Synthesis and NMR characterisation of novel highly cyclised polyprenoid hydrocarbons from sediments

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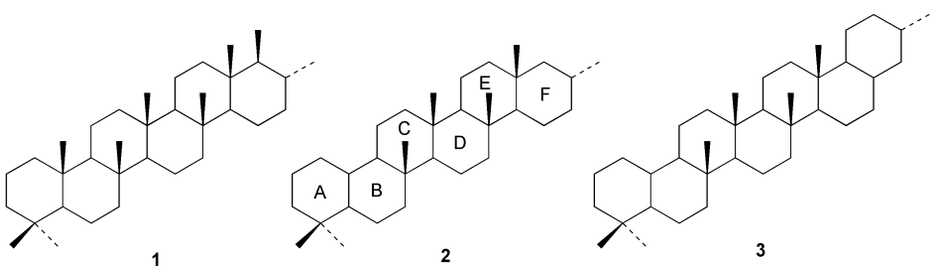
We report here on the identification of two novel hexacyclic alkanes (C_{33} and C_{35}) occurring in bitumen. The C_{35} compound **1** was identified by comparison with a standard obtained by synthesis involving a biomimetic proton-induced extensive cyclisation of an acyclic heptaprenoid. This cascade cyclisation allows the formation of eleven asymmetric centres present in the natural compound in only one step. The C_{33} analogue **2** was identified by NMR studies after isolation from the saturated hydrocarbon fraction of a bituminous rock. Both compounds are "orphan" molecular fossils of biological lipids of unknown origin formed by the extensive cyclisation of higher regular polyprenoids.

Introduction

Highly cyclised compounds based on regular polyprenoid skeletons have recently been shown to occur in various sediments as monoaromatic hydrocarbons[1,2] and sulfides.[3] The biological precursors from which these molecular fossils originate remain, however, to be discovered. According to their specific structure, these precursors were thought to derive from the proton-induced and/or enzymatic cyclisation of all-*trans* regular isoprenoids. The possible presence in the sedimentary record of aliphatic hydrocarbons structurally related to these compounds,[1,4] as suggested by mass spectrometry analyses, was recently confirmed by the identification of a C_{37} heptacyclic alkane **3** isolated from a bituminous rock from a Spanish quarry.[5] Mass spectral investigation of the saturated hydrocarbon fraction from the same sample further revealed an extended series of related polycyclic polyprenoid alkanes, which may be either complete polyprenoids or dealkylated analogues. These assumptions have now been confirmed by unambiguous identification of two polycyclic alkanes belonging to this series (**1**, **2**). The hexacyclic C_{35} compound **1** was characterised by comparison with a standard obtained by synthesis involving the biomimetic proton-induced cyclisation of an acyclic regular heptaprenoid. A lower C_{33} homologue **2** was isolated from the saturated hydrocarbon fraction of a bituminous rock from a Spanish quarry and identified by NMR studies.

Results

Gas chromatography–mass spectrometry (GC-MS) investigation of the saturated hydrocarbon fraction of bituminous rocks from the Aquitaine basin (Lameignère quarry; France)[6] revealed the presence of a series of high molecular weight polycyclic hydrocarbons possessing up to 8 rings. These saturated hydrocarbons display characteristic and information-rich mass spectra, showing two main series of fragments, each regularly shifted by 68 Da. As the shift of 68 Da was likely to correspond to an isoprene unit (C_5H_8), these compounds were thought to have regular polycyclic polyprenic structures. Thus, considering the mass spectra of the hexacyclic compounds, which constitute the main series, one can observe a common fragmentation pattern showing fragments at *m*/*z* 191, 259, 327 and 395 which remain unchanged within the series, whereas a second fragmentation pattern (*m*/*z* 149+14*n*, 217+14*n*, 285+14*n*, $n = 0$ for C_{34}) was found to depend on the molecular mass of the considered compound. The structure proposed for the hexacyclic compounds, as illustrated in Fig. 1(a), can easily account for the two fragmentation patterns observed: the first set of ions (*m*/*z* 191, 259, 327 and 395) can result from the fragmentation of the left part of the structures whereas the second set (*m*/*z* 149+14*n*, 217+14*n*, 285+14*n*) increases with the length of the side chain and can originate from the fragmentation of the right part of the structure. Based on these hypotheses, structure **1** was ascribed to the hexacyclic C_{35} ($n = 1$, Fig. 1(a)) homologue occurring in Lameignère bitumen. As this compound was present in the complex bitumen fractions in amounts too small to allow isolation and characterisation by NMR, synthesis of a standard has been carried out.



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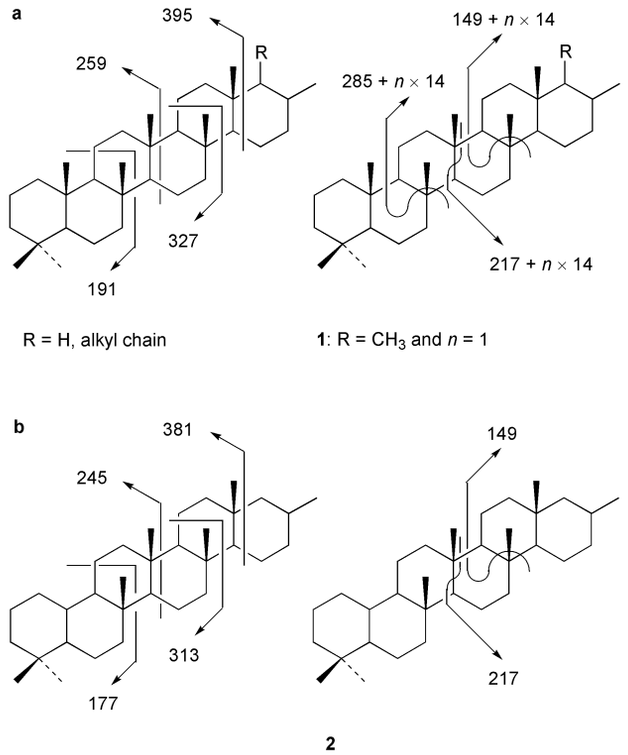


Fig. 1 (a) Generic mass spectrometry fragmentation pattern of the hexacyclic polyprenoid saturated hydrocarbons and (b) of compound **2**. The absolute configuration chosen is that determined for related monoaromatic compounds identified in sediments.[1,2]

Synthesis of hexacyclic polyprenoid C_{35} hydrocarbon 1 and identification in bitumen sample

The strategy used for the preparation of the C_{35} hexacyclic hydrocarbon **1** involves the extensive cyclisation of an all-*trans* C_{35} regular heptaprenoid along its whole structure. Such cascade cyclisations described in the literature can be either cationic-[2,7] or radical-induced.[8] The method adopted here was developed in recent years by Vlad[7c] and consists of a proton-induced cyclisation using a superacid ($HFSO_3$) generating in our case 11 asymmetric centres at one go. Thus, it was envisaged that the synthesis of compound **1** could be achieved by a cascade of six successive cyclisations from the acyclic all-*trans* heptaprenoic acid methyl ester **12** (Fig. 2) followed by reduction of the methyl ester and of the residual double bond. Synthesis of the isoprenoid ester **12** relies on a method previously described for the synthesis of squalene and involving the head to tail coupling of two adequately functionalized C_{20} and C_{15} isoprenoid units (**9** and **7**, respectively; Fig. 2).[9,10] The phenyl thioether **9** was synthesised from commercial all-*trans* geranylgeraniol **8** following standard procedures.[9,10] Synthesis of the chloride **7** from farnesol **4** (Fig. 2) first requires functionalisation at the head of the isoprenoid skeleton to allow incorporation of chlorine. For this purpose, the benzyl ether-protected farnesol **5**[11] was oxidised regioselectively at the terminal *trans* methyl group with SeO_2 in the presence of *tert*-butyl hydroperoxide to give (2*E*,6*E*,10*E*)-1-benzyloxy-3,7,11-trimethyldodeca-2,6,10-trien-12-ol **6**.[12] The latter was converted into the chloride **7** by treatment with toluene-*p*-sulfonyl chloride in the presence of triethylamine and 4-dimethylaminopyridine.[13] NMR studies comprising homonuclear (^1H–^1H, COSY and NOESY) and heteronuclear (^1H–^{13}C, HMQC and HMBC) correlation experiments were performed on the chloride **7** in order to confirm the location of the chlorine atom on the *trans* terminal methyl group. Thus, the absence of nuclear Overhauser effects (NOEs) between vinylic protons and vinylic methyl groups unambiguously confirmed that all the double bonds are *trans*. Location of the chlorine atom at position 12 was established by the occurrence of NOEs between the vinylic proton at position 10 and the allylic protons (α to chlorine atom) at position 12. Head to tail coupling of C_{20} and C_{15} isoprenoids was achieved by reacting the chloride **7** with the sulfur-stabilised allylic carbanion obtained by reaction of the phenyl thioether **9** with *n*-butyllithium,[9] yielding the C_{35} isoprenoid **10**. Final deprotection was performed with lithium in ethylamine. The resulting C_{35} polyprenol **11** was characterised by 1D- and 2D-NMR studies. Interpretation of ^{13}C and DEPT spectra allowed us to establish that the carbon chemical shifts of one of the geminal vinylic methyl groups has a value of 28 ppm whereas the values for all the other vinylic methyl groups appear in the 15–18 ppm range. This is in accordance with the fact that all the double bonds are *trans*[14] and was further corroborated by the interpretation of the NOESY correlation pattern which, in all cases but one (*i.e.* methyl 28), does not show any NOE between vinylic protons and vinylic methyl groups.

Several attempts were carried out to cyclise directly the polyprenol **11** with fluorosulfonic acid in 1-nitropropane at −78 °C as described by Vlad[7c] for the cyclisation of lower polyprenols, but were unsuccessful due to predominant polymerisation and dehydration reactions. In order to limit the extent of side-reactions, cyclisation was finally performed on the all-*trans* heptaprenoic acid methyl ester **12**. The latter was obtained by oxidation of the polyprenol **11** to the related aldehyde (MnO_2) followed by further oxidation to the corresponding methyl ester **12** (NaCN, MnO_2, MeOH)[15] by a mild procedure which avoids isomerisation of the double bond located at position 2. The cyclisation was performed by slow addition of a diluted solution of the ester **12** in 1-nitropropane to a solution of fluorosulfonic acid in 1-nitropropane at −78 °C. Work-up of the reaction mixture, followed by chromatography on a silica gel column, afforded a complex mixture of monoesters comprising, as evidenced by GC-MS analysis, numerous partly cyclised isoprenoid esters but also a small amount of the hexacyclic ester **13** which could be isolated in 1% yield by reversed phase HPLC.

The structure of the ester **13** was confirmed by NMR studies comprising homonuclear (^1H–^1H, COSY and NOESY) and heteronuclear (^1H–^{13}C, HMQC and HMBC) correlation experiments. Interpretation of the DEPT spectrum, as well as of heteronuclear (^1H–^{13}C, HMQC and HMBC) correlation experiments, allowed us to determine clearly the carbon skeleton and, in particular, that each ring junction methyl group is separated from its neighbouring methyl groups by one common remote (^3J) connection with a methine (Fig. 3). Stereochemical information was obtained from the NOESY experiment (Fig. 3). Occurrence of NOEs between each ring-junction methyl group and its neighbouring methyls clearly shows that they are all axial and located on the same side of the molecule. Furthermore, the occurrence of NOEs between H-2/H-6 and H-2/H_{ax}-8 indicates that H-2 is α and, therefore, that the methoxycarbonyl group is β. Combination of the results from all the experiments finally allowed us to establish firmly the structure and to assign all the ^1H and ^{13}C chemical shifts (Table 1). Additional structural information was obtained from the interpretation of the ^{13}C chemical shifts (Table 1). Thus, the *trans*,*transoid*,*trans* stereochemistry of all the ring junctions, as well as the fact that all the ring junction methyl groups are located on the same side of the molecule, was further supported by the ^{13}C chemical shifts of these methyls. Their values, below 20 ppm, indicate therefore the occurrence of γ-gauche effects, as expected for axial methyl groups on an all-*trans* polycyclic structure.[16] Moreover, the strong deshielding of the ring junction methines and of the methylenes bearing axial protons *trans* relative to axial methyl groups ($57 < \delta < 62$ ppm and $40 < \delta < 43$ ppm, respectively) shows the absence of γ-gauche effects, which is also in accordance with an all-*trans* structure.[16]

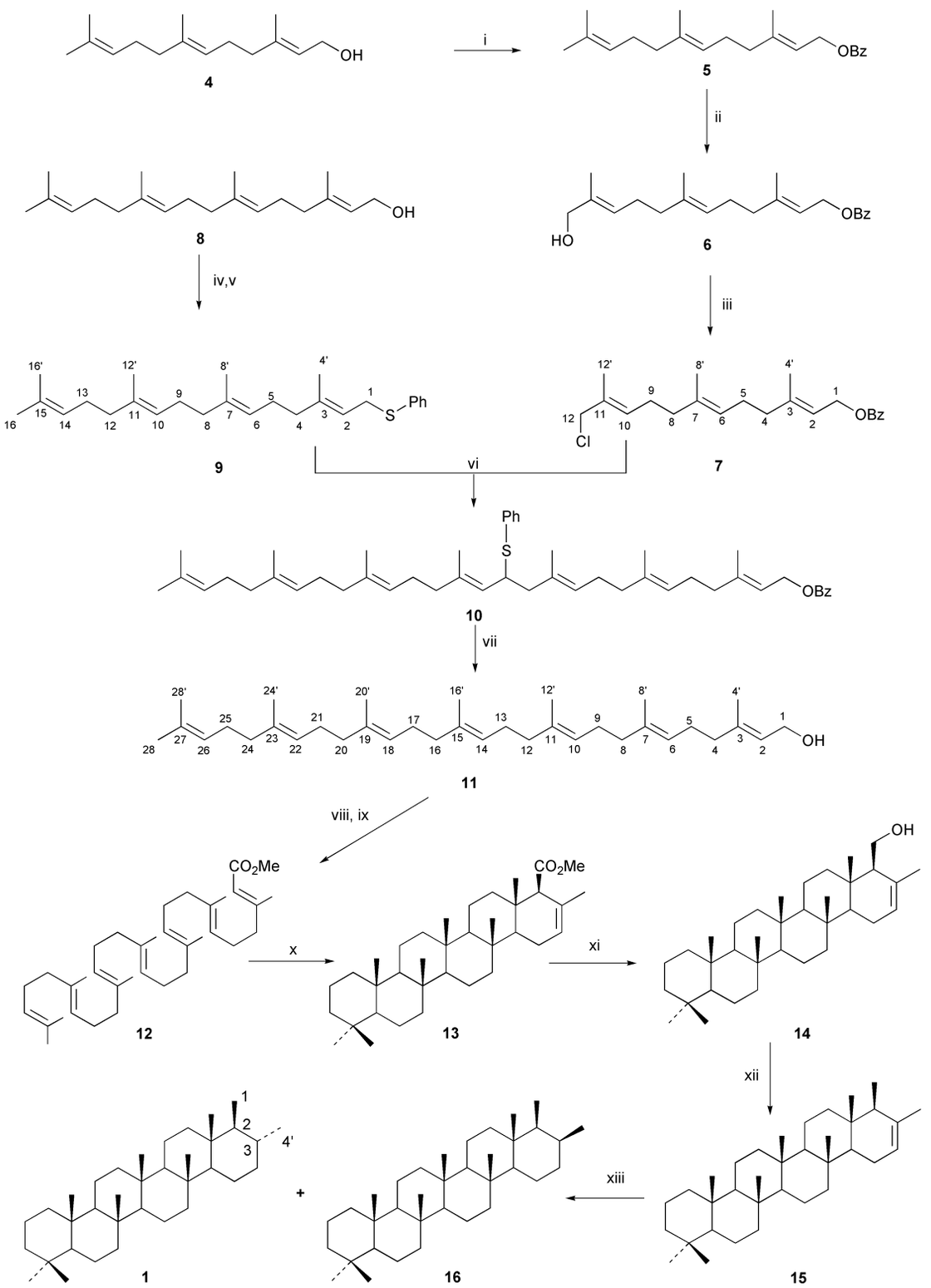


Fig. 2 Synthesis of the hexacyclic polyprenoid hydrocarbon **1**. *Reagents and conditions*: i, benzyl chloride, NaOH, DMSO; ii, SeO_2, Bu^{t}OOH, CH_2Cl_2; iii, *p*TsCl, Et_3N, DMAP, CH_2Cl_2; iv, $MeSO_2Cl$, Et_3N, CH_2Cl_2; v, PhSH, MeLi, Et_2O; vi, **9**, Bu^{n}Li, DABCO, **7**, THF, −78 °C; vii, Li, $EtNH_2$, −78 °C; viii, MnO_2, hexane; ix, MnO_2, NaCN, AcOH, MeOH; x, $HFSO_3$, 1-nitropropane, −78 °C; xi, $LiAlH_4$, THF; xii, (a) $MeSO_2Cl$, Et_3N, CH_2Cl_2; (b) $LiAlH_4$, THF; xiii, H_2, PtO_2, AcOEt.

Reduction of the ester **13** *via* alcohol **14** and alkene **15** following standard procedures yielded an equimolar mixture (as determined by GC) of two hexacyclic alkanes **1** and **16**. All our attempts to separate these two isomers using various reversed phase HPLC columns were unsuccessful. Comparison of the mass spectra of the synthetic isomers with those of the C_{35} hexacyclic alkane occurring in Lameignère bitumen, as well as co-injection experiments on two GC columns coated with different phases clearly established that only one of the synthetic isomers is present in the bitumen sample. Since the bitumen sample originates from a "mature" petroleum, and has undergone rather high thermal stress, the thermodynamically more stable configurations at asymmetric centres of polycyclic hydrocarbons are strongly favoured.[17] Thus, it can be proposed that methyl 4′ is equatorial and, therefore that the hydrocarbon **1** corresponds to the geological compound. This hypothesis is

Table 1 ^{13}C and ^{1}H NMR data of ester **13** (500 MHz, C_6D_6, δ relative to TMS)

C atom[a]	δ_C (ppm)	δ_H (ppm)	C atom[a]	δ_C (ppm)	δ_H (ppm)
1	173.0	—	16	42.2	0.81(α), 1.73(β)
2	62.9	3.08 s	16′	17.9	0.80 s
3	129.5	—	17	17.6	1.31(β), 1.52(α)
4	124.0	5.50	18	61.3	0.67
4′	21.6	1.77	19	38.2	—
5	23.2	1.91(α), 1.91(β)	20	42.1	0.82(α), 1.73(β)
6	55.2	1.15	20′	17.7	0.83 s
7	36.9	—	21	18.9	1.52(α), 1.34(β)
8	42.4	1.49(α), 1.87(β)	22	57.0	0.78
8′	15.9	1.19 s	23	37.9	—
9	17.9	1.28(β), 1.45(α)	24	40.2	0.77(α), 1.68(β)
10	61.9	0.71	24′	16.8	0.85 s
11	37.9	—	25	19.2	1.64(β), 1.41(α)
12	42.1	1.64(β), 0.82(α)	26	42.6	1.18(α), 1.41(β)
12′	16.8	0.85 s	27	33.6	—
13	17.5	1.29(β), 1.46(α)	28	33.8	0.92 s
14	61.5	0.64	28′	21.8	0.88 s
15	37.9	—	OCH_3	50.8	3.40 s

[a] For C numbering, see Fig. 3.

Table 2 ^{13}C and ^{1}H NMR data of hydrocarbon **2** (500 MHz, C_6D_6, δ relative to TMS)

C atom[a]	δ_C (ppm)	δ_C (ppm)	C atom[a]	δ_C (ppm)	δ_C (ppm)
2	55.4	0.76(α), 1.29(β)	16	41.4	0.81(α), 1.76(β)
3	27.9	1.51(β)	16′	17.7	0.88 s
4	36.9	0.88(α), 1.78(β)	17	20.3	1.09(β), 1.61(α)
4′	23.2	0.90	18	53.6	0.69(α)
5	21.2	1.21(α), 1.54(β)	19	36.8	—
6	58.6	0.73	20	39.6	0.88(α), 1.81(β)
7	35	—	20′	14.3	0.77 s
8	44.1	1.14(α), 1.53(β)	21	21.6	1.28(β), 1.58(α)
8′	21.2	0.93 s	22	52.3	0.75(α)
9	17.7	1.36(α), 1.26(β)	23	35.3	1.11(β)
10	62.2	0.74	24	31.5	0.67(α), 1.86(β)
11	37.7	—	25	22.6	1.43(β), 1.56(α)
12	42.2	0.84(α), 1.73(β)	26	42.6	1.19(α), 1.38(β)
12′	17.3	0.84 s	27	33.2	—
13	17.7	1.36(β), 1.53(α)	28	30.8	0.93 s
14	59.6	0.70(α)	28′	20.3	0.87 s
15	37.4	—			

[a] For C numbering, see Fig. 4.

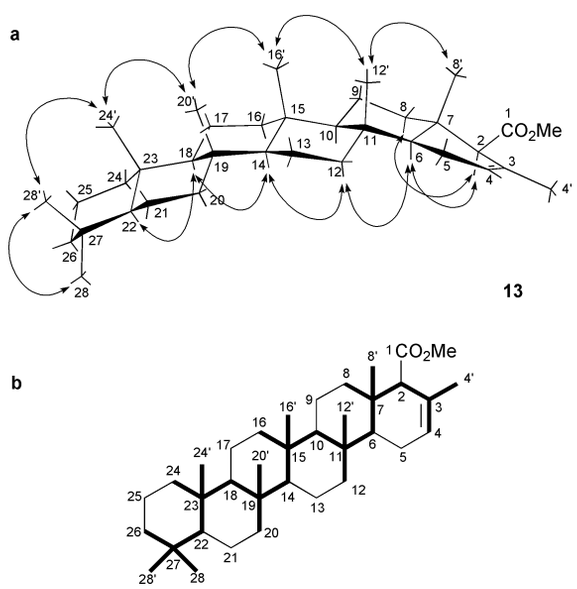


Fig. 3 (a) Spatial representation of **13** showing the most important NOEs observed. (b) Carbon sequence (bold) established from inverse long-range ^{1}H–^{13}C correlation experiment. The absolute configuration of compound **13** is not known. The absolute configuration represented in this figure is that determined for related monoaromatic compounds identified in sediments.[1,2]

further corroborated by previous studies on C_{20} regular tricyclic polyprenoid hydrocarbons having the same partial structure (ring E and F) in which equatorial methyls 1 and 4′ strongly predominate in mature samples.[18]

Isolation and identification of demethylated hexacyclic C_{33} hydrocarbon 2

In the course of investigation of bituminous rocks from a Spanish quarry (Maestu),[19] GC-MS analysis of the alkane fraction showed clear evidence for the occurrence of the polyprenoid polycyclic saturated hydrocarbons, including compound **1**, already encountered in bitumens from Lameignère (France). GC-MS analyses also revealed the presence of other related series of polycyclic hydrocarbons (6 to 8 rings), showing a similar fragmentation pattern with two sets of main fragments, each regularly shifted by 68 Da. However, as the main set of fragments was shifted by 14 Da [Fig. 1(b)] as compared to the major hydrocarbons occurring in Lameignère samples, it was likely that these new compounds were demethylated structures.

The fraction showed, at the beginning of the gas chromatogram, a predominant complex mixture of low molecular weight hydrocarbons characteristic of strongly biodegraded bitumens.[20] The minor heavy saturated hydrocarbons, including the polycyclic polyprenoid hydrocarbons, could, however, be enriched by successive precipitation in acetone. A sequence of chromatographic separation steps involving different types of reversed phase HPLC columns was performed on the precipitated fraction and allowed us to isolate approximately 1 mg of a hexacyclic demethylated compound **2** (96% purity estimated by GC, [M^+] 452 $C_{33}H_{56}$). Characterisation of hydrocarbon **2** was carried out by 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, which permitted us to assign the signals of all the protons and all the carbon atoms (Table 2) and to establish unambiguously its structure.

NMR characterization of hydrocarbon 2. ^{13}C-NMR ^{1}H noise-decoupled and DEPT spectra of compound **2** show the presence of 7 methyl, 14 methylene, 7 methine and 5 quaternary carbons. Furthermore, the ^{1}H-NMR spectrum reveals the presence of 6 methyl singlets and one methyl doublet, which suggests a lack of two methyls on this compound as compared to the spectrum of the complete C_{35} hexacyclic regular polyprenoid hydrocarbon **1**. The carbon skeleton was established using ^{1}H–^{13}C long-range ($^{2,3}J$) couplings, the most intense of which are observed from the methyl groups. Among the methyls, two 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 to each other. The sector formed by these geminal methyl groups (*i.e.*, atoms 22, 26, 27, 28 and 28′) appears to be cut off from the remaining part of the molecule (Fig. 4). Indeed, C-22 does not show remote connections with any remaining methyl groups, thus suggesting the absence of the 24′-Me at C-23. All the other methyl groups (*i.e.* 4′, 8′, 12′, 16′, 20′ methyl groups) are linked two-by-two to each other through one common remote connection (^{3}J) with a methine or with a methylene (C-2). ^{1}H–^{1}H COSY and NOESY spectra allowed us to link the sector of the geminal methyl groups to that of the other methyl groups (Fig. 4) and to assign the signals of the remaining atoms. Indeed, as compared to a regular hexacyclic polyprenoid

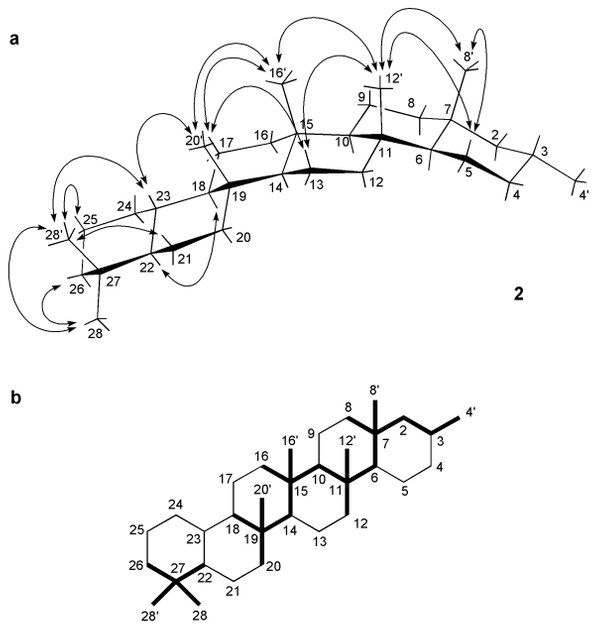


Fig. 4 (a) Spatial representation of **2** showing the most important NOEs observed. (b) Carbon sequence (bold) established from inverse long-range ^{1}H–^{13}C correlation experiment. The absolute configuration of compound **2** is not known. The absolute configuration represented in this figure is that determined for related monoaromatic compounds identified in sediments.[1,2]

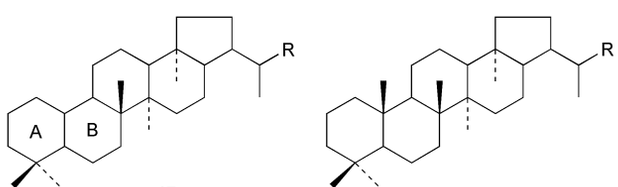
hydrocarbon, interpretation of the NOESY correlation pattern afforded evidence that the missing methyl has been replaced by a hydrogen at C-23 (presence of NOEs between 28′-Me/H-23, 20′-Me/H-23). Moreover, NOEs observed between 28′-Me/H-23, H-23/20′-Me, 20′-Me/16′-Me, 16′-Me/12′-Me, 12′-Me/8′-Me reveal that 28′-Me, H-23, 20′-Me, 16′-Me, 12′-Me and 8′-Me are all on the same side of the molecule, thus confirming its regular polyprenoid structure. No correlation between methyls 8′, 12′, 16′, 20′, proton 23 and protons 6, 10, 14, 18, 22 on ring junctions could be observed in the NOESY spectrum. This is in agreement with the fact that all the ring junctions are *trans*.

The underscoring of γ-gauche effects also supports the *trans*, *transoid*,*trans* stereochemistry.[16] The methyl groups 20′-Me, 16′-Me, 12′-Me, 8′-Me, the methine C-23 and the methylene groups C-5, C-9, C-13, C-17, C-21, C-25 show strong γ-gauche interactions, on account of the strong shielding of their carbon atoms ($\delta^{13}C < 21$ ppm for the methyl groups, $\delta^{13}C = 35$ ppm for the methine and $\delta^{13}C < 22.5$ ppm for the methylenes) together with the deshielding of the corresponding axial protons belonging to the methylene groups ($1.1 < \delta^{1}H < 1.4$ ppm). Conversely, low-field ^{13}C chemical shifts (as well as corresponding high-field ^{1}H chemical shifts) indicate the absence of γ-gauche effects. This is the case for the methines C-6, C-10, C-14, C-18, C-22 and methylenes C-2, C-8, C-12, C-16, C-20 ($52 < \delta^{13}C < 62$ ppm for methine and $40 < \delta^{13}C < 55$ ppm for methylene carbons, $\delta^{1}H < 0.75$ ppm for axial methine protons and $\delta^{1}H < 1.1$ ppm for axial methylene protons). These observations are in agreement with an axial orientation for these quoted methyl groups, as well as for the methines C-6, C-10, C-14, C-18, C-22 which are *trans* relative to the methyl groups. The low-field ^{13}C chemical shift value for methyl 28 is characteristic for an equatorial geminal methyl group. Similarly, the ^{13}C chemical shift value of 23.3 ppm for the methyl 4′—as compared to those of axial methyl groups which do not exceed 21 ppm—suggests an equatorial position. The equatorial position of methyl 4′ is particularly difficult to prove since no clear NOE could be observed with any of the protons located on C-atoms 2, 3, 4′, 8′. This is certainly due to the fact that the H-3 signal is very broad due to numerous couplings with its neighbouring protons and to distortion of ring F. Nevertheless, no NOEs were observed between the methyl groups 4′ and 8′ as expected for an equatorial position for 4′. Furthermore, the shielding of the C-3 carbon atom combined with the deshielding of the proton at C-3, which reveal the presence of γ-gauche effects between H-3 and Me-8′, is also in agreement with an equatorial position for Me-4′.

Discussion

The two new hydrocarbons **1** and **2** clearly result from the diagenetic transformation, by various processes known to occur in the subsurface, of polycyclic polyprenoid precursors formed by proton-induced cyclisation of regular all-*trans* unsaturated polyprenoids (Fig. 5). The presence of higher homologues of **1** and **2** bearing extended side-chains may be explained by incomplete cyclisation of higher polyprenoids.

GC-MS analysis of several biodegraded bitumen samples containing the newly-identified hydrocarbons and having undergone weathering to various extents shows that the relative amount of demethylated polycyclic polyprenoid alkanes (such as **2** and **3**) increases with the extent of weathering as compared to the amount of the related hydrocarbons from the regular series (such as **1**).[19] A similar situation is observed in the case of hopanes[21a] which are ubiquitous molecular fossils of a widespread family of bacterial lipids, the biohopanoids.[21b] Exposure of bitumen samples to biodegradation indeed results in an increase of the relative amount of demethylated compounds **17** as compared to their regular counterparts **18**.[21] By analogy,[22c]



it may be inferred that the demethylated polyprenoid hydrocarbons are biotransformation products of the hydrocarbons from the regular series. Alternatively, biodegradation may have resulted in a preferential removal of the hydrocarbons from the regular series, leading therefore to a relative enrichment of the more resistant demethylated structures, already present in low concentrations in the original, unweathered bitumen.[22b] Concerning the origin of the latter, it can be proposed either that the missing methyl groups were already absent from the biological precursors, or that functionalized groups were present at these positions and lost by various diagenetic transformation processes.[5] It cannot be excluded that functionalisation of methyl groups on the precursor polyprenoid was induced by microbial oxidation processes occurring at the earliest stages of diagenesis.[23] It is striking in this respect that the demethylated hopanes have also lost the methyl group located at the A–B ring junction.[22a] As the partial structural subunit constituted by the A–B–C ring system is common to hopanoids and polycyclic polyprenoid alkanes, it can be inferred that this partial structure may be recognised and demethylated by bacteria involved in the biodegradation of bitumen exposed to atmospheric conditions. Alternatively, the absence of the methyl group at the A–B ring junction may increase resistance towards biodegradation not only of hopanoids but also of various structurally related polycyclic polyterpenoids.

Experimental

General

NMR spectra were recorded on a Bruker ARX-500 or on a

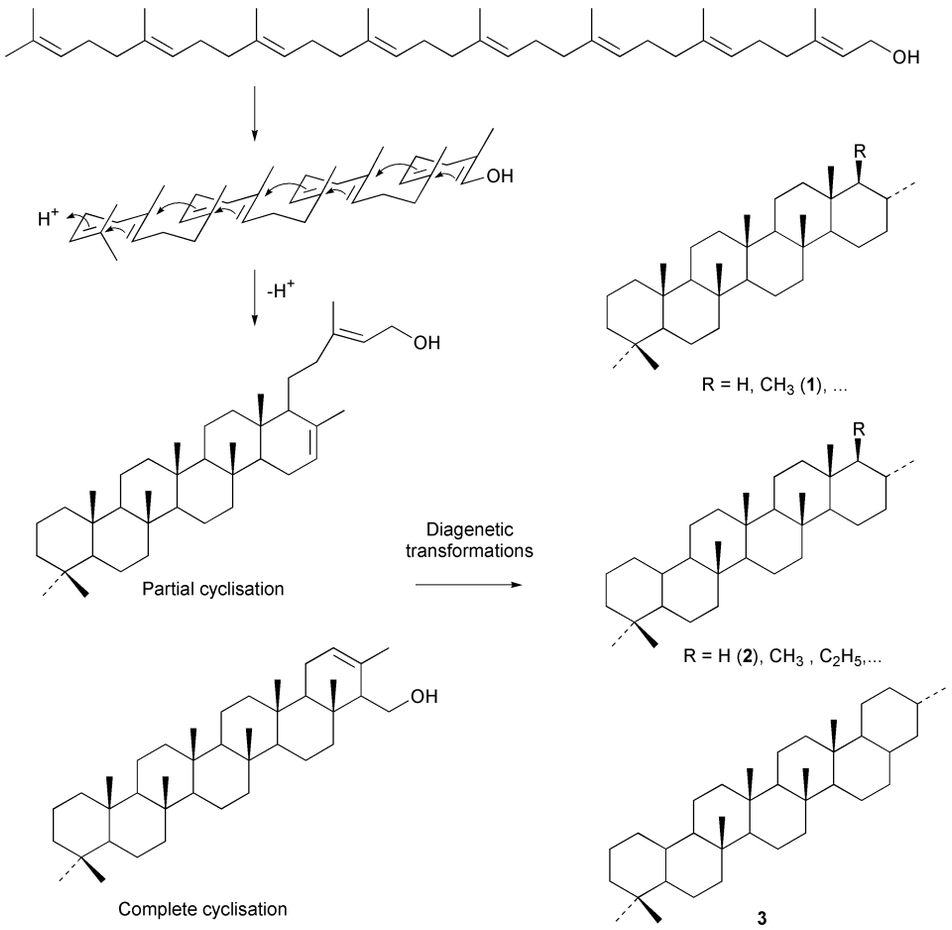


Fig. 5 Possible origin of the novel highly cyclised polyprenoid hydrocarbons by diagenetic transformation of biological polycyclic polyprenoid lipids (modified from Schaeffer *et al.*[1])

Bruker WP 200 SY spectrometer operating at observation frequencies of 500 MHz (resp. 200 MHz) for ^{1}H and 125 MHz for ^{13}C nuclei and data were recorded at 300 K. Chemical shifts (δ) are reported in ppm from tetramethylsilane, using the solvent (C_6D_6: $\delta^{1}H$ 7.16, $\delta^{13}C$ 128.0 or $CDCl_3$: $\delta^{1}H$ 7.26, $\delta^{13}C$ 77.0) as internal reference. *J* values are given in Hz. 2D-NMR studies including homonuclear (^{1}H–^{1}H, COSY and NOESY) and heteronuclear (^{1}H–^{13}C, HMQC and HMBC) correlation experiments were performed on the Bruker ARX-500 spectrometer.

Gas chromatography–mass spectrometry analyses were carried out either on a Finnigan MAT INCOS 50 spectrometer connected to a Varian 3400 gas chromatograph equipped with an on-column injector and with a DB-5 J&W column (30 m × 0.25 mm, 0.1 μm film thickness), or on a Finnigan MAT TSQ 700 spectrometer connected to a Varian 3400 gas chromatograph (on-column injector, DB-5 J&W column, 60 m × 0.25 mm, 0.1 μm film thickness). Mass spectra were produced at 70 eV and helium was used as carrier gas. Co-injection experiments were carried out using both an apolar column (DB5 J&W, 60 m × 0.25 mm, 0.1 μm film thickness) and a more polar column (DB17 J&W, 30 m × 0.25 mm, 0.15 μm film thickness).

Probe mass spectra were recorded on the Finnigan MAT TSQ 700 spectrometer.

Separation of alkane fractions. Isolation of hexacyclic alkane 2

The crushed rock sample (Maestu quarry, Spain; Lameignère quarry; France) was extensively extracted with CH_2Cl_2–CH_3OH (1 : 1, v/v). Chromatography of the organic extract on a silica gel column yielded the saturated hydrocarbon fraction using hexane as eluent. Successive washing of the alkane fraction from the Spanish sample with acetone followed by sonication allowed extraction of the major part of the soluble low molecular weight constituents and consequently enrichment of the remaining precipitated alkane fraction in polycyclic polyprenoid hydrocarbons. For the isolation of the hexacyclic compound **2** ([M^+] 452, $C_{33}H_{56}$), a sequence of chromatographic separation steps on different HPLC columns was performed on the precipitated alkane fraction. Separation on a Du Pont Zorbax ODS column (250 × 21.2 mm, 8 μm, 80 Å; CH_3OH–$CHCl_3$ 50 : 50 v/v; 20 ml min^{-1}) allowed us to obtain a fraction enriched in compound **2**. Additional fractionation by reversed phase HPLC (Du Pont Zorbax ODS 250 × 9.4 mm, 5 μm, 60 Å; CH_3OH–$CHCl_3$ 55 : 45 v/v; 5 ml min^{-1}; Vydac C_{18} 250 × 4.6 mm, 5 μm, 300 Å; CH_3OH–$CHCl_3$ 65 : 35 v/v; 1 ml min^{-1}) led to the isolation of 1 mg of compound **2** with a purity of 96% (GC).

Compound **2**: MS (EI, 70 eV): 452(M^+, 59%), 437(36), 313(62), 287(3), 271(4), 259(6), 245(76), 231(9), 217(30), 203(7), 191(19), 177(42), 163(28), 149(56), 135(28), 121(37), 109(63), 95(90), 81(100), 69(56), 55(59).

Synthesis of hexacyclic polyprenoid alkane 1

(2*E*,6*E*,10*E*)-1-Benzyloxy-3,7,11-trimethyldodeca-2,6,10-triene 5. To a solution of sodium hydroxide (4 g; 0.1 mol) in DMSO (100 ml) were added 15 g (0.07 mol) of farnesol [(2*E*,6*E*,10*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol] **4**. After 2 h at room temperature, a solution of benzyl chloride (10 g; 0.08 mol) in DMSO (20 ml) was slowly added. The reaction mixture was kept under argon at room temperature for 12 h after which the mixture was poured into water and extracted with CH_2Cl_2 (3×). The crude extract was adsorbed on to silica gel and chromatographed on a silica gel column (CH_2Cl_2–hexane 50 : 50 v/v) yielding 19.8 g (91%) of (2*E*,6*E*,10*E*)-1-benzyloxy-3,7,11-trimethyldodeca-2,6,10-triene

5. NMR δ_H ($CDCl_3$, 200 MHz): 1.60 (6H, s, 2 × CH_3), 1.65 (3H, s, CH_3), 1.68 (3H, s, CH_3), 1.92–2.17 (8H, m, H allylic), 4.03 (2H, d, *J* 6.5 Hz, H-1), 4.51 (2H, s, -CH_2-O-CH_2-Phe), 5.06–5.12 (2H, m, H vinylic), 5.41 (1H, t, *J* 6.0 Hz, H-2), 7.27–7.36 (5H, m, H aromatic). MS (EI, 70 eV): 312(M^+, 1%), 221(15), 204(12), 191(25), 189(24), 161(21), 135(41), 121(45), 107(47), 91(100), 69(94), 55(18).

(2*E*,6*E*,10*E*)-1-Benzyloxy-3,7,11-trimethyldodeca-2,6,10-trien-12-ol 6. Selenium dioxide (180 mg, 1.6 mmol) was added to a mixture of a 70% aqueous solution of *tert*-butyl hydroperoxide (7.3 g; 81 mmol) and CH_2Cl_2 (25 ml). The mixture was stirred for 30 min at room temperature and a solution of (2*E*,6*E*,10*E*)-1-benzyloxy-3,7,11-trimethyldodeca-2,6,10-triene **5** (8.4 g; 27 mmol) in CH_2Cl_2 (20 ml) was added. After 72 h at room temperature, toluene was added and the mixture concentrated under reduced pressure, diluted with water and extracted with CH_2Cl_2. Chromatography on a silica gel column (CH_2Cl_2–diethyl ether 9 : 1 v/v) yielded 2.8 g (32%) of (2*E*,6*E*,10*E*)-1-benzyloxy-3,7,11-trimethyldodeca-2,6,10-trien-12-ol **6**. NMR δ_H ($CDCl_3$, 200 MHz): 1.60 (3H, s, CH_3), 1.65 (6H, s, 2 × CH_3), 1.92–2.17 (8H, m, H allylic), 3.97 (2H, s, H-12), 4.03 (2H, d, *J* 6.5 Hz, H-1), 4.50 (2H, s, -CH_2-O-CH_2-Ph), 5.12 (1H, m, H vinylic), 5.30–5.40 (2H, m, H vinylic), 7.29–7.36 (5H, m, H aromatic). MS (EI, 70 eV): 328(M^+, <1%), 310(<1), 219(3), 189(6), 161(7), 138(12), 123(21), 107(23), 95(41), 91(100), 69(35), 55(10).

(2*E*,6*E*,10*E*)-1-Benzyloxy-3,7,11-trimethyl-12-chlorododeca-2,6,10-triene 7. 4-Dimethylaminopyridine (1.1 g; 9 mmol), toluene-*p*-sulfonyl chloride (2.2 g; 11 mmol) and triethylamine (2 ml; 1.4 g; 14 mmol) were added to a solution of (2*E*,6*E*,10*E*)-1-benzyloxy-3,7,11-trimethyldodeca-2,6,10-trien-12-ol **6** (3.0 g; 9 mmol) in anhydrous CH_2Cl_2 (20 ml). The solution was kept under argon at room temperature for 12 h. The mixture was then poured into water and extracted with CH_2Cl_2. The crude extract was chromatographed on a silica gel column (CH_2Cl_2) to give (2*E*,6*E*,10*E*)-1-benzyloxy-3,7,11-trimethyl-12-chlorododeca-2,6,10-triene **7** (2.2 g, 69%). NMR δ_H ($CDCl_3$, 500 MHz): 1.59 (3H, s, CH_3-4′), 1.64 (3H, s, CH_3-8′), 1.72 (3H, s, CH_3-12′), 1.98–2.26 (8H, m, H allylic), 4.01 (2H, s, H-12), 4.03 (2H, d, *J* 7.0 Hz, H-1), 4.50 (2H, s, -CH_2-O-CH_2-Phe), 5.12 (1H, t, *J* 5.5 Hz, H-6), 5.41 (1H, t, *J* 7.0 Hz, H-2), 5.50 (1H, t, *J* 6.5 Hz, H-10), 7.25–7.36 (5H, m, H aromatic); δ_C ($CDCl_3$, 125 MHz): 14.13 (C-12′), 15.96 (C-4′), 16.52 (C-8′), 26.32 and 26.66 (C-5 and C-9), 39.04 and 39.73 (C-4 and C-8), 52.70 (C-12), 66.50 (C-1), 71.99 (-O-*C*H_2-Phe), 120.64 (C-6), 124.35 (C-2), 128.30–129.20 (C-aromatic), 130.67 (C-10), 131.53 (C-11), 134.50 (C-3), 142.67 (C-7). MS (EI, 70 eV): 347(M^+, 1%), 329(1), 311(4), 293(3), 241(33), 239(93), 225(1), 205(20), 203(100), 171(34), 135(42), 107(44).

(2*E*,6*E*,10*E*,14*E*)-1-Phenylsulfanyl-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraene 9. (1) Triethylamine (760 mg; 7.5 mmol) and, after 30 min, methanesulfonyl chloride (855 mg; 7.5 mmol) were added at room temperature under argon to a solution of (2*E*,6*E*,10*E*,14*E*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-ol **8** (1.3 g; 4.5 mmol) in anhydrous CH_2Cl_2 (25 ml). After 16 h, the reaction was quenched with water and extracted with CH_2Cl_2 (3×), dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude extract was rapidly chromatographed on silica gel (CH_2Cl_2) and immediately used without further purification.

(2) A solution of methyllithium in THF (12.5 mmol; 1 M solution) was added dropwise under argon to a cold solution (0 °C) of thiophenol (1.1 g; 10 mmol) in anhydrous diethyl ether (20 ml). After 1 h, the crude mixture obtained from step (1), dissolved in 20 ml anhydrous diethyl ether, was slowly added. The resulting mixture was allowed to warm to room temperature and was stirred under argon for a further 12 h. The reaction mixture was quenched with water and extracted with diethyl ether (3×). Chromatography of the crude mixture on silica gel (hexane–CH_2Cl_2 8 : 2 v/v) gave 1.0 g (58%) of (2*E*,6*E*,10*E*,14*E*)-1-phenylsulfanyl-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraene **9**. NMR δ_H ($CDCl_3$, 200 MHz): 1.60 (12H, s, 4 × CH_3), 1.69 (3H, s, CH_3-4′), 1.93–2.09 (12H, m, H allylic), 3.55 (2H, d, *J* 7.5 Hz, H-1), 5.05–5.15 (3H, m, H vinylic), 5.32 (1H, t, *J* 7.5 Hz, H vinylic), 7.32–7.44 (5H, m, H aromatic). MS (EI, 70 eV): 382(M^+, 8%), 313(9), 273(13), 257(2), 229(8), 204(10), 177(16), 149(18), 135(21), 109(26), 95(29), 81(92), 69(100).

(all-*E*)-1-Benzyloxy-3,7,11,15,19,23,27-heptamethyl-13-phenylsulfanyloctacosa-2,6,10,14,18,22,26-heptaene 10. A solution of (2*E*,6*E*,10*E*,14*E*)-1-phenylsulfanyl-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraene **9** (500 mg; 1.3 mmol) and 1,4-diazabicyclo[2.2.2]octane (180 mg; 1.6 mmol) in anhydrous THF (10 ml) was treated with *n*-butyllithium (1.9 mmol) under argon at −78 °C. After 30 min, a solution of (2*E*,6*E*,10*E*)-1-benzyloxy-3,7,11-trimethyl-12-chlorododeca-2,6,10-triene **7** (695 mg; 2 mmol) in anhydrous THF (5 ml) was added to the red solution. The reaction mixture was allowed to warm to room temperature. After 3 h, the reaction mixture was cooled to −78 °C and the reaction quenched with methanol. Extraction with diethyl ether followed by chromatography over silica gel (CH_2Cl_2–hexane 1 : 1 v/v) yielded (all-*E*)-1-benzyloxy-3,7,11,15,19,23,27-heptamethyl-13-phenylsulfanyloctacosa-2,6,10,14,18,22,26-heptaene **10** (480 mg; 53%). NMR δ_H ($CDCl_3$, 200 MHz): 1.35 (3H, s, CH_3), 1.59 (15H, s, 5 × CH_3), 1.64 (3H, s, CH_3-4′), 1.68 (3H, s, CH_3), 1.80–2.12 (22H, m, H allylic), 3.95–4.07 (3H, m, H-13 and H-1), 4.50 (2H, s, -CH_2-O-CH_2-Phe), 4.94–5.23 (6H, m, H vinylic), 5.40 (1H, t, *J* 6.5 Hz, H-2), 7.21–7.43 (10H, m, H aromatic). MS (EI, 70 eV): 692(M^+, 1%), 623(0.5), 601(0.3), 583(5), 475(29), 407(8), 381(33), 339(5), 271(13), 173(45), 121(47), 105(89), 69(100).

(all-*E*)-3,7,11,15,19,23,27-Heptamethyloctacosa-2,6,10,14,18,22,26-heptaen-1-ol 11. Small portions of lithium containing 1% sodium (115 mg; 18 mmol) were added to dry ethylamine (10.5 ml) under argon at −78 °C. The resulting blue solution was allowed to warm to 0 °C to ensure complete dissolution of lithium. After 1 h, the reaction mixture was cooled to −78 °C and a solution of (all-*E*)-1-benzyloxy-3,7,11,15,19,23,27-heptamethyl-13-phenylsulfanyloctacosa-2,6,10,14,18,22,26-heptaene **10** (250 mg; 0.36 mmol) in anhydrous THF was added. After 2 h, the reaction was quenched with sodium benzoate at −78 °C (disappearance of the blue colour) and the mixture allowed to warm to room temperature. Methanol was added and the mixture poured into water. Extraction with CH_2Cl_2 followed by chromatography over silica gel (CH_2Cl_2) afforded (all-*E*)-3,7,11,15,19,23,27-heptamethyloctacosa-2,6,10,14,18,22,26-heptaen-1-ol **11** (146 mg, 82%). NMR δ_H ($CDCl_3$, 500 MHz) in accordance with published data in the literature:[24] 1.60 (18H, s, CH_3-8′, CH_3-12′, CH_3-16′, CH_3-20′, CH_3-24′, CH_3-28′), 1.68 (6H, s, CH_3-4′, CH_3-28), 1.97–1.99 (10H, m, H allylic), 2.00–2.12 (14H, m, H allylic), 4.15 (2H, d, *J* 6.5 Hz, H-1), 5.11 (6H, m, H vinylic, H-6, H-10, H-14, H-18, H-22, H-26), 5.32 (1H, t, *J* 6.5 Hz, H vinylic, H-2); δ_C ($CDCl_3$, 125 MHz): 15.76 (C-8′, C-12′, C-16′, C-20′, C-24′), 16.04 (C-4′), 17.43 (C-28′), 25.44 (C-28), 26.10–26.50 (C-5, C-9, C-13, C-17, C-21, C-25), 39.32–39.48 (C-4, C-8, C-12, C-16, C-20, C-24), 59.18 (C-1), 123.10–124.20 (C-2, C-6, C-10, C-14, C-18, C-22, C-26), 131.00 (C-27), 134.65–135.20 (C-7, C-11, C-15, C-19, C-23), 139.64 (C-3). MS (EI, 70 eV): 494(M^+, 3%), 476(4), 407(3), 339(5), 271(9), 203(16), 189(18), 161(25), 135(38), 84(95), 69(100).

(all-*E*)-3,7,11,15,19,23,27-Heptamethyloctacosa-2,6,10,14,18,22,26-heptaenoic acid methyl ester 12. (1) A suspension of manganese dioxide (870 mg; 10 mmol) in a solution of (all-*E*)-

3,7,11,15,19,23,27-heptamethyloctacosa-2,6,10,14,18,22,26-heptaen-1-ol **11** (100 mg; 0.2 mmol) in hexane (20 ml) was stirred for 1 h at 0 °C. Filtration of the reaction mixture over silica gel using CH_2Cl_2 yielded crude (all-*E*)-3,7,11,15,19,23,27-heptamethyloctacosa-2,6,10,14,18,22,26-heptaen-1-al which was used without further purification.

(2) Crude (all-*E*)-3,7,11,15,19,23,27-heptamethyloctacosa-2,6,10,14,18,22,26-heptaen-1-al was added to a suspension of manganese dioxide (870 mg; 10 mmol) in methanol (20 ml). Sodium cyanide (100 mg; 2 mmol) and a few drops of acetic acid were added. After 17 h, the reaction mixture was centrifuged to remove the manganese dioxide and the organic phase was concentrated, water was added and the mixture extracted with CH_2Cl_2. The mixture was chromatographed over silica gel (CH_2Cl_2–hexane 1 : 1 v/v) to give (all-*E*)-3,7,11,15,19,23,27-heptamethyloctacosa-2,6,10,14,18,22,26-heptaenoic acid methyl ester **12** (84 mg; 80%) as a colourless oil. NMR δ_H ($CDCl_3$, 200 MHz): 1.48 (3H, s, CH_3), 1.59 (15H, s, 5 CH_3), 1.62 (3H, s, CH_3), 1.79–2.09 (24H, m, H allylic), 2.18 (3H, br s, CH_3-4′), 3.65 (3H, s, -O-C*H*$_3$), 4.92–5.24 (6H, m, H vinylic), 5.67 (1H, m, H vinylic, H-2). MS (EI, 70 eV): 522(M^+, 7%), 491(3), 453(7), 385(4), 341(3), 285(4), 257(7), 203(16), 189(23), 161(32), 135(58), 121(50), 95(38), 81(75), 69(100).

Hexacyclic ester 13. A solution of (all-*E*)-3,7,11,15,19,23,27-heptamethyloctacosa-2,6,10,14,18,22,26-heptaenoic acid methyl ester **12** (124 mg; 0.24 mmol; 0.015 M) in dry 1-nitropropane was slowly added (5 ml min^{-1}) to a solution of fluorosulfonic acid (12 mmol; 0.5 M) in dry 1-nitropropane at −78 °C under argon. After 2 h, the reaction was quenched with triethylamine (disappearance of the initial yellowish colour). Extraction with CH_2Cl_2 followed by chromatography over silica gel (CH_2Cl_2) yielded 60 mg of a crude mixture ("monoester" fraction). Further fractionation by reversed phase HPLC (Du Pont Zorbax ODS 250 × 9.4 mm, 5 μm; acetone–water 95 : 5 v/v) allowed isolation of 1.2 mg (1%) of the hexacyclic ester **13**, whose structure was unambiguously established by 1D- and 2D-NMR studies including homonuclear (^1H–^1H, COSY and NOESY) and heteronuclear (^1H–^{13}C, HMQC and HMBC) correlation experiments (see text and Table 1). MS (EI, 70 eV): 522(M^+, 10%), 507(3), 448(4), 396(20), 327(86), 281(7), 259(49), 191(70), 177(27), 163(37), 121(61), 109(65), 95(100), 81(96), 69(98), 55(56).

Hexacyclic alcohol 14. Lithium aluminium hydride in excess was added to a solution of the ester **13** (2.5 mg; 4.8 μmol) in anhydrous tetrahydrofuran (5 ml). The mixture was stirred and refluxed under argon for 2 h. Extraction with CH_2Cl_2 (3×) followed by thin-layer chromatography (SiO_2, CH_2Cl_2) yielded 2 mg (85%) of the alcohol **14**. NMR δ_H (C_6D_6, 500 MHz): 0.78 (3H, s, CH_3), 0.80 (3H, s, CH_3), 0.84 (6H, s, 2 × CH_3), 0.89 (3H, s, CH_3), 0.91 (3H, s, CH_3), 0.93 (3H, s, CH_3), 1.76 (3H, s, CH_3-4′), 3.73 (1H, dd, *J* 11.0 and 4.0 Hz, H-1), 3.81 (1H, dd, *J* 11.0 and 3.0 Hz, H-1), 5.51 (1H, m, H-4). MS (EI, 70 eV): 494(M^+, 12%), 479(5), 396(38), 381(12), 341(8), 327(18), 259(66), 191(38), 175(18), 163(30), 149(28), 121(56), 95(94), 81(100), 69(96).

Hexacyclic alkene 15. Triethylamine and methanesulfonyl chloride were added (under argon) in excess to a solution of the hexacyclic alcohol **14** (2 mg; 4 μmol) in dry CH_2Cl_2. After 4 h, the reaction mixture was poured on ice and extracted with CH_2Cl_2 (3×). The mixture obtained was rapidly filtered through silica gel (CH_2Cl_2). The resulting crude methanesulfonate derivative was reduced under argon with an excess of lithium aluminium hydride in dry THF (reflux). After 2 h, the reaction mixture was poured on to ice and extracted with CH_2Cl_2. The residue was chromatographed on silica gel (TLC, hexane) to give 1.3 mg (67%) of the hexacyclic alkene **15**. MS (EI, 70 eV): 478(M^+, 5%), 464(4), 396(38), 327(15), 259(78), 191(20), 163(28), 135(42), 121(72), 107(80), 95(100), 81(95), 69(88), 55(80).

Hexacyclic C_{35} alkanes 1 and 16. The hexacyclic alkene **15** (1.3 mg; 2.7 μmol) dissolved in ethyl acetate was stirred with platinum dioxide under a H_2 atmosphere for 6 h. After purging with argon and filtration of the reaction mixture through a small silica gel column, the mixture was subjected to reversed phase HPLC (Du Pont Zorbax ODS 250 × 4.6 mm, 5 μm; acetone), yielding 900 μg (69%) of a mixture of the two isomers separable by GC (1 : 1 ratio) but not by HPLC. NMR spectra were measured on the mixture of two isomers **1** and **16**. NMR δ_H (C_6D_6, 500 MHz): 0.83 (3H, s, CH_3), 0.84 (3H, s, CH_3), 0.85 (6H, s, 2 × CH_3), 0.86 (3H, s, CH_3), 0.88 (3H, s, CH_3), 0.93 (3H, s, CH_3), 0.90 (d, *J* 7.0 Hz, CH_3 isomer a), 1.09 (d, *J* 6.5 Hz, CH_3 isomer b), 1.10 (3H, d, *J* 6.5 Hz, CH_3); isomer **1**: MS (EI, 70 eV): 480(M^+, 36%), 465(18), 395(12), 327(22), 299(38), 259(48), 231(80), 191(64), 163(100), 123(50), 109(62), 95(86), 81(82), 69(78), 55(72); isomer **16**: MS (EI, 70 eV): 480(M^+, 42%), 465(22), 395(72), 327(28), 299(20), 259(38), 231(42), 191(48), 163(56), 123(58), 109(64), 95(100), 81(98), 69(96), 55(64).

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