Sedimentary Evidence for a Diaromatic Carotenoid with an Unprecedented Aromatic Substitution Pattern

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A novel diaryl isoprenoid ($C_{40}H_{66}$), possessing a 3,4,5-/2,3,6-trimethyl aromatic substitution pattern, which is attributed to a presently unknown strain of photosynthetic green sulfur bacteria, has been identified in a carbonaceous sedimentary rock.

The 'aromatic hydrocarbon' fraction of the extractable organic matter from Palaeozoic sediments and related oils from the Western Canada and Williston Basins (northern America, 400-300 × 10^6 years)[1] contains three diaryl isoprenoids and a series of diagenetically-derived counterparts thereof.[2] The structures of the two less abundant diaryl isoprenoids have recently been identified[3] as isorenieratane **1** and renieratane **2** by comparison of mass spectra and relative GC retention time data with those of authentic standards.[4] **1** and **2** are thought to be formed from their precursors, the diaromatic carotenoids isorenieratene and renieratene, respectively, by a selective hydrogenation of the poly-unsaturated isoprenoidal alkyl chain upon sediment burial.[4]

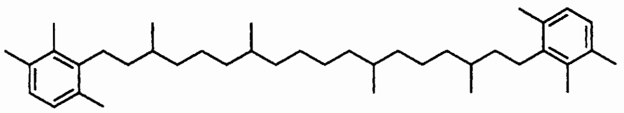
The most abundant diaryl isoprenoid, however, exhibited a GC retention behaviour that did not match those of previously identified diaryl isoprenoids.[4] Some structural information on this compound was obtained from its mass spectrum obtained by GC-MS analysis [m/z 546(25), 133(72), 134(100)]. Mass spectra of diaryl isoprenoids reported so far exhibit a base peak of m/z 133,[4] corresponding to cleavage β to the aromatic ring. The higher intensity of the m/z 134 fragment ion, which results from a McLafferty rearrangement, indicates an alternative substitution pattern with (an) additional vacant *ortho* and/or *para* position(s) on the aromatic ring(s).[5]

Here, we report the elucidation of the aromatic substitution pattern of the major diaryl isoprenoid by ^{1}H NMR. The component was isolated from the 'aromatic hydrocarbon' fraction of a sedimentary rock extract of the Exshaw Formation by reversed-phase HPLC using a Polygosyl preparative column by successive elutions with MeOH–EtOAc (50/50) and MeOH–EtOAc (80/20). The purest fraction (3.3 mg), consisting of 46% (corresponding to 0.01% of total organic matter) of the most abundant diaryl isoprenoid, was analysed by ^{1}H NMR (Table 1). To assign its aromatic substitution pattern, ^{1}H NMR data of this component were compared with those of the six possible isomers of ethyltrimethylbenzene, prepared by Friedel–Crafts alkylation of various trimethylbenzenes (1,2,3-, 1,2,4-, and 1,2,5-trimethylbenzene) with ethylbromide and $AlCl_3$. The individual isomers were isolated from the reaction mixture using reversed-phase HPLC using a Polygosyl preparative column with MeOH–H_2O (80/20) as the eluent. All six isomers were fully characterized by ^{1}H NMR (Table 1). Mass spectral and GC retention index data are published elsewhere.[3] The dominant diaryl isoprenoid present in the sediment extract of the Exshaw Formation was, thus, identified as having a 3,4,5-/2,3,6-trimethyl aromatic substitution pattern **3**. The ^{1}H NMR values found for the 2,3,6-substituted aromatic ring are in agreement with earlier published data for isorenieratane.[4] Also, the substitution pattern is reflected by the higher ratio of m/z 134/133 in its mass spectrum. The presence of two vacant *ortho*-positions in the 3,4,5-trimethyl substituted aromatic ring flavours the

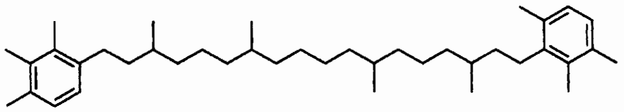
Table 1 ^{1}H NMR data for the isolated diaryl isoprenoid and the alkylbenzene standards prepared; shifts are the δ values in $CDCl_3$

Methyl substitution pattern[a]	Ar–H	Ar–H[b]	Ar–CH_3	Ar–CH_2^-	–CH_3
Diaryl isoprenoid **3**					
2,3,6	s, 2 H, 6.90	d, 1 H, 7.5 Hz, 6.96 d, 1 H, 7.5 Hz, 6.93	s, 3 H, 2.28 s, 3 H, 2.24 s, 3 H, 2.21	m, 2.64	d, 6.6 Hz, 0.98[c]
3,4,5	s, 2 H 6.83	s, 2 H, 6.91	s, 6 H, 2.26 s, 3 H, 2.13	m, 2.56	d, 6.4 Hz, 0.92[c]
Ethyltrimethylbenzenes					
2,4,6	s, 2 H, 6.83	s, 2 H, 6.78[d]	s, 6 H, 2.29 s, 3 H, 2.24	q, 2 H, 7.5 Hz, 2.62	t, 3 H, 7.5 Hz, 1.10
3,4,5,	s, 2 H, 6.85	s, 2 H, 6.83	s, 6 H, 2.27 s, 3 H, 2.14	q, 2 H, 7.6 Hz, 2.55	t, 3 H, 7.6 Hz, 1.21
2,4,5	s, 1 H, 6.92 s, 1 H, 6.91	d, 1 H, 0.2 Hz, 6.90[d] d, 1 H, 0.2 Hz, 6.86[d]	s, 3 H, 2.24 s, 3 H, 2.21 s, 3 H, 2.20	q, 2 H, 7.6 Hz, 2.56	t, 3 H, 7.6 Hz, 1.18
2,3,5	s, 2 H, 6.84	d, 1 H, 1.9 Hz, 6.83[d] d, 1 H, 1.9 Hz, 6.80[d]	s, 3 H, 2.26 s, 3 H, 2.25 s, 3 H, 2.16	q, 2 H, 7.5 Hz, 2.61	t, 3 H, 7.5 Hz, 1.18
2,3,6	s, 2 H, 6.90	d, 1 H, 8.1 Hz, 6.93[d] d, 1 H, 7.5 Hz, 6.91[d]	s, 3 H, 2.29 s, 3 H, 2.25 s, 3 H, 2.22	q, 2 H, 7.5 Hz, 2.68	t, 3 H, 7.5 Hz, 1.11
2,3,4	d, 1 H, 7.9 Hz, 6.95 d, 1 H, 7.7 Hz, 6.92	d, 1 H, 8.1 Hz, 6.96 d, 1 H, 7.9 Hz, 6.93	s, 3 H, 2.26 s, 3 H, 2.22 s, 3 H, 2.19	q, 2 H, 7.5 Hz, 2.63	t, 3 H, 7.5 Hz, 1.18

[a] For reasons of convenience to the reader the numbering of the methyl substitution pattern has been chosen relative to the alkyl chain. [b] Shifts are δ values of aromatic protons in [$^2H_6$]benzene. [c] Shifts are δ values of isoprenoid methylgroups nearby the aromatic ring. [d] Coupling constants of aromatic protons with ethyl group decoupled.



1



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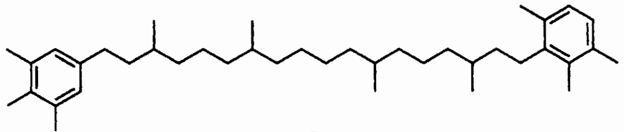
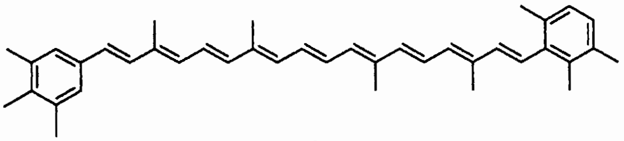


3



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McLafferty rearrangement, resulting in an increase of the relative intensity of *m/z* 134. This effect is, however, mediated by the presence of the 2,3,6-substituted aromatic ring in the molecule. In agreement with the GC retention behaviour of 1-ethyl-3,4,5-trimethylbenzene,[3] which elutes before 1-ethyl-2,3,6-trimethylbenzene, component **3** elutes before isorenieratane **1**, possessing a 2,3,6-/2,3,6- aromatic substitution pattern.

The carbon skeleton of **3** is comparable to that of isorenieratane **1** and renieratane **2**, which have been reported to be derived form the diaromatic carotenoids isorenieratene and renieratene. Therefore, it is proposed that **3** originates from diaromatic carotenoid **4** with the unprecedented 3,4,5,-trimethyl substitution pattern. An origin from other diaromatic carotenoids, involving shifs of methyl groups on the aromatic ring, is deemed unlikely since no other structural isomers are encountered in the sedimentary rock extract. Isorenieratene is a specific carotenoid biosynthesized by photosynthetic green sulfur bacteria.[6] These obligatory anaerobic organisms have a distinctive biochemistry leading to a biomass enriched in ^{13}C.[7] Indeed, isorenieratane **1** from sediments and oils from the Western Canada and Williston Basins was found to be enriched in ^{13}C corroborating its origin from photosynthetic green sulfur bacteria.[3] A similar carbon isotope anomaly was observed for **3**,[3] suggesting that the novel diaromatic carotenoid **4** is also derived from photosynthetic sulfur bacteria, although it has not been reported to occur in contemporary species of these bacteria nor in other organisms.

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