

The fate of carotenoids in sediments: An overview

Jaap S. Sinninghe Damsté and Martin P. Koopmans

Netherlands Institute for Sea Research (NIOZ), Department of Marine Biogeochemistry and Toxicology, P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands

Abstract: Despite carotenoids being abundant natural products, there are only scattered literature reports of carotenoid derivatives (mainly in the form of their 'perhydro' derivatives) in ancient sediments and petroleum. This was thought to be due to the sensitivity of carotenoids toward oxygen and their preservation potential in sediments was judged to be low. Detailed studies in the past years have indicated, however, that the fossil 'perhydro' derivatives of carotenoids represent only the tip of the iceberg and that a number of reactions occurring with the polyene chain (i.e. expulsion of toluene and xylene, cyclisation and aromatisation, reaction with reduced inorganic sulfur species, hydrogenation, C-C bond cleavage) determine the fate of carotenoids in ancient sediments and lead to complex mixtures of carotenoid derivatives. In addition, aromatisation of cyclohexenyl moieties (as present in e.g. β -carotene) occurs. An overview of these reactions is given.

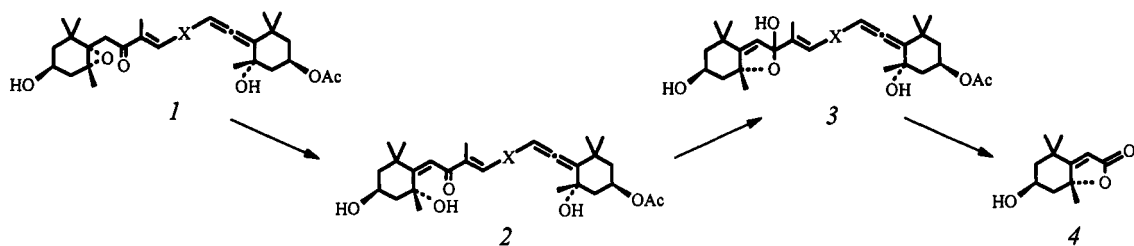
INTRODUCTION

Only a minute fraction of the organic matter biosynthesised by marine photosynthetic organisms reaches the sea floor and ultimately becomes buried in ancient sediments. This is a process which leads to loss of labile biosynthetic products and carotenoids were generally thought to be included in this class of components (ref. 1). Recently, however, it has become clear that the potential for preservation of carotenoids is actually larger than anticipated and remnants of carotenoids are present in ancient rocks and crude oils as old as 450 million years. This paper is intended to provide a brief overview of the occurrence of sedimentary products derived from carotenoids in the geological record and the reactions determining their distribution. For detailed reviews of the older literature and of carotenoids in ancient lake sediments see refs. 2-4.

INTACT CAROTENOIDS AND THEIR EARLY DIAGENETIC PRODUCTS

The occurrence of unaltered carotenoids was first reported in lake sediments in 1923 (ref 5) and in marine sediments in 1930 (ref. 6). Ever since, a variety of fossil carotenoids has been identified (e.g. refs. 7-29). The oldest intact carotenoids reported are of Miocene age: isorenieratene in a Messinian (6 Ma) marl from Italy (ref. 12) and an unspecified diaromatic carotenoid from a Lower Miocene (c. 20 Ma) clay from the Blake-Bahama basin in the Western North Atlantic Ocean (ref. 13). In general, however, concentrations of intact carotenoids decrease significantly with depth, with a preferential loss of oxygen-containing (hydroxy-, oxo-, epoxy) carotenoids (refs. 8-11). Since carotenoids can be easily oxidised, a low oxygen concentration in both the water column and the sediment is the most important factor for preservation of carotenoids in sediments (ref. 4).

Careful studies of Repeta and co-workers (refs. 16-21) have indicated that a number of degradation reactions determine the fate of the most abundant carotenoid on earth (ref. 3), fucoxanthin 1,



Degradation pathway of fucoxanthin 1, ultimately leading to loliolide 4. After ref. 21.

and related oxygen-containing carotenoids. In the water column, hydrolysis to fucoxanthinol 2 by zooplankton and other marine herbivores occurs. In surface sediments, dehydration at the 5' position, probably microbially mediated, and later on at the 3 and 3' positions takes place. The major degradation route in surface sediments, however, is epoxide opening, formation of the hemiketal 3 and production of loliolide 4. This is consistent with the high concentrations of loliolide in Namibian shelf sediments (up to 2 % of the total organic matter), where it was first identified (ref. 29). In the anoxic sediments from the Peruvian upwelling region the transformation of fucoxanthin into loliolide is nearly complete at 20 cm depth in the sediment, indicating a very rapid conversion. Carotenes and β -cyclic xanthophylls are degraded more slowly to dihydroactinidiolide 5 and isololiolide 6 through epoxidation of the 5,6 unsaturation. The preferential removal of caroteno-5,6-epoxides provides an explanation at the molecular level for the post-depositional increase in carotene/xanthophyll ratios reported earlier (see ref. 3 for a summary). In lacustrine systems the carotenoid composition may also be dramatically altered both within the water column and in the sediment (refs. 22-23).

Despite the rapid diagenetic transformation reactions that may occur with fossil carotenoids, these compounds have successfully been used to study past changes in depositional environments, predominantly in anoxic lakes and seas (refs. 4, 24-28). For example, in the present day Black Sea, the diaromatic carotenoid isorenieratene, derived from green sulfur bacteria (Chlorobiaceae), was isolated from water samples at 80 m from the surface (ref. 30). Since Chlorobiaceae are photoautotrophic organisms that are strictly anaerobic and require hydrogen sulphide, the presence of this carotenoid indicates the overlap of photic and anoxic zones. The fossil record of isorenieratene in Black Sea sediments has been used to assess these conditions in the past and, thus, provides a reconstruction of variations in the depth of the chemocline (ref. 26).

FURTHER DIAGENETIC REACTIONS OF FOSSIL CAROTENOIDS

Hydrogenation The first indication that carotenoid carbon skeletons can also be preserved in ancient sediments came from the identification of the "perhydro" derivatives of β -carotene (7; ref. 31), γ -carotene (8; ref. 32), lycopene (9; ref. 33), β -isorenieratene (10; ref. 34), chlorobactene (11; ref. 35) and the diaromatic carotenoids, isorenieratene, renieratene and renierapurpurin (12-14; ref. 36). Subsequently, 7, 8, 10, 12 and 13 have also been identified in crude oils (refs. 37-41) and, in particular cases, β -carotene 7 may represent the single most abundant alkane in crude oils (refs. 37, 42) (Fig. 1). In these cases, it is likely that organisms like *Dunaliella salina* that produce high concentrations of β -carotene in saline environments populated by restricted biological assemblages have contributed significantly to the sedimentary organic matter (ref. 43). These findings strongly suggested that these molecular fossils were formed through hydrogenation of the polyene chain of the preserved carotenoids, although lycopane 9 is more likely derived from lycopadiene biosynthesised by the freshwater alga *Botryococcus braunii* race L (ref. 44) or by an unknown marine photoautotroph (ref. 45). Partial hydrogenation of zeaxanthin and canthaxanthin has been observed in sediments from the Cariaco Trench (ref. 9). No evidence for partial saturation of carotenoids was found in much younger sediments of the Peru Margin (ref. 21), although recently partially hydrogenated β -carotene derivatives were identified in these sediments (ref. 46).

The analysis of fossil 'perhydro' carotenoids may also provide clues to the structure of carotenoids that once existed but have become extinct during evolution. In a suite of samples from the Williston Basin the 'perhydro' carotenoids were dominated by a diaryl isoprenoid 15 possessing the unprecedented 2,3,4-trimethyl substitution pattern for the aromatic ring in combination with the more

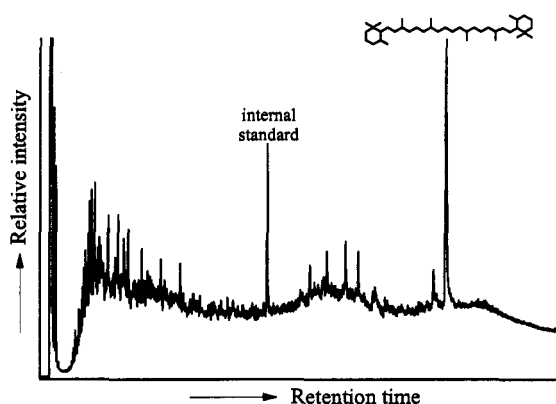


Fig. 1 Gas chromatogram of the apolar fraction from a crude oil from Albania. β -carotane is the most abundant compound of the oil, amounting to 7.4 mg/g oil.

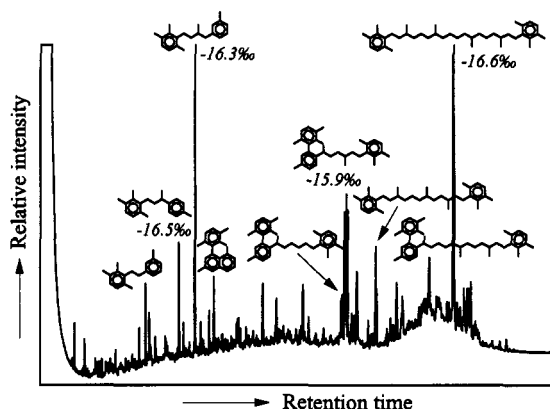


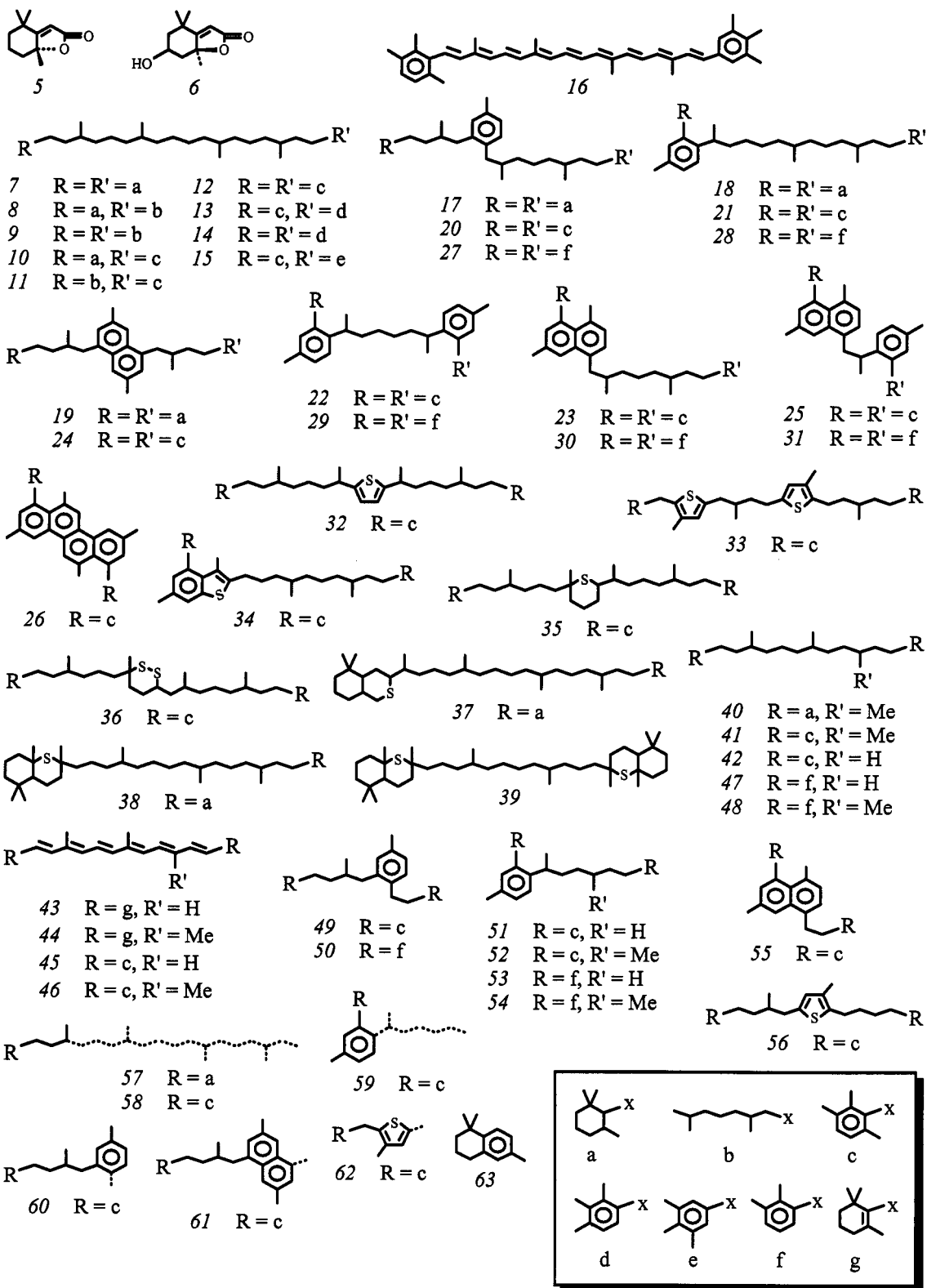
Fig. 2 Gas chromatogram of the aromatic hydrocarbon fraction of an extract from the Kimmeridge Clay Formation (c. 150 Ma). Stable carbon isotopic compositions are shown in italics, and differ c. 15‰ from algal lipids. After ref. 52.

common 1,2,5-trimethyl substitution pattern (refs. 39, 40, 47). Since shifts of methyl groups of aromatic rings during diagenesis were deemed highly unlikely, this suggested a novel carotenoid *16* as precursor. Since this carotenoid has not been reported to occur in nature and diaryl isoprenoid *15* is distinct from other molecular fossils in its carbon isotopic composition, it was speculated that this may be caused by an origin from a now extinct species of green sulfur bacteria.

Cyclisation and aromatisation Recently a suite of diagenetic products of β -carotene (e.g. *17-19*) and isorenieratene (e.g. *20-26*) has been identified with structures containing one to four additional aromatic rings (refs. 48-56). Most of these components have only been characterised by mass spectrometry but in specific cases key structures have been isolated from sediment extracts and fully characterised by NMR (refs. 48, 56). Additional evidence in case of the products derived from isorenieratene comes from their stable carbon isotopic composition. Isorenieratene is biosynthesised by green sulfur bacteria through the reversed TCA-cycle, which leads to much less fractionation against ^{13}C , than in organisms using rubisco (ref. 57). Sedimentary isorenieratane *12* is, therefore, typically 15‰ (see for a definition ref. 58) enriched relative to lipids derived from algae (refs. 48-54) and often possesses a unique "isotopic niche". The identical carbon isotopic compositions of isorenieratane and other C_{40} isorenieratene derivatives thus confirms their origin from isorenieratene (Fig. 2). The structures of these diagenetic products strongly suggest that they have been formed by a sequence of cyclisation, aromatisation and hydrogenation reactions occurring with the polyene system (Fig. 3). Initial cyclisation seems to occur mainly after appropriate *trans-cis* isomerisation at two specific sites, which favours a Diels-Alder reaction (ref. 49).

Aromatisation of the cyclohexenyl moiety In addition to hydrogenation of the cyclohexenyl moieties of carotenoids such as β -carotene, aromatisation with concomitant loss and transfer of methyl groups is also thought to occur. In combination with cyclisation, aromatisation and hydrogenation reactions of the polyene chain, this leads to a number of specific products (e.g. *27-31*) (refs. 55-56). An origin of *27-31* from an unprecedented C_{38} diaromatic carotenoid can, however, not be excluded (ref. 55). Alternatively, a 1,2-shift of a geminal methyl group of β -carotene may occur during aromatisation as has been simulated in the laboratory (ref. 41). In this way, β -isorenieratane *10* present in a North Sea crude oil is thought to be derived from aromatisation and hydrogenation of β -carotene and not from hydrogenation of β -isorenieratene (ref. 41). This was corroborated by its stable carbon isotopic composition which was identical to that of β -carotane in the same oil but 15‰ depleted relative to that of isorenieratane derived from green sulfur bacteria, which are also known to biosynthesise β -isorenieratene.

Intramolecular sulfurisation Various sedimentary organic sulfur compounds possessing the isorenieratane (e.g. *32-36*) and β -carotane carbon skeleton (e.g. *37-39*) have been identified by mass spectrometry and Raney Ni desulfurisation (refs. 49, 59-60). They occur as thiophenes, dithiophenes, bithiophenes,



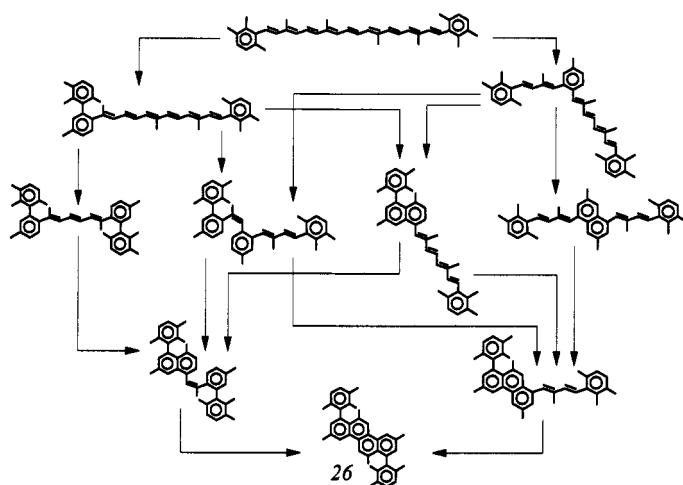


Fig. 3 Overview of formation pathways of C_{40} cyclised and aromatised diagenetic products of isorenieratene. Cyclisation occurs via a Diels-Alder reaction, after *trans-cis* isomerisation at two specific sites. After ref. 49.

benzo[*b*]thiophenes and cyclic (poly)sulfides (thianes and dithianes). Sedimentary organic sulfur compounds result from a reaction of reduced inorganic sulfur species, formed by sulphate reducing bacteria, and functionalised lipids during early diagenesis ('natural vulcanisation'; see for a review ref. 61). Obviously, the polyene system of carotenoids is prone to natural sulfurisation as well and, after the subsequent reduction of double bonds, leads to the formation of sulfur compounds with carotenoid skeletons.

Incorporation into macromolecular organic matter The largest fraction (> 90%) of the sedimentary organic matter is composed of 'kerogen', macromolecular organic matter which is insoluble in water and common organic solvents. In addition, the extractable organic matter also contains macromolecular aggregates. These fractions often contain sequestered molecular fossils which can be released by chemical and thermal degradation methods. The polyene system of carotenoids has been shown to be prone to reactions leading to sequestration. The most common of these reactions is natural vulcanisation but, in this case, operating in an intermolecular fashion and leading to cross-linking of carbon skeletons through (poly)sulphide linkages. A variety of 'perhydro' carotenoids, *i.e.* lycopane 9, β - and γ -carotane 7-8, β -isorenieratane 10, chlorobactane 11, isorenieratane 12, renieratane 13 and diaryl isoprenoid 15 have been released from soluble macromolecular aggregates (refs. 40, 49, 57, 60-70) and from kerogen (ref. 71) by various desulfurisation methods. Isorenieratane and β -carotane were also released by selective cleavage of polysulfide bonds, indicating that carotenoid skeletons may also be bound only via polysulfide linkages (refs. 63, 72-73). However, the amounts released in this way are much lower since carotenoid skeletons are likely to be bound via several S-linkages and the chance that these are only polysulfide linkages is small. In addition, diagenetic products of isorenieratene formed by cyclisation/aromatisation (*e.g.* 20-21) have also been found upon desulfurisation of geomacromolecules (ref. 49, 69)

Thermal degradation has also been used to analyse sequestered carotenoids. The high abundance of 1,2,3,4-tetramethylbenzene (TMB) in flash pyrolysates of kerogens (refs. 38-40, 74-77) has been explained by its formation through β -cleavage of a benzene ring of aromatic carotenoids incorporated into the kerogen. TMB can be derived from the diaromatic carotenoids isorenieratene, renieratene, renierapurpurin and 16, or from the monoaromatic carotenoids, chlorobactene and okenone. However, a distinction can be made based on the distribution of γ -cleavage products in the flash pyrolysates and the ^{13}C content of the β - and γ -cleavage products. 2-Ethyl-1,3,4-trimethylbenzene is often a dominant C_5 alkylbenzene in pyrolysates of kerogens (refs. 40, 76) consistent with a derivation from isorenieratene or chlorobactene, specific carotenoids of green sulfur bacteria. TMB and 2-ethyl-1,3,4-trimethylbenzene released after off-line pyrolysis of a kerogen isolated from the Duvernay Formation also had anomalously high ^{13}C contents consistent with this explanation (refs. 39-40). In contrast, the ^{13}C content of TMB in a pyrolysate of a

kerogen isolated from Indian Ocean surface sediments is identical to that of algal lipids, excluding an origin from isorenieratene (ref. 78), indicating that there are multiple origins for TMB. The anomalously high concentration of TMB and 2-ethyl-1,3,4-trimethylbenzene in petroleum from the Williston Basin (ref. 40) and their high ^{13}C contents indicates that these products are also formed from macromolecularly-bound aromatic carotenoids during cracking in the subsurface. Similar observations have been made for products derived from diaromatic carotenoid 16 (i.e. 1,2,3,5-tetramethylbenzene and 5-ethyl-1,2,3-trimethylbenzene; ref. 40). Evidence for macromolecularly-bound carotenoids in kerogen was also obtained by analysis of KMnO_4 degradation products, which contained 2,2-dimethylsuccinic acid and 2,2-dimethylglutaric acid, thought to be derived from β -carotene related structures in the kerogen matrix (ref. 79).

Although most studies have shown that sulfur bonding is important for sequestration of carotenoids in macromolecular fractions, a combined chemical and thermal degradation study of diaromatic carotenoid skeletons in a sediment of the Duvernay Formation has indicated that only a minor fraction is bound solely via S-linkages (ref. 40), indicating that alternative modes of binding such as oxygen or carbon linkages are involved as well. Quantitative analysis of all organic matter fractions from this sediment revealed that the pool of diaromatic carotenoids initially biosynthesised was almost completely (ca. 99%) incorporated into macromolecular fractions (ref. 40), demonstrating the importance of carotenoid sequestration during diagenesis.

Expulsion of toluene and xylene A C_{33} pseudohomologue of β -carotene (lexane 40) has been tentatively identified in various sediments and petroleum (refs. 37, 55, 80-81). The identical ^{13}C compositions of lexane and β -carotene in the saturated hydrocarbon fraction of a gilsonite (Utah) suggested that both derive from β -carotene (ref. 81). C_{33} (41) and C_{32} (42) pseudohomologues of isorenieratene have also been reported both as free components or in a S-bound form (refs. 49-53, 69, 71). Mild heating of β -carotene produces C_{32} (phylene 43) and C_{33} (lexene 44) 'carotenoids' and *m*-xylene and toluene, respectively (e.g. refs. 82-85) by a pericyclic reaction *via* an eight-membered ring transition state of the polyene chain (refs. 85-86). Since this mechanism does not involve the cyclohexenyl moieties, it is postulated that isorenieratene can undergo similar reactions resulting in formation of C_{32} and C_{33} diaromatic 'carotenoids' 45 and 46 (refs. 49, 69). Further expulsion of *m*-xylene or toluene from 43-46 is less likely because only six double bonds remain in the isoprenoid chain, complicating formation of a sterically unhindered eight-membered ring transition state (ref. 86). It is thought that these expulsion reactions also occur in immature sediments and that subsequent hydrogenation leads to compounds 40-42. The cyclisation, aromatisation and sulfuration reactions that led to the C_{40} diagenetic products of isorenieratene and β -carotene also occur with C_{32} and C_{33} 'carotenoids' 43-46 as indicated by the identification of compounds 47-56.

C-C bond cleavage A wide variety of products (e.g. 57-62), which can be thought to be derived from C-C bond cleavage of fossil carotenoid derivatives, have been identified (refs. 37, 40, 49, 87-89). These include the pseudo homologous series 57-58, whose structures are sub-units of the 'perhydro' carotenoids β -carotene 7 and isorenieratene 12. Other products are formed from carotenoid units which have undergone cyclisation and aromatisation, expulsion or sulfuration reactions. These products are probably not directly formed from fossil carotenoids but after initial incorporation of carotenoids in high-molecular-weight fractions and subsequent C-C bond cleavage during increasing thermal stress due to burial in the subsurface (refs. 38, 40, 49). Some of these products may be abundant constituents (e.g. Fig. 2). The aryl isoprenoids 58 were actually the first sedimentary isorenieratene products firmly identified, revealing the abundant presence of green sulfur bacteria in ancient palaeoenvironments (refs. 88-89).

Another short-chain sedimentary product of β -carotene is ionene 63. Ionene is formed as a major product upon mild heating of β -carotene (ref. 83) and has also been identified in sediments (ref. 90), suggesting that a similar reaction takes place in Nature.

CONCLUSIONS

Intact carotenoids have been identified in sediments up to 20 Ma old. However, concentrations rapidly decrease with depth and only in specific circumstances (anoxic lakes or seas) can substantial amounts of carotenoids be preserved in the fossil record. During burial of sediments a number of reactions can occur with carotenoids and remnants of these natural products have been identified in sediment and petroleum of

up to 450 Ma. The time scales of these reactions can differ significantly. For example, in anoxic sediments fucoxanthin is almost quantitatively transformed into loliolide within a few thousand years, whereas other reactions take much longer. During diagenesis, the polyene chain of carotenoids is subjected to a number of reactions which include hydrogenation, cyclisation and aromatisation, expulsion, natural vulcanisation and C-C bond cleavage. Cylohexenyl moieties of carotenoids become either hydrogenated or aromatised with loss and/or transfer of methyl groups. These reactions lead to complicated mixtures of products derived from one carotenoid. Despite this complexity the analysis of carotenoid derivatives in sediments and petroleum may be essential in deciphering past depositional environments. Isorenieratene derivatives indicate the overlap of the photic and anoxic zones and are probably among the most valuable molecular fossils for the molecular palaeontologist. Their presence in numerous organic matter-rich sediments (ref. 34, 49) probably indicates that euxinic conditions (i.e. free H₂S in the water column) have been important for the formation of petroleum source rocks.

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