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Sulphur-bound steroid and phytane carbon skeletons in geomacromolecules: Implications for the mechanism of incorporation of sulphur into organic matter

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Abstract—Sulphur-bound steroid and phytane moieties in macromolecules present in the polar fractions of six immature samples (both crude oils and sediment extracts) have been analyzed using S-selective chemolysis methods and analytical pyrolysis. The identifications of the methylthioethers released from the macromolecule-containing fractions after MeLi/MeI treatment are based on comparison of mass spectral data and chromatographic data with those for synthesized methylthioethers. Evidence is presented that di- or polysulphide linkages are present in geomacromolecules in both sediments and oils and that the location of di- or polysulphide linkages in macromolecularly S-bound moieties is the same as that of monosulphide linkages. Macromolecularly S-bound phytanyl moieties are chiefly bound with S linkages located at the tertiary positions of their carbon skeletons, which indicates that the S incorporation mechanism(s) involve(s) intermediate carbocations. The macromolecularly S-bound steroids are bound with S linkages located mainly at C-2, C-3, C-4, or C-5 of their carbon skeletons, which indicates that the S incorporation took place into sterenes or steradienes—the dehydration products of stanols and stenols, respectively. However, it remains possible that the macromolecularly S-bound steroids with an axial S linkage at C-3 are, in part, resulting from a S_N2 reaction of inorganic S species with steryl esters or stanols.

INTRODUCTION

IN RECENT YEARS, compelling evidence accumulated that inorganic S species (H_2S, HS_x^-) react with functionalized (bio)lipids during the early stages of diagenesis (for a review, see SINNINGHE DAMSTÉ and DE LEEUW, 1990). The widespread occurrence of low-molecular-weight organic S compounds (OSC) and S-bound moieties in geomacromolecules (TEN HAVEN et al., 1990; SINNINGHE DAMSTÉ and DE LEEUW, 1990; KOHNEN et al., 1991b) testifies to the ubiquity of this reaction. Numerous methods exist today which can release S-bound biomarkers from geomacromolecules present in polar, asphaltene, and kerogen fractions (ADAM et al., 1991; HOFMANN et al., 1992; KOHNEN et al., 1991b; RICHNOW et al., 1993; SCHMID, 1986; SINNINGHE DAMSTÉ et al., 1988, 1990a; TRIFILIEFF, 1987).

It has been demonstrated that the analysis of low-molecular-weight OSC and S-rich macromolecules in sediments and oils provides valuable geochemical information concerning the palaeoenvironment of deposition (SINNINGHE DAMSTÉ et al., 1989a,b, 1990b; KOHNEN et al., 1990b, 1991c). In a recent communication, we reported on the "reconstruction" of the precursors of OSC and macromolecularly S-bound moieties present in a Miocene bituminous marl from the Vena del Gesso Basin (KOHNEN et al., 1992a). The positions of functional groups (e.g., double bonds) in the alleged precursors (i.e., palaeobiochemicals or their diagenetic products) are assumed to correspond to the position(s) of the C-S bond(s) in the corresponding OSC and macromolecularly S-bound moieties (SINNINGHE DAMSTÉ et al., 1989a; KOHNEN et al., 1991b, 1992a). However, the mechanisms involved in the reaction of functionalized lipids with inorganic S species are still poorly understood. A better understanding of these reactions is required, since they control the ultimate position of a C-S bond in a sulphurized lipid. This positional information is judged valuable to "reconstruct" palaeobiochemicals and thus is of importance for retrieval of information concerning the palaeocommunity and the palaeoenvironmental conditions (KOHNEN et al., 1992a).

Here we report on the positions of C-S bonds in S-bound steroid and phytane moieties in macromolecules present in the polar fractions of a suite of organic S-rich sediment extracts and crude oils. By means of S-selective chemolysis, experiments using MeLi/MeI (KOHNEN et al., 1991b) data were obtained on the positions of di- or polysulphide linkages in macromolecularly S-bound steroid and phytane moieties. Analytical pyrolysis of the treated and untreated macromolecule-containing fractions revealed additional details on the positions of the monosulphide linkages. This study is focused on macromolecularly S-bound steroid and phytane moieties since they are widespread and their precursors are likely to be the common sterols and C_{20} isoprenoid alcohols or their diagenetic products (KOHNEN et al., 1992a). This enabled us to compare the positions of the functional group in the precursor with that of the S linkage in the product and hence to elaborate on the reaction mechanism(s) involved in the natural sulphurization process.

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EXPERIMENTAL

Samples and Geological Setting

The Northern Apennines Marl (NAM-1) is from Miocene strata in the Perticara Basin (Italy) which consist of gypsum deposits interbedded with bituminous marl layers. A 40-cm-thick marl layer interbedded between gypsum layers was sampled. The composition of the saturated hydrocarbon, "aromatic hydrocarbon," and polar fractions from another marl layer from the same outcrop in the Perticara Basin was described by TEN HAVEN et al. (1985) and SINNINGHE DAMSTÉ et al. (1986, 1989b, 1990a). Examination of these fractions from the extract of this sample indicated, however, that it is rather different in composition (M. E. L. Kohnen, unpubl. data).

Two bituminous marl samples (4A and 7A3) from the Vena del Gesso Basin in the Northern Appennines (Italy) were taken from fresh outcrops of different marly layers. The geological setting of this Messinian (Upper Miocene) basin is described in detail by VAI and RICCI LUCCHI (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. A detailed geochemical description of the composition of the saturated hydrocarbon, the "aromatic hydrocarbon," polar and asphaltene fractions of the Vena del Gesso Marl-4A and, in less detail, of the Vena del Gesso Marl-7A3 is given in KOHNEN et al. (1991a,b,c, 1992a,b). The immature character of the organic matter of these nonevaporitic bituminous marls is illustrated by the low reflectance (average R_0 = 0.25%) of the trace indigenous vitrinite particles in Vena del Gesso Marl-4A.

The Rozel Point Oil (RPO) is from a shallow reservoir in the northwestern part of Utah (USA). Its source rock is thought to be a playa lake deposit (MEISSNER et al., 1984). Bulk properties of the oil and a detailed characterization of both the saturated hydrocarbon and the "aromatic hydrocarbon" fractions have been reported by SINNINGHE DAMSTÉ et al. (1987, 1989b) and TEN HAVEN et al. (1988).

The Sicily Seep Oil (SSO-E2) seeps out of Upper Miocene marl layers interbedded with evaporites deposited in an environment analogous to that described for the Northern Apennines Marl (TEN HAVEN et al., 1985, 1988). The geological setting and a description of the saturated hydrocarbon and the "aromatic hydrocarbon" fractions are given by PALMER and ZUMBERGE (1981), TEN HAVEN et al. (1988), SINNINGHE DAMSTÉ et al. (1989b), and DE LEEUW and SINNINGHE DAMSTÉ (1990).

The Chinese oil (Jianghan Oil B-1) is from a shallow reservoir (ca. 600 m) in the Jianghan Basin. This immature oil is thought to be derived from a hypersaline lacustrine source rock. The location of the well site and a description of the saturated hydrocarbon and the "aromatic hydrocarbon" fractions are presented by SHENG et al. (1987), BRASSELL et al. (1988), and SINNINGHE DAMSTÉ et al. (1989b).

Extraction and Fractionation

The sediment samples were freeze-dried and subsequently 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. The extract was taken up in CH_2Cl_2 and was washed with water using a separatory funnel. The water-layer was re-extracted four times 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 or the oil samples in a minimum volume CH_2Cl_2 and subsequently adding a 40-fold excess of *n*-heptane. After 8 h the asphaltenes flocculated completely and the precipitate (asphaltene fraction) and supernatant (maltene fraction) were separately collected. 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) and 150 mL methanol/CH_2Cl_2 (1:1, v/v; the polar fraction).

Raney Nickel Desulphurisation/Hydrogenation

Several fractions were desulphurized with Raney Ni and subsequently hydrogenated as described elsewhere (SINNINGHE DAMSTÉ et al., 1988). The hydrocarbons formed were isolated from the reaction mixture using column chromatography over Al_2O_3 and were subsequently analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

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

Polar fractions were treated with MeLi/MeI as described previously (KOHNEN et al., 1991b). After reaction with MeLi/MeI, the polar fractions were separated into an apolar fraction (released compounds) and a polar fraction (residue) using column chromatography as described above.

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 S-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 programmed to 130°C at 10°C/min, and then at 4°C/min to 320°C, at which it was held for 40 min.

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). The carrier gas was helium. The samples were injected at 50°C, and the oven was programmed to 130°C at 20°C/min, and then at 4°C/min to 300°C, where it was held for 10 min.

Curie-Point Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS)

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 were the same as described above for GC-MS. The samples, dissolved in CH_2Cl_2, were applied to a ferromagnetic wire with a Curie temperature of 610°C using a syringe, and the solvent was evaporated. 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). The carrier gas was helium.

Synthesis of Reference Compounds

1-(Methylthio)-phytane and 3-(methylthio)-phytane were prepared as follows. To a stirred solution of phytol (3 mmol) in 5 mL dry tetrahydrofuran, 4 mmol of BuLi (2.5 M in hexane) was slowly added at −70°C under a nitrogen atmosphere. After slowly raising the temperature to 0°C, 3 mmol CS_2 was added and stirring was continued for 45 min. Subsequently, 4 mmol CH_3I was added at room temperature and the solution was stirred for 3 h (RAUTENSTRAUCH, 1971; TAGUCHI and NAKAO, 1972). The initially formed methylated dithiocarbonate derivative underwent a sigmatropic rearrangement to give 3-[thio(methylthio)carbonyl]-phytane. After addition of water, the reaction mixture was extracted several times with diethyl ether. The combined diethyl ether extracts were washed three times with water and once with NaCl-saturated water, dried on $MgSO_4$, and evaporated to dryness yielding a yellow liquid. To an aliquot of this crude product mixture (290 mg) dissolved in 1 mL

hexane, 1.2 mL of a 1 M NaOEt solution was added at 15°C, and after stirring for 30 min. 100 μL CH_3I was added. After stirring for an additional 1.5 h, 10 mL NH_4Cl-saturated water was added, and the reaction mixture was extracted several times with hexane. The extract was dried on $MgSO_4$ and evaporated to dryness (yield 241 mg) and subsequently hydrogenated in 20 mL ethyl acetate and 1 mL acetic acid with 140 mg PtO_2 under hydrogen. An additional batch of PtO_2 (140 mg) was added after 48 h and stirring was continued for 24 h. The reaction mixture was filtered and the PtO_2 was washed several times with hexane. The obtained reaction mixture was subsequently washed twice with $NaHCO_3$-saturated water and twice with NaCl-saturated water, dried on $MgSO_4$, and evaporated to dryness yielding a colourless oil (yield 99 mg) which contained 1-(methylthio)-phytane in addition to the expected 3-(methylthio)-phytane. The methylthio-phytanes were isolated from the reaction mixture by preparative thick layer chromatography [SiO_2; hexane/ethyl acetate (98:2, v/v) as developer; R_f = 0.66–0.74] and subsequently separated from each other by HPLC on a preparative octadecyl-silane bonded-phase column (25 cm × 8 mm; polygoSil60-C_{18}; 10 μm) using methanol/water (93/7, v/v) as an eluent (4 mL/min), which afforded 10.5 mg 1-(methylthio)-phytane (overall yield ca. 4%) and 3.6 mg 3-(methylthio)-phytane [overall yield ca. 1%; both ca. 99% pure (GC)].

^{1}H- and ^{13}C-NMR spectra of both the 1-(methylthio)-phytane and the 3-(methyl)-phytane confirmed their structures: ^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 1-(methylthio)-phytane, 0.83–0.90 (m, 15H (C-16–C-20 CH_3's)), 1.00–1.46 (m, 22H (C-3–C-15)), 1.47–1.58 (m, 2H (C-2)), 2.10 (s, 3H(S-CH_3)), 2.42–2.58 (m, 2H(C-1)); 3-(methylthio)-phytane, 0.83–0.88 (m, 12H (C-16, C-18–C-20 CH_3's)), 0.92 (t, J = 7 Hz, 3H (C-1 CH_3)), 1.00–1.46 (m, 21H (C-4–C-15)), 1.19 (s, 3H (C-17 CH_3)), 1.48–1.57 (m, 2H (C-2)), 1.93 (s, 3H (S-CH_3)); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 1-(methylthio)-phytane, 15.56 (S-CH_3), 19.37, 19.43 (C-17), 19.67–19.79 (C-18, C-19), 22.63, 22.72 (C-16, C-20), 24.40 (C-13), 24.47, 24.49 (C-5), 24.80 (C-9), 27.99 (C-15), 32.12 (C-3), 32.20, 32.21 (C-1), 32.78, 32.80 (C-7, C-11), 36.39, 36.48 (C-2), 37.07, 37.11 (C-4), 37.27–37.49 (C-6, C-8, C-10, C-12), 39.37 (C-14); 3-(methylthio)-phytane, 8.60 (C-1), 10.30 (S-CH_3), 19.63, 19.71 (C-18, C-19), 21.49, 21.50 (C-5), 22.56, 22.67 (C-16, C-20), 24.42 (C-13), 24.75, 24.77 (C-17), 24.99 (C-9), 27.93 (C-15), 31.66 (?), 32.66, 32.68 (C-7), 32.73, 32.75 (C-11), 37.23 (C-2), 37.34, 37.39, 37.55, 37.64 (C-6, C-8, C-10, C-12), 39.20 (C-4), 39.32 (C-14), 47.84 (C-3). The ^{13}C-NMR assignments are tentative and were made by comparison with the ^{13}C-NMR spectrum of phytane (YON, 1981) and by using the additivity rules (LINDEMANS and ADAMS, 1971). A number of carbon atoms gave rise to two signals in the NMR spectrum because the synthesis yielded a pair of diastereomers (C-3 is a chiral centre) for each methylthio-phytane.

Three stereomeric 3-(methylthio)-cholestanes (XXII–XXIV; Fig. 5) were synthesized from appropriate cholestanol isomers by derivatization of the cholestanols into corresponding cholestanyl tosylates and a subsequent substitution reaction (S_N2 reaction) of the tosyl group with a methylthio group. 20R-5α(H),14α(H),17α(H)-cholestan-3α-ol, 20R-5α(H),14α(H),17α(H)-cholestan-3β-ol, and 20R-5β(H),14α(H),17α(H)-cholestan-3β-ol were used to obtain 3β - methylthio - 20R - 5α (H), 14α (H), 17α (H) - cholestane, 3α-methyl-thio-20R-5α(H),14α(H),17α(H)-cholestane and 3α-methylthio-20R-5β(H),14α(H),17α(H)-cholestane, respectively. The stereomeric cholestanols were synthesized as follows: 20R-5α(H),14α(H),17α(H)-cholestan-3α-ol and 20R-5α(H),14α(H),17α(H)-cholestan-3β-ol were obtained by reduction of 20R-5α(H),14α(H),17α(H)-cholestan-3-one using $NaBH_4$ (BRUCE and RALLS, 1943). Yields are ca. 14 wt% for cholestan-3α-ol and ca. 82 wt% for cholestan-3β-ol. 20R-5β(H),14α(H),17α(H)-cholestan-3β-ol was obtained via catalytic hydrogenation of cholesterol (20R-14α(H),17α(H)-cholest-5-en-3β-ol) which, prior to hydrogenation, was derivatized to its acetate ester and subsequent saponification of the reaction mixture (BRUCE and RALLS, 1943). After crystallisation of the 5α(H)-cholestan-3β-ol, the 5β(H)-cholestan-3β-ol isomer was isolated from the reaction mixture using a SiO_2 column (l = 30 cm, i.d. 3 cm) by elution with 250 mL ethyl acetate/hexane (1:9, v/v; overall yield ca. 6 wt%).

As an example, the synthesis of 3β-methylthio-20R-5α(H), 14α(H),17α(H)-cholestane is described. To a stirred solution of 20R-5α(H),14α(H),17α(H)-cholestan-3α-ol (0.1 mmol) in 0.6 ml anhydrous pyridine, p-toluene sulphonylchloride (0.2 mmol) was added at 50°C under nitrogen, and stirring was continued for 4 h at 60°C (modified after NACE, 1952). After cooling, 10 mL diethyl ether and 5 mL 1 M HCl solution were added. This mixture was extracted several times with diethyl ether. The combined extract was washed with 5 mL NaCl-saturated 1 M HCl solution (×2), with 5 mL $NaHCO_3$-saturated water, and with 5 mL NaCl-saturated water (×2), and was subsequently dried over anhydrous $MgSO_4$ and evaporated to dryness to give a light yellow oil. Fractionation of the reaction mixture by column chromatography (SiO_2, l = 30 cm, i.d. 3 cm) using ethyl acetate/hexane (1:19, v/v) as eluent yielded ca. 4 mg of the tosylate ester. To a solution of this tosylate in 0.2 mL propan-2-ol/toluene, 0.3 mmol sodium thiomethoxide was added at 100°C under nitrogen and stirred for 1.5 h, yielding the methylthio derivative (modified after JONES et al., 1968).

RESULTS

The so-called "polar" fraction isolated from S-rich sediment extracts and oils contains apart from low-molecular-weight polar compounds (e.g., alcohols, acids, ketones) also high-molecular-weight substances (MW > ca. 800) which consist of S cross-linked low-molecular-weight units (ADAM et al., 1991; KOHNEN et al., 1991b; RICHNOW et al., 1993; SCHMID, 1986; SINNINGHE DAMSTÉ et al., 1988, 1990a; TRIFILIEFF, 1987). The polar fractions of the selected samples were treated with a novel chemolysis method (MeLi/MeI; KOHNEN et al., 1991b) which cleaves selectively and quantitatively di- or polysulphide linkages and subsequently derivatises the cleavage products to the corresponding methylthio-ethers. Analysis of the cleavage products using GC-MS yielded details on the location of di- or polysulphide linkages in macromolecularly S-bound steroid and phytane moieties. However, we emphasize that these chemolysis experiments reveal only information on the linkage-sites for those units which are bound via di- or polysulphide linkage(s), exclusively. On the other hand, knowledge on the location of monosulphide linkages is scant, since there exist no methods, at present, to selectively cleave monosulphide linkages. However, we hypothesize that both polysulphide and monosulphide linkages are at the same positions in macromolecularly S-bound moieties, since the position of a S linkage is likely to be controlled by the position of the functional group in its biological precursor. In order to prove this hypothesis, a few selected polar fractions before and after removal of di- or polysulphide bound units were analyzed using Py-GC-MS.

Selective Chemolysis of Di- and Polysulphide Linkages Using MeLi/MeI

Methylthioethers with a phytane carbon skeleton

In a recent communication, we reported on the presence of mono(methylthio)-phytanes (II–VI, VIII; Fig. 2) in the mixture of methylthio-ethers formed after MeLi/MeI-treatment of the polar fraction from a bituminous marl from the Vena del Gesso Basin (Marl-4A; KOHNEN et al., 1991b). The distribution of the various structural isomers is displayed by a mass chromatogram of their molecular ion at m/z 328 ($C_{21}H_{44}S$; Fig. 1a). The major isomers were tentatively

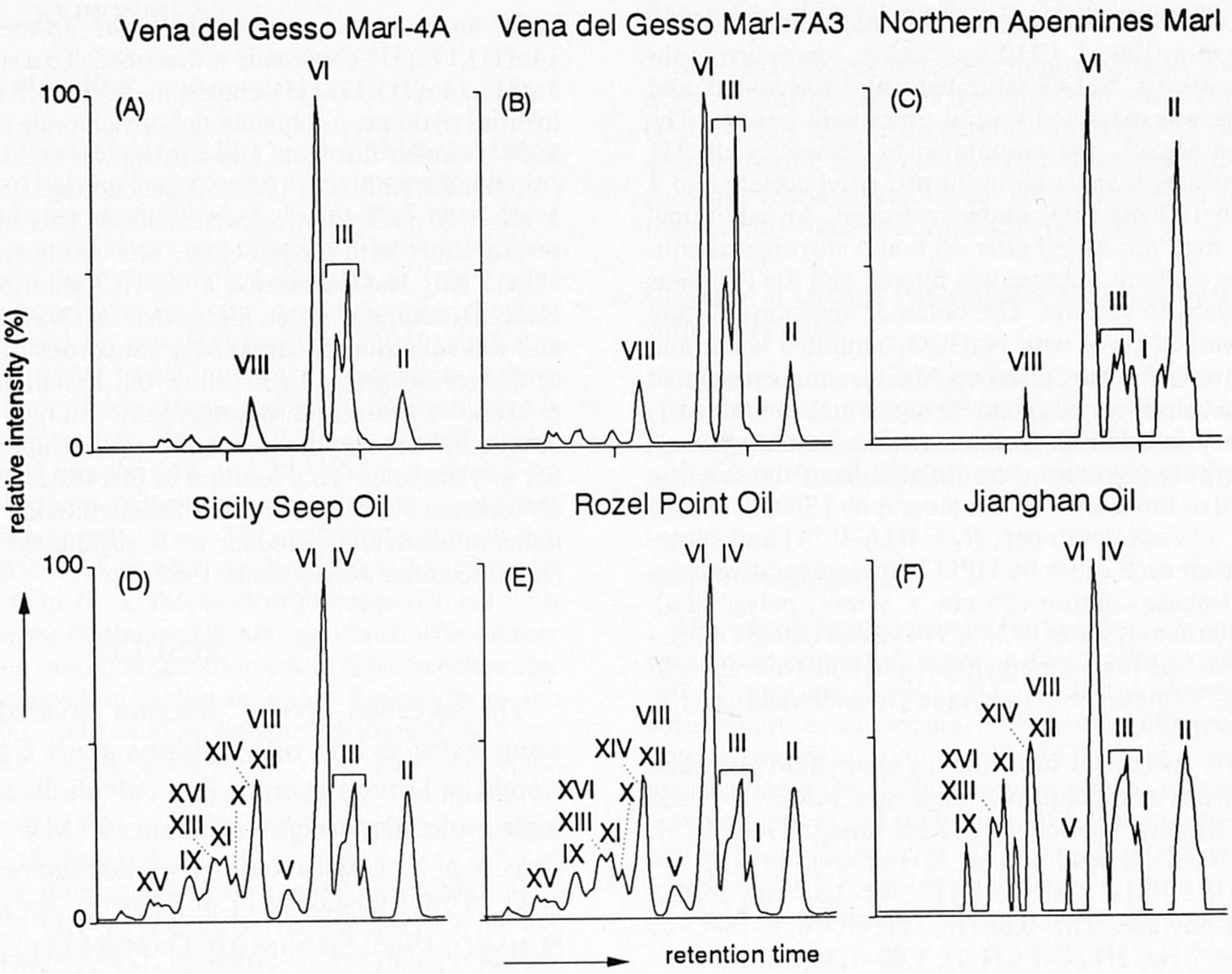

FIG. 1. Partial mass chromatograms of m/z 328 showing the distribution of the methylthio-phytanes formed after MeLi/MeI treatment of polar fractions from the indicated samples. Assignments of labeled peaks are given in Fig. 2.

identified on the basis of Raney Ni desulphurisation, relative GC-retention time, and mass spectral data (KOHNEN et al., 1991b). Here we confirm the preliminary tentative identifications of the 1-(methylthio)-phytane (II; Fig. 2) and the major 3-(methylthio)-phytane (VI; Fig. 2). Both compounds displayed the same mass spectra as those of synthesized standards and coeluted with the corresponding standards upon coinjection experiments on a CP-Sil 5 capillary column.

The m/z 328 mass chromatograms of the samples analyzed are shown in Fig. 1. It should be emphasized that the relative distribution of these various methylthio-phytanes as exemplified by the m/z 328 mass chromatogram is biased, since the relative intensity of this ion in the mass spectra of the different structural isomers varies considerably (KOHNEN et al., 1991b). The distributions of Vena del Gesso Marls 4A and 7A3 and Northern Apennines Marl (Fig. 1a–c) are rather similar to each other and reveal that the methylthio-phytanes are dominated by II, III, VI, and VIII (for structures see Fig. 2). The major isomer (VI) possesses its methylthio-group at a tertiary carbon atom (C-3) of its carbon skeleton. The dominance of this isomer is even more pronounced than is suggested by the m/z 328 mass chromatogram, since the relative intensity of the $M^{+\cdot}$ ion in its mass spectrum is significantly lower than that of the molecular ions in the spectra of the other isomers (KOHNEN et al., 1991b). The distributions in the Sicily Seep Oil, Rozel Point Oil, and Jianghan Oil (Fig. 1d–f) are quite similar to each other but differ significantly from the other samples analyzed (Fig. 1). The cluster of methylthio-phytanes from the Sicily Seep Oil, for example, shows other major isomers in addition to the dominant isomers encountered in the Vena del Gesso Marl-4A. The structural identities of these methylthio-phytanes are deciphered from the m/z values of the major S-containing fragment ions present in their mass spectra (m/z 61 + 14n, n = 0–18; cf. KOHNEN et al., 1991b) and their relative GC-retention times. Mass chromatography using these characteristic ions unravels the mixture of methylthio-phytanes represented by the complex cluster in the TIC (Fig. 2). It is evident that the major isomers are those with the methylthio-group located at a tertiary carbon atom of the phytane skeleton (i.e., at C-3, C-7, C-11, and C-15; IV, VI, XII, and XIV). It is of note that we have not been able to trace isomers with the methylthio-group at C16, C18, C19, and C20. Several structural isomers are represented by two unresolved peaks in the appropriate mass chromatograms, which is thought to be a reflection of the presence of diastereomers (Fig. 2).

Among the GC-amenable products released from the polar fraction of the Sicily Seep Oil after treatment with MeLi/MeI, a series of compounds was encountered that contain two S atoms per molecule according to their intensity in the FPD chromatogram relative to compounds containing one S atom. Results from Raney Ni desulphurisation of this methylthio-ether fraction and relative GC-retention times indicate that these compounds possess a phytane carbon skeleton. Two typical mass spectra of these novel compounds are shown in Fig. 3. Their mass spectra exhibit a molecular ion at m/z 354 ($C_{21}H_{36}S_2$) and characteristic features of methylthio-ethers being the $M^{+\cdot}$-47 fragment ion at m/z 307 (loss of S-CH_3) and the minor rearrangement ion at m/z 48 (CH_3SH). The relative intensity of the M + 2 isotope peak of about 10% of the intensity of the molecular ion is consistent with two S atoms per molecule. All spectra exhibit a S-con-

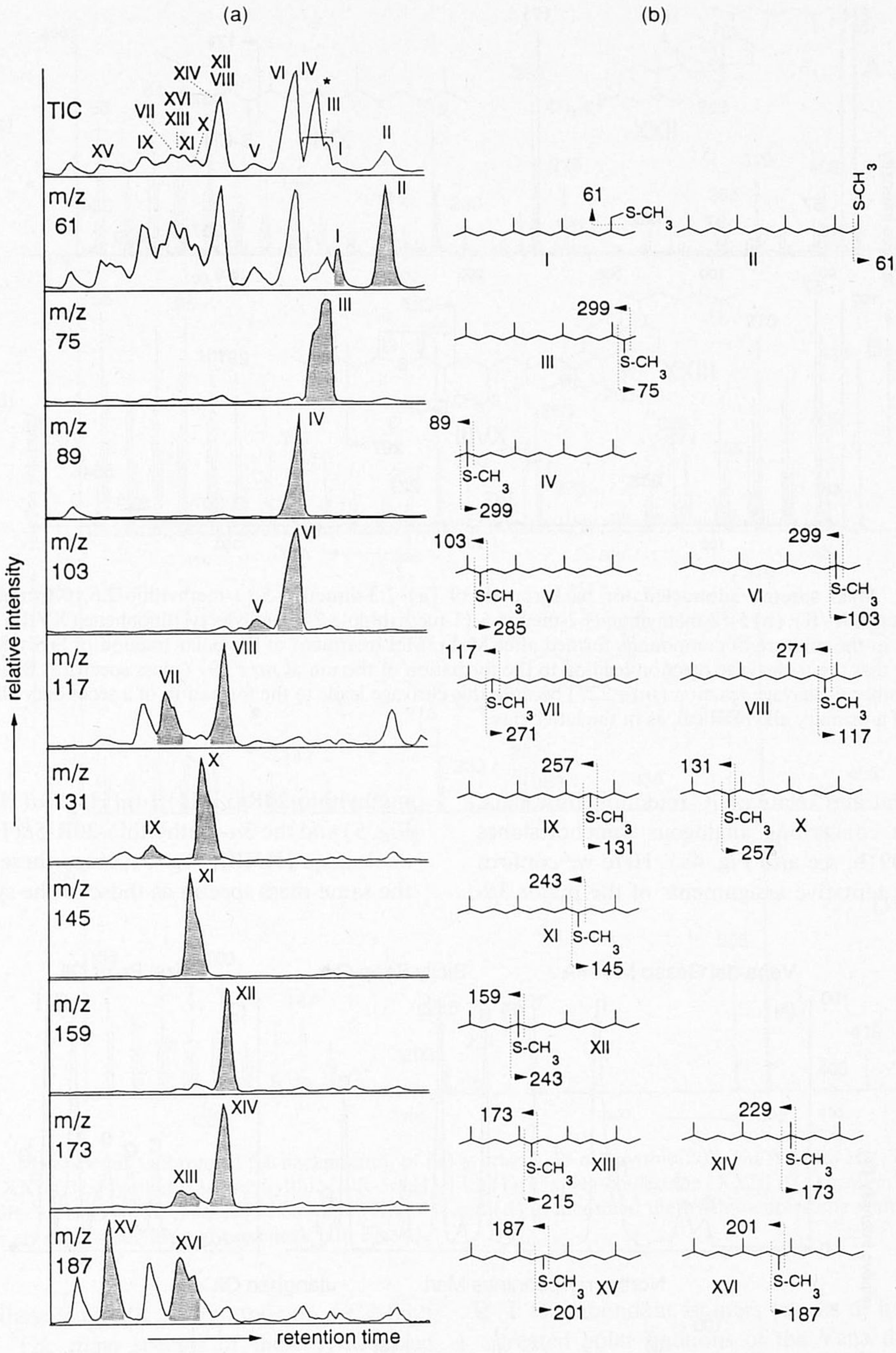

FIG. 2. (a) Partial TIC and mass chromatograms of diagnostic m/z values for methylthio-phytanes of the methylthioether fraction isolated after treatment of the polar fraction of the Sicily Seep Oil with MeLi/MeI. The peak labeled with an asterisk represents the internal standard, 5,5-D_2-3-methylicosane. (b) Structures of methylthiophytanes.

taining fragment ion at m/z 125 (C_7H_9S), which suggests the presence of an alkylated thiopene group. The major fragment ion at m/z 171, which according to its accurate m/z value contains two S atoms ($C_8H_{11}S_2$), reveals that the methylthio-group is located at the α-carbon atom(s) of the long alkyl side-chain of the thiophene ring. Hence, the major compound is tentatively assigned as 2,3-dimethyl-5-(1-methylthio-2,6,10-trimethylundecyl)thiophene (XVII; Fig. 3a). The other, earlier eluting, isomers show in addition to the presumably secondary ion at m/z 171 other fragment ions that also possess two S atoms (e.g., m/z 227 and 297 in Fig. 3b), which probably result from β-cleavage reactions of the alkyl substituents. Therefore, these compounds are tentatively assigned as XVIII–XXI (Fig. 3 and Appendix).

Methylthioethers with a steroid carbon skeleton

The mono(methylthio)-cholestanes released from the polar fraction of Vena del Gesso Marl-4A have been previously tentatively identified on the basis of Raney Ni desulphurisation

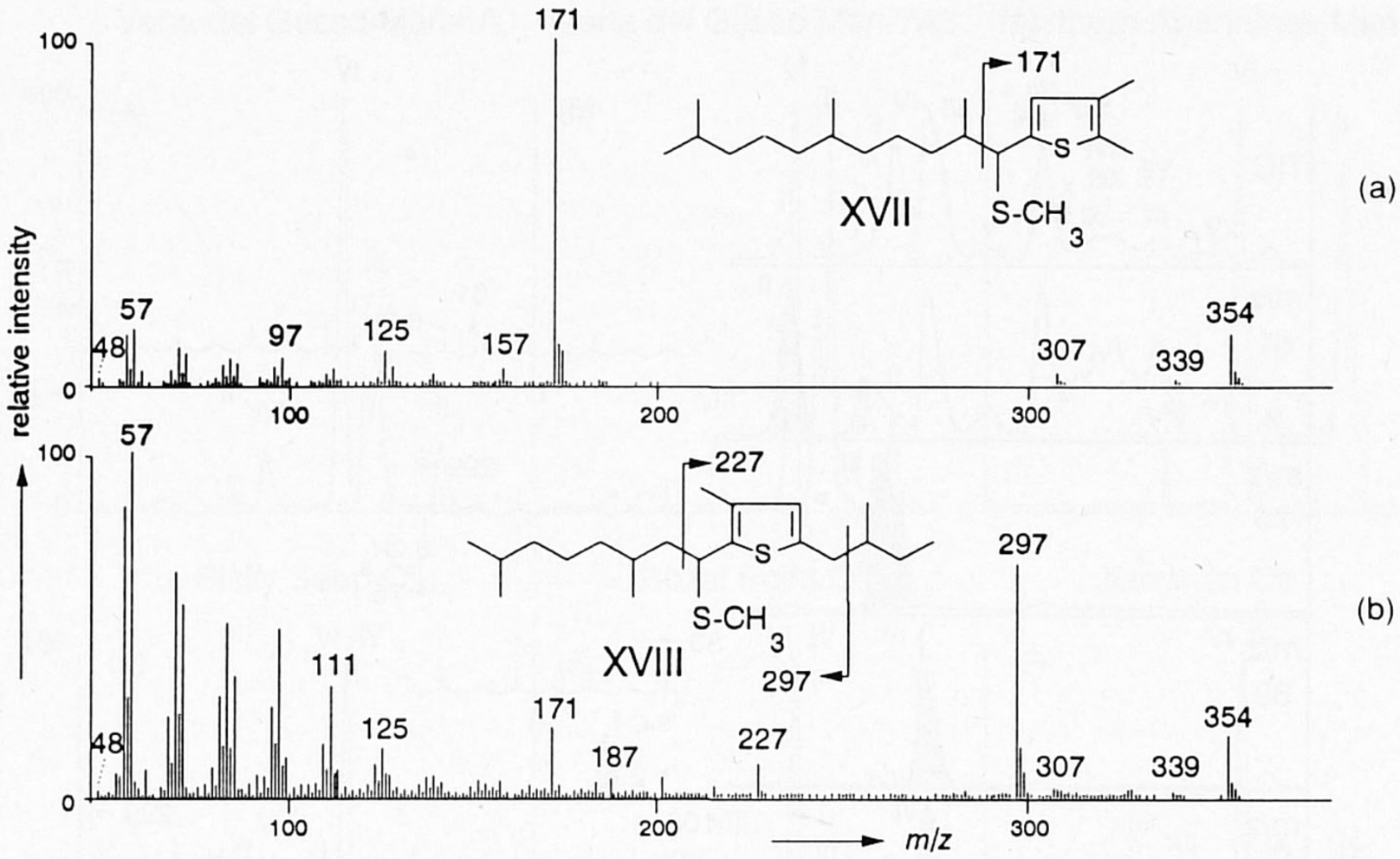

FIG. 3. Mass spectra, subtracted for background, of (a) 2,3-dimethyl-5-(1-methylthio-2,6,10-trimethylundecyl)thiophene (XVII); (b) 5-(2-methylbutyl)-2-methyl-5-(1-methylthio-3,7-dimethyloctyl)thiophene (XVIII) tentatively identified in the mixture of compounds formed after MeLi/MeI treatment of the polar fraction of Sicily Seep Oil. It is of note that the β-cleavage reaction leading to the formation of the ion at m/z 297 (mass spectrum B) is favoured over the other β-cleavage reaction (m/z 227) because this cleavage leads to the formation of a secondary alkyl radical instead of a primary alkyl radical, as in the latter case.

results, mass spectral and relative GC-retention time data, and literature data concerning analogous thiocholestanes (KOHNEN et al., 1991b; see also Fig. 4a). Here we confirm unambiguously the tentative assignments of the major 3β-methylthio-20R-5α(H),14α(H),17α(H)-cholestane (XXII; Fig. 5) and the 3α-methylthio-20R-5β(H),14α(H),17α(H)-cholestane (XXIII; Fig. 5), since these compounds exhibit the same mass spectra as those of the synthesized standards

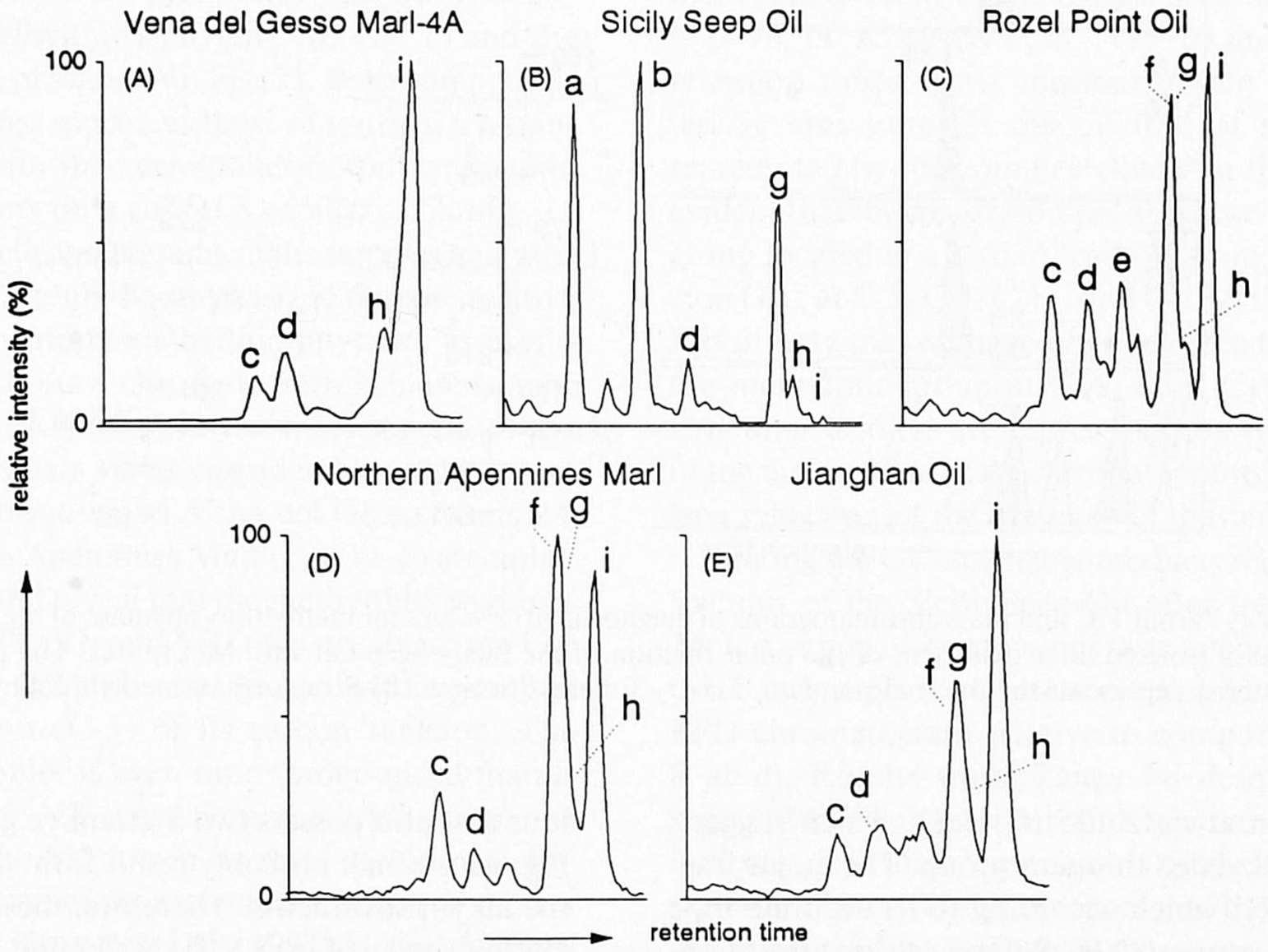

FIG. 4. Partial mass chromatograms of m/z 418 showing the distribution of the methylthio-cholestanes formed after MeLi/MeI treatment of polar fractions from the samples indicated. Key: a = a methylthio-cholestane with an equatorial methylthio-group; b = a methylthio-cholestane with an axial methylthio-group; c = 4α-methylthio-5β(H),14α(H),17α(H)-cholestane (tentative); d = 3α-methylthio-20R-5β(H),14α(H),17α(H)-cholestane (XXIII); e = a methylthio-5α(H),14β(H),17β(H)-cholestane; f = an unidentified methylthio-cholestane; g = 3α-methylthio-20R-5α(H),14α(H),17α(H)-cholestane (XXIV); h = 2α-methylthio-20R-5α(H),14α(H),17α(H)-cholestane (tentative); i = 3β-methylthio-20R-5α(H),14α(H),17α(H)-cholestane (XXII).

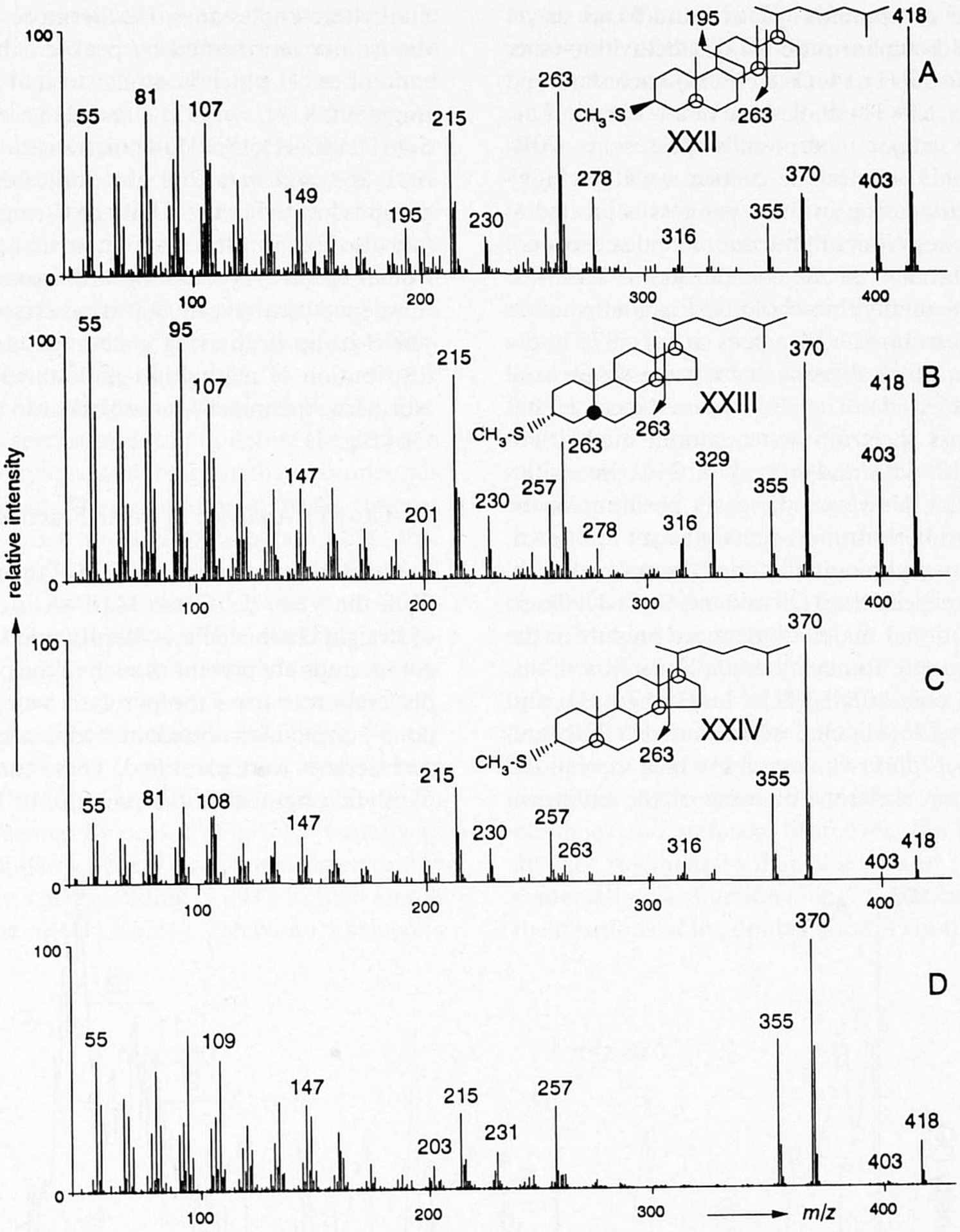

FIG. 5. Mass spectra, subtracted for background, of (a) synthetic 3β-methylthio-20R-5α(H),14α(H),17α(H)-cholestane (XXII); (b) synthetic 3α-methylthio-20R-5β(H),14α(H),17α(H)-cholestane (XXIII); (c) synthetic 3α-methylthio-20R-5α(H),14α(H),17α(H)-cholestane (XXIV); and (d) unidentified methylthio-cholestane which probably possesses an axial methylthio-group (peak b in Fig. 4).

and coelute with these synthetic compounds on a CP-Sil 5 capillary column. The mass spectra of these synthesized compounds are presented in Fig. 5a and b.

The m/z 418 mass chromatograms of the samples analyzed are shown in Fig. 4. We emphasize that the relative abundance of a particular isomer suggested by the m/z 418 mass chromatogram is biased, since the relative intensity of this ion in the mass spectra of the different isomers is highly variable (Fig. 5). The distribution of the methylthio-cholestanes released from the Vena del Gesso Marl-7A3 (not shown) is similar to that of Vena del Gesso Marl-4A. The m/z 418 traces of the other samples differ significantly from that of Vena del Gesso Marl-4A. It is noteworthy that for all the samples analyzed the distributions of the methylthio-24-methylcholestanes and methylthio-24-ethylcholestanes are similar to that of the methylthio-cholestanes (as revealed by m/z 432 and 446 mass chromatograms).

The abundant isomers (peaks d, h, i) in the MeLi/MeI treated polar fractions of the Vena del Gesso samples are minor in the Sicily Seep Oil (Fig. 4a and b). In the latter sample, the last eluting major compound (peak g) displays the same mass spectrum as the synthesized 3α-methylthio-20R-5α(H),14α(H),17α(H)-cholestane (XXIV; Fig. 5). This spectrum is characterized by a minor M^+ ion and a base peak at m/z 370, in contrast to the mass spectra of methylthio-cholestanes XXII and XXIII (Fig. 5). The observed differences in degree of elimination of the methylthio-group during mass spectral fragmentation induced upon electron impact is likely to be related to the stereochemistry of the methylthio-group; methylthio-cholestane XXIV possesses an axial methylthio-group whereas the other two synthesized isomers, XXII and XXIII, contain an equatorial methylthio-group. Coinjection experiments with the synthetic standard unequivocally confirmed the presence of XXIV. The other two

earlier eluting major compounds (peaks a and b) are as yet unknown. Raney Ni desulphurization of this methylthio-ether fraction afforded 20R-5β(H),14α(H),17α(H)-cholestane and 20R-5α(H),14α(H),17α(H)-cholestane in a 1:10 ratio, suggesting that these major compounds possess a 20R-5α(H),14α(H),17α(H)-cholestane carbon skeleton. However, if the methylthio-group in these isomers is located at C-4, C-5, or C-6, Raney Ni desulphurisation and subsequent hydrogenation might alter the stereochemistry at C-5. The mass spectrum of the methylthio-cholestane isomer which is represented by peak a shows a base peak at m/z 370 and a small molecular ion, which suggests that it possesses an axial methylthio-group (Fig. 5d). The other isomer (peak b) has according to its mass spectrum an equatorial methylthio-group (i.e., m/z 418, 55% and m/z 370, 25%, intensities relative to base peak). However, the exact positions of the methylthio-groups in both isomers remain as yet unknown.

Apart from the methylthio-cholestanes (peaks c, d, g, h, i) encountered in the Sicily Seep Oil and the Vena del Gesso sediments, two additional major isomers are present in the Rozel Point Oil (Fig. 4c). Raney Ni desulphurisation of this fraction yielded not only 20R-5β(H),14α(H),17α(H)- and 20R-5α(H),14α(H),17α(H)-cholestanes but also 20R- and 20S-5α(H),14β(H),17β(H)-cholestanes which reveal the identity of the carbon skeletons of some of the unknown methylthio-cholestanes. Furthermore, the mass spectrum of the isomer represented by peak e exhibits a m/z 263/264 ratio of ca. 1, which is similar to that for the corresponding fragments (i.e., m/z 217 and 218) in the mass spectra of 5α(H),14β(H),17β(H)-cholestanes. The fragment ions m/z 263 and m/z 264 also indicate that the methylthio-group is located in the A-, B-, or C-ring of its carbon skeleton (see also Fig. 5a). It is also of note that an abundant unknown isomer (peak f) is coeluting with isomer XXIV (peak g). Its mass spectrum reveals that it possesses an equatorial methylthio-group in the ring system of its carbon skeleton. The distribution of methylthio-cholestanes of Jianghan Oil and Northern Apennines Marl is similar to that of the Rozel Point Oil (Fig. 4).

Py-GC-MS Analysis of Polar Fractions

The flash pyrolysate (610°C) of the polar fraction isolated from the Vena del Gesso Marl-4A is dominated by a series of straight chain alcohols, sterols, and stanols (Fig. 6). These compounds are present as such in the polar fraction and simply evaporate from the pyrolysis wire. In addition to these polar compounds abundant hydrocarbons such as phytenes and sterenes were identified. These compounds must have a pyrolytic origin since the presence of hydrocarbons as such

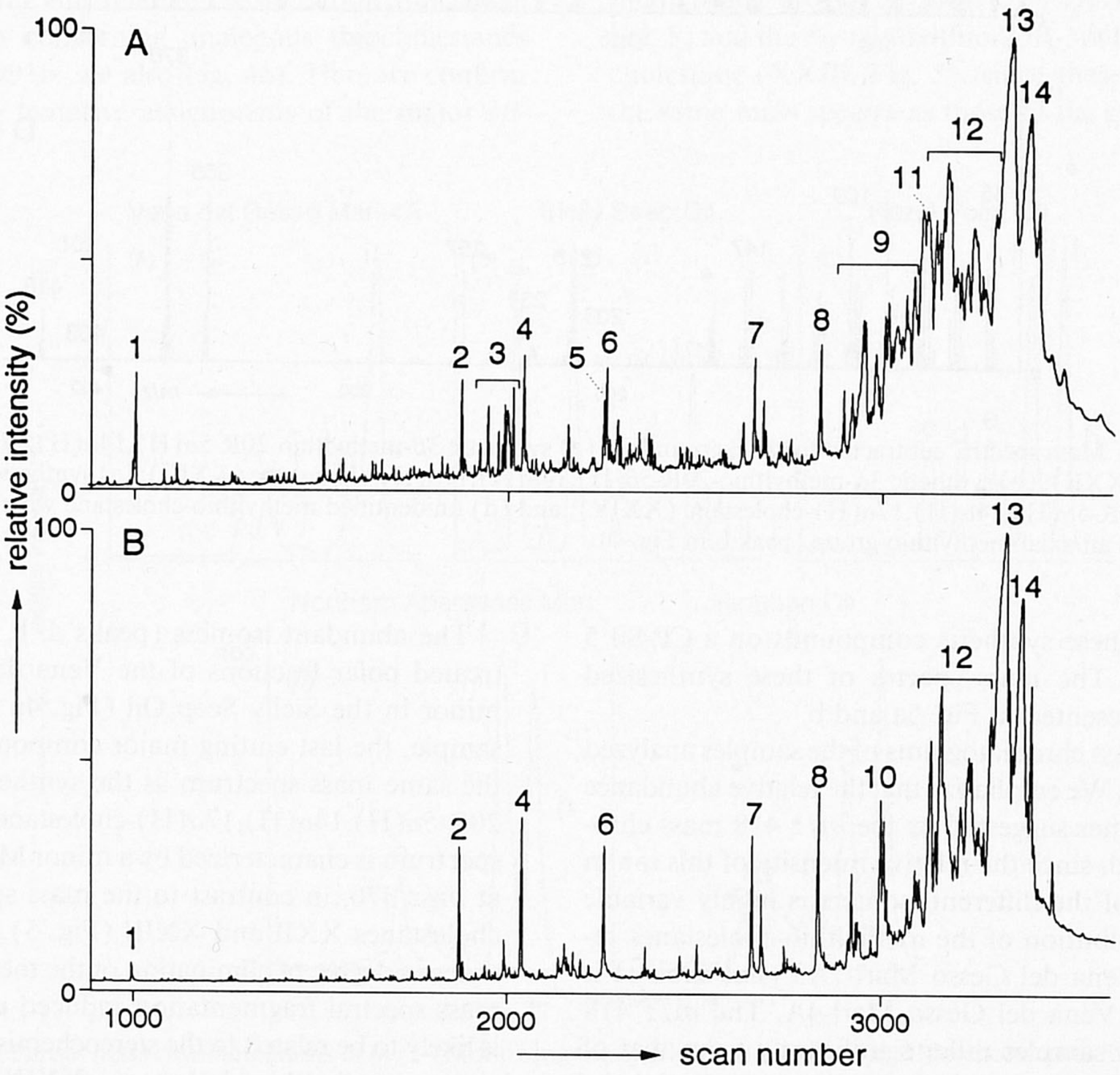

FIG. 6. TIC of flash pyrolysates (610°C) of (a) untreated polar fraction of Vena del Gesso Marl-4A and (b) residue after Raney Ni desulphurisation of the polar fraction of Vena del Gesso Marl-4A. Key: 1 = 1,2,3,4-tetramethylbenzene; 2 = prist-1-ene; 3 = phytenes; 4 = hexadecan-1-ol; 5 = 3-(3,7,11,15-tetramethylhexadecane)thiol; 6 = octadecan-1-ol; 7 = docosan-1-ol; 8 = tetracosan-1-ol; 9 = C_{27}–C_{29} sterenes; 10 = hexacosan-1-ol; 11 = dinosterene; 12 = C_{27}–C_{29} sterols and stanols; 13 = dinosterol; and 14 = dinostanol.

in the polar fraction is excluded by the chromatographic fractionation procedure used to isolate this fraction. To assess whether these pyrolysis products are derived from S-bound moieties a selective cleavage of C-S bonds by a Raney Ni desulphurization of this polar fraction was performed. After removal of the released hydrocarbons the residue was pyrolysed under the same conditions. This pyrolysate contained neither phytenes nor sterenes in significant amounts, which indicates that these compounds in the pyrolysate of the untreated polar fraction are derived from phytane and steroid carbon skeletons bound via one or more unspecified S linkages to macromolecules (cf. Fig. 6a and b).

The distribution of the phytenes is shown in Fig. 7a. Unfortunately, the mass spectrum does not define the position of a double bond in a phytene, and therefore these compounds were not further identified. The distribution of the C_{27} sterenes is displayed by a m/z 370 mass chromatogram (Fig. 7b). The major isomers are 20R-5α(H),14α(H),17α(H)- and 20R-5β(H),14α(H),17α(H)-cholest-2-ene and 20R-5α(H),14α(H),17α(H)- and 20R-5β(H),14α(H),17α(H)-cholest-3-ene (peaks 1, 2, 4, and 6). In addition, minor amounts of cholest-4-ene and cholest-5-ene were detected. Apart from the first eluting compound (peak 1, Fig. 7), the cholestenes were identified by comparing their mass spectra with literature data (PHILIP, 1985; PEAKMAN et al., 1992). The cholestene represented by peak 1 (Fig. 7) is tentatively identified as 5β(H)-cholest-2-ene, since its mass spectrum is similar to that of the corresponding 5α(H)-isomer and it elutes earlier than the 5α(H) isomer, behaviour analogous to the 5β(H)- and 5α(H)-stereomers of cholest-3-ene. In the pyrolysate of the Sicily Seep Oil polar fraction, the cholest-2-enes and cholest-3-enes are also the dominant C_{27} sterenes and cholest-4-ene and cholest-5-ene are minor pyrolysis products. It is also of note that the other homologues sterenes (C_{28} and C_{29}) exhibit similar distributions, as revealed by mass chromatograms of their molecular ions.

In addition to these hydrocarbons, minor amounts of alkylthiols with a phytane and sterane skeleton were detected among the pyrolysis products from the Vena del Gesso Marl-4A (Fig. 6a). The major alkylthiol possessing a phytane carbon skeleton was tentatively identified as 3-(3,7,11,15-tetramethylhexadecane)thiol on the basis of its mass spectrum and its GC-retention time (peak 5, Fig. 6a). According to their mass spectra, the thiosterols have the thiol-functionality in either the A-, B-, or C-ring. It is of note that the presumed location of the thiol group in these pyrolysis products is the same as that of the methylthio-groups in the corresponding methylthio-ethers released from this particular polar fraction after MeLi/MeI treatment.

After MeLi/MeI treatment and subsequent removal of the released methylthio-ethers, the residue of the polar fraction Vena del Gesso Marl-4A contains only steroid and phytanyl moieties which are bound via monosulphide linkages. Pyrolysis of this residue still yields considerable amounts of phytenes and sterenes. Moreover, the distribution of these alkenes is similar to that observed in the pyrolysate of the untreated polar fraction (Fig. 7). Hence, if one assumes that the positions of the double bond(s) in the pyrolysis products

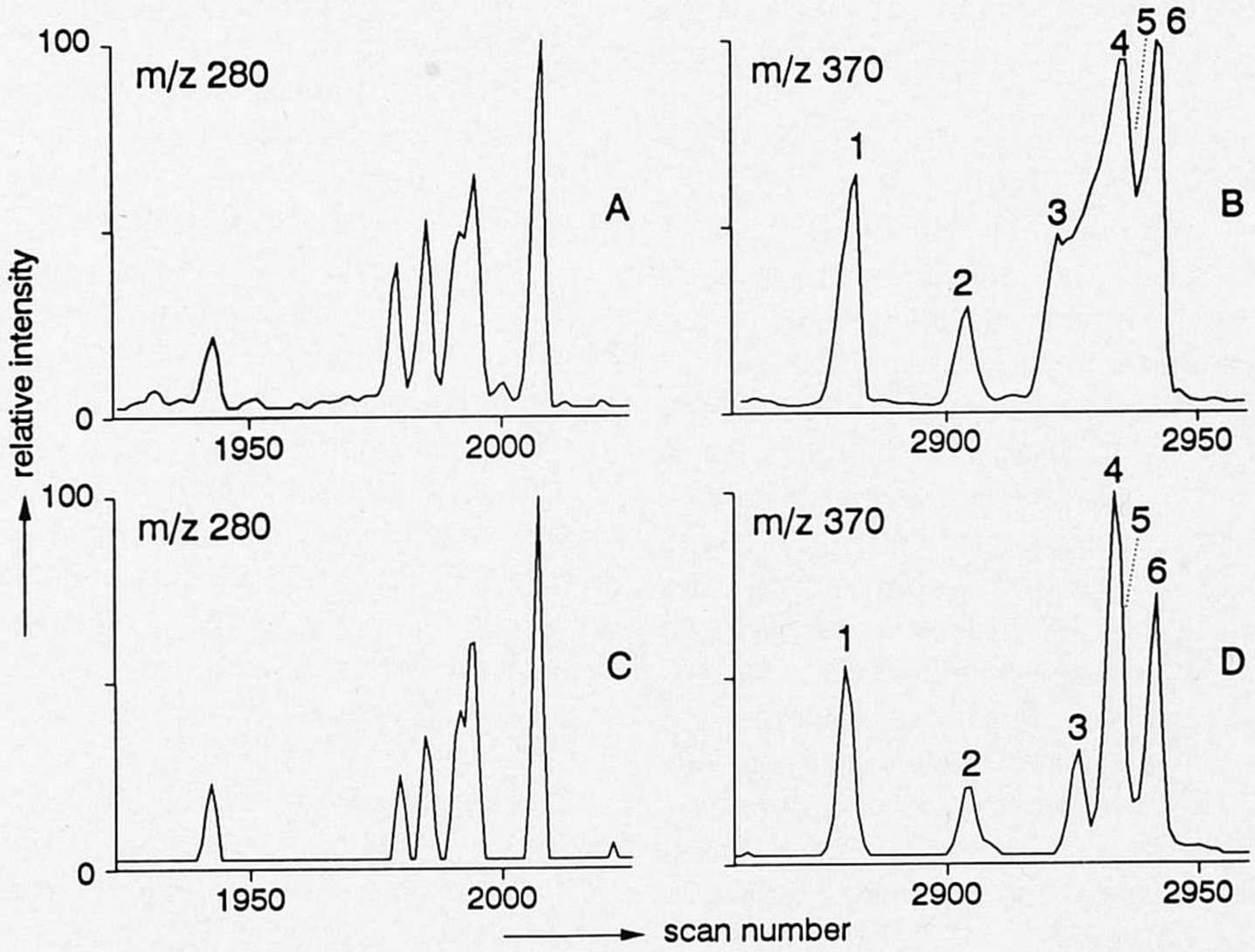

FIG. 7. Partial mass chromatograms of the pyrolysate of the polar fraction Vena del Gesso Marl-4A; (a) m/z 280 exhibiting the distribution of the phytenes; (b) m/z 370 exhibiting the distribution of the cholestenes and partial mass chromatograms of the pyrolysate of the residue of the MeLi/MeI treated polar fraction Vena del Gesso Marl-4A; (c) m/z 280; (d) m/z 370. It is emphasized that this residue comprises only monosulphide-bound moieties in the macromolecular matrix. Moreover, the di- or polysulphide-linked units comprise a significant portion (ca. 50 wt%) of the total amount of macromolecularly S-bound units in the Vena del Gesso Marl-4A (KOHNEN et al., 1991a). Key: 1 = 20R-5β(H),14α(H),17α(H)-cholest-3-ene; 2 = 20R-5β(H),14α(H)-cholest-2-ene; 3 = 20R-14α(H),17α(H)-cholest-4-ene; 4 = 20R-5α(H),14α(H),17α(H)-cholest-2-ene; 5 = 20R-14α(H),17α(H)-cholest-5-ene; 6 = 20R-5α(H),14α(H),17α(H)-cholest-3-ene.

are related to the positions of the former C-S linkage(s), these similar distributions indicate that the monosulphide linkages are at the same positions as the polysulphide linkages in the carbon skeletons of the macromolecularly bound moieties. It is also of note that the pyrolysate of the MeLi/MeI-treated fraction does not contain alkylthiols, in contrast to the pyrolysate of the untreated fraction. This indicates that the alkylthiols in the pyrolysate are derived from units which are linked with a di- or polysulphide linkage to the macromolecular matrix, consistent with the suggestion of SCHMID (1986).

DISCUSSION

Incorporation of Inorganic Sulphur Species into Functionalized Lipids

Detailed prior studies have shown that the location of C-S bonds in low-molecular-weight OSC in many cases can be related to the specific positions of functional groups (e.g., double bonds) in the alleged biological precursors of these compounds (e.g., SINNINGHE DAMSTÉ et al., 1989a; KOHNEN et al., 1991b). The data presented here on the chemolysis products released from S-rich macromolecules justify the extension of this concept to macromolecularly S-bound moieties. It should be noted that these chemolysis experiments reveal only information about the positions of di- or polysulphide linkages in macromolecules. Py-GC-MS analysis of an untreated and a MeLi/MeI treated polar fraction of Vena del Gesso Marl-4A which yielded for both fractions similar phytene and sterene distribution patterns (Fig. 7) revealed, however, that both polysulphide and monosulphide linkages are at the same positions in the macromolecularly S-bound moieties.

In the Vena del Gesso Marls 4A and 7A3 and the Northern Apennines Marl the macromolecularly S-bound phytanyl moieties are bound with S linkages located at C-1, C-2, C-3, C-4, and C-17, with C-3 as the far most dominant position (Fig. 1). These sites of bonding are in full agreement with the incorporation of inorganic S species into phytol-derived phytadienes (XXV, XXVI; KOHNEN et al., 1991b). The presence of a S linkage at C-17, especially, indicates that S reacted with phytol-derived phytadienes instead of phytol, since it is unlikely that the double bond in phytol shifted to that position. We cannot rule out the possibility that the isomers with the S linkage at C-1 are, at least in part, formed via an S_N2-reaction of HS_x^- with a phytolester (DE LEEUW and SINNINGHE DAMSTÉ, 1990). Reactions of inorganic S species with α,β-unsaturated acids or aldehydes at relatively low temperatures (15–45°C) are possible (VAIRAVAMURTHY and MOPPER, 1987, 1989; ROWLAND et al., 1992). Sulphurization reactions of phytenic acid and phytenal, both occurring in sediments (e.g., BOON et al., 1975; RONTANI et al., 1990), could therefore yield the macromolecularly S-bound phytane carbon skeleton. However, it is difficult to imagine how the oxygen functionalities are removed with retention of the intact carbon skeleton. Therefore, this alternative is considered unlikely.

From the apparent preference of the C-3 position we conclude that S addition to a double bond probably involves an intermediate carbocation (or a polarisation of the π-electron cloud of the double bond) since such an ion will be at the most substituted position (C-3) according to Markovnikov's addition rules. Subsequently, this carbocation will be attacked by a nucleophilic S species resulting in a C-S bond at C-3. This addition reaction will be completed by attack of this reactive intermediate (e.g., thiol group) to another functionality (e.g., double bond) in either an intermolecular fashion leading to macromolecularly bound phytanyl moieties or an intramolecular fashion leading to cyclic OSC. In the case of the phytol-derived phytadienes, the preferred formation of a C-S bond at C-3 excludes the subsequent formation of cyclic S compounds, since the remaining double bond is not in the correct position to enable ring closure and stabilize the reactive intermediate OSC (KOHNEN et al., 1991b). Therefore, this major putative intermediate reacts intermolecularly and is accumulated in the macromolecules. On the other hand intramolecular incorporation of S into phytol-derived phytadienes does lead to the formation of the widespread cyclic OSC (XXVII–XXXII, Fig. 8; SINNINGHE DAMSTÉ and DE LEEUW, 1990). However, in their formation an energetically unfavourable secondary carbocation has to be involved. All this finds its reflection in the tremendous dominance of the macromolecularly S-bound phytanes over the low-molecular-weight OSC with a phytane carbon skeleton in, for example, the Vena del Gesso Marl-4A (Fig. 8).

The C-S linkages in the macromolecularly bound phytanyl moieties in the Sicily Seep Oil, Rozel Point Oil, and Jianghan Oil are at almost all positions of the carbon skeleton except C-16, C-18, C-19, and C-20. Geranyl-geraniol (XXXIII) and possibly also 2,6-phytadienol and 2,10-phytadienol or their diagenetic products (i.e., polyunsaturated phytenes) are likely substrates for the incorporation of S leading to the formation of these macromolecularly S-bound phytanyls. Hence, S incorporation occurs at isolated double bonds, which under marine surface sediment conditions seems to require some kind of catalysis.[§] Although the C-S linkages are located at almost any position of the phytane carbon skeleton, it is evident that there is a profound preference for the most substituted positions (i.e., C-3, C-7, C-11, and C-15). This once again reveals that S addition to a double bond involves an intermediate carbocation. The presence, although in relatively minor amounts, of phytanyl moieties with S linkages at C-5, C-9, and C-13 cannot straightforwardly be explained in this way. Preceding the incorporation of S at these positions, the double bonds of the invoked precursors would have to shift to these positions, isomerizations which would entail unfavourable secondary carbocations (DE LEEUW et al., 1989).

The macromolecularly S-bound isoprenoid thiophenes (XVII–XXI) in the Sicily Seep Oil are an example of macromolecularly S-bound phytane carbon skeletons in which an additional S atom is present resulting from an intramolecular incorporation of S. Geranyl-geraniol derived polyenes are thought to be the precursors of these moieties. It is striking that in all compounds identified, the intermolecular S linkages

[§] Recently, however, DE GRAAF et al. (1992) demonstrated under simulated natural conditions that isolated double bonds of alk-1-enes react with inorganic polysulphides at 20°C.

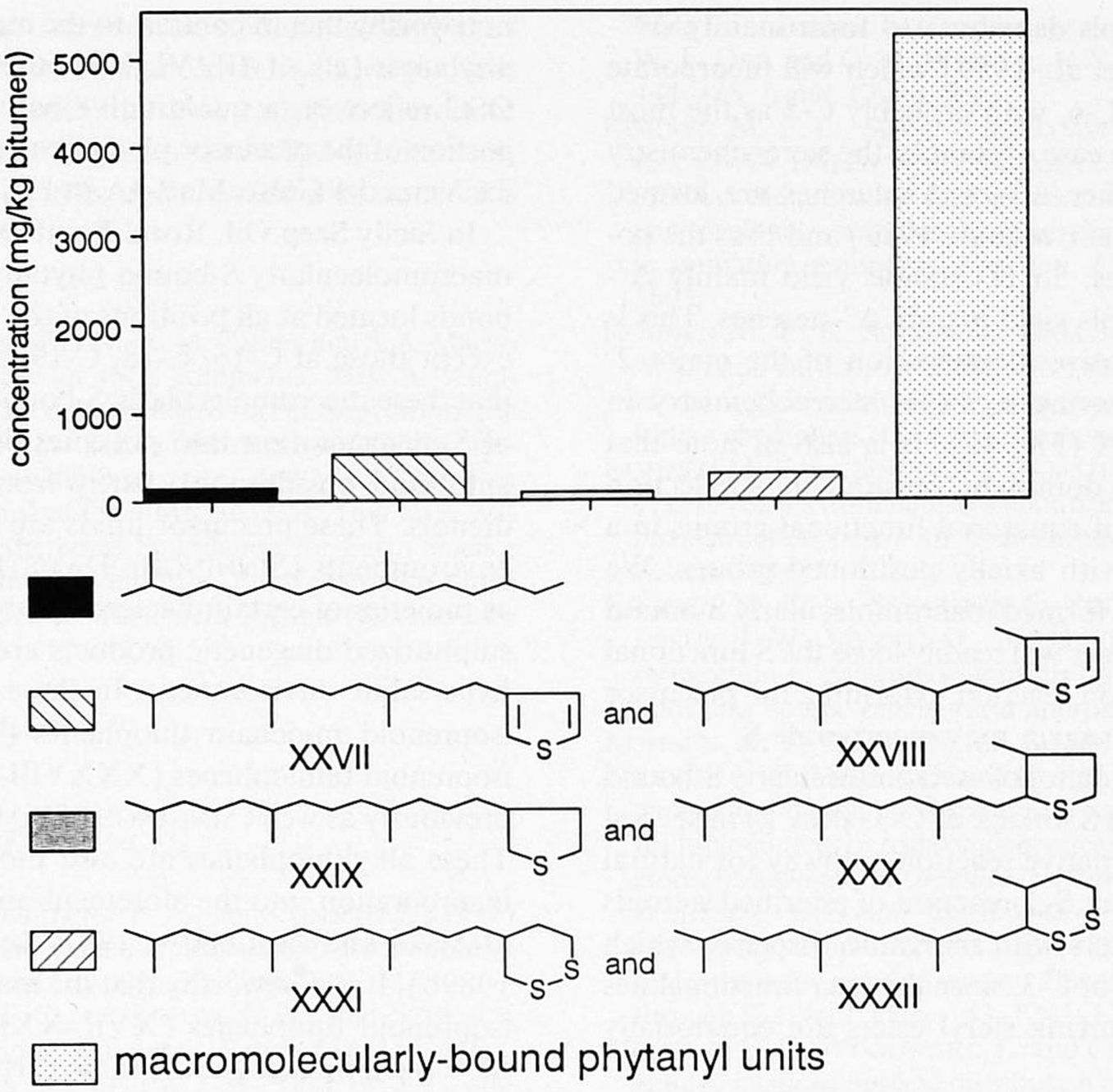

FIG. 8. The distribution of the phytane carbon skeleton over the different modes of occurrence in the Vena del Gesso Marl-7A3. It is evident that the major portion of the amount of phytane carbon skeletons is present as macromolecularly S-bound moieties. Di- or polysulphide bound phytanyl moieties with the S linkages at C-1, C-2, C-3, C-4, and C-17 comprise ca. 40 wt% of all macromolecularly S-bound phytanyl moieties, and the ones with the S linkage located at C-3 are by far dominant (ca. 80 wt%). The particular compounds were analyzed quantitatively by GC using internal standards as described previously (KOHNEN et al., 1990b).

are located at the α-carbon atom(s) of the alkyl side-chains of the thiophene-ring. This positional relationship may be caused by anchimeric assistance of a S-containing functional group activating the α-carbon atom(s) for further sulphurization.

Invoking phytol-derived phytadienes and geranyl-geraniol-derived polyenes as precursors for the macromolecularly S-bound phytane carbon skeletons is apparently inconsistent with the fact that neither methylthio-phytenes have been detected in the MeLi/MeI treated polar fractions nor significant amounts of phytadienes or polyenes have been detected in the pyrolysates of these polar fractions. Therefore, we speculate that the remaining double bonds of these sulphurized lipids have been hydrogenated. We emphasize that such hydrogenation reactions should occur after sulphurization of the organic matter, since KOHNEN et al. (1992b) have demonstrated for the immature Vena del Gesso sediments that saturated hydrocarbons are biosynthesized as such and were not formed by hydrogenation of alkenes. These latter compounds were not hydrogenated but acted as substrates for sulphurization. In this respect, it also noteworthy that in Recent Black Sea sediments, in which sulphate reduction is still taking place, hydrogenation of already sulphurized lipids does not occur, as revealed by the presence of polyunsaturated C_{25} and C_{30} highly branched isoprenoid thiolanes (KOHNEN et al., 1990a) and macromolecularly S-bound C_{30} highly branched isoprenoid alkenes (S. Wakeham and M. E. L. Kohnen, unpubl. data).

Analysis of the macromolecularly S-bound steroids revealed that the C-S linkages are also located at specific positions of their carbon skeletons, which is consistent with the work of ADAM et al. (1991). By means of chemolysis experiments it is established that the C-3 position of both the 5α(H)- and 5β(H)-steranes is a dominant position for intermolecular S linkages. Additionally, the presence of S linkages at C-2 or C-4 is tentatively inferred. Circumstantial evidence for the location of C-S bonds is obtained by Py-GC-MS analysis of the polar fractions. The presence of Δ^2-, Δ^3-, Δ^4-, and Δ^5-sterenes in the pyrolysates of S-rich macromolecules indicates that the S linkages are located at C-2, C-3, C-4, and C-5 of the S-bound steroidal moieties. The postulated macromolecularly S-bound 5α(H)-steranes with the C-S bond at C-2 are expected to generate not only Δ^2- but also Δ^1-sterenes upon pyrolysis. However, Δ^1-5α(H)-sterenes are thermodynamically less stable than the corresponding Δ^2-sterenes (DE LEEUW et al., 1989), which might explain the absence of Δ^1-sterenes in the pyrolysates.

We suggest that the S-bound steroid carbon skeletons result from a reaction of inorganic S species with the double bonds of sterenes or steradienes: the dehydration products of stanols and stenols, respectively. The presence of S linkages at C-2, C-3, C-4, and possibly at C-5 provides compelling evidence

for this hypothesis. Sterols dehydrate to form mainly $\Delta^{3,5}$-steradienes (DE LEEUW et al., 1989) which will incorporate S at C-3, C-4, C-5, and C-6, with probably C-5 as the most preferred position. In the case of stanols, the stereochemistry at C-5 determines whether Δ^2- or Δ^3-sterenes are formed upon dehydration (DE LEEUW et al., 1989) and thus the position of the C-S linkages; 5α(H) stanols yield mainly Δ^2-sterenes and 5β(H) stanols yield mainly Δ^3-sterenes. This is consistent with the tentative identification of the major 2-methylthio-cholestane having a 5α(H) stereochemistry in Vena del Gesso Marl-4A (Fig. 4a). It is also of note that equatorial S linkages are dominant, presumably a reflection of the thermal stability of equatorial functional groups in a ring system compared with axially positioned groups. We speculate that eventually formed macromolecularly S-bound steroids with axial S linkages will readily loose the S functional group via a 1,2-elimination reaction, reforming the precursor sterene, which then once again may incorporate S.

The considerable abundance of macromolecularly S-bound cholestanes with an axial S linkage at C-3 (peak g) in several samples hints at an alternative reaction pathway for natural sulphurization. That is, an S_N2 reaction of esterified steroids or steroidal sulphate esters with inorganic S species which will yield axial S linkages at C-3, since the ester functionalities at C-3 in naturally occurring steryl esters are equatorially positioned.

The large variations in positions and stereochemistry of the S linkages in macromolecularly S-bound steroids are probably a reflection of the many different substrates that have the ability to react with S: e.g., Δ^2-, Δ^3-, Δ^5-sterenes, $\Delta^{3,5}$-steradienes, steryl esters, and steroidal sulphate esters. Hence, the timing (i.e., before or after hydrolysis and subsequent dehydration of steryl esters) of sulphurization should have a tremendous influence on the ultimate positions and stereochemistry of the intermolecular S linkages. The timing of sulphurization is controlled by the amount of reactive Fe in the sediment. When this is exhausted, the organic matter will react with inorganic sulphur species.

Macromolecularly S-Bound Phytanyl Moieties as Biomarkers

As mentioned above, the presence of macromolecularly S-bound phytanyls with S linkages at C-1, C-2, C-3, C-4, and C-17 is thought to be a reflection of the presence of phytol-derived phytadienes in the palaeoenvironment. Hence, these macromolecularly bound compounds can be considered biomarkers for photosynthetic organisms. The major C_{20} isoprenoid thiophenes XXVII and XXVIII, which have been identified previously in these particular samples, are also thought to be the result of S incorporation into phytol-derived phytadienes (BRASSELL et al., 1986; SINNINGHE DAMSTÉ et al., 1986; RULLKÖTTER et al., 1988). These OSC are the common isoprenoid thiophenes found in many normal marine salinity sediments (BRASSELL et al., 1986; RULLKÖTTER et al., 1988; TEN HAVEN et al., 1990) and have been used by several investigators as indicators of palaeoenvironmental change (SINNINGHE DAMSTÉ and DE LEEUW, 1987; SINNINGHE DAMSTÉ et al., 1989b, 1990b; KOHNEN et al., 1990b; DE LEEUW and SINNINGHE DAMSTÉ, 1990). However, it is noteworthy that in contrast to the macromolecularly S-bound phytanyls (e.g., I–III, VI, VIII) these low-molecular-weight OSC reflect on a quantitative basis only a relatively small portion of the precursor phytadiene pool, as clearly illustrated for Vena del Gesso Marl-4A in Fig. 8.

In Sicily Seep Oil, Rozel Point Oil, and Jianghan Oil, the macromolecularly S-bound phytanyl moieties have the C-S bonds located at all positions of the phytane carbon skeleton except those at C-16, C-18, C-19, and C-20. It is suggested that these macromolecularly S-bound phytanyls are the result of S incorporation into polyenes derived from geranyl-geraniol and possibly also partly from $\Delta^{2,6}$- and $\Delta^{2,10}$-phytadienols. These precursor lipids are restricted to hypersaline environments (SINNINGHE DAMSTÉ and DE LEEUW, 1987) as moieties of certain bacteriochlorophylls. Therefore, these sulphurized diagenetic products are potential markers for a hypersaline environment. In these three samples, so-called isoprenoid midchain thiophenes (XXXIV–XXXVII) and isoprenoid bithiophenes (XXXVIII–XL) have been detected previously as well (SINNINGHE DAMSTÉ et al., 1987, 1989b). These alkylthiophenes are also thought to be formed by S incorporation into the aforementioned polyenes (SINNINGHE DAMSTÉ and DE LEEUW, 1987; SINNINGHE DAMSTÉ et al., 1989b). It is noteworthy that the macromolecularly S-bound isoprenoid thiophenes (XVII–XXI) in the Sicily Seep Oil have the S linkages at the same carbon atoms as those in the isoprenoid bithiophenes (XXXVIII–XL). Therefore these macromolecularly S-bound moieties are considered to be diagenetic products of geranyl-geraniol, like the bithiophenes, and are thus molecular markers for hypersaline palaeoenvironments. The presence of both the macromolecularly S-bound phytanyl moieties (IV, XII, XIV) and the midchain isoprenoid thiophenes in Rozel Point Oil, Sicily Seep Oil, and Jianghan Oil is in agreement with the inferred provenance of these three oils from hypersaline source rocks (TEN HAVEN et al., 1988; SINNINGHE DAMSTÉ et al., 1989b).

CONCLUSIONS

Di- or polysulphide-linked biomarkers are present in both sediment extracts and crude oils. The location of di- or polysulphide linkages in macromolecularly S-bound moieties is the same as that of monosulphide linkages. Macromolecularly S-bound phytanyl moieties are chiefly bound with S linkages located at the tertiary positions of their carbon skeletons, which indicates that the S incorporation mechanism involves intermediate carbocations. The position of the double bonds in the precursors controls the position of the S linkages. That is, phytol-derived phytenes yield mainly macromolecularly S-bound phytanyl moieties with a S linkage mainly at C-3, and geranyl-geraniol-derived polyenes mainly accumulate in macromolecules bound with S linkages at positions C-3, C-7, C-11, or C-15. Hence, these different types of macromolecular S-bound phytanyl moieties might be used as molecular markers for normal marine environments in the case of the ones which are bound at C-3, and as markers for hypersaline environments in the case of those which are bound at C-7, C-11, C-15. Macromolecularly S-bound steroids are chiefly bound with S linkages located at C-2, C-3, C-4, or C-5 of their carbon skeletons, which indicates that S incor-

poration takes place into sterenes or steradienes and thus after dehydration of sterols or stanols. It is, however, not possible to rule out the possibility that esterified sterols or stanols react with inorganic S species via an S_N2 mechanism, yielding macromolecularly S-bound steroids with an axial S linkage at C-3.

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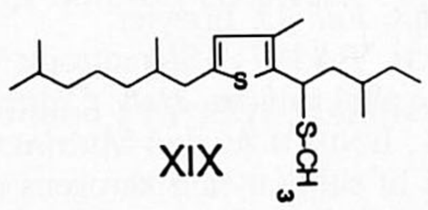
Appendix



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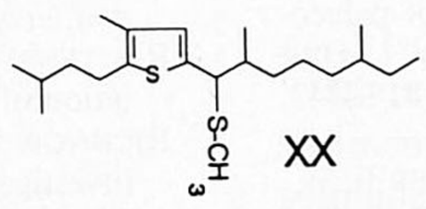

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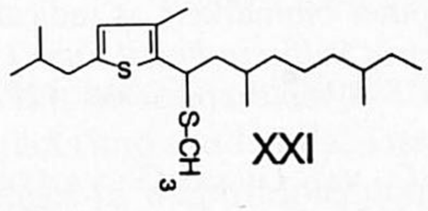

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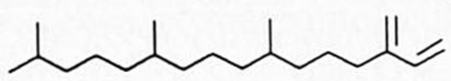

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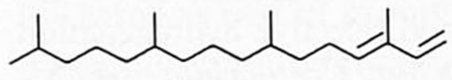

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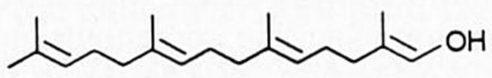

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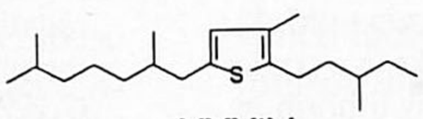

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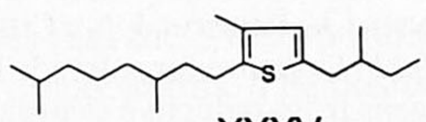

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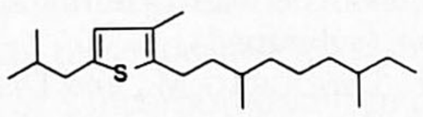

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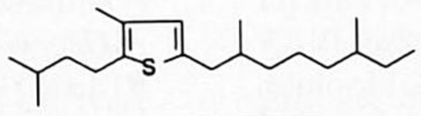

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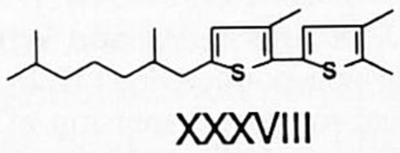

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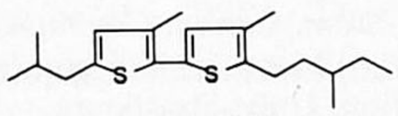

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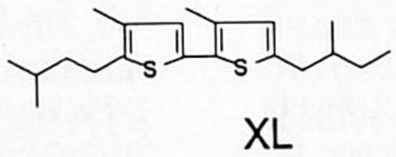

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