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Novel Aromatic Carotenoid Derivatives from Sulfur Photosynthetic Bacteria in Sediments

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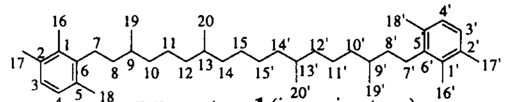
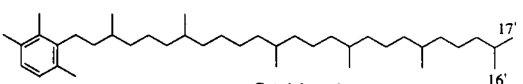
Abstract: Novel aromatic carotenoid derivatives **7** and **8** have been identified by synthesis of reference compounds in a H_2/PtO_2-hydrogenated fraction from the organic extract of a lake sediment (lake Cadagno, Switzerland). They derive from unreported carotenoids presumably biosynthesized by purple sulfur photosynthetic bacteria (*Chromatiaceae*) and represent potential biomarkers to establish the occurrence of photic zone anoxia in ancient ecosystems. © 1997 Elsevier Science Ltd.

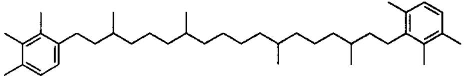
Since the first identification in Paris Basin sediments of C_{40} diaryl isoprenoids **1**-**3**[1] resulting from the diagenetic reduction of the corresponding diaromatic carotenoids isorenieratene, renieratene and renierapurpurin,[2] many studies have shown that isorenieratene transformation products occur in a wide range of organic-rich sediments.[3] Recently, Hartgers *et al.*[4] isolated and characterized an additional C_{40} diaryl isoprenoid (**4**) with a methyl substitution pattern for one of the aromatic rings which could not be related to any known precursor carotenoid but whose origin could be attributed to *Chlorobiaceae*, based on carbon isotopic measurements.[5] In recent years, the identification of molecular fossils from sulfur photosynthetic bacteria (i.e. aryl isoprenoids and transformation products of bacteriochlorophylls) in sediments or petroleum has become of major interest for palaeoreconstruction as they represent unique tools to determine the presence of an anoxic zone extending up to the photic zone of ancient ecosystems.[3b-f; 6]

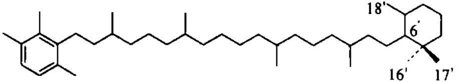


φ,φ-carotane **1** (isorenieratane)

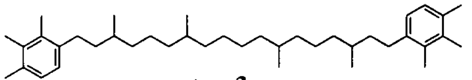


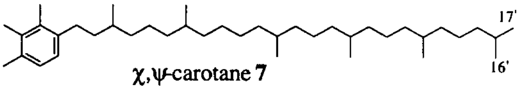
φ,ψ-carotane **5** (chlorobactane)


φ,χ-carotane **2** (renieratane)

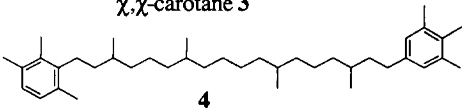


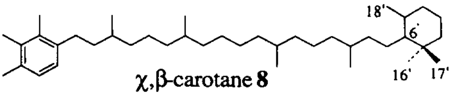
φ,β-carotane **6** (β-isorenieratane)


χ,χ-carotane **3**



χ,ψ-carotane **7**


4



χ,β-carotane **8**

We report here the identification by synthesis of novel aromatic carotenoid derivatives in a lake bottom sediment (lake Cadagno, Switzerland).[7] The organic matter recovered by DCM/MeOH extraction was prefractionated by liquid chromatography on silica gel into an apolar (hexane/DCM 4:1 v/v) and a more polar fraction (DCM/MeOH 1:1 v/v). The apolar fraction was subjected to TLC using hexane as developer to afford an alkane + alkene fraction (R_f 1-0.8), an aromatic fraction (R_f 0.8-0.1) and a more polar fraction (R_f<0.1);

the latter contained, in particular, partly reduced β,β-carotene and isorenieratene as indicated by MS. GC-MS investigation of this fraction after hydrogenation with H_2/PtO_2 revealed five C_{40} components characterized by base peaks at *m/z* 133 or 134 and corresponding to two C_{40} monoaromatic compounds (M^+ 554), two C_{40} monoaromatic compounds having an additional degree of unsaturation (M^+ 552) and one C_{40} diaromatic compound (M^+ 546). Mass spectral comparison and GC coinjection experiments with an authentic standard[1] allowed us to establish that the diaromatic compound corresponds to isorenieratane **1**. In addition, determination of the stable carbon isotopic composition ($\delta^{13}C$) of the five H_2/PtO_2-hydrogenated carotenoids indicated that the minor of the two monoaromatic compounds and the minor of the M^+ 552 compounds share the same origin as isorenieratane ($\delta^{13}C$ = -27‰) and likely correspond to chlorobactane **5** ($\delta^{13}C$ = -28‰) and β-isorenieratane **6** ($\delta^{13}C$ = -26‰) the precursor carotenoids of which are biosynthesized by *Chlorobiaceae*.[2b-c] This hypothesis was further confirmed by synthesis of standards using Wittig reactions on crocetindialdehyde or on β-apo-8'-carotenal followed by catalytic hydrogenation of the carotenoids formed.[8]

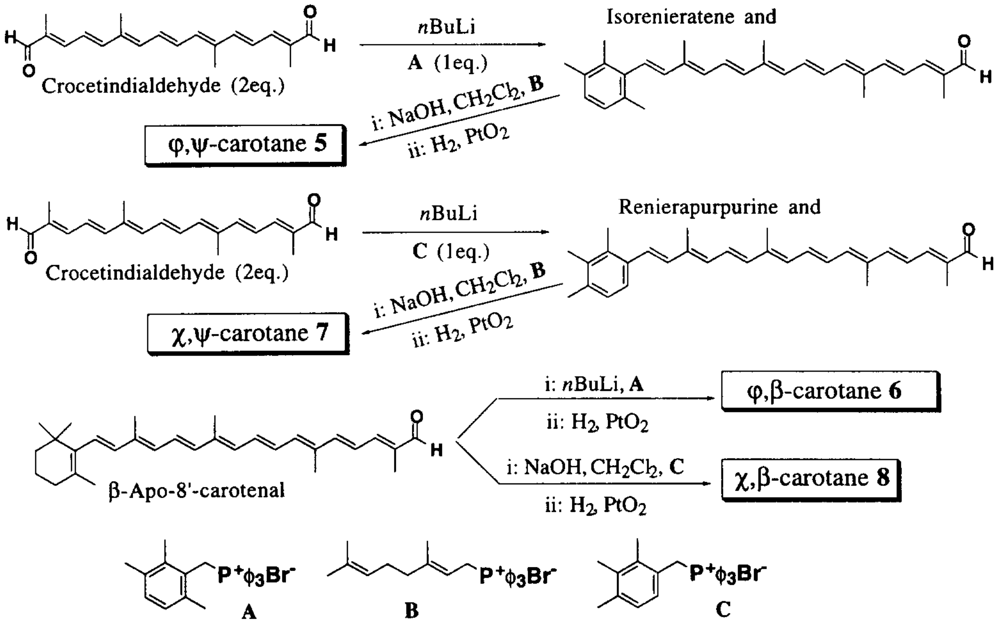


Figure 1: Synthetic scheme for carotanoids **5** - **8**; for synthesis of **A** and **C**, see reference 1.

In addition, the $\delta^{13}C$ value of the hydrogenated okenone[9] ($\delta^{13}C$ = -45‰) was close to the values of the major monoaromatic compound ($\delta^{13}C$ = -44‰) and to the major of the M^+ 552 compounds ($\delta^{13}C$ = -42‰), suggesting a common biological origin (i.e. from *Chromatiaceae*) and the same substitution pattern as okenone. This was confirmed by synthesis of χ,ψ-carotane **7** and χ,β-carotane **8** using identical methods as for synthesis of **5** and **6**[10] (Fig. 1). Identification of the different carotanoids was established by comparison of their mass spectra and relative retention times in GC of the synthesized standards with those of the naturally-occurring compounds. Regarding the possible carotenoid precursors of **7** and **8**, it can be envisaged that **7** derives from the early diagenetic transformation of okenone, which has an identical hydrocarbon skeleton. This transformation would imply elimination of the methoxy group and reduction of the ketone functionality. The carotenoids tethyatene (3,4-didehydro-β,χ-carotene) or tedanin (3-hydroxy-2,3-didehydro-β,χ-caroten-4-one), which occur in the sea sponges *Tedania digitata* and *Clathria frondifera*[11] are structurally related to compound **8**. However, given the $\delta^{13}C$ values of compounds **7** and **8**, specific for *Chromatiaceae*, it can be envisaged that, as for chlorobactene and β-isorenieratene biosynthesized by *Chlorobiaceae*, they could originate from χ,ψ-carotene and χ,β-carotene precursors respectively which are not known to date in living organisms[12] and still need to be discovered in purple sulfur photosynthetic bacteria.

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5. The recent progress in determining the stable carbon isotopic compositions of individual components by GC-irmMS allows the link to be established between sedimentary aromatic carotenoid-derivatives and their biological precursors, the green sulfur photosynthetic bacteria (i.e. *Chlorobiaceae*) as these strictly anaerobic microorganisms use the reverse tricarboxylic acid cycle for carbon fixation (Quandt, I.; Gottschalk, G.; Ziegler, H.; Stichler, W. *FEMS Microbiol. Lett.* **1977**, *1*, 125-128; Sirevåg, R.; Buchanan, B.B.; Berry, J.A.; Troughton, J.H. *Arch. Microbiol.* **1977**, *112*, 35-38). This results in an significant enrichment in ^{13}C of their biomass ($\delta^{13}C$ = -27‰ for isorenieratane **1** in lake Cadagno sediment) relative to the lipids from other (micro)organisms such as phytoplankton ($\delta^{13}C$ -33 to -35‰) or higher plants ($\delta^{13}C$ -31 to -33‰). $\delta^{13}C=10^3[(R_x-R_s/R_s)]$, where $R={}^{13}C/{}^{12}C$, x: sample, s: PDB standard and R_s=0.0112372.
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7. Lake Cadagno (Swiss Alps) is characterized by extensive sulfate reduction resulting in the formation of anoxic bottom waters extending up to the photic zone (Bernasconi, S.; Hanselmann, K. In *Organic Geochemistry: Developments and Applications to Energy, Climate, Environment and Human History.*

Grimalt, J.O. *et al.* Eds.; A.I.G.O.A.: San Sebastian, 1995; pp. 1169-1170). This particular ecosystem allows populations of purple and green sulfur photosynthetic bacteria (i.e. *Chromatiaceae* and *Chlorobiaceae* respectively) to thrive during certain periods of the year (Züllig, H. *Schweiz Z. Hydrol.* **47**, 87-125.

8. Catalytic hydrogenation in ethyl acetate led to the formation of carotanoid isomers, which were not resolved by GC (i.e. presence of a single peak), but result in a broadening or splitting (<1Hz) of the ^{1}H-NMR signals of the non-aromatic methyl groups.

   Analytical data

   φ,ψ-carotane **5**: ^{1}H-NMR (500MHz, CD_2Cl_2) δ ppm: 6.85 (s, 2H, H-3 and H-4), 2.66 (br ddd, 1H, *J*=13.0, 13.0, 5.0Hz, H-7a or H-7b), 2.56 (br ddd, 1H, *J* =13.0, 13.0, 5.0Hz, H-7a or H-7b), 2.26 (s, 3H, CH_3-aros), 2.22 (s, 3H, CH_3-aros), 2.20 (s, 3H, CH_3-aros), 1.60-1.03 (38H), 0.99 (br d, 3H, *J* =6.5Hz, H-19), 0.87 (br d, 6H, *J* =6.5Hz, H-16' and H-17'), 0.86 (d, 3H, *J* =6.5Hz, H-20), 0.84 (br d, 9H, *J* =6.5Hz, H-20', H-19' and H-18'). GC-MS (EI, 70eV), *m/z* (rel. int.): 554 $[M^+]$ (15), 147(3), 134(100), 133(82), 120(10), 119(10), 85(3), 71(8), 57(14).

   φ,β-carotane **6**: ^{1}H-NMR (500MHz, CD_2Cl_2) δ ppm: 6.85 (s, 2H, H-3 and H-4), 2.66 (ddd, 1H, *J*=13.0, 13.0, 5.0Hz, H-7a or H-7b), 2.56 (ddd, 1H, *J* =13.0, 13.0, 5.0Hz, H-7a or H-7b), 2.26 (s, 3H, CH_3-aros), 2.22 (s, 3H, CH_3-aros), 2.20 (s, 3H, CH_3-aros), 1.90 (m, 1H, H-6'), 1.60-1.03 (37H), 0.99 (br d, 3H, *J* =6.5Hz, H-19), 0.94 (br s, 3H, H-16' or H-17'), 0.88-0.83 (15H, H-20, H-16' or 17', H-18', H-19' and H-20'). GC-MS (EI, 70eV), *m/z* (rel. int.): 552 $[M^+]$ (15), 173(1), 147(3), 134(100), 133(76), 125(1), 123(2), 120(10), 119(11), 83(5), 69(14), 55(11).

9. *Chromatium okenii*, the predominant species of purple sulfur photosynthetic bacterium in lake Cadagno, biosynthesizes okenone (1'-methoxy-1',2'-dihydroxy-χ,ψ-caroten-4'-one) as the main carotenoid.[7] The $\delta^{13}C$ of the H_2/PtO_2 hydrogenated okenone in the sediment ($\delta^{13}C$ = -45‰) indicates a significant depletion in ^{13}C relative to the lipids from higher plants and phytoplankton.[5]

10. Analytical data

    χ,ψ-carotane **7**: ^{1}H-NMR (500MHz, CD_2Cl_2) δ ppm: 6.88 (d, 1H, *J*=7.5Hz, H-4 or H-5), 6.85 (d, 1H, *J* =7.5Hz, H-4 or H-5), 2.60 (ddd, 1H, *J*= 14.0, 11.0, 5.5Hz, H-7a or H-7b), 2.52 (ddd, 1H, *J*=11.0, 11.0, 5.0Hz, H-7a or H-7b), 2.24 (s, 3H, CH_3-aros), 2.20 (s, 3H, CH_3-aros), 2.17 (s, 3H, CH_3-aros), 1.55-1.03 (38H), 0.95 (br d, 3H, *J* =6.5, H-19), 0.87 (br d, 6H, *J* =6.5Hz, H-16' and H-17'), 0.85 (br d, 12H, *J* =6.6Hz, H-20, H-18', H-19' and H-20'). GC-MS (EI, 70eV), *m/z* (rel. int.): 554 $[M^+]$ (15), 173(2), 147(3), 134(100), 133(83), 120(8), 119(13), 85(3), 71(8), 57(13).

    χ,β-carotane **8**: ^{1}H-NMR (500MHz, CD_2Cl_2) δ ppm: 6.88 (d, 1H, *J*=7.5Hz, H-4 or H-5), 6.85 (d, 1H, *J* =7.5Hz, H-4 or H-5), 2.61 (ddd, 1H, *J*=13.5, 11.0, 5.0Hz, H-7a or H-7b), 2.52 (ddd, 1H, *J*=11.5, 11.5, 4.5Hz, H-7a or H-7b), 2.24 (s, 3H, CH_3-aros), 2.20 (s, 3H, CH_3-aros), 2.17 (s, 3H, CH_3-aros), 1.90 (m, 1H, H-6'), 0.95 (br d, 3H, *J* =6.5Hz, H-19), 0.94 (br s, 3H, H-16' or H-17'), 0.87 (br s, 3H, H-16' or H-17'), 0.87 (br d, 3H, *J* =7.5Hz, H-18'), 0.85 (br d, 9H, *J* =6.5Hz, H-20, H-19'and H-20'), 1.55-1.03 (37H). GC-MS (EI, 70eV), *m/z* (rel. int.): 552$[M^+]$ (15), 173(3), 134(100), 133(75), 123(2), 120(8), 119(13), 83(5), 69(13), 55(10).

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