

The occurrence and identification of series of organic sulphur compounds in oils and sediment extracts. I. A study of Rozel Point Oil (U.S.A.)

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Abstract—A number of novel series of organic sulphur compounds (OSC) have been identified in several oils and sediment extracts including Rozel Point Oil (Box Elder County, Utah, U.S.A.; Miocene). This oil contains these series in abundance, and the OSC were therefore studied in detail. The "aromatic" fraction was fractionated using column chromatography, and the fractions obtained were analyzed by GC with simultaneous FID and FPD detection and by GC-MS. Series of isoprenoid thiophenes, isoprenoid thiolanes, isoprenoid bithiophenes, isoprenoid thienylthiolanes, isoprenoid benzothiophenes, thiophene and thiolane steranes, 2,5-di-*n*-alkylthiolanes, 2,6-di-*n*-alkylthianes and 2,4-di-*n*-alkylbenzo(*b*)thiophenes have been identified. The identifications were based on chromatographic and mass spectral data, response on the FPD, carbon skeleton determination by desulphurization and in some cases on mass spectral data from, and coinjections with, synthetic compounds. Together, almost 1000 OSC have been identified in the "aromatic" fraction of this oil. Because the carbon skeletons of the OSC identified have the same structures as the well known hydrocarbons from geological materials (isoprenoid hydrocarbons, steranes, *n*-alkanes) these compounds are thought to be products of sulphur incorporation reactions into specific precursors.

INTRODUCTION

IN SEVERAL OILS and sediments organic sulphur compounds (OSC) are present in abundance. This abundance contrasts with the relatively low concentration of organically-bound sulphur in the biomass, the precursor of these geological materials. Recently we have reported that this enrichment in OSC correlates with a hypersaline environment of deposition of the sediment or source rock (SINNINGHE DAMSTÉ *et al.*, 1986; TEN HAVEN *et al.*, 1987). This correlation should therefore also hold to a large extent for the source rock of Rozel Point Oil (RPO), an oil from the north-western part of Utah, U.S.A. MEISSNER *et al.* (1984) reported that the organic matter of the source rock of RPO was originally deposited in a playa lake environment. The structures and distribution patterns of the hydrocarbons present in the oil, as reported by TEN HAVEN *et al.* (1987), are thought to be characteristic for a hypersaline environment of deposition, *e.g.* even-over-odd carbon number predominance of the *n*-alkanes, low pristane/phytane ratio (<0.1) and the presence of a series of extended hopanes and hopenes maximizing at C₃₅ (TEN HAVEN *et al.*, 1985, 1987).

The extremely high amount of organic sulphur in RPO (14%) has been reported by THOMPSON (1981). This enabled us to study in detail the structures of the OSC. Since the same series have been encountered in several other oils and sediment extracts, it was thought that the identification of the individual structures might help us to understand their origin. Once the origin is understood their application as markers for several environmental settings and diagenesis can be evaluated. A preliminary report on several isoprenoid OSC in RPO has been published elsewhere (SINNINGHE DAMSTÉ and DE LEEUW, 1987). It should be noted

that similar studies on OSC present in another RPO sample and in a French oil have been performed simultaneously by the Organic Geochemistry Unit in Strasbourg, France (SCHMID, 1986; SCHMID *et al.*, 1987).

EXPERIMENTAL

Sample. The Rozel Point Oil field is located in Box Elder County, Utah (U.S.A.). Various oil occurrences are known in this area, including seeps and reservoirs at shallow depths, which produce heavy oils with an unusually high sulphur content up to 14 percent by weight (Utah Geological and Mineralogical Survey, pers. commun.).

The sample described here (West Rozel #2) is from an Amoco wildcat well (T8N, R8W, section 15) in the great Salt Lake. This oil is a well sample from fractured Tertiary basalt at a depth interval between 810 and 825 m. Bulk properties include an API gravity of 4.8, a sulphur content of 7.5 weight percent and a pour point of 24°C (M. D. LEWAN, pers. commun.).

Fractionation. The oil was fractionated by column chromatography as described previously for a sediment extract (SINNINGHE DAMSTÉ *et al.*, 1986). Briefly, the oil (214.8 mg) was separated into "saturated" (3.0 mg), "aromatic" (68.4 mg) and "polar" compounds (94.0 mg) using pentane, toluene and toluene/methanol (1:1) as eluents. The "aromatic" fraction (≈50 mg) was further separated on an alumina column (25 cm, i.d. = 1 cm) using hexane (90 ml), hexane/toluene (9:1) (25 ml) and toluene (25 ml), respectively, as eluents. The first six sub-fractions, named RPO 1-6 (Table 1), were analyzed by gas chromatography (GC) using flame ionization (FID) and flame photometric detection (FPD) and by gas chromatography-mass spectrometry (GC-MS). At a later stage the fractionation of the "aromatic" fraction was scaled up to obtain larger quantities of the various sub-fractions, enabling NMR analysis and desulphurization experiments. Thus, 3.4 g of the "aromatic" fraction from 7.2 g RPO was fractionated on a column with alumina (60 cm, i.d. = 3.5 cm) using hexane (1.80 l), hexane/toluene (9:1) (0.72 l) and toluene (0.72 l) as eluents respectively. A large number of sub-fractions was obtained in order to relate more accurately the desulphurization

TABLE 1: SEPARATION SCHEME OF THE AROMATIC FRACTION.

cut	major compound type(s)	volume (ml)	eluent ^a
prewash	-	20	H
1	isoprenoid thiophenes isoprenoid thiolanes thiophene steranes	35	H
2	2,6-di- <i>n</i> -alkylthianes	20	H
3	2,6-di- <i>n</i> -alkylthianes 2,5-di- <i>n</i> -alkylthiolanes	20	H
4	2,5-di- <i>n</i> -alkylthiolanes thiolane steranes	5	H/T(9:1)
5	2,4-di- <i>n</i> -alkylbenzothiophenes isoprenoid bithiophenes	5	H/T(9:1)
6	isoprenoid bithiophenes isoprenoid benzothiophenes	5	H/T(9:1)

^a H = hexane, T = toluene

products with the starting OSC. Where the composition of such a fraction was very similar to that of one of the fractions RPO 1-6, it is referred to as RPO 1'-6'.

The "aromatic" fraction (68.4 mg) was also separated into a so-called low molecular weight "aromatic" fraction (LMWA) (19.0 mg) and a high molecular weight "aromatic" fraction (HMWA) (43.3 mg) on a column (25 cm, i.d. = 1 cm) of activated (1 h at 150°C) alumina using 50 ml hexane/toluene (9:1) and 50 ml toluene, respectively. The HMWA fraction contained hardly any GC amenable compounds and is thought to consist of asphaltenes.

The isolation procedures were repeated several times for this oil, related samples and synthetic compounds. The recovery and reproducibility were good and no artefact formation was apparent.

Synthesis. *cis*-2-Methyl-5-tridecylthiolane and *cis*-2-dodecyl-5-ethylthiolane were obtained previously as side products during the catalytic hydrogenation of the corresponding thiophenes (SINNINGHE DAMSTÉ *et al.*, 1986). To obtain the *trans* isomers 2-(5-methylthienyl)dodecyl ketone and 2-(5-ethylthienyl)undecyl ketone were subjected to an ionic hydrogenation (PARNES *et al.*, 1977). The alkylthienyl ketones (20 mg) were mixed with 50 μ l triethylsilane, and 100 μ l of a 3% solution of BF₃ etherate in trifluoroacetic acid was slowly added at -10°C. The reaction time was 1 h. 2,5-Diheptylthiolane was synthesized as follows: Thiophene was coupled with heptanoic acid in refluxing toluene with P₂O₅ as dehydrating agent. The resulting ketone was reduced to 2-heptylthiophene via a simplified Wolf-Kishner reduction (KING and NORD, 1949). 2-Heptylthiophene was subsequently coupled with heptanoic acid in refluxing toluene with P₂O₅ as dehydrating agent. Wolf-Kishner reduction of the resulting ketone afforded 2,5-diheptylthiophene, which was hydrogenated to 2,5-diheptylthiolane using both ionic hydrogenation as well as hydrogenation in ethyl acetate with Pd/C (10%) as catalyst.

3-Methyl-2-(3,7,11-trimethyldodecyl)thiolane was synthesized from the corresponding thiophene, synthesized previously (BRASSELL *et al.*, 1986), using both Pd/C (10%) as well as triethylsilane under the conditions described above.

Raney Ni desulphurization. Typically, 20 mg of a sub-fraction was dissolved in 3.5 ml abs. ethanol together with 0.5 ml of a suspension (0.4 mg/ml) of Raney Ni (W-6; BILICA and ADKINDS, 1955) and heated under reflux for 1.5 h. The desulphurization products (yield \approx 70%) were isolated by centrifugation and subsequent extraction with diethylether (2.5 ml, \times 4). The combined extracts were washed (\times 3) against NaCl-saturated, double-distilled H₂O, dried with MgSO₄ and evaporated to dryness using a rotating evaporator at 20°C. The extract was taken up into a small volume of CH₂Cl₂ and chromatographed over silica (5 cm \times 5 mm) using CH₂Cl₂ as eluent. In some cases the desulphurization products were

further separated by preparative thin layer chromatography on silica plates using *n*-hexane as developer. The desulphurization products were analyzed by GC-FID, GC-MS and in some cases with ¹H-NMR.

¹H-NMR. ¹H-NMR spectra were measured at 200 MHz with a Nicolet NT 200 WB spectrometer using the FT-technique. CDCl₃ and CD₃COCD₃ were used as solvents.

Gas chromatography. GC was carried out on a Varian 3700 gas chromatograph using a DB-5 (30 m \times 0.26 mm i.d., film thickness = 0.1 μ m) capillary column. The flow was splitted in a ratio 1:4 to the flame ionization and flame photometric detector, respectively. Helium was used as carrier gas. Samples were injected splitless at 70°C. After two minutes the oven temperature was programmed to 130°C at 10°C/min. From 130°C it was programmed at 4°C/min to 300°C.

GC of the desulphurized fractions was carried out on a Carlo Erba 4160 instrument equipped with a flame ionization detector and an on-column injector. A fused silica capillary column (25 m \times 0.34 mm i.d.) coated with CP-Sil 5 (film thickness = 0.12 μ m) was used with helium as carrier gas. Samples were injected at 100°C in ethyl acetate, and the oven temperature was programmed to 320°C at 4°C/min.

Gas chromatography-mass spectrometry. GC-MS was carried out on a Varian-MAT 44 instrument. Details of the conditions used have been described previously (SINNINGHE DAMSTÉ *et al.*, 1986). GC-HRMS was performed on a HP-5890 A gas chromatograph coupled with a VG-70S mass spectrometer at a resolution of 5000. Mass spectra were recorded at 70 eV with a mass range from *m/z* 50 up to *m/z* 500 with a cycle time of 1 s.

RESULTS

The FPD and FID gas chromatograms of the LMWA fraction of the RPO are shown in Fig. 1. This fraction contained most of the GC-amenable compounds of the "aromatic" fraction and all compounds discussed hereafter. The letters correspond to the classes of compounds in Table 2. The numbers indicate the total number of carbon atoms. It is evident that the "aromatic" fraction is very complex and contains virtually only OSC. Therefore, a further separation step was performed. Figure 2 shows the FPD chromatograms of the sub-fractions RPO 1-6 (Table 1). The peak indications also correspond with those given in Table 2. In Table 2 a summary of the range of occurrence of the various classes of compounds and the chain length distribution of the individual classes within a certain fraction is also given.

Isoprenoid thiophenes

A series of isoprenoid thiophenes (alkylthiophenes with an isoprenoidal carbon skeleton) have been identified on the basis of mass spectral data and relative retention times of the compounds and their desulphurized products, and by comparison of mass spectra and retention times with those of synthetic compounds (BRASSELL *et al.*, 1986; SINNINGHE DAMSTÉ *et al.*, 1987). The isoprenoid thiophenes showed up in fraction RPO 1 (Fig. 2). Their distribution is exemplified by Fig. 3, which shows a mass chromatogram of *m/z* 125, a frequently observed ion in the mass spectra of isoprenoid thiophenes. The major members are the C₂₀ thiophenes I-V (see Appendix for structures and names) as described elsewhere (SINNINGHE DAMSTÉ

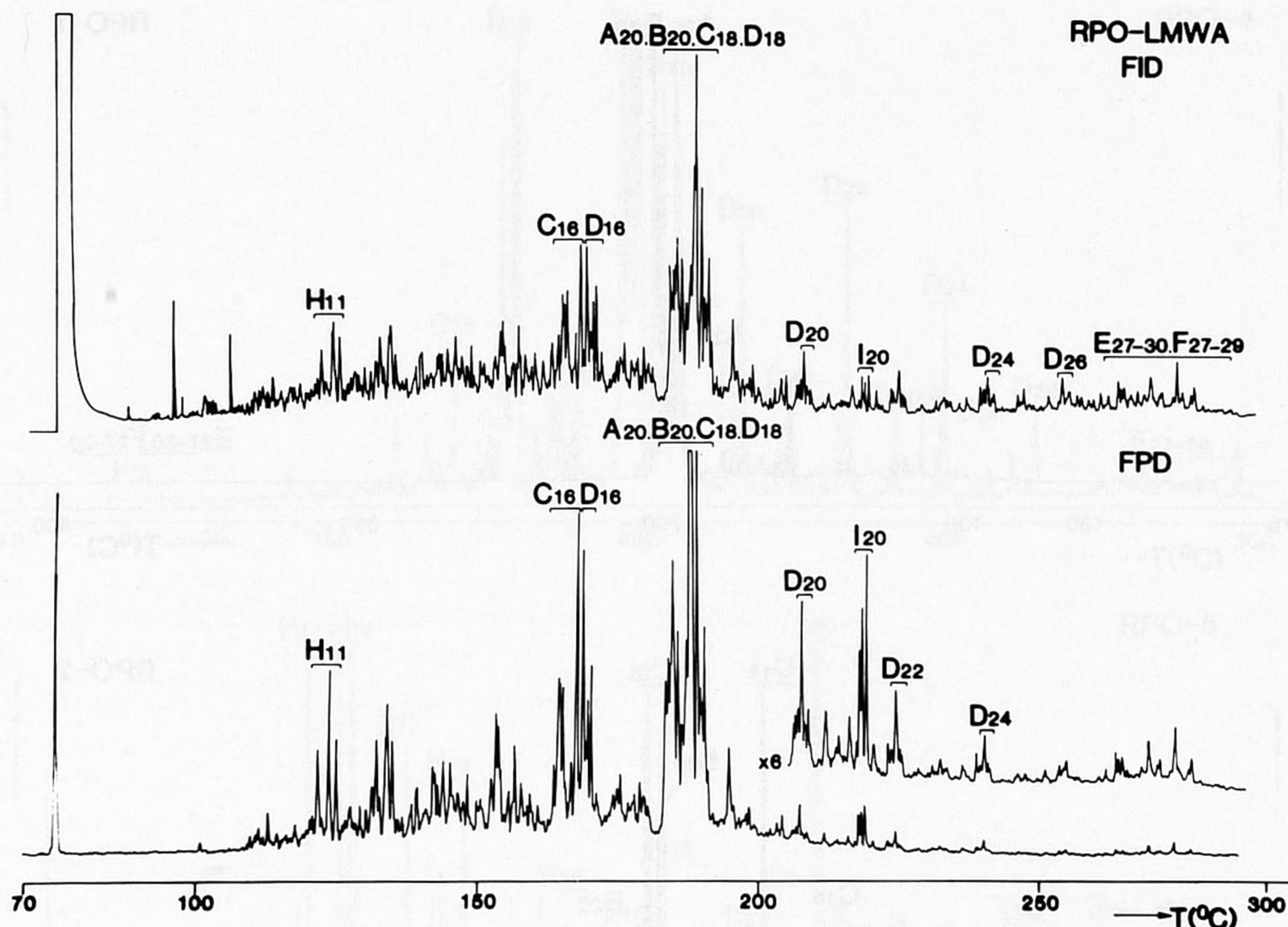


FIG. 1. FID (upper trace) and FPD (lower trace) chromatogram of the low molecular weight "aromatic" fraction of RPO. The letters correspond to classes listed in Table 2. The numbers indicate the total number of carbon atoms of the particular compound class.

and DE LEEUW, 1987). The GC-HRMS data supported these identifications (Table 3). Additional evidence for the assignments was obtained by Raney Ni desulfurization of particular fractions of the RPO which contained these C_{20} isoprenoid thiophenes as primary constituents: a mixture of hydrocarbons dominated by phytane resulted. The occurrence of I in RPO was recently confirmed by coinjection with a synthetic standard (SINNINGHE DAMSTÉ *et al.*, 1987).

Three relatively abundant C_{15} thiophenes (VI–VIII) were identified on the basis of mass spectral data, relative retention times and the formation of farnesane upon desulfurization.

The C_{30} and C_{35} thiophenes were also relatively abundant. The mass spectra of four C_{30} thiophenes (IX–XII) were characterized by a molecular ion at m/z 448 and a presumed secondary fragment at m/z 125. In addition, characteristic fragment ions resulting from β -cleavages of the alkyl substituents, m/z 167 and 405 (IX), m/z 181 and 391 (X), m/z 237 and 335 (XI) (Fig. 4A) and m/z 251, 252 and 321 (XII) were present. Desulfurization of the mixture yielded only one C_{30} hydrocarbon, squalane.

The rearrangement ion m/z 252 in the mass spectrum of XII further supports this skeleton structure. This ion can be explained by a McLafferty rearrange-

TABLE 2: DISTRIBUTION OF OSC OVER SEVERAL FRACTIONS AND WITHIN FRACTIONS.

COMPOUND CLASS	RPO LMWA	RPO 1	RPO 2	RPO 3	RPO 4	RPO 5	RPO 6
A isoprenoid thiophenes	C_{15} - C_{40} (C_{20})	C_{15} - C_{40} (C_{20})	C_{20} - C_{21} (C_{20})	-	-	-	-
B isoprenoid thiolanes	C_{20}	C_{15} , C_{20} , C_{30} (C_{20})	C_{20}	C_{20}	C_{20}	-	-
C 2,6-di- <i>n</i> -alkylthianes	C_{11} - C_{28} (C_{18})	C_{11} - C_{18} (C_{16})	C_{13} - C_{22} (C_{18})	C_{15} - C_{26} (C_{18})	C_{15} - C_{26} (C_{18})	-	-
D 2,5-di- <i>n</i> -alkylthiolanes	C_{11} - C_{28} (C_{18})	-	C_{12} - C_{16} (C_{14})	C_{14} - C_{21} (C_{18})	C_{14} - C_{27} (C_{18})	C_{16} - C_{31} (C_{24})	C_{22} - C_{27} (C_{24})
E thiophene steranes	C_{27} - C_{30} (C_{29})	C_{27} - C_{30} (C_{29})	C_{27} - C_{29} (C_{29})	-	-	-	-
F thiolane steranes	C_{27} - C_{29} (C_{29})	C_{27} - C_{29} (C_{29})	-	C_{27} - C_{29} (C_{28})	C_{27} - C_{28} (C_{28})	C_{27} - C_{29}	C_{27} - C_{29}
G isoprenoid benzothiophenes	C_{20}	-	-	-	-	C_{20}	C_{20}
H 2,4-di- <i>n</i> -alkylbenzothiophenes	C_{11} - C_{24} (C_{12})	-	-	-	-	C_{11} - C_{29} (C_{12})	C_{11} - C_{26} (C_{14})
I isoprenoid bithiophenes	C_{15} , C_{18} - C_{23} (C_{20})	-	-	-	-	C_{20}	C_{15} , C_{18} - C_{23} (C_{20})
J isoprenoid (thienyl)alkylthiophenes	C_{20}	-	-	-	-	C_{20}	C_{20}
K isoprenoid thienylthiolanes	C_{20}	-	-	-	-	C_{20}	C_{20}

Carbon numbers in parentheses indicate maxima in carbon number distributions.

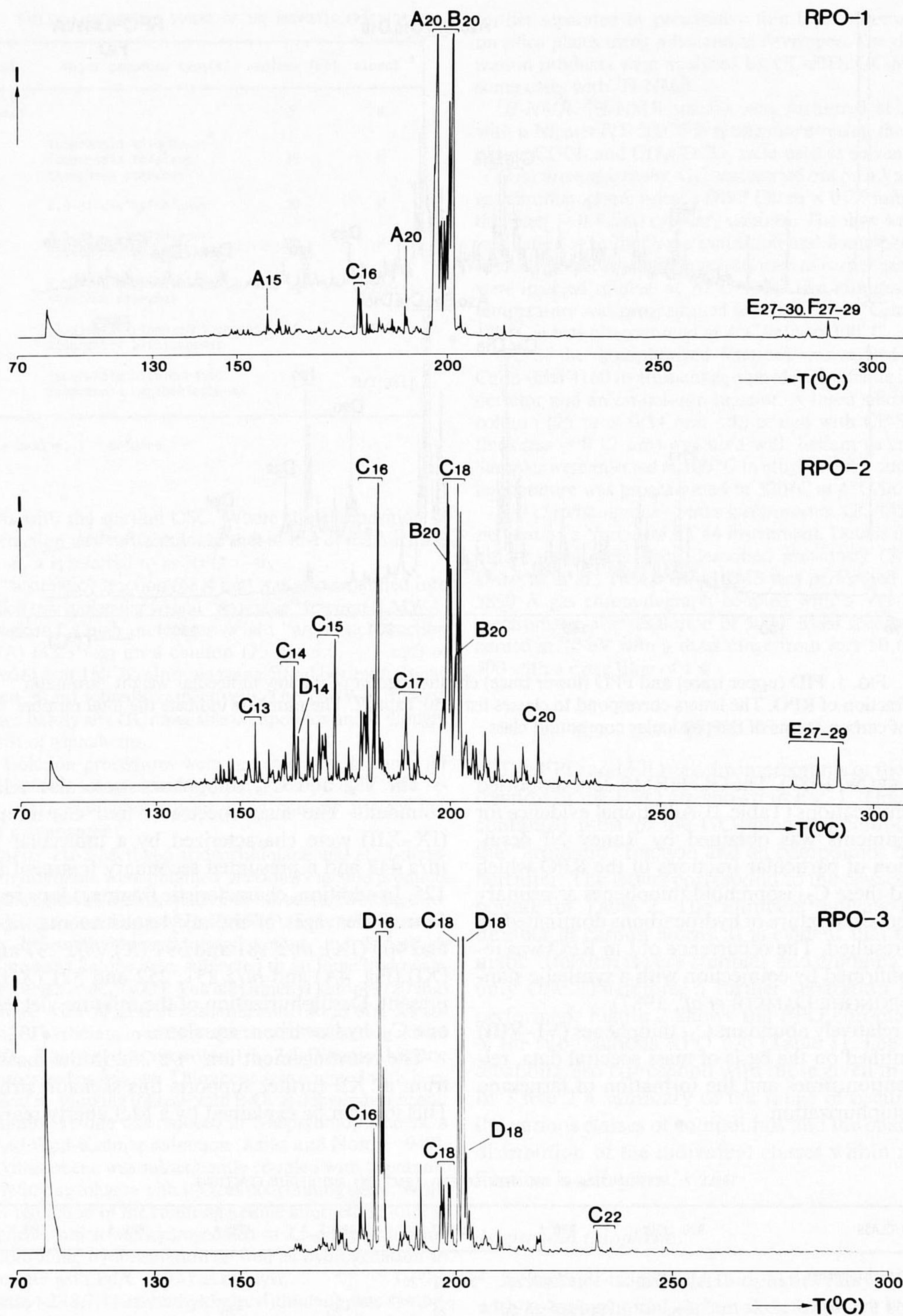


FIG. 2. FPD chromatograms of sub-fractions of the "aromatic" fraction of RPO. The peak designations refer to compounds as indicated in Fig. 1.

ment. The intensity enhancement compared with other isoprenoid thiophenes is thought to be due to abstraction of a tertiary hydrogen from the isoprenoid side chain in the six-membered ring transition state. Such an intensity enhancement of the rearrangement ion, although less pronounced, is also apparent in the mass spectra of 2-(3-methylbutyl)thiophene, 2-ethyl-5-(3-

methylbutyl)thiophene and 2,5-di-(3-methylbutyl)thiophene as compared with those of 2-butylthiophene, 2-butyl-5-ethylthiophene and 2,5-dibutylthiophene, respectively (FOSTER *et al.*, 1964). Similar rearrangement ions were observed in the mass spectra of XIII, XIV and XV as reported before (SINNINGHE DAMSTÉ *et al.*, 1986). These isoprenoid thiophenes all

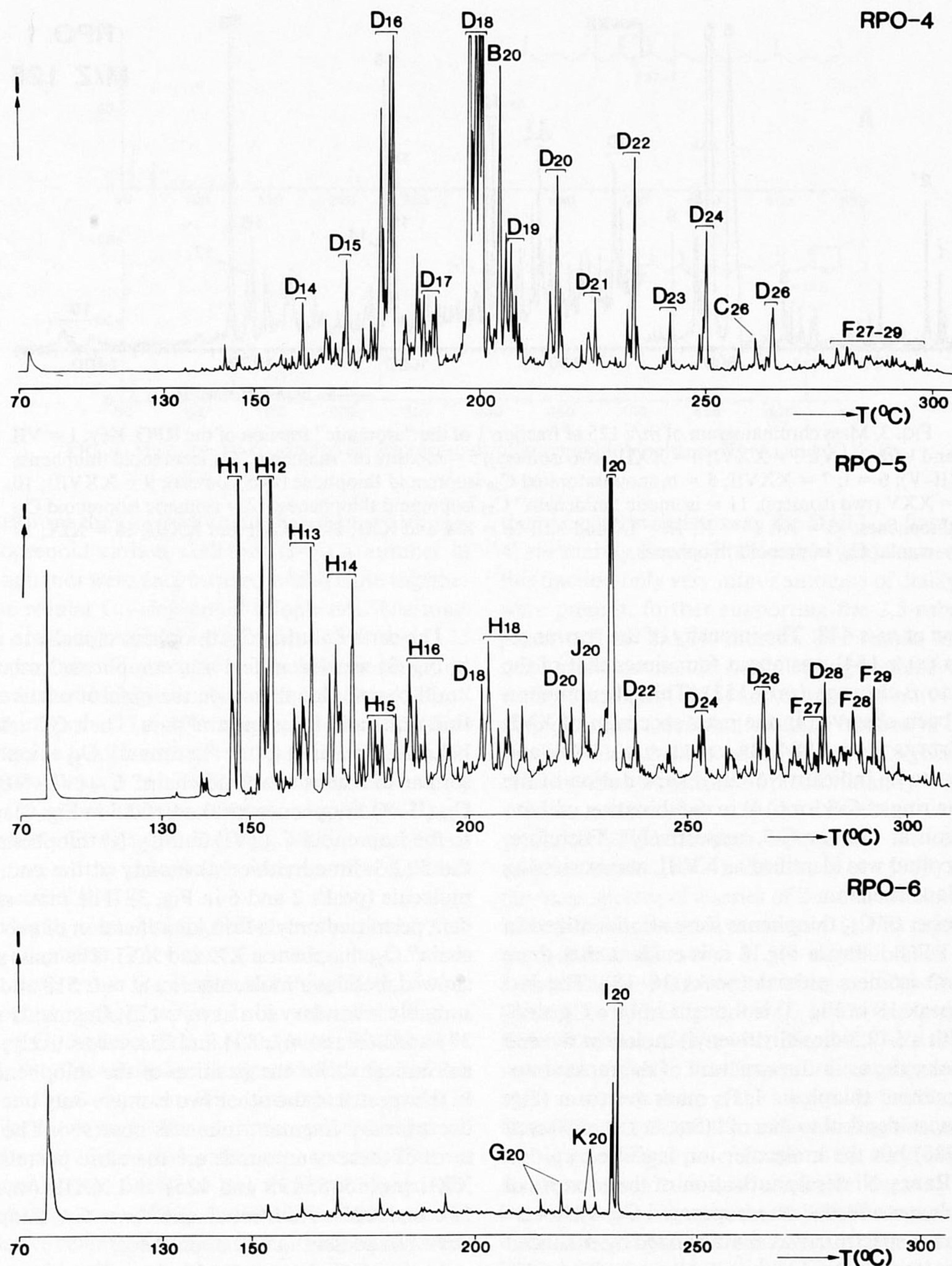
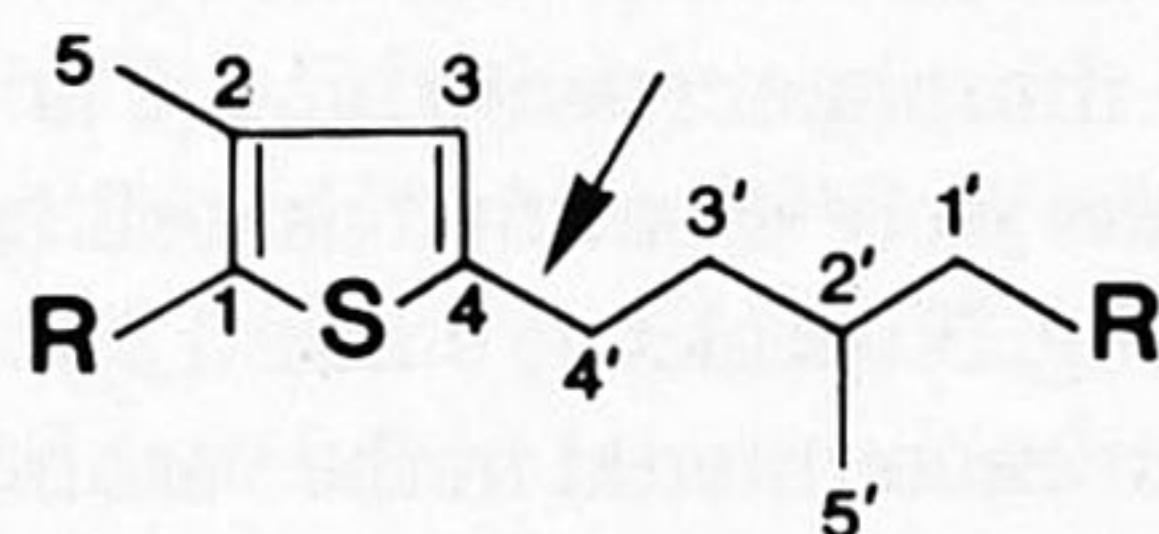


FIG. 2. (Continued)

have a specific part of their structure in common: A tail-to-tail linkage of the isoprenoid carbon skeleton at a particular site of the skeleton as indicated below.



Another observation supports the conclusion that these C_{30} thiophenes have a squalane skeleton: An isoprenoid C_{30} thiophene with a 5-(2,3-dimethyl)thienyl

moiety such as in the structure of I and VI was not present. Because squalane exhibits a tail-to-tail linkage at the centre of the molecule, no such compound can be constructed with squalane as the carbon skeleton. On the basis of these results and considerations the isoprenoid C_{30} thiophenes were identified as IX, X, XI and XII.

Two less abundant C_{30} thiophenes eluted somewhat later than the others. The mass spectrum of one shows ions at m/z 111 (base peak), 139, 181, 209 and 448. It was tentatively identified as XVI, also with the squalane skeleton. The mass spectrum of the other was characterized by ions at m/z 154 and 153 and a mo-

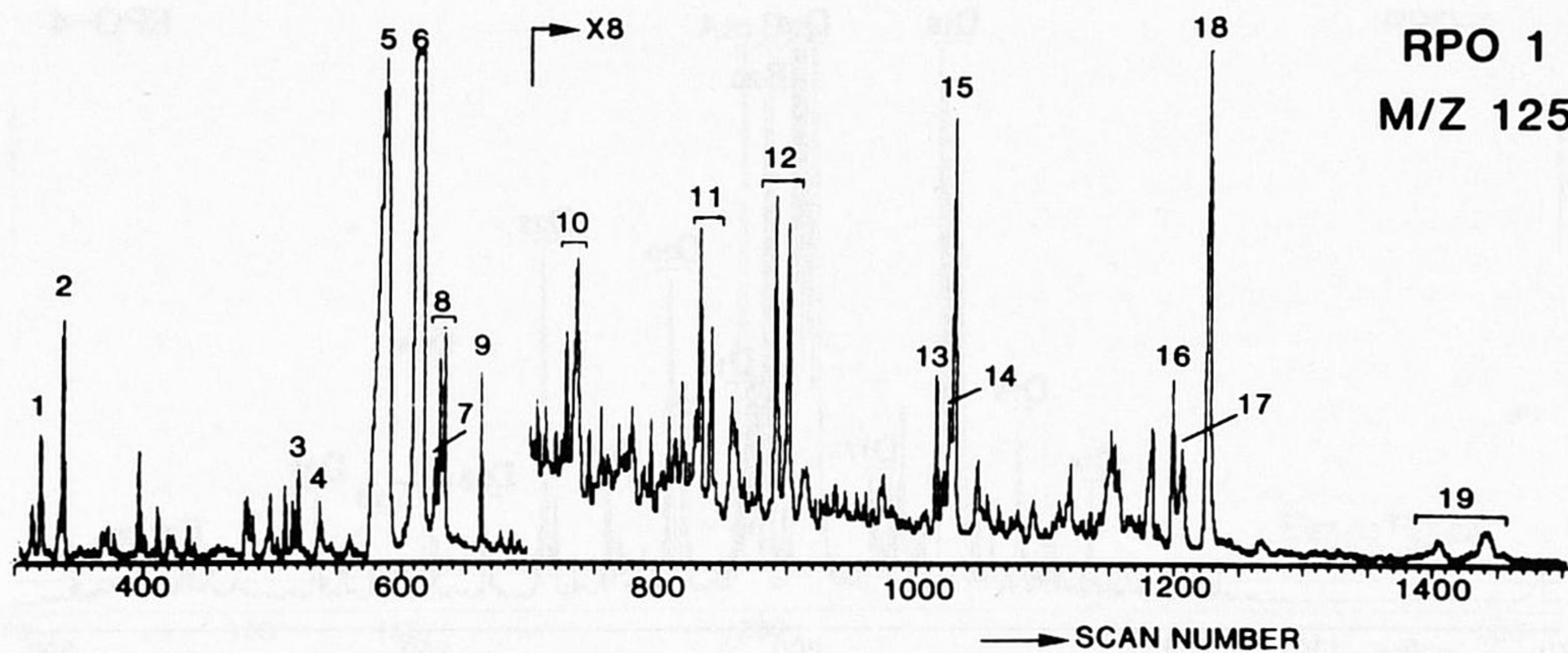


FIG. 3. Mass chromatogram of m/z 125 of fraction 1 of the "aromatic" fraction of the RPO. Key: 1 = VII and VIII; 2 = VI; 3 = XXVI; 4 = XXIV (two isomers); 5 = mixture of "midchain" C_{20} isoprenoid thiophenes (II–V); 6 = I; 7 = XXVII; 8 = monounsaturated C_{20} isoprenoid thiophene (two isomers); 9 = XXVIII; 10 = XXV (two isomers); 11 = isomeric "midchain" C_{25} isoprenoid thiophenes; 12 = isomeric isoprenoid C_{26} thiophenes; 13 = XI; 14 = X; 15 = IX and XII; 16 = XX and XXI; 17 = XXII and XXIII; 18 = XIX; 19 = regular C_{40} isoprenoid thiophenes.

lecular ion at m/z 448. The intensity of the rearrangement ion (m/z 154) was about four times that of the ion due to β -cleavage (m/z 153). This phenomenon has also been observed in the mass spectrum of XVII (RULLKÖTTER *et al.*, 1984; BRASSELL *et al.*, 1986) and is thought to be indicative of alkyl substitution of the thiophene ring at C-3 (or C-4) in combination with no substitution at C-2 (or C-5, respectively). Therefore, this compound was identified as XVIII, also possessing the squalane skeleton.

A number of C_{35} thiophenes were also identified in fraction RPO 1. From Fig. 3 it is evident that there are several isomers present (peaks 16–18). The last eluting (peak 18 in Fig. 3) is thought to be a C_{35} thiophene with a 5-(2,3-dimethylthienyl) moiety at the end of the molecule, as in the structure of the most abundant isoprenoid thiophene I. Its mass spectrum (Fig. 4B) is almost identical to that of I (SINNINGHE DAMSTÉ *et al.*, 1986) but the molecular ion is shifted up 210 daltons. Raney Ni desulphurization of the mixture of C_{35} thiophenes afforded one isoprenoid C_{35} hydrocarbon. Its mass spectrum was characterized by enhanced intensities (as compared with an n -alkane) of m/z 113, 127, 183, 197 and 253, which suggests a regular head-to-tail isoprenoid skeleton. Based on these results this compound was identified as XIX.

The earlier eluting C_{35} thiophenes (peaks 16 and 17 in Fig. 3) were identified as a complicated mixture of "mid-chain" thiophenes on the basis of relative retention times and mass spectral data. Their GC retention behaviour relative to the "terminal" C_{35} thiophene is similar to that of the "mid-chain" C_{15} (VII–VIII) and C_{20} (II–V) thiophenes (peaks 1 and 5 in Fig. 3) relative to the isoprenoid C_{15} (VI) and C_{20} (I) thiophenes with the 5-(2,3-dimethylthienyl) moiety at the end of the molecule (peaks 2 and 6 in Fig. 3). The mass spectral data permitted only a firm identification of two "mid-chain" C_{35} thiophenes: XX and XXI. The mass spectra showed, besides a molecular ion at m/z 518 and a presumably secondary ion at m/z 125, fragments at m/z 377 and 265 and m/z 391 and 251, respectively, which are indicative for the position of the thiophene ring. In the spectra of the other two isomers only one of the two primary fragment ions was observed. The structures of these compounds are therefore postulated as XXII (m/z 518, 195 and 125) and XXIII (m/z 518, 181 and 125). Additional unknown C_{35} thiophenes were also present in low abundance.

At the end of the m/z 125 chromatogram of RPO 1 (Fig. 3) two broad peaks are present. These are ascribed to C_{40} isoprenoid thiophenes. The identifications are, however, tentative because no molecular ions were observed in the mass spectra or those of their desulphurization product, thought to be the regular C_{40} isoprenoid hydrocarbon (enhanced intensities of m/z 113, 127, 183 and 197).

Isoprenoid thiophenes with C_{18} – C_{19} and C_{21} – C_{28} carbon numbers were identified as well (some of them are shown in Fig. 3 (peaks 4, 9 and 12)). A number of these have been encountered in the "aromatic" fraction of an extract of a marl layer from the Northern Apennines (Italy; SINNINGHE DAMSTÉ *et al.*, 1986). Desulphurization confirmed the proposed structures. The distribution of these thiophenes in RPO is similar to that in Northern Apennines Marl.

TABLE 3: EXACT MASS MEASUREMENTS OF PRINCIPAL IONS IN THE MASS SPECTRA OF TWO MAJOR ORGANIC SULPHUR COMPOUNDS.

COMPOUND	m/z value	fragment	exact mass	measured mass	ΔM
I	308 (M^+)	$C_{20}H_{36}S$	308.2538	308.2526	0.0012
	125	C_7H_9S	125.0425	125.0423	0.0002
XXXIII	284 (M^+)	$C_{18}H_{36}S$	284.2438	284.2525	0.0013
	227 (r) ^a	$C_{14}H_{27}S$	227.1834	227.1840	-0.0006
	143 (t) ^a	$C_8H_{15}S$	143.0895	143.0887	0.0008
	101 (q) ^a	C_5H_9S	101.0425	101.0419	0.0006

^a letters refer to fragmentation pathways outlined in Fig. 5.

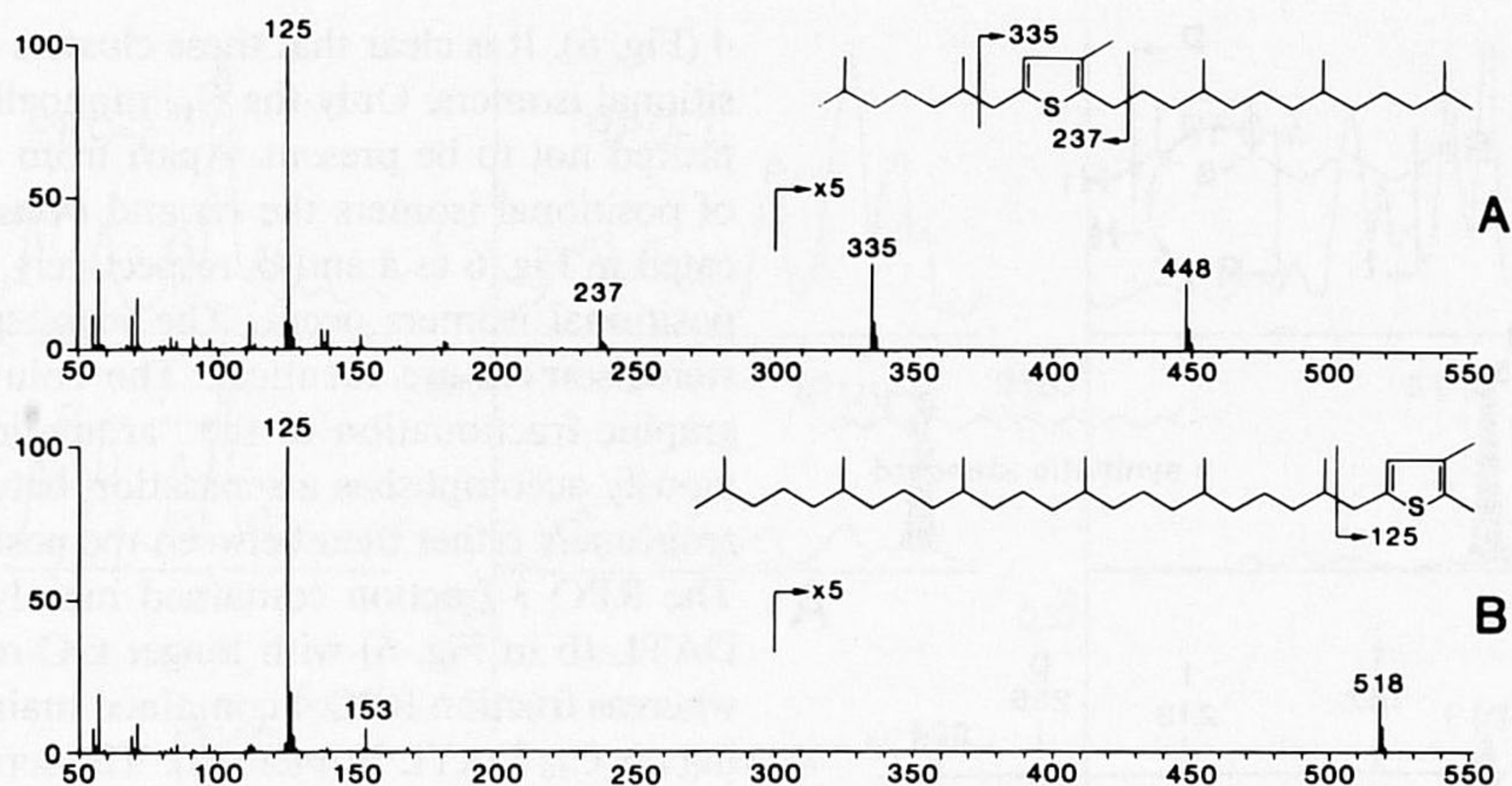


FIG. 4. Mass spectra (corrected for background) of isoprenoid thiophenes XI (A) and XIX (B).

Apart from the C_{20} isoprenoid thiophenes with regular isoprenoid carbon skeletons (I–V) a number of C_{20} thiophenes were encountered which elute together with the regular C_{19} isoprenoid thiophenes. The mass spectra are characterized by m/z values 308, 195, 125 and several other fragment ions. The relative retention times indicate a highly branched carbon skeleton. Upon desulphurization 2,6,10-trimethyl-7-(3-methylbutyl)dodecane (which coelutes with pristane; YON *et al.*, 1982; ROWLAND *et al.*, 1985) was formed, thus establishing the carbon skeleton of these compounds. Based on synthesis (SINNINGHE DAMSTÉ *et al.*, unpublished results) two of the compounds were identified as diastereomers of XXIV.

Two highly branched C_{25} thiophenes were also tentatively identified. They eluted at about the same retention time as the C_{23} isoprenoid thiophenes, which is analogous to the retention behaviour of the highly branched C_{25} hydrocarbon 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane compared with that of the isoprenoid hydrocarbons. The mass spectra exhibit a molecular ion at m/z 378 and two prominent fragment ions at m/z 125 and 265. The mass spectra of these two isomers are identical to those published by RULLKÖTTER *et al.* (1982). Based on the mass spectral and relative retention time data the compounds were tentatively identified as stereoisomers of XXV.

2,5-Di-*n*-alkylthiolanes (DATL)

A series of C_{11} – C_{28} 2,5-di-*n*-alkylthiolanes (DATL) (XXIX, XXX) was identified from mass spectral data, relative retention times, coinjection with several synthetic C_{18} DATL, and desulphurization experiments. Evidence for the 2,5-substitution pattern was obtained by desulphurization of fraction RPO 4', which consisted of a complicated mixture of DATL. Mass spectral analysis showed (see below) that this sub-fraction contained only DATL, except for one isoprenoid C_{20} thiolane and a mixture of thiolane steranes (Fig. 2; RPO 4). Desulphurization gave a mixture strongly dominated by *n*-alkanes with an even-over-odd carbon number predominance. This experiment established

that the carbon skeletons of the DATL in fraction RPO 4' are mainly linear. It should be emphasized that in this fraction only very minor amounts of dialkylthianes were present, further supporting the 2,5-substitution pattern of the DATL present. The results of the desulphurization are in complete agreement with the work of SCHMID (1986) and SCHMID *et al.* (1987) who desulphurized an aliphatic sulfide fraction of the Marvejols crude oil (Alès basin, France; Oligocene) and also obtained a mixture of predominantly *n*-alkanes.

Mass spectra of several low molecular weight ($C_{tot} < C_{10}$) DATL have been reported by KHVOSTENKO and SULTANOV (1964). ZIMINA *et al.* (1960) reported the mass spectra of a series of 2-*n*-alkylthiolanes up to 2-hexylthiolane and ZAIKIN *et al.* (1978) reported the mass spectra of a series of 3-*n*-alkylthiolanes up to 3-hexylthiolane. Mass spectra of several C_{18} – C_{23} DATL (SCHMID, 1986; SCHMID *et al.*, 1987) and 2-methyl-5-tridecylthiolane (SINNINGHE DAMSTÉ *et al.*, 1986) were reported recently. The features of these spectra are in agreement with those of the long-chain DATL reported here. They are characterized by the fragmentations exemplified in Fig. 5; α -cleavage of the alkyl side chains results in ions p and q. The relative intensity of these ions in the mass spectrum of a DATL depends on the ratio of the length of the side chains. The spectrum of XXXI (Fig. 5A) exhibits a M-29 fragment (p) of low intensity. This ion becomes more important as the length of the shorter alkyl side chain increases (Fig. 5). Secondary fragmentations then become more important. In the spectrum of, *e.g.* XXXII (Fig. 5C) m/z 87, which probably results from loss of both alkyl side chains accompanied by transfer of one hydrogen to the thiolane ring, is the base peak. Cleavage through the thiolane ring accompanied by transfer of a hydrogen (fragmentation r, Fig. 5) results in an ion with an m/z value of 42 daltons less than ion p (or q). A displacement reaction, probably *via* a five-membered-ring transition state, results in fragmentation t (Fig. 5). This ion has an m/z value of 42 daltons more than ion q (or p if the side chain is long enough). The elemental composition of the molecular ion and various fragment ions of several C_{18} DATL present in RPO, resulting

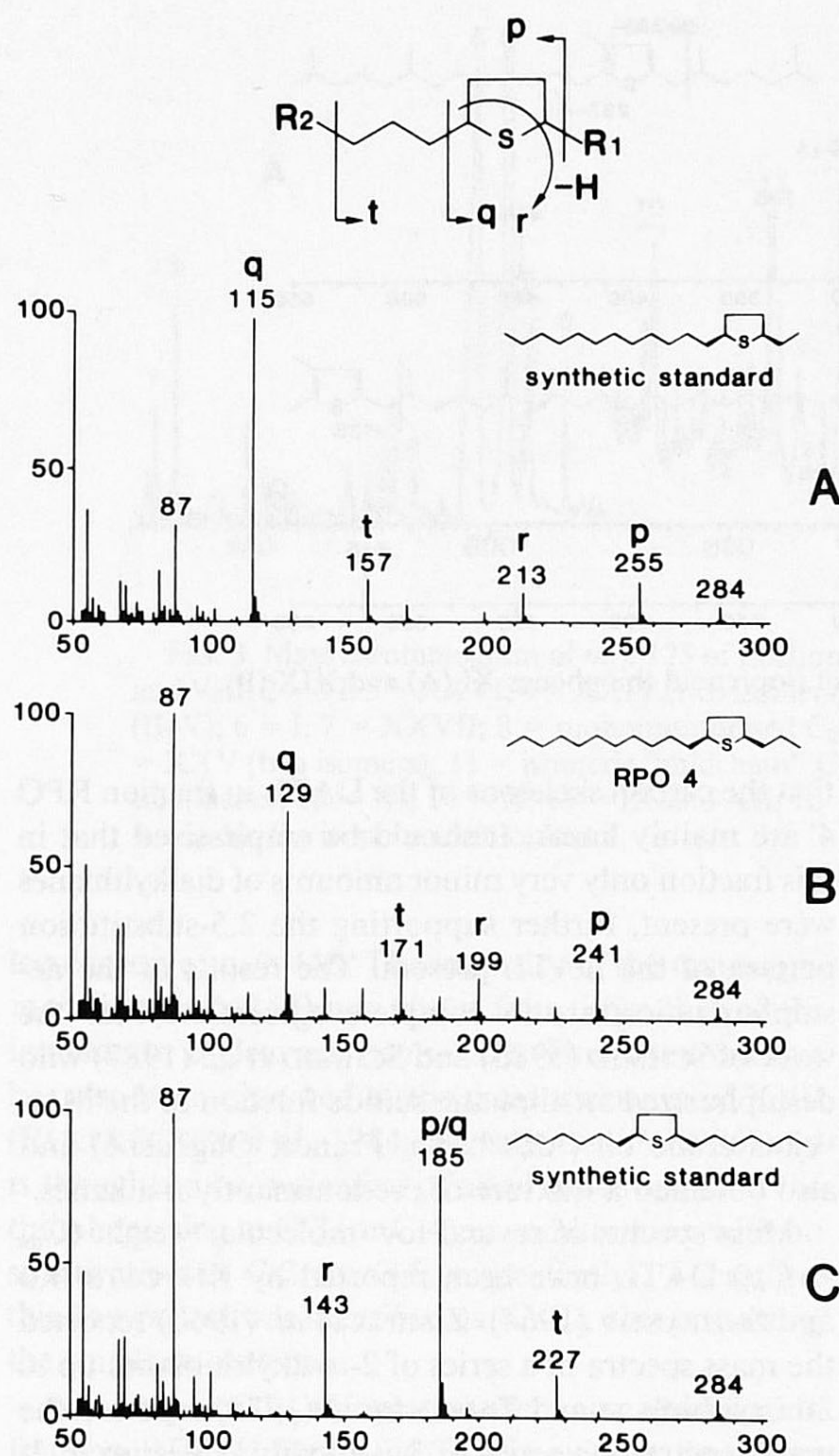


FIG. 5. Mass spectra (corrected for background) of: (A) *trans*-2-dodecyl-5-ethylthiolane (XXXI), (B) *trans*-2-butyl-5-decylthiolane (XXXIV) and (C) *trans*-2,5-diheptylthiolane (XXXII). The letters refer to fragmentation pathways exemplified in the general structure at the top of this figure.

from the proposed fragmentation pathways, is supported by GC-HRMS (Table 3; XXXIII as an example).

Mass chromatography using the characteristic ions p and q seemed suitable for identifying the positional isomers of DATL with a certain number of carbon atoms. The m/z values of ions r and t, however, fall in the same $101 + 14n$ series as those of p and q. This complicated to some extent the identification of the various DATL isomers. For instance, m/z 143 corresponds to both ion t of XXXIII (for a mass spectrum see SINNINGHE DAMSTÉ *et al.*, 1986) and to ion q of XXXIV. The several positional isomers of a DATL cluster representing a certain chain length exhibit, however, slight differences in retention time. Therefore, it is possible to match the several mass chromatograms representing the corresponding p and q fragment ions. The m/z values of ions p and q have to follow the equation $p + q - M = 86$ where M is the molecular ion. This method of identification is exemplified for the C_{16} DATL clusters observed in fractions RPO 2–

4 (Fig. 6). It is clear that these clusters contain six positional isomers. Only the C_{16} monoalkylthiolane appeared not to be present. Apart from the occurrence of positional isomers the *cis* and *trans* isomers, indicated in Fig. 6 as a and b, respectively, of virtually all positional isomers occur. The mass spectra of these stereoisomers are identical. The column chromatographic fractionation of the "aromatic" fraction obviously accomplishes a separation between these stereoisomers rather than between the positional isomers. The RPO 3 fraction contained mainly the *trans* C_{16} DATL (b in Fig. 6) with longer GC retention times, whereas fraction RPO 4 contained mainly earlier eluting *cis* C_{16} DATL isomers (a). The separation on the CP-Sil 5 column between the several C_{16} DATL isomers with the same sterical configuration was more effective for the *cis*-series. The separation between the positionally-isomeric C_{16} DATL decreased as the length of the shortest alkyl side chain increased.

The identification of the *cis* and *trans* isomers is based on the synthesis of several isomeric C_{18} standards and their coinjections (Figs. 7A–D). Three C_{18} DATL have been synthesized: XXXIII, XXXI (SINNINGHE DAMSTÉ *et al.*, 1986) and XXXII. These standards were obtained from the corresponding thiophenes by two different synthetic pathways. The *cis* isomers were obtained exclusively (>90%) after catalytic hydrogenation with Pd/C, whereas a mixture (1:1) of the *cis* and *trans* isomers was obtained applying an ionic hydrogenation with triethylsilane (PARNES *et al.*, 1977). The *cis* isomer of XXXII coeluted with the first eluting compound of the mixture of stereoisomers obtained by ionic reduction of 2,5-diheptylthiophene. Both the *cis* and the *trans* isomers of the synthetic XXXIII and XXXI coeluted with each other and with the corresponding positional isomers in fractions RPO 3 and 4. The mass spectra of the *cis* and *trans* isomers of XXXI, XXXII, and XXXIII were identical.

Proof of the fact that ionic hydrogenation indeed yielded a pair of coeluting *cis* and *trans* isomers was obtained from the formation of sulfoxides in the synthesis of the DATL XXXI, XXXII and XXXIII. In all three syntheses, using ionic hydrogenation as the last reaction step, a pair of two GC-separable isomeric, probably *cis* and *trans*, sulfoxides with the same carbon skeletons as the corresponding thiolanes were formed as side products. These pairs of isomeric sulfoxides exhibited identical mass spectra. The ratio of the two sulfoxides formed in the ionic reduction of 2,5-diheptylthiophene was the same as that of *cis* and *trans* 2,5-diheptylthiolane. Hydrogenation with Pd/C of 2,5-diheptylthiophene led to the formation of only one sulfoxide (and one thiolane). These two facts indicated that the *cis* and *trans* sulfoxides were probably formed via the *cis* and *trans* DATL. Because of the formation of two isomeric sulfoxides in the synthesis of thiolanes XXXI and XXXII using ionic hydrogenation, it seems highly probable that *cis* and *trans* DATL were formed but coeluted on the column used.

The other thiolane clusters in fractions RPO 2–5

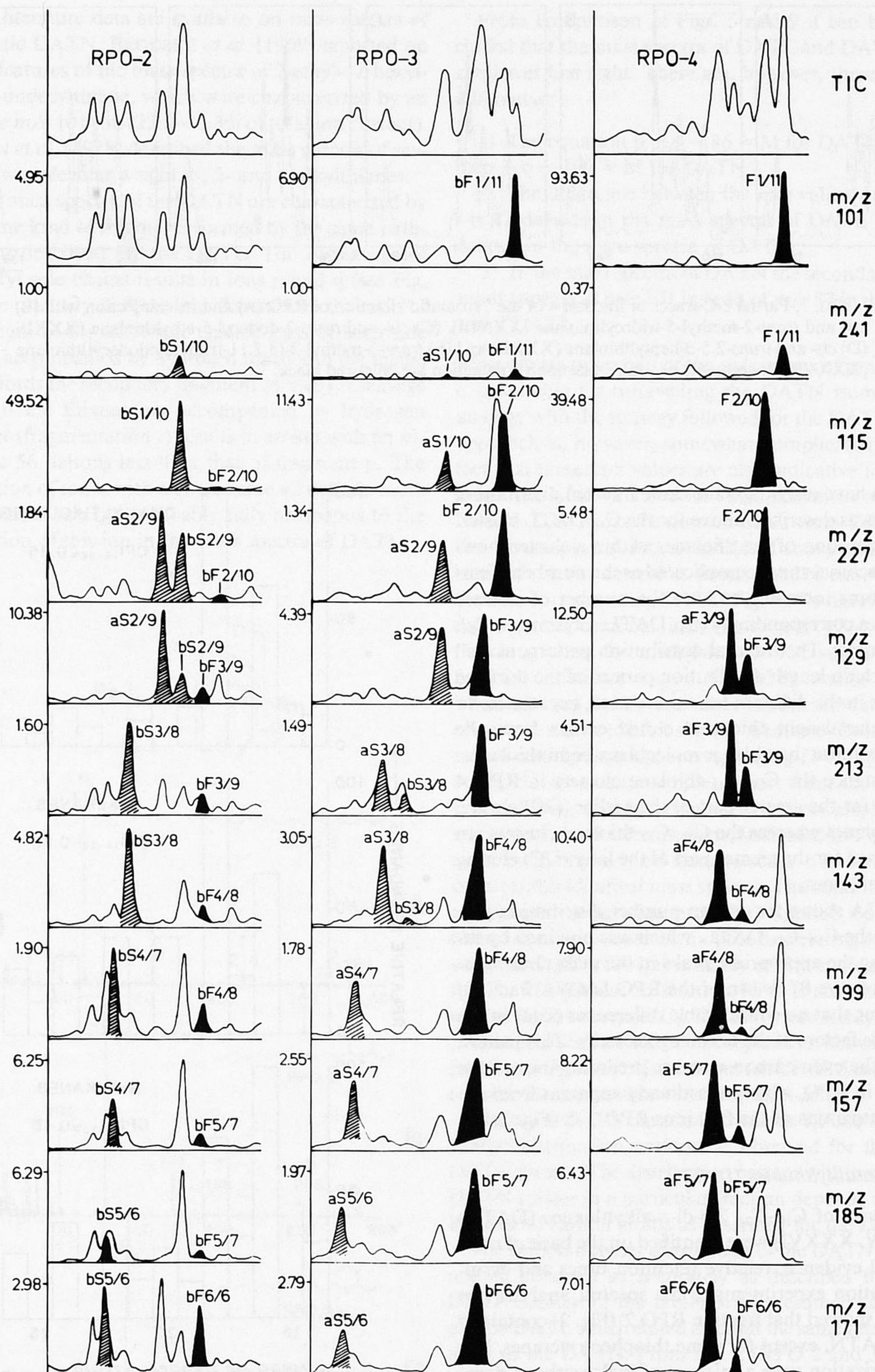


FIG. 6. Mass chromatograms of diagnostic m/z values for DATL (black peaks) and DATN (shaded peaks) of that particular area of the TIC of fractions 2, 3 and 4 of the "aromatic" fraction of the RPO, in which the C_{16} DATL and DATN elute. The symbol indications refer to particular isomers (see XXIX, XXX, XXXV and XXXVI, key: F = five membered ring, S = six membered ring, x and y = number of carbon atoms in alkyl side chains, a = *cis*, b = *trans*).

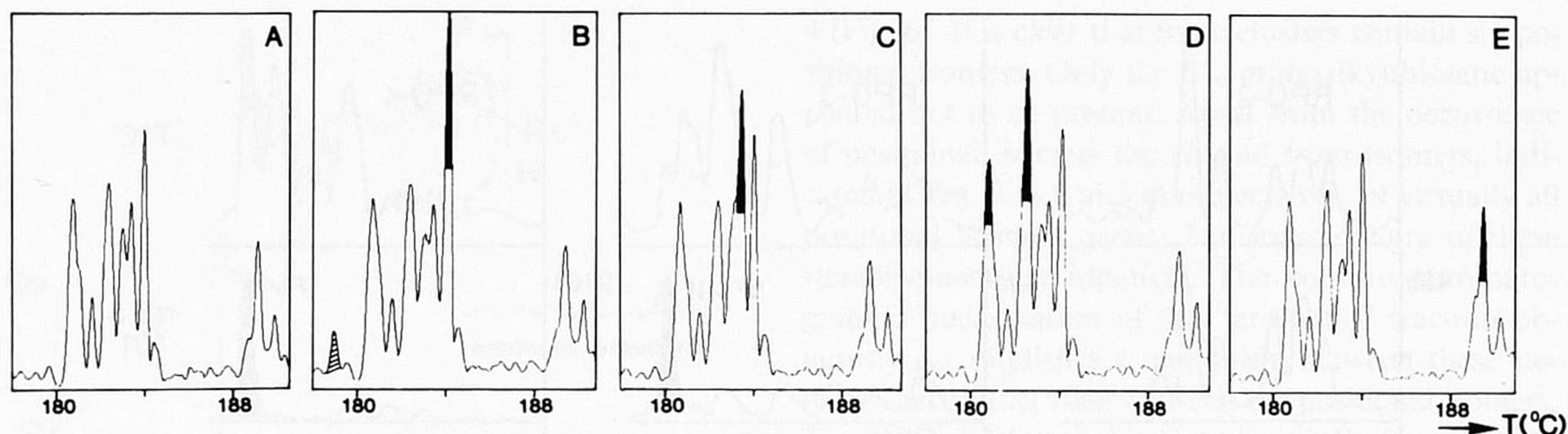


FIG. 7. Partial GC-traces of fraction 4 of the "aromatic" fraction of RPO (A) and its coinjection with (B) *cis*- and *trans*-2-methyl-5-tridecylthiolane (XXXIII), (C) *cis*- and *trans*-2-dodecyl-5-ethylthiolane (XXXI), (D) *cis*- and *trans*-2,5-diheptylthiolane (XXXII) and (E) *trans*-3-methyl-2-(3,7,11-trimethyldodecyl)thiolane (XXXVII). Increments of peak areas upon coinjection are coloured black.

(Fig. 2) have essentially the same internal distribution patterns as described above for the C_{16} DATL cluster. The separation of the thiolanes within a cluster, however, becomes more complicated as the number of carbon atoms increases because the number of isomers increases correspondingly (C_{16} DATL; 12 isomers; C_{24} , 20 isomers). The internal distribution pattern, as well as the chain length distribution pattern of the thiolane clusters in the different fractions varied, because lower molecular weight thiolanes eluted earlier from the Al_2O_3 column than higher molecular weight thiolanes. For instance the C_{14} - C_{18} thiolane clusters in RPO 4 consist for the greater part of the earlier (GC) eluting *cis*-thiolanes whereas the C_{19} - C_{27} thiolane clusters are composed for the greater part of the later (GC) eluting *trans*-thiolanes.

Fig. 8A shows the carbon-number distribution pattern of the C_{14} - C_{26} DATL, which was obtained by integrating the appropriate peaks in the mass chromatograms of $m/z 87 + 14n$ of the RPO LMWA fraction, assuming that no considerable differences occur in the response factors of the homologous series. This pattern shows the even carbon number predominance of the DATL in RPO, which was already apparent from the chromatograms of the fractions RPO 3-5 (Fig. 2).

2,6-Di-*n*-alkylthianes (DATN)

A series of C_{11} - C_{28} 2,6-di-*n*-alkylthianes (DATN) (XXXV, XXXVI) were identified on the basis of mass spectral evidence, relative retention times and desulphurization experiments. Mass spectral analysis (see below) showed that fraction RPO 2' (Fig. 2) contained only DATN, except for some thiophene steranes. Desulphurization gave a mixture of hydrocarbons dominated by *n*-alkanes with even-over-odd carbon number predominance. This experiment established that the DATN are substituted exclusively at positions 2 and 6 of the thiane ring. It should be emphasized that in this fraction only very minor amounts of DATL were present. The isomer distribution patterns were studied in detail by GC-MS.

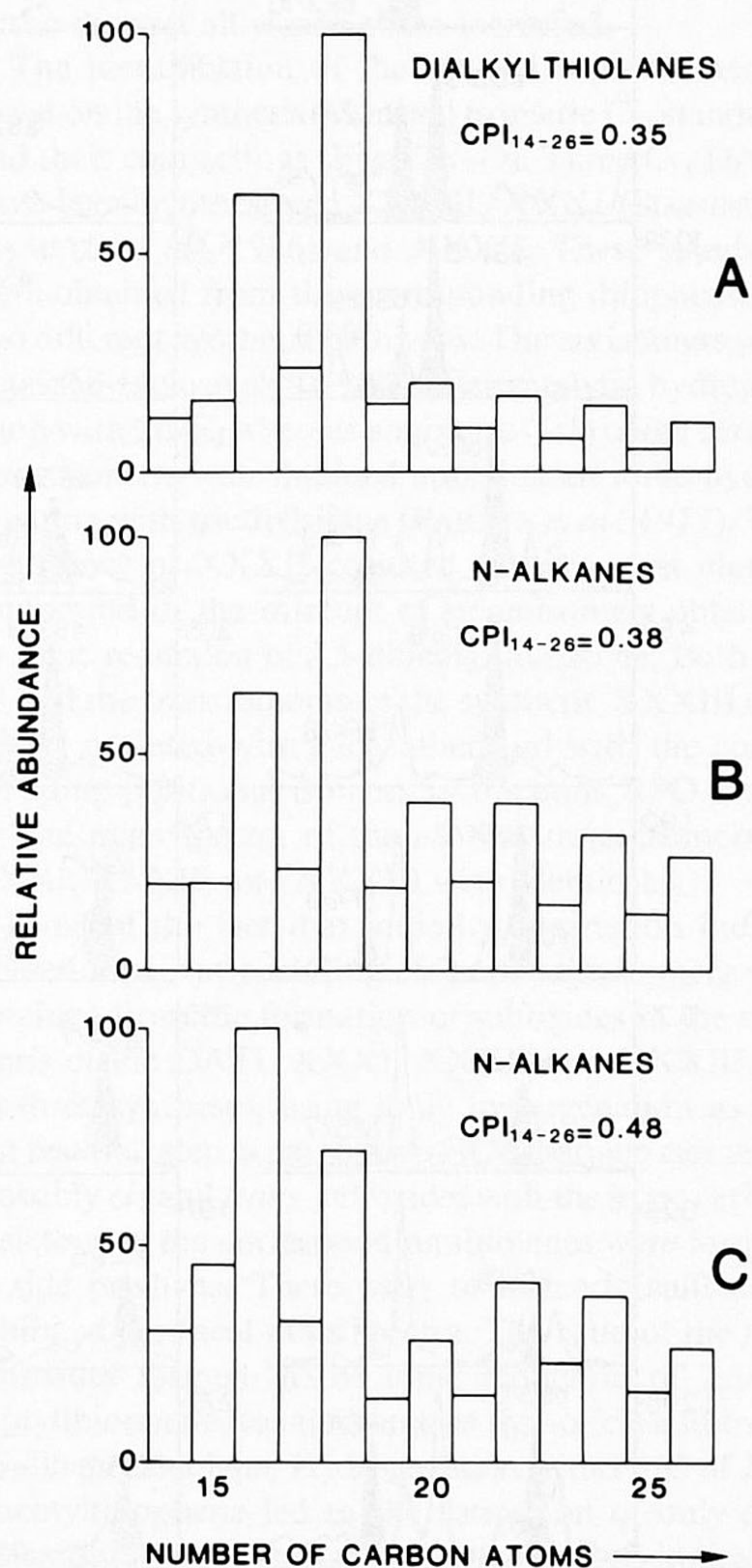


FIG. 8. Carbon number distribution patterns (C_{14} - C_{26}) of: (A) DATL obtained by integrating the appropriate peaks in the mass chromatograms of $m/z 87 + 14n$ (n depends on the carbon number) of the LMWA fraction of the RPO, (B) *n*-alkanes after desulphurization of the aromatic fraction of the RPO. This pattern represents the distribution pattern of DATL plus DATN, (C) *n*-alkanes in the adduct of the aliphatic fraction of RPO.

No literature data are available on mass spectra of synthetic DATN. BRODSKII *et al.* (1969) reported on some features of the mass spectra of 2-ethyl-, 2-hexyl- and 2-undecylthiane, which were characterized by an intense m/z 101 ion (23.8–33.3% of total ion current). ZAIKIN *et al.* (1978) described the mass spectra of several low molecular weight 2-, 3- and 4-alkylthianes.

The mass spectra of the DATN are characterized by the same kind of fragments formed by the same pathways as described for the DATL. The α -cleavage of the alkyl side chains results in ions p and q (see Fig. 9). The intensity of these ions depends on the ratio of the length of the alkyl side chains. Loss of both side chains accompanied by hydrogen transfer to the thiane ring affords the secondary fragment m/z 101. Cleavage through the thiane ring accompanied by hydrogen transfer (fragmentation r) results in an ion with an m/z value 56 daltons less than that of fragment p. The formation of ion t, with an m/z value 42 daltons more than that of ion q, is probably fully analogous to the formation of this ion in the mass spectra of DATL.

From comparison of Figs. 5 and 9 it can be concluded that the mass spectra of DATL and DATN are similar at first sight. There are, however, three major differences:

- 1) The equation $p + q - 86 = M$ for DATL has to be $p + q - 100 = M$ for DATN.
- 2) The difference between the m/z values of p and r is 42 daltons in the mass spectra of DATL but 56 daltons in the mass spectra of DATN.
- 3) In the mass spectra of DATN the secondary fragment appears at m/z 101 instead of m/z 87 in the spectra of DATL.

Mass chromatography of the m/z values of ions p and q is suitable for unravelling the DATN isomers, by analogy with the strategy followed for the DATL. This approach is, however, somewhat complicated by the fact that these m/z values are also indicative for fragment ions of DATL and for fragment ions r and t of DATN. DATN can be discriminated from DATL by their mass spectral data as indicated above and on the basis of relative retention times. DATN elute earlier than the corresponding DATL (Fig. 6). To discriminate the several positional isomers of a DATN cluster matching mass chromatograms of m/z values which fulfil the equation $p + q - 100 = M$ have to be traced. The positional DATN isomers with the same number of carbon atoms exhibit slightly greater differences in retention time than the corresponding DATL (cf. Fig. 6; C_{16} cluster as an example). Apart from the occurrence of five positional isomers (the monoalkylthiane was absent) both stereoisomers, indicated in Fig. 6 by a and b, of all positional isomers occur. The stereoisomers exhibit identical mass spectra. The column chromatographic separation procedure applied led to an almost complete separation of the first eluting (GC) a-series of C_{16} DATN (presumably *cis* by analogy with the DATL) present in fraction RPO 2 and the later eluting (GC) b-series of C_{16} DATN (presumably *trans*) present in fraction RPO 3. The separation between the positional C_{16} DATN isomers on the CP-Sil 5 capillary column was somewhat better for the a-series.

The other DATN clusters in fractions RPO 2–4 have similar distribution patterns as observed for the C_{16} DATN cluster. The distribution pattern within a given DATN cluster in a particular fraction depends on the number of carbon atoms as described for the DATL.

The carbon number distribution of the DATN could not be obtained as accurately as described for the DATL because of the presence of presumed *iso* and *anteiso* DATL which eluted at about the same retention times as the DATN. From Fig. 2 (RPO 2 and 3) it is obvious, however, that the DATN also show an even carbon number preference. Figure 8B shows the carbon number distribution of the C_{14} – C_{26} *n*-alkanes, observed after desulphurization of the "aromatic" fraction of RPO. This pattern reflects those of DATL and DATN added to each other. Because the concentrations of DATL and DATN are of the same order of magnitude (Fig. 1) and this distribution pattern resembles that of

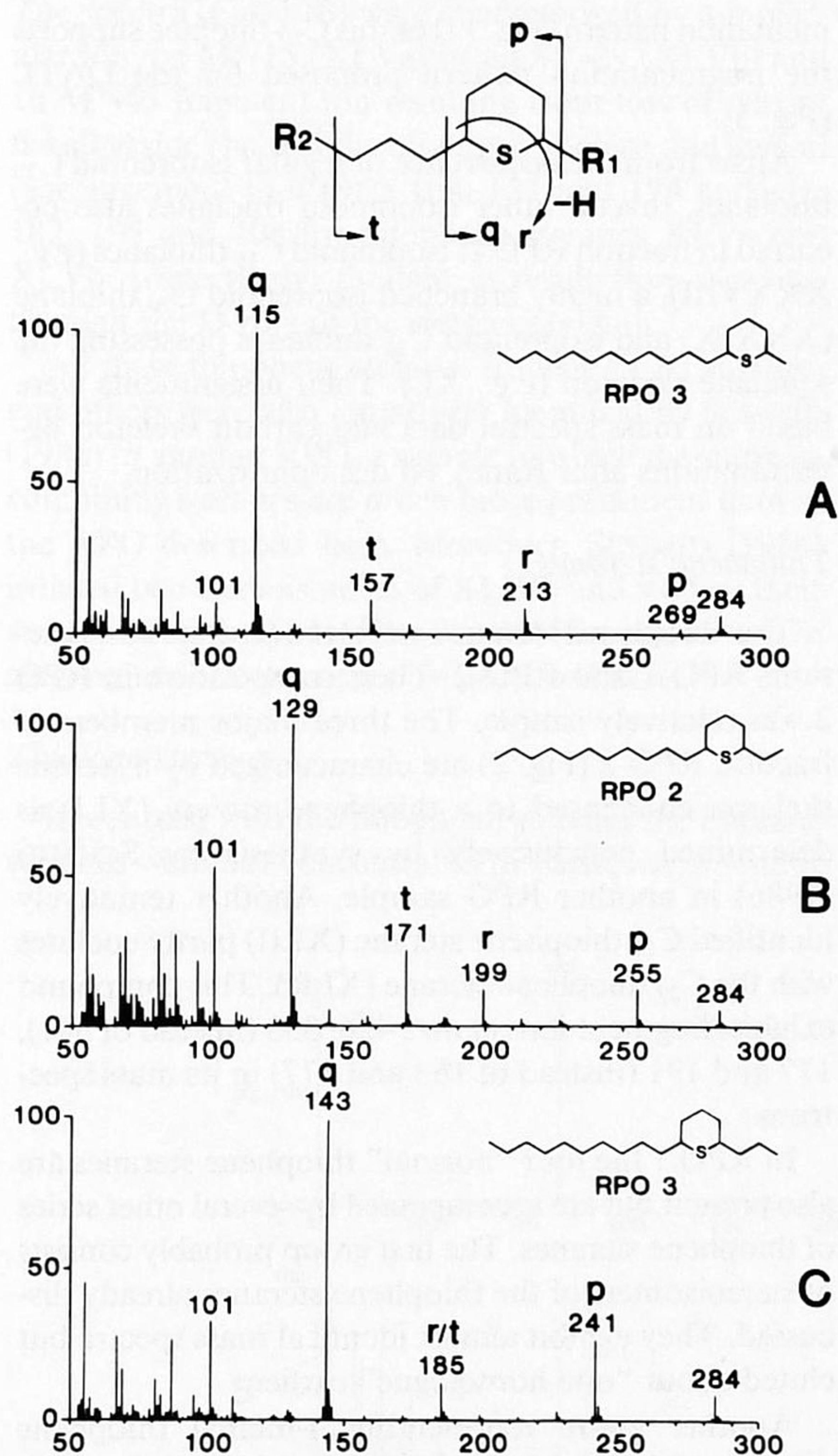


FIG. 9. Mass spectra (corrected for background) of: (A) 2-dodecyl-6-methylthiane, (B) 6-ethyl-2-undecylthiane and (C) 2-decyl-6-propylthiane. The letters refer to fragmentation pathways exemplified in the general structure shown.

the DATL (Fig. 8A) it may be concluded that the distribution pattern of DATN is similar to that of the DATL.

Isoprenoid thiolanes

A complicated mixture of presumably C_{20} isoprenoid thiolanes occurs in RPO. These compounds elute at about the same retention times as the C_{18} DATL and DATN as shown in Fig. 10. This figure shows the high resolution mass chromatograms of m/z 284.2538 ($C_{18}H_{36}S$) and m/z 312.2851 ($C_{20}H_{40}S$) of the LMWA fraction of RPO. This retention behaviour is indicative of the isoprenoid nature of these compounds. This assignment was supported by desulphurization of a sub-fraction of RPO 1, a fraction which did not contain any isoprenoid thiophenes; phytane was the most abundant compound.

The identification of these thiolanes was complicated by the complex composition of this compound class. Upon fractionation of the "aromatic" fraction of RPO the isoprenoid C_{20} thiolanes showed up in fractions RPO 1–4. However, the larger part eluted in fraction RPO 1 (Fig. 2). This complex C_{20} -cluster of isoprenoid thiolanes could not be separated by GC into individual compounds, thus preventing the acquisition of pure mass spectra. The presence of both *cis* and *trans* isomers is a possible explanation for the higher degree of complexity when compared with that observed for the isoprenoid thiophenes. Also, the possible presence of isoprenoid C_{20} thianes in this cluster cannot be ruled out. Nevertheless, a limited number of isoprenoid thiolanes could be identified. Some have been described before (SINNINGHE DAMSTÉ and DE LEEUW, 1987).

An isoprenoid thiolane present in RPO 4 could be identified as XXXVII. This compound has been reported previously to occur in a sediment extract from

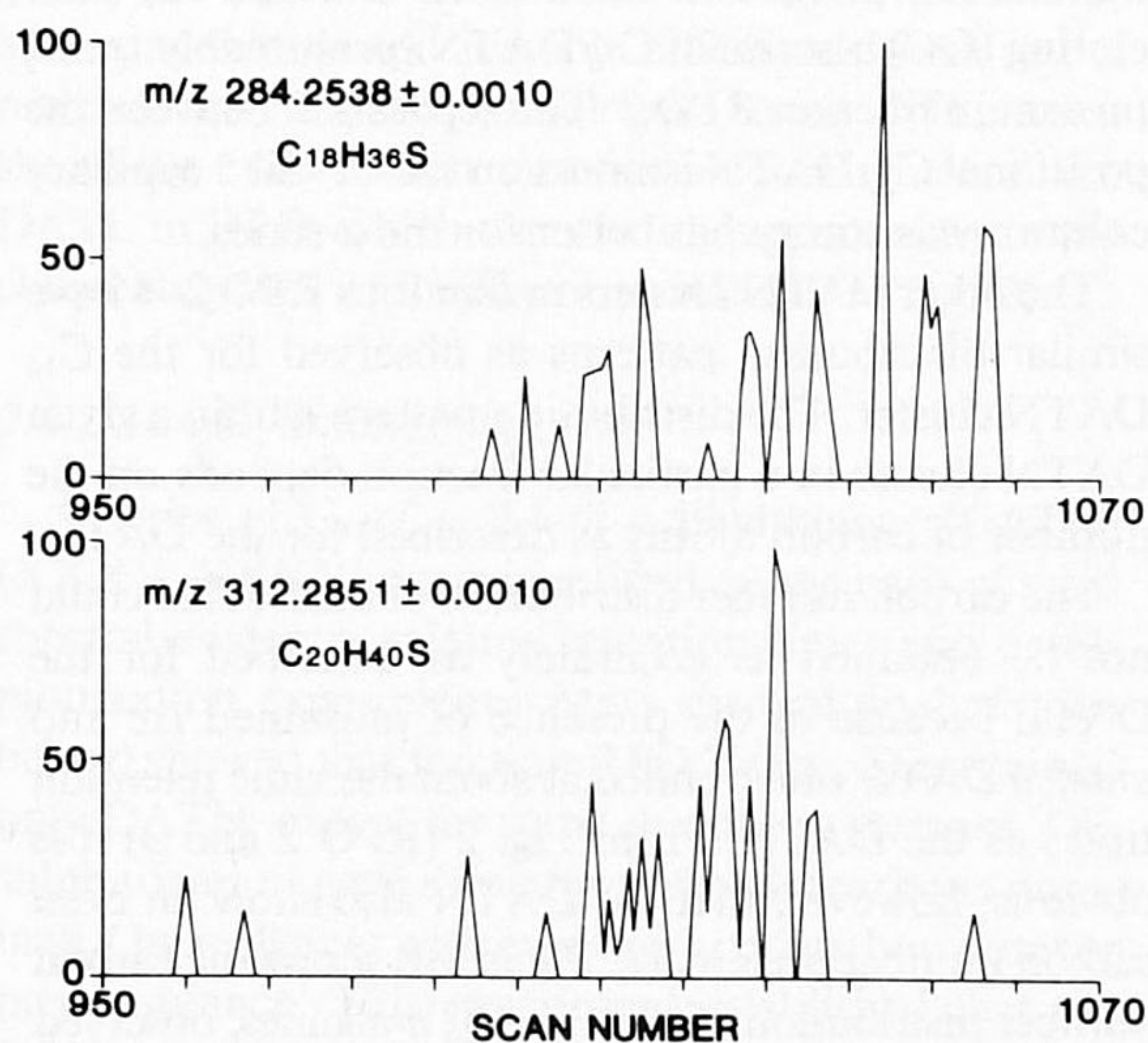


FIG. 10. High resolution mass chromatogram of m/z 284.2538 \pm 0.0010 ($C_{18}H_{36}S$) and m/z 312.2851 \pm 0.0010 ($C_{20}H_{40}S$) of the LMWA fraction of the RPO, showing the coelution of the isoprenoid C_{20} thiolanes with the C_{18} DATL and DATN.

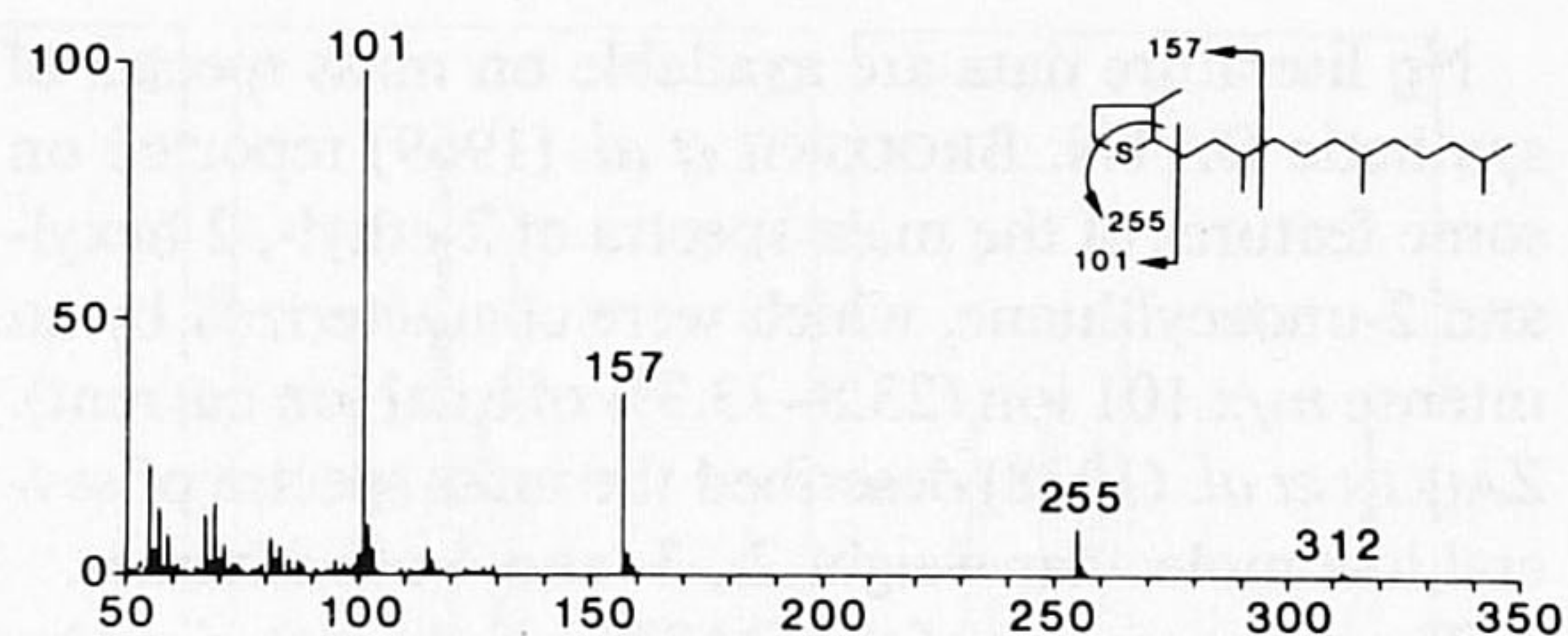


FIG. 11. Mass spectrum (corrected for background) of *trans*-3-methyl-2-(3,7,11-trimethyldodecyl)thiolane (XXXVII).

a marl layer in the Northern Apennines (SINNINGHE DAMSTÉ *et al.*, 1986). Coinjection of fraction RPO 4 with a synthetic standard proved this assignment (Figs. 7A and E). The standard was obtained by ionic hydrogenation of XXVII, synthesized previously (BRASSELL *et al.*, 1986). This hydrogenation yielded a mixture of isoprenoid thiolanes of which presumably *trans*-3-methyl-2-(3,7,11-trimethyldodecyl)thiolane was the primary constituent; *cis*-3-methyl-2-(3,7,11-trimethyldodecyl)thiolane was obtained by hydrogenation of XXVII with Pd/C. The mass spectral fragmentation pattern (Fig. 11) of this C_{20} -thiolane supports the fragmentation pattern proposed for the DATL (Fig. 5).

Apart from the occurrence of regular isoprenoid C_{20} thiolanes, several other isoprenoid thiolanes also occurred in fraction RPO 1: Isoprenoid C_{15} thiolanes (*e.g.*, XXXVIII), a highly branched isoprenoid C_{20} thiolane (XXXIX) and isoprenoid C_{30} thiolanes possessing the squalane skeleton (*e.g.*, XL). Their assignments were based on mass spectral data and carbon skeleton determinations after Raney Ni desulphurization.

Thiophene steranes

The thiophene steranes were concentrated in fractions RPO 1 and RPO 2. Their composition in RPO 2 was relatively simple. The three major members of fraction RPO 2 (Fig. 2) are characterized by a sterane skeleton condensed to a thiophene moiety (XLI) as determined conclusively by synthesis by SCHMID (1986) in another RPO sample. Another tentatively identified C_{29} thiophene sterane (XLII) partly coelutes with the C_{29} thiophene sterane (XLIc). This compound exhibits fragment ions at m/z 426, 355 (instead of 341), 177 and 191 (instead of 163 and 177) in its mass spectrum.

In RPO 1 the four "normal" thiophene steranes are also present but are accompanied by several other series of thiophene steranes. The first group probably consists of stereoisomers of the thiophene steranes already discussed. They exhibit almost identical mass spectra but eluted about "one homologue" earlier.

Another group representing 4-methyl thiophene steranes (XLIII) was also encountered in this fraction. Their structures are characterized by a condensed thiophene moiety and a methyl group at C-4 of the sterane skeleton. The mass spectra show a molecular

ion, a base peak at m/z 355 and two smaller fragment ions at m/z 163 and 177. These last two fragment ions are important because their presence suggested that the methyl group was not located at positions 14 or 22 of the original sterane skeleton since the ions m/z 177 and 191 were not enhanced in the mass spectra.

Two other groups of thiophene steranes were also tentatively identified. Both are characterized by the presence of a thiophene moiety in the side chain. The mass spectra of XLIV were characterized by the molecular ion and by one major fragment ion either at m/z 153 or 167 (Fig. 12A) originating from β -cleavage. Two stereoisomers of every structural isomer were encountered. Circumstantial evidence for the assignment of XLIV b and c was obtained by Raney Ni desulphurization. These four isomeric C_{29} thiophene steranes were concentrated in one particular fraction, which did not contain other thiophene steranes. Desulphurization of this fraction afforded a mixture of C_{29} steranes dominated by the $5\alpha(H),14\beta(H),17\beta(H)$ -isomers.

The mass spectra of the other group of thiophene steranes (XLV) exhibited more complicated spectra. The spectra (Fig. 12B) were characterized by a molecular ion, an M^+-15 (XLVa) or an M^+-29 (XLVb) and an M^+-43 fragment ion resulting from loss of part of the alkyl side chain of the thiophene moiety, and several rearrangement ions (m/z 168, 181 and 194 and m/z 182, 195 and 208 for thiophene steranes XLVa and XLVb, respectively) thought to result from cleavage through the D-ring of the sterane skeleton.

All these thiophene steranes (except XLII) and several others were also tentatively identified by SCHMID (1986) in another RPO, a sample in which the sulphur-containing steranes are much more prominent than in the RPO described here. Moreover, SCHMID (1986) isolated two stereoisomers of XLIVb and studied their structures with 1H -NMR. The NMR spectra are in agreement with the structure proposed here.

Thiolane steranes

In contrast with the thiophene steranes the thiolane steranes were not concentrated in particular fractions

(Table 2). They consisted of a complicated mixture, probably because of the presence of additional stereoisomers as compared with the thiophene steranes. The mass spectra (see SCHMID, 1986) of an abundant thiolane steranes series (XLVI) are characterized by an ion at m/z 331 resulting from α -cleavage of the alkyl side chain and a base peak at m/z 115, 129 and 143 for the C_{27} , C_{28} and C_{29} members, respectively. The formation of these latter ions is probably analogous to fragmentation r (Fig. 5), *i.e.* fragmentation through the thiolane ring. An additional ion at m/z 99 (C_5H_7S) was observed. Several other series, described by SCHMID (1986), were present.

2,4-Di-*n*-alkylbenzo(b)thiophenes (DABT)

Series of 2,4-di-*n*-alkylbenzo(b)thiophenes (DABT; XLVII) were identified from mass spectral, 1H -NMR and relative retention time data and desulphurization experiments. Upon fractionation the DABT were concentrated in fractions RPO 5 and 6. Fraction RPO 5 especially contains a considerable portion of DABT (Fig. 2).

The 2,4-dialkyl substitution of the DABT was confirmed by desulphurization of fraction RPO 5'. GC and GC-MS data revealed the presence of all isomeric 1,2-di-*n*-alkylbenzenes (DAB) ranging from C_{11} up to C_{26} . The mass spectra of the DAB are characterized by one or two ions, resulting from β -cleavage(s) of the alkyl side chain(s). These ions were used to identify the several isomers in a cluster of DAB. The retention behaviour of the C_{11} to C_{16} DAB was in accordance with literature data (SOJÁK *et al.*, 1977; Fig. 13). The aromatic signals in the 1H -NMR spectra (in CD_3COCD_3) of the desulphurized fraction were consistent with the 1,2-substitution pattern. The aromatic signal consisted of a very complicated but symmetric signal ranging from $\delta = 7.05$ up to $\delta = 7.17$, which is indicative for ortho-substituted benzenes.

The aromatic signals in the 1H -NMR spectra of the original fraction (fraction RPO 5') were in agreement with the 2,4-substitution pattern of the DABT. These signals were characterized by two broad peaks ($\delta = 7.07$

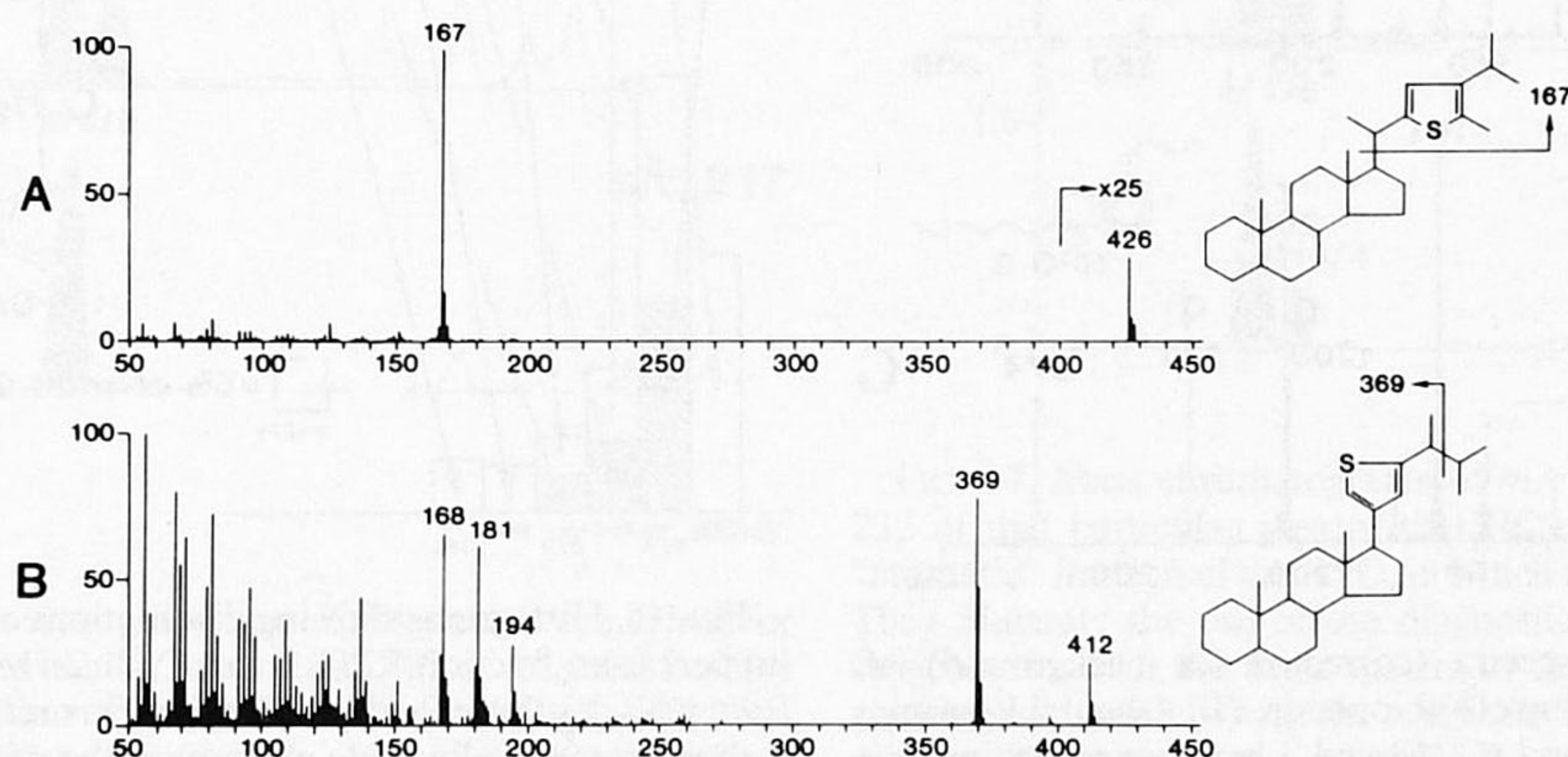


FIG. 12. Two selected mass spectra of tentatively identified steranes (XLIVb and XLVa) with a thiophene moiety in the side chain.

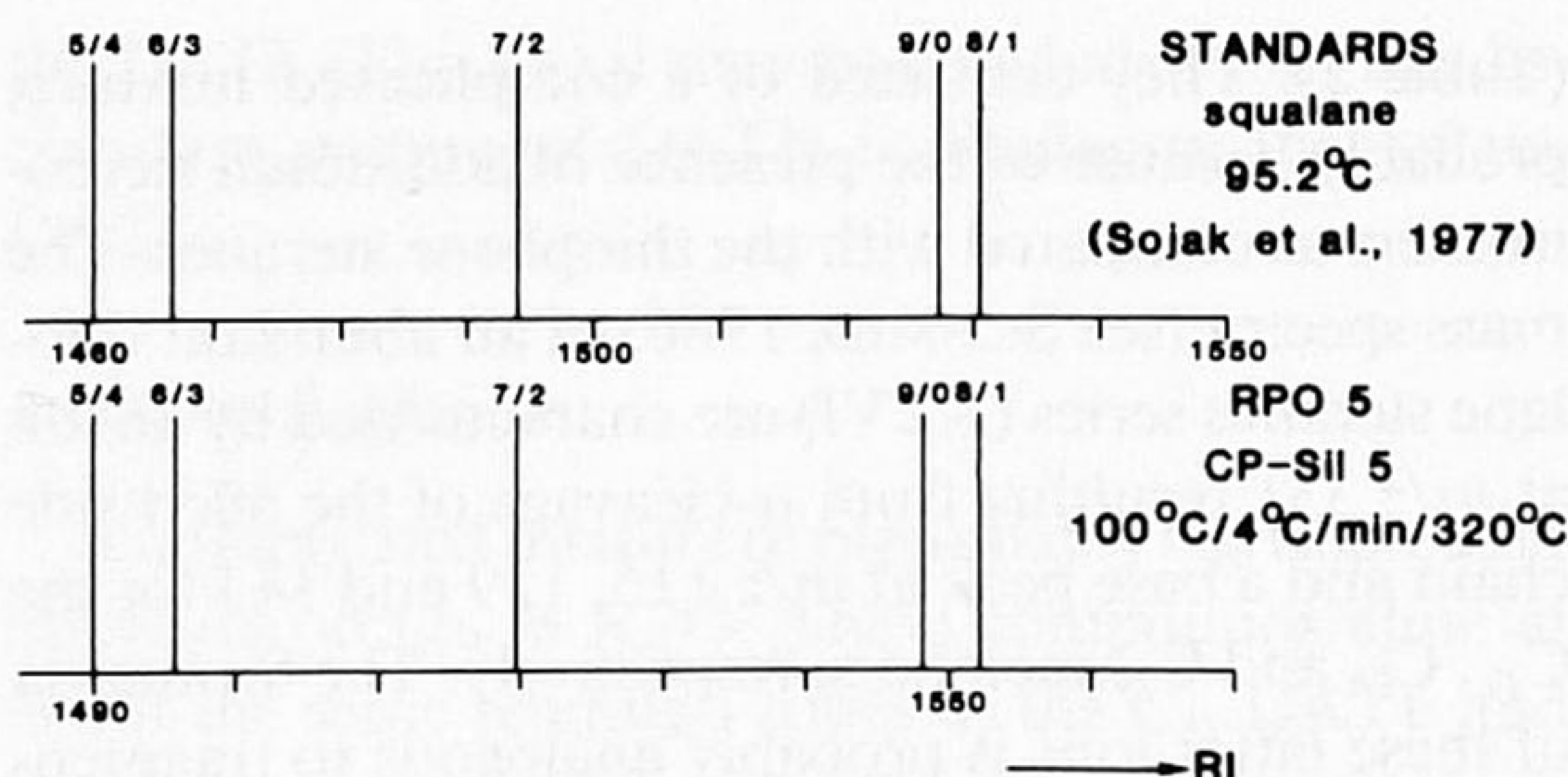


FIG. 13. Comparison of retention indices of the C_{15} 1,2-DAB obtained by desulphurization of the C_{15} 2,4-DABT of fraction 5' of the "aromatic" fraction of the RPO and those of standards as reported by SOJAK *et al.* (1977). Numbers refer to the number of carbon atoms of alkyl side chain(s) of positional isomers of the C_{15} DAB at positions 1 and 2, respectively.

and 7.10, $J \approx 6.5$ Hz), thought to reflect a double doublet of the proton at C-5, a triplet ($\delta = 7.17$, $J = 7.4$ Hz), assigned to the proton at C-6, a singlet ($\delta = 7.25$)

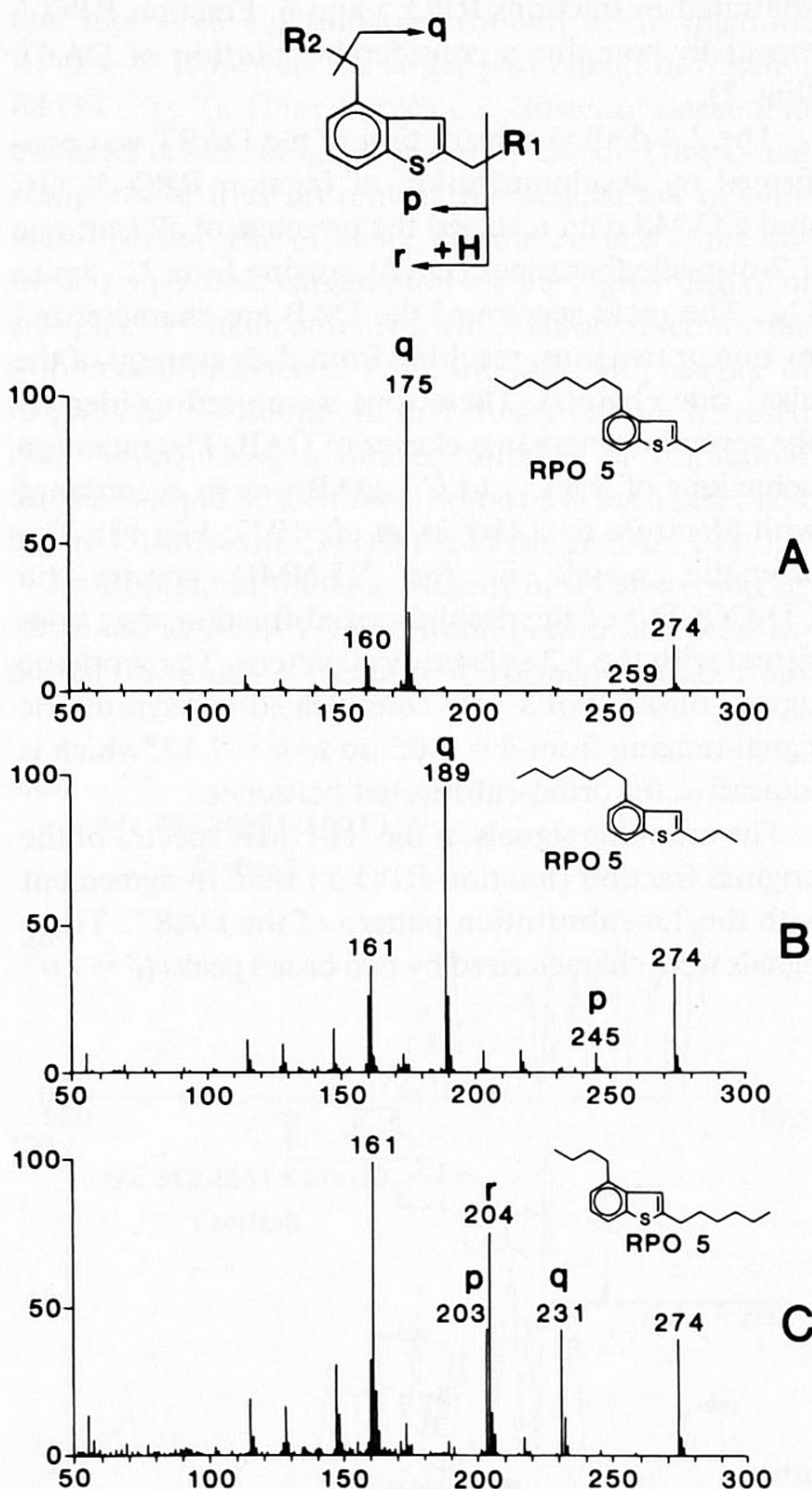


FIG. 14. Mass spectra (corrected for background) of: (A) 2-ethyl-4-octylbenzo(*b*)thiophene, (B) 4-heptyl-2-propylbenzo(*b*)thiophene and (C) 4-butyl-2-hexylbenzo(*b*)thiophene (XLIX). The letters refer to fragmentation pathways exemplified in the general structure shown.

assigned to the proton at C-3 and two broad peaks ($\delta = 7.58$ and 7.62 , $J \approx 7.6$ Hz), thought to reflect a double doublet of the proton at C-7.

Figure 14 shows the mass spectra of three isomeric 2,4-DABT. They are characterized by a molecular ion, one or two ions resulting from β -cleavage of the alkyl side chain(s) (fragments p and q in Fig. 14), and sometimes an ion resulting from a McLafferty rearrangement (fragment r, Fig. 14). Two secondary fragment ions at m/z 160 and 161 caused by loss of the alkyl side chains as alkyl radical and an alkene and two alkyl radical ions, respectively, were also frequently observed. The rearrangement ion (fragment r) could be due to a McLafferty rearrangement of both alkyl side chains. However, a rearrangement of one of these two was particularly favoured because, at the most, one such ion was observed in the spectra of DABT. To establish which alkyl side chain was involved in this rearrangement, the relative concentrations of both the C_{16} 2,4-DABT as well as their desulphurization products, the C_{16} 1,2-DAB were compared (Fig. 15). Because a particular 2,4-DABT upon desulphurization gives rise to a particular 1,2-DAB their relative concentrations (% of total 1,2-DAB or 2,4-DABT) have to be the same and therefore the 2,4-DABT could all be assigned. These assignments, in combination with the mass spectral data, indicated that the alkyl side chain at C-2 in the 2,4-DABT was involved in the rearrangement (Fig. 14).

All positional isomers of 2,4-DABT with a given number of carbon atoms were encountered. Figure 16 shows the presence of the C_{18} 2,4-DABT using the appropriate mass chromatography. The McLafferty rearrangement of the alkyl side chain at C-2 of the benzothiophene was indispensable in distinguishing the

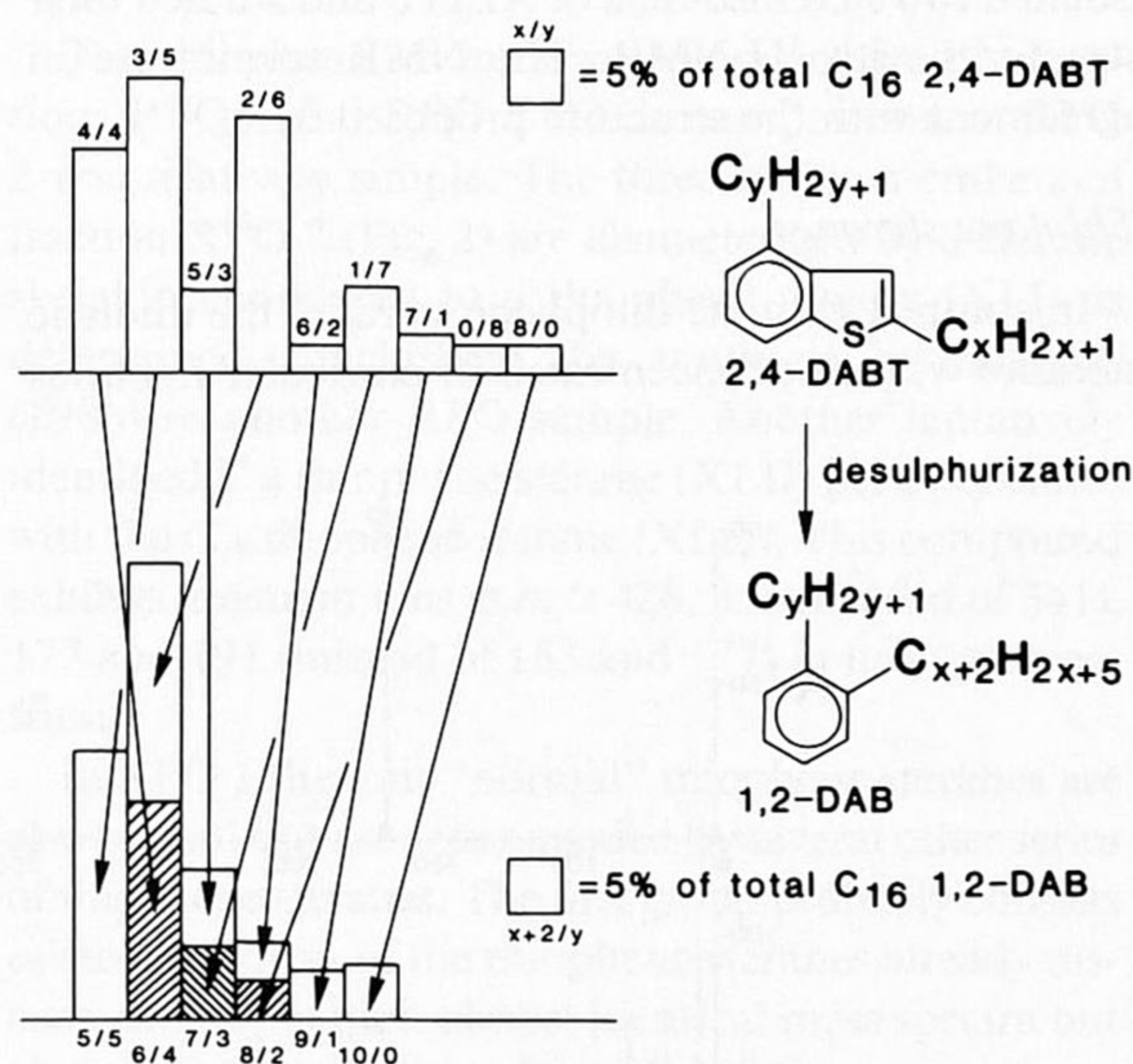


FIG. 15. Histograms showing distributions of C_{16} 2,4-DABT (upper) from fraction RPO 5' and C_{16} 1,2-DAB (lower) from Raney Ni desulphurization. The numbers refer to number of carbon atoms of alkyl side chain(s) of the positional isomers of the C_{16} DABT at position 2 and 4, respectively, and of the C_{16} DAB at position 1 and 2, respectively.

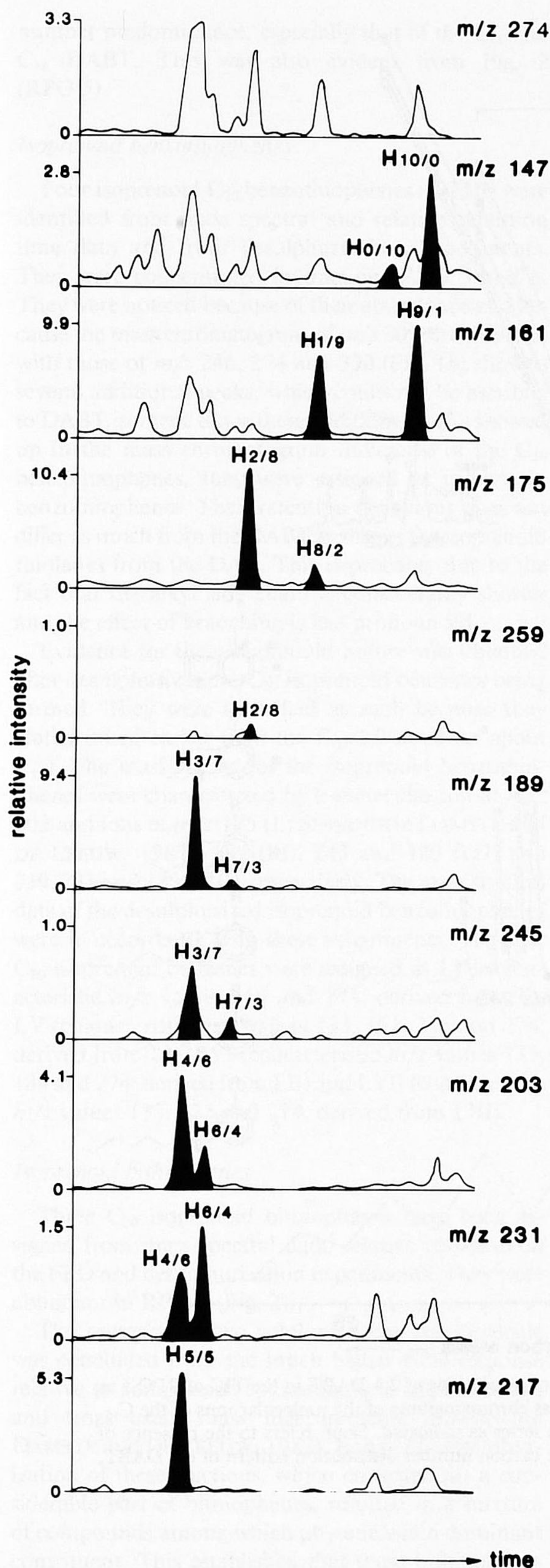


FIG. 16. Mass chromatograms of diagnostic m/z values for DABT of that particular area of the TIC of fraction 5 of the "aromatic" fraction of the RPO, in which the C_{18} DABT (molecular weight 274, upper) elute. Letters refer to Table 2. Numbers indicate the number of carbon atoms of alkyl side chain(s) at positions 2 and 4 of the DABT, respectively.

two isomers with the same alkyl substituents but at the other positions. For instance, mass chromatography of m/z 204 and 232 (Fig. 17) readily indicated the identification of XLVIII and XLIX. The absence of a prominent rearrangement ion in the mass spectra of 2,4-DABT was also helpful in their identification.

The retention data for several abundant DABT homologous series are plotted in Fig. 18. From these plots and the inserts, which show the mass chromatograms of the m/z values of the C_{16} , C_{18} , C_{20} and C_{22} DABT molecular ions, it can be seen that all 2,4-DABT patterns are essentially similar to the C_{18} DABT cluster. The mass chromatogram of m/z 302, corresponding to the molecular ion of C_{20} DABT, revealed the presence of several additional compounds. They were ascribed to C_{20} isoprenoid benzothiophenes (see below). In parallel with our identifications of DABT these compounds were also identified by PERAKIS (1986) in a very immature sediment from Maroc and in another RPO sample by synthesis of all possible C_{18} -DABT including 2- and 4-decylbenzo(*b*)thiophene. The mass

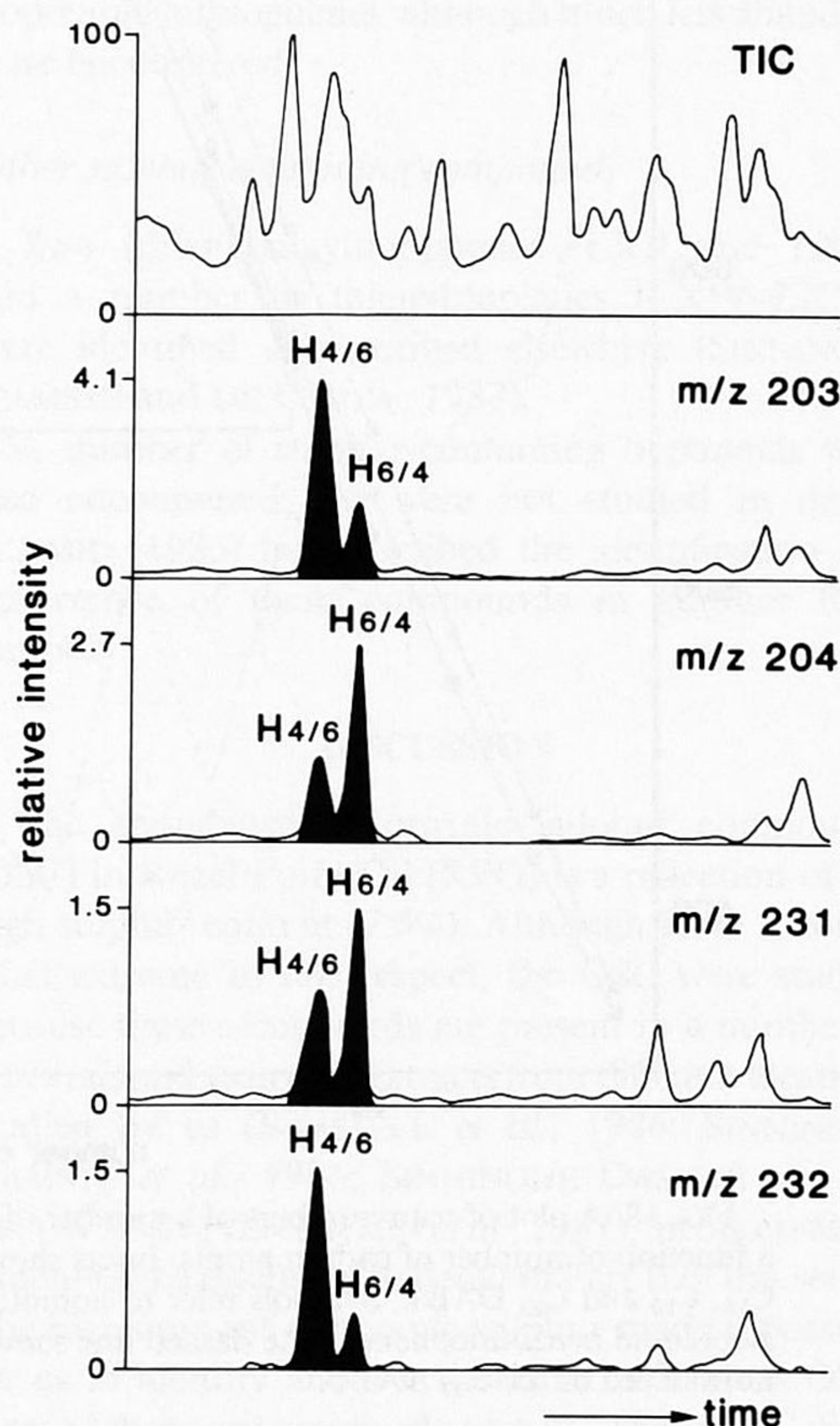


FIG. 17. Mass chromatograms of m/z 203, 204, 231 and 232 of that particular area of the TIC of fraction 5 of the "aromatic" fraction of the RPO, in which the C_{18} DABT elute. They illustrate the use of the diagnostic value of the rearrangement ion in the mass spectra of the DABT for distinguishing two isomers with the same alkyl substituents but at different positions on the molecule. Letters refer to Table 2. Numbers indicate number of carbon atoms of alkyl side chains at positions 2 and 4 of the DABT, respectively.

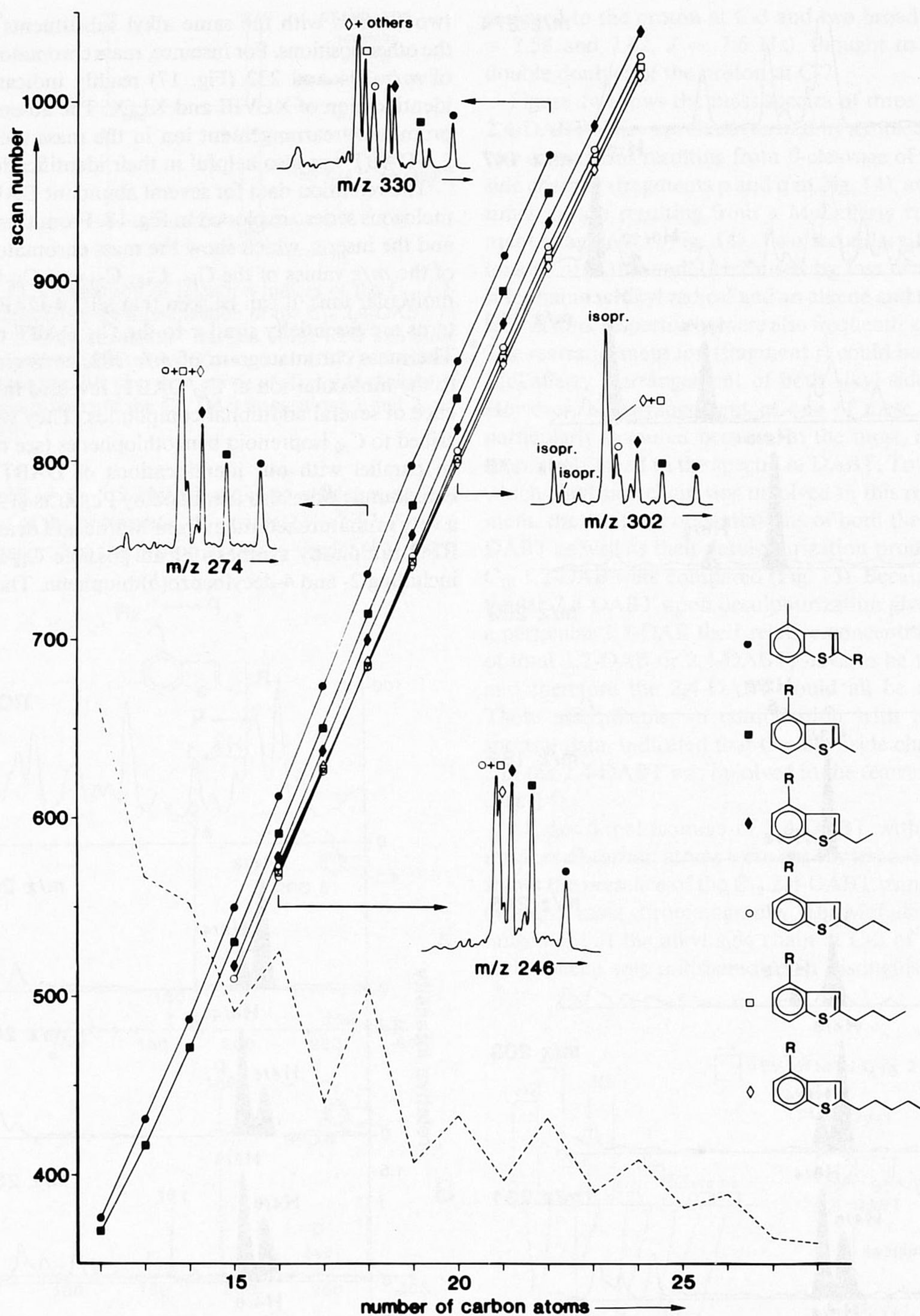


FIG. 18. A plot of scan numbers of a number of homologous series of 2,4-DABT in the TIC of RPO 5 as a function of number of carbon atoms. Insets show mass chromatograms of the molecular ions of the C_{16} , C_{18} , C_{20} and C_{22} DABT. Symbols refer to homologous series as indicated. Isopr. refers to the presence of isoprenoid benzothiophenes. The dashed line shows the carbon number distribution pattern of the DABT, normalized to the C_{12} DABT.

spectra and relative retention times of the synthetic compounds are in complete agreement with our results.

The carbon number distribution pattern of the DABT as obtained by integration of the mass chromatograms of the m/z values of the respective molecular ions is presented in Fig. 18 (dotted line). The way

in which it was calculated favours the lower molecular weight DABT because they give rise to less fragmentation than higher molecular weight DABT and hence the molecular ions constituted a greater part of the total ion yield of their mass spectra. This phenomenon does not affect extensively the even-over-odd carbon

number predominance, especially that of the C₁₆ and C₁₈ DABT. This was also evident from Fig. 2 (RPO 5).

Isoprenoid benzothiophenes

Four isoprenoid C₂₀ benzothiophenes (L-LIII) were identified from mass spectral and relative retention time data and from desulphurization experiments. They were concentrated in fractions RPO 5 and 6. They were noticed because of their abundance and because the mass chromatogram of *m/z* 302 as compared with those of *m/z* 246, 274 and 330 (Fig. 18) showed several additional peaks, which could not be ascribed to DABT isomers. Since these additional peaks showed up in the mass chromatogram indicative of the C₂₀ benzothiophenes, they were assigned as isoprenoid benzothiophenes. Their retention behaviour does not differ as much from the DABT as that of the isoprenoid thiolanes from the DAT. This is probably due to the fact that the alkyl side chain is considerably shorter and the effect of branching is less pronounced.

Evidence for their isoprenoid nature was obtained after desulphurization, C₂₀ isoprenoid benzenes being formed. They were identified as such because they eluted much earlier than the C₂₀ 1,2-DAB (at about C₁₈). The mass spectra of the isoprenoid benzothiophenes were characterized by a molecular ion at *m/z* 302 and ions at *m/z* 175 (L; SINNINGHE DAMSTÉ and DE LEEUW, 1987), 189 (LI), 245 and 189 (LII) and 259, 231 and 189 (LIII), respectively. The mass spectral data of the desulphurized isoprenoid benzothiophenes were in accordance with these assignments. The four C₂₀ isoprenoid benzenes were assigned as LIV (characteristic *m/z* values 119 and 274; derived from L), LV (characteristic *m/z* values 133, 161, 245 and 274; derived from LI), LVI (characteristic *m/z* values 133, 189 and 274; derived from LII) and LVII (characteristic *m/z* values 133, 175 and 274; derived from LIII).

Isoprenoid bithiophenes

Three C₂₀ isoprenoid bithiophenes have been assigned from mass spectral data, relative response on the FPD and desulphurization experiments. They were abundant in RPO 6 (Fig. 2).

The presence of two sulphur atoms per molecule was concluded from the much higher FPD response relative to compounds containing one sulphur atom, and from exact mass measurements (SINNINGHE DAMSTÉ and DE LEEUW, 1987). Raney Ni desulphurization of these fractions, which consisted for a considerable part of bithiophenes, resulted in a mixture of compounds among which phytane was a dominant constituent. This established that these bithiophenes possess a phytane carbon skeleton.

Mass spectral data (Fig. 19) together with the above presented results led to their conclusive identification (SINNINGHE DAMSTÉ and DE LEEUW, 1986). They were identified as LVIII, LIX and LX. Their presence was also noted by SCHMID (1986) in another RPO sample.

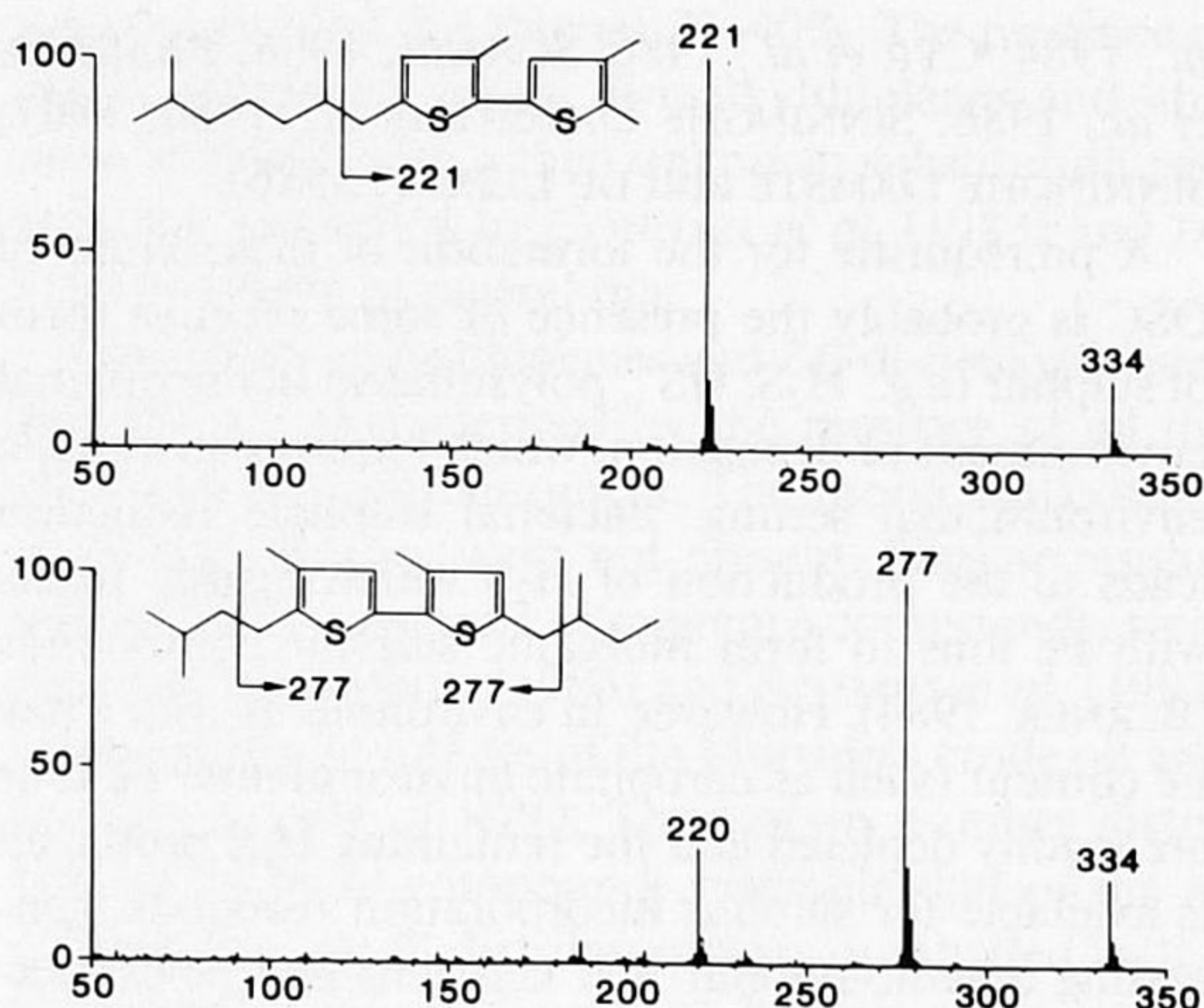


FIG. 19. Mass spectra (corrected for background) of isoprenoid bithiophenes LX (upper) and LIX (lower).

A C₁₅ isoprenoid bithiophene (LXI) was also encountered. Its mass spectrum was characterized by a molecular ion at *m/z* 264 and a fragment ion at *m/z* 211. In addition several other C₁₈, C₁₉, C₂₁ and C₂₂ isoprenoid bithiophenes, although much less abundant, were encountered.

Other sulphur containing compounds

Two (thienyl)alkylthiophenes (LXII and LXIII) and a number of thienylthiolanes (LXIV-LXVII) were identified as described elsewhere (SINNINGHE DAMSTÉ and DE LEEUW, 1987).

A number of sulphur-containing hopanoids were also encountered but were not studied in detail. SCHMID (1986) has described the identification and occurrence of these compounds in another RPO sample.

DISCUSSION

The abundance of organic sulphur compounds (OSC) in Rozel Point Oil (RPO) is a reflection of the high sulphur content (7.5%). Although RPO is somewhat extreme in this respect, the OSC were studied because these compounds are present in a number of other oils and sediment extracts from different locations studied by us (BRASSELL *et al.*, 1986; SINNINGHE DAMSTÉ *et al.*, 1986; SINNINGHE DAMSTÉ and DE LEEUW, 1987; TEN HAVEN *et al.*, 1987), an observation which will be discussed in detail in Part II of this series. The high amount of organic sulphur made it possible for us to identify about a thousand individual OSC, most of them not previously known. These OSC differ essentially from most of the OSC previously identified in oils (*e.g.*, THOMPSON, 1981; AKSENOV and KAMYANOV, 1981; HUGHES, 1984) because they have higher molecular weights and their structures seem to be related to certain bio- and/or geochemical precursors, thereby providing clues to their origin. The identification of these kinds of OSC started only recently (PAYZANT *et al.*, 1983, 1985, 1986; VALISOLALAO *et*

al., 1984; CYR *et al.*, 1986; SCHMID, 1986; BRASSELL *et al.*, 1986; SINNINGHE DAMSTÉ *et al.*, 1986, 1987; SINNINGHE DAMSTÉ and DE LEEUW, 1986).

A prerequisite for the formation of these kinds of OSC is probably the presence of some reactive form of sulphur (*e.g.*, H₂S, HS⁻, polysulfides) in the original environment of deposition, which requires an euxinic environmental setting. Bacterial sulphate reduction leads to the production of H₂S which readily reacts with Fe ions to form inorganic sulphur compounds (BERNER, 1984). However, in environments with a low Fe content (such as carbonate environments) Fe ions are readily depleted and the remaining H₂S produced is available for sulphur incorporation reactions, generating organic-sulphur-rich kerogens and/or extractable OSC (GRANSCH and POSTHUMA, 1974). This mechanism explains the fact that crude oils from carbonate/evaporite source rocks exhibit a consistently higher sulphur content (TISSOT and WELTE, 1984; JONES, 1984) and our observation that the high abundance of OSC in our samples (SINNINGHE DAMSTÉ *et al.*, 1986; TEN HAVEN *et al.*, 1987) seems to correlate with their hypersaline environment of deposition.

The OSC in RPO can be divided into three groups, *i.e.*, those with an isoprenoidal carbon skeleton (thiophenes, thiolanes, benzothiophenes, bithiophenes, (thienyl)alkylthiophenes and thienylthiolanes), those with a steroidal carbon skeleton (thiophene and thiolane steranes) and those possessing a linear carbon skeleton (2,5-di-*n*-alkylthiolanes, 2,6-di-*n*-alkylthianes and 2,4-di-*n*-alkylbenzothiophenes).

Isoprenoid OSC

The group of OSC with an isoprenoid carbon skeleton is dominant in RPO. Isoprenoid thiophene I is the most abundant OSC in the LMWA fraction of RPO. The carbon number distribution patterns of the various classes of OSC comprising this group are dominated by the C₂₀ members (Table 2). This fact, together with the structural resemblance of all these C₂₀ isoprenoid compounds (they all possess a phytane carbon skeleton) prompted us to propose a common precursor for these compounds (SINNINGHE DAMSTÉ and DE LEEUW, 1987). Sulphur incorporation into mono- and polyunsaturated C₂₀ isoprenoid alcohols or their diagenetic products is proposed to lead to the formation of thiophenes I–V. The presence of additional double bonds in the alkyl side chain of these thiophenes may result in incorporation of another sulphur atom, leading to the formation of bithiophenes (LVIII–LX) and (thienyl)alkylthiophenes (LXII–LXIII), or in a ring closure reaction followed by aromatization, leading to the formation of isoprenoid benzothiophenes (L–LIII). The isoprenoid thiolanes (*e.g.*, XXXVII) and thienylthiolanes (LXIV–LXVIII) may be intermediate compounds. The precursors proposed for these reactions, unsaturated C₂₀ isoprenoid alcohols, are widely distributed in the geosphere. The ubiquitous esterifying

alcohol of (bacterio)chlorophylls *a* and *b*, phytol, would, upon sulphur incorporation, give rise to “terminal” C₂₀ isoprenoid thiophenes I, XXVII and XVII. These compounds have been observed in a number of sediment extracts, including those from “normal” oceanic sediments (BRASSELL *et al.*, 1986). The availability of polyunsaturated C₂₀ isoprenoid alcohols, precursors proposed for the “mid-chain” thiophenes II–V, the “mid-chain” thiolanes, the bithiophenes, the (thienyl)alkylthiophenes, the thienylthiolanes and the benzothiophenes, is, however, restricted to environments where photosynthetic sulphur bacteria and archaeobacteria contribute significantly to the biomass. Hypersaline environments, such as sabkhas, are good examples in this respect.

These sulphur incorporation reactions occur at an early stage of diagenesis (BRASSELL *et al.*, 1986); C₂₀ isoprenoid thiophenes are already observed in a gypsum crust containing layers of living photosynthetic sulphur bacteria overlying a sulphate reducing zone (H. C. GOOSSENS, unpublished results).

The other isoprenoid OSC are thought to be formed by the same mechanism, starting with other substrates. So, the C₁₅ isoprenoid thiophenes (VI–VIII), thiolanes (*e.g.*, XXXVIII) and bithiophene (LXI) are thought to be formed from sulphur incorporation into farnesol (or its diagenetic products), the C₃₀ isoprenoid thiophenes (IX–XII, XVI, XVIII) and thiolanes (*e.g.*, XL) from sulphur incorporation into squalenes and/or hydrosqualenes, and the highly branched C₂₀ and C₂₅ thiophenes (XXIV and XXV) and C₂₀ thiolane (XXXIX) from sulphur incorporation into the highly branched C₂₀ and C₂₅ alkenes, widely distributed in the geosphere (ROWLAND *et al.*, 1985; ROBSON and ROWLAND, 1986). The regular C₃₅ (XIX–XXII) and C₄₀ isoprenoid thiophenes may result from sulphur incorporation into C₃₅ and C₄₀ polyprenols or their diagenetic products, bacterial compounds proposed to be precursors of the corresponding regular isoprenoid hydrocarbons occurring in certain petroleum (ALBAIGÉS, 1980; ALBAIGÉS *et al.*, 1985). These hydrocarbons were relatively abundant in Amposta-marino crude oil (Spain; sulphur content 4.3%; MOLDOWAN *et al.*, 1986). Its source rock is thought to have been deposited in a hypersaline carbonate environment (ALBAIGÉS, 1980). The other isoprenoid thiophenes (C₁₈–C₁₉, C₂₁–C₂₈) and bithiophenes (C₁₈–C₁₉, C₂₁–C₂₃) are less obviously related to particular precursors but may find an origin in unsaturated isoprenoid alkenes biosynthesized by archaeobacteria (SINNINGHE DAMSTÉ *et al.*, 1986).

Further evidence for the existence of this sulphur incorporation mechanism is the distribution of the saturated isoprenoid hydrocarbons (*e.g.*, farnesane, phytane, squalane, 2,6,10-trimethyl-7-(3-methylbutyl)dodecane, regular C₃₅ and C₄₀ isoprenoid hydrocarbons), which are thought to have been formed from the same precursors as those proposed for the isoprenoid OSC. The carbon number distribution pattern of these isoprenoid hydrocarbons is remarkably similar

with those of the isoprenoid thiophenes; both are for instance dominated by the C₂₀ member(s).

Steroid OSC

The OSC with a steroidal carbon skeleton also occur in RPO. The thiophene steranes XLIIa–c were conclusively identified by synthesis by SCHMID (1986). Apart from these thiophene steranes several other groups (XLIII–XLV) were tentatively identified. The composition of the thiolane steranes occurring in RPO is very complex. Some of these could be tentatively identified, because they are saturated counterparts of the thiophene steranes (XLI). More work on these types of thiolane steranes has been reported recently (SCHMID, 1986).

The origin of these compounds is as yet unclear. CYR *et al.* (1986) suggested a biosynthetic origin for a series of hopanoid OSC. However, an early diagenetic sulphur incorporation into *e.g.* hop-17(21)-enes and Δ^{22} -sterols and/or sterenes also may explain the formation of the hopanoid and steroid OSC, respectively. VALISOLALAO *et al.* (1984) identified a C₃₅ pentacyclic triterpenoid of the hopane series containing a thiophene ring in its side chain in immature sediments and suggested an origin from incorporation of bacterially-formed sulphur into a suitable precursor, *e.g.* bacteriohopanetetrol, supporting this view. A formation of thiophene steranes due to a reaction of elemental sulphur at elevated temperatures with steranes is less likely (SCHMID, 1986).

Linear OSC

The series of OSC with a linear carbon skeleton are all characterized by a continuous carbon number distribution from C₁₁ to C₂₈, with an even-over-odd carbon number predominance. The starting and end points of these distributions, however, are both determined by the experimental conditions used. Desulphurization experiments established that higher molecular weight members of these series are probably present.

It was recognized earlier, especially by several Russian investigators, that monocyclic sulfides are predominant types of OSC in certain oils (GAL'PERN, 1971, 1976; POLYAKOVA *et al.*, 1978; AKSENOV and KAMYANOV, 1981). Some of the structures of the lower molecular weight monocyclic sulfides, called thiophanes, were identified already at the beginning of this century (MABERY and QUALE, 1900, 1906). These identifications were subsequently confirmed and those of other low molecular weight monocyclic sulfides (C₄–C₈) reported (GAL'PERN, 1971; THOMPSON, 1981). AKSENOV and KAMYANOV (1981) described the C₁₀–C₂₀ monocyclic sulfides in the 300–325°C cut of a West Siberian petroleum as primarily 2-*n*-alkyl or 2,5-di-*n*-alkyl monocyclic sulfides because of the formation of *n*-alkanes on desulphurization. Based on literature data the thiolanes were estimated to be 60–70% of the monocyclic sulfides in the middle cuts distilled from various

petroleums, and the thianes 30–40%. The presence of higher molecular weight di-*n*-alkylthiolanes and -thianes, although with a then unknown substitution pattern, was described by CONNAN *et al.* (1983) and DE LEEUW (1986) in several oils.

The 2,5-di-*n*-alkylthiolanes and 2,6-di-*n*-alkylthianes in RPO are characterized by the presence of all the structural and stereoisomers. The mono *n*-alkylthiolanes and -thianes were not present. Similar results, also based on synthesis of reference compounds, were reported by SCHMID (1986) and SCHMID *et al.* (1987) on the aliphatic sulfides of the Marvejols crude oil and another sample of RPO. The carbon number distributions of these compounds resemble that of the *n*-alkanes (Fig. 8); all distributions are dominated by the C₁₆ and C₁₈ members and exhibit an even-over-odd carbon number predominance. This observation is comparable with that made for the isoprenoid thiophenes and isoprenoid hydrocarbons whose distributions also resemble each other, suggesting a similar process of formation. Appropriate precursors for the DATL and DATN in such a process would be *n*-alkenes or polyunsaturated *n*-alkenes, compounds which are not so widely distributed as unsaturated isoprenoid alkanes. However, unsaturated fatty acids or alcohols (after dehydration) might also play a role as precursors of DATL and DATN. After sulphur incorporation into an unsaturated fatty acid, the acid group of the resulting intermediate product would be reduced to explain the even carbon number predominance of the DATL and DATN. Although this reaction is not a likely one (NISHIMURA and BAKER, 1986), it has been postulated (WELTE and EBHARDT, 1968; KVENVOLDEN, 1970; ALBAIGÉS and TORRADAS, 1974; SEIFERT, 1975; SHENG *et al.*, 1980) to explain the even-over-odd carbon number predominance of the *n*-alkanes sometimes observed. RPO is also characterized by a strong even carbon number predominance of the *n*-alkanes (TEN HAVEN *et al.*, 1987). Recently, VERNE-MISMER *et al.* (1986) also proposed a sedimentary reduction of carboxylic groups in porphyrins.

The inference that DATL and DATN are formed during early diagenesis, cannot be made definitely at the moment for several reasons. First, although the formation of isoprenoid thiophenes at the early stages of diagenesis has been shown convincingly (BRASSELL *et al.*, 1986), the formation of DATL and DATN has not been observed. Furthermore, the presence of all structural isomers suggests that the double bond in the precursors was located at every possible position. Another possibility might be that, during thermal stress, all structural isomers of DATL and DATN were formed from one particular compound due to shifting of the carbon-sulphur bond. Thirdly, SCHMID (1986) has demonstrated that di-*n*-alkylthiophenes can be formed from a reaction between *n*-alkanes and *n*-alkenes with elemental sulphur at 200°C. SCHMID (1986) stated that the reaction conditions were too vigorous to get thiolanes. However, at lower reaction temperatures no reaction was observed. Therefore, there is some

doubt in the geochemical significance of these model experiments. The origin of the DATL and DATN remains, therefore, far from being understood.

The series of 2,4-di-*n*-alkylbenzo(*b*)thiophenes is also ranked under the OSC with a linear carbon skeleton since the substitution pattern suggests a relationship with the 2,5-di-*n*-alkylthiophenes. These compounds are present in only relatively small amounts in RPO but are much more important in an extract from a marl layer in the Northern Apennines (Italy) (SINNINGHE DAMSTÉ *et al.*, 1986). The 2,4-DABT can be formed from these structurally related 2,5-di-*n*-alkylthiophenes *via* ring closure followed by aromatization. Whether this mechanism operates in the geosphere cannot be answered at this moment. Because DABT were formed when alkanes are heated at 250°C in the presence of elemental sulphur (SCHMID, 1986), PERAKIS (1986) suggested that the DABT might be formed from such a reaction. The carbon number distribution pattern of the 2,4-DABT (Fig. 18) is characterized by an even carbon number predominance just as that of the *n*-alkanes, DATN and DATL. In addition to the lower molecular weight DABT, the C₁₆ and C₁₈ members are present in abundance.

It is tempting to assume that upon diagenesis DATL and DATN are intermediates in the formation of dialkylthiophenes and that the dialkylthiophenes in their turn are intermediates in the formation of DABT. The similarities between the distribution patterns of DATL, DATN and DABT are in favour of such a hypothesis. Laboratory experiments in which the origin and formation of OSC and their presumed diagenetic pathways are simulated are presently carried out at this laboratory. Part II of this series will deal with the widespread occurrence of these OSC in the geosphere.

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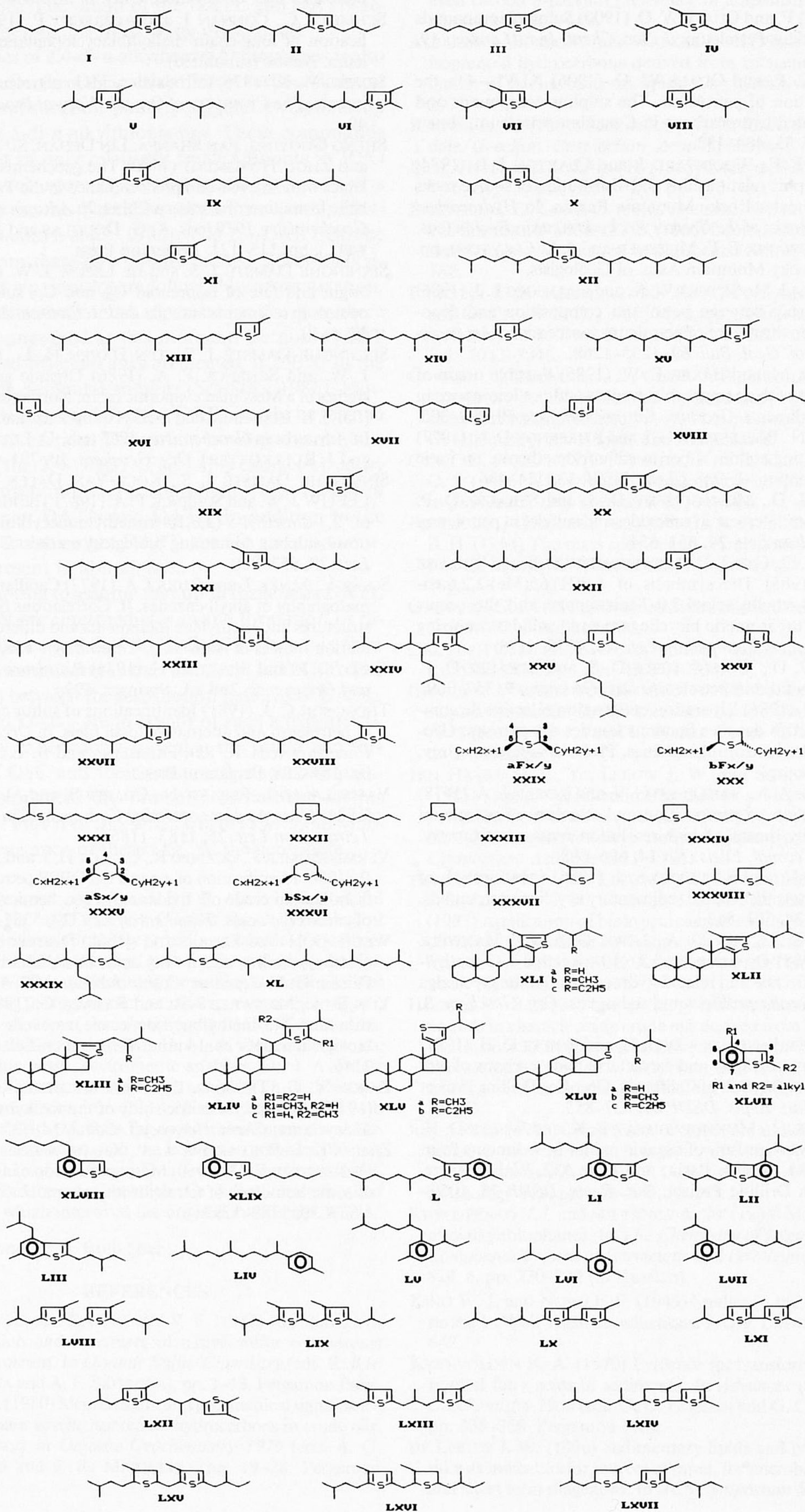
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APPENDIX



NAMES OF COMPOUNDS DRAWN			
		XXXIX	2,3-dimethyl-5-(5-(2,6,10-trimethylundecyl))thiolane
I	2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiophene	XL	5-(2-methylpropyl)-3-methyl-2-(3,8,12,16-tetramethylheptadecyl)thiolane
II	2-(3,7-dimethyloctyl)-5-(2-methylbutyl)-3-methylthiophene	XLIIa	5'-(3-methylbutyl)-4'-methyl-androst-16-eno[16,17- <i>b</i>]thiophene
III	5-(2,6-dimethylheptyl)-2-(3-methylpentyl)-3-methylthiophene	XLIIb	5'-(2,3-dimethylbutyl)-4'-methyl-androst-16-eno[16,17- <i>b</i>]thiophene
IV	5-(2,6-dimethyloctyl)-2-(3-methylbutyl)-3-methylthiophene	XLIIc	5'-(2-ethyl-3-methylbutyl)-4'-methyl-androst-16-eno[16,17- <i>b</i>]thiophene
V	2-(3,7-dimethylnonyl)-5-(2-methylpropyl)-3-methylthiophene	XLII	5'-(2-(3,4-dimethylpentyl))-4'-methyl-androst-16-eno[16,17- <i>b</i>]thiophene
VI	5-(2,6-dimethylheptyl)-2,3-dimethylthiophene	XLIIIa	5'-(2,3-dimethylbutyl)-4,4'-dimethyl-androst-16-eno[16,17- <i>b</i>]thiophene
VII	5-(2-methylbutyl)-2-(3-methylbutyl)-3-methylthiophene	XLIIIb	5'-(2-ethyl-3-methylbutyl)-4,4'-dimethyl-androst-16-eno[16,17- <i>b</i>]thiophene
VIII	2-(3-methylpentyl)-5-(2-methylpropyl)-3-methylthiophene	XLIVa	20-(2'-(4'-isopropyl)thienyl)pregnane
IX	5-(2-methylpropyl)-3-methyl-2-(3,8,12,16-tetramethylheptadecyl)thiophene	XLIVb	20-(5'-(3'-isopropyl-2'-methyl)thienyl)pregnane
X	2-(3-methylbutyl)-3-methyl-5-(2,7,11,15-tetramethylhexadecyl)thiophene	XLIVc	20-(2'-(4'-isopropyl-3'-methyl)thienyl)pregnane
XI	5-(2,7-dimethylheptyl)-3-methyl-5-(4,8,12-trimethyltridecyl)thiophene	XLVa	17-(4'-(2'-(2-(3-methylbutyl)))thienyl)androstane
XII	2-(3,7-dimethyloctyl)-3-methyl-5-(3,7,11-trimethyl-dodecyl)thiophene	XLVb	17-(4'-(2'-(3-(2-methylpentyl)))thienyl)androstane
XIII	4-methyl-2-(3,7,11,15-tetramethylhexadecyl)thiophene	XLVIa	5'-(3-methylbutyl)-4'-methyl-androstano[16,17- <i>b</i>]thiolane
XIV	2,3-dimethyl-5-(3,7,11,15-tetramethylhexadecyl)thiophene	XLVIb	5'-(2,3-dimethylbutyl)-4'-methyl-androstano[16,17- <i>b</i>]thiolane
XV	4-methyl-2-(3,7,11-trimethyl-dodecyl)thiophene	XLVIc	5'-(2-ethyl-3-methylbutyl)-4'-methyl-androstano[16,17- <i>b</i>]thiolane
XVI	4-methyl-2-(2,6,11,15,19-pentamethylicosyl)thiophene	XLVII	2,4-di- <i>n</i> -alkylbenzo(<i>b</i>)thiophenes
XVII	3-(4,8,12-trimethyltridecyl)thiophene	XLVIII	2-butyl-4-hexylbenzo(<i>b</i>)thiophene
XVIII	2-(2-methylpropyl)-4-(4,9,13,17-octadecyl)thiophene	XLIX	4-butyl-2-hexylbenzo(<i>b</i>)thiophene
XIX	5-(2,6,10,14,18,22-hexamethyltricosyl)-2,3-dimethylthiophene	L	2-(3,7-dimethyloctyl)-3,6-dimethylbenzo(<i>b</i>)thiophene
XX	2-(3,7-dimethylnonyl)-3-methyl-5-(2,6,10,14-tetramethylpentadecyl)thiophene	LI	4-(2,6-dimethylheptyl)-2,3,6-trimethylbenzo(<i>b</i>)thiophene
XXI	5-(2,6-dimethyloctyl)-3-methyl-2-(3,7,11,15-tetramethylhexadecyl)thiophene	LII	2-(3-methylbutyl)-4-(2-methylbutyl)-3,6-dimethylbenzo(<i>b</i>)thiophene
XXII	2-(3-methylpentyl)-3-methyl-5-(2,6,10,14,18-pentamethylnonadecyl)thiophene	LIII	2-(3'-methylpentyl)-4-(2-methylpropyl)-3,6-dimethylbenzo(<i>b</i>)thiophene
XXIII	5-(2-methylbutyl)-3-methyl-2-(3,7,11,15,19-pentamethylicosyl)thiophene	LIV	1-(2-(6,10-dimethylundecyl))-4-methylbenzene
XXIV	2,3-dimethyl-5-(5-(2,6,10-trimethylundecyl))thiophene	LV	1-(2-butyl)-2-(2,6-dimethylheptyl)-4-methylbenzene
XXV	2,3-dimethyl-5-(7-(2,6,10,14-tetramethylpentadecyl))thiophene	LVI	2-(2-methylbutyl)-1-(2-(6-methylheptyl))-4-methylbenzene
XXVI	2-(3,7-dimethyloctyl)-5-(2-methylpropyl)-3-methylthiophene	LVII	1-(2-(6-methyloctyl))-2-(2-methylpropyl)-4-methylbenzene
XXVII	3-methyl-2-(3,7,11-trimethyl-dodecyl)thiophene	LVIII	5'-(3-methylpentyl)-5-(2-methylpropyl)-3,4'-dimethyl-2,2'-bithiophene
XXVIII	3,5-dimethyl-2-(3,7,11-trimethyl-dodecyl)thiophene	LIX	5-(2-methylbutyl)-5'-(3-methylbutyl)-3,4'-dimethyl-2,2'-bithiophene
XXIX	<i>cis</i> -2,5-di- <i>n</i> -alkylthiolanes	LX	5-(2,6-dimethylheptyl)-3,4',5'-trimethyl-2,2'-bithiophene
XXX	<i>trans</i> -2,5-di- <i>n</i> -alkylthiolanes	LXI	5-(2-methylpropyl)-2,4',5'-trimethyl-2,2'-bithiophene
XXXI	2-dodecyl-5-ethylthiolane	LXII	2-(4-(2',3'-dimethyl-5'-thienyl)-3-methylbutyl)-5-(2-methylpropyl)-3-methylthiophene
XXXII	2,5-diheptylthiolane	LXIII	5-(3-(2',3'-dimethyl-5'-thienyl)-2-methylpropyl)-2-(3-methylbutyl)-3-methylthiophene
XXXIII	2-methyl-5-tridecylthiolane	LXIV	5-(2,6-dimethylheptyl)-2-(2',3'-dimethyl-5'-thienyl)-3-methylthiolane
XXXIV	2-butyl-5-decylthiolane	LXV	5-(5'-(2-methylbutyl)-3'-methyl-2'-thienyl)-2-(3-methylbutyl)-3-methylthiolane
XXXV	<i>cis</i> -2,6-di- <i>n</i> -alkylthianes	LXVI	2-(2'-(3-methylbutyl)-3'-methyl-5'-thienyl)-5-(2-methylbutyl)-3-methylthiolane
XXXVI	<i>trans</i> -2,6-di- <i>n</i> -alkylthianes	LXVII	2-(2'-(3-methylpentyl)-3'-methyl-5'-thienyl)-5-(2-methylpropyl)-3-methylthiolane
XXXVII	3-methyl-2-(3,7,11-trimethyl-dodecyl)thiolane		
XXXVIII	5-(2,6-dimethylheptyl)-2,3-dimethylthiolane		