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Diagenetic and catagenetic products of isorenieratene: Molecular indicators for photic zone anoxia

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Abstract—A wide range of novel diagenetic and catagenetic products of the diaromatic carotenoid isorenieratene, a pigment of the photosynthetic green sulphur bacteria *Chlorobiaceae*, has been identified in a number of sedimentary rocks ranging from Ordovician to Miocene. Compound identification is based on NMR, mass spectrometry, the presence of atropisomers, and stable carbon isotopes. Atropisomers contain an axially chiral centre which, in combination with other chiral centres, results in two or more diastereomers that can be separated on a normal GC column. *Chlorobiaceae* use the reverse TCA cycle to fix carbon, so that their biomass is enriched in ¹³C. High ¹³C contents of isorenieratene derivatives therefore support their inferred origins.

Isorenieratene derivatives include C₄₀, C₃₃, and C₃₂, diaryl isoprenoids and short-chain aryl isoprenoids with additional aromatic and/or S-containing rings. C₃₃ and C₃₂ compounds are diagenetic products of C₃₃ and C₃₂ “carotenoids” formed from isorenieratene during early diagenesis through expulsion of toluene and *m*-xylene, respectively. Cyclisation of the polyene acyclic isoprenoid chain can proceed via an intramolecular Diels–Alder reaction, followed by aromatisation of the newly formed ring. Sulphurisation is also an important process during early diagenesis, competing with expulsion and cyclisation. Sulphur-bound isorenieratene is released during progressive diagenesis, due to cleavage of relatively weak S–S and C–S bonds. Cleavage of C–C bonds during aromatisation of newly formed rings and during catagenesis yields short-chain compounds. The inherent presence of a conjugated double bond system in carotenoids implies that similar diagenetic and catagenetic reactions can occur with all carotenoids.

Chlorobiaceae live at or below the oxic/anoxic boundary layer and require both light and H₂S. The presence of isorenieratene or its diagenetic and catagenetic products in ancient sedimentary rocks and crude oils is therefore an excellent indication for photic zone anoxia in the depositional environment. Diagenetic and catagenetic products of isorenieratene are expected to find applications in reconstruction of palaeoenvironments and in oil–oil and oil–source rock correlation studies. Their presence in several petroleum source rocks suggests that anoxia is an important environmental parameter for the preservation of organic matter.

1. INTRODUCTION

It is generally accepted that anoxic depositional environments favour the preservation of organic matter (Demaison and Moore, 1980), albeit that Pedersen and Calvert (1990) have recently challenged this. Anoxic conditions in the marine environment occur when the supply of oxygen in bottom waters is limited by restricted water circulation arising from density or temperature stratification, or by contact of an oxygen-minimum layer with the seafloor (e.g., on continental shelves and slopes). Since anoxia and thus preservation of organic matter can lead to formation of petroleum source rocks, it is important to be able to recognise (ancient) anoxic basins. Hence, recognition of biomarkers in sedimentary rocks and oils that are unambiguously related to anoxic depo-

sitional conditions is a challenge for petroleum geochemistry and organic geochemistry alike.

Green sulphur bacteria (*Chlorobiaceae*) are photoautotrophic organisms that are strictly anaerobic and require both light and H₂S. Their presence attests to an anoxic water layer reaching into the photic zone, and euxinic conditions. *Chlorobiaceae* produce two types of characteristic pigments (bacteriochlorophylls and isorenieratene) that can be used to trace their distribution in the geological record. The bacteriochlorophylls *c*, *d*, and *e* are unique compared to other (bacterio)chlorophylls in that their tetrapyrrole nucleus can contain as many as thirty-eight carbon atoms (Scheer, 1991). The diaromatic carotenoid isorenieratene (**I**; see Appendix A) comprises an irregular (tail-to-tail) isoprenoid chain, and a specific 1-alkyl-2,3,6-trimethyl substitution pattern for both its aromatic rings (Liaaen-Jensen, 1978a,b). The high ¹³C content of isorenieratene and the bacteriochlorophylls *c*, *d*, and *e* determined, respectively, from octadecahydroisorenieratene (or isorenieratane; **II**) (Kohnen et al., 1992; Sinninghe Damsté et al., 1993b; Hartgers et al., 1994c) and the breakdown products of the extended porphyrins (Grice et al., 1995), can be attributed to carbon fixation via the

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reverse tricarboxylic acid (TCA) cycle used by *Chlorobiaceae* (Quandt et al., 1977; Sirevag et al., 1977; Schidlowski et al., 1984). Isorenieratene and the bacteriochlorophylls *c*, *d*, and *e* and their diagenetic and catagenetic products are, therefore, excellent biomarkers for anoxic depositional environments with a relatively shallow oxic water column.

Isorenieratene has been identified in several immature marine sediments (Cardoso et al., 1978; Brassell et al., 1983; Repeta, 1993; Keely et al., 1995). The fossilisation potential of the isorenieratene carbon skeleton was first shown by Schaefflé et al. (1977), who described the isolation and identification (by synthesis of an authentic standard) of isorenieratane (II) from a Jurassic shale from the Paris Basin. They proposed that isorenieratane originated from isorenieratene by saturation of the double bonds in the isoprenoid chain. Isorenieratane (Requejo et al., 1992; Hartgers et al., 1994b,c) and diaryl isoprenoids with unspecified aromatic substitution patterns (Chou and Wood, 1986; Schwark and Püttmann, 1990; Requejo et al., 1992) have subsequently been reported in various samples. In an Uzbek oil of unspecified age, Ostroukhov et al. (1982) tentatively identified a series of 1-alkyl-2,3,6-trimethylbenzenes (III). Summons and Powell (1986, 1987) identified 1-alkyl-2,3,6-trimethylbenzenes in oils of Palaeozoic age by synthesis of authentic standards, and found them to be enriched in ^{13}C by 7–8‰ compared to alkanes in the same oils. These carbon isotopic data, together with a distribution pattern ranging from C_{13} to C_{31} , wherein the C_{17} , C_{23} , and C_{28} members are present only in low abundance, led them to propose an origin from isorenieratene for these aryl isoprenoids. Hartgers et al. (1994c) have shown that aryl isoprenoids are derived from thermal breakdown of isorenieratene incorporated into high-molecular-weight (HMW) organic matter, rather than from thermal breakdown of free isorenieratane. Several other workers have recognised 1-alkyl-2,3,6-trimethylbenzenes (Clark and Philp, 1989; Xinke et al., 1990; Fowler, 1992; Requejo et al., 1992; Hartgers et al., 1994b) and alkyltrimethylbenzenes with other or unspecified aromatic substitution patterns (Schwark and Püttmann, 1990; Clayton et al., 1992; Requejo et al., 1992, 1995; Hartgers et al., 1994b; Guthrie and Pratt, 1995) in sedimentary rocks and crude oils. Summons and Powell (1992) claimed the identification of a variety of aryl isoprenoid isomers in Siberian Platform oils of Late Proterozoic age, a finding of particular interest because it would represent the oldest report of *Chlorobiaceae* in the fossil record. However, evidence for the identification is poor, and the inferred isomerisation of the isoprenoid chain (Summons and Powell, 1992) seems unlikely.

The nine conjugated double bonds of isorenieratene make it highly susceptible to reaction with reduced inorganic sulphur species (e.g., de Graaf et al., 1992; Schouten et al., 1994) that are present in abundance in the anoxic water column and the top layers of the sediment. Incorporation of sulphur into isorenieratene results in the formation of multiple S-linked isorenieratane moieties in HMW fractions such as the polar and asphaltene fractions and the kerogen during early diagenesis (Sinninghe Damsté et al., 1989; Kohnen et al., 1990). In the maltene fraction of immature ($R_0 \approx 0.25\%$) marlstones from the Vena del Gesso Basin in northern Italy

diaryl isoprenoids were present solely as S-bound moieties in macromolecular aggregates (Kohnen et al., 1991c; Kenig et al., 1995). A similar observation was made for β -carotane by Adam et al. (1993), who liberated this compound after desulphurisation of a HMW fraction of a S-rich oil that did not contain free β -carotane in the saturated hydrocarbon fraction. The early diagenetic fate of isorenieratene can be reconstructed from studies in the Black Sea. *Chlorobiaceae* were isolated from the chemocline and were shown to contain isorenieratene (Overmann et al., 1992), which was also identified in suspended particulate organic matter from waters at and below the chemocline (Repeta et al., 1989). In the top layers of the sediment, both free isorenieratene (Repeta, 1993) and isorenieratene sulphur bound in geomacromolecules (Sinninghe Damsté et al., 1993b; Wakeham et al., 1995) were identified. The portion of isorenieratene sequestered in S-rich geomacromolecules increases from 15% in the top sediment layer (Unit I) to 95% at 40–60 cm depth (Unit IIb), suggesting a progressive sulphurisation of isorenieratene with increasing depth (cf. Sinninghe Damsté et al., 1993b; Repeta, 1993; Wakeham et al., 1995).

Sulphur sequestration of isorenieratene has also been observed in other samples. There are various reports on the identification of isorenieratane (Kohnen et al., 1992; Kenig et al., 1995; Schaeffer et al., 1995a,b; Sinninghe Damsté et al., 1993b, 1995a) and diaryl isoprenoids with unspecified aromatic substitution patterns (Sinninghe Damsté et al., 1990; Kohnen et al., 1991b,c) in S-rich geomacromolecules. Several studies have reported a high abundance of 1,2,3,4-tetramethylbenzene (TMB) in flash pyrolysates of kerogens (Douglas et al., 1991; Hartgers et al., 1991, 1994a,b,c; Requejo et al., 1992; Gelin et al., 1995), and proposed that it is formed by β -cleavage of a benzene ring of a diaromatic carotenoid incorporated into the kerogen. In a comparative study of immature kerogens and coals, Hartgers et al. (1994a,c) proposed that TMB is a ‘‘pyrolytic’’ biomarker for macromolecularly bound diaromatic carotenoids. However, the ^{13}C content of TMB in flash pyrolysates of kerogens isolated from Indian Ocean surface sediments is identical to that of algal lipids, excluding an origin from isorenieratene (Hoefs et al., 1995). Moreover, it should be noted that TMB is not a biomarker for macromolecularly bound isorenieratene per sé. β -cleavage of a benzene ring of macromolecularly bound renieratene and renierapurpurin, diaromatic carotenoids with a 2,3,4/2,3,6- and 2,3,4/2,3,4-trimethyl substitution pattern for their aromatic rings, respectively, or of the monoaromatic carotenoid chlorobactene also yields TMB. However, a distinction between the aromatic carotenoids can be made based on the $\delta^{13}\text{C}$ values of the β - and γ -cleavage products, and the distribution of γ -cleavage products in the flash pyrolysates. Hartgers et al. (1994b,c) found that TMB and 1-ethyl-2,3,6-trimethylbenzene (γ -cleavage product of isorenieratane) released after off-line pyrolysis of a kerogen isolated from the Duvernay Formation had anomalously high ^{13}C contents. 1-Ethyl-2,3,6-trimethylbenzene is often a dominant C_5 alkylbenzene in kerogens containing macromolecularly bound isorenieratene (Hartgers et al., 1994a,c).

Here, we report on the discovery of a wide range of un-

precedented diagenetic and catagenetic products of isorenieratene in rock samples from different geographical locations ranging through the Phanerozoic. They include S-containing compounds, polyaromatic compounds with up to four additional aromatic rings, shorter-chain analogues with a C₃₂ or C₃₃ carbon skeleton, and aryl isoprenoids with additional aromatic rings or S-containing moieties. These compounds are all related to isorenieratene and can therefore be regarded as indicators for photic zone anoxia in ancient depositional environments. The effect of progressive diagenesis and early catagenesis on the amounts and distributions of these compounds was studied by hydrous pyrolysis of an immature ($R_0 \approx 0.25\%$) S-rich sedimentary rock from the Gessoso-solfifera Formation (Messinian) that contains abundant macromolecularly bound isorenieratene.

2. EXPERIMENTAL

2.1. Hydrous Pyrolysis

A sample of the 1.2 m thick marl layer of evaporitic cycle IV of the Gessoso-solfifera Formation, Messinian, in the Vena del Gesso Basin, northern Italy, was subjected to artificial maturation by hydrous pyrolysis, as described by Lewan (1993). In short, a 1 L Hastelloy-C276 reactor was filled with rock chips (90–100 g) and distilled water (475 g). The remaining volume was purged and filled with helium (2.4 bar). Aliquots of the marl sample were heated isothermally for 72 h at 160, 180, 200, 220, 239, 260, 280, 300, and 330°C, respectively, with their temperatures monitored at 30 s intervals. Standard deviations were between ± 0.2 and 0.3°C ($\pm 0.6^\circ\text{C}$ at 160°C). The experiments at 300 and 330°C generated an expelled oil that was recovered from the water surface with a pipette. The reactor walls and the rock chips were rinsed with benzene to recover any sorbed oil films, which occurred in experiments at 260°C and higher temperatures. 95% or more of the originally loaded rock chips were recovered from all experiments. The residual rock chips were dried in a vacuum oven ($T \leq 50^\circ\text{C}$).

2.2. Extraction and Fractionation

Samples were freeze-dried and ultrasonically or Soxhlet extracted with dichloromethane/methanol (7.5:1 v/v). For the artificially matured samples, the sorbed oil and expelled oil (if present) were combined with the extract. Extracts were separated into a maltene and an asphaltene fraction by repeated (3 times) precipitation in *n*-heptane. An aliquot of the maltene fraction (ca. 250 mg), with an added standard [2-methyl-2-(4,8,12-trimethyltridecyl)chroman] for quantitative analysis, was fractionated by column chromatography with alumina into an apolar and a polar fraction by elution with *n*-hexane/dichloromethane (9:1 v/v) and dichloromethane/methanol (1:1 v/v), respectively. Further separation of the apolar fraction by argentatious thin-layer chromatography, using *n*-hexane as a developer, yielded the polyaromatic fraction ($R_f = 0.0$ – 0.4). When less complex fractions were required (e.g., for isotope-ratio-monitoring gas chromatography-mass spectrometry), two separate fractions were collected (A3 and A4 fraction; $R_f = 0.05$ – 0.4 and $R_f = 0.0$ – 0.05 , respectively).

2.3. Desulphurisation

Polar fractions were desulphurised with Raney Ni and subsequently hydrogenated (Sinninghe Damsté et al., 1988b). Before desulphurisation, a known amount of a synthetic standard [2,3-dimethyl-5-(1',1'-d₂-hexadecyl)thiophene] was added to 10–20 mg of the polar fraction. The released hydrocarbons were isolated from the desulphurised polar fraction by column chromatography using alumina.

2.4. Gas Chromatography

Gas chromatography (GC) was performed using a Hewlett-Packard 5890 instrument equipped with an on-column injector. A fused silica capillary column (25 m \times 0.32 mm) coated with CP Sil-5 (film thickness 0.12 μm) was used with helium as carrier gas. The column effluent was monitored by both a flame ionisation (FID) and a sulphur-selective flame photometric (FPD) detector, using a stream-splitter at the end of the column (split ratio FID:FPD = 1:2). 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 20 min.

2.5. 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 40–800 and a cycle time of 1.8 s (resolution 1000). The gas chromatograph was equipped with a fused silica capillary column (25 m \times 0.32 mm) coated with CP Sil-5 (film thickness 0.12 μm). Helium was used as carrier gas. 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 310°C, at which it was held for 20 min.

2.6. Isotope-Ratio-Monitoring Gas Chromatography-Mass Spectrometry

The DELTA-C irm-GC-MS system is, in principle, similar to the DELTA-S system described previously (Hayes et al., 1990). The gas chromatograph (Hewlett-Packard 5890) was equipped as for GC-MS analyses above with helium as carrier gas. Samples were injected on-column at 70°C and the oven was programmed as for GC analyses above. Isotopic values were calculated by integrating the mass 44, 45, and 46 ion currents of the peaks produced by combustion of the chromatographically separated compounds and of CO₂-spikes generated by admitting CO₂ of a known ¹³C content at regular intervals into the mass spectrometer. Values were determined at least in duplicate. Results were averaged to obtain mean values and to calculate standard deviations. The stable carbon isotope compositions are reported in the delta notation against the PDB ¹³C standard.

2.7. Quantitation

Diagenetic and catagenetic products of isorenieratene in the polyaromatic fraction and those released after desulphurisation of the polar fraction were quantitated by integration of a summed mass chromatogram of their major fragment ions (m/z 133 + 237 + 287) and the main fragment ions of the standards, 2-methyl-2-(4,8,12-trimethyltridecyl)chroman (m/z 147) and 6,6-d₂-3-methylheneicosane (m/z 57), respectively. Corrections were made to account for the intensity of the fragment ions relative to the total ion current in the spectra of the compounds quantitated and of the standard. Since mass spectrometric detection of compounds gives a molar response, a factor was introduced taking into account the molecular weights of the compounds quantitated and the standard to obtain absolute amounts ($\mu\text{g/g}$ TOC).

3. RESULTS

The rock samples used in this study originate from different depositional environments and geographical locations and range from Ordovician to Miocene. They were selected because previous work in our laboratory had shown that they contain abundant isorenieratene derivatives. An overview of the samples, some selected characteristics and references are given in Table 1. Our aim was to identify as many diagenetic and catagenetic products of isorenieratene as possible, in order to extend the use of isorenieratene as indicator for

Table 1. Sample description.

Formation name	Location	Age	Lithology	Setting	Kerogen Type	TOC (%)	R _o (%)	Reference
Gessoso-solfifera	Italy	Late Miocene	Marlstone	Lagoonal	II-S	2.0	0.25	Vai and Ricci Lucchi (1977)
Menilite	Poland	Oligocene	Black shale	Flysch	II-S	17.2	±0.25	Köster <i>et al.</i> (1995a)
Canje	Guyana	Late Cretaceous	Calcareous shale	Shallow marine	II	3.3	±0.5	
Kimmeridge Clay	UK	Late Jurassic	Oil shale	Shallow marine	II-S	28.9	0.42 ^a	Oschmann (1991)
Calcaires en Plaquettes	France	Late Jurassic	Laminated limestone	Carbonate platform	I-S	6.2	±0.3	Tribovillard <i>et al.</i> (1992)
Oxford Clay	UK	Late Jurassic	Claystone/shale	Shallow marine	II	16.6	0.38 ^a	Kenig <i>et al.</i> (1994)
Schistes Cartons (G6-5-6)	France	Early Jurassic	Marlstone	Shallow marine	II	8.4	0.43 ^a	MacKenzie <i>et al.</i> (1980)
Schistes Cartons (JAA) ^b	France	Early Jurassic	Laminated black shale	Shallow marine	II	12.2	0.42 ^a	MacKenzie <i>et al.</i> (1980)
Allgäu	Germany	Early Jurassic	Marlstone	Restricted local basin	II	11.0	0.43 ^a	Köster <i>et al.</i> (1995b)
Hauptdolomit	Germany	Late Triassic	Laminated black marlstone	Carbonate platform	I-S	28.8	±0.4 ^c	Köster <i>et al.</i> (1988)
Kössen Marl	Hungary	Late Triassic	Marlstone	Shallow marine	II-S	5.3	±0.4 ^c	Clayton and Koncz (1994)
Minnelusa	USA	Late Carboniferous	Marlstone	Shallow marine	II-S	20.9	±0.6 ^c	Clayton <i>et al.</i> (1992)
Exshaw	Canada	Early Carboniferous	Black shale	Shallow marine	II	15.9	0.43	Allen and Creaney (1991)
Duvernay	Canada	Late Devonian	Laminated limestone	Marine	II	9.0	±0.4	Requejo <i>et al.</i> (1992)
Boas Oil Shale	Canada	Late Ordovician	Oil shale	Shallow marine	II	6.3	0.47 ^a	McCracken and Nowlan (1989)
Womble	USA	Ordovician	Laminated cherty shale	Marine	II	14.6	na ^d	Douglas <i>et al.</i> (1991)

^a Estimated value determined from the Pristane Formation Index (Goossens *et al.*, 1988a,b).

^b Jouy-aux-Arches.

^c Approximate value determined from several independent measures of thermal maturity.

^d Not applicable.

photic zone anoxia in ancient depositional environments. The many novel isorenieratene derivatives encountered can be divided into three groups according to their carbon number, namely (1) C₄₀, (2) C₃₂ and C₃₃, and (3) short-chain compounds, respectively. Further groupings were based on the presence of additional aromatic rings and S-containing rings.

3.1. Structural Identifications

A detailed description of the identification of diagenetic and catagenetic products of isorenieratene is given in Appendix B. To avoid duplications, the various aspects of the identifications are discussed in the text only once. Identification is based on mass spectrometry, NMR, co-injection with an authentic standard, and the presence of atropisomers. For clarity we use the numbering system of Fresenius (1989) to denote specific carbon atoms of the isorenieratene skeleton (cf. I in Appendix A).

3.1.1. Atropisomers

Before discussing the structures of the diagenetic and catagenetic products of isorenieratene, an important aspect of their structural identification should be introduced, i.e. the recognition of atropisomers. The term atropisomers refers to stereoisomeric compounds that result from restricted rotation around a C-C single bond, which are configurationally stable at room temperature (Mislow, 1966; March, 1985). Some of the isorenieratene derivatives contain a biphenyl or phenyl-naphthyl moiety. Rotation around the phenyl-phenyl or phenyl-naphthyl C-C bond in these molecules is severely hindered because of the presence of *ortho*, and, to a lesser

extent (Theilacker and Hopp, 1959; Ling and Harris, 1964; Wolf *et al.*, 1995), *meta*, and *para* methyl and/or alkyl groups. These substituents cause excessive nonbonded interactions when they are forced to "pass" each other in the transition state. As a result, two stable configurations exist in which the two aromatic ring systems are almost perpendicular. Neither the phenyl nor naphthyl groups possess a mirror plane perpendicular to the plane of the aromatic ring, given the 2,3,6-trimethyl substitution pattern for the aromatic rings and the isoprenoid character of the chain. Thus, the two stable configurations are atropisomers and the molecule is axially chiral (Mislow, 1966; March, 1985). If it also contains an asymmetric carbon atom, two diastereomers result which may be separated on a capillary GC column with a normal apolar stationary phase, appearing as peaks of approximately equal intensity.

The high activation energy barrier that separates atropisomers results in restricted interconversion, at a rate that depends on the activation energy and the temperature. Molecular mechanics calculations indicate that the activation energies for rotation around the phenyl-phenyl C-C bond in 2,3,5',6-tetramethyl-2'-(2-butyl)biphenyl (IV) and the phenyl-naphthyl C-C bond in 3,8-dimethyl-1-(2,3,6-trimethylphenyl)naphthalene (V) are 138–168 and 180–210 kJ/mol, respectively (van Duin *et al.*, 1996). The latter value is higher, probably due to the enhanced rigidity of V compared to IV. A pseudohomologous series of 2,3,5',6-tetramethyl-2'-alkylbiphenyls was identified in the Allgäu Formation (see below), which can be recognised by the main fragment ion at *m/z* 237, corresponding to cleavage β to the biphenyl moiety (Fig. 1). The C₂₀ component and the higher pseudohomologues of this series contain an asymmetric car-

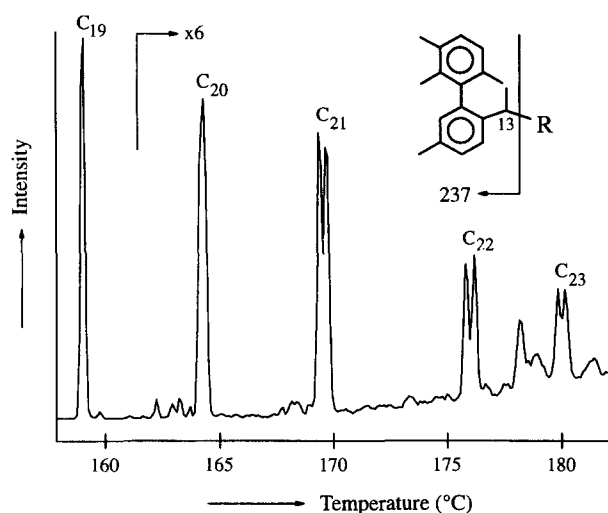


FIG. 1. Partial m/z 237 mass chromatogram of the polyaromatic fraction of the sample from the Allgäu Formation, showing the gas chromatographic separation of the atropisomers of the C_{20} - C_{23} members of the 2,3,5',6'-tetramethyl-2'-alkylbiphenyls in an approximate 1:1 ratio. The C_{19} ($R = CH_3$) member is reflected by one peak because it does not contain an asymmetric carbon atom.

bon atom at C-13, which, in combination with the axially chiral biphenyl moiety, results in two diastereomers that are separated in GC analysis (Fig. 1). Thus, the elution temperature of these compounds (159–180°C) is insufficient to overcome the high activation energy barrier for rotation around the phenyl-phenyl C-C bond. It should be noted that separation of the atropisomers is dependent on both the activation energy for rotation and differences in their physical properties. An axially chiral centre seems important for separation of these diastereomers, because acyclic compounds with two (or more) asymmetric carbon atoms (e.g. squalane) are not or hardly separated by capillary GC using a normal apolar stationary phase.

Competition between the half-life and the retention time of atropisomers will determine their appearance in the GC. This was shown by König et al. (1993), who carried out isothermal GC using capillary columns coated with modified cyclodextrins, allowing separation of enantiomeric structures, and determined the separation of the two atropisomers of 2,2'-diisopropylbiphenyl as a function of column temperature.

3.1.2. C_{40} compounds

Isorenieratane. We encountered isorenieratane (II) in all samples, except that from the Gessoso-solfifera Formation. Schwark and Püttmann (1990) detected "isorenieratane or its positional isomers" as a broadened peak, and concluded that this was caused by decomposition reactions during GC analysis. However, peak broadening probably resulted from the GC operating in the isothermal ($T = 300^\circ\text{C}$) mode during elution of the diaryl isoprenoid, and from the presence of numerous diastereomers. Isorenieratane isolated from the Exshaw Formation also showed peak broadening during GC

analysis, and was shown by NMR to contain four isomerised chiral carbon atoms (W. A. Hartgers and J. S. Sinnighe Damsté, unpubl. data).

Diaryl isoprenoids with one additional aromatic ring. Two diaryl isoprenoids with one additional aromatic ring have been identified (VI and VII). The position of the additional aromatic ring in VI was established by ^1H and ^{13}C NMR (Sinnighe Damsté et al., 1995b). In the mass spectrum of VII (Fig. 2a), the higher intensity of the fragment ion at m/z 237 vs. that at m/z 133 is explained by the fact that it is also α to the tertiary carbon atom C-13. The fragment ions at m/z 222 and 207 probably result from consecutive loss of CH_3 from the main fragment ion at m/z 237. Polychlorinated biphenyls (PCBs) can lose Cl^\cdot or even Cl_2 consecutively during mass spectral fragmentation, and the corresponding fragment ions have a higher relative intensity if the PCB contains at least two chlorine atoms in *ortho* positions (Safe and Hutzinger, 1971, 1972). This is probably due to the production, upon electron impact, of ions with a high internal energy caused by steric strain associated with restricted rotation around the phenyl-phenyl C-C bond. Consecutive loss of Cl^\cdot or Cl_2 results in an energy gain through release of steric strain (Safe and Hutzinger, 1971, 1972). Alkylated biphenyls that exhibit hindered rotation around the phenyl-phenyl C-C bond show consecutive loss of methyl or

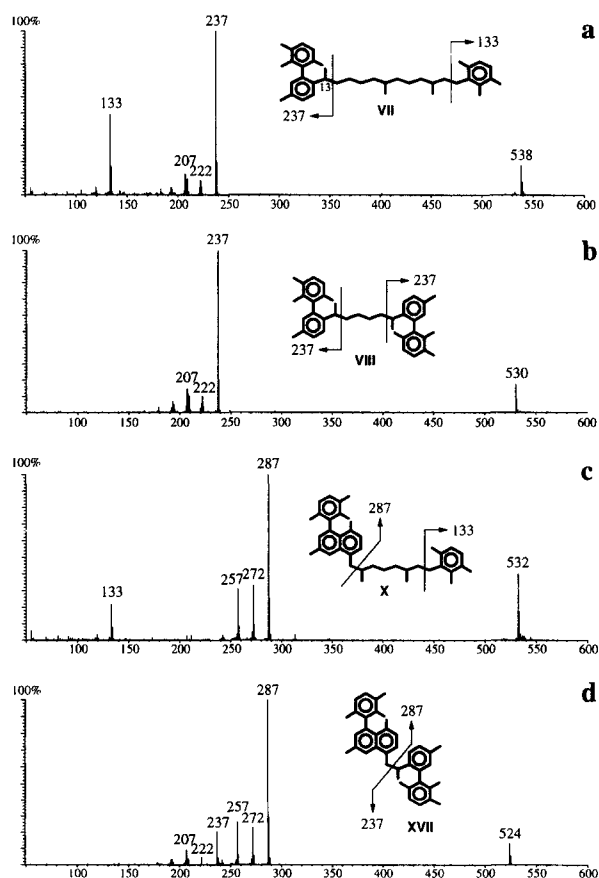


FIG. 2. Mass spectra (subtracted for background) of some C_{40} cyclised and aromatised diagenetic products of isorenieratene.

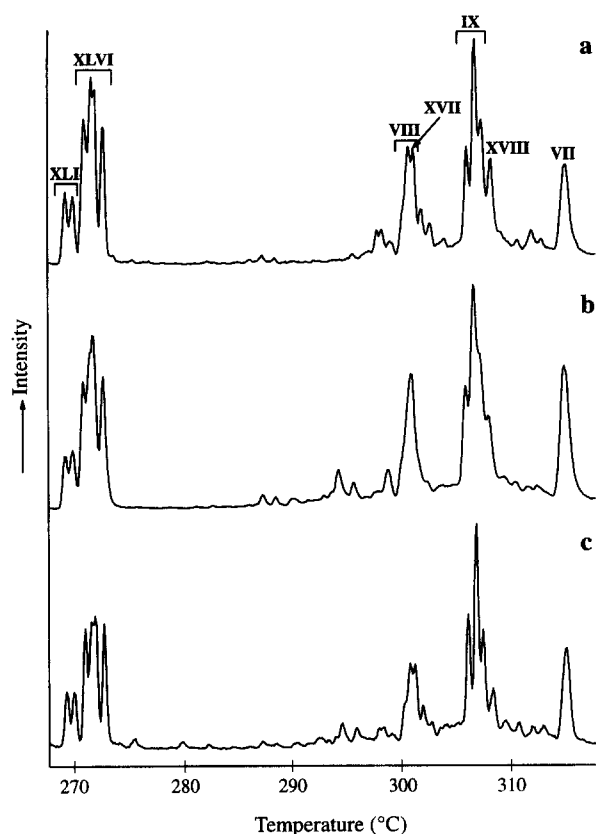


FIG. 3. Partial m/z 237 mass chromatograms of the polyaromatic fractions of the samples from the (a) Allgäu, (b) Kimmeridge Clay, and (c) Schistes Cartons (G6-5-6) Formations, showing compounds containing a biphenyl moiety. The gas chromatographic separation of the atropisomers of **VIII**, **IX**, **XLI**, and **XLVI** can be discerned. Roman numbers refer to structures in Appendix A.

alkyl radicals from their molecular ions (Wolf, 1993; Wolf et al., 1995).

Diaryl isoprenoids with two additional aromatic rings. These compounds can be divided into four groups: those with (1) two additional benzene rings (**VIII** and **IX**), (2) a naphthalene moiety (**X–XIII**), (3) an acenaphthene moiety (**XIV**), and (4) a naphthalene moiety and a cyclohexadienyl moiety (**XV** and **XVI**). Mass spectra of **VIII** and **X** are shown in Fig. 2b and c, respectively. The similarity of the distribution of the diastereomers of **VIII** and **IX** in three samples from different locations, and of variable age and maturity (Fig. 3) indicates that they represent atropisomers.

Identification of a naphthalene moiety in **X**, **XII**, and **XIII** is supported by their accurate masses, which differ from that of isorenieratane by 14.10 amu. This is consistent with a difference of fourteen hydrogen atoms, and not a methylene group, which would have resulted in a difference of 14.02 amu. Schwark and Püttmann (1990) reported a C_{39} diaryl isoprenoid with a regular head-to-tail isoprenoid chain and an unknown substitution pattern for the aromatic rings in the Permian Kupferschiefer. Their tentative assignment was based on mass spectral data that revealed a molecular ion at m/z 532. However, it seems more likely that this compound

is a C_{40} diaryl isoprenoid with an additional naphthalene moiety, since loss of a methylene group from the acyclic isoprenoid chain seems improbable. The intensity of the molecular ion in their mass spectrum (55%) is much higher than that of the C_{40} diaryl isoprenoid they report (21%), despite the structural similarity of the presumed C_{39} and C_{40} diaryl isoprenoids. This supports the presence of an additional naphthalene moiety in their compound, which is further substantiated by a small (7%) fragment ion at m/z 183.

The m/z 287 mass chromatogram of the polyaromatic fraction of the sample from the Kimmeridge Clay Formation (Fig. 4a) shows that the peaks attributed to **X** and **XI** do not occur in the 1:1 distribution expected for two atropisomers. Moreover, different distributions are found in the Allgäu Formation (Fig. 4b) and in sample G6-5-6 from the Schistes Cartons Formation (Fig. 4c), thereby fully excluding the possibility that **X** and **XI** are atropisomers. Their peak assignments were made based on the intensity of the fragment ions at m/z 272 and 257 relative to that at m/z 287. The main fragment ion at m/z 287 has a high internal energy because of restricted rotation around the phenyl-naphthyl C–C bond. The 1-phenylnaphthalene backbone of **X** is anti-

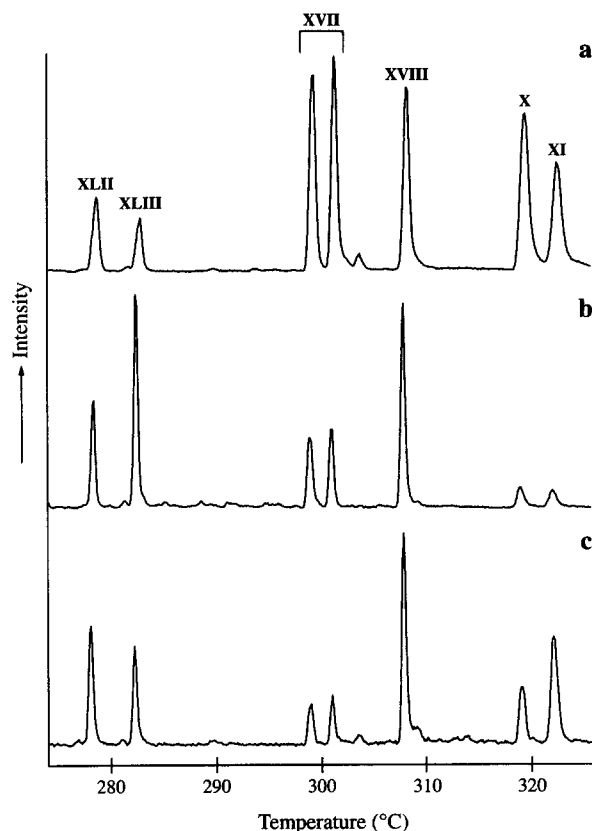


FIG. 4. Partial m/z 287 mass chromatograms of the polyaromatic fractions of the samples from the (a) Kimmeridge Clay, (b) Allgäu, and (c) Schistes Cartons (G6-5-6) Formations, showing compounds containing a phenyl-naphthyl moiety. The gas chromatographic separation of the atropisomers of **XVII** in a consistent approximate 1:1 distribution can be discerned. Roman numbers refer to structures in Appendix A.

pated to have a higher rotational energy barrier than the 2-phenylnaphthalene backbone of **XI**, because of the hindering position of C-20 in **X**. Therefore, the fragment ions at m/z 272 and 257 are expected to have a higher intensity relative to m/z 287 in the mass spectrum of **X**. It was thus concluded that the last eluting peak in the m/z 287 mass chromatogram corresponds to **XI** (Fig. 4).

Diaryl isoprenoids with three additional aromatic rings.

These compounds can be divided into two groups: compounds with a naphthalene moiety and an additional benzene ring (**XVII** and **XVIII**), and those with a condensed aromatic ring system (**XIX** and **XX**). The mass spectrum of **XVII** is shown in Fig. 2d.

The peak assignments of **XVII** and **XVIII** were made based on the presence of atropisomers and the intensity of the fragment ions at m/z 272 and 257 relative to that at m/z 287 (cf. **X** and **XI**). The m/z 287 mass chromatogram shows three peaks with a molecular ion at m/z 524, attributed to **XVII** and **XVIII** (Fig. 4). The mass spectra of the first two are virtually identical, but that of the third has less intense fragment ions at m/z 257 and 272. The similarity in the mass spectra of the first two peaks, combined with their approximate 1:1 distribution in three samples of different locations, age, and maturity (Fig. 4) strongly suggests that they are atropisomers. Thus, the third peak represents the other structure containing a naphthalene moiety and an additional benzene ring. Both structures comprise an asymmetric carbon atom and a biphenyl moiety with restricted rotation around the phenyl-phenyl C-C bond. Rotation around the phenyl-naphthyl C-C bond in the 1-phenylnaphthyl moiety of **XVII** is severely hindered, but in the 2-phenylnaphthyl moiety of **XVIII** it can rotate more freely due to lack of direct interaction of bulky *ortho* groups. Therefore, **XVIII** is probably represented by the third peak in the cluster with molecular ion at m/z 524 in the m/z 287 mass chromatogram, whereas the axially chiral centre in **XVII** results in the first two separate peaks (Fig. 4). This identification is supported by the relative intensities of the fragment ions at m/z 257 and 272 in their mass spectra, associated with restricted rotation around the phenyl-naphthyl C-C bond (cf. **X** and **XI**). Also, peak assignments of **XVII** and **XVIII** are consistent with those of **X** and **XI**, in that the compounds with the 1-phenylnaphthyl moiety elute before their counterparts with the 2-phenylnaphthyl moiety.

Diaryl isoprenoids with four additional aromatic rings.

In this group, one compound has been identified (**XXI**). Comparison of the accurate masses of the molecular ions of isorenieratane and **XXI** (m/z 520) reveals a difference of 0.18 amu, corresponding to twenty-two hydrogen atoms. Within experimental error, this is consistent with an elemental formula $C_{40}H_{40}$.

Diaryl isoprenoids with non-aromatic rings. Several compounds were recognised with major fragment ions that resembled those of compounds discussed above, but with mass spectra that did not match any diaryl isoprenoids with additional aromatic rings. We believe that they represent structures intermediate between isorenieratene and its aromatic derivatives, containing one or more (unsaturated) cyclohexyl moieties, possibly in combination with aromatic rings.

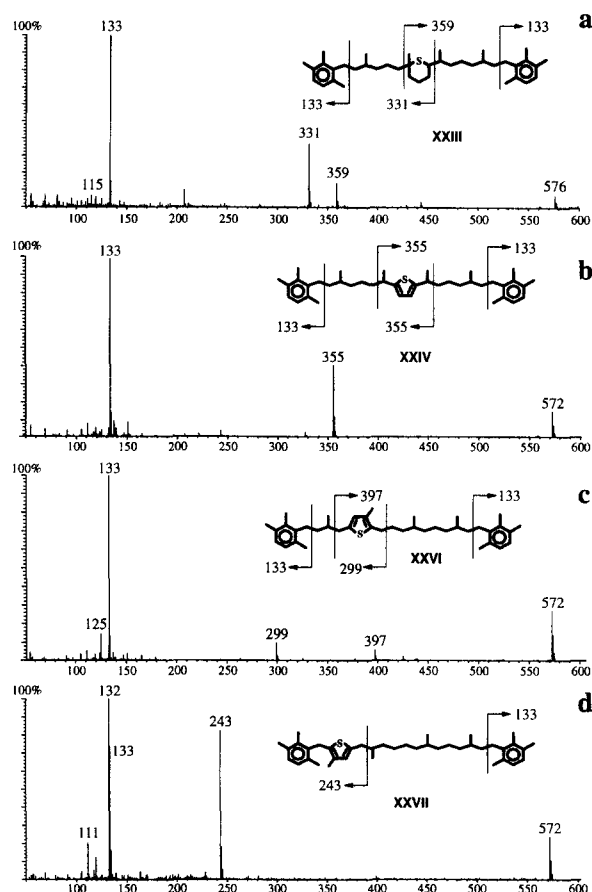


Fig. 5. Mass spectra (subtracted for background) of some C_{40} S-containing diagenetic products of isorenieratene.

A typical example of a diaryl isoprenoid with a saturated cyclohexyl moiety is **XXII**, which has a molecular ion at m/z 528 and major fragment ions at m/z 237 and 291. These ions suggest a structure like **XXII** based on its similarity to **XVII**.

Diaryl isoprenoids containing one sulphur atom. Compounds identified with one sulphur atom include a thiane (**XXIII**), five thiophenes (**XXIV**–**XXVIII**), two benzo[*b*]thiophenes (**XXIX** and **XXX**), and a compound with a thiophene ring and a benzene ring (**XXXI**). The identification of these compounds is mainly based on mass spectrometry (e.g., Fig. 5) and detection with a S-selective detector (FPD).

XXIII is reflected by two peaks in the gas chromatogram, corresponding to *cis-trans* isomers. Their elution order is assumed to be the same as that of the *cis-trans* isomers of 2,6-di-*n*-alkylthianes (Sinninghe Damsté et al., 1987), i.e., *cis* elutes before *trans*. The sulphur atom is attached at one side to a tertiary carbon atom, consistent with Markovnikov's rule (de Graaf et al., 1992). Formation of a thiane with sulphur attached to two tertiary carbon atoms apparently does not take place, although Markovnikov's rule favours this reaction.

Formation of the fragment ion at m/z 132, the base peak

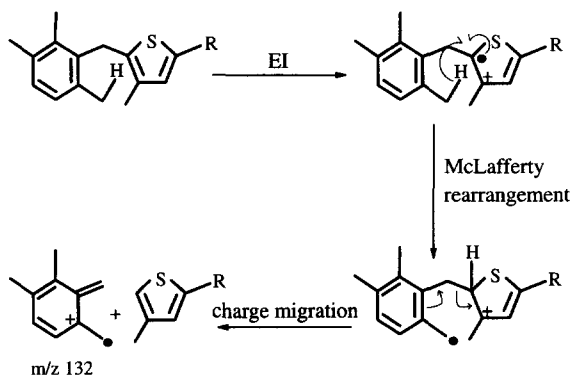


FIG. 6. McLafferty rearrangement leading to the fragment ion at m/z 132 in the mass spectra of **XXVII**, **XXX–XXXIII**, **XXXV**, **XLIX**, **L**, **LXI**, and **LXII**.

in the mass spectrum of **XXVII**, is thought to result from a McLafferty rearrangement involving hydrogen transfer from the benzene ring to the thiophene ring (Fig. 6). This rearrangement also occurs in methylated diphenylmethanes where the ion intensity is proportional to the number of *ortho* methyl groups, probably due to increased availability of hydrogen atoms (McLafferty and Stauffer, 1989).

The fragment ion at m/z 308 in the mass spectrum of **XXX** results from a McLafferty rearrangement favoured by a vacant *ortho* and *para* position of the benzene ring and the tertiary carbon atom C-13' to which the γ -hydrogen atom is attached (cf. Kingston et al., 1988; Sinnighe Damsté et al., 1988a).

Diaryl isoprenoids containing two sulphur atoms. This group comprises two dithiophenes (**XXXII** and **XXXIII**), a thieno[3,2-*b*]thiophene (**XXXIV**), a compound containing a benzo[*b*]thiophene moiety, a thiophene ring (**XXXV**), and a 1,2-dithiane (**XXXVI**).

The fragment ion at m/z 194 in the mass spectrum of **XXXIV** results from cleavage β to both sides of the thieno[3,2-*b*]thiophene moiety, and is analogous to the fragment ion at m/z 220 in the mass spectrum of a bithiophene with a phytane carbon skeleton (Sinnighe Damsté and de Leeuw, 1987). Stabilisation energy associated with the fully conjugated fragment ion favours the double cleavage.

3.1.3. C_{32} and C_{33} compounds

Several compounds were encountered with mass spectral characteristics (e.g., m/z 133, 237, 287) that suggest structures similar to those described above, but with molecular ions ($420 \leq M^{+} \leq 474$) consistent with diagenetic products of C_{32} and C_{33} "carotenoids" (**XXXVII** and **XXXVIII**) formed from isorenieratene by expulsion of *m*-xylene and toluene, respectively (see below). The carbon numbering system (Fresenius, 1989) for isorenieratene is applied in a modified form to **XXXVII** and **XXXVIII**, based on their structural similarities. This does not imply that the absent carbon atoms are necessarily the atoms lost upon expulsion.

C_{32} compounds. C_{32} diagenetic products of **XXXVII** include a diaryl isoprenoid (**XXXIX**), two compounds with

an additional benzene ring (**XL** and **XLI**), two compounds with a naphthalene moiety (**XLII** and **XLIII**), and a thiophene (**XLIV**). Mass spectra of **XXXIX–XLI** and **XLIII** are shown in Fig. 7.

XXXIX was recently reported after desulphurisation of polar fractions and kerogens of immature marlstones from the Gessoso-solfifera Formation (Kenig et al., 1995; Schaeffer et al., 1995a). The relative intensity of the fragment ion at m/z 134 in the mass spectrum of **XXXIX** is lower than in those of isorenieratene and the C_{33} diaryl isoprenoid (see below). This is due to the absence of methyl group C-19', so that the γ -hydrogen atom is attached to a secondary instead of a tertiary carbon atom, favouring the McLafferty rearrangement in isorenieratene and the C_{33} diaryl isoprenoid (cf. Kingston et al., 1988; Sinnighe Damsté et al., 1988a).

XLI appears as two peaks of equal intensity in the m/z 237 mass chromatogram (Fig. 3). These diastereomers result from its axial chirality and an asymmetric carbon atom. The high activation energy for rotation around the phenyl-phenyl C-C bond (138–168 kJ/mol; van Duin et al., 1996) and the relatively low elution temperature (269–270°C) prevent interconversion of the atropisomers. The constant 1:1 distribution of the diastereomers in samples from different geographical locations, age, and maturity (Fig. 3) further sup-

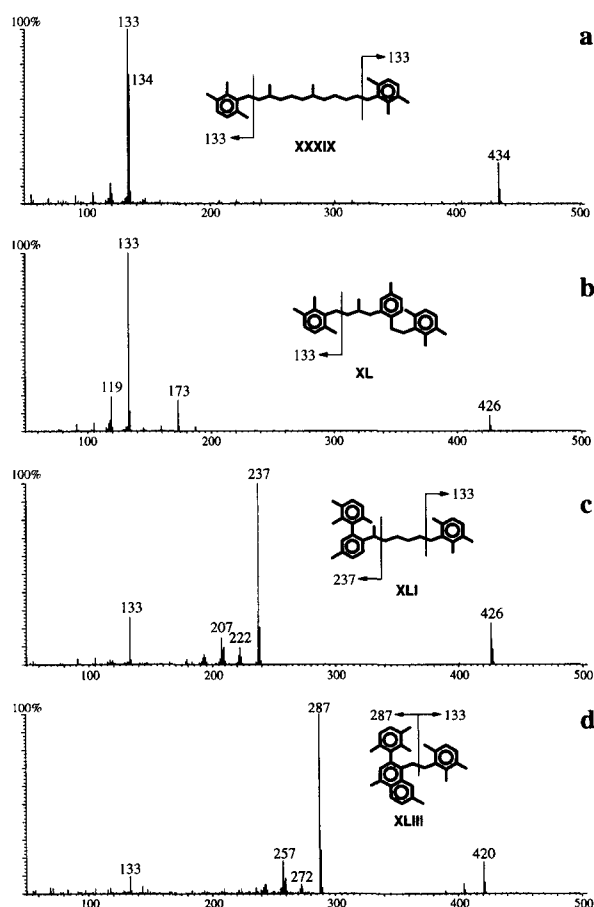


FIG. 7. Mass spectra (subtracted for background) of some C_{32} diagenetic products of isorenieratene.

ports their identification as atropisomers. Schwark and Püttmann (1990) reported an unknown compound with a molecular ion at m/z 426 and a fragment ion at m/z 133 in the Permian Kupferschiefer. This may be **XL**, because they tentatively identified isorenieratane in the same sample.

The GC separation of the two atropisomers of **XLI** enabled determination of the activation energy for rotation around the phenyl-phenyl C-C bond, using the approach of König et al. (1993). They employed isothermal GC using capillary columns coated with modified cyclodextrins, which separated enantiomeric structures, to calculate the rate of interconversion of the two atropisomers of 2,2'-diisopropylbiphenyl as a function of temperature. Their data and the activation energy for rotation around the phenyl-phenyl C-C bond of 2,2'-diisopropylbiphenyl determined by Wolf (1993) make it possible to distinguish three stages of atropisomer peak separation and their respective ratios of half-life ($t_{1/2}$) to retention time (t_r), namely, (1) plateau between peaks $\leq 10\%$ of peak height ($t_{1/2} \geq 2 * t_r$), (2) plateau between peaks $\cong 50\%$ of peak height ($t_{1/2} \cong t_r$), and (3) one peak ($t_{1/2} \leq 0.5 * t_r$). Thus, the peak shapes at different temperatures enable estimation of the half-life of the atropisomers which is related to the activation energy by the equation

$$t_{1/2} = (\ln 2/A) * \exp(E/RT), \quad (1)$$

where $t_{1/2}$ = half-life, A = frequency factor, E = activation energy, R = gas constant, and T = column temperature. Isothermal GC analyses of the polyaromatic fraction of the sample from the Kimmeridge Clay Formation at eight temperatures from 260 to 320°C showed that at 260°C the two peaks are fully separated, at 270°C the plateau between the peaks is approximately 10% of the peak height, at 300°C the plateau is approximately 50% of the peak height, and at 320°C one broad peak results (Fig. 8). Given A , which is known from molecular mechanics calculations (van Duin et al., 1996), the activation energy is 155 kJ/mol, within the range (138–168 kJ/mol) calculated for **IV** using molecular mechanics (van Duin et al., 1996). Apparently, the elongated alkyl chain present in **XLI** but absent in **IV** has little influence on the activation energy for rotation around the phenyl-phenyl C-C bond.

Neither **XLII** nor **XLIII** contains an asymmetric carbon atom in addition to the axially chiral centre at the phenyl-naphthyl C-C bond, so that they do not occur as diastereomers. The m/z 287 mass chromatogram reveals **XLII** and **XLIII** with variable relative intensities for different samples, proving that they are not atropisomers (Fig. 4). As with **X** and **XI**, peak assignment of **XLII** and **XLIII** is based on the intensity of the secondary fragment ions at m/z 257 and 272 relative to the main fragment ion at m/z 287. The mass spectrum of the first eluting compound has more intense fragment ions at m/z 257 and 272 than that of the second, suggesting that it is **XLII**. This proposed elution order for **XLII** and **XLIII** matches those of **X** and **XI** and **XVII** and **XVIII**, where the compound with a 1-phenyl-naphthyl moiety elutes before that with a 2-phenyl-naphthyl moiety.

C₃₃ compounds. **C₃₃** diagenetic products of **XXXVIII** comprise a **C₃₃** diaryl isoprenoid (**XLV**), a **C₃₃** diaryl isoprenoid with an additional benzene ring (**XLVI**), and four

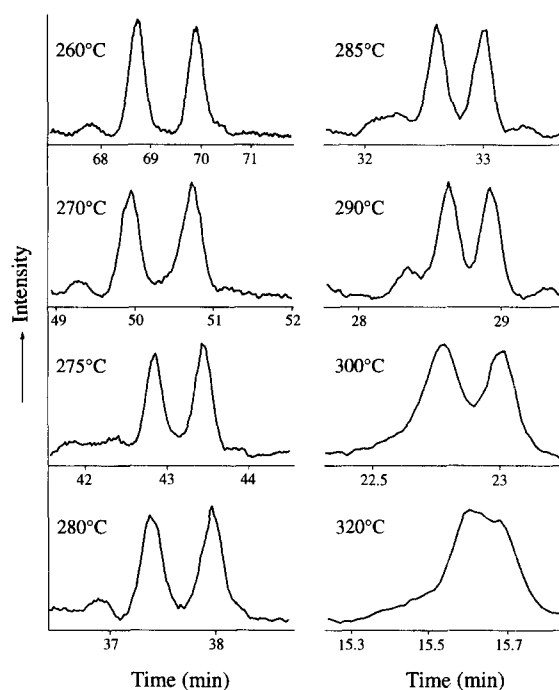


FIG. 8. Isothermal, partial gas chromatograms of the polyaromatic fraction of the sample from the Kimmeridge Clay Formation at various temperatures, showing the increasing height of the plateau between the peaks representing the atropisomers of **XLI** with increasing temperature.

thiophenes (**XLVII–L**). Mass spectra of **XLV–XLVII** and **XLIX** are shown in Fig. 9.

XLV was recently reported after desulphurisation of polar fractions and kerogens of immature marlstones from the Gessoso-solfifera Formation (Kenig et al., 1995; Schaeffer et al., 1995a). The McLafferty fragment ion at m/z 134 has a higher relative intensity than in the mass spectrum of **XXXIX**, and approximately the same as in isorenieratane (see above).

XLVI comprises an axially chiral centre and two asymmetric carbon atoms giving rise to four diastereomers, reflected in the m/z 237 mass chromatogram by four peaks of approximately equal intensity (Fig. 3). The absence of **C₃₃** diaryl isoprenoids with an additional benzene ring involving C-11 to C-9' and with a naphthalene moiety is due to the C-20 and C-19' methyl groups that preclude aromatisation.

The absence of a centre of symmetry in the **C₃₃** diaryl isoprenoid carbon skeleton leads to different compounds from formation of thiophene rings at C-8 to C-11 (**XLIX**) vs. C-14 to C-8' (**L**). Upon cleavage β to the thiophene ring, the former yields a fragment ion at m/z 243 and the latter at m/z 257. In **L** this cleavage is also α to a tertiary carbon atom, which may explain its higher relative intensity.

3.1.4. Short-chain compounds

This group consists of compounds derived from C-C bond cleavage of **C₃₂**, **C₃₃**, and **C₄₀** compounds. They will be differentiated as (1) aryl isoprenoids (**III**), (2) aryl isoprenoids

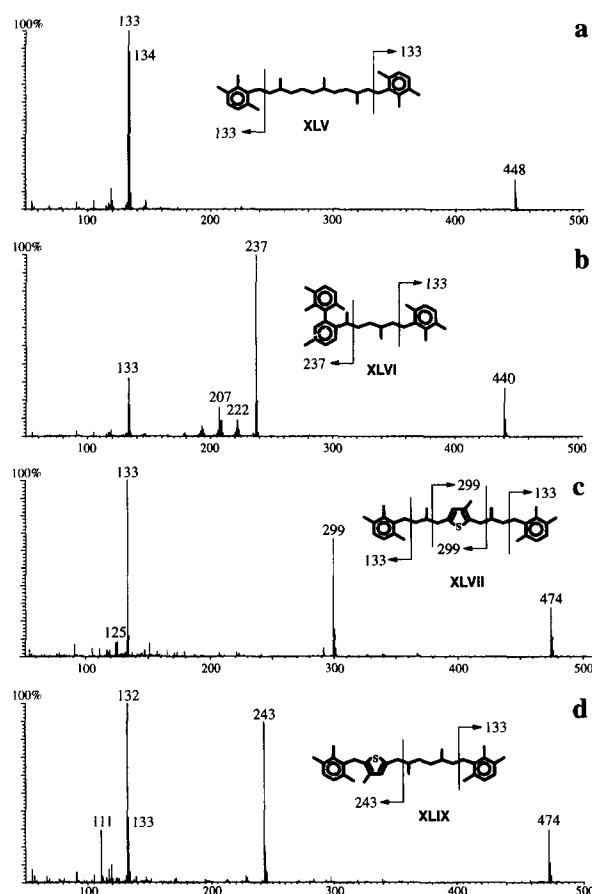


Fig. 9. Mass spectra (subtracted for background) of some C_{33} diagenetic products of isorenieratene.

with additional aromatic rings (LI–LX), and (3) *S*-containing aryl isoprenoids (LXI and LXII). Mass spectra of LI, LIII, LX and LXII are shown in Fig. 10.

Aryl isoprenoids. A pseudohomologous series of aryl isoprenoids occurred in almost all samples with a distribution pattern containing low abundances of C_{12} , C_{17} , and C_{23} members. Summons and Powell (1992) claimed identification of a variety of C_{14} – C_{20} aryl isoprenoid isomers in Siberian Platform oils of Late Proterozoic age. To explain the large number of isomers, they suggested methyl shifts in the isoprenoid chain. In the present study, however, no evidence was found for such isomerisations of aryl isoprenoids. Recently we found evidence for the formation of aryl isoprenoids from β -carotene via aromatisation of a cyclohexenyl moiety and subsequent C–C bond cleavage of the isoprenoid chain (Koopmans et al., 1996c). This indicates that aryl isoprenoids can only be taken as catagenetic products of isorenieratene when they are significantly enriched in ^{13}C (by ca. 15‰) compared to algal lipids.

Aryl isoprenoids containing one additional aromatic ring. These compounds can be divided in four groups that differ in the position of the additional benzene ring in the isoprenoid chain and in their carbon skeleton, dependent on their formation from C_{32} , C_{33} , and C_{40} precursors. The first group

has an additional benzene ring in the ‘‘biphenyl position’’ (LI). The other three groups have an additional benzene ring at various positions in the isoprenoid chain (LII–LIV).

LI include C_{19} – C_{23} components easily distinguished in a mass chromatogram of their main fragment ion at m/z 237 (Fig. 1), and C_{16} – C_{18} components recognised from their retention times and characteristic (alkylated biphenyl-like) mass spectra, which contain fragment ions generated by consecutive loss of methyl radicals from the molecular ion (Wolf, 1993; Wolf et al., 1995). The doubly ionised fragments, for instance at $(M^{+} - n * 15)/2$, in the mass spectra of LI are diagnostic of polyaromatic hydrocarbons (Safe and Hutzinger, 1973). The C_{20} – C_{23} components possess both an axially chiral centre and an asymmetric carbon atom, which gives rise to two diastereomers. For the C_{21} – C_{23} components these are separated by gas chromatography in a 1:1 distribution typical of atropisomers, whereas the C_{20} diastereomers almost coelute, as judged from the slightly broadened peak (Fig. 1).

C_{18} ($R = H$) and C_{19} ($R = CH_3$) components of LII, C_{21} ($R = H$), C_{22} ($R = CH_3$), and C_{24} ($R = C_3H_7$) components of LIII, and C_{26} ($R = H$) and C_{27} ($R = CH_3$) components of LIV were identified. Their mass spectra show enhanced

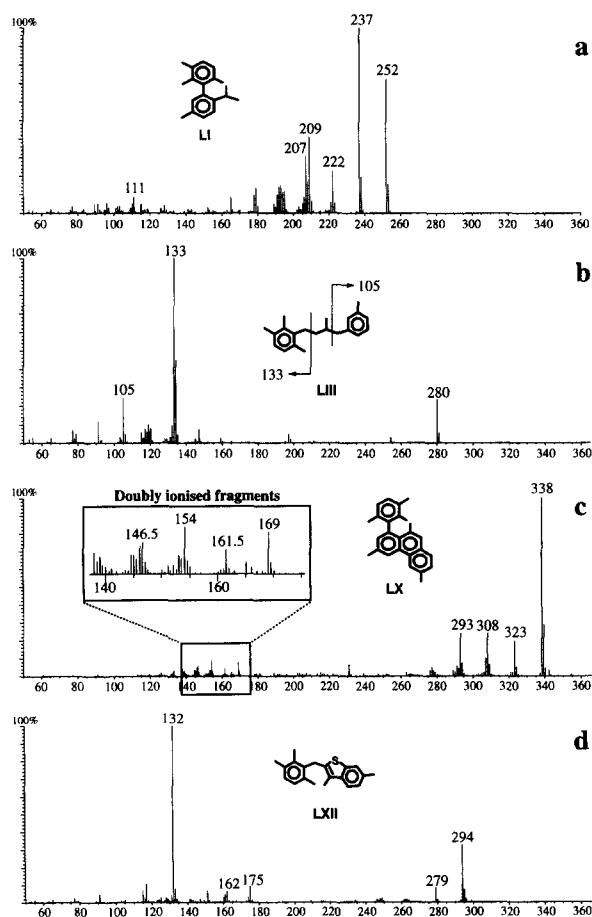


Fig. 10. Mass spectra (subtracted for background) of some short-chain isorenieratene derivatives.

peaks at m/z 105 or 119 resulting from cleavage β to one or both sides of the additional benzene ring. Four unknown compounds with molecular ions at m/z 266, 280, 294, and 336 in the m/z 133 mass chromatogram of the aromatic fraction of a sample from the Permian Kupferschiefer have been reported (Schwark and Püttmann, 1990). The second component ($M^{+} = 280$) was the most prominent and appears to be the C_{21} diaryl isoprenoid **LIII** ($R = H$), because (1) no other major fragment ions were reported, which excludes the possibility of a biphenyl structure, (2) it is prominent in our samples as well, and (3) isorenieratene was also tentatively identified. Diaryl isoprenoids **LIII** with molecular ions at m/z 280, 294, and 322 were also found in a Devonian oil from the Pripyat Basin (Belorussia) that contains abundant aryl isoprenoids (J.L. Clayton, pers. commun.).

Aryl isoprenoids containing two additional aromatic rings. These compounds can be divided into two groups: aryl isoprenoids with a naphthalene moiety involving (1) C-7 to C-15' (**LV** and **LVI**) and (2) C-11 to C-11' (**LVII**–**LIX**).

Mass spectra of **LV** and **LVI** show no major fragment ions indicative of the structural moieties encountered in other isorenieratene derivatives (e.g., m/z 133 and 237). However, three features are typical of alkylated polyaromatic hydrocarbons (Safe and Hutzinger, 1973). First, the molecular ion is the base peak, which testifies to the stability of the molecule under electron impact. Second, a number of doubly ionised fragments is observed (e.g., at m/z 137 and 144). Third, the spectra exhibit strong ($M^{+} - n \cdot 15$) fragment ions from consecutive loss of methyl radicals. All four compounds contain an axially chiral centre, but there are no diastereomers because none contains an asymmetric carbon atom. They elute in the order **LVI** ($R = H$), **LVI** ($R = CH_3$), **LV** ($R = H$), and **LV** ($R = CH_3$), based on the intensity of the fragment ions ($M^{+} - n \cdot 15$) relative to the molecular ion (see also identifications of **X**, **XI**, **XVII**, **XVIII**, **XLII**, and **XLIII**). The elution order of **LV** and **LVI**, with the 2-phenylnaphthyl compounds eluting prior to their 1-phenylnaphthyl counterparts, differs from that of the corresponding C_{32} (**XLII** and **XLIII**) and C_{40} (**X** and **XI**) compounds. Perhaps the structural difference between the isomers is more pronounced for the short-chain compounds, whereas for the C_{32} and C_{40} compounds the retention time is predominantly influenced by the alkyl chain.

Aryl isoprenoids containing three additional aromatic rings. One aryl isoprenoid with a phenanthrene moiety has been identified (**LX**). Its mass spectrum (Fig. 10c) shows characteristics similar to those of **LV** and **LVI** (cf. Safe and Hutzinger, 1973), indicative of alkylated polyaromatic hydrocarbons. No diastereomers exist due to the absence of asymmetric carbon atoms.

Sulphur-containing aryl isoprenoids. This group of compounds is mainly comprised of aryl isoprenoids with a thiophene ring or cyclic sulphide moiety, although one compound with a benzo[*b*]thiophene (**LXII**) moiety has been identified. The S-containing ring occurs at various positions in the isoprenoid chain, yielding different families of compounds. Identification of these compounds in the Calcaires en Plaquettes Formation is supported by production of abun-

dant aryl isoprenoids upon desulphurisation, as discussed elsewhere (van Kaam-Peters et al., 1996b).

One family of aryl isoprenoids with a thiophene ring (**LXI**) is easy to recognise by an abundant fragment ion at m/z 132 (Fig. 6) and a fragment ion at m/z 243 resulting from cleavage β to the thiophene ring. It cannot be presumed that the alkyl side chain of **LXI** is a tail-to-tail isoprenoid chain (cf. **XXVII**), because it may be formed by C-C bond cleavage of **XLIX** or **L**, which possess different alkyl chains. Cleavage products of **L**, however, would be distinguished by a fragment ion at m/z 257.

3.2. Isorenieratene Derivatives in Polar Fractions

Comparison of S-bound isorenieratene derivatives present in the polar fractions of samples from the Gessoso-solfifera, Schistes Cartons, Allgäu, and Boas Oil Shale Formations was made after Raney Ni desulphurisation followed by GC-MS analysis of the released hydrocarbons. The distributions of these products are revealed by partial summed mass chromatograms of fragments (m/z 133 + 237 + 287) that represent the most important ions in the mass spectra of most isorenieratene derivatives (Fig. 11).

Released hydrocarbons include a series of aryl isoprenoids (C_{15} – C_{27}), diaryl isoprenoids (**II**, **XXXIX**, and **XLV**), and diaryl isoprenoids with an additional benzene ring (**VI** and **VII**). C_{32} and C_{33} diaryl isoprenoids with an additional benzene ring and diaryl isoprenoids with more than one additional aromatic ring are present in low amounts.

3.3. ^{13}C Content of Diagenetic and Catagenetic Products of Isorenieratene

Important circumstantial evidence for the genetic relationship of **II**–**LXII** with isorenieratene is provided by their ^{13}C content (Table 2). Comparison of $\delta^{13}C$ values of **II**–**LXII** and components [phytane and 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman] presumed to be derived from algae living in the upper part of the water column (Sinninghe Damsté et al., 1993a) and using Rubisco for CO_2 fixation (Hayes, 1993) reveals a consistent enrichment of ca. 15‰ for **II**–**LXII** in samples ranging from Ordovician to Recent (Table 2 and Fig. 12).

The complex composition of the A3 and A4 fractions (see section 2) containing the diagenetic and catagenetic products of isorenieratene may introduce several problems during isotope-ratio-monitoring GC-MS. First, coelution of compounds will complicate peak definition (Ricci et al., 1994). Coelution of the diagenetic and catagenetic products of isorenieratene and compounds not originating from *Chlorobiaceae* may introduce errors of up to several permil, although GC-MS analyses indicate that such coelutions are rare. Second, subtraction of the appropriate background value is difficult when peaks elute in a "hump." In both cases systematic errors will be made; i.e., a low standard deviation does not guarantee that the value is reliable. However, the difference in $\delta^{13}C$ values between diagenetic and catagenetic products of isorenieratene and compounds derived from algae (ca. 15‰) is much larger than these errors, so they can still be used to characterise an origin from *Chlorobiaceae*.

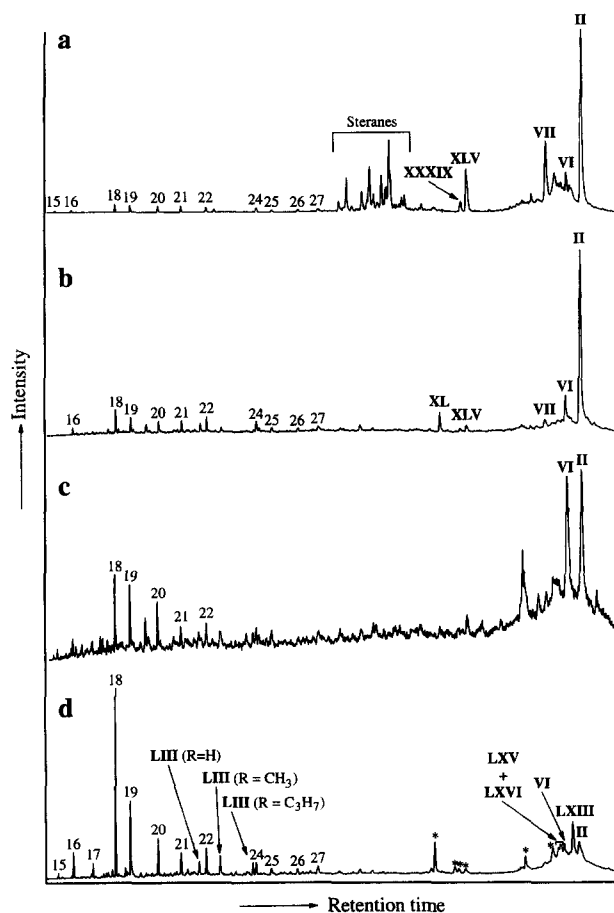


FIG. 11. Summed (m/z 133 + 237 + 287) mass chromatograms of the desulphurised polar fractions of the samples from the (a) Gessoso-solfifera, (b) Schistes Cartons, (c) Allgäu, and (d) Boas Oil Shale Formations. Aryl isoprenoids are indicated by numbers that represent the total number of carbon atoms. Roman numbers refer to structures in Appendix A. Compounds with an unidentified aromatic substitution pattern are denoted with an asterisk.

$\delta^{13}\text{C}$ values of diagenetic and catagenetic products of isorenieratene in each sample fall within a 2–3‰ range. Thus, there are no systematic differences in $\delta^{13}\text{C}$ values between compounds with different carbon skeletons or type or number of S-containing or aromatic rings. This is in agreement with the findings of Freeman et al. (1994) and Sinninghe Damsté et al. (1995b) that diagenetic aromatisation is not accompanied by isotopic fractionation.

Schouten et al. (1995) found that laboratory hydrosulphurisation of a model compound (1-decene) was accompanied by a kinetic isotope effect, producing sulphur compounds that were initially depleted in ^{13}C . They argued that a lack of fractionation effects in other studies of free and sulphurised sedimentary lipids (Kohnen et al., 1992; Schouten et al., 1996) could be explained by complete sulphurisation of functionalised lipids, in agreement with our results. Hartgers et al. (1994c) found that free isorenieratene was enriched in ^{13}C relative to its macromolecularly bound counterpart by ca. 12‰, a difference ascribed to a kinetic isotope effect during formation of geomacromolecules. The

lack of such a difference here may indicate that all available isorenieratene has been incorporated into macromolecules from which it is only released at a later stage of diagenesis. The absence of free isorenieratane in immature sedimentary rocks ($R_0 \approx 0.25\%$) from the Vena del Gesso Basin, northern Italy, and the high amounts of isorenieratane released after desulphurisation of polar fractions, asphaltenes, and kerogen (Kohnen et al., 1991c; Kenig et al., 1995; Schaeffer et al., 1995a; Koopmans et al., 1996a) supports this interpretation.

3.4. Diagenetic Products of a Diaromatic Carotenoid with a 3,4,5-/2,3,6-trimethyl Substitution Pattern

Recently, Hartgers et al. (1993) identified a C_{40} diaryl isoprenoid with an unprecedented 3,4,5-/2,3,6-trimethyl substitution pattern for the aromatic rings (LXIII) in the Mississippian Exshaw Formation. This compound is thought to derive from a diaromatic carotenoid with the same carbon skeleton (LXIV), biosynthesised by photosynthetic sulphur bacteria that may be extinct (Hartgers et al., 1993, 1994c). Because LXIV possesses a conjugated double bond system identical to that of isorenieratene, we anticipate that diagenetic and catagenetic products analogous to those formed from isorenieratene can be identified.

Both isorenieratane and LXIII were identified in the Boas Oil Shale, Womble, Exshaw, and Duvernay Formations. An origin for LXIII from photosynthetic sulphur bacteria is supported by its high ^{13}C content ($\delta^{13}\text{C} = -17.2 \pm 1.1\%$; Table 2) in the Boas Oil Shale Formation. This sample also contains two diaryl isoprenoids with a 3,4,5-/2,3,6-trimethyl substitution pattern and an additional aromatic ring (LXV and LXVI). These elute earlier than VI, compatible with the elution order of the diaryl isoprenoids LXIII and isorenieratane. LXIII, LXV, and LXVI were also released after desulphurisation of the polar fraction of the sample from the Boas Oil Shale Formation (Fig. 11d).

Because of the asymmetric carbon skeleton of LXIV, different compounds result when an additional aromatic ring is present at either C-11 to C-15' or C-15 to C-11' (LXV and LXVI). This complication also applies to other diagenetic products of LXIV. The input of two structurally distinct diaromatic carotenoids (I and LXIV) into the sediment, one with an asymmetric carbon skeleton, severely complicates identification and distinction of their diagenetic and catagenetic products.

3.5. Artificial Maturation Experiments

To study the influence of thermal maturity on the amounts and distributions of isorenieratene derivatives in polyaromatic and HMW fractions, artificial maturation experiments were conducted with a marl sample from the Gessoso-solfifera Formation using hydrous pyrolysis at temperatures between 160 and 330°C.

Partial summed (m/z 133 + 237 + 287) mass chromatograms of the polyaromatic fractions of the original sample and the samples artificially matured at 160, 260, and 300°C are shown in Fig. 13. The distribution of isorenieratene deriv-

Table 2. $\delta^{13}\text{C}$ values (‰) of dia- and catagenetic products of isorenieratene.

	Kimberidge Clay	Calcaires en Plaquettes	Allgäu	Gessoso-solfifera ^a	Gessoso-solfifera ^b	Boas Oil Shale
II	-16.6±0.3	-19.0±0.8	-19.0±0.3	-16.4±1.0	-13.7±0.8	
III (C₁₆)						-18.9±0.3
VI	-16.3±0.2		-18.7±0.7			
VII	-17.3±0.3					
VIII+XVII	-17.9±0.4					
IX	-15.3±0.5					
X+XIV	-19.9±1.4					
XVIII	-14.7±0.5		-21.3±1.2			
XXIV		-19.2±0.3				
XXV		-19.6±0.6				
XXVI		-21.2±0.5				
XXXIX					-13.5±3.0	
XL			-19.0±1.1			
XLIII			-20.8±0.4			
XLV	-15.9±0.3			-15.8±0.8	-13.4±1.0	
XLVI	-17.7±0.8					
LI (R=H)					-16.2±0.7	
LI (R=i-Pr)					-15.9±1.0	
LIII (R=H)	-16.3±0.2					
LV (R=H)	-18.6±1.8					
LXIII						-17.2±1.1
Phytane	-31.2±0.2	-30.5±0.1	-33.5±0.2	-30.1±0.3 ^c	-29.3±0.7	-34.2±0.3
Chroman^d	-33.1±0.5	-32.6±0.2	-33.6±0.2	-28.7±0.3	-26.3±0.2	

^a Polyaromatic fraction of the sample artificially matured by hydrous pyrolysis at 239°C.

^b Desulphurised polar fraction of unheated sample.

^c Phytane in the sample artificially matured by hydrous pyrolysis at 260°C.

^d 2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman.

atives in the original sample is dominated by **VII** (3 $\mu\text{g/g}$ TOC), whereas isorenieratane (**II**) is absent (Fig. 13a). Mild heating (160°C) results in release of low amounts of isorenieratene derivatives with molecular ions 2 or 4 amu less than expected, suggesting the presence of additional rings or double bonds (Fig. 13b). These compounds disappeared upon hydrogenation of the A3 fraction of the sample heated at 200°C whereas the relative amount of isorenieratane increased, indicating that they are unsaturated isorenieratene derivatives. After heating between 239 and 300°C, isorenieratane (**II**) is the most abundant isorenieratene derivative (79 and 61 $\mu\text{g/g}$ TOC at 260 and 300°C, respectively; Fig. 13c and d). A wide range of polyaromatic diagenetic products of isorenieratene and some S-containing compounds are also present. At 330°C only small amounts of isorenieratene derivatives remain, presumably due to their thermal degradation.

Summed (m/z 133 + 237 + 287) mass chromatograms of the desulphurised polar fractions of the same samples are shown in Fig. 14. Desulphurisation of the polar fraction of the original sample yields isorenieratane (66 $\mu\text{g/g}$ TOC), intermediate amounts of **VI**, **VII**, and the C₃₂ and C₃₃ diaryl isoprenoids **XXXIX** and **XLV**, and low amounts of C₁₅–C₂₇ aryl isoprenoids (Fig. 14a). As temperatures are raised to 300°C, the amounts of these compounds first increase and then decrease, but no other isorenieratene derivatives with more than one additional aromatic ring are released. However, at 300°C **IX** is present in low abundance (6 $\mu\text{g/g}$ TOC). At 330°C no isorenieratene derivatives could be detected. The concentration of isorenieratane is highest at 200°C (1.0 $\times 10^3$ $\mu\text{g/g}$ TOC; Fig. 15a). It is the most abundant compound below 300°C, but at higher temperatures

VI predominates. The relative amounts of aryl isoprenoids increase progressively, but their absolute amounts remain low (C₁₈ = 7 $\mu\text{g/g}$ TOC at 300°C). They are dominated by C₁₆ and C₁₈ members.

In the original sample isorenieratane is solely present as a S-bound moiety in the polar fraction (Fig. 15) and in other HMW fractions (i.e., asphaltenes and kerogen). The thermal release of S-bound isorenieratane can be examined by plotting the ratio [polar S-bound]/[polar S-bound + free] for isorenieratane against maturation temperature (Fig. 15b). Isorenieratane is predominantly present as a S-bound moiety up to 239°C. At higher temperatures it is released, so that the percentage of S-bound isorenieratane decreases rapidly to zero at 330°C.

4. DISCUSSION

4.1. Diagenetic and Catagenetic Pathways of Isorenieratene

Hartgers et al. (1994c) presented a concise schematic overview of diagenetic and catagenetic pathways of isorenieratene, stating that it is predominantly (>99%) incorporated into macromolecular substances via multiple S-, O-, and/or C-links. Progressive diagenesis and catagenesis results in the release of aryl isoprenoids from these HMW substances. The small percentage of isorenieratene not incorporated into HMW fractions was held to account for the free isorenieratene in sedimentary rocks and crude oils (Hartgers et al., 1994c). This scheme does not include LMW S-containing or aromatised C₃₂, C₃₃, or C₄₀ compounds, which is our aim here.

There are three principal early diagenetic pathways for

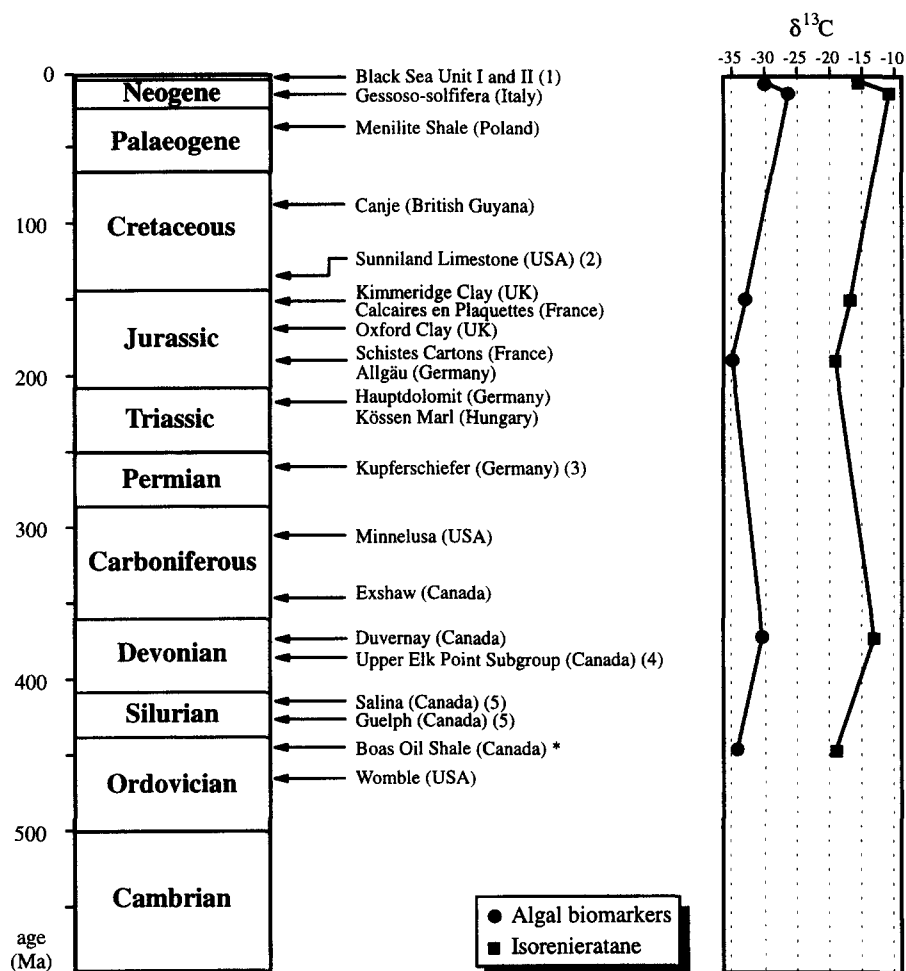


FIG. 12. Occurrence of isorenieratene derivatives through time. $\delta^{13}\text{C}$ values of isorenieratane and algal biomarkers [phytane and 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman] in samples of different age display a constant difference of ca. 15‰. Key: * = isotope value of C_{16} aryl isoprenoid. Numbers in brackets refer to previous studies: (1) Sinninghe Damsté et al. (1993b); (2) Xinke et al. (1990); (3) Schwark and Püttman (1990); (4) Clark and Philp (1989); (5) Summons and Powell (1987).

isorenieratene. First, cyclisation with subsequent aromatisation of the isoprenoid chain can occur. Second, toluene or *m*-xylene can be expelled yielding C_{33} or C_{32} "carotenoids." Third, reduced inorganic sulphur species (either monosulphides or polysulphides) can react with isorenieratene in an intramolecular and intermolecular fashion to generate LMW and HMW products (Sinninghe Damsté et al., 1989, 1993b; de Graaf et al., 1992; Kohnen et al., 1992; Schouten et al., 1994; Kenig et al., 1995; Schaeffer et al., 1995a). In the following sections these reactions will be discussed, together with C-C bond cleavage that occurs during later stages of maturation.

4.1.1. Cyclisation and aromatisation

Formation of additional aromatic rings in isorenieratene derivatives seems to take place at specific sites in the acyclic isoprenoid chain. Five different diaryl isoprenoids with one additional aromatic ring can be anticipated (Sinninghe Dam-

sté et al., 1995b), but only two are encountered as major components (VI and VII). It appears that they form from isorenieratene by cyclisation and aromatisation of a part of the acyclic isoprenoid chain. Cyclisation probably proceeds in two steps (Fig. 16). First, *trans-cis* isomerisation of one double bond within the all-*trans* conjugated double bond system of isorenieratene must occur to form a six-membered ring transition state. Such isomerisation reactions do occur during diagenesis, given the presence of isorenieratene and one of its stereoisomers, containing one *cis* double bond, in an immature marlstone from the Gessoso-solfifera Formation (Keely et al., 1995). Second, an intramolecular Diels-Alder reaction takes place where the diene and the dienophile are adjacent double bonds in the six-membered ring transition state, as proposed earlier for the thermal degradation of polyvinyl chloride (e.g., Tüdös et al., 1974; O'Mara, 1977; Starnes and Edelson, 1979) and β -carotene (Ishiwatari, 1980). The resulting cyclohexadienyl moieties probably aromatise fast because of the energy gain associated with

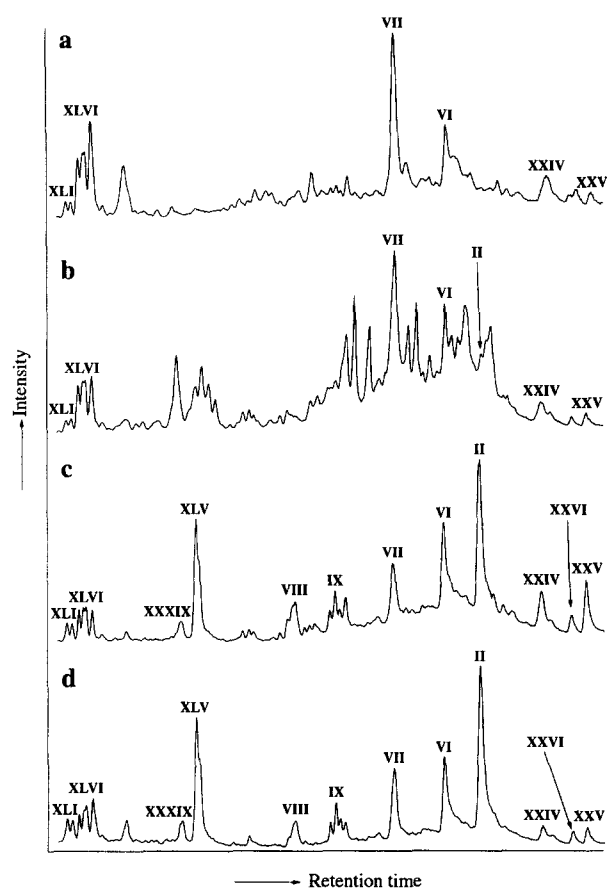


FIG. 13. Partial summed (m/z 133 + 237 + 287) mass chromatograms of the polyaromatic fractions of (a) the original sample from the Gessoso-solfifera Formation, and the samples artificially matured at (b) 160°C, (c) 260°C, and (d) 300°C. Roman numerals refer to structures in Appendix A.

restoration of a fully conjugated double bond system. The Diels-Alder reaction can only result in cyclisation involving C-11 to C-15' and C-7 to C-12, yielding compounds with a ring in the isoprenoid chain at the position found in VI and VII (Fig. 16). The dominance of VI over VII usually observed is probably due to steric hindrance associated with formation of the latter. The positions of the additional benzene rings in VIII and IX are identical to those in VII and VI. Thus, even when two additional benzene rings are present they occupy the two preferred positions discussed above, suggesting that they form by the same cyclisation process.

It can be argued, however, that the double bonds in the isoprenoid chain of isorenieratene form a conjugated system and are therefore not localised as depicted in Fig. 16. Delocalisation of double bonds would, in principle, enable cyclisation involving C-10 to C-15 and C-12 to C-14', even though the six-membered ring transition states only comprise two double bonds. The six-membered ring transition states of the cyclisation reactions involving C-10 to C-15 and C-12 to C-14' are thought to have higher activation energies than those involving C-7 to C-12 and C-11 to C-15', however, because they imply that the molecule is either a biradi-

cal or an ionic species. Indeed, C₄₀ diaryl isoprenoids with an additional aromatic ring involving C-10 to C-15 and C-12 to C-14' have only been found in low abundance in the Kimmeridge Clay Formation (van Kaam-Peters et al., 1996a). It can therefore be assumed that cyclisation via six-membered ring transition states that contain three double bonds represent the major formation pathway of additional aromatic rings in isorenieratene. Cyclisation involving C-20 to C-14' is improbable because C-20 is sp³ hybridised and not part of the conjugated system.

Formation of diaryl isoprenoids with an additional naphthalene moiety probably occurs in a similar fashion (Fig. 17). The cyclisation products shown in Fig. 16 serve as precursors. First, a *trans-cis* isomerisation reaction must occur to form a six-membered ring transition state. Second, the double bonds in the cyclohexadienyl moiety must shift to form a conjugated system and enable a Diels-Alder reaction. Alternatively, and more likely, the ring may have aromatised, which also enables a Diels-Alder reaction. X can form from the precursors of both VI and VII, whereas XII can only

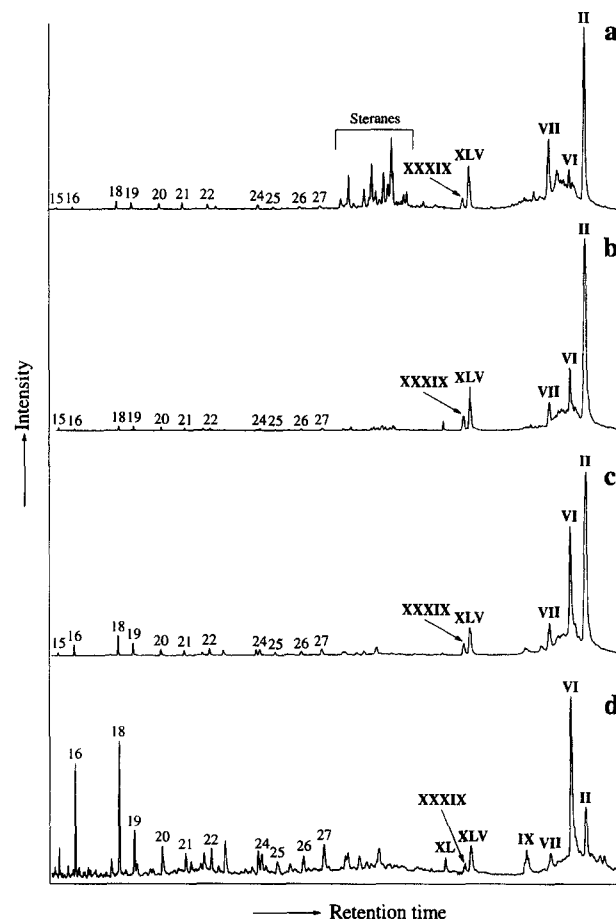


FIG. 14. Summed (m/z 133 + 237 + 287) mass chromatograms of the desulphurised polar fractions of (a) the original sample from the Gessoso-solfifera Formation, and the samples artificially matured at (b) 160°C, (c) 260°C, and (d) 300°C. Aryl isoprenoids are indicated by numbers that represent the total number of carbon atoms. Roman numerals refer to structures in Appendix A.

form from the precursor of **VI** (Fig. 17). **XI** and **XIII** cannot form from either precursor. They require a ten-membered ring transition state and are probably formed directly from isorenieratene (Fig. 18). **XI** is abundant in the Kimmeridge Clay Formation, and is more abundant than **X** in sample G6-5-6 from the Schistes Cartons Formation (Fig. 4), indicating the importance of its formation pathway. However, direct comparison of the amounts of **XI** and **X** is inherently dangerous, because the precursor of **X** can undergo more subsequent cyclisation reactions. The factors controlling formation of compounds with either a 1- or 2-phenylnaphthalene moiety (e.g., **X** and **XI**) are presently unclear.

Formation of the diaryl isoprenoid with a phenanthrene moiety (**XX**) from the aromatised precursor of **IX**, **X**, or **XII** (Fig. 19) requires a sequence of reactions similar to that responsible for formation of diaryl isoprenoids with a naphthalene moiety from the aromatised precursors of **VI** and **VII** (cf. Fig. 17). We propose, following identification of **VII**, **X**, **XVII**, and **XX**, that the diaryl isoprenoid with four additional aromatic rings (**XXI**) is the expected end point of the cyclisation and aromatisation process described above (see Figs. 16, 17, and 19).

The cyclohexadienyl moiety of **XV** and **XVI** is probably also the product of a Diels-Alder reaction. Subsequent aromatisation of this ring is precluded by the quaternary carbon atom C-9'. A similar observation is made for **XIV**, where the quaternary carbon atom C-13' seems to preclude

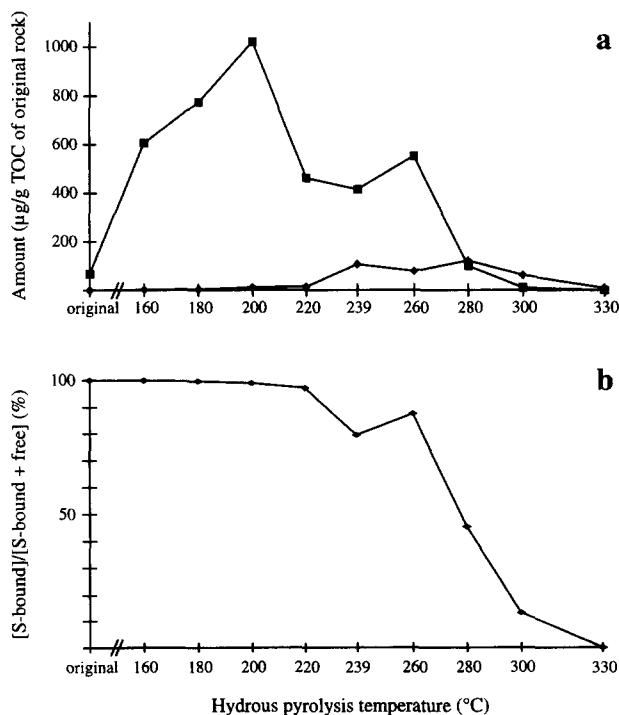


FIG. 15. (a) Generation profiles of free isorenieratane in the polyaromatic fraction (diamonds) and S-bound isorenieratane in the polar fraction (squares) of artificially matured samples from the Gessosolfifera Formation as a function of maturation temperature. (b) [Polar S-bound]/[polar S-bound + free] ratio for isorenieratane as a function of maturation temperature.

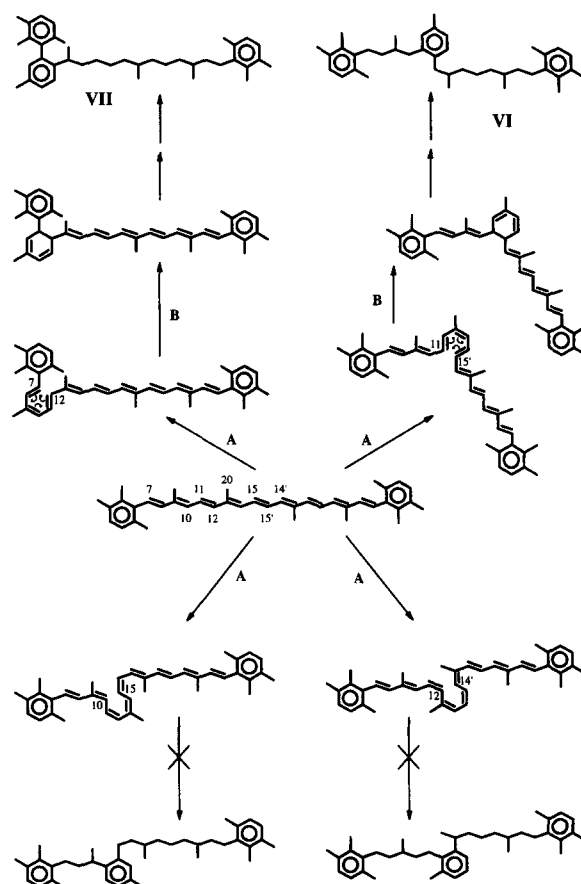


FIG. 16. Formation of diaryl isoprenoids with an additional benzene ring. Cyclisation of isorenieratene proceeds in two steps: (A) *trans-cis* isomerisation of a double bond, (B) intramolecular Diels-Alder reaction. It is unlikely that the Diels-Alder reaction proceeds via the other two six-membered ring transition states, because these have a high activation energy due to the unfavourable positions of the double bonds.

aromatisation of the acenaphthene moiety. However, **XIX** is probably the aromatised counterpart of **XIV**, formed via loss of methyl group C-20'. Corresponding aromatised counterparts of **XV** and **XVI** were not found.

The cyclisation and aromatisation reactions of isorenieratene are summarised in Fig. 20. The double bonds indicated are actually absent in the diagenetic products. They must be hydrogenated at some stage of diagenesis.

4.1.2. Expulsion of *m*-xylene and toluene

Mild heating of β -carotene (**LXVII**) produces C_{32} (phillene; **LXVIII**) and C_{33} (lexene; **LXIX**) "carotenoids" plus *m*-xylene and toluene, respectively (e.g., Kuhn and Winterstein, 1933; Day and Erdman, 1963; Edmunds and Johnstone, 1965; Byers and Erdman, 1983). Jiang and Fowler (1986) tentatively identified fully hydrogenated lexene (or lexane; **LXX**), the C_{33} pseudohomologue of β -carotene (**LXXI**), in an oil from northwestern China. **LXX** and a series of 2,2,6-trimethyl-1-alkylcyclohexanes were identified in Canadian oils (Fowler et al., 1993). The identical ^{13}C

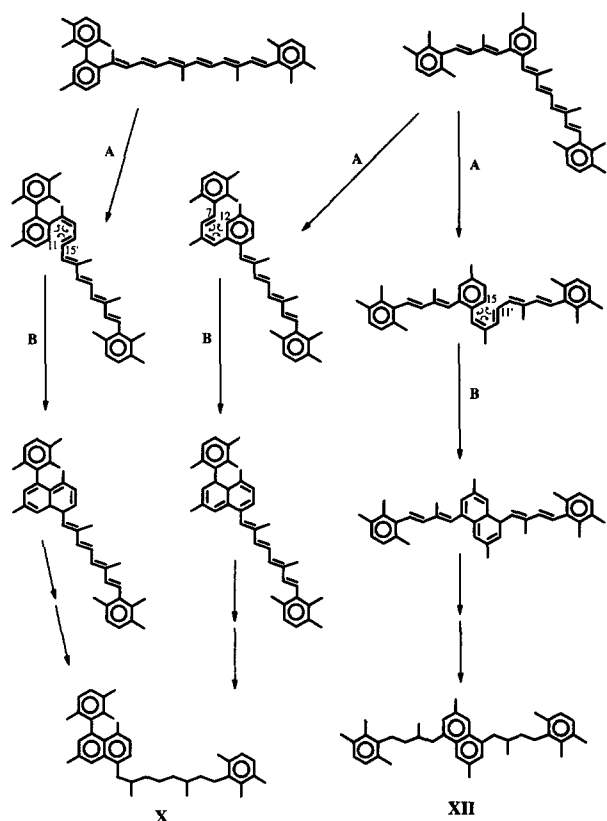


FIG. 17. Formation of diaryl isoprenoids with a naphthalene moiety. Cyclisation of the aromatised precursors of VI and VII, which serve as starting products, proceeds in two steps: (A) *trans-cis* isomerisation of a double bond, (B) intramolecular Diels-Alder reaction.

compositions of LXX and LXXI in the saturated hydrocarbon fraction of a gilsonite from Utah, USA, suggested a common C₄₀ carotenoid precursor (Schoell et al., 1994). The fragment ions at m/z ($M^+ - 106$) and ($M^+ - 92$) in the mass spectrum of β -carotene are thought to originate from loss of *m*-xylene and toluene, respectively, either during mass spectral fragmentation, or during heating in the ion source and subsequent ionisation of the degradation products (e.g., Vetter et al., 1971; Enzell and Wahlberg, 1980).

A pericyclic reaction via an eight-membered ring transition state comprising carbon atoms from the acyclic isoprenoid chain leads to expulsion of *m*-xylene and toluene from β -carotene (Vetter et al., 1971; Byers and Erdman, 1983). Since this mechanism does not involve the cyclohexenyl moieties, isorenieratene can undergo similar reactions, as proposed recently by Kenig et al. (1995). Cleavage only occurs between carbon atoms linked by double bonds in the polyene isoprenoid chain (Vetter et al., 1971), which, for isorenieratene, implies that only one C₃₂ (XXXVII) and one C₃₃ "carotenoid" (XXXVIII) can be formed. There are four possibilities for the expulsion of toluene from isorenieratene, and only two for *m*-xylene (cf. Vetter et al., 1971; Enzell and Wahlberg, 1980). Although steric hindrance associated with formation of the eight-membered ring transition state

may also be influential, in most samples C₃₃ compounds are indeed more abundant than C₃₂ compounds. The cyclisation, aromatisation and sulphurisation reactions that led to the C₄₀ diagenetic products of isorenieratene can also occur with XXXVII and XXXVIII. Further expulsion of *m*-xylene or toluene from XXXVII and XXXVIII is less likely because only six double bonds remain in the isoprenoid chain, complicating formation of a sterically unhindered eight-membered ring transition state (Vetter et al., 1971). Thermal degradation of philene (LXVIII) and lexene (LXIX) under laboratory conditions indeed proceeds considerably slower than thermal degradation of β -carotene (LXVII) (Byers and Erdman, 1983). A C₂₈ "carotenoid" may be formed via a similar reaction, involving expulsion of either 1,5- or 1,6-dimethylnaphthalene via a twelve-membered ring transition state. Small amounts of dimethylnaphthalenes were reported in thermal degradation experiments with β -carotene (Day and Erdman, 1963; Byers and Erdman, 1983). Schaeffer et al. (1995a) found small amounts of a C₂₈ diaryl isoprenoid after desulphurisation of a S-rich kerogen.

The additional benzene rings in the C₃₂ and C₃₃ compounds XI, XII, and XIII are in the same positions as in the C₄₀ compounds VI and VII, suggesting either that (1) cyclisation and aromatisation occurs at preferred parts of the isoprenoid chain which are "recognisable" in C₃₂ and C₃₃ "carotenoids" or (2) the conjugated double bond system remaining after cyclisation of isorenieratene is large enough to facilitate expulsion of *m*-xylene or toluene. The first explanation is consistent with proposed cyclisation reactions for

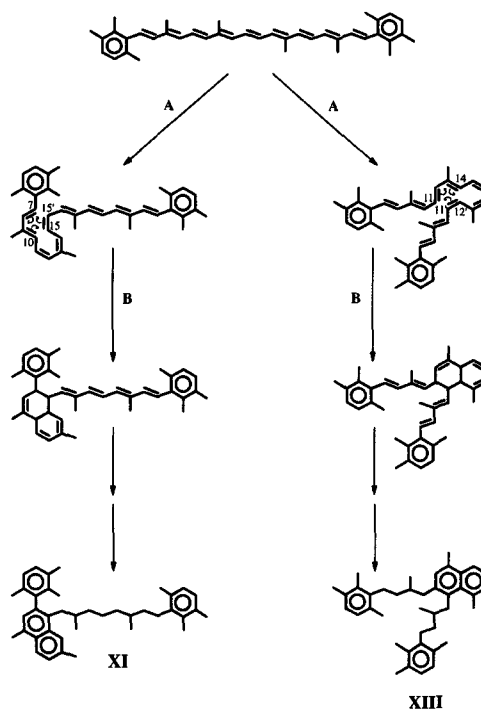


FIG. 18. Formation of diaryl isoprenoids with a naphthalene moiety via a ten-membered ring transition state. Cyclisation of isorenieratene occurs in two steps: (A) three *trans-cis* isomerisation reactions of double bonds, (B) intramolecular Diels-Alder reaction.

isorenieratene (Fig. 16), whereas in the second cyclisation of isorenieratene may produce the unsaturated "precursor" of VI, leaving a conjugated double bond system that is insufficient to enable expulsion of *m*-xylene or toluene (Vetter et al., 1971). Therefore, XL, XLI, and XLVI are held to form through cyclisation and aromatisation of XXXVII and XXXVIII (cf. Fig. 16), i.e., after expulsion of *m*-xylene or toluene from isorenieratene.

4.1.3. Sulphurisation

No carotenoids of biological origin with sulphur directly bound to their carbon skeleton have been reported (Liaaen-Jensen, 1990), suggesting that S-containing diaryl isoprenoids are diagenetic products of isorenieratene. Reaction of isorenieratene with reduced inorganic sulphur species during early diagenesis (sulphurisation) is probably particularly efficient because isorenieratene contains nine conjugated double bonds all available for reaction (Sinninghe Damsté et al., 1989; de Graaf et al., 1992; Schouten et al., 1994). Sulphurisation can proceed in an intermolecular and/or an intramolecular fashion.

Intermolecular sulphurisation of isorenieratene results in

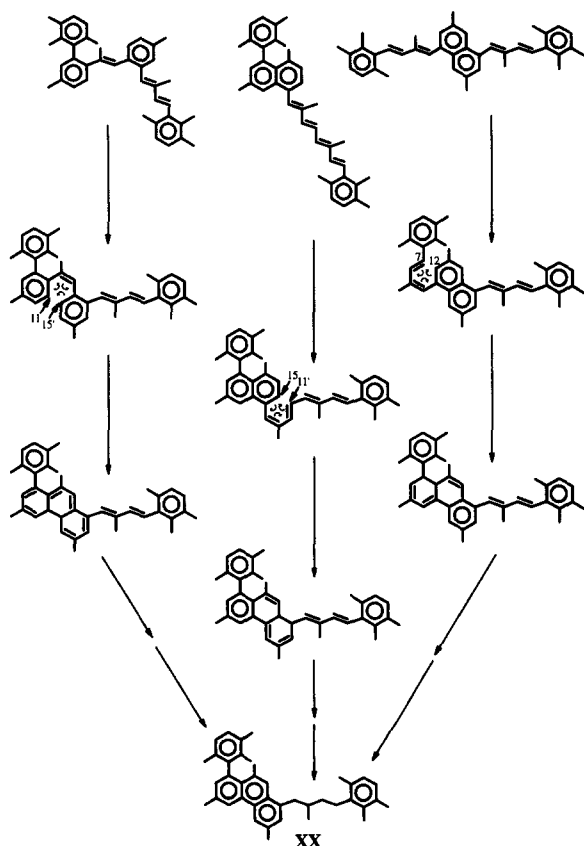


FIG. 19. Formation of a diaryl isoprenoid with a phenanthrene moiety. Cyclisation of the aromatised precursors of IX, X, and XII, which serve as starting products, proceeds in two steps: (A) *trans-cis* isomerisation of a double bond, (B) intramolecular Diels-Alder reaction.

the formation of cross-linked structures that make up part of geomacromolecules. The nine conjugated double bonds of isorenieratene allow for abundant formation of sulphur links. The number of sulphur links and the average molecular weight of geomacromolecules are positively correlated (Sinninghe Damsté et al., 1990; Kohnen et al., 1991b), so that isorenieratene is probably mainly incorporated into the kerogen. This is supported by the artificial maturation experiments with the marl sample from the Gessoso-solfifera Formation. Desulphurisation of the polar fraction of the unheated sample yields relatively low amounts of isorenieratane (Fig. 15a). When the sample is heated to 200°C, large amounts of S-bound isorenieratane are present in the polar fraction. This increase suggests that S-bound isorenieratane in the kerogen is released as part of smaller S-linked structures that reside in the polar fraction, by the thermal cleavage of relatively weak S-S and C-S bonds (Koopmans et al., 1996a).

Intramolecular sulphurisation can yield free isorenieratene derivatives containing one or more sulphur atoms (e.g., thiophenes). However, it seems more likely that intramolecular and intermolecular sulphurisation occur simultaneously, so that free S-containing isorenieratene derivatives are not expected to be abundant. Indeed, such compounds have not been reported, whereas isorenieratane has been released after desulphurisation of the polar fraction of several samples (Kohnen et al., 1992; Sinninghe Damsté et al., 1993b, 1995a; Kenig et al., 1995; Schaeffer et al., 1995a,b). An alternative formation pathway of free S-containing isorenieratene derivatives is via thermal degradation of (poly)sulphide-bound isorenieratane, which can yield alkylthiophenes (cf. Krein and Aizenshtat, 1994; Schouten et al., 1994; Koopmans et al., 1995).

Five structural isomers of diaryl isoprenoids with one thiophene ring (XXIV–XXVIII) were identified. These represent all possible isomers, if thiophenes involving C-19, C-20, C-19', and C-20' are disregarded. This suggests that many dithiophenes and even terthiophenes can be expected to form during diagenesis. Although a maximum of fifteen dithiophene isomers can be formed, only two were identified (XXXII and XXXIII). This may be due to their low abundance which makes identification difficult. Moreover, several of the dithiophene isomers will probably not elute from the capillary column.

Comparison of distributions of S-bound isorenieratene derivatives in the polar fraction of samples from the Gessoso-solfifera, Schistes Cartons, Allgäu, and Boas Oil Shale Formations (Fig. 11) reveals that C₃₂ and C₃₃ diaryl isoprenoids with an additional aromatic ring and diaryl isoprenoids with more than one additional aromatic ring are present in low amounts, probably because the sulphur bridges that link their precursors to the macromolecular network prevent cyclisation reactions. Isorenieratane (II) is much more abundant than other isorenieratene derivatives compared to the polyaromatic fractions of the same samples (see below), which may indicate that the conjugated double bond system of sulphurised isorenieratene is too small to enable expulsion or cyclisation reactions. Alternatively, the number of double bonds in isorenieratene (compared to its cyclised diagenetic

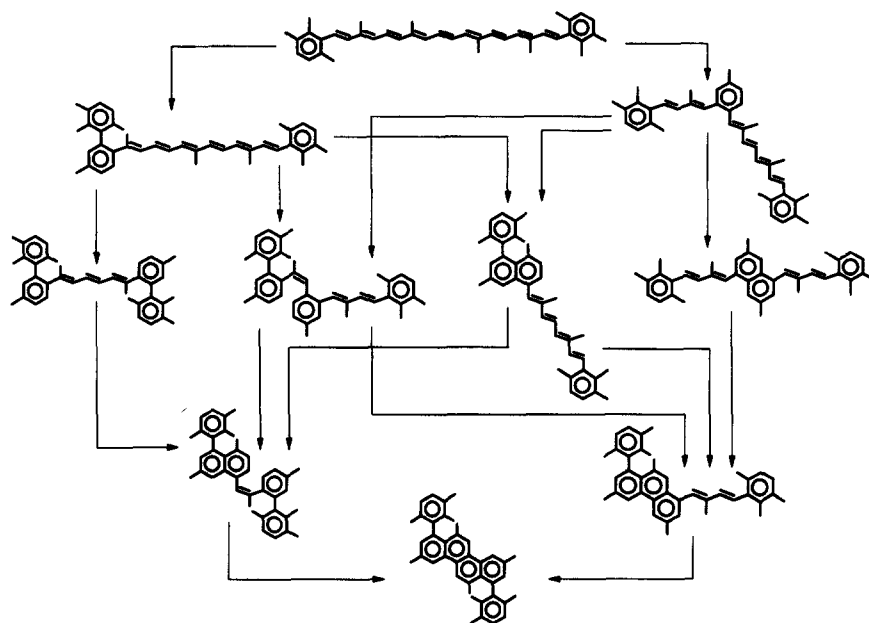


FIG. 20. Overview of formation pathways of C_{40} cyclised and aromatised diagenetic products of isorenieratene. This scheme does not include compounds comprising naphthalene moieties formed via ten-membered ring transition states. Their formation pathways are outlined in Fig. 18.

products) makes it more susceptible to incorporation into a S-rich macromolecular network.

4.1.4. Interplay of Early Diagenetic Reactions

Studies of Recent Black Sea sediments have shown that incorporation of isorenieratene into a S-rich macromolecular network proceeds quickly in the upper sediment layers (cf. Sinninghe Damsté et al., 1993b; Repeta, 1993; Wakeham et al., 1995). Identification of **VI**, **VII**, **XXXIX**, and **XLV** in the desulphurised polar fraction of an immature marlstone from the Gessoso-solfifera Formation (Fig. 14a) suggests that cyclisation and expulsion can occur before sulphurisation, although it cannot exclude such reactions after incorporation into a macromolecular network. However, **XXX**, **XXXV**, and **LXII** strongly suggest that sulphurisation preceded cyclisation, which is unlikely to proceed through C-10 to C-15 in isorenieratene (Fig. 16). Thus, cyclisation with subsequent aromatisation, expulsion, and sulphurisation are probably competing processes during early diagenesis, probably dependent on the availability of reduced inorganic sulphur species which may 'quench' isorenieratene before any pericyclic reactions (expulsion and cyclisation) can occur. However, sulphur is not a prerequisite for expulsion and cyclisation reactions, because cyclised and aromatised diagenetic products of β -carotene have been identified in a sample from the Green River Formation that contains only small amounts of organic sulphur (Koopmans et al., 1996b). In summary, the three competing processes probably succeed each other. For instance, **XLI** may be formed via several different pathways (Fig. 21). These pathways should include one cyclisation and one expulsion reaction. Sulphurisation

is not required, but can occur at any stage of the process (Fig. 21).

The diagenetic and catagenetic products of isorenieratene identified in this study mark the first recognition of cyclisation and aromatisation of polyunsaturated acyclic moieties in biomarkers during sediment diagenesis. These reactions are not restricted to isorenieratene. The conjugated double bond systems of other carotenoids are anticipated to experience similar diagenetic reactions, as observed here for the diaromatic carotenoid with the 3,4,5-/2,3,6-trimethyl substitution pattern. Analysis of a sample from the Green River Formation (Mahogany Zone) reveals a wide range of previously unrecognised cyclised and aromatised diagenetic products of β -carotene that are structurally similar to the compounds described here (Koopmans et al., 1996b). These are likely formed by comparable pathways.

4.1.5. C-C bond cleavage

It appears that short-chain compounds form from C-C bond cleavage of C_{32} , C_{33} , and C_{40} isorenieratene derivatives. For example, **LII** probably originates from the C-11 to C-1' part of **XL**, and **LIV** can only be formed from C-C bond cleavage of **VI**. Random C-C bond cleavage probably only takes place at relatively high levels of thermal maturity (Requejo et al., 1992), comparable to the formation of alkanes and aromatics from kerogen, although perhaps facilitated by methyl branches and aromatic moieties in the isoprenoid chain. Aryl isoprenoids typically represent the only discernible remnants of isorenieratene in mature sedimentary rocks and crude oils (e.g., Ostroukhov et al., 1982; Summons and Powell, 1986, 1987), although they also occur in immature

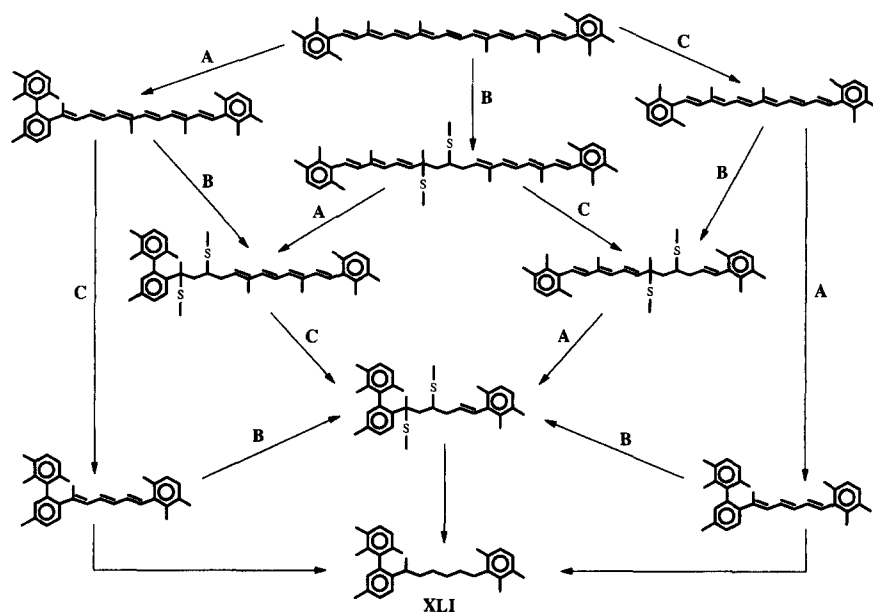


FIG. 21. Possible formation pathways of **XLI**, including (A) cyclisation, (B) sulphurisation, and (C) expulsion reactions.

sedimentary rocks as part of S-rich geomacromolecules (Hartgers et al., 1994c).

C_{12} , C_{17} , and C_{23} aryl isoprenoids cannot be formed by single C-C bond cleavage of isorenieratane. However, in **XXXIX** and **XLV** single C-C bond cleavage results in aryl isoprenoids with isoprenoid chains different from those formed from isorenieratane. For example, C_{12} and C_{17} aryl isoprenoids can result from thermal breakdown of **XXXIX**, and C_{17} and C_{23} aryl isoprenoids may be similarly derived from **XLV**. Compounds comprising additional aromatic rings preclude formation of aryl isoprenoids with up to thirty-one carbon atoms. For instance, C-C bond cleavage of **VI** can, at most, yield a C_{18} aryl isoprenoid.

There is a discrepancy between the distributions of aryl isoprenoids and their counterparts with additional aromatic rings. For the former a typical pattern includes compounds in the range C_{13} – C_{31} with the short-chain components dominant (Summons and Powell, 1986, 1987), a distribution that seems to indicate random C-C bond cleavage. For the latter the distributions are restricted to compounds apparently formed by α ($R = H$) and β ($R = CH_3$) cleavage (see Appendix A). This suggests that they form from C_{32} , C_{33} , and C_{40} precursors by two principal processes: (1) cleavage of an alkyl side chain associated with aromatisation of unsaturated rings (cf. Starnes and Edelson, 1979) and (2) C-C bond cleavage β to the aromatic ring system.

4.2. Maturity-Related Changes in Abundance and Distribution of Isorenieratene Derivatives

The increasing amount of S-bound isorenieratane in the polar fraction of the sample from the Gessoso-solfifera Formation with increasing artificial maturation temperature up to 200°C (Fig. 15a) is probably due to the cleavage of rela-

tively weak S-S and C-S bonds in the kerogen, resulting in the release of smaller S-linked structures that contain isorenieratane and end up in the polar fraction (Koopmans et al., 1996a). After heating at temperatures higher than 200°C, the amount of S-bound isorenieratane in the polar fraction decreases, probably due to the thermal cleavage of the remaining S-S and C-S bonds. This process can be monitored by plotting the ratio [polar S-bound]/[polar S-bound + free] as a function of temperature (Fig. 15b), which shows that considerable release of S-bound isorenieratane only occurs at 280°C and higher temperatures. It is obvious from Fig. 15a that S-bound isorenieratane is not thermally released exclusively as free isorenieratane. The far greater part is probably released as cyclised and aromatised or S-containing derivative of isorenieratene as a means of stabilisation.

Isorenieratane (**II**) and **VI** are abundantly present in the desulphurised polar fractions of the samples from the artificial maturation series (Fig. 14). **II** is presumed to be linked via more sulphur bonds than **VI** because of the greater number of double bonds in isorenieratene compared to the precursor of **VI**, suggesting that the **VI/II** ratio in the desulphurised polar fraction would decrease with increasing maturation temperature. However, it increases from 0.1 in the original sample to 2.4 at 300°C. There are two possible explanations for this discrepancy. First, thermal cleavage of sulphur bonds linking isorenieratene may produce radical sites within the isoprenoid chain that may induce cyclisation and subsequent aromatisation as a means of stabilisation. This stabilisation reaction may be enhanced at higher temperatures, yielding greater amounts of diaryl isoprenoids with an additional aromatic ring upon desulphurisation. However, it is unlikely that this process would yield **VI** exclusively, because the radical sites would be expected at various positions. Second, if the polar fraction contains isore-

neratene or its partly hydrogenated counterparts, increasing maturation temperatures may induce the cyclisation reactions shown in Fig. 16. Keely et al. (1995) found isorenieratene in immature marlstones from the Gessoso-solfifera Formation. Desulphurisation and subsequent hydrogenation of a polar fraction containing isorenieratene or its partly hydrogenated counterparts would yield isorenieratane. However, the ratio of VII to II does not increase with increasing temperature, nor does the ratio $(XXXIX + XLV)/II$, which could serve as an indicator for the extent of expulsion.

The maturity-related trends in the amounts and distributions of isorenieratene derivatives deduced from the artificial maturation experiments can be compared to those in natural samples by examination of polyaromatic and desulphurised polar fractions of four samples with different degrees of thermal maturity. These are from the (1) Gessoso-solfifera ($R_o \approx 0.25\%$), (2) Schistes Cartons (Paris Basin G6-5-6; $R_o = 0.43\%$), (3) Allgäu ($R_o = 0.43\%$), and (4) Boas Oil Shale Formations ($R_o = 0.47\%$). Vitrinite reflectance values for the last three samples were determined via the Pristane Formation Index (Goossens et al., 1988a,b).

Partial summed (m/z 133 + 237 + 287) mass chromatograms of the polyaromatic fractions of these four samples (Fig. 22) reveal no obvious trends. The absence of isorenieratane in the Gessoso-solfifera Formation is attributed to its initial incorporation via sulphur linkages in HMW fractions. The other three samples do not contain Type II-S kerogen, so that it is unclear whether the presence of isorenieratene in their polyaromatic fractions reflects their lower sulphur contents or higher levels of thermal maturity. Samples from the Schistes Cartons and Allgäu Formations possess similar distributions of isorenieratene derivatives (Fig. 22b and c), but the amounts of C_{32} and C_{33} compounds relative to isorenieratane in the latter is about twice that in the former. It also contains about twice the amount of C_{40} cyclised and aromatised isorenieratene derivatives relative to isorenieratane, suggesting that pericyclic reactions (i.e., expulsion and cyclisation) were more prominent during early diagenesis of the Allgäu than of the Schistes Cartons Formation. The ratio of C_{40} compounds to C_{32} and C_{33} compounds is probably not a function of thermal maturity, because it is fixed during early diagenesis given the low activation energy of the expulsion reaction (Byers and Erdman, 1983). The proportion of cyclised and aromatised products relative to diaryl isoprenoids is probably governed by similar controls, because cyclisation and expulsion are both pericyclic reactions. In the Boas Oil Shale Formation, concentrations of C_{32} , C_{33} , and C_{40} isorenieratene derivatives are low ($<30 \mu\text{g/g TOC}$). The abundance of aryl isoprenoids ($C_{15} = 41 \mu\text{g/g TOC}$; not shown in Fig. 22d) may be related to the higher level of thermal maturity, because aryl isoprenoids are the only recognisable remains of isorenieratene previously identified in ancient sedimentary rocks and crude oils (e.g., Summons and Powell, 1986, 1987). The high amount of aryl isoprenoids in the Boas Oil Shale suggests that C-C bond cleavage is more important with increasing thermal maturity, in agreement with data of Requejo et al. (1992).

Summed mass chromatograms (m/z 133 + 237 + 287) of the desulphurised polar fractions (Fig. 11) show an in-

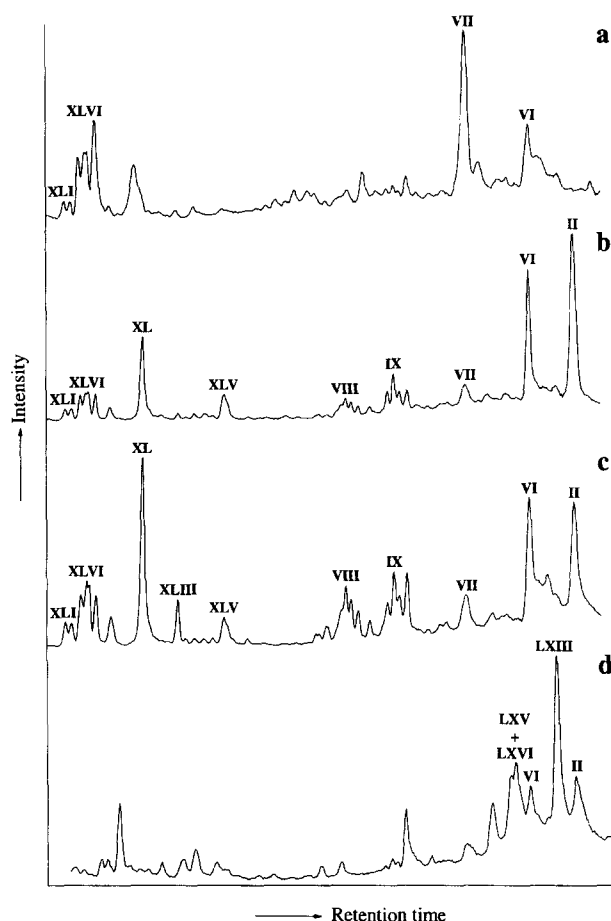


FIG. 22. Partial summed (m/z 133 + 237 + 287) mass chromatograms of the polyaromatic fractions of the samples from the (a) Gessoso-solfifera, (b) Schistes Cartons, (c) Allgäu, and (d) Boas Oil Shale Formations. Roman numbers refer to structures in Appendix A.

crease in short-chain compounds, particularly aryl isoprenoids, with increasing thermal maturity. The VI/II ratio does not increase with increasing thermal maturity as in the maturation series from the Gessoso-solfifera Formation (Fig. 14). 30% of isorenieratane in the Schistes Cartons Formation and 7% in the Allgäu Formation is sulphur bound. For the C_{40} diaryl isoprenoid LXIII in the Boas Oil Shale Formation this percentage is 21. These values suggest that these samples represent a level of thermal maturity comparable to the sample from the Gessoso-solfifera Formation artificially matured at temperatures higher than 280°C (cf. Fig. 15b). However, the amount of reduced sulphur species present during early diagenesis of these sediments may also influence these percentages.

4.3. Palaeoenvironmental Significance of Isorenieratene Derivatives

Diagenetic and catagenetic products of isorenieratene provide important information on the anoxic conditions of the depositional environment, especially in sedimentary rocks and crude oils that do not contain isorenieratane. They are

potentially valuable tools in oil-oil and oil-source rock correlation and biomarker studies because of their specific carbon skeletons and anomalously high ^{13}C contents. Molecular palaeontology (i.e., recognition of biological sources of sedimentary organic compounds) is severely complicated by the diagenetic reactions of isorenieratene, because it is the precursor of many products. Precise identifications of carbon skeletons and ^{13}C contents of these products is required, because other carotenoids (e.g., β -carotene) can undergo the same diagenetic transformations leading to similar, albeit not identical, suites of diagenetic products (Koopmans et al., 1996b).

In the present study, isorenieratene derivatives have been identified in sedimentary rocks ranging from Ordovician to Miocene (Fig. 12). This suggests that in the past anoxygenic photosynthesis has been a more common process. The only contemporary example of a marine basin where anoxygenic photosynthesis takes place is the Black Sea (Repeta et al., 1989). The presence of isorenieratene derivatives in the samples studied also shows that anoxic conditions can be associated with formation of organic-rich sediments, given that all samples contain more than 5% TOC except those from the Gessoso-solfifera and Canje Formations. Petroleum source rocks containing isorenieratene derivatives are from the Duvernay, Exshaw, Schistes Cartons, Kimmeridge Clay, and Menilite Formations (Klemme and Ulmishek, 1991). Thus, euxinic conditions with anoxia extending into the photic zone favour preservation of organic matter, and can accompany formation of petroleum source rocks. However, our data cannot resolve the relative importance of anoxia and productivity for the preservation of organic matter (cf. Demaison and Moore, 1980; Pedersen and Calvert, 1990).

Several sedimentological (e.g., presence of lamination), geochemical (e.g., S/C ratio, degree of pyritisation, trace metal concentrations), and palaeontological (e.g., biofacies analysis) indicators are commonly used to assess bottom water anoxia (see Arthur and Sageman, 1994, and Wignall, 1994, for reviews). However, these indicators cannot provide information on the thickness of the oxic and anoxic part of the water column. The strength of the isorenieratene derivatives as palaeoenvironmental indicators is that they give an indication of the position of the chemocline in the water column (cf. Black Sea; Sinninghe Damsté et al., 1993b), because light penetration is limited to a certain depth depending on local conditions. A relatively small oxic water column implies degradation of organic matter at a relatively low rate, and thus enhanced preservation of organic matter, because it is generally believed that the rate of aerobic degradation is lower than the rate of anaerobic degradation (e.g., Peters and Moldowan, 1993). The palaeoenvironmental significance of isorenieratene derivatives in relation to the geological setting of the samples studied will be further elaborated in a separate paper.

5. CONCLUSIONS

A wide range of novel diagenetic and catagenetic products of the diaromatic carotenoid isorenieratene has been identified in a number of sedimentary rocks ranging from Ordovi-

cian to Miocene. Their identification is based on NMR, mass spectrometry, the presence of atropisomers, and stable carbon isotopes. $\delta^{13}\text{C}$ values of diagenetic and catagenetic products of isorenieratene and compounds derived from algae living in the upper part of the water column show a consistent difference of ca. 15‰ throughout the Phanerozoic. This supports an origin from *Chlorobiaceae* because these organisms use the reverse TCA cycle to fix carbon, leading to biomass anomalously enriched in ^{13}C .

Diagenetic and catagenetic products of isorenieratene include C_{40} , C_{33} , and C_{32} diaryl isoprenoids and short-chain aryl isoprenoids with additional aromatic and/or S-containing rings. The reactions by which these compounds are formed include cyclisation with subsequent aromatisation, expulsion, sulphurisation, hydrogenation, and C-C bond cleavage. Cyclisation of the acyclic isoprenoid chain is proposed to occur via an intramolecular Diels-Alder reaction. C_{33} and C_{32} compounds are diagenetic products of C_{33} and C_{32} "carotenoids" formed from isorenieratene by expulsion of toluene and *m*-xylene, respectively. Sulphurisation during early diagenesis sequesters isorenieratene predominantly in the kerogen, from which it is released during progressive diagenesis as indicated by artificial maturation experiments. C-C bond cleavage of isorenieratene derivatives is governed by two processes: (1) cleavage β to the additional aromatic ring system and (2) loss of an alkyl chain associated with aromatisation of a newly formed ring. Similar reactions can occur with other carotenoids, as shown by the identification of novel diagenetic products of a diaromatic carotenoid with a 3,4,5-/2,3,6-trimethyl substitution pattern for the aromatic rings.

Sulphurisation, expulsion, and cyclisation with subsequent aromatisation are probably competing processes during early diagenesis. Incorporation of (partly cyclised) isorenieratene into a S-rich macromolecular network diminishes the possibilities for further expulsion and cyclisation reactions due to lack of a conjugated double bond system sufficiently large for these reactions. Therefore, the availability of reduced inorganic sulphur species during early diagenesis may (partly) determine the fate of isorenieratene. The extent of expulsion and cyclisation with subsequent aromatisation is probably already established during early diagenesis, and increasing maturation does not profoundly influence the product distribution.

Since isorenieratene is uniquely biosynthesised by the photosynthetic green sulphur bacteria *Chlorobiaceae*, the presence of any diagenetic and catagenetic products of isorenieratene in sedimentary rocks and crude oils indicates photic zone anoxia in the depositional environment. These compounds are expected to be useful in palaeoenvironmental reconstruction and in oil-oil and oil-source rock correlation studies. Their presence in several petroleum source rocks suggests that anoxia is important for the preservation of organic matter.

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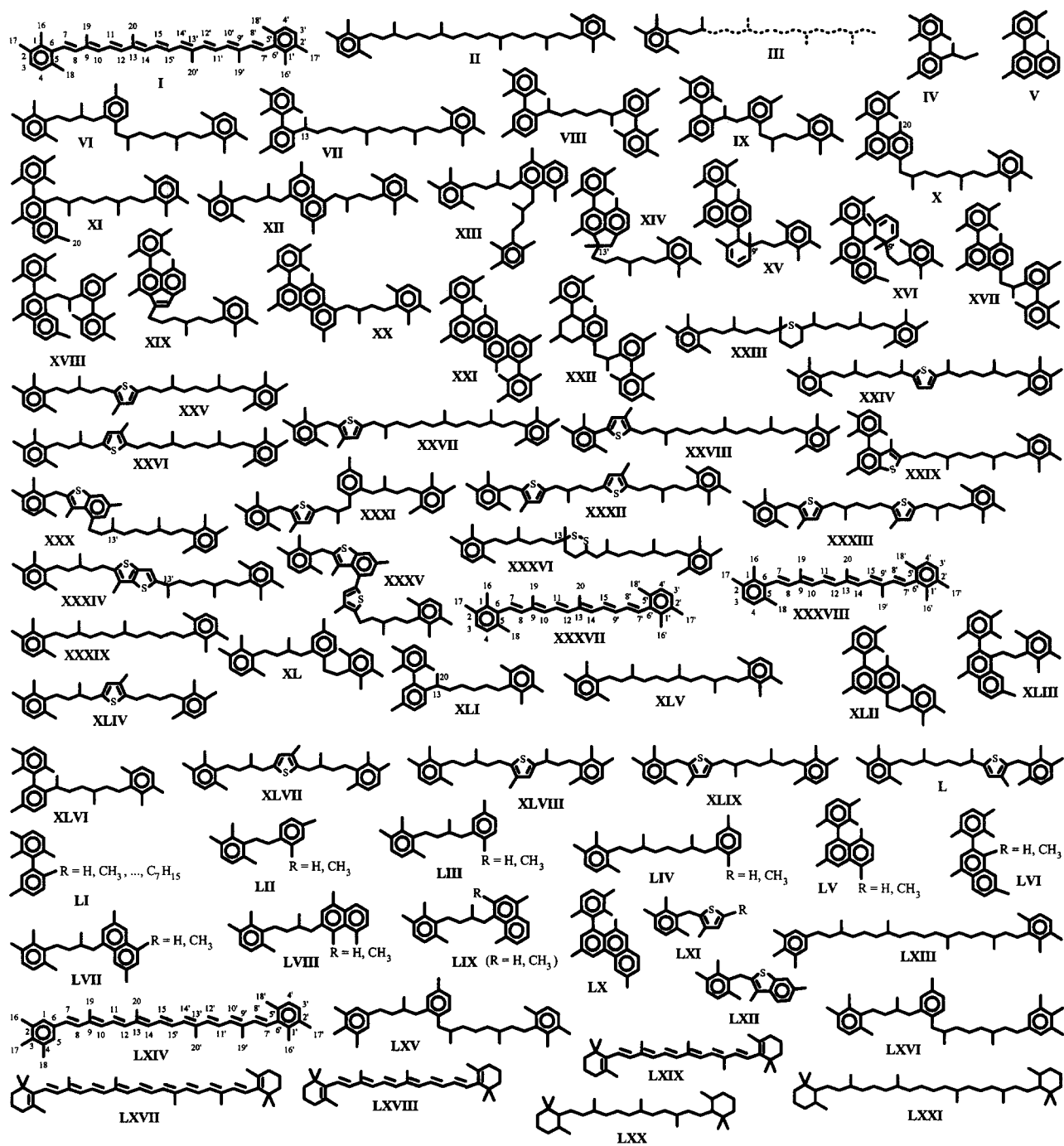
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Appendix A



Appendix B. Details of structural identifications of II-LXII.

Structure	Formula	M^+	Diagnostic fragment ions (intensity)	Cyclisation ^a	Atropisomers	Further details
C₄₀ compounds						
II	C ₄₀ H ₆₆	546(29)	133 ^b (100), 134 ^c (91)	No	No	Co-injection with authentic standard
VI	C ₄₀ H ₅₈	538(20)	119 ^d (22), 133 ^b (100), 134 ^c (36), 173(12)	11-15' (Fig. 16)	No	¹ H and ¹³ C NMR (see Appendix C and Sinnighe Damsté <i>et al.</i> , 1995b)
VII	C ₄₀ H ₅₈	538(17)	133 ^b (49), 207 ^c (12), 222 ^f (9), 237 ^g (100)	7-12 (Fig. 16)	No ^b	Mass spectrum in Fig. 2a
VIII	C ₄₀ H ₅₀	530(17)	207 ^c (15), 222 ^f (10), 237 ^g (100)	7-12 (Fig. 16)	Yes ⁱ	Mass spectrum in Fig. 2b
IX	C ₄₀ H ₅₀	530(1)	133 ^b (17), 207 ^c (11), 222 ^f (7), 237 ^g (100), 294 ^h (2)	7-12, 15-11' (Fig. 16)	Yes (1:2:1) ^k	
X	C ₄₀ H ₅₂	532(40)	133 ^b (22), 257 ^c (31), 272 ^f (33), 287 ^g (100)	7-12, 11-15' (Fig. 17)	No ^b	Mass spectrum in Fig. 2c
XI	C ₄₀ H ₅₂	532(76)	133 ^b (37), 257 ^c (23), 272 ^f (7), 287 ^g (100)	7-15', 10-15 (Fig. 18)	No ^b	
XII	C ₄₀ H ₅₂	532(54)	133 ^b (100), 183 ^m (65)	11-15', 15-11' (Fig. 17)	No	
XIII	C ₄₀ H ₅₂	532(9)	133 ^b (100), 183 ^m (26)	11-11', 14-12' (Fig. 18)	No	
XIV	C ₄₀ H ₅₀	530(37)	133 ^b (59), 283 ⁿ (13), 298 ^l (6), 313 ^o (100)	7-12, 11-15' (Fig. 17), 10-13' ^o	No ^b	
XV	C ₄₀ H ₄₆	526(8)	133 ^b (59), 147 ^p (28), 235(13), 379 ^q (100)	7-12, 11-15' (Fig. 17), 14'-9'	Yes (1:1) ^q	
XVI	C ₄₀ H ₄₆	526(9)	133 ^b (58), 147 ^p (22), 235(8), 379 ^q (100)	7-15', 10-15 (Fig. 18), 14'-9'	Yes (1:1) ^q	
XVII	C ₄₀ H ₄₄	524(13)	207 ^c (9), 222 ^f (5), 237 ^g (20), 257 ^h (26), 272 ^f (23), 287 ^g (100)	7-12, 11-15' (Fig. 17), 12'-7' (Fig. 16)	Yes (1:1)	Mass spectrum in Fig. 2d
XVIII	C ₄₀ H ₄₄	524(23)	207 ^c (18), 222 ^f (9), 237 ^g (64), 257 ^h (15), 272 ^f (5), 287 ^g (100)	7-15', 10-15 (Fig. 18), 12'-7' (Fig. 16)	No ^f	
XIX	C ₃₉ H ₄₆	514 ^{4*} (100)	133 ^b (12), 280 ^r (8), 295 ^s (14), 310 ^t (29), 325(10)	7-12, 11-15' (Fig. 17), 10-13' ^o	No ^b	
XX	C ₄₀ H ₄₆	526(100)	133 ^b (67), 321 ^c (17), 336 ^l (1), 351 ^v (71)	7-12, 11-15', 15-11' (Fig. 19)	No ^b	
XXI	C ₄₀ H ₄₀	520 ^l (100)	nd ^w	7-12, 11-15', 15-11', 12'-7'	No ^b	
XXII	C ₄₀ H ₄₈	528(12)	207 ^c (27), 222 ^f (11), 237 ^g (100), 261 ^c (12), 276 ^l (11), 291 ^u (92)	7-12, 11-15' (Fig. 17), 12'-7' (Fig. 16)	Yes ^y	Similarity to XVII; see also van Kaam-Peters <i>et al.</i> (1996a)
XXIII ^z	C ₄₀ H ₆₄ S	576(6)	115 ^{aa} (7), 133 ^b (100), 331 ^{ab} (36), 359 ^{ab} (14)	No	No	Mass spectrum in Fig. 5a
XXIV	C ₄₀ H ₆₀ S	572(14)	133 ^b (100), 355 ^{ac} (40)	No	No	Mass spectrum in Fig. 5b
XXV	C ₄₀ H ₆₀ S	572(31)	125 ^{ad} (23), 133 ^b (100), 313 ^{ac} (3), 314 ^c (3), 327(4), 383 ^{ac} (5)	No	No	
XXVI	C ₄₀ H ₆₀ S	572(26)	125 ^{ad} (14), 133 ^b (100), 299 ^{ac} (9), 397 ^{ac} (6)	No	No	Mass spectrum in Fig. 5c
XXVII	C ₄₀ H ₆₀ S	572(23)	111 ^{ae} (20), 132 ^{ad} (100), 133 ^b (74), 243 ^{ac} (82)	No	No	Mass spectrum in Fig. 5d
XXVIII	C ₄₀ H ₆₀ S	572(63)	132 ^{ad} (55), 133 ^b (71), 229 ^{ac} (100)	No	No	
XXIX	C ₄₀ H ₅₄ S	566(38)	133 ^b (100), 293 ^{ab} (17)	7-12 (Fig. 16)	No ^b	
XXX	C ₄₀ H ₅₄ S	566(90)	132 ^{ad} (53), 133 ^b (100), 175 ^{ae} (15), 307 ^{ab} (11), 308 ^c (11)	10-15	No	
XXXI	C ₄₀ H ₅₂ S	564(37)	119 ^d (6), 132 ^{ad} (13), 133 ^b (100), 243 ^{ac} (13), 244 ^c (17), 389 ^b (5)	15-11' (Fig. 16)	No	
XXXII	C ₄₀ H ₅₄ S ₂	598(31)	111 ^{ae} (33), 132 ^{ad} (55), 133 ^b (100), 243 ^{ac} (59), 409 ^{ac} (7)	No	No	
XXXIII	C ₄₀ H ₅₄ S ₂	598(45)	111 ^{ae} (31), 132 ^{ad} (59), 133 ^b (100), 243 ^{ac} (72), 299 ^{ac} (20), 423 ^{ac} (24)	No	No	
XXXIV	C ₄₀ H ₅₆ S ₂	600(55)	133 ^b (100), 194 ^{ah} (16), 383 ^{ab} (56), 411 ^{ah} (10)	No	No	
XXXV	C ₄₀ H ₄₈ S ₂	592(100)	132 ^{ad} (9), 133 ^b (65), 403 ^{ac} (26), 460 ^{ah} (6)	10-15	No	
XXXVI	C ₄₀ H ₆₄ S ₂	608(2)	133 ^b (100), 359 ^{am} (2), 575 ^{am} (5)	No	No	
C₃₂ compounds						
XXXIX	C ₃₂ H ₅₀	434(23)	133 ^b (100), 134 ^c (73)	No	No	Mass spectrum in Fig. 7a
XL	C ₃₂ H ₄₂	426(7)	119 ^d (18), 133 ^b (100), 173(17)	11-9' (cf. Fig. 16)	No	Mass spectrum in Fig. 7b
XLI	C ₃₂ H ₄₂	426(22)	133 ^b (25), 207 ^c (14), 222 ^f (9), 237 ^g (100)	7-12 (cf. Fig. 16)	Yes (1:1)	Mass spectrum in Fig. 7c
XLII	C ₃₂ H ₃₆	420(21)	133 ^b (?) ^{ao} , 257 ^c (20), 272 ^f (6), 287 ^g (100)	7-12, 11-9' (cf. Fig. 17)	No	
XLIII	C ₃₂ H ₃₆	420(17)	133 ^b (9), 257 ^c (17), 272 ^f (5), 287 ^g (100)	7-9', 10-15 (cf. Fig. 18)	No	Mass spectrum in Fig. 7d
XLIV	C ₃₂ H ₄₄ S	460(67)	133 ^b (100), 285 ^{ac} (66), 299 ^{ac} (25)	No	No	
C₃₃ compounds						
XLV	C ₃₃ H ₅₂	448(16)	133 ^b (100), 134 ^c (88)	No	No	Mass spectrum in Fig. 9a
XLVI	C ₃₃ H ₄₄	440(26)	133 ^b (31), 207 ^c (15), 222 ^f (8), 237 ^g (100)	7-12 (cf. Fig. 16)	Yes (1:1:1:1)	Mass spectrum in Fig. 9b
XLVII	C ₃₃ H ₄₆ S	474(27)	125 ^{ad} (9), 133 ^b (100), 299 ^{ac} (66)	No	No	Mass spectrum in Fig. 9c
XLVIII	C ₃₃ H ₄₆ S	474(40)	133 ^b (100), 139 ^{ad} (16), 285 ^{ac} (9), 327 ^{ac} (57), 341 ^{ad} (38)	No	No	
XLIX	C ₃₃ H ₄₆ S	474(28)	111 ^{ae} (29), 132 ^{ad} (100), 133 ^b (36), 243 ^{ac} (90)	No	No	Mass spectrum in Fig. 9d
L	C ₃₃ H ₄₆ S	474(35)	125 ^{ad} (42), 132 ^{ad} (75), 133 ^b (57), 257 ^{ac} (100)	No	No	
Short-chain compounds						
LI	C ₁₆ H ₁₇ R ^{av}	252(72)	111 ^{ae} (8), 207 ^c (30), 209 ^{ac} (41), 222 ^{av} (22), 237 ^{aw} (100)	7-12 (cf. Fig. 16)	Yes ^{aa} (1:1)	Mass spectrum in Fig. 10a
LII	C ₁₈ H ₂₁ R ^{av}	252(22)	119 ^d (79), 133 ^b (100)	11-9' (cf. Fig. 16)	No	
LIII	C ₂₁ H ₂₇ R ^{av}	280(23)	105 ^b (24), 133 ^b (100)	11-15' (cf. Fig. 16)	No	Mass spectrum in Fig. 10b
LIV	C ₂₆ H ₃₇ R ^{av}	364(11)	119 ^d (61), 133 ^b (100)	15-11' (cf. Fig. 16)	No	
LV	C ₂₁ H ₂₁ R ^{av}	288(100)	144 ^{aa} (11), 243 ^{ab} (32), 258 ^{av} (29), 273 ^{aw} (43)	7-12, 11-15' (cf. Fig. 17)	No	
LVI	C ₂₁ H ₂₁ R ^{av}	288(100)	144 ^{aa} (3), 243 ^{ab} (28), 258 ^{av} (17), 273 ^{aw} (32)	7-15', 10-15 (cf. Fig. 18)	No	
LVII-LIX ^{ba}	C ₂₆ H ₃₁ R ^{ab}	344(47)	133 ^b (54), 169 ^b (100), 170 ^c (26)	11-15', 15-11' (cf. Fig. 17); 11-11', 14-12' (cf. Fig. 18); 11-11', 12-14' (cf. Fig. 18)	No	
LX	C ₂₆ H ₂₆	338(100)	154 ^{aa} (9), 169 ^{ab} (8), 293 ^{ac} (23), 308 ^{av} (23), 323 ^{aw} (19)	7-12, 11-15', 15-11' (cf. Fig. 19)	No	Mass spectrum in Fig. 10c
LXI	C ₁₅ H ₁₇ SR ^{bc}	272(22)	132 ^{ad} (100), 243 ^{ac} (8)	No	No	
LXII	C ₂₀ H ₂₂ S	294(32)	132 ^{ad} (100), 162 ^{ad} (6), 175 ^{ab} (9), 279 ^{ad} (7)	10-15	No	Mass spectrum in Fig. 10d

Appendix B. (Continued).

- ^a Carbon atoms connected by a C-C bond formed by cyclisation are indicated by their carbon number (cf. I in Appendix I).
- ^b Cleavage β to benzene ring.
- ^c McLafferty rearrangement.
- ^d Cleavage β to both sides of additional benzene ring.
- ^e Loss of two $\bullet\text{CH}_3$'s from the main fragment ion, indicating severely hindered rotation around a C-C single bond (cf. Safe and Hutzinger, 1971, 1972; Wolf, 1993; Wolf *et al.*, 1995).
- ^f Loss of $\bullet\text{CH}_3$ from the main fragment ion, indicating severely hindered rotation around a C-C single bond (cf. Safe and Hutzinger, 1971, 1972; Wolf, 1993; Wolf *et al.*, 1995).
- ^g Cleavage β to biphenyl moiety.
- ^h Despite the presence of several chiral centres this compound appears as a single peak in the gas chromatogram, probably due to the high elution temperature that causes rapid interconversion of the atropisomers.
- ⁱ VIII contains two biphenyl moieties and two asymmetric carbon atoms. It appears as a broad, asymmetric peak in the m/z 237 mass chromatogram (Fig. 3), which indicates that its biphenyl moieties still maintain some axially chiral character at the elution temperature (301°C).
- ^j M/z (M^+ -236): loss of biphenyl moiety *via* a McLafferty rearrangement.
- ^k Distribution arising from partial coelution of two of four diastereomers.
- ^l Cleavage β to naphthalene moiety.
- ^m Cleavage β to both sides of naphthalene moiety.
- ⁿ Cleavage α to acenaphthene moiety and carbon atom C-13', explaining the high intensity of this fragment ion compared to that at m/z 133.
- ^o The mechanism of formation of the five-membered ring is presently not understood.
- ^p Cleavage α to cyclohexadienyl moiety.
- ^q Similar 1:1 distributions are found for the samples from the Kimmeridge Clay and Schistes Cartons Formations, and for an artificially matured sample (239°C) from the Gessoso-solfifera Formation, supporting their identification as atropisomers.
- ^r Due to the lower rotational barrier of the 2- compared to the 1-phenylnaphthalene moiety (cf. XVII), the number of diastereomers is twice as low, resulting in one peak in the m/z 287 mass chromatogram (Fig. 4).
- ^s The molecular mass of XIX is lower than that of a fully aromatised C_{40} diaryl isoprenoid. Therefore, it is concluded that XIX is a C_{39} compound.
- ^t The high intensity of the molecular ion is indicative of polyaromatic character (Safe and Hutzinger, 1973).
- ^u Cleavage β to acenaphthylene moiety.
- ^v Cleavage β to phenanthrene moiety.
- ^w Interference from other isorenieratene derivatives made it difficult to obtain a pure mass spectrum.
- ^x Cleavage β to tetrahydronaphthalene moiety.
- ^y The m/z 291 mass chromatogram contains several peaks with a molecular at m/z 528. Due to the presence of three asymmetric carbon atoms and two axially chiral centres, it is unclear if these peaks represent atropisomers. However, the elution temperature (279-282°C) is probably low enough to prevent rapid interconversion of atropisomers.
- ^z *Trans* isomer.
- ^{aa} Cleavage α to both sides of thiane ring.
- ^{ab} Cleavage α to thiane ring.
- ^{ac} Cleavage β to thiophene ring.
- ^{ad} Cleavage β to both sides of thiophene ring.
- ^{ae} M/z (243-132).
- ^{af} McLafferty rearrangement, indicating the presence of an aromatic moiety separated from a terminal benzene ring by a methylene group (Fig. 6).
- ^{ag} This fragment ion is probably due to coelution with XXVII.
- ^{ah} Cleavage β to benzo[*b*]thiophene moiety.
- ^{ai} M/z (307-132).
- ^{aj} Cleavage β to both sides of thieno[3,2-*b*]thiophene moiety.
- ^{ak} Cleavage β to thieno[3,2-*b*]thiophene moiety. The fragment ion at m/z 383 has a higher intensity than that at m/z 411, because it is α to the quaternary carbon atom C-13'.
- ^{al} M/z (M^+ -132): McLafferty rearrangement followed by charge retention instead of charge migration (cf. Fig. 6).
- ^{am} Cleavage α to 1,2-dithiane moiety and α to quaternary carbon atom C-13, after loss of HS^\bullet radical.
- ^{an} M/z (M^+ -33): loss of HS^\bullet , a fragmentation characteristic of 1,2-dithianes (Kohnen *et al.*, 1991a).
- ^{ao} The relative intensity of the fragment ion at m/z 133 could not be determined due to coelution with the more abundant XL.
- ^{ap} M/z (M^+ -133).
- ^{aq} M/z (257-132).
- ^{ar} $\text{R} = \text{H}, \dots, \text{C}_7\text{H}_{15}$. Listed mass spectrum belongs to $\text{R} = \text{CH}(\text{CH}_3)_2$ component.
- ^{as} Doubly ionised fragment ion, diagnostic of polyaromatic hydrocarbons (Safe and Hutzinger, 1973).
- ^{at} Loss of three $\bullet\text{CH}_3$'s from the molecular ion, indicating severely hindered rotation around a C-C single bond (cf. Safe and Hutzinger, 1971, 1972; Wolf, 1993; Wolf *et al.*, 1995).
- ^{au} M/z (M^+ -43): loss of isopropyl moiety.
- ^{av} Loss of two $\bullet\text{CH}_3$'s from the molecular ion, indicating severely hindered rotation around a C-C single bond (cf. Safe and Hutzinger, 1971, 1972; Wolf, 1993; Wolf *et al.*, 1995).
- ^{aw} Loss of $\bullet\text{CH}_3$ from the molecular ion, indicating severely hindered rotation around a C-C single bond (cf. Safe and Hutzinger, 1971, 1972; Wolf, 1993; Wolf *et al.*, 1995).
- ^{ax} The C_{20} - C_{23} components possess both an axially chiral centre and an asymmetric carbon atom, which gives rise to two diastereomers. The C_{16} - C_{19} components do not have an asymmetric carbon atom, hence they occur as a single peak in the gas chromatogram.
- ^{ay} $\text{R} = \text{H}, \text{CH}_3$. Listed mass spectrum belongs to $\text{R} = \text{CH}_3$ component.
- ^{az} $\text{R} = \text{H}, \text{CH}_3, \text{C}_3\text{H}_7$. Listed mass spectrum belongs to $\text{R} = \text{H}$ component.
- ^{ba} LVII-LIX are represented in the gas chromatogram by a broad, asymmetric peak (not shown), which suggests that they are coeluting.
- ^{bb} $\text{R} = \text{H}, \text{CH}_3$. Listed mass spectrum belongs to $\text{R} = \text{H}$ component.
- ^{bc} Listed mass spectrum belongs to $\text{R} = \text{C}_3\text{H}_7$ component.
- ^{bd} M/z (M^+ -15).

Appendix C: ¹H and ¹³C NMR data of VI.⁸

chemical shift			chemical shift				
carbon atom	$\delta^1\text{H}$ (ppm)	$\delta^{13}\text{C}$ (ppm) ^b	carbon atom	$\delta^1\text{H}$ (ppm)	$\delta^{13}\text{C}$ (ppm) ^b		
CH3	C-16	2.17 (s) ^c	15.2 ^d	CH	C-3	6.89 (s) ^o	127.1 ^{m,c,d}
	C-17	2.23 (s) [^]	20.7 ^{f,d,e}		C-4	6.89 (s) ^o	127.4 ^{m,c,d}
	C-18	2.27 (s) [^]	19.9 ^{f,e}		C-9	1.86 (m) ^k	34.7 ^o
	C-19	1.00 (d, J = 6.7 Hz)	19.5 ^{^,f}		C-12	6.94 (bm)	130.6 ^{^,e,g}
	C-20	2.23 (s)	21.0 ^{f,g}		C-14	6.92 (dd, J = 7.7&1.6 Hz)	126.2 [^]
	C-16'	2.19 (s) ^{^,h}	15.2 ^d		C-15	6.99 (d, J = 7.7 Hz)	130.1 ^{^,g}
	C-17'	2.25 (s) ^{^,h}	20.7 ^{f,d,e}		C-3'	6.89 (s) ^o	127.1 ^{m,c,d}
	C-18'	2.29 (s) ^{^,h}	19.9 ^{f,e}		C-4'	6.89 (s) ^o	127.4 ^{m,c,d}
	C-19'	0.97 (d, J = 6.7 Hz) ^{ij}	19.6 ^{^,f}		C-9'	1.52 (m) ^k	33.6 ^o
	C-20'	0.85 (d, J = 6.7 Hz) [^]	19.5 ^{^,f}		C-13'	1.70 (m) ^k	35.4 ^o
CH2	C-7	2.60 (m), 2.76 (m) ^k	27.9	C	C-1	-	133.3 ^q
	C-8	1.36 (m), 1.53 (m) ^k	n.d. ^l		C-2	-	134.7 ^{v,q}
	C-10	2.42 (m), 2.71 (m) ^k	40.0		C-5	-	134.3 ^{v,q}
	C-7'	2.57 (m), 2.66 (m) ^{k,m}	27.6		C-6	-	139.4 ^{u,q}
	C-8'	1.37 (m), 1.53 (m) ^k	n.d.		C-11	-	139.7
	C-10'	1.19 (m), 1.40 (m) ^k	n.d.		C-13	-	134.4 ^{v,g}
	C-11'	1.28 (m), 1.47 (m) ^k	24.7 ⁿ		C-1'	-	133.3 ^q
	C-12'	1.19 (m), 1.40 (m) ^k	n.d.		C-2'	-	134.7 ^{v,q}
	C-14'	2.34 (m), 2.63 (m) ^k	n.d.		C-5'	-	134.3 ^{v,q}
					C-6'	-	139.2 ^{u,q}
					C-15'	-	136.6

Assignments may be interchanged.

^a Determined on a Bruker AMX-400 Mhz NMR spectrometer in CDCl₃.

^b Multiplicity of signals assessed by an attached proton test.

^c Determined by a ¹H-¹³C correlation.

^d In agreement with shifts of 1,2,3,4-tetramethylbenzene: CH₃ at C-1 (20.5), CH₃ at C-2 (15.6), C-5 and C-6 (126.9).

^e ¹H-¹³C correlation shows correlation with aromatic methyl.

^f ¹H-¹³C correlation shows correlation with aliphatic methyl.

^g In agreement with the shifts of 1,2,4-trimethylbenzene: CH₃ at C-4 (20.9), C-3 (130.5), C-4 (135.1), C-5 (126.6) and C-6 (129.6).

^h In agreement with δ aromatic CH₃'s of 1-isopentyl-2,3,6-trimethylbenzene (δ = 2.28, 2.24, 2.20; Summons and Powell, 1987), aryl isoprenoids (δ = 2.30, 2.25, 2.20; Summons and Powell, 1987) and isorenieratane (δ = 2.28, 2.23, 2.20; Schaeffé *et al.*, 1977).

ⁱ In agreement with δ CH₃ at C-3' of 1-isopentyl-2,3,6-trimethylbenzene (d, δ = 0.98; Summons and Powell, 1987), aryl isoprenoids (d, δ = 0.98; Summons and Powell, 1987) and isorenieratane (d, δ = 0.98; Hartgers *et al.*, 1993).

^j Average of four doublets due to the presence of four diastereomers.

^k Assignments established by a COSY spectrum and long-range ¹H-¹³C correlations.

^l n.d. = not determined.

^m In agreement with δ CH₂ at C-1' of 1-isopentyl-2,3,6-trimethylbenzene (δ = 2.61; Summons and Powell, 1987) and isorenieratane (δ = 2.64; Hartgers *et al.*, 1993).

ⁿ In agreement with δ CH₂ at C-5', C-9', C-11' in 1-methyl-3-phytanylbenzene (δ = 24.4-24.8; Sinnighe Damsté *et al.*, 1988a).

^o In agreement with δ aromatic protons of 1-ethyl-2,3,6-trimethylbenzene (s, δ = 6.90; Hartgers *et al.*, 1993), 1-isopentyl-2,3,6-trimethylbenzene (s, δ = 6.89; Summons and Powell, 1987), aryl isoprenoids (s, δ = 6.91; Summons and Powell, 1987) and isorenieratane (s, δ = 6.92; Schaeffé *et al.*, 1977).

^p In agreement with δ CH at C-3' in 1-methyl-3-phytanylbenzene (δ = 33.4; Sinnighe Damsté *et al.*, 1988a).

^q In agreement with shifts calculated by the additivity principle and using the shifts of 1,2,3,4-tetramethylbenzene, 1,3-dimethylbenzene and 1-methyl-3-phytanylbenzene (Sinnighe Damsté *et al.*, 1988a): C-1 (132.8), C-2 (134.8), C-3 (126.8), C-4 (126.7), C-5 (133.7) and C-6 (139.2).