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SCIENTIFIC COMMENT

Restricted utility of aryl isoprenoids as indicators for photic zone anoxia

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Abstract—In a North Sea oil, the carotenoid derivatives β-carotane, β-isorenieratane, and isorenieratane were identified, together with a series of aryl isoprenoids with a 2,3,6-trimethyl substitution pattern for the aromatic ring. The $\delta^{13}C$ values of β-carotane and β-isorenieratane are similar, whereas isorenieratane is ca. 15‰ heavier. This suggests that β-isorenieratane is not derived from β-isorenieratene biosynthesised by *Chlorobiaceae,* but from aromatisation of β-carotene. This was confirmed by laboratory aromatisation of partially hydrogenated β-carotene, which yielded β-isorenieratane as the main product. The aryl isoprenoids, which can be formed by C-C bond cleavage of both isorenieratane and β-isorenieratane, have a mixed isotopic signature in the oil. These results indicate that mere identification of aryl isoprenoids, without determination of their $\delta^{13}C$ values, cannot be used to assess the presence of *Chlorobiaceae,* and, thus, photic zone anoxia in the depositional environment.

1. INTRODUCTION

The occurrence of aryl isoprenoids with a 2,3,6-trimethyl substitution pattern for the aromatic ring and a tail-to-tail isoprenoid chain (I; see Appendix) in sedimentary rocks and crude oils is often taken as an indication for the presence of brown-coloured photosynthetic green sulphur bacteria (*Chlorobiaceae*) and photic zone anoxia in the depositional environment (Summons and Powell, 1986, 1987; Clark and Philp, 1989; Yu Xinke et al., 1990; Fowler, 1992; Requejo et al., 1992; Hartgers et al., 1994a,b; Koopmans et al., 1996). The aryl isoprenoids are thought to form from C-C bond cleavage of the isoprenoid chain of isorenieratene (II) and β-isorenieratene (III), both biosynthesised by Chlorobiaceae (Liaaen-Jensen, 1978a,b; de Wit and Caumette, 1995), that are bound in high-molecular-weight organic matter fractions (e.g., kerogen) of the sediment (Requejo et al., 1992; Hartgers et al., 1994b). The origin of aryl isoprenoids from *Chlorobiaceae* can be confirmed by their ^{13}C contents, because *Chlorobiaceae* fix CO_2 via the reverse tricarboxylic acid cycle (Sirevåg and Ormerod, 1970) leading to biomass anomalously enriched in ^{13}C (Quandt et al., 1977; Sirevåg et al., 1977). However, ^{13}C contents of aryl isoprenoids are seldom reported because these compounds are usually present only in low abundance in complex mixtures.

Here, we reveal a diagenetic origin of β-isorenieratane (IV) from the ubiquitous carotenoid β-carotene (V). Evidence comes from (1) stable carbon isotope measurements of β-carotane (VI), β-isorenieratane, and isorenieratane (VII) in a North Sea oil, and (2) laboratory formation of β-isorenieratane from β-carotene. Because C-C bond cleavage of both macromolecularly bound isorenieratane and β-isorenieratane yields aryl isoprenoids, these compounds have a mixed origin as confirmed by their ^{13}C content in the oil. This indicates that aryl isoprenoids cannot be used as markers for *Chlorobiaceae* and, thus, photic zone anoxia, unless they are significantly enriched in ^{13}C compared to algal lipids.

2. EXPERIMENTAL

2.1. Fractionation

40.1 mg of a North Sea oil, to which a mixture of three standards was added for quantitative analysis, was chromatographed with Al_2O_3 to obtain the apolar fraction (hexane/dichloromethane 9:1 v/v). Further separation of the apolar fraction by argentatious thin-layer chromatography yielded the saturated hydrocarbon fraction (R_f = 0.78–1.00), the monoaromatic hydrocarbon fraction (R_f = 0.29–0.78), and the polyaromatic hydrocarbon fraction (R_f = 0.06–0.29). For stable carbon isotope measurements, some fractions were further chromatographed. The saturated hydrocarbon fraction was eluted over silica-lite with cyclohexane to remove linear alkanes. The monoaromatic hydrocarbon fraction was first eluted over silica-lite with cyclohexane to remove linear alkylbenzenes, and then over Al_2O_3 with hexane and hexane/dichloromethane (9:1 v/v). The latter fraction contained the aryl isoprenoids and some C_{40} carotenoid derivatives.

2.2. Laboratory Formation of β-Isorenieratane from β-Carotene

4.5 mg of β-carotene (Acros Chimica) was hydrogenated with H_2/PtO_2 for 4 h. Column chromatography using Al_2O_3 (hexane/dichloromethane 1:1 v/v) yielded the hydrogenated products (78%). These were dissolved in dry toluene, and 26.0 mg of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) was added. The mixture was allowed to reflux for 24 h. The reaction mixture was chromatographed with hexane/dichloromethane (9:1 v/v) over Al_2O_3.

2.3. Gas Chromatography

Gas chromatography (GC) was performed using a Hewlett-Packard 5890 instrument, equipped with an on-column injector. A fused

Table 1. Absolute amounts of several carotenoid derivatives. Roman numbers refer to structures in the Appendix.

Compound	Amount (μg/g oil)
β-carotane (VI)	$1.1x10^3$
β-isorenieratane (IV)	$1.3x10^2$
IX	$2.6x10^2$
Isorenieratane (VII)	$2.1x10^2$
C_{19} aryl isoprenoid (I)	$2.7x10^2$
C_{19} cyclohexane (VIII)	$4.3x10^2$

silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness 0.12 μm) was used with helium as carrier gas. For the co-injection experiments, a capillary column (30 m × 0.25 mm) coated with DB-1701 (film thickness 0.25 μm) was also used. The column effluent was monitored by a flame ionisation detector. Samples were injected at 70°C and the oven was subsequently 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.

2.4. 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 configuration and conditions of the gas chromatograph were the same as for GC described above.

2.5. 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 analyses with helium as carrier gas. Fractions were injected on-column at 70°C and the oven was programmed as for GC analyses. 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 CO_2-spikes generated by admitting CO_2 of a known ^{13}C 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 ^{13}C standard.

3. RESULTS AND DISCUSSION

The oil was fractionated by column chromatography into a saturated hydrocarbon fraction, a monoaromatic hydrocarbon fraction, and a polyaromatic hydrocarbon fraction. The saturated hydrocarbon fraction contains a substantial amount (Table 1) of β-carotane, and a series of 1-alkyl-2,2,6-trimethylcyclohexanes (VIII) first identified by Anders and Robinson (1971). The monoaromatic hydrocarbon fraction contains β-isorenieratane (IV), an aromatised diagenetic product of β-carotene (IX), and a series of aryl isoprenoids with a distribution similar to that of the 1-alkyl-2,2,6-trimethylcyclohexanes. The polyaromatic hydrocarbon fraction contains isorenieratane.

After further chromatographic steps (see section 2), the ^{13}C contents of β-carotane, β-isorenieratane, isorenieratane, IX and the C_{19}–C_{21} aryl isoprenoids were measured (Fig. 1). The $\delta^{13}C$ values of β-carotane and its aromatic counterpart IX are almost identical. The $\delta^{13}C$ values of isorenieratane and phytane, which is taken as a marker for algae living in the upper part of the water column and using Rubisco for CO_2 fixation (Hayes, 1993), differ by ca. 16‰. This is in agreement with values obtained from a suite of carbonaceous rocks throughout the Phanerozoic (Koopmans et al., 1996). However, the $\delta^{13}C$ value of β-isorenieratane, which was expected to be similar to that of isorenieratane because their unsaturated counterparts β-isorenieratene and isorenieratene are biosynthesised by *Chlorobiaceae*, is close to that of β-carotane. In addition, the C_{19}, C_{20}, and C_{21} aryl isoprenoids are ca. 10‰ lighter than isorenieratane.

In order to explain the similarity of the $\delta^{13}C$ values of β-carotane and β-isorenieratane, an attempt was made to form β-isorenieratane from β-carotene in the laboratory (Fig. 2). β-carotene was hydrogenated, which yielded β-carotane and a suite of monounsaturated and diunsaturated counterparts. This mixture was aromatised with DDQ, which yielded β-isorenieratane (10%) as the main product. β-isorenieratane was identified by comparison of its mass spectrum with that of an authentic standard, and by co-injection experiments using two different capillary columns. Apparently, a geminal

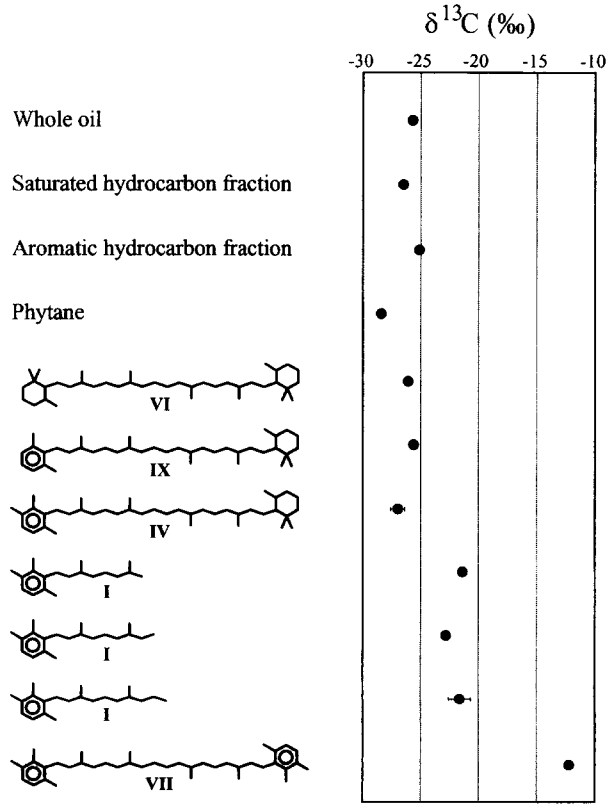

FIG. 1. $\delta^{13}C$ values of β-carotane, β-isorenieratane, isorenieratane, IX, phytane, several aryl isoprenoids, and bulk fractions. When error bars are not shown, the standard deviation (n = 2) falls within the range given by the markers. The $\delta^{13}C$ value of β-isorenieratane is similar to that of β-carotane, but differs strongly from that of isorenieratane. The aryl isoprenoids have values intermediate between those of β-isorenieratane and isorenieratane.

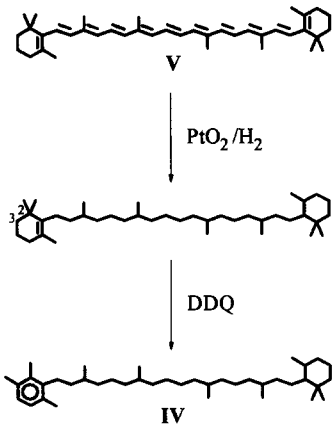

FIG. 2. Scheme for the laboratory transformation of β-carotene into β-isorenieratane.

methyl group of the cyclohexenyl moiety has shifted exclusively from the 2 to the 3 position during aromatisation (Fig. 2), in agreement with literature data on aromatisation reactions with DDQ of similar compounds (Braude et al., 1960; Fu and Harvey, 1978). These results indicate that β-isorenieratane present in sedimentary rocks and crude oils cannot be used as a marker for Chlorobiaceae. It should be noted that, in principle, isorenieratane can be formed from β-carotene by aromatisation of both cyclohexenyl moieties. However, the different $\delta^{13}C$ values of β-carotane and isorenieratane in the North Sea oil (Fig. 1) indicate that this is not an important process.

Aryl isoprenoids with a 2,3,6-trimethyl substitution pattern for the aromatic ring are thought to form from C-C bond cleavage of the isoprenoid chain of macromolecularly bound isorenieratene and β-isorenieratene (Requejo et al., 1992; Hartgers et al., 1994b). Thus, our results suggest that aryl isoprenoids can be formed indirectly from β-carotene during sediment burial, as supported by their $\delta^{13}C$ values in the oil (Fig. 1). This implies that the occurrence of aryl isoprenoids in sedimentary rocks and crude oils cannot be taken as an indication for the presence of *Chlorobiaceae* in the depositional environment, unless they are significantly enriched in ^{13}C compared to algal lipids. Interestingly, β-carotene was originally proposed as the precursor of the aryl isoprenoids (Ostroukhov et al., 1982). This hypothesis was later questioned (Summons and Powell, 1986), because synthesis of aryl isoprenoids by aromatisation of β-ionone (Ostroukhov et al., 1982) resulted in two isomers with different trimethyl substitution patterns for the aromatic ring. However, the fate of geminal methyl groups during aromatisation reactions is merely a result of the reagent used (Fu and Harvey, 1978).

Our results also could explain two apparent anomalies. First, the C_{31} aryl isoprenoid is present in the oil under study and has been reported before as the ultimate member of the series in other sedimentary rocks and crude oils (Summons and Powell, 1986, 1987; Requejo et al., 1992), although the formation of this component from isorenieratane requires an unlikely cleavage α to the benzene ring. However, cleavage α to the cyclohexyl moiety of β-isorenieratane is facile, and results in the formation of the C_{31} aryl isoprenoid. Second, the difference in $\delta^{13}C$ values of the aryl isoprenoids and the saturated hydrocarbon fraction reported by Summons and Powell (1986) is only 6–9‰. A similar difference is found in this study (4–5‰), but it is much smaller than the difference expected when the aryl isoprenoids would be exclusively formed from isorenieratane (ca. 15‰; Koopmans et al., 1996). This can be explained by the formation of aryl isoprenoids from β-isorenieratane, and thus, indirectly, from β-carotene. Therefore, it seems likely that the aryl isoprenoids reported by Summons and Powell (1986) were partly derived from β-carotene.

4. CONCLUSIONS

$\delta^{13}C$ values of β-carotane, β-isorenieratane, and isorenieratane in a North Sea oil suggest a genetic relationship of β-carotane and β-isorenieratane. This is supported by laboratory aromatisation of β-carotene, which yielded β-isorenieratane. The $\delta^{13}C$ values of the aryl isoprenoids with a 2,3,6-trimethyl substitution pattern for the aromatic ring are intermediate between those of β-isorenieratane and isorenieratane, suggesting a mixed source. These results indicate that aryl isoprenoids in sedimentary rocks and crude oils do not indicate the presence of *Chlorobiaceae,* and, thus, photic zone anoxia in the depositional environment, unless they are significantly enriched in ^{13}C compared to algal lipids. Particularly the co-occurrence of aryl isoprenoids and β-carotane should be viewed with caution.

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Appendix