungeons Lane, 0-3 cm) are given in Table 4.6. The sediment contained 1331  $\mu\text{gg}^{-1}$  dry weight of hydrocarbons of which 55% was saturated hydrocarbons and 45% aromatic hydrocarbons. Davies and Wolff (1990) found concentrations of 80-400  $\mu\text{gg}^{-1}$  dry weight in sediments from nearby Widnes, collected a day after the TJP spill. These concentrations can be compared to values of ca. 100  $\mu\text{gg}^{-1}$  dry weight from Mersey (Eastham Ferry) sediments collected in 1984 (Readman et al., 1986) and a range of 5-100  $\mu\text{gg}^{-1}$  for estuarine sediments in general (Farrington and Meyers, 1975). Thompson and Eglinton (1978) reported ca. 100  $\mu\text{gg}^{-1}$  dry weight in the Severn Estuary, whilst lower levels (10 and 42  $\mu\text{gg}^{-1}$ ) were reported in the less industrialised Dee and Tamar Estuaries

**TABLE 4.6: CONCENTRATION OF HYDROCARBONS IN TJP OIL (% OF TOTAL OIL) AND MERSEY SEDIMENTS ( $\mu\text{g g}^{-1}$  DRY WEIGHT)**

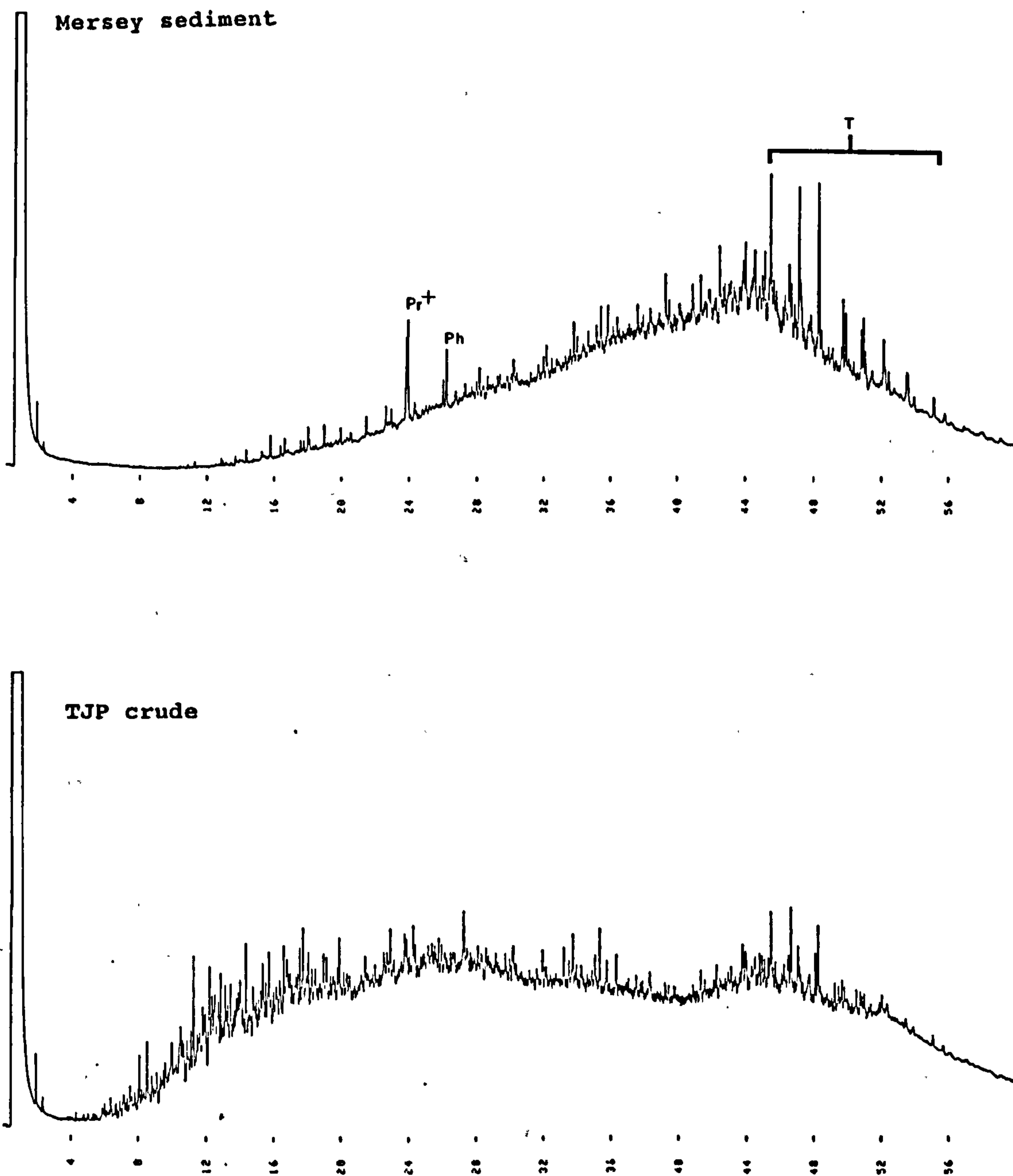
<u>FRACTION</u>	<u>SAMPLE</u>	
	TJP Crude	Mersey Sediment
Total organic extract	-	2669
Total saturates	23	730
Total aromatics	27	601

(Readman et al., 1986).

In contrast, concentrations of hydrocarbons in oil polluted sediments range at least up to 12400  $\mu\text{g g}^{-1}$  dry weight (Farrington and Meyers, 1975) and levels of 53660  $\mu\text{g g}^{-1}$  were reported in Humber Estuary sediments 5 months after a spill of 6000 tonnes of Nigerian crude oils (Jones et al., 1986). However, in most of these studies the spilled oil was of "average" composition, i.e. ca. 86% hydrocarbons with 57% saturated hydrocarbons and 29% aromatic hydrocarbons (average of 517 crude oils, Tissot and Welte, 1984). In contrast, TJP is a heavy asphaltic oil with a low hydrocarbon content (50%) and especially low saturated hydrocarbon content (23%; Table 4.7). This is typical of the heavy oils of Venezuela (Tissot and Welte, 1984; de Audemard et al., 1987) which characteristically have high asphaltene, resin and NSO content.

Thus, although "oiling" of sediments is often characterised by an elevation of the saturated hydrocarbon concentration, this may not be the case when the oil is a heavy asphaltic oil such as the TJP. This may explain why Davies and Wolff (1990) were unable to detect TJP crude residues in a freshly oiled Mersey sediment, even though the oil was visible.

GC analysis of saturated hydrocarbons from both the Mersey sediment and TJP crude exhibited predominant UCMs (Figure



**FIGURE 4.22. GAS CHROMATOGRAMS OF MERSEY SEDIMENT AND TIA JUANA PESADO CRUDE ALIPHATIC FRACTIONS**  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)  
 (Pr<sup>+</sup> = pristane + co-eluting compounds, Ph = phytane  
 T = triterpane compounds)

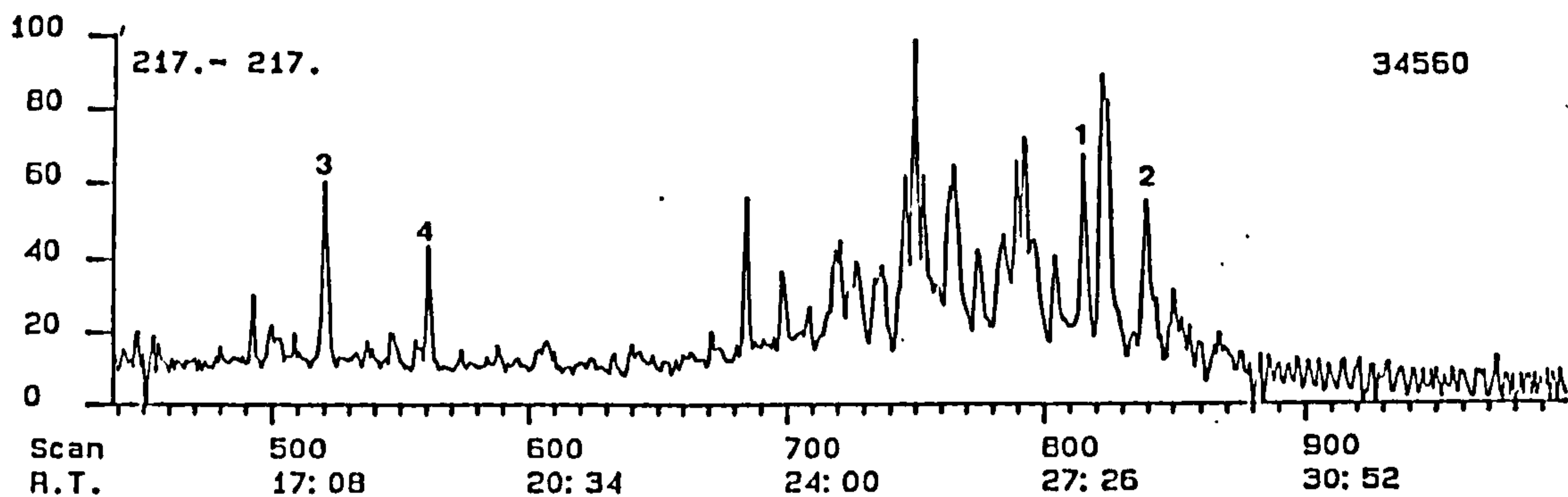


4.22) with a few resolved components which are mainly terpanes (identified by relative retention times). Otherwise, the two UCM profiles were very different.

#### 4.4.2.3 GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Qualitatively the distributions of steranes were similar in both oil and sediment (Figures 4.23 and 4.24). Which is not entirely unexpected since both have been biodegraded. For example the  $C_{29}\alpha\alpha$  20S and 20R sterane distribution (Figure 4.23; peaks 1 and 2 respectively) has been altered such that the more readily biodegraded 20R isomer (peak 2) has been reduced from the usual 60% abundance (relative to total R and S) to ca. 48%. However, there are also quantitative differences in the ratio of certain low molecular weight steranes (peaks 3 and 4; Figure 4.23) compared to the higher molecular weight steranes (e.g. peaks 1 and 2) which are reflected in the absolute concentrations of these compounds (Davies and Wolff, 1990). This suggests that the sterane profile of the sediment is not dominated by a contribution from the TJP crude. This is confirmed by the triterpane distributions (Figures 4.25 and 4.26) which show significant differences between the sediment and crude, notably an enhanced tricyclic terpane content (e.g. peak 1; Figure 4.25) and an unidentified component (peak 2) in the oil which is not detected in the sediment (see also Davies and Wolff, 1990). The concentration of demethylated triterpanes (Figure 4.26) is

Mersey sediment



TJP crude

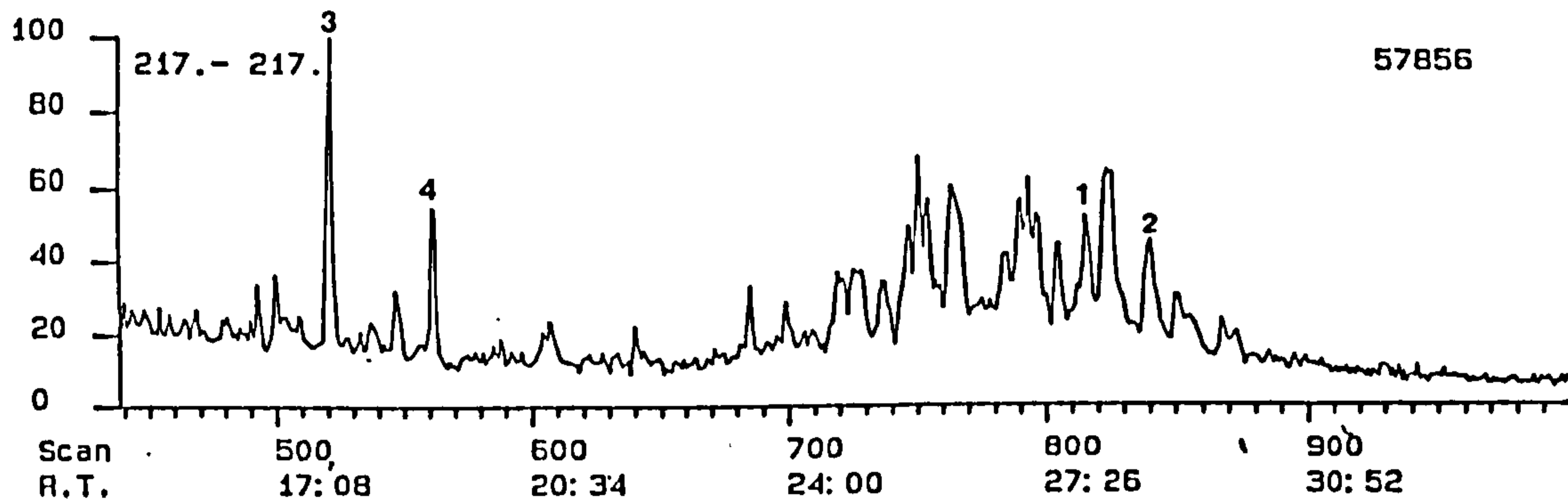
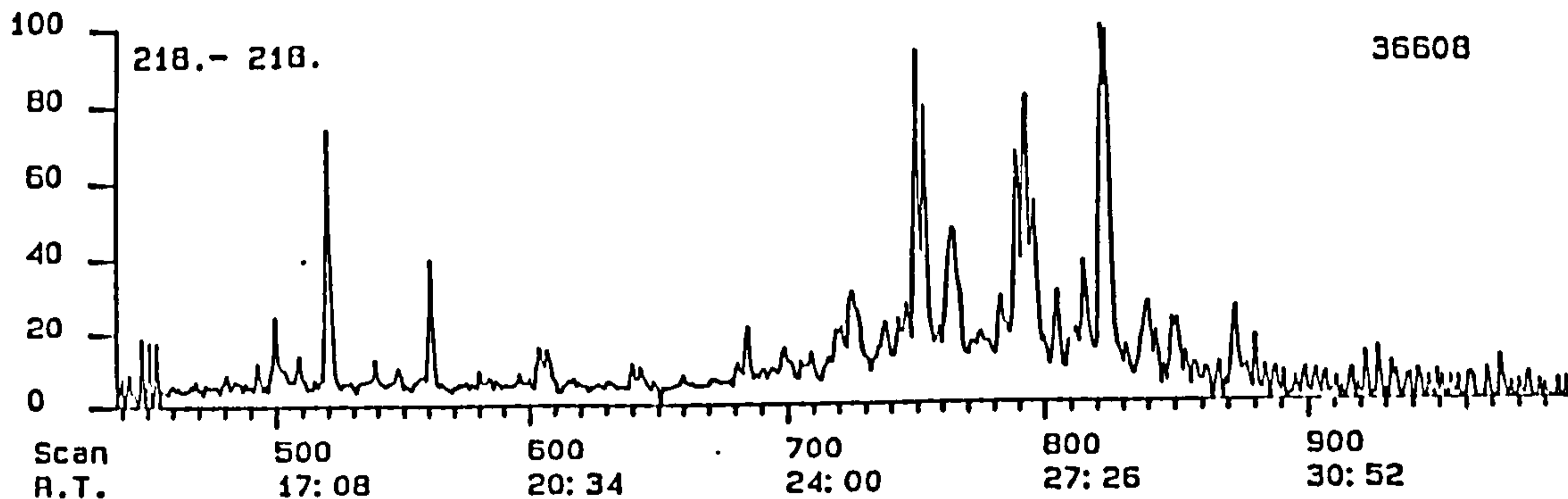


FIGURE 4.23. M/Z 217 MASS CHROMATOGRAM FOR MERSEY SEDIMENT AND TJP CRUDE ALIPHATIC FRACTIONS (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)

Mersey sediment



TJP crude

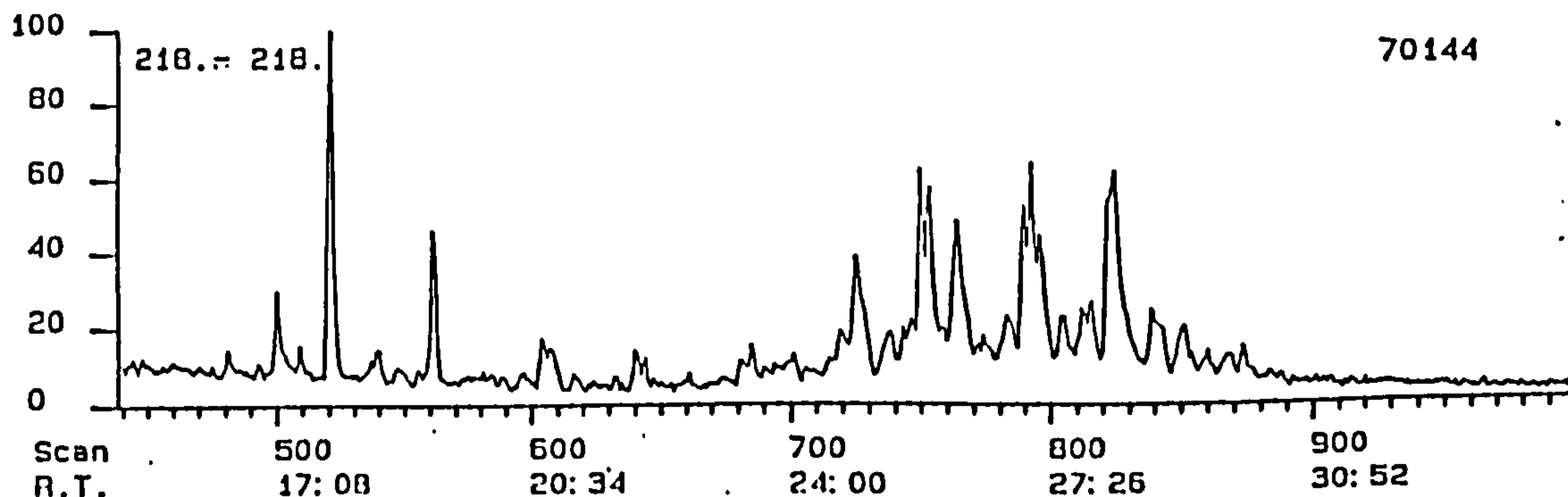
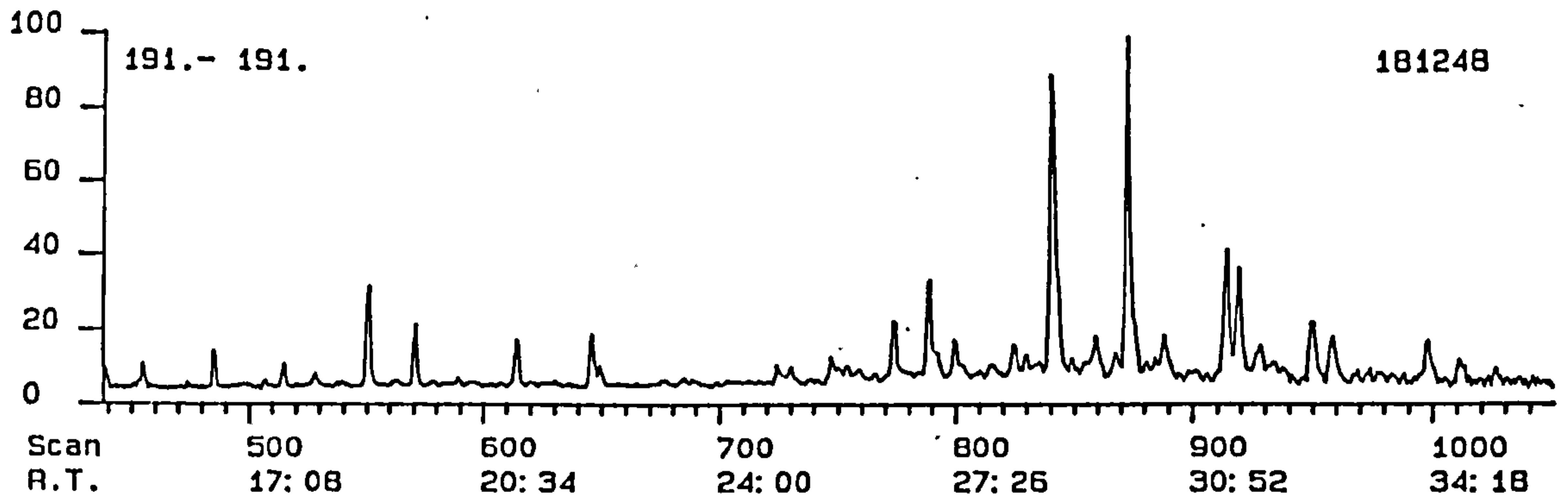


FIGURE 4.24. M/Z 218 MASS CHROMATOGRAM FOR MERSEY SEDIMENT AND TJP CRUDE ALIPHATIC FRACTIONS (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)

Mersey sediment



TJP crude

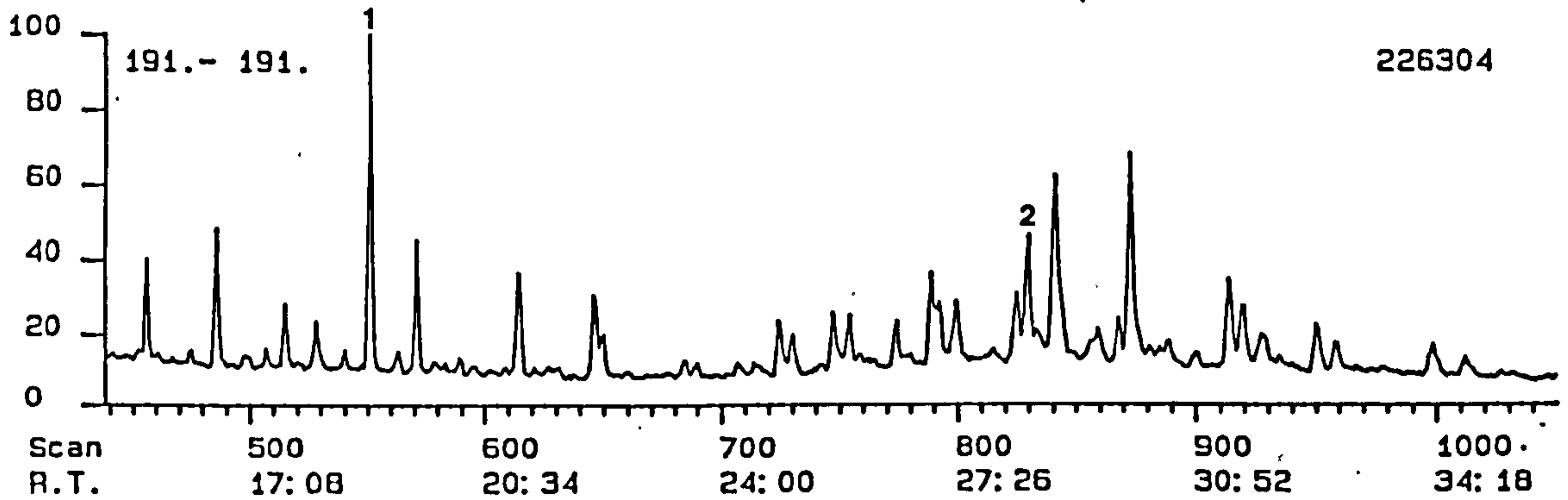
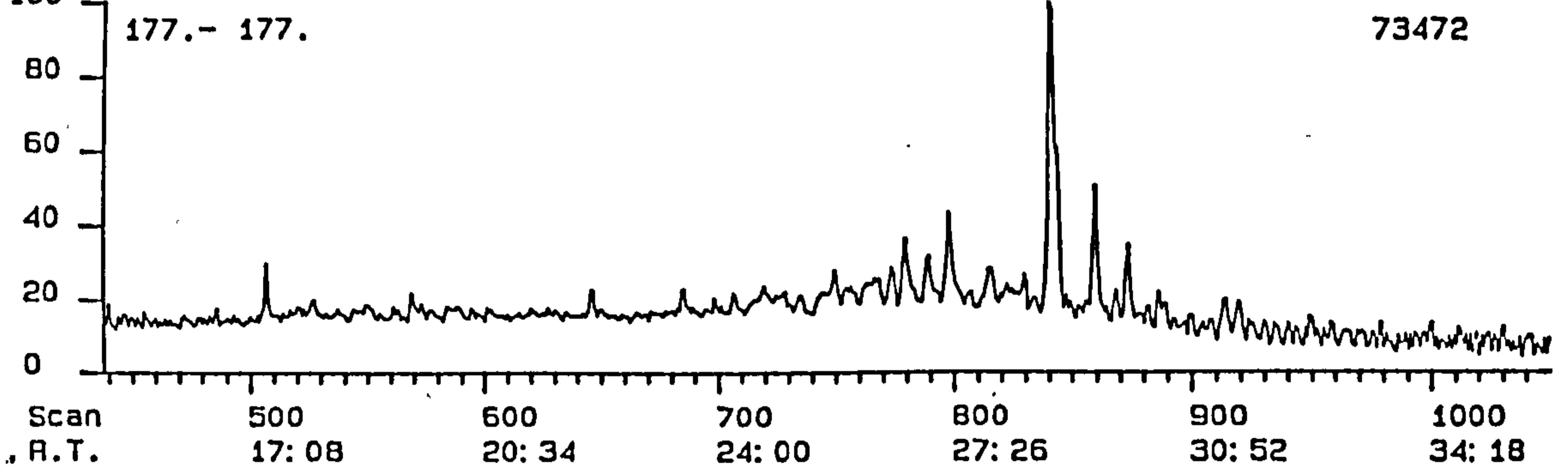


FIGURE 4.25. M/Z 191 MASS CHROMATOGRAM FOR MERSEY SEDIMENT AND TJP CRUDE ALIPHATIC FRACTIONS (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)

Mersey sediment



TJP crude

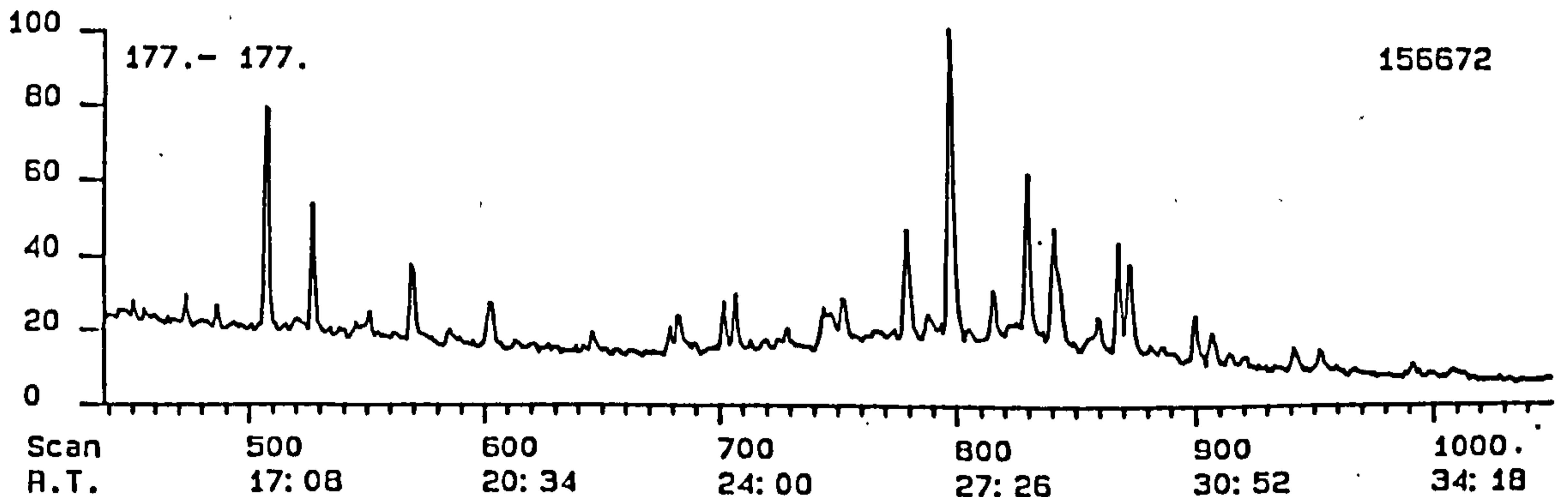


FIGURE 4.26. M/Z 177 MASS CHROMATOGRAM FOR MERSEY SEDIMENT AND TJP CRUDE ALIPHATIC FRACTIONS (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)



also higher in the crude (Davies and Wolff, 1990; a feature typical of biodegraded oils).

Thus the biomarker evidence is inconclusive but suggests that TJP hydrocarbons have not made a major contribution to the sediment. In order to provide complementary data, an examination of the sediment and oil UCMs was made.

#### 4.4.2.4 OXIDATIVE DEGRADATION

Oxidative degradation of TJP crude and sediment saturated hydrocarbons (both 80% unresolved) produced the yields shown in Table 4.7. Also included is data for the hydrocarbon feedstock of TJP lubricating oil (Shell naphthenic lubricating oil, Gough, 1989) since this is refined on Merseyside. Most of the UCM hydrocarbons in the sediment are less readily oxidised than those in either oil (40% *cf.* ca. 70%). If all the oxidisable (40%) UCM hydrocarbons in the sediment were from TJP crude then a maximum of 57% of the saturated hydrocarbons in the sediment could originate from TJP crude. There is no evidence for this from GC and GCMS, but these may not be good guides due to the low hydrocarbon content of TJP. When equal amounts of saturated hydrocarbons from TJP crude and the sediment were mixed and analysed by GC, the resulting profile was almost identical to that of the original sediment (Figure 4.27). This infers that GC alone could not reveal even this level of contamination.

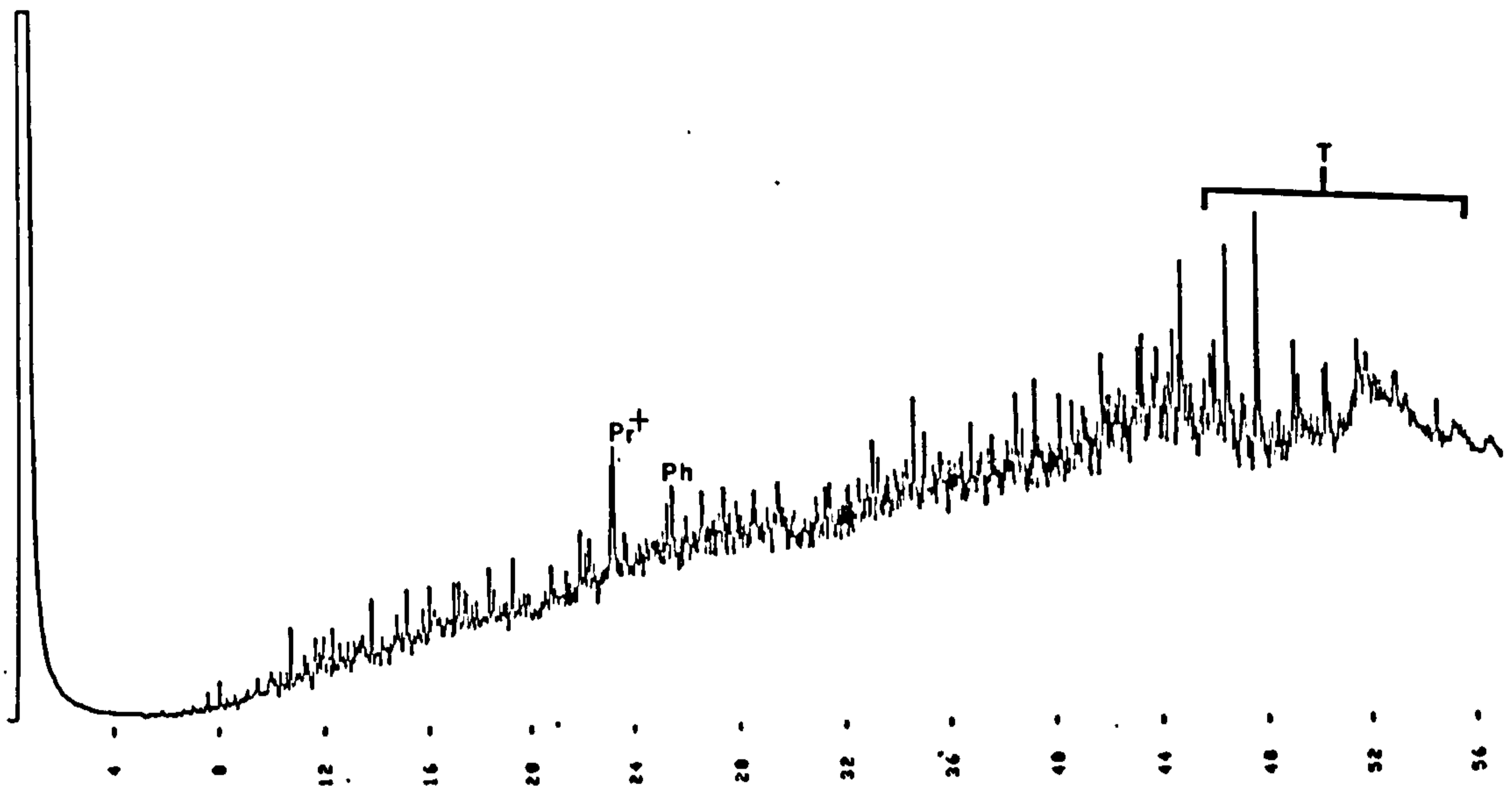


**TABLE 4.7: OXIDATION PRODUCT YIELDS FROM TJP OILS AND MERSEY SEDIMENTS**

<b>SAMPLE</b>	<b>% SATURATES IN ORIGINAL SAMPLE</b>	<b>TOTAL YIELD FROM OXIDATION (%)</b>	<b>ISOLATED<sup>a</sup> OXIDATION PRODUCTS (%)</b>
Sediment	55	95	40
TJP crude	23	87	71
TJP lube oil <sup>b</sup>	66	73	69

<sup>a</sup> Refers to that fraction not eluting with hexane from column chromatography and includes acids, ketones and lactones

<sup>b</sup> Gough and Rowland (1990); Gough (1989).



**FIGURE 4.27. GAS CHROMATOGRAM OF A MIXTURE OF THE ALIPHATIC HYDROCARBONS FROM MERSEY SEDIMENT (50%) AND TJP CRUDE (50%)**  
 (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)  
 (Pr<sup>+</sup> = pristane + co-eluting compounds, Ph = phytane  
 T = triterpane compounds)

Visual examination of the oxidation product profiles of TJP crude and the sediment by mass chromatography showed the two profiles to be qualitatively similar to each other (Figures 4.28 and 4.29) and similar to that of the TJP lubricating oil (Gough, 1989).

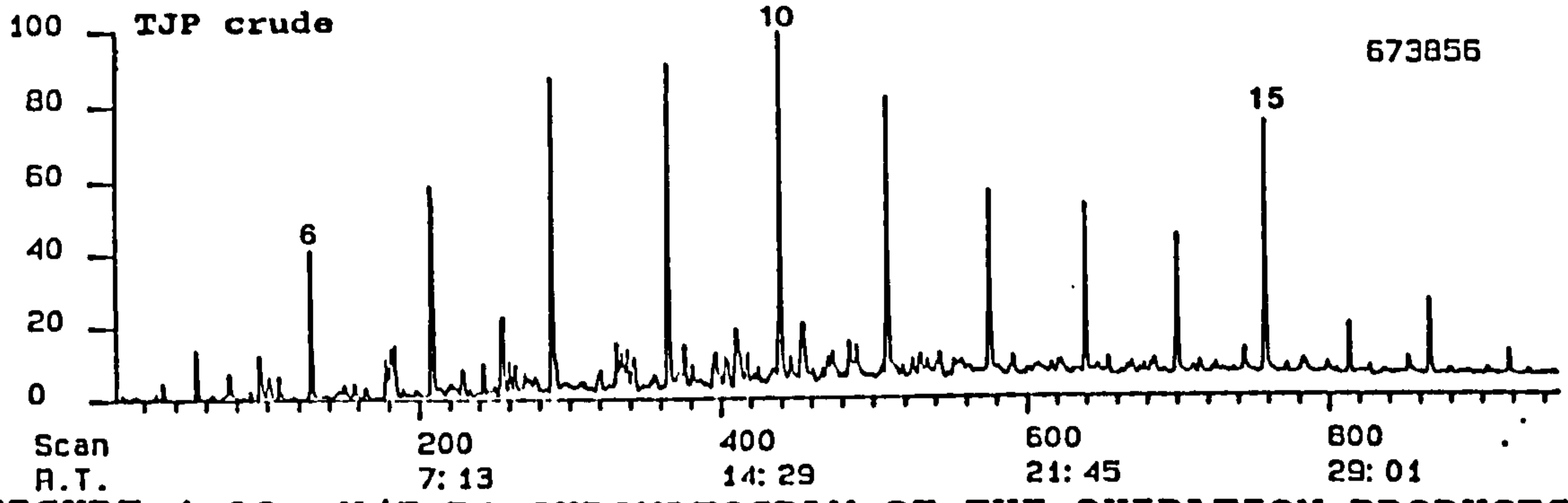
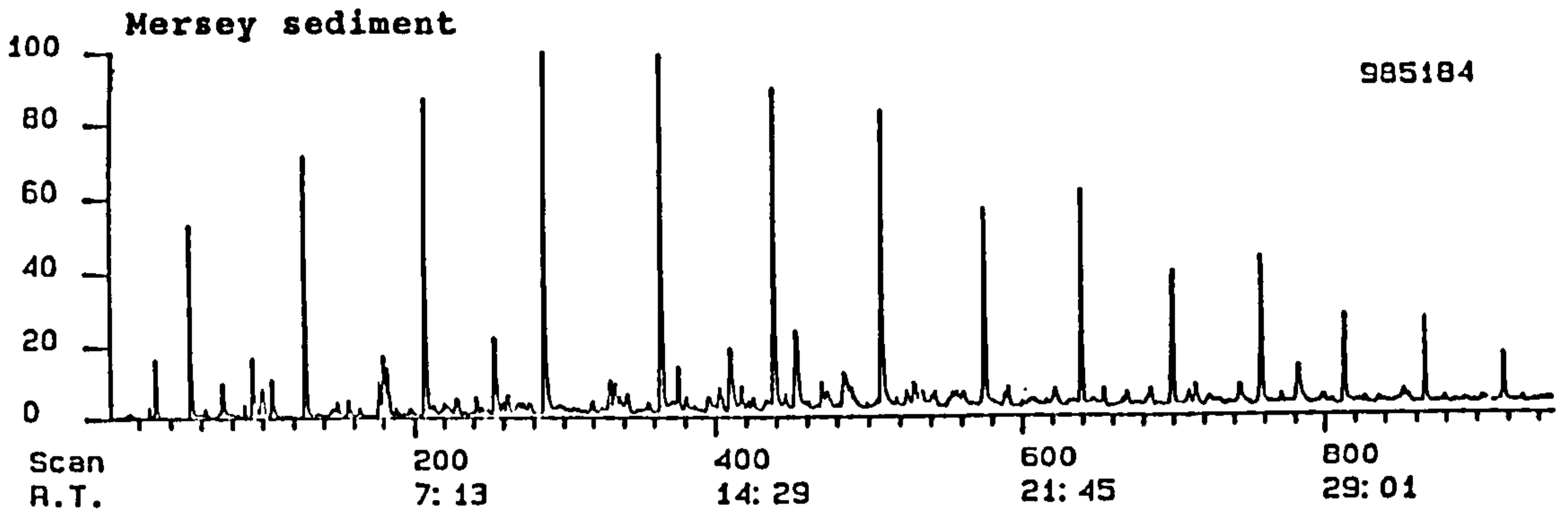
The data were then subjected to more rigorous statistical analyses in conjunction with the data from the Sullom Voe case study and, in order to expand the data base additional sets of samples were analysed. These included duplicate oxidations of 2 mg samples of Silkolene lubricating oil, an oxidation of 50 mg of the same oil, oxidation of two acute oil spills studied by Gough (1989) and Gough and Rowland (1990) and a repeat GCMS analysis of one of the samples. Gravimetric data for all the samples included in this data set are given in Table 4.8.

#### **4.4.3 STATISTICAL ANALYSIS OF UCM OXIDATION DATA**

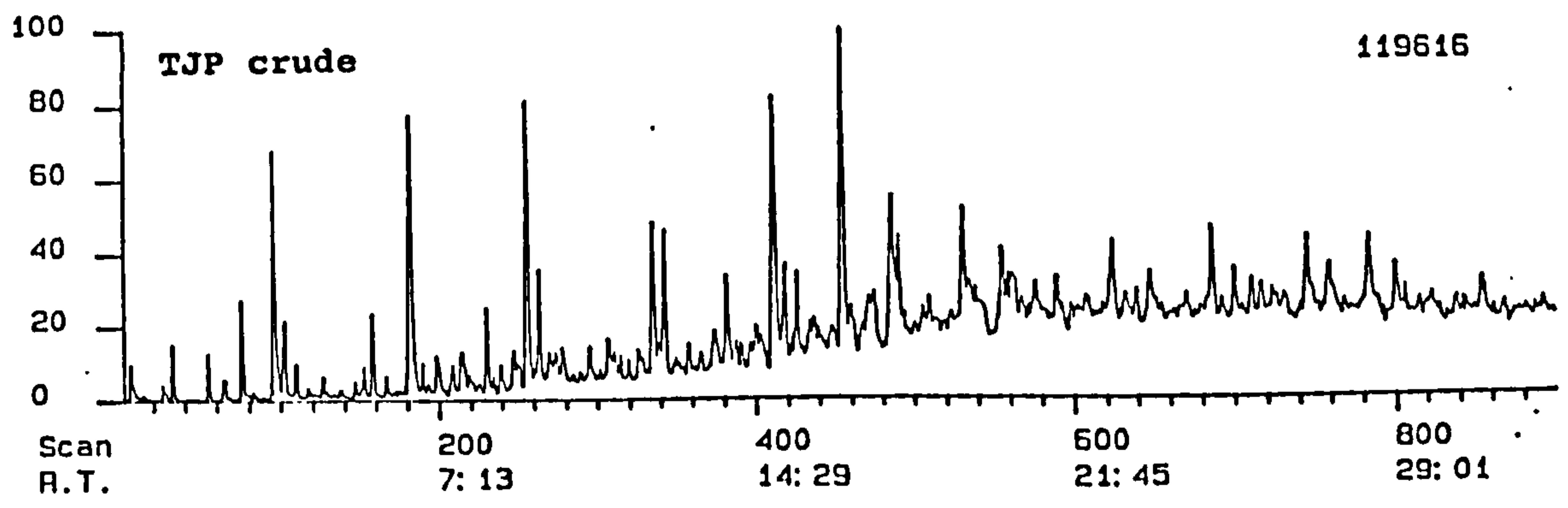
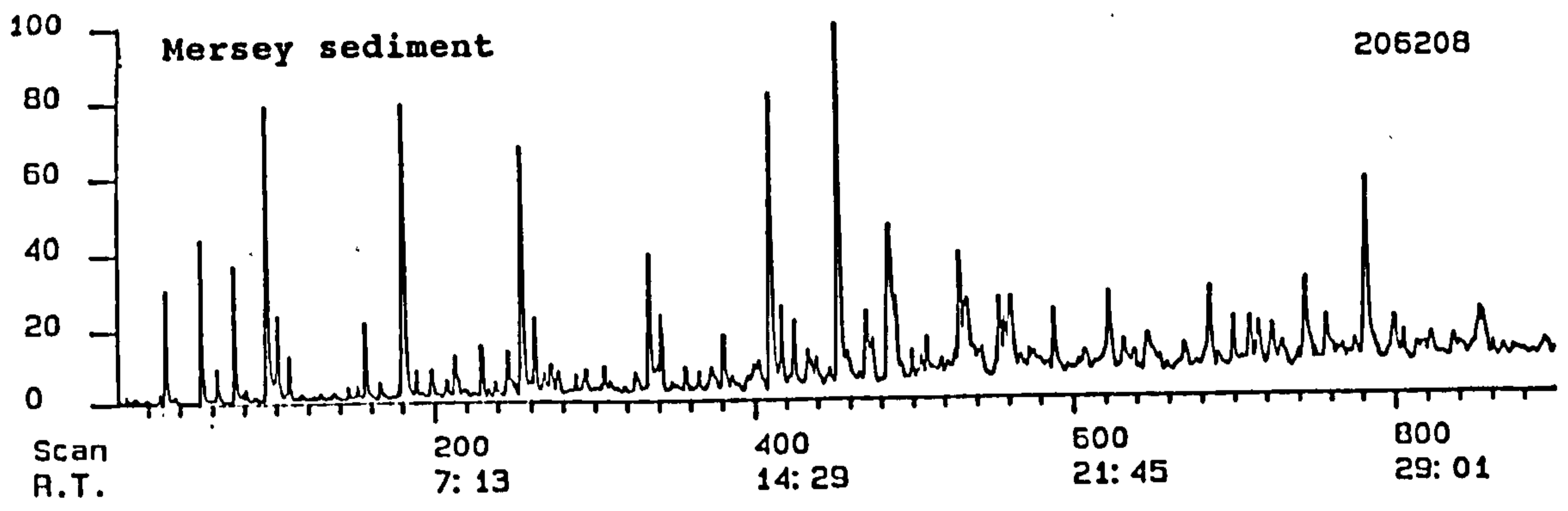
##### **4.4.3.1 CLUSTER ANALYSIS AND MULTIDIMENSIONAL SCALING**

Each compound class in the oxidation product profiles was quantified, the resulting data analysed by the DB-MATCH program and then subjected to cluster analysis and MDS. The results of this are shown in two ways,

- (i) a hierarchical dendrogram which indicates groups of samples and the degree of their association,



**FIGURE 4.28. M/Z 74 CHROMATOGRAM OF THE OXIDATION PRODUCTS OF MERSEY SEDIMENT AND TJP CRUDE UCMS (J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas) (numbers indicate n-acid chain length)**



**FIGURE 4.29. SUMMED M/Z 58 + 99 CHROMATOGRAM OF THE OXIDATION PRODUCTS OF MERSEY SEDIMENT AND TJP CRUDE UCMS (J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas)**



**TABLE 4.8: GRAVIMETRIC DATA FOR SAMPLES ANALYSED BY MULTIVARIATE STATISTICS**

No.	SAMPLE	ALKANES (%)	UCMs (%)	TOTAL Yield (%)	OXIDISED Yield (%)
1,2	Silkolene 150 (2mg)	78	86	94	-
3	Silkolene (50mg)	79	86	95	71
4	Amoco tank oil	16	68	87	-
5	Newgale beach oil	16	21	75	-
6	Sivand cargo	55	76	83	-
7	Humber sediment	43	87	83	-
8	Mersey sediment repeat GCMS analysis				
9	Sullom Voe sediment	66	9	95	70
10	Ninian crude oil	52	31	89	55
11	Bernicia fuel oil	28	62	84	69
12	Mersey sediment	55	80	95	40
13	TJP crude oil	22	85	87	71

and,

(ii) a two dimensional plot of samples which indicates the relative positions of the samples. This can show both how samples within a group are related and how close each sample is to the next group.

Thus both forms of data presentation complement each other and therefore, should be studied at the same time.

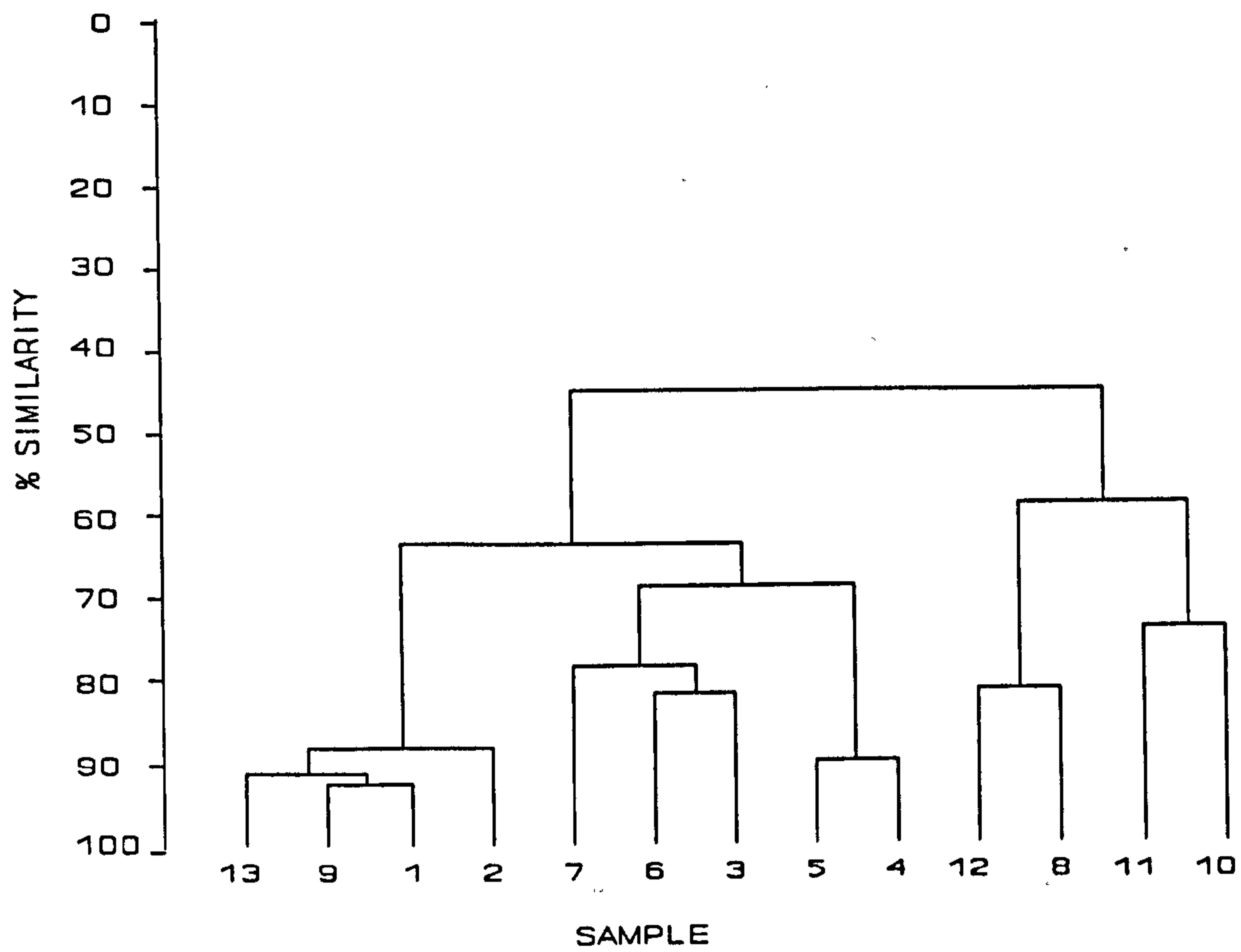
A technique often used in statistical analyses of this type is standardisation of the data (Smith, 1991). This involves calculating the mean and standard deviation for each variable and dividing each number by one of them or by subtracting the mean and dividing by the standard deviation. The aim of this treatment of the data is to prevent any one variable having a disproportionately large effect upon the end result. However, it is unclear if a data set with only thirteen samples (including duplicates) is large enough for this to work and results from this study suggest not (see end of this section). Thus the initial analyses were performed without standardisation.

#### *Samples 1-3: Reproducibility Study*

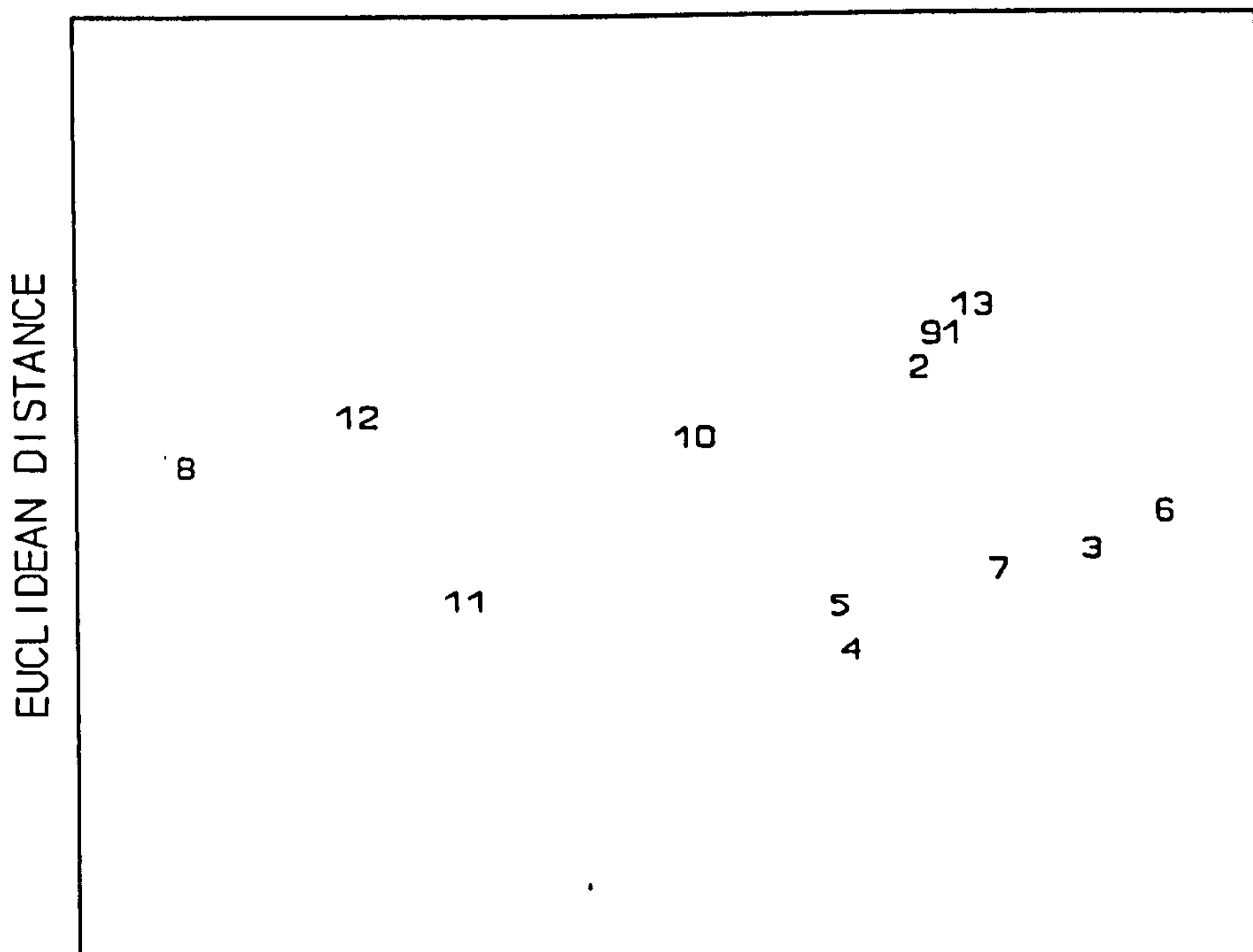
The two oxidations of 2 mg of Silkolene lubricating oil (samples 1 and 2, Figure 4.30) show that the reproducibility of the method at this level is in the region of >88%, though further replication would be

**KEY TO SAMPLE NUMBERS IN FIGURE 4.30**

No.	SAMPLE
1	Silkolene 150 (2mg)
2	Silkolene 150 (2mg)
3	Silkolene 150 (50mg)
4	Amoco tank oil
5	Newgale beach oil
6	Sivand cargo
7	Humber sediment
8	Mersey Sediment repeat GCMS analysis
9	Sullom Voe sediment
10	Ninian crude oil
11	Bernicia fuel oil
12	Mersey sediment
13	TJP crude oil



EUCLIDEAN DISTANCE



**FIGURE 4.30. RESULTS OF CLUSTER AND MDS ANALYSES OF THE OXIDATION PRODUCTS FROM SEVERAL CASE STUDIES WITH NO STANDARDISATION (For sample identities see key opposite)**



desirable, this was precluded by time constraints. Comparison of these samples with the oxidation of 50 mg of the same oil (sample 3) show poor agreement. This is probably due to the different oxidising environment experienced by the substrate (viz. relatively more solvent in the 2 mg oxidation; see experimental).

#### *Samples 4 and 5: Amoco Tank Spill*

One of the oil spills investigated by Gough and Rowland (1990) was a well documented spillage of oil from an Amoco tank at Milford Haven and was chosen by those authors because the oil had a known source (thus the oil and sediment hydrocarbons can almost be regarded as duplicates). These samples cluster together with >90% similarity, well away from other samples (4 and 5; Figure 4.30).

#### *Samples 6 and 7: Humber Spill*

Gough and Rowland (1990) also studied a spill of Nigerian Crude oils from the tanker *Sivand* into the Humber estuary (samples 6 and 7). These showed a correlation of ca. 78%. The scale of dissimilarity is in agreement with previous studies of this oil spill (Jones et al., 1986) and suggests that there have been other inputs of hydrocarbons to the Humber sediment in addition to the *Sivand* oil.

### *Samples 9-11: Sullom Voe*

The Sullom Voe sediment (sample 9) clusters with the Silkolene lubricating oil samples (1 and 2) and the biodegraded TJP Crude (sample 13) but well away from the suspected sources (Ninian Crude, sample 10 and *Bernicia* fuel oil, sample 11). This suggests that the UCM in Sullom Voe sediments is most likely due to a chronic accumulation from weathered hydrocarbon sources such as the hydrocarbons in aqueous road run-off, which often exhibits a strong UCM (Bomboi and Hernandez, 1991). This result contradicts the biological marker data which indicated that the *Bernicia* fuel oil was a more likely source, though this evidence was not conclusive (see Section 4.4.1.3).

### *Samples 8, 12 and 13: Mersey Oil Spill*

The samples from the Mersey spill (12 and 13) were not similar (<50%) and this is in agreement with the GC and GCMS results (Sections 4.4.2.2 and 4.4.2.3; Davies and Wolff, 1990). These showed the UCM distributions to be very different and that the oil contained relatively high proportions of tricyclic triterpenoids and demethylated hopanes compared with the sediment. Interestingly, this method fails to provide any evidence for the presence of TJP Crude in the Mersey sediment, yet detected the presence of the *Sivand* oil in the Humber sediments which are also known to already contain a hydrocarbon burden (Readman et

al., 1986). This emphasises the effect of the background hydrocarbon loading of the Mersey sediments on the relatively low saturated hydrocarbon content of TJP crude.

Repeat GCMS analysis of the same sample (samples 8 and 12; Figure 4.30) show the level of reproducibility to be 80% which is less than that for the two Silkolene oxidations and may suggest that this is a worst case example. If this is taken into account for the other samples, the existing clusters remain intact. The difference between samples 8 and 12 is likely to be a function of peak height integration. In conclusion, all samples known to be identical gave similarity values >80% and two known to be fairly similar (78%).

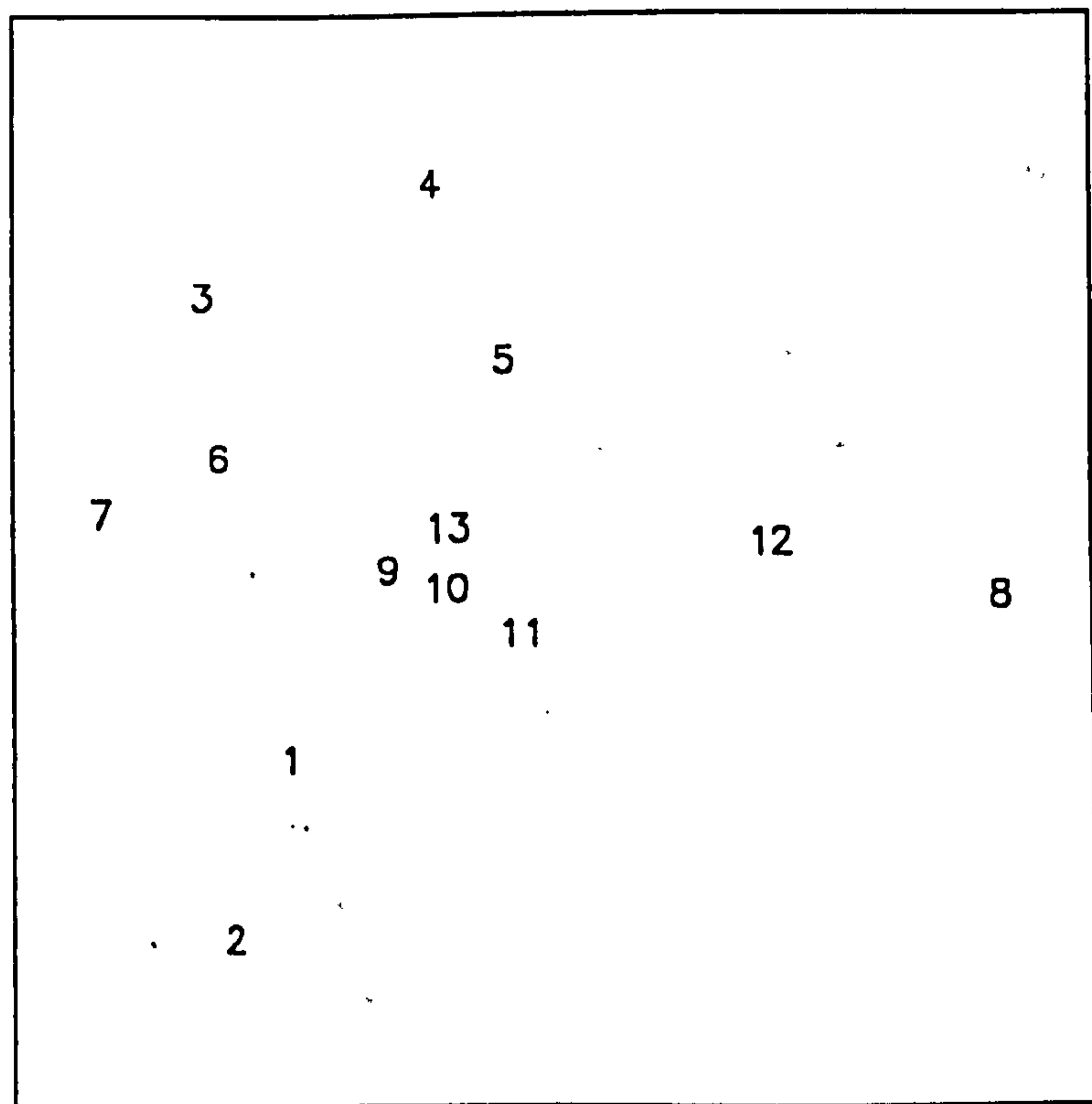
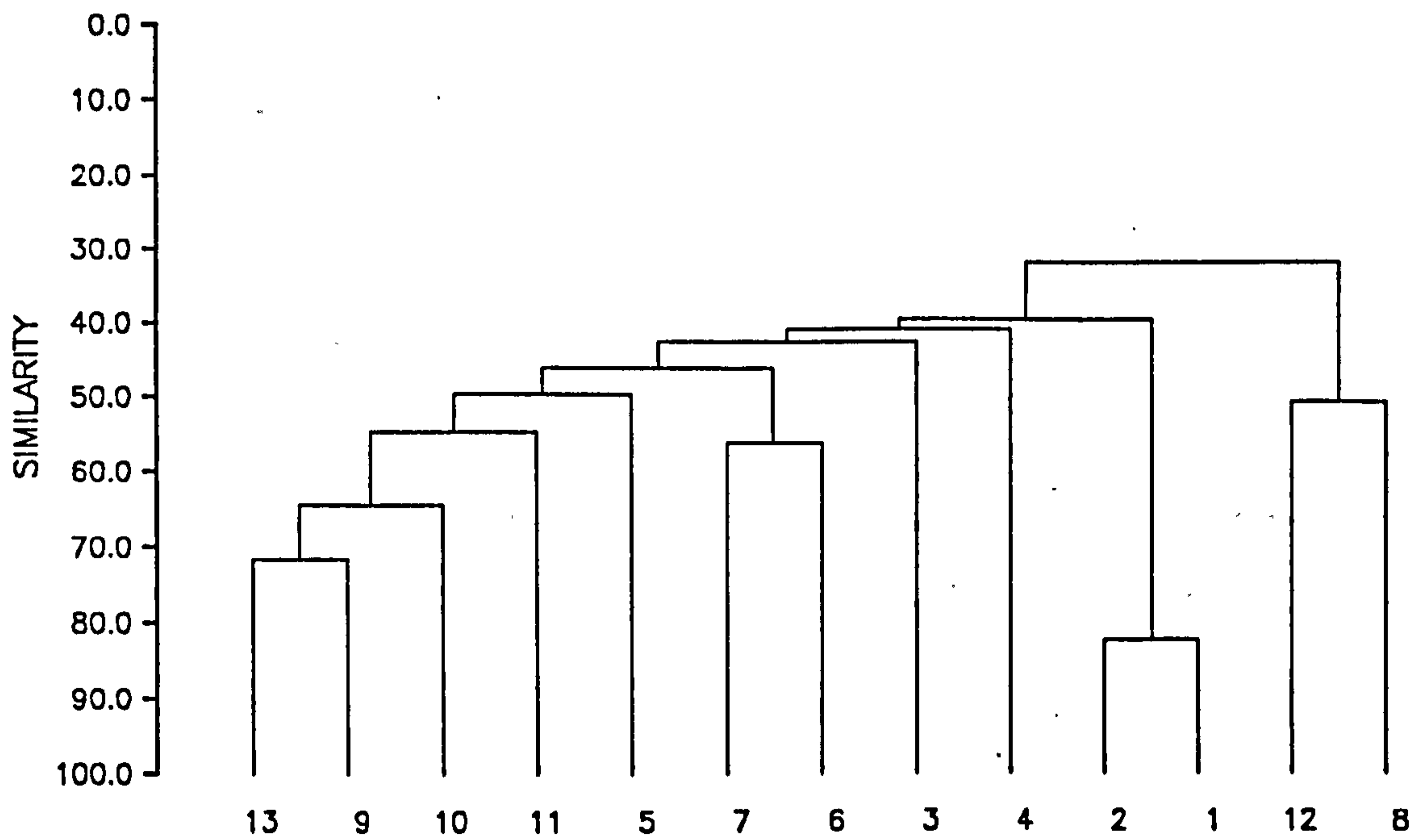
Subsequent re-analysis of the same data but after standardisation (division by the mean) produced results which appear to be less convincing (Figure 4.31). It is interesting that similar clusters still form within the data set, i.e. samples 1 and 2, 12 and 8, 6 and 7, although some are broken apart, e.g. samples 4 and 5; however each cluster exhibits less similarity between individual members of the cluster. For example, in the analysis without standardisation, samples 1 and 2 were 90% similar (Figure 4.30) but this has decreased to 80% with standardisation (Figure 4.31). More strikingly, samples 4 and 5 have decreased from 90% to ca. 40% similarity. It would appear therefore, with the prior knowledge available for the



**KEY TO SAMPLE NUMBERS IN FIGURE 4.31**

<b>No.</b>	<b>SAMPLE</b>
1	Silkolene 150 (2mg)
2	Silkolene 150 (2mg)
3	Silkolene 150 (50mg)
4	Amoco tank oil
5	Newgale beach oil
6	Sivand cargo
7	Humber sediment
8	Mersey Sediment repeat GCMS analysis
9	Sullom Voe sediment
10	Ninian crude oil
11	Bernicia fuel oil
12	Mersey sediment
13	TJP crude oil





**FIGURE 4.31. RESULTS OF CLUSTER AND MDS ANALYSIS OF THE OXIDATION PRODUCTS FROM SEVERAL CASE STUDIES WITH STANDARDISATION OF THE DATA (for sample identities see key opposite)**

samples within the database, that, in this case, standardisation of the data does not help the analysis, presumably due to the relatively small number of samples.

#### 4.4.3.2 PRINCIPAL COMPONENTS ANALYSIS

As discussed in section 4.3 the small numbers of samples and large number of variables involved in the case studies make statistical analysis by MDS the most appropriate method for comparing the data. The foregoing section shows that this approach is valid, and gives good reproducibility. The advantages of the above approach include the provision of both a two dimensional distances plot and the cluster analysis dendrogram from which a quantitative measure (% similarity) of difference is obtained. However, one of the disadvantages of this method is that no measure of the relative influence of the different variables (*i.e.* *n*-acids, lactones and ketones) to the overall differences between samples is provided. This information is provided by PCA, where the effects are expressed by so-called "loading" values *i.e.* those variables with the greatest loading being responsible for the greatest difference between samples. However, PCA is usually deemed inappropriate for analysis of small sample sets (Tabachnick and Fidell, 1989).

It was, therefore, a considerable surprise when application of a PC-based PCA software package (UNSCRAMBLER II<sup>®</sup>) to the

environmental data set studied above (section 4.4) produced a score plot (Figure 4.32; cf. MDS distance plot) which was strikingly similar to the MDS analysis. The orientation of the MDS and PCA plots are different but the groupings of samples are remarkably similar. It is apparent therefore, unless this is an extreme coincidence, that the PCA package which uses a newly developed algorithm (Tyssø et al., 1987) is appropriate for this size and type of data set. The importance of this conclusion is that a measure of the relative influence of the variables could now be obtained (note, however, that the PCA analysis does not supplant MDS since no cluster analysis was available with the PCA package).

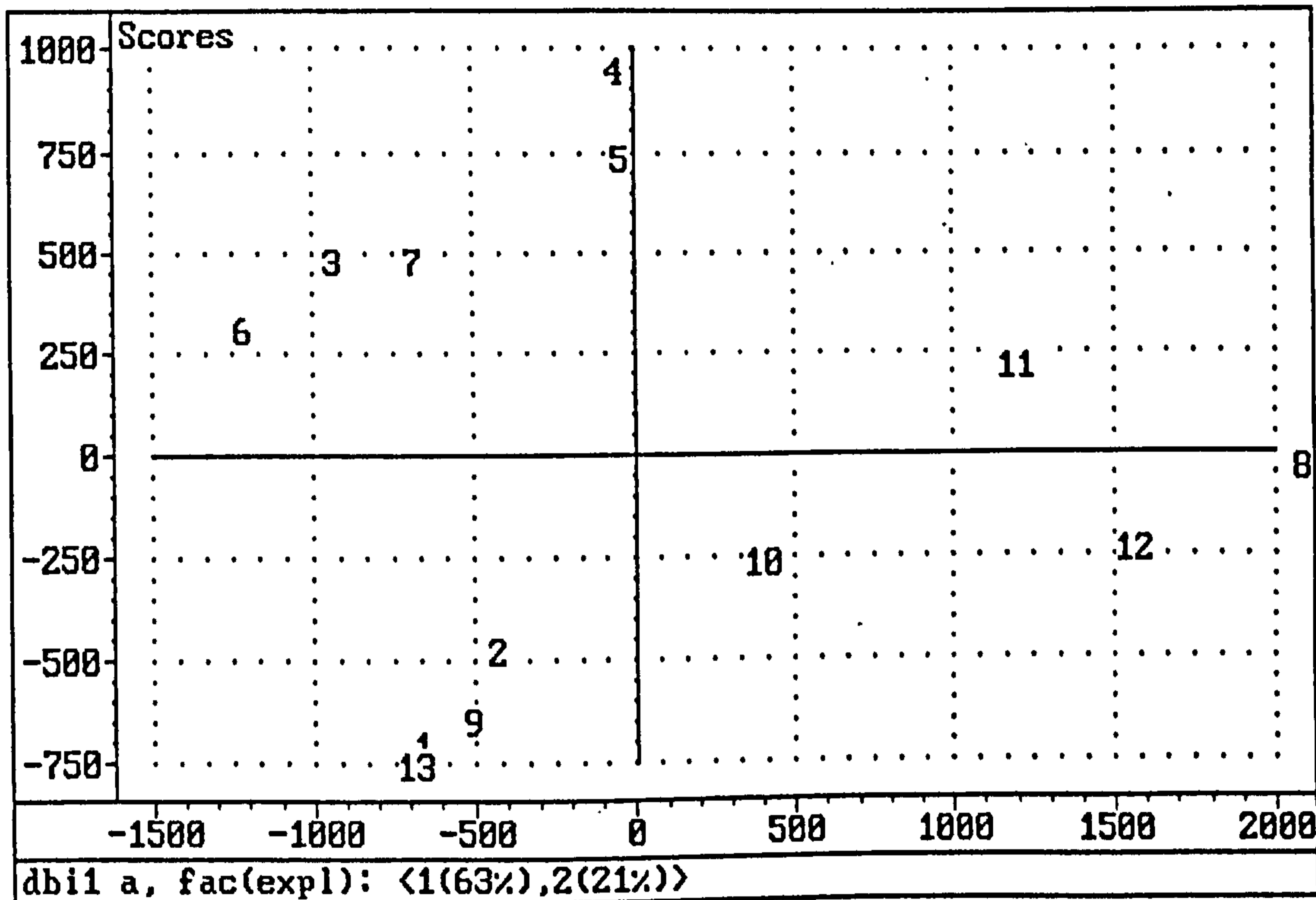
Table 4.9 shows that the range of acid concentrations is considerably greater than that of either the lactones or ketones. A small percentage change in the acid concentration therefore has quite a large influence on the MDS similarity measure (*i.e.* Euclidean distance) between samples. PCA of the data demonstrated this since PC1 and PC2 accounted for 84% of the total variation (PC1 = 63%). Within PC1 the greatest loading is found for the first 50 variables which are all *n*-acids. This is shown diagrammatically in Figure 4.33 where the magnitude of either positive or negative deviation on the y-axis indicates the influence of the variable on the difference between samples. It is clear that variables 1-50 (*n*-acids) have the greatest influence. The influence of PC2 is not

**KEY TO SAMPLE NUMBERS IN FIGURE 4.32**

No.	SAMPLE
1	Silkolene 150 (2mg)
2	Silkolene 150 (2mg)
3	Silkolene 150 (50mg)
4	Amoco tank oil
5	Newgale beach oil
6	<i>Sivand</i> cargo
7	Humber sediment
8	Mersey Sediment repeat GCMS analysis
9	Sullom Voe sediment
10	Ninian crude oil
11	<i>Bernicia</i> fuel oil
12	Mersey sediment
13	TJP crude oil



PCA



MDS

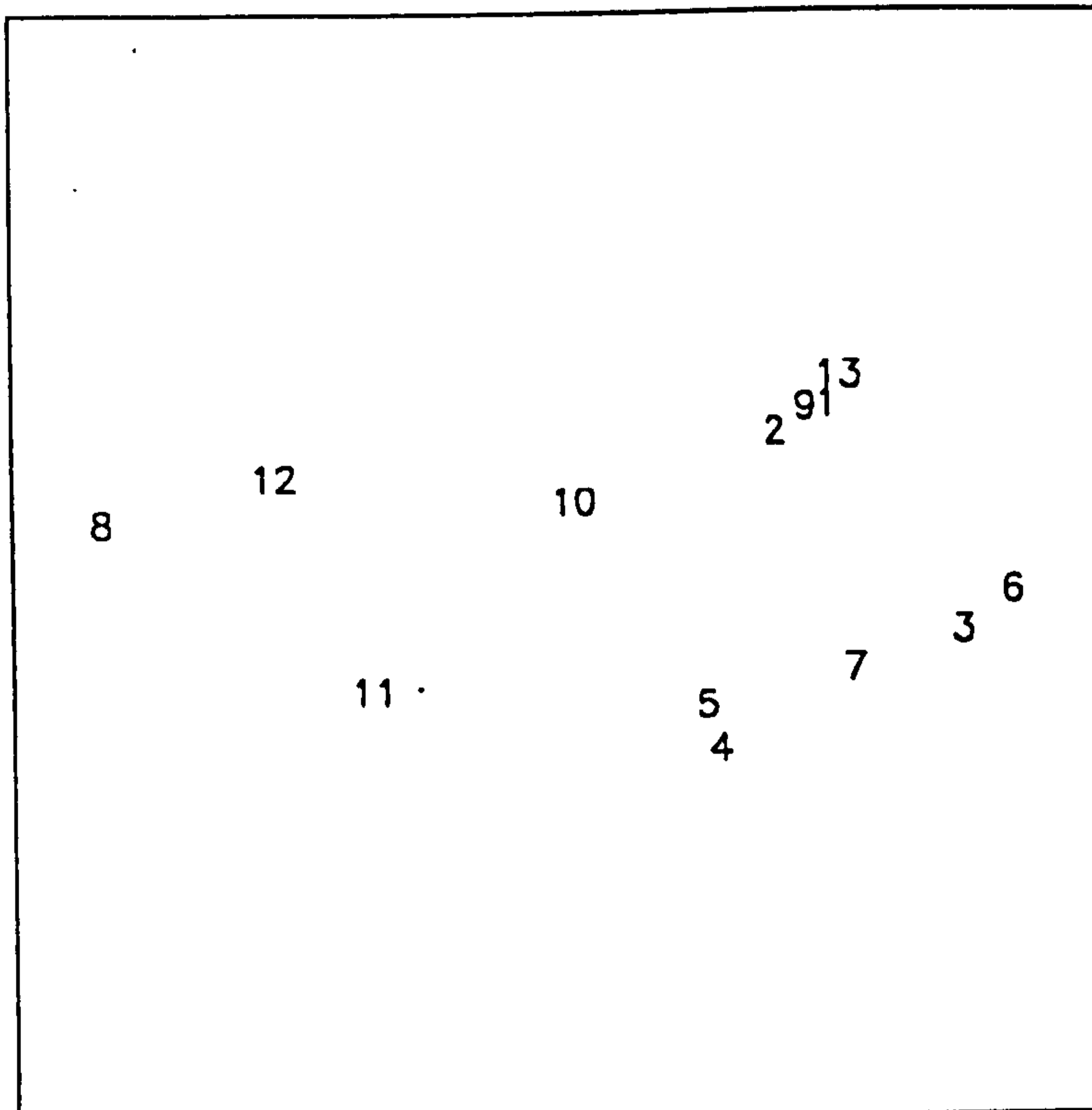


FIGURE 4.32. COMPARISON OF THE TWO DIMENSIONAL PLOTS PRODUCED BY MDS AND PCA (For sample identities see key opposite)

**TABLE 4.9: CONCENTRATION OF OXIDATION PRODUCT GROUPS ( $\mu\text{g mg}^{-1}$  OF OXIDATION PRODUCTS)**

SAMPLE	ACIDS	LACTONES	KETONES	ACIDS : LACTONES : KEIONES
Silkolene 2mg <sup>1</sup>	19.3	3.6	5.1	5.4 : 1.0 : 1.4
Silkolene 2mg <sup>2</sup>	25.5	4.8	7.6	5.3 : 1.0 : 1.6
Silkolene 50mg	34.1	6.4	5.1	6.7 : 1.3 : 1.0
Amoco tank oil	52.9	6.6	5.2	10.0 : 1.3 : 1.0
Newgale beach	46.4	7.2	3.7	12.5 : 1.9 : 1.0
Sivand cargo oil	30.3	7.7	5.6	5.4 : 1.4 : 1.0
Humber Estuary sediment	38.2	9.9	8.6	4.4 : 1.2 : 1.0
Sullom Voe station 6	21.4	2.3	2.3	9.3 : 1.0 : 1.0
Ninian crude	35.6	3.7	3.1	11.5 : 1.2 : 1.0
Berricia fuel oil	52.9	3.7	6.2	14.3 : 1.0 : 1.7
TJP crude	17.5	1.2	1.2	14.6 : 1.0 : 1.0
Mersey Estuary sediment	64.6	3.9	2.7	23.9 : 1.4 : 1.0
Mersey sediment repeat GCMS analysis	80.1	5.0	3.8	21.1 : 1.3 : 1.0

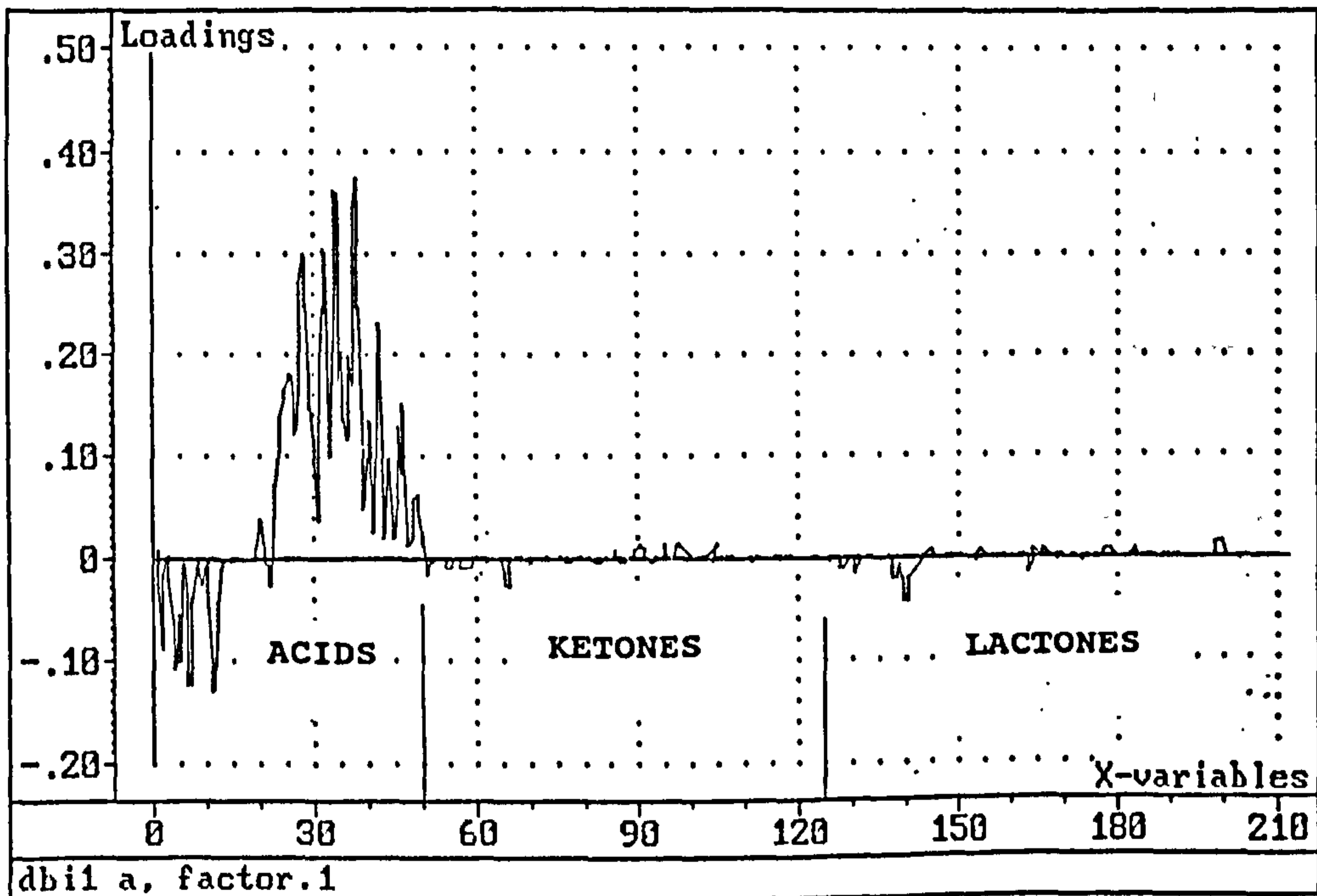


FIGURE 4.33. LOADINGS PLOT FOR PC 1 FROM PRINCIPAL COMPONENTS ANALYSIS OF THE DATA

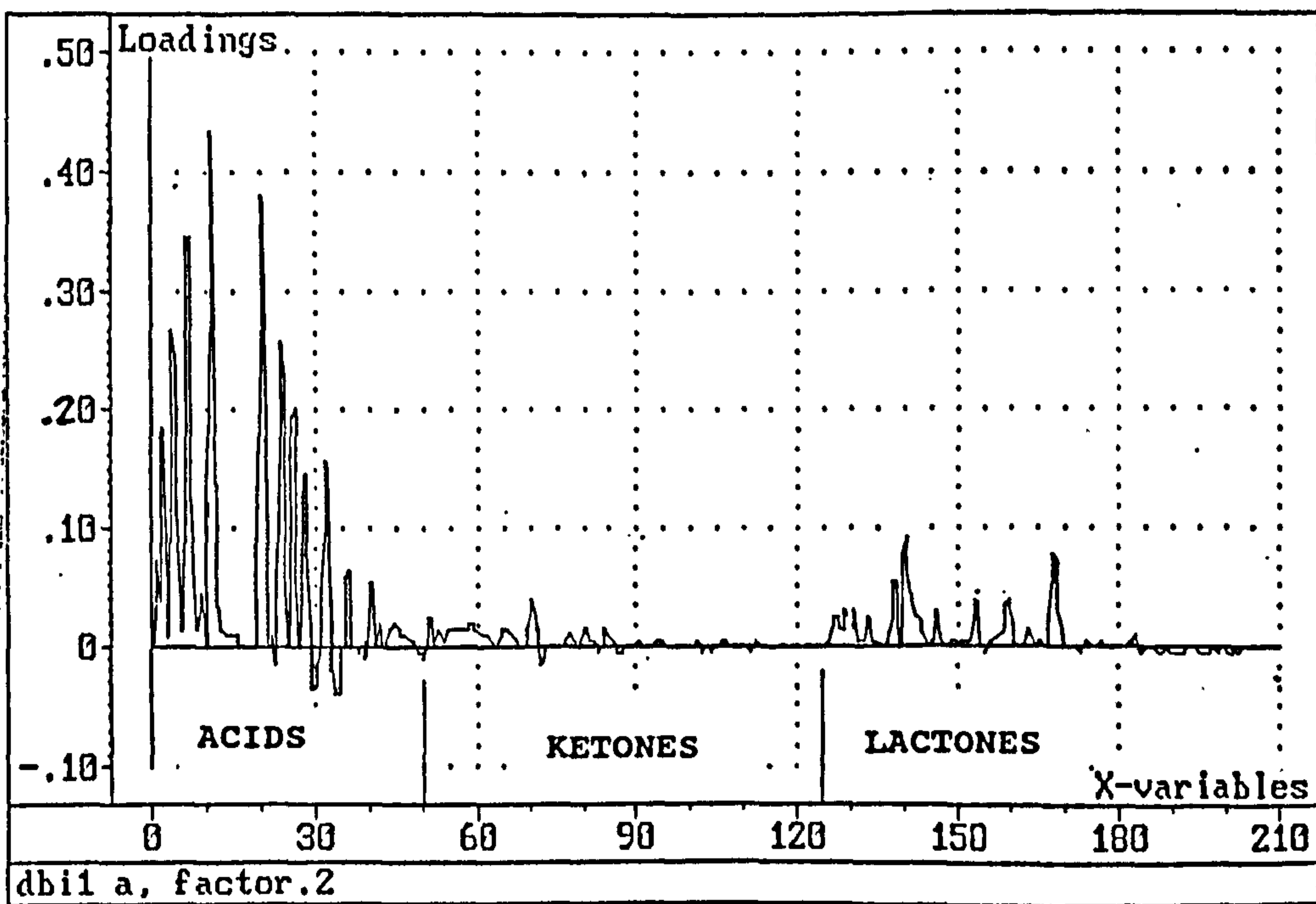


FIGURE 4.34. LOADINGS PLOT FOR PC 2 FROM PRINCIPAL COMPONENTS ANALYSIS OF THE DATA

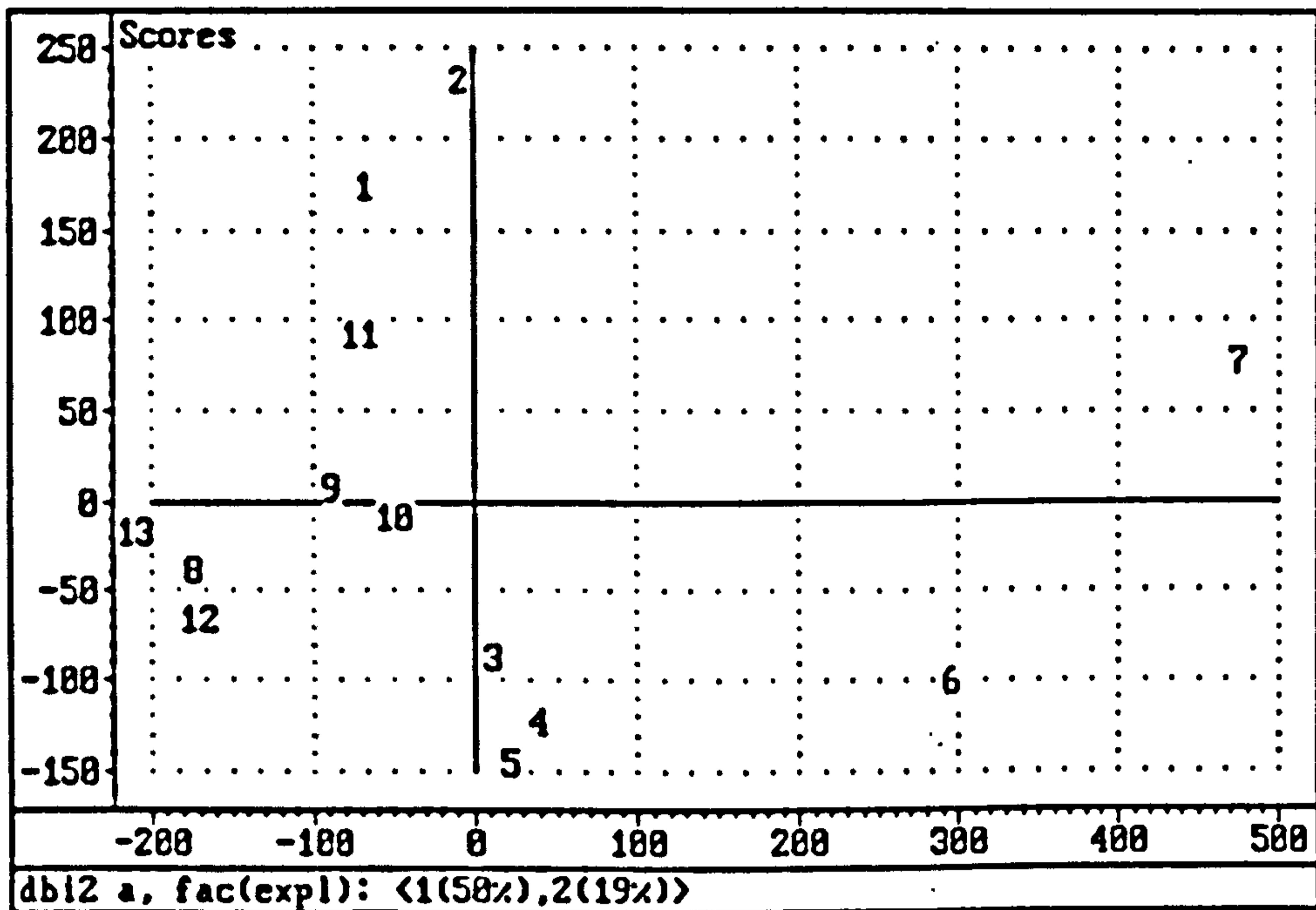
negligible (21%) as shown in Figure 4.34, and again the major loading is due to the *n*-acid oxidation products. Thus, analysis of the loading plots confirms that the groupings in Figure 4.32 are due mainly to differences in the *n*-acid distribution. When the *n*-acid data are removed from the data matrix a somewhat different scores plot is obtained (Figure 4.35). The duplicate oxidations (1 and 2), repeat GCMS analysis (8 and 12) and known correlated samples (Amoco tank oil [4], Newgale beach [5]) remain well grouped together. However samples 6 and 7 (*Sivand* oil and Humber sediment) are no longer close to one another. This suggests that the UCM precursors of the lactones and ketones in the Humber sediment were not all from the *Sivand*, whilst the UCM precursors of the *n*-acids were dominated more by the *Sivand* UCM. Interestingly, for the Mersey case study the opposite appears to be true; viz. the Mersey sediment UCM contains similar lactone and ketone precursors to TJP crude whereas the UCM precursors of the *n*-acids in the Mersey sediment are quite different to the TJP crude UCM and are probably dominated by *n*-acid precursors from the background contamination of the Mersey.

If the same logic is applied to the Sullom Voe study (samples 9-11) the Ninian Crude UCM (10) seems to have a similar lactone and ketone distribution (Figure 4.35) to that of the sediment, whilst the *n*-acids in the oxidised sediment UCM are apparently mainly from another source (Figure 4.32), possibly background hydrocarbons from



**KEY TO SAMPLE NUMBERS IN FIGURE 4.35**

<b>No.</b>	<b>SAMPLE</b>
1	Silkolene 150 (2mg)
2	Silkolene 150 (2mg)
3	Silkolene 150 (50mg)
4	Amoco tank oil
5	Newgale beach oil
6	Sivand cargo
7	Humber sediment
8	Mersey Sediment repeat GCMS analysis
9	Sullom Voe sediment
10	Ninian crude oil
11	Bernicia fuel oil
12	Mersey sediment
13	TJP crude oil



**FIGURE 4.35. TWO DIMENSIONAL PCA SCORES PLOT FOR ANALYSIS OF THE DATABASE EXCLUDING *n*-ACIDS DATA (For sample identities see key opposite)**

aqueous road-run off. These results appear to make sense, since, for example, in each case study it is known that the source oils contributed to the sediments (the Sivand spill did impact the Humber sediment [Jones et al., 1986], the TJP visually contaminated the Mersey sediments [Davies and Wolff, 1990] and Sullom Voe oil terminal does discharge Ninian crude contaminated water [Dodd and Howells, 1985]) but they are not the only, or necessarily major, source of UCM hydrocarbons in each case. Thus, it seems reasonable that the UCMs in each sediment comprise mixtures of hydrocarbons from several sources and, like the biomarkers and other hydrocarbons, the UCMs have different compositions and make different contributions to the final UCM oxidation product profiles.

The fact that UCMs are quantitatively more important than individual hydrocarbons classes (e.g. biomarkers) led to the original proposition that UCMs might better reflect the sources of contamination. This appears to be invalid. The argument has come full circle in that UCM "fingerprinting" suffers from similar limitations to biomarker "fingerprinting". The importance, at present, lies in the additional information provided by the method, as originally suggested by Gough and Rowland (1990). Thus, it would appear that in studies such as these, a combined approach which utilises biomarker, UCM and any other data available is more likely to produce the answer closest to the truth!

#### 4.4.4 THE SILJAN RING

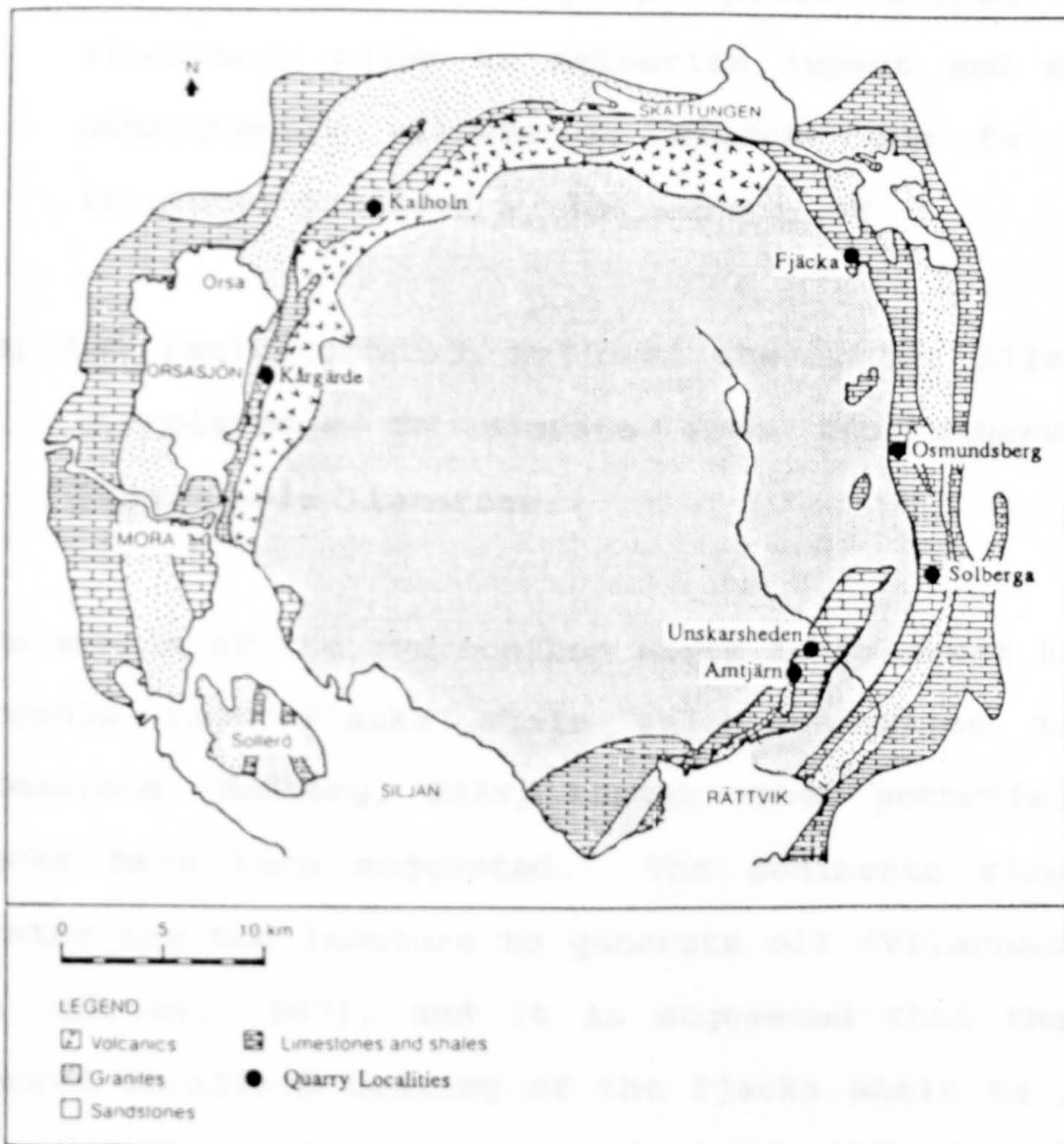
The initial success of the MDS/cluster analysis method in the foregoing environmental case studies led the author to consider application to a geochemical problem, where conventional techniques had been unable to provide an answer.

##### 4.4.4.1 GENERAL

The Siljan Ring (Sweden; Figure 4.36) is widely accepted as a meteorite impact crater (Svensson, 1973; Rondot, 1975; Thorsland and Auton, 1975; Jaanusson, 1982; Vlierboom et al., 1986; Hedberg, 1988 and Konor et al., 1988). Impact is thought to have occurred in the late Devonian (360 x10<sup>6</sup> years BP; Bottomley et al., 1978). This region has been of interest to geologists for many years (e.g. Linnaeus, 1734; Tilas, 1740; cited in Hedberg, 1988) and interest then, as now has centred around the occurrences of sporadic petroleum or "Rock oil", especially within the limestones, in this mainly igneous region. In the field, bituminous seeps are observed oozing from fractures, stylolites or partially cement-filled cavities (Middleton, 1990). Also where limestone outcrops occur, staining can be seen as a result of hydrocarbon infiltration of the fine grained matrix (Middleton, 1990).

Middleton (1990) suggested that the hydrocarbons could





**FIGURE 4.36. SIMPLIFIED GEOLOGICAL MAP OF THE SILJAN RING (Middleton, 1990)**

occur by either,

- (a) their being already in place within the limestones prior to meteorite impact and were subsequently allowed to migrate due to the fractures produced by the impact, or
- (b) the faulting which followed the impact allowed hydrocarbons to migrate into the otherwise impermeable limestone.

The source of the hydrocarbon seeps is presumed to be the organic rich Fjacka shale which underlies the Boda limestone (Hedberg, 1988) though other potential source rocks have been suggested. The sediments ringing the crater are too immature to generate oil (Vlierboom, cited in Shirley, 1987), and it is suggested that the impact caused localised heating of the Fjacka shale to liberate hydrocarbons which have migrated to the overlying limestone. However, a maximum burial depth for the Boda limestone, prior to impact, of 2.5 km (Middleton, 1990 and references therein) may have heated the Fjacka shale sufficiently to produce some hydrocarbons. An alternative theory, and the reason for much renewed interest in the Siljan ring, is that of an abiogenic origin for oil and gas (see Section 3.1; Gold 1979). Gold (1979) has suggested that the meteorite impact produced fracturing which reached the mantle, allowing outgassing of methane and subsequent



hydrocarbon formation. The Siljan Ring is a good test site for these theories since the region is mainly underlain by granite rather than sedimentary rocks (Gold, 1986).

Previous attempts to correlate the hydrocarbon seeps with their proposed source rocks by "traditional" biomarker analysis have been hampered by the severe biodegradation and/or weathering of the samples (Hedberg, 1988). In this study, UCM fingerprinting was attempted in order to correlate the oil seeps with their potential source rocks.

#### 4.4.4.2 GRAVIMETRY AND GAS CHROMATOGRAPHY

Gravimetric data and sample descriptions are given in Table 4.10. The samples yielded widely differing concentrations of aliphatic hydrocarbons (23-50% of total organic extract; Table 4.10) but all exhibited a broad UCM (KI 1100-3500; Figure 4.37) with samples 4, 5 and 6 possessing insignificant amounts of resolved compounds. Samples 1 and 2 are, as expected, very similar by GC (Figure 4.37), although the relative amounts of  $n$ -C<sub>17</sub> and pristane appear different and as yet the reason for this is unclear. The amount of  $n$ -C<sub>19</sub> also appears to be enhanced in sample 2.

Sample 3 exhibits relatively enhanced levels of the lower molecular weight  $n$ -alkanes ( $n$ -C<sub>15-17</sub>), with high levels of pristane.

TABLE 4.10: SAMPLE DESCRIPTIONS AND GRAVIMETRIC DATA FOR SILJAN RING SAMPLES

No	Sample Descriptions/Location (Figure 4.34)	Age	ALIPHATICS									
			Amount Extract Used (mg)	Aliphatic hydrocarbons mg (%)	Aromatic hydrocarbons mg (%)	UA(mg) (%) of aliphatics	Demethylated UNA mg (%) of aliphatics	TUA mg (%) of UNA	TUNA mg (%) of UNA	Amount Oxidized (mg)	Recovered Material mg (%)	
1	Fjacks Oil Shale, Organic rich layer/Omsundberg Quarry	Ordovician	40	13(33)	15(38)	6.3(49)	5.8(45)	2.5(43)	2.5(43)	2.0	1.6(80)	
2	Fjacks Oil Shale, Whole/Omsundberg Quarry	Ordovician	44	11(25)	14(32)	5.3(48)	4.1(37)	1.9(46)	2.1(51)	2.0	1.8(90)	
3	Calcareous Nodule, Oil Stained/Kalholm Quarry	Silurian	14	6(43)	3(21)	3.0(50)	2.4(40)	0.2(8)	2.0(83)	2.0	1.5(75)	
4	Coral Reef, Oil Stained/Umsarheden Quarry	Ordovician	30	7(23)	5(17)	3.0(43)	3.1(44)	1.0(32)	1.9(61)	1.9	1.5(79)	
5	Alum Shale, Whole/ - not known	Cambrian	20	10(50)	5(25)	2.8(28)	6.0(60)	2.5(42)	3.2(53)	2.0	1.8(90)	
6	Oil Seep/Solberga	N/A	29	10(34)	8(28)	4.5(45)	4.1(41)	1.8(44)	2.0(49)	2.0	1.6(80)	

N/A = Not Applicable



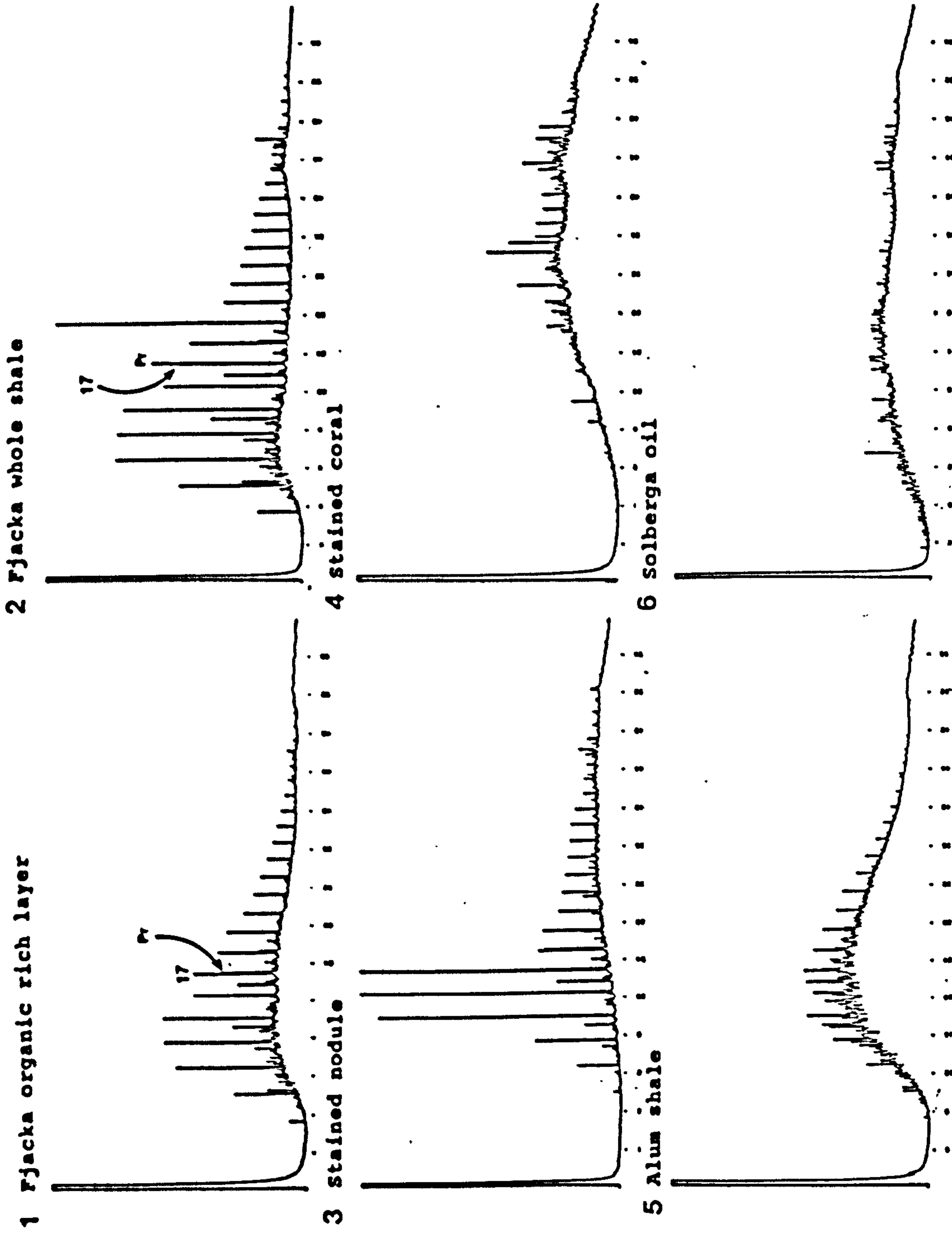


FIGURE 4.37. GAS CHROMATOGRAMS OF THE ALIPHATIC FRACTIONS ISOLATED FROM THE SILJAN RING SAMPLES (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas) (numbers indicate chain length, Pr = pristane)

Removal of the resolved components with urea highlighted the relatively high levels of acyclic isoprenoids in samples 2 and 3, and to a slightly lesser extent sample 1 (Figure 4.38). All the samples yielded ca. 40% of UNA from the aliphatic fraction (Table 4.10) except for the Alum shale (sample 5), which yielded 60% of UNA (Table 4.10). Thiourea adduction was used in an attempt to remove these acyclic isoprenoid compounds and, for the sake of consistency, all samples were treated, even those which exhibited little or no isoprenoids. The procedure was moderately successful, especially in samples 1 and 3 (Figure 4.39) and to a lesser extent with sample 2. Unfortunately, each adduction removes some UCM, thus a compromise has to be reached between the level of resolved compounds and the amount of material remaining. However in each sample, the thio-urea adduction highlighted the presence of steranes and triterpanes (KI 2700-KI 3200; Figure 4.39).

#### 4.4.4.3 GAS CHROMATOGRAPHY MASS SPECTROMETRY

GCMS of sterane distributions (Figure 4.40) highlights the expected similarity between samples 1 and 2. Sample 3 also has some similarity to the Fjacka shale, in that the (20R)  $C_{29}\alpha\alpha\alpha$  isomer (peak 8) is dominant, but the level of the  $C_{29}\beta\beta$  isomers appears to be enhanced compared to the (20S)  $C_{29}\alpha\alpha\alpha$  isomer (Figure 4.40). Sample 4, the stained coral, exhibits some similarities to the Solberga oil, with the

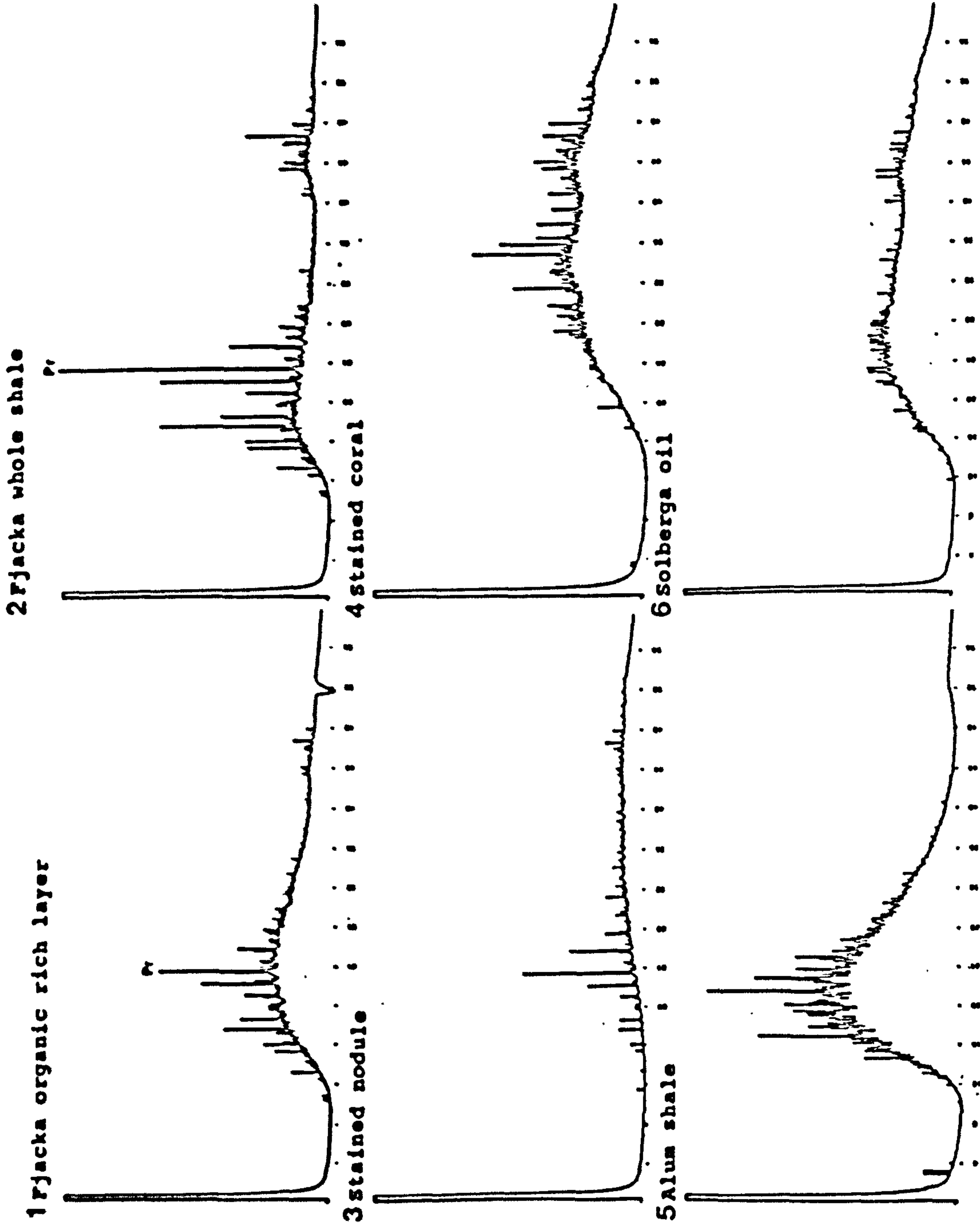


FIGURE 4.38. GAS CHROMATOGRAMS OF THE ALIPHATIC UNSATURATED FRACTIONS ISOLATED FROM THE SILJAN RING SAMPLES

(J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas) (Pr = pristane).

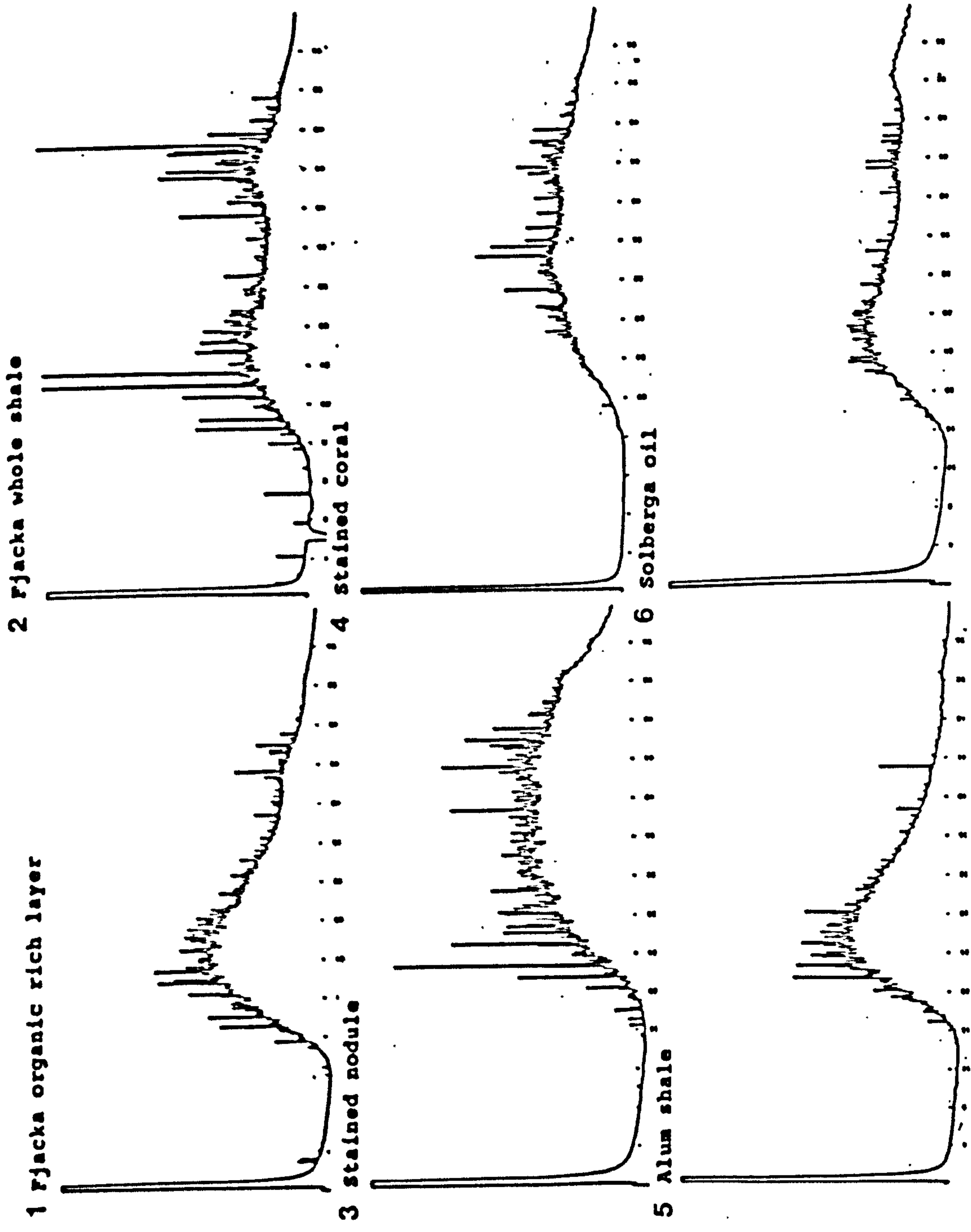


FIGURE 4.39. GAS CHROMATOGRAMS OF THE ALIPHATIC TUNA FRACTIONS ISOLATED FROM THE SILJAN RING SAMPLES

(J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)

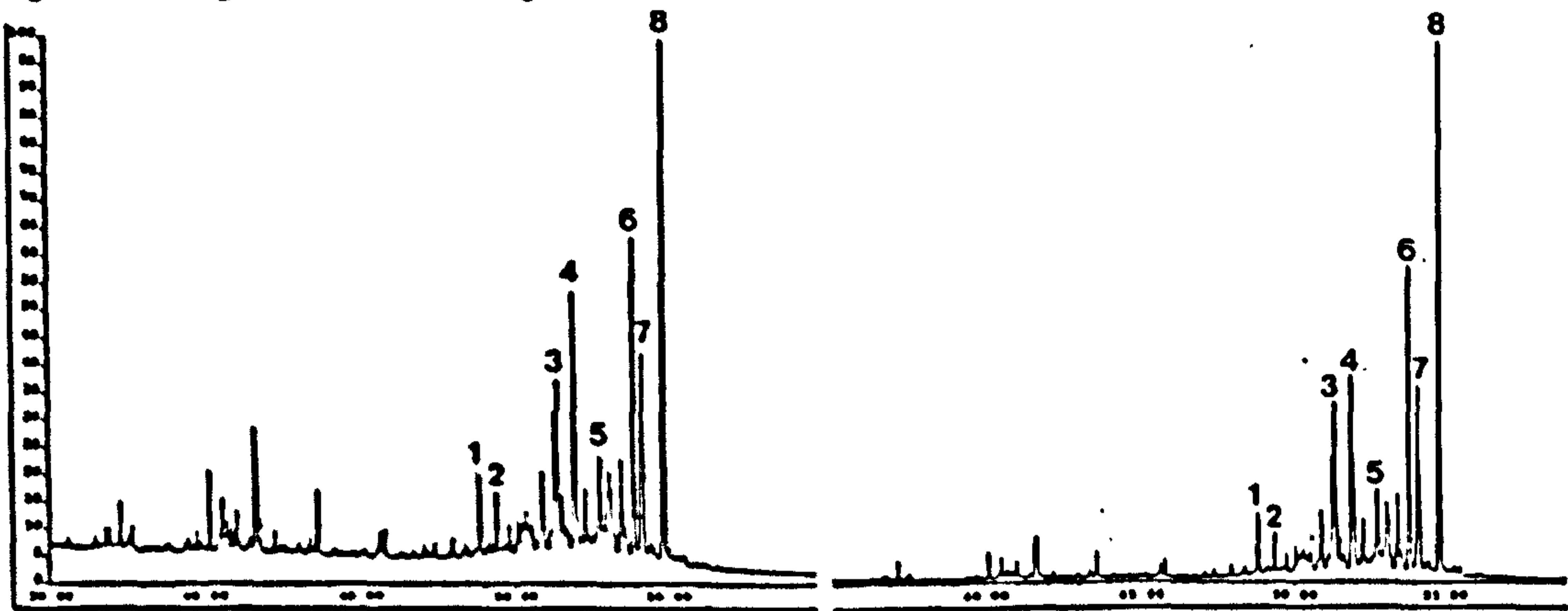


# KEY TO M/Z 217 PEAK ASSIGNMENTS

Peak #	Assignment
1	C <sub>27</sub> βα 20S Diasterane
2	C <sub>27</sub> βα 20R Diasterane
3	C <sub>27</sub> ααα 20S
4	C <sub>27</sub> ααα 20R
5	C <sub>28</sub> ααα 20S
6	C <sub>29</sub> ααα 20S
7	C <sub>29</sub> αββ (20S + 20R)
8	C <sub>29</sub> ααα 20R

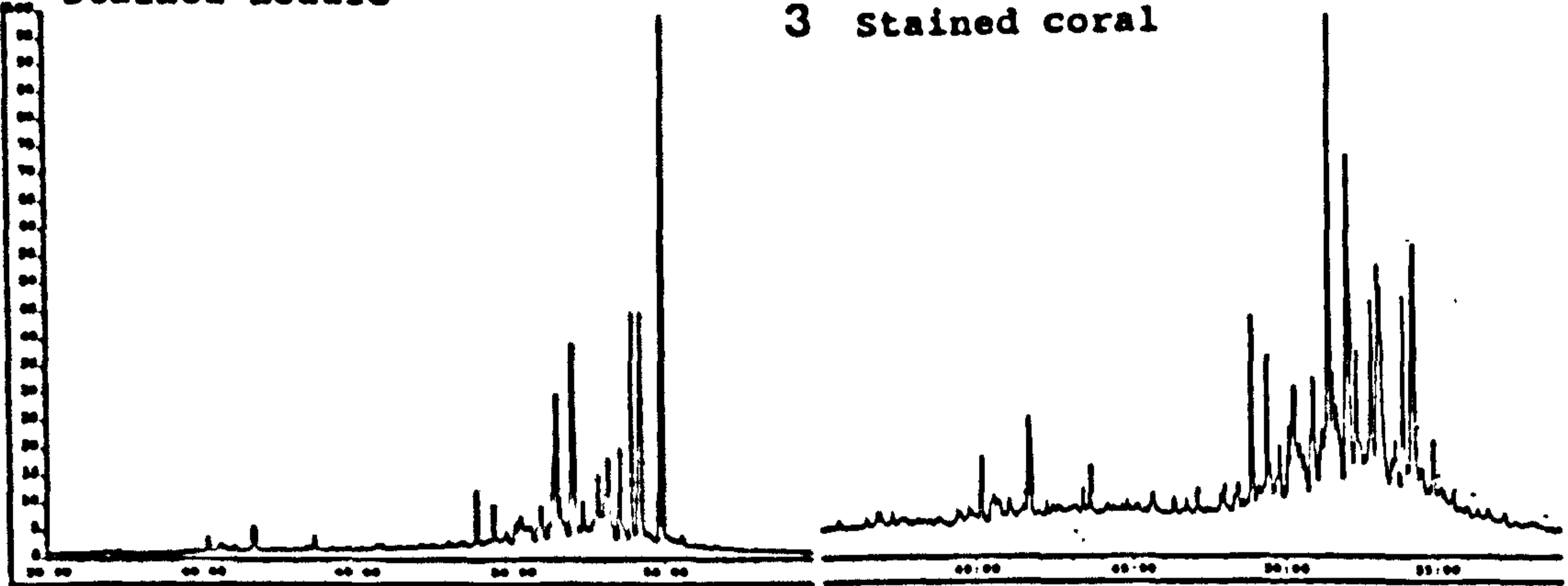
1 Fjacka organic rich layer

2 Fjacka whole shale



3 Stained nodule

3 Stained coral



5 Alum shale

6 Solberga oil

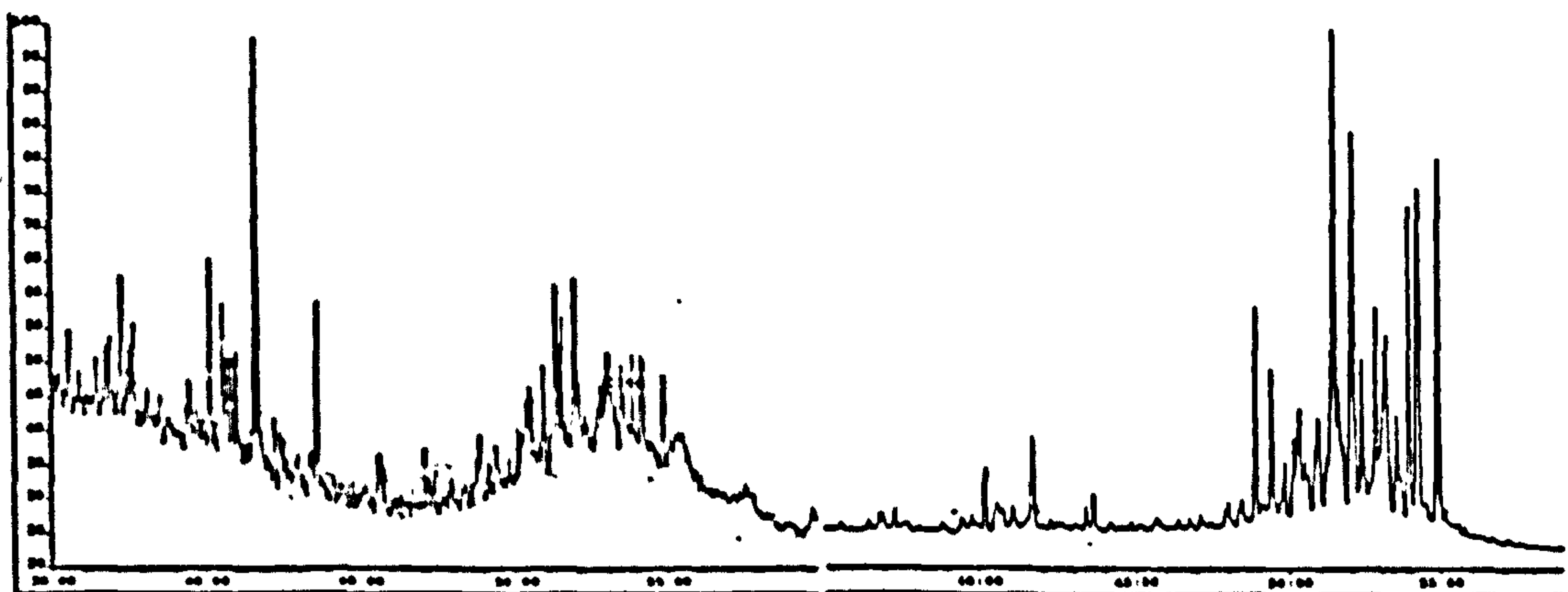


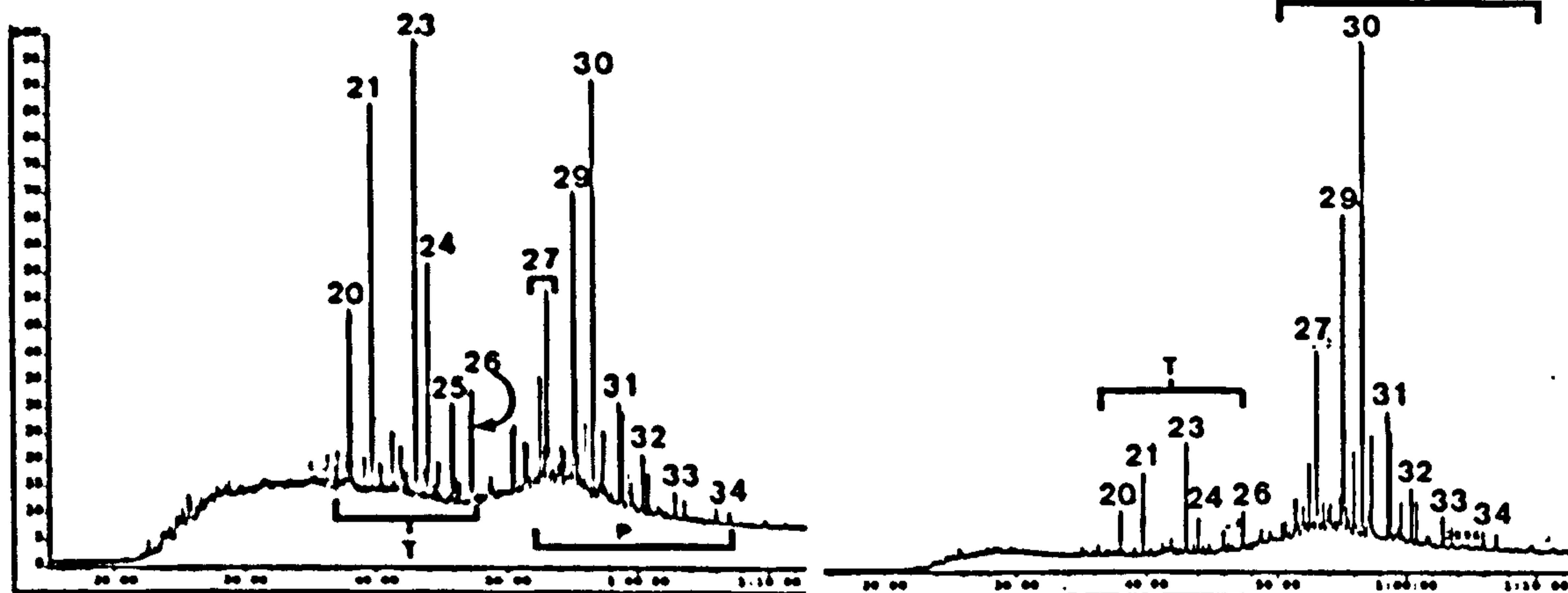
FIGURE 4.40. M/Z 217 CHROMATOGRAMS OF THE ALIPHATIC FRACTIONS ISOLATED FROM THE SILJAN RING SAMPLES (J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas) (for peak identities see key opposite)

(20S)  $C_{27}\alpha\alpha\alpha$  isomer being dominant. An important difference, however, is the enhanced level of (20R)  $C_{29}\alpha\alpha\alpha$  in the borehole hydrocarbons, which is absent in the coral sample. The Alum shale shows a low level of steranes, with those that are present appearing to correspond to  $\beta\beta$  isomers, indicating a high degree of maturity. Thus, while the sterane distributions show similarities between certain samples, there are also important differences, making it difficult to draw any firm conclusions.

Examination of the triterpane distributions gives similar results to that of the steranes (Figure 4.41). Samples 1 and 2 exhibit the expected similarity, except that the whole shale produces a distribution lower in tricyclic terpanes, suggesting that there are pentacyclic triterpanes present in fractions of the shale other than the so called organic rich layer. Sample 3 again shows some similarity to the Fjacka shale, but again with important differences, namely the relative proportions of  $C_{29}$  and  $C_{30}$  hopanes and  $C_{23}$  and  $C_{24}$  tricyclic compounds (Figure 4.41). Similarly sample 4 again has greatest affinity with the Solberga oil (sample 6), except for the relative amounts of  $C_{29}$  and  $C_{30}$  hopanes and the Alum shale (sample 5) appears to be devoid of pentacyclic compounds, although a few tricycles appear to be present. This is presumably attributable to the high maturity of the Alum shale.

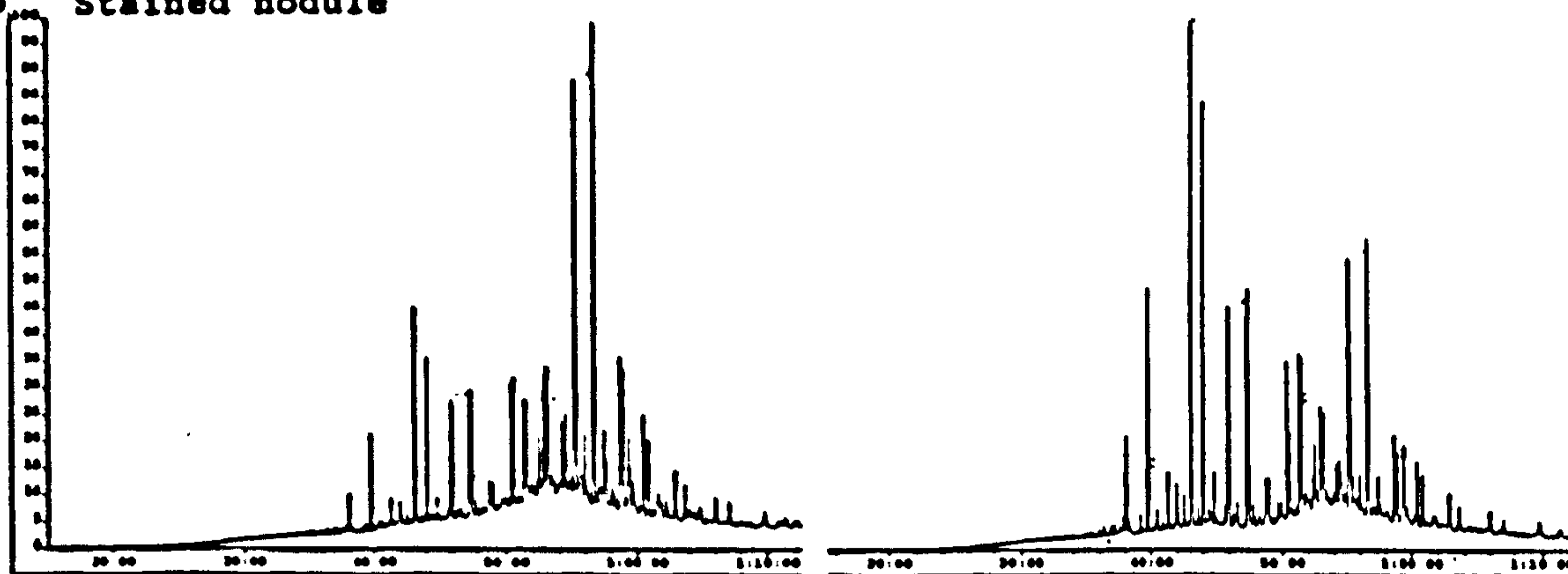
1 Fjacka organic rich layer

2 Fjacka whole shale



3 Stained nodule

4 Stained coral



5 Alum shale

6 Solberga oil

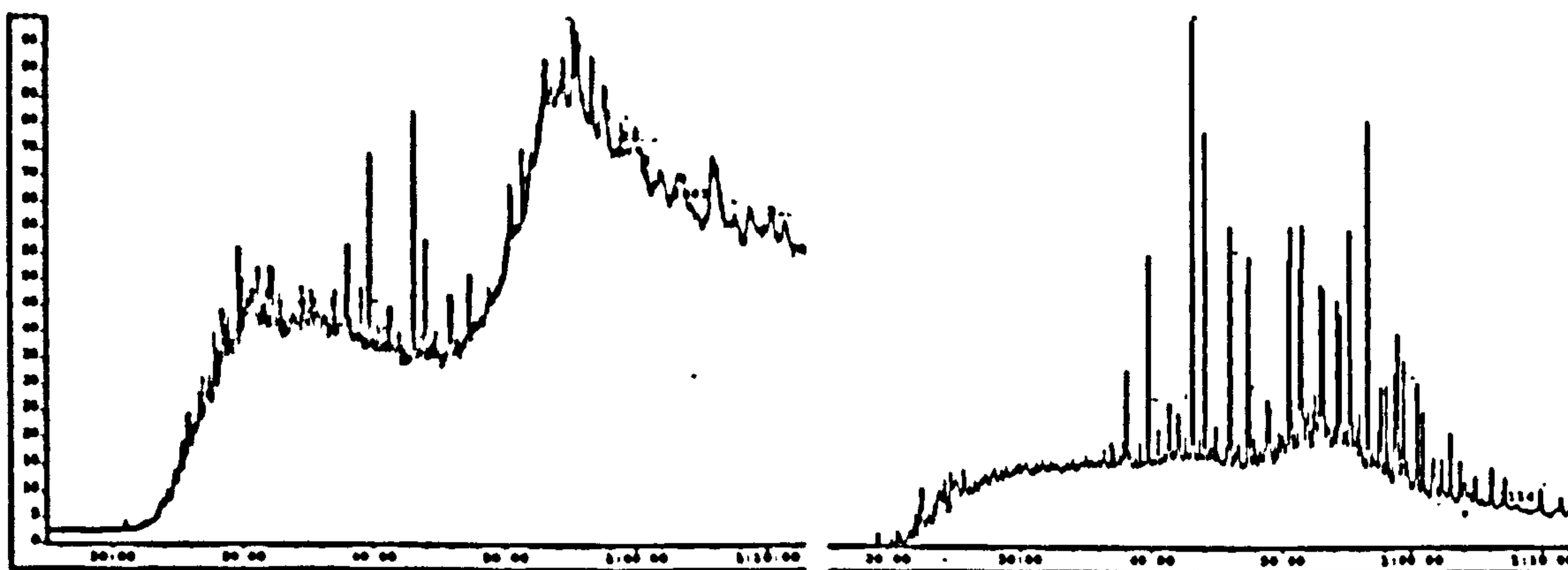


FIGURE 4.41. M/Z 191 CHROMATOGRAMS OF THE ALIPHATIC FRACTIONS ISOLATED FROM THE SILJAN RING SAMPLES (J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas) (numbers refer to carbon number, T = tricyclic compounds, P = pentacyclic compounds)



#### 4.4.4.4 OXIDATIVE DEGRADATION

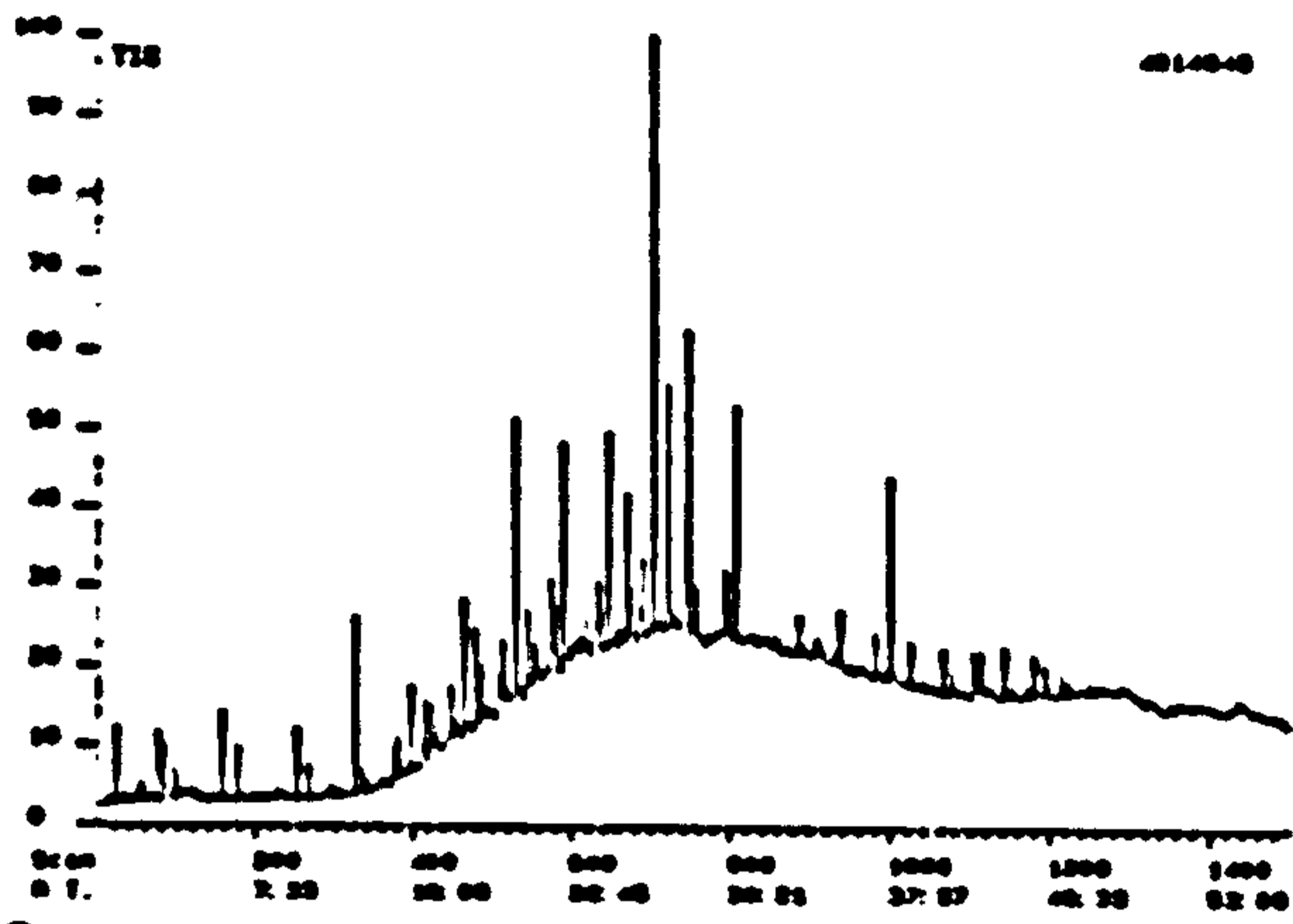
The lack of any conclusive correlations from the biomarker analyses suggested the use of oxidative degradation of the isolated UCMs, in an attempt to gain further information on the possible origins of the hydrocarbon seeps. Thus, each UCM was subjected to oxidation as previously described, with good recoveries (Table 4.10), though the amount of resolved material by GC was still low (ca. 20%). These distributions are in agreement with the biomarker data in that samples 1 and 2 while similar (Figure 4.42), do exhibit some differences, and samples 3 and 4 appear very different to the rest. This is further highlighted by examination of the carboxylic acid profiles ( $m/z$  74; Figure 4.43). All the samples appear to exhibit a strong odd over even predominance, with the  $C_{13}$  and  $C_{15}$  acids predominating. Analysis of  $\gamma$ -lactones ( $m/z$  99; Figure 4.44) and ketones ( $m/z$  58; Figure 4.45) show greater differences between the samples, though these components are present in only relatively minor amounts.

In an attempt to judge the similarity of the samples, the data was subjected to statistical analysis as before.

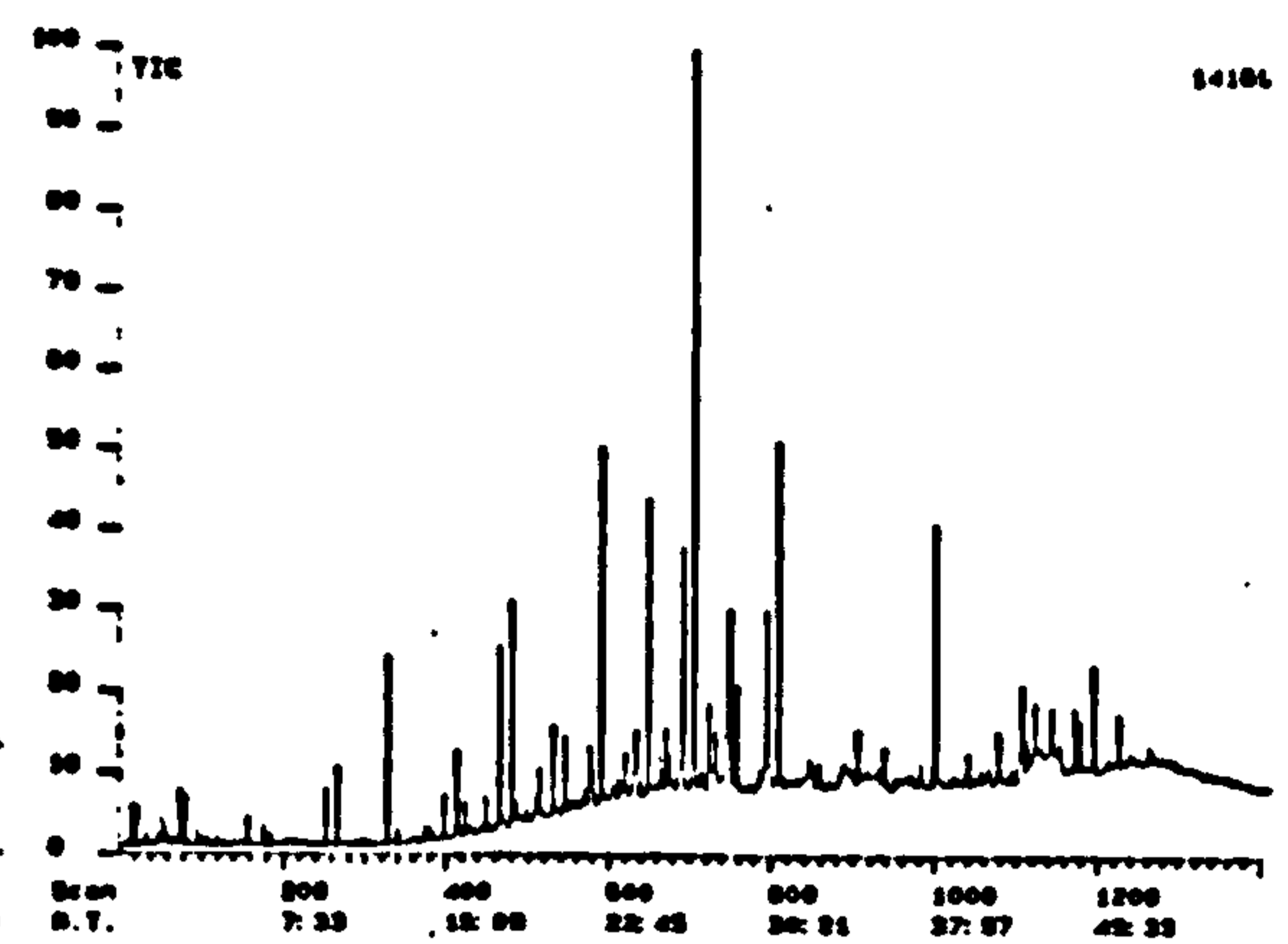
#### 4.4.4.5 STATISTICAL ANALYSIS

Comparison of the chromatographic data using the DBMATCH program and subsequent statistical analysis produced the

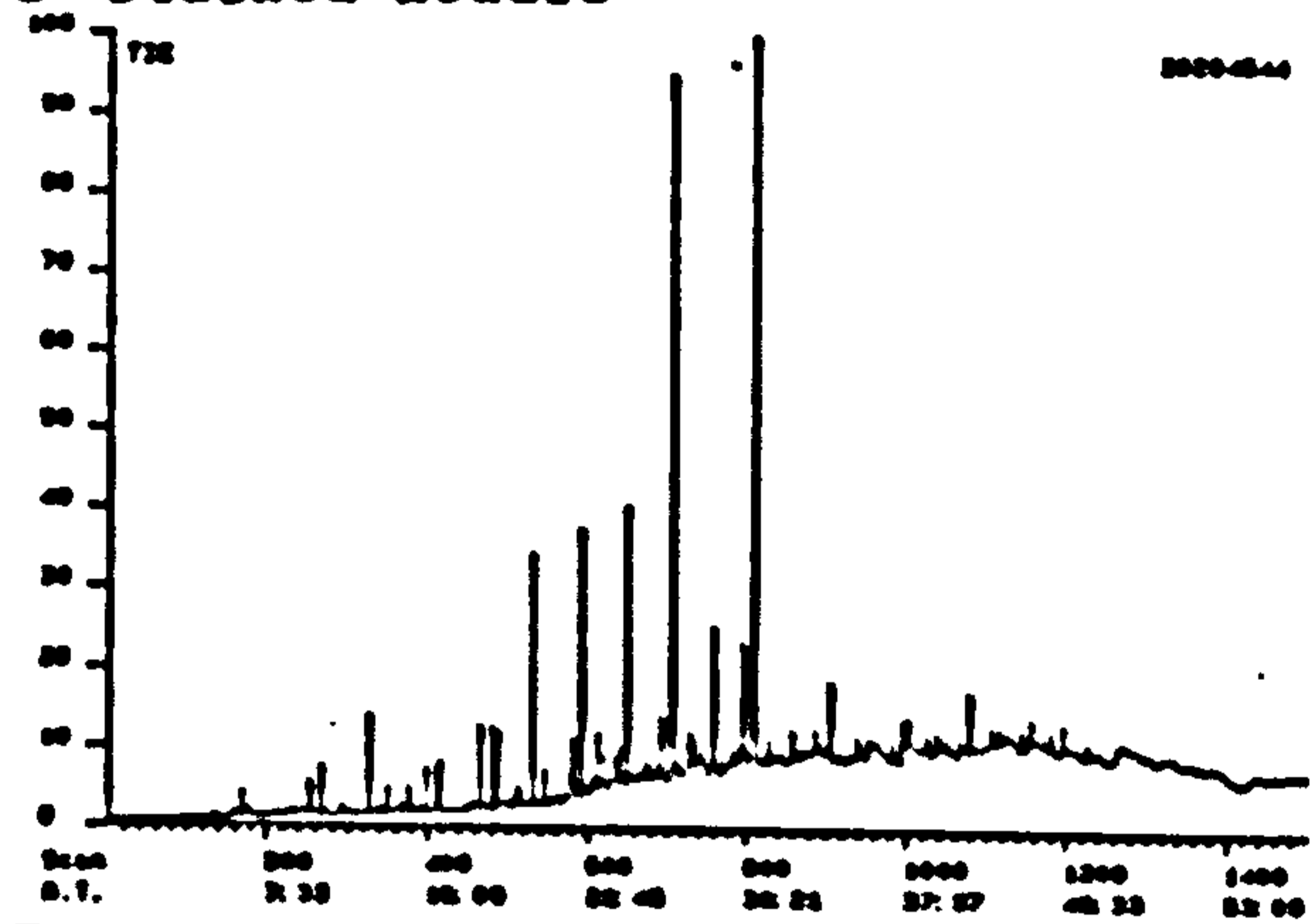
1 Fjacka organic rich layer



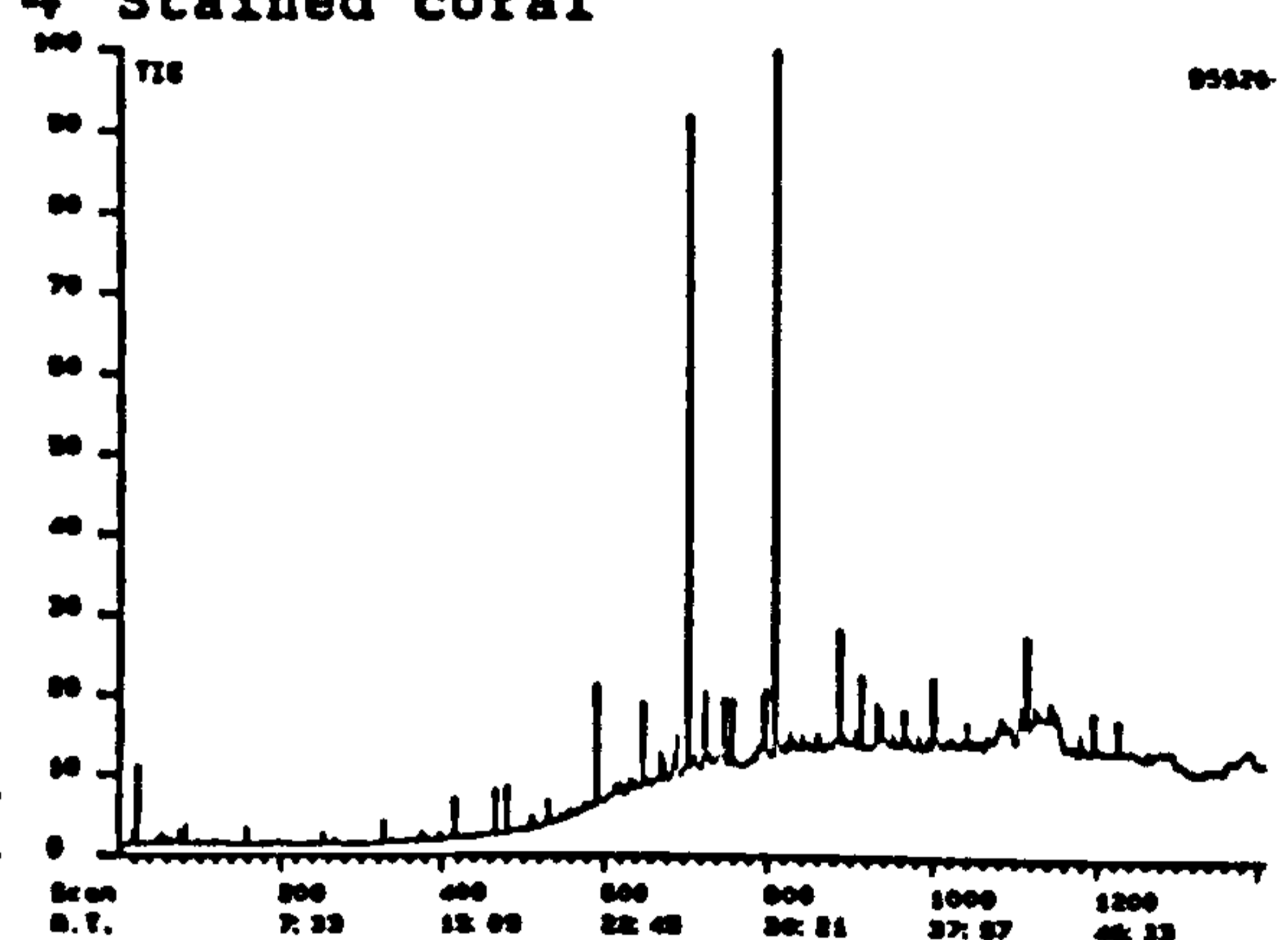
2 Fjacka whole shale



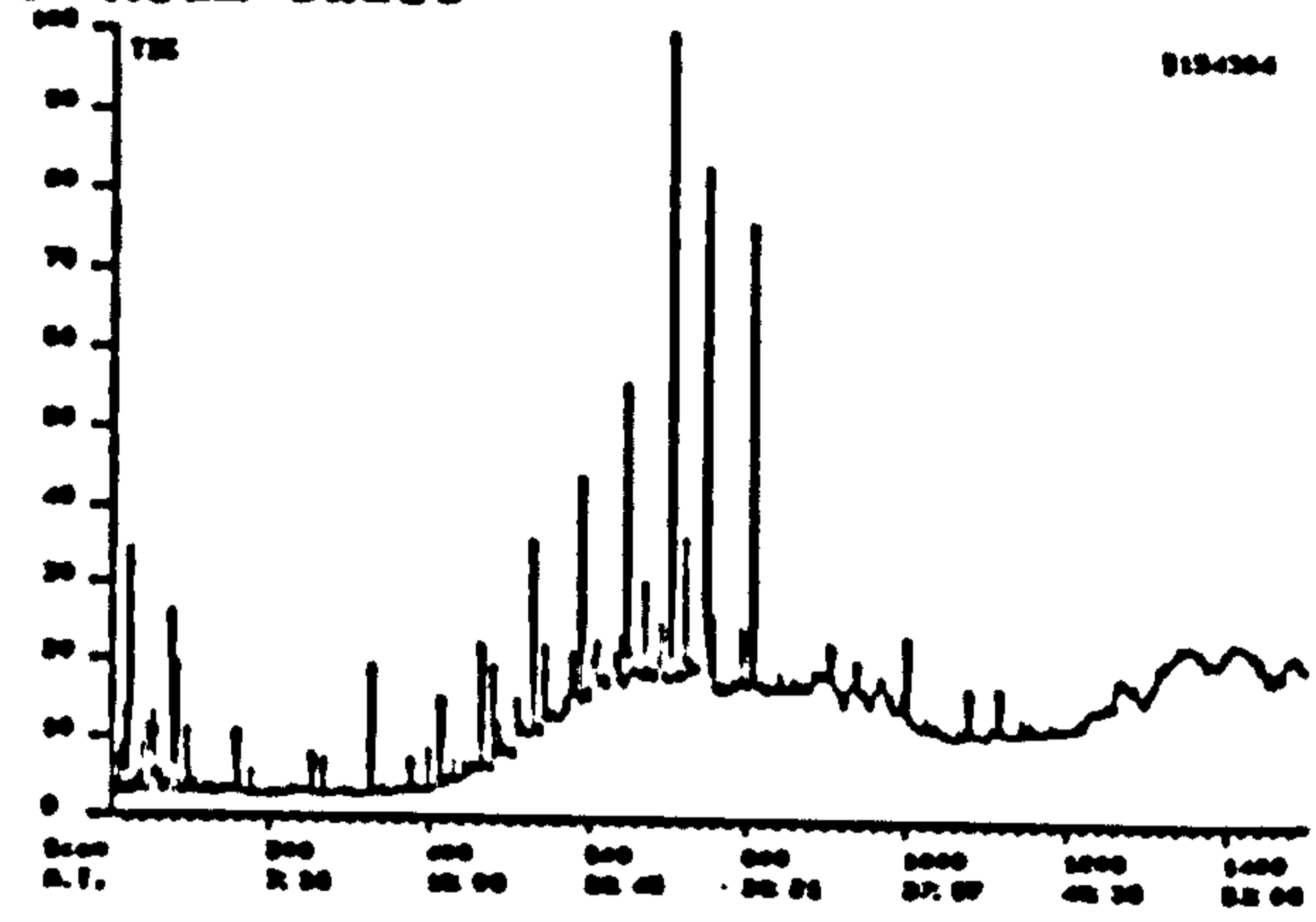
3 Stained nodule



4 Stained coral



5 Alum shale



6 Solberga oil

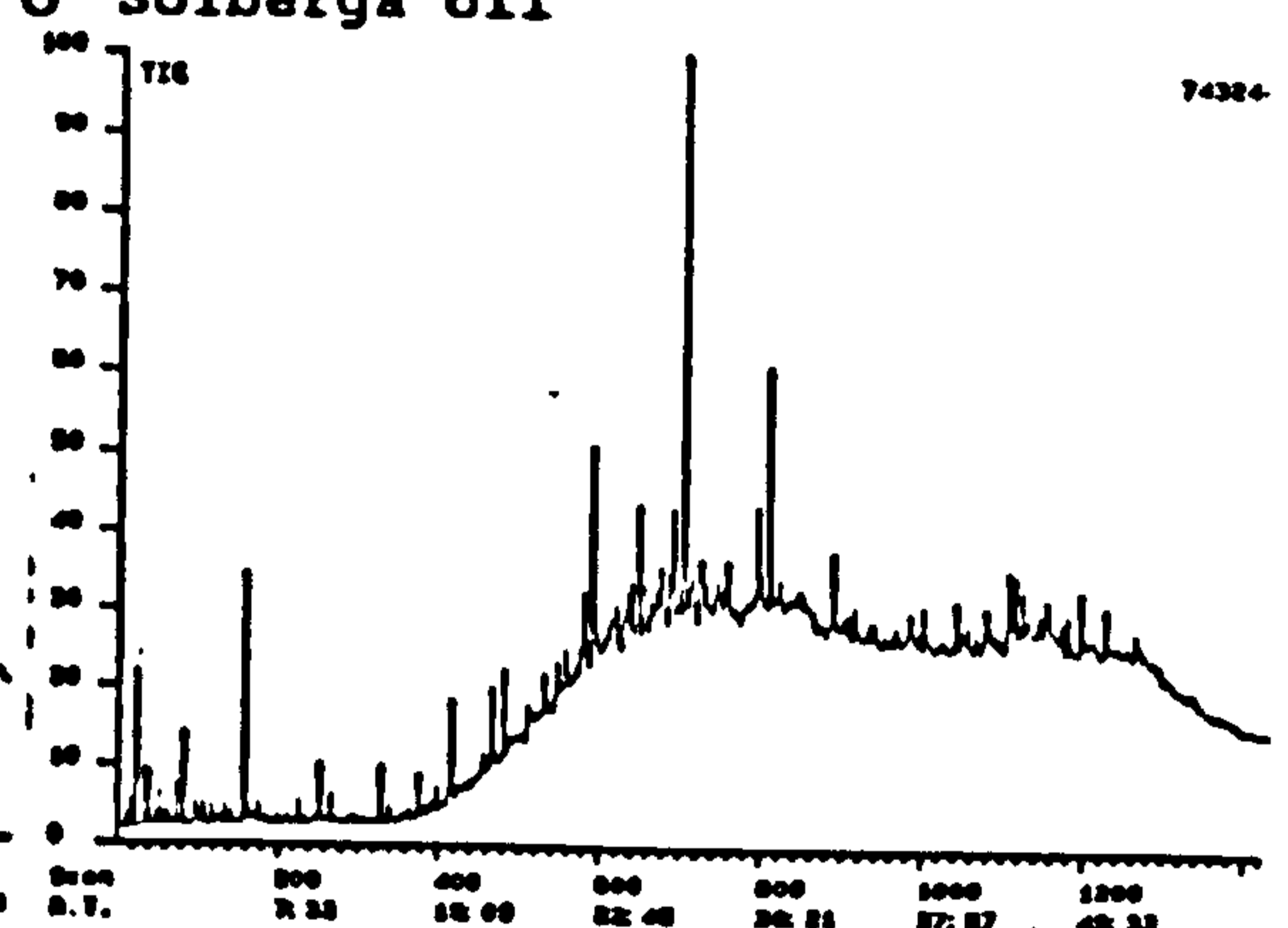
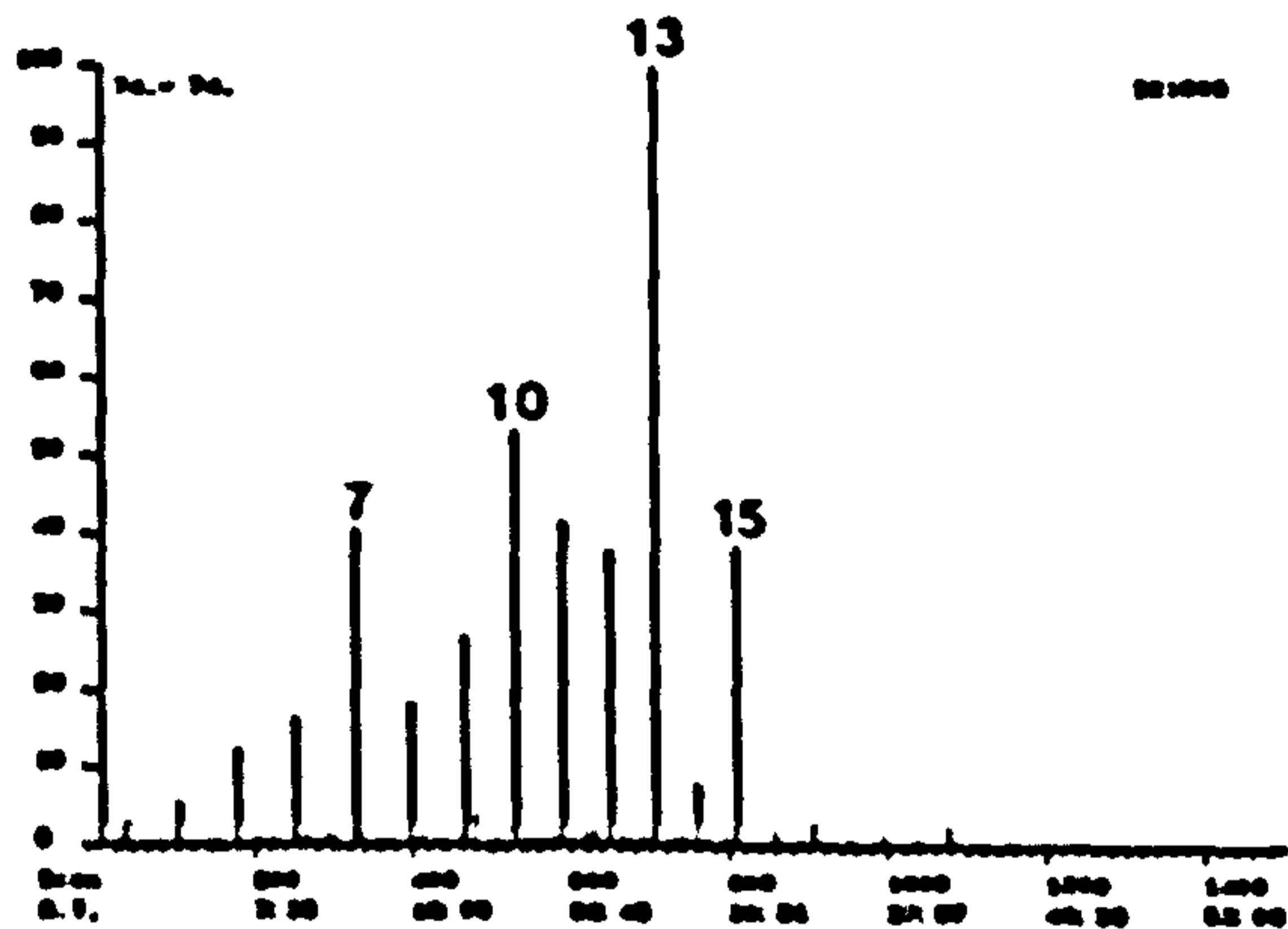
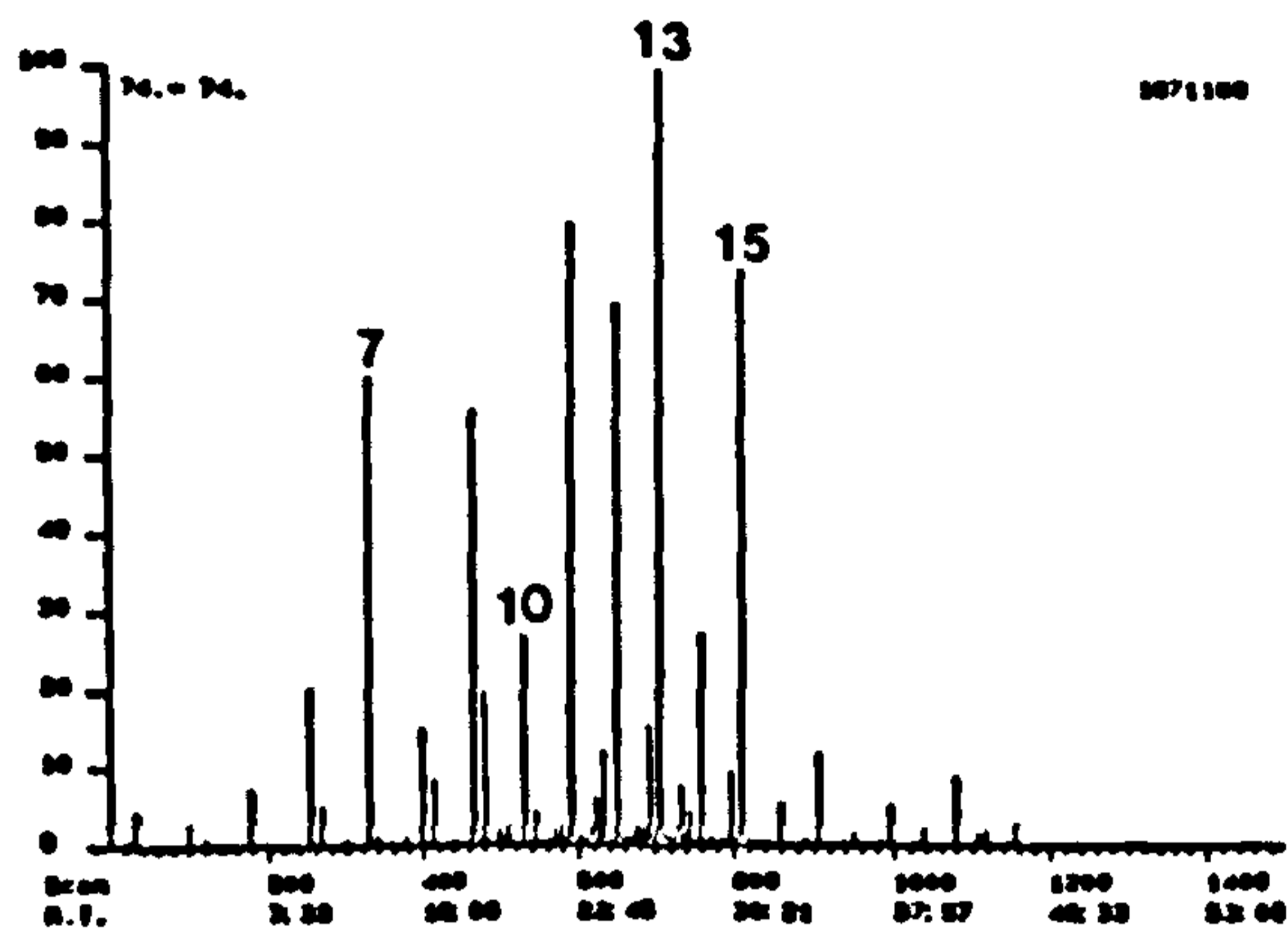


FIGURE 4.42. TOTAL OXIDATION PRODUCTS FOR UCMS ISOLATED FROM THE SILJAN RING SAMPLE (J&W DB-5, 25m, 40-300°C @ 5°C/min., H<sub>2</sub> carrier gas)

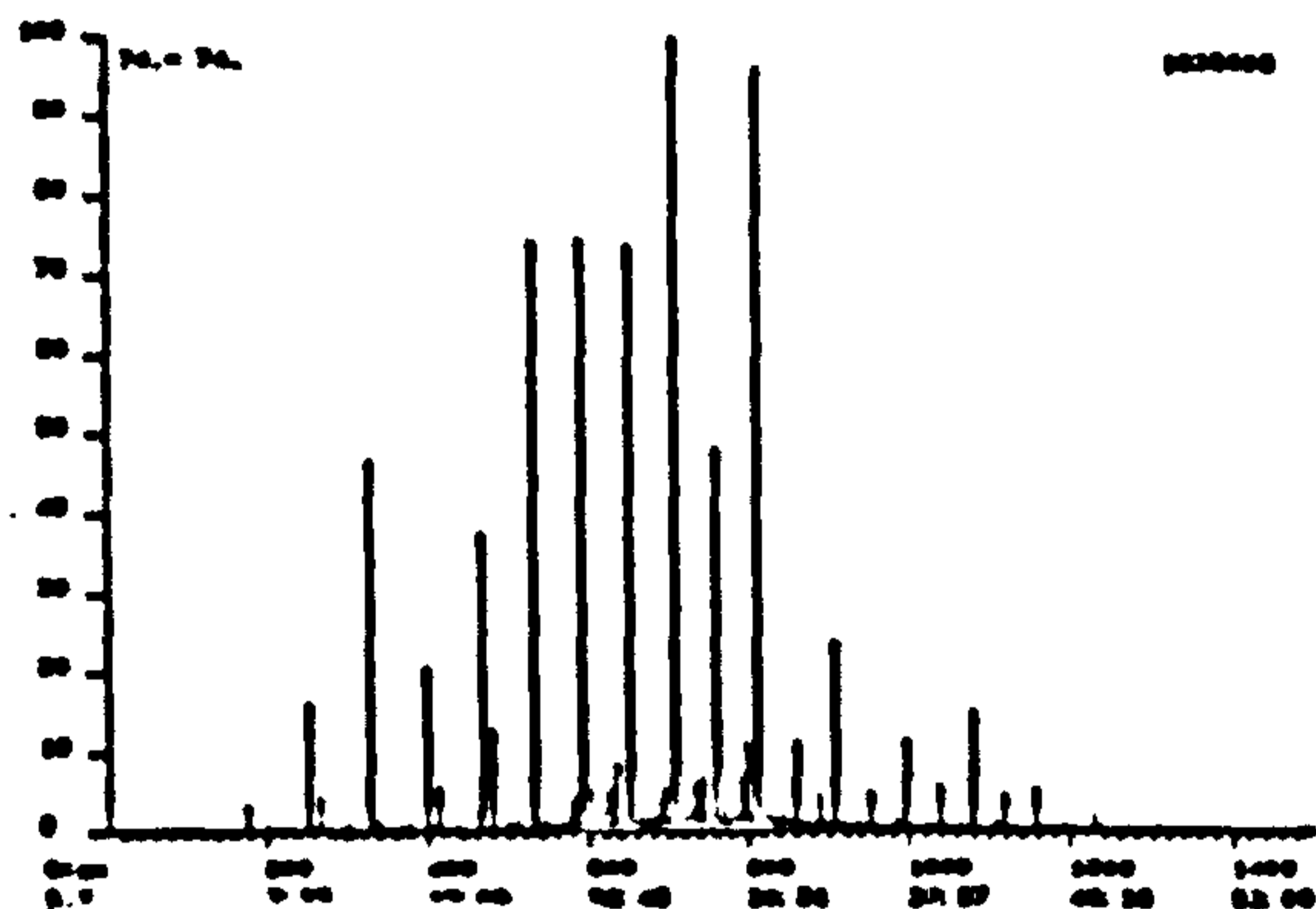
1 Fjacka organic rich layer



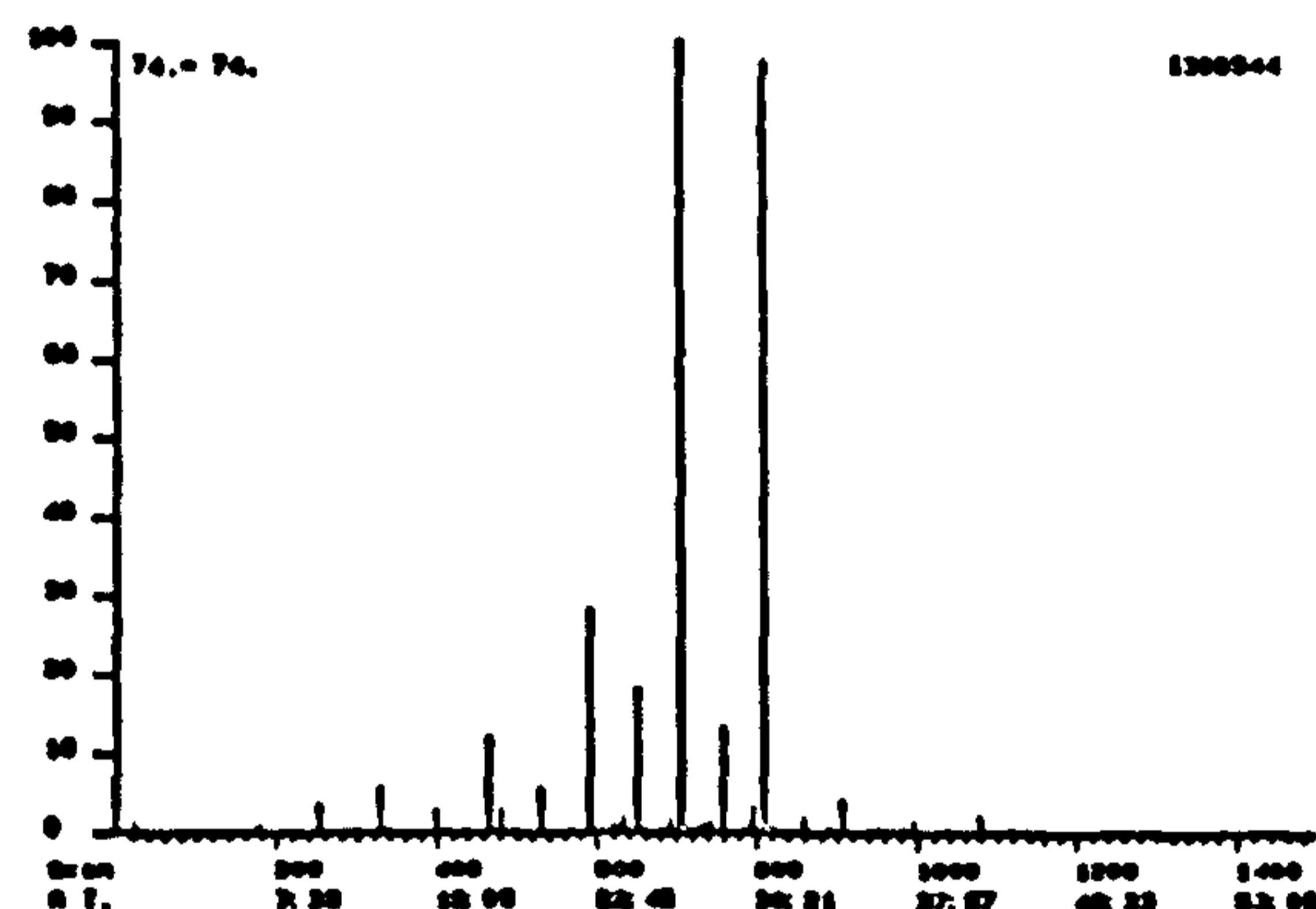
2 Fjacka whole shale



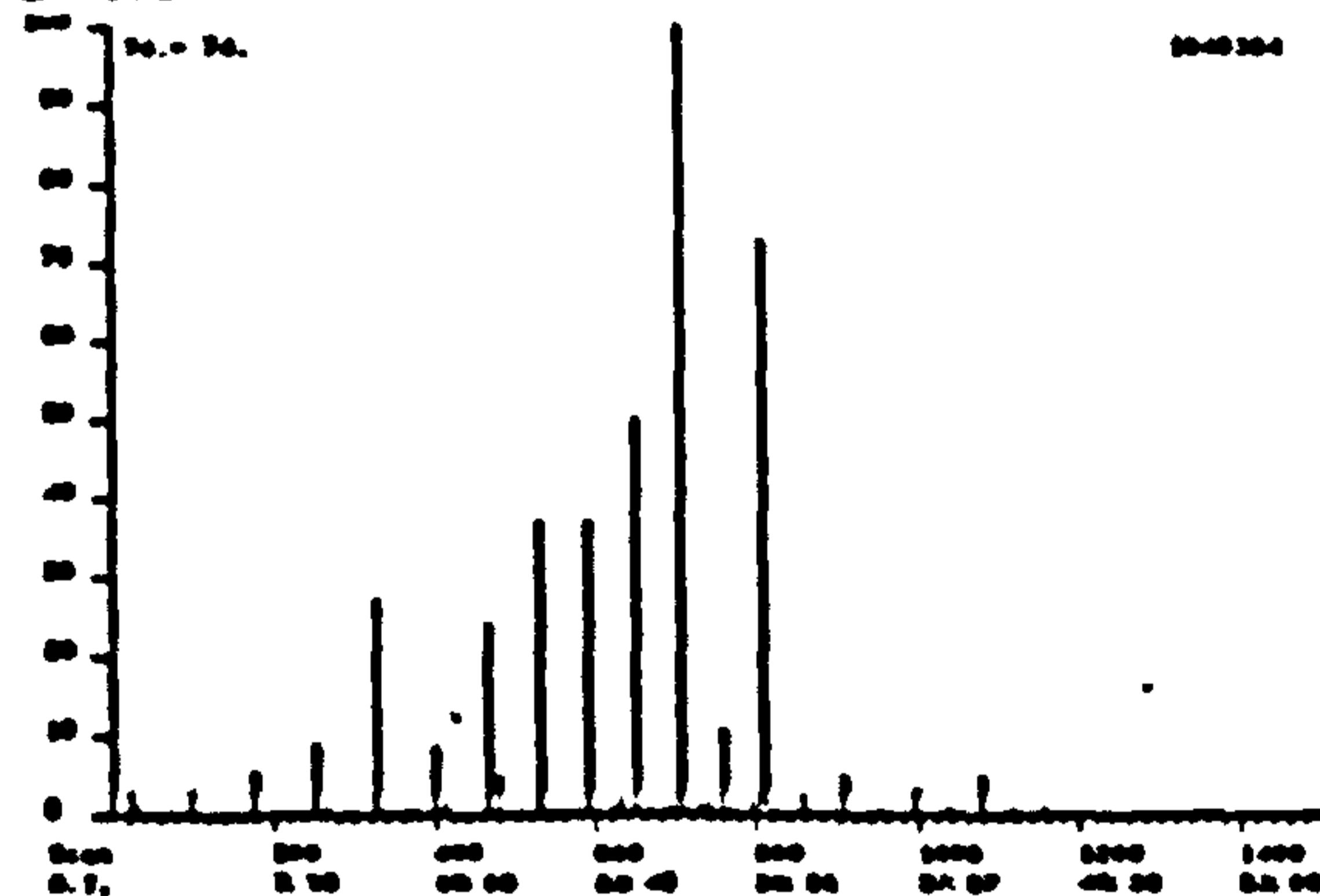
3 Stained nodule



4 Stained coral



5 Alum shale



6 Solberga oil

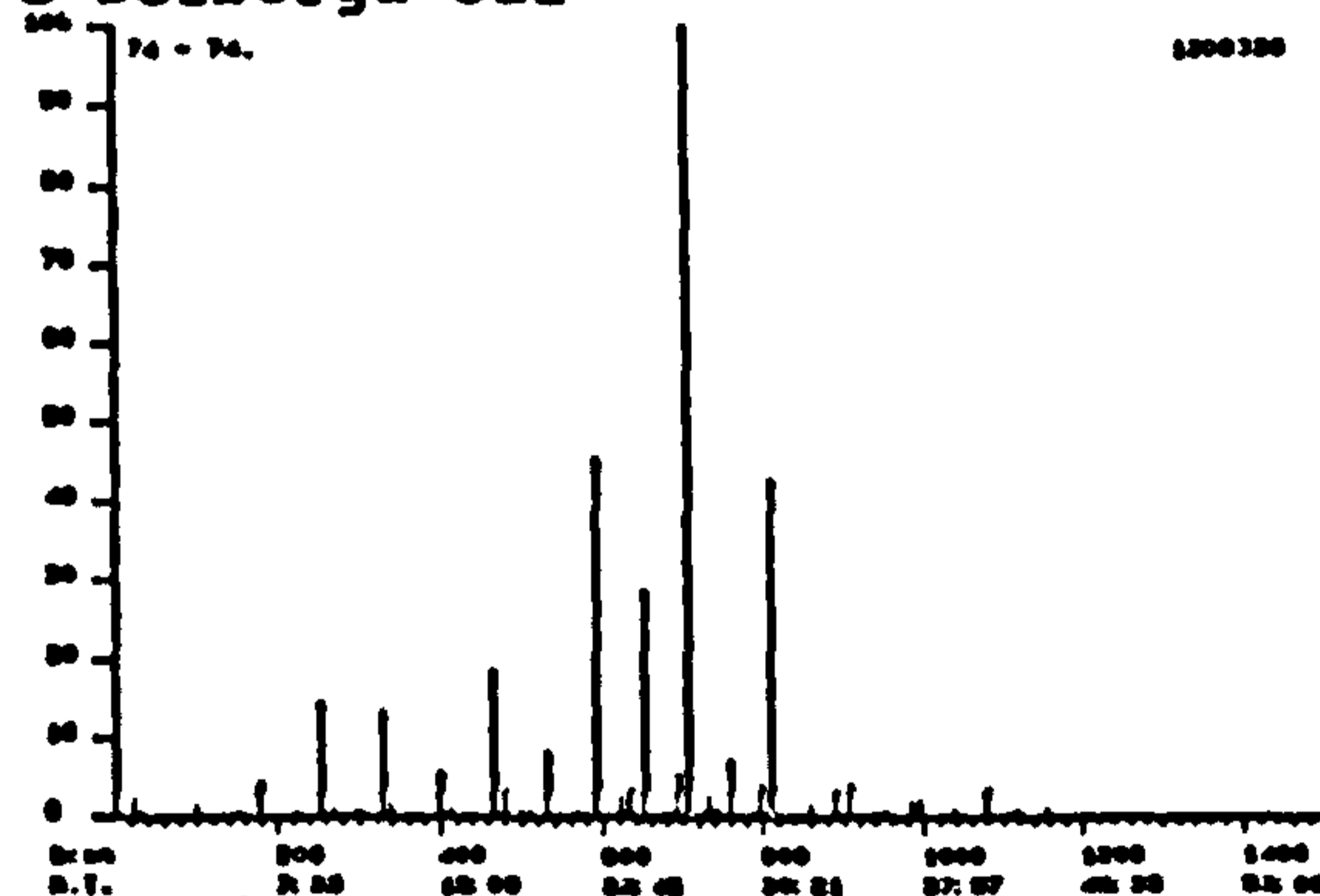
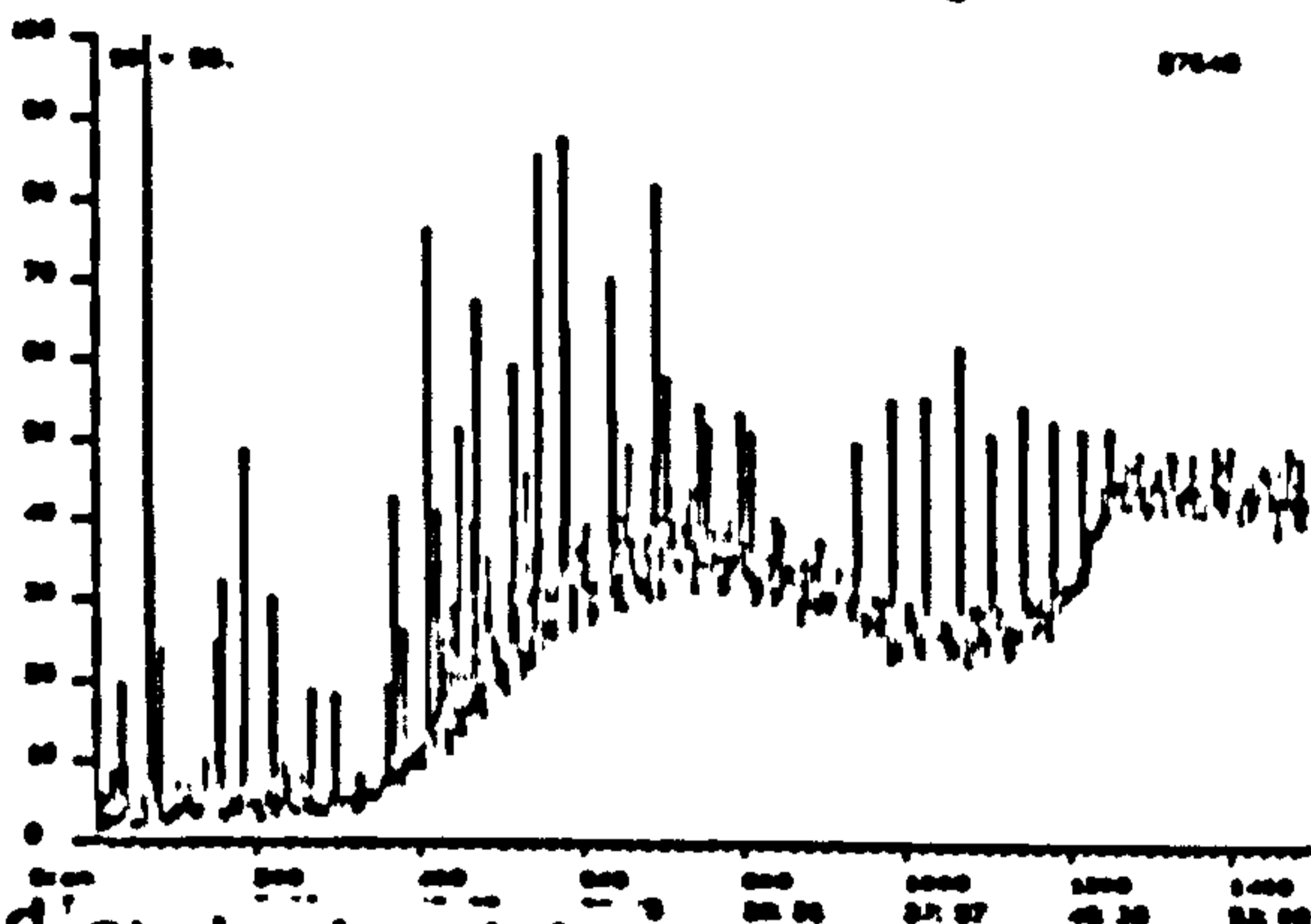
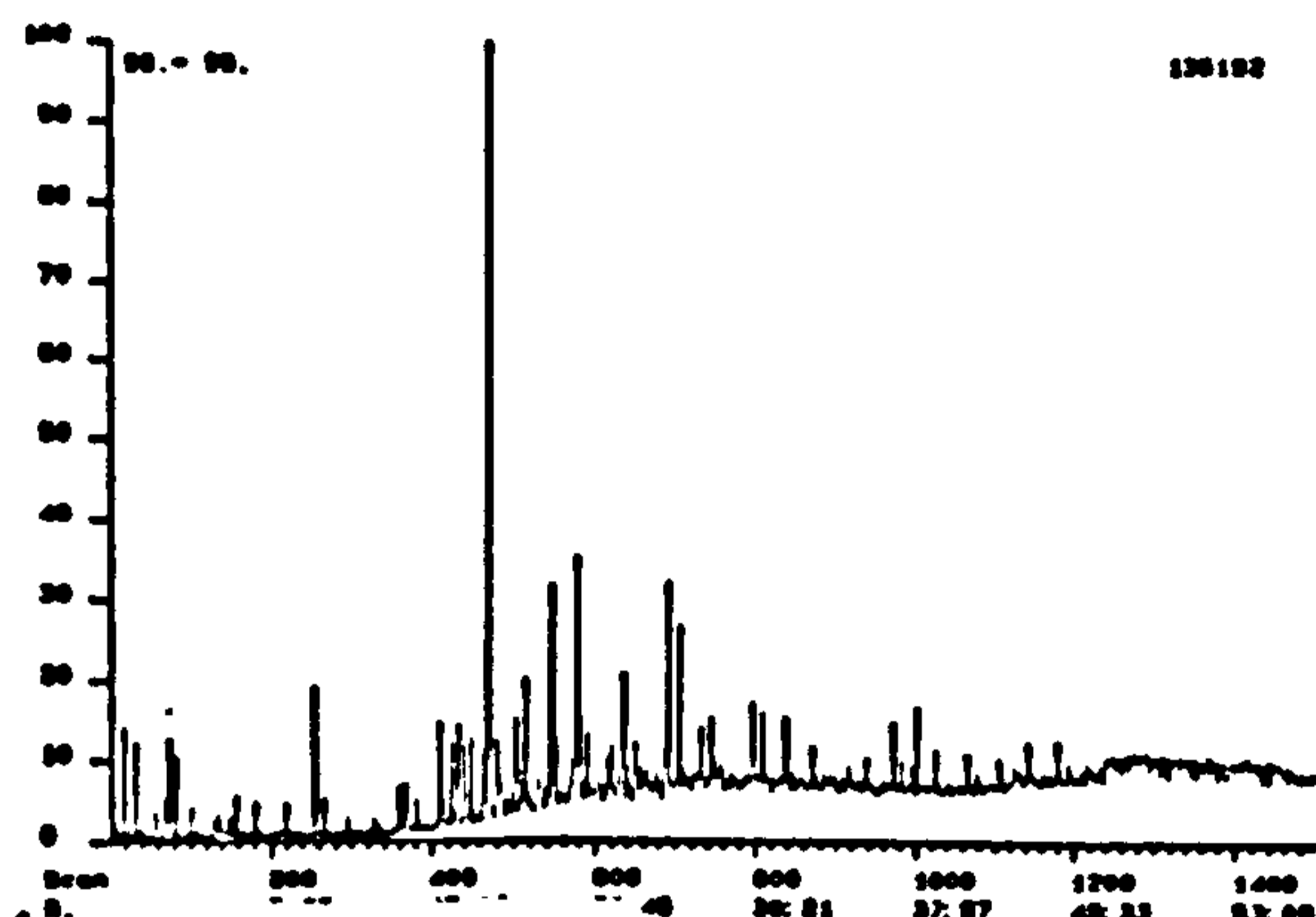


FIGURE 4.43. M/Z 74 CHROMATOGRAM FOR THE OXIDATION PRODUCTS OF UCMS ISOLATED FROM THE SILJAN RING SAMPLES (J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas) (numbers refer to n-acid chain length)

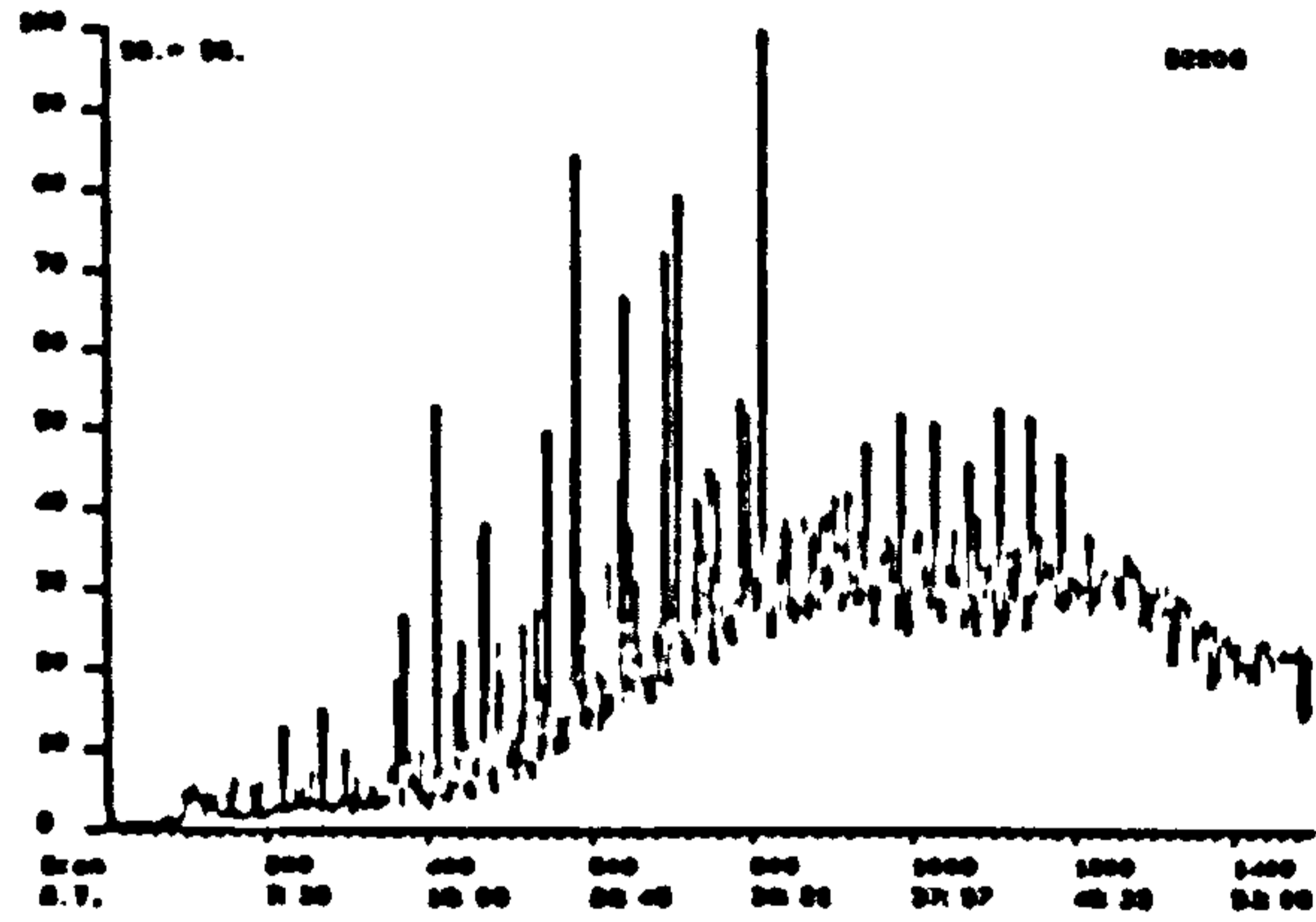
1 Fjacka organic rich layer



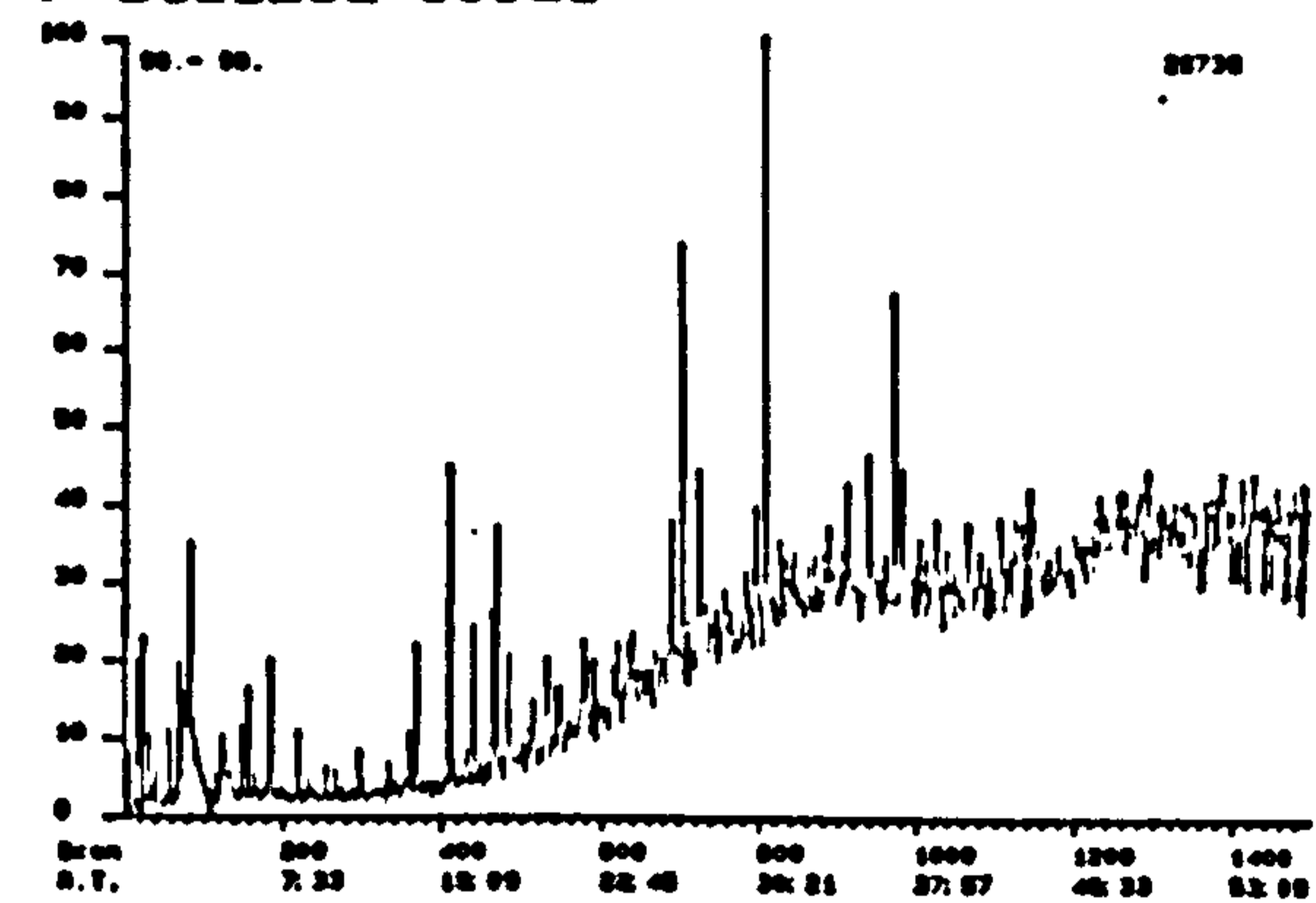
2 Fjacka whole shale



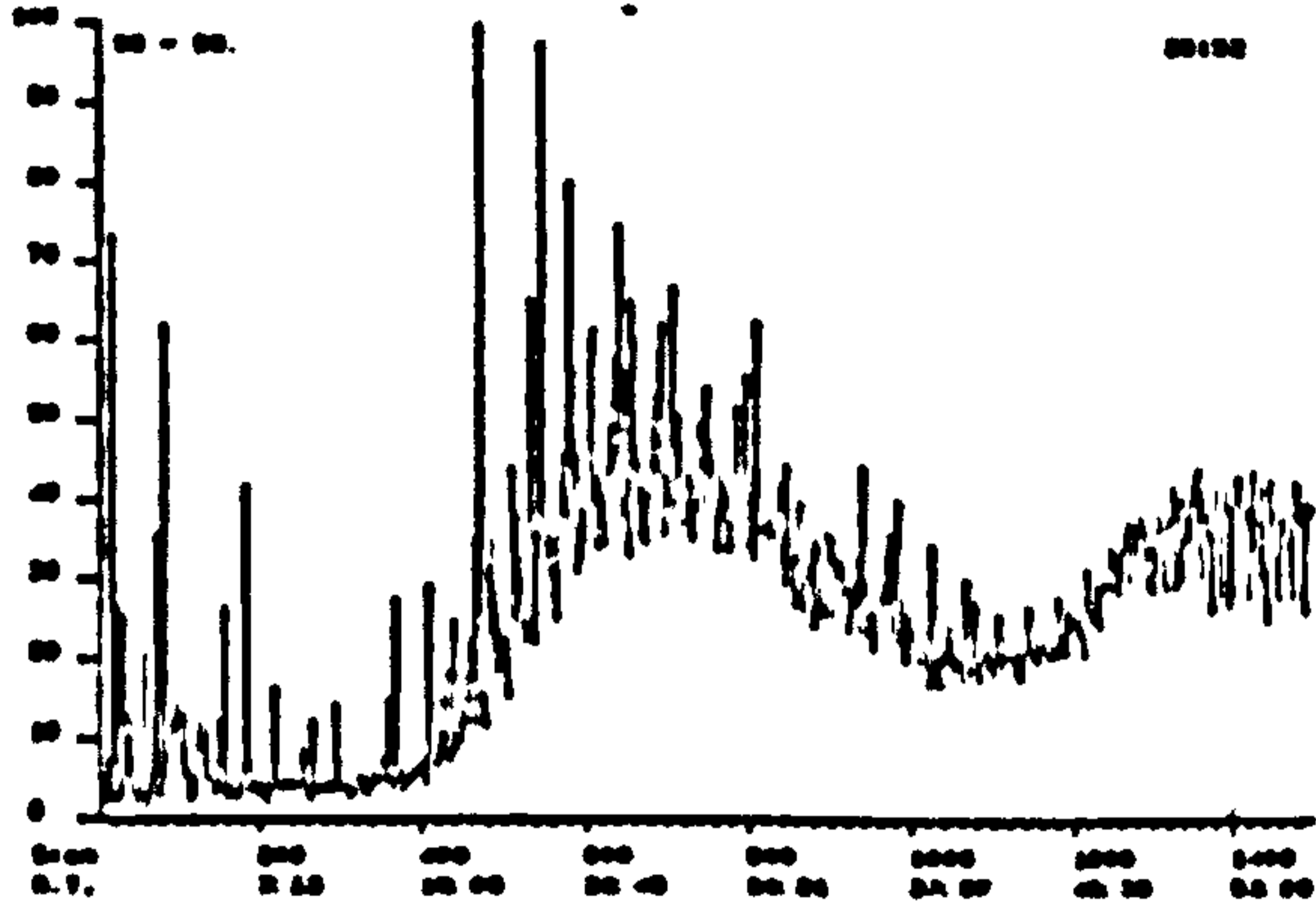
3 Stained nodule



4 Stained coral



5 Alum shale



6 Solberga oil

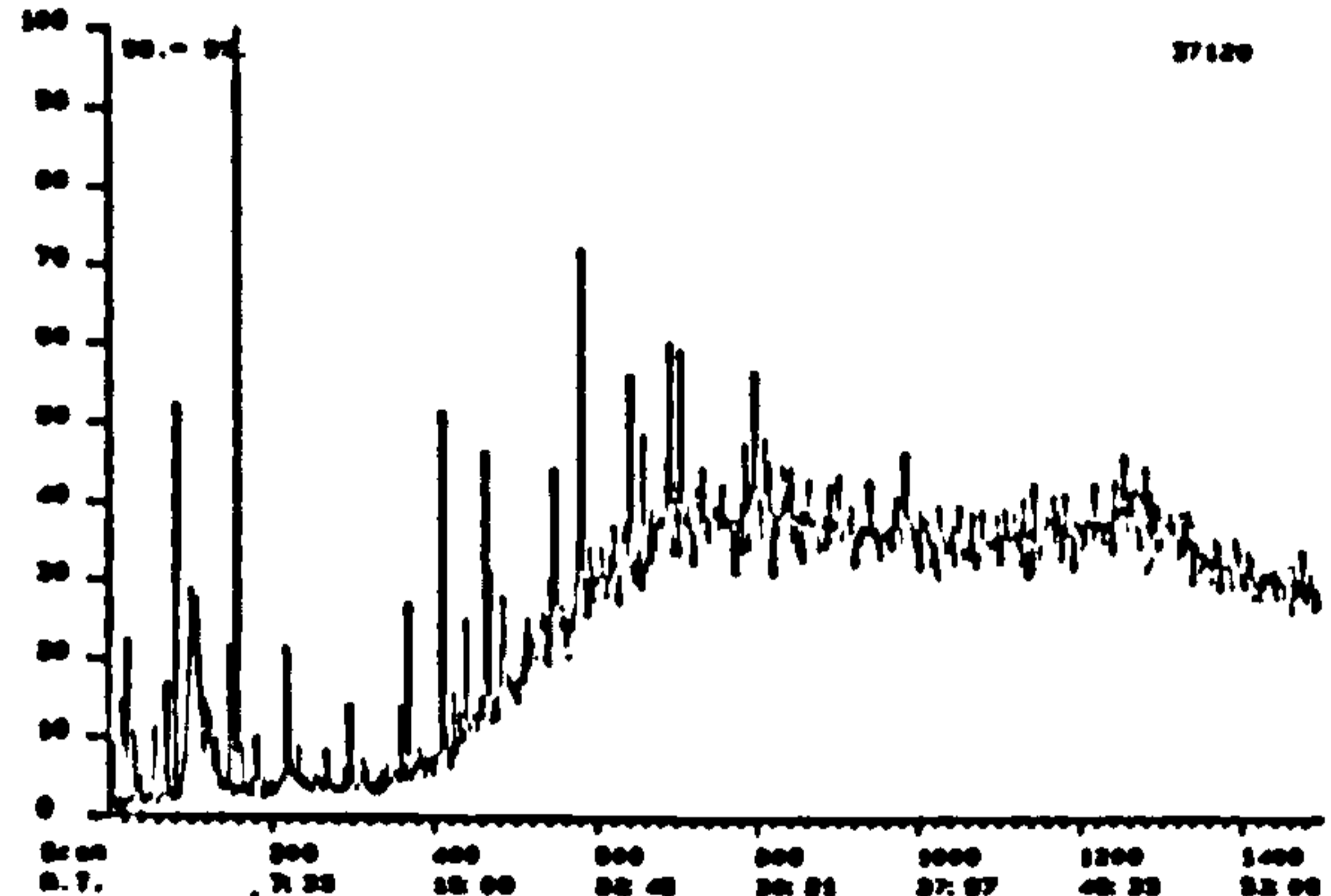


FIGURE 4.44. M/Z 99 CHROMATOGRAM FOR THE OXIDATION PRODUCTS OF UCMS ISOLATED FROM THE SILJAN RING SAMPLES (J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas)



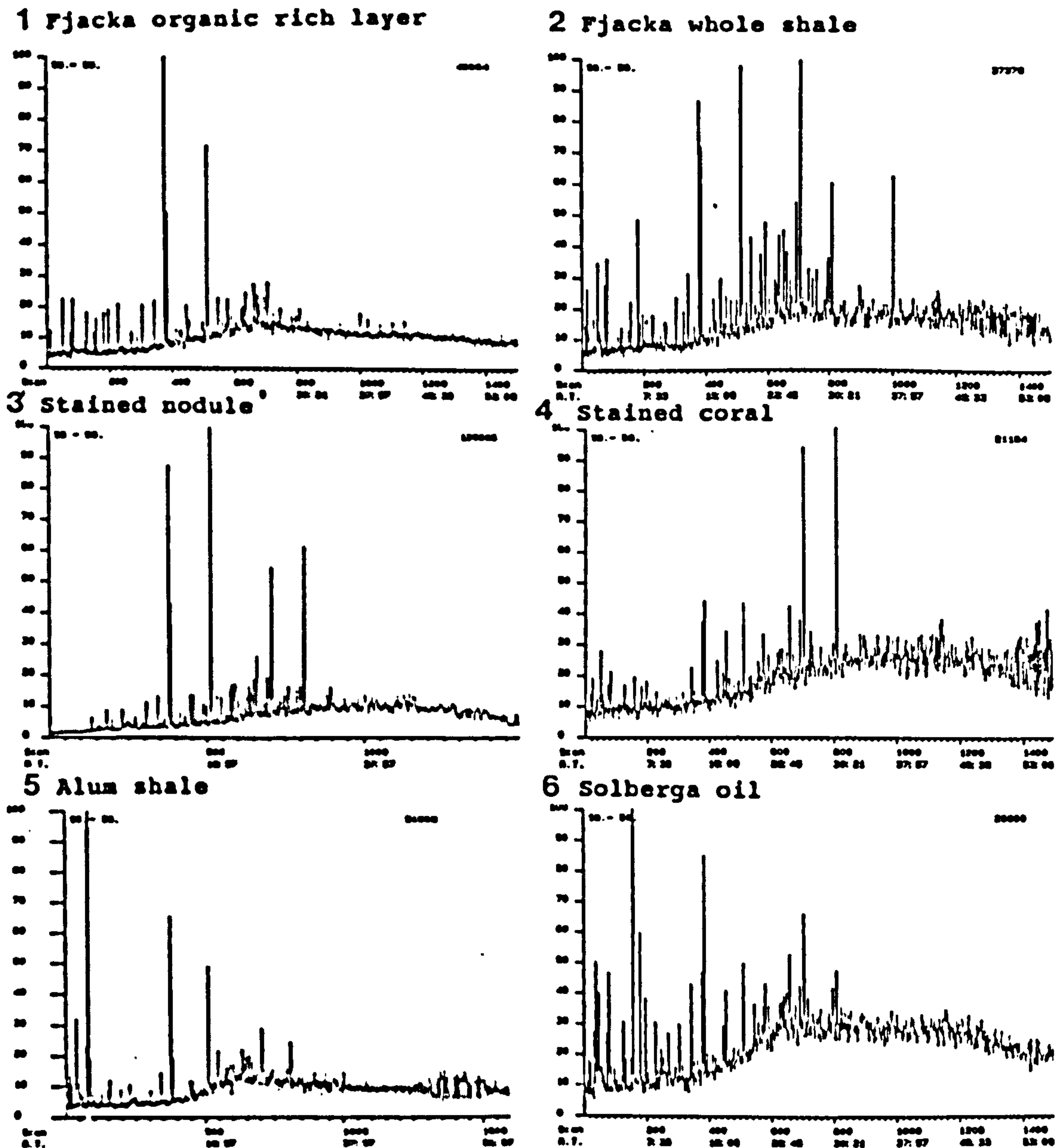
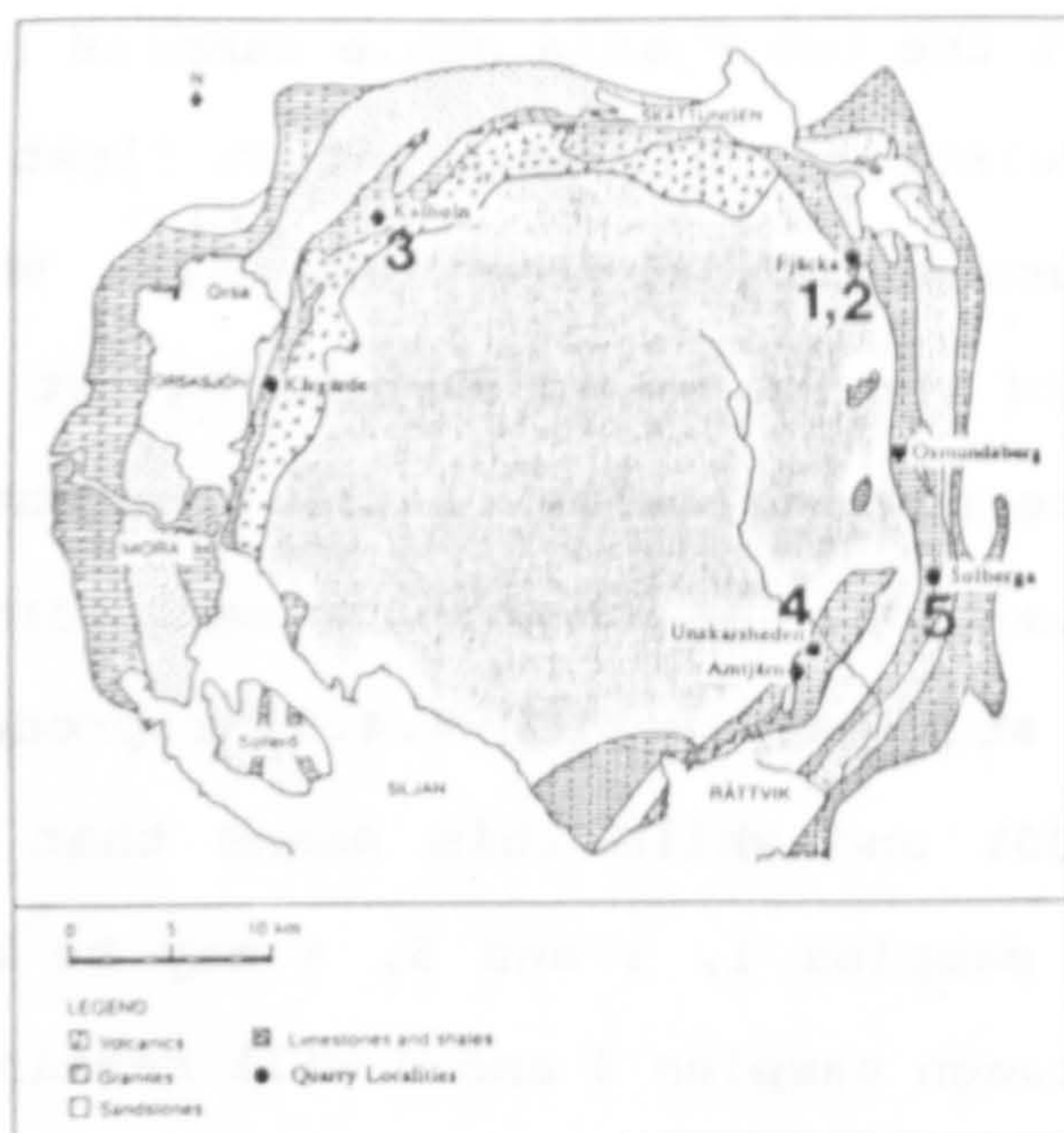


FIGURE 4.45. M/Z 99 CHROMATOGRAM FOR THE OXIDATION PRODUCTS OF UCMS ISOLATED FROM THE SILJAN RING SAMPLES (J&W DB-5, 25m, 40-300°C @ 5°C/min., He carrier gas)

results shown in Figure 4.46. Taking into consideration the previous data (e.g. biomarker analysis) it is not surprising that the two Fjacka shale samples (samples 1 and 2) do not correlate as well as might at first be expected. The strong correlation between the Alum shale and the organic layer of the Fjacka is surprising but it is unclear what effect the high maturity of the Alum shale will have upon UCM composition. It is worth remembering that in the previous case studies (section 4.4.3) reproducibility was shown to be 80% and while this means that the relative clustering of samples 1, 2 and 5, 6 may be affected, the difference between samples 3 and 4 will remain great. Most notable, however, is the strong dissimilarity of the two seep samples (3 and 4), both with each other (44% similar) and with the rest of the samples (only 27% similar).

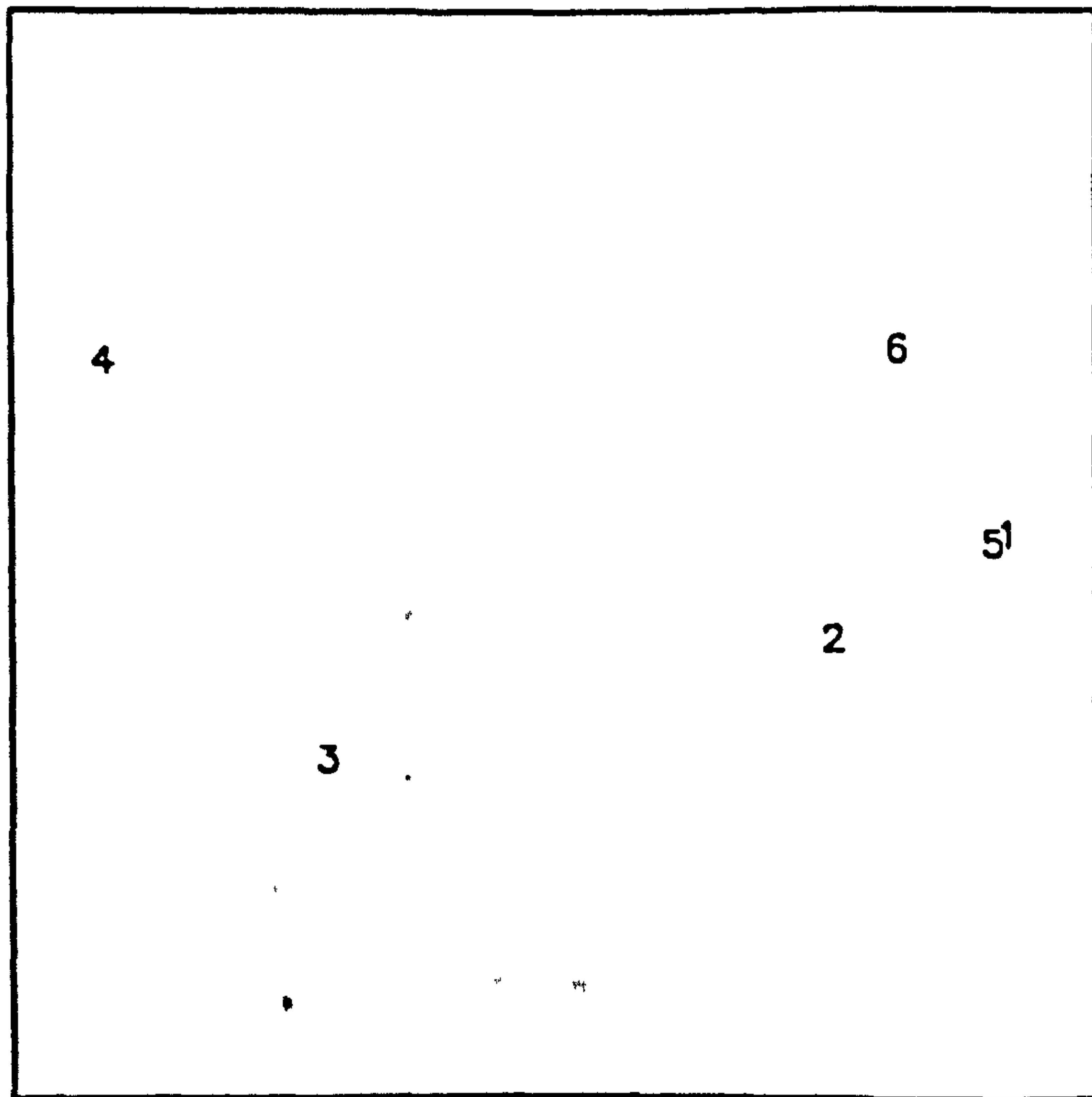
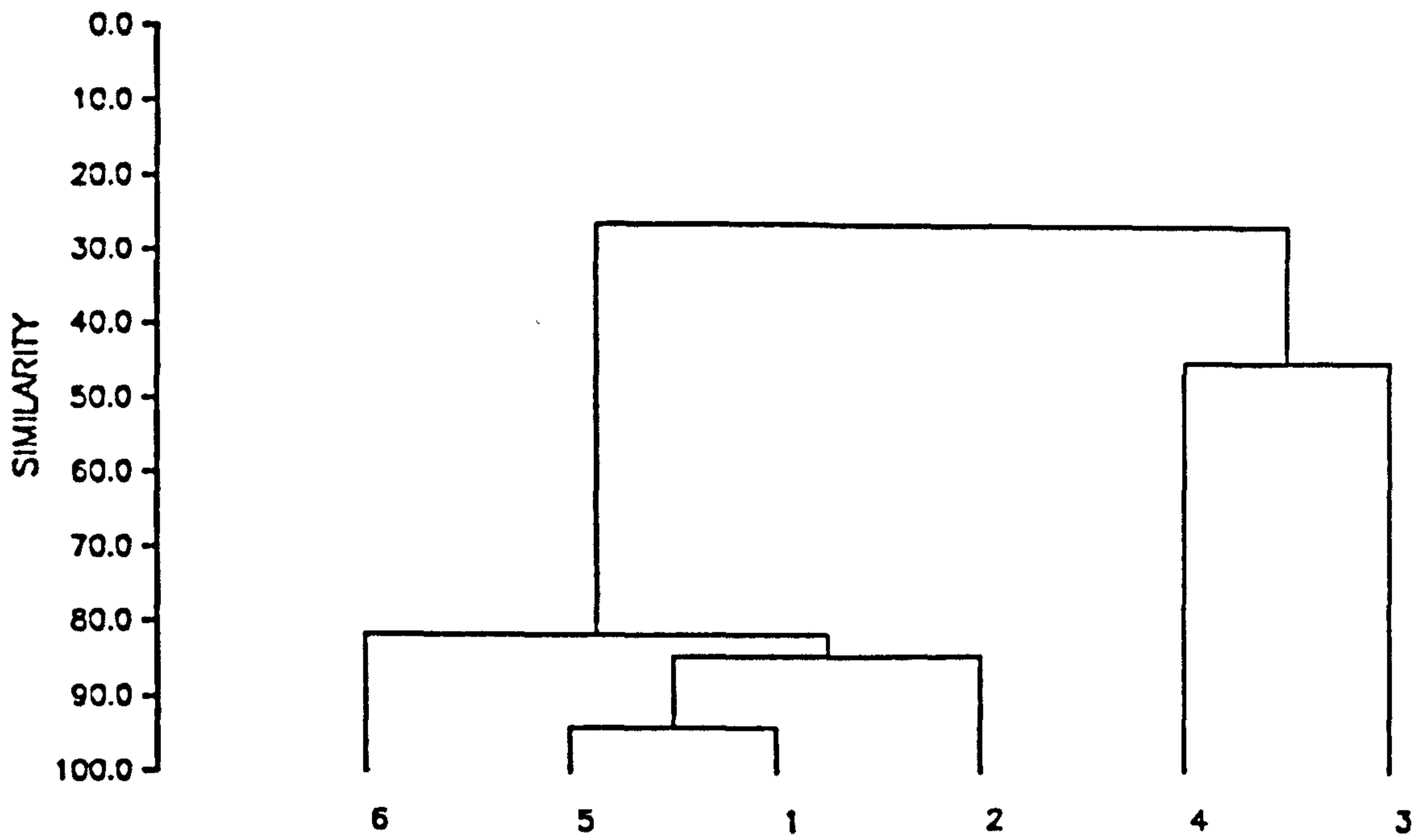
Comparison of the chromatographic data using the Peak match program, which produces fewer variables (only those peaks present in all samples), produced essentially the same results (Figure 4.47).

It is unclear at present as to why the two seep samples are so different from the other samples. Indeed, when this data set was analysed with the samples discussed earlier (section 4.4), the extreme difference of these two samples caused all 17 other samples to correlate so strongly that they coincided on the 2-D plot. It is conceivable that these differences are caused by some effect during



### KEY TO SILJAN RING SAMPLES

sample #	Identity
1	Fjacka Shale (Organic rich layer)
2	Fjacka Shale (Whole shale)
3	Stained Calcareous nodule
4	Stained Coral reef
5	Alum Shale
6	Solberga Oil



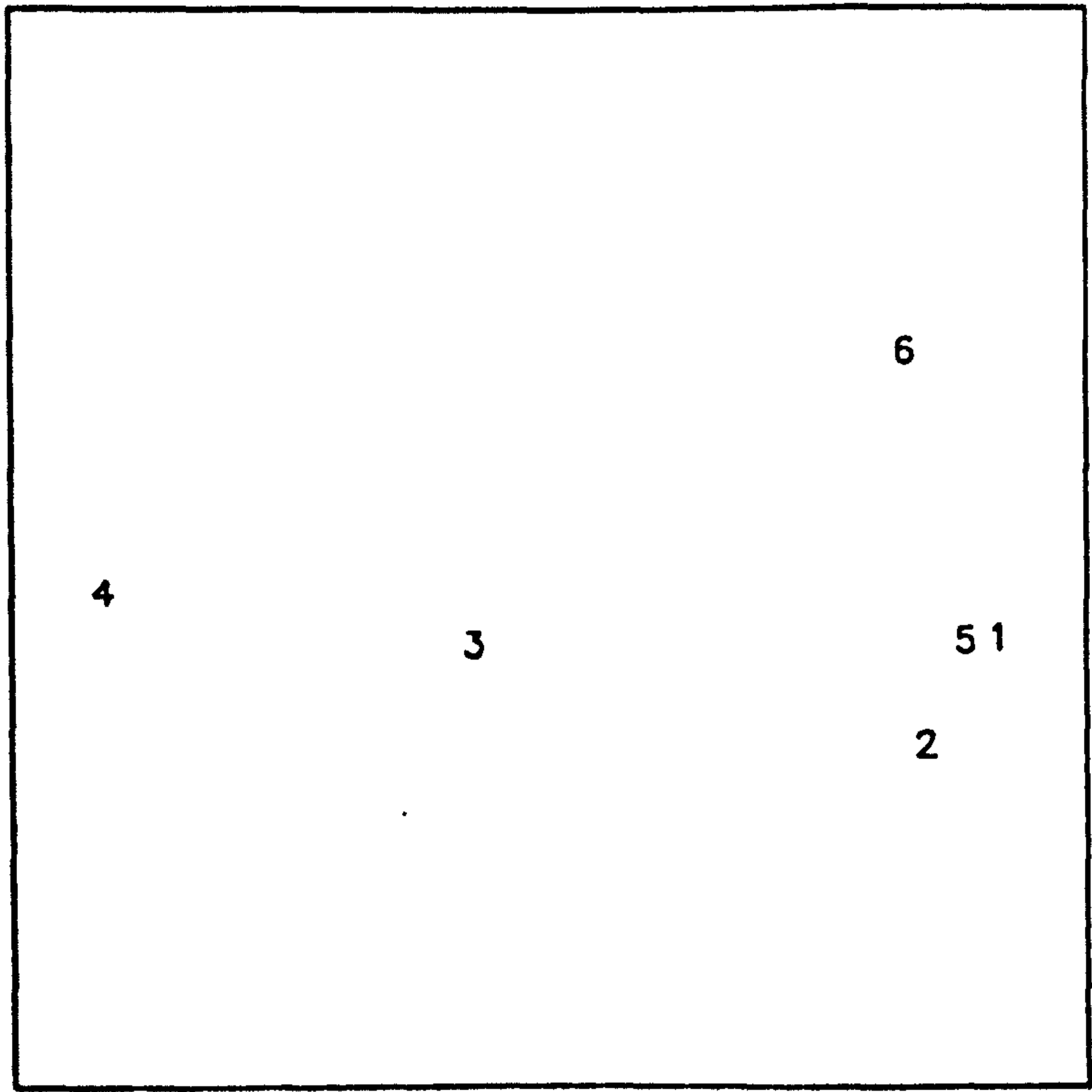
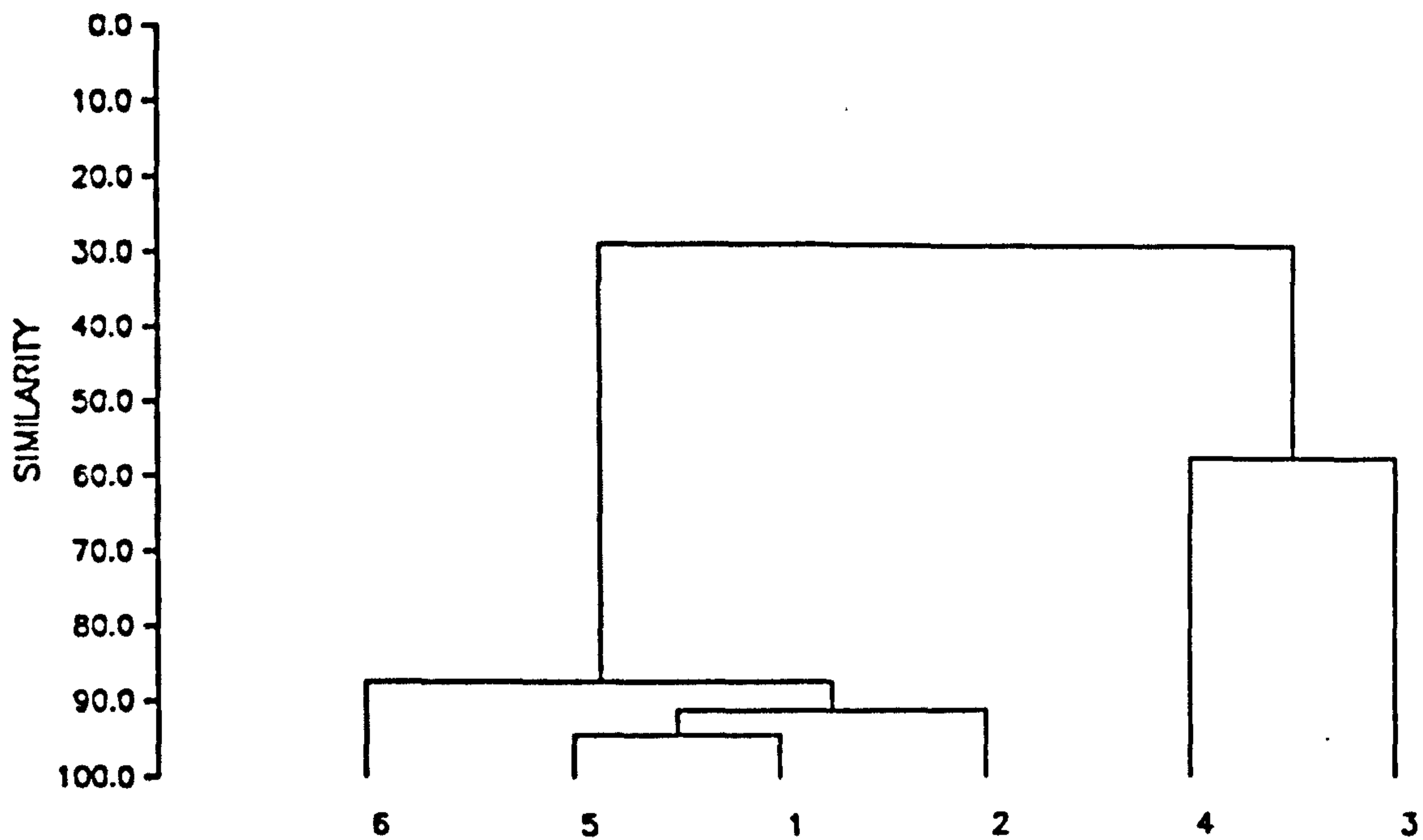
**FIGURE 4.46. RESULTS OF STATISTICAL ANALYSES OF THE UCM OXIDATION PRODUCTS FOR THE SILJAN RING SAMPLES: CHROMATOGRAMS COMPARED USING THE DBMATCH PROGRAM (for sample identities and locations see key opposite)**





#### KEY TO SILJAN RING SAMPLES

sample #	Identity
1	Fjacka Shale (Organic rich layer)
2	Fjacka Shale (Whole shale)
3	Stained Calcareous nodule
4	Stained Coral reef
5	Alum Shale
6	Solberga Oil



**FIGURE 4.47. RESULTS OF STATISTICAL ANALYSES OF THE UCM OXIDATION PRODUCTS FOR THE SILJAN RING SAMPLES: CHROMATOGRAMS COMPARED USING THE PEAK MATCH PROGRAM (for sample identities and locations see key opposite)**

oxidation, but it would appear too strong a coincidence for both seep samples to be affected rather than one seep and one shale sample. In addition, both samples gave good recoveries of material after oxidation (Table 4.10) and a reasonable distribution of oxidation products (Figures 4.42-4.45) which might not be expected if say over or under oxidation had occurred. Thus, with the information available at present, there is no reason to suspect that these differences are anything but genuine.

#### 4.5 SUMMARY

Oxidative degradation of UCMs, followed by multivariate statistical analysis of the oxidation product profiles has been used in an attempt to fingerprint hydrocarbon residues in cases where conventional biomarker analysis has proved inconclusive. Initial validation of a microscale method gave good reproducibility, and the method appears to correlate sediment hydrocarbons with the likely source oil, based on evidence from known oil spills (Section 4.4.3). Results from the analysis of Sullom Voe sediments suggests that the low level of hydrocarbon contamination present in these samples is due mostly to a chronic accumulation of general background hydrocarbons. Subsequent attempts to correlate two oil seep samples in the Siljan ring region, Sweden, with their possible source, failed to produce any correlation, but instead suggested a large difference between the seep samples and any of the suggested sources.

However, the differences observed do appear to be supported by the biomarker analysis.



## **C H A P T E R F I V E**

### **OVERALL CONCLUSIONS AND FUTURE RESEARCH**

*This study has extended present knowledge of UCM composition and suggested a mechanism for UCM formation. Furthermore, quantitative and statistical analysis of UCM oxidation products has been shown to be useful for oil identification. There is still much to be learned about UCMs and the subject should provide a fruitful area for further research. Some possible approaches are suggested.*

Now, in perusing what follows, the reader should bear in mind not only the general circuit as adumbrated above, with many sidetrips and tourist traps, secondary circles and skittish deviations but also the fact that far from being an indolent partie de plaisir, our tour was a hard, twisted teleological growth whose sole raison d'être (these French clichés are symptomatic) was to keep my companion in passable humour from kiss to kiss.

V.V. Sirin, Taina (p. 260)  
translated from the Russian by  
Vivian Darkbloom as Tatyana (p. 19)

## OVERALL CONCLUSIONS AND FUTURE RESEARCH

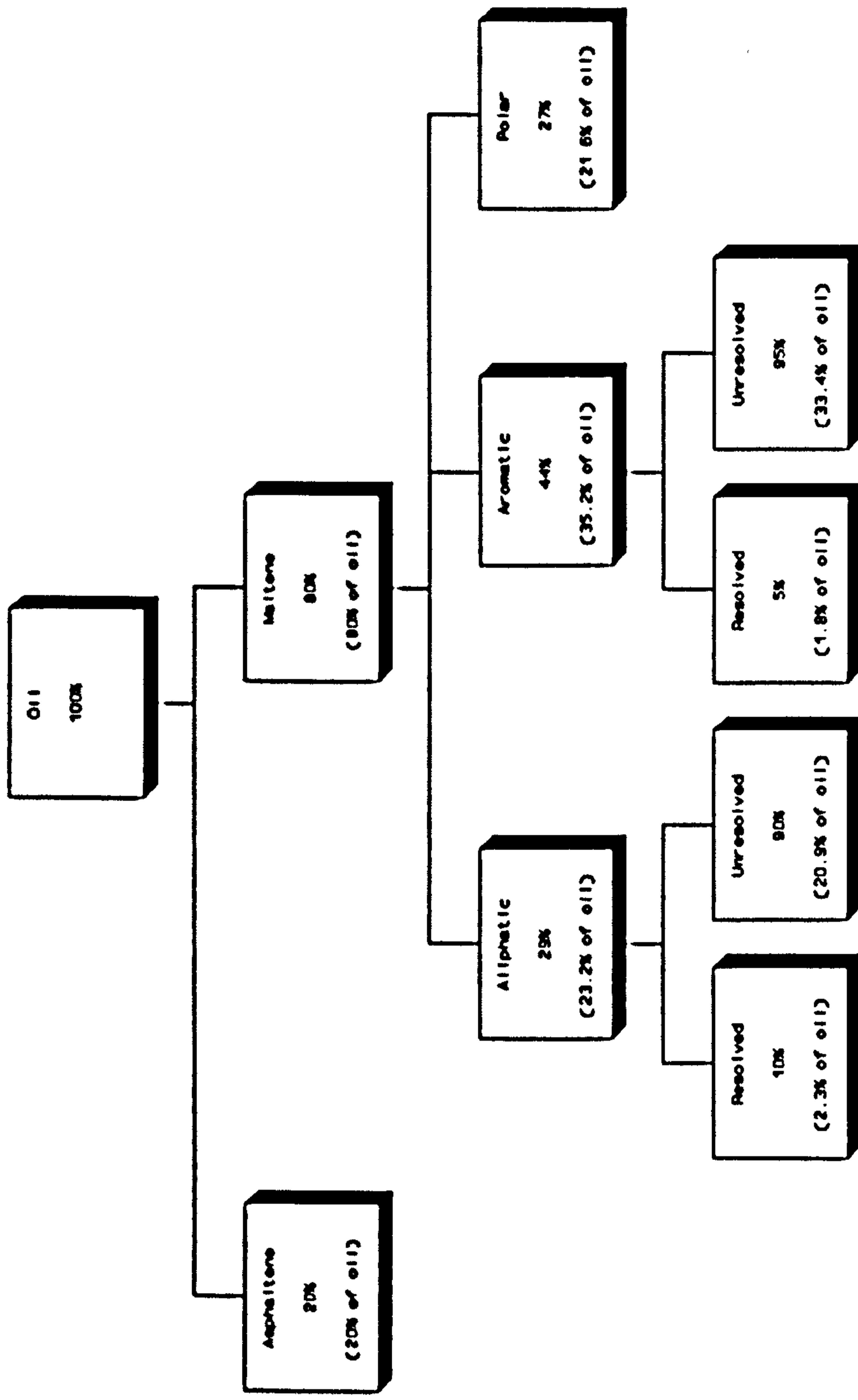
### 5.1 OVERALL CONCLUSIONS

Although crude oil has been used and studied for many years, very little is still known about the majority of its components. For example, if we consider a heavy asphaltic crude oil (Tia Juana Pesado, Venezuela) the fractions of the oil we know little or nothing about in terms of composition (asphaltenes, aliphatic and aromatic UCM and polar compounds) total 95% of the crude (Figure 5.1). Central to this are the aliphatic and aromatic UCMs (21% and 33% of the oil, respectively) since it is these fractions that often assume a greater importance due to weathering and refining. While this oil is an "extreme" example, with respect to petroleum typically produced worldwide, the aliphatic UCM can account for 15-20% of a fresh "average" crude oil.

Despite the ubiquitous and often abundant nature of the UCM, little is known of its detailed composition (especially for the aromatic UCM) and of possible precursors to UCM compounds, although a petrogenic origin is widely accepted.

The main aims of this study were threefold:-

- (1) To elucidate the UCM composition by analysis of an



**FIGURE 5.1. TIA JUANA PESADO CRUDE OIL COMPOSITIONAL DATA**



aromatic UCM.

- (2) To investigate possible precursor compounds, which during catagenesis may yield a UCM.
- (3) To use information about aliphatic UCM composition in conjunction with multivariate statistical techniques to investigate the fingerprint potential of UCMs.

Due to the difficulty of removing resolved aromatic compounds (cf. urea adduction) an in-reservoir biodegraded crude oil, Tia Juana Pesado (Venezuela) was chosen as this exhibited an aromatic fraction which was 95% unresolved by GC (KI 1200-KI 3500+). Conventional GCMS indicated the presence of alkylbenzene and naphthoaromatic compounds, however, no detailed molecular structural information could be deduced and the data was not quantitative.

With only limited information having been gained from GC and GCMS analysis, the aromatic UCM was subjected to analysis by a variety of techniques including IR, UV, UVF, <sup>1</sup>H- and <sup>13</sup>C-NMR, FDMS and elemental analysis. IR and NMR proved useful in determining bulk characteristics, which suggested a highly aliphatic nature for the aromatic UCM and little evidence for long chain alkylbenzene structures, contradicting the historical view that these were major components of the aromatic UCM. These techniques also

provided good evidence for the presence of naphthenoaromatic compounds. FDMS allowed the distributions of a wide range of compound classes to be examined. However, no one group appeared to dominate the UCM, although instrument resolution was insufficient to prevent overlap between various compound classes.

Whilst the information gained from these techniques was useful, they yielded limited detailed structural information. As a result, an alternative approach was adopted which utilised chemical degradation of the UCM compounds. Oxidation with  $\text{CrO}_3$  produced good yields of recovered material (>80%) of which 25% was resolved by GC. The major oxidation products were *n*-monocarboxylic acids ( $\text{C}_5$ - $\text{C}_{23}$ ) with some odd over even predominance. These could result from oxidative cleavage at benzylic or tertiary carbons. The presence of ketones and lactones as minor oxidation products is seen as evidence for methyl branching of the aliphatic side chain.

The lack of specificity of  $\text{CrO}_3$  as an oxidant led to an alternative more specific reagent,  $\text{RuO}_4$ , to be used. This oxidant is known to attack aromatic rings specifically whilst leaving aliphatic side chains intact.  $\text{RuO}_4$  oxidation produced good yields of recovered material (48-98%) but surprisingly few resolved components (5% by GC) which were predominantly *n*-carboxylic acids  $\text{C}_9$ - $\text{C}_{22}$  with a strong even over odd predominance. Interestingly, certain chain



lengths were absent, namely  $C_{17}$ ,  $C_{19}$ , and  $C_{21}$ , which, due to the mode action of  $RuO_4$ , correspond to actual chain lengths of  $C_{16}$ ,  $C_{18}$ , and  $C_{20}$  respectively. In addition, the  $m/z$  74 mass chromatogram, used to detect carboxylic acid methyl esters, exhibited a strong UCM. The composition of this unresolved portion was investigated by first isolating the acid fraction via a hydrolysis and back extraction and then subjecting the isolated fraction to NMR, GCMS and other bulk characterisation techniques. These confirmed the presence of acids and the possibility of diacids being present. These diacids could reasonably be expected to occur due to the presence of naphthenoaromatics in the UCM, and this was further supported by the presence of dimethylphthalate in the chromic acid oxidation products. There was also evidence from GCMS of the  $RuO_4$  oxidation products that the aliphatic side chains could possess cyclic moieties. Thus, it is proposed that the aromatic UCM consists of mainly naphthenoaromatic compounds with branched aliphatic side chains which may or may not contain a cyclic moiety. Such compounds consisting of a tetralin structure with a monoalkyl branched side chain, terminating in 0-2 cyclohexyl rings between  $C_{20}$  and  $C_{30}$  were shown, via a purpose written computer program, to be able to exist as at least, 1,213 isomers. Under typically employed GC conditions (DB-5 (J&W), 25m, 40-300°C 5°C min<sup>-1</sup>),  $C_{20}$  to  $C_{30}$  n-alkanes require 17 minutes (1020 seconds) for complete elution. In this region, baseline peak width was estimated at ca. 10 seconds, thus allowing for elution of 102

compounds with baseline resolution. Therefore, it is apparent that compounds of this type could readily account for the unresolved nature of the UCM. These findings are in agreement with those of Rossini et al. (1953) who, after exhaustive research into petroleum hydrocarbons (API Research Project 6), proposed that mononuclear, and at higher molecular weights, dinuclear aromatic compounds "consisting of one [or two] aromatic ring[s], with one, two or three cycloparaffin rings, probably most frequently condensed, together with paraffin side or connecting groups as appropriate ..." were the major aromatic compounds. Thus, nearly 40 years after this work was first published, the methods used in this study appear to confirm their conclusions.

A common principle within organic geochemical studies is that of "retro-analysis", i.e. to start with a known structure and attempt to formulate ideas about possible precursor compounds. Similarly, the structural information gained from the aliphatic (Gough 1989; Gough and Rowland, 1990) and the aromatic UCM (this study) would appear to lend itself to this type of approach. Acceptance of a petrogenic origin for the UCM suggests that formation of this complex mixture occurs during catagenesis and that the precursor compounds are in some way associated with the kerogen.

Laboratory maturation of kerogen (hydrous pyrolysis)



produces resolved compounds which often are associated with a significant UCM, although this is frequently ignored. While this suggests that the UCM does indeed have kerogen based precursors, it does not yield information as to what type of structures these may be. In order to investigate this further, two types of material were subjected to artificial maturation to see if one or both could yield a UCM.

The first, a man-made polymer (Alkathene) yielded both resolved and unresolved compounds with the latter containing both saturated and unsaturated components. Conventional analytical techniques indicated that the saturated unresolved compounds contain a high degree of branching and/or cyclicity, while the unsaturated fraction appeared to be predominantly alkenes with some aromatics, and a much reduced level of tertiary centres. Thus, from this evidence, it certainly appears that the saturated UCM consists of structures similar to those proposed by Gough and Rowland (1990) i.e. a cyclic moiety with a branched alkyl side chain. Chemical oxidation of this UCM produced a series of resolved compounds, a result similar to that from petrogenic UCMs. However, these resolved components contain a relatively high proportion of *n*-diacids and ca. 70% resolved material (compared with 20% from petrogenic UCMs). Both these factors suggest a degree of over oxidation, possibly due to this UCM comprising more simply branched structures than petrogenic UCMs.

The second material subjected to artificial maturation was cutan, isolated from the cuticle of *Agave americana*. This type of macromolecule, proposed to be geochemically resistant, has received wide interest as an alternative means to kerogen formation. Many workers have subjected this material to hydrous pyrolysis and shown the formation of resolved compounds but, again, the presence or otherwise of a UCM appears to have been ignored. The experiments in this study indeed produced a series of *n*-alkanes in high abundance, but removal of these showed the presence of a UCM. This unresolved portion could be further fractionated into hydrocarbon and non-hydrocarbon fractions, of which the latter appeared to contain the more significant UCM. Instrumental analysis of the non-hydrocarbon fraction indicated the presence of functionalised compounds (aldehydes, ketones etc.) while the hydrocarbon fraction comprised branched, cyclic and to a lesser extent aromatic compounds.

Chemical oxidation of the hydrocarbon fraction again produced a series of resolved components but again these included a relatively high proportion of *n*-diacids, though these were not as dominant as in the Alkathene experiments. Importantly, the oxidation products also included  $\gamma$ -lactones and ketones which were absent in the Alkathene experiment, but have been found in every oil UCM oxidised to date.



These experiments show that a polymethylenic chain will, upon artificial maturation yield a UCM. However neither of the UCMs produced in this study are exactly like those of oils, the cutan macromolecule appears to contain structures and/or functionalities which, when pyrolysed produce a UCM more similar to those in oils than the Alkathene.

Thus, it would appear possible that the type of organic matter incorporated into the kerogen structure plays an important role in UCM formation and composition. If this is the case, the UCM should contain some palaeoenvironmental information which may then allow different UCMs to be distinguished on the basis of their content or in this case, oxidation products.

The correlation of biodegraded oils, both with each other and their source rocks, or with a fresh crude oil has long been a problem in exploration geochemistry and pollution studies. Commonly, the so called biomarkers have been used, but these may be altered beyond recognition in heavily degraded oils. However, a common feature of biodegraded and weathered oils is a prominent UCM and in this study it is UCM composition via chemical oxidation, that has been used to correlate sediments contaminated with oil with the likely source oil. This involves comparison of complex chromatograms necessitating the use of multivariate statistical techniques and various purpose written computer programs. This approach has two major advantages; first it

allows a large number of samples to be compared relatively quickly and secondly, the final analysis/result is non-subjective.

Application of this method to a variety of environmental case studies and reproducibility tests produced encouraging results, with samples known to be the same correlating well. A surprising result from these studies is that analysis of the data by principal components analysis, a technique previously thought to be unsuitable, produced results which are in close agreement with those from multi-dimensional scaling. This allows the variables responsible for the majority of variation, namely the n-acids to be identified. Removal of these components from the data matrix with subsequent re-analysis by PCA produces some different but not altogether unreasonable correlations within the samples. This indicates that in some cases the n-acids are responsible for the similarity between samples but in others they produce a difference which may be misleading. Thus the UCM "fingerprinting" method may be subject to similar problems as those associated with biomarker analysis in that a confused result occurs when mixtures are present.

The early success of the method led to it being applied in an attempt to correlate two oil seep samples from the Siljan Ring region, central Sweden with their proposed sources. This area is of interest due to the presence of



oil staining in a predominantly igneous region. While the samples are predominantly UCMs, the method fails to find any real correlations, except that the two samples of staining are very different from any of their proposed sources.

The UCM is quantitatively important in most oils and for this reason more detailed studies of the compounds present should be carried out, both to increase our knowledge of oil composition in general and for a better understanding of oil formation. As this study has shown, artificial maturation of polymethylenic chains can yield a UCM, but the end result is different depending upon the starting material. A combination of structural and source information may lead to the UCM supplying additional geochemical information, not only as a fingerprint but about maturity or palaeoenvironment.

## **5.2 PROPOSALS FOR FUTURE RESEARCH**

Suggestions for further research suggested here follow on from the results of this study and as such relate to the three general areas covered by this work, namely, UCM composition, origin and possible geochemical information.

1. Further information on UCM composition, both aliphatic and aromatic, is required. For the aliphatic UCM, alternative oxidants should be investigated. These

are few, but one is chromyl chloride ( $\text{CrO}_2\text{Cl}_2$ ) which is soluble in aprotic solvents (e.g. DCM; Cainelli and Cardillo, 1984). In addition, other bulk characteristics could be determined by techniques such as NMR and FDMS (or FIMS).

For the aromatic UCM, ruthenium tetroxide proved a good oxidant due to its specificity but there was a very significant proportion of the oxidation products which were oxidised but still unresolved. These were only briefly investigated in this study but are obviously important with respect to UCM structure. One method of analysis might be to adduct the *n*-acids with urea and then examine the unresolved fraction by either instrumental techniques (e.g. NMR, FABMS) or chemically by further oxidation.

2. It appears that all UCM studies so far, have concentrated on the whole fraction but the UCM is unresolved because it is such a complex mixture. If, however, it could in some way be fractionated, it may be possible to achieve better resolution or oxidation. One method may be GPC and this was pursued to some extent by Gough (1989). This would produce a fractionation by size which may then yield more specific information when analysed. A second technique is the so called GC "heart-cut" (e.g. Schaefer and Holtkemeier, 1988). This generally

involves taking a small cut from the GC analysis and then re-analysing this fraction isothermally on a different stationary phase, or on a slow temperature ramp. Coupled with mass spectrometry this could prove to be a very powerful tool. Thirdly, the technique of micro-scale distillation (e.g. Mayo et al., 1991) could prove a viable way of fractionating the UCM.

3. A method which may be useful in order to resolve some compounds from the aliphatic UCM is catalysed rearrangements, e.g. the Wagner-Meerwein rearrangement. Although generally used for functionalised compounds, alkanes, when treated with a Lewis acid and an initiator can undergo rearrangement (March, 1985) e.g. a C<sub>10</sub> tricyclic compound will be converted to adamantane (Nomura et al.; March, 1985).
4. A feature of this study was the attempted comparison of degraded oils with relatively fresh ones which necessitated isolation of the UCM by urea and thiourea adduction. While urea adduction is a relatively straight forward and well established technique, thiourea adduction tends to be more unpredictable in nature. While alternatives to urea exist (i.e. molecular sieves) there appears to be none for thiourea. Zeolites are an obvious starting point, and although there are none commercially available with



the correct pore size (7.5-8 Å) it may be possible through collaboration with other institutions to design and synthesise appropriate candidates. Another promising area may be the use of "silicalite" (e.g. Fisher et al., 1991). Since both urea and thiourea appear to remove some UCM, it is necessary to establish the effects of these techniques on the resulting oxidation product profile but this cannot be achieved until the two methods are sufficiently standardised.

5. The use of two aliphatic polymers in this study to produce UCMs has shown that this type of material may play a role in UCM (and oil) formation. Further studies should now be undertaken involving different polymers, both abiogenic (e.g. a highly cross linked polythene) and biogenic (e.g. algaenans, suberans etc.) and possibly mixtures to try and understand the types of precursor structures involved. Also, further characterisation of the unsaturated and non-hydrocarbon fractions produced in this study may help to show the mechanisms by which these UCMs formed.
6. A parameter often important in petroleum geochemistry is that of maturity. Little, if anything, appears to be known about how maturity affects the UCM in terms of composition and if any information is contained within the UCM which could be used as a maturity



parameter. There are reported occurrences of igneous dykes artificially maturing organic matter through the oil window (e.g. Ambler, 1989) and a suite of samples, moving towards the dyke would provide a maturation sequence in which the UCM could be analysed.

7. The fingerprinting studies in Chapter 4 indicate that the lactone and ketone oxidation products may contain some specific information, possibly for maturation and palaeoenvironmental studies. If these could be isolated from the rest of the oxidation products, analysis using chiral GC columns (e.g. cyclodextrin stationary phases) may prove interesting.
8. The case studies in Chapter 4 involved the comparison of biodegraded oils with their possible fresh crude oil precursor. It has been generally assumed that little change occurs, with respect to the bulk of the UCM, during biodegradation of the oil but this is by no means certain. Thus, a suite of samples of known biodegradation states, or even one produced in the laboratory, should be obtained and the UCM from each oxidised and the resulting products compared.

## **C H A P T E R S I X**

### **EXPERIMENTAL DETAILS**

*This chapter describes the analytical procedures used in this study.*

## EXPERIMENTAL

### 6.1 GENERAL PROCEDURES

All glassware was pre-cleaned in either chromic acid or a solution of "Neutracon" (2%), rinsed thoroughly in hot tap water and distilled water and dried (110°C).

Solvents were Rathburns (Walkerburn, U.K.) HPLC or glass distilled grades and purity was routinely checked by rotary evaporation (Buchi, 40°C; 100 cm<sup>3</sup>), transfer to a vial, evaporation to 50 µl (N<sub>2</sub>) and analysis of an aliquot (0.5 µl) by gas chromatography (GC).

Silica gel (BDH, 60-120 mesh) and aluminium oxide (BDH, Grade 1, neutral) adsorbents used for chromatographic separations were Soxhlet extracted (DCM, 24 hr) and dried (40°C) prior to activation. Adsorbents with the required percentage water were prepared according to Gough (1989) by activation (12 hr; SiO<sub>2</sub> @ 185°C, Al<sub>2</sub>O<sub>3</sub> @ 450°C), cooling in a desiccator, addition of water (millipore grade) and homogenisation by mechanical shaking (3-5 hr). TLC plates were prepared on solvent-washed glass plates (20 cm x 20 cm) spread with an aqueous slurry of silica gel (Merck 60 G, kieselgel). Argentatious TLC plates were prepared similarly from a slurry of silica gel made with an aqueous solution of silver nitrate (10% w/v). Freshly spread plates were dried (120°C, 1 hr), pre-eluted (ethyl acetate,

the top 5 cm of adsorbent was removed and the plate re-activated (120°C, 1 hr) prior to use. Argentatious plates were stored in the dark.

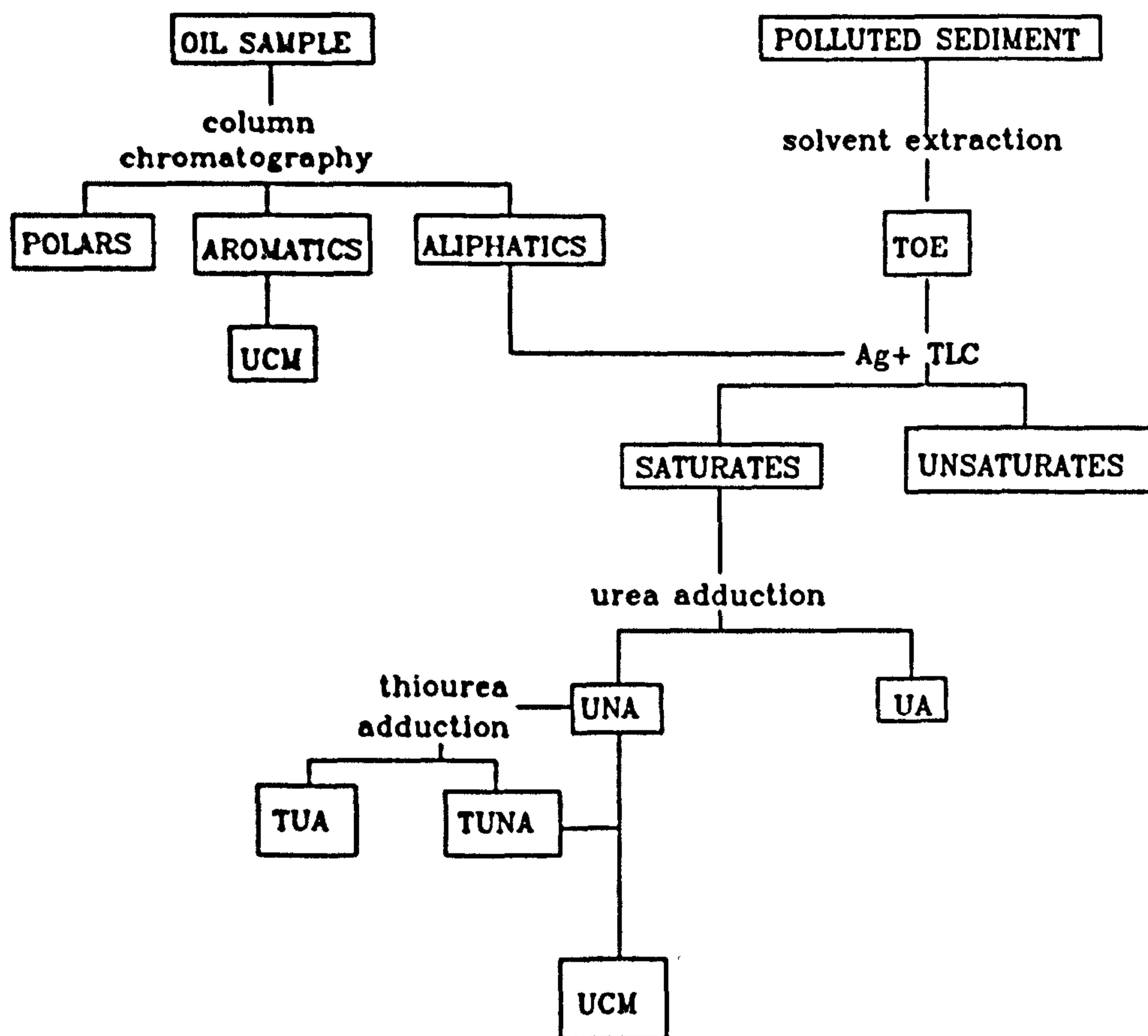
Concentrated hydrochloric acid, mercury, anhydrous sodium sulphate, cotton wool and anti-bumping granules were all pre-extracted (DCM) prior to use.

## 6.2 ISOLATION OF ALIPHATIC AND AROMATIC UCM HYDROCARBONS

The lubricating oil used in this study was the same as that used by Gough, (1989), (Silkolene 150). Samples of Sullom Voe sediment (Garths Voe), Ninian Crude oil, Mersey sediment (Dungeon's lane) and Tia Juana Pesado crude oil were supplied by the Field Studies Council Research Centre (F.S.C.R.C.), and a sample of the Esso Bernicia fuel oil was supplied by the operator of the Sullom Voe oil terminal.

Isolation of aliphatic and aromatic UCMs from oil samples followed the scheme outlined in Figure 6.1. This involved fractionation of the oil by open column liquid solid chromatography (70 cm x 2 cm i.d.) packed with a hexane slurry of silica gel (60-120 mesh; 5% H<sub>2</sub>O-SiO<sub>2</sub>; 40g) under aluminium (Grade 1 - neutral; 1.5% H<sub>2</sub>O-Al<sub>2</sub>O<sub>3</sub>; 20g). Lubricating oil (ca. 2g) was applied in hexane (2 cm<sup>3</sup>) and TJP (ca. 200 mg) was pre-adsorbed onto aluminium oxide and added to the top the column. The column was eluted with





**FIGURE 6.1. ISOLATION OF ALIPHATIC AND AROMATIC UCMS**

hexane (178 cm<sup>3</sup>), DCM (200 cm<sup>3</sup>) and methanol (200 cm<sup>3</sup>) to provide aliphatic, aromatic and polar fractions respectively. TJP Crude was chromatographed according to the method of Davies and Wolff (1990) by elution with hexane (170 cm<sup>3</sup>) to yield an aliphatic fraction and hexane/toluene (3:1; 170 cm<sup>3</sup>) and hexane/toluene (1:1; 170 cm<sup>3</sup>) to yield an aromatic fraction. Following solvent evaporation (Buchi, 40°C) and N<sub>2</sub> blow down, the fractions were weighed.

The aliphatic fraction derived from column chromatographic separation was further purified by argentation TLC with hexane as the mobile phase. The plate was visualised with dichlorofluorescein (0.5% in methanol) and examined under UV light (364 nm). The alkane band was removed, transferred to a small column, and the alkanes desorbed with hexane (2 column volumes) and DCM (1 column volume) and weighed. The separation efficiency of the silver ion TLC plates was monitored by simultaneous analysis of a standard mix consisting of *n*-eicosane (*n*-C<sub>20</sub>), *n*-eicos-1-ene (*n*-C<sub>20:1</sub>), 1-phenyldecane and anthracene, (R<sub>f</sub> values 0.7-0.8, 0.5-0.6, 0.5-0.6, 0.3-0.35 and 0.05-0.2, respectively).

Normal and monomethyl branched alkanes were removed from the total alkane fraction by urea adduction. The total alkanes (ca. 50 mg) were dissolved in hexane/acetone (2:1, 10 cm<sup>3</sup>) and a saturated solution of urea in methanol added dropwise (ca. 1 cm<sup>3</sup>). Solvent was removed (N<sub>2</sub> blow down)

and the process repeated twice. Non-adduct was recovered by the addition of hexane (10 cm<sup>3</sup>), ultrasonic agitation (Soniprep bath, 30 s) and centrifugation (2,000 rpm, 10 min). The supernatant was filtered through a defatted cotton wool plug and the process repeated with fresh solvent. The extracts were combined, evaporated (Buchi, 40°C) and the UNA alkanes weighed.

The urea adduct (UA) was recovered by the addition of water (millipore grade, 10 cm<sup>3</sup>), transfer to a separating funnel and extraction with hexane (3 x 10 cm<sup>3</sup>). The extract was dried (anh. Na<sub>2</sub>SO<sub>4</sub>), solvent removed (Buchi, 40°C; N<sub>2</sub> blowdown) and UA fraction weighed.

The UNA fraction provided the aliphatic UCM. The aromatic UCM was the aromatic fraction taken directly from column chromatography.

### **6.3 ISOLATION OF ENVIRONMENTAL UCM HYDROCARBONS**

Sediment samples were supplied frozen in aluminium containers. The thawed samples were solvent extracted following the methods of Douglas et al. (1981). Sediment (ca. 50 g wet weight) was extracted with methanol (40 cm<sup>3</sup>) by ultrasonic agitation (Soniprep<sup>®</sup> 150 probe, 2 x 5 min, stirring in between) 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 cm<sup>3</sup>) was added, and the organic fraction collected. The aqueous layer was further extracted with DCM (3 x 15 cm<sup>3</sup>), the solvent extracts combined, dried (anh. Na<sub>2</sub>SO<sub>4</sub>), concentrated (Buchi, 40°C, N<sub>2</sub> blowdown) and weighed. The total alkane fraction of each sediment and reference oil were isolated directly by argentatious TLC, and urea adduction.

The Ninian crude oil UNA fraction still contained predominantly resolved compounds (acyclic isoprenoids) and was therefore subjected to thiourea adduction, according to the method of Rubinstein and Strausz, (1979). The aliphatic UNA fraction (50 mg) was de-sulphurised with spongy copper (Blumer, 1957), and dissolved in chloroform (12.5 cm<sup>3</sup>) and a saturated solution of thiourea in methanol added (1 cm<sup>3</sup>). The solution was warmed to achieve complete dissolution of the thiourea crystals and the solution allowed to cool slowly at ambient temperature and then at 4°C. This caused the formation of needle shaped crystals. Non-adducted hydrocarbons (TUNA) were recovered by the addition of hexane, (10 cm<sup>3</sup>) and ultrasonic agitation (Soniprep bath, 30s) and the supernatant filtered through defatted cotton wool. The above procedure was repeated with a further 12.5 cm<sup>3</sup> chloroform (forming needle-like crystals) and 5 cm<sup>3</sup> chloroform (forming rhombic crystals). The thiourea adducted (TUA) fraction was recovered by solubilisation of the crystals in warm water (millipore grade, 5 cm<sup>3</sup>) followed



by extraction with hexane (2 x 10 cm<sup>3</sup>), drying (anh. Na<sub>2</sub>SO<sub>4</sub>) and concentration (Buchi, 40°C, N<sub>2</sub> blowdown). The thiourea non-adducted (TUNA) fraction was utilised as the UCM.

#### 6.4 OXIDATION OF HYDROCARBON UCMs AND MODEL COMPOUNDS

##### 6.4.1 CHROMIUM TRIOXIDE OXIDATIONS

Aliphatic and aromatic UCM material was oxidised with chromium trioxide (CrO<sub>3</sub>, Aldrich) following the method of Gough (1989). The sample (ca. 50 mg) was added to glacial acetic acid (May and Baker, AR grade, 10 cm<sup>3</sup>) in a two necked RBF (25 cm<sup>3</sup>) equipped with a reflux condenser. The solution was pre-heated to 70°C (± 2°C, water bath) with stirring for 5 min, followed by the addition of the oxidant (CrO<sub>3</sub>) at a 10:1 molar ratio of oxidant to substrate, assuming 352 g mole<sup>-1</sup> (C<sub>25</sub>H<sub>52</sub>) for the aliphatic fraction and 344 g mole<sup>-1</sup> (C<sub>25</sub>H<sub>44</sub>) for the aromatic UCM. The solution was maintained at 70°C, with stirring (60 min), cooled (iced water bath), and transferred to a separating funnel with water (millipore, 10 cm<sup>3</sup>) and DCM (10 cm<sup>3</sup>). The organic layer was removed and the aqueous layer re-extracted with DCM (2 x 10 cm<sup>3</sup>). The combined organic extract was back washed (millipore grade water, 2 x 10 cm<sup>3</sup>), dried (anh. Na<sub>2</sub>SO<sub>4</sub>) and the volume reduced to near dryness (Buchi, 40°C). The extract was then hydrolysed using methanolic KOH (10% KOH/Methanol w/v) under reflux (30 min), cooled at ambient temperature, acidified to pH 1 (conc. HCl), water

added (millipore grade, 5 cm<sup>3</sup>) and the total hydrolysed material extracted into DCM (1 x 10 cm<sup>3</sup>, 2 x 5 cm<sup>3</sup>). The combined extracts were dried (anh. Na<sub>2</sub>SO<sub>4</sub> column), methylated using BF<sub>3</sub>/methanol complex (BDH, 14%, 10 cm<sup>3</sup>) under reflux for 5 min and the total methylated products recovered by addition of water (10 cm<sup>3</sup>) and extraction with DCM (1 x 10 cm<sup>3</sup>, 2 x 5 cm<sup>3</sup>). The Combined DCM extract was backwashed with water (2 x 10 cm<sup>3</sup>), dried (anh. Na<sub>2</sub>SO<sub>4</sub> column) and solvent removed (Buchi, 40°C; N<sub>2</sub> blow down).

In certain cases, oxidation was carried out on 2 mg of UCM, necessitating scaled down apparatus (Wheaton; Figure 6.2). Oxidation was carried out following the method above except that the oxidation was carried out in 1 cm<sup>3</sup> of glacial acid, solvent volumes were kept to a minimum and all solvent removal was by N<sub>2</sub> blowdown only. As many of the steps as possible were carried out in the same glassware.

#### 6.4.2 RUTHENIUM TETROXIDE OXIDATIONS

The aromatic UCM (ca. 50 mg) in chloroform (4 cm<sup>3</sup>) was placed in a conical flask (100 cm<sup>3</sup>) and ruthenium(III) chloride trihydrate (2.6 mg), sodium periodate (3.4 mg), acetonitrile (4 cm<sup>3</sup>) and water (6 cm<sup>3</sup>) added. The flask was then sealed as much as possible whilst still including a mechanical stirrer, and kept at 35°C for 24 hours (Figure 6.3). The reaction supernatant was transferred to a separating funnel for extraction and the solid residue

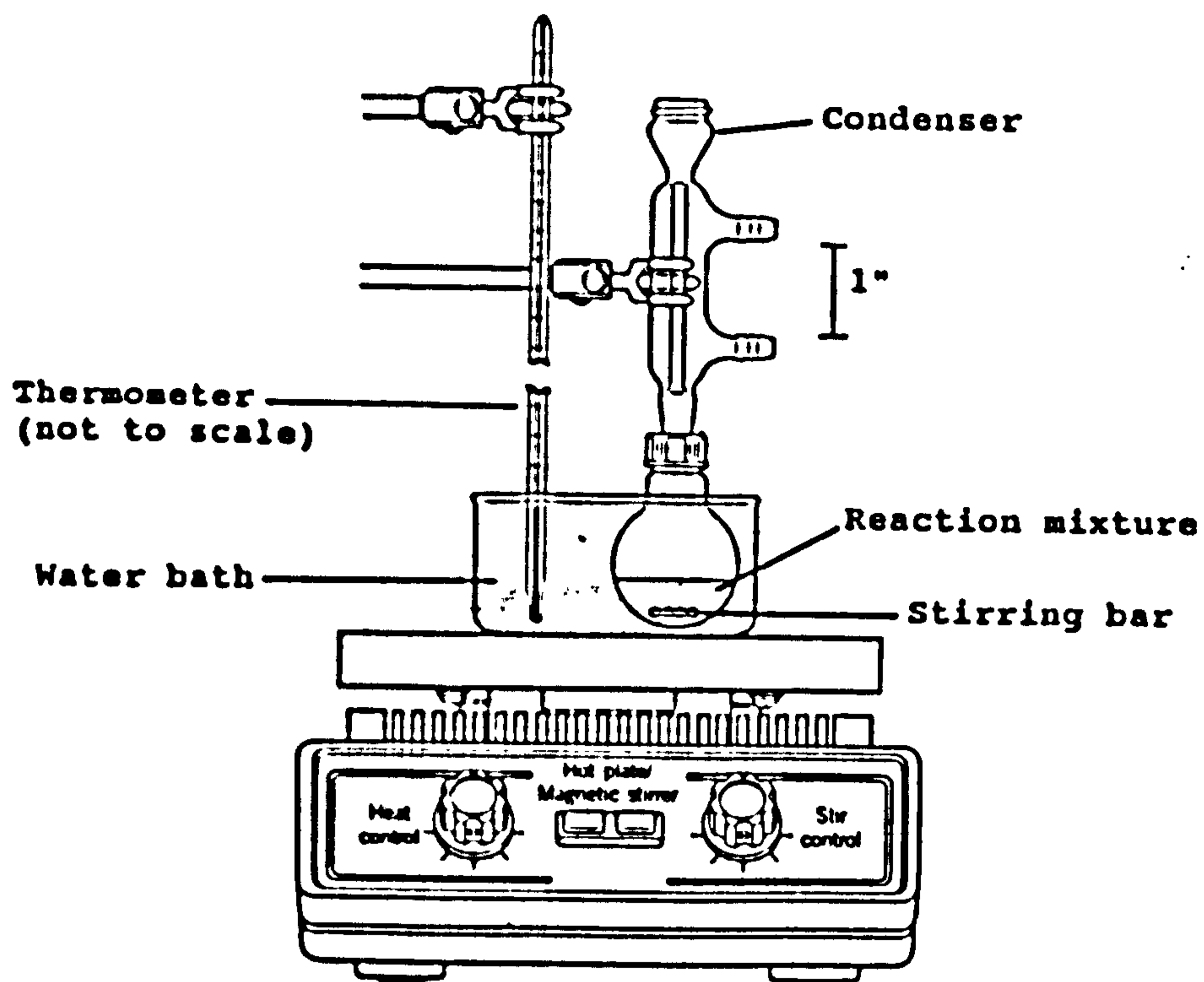


FIGURE 6.2. MICROSACLE (WHEATON) GLASSWARE USED TO OXIDISE 2 MG SAMPLES

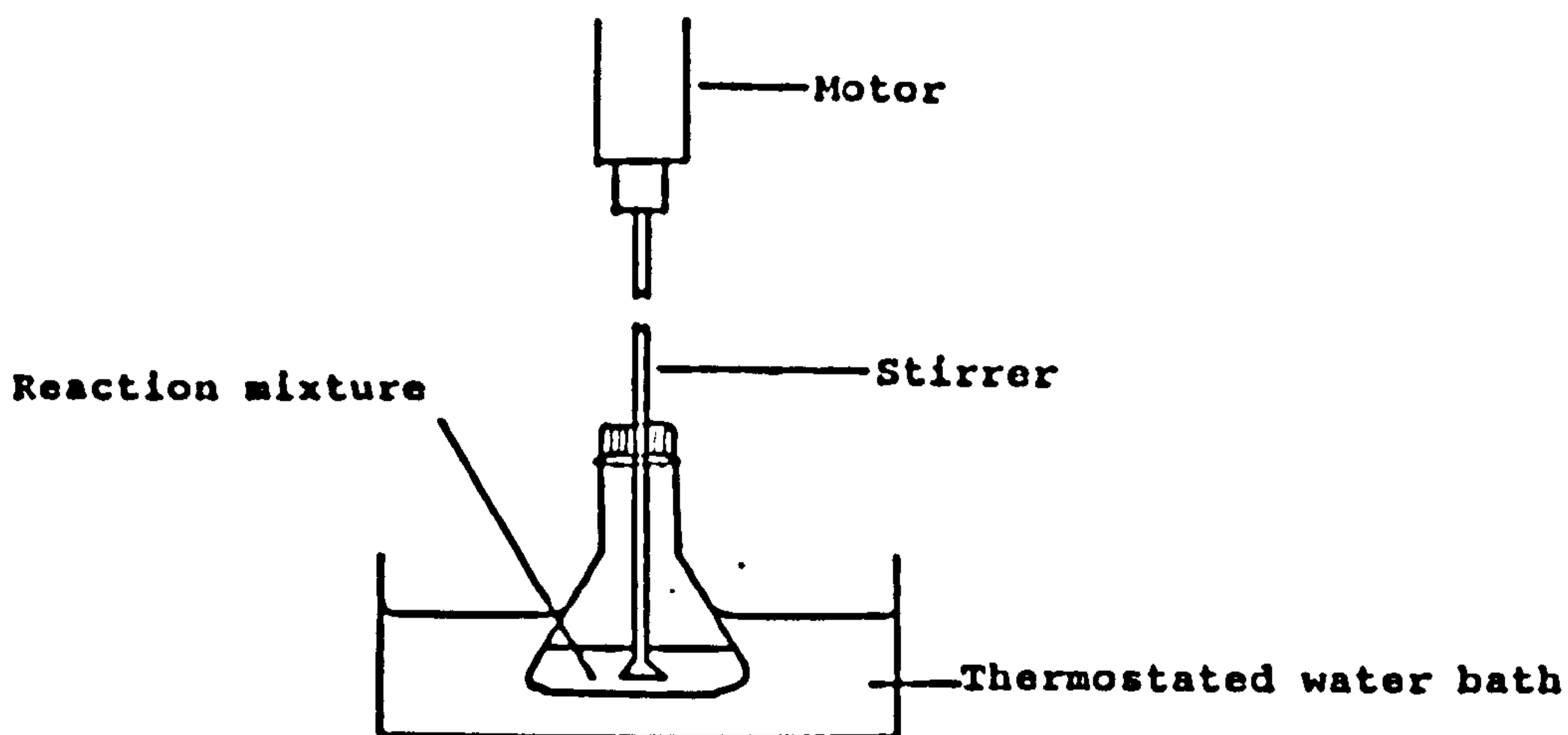


FIGURE 6.3. EQUIPMENT USED FOR RUTHENIUM TETROXIDE OXIDATIONS



extracted with DCM (2 x 10 cm<sup>3</sup>) by ultrasonic (Soniprep bath, 30s) and centrifugation (2000 rpm, 10 min). The combined extracts were dried (anh. Na<sub>2</sub>SO<sub>4</sub>) and filtered through a thin layer of pre-extracted fullers earth in a buchner funnel. The filtrate was methylated as before, dried (anh. Na<sub>2</sub>SO<sub>4</sub>) and weighed.

In certain circumstances, the oxidised material was separated from the unoxidised by argentatious TLC as previously described.

#### 6.5 HYDROUS PYROLYSIS

Hydrous pyrolysis of Alkathene was performed by Dr. S. Petch (Newcastle University, U.K.) at 350°C for 24 hours and 48 hours, and on a sample of the aliphatic biopolymer from *Agave americana* at 350°C for 48 hours. In each case the samples were placed in stainless steel bomblets and distilled water (5 cm<sup>3</sup>) added. The bomblets were placed in a Parr bomb (500 cm<sup>3</sup>) to which distilled water (150 cm<sup>3</sup>) was added. The bomb was sealed and heated to the required temperature for the time chosen. On cooling, the contents of the bomblet were filtered (Buchner funnel, pre-extracted filters) and the bomblet washed successively with distilled water, methanol and DCM. The solid residue was similarly washed with DCM (75 cm<sup>3</sup>). The aqueous and organic layers were separated and the aqueous/methanol phase washed with dichloromethane (3 x 50 cm<sup>3</sup>). The combined organic fraction



was concentrated (Buchi, 35°C), transferred to a vial and blown down (N<sub>2</sub>).

A similar sample to that above of the hydrous pyrolysate (365°C, 72 hours) from *A. americana* cutan was obtained from Dr J. W. de Leeuw (Delft University, The Netherlands).

Pyrolysate UCMs were isolated by urea adduction.

## **6.6 ANALYSES**

### **6.6.1 ELEMENTAL ANALYSIS**

Elemental analysis (C,H,N,S) of the aromatic UCM was performed by Dr. R. Raiswell (Department of Earth Sciences, University of Leeds) on a Carlo Erba 1106 elemental analyser.

### **6.6.2 GAS CHROMATOGRAPHY (GC)**

Gas chromatography was carried out on a Carlo Erba 5300 Mega series gas chromatograph fitted with a 25 m fused silica capillary column (0.32 mm i.d.) coated with DB-5 stationary phase (J&W inc. U.S.A.). The oven was typically programmed from 40°C to 300°C at 5°C min<sup>-1</sup>, and held at 300°C for 10 minutes. Hydrogen was the carrier gas (2 cm<sup>3</sup> min<sup>-1</sup>, measured at 250°C). For FID, the detector parameters were optimised for response to n-alkanes to (Temperature, 320°C;

H<sub>2</sub>, 45 KPa; Air, 180 KPa).

For FPD (sulphur selective), response was optimised for dibenzothiophene to (Temperature, 200°C; H<sub>2</sub>, 142 KPa, [100cm<sup>3</sup> min<sup>-1</sup>]; Air, 1.75 kg/cm<sup>3</sup> [120 cm<sup>3</sup> min<sup>-1</sup>]; Excitation, 6.0; INPUT, 10; LINEAR, OUT; Attenuation, 16).

Column performance was monitored daily by injection of an alkane mixture which included *n*-hexadecane, *n*-heptadecane, pristane and a range to *n*-dotriacontane and occasionally, a Grob-type mix was also injected to monitor column activity.

All chromatograms were recorded using a Shimadzu C-R3A chromatopac computing integrator. Estimates of percentage resolved compounds versus percentage unresolved were determined electronically using a time slice area program (Appendix I).

### 6.6.3 GAS CHROMATOGRAPHY-ELECTRON IMPACT MASS SPECTROMETRY (GC-EIMS)

Analyses of selected samples were performed using a Carlo Erba 5300 mega series gas chromatograph coupled to a Kratos MS25 double focusing magnetic sector mass spectrometer. GC conditions were as in Section 6.6.1 except that helium was used as carrier gas with the capillary column led directly

via a heated transfer line to the mass spectrometer source (ionisation voltage, 40eV; Filament emission current, 400 $\mu$ A; Source temperature, 250°C). Spectra (50-500+ Daltons) were collected each second using a Tektronix data system with DS90 software (rev. 5).

#### 6.6.3.1 SELECTED ION MONITORING (SIM)

For certain examples, concentrations of biomarker compounds were determined using selected ion monitoring for the fragment ions  $m/z$  191 and  $m/z$  217, with quantification by comparison of peak areas with that from 2,2,4,4-tetra deuteriocholestane ( $m/z$  221; Dr G Wolff, Liverpool University) assuming relative response factors of 1. Measurements were carried out on a Hewlett Packard MSD GCMS system (injector temperature, 250°C; auto injection, 0.5 $\mu$ l; electron multiplier, 200 mV; scan rate, 1.72 cycles/s with Chemstation software.

#### 6.6.4 FIELD DESORPTION MASS SPECTROMETRY (FDMS)

FDMS was performed by M-SCAN Inc., USA and V.G. Analytical Ltd., U.K., using a VG ZAB-2SE double focusing mass spectrometer (accelerating voltage, 8KV; extraction voltage, 5KV; mass range, 50 - 1550 Daltons). Calibration was either CsI in FAB mode or polystyrene. The sample (250nl, neat or in solvent (DCM), was applied to the probe



wire by syringe and 4-5 scans averaged to give the final spectrum.

#### **6.6.5 ULTRA VIOLET SPECTROSCOPY**

UV absorbance spectra for selected aromatic fractions were recorded using a Perkin Elmer Lambda 7 UV/visible spectrometer. Samples in hexane were measured against a solvent blank.

#### **6.6.6 ULTRA VIOLET FLUORESCENCE SPECTROSCOPY**

UVF spectra were recorded using a Perkin Elmer MPF-3 fluorescence spectrometer in synchronous scanning mode (initial  $\lambda_{ex}$  = 200 nm,  $\Delta\lambda$  = 43 nm; Mille et al., 1988).

#### **6.6.7 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY**

<sup>1</sup>H- and <sup>13</sup>C-NMR spectra of samples were in deuteriated chloroform (TMS reference) were recorded using a Jeol 270 MHz high resolution FT-NMR.

#### **6.6.8 INFRA RED SPECTROSCOPY**

IR spectra of either neat liquid films (NaCl discs) or solutions (CCl<sub>4</sub>) were recorded on a Perkin Elmer 298 infra red spectrometer. Spectra were calibrated with a polystyrene film.



## 6.7 PREPARATION OF AUTHENTIC COMPOUNDS

Isomeric mixtures of 7-hexylnonadecene (7-HN), 9-(2-cyclohexylethyl)heptadecene (9-CEH) and 9-(2-phenylethyl)heptadecene (9-PEH) were prepared by Mr A Tonkin (Polytechnic South West, U.K.) following the method of Gough (1989). Each isomeric mixture was then hydrogenated by bubbling hydrogen through the sample in hexane, over a catalyst (5% Pd on carbon; 1 mg/5 mg of sample; Table (6.1)). The purity of the products was monitored by GC (Table 6.1) and IR.

## 6.8 UCM FINGERPRINTING

The overall procedure is outlined in Figure 6.4.

### 6.8.1 ANALYSIS OF SAMPLES

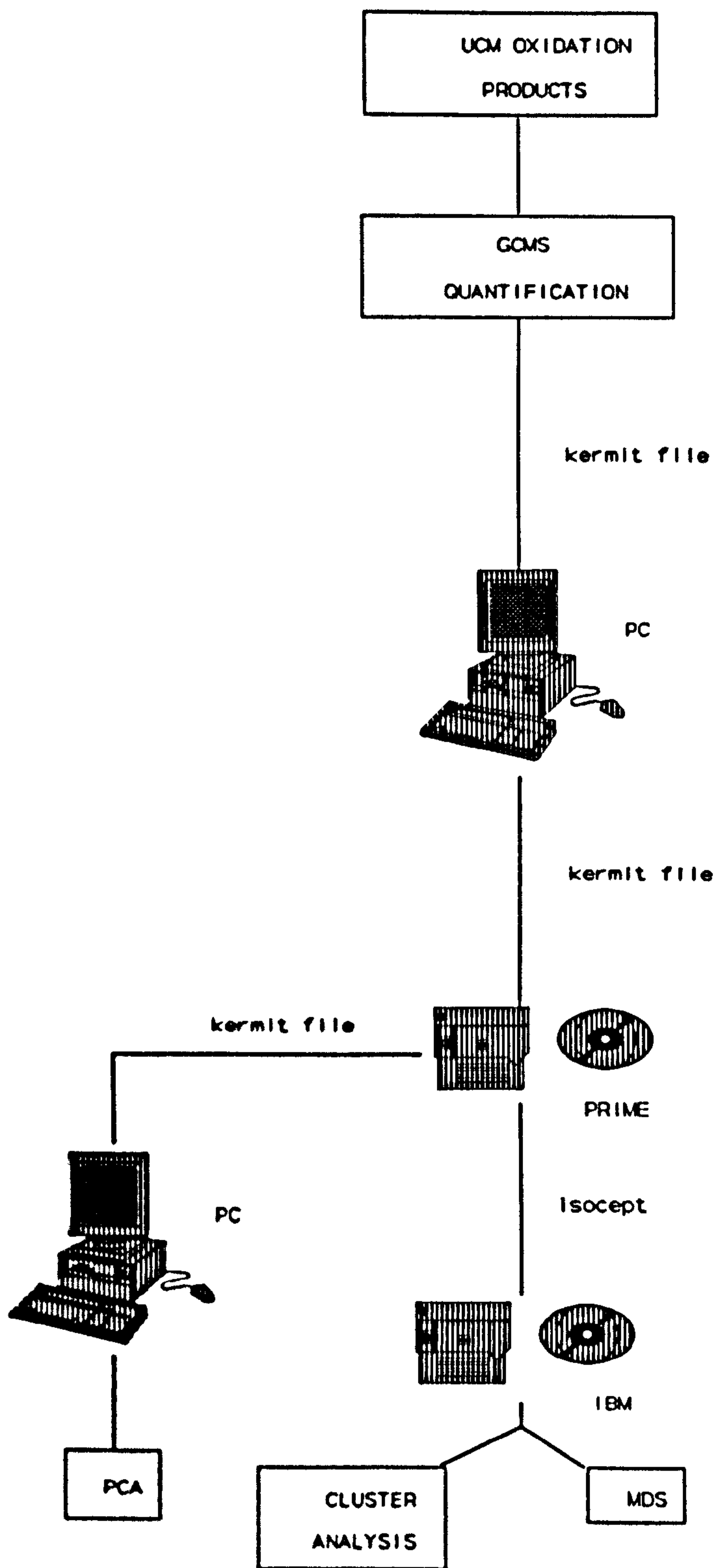
Each sample of oxidation products was weighed using a 6 decimal place balance with a reproducibility previously found to be  $\pm 0.0061\%$  (% standard deviation 0.00031) and then solvent (DCM; 50  $\mu\text{l mg}^{-1}$ ) and two standards ( $d_1$ -naphthalene and perylene; 5  $\mu\text{g mg}^{-1}$  each) added prior to analysis by GCMS. Mass chromatography was then used to measure *n*-carboxylic acids ( $m/z$  74),  $\gamma$ -methyl- $\gamma$ -lactones ( $m/z$  99) alkyl ketones ( $m/z$  58) and the two internal standards  $d_1$ -naphthalene ( $m/z$  136) and perylene ( $m/z$  252). Each mass chromatogram was integrated using the DS90

TABLE 6.1: PREPARATION OF AUTHENTIC REFERENCE COMPOUNDS

Starting Material	Amount (mg)	Amount Catalyst (mg)	KI* Starting Material	Amount Recovered (mg)	KI Products*		GC Purity
					This Study	Gough (1989)	
7-HN	66.1	13	2359*	65	2359*	2360	90%
9-CEH	46.8	9	2410	42.2	2420	2420	91%
9-PEH	43.3	8.5	2435	31.6	2447	2444	95%

\* measured using an n-alkane standard mix (n-C<sub>6</sub> to n-C<sub>20</sub>)  
J & W DB-5 40-300°C @ 5°C min<sup>-1</sup>, H<sub>2</sub> carrier gas

• suggests already hydrogenated



**FIGURE 6.4. OUTLINE OF THE PROCEDURE USED FOR UCM FINGERPRINTING**

software to give retention time, scan number, peak area and peak height of each significant (> 2% above baseline) component.

#### 6.8.2 COMPUTER MANIPULATION OF DATA

The data for each sample were downloaded to an IBM compatible PC from the Data General datasystem via a KERMIT file transfer program. The retention times (scan number) and peak height information for each class of compound in each sample was then used in two purpose-written computer programs (Appendix III), 1124XoutlinedFigures 6.5 and 6.6. The first, "PEAK MATCH",\*p1180Xprovided information on those peaks present in all samples, while the second, "DBMATCH", compared each sample with all others and any peaks absent in a sample were assigned a height of zero. Thus, in each case, all the samples effectively had the same number of components. A window of  $\pm 5s$  retention was used for a positive match.

The heights for the resulting "matched" peaks were formed into a data matrix of n rows and p columns, where n = number of peaks and p = number of samples. The compound classes were arranged as distinct groups down the columns, in the order n-carboxylic acids,  $\gamma$ -lactones and ketones, with the peaks in each class in elution order. This data matrix was constructed on the Polytechnic South West PRIME mainframe computer and transferred to an IBM mainframe at



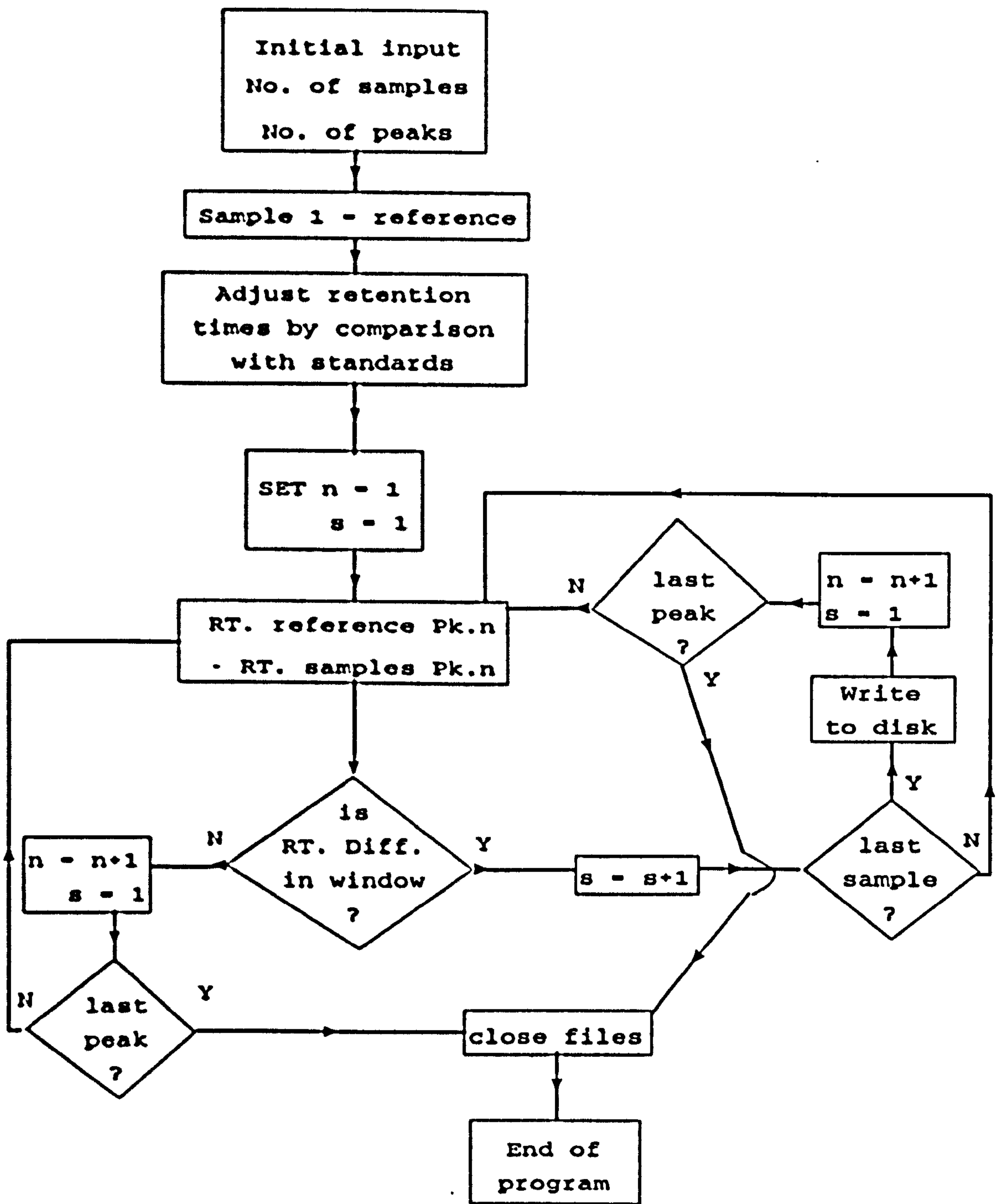


FIGURE 6.5. OUTLINE OF THE PURPOSE WRITTEN PEAK MATCH PROGRAM  
(Y = Yes, N = No)

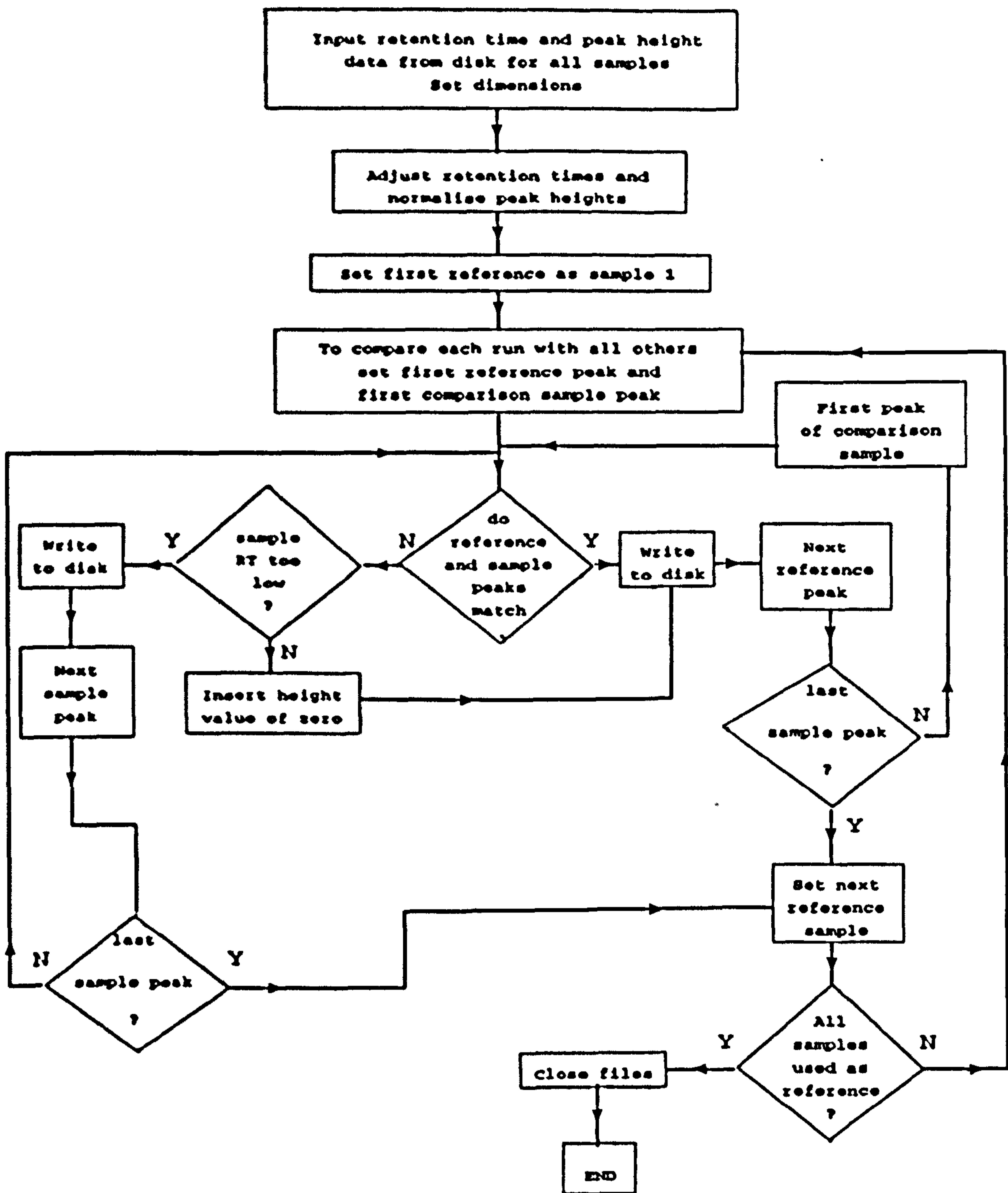


FIGURE 6.6. OUTLINE OF THE PURPOSE WRITTEN DBMATCH PROGRAM  
(Y = Yes, N = No)

Plymouth Marine Laboratory (PML) via the Isocept file transfer procedure. Inter-sample Euclidean distances were then calculated, using a purpose-written program (M. Carr, PML; Appendix IV) within the SAS statistical package, according to:-

$$D_{ij} = \left( \sum_{n=1}^p (X_{in} - X_{jn})^2 \right)^{1/2}$$

where  $D_{jk}$  = Euclidean distance between samples j and k

$X_{ij}$  = value of the ith variable for sample j

$X_{ik}$  = value of the ith variable for sample k

The resulting distance matrix was split and the lower triangle used as input to cluster analysis and multidimensional scaling programs, producing similarity dendrograms and two-dimensional scatter plots, respectively.

The initial data matrix was also used in principal components analysis using a commercially available software package, UNSCRAMBLER II (ver. 3.13; CAMO A/S, Trondheim, Norway).

**R E F E R E N C E S**



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**A P P E N D I X O N E**  
**UCM INTEGRATION**



## APPENDIX ONE

The percentage of resolved and unresolved peaks in GC chromatograms was estimated by electronic integration using the Shimadzu C-R3A integrator. This involved manual peak detection and use of the "time slice area measurement" function and programming the integrator in BASIC to sum integral areas for each "time slice".

### i) RESOLVED PEAKS

Parameters:           width: 5  
                          drift: 10 000  
                  attenuation: 5  
                          method: 0840  
                          slope: automatic  
          minimum area : 5 000 - 20 000

Time program: 0.01 L. on  
                  2.00 L. off

### ii) TOTAL (UCM + resolved peaks; for a UCM extending from 12 minutes to end of run)

Parameters:           width: 50  
                          drift: 1 x10<sup>11</sup>  
                  attenuation: 5  
                          method: 4010  
                          slope: automatic  
          minimum area: 5 000

Time program:           0.01 L. on  
                          2.00 L. off  
                          12.00 pk. U  
                          36.00 pk. D  
                          60.00 pk. off

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

**A P P E N D I X T W O**  
**CALCULATION OF T-BRANCH ISOMERS**

## APPENDIX TWO

The possible numbers of structural isomers of naphthenoaromatic compounds with monoalkyl branched side chains were calculated using a purpose written (C.A. Lewis, 1991) program in "Quick Basic".

```

*****
/* PROGRAM T-BRANCH CALCULATES THE NUMBER OF ISOMERIC T-BRANCHED ALKANES, *
/* EXCLUDING STEREOISOMERS, BETWEEN THE LIMITS CHOSEN *
/* WRITTEN BY C.A. LEWIS, POLYTECHNIC SOUTH WEST, U.K. 1991 *
/* FREE FOR NON-PROFIT, AS LONG AS HEADER RETAINED *
/* VERSION 2.00 *
/* 11/10/91 *
*****
DEFINT I-N
DECLARE FUNCTION GTTXLS (In$, prompt$)
DECLARE FUNCTION GTINTX (In, min, max, prompt$)
CLS
lowX = 10: highX = 35: Totalisomers = 0
outdevice$ = "S": filename$ = "TBRANCH.DAT": asym$ = "S"

prompt$ = "Lowest carbon number alkane"
lowX = GTINTX(lowX, 1, 100, prompt$)
prompt$ = "Highest carbon number alkane"
highX = GTINTX(highX, lowX + 1, 101, prompt$)
prompt$ = "Is the molecule asymmetric or symmetric (A or S)"
asym$ = UCASE$(GTTXLS(asym$, prompt$))
IF asym$ = "S" THEN
  MaxChain = INT((highX - 1) / 3)
  asymtitle$ = "MOLECULE TOTALLY SYMMETRICAL"
ELSEIF asym$ = "A" THEN
  asym$ = "Y"
  prompt$ = "Is the molecule asymmetric at both ends (Y or N)"
  asym$ = UCASE$(GTTXLS(asym$, prompt$))
  IF asym$ = "N" THEN
    asym$ = "A"
    MaxChain = INT((highX - 1) / 2)
    asymtitle$ = "MOLECULE ASYMMETRIC AT ONE END ONLY"
  ELSE
    asym$ = "AA"
    MaxChain = highX - 1
    asymtitle$ = "MOLECULE ASYMMETRIC AT BOTH ENDS"
  END IF
ELSE
  PRINT "You blew it!"
END
END IF
BlankSpace = 9 + (4 * MaxChain)

Startover:
prompt$ = "Output data to disk, printer or screen (D, P or S)"
outdevice$ = UCASE$(GTTXLS(outdevice$, prompt$))
outnum = FREEFILE
SELECT CASE outdevice$
CASE "D"
  prompt$ = "What filename"
  filename$ = GTTXLS(filename$, prompt$)
  OPEN filename$ FOR OUTPUT AS #outnum
CASE "P"
  outdevice$ = "lpt1:"
  OPEN outdevice$ FOR OUTPUT AS #outnum
  PRINT #outnum, CHR$(27) + CHR$(64); 'reset printer
  WIDTH #outnum, 255 'set width, 255 special
  PRINT #outnum, CHR$(27) + CHR$(77); 'font
  PRINT #outnum, CHR$(27) + CHR$(50); 'line spacing
  PRINT #outnum, CHR$(27) + CHR$(108) + CHR$(10); 'left margin
  PRINT #outnum, CHR$(27) + CHR$(81) + CHR$(10); 'right margin
  PRINT #outnum, CHR$(27) + CHR$(78) + CHR$(4); 'bottom margin
  FOR I = 1 TO 6
    PRINT #outnum, " "
  NEXT I

```

```

CASE "S"
  outdevice$ = "scrn:"
  OPEN outdevice$ FOR OUTPUT AS #outnum
CASE ELSE
  PRINT "Sorry that is unacceptable, try again"
  outdevice$ = "S"
  GOTO Startover
END SELECT

PRINT #outnum, ""
PRINT #outnum, SPC(5); "NUMBER OF T-BRANCH ALKANES, EXCLUDING STEREOISOMERS"
PRINT #outnum, SPC(5); asymtitle$
PRINT #outnum, " "
PRINT #outnum, " Total"; SPC(((Blankspace - 23) / 2) + 3); "Branch Carbons"
PRINT #outnum, "Carbons"; SPC(2);
FOR i = 1 TO MaxChain
  PRINT #outnum, USING "##"; i;
  PRINT #outnum, SPC(2);
NEXT i
PRINT #outnum, SPC(4); "Total"
PRINT #outnum, STRING$(Blankspace + 9, "-")
FOR i = low% TO high%
  SumIsomers = 0
  IF asym$ = "S" THEN
    MaxChain = INT((i - 1) / 3)
  ELSEIF asym$ = "A" THEN
    MaxChain = INT((i - 1) / 2)
  ELSEIF asym$ = "AA" THEN
    MaxChain = i - 1
  END IF
  PRINT #outnum, SPC(3);
  PRINT #outnum, USING "##"; i;
  PRINT #outnum, SPC(4);
  FOR j = 1 TO MaxChain
    IF asym$ = "S" THEN
      NumIsomers = CINT((i - j + .001) / 2) - j
    ELSEIF asym$ = "A" THEN
      NumIsomers = i - (j * 2)
    ELSEIF asym$ = "AA" THEN
      NumIsomers = MaxChain - j + 1
    END IF
    SumIsomers = SumIsomers + NumIsomers
    PRINT #outnum, USING "##"; NumIsomers;
    PRINT #outnum, SPC(2);
  NEXT j
  PRINT #outnum, SPC(Blankspace - 4 - (4 * MaxChain));
  PRINT #outnum, USING "###"; SumIsomers
  TotalIsomers = TotalIsomers + SumIsomers
NEXT i
PRINT #outnum, SPC(Blankspace + 3); "====="
PRINT #outnum, SPC(Blankspace + 4);
PRINT #outnum, USING "###"; TotalIsomers
PRINT #outnum, ""
PRINT #outnum, "Total isomers including unbranched parent = ";
PRINT #outnum, USING "###"; TotalIsomers + high% - low% + 1
IF outdevice$ = "lpt1:" THEN
  PRINT #outnum, CHR$(12)
END IF
CLOSE
END

```



**A P P E N D I X T H R E E**  
**BASIC PROGRAMS TO COMPARE CHROMATOGRAMS**

### APPENDIX THREE

Two BASIC programs were written to compare the peaks present in a chromatogram by retention time. These programs are available for free, non-profit use provided proper credit is given.

1) **Peak Match.** Compares chromatograms and records only those peaks present in all samples.

```
300 INPUT "DO YOU WANT TO PROCESS YOUR DATA NOW?  ",P$
310 IF P$="Y" THEN 330
315 IF P$="N" THEN 1080
320 END
330 CLS
335 INPUT "NUMBER OF SAMPLES ?","M
336 INPUT "NUMBER OF PEAKS ?","N
337 CLS
340 PRINT "PROCESSING DATA"
345 PRINT
350 PRINT
355 REM READ DATA FROM DISK TO MAIN PROGRAM
360 OPEN "I",#1,"A:\BASIC\DATA"
365 DIM SRT1(M),SRT2(M),RRT1(M),RRT2(M)
367 DIM SA1(M),SA2(M)
370 DIM RRTX(M),SCAND(M),RTC(M)
375 DIM RT(M,N),A(M,N),CAR(M,N)
380 FOR J=1 TO M
390 FOR I=1 TO N
400 INPUT #1,RT(J,I),A(J,I)
405 IF RT(J,I)>1700 THEN 420
410 NEXT I
420 INPUT #1,SRT1(J),SA1(J)
425 INPUT #1,SRT2(J),SA2(J)
430 NEXT J
435 REM M=No OF SAMPLES, N=No OF PEAKS
440 REM RT=RET.TIME, A=AREA, S=STANDARD
450 CLOSE #1
451 TSRT1=0
452 TSRT2=0
453 FOR J=1 TO M
454 TSRT1=TSRT1+SRT1(J)
455 TSRT2=TSRT2+SRT2(J)
456 NEXT J
457 ASRT1=TSRT1/M
458 ASRT2=TSRT2/M
460 REM ADJUSTMENT OF RET. TIMES
461 DIM CRT(M,N)
462 OPEN "A:\BASIC\MATCHED PEAKS" FOR OUTPUT AS #2
464 OPEN "A:\BASIC\NON-MATCHED PEAKS" FOR OUTPUT AS #3
470 FOR J=1 TO M
475 RRT1(J)=ASRT1-SRT1(J)
477 RRT2(J)=ASRT2-SRT2(J)
480 RRTX(J)=RRT2(J)-RRT1(J)
482 SCAND(J)=SRT2(J)-SRT1(J)
484 RTC(J)=RRTX(J)/SCAND(J)
```

```

490 NEXT J
500 FOR J=1 TO M
510 FOR I=1 TO N
520 CRT(J,I)=((RT(J,I)-SRT1(J))*RTC(J))+RRT1(J)+RT(J,I)
525 IF CRT(J,I)>1700 THEN 540
530 NEXT I
540 NEXT J
550 FOR I=1 TO N
560 CRT(1,X)=RT(1,I)
565 IF CRT(1,X)>1700 THEN 580
570 NEXT I
580 REM COMPARISON OF RETENTION TIMES AND PEAK MATCHING
585 INPUT "REQUIRED SCAN WINDOW ?",W
590 Z=0
600 X=1
610 J=1
620 FOR I=1 TO N
630 D=CRT(1,X)-CRT(J,I)
640 IF D>W THEN 720
650 IF D<-W THEN 800
660 CRT(J,K)=CRT(J,I)
670 A(J,K)=A(J,I)
675 REM TO COMPARE SAME PEAK NEXT SAMPLE
680 P=J
690 J=P+1
700 IF J>M THEN 900
710 GOTO 620
720 NEXT I
740 REM TO MOVE TO NEXT PEAK IN REFERENCE
750 L=X
760 X=L+1
770 IF X>N THEN 1020
780 GOTO 610
800 REM TO PRINT NON-MATCHED PEAKS
820 FOR J=1 TO M
830 PRINT #3,J,CRT(J,X),A(J,X)
840 NEXT J
860 GOTO 740
900 REM USE OF MATCHED PEAKS
910 Z=Z+1
920 FOR J=1 TO M
930 CAR(J,K)=A(J,K)/SA1(J)*1000
940 NEXT J
960 FOR J=1 TO M
970 PRINT #2,J,CRT(J,K),CAR(J,K)
980 NEXT J
1000 GOTO 740
1020 PRINT "END OF PEAK MATCHING"
1025 Q=N-Z
1030 CLOSE #2
1035 CLOSE #3
1040 PRINT
1050 PRINT "MATCHED PEAKS ARE STORED IN B:MATCHED PEAKS"
1060 PRINT
1070 PRINT "NON-MATCHED PEAKS ARE STORED IN B:NON-MATCHED
PEAKS"

```

```

1080 PRINT
1090 INPUT "DO YOU REQUIRE OUTPUT AT THIS TIME?      ",O$
1100 IF O$="Y" THEN 1150
1105 IF O$="N" THEN 1610
1110 END
1150 REM OUTPUT OF RESULTS TO PRINTER
1160 REM OUTPUT OF MATCHED PEAKS
1170 LPRINT "          ", "MATCHED PEAKS"
1180 LPRINT
1190 LPRINT "          ", "CORR. SCAN NO.", "          ", "CORR. PEAK
AREA"
1200 LPRINT
1201 Z=1
1205 LPRINT "SAMPLE      ";Z
1206 LPRINT
1220 OPEN "I",#2,"B:MATCHED PEAKS"
1250 IF EOF(2) THEN 1300
1260 INPUT #2,J,CRT(J,K),CAR(J,K)
1270 IF J=Z THEN LPRINT "          ",CRT(J,K), "          ",CAR(J,K)
1280 GOTO 1250
1300 CLOSE #2
1301 Z=Z+1
1305 IF Z>M THEN 1330
1310 LPRINT "SAMPLE      ";Z
1315 LPRINT
1320 GOTO 1220
1330 CLOSE #2
1340 LPRINT
1350 PRINT "END OF MATCHED PEAKS"
1360 PRINT
1370 INPUT "DO YOU REQUIRE NON-MATCHED PEAKS?      ",B$
1380 IF B$="Y" THEN 1400
1390 GOTO 1610
1400 REM OUTPUT ON NON-MATCHED PEAKS
1410 LPRINT "          ", "NON-MATCHED PEAKS"
1420 LPRINT
1430 LPRINT "          ", "CORR. SCAN NO.", "          ", "NON-CORR.
PEAK AREA"
1440 LPRINT
1445 Z=1
1450 LPRINT "SAMPLE      ";Z
1451 LPRINT
1455 DIM PRT(M,X),P(M,X)
1460 OPEN "I",#3,"B:NON-MATCHED PEAKS"
1490 IF EOF(3) THEN 1530
1500 INPUT #3,J,PRT(J,X),P(J,X)
1510 IF J=Z THEN LPRINT "          ",PRT(J,X), "          ",P(J,X)
1520 GOTO 1490
1530 CLOSE #3
1535 Z=Z+1
1540 IF Z>M THEN 1570
1545 LPRINT "SAMPLE      ";Z
1550 LPRINT
1555 GOTO 1460
1570 CLOSE #3
1580 PRINT

```



```

1590 PRINT "END OF NON-MATCHED PEAKS"
1600 PRINT
1610 PRINT "YOU HAVE BEEN USING PEAK MATCH"
1620 PRINT "COPYRIGHT A.REVILL, PSW, 1990"
1630 END

```

2) DBMATCH. Compares each chromatogram with all others and any peak not present is inserted with an integral value of zero.

```

10 CLS
20 INPUT "NUMBER OF SAMPLES ?",M
30 INPUT "NUMBER OF PEAKS ?",N
40 CLS
50 PRINT "PROCESSING DATA"
60 PRINT
70 PRINT
80 REM READ DATA FROM DISK TO MAIN PROGRAM
100 DIM SRT1(M),SRT2(M),RRT1(M),RRT2(M)
110 DIM SA1(M),SA2(M)
120 DIM RRTX(M),SCAND(M),RTC(M)
130 DIM RT(M,N),A(M,N),CAR(M,N),CRT(M,N)
135 OPEN "I",#1,"A:\BASIC\DATA"
140 FOR J=1 TO M
150 FOR I=1 TO N
160 INPUT #1,RT(J,I),A(J,I)
170 IF RT(J,I)>1700 THEN 190
180 NEXT I
190 INPUT #1,SRT1(J),SA1(J)
200 INPUT #1,SRT2(J),SA2(J)
210 NEXT J
220 CLOSE #1
230 REM ADJUSTMENT OF RET. TIMES
232 TSRT1=0
234 TSRT2=0
236 FOR J=1 TO M
238 TSRT1=TSRT1+SRT1(J)
240 TSRT2=TSRT2+SRT2(J)
242 NEXT J
244 ASRT1=TSRT1/M
246 ASRT2=TSRT2/M
250 FOR J=1 TO M
260 RRT1(J)=ASRT1-SRT1(J)
270 RRT2(J)=ASRT2-SRT2(J)
280 RRTX(J)=RRT2(J)-RRT1(J)
290 SCAND(J)=SRT2(J)-SRT1(J)
300 RTC(J)=RRTX(J)/SCAND(J)
310 NEXT J
320 FOR J=1 TO M
330 FOR I=1 TO N
340 CRT(J,I)=((RT(J,I)-SRT1(J))*RTC(J))+RRT1(J)+RT(J,I)
345 CAR(J,I)=(A(J,I)/SA1(J))*1000
350 IF CRT(J,I)>1700 THEN 370
360 NEXT I
370 NEXT J
380 REM TO COMPARE EACH RUN WITH ALL OTHERS

```

```

385 INPUT "REQUIRED SCAN WINDOW ?",W
390 X=1
400 Y=1
405 OPEN "O",#2,"A:\BASIC\MATCHED"
410 FOR J=1 TO M
420 FOR I=1 TO N
425 D=CRT(X,Y)-CRT(J,I)
430 IF CRT(X,Y)>1700 THEN 1000
440 IF D>W THEN 1000
450 IF D<-W THEN 2000
460 PRINT #2,J,CRT(J,I),CAR(J,I)
470 Y=Y+1
480 GOTO 1020
1000 IF CRT(J,I)>1700 THEN 1030
1010 PRINT #2,J,CRT(J,I),CAR(J,I)
1020 NEXT I
1030 PRINT #2,J,CRT(J,I),CAR(J,I)
1040 Y=1
1045 PRINT "SAMPLE ",X,"J,"COMPLETED"
1050 NEXT J
1060 GOTO 3000
2000 PRINT #2,J,CRT(X,Y),0
2010 Y=Y+1
2030 GOTO 425
3000 CLOSE #2
3010 X=X+1
3020 IF X>M THEN 5000
3030 OPEN "I",#2,"A:\BASIC\MATCHED"
3040 FOR J=1 TO M
3050 FOR I=1 TO N
3060 INPUT #2,J,CRT(J,I),CAR(J,I)
3070 IF CRT(J,I)>1700 THEN 3090
3080 NEXT I
3090 NEXT J
4000 CLOSE #2
4010 GOTO 400
5000 PRINT "END OF PROCESSING"
5010 END

```

**A P P E N D I X F O U R**  
**C A L C U L A T I O N O F E U C L I D E A N D I S T A N C E S**

## APPENDIX FOUR

Prior to cluster and MDS analyses between sample Euclidean distances were calculated using a purpose written program (M.Carr, P.M.L., 1991) within the SAS statistical package on an IBM mainframe computer. The example shown is for a data matrix of 5 samples each with 84 peaks.

```
cms filedef xxx disk statdat file a1;

data data1;
infile xxx;
input sa1-sa5
run;

proc transpose data=data1 out=data1;
run;

data data1;
set data1;
drop _name_;
run;

proc means data=data1 noprint;
output out=data2 mean=m1-m84 std=sd1-sd84;

data data2;
set data2;
array mm{84} m1-m84;
array ssdd{84} sd1-sd84;
do i=1 to 84;
  mean=mm{i}; sdev=ssdd{i}; output;
end;
keep mean sdev;
run;

proc transpose data=data1 out=data1;
run;

data data1;
set data1;
drop _name_;
run;

data data3;
merge data1 data2;
run;

data data3;
set data3;
array v{5} col1-col5;
do i=1 to 5;
  v{i} = (v{i} - mean);
end;
drop mean sdev i;
run;
```



```

data data3;
set data3;
array v{5} col1-col5;
array dist{25} d1-d25;
do i=1 to 5;
do j=1 to 5;
  dist{ (i-1)*5+j }=( v{i}-v{j} ) ** 2;
end;
end;
drop i j;
run;

```

```

proc means data=data3 noprint;
var d1-d25;
output out=data4 sum=s1-s25;
run;

```

```

data data4;
set data4;
array ss{25} s1-s25;
do i=1 to 25;
  ss{i}=sqrt(ss{i});
end;
drop i;
run;

```

```

data data5;
set data4;
array ss{25} s1-s25;
array cc{5} c1-c5;
do i=1 to 5;
do j=1 to 5;
  if j<1 then cc{j}=ss{ (i-1)*5 + j };
end;
output;
end;
keep c1-c5;
run;

```

```

cms filedef yyy disk statdat dissim a1;
data _null_;
set data5;
file yyy;
if _n_>1 then put c1-c5;
run;

```

**CONFERENCES AND PRESENTATIONS**

## CONFERENCES AND PRESENTATIONS

### i) CONFERENCES

British Organic Geochemistry Society meeting, July, 1989,  
Liverpool, U.K.

14th International meeting on Organic Geochemistry,  
September 18-22, 1989, Paris, France.

4th Workshop on the chemistry and analysis of environmental  
hydrocarbons, April 19-21, 1990, Strasbourg, France.

British Organic Geochemistry Society meeting, July, 1990,  
Bideford, Devon, U.K.

13th International symposium on capillary chromatography,  
May 13-16, 1991, Riva del Garda, Italy.

15th International meeting on organic geochemistry,  
September 16-20, 1991, Manchester, U.K.

### ii) POSTERS

Revill, A.T.; Carr, M.R. and Rowland, S.J. *The use of capillary gas chromatography and multidimensional scaling to fingerprint unresolved complex mixtures of hydrocarbons.* Presented at the 13th International symposium on capillary

chromatography, Riva del Garda, Italy, 1991.

Revill, A.T.; Carr, M.R. and Rowland, S.J. *The use of multidimensional scaling to fingerprint unresolved complex mixtures of hydrocarbons.* Presented at the 15th International meeting on organic geochemistry, Manchester, U.K., 1991.

### iii) ORAL PRESENTATIONS

Revill A.T.; Gough, M.A. and Rowland, S.J. *Fingerprinting of unresolved complex mixtures of hydrocarbons.* Presented at The 4th workshop on the chemistry and analysis of environmental hydrocarbons, Strasbourg, France, 1990.

Revill, A.T. *Fingerprinting UCMS in Sullom Voe sediments.* Presented at the British Organic Geochemistry Society meeting, 1990, Bideford, U.K.

### iv) PUBLICATION

Revill, A.T.; Carr, M.R. and Rowland, S.J. (1992) Use of oxidative degradation followed by capillary GC-MS and multidimensional scaling analysis to fingerprint unresolved complex mixtures of hydrocarbons *J. Chromatogr.* **589**, 281-286.