Notes

Nostocyclyne A, a Novel Antimicrobial Cyclophane from the Cyanobacterium *Nostoc* sp.

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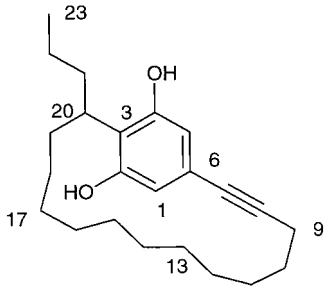
A novel acetylene-containing para-[14]-cyclophane, nostocyclyne A (**1**), possessing antimicrobial activity, is the major active metabolite of the natural bloom of the cyanobacterium *Nostoc* sp. (TAU strain IL-220). Homonuclear and heteronuclear 2D NMR techniques as well as HREIMS determined the gross structure of **1**.

Cyanobacteria are well recognized as a rich source of biologically active natural products.[1,2] Many of these natural products are of peptidic nature.[3] Biologically active polyketides are isolated, less frequently, from cyanobacteria, but these usually possess unique structures.[4−8] Cyanobacteria are the only organisms that are reported to produce natural cyclophanes.[9,10] We report here the isolation and structure elucidation of a novel acetylene-containing cyclophane, nostoccyclyne A (**1**), from a terrestrial *Nostoc* sp.[11] (TAU strain IL-220) that possesses moderate antibacterial activity.



Nostocyclyne A (**1**)

The massive growth of *Nostoc* sp. (TAU strain IL-220) was collected from the ground of a greenhouse in Newe Monosson, Israel. The cyanobacterial growth was collected following the observation that the death of plants of the fern *Adiantum capillus veneris* was associated with the cyanobacterial bloom. The freeze-dried cells from the natural bloom were extracted with 70% methanol in water. The methanol was removed under reduced pressure, and the resulting water solution was extracted with chloroform (3 ×). The crude extract obtained, after evaporation of the chloroform, was subjected to repeated chromatography on Sephadex LH-20 to obtain almost pure **1** (26 mg). Final purification on a preparative Alltech C_{18} HPLC column afforded pure **1** (10.6 mg, 0.05% of crude extract). The pure clonal strain (IL-220-1), mass cultured in the laboratory,[12] failed to produce nostocyclyne A (**1**) or related metabolites.

Nostocyclyne A (**1**) is an optically active natural product with an $[\alpha]_D$ value of −7.1° (*c* 0.7, $CHCl_3$). HREIMS measurements of nostocyclyne A (**1**) presented a molecular ion at *m/z* 342.2554, which fits the molecular formula $C_{23}H_{34}O_2$ (Δ 0.5 mDa). This formula accounts for seven double-bond equivalents. Six degrees of the unsaturation were attributed to phenyl and acetylene moieties, while the seventh was attributed to a carbocycle. The IR spectrum, 3599 (sh, OH stretch), 3330 (br, OH stretch), 3020 (sh, aromatic C−H stretch), 2928 and 2855 (methylene and methyl C−H stretch), 2210 (w, triple bond stretch) cm^{-1}, advised us of the presence of aromatic and acetylenic moieties. The ^1H NMR spectrum revealed signals for two aromatic proton singlets (δ 6.42 and 6.35 s), one benzylic proton (δ 3.14 tt), one terminal methyl (δ 0.89 t), and 26 methylene protons (δ 2.35−0.75) Twenty-three carbon signals were revealed in the ^{13}C NMR spectrum. Six of these signals were attributed to a tetrasubstituted diphenolic system. Two other signals (δ 91.9 and 82.2 s) were assigned to a triple bond. On the basis of a DEPT experiment, the remaining signals were assigned to one methine carbon, 13 methylenes, and one methyl carbon.

Interpretation of the COSY map (See Table 1) furnished two fragments—CH_2(9) to CH_2(13) and CH_2(17) to CH_3-(23)—that could not be connected due to the overlapping of the signals between δ 1.00 and 0.70 ppm. All of the ^1J H−C connectivities were assigned by an HMQC experiment (see Table 1). Long-range H−C correlations from an HMBC experiment (see Table 1) allowed the connection of CH_2(13) to CH_2(17), CH_2(9) to the acetylene, the acetylene to the phenyl residue, and, finally, the phenyl residue to the benzylic methine. Some ^4J H−C connectivities (H-9 and H-9′ with C-6 and H-1 and H-5 with C-20) and ^5J H−C connectivities (H-9 and H-9′ with C-1 and C-5) were observed in the unsaturated residues of the molecule, along with the expected ^2J and ^3J connectivities. On the basis of the arguments described above, structure **1** was assigned to nostocyclyne A.

Nostocyclyne A (**1**) is believed to be a polyketide metabolite derived from the assembly of 12 acetate units (Figure 1). The last four acetate units of the linear polyketide could cyclize to give a six-membered ring, and a bond could form between carbons 3 and 20. Subsequent decarboxylation,

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Table 1. NMR Data of Nostocyclyne A (**1**) in $CDCl_3$[a]

carbon no.	δ_C, mult.[b]	δ_H, mult.,[b] J (in Hz)	H−H correlations[c]	H−C correlations[d]
1	111.1 d	6.42 s		H-5, H-9, H-9′
2	155.0 s			H-1, H-20
3	117.9 s			H-1, H-5, H-20, H-21, H-21′
4	156.0 s			H-5, H-20
5	112.0 d	6.35 s		H-1, H-9, H-9′
6	122.7 s			H-1, H-5, H-9, H-9′
7	82.2 s			H-1, H-5, H-9, H-9′
8	91.9 s			H-9, H-9′, H_2-10
9	19.2 t	2.32 dt, 17.0, 6.4	H-9′, H_2-10	H_2-10, H_2-11
		2.37 dt, 17.0, 5.7	H-9, H_2-10	
10	26.6 t	1.61 m	H-9, H-9′, H_2-11	H-9, H-9′, H_2-11, H_2-12
11	28.3 t	1.45 m	H_2-10, H_2-12	H-9, H-9′, H_2-13
12	28.6 t	1.38 m	H_2-11, H_2-13	H_2-10, H2-11
13	29.9 t	1.13 m	H_2-12[e]	H_2-10, H_2-11, H-14, H_2-15
14	29.6 t	0.80 m, 0.95 m	*e*	H_2-12
15	29.7 t	0.98 m	*e*	H_2-13, H-17
16	29.3 t	0.79 m	*e*	
17	28.1 t	0.77 m, 0.90 m	*e*, H-18, H-18′	H_2-16, H-18′, H-19′
18	27.6 t	1.05 m, 1.31 m	H-17, 17′, H-19, 19′	H_2-16, H-17′, H_2-19, H-20
19	31.7 t	1.63 m, 1.85 m	H-18, 18′, H-20	H_2-17, H_2-18, H-20, H_2-21
20	36.2 d	3.14 tt, 10.1, 5.6	H-19, 19′, H-21, 21′	H-1, H-5, H-18, H_2-21, H_2-22
21	36.6 t	1.58 m, 1.90 m	H-20, H_2-22	H-20, H_2-22, H_3-23
22	21.5 t	1.30 m	H-21, 21′, H_3-23	H-20, H_2-21, H_3-23
23	14.2 q	0.89 t, 7.3	H_2-22	H_2-21, H_2-22

[a] 500 MHz for ^1H and 125 MHz for ^{13}C. [b] Multiplicity. [c] From COSY experiment. [d] From HMBC experiment. [e] Could not be determined due to signal overlapping.

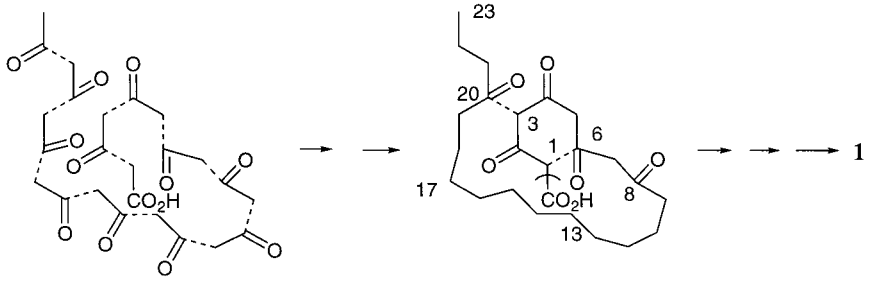

Figure 1. Suggested biosynthesis of nostocyclyne A (**1**).

elimination of three molecules of water, and tautomerization, to the diphenolic system, could produce the final natural product, **1**.

Nostocyclyne A (**1**) was not active against *Staphyloccocus albus* and *Escherichia coli* at 100 μg/6 mm disk but presented MIC values of 12.5 μg/disk against *S. aureus* and 10 μg/disk against *Bacillus subtilis*.[13] Nostocyclyne A (**1**) shows a weak inhibition of photosynthesis (5% at 100 μg/mL) in green alga.[14]

Experimental Section

Instrumentation. IR spectra were recorded on a Nicolet FTIR in $CHCl_3$ or neat. Low- and high-resolution MS were recorded on a Fisons VG AutoSpecQ M 250 instrument. UV spectra were recorded on a Kontron 931 plus spectrophotometer. Optical rotaions were measured on a Jasco P-1010 polarimeter. NMR spectra were recorded on a Bruker ARX-500 spectrometer at 500.136 MHz for ^1H and 125.76 MHz for ^{13}C. ^1H, ^{13}C, DEPT, COSY-45, 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.

Culture Conditions. An edaphic form of *Nostoc* sp., designated Tel Aviv University (TAU) strain number IL-220-1, was isolated from a soil sample collected at a greenhouse in Newe Monosson, Israel. A clonal strain was purified on BG-11 agar medium.[12] The isolate is currently maintained in the culture collection at Tel Aviv University. The cyanobacterium was cultured in 20 L glass bottles containing a BG-11 medium.[12] Cultures were illuminated continuously at an intensity of 75 μmol quanta/M^2/s from fluorescent tubes and aerated with 0.5% CO_2 in air (1 L/min) at an incubation temperature of 25 °C for 30−35 days. The cells were harvested using a continuous-flow centrifuge. Yields of lyophilized cells typically ranged from 0.15 to 0.3 g/L of culture.

Isolation Procedure. The naturally collected freeze-dried cells (50 gr) were extracted with 7:3 MeOH:H_2O (×3). The filtered extract was concentrated under reduced pressure. The crude extract (21 g) was partitioned between $CHCl_3$ and water (×3). The lipophilic layer (2.7 g) was applied to a Sephadex LH-20 column (i.d. × h, 5 × 40 cm) eluted with 2:1 $CHCl_3$−MeOH (8 fractions, each of 50 mL). Fraction 7 was applied to the same column eluted with 1:1:1 $CHCl_3$−MeOH−petroleum ether (9 fractions, each of 50 mL). Fraction 5 was applied to the same column eluted with 1:2 $CHCl_3$−petroleum ether (8 fractions, each of 50 mL). Fraction 5 (26 mg), containing almost pure **1** (by NMR), was applied to a preparative HPLC column (Alltech Econosil C_{18}, 10 μm, 250 × 22.5 mm). The column was eluted with a 60:25:15 MeOH−acetonitrile−H_2O solution (5 mL/min) and monitored by UV (263 nm). Pure **1** (10.6 mg, 0.05% of crude extract) was eluted from the column with a retention time of 62.2 min. A similar procedure was used for the isolation of metabolites from the cells of strain IL-220-1, but **1** was not present in the extracts of this cultured strain.

Nostocyclyne A (1): colorless oil; $[\alpha]^{25}_D$ −7.1° (*c* 0.7, $CHCl_3$); UV ($CHCl_3$) λ_{max} (ϵ) 221 (39550), 262 (19000), 295 (5500) nm; IR ($CHCl_3$) 3599 (sh) 3330 (br), 3020 (sh) 2928, 2855, 2210 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* (rel intensity) 342 (M^+, 100), 300 (170), 279 (150), 207 (45), 149 (35); HREIMS *m*/*z* 342.2554 (M^+, calcd for $C_{23}H_{34}O_2$, 342.2558).

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