Prenostodione, a Novel UV-Absorbing Metabolite from a Natural Bloom of the Cyanobacterium *Nostoc* Species

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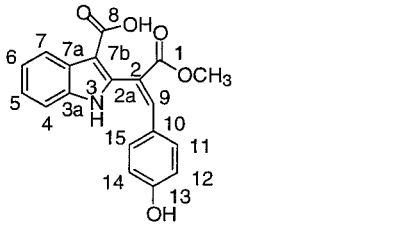
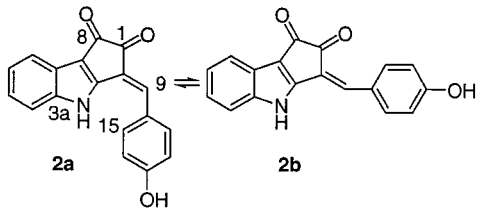
A novel UV-absorbing natural product, prenostodione (**1**), has been isolated from a natural bloom of the cyanobacterium *Nostoc* sp. (TAU strain IL-235). Homonuclear and heteronuclear 2D NMR techniques as well as HREIMS determined the gross structure of **1**.

Cyanobacteria sunscreen pigments were first reported more than 150 years ago.[1] Recently, the structure of two ultraviolet sunscreen pigments, scytonemin[2] and nostodione A,[3] were described. Scytonemin and nostodione A likely derive from condensation of tryptophan- and phenylpropanoid-derived subunits. Herein, we report the structure of a new UV-absorbing natural product, prenostodione (**1**). The structure of prenostodione (**1**), which we propose to be a precursor of nostodione A and scytonemin, enlightens the biosynthetic pathway of these pigments.



Prenostodione (**1**)



Nostodione A (**2**)

Nostoc sp.,[4] TAU strain IL-235, appears as clumps of green-yellow gelatinous material. Young colonies are initially attached to the rocky river bed. When mature, they fill with air, detach, and float as yellow clumps on the water surface.

In the summer of 1999 we collected many of the cyanobacterium clumps. Extraction of the dried cells (146 g) afforded 31.2 g of crude extract. The hydrophilic and lipophilic extracts yielded two UV-absorbing pigments. The major one was identified as a mixture of the two isomers (**2a** and **2b**) of the known nostodione A (**2**, 18.2 mg, 0.012% of dry cell weight).[3] The minor pigment was isolated from the lipophilic extract by successive gel-filtration on Sephadex LH-20 and reversed-phase HPLC and was named prenostodione (**1**, 3.5 mg, 0.002% of dry cell weight).

The molecular formula of prenostodione (**1**) was deduced to be $C_{19}H_{15}NO_5$ from the HREIMS molecular ion at *m/z* 337.0931 (Δ 0.5 mDa) and corresponded to 13 deg of unsaturation. The UV spectrum showed four maxima at 217, 230, 287, and 318 nm, resembling the superimposed UV spectra of *trans*-*p*-coumaric acid (maxima at 227 and 311 nm)[5] and indole carboxylic acid (maxima at 223 and 288 nm).[6] The ^{1}H NMR spectrum of **1** (Table 1) revealed one set of aromatic protons (as opposed to two sets in **2**) and a carboxymethyl group, which clearly pointed to a similarity in the structure of both compounds (**1** and **2**). The most significant differences in the ^{13}C chemical shifts between **1** and **2** were observed for C-1, C-2a, C-7b, C-8, and C-9 pointing to two carboxy groups in **1** instead of the α-diketone moiety in **2**.

Analysis of the COSY and HMQC experiments allowed the assignment of the protonated carbons of **1**. The carbon skeleton was assembled using the data from an HMBC experiment. The structure of the indole moiety was assembled through correlations of NH-3 with carbons 2a, 3a, 7a, 7b, and 8;[7] H-4 with carbons 6 and 7a; H-5 with carbons 3a and 7; H-6 with carbons 4 and 7a; and H-7 with carbons 3a and 5. The structure of the coumaric acid moiety was deduced from correlations of the methoxyl protons with carbon 1; H-9 with carbons 1, 2, and 11/15; H-11/15 with carbons 9, 15/11, 12/14, and 13; and H-12/14 with carbons 10, 14/12, and 13. The indole carboxylic acid and coumaric acid moieties were assembled through the correlation of H-9 with carbon 2a. Finally, the *E*-configuration of the 2,9-double bond was deduced from the NOE data (ROESY experiment), particularly the correlation between the methoxy protons and H-9 and the correlation between H-11/15 and H-12/14 and NH-3.

Prenostodione (**1**) is considered to be a precursor of nostodione A and scytonemin because it appears as a single isomer. A degradation product of **2**, on the other hand, is expected to produce two different isomers. The initial step of the biosynthesis is proposed to be an ionic or biradical coupling of the enamine methine of indolcarboxylic acid with the ketone of *p*-hydroxyphenylpyruvic acid. Water elimination, from the coupling product, produces the prenostodione skeleton. Reductive coupling of the two carboxyl moieties affords the cyclic diketone moiety of nostodione A. Reductive coupling of the C-8 ketone of two nostodione A molecules followed by water elimination produces scytonemin.

Experimental Section

Instrumentation. IR spectra were recorded on a Nicolet FTIR in $CHCl_3$ or neat. Low- and high-resolution mass spectra were recorded on a Fisons VG AutoSpecQ M 250 instrument.

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Table 1. NMR Data of Prenostodione (**1**) and Nostodione A Isomers (**2a** and **2b**)[a]

	1[b]		**2a**[b]		**2b**[b]	
position	δ_C	δ_H, J (Hz)	δ_C	δ_H, J (Hz)	δ_C	δ_H, J (Hz)
1	166.9 s		193.5 s		192.7 s	
1-OMe	52.1 q	3.63 s				
2	120.4 s		119.2 s		119.2 s	
2a	139.3 s		158.6 s		164.7 s	
3		11.82 s		12.23 s		13.02 s
3a	135.8 s		140.9 s		140.9 s	
4	112.1 d	7.37 d 8.8	114.3 d	7.67 d 8.0	113.3 d	7.56 d 8.1
5	122.5 d	7.20 m	126.6 d	7.41 dd 8.0, 7.9	126.1 d	7.37 dd 8.1, 7.9
6	121.2 d	7.18 m	124.1 d	7.33 dd 7.9, 7.8	123.7 d	7.27 dd 7.9, 7.7
7	121.2 d	8.03 d 8.7	120.9 d	7.83 d 7.8	121.2 d	7.76 d 7.7
7a	127.1 s		121.0 s		121.8 s	
7b	105.8 s		123.8 s		119.5 s	
8	165.8 s		177.2 s		176.1 s	
9	142.3 d	7.78 s	129.1 d	7.29 s	132.1 d	7.26 s
10	124.8 s		124.6 s		125.4 s	
11,15	132.3 d	6.84 d 8.7	131.9 d	7.69 d 8.7	134.2 d	8.08 d 8.7
12,14	115.8 d	6.58 d 8.7	116.7 d	6.96 d 8.7	115.9 d	6.89 d 8.7
13	159.6 s		160.1 s		160.6 s	
13-OH		9.99 s		10.30 s		10.35 s

[a] Dissolved in DMSO-d_6. [b]Assignment by HMQC and HMBC (J = 8 Hz) experiments.

UV spectra were recorded on a Kontron 931 plus spectrophotometer. NMR spectra were recorded on a Bruker ARX-500 spectrometer at 500.136 MHz for ^{1}H and 125.76 MHz for ^{13}C. ^{1}H, ^{13}C, DEPT, COSY-45, ROESY, HMQC, and HMBC spectra were recorded using standard Bruker pulse sequences. HPLC separations were performed on an ISCO HPLC system (model 2350 pump and model 2360 gradient programmer) equipped with an Applied Biosystem Inc. diode-array detector.

Biological Material. *Nostoc* sp., TAU strain IL-235, was collected from the spring pool of the Banyas stream, one of the tributaries that make up the Jordan River, in Israel.

Isolation Procedure. The naturally collected, freeze-dried cells (146 g) were extracted with MeOH−H_2O (7:3) (×3) and then with MeOH−chloroform (1:1) (×3). The filtered extracts were concentrated under reduced pressure to afford 31.2 g of crude extracts. The hydrophilic and lipophilic extracts yielded two UV-absorbing pigments. The major one was identified as a mixture of the two isomers (**2a** and **2b**) of the known nostodione A (**2**, 18.2 mg, 0.012% of dry cell weight).[3] The minor pigment was isolated from the lipophilic extract (MeOH−$CHCl_3$, 1:1) by successive separations by Sephadex LH-20 gel-filtration and HPLC. The lipophilic extract (2.9 g) was applied to a Sephadex LH-20 column (i.d. × h, 5 × 40 cm) and eluted with $CHCl_3$−MeOH (1:1) (8 fractions, each of 50 mL). Fraction 7 was applied to a preparative HPLC column (Alltech Econosil C_{18}, 10 μm, 250 × 22.5 mm). The column was eluted with a MeOH−H_2O (60:40) solution (5 mL/min) and monitored by UV (230 nm). Pure **1** (3.5 mg, 0.002% of dry cell weight) was eluted from the column with a retention time of 25.3 min and was named prenostodione.

Prenostodione (1): yellow oil; UV (MeOH) λ_{max} (ϵ) 217 (29 200), 230 (24 150), 287 (17 000), 318 (15 640) nm; IR (MeOH) 3664, 3532, 2975, 2874, 1692, 1604, 1513, 1441 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* (rel int) 337 (M^+, 100), 304 (95), 293 (40), 291 (35), 260 (65), 233 (80), 204 (40), 137 (45), 81 (40), 44 (40); HREIMS *m*/*z* 337.0931 (M^+, calcd for $C_{19}H_{15}NO_5$, 337.0950); 304.0604 ([M − MeOH − H]$^+$, calcd for $C_{18}H_{10}NO_4$, 304.0609); 233.0820 ([M − CO_2H − CO_2-CH_3]$^+$, calcd for $C_{16}H_{11}NO$, 233.0840).

Supporting Information Available: NMR data (^{1}H, ^{13}C, HMQC, HMBC, and ROESY) of **1** in DMSO-d_6 and EIMS. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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(7) A similar correlation between NH-3 and C-8 was observed in the HMBC spectrum of compound **2**.

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