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Geomimetic Autoxidation of Biohopanoids : a Route to *Bis*-hopanoids, Potential New Sedimentary Molecular Fossils

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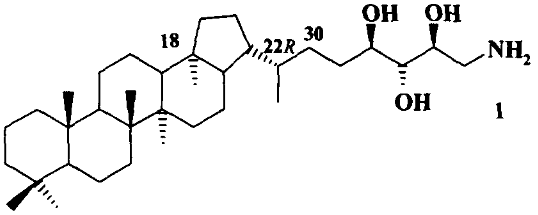
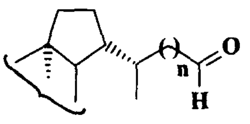
Abstract: Representative biohopanoids **1** and/or **4** were submitted to geomimetic autoxidation as in water or in benzene, as well as without solvent, leading interestingly in the case of benzene to the formation of C_{64} novel *bis*-hopanoids which might be precursors of a still unknown series of sedimentary molecular fossils and, in the latter case, to the formation of a saturated hydrocarbon hopanoid. This last result should provide a new look on the formation of hydrocarbon geohopanoids believed so far to arise exclusively from reductive environments.

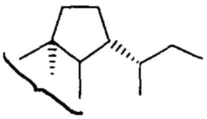
The molecular fossils of bacterial triterpenoids of the hopane series are ubiquitously represented in sediments. In order to understand the involvement of dioxygen in the chemical transformation of biohopanoids into their counterparts found in sediments, the geohopanoids, we initiated autoxidation experiments on a representative bacterial hopanoid, the aminobacteriohopanetriol **1**, as well as on its primary degradation product, the *trisnor*-aldehyde **4**.[1] These experiments fulfilled our expectations as they enabled under very mild conditions the transformation of both compounds into an array of degradation products, all of geochemical value. Despite their performance, they suffered from a serious handicap, as they had to be run in pyridine, the best solvent for the highly insoluble amphiphilic aminotriol **1**, but one quite devoid of any geomimetic significance.



1



n = 0 **2**
n = 1 **3**
n = 2 **4**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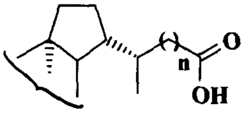

n = 0 **5**
n = 1 **6**
n = 2 **7**

Concerned in the first place with matching geochemical conditions prevailing in an aqueous oxic environment, we ran preliminary experiments starting from **1** under the same conditions as before (*i.e.* O_2 atmosphere, Cu_2Cl_2),[1] but instead of using pyridine as a solvent we used either distilled boiling water alone during 14 h or water in the presence of dipalmitoylphosphatidylcholine (DMPC) as a homogenizing agent during 5 days at 70°C.[2] In both cases, the starting material was mostly recovered unchanged (isolated as its tetracetylated derivative after Ac_2O:Py treatment), only traces (<5 %) of degradation products being isolated in the form of a mixture of aldehydes **3** and **4** in a *ca.* 1:3 ratio as determined by GC. These poorly encouraging results enticed us to interest ourselves in autoxidations run either in benzene, a solvent that would mimic hydrophobic media, or in the complete absence of any solvent.

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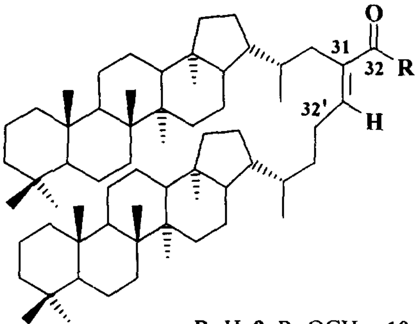
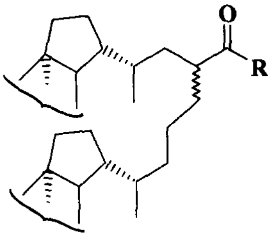
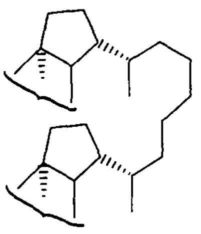
Heating aminotriol **1** under conditions we previously selected[1], but replacing pyridine by benzene, resulted in a much slower oxidative degradation, the starting material being recovered after acetylation in the form of its tetracetate in 70 % yield after TLC, together with small amounts (10 %) of the three aldehydes **2-4** in a 1:2:2 ratio (from GC) as well as the corresponding carboxylic acids **5-7**, isolated as methyl esters in 10 % yield in a 1:2:2 GC ratio after CH_2N_2 treatment. The low solubility of **1** in benzene certainly accounts partly for these differences in reactivity. Instead of repeating experiments on aminotriol **1** under forcing conditions, we preferred to start from the C_{32} aldehyde **4**, already recognized as the key-intermediate towards a series of oxidized hopanoids of geochemical interest.[1]



R=H, **9**; R=OCH_3, **10**



R=H, **11**; R=OCH_3, **12**



13

In a typical experiment, aldehyde **4** (20 mg, >99 % pure on GC) was refluxed under O_2 (1atm) and strong stirring in dry benzene (5 ml) during 5 days in the presence of Cu_2Cl_2 (10 mg) and 4 Å molecular sieves (*ca.*20 mg). The reaction mixture was then purified after filtration by TLC (Cy:EtOAc, 98:2, v/v) to yield, next to a fraction (3 mg) of the same polarity as the starting product appearing on GC to contain a 1:9 mixture of the two aldehydes **3** and **4**, two major fractions, one on the base line, the other at R_f= 0.5 as well as two minor fractions at R_f= 0.9 and 0.7. The principal fraction (4 mg) at R_f= 0.5 appeared at first glance in ^{1}H-NMR as an equimolar mixture of two simple hopanoids, exhibiting in particular two shielded singlets at 0.699 and 0.746 ppm characteristic of 18α hopanoid methyl groups as well as two doublets at 0.952 and 0.968 ppm characteristic of 22*R* methyl groups. Indeed, resonances at *ca.* 0.70 and 0.95 ppm are encountered for instance in hopanal **4**, more deshielded ones at *ca.* 0.74 and 0.97 ppm being found in the spectrum of its *nor*-derivative **3** which possesses a functionality closer to the hopane framework. In view of the impossibility to separate by TLC or by HPLC two compounds, we concluded that the fraction contained in fact a single product of structure **9**[3], belonging to a *bis*-hopanoid series never reported so far and corresponding to the product of aldolization and crotonization of the starting aldehyde. Support to the dimeric structure was obtained by repeating the synthesis of **9** under more standard chemical methods. After several assays under basic or acidic conditions, the condensation leading to **9** proved to work best using the procedure described by Casnati *et al.*[4] Additional structural proof came from UV spectroscopy as well as mass spectrometry, the molecular mass of **9** being in particular well observable at m/z = 890 whereas UV data proved characteristic of an α,β-unsaturated aldehyde. The *E* configuration of **9** with its two hopanyl moieties on the same side of the double bond was unambiguously established in ^{1}H-NMR by the very strong (20 %) nOe observed between the aldehyde proton and the vinylic proton at C-32'.

The other important fraction, localized on the base line, yielded after CH_2N_2 treatment and a further TLC (Cy:EtOAc, 99:1, v/v) not only a mixture of the methyl esters from the carboxylic acids **5-7** (3 mg, R_f= 0.2) in the respective 0.1:1:2 GC ratio but also, interestingly at a higher R_f (0.35), a minor product isolated in 4 % yield corresponding to the methyl ester of *bis*-hopanoid structure **10**. The configuration of **10** could not be deduced, contrary to **9**, from nOe investigations in ^{1}H-NMR but was conforted from its chemical correlation with the methyl ester prepared in 60 % yield after CH_2N_2 treatment of the carboxylic acid obtained by oxidation of aldehyde **9** using sodium chlorite as an oxidant and 2-methyl-2-butene as a chlorine trap.[5a] Under these conditions this oxidation reaction has been recognized as stereospecific;[5b] it overtook also by far the method described by Corey *et al.*[5c] which proved inefficient in our case.

From the two minor fractions, only one ($R_f = 0.6$, isolated in 2 % yield) appeared as a pure compound and corresponded to the stereomer of aldehyde **9** (*i.e.* the *Z* isomer), the other fraction almost at the solvent front appearing in GC as a complex mixture of hydrocarbons.

Experiments without solvent were run after depositing a film of aldehyde **4** from its $CHCl_3$ solution on the surface of either a Pyrex round-bottomed flask or a teflon-covered pan. In both cases, samples were kept in the dark and in the presence of air at 110°C for 5 days, giving after TLC (Cy:EtOAc, 98:2, v/v) a minor fraction containing rest of the starting hopanoid (5 %) next to two fractions, one on the base line and another one located on the solvent front. After CH_2N_2 treatment and further TLC, the base line proved to contain a mixture of the methyl esters of the acids **6** and **7** in the respective 1:5 ratio from GC. The apolar fraction appeared in GC essentially represented (90 %) by a single peak corresponding to homohopane **8**.[3] The structure of this latter hydrocarbon was confirmed by preparing it independently in 50 % yield using on aldehyde **4** the classical decarbonylation procedure over the Wilkinson's catalyst.[6]

As can be seen, these two sets of experiments performed either in benzene or without solvent appeared less sluggish and more interesting than those run in water, leading in one case to the discovery of compounds from the C_{64} *bis*-hopanoid series and, in the other case, to a saturated hydrocarbon in C_{31} obtained under oxic conditions. These experiments appeared in addition complementary as no traces of dimeric hopanoids were detected in the solid phase investigations whereas only traces of **8** among a complex hydrocarbons mixture could be seen in GC in the case of autoxidation experiments run in benzene.

Focusing first on the autoxidations without solvent, we demonstrated that the degradation pattern did not depend on the nature of the support used (Pyrex or teflon). Obtention of appreciable quantities of the saturated hydrocarbon **8** under oxic conditions appeared at first sight surprising. Although we did not investigate the mechanism of its formation, the latter homohopane probably arose from a carboxylic precursor or even a C_{32} peracid, thermal conversions of carboxylic acids into saturated hydrocarbons with one carbon atom less having already been reported in the literature.[7] Formation of a saturated hydrocarbon starting from the immediate degradation product from a biohopanoid under oxic conditions has never been reported so far to our knowledge. It will certainly provide a new look on the origin of geohopanoid hydrocarbons believed so far to arise from reductive environments.[8]

In the case of experiments in benzene, we observed as a major product the compound deriving from the aldolization and crotonization of aldehyde **4**. Neither O_2, nor Cu_2Cl_2 proved here necessary to the obtention of *bis*-hopanoid **9**. Indeed, when we repeated the experiment in benzene and under argon gas in the presence only of molecular sieves, we got roughly the same amount in **9**. On the contrary the role of the molecular sieves, probably both as dehydrating agents as well as a catalyst, appeared essential as an experiment run without them was not conclusive. As molecular sieves are not in contradiction at all with a sedimentary environmental medium, matching certain types of zeolites, we can anticipate that members from the new *bis*-hopanoids decribed above are likely to occur in some sediments. Such derivatives have never been so far reported. This may come from the very simple fact they are not detectable by the standard analytical GC-MS analyses used by geochemists looking for lipids of lower molecular weight.

In order to obtain other *bis*-hopanoid references susceptible to be encountered in sediments *i.e.* that could reasonably arise from diagenetic maturations in sediments, *bis*-hopanoid **9** was hydrogenated first under H_2 (1atm) over Pd/C in isopropanol into the aldehydes **11** in 60 % yield. The latter compounds were submitted then to the same type of heating experiments under solvent-free conditions we used to convert C_{32} aldehyde **4** into the corresponding carboxylic acid as well as into the saturated hydrocarbon **8**. After 5 days at 115°C at the air, (31*R,S*) *bis*-hopanals **11** were transformed in the same way not only into the corresponding carboxylic acids isolated as methyl esters **12** after CH_2N_2 treatment, both isomers at C-31 being separable by TLC (Cy:EtOAc, 99:1, **12a**, 10 % yield, $R_f = 0.3$; **12b**, 5 % yield, $R_f = 0.2$), but also into the desired *bis*-hopanoid hydrocarbon **13** in C_{63} (15 % yield).

With these references in hand, the final decisive step lies of course in the recognition of such new potential molecular fossils in sediments.

Acknowledgements

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References and notes

1. Bisseret. P.; Rohmer, M. *Tetrahedron Lett.* **1995**, *36*, 7077-7080 and references cited therein.

2. In this case, at first, a stable suspension of aminotriol **1** could be realized in the presence of an excess (ca. 7eq.) of DMPC and Cu_2Cl_2 (5eq.) by prolonged sonication. An essay realized without copper salts did not lead to a stable homogenous medium.

3. All new compounds afforded analytical data in accordance with their structures. *Selected analytical data* (^{1}H-NMR: 250 MHz, $CDCl_3$):

8: ^{1}H-NMR δ 0.703 (3H, s), 0.791 (3H, s), 0.806 (3H, t, J=7.5 Hz), 0.815 (3H, s), 0.846 (3H, s), 0.910 (3H, d, J=6.5 Hz), 0.951 (6H, s).

9: ^{1}H-NMR δ 0.699 (3H, s), 0.746 (6H, s), 0.791 (6H. s), 0.816 (6H, s), 0.846 (6H, s), 0.952 (3H, d, J=6.5 Hz), 0.954 (12H, s), 0.968 (3H, d, J=6.5 Hz), 2.3 (4H, m), 6.44 (1H, t, J=7.5 Hz), 9.349 (1H, s); ^{13}C-NMR (63 MHz, $CDCl_3$) δ 15.86, 15.93, 16.53, 16.63, 18.73, 19.54, 19.99, 20.99, 21.62, 22.85, 22.98, 24.01, 26.29, 27.78, 30.59, 33.28, 33.33, 33.43, 33.75, 33.79, 34.67, 36.85, 37.03, 37.44, 40.37, 41.64, 41.71, 41.75, 41.85, 42.15, 44.38, 44.42, 46.02, 47.37, 49.37, 49.39, 50.48, 54.52, 54.79, 56.18, 142.88, 156.62, 195.65; MS (electronic impact) m/z (%) 890 (M^+, 2 %), 875 (3 %), 857 (4 %), 669 (30 %), 651 (28 %), 395 (40 %), 369 (10 %), 367 (20 %), 191 (100 %); UV (Cy) λmax: 229 nm, ε=7990.

10: ^{1}H-NMR δ 0.696 (3H, s), 0.737 (3H, s), 0.791 (6H, s), 0.816 (6H, s), 0.846 (6H, s), 0.951 (12H, s), 3.696 (3H, s), 6.75 (1H, t, J=7.5 Hz).

12a: ^{1}H-NMR δ 0.645 (3H, s), 0.686 (3H, s), 0.785 (6H, s), 0.807 (6H, s), 0.841 (3H, s), 0.939 (12H, s), 3.634 (3H, s); MS (*i*-butane CI) m/z (%) 923 (M^++1, 16 %), 455 (33 %), 369 (23 %), 329 (30 %), 287 (100 %), 233 (40 %), 191 (88 %).

12b: ^{1}H-NMR δ 0.692 (3H, s), 0.707 (3H, s), 0.791 (6H, s), 0.815 (6H, s), 0.846 (6H, s), 0.948 (12H, s), 3.653 (3H, s); MS (*i*-butane CI) m/z (%) 923 (M^++1, 8 %), 879 (8.5 %), 861 (9 %), 701 (11 %), 657 (11 %), 455 (15 %), 396 (13 %), 369 (50 %), 287 (65 %), 233 (26 %), 191 (100 %).

13: ^{1}H-NMR δ 0.692 (6H, s), 0.784 (6H, s), 0.806 (6H, s), 0.804 (6H, s), 0.941 (12H,s); MS (*i*-butane CI) m/z (%) 863 (2 %, M^+-1), 571 (10 %), 439 (6 %), 369 (7 %), 343 (16 %), 329 (31 %), 287 (100 %), 279 (28 %), 252 (26 %), 191 (50 %), 177 (52 %).

4. Casnati, G.; Pochini, A.; Salerno, G.; Ungaro, R. *Tetrahedron Lett.* **1974**, *12*, 959-962. This procedure using 2,4,6-trimethylphenoxy magnesium bromide as an aldolization and crotonization catalyst worked with a modest yield of 40 %, overtaking nevertheless other procedures like the one using boric acid as a catalyst described in: Offenhauer, R., D.; Nelsen, S., F. *J. Org. Chem.* **1968**, *33*, 775-777 which led to sluggish reactions, probably because of the high molecular weight of the required dimeric hopanoid.

5.(a) Salerno, G.: Ungaro, R.; Krus, G., A.; Roth, B. *J. Org. Chem.* **1980**, *45*, 4825-4830; (b) Bal, B., S.; Childers, W., E.; Pinnick Jr.; Pinnick, H., W. *Tetrahedron*, **1981**, *37*, 2091-2096; (c) Corey, E., J.; Gilman, N., W.; Ganem, B., E. *J. Am. Chem. Soc.* **1968**, *90*, 5616-5617; (d) The high chemical shift of the vinylic proton in **10** is in addition characteristic of *E*-isomers of trisubstituted unsaturated methyl esters as debated in: Morimoto, Y.; Shirahama, H. *Tetrahedron* **1996**, *52*, 10631-10652.

6. Baird, M., C.; Nyman, C., J.; Wilkinson, G. *J. Chem. Soc.* A **1968**, 348-351.

7. Barton, D., H., R.; Dowlatshahi, H., A.; Motherwell, W. B.; Villemin, D. *J. Chem. Soc. Chem. Comm.* **1980**, 732-733.

8. See for instance: Ourisson, G.; Albrecht, P. *Acc. Chem. Res.* **1992**, *25*, 398-402.

9. An attempt to decarbonylate aldehydes **11** over Wilkinson's catalyst, *i.e.* using the same procedure we used on **4**, failed completely.

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