Novel Extracellular Diterpenoids with Biological Activity from the Cyanobacterium *Nostoc commune*

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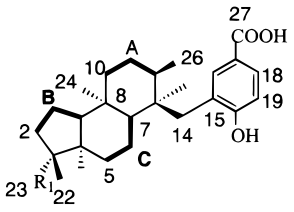
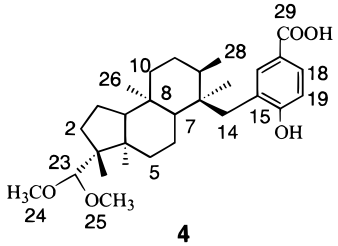
Received June 21, 1999

Five novel extracellular metabolites with an unprecedented diterpenoid skeleton, 5-[(5-carboxy-2-hydroxy)-benzyl]-11-hydroxymethyl-2,5,6,8a,11-pentamethyldodecahydrocyclopenta⟨*a*⟩naphthalene (**1**), 5-[(5-carboxy-2-hydroxy)benzyl]-11-formyl-2,5,6,8a,11-pentamethyl-dodecahydrocyclopenta⟨*a*⟩naphthalene (**2**), 5-[(5-carboxy-2-hydroxy)benzyl]-11-carboxy-2,5,6,8a,11-pentamethyl-dodecahydrocyclopenta⟨*a*⟩naphthalene (**3**), 5-[(5-carboxy-2-hydroxy)benzyl]-11-dihydroxymethyl-2,5,6,8a,11-pentamethyldodecahydrocyclopenta⟨*a*⟩-naphthalene (**4**), and 5-[(5-carboxy-2-hydroxy)benzyl]-11-acetyl-2,5,6,8a-tetramethyldodecahydrocyclopenta-⟨*a*⟩naphthalene (**5**), have been isolated from the culture medium of the terrestrial cyanobacterium *Nostoc commune* by means of bioguided isolation. The molecules were designated as comnostins A−E. The structures were determined by spectroscopic methods, mainly NMR and mass spectrometry. The relative stereochemistry of comnostin A was confirmed by single-crystal X-ray structure analysis. All comnostins showed antibacterial activities. Additionally, cytotoxic and molluscicidal activities were found for comnostin B.

Cyanobacteria are known to produce a number of interesting secondary metabolites, including peptides, alkaloids, nucleosides, and lactones.[1] Nevertheless, the occurrence of diterpenoids in cyanobacteria is rather uncommon. To our knowledge there are only two reports. Prinsep et al. described the isolation of tolypodiol, a diterpenoid isolated from the cells of *Tolypothrix nodosa*.[2] Recently, we described the structure of noscomin, the first reported extracellulary diterpenoid from the terrestrial cyanobacterium *Nostoc commune* Vaucher.[3] In continuation of our studies of this cyanobacterial strain, comnostins A−E (**1**−**5**), five biologically active metabolites with an unprecedented diterpenoid skeleton, have been isolated from the culture medium.



1 R_1 = CH_2OH
2 R_1 = CHO
3 R_1 = COOH



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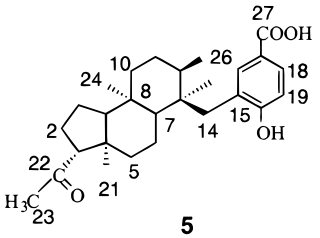

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Results and Discussion

Nostoc commune (EAWAG 122b) was cultured in an inorganic medium. The culture medium was separated from the cells and subjected to a solid-phase extraction on Amberlite XAD-2 resin. The resin was subsequently eluted with MeOH. The biologically active MeOH extract was fractionated on Sephadex LH-20. The final purification steps were performed by reversed-phase HPLC as outlined in the Experimental Section.

The molecular formula of comnostin A (**1**) was deduced as $C_{27}H_{40}O_4$ from ESIMS and EIMS in combination with ^{13}C NMR spectroscopy. The negative ESIMS gave the $[M - H]^-$ ion peak at *m/z* 427. The IR spectrum revealed absorption bands for hydroxy (3300 cm^{-1}), carboxy (1710 cm^{-1}), and aromatic (1604 and 1520 cm^{-1}) moieties. The ^1H NMR spectrum contained signals for five methyl groups, four tertiary (δ 0.86, 0.94, 0.99, 1.00, each s) and one secondary (δ 0.98 d, J = 5.5 Hz) C-atoms, as well as signals indicating a 1,2,4-substituted aromatic ring (δ 6.93, d, J = 8.4 Hz; δ 7.75, br d, J = 8.4 Hz; δ 7.89, br s). Furthermore, the methylene protons of a secondary alcohol group (δ 3.28 d, 3.60, each d, J = 10.4 Hz) could be detected in addition to a number of aliphatic signals. The ^{13}C spectrum of **1** showed the presence of one carboxy group (δ_C 167.8, s) and the previously deduced secondary alcohol moiety (δ_C 69.8, t). The observation of three low-field quaternary carbon signals (δ_C 122.0, 126.2, 161.4, each s) and three methine

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10.1021/np9903090 CCC: $19.00
Published on Web 02/16/2000

carbon signals (δ_C 130.1, 115.6, 135.5, each d) confirmed the presence of a trisubstituted aromatic ring.

Analysis of the DQF COSY and the TOCSY spectra revealed spin-system A (H_3-26, H-12, H_2-11, H_2-10), spin-system B (H_1-9, H_2-1, H_2-2), and spin-system C (H-7, H_2-6, H_2-5). The carbon skeleton was assembled by using the results of an HMBC experiment. In particular, the spin systems B and C were connected by two fragments. One was determined by the correlations observed between H-7 (δ 1.02, m, β) and C-8 (δ_C 37.9, s) and between C-9 (δ_C 59.3, d), C-8 (δ_C 37.9, s), C-7 (δ_C 52.1, d) and H_3-24 (δ 1.00, s). In addition the correlations observed between H_3-21 (δ 0.94, s) and C-5 (δ_C 35.4, t), C-4 (δ_C 48.2, s), and C-3 (δ_C 56.0, s), which in turn showed correlations to H_3-22 (δ 0.99, s), H_2-23 (δ 3.28, 3.60, each d, J = 10.4 Hz), H_2-2 (δ 1.63, m, β, δ 1.80, m, α), and H_2-1 (δ 1.27−1.43), completed the connectivities between spin-systems B and C.

HMBC couplings from H_2-14 (δ 2.66, 2.84, each d, J = 14.0 Hz) to C-7 (δ_C 52.1, d), C-13 (δ_C 42.0, s), C-12 (δ_C 37.0, d), C-15 (δ_C 126.2, s), C-16 (δ_C 135.5, s), C-20 (δ_C 161.4, s), and C-25 (δ_C 17.7, q) established the connectivity of the aromatic ring via a CH_2-group (C-14) to C-13 (δ_C 42.0, s), which, in turn, is the connecting carbon between spin-systems A and C. Interactions between H_2-14 and C-20 located the aromatic hydroxy-substituted carbon (δ_C 161.4, s). Further, the correlations from H-16 (δ 7.89, s) and H-18 (δ 7.75, br d, J = 8.4 Hz) to C-27 (δ_C 167.8, s) enabled us to position the acid moiety at C-17.

The relative stereochemistry structure of **1** was determined by a 2D-TROESY experiment. The spectrum exhibited the presence of ROEs, indicating that CH_3-21, CH_2-23, CH_3-24, and CH_3-25 were oriented on the same face of the diterpenoid plane (α), while H-7, H-9, CH_3-22, and CH_3-26 were in the opposite plane (β). This deduction was subsequently confirmed by a single-crystal X-ray structure analysis (Figure 1).

Comnostins B−E (**2**−**5**) were closely related to comnostin A. The molecular formula was deduced as $C_{27}H_{38}O_4$ for comnostin B (**2**), $C_{27}H_{38}O_5$ for comnostin C (**3**), $C_{29}H_{44}O_5$ for comnostin D (**4**), and $C_{27}H_{38}O_4$ for comnostin E (**5**) by ESIMS and EIMS as well as NMR spectroscopy. Comnostin B showed an aldehyde (C-23, δ_C 205.2, d; H-23, δ 9.6, s) and comnostin C a carboxy group (δ_C 181.4, s) instead of the secondary alcohol group in position 23 of comnostin A.

Comnostin D revealed an acetal group (C-23, δ_C 114.9, d; C-24, δ_C 60.1, q, H_3-24, δ 3.37, s; C-25, δ_C 56.7, q; H_3-25, δ 3.48, s) at position 23. It is possible that the acetal is generated by methylation of the aldehyde group of comnostin B during the extraction procedure with MeOH. Therefore, comnostin D may be an artifact of isolation and not a genuine natural product. Compared with comnostin A, comnostin E lacks the methyl group in position 22. An acetyl group replaces the secondary alcohol group (C-22, δ_C 226.7, s; C-23, δ_C 13.7, q; H-23, δ 1.03, s).

LC NMR Experiments. To exclude potential uncertainties from possibly incomplete axenic cultures, the cyanobacterial origin of the presented compounds was proven by conducting LC NMR experiments on the DCM−MeOH (2:1) extract of lyophylized cyanobacterial cells. The diterpenoid skeleton could be detected as a major compound in the cell material but in a minor concentration in comparison to the extracellular isolates. For the LC NMR study, the crude DCM−MeOH (2:1) extract was separated into six fractions by VLC over Si gel employing an *n*-hexane−EtOAc (80:20 to 20:80) gradient. Fraction 3 (*n*-hexane−EtOAc, 60:40) was investigated by LC NMR using the on-flow method (200-μg injection, RP_{18}, MeCN−H_2O, 60:40, flow 0.7 mL/min). The diterpenoid skeleton could be identified by ^{1}H NMR spectroscopy. As these medium-derived compounds were found to occur in the cell extracts in a minor concentration, it is suggested that they are actively released into the culture medium.

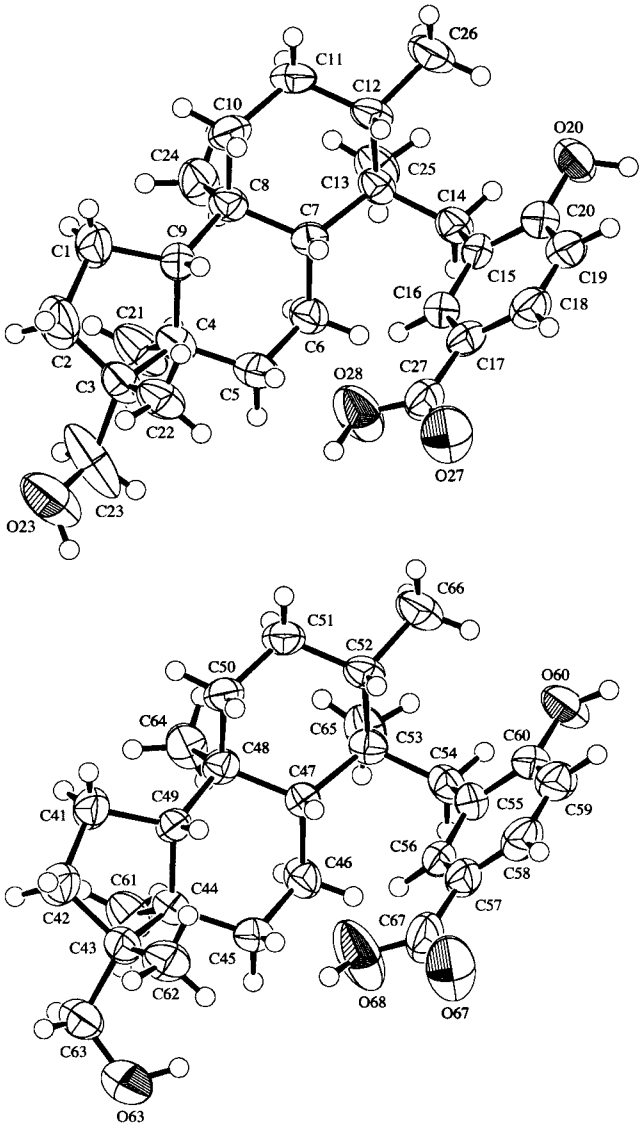

Figure 1. ORTEP drawing of the two independent molecules of **1** at 297 K, with 50% probability ellipsoids. H atoms are represented by circles of arbitrary radius.

Bioactivity. Comnostins C and E display a selective potent antibacterial activity. Comnostin C had a MIC value for *Escherichia coli* equal to tetracycline, and comnostin E had a MIC value for *Staphylococcus epidermidis* equal to chloramphenicol. Moderate antibacterial activity against *Bacillus cereus* could be detected for comnostins A−E, against *S. epidermidis* for comnostins A−D, and against *E. coli* for comnostins A, B, and D. Comnostin B additionally showed moderate cytotoxic activity in KB and Caco-2 cell assays and a strong molluscicidal effect against *Biomphalaria glabrata*. Details are listed in Table 1.

Considering them as actively released compounds and regarding the results of the biological testing, it may be concluded that comnostins A−E play a special role in the defense mechanism against enemies or other competitors.

Experimental Section

General Experimental Procedures. Optical rotation was recorded with a Perkin-Elmer 242 polarimeter using MeOH

Table 1. Biological Activities of the Isolates

	antibacterial activity (MIC, ppm)			molluscicidal activity (MIC, ppm)	cytotoxicity (ED_{50}, ppm)	
compound	*B. cereus*	*S. epidermdis*	*E. coli*	*B. glabrata*	KB cells	Caco-2 cells
comnostin A (**1**)	32	16	128			
comnostin B (**2**)	32	16	128	20	0.40	0.18
comnostin C (**3**)	32	16	64			
comnostin D (**4**)	16	32	128			
comnostin E (**5**)	128	4				
chloramphenicol	8	4				
tetracycline			64			
$CuSO_4$				100		
podophyllotoxin					0.01	0.02

as solvent. The IR spectra were measured on a Perkin-Elmer system 2000 FT-IR infrared spectrometer as a pressed KBr disk. The UV spectra were recorded in MeOH using a UVIKON 930 spectrophotometer. ESIMS spectra were measured on a Finnigan TSQ 7000 mass spectrometer and EIMS spectra on a Hitachi-Perkin-Elmer-RMUGM mass spectrometer at 70 eV. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AMX-300 spectrometer operating at a basic frequency of 300 MHz, using the solvent signal [CD_3OD, ^{1}H δ 3.31, ^{13}C δ 49.0 or $(CD_3)_2CO$, ^{1}H δ 2.05, ^{13}C δ 29.8 and 206.0] as a reference. HPLC separations were performed with a Waters model 590 pump connected to a Rheodyne HPLC injector, a Knauer variable wavelength detector, and a Knauer HPLC column (Hypersil ODS, 3 μm, 250 × 16 mm). LC NMR experiments were outlined on a Varian 500 MHz spectrometer, which was connected online to a Varian HPLC system with an analytic RP_{18} column (Grom ODS, 5 μm, 250 × 4 mm). MeOD as solvent was used as a reference (MeOD, ^{1}H δ 3.31).

Sephadex LH-20 (Pharmacia) was used for open column chromatography (column 4 × 100 cm). For TLC controls, RP_{18} F_{254} precoated sheets (0.25 mm, Merck) were used. All solvents used were HPLC grade.

Organisms and Culture Conditions. *Nostoc commune* Vaucher, designated strain EAWAG 122b, was isolated from a sample collected at Mellingen, Switzerland, 1965. The culture is deposited in the Culture Collection of Algae at the Swiss Federal Institute for Water Resources and Water Pollution Control (EAWAG), Dübendorf, Switzerland. The cyanophyte was cultivated in 10-L glass bottles containing a modified inorganic culture medium (Z).[4] The cultures were illuminated continuously with fluorescent lamps (Philips TLM/33 Rs 40 W) at 29 μmol/s/m^2, aerated with a mixture of 2% CO_2 in air, and incubated at a temperature of 24 ± 1 °C. The cyanobacterial cultures were harvested after 25−30 days. The supernatant was separated from the cells by filtration and adsorbed on a column filled with 250 g Amberlite XAD-2 resin (nonpolar, surface area 330 m^2/g). Subsequently, the column was washed with MeOH. This MeOH extract was dried in vacuo at temperatures below 35 °C.

Crystallographic Analysis of 1.[5] A crystal with the composition $C_{27}H_{40}O_4 \cdot H_2O$ obtained from MeOH, was used for an X-ray structure determination. All measurements were made on a Rigaku AFC5R diffractometer using graphite-monochromated Cu Kα radiation (λ = 1.54178 Å) and a 12-kW rotating anode generator. The intensities were collected using $\omega/2\theta$ scans, and three frequently measured standard reflections remained stable throughout the data collection. The data collection included the measurement of the Friedel opposites of all symmetry-unique reflections. The intensities were corrected for Lorentz and polarization effects, but not for absorption. The space group was determined from packing considerations, a statistical analysis of intensity distribution and the successful solution and refinement of the structure. Friedel pairs were not merged. The structure was solved by direct methods using SHELXS86,[6] which revealed the positions of all non-hydrogen atoms. The asymmetric unit contains two comnostin A molecules and two water molecules. The comnostin A molecules are of the same absolute configuration and differ principally only in the orientation of the hydroxy-methyl substituents. The difference in torsion angles about the C(3)−C(23) bond in molecule A and C(43)−C(63) bond in molecule B is about 103°. The non-hydrogen atoms were refined anisotropically. All of the H-atoms, except those of the hydroxy groups and water molecules, were fixed in geometrically calculated positions [d(C−H) = 0.95 Å]. The hydroxy and water H-atoms were fixed in the positions indicated by a difference electron density map. The distances and angles, but not the orientation, of the hydroxy H-atoms were then geometrically optimized. Each H-atom was assigned a fixed isotropic displacement parameter with a value equal to 1.2U_{eq} of the atom to which it was bonded. Refinement of the structure was carried out on F using full-matrix least-squares procedures, which minimized the function $\sum w(|F_o| - |F_c|)^2$, where $w = [\sigma^2(F_o) + (0.005F_o)^2]^{-1}$. A correction for secondary extinction was applied. Data collection and refinement parameters are given in Table 4. A view of the molecule is shown in Figure 1. All calculations were performed using the TEXSAN crystallographic software package.[7] The absolute configuration has not been determined. The enantiomorph used in the refinement was chosen arbitrarily.

Isolation of Comnostins A−E. The MeOH extract (1.2 g) obtained from 85 L of microscopically cell-free culture medium was applied to a gel-filtration column (4 × 100 cm, Sephadex LH-20, Pharmacia). Elution was carried out with 1.5 L of MeOH−H_2O (1:1), 1 L of MeOH−H_2O (75:25), 1 L of MeOH, 500 mL of MeOH−Me_2CO (1:1), and 200 mL of Me_2CO 100% to obtain 16 fractions (20−40 mg). Bioactive fraction 9 (79 mg, eluted with MeOH−H_2O, 75:25) was subjected to reversed-phase HPLC using MeOH−MeCN−H_2O (63:25:12) as an eluent to yield comnostin C as a pure compound (3.5 mg) in addition to fractions 1−3. Of these fractions, one (fraction 2) was rechromatographed on reversed-phase material with MeCN−H_2O (80:20) as eluent to give comnostin A (8.2 mg) and comnostin B (9.1 mg). Purification of the bioactive fraction 7 (50 mg, eluted with MeOH−H_2O, 1:1) on reversed-phase HPLC using MeOH−H_2O (40:60) as an eluent, gave comnostin D (3.3 mg) and comnostin E (4.0 mg).

Antibacterial Assay. The MIC determinations for comnostins A−E were performed as previously described.[8] Test organisms were *B. cereus* (ATCC 10702, Gram-positive), *S. epidermidis* (ATCC 12228, Gram-positive), and *E. coli* (ATCC 25922, Gram-negative).

Cytotoxic Assay. The ED_{50} values[9] for comnostin B were assessed using a KB cell line (ATCC CCL 17) as well as a Caco-2 cell line (ATCC HTB-37). The volumes were modified for cultivation in 24-well plates with MEM (21090−022; Gibco, Life Technologies, Switzerland) for KB cells and DMEM (31966−021, Gibco, Life Technologies, Switzerland) for Caco-2 cells.

Molluscicidal Assay. The MIC determination for comnostin B was performed with freshwater snails of the species *B. glabrata*.[10]

Physical and Spectroscopic Data. Comnostin A (1): white, amorphous solid (8.2 mg); mp 255 °C; $[\alpha]^{25}_D$ +44° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3300, 1710, 1604, 1520 cm^{-1}; UV λ_{max} (MeOH) 252 nm; ^{1}H NMR [300 MHz, $(CD_3)_2CO$] and ^{13}C NMR [75.5 MHz, $(CD_3)_2CO$], see Tables 2 and 3; EIMS *m/z* (rel int) $[M]^+$ absent, 411 $[M - OH]^+$ (<1), 382 $[M - COOH - H]^+$ (<1), 367 $[M - CO_2 - H]^+$ (<1), 277 $[M - C_8H_7O_3]^+$ (100), 259

Table 2. ^{1}H NMR Spectral Data of **1**−**5** (300 MHz, δ ppm, J Hz)

H	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[b]
1	1.27−1.43[c]	1.37−1.49[c]	1.42−1−46[c]	1.34−1−45[c]	1.75−1.82[c]
2	1.63 (1H, m, β)	1.64 (1H, m, β)	1.27 (1H, m, β),	1.16 (1H, m, β),	2.03 (1H, m, β)
	1.80 (1H, m, α)	1.73 (1H, m, α)	2.48 (1H, m, α)	1.68 (1H, m, α)	2.45 (1H, m, α)
3					1.81−1.86[c]
5	1.14 (1H, m, β)	1.08 (1H, m, β)	1.79 (1H, m, β)	1.52 (1H, m, β)	1.20 (1H, m, β)
	1.43 (1H, m, α)	1.15 (1H, m, α)	1.84 (1H, m, α)	1.63 (1H, m, α)	1.33 (1H, m, α)
6	1.63 (1H, m, α)	1.66 (1H, m, α)	1.63 (1H, m, α)	1.48 (1H, m, α)	1.60 (1H, m, α)
	2.05 (1H, m, β)	2.13 (1H, m, β)	2.15 (1H, m, β)	2.01 (1H, m, β)	2.13 (1H, m, β)
7	1.02 (1H, m, β)	1.04−1.08[c]	0.96 (1H, m, β)	0.86−0.87[c]	0.89−0.90[c]
9	1.45 (1H, m, β)	1.38 (1H, m, β)	1.40(1H, m, $^{\beta)}$	1.30 (1H, m, β)	1.43 (1H, m, β)
10	1.24−1.32[c]	1.28−1.34[c]	1.33−1.38[c]	1.22−1.29[c]	1.57−1.61[c]
11	1.16−1.33[c]	1.17−1.39[c]	1.29−1.31[c]	1.17−1.38[c]	1.27−1.32[c]
12	1.27[c]	1.25[c]	1.25[c]	1.22[c]	1.33[c]
14	2.66 d (14.0)	2.65 d (14.1)	2.62 d (13.9),	2.61 d (14.1),	2.65 d (14.0),
	2.84 d (14.0)	2.88 d (14.1)	2.81 d (13.9)	2.86 d (14.1)	2.80 d (14.0)
16	7.89 br s	7.99 br s	7.84 br s	7.77 br s	7.83 br s
18	7.75 br d (8.4)	7.76 br d (8.4)	7.70 br d (8.3)	7.65 br d (8.4)	7.50 br d (8.4)
19	6.93 d (8.4)	6.95 d (8.4)	6.78 d (8.3)	6.72 d (8.4)	6.79 d (8.4)
21	0.94 s	0.98 s	1.16 s	1.16 s	0.96 s
22	0.99 s	0.92 s	0.94 s	0.84 s	
23	3.28 d (10.4)	9.60 s		4.15 s	1.03 s
	3.60 d (10.4)				
24	1.00 s	0.95 s	0.93 s	3.37 s	0.93 s
25	0.86 s	0.87 s	0.85 s	3.48 s	0.86 s
26	0.98 d (5.5)	1.02 d (6.2)	1.01 d (6.0)	0.88 s	1.07 d (7.5)
27				0.80 s	
28				0.96 d (5.5)	

[a] Measured in $(CD_3)_2CO$. [b] Measured in MeOD. [c] Multiplicity and intensity of the signals are unclear due to overlapping.

Table 3. ^{13}C NMR Spectral Data of **1**−**5** (75.5 MHz, δ ppm)[a]

C	**1**[b]	**2**[b]	**3**[c]	**4**[c]	**5**[c]
1	20.6 t	20.4 t	20.0 t	20.5 t	20.1 t
2	34.5 t	43.2 t	32.7 t	34.5 t	37.6 t
3	56.0 s	59.1 s	57.7 s	52.9 s	51.6 s
4	48.2 s	46.9 s	46.6 s	47.0 s	40.7 s
5	35.4 t	36.9 t	34.4 t	35.5 t	37.2 t
6	20.7 t	20.4 t	20.9 t	20.8 t	22.9 t
7	52.1 d	51.8 d	52.3 d	52.5 d	51.6 d
8	37.9 s	38.0 s	38.3 s	38.3 s	38.7 s
9	59.3 d	58.8 d	58.3 d	58.9 d	51.6 d
10	40.7 t	40.4 t	40.8 t	41.1 t	40.5 t
11	28.0 t	29.0 t	28.4 t	28.4 t	28.5 t
12	37.0 d	37.0 d	37.3 d	37.5 d	37.0 d
13	42.0 s	42.0 s	42.4 s	42.4 s	42.7 s
14	37.1 t	37.0 t	37.4 t	37.4 t	37.9 t
15	126.2 s	126.1 s	126.6 s	126.6 s	127.8 s
16	135.5 d	135.4 d	135.9 d	135.8 s	136.0 d
17	122.0 s	121.9 s	121.8 s	122.6 s	122.2 s
18	130.1 d	130.1 d	130.4 d	130.3 d	130.4 d
19	115.6 d	115.7 d	115.6 d	115.5 d	115.5 d
20	161.4 s	161.5 s	162.5 s	162.8 s	162.1 s
21	18.3 q	20.2 q	17.2 q	17.8 q	24.0 q
22	23.5 q	19.7 q	23.7 q	16.9 q	226.7 s
23	69.8 t	205.2 s	181.4 s	114.9 d	13.7 q
24	16.7 q	16.6 q	19.3 q	60.1 q	16.9 q
25	17.7 q	17.8 q	18.3 q	56.7 q	18.2 q
26	17.8 q	18.3 q	17.9 q	17.9 q	18.3 q
27	167.8 s	167.8 s	170.5 s	18.3 q	171.2 s
28				17.5 q	
29				171.1 s	

[a] Multiplicity by DEPT. [b] Measured in $(CD_3)_2CO$. [c] Measured in MeOD.

Table 4. Crystallographic Data for **1**

empirical formula	$C_{27}H_{40}O_4 \cdot H_2O$
formula weight	446.62
crystal color, habit	colorless, tablet
crystal dimens (mm)	0.15 × 0.40 × 0.40
temperature (K)	297 (1)
crystal system	triclinic
space group	$P1$
Z	2
reflns for cell determination	25
2θ range for cell determination (deg)	93−96
a (Å)	11.3260 (8)
b (Å)	16.293 (1)
c (Å)	6.625 (2)
α (deg)	90.04 (1)
β (deg)	92.28 (1)
γ (deg)	84.489 (5)
V (Å^3)	1215.8 (3)
F(000)	488
D_x (g cm^{-3})	1.220
μ(Mo Kα) (mm^{-1})	0.655
$2\theta_{(max)}$ (deg)	120
refln ranges	$-12 < h < 12$; $-18 < k < 18$; $-7 < l < 7$
total no. of reflns measured	7684
no. of symmetry independent reflns	7684
no. of reflns used [$I > 2\sigma(I)$]	7322
no. of params refined	575
R	0.0620
R_w	0.0654
goodness of fit	4.815
secondary extinction coeff.	5.6(6) × 10^{-6}
final Δ_{max}/σ	0.002
$\Delta\rho$ (max; min) (e Å^{-3})	0.44; −0.34

$[M - C_8H_7O_3 - H_2O]^+$ (65), 151 $[M - C_{18}H_{33}O]^+$ (50), 107 $[M - C_{19}H_{33}O - CO_2]^+$ (33); ESIMS m/z (rel int) $[M - H]^-$ 427 (100).

Comnostin B (**2**): white, amorphous solid (9.1 mg); mp 258 °C; $[\alpha]^{25}_D$ +45° (c 0.1, MeOH); IR (KBr) ν_{max} 3283, 1704, 1602, 1512 cm^{-1}; UV λ_{max}(MeOH) 251 nm; ^{1}H NMR [300 MHz, $(CD_3)_2CO$] and ^{13}C NMR [75.5 MHz, $(CD_3)_2CO$], see Tables 2 and 3; EIMS m/z (rel int) $[M]^+$ absent, 409 $[M - OH]^+$ (<1), 381 $[M - COOH]^+$ (<1), 275 $[C_8H_7O_3]^+$ (100), 247 $[M - C_8H_7O_3 - CO]^+$ (51), 151 $[M - C_{19}H_{31}O]^+$ (64), 107 $[M - C_{19}H_{31}O]^+$ (24); ESIMS m/z (rel int) $[M - H]^-$ 425 (100).

Comnostin C (**3**): white, amorphous solid (3.5 mg); mp 252 °C; $[\alpha]^{25}_D$ +37° (c 0.1, MeOH); IR (KBr) ν_{max} 3350, 1715, 1620, 1525 cm^{-1}; UV λ_{max}(MeOH) 251 nm; ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75.5 MHz, CD_3OD), see Tables 2 and 3; EIMS m/z (rel int) $[M]^+$ absent, 425 $[M - OH]^+$ (<1), 396 $[M - COOH - H]^+$ (<1), 381 $[M - CO_2 - OH]^+$ (<1), 291 $[M - C_8H_7O_3]^+$ (2), 274 $[M - C_8H_7O_3 - OH]^+$ (81), 273 $[M - C_8H_7O_3 - H_2O]^+$ (8), 274 $[M - C_8H_7O_3 - OH]^+$ (273), 151 $[M - C_{19}H_{31}O_2]^+$ (22), 107 $[M - C_{19}H_{31}O_2 - CO_2]^+$ (48); ESIMS m/z (rel int) $[M - H]^-$ 441 (100).

Comnostin D (**4**): yellow, amorphous solid (3.3 mg); mp 251 °C; $[\alpha]^{25}_D$ +21° (c 0.1, MeOH); IR (KBr) ν_{max} 3350, 1710, 1622,

1525 cm^{-1}; UV λ_{max}(MeOH) 246 nm; ^1H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75.5 MHz, CD_3OD), see Tables 2 and 3; EIMS *m/z* (rel int) $[M]^+$ absent, 429 $[M - CO_2 - H]^+$ (<1), 321 $[M - C_8H_7O_3]^+$ (<1), 151 $[M - C_{21}H_{37}O_2]^+$ (4), 107 $[M - C_{21}H_{37}O_2 - CO_2]^+$ (19); ESIMS *m/z* (rel int) $[M - H]^-$ 471 (100).

Comnostin E (5): yellow amorphous solid (4.0 mg); mp 256 °C; $[\alpha]^{25}_D$ +15° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3345, 1722, 1623, 1525 cm^{-1}; UV λ_{max}(MeOH) 247 nm; ^1H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75.5 MHz, CD_3OD), see Tables 2 and 3; EIMS *m/z* (rel int) $[M]^+$ absent, 409 $[M - OH]^+$ (<1), 382 $[M - CO_2]^+$ (<1), 381 $[M - COOH]^+$ (<1), 380 $[M - COOH - H]^+$ (<1), 365 $[M - CO_2 - OH]^+$ (<1), 275 $[M - C_8H_7O_3]^+$ (36), 257 $[M - C_8H_7O_3 - H_2O]^+$ (6), 151 $[M - C_{19}H_{31}O]^+$ (6), 107 $[M - C_{19}H_{31}O - CO_2]^+$ (54); ESIMS (MeOH) *m/z* (rel int) $[M - H]^-$ 425 (100).

Acknowledgment. We thank Dr. Hans-Rudolf Bürgi, Dr. Marianne Bosli, and Mr. Frank Sunder (EAWAG, Dübendorf/Switzerland) for providing and cultivating the cyanobacterial material; Dr. Oliver Zerbe (ETH Institute of Pharmaceutical Sciences) for assistance in NMR measurements; Dr. Engelbert Zass (ETH Chemistry Department) for performing literature searches; Mr. Oswald Greter and Dr. Walter Amrein (ETH Chemistry Department, Mass Spectral Service) for recording mass spectra; and Mr. Michael Wasescha for technical assistance and performing KB cell assays.

Supporting Information Available: X-ray crystallographic data for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP9903090