Hdb Env Chem Vol. 2, Part N (2006): 127–163
DOI 10.1007/698_2_005

Published online: 6 October 2005

Biological Markers for Anoxia in the Photic Zone of the Water Column

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Abstract In this chapter we review the current state of knowledge on the occurrence of anoxic conditions in the water column of past depositional systems using biomarkers. The recognition of such conditions is important for a better understanding of the deposition of petroleum source rocks and periods of widespread water column anoxia in the geological past (so-called oceanic anoxic events). The most reliable biomarkers for this purpose are specific pigments and bacteriochlorophylls derived from photosynthetic green and purple sulfur bacteria, which require both light and sulfide. These proxies for water column anoxia have been well tested in present-day lakes, fjords and stratified basins. With increasing burial in the sediment, the pigments and bacteriochlorophylls undergo a myriad of transformation reactions, but the products are in most cases still specific enough to use them as environmental indicators. In this way, the occurrence of photic zone euxinia has been demonstrated for a wide variety of settings (e.g. shelf seas, enclosed basins, open ocean systems) and during geological eras as old as the Precambrian.

Keywords Ancient depositional environments · Green sulfur bacteria · Molecular fossils · Sediments

Abbreviations

Fmo	Fenna–Matthews–Olsen protein
OM	organic matter
OC	organic carbon
GSB	green sulfur bacteria
PSB	purple sulfur bacteria
TCA	tricarboxylic acid
ATP	adenosine triphosphate
$\Delta\delta^{13}C$	$\delta^{13}C$ of the cell material relative to the $\delta^{13}C$ of CO_2
ε_p	isotopic fractionation factor
HPLC-PDA-MS	high performance liquid chromatography-photo diode array-mass spectroscopy
NMR	nuclear magnetic resonance
TMB	1,2,3,4-tetramethylbenzene
Me,Et maleimide	methyl ethyl maleimide
n-Pr	normal-propyl
i-Bu	iso-butyl
OAE	oceanic anoxic event
ODP	Ocean Drilling Project

1 Introduction

Vast amounts of organic matter (OM) have been buried in sediments over geological time, but only a small fraction has been transformed into petroleum. Today's human population relies critically on this geological transformation product of sedimentary OM for their ever-increasing energy demand. In the present-day oceans hot-spots for accumulation of OM sediments are restricted to those highly productive shelf areas that have a large input of

nutrients, either by upwelling or input from rivers, or to anoxic basins such as the Black Sea [1]. It is believed that anoxic conditions of the water column play an important role in the preservation of oil-prone organic matter in sediments because aerobic degradation of OM is generally thought to be more efficient than anaerobic degradation [1]. Primary productivity [2] and sedimentation rate [3] may also exert an important control on OC accumulation rates, but in both cases there is a direct link to anoxia. Increased primary productivity increases the oxygen demand in the water column and pore waters of surface sediments and can ultimately induce anoxia. Sediments deposited in the oxygen minimum zone of the Arabian Sea have higher OC accumulation rates than those deposited above and below the oxygen minimum zone, even though the primary productivity in the photic zone is the same [4]. Hedges and co-workers [5, 6] showed that the time OM is exposed to oxygen (i.e. oxygen exposure time) is the primary control on the flux of OM to the deeper sediment and in this way explained the observed relationship with sedimentation rate.

There are indications that in the geological past the deposition of OC-rich sediments was more widespread during specific time periods [7] and also in settings (e.g. shelf seas and open marine basins) that are quite different from those in the present-day ocean where we find high OC accumulation rates. In these cases, it becomes more difficult to understand how petroleum source rocks were formed since present-day analogs do not exist. Geochemists, therefore, try to reconstruct the depositional conditions of these petroleum source rocks by using specific indicators (so-called proxies) for anoxic depositional conditions. For example, some trace metals (e.g. V, Ag and Cd) are selectively enriched relative to Al under anoxic conditions and can be used to trace anoxic conditions in past depositional systems [8]. Specific biomarkers (i.e. organic compounds found in sediments and oils which can unambiguously be related to specific lipids derived from characteristic organisms or groups of organisms) derived from organisms thriving under anoxic conditions can also be used [9].

It is often difficult to discriminate between environments having anoxic bottom waters and those in which only the pore waters of the sediment are anoxic since the microorganisms and processes in anoxic bottom waters and anoxic shallow sediments are often the same. Therefore, geochemists are interested in proxies that can be unambiguously related to water column anoxia. These are now available from the proxies derived from phototrophic anoxygenic bacteria which thrive in anoxic waters receiving light. In shallow aquatic settings such communities can be found at the water/sediment interface, but in most settings light does not reach the bottom and, consequently, the presence of photosynthetic anoxygenic bacteria indicates an almost complete anoxic water column.

Although cellular remains of photosynthetic anoxygenic bacteria are usually not found in sediments, specific organic components (i.e. carotenoids and

bacteriochlorophylls) are, albeit often in a diagenetically altered form, and thus provide information on the redox state of the environment at the time of deposition. These biomarkers (the diagenetic products of characteristic natural products) have been found in sediments as old as the Paleoproterozoic and can even be traced back in petroleum. Here, we review the current state of knowledge with respect to biomarkers for photic zone anoxia in the water column. In this review we will limit ourselves to two important microbial groups: the green and purple sulfur bacteria and, specifically, to those that contain characteristic organic components that can be retrieved from the sedimentary record.

2
Taxonomy, Physiology, and Ecology of Anoxygenic Phototrophic Sulfur Bacteria

Phototrophic sulfur bacteria develop in anoxic environments in which light and reduced sulfur compound occur simultaneously. For this review two groups are important: the green sulfur bacteria (GSB; the family *Chlorobiaceae*) and the purple sulfur bacteria (PSB). This latter group is comprised of two families: the Chromatiaceae and the Ectothiorhodospiraceae. This latter family, however, does not contain characteristic organic compounds which can be traced back in the geological record and will not be considered here. There exists a variety of reviews on the microbiology of these groups [10–13]. Here we will only briefly describe some relevant aspects.

The GSB represent a phylogenetically isolated group within the domain Bacteria. They are presently divided into six genera: *Chlorobium*, *Ancalochloris*, *Chlorobaculum*, *Chloroherpeton*, *Pelodictyon*, and *Prosthecochloris* [14]. This classification was until recently based on morphology, motility and the presence or absence of gas vesicles in the cell [11]. Recent phylogenetic characterization using the 16S ribosomal DNA gene and fmo (Fenna–Matthews–Olsen protein) genes, however, indicated that the conventional taxonomy was not congruent with the phylogenetic tree: species from different genera were phylogenetically closely related, whereas some species from the same genus varied substantially in the 16S rDNA and fmo gene composition, requiring a substantial revision of the conventional taxonomy [15].

The Chromatiaceae family of PSB is presently comprised of 24 genera [14]. Together they form a well-defined phylogenetic cluster in the gamma proteobacteria group [10] that is quite closely related to the other family of PSB, the *Ectorhodospiraceae*. Within the Chromatiaceae a major phylogenetic branch contains only marine and halophilic species.

GSB and PSB require both light and a reduced form of sulfur, either sulfide or thiosulfate for growth. The GSB are non-motile, strictly anaerobic, and obligately phototrophic bacteria. Some GSB contain gas vacuoles which can be

used to control buoyancy. They are autotrophic as they use carbon dioxide as their sole carbon source. Some simple organic components (e.g. acetate) can also be photoassimilated. Sulfide is used as an electron donor and is oxidized to sulfate. Depending on the sulfide concentration and light intensity, elemental sulfur may be formed as an intermediate product. The *Chromatiaceae* family of PSB form elemental sulfur as the oxidation product from sulfide or thiosulfate and store this internally in their cell. Because they are able to oxidize this further photoautotrophically to sulfate, the presence of elemental sulfur inside their cell provides the cell with a reservoir of photosynthetic electrons. Two major physiological groups are discriminated within the PSB. One group is comprised of strict anaerobes which are inhibited by oxygen, are obligately phototrophic and which require sulfide. Some simple organics (acetate and pyruvate) can be photoassimilated if sulfide and CO_2 are present. The other group is metabolically much more versatile and can photoassimilate organic substrates and use organic substrates as electron donors in the absence of sulfide.

GSB and PSB form dense layers in a variety of aquatic ecosystems such as lakes, lagoons, stratified marine basins and sediments receiving light. They are predominantly mesophilic although some thermophilic species (e.g. *Chlorobaculum tepidum*, *Chromatium tepidum*) are known. In an overview, van Gemerden and Mas [16] reported cell densities for over 60 different lakes and 7 sediment ecosystems containing phototrophic sulfur bacteria. Densities between 10^4 and 10^7 cells ml^{-1} and bacteriochlorophyll concentrations of 10–1000 $\mu g\, L^{-1}$ are common in these settings. *Chromatiaceae* are typically found in freshwater and marine environments but not in hypersaline waters, where their sister group, the *Ectothiorhodospiraceae*, are dominant. Because of their metabolic requirements (see above), the phototrophic sulfur bacteria are limited to those environments where light reaches anoxic, sulfide-containing bottom layers. Because light and sulfide usually occur in opposing gradients, a niche of PSB and GSB only exists if there is a zone of overlap and this is only possible if the chemical gradient of sulfide is stabilized against vertical mixing.

In pelagic environments such as lakes, lagoons, fjords and stratified marine basins, chemical gradients are often stabilized by density differences between the oxic and anoxic water layers. Such density differences are either the result of thermal (and thus seasonal) stratification or substantial differences in the salt concentration of the surface and bottom waters. In these stratified systems, bottom waters become oxygen-depleted due to the remineralization of descending organic matter-containing particles by heterotrophic bacteria. If all the oxygen is consumed, sulfate-reducing bacteria produce sulfide, resulting in a plate of phototrophic sulfur bacteria in the water column of lakes and stratified marine basins which can extend over vertical distances of 10 cm [16, 17] up to 30 m [18], depending on the steepness of the chemical and light gradients. Although much more common in

present-day stratified lakes and fjords, layers of phototrophic sulfur bacteria can also develop in stratified marine basins such as the Black Sea [18]. As we will discuss below, such systems may have been much more common in the geological past.

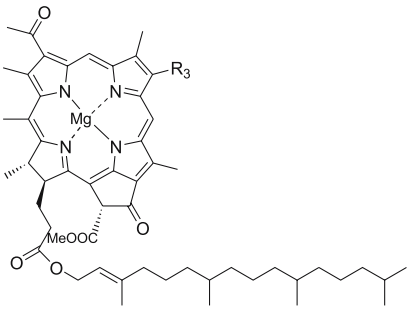
3
Specific Pigments of Green and Purple Sulfur Bacteria

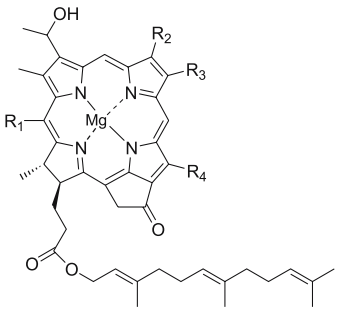
The characteristic organic components found in GSB and PSB are directly related to their distinct physiology, i.e. anoxygenic photosynthesis. To absorb light they use chlorophylls and accessory pigments (carotenoids). Since the GSB and PSB occupy distinct ecological niches and receive light in much lower intensities and with a different electromagnetic spectrum than at the surface, they biosynthesize different pigments from those in oxygenic phototrophs, i.e. algae and cyanobacteria living in the upper part of the photic zone.

The PSB are characterized by the presence of bacteriochlorophyll-*a* or, in some cases, bacteriochlorophyll-*b* (see Scheme 1 for chlorophyll structures) and a number of characteristic carotenoids (Table 1) of which okenone (**1**, see Scheme 2 for carotenoid structures), an aromatic carotenoid, is of special geochemical interest since it is highly specific for PSB and can be traced through the geological record. This carotenoid has been reported in at least eight different genera of PSB (Table 1) and occurs in species from various branches in the phylogenetic tree of the *Chromatiaceae*. From the analysis of sediments of Lake Cadagno, a Swiss alpine stratified lake, Schaeffer et al. [19] suggested two other aromatic carotenoids (**2** and **3**) with the 2,3,4-trimethyl aromatic substitution pattern[1] also found in okenone. Their carbon isotopic composition suggests that they derive from *Chromatiaceae*, thriving at the shallow chemocline of Lake Cadagno. However, these pigments have not been identified so far in cultures of PSB.

The GSB contain a distinct suite of pigments: bacteriochlorophyll-*c*, -*d* or -*e* in combination with the aromatic carotenoids chlorobactene (**5**) and isorenieratene (**7**) (Table 1), sometimes in combination with smaller amounts of β-isorenieratene (**6**) and β-carotene. All these aromatic carotenoids are characterized by the 2,3,6-trimethyl substitution pattern of the aromatic ring. The distinct pigment composition of GSB results from their ecological niche which differs from that of the PSB; GSB can grow at lower light intensities than the PSB as can be clearly seen in anoxic, stratified lakes where often a purple bacterial "plate" (representing the PSB) overlays a green plate (representing the GSB) at the chemocline. Consequently, GSB also receive light

[1] Throughout this review we will use, for reasons of convenience, this numbering system and apply it also for diagenetic products of aromatic carotenoids even if this results in nomenclature not in agreement with general IUPAC rules.

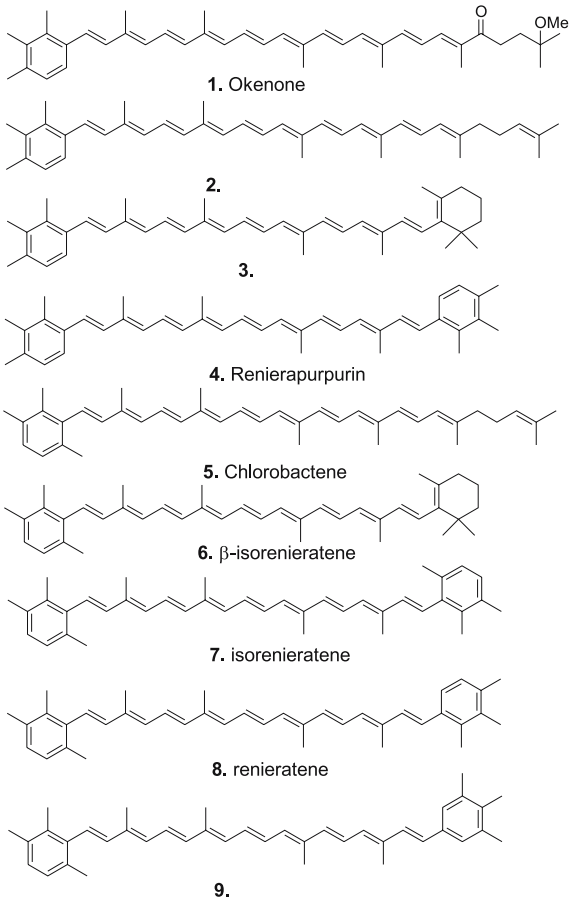




bacteriochlorophyll *a* R_3 = ethyl
bacteriochlorophyll *b* R_3 = vinyl

bacteriochlorophyll *c* R_1= Me, R_2 = Me, R_3 = Et, Pr, *i*-Bu, R_4 = Me, Et
bacteriochlorophyll *d* R_1= H, R_2 = Me, R_3 = Et, Pr, *i*-Bu, R_4 = Me, Et
bacteriochlorophyll *e* R_1= Me, R_2 = Me, R_3 = CHO, Pr, *i*-Bu, R_4 = Et

Scheme 1 Bacteriochlorophyll's of GSB and PSB



Scheme 2 Aromatic carotenoids

Table 1 Pigment composition of the major species[a] of PSB

Genus[a]	Species	Bacterio-chlorophyll	Major carotenoids[b]	Refs
Chromatium	*C. okenii*	*a*	Ok	[10]
	C. weissei	*a*	Ok	[10]
Allochromatium	*A. vinosum*	*a*	Sp, Ly, Rh	[10, 99]
	A. minutissimum	*a*	Sp, Ly, Rh	[10, 99]
	A. warmingii	*a*	Rl	[10, 99]
Halochromatium	*H. salexigens*	*a*	Sp, Ly, Rh	[10, 99]
	H. glycolicum	*a*	Sp	[10]
Isochromatium	*I. buderi*	*a*	Rl	[10]
Lamprobacter	*L. modestohalophilus*	*a*	Ok	[10]
Lamprocystis	*L. roseopersicina*	*a*	Rl	[10]
	L. purpurea	*a*	Ok	[99]
Marichromatium	*M. gracile*	*a*	Sp, Ly, Rh	[10, 99]
	M. purpuratum	*a*	Ok	[10, 99]
Rhabdochromatium	*R. marinum*	*a*	Ly	[10]
Thermochromatium	*T. tepidum*	*a*	Sp, Ly, Rh, Rv	[10, 99]
Thioalkalicoccus	*T. limnaeus*	*a*	Ts	[10]
Thiobaca	*T. trueperi*	*a*	Ly	[10]
Thiocapsa	*T. roseopersicina*	*a*	Ok, Sp	[10, 99]
	T. rosea	*a*	Sp	[10, 99]
	T. pendens	*a*	Sp	[10]
	T. litoralis	*a*	Sp	[10]
	T. marina	*a*	Ok	[100]
	T. sp.	*a*	Sp	[99]
Thiococcus	*T. pfennigii*	*a*	Ts	[10]
Thiocystis	*T. gelatinosa*	*a*	Ok	[10, 99]
	T. minor	*a*	Ok	[10, 99]
	T. violacea	*a*	Rl	[10, 99]
	T. violascens	*a*	Rl	[10]
Thiodictyon	*T. elegans*	*a*	Rl	[10]
	T. bacillosum	*a*	Rl	[10]
Thioflavicoccus	*T. mobilis*	*a*	Ts	[10]
Thiohalocapsa	*T. halophila*	*a*	Ok	[10, 99]
Thiolamprovum	*T. pedioforme*	*a*	Sp	[10]
Thiopedia	*T. rosea*	*a*	Ok	[10]
Thiorhodococcus	*T. minor*	*a*	Sp	[10]
Thiorhodovibrio	*T. winogradskyi*	*a*	Sp	[10]
Thiospirillum	*T. jenense*	*a*	Rh, Ly	[10]

[a] Classification according to Bergeys 2004;

[b] Key: Ok = okenone (**1**), Sp = spirilloxanthin, Ly = lycopene, Rh = rhodopin, Rv = rhodovibrin, Rl = rhodopinal, Ts = tetrahydro-spirilloxanthin [10, 99, 100]

with a different electromagnetic spectrum and have a different pigment composition accordingly. Both the specific bacteriochlorophylls (*c*, *d* and *e*) as well as the aromatic carotenoids are able to absorb light in the maxima of the electromagnetic spectrum of light in water at greater depth.

Within the GSB, there is a profound distinction in pigment composition between the green and brown colored GSB (Table 2). The green colored strains contain bacteriochlorophyll-*c* or -*d* and chlorobactene as the major pigments, whereas the brown colored species contain bacteriochlorophyll-*e* and isorenieratene. This distinction does not follow the molecular phylogeny and taxonomy of the GSB; i.e. some single species have both green and brown strains (Table 2). However, there is a clear relationship with the ecology of the strains; brown-colored GSB are often found in niches receiving even lower light intensities than those of the green colored GSB and, consequently, the specific pigment composition of the brown colored strains is interpreted as a further adaptation to reduced light intensity and alteration of the electromagnetic spectrum. With this specific set of pigments a strain of *Chlorobium phaeobacteroides* is capable of performing photosynthesis at a depth of ca. 80 m in the water column of the Black Sea [20–22]. GSB can also be part of so-called phototrophic con-

Table 2 Pigment composition of the major species[a] of GSB

Genus[a]	Species	Color	Bacterio-chlorophyll	Major carotenoids[b]	Refs
Chlorobium	*C. limicola*	Green	*c* or *d*	Cb	[11, 15, 99]
		Brown	*e*	Iso	[15]
	C. clathratiforme	Green	*c* or *d*	Cb	[11]
		Brown	?	Iso	[15]
	C. luteolum	Green	*c* or *d*	Cb	[11, 15]
	C. phaeobacteriodes	Brown	*e*	Iso	[11, 15, 99]
	C. phaeovibriodes	Brown	*e*	Iso	[11, 15]
Ancalochloris	*A. perfilievii*				
Chlorobaculum	*C. tepidum*	Green	*c*	Cb	[15]
	C. chlorovibrioides	Green	*c* or *d*	Cb	[11]
	C. limnaeum	Green	*c*	Cb	[15]
	C. parvum	Green	*d*	Cb	[15]
	C. thiosulfatophilum	Green	*c*	Cb	[15]
	"Clathrochloris sulfurica"	Green	*c* or *d*	Cb	[11]
Chloroherpeton	*C. thalassium*	Green	*c* or *d*	Ga	[11]
Pelodictyon	*Pelodictyon phaeum*	Brown	*e*	Iso	[11]
Prosthecochloris	*P. aestuarii*	Green	*c* or *d*	Cb	[11, 15]
	P. vibrioformis	Green	*c* or *d*	Cb	[11, 15, 99]
	P. phaeoaestuarii	Brown	*e*	Iso	[11]

[a] Classification according to Bergeys [14];
[b] Key: Cb = chlorobactene (5), Iso = Isorenieratene (7), Ga = γ carotene [11, 15, 99]

sortia. Glaeser and Overmann [23] showed that the phototrophic consortium "*Pelochromatium roseum*", which constituted 88% of the GSB (belonging to the brown colored strains) at the chemocline, did contain bacteriochlorophyll-*e* but, surprisingly, no isorenieratene or chlorobactene.

There are two other diaromatic carotenoids known to occur in nature; renieratene (**8**) and renierapurpurin (**4**). Although their structures resemble those of the aromatic carotenoids found in phototrophic sulfur bacteria, they have never been found in cultures to the best of our knowledge. They are thought to derive from sponges or sponge symbionts.

4 Carbon Isotopic Fractionation of Anoxygenic Phototrophic Bacteria

Both the GSB and the PSB are chemoautotrophic bacteria that use CO_2 as their carbon source. In *Chromatiaceae*, CO_2 is assimilated using the reductive pentose phosphate or Calvin cycle in which the first step is the fixation of CO_2 with the enzyme RuBisCo. Culture experiments with different species of *Chromatiaceae* have consistently shown a depletion of 20–23‰ for the cell material relative to CO_2 [24–27], in line with fractionations observed for autotrophic algae using the Calvin cycle. Carotenoids of PSB are approximately 3–5‰ depleted relative to the cell material [24, 27]. The lipids in a culture of *Thiocapsa roseopersicina* were found by van der Meer et al. [28] to be slightly enriched relative to the cell material and attributed this to the operation of alternative (i.e. non-Calvin) biochemical pathways.

GSB fix CO_2 through a completely different pathway: the reversed (or reductive) tricarboxylic acid (TCA) cycle. Compared with the Calvin cycle operated by the *Chromatiaceae* only slightly more than half of the amount of ATP is required per molecule of CO_2 in the reverse TCA cycle. Together with their specific pigment composition, this explains the low light adaptation of the GSB. The enzymes involved in the CO_2 fixation in the reverse TCA cycle only discriminate modestly against ^{13}C. Consequently, the cell material of GSB is isotopically heavy. Using cultures, Quandt et al. [25] and Sirevag et al. [26] reported $\Delta\delta^{13}C$ values ($\delta^{13}C$ of the cell material relative to the ^{13}C content of the CO_2) of 2.5 to 12.2‰.

Van der Meer et al. [28] determined the $\delta^{13}C$ of carotenoids, the esterifying alcohols of its bacteriochlorophyll-*c* and -*a* (farnesol and phytol, respectively) and fatty acids in a culture of *Chlorobium limicola*. The isoprenoid lipids (chlorobactene, farnesol and phytol) were ca. 2–3‰ enriched, whereas the straight-chain lipids were strongly enriched (11–16‰) relative to the total cell material. This trend is opposite that commonly observed where straight-chain lipids are ^{13}C-depleted compared to isoprenoid lipids, which in turn are ^{13}C-depleted compared to total cell material [29], which is a consequence of the biochemical pathway of lipid biosynthesis. This trend, however, can be ex-

plained by the specific biochemical pathways of the reverse TCA cycle. Recently, van Breugel et al. [30] determined ε_p (the isotopic fractionation factor) between the pigment isorenieratene and CO_2 in a natural population of GSB residing at the chemocline at ca. 4 m water depth of a stratified Norwegian fjord. These authors found a consistent ε_p of 4 ± 1‰, which was independent of the CO_2 and isorenieratene concentration. These data fit well with those of culture experiments [25, 26] and the ε_p of a natural population of GSB in the Black Sea. Glaeser and Overmann [23] reported a difference of 7‰ between CO_2 and farnesol, the esterifying alcohol of bacteriochlorophyll-*e* from a dense layer of the photoautotrophic consortium "*Pelochromatium roseum*", which was partially composed of brown-colored GSB, at the chemocline of a temperate holomictic lake (Lake Dagow, Brandenburg, Germany). These data indicate that pigments derived from GSB are generally enriched in ^{13}C relative to organic components derived from most other microorganisms and this has been very useful in the characterization of diagenetic products of pigments of GSB (see below).

Because both PSB and GSB reside at the chemocline of stratified basins, the CO_2 they fix is generally not completely derived from CO_2 from the atmosphere with a $\delta^{13}C$ of ca. – 8‰, but it is also partly derived from remineralization of organic matter below the chemocline. This so-called respired CO_2 is much more depleted in ^{13}C and, consequently, $\delta^{13}C$ of CO_2 at the chemocline might be as depleted as – 19‰ [31–35] in lakes and fjords. Although GSB fractionate to a minor extent (see above), such depleted $\delta^{13}C$ values for the CO_2 may still result in relatively light values for GSB pigments. Indeed, $\delta^{13}C$ values of up to – 30‰ have been reported for isorenieratene [19, 30, 36]. For PSB, which use the Calvin cycle, the presence of respired CO_2 at the chemocline may result in quite depleted $\delta^{13}C$ values; values as depleted as – 45‰ have been reported for okenone [19, 36].

5
Photic Zone Anoxia in Recent Depositional Environments and its Manifestation in the Sedimentary Record

The occurrence of phototrophic sulfur bacteria in environments which are characterized by an overlap of the photic and anoxic zones has been documented for a variety of settings (see for a review [16]). Here we will illustrate their ecological occurrence in four different settings for which also paleoecological information is available.

5.1
Mahoney Lake

Mahoney Lake is a small (800×400 m) and shallow (max. depth 18 m) meromictic lake in British Columbia (Canada, 49° N, 119° W) with a steep

chemocline at 6.6 m (Fig. 1a). PSB form a dense, 10-cm-thick layer at the chemocline [17]. The dominant (98%) species, *Lamprocystis purpurea* formerly known as *Amoebobacter purpureus*, reached a maximum cell num-

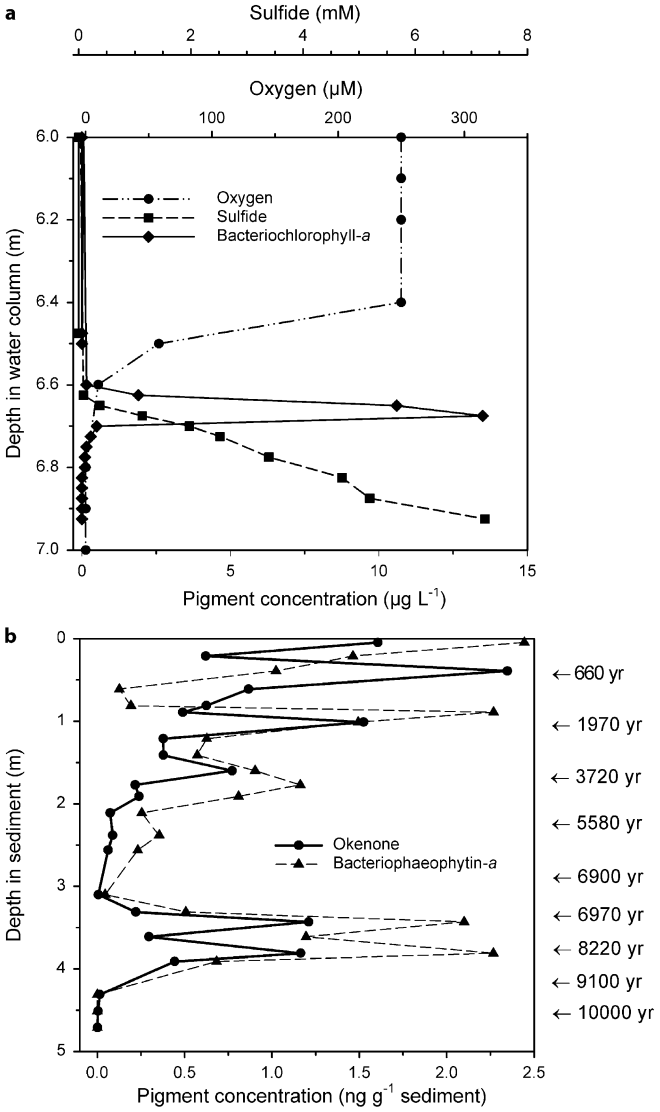


Fig. 1 **a** Vertical distribution of oxygen, sulfide, and bacteriochlorophyll-*a* derived from PSB in the present-day water column of Mahoney Lake in September 1989. Data are from [17]. **b** Concentration profiles of the PSB pigments okenone (**1**) and bacteriophaeophytin-*a*, a diagenetic product of bacteriochlorophyll-*a*, in the sediments of Mahoney Lake. Radiocarbon dates (in yr) are indicated. Data are from [37]

ber of 4×10^8 cells ml^{-1}. Extremely high concentrations of up to 21 mg bacteriochlorophyll-*a* L^{-1} were found in this layer and okenone (**1**) was detected as the dominant carotenoid [17]. Anoxygenic photosynthesis was limited by sulfide at the top of the layer, whereas the substantially reduced irradiance resulted in light limitation for most of the cell population below. GSB are not present in this lake and are probably inhibited by the high sulfide concentrations. Pigment analysis revealed that these specific conditions were common during the Holocene development of the lake; both fossil bacteriochlorophyll-*a* as well as okenone were identified in various strata (Fig. 1b) [37]. This was supported by fossil DNA data which revealed four different 16S rRNA gene sequences falling in the group of *Chromatiaceae*. The sequence from sediments 9100-year-old was 99.2% identical to the 16S rRNA gene sequence of *Lamprocystis purpurea* isolated from the present-day chemocline of the lake. At other sediment intervals other dominant 16S rRNA gene sequences were found that were related to *Marichromatium* and *Thiorhodovibrio* species, indicating that the PSB population varied substantially during the Holocene.

5.2 Ace Lake

Post-glacial Ace Lake is a small (200 × 300 m) and shallow (max. depth 25 m), meromictic, saline lake in the Vestfold Hills of eastern Antarctica (68° S, 78° E) and usually covered by ice for about 11 months of the year. It contains anoxic, sulfidic, sulfate-depleted, and methane-saturated bottom waters (Fig. 2a) [38]. The euxinic chemocline at 11.7 m depth is colonized by obligate anoxygenic photolithotrophic GSB [39] with the brown-colored *Chlorobium phaeovibrioides* DSMZ 269^T as their closest relative (99.6% sequence similarity) [40]. The concentration of a characteristic carotenoid for GSB, chlorobactene (**5**), peaked at the chemocline with concentrations of 4 mg l^{-1} (Fig. 2a). Smaller amounts of isorenieratene (**7**) and β-isorenieratene (**6**) were also found. This is somewhat surprising since *C. phaeovibrioides* is a brown strain of GSB and hence would be expected to have isorenieratene as its most abundant carotenoid (Table 2). However, some species of GSB are now known to have both green and brown colored strains (Table 2). PSB were not encountered in this lake, probably due to the relatively low light intensity at the chemocline. Analysis of fossil carotenoids in a sediment core covering the last 10.5 ka revealed that almost immediately after marine waters entered the paleo freshwater lake as a result of post-glacial sea-level rise, Ace Lake became meromictic with the formation of sulfidic bottom waters and a chemocline colonized by GSB (Fig. 2b). The carbon isotopic composition of fossil chlorobactene (ca. – 18‰) confirmed its origin from GSB [41]. Analysis of fossil 16S rRNA genes revealed that the biological source of chlorobactene throughout the Holocene was identical to that residing at the present-day chemocline.

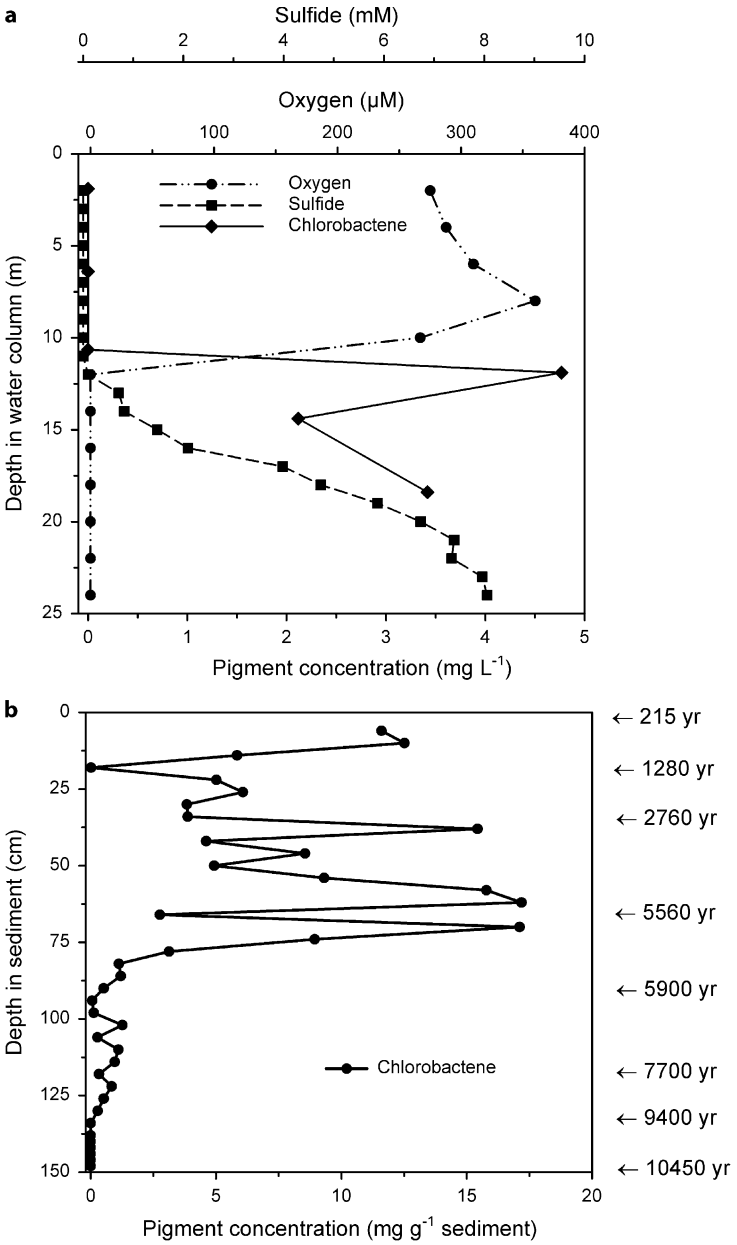


Fig. 2 **a** Vertical distribution of oxygen, sulfide, and chlorobactene derived from GSB in the present-day water column of Ace Lake (November 2000). Data are from [38, 40]. **b** Concentration profiles of the GSB pigments chlorobactene (**5**) in the sediments of Ace Lake. Radiocarbon dates (in yr) are indicated. Data are from [40]

5.3
Kyllaren Fjord

Kyllaren is a small (ca. 0.35 km^2), 29 meter deep fjord on the west coast of Norway (62° S, 5° E). It is connected to the Norwegian Sea through a shallow inlet which consists of a tidal flat and a 1–2 m deep water channel, depending on the tide. Consequently, bottom water exchange is restricted, resulting in a permanent halocline/chemocline at 2–5 m water depth, at which a population of GSB resides. GSB carotenoids detected in the water column of Kyllaren fjord are dominated throughout the year by isorenieratene and β-isorenieratene, peaking at the chemocline (Fig. 3a), with concentrations up to 9.2 and 3.0 $\mu g\ L^{-1}$, respectively [30]. The stable carbon isotope composition of isorenieratene and β-isorenieratene vary from – 24 to – 30‰, which, in combination with the strongly depleted $\delta^{13}C$ values of CO_2 at the chemocline (– 20 to – 27‰), confirms their origin from GSB. The presence of fossil carotenoids in the upper 20 cm of sediments (Fig. 3b), indicate that GSB and, surprisingly, also PSB, as indicated by the presence of okenone **1**, thrived at the chemocline for the last 200 yr [42]. Their isotopic compositions (– 25‰ for isorenieratene and – 39‰ for okenone) are in agreement with these assignments of the biological sources for these fossil carotenoids [42], considering that these are relatively light due to the prominence of the respired CO_2 recycling process [31]. During the last 200 years there has been a tendency of GSB to become more abundant than PSB (Fig. 3b) in this fjord. This may result from the increased primary productivity in the fjord, induced by an increase in nutrient load [42]. The increase in algal biomass is thought to have affected the light intensity and light quality at the chemocline, inducing a shift in the composition of the phototrophic bacteria.

5.4
Black Sea

The Black Sea is the world's present-day largest anoxic basin. It has an area of 422 000 km^2 and its maximum depth is ca. 2200 m. It is a restricted basin because of the shallow connection to the Mediterranean Sea (Strait of Bosporus), which substantially limits the mixing of low salinity surface water with the high salinity (and therefore more dense) bottom water. This results in a strong density stratification with an associated chemocline. In 1989, Repeta and co-workers [22] showed that, although the chemocline is much deeper than in stratified lakes and fjords, the Black Sea's deep chemocline (at ca. 80 m) still provided a niche for GSB. This was revealed by the analysis of the characteristic pigments, bacteriochlorophyll-*e*, isorenieratene, and β-isorenieratene in the water column (Fig. 4a), which all peaked at ca. 80 m depth. Overmann et al. [43] showed by cultivation that these GSB are brown-colored *Chlorobium phaeobacteroides* strains, which are adapted to

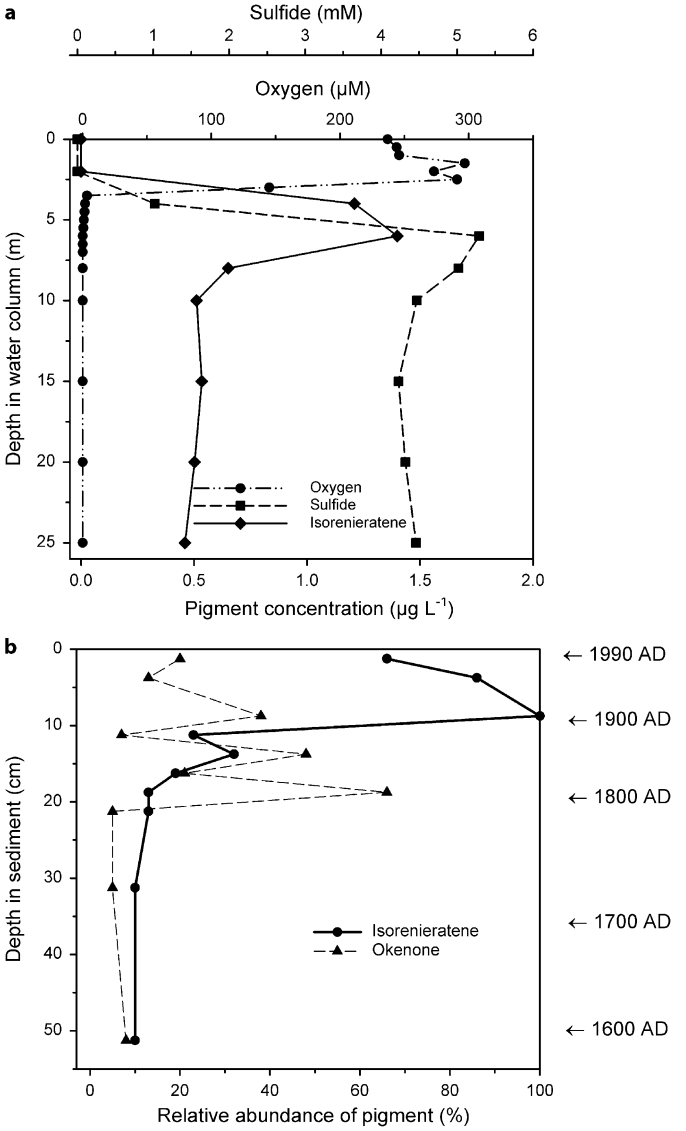


Fig. 3 **a** Vertical distribution of oxygen, sulfide, and isorenieratene derived from GSB in the present-day water column of Kyllaren fjord (August 2002). Data are from [30, 37]. **b** Concentration profiles (in relative abundance) of the GSB and PSB pigments isorenieratene (**7**) and okenone (**1**) in the sediments of Kyllaren fjord. Estimated dates of sediment intervals (in yr), based on ^{210}Pb, are indicated. Data are from [42]

the very low light intensities (ca. 0.003 μmol Quanta m^{-2} s^{-1}) at the chemocline. At these low light intensities, it is estimated that each bacteriochlorophyll molecule will absorb a photon only once every 8 hours. Consequently,

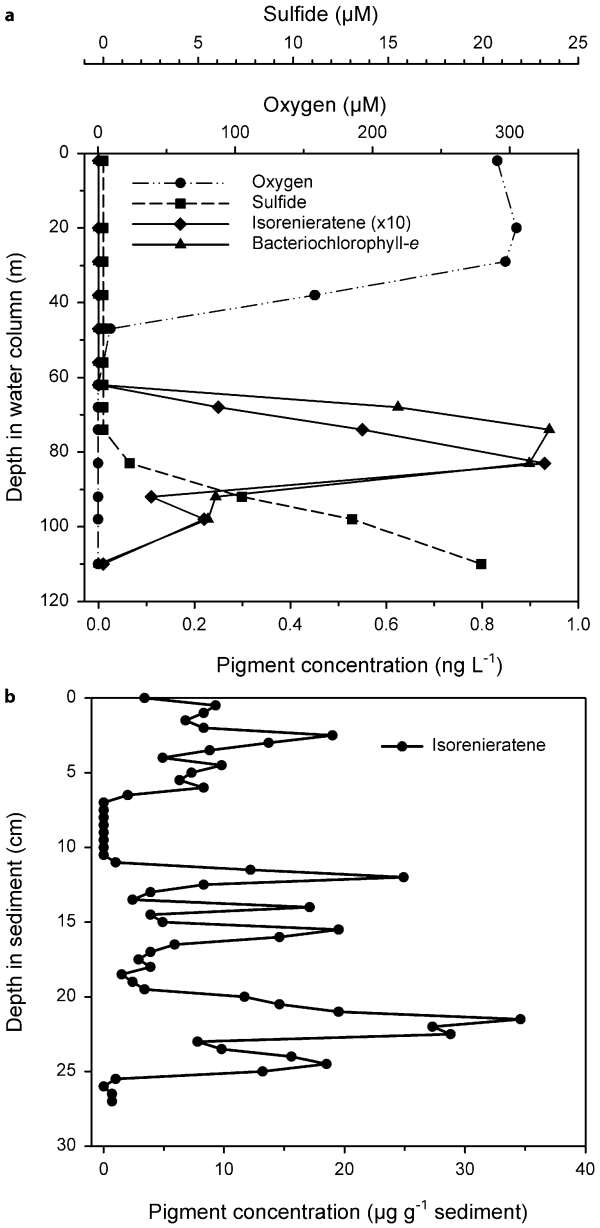


Fig. 4 **a** Vertical distribution of oxygen, sulfide and the GSB pigments bacteriochlorophyll-*e* and isorenieratene in the present-day water column of Black Sea (May 1988). Data were obtained from Station BS2-2 of the RV Knorr cruise 134, Leg 9 and are from [22]. **b** Concentration profiles of the GSB pigments isorenieratene (**7**) in the unit-1 sediments of the Black Sea from the same station. The core reflects ca. 1500 yr of sedimentation. Data are from [44]

these GSB have to be extremely efficient with respect to energy. Indeed, in comparison with the type strain of *C. phaeobacteroides*, the GSB isolated from the Black Sea chemocline have a very low maintenance energy requirement and an increased concentration of light-harvesting pigments [43]. Repeta [44] and Sinninghe Damsté et al. [45] showed that the present overlap of the photic and sulfidic zone has not always been the case in the last 8200 years. Repeta [44] provided high-resolution data (Fig. 4b) of intact isorenieratene in the sediments, which revealed substantial fluctuations related to the depth of the chemocline. Sinninghe Damsté et al. [45] showed that fossil isorenieratene is indeed derived from GSB since its $\delta^{13}C$ values are relatively enriched, consistent with the use of the reverse TCA cycle of the GSB.

These four cases provide insight in the type of environments in which the GSB and PSB can thrive and indicate that the pigments of these types of bacteria can become part of the sediments and, thus, can provide insight into past communities of phototrophic bacteria. Since these bacteria require both light and sulfide, this provides important information on past water column anoxia.

6 Ancient Sediments Containing Intact Pigments from Phototrophic Sulfur Bacteria

In the previous section we have seen that intact pigments of GSB and PSB are preserved in sediments up to 9 ka. This is already surprising since both carotenoids and bacteriochlorophylls are labile components that undergo rapid transformation reactions during burial (so-called dia- and catagenesis) [44, 46]. In the next section, we will describe these pathways in detail, but first we will discuss the occurrence of intact pigments in ancient sediments.

The oldest well-documented occurrence of intact pigments from GSB and PSB is in the Upper Miocene Gessosso-Solfifera Formation in Italy. Maxwell and co-workers [47, 48] identified intact isorenieratene by HPLC-PDA-MS in the marl layers of this 6 Ma year-old formation. It was accompanied by smaller amounts of *cis*-isorenieratene, obviously formed by *trans-cis* isomerization of the double bond(s) of isorenieratene, which are all *trans*. Intact isorenieratene was also identified in Pliocene sapropels ca. 3 Ma years old, deposited in the Eastern Mediterranean Sea [49]. The amount of intact isorenieratene (summed all-*trans* and *cis* isomers) ranged from non-detectable at the base and top of a sapropel up to 140 $\mu g\, g^{-1}$ sediment in the central part of the sapropels.

Isorenieratene accumulation rates at a central and western site in the Eastern Mediterranean Basin are remarkably similar and increase sharply to levels of up to 3.0 $mg\, m^{-2}\, yr^{-1}$ in the central part of the sapropel and then drop to low levels. Since Ba/Al ratios indicate enhanced paleoproductivity during

sapropel formation, these data support previously proposed models, according to which increased productivity is the driving force for the generation of euxinic conditions [50]. Apart from intact isorenieratene, these sapropels, which are buried 50–100 m in the subsurface, also contain components that have been interpreted as diagenetic derivatives of isorenieratene [51, 52]. They are formed by cyclization reactions involving the polyene system of double bonds in these carotenoids. The following section will discuss some major diagenetic products of aromatic carotenoids that have been identified so far.

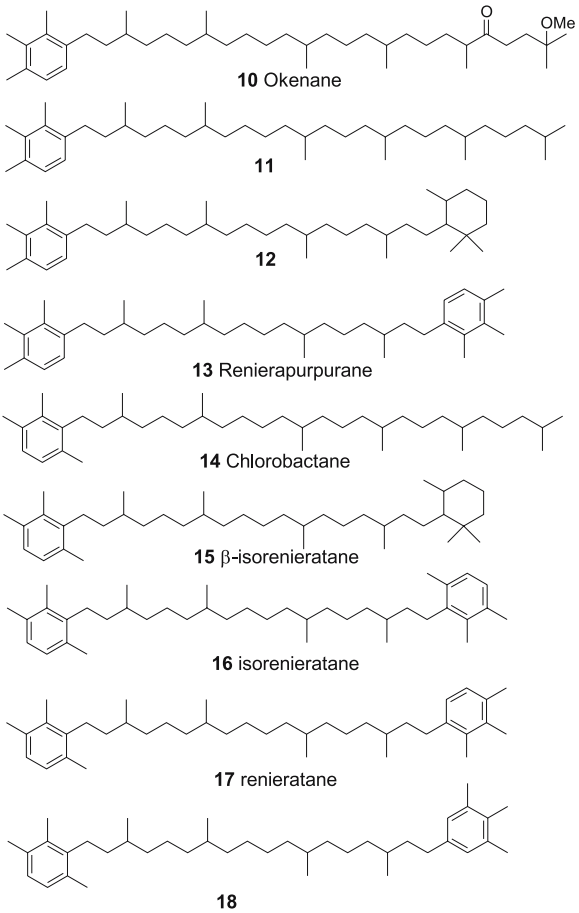
7
Dia- and Catagenesis of Carotenoid Markers for Photic Zone Anoxia

The first indication that carotenoid carbon skeletons can be preserved in ancient sediments came from the identification of three perhydro diaromatic carotenoids (**16**, **13**, and **17**; see Scheme 3 for structures of perhydro aromatic carotenoids) in the Schistes Carton of the Paris Basin [53]. Summons and Powell [54, 55] subsequently identified two series of aryl isoprenoids (**19** and **20**; see Scheme 4 for diagenetic products of aromatic carotenoids) in sediments and petroleum. These series of components had the 2,3,6- and 2,3,4-trimethyl substitution pattern of the aromatic moiety, which suggested a relationship with isorenieratene (**7**) or chlorobactene (**5**) and okenone (**1**) or renierapurpurin (**4**), respectively, through hydrogenation and C – C bond cleavage. Since the series of 2,3,6-trimethyl aryl isoprenoids extended to C_{30} (and not to C_{40} as would be expected for a chlorobactene origin), it was likely that these products were derived from isorenieratene. Summons and Powell [54, 55] also showed that the $\delta^{13}C$ of the 2,3,6-trimethyl aryl isoprenoids was relatively enriched, lending further support to their derivation from isorenieratene produced by GSB using the reverse TCA cycle (see above).

Subsequently, a multitude of dia- and catagenetic products of aromatic carotenoids has been identified (see for a review [46]) and the processes by which they are formed are now reasonably well understood. Isolation of key components and elucidation of their structure by high-field NMR techniques and the determination of their ^{13}C content has been essential in this respect. For proper assessment of photic zone anoxia in past depositional settings, it is important to understand these dia- and catagenetic pathways as discussed below.

7.1
Hydrogenation

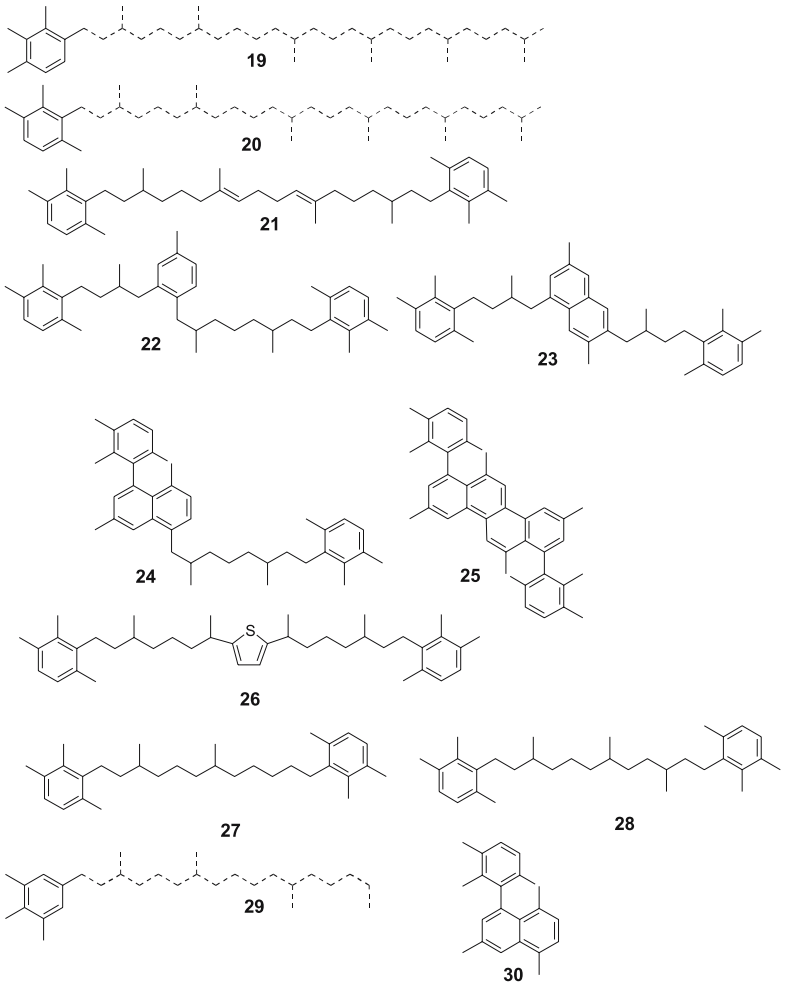
The first indication that intact aromatic carotenoid carbon skeletons can be preserved in ancient sediments came from the identification of the



Scheme 3 Perhydro aromatic carotenoids

"perhydro" derivatives of the diaromatic carotenoids, isorenieratene, renieratene, and renierapurpurin (**13**, **16**, **17** [53]), β-isorenieratene (15 [56]), and chlorobactene (**14** [57]). Subsequently, **13**, **15**, **16**, and **17** have also been identified in crude oils [58–62]. These findings strongly suggested that these molecular fossils were formed through hydrogenation of the polyene chain of the preserved carotenoids. Schaeffer et al. [63] recently identified some partially hydrogenated derivatives of isorenieratene and okenone (e.g. **21**) in surface sediments of Lake Cadagno, indicating that hydrogenation may occur during the first phases of diagenesis.

The analysis of fossil "perhydro" carotenoids may also provide clues as 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 **18** possessing



Scheme 4 Examples of dia- and catagenetic products of aromatic carotenoids

the unprecedented 3,4,5-trimethyl substitution pattern for the aromatic ring in combination with the more common 2,3,6-trimethyl substitution pattern [59–61]. Since shifts of methyl groups of aromatic rings during diagenesis were deemed highly unlikely, this suggested a novel carotenoid **9** (Scheme 2) as precursor. Since this carotenoid has not been reported to occur in nature and diaryl isoprenoid **18** is distinct from other molecular fossils in its carbon isotopic composition, it was speculated that this may be derived from a now extinct species of GSB.

7.2 Cyclization and Aromatization

A large suite of diagenetic products of isorenieratene (e.g. **22**–**25**) and other aromatic carotenoids has been identified with structures containing one to four additional, in most cases aromatic, rings [52, 64–67]. Most of these components have only been characterized by mass spectrometry, but in specific cases key structures (**22**–**23**) have been isolated from sediment extracts and fully characterized by NMR [52, 64]. The identical carbon isotopic compositions of isorenieratane and these C_{40} isorenieratene derivatives confirm their diagenetic relationship (Fig. 5). The structures of these diagenetic products strongly suggest that they have been formed by a sequence of cyclization, aromatization, and hydrogenation reactions occurring within the polyene system (Fig. 6). Initial cyclization seems to occur mainly after appropriate *trans-cis* isomerization at two specific sites, which favors a Diels–Alder reaction [65].

These pathways are supported by calculations using molecular dynamics simulation [68] which allowed the entire cyclization pathway to be simulated, including stable intermediates as well as energy barriers related to transition states. The simulations indicated that the formation of tetracyclic isorenieratene derivatives is likely to occur via an A-ring initiated reaction mechanism,

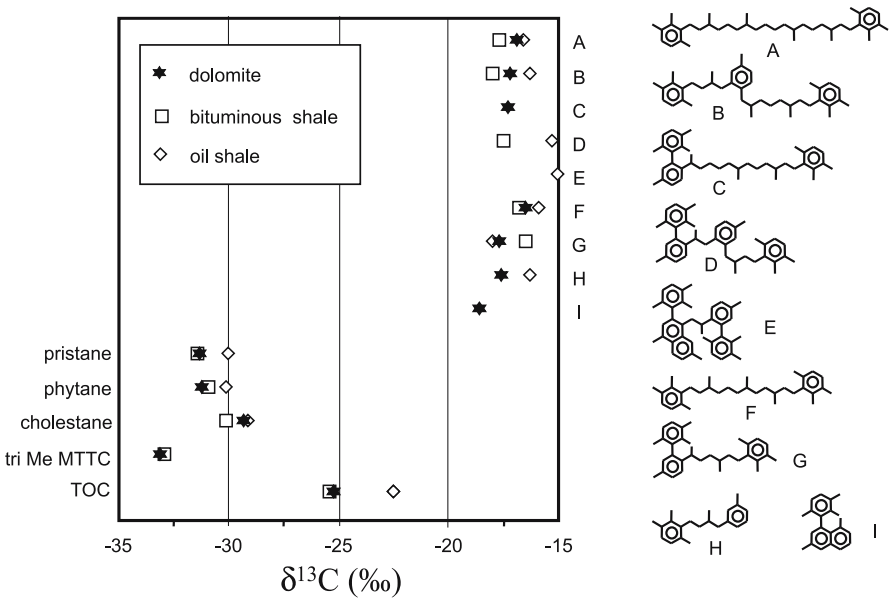


Fig. 5 Stable carbon isotopic composition of isorenieratene derivatives and some algal biomarkers present in three sedimentary facies of the Lower Kimmeridge Clay Fm. [118]

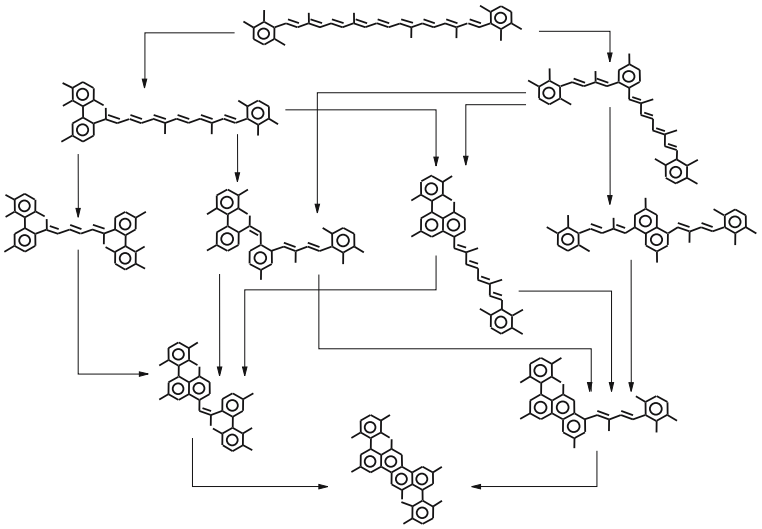


Fig. 6 Cyclization and aromatization of isorenieratene resulting in the formation of diagenetic derivatives [49]

as the reaction product resulting from A-ring closure is more stable than that derived from B-ring closure. Furthermore, the A-ring initiated tetracyclization pathways contain one fewer high-energy hydrogen shift step than their B-ring initiated counterparts, indicating that B-ring initiated cyclization is more likely to result in the formation of monoaromatic compounds. These observations are in excellent agreement with observed distributions of isorenieratene derivatives in sediments.

7.3 Aromatization of the Cyclohexenyl Moiety

In addition to hydrogenation of the cyclohexenyl moieties of carotenoids, as in β-isorenieratene, aromatization with concomitant transfer of methyl groups is also thought to occur. A 1,2-shift of a geminal methyl group of β-carotene may occur during aromatization as has been simulated in the laboratory [62]. In this way, β-isorenieratane (**15**) present in a North Sea crude oil is thought to be derived from aromatization and hydrogenation of β-carotene and not from hydrogenation of β-isorenieratene [52, 62]. 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 biosynthesize β-isorenieratene. In this way, aryl isoprenoids may also partly derive from β-carotene and, indeed, the aryl isoprenoids in the North Sea crude oil studied by Koopmans et al. [62] had a carbon isotopic compo-

sition indicating a mixed origin from both β-carotene and isorenieratene. Therefore, aryl isoprenoids should be used with caution and we recommend that their $\delta^{13}C$ value should be determined to be assured of an origin from *Chlorobiaceae*.

7.4 Intramolecular Sulfurization

Various sedimentary organic sulfur compounds possessing the isorenieratane skeleton have been identified by mass spectrometry and Raney Ni desulfurization [65]. They occur as thiophenes (e.g. **26**), dithiophenes, bithiophenes, 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 sulfate reducing bacteria, and functionalized lipids during early diagenesis ("natural sulfurization"; see for a review [69]). Obviously, the polyene system of carotenoids is prone to natural sulfurization as well and, after the subsequent reduction of double bonds, leads to the formation of sulfur compounds with aromatic carotenoid skeletons.

7.5 Incorporation into Macromolecular Organic Matter

The largest fraction (> 90%) of 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 aromatic carotenoids has been shown to be prone to reactions leading to sequestration. The most common of these reactions is natural sulfurization but, in this case, operating in an intermolecular fashion and leading to cross-linking of carbon skeletons through (poly)sulfide linkages. A variety of "perhydro" aromatic carotenoids, β-chlorobactane **14**, isorenieratane **15**, isorenieratane **16**, renieratane **17**, and diaryl isoprenoid **18** have been released from soluble macromolecular aggregates [45, 56, 59, 65, 70–80] and from kerogen [81] by various desulfurization methods. Isorenieratane was also released by selective cleavage of polysulfide bonds, indicating that this carotenoid skeleton may also be bound only via polysulfide linkages [65, 71, 82]. 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 cyclization/aromatization (e.g. **22**) have also been found upon desulfurization of geomacromolecules [65, 77].

Thermal degradation has also been used to analyze aromatic carotenoids sequestered in high-molecular-weight material. The high abundance of 1,2,3,4-tetramethylbenzene (TMB) in flash pyrolysates of kerogens [58, 59, 61, 83–85] 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 almost all mono- and diaromatic carotenoids depicted in Scheme 2. 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. 1-Ethyl-2,3,6-trimethylbenzene is often a dominant C_5 alkylbenzene in pyrolysates of kerogens [59, 84] consistent with a derivation from isorenieratene or chlorobactene. TMB and 1-ethyl-2,3,6-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 [59, 61]. Pedentchouk et al. [86] showed that 1,2,4-trimethylbenzene in pyrolysates may also be (partially) derived from kerogen-bound isorenieratene.

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 [87], indicating that there are multiple origins for TMB. The anomalously high concentration of TMB and 1-ethyl-2,3,6-trimethylbenzene in petroleums from the Williston Basin [59] 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 **9** (i.e. 1,3,4,5-tetramethylbenzene and 1-ethyl-3,4,5-trimethylbenzene [59]).

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 [59], 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 biosynthesized was almost completely (ca. 99%) incorporated into macromolecular fractions [59], demonstrating the importance of aromatic carotenoid sequestration during diagenesis.

7.6 Expulsion of Toluene and Xylene

C_{32} (**27**) and C_{33} (**28**) pseudohomologs of isorenieratane have been reported both as free components or in a S-bound form [65, 66, 77]. Mild heating of β-carotene produces C_{32} and C_{33} "carotenoids" and *m*-xylene and toluene, respectively (e.g. [88]) by a pericyclic reaction via an eight-membered ring

transition state of the polyene chain. Since this mechanism only involves the polyene system of the carotenoid, it is postulated that isorenieratene and other aromatic carotenoids can undergo similar reactions, resulting in the formation of C_{32} and C_{33} diaromatic "carotenoids" [65, 67]. It is thought that these expulsion reactions also occur in immature sediments and that subsequent hydrogenation leads to compounds **27**–**28**. The cyclization, aromatization, and sulfurization reactions that lead to the C_{40} diagenetic products of isorenieratene also occur with C_{32} and C_{33} "carotenoids".

7.7
C – C Bond Cleavage

A wide variety of putative products from C – C bond cleavage of fossil aromatic carotenoid derivatives have been identified [65]. These include the pseudo-homologous series of aryl isoprenoids **19**, **20**, and **29**, whose structures are sub-units of the "perhydro" aromatic carotenoids [54, 55, 59, 89]. Other products (e.g. **30**) are formed from carotenoid units which have undergone cyclization and aromatization, expulsion, or sulfurization reactions. These products are probably not directly formed from fossil carotenoids but rather form 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 [58, 65].

8
Dia- and Catagenesis of Bacteriochlorophyll Markers for Photic Zone Anoxia

Bacteriochlorophylls are subjected to substantial alterations during sediment dia- and catagenesis, ultimately resulting in the formation of porphyrins which can still be found in petroleum. This has been the subject of various reviews (e.g. [90]) and thus will not be covered here. Members of the bacteriochlorophylls *c*, *d*, and *e* are characterized by the presence of additional carbon atoms at position C-8 (leading to e.g. propyl and *i*-butyl substitution), C-12 and C-20 of the chlorophyll skeleton (Scheme 1) in comparison with chlorophyll *a*. This higher and characteristic degree of alkylation can be found in diagenetic products of the bacteriochlorophylls (e.g. [91, 92]) and in this way photic zone anoxia in past depositional systems can be assessed.

Oxidative degradation productions of bacteriochlorophylls have also been recognized in the fossil record. Grice et al. [93] identified maleimides (1*H*-pyrrole-2,5-diones) as degradation products of photosynthetic tetrapyrrole pigments for the first time in ancient marine sediments. The maleimides had a relatively simple distribution, dominated by Me,Et maleimide, which is predominantly derived from chlorophyll of planktonic origin. Me,*n*-Pr and Me,*i*-Bu maleimides, present in low abundance, were thought on structural

grounds to be derived from the bacteriochlorophylls *c*, *d*, or *e* of *Chlorobiaceae*. This was confirmed by their enrichment in ^{13}C resulting from the reversed TCA cycle employed by the GSB. The structurally more specific Me,*i*-Bu maleimide was slightly more enriched in ^{13}C than Me,*n*-Pr maleimide, suggesting that the latter is partly derived from phytoplanktonic chlorophyll. These results provide evidence for the existence in both depositional settings of microbial communities containing *Chlorobiaceae*. It remains enigmatic when the maleimides are formed: by oxidation of the bacteriochlorophylls at the time of deposition, during weathering or during sample processing.

9
Evidence for Photic Zone Anoxia in Ancient Depositional Environments

The dia- and catagenetic products of the pigments of GSB have been used to assess photic zone anoxia in a wide variety of settings. Table 3 provides a literature overview of the depositional environments for which molecular paleontological evidence exists for an overlap of the photic and sulfidic zone. It also specifies whether this information is based on fossil carotenoid derivatives or on fossil bacteriochlorophyll derivatives or both. In most cases, the evidence is based on the presence of carotenoid derivatives, probably because they are easier to analyze than the bacteriochlorophyll derivatives. In most instances where intact aromatic carotenoid derivatives were found, products derived from isorenieratene and not from chlorobactene or β-isorenieratene were encountered. On the basis of the relatively high abundance of isorenieratene in the brown strains of the GSB (Table 2), this indicates a relatively deep chemocline in the photic zone.

Chlorobactene derivatives were only encountered in a few settings: during OAE-2 in the Tarfaya and Cape Verde (DSDP Site 367) basins and during the Toarcian anoxic event in the Paris Basin (Schistes Carton Formation) and SW Germany (Posidonia Shale) and the 1.64 Ga Barney Creek Formation [94]. In these latter deposits the hydrogenated form of okenone, okenane, derived from PSB was also found. This would indicate even shallower anoxic conditions in the photic zone. Dia- and catagenetic products of the hypothetical diaromatic carotenoid (**9**) with the 3,4,5-trimethyl substitution pattern of one of the rings is only found in sediments of 450 to 310 Ma in age, but were not present in the Paleoproterozoic Barney Creek Formation.

Most of the settings for which we have evidence for photic zone anoxia were apparently highly productive shelf seas with paleo water depths of 100–300 m (the so-called continental or epeiric seas). The stratification in these systems is believed to be seasonally controlled [95] and photic zone anoxia is probably highly dynamic. The Oxford Clay is probably a typical example of this type of setting since in the same stratum both delicately preserved fossils of benthic organisms requiring oxygen and isorenieratene

Table 3 Molecular paleontological evidence for photic zone anoxia in ancient depositional systems

Black shale	Geological age	Aromatic carotenoid[a] derivatives	Bacterio-chlorophyll derivatives[b]	Refs
Sediments				
Eastern Mediterranean sapropels ODP 964, 967, 969 leg 160	Pliocene	7		[49–52]
Sdom Fm, Dead Sea (Israel)	Miocene/Pliocene	7		[101]
Gessoso-Solfifera Fm. (Italy)	Messinian	7	P	[47, 48, 62, 72, 76, 77]
Gibellina Marl (Sicily, Italy)	Messinian	7, 5		[70]
Menilite Fm. (Poland)	Oligocene	7		[65, 102]
Mulhouse Basin (France)	Oligocene		P	[92, 103]
Qianjiang Fm., Jianghan Basin (China)	Middle/Late Eocene	7, 6, 5		[104]
Messel (Germany)	Eocene		P	[91]
Xingouzhui Fm., Jianghan Basin, (China, 113°E)	Paleocene-Early Eocene	7		[104]
Deep Ivorian Basin (ODP 959)	Coniacan/Santonian	7, 5		[105]
Canje Fm. (British Guyana)	Late Turonian	7		[65]
OAE-2				
Tarfaya (Morocco)	Cenomanian-Turonian	7, 5		[56, 79, 80, 106]
Cape Verde Basin, North Atlantic (DSDP 367)	Cenomanian-Turonian	7, 5	P, Ma	[56, 79, 107]
Cape Verde Rise, North Atlantic (DSDP 368)	Cenomanian-Turonian		P, Ma	[107]
Demerara Rise, North Atlantic (DSDP 144)	Upper Cenomanian	7, 5		[56, 79]
Cape Hatteras, North Atlantic (DSDP 603B)	Cenomanian-Turonian	7	Ma	[107, 108]
Western Interior Seaway (USA)	Cenomanian-Turonian	7		[109]
Bahloul Fm. (Tunesia)	Cenomanian-Turonian	7	P, Ma	[107]
Livello Bonarelli (Italy)	Cenomanian-Turonian		P, Ma	[107]
Exmouth Plateau, Indian Ocean (ODP 763C)	Cenomanian-Turonian		Ma	[107]

Table 3 Continued

Black shale	Geological age	Aromatic carotenoid[a] derivatives	Bacterio-chlorophyll derivatives[b]	Refs
OAE-1a				
Livello Selli (Italy)	Early Aptian	7	P, Ma	[107, 110]
Maculungo Shale, Kwanza Basin (West Africa, 10°S, 14°E)	Barremian	7		[86]
Sunniland Limestone Fm (USA)	Early Cretaceous	AI		[111]
Calcaires en Plaquettes Fm. (France)	Late Jurassic	7, 5		[57, 65, 97]
Kimmeridge Clay Fm (UK)	Late Jurassic	7, 4, 8		[52, 65, 112]
Toarcian OAE				
Schistes Cartons (France)	Toarcian	7	P	[53, 65, 107]
Posidonia Shale (Germany)	Toarcian	7, 5, 6	P	[107, 113, 114]
Whitby Fm. (UK)	Toarcian	AI		[115]
Marche-Umbria Basin (Italy)	Toarcian		P, Ma	[107]
Oxford Clay Fm (UK)	Middle Callovian	7		[96]
Allgäu Fm. (Germany)	Early Jurassic (Toarcian)	7		[65, 116]
Hauptdolomit (Germany)	Late Triassic	7		[65]
Kőssen Marl (Hungary)	Late Triassic	7		[65]
Sepiano Shale (Switzerland)	Middle Triassic		P, Ma	[117, 118]
Kupferschiefer (Germany)	Late Permian	7	P	[66, 93, 118–122]
Minnelusa Fm (USA)	Late Carboniferous	7		[65]
Lodegepole Fm (Western Canada Basin)	Early Carboniferous	AI		[58]
Exshaw Fm (Western Canada Basin)	Early Carboniferous	9, 7		[58, 65]
Bakken Fm (Williston Basin, Canada)	Early Carboniferous	AI		[58]
Holy Cross Mountains (Poland)	Late Devonian	9, 7		[123]

Table 3 Continued

Black shale	Geological age	Aromatic carotenoid[a] derivatives	Bacterio-chlorophyll derivatives[b]	Refs
Duvernay Fm. (Canada)	Late Devonian	9, 7, 8		[58–60]
Ratner Shale (Western Canada Basin)	Middle Devonian	AI		[58]
Keg River Fm. (Canada)	Middle Devonian	9, 7		[98, 124]
Salina, Michigan Basin (Canada)	Silurian	AI		[54, 55]
Maquoketa, Illinois Basin (USA)	Late Ordovician	AI		[125]
Boas Oil Shale (Canada)	Late Ordovician	9, 7		[126]
Decorah Fm. (USA)	Middle Ordovician	7		[127]
Womble Shale (USA)	Middle Ordovician	7, 9		[126]
Barney Creek Fm. (Australia)	Paleoproterozoic	4, 5, 1, 8, 7		[94]
Oils				
Sunniland Limestone Fm (USA)	Early Cretaceous	AI		[111]
Lodegepole Fm (Western Canada Basin)	Carboniferous	AI		[58]
Exshaw Fm (Western Canada Basin)	Carboniferous	AI		[58]
Bakken Fm (Williston Basin, Canada)	Carboniferous	AI		[58]
Duvernay (Western Canada Basin)	Upper Devonian	9, 7		[58–60]
Ratner Shale (Western Canada Basin)	Middle Devonian	AI		[58]
Keg River (Western Canada Basin)	Middle Devonian	AI		[54, 55]
Pripyat River (Belarussia)	Devonian	9, 7		[67]
Salina (Michigan Basin, Canada)	Silurian	AI		[54, 55]

[a] numbers refer to carotenoids indicated in Scheme 1, AI = aryl isoprenoids;
[b] P = prophyrins, Ma = maleïmides

derivatives occur. This has been explained by the concept of intermittent anoxia [96, 97]. The problem with the understanding of these settings is that we have no contemporary analogue for these systems.

Some of the settings where photic zone anoxia occurred in the geological past represent deep water settings (e.g. Mediterranean Sea, Atlantic Ocean during OAE-2, and the Ivorian Basin) and these cases likely represent more or less stagnant basins for which the present-day Black Sea can serve as a modern analogue. The Eastern Mediterranean basin is probably the most comparable to the present-day Black Sea as it is also a land-locked basin with a shallow sill.

10 The Fossil Record of Green and Purple Sulfur Bacteria

The occurrence of diagenetic products derived from the pigments of GSB also provides an insight into the evolution of these bacteria. The oldest reported occurrence of GSB is from the Paleoproterozoic Barney Creek Formation. Both isorenieratane and chlorobactane were reported in these 1.64 Ga deposits, testifying to the existence of an adaptation to different light regimes already present at that time [94]. Unfortunately, due to the high background of organic constituents in these very old sediments, it has been impossible to measure the $\delta^{13}C$ values of individual carotenoid derivatives and, in this way, unambiguously confirm their origin from GSB. However, since their structures are so specific and their occurrence fits so well to the assumed anoxicity of the ocean at that time [94], there remains little doubt that GSB indeed thrived in the Paleoproterozoic.

A large gap in the fossil record falls from 1.64 Ga to ca. 0.45 Ga with no reported occurrences of fossil molecules derived from GSB. However, from the Ordovician up to the present-day numerous occurrences of GSB have been documented (Table 3) and in many cases it has been shown that the carotenoid derivatives bear the ^{13}C signature of the reverse TCA cycle (Fig. 7). This indicates that for at least 450 Ma the GSB biosynthesized isorenieratene through a specific biochemical pathway. This shows that lipid biochemistry can be very conservative and, consequently, that lipid biomarkers can indeed be used for tracing back specific organisms on time scales of hundreds to even thousands of millions of years.

An interesting phenomenon in the evolution of the GSB is the existence of the hypothetical carotenoid **9** with the 3,4,5-trimethyl substitution pattern of one of the rings. The structural resemblance of its diagenetic derivatives to isorenieratene (**7**) in combination with its distinct isotopic signature [59, 67] confirms its origin from GSB. However, diagenetic products from this carotenoid have only been found in sediments and oils from the Ordovician to the Devonian (Table 3) and have not been found in any samples

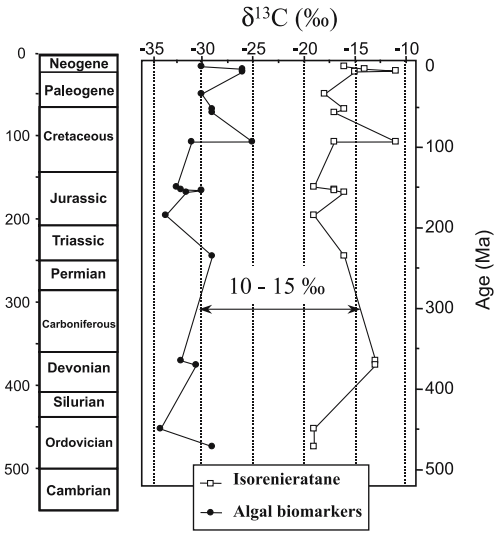


Fig. 7 Stable carbon isotopic composition of isorenieratene derivatives through time

of younger age. This suggests that the group of GSB capable of biosynthesizing this carotenoid had become extinct during evolution of the GSB. We can only speculate why. Perhaps the carotenoid with its odd substitution pattern has slightly different light absorption characteristics. It is known that the light intensity of the sun reaching the Earth's surface has increased over geological time and, perhaps, the specific carotenoid helped GSB to cope with this in their specific ecological niches. However, it is peculiar that derivatives of this carotenoid have not been found in the much older Paleoproterozoic sediments (Summons, per commun.), although isorenieratene derivatives are only minor components, due to the assumed shallow anoxic conditions [94]. Behrens et al. [98] noted that in sediments from the lower Keg River Formation derivatives from carotenoid **9** were predominant, whereas in sediment from the Upper Keg River only derivatives of isorenieratene **7** occurred. They attributed this to a change in the population of *Chlorobiaceae* resulting from a change in salinity or light availability.

The sedimentary record of PSB is much more sketchy. Derivatives of okenone are much less common, although aryl isoprenoids with the 2,3,4-trimethyl aryl substitution pattern have been widely reported. The perhydro derivative of okenone has, however, been found in the Paleoproterozoic Barney Creek Fm., indicating that ancestors of this group within the proteobacteria existed at that time and probably had the same physiology as the present day organisms.

11 Conclusions

Both intact pigments and bacteriochlorophyll of green and purple sulfur bacteria and their diagenetic and catagenetic derivatives can provide information on the spatial overlap of the photic and euxinic zone in past depositional systems. This is important in understanding the processes and conditions associated with burial of organic matter in sediments. In contrast to present day oceanic settings, photic zone anoxia occurred quite commonly during certain periods in the geological past and this often resulted in the deposition of black shales, the precursor of petroleum.

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