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Occurrence of Precursors of Regular Tricyclopolyprenoids in Recent Sediments

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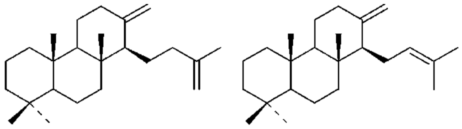
ABSTRACT



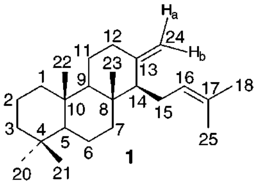
Two novel C_{24} tricyclic alkenes of the cheilanthane series have been isolated from the solvent extract of a sulfur-rich recent sediment and their structures determined by NMR studies. They represent the first example of functionalized tricyclic hydrocarbons structurally related to the tricyclopolyprenanes widespread in ancient sediments and petroleum identified in recent sediments.

Despite the ubiquity of extended series ($C_{19}-C_{45}$) of tricyclopolyprenanes in the geosphere,[1] the nature of their functionalized precursor molecules and, hence, of their biological precursors has never been identified clearly in the biosphere, and only a very few living organisms (i.e. ferns, marine sponges, and nudibranchs) are known to contain tricyclic terpenoids formed by cyclization of regular polyprenoids.[2] These sedimentary tricyclic hydrocarbons represent, therefore, one of the most intriguing examples of orphan molecular fossils.

We report here on the identification by NMR studies of two C_{24} tricyclic diunsaturated norsesterterpenoid hydrocarbons of the cheilanthane series which have been isolated from the solvent extract of a recent organic-rich and sulfur-rich sediment from Lake Cadagno (near Andermatt, Switzerland).

A coupled gas chromatography−mass spectrometry (GC-MS) investigation of the conventional “aromatic” hydrocarbon fraction from a surface sediment of Lake Cadagno revealed the presence of two predominant compounds with unknown mass spectra characterized by a molecular ion at m/z 328 ($C_{24}H_{40}$) and a prominent fragment at m/z 191 indicative of a polycyclic terpenoid structure.

Further fractionation of the hydrocarbons using reverse-phase HPLC and argentation TLC allowed isolation of the pure compounds **1** and **2** (ca. 1 mg of each, isolated from ca. 25 g of total solvent extract).



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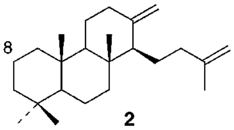

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First, structural information was obtained by catalytic hydrogenation (H_2, PtO_2, AcOEt) of an aliquot of **1** and **2**, which yielded, in both cases, the same isomeric pair of tricyclic hydrocarbons (M^+ at m/z 332) in a 1:3 ratio. Both hydrogenated products had identical mass spectra, and it could be determined by GC coelution experiments and by mass spectral comparison with a synthesized standard[3] that the first-eluting compound corresponded to 19-*nor*-13βH,14αH-

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[1] See for example: (a) Moldowan, J. M.; Seifert, W. K.; Gallegos, E. J. *Geochim. Cosmochim. Acta* **1983**, *47*, 1531−1535. (b) De Grande, S. M. B.; Aquino Neto, F. R.; Mello, M. R. *Org. Geochem*. **1993**, *20*, 1039−1047.

[2] Hanson, J. R. *Nat. Prod. Rep*. **1992**, *2*, 481−489.

[3] Heissler, D.; Ocampo, R.; Albrecht, P.; Riehl, J. J.; Ourisson, G. *J. Chem. Soc., Chem. Commun.* **1984**, 496−498.

[4] Chicarelli, M. I.; Aquino Neto, F. R.; Albrecht, P. *Geochim. Cosmochim. Acta* **1988**, *52*, 1955−1959.

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cheilanthane. On the basis of its relative retention time, the structure of the second eluting isomer was consistent with 19-*nor*-13αH,14αH-cheilanthane.[4]

To further characterize the structure of the terpadienes, 1D and 2D homonuclear (COSY, NOESY) and heteronuclear (HSQC, HMBC) NMR studies were carried out on compound **1** (Table 1), which allowed the location of the $\Delta^{13(24)}$ and Δ^{16} double bonds to be assigned unambiguously. The 19-*nor*,14αH-cheilantha-13(24),16-diene structure of compound **1** was deduced from the presence of six methyl, nine methylene, four methine, and five quaternary atoms, as well as from the ^{1}H−^{1}H COSY and carbon connectivity established by ^{1}H−^{13}C correlation experiments. In the ^{1}H NMR spectrum, four of the six methyl groups appear in the high-field range, corresponding to the methyl groups of the tricyclic moiety of the hydrocarbon skeleton, and the two remaining methyl groups are more deshielded (i.e. vinylic methyl group). Furthermore, the presence of two double bonds is indicated by the low-field resonances of one vinylic proton (H-16) and two exocyclic methylene protons (H-24_a and H-24_b; $J_{24a/24b}$ = 1.7 Hz). The *all-trans* and 14αH stereochemistry could be determined, given the presence of nuclear Overhauser effects for H-5/H-9, H-9/H-14, 22-CH_3/23-CH_3, H-24_a/H-12β, and H-24_b/H-16.[5]

Table 1. ^{13}C and ^{1}H NMR Assignments for 19-*nor*,14αH-Cheilantha-13(24),16-diene **1** (125.8 and 500.1 MHz, $CDCl_3$, 300 K)

C	$\delta(^{13}C)$, ppm	$\delta(^{1}H)$, ppm		C	$\delta(^{13}C)$, ppm	$\delta(^{1}H)$, ppm	
1	40.09	1.66	0.81	13	148.82		
2	18.65	~1.6	~1.4	14	57.65	1.66	
3	42.00	1.36	1.13	15	22.50	2.20	1.96
4	33.23			16	125.06	5.04	
5	56.41	0.84		17	130.48		
6	19.10	1.58 (α)	1.36 (β)	18	25.74	1.65	
7	40.74	1.86 (β)	1.18 (α)	20	33.33	0.86	
8	39.80			21	21.43	0.80	
9	60.07	1.05		22	16.24	0.81	
10	37.82			23	15.31	0.70	
11	23.03	1.68 (α)	1.33 (β)	24	106.81	4.79 (a)	4.46 (b)
12	38.05	2.36 (β)	1.96 (α)	25	17.84	1.62	

With regard to compound **2**, most of the chemical shifts obtained in the ^{1}H NMR spectrum were identical with those of compound **1**. However, the presence of two additional signals in the high-field range (H-18_a, 4.68 ppm; H-18_b, 4.65 ppm) indicative of an exocyclic methylene group, the absence of the vinylic H-16 signal, and the presence of a single vinyl methyl group (25-CH_3, 1.72 ppm) indicated a 19-*nor*,14α-(H)-cheilantha-13(24),17-diene structure for compound **2**.

The compounds **1** and **2** identified in the present study represent the first report of sedimentary functionalized tricyclopolyprenoids $>C_{20}$ in recent sediments, and the structures of their hydrocarbon skeletons indicate that they likely result from the enzyme-mediated cyclization of the acyclic regular pentaprenol **3** (Figure 1).[6] Following cyclization, the tricyclic alcohol **4** might have undergone further transformation(s) upon diagenesis, including side-chain shortening by oxidation and decarboxylation of the terminal carbon of the side chain and double-bond migration, leading finally to the tricyclic alkenes **1** and **2**.

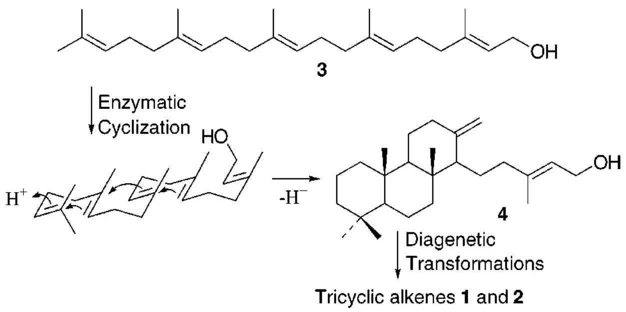

Figure 1. Possible mode of formation for compounds **1** and **2**.

Given the structural similarities between compounds **1** and **2**, it might be anticipated that they (or their functionalized precursor molecules) were biosynthesized by a common organism. However, analysis by coupled gas chromatography−isotope ratio monitoring mass spectrometry (GC-irmMS) of the “aromatic” fraction from the solvent extract of Lake Cadagno sediments revealed that compounds **1** and **2** have significantly distinct stable carbon isotopic values ($\delta(^{13}C)$), which differ by ca. 10‰ ($\delta(^{13}C)$ of −30‰ and −40‰ for compounds **1** and **2**, respectively), indicating a different biological origin.[7] In Lake Cadagno, ^{13}C-depleted values between −40‰ and −45‰ are found for components biosynthesized by purple sulfur photosynthetic bacteria (*Chromatiaceae*),[8] suggesting that compound **2** originates from such organisms. On the other hand, lipids from green sulfur photosynthetic bacteria (*Chlorobiaceae*) have $\delta(^{13}C)$ of ca. −27‰, which is 3‰ more enriched in ^{13}C relative to compound **1**. However, since upon diagenesis compound **1** can partly originate from compound **2** via migration of the Δ^{17} double bond into the more substituted Δ^{16} position, it is possible that the $\delta(^{13}C)$ value measured for compound **1** corresponds to a mixed value, and the original $\delta(^{13}C)$ value of compound **1** might therefore have been more ^{13}C-enriched, falling in the same range as the values typical for lipids from *Chlorobiaceae*. Consequently, although further work is required to verify this hypothesis, it can be proposed that compounds **1** and **2** may represent novel biological markers for green and purple sulfur photosynthetic bacteria, at least in the sediments from Lake Cadagno.

Since the first discovery of cheilanthatriol in a fern,[9] the structure of only very few compounds related to the cheilanthane series and having a hydrocarbon skeleton with more than 20 carbon atoms (i.e. luteone,[10] 17(*Z*)-cheilantha-

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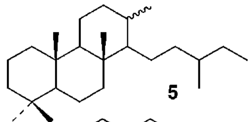
(5) Since the configuration assigned is relative, the structures are arbitrarily represented with the same configuration as luteone (see ref 8).

(6) Schaeffer, P.; Poinsot, J.; Hauke, V.; Adam, P.; Wehrung, P.; Trendel, J. M.; Albrecht, P.; Dessort, D.; Connan, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *11*, 1166−1169.

13(24),17-diene-6α,19-diol, and 17(*Z*)-cheilantha-13(24),17-diene-1*β*,6α,19-triol[11]) have been characterized among the constituents of living organisms.

Unlike the tricyclic norsesterterpenoids **1** and **2**, these compounds bear oxygenated functionalities on the rings A and/or B of the polycyclic moiety of the hydrocarbon skeleton, and it seems therefore improbable that they represent the functionalized precursors of compounds **1** and **2** and, hence, that they derive from such biological precursors. Notably, although the $\delta(^{13}C)$ values determined for lipids of higher plant origin in Lake Cadagno (i.e. between −29‰ and −32‰) are close to the $\delta(^{13}C)$ value of compound **1** (see above), an origin from higher plants can be ruled out on the basis of structural grounds.

It can thus be proposed that they originate from different precursor molecules not functionalized on rings A and B. In this respect, tricyclic terpanes derived from the cyclization of regular polyprenoids are commonly found as homologous series ($C_{19}-C_{45}$) of saturated hydrocarbons[12] (e.g. **5**), aromatic hydrocarbons[13] (**6**), sulfides[14] (**7**), or carboxylic acids[15] (**8**) in ancient sediments and petroleum. It has been hypothesized that they could be formed by the diagenetic transformation of precursors resulting from the *trans-anti-trans* enzyme-mediated cyclization of polyprenols in an all-chair conformation,[16] similarly to the tricyclic alkenes **1** and **2** identified in the present study.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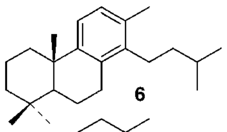
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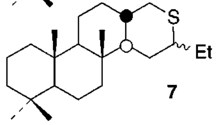

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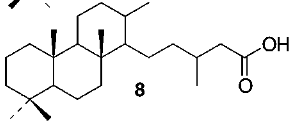

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Furthermore, among sedimentary compounds of the tricyclopolyprenane series, the occurrence of carboxylic acids (e.g. **8**) and sulfides (**7**) is indicative of precursors functionalized on the side chain and at C-24, respectively, whereas there is no evidence thus far for the presence of ring A and/or ring B functionalized molecules within sedimentary lipids.

It therefore appears that the precursor molecule(s) of the widespread sedimentary tricyclopolyprenane series and of the two cheilanthane derivatives **1** and **2**, although still unknown, may be structurally close and may be formed by the enzymatic cyclization of common functionalized precursors (i.e. regular polyprenols) via similar processes. It is, however, striking that lower or higher homologues of **1** and **2** could not be detected among the lipids from Lake Cadagno sediments. Further work, including a detailed lipid investigation of cultured green and purple sulfur photosynthetic bacteria, is currently underway in order to determine the possible presence of tricyclic derivatives in these organisms.

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(7) The stable carbon isotopic composition of lipids depends on the $\delta(^{13}C)$ value of the carbon source(s) and on the biosynthetic pathways used by living organisms for carbon fixation. The $\delta(^{13}C)$ values of individual components have been determined by GC-irmMS and make it possible to establish links between sedimentary lipids and their biological precursors.

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(12) See e.g.: (a) Aquino Neto, F. R.; Restlé, A.; Connan, J.; Albrecht, P.; Ourisson, G. *Tetrahedron Lett.* **1982**, *23*, 2027−2030. (b) Ekweozor, C. M.; Strausz, O. P. In *Advances in Organic Geochemistry*; Bjorøy, M. et al., Eds.; Wiley: Chichester, U.K., 1981; pp 746−766. (c) Aquino Neto, F. R.; Trendel, J. M.; Restlé, A.; Connan, J.; Albrecht, P. In *Advances in Organic Geochemistry*; Bjorøy, M. et al., Eds.; Wiley: Chichester, U.K., 1981; pp 659−667.

(13) See e.g.: (a) Azevedo, D. A.; Aquino Neto, F. R.; Simoneit, B. R. T.; Pinto, A. C. *Org. Geochem.* **1992**, *18*, 9−16. (b) Azevedo, D. A.; Aquino Neto, F. R.; Simoneit, B. R. T. *Org. Geochem.* **1994**, *22*, 991−1004. (c) Boreham, C. J.; Wilkins, A. L. *Org. Geochem.* **1995**, *23*, 461−466.

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