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Identification of carotenals in sediments

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Abstract

High performance liquid chromatography with photodiode array UV–Vis detection and atmospheric pressure chemical ionization mass spectrometry was used to analyze sediment samples from the meromictic Ace Lake (Antarctica), as well as a Pliocene sapropel from the Mediterranean Sea and a Miocene marl from the Vena del Gesso formation (Italy). The Ace Lake samples contained intact chlorobactene, isorenieratene, γ- and β-carotene and two series of carotenoid breakdown products. The first and more abundant series was identified as aryl (ϕ)-carotenals of increasing chain length (C_{13}, C_{15}, C_{18}, C_{20}, C_{22}, C_{25}, C_{27}, C_{30}, and C_{32}) on the basis of their UV–Vis and mass spectra. They appear to be formed from chlorobactene through oxidative cleavage of the double bonds of the polyene chain. The minor series (β-carotenals) was also identified, and may be formed from γ-carotene in a similar manner. Intact isorenieratene and the ϕ-carotenal series were also found in the Mediterranean Sea sapropel. Only a trace of the C_{32} ϕ-carotenal could be detected, suggesting isorenieratene as the predominant source of these ϕ-carotenals. Additionally, intact β-carotene and C_{13}–C_{30} β-carotenals were found in the sapropel. The absence of the C_{32} β-carotenal strongly suggests these breakdown products are derived from β-carotene. The Vena del Gesso marl contained both intact isorenieratene and the C_{13}–C_{25} ϕ-carotenals. The absence of the longer chain length ϕ-carotenals from this sample may indicate more advanced oxidation of isorenieratene. The carotenals are likely formed during storage of samples and/or extracts under non-ideal conditions, i.e., not frozen and exposed to oxygen although they may also be formed by diagenetic processes. They can be relatively abundant and sometimes be an order of magnitude higher in concentration than the parent carotenoid. Hence, not including the ϕ-carotenals could lead to an underestimation of the persistence of photic zone euxinia.
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1. Introduction

Carotenoids are abundant natural products, but were long considered not to be useful as biomarkers due to their sensitivity towards oxygen and low preser-

vation potential in sediments (Brassell, 1993). However, in recent years several studies have indicated the presence of both intact and diagenetically altered carotenoids in sediments dating back as far as the Miocene (see Sinninghe Damsté and Koopmans, 1997 for a review). Of specific interest are carotenoids diagnostic of past environmental conditions, such as chlorobactene and isorenieratene. These aromatic carotenoids are specifically produced by green sulfur

bacteria (Chlorobiaceae), which are strictly anaerobic organisms that require sulfide. Therefore, the presence of these compounds indicates the overlap of photic and anoxic zones and can be used to recognise the presence of these conditions in the past (e.g. Repeta, 1993; Sinninghe Damsté et al., 1993). Isorenieratene (**1**, Fig. 1) and β-isorenieratene (**3**) occur exclusively in the brown-coloured strains of photosynthetic green sulfur bacteria (de Wit and Caumette, 1995). Chlorobactene (**2**) occurs predominantly in the green-coloured strains such as *Chlorobium limicola* (de Wit and Caumette, 1995).

The occurrence of unaltered bacterial carotenoids in lake sediments was first reported at the beginning of the 20th century (for an overview, see Sinninghe Damsté and Koopmans, 1997). Isorenieratene, identified in a Messinian (6 Ma) marl from Italy by Keely et al. (1995), and an unspecified diaromatic carotenoid from a Lower Miocene (ca. 20 Ma) clay from the Blake-Bahama basin in the Western North Atlantic Ocean (Cardoso et al., 1978), represent the oldest intact carotenoids reported. In general, concentrations of intact carotenoids, especially oxygen-containing ones, decrease rapidly with depth and only under anoxic conditions can substantial amounts of intact

carotenoids be preserved in sediments (Watts and Maxwell, 1977; Brassell et al., 1980; Louda and Baker, 1981).

Diagenetic products of the bacterial carotenoids have been found in sediments throughout the Phanerozoic. During diagenesis, the polyene chain can be altered by a number of reactions, which include reduction, cyclization and aromatization, expulsion, sulfurization, and C–C bond cleavage. Cyclohexenyl moieties of carotenoids become either reduced or aromatized with loss and/or transfer of methyl groups (reviewed by Sinninghe Damsté and Koopmans, 1997). Recently, Schaeffer et al. (2001) described a series of partially reduced derivatives of the aromatic carotenoids in recent sediments, where up to eight double bonds of the polyene chain were reduced. Often, dia- and catagenic bacterial carotenoid derivatives were identified on the basis of both structural features and stable carbon isotopic composition. As green sulfur bacteria biosynthesize carotenoids via the reversed tricarboxylic acid-cycle, these compounds are typically 15‰ enriched relative to lipids derived from algae (Koopmans et al., 1996; Grice et al., 1996a). The identical carbon isotopic composition of diagenetic derivatives to the parent carotenoid thus confirms their origin.
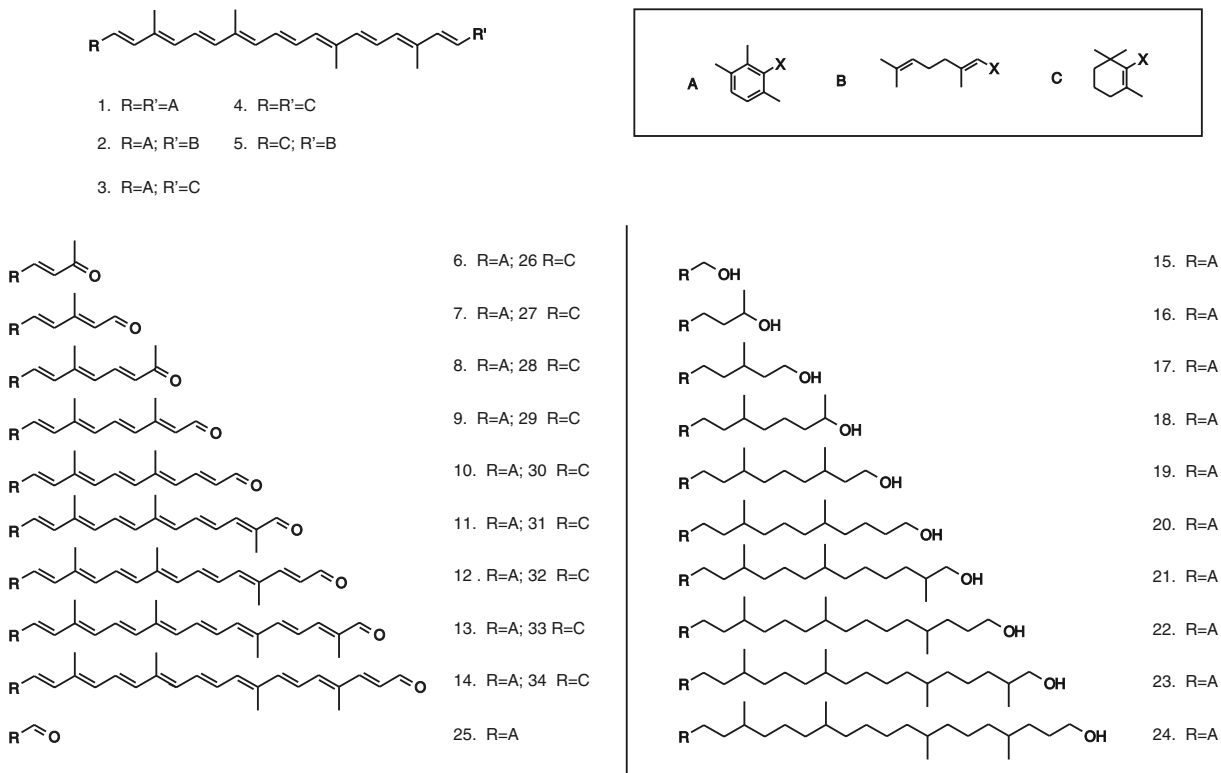

Fig. 1. Structures of carotenoids and carotenoid derivatives referred in the text.

Although much is known about carotenoid derivatives in ancient sediments and petroleum, knowledge of the very early diagenetic processes that affect the distribution of carotenoids and their breakdown products is limited. This is partially due to the fact that intact carotenoids, and likely the early diagenetic products, are not amenable to the traditional analytical tools of organic geochemistry, i.e., gas chromatography (GC) and GC–mass spectrometry (MS). Here, high performance liquid chromatography combined with photodiode array UV–Vis detection and atmospheric pressure chemical ionization mass spectrometry (HPLC/APCI-MS) was used to analyze sediment samples from the meromictic Ace Lake (Antarctica), a Pliocene sapropel from the Mediterranean Sea and a Miocene Marl layer from the Vena del Gesso (VDG) formation. The direct analysis of intact bacterial carotenoids is discussed, as well as the identification of two series of oxidation products, the carotenals, and the geochemical implications of these findings.

2. Methods

2.1. Samples

The three sample locations were chosen on the basis of the reported presence of intact isorenieratene or chlorobactene, or diagenetic products thereof (Volkman et al., 1988; Keely et al., 1995; Bosch et al., 1998).

A 25 cm core was taken at the centre of Ace Lake in 1992, sectioned and stored frozen. Four samples were taken from depths of 6.6–8.3, 11.6–13.3, 16.6–18.3 and 21.6–23.3 cm (referred to below as samples AL-2 to AL-5, respectively). The average sedimentation rate was estimated to be around 0.18 mm/year (Roberts and McMinn, 1999), indicating that the deepest sample was deposited approximately 1300 year B.P. and the sediment samples represent an average of approximately 100 years (except for the top sample). Volkman et al. (1988) reported the presence of chlorobactene throughout the core. In addition, a sediment grab sample was taken near the centre of the lake at 25 m water depth. In 2002, a 150 cm core was taken at the depocentre of the lake (water depth 24.7 m) and stored in the dark at −40 °C until sampling (Coolen et al., 2004). The frozen sediment core was sliced in 2 cm horizontal fragments and 41 out of 74 slices with 2 cm space intervals were analyzed.

A subsample (IV-1-3 as described by Kenig et al., 1995) of the marl bed of cycle IV of the Messinian VDG sediments (Vai and Lucchi, 1977; Sinninghe Damsté et al., 1995) with a TOC of 1.28% was analysed. Keely et al. (1995) reported an average concentration of isorenieratene in horizon IV of ca. 300 μg/g TOC, as determined by HPLC with UV–Vis detection and LC–MS.

A Pliocene sapropel recovered during Ocean Drilling Program (ODP) leg 160 (specifically, sample 160-969E-6H-6, 34–35 cm) in the Eastern Mediterranean Sea was also analyzed. Bosch et al. (1998) and Passier et al. (1999) first reported the presence of isorenieratene derivatives, suggesting the photic zone was euxinic for a significant period of time during deposition of the sapropel layers. Photic zone euxinia during deposition of this sapropel was confirmed by Menzel et al. (2002), who found intact isorenieratene in this and in two additional time-equivalent sapropels.

2.2. Extraction and fractionation

The Ace Lake sediments were ultrasonically extracted using mixtures of chloroform and methanol. The extracts were separated using a column packed with Al_2O_3 to obtain an apolar and a polar fraction using hexane/dichloromethane (9:1, v/v) and dichloromethane/methanol (1:1, v/v) as eluents, respectively. Portions of the polar fractions were silylated using BSTFA/pyridine and analysed using GC, GC–MS and isotope-ratio-monitoring (irm)-GC–MS. A part of the polar fraction was also reduced using Raney Nickel (Sinninghe Damsté et al., 1989) and analysed using GC and GC–MS. A sub-fraction of the polar fraction, containing apolar carotenoids, from here on referred to as the carotenoid fraction, was isolated using column chromatography (silica as stationary phase, 3 column volumes dichloromethane as eluent) and analysed using HPLC/APCI-MS.

Both the VDG IV-1-3 sample and the ODP 160 sample were ultrasonically extracted using acetone. From these extracts a carotenoid fraction was prepared using a silica column and dichloromethane as eluent as described above for the Ace Lake sediments.

2.3. HPLC–MS

Carotenoid fractions were analysed with an HP 1100 series LC–MS equipped with an auto-injector, photodiode array detector, mass detector, and Chemstation chromatography manager software. Separation was achieved using a HP XDB-C_{18} column (2.1 × 120 mm, 5 μm particles), maintained at 30 °C, with a gradient from methanol/water (4:1, v/v) to acetone/methanol/water (19:1:1, v/v/v) in 50 min. The total run time was 60 min. Detection was achieved using in-line UV–Vis detection and APCI-MS of the eluent. Conditions for APCI-MS were as follows: nebulizer pressure was set at 40 psi, the vaporizer temperature was 400 °C, drying gas (N_2) was delivered at 8 l/min, the capillary voltage was −3 kV and the corona needle was set at 7 A. Compounds were identified from their retention times, UV–Vis spectra (250–700 nm) and APCI-mass spectra (m/z 100–1000). Isorenieratene was quantified from its

UV–Vis response by comparing the peak area at 454 nm to peak areas at 454 nm of known amounts of an authentic β-carotene standard (Aldrich). Chlorobactene was quantified by comparing its UV–Vis response at 462 nm to the UV–Vis response of known amounts of β-carotene (**4**) standard and correcting for the difference in extinction coefficients. The C_{20}–C_{32} φ-carotenals were quantified by comparing their UV–Vis response to the UV–Vis response of known amounts of authentic retinal (C_{20} β-carotenal; Aldrich) as discussed below.

2.4. GC

GC was performed using a Hewlett–Packard 5890 series II chromatograph equipped with an on-column injector and fitted with a 25 m × 0.32 mm fused silica capillary column coated with CP-Sil 5 (film thickness 0.12 μm). Helium was used as carrier gas and the oven was programmed from 70 to 130 °C at 20 °C/min, followed by an increase of 4 °C/min to 320 °C (15 min hold time). Compounds were detected using a flame ionization detector (FID).

2.5. GC–MS

GC–MS analysis was performed using the GC conditions described above. The column was directly inserted into the electron ionization source of a VG Autospec Ultima mass spectrometer, operated with a mass range of m/z 40–800, a cycle time of 1.8 s and ionization energy of 70 eV.

2.6. Irm-GC–MS

Irm was performed using a DELTA-C system (Schouten et al., 1998), equipped with an on-column injector and fitted with a 25 m × 0.32 mm fused silica capillary column coated with CP-Sil 5 (film thickness 0.12 μm). Helium was used as carrier gas and the oven was programmed from 70 to 130 °C at 20 °C/min, followed by an increase of 4 °C/min to 320 °C (20 min hold time). Isotopic values were calculated by integrating the m/z 44, 45 and 46 ion currents of the peaks produced by combustion of the chromatographically separated compounds and those of CO_2 standard spikes admitted at regular intervals. Each sample was analysed in duplicate and the results presented as an average and its standard deviation.

3. Results and discussion

3.1. The φ-carotenal series

HPLC/APCI-MS analysis of the carotenoid fractions of the Ace Lake core and grab sample (e.g. Fig. 2) re-
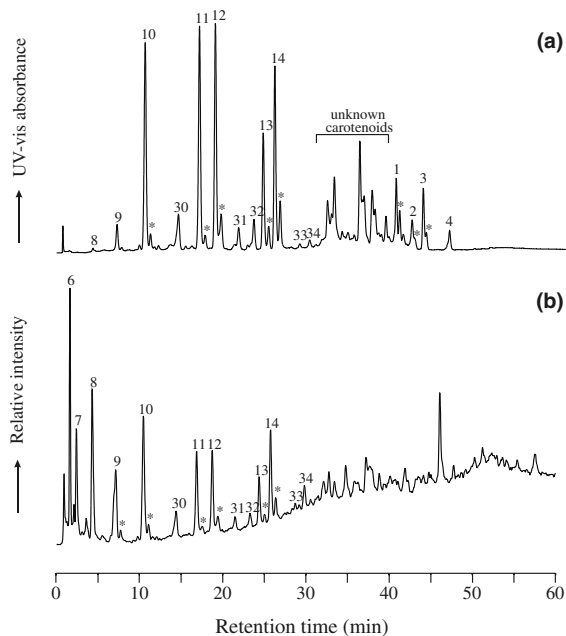
Fig. 2. UV chromatogram at 436 nm (A) and total ion current chromatogram (m/z 100–1000) (B) of the carotenoid fraction of AL-4. Numbers refer to structures in Fig. 1. Peaks marked * are tentatively identified as *cis*-isomers of peaks eluting immediately before them.
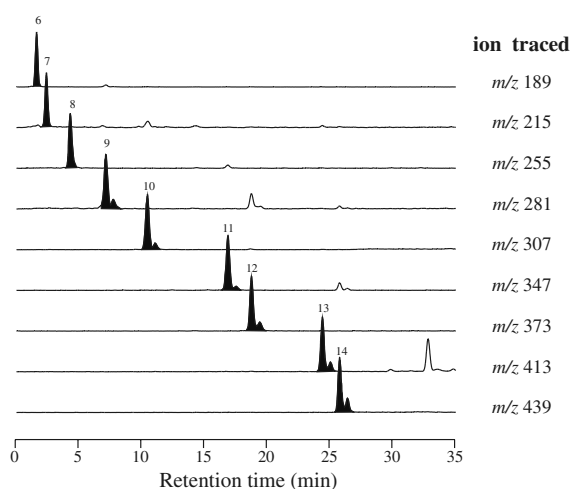
Fig. 3. Mass chromatograms of [M + H]$^+$ ions of φ-carotenals. Numbers refer to structures in Fig. 1. Peaks marked * are tentatively identified as *cis*-isomers of the respective φ-carotenals.

vealed a series of components (**6**–**14**) with both increasing molecular weight and increasing UV–Vis absorption maximum as retention time increased {[M + H]$^+$ from m/z 189 to 439 (Fig. 3) and λ_{max} from 342 to 478 nm,
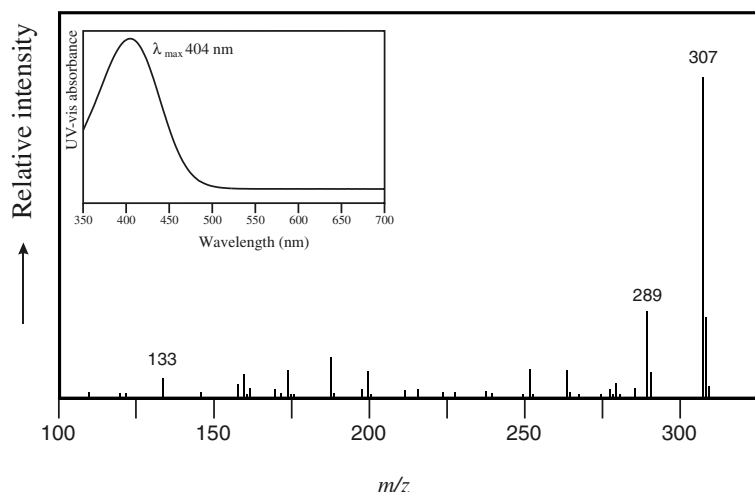
Fig. 4. APCI mass spectrum and UV–Vis spectrum (inset) of the C_{22} φ-carotenal (**10**). Spectra are corrected for background.

respectively}. The distribution of molecular weights suggested a pseudo homologous series of isoprenoids, while the concurrent red shift of λ_{max} to longer wavelengths indicated an increasing number of conjugated double bonds. The mass spectra all showed a major fragment ion at $[M + H]^+ - 18$ (e.g. Fig. 4), indicative of a loss of either an hydroxyl, aldehyde or keto function as water.

Reduction of an aliquot of the polar fractions with Raney Nickel was used to elucidate the carbon skeleton of this series of compounds. This reagent saturates double bonds and reduces aldehyde and keto functions to primary and secondary alcohols, respectively. GC–MS analysis of the reduced fractions revealed a matching series of 1,2,5-trimethylphenyl isoprenoid primary alcohols (C_{10}–C_{27}; **15**–**24**), of which the identity of the C_{15} aryl isoprenoid alcohol was confirmed by co-injection with a 3-methyl-5-(2′,3′6′-trimethylphenyl)pentan-1-ol authentic standard (kind gift from Dr. P. Schaeffer, University of Strasbourg). Mass spectra of these compounds are characterised by M^+, $M^+ - 15$ (–CH$_3$) and $M^+ - 90$ (–OTMSi) and a base peak at m/z 133. Based on this, the structures of the unknown compounds detected by LC–MS were established as a series of aryl (φ) carotenoid derivatives with either a primary alcohol or aldehyde function. The molecular weights excluded the possibility of a series of φ-carotenols, but were compatible with the calculated molecular weights of a series φ-carotenals and φ-carotenones. Hence, the unknown series was identified as structures **6**–**14** and **25** (Fig. 1).

Further evidence for the identity of the φ-carotenals was obtained from their UV–Vis spectra (e.g. Fig. 4). The contribution of an φ-end group to the UV–Vis spectrum is identical to that of a β-ring and the measured UV–Vis absorption maxima of the φ-carotenals indeed closely match the reported UV–Vis absorption maxima

of β-carotenals (Britton, 1995; Fig. 6). The red shift of approximately 10 nm in λ_{max} of the φ-carotenals compared to those of their β-carotenal counterparts of equal carbon number can be explained by the difference in polarity of the HPLC eluent, containing acetone, and the reference solvent, petroleum ether (Britton, 1995).

Direct GC–MS analysis of the polar fraction showed the presence of a major early eluting peak, tentatively identified as the C_{10} φ-carotenal from its mass spectrum (McLafferty and Stauffer, 1989). The C_{10} φ-carotenal was probably not detected with LC–MS because it eluted with the injection peak under the LC conditions used. The irm-GC–MS analysis revealed that this compound was relatively enriched in ^{13}C ($\delta^{13}C$ value of $-16.7 \pm 1.0‰$), similar to the intact carotenoids (chlorobactene, isorenieratene, β- and γ-carotene) previously determined as their hydrogenated products in these sediments by Schouten et al. (2001). Based on the structural resemblance and the similar enrichment in ^{13}C to the intact carotenoids, it appears that this series of φ-carotenals represents a group of products related to chlorobactene or isorenieratene.

In the case of several of the φ-carotenals (C_{20}–C_{32}) a second, much smaller peak, eluting immediately after the main peak (Fig. 3), was observed. These peaks had identical mass spectra to the main peak, but UV–Vis spectra that showed a blue shift of 4–8 nm in λ_{max}. Such a shift is typical of *cis*-isomers of carotenoids (Britton, 1995) and these compounds were therefore tentatively identified as *cis*-isomers of the respective φ-carotenals.

3.2. The β-carotenal series

In addition to the φ-carotenals, a minor series of peaks (**26**–**34**, Figs. 2 and 6) was also observed in the Ace Lake carotenoid fractions. As was the case with

the φ-carotenals, the distribution of molecular weights ($[M + H]^+$ from 193 to 443; Fig. 6) and the increase in UV–Vis absorption maximum with increasing molecular weight suggested a pseudo homologous series of isoprenoids with polyene chromophores of increasing chain length (λ_{max} from 386 to 464 nm for peaks where determination was possible; Fig. 5). Again, mass spectra with a major fragment ion at $[M + H]^+ - 18$ were observed, indicating the presence of either a hydroxyl, aldehyde or keto function. Molecular weights were equal to those calculated for a series of β-carotenals. Indeed, the identity of the C_{20} β-carotenal (29) was confirmed by co-injection with an authentic standard (retinal) and this

second series of unknown compounds detected by LC–MS was thus identified as a series of β-carotenals.

As expected, the UV–Vis spectra closely matched the UV–Vis spectra observed for the φ-carotenals. The slight red shift in observed λ_{max} of the β-carotenals compared to the λ_{max} of their φ-carotenal counterparts is attributed to the polarity of the LC gradient. Under the LC conditions used the β-carotenals elute slightly later than the φ-carotenals, resulting in an eluent containing more acetone at the moment of UV–Vis detection. Acetone is known to cause such a red shift (Britton, 1995).

As was the case for the φ-carotenals, a second, much smaller peak was observed just before the main peak for some of the β-carotenals (Fig. 5). These signals were often too small to obtain a UV–Vis spectrum, but in the few cases where this was possible a small shift of 4–8 nm of the λ_{max} towards longer wavelength was observed. Therefore, it seems unlikely that these peaks represent cis-isomers of the β-carotenals as cis-isomers typically show a shift in λ_{max} toward shorter wavelengths. However, these peaks may represent carotenal isomers with a ψ-end group. Such ψ-carotenals would have identical molecular weights to their β-carotenal counterparts and would be expected to elute before the β-carotenals, based on the order of elution of the intact carotenoids (see below) in the reversed-phase LC separation used.

3.3. Distribution of carotenals and intact carotenoids in Ace Lake

The presence of intact bacterial carotenoids was also investigated in the Ace Lake samples in order to determine the precursors of the carotenals. All the extracts contained intact chlorobactene, isorenieratene, β-isorenieratene, β-carotene, and γ-carotene (5, Fig. 1; cf. Schouten et al., 2001; see also Fig. 2) with the exception of AL-4, which did not contain γ-carotene. In most cases, a second component, with an identical mass spectrum, eluted directly after each of the main carotenoids. These components had slightly deviating UV–Vis spectra, with the λ_{max} showing a blue shift of approximately 4 nm compared to the λ_{max} of the main peak and a less pronounced fine structure. Based on these observations, these peaks were assigned as cis-isomers of the respective intact carotenoids (Britton, 1995). The occurrence of a cis-isomer of isorenieratene has previously been reported in the Vena del Gesso sediments by Keely et al. (1995) and was later confirmed by Putschew et al. (1998). Although Putschew et al. (1998) determined that the cis-isomer was naturally occurring and not an artifact of sample treatment and storage, the latter possibility cannot be excluded here.

In order to determine the abundance of the series of carotenals relative to the intact carotenoids, we quantified the carotenals where possible. Quantification based
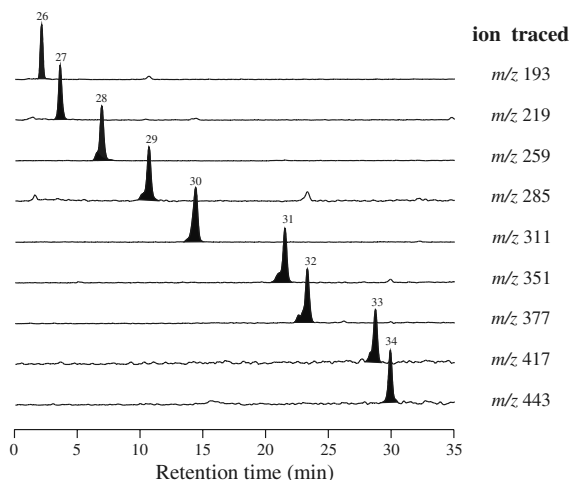

Fig. 5. Mass chromatograms of $[M + H]^+$ ions of β-carotenals. Numbers refer to structures in Fig. 1. Shoulders indicated with ● are tentatively identified as ψ-carotenals.
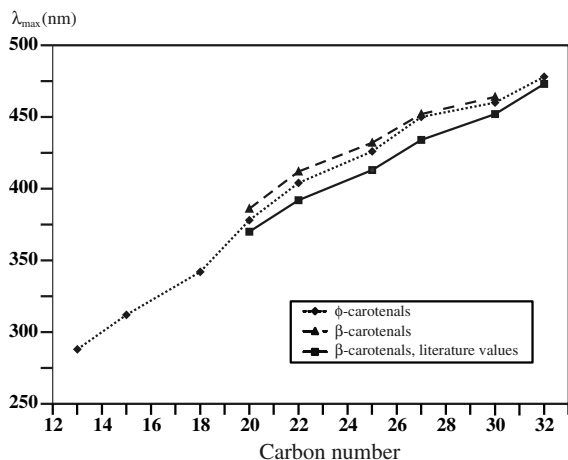

Fig. 6. Measured UV–Vis absorption maxima (λ_{max}) of both the φ- and β-carotenals. Also shown are maxima for the β-carotenals in petroleum ether (Britton, 1995).

on MS response would be inaccurate as the ionization efficiencies are unknown and likely to be different for each carotenal. A further complication for MS quantification is that fragment ions appear to be much more numerous for the short-chain carotenals than for the long-chain carotenals. Quantification of the carotenals based on UV–Vis response is also complicated as each has a different λ_{max} and extinction coefficient (generally increasing with increasing polyene chain length). An authentic standard (retinal) was only available for the C_{20} β-carotenal. It was used to construct an external standard curve based on UV–Vis response at its λ_{max} of 386 nm. Unfortunately, it co-elutes with the C_{22} φ-carotenal, which can not be quantified in this manner. However, as both the aromatic φ- and β-end groups have identical contributions to UV–Vis response of carotenoids, this standard curve can be used to estimate amounts of the C_{20} φ-carotenal.

Based on the known extinction coefficients of the C_{20} and C_{25}–C_{32} β-carotenals (Isler, 1971; Britton, 1995) and assuming the extinction coefficients for φ-carotenals are identical to those of β-carotenals, we were also able to estimate concentrations of the C_{25}–C_{32} φ-carotenals. For the C_{22} φ-carotenals, an extinction coefficient was estimated based on interpolation between the extinction coefficients of the C_{20} and C_{25} β-carotenals. For each φ-carotenal, the UV–Vis peak area was determined at its λ_{max}, after which the peak area was corrected for the difference in UV–Vis response between the φ-carotenal and the C_{20} β-carotenal based on the ratio of their extinction coefficients. The corrected UV–Vis response was then compared to that of known amounts of the C_{20} β-carotenal (retinal) to estimate the concentration of the φ-carotenal. It is accepted that there are several poten-

tial problems with this approach: the reported extinction coefficients are determined in pure reference solvents, and the effect of the mixed HPLC mobile phase on the extinction is unknown. Due to the use of a gradient in the LC separation, the solvent effect will be slightly different for each carotenal. However, this method will still provide a reasonable estimate of the order of magnitude of the carotenal concentrations and their relative contributions.

The concentrations of the quantified φ-carotenals and isorenieratene, (combining both *cis*- and all-*trans*-isomers) are shown in Fig. 7 for each of the Ace Lake samples. A similar distribution pattern is apparent for AL-2, -3, and -4, where the C_{20}, C_{25}, C_{27} and C_{32} φ-carotenals are present in concentrations approximately equal to the isorenieratene concentration, while the C_{22} φ-carotenal concentration is up to twice as high and the C_{30} φ-carotenal concentration is approximately 30–50% less. The deepest sample (AL-5) clearly contains substantially more intact isorenieratene and chlorobactene than the shallower samples. Similarly, Schouten et al. (2001) reported higher relative concentrations for all intact carotenoids related to photosynthetic green sulfur bacteria in this sample and speculated that this may be due to higher water sulfate concentrations at the time of deposition. Compared to the intact carotenoid concentrations in AL-5, the φ-carotenals are present at relatively low levels, yet their absolute concentrations are similar to those observed in the three shallower Ace Lake samples. A somewhat different distribution pattern is also observed among the φ-carotenals in AL-5. The C_{20}–C_{32} φ-carotenals are present in approximately equal concentrations, while the C_{32} φ-carotenal is present at a much higher level.
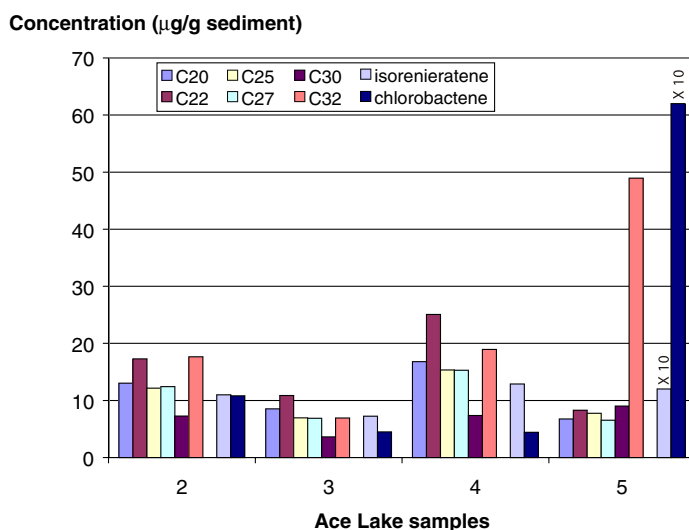

Fig. 7. Concentrations of intact isorenieratene and six quantified φ-carotenals in Ace Lake sediments.

3.4. Distribution of carotenals and intact carotenoids in ancient sediments

The Pliocene Mediterranean Sea sapropel contained the C_{13}–C_{32} φ-carotenals, although only a relatively small amount of the C_{32} φ-carotenal could be detected (Table 1), as well as the C_{13}–C_{30} β-carotenals. In addition to these carotenals, β-carotene and a considerable amount of intact isorenieratene (Table 1) were found. Bosch et al. (1998) have determined the presence of several diagenetic products of isorenieratene in this sapropel and isorenieratane was found after Raney nickel desulfurization. Menzel et al. (2002) reported the presence of intact isorenieratene in these Eastern Mediterranean sapropels, further confirming the presence of an euxinic water column during sapropel formation.

The Vena del Gesso marl contained both the C_{13}–C_{27} φ-carotenals and intact isorenieratene (Table 1). An isorenieratene concentration of 625 μg/g TOC was measured here, showing good agreement with the previously reported concentration of 800 μg/g TOC by Keely et al. (1995) in the same sediment. The concentrations of the φ-carotenals are relatively low. Neither the

β-carotenals, nor other intact carotenoids were detected in this sediment.

3.5. Formation of carotenals

The carotenals identified here have never been reported to occur naturally in bacteria or algae and so it is likely that they are formed during senescence and sedimentation of bacterial or algal biomass, post-depositional oxidation or during sample storage or processing. Based on the structural similarity of the carotenals to the intact carotenoids, it appears likely that the former are formed by oxidative cleavage of the double bonds of the polyene chain of the carotenoids (Fig. 8). In this manner, the C_{10}–C_{30} φ-carotenals could theoretically originate from chlorobactene, isorenieratene, or β-isorenieratene. Importantly, however, the C_{32} φ-carotenal can only originate from chlorobactene. The double bond that would have to be oxidized to form this specific φ-carotenal is only present in chlorobactene as part of the ψ (acyclic) end group. Similarly, the C_{13}–C_{30} β-carotenals may be formed from either β-carotene, γ-carotene, and even β-isorenieratene, but the C_{32} β-carotenal can only originate from γ-carotene.

Further support for the origin of the carotenals can be found in their distribution in relationship to their proposed precursor carotenoids. In the Ace Lake samples, it is striking that the C_{32} φ-carotenal is of greatest relative abundance in the sample (AL-5) in which chlorobactene is also of greatest relative abundance (Schouten et al., 2001). In addition, the small peaks eluting just before the main β-carotenal peaks (Fig. 6) and which may represent ψ-carotenals, are much more dominant in those Ace Lake sediment samples where γ-carotene was detected (Schouten et al., 2001). Such ψ-carotenals may be formed from either γ-carotene or chlorobactene in a similar manner as shown for the β- and φ-carotenals in Fig. 8. While the Mediterranean

Table 1
Isorenieratene and φ-carotenal concentrations (μg/g) in ancient sediments

Sediment	ODP 160-969E-6H-6	VDG IV-1-3
Isorenieratene[a]	117	8
C_{20} φ-carotenal	0.5	0.1
C_{22} φ-carotenal	0.7	0.1
C_{25} φ-carotenal	0.8	0.1
C_{27} φ-carotenal	0.8	0.1
C_{30} φ-carotenal	0.9	ND[b]
C_{32} φ-carotenal	0.1	ND[b]

[a] Sum of isorenieratene and *cis*-isorenieratene.
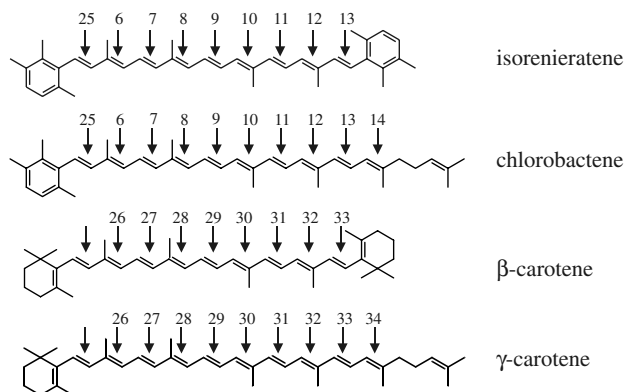[b] ND, not detected.

Fig. 8. Schematic of proposed formation of carotenals from their proposed parent carotenoids. Numbers refer to the carotenal formed (structures in Fig. 1) if oxidative cleavage were to occur at the double bond indicated by the arrow.

Sea sapropel contained the complete series of φ-carotenals, only a trace of the C_{32} φ-carotenal could be detected (0.1 μg/g sediment of C_{32} φ-carotenal versus 0.5–0.9 μg/g sediment of each of the C_{20} and C_{25}–C_{30} φ-carotenals), suggesting isorenieratene as the predominant source of these sedimentary φ-carotenals in this case. Indeed, intact isorenieratene (both *cis*- and *trans*-isomers) was found in the Mediterranean Sea sapropel in a concentration comparable to the deepest Ace Lake sample (AL-5), while chlorobactene could not be detected. In addition, the fact that all β-carotenals except the C_{32} β-carotenal were found, strongly suggests these breakdown products derive only from β-carotene, which was indeed detected in the sapropel.

The question remains as to the stage at which the parent carotenoids are oxidized to carotenals. The proposed precursors for the β-carotenals, β-carotene and γ-carotene, are present in oxic waters with relatively high amounts of light, conditions which are excellent for photochemically induced oxidations such as, for example, those described by Rontani et al. (1998). Among other reactions, they described the oxidative cleavage of the double bond of phytol to form 6,10,14-trimethylpentadecan-2-one. Such an oxidative cleavage could produce the β-carotenals from the proposed precursor carotenoids. However, such a mechanism could not explain the formation of the φ-carotenals. Isorenieratene and chlorobactene are biosynthesized by photosynthetic green sulfur bacteria, which occur exclusively under anoxic conditions, ruling out photochemical oxidation as a formation mechanism for the φ-carotenals. The bottom waters and sediment of Ace lake have been stratified and anoxic for more than 4000 years (Fulford-Smith and Sikes, 1996) and light penetrates only the first 2 m of the water column. Hence, oxidation of aromatic carotenoids by singlet oxygen and ultraviolet radiation during senescence and sedimentation is unlikely to occur in this particular setting.

To investigate whether the oxidation of the parent carotenoids occurred after deposition of the sediment, samples of a core taken from the depo-centre of Ace Lake in 2002 and stored frozen until sub-sampling, were extracted and analyzed immediately. All intact carotenoids previously found in AL-2 to -5 were present (Coolen et al., 2004); however no carotenals could be detected in any of the samples. This clearly shows that, in the Ace Lake samples, the oxidation of carotenoids to their carotenals is not a diagenetic process or an artefact of sample preparation. At this point it seems most likely that the formation of the carotenals occurs during storage of samples and/or extracts under non-ideal conditions, i.e., not frozen and exposed to oxygen. The extracts of the Ace Lake samples from 1992 had been stored cooled and in the dark for several years prior to LC–MS analysis, providing ample time for oxidative degradation to occur. Mordi et al.

(1991) showed the formation of the complete series of β-carotenals by reacting β-carotene in benzene with molecular oxygen at 30 °C in the dark for 24 h. Such a self-initiated auto-oxidation could well be responsible for the formation of the carotenals identified here. Indeed, small amounts of β-carotenals were observed in a β-carotene standard, which had been stored at −4 °C for several months, confirming the likelihood of this scenario.

In the cases of the VDG sediment and the Pliocene Mediterranean Sea sapropel, extracts were freshly prepared prior to analysis. However, the VDG sediment had been stored in dried form at room temperature for several years and the sapropel sample was obtained from a core stored cool at 4 °C, but not frozen in the ODP repository in Bremen. It is, therefore, possible that the carotenals were formed in these sediments during storage, although a diagenetic mode of formation cannot be ruled out completely. Schaeffer et al. (2001) reported a C_{18} aryl (χ) isoprenoid ketone in sediments from the anoxic sulfur-rich lake Cadagno, Switzerland. This compound was apparently formed by reduction of the three double bonds of a C_{18} χ-carotenal, which, in turn, was proposed to have been formed from okenone. In this case the carotenal must have formed under anoxic conditions either during sedimentation or in the very early stage of diagenesis.

Some carotenoid degradation processes under anoxic conditions have been described. Repeta (1989) described the degradation of fucoxanthin to loliolide via an intramolecular rearrangement leading to fragmentation without the addition of oxygen. Degradation of β-carotene or diatoxanthin to loliolide according to this scheme requires oxidation of the molecule to an epoxide through microbially mediated fermentation reactions (Repeta, 1989; Baker and Louda, 1982). Perhaps such microbial fermentative processes in the anoxic parts of the water column could also play a role in the formation of the carotenals identified here. In addition, Grice et al. (1996b) described a series of maleimides (1*H*-pyrrole-2,5-diones), derived from the tetrapyrrole moiety of (bacterio)chlorophylls. These compounds may be products formed by oxidative cleavage of double bonds under anoxic conditions. Such a mechanism may also be responsible for the formation of the carotenals described here.

4. Conclusions

We have identified two novel series of oxidative carotenoid degradation products in sediments, φ- and β-carotenals. It appears the φ-carotenals are formed from isorenieratene and chlorobactene and the β-carotenals from β-carotene and β-isorenieratene during storage of sediments and/or extracts under non-ideal

conditions, i.e., not frozen and exposed to oxygen. However, the possibility that these compounds can be formed in diagenetic processes cannot be ruled out completely.

Regardless of the mechanism of their formation, it is clear that these novel compounds can be present in summed concentrations exceeding those of their intact parent carotenoid by an order of magnitude. In three (AL-2, AL-3, and AL-4) of the Ace Lake extracts, the concentration of each of the quantified φ-carotenals alone is comparable to the concentration of intact parent carotenoid. If similar concentrations are assumed for those φ-carotenals that were not quantified, which seems reasonable based on their intensity in the TIC chromatogram (Fig. 2), the total level of φ-carotenals would be approximately 10-fold higher than the level of isorenieratene, the most dominant intact carotenoid present in these samples. Not including the φ-carotenals could thus lead to a serious underestimation of the persistence of photic zone euxinia.

Finally, our analyses of the Ace Lake sediments clearly show that the carotenals are formed due to improper storage of sediments and/or extracts. The sensitivity of carotenoids towards light and oxygen has been well known for many years. Researchers investigating, for instance, the occurrence of photic zone anoxia through the biomarker carotenoids of Chlorobiaceae should take care that carotenoid analysis is performed under carefully controlled circumstances and that samples earmarked for such a study should be stored under different conditions than may be usual in organic geochemical investigations.

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