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LETTERS

A sedimentary tetrahydrophenanthrene derivative of tetrahymanol

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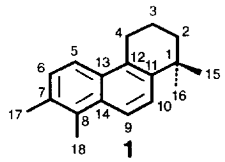
Abstract: A novel tetrahydrophenanthrene derivative **1** (1,1,7,8-tetramethyl-1,2,3,4-tetrahydrophenanthrene), formed from the triterpenoid tetrahymanol **2a** by dehydration, ring A and B degradation and aromatisation during sediment diagenesis, has been isolated from a 180 Ma old marine sedimentary rock and identified by ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry. © 1999 Elsevier Science Ltd. All rights reserved.

Aromatisation of triterpenoids and steroids during sediment diagenesis has been extensively studied.[1] In general, aromatisation starts in the ring containing the oxygenated function. For example, aromatisation in higher plant triterpenoids, such as lupeol and β- and α-amyrin starts in ring A and proceeds stepwise towards ring D.[2-4] Alternatively, the triterpenoids may initially undergo loss of ring A and subsequently aromatise from ring B to D. Accordingly, mono- to triaromatic de-A lupanes[3] (**3-4**), de-A oleananes[4] (**5-6**) and de-A ursanes[4d] have been identified in various sediments. Similarly, de-A monoaromatic steroids[5] (e.g. **7**) can be produced during diagenesis. Here we provide evidence that diagenesis of the triterpenoid tetrahymanol (**2a**) may lead to loss of both the A- and B-rings and aromatisation of the C- and D-rings with concomitant loss or transfer of angular



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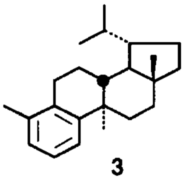

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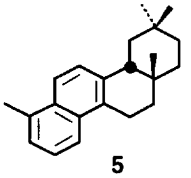

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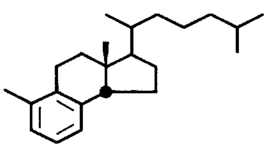

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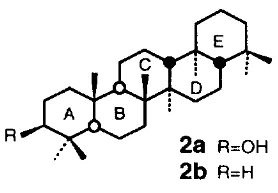
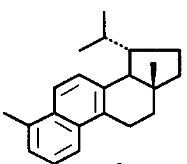


2a R=OH
2b R=H



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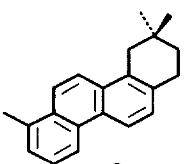

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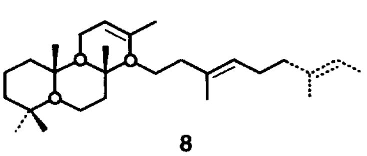

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Table 1. ^1H and ^{13}C data of 1,1,7,8-tetramethyl-1,2,3,4-tetrahydrophenanthrene (**1**).

C-number	H-shift	C-shift			
		CH_3	CH_2	CH	C
1	-				34.01
2	1.73 (m)		38.87		
3	1.94 (m)		19.58		
4	3.11 (t, J=6.5 Hz)		26.92		
5	7.77 (bd, J=8.7 & 0.8 Hz)			120.96	
6	7.30 (d, J=8.5 Hz)			128.83	
7	-				132.05
8	-				131.39
9	7.88 (bd, J=9.0 & 0.8 Hz)			121.72	
10	7.50 (d, J=9.1 Hz)			124.90	
11	-				141.13
12	-				130.98[a]
13	-				131.02[a]
14	-				130.87
15	1.36 (s)	31.43			
16	1.36 (s)	31.43			
17	2.46 (s)	20.54			
18	2.58 (s)	14.64			

[a] assignments may be interchanged

methyl groups.

During examination of the aromatic hydrocarbon fraction of solvent extracts of marlstones from the Lower Jurassic Allgäu Formation by GC-MS, we encountered an abundant aromatic component **1** with a mass spectrum[6] dominated by a molecular ion at *m/z* 238, a base peak at *m/z* 223, and fragment ions at *m/z* 208, 193, 179 and 123. This spectrum is identical to that of a C_4 alkylated tetrahydrophenanthrene tentatively identified in various sedimentary rocks.[7] To unequivocally establish the structure of **1**, it was isolated from the extract by column chromatography on alumina and subsequent preparative GC.[8] A fraction (0.9 mg) consisting of 85% of **1** (the other 15% consisted of a complex mixture of hydrocarbons, none representing more than 1% of the total fraction) was analysed by high field ^1H and ^{13}C NMR[9] (Table 1) which led to complete assignment of proton and carbon chemical shifts. Carbon multiplicities were established by an APT spectrum and revealed that **1** contains 18 carbon atoms with six aromatic and one aliphatic C_q, four aromatic CH, three CH_2 and four CH_3 units. The proton spectrum showed four aromatic protons, two CH_3 units attached to an aromatic system, one CH_2 attached

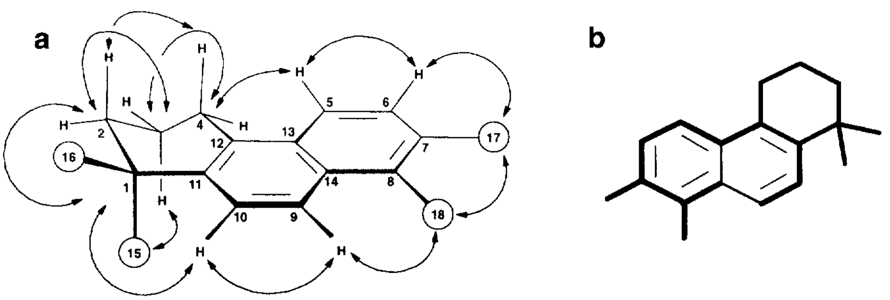


Fig. 1: NOESY and one dimensional NOE interactions (**a**) and connectivities from an inverse long range ^1H-^{13}C correlation experiments (**b**) for **1**.

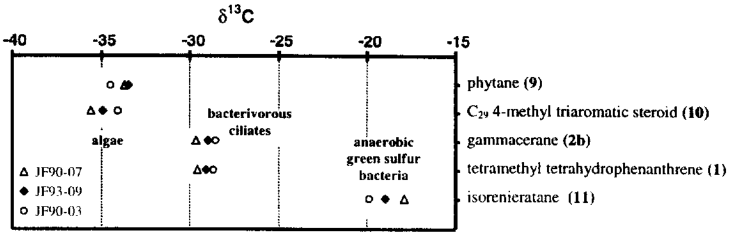
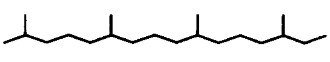


Fig. 2: Stable carbon isotopic compositions of tetramethyl tetrahydrophenantrene (**1**), gammacerane (**2b**), and other chemical fossils. Phytane (**9**) is derived from the side chain of algal chlorophyll-a,[1] the C_{29} 4-methyl triaromatic steroid (**10**) from dinosterol[14a] from dinoflaggelates, and isorenieratane (**11**) from isorenieratene,[14b] a diaromatic carotenoid of green sulfur bacteria.

to an aromatic ring system (represented by a triplet at δ=3.11 ppm), two aliphatic CH_2 groups, and two identical methyl groups attached to an aliphatic C_q (Table 1). This would indeed be in agreement with a C_4 alkylated 1,2,3,4-tetrahydrophenanthrene. ^1H-^1H COSY, long-range COSY, NOESY and one dimensional NOE experiments (Fig. 1a) and inverse ^1H-^{13}C correlation experiments (HMQC and HMBC; Fig. 1b) established the full structure of **1**.

Co-injection of **1** isolated from the Allgäu Formation marlstone with an aromatic hydrocarbon fraction of the Permian Tasmanite Oil Shale revealed that the C_4 alkylated tetrahydrophenanthrene tentatively identified in this shale[7a] is indeed **1**. Due to the dominance of saturated and monoaromatic tricyclic terpanes in the Tasmanite oil shale, the diaromatic tricylic terpenoid **1** was proposed to derive from aromatisation of tricyclopolyprenols **8**.[7a] This explanation was supported by the tentative identification of C_{17} and C_{19} homologues of **1**, which contained a hydrogen and an ethyl group at C-18, respectively. These homologues are absent in the marlstones from the Allgäu Formation. Other diagenetic derivatives of tricyclopolyprenols are present only in very low quantities. Therefore, it is likely that in these sediments **1** is derived from another precursor. We propose for various reasons that it is formed during sediment diagenesis from the triterpenoid tetrahymanol (**2a**). Firstly, gammacerane (**2b**), a well known diagenetic product of tetrahymanol,[10] is the most abundant saturated hydrocarbon, clearly indicating a significant input of tetrahymanol into the sediments. Secondly, the concentrations of gammacerane (**2b**) and **1** are positively correlated (r^2=0.90), suggesting that they derive from the same precursor. Thirdly, the $\delta^{13}C$ values[11] of **1** and gammacerane are identical and significantly different from the chemical fossils derived from algae and green sulfur bacteria (Fig. 2). Anaerobic ciliates, feeding predominantly on bacteria, biosynthesise tetrahymanol (**2a**) instead of sterols as membrane rigidifiers.[12] Therefore, tetrahymanol and its diagenetic products indicate the presence of bacterivorous ciliates.[10b-c] Their biosynthetic products are enriched in ^{13}C relative to those of algae since they probably fed in part on green sulfur bacteria, which are significantly enriched in ^{13}C since they fix carbon dioxide by the reversed tricarboxylic acid cycle.[10c] The close match of the carbon isotopic composition of gammacerane (**2b**) and **1** in three different samples thus provides circumstantial evidence that they both derive from bacterivorous ciliates.

At present it is not exactly clear how the conversion from tetrahymanol into **1** takes place. It is anticipated that this process starts by formation of gammacer-2-ene by dehydration of tetrahymanol.[10b] Subsequent aromatisation of ring A with concomitant opening of ring B will yield an intermediate compound,[13] which upon further aromatisation of ring C and D with simultaneous elimination of ring A and B and a methyl transfer



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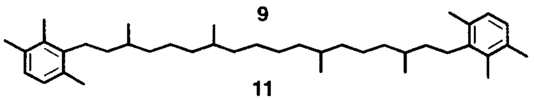

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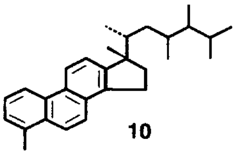

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reaction will render **1**. Confirmation of this novel pathway of triterpenoid diagenesis is required by the full structural identification of intermediate products.

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8. Preparative gas chromatography was performed on a HP 6890 gas chromatograph equipped with a Gerstel temperature programmable injector, a 25 m x 0.32 mm i.d. CP-Sil 5CB capillary column ($d_f = 0.52$ μm) and a Gerstel preparative fraction collection system cooled with a cryostatic bath at 10°C. Details of the trapping procedure have been described in Eglinton, T.I.; Aluwihare L.I., Bauer, J.E.; Druffel E.R.M.; McNichol A.P. *Anal. Chem.* **1996**, *68*, 904-912. 330 injections were performed to trap sufficient material.
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