should prove to be a versatile 2π ligand. An isolobal analog, $[(CO)_3Fe]_3(\mu\text{-}H)_3CR$, is well-known.[17] Other instances where a planar skeleton distorts under the strain of bridging groups must exist. Recently Schaefer and Xie had suggested a hexabridged D_{6h} structure for C_6Li_6.[18] It is unlikely that higher analogs of these would prefer D_{nh} structures.[19]

The $B_3H_6^+$ (**6**) is a highly favored ion. Equations 1 and 2 compare the extra stability of **6** against the cyclopropenium ion, **1**. It should be possible to observe $B_3H_6^+$ experimentally.

$$2B_3H_6^+ + 2C_3H_6 \rightarrow 2C_3H_3^+ + 3B_2H_6 \qquad \Delta E = 58.70 \text{ kcal/mol} \quad (1)$$

$$B_3H_6^+ + C_3H_6 \rightarrow C_3H_3^+ + B_3H_9 \qquad \Delta E = 41.59 \text{ kcal/mol} \quad (2)$$

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Supplementary Material Available: Table of geometric parameters of structures **1-18** at the HF/6-31G*, MP2/6-31G*, and QCISD(T)/6-31G* levels (3 pages). This supplementary material is provided in the archival edition of the journal, which is available in many libraries. Alternatively, ordering information is given on any current masthead page.

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Cylindrospermopsin: A Potent Hepatotoxin from the Blue-Green Alga *Cylindrospermopsis raciborskii*

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Hepatoenteritis in humans caused by toxic cyanobacterial blooms in domestic water supplies that have become eutrophic is a growing concern. *Microcystis aeruginosa* is the most frequently implicated blue-green alga in these poisonings,[1] and the hepatotoxins associated with this cyanophyte are cyclic heptapeptides known as microcystins.[2] Circumstantial evidence is slowly emerging linking toxic *Microcystis* blooms with a higher incidence of liver cancer among populations in Third World countries such as China that depend on surface drinking water.[3] In 1979, however, an outbreak of hepatoenteritis on Palm Island in northern Queensland, Australia, was traced to a different cyanobacterium, *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju, a species that had not been previously found to be toxic.[4] We report here the isolation and gross structure determination of an unusual alkaloid, cylindrospermopsin, which is hepatotoxic with symptoms indistinguishable from those originally described for the cyanobacterial extract.[4,5]

C. raciborskii was grown in culture as previously described.[4] An aqueous extract (0.9% NaCl) of the ultrasonicated, freeze-dried alga (0.7 g) was fractionated (bioassay-guided) by successive gel filtration on Toyopearl HW40F with 1:1 MeOH/H_2O and reversed-phase HPLC on C18 with 5% MeOH in H_2O to give white microcrystals of cylindrospermopsin (**1**, $C_{15}H_{21}N_5O_7S$; positive ion HRFABMS, glycerol matrix: MH^+ m/z 416.1236, Δ = 0.4 mmu), in 0.5% yield, $[\alpha]_D$ -31° (H_2O, c 0.1), as the only detectable hepatotoxin. The intense negative ion FABMS ($M - H^-$ m/z 414) and UV spectrum in H_2O [λ_{max} 262 (ϵ 5800), sh 290 nm (2100)] was consistent with **1** being a substituted uracil. Comparison of the ^{13}C chemical shifts in both D_2O and H_2O (Figure 1) and $^1J_{CH}$ for C-5 (175 Hz) with values reported for uracil[6] indicated that the substitution was on C-6. The toxin appeared to be a sulfate ester since the air CIDMS of the MH^+ ion (FAB mode) showed fragment ions at m/z 336.1688 ($C_{15}H_{22}N_5O_4$, Δ = -1.6 mmu), 318, 274 [MH - (hydroxymethyl)uracil]$^+$, 194, and 176 for the loss of SO_3 and H_2SO_4 from the MH^+ and [MH - (hydroxymethyl)uracil]$^+$ ions.

Detailed analysis of the 500-MHz ^1H and 125-MHz ^{13}C NMR spectra in D_2O, aided by two-dimensional COSY, HMQC, and HMBC experiments, enabled us to assign all of the ^1H and ^{13}C signals and to propose the structure shown in Figure 1. Chemical shifts suggested that nitrogen was attached to the carbons resonating at 45.0 (C-10), 48.3 (C-15), 53.6 (C-8), and 57.9 (C-14) ppm whereas oxygen was present on the carbons absorbing at 70.7 (C-7) and 78.2 (C-12) ppm. Isotope shifts for the C-7, C-8, and C-15 signals in H_2O (see $\Delta\delta_C$ values in Figure 1) established that NH's were on C-8 and C-15 and an OH group was on C-7. The sulfate group was therefore attached to C-12, and its placement here was supported by the CIDMS data. The coupling constants (J_{trans} = 11.1-11.8 Hz, J_{cis} = 2.0-3.9 Hz, and J_{gem} = -13.9 ± 0.5 Hz) associated with the signals for the protons on C-8, C-9 (28.5 ppm), C-10, C-11 (36.3 ppm), C-12, C-13 (39.8 ppm), and C-14 showed that these nuclei were located in six-membered rings which required that (1) the same nitrogen be connected to C-10 and C-14, (2) the sulfate ester group on C-12 be oriented axially, and (3) the methyl substituent on C-13 be equatorially disposed. The proton on C-8 (3.87 ppm) and one of the protons on C-15 (3.84 ppm) were coupled (HMBC cross peaks) to a guanidino carbon resonating at 156.5 ppm, and this meant that the toxin was a

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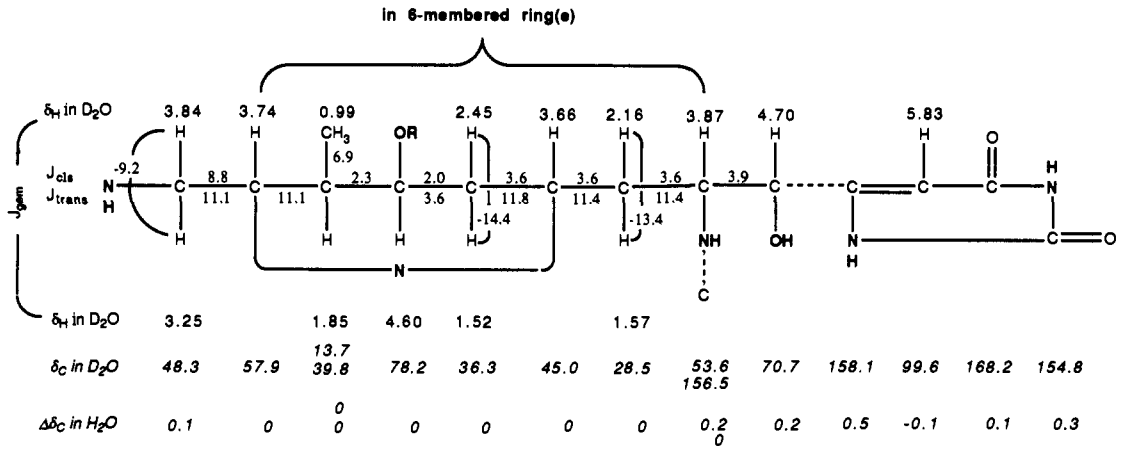
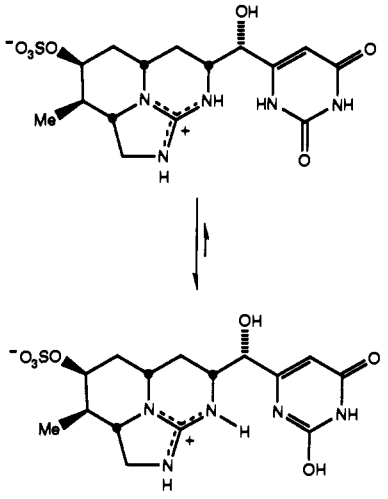
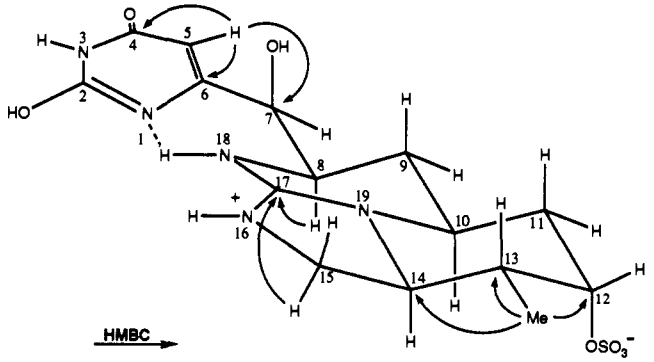


Figure 1. ^{1}H and ^{13}C chemical shift and ^{1}H-^{1}H coupling constant assignments for cylindrospermopsin.

tricyclic guanidine as depicted in **1**. The uracil ring had to be attached to C-7 since HMBC cross peaks were clearly seen between H-5 and C-4, C-6, and C-7 (**1a**). The ^{13}C-^{13}C COSY spectrum of uniformly 80+% ^{13}C enriched **1**, isolated from alga grown on $NaH^{13}CO_3$ (99%), confirmed the gross structure (see supplementary material).



1



1a

The ^{13}C signals for C-2 and C-6 were broad, and their chemical shifts, unlike the ones for uracil, were very sensitive to small changes in pH around 7, hinting that N-1/C-2 might have an enol rather than an amide structure. Favoring the enol tautomer could be a consequence of the uracil and guanidine units being coplanar with 18(N)-H hydrogen-bonded to N-1. If so, then the NOESY spectrum (NOE correlations: 5-H ↔ 7-H; 7-H ↔ 8-H; 9-H_{eq} ↔ 11-H_{eq}; 10-H ↔ 14-H; 13-H ↔ 11-H_{ax}, 12-H, and 15-H (cis); and 13-CH_3 ↔ 12-H, 14-H, and 15-H_2) suggests that the toxin has the relative stereochemistry shown in **1a**.

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Supplementary Material Available: 500-MHz ^{1}H and 125-MHz ^{13}C NMR spectra of **1** in D_2O, ^{1}H-^{13}C HMQC, ^{1}H-^{13}C HMBC, and ^{1}H-^{1}H NOESY spectra of **1** in D_2O, ^{13}C-^{13}C COSY spectrum (D_2O) of [U-^{13}C]-**1**, and ^{13}C NMR spectrum of [U-^{13}C,^{15}N]-**1**[7] in D_2O (7 pages). Ordering information is given on any current masthead page.

Role of Peptide Conformation in Asparagine-Linked Glycosylation

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The first committed step in the biosynthesis of N-linked glycoproteins is catalyzed by the membrane-associated enzyme oligosaccharyltransferase (OT) and involves the cotranslational transfer of a complex carbohydrate from a lipid-linked pyrophosphate donor to the side-chain nitrogen of an asparagine (Scheme I).[1] The primary peptide sequence requirements for the process include a minimum -Asn-Xaa-Ser/Thr- tripeptide recognition motif.[2] This transformation is intriguing in that it formally involves nucleophilic attack by an asparagine primary amide nitrogen and displays remarkable selectivity considering the competing functionality in the peptidyl substrates in which the "reactive" asparagine is localized. Given these considerations and the recent proposal that the unique reactivity of asparagine may arise from a conformational bias resulting in the formation of the Asx turn,[3,4] we have synthesized and evaluated conformationally *constrained*[5] peptide substrates in order to probe the

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