New Antibacterial Metabolites from the Cyanobacterium *Nostoc commune* (EAWAG 122b)

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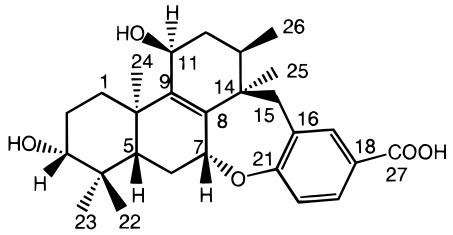
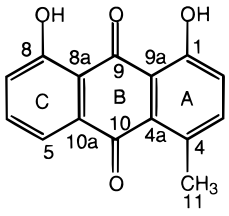
Two new compounds, a diterpenoid and an anthraquinone, as well as an indane derivative, which is reported as a natural product for the first time, have been isolated from the cells of the cultured cyanobacterium *Nostoc commune* (EAWAG 122b) by means of bioguided isolation. The structures were determined by spectroscopic methods, mainly NMR, infrared spectroscopy, and mass spectrometry. All isolates exhibit antibacterial activity.

Terrestrial and marine blue-green algae have proven to be an extremely valuable source of novel bioactive agents.[1] We recently reported the structures of noscomin[2] and comnostins A−E.[3] These bioactive compounds were isolated from the culture medium of the cyanobacterium *Nostoc commune* Vaucher (EAWAG 122b). In a continuing investigation of this cyanobacterium, we report here the isolation, structure elucidation, and biological activity of three more compounds, 8-[(5-carboxy-2,9-epoxy)benzyl]-2,5-dihydroxy-1,1,4a,7,8-pentamethyl-1,2,3,4,4a,6,7,8,9,10,10a-dodecahydrophenanthrene (**1**), 1,8-dihydroxy-4-methylanthraquinone (**2**), and 4-hydroxy-7-methylindan-1-one (**3**) from the cultured cells.



1



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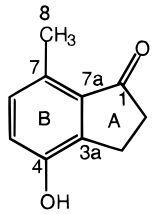

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ESIMS of **1** gave the $[M - H]^-$ ion peak at *m*/*z* 439.2, and EIMS gave the $[M]^+$ ion peak at *m*/*z* 440.1. The IR spectrum revealed bonds for hydroxyl (3416 cm^{-1}), carboxyl (1686 cm^{-1}), and aromatic (1603 and 1546 cm^{-1}) groups. The ^1H NMR spectrum contained signals for five methyl groups, four tertiary (δ 0.87, 1.08, 1.11, 1.20, each s) and one secondary (δ 1.14, d, J = 6.9 Hz) as well as signals indicative of a 1,2,4-substituted aromatic ring (δ 6.9, d, J = 8.2 Hz, δ 7.73, d, J = 1.9 Hz, δ 7.77, dd, J = 1.9, 8.2 Hz). Three protons attached to oxygenated carbons (δ 3.21, dd, J = 5.0, 11.2 Hz; 4.76, m; 4.25 m) could be detected, in addition to a number of aliphatic signals (see Table 1).

The ^{13}C NMR spectral data of **1** showed the presence of one carboxy group (δ_C 170.2, s), three tertiary (δ_C 64.8, 77.1, 79.5, each d) aliphatic oxygen-substituted C atoms, and one C=C double bond (δ_C 138.5, 146.9, each s). Furthermore, the observation of three low-field quaternary carbon signals (δ_C 126.9, 127.0, 163.8, each s) and three methine carbon signals (δ_C 121.6, 130.6, 133.6, 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-13, H_2-12, and H-11), spin system B (H_2-1, H_2-2, H-3), and spin system C (H-7, H_2-6, H-5).

The structure was assembled by analysis of an HMBC experiment. In particular, the spin systems B and C were connected by two fragments. One was determined by the correlations between H-7 (δ 4.76, m) and C-8 (δ_C 138.5, s) as well as C-9 (δ_C 146.9, s) and between C-1 (δ_C 36.2, t), C-5 (δ_C 45.6, d), C-9 (δ_C 146.9, s), C-10 (δ_C 39.8, s), and H_3-24 (δ 1.20, s). The second is generated on the basis of interactions from the quaternary carbon C-4 (δ_C 39.9, s) to H_3-22 (δ 0.87, s) and H_3-23 (δ 1.11, s), which both further correlated to C-3 (δ_C 79.5, d) and C-5 (δ_C 45.6, d). The spin systems A and C were connected by a fragment determined to be due to the correlations H-7 (δ 4.76, m) to C-8 (δ_C 138.5, s) and C-14 (δ 41.4, s) as well as H-13 (δ 2.01, m) to C-8 (δ 138.5, s). The ring skeleton of the diterpenoid was completed by the correlations between H-11 (δ 4.25, m) and H_2-12 (δ 1.44, dd J = 2.9, 5.8) to C-9 (δ_C 146.9, s). Couplings from H_2-15 (δ 2.62; 2.77, each d, J = 14.1 Hz) to C-8 (δ_C 138.5, s), C-13 (δ_C 34.4, d), C-14 (δ_C 41.4, s), C-16 (δ_C, 127.0, s), C-21 (δ_C 163.8, s), and CH_3-25 (δ_C 26.4, q) established that the aromatic ring was attached via a CH_2 group (δ_C 38.2, t). Correlations between H-7 (δ 4.76, m) and C-21 (δ_C 163.8, s) determined the ether connection between the aromatic ring system and the diterpenoid skeleton.

Further correlations from C-27 (δ_C 170.2, s) to H-17 (δ 7.73, d, J = 1.9 Hz) and H-19 (δ 7.77, dd, J = 1.9, 8.2 Hz) enabled us to position the carboxy group at C-18 (δ_C 126.9,

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Table 1. ^{1}H NMR Spectral Data of **1**−**3** (δ ppm, J Hz, MeOD)

H	**1**[a]	**2**[b]	**3**[a]
1	1.26 (ddd, 4.4, 11.3, 17.5, β)		
	2.05 (ddd, 3.2, 12.7, 17.5, α)		
2	1.73 (m)	7.21 (d, 8.7)	2.52 (dd, 5.5, 18.1)
			3.15 (dd, 5.5, 18.1)
3	3.21 (dd, 5.0, 11.2)	7.49 (d, 8.7)	2.26[c]
			1.63[c]
3a			
4			
4a			
5	1.68 (dd, 3.2, 7.3)	6.76 (dd, 2.7, 8.0)	7.38 (d, 8.5)
6	1.98 (d, 14.1, α)	6.74 (br d, 8.0)	6.81 (d, 8.5)
	2.21 (d, 14.1, β)		
7	4.76 (m)	6.70 (dd, 2.7, 8.0)	
7a			
8			2.38 (s)
8a			
9			
9a			
10			
10a			
11	4.25 (m)	2.60 (s)	
12	1.44 (dd, 2.9, 5.8)		
13	2.01 (m)		
14			
15	2.62 (d, 14.1)		
	2.77 (d, 14.1)		
16			
17	7.73 (d, 1.9)		
18			
19	7.77 (dd, 1.9, 8.2)		
20	6.90 (d, 8.2)		
21			
22	0.87 (s)		
23	1.11 (s)		
24	1.20 (s)		
25	1.08 (s)		
26	1.14 (d, 6.9)		
27			

[a] Measured at 300 MHz. [b] Measured at 500 MHz.

Table 2. ^{13}C NMR Data of Compounds **1**−**3** (δ ppm, MeOD)

C	**1**[a,c]	**2**[b,c]	**3**[a,d]
1	36.2 t	161.1 s	207.4 C
2	28.5 t	125.9 d	33.4 CH_2
3	79.5 d	139.4 d	31.3 CH_2
3a			117.6 C
4	39.9 s	128.3 s	162.3 C
4a		134.4 s	
5	45.6 d	119.1 d	118.7 CH
6	29.3 t	117.8 d	136.2 CH
7	77.1 d	116.5 d	129.2 C
7a			143.2 C
8	138.5 s	149.3 s	18.1 CH_3
8a		120.9 s	
9	146.9 s	191.2 s	
9a		115.2 s	
10	39.8 s	184.8 s	
10a		150.5 s	
11	64.8 d	22.5 q	
12	38.6 t		
13	34.4 d		
14	41.4 s		
15	38.2 t		
16	127.0 s		
17	133.6 d		
18	126.9 s		
19	130.6 d		
20	121.6 d		
21	163.8 s		
22	28.7 q		
23	16.5 q		
24	21.8 q		
25	26.4 q		
26	17.6 q		
27	170.2 s		

[a] Measured at 75 MHz. [b] Measured at 125 MHz. [c] Multiplicities were determined by a DEPT 135 experiment. [d] Multiplicities were determined by a HMQC experiment.

s). Correlations between H_2-15, H-7, and C-21 located the aromatic hydroxy-substituted carbon at C-21.

The relative stereochemistry of **1** was determined by a 2D TROESY experiment. The TROESY spectrum exhibited the presence of ROEs indicating that H-13, H_3-23, H_3-24, and H_3-25 were oriented on the same face of the diterpenoid plane (α), while H-3, H-5, H-7, and H_3-22 were in β-position. Comprising the same diterpenoid skeleton with an additional ether ring connection, compound **1** is structurally related to noscomin.[2]

EIMS of **2** gave the $[M + H]^+$ ion peak at m/z 255.2. The ^{1}H NMR spectrum of **2** contained signals for one methyl group attached to an aromatic ring (δ 2.60, s), as well as two aromatic doublets (δ 7.21, d, J = 8.7 Hz; 7.49, d, J = 8.7 Hz) and three aromatic double doublets (δ 6.70, dd, J = 2.7, 8.0 Hz; 6.74, br d, J = 8.0 Hz; 6.76, dd, J = 2.7, 8.0 Hz) (see Table 1). In the ^{13}C NMR spectrum a total of 15 carbon signals could be observed. The spectrum shows the presence of one methyl group (δ_C 22.5, q), two carbonyl groups (δ_C 191.2, 184.8, each s), and two aromatic oxygen-substituted carbons (δ_C 149.3, 161.1, each s). Additionally, five low-field methine carbon signals (δ_C 116.5, 117.8, 119.1, 125.9, 139.4, each d) and four low-field quaternary carbon signals (δ_C 115.2, 120.9, 134.4, 150.5, each s) could be detected (see Table 2).

The structure was assembled from HMBC experiment results. Ring A was established by the couplings from H_3-11 (δ 2.60, s) to C-4a (δ_C 134.4, s), C-4 (δ_C 128.3, s), and C-3 (δ_C 139.4, d); from H-3 (δ 7.49, d, J = 8.7 Hz) to C-1 (δ_C 161.1, s), C-4a (δ_C 134.4, s), and C-4 (δ_C 128.3, s), as well as from H-2 (δ 7.21, d, J = 8.7 Hz) to C-9a (δ_C 115.2, s). Correlations observed from H-5 (δ 6.76, dd, J = 2.7, 8.0 Hz) to C-10a (δ_C 150.5, s), C-6 (δ_C 117.8, d), and C-7 (δ_C 116.5, d), from H-6 (δ 6.74, br d, J = 8.0 Hz) to C-7 (δ_C 116.5, d), C-8 (δ_C 149.3, s), and C-10a (δ_C 150.5, s), and from H-7 (δ 6.70, dd, J = 2.7, 8.0 Hz) to C-6 (δ_C 117.8, d), C-5 (δ_C 119.1, d), C-8 (δ_C 149.3, s), and C-8a (δ_C 120.9, s) generated ring C. An additional correlation could be observed between H-5 (δ 6.76, dd, J = 2.7, 8.0 Hz) and C-10 (δ_C 184.8, s).

The 1 hydroxy−4 methyl substitution pattern of ring A instead of a 1 methyl−4 hydroxy was proven by infrared spectroscopy, which showed two separated carbonyl bonds at 1608 cm^{-1} belonging to the chelated carbonyl group C-9 and at 1740 cm^{-1} belonging to the free carbonyl group at C-10.

To our knowledge compound **2** is the first anthraquinone isolated from a cyanobacterium.

EIMS of **3** gave the $[M + H]^+$ ion peak at m/z 163.1. The ^{1}H NMR of **3** contained signals for one methyl group (δ 2.38, s) attached to an aromatic ring as well as two aromatic (δ 6.81; 7.38 each d, J = 8.5 Hz) and two aliphatic doublets (δ 2.52, 3.15, dd, J = 5.5, 18.1 Hz: see Table 1). The ^{13}C NMR data were assigned indirectly by analyzing HMQC and HMBC spectra (see Table 2). These data comprise typical shifts for five low-field quaternary (δ_C 207.4, 162.3, 129.2, 143.2, 117.6, each s) and two low-field tertiary (δ_C 118.7, 136.2, each d) carbons, two aliphatic CH_2 moieties (δ_C 31.3, 33.4, each t), and one methyl group (δ_C 18.1, q).

An HMBC experiment established ring B by the couplings from H-5 (δ 7.38, d, J = 8.5 Hz) to C-4 (δ_C 162.3, s),

Table 3. Biological Activities of the Isolates

compound	antibacterial activity [MIC] (ppm)	
	S. epidermidis	*B. cereus*
1	4	128
2	32	32
3	128	64
chloramphenicol	4	8

C-6 (δ_C 136.2, d) and C-7 (δ_C129.2, s), from H_3-8 (δ 2.38, s) to C-7 (δ_C 129.2, s), C-7a (δ_C 143.2, s), and C-6 (δ_C 136.2, d), and from H-6 (δ 6.81, d, J = 8.5 Hz) to C-7a (δ_C 143.2, s). Correlations observed from H-2 (δ 2.52, 3.15, dd, J = 5.5, 18.1 Hz, each) to C-3 (δ_C 31.3, t), C-3a (δ_C 117.6, s), and C-1 (δ_C 207.4, s) and from H-3 (δ 1.63, 2.66) to C-4 (δ_C 162.3, s), C-2 (δ_C 33.4, t), and C-7a (δ_C 143.2, s) generated ring A. Although **3** is known as a synthetic compound,[4] this is the first report of **3** as a natural product.

Compound **1** displays a selective potent antibacterial activity against *Staphylococcus epidermidis* equal to chloramphenicol. Moderate antibacterial activity against *S. epidermidis* could be detected for compounds **2** and **3** and against *Bacillus cereus* for compounds **1**, **2**, and **3**. Data are listed in Table 3.

Experimental Section

General Experimental Procedures. Optical rotation was recorded with a Perkin-Elmer 242 polarimeter using MeOH as solvent. The IR spectra were measured on a Perkin-Elmer System 2000 FT-IR infrared spectrometer as liquid films on a pressed KBr disk. The UV spectra were recorded in MeOH using a Uvicon 930 spectrophotometer. ESIMS spectra were measured on a Finnigan TSQ 7000 mass spectrometer; 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 or a Bruker AMX-500 spectrometer operating at a basic frequency of 300 or 500 MHz, respectively, using solvent (CH_3OH, ^{1}H δ 3.31, ^{13}C δ 49.0) as a reference. HPLC separations were performed with a Merck-Hitachi pump connected to a Rheodyne HPLC injector, a Merck variable wavelength monitor, and a Knauer HPLC column (Hypersil ODS, 5 μm, 250 × 16 mm). Si gel was used for open column chromatography and VLC. For TLC controls, RP-18 F_{254} precoated sheets (0.25 mm, Merck) were used. All solvents were HPLC grade.

Organisms and Culture Conditions. *N. 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 cyanobacterium was cultivated in 10 L glass bottles containing a modified inorganic culture medium (Z).[5] The cultures were illuminated continuously with fluorescent lamps (Philips TLM/33 Rs 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 cultures were harvested after 25−30 days, filtered, and lyophylized.

Isolation. The 70:30 MeOH/H_2O extract obtained from 43 g of lyophylized cell material was subjected to Si gel vacuum liquid chromatography. Elution was carried out with a step gradient hexane/EtOAc (90:10 to 10:90) mixture to obtain 11 fractions. Fractions 5 and 6 exhibited antibacterial activity. Bioactive fraction 6 (80 mg, eluted with 1:1 hexane/EtOAc) was subjected to Si gel open column chromatography using 30:70 $CHCl_3$/MeOH as eluent to yield five fractions. Of these fractions, one (fraction 4) was purified by means of reversed-phase chromatography (MeCN/H_2O, 60:40) and gave **1** (4 mg). Bioactive fraction 5 was subjected to Si gel open column chromatography using $CHCl_3$/MeOH (45:55) as eluent to yield eight fractions. Of these fractions, one (fraction 5) was purified by means of reversed-phase chromatography (MeOH/H_2O, 65:35) and gave **2** (3 mg) and **3** (3 mg).

Antibacterial Assay. The MIC determination for **1**, **2**, and **3** was performed, as previously described.[6] Test organisms were *B. cereus* (ATCC 10702) and *S. epidermidis* (ATCC 12228).

Physical and Spectroscopic Data

Compound 1: white amorphous solid (4 mg); $[\alpha]^{20}_D$ +15° (*c* 0.1, MeOH); UV (MeOH) λ_{max} 280 nm; ^{1}H NMR (500 MHz, MeOD) and ^{13}C NMR (125 MHz, MeOD) see Tables 1 and 2; EIMS (MeOH) *m/z* (rel int) 440 $[M]^+$ (1), 422 $[M - H_2O]^+$ (<1), 389 $[M - 3\ OH]^+$ (<1), 361 $[M - 2\ OH - COOH]^+$ (<1), 289 $[M - C_8H_7O_3]^+$ (<1), 271 $[M - C_8H_7O_3 - H_2O]^+$ (2), 163 $[M - C_{18}H_{29}O_2]^+$ (<1), 119 $[M - C_{18}H_{29}O_2 - CO_2]^+$ (1); ESIMS (MeOH) *m/z* (rel int) $[M - H]^+$ 439.2 (100).

Compound 2: red oil (3 mg); IR (KBr disk) ν_{max} 3387, 1740, 1608 cm^{-1}; UV (MeOH) λ_{max} 276 nm; ^{1}H NMR (300 MHz, MeOD) and ^{13}C NMR (75 MHz, MeOD) see Tables 1 and 2; EIMS 254 $[M]^+$ (6), 239 $[M - CH_3]^+$ (28), 225 $[M - H - CO]^+$ (7), 207 $[M - H - CO - H_2O]^+$ (9), 189 $[M - H - CO - 2H_2O]^+$ (9).

Compound 3: yellow oil (3 mg); ^{1}H NMR (300 MHz, MeOD) and ^{13}C NMR (75 MHz, MeOD) see Tables 1 and 2; EIMS 163 $[M + H]^+$ (42), 147 $[M + H - CH_3]^+$ (26), 135 $[M + H - CO]^+$ (93).

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