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Occurrence of two novel benzothiophene hopanoid families in sediments

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Summary—Two novel families of C_{34}–C_{35} benzothiophene hopanoids have been identified in laminated marlstones and limestones of Hauptdolomit (Germany, Upper Triassic), Calcaires en Plaquettes (France, Upper Jurassic) and Ghareb (Jordan, Upper Cretaceous) Formations. Structures were assigned on the basis of Raney nickel desulphurization, deuteriated nickel boride desulphurization, mass spectral data and GC behaviour. Based on quantitative analysis, a precursor–product relationship between thiophene hopanoids and benzothiophene hopanoids seems likely. This means that the novel hopanoids are not formed by sulphur incorporation into benzohopanes, but instead cyclization and aromatization of the hopanoid side chain occur after sulphur incorporation.

Key words—aromatization, benzohopanes, benzothiophene hopanoids, thiophene hopanoids, organic sulphur compounds

INTRODUCTION

C_{30+} hopanoid biomarkers are widespread in the geosphere. During diagenesis the precursor bacteriohopanepolyol derivatives (Rohmer *et al.*, 1993) are subjected to different chemical reactions leading to a myriad of structures. For example, thiophene hopanoids (I, see Appendix), products of early diagenetic sulphur incorporation, have been reported by Valisolalao *et al.* (1984) and Sinninghe Damsté *et al.* (1989). Benzohopanes (II) are probably formed by side chain cyclization and aromatization (Hussler *et al.*, 1984). Recently, it has become clear that side chain cyclization and aromatization of extended hopanoids can also give rise to a second series of benzohopanes with the aromatic ring condensed to the D and E ring (III) (Schaeffer *et al.*, 1995). Here we report on two novel families of benzothiophene hopanoids (IV, V) which are diagenetically formed by both sulphur incorporation and aromatization reactions.

EXPERIMENTAL

Sample descriptions

Laminated marlstones (TOC = 23.6%) and carbonates (TOC = 0.3%) of the Hauptdolomit Formation are from Norian (Upper Triassic) strata in the Northern Calcareous Alps. These sediments were deposited in restricted, subtidal areas of extended carbonate platforms surrounding the Tethys ocean (Köster *et al.*, 1988). Samples were taken from outcrops near a former oil shale mine in the Isar valley (Schröfeln, Bavaria/Germany).

Bituminous coccolithic limestones (TOC = 6.2%) of the Kimmeridgian (Upper Jurassic) Calcaires en Plaquettes Formation were taken from a quarry near the village of Orbagnoux (French Southern Jura, 50 km southwest of Geneva). These parallel laminites are found in a sedimentary sequence together with undulating laminites, massive limestones and laminated limestones, all deposited in a lagoonal environment (Tribovillard *et al.*, 1992; Bernier, 1984).

The organic-rich marlstones (TOC = 17.9%) of the Upper Cretaceous Ghareb Formation are from the El Lajjun deposit in Central Jordan. This near surface (i.e. exploitable by open-cast mining) deposit is located 100 km south of Amman. Sedimentation of the Ghareb oil shales took place in a number of restricted small basins on the edge of the Tethys ocean. Further geological background information on the El Lajjun deposit and other Ghareb oil shale deposits is given by Hufnagel (1984).

Extraction and fractionation

The powdered samples (*ca* 100 g) were Soxhlet extracted with methanol/dichloromethane (1:7.5, v/v) for 24 h. Asphaltenes were removed from the extracts by precipitation in *n*-heptane. Aliquots of the maltene fractions (*ca* 250 mg), to which a mixture of four standards was added for quantitative analysis (Kohnen *et al.*, 1990), were separated into two

fractions using a column (25 cm x 2 cm; column volume 35 ml) packed with alumina (activated for 2.5 h at 150°C) by elution with *n*-hexane/dichloromethane (9:1, v/v; 150 ml; "apolar fraction") and dichloromethane/methanol (1:1, v/v; 150 ml; "polar fraction"). Aliquots (*ca* 10 mg) of the apolar fractions were further separated by argentation thin layer chromatography using hexane as developer. The $AgNO_3$-impregnated silica plates (20 x 20 cm; thickness 0.25 mm) were prepared by dipping them in a solution of 1% $AgNO_3$ in $MeOH/H_2O$ (4:1, v/v) for 45 s and subsequent activation at 120°C for 1 h. Four fractions (A1, R_f = 0.85–1.00; A2, R_f = 0.35–0.85; A3, R_f = 0.06–0.35; A4, R_f = 0–0.06) were scraped off the TLC plate and ultrasonically extracted with ethyl acetate (× 3).

Raney nickel desulphurization

The A3 fraction was dissolved in 4 ml ethanol together with 0.5 ml of a suspension of Raney nickel (0.5 g/ml ethanol) and refluxed under a nitrogen stream for 1.5 h. The desulphurization products were isolated by centrifugation and subsequent extraction with dichloromethane (× 4). The combined extracts were washed (× 3) against NaCl-saturated, double-distilled H_2O, dried with $MgSO_4$ and evaporated to dryness. The extract was taken up into a small volume of ethylacetate (2 mg extract/ml) to be analysed by GC and GC–MS.

Desulphurization using deuteriated nickel boride

The A3 fraction was dissolved in 4 ml of a dry MeOD/tetrahydrofuran mixture (1:1, v/v). Subsequently, 100 mg of anhydrous $NiCl_2$ was added under a nitrogen stream. The solution was stirred and 100 mg $NaBD_4$ was added slowly. The reaction mixture was refluxed under a stream of nitrogen for 1 h. The desulphurization products were isolated by centrifugation and subsequent extraction (× 3) with a dichloromethane/methanol mixture (1:1, v/v). The combined extracts were then treated as above.

Gas chromatography

Gas chromatography (GC) was performed using a Carlo Erba 5300 or a Hewlett–Packard 5890 instrument, both 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. Compounds analysed on the Carlo Erba 5300 were detected by a flame ionization detector (FID). Compounds analysed on the Hewlett–Packard 5890 were detected by both a flame ionization detector (FID) and a sulphur-selective flame photometric detector (FPD), using a stream-splitter with a split FID:FPD ratio = *ca* 1:2. The samples were injected at 70°C and the oven was programmed to 130°C at 20°C/min and then at 4°C/min to 320°C, at which it was held for 15 min.

Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) was carried out on a Hewlett–Packard 5890 gas chromatograph interfaced to a VG Autospec Ultima mass spectrometer operated at 70 eV with a mass range m/z 50–800 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 0.12 μm). Helium was used as carrier gas. The samples were injected at 60°C and the oven was programmed to 130°C at 20°C/min and then at 4°C/min to 320°C, at which it was held for 15 min.

Quantitation

Benzohopanes II and thiophene hopanoids were quantified by integration of their peaks in the FID-trace and comparison with the internal standard. The most abundant members of the benzothiophene hopanoids and benzohopanes III were quantified in the same way. Remaining members of these series were quantified by integration of M^+ and $M^+ - 15$ mass chromatograms and comparison with the earlier established concentrations of the most abundant members, assuming that the relative contributions of these ions to the total ion current do not change significantly from one member to another.

RESULTS

GC and GC–MS analyses of diaromatic (A3) fractions of a marlstone and carbonate of the Hauptdolomit Formation, a marlstone of the Ghareb Formation and a coccolithic limestone of the Calcaires en Plaquettes Formation (CPF) revealed a number of novel compounds eluting at the end of the gas chromatograms (e.g. Fig. 1). The response on the sulphur-selective FPD indicated that they probably contain one sulphur atom, in accordance with the mass deficiency of the molecular ions and the M^+, $M^+ + 1$ and $M^+ + 2$ distributions in their mass spectra. In the Ghareb marlstone only one of the novel compounds, characterized by the mass spectrum shown in Fig. 2(c), was present. Upon Raney nickel desulphurization this component disappeared and an equal amount of the C_{35} benzohopane II emerged. The benzohopane was identified by comparison of its mass spectrum with that published (Hussler *et al.*, 1984). Since benzohopanes were absent in the original A3 fraction this established the benzohopane carbon skeleton of type II for the novel compound. Besides this novel compound, the coccolithic limestone of the CPF and the marlstone of the Hauptdolomit Formation contained a novel family of compounds characterized by the mass spectra shown in Fig. 3(b) and (c). Raney nickel desulphurization transformed them into the C_{34}–C_{35} benzohopanes III recently identified by Schaeffer *et al.* (1995). Finally, the A3 fraction of the Hauptdolomit carbonate was desulphurised using deuteriated nickel boride

(Schouten *et al.*, 1993). This A3 fraction contained two members of each novel hopanoid family [for mass spectra see Figs 2(b) and (c) and 3(b) and (c)], and also series of benzohopanes II and III (Fig. 1). These latter components usually end up in the A2 fraction. After desulphurization the novel compounds had largely disappeared and the C_{35} and C_{34} members of benzohopanes II and III, respectively, had significantly increased (Fig. 1), which could be accounted for by the decrease in concentration of the novel compounds. These desulphurization experiments established the benzohopane carbon skeleton of type II for compounds with the mass spectra shown in Fig. 2(b) and (c), and the benzohopane carbon skeleton of type III for compounds with the mass spectra shown in Fig. 3(b) and (c).

The molecular ions at m/z 488 and 502 in the mass spectra of the novel components indicate molecular formulae of $C_{34}H_{48}S$ and $C_{35}H_{50}S$. This suggests the presence of a thiophene moiety condensed to the aromatic ring of both benzohopane isomers. Indeed, the mass spectra of the novel compounds show a similarity with those of benzohopanes II and III [Figs 2(a) and 3(a)]. Major fragments in the spectra of the novel hopanoids have shifted an equal number of mass units compared to the major fragments in the spectra of the resultant benzohopanes (see Hussler *et al.*, 1984; Schaeffer *et al.*, 1995) due to the presence of the thiophene group. The incorporated sulphur atom, thus, has little effect on the fragmentation pathway. Differences between the spectra of the benzohopanes and the novel hopanoids, besides the consistent shift of the major fragments, largely concern the relative intensities of the fragments. The spectra of the benzothiophene hopanoids are dominated by their molecular ions, whereas benzohopanes II and III have a base peak at m/z 191 and $M^+ - 15$, respectively, this latter probably mainly due to loss of the methyl group at position 18.

For every benzohopane skeleton two different benzothiophene hopanoid isomers (IV–VII) can be envisaged. Dreiding molecular models indicate that

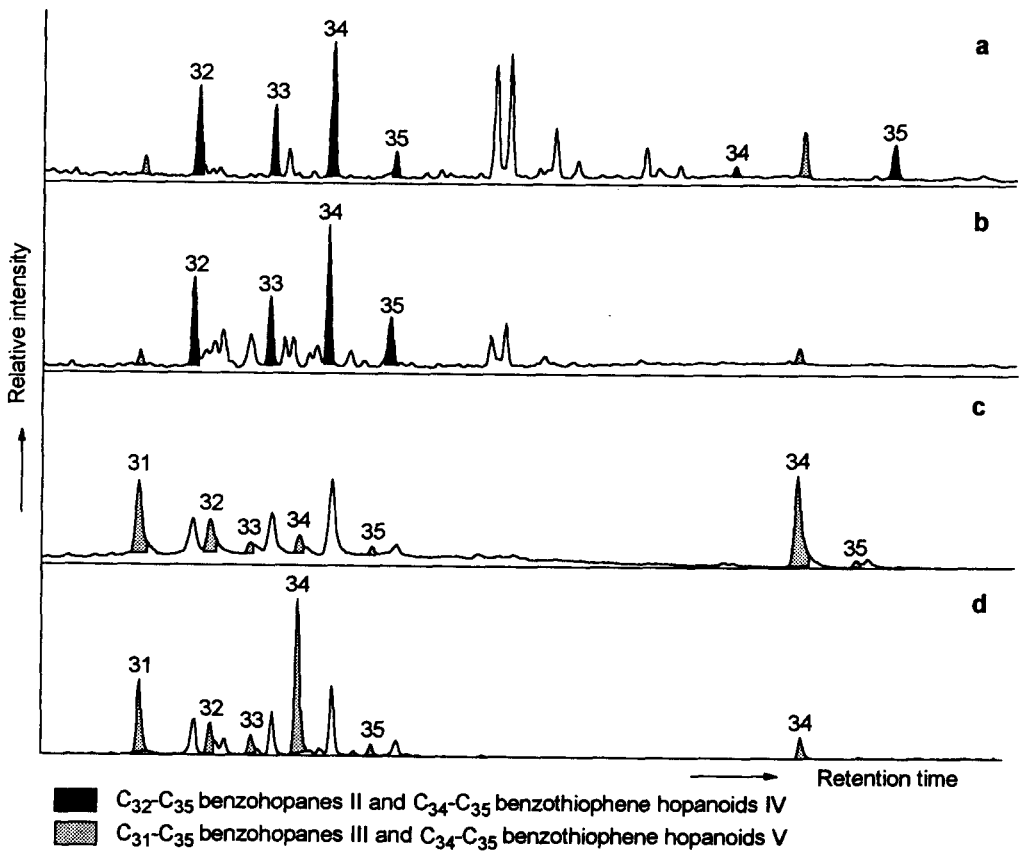

Fig. 1. Distributions of benzohopanes and benzothiophene hopanoids in the A3 fraction of the Hauptdolomit carbonate before and after desulphurization. FID trace (a) before and (b) after deuteriated nickel boride desulphurization. Summed mass chromatogram of m/z 403 + 417 + 431 + 445 + 448 + 449 + 459 + 462 + 463 + 473 + 487 ($M^+ - 15$ fragments of benzohopanes III and benzothiophene hopanoids V) (c) before and (d) after deuteriated nickel boride desulphurization. Note that $M^+ - 15$ fragments of benzohopanes with only one or two deuterium atoms are not shown, because they coincide with M^+ and $M^+ + 1$ fragments of the more abundant benzohopanes II and benzothiophene hopanoids IV.

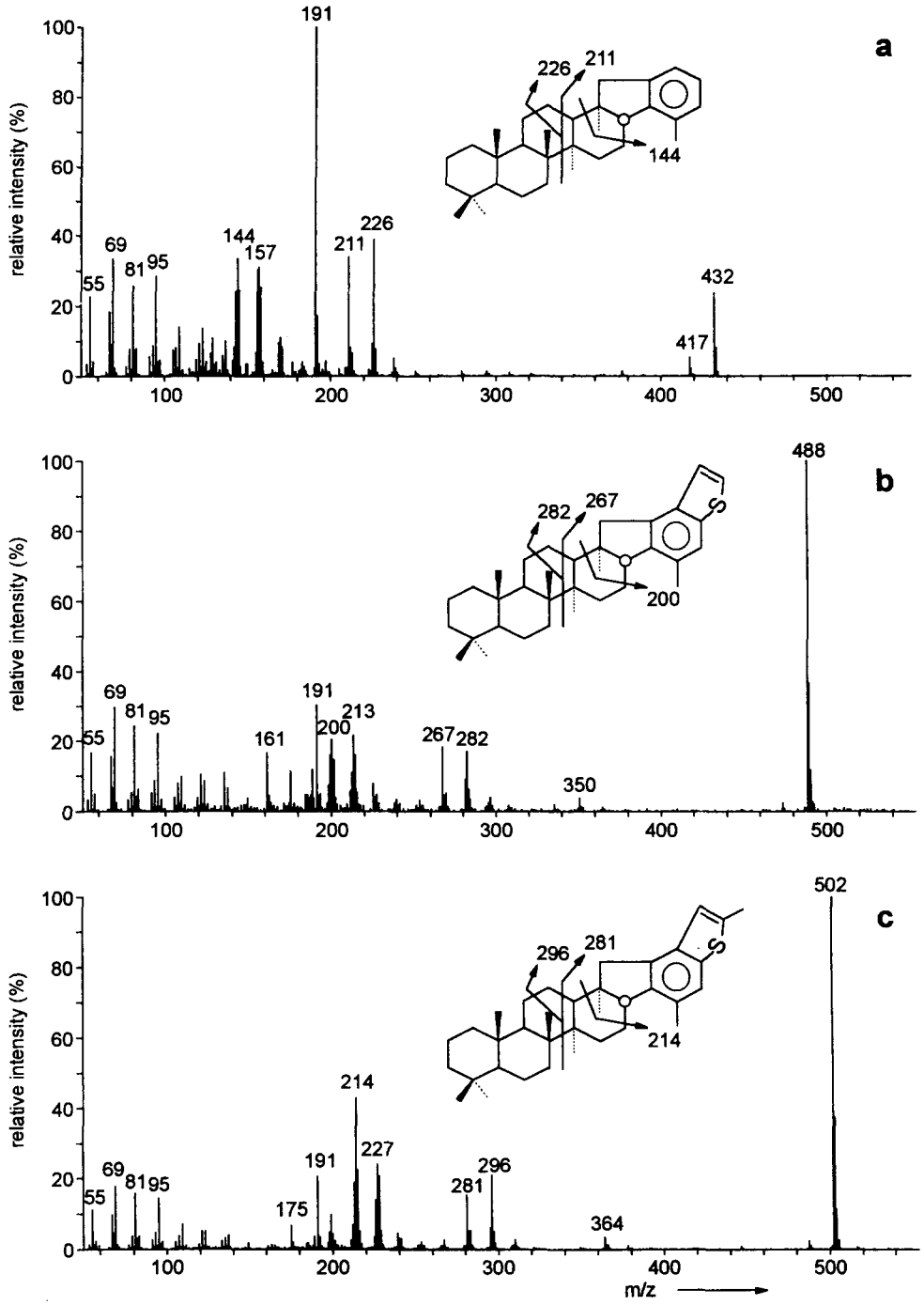

Fig. 2. Mass spectra of (a) C_{32} member of benzohopanes II, (b) C_{34} benzothiophene hopanoid IVa, (c) C_{35} benzothiophene hopanoids IVb.

structures VI and VII experience far more steric stress than IV and V. Structure VI can probably be excluded as a possible isomer because of high ring stress. Mass spectral data, however, provide sufficient evidence to assign IV and V as the actual structures of the novel hopanoids. Their mass spectra are perfectly explained by these isomers (Figs 2 and 3), whereas for isomers VI and VII different fragmentations are expected. In case of isomer VIb the $M^+ - 15$ fragment is expected to be a major peak in its mass spectrum, because at both sides of the thiophene moiety, β cleavage will lead to loss of a methyl group, especially at position 18. Clearly the spectrum shown in Fig. 2(c) does not fit this feature. Isomers VIIb–c are rejected because they can

not possibly account for the high intensity of the m/z 228 and 242 peak [Fig. 3(b) and (c)]. In addition, β cleavage at both sides of the thiophene moiety, especially at position 14, is expected to yield a $M^+ - 15$ fragment that surpasses the molecular ion in intensity. This is clearly not the case [Fig. 3(b) and (c)].

Additional evidence for the structural positions of the sulphur atoms in the novel hopanoids was obtained from the mass spectra of the deuteriated benzohopanes (Fig. 4) released by deuteriated nickel boride desulphurization. Each of the released benzohopanes had incorporated four deuterium atoms. Because of coelution with the free benzohopanes it was not possible to get pure spectra of the deuterated benzohopanes; it is, however, clear that the deuterium atoms are not located at the carbon atoms

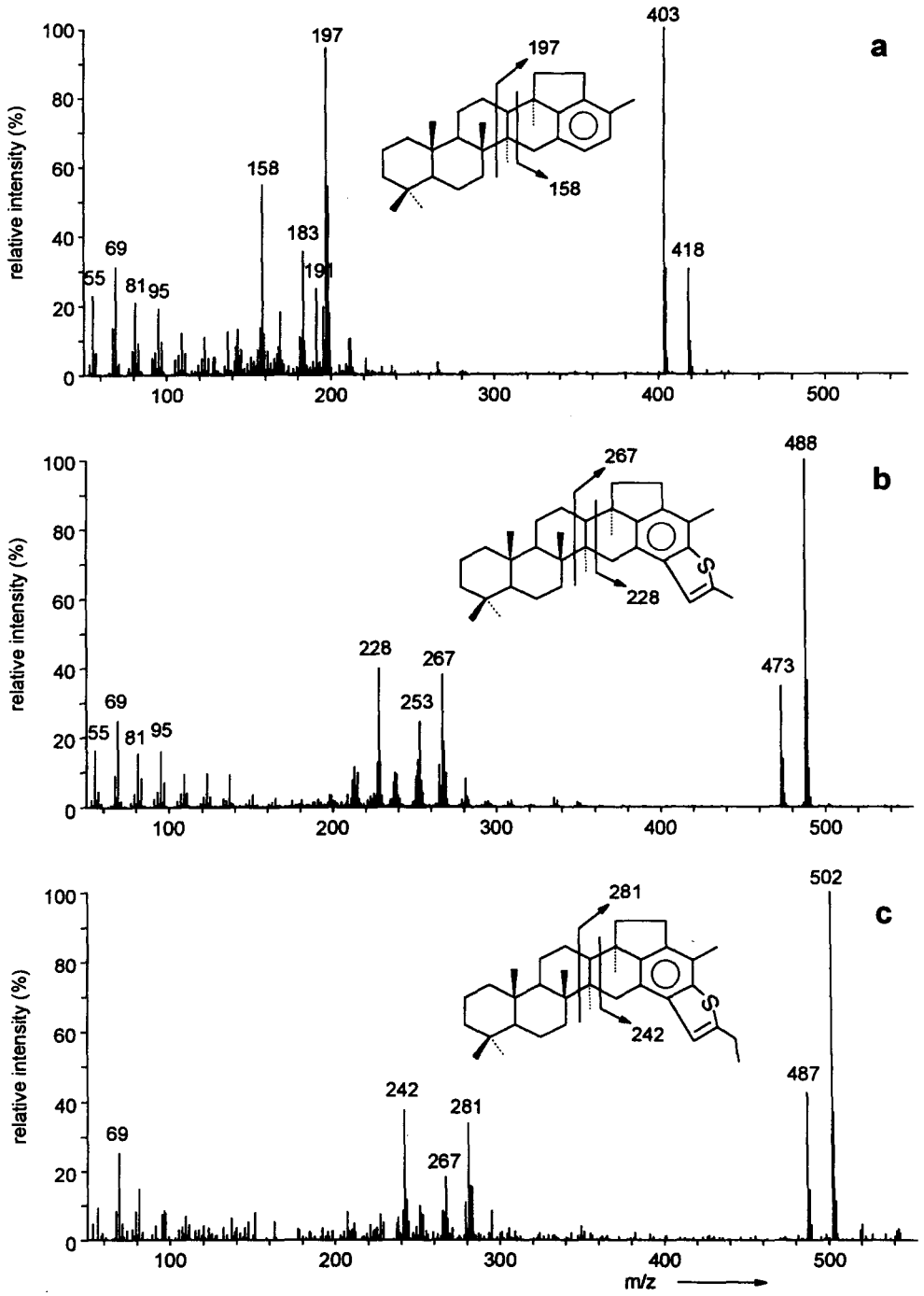

Fig. 3. Mass spectra of (a) C_{31} member of benzohopanes III, (b) C_{34} benzothiophene hopanoid Vb, (c) C_{35} benzothiophene hopanoid Vc.

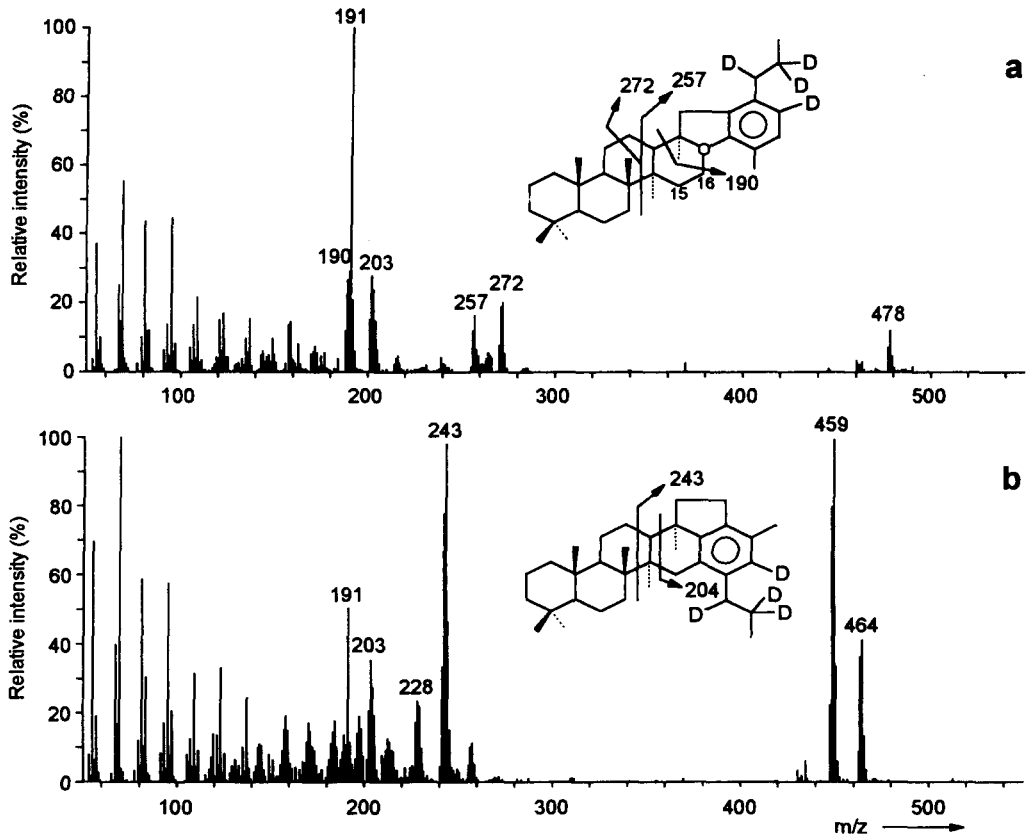

Fig. 4. Mass spectra of deuterated benzohopanes obtained after desulphurization of novel benzothiophene hopanoids. (a) Deuterated C_{35} member of benzohopanes II; (b) deuterated C_{34} member of benzohopanes III. Spectra were taken at one or two scans before peak maxima in order to reduce the signal of the coeluting non-deuterated benzohopanes.

of the A, B (or C-ring or their methyl substituents) nor at C–15 and C-16 of the type II compound. That is the fragments created by cleaving the D-ring and representing the E-ring plus adjoining atoms had gained four mass units, whereas, for example, mass fragment 191 did not shift (Fig. 4). This confirms the presence of a thiophene moiety condensed to the aromatic ring. Since the mass spectra of benzohopanes possessing less than 34 carbon atoms, and without any sulphur bearing equivalents, had not changed after the experiment, it is certain that in the procedure used hydrogen atoms of the aromatic ring do not exchange with deuterium.

The C_{36} ring A methylated benzothiophene hopanoid VIII, which elutes after the C_{35} benzothiophene hopanoid IVb, was tentatively identified in two of the samples. Its mass spectrum is nearly identical to that of IVb, except that it has a molecular ion at m/z 516 instead of m/z 502 and a major fragment at m/z 205 instead of m/z 191. Because on most columns 2β methylated hopanoids elute close to their non-methylated analogs and 3β methylated hopanoids have longer retention times (Rohmer *et al.*, 1993, and references therein), the C_{36} ring A methylated benzothiophene hopanoid probably has a 3β methyl substituent.

It is noted that the identification of the benzothiophene hopanoids should be considered tentative since no authentic standards were used.

DISCUSSION

The formation of benzothiophene hopanoids is structurally limited to the higher members of the homohopane series. At least 34 carbon atoms are required to form a benzothiophene hopanoid of type IV, and at least 33 to form the type V compounds. There are two pathways by which the benzothiophene hopanoids can be formed (Fig. 5). Either sulphur is incorporated into benzohopanes, or sulphur is incorporated prior to cyclization and aromatization of the hopanoid side chain. Because of the fixed position of a thiophene moiety in the side chain, subsequent cyclization and aromatization of the side chain can only lead to compounds IVa and b in the case of thiophene hopanoids Ib and c, and to compounds Va, b and c in the case of thiophene hopanoids Id, e and f (Fig. 5). For the same reason the C_{36} ring A

methylated thiophene hopanoid IX can only give rise to benzothiophene hopanoid VIII.

In order to determine how the benzothiophene hopanoids were formed, their distributions were compared with those of the benzohopanes and thiophene hopanoids within the same sample (Fig. 6). Distributions of C_{34}–C_{35} benzohopanes of both types differ significantly from those of the corresponding benzothiophene hopanoids. On the other hand, the distributions of thiophene hopanoids resemble those of the benzothiophene hopanoids, suggesting a precursor–product relationship. The concentrations of benzothiophene hopanoids are substantially lower than those of the thiophene hopanoids, which may explain the apparent absence of benzothiophene hopanoids derived from some of the least abundant thiophene hopanoids. These data seem to indicate that the benzothiophene hopanoids are formed subsequent to or perhaps simultaneously with the process of sulphur incorporation.

Desulphurization of the asphaltenes and polar fractions of the samples studied did not yield any benzohopane. This is also an indication that the benzothiophene hopanoids are not formed by sulphur incorporation into benzohopanes, otherwise there would be no reason why the benzohopanes are not incorporated via sulphur linkages into macromolecules. Finally, we favour a mechanism of sulphur incorporation prior to cyclization and aromatization, because sulphur incorporation is known to occur shortly after sediment deposition (e.g. Wakeham *et al.*, 1995), whereas aromatization usually takes place at a later stage of diagenesis.

The Hauptdolomit carbonate displays a hopanoid distribution somewhat different from the other samples (Fig. 6) i.e. most of its series are dominated by the C_{34} member, and the C_{34} benzothiophene hopanoid IVa is relatively abundant, but its presumed precursor, the C_{34} thiophene hopanoid Ib, was not identified. At present we cannot offer an explanation for this.

In the Jurf ed Darawish oil shales benzohopanes II can be detected, provided that at least some of the free hopanes have isomerized to the 17α,21β(H) configuration (Sinninghe Damsté *et al.*, 1995). Therefore, these benzohopanes probably originated from 17α,21β(H) hopanes only. By analogy, benzothiophene hopanoids IV probably only derive from 17α,21β(H) thiophene hopanoids. In the four samples investigated the 17β,21α(H) and 17β,21β(H) thiophene hopanoids are absent, or minor, compared to the 17α,21β(H) isomers.

Benzothiophene hopanoids have so far been identified in laminated marlstones and carbonates of the Hauptdolomit Formation (Upper Triassic, Northern Calcareous Alps), coccolithic limestones of the Calcaires en Plaquettes Formation (Upper Jurassic, Orbagnoux, France) and El Lajjun oil shales of the Ghareb Formation (Upper Cretaceous, Jordan). The Jurf ed Darawish oil shales studied by Kohnen *et al.* (1990), likewise the El Lajjun sediments, belong to the Ghareb Formation. Therefore, these Jurf ed Darawish samples were re-examined for hopanoid

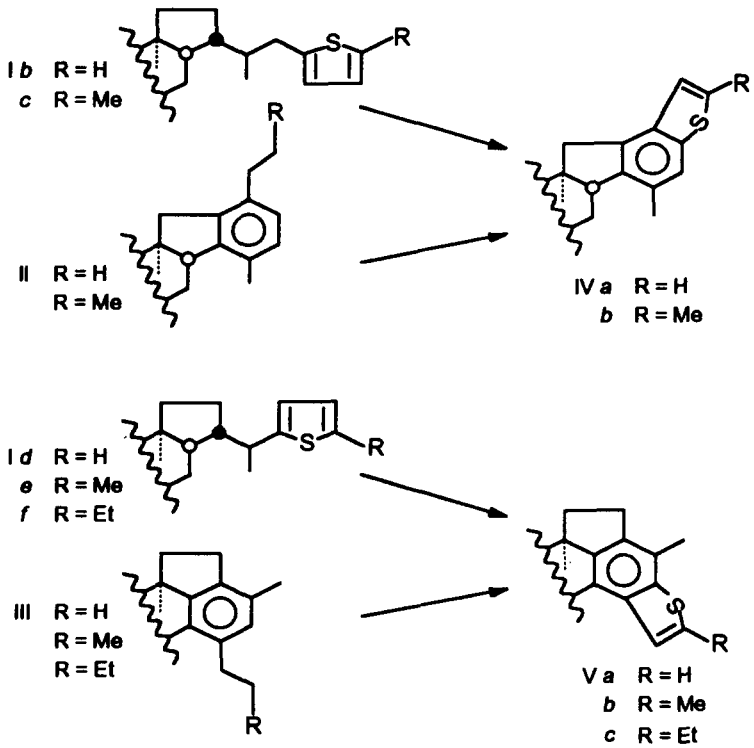

Fig. 5. Hypothetical precursor–product relationships in the formation of benzothiophene hopanoids.

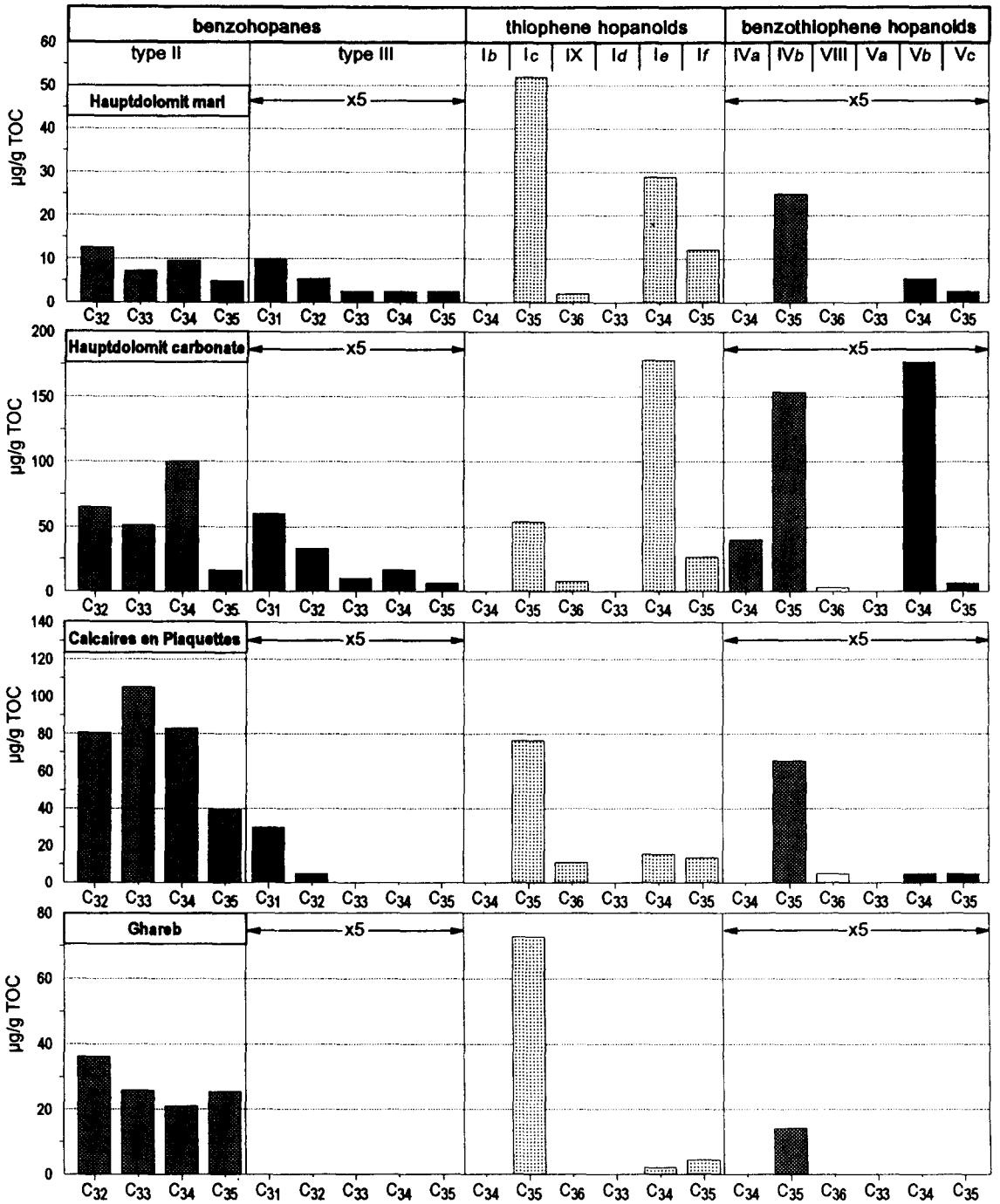

Fig. 6. The concentrations of benzohopanes, thiophene hopanoids and benzothiophene hopanoids in the four investigated samples.

biomarkers. It appeared that the novel benzothiophene hopanoids were absent, and that thiophene hopanoids were different from those in the other samples. In the carbonate-rich facies of the Jurf ed Darawish sediments 17β,21β(H) thiophene hopanoids are dominant over their 17α,21β(H) counterparts. Since benzothiophene hopanoids are thought to derive from 17α,21β(H) thiophene hopanoids only, their absence here can be understood. In the more argillaceous facies, and in the phosphorite, 17α,21β(H) thiophene hopanoids are more pronounced, but they are dominated by the C_{35} member with a terminal thiophene ring (Ia), which is not a potential precursor of the novel benzothiophene hopanoids. Nevertheless, thiophene hopanoid Ic is present and its presumed derivative IVb is not. This is

possibly due to a lower maturity of the Jurf ed Darawish sediments as compared to the other samples studied. Alternatively, if thiophene hopanoids and benzothiophene hopanoids are simultaneously formed and not related to differences in maturity, the presence of Ic and the absence of IVb may be indicative of a precursor bacteriohopanepolyol derivative possessing such functionalities that enable the formation of a thiophene ring but have no potential to form a benzothiophene group. For example, dehydration of bacteriohopanepentol Xa (Rohmer *et al.*, 1993) may yield a derivative containing one or more double bonds in the side chain plus a double bond at C22(29) which, through isomerization via tertiary carbon atoms, can move into the D or E-ring. Such a derivative may be a precursor of predominantly benzothiophene hopanoids. On the contrary, dehydration of bacteriohopanetetrol Xb (Rohmer *et al.*, 1993) will yield a derivative containing unsaturations only in the side chain, since isomerization of the double bonds cannot proceed via C31. Such a derivative may form thiophene hopanoids, whereas the formation of benzothiophene hopanoids may not be possible.

CONCLUSIONS

Two novel families of C_{34}–C_{35} benzothiophene hopanoids have been tentatively identified in several laminated marlstones and limestones. These compounds are probably formed by side chain cyclization and aromatization of C_{34}–C_{35} thiophene hopanoids. Apart from the availability of reduced inorganic sulphur species, a certain level of maturity may be required to form the novel hopanoids from their precursor bacteriohopanepolyol derivatives. However, if sulphur incorporation, cyclization and aromatization take place simultaneously, both thiophene hopanoids and benzothiophene hopanoids can be formed soon after organic matter deposition. In that case, the presence of benzothiophene hopanoids in sediments may be indicative of specific precursor bacteriohopanepolyol derivatives.

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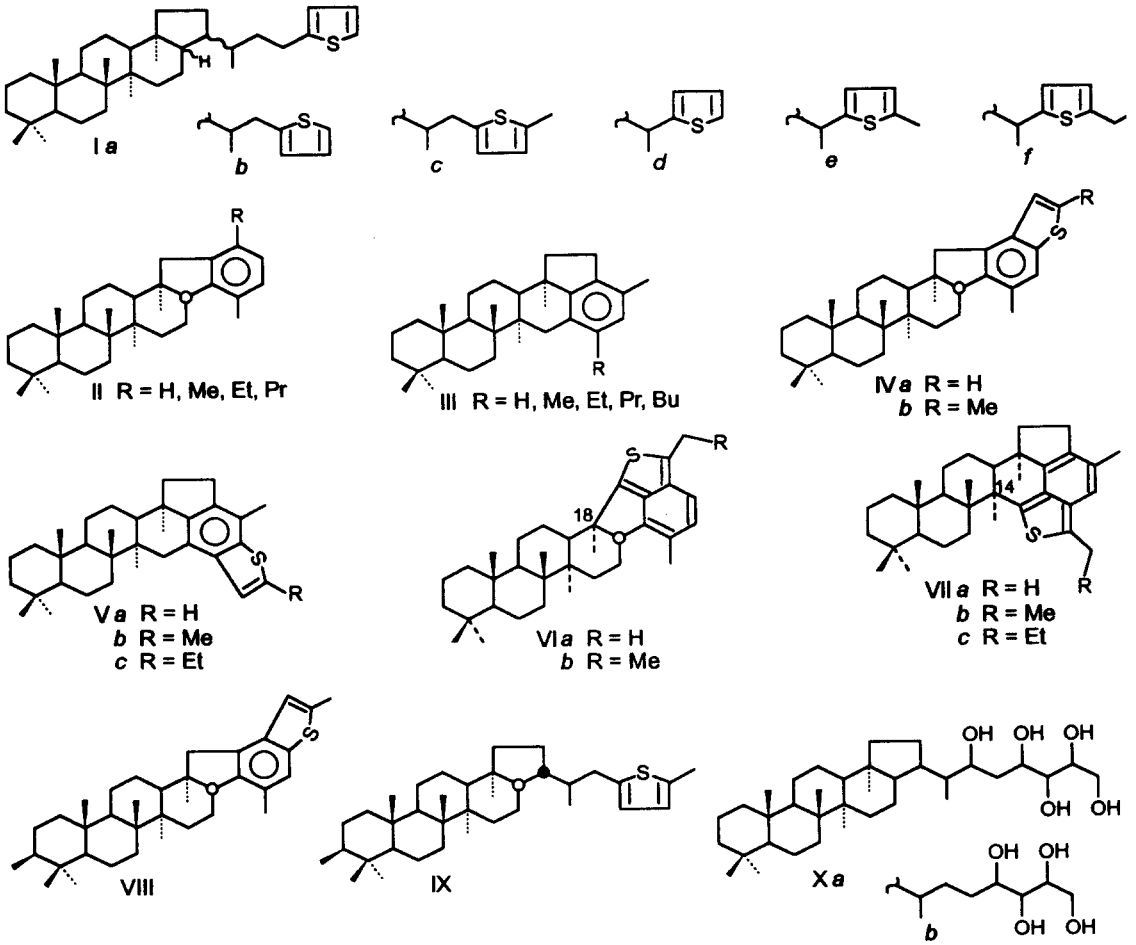
APPENDIX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III R = H, Me, Et, Pr, Bu
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b R = Me
V a R = H
b R = Me
c R = Et
VI a R = H
b R = Me
VII a R = H
b R = Me
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IX
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