Cornutin A and B: Novel Diterpenoid Repellents of Leafcutter Ants from *Cornutia grandifolia*

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Two novel *neo*-clerodane diterpenoids, distinguished by a unique ether linkage spanning C-1 and C-12, have been isolated from the leaves of *Cornutia grandifolia*. These two highly oxidized diterpenoids are significant repellents of the leafcutter ant *Acromyrmex octospinosus* in laboratory bioassays. Two simpler *neo*-clerodanes isolated from the same plant, identified as the parent carboxylic acids to the methyl esters ajugarin-IV and deacetylajugarin-IV, showed no significant activity in this bioassay at comparable concentrations.

Throughout the tropical and subtropical Americas, the most destructive insect pests are leafcutting ants (Hymenoptera, Formicidae, Attini).[1] In contrast to the ants' apparently catholic interest in cultivated plants, many plant species in native forests suffer little from leafcutter attack.[2] We have advanced the premise that leafcutter foraging preferences can be used to screen native plants in the field for the presence of bioactive natural products,[3] and we have investigated species that leafcutters avoid in an effort to characterize natural plant defenses against these insects.[4] Defensive chemicals could embody ant-repellent or formicidal activities or, because the leafcutters use collected plant material as a culture medium for the fungus that serves as their principal food, might have antifungal activity.[5] Our approach has resulted in isolation of a variety of natural products, with a range of these biological activities. In this paper, we report our most recent study in this series, an investigation of *Cornutia grandifolia* that has uncovered a pair of novel pentacyclic *neo*-clerodane diterpenoids with significant ant-repellent activities.

Results and Discussion

A sample of *C. grandifolia* was collected in Panama, after our field bioassay[3] suggested that fresh leaves were highly repellent to a local leafcutter ant, *Acromyrmex octospinosus* (Reich). A crude chloroform extract of the leaves was first subjected to dry column chromatography over silica gel with an EtOAc/hexane gradient, providing two fractions with intriguing patterns of downfield resonances in their ^{1}H NMR spectra. Repeated flash column chromatography of these fractions, and final purification on reverse phase C18 Sep-Pak cartridges, gave pure samples of four compounds. Two ultimately were characterized as cornutins A and B, and two were identified as parent carboxylic acids to known ajugarin-IV methyl esters.

The molecular formula of cornutin A was established as $C_{24}H_{30}O_8$ by high-resolution mass spectrometry (m/z 446.1963 $[M]^+$), implying 10 degrees of unsaturation. From a cursory inspection of the ^{13}C NMR spectrum, five could be attributed to two carbon–carbon double bonds and three carbonyl groups (one ketone and two acetates),

Table I. Proton and Carbon NMR Data for Cornutin A

C/H	^{13}C	^{1}H	long-range ^{1}H–^{13}C correlations
1	64.81 (d)	4.45 (ddd, 6.4, 10.0, 10.4)	9
2	46.57 (t)	2.55 (dd, 10.0, 15.5)	1, 3, 10
		3.08 (dd, 6.4, 15.5)	1, 3, 4, 10
3	202.52 (s)		
4	69.27 (s)		
5	41.09 (s)		
6	73.09 (d)	4.82 (d, 9.6)	4, 5, 19, 6-Ac
7	72.64 (d)	5.04 (dd, 9.6, 11.1)	
8	47.03 (d)	1.57 (m)	7, 9, 17, 20
9	36.63 (s)		
10	51.80 (d)	1.87 (d, 10.4)	5, 9, 19, 20
11	43.70 (t)	1.82 (dd, 6.7, 13.7)	9, 12, 13, 20
		2.10 (dd, 1.9, 13.7)	9, 10, 20
12	67.70 (d)	5.14 (m)	1, 9, 11, 13, 16
13	126.83 (s)		
14	108.70 (d)	6.27 (dd, 1.5, 1.7)	13, 15, 16
15	143.47 (d)	7.42 (dd, 1.7, 1.7)	13, 16
16	138.28 (d)	7.30 (dd, 1.5, 1.5)	14, 15
17	9.67 (q)	0.90 (d, 6.7)	7, 8, 9
18	50.94 (t)	2.57 (d, 4.5)	3, 4, 5
		3.43 (d, 4.5)	4, 5
19	15.25 (q)	1.15 (s)	4, 5, 6, 10
20	18.68 (q)	0.96 (s)	8, 9, 10, 11
6-OAc	169.99 (s)		
7-OAc	170.30 (s)		
	20.57 (q)	1.97 (s)	
	20.63 (q)	2.02 (s)	

suggesting a pentacyclic system. It was assumed that the main C_{20} skeleton probably was diterpenoid in origin.

In addition to the two acetate groups, 20 other carbons could be identified by ^{13}C NMR and DEPT experiments (Table I). Five of these were quaternary carbons (one carbonyl, two aliphatic, one oxygenated aliphatic, and one olefinic). The remaining resonances implied the presence of three aliphatic methyl groups, three methylene units (two aliphatic and one oxygenated aliphatic), and nine methine carbons (two aliphatic, four aliphatic and oxygenated, one olefinic, and two both olefinic and oxygenated).

One of the five ring systems was identified as a β-substituted furan moiety, based on a pattern arising from aromatic resonances at δ 6.27, 7.30, and 7.42 in the ^{1}H NMR spectrum (Table I). In addition, a ^{1}H–^{1}H COSY experiment revealed an allylic coupling between one α-furan hydrogen (δ 7.30) and an oxygenated methine hydrogen (δ 5.14), which was also coupled to two methylene H's (δ 1.82 and 2.10). These data led to assembly of partial structure A.

Partial structures B, C, and D also were indicated by coupling correlations from the ^{1}H–^{1}H COSY spectrum. One spin system, proceeding from the doublet methyl

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(2) Rockwood, L. L. *Ecology* **1976**, *57*, 48.
(3) Hubbell, S. P.; Howard, J. J.; Wiemer, D. F. *Ecology* **1984**, *65*, 1067.
(4) For recent publications in this series, cf.: (a) Chen, T.-B.; Wiemer, D. F. *J. Nat. Prod.* **1991**, *54*, 1612. (b) Green, T. P.; Wiemer, D. F. *Phytochemistry* **1991**, *30*, 3759. (c) Green, T. P.; Galinis, D. L.; Wiemer, D. F. *Phytochemistry* **1991**, *30*, 1649. (d) Roussis, V.; Ampofo, S. A.; Wiemer, D. F. *Phytochemistry* **1990**, *29*, 1787; (e) Hammond, G. B.; Baenziger, N. C.; Wiemer, D. F. *Phytochemistry* **1990**, *29*, 783.
(5) Howard, J. J.; Cazin, J., Jr.; Wiemer, D. F. *J. Chem. Ecol.* **1988**, *14*, 59.

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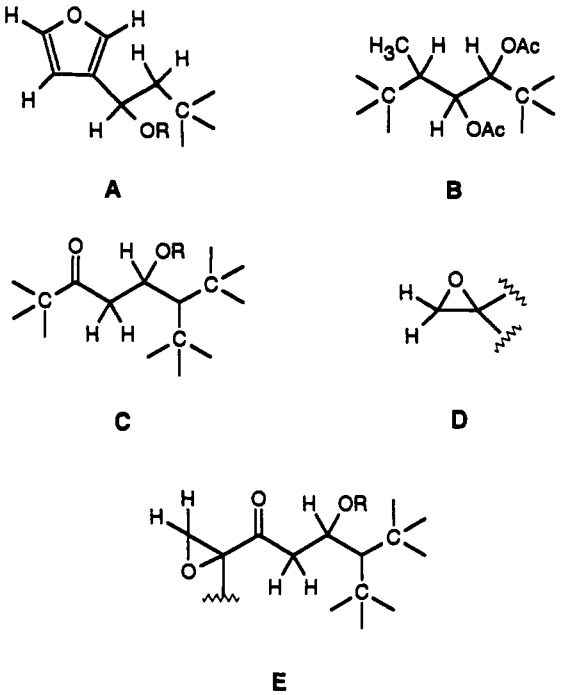


resonance (δ 0.90) through its geminal methine (δ 1.57) and two downfield resonances (δ 5.04 and 4.82, respectively), was easily attributed to the partial structure B. The chemical shifts and the J_{gem} for the two methylene hydrogens (δ 2.55 and 3.08, J = 15.5 Hz) in structure C implied that this methylene was next to the ketone carbonyl, a conclusion later confirmed by a selective INEPT experiment.[6] Structure D was deduced from observation of two doublets (δ 2.57 and 3.43) with characteristic epoxide geminal coupling (4.5 Hz).

The other two methyl groups (δ 0.96 and 1.15) must be connected to quaternary carbons. Of special value was observation in the COSY spectrum of long-range W-coupling between one CH_3 (δ 0.96) and one methylene hydrogen (δ 1.82) in partial structure A, which suggested that this methyl group was a substituent on the quaternary carbon of this fragment.

A series of selective INEPT experiments (Table I) was employed to join these partial structures through the bridging quaternary carbons. The observation of one quaternary carbon (69.27 ppm) upon irradiation of one methylene H (δ 3.08) assigned in structure C and the epoxide H's (δ 2.57 and 3.43) in structure D allowed their combination into structure E. The gross structure of the A and B rings was established by (1) observation of a quaternary carbon (36.63 ppm) upon irradiation of the bridgehead methine hydrogen (δ 1.87) in E, the methine hydrogen (δ 1.57) in B, and one methyl group (δ 0.96) and (2) observation of two quaternary carbons (41.09 and 69.27 ppm) upon irradiation of the bridgehead methine hydrogen (δ 1.87) in E, the methine hydrogen (δ 4.82) in B, and the remaining singlet methyl group (δ 1.15).

Experiments demonstrating correlations between the oxygenated methine hydrogen (δ 5.14) in structure A and the oxygenated methine carbon (64.81 ppm) in the A ring, and between two methylene H's (δ 1.82 and 2.10) in structure A and the quaternary carbon (36.63) in the B ring, required an unusual C-ring connectivity in this compound. By combining the information observed in these experiments with the additional selective INEPT experiments listed in Table I, a bridged clerodane skeleton was

(6) Bax, A. *J. Magn. Reson.* 1984, *57*, 314.

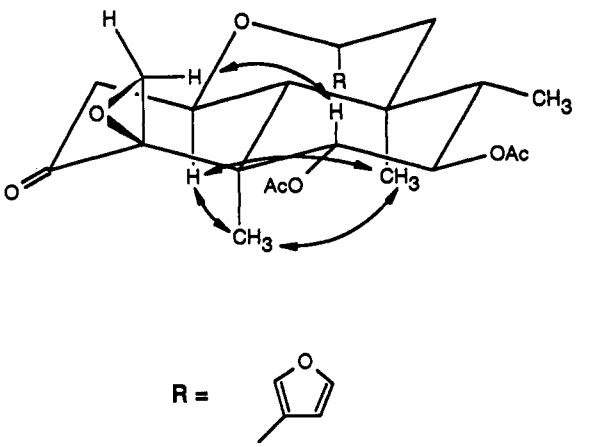
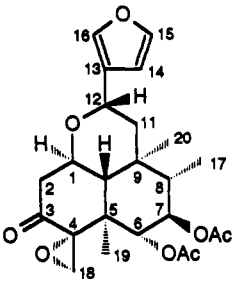


Figure 1. Selected NOE correlations for cornutin A.

assigned as shown in compound 1.

The relative stereochemistry of eight of the nine stereogenic centers in compound 1 could be assigned readily on the basis of coupling constants and a NOESY experiment. Large coupling constants between H-1 and H-10 (10.4 Hz) required that H-1 and H-10 be found in axial α- and β-orientations, respectively. Furthermore, diaxial couplings between H-6 and H-7 (9.6 Hz) and between H-7 and H-8 (11.1 Hz) required that both acetates and the methyl group must be equatorial. Therefore, H-6, H-7, and H-8 must be in β-, α-, and β-orientations, respectively. A NOESY experiment exhibiting correlations among Me-19, Me-20, and H-1 suggested that Me-19 and Me-20 should be in axial positions to produce these through-space interactions (Figure 1). Finally, an NOE observed between one of the H-18 hydrogens (δ 3.43) and H-6 (δ 4.82) established the stereochemistry of C-4.



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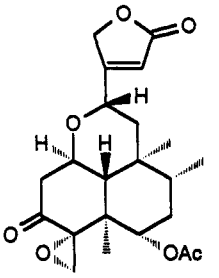

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For the remaining stereogenic center, C-12, observation of 6.7- and 1.9-Hz couplings between H-12 and the H-11 hydrogens implied that H-12 should be in a β-orientation. In the NOESY experiment, a weak correlation between H-12 and H-10 was observed, while no correlations were detected between H-12 and either H-1 or Me-20. Together, these results led to the stereochemical assignment shown in structure 1. Experiments with cornutin B provide further, albeit circumstantial, support for this assignment.

The high-resolution mass spectrum of cornutin B revealed a molecular ion peak at m/z 404.1840, corresponding to a composition of $C_{22}H_{28}O_7$. Both the ^1H and ^{13}C NMR spectra exhibited many signals similar to those assigned to the A, B, and C rings of compound 1, with the notable lack of one acetate and replacement of the furan moiety by a γ-lactone. The single acetate substituent was assigned at C-6 based on observation of coupling between the H-6 (δ 4.84) and two H-7 hydrogens (δ 1.56) in the ^1H–^1H COSY experiment. This assignment was confirmed by observation of C-6 (70.69 ppm) upon irradiation of H-10

Table II. Proton and Carbon NMR Data for Cornutin B

C/H	^{13}C	^{1}H	long-range ^{1}H–^{13}C correlations
1	66.41 (d)	4.35 (ddd, 6.3, 10.0, 10.6)	9, 12
2	46.46 (t)	2.51 (dd, 10.0, 15.5)	
		3.04 (dd, 6.3, 15.5)	1, 3, 4, 10
3	202.24 (s)		
4	69.11 (s)		
5	39.95 (s)		
6	70.69 (d)	4.84 (m)	4, 5, 19, 6-Ac
7	32.09 (t)	1.56 (m)	5, 6, 8, 9
		1.56 (m)	
8	41.77 (d)	1.50 (m)	7, 9, 20
9	36.10 (s)		
10	51.66 (d)	1.76 (d, 10.6)	1, 2, 4, 5, 6, 8, 9, 19, 20
11	42.33 (t)	1.83 (dd, 7.3, 14.0)	8, 9, 12, 13, 20
		1.95 (dd, 2.4, 14.0)	
12	69.46 (d)	4.94 (m)	1, 9, 11, 13, 14
13	171.07 (s)		
14	115.32 (d)	6.02 (m)	12, 13, 15, 16
15	172.84 (s)		
16	71.06 (t)	4.85 (brd s)	
		4.85 (brd s)	
17	14.06 (q)	0.90 (d, 6.2)	7, 8, 9
18	50.78 (t)	2.56 (d, 4.7)	
		3.33 (d, 4.7)	4, 5
19	14.06 (q)	1.12 (s)	4, 5, 6, 10
20	16.66 (q)	1.02 (s)	8, 9, 10, 11
6-OAc	170.08 (s)		
	20.99 (q)	2.00 (s)	

(δ 1.76) in the selective INEPT experiment. The presence of a conjugated γ-lactone moiety, bearing an α-hydrogen as in the ajugarins,[7,8] was indicated by ^{1}H NMR resonances at δ 6.02 (1 H, m, H-14) and 4.85 (2 H, brd s, H-16) and ^{13}C NMR resonances at 171.07 (s, C-13), 115.32 (d, C-14), 172.84 (s, C-15), and 71.06 ppm (t, C-16). This also was supported by selective INEPT experiments (cf. Table II).

The relative stereochemistry of the stereogenic centers in the A, B, and C rings of this compound was assigned analogous to those of compound 1, through analysis of comparable coupling constants (Table II) and/or NOE effects. Even though the coupling constants between H-6 and H-7, and between H-7 and H-8, could not be determined due to overlapping resonances in the ^{1}H NMR spectrum, an NOE correlation among H-6, H-8, and H-10 was apparent in the NOESY spectrum. This allowed assignment of the relative stereochemistry at C-6 and C-8.

For assignment of the C-12 stereochemistry, the observed coupling constants (7.3 and 2.4 Hz) suggested an equatorial hydrogen, but may not be completely unambiguous. Fortunately, crystalline cornutin B was obtained from dichloromethane by slow evaporation, and a single-crystal x-ray diffraction analysis was conducted on this material. This analysis confirmed the gross structure and stereochemical detail proposed on the basis of spectral data, allowing a complete structure assignment as shown in Figure 2. In particular, the γ-lactone substituent at C-12 is in an axial α-orientation. Unfortunately, the lack of a heavy atom precluded determination of the absolute stereochemistry.

Finally, two related diterpenoids were isolated from these fractions in comparable amounts. Their EI mass spectra showed fragment ion peaks at m/z 374 ($M^+ - H_2O$) for compound 3 and 332 ($M^+ - H_2O$) for compound 4, suggesting less highly functionalized diterpenoids. The ^{1}H and ^{13}C NMR spectra of compounds 3 and 4 were nearly

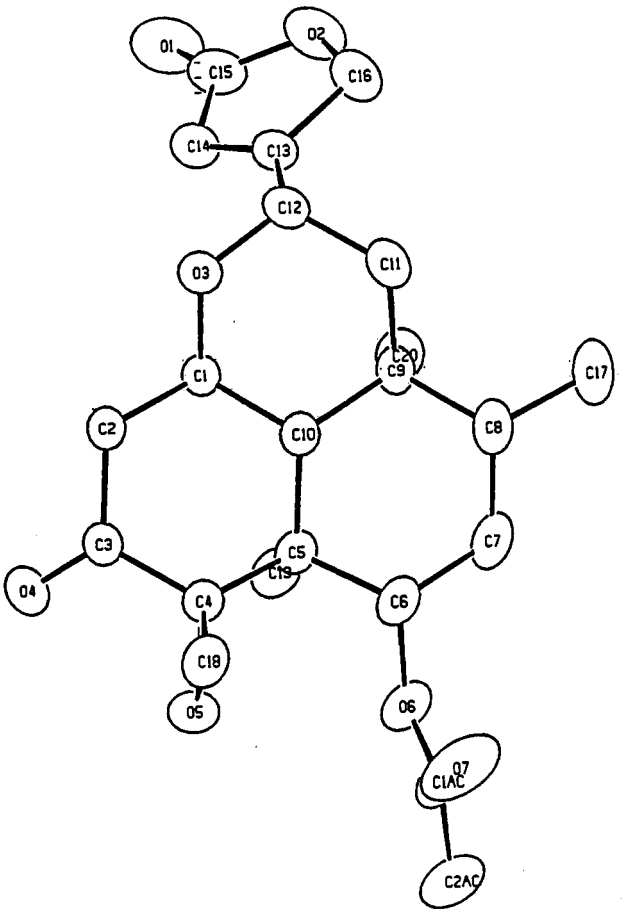
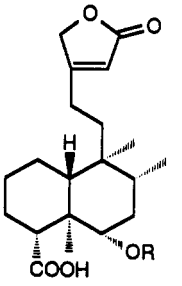


Figure 2. ORTEP drawing of cornutin B.

identical to those reported for the diterpenoids ajugarin-IV[7] and deacetylajugarin-IV,[8] respectively, although we isolated the C-18 carboxylic acids instead of the methyl esters previously reported.



3 R = Ac
4 R = H

The absolute stereochemistry of compound 3 was established by treating a small sample with diazomethane to obtain the corresponding methyl ester. The NMR spectra of this methyl ester were identical to those previously reported for ajugarin-IV,[7] and the rotation had the same sign, confirming identification of our compound as a *neo*-clerodane. Although it does not rigorously prove such an assignment, the co-occurrence of this *neo*-clerodane with cornutins A and B suggests that all belong to the same enantiomeric series.

Cornutins A and B belong to an extensive diterpene family,[9] including the corymbotins we recently reported

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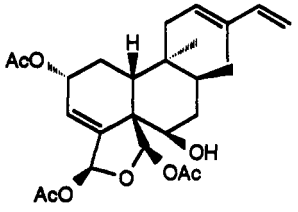
(8) Shimomura, H.; Sashida, Y.; Ogawa, K. *Chem. Pharm. Bull.* **1989**, *37*, 988.
(9) Hanson, J. R. *Nat. Prod. Rep.* **1988**, 211.

Table III. Ant-Repellency Bioassay Data[a]

	[mg/g][b] (flakes)	[mg/g][c] (leaves)	no. flakes taken: control	no. flakes taken: test	P
1	0.33	≥0.83	31	10	<0.001
2	0.33	≥1.44	30	13	<0.01
3	0.50	≥2.17	30	19	N.S.
4	0.50	≥0.72	30	27	N.S.

[a] All bioassays were conducted with *Acromyrmex octospinosus*. [b] Bioassay concentrations in terms of milligrams of compound added to grams of rye flakes. [c] Natural concentrations estimated from recovered natural products.

(e.g. 5),[4a] as well as a variety of natural insecticides and insect repellents such as the well-known ajugarins.[10] However, formation of a C ring by an ethereal linkage between C-1 and C-12 is without precedent in the clerodanes. Furthermore, as shown in Table III, both cornutin A and B demonstrate significant activity in ant-repellency bioassays[11] done with a captive colony of *A. octospinosus*, while compounds 3 and 4 show no significant activity in parallel bioassays at comparable concentrations. Because significant activity is apparent at concentrations below those we recovered from the plant, it is likely that the cornutins play a significant role in defense of this species against leafcutter attack. It is tempting to speculate that their activity springs from the unusual C ring. However, because we previously have shown that kolavanol (6), a minimally oxidized and simply bicyclic member of this diterpene family, is repellent to the related leafcutter *Atta cephalotes*,[12,13] further studies to identify the molecular features required for repellency would be attractive.



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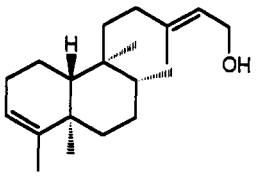

6

Experimental Section

The NMR spectra (^1H and ^{13}C) were recorded on $CDCl_3$ solutions with an internal TMS standard. Both low- and high-resolution EIMS were obtained at 70 eV. Melting points were measured with a Thomas-Hoover melting point apparatus and are uncorrected.

Plant Collection. *C. grandifolia* leaves were collected on the Rodman Naval Ammunition Supply Depot (ca. 10 km west of Panama City, Panama), air-dried at ambient temperature, and stored in plastic bags until extracted. Voucher specimens (J. J. Howard no. 162) have been deposited at the National Museum, Panama City, Panama, and at the Missouri Botanical Gardens, St. Louis, MO.

Isolation. Dried *C. grandifolia* leaves (120 g) were ground in a Waring blender and then extracted with $CHCl_3$ (2 L) using a Soxhlet extractor. After concentration of the $CHCl_3$ extract in vacuo, approximately 8 g of residue remained. This residue was subjected to dry column chromatography on silica gel, with a solvent gradient from 10% EtOAc/hexane to 100% EtOAc. Fractions were collected with every 10% increment in the EtOAc concentration.

Inspection of ^1H NMR spectra of the crude fractions suggested that the most interesting components were located in fractions eluting with 40 and 50% EtOAc/hexane. Because these fractions exhibited similar features on TLC, they were combined. After repeated flash column chromatography on silica gel with 1% HOAc in EtOAc/hexane solvent gradient (15–50% EtOAc), compounds 1–4 were obtained in nearly pure form. Final purification of each compound on C18 Sep-Pak cartridges, eluting with 85% MeOH in H_2O, provided pure compounds 1 (57 mg), 2 (85 mg), 3 (103 mg), and 4 (23 mg).

Cornutin A (1): oil; $[\alpha]^{25}_D = 0.40°$ ($c = 0.050$, $CDCl_3$); ^1H and ^{13}C NMR data, cf. Table I; EIMS m/z (relative intensity) 446 (8), 404 (1), 371 (3), 327 (5), 95 (100), 67 (15); HREIMS calcd $C_{24}H_{30}O_8$ 446.1940, found 446.1962.

Cornutin B (2): white crystals; mp 235–237 °C; $[\alpha]^{25}_D = 30.53°$ ($c = 0.075$, $CDCl_3$); ^1H and ^{13}C NMR data, cf. Table II; EIMS m/z (relative intensity) 404 (5), 389 (2), 360 (4), 344 (6), 259 (7), 43 (100); HREIMS calcd $C_{22}H_{28}O_7$ 404.1835, found 404.1840.

X-ray Diffraction Analysis of Cornutin B (2). X-ray diffraction intensity data were obtained from a white crystal (0.80 × 0.30 × 0.50 mm), using an Enraf-Nonius CAD4 diffractometer. Graphite monochromatized Cu radiation [λ(av) = 1.5418 Å] was used at 295 K. Data collection parameters: ω scan 1.0 + 0.14 tan (θ), background 25% below and above range; peak/background counting time 2/1; scan speed 1.65–4.1 deg/min depending on intensity; hemisphere collected from 2 to 75° 2θ. Measured reflections: total = 4538; net averaged = 4153, used in refinement ($>3\sigma$) = 3949, agreement among equivalent reflections (on F) = 1.9%. Intensities were corrected for absorption (by empirical method) max/min 1.00/0.97, but not for decay (<4%). The cell dimensions [$a = 11.7788$ (14), $b = 9.1063$ (13), and $c = 9.8861$ (12) Å; $V = 1014.8$ (4) Å^3] were obtained from 25 reflections between 70 and 80° 2θ. With an empirical formula of $C_{22}H_{28}O_7$ and a formula weight of 404.46, Z equals 2, the space group was $P21$, and D_x equals 1.324 g/cm^3.

The structure was solved using MULTAN and electron density difference maps. Full-matrix refinement was carried out anisotropically on all non-hydrogen atoms. Idealized positions were calculated for the hydrogen atoms and remained fixed during refinement. The total parameters were 261. The residual electron density was <0.404 e/Å^3. Refinement was continued until parameter shift/esd < 0.08. The SDOUW = 1.259. Final R values of $R_1 = 0.055$ and $R_2 = 0.079$ were obtained.

Compound 3: white solid; mp 267–270 °C dec; $[\alpha]^{25}_D = -9.14°$ ($c = 0.070$, $CDCl_3$); ^1H NMR, δ 0.79 (3 H, s, Me-20), 0.82 (3 H, d, $J = 6.4$ Hz, Me-17), 1.10 (1 H, dd, H-10), 1.19 (3 H, s, Me-19), 1.22–1.92 (9 H, m, CH_2), 1.95 (3 H, s, 6-OAc), 2.00 (1 H, m, CH_2), 2.12 (1 H, dd, $J = 12.7$, 3.2 Hz, H-4), 2.12 and 2.15 (2 H, m, H-12), 4.59 (1 H, dd, $J = 11.5$, 4.3 Hz, H-6), 4.75 (2 H, brd s, H-16) and 5.84 (1 H, brd s, H-14); ^{13}C NMR 180.59 (s), 173.88 (s), 170.53 (s), 170.24 (s), 115.22 (d), 80.31 (d), 73.04 (t), 55.27 (d), 48.40 (d), 42.05 (s), 38.53 (s), 34.97 (t), 34.44 (d), 31.81 (t), 25.68 (t), 25.62 (t), 21.96 (t), 20.95 (q), 20.43 (t), 17.97 (q), 15.39 (q), 10.34 (q); EIMS m/z (relative intensity) 374 ($M^+ - H_2O$, 0.1), 332 (3), 314 (11), 286 (10), 221 (70), 55 (100).

Compound 4: oil; $[\alpha]^{25}_D = -22.17°$ ($c = 0.023$, $CDCl_3$); ^1H NMR δ 0.76 (3 H, s, Me-20), 0.83 (3 H, d, $J = 6.1$ Hz, Me-17), 1.00 (1 H, dd, $J = 11.4$, 3.2 Hz, H-10), 1.12 (3 H, s, Me-19), 1.21–1.90 (12 H, m, CH_2), 2.11 (1 H, dd, $J = 12.2$, 3.4 Hz, H-4), 2.14 and 2.26 (2 H, m, H-12), 3.52 (1 H, dd, $J = 10.0$, 5.2 Hz, H-6), 4.75 (2 H, brd s, H-16) and 5.84 (1 H, brd s, H-14); ^{13}C NMR 180.82 (s), 173.84 (s), 170.30 (s), 115.27 (d), 78.01 (d), 73.01 (t), 55.69 (d), 48.44 (d), 44.25 (s), 38.45 (s), 36.02 (t), 35.05 (d), 34.99 (t), 25.86 (t), 25.37 (t), 22.05 (t), 20.66 (t), 17.91 (q), 15.63 (q), 9.41 (q); EIMS m/z (relative intensity) 332 ($M^+ - H_2O$, 0.5), 314 (5), 286 (3), 221 (40), 175 (100), 55 (59).

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Supplementary Material Available: ^1H and ^{13}C NMR spectra for compounds 1 and 2 and crystal data for compound 2 (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Identification of 12 Hepatotoxins from a Homer Lake Bloom of the Cyanobacteria *Microcystis aeruginosa*, *Microcystis viridis*, and *Microcystis wesenbergii*: Nine New Microcystins

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Eleven minor components were isolated, together with microcystin-LR (LR, 1, Scheme I) as the principal toxin (ca. 90% of the toxic components), from *Microcystis* cyanobacteria (blue-green algae) collected from Homer Lake (Illinois) in the summer of 1988. The components were characterized by amino acid analysis and HRFABMS, FABMS/MS, ^1H NMR, and UV spectroscopic methods as microcystins-RR (2) and -YR (3) (Scheme I) and nine new microcystins. The structures of seven new microcystins were assigned as [DMAdda^5]microcystin-LR (4), [Dha^7]microcystin-LR (5), microcystin-FR (6), microcystin-AR (7), microcystin-M(O)R (8), [Mser^7]microcystin-LR (9), and microcystin-WR (12). Compound 4 is the first microcystin containing 9-*O*-demethyl-Adda, while phenylalanine, *N*-methylserine, and tryptophan are also new variations in amino acid components of microcystins. Compound 11 was deduced to be a (C_3H_7O) monoester of the α-carboxyl on the Glu unit of LR (1). New microcystin 11 caused no apparent toxic effects in mice dosed ip at 1 mg/kg, while the others had LD_{50}'s of 90–800 μg/kg.

The microcystins[1] are well-known cyclic heptapeptide heptatoxins obtained from cyanobacteria (blue-green algae), which grow worldwide in fresh and brackish waters and cause animal and human water-based toxicosis.[2,3] Nine chemically defined microcystins (1–3 and 13–18, **Scheme** I) have been isolated from the genera *Microcystis*,[4-12] *Anabaena*,[7,8] and *Oscillatoria*.[3,8,13] *Microcystis* is the most common producer of these hepatotoxins, and microcystin-LR (LR, 1, Scheme I) occurs most often.[2,3] The structures of the microcystins differ primarily in the variations in the two L-amino acids at positions 2 and 4 and secondarily in the absence of the methyl groups on D-*erythro*-β-methylaspartic acid (D-MeAsp) and/or *N*-methyldehydroalanine (Mdha) (Scheme I).[2] Nodularin[14] (19, Scheme I), isolated from *Nodularia spumigena*, is thus far the only related cyclic pentapeptide, and it possesses similar hepatotoxicity.[15] Hepatotoxic *Aphanizomenon* and *Gomphosphaeria* species have also been reported.[16] The recently reported[17] inhibition of protein phosphatases 1 and 2A by these toxins makes them important biological tools.

The most unusual feature of nodularin and microcystins is the C_{20} amino acid, (2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda),[14] which plays an important role in their toxicity. Hydrogenation or ozonolysis of the diene system in the Adda unit gives an inactive product,[18] and the stereoisomer at the Δ^6

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