PERGAMON

Organic Geochemistry 30 (1999) 379–383

Organic Geochemistry

Note

17(*E*)-13α(*H*)-Malabarica-14(27),17,21-triene, an unexpected tricyclic hydrocarbon in sediments

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Received 8 January 1999; accepted 28 January 1999

(Returned to author for revision 21 January 1999)

Abstract

17(*E*)-13α(*H*)-malabarica-14(27),17,21-triene has been isolated from the solvent extract of a recent sediment from a sulfur-rich meromictic lake (Lake Cadagno, Switzerland) and its structure unambiguously established by NMR studies. This tricyclic triterpenoid, with a rare hydrocarbon skeleton, may originate from algal or bacterial biological precursors which remain to be discovered. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: 17(*E*)-13α(*H*)-Malabarica-14(27),17,21-triene; Tricyclic triterpenoids; NMR identification; Sulfur-rich environments; Lake Cadagno

Tetra- and pentacyclic triterpenoids derived from the enzymatic cyclization of squalene or epoxysqualene are widespread in both eu- and pro-caryotes and contribute to a large extent to the lipids accumulated in the sediments after the decay of living organisms. For example, Ourisson and Albrecht (1992) have estimated that the fossilized hydrocarbon skeletons of the pentacyclic hopanoids biosynthesized by bacteria may represent the most abundant natural products on earth. Similarly, the tetracyclic hydrocarbon skeletons of steroids, which occur in all eucaryotes, can be detected among the lipids from the organic matter of nearly all sediments. Biosynthetic derivatives of squalene with a tricyclic hydrocarbon skeleton are, however, restricted in living organisms to only a few examples. The tricyclic hydrocarbon skeleton of malabaricane has indeed been identified in the form of ketone, alcohol or epoxide derivatives in higher plants such as *Ailathus malabarica*, *Pistacia lentiscus* and *Pyrethrum santolinoides* (Paton et al., 1979; Marner et al., 1991; Jakupovic et al., 1987), in some ferns (Ageta et al., 1982; Masuda et al., 1989) and possibly in the marine sponge *Japsis stellifera* (Ravi et al., 1981; Ravi and Wells, 1982); however, in the latter case, according to McCabe et al. (1982), the triterpenoids described by Ravi and co-workers could rather correspond to isomalabaricane derivatives. To the best of our knowledge, malabaricane derivatives—or their diagenetic transformation products—have, however, never been identified so far in sediments.

We report here the identification of the tricyclic alkene **1**, which has been identified by NMR studies after isolation from the solvent extract of a recent (< 200y) sediment from the meromictic Lake Cadagno (for a detailed description of the geology and biogeochemistry of Lake Cadagno, see Bernasconi and Hanselmann, 1995; Züllig, 1985; Putschew et al., 1996).

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PII: S0146-6380(99)00010-8

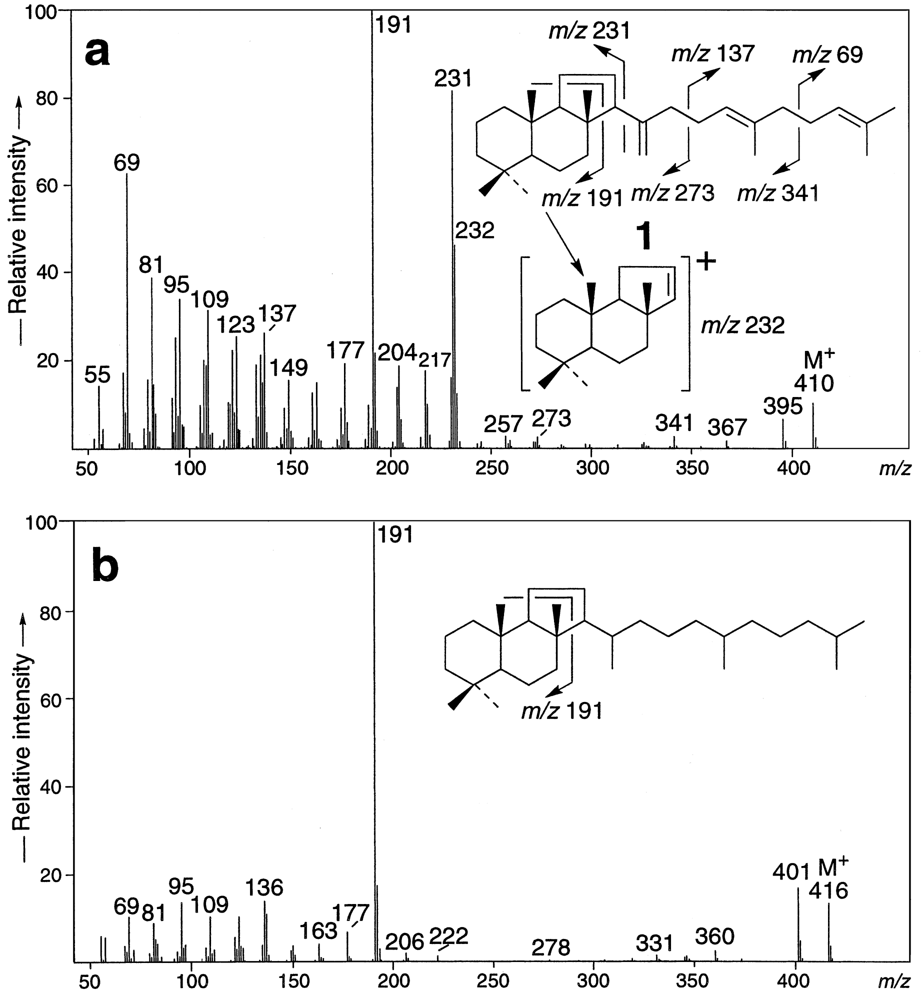
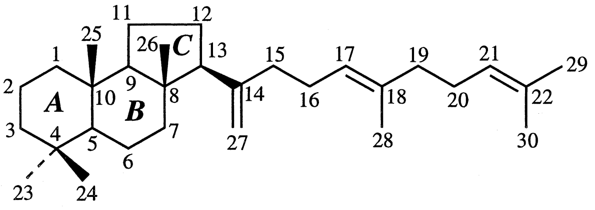
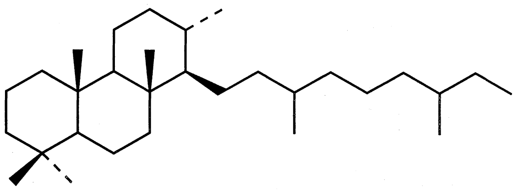


Fig. 1. Mass spectra of (a) 17(*E*)-13α(*H*)-malabarica-14(27),17,21-triene **1** and (b) of the major tricyclic alkane obtained by catalytic hydrogenation of **1**. Conditions; Finnigan MAT TSQ 700, EI, 70 eV.



(17E)-13α(H)-malabarica-14(27),17,21-triene **1**



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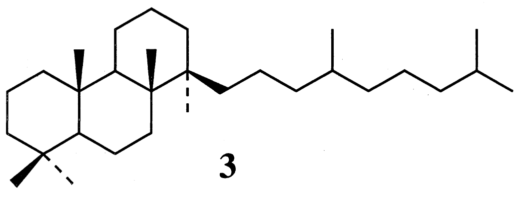

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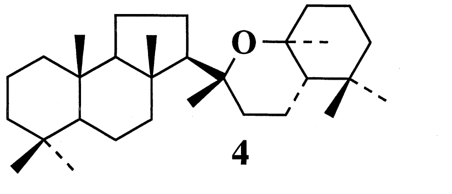

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Silica gel liquid chromatography of the solvent extract (acetone, ×3; CH_2Cl_2/MeOH 3:1 v/v, ×4; ca. 30 g of extract recovered) of a superficial sediment from Lake Cadagno yielded an apolar fraction (ca. 3% yield after removal of S_0, elution with a 4:1 v/v mixture of hexane/CH_2Cl_2), which was further fractionated (silica gel, elution with hexane) into an (alkane + alkene) fraction (68% yield) and a more polar fraction containing aromatic hydrocarbons and polyunsaturated alkenes (14% yield). Coupled gas chromatography–mass spectrometry (GC–MS) of the latter fraction revealed the presence of several C_{30} polyunsaturated isoprenoids, including squalene and a minor hydrocarbon with the same molecular ion at *m/z* 410 corresponding to the molecular formula $C_{30}H_{50}$. Its mass spectrum (Fig. 1a) shows intense fragments at *m/z* 191 and 231, suggesting a polycyclic hydrocarbon skeleton. Further structural information was obtained by catalytic hydrogenation (H_2/PtO_2, ethyl acetate) of an aliquot of pure compound **1** which was isolated after sub-fractionation of the (aromatic + polyunsaturated hydrocarbon fraction) using liquid chromatography (silica gel, hexane), followed by reverse phase HPLC separation (Zorbax ODS, 250 mm × 4.6 mm; 5 μm; acetone/methanol 1:1; Vydac C_{18}, 250 mm×4.6 mm; 5 μm; methanol) of the sub-fractions enriched in compound **1**. Following hydrogenation, an isomeric mixture of two tricyclic hydrocarbons with almost identical mass spectra was obtained (Fig. 1b). These spectra are also very similar to the spectra of authentic standards of the C_{30} tricyclopolyprenane **2** which is widespread in geological samples (Heissler et al., 1984) and of the tricyclic hydrocarbon **3** obtained by a Wolf-Kishner reduction of (20ξ)-13,17-secodammarane-13,17-dione (Aquino Neto et al., 1986). However, as shown by GC coelution experiments on an apolar capillary column (DB5, J&W, 30 m×0.5 mm; 0.1 μm film thickness), neither **2**, nor **3** had the same retention time as the hydrogenated hydrocarbons.

Finally, compound **1** was unambiguously identified as 17(*E*)-13α(*H*)-malabarica-14(27),17,21-triene **1** by 1D and 2D homonuclear (COSY, NOESY) and heteronuclear (HSQC and HMBC) NMR studies (Table 1) performed on ca. 1 mg of isolated hydrocarbon.

Table 1
^{13}C (125.8 MHz) and ^{1}H (500.1 MHz) NMR data for 17(*E*)-13α(*H*)-malabarica-14(27),17,21-triene **1** (Bruker ARX 500; in $CDCl_3$; δ relative to TMS)

C	δ ^{13}C ppm	δ ^{1}H ppm	
1	40.01	1.42 (β)	0.88 (α)
2	19.46^{a}	1.63 (β)	1.40 (α)
3	42.51	1.37 (β)	1.14 (α)
4	32.99		
5	57.43	0.83	
6	18.35	1.55 (α)	1.37 (β)
7	41.07	1.77 (β)	1.23 (α)
8	43.75		
9	63.25	1.26	
10	37.01		
11	19.45^{a}	1.48	1.27
12	25.29	~1.63	1.75
13	57.12	2.04	
14	149.39		
15	37.55	2.04	1.95
16	26.66^{b}	2.0–2.1	2.0–2.1
17	124.33^{c}	5.11	
18	134.94		
19	39.68	1.97	1.97
20	26.94^{b}	2.0–2.1	2.0–2.1
21	124.24^{c}	5.09	
22	131.29		
23	33.65	0.84	
24	21.46	0.81	
25	15.35	0.84	
26	15.07	0.64	
27	109.82	4.89 (b)	4.73 (a)
28	16.03	1.60	
29	17.69	1.60	
30	25.71	1.68	

$^{a–c}$ Assignments interchangeable.

In particular, the nature of the polycyclic hydrocarbon skeleton, containing two six-membered rings (rings A and B) and one five-membered ring (ring C), could be established given the presence of seven methyl, twelve methylene, five methine and six quaternary carbon atoms, as well as from the COSY and ^{1}H–^{13}C correlation experiments and from the carbon connectivity deduced from the HMBC experiment. The presence of nuclear Overhauser effects (Fig. 2) between

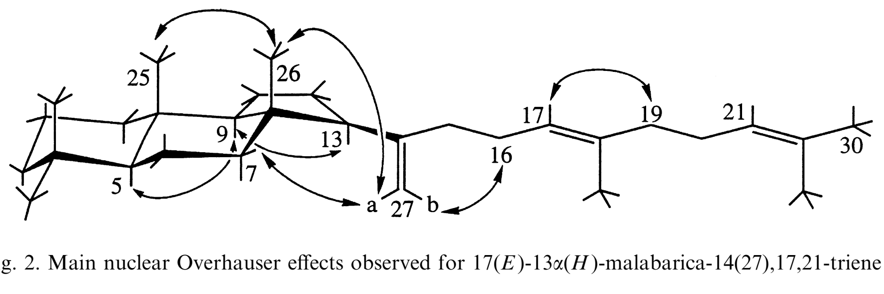

Fig. 2. Main nuclear Overhauser effects observed for 17(*E*)-13α(*H*)-malabarica-14(27),17,21-triene **1**.

H-5/H-9, H-9/H-13 and 25-CH_3/26-CH_3 allowed us to determine the all-*trans* stereochemistry of the polycyclic ring skeleton, and nuclear Overhauser effects between H-17 and H-19 (Fig. 2) indicate that the Δ^{17} double bond is *E*. In addition, the position of the exomethylene group on C-14 is indicated by the presence of nuclear Overhauser effects between H-27a/H-7β, H-27a/26-CH_3 and H-27b/H-16 (Fig. 2). Furthermore, the formation of predominantly 2 isomers upon H_2/PtO_2 hydrogenation (see above) is in agreement with the presence of a double bond in position 14 (i.e. formation of 14*R* and 14 *S* isomers).

Since the first identification of triterpenes with the malabaricane hydrocarbon skeleton in the trunk of *Ailanthus malabarica* by Chawla and Dev (1967), several species of higher plants, ferns and possibly marine sponges have been shown to contain malabaricane derivatives which occur with oxygenated functionalities. There is, however, only one example of a living organism where hydrocarbons of the malabaricane series, namely 13β(*H*)-malabaricatriene and 13α(*H*)-malabaricatriene **1** (ratio 13β/13α = 7.5), have been identified after isolation from the fern *Lemnaphyllum microphyllum* var. *obovatum*. (Masuda et al., 1989). Regarding the possible biological source(s) for 13α(*H*)-malabaricatriene **1** detected in sediments, the stable carbon isotopic composition of compound **1** present in Lake Cadagno sediment ($\delta^{13}C \sim -33‰$) falls in the same range as the $\delta^{13}C$ values determined for (phyto)planktonic lipids ($\delta^{13}C$ between −33 and −35‰), but is also close to the values measured for higher plant *n*-alkanes ($\delta^{13}C$ between −31 and −33‰). An origin from the latter source seems, however, rather unlikely for the following reasons. Indeed, except for colysanoxide **4** which has been identified in the ferns *Colysis elliptica* and *C. pothifolia* (Ageta et al., 1982), the 13β(*H*) configuration is the sole or the predominant configuration found for malabaricane derivatives in living organisms; furthermore, 13β(*H*)-malabaricatriene, which has a mass spectrum clearly distinct from that of the 13α(*H*) isomer **1** (i.e. base peak at *m*/*z* 231 instead of 191; Masuda et al., 1989) could not be detected in the Lake Cadagno extract. Moreover, a compound with a mass spectrum identical to that of 17(*E*)-13α(*H*)-malabarica-14(27),17,21-triene **1** was detected among the major unsaturated hydrocarbons of marine sediments from the Cariaco basin (Wakeham, 1990) and among the apolar lipids of anoxic surface sediments from the Arabian Sea (Sinninghe Damsté, unpublished results). The possible presence of malabaricatriene(s)—these identifications being based on mass spectral comparison—in these two geological settings, where the biomarker signature is dominated by compounds of predominantly algal/bacterial origin and where the contribution of higher plant derivatives is of only minor importance, also suggests that the biological precursor(s) of the malabaricatriene(s) found in sediments are not ferns. In this respect, it is also noteworthy that Lake Cadagno, the Cariaco basin and the Arabian Sea are sulfur-rich ecosystems characterized by the presence of anoxic bottom waters and sediments. We therefore propose that the hydrocarbon skeleton of malabaricatriene found in these sediments may originate from an unknown, but possibly widespread, biological source able to thrive in particular ecosystems (i.e. highly anoxic and rich in reduced sulfur species) and which remains to be discovered.

Acknowledgements

We thank E. Mastio and P. Wehrung for the GC-MS and GC-irmMS analyses, R. Graff for the NMR measurements, J. Blanzat for his contribution to the sampling of the sediment from Lake Cadagno and P. Adam for helpful discussions. Prof. J.R. Maxwell is thanked for the review of this manuscript.

Associate Editor—**A.G. Douglas**

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