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## Structures of Two Bacteriohopanoids with Acyclic Pentol Side-Chains from the Cyanobacterium *Nostoc* PCC 6720

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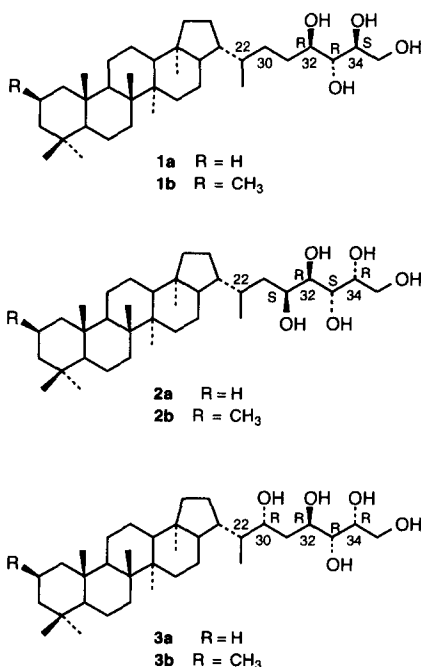
**Abstract:** Two bacteriohopanepentols **2** and **3** have been isolated from the cyanobacterium *Nostoc* PCC 6720. Their structures were elucidated by  $^1\text{H}$ ,  $^{13}\text{C}$ -NMR and mass spectrometry. The absolute configurations of the acyclic 1,2- and 1,2-/1,3-mixed pentol side-chains were determined by bichromophoric exciton coupled dichroism on microgram scale. An improved first step anthroylation in the two-step derivatization is also described.

### INTRODUCTION

Pentacyclic triterpenoids of the hopane series are commonly found in bacteria where they play an important role in maintaining membrane stability.<sup>1</sup> The most common compounds found to date are the C<sub>35</sub> bacteriohopanepolyols in which an additional sugar-derived acyclic C<sub>5</sub> unit is linked to the isopropyl group of the hopane framework, e.g., aminobacteriohopanetriol (**1a** with terminal amino instead of hydroxyl group) and composite hopanoids where bacteriohopanetetrol **1a** is linked to other polar moieties usually derived from carbohydrates.<sup>2</sup> Whereas polyol side-chains with free primary amino groups are quite common, free polyols have been rarely reported, the main free polyols being tetrols **1** from the *Acetobacter* species.<sup>3</sup> Bacteriohopanepentols have been found in one case only, namely **2** and **3**, which differ one from each other by the presence of 1,2- or of mixed 1,2/1,3 polyol type side-chains (**Figure 1**);<sup>4</sup> they were isolated from a

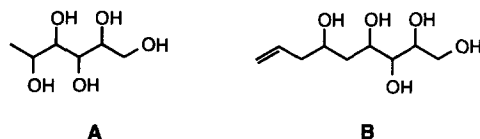
cyanobacterium of the genus *Nostoc*. However, because of problems in *Nostoc* mass production and in the isolation of the polyols, only sub-mg amounts were available for each compound.

Moreover, the absolute stereochemical assignment of the hopanoid side-chain presents considerable difficulties similarly to many other acyclic molecules because of their conformational instability. Establishment of the structures of **2** and **3** would contribute significantly in understanding the origin of the additional polyhydroxylated C5 unit of hopanoids, as well as the biogenesis of the bacteriohopane skeleton and the effect of side-chain configurations on the stabilization of phospholipid bilayers.



**Figure 1.** Structures of bacteriohopanetetrol **1** and bacteriohopanepentols **2** and **3**.

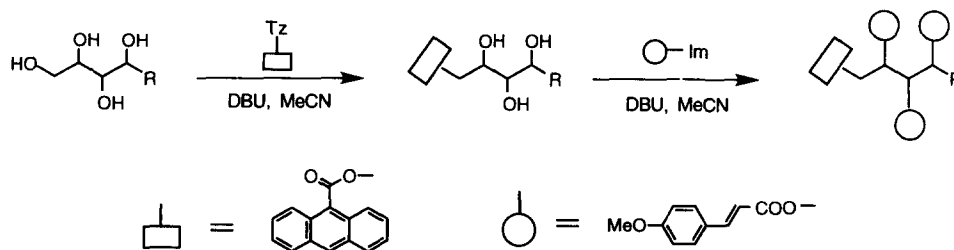
The CD library of bichromophoric derivatives of acyclic 1,2-polyols, i.e., 1,2,3,4,5-pentols of “Type A” and 1,2-/1,3-mixed polyols, i.e., 1,2,3,4,6-pentols of “Type B” have recently been set up.<sup>5-9</sup> Each CD curve of the library can be rationalized by taking into account the pairwise additivity of “basis set” interchromophoric couplings and conformational equilibria (<sup>1</sup>H NMR<sup>5</sup>). The CD spectra of all stereoisomers are characteristic for each solvent employed, namely, methylcyclohexane (MC) and acetonitrile (AN), and in most cases are predictable for each configurational pattern. The CD curves can thus be used as reference curves for stereochemical assignment of unknown 1,2- or 1,2-/1,3-mixed pentols. Following the configurational determination of several aminobacteriohopanepolyols<sup>6-8</sup> by microscale bichromophoric exciton coupled CD, the method has been applied to newly isolated bacteriohopanepentols **2** and **3**.



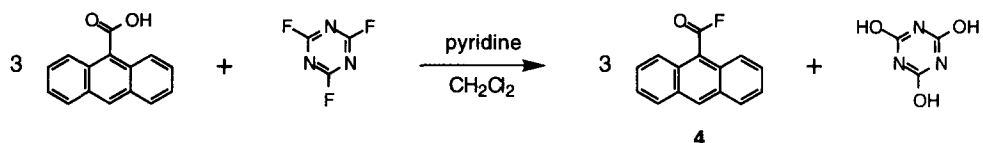
## RESULTS AND DISCUSSION

**Isolation of Bacteriohopanepoyls 1-3.** The hopanoid composition of *Nostoc* PCC 6720 was qualitatively similar to that of the *Nostoc* B-1452-12b strain which was analyzed earlier.<sup>10</sup> The presence of hopanoids was checked by our classical H<sub>5</sub>IO<sub>6</sub>/NaBH<sub>4</sub> treatment of the crude extract followed by TLC and acetylation,<sup>11</sup> which yielded three bacteriohopanederivatives with degraded side-chains: the monoacetates of (*22R*)-tetranorbacteriohopane-31-ol and (*22R*)-trinorbacteriohopane-32-ol, and the diacetate of (*22R*)-trinorbacteriohopane-30,32-diol. The presence of a mixture of 2 $\beta$ -methylhopanoids and their non-methylated homologues was confirmed by GC/MS. These three derivatives corresponded to bacteriohopanetetrol **1** and two bacteriohopanepentols **2** and **3**. Each bacteriohopanepolyol was a mixture of the non-methylated hopanoid as minor compound accompanied by larger amounts of its 2 $\beta$ -methyl homologue. This methylation pattern of the hopane skeleton is quite often observed in cyanobacteria as well as in the related Prochlorophyte *Prochlorothrix hollandica*.<sup>12</sup> All structures, with the exception of the stereochemistry of the side-chain, were determined by spectroscopic methods (<sup>1</sup>H- and <sup>13</sup>C-NMR, mass spectrometry) and the comparison of these data with those of previously isolated hopanoids or synthetic 2 $\beta$ -methylhopanoids such as 2 $\beta$ -methylhopane-22-ol and 2 $\beta$ -methylhopane-22(29)-ene.<sup>10,12,13</sup>

**Bichromophoric Derivatization of Bacteriohopanepentols 2 and 3.** In the preceding paper,<sup>9</sup> the primary and secondary hydroxyl groups of reference compounds were treated with 9-anthroyl tetrazole and *p*-methoxycinnamoyl imidazole, respectively (**Scheme 1**). However, the yield of the first anthroylation step was only ~50%. In view of the limited microgram availability of the bacteriohopanepentols, and their poor solubility in organic solvents, the anthroylation step had to be improved. A search for various reagents, solvents and reaction conditions showed 9-anthroyl fluoride **4**, readily prepared by the Olah procedure<sup>14</sup> (**Scheme 2**) to be an excellent reagent for the selective acylation of terminal hydroxyl groups in the presence of secondary hydroxyls. Although the reactivity of **4** is similar to 9-anthroyl chloride used before,<sup>5</sup> the smaller size of the fluorine atom facilitates attachment of the bulky anthroyl group to the terminal hydroxyl group.

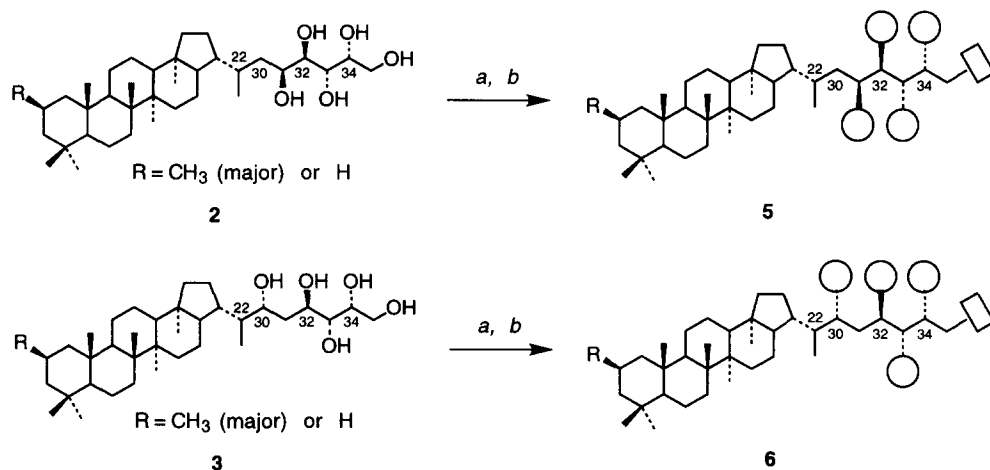


**Scheme 1.** Bichromophoric derivatization of polyols.



**Scheme 2.** Preparation of 9-anthroyl fluoride.

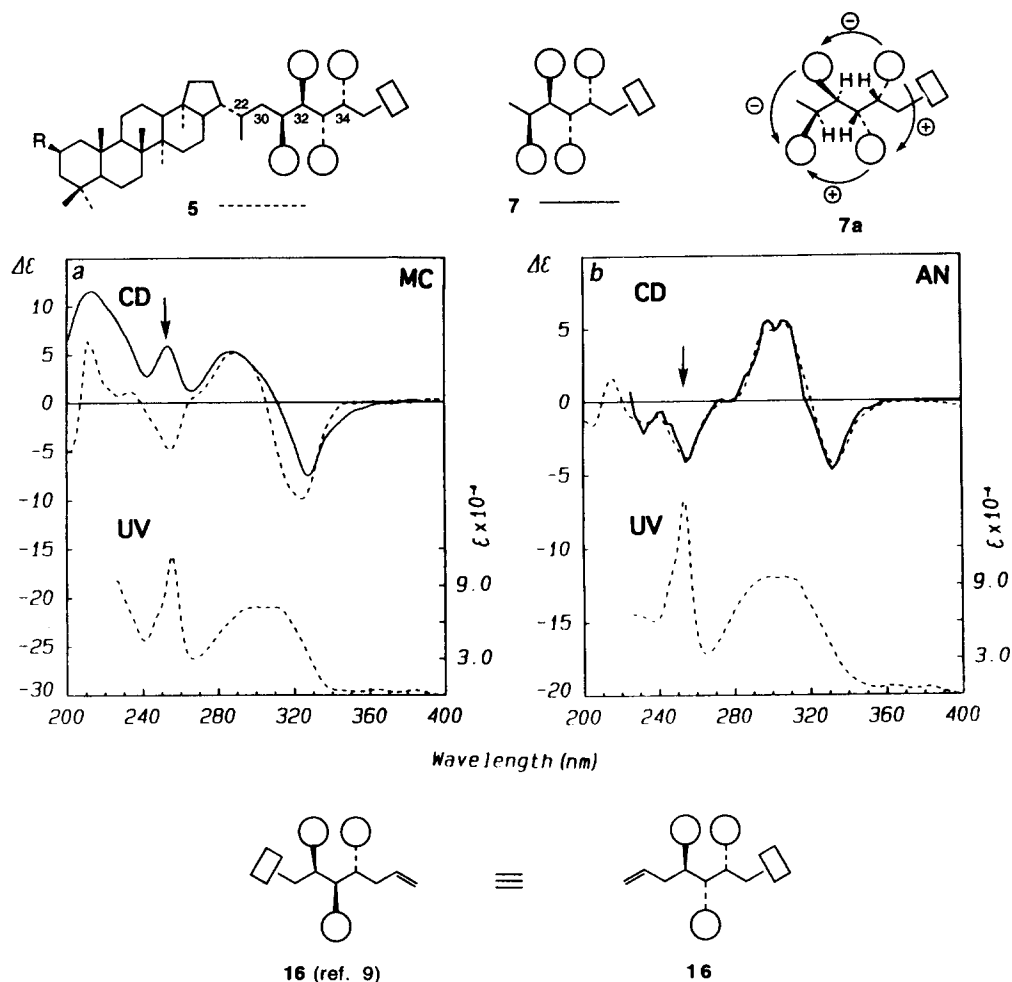
9-Anthroyl fluoride **4** was used under two slightly different reaction conditions, both of which afforded the anthroates in higher and reproducible yields: i) 9-anthroyl fluoride/DMAP/pyridine, yield 82%; ii) 9-anthroyl fluoride/DBU/MeCN, yield 80%. However, protocol (i) proved to be more suited for microscale derivatization, because of formation of far less side products and ease of purification, e.g., absence of bisanthroate, small amount of which was detected by protocol (ii). The treatment of 9-anthracene carboxylic acid with 2 equiv. of cyanuric fluoride in the presence of 1 equiv. of pyridine gave 9-anthroyl fluoride **4** as a yellow solid, 90% yield, which can be stored at room temperature for a long period. In all cases the bacteriohopanepentols (ca. 100 micrograms) were treated with **4** in pyridine and a catalytic amount of DMAP in silylated vials because of their minimal surface absorbability. After purification, *p*-methoxycinnamoylation was performed with *p*-methoxycinnamoyl imidazole to afford derivatives **5** and **6**, which were further purified by HPLC prior to CD measurements (**Scheme 3**).



**Scheme 3.** a) 9-anthroyl fluoride, DMAP, Py, rt, overnight;  
b) *p*-methoxycinnamoyl imidazole, DBU, MeCN, rt, overnight.

**CD of Bacteriohopanepentol Derivatives 5, 6 and Absolute Configurational Assignments.** Stereochemical assignment of hopanepentols **2** and **3** was performed by comparison of the CD spectra of corresponding derivatives **5** and **6** with the reference spectra in the CD library.<sup>5-9</sup> Hopanepentol **2** contains the 1,2-pentol moiety of type A. A comparison of the CD of its bichromophoric derivative **5** with the reference CD of 1,2,3,4,5-pentols<sup>5-8</sup> indicates that the sole matching similarity exists with the reference CD of **7** derived

from D-galactose, especially in AN (**Figure 2b**, **Table 1**). As mentioned earlier,<sup>9</sup> in some cases a good resemblance with a reference curve could be seen only in one of the two solvents, AN or MC. If this is the case, the conclusion should be based on the CD measured in the solvent which gives the better match. The CD of **5** in MC is an example of such a case; the similarity with reference curve of **7** is not as good as in AN (**Figure 2a**), while the CEs at 252 nm are even of opposite signs. It is possible that in this case the influence of the hydrophobic triterpenic nucleus on the side chain conformation becomes more pronounced. However, the excellent agreement seen in AN clearly shows that the configuration of **5** is identical with that of the D-galactose derivative **7**, namely, **31S**, **32R**, **33S**, **34R**. The NMR conformational analysis of **7**<sup>5</sup> revealed that in CD<sub>3</sub>CN it adopts exclusively the extended conformation **7a** (**Figure 2**). The fact that the cinnamate/cinnamate exciton coupling around 310 nm is weak<sup>5</sup> in both solvents is due to the additive effects of



**Figure 2.** CD and UV spectra of derivatives of bacteriohopanepentol **5** (dashed) and D-galactose **7** (solid) in methylcyclohexane and acetonitrile.

**Table 1.** CD ( $\lambda_{\text{ext}}$  nm/ $\Delta\epsilon$ ) data of bichromophoric derivatives **5-7** and **44** in methylcyclohexane and acetonitrile.

| Entry | Compd     | CD [ $\lambda_{\text{ext}}$ ( $\Delta\epsilon$ )] in MC | CD [ $\lambda_{\text{ext}}$ ( $\Delta\epsilon$ )] in AN |
|-------|-----------|---|---|
| 1     | <b>5</b>  | 253 (-4.8), 287 (+5.3),<br>323 (-9.9)                   | 253 (-3.9), 298 (+4.8),<br>306 (+5.3), 332 (-4.3)       |
| 2     | <b>6</b>  | 253 (+0.6), 315 (+5.0)                                  | 259 (-3.5), 281 (-6.4),<br>319 (+16.9)                  |
| 3     | <b>7</b>  | 254 (+5.9), 287 (+5.7),<br>327 (-7.5)                   | 253 (-4.3), 298 (+5.7),<br>306 (+5.5), 332 (-4.7)       |
| 4     | <b>44</b> | 252 (+2.0), 316 (+5.4)                                  | 257 (-3.0), 279 (-5.3),<br>318 (+16.0)                  |

two 1,3-pairwise interactions with opposite chirality (C-2/C-4 and C-3/C-5 cinnamate pairs) and two 1,2-interactions also with opposite chirality (C-2/C-3 and C-4/C-5 cinnamate pairs).

Hopanepentol derivative **5** is the higher 1,2-*syn* and 1,3-*anti* extended homologous polyol of 1,2-tetrol derivative **16** (see Figure 4, **16** in ref. 9). Its CD spectrum is quite different from that of **16**, which shows a well-defined negative couplet with A value -57 in MC and A value -51 in AN.<sup>9</sup> This is consistent with the systematic trends seen in homologous 1,2-polyols.<sup>5-8</sup> Namely, if the 1,2-*syn* extension gives rise to an additional 1,3-*anti* CD pairwise interaction, the CD of the higher homolog and the parent polyol are distinctly different.

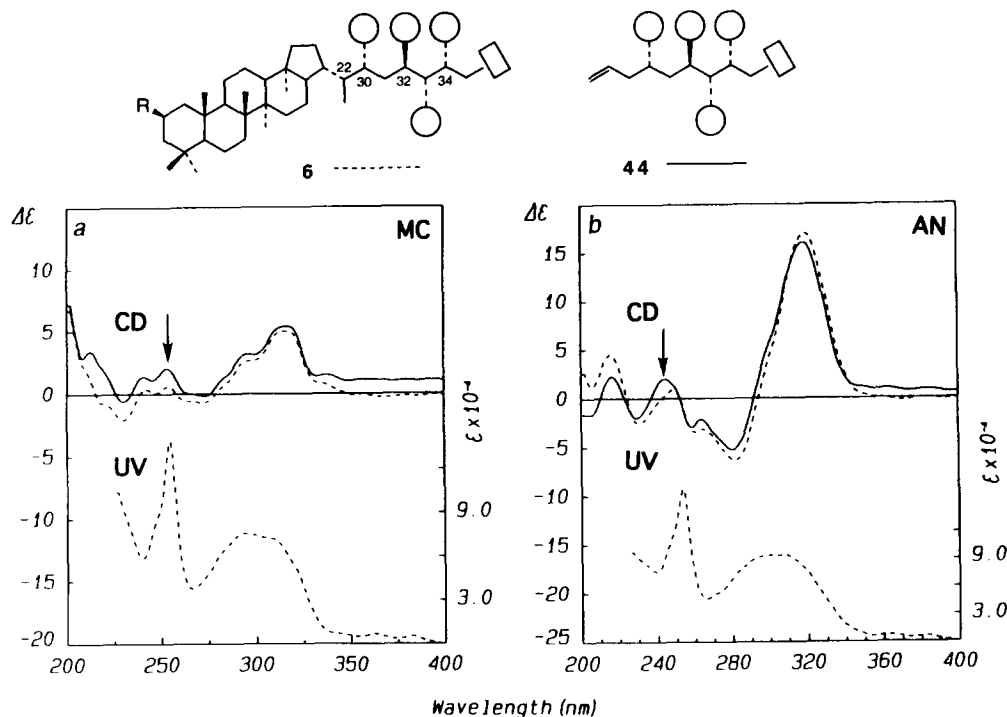
The pentol derivative **6** belongs to the mixed type **B**, 1,2-/1,3- polyol, with the same 1,2,3,4,6- pattern of the synthetic pentols **37-44** described in the preceding paper.<sup>9</sup> The comparison of CD spectra of **6** in two solvent with different polarity, namely, MC and AN (**Figure 3**, **Table 1**) with those of **37-44** unambiguously revealed that only **6** and **44** show very close resemblance, the overall curve shape and intensity being almost superimposable. This shows that the absolute configuration of the side chain of **6** and the parent bacteriohopanepentol **3** should be assigned **30R**, **32R**, **33R**, **34R**.

This conclusion follows from the results presented in the previous paper,<sup>9</sup> namely, the summation of all pair-wise exciton coupled interactions present in the various conformations in equilibrium give rise to CD curves which are unique for each configurational pattern and solvent.

The CD spectra of hopanepentol **6** and acyclic reference pentol **44** are almost superimposable in both MC and AN; however, the spectra in two solvents are quite different. The similar effects on CD of **6** and **44** by changing the solvent polarity show indeed that the acyclic moieties in both compounds are undergoing similar solvent-dependent conformational changes because of the identical configurations.

The weak positive Cotton effects (CE) in the 280-320 nm region is the additive result of all pairwise cinnamate/cinnamate interactions. The weak band at 252 nm is a result of partial canceling of the positive CE due to 35-anthroate/34 $\beta$ -cinnamate interaction and the negative CE arising from the 35-anthroate/33 $\beta$ -cinnamate coupling. Compared with the lower homologous tetrol derivative **16** (see Figure 4, **16** in ref. 9), the CD of **6**

is quite different in 280-320 nm region, which further confirms the general trend in CD by 1,3-*anti* polyols extension described earlier.<sup>9</sup>



**Figure 3.** CD and UV spectra of derivatives of bacteriohopanepentol **6** (dashed) and D-galactose **44** (solid) in methylcyclohexane and acetonitrile.

As the **32R**, **33R**, **34S** absolute configuration of bacteriohopanepentol **1**, which is found in practically all bacteriohopanepentols, could be established by <sup>1</sup>H-NMR comparisons of its tetraacetate with the eight hemisynthetic diastereoisomers,<sup>15</sup> its structure was not further investigated by CD. This stereochemistry, together with results of <sup>13</sup>C-acetate or <sup>13</sup>C-glucose incorporation into several bacterial hopanoids,<sup>16,17</sup> shows that the C5 side-chain is formed by attachment of a D-ribose derivative via C-5 to the isopropyl group of the hopane skeleton.

One of the most striking features of the two pentols **2** and **3** isolated from the genus *Nostoc* is the **34R** configuration. No other tetrol could be detected in the tetrol fraction from *Nostoc*, especially not the **32R**, **33R**, **34R** isomer which is already known from *Acetobacter* species<sup>3</sup> and possesses the same configuration at C-34 as the two pentols. From the examination of the methyl region of the acetoxy groups, if such an isomer would be present, it would represent less than 5% of the tetrol fraction.

There is no direct experimental proof concerning the origin of the compounds with **32R**, **33R**, **34R** configuration. They could derive either from direct linkage between a D-arabinose derivative and a triterpenic

precursor, or from the isomerization of a possible common precursor, such as 29-(5'-riboseyl)hopane, which could lead to isomerization in a position of the carbonyl group to the 34*R* as well as to the 34*S* series.<sup>2</sup>

The biogenetic significance of the pentols is yet unknown. Highly oxidized side-chains are also known in the aminopolyol series, *e.g.* aminobacteriohopanetetrol and aminobacteriohopanepentol.<sup>7</sup> It has still to be tested whether such compounds result from trapping by water addition of cationic intermediates of the enzymatic coupling reaction and whether they might be precursors for tetrols or represent products of oxidative catabolism of the tetrols.

## CONCLUSION

The structures of two bacteriohopanepentols isolated from the cyanobacterium *Nostoc* PCC 6720 have been determined. The acyclic 1,2- and 1,2-/1,3-mixed side-chain configurations were determined by bichromophoric circular dichroic spectroscopy involving anthroylation and *p*-methoxycinnamoylation steps; an improved anthroylation step leading to higher yields is described. This microscale bichromophoric CD method can be used as a general method for the configurational assignments of acyclic 1,2-/1,3-mixed polyols. Establishment of these compounds with unique pentol side-chains should contribute in understanding the biogenesis and role of these polyhydroxylated bacteriohopanoids.

## EXPERIMENTAL SECTION

**General.** Solvents used for reactions were reagent grade. Anhydrous solvents were freshly distilled (pyridine, CH<sub>2</sub>Cl<sub>2</sub> and acetonitrile from CaH<sub>2</sub>). Unless otherwise mentioned, reagents were obtained from commercial sources and were used as such. Microscale reactions were carried out in silylated vials under nitrogen atmosphere. Thin-layer chromatography (TLC) was used for monitoring reactions, by using Analtech Silica Gel GHLF plates (250 μm thick).

ICN silica gel (32-63 mesh) was employed for flash chromatography. HPLC purifications were performed using waters HPLC system equipped with a diode array detector. Solvents used for chromatographic separation were HPLC grade.

All <sup>1</sup>H and <sup>13</sup>C NMR spectra were obtained in CDCl<sub>3</sub> on a Varian VXR 65, 250 and 400 and are reported in parts per million (δ) relative to CHCl<sub>3</sub> (7.24 ppm) as an internal reference, with coupling constants (J) reported in hertz (Hz). FAB-MS was measured on a JEOL JMS-DX 303 HF mass spectrometer. UV-VIS and CD spectra were recorded in methylcyclohexane and acetonitrile solutions on a Perkin-Elmer Lambda 4B UV/VIS spectrophotometer and JASCO J-720 spectropolarimeter respectively. Smoothing and other manipulation of spectra were carried out with software developed in house: DFT (Discrete Fourier Transform) procedure for smoothing.

### **Culture Conditions and Isolation of Hopanoids**

*Nostoc* PCC 6720 (Pasteur Culture Collection, Paris) was grown autotrophically in a mineral medium in presence of CO<sub>2</sub> enriched air.<sup>18</sup> Analytical procedures and spectroscopic identifications were as described



previously.<sup>19</sup> A small sample of freeze-dried cells (0.1g) was extracted with CHCl<sub>3</sub>/CH<sub>3</sub>OH, and the crude extract treated by H<sub>5</sub>IO<sub>6</sub>/NaBH<sub>4</sub> in order to detect the presence of hopanoids by GLC.<sup>11</sup> The CHCl<sub>3</sub>/CH<sub>3</sub>OH extract of a large sample of freeze-dried cells (4g) was acetylated and separated by TLC (CHCl<sub>3</sub>, 2 migrations) to give the tetraacetates of bacteriohopanetetrols **1a** and **1b** (R<sub>f</sub> = 0.61, 0.9 mg), the pentaacetates of bacteriohopanepentols **2a** and **2b** (R<sub>f</sub> = 0.61, 1.1 mg), and of the pentols **3a** and **3b** (R<sub>f</sub> = 0.47, 1.4 mg) (Fig. 1).

Free polyols were obtained from the corresponding pentaacetates after treatment with basic Amberlyst A-26 in THF/MeOH (1:1, v/v)<sup>20,21</sup> and were characterized by mass spectrometry.

### **Identification of compounds 1, 2 and 3**

All hopanoids were isolated as a mixture of non-methylated hopanoids and 2β-methylhopanoids which could not be separated one from another neither by TLC, nor by reverse phase HPLC. The ratio of the non-methylated hopanoid and its 2β-methyl homologue was evaluated by <sup>1</sup>H-NMR spectroscopy by comparison with the relative intensity of the methyl signals and by mass spectrometry by comparison of the relative intensities of signals obtained by the same fragmentation. Assignments of the signals from the hopane skeleton were made according to earlier work,<sup>13d</sup> those of the side-chain signals were obtained by selective <sup>1</sup>H/<sup>1</sup>H decoupling for <sup>1</sup>H-NMR spectra and by <sup>1</sup>H/<sup>13</sup>C decoupling experiments (for <sup>13</sup>C-NMR spectra). In the <sup>1</sup>H-NMR spectra, methyl signals of the non-methylated hopanoid are labelled with \*. The signals from the side-chain protons were identical for both methylated and non-methylated compounds. Because of their low intensity, the signals of the minor non-methylated compound were not described in <sup>13</sup>C-NMR spectra.

**Tetraacetates of bacteriohopane-32,33,34,35-tetrols 1a and 1b** (in a 3:7 ratio). <sup>1</sup>H-NMR (250 MHz); δ 0.686 (3H, s, 18α-CH<sub>3</sub>), \*0.790 (3H, s, 4β-CH<sub>3</sub>), 0.801 (3H, d, J = 6.5 Hz, 2β-CH<sub>3</sub>), 0.828 (3H, s, 4β-CH<sub>3</sub>), 0.881 and 0.890 (6H, 2s, 4α- and 10β-CH<sub>3</sub>), 0.901 (3H, d, J = 6.5 Hz, 22-CH<sub>3</sub>), 0.933 (6H, 1s, 8β- and 14α-CH<sub>3</sub>), \*0.944 (6H, 2s, 8β- and 14α-CH<sub>3</sub>), 2.046 (3H, s, CH<sub>3</sub>COO-), 2.067 (3H, s, CH<sub>3</sub>COO-), 2.074 (3H, s, CH<sub>3</sub>COO-), 2.078 (3H, s, CH<sub>3</sub>COO-), 4.14 (1H, dd, J<sub>35a,35b</sub> = 12.5 Hz, J<sub>34,35a</sub> = 6.5 Hz, 35-Ha), 4.38 (1H, dd, J<sub>35a,35b</sub> = 12.5 Hz, J<sub>34,35b</sub> = 2.5 Hz, 35-Hb), 5.03 (1H, dt, J<sub>32,33</sub> = 4.5 Hz, J<sub>31,32</sub> = 9.5 Hz, 32-H), 5.22 (1H, dd, J<sub>32,33</sub> = 4.5 Hz, J<sub>33,34</sub> = 6.0 Hz, 33-H), 5.27 (1H, dt, J<sub>34,35b</sub> = 1.5 Hz, J<sub>34,35a</sub> = J<sub>33,34</sub> = 6.5 Hz, 34-H).

**Pentaacetates of bacteriohopane-31,32,33,34,35-pentols 2a and 2b** (in a 2:8 ratio). <sup>1</sup>H-NMR (250 MHz); δ 0.688 (3H, s, 18α-CH<sub>3</sub>), \*0.789 (3H, s, 4β-CH<sub>3</sub>), 0.808 (3H, d, J = 6.5 Hz, 2β-CH<sub>3</sub>), 0.817 (3H, s, 4β-CH<sub>3</sub>), 0.883 and 0.892 (6H, 2s, 4α- and 10β-CH<sub>3</sub>), 0.919 (3H, d, J = 6.5 Hz, 22-CH<sub>3</sub>), 0.933 (6H, 2s, 8β- and 14α-CH<sub>3</sub>), 2.025 (3H, s, CH<sub>3</sub>COO-), 2.038 (3H, s, CH<sub>3</sub>COO-), 2.073 (3H, s, CH<sub>3</sub>COO-), 2.089 (3H, s, CH<sub>3</sub>COO-), 2.111 (3H, s, CH<sub>3</sub>COO-), 4.12 (1H, dd, J<sub>35a,35b</sub> = 12 Hz, J<sub>34,35a</sub> = 7.4 Hz, 35-Ha), 4.36 (1H, dd, J<sub>35a,35b</sub> = 12 Hz, J<sub>34,35b</sub> = 3.2 Hz, 35-Hb), 5.10 (1H, m, 31-H), 5.22 (1H, dd, J<sub>32,33</sub> = 6.7 Hz, J<sub>31,32</sub> = 4.0 Hz, 32-H), 5.30 (1H, ddd, J<sub>34,35a</sub> = 7.4 Hz, J<sub>34,35b</sub> = 3.3 Hz, J<sub>33,34</sub> = 4.0 Hz, 34-H), 5.33 (1H, dd, J<sub>33,34</sub> = 4.0 Hz, J<sub>32,33</sub> = 6.5 Hz, 33-H).

<sup>13</sup>C-NMR (65 MHz); δ 15.9, 16.2, 16.4 (C-26, C-27, C-28), 19.9 (C-6, C-9 and CH<sub>3</sub>COO-), 20.7 (CH<sub>3</sub>COO-), 20.8 (CH<sub>3</sub>COO-), 20.9 (2 CH<sub>3</sub>COO-), 21.8 (C-25), 22.0 (C-11), 22.9 (C-16), 23.2 (methyl at C-2β), 24.3 (C-12), 24.8 (C-2), 26.1 (C-24), 28.0 (C-20), 31.0 (C-23), 32.5 (C-4), 32.6 (C-7), 33.7 (C-15

and C-22), 35.6 (C-30), 37.8 (C-10), 41.6 (C-19), 41.8 (C-14), 41.9 (C-8), 44.3 (C-18), 45.2 (C-1), 46.3 (C-21), 49.68 and 49.74 (C-9 and C-13), 49.81(C-3), 51.0 (C-5), 54.5 (C-17), 61.8 (C-35), 69.5, 69.9, 70.1, 71.7 (C-31, C-32, C-33 and C-34). Signals of the carbonyl groups were not observed because of the low amounts.

MS (\* ions from non-methylated hopanoid):  $m/z$  786 ( $M^+$ , 0.2%), 551 (ring C cleavage, 52%), 491 (551-AcOH, 8%), 431 (551-2AcOH, 6%), 383 ( $M^+$ -side-chain, 16%), \*369 ( $M^+$ -side-chain, 4%), 205 (ring C cleavage, 100%), \*191 (ring C cleavage, 33%).

**Bacteriohopane-31,32,33,34,35-pentols 2a and 2b.** MS:  $m/z$  576 ( $M^+$ , 1%), 561 ( $M^+$ -Me, 1%), 558 ( $M^+$ -H<sub>2</sub>O, 1%), 543 (558-Me, 2%), 525 (543-H<sub>2</sub>O, 1%), 383 ( $M^+$ - side-chain, 14%), \*369 ( $M^+$ - side-chain, 5%), 341 (ring C cleavage, 9%), 323 (341-H<sub>2</sub>O, 14%), 205 (ring C cleavage, 100%), \*191 (ring C cleavage, 38%).

**Pentaacetates of bacteriohopane-30,32,33,34,35-pentols 3a and 3b** (in a 1:5 ratio). <sup>1</sup>H-NMR (250 MHz);  $\delta$  0.754 (3H, s, 18 $\alpha$ -CH<sub>3</sub>), 0.820 (3H, d,  $J$  = 6.5 Hz, 2 $\beta$ -CH<sub>3</sub>), 0.828 (3H, s, 4 $\beta$ -CH<sub>3</sub>), 0.882 and 0.890 (6H, 2s, 4 $\alpha$ - and 10 $\beta$ -CH<sub>3</sub>), 0.919 (3H, d,  $J$  = 6.5 Hz, 22-CH<sub>3</sub>), 0.933 (6H, 2s, 8 $\beta$ - and 14 $\alpha$ -CH<sub>3</sub>), 1.993 (3H, s, CH<sub>3</sub>COO-), 2.037 (3H, s, CH<sub>3</sub>COO-), 2.054 (3H, s, CH<sub>3</sub>COO-), 2.090 (3H, s, CH<sub>3</sub>COO-), 2.100 (3H, s, CH<sub>3</sub>COO-), 4.13 (1H, dd,  $J_{35a,35b}$  = 12.3 Hz,  $J_{34,35a}$  = 6.4 Hz, 35-Ha), 4.36 (1H, dd,  $J_{35a,35b}$  = 12.3 Hz,  $J_{34,35b}$  = 2.4 Hz, 35-Hb), 4.96 (1H, m, 30-H), 5.08 (1H, m, 32-H), 5.22 (1H, td,  $J_{33,34}$  =  $J_{34,35a}$  = 6.4 Hz,  $J_{34,35b}$  = 2.4 Hz, 34-H), 5.26, (1H, dd,  $J_{32,33}$  = 3.8 Hz,  $J_{33,34}$  = 6.3Hz, 33-H).

<sup>13</sup>C-NMR (65 MHz);  $\delta$  14.4 (C-29), 15.7, 16.2, 16.4 (C-26, C-27, C-28), 19.9 (C-6), 20.7 (CH<sub>3</sub>COO-), 20.8 (2 CH<sub>3</sub>COO-), 20.9 (CH<sub>3</sub>COO-), 21.2 (CH<sub>3</sub>COO-), 21.8 (C-25), 21.9 (C-11), 22.7 (C-16), 23.2 (methyl at C-2 $\beta$ ), 24.3 (C-12), 24.8 (C-2), 26.10 (C-24), 26.13 (C-20), 26.7 (C-31), 31.1 (C-23), 32.5 (C-4), 32.6 (C-7), 33.7 (C-15), 37.8 (C-10), 38.7 (C-22), 41.65 (C-19), 41.72 (C-14), 42.0 (C-8), 43.2 (C-21), 44.6 (C-18), 45.2 (C-2), 49.68 and 49.74 (C-9 and C-13), 49.81 (C-3), 51.0 (C-5), 54.4 (C-17), 62.1 (C-35), 68.4 (C-32), 69.4 (C-34), 71.6 (C-30 and C-33). The signals of the carbonyl groups were not observed because of the low amounts.

MS (\* ion from non-methylated hopanoid):  $m/z$  786 ( $M^+$ , 0.1%), 726 ( $M^+$ -AcOH, 3%), 551 (ring C cleavage, 1%), 491 (551-AcOH, 51%), 431 (551-2AcOH, 12%), 383 ( $M^+$ -side-chain, 11%), 382 ( $M^+$ -side-chain-H, 34%), 205 (ring C cleavage, 100%), \*191 (ring C cleavage, 18%).

**Bacteriohopane-30,32,33,34,35-pentols 3a and 3b.** MS:  $m/z$  576 ( $M^+$ , 0.5%), 558 ( $M^+$ -H<sub>2</sub>O, 1%), 543 (558-Me, 2%), 383 ( $M^+$ - side-chain, 17%), 382 ( $M^+$ - side-chain -H, 3%), 341 (ring C cleavage, 3%), 323 (341-H<sub>2</sub>O, 3%), 205 (ring C cleavage, 100%), \*191 (ring C cleavage, 18%).

**9-Anthroly fluoride (4).** To a solution of cyanuric fluoride (327 mg, 2.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 ml) was added 9-anthracenecarboxylic acid (269 mg, 1.2 mmol) and pyridine (0.1 ml, 1.2 mmol). After the reaction mixture was stirred for 4 h at room temperature, the mixture was poured into ice-water, extracted with CH<sub>2</sub>Cl<sub>2</sub> and dried over MgSO<sub>4</sub>. After filtration, the filtrate was concentrated in *vacuo* to give a yellow residue, which

can be further purified by column chromatography on silica gel (hexane-AcOEt, 2:1). A yellow solid (244 mg) was obtained in 90% yield. M.S. :  $m/z$  242 (M+18)<sup>+</sup>.

**General procedure for microscale bichromophoric derivatization of bacterio-hopanepentols 2 and 3 to 5 and 6.**

i) **Anthroylation:** Each sample of hopanepentols (~100  $\mu$ g, 173 nmol) was dried in a silylated vial, dissolved in 150  $\mu$ l dry pyridine and stirred for overnight at room temperature with 9-anthroyl fluoride (116  $\mu$ g, 519 nmol) and catalytic amount of DMAP. The crude reaction mixture was purified by a small flash column on silica gel (MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 8:92). The fractions were concentrated in a silylated vial for next step, cinnamoylation.

ii) **Cinnamoylation:** To the 1-anthroate in acetonitrile (150  $\mu$ l) was added excess *p*-methoxycinnamoyl imidazole (0.5 mg) and DBU (0.3  $\mu$ l). The reaction mixture was stirred at room temperature for overnight. The final product was purified by HPLC (5 $\mu$  altech silica gel, AcOEt/Hexane, 30:70), characterized by UV, FAB-MS and submitted to CD measurements.

**35-Anthroate-31,32,33,34-tetra-*p*-methoxycinnamate hopanepentol (5).** FAB-MS  $m/z$  1241 (M<sup>+</sup>) (R = CH<sub>3</sub>, major). CD: 254 (+5.9), 287 (+5.7), 327 (-7.5) in MC; 253 (-4.3), 298 (+5.7), 306 (+5.5), 332 (-4.7) in AN.

**35-Anthroate-30,32,33,34-tetra-*p*-methoxycinnamate hopanepentol (6).** FAB-MS  $m/z$  1241 (M<sup>+</sup>) (R = CH<sub>3</sub>, major). CD: 253 (-4.8), 287 (+5.3), 323 (-9.9) in MC; 253 (-3.9), 298 (+4.8), 306 (+5.3), 332 (-4.3) in AN.

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