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35-O-β-6-Amino-6-deoxyglucopyranosyl Bacteriohopanetetrol, a Novel Triterpenoid of the Hopane Series from the Cyanobacterium *Synechocystis* sp. PCC 6714.

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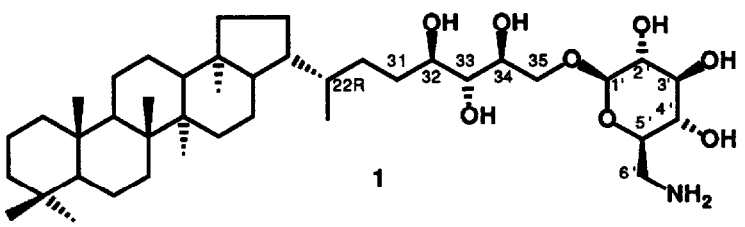
Abstract. A novel hopanoid, 35-O-β-6-amino-6-deoxyglucopyranosyl-bacteriohopanetetrol has been isolated from the cyanobacterium *Synechocystis* sp. PCC 6714.

Cyanobacteria of the genus *Synechocystis* are capable of synthesizing triterpenoids of the hopane series.[1] These lipids act as bacterial membrane stabilizers,[2] and we reported in a recent paper their localization in the cell wall and in the thylakoids of this cyanobacterium.[3] In this paper, we describe now the full structure of a novel bacteriohopanetetrol glycoside which was the sole bacteriohopane derivative isolated from this microorganism.



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Synechocystis sp. PCC 6714 (Pasteur Culture Collection) was grown photoautotrophically as earlier reported.[3] The freeze-dried cells (10g) were extracted with $CHCl_3/CH_3OH$ (2:1), and the extract was either submitted to our $H_5IO_6/NaBH_4$ treatment to evaluate the hopanoid content by GLC[1,4] (diplopterol: 15μg/g, dry weight; bacteriohopane derivatives: 730μg/g), or directly acetylated in order to obtain bacteriohopanepolyols with intact side chains.[5] TLC (cyclohexane/EtOAc, 3:7) afforded thus the heptaacetate of the glycoside **1** (R_f=0.30, 1.2 mg/g) as a novel complex hopanoid. Both procedures yielded similar hopanoid concentrations, excluding the presence of other bacteriohopanepolyol derivatives in significant amounts. An aliquot of the extract was acetylated with deuterated acetic anhydride. The proton NMR spectrum of the resulting heptaacetate showed no methyl signals in the 2 ppm region, indicating that all hydroxy and amino groups were free in the native glycoside.

The structure of the novel hopanoid was deduced from the ^{1}H and ^{13}C NMR spectra as well as from the direct inlet electron impact mass spectrum of the heptaacetate.[6] The singlets in the methyl region of the ^{1}H-NMR spectrum as well as the signals below 60 ppm of the ^{13}C-NMR spectrum characterized the triterpenic hopane skeleton.[7] The triplet at 6.46 ppm belonged to a proton slowly exchangeable in $^{2}H_2O$ and betrayed the presence of an AcNH- moiety linked to a methylene group. $^{1}H/^{1}H$ Homonuclear correlation (COSY) permitted to determine the presence of an acetylated 6-amino-6-deoxyglucopyranose residue. The $^{1}H/^{1}H$ coupling constants corresponded all to *trans*-diaxial protons, suggesting a chair conformation of the hexose with all substituents in equatorial position, including that on the anomeric carbon, and revealing thus the stereochemistry of a β-glycosidic bond. The ^{1}H- and ^{13}C-NMR data of the bacteriohopanetetrol moiety were similar to those of other known glycosides.[7,8] The two most shielded protons at 3.75 and 3.94 ppm corresponded to the C-35 methylene group bearing the glycosyl residue, whereas the three protons resonating at lower field characterized the C-32, C-33 and C-34 methines bearing acetoxy groups. The glycosidic sructure was further supported by mass spectrometry. Next to the molecular ion at m/z 1001 and typical fragmentations of the hopane skeleton at m/z 369 (loss of the side-chain) and 191 (ring C cleavage), the main ions at m/z 655 and 330 arose from fragmentations induced by the glycosidic bond.[5b,8]

Full confirmation of the structure was obtained by cleavage of the glycoside. Methanolysis in presence of dry HCl followed by acetylation[9] yielded on the one hand tetraacetoxybacteriohopane of 32*R*, 33*R* and 34*S* configuration as determined by comparison of its ^{1}H-NMR spectrum with those of all synthetic acetylated bacteriohopanetetrol diastereoisomers,[10] and on the other hand the tetraacetates of the α- and β-methylglycosides of 6-amino-6-deoxyglucopyranose in a 1/1 ratio. These hexose derivatives were identified by direct comparison (TLC, GLC, GLC/MS and ^{1}H-NMR spectroscopy) with the corresponding carbohydrates obtained from the same acid catalyzed methanolysis of kanamycin A followed by acetylation.[11]. 6-Amino-6-deoxyglucose is a carbohydrate found rather rarely, mainly in aminoglycoside antibiotics related to gentamycin and kanamycin.[12] Whether it confers specific physiological properties to glycoside **1** has to be determined.

The identification of a novel hopanoid from *Synechocystis* is not too surprising. Very few Eubacteria species (only 12) have been thoroughly investigated for their complex hopanoid content. The apparent diversity of the bacteriohopanepolyol structures found until now is by far not as rich as that observed for sterols in eukaryotes, in plants or algae, and especially in marine invertebrates.[13] Indeed many variations on the bacteriohopanepolyol framework[14] (introduction of double bonds, methylations of ring A) or on the side chain (number of hydroxyl groups, replacement of the C-35 hydroxyl group by an amino group, presence of amino acid or carbohydrate moieties) might be allowed, provided the basic structural features (amphiphilic character, planar ring system, length of the hydrophobic part permitting the inclusion in phospholipid bilayers of biological membranes) required for a membrane stabilizer are fulfilled.[15]

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6. NMR spectra were recorded on a Bruker AC250 spectrometer at 300K in C^2HCl_3 using $CHCl_3$ (δ=7.260 ppm) as internal standard for ^{1}H-NMR and $^{13}C^2HCl_3$ (δ=77.0 ppm) for ^{13}C-NMR. Assignments were made according to the literature,[7] by homonuclear $^1H/^1H$ correlation and selective decoupling experiments. Numbering of the triterpenic ring system is as usual.[7] Direct inlet electron impact (70ev) mass spectrometry was done on a Finnigan TSQ 70 apparatus, and GLC/MS on a LKB 9000S spectrometer.
   Identification of the heptaacetate of hopanoid **1**.
   ^{1}H-NMR: δ/ppm = 0.690 (3H, s, 18α-CH_3), 0.789 (3H, s, 4β-CH_3), 0.812 (3H, s, 4α-CH_3), 0.844 (3H, s, 10β-CH_3), 0.907 (3H, d, J=6.5Hz, 22R-CH_3), 0.945 (6H, s, 8α and 14β-CH_3), 1.989(3H, s, **CH3**CONH-), 2.009 (3H, s, **CH3**CO_2-), 2.029 (3H, s, **CH3**CO_2-), 2.056 (3H, s, **CH3**CO_2-), 2.066 (3H,s, **CH3**CO_2-), 2.070 (3H, s, **CH3**CO_2-), 2.082 (3H, s, **CH3**CO_2-), 3.38 (1H, m, 6'-H_a), 3.54 (2H, m, 5'-H and 6'-H_b), 3.75 (1H, dd, $J_{34,35a}$=5.5Hz, $J_{35a,35b}$=11.5Hz, 35-H_a), 3.94 (1H, d, $J_{34,35b}$=4Hz, $J_{35a,35b}$=12Hz, 35-H_b), 4.51 (1H, d, $J_{1',2'}$=8Hz, 1'-H), 4.90 (1H, t, $J_{3',4'}$=$J_{4',5'}$=9.5Hz, 4'-H), 4.96 (1H, dd, $J_{1',2'}$=8Hz, $J_{2',3'}$=9.5Hz, 2'-H), 5.06 (1H, m, 32-H), 5.17 (1H, t, $J_{2',3'}$=$J_{3',4'}$=9.5Hz, 3'-H), 5.19 (2H, m, 33-H, 34-H), 6.46 (1H, t, $J_{6'a,NH}$=$J_{6'b,NH}$=6Hz, -NH-). ^{13}C-NMR : δ/ppm = 15.9 (C-25 and C-28), 16.5 and 16.6 (C-26 and C-27), 18.7 (C-2 and C-6), 19.9 (C-29), 20.6 (2 **CH3**CO-), 20.7 (**CH3**CO-), 20.9 (2 **CH3**CO-), 21.0 (C-11), 21.1 (**CH3**CO-), 21.6 (C-24), 22.8 (C-16), 23.0 (**CH3**CO-), 24.0 (C-12), 26.3 (C-31), 27.6 (C-20), 30.9 (C-30), 33.3 (C-4 and C-7), 33.4 (C-23), 33.7 (C-15), 36.2 (C-22), 37.4 (C-10), 39.2 (C-6'), 40.4 (C-1), 41.6 (C-19), 41.7 (C-8), 41.8 (C-14), 42.1 (C-3), 44.4 (C-18), 46.0 (C-21), 49.3 (C-13), 50.5 (C-9), 54.5 (C-17), 56.2 (C-5), 66.5 (C-35), 68.9 (C-4'), 70.6 (C-34), 70.9 (C-2'), 72.0 (C-32), 72.2 (C-33), 72.7 (C-3'), 72.9 (C-5'), 169.2, 169.7, 170.0, 170.1, 170.2, 170.5, 170.7, (7 CH_3**CO**-). MS (direct inlet, electron impact) : m/z = 1001 (M^+, 2%), 942 (M^+-$AcNH_2$, 1%), 941 (M^+-AcOH, 1%), 882 (M^+-AcOH-$AcNH_2$, 0.5%), 822 (M^+-2AcOH-$AcNH_2$, 1%), 762 (M^+-3AcOH-$AcNH_2$, 1%), 671 (cleavage of the glycosidic bond between C-1' and oxygen atom at C-35, 1%), 655 (cleavage of the glycosidic bond between C-35 and oxygen atom at C-35, 11%), 642 (1%), 474 (1%), 369 (M^+-side chain,), 330 (cleavage of the

glycosidic bond between C-1' and oxygen atom at C-35, 100%), 191 (ring C cleavage, 10%). MS (direct inlet, chemical ionization using isobutane as reactant gas): m/z = 1002 $(M+H)^+$.

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9. The heptaacetate of **1** (4mg) was treated for 16h at 100°C with a 10% solution of dry HCl in CH_3OH. The reaction mixture was taken to dryness, acetylated and separated by TLC (cyclohexane/EtOAc, 7:3) into tetraacetoxybacteriohopane (R_f=0.22, 1.8mg) and the acetylated methylglycosides of 6-amino-6-deoxyglucopyranose on the base line. These were further separated ($CHCl_3/CH_3OH$, 98:2, 3 migrations) into tetraacetoxy-α-methylglycoside (R_f=0.86, 0.2mg) and tetraacetoxy-β-methylglycoside (R_f=0.82, 0.2mg).
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11. Kanamycin A (30mg) was treated for 16h at 100°C with a 10% solution of dry HCl in CH_3OH. Evaporation of the reagent, acetylation and TLC separation yielded among other expected carbohydrate derivatives the tetraacetylated α- and β-methyl glycosides of 6-amino-6-deoxyglucose.
Identification of the tetraacetate of α-methyl-6-amino-6-deoxyglucopyranoside. ^{1}H-NMR: δ/ppm = 2.004 (6H, s, 2C**H**$_3$CO-), 2.060 (3H, s, **CH**$_3$CO-), 2.078 (3H, s, **CH**$_3$CO-), 3.37 (1H, m, 6-H_a), 3.56 (1H, ddd, $J_{5,6b}$=3Hz, $J_{5,6a}$=5.5Hz, $J_{4,5}$=10Hz, 5-H), 4.84 (1H, dd, $J_{1,2}$=4Hz, $J_{2,3}$=10Hz, 2-H), 4.88 (1H, t, $J_{3,4}$=$J_{4,5}$=10Hz, 4-H), 4.92 (1H, d, $J_{1,2}$=3.5Hz, 1-H), 5.47 (1H, t, $J_{2,3}$=$J_{3,4}$=10Hz, 3-H), 5.79 (1H, t, $J_{6a,NH}$=$J_{6b,NH}$=6Hz, -NH-). ^{13}C-NMR: δ/ppm = 20.7 (3C**H**$_3$CO-), 23.3 (**CH**$_3$CO-), 38.9 (C-6), 55.5 (**CH**$_3$O-), 67.8 (C-5), 69.3 (C-4), 69.9 (C-3), 71.0 (C-2), 96.8 (C-1), 170.0, 170.1 and 170.3 (4CH_3**C**O-).
Identification the tetraacetate of β-methyl-6-amino-6-deoxyglucopyranoside. ^{1}H-NMR: δ/ppm = 2.000 (6H, s, 2C**H**$_3$CO-), 2.054 (3H, s, **CH**$_3$CO-), 2.056 (3H, s, **CH**$_3$CO-), 3.51 (3H, s, **CH**$_3$O-), 3.52 (3H, m, 5-H and 6-H_a), 3.61 (1H, m, 6-H_b), 4.42 (1H, d, $J_{1,2}$=8Hz, 1-H), 4.92 (1H, t, $J_{3,4}$=$J_{4,5}$=9.5Hz, 4-H), 4.96 (1H, dd, $J_{1,2}$=8Hz, $J_{2,3}$=10Hz, 2-H), 5.19 (1H, t, $J_{2,3}$=$J_{3,4}$=9.5Hz, 3-H), 5.80 (1H, t, $J_{6a,NH}$=$J_{6b,NH}$=5.5Hz, -NH-). ^{13}C-NMR: δ/ppm = 20.6 (**CH**$_3$COO-), 20.7 (2**CH**$_3$OO-), 23.2 (**CH**$_3$CONH-), 39.0 (C-6), 57.2 (CH_3O-), 68.8 (C-4), 71.3 (C-2), 72.4 (C-3), 72.7 (C-5), 101.8 (C-1), 169.4, 169.8, 170.1 and 170.2 (4CH_3**C**O-). GLC/MS: m/z = 330 (M^+-CH_3OH, 0.5%), 289 (M^+-CH_2NHAc, 7%), 241 (M^+-2AcOH, 7%), 200 (M^+-CH_3O-CH_2NHAc-AcOH, 11%), 139 (M^+-CH_2NHAc-2AcOH-CH_3O, 12%), 43 (100%).
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