# Bacteriohopanehexol, a New Triterpene from the Marine Sponge *Petrosia* Species

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A new bacteriohopanoid, bacteriohopanehexol was isolated from the marine sponge *Petrosia* sp., and its structure **1a** was established on the basis of the NMR and MS spectroscopic data of its hexaacetate derivative **1b**. Bacteriohopanehexol is most likely a bacterial metabolite.

Hopanoid triterpenes are divided into two groups, the eukaryotic $C_{30}$ plant hopanes and the prokaryotic $C_{35}$ bacteriohopanes.[1] Plant hopanoids are present in a few scattered taxa of higher plants as well as in several cryptogames, e.g., ferns. The $C_{35}$-bacteriohopanes (geohopanes), on the other hand, are very common in the organic matter of all sediments and petroleums, independently of their age, nature, and origin.[2] The discovery of the $C_{35}$ bacteriohopanetetrols in *Acetobacter aceti* ssp. *xylinum*[3] suggested the prokaryotic origin of the geohopanoids. The bacteriohopanoids play an important role in maintaining membrane stability in bacteria similar to the role of sterols in eukaryotes.

All major prokaryotic hopanoid triterpenoids possess a $C_5$ polyhydroxylated unit with the 32*R*,33*R*,34*S* absolute configuration as in D-ribose. Thus, D-ribose is assumed to be the precursor of these additional five C atoms of the bacteriohopanoid side chain.[1,4]

Triterpenes of marine origin are rare,[5] and most of the known ones consist of two separate oxygenated polycyclic halves like in the sponge metabolites, the sipholanes,[6] and the sodwanones.[7] Two pentacyclic triterpenes of the hopane series were, however, isolated from a cyanobacterium of the genus *Nostoc*.[8]

Sponges are well-known for their symbiont microorganisms, which, in many cases, are the source of secondary metabolites.[5] Therefore, the real source of the bacteriohopanoid **1a** from a *Petrosia* sp. described hereafter might be bacteria or cyanobacteria living in the sponge. The MeOH extract of the *Petrosia* sp. (experimental) collected in Dahlak archipelago, Eritrea, afforded after several chromatographies on Sephadex LH-20, eluting with MeOH, a highly polar compound (**1a**) (0.02% dry wt).

Final purification of compound **1a** was achieved after silica gel chromatography of its peracetate **1b** (experimental), which analyzed for $C_{47}H_{74}O_{12}$ by HREIMS (*m*/*z* 830.5157). As compound **1b** exhibited six acetates in the $^1$H NMR spectrum (Table 1), the natural compound (**1a**) has to be $C_{35}H_{62}O_6$, with five degrees of unsaturation. In the absence of double bonds ($^{13}$C NMR data, Table 1) **1a** has to be pentacyclic.

Besides the six acetate methyl groups, **1b** possesses seven additional methyl groups (Table 1). The $^{13}$C NMR data of **1b** also indicated the presence of five methinoxy and one methyleneoxy groups, carrying the six acetates, eleven additional methylenes, five methines, and five quaternary carbon atoms.

COSY and HMBC correlations (Table 1) suggested a 1,2,3,4-tetraacetoxy-7-methylheptane chain as the major polar functionality of the compound (C-22 to C-35).

The NMR spectra also implied two equatorial acetate groups on cyclohexane rings (on C-6 and -11, $J_{5ax,6ax} = J_{7ax,6ax} = 11$ Hz and $J_{11ax,9ax} = J_{11ax,12ax} = 11$ Hz) according to the two double triplet signals of the corresponding methine protons.

Most important for the structure elucidation were the CH correlations (HMBC) from the two ring C*H*OAc protons and especially from the six methyl groups of the pentacyclic skeleton to their neighbor methine and methylene groups (Table 1). The latter correlations enabled the complete $^{13}$C-resonance line assignment of the carbon atoms of the pentacyclic skeleton.

All the above data suggested for **1b** the hopanoid structure, which was confirmed by comparison of the $\delta_C$ values with those of hopane (Table 1).[9]

Comparison of the $\delta_C$ values of **1b** and hopane (Table 1) pointed clearly to the same stereochemistry of the pentacyclic skeleton as well as to structural changes in the C-6 and C-11 vicinity. Overlapping signals in the $\delta$ 5.17−5.26 ppm region in the $^1$H NMR spectrum (in $CDCl_3$) made interpretation of the NMR spectra difficult, a problem that was solved by repeating the measurements in $C_6D_6$. Especially from the HMBC correlations (Table 1, e.g., correlations between $CH_3$-25, -26 and H-11 to C-9, and correlations between $CH_3$-23, -24 and H-6 to C-5) it became clear that the two skeletal acetate groups are located at C-6 and C-11.

Furthermore, the equatorial C-6α and C-11α orientation of the two acetates, suggested by the coupling constants of the H-6$\beta$ and H-11$\beta$ protons (Table 1) together with the hopanoid stereochemistry, was strongly supported by NOEs measured between H-6$\beta$ and $CH_3$'s 24, 25, 26 and H-7$\beta$ as well as between H-11$\beta$ and $CH_3$'s 25, 26 and H-13$\beta$. Final confirmation of the suggested 6α,11α-diacetoxy hopanoid structure came from the MS fragmentations (Scheme 1), which were in full agreement with known fragmentations of hopanes.[10]

The absolute stereochemistry of the side chain of bacteriohopanes was established by Bisseret, who synthesized the eight possible diastereomers,[11] which could be distinguished well by their $^1$H NMR spectra. Comparison of the $\delta_H$ values of **1b** with the values of the synthetic acetates[4] suggested also for bacteriohopanehexol (**1a**) the 22*R*,[12]32*R*,-33*R*,34*S* structure. Assuming, as discussed above, a bacterial origin of **1a**, tentatively, the same bacterial absolute configuration is also suggested for **1a**.

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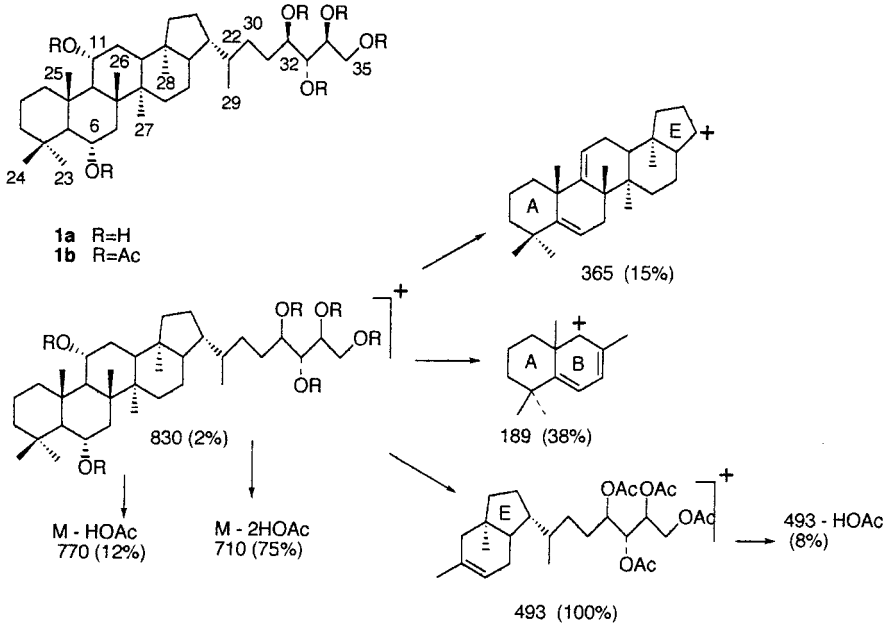
**Table 1.** NMR Data of Bacteriohopanehexol Hexaacetate (**1b**) (125 and 500 MHz, in $CDCl_3$)[a]

| | $\delta_C$ (mult) | | | COSY | |
|---|---|---|---|---|---|
| C no. | **1** | hopane[b] | $\delta_H$ (mult)[e] | **1** | HMBC |
| 1 | 41.7 t | 40.4 t | 1.69 (2H) | | |
| 2 | 18.7 t | 18.8 t | 1.35, 1.54 | | |
| 3 | 42.4 t | 42.2 t | 1.20, 1.33 | | |
| 4 | 33.2 s | 33.3 s | | | |
| 5 | 56.9 d | 56.2 d | 1.19 d (11 Hz) | 5.16 | 6 |
| 6 | 71.5 d | 18.8 t | 5.16 dt (4.0,11Hz) | 1.19, 1.46 | 7[c] |
| 7 | 41.2 t | 33.4 t | 1.46 (2H) | 5.16 | 5,6,9,14,Me26 |
| 8 | 41.4 s | 41.9 s | | | |
| 9 | 52.1 d | 50.5 d | 1.68 d (10.5 Hz) | 5.23 | 8,10,11,Me26 |
| 10 | 41.4 s | 37.5 s | | | |
| 11 | 71.9 d | 21.0 t | 5.23 dt (6.0,10.5Hz) | 1.68,1.47,1.73 | 9,12[c] |
| 12 | 31.2 t | 24.0 t | 1.47, 1.73 | 5.23, 1.41 | |
| 13 | 46.8 d | 49.4 d | 1.41 | 1.47, 1.73 | |
| 14 | 43.9 s | 41.8 s | | | |
| 15 | 33.4 t | 33.7 t | 1.20, 1.28 | 1.52, 1.70 | |
| 16 | 22.5 t | 22.7 t | 1.52, 1.70 | 1.20 | |
| 17 | 54.4 d | 54.7 d | 1.25 | | |
| 18 | 43.9 s | 44.4 s | | | |
| 19 | 41.2 t | 41.7 t | 0.92 | | |
| 20 | 27.5 t | 27.7 t | 1.42, 1.78 | | |
| 21 | 45.6 d | 47.9 d | 1.73 | | |
| 22 | 36.1 d | 32.0 d | 1.44 | 0.91 | |
| 23 | 35.9 q | 33.4 q | 1.05 s (3H) | | 3,4,5,24 |
| 24 | 22.6 q | 21.6 q | 0.85 s (3H) | | 3,4,5,23 |
| 25 | 18.5 q | 15.9 q | 1.05 s (3H) | | 1,5,9,10 |
| 26 | 18.8 q | 16.6 q | 1.13 s (3H) | | 7,8,9,14 |
| 27 | 16.5 q | 16.7 q | 1.00 s (3H) | | 8,13,14,15 |
| 28 | 15.4 q | 15.9 q | 0.68 s (3H) | | 13,17,18,19 |
| 29 | 19.8 q | 22.9 q | 0.91 d (3H) (6 Hz) | 1.44 | 21,22,30 |
| 30 | 30.8 t | 23.9 q | 1.01, 1.40 | | |
| 31 | 26.1 t | | 1.50, 1.62 | 5.02 | |
| 32 | 72.2 d | | 5.02 dt (9.5, 4.4 Hz) | 5.20,1.50,1.62 | 30,31,33,34 |
| 33 | 71.7 d | | 5.20 dd (4.4,5.8 Hz) | 5.02,5.25 | 31,32[c],34,35 |
| 34 | 69.6 d | | 5.25 dt (2.5, 6.3 Hz) | 5.20,4.15,4.38 | 33,35[c] |
| 35 | 62.2 t | | 4.15 dd (6.6,12.3 Hz) | 5.25 | 34 |
| | | | 4.38 dd (2.5,12.3 Hz) | | |
| OAc[d] | 20.6 q | 169.6 s | 1.987 s | | |
| OAc | 20.7 q | 169.9 s | 2.025 s | | |
| OAc | 20.8 q | 170.1 s | 2.033 s | | |
| OAc | 20.9 q | 170.2 s | 2.051 s | | |
| OAc | 22.01q | 170.3 s | 2.061 s | | |
| OAc | 22.02 q | 170.6 s | 2.064 s | | |

[a] Assignment assisted by running DEPT and HMQC spectra. [b] Values from ref 9. [c] Additional HMBC correlations obtained by emphasis on $J = 4$ Hz instead of 8 Hz. [d] The acetate resonances may be interchanged. [e] The coupling constant assignments in the 4.15−5.26 ppm region are based in part on the values obtained from spectra measured in $C_6D_6$ ($\delta_H$ 1.29 d, H-5; 5.46 dt, H-6; 1.55 and 1.65 m, 2H-7; 1.75 d, H-9; 5.50 dt, H-11; 1.58 and 1.95 m, H-12; 5.37 dt, H-32; 5.62 dd, H-33; 5.58 dt, H-34; 4.26 dd and 4.55 dd, 2H-35).

**Scheme 1**



## Experimental Section

**General Experimental Procedures.** UV spectra were obtained with a Varian Cary 219 spectrophotometer, and IR spectra with a Bruker Vector 22 spectrophotometer. $^1$H NMR, $^{13}$C NMR, and 2D NMR spectra were recorded on a Bruker ARX-500 spectrometer with TMS as internal standard. MS data were recorded on a Fisons Autospec-Q spectrometer.

**Animal Material.** The sponge was collected near Durgam Island, in Dahlak archipelago, Eritrea, using scuba at a depth of 10 m during October 1995. A voucher sample is deposited at the Zoological Museum, Tel Aviv University (no. ET-145, SP 25102). The sponge has a hard pink exterior and a soft yellow interior and is closest to *Petrosia* sp. (Demospongiae, order Haplosclerida, family Petrosiidae). It has predominantly strongyles and could be considered a *Strongylophora*, but the two genera grade into each other. There was no matching description; thus, it is possible that this sponge is a new species.

**Extraction and Isolation.** After collection, the sponge was immediately frozen and kept at −20 °C. The frozen sponge was thawed and then extracted with a mixture of MeOH−EtOAc (1:1) × 3 to give a dark brown gum (300 mg). Solvent partition[11] between aqueous MeOH and hexane, $CCl_4$, and $CHCl_3$ gave crude **1a** (60 mg) in the $CHCl_3$ phase.

The latter material was chromatographed three times, sequentially, on Sephadex LH-20 eluted with MeOH to give **1a** (10 mg) as an amorphous powder. For further purification **1a** was acetylated with a mixture of $Ac_2O$−pyridine (1 mL) overnight at room temperature. The residue after evaporation was chromatographed on Si gel eluted with hexanes−EtOAc to give the pure hexaacetate **1b** (5 mg) as a solid oil.

**Bacteriohopanehexol hexaacetate (1b):** $[\alpha]_D$ + 34° (*c* 0.02, $CHCl_3$); IR (neat) 3500, 2900, 1720, 1200 cm$^{-1}$; EIMS *m*/*z* (%) 830 (2), 770 (12), 710 (75), 493 (100), 433 (8), 365 (15), 253 (12), 189 (38), HREIMS *m*/*z* 830.5157 (Δmmu = −0.2); NMR data, see Table 1.

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(12) The 22*R* configuration is common for all known hopanoids.

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