Identification of Two C-10 Demethylated C_{28} Hopanes in Biodegraded Petroleum

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Two C-10 demethylated C_{28} hopanes have been isolated from a biodegraded asphalt and characterised by mass spectrometry and single-crystal *X*-ray diffraction; their structures reveal a major microbiological pathway of degradation of the widespread hopane triterpene skeleton.

The precise structural identification of molecular fossils occurring in sediments and petroleums has proved extremely useful for the understanding of the origin of geological organic matter and the transformations taking place in the subsurface; in particular the biomarkers, which are in a structural sense information-rich compounds deriving from often specific biological precursors, are being increasingly used in petroleum exploration.[1] Among the latter, the ubiquitous hopanes essentially originate from polar lipidic membrane constituents of procaryotic organisms.[2]

Increasing interest has been given recently to demethylated triterpene hydrocarbon skeletons.[3—12] Some of them show in their mass spectra an intense peak at *m/z* 177 and appear to be homologues belonging to series frequently encountered in the alkane mixtures of heavy crude oils which have been naturally biodegraded in their reservoirs, as shown by the 177 mass fragmentogram distribution obtained in the course of GC-MS studies.[4—7] These series often parallel that of the regular hopane series found in undegraded oils which display an important *m/z* 191 ion. On the basis of the mass spectral and ^{1}H NMR data of a C_{29} member of one homologous series isolated by preparative GC from an asphalt, it has been proposed that these series of demethylated triterpene hydrocarbons detected in *m/z* 177 fragmentograms are most likely 25-nor-17α-hopanes and 25-nor-21β-hopanes.[6] However, the conclusive structural elucidation of these components remained to be carried out. One of the major problems encountered in accurate structural determination of these alkanes lies in the difficulty of obtaining sufficient quantities of pure compounds from the highly complex hydrocarbon mixtures. We report here the first unambiguous characterisation of two 25,30-dinor-17α-hopanes (**1**) and (**2**) isolated from a heavy oil-impregnated sandstone (Loufika outcrop, Congo).

The asphalt (10% of sandstone) was extracted from the rock by a mixture of toluene–methanol (3 : 1). After de-asphalting with hexane, the hexane-soluble material (28% of asphalt) was fractionated on silica gel columns and the alkane fraction (10% of asphalt) obtained by elution with hexane was further separated by reverse-phase HPLC [RP-18; elution with ethanol–chloroform (80 : 20) or methanol–chloroform (85 : 15)]. This procedure led to the isolation of one major (**1**) (16 mg, 600 p.p.m. of asphalt) and one minor (**2**) (2 mg, 75 p.p.m. of asphalt) pure compound (>99% by GC).

The mass spectra† of both compounds, which are quite similar, exhibit a molecular ion at *m/z* 384 corresponding to $C_{28}H_{48}$ and a prominent base peak at *m/z* 177 without other intense ion fragments. This is consistent with a triterpene hydrocarbon which has lost a methyl group in the *A/B* portion as well as in the *D/E* moiety of the pentacyclic skeleton and which has an ethyl substituent in the *D/E* part of the molecule, as suggested by the presence of the fragmention at *m/z* 355 (Figure 1).

The complete structures and stereochemistry of (**1**) and (**2**) were established by *X*-ray analyses‡ of monocrystals obtained by recrystallisation from a mixture of methanol–dichloromethane. Both products have lost the 25-methyl group, but have kept identical stereochemistry at C-10 as compared with the undegraded skeleton; configurations at C-17, C-18, and C-21 are α. They differ from each other by the location of the 28-methyl group, which in the case of the minor compound (**2**) has migrated from the 18α to the 17α position, as in the case of the C_{27} rearranged hopane.[13]

It should be noted that these compounds have extremely close retention times in GC and may therefore remain unresolved under standard analytical conditions [DB5 J&W, 30 m × 0.25 mm × 0.1 μm, H_2 : retention time just slightly shorter for (**1**)]. Compound (**2**) probably belongs to a series of demethylated rearranged hopanes which in most cases remain undetected.

The origin of the 25-norhopanes is still controversial. Although there is ample evidence that most of the C-10 demethylated hopanes are biotransformation products of the normal hopane series occurring in crude oils,[3d,5—8] their presence in mature and immature source rocks[11,12] (more than sixty samples[12]) strongly suggests that they could not only be formed during the biodegradation of the oil in the reservoir itself, but also at a much earlier stage during diagenesis in the sediment. Their preservation in biodegraded oils might therefore result from a concentration of minor pre-existing compounds as a result of selective removal of more easily biodegradable polycyclic alkanes (*e.g.*, regular hopanes).

† *Mass spectrometry data* (KRATOS MS80RF, 1000 R.P.) EI (70 eV), *m/z* (rel. int.) for: (**1**) 384(M^+, 34%), 369(14), 355(5), 260(3), 245(2), 217(2), 205(3), 204(3), 193(9), 192(14), 191(11), 177(100), 163(28), 149(14), 135(15), 123(30), 122(26), 121(16), 109(36), 107(19), 95(43), 81(47), 69(38); (**2**) 384(M^+, 29%), 369(9), 355(4), 260(1), 245(1), 217(1), 205(4), 204(1), 193(4), 192(8), 191(6), 177(100), 163(26), 149(14), 135(13), 123(18), 122(15), 121(11), 109(24), 107(14), 95(24), 81(27), 69(19).

‡ *Crystal data* for (**1**): $C_{28}H_{48}$, M = 384.81, orthorhombic system, space group $P2_12_12_1$ Z = 4, a = 21.979(12), b = 14.538(9), c = 7.510(5) Å, U = 2400 Å^3, D_c = 1.065, μ = 4.3 cm^{-1} (λ = 1.5418 Å). Intensity measurements were made with a 4-circle, graphite monochromated Philips diffractometer, using the following scan conditions: mode θ/2θ, speed 0.05° s^{-1}, width 1.2°. From 2514 measured unique reflections, 1919 were used [$I > 3\sigma(I)$]. The structures were solved by direct methods [17] and refined by the large blocks method,[18] minimizing $\Sigma w(\Delta F)^2$. The hydrogen atoms were located on difference-Fourier maps and refined with constraints[19] close to their theoretical positions, with an isotropic thermal factor equal to that of the carbon atom. Final conventional R = 0.053 and final weighting scheme $w = [\sigma^2(F) + KF^2]^{-1}$, where K = 0.0032 and σ from counting statistics. For (**2**): $C_{28}H_{48}$, M = 384.81, monoclinic, $P2_1$, Z = 2, a = 14.945(10), b = 7.843(6), c = 10.486(8) Å, β = 104.97(8)°, U = 1186 Å^3, D_c = 1.077, μ = 4.3 cm^{-1} (λ = 1.5418 Å). From 2358 measured unique reflections 1723 were used [$I > 3\sigma(I)$]. Final conventional R = 0.047, $w = [\sigma^2(F) + KF^2]^{-1}$, where K = 0.0013. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

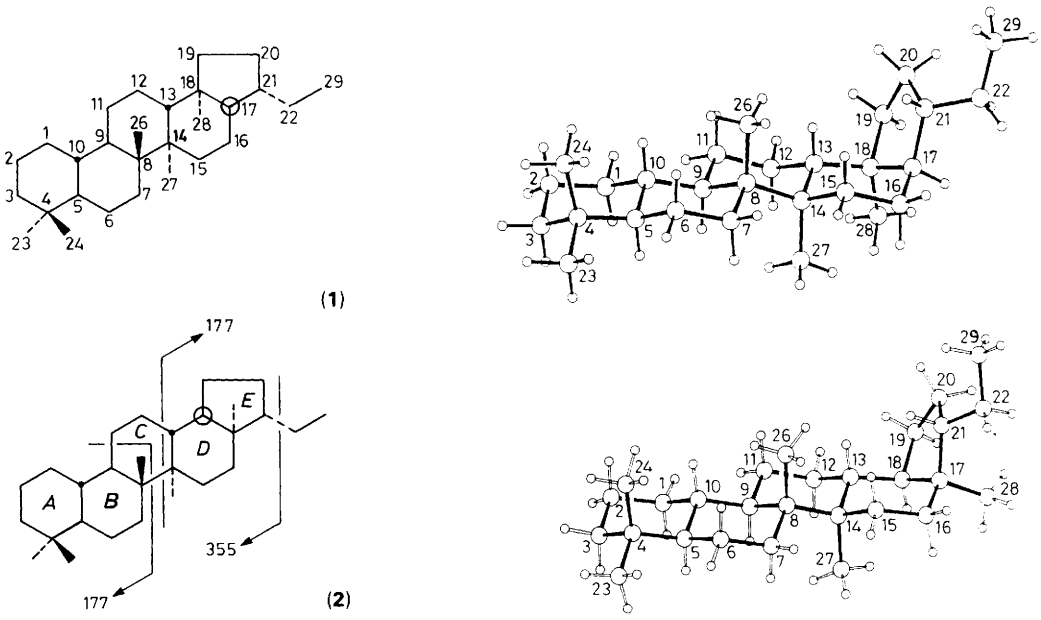

Figure 1. Structures of the two 25-norhopanes from Loufika asphalt obtained by *X*-ray diffraction.

Although laboratory biodegradation studies have as yet failed to produce C-10 demethylated hopanes, but have instead suggested a selective attack on the side chain,[14] the methyl-25 appears to be one of the preferential sites of enzymatic attack on the hopane skeleton, which could lead, depending on the redox conditions prevailing in the sediment (or in the reservoir), either to 25-norhopanes or to the complete degradation of the skeleton. Lack of this methyl group could eventually protect 25-norhopanes from further bacterial degradation as compared to regular hopanes, which would explain their selective preservation and concentration in heavily biodegraded crude oils. Their occurrence in the latter is, however, not absolutely general. Indeed, some of these oils show complete removal of the regular hopanes without detectable 25-norhopane appearance;[15] in some cases other hopane-derived compounds, *e.g.*, 8,14-secohopanes,[16] even become predominant.

In another context, it has been proposed that these demethylated hopanes may be useful maturity parameters for severely biodegraded petroleums for which the usual biomarkers either are totally removed or show preferential degradation of isomers, thus becoming unsuitable for maturity evaluation.[7]

We thank the Société Nationale Elf-Aquitaine (Production) for financial support and supply of geological samples; F. J. Fago and J. M. Moldowan (Chevron Oil Field Research Company) for helpful information.

Received, 7th December 1989; Com. 9/05226G

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