A Novel Extracellular Diterpenoid with Antibacterial Activity from the Cyanobacterium *Nostoc commune*

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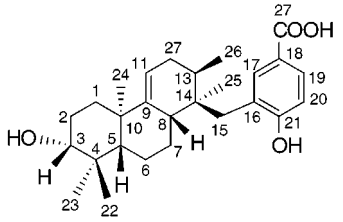
A novel extracellular metabolite with an unprecedented diterpenoid skeleton, 8-[(5-carboxy-2-hydroxy)-benzyl]-2-hydroxy-1,1,4a,7,8-pentamethyl-1,2,3,4,4a,6,7,8,8a,9,10,10a-dodecahydrophenanthrene, has been isolated from the culture medium of the terrestrial cyanobacterium *Nostoc commune* Vaucher (EAWAG 122b) by means of bioguided isolation. The molecule was designated as noscomin. The structure was determined by spectroscopic methods, mainly NMR and mass spectrometry. Noscomin exhibited antibacterial activity against *Bacillus cereus*, *Staphylococcus epidermidis*, and *Escherichia coli*.

Cyanobacteria are known to be a rich source of secondary metabolites with a wide variety of biological activities including toxins, antibiotics, fungicides, and antineoplastic agents.[1] The majority of these metabolites have been found in association with the cyanobacterial cells. The occurrence of terpenoids in cyanobacteria is rather uncommon. A few examples are the triterpenoid bacteriophanes[2] isolated from several species of cyanobacteria. The antifungal hapalindoles,[3,4] hapalindolinones,[5] and ambiguines[6] as well as the welwitindolinones[7] are metabolites of mixed biosynthetic origin containing isoprene units and have been found in several species of the family Stigonemataceae. The only diterpenoid compound reported from cyanobacteria is the antiinflammatory tolypodiol[8] from *Tolypothrix nodosa*.

This paper describes the isolation of noscomin (**1**), a novel antibacterial metabolite with an unprecedented diterpenoid skeleton, from the culture medium of a cultured *Nostoc commune* Vaucher strain. This strain was selected on the basis of a biological screening of 43 different cyanobacterial cultures.



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 step was performed by reversed-phase HPLC as outlined in the Experimental Section.

Noscomin gave the $[M - H]^-$ ion peak at *m/z* 425.2 by ESIMS. The IR spectrum revealed bonds for hydroxyl (3416 cm^{-1}), carboxyl (1686 cm^{-1}), and aromatic (1603 and 1546 cm^{-1}) moieties. The ^1H NMR spectrum contained signals for five methyl groups, four tertiary (δ 0.67, 0.88, 0.89, 1.11, each s), one secondary (δ 1.05 d, J = 6.7 Hz) as well as signals indicative of a 1,2,4-substituted aromatic ring (δ 6.74 d, J = 8.3 Hz, δ 7.70 d, J = 6.6 Hz, δ 7.80 s). Furthermore, one proton attached to an oxygenated carbon (δ 3.19 dd J = 4.7, 11.1 Hz), and an olefinic CH resonance (δ 5.38 m) could be detected in addition to a number of aliphatic signals. The ^{13}C NMR spectral data of **1** showed the presence of one carboxy group (δ_C 172.0 s), one tertiary aliphatic oxygen-substituted C atom (δ_C 79.8 d), and one C=C double bond (δ_C 116.4 d, 152.1 s). The observation of three quaternary carbon signals (δ_C 126.9 s, 161.1 s, 127.8 s) and three methine carbon signals (δ_C 130.1 d, 115.3 d, 135.3 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, H-11), spin system B (H_3-1, H_2-2, H_2-1), and spin system C (H-8, H_2−7, H_2-6, H-5).

The structure was assembled by 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-8 (δ 2.44, m, β) and C-9 (δ_C 152.1 s) and between C-1 (40.8 t), C-5 (δ_C 45.7 d), C-9 (δ_C 152.1 s), C-10 (δ_C 39.2 s), and H3−24 (δ 1.11, s). The second is generated on the basis of interactions from the quaternary carbon C-4 (δ_C 40.2 s) to H_3-22 (δ 0.89 s) and H_3-23 (δ 0.88, s), which both further correlated to C-3 (δ 79.8 d) and C-5 (δ 45.7 d). Couplings from H_2-15 (δ 2.46, d, J = 13.2 Hz; δ 2.89, d, J = 13.2 Hz) to C-8 (δ_C 39.8 d), C-13 (δ_C 34.5 d), C-14 (δ_C 39.3 s), C-16 (δ_C 127.8 s), C-21 (δ_C 161.1 s), and C-25 (δ_C 15.8 q) established that the aromatic ring was attached by a CH_2 group (CH_2-15) to C-14, which is also the connecting carbon between the spin systems A and C.

Further correlations from C-27 (δ_C 172.0 s) to H-17 (δ 7.80 s) and H-19 (δ 7.70, d, J = 6.6 Hz) enabled us to position the carboxy group at C-18 (δ_C 126.9 s). Interactions between H_2-15 and C-21 located the aromatic hydroxy-substituted carbon (δ_C 161.1 s). The relative stereoconfigurational structure of **1** was determined by a 2D-t-ROESY experiment. The t-ROESY spectrum exhibited the presence of ROEs indicating that H-13, CH_3-23, CH_3-24, and CH_3-25 were oriented on the same face of the diterpene plane (α), while H-3, H-5, H-8, CH_2-15, CH_3-22, and CH_3-26 were in the β-position.

Noscomin showed antibacterial activity against *Bacillus cereus* (MIC 32 ppm), *Staphylococcus epidermidis* (MIC 8 ppm), and *Escherichia coli* (MIC 128 ppm). These MIC

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values for noscomin are comparable with those obtained for the standards chloramphenicol (*B. cereus*, MIC 8 ppm; *S. epidermidis*, MIC 4 ppm) and tetracycline (*E. coli*, MIC 64 ppm).

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 a pressed KBr disk. The UV spectrum was 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 solvent (CD_3OD, ^{1}H δ 3.31, ^{13}C δ 49.0) as a reference. HPLC separations were performed with a Waters model 590 pump connected to a Rheodyne HPLC injector, a Knauer variable wavelength monitor, and a Knauer HPLC column (Hypersil ODS, 3 μm, 250 × 16 mm). 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 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 cyanophyte was cultivated in 10-L glass bottles containing a modified inorganic culture medium (*Z*).[9] 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 decanting and adsorbed on a column filled with 250 g of Amberlite XAD-2 resin (nonpolar, surface area 330 m^2/g). Subsequently, the column was washed with MeOH.

Isolation of Noscomin (1). The MeOH extract (1.2 g) obtained from 85 L 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 MeOH/H_2O 1:1, 1 L of MeOH/H_2O 75:25, 1 L of MeOH, 500 mL of MeOH/acetone 1:1, and 200 mL of acetone 100% to obtain 16 fractions (20−40 mg). Bioactive fraction 14 (40 mg, eluted with MeOH/H_2O 75:25) was subjected to reversed-phase HPLC using MeOH/ACN/H_2O 63:25:12 as an eluent to yield **1** (7.6 mg) as an amorphous white solid, pure by TLC analysis (RP-18, ACN/H_2O 7:3, R_f = 0.33).

Antibacterial Assay. The MIC determinations for noscomin were performed as previously described.[10] Test organisms were *B. cereus* (ATCC 10702, Gram-positive), *S. epidermidis* (ATCC 12228, Gram-positive), and *E. coli* (ATCC 25922, Gram-negative).

Noscomin: white amorphous solid (7.6 mg): $[\alpha]^{25}_D$ +16° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3416, 1686, 1603, 1546 cm^{-1}; UV λ_{max}^{MeOH} 282 nm; EIMS (MeOH) *m/z* (rel int) $[M]^+$ absent, 409 $[M - OH]^+$ (<1), 380 $[M - COOH - H]^+$ (<1), 365 $[M - CO_2 - OH)]^+$ (<1), 275 $[M - C_8H_7O_3]^+$ (75), 257 $[M - C_8H_7O_3 - H_2O]^+$ (97), 107 $[M - C_{19}H_{31}O]^+$ (100); ESIMS (MeOH) *m/z* (rel int) $[M - H]^-$ 425.2 (100); ^{1}H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75.5 MHz, CD_3OD), see Table 1.

Table 1. ^{1}H and ^{13}C NMR Chemical Shift Assignments (ppm) of **1**

position	^{1}H NMR chemical shift δ (mult, *J* (Hz))	^{13}C NMR chemical shift δ[a]
1	1.37 (1H, m, β), 1.93 (1H, m, α)	40.8 t
2	1.66 (1H, m, β), 1.84 (1H, m, α)	20.8 t
3	3.19 (1H, dd, *J* = 4.7, 11.1 Hz)	79.8 d
4		40.2 s
5	1.44 (1H, m, β)	45.7 d
6	1.61−1.69 (2H)[b]	28.9 t
7	1.25 (1H, m, α), 1.29 (1H, m, β)	20.4 t
8	2.44 (1H, m, β)	39.8 d
9		152.1 s
10		39.2 s
11	5.38 (1H, m)	116.4 d
12	1.67 (1H, m, β), 1.70 (1H, m, α)	31.6 d
13	1.55 (1H, m, α)	34.5 d
14		39.3 s
15	2.46 (1H, d, *J* = 13.2), 2.89 (1H, d, *J* = 13.2)	35.2 t
16		127.8 s
17	7.80 (1H, s)	135.3 d
18		126.9 s
19	7.70 (1H, d, *J* = 8.3)	130.1 d
20	6.74 (1H, d, *J* = 8.3)	115.3 d
21		161.1 s
22	0.89 (3H, s)	28.2 q
23	0.88 (3H, s)	15.9 q
24	1.11 (3H, s)	25.9 q
25	0.67 (3H, s)	19.5 q
26	1.05 (3H, d, *J* = 6.7)	15.8 q
27		172.0 s

[a] Multiplicities determined by DEPT 135 NMR experiments. [b] Signal pattern unclear due to overlapping.

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