A heptacyclic polyprenoid hydrocarbon in sediments: a clue to unprecedented biological lipids†

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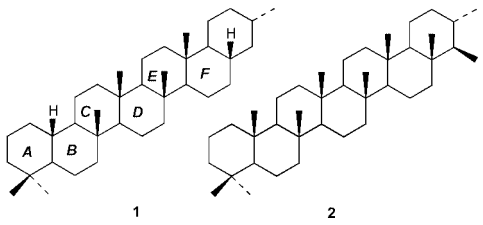
A novel heptacyclic C_{37} alkane presenting a regular polyprenoid structure has been isolated from a biodegraded bituminous rock and identified by NMR; this hydrocarbon is the most highly cyclized polyprenoid alkane ever completely identified; the absence of specific methyl groups give clues to unprecedented biological precursor lipids resulting from the extensive cyclization of regular octaprenoids.

Polycyclic hydrocarbons of high molecular weight (> C_{30}) with a regular polyprenic skeleton identified in sediments and petroleum were for a long time restricted to tricyclopolyprenanes.[1] Recently, the structures and the occurrence of highly cyclized compounds based on regular polyprenoid skeletons could be established in various sediments; they were essentially identified as monoaromatic hydrocarbons[2,3] and sulfides.[4] Their regular polyprenoid structure strongly suggests that the biological precursors from which these molecular fossils originate, most likely derive from proton-induced enzymatic cyclization of all-*trans* regular isoprenoids. Biological precursors have not been observed until now in living organisms and so these molecular fossils would appear to belong to a new class of biolipids whose origin and function remain unclear. More recently, investigation of the saturated hydrocarbon fraction of sediments from various origins revealed the occurrence of new sedimentary polycyclic alkanes (four to seven rings) which were suggested to have a complete regular polyprenoid structure according to mass spectral data.[2,5] We report here on the first unambiguous identification of a heptacyclic alkane **1**, which is related to this new series of saturated hydrocarbons.



1 **2**

The studied sample was collected from a quarry (Maestu, Spain) which consisted of a calcareous reservoir rock of Campanian age impregnated by a heavy, partially biodegraded, petroleum.[5a] The crushed rock sample was extensively extracted with $CHCl_3$–CH_3OH (1/1, v:v) and the resulting extract chromatographed (SiO_2 / hexane) yielding the saturated hydrocarbon fraction. The major part of the low molecular weight constituents of the alkane fraction was removed by precipitating successively the heavy saturated hydrocarbons, including the polycyclic polyprenoid hydrocarbons, with acetone. Consecutive reverse-phase HPLC fractionation performed on the precipitated fraction led to the isolation of *ca.* 1 mg of the heptacyclic compound **1** with a purity of 91% (GC).

The EIMS spectrum of product **1**‡ exhibits a molecular ion at *m/z* 506 corresponding to the formula $C_{37}H_{62}$. Two characteristic fragmentation patterns composed of two sets of major fragments regularly shifted by 68 Da (one isoprene unit) were observed (*m/z* 177, 245, 313 and *m/z* 135, 203, 271) and were consistent with a polycyclic polyterpenoid structure.

1D- and 2D-NMR studies including homonuclear (^{1}H–^{1}H, COSY and NOESY) and heteronuclear (^{1}H–^{13}C, HMQC and HMBC) correlation experiments allowed us to assign the signals of all the protons and all the carbon atoms (ESI)† and to establish unequivocally the structure of the isolated compound.

The ^{13}C NMR spectrum of **1** shows 37 resonances, resolved into 7 methyl, 16 methylene, 9 methine and 5 quaternary carbon signals (as deduced from DEPT spectra and ^{1}H–^{13}C correlation experiments). The ^{1}H NMR spectrum also reveals 7 methyl signals, including 6 singlets and one doublet. A part of the carbon skeleton [Fig. 1(b)] could be established using ^{1}H–^{13}C long-range ($^{2,3}J$) couplings, the most intense of which are observed from the methyl groups. Two of the methyls exhibit remote connections ($^{2,3}J$) to each other as well as to the same quaternary, methine and methylene carbons, which implies that they must be geminal. The sector formed by these geminal methyl groups (*i.e.* atoms 26, 30, 32 and 32′) appears to be isolated from the rest of the molecule. Indeed, C-26 does not show remote connections with any remaining methyl groups, thus suggesting the absence of the Me-28′ at C-27. The nuclear Overhauser effects (NOE) observed between H-27 and protons of both 32′- and 24′-methyl groups [Fig. 1(a)] confirm the presence of a hydrogen atom at C-27 instead of a methyl group.

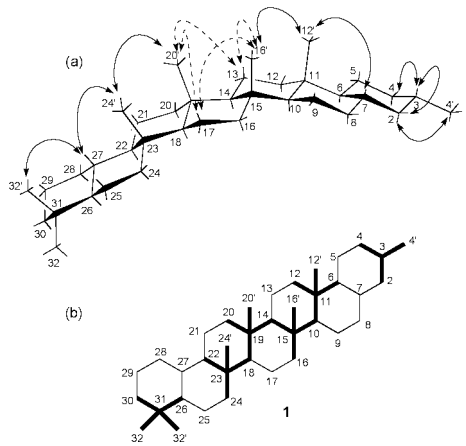


Fig. 1 (a) Spatial representation of **1** showing the most important NOEs observed. (b) Carbon sequence (bold) established from inverse long-range ^{1}H–^{13}C correlation experiment. Numbering is based on that of an acyclic octaprenol. The absolute configuration of compound **1** is not known and has been chosen arbitrarily.

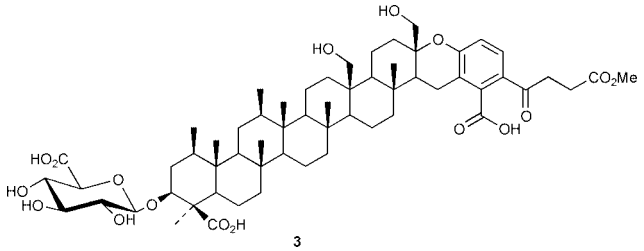
† Electronic supplementary information (ESI) available: ^{13}C and ^{1}H NMR data for hydrocarbon **1**. See http://www.rsc.org.suppdata/cc/b0/b001804j/

The central part of the molecule is constituted by the 12′, 16′, 20′ and 24′ methyl groups which are linked two by two to each other through one remote connection (^3J) with a methine (C-10, C-14 and C-18).

The remote connections of the last methyl group 4′ with the methylene (C-2 and C-4) and the methine (C-3) carbons confirm the position of substitution for this ring. This sequence Me-4′, C-2, C-3, C-4 also forms an isolated sector as demonstrated by the ^{1}H–^{13}C long-distance correlation experiment. However, linkage of this sector to the main structure, as well as final assignment of all remaining ^{1}H and ^{13}C chemical shifts was made possible through the COSY and NOESY experiments. Thus, the NOE observed between the proton H-7 and the protons of the 12′-methyl group demonstrates the lack of a methyl group (replaced by a H atom) on the ring junction at C-7. The ^{1}H–^{1}H COSY connectivities between H-7 and the protons of the methylene carbon C-2 confirm the sequence Me-4′, C-2, C-3, C-4 on the skeleton.

Stereochemical assignments can be made from the NOESY data: the NOEs observed between Me-32′/H-27, H-27/Me-24′, Me-24′/Me-20′, Me-16′/Me-12′, Me-12′/H-7 afford evidence that Me-32′, H-27, Me-24′, Me-20′ on one hand and Me-16′, Me-12′, H-7 on the other hand are on the same side of the molecule. Moreover, as no correlation between protons 7, 12′, 16′, 20′, 24′, 27 and protons 6, 10, 14, 18, 22, 26 could be detected on the NOESY spectrum, all the ring junctions are likely to be *trans* along the whole structure. As a result of all the NOEs reported in Fig. 1(a), both sectors comprising, respectively, Me-32′, H-27, Me-24′, Me-20′ and Me-16′, Me-12′, H-7, H-3 present *trans–transoid–trans* stereochemistries. Unfortunately, the determination of the global relative stereochemistry is hindered by the superposition of ^{1}H and ^{13}C chemical shifts of Me-16′ and Me-20′, as well as those of methylene groups C-13 and C-17. Thus, NOEs are indeed observed between the group of overlapping axial protons from methylene groups C-13 and/or C-17 and the overlapping methyl groups C-20′/C-16′ [indicated by dashed arrows on Fig. 1(a)] but individual interactions cannot be distinguished. This feature is not surprising in the case of highly symmetrical polycyclized regular polyprenoids since methyls 16′ and 20′, as well as methylenes 13 and 17, located in the 'middle' of the structure (*i.e.* far from demethylated positions which induce dissymmetry) have almost identical environments. The uncertainty concerning the global relative stereochemistry which arose from the interpretation of the NOESY spectrum could, however, be cleared by interpreting the chemical shift values. A *trans–transoid–trans* stereochemistry along the whole structure of compound **1** is indeed strongly supported by the underscoring of γ-*gauche* effects reflected by the chemical shifts of ^{1}H and ^{13}C proton and carbon signals.[6] In particular, the resonances of axial methylene protons Hβ-13 and Hβ-17 appear at high field (δ_H 1.35) and those of their related carbons at very low field (δ_C 17.4). This is concordant with triple γ-*gauche* interactions with the methyl groups. Furthermore, the strong deshielding of the methine carbon C-14 (δ_C 61.7), as well as the shielding of the corresponding proton (δ_H 0.73) can be taken as firm evidence for an absence of γ-*gauche* effects and thereby confirm the global relative stereochemistry with all the ring-junction axial methyl groups on the same side of the molecule. An equatorial position can reasonably be envisaged for methyl 4′ given the NOE between protons of Me-4′ and the axial proton located on C-2 and those between H-3 and H-4β, H-3 and H-2β.

From its structure, it appears that the new hydrocarbon **1**, which represents the most highly cyclized polyprenoid alkane ever identified, is likely to result from the diagenetic transformation of polycyclic polyprenoid precursors formed by proton induced cyclization of regular all-*trans* octaprenoids.[2] Indeed, the isolated compound definitely corresponds to a demethylated counterpart of a complete polycyclic octaprenoid hydrocarbon such as **2**. Until now, only one example of a polycyclic biological lipid **3** with more than four rings has been



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identified in a living organism (bacterium *Streptomyces argenteolus*).[7] This bacterium can probably not account solely for the large sedimentary record of highly cyclized polyprenoid structures.[2,4,5] Therefore, the biological organisms able to biosynthesize the precursors of **1** still remain to be discovered. A wider survey of the occurrence of these new polyprenoid hydrocarbons in sediments could eventually pin-point the type of actual natural environments likely to contain their source-organism(s) and orient further research for their discovery.

Some structural features of the unknown biological precursors can, however, already be inferred from the structure of the molecular fossil **1**. Thus, the absence of three methyl groups compared to a complete octaprenoid hydrocarbon suggests either that these methyl groups were absent within the biological precursor or, that these positions were functionalized (*e.g.* **3**) and susceptible to various diagenetic processes (*e.g.* by decarboxylation). It can, however, not be excluded, that functionalization of methyl groups on the precursor polyprenoid was induced by microbial oxidation processes occurring at the earliest stages of diagenesis.[8]

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Notes and references

‡ *MS data* (Finnigan MAT TSQ 700) EI (70 eV), *m/z* (rel. int.) for **1**: 506([M$^+$] $C_{37}H_{62}$, 59%), 491(14), 313(36), 271(7), 259(3), 245(36), 231(15), 217(4), 203(100), 190(8), 177(16), 163(22), 149(23), 135(33), 121(32), 109(57), 95(71), 81(59), 69(31), 55(28).

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