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Di- or polysulphide-bound biomarkers in sulphur-rich geomacromolecules as revealed by selective chemolysis*

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Abstract—Three types of sulphur-rich high–molecular-weight material in the alkylsulphide, the polar, and the asphaltene fractions isolated from the bitumen of an immature bituminous shale from the Vena del Gesso basin (Italy) were desulphurised using Raney Ni and were treated with MeLi/MeI, a chemical degradation method which cleaves selectively and quantitatively di- or polysulphide linkages. The products formed were characterised by gas chromatography-mass spectrometry. Raney Ni desulphurisation revealed that these S-rich macromolecules are in substantial part composed of sulphur-linked biomarkers with linear, branched, isoprenoid, steroid, hopanoid, and carotenoid carbon skeletons. MeLi/MeI treatment provided evidence that a major part of the total amount of macromolecularly bound biomarkers are linked via di- or polysulphide moieties to the macromolecular network. Since the di- or polysulphide linkages are attached at specific positions of the bound biomarkers it is proposed that they are formed by intermolecular incorporation reactions of HS_x^- into low–molecular-weight functionalised biological lipids during early diagenesis. The different properties (solubility and molecular weight) of the sulphur-rich macromolecules in the alkylsulphide, the resin, and the asphaltene fractions can be explained simply by differences in degree of sulphur cross-linking.

INTRODUCTION

RECENT STUDIES HAVE provided evidence that organically bound sulphur in geomacromolecules is formed during early diagenesis via inter- and intramolecular incorporation of reduced inorganic sulphur species into functionalised biosynthetic lipid moieties, which were or have become part of a macromolecular structure (BRASSELL et al., 1986; DE LEEUW and SINNINGHE DAMSTÉ, 1990; SINNINGHE DAMSTÉ et al., 1988b, 1989a,b, 1990). The identification of sulphur-containing moieties in geomacromolecules at a molecular level has been mainly focused on the thermally more stable forms of organic sulphur such as thiophene and benzothiophene moieties (SINNINGHE DAMSTÉ et al., 1988a, 1989a, 1990; PAYZANT et al., 1988). Thermally less-stable forms of organic sulphur such as mono-, di-, and polysulphide moieties are also thought to be present (TISSOT and WELTE, 1984; ORR, 1986). Sulphur-sulphur (average bond energy ca. 250 kJ/mol) and carbon-sulphur (ca. 275 kJ/mol) linkages have a relatively low bond energy as compared to carbon-carbon linkages (ca. 350 kJ/mol). Consequently the abundance of mono, di-, and polysulphide linkages will lower the mean activation energy for thermal degradation of geomacromolecules. Several workers (ORR, 1986, and references therein) have followed this line of thought in explaining the phenomenon that sulphur-rich high–molecular-weight organic matter begins to generate oil at a significantly lower thermal exposure level than sulphur-lean organic matter. However, data on a molecular level concerning the presence of mono, di-, and polysulphide linkages in geomacromolecules are fairly limited.

Recently, PAYZANT et al. (1988) identified cyclic monosulphides (i.e., alkylthiolanes and alkylthianes) in the pyrolysis oil of extracted Athabasca asphaltenes. GEORGE and GORBATY (1989) and KELEMEN et al. (1990) demonstrated the occurrence of mono(poly)sulphide moieties in heavy oils, asphaltenes, and coals by X-ray absorption near edge structure spectroscopy and X-ray photoelectron spectroscopy, respectively. Lately, the presence of bound cyclic monosulphide moieties in sulphur-rich resins was shown by flash-pyrolysis GC-MS (SINNINGHE DAMSTÉ et al., 1990). It was further demonstrated that these cyclic sulphides as well as hydrocarbon units are bound via unspecified sulphide linkages. SCHMID (1986) demonstrated the presence of disulphide-linked steroids in the resin fraction of the Rozel Point oil by using $LiAlH_4$, although it was mentioned by the author that this reagent also cleaves C-S bonds to some extent. The recent identification of low–molecular-weight cyclic di- and polysulphides in immature sediments (KOHNEN et al., 1989, 1991) suggests that it is not unlikely that these sulphur moieties are also present in macromolecules as acyclic linkages. In this paper we report the identification of di- or polysulphide linkages in macromolecules present in the so-called alkylsulphide fraction as well as in the polar (containing sulphur-rich resins) and asphaltene fractions from an immature sediment (Vena del Gesso basin) by a chemical degradation method which cleaves di- or polysulphide bonds selectively.

EXPERIMENTAL

Sample and Geological Setting

The sample investigated was collected in the Vena del Gesso basin in the Northern Appenines (Italy). The geological setting of this Messinian (Upper Miocene) basin is described in detail by VAI and RICCI LUCHI (1977). In brief, the evaporitic basin is filled with thick (35 m) beds of coarse crystalline gypsum associated with thinner carbonate and shaly (euxinic) intercalations. VAI and RICCI LUCCHI (1977)

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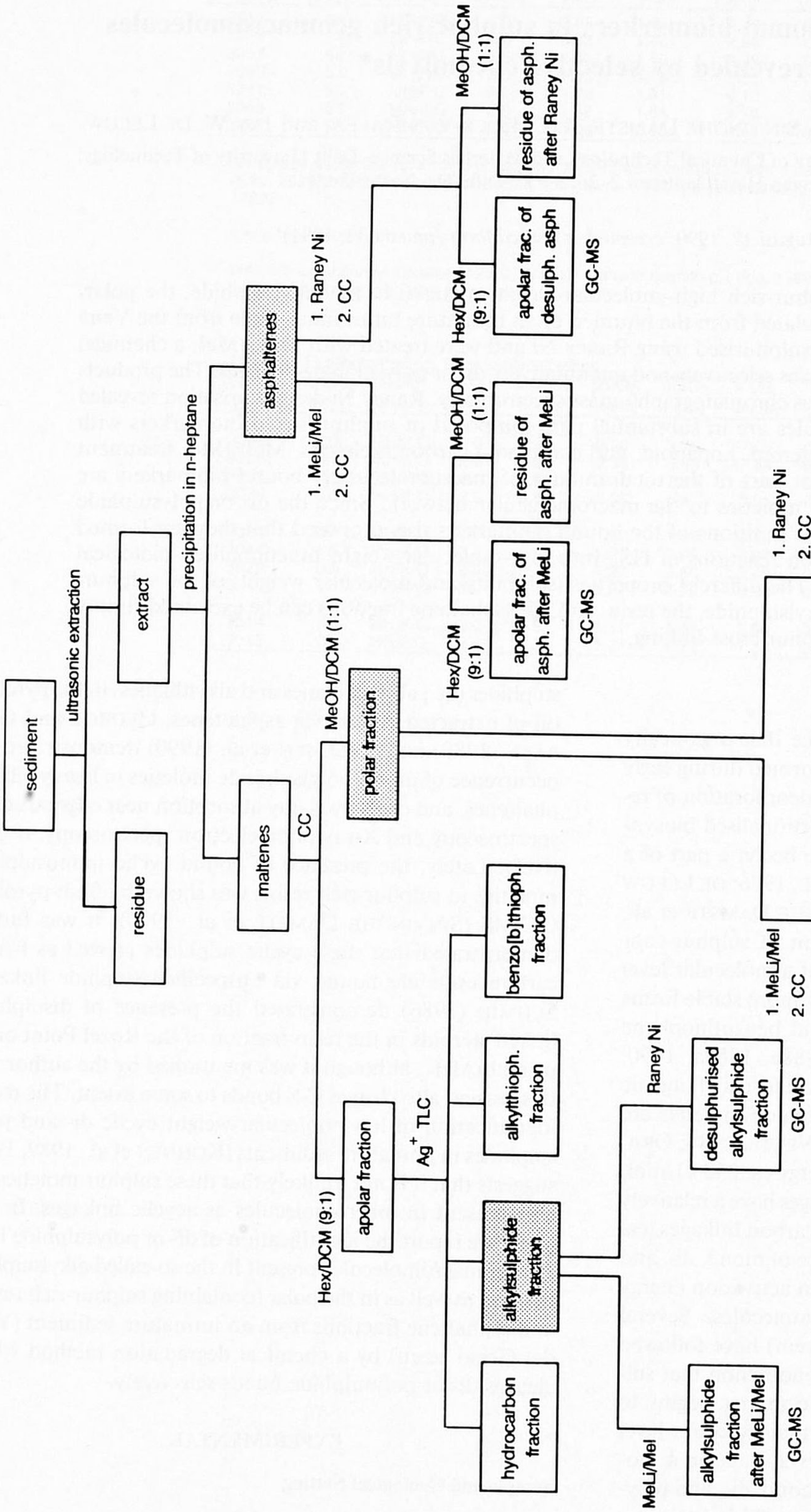

FIG. 1. Analytical flow diagram. The macromolecule-containing fractions studied are shaded.

found a sedimentary sequence comprising 6 facies which is repeated 14 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 investigated sample (VDG-4A) was taken from a fresh outcrop of the above-mentioned non-evaporitic bituminous shales from the fourth cycle. The immature character of these bituminous shales is illustrated by the low reflectance (average R_o = 0.25%) of the trace indigenous vitrinite particles in this sample.

Extraction and Fractionation

The freeze-dried shale sample was powdered in a rotary disc mill and ultrasonically extracted with methanol (×1), methanol/CH_2Cl_2 (1:1, v/v; ×1), and CH_2Cl_2 (×5), respectively. The bitumen was obtained by removing the solvent with a rotary evaporator at 30°C and weighed (0.46 g; 0.2 wt% of whole rock). After water was added, the extract was separated, using a separating funnel, into a CH_2Cl_2 layer and a methanol/H_2O (1:1, v/v) layer; the latter was re-extracted twice with CH_2Cl_2. The combined CH_2Cl_2 layers were dried with anhydrous Na_2SO_4 and evaporated to dryness. Separation of the asphaltenes from the maltenes was achieved by dissolving the extract in a minimum volume CH_2Cl_2 and subsequently adding a 40-fold excess of *n*-heptane (Fig. 1). After >8 h the asphaltenes flocculated out completely and the precipitate (asphaltene fraction, 17 wt% of total extract; Table 1) and supernatant (maltene fraction, 83 wt% of extract) were separately collected. The asphaltene fraction was purified by repeating the precipitation method two times more. An aliquot (ca. 200 mg) of the maltene fraction was fractionated on a column (25 cm × 2 cm; V_0 = 35 mL) packed with alumina (activated for 2.5 h at 150°C) by elution with 150 mL hexane/CH_2Cl_2 (9:1, v/v; the apolar fraction, ca. 13 wt% of the maltene fraction) and 150 mL methanol/CH_2Cl_2 (1:1, v/v; the polar fraction, ca. 63 wt% of the maltene fraction; Table 1). An aliquot of the apolar fraction (ca. 10 mg) was further separated by argentation thin layer chromatography (Ag^+-TLC) (KOHNEN et al., 1990). Four bands, R_f 0.8–1.0 (fraction A1), R_f 0.5–0.8 (fraction A2), R_f 0.1–0.5 (fraction A3), and R_f 0.0–0.1 (fraction A4), were scraped of the TLC plate, ultrasonically extracted with ethyl acetate (×3), and subsequently analysed by GC and GC-MS.

Gas Chromatography

Gas chromatography (GC) was performed using a Carlo Erba 5300 instrument, equipped with an on-column injector. A fused silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness 0.12 μm) was used with helium as carrier gas. The column effluent was monitored by 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 subsequently the oven was 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.

Table 1. Relative abundances of the macromolecule-containing fractions in the total extract (VDG-4A) and the hydrocarbon and methylthioether yields of these fractions after Raney Ni desulphurisation and MeLi/MeI treatment, respectively

	wt.% of total extract	hydrocarbon yield (wt.%)* Raney Ni treatment	methylthioether yield (wt.%)* MeLi/MeI treatment
alkylsulphide fraction	6	*c.* 80	*c.* 90**
polar fraction	53	17.5	9.6
asphaltene fraction	17	1.0	9.1

*Yield in wt.% of the total untreated fraction. **The alkylsulphide fraction contains alkylthiolanes which are not derivatised to methylthioethers.

Gas Chromatography-Mass Spectrometry

Gas chromatography-mass spectrometry (GC-MS) was carried out on a Hewlett-Packard 5480 gas chromatograph interfaced to 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 × 0.32 mm) coated with CP Sil-5 (film thickness = 0.2 μm). Helium was used as carrier gas. The samples were injected at 50°C, and subsequently the oven was programmed to 130°C at 20°C/min and then at 4°C/min to 300°C, at which it was held for 10 min.

Curie-point Pyrolysis-Gas Chromatography-Mass Spectrometry

Py-GC-MS analyses were performed on a Hewlett-Packard 5840 gas chromatograph interfaced to a VG-70S mass spectrometer using a FOM-3LX unit for pyrolysis. The operating conditions of the MS are the same as described above for GC-MS. The samples were applied to a ferromagnetic wire with a Curie temperature of 610°C. The gas chromatograph, equipped with a cryogenic unit, was programmed from 0°C (5 min) to 320°C (20 min) at a rate of 3°C/min. Separation was achieved using a fused-silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness 0.45 μm). Helium was used as the carrier gas.

Raney Nickel Desulphurisation/Hydrogenation

Appropriate fractions (see Fig. 1) were desulphurised with Raney Ni and subsequently hydrogenated as described previously (SINNINGHE DAMSTÉ et al., 1988b). The resultant hydrocarbons isolated from the reaction mixture using column chromatography over Al_2O_3 were analysed by GC and GC-MS.

Cleavage of Di- and Polysulphide Linkages by Treatment with MeLi/MeI

In a typical MeLi/MeI chemical degradation experiment (modified after ELIEL et al., 1976) the sample (ca. 30 mg) was dissolved in diethylether, stirred under nitrogen, and cooled to 0°C. Through a septum, MeLi (ca. 0.5 mmol) was gradually added and, after stirring for 15 min, tetramethylethylenediamine (ca. 0.5 mmol) was added. Stirring was continued at 15°C for 2 h, and subsequently MeI (ca. 0.5 mmol) was gradually added through a septum at 0°C over a 20 min period. Stirring was continued at 0°C for 2 h, and then a sodium chloride solution was added and the mixture was extensively extracted with diethyl ether.

After reaction with MeLi/MeI, the polar fraction and the asphaltene fraction from the Vena del Gesso bitumen were separated into an apolar fraction (released compounds) and a polar fraction (residue) using column chromatography as described above (Fig. 1). The apolar fraction obtained from the treated resins (Fig. 1) was subsequently further separated by thin layer chromatography (TLC) using hexane/ethyl acetate (99:1, v/v) as developer. The silica plates (20 × 20 cm; thickness 0.25 mm) were pre-activated at 120°C for 2 h. Three fractions (I, R_f = 0.7–1.0; II, R_f = 0.4–0.7; III, R_f = 0.05–0.4) were scraped off the TLC plate, ultrasonically extracted with ethyl acetate (×3), and analysed by GC-FID/FPD and GC-MS.

Derivatisation of Thiol Groups to Deuterated Methylthio Groups with C_2H_5ONa/CD_3I

In a typical experiment, the sample (ca. 30 mg) was dissolved in ethanol and stirred under nitrogen at room temperature. Through a septum, a solution of sodium ethoxide (ca. 0.5 mmol) in ethanol was added. After stirring for 5 min, ca. 0.5 mmol CD_3I was added through the septum. After 8 h an excess aqueous NaCl/HCl solution was added and the mixture was extensively extracted with diethyl ether.

Synthesis of Reference Compounds

Di(1-octadecyl)disulphide and di(3-cholest-5-enyl)disulphide were obtained by oxidation of 1-octadecanethiol (Aldrich) and thiocholesterol (Aldrich), respectively. In brief, a solution of the alkylthiol

in methanol was stirred with potassium carbonate at room temperature for 2 h while oxygen was bubbled through the solution (CHIU-HONG LIN et al., 1982). Di(1-octadecyl)tetrasulphide and di(3-cholest-5-enyl)tetrasulphide were synthesised by a reaction of the aforementioned alkylthiols with sulphur monochloride as described by SCHWAB et al. (1979). 2-nonyl-5-pentadecylthiophene, 2-nonyl-5-pentadecylthiolane, and 3-methyl-6-tridecyl-1,2-dithiane were synthesised previously (SINNINGHE DAMSTÉ et al., 1989b; KOHNEN et al., 1991). Di(1-dodecyl)sulphide was obtained from Aldrich.

2-(methylthio)-hexadecane was synthesised as follows: hexadecan-2-ol (Aldrich) was reacted with *p*-toluene sulfonylchloride under reflux (48 h) in anhydrous pyridine (NACE, 1952). The resulting tosylate was treated with sodium thiomethoxide in refluxing propan-2-ol/toluene (3:4, v/v) for 2 h yielding 2-(methylthio)-hexadecane (JONES et al., 1968).

RESULTS

ELIEL et al. (1976) reported a method involving MeLi/MeI that cleaves the S-S bond of a disulphide moiety and subsequently derivatises the cleavage products into the corresponding methylthioethers with high yields (Fig. 2a). The present study investigated whether this method is suitable for identifying disulphide moieties in geomacromolecules. To this end several model compounds were treated with MeLi/MeI in order to test its reactivity towards different forms of organic sulphur.

Soluble organic matter encountered in sediments comprises, in addition to low–molecular-weight compounds, two analytically defined fractions which contain macromolecules, namely the "resin" and "asphaltene" fractions (TISSOT and WELTE, 1984). Resins are defined as the high–molecular-weight (MW > ca. 800) substances soluble in light hydrocarbons (TISSOT and WELTE, 1984). Using conventional separation methods (liquid-solid chromatography) for the fractionation of crude oils and bitumens it is usually assumed that resins elute in the polar fraction (NSO fraction). However, SINNINGHE DAMSTÉ et al. (1988b) reported that the "aromatic hydrocarbon" fraction of immature sulphur-rich crude oils and bitumens also contains substantial amounts of high–molecular-weight, non-GC amenable substances (resins per definition). In order to overcome this undesirable partition of resin material over two fractions, a separation procedure was performed in which all the resins present are collected in one fraction, the so-called "polar" fraction (Fig. 1). It should be noted that apart from the resins this polar fraction also contains low–molecular-weight polar compounds (e.g., alcohols, acids, ketones). Asphaltenes are defined as the high–molecular-weight (MW > ca. 800) substances which are, in contrast with the resins, not soluble in light hydrocarbons (TISSOT and WELTE, 1984).

A polar (resin) and an asphaltene fraction isolated from the bitumen (Fig. 1) of an immature bituminous shale from the Messinian Vena del Gesso basin (Northern Appenines, Italy) were treated with MeLi/MeI.

Model Compounds

In order to assess the selectivity of the MeLi/MeI method with respect to the cleavage of different types of sulphur linkages, several synthesised standards were treated with MeLi/MeI. Acyclic mono-, di-, and tetrasulphides, cyclic mono- and disulphides, an alkylthiophene, and alkylthiols possessing

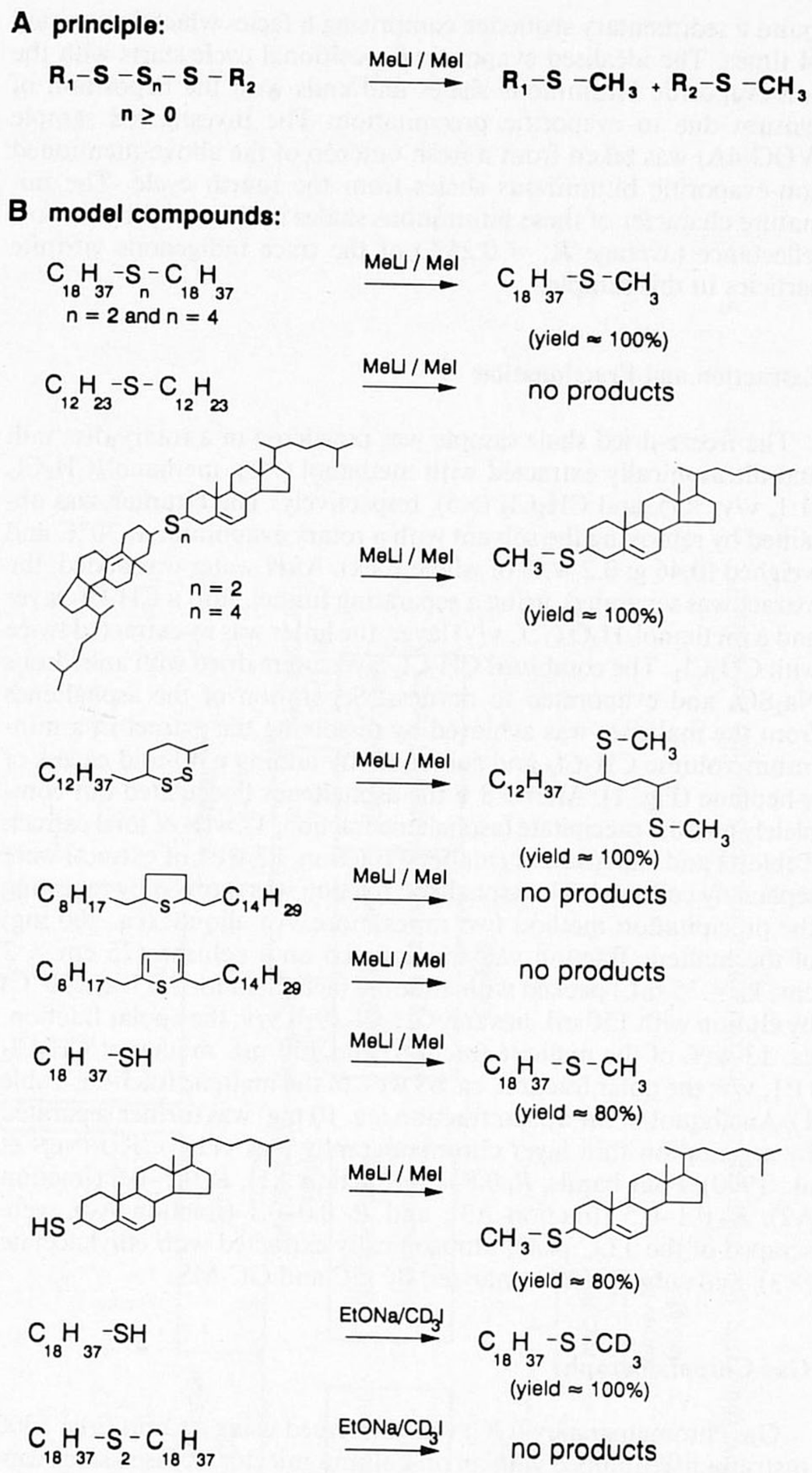

FIG. 2. (a) Cleavage of di- and polysulphide bonds in macromolecular organic matter by MeLi/MeI. (b) Model compounds treated with MeLi/MeI and C_2H_5ONa/CD_3I.

different carbon skeletons were treated with MeLi/MeI (Fig. 2b). The S-S linkages in di- and tetrasulphides were selectively and quantitatively cleaved, and the corresponding methylthioethers were formed. On the other hand, cyclic and acyclic monosulphides and alkylthiophenes were not affected by reaction with MeLi/MeI. In the case of alkylthiols a partial conversion (yield ca. 80%) to the corresponding methylthioethers was observed.

Resins

In order to assess all the sulphur-linked structural units in the sulphur-rich resins, the polar fraction was initially desulphurised using Raney Ni (SCHMID, 1986; DE LEEUW and SINNINGHE DAMSTÉ, 1990; SINNINGHE DAMSTÉ et al., 1988b, 1990). Raney Ni cleaves C-S bonds selectively and quantitatively. Desulphurisation of the polar fraction (containing the resins) yielded ca. 17.5 wt% hydrocarbons (Table 1) dom-

inated by *n*-alkanes (C_{15}–C_{28}), phytane, steroid hydrocarbons [amongst which 20R-5β(H),14α(H),17α(H)- and 20R-5α(H),14α(H),17α(H)-cholestanes and -24-ethyl-cholestanes, 20R-4α,23,24-trimethyl-5α(H),14α(H),17α(H)-cholest-22-ene (dinosterene) and dinosterane are dominant], and a diaromatic carotenoid (Fig. 3b).

To assess whether these lipids were bound via monosulphide or di(poly)sulphide linkages, or both, the polar fraction was treated with MeLi/MeI. Figure 4b shows the FID chromatogram of the apolar fraction (9.6 wt% of polar fraction; Table 1) obtained after MeLi/MeI treatment of the polar fraction. Comparison of the FID and FPD traces (not shown)

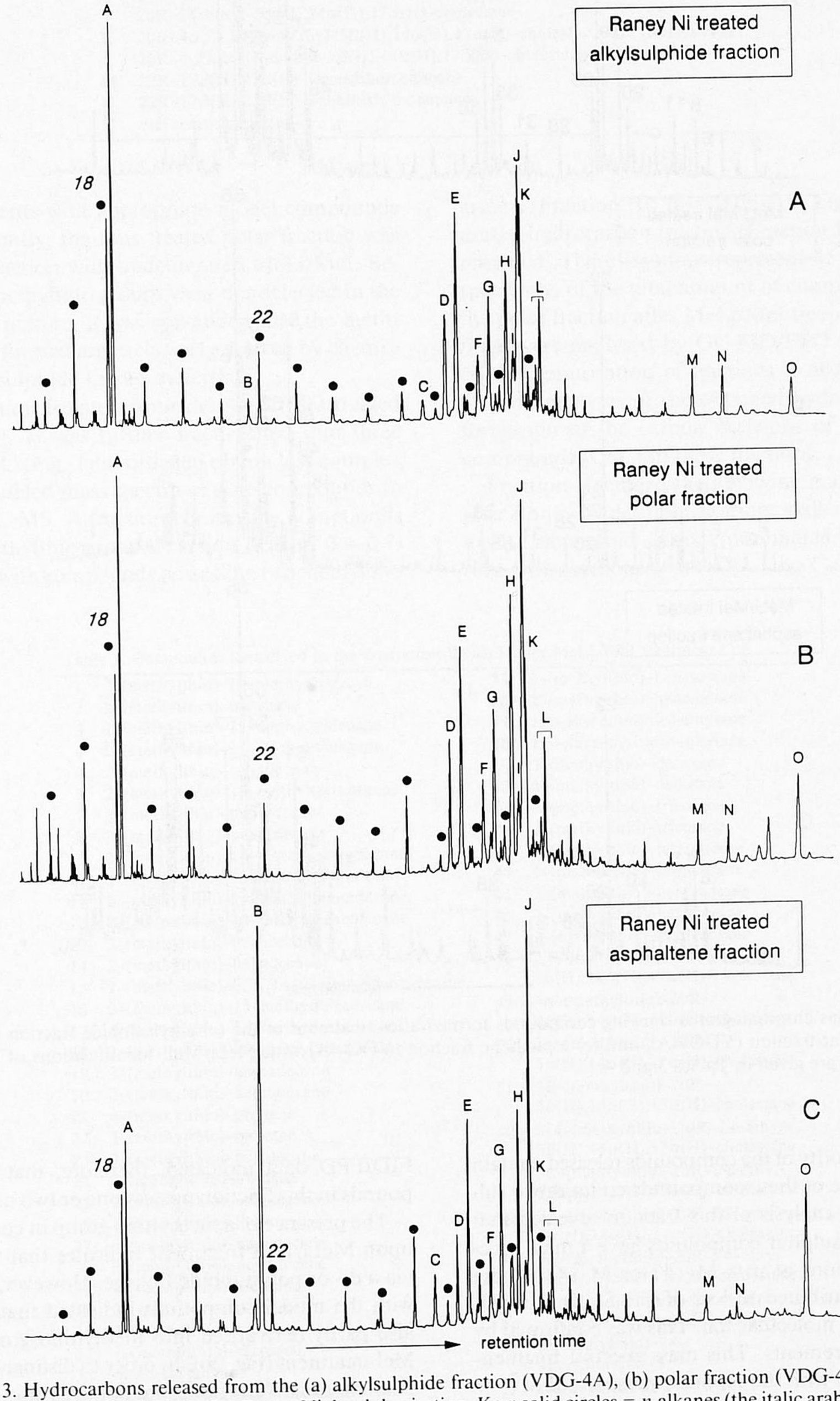

FIG. 3. Hydrocarbons released from the (a) alkylsulphide fraction (VDG-4A), (b) polar fraction (VDG-4A), and (c) asphaltene fraction (VDG-4A) after Raney Ni desulphurisation. Key: solid circles = *n*-alkanes (the italic arabic numbers indicate the number of carbon atoms of *n*-alkanes). Identifications of character-labeled compounds are given in Table 2.

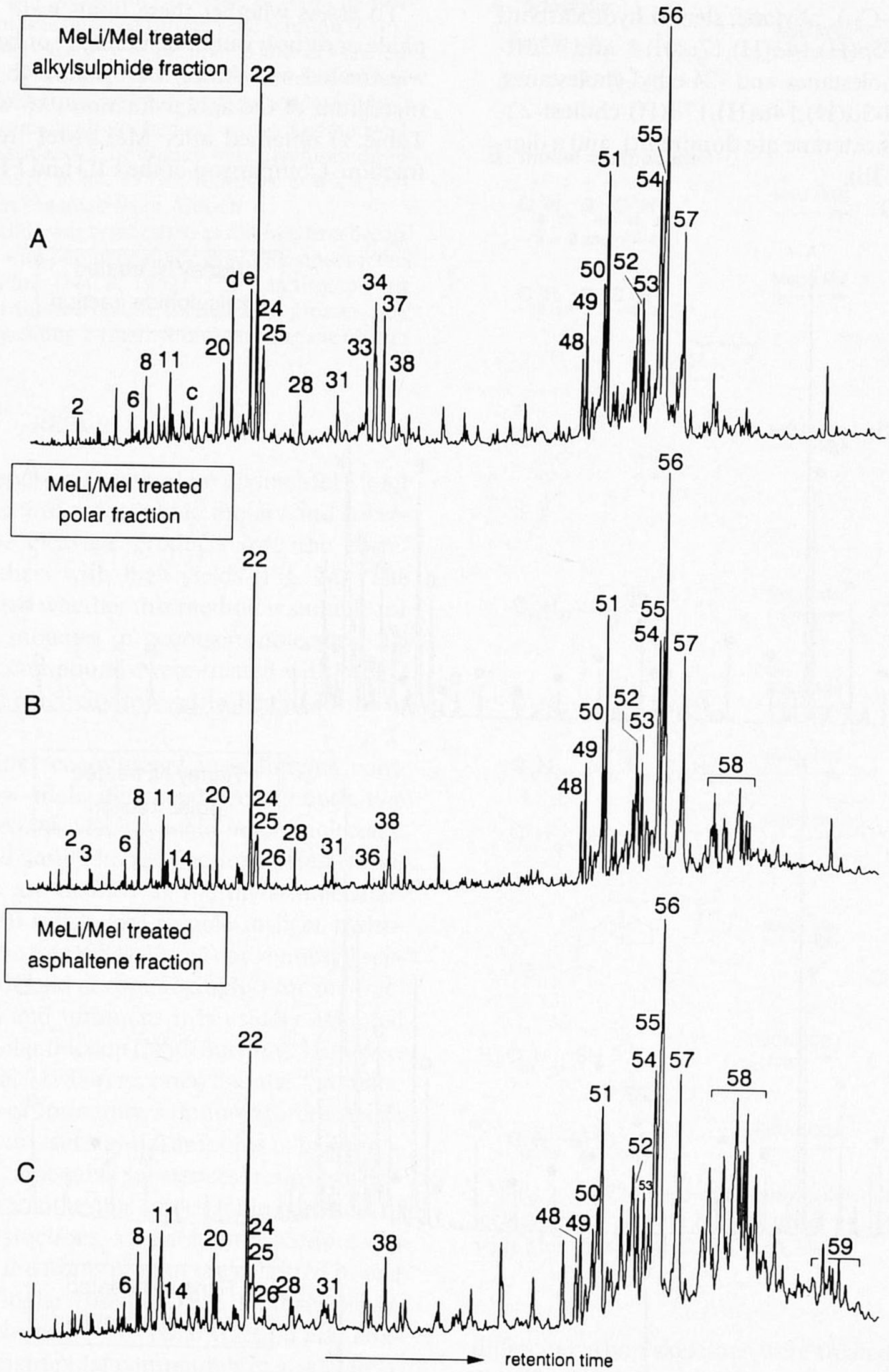

FIG. 4. Gas chromatograms showing compounds formed after treatment of the (a) alkylsulphide fraction (VDG-4A), (b) polar fraction (VDG-4A), and (c) asphaltene fraction (VDG-4A) with MeLi/MeI. Identifications of labeled compounds are given in Tables 3 and 4.

indicated that the majority of the compounds released contain sulphur and that some of these compounds contain two sulphur atoms. GC-MS analysis of this fraction revealed that all the major organic sulphur compounds have a mass spectrum with fragment ions at m/z $M^{+\cdot}$-47 or $M^{+\cdot}$-48. These fragment ion were attributed to loss of $CH_3S\cdot$ or CH_3SH, respectively, from the molecular ion. This was confirmed by accurate mass measurements. This mass spectral fragmentation is in agreement with the reported presence of $M^{+\cdot}$-47 fragment ions in mass spectra of di(methylthio)alkanes (FRANCIS and VELAND, 1981). The mass spectral and GC-FID/FPD data indicated, therefore, that the major compounds in this fraction possess one or two methylthio groups.

The presence of a methylthio group in compounds formed upon MeLi/MeI treatment indicates that they were bound via a di- or polysulphide linkage. However, the experiments with the model compounds indicated that thiol groups are also partly derivatised into methylthio groups upon MeLi/MeI treatment (Fig. 2b). In order to distinguish between these possible origins the polar fraction was also treated with deuterated MeLi/sodium ethoxide. This reaction exclusively derivatises thiol groups to deuterated methylthio groups as re-

Table 2. Compounds identified in the fractions obtained after Raney Ni treatment.

A	2,6,10,14-tetramethylhexadecane (phytane)
B	6,6-d_2-3-methyleicosane (internal standard)
C	2,6,10,15,19,23-hexamethyltetracosane (squalane)
D	20R-5β(H),14α(H),17α(H)-cholestane
E	20R-5α(H),14α(H),17α(H)-cholestane
F	20R-24-methyl-5β(H),14α(H),17α(H)-cholestane
G	20R-24-methyl-5α(H),14α(H),17α(H)-cholestane
H	20R-24-ethyl-5β(H),14α(H),17α(H)-cholestane
I	20R-4,24-dimethyl-5β(H),14α(H),17α(H)-cholestane
J	20R-24-ethyl-5α(H),14α(H),17α(H)-cholestane
K	20R-4α,23,24-trimethyl-5β(H),14α(H),17α(H)-cholest-22-ene (dinosterene)
L	20R-4,23,24-trimethyl-5β(H),14α(H),17α(H)-cholestane (dinosterane)
M	22R-17β(H),21β(H)-tetrakishomohopane
N	22R-17β(H),21β(H)-pentakishomohopane
O	diaromatic carotenoid

vealed by experiments with appropriate model compounds (Fig. 2b). Subsequently, the thus treated polar fraction was subjected to the reaction with undeuterated MeLi/MeI. Because deuterated methylthio groups were not detected in the ultimate reaction mixture, it was concluded that the methylthio compounds formed are exclusively derived by chemolysis of di- or polysulphide linked moieties.

The apolar fraction isolated from the MeLi/MeI treated polar fraction (Fig. 1) was further fractionated into three fractions using TLC (Fig. 1) in order to obtain less complex mixtures which enabled mass spectra of pure compounds to be obtained by GC-MS. A fraction comprising compounds possessing one methylthio group (Fraction II, R_f = 0.4–0.7; Fig. 5a), a fraction with compounds possessing two methylthio groups (Fraction III, R_f = 0.05–0.4; Fig. 5b), and an unsaturated hydrocarbon fraction (Fraction I, R_f = 0.7–1.0) were obtained. These fractions represent 45, 48, and 7 wt%, respectively, of the total amount of compounds released from the polar fraction after MeLi/MeI treatment. The TLC fractions were analysed by GC-FID/FPD and GC-MS. Raney Ni desulphurisation of fractions II and III and subsequent GC-MS analyses of the obtained hydrocarbons yielded information of the carbon skeletons of the organic sulphur compounds (OSC) in these fractions.

Fraction II yielded the following main compound classes after Raney Ni desulphurisation: *n*-alkanes ranging from C_{15} to C_{28}; isoprenoid alkanes (dominated by phytane); and steroid hydrocarbons [with C_{27}, C_{28}, C_{29} 20R-5β(H),14α(H),

Table 3. Compounds identified in the fractions obtained after MeLi/MeI treatment.

1	2-(methylthio)-10-methyldodecyl
2	2-(methylthio)-tridecane
3	2-(methylthio)-12-methyltridecane
4	2-(methylthio)-11-methyltridecane
5	2-(methylthio)-tetradecane
6	2-(methylthio)-13-methyltetradecane
7	3-(methylthio)-pentadecane
8	2-(methylthio)-pentadecane
9	3-(methylthio)-14-methylpentadecane
10	3-(methylthio)-13-methylpentadecane
11	2-(methylthio)-14-methylpentadecane
12	2-(methylthio)-13-methylpentadecane
13	3-(methylthio)-hexadecane
14	2-(methylthio)-hexadecane
15	2-(methylthio)-6,10,14-trimethylpentadecane
16	3-(methylthio)-15-methylhexadecane
17	3-(methylthio)-14-methylhexadecane
18	2-(methylthio)-15-methylhexadecane
19	3-(methylthio)-heptadecane
20	2-(methylthio)-heptadecane
21	4-(methylthio)-phytane
22	3-(methylthio)-phytane
23	3-(methylthio)-octadecane
24	2-(methylthio)-phytane
25	2-(methylthio)-octadecane
26	1-(methylthio)-phytane
27	3-(methylthio)-nonadecane
28	2-(methylthio)-nonadecane
29	4-(methylthio)-eicosane
30	3-(methylthio)-eicosane
31	2-(methylthio)-eicosane
32	1-(methylthio)-eicosane
33	internal standard
34	2,5-di(methylthio)-octadecane*
35	3-(methylthio)-heneicosane
36	2-(methylthio)-heneicosane
37	1,4-di(methylthio)-phytane*
38	1,3-di(methylthio)-phytane
39	3-(methylthio)-docosane
40	2-(methylthio)-docosane
41	3-(methylthio)-tricosane
42	2-(methylthio)-tricosane
43	3-(methylthio)-tetracosane
44	2-(methylthio)-tetracosane
45	2-(methylthio)-pentacosane
46	2-(methylthio)-hexacosane
47	2-(methylthio)-heptacosane
48	2/4-(methylthio)-20R-5β(H),14α(H),17α(H)-cholestane
49	3α-(methylthio)-20R-5β(H),14α(H),17α(H)-cholestane
50	2α-(methylthio)-20R-5α(H),14α(H),17α(H)-cholestane
51	3β-(methylthio)-20R-5α(H),14α(H),17α(H)-cholestane
52	2/4-(methylthio)-20R-24-ethyl-5β(H),14α(H),17α(H)-cholestane
53	3α-(methylthio)-20R-24-ethyl-5β(H),14α(H),17α(H)-cholestane
54	2α-(methylthio)-20R-24-ethyl-5α(H),14α(H),17α(H)-cholestane
55	3β-(methylthio)-20R-24-ethyl-5α(H),14α(H),17α(H)-cholestane
56	mono(methylthio)-dinosterene
57	mono(methylthio)-dinosterane
58	di(methylthio)-24-ethylcholestanes
59	tri(methylthio)-24-ethylcholestanes

*derived from alkyldithianes present in the alkylsulphide fraction

Table 4. Compounds identified in the alkylsulphide fraction.

a	2-decyl-5-methylthiolane
b	2-methyl-5-undecylthiolane
c	2-dodecyl-5-methylthiolane
d	2-methyl-5-tridecylthiolane
e	3-methyl-5-(3,7,11-trimethyldodecyl)thiolane
f	3-methyl-6-tridecyl-1,2-dithiane (cis and trans isomers)
g	4-methyl-3-(3,7,11-trimethyldodecyl)1,2-dithiane
h	4-(4,8,12-trimethyltridecyl)-1,2-dithiane
i	2,3-dimethyl-5-(1,1-d_2-hexadecyl)thiophene (internal standard)
j	2-heptadecyl-5-methylthiolane
k	2-methyl-5-nonadecylthiolane
l	hopanoid sulphide

17α(H) and 20R-5α(H),14α(H),17α(H) regular steranes and 20R-4α,23,24-trimethyl-5α(H),14α(H),17α(H)-cholest-22-ene(dinosterene) and dinosterane dominating]. Minor compound classes comprise methyl-branched alkanes ranging from C_{15} to C_{19}, 17β(H),21β(H)-homohopane, and 17β(H),21β(H)-tetrakishomohopane. Fraction III yielded (after Raney Ni desulphurisation) 2,6,10-trimethyldodecane (farnesane), phytane, 20R-24-ethyl-5β(H),14α(H),17α(H)-cholestane, and 20R-24-ethyl-5α(H),14α(H),17α(H)-cholestane as major compounds. Minor compounds encountered in the desulphurised Fraction III are a series of *n*-alkanes (C_{15}–C_{28}), methyl-branched alkanes (C_{15}–C_{19}), C_{27} and C_{28} regular steranes, dinosterene, dinosterane, and a di-aromatic carotenoid.

In the following sections the identification of the methylthioethers encountered in Fractions II and III (prior to Raney Ni desulphurisation), arranged according to their type of carbon skeletons, will be discussed.

Mono(methylthio) n-*alkanes*

The compounds giving rise to the homologous series of peaks in the gas chromatogram of Fraction II (Fig. 5a; peaks 2, 14, 20, 25, 28, 30, 36, 40, and 42) are identified as mono(methylthio) *n*-alkanes. The mass spectra of these compounds are characterised by a molecular ion at m/z 230 + 14 · *n* (*n* = 0–12); a series of sulphur-containing fragment ions at m/z 61, 75 (base peak), 89, and 103; minor fragment ions at m/z $M^{+\cdot}$-48, m/z $M^{+\cdot}$-47, and two series of hydrocarbon fragment ions (C_nH_{2n+1} and C_nH_{2n-1}) (e.g., Fig. 6a). The fragment ions at m/z $M^{+\cdot}$-48 and m/z $M^{+\cdot}$-47 are due to loss of, respectively, CH_3SH and $CH_3S\cdot$ from the molecular ion. Mass spectra of dialkylsulphides and of methylthioalkanes show characteristic fragment ions associated with β-cleavage with respect to the sulphur atom (BUDZIKIEWICZ et al., 1965; FRANCIS and VELAND, 1981). Consequently, the major sulphur-containing fragment ion at m/z 75 and the

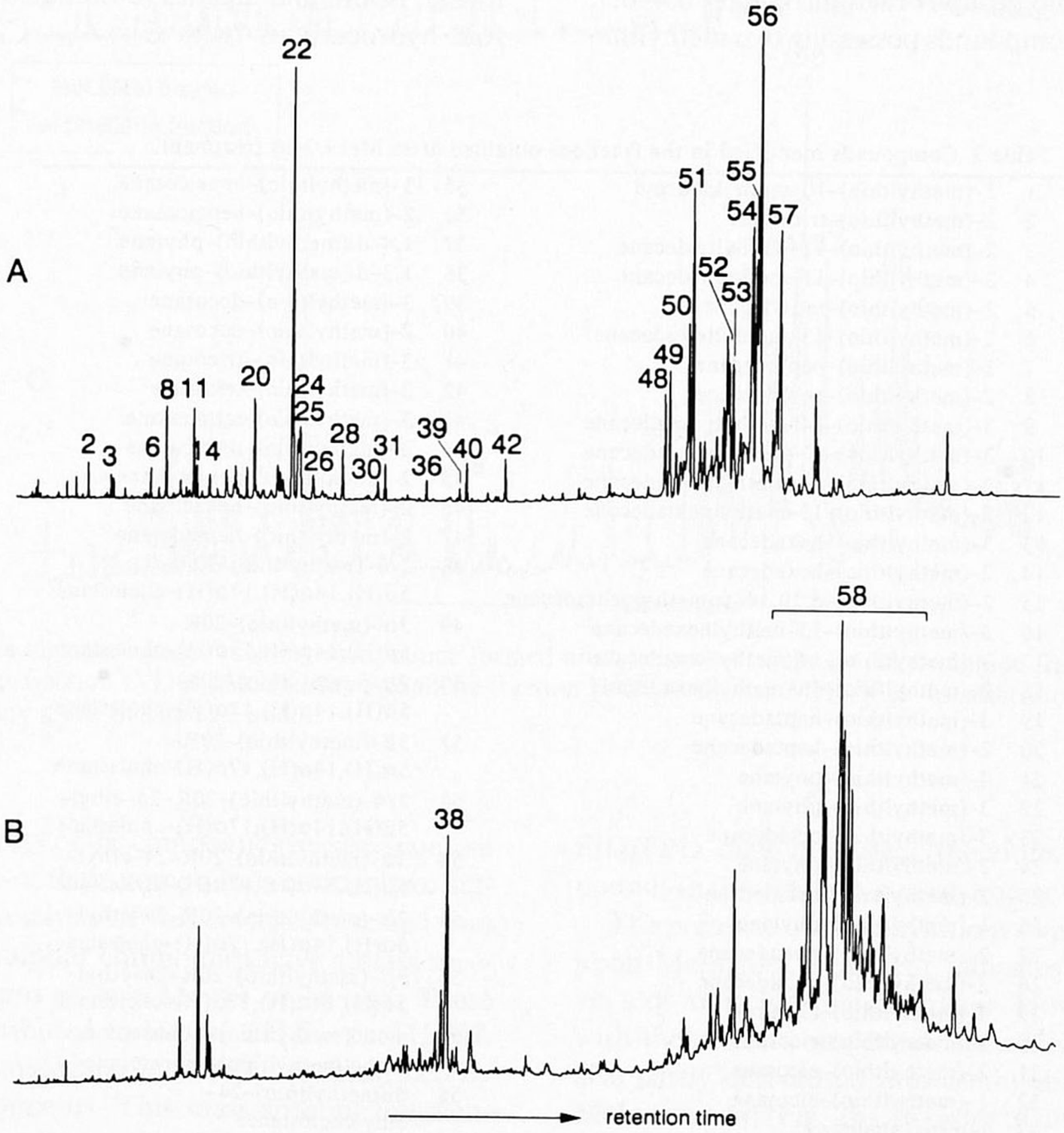

FIG. 5. Gas chromatograms of (a) Fraction II and (b) Fraction III isolated from the mixture of methylthioethers formed after MeLi/MeI treatment of the polar fraction (VDG-4A) using TLC. Identifications of labeled compounds are given in Table 3.

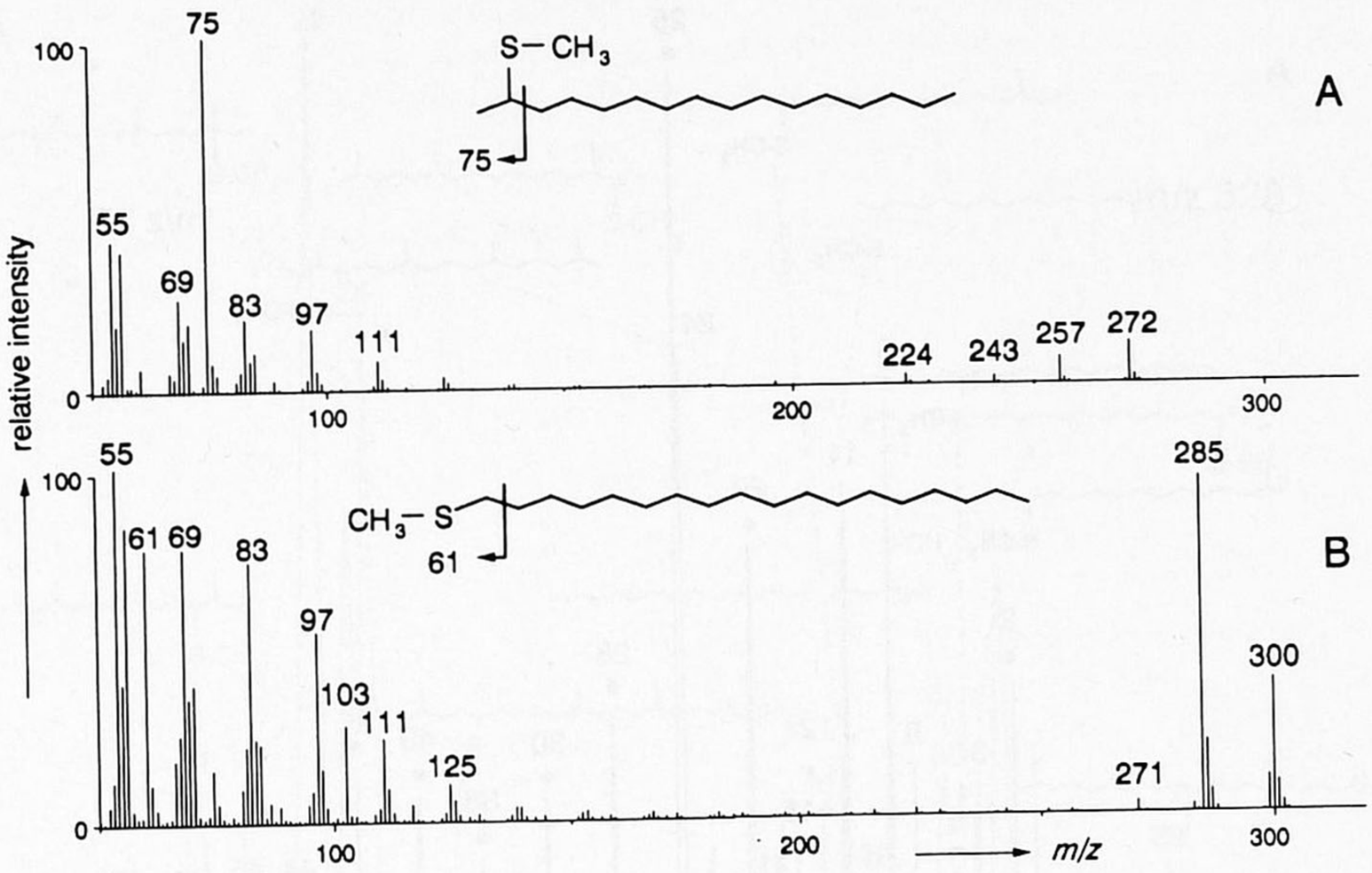

FIG. 6. Mass spectra of (a) synthesised 2-(methylthio)-hexadecane and (b) synthesised 1-(methylthio)-octadecane.

fragment ions at m/z $M^{+\cdot}$-15 indicated that this homologous series consists of 2-(methylthio)-alkanes. To unambiguously assess their structures the 2-(methylthio)-hexadecane was synthesised. The mass spectrum of the synthesised 2-(methylthio)-hexadecane (Fig. 6a) is identical with that of the tentatively identified 2-(methylthio)-hexadecane encountered in fraction II. Coinjections with the standard on two capillary columns coated with different stationary phases unequivocally established the presence of 2-(methylthio)-hexadecane in Fraction II. The mass chromatogram of m/z 75 reveals the distribution of the 2-(methylthio)-*n*-alkanes in Fraction II (Fig. 7a).

Figure 8a shows a partial mass chromatogram of m/z 328 ($M^{+\cdot}$ of the C_{20} methylthio-alkanes) of Fraction II. Two clusters of peaks are observed. The major structural isomer (peak 31; Fig. 8a) eluting in the second cluster is identified as 2-(methylthio)-eicosane. In addition to this major compound other minor methylthioethers possessing an eicosane carbon skeleton are encountered (peaks 29, 30, and 32). The last eluting compound (peak 32; Fig. 8a) shows a mass spectrum which is characterised by a molecular ion at m/z 328, a series of sulphur-containing fragment ions at m/z 61, 75, 89, 103, 117, and 313 ($M^{+\cdot}$-15), two series of hydrocarbon fragment ions (C_nH_{2n+1} and C_nH_{2n-1}), and minor fragment ions at m/z 252 ($M^{+\cdot}$-CH_3SH) and m/z 253 ($M^{+\cdot}$-CH_3S). The major sulphur-containing fragment ion at m/z 61 indicated that this compound is 1-(methylthio)-eicosane. From the linear relation between retention time and number of carbon atoms of a homologous series and mass spectral data, a minor series of 1-(methylthio)-*n*-alkanes (C_{15}–C_{23}) has been assigned. In order to confirm this tentative identification of this homologous series, 1-(methylthio)-octadecane was synthesised. This compound shows the same mass spectrum (Fig. 6b) as that of the tentatively identified 1-(methylthio)-octadecane in Fraction II and co-eluted with that component upon coinjection experiments. It is noteworthy that the intensity of the sulphur-containing fragment ions in the mass spectrum of 1-(methylthio)-octadecane decreases progressively in going from m/z 61 to m/z 89 and then increases again with m/z 103, which is presumably due to stabilization of this latter ion in a five-membered ring.

Mass spectra of the minor compounds (peaks 29 and 30; Fig. 8a) eluting before 2-(methylthio)-eicosane also exhibit the characteristic fragment ions of methylthio-eicosanes. The major sulphur-containing fragment ions in their mass spectra are diagnostic for the position of the methylthio group. Compound 29 is tentatively identified as 4-(methylthio)-eicosane (major fragment ions at m/z 103 and m/z 285) and compound 30 is assigned as 3-(methylthio)-eicosane (ions at m/z 89 and m/z 299). A partial mass chromatogram of m/z 89 shows the distribution of the minor homologous series of 3-(methylthio)-*n*-alkanes in Fraction II (Fig. 7b). In addition to 4-(methylthio)-eicosane, only 4-(methylthio)-docosane has been detected in minute amounts.

Mono(methylthio) branched alkanes

The mass chromatogram m/z 75 (Fig. 7a) revealed that, in addition to the 2-(methylthio)-*n*-alkanes, other major 2-(methylthio)-alkanes are present in Fraction II. Their relative retention times with respect to 2-(methylthio)-*n*-alkanes, together with the presence of 2-methylalkanes and 3-methylalkanes in the corresponding desulphurised Fraction II, indicated that they are 2-(methylthio)-(ω-1)-methylalkanes (C_{14}–C_{17}) and 2-(methylthio)-(ω-2)-methylalkanes (C_{13}, C_{14} and C_{16}). Minute amounts of 3-(methylthio)-14-methylpentadecane and 3-(methylthio)-13-methylpentadecane have also been detected in Fraction II.

Mono(methylthio)-phytanes

The compounds giving rise to the first cluster of peaks in the mass chromatogram of m/z 328 (Fig. 8a) show mass spectra with an $M^{+\cdot}$ ion at m/z 328 and minor fragment ions

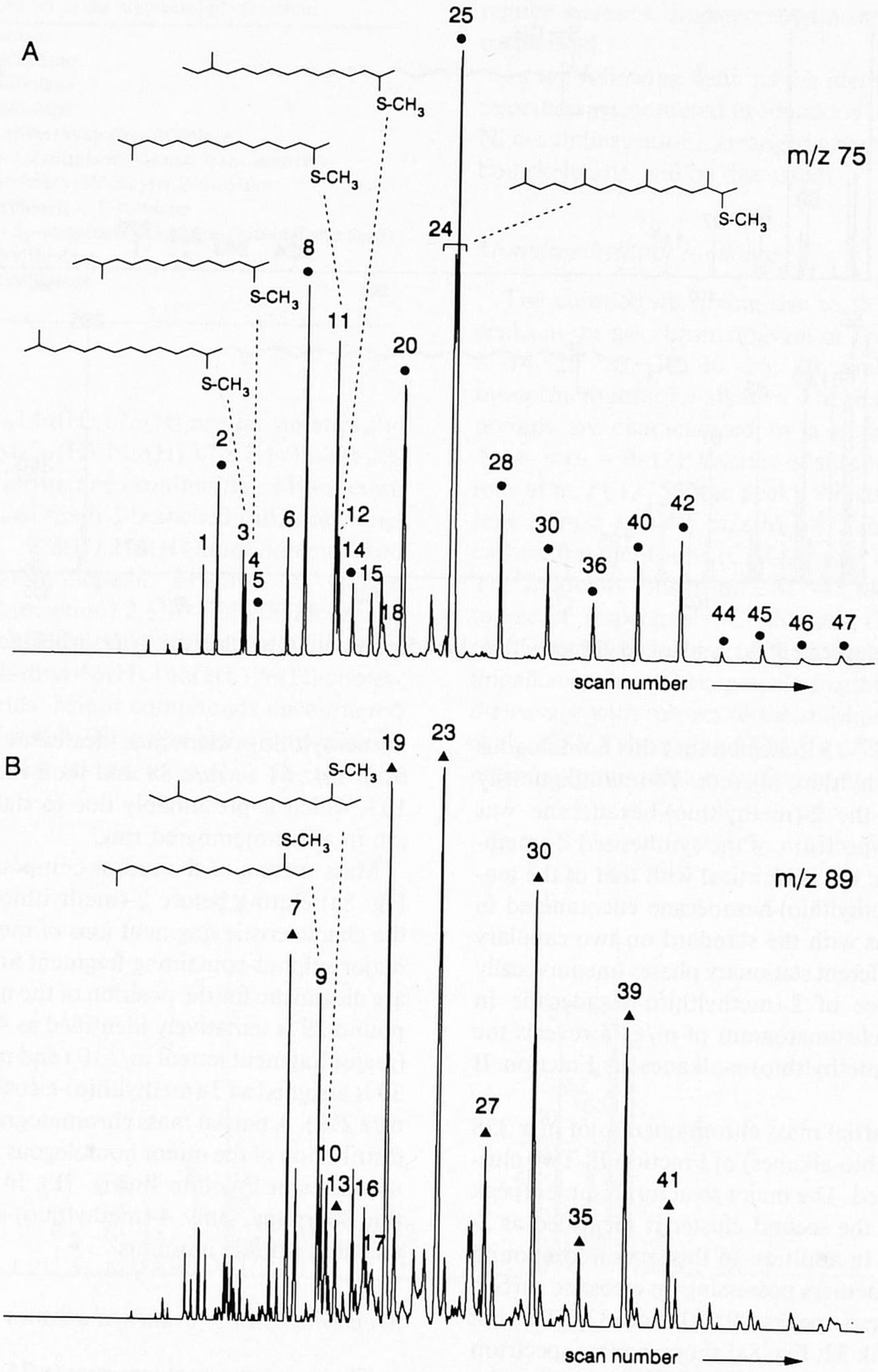

FIG. 7. (a) Partial mass chromatogram of m/z 75 showing the distribution of the 2-(methylthio)-alkanes (indicated by dots) formed after MeLi/MeI treatment of the polar fraction from the VDG-4A total extract. (b) Partial mass chromatogram of m/z 89 showing the distribution of the 3-(methylthio)-alkanes (indicated by triangles) formed after MeLi/MeI treatment of the polar fraction from the VDG-4A total extract. Identifications of labeled compounds are given in Table 3.

at m/z 280 and m/z 281. This indicated that these compounds are also methylthioethers possessing a C_{20} carbon skeleton. The relative retention times of these compounds suggested that they possess an isoprenoid carbon skeleton. Moreover, phytane is a major desulphurisation product of Fraction II. Consequently, these compounds are tentatively identified as methylthio-phytanes. The major compound (peak 22) shows a mass spectrum with a molecular ion at m/z 328, major sulphur containing fragment ions at m/z 103 and m/z 299 ($M^{+\cdot}$-29), two series of hydrocarbon fragment ions (C_nH_{2n+1} and C_nH_{2n-1}), and fragment ions at m/z 280 ($M^{+\cdot}$-CH_3SH) and m/z 281 ($M^{+\cdot}$-CH_3S) (Fig. 9c). The fragment ions at m/z 103 and m/z 299 are indicative of a methylthio group at the C-3 position of the phytanyl carbon skeleton. Therefore, this major compound was tentatively identified as 3-(methylthio)-phytane.

The positions of the methylthio groups in the other relatively minor methylthio-phytanes were deduced from the m/z values of the major sulphur-containing fragment ions shown in their mass spectra and from their relative retention times. The compound giving rise to peak 21 (Fig. 8a) exhibits major fragment ions at m/z 271 and m/z 117 in its mass

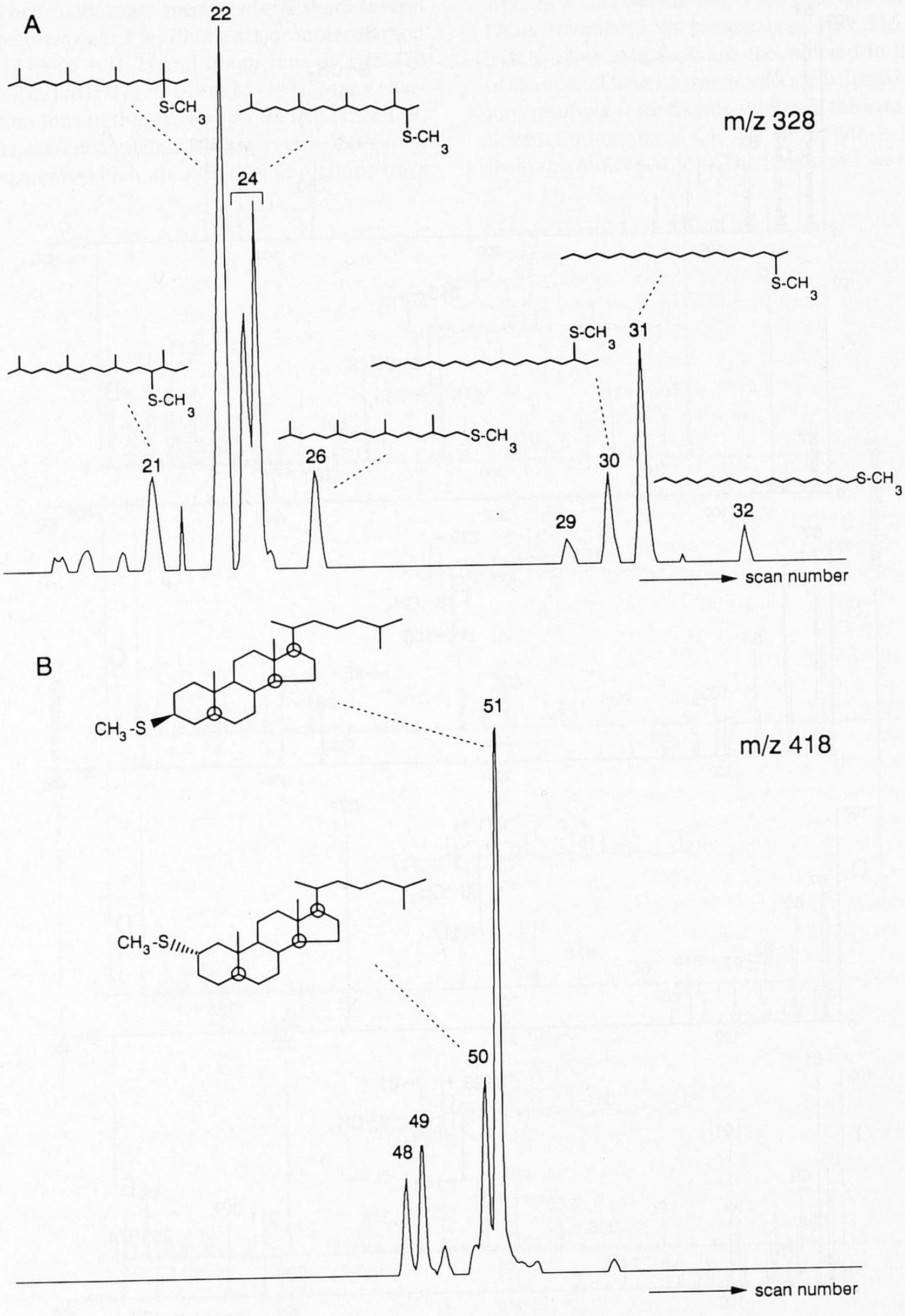

FIG. 8. (a) Partial mass chromatogram of m/z 328 showing the distribution of the C_{20}-methylthio-alkanes formed after MeLi/MeI treatment of the VDG-4A polar fraction. (b) Partial mass chromatogram of m/z 418 showing the distribution of the methylthio-cholestanes formed after MeLi/MeI treatment of the VDG-4A polar fraction. Identifications of labeled compounds are given in Table 3.

spectrum (Fig. 9d) which suggested that this compound is 4-(methylthio)-phytane. The two compounds (peak 24; Fig. 8a) eluting just after 3-(methylthio)-phytane have identical mass spectra (e.g. Fig. 9b), showing a base peak at m/z 75 and a characteristic fragment ion at m/z 313 (M^{+}-15). Therefore, these compounds were assigned as stereoisomeric 2-(methylthio)-phytanes. The structure of the last eluting methylthio-phytane (peak 26, Fig. 8a) was tentatively assigned as 1-(methylthio)-phytane because of the major m/z 61 fragment ion in its mass spectrum (Fig. 9a). It should be emphasised that the relative distribution of these various methylthio-phytanes displayed by the m/z 328 mass chromatogram

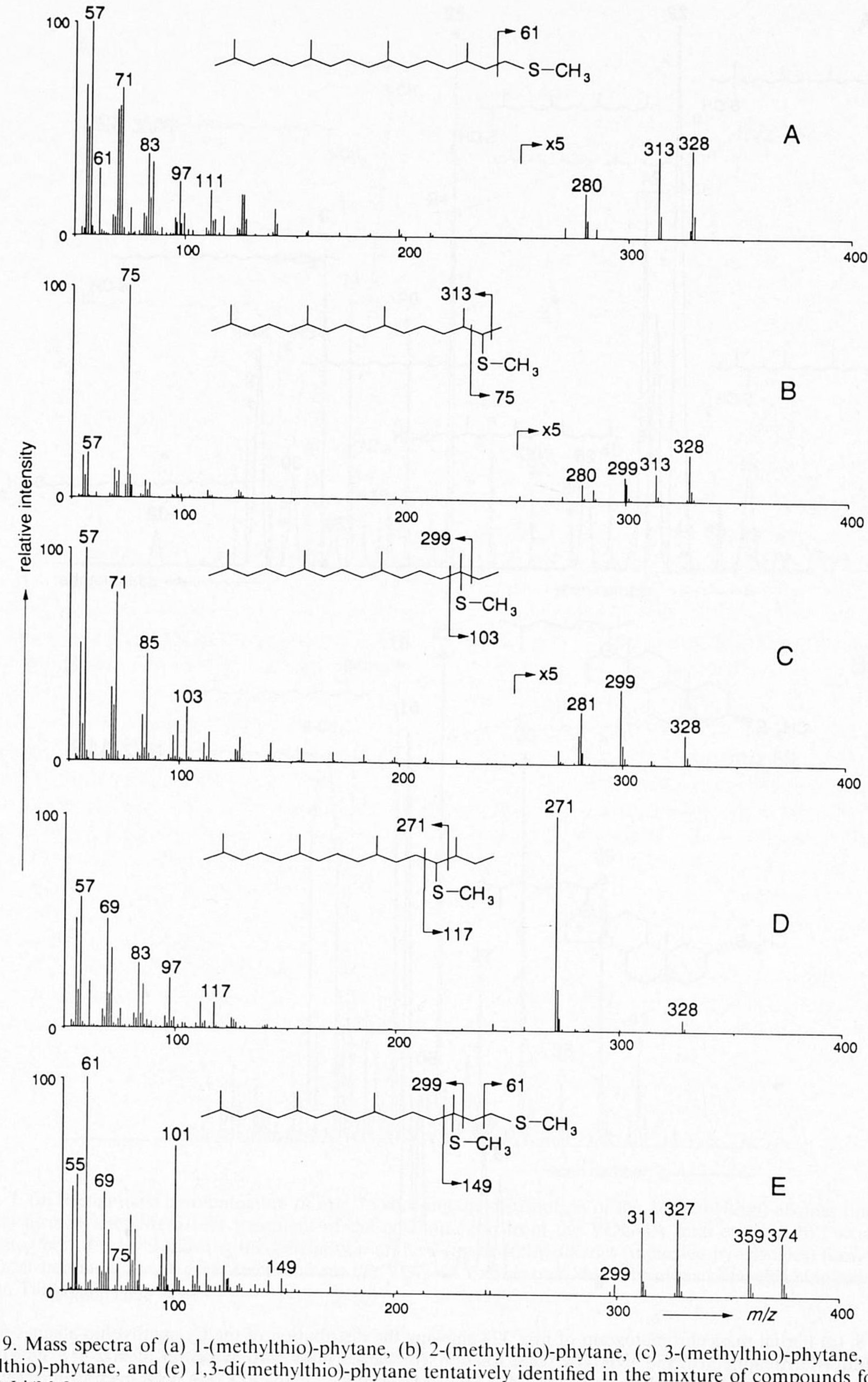

FIG. 9. Mass spectra of (a) 1-(methylthio)-phytane, (b) 2-(methylthio)-phytane, (c) 3-(methylthio)-phytane, (d) 4-(methylthio)-phytane, and (e) 1,3-di(methylthio)-phytane tentatively identified in the mixture of compounds formed after MeLi/MeI treatment of the polar fraction (VDG-4A).

is biased since the relative intensity of the $M^{+\cdot}$ ion in the mass spectra of the different structural isomers varies considerably. From the gas chromatogram of Fraction II it is evident that 3-(methylthio)-phytane is by far the most abundant isomer (Fig. 5a).

Mono(methylthio)-steranes

Major compounds in Fraction II (peaks 48–56; Fig. 5a) are tentatively identified as mono(methylthio)-steranes on the basis of the Raney Ni desulphurisation results and mass

spectral data. They show mass spectra which share several characteristics features (e.g., Fig. 10a): a major molecular ion at m/z 418 + 14 · n (n = 0–2) and major ions at m/z 370 + 14 · n ($M^{+\cdot}$-48) and m/z 371 + 14 · n ($M^{+\cdot}$-47). Many characteristic fragment ions in these mass spectra (e.g., m/z 149, 151, and 217) are also encountered in mass spectra of steranes. In addition, fragments which are offset by 46 daltons (m/z 149, 217, 232 versus m/z 195, 263, 278) are also present. Other fragment ions present (e.g., m/z 215, 230, 257, and 316 + 14 · n, n = 0–2) are encountered in the mass spectra of sterenes. These fragment ions are believed to be secondary ions resulting from fragmentation of the ions that are formed upon elimination of CH_3SH (m/z 370 + 14 · n, n = 0–2) from the molecular ion. The fragment ions at m/z 263 (m/z

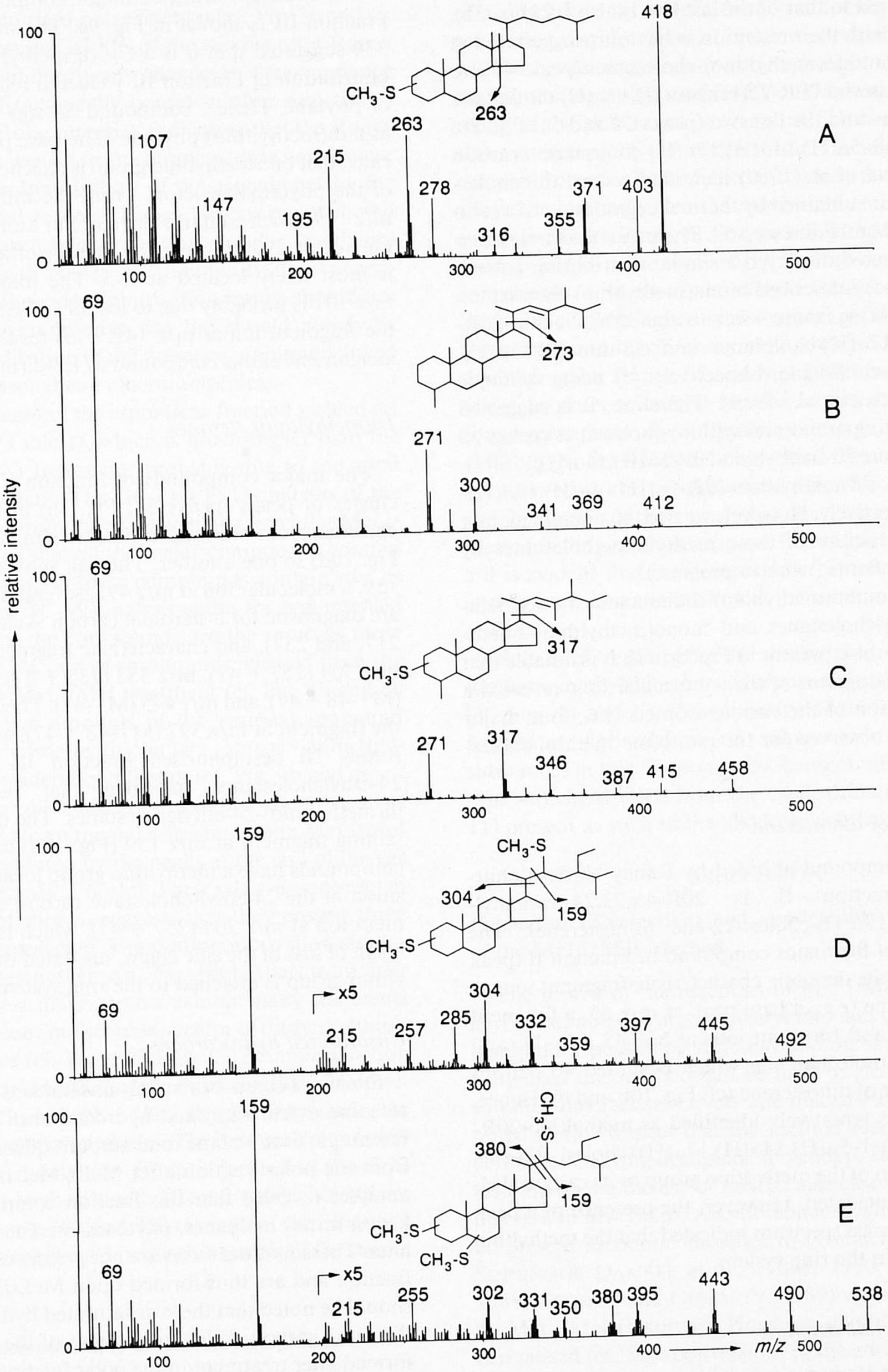

FIG. 10. Mass spectra of (a) 3β-methylthio-20R-5α(H),14α(H),17α(H)-cholestane, (b) dinosterene, (c) methylthiodinosterene, (d) di(methylthio)-24-ethylcholestane, and (e) tri(methylthio)-24-ethylcholestane.

217 + 46) and m/z 195 (m/z 149 + 46) indicated that the methylthio group is located in the A/B ring.

The presence of four major mono(methylthio)-cholestanes in Fraction II is illustrated by a partial mass chromatogram of their molecular ion m/z 418 (Fig. 8b). Desulphurisation of Fraction II afforded 20R-5β(H),14α(H),17α(H)-cholestane and 20R-5α(H),14α(H),17α(H)-cholestane in a 1:2 ratio. The relative abundance of the first two eluting methylthio-cholestanes compared to that of the last two is also 1:2 (Fig. 3b). This, together with their retention behaviour, suggested that the first eluting mono(methylthio)-cholestanes (peaks 48 and 49; Fig. 8b) possess a 20R-5β(H),14α(H),17α(H)-cholestane carbon skeleton, and the last two (peaks 50 and 51; Fig. 8b) possess a 20R-5α(H),14α(H),17α(H)-cholestane carbon skeleton. MYCKE et al. (1989) identified several thiocholestanes in a mixture obtained by thermal degradation of a resin fraction of the Rozel Point seep oil. The major thiocholestanes (4 isomers) released displayed a similar distribution pattern as that of the above-described mono(methylthio)-cholestanes. The last two eluting isomers were assigned as 2α-thio-20R-5α(H),14α(H),17α(H)-cholestane and 3β-thio-20R-5α(H),14α(H),17α(H)-cholestane, respectively, by using synthetic standards (MYCKE et al., 1989). Therefore, it is suggested that the last eluting mono(methylthio)-cholestanes (peaks 50 and 51, Fig. 8b) are 2α-methylthio-20R-5α(H),14α(H),17α(H)-cholestane and 3β-methylthio-20R-5α(H),14α(H),17α(H)-cholestane, respectively. However, we need to unambiguously elucidate the structure of these methylthio-cholestanes by synthesis of standards (work in progress).

Apart from mono(methylthio)-cholestanes, mono(methylthio)-24-methylcholestanes and mono(methylthio)-24-ethylcholestanes are also present in Fraction II. It is notable that the mass chromatograms of their molecular ions revealed a similar distribution of the various isomers (i.e., four major isomers) as that observed for the mono(methylthio)-cholestanes.

Mono(methylthio)-dinosterene

The major compound afforded by Raney Ni desulphurisation of Fraction II is 20R-4α,23,24-trimethyl-5α(H),14α(H), 17α(H)-cholest-22-ene (dinosterene). The mass spectrum of the major compound in Fraction II (peak 56; Fig. 5a) exhibits the same characteristic fragment ions as that of dinosterene (e.g., a base peak at m/z 69, a fragment ion at m/z 271, and fragment ions at $M^{+\cdot}$-43, $M^{+\cdot}$-71, and $M^{+\cdot}$-112) and a molecular ion which is shifted 46 daltons compared to that of dinosterene (cf. Fig. 10b and c). Hence, this compound is tentatively identified as methylthio-20R-4α,23,24-trimethyl-5α(H),14α(H),17α(H)-cholest-22-ene. The exact position of the methylthio group in its carbon skeleton is not yet elucidated. However, the presence of a peak at m/z 317 in its mass spectrum indicated that the methylthio group is located in the ring system.

Mono(methylthio)-dinosterane

Compound 57 (Fig. 5a) shows a mass spectrum with a molecular ion at m/z 460, a major fragment ion at m/z 414 ($M^{+\cdot}$-48), and a series of fragment ions also encountered in that of dinosterane, including the ion at m/z 98 with the characteristic enhanced intensity (SUMMONS et al., 1987). Consequently, this compound is tentatively assigned as a mono(methylthio)-dinosterane.

Di(methylthio)-phytane

The mass spectrum of major compound 38 (Fig. 5b) in Fraction III is shown in Fig. 9e. The molecular ion at m/z 374 suggested that it is a C_{20} di(methylthio)-alkane. Desulphurisation of Fraction III yielded a relatively high amount of phytane. Hence, compound 38 was tentatively assigned as a di(methylthio)-phytane. The base peak at m/z 61 indicated that one methylthio group is attached at the C-1 position of the phytanyl skeleton. From the minor fragment ion at m/z 149 which contains two sulphur atoms and the fragment ion at m/z 299, it is inferred that the other methylthio group is most likely located at C-3. The major fragment ion at m/z 101 is probably due to loss of CH_3SH (48 daltons) from the fragment ion at m/z 149. This resulted in the tentative assignment of this compound as 1,3-di(methylthio)-phytane.

Di(methylthio) steranes

The major compounds of Fraction III giving rise to the cluster of peaks (peaks 58; Fig. 5b) at the end of the gas chromatogram of Fraction III show similar mass spectra (e.g., Fig. 10d) to one another. They all show a base peak at m/z 159, a molecular ion at m/z 492, several fragment ions which are diagnostic for a steroidal carbon skeleton (e.g., m/z 215, 217, and 257), and characteristic fragment ions at m/z 285, m/z 304 (257 + 47), m/z 332 (285 + 47), m/z 333, m/z 397 ($M^{+\cdot}$-48 − 47), and m/z 445 ($M^{+\cdot}$-47). Their molecular weight, the fragment at m/z 397 ($M^{+\cdot}$-48 − 47), and the fact that the Raney Ni desulphurised Fraction III is dominated by 24-ethylcholestanes indicated that they are probably di(methylthio)-24-ethylcholestanes. The major sulphur-containing fragment at m/z 159 (Fig. 10d) indicated that these compounds have a methylthio group located at the C-22 position of the 24-ethyl-cholestane carbon skeleton. The fragment ion at m/z 304 (257 + 47), which is thought to be the result of loss of the side chain, suggested that the other methylthio group is attached to the ring system.

Unsaturated hydrocarbons

Fraction I comprises mainly unsaturated hydrocarbons and to some extent saturated hydrocarbons. This fraction represents ca. 6 wt% of the total amount of compounds released from the polar fraction after MeLi/MeI treatment. GC-MS analyses revealed that this fraction comprises mainly *n*-alkenes, minor *n*-alkanes, phytenes, sterenes, and minor steranes. These hydrocarbons are not present as such in the polar fraction and are thus formed upon MeLi/MeI treatment. It should be noted that these unsaturated hydrocarbons possess the same carbon skeletons as those of the methylthioethers formed after treatment of the polar fraction with MeLi/MeI. Therefore, it is likely that these compounds are formed by elimination reactions during the treatment with MeLi/MeI. Such elimination reactions were not observed in the exper-

iments with the model compounds, which is, at present, not completely understood.

Asphaltenes

Raney Ni desulphurisation of the asphaltene fraction yielded a mixture of hydrocarbons (ca. 1 wt% of asphaltene fraction; Table 1) comprising the same compounds as observed in the desulphurised polar fraction (cf. Fig. 3b and c). However, flash pyrolysis-GC-MS of the residue after Raney Ni treatment revealed hardly any changes in the abundance and composition of organically bound sulphur moieties as compared to the starting material, indicating that the Raney Ni desulphurisation was far from complete (data not shown). The incomplete desulphurisation of the asphaltenes by the heterogenic dispersed reactant Raney Ni can be attributed to the poor solubility of these macromolecules in ethanol (the solvent used with Raney Ni desulphurisation experiments). Therefore, these desulphurisation results should not be interpreted in a quantitative way but should merely be considered as an indication of the presence of sulphur-linked biomarkers in the asphaltene macromolecules.

MeLi/MeI treatment of the asphaltene fraction yielded ca. 9.1 wt% products (Table 1), which is much higher than the yield after Raney Ni treatment, probably due to the good solubility of this reagent. The GC-FID/FPD analyses of the compounds released from the asphaltene fraction after MeLi/MeI reaction showed that all the major compounds contain sulphur and that some of these compounds contain two or three sulphur atoms. GC-MS analysis of this fraction revealed that the majority of the compounds are the same as those encountered in the fraction of compounds released from the polar fraction after MeLi/MeI treatment (cf. Fig. 4b and c). However, the relative amounts of the various compound classes (e.g., mono(methylthio)ethers versus di(methylthio)ethers) are considerably different (cf. Fig. 4b and c).

The compounds which are not detected in the mixture of compounds formed from the polar fraction upon MeLi/MeI treatment are represented by the peaks at the end of the gas chromatogram (peak 59; Fig. 4c). Their relative response on the FPD trace (not shown) suggests that they possess three sulphur atoms per molecule. These compounds show similar mass spectra to one another (e.g., Fig. 10e). Apart from their molecular ion, their mass spectra exhibit many fragments which are also present in the mass spectra of di(methylthio)-24-ethylcholestanes (cf. Fig. 10d and e). The molecular ion is 46 daltons higher than that of the di(methylthio)-24-ethylcholestanes, which indicates that these compounds are tri(methylthio)-24-ethylcholestanes. The base peak at m/z 159 indicates that one methylthio group is located at the C-22 position in analogy to the di(methylthio)-24-ethylcholestanes. The fragments at m/z 380 and 350 reveal that the other two methylthio groups are located in the ring system of the steroid carbon skeleton.

Sulphur-rich Macromolecules in the Alkylsulphide Fraction

The most polar sub-fraction (alkylsulphide fraction) of the "apolar" fraction of the bitumen was found to contain a high concentration of material which revealed its presence as an unresolved envelope in the gas chromatogram (Fig. 11). GC-FID/FPD analysis (not shown) indicated that this "hump" comprises sulphur-containing compounds. Mass chromatograms of m/z values, e.g., 57 and 217, diagnostic for aliphatic compounds and steranes, respectively, mimicked the shape of the "hump" as displayed in the TIC-trace (Fig. 12). Since the fractionation procedure employed precluded the presence of hydrocarbons as such in the alkylsulphide fraction, it was concluded that the "hump" is due to thermal degradation of sulphur-containing high–molecular-weight material during gas chromatographic analysis. VOLKMAN et al. (1986) also reported the presence of a "sulphur-rich hump" in the gas chromatograms of the hydrocarbon fractions of sediment samples from Ace Lake. In order to study the nature of these alleged macromolecules the alkylsulphide fraction was desulphurised with Raney Ni and treated with MeLi/MeI (Table 1). It should be noted that apart from high–molecular-weight compounds, low–molecular-weight OSC such as alkylthiolanes and alkyldithianes are also present in this fraction. The latter compounds will also yield hydrocarbons and methylthioethers after Raney Ni and MeLi/MeI treatment, respectively. In order to correct for this an internal standard was added prior to the chemolysis experiments.

Raney Ni desulphurisation yielded the same compounds as observed in the desulphurised polar fraction (cf. Fig. 3a and b). It should be noted that the "hump" is no longer present in the gas chromatogram of this fraction. From Table 5 it is evident that the compounds formed after desulphurisation are mainly derived from the sulphur-rich macromolecules.

MeLi/MeI treatment of the alkylsulphide fraction yielded the same methylthioethers as reported for the polar fraction (cf. Fig. 4a and b). The majority of the methylthioethers formed are derived from the sulphur-rich macromolecular substances in this fraction; a few compounds, e.g., 34 and 37 (Fig. 4a), are derived from the cyclic dithianes (f and g; Fig. 11) present as such in the alkylsulphide fraction.

DISCUSSION

Evaluation of Selectivity and Applicability of the MeLi/MeI Method

The results of the reactions of MeLi/MeI with synthetic acyclic mono-, di-, and tetrasulphides and cyclic mono- and disulphides possessing different carbon skeletons (Fig. 2B) show that this method can be used to cleave di- and polysulphide linkages selectively and quantitatively. It should be noted in this respect that the method reported by SCHMID (1986) for cleaving disulphide moieties in sulphur-containing resin involving the use of $LiAlH_4$ also cleaves monosulphide linkages in low yields. Alkylthiophenes which are known to be present as bound moieties in geomacromolecules (e.g., SINNINGHE DAMSTÉ et al., 1988a, 1990; PAYZANT et al., 1988; GEORGE and GORBATY, 1989) were also not affected by MeLi/MeI treatment. However, alkylthiols are partially derivatised (ca. 80% conversion) to the corresponding methylthioethers by treatment with MeLi/MeI. This signifies that methylthio groups in MeLi/MeI chemolysis products released from fractions containing macromolecules may not be ex-

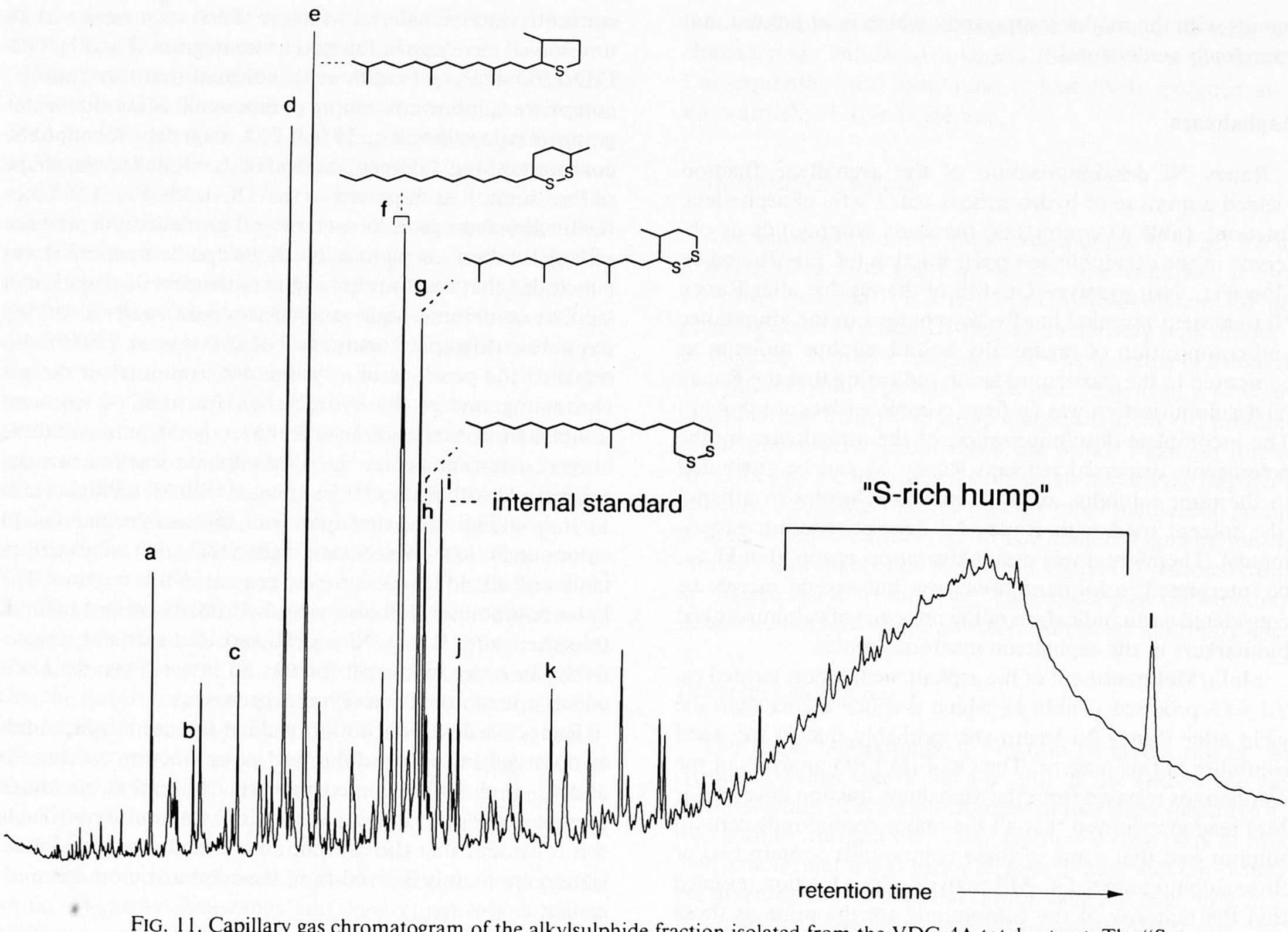

FIG. 11. Capillary gas chromatogram of the alkylsulphide fraction isolated from the VDG-4A total extract. The "S-rich hump" is due to thermal decomposition of sulphur-rich high–molecular-weight material in the capillary column. Identifications of labeled compounds are given in Table 4.

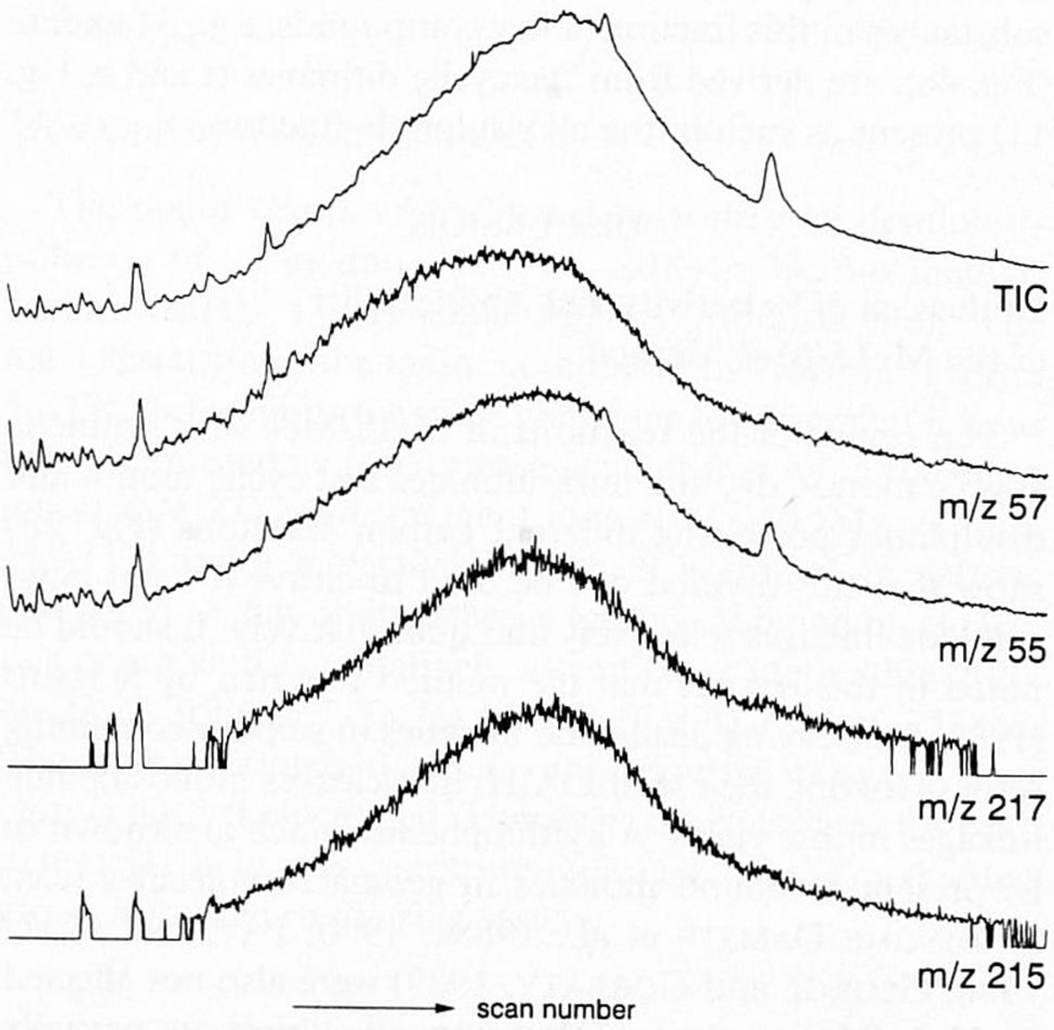

FIG. 12. GC-MS analysis of the "sulphur-rich hump" showing that the partial mass chromatograms of m/z 55, 57, 215, and 217 are mimicking the shape of the hump displayed in the partial TIC trace. The "hump" is due to thermal degradation of sulphur-containing high–molecular-weight material during gas chromatographic analysis.

clusively derived from di- or polysulphide linkages but may also be derived from free thiol groups. However, the possibility that mono(methylthio)ethers formed upon treatment of polar and asphaltene fractions are derived from non-bound alkylthiols can be ruled out since no low–molecular-weight alkylthiols are present in these fractions using the fractionation procedure described. In the case of compounds with two methylthio groups the possibility remains that one methylthio group is derived from a free thiol group present in the di(poly)sulphide-bound unit. In order to discriminate these origins a method which derivatises thiol groups exclusively to the corresponding deuterated methylthioethers can be employed prior to MeLi/MeI treatment as shown in this study. In summary, it can be concluded that the MeLi/MeI chemical degradation method is highly appropriate to identify the presence of di- and polysulphide linkages in soluble geomacromolecules.

Apart from recognition of di- and polysulphide linkages in macromolecules, the usage of this MeLi/MeI method also provides information on the positions of di- or polysulphide bonds between the released molecule and the macromolecular matrix. This knowledge leads to a better insight into the structure of sulphur-rich geomacromolecules, which in turn contributes to a better understanding of their formation during early diagenesis. Because this method does not cleave C-

Table 5. Relative abundance of selected compounds in the alkylsulphide fraction and in the Raney Ni desulphurised alkylsulphide fraction

	alkylsulphide fraction		Raney Ni treated alkylsulphide fraction	
	peaks integrated (Fig. 11)	relative amount*	peaks integrated (Fig. 4A)	relative amount*
compounds possessing an n-C_{18} carbon skeleton	d and f	4.7	peak labeled 18	6.3
compounds possessing a phytane carbon skeleton	e, h and g	4.9	A	12.4
compounds possessing a cholestane carbon skeleton	-	0	D and E	14.8

*(peak area compound(s))/(peak area internal standard)

S bonds, the location of the di- or polysulphulphide linkages is preserved by the position of the methylthio group in the released structural unit. Electron impact–induced mass spectrometric fragmentation reactions of the so-produced methylthioethers are preferentially related to cleavages associated with the methylthio group (e.g., Fig. 9). This enables in many cases a facile determination of the position of the methylthio group in the carbon skeleton by mass spectrometry. The $LiAlH_4$ method (SCHMID, 1986) used for cleaving disulphide moieties in sulphur-containing resins releases the disulphide-linked compounds as thiols. The mass spectra of alkylthiols are in many cases significantly less diagnostic with respect to the position of the thiol group than those of the corresponding methylthioethers. Upon electron impact–induced fragmentation hydrogen sulphide is preferentially eliminated from alkylthiols in analogy to dehydration of alcohols (BUDZIKIEWICZ et al., 1965). The positions of the double bonds in the thus formed alkene fragments, which are informative on the position of the thiol group in the parent molecule, are difficult to determine by mass spectral analysis. It is possible to overcome this problem since the formed thiols can be derivatised to methylthioethers, but such a second reaction step will introduce the possibility of oxidation or elimination of the thiol groups.

Structure of Sulphur-rich Geomacromolecules

The structure of sulphur-rich resins and asphaltenes in immature crude oils and bitumens has been studied in some detail (SCHMID, 1986; SINNINGHE DAMSTÉ et al., 1988b, 1989a, 1990; PAYZANT et al., 1988; GEORGE and GORBATY, 1989; MYCKE et al., 1989; KELEMEN et al., 1990). SCHMID (1986) and SINNINGHE DAMSTÉ et al. (1988b, 1990) concluded that the compounds released from a resin fraction upon Raney Ni desulphurisation are the building blocks of the macromolecular network which are connected to each other by one or more sulphur linkages. MYCKE et al. (1989) used deuterated Raney Ni, which yielded information on the number of sulphur-bridges and also to some extent indicated the position(s) of the sulphur-bridge(s) in the structural units. Flash Py-GC-MS studies also provided information on the carbon skeletons of the sulphur-bound structural units of sulphur-rich resins (SINNINGHE DAMSTÉ et al., 1990). Moreover, evidence was presented that in addition to intermolecular (acyclic) sulphur linkages, intramolecular (cyclic) sulphur linkages (i.e., thiolanes, thianes, and thiophenes) are also present in sulphur-rich resins. The analyses of resin fractions using flash Py-GC-MS employing different flash pyrolysis temperatures provided circumstantial evidence that some units are bound with multiple sulphur linkages (SINNINGHE DAMSTÉ et al., 1990).

Usage of flash Py-GC-MS (SINNINGHE DAMSTÉ et al., 1989a) and off-line pyrolysis with subsequent GC-MS analyses of the trapped pyrolysate (PAYZANT et al., 1988) provided evidence for the presence of benzothiophene, thiophene, and, to some extent, thiolane moieties in asphaltenes. GEORGE and GORBATY (1989) used X-ray spectroscopy in the characterisation of an asphaltene and found evidence for the presence of both thiophene and sulphide moieties.

In summary, the analytical techniques described above fail to characterise properly the intermolecular sulphur bridges (mono-, di-, or polysulphide) in sulphur-rich geomacromolecules. Furthermore, knowledge about the exact positions of the sulphur linkages in sulphur cross-linked units is also fairly limited.

Raney Ni desulphurisation of the alkylsulphide-, polar-, and asphaltene fractions of the Vena del Gesso sample yielded the same hydrocarbons for all fractions, although with small variations in their distribution patterns (Fig. 3). This shows that the sulphur-bound structural units in the three macromolecule-containing fractions are similar. These structural units apart from the major dinosterene have been reported to occur in sulphur-rich resins (SCHMID, 1986; SINNINGHE DAMSTÉ et al., 1988b, 1990; DE LEEUW and SINNINGHE DAMSTÉ, 1990).

The products released from the alkylsulphide, polar, and asphaltene fractions after MeLi/MeI treatment make up a considerable portion of the total amount of sulphur-linked compounds (e.g., for the polar fraction, ca. 50%; Table 1). It is self-evident that they possess the same carbon skeletons as the products obtained by Raney Ni desulphurisation (cf. Figs. 3 and 4). The compounds released possess one to three methylthio groups, which shows that they are bound via one or more di- or polysulphide linkages, and the sulphur-linked compounds not released are consequently bound with at least one monosulphide linkage and possibly with (an) additional (poly)sulphide linkage(s). The relative abundance of compounds possessing more than one methylthio group, in particular those with a 24-ethylcholestane carbon skeleton, increases significantly from the alkylsulphide, to the resin, up

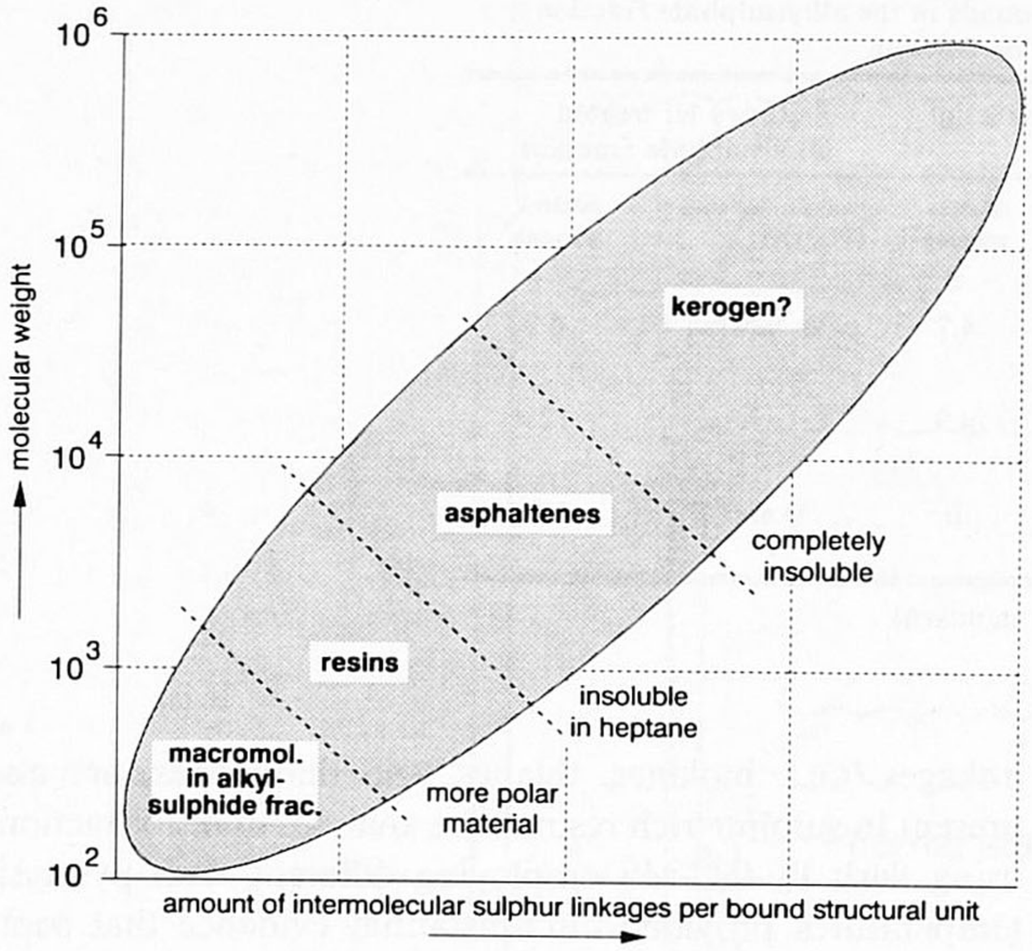

FIG. 13. Relationship between the degree of intermolecular sulphur cross-linking and molecular weight for sulphur-rich macromolecules (modified after SINNINGHE DAMSTÉ et al., 1990).

to the asphaltene fraction (Fig. 4). These compounds can be considered as "cross-links" in the macromolecule, whereas the mono(methylthio) compounds are end groups. Although the average amount of mono-sulphide linkages per bound structural unit is still unknown, this varying relative abundance of units bound with more than one di- or polysulphide linkage indicates significant differences in the degree of sulphur cross-linking of the three types of macromolecules. It is thought that there is a direct relationship between the degree of intermolecular sulphur cross-linking and the molecular weight and solubility of sulphur-rich macromolecules (Fig. 13; SINNINGHE DAMSTÉ et al., 1990). This paper presented direct evidence at a molecular level for this hypothesis. The most sulphur cross-linked macromolecules, i.e., the asphaltenes, are the least soluble. On the other hand, the macromolecules in the alkylsulphide fraction which are the least sulphur cross-linked, are the most soluble. The relative abundance of "cross-links" in the alkylsulphide, the resin, and the asphaltene fractions suggests that the numbers of structural units connected to each other are limited to 2 or 3, 3 to 5, and 5 to 7, respectively. In the case of the resins this results in an estimation of the molecular weight which is in the same range as the average molecular weight (ca. 2000) of the sulphur-rich resins in the Rozel Point oil determined by gel permeation chromatography (MYCKE et al., 1989).

In summary, the alkylsulphide, polar, and the asphaltene fractions of the Vena del Gesso bitumen comprise sulphur-rich macromolecules which consist of structural units connected to each other at specific positions with mono-, di-, or polysulphide linkages. These units possess carbon skeletons identical to the well-known geologically occurring hydrocar-

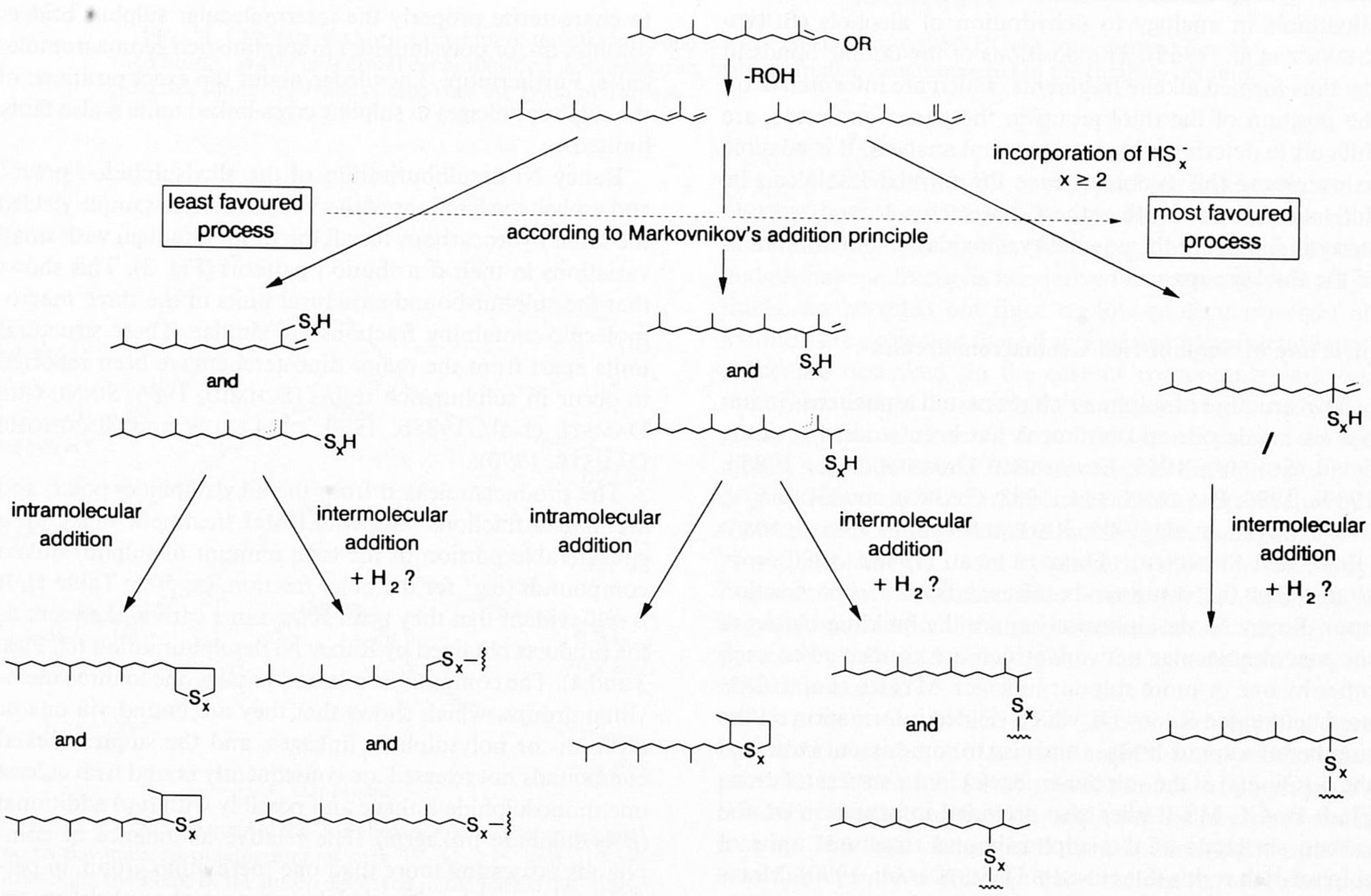

FIG. 14. Diagenetic scheme showing the possible origin of cyclic OSC and macromolecularly S-bound compounds possessing a phytane carbon skeleton.

bon biomarkers: e.g., *n*-alkanes, isoprenoid alkanes, steranes, and carotenoids. The structural differences of the macromolecules present in the three studied macromolecule-containing fractions are attributable solely to the degree of intermolecular sulphur cross-linking.

Origin of Sulphur-rich Geomacromolecules

With respect to the origin of sulphur-rich macromolecules it is thought that they are formed by intermolecular incorporation reactions of reduced sulphur species into low–molecular-weight functionalised biological lipids (BRASSELL et al., 1986; DE LEEUW and SINNINGHE DAMSTÉ, 1990; SINNINGHE DAMSTÉ et al., 1988b, 1989a,b, 1990). Consequently, the position of the sulphur linkages is determined by the position(s) of the functionality(ies) in the biological precursor molecules. The results of this study provide strong evidence for this hypothesis since the sulphur linkages are attached at specific positions of the structural units. For instance, the major phytanyl structural unit is linked at the C-3 position via a di- or polysulphide bond. This is interpreted as a reflection of reaction of chlorophyll-derived phytadienes with reduced sulphur species during early diagenesis (Fig. 14). Addition of HS_x^- to a double bond probably involves an intermediate carbocation. The preferentially formed ion will be the most substituted one (Markownikov's rule). The resulting sulphur compound (I) is thought to react with functionalities of other compounds resulting in the formation of high–molecular-weight substances ("natural vulcanisation"). On the other hand, providing that other suitable functionalities are present within the molecule, the intermediate sulphide will undergo an intramolecular reaction more readily than an intermolecular reaction. In the case of the reaction of chlorophyll-derived phytadienes with reduced sulphur species the C-S bond will preferentially form at the C-3 position (Fig. 14). A subsequent intramolecular reaction of compound I (Fig. 14) leading to the formation of a stable cyclic sulphur compound is not possible because of the unfavourable position of the remaining double bond. Consequently, the intermediate sulphur compound will react exclusively in an intermolecular fashion leading to a macromolecularly bound phytanyl moiety. In addition to the above-mentioned phytadienes, phytol-derived phytenals which have been encountered in sediments and particulate matter in the water column are also suitable precursors for the formation of sulphur-linked phytanyl moieties as encountered in the Vena del Gesso resin. Michael-addition of H_2S or HS_x^- to these compounds will also preferentially lead to a C-S bond at the C-3 position of the phytanyl moiety. However, this compound should subsequently lose its aldehyde functionality, in one way or the other.

In addition to these C-3 linked phytanyl structural units, phytanyl structural units that are linked to the macromolecule with two di- or polysulphides moieties at C-3 and C-1 are also present. Phytadienes cannot be considered as the precursors for these units since addition of sulphur to these dienes will not lead to a C-S bond preferentially at the C-1 position according the Markownikov's rule. On the other hand, a Sn_2-reaction of HS_x^- with a phytolester leads to the formation of a C-S bond at the C-1 position (DE LEEUW and SINNINGHE DAMSTÉ, 1990).

The major di- or polysulphide-linked *n*-alkyl moieties in the studied resin have the di- or polysulphide linkage at the C-2 position. This indicates that the precursors for these units are *n*-alk-1-enes which are most likely derived from dehydration of ubiquitously occurring *n*-alkanols. The presumed reaction of reduced sulphur species with *n*-alk-1-enes indicates that these abiogenic incorporation reactions also occur with isolated double bonds. Since these incorporation reactions take place under the mild conditions (low temperature) prevailing during the deposition of marine sediments, they require some sort of catalysis.

The major steroid structural units are linked at either the C-3 or the C-2 position with a di- or polysulphide moiety to a macromolecule. It is believed that this reflects a reaction of HS_x^- with Δ^2-sterenes which are formed after dehydration of the 3-OH group of 5α(H)-stanols. The 24-ethylcholestanyl structural units which are connected to a macromolecule with two di- and/or polysulphide moieties possess the additional linkage at the C-22 position. In sediments 24-ethylcholestadienes with one double bond at C-22 have been observed (e.g., MCEVOY et al., 1981). These compounds are suitable substrates for intermolecular HS_x^- incorporation leading to the formation of 24-ethylcholestanyl structural units which are macromolecularly bound with two di- or polysulphide moieties. Steric hindrance may explain why sulphur addition to the Δ^{22} double bond exclusively yields a C-S bond at the C-22 position.

CONCLUSIONS

1) The MeLi/MeI method is a suitable method to investigate di- or polysulphide-bound biomarkers in geomacromolecules.
2) MeLi/MeI treatment in combination with Raney Ni desulphurisation revealed that the macromolecule-containing fractions of the sulphur-rich bitumen from the Vena del Gesso shale (VD-4A) are in substantial part composed of monomers (biomarkers) with linear, branched, isoprenoid, steroid, hopanoid, and carotenoid carbon skeletons connected to each other by mono- and di- or polysulphide linkages at specific positions.
3) The different properties (solubility and molecular weight) of sulphur-rich macromolecules in the alkylsulphide, the polar, and the asphaltene fractions can be explained solely by differences in degree of sulphur cross-linking.
4) The di- or polysulphide-linked macromolecules are the result of incorporation reactions in an intermolecular fashion of inorganic polysulphides into low–molecular-weight functionalised biological lipids during the early stages of diagenesis.

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