Pergamon

*Tetrahedron Letters*, Vol. 37, No. 11, pp. 1791-1794, 1996

0040-4039/96 $15.00 + 0.00

S0040-4039(96)00133-5

# Relative Configurations of Carbapseudopentose Moieties of Hopanoids of the Bacterium *Zymomonas mobilis* and the Cyanobacterium '*Anacystis montana*'

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**Abstract:** A bacteriohopanetetrol cyclitol ether with a new configuration of its carbapseudopentose moiety has been isolated from the cyanobacterium '*Anacystis montana*' and compared to a similar hopanoid previously isolated from *Zymomonas mobilis*. As shown by two dimensional $^1$H-NMR spectroscopy using Nuclear Overhauser Effect correlations, both compounds were diastereomers and differed from a third stereomer found in the biphytanyl lipids of the thermoacidophilic archaebacterium *Sulfolobus* sp.

Triterpenoids of the hopane series present a huge diversity of side-chain structures.[1] In several bacteriohopanetetrol or bacteriohopanepentol derivatives, a carbapseudopentose moiety is linked via an ether bond to the C-35 hydroxy group of the polyhydroxylated side-chain.[2] For all such bacteriohopanepolyol ethers isolated to date, the identity of the $^1$H- and $^{13}$C-NMR data concerning the cyclitol moiety pointed toward the existence of a single diastereomer. Tentative identification of the configuration has been already attempted for the compound isolated from *Zymomonas mobilis*,[3] but could not be confirmed in this study. We wish to report here the isolation and identification of a new bacteriohopanetetrol cyclitol ether from the cyanobacterium '*Anacystis montana*',[4] and the determination of the relative configurations of the two related carbapseudopentose series found in the tetrol ethers from '*A. montana*' on the one hand and from the previously investigated bacteria on the other hand.

TLC separation of the acetylated $CHCl_3/CH_3OH$ extract from '*A. montana*', afforded diplopterol and the mixture of acetylated hopanoids **1** and **2**.[5] All data ($^1$H- and $^{13}$C-NMR, mass spectrometry) were quite similar to those of the heptaacetate of the bacteriohopanetetrol cyclitol ether previously isolated and led to assign the same planar structure to both compounds. Comparison of $^1$H-NMR spectra of the carbapseudopentose moieties from the two hopanoids isolated from '*A. montana*' or *Z. mobilis* showed however significant differences for chemical shifts and coupling constants (Tab. 1), suggesting the structures of two diastereomers. One of the most striking features was the presence of a $^4$J W coupling constant (1,5Hz) between the H-1 and the H-4 protons in the '*A. montana*' hopanoid, which was not observed in the *Z. mobilis* hopanoid. This suggested different configurations at C-1 in the two carbapseudopentoses. Two-dimensional Nuclear Overhauser Effect correlation experiments (NOESY) carried out on both compounds[6] allowed to determine the relative configurations, which differ one from another at C-1 and C-5 (Fig. 1).

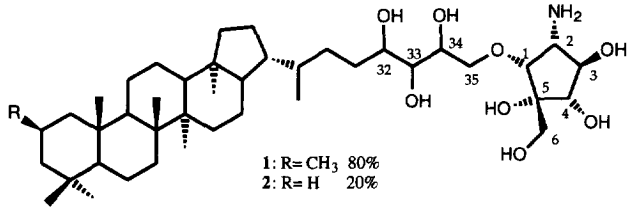
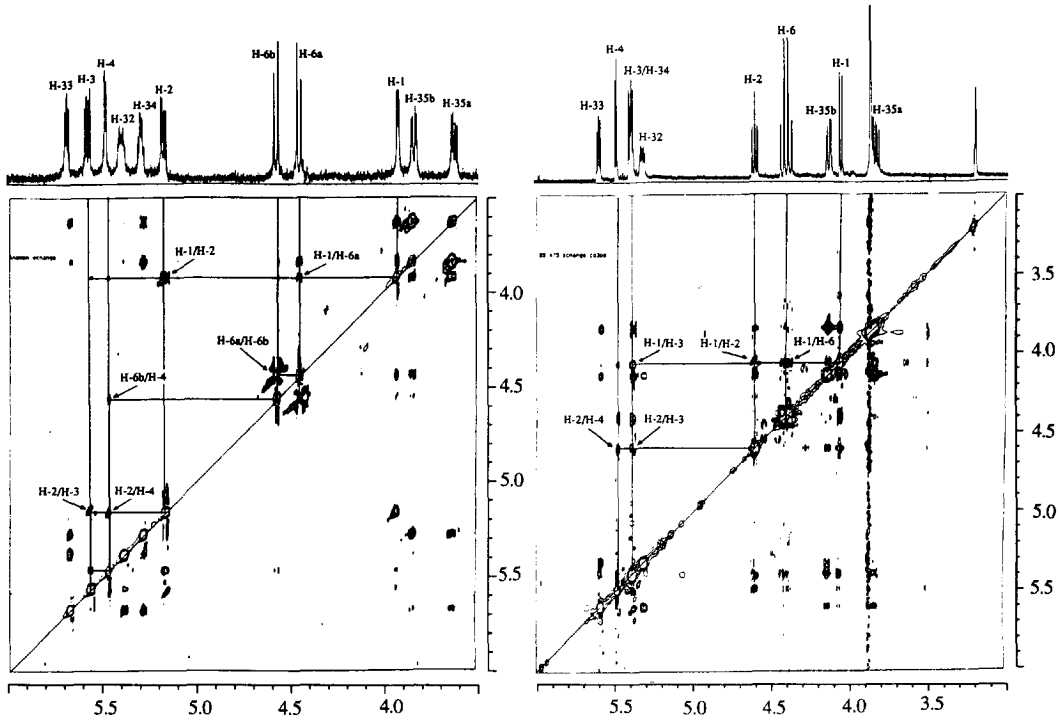
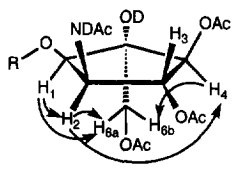
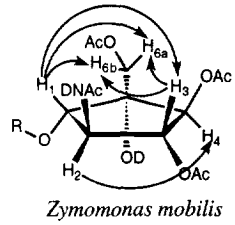



Figure 1. NOE observed between protons of cyclitol moieties of hopanoids isolated from '*Anacystis montana*' and *Zymomonas mobilis* after $CD_3OD$ exchange.

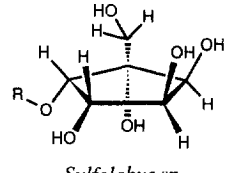



*'Anacystis montana'*




*Zymomonas mobilis*




*Sulfolobus* sp.

Table 1. Comparison of $^1$H-NMR spectra ($C_6D_6$) of bacteriohopanetetrol cyclitol ethers isolated from '*Anacystis montana*' and *Zymomonas mobilis*

| | *'Anacystis montana'* | | *Zymomonas mobilis* | |
|---|---|---|---|---|
| | δ (ppm) | J (Hz) | δ (ppm) | J (Hz) |
| H-1 | 3.80 (dd) | $J_{1-2}$ = 4.5<br>$J_{W1-4}$ = 1.5 | 4.06 (d) | $J_{1-2}$ = 7.5 |
| H-2 | 5.22 (ddd) | $J_{2-1}$ = 4.5<br>$J_{2-NH}$ = 8.5<br>$J_{2-3}$ = 9.5 | 4.27 (q) | $J_{2-1}$ = 7.5<br>$J_{2-NH}$ = 7.5<br>$J_{2-3}$ = 7.5 |
| H-3 | 5.63 (dd) | $J_{3-2}$ = 9.5<br>$J_{3-4}$ = 5.0 | 5.25 (dd) | $J_{3-2}$ = 7.5<br>$J_{3-4}$ = 5.5 |
| H-4 | 5.45 (dd) | $J_{4-3}$ = 5.0<br>$J_{W4-1}$ = 1.5 | 5.61 (d) | $J_{4-3}$ = 5.5 |
| NH | 6.70 (d) | $J_{NH-2}$ = 8.5 | 5.49 (d) | $J_{NH-2}$ = 7.5 |
| H-6a | 4.37 (d) | $J_{6a-6b}$ = 11.5 | 4.34 (d) | $J_{6a-6b}$ = 11.5 |
| H-6b | 4.45 (d) | $J_{6b-6a}$ = 11.5 | 4.45 (d) | $J_{6b-6a}$ = 11.5 |

Incorporation of $^{13}$C labelled D-glucose into the hopanoids of *Z. mobilis* and *Methylobacterium fujisawaense* or of $^{13}$C labelled acetate into those of *Methylobacterium organophilum* showed that the five-membered ring of the carbapseudopentose is made by formation of a carbon/carbon between C-1 and C-5 of a D-hexose skeleton. A hypothetical biogenetic pathway could be proposed starting from D-fructose and resembling that involved in the formation of *myo*-inositol from D-glucose. The numbering of the carbapseudopentose five-membered ring is thus directly derived from the D-glucose numbering, and the absolute configurations, *i.e.* (1*R*, 2*R*, 3*R*, 4*S*, 5*S*) for the '*Anacystis montana*' hopanoid and (1*S*, 2*R*, 3*R*, 4*S*, 5*R*) for the *Z. mobilis* hopanoid, correspond to those expected from D-glucose and D-fructose as precursors.[7]

Recently a revised structure has been proposed for the nonitol found in the polar heads of the biphytanyl lipids of the thermoacidophilic archaebacterium *Sulfolobus* sp. This nonitol consists of glycerol linked via an ether bond to a carbapseudopentose with same carbon skeleton and hydroxylation pattern as those found for the two former carbapseudopentoses in eubacteria but with a different configuration.[8] It remains now to be tested whether all these pseudopentoses from eubacteria and archaebacteria are biogenetically related.

**Acknowledgements.** We thank D. Le Nouen and R. Graff for NMR measurements, Elf Aquitaine, the Centre National de la Recherche Scientifique and the European Community generic project '*Biotechnology of Extremophiles*' (contract BIO-CT93-02734) for financial support.

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4. '*A. montana*' CCAP 1405/3 (Culture Collection of Algae and Protozoa, Cambridge, UK) is named in inverted commas because of its unclear taxonomic position. This cyanobacterium belongs probably to an intermediate group between *Synechococcus* and *Synechocystis* according to the current classification of cyanobacteria (Rippka, R.; Dernalles, J.; Waterbury, J.; Hordman, M.; Stanier, R. *J. Gen. Microbiol.* **1979**, *III*, 1-61). It was grown at 23°C in a 6l fermenter for 17 days under artificial light during 18h/24h (the first five

days the lighting was raised to 3300 lux (473nm) by 6 fluorescent lamps, then to 4800 lux by 8 lamps) in a mineral medium ($MgSO_4.7H_2O$, 0.25g.$l^{-1}$; $K_2HPO_4$, 1.0g.$l^{-1}$; $Ca(NO_3)_2.4H_2O$, 0.025g.$l^{-1}$; $KNO_3$, 1.0 g.$l^{-1}$; sodium citrate.$2H_2O$, 0.165g.$l^{-1}$; $Fe_2(SO_4)_3.6H_2O$, 4 mg.$l^{-1}$; $H_3BO_3$, 2.86 mg.$l^{-1}$; $MnCl_2.4H_2O$, 1.81 mg.$l^{-1}$; $ZnSO_4.7H_2O$, 0.22 mg.$l^{-1}$; $MoO_3$, 0.17mg.$l^{-1}$; $CuSO_4.5H_2O$, 0.8 mg.$l^{-1}$) supplemented with air containing 1% $CO_2$. Yield: 1,17g/l (freeze-dried cells).

5. Diplopterol (50 µg/g, freeze-dried cells; $R_f$=0,50) and the mixture of the heptaacetates of hopanoids **1** and **2** (220 µg/g; $R_f$=0.10) were isolated by TLC (cyclohexane/ethyl acetate, 1:1) from the acetylated $CHCl_3/CH_3OH$ extract. Acetylated tetrol ethers **1** and **2** ($R_T$=21 min) were purified by reverse phase HPLC on a Dupont Zorbax ODS column (250x4.6mm) using methanol as eluent (1ml/min) and a Spectra Physics 6040 differential refractometer as detector.

$^1$H-nmr spectrum of acetylated **1** (250MHz, 298K, $CDCl_3$) δ 0,687 (3H, s, 18α-$CH_3$); 0,827 (3H, d, J=6,5Hz, $CH_3$); 0,828 (3H, s, $CH_3$); 0,890 (6H, 2s, 2 $CH_3$); 0,89 (3H, d, J=6,5Hz, 22*R*-$CH_3$); 0,934 (6H, 2s, 2 $CH_3$); 1,994 (3H, s, NH-CO-**$CH_3$**); 2,064 (3H, s, -OCO**$CH_3$**); 2,072 (3H, s, -OCO**$CH_3$**); 2,080 (3H, s, -OCO**$CH_3$**); 2,102 (3H, s, -OCO**$CH_3$**); 2,114 (3H, s, -OCO**$CH_3$**); 2,130 (3H, s, -OCO**$CH_3$**); 2,146 (6H, 2s, 2 -OCO**$CH_3$**); 2,62 (1H, dd, $J_{35a\text{-}35b}$=10,5Hz, $J_{35a\text{-}34}$=5,0Hz, $H_a$-35); 3,15 (1H, s **OH**); 3,72 (1H, dd, $J_{w1'\text{-}4'}$=1,5Hz, $J_{1'\text{-}2'}$=4,5Hz, H-1'); 3,77 (1H, dd, $J_{35b\text{-}35a}$=10,5Hz, $J_{35b\text{-}34}$=4,0Hz, $H_b$-35); 4,16 (1H, d, $J_{6a'\text{-}6b'}$=11,5Hz, $H_a$-6'); 4,24 (1H, d, $J_{6b'\text{-}6a'}$=11,5Hz, $H_b$-6'); 4,75 (1H, ddd, $J_{2'\text{-}3'}$=9,5Hz, $J_{2'\text{-}NH}$=8,5Hz, $J_{2'\text{-}1'}$=4,5Hz, H-2'); 5,06 (2H, m, H-34 and H-32); 5,07 (1H, dd, $J_{4'\text{-}1'w}$=1,5Hz, $J_{4'\text{-}3'}$=5,0Hz, H-4'); 5,23 (1H, dd, $J_{3'\text{-}2'}$=9,5Hz, $J_{3'\text{-}4'}$=5,0Hz, H-3'); 5,35 (1H, dd, $J_{33\text{-}32}$=5,5Hz, $J_{33\text{-}34}$=4,5Hz, H-33); 6,48 (1H, d, $J_{NH\text{-}2'}$=8,5Hz, NH-CO-CH3).

$^{13}$C-nmr spectrum of acetylated mixture of **1** and **2** (65 MHz, 298K, $CDCl_3$) δ 15,9 (°C-25 and C-28); 16,2 (C-27); 16,4 (C-26); 18,7 (°C-2 and °C-6); 19,9 (*C-6); 19,9 (C-29); 20,7; 20,8; 20,9; 21,0 (6 **$CH_3$**CO); 20,9 (C-11); 21,8 (*C-25); 21,9 (°C-24); 22,8 (C-16); 22,9 (2β-Me); 23,2 (**$CH_3$**CONH); 24,2 (C-12); 24,8 (*C-2); 25,6 (C-31); 26,1 (*C-24); 27,5 (C-20); 31,0 (C-30); 31,0 (*C-23); 32,4 (*C-4); 32,6 (*C-7); 33,3 (°C-4, °C-7 and °C-23); 33,8 (C-15); 36,1 (C-22); 37,5 (°C-10); 37,8 (*C-10); 40,3 (°C-1); 41,7 (C-8 and C-19); 41,8 (C-14); 41,9 (°C-3); 44,4 (C-18); 45,2 (*C-1); 45,9 (C-21); 49,7 (C-13); 49,8 (*C-3); 50,5 (C-9); 51,0 (*C-5); 54,1 (C-2'); 54,5 (C-17); 56,2 (°C-5); 63,4 (C-6'); 70,8 (C-35 and C-32); 71,6 (C-33); 72,2 (C-34); 79,8 (C-5'); 80,3; 80,9 (C-3' and C-4'); 83,9 (C-1'); 169,6; 170,1; 170,4; 170,5; 170,6 (2); 171,0 (7 $CH_3$**C**O). Data concerning the spectrum of non-methylated hopanoid **2** were labelled with °, those of the methylated one **1** with *. Data without superscript are common to both compounds.

Mass Spectrum of acetylated mixture of compounds **1** and **2.** Electron Impact m/z 1015 ($M_1^+$, 45%); 1001 ($M_2^+$, 16%); 1000 ($M_1^+$-$CH_3$; 9%); 986 ($M_2^+$-$CH_3$; 9%); 956 ($M_1^+$-$AcNH_2$, 7%); 955 ($M_1^+$-AcOH, 14%); 942 ($M_2^+$-$NH_2$Ac, 7%); 941 ($M_1^+$-AcOH-$CH_3$, 8%); 941 ($M_2^+$-AcOH, 14%); 896 ($M_1^+$-AcOH-$AcNH_2$, 4%); 882 ($M_2^+$-AcOH-$AcNH_2$, 1%); 780 (ring C cleavage, 23%); 669 ($M_1$ ether cleavage between C-35 and O-35, 12%); 655 ($M_2$ ether cleavage between C-35 and O-35, 4%); 383 ($M_1^+$-side chain, 7%); 369 ($M_2^+$-side chain, 3%); 330 (ether cleavage between O-35 and C-1', 24%); 205 ($M_1$ ring C cleavage, 40%); 191 ($M_2$ ring C cleavage, 26%). Chemical Ionisation (*i*-butane) m/z 1016 $(M_1+H)^+$22%; 1002 $(M_2+H)^+$6%.

Ratio of compounds **1** and **2** (about 3:1) was determined by comparison of respective relative intensities of signals of carbon atoms in $^{13}$C nmr and those of signals of molecular ions in mass spectroscopy.

6. NOESY two dimensional $^1$H/$^1$H correlations were carried out in $C_6D_6$ with 10% $CD_3OD$ for proton exchange in the NH group in order to provide transfer of polarity and simplify the two dimensional spectra.

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(*Received in France* 12 *January* 1996; *accepted* 23 *January* 1996)