



Quantitative assessment of mono- and polysulphide-linked carbon skeletons in sulphur-rich macromolecular aggregates present in bitumens and oils*

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Abstract—Polar fractions of three immature sulphur-rich sediments and four sulphur-rich oils, all of Miocene age, were studied using two selective chemolytic methods, namely methyl lithium/methyl iodide, which selectively cleaves polysulphide bonds, and Raney nickel or nickel boride which cleave both mono- and polysulphide bonds. In this way the amount of polysulphide-linked *vs* mono- and polysulphide-linked carbon skeletons was assessed. Steranes, phytane and gammacerane are bound by polysulphide linkages in large amounts (30% or more) in the immature Vena del Gesso sediment whereas squalane, isorenieratane and C₃₅ hopane carbon skeletons are hardly bound at all by polysulphide-bonds only. The amounts of polysulphide-bound carbon skeletons are much lower in samples from the Northern Apennines and the Monterey Formation and consist predominantly of *n*-alkanes. Phytane and steranes are relatively more polysulphide-linked than β -carotane and *n*-alkanes in the oils. This phenomenon may be explained in part by the number of carbon-sulphur bonds by which they are bound to the macromolecular moieties, since it is statistically unlikely that moieties bound by several sulphur-bonds are linked exclusively by polysulphide-bonds. Other explanations, however, such as differences in the timing and nature of the sulphur incorporation processes have also to be considered.

Key words—organic sulphur compounds, bitumen, desulphurization, polysulphide linkages, monosulphide linkages, macromolecules

INTRODUCTION

Investigations of sulphur-rich geomacromolecules in sediments and petroleum are important for understanding the nature and origin of sulphur-containing lipids, since this may enable one to reconstruct paleobiochemical inputs from organic sulphur compounds (OSC) (e.g. Kohnen *et al.*, 1992). Furthermore, it is thought that sulphur-rich organic matter generates oil at a significantly lower maturity level than sulphur-poor organic matter (Orr, 1986). Investigations of sulphur-rich geomacromolecules are thus valuable both for paleoenvironmental studies as well as for petroleum generation studies. Due to the development of new analytical techniques much more information on OSC has become available. For example, XANES-spectroscopy allows for the direct detection and quantification of different forms of inorganic and organic sulphur (e.g. thiophene,

sulphide, sulphoxide, sulphate etc.; George and Gorbaty, 1989; Waldo *et al.*, 1991; Eglinton *et al.*, 1994). Curie-point pyrolysis breaks macromolecular networks into smaller molecules which can be analyzed by gas chromatography-mass spectrometry and thus gives information concerning specific moieties in the network (e.g. Sinninghe Damsté *et al.*, 1989a; Eglinton *et al.*, 1990).

The use of different chemical degradation methods allows for the selective release of carbon skeletons bound to the macromolecular matrix by C-S bonds. For instance, LiAlH₄ (Adam *et al.*, 1991, 1992, 1993) and MeLi/MeI (Kohnen *et al.*, 1991, 1993) selectively cleave sulphur-sulphur bonds. Based on the presence of specific functional groups (thiol and methylthio groups, respectively) the position and amount of the carbon-sulphur bonds can be determined. Other reagents such as Raney nickel (Hauptmann and Walter, 1961), nickelocene (Richnow *et al.*, 1992), Li/EtNH₂ (Hoffman *et al.*, 1992) and nickel boride (Schouten *et al.*, 1993a) cleave both S-S and C-S bonds. Products are released as hydrocarbons or, by using deuterated reagents, as deuterated compounds.

In this paper we report the results of chemical

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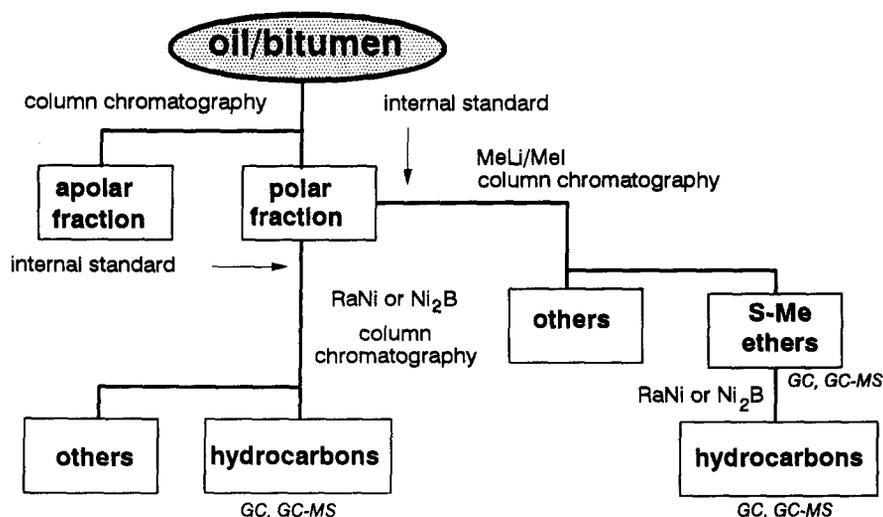


Fig. 1. Analytical flow diagram of the chemical degradation techniques used on the studied oils and sediments.

degradation of polar fractions from four oils and three sediment extracts. These were treated both by MeLi/MeI, to release polysulphide-linked carbon skeletons, and by Raney nickel or nickel boride to release both mono- and polysulphide-linked carbon skeletons. Internal standards were used to quantify the products released. In this way a qualitative and quantitative assessment of the mono- and polysulphide-linked carbon skeletons has been obtained.

EXPERIMENTAL

Chemical degradation (Fig. 1)

The polar fractions (listed in Table 1) were isolated as described by Kohnen *et al.* (1991). After adding known amounts of an internal standard [2,3-dimethyl-5-(1,1-d₂-hexadecyl)thiophene] the fractions were desulphurized either with Raney nickel (Sinninghe Damsté *et al.*, 1990) or by nickel boride (Schouten *et al.*, 1993a) and subsequently hydrogen-

ated. The hydrocarbons released were isolated by column chromatography on Al₂O₃ and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The polar fractions were, after addition of the internal standard, also treated with MeLi/MeI as described by Kohnen *et al.* (1991). The released methylthioethers were isolated by column chromatography using Al₂O₃ as stationary phase and 3 column volumes of hexane/dichloromethane (9:1 v/v) as eluent. The methylthioether fractions were analyzed by GC and GC-MS. Furthermore, they were desulphurized by either Raney nickel or nickel boride. The resulting alkane fractions were analyzed by GC and GC-MS.

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

Table 1. Data of the four oils and three sediments studied

| Name | Sediment/ oil | Polars (wt%) | Saturates (wt%) | Desulphur. (wt%) | MeLi/MeI (wt%) | MeLi/MeI: Des.* (%) | Reference |
|----------------------------|------------------|-----------------|--------------------|---------------------|-------------------|------------------------|--|
| Rozel point seep | Oil | 57 | 1 | 16 | 3.4 | 21 | Sinninghe Damsté <i>et al.</i> (1987) |
| Sicily seep | Oil | 57 | 3 | 5.7 | 0.6 | 10 | Sinninghe Damsté <i>et al.</i> (1988) |
| Jiangnan | Oil | 52 | 3 | 8.8 | 2.1 | 23 | Sinninghe Damsté <i>et al.</i> (1988) |
| Amposta | Oil | 40 | 16 | 6.4 | 2.0 | 31 | Sinninghe Damsté <i>et al.</i> (1988) |
| Vena del Gesso | Sediment | 35 | 1 | 6.0 | 2.5 | 41 | Kohnen <i>et al.</i> (1992) |
| Northern Appenines Marl | Sediment | 28 | 3 | 3.9† | 0.8 | 21 | Sinninghe Damsté <i>et al.</i> (1986) |
| Monterey formation | Sediment | 25 | 2 | 1.0† | 0.3 | 25 | Schouten <i>et al.</i> (1995) |

*Amount released by MeLi/MeI divided by amount released by RaNi or Ni₂B.

†After removal of sulphoxides.

helium as carrier gas. A flame ionization detector (FID) and a sulphur-selective flame photometric detector (FPD) were used simultaneously with a stream-splitter (split ratio FID:FPD = 1:2). The samples (in ethyl acetate) were injected at 75°C and the oven was subsequently programmed to 130°C at 20°C/min and then at 4°C/min to 320°C then held for 20 min.

Gas chromatography-mass spectrometry

GC-MS was performed with a Hewlett-Packard 5480 gas chromatograph interfaced to a VG-70S mass spectrometer or a Hewlett-Packard 5890 gas chromatograph interfaced to a VG-Autospec Ultima mass spectrometer. Both spectrometers were operated at 70 eV with a mass range m/z 40–800 and a cycle time of 1.8 s (resolution 1000). The chromatographs were 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. Samples were injected on column 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 and held there for 10 min.

RESULTS

The polar fractions of the four oils and three sediment extracts (Table 1) were treated separately with MeLi/MeI and with Raney nickel or nickel boride (Fig. 1). The first reagent selectively releases polysulphide-bound carbon skeletons (Kohnen *et al.*, 1991) while the latter releases all sulphur-bound carbon skeletons. Addition of an internal standard [2,3-dimethyl-5-(1,1-d₂-hexadecyl)thiophene] enabled quantification of the carbon skeletons released by Raney nickel desulphurization of the polar fraction. Due to the often complex composition of the methylthioether fractions, quantification was only performed after desulphurization of the methylthioether fractions. This may introduce an error in the quantitation of the polysulphide-linked carbon skeletons since it is possible that monosulphide-linked oligomers, consisting of two or more carbon skeletons bonded to each other by monosulphide-linkages only, are present in the methylthioether fractions through cleavage of a polysulphide-linkage, which connected these oligomers to the macromolecular matrix. These oligomers, which are not GC-amenable and thus difficult to analyze, will upon desulphurization add to the pool of polysulphide-bound carbon skeletons whereas they represent only "indirectly" polysulphide-bound carbon skeletons. An estimate of the error thus introduced was obtained by comparing the amounts of gammacerane in the VDG methylthioether fraction before and after desulphurization. About 90% (±10%) of the gammacerane present in the desulphurized methylthioether fraction is actually present as 3-methylthioethergammacerane which means that the amount of macromolecular-bound gammacerane is fairly

small (*ca* 10%). The reported amounts of polysulphide-linked carbon skeletons, therefore, represent reasonable estimates though they have to be considered as maximum values.

Sediments

The MeLi/MeI treatment of the polar fraction of a Vena del Gesso marl yielded a methylthioether fraction in higher amounts than the free hydrocarbon fraction (Table 1). The composition of the methylthioether fraction [Fig. 2(A)] is somewhat different to that of another marl sample of the Vena del Gesso basin described by Kohnen *et al.* (1991, 1993); 3-(methylthio)phytane is the dominant compound. Furthermore, *n*-alkanes with a methylthio group predominantly at C-2 are present. C₂₇–C₂₉ steranes, dinosterane and dinoster-22-ene, with the methylthio group mostly at C-3, dominate the steroids. A gammacerane methylthioether is tentatively identified. Based on the position of the functional group in the precursor lipid, tetrahymanol (e.g. ten Haven *et al.*, 1989), the methylthio group is tentatively located at C-3. Low amounts of C₃₂–C₃₅ hopanes (maximum C₃₄) with one methylthio group are also present. The mass spectrum of the C₃₄ hopanoid methylthioether, shows a small m/z 75 fragment, indicating that the methylthio group is located at the penultimate carbon of the side-chain.

Desulphurization of the methylthioether fraction yielded phytane, *n*-alkanes, steranes and pentacyclic triterpenoids [Fig. 2(B)]. The C₂₄₊ *n*-alkanes are present in low amounts and have an even-over-odd carbon number predominance. The dominant steroids are C₂₇–C₂₉ steranes, 4-desmethyl dinosterane, dinoster-22-ene and dinosterane. The pentacyclic triterpenoids consist mainly of gammacerane and small amounts of C₃₂–C₃₅ hopanes (maximum C₃₄). These results compare well with the carbon skeleton distribution of the desulphurized polar fraction [Fig. 2(C)]. The major difference is the presence of relatively large amounts of isorenieratane and a (partially) hydrogenated derivative in the desulphurized polar fraction which are absent from the (desulphurized) methylthioether fraction.

Quantitation shows that in the Vena del Gesso sediment the lower-carbon number *n*-alkanes (less than 28 carbons) are *ca* 30% bound by polysulphide-bonds [Fig. 3(C)]. The higher carbon number *n*-alkanes (C₂₈–C₃₄) are only *ca* 10% polysulphide-bound. For steranes this proportion averages *ca* 20%. Squalane and isorenieratane are not bound only by polysulphide-linkages. Gammacerane is exceptional in that it is *ca* 80% polysulphide-bound. In contrast, the C₃₅ hopane is bound by polysulphide-linkages to an extent of only 10%. This contrast is also demonstrated in Fig. 3(D) which shows the distribution of the pentacyclic triterpenoids in the Vena del Gesso sample. 22,29,30-Trisnorhopane, gammacerane and the C₃₃ and C₃₄ hopanes are more than 50% bound by polysulphide linkages.

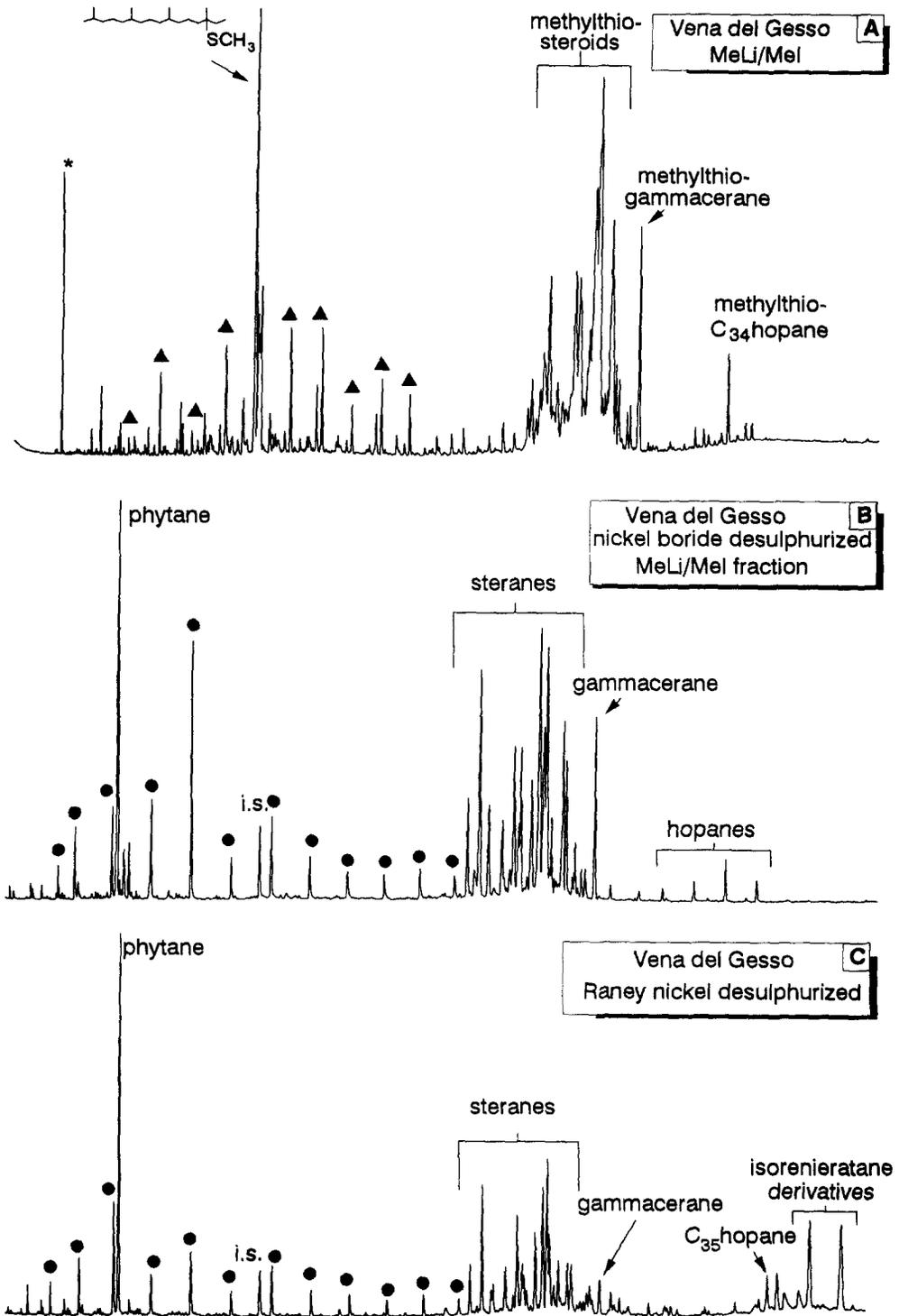


Fig. 2. Gas chromatogram of the product mixture obtained by (A) MeLi/MeI treatment of the Vena del Gesso polar fraction (B) desulphurization of the MeLi/MeI products and (C) desulphurization of the Vena del Gesso polar fraction. * = contamination; i.s. = internal standard; \bullet = n-alkanes; \blacktriangle = 2-methylthioalkanes.

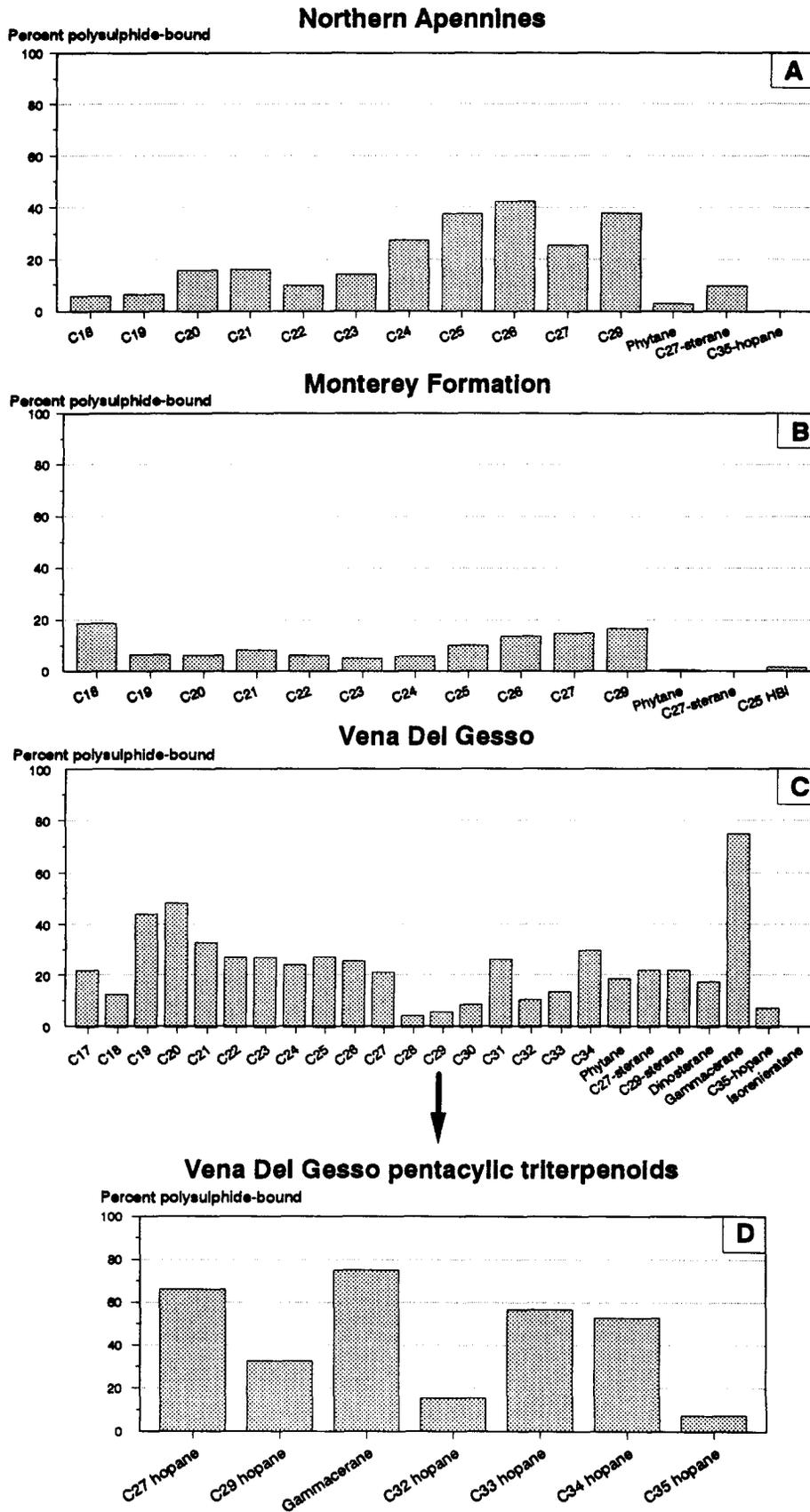


Fig. 3. Bar diagrams showing the relative amounts of polysulphide *vs* poly- + monosulphide-bound carbon skeletons in the three sediments studied.

MeLi/MeI treatment of the other two sediments yielded a more complex mixture of methylthioethers in small amounts (Table 1). Methylthioethers with phytane and cholestane skeletons were identified in

both the Northern Apennines Marl and the Monterey sample (see also Kohnen *et al.*, 1993).

The alkanes released from the desulphurized methylthioether fraction of the Northern Apennines

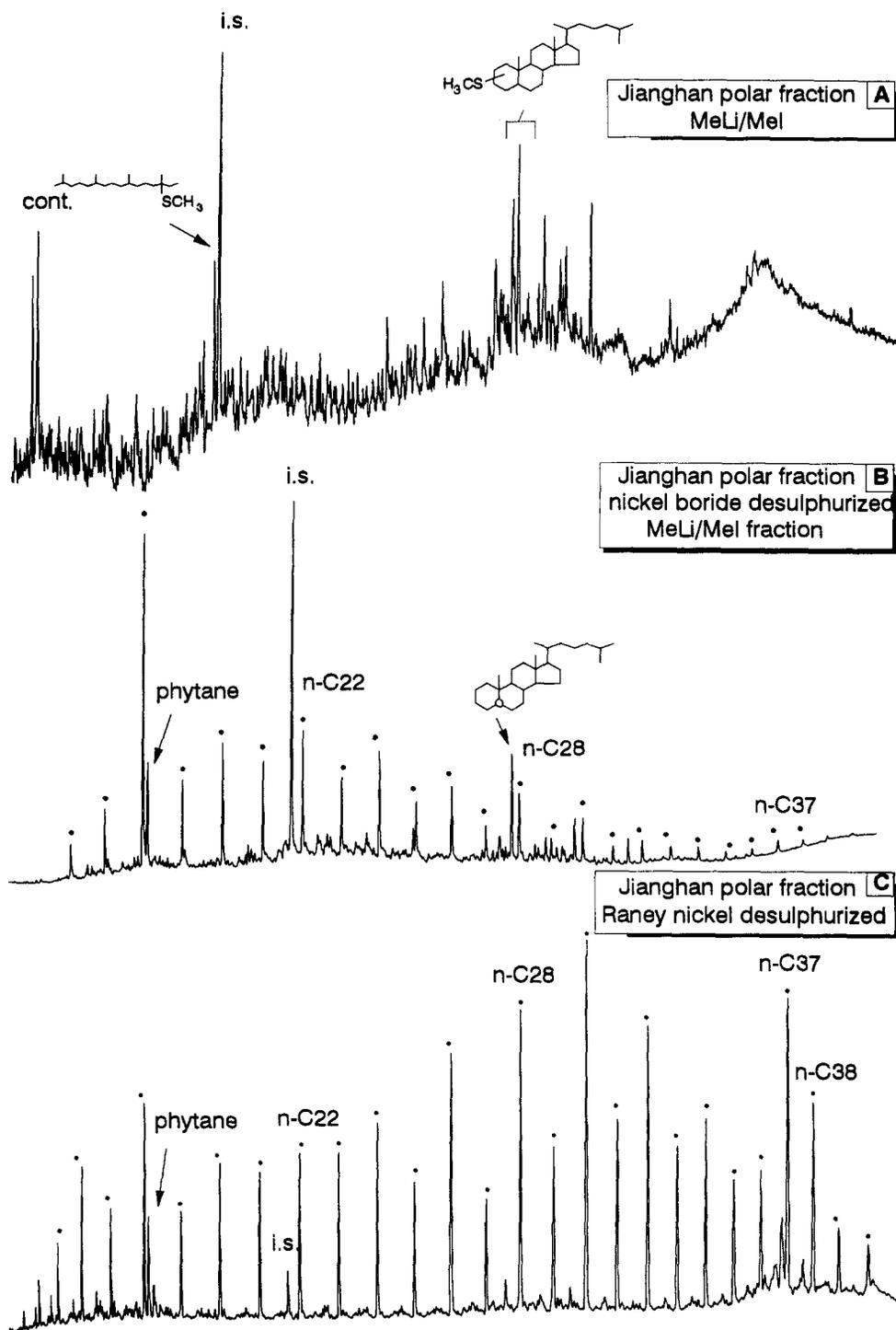
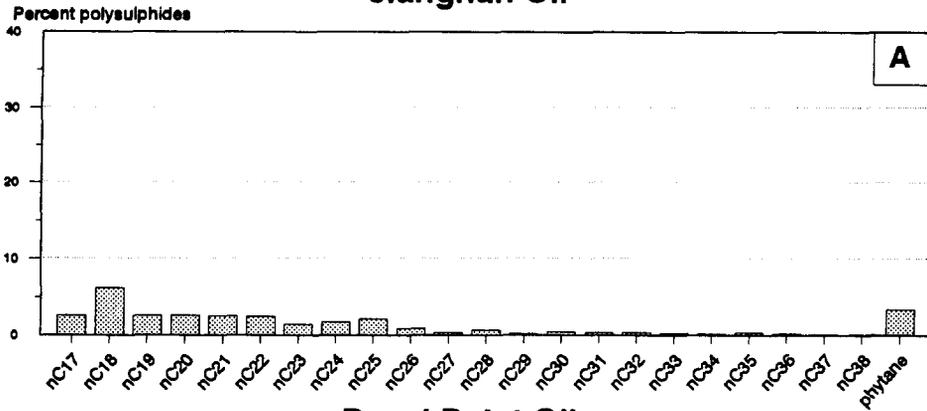
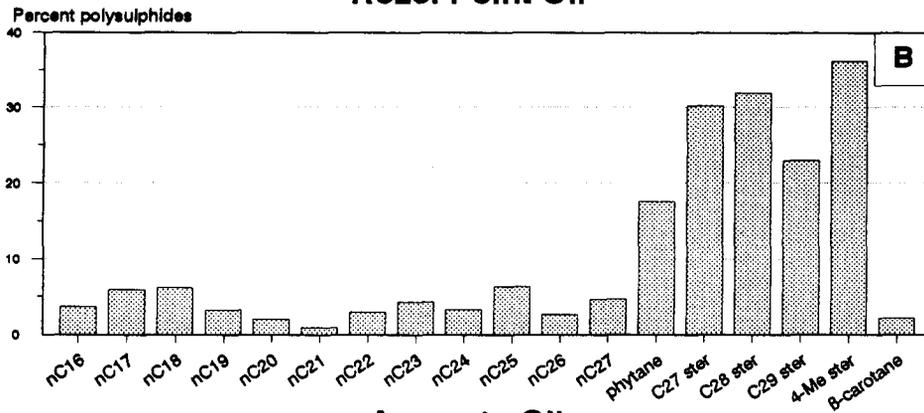


Fig. 4. Gas chromatogram of the product mixture obtained by (A) MeLi/MeI treatment of the Jiangnan polar fraction (B) desulphurization of the MeLi/MeI products and (C) desulphurization of the polar fraction of the Jiangnan oil. * = contamination; i.s. = internal standard; ● = n-alkanes.

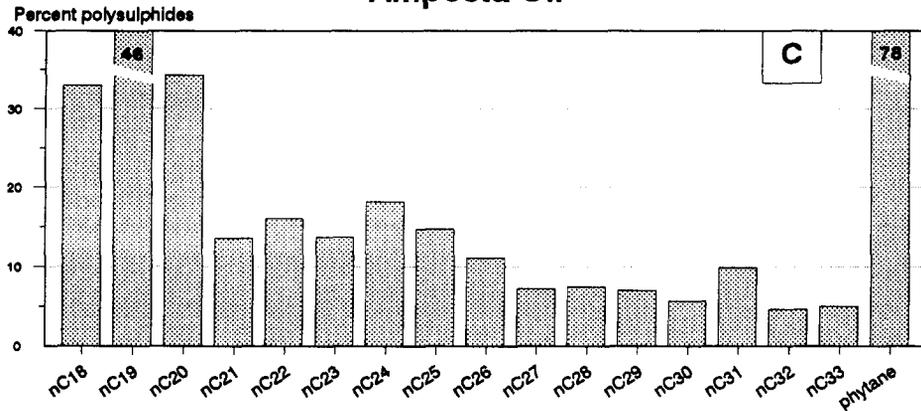
Jlanghan Oil



Rozel Point Oil



Amposto Oil



Sicily Seep Oil

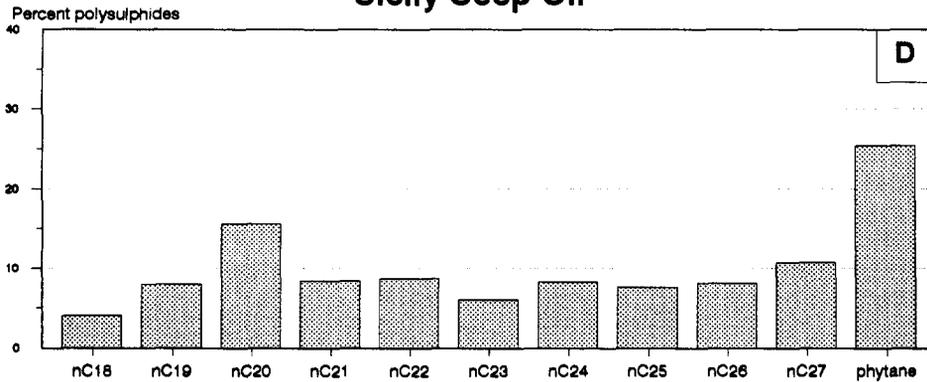


Fig. 5. Bar diagrams showing the relative amounts of polysulphide *vs* poly- + monosulphide-bound carbon skeletons in the four oils studied.

Marl were phytane, *n*-alkanes and 5 α - and 5 β -cholestane. *N*-alkanes are the main products from the Monterey sample. Phytane, steranes and a C₂₅ highly branched isoprenoid (2,6,10,14-tetramethyl-7-(3'-methylpentyl)pentadecane; C₂₅ HBI) are present in trace amounts. The C₂₅ HBI was not detected as a methylthioether due perhaps to small amounts and the multiple isomers present of methylthioethers with a C₂₅ HBI carbon skeleton. The types of carbon skeletons in the desulphurized methylthioether fraction are similar to those found in the desulphurized polar fraction.

Quantification revealed that the *n*-alkanes from the Northern Apennines Marl and Monterey samples had a different pattern of sulphur-linkages than did the Vena del Gesso sediment (Fig. 3). Higher carbon number *n*-alkanes (>24 carbons) are polysulphide-bound to a greater extent than the lower carbon number *n*-alkanes. Phytane, steranes and the C₂₅ HBI are polysulphide-bound to a minor extent only. Polysulphide-bound C₃₅ hopane is absent from the Northern Apennines Marl sample.

Oils

The gas chromatogram of the product mixture from the MeLi/MeI treated polar fraction of the Jiangnan oil shows its complex nature [Fig. 4(A)]. 3-Methylthiophytane and 3-methylthiocholestane were identified as discrete components. Mass chromatograms of *m/z* 75, 89, 103 etc. revealed the presence of *n*-alkanes with methylthio groups at C-2, C-3, C-4, etc. *n*-Alkanes with a methylthio group at the terminal carbon atom are, however, present in relatively low abundance.

The desulphurized methylthioether fraction of the Jiangnan oil consists predominantly of *n*-alkanes and phytane [Fig. 4(B)]. Linear carbon skeletons are now clearly present, in contrast to the complex mixture of methylthioethers in the MeLi/MeI treated polar fraction [Fig. 4(A)]. The types of carbon skeletons in the desulphurized methylthioether fraction are similar to those obtained by desulphurization of the total polar fraction [Fig. 4(C)], although the distribution is different.

Quantification of the different fractions of the Jiangnan oil shows that the higher *n*-alkanes (C₂₆–C₃₈) are polysulphide-bound to less than 1% [Fig. 5(A)]. The lower *n*-alkanes and phytane are polysulphide-bound up to 7%.

In the case of the Rozel Point seep oil the product mixture after MeLi/MeI-treatment was dominated by C₂₇–C₃₀ steranes with a methylthio group at C-2, C-3 and C-4 (see also Kohnen *et al.*, 1993). Furthermore, phytane with a methylthio group at C-3 was a major product. The gas chromatogram does not reveal specific straight chain methylthioethers although mass chromatography reveals their presence as a homologous series as for the Jiangnan oil. This indicates that methylthioethers with a straight chain

carbon skeleton are present but as a complex mixture of many isomers.

The desulphurized methylthioether fraction of the Rozel Point seep oil is dominated by C₂₇–C₃₀ steranes, phytane and small amounts of *n*-alkanes and β -carotane. The presence of β -carotane indicates that this carbon skeleton is also released as a (poly)-methylthio derivative on MeLi/MeI treatment of the polar fraction but as a complex mixture and in low abundance. The types of carbon skeletons in the desulphurized methylthioether fraction is similar to that of the desulphurized polar fraction although their distribution is different.

The quantitative data for the Rozel Point seep oil [Fig. 5(B)] show that phytane and steranes are up to 30% exclusively polysulphide-bound. The *n*-alkanes and β -carotane, however, are for the greater part bound by at least one monosulphide bond since they are only less than 10% polysulphide-bound.

The methylthioether fractions of the Sicily seep and Amposta oils are similar in composition to the Jiangnan oil, i.e. phytane and *n*-alkanes are the main carbon skeletons. Furthermore the desulphurized methylthioether fractions have carbon skeletons comparable to those present in the desulphurized polar fraction.

Quantitation of the fractions shows that in the Amposta oil the high carbon-number *n*-alkanes are only polysulphide-bound to a minor extent whilst lower *n*-alkanes and phytane are polysulphide-bound to a significant extent [up to 78%; Fig. 5(C)]. In the Sicily seep oil the *n*-alkanes are in general exclusively polysulphide-bound up to 15%, in contrast to phytane at ca 25% [Fig. 5(D)].

DISCUSSION

Sediments

Quantitative analyses of the compounds in the desulphurized methylthioether fraction, and the desulphurized polar fraction, revealed substantial differences in the relative amount of polysulphide linkages per carbon skeleton. Phytane and the steranes are bound predominantly by one sulphur link since phytane and steranes possessing 2 or more methylthioether groups, as reported by Kohnen *et al.* (1991) in another marl sample treated in a similar way, were present in low abundance in our Vena del Gesso sample. For these compounds an average of 20% of the sulphur-linkages are polysulphidic in nature (Fig. 3). From the products released by MeLi/MeI, the amount of compounds bound by several (poly)-sulphur linkages can be calculated. For instance, a carbon skeleton bound by two sulphide-linkages of which 20% each are polysulphidic in nature, should yield ca 4% ($0.2 \times 0.2 \times 100\%$) of the compound on treatment with MeLi/MeI. Similarly, for compounds bound by three sulphur linkages this figure is ca 0.8% and for compounds bound by four

sulphur linkages it is *ca* 0.2%. Thus, compounds bound by multiple sulphur linkages will be trace components in the methylthioether fraction. This is probably the reason for the very low amounts of exclusively polysulphide-bound squalane, isorenieratane and C₃₅ hopane skeletons compared to the sterane and phytane skeletons in the Vena del Gesso sediment. These compounds have biochemical precursors which possess multiple functionalities. The C₃₅ hopane carbon skeleton is derived from bacteriohopanetetrol (Valisolalao *et al.*, 1984), isorenieratane from isorenieratene (Kohnen *et al.*, 1992; Sinninghe Damsté *et al.*, 1993) and squalane from squalene. The double bonds, present as such or diagenetically formed, reacted with sulphur and formed a structure linked by multiple sulphur-bonds to a macromolecular network. Statistically, this means that only a small part of the molecules will be linked exclusively to polysulphide-bonds.

Gammacerane is the most abundant (*ca* 78%) exclusively polysulphide-bound compound (Fig. 3) in the Vena del Gesso sediment. The precursor for this compound is probably tetrahymanol (e.g. ten Haven *et al.*, 1989) or a diagenetic derivative. Since this molecule possess only one functional group, it is not surprising that it is bound by one sulphur-link only. The percentage of polysulphide linkage is substantially higher than for most of the other carbon skeletons linked by one sulphur-bond (Fig. 3). This difference must thus have been caused by factors other than the number of sulphur-linkages per compound. One factor may be the timing of sulphur incorporation into the different precursor lipids. It has been shown recently *via* simulation experiments that several sulphur species may react with functionalized lipids. Hydrogen sulphide (Fukushima *et al.*, 1992) and especially hydrogen polysulphides (de Graaf *et al.*, 1992, 1995; Krein and Aizenshtat, 1994; Schouten *et al.*, 1994) are species which may react with lipids under varying environmental conditions and form sulphur compounds in different ways. Thus, depending on the availability of the precursor molecule and the reactive sulphur species present, different relative amounts of polysulphide-bound carbon skeletons may be formed. Another possibility is that the type of functional group (e.g. ketone, aldehyde, mid-chain double bond, terminal double bond, etc.) which reacted with sulphur may be one of the controlling factors for the amount of polysulphide- *vs* monosulphide-linkages. Simulation experiments by de Graaf *et al.* (1995), Schouten *et al.* (1994) and Krein and Aizenshtat (1994) revealed that there are substantial differences in the types of sulphur-linkage in the sulphur compounds formed from different substrates.

The differences in the distributions of the pentacyclic hopanoids in the Vena del Gesso sample are striking (Fig. 3). Gammacerane appears to be bound by only one sulphur-bond. The precursor for the C₃₂-C₃₅ hopanes is the C₃₅ hopanetetrol (or deriva-

tives). It might be expected that hopanes with lower carbon numbers, and thus shorter side-chains, have fewer sulphur-links and are thus to a greater extent polysulphide-bound. This is true for the C₃₃ and C₃₄ hopanes but not for the C₃₂ hopane. This molecule may thus have another precursor (e.g. C₃₂ hopenes) with a different number and types of functionality. The C₂₇ and C₂₉ hopanes probably have different precursors than the extended hopanes. Possibly their precursors incorporated sulphur at different times or possessed different functional groups from the other homologs.

In the Northern Apennines marl and Monterey sediment carbon skeletons are bound by polysulphide-links to a much lesser extent than those in the Vena del Gesso sediment. Possibly the (in)organic sulphur species differed in the respective depositional environments, or the amount of polysulphides decreased in the Northern Apennines Marl and the Monterey Formation through diagenesis since polysulphides bonds are weaker than monosulphide bonds. It is striking that the *n*-alkane skeletons occur more exclusively polysulphide-bound than do sterane, phytane or the C₂₅ HBI skeletons. This may be explained by the difference in the number of functionalities, but other explanations such as differences in timing of sulphur incorporation and the type of functional groups may also have to be invoked.

Oils

It may be surprising to find relatively high amounts of carbon skeletons exclusively bound *via* polysulphide-links in the oils studied. Such bonds may be relatively easy to cleave during oil-generation. The oils studied are, however, "low thermal stress" oils. For instance, the sterane biomarkers in the Rozel Point seep oil do not show a high degree of "thermally-induced isomerization" and thus there was a potential for polysulphide-bonds to survive. In fact these oils may not be "classical" oils in that they are not generated from kerogens on thermal stress; i.e. they may simply represent sulphur-rich bitumens (Sinninghe Damsté *et al.*, 1989b).

The methylthioether fractions of the Sicily seep, Jiangnan and Amposta oils consist mainly of a complex mixture of methylthioethers with a straight-chain skeleton. These results compare well with those of Sinninghe Damsté *et al.* (1989b) who showed that in the apolar fractions the thiolane distributions are dominated by those possessing *n*-alkane skeletons. This suggests that the precursor lipids were *n*-alkenes possessing double bonds which were more or less randomly distributed over the carbon skeletons. The relatively low abundance of *n*-alkanes with a methylthio group at the terminal carbon atom indicates that the inorganic sulphur species reacted primarily according to Markovnikov's rule (Kohnen *et al.*, 1993; de Graaf *et al.*, 1992). Alternatively the sulphur-links were mostly at the penultimate carbon atom to begin with, as is the case of the highly

immature Vena del Gesso sediment, but diagenesis, elimination of the polysulphide links could be followed immediately by addition (according to Markovnikov's rule) of the adjacent nearby and reactive polysulphide-moiety. This could spread the polysulphide-links throughout the *n*-alkyl chain. This process may happen during early diagenesis since the oils are of low thermal maturity.

The C₃₇ and C₃₈ *n*-alkanes are present in relatively high abundance in the desulphurized polar fractions of, for instance, the Jiangnan oil (Fig. 4). The same holds for β -carotane in the Rozel Point seep oil. Their amounts in the desulphurized methylthioether fraction are, in contrast, low. The reason for this becomes clear when their precursors are considered. The C₃₇ and C₃₈ *n*-alkanes are probably derived from alkenones possessing 2 or 3 double bonds (Schouten *et al.*, 1993b). Both the double bonds and the keto-group can react and form an alkyl moiety linked by several sulphur-bonds to a macromolecular network. The same holds for β -carotane which is derived from the polyene β -carotene.

Steranes and phytane are polysulphide-bound in significant amounts (*ca* 15–35%) in the Rozel Point seep oil. This result is at variance with the data of Adam *et al.* (1992) who concluded that only 3% of the sulphide-bonds of mono-linked steroids are polysulphidic in nature. Reasons for this discrepancy may be either differences in the macromolecular fraction studied ("non-polar macromolecular fraction" *vs* "polar fraction") or the different reagents used for the selective cleavage of S—S bonds (LiAlH₄ *vs* MeLi/MeI). LiAlH₄ under the conditions used may not quantitatively cleave di- and polysulphide-bound carbon skeletons in polar fractions, in contrast to MeLi/MeI (Kohnen *et al.*, 1991).

CONCLUSIONS

Chemolytic treatments of polar fractions of four oils and three sediments have released substantial amounts of products. Many of these compounds can be related to specific functionalized precursors which reacted with sulphur to form macromolecular aggregates. In particular sediments C₃₃ and C₃₅ hopanes, gammacerane and *n*-alkanes are bound by polysulphide-links, sometimes in large amounts. Other mono-linked carbon skeletons, such as steranes and phytane are significantly less polysulphide-bound. This may be due to the type of functional groups which reacted with sulphur, and the timing of the sulphurization reaction(s), during deposition. Isorenieratane, squalane and the C₃₅ hopane carbon skeletons are bound by several sulphur-linkages in the polar fraction, of which at least one is a mono-sulphide bond. Results obtained for the suite of oils indicate that phytane and steranes can for a significant part be bound by polysulphide-bonds, in contrast to C₂₅–C₃₈ *n*-alkanes and β -carotane. Since relatively many polysulphide-bonds are present in

these oils, it is suggested that they did not undergo any significant thermal stress and that these oils, in essence, are sulphur-rich bitumens as suggested before (Sinninghe Damsté *et al.*, 1989b).

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