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From Bio- to Geohopanoids: an Efficient Abiotic Passage Promoted by Oxygen in the Presence of Cuprous Chloride

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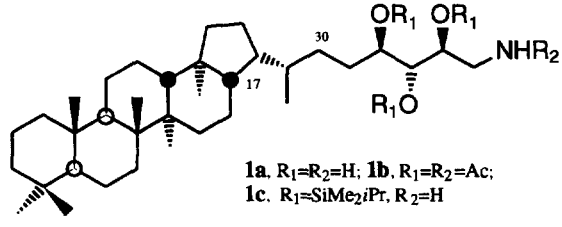
Abstract: In the presence of cuprous chloride, the representative biohopanoid **1a** was smoothly converted by oxidation in pyridine into aldehydes, ketones and carboxylic acids with the same skeletons as those of molecular fossils ubiquitously found in the organic matter of sediments.

With their polyhydroxylated C_5 side-chains and their all-*trans* triterpenic skeleton, biohopanoids such as the representative aminotriol **1a** have asserted themselves in the past twenty years as an important class of natural products specific to many bacteria of diverse origin.[1] They have been so far almost exclusively isolated from axenic bacterial strains cultivated in the laboratory, many of them being representative of microbial populations in sedimentary environments. Indeed, biohopanoids are obviously also widespread in microorganisms from natural sites, as witness their many chemical fossils called geohopanoids, encountered in every sediments and which often possess closely related skeletons but shorter less-functionalized side-chains.[2] If the bio- to geohopanoids relation is in this sense undeniable, the detailed mechanisms that govern the transformations of bacterial triterpenoids into geohopanoids remain largely obscure. We already have engaged ourselves with success in geomimetic experiments involving heating of a type-biohopanoid in molten sulphur to account for the epimerization at C-17 found in the skeletons of geohopanoids from older sediments;[3] in this work, we present simple and smooth autoxidation experiments performed on the widespread biohopanoid **1a** that led to an efficient degradative stripping of its polyfunctionalized side-chain, giving two series of hopanoids of geochemical value.

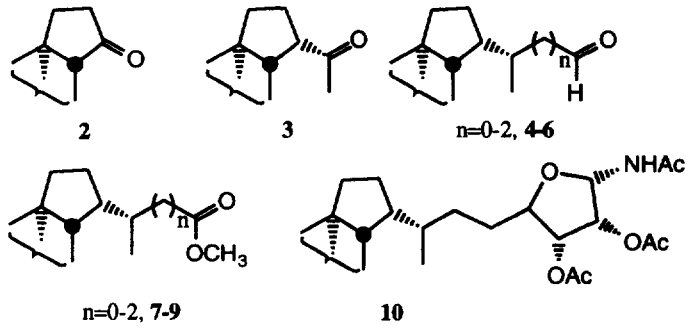


1a, R_1=R_2=H; **1b**, R_1=R_2=Ac;
1c, R_1=$SiMe_2iPr$, R_2=H

We first studied the fate of aminotriol **1a** under oxic conditions (O_2, 1Atm) using pyridine as a solvent and in the presence of cuprous chloride (Cu_2Cl_2), *i.e.* under conditions known for to the oxidation of a primary aliphatic amino group.[4] To ensure a minimum of 85% conversion of the starting material, the reaction mixture was kept 14h at 70°C.[5] After filtration of the inorganic salts and evaporation of the solvents, the solid residue was

submitted to a silicagel column chromatography, giving after elution with CH_2Cl_2 in 30% yield an apolar fraction and, in 60% yield, after elution with $CHCl_3/CH_3OH$ (4/1, v/v) a series of polar compounds. The apolar fraction could be directly analyzed in gc-ms by comparison with reference compounds available in our laboratory. It consisted in *trisnor*hopanone **2** as a minor product (1% of the fraction) and in the four oxo-derivatives **3-6** as major products in the following percentages in the fraction: **3** (15%), **4** (30%), **5** (34%), **6** (20%). Confirmation of the stucture of these compounds was obtained by 250MHz ^{1}H-nmr after further silicagel tlc purification of the fraction, yielding after two migrations in cyclohexane/$CHCl_3$ (2/1, v/v) in order of decreasing polarity **4** (R_f=0,6), a mixture of **5** and **6** (R_f=0,5), adiantone **3** (R_f=0,4) and finally *trisnor*hopanone **2** (R_f=0,25).

As far as the polar compounds are concerned, they were separated after CH_2N_2 treatment and acetylation (Ac_2O/Py, 1/1, v/v; 12h, 20°C) on silicagel tlc not only into rest (15%) of the starting material in the form of its tetraacetylated derivative **1b** accompanied in 10% yield by its immediate oxidation product isolated as a triacetate **10**, but also interestingly into a series of methyl-esters **7-9** in a 5/3/2 respective ratio and readily identified in gc-ms by comparison with reference compounds. The ribosylamine derivative **10** exhibited in ^{1}H-nmr[6] an anomeric proton at 6ppm with a coupling constant of 5Hz with its neighbouring proton on the C_5 ring characteristic of an α anomer, in agreement with the reported formation of only the α anomer after acetylation of D-ribosylamine.[7]



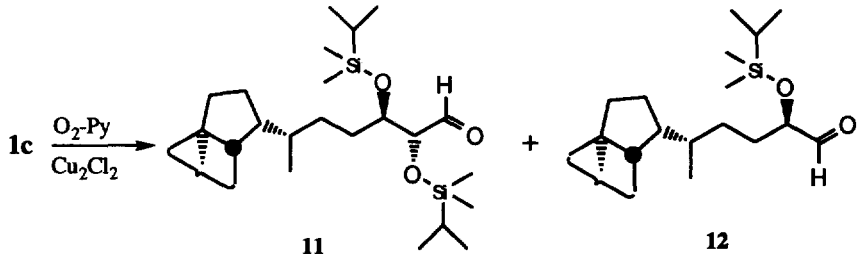
2 **3** n=0-2, **4-6**

n=0-2, **7-9** **10**

The presence of copper salts appeared necessary to ensure an efficient degradative stripping of the acyclic moiety of aminobacteriohopanetriol **1a** as demonstrated by an independent experiment run under the same conditions but without cuprous salt. In this case, the starting material, isolated as its tetraacetylated derivative, was recovered in 95% yield. Indeed, as already reported occasionally in the literature,[8] the copper salts assist the cleavage of the vicinal hydroxy functions in **1a**, giving access to the C_{32} aldehyde **6**. The intermediate key-position of this latter aldehyde in the degradation of aminotriol **1a** could be checked further by submitting it to our typical oxidizing procedure. It was converted almost totally (>98%) into the same series of compounds as in the case of **1a**, *i.e.* for two third into the shorter derivatives **2-5** and for the rest into carboxylic acids identified as the methyl-esters

derivatives **7-9** in the respective 2/1/1 ratio. For the degradation of aldehyde **6**, the rôle of the cuprous salt did not prove to be essential as the same degradation pattern was observed in an autoxidation experiment run without it. Its oxidation appeared to follow pathways already reported for aliphatic enolizable aldehydes, an initial attack of oxygen on the aldehyde group leading to carboxylic acids with the same number of carbon atoms whereas an attack next to this group generated a carbonyle derivative with one carbon atom less.[9] The lack of formation of the aldehyde in C_{28} results probably from the tendency of the C_{29} oxo-derivative **3** to react with oxygen under its most stable tetrasubstituted enol form.

By curiosity we extended our autoxidation conditions to hopanoid **1c** of easy access by treatment of the free aminotriol with excess of isopropyldimethylchlorosilane in pyridine followed by a SiO_2 tlc purification as already detailed.[10] We were pleased to discover that the starting compound was converted in 55% yield into the two immediate shorter homologues **11** and **12** in a 3:2 ratio.[6] The isopropyldimethylsilyl protection proved to offer here a moderate resistance to the oxidizing conditions, making it particularly suitable to track aldehydo-derivatives with C_{33} and C_{34}-biohopanoid frameworks.



11 **12**

The oxidizing conditions we chose proved very efficient to convert within a few hours and at a moderate temperature the type-biohopanoid **1a** into an array of degraded hopanoids which are all unknown presently in living organisms, except the norhopanone **3** that has been found so far essentially in a few ferns from the genus *Adiantus*.[11] Indeed, the three methyl-esters **7-9** are derivatives from carboxylic acids widespread in sediments of various age and origin as they are not only present in very immature ones like lacustrine deposits or soils,[12] but are also found in ancient sediments with a well preserved structural integrity, with the exception of their configuration at C-17 which tends to epimerize to the more thermodynamically stable 17α(H) one when experiencing diagenetic maturation.[13] The carbonyl-derivatives **2-6**, although of exceptional occurence in sediments,[2b,13] represent the reduced counterpart of the former carboxylic acids (for aldehydes **4-6**) or oxidized counterpart of series of saturated geohopanoid hydrocarbons which are once again nearly ubiquitous in sediments.[2a,12,13] The absence of any C_{28} hopanoid in our oxidation experiments accounts for the absence of C_{28} geohopanoids.

Apart from the fact that copper salts are usually only present in very low amounts in sediments,[14] the geomimetic character of our experiments is on the whole at this stage open to

criticism as they were, in the first place, run in pyridine, a solvent which nevertheless guarantees the complete solubilization of **1a** reknown for its reluctance to solubilize in nearly every solvent including water. Despite of that, our study militates for the essential and early degradative action of oxygen on biohopanoids once in the presence of copper salts, as well as for the importance of the C_{32} aldehyde **6** as an intermediate in this degradative process. From an environmental view point, it enlightens the strong competition of abiotic *versus* biological processes offered by oxic or even probably suboxic conditions on the degradative stripping of the side-chains of biohopanoids. Contrary to sulphur,[3] oxygen did not appear to affect at all the biohopanoid type all-*trans* skeleton, as even trisnorhopanone **2**, of high epimerizability at C-17, was found as a pure 17β(H) epimer by nmr after SiO_2 tlc isolation.

We are now conducting further autoxidation experiments on **1a** under more geomimetic conditions, switching cuprous chloride to iron salts, which are more commonly encountered in sediments, and eliciting water in the presence of phospholipids as a medium.

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5. *Typical procedure*: To aminotriol **1a** (10mg) in solution in pyridine (3ml) at 70°C was added cuprous chloride pure at 99% (7mg). After 14h of stirring at the same temperature under O_2 (1Atm), the reaction was treated as indicated in the text.
6. All new compounds afforded analytical data in accordance with their structures. *Selected analytical data*: **10**: ^{1}H-nmr (250MHz, $CDCl_3$) δ 0.688 (3H, s), 0.814 (3H, s), 0.845 (3H, s), 0.947 (6H, s), 2.041 (3H, s), 2.099 (3H, s), 2.124 (3H, s), 4.01 (1H, m), 5.12 (1H, t, J=0.5Hz), 5.29 (1H, t, J=5.0Hz), 5.99 (1H, dd, J=5.O&9.5Hz), 6.20(1H, d, J=9.5Hz); ms (electronic impact) m/z (%) 669 (M^+, 5%), 610 (4%), 490 (5%), 448 (50%), 434 (15%), 389 (17%), 369 (15%), 329 (12%), 269 (20%), 191 (100%). **11**: ^{1}H-nmr (250MHz, $CDCl_3$) δ 0.054 (3H, s), 0.061 (3H, s), 0.074 (6H, s), 0.698 (3H, s), 0.792 (3H, s), 0.815 (3H, s), 0.847 (3H, s), 0.951 (3H, s), 3.83 (2H, m), 9.60 (1H, d, J=2Hz). **12**: ^{1}H-nmr (250MHz, $CDCl_3$) δ 0.079 (3H, s), 0.084 (3H, s), 0.697 (3H, s), 0.792 (3H, s), 0.816 (3H, s), 0.949 (6H, s), 3.92 (1H, dt, J=1.7&6Hz), 9.58 (1H, d, J=1.7Hz).
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