the dark black-brown residue was dissolved in MeOH. The residue was purified by using reversed-phase HPLC (70:30 MeOH–H_2O) to afford 3.0 mg of compound 7 (34.3% isolated yield based on the amount of 1 used; t_R 8.5 min), as well as a number of unidentified minor components. Although the starting material employed in this experiment was only >90% pure, the amount of product obtained indicated that most of it must be derived from 1. The ^1H NMR, ^{13}C NMR, and EIMS data for 7 were identical with those reported in the literature for (–)-regiolone,[10] while the $[\alpha]_D$ (–5.6° in EtOH at 31 °C) was comparable to the literature value (–3.3° in EtOH; temperature not reported).

Acetylation of Preussomerin D (4). The procedure described above for compound 1 was repeated, using 1.7 mg of 4. In this case, 1.4 mg (67% yield) of diacetylated product was obtained, and no HPLC purification was necessary: ^1H NMR (acetone-d_6) 7.56 (dd, J = 7.8, 1.2, 1 H), 7.52 (t, J = 7.8, 1 H), 7.45 (d, J = 10.0, 1 H), 7.22 (dd, J = 7.8, 1.2, 1 H), 7.10 (d, J = 9.0, 1 H), 6.93 (d, J = 9.0, 1 H), 6.67 (d, J = 10.0, 1 H), 6.52 (m, 1 H), 4.03 (d, 4.2, 1 H), 3.90 (dd, J = 4.2, 1.6, 1 H), 2.25 (s, 3 H), 2.21 (s, 3 H).

Oxidation of Preussomerins A and E (1 and 5). A solution of 1 (1.8 mg in 50 μL of acetone) was added to 1 mL of CH_2Cl_2 and combined with NaOAc (6.7 mg), PCC (10.6 mg), and Florisil (25 mg) in a 1-dram vial.[14] After stirring at room temperature for 4 h, no starting material remained. At this point, Et_2O (2 mL) was added. Upon stirring for 10 additional min, the supernatant solution was decanted. The residual solids were washed with Et_2O (3 × 2 mL), and the washings were combined with the supernatant solution and passed through a Pasteur pipet column of Florisil, eluting progressively with Et_2O (10 mL), $CHCl_3$ (5 mL), 1:9 MeOH–$CHCl_3$ (5 mL), and MeOH (10 mL). The product 8 (0.9 mg; 50% yield) was obtained upon evaporation of the $CHCl_3$ and MeOH–$CHCl_3$ fractions. Reaction of 5 under identical conditions afforded the same product: HPLC t_R 24.9 min (70:30 MeOH–H_2O); ^1H NMR (acetone d_6; assignments proposed by analogy to those in Table I) 7.59 (dd, J = 7.6, 1.2, H-9′), 7.54 (dd, J = 8.1, 7.6, H-8′), 7.47 (d, J = 10.0, H-2′), 7.22 (d, J = 9.0, H-7), 7.20 (dd, J = 7.8, 1.2, H-7′), 7.05 (d, J = 9.0, H-8), 6.68 (d, J = 10.0, H-3′), 4.53 (d, J = 3.9, H-3), 4.05 (d, J = 4.2, H-2); EIMS (70 eV) m/z 362 (M^+; rel int 100), 333 (6), 289 (2), 174 (53), 165 (16), 125 (45).

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Supplementary Material Available: Selected ^1H NMR spectra (for preussomerins B and F), ^{13}C NMR spectra (for preussomerins A–F), and ORTEP representations of preussomerin A (9 pages). Ordering information is given on any current masthead page.

Structures of Nostocyclophanes A–D

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Nostocyclophanes A–D are the cytotoxins associated with the blue-green alga *Nostoc linckia* (Roth) Bornet ex Bornet & Flahault (UTEX B1932). The gross structures of these [7.7]paracyclophanes have been elucidated by mass and NMR spectral analyses and the relative and absolute stereochemistry of nostocyclophane D determined by X-ray crystallography. Since the CD spectra of the four compounds are essentially identical, nostocyclophanes A–D are proposed to have the same stereochemistry. The sugar unit in nostocyclophanes A and B has been shown to be D-glucose by semisynthesis of nostocyclophane B 9-*O*-(2,3,4,6-tetra-*O*-acetyl)-β-D-glucopyranoside from nostocyclophanes B and D.

In a preliminar paper we reported the isolation and structure determination of the first naturally occurring [*m.n*]paracyclophanes.[1] Cytotoxins associated with two species of terrestrial blue-green algae belonging to the Nostocaceae, e.g., *Nostoc linckia* (Roth) Bornet ex Bornet & Flahault (UTEX B1932) and *Cylindrospermum licheniforme* Kützing (ATCC 29204), were found to be [7.7]-paracyclophanes. The total structure of nostocyclophane D, the major chlorine-containing [7.7]paracyclophane in the *N. linckia*, was established by an X-ray crystallographic study. We describe here the isolation and structure determination of three minor [7.7]paracyclophanes, nostocyclophanes A–C, from this alga.

Results and Discussion

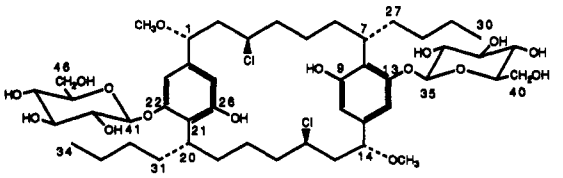
In an evaluation of extracts of blue-green algae for antitumor activity, the lipophilic (1:1 methylene chloride/2-propanol) extract of terrestrial *N. linckia* UTEX B1932 was found to be cytotoxic against KB (human nasopharyngeal carcinoma) and LoVo (human colon adenocarcinoma) cell lines at 13 μg/mL. When a 70% ethanol/water extract of the freeze-dried cyanophyte was subjected to flash reversed-phase chromatography on C-18, a mixture of cytotoxic [7.7]paracyclophanes (MIC 5 μg/mL) was obtained that could be separated by reversed-phase HPLC on C-18 into nostocyclophanes A–D in yields of 0.01, 0.02, 0.01, and 0.13%, respectively. The major compound, nonstocyclophane D, was found to be cytotoxic at 0.5 μg/mL (IC_{50}); the three minor compounds, nostocyclophane A–C, were cytotoxic at 1–2 μg/mL (IC_{50}s).

Nostocyclophane D. The positive-ion fast atom bombardment mass spectrum, which displayed a 10:6.5:1 MH^+ ion cluster at m/z 653/655/657, and the field-desorption mass spectrum, which exhibited a 10:6.5:1 M^+ ion cluster at m/z 652/654/656, indicated that nostocyclophane D had a molecular weight of 652 D and possessed two chlorine atoms. Only 18 carbon signals could be seen in its ^{13}C NMR spectrum (one obscured by solvent signal but clearly visible in DEPT spectrum), however, and this implied that the molecule had a 2-fold axis of symmetry and, therefore, 36 carbon atoms. Of the 54 protons in the molecule, 50 were nonexchangeable (attached to carbon), since DEPT experiments showed the presence of two methyl, seven

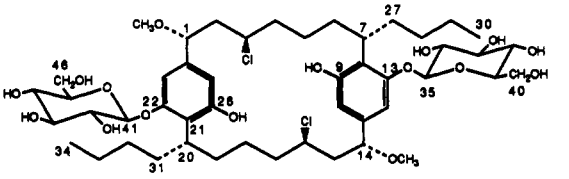
(1) Moore, B. S.; Chen, J.-L.; Patterson, G. M. L.; Moore, R. E.; Brinen, L. S.; Kato, Y.; Clardy, J. *J. Am. Chem. Soc.* **1990**, *112*, 4061.

0022-3263/91/1956-4360$02.50/0

A

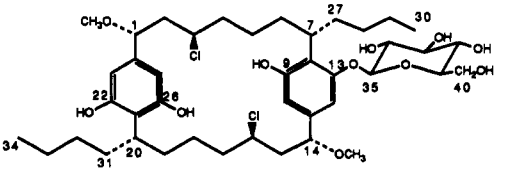


major conformer

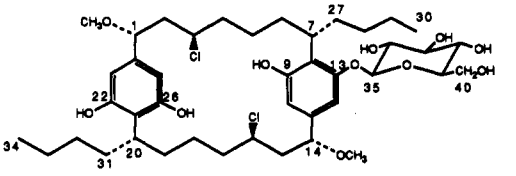


minor conformer

B

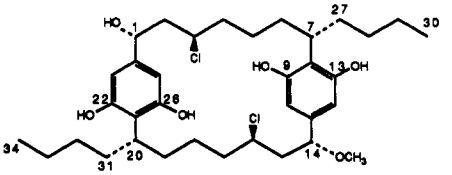


major conformer

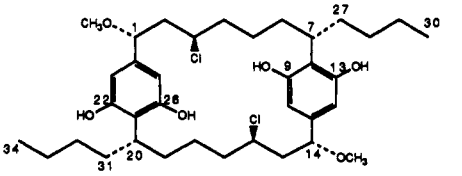


minor conformer

C



D



methylene and five methine carbon signals, and four were exchangeble, since the ^1H NMR spectrum displayed two phenolic OH-type signals, which disappeared in MeOH-d_4 and showed no carbon correlations in the HMQC spectrum. Finally, nostocyclophane D possessed six oxygen atoms, where four were located in phenolic OH groups and the other two situated in two equivalent methoxyl groups (6 H singlet at 3.05 ppm). From these data nostocyclophane D had to have the molecular formula $C_{36}H_{54}Cl_2O_6$.

The ^{13}C–^{13}C COSY-X spectrum of uniformly ^{13}C-enriched nostocyclophane D allowed us to determine the gross structure unambiguously. Its relative and absolute stereochemistry was established by an X-ray crystallographic study and a computer-generated perspective view of the molecule was reported in our paper.[1] The X-ray model was found to have molecular, but not crystallographic, 2-fold symmetry.

Nostocyclophane C. From its field-desorption mass spectrum (10:6.5:1 MH^+ ion cluster at m/z 639/641/643), nostocyclophane C differed from nostocyclophane D by only 14 mass units, i.e., by the mass of a methylene, strongly suggesting that it had the molecular composition $C_{35}H_{52}O_6Cl_2$. The ^1H and ^{13}C NMR spectra of nostocyclophane C, compared with those of nostocyclophane D, were more complex, since the molecule lacked symmetry, which resulted in a doubling of many of the signals. For example, in the ^1H NMR spectrum of nostocyclophane C four phenolic OH signals and four aromatic proton signals could now be seen and in the ^{13}C NMR spectrum 12 aromatic carbon signals were present. The most diagnostic features, however, were the presence of an additional exchangeable proton signal at 5.10 ppm and a *single* 3 H signal for a methoxyl group. The placement of the extra hydroxyl group on C-1 was corroborated by a ^1H–^1H COSY experiment. The proton on C-1, which exhibited a doublet of triplets at 4.42 ppm, was coupled to the exchangeable proton at 5.10 ppm (3.5 Hz) and two protons of a methylene group at 1.81 and 2.01 ppm (3.5 and 10.5 Hz, respectively). The protons of the C-2 methylene were in turn coupled to the proton of the chlorine-bearing methine at 2.83 ppm. The proton on C-1 showed a one-bond correlation with the carbon signal at 70.4 ppm. When the ^{13}C NMR spectra of nostocyclophanes C and D were compared, only the chemical shifts of C-1 (70.4 ppm for C vs 80.9 ppm for D) and the carbons in the vicinity of C-1, viz C-2, C-3, and C-21 to C-26, were significantly different. These data indicated that nostocyclophane C was 1-*O*-desmethylnostocyclophane D.

Nostocyclophanes A and B. Inspection of the ^1H NMR spectra of nostocyclophanes A and B suggested that the two compounds were di- and monoglycosides of nostocyclophane D, respectively.

Detailed NMR studies on the heptaacetate derivative of nostocyclophane B indicated that glucose was the sugar unit and furthermore that it was attached β to one of the phenolic groups. All of the vicinal proton coupling constants for the cyclic portion of the sugar unit were 8–10 Hz. In addition, the proton signals at 3.95 (H-40b) and 4.24 (H-40a) ppm, both doublets of doublets showing geminal coupling to each other (−12.5 Hz) and small vicinal coupling (1.3 and 3.9 Hz, respectively) to the ring proton at 2.97 ppm (H-39), established that a hydroxymethyl group was present in the sugar unit. The positive ion FABMS of nostocyclophane B, which exhibited a 10:6.5:1 MH^+ ion cluster at m/z 815/817/819, supported the conjecture that nostocyclophane B was nostocyclophane D β-glucopyranoside. The sugar unit was rigorously shown to be D-glucose by semisynthesis of nostocyclophane B heptaacetate from nostocyclophane D. By use of a modified Koenigs–Knorr reaction,[2] nostocyclophane D was reacted with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of cadmium carbonate to form nostocyclophane D 9-*O*-(2,3,4,6-tetra-*O*-acetyl)-β-D-glucopyranoside. Acetylation of the latter compound produced semisynthetic nostocyclophane B heptaacetate, which had NMR and CD spectra that were identical with those of the

(2) Conrow, R. B.; Bernstein, S. *J. Org. Chem.* **1971**, *36*, 863.

heptaacetate obtained from acetylation of nostocyclophane B.

Glucose also accounted for the two sugar units in nostocyclophane A. A detailed NMR examination of the decaacetate established that both sugar units were identical, since only one set of sugar signals could be observed. Both sugar units were probably D-glucose and attached β to two of the phenolic groups, since the chemical shifts and coupling constants for the protons of the acetylated sugar units were essentially the same as those observed for nostocyclophane D-9-*O*-(2,3,4,6-tetra-*O*-acetyl)-β-D-glucopyranoside. The FAB mass spectrum supported the di-β-D-glucopyranose structure for nostocyclophane A by showing a 10:6.5:1 MH^+ ion cluster at m/z 977/979/981 ($C_{48}H_{74}O_{16}Cl_2$).

^1H and ^{13}C NMR spectra indicated that nostocyclophanes A and B existed in two conformations in solution. The structure of each conformational isomer could be elucidated by 2D NOESY spectral analysis. The regiochemistry of the two glucose units in the major conformer of nostocyclophane A were found to be nonidentical; one of the anomeric protons (H-35) showed NOE correlations to an aromatic proton (H-10) and the benzylic methine proton H-14 whereas the other anomeric proton (H-41) showed an NOE to an aromatic proton (H-23) but not to the benzylic methine proton H-1. The NOESY data also rigorously established that the glucose units were on different aromatic rings rather than on the same one. The minor conformer of nostocyclophane A displayed only one set of glucose signals because of its 2-fold symmetry; the signal for the equivalent anomeric protons H-35,41 showed NOE correlations to the signals for the aromatic protons H-10,23 and benzylic protons H-1,14.

The major conformer of the monoglycoside nostocyclophane B also exhibited NOEs from the anomeric proton H-35 to both H-10 and H-14; however, the anomeric proton H-35 of the minor conformer gave an NOE correlation to H-10 only and not one to H-14.

Interestingly, only one conformational isomer could be detected in the ^1H NMR spectrum of either nostocyclophane A decaacetate or nostocyclophane B heptaacetate when the derivative was examined immediately after isolation from the acetylation reaction mixture. The regiochemistry of the single conformational isomer for each derivative was not deduced, but is suspected to be the same as that depicted for the major conformer of the corresponding unacetylated compound. On standing in solution, a minor conformational isomer slowly formed in the case of nostocyclophane B heptaacetate.

Absolute Stereochemistry. Nostocyclophanes A–D were found to exhibit similar UV and CD spectra. All of the CD spectra revealed a strongly negative peak around 212–218 nm with a shoulder extending from 220 to 240 nm and two weakly negative, broad peaks in the 260–280 nm region. This CD behavior strongly suggested that the four compounds had the same absolute stereochemistry, viz 1*R*,3*R*,7*S*,14*R*,16*R*,20*S* as shown, since X-ray crystallographic analysis established that nostocyclophane D had this absolute stereochemistry and nostocyclophanes B and D could be converted into optically identical samples of nostocyclophane B heptaacetate.

Experimental Section

Spectral Analysis and General Procedures. ^1H NMR chemical shifts are referenced in DMSO-d_6 to the residual DMSO-d_5 signal (2.49 ppm), in benzene-d_6 to the residual benzene-d_5 signal (7.15 ppm), and in $CDCl_3$ to TMS (0 ppm); ^{13}C chemical shifts are referenced in DMSO-d_6, benzene-d_6, and chloroform-d to the solvent signals (39.5, 128, and 77 ppm, respectively). Methyl, methylene, and methine carbons were distinguished by a DEPT experiment. Homonuclear ^1H connectivities were determined by using the COSY experiment. Homonuclear ^1H NOEs were obtained by Hypercomplex Phase-Sensitive NOESY experiments using a 3-s recycling delay and 500-ms mixing period. Heteronuclear ^1H–^{13}C connectivities were determined by proton-detected HMQC[3] and HMBC[4] experiments. UV and CD spectra were recorded in MeOH at 25 °C. Unless otherwise noted optical rotations were determined in MeOH at 25 °C. Analytical HPLC was carried out using a diode array UV detector linked to a computer. Mass spectra were determined in the fast atom bombardment (FAB) and field desorption (FD) modes.

Culture Conditions. *N. linckia* UTEX B1932 was purchased from the University of Texas Culture Collection of Algae. The cyanophyte was cultured in 20-L glass bottles containing a modified inorganic medium, designated A_3M_7.[5] Prior to autoclaving, the pH of the medium was adjusted to 7.0 with sodium hydroxide. Cultures were illuminated continuously at an incident intensity of 300 μeinstein m^{-2} s^{-1} from banks of cool-white fluorescent tubes, aerated at a rate of 5 L/min with a mixture of 0.5% CO_2 in air and incubated at a temperature of 24 ± 1 °C. After 18–20 days the alga was harvested by filtration. Yields of lyophilized cells averaged 0.16 g/L of culture.

Isolation. Freeze-dried alga (25.8 g) was extracted twice with 4 L and 2 L of 7:3 ethanol/water for 24 h at room temperature. The combined dark green extract was concentrated in vacuo to 350 mL (contains about 5 g of solid) and the concentrate applied to a 25 × 4.6 cm column of ODS silica gel. The chromatogram was eluted with water (1.5 L), 1:1 methanol/water (1.5 L), 4:1 methanol/water (2 L), and methanol (2 L). The 4:1 methanol/water fraction, which accounted for most of the cytotoxicity against KB cells, was evaporated. The residue (1 g) was passed through a 500-mg flash C-18 BondElut in 100-mg portions, using 15 mL of methanol for each portion. Evaporation in vacuo gave material that was dissolved in 4 mL of methanol (filtered) and subjected to HPLC (250–300 mL/injection) on a 10 × 250 mm column of Econosil C-18 (10 m) with 80:20:0.05 MeOH/H_2O/CF_3CO_2H at flow rate of 1.5 mL/min. Separation was monitored by continuous UV analysis at 228 nm. Nostocyclophanes were found in fractions that were eluted at 12.5 min (A, 3 mg, 0.01%), 26.5 min (B, 6 mg, 0.02%), 30.0 min (C, 3 mg, 0.01% yield), and 47.0 min (D, 35 mg, 0.13%).

Nostocyclophane A: $[\alpha]_D$ −12.0° (c 0.1); UV λ_{max} 207 nm (ϵ 55 300), 225 (13 400), 274 (2370), 281 (2330); CD $[\theta]_{212}$ −53 100, $[\theta]_{222}$ −17 100, $[\theta]_{230}$ −9940, $[\theta]_{270}$ −3430, $[\theta]_{280}$ −3430; positive-ion FABMS 977/979/981 (10:6.5:1 MH^+ ion cluster[6] for $C_{48}H_{74}O_{16}Cl_2$).

^1H NMR (DMSO-d_6) exists in two conformations in a ratio ~2:1 at 25 °C. Major conformer: δ (multiplicity, J in Hz, assignment) 9.06 (s, OH on C-26), 9.01 (s, OH on C-13), 6.60 (s, H-10), 6.38 (s, H-23), 6.31 (s, H-12,25), 5.04 (d, 5.0, OH on C-42), 5.01 (d, 6.1, OH on C-36), 4.96 (d, 4.4, OH on C-37,38,43, or 44), 4.94 (d, 5.5, OH on C-37,38,43, or 44), 4.92 (d, 4.4, OH on C-37,38,43, or 44), 4.88 (d, 3.9, OH on C-37,38,43, or 44), 4.91 (d, 7.7, H-41), 4.48 (d, 7.8, H-35), 4.46 (dd, 6.1/5.0, OH on C-40), 4.34 (dd, 5.8, OH on C-46), 4.14. (dd, 11.1/6.1, H-14), 4.10 (dd, 10.5/3.3, H-1), 3.65 (m, 5.0/−11.1, H-40), 3.54 (m, H′-40), 3.58/3.48 (m, H_2-46), 3.35 (m, H-7), 3.20 (m, H-36,37,38,39,42,43,44,45), 3.09 (m, H-20), 3.06 (s, OMe on C-1,14), 2.86 (m, H-3), 2.72 (m, H-16), 2.07/1.75 (m, H_2-2,15), 2.05/1.60 (m, H_2-31), 1.88/1.45 (m, H_2-27), 1.81/1.22 (m, H_2-19), 1.80/1.45 (m, H_2-6), 1.48/1.40 (m, H_2-4,17), 1.45/0.48 (m, H_2-18), 1.45/0.42 (m, H_2-5), 1.26/1.10 (m, H_2-28,32), 1.22/1.14 (m, H_2-29,33), 0.79 (t, 7.0, H_3-34), 0.75 (t, 7.2, H_3-30).

Minor conformer: δ (multiplicity, J in Hz, assignment) 9.02 (s, OH on C-13,26), 6.38 (s, H-10,23), 6.31 (s, H-12,25), 5.05 (d, 5.0, OH on C-36,42), 4.86 (d, 7.7, H-35,41), 4.28 (dd, 5.5/5.0, OH at C-40,46), 4.14 (dd, 10.6, H-1,14), 3.57/3.44 (m, H_2-40,46), 3.10

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(4) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.
(5) Moore, R. E.; Cheuk, C.; Yang, X.-Q. G.; Patterson, G. M. L.; Bonjouklian, R.; Smitka, T. A.; Mynderse, J. S.; Foster, R. S.; Jones, N. D.; Swartzendruber, J. K.; Deeter, J. B. *J. Org. Chem.* **1987**, *52*, 1036.
(6) In the FAB mass spectrum of each nostocyclophane, using glycerol as the matrix, the relative intensity of the largest peak in the protonated molecular ion cluster is approximately 5–10% that of the base peak for the spectral region above m/z 300.

(m, H-7,20), 2.86 (m, H-3,16), 2.15/1.60 (m, H_2-27,31), 1.80/1.22 (m, H_2-6,19), 1.73/0.52 (m, H_2-5,18), 0.79 (t, 7.0, H_3-30,34).

Nostocyclophane A Decaacetate. A mixture of 2 mg of nostocyclophane A and 1 mL of acetic anhydride/pyridine (1:1) was stirred at room temperature overnight. After evaporation of the volatiles under reduced pressure, nostocyclophane A decaacetate was found to be the sole product; positive-ion FABMS 1379/1399/1401 (10:6.5:1 MH^+ ion cluster for $C_{68}H_{94}O_{26}Cl_2$).

^1H NMR (benzene-d_6): δ (multiplicity, J in Hz, assignment) 7.101/7.089/6.998/6.995 (s, H-10,12,23,25), 5.58 (dd, 8.0/9.3, H-36,42), 5.37 (dd, 9.3/9.5, H-37,43), 5.29 (t, 9.6, H-38,44), 5.13 (d, 8.0, H-35,41), 4.67 (dd, 10.6/3.6, H-1,14), 4.26 (dd, 3.7/−12.5, H-40a,46a), 3.91 (dd, −12.5/1.7, H-40b,46b), 3.58/3.45 (m, 10.5/5.5, H-3,16), 3.02/3.01 (s, OMe on C-1,14), 2.84 (ddd, 9.5/3.7/1.7, H-39,45), 2.45/1.80 (m, H-2,15), 2.23 (m, H-7,20), 1.81/1.38 (m, H-27,31), 1.77/1.74/1.711/1.705/1.70/1.67 (2:2:2:1:2:1 s, 10OAc), 1.72/1.30 (m, H-6,19), 1.64/1.50 (m, H-4,17), 1.48/0.95 (m, H-5,18), 1.27 (m, H-29,33), 1.14 (m, H-28,32), 0.86 (t, 6.8, H-30,34). Only one conformational isomer appears to be present (compare with nostocyclophane B heptaacetate).

Nostocyclophane B: $[\alpha]_D$ −3.7° (c 0.25); UV λ_{max} 207.6 nm (ϵ 68 800), 225 (17 000), 275 (2970), 282 (2960); CD $[\theta]_{214}$ −50 700, $[\theta]_{226}$ −18 500, $[\theta]_{230}$ −13 600, $[\theta]_{270}$ −3510, $[\theta]_{280}$ −3900; positive-ion FABMS 815/817/819 (10:6.5:1 MH^+ ion cluster[6] for $C_{42}H_{64}O_{11}Cl_2$).

^1H NMR (DMSO-d_6) exists in two conformations in a ratio ~1:1.5 at 25 °C. Major conformer: δ (multiplicity, J in Hz, assignment) 9.02 (s, OH on C-13), 8.89 (s, OH on C-26), 8.86 (s, OH on C-22), 6.36 (s, H-10), 6.32 (s, H-12), 6.16 (s, H-25), 6.11 (s, H-23), 5.03 (d, 5, OH on C-36), 4.96 (br, OH on C-37,38), 4.90 (d, 7.5, H-35), 4.38 (dd, OH on C-40), 4.08 (dd, H-14), 4.05 (dd, H-1), 3.56/3.40 (m, H_2-40), 3.20 (m, H-36,37,38,39), 3.12 (m, H-7), 3.10 (m, H-20), 3.04 (s, OMe on C-1,14), 2.84 (m, H-16), 2.80 (m, H-3), 2.10/1.68 (m, H_2-2), 2.10/1.59, (m, H_2-27), 2.06/1.85 (m, H_2-15), 1.85/1.55 (m, H_2-6,19), 1.82/1.42 (m, H_2-31), 1.58/1.42 (m, H_2-4,17), 1.46/0.53 (m, H_2-5), 1.27/0.49 (m, H_2-18), 1.22/1.13 (m, H_2-29,33), 1.18/1.02 (m, H_2-28,32), 0.79 (t, 6.5, H_3-30), 0.77 (t, 6.8, H_3-34).

Minor conformer: δ (multiplicity, J in Hz, assignment) 9.02 (s, OH on C-13), 8.91 (s, OH on C-26), 8.84 (s, OH on C-22), 6.58 (s, H-10), 6.32 (s, H-12), 6.14 (s, H-25), 6.11 (s, H-23), 5.06 (d, 5, OH on C-36), 4.48 (d, 7.2, H-35), 4.48 (dd, OH on C-40), 4.12 (dd, H-14), 4.04 (dd, H-1), 3.65/3.53 (m, H_2-40), 3.20 (m, H-36,37,38,39), 3.10 (m, H-7,20), 3.04 (s, OMe on C-1,14), 2.77 (m, H-3), 2.70 (m, H-16), 2.14/1.59 (m, H_2-6), 2.08/1.82 (m, H_2-15), 2.06/1.78 (m, H_2-2), 1.85/1.50 (m, H_2-19), 1.72/1.35 (m, H_2-27,31), 1.49/1.38 (m, H_2-4,17), 1.33/0.45 (m, H_2-5), 1.30/0.58 (m, H_2-18), 1.20/1.11 (m, H_2-29,33), 1.18/1.02 (m, H_2-28,32), 0.77 (t, 6.5, H_3-34), 0.75 (t, 6.8, H_3-30).

^{13}C NMR (DMSO-d_6) major conformer: δ 157.9 (s, C-9), 157.5 (s, C-26), 155.7 (s, C-13), 155.1 (s, C-22), 138.4 (s, C-11), 138.2 (s, C-24), 119.9 (s, C-8), 118.1 (s, C-21), 107.0 (d, C-12), 101.0 (d, C-10), 107.0 (d, C-25), 102.6 (d, C-23), 99.1 (d, C-35), 81.0 (d, C-14), 80.8 (d, C-1), 77.1 (d, C-36), 76.3 (d, C-37), 73.6 (d, C-39), 69.6 (d, C-38), 63.0 (d, C-16), 62.7 (d, C-3), 60.6 (t, C-40), 55.7 (q, OMes at C-1,14), 45.9 (t, C-15), 45.8 (t, C-2), 40.2 (t, C-4,17), 35.0 (d, C-7,20), 32.7 (t, C-19), 32.6 (t, C-27,31), 30.3 (t, C-32), 30.1 (t, C-28,), 26.7 (t, C-5,18), 22.2 (t, C-29,33), 14.0 (q, C-30,34).

Minor conformer: δ 157.4 (s, C-9), 110.0 (d, C-12), 109.0 (d, C-10), 107.5 (d, C-25), 104.5 (d, C-35), 76.8 (d, C-36), 29.6 (t C-28,).

Nostocyclophane B Heptaacetate. A mixture of 4 mg of nostocyclophane B and 1 mL of acetic anhydride/pyridine (1:1) was stirred at room temperature overnight. After evaporation of the volatiles under reduced pressure, nostocyclophane B heptaacetate was found to be the sole compound in the residue: CD $[\theta]_{212}$ −3170, $[\theta]_{220}$ −1940, $[\theta]_{228}$ + 151, $[\theta]_{233}$ −654, $[\theta]_{268}$ −1560, $[\theta]_{272}$ −1550; positive-ion FABMS 1109/1111/1113 (10:6.5:1 MH^+ ion cluster for $C_{56}H_{78}O_{18}Cl_2$).

The ^1H NMR spectrum of the freshly prepared heptaacetate initially showed signals for a single (major) conformational isomer; however, as a solution of the heptaacetate was allowed to stand, even in the freezer, signals for a minor conformational isomer slowly appeared. ^1H NMR (benzene-d_6) of major conformer: δ (multiplicity, J in Hz, assignment) 7.20/7.19/7.07/7.05 (s, H-10,12,23,25), 5.55 (dd, 7.9/9.3, H-36), 5.37 (dd, 9.3/9.6, H-37), 5.27 (t, 9.6, H-38), 5.14 (d, 7.9, H-35), 4.63 (m, H-1,14), 4.24 (dd, −12.5/3.9, H-40a), 3.95 (dd, −12.5/1.3, H-40b), 3.51/3.36 (m, H-3,16), 3.08 (s, OMe on C-14), 3.04 (s, OMe on C-1), 2.97 (ddd, 9.6/3.9/1.3, H-39), 2.42/1.81 (m, H-2,15), 2.21 (m, H-7,20), 1.80/1.37 (m, H-27,31), 1.78/1.75/1.74/1.73/1.72/1.70/1.69 (7 s, OAc), 1.70/1.25 (m, H-6,19), 1.65/1.58 (m, H-4,17), 1.40/1.01 (m, H-5,18), 1.35/1.27 (m, H-28,32), 1.28 (m, H-29,33), 0.86 (t, 7.2, H-30), 0.82 (t, 7.2, H-34).

Minor conformer: δ (multiplicity, J in Hz, assignment) 7.19/7.18/7.05/7.04 (s, H-10,12,23,25), 5.60 (dd, 7.2/9.2, H-36), 5.09 (d, 7.2 H-35), 4.75 (dd, −12.4/3.0, H-40a), 4.35 (dd, −12.4/2.0, H-40b), 3.19 (s, OMe on C-14), 3.06 (s, OMe on C-1), 2.11/1.95/1.83/1.754/1.749/1.74/1.72, (s, OAc), 0.83 (t, 7.2, H-30,34).

Preparation of Nostocyclophane B Heptaacetate from Nostocyclophane D. (1) 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl Bromide. A solution of β-D-glucopyranose pentaacetate (500 mg) in 2 mL of 30% HBr/HOAc was allowed to stand in the refrigerator overnight and the solvent evaporated. The residue was dissolved in 3 mL of chloroform, the solution washed with cold saturated $NaHCO_3$ solution followed by water and dried over sodium sulfate, and the solvent evaporated. The residual syrup in 2.5 mL of absolute EtOH was allowed to stand in the cold for 2 h to give crystals of the bromide (450 mg, 85%) that were separated by filtration and dried: mp 87–89 °C; $[\alpha]_D$ +195° (c 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ (multiplicity, J in Hz, assignment) 6.60 (d, 4.0, H-1), 5.55 (dd, 9.7, H-4), 5.15 (dd, 10.0/9.7, H-3), 4.82 (dd, 10.0/4.0, H-2), 4.32 (dd, −10.5/3.7, H-6), 4.30 (ddd, 9.7/4.1/3.7, H-5), 4.15 (dd, −10.5/4.1, H-6′), 2.10 (s, OAc), 2.09 (s, OAc), 2.05 (s, OAc), 2.03 (s, OAc). The ^{13}C NMR spectrum of the bromide was essentially identical with that described in the literature.7

(2) Nostocyclophane D 9-*O*-(2,3,4,6-Tetra-*O*-acetyl)-β-D-glucopyranoside. A mixture of nostocyclophane D (4.0 mg, 0.006 mmole), cadmium carbonate (3.5 mg, 0.02 mmol), and 5 mL of toluene was boiled until ~3.5 mL of toluene had been removed. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (12 mg, 0.029 mmole) in 2 mL of toluene was added dropwise to the stirred mixture over 15 min as an equal volume of toluene was distilled from the flask. For the next 3 h, toluene was added dropwise to the mixture as an equal amount of the toluene was removed from the mixture by distillation. Then the mixture was refluxed for 40 h and finally filtered through an ACRO LC13 filter (0.2 μm). The filtrate was evaporated, the residue was dissolved in chloroform, and the solution was washed with 1 M sodium bicarbonate followed by water. The solvent was evaporated, and a filtered methanolic solution of the residual material was subjected to HPLC purification on a 10 × 250 mm column of Econosil C-18 (10 m) with 80:20 CH_3CN/H_2O at flow rate of 2.5 mL/min. Separation was monitored by continuous UV analysis at 228 nm. The glycoside was found in a fraction that eluted at 23 min (0.85 mg, 14.1%); unreacted nostocyclophane D eluted at 20 min (2.5 mg, 62.5%). The ^1H NMR spectrum of the glycoside in $CDCl_3$ showed aromatic proton signals at 6.52, 6.34, 6.25, and 6.18 ppm, methoxy proton signals at 3.24 and 3.20 ppm, and acetate signals at 2.21, 2.05, 2.04, and 2.03 ppm.

(3) Nostocyclophane B Heptaacetate. The semisynthesized nostocyclophane D 9-*O*-(2,3,4,6-tetra-*O*-acetyl)-β-D-glucopyranoside was treated with 0.5 mL of 1:1 acetic anhydride/pyridine at room temperature overnight. After evaporation of the volatiles in vacuo, the residue was dissolved in chloroform and the solution washed with 1 M sodium bicarbonate followed by water, dried with sodium sulfate, and evaporated. A methanolic solution of the residue was subjected to HPLC on a 10 × 250 mm column of Econosil C-18 (10 m) with 85:15 CH_3CN/H_2O at flow rate of 2.5 mL/min. The product eluted at 30 min (0.8 mg, 83.4%), and its ^1H NMR and CD spectra were identical with those of nostocyclophane B acetate described previously.

Nostocyclophane C: $[\alpha]_D$ −5.53° (c 0.27); UV λ_{max} 209 nm (ϵ 47 200), 226 (15 800), 276 (3000), 283 (2960); CD $[\theta]_{216}$ −33 600, $[\theta]_{230}$ −13 500, $[\theta]_{270}$ −2520, $[\theta]_{280}$ −3030; FDMS m/z 639/641/643 (10:6.5:1 MH^+ ion cluster[6] for $C_{35}H_{52}O_6Cl_2$).

^1H NMR (DMSO-d_6): δ (multiplicity, J in Hz, assignment) 8.81 (s, phenolic OH at C-13), 8.79 (s, phenolic OH at C-26), 8.72 (s, phenolic OH at C-22), 8.71 (s, phenolic OH at C-9), 6.22 (d, 1.1, H-23), 6.15 (d, 1.6, H-12), 6.11 (d, 1.1, H-25), 6.11 (d, 1.6, H-10),

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5.10 (d, 3.5, OH on C-1), 4.42 (dt, 10.5/3.5, H-1), 4.04 (dd, 10.5/4.0, H-14), 3.10 (m, H-7), 3.07 (m, H-20), 3.04 (s, OMe on C-14), 2.83 (m, 10.7, H-3,16), 2.08 (m, -13.9/10.5/4.3, H-15), 2.01 (m, -13.9/10.5/3.7, H-2), 1.84 (m, -13.9/4.0, H-15), 1.84/1.56 (m, H_2-6,19), 1.81 (m, -13.9/3.5, H-2'), 1.60/1.50 (m, H_2-17), 1.60/1.45 (m, H_2-4), 1.81/1.39 (m, H_2-27,31), 1.52/0.55 (m, H-5,18), 1.25 (m, H_2-29,33), 1.18 (m, H_2-28,32), 0.77/0.76 (t, H_3-30,34).

^{13}C NMR (DMSO-d_6): δ 157.4 (s, C-13), 157.2 (s, C-26), 155.6 (s, C-9), 155.3 (s, C-22), 142.0 (s, C-24), 138.2 (s, C-11), 115.9 (s, C-8), 115.0 (s, C-21), 107.1 (d, C-12), 105.6 (d, C-25), 103.4 (d, C-23), 102.7 (d, C-10), 80.8 (d, C-14), 70.4 (d, C-1), 63.2 (d, C-3), 62.7 (d, C-16), 55.6 (q, OMe on C-14), 47.7 (t, C-2), 45.4 (t, C-15), 40.3 (t, C-4,17), 34.8 (d, C-7), 34.7 (d, C-20), 32.6 (t, C-6,19), 32.4 (t, C-27,31), 30.0/29.9 (t, C-28,32), 26.6 (t, C-5,18), 22.1 (t, C-29,33), 13.9 (q, C-30,34).

Nostocyclophane D. Recrystallized from aqueous EtOH, mp 242-3 °C: $[\alpha]_D$ +10.8° (c 0.4); UV λ_{max} 215 nm (ε 19 600), 228 (13 500), 276 (2080), 283 (2110); CD $[\theta]_{218}$ -28 200, $[\theta]_{230}$ -14 900, $[\theta]_{270}$ -2230, $[\theta]_{280}$ -2600; positive-ion FABMS m/z 653/655/657 (10:6.5:1 MH^+ ion cluster[6] for $C_{36}H_{55}O_6Cl_2$), 637/639/641 (M - Me), 620/622/624 (M - MeOH), 588/590/592 (M - 2 MeOH), 553/555 (M - 2 MeOH - Cl), and 518 (M - 2 MeOH - 2 Cl) at m/z 627, 620, 588, 553, and 518; FDMS m/z 652/654/656 (10:6.5:1 MH^+ ion cluster for $C_{36}H_{54}O_6Cl_2$).

^{1}H NMR (DMSO-d_6): δ (multiplicity, J in Hz, assignment) 8.82/8.80 (s, phenolic OH), 6.15/6.10 (s, H-10,12,23,25), 4.05 (dd, 10.7/3.3, H-1,14), 3.10 (tt, 10.5/3.2, H-7,20), 3.05 (s, OMe on C-1,14), 2.82 (tt, 10.5/3.0, H-3,16), 2.08 (m, -11.3/10.3/3.3, H_a-2,15), 1.85 (m, -11.3/10.7/3.0, H_b-3,15), 1.85/1.58 (m, H_2-6,19), 1.81/1.36 (m, H_2-27,31), 1.62/1.51 (m, H_2-4,17), 1.48/0.54 (m, H_2-5,18), 1.23 (m, H_2-29,33), 1.18 (m, H_2-28,32), 0.77 (t, 7.2, H_3-30,34).

^{13}C NMR (DMSO-d_6): δ 157.5 (s, C-13,26), 155.6 (s, C-9,22), 138.0 (s, C-11,24), 116.1 (s, C-8,21), 107.3 (d, C-12,25), 102.8 (d, C-10,23), 80.9 (d, C-1,14), 62.6 (d, C-3,16), 55.6 (q, OMe on C-1,14), 45.5 (t, C-2,15), 40.3 (t, C-4,17), 34.8 (d, C-7,20), 32.7 (t, C-6,19), 32.5 (t, C-27,31), 30.0 (t, C-28,32), 26.5 (t, C-5,18), 22.1 (t, C-29,33), 13.9 (q, C-30,34).

Uniform Enrichment of Nostocyclophane D. *N. linckia* UTEX B1932 was grown in a 10-L glass bottle on 5.0 g of $NaH^{13}CO_3$ (99 atom %) and 4.0 g of $Na^{15}NO_3$ (99 atom %) as previously described.[8] After 28 days the 8-L culture (alga and medium) was lyophilized. Extraction and workup resulted in the isolation of 2 mg of labeled nostocyclophane D; inspection of its ^{13}C NMR spectrum indicated 37% uniform enrichment.

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Supplementary Material Available: ^{1}H NMR and CD spectra of nostocyclophanes A-D, ^{13}C NMR spectrum of D, and two-dimensional NOESY spectra of A and B (9 pages). Ordering information is given on any current masthead page.

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General Base Catalysis by Hydroxocopper(II) Ion and Existence of Addition Intermediate in Hydrolysis of *m*-(2-Imidazolylazo)phenyl *p*-Toluenesulfonate

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Rates of the hydrolysis of *m*-(2-imidazolylazo)phenyl p-toluenesulfonate (1) were measured in the presence of Cu(II) ion. Saturation behavior was observed for the dependence on [Cu(II)] of the absorbance (Abs) of 1 or that of the pseudo-first-order rate constant (k_0). The formation constant measured from the dependence on [Cu(II)] of k_0 was much smaller than that of Abs. The binding constant reflected in the Abs data indicates the formation of a 1:1-type complex. The binding constant estimated with the k_0 values may be related to the formation of a 2:1-type complex. This possibility, however, is excluded on the basis of the dependence of the binding constants on pH and the dependence of Abs on [Cu(II)]. Instead, the saturation kinetic behavior agrees with the shift of the rate-determining step between the formation and the breakdown processes of an intermediate upon increase in [Cu(II)]. On the basis of the kinetic data, it is shown that the 1:1-type complex is hydrated to form an addition intermediate, which is subsequently converted into the hydrolysis products, and that hydroxocopper(II) ion participates as a general-base catalyst in the rate-controlling proton-transfer process.

Collection of proofs of existence or nonexistence of intermediates is among the most important tasks in the studies of reaction mechanisms. For nucleophilic substitution on the derivatives of phosphorus oxy acids or sulfur oxy acids, whether the reaction proceeds through the addition-elimination process involving a pentacovalent trigonal-bipyramidal intermediate or through the concerted process involving simultaneous attack of the nucleophile and cleavage of the leaving group has been the center of the mechanistic studies.[1] Results of some recent intensive investigations supported the concerted mechanism in the transfer of phosphoryl, sulfuryl, or sulfonyl groups between various nucleophiles.[2-11] For nucleophilic reactions on aryl

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