

Identification and geochemical significance of cyclic di- and trisulphides with linear and acyclic isoprenoid carbon skeletons in immature sediments*

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Abstract—Homologous series (C₁₅–C₂₄) of novel 3-*n*-alkyl-1,2-dithianes and 3-*n*-alkyl-6-methyl-1,2-dithianes have been identified in immature sediments. The identification of these compounds was based on comparison of mass spectra and chromatographic data with those of synthesized 3-methyl-6-tridecyl-1,2-dithiane. In addition, 4-methyl-3-(3,7,11-trimethyldodecyl)-1,2-dithiane, 4-(4,8,12-trimethyltridecyl)-1,2-dithiane, 5-methyl-4-(3,7,11-trimethyldodecyl)-1,2,3-trithiepane, and a 1,2-dithiane possessing a pentakishomohopane carbon skeleton were tentatively assigned on the basis of mass spectral characteristics, selective chemolysis, and desulphurisation. The occurrence of these cyclic di- and trisulphides with linear, acyclic isoprenoid and hopanoid carbon skeletons in thermally immature sediments indicates that inorganic polysulphides are incorporated into functionalised lipids during the early stages of diagenesis.

INTRODUCTION

OVER THE LAST YEARS a large number of organic sulphur compounds (OSC) have been identified in crude oils and sediments (for a review see SINNINGHE DAMSTÉ and DE LEEUW, 1990). These studies have shed light on the origin of OSC and their fate in the course of geological evolution (SINNINGHE DAMSTÉ and DE LEEUW, 1987; SINNINGHE DAMSTÉ et al., 1989a; KOHNEN et al., 1990b). However, a further understanding of the origin and diagenetic pathways of OSC is a prerequisite for the application of these compounds as molecular markers to characterize depositional environments and as molecular maturity indicators.

Various investigators (VALISOLALAO et al., 1984; BRASSELL et al., 1986; SINNINGHE DAMSTÉ et al., 1987, 1988, 1989a; KOHNEN et al., 1990a,b) have provided compelling evidence that OSC are formed during the early stages of diagenesis as a result of incorporation of sulphur (H₂S, polysulphides) into specific functionalised precursors. However, whether H₂S or polysulphides are the main inorganic sulphur species that are incorporated is still a matter of debate. The isotopic composition of organically bound sulphur led NISSENBAUM and KAPLAN (1972) to suggest that sulphur enrichment of the organic matter may be due to reactions of the organic matrix with H₂S produced by dissimilatory sulphate reduction. AIZENSHTAT et al. (1983) have shown that the amount of organically bound sulphur in protokerogens of Solar Lake sediments increased with depth. They attributed this to a reaction of immature organic matter with polysulphides. FRANCOIS (1987) has reported a strong increase of S/C ratios of organic matter from a near-shore sediment core with depth and proposed reaction of the organic matter with H₂S and/or polysulphides. Recently, evidence was presented for the

formation of 3-mercaptopropionic acid in Recent marine sediments by an abiotic reaction of H₂S with acrylic acid (VAIRAVAMURTHY and MOPPER, 1987). LALONDE et al. (1987) demonstrated, by means of theoretical calculations, that the more catenated sulphur species (e.g., polysulphides) are those sulphur species which are the most reactive ones to appropriate functionalised lipids (e.g., conjugated ene carbonyls). Simulation experiments have shown that glucose reacts readily with both H₂S and polysulphides at ambient temperatures to form reaction products that yield several thiophenes upon pyrolysis (MOERS et al., 1988). SINNINGHE DAMSTÉ et al. (1989a) have proposed a hypothetical model in which addition of H₂S to double bonds (or other reactive functionalities) and a subsequent intramolecular addition of the resulting thiol to another double bond can lead to OSC. The presence of di- or polysulphide linkages in sulphur-rich geomacromolecules was demonstrated by KOHNEN et al. (1991b) using a selective chemical degradation method. The same authors rationalised the presence of these acyclic di- or polysulphide moieties as a reaction of polysulphides with functionalised lipids during early diagenesis.

In a recent preliminary communication (KOHNEN et al., 1989), we have reported the occurrence of cyclic disulphides with linear and acyclic isoprenoid carbon skeletons and a cyclic trisulphide with an acyclic isoprenoid carbon skeleton. These results are now described herein in full along with further data on hopanoid carbon skeletons possessing a disulphide moiety. These findings provide evidence at a molecular level that inorganic polysulphides are involved in the formation of OSC.

EXPERIMENTAL

Samples

The sediment samples studied originate from the Vena del Gesso basin (northern Apennines, Italy) and from the Peru upwelling area.

The geology of the Messinian Vena del Gesso basin has been extensively described by VAI and RICCI LUCHI (1977, and references

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cited therein). In brief, this evaporitic basin is filled with very thick (35 m) beds of coarse crystalline gypsum associated with thinner carbonate and shaly (euxinic) intercalations. VAI and RICCI LUCCHI (1977) found a sedimentary sequence comprised of six facies which is repeated fourteen times. The idealised evaporitic depositional cycle starts with the non-evaporitic bituminous shales and ends with the deposition of gypsum due to evaporitic precipitation. The samples investigated were taken from fresh outcrops of the above mentioned nonevaporitic bituminous shale intercalations (smelling strongly of H₂S). Samples VDG-JE, VDG-4A, VDG-7A are from different shaly intercalations. Sample VDG-7A comprises three subsamples (7A1, 7A2, 7A3) from different horizons of the same shaly intercalation (thickness ca. 1.2 m). The immature character of these bituminous shales is reflected by the low mean vitrinite reflectance ($R_0 \approx 0.25\%$) of the indigenous vitrinite particles encountered in trace amounts in sample VDG-JE.

The other samples are from ODP Site 684 (112-684C-4H-3, 30–37 cm and 112-684C-4H-7, 30–37 cm) located in the Trujillo basin within the Peru upwelling area. Geological background information has been summarised by TEN HAVEN et al. (1990). In brief, these samples were taken from Unit 3 (Pliocene), which is characterised by dark olive grey to black homogeneous to mottled bioturbated diatomaceous mud. Detailed analyses of the extractable lipids indicated that the organic matter is of predominantly marine planktonic and bacterial origin (TEN HAVEN et al., 1990). The very immature character of these samples is reflected by the fact that extractable steroids are almost solely present as ketones and free and bound alcohols, but not as hydrocarbons (TEN HAVEN et al., 1990).

Extraction and Fractionation

At Jülich the sediment samples of the Peru upwelling area were extracted and the resulting extracts were fractionated (see TEN HAVEN et al., 1990). At Delft the thus obtained "aromatic hydrocarbon" fractions were reanalysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) and then desulphurised with Raney Ni. The samples of the Vena del Gesso basin were powdered in a rotary disc mill and ultrasonically extracted with methanol ($\times 1$), methanol/dichloromethane (1:1, v/v, $\times 1$), and dichloromethane ($\times 5$), respectively. The bitumen (0.11–0.21 wt% of whole rock) was obtained by removing the solvent with a rotary evaporator at 30°C.

The maltene fraction was isolated from the bitumen by precipitating the asphaltenes in *n*-heptane. This fraction (ca. 250 mg) was further separated into two fractions with a column (25 cm \times 2 cm; $v_0 = 35$ mL) packed with alumina (activated for 2.5 h at 150°C) by elution with hexane/dichloromethane (9:1, v/v; 150 mL; the apolar fraction) and methanol/dichloromethane (1:1, v/v; 100 mL; the polar frac-

tion). The apolar fraction (ca. 10 mg) was then separated by thin layer chromatography (TLC) on silica gel impregnated with AgNO₃ (KOHNE et al., 1990c). Three bands, R_f 0.8–1.0 (fraction X1), R_f 0.2–0.8 (fraction X2), and R_f 0.0–0.2 (fraction X3) were scraped off the TLC plate, ultrasonically extracted with ethyl acetate ($\times 3$), and subsequently analysed by GC and GC-MS. In the case of samples VDG-4A and VDG-7A(1–3), four bands— R_f 0.8–1.0 (fraction X1), R_f 0.7–0.8 (fraction X2), R_f 0.2–0.7 (fraction X3), and R_f 0.0–0.2 (fraction X4)—were scraped off the TLC plate and subsequently analysed as described above.

Gas Chromatography

GC was performed on a Carlo Erba 5300 instrument equipped with an on-column injector. A fused silica capillary column (25 m \times 0.32 mm) coated with CP Sil-5 (film thickness 0.12 μ m) was used with helium as the carrier gas. A more polar column (DB17) was also used for coinjection experiments. Detection was accomplished with both a flame ionization detector (FID) and a sulphur-selective flame photometric detector (FPD), using a stream-splitter at the end of the column (split ratio FID:FPD = 1:2). The samples dissolved in ethyl acetate were injected at 70°C and the oven was subsequently programmed to 130°C at 10°C/min and then at 4°C/min to 320°C, at which it was held for 20 min.

Gas Chromatography-Mass Spectrometry

GC-MS was carried out on a Hewlett-Packard 5480 gas chromatograph connected with a VG-70s mass spectrometer operated at 70 eV with a mass range m/z 40–800 and a cycle time of 1.8 s (resolution 1000). The gas chromatograph was equipped with a fused silica capillary column (25 m \times 0.32 mm) coated with CP Sil-5 (film thickness = 0.2 μ m). Helium was used as the carrier gas. The operating conditions were the same as those described above. Exact mass measurements were performed by peak matching using selected masses from perfluorkerosene as the reference at a resolution of 5000.

Raney Ni Desulphurisation

Several fractions were desulphurised with Raney Ni and subsequently hydrogenated over PtO₂ (SINNINGHE DAMSTÉ et al., 1988). The thus obtained hydrocarbons were analysed by GC and GC-MS.

MeLi/MeI Treatment

The alkylsulphide fraction (i.e., fraction X4) of VDG-4A was degraded, with MeLi/MeI as described elsewhere (KOHNE et al., 1991b). The resulting reaction mixture was analysed by GC-MS.

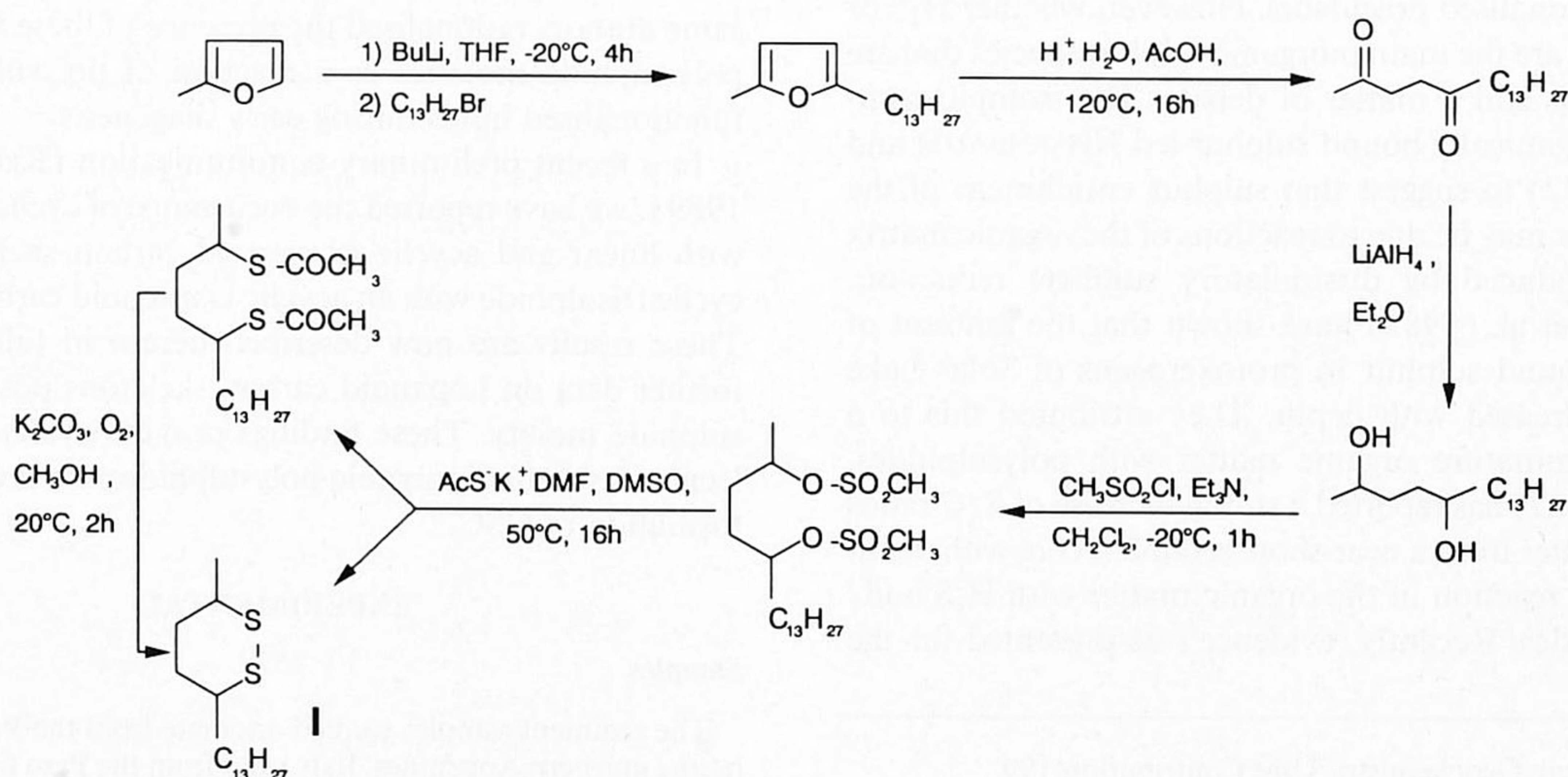


FIG. 1. Summary scheme for the synthesis of a mixture of *cis* and *trans* 3-methyl-6-tridecyl-1,2-dithiane (I).

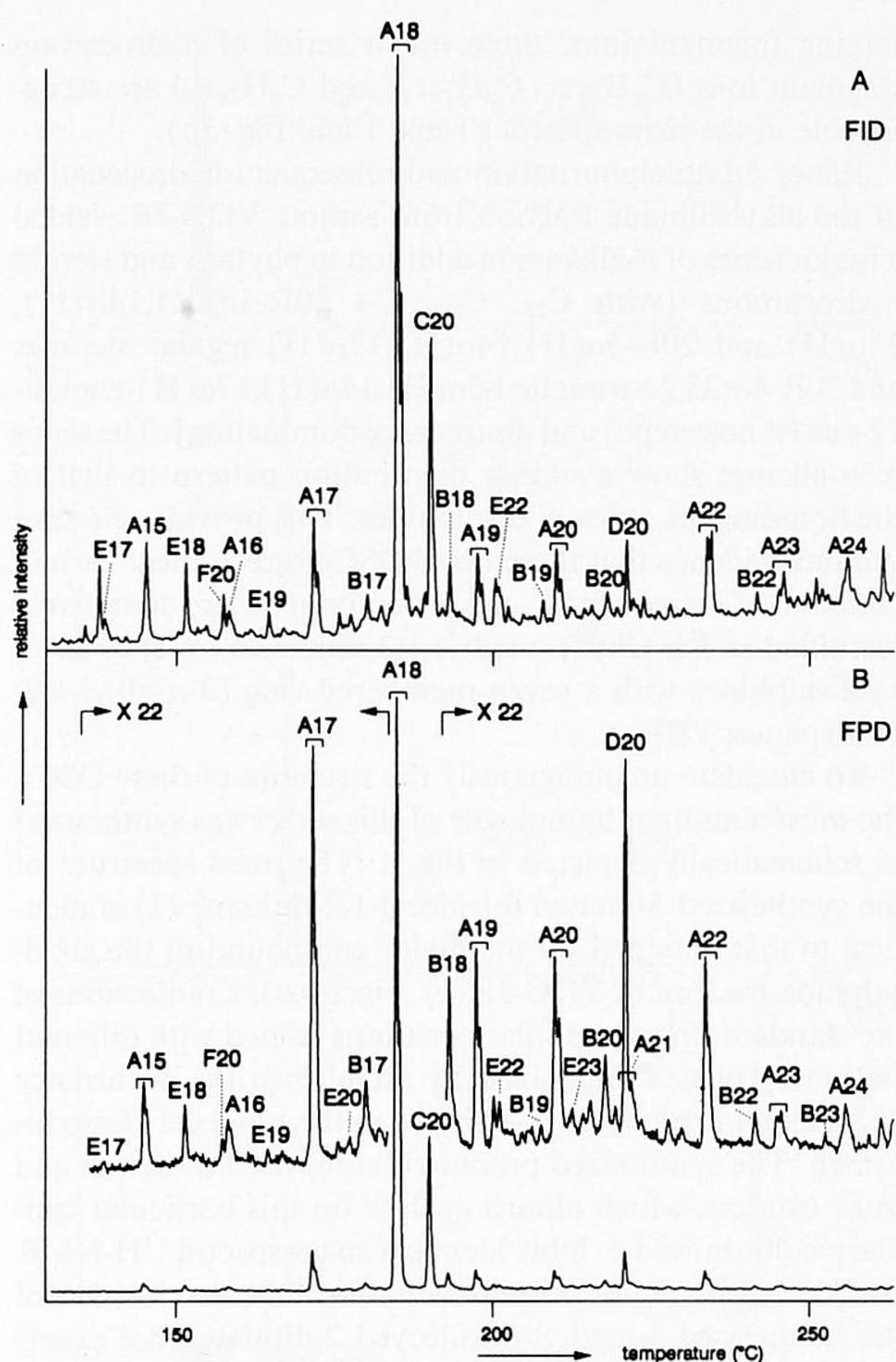


FIG. 2. Partial FID (upper trace) and FPD (lower trace) chromatograms of the alkylsulphide fraction ($R_f = 0.0-0.2$) isolated from the extract of sediment sample VDG-JE. Key: A = 3-*n*-alkyl-6-methyl-1,2-dithianes (VII); B = 3-*n*-alkyl-1,2-dithianes; C = 4-methyl-3-(3,7,11-trimethyldodecyl)-1,2-dithiane (XI); D = 5-methyl-4-(3,7,11-trimethyldodecyl)-1,2,3-trithiepane (XVI); E = 2-*n*-alkyl-5-methylthiolanes; F = 3-methyl-2-(3,7,11-trimethyldodecyl)thiolane (III). Arabic numbers indicate the number of carbon atoms of the particular compound.

Synthesis of Authentic Standard

3-methyl-6-tridecyl-1,2-dithiane (I; see Appendix) was prepared as outlined in Fig. 1. Metallation of 2-methylfuran with butyllithium in tetrahydrofuran (THF) solution at -25°C yielded 2-lithio-5-methylfuran, which on condensation with 1-bromotridecane dissolved in THF gave 2-methyl-5-tridecylfuran (BÜCHI and WÜEST, 1965). Hydrolysis for 6 h at 120°C (BÜCHI and WÜEST, 1965) of this crude product in glacial acetic acid containing some aqueous sulphuric acid (ca. 7%) yielded 2,5-octadecanedione. Purification was performed by recrystallisation from hexane (yield 36%). This diketone was reduced with LiAlH_4 in diethyl ether (yield 71%). The obtained diol was converted to the 2,5-dimesylate ester (yield 80%) containing methanesulfonyl chloride in a CH_2Cl_2 solution with a small amount of triethylamine (CHIU-HONG LIN et al., 1982). Reaction of the dimesylate with potassium thioacetate dissolved in dimethyl sulphoxide/dimethylformamide (1:1; CHIU-HONG LIN et al., 1982) in addition to 2,5-di-(thioacetyl)octadecane yielded 3-methyl-6-tridecyl-1,2-dithiane (I). Hydrolysis of this crude product dissolved in methanol with potassium carbonate in an oxygen atmosphere (CHIU-HONG LIN et al., 1982) resulted in the formation of the 3-methyl-6-tridecyl-1,2-dithiane (I; yield 25%). Upon purification of the product mixture with column chromatography (Al_2O_3 , *n*-hexane) two fractions were

obtained containing a mixture of two *cis* and *trans* isomers of 3-methyl-6-tridecyl-1,2-dithiane (I) in different abundances as revealed by GC and GC-MS. $^1\text{H-NMR}$ analyses (Varian VXR-400s) of both fractions confirmed the structure of the synthesized product: $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ (ppm relative to TMS): 0.88 [*t*, $J = 7$ Hz (H at C-13')]; 1.19 [*d*, $J = 7$ Hz (CH_3 at C-3 of *trans* isomer)]; 1.35 [*d*, $J = 7$ Hz, CH_3 at C-3 of *cis* isomer]; 2.68 [*m*, H at C-3 of *cis* isomer]; 2.80 [*m*, H at C-3 of *trans* isomer]; 2.94 [*m*, ^1H , H at C-6 of *cis* and *trans* isomer]. The sum of the intensities of the signals at 2.68 and 2.80 ppm and at 1.19 and 1.35 ppm observed in both fractions represent ^1H and ^3H , respectively. A significant sharpening of the resonances at 1.35 and 2.68 was observed at elevated temperatures (50 vs. 20°C) due to an averaging of the signals of the two conformers of the *cis* isomer as a result of more rapid interconversion. Further confirmation of the assignment of the NMR signals was accomplished by COSY and proton-decoupling experiments. These data enabled the assignment of the compound eluting earlier on the GC column as the *trans* isomer.

2,5-Di(methylthio)-octadecane (Fig. 6B) was obtained by MeLi/MeI treatment of the synthesized 3-methyl-6-tridecyl-1,2-dithiane as described above.

RESULTS

Identification of Cyclic Disulphides with Linear Carbon Skeletons

Fig. 2 shows partial FID and FPD chromatograms of the alkylsulphide fraction (fraction X3) isolated from the extract of the bituminous shale (VDG-JE) by column chromatography and subsequent argentation thin layer chromatography. GC-MS analyses revealed that this fraction contains a series of 2-*n*-alkyl-5-methylthiolanes ($\text{C}_{15}-\text{C}_{24}$; II) and a C_{20} isoprenoid thiolane (III), both previously identified in a number of oils and sediment extracts (SCHMID et al., 1987; SINNINGHE DAMSTÉ et al., 1986, 1987, 1989a,b). However, the major compounds giving rise to the series of peaks labelled "A" are members of an as yet unknown class of OSC (Fig. 2). These OSC occur as doublets of peaks, just separated from each other (Fig. 2). Mass spectra of the two compounds comprising such a doublet are identical.

Based on the difference in response of these compounds by FPD and FID (Fig. 2) compared with those of the alkylthiolanes and based on exact mass measurements (Table 1), it is concluded that these novel OSC contain two sulphur atoms per molecule. The elemental composition is $\text{C}_n\text{H}_{2n}\text{S}_2$ ($n = 15-24$) and the molecular weight is $274 + 14 \cdot n$ ($n = 0-9$). The C_{18} homologues (A18; Fig. 2) are the most abundant members of these series.

The mass spectra of these OSC show a characteristic major $\text{M}^+ - 33$ fragment (e.g., mass spectrum of the C_{18} homologue;

Table 1. Exact mass measurements of characteristic ions in the mass spectrum of 3-methyl-6-tridecyl-1,2-dithiane (peak A18; Fig. 2)

m/z	atomic composition	exact mass	measured mass	ΔM (mDa)
316	$\text{C}_{18}\text{H}_{36}\text{S}_2$	316.2258	316.2262	-0.4
283	$\text{C}_{18}\text{H}_{35}\text{S}$	283.2459	283.2459	0.0
227	$\text{C}_{14}\text{H}_{27}\text{S}$	227.1833	227.1824	1.1
185	$\text{C}_{11}\text{H}_{21}\text{S}$	185.1364	185.1356	0.8
143	$\text{C}_8\text{H}_{15}\text{S}$	143.0894	143.0879	1.5
133	$\text{C}_6\text{H}_9\text{S}_2$	133.0146	133.0143	0.3
101	$\text{C}_6\text{H}_9\text{S}$	101.0425	101.0368	5.7
57	C_4H_9	57.0704	57.0708	-0.4
55	C_4H_7	55.0548	55.0553	-0.5

Fig. 3b). Mass measurements of this fragment ion (Table 1) reveal that it results from loss of SH from the molecular ion. Such a fragmentation pathway is also reported in mass spectra of 1,3-dithiane (IV), 2-methyl-1,3-dithiane, 2,2-dimethyl-1,3-dithiane, 2-phenyl-1,3-dithiane (BOWIE and WHITE, 1972, and references cited therein), and of dimethyl disulphide, diallyl disulphide, methyl phenyl disulphide, diphenyl disulphide, 1,2-dithiolane-3-formic acid (V), 1,2-dithiane (VI), and 1,2-dithiane-4-formic acid (BOWIE et al., 1966). Other sulphur-containing fragment ions with an elemental composition of $C_nH_{2n-1}S$ are encountered at m/z 101, 143, and $185 + 14 \cdot n$ (C_{15} homologue $n = 0$, C_{16} homologue $n = 1$, etc.) in the mass spectra of the novel OSC in the VDG-JE sample (Table 1). Among these three ions the one at m/z 101 is the most abundant (Fig. 3b). CORINA et al. (1979) reported that a fragment ion at m/z 101 of nonsubstituted cyclic disulphides may be due to the formation of the cyclic ion C_5H_9S by expulsion of $(CH_2)_{n-5}SH$ from the molecular ion. Such a cyclic ion (C_5H_9S) is in agreement with the elemental composition of the m/z 101 fragment ion (Table 1). Only a minor fragment ion at m/z 133 ($C_5H_9S_2$) contains two sulphur atoms (Table 1). Apart from these sulphur-con-

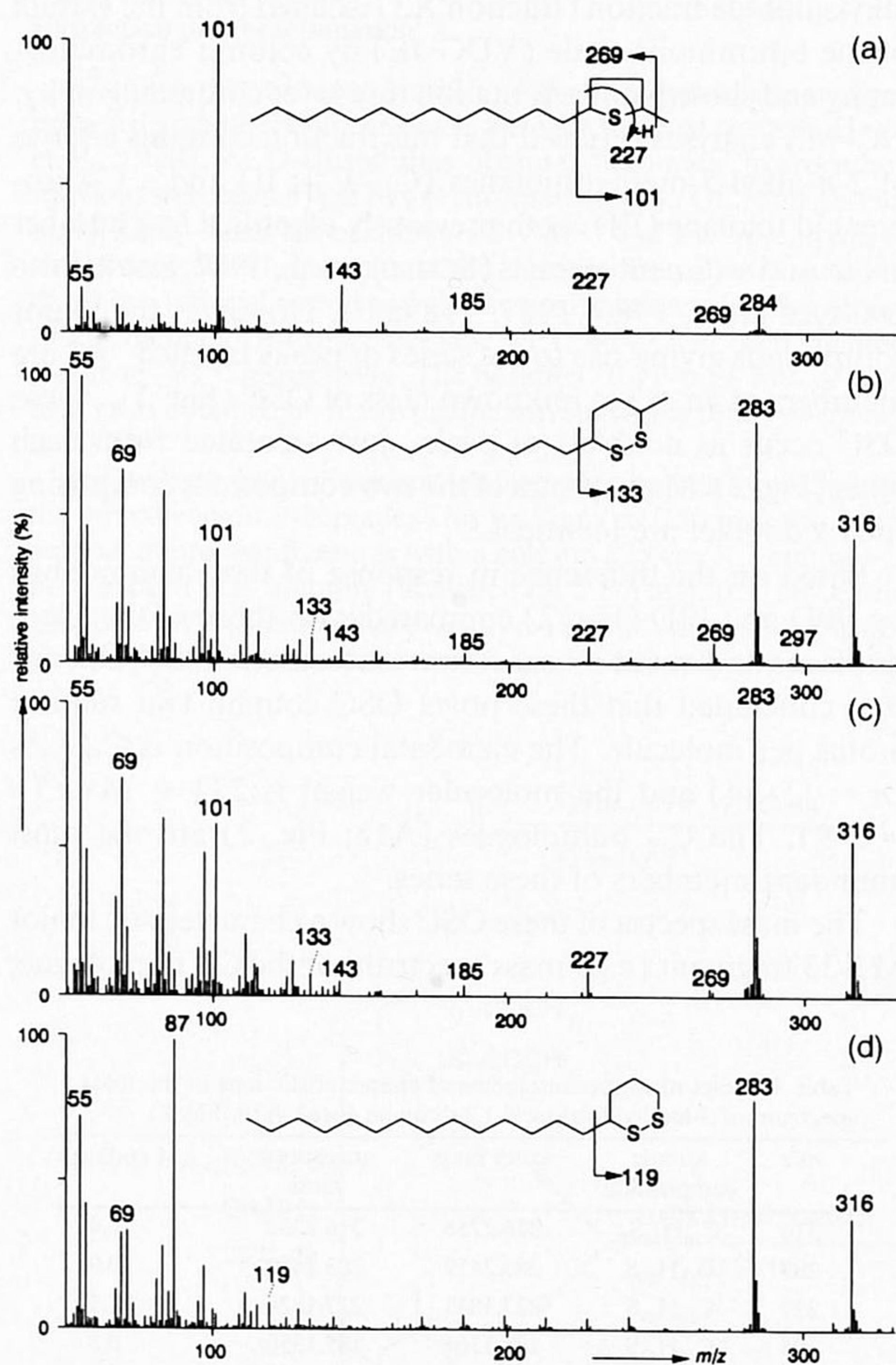


FIG. 3. Mass spectra (subtracted for background) of (a) 2-methyl-5-tridecylthiolane, (b) 3-methyl-6-tridecyl-1,2-dithiane (I) encountered in sample VDG-JE, (c) authentic 3-methyl-6-tridecyl-1,2-dithiane, (d) 3-tetradecyl-1,2-dithiane.

taining fragment ions, three major series of hydrocarbon fragment ions (C_nH_{2n+1} , C_nH_{2n-1} , and C_nH_{2n-3}) are recognizable in the mass spectra (Table 1 and Fig. 3b).

Raney Ni desulphurisation and subsequent hydrogenation of the alkylsulphide fraction from sample VDG-JE yielded a major series of *n*-alkanes in addition to phytane and steroid hydrocarbons [with C_{27} , C_{28} , C_{29} 20R-5 β (H),14 α (H), 17 α (H) and 20R-5 α (H),14 α (H),17 α (H) regular steranes and 20R-4 α ,23,24-trimethyl-5 α (H),14 α (H),17 α (H)-cholest-22-ene (dinosterene) and dinosterane dominating]. The series of *n*-alkanes show a similar distribution pattern to that of the homologous series of disulphides. This provides circumstantial evidence that these novel OSC possess linear carbon skeletons. Consequently, these compounds are tentatively identified as 3-*n*-alkyl-6-methyl-1,2-dithianes (VII) or as alkylsulphides with a seven-membered ring (3-*n*-alkyl-1,2-dithiepanes; VIII).

To elucidate unambiguously the structure of these OSCs, the most abundant homologue of this series was synthesized as schematically depicted in Fig. 1. The mass spectrum of the synthesized 3-methyl-6-tridecyl-1,2-dithiane (I) is identical to that obtained for the major compound in the alkylsulphide fraction of VDG-JE (cf. Fig. 3b,c). Coinjections of the standard on two capillary columns coated with different stationary phases unequivocally established the occurrence of 3-methyl-6-tridecyl-1,2-dithiane in the Vena del Gesso bitumen. The synthesized product is comprised of the *cis* and *trans* isomers, which almost coelute on this particular capillary column and exhibit identical mass spectra. 1H -NMR analyses of fractions enriched with one of the two isomers of the synthesized 3-methyl-6-tridecyl-1,2-dithiane (see experimental section) revealed that the first eluting compound is the *trans* isomer and the latter one is the *cis* isomer. Hence, the compounds of the doublets observed in the alkylsulphide fraction correspond to the *trans* and *cis* 3-methyl-6-*n*-alkyl-1,2-dithianes, respectively.

Unravelling the fragmentation pathway of 3-methyl-6-tridecyl-1,2-dithiane (I) upon electron impact ionization mass spectrometry was accomplished by detection of daughter ions of selected parent ions in the linked scan mode with a constant B/E ratio. These analyses revealed that the ions at m/z 283 ($M^+ - 33$) and 133 ($C_5H_9S_2$) are daughter ions of the molecular ion (m/z 316) and the ions at m/z 101, 143, and 227 are daughter ions of the fragment ion at m/z 283. Thus, elimination of SH from the molecular ion leads to an ion with an elemental composition of $C_{18}H_{35}S$, which yields secondary fragment ions at m/z 101, 143, and 227. It should be noted that these secondary ions are also key fragment ions in the mass spectrum of 2-methyl-5-tridecylthiolane (IX; SINNINGHE DAMSTÉ et al., 1986), which is a reflection of their structural similarity (i.e., both compounds have an octadecane carbon skeleton and S-C bonds at C-2 and C-5; cf. Fig. 3a,c).

Fig. 4a shows a partial mass chromatogram of the molecular ion (m/z 316) of the C_{18} 1,2-dithianes in the alkylsulphide fraction from the Vena del Gesso bitumen (VDG-7A1). The C_{18} cluster consists of the *trans* (peak 1 in Fig. 4a) and the *cis* (peak 2 in Fig. 4a) isomers of 3-methyl-6-tridecyl-1,2-dithiane and an additional compound (peak 5 in Fig.

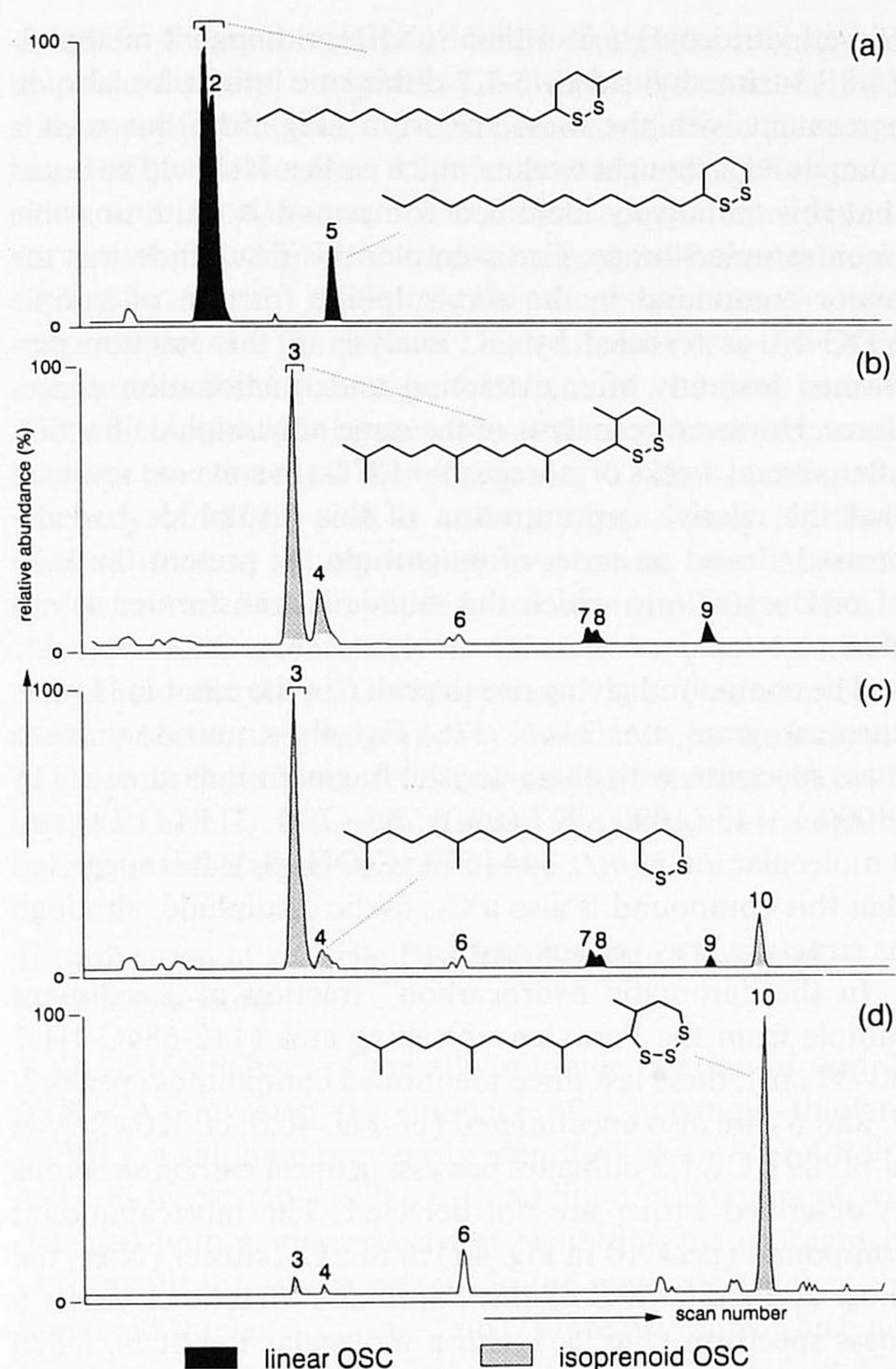


FIG. 4. Partial mass chromatograms of (a) m/z 316 showing the distribution of the C_{18} 1,2-dithianes present in the alkylsulphide fraction VDG-7A1, (b) m/z 344 + 376 showing the distribution of the C_{20} 1,2-dithianes present in the alkylsulphide fraction VDG-7A1, (c) m/z 344 + 376 showing the distribution of the C_{20} 1,2-dithianes and 1,2,3-trithiepanes present in alkylsulphide fraction VDG-7A2, (d) m/z 344 + 376 showing the distribution of the C_{20} 1,2-dithianes and 1,2,3-trithiepanes present in the "aromatic hydrocarbon" fraction isolated from the extract of the Peru upwelling area sediment sample (112-684C-4H-7, 30-37 cm). Identifications of labeled compounds: 1 = *trans* 3-methyl-6-tridecyl-1,2-dithiane; 2 = *cis* 3-methyl-6-tridecyl-1,2-dithiane; 3 = *trans* and *cis* 4-methyl-3-(3,7,11-trimethyldodecyl)-1,2-dithiane; 4 = 4-(4,8,12-trimethyltridecyl)-1,2-dithiane; 5 = 3-tetradecyl-1,2-dithiane; 6 = C_{20} 1,2-dithiane with unknown structure; 7 = *trans* 3-methyl-6-pentadecyl-1,2-dithiane; 8 = *cis* 3-methyl-6-pentadecyl-1,2-dithiane; 9 = 3-hexadecyl-1,2-dithiane; 10 = 5-methyl-4-(3,7,11-trimethyldodecyl)-1,2,3-trithiepane.

4b), which elutes later. This latter compound shows a mass spectrum which is also characterised by an intense M^+-33 fragment ion and a molecular ion at m/z 316 (Fig. 3d). The three major series of hydrocarbon fragment ions as described for the mass spectrum of 3-methyl-6-tridecyl-1,2-dithiane are also present, but the ions at m/z 133 and 101 are now shifted to 119 and 87, which suggests that the 1,2-dithiane is now only monosubstituted. Hence, this compound is tentatively identified as 3-tetradecyl-1,2-dithiane (X). This assignment is supported by the retention behaviour of this monosubstituted compound relative to that of 3-methyl-6-tridecyl-1,2-

dithiane. This is analogous to that of the monosubstituted 2-*n*-alkylthiolanes, which also elute later than the corresponding 2-*n*-alkyl-5-methylthiolanes (SINNINGHE DAMSTÉ et al., 1987).

From the approximately linear relationship between retention time and the number of carbon atoms for components comprising a homologous series and mass spectral data, two homologous series of 3-*n*-alkyl-6-methyl-1,2-dithiane (C_{15} – C_{24} ; *trans* and *cis* isomers) and 3-*n*-alkyl-1,2-dithianes (C_{15} – C_{24}) were identified. Table 2 shows an overview of the samples studied and the occurrence of 1,2-dithianes possessing a linear carbon skeleton in these samples.

Identification of Cyclic Di- and Trisulphides with an Acyclic Isoprenoid Carbon Skeleton

Mass chromatography of the molecular ions of the cyclic disulphides ($274 + n.14$) revealed the presence of C_{20} compounds (peaks 3 and 4 in Fig. 4b), eluting much earlier than the C_{20} dithianes with linear carbon skeletons (peaks 7, 8, and 9 in Fig. 4b). Raney Ni desulphurisation of this alkylsulphide fraction (VDG-7A1) afforded a substantial amount of phytane relative to the *n*-alkanes, indicating that these compounds possess a phytane carbon skeleton. The mass spectra (Fig. 5b) of the first eluting compounds, represented by the rather broad peak 3, are identical and exhibit a molecular ion at m/z 344, an intense M^+-33 ion, a minor fragment ion which contains two sulphur atoms at m/z 133 ($C_5H_9S_2$), and a series of hydrocarbon fragment ions which are also present in the mass spectra of the linear 1,2-dithianes. Other characteristic fragment ions are present at m/z 101, 157, and 255. It is assumed that these latter ions are daughter ions of the M^+-33 fragment ion by analogy with the fragmentation pathway described for the linear C_{18} 1,2-dithiane. These fragment ions are also present in the mass spectrum of 3-methyl-2-(3,7,11-trimethyldodecyl)thiolane (III; cf. Fig. 5a,b). These compounds are believed, therefore, to be *trans* and *cis* 4-methyl-3-(3,7,11-trimethyldodecyl)-1,2-dithiane (XI). Treatment of this alkylsulphide fraction with MeLi/MeI yielded supporting evidence for this tentative identification. This chemical degradation method cleaves selectively di- or polysulphide linkages and subsequently derivatises the cleavage products to their corresponding methylthioethers (KOHEN et al., 1991b). A major amount of 1,4-di(methylthio)-phytane (XII; Fig. 6) was tentatively identified

Table 2. Cyclic di- and trisulphides encountered in samples studied

sample	TOC (%)	1	2	3	4	5
VDG-JE	n.a.	C_{16} – C_{24}	C_{15} – C_{24}	XI	XVI	n.d.
VDG-4A	n.a.	C_{17} – C_{22}	C_{17} – C_{22}	XI, XIII	n.d.	n.d.
VDG-7A1	1.7	C_{17} – C_{22}	C_{17} – C_{22}	XI, XIII	n.d.	XVIII
VDG-7A2	1.3	C_{16} – C_{20}	C_{16} – C_{20}	XI, XIII	XVI	XVIII
VDG-7A3	1.2	C_{16} – C_{20}	C_{16} – C_{20}	XI, XIII	XVI	XVIII
ODP 112-684C-4H-3, 30-37cm	7.5	n.d.	C_{19} – C_{20}	XI, XIII	XVI	n.d.
ODP 112-684C-4H-7, 30-37cm	4.1	n.d.	n.d.	XI, XIII	XVI	n.d.

Key: n.a. = not analysed, n.d. = not detected, 1 = 2-*n*-alkyl-1,2-dithianes, 2 = 3-*n*-alkyl-6-methyl-1,2-dithianes, 3 = 1,2-dithianes possessing a phytane carbon skeleton, 4 = 1,2,3-trithiepane possessing a phytane carbon skeleton, 5 = 1,2-dithiane possessing a hopanoid carbon skeleton.

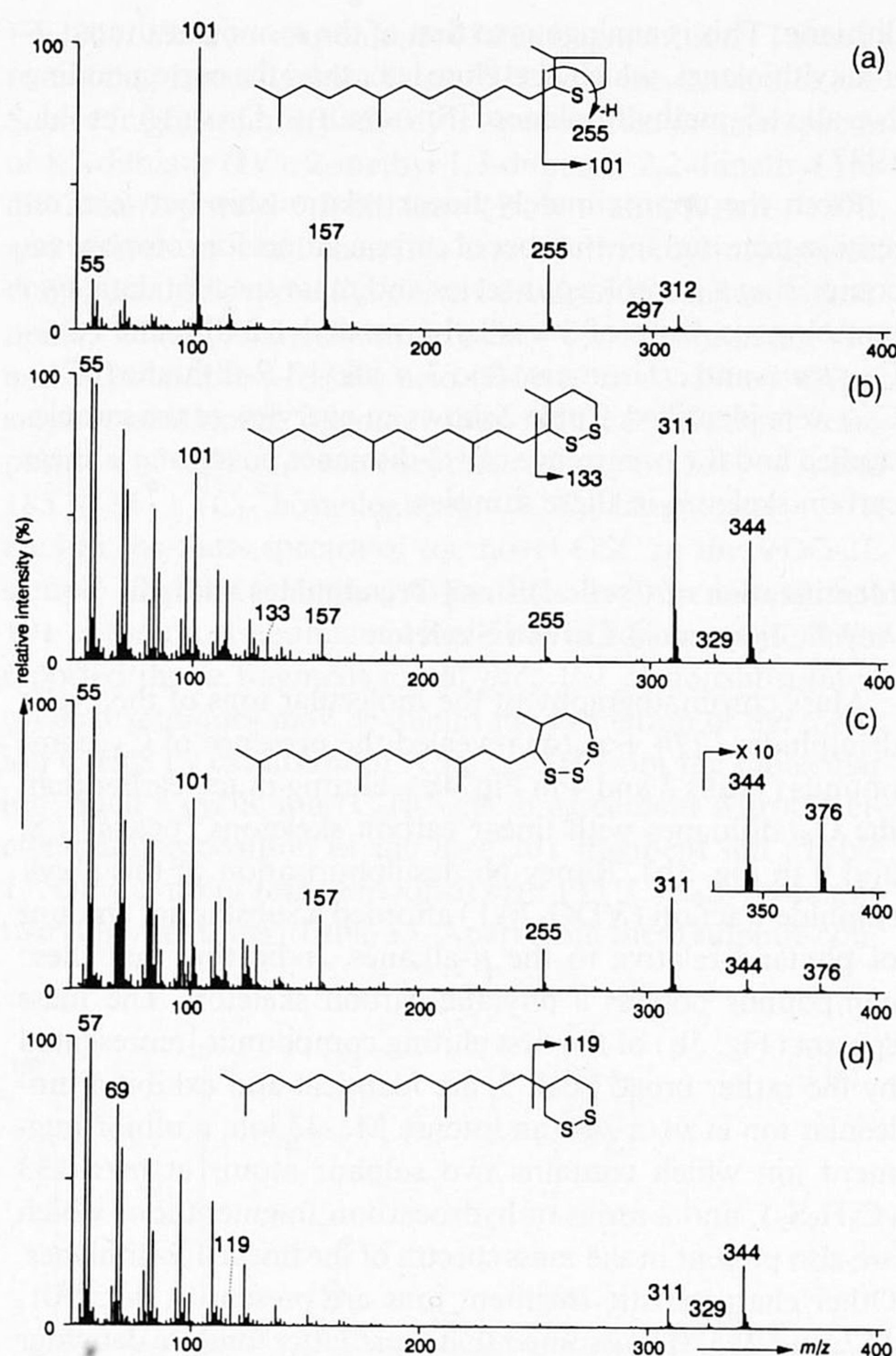


FIG. 5. Mass spectra (subtracted for background) of (a) 3-methyl-2-(3,7,11-trimethyldodecyl)thiolane, (b) 4-methyl-3-(3,7,11-trimethyldodecyl)-1,2-dithiane, (c) 5-methyl-4-(3,7,11-trimethyldodecyl)-1,2,3-trithiepane, (d) 4-(4,8,12-trimethyltridecyl)-1,2-dithiane.

by GC-MS analysis in the alkylsulphide fraction treated with MeLi/MeI. Although the mass spectrum of this di(methylthio)-phytane is not fully understood, it shows the same characteristics as that of the synthesized 2,5-di(methylthio)-octadecane (cf. Fig. 6a,b). Both spectra exhibit major fragment ions associated with β -cleavage with respect to the sulphur atom(s) (m/z 61, 163, 271 vs. m/z 75, 163, 243) and a major secondary fragment ion at m/z 101. In addition, both spectra show a major rearrangement ion [m/z 74 vs. m/z 88 ($C_nH_{2n}S$); Fig. 6]. The positions of the methylthio groups in the carbon skeleton of this 1,4-di(methylthio)-phytane are the same as the positions of the S-C bonds of the tentatively assigned 4-methyl-3-(3,7,11-trimethyldodecyl)-1,2-dithiane (XI), which thus supports its identification.

The minor compound (peak 4 in Fig. 4b,c) eluting just after this isoprenoid disulphide shows a mass spectrum similar to that of 4-methyl-3-(3,7,11-trimethyldodecyl)-1,2-dithiane (cf. Fig. 5b,d). However, an important difference is the minor fragment ion at m/z 119 which contains two sulphur atoms. Based on its mass spectrum and its relative GC retention time, this compound is tentatively identified as 4-(4,8,12-

trimethyltridecyl)-1,2-dithiane (XIII), although 3-methyl-3-(4,8,12-trimethyltridecyl)-1,2-dithiolane might be also in agreement with the mass spectrum (Fig. 5d); but such a compound is thought to elute much earlier. It should be noted that this tentatively identified compound is quite unstable upon sample storage. For example, this disulphide was the major compound in the alkylsulphide fraction of sample VDG-4A as revealed by GC analysis of this fraction performed instantly after extraction and fractionation procedures. However, reanalysis of the same alkylsulphide fraction after several weeks of storage at -18°C (in solvent) revealed that the relative concentration of this disulphide had decreased almost an order of magnitude. At present the kind of product(s) into which this sulfide is transformed is not clear.

The compound giving rise to peak 6 in the combined mass chromatogram m/z 344 + 376 (Fig. 4b, c, and d) shows a mass spectrum with characteristic fragment ions at m/z 115 (100%), 143 (18%), 227 (9%), 255 (7%), 311 (11%), and a molecular ion at m/z 344 (57%; $C_{20}H_{40}S_2$). It is suggested that this compound is also a C_{20} cyclic disulphide, although its structure is as yet unknown.

In the "aromatic hydrocarbon" fraction of a sediment sample from the Peruvian upwelling area (112-684C-4H-7 30-37 cm), these last three mentioned compounds (peaks 3, 4, and 6) are also encountered (cf. Fig. 4b,d; cf. KOHNEN et al., 1989). C_{20} 1,2-dithianes possessing linear carbon skeletons as described earlier are not detected. The most abundant compound (peak 10 in Fig. 4d) in this C_{20} cluster (N.B., the most abundant OSC of this "aromatic" fraction) shows a mass spectrum (Fig. 5c) with a molecular ion at m/z 376 and characteristic ions at m/z 101, 157, 255, 311, and 344 similar to those present in the mass spectrum of the earlier mentioned 4-methyl-3-(3,7,11-trimethyldodecyl)-1,2-dithiane (XI; cf. Fig. 5b,c). The $M^+ - 32$ fragment ion (m/z 344) is characteristic for this compound. Loss of 32 daltons is re-

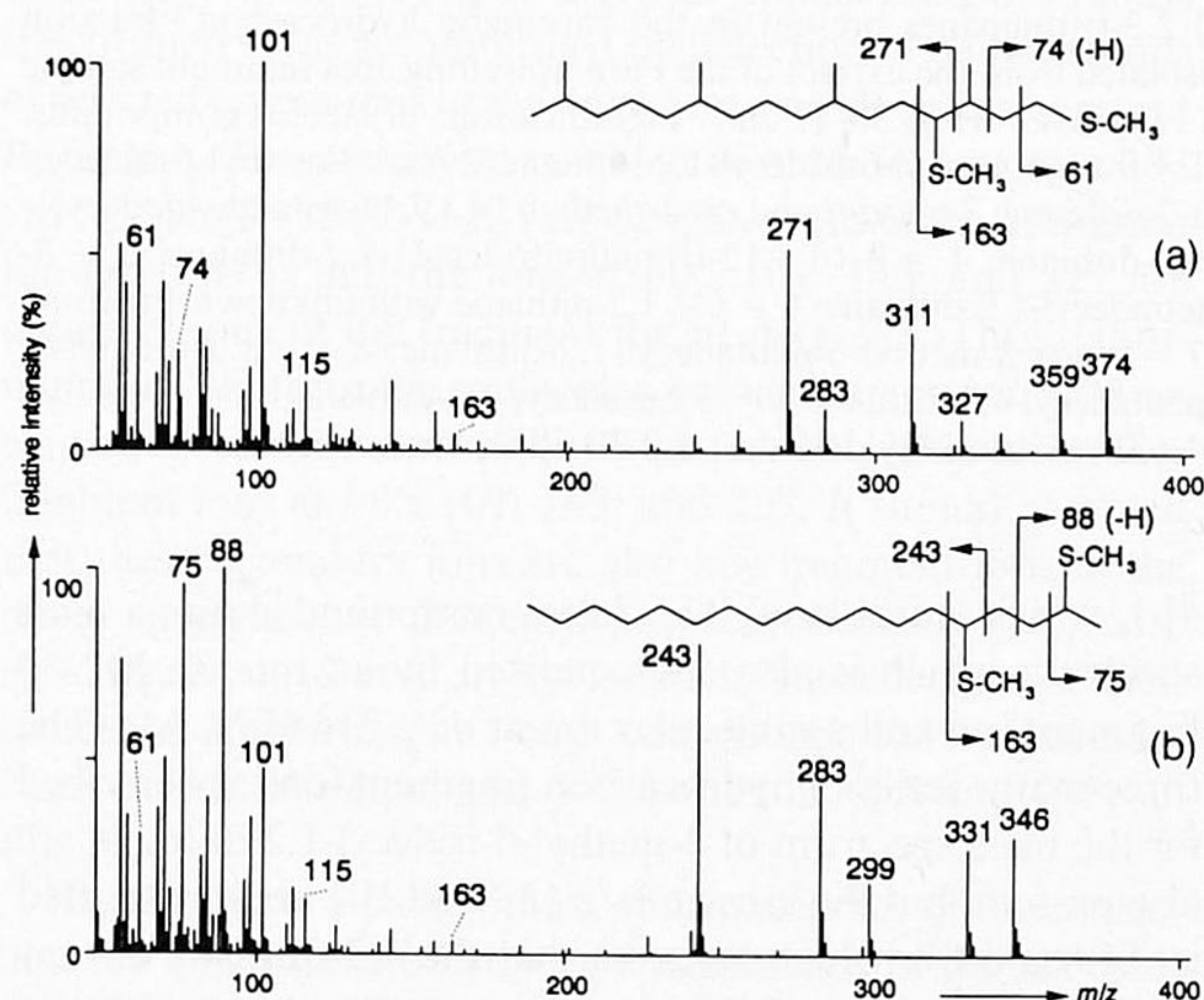


FIG. 6. (a) Mass spectrum (subtracted for background) of 1,4-di(methylthio)phytane. This compound is present in significant amounts in the alkylsulphide fraction VDG-4A after treatment with MeLi/MeI. (b) Mass spectrum of synthesized 2,5-di(methylthio)-octadecane.

ported in the mass spectra of diallyl disulphide, dibenzyl disulphide, diphenyl disulphide, 1,2-dithiane-3-formic acid, 1,2-dithiane-4-formic acid, 1,2-dithiolane-formic acid (BOWIE et al., 1966), lenthionine (1,2,3,5,6-pentathiepane, XIV; MORITA and KOBAYASHI, 1966), dioctadecyl tetrasulphide (SCHWAB et al., 1979), and 1,5-dihydro-2,3,4-benzotrithiepin (XV; SINGH et al., 1988). Another diagnostic feature of this mass spectrum is the relatively high intensity (ca. 16% of the magnitude of the parent peak) of the $M^+ + 2$ peak at m/z 378 which points to the presence of three sulphur atoms and suggests an elemental composition of $C_{20}H_{40}S_3$ (calculated intensity of $M^+ + 2$ peak is 15.6%). Upon Raney Ni desulphurisation and subsequent hydrogenation, this "aromatic hydrocarbon" fraction yielded almost only phytane. Hence, this novel OSC is tentatively identified as 5-methyl-4-(3,7,11-trimethyldodecyl)-1,2,3-trithiepane (XVI). This compound is also present, although in lower abundance, in the bitumen of several of the Vena del Gesso samples studied (Fig. 4C; see also Table 2). It should be noted that the cyclic trisulphide is also unstable upon sample storage.

Identification of a Cyclic Disulphide with a Hopanoid Carbon Skeleton

GC-MS analyses of the alkylsulphide fraction of sample VDG-7A1 revealed the presence of a hopanoid thiolane (XVII), a sulphide previously identified in the Rozel Point seep oil by SCHMID (1986). In addition, a compound was detected with a mass spectrum exhibiting many fragment ions identical to those present in the mass spectrum of the hopanoid thiolane (cf. Fig. 7a,b). Moreover, ions which are offset by 32 daltons (m/z 291, 512 vs. m/z 323, 544; cf. Fig. 7a,b) are also present, which, in combination with its molecular ion (m/z 544), point to a hopanoid disulphide. The presence of the fragment ion at m/z 369 indicates that the sulphur atoms are present in the alkyl side chain of the pentakishomohopane skeleton. Although a fragment ion at m/z 119 ($C_4H_7S_2$) was not detected, the abundance of the fragment ion m/z 87 (C_4H_7S) indicates that the cyclic disulphide

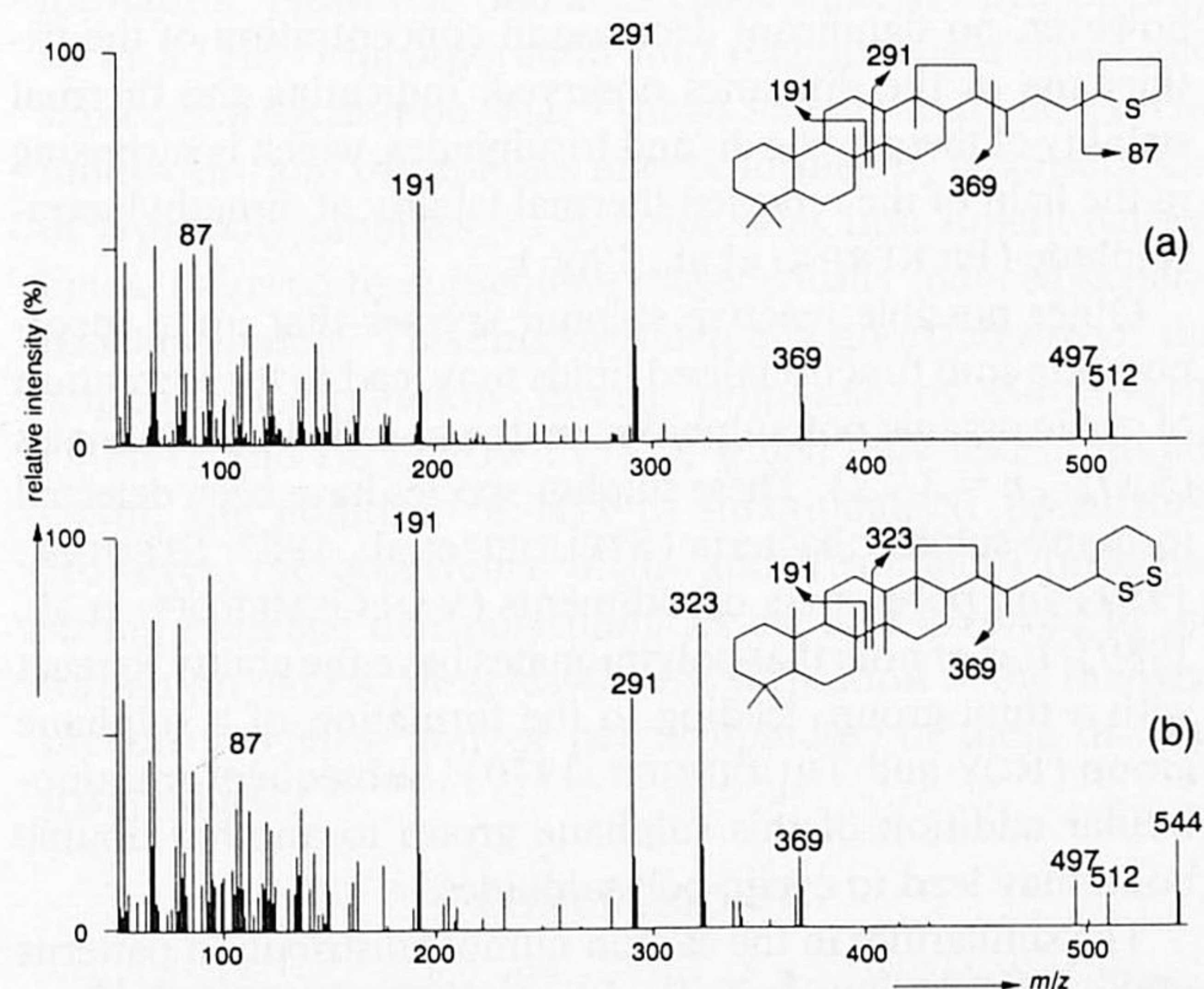


FIG. 7. Mass spectra (subtracted for background) of (a) hopanoid thiolane and (b) tentatively identified hopanoid 1,2-dithiane.

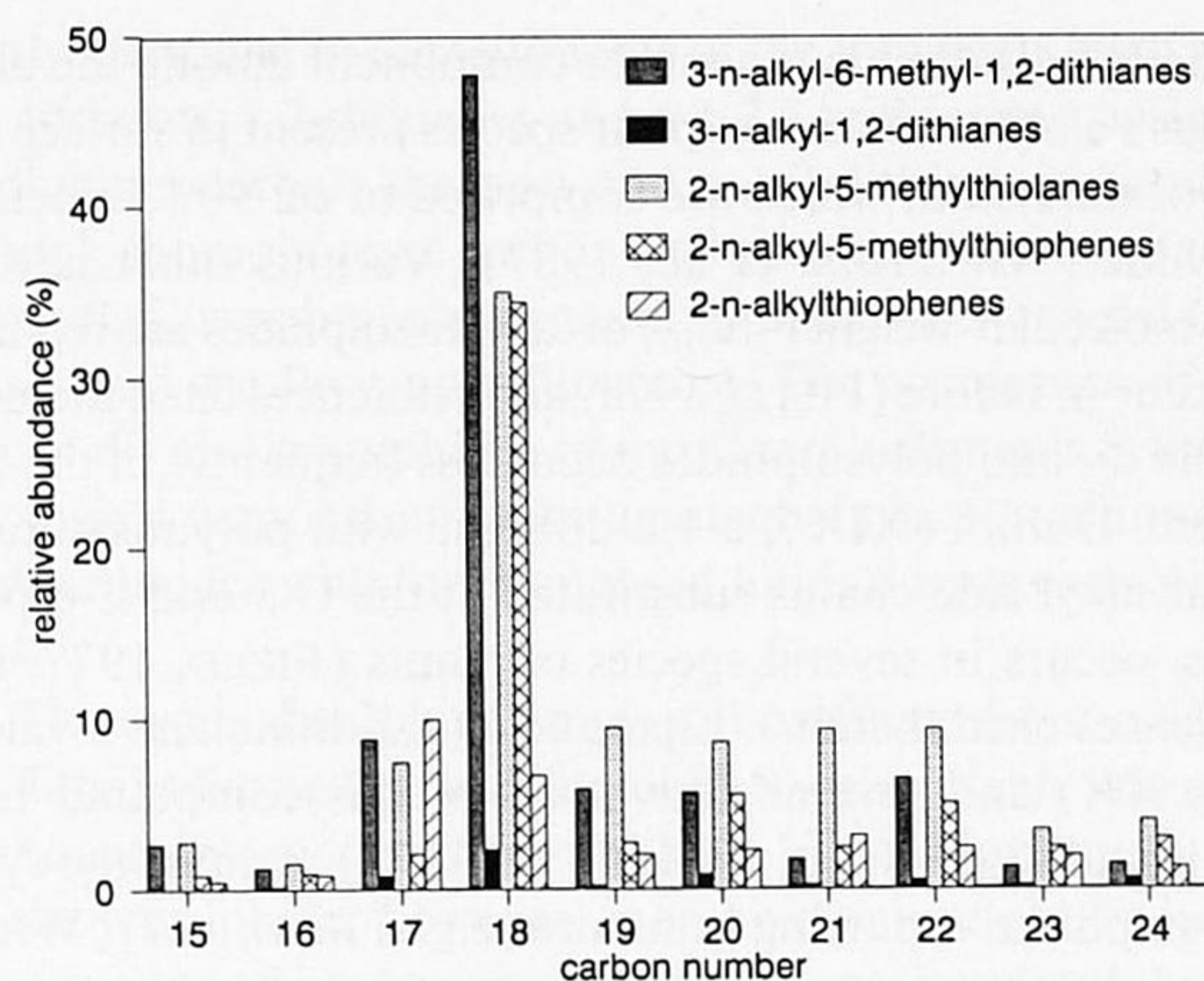


FIG. 8. Carbon number distributions of the various classes of OSC indicated encountered in the extract of sample VDG-JE.

moiety is monosubstituted and thus is located at the end of the side chain. The formation of the fragment ion m/z 87 is thought to be analogous to that observed in the mass spectrum of 3-tetradecyl-1,2-dithiane (Fig. 3d). It is noteworthy in this case that the ion at m/z 119 is small relative to that observed at m/z 87 (Fig. 3d). Hence, this compound was tentatively assigned as hopanoid dithiane XVIII. It is presently not understood why the $M^+ - 33$ fragment ion characteristic for 1,2-dithianes possessing linear and acyclic isoprenoid carbon skeletons is not observed in the mass spectrum of this hopanoid dithiane.

Carbon Number Distribution of OSC with Linear Carbon Skeletons

The carbon number (C_{15} – C_{24}) distributions of the 3-*n*-alkyl-6-methyl-1,2-dithianes, 3-*n*-alkyl-1,2-dithianes, 2-*n*-alkyl-5-methylthiolanes, 2-*n*-alkyl-5-methylthiophenes, and 2-*n*-alkylthiophenes present in the bitumen from sample VDG-JE are depicted in Fig. 8. The thiophenes are present in the X2 fraction of the bitumen. Except for a few discrepancies (e.g., C_{17} 2-*n*-alkylthiophene), the distributions of the various OSC classes are fairly similar (Fig. 8). All the series show a predominance of the C_{18} homologue, and the monosubstituted isomers are mostly subsidiary to the disubstituted isomers of the various classes of OSC. This latter aspect provides an explanation for the virtual absence (i.e., concentration below detection limit) of 2-*n*-alkylthiolanes, since the disubstituted thiolanes are present in minor absolute quantities. However, it is noteworthy that in contrast with the 1,2-dithianes and thiolanes, the homologous series of thiophenes range from C_{15} to C_{40} . In addition to the earlier mentioned alkylthiophene isomers, the so-called "mid-chain" isomers and the 2-*n*-alkyl-5-ethylthiophenes have been encountered as well.

DISCUSSION

Disulphide moieties and, to a much lesser extent, trisulphide moieties are common in the biosphere. Among the amino acids, cystine and homocystine contain disulphide linkages (FIELD, 1977, and references cited therein).

Dimethyl disulphide is a minor component among the ubiquitous volatile reduced sulphur species present in surface waters of the ocean, which are comprised of ca. 90% dimethylsulphide (WAKEHAM et al., 1987). Various other acyclic, low-molecular-weight ($<C_{10}$) di- and trisulphides are reported to occur in nature (FIELD, 1977, and references cited therein). Cyclic di- and polysulphides occur less frequently in the biosphere. Dithiin (XIX), a 1,2-dithiane with polyunsaturated linear alkyl side chains substituted at the C-3 and C-6 positions, occurs in several species of plants (FIELD, 1977, and references cited therein). Lipoic acid (1,2-dithiolane-3-valeric acid; XX) and several derivatives of this compound have also been found (REED, 1961; FIELD, 1977). Among the cyclic polysulphides occurring in nature (e.g., FIELD, 1977; WRATTEN and FAULKNER, 1976; ANTHONI et al., 1980) are compounds which possess di- and/or trisulphide moieties—e.g., 1,2,3,5,6-pentathiepane (XIV; red algae), 5-methylthio-1,2,3-trithiane (XXI; green algae).

Only a few literature reports deal with the occurrence of organic di- or polysulphides in the geosphere. SCHMID (1986) tentatively identified steroids (C_{27} – C_{30}) with a disulphide moiety (XVII) in an immature sulphur-rich oil. KAWKA and SIMONEIT (1987) reported the occurrence of CH_2S_4 (tetrathiolane), $(CH_2)_2S_4$ (tetrathianes, two unidentified isomers), CH_2S_5 (pentathiane), $(CH_2)_2S_5$ (pentathiepane, two unidentified isomers), and CH_2S_6 (hexathiepane) in the extract of a sample from an active deep-sea chimney venting hot hydrothermal waters (290–300°C). A thermal source was given preference over a biosynthetic source for the origin of these cyclic polysulphides. It is noteworthy to mention that CIERESZKO and YOUNGBLOOD (1971) reported the presence of *n*-hexadecyl and *n*-octadecyl disulphides in an artificially prepared sediment produced by the anaerobic decomposition of *Pseudoplexaura porosa* (sea rod). The investigators were unable to obtain the disulphides from hexane extracts of dried fresh *Pseudoplexaura porosa* and thus assumed that the disulphides were formed in the sediment from some component of the polar fraction.

The structure of the di- and trisulphides occurring in the biosphere are not related to the structure of the sedimentary di- and trisulphides reported in this paper. Therefore, it is suggested, analogous to the presumed origin of the other classes of OSC present in sediments and crude oils, that these novel classes of OSC are the result of abiotic sulphur incorporation into specific functionalised lipids during early diagenesis. For example, phytadienes, which are the putative precursors of the isoprenoid thiolanes (e.g., III; BRASSELL et al., 1986; SINNINGHE DAMSTÉ and DE LEEUW, 1987), may also act as suitable substrates for the formation of the C20 isoprenoid di- and trisulphides (XI and XVI).

Whether H_2S or polysulphides or both are incorporated into functionalised lipids during early diagenesis is still unclear. All the previously reported OSC occurring in sediments and crude oils have in common that they can be formed by H_2S (HS^-) addition to a double bond and a subsequent intramolecular cyclisation (SINNINGHE DAMSTÉ et al., 1989a). The alkylated 1,2-dithianes reported herein may also be formed by addition of two H_2S molecules to two double bonds and a subsequent oxidation of the intermediate dithiol, al-

though this seems rather unlikely in an anoxic environment. However, the cyclic trisulphide cannot be viewed to result from H_2S incorporation into functionalised lipids. Therefore, its presence strongly indicates that other sulphur species are involved in their formation.

Inorganic polysulphides occurring in natural environments (BOULÈGUE et al., 1982, and references cited therein; LUTHER et al., 1986) may be suitable sulphur species for the formation of trisulphides. Inorganic polysulphides are formed in nature by either reaction of H_2S with elemental sulphur or by both biotic and abiotic oxidation of H_2S (BOULÈGUE et al., 1982; VAN GEMERDEN et al., 1989). In natural aqueous solutions ($6 < pH < 8$) the predominant polysulphides are HS_4^- , HS_5^- , S_4^{2-} , and S_5^{2-} (BOULÈGUE et al., 1982). This seems to be in contradiction with the occurrence of organic di- and trisulphides, since these compounds possess two or three sulphur atoms adjacent to each other. However, it is known that organic polysulphides are thermally unstable (e.g., PICKERING and TOBOLSKY, 1972; FIELD, 1977). PICKERING et al. (1966) have studied the thermal decomposition of dimethyl tetrasulphide, which was found to undergo a facile disproportionation at 80°C to give, besides various other products, dimethyl trisulphide. The thermal instability of organic polysulphides is related to the low dissociation energies of polysulphide linkages (C-C ca. 350 KJ/mol; C-S ca. 275 KJ/mol; S-S ca. 147 KJ/mol). Therefore, it is suggested that initially formed organic cyclic tetra- and/or pentasulphides will easily degrade into tri- and disulphides during prograding diagenesis. In this context it is interesting to note that the younger (Pliocene) and less deeply buried Peruvian upwelling area sediments contain almost exclusively cyclic trisulphides in contrast to the older (Upper Miocene) and once more deeply buried Vena del Gesso sediments, which contain mainly cyclic disulphides. Although both sediments can be classified as immature, it is irrefutable that the older and once more deeply buried Vena del Gesso sediments have experienced more geological history and, hence, more diagenetic changes. In an attempt to simulate diagenetic processes, the alkylsulphide fraction of VDG-7A3 was refluxed in toluene (ca. 111°C) for 35 h under nitrogen. There was, however, no significant decrease in concentration of the tri-thiepane or the dithianes observed, indicating the thermal stability of these cyclic di- and trisulphides, which is surprising in the light of the reported thermal lability of dimethyl tetrasulphide (PICKERING et al., 1966).

Other possible reactive sulphur species that upon incorporation into functionalised lipids may lead to the formation of cyclic organic polysulphides are the so-called polythionates ($S_nO_6^{2-}$, $n = 3$ –22). These sulphur species have been detected in many sulphur bacteria (STEUDEL et al., 1987; STEUDEL, 1989) and porewaters of sediments (VAN GEMERDEN et al., 1989). It is of note that polythionates have the ability to react with a thiol group, leading to the formation of a sulphane group (ROY and TRUDINGER, 1970). Subsequent intramolecular addition of this sulphane group to another double bond may lead to cyclic polysulphides.

The similarities in the carbon number distribution patterns of the alkyl-1,2-dithianes, alkylthiolanes, and alkylthiophenes possessing linear carbon skeletons suggest that there is a dia-

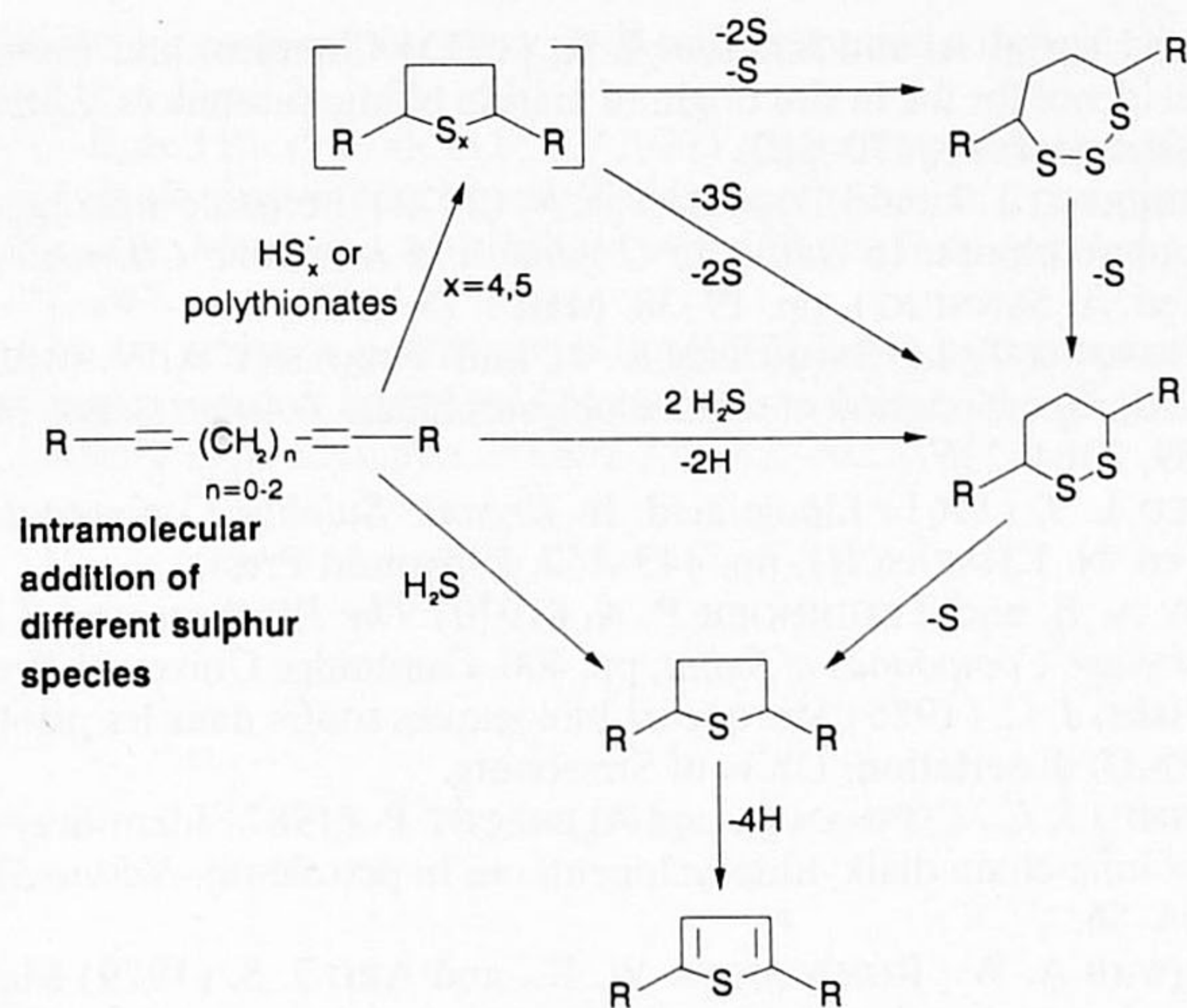


FIG. 9. Scheme for the formation of thiolanes, 1,2-dithianes, 1,2,3-trithiepanes, and thiophenes resulting from intramolecular incorporation of H_2S or polysulphides followed by diagenetic induced skeletal rearrangements. The penta- and the tetrasulphides are thought to be intermediates in the formation of tri- and disulphides since HS_4^- , HS_5^- , S_4^{2-} , and S_5^{2-} are the predominant inorganic polysulphides in natural aqueous solutions ($6 < \text{pH} < 8$; BOULÈGUE et al., 1982). These cyclic penta- and tetrasulphides have not yet been identified, possibly due to their unstable nature.

genetic relationship between these various classes of OSC (Fig. 8). Moreover, by analysis of appropriate fractions of sample VDG-7A2 by isotope-ratio-monitoring gas chromatography-mass spectrometry, we have shown that all the OSC with a phytane carbon skeleton (thiophene XXIII, thiolane III, 1,2-dithiane XI, 1,2,3-trithiepane XVI) are isotopically identical, which indicates that these OSC probably have the same precursor(s) (e.g., phytadiene) and may be diagenetically related to each other (KOHEN et al., 1991a). It has already been suggested by various authors (e.g., SINNINGHE DAMSTÉ et al., 1989a; KOHEN et al., 1990b) that thiophenes are diagenetic products of thiolanes. On the basis of the results presented in this paper, it is suggested that 1,2-dithianes and 1,2,3-trithiepanes are possible intermediates in the thiolane formation. However, thiolanes can also be formed directly by H_2S (HS^-) incorporation into functionalised lipids and subsequent cyclisation. Fig. 9 shows schematically how cyclic mono-, di-, and trisulphides may be formed by incorporation of H_2S , polysulphides, or polythionates into functionalised lipids, followed by subsequent diagenetically induced skeletal rearrangements. This model is actually an extension of the sulphur incorporation model proposed by SINNINGHE DAMSTÉ and DE LEEUW (1987), which only takes into account, the addition of H_2S to functionalised precursors. However, the occurrence of di- and trisulphides furnishes strong evidence that polysulphides are also involved in the formation of OSC or at least in the formation of the invoked precursors (tetra- and/or pentasulphides) of these di- and trisulphides.

CONCLUSIONS

Homologous series (C_{15} – C_{24}) of 3-*n*-alkyl-1,2-dithianes and 3-*n*-alkyl-6-methyl-1,2-dithianes have been unambigu-

ously identified in the sediments of the Vena del Gesso basin. In addition, 1,2-dithianes and a 1,2,3-trithiepane possessing a phytane carbon skeleton and a 1,2-dithiane possessing a pentakishomohopane carbon skeleton have been tentatively identified in sediments originating from the Vena del Gesso basin and the Peru upwelling area. The occurrence of these cyclic di- and trisulphides in immature sediments is the first geological demonstration for a potential reaction of inorganic polysulphides with functionalised lipids during early diagenesis.

The cyclic disulphides may still originate from oxidation of dithiols formed by reaction of H_2S with suitable precursors, although this seems rather unlikely. For the formation of the cyclic trisulphide, however, inorganic polysulphides have to be invoked. The occurrence of inorganic polysulphides is known to be sensitive towards variations in pH and Eh, and organic di- and trisulphides, reflecting the presence of inorganic polysulphides in the palaeoenvironment, may thus prove to be potential tools for the reconstruction of palaeoenvironmental conditions. Similarities in carbon number distribution patterns of 1,2-dithianes, thiolanes, and thiophenes with linear carbon skeletons suggest that these cyclic tri- and disulphides are intermediates in the formation of thiolanes.

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APPENDIX

