(dd, J = 7.1 and 4.0 Hz, H), 3.56 (m, 3 H), (D_2O/NaOD, room temperature δ 7.86 (s, H), 5.7 (d, J = 6.5 Hz, H), 4.68 (m, H), 4.20 (m, 2 H), 3.79 (dd, J = 12.6 and 3.4 Hz, H), 3.89 (dd, J = 12.6 and 2.9 Hz, H); ^{13}C NMR (DMSO- d_6, room temperature) δ 156.85 (s, +, C-6), 153.72 (s, +, C-2), 151.39 (s, +, C-4), 135.68 (d, J = 214.5 Hz, C-8), 116.73 (s, +, C-5), 86.4 (d, –, J = 164.4 Hz, C-1′), 85.27 (d, – J = 148.3 Hz, C-2′), 73.77 (d, –, J = 148 Hz, C-3′), 70.45 (d, –, J = 150.4 Hz, C-4′), 60.47 (t, +, J = 141 Hz, C-5′). Anal. Calcd for $C_{10}H_{13}N_5O_5 \cdot 2H_2O$: C, 37.62; H, 5.37; N, 21.93. Found: C, 37.61; H, 4.92; N, 21.53.

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Scytonemin A, a Novel Calcium Antagonist from a Blue-Green Alga

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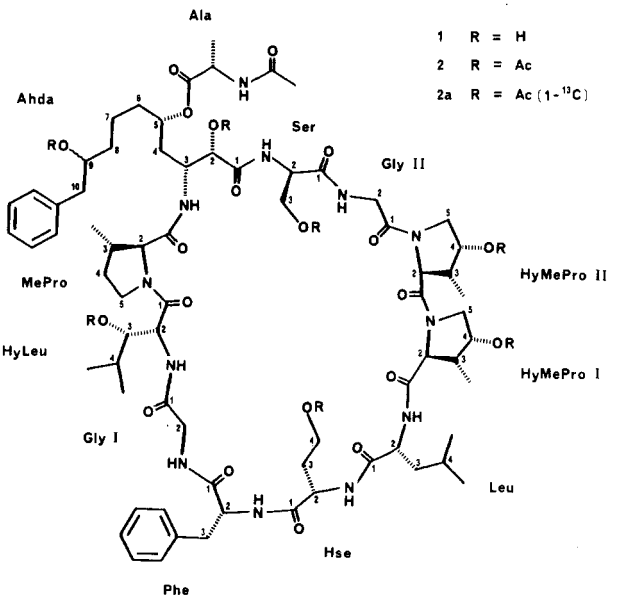
A novel cyclic peptide, scytonemin A, possessing potent calcium antagonistic properties is a major metabolite of the cultured cyanophyte *Scytonema* sp. (strain U-3-3). Vigorous acid hydrolysis of scytonemin A leads to L-alanine, 2 equiv of glycine, L-homoserine (Hse), D-(2*R*,3*S*)-*threo*-3-hydroxyleucine (HyLeu), D-leucine, D-serine, L-(2*S*,3*S*)-*trans*-3-methylproline (MePro), 2 equiv of L-(2*S*,3*R*,4*R*)-4-hydroxy-3-methylproline (HyMePro), D-phenylalanine, and (2*S*,3*R*,5*S*)-3-amino-2,5,9-trihydroxy-10-phenyldecanoic acid (Ahda). Mild acid hydrolysis results in predominantly two acyclic peptides, viz. Ser-Gly-HyMePro-HyMePro-Leu-Hse and Phe-Gly-HyLeu-MePro-Ahda. Still milder hydrolysis results in selective cleavage of the homoseryl amide bond in scytonemin A to give an acyclic peptide, Phe-Gly-HyLeu-MePro-Ahda-Ser-Gly-HyMePro-HyMePro-Leu-Hse, with an *N*-acetylalanyl unit attached via an ester linkage to C-5 of Ahda and a homoseryl lactone unit at the carboxyl terminus. State-of-the-art NMR and MS techniques have been used to determine the total structures of scytonemin A and the degradation products.

The blue-green algae have until recently been largely overlooked as a source of new pharmaceuticals and agrochemicals. Malyngolide,[1] majusculamide C,[2] cyanobacterin,[3] hapalindole A,[4] and scytophycins A and B[5] are examples of bioactive agents from this ubiquitous group of prokaryotic organisms which have already been described. We report here the isolation and structure elucidation of an unusual cyclic peptide, scytonemin A (1), from a *Scytonema* sp. (strain U-3-3) (Scytonemataceae)[6] which possesses potent calcium antagonistic properties.[7]



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(6) U-3-3 was originally identified as a *Plectonema* sp. (Geitler, L. In *Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz*; Rabenhorst, L., Ed.; Koeltz Scientific Books: Wiesbaden, 1985 (reprint); Vol. 14.] since heterocysts could not be detected in the original algal isolate or any subculture grown under the conditions described in ref 4b. When U-3-3 was grown on ^{15}N-enriched nitrate, however, heterocysts developed in the cultured alga which meant the cyanophyte was a *Scytonema* sp. Interestingly heterocysts disappeared when the heterocystous U-3-3 was regrown on ^{14}N-nitrate.

(7) On atria calcium antagonistic effects were observed at 5 μg/mL but not at 2.5 μg/mL; by comparison diltiazem was active at 2.5 μg/mL. On rat portal vein calcium blocking was observed at 20 μg/mL but not at 10 μg/mL; diltiazem showed activity at 0.5 μg/mL. Scytonemin A showed weak activity against a wide spectrum of bacteria and fungi; for example, activity was observed against *Mycobacterium ranae* at 1 μg/mL (MIC) but not at 0.5 μg/mL (MIC). By comparison gentamycin showed activity against *M. ranae* at 0.5 μg/mL (MIC). Weak antiprotozoal activity was noted against *Trichomonas vaginalis* and *Tritrichomonas foetus* at 1.56 and 3.12 μg/mL, respectively; metronidazole showed activity at 0.78 μg/mL. Some activity was observed against coccidia (*Eimeria tenella*) at 2.5 μg/mL but not at 1.25 μg/mL. Scytonemin A was mildly cytotoxic (IC50 = 2.9 μg/mL against CCRF-CEM).

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Results and Discussion

The *Scytonema* sp. (U-3-3) was isolated from a soil sample collected on Majuro Atoll in the Marshall Islands. A pure clonal strain was selected by repeated subculture on solid media and grown in mass culture in the laboratory.[4b] The freeze-dried cells were extracted with 70% aqueous ethanol and the resulting extract was desalted and chromatographed on Amberlite XAD-2 resin. The fractions that exhibited antibiotic activity were pooled and subjected to reverse-phase HPLC on ODS-2 (75% MeOH/H_2O) to give, after lyophilization, scytonemin A as a white amorphous powder in 0.45% yield.

Gross Structure. The positive ion and negative ion fast atom bombardment (FAB) mass spectra showed $(M + H)^+$ and $(M - H)^-$ peaks at m/z 1463 and 1461, respectively, indicating a molecular weight of 1462 for scytonemin A.

In the ^{13}C NMR spectrum (DMSO-d_6), a total of 13 carbonyl signals could be seen between 172.3 and 166.7 ppm. By using the refocused INEPT[8] pulse sequence, 31 methine, 16 methylene, and 9 methyl signals were identified. These signals, along with two additional quaternary carbon signals at 138.1 and 139.9 ppm, indicated that a total of 71 carbons were present in scytonemin A.

The FT-IR spectrum in $CHCl_3$ showed an intense amide carbonyl band at 1657 cm^{-1} and an ester carbonyl band at 1738 cm^{-1}, and the UV spectrum in MeOH gave a λ_{max} nm (ϵ) at 210 (52700) along with less intense bands at 253, 259 (1000), and 263, typical for a phenyl chromophore. From these data, scytonemin A appeared to be a phenylalanine-containing polypeptide. Since the compound gave a negative response to ninhydrin, however, scytonemin A was either a cyclic peptide or an acyclic peptide with a protected N-terminus.

The peptidal nature was further supported by the ^1H NMR spectrum in DMSO-d_6 (Table I), which showed the presence of nine amide NH signals between 7.6 and 8.5 ppm and a complex set of multiplets between 5.4 and 2.8 ppm. The spectrum also showed multiplets in the aromatic region for two phenyl groups, doublets between 1.26 and 0.82 ppm for eight aliphatic methyl groups, and a singlet at 1.82 ppm for an acetate group.

The number of nitrogens and oxygens was determined by a combination of chemical degradation (acid hydrolysis), derivatization (acetylation), and 2D NMR experiments. Since scytonemin A possessed nine secondary amide groups and produced two different proline compounds on acid hydrolysis, at least eleven nitrogens had to be present. The 2D-COSY spectrum of the intact peptide (DMSO-d_6), however, indicated the presence of a third substituted proline unit, bringing the total number of nitrogens in the molecule to 12. Since 12 of the 13 carbonyls were amide carbonyls, the 13th one had to be an ester functionality to account for the IR band at 1738 cm^{-1}.

One of the carbonyls was in an acetyl group. Acetylation with Ac_2O in pyridine overnight resulted in **2**, the NMR spectrum of which (Table II) depicted the presence of seven additional acetate methyl signals. This meant that scytonemin A possessed 7 hydroxyl groups and that a total of 21 oxygens were present in the molecule. The total number of hydrogens was, therefore, 106, i.e. the sum of the protons found in 31 methine, 16 methylene, 9 methyl, 9 secondary amide, and 7 hydroxyl groups.

These data, coupled with the molecular weight of 1462 determined by FAB mass spectrometry, indicated the

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Table I. Correlated ^{13}C and ^1H NMR Spectral Data for Scytonemin A in DMSO-d_6

amino acid unit	assgnmnt	$^{13}C^{a,b}$ (mult)	$^1H^c$
Ser	C2	54.91 (d)	4.25
	C3	61.41 (t)	3.69, 3.57
	OH		4.81
	NH		7.76
Ahda	C2	72.87 (d)	3.94
	C3	50.74 (d)	4.24
	C4	36.45 (t)	1.44, 1.41
	C5	71.03 (d)	4.73
	C6	34.24 (t)	1.80, 1.72
	C7	20.62 (t)	1.42
	C8	39.79 (t)	1.33, 1.25
	C9	70.86 (d)	3.60
	C10	43.64 (t)	2.61, 2.59
	C1′	139.74 (s)[d]	
	C2′,6′	128.09 (d)[e]	7.1–7.3
	C3′,5′	128.98 (d)[f]	7.1–7.3
	C4′	125.65 (d)[g]	
	C2-OH		5.34
	C9-OH		4.46
	NH		8.40
Ala	C2	47.82 (d)	4.12
	C3	17.02 (q)	1.23
	NH		8.18
acetate	CH_3	22.21 (q)	1.82
MePro	C2	62.24 (d)	4.07
	C3	42.28 (d)	2.05
	C4	32.05 (t)	2.05, 1.50
	C5	43.64 (t)	3.69, 3.50
	CH_3	18.28 (q)	1.02
HyLeu	C2	52.32 (d)	4.72
	C3	75.58 (d)	3.39
	C4	30.13 (d)	1.51
	CH_3	19.55 (q)	0.88
	CH_3	18.44 (q)	0.87
	OH		4.06
	NH		7.68
Gly I	C2	46.24 (t)	3.85, 3.78
	NH		8.06
Phe	C2	54.14 (d)	4.45
	C3	37.06 (t)	3.06, 2.83
	C1′	137.90 (s)[d]	
	C2′,6′	127.94 (d)[e]	7.1–7.3
	C3′,5′	129.33 (d)[f]	7.1–7.3
	C4′	126.20 (d)[g]	7.1–7.3
	NH		7.68
HSer	C2	50.49 (d)	4.21
	C3	34.93 (t)	1.78, 1.73
	C4	57.61 (t)	3.27
	OH		4.37
	NH		7.89
Leu	C2	48.18 (d)	3.92
	C3	41.08 (t)	1.70
	C4	24.40 (d)	1.56
	CH_3	20.62 (q)	0.79
	CH_3	23.42 (q)	0.88
	NH		7.82
HyMePro I	C2	67.02 (d)	3.79
	C3	36.56 (d)	2.16
	C4	71.33 (d)	4.07
	C5	56.31 (t)	3.56, 3.30
	CH_3	11.36 (q)	1.01
	OH		5.12
HyMePro II	C2	65.57 (d)	3.92
	C3	41.71 (d)	2.14
	C4	71.17 (d)	4.14
	C5	53.95 (t)	3.63, 3.59
	CH_3	10.52 (q)	0.99
	OH		5.20
Gly II	C2	42.28 (t)	3.81, 3.70
	NH		7.76

[a] Chemical shifts of carbonyl carbons: δ 172.46, 172.27, 172.18 (2), 171.55, 171.39, 171.10, 170.33, 170.09, 169.39, 169.18, 168.65, 166.78. [b] DMSO-d_6 as internal standard (δ 39.50). [c] Residual DMSO-d_5 as internal standard (δ 2.49). [d-g] Values may be interchanged.

Table II. Correlated ^{13}C and ^{1}H NMR Spectral Data for 2 in $CDCl_3$

amino acid unit	assgnmnt	$^{13}C^{a-c}$ (mult)	$^{1}H^{d}$ (mult; J, Hz)	amino acid unit	assgnmnt	$^{13}C^{a-c}$ (mult)	$^{1}H^{d}$ (mult; J, Hz)
Ser	C2	52.79 (d)	4.817 (m)	Phe	C2	52.94 (d)	4.742 (dt; 7.1, 4.3)
	C3	63.92 (t)	4.508 (dd; −11.5, 7.4)		C3	37.05 (t)	3.016 (br dd; −14.5, 4.3)
			4.384 (dd; −11.5, 5.1)				2.941 (dd; −14.5, 7.2)
	NH		7.826 (br d; 7.2)		C1′	137.22 (s)[f]	
Ahda	C2	79.32 (d)	4.914 (d; 3.2)		C2′,6′	128.19 (d)[g]	7.13 (m)
	C3	46.30 (d)	4.334 (m)		C3′,5′	129.19 (d)[h]	7.26 (m)
	C4	33.88 (t)	1.937 (dt; −12.2, 2.9)		C4′	126.35 (d)[h]	7.19 (m)
	C5	71.32 (d)	4.862 (m)		NH		6.795 (br d; 7.0)
	C6	34.69 (t)[e]	1.58 (m)	HSer	C2	50.55 (d)	4.513 (q; 7.5)
	C7	29.51 (t)	1.26 (m)		C3	29.94 (t)	2.196 (dq; −14.3, 7)
	C8	33.08 (t)[e]	1.53 (m)				1.731 (dt; −14.3, 7)
	C9	78.34 (d)	5.014 (p)		C4	61.22 (t)	4.04 (m)
	C10	40.45 (t)	2.830 (dd; −13.9, 7.3)				3.94 (m)
			2.748 (dd; −13.9, 6.3)		NH		7.712 (br d; 7.5)
	C1′	136.66 (s)[f]		Leu	C2	50.55 (d)	4.596 (dt; 7.5, 1.5)
	C2′,6′	128.33 (d)[g]	7.14 (m)		C3	41.27 (t)	1.990 (m)
	C3′,5′	129.19 (d)[h]	7.26 (m)				1.395 (m)
	C4′	126.46 (d)[h]	7.20 (m)		C4	24.75 (d)	1.640 (m)
			6.866 (br d; 9.7)		CH_3	23.37 (q)	0.852 (d; 6.5)
Ala	C2	48.23 (d)	4.514 (p; 7.3)		CH_3	21.02 (q)	0.944 (d; 6.5)
	C3	17.84 (q)	1.368 (d; 7.3)		NH		7.282 (br d; 7.5)
	NH		6.130 (d; 7.3)	HyMePro I	C2	66.49 (d)	3.800 (d; 3.9)
N-acetate	CH_3	22.91 (q)	1.978 (s)		C3	40.37 (d)	2.552 (m)
MePro	C2	67.28 (d)	3.865 (d; 8.8)		C4	74.79 (d)	5.265 (br t; 4)
	C3	35.23 (d)	2.552 (m)		C5	54.00 (t)	3.833 (dd; −11.8, 4)
	C4	32.16 (t)	2.552 (m)				3.675 (br d; −11.8)
			1.650 (m)		CH_3	10.25 (q)	1.033 (d; 6.8)
	C5	46.09 (t)	3.741 (m)	HyMePro II	C2	62.53 (d)	4.088 (d; 8.8)
			3.673 (m)		C3	38.49 (d)	2.386 (m)
	CH_3	18.16 (q)	1.117 (d; 7.0)		C4	74.79 (d)	5.225 (br t; 4.1)
HyLeu	C2	51.09 (d)	5.028 (dd; 8.2, 4.0)		C5	52.34 (t)	3.902 (dd; −11.7, 4.1)
	C3	74.14 (d)	4.990 (dd; 7, 4.0)				3.480 (br d; −11.7)
	C4	28.99 (d)	1.821 (oct; 7)		CH_3	11.23 (q)	1.093 (d; 7.2)
	CH_3	19.18 (q)	0.882 (d; 7)	Gly II	C2	43.56 (t)	3.939 (dd; −16.5, 7.1)
	CH_3	17.45 (q)	1.000 (d; 7)				3.703 (dd; −16.5, 8.0)
	NH		7.564 (br d; 8.2)		NH		7.342 (br t)
Gly I	C2	41.81 (t)	4.000 (dd; −16.6, 8.1)				
			3.917 (dd; −16.6, 8.1)				
	NH		7.440 (br t; 8.1)				

[a] Chemical shifts of acetates introduced by acetylation: ^{13}C carbonyls[i] δ 171.08, 170.69 (2), 170.51 (2), 170.26, 169.93; ^{13}C methyls δ 21.02, 20.92, 20.75 (3), 20.60, 20.51; ^{1}H methyls δ 2.151, 2.106, 2.060, 2.025, 2.000, 1.968, 1.962. [b] Carbon-13 chemical shifts of carbonyls belonging to scytonemin A: δ 172.17, 171.96 (2), 171.47, 170.51, 170.42, 170.10, 169.56 (2), 168.62, 168.46, 167.88, 166.90. [c] $CDCl_3$ solvent peak used as internal reference δ 77.00. [d] Residual $CHCl_3$ used as internal reference δ 7.240. [e,f] Chemical shifts may be interchanged. [g] Proton chemical shift assignments are based on a long-range COSY experiment which shows cross peaks between the C2′,6′ protons and the C3 protons of Phe or the C10 protons of Ahda. [h] Chemical shifts may be interchanged. [i] Values obtained from the ^{13}C spectrum of **2a**.

molecular composition $C_{71}H_{106}N_{12}O_{21}$ [calcd 1463.7674; obsd 1463.7699 for $(M + H)^+$].

Acid hydrolysis of the intact peptide with 6 N HCl gave several ninhydrin-positive components, all amino acids, which were isolated by repeated reverse-phase HPLC on ODS-3 (0.1% TFA/H_2O). Serine (Ser), alanine (Ala), glycine (Gly), leucine (Leu), and phenylalanine (Phe) were identified by comparison of their ^{1}H NMR spectra in 1 N DCl in D_2O and R_f values on silica gel TLC (4:1:2 *n*-BuOH/HOAc/H_2O) with those of authentic samples. Homoserine (Hse), isolated as the lactone, was identified by spectral comparison (^{1}H NMR and CD) with synthetic homoserine lactone. *threo*-3-Hydroxyleucine (HyLeu) was identified by comparison of the amino acid's R_f value on paper chromatography and the ^{1}H and ^{13}C NMR data with published data.[9]

A quantitative amino acid analysis of the 6 N HCl hydrolyzate gave the following molar proportions (in order of elution): Ser (1), Hse/Hse lactone (1), HyLeu (1), Gly (2), Ala (1), Leu (1), and Phe (1). Scytonemin A, therefore, possessed two glycyl units and only one of each of the other amino acid residues above.

The remaining compounds in the 6 N HCl acid hydrolyzate gave faint yellow responses to ninhydrin. Repeated reverse-phase HPLC resulted in the isolation of 3-methylproline (MePro) and 4-hydroxy-3-methylproline (HyMePro) in a 1:2 ratio, respectively, suggesting the presence of one MePro and two HyMePro units in scytonemin A. This was corroborated by 2D-COSY spectroscopy. 4-Hydroxy-3-methylproline appeared to be a new amino acid,[10] whereas 3-methylproline had been previously reported to be a constituent of the antibiotic bottromycin[11] and the mycotoxin roseotoxin.[12]

The structures of the two prolines were determined in

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(10) An isomeric amino acid, (2*S*,3*S*,4*S*)-3-hydroxy-4-methylproline, has been isolated from the echinocandins: (a) Benz, F.; Knusel, F.; Nuesch, J.; Treichler, H.; Voser, W.; Nyfeler, R.; Keller-Schierlein, W. *Helv. Chim. Acta* **1974**, *57*, 2459. (b) Keller-Juslen, C.; Kuhn, M.; Loosli, H. R.; Petcher, T. J.; Weber, H. P.; vonWartburg, A. *Tetrahedron Lett.* **1976**, 4147. (c) Koyama, G. *Helv. Chim. Acta* **1974**, *57*, 2477. (d) Kurokawa, N.; Ohfune, Y. *J. Am. Chem. Soc.* **1986**, *108*, 6041.

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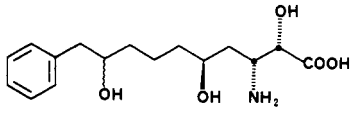
(12) Engstrom, G. W.; DeLance, J. V.; Richard, J. L.; Baetz, A. L. *J. Agric. Food Chem.* **1975**, *23*, 244.

^{1}H NMR studies. The chemical shifts and coupling constants for MePro agreed well with those reported by Mauger et al.[13] for *trans*-3-methylproline and the ones for HyMePro were in good agreement with some of the values observed for commercial *trans*-4-hydroxy-L-proline. NOE experiments confirmed that MePro was *trans*-3-methylproline, since irradiation of the methyl protons produced a positive NOE (3.6%) for the H-2 signal and irradiation of H-2 produced a positive NOE (6.5%) in the Me signal. Identical NOEs were seen for HyMePro when the C-2 and methyl protons were irradiated, respectively. Moreover, irradiation of H-4 of HyMePro showed a positive NOE (6.7%) to H-3, indicating that the substituents on C-3 and C-4 were cis to each other. Finally the relative stereochemistries of the MePro and HyMePro residues in the intact peptide (1) were concluded to be the same as in the free amino acids, since irradiation of the C-3 methyl group in each of these units produced a negative NOE in the H-2 signal; however, no NOE was seen in the H-4 signal for each of the two HyMePro units.

The most unusual component in the acid hydrolyzate of scytonemin A was 3-amino-2,5,9-trihydroxy-10-phenyldecanoic acid (Ahda) (**3**).[14] This novel β-amino acid



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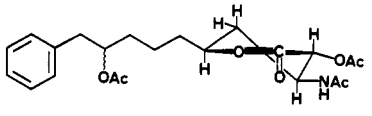
was isolated by acid hydrolysis with refluxing 1.5 N HCl for 21 h followed by gel filtration of the hydrolyzate on Sephadex G-10. The positive ion FAB mass spectrum gave a $(M + H)^+$ ion at m/z 312, which was consistent with the molecular formula $C_{16}H_{25}NO_5$.

Acetylation of **3** gave, after workup and HPLC, lactone **4** in about 62% yield. The derivative was clearly a tri-



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acetate according to the ^{1}H NMR spectrum (3-H singlets at 1.95, 1.98, and 2.17 ppm) and EI mass spectrum (fragment ions at m/z 359, 300, and 240 for successive losses of HOAc, acetamide, and HOAc). The structure of **4** was determined primarily by ^{1}H–^{1}H decoupling studies. The H-2 signal was a sharp doublet at 5.09 ppm. Large coupling (11.2 Hz) between H-2 and H-3 indicated that the substituents on C-2 and C-3 were oriented pseudoequatorially. The proton on C-3 was coupled (7.4 Hz) to the amide proton at 5.58 ppm and also to two protons on C-4, i.e. to H_{ax}-4 (1.65 ppm) by 12.4 Hz and to H_{eq}-4 (2.35 ppm) by 4.0 Hz. H_{ax}-4 and H_{eq}-4 were coupled in turn to H-5 (4.41 ppm) by 12.4 and 4.0 Hz, respectively, indicating that C-6 was attached pseudoequatorially to C-5. The signals for the two nonequivalent protons on C-10 were doublets of doublets (2.77 and 2.85 ppm), showing 6.0 and 6.5 Hz coupling, respectively, to H-9 (5.03 ppm). The ^{1}H NMR analysis described above indicated that Ahda had the relative stereochemistry as shown in **3**.

Having determined the gross structures of all of the amino acid residues in the molecule, studies were now carried out to sequence the various units into a total structure.

Difference NOE studies of the intact peptide in DMSO-d_6 led to partial structures Ac-Ala, Phe-Gly(I) and HyMePro(I)-Leu. Irradiation of the Ala amide proton at 8.18 ppm (Figure 1a) gave a 2% negative NOE in the acetate methyl signal at 1.82 ppm. This indicated that the acetate group in scytonemin A was attached to the Ala nitrogen. Ala, therefore, had to be either the N-terminus of a cyclic depsipeptide or the N-terminus of a side chain connected to a cyclic peptide through an ester linkage. Irradiation of the Gly(I) amide proton at 8.06 ppm (Figure 1b) resulted in a 6% negative NOE in the signal for the α proton of Phe (4.45 ppm), indicating that the carbonyl of Phe was attached to the nitrogen of Gly(I). Similarly, irradiation of the Leu amide proton at 7.82 ppm (Figure 1c) induced a 6% negative NOE in the signal for the α proton of HyMePro(I) (3.79 ppm), establishing that the carbonyl of HyMePro(I) was connected to the nitrogen of Leu.

The amide proton signals of Ser and Gly(II) in **1** overlapped at 7.76 ppm. Irradiation of these two protons resulted in negative NOEs in the signals for the α proton of Ser and the C-2 proton of Ahda. Subsequent irradiation of the C-2 proton of Ahda, which overlapped at 3.92 ppm with the α proton of HyMePro (II), resulted in negative enhancements of the signals for the Ahda amide proton (8.40 ppm), the methyl group in HyMePro (II) (0.99 ppm), and the Ser amide proton (doublet at 7.76 ppm, J = 7.2 Hz). The carbonyl of Ahda was, therefore, attached to the nitrogen of Ser. This was corroborated by irradiation of the Ser amide proton of derivative **2** in $CDCl_3$ (7.83 ppm), which produced a positive NOE in the C-2 proton signal of Ahda (4.91 ppm). Irradiation of the Ahda amide proton (6.87 ppm) in **2** induced a positive NOE in the signal for the α proton of MePro (3.87 ppm). These data suggested that the partial sequence MePro-Ahda-Ser was present in **1**.

The signals for the amide protons of HyLeu and Phe in **1** overlapped at 7.68 ppm. Irradiation of these signals resulted in negative NOEs in the signals for the α proton of Hse and both methylene protons of Gly(I). Since the sequence Phe-Gly(I) had already been established, the NOEs suggested that the amide proton of HyLeu was responsible for the effect seen in the signals for the methylene protons of Gly(I) and that the amide proton of Phe had given rise to the enhancement seen in the signal for the α proton of Hse. This meant that the sequence Hse-Phe-Gly(I)-HyLeu was present in scytonemin A.

Since NOEs were not observed on the amino sides of any of the proline residues when the proline α protons were irradiated or on the amino side of Hse when the Hse amide proton (7.89 ppm) was irradiated, none of the partial structures could be expanded further by NOE methods.

Attempts to sequence the amino acids in the intact molecule by using FAB mass spectrometry and tandem mass spectrometry were unsuccessful. The cleavage of the side chain preempted any informative fragmentation about the cyclic portion. The positive ion FAB mass spectrum of scytonemin A showed an abundant fragment ion at m/z 1332, which resulted from loss of *N*-acetylalanine from the $(M + H)^+$ ion (m/z 1463), and this suggested that the *N*-acetylalanyl unit was attached to one of eight hydroxyl groups. The collisional activated decomposition (CAD) spectra[15] of both the ions of m/z 1463 and 1332 were determined in the tandem mass spectrometry (MS/MS) mode, but little fragmentation information was obtained

(13) Mauger, A. B.; Irreverre, F.; Witkop, B. *J. Am. Chem. Soc.* **1966**, *88*, 2019.

(14) Interestingly **3** appears to be biogenetically related to 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, a novel amino acid component of cyanogenosin-LA, a potent hepatotoxin isolated from South African *Microcystis aeruginosa*. Botes, D. P.; Tuinman, A. A.; Wessels, P. L.; Viljoen, C. C.; Kruger, H.; Williams, D. H.; Santikarn, S.; Smith, R. J.; Hammond, S. J. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2311.

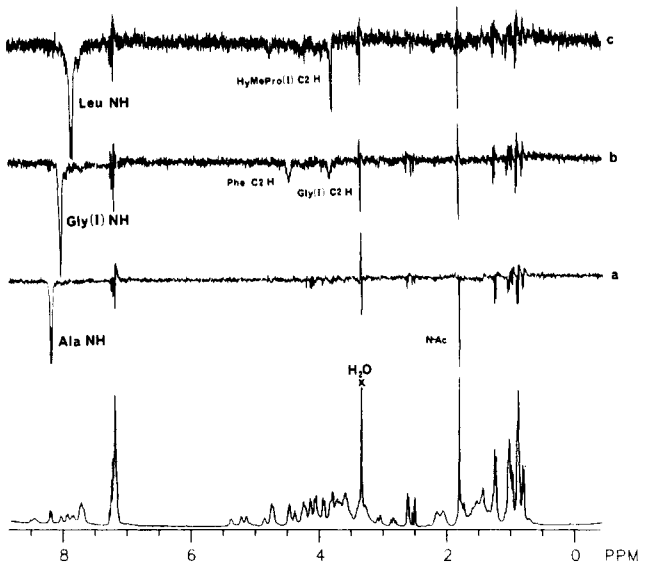


Figure 1. ^{1}H NMR spectrum of scytonemin A in DMSO-d_6 with NOEs resulting from (a) irradiation of the alanine amide proton to give a 2% negative enhancement of the acetate methyl signal, (b) irradiation of the glycine(I) amide proton to give negative enhancements of the signals for the C2 proton of phenylalanine (6%) and the C2 proton (3.80 ppm) of glycine(I) (5%), (c) irradiation of the leucine amide proton to give a 6% negative enhancement of the signal for the C2 proton of 4-hydroxy-3-methylproline(I).

except for conformation of the loss of 131 amu from the ion at m/z 1463.

Scytonemin A was next hydrolyzed to obtain large acyclic peptides which were analyzed by mass spectrometry. In one experiment, hydrolysis with 0.6 N HCl at 65 °C yielded two major fragments, viz. Ser-Gly-HyMePro-HyMePro-Leu-Hse and Phe-Gly-HyLeu-MePro-Ahda, which were separated by gel filtration.

The crude, faster moving hexapeptide was acetylated with pyridine/Ac_2O and esterification was attempted with CH_2N_2. The acetylated product was then purified by reverse-phase HPLC to yield **5**. Degradation product **5** was shown by ^{1}H NMR analysis ($CDCl_3$) to contain Hse, Leu, both HyMePro residues, Gly, and Ser. The NMR spectrum, however, exhibited only four acetate methyl signals and lacked a methyl ester signal. These data, coupled with a 0.30 ppm downfield shift in the signal for the C4 protons of Hse, suggested that Hse was at the carboxyl terminus and had lactonized. Although somewhat obscured by a broad ester carbonyl band centered at 1738 cm^{-1}, the FT-IR spectrum showed a small inflection at 1780 cm^{-1} for the γ-lactone carbonyl.

Irradiation of the Ser amide proton of **5** in $CDCl_3$ resulted in a positive NOE in one of the acetate methyl signals (1.82 ppm), thereby placing Ser at the N-terminus. Of the remaining amide protons, only irradiation of the one for Leu resulted in a NOE and this was to one of the HyMePro C2 protons.

The positive ion FAB spectrum of **5** showed a $(M + H)^+$ peak at m/z 781, which was consistent with the proposed molecular formula $C_{35}H_{52}N_6O_{14}$. The $(M + H)^+$ ion underwent collision-activated decomposition[15] to give the spectrum shown in Figure 2a. The CAD spectrum not only established the sequence of the amino acids in **5** but

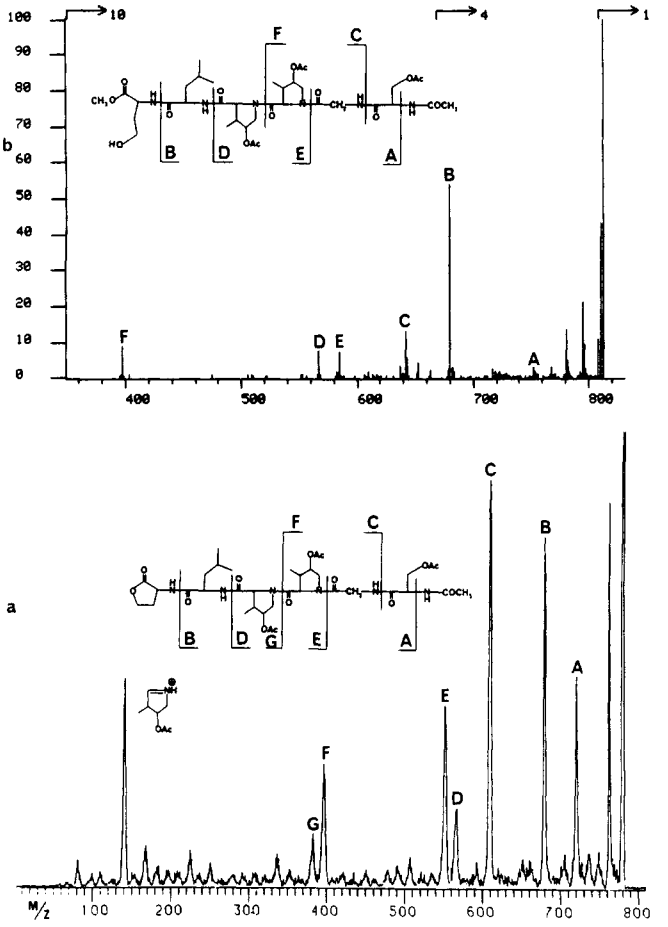
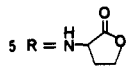
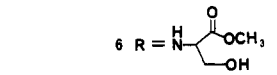


Figure 2. (a) Collision activated decomposition (CAD) mass spectrum of the $(M + H)^+$ ion (m/z 781) of compound **5** showing fragmentations from the acetylated amino terminus [ions A (m/z 721), C (610), E (553), G (384)] and lactonized carboxyl terminus [ions B (m/z 680), D (567), F (398)]. (b) Linked scan mass spectrum of $(M + H)^+$ ion (m/z 813) of compound **6** showing 31–32 amu increase in mass (due to addition of methanol to the carboxyl terminus) of fragment ions resulting from losses from the amino terminus. Ions A, C, and E are found at m/z 752, 642, and 585, respectively, whereas ions B, D, and F are found at the same positions, viz. m/z 680, 567, and 398, respectively.

added proof of the homoseryl lactone at the carboxyl terminus. Upon standing in methanol, **5** underwent methanolysis to give an equilibrium mixture of **5** and **6**.

AcHN-Ser-Gly-HyMePro(OAc)-HyMePro(OAc)-Leu-R

5 R = 

6 R = 

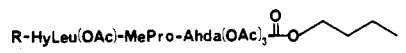
Compound **6** exhibited a $(M + H)^+$ ion at m/z 813 which gave the linked scan spectrum[16] shown in Figure 2b. The fragments shown in the CAD spectra were only consistent with the sequence Ser-Gly-HyMePro-HyMePro-Leu-Hse.

The crude, slower moving pentapeptide from gel filtration of the 0.6 N HCl hydrolyzate was rechromatographed on a small cellulose column by using 4:2:1 *n*-BuOH/H_2O/HOAc as the eluant. An appreciable amount of a tripeptide accompanied the pentapeptide. Acetylation of all of the fractions containing Ahda and Phe followed by attempted esterification with diazomethane and HPLC resulted in the isolation of **7**. ^{1}H NMR analysis showed that **7** was a butyl ester, not a methyl ester as expected from diazomethane treatment. Apparently the butyl ester

(15) (a) Eckart, K.; Schwarz, H.; Tomer, K. B.; Gross, M. L. *J. Am. Chem. Soc.* **1985**, *107*, 6765. (b) Tomer, K. B.; Grow, F. W.; Gross, M. L.; Kopple, K. D. *Anal. Chem.* **1984**, *56*, 880. (c) Lippstreu-Fischer, D. L.; Gross, M. L. *Anal. Chem.* **1985**, *57*, 1174.

(16) Jennings, K. R.; Mason, R. S. In *Tandem Mass Spectrometry*; McLafferty, F. W.; Ed.; John Wiley: New York, 1983; pp 197–222.

had been formed during the cellulose chromatography of the hydrolyzate. The NMR spectrum clearly showed that **7** contained Ahda, MePro, HyLeu, Gly, and Phe.

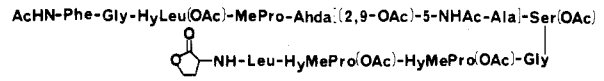


7 R = AcHN-Phe-Gly **8** R = AcHN

Tripeptide **8** was isolated from the HPLC fraction immediately following **7**. This compound was also a butyl ester. NOEs, depicted in Figure 3, established the sequence of **8** as Ac-HyLeu(Ac)-MePro-Ahda(Ac)$_3$-OBu.

The positive ion FAB mass spectra of peptides **7** and **8** exhibited $(M + H)^+$ peaks at m/z 1022 and 818, respectively. The CAD spectrum of the $(M + H)^+$ ion of **7** showed that sequential losses of *N*-acetyl-Phe (m/z 833), Gly (m/z 776), and *O*-acetyl-HyLeu (m/z 605) were occurring from the N-terminus which meant that the sequence of **7** was Ac-Phe-Gly-HyLeu(Ac)-MePro-Ahda-(Ac)$_3$-OBu.

Since difference NOE studies had already shown that the partial structures Ahda-Ser and Hse-Phe were present in scytonemin A, the sequence of the 11 amino acids in the cyclic peptide portion of the molecule was concluded to be as shown in **1**. Further proof was obtained when scytonemin A was treated with 1.2 N methanolic HCl for 62 h at room temperature in an attempt to hydrolyze the *N*-acetylalanyl ester side chain selectively. Cleavage of the Hse-Phe amide bond was found to proceed more readily. When the hydrolysis mixture was acetylated, treated with CH_2N_2, and separated by reverse-phase HPLC, **9** was obtained. Compound **9** had an NMR spectrum that was



9

quite similar to that of **2**. It showed eight acetate signals, but lacked a signal for a methyl ester. The major differences between the two spectra were mostly in the signals assigned to Phe, Gly(I), Hse, and Leu. The γ protons of Hse had the same chemical shift as seen in **5**. This, together with the lack of a methyl ester signal, indicated that the Hse unit was at the carboxyl terminus and had lactonized. The signal for the Phe amide proton was shifted downfield from 6.8 ppm in **2** to 7.41 ppm in **9**. Irradiation of this signal resulted in a weak positive NOE to one of the acetate methyl signals and this suggested that Phe was at the N-terminus. These results provided further evidence for the Hse-Phe connectivity in scytonemin A.

The positive ion FAB spectrum of **9** gave the expected $(M + H)^+$ ion at m/z 1757 and showed a large number of sequence ions. Successive losses of Ac-Phe, Gly, and HyLeu(Ac) from the N-terminus of the $(M + H)^+$ ion gave rise to ions at m/z 1570, 1512, and 1341, respectively. The ion at m/z 1341 then showed loss of 131 mass units for *N*-acetylalanine to give an ion at m/z 1210.

The mass spectral data and homoseryl lactone nature of **9** ruled out the placement of the *N*-acetylalanyl side chain on either the hydroxyl of Hse or HyLeu. A complete assignment of the ^1H spectrum of **1** in DMSO-d_6 via COSY and RELAY[17] 2D NMR experiments at 300 MHz as well as a SECSY experiment at 600 MHz[18] allowed identification of all the signals for methine and methylene protons on carbons bearing hydroxyls as well as their connectivities

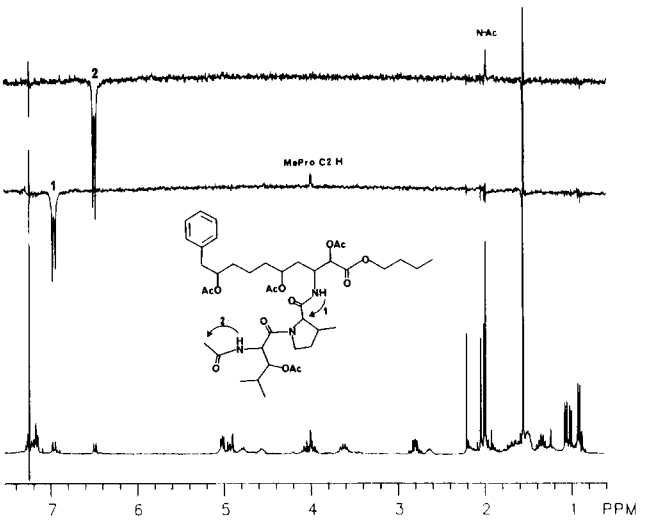


Figure 3. Difference ^1H NOE spectra for compound **8** in $CDCl_3$ showing (1) irradiation of the amide proton of Ahda to give a positive enhancement of the C2 proton of 3-methylproline and (2) irradiation of the amide proton of HyLeu to give a positive enhancement of the *N*-acetate methyl signal.

to hydroxyl signals. The hydroxyl proton signals were also assigned by a deuterium exchange experiment and by a difference spectrum where the unexchanged sample was irradiated on and off the water resonance.

The H-5 signal for Ahda (4.72 ppm) appeared at much lower field than any of the other signals for protons on hydroxyl-bearing carbons, more than 0.5 ppm downfield compared with the H-4 signals for the two HyMePro residues and more than 1 ppm downfield from either the H-9 signal for Ahda or the β proton signals for Ser. In addition to a low chemical shift, the H-5 signal of Ahda did not show a coupling to a hydroxyl proton. These data confirmed that C-5 of Ahda was the site of the *N*-acetylalanine ester attachment.

To rule out further the placement of the ester group at other positions, scytonemin A was acetylated with acetic anhydride-*1,1'*-^{13}C to give **2a**. The ^1H spectra of **2** and **2a** were then compared to find out which ester-bearing methine and methylene signals showed additional 2–3.5 Hz ^3J ^1H–^{13}C couplings. The H-2 signal for Ahda and the H-4 signal for each of the two HyMePro residues clearly showed an extra 2.8–3.0-Hz coupling. To visualize the other ^3J ^1H–^{13}C couplings, a proton spectrum was determined in which the carbonyl region of the ^{13}C spectrum was noise-decoupled. Subtraction of this spectrum from the undecoupled spectrum resulted in a difference spectrum which revealed the positions of all the protons coupled to acetate carbonyl carbons (Figure 4). Signals for protons on the C-3 and C-4 methylenes of Ser and Hse, respectively, could also be seen. The signals for H-9 of Ahda and the H-3 of HyLeu were overlapped in the difference spectrum, however, but were separated in a homonuclear 2D *J*-resolved spectrum where ^3J ^1H–^{13}C couplings of 3.3 and 2.9 Hz, respectively, were revealed in the F2 dimension.[19] Clearly missing in the difference spectrum (Figure 4) was a signal for H-5 of Ahda. These data unambiguously pointed to the oxygen on C-5 of Ahda as the site of attachment for the *N*-acetylalanyl unit.

We concluded, therefore, that scytonemin A had to have the gross structure depicted in **1**.

(17) Bax, A.; Drobny, G. *J. Magn. Reson.* **1985**, *61*, 306.
(18) Nagayama, K.; Wuthrich, K.; Ernst, R. R. *Biochem. Biophys. Res. Commun.* **1983**, *36*, 1613.
(19) Bax, A.; Marzilli, L. G.; Summers, M. F. *J. Am. Chem. Soc.* **1987**, *109*, 566.
(20) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591.

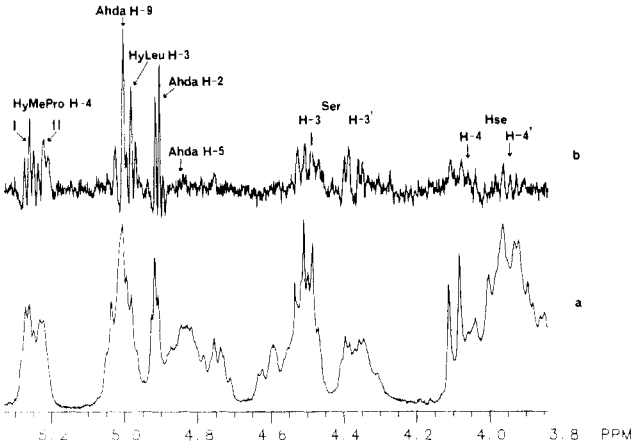


Figure 4. ^1H NMR spectrum of compound **2a**. (a) Undecoupled spectrum showing the region 3.84–5.33 ppm. (b) ^{13}C-decoupled difference spectrum of the same spectral region indicating the positions of all protons which have ^3J ^1H–^{13}C coupling to the labeled acetate carbonyl carbons. H-5 of Ahda lacks such a coupling.

Absolute Stereochemistry. With use of Marfey's procedure,[20] the absolute configurations of Ala and Hse were determined to be L and those of Leu and Ser to be D. The CD spectrum of the Hse lactone provided additional proof that Hse was L. The absolute configuration of Phe was shown to be D from its optical rotation and R_f value on a chiral TLC plate.

HyLeu was concluded to be a D amino acid on examination of the CD spectrum of the fluorescamine condensation product, which showed a positive sign for the peak at 317 nm. According to the literature,[21] a primary α-amino acid condenses with fluorescamine to form a pyrrolinone-type derivative which exhibits two prominent UV absorption maxima, one in the 380–390 nm (ϵ 6000–7000) region and the other in the 270–280 nm (ϵ 18 000–20 000) region. The UV peak in the 270–280-nm region gives rise to a split Cotton effect in the CD spectrum and the peak at longer wavelength for the split Cotton effect, which is found in the 310–325-nm region, always has a positive sign for D and a negative sign for L α-amino acids.[21d] Since HyLeu was threo, its absolute configuration had to be 2*R*,3*S*.

All three proline units in scytonemin A were found to have the L configuration. MePro and HyMePro were determined to be L amino acids on the basis of the CD spectra of the fluorescamine condensation products, which showed negative signs for the peak at 302–303 nm. According to the literature,[22] a secondary α-amino acid condenses with fluorescamine to form an amino enone derivative which exhibits three absorption maxima in the UV spectrum and Cotton effects in the CD spectrum centered at approximately the same wavelengths. In all cases studied the sign of the intense peak around 300–305 nm is negative for derivatives obtained from L amino acids and positive for those derived from D amino acids. HyMePro, for example, formed a derivative which showed UV peaks at 270 (ϵ 8300) and 310 nm (21 800) with a shoulder at 350 (11 500). The MePro derivative exhibited essentially the same UV spectrum. Although it is not clear from Toome et al.,[22] both of the UV peaks at 270 and 310 nm appear to be giving rise to the split Cotton effect in the CD spectrum. The CD spectrum of HyMePro displayed a typical curve for a split Cotton effect, viz. a negative peak at 302 nm and a positive peak at 270 nm with a null at 288 nm. The MePro derivative showed the same CD curve. The absolute stereochemistries of MePro and HyMePro were therefore 2*S*,3*S* and 2*S*,3*R*,4*R*, respectively.

The absolute stereochemistry of C2, C3, and C5 in Ahda were determined to be 2*S*,3*R*,5*S* by a CD study of δ-lactone **4**. ^1H NMR analysis clearly showed that the lactone ring existed in a half-chair conformation in solution. The CD curve of **4** in methanol showed a positive peak at 225 nm, indicating that the δ-lactone ring has the absolute configuration depicted.[23,24] At this writing the stereochemistry of C9 in Ahda is unknown.

Scytonemin A therefore has the absolute stereochemistry depicted in **1**. The C-9 carbon in the Ahda unit is the only asymmetric carbon in the entire scytonemin A molecule of unknown chirality. We intend to report it in a future publication dealing with the total structure of scytonemin B, a related cyclic peptide from a different *Scytonema* sp. (BX-5-1).

Experimental Section

Spectral Analysis. NMR spectra were determined at 300 MHz for proton and 75 MHz for carbon-13. Proton chemical shifts are referenced in chloroform-*d* to the residual chloroform signal (7.24 ppm), in dimethyl sulfoxide-d_6 to the residual DMSO-d_5 signal (2.49 ppm), and in D_2O to internal *p*-dioxane (3.75 ppm from DSS); carbon chemical shifts are referenced in chloroform-*d* and DMSO-d_6 to the solvent peaks (77.0 and 39.5 ppm, respectively) and in D_2O to internal dioxane (66.5 ppm from DSS). Homonuclear ^1H and heteronuclear ^1H–^{13}C connectivities were determined by using phase-cycled 16-step COSY and CSCM experiments, respectively, as described by Bax.[25] Long-range connectivities were determined by using the same basic pulse sequence with the addition of a 120-ms delay for evolution of small couplings.[26] Relayed coherence transfer (RCT) spectra were obtained by using the pulse sequence and phase cycling described by Bax and Drobny;[17] two separate experiments using refocusing delays (τ/2) of 18 and 25 ms were sufficient to unambiguously map all of the amino acid spin systems.

Quantitative homonuclear ^1H NOEs were obtained for a degassed sample of scytonemin A (15.6 mM) in DMSO-d_6 by saturating selected amide protons for 2 s using 32 dB of decoupler power in the hetero mode, followed by data acquisition (decoupler off) and a recycle delay of 2 s. A total of 8000 transients were collected at 24 ± 1 °C in 16K data points for each irradiated proton(s). The FIDs were multiplied by an exponential function, Fourier transformed, and subtracted from an off resonance control spectrum. Integration of the resulting NOE difference spectra using the phenylalanine C3 proton at 2.83 ppm as a 1-H reference led to values for the NOEs. The values in percent were not corrected for partial saturation.[27] Qualitative homonuclear ^1H–^1H NOEs were obtained for samples in $CDCl_3$ by selective irradiation for 2 s using 30–32 dB of gated decoupler power (hetero mode) followed by data acquisition (decoupler off) with no recycle delay.

(21) (a) Toome, V.; Wegrzynski, B.; Reymond, G. *Biochem. Biophys. Res. Commun.* **1976**, *69*, 206. (b) Kovacs, K. L. *Biochem. Biophys. Res. Commun.* **1979**, *86*, 995. (c) Toome, V.; Wegrzynski, B. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 447. (d) Toome, V.; Wegrzynski, B. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 1496.

(22) (a) Toome, V.; Wegrzynski, B.; Dell, J. *Biochem. Biophys. Res. Commun.* **1976**, *71*, 598. (b) Toome, V.; Wegrzynski, B.; Dell, J. *Heterocycles* **1977**, *7*, 787.

(23) (a) Wolf, H. *Tetrahedron Lett.* **1966**, 5151. (b) Korver, O. *Tetrahedron* **1970**, *26*, 2391. (c) Legrand, M.; Bucourt R. *Bull. Soc. Chim. Fr.* **1967**, 2241. (d) Moore, R. E.; Bartolini, G.; Barchi, J.; Bothner-By, A. A.; Dadok, J.; Ford, J. *J. Am. Chem. Soc.* **1984**, *104*, 3776.

(24) The presence of equatorial acetoxyl groups on the δ-lactone does not affect the sign of the Cotton effect in the CD curve; e.g. compare the CDs of δ-lactone **17** and the corresponding diacetate in ref 23d. Also see: Moore, R. E. *Prog. Chem. Org. Nat. Prod.* **1985**, *48*, 81.

(25) Bax, A. *Two-Dimensional Nuclear Magnetic Resonance in Liquids*; Delft University Press: Delft, Holland, 1982.

(26) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542.

(27) Jones, C. R.; Sikana, C. T.; Hehir, S.; Kuo, M.; Gibbons, W. A. *Biophys. J.* **1978**, *24*, 815.

Subtraction of this on-resonance FID from an off-resonance FID resulted in a difference FID which after processing gave an NOE difference spectrum.

The ^{13}C-decoupled difference spectrum was acquired by using a homebuilt ^{13}C probe in which the proton decoupler coil was used for the observe channel. The ^{13}C decoupling unit consisted of a PTS 160 frequency synthesizer, a Tecmag DECkit-2 decoupler interfaced with the spectrometer pulse programmer, and an ENI 420 amplifier. The amplifier output was attenuated to give approximately a 0.8-KHz decoupler field which was centered on the carbonyl region of the carbon spectrum. Broad-band modulation was used which gave complete decoupling of the carbonyls (evidenced by complete collapse of the acetate methyl doublets) with a minimum disturbance of the lock. The proton spectra were obtained in an interleaved manner by alternating scans with and without ^{13}C decoupling and storing the files in separate memory locations. A total of 400 16K FIDs were collected in each file, apodized with 0.3-Hz exponential line broadening, and subtracted to yield the difference spectrum.

The 2D homonuclear J-resolved spectrum consisted of a 64 × 8192 data matrix with 128 scans per t_1 value. The FIDs were multiplied with 1.9 Hz of exponential line narrowing and 1.2 Hz of Gaussian line broadening and Fourier transformed. A 1024-point window corresponding to the 5.38–4.30 ppm region of the F2 domain was then transposed. Multiplication with a double exponential (DM = 3) and zero-filling resulted in a final 1024 × 256 F2F1 data matrix which was then symmetrized. The digital resolution was 0.3 Hz/point in both F2 and F1.

FAB and CAD mass spectra were obtained on a Kratos MS-50 triple analyzer mass spectrometer[28] using previously described experimental conditions.[15]

Optical rotations were measured at 20 °C at the sodium D line. Circular dichroism spectra were determined on a Cary 61 spectropolarimeter.

Culture Conditions. *Scytonema* sp. (strain U-3-3) was isolated from a soil sample collected at Majuro Atoll in the Marshall Islands and grown in mass culture in the laboratory by using the same conditions described for *Hapalosiphon fontinalis*.[4b]

Isolation. Freeze-dried alga (28.6 g) was extracted with 3 L of 70% EtOH for 24 h at 25 °C. The crude extract was concentrated to 500 mL under reduced pressure at 25 °C and the concentrate lyophilized to yield 4.87 g of a dark green powder. Reextraction of the alga with 1.4 L of 70% EtOH gave an additional 0.59 g of extract (red-brown powder) which was combined with the first batch.

The 5.46 g of extract was dissolved in 125 mL of water and the solution was filtered through cotton and applied to a 8 × 42 cm column of Amberlite XAD-2 resin. The column was eluted with 2.5 L of water followed by 2-L portions of each of the following EtOH/H_2O mixtures: 40% EtOH, 60% EtOH, 80% EtOH, and 100% EtOH. Fifteen 500-mL fractions were collected and aliquots of each fraction were assayed for antimicrobial activity (*B. subtilis*).[7] The assay showed that fractions 6–14 were active, with most of the activity in fractions 9–11. Fractions 9–11 were combined, concentrated in vacuo, and lyophilized to give 195 mg of a flocculent yellow powder. Final purification was achieved by reverse-phase HPLC on a 1 × 50 cm column of Whatman Partisil ODS-2 with 75% MeOH/H_2O (12-mg portions, 4 mL/min flow rate) to give after lyophilization, 130 mg (0.46% yield, t_R 15 min) of scytonemin A (1), as a white powder: $[\alpha]_D$ +38.8° (*c* 0.04, MeOH); UV (MeOH) λ_{max} 210 nm (ϵ 52 700), 259 (1000); IR ($CHCl_3$) ν_{max} 3324, 3021, 1738, 1659, 1540, 1454 cm^{-1}; high resolution FABMS, m/z 1463.7699 $(M + H)^+$ (calcd for $C_{71}H_{107}N_{12}O_{21}$ 1463.7674); ^1H and ^{13}C NMR, see Table I.

Acetylation. A mixture of 11 mg of scytonemin A in 2 mL of acetic anhydride and 1 mL of pyridine was allowed to stir overnight. The excess reagents were removed under reduced pressure and the residue was dissolved in 2 mL of CH_2Cl_2 and washed successively with 2 mL of dilute HCl, saturated $NaHCO_3$, and brine. The organic layer was then passed through a small cotton filter and evaporated to dryness to yield 12.5 mg of a yellow glass. The dried residue was dissolved in MeOH, filtered, and chromatographed by reverse-phase HPLC on a Whatman 1 × 50 cm ODS-3 column using 65% MeOH/H_2O as the eluant. At a flow rate of 4 mL/min the major peak eluting at 25 min afforded, after lyophilization, 10.5 mg of scytonemin A heptaacetate (2) as a clear glass: $[\alpha]_D$ +44.3° (*c* 0.03, $CHCl_3$); TLC R_f (silica gel, 1:9 EtOH/EtOAc), 0.38.

Acetylation of 9.9 mg of scytonemin A using 2 mL of acetic anhydride-d_6 (99+ atom % D) resulted in 11.2 mg of the deuteriated heptaacetate. The ^1H NMR spectrum ($CDCl_3$) showed only one acetate methyl signal at 1.978 ppm, assignable to the natural acetate in scytonemin A.

In a third experiment a mixture of 6.8 mg of scytonemin A, 100 μL of acetic anhydride-*1,1'*-^{13}C (99+ atom %), and 80 μL of pyridine was stirred in a 0.5-mL Wheaton vial at room temperature for 40 h and then evaporated under reduced pressure. Workup yielded 6.3 mg of CH_2Cl_2 soluble material which gave after HPLC 4.6 mg of scytonemin A heptaacetate-*1*-^{13}C (**2a**) as a clear glass: ^1H NMR ($CDCl_3$) acetate signals (multiplicity, $^2J_{CH}$ in Hz) δ 2.138 (d, 7.1), 2.114 (d, 7.0), 2.054 (d, 6.8), 2.018 (d, 6.5), 2.000 (d, 6.8), 1.969 (s), 1.964 (2 d, 6.7).

Hydrolysis. (A) With 6 N Hydrochloric Acid. For amino acid analysis, 60 μg of scytonemin A in 0.5 mL of 6 N HCl containing a small amount of phenol was heated to 110 °C for 30 h in a sealed tube. Analysis indicated the following primary α-amino acids and the relative amounts: Ser (1), Hse/Hse lactone (1), HyLeu (1), Gly (2), Ala (1), Leu (1), and Phe (1).

In a second experiment, a degassed solution of 23.2 mg of scytonemin A in 2.0 mL of freshly distilled constant boiling HCl in a sealed tube was heated to 110 °C for 30 h. The mixture was evaporated in vacuo and the thoroughly dried residue was dissolved in 250 μL of 0.1% trifluoroacetic acid (TFA) in water. Reverse-phase HPLC on a Whatman 1 × 50 cm ODS-3 column using 0.1% TFA as the eluant gave, at a flow rate of 4 mL/min, seven major fractions (peaks) with t_R 8.1, 9.5, 10.1, 12.0, 15.5, 17.5, and 26.7 min. ^1H NMR analysis of the amino acids in these seven fractions gave the following results: Ala, Ser, Gly, Hse (8.1 min); Hse, Hse lactone (9.5); HyMePro (10.1); HyLeu (12.0); MePro (15.5); Leu (17.5); Phe (26.7).

The crude Hse/Hse lactone fraction was rechromatographed on a 1 × 50 cm Whatman PAC column with 0.1% TFA to give 1.5 mg of Hse lactone: CD (H_2O) $[\Theta]_{218}$ +1000; R_f 0.51 (TLC, cellulose, 4:1:1 *n*-BuOH/H_2O/HOAc); ^1H NMR (D_2O + DCl) δ 4.47 (m, H2), 2.81 (m, H3), 2.45 (br p, J = 11 Hz, H3'), 4.43 (m, H4), 4.61 (br t, J = 9.5 Hz, H4'). The CD spectrum (H_2O) of synthetic L Hse lactone HCl, mp 217–220 °C, shows a positive Cotton effect, $[\Theta]_{218}$ +2900.

(B) With 1.5 N Hydrochloric Acid. A solution of 200 mg of scytonemin A in 15 mL of 1.5 N HCl was refluxed under nitrogen for 21 h. After cooling to room temperature, 10 mL of water was added and the solution was evaporated to dryness under reduced pressure. The process was repeated two more times to remove traces of HCl. The hydrolyzate was then dissolved in 5 mL of water and the solution lyophilized. The freeze-dried material was then dissolved in 3 mL of 0.2 N HOAc and chromatographed in 1-mL portions on a 2.2 × 25 cm column of Sephadex G-10 (0.2 N HOAc, 2.5 mL/min). After 25 mL of effluent had passed, 75 × 1.25 mL fractions were collected. Analysis by TLC and ^1H NMR showed that fractions 30–44 were mixtures of Ahda and Phe while fractions 45–52 were pure Ahda. Fractions 14–29 were found to be complex mixtures of small peptides and the remaining amino acids. Fractions 30–44 were combined and rechromatographed on Sephadex G-10 to give 18 mg of pure Phe and additional Ahda. The total yield of Ahda was 14.6 mg.

Phe showed an R_f value of 0.47 when analyzed by TLC on Chiralplate (Macherey-Nagel, Germany) with 1:1:4 MeOH/H_2O/CH_3CN. R_f values for authentic compounds: D-Phe (0.47) and L-Phe (0.59).

3-Amino-2,5,9-trihydroxy-10-phenyldecanoic acid (Ahda) had the following properties: $[\alpha]_D$ +7° (2:1 EtOH/H_2O, *c* 0.1); R_f 0.43 (TLC, silica gel, 8:4:1 MeOH/*n*-BuOH/HOAc); FABMS (glycerol), m/z 312 $(M + H)^+$; ^1H NMR (D_2O) δ 4.46 (d, J = 4.5 Hz, H2), 3.71 (q, J = 6.2 Hz, H3), 1.87 (br t, J = 6.2 Hz, H4 and H4'), 3.92 (m, H5), 1.54 (br m, 6 H on C6, C7, and C8), 3.97 (m, H9), 2.85 (dd, J = −14.1 and 7.7 Hz, H10), 2.73 (dd, J = −14.1 and 5.1, H10'), 7.37–7.27 (br m, 5 H on aromatic ring); ^{13}C NMR (D_2O) δ 176.2

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(s, C1), 71.1 (d, C2), 51.1 (d, C3), 36.2 (t, C4), 67.5 (d, C5), 35.4 (t), 20.9 (t, C7), 35.3 (t), 72.3 (d, C9), 42.6 (t, C10), 139.0 (s, C1′), 128.5 (d, C2′ and C6′), 129.4 (d, C3′ and C5′), 126.3 (d, C4′).

Fractions 14–29 from the Sephadex G-10 gel filtration were combined, lyophilized, and rechromatographed on a 2 × 26.5 cm column of powdered cellulose (*n*-BuOH/H_2O/HOAc, 4:1:1, 0.5 mL/min). After 38 mL of effluent had passed, 70 × 2.5 mL fractions were collected and analyzed by TLC and ^1H NMR. Fractions 20–23 consisted of a 1:1 mixture of MePro and HyLeu while fractions 27–29 contained pure HyMePro which after recrystallization from EtOH/H_2O amounted to 13.4 mg.

(2*S*,3*R*,4*R*)-4-Hydroxy-3-methyl-L-proline had the following properties: mp 255–260 °C dec; $[\alpha]_D$ +10.3° (H_2O, *c* 0.01); R_f 0.29 (TLC, silica gel, 4:1:1 *n*-BuOH/H_2O/HOAc); ^1H NMR (D_2O) δ 3.82 (d, *J* = 11.0 Hz, H2), 2.54 (ddq, *J* = 11.0, 6.7, 3.7 Hz, H3), 4.44 (br t, *J* = 3.7 Hz, H4), 3.54 (dd, *J* = −12.7, 3.7 Hz, H5), 3.36 (dd, *J* = −12.7, 0.8 Hz, H5), 1.24 (d, *J* = 6.9 Hz, Me); ^{13}C NMR (D_2O) δ 173.5 (s, C1), 64.1 (d, C2), 43.1 (d, C3), 72.6 (d, C4), 52.5 (t, C5), 11.1 (q, Me).

Separation of MePro and HyLeu was accomplished by reverse-phase HPLC on a 1 × 50 cm Whatman PAC column with 0.1% TFA in H_2O which yielded, after lyophilization, 8.0 mg of MePro and 7.8 mg of HyLeu. HyLeu could be crystallized from EtOH/EtOAc, but all attempts to crystallize MePro failed in our hands.

(2*R*,3*S*)-*threo*-3-Hydroxy-D-leucine had the following properties: mp 215–217 °C; $[\alpha]_D$ 0° (H_2O, *c* 0.01), $[\alpha]_D$ −8° (5 N HCl, *c* 0.01);[29] R_f 0.48 (ascending paper, Whatman #4, 8:6:1:1 *n*-BuOH/H_2O/acetone/concentrated NH_4OH) [lit.[9b] R_f 0.44 for L-threo and 0.30 for L-erythro]; ^1H NMR (D_2O) δ 4.02 (d, *J* = 3.0 Hz, H2), 3.82 (dd, *J* = 8.4 and 3.0 Hz, H3), 1.76 (octet, *J* = 7.0 Hz, H4), 1.00 (d, *J* = 6.7 Hz, Me), 0.96 (d, *J* = 6.7 Hz, Me); ^{13}C NMR (D_2O) δ 173.1, (s, C1), 56.8 (d, C2), 75.0 (d, C3), 30.2 (d, C4), 18.4 (q, Me), 17.4 (q, Me) [lit.[9c] δ 173.2, 56.8, 74.8, 30.1, 18.3, 17.2 for L-threo and δ 171.6, 57.0, 76.0, 30.1, 18.4 (2C) for L-erythro using 66.5 ppm for dioxane reference].

(2*S*,3*S*)-*trans*-3-Methyl-L-proline had the following properties: $[\alpha]_D$ +3° (5 N HCl, *c* 0.03) [lit.[30] +4.6° (5 N HCl, *c* 1]; R_f 0.34 (TLC, silica gel, 4:1:1 *n*-BuOH/H_2O/HOAc); ^1H NMR (D_2O) δ 3.94 (d, *J* = 8.7 Hz, H2), 2.54 (m, H3), 2.26 (m, H4), 1.74 (m, H4′), 3.48 (m, H5), 3.40 (m, H5′), 1.27 (d, *J* = 7.3 Hz, Me); ^{13}C NMR (D_2O) δ 173.6 (s, C1), 62.4 (d, C2), 38.0 (d, C3), 32.1 (t, C4), 45.0 (t, C5), 17.4 (q, Me).

(C) With 0.6 N Hydrochloric Acid. A stirred solution of 21.3 mg of scytonemin A in 5 mL of 0.6 N HCl was heated under a nitrogen atmosphere at 65–70 °C for 9.5 h. At this time the reaction mixture showed a faint response to ninhydrin. The solution was allowed to stir at room temperature overnight, diluted with water, and evaporated (lyophilized) as described above to remove all traces of HCl. The dried residue was dissolved in 1 mL of 0.2 N acetic acid and chromatographed on a 21 × 2.2 cm column of Sephadex G-10. Fifteen fractions were collected by monitoring UV absorbance at 254 nm. Fractions 4–6 (20–26 min using a flow rate of 1.5 mL/min) were combined on the basis of similar R_f values and ninhydrin coloration on TLC (silica gel, 4:2:1 *n*-BuOH/H_2O/HOAc). Fractions 7–10 were also combined.

Combined fractions 4–6 were lyophilized to yield 4 mg of a clear glass which was dissolved in 2 mL of MeOH. Triethylamine was then added until the pH was 9. The mixture was stirred and 0.2 mL of acetic anhydride was added. TLC analysis after 1 min showed negative ninhydrin responses for the peptide fragments. The mixture was evaporated after 15 min and the residue was then completely acetylated with 2:1 acetic anhydride/pyridine overnight at room temperature.[31] The crude acetylation product was next dissolved in 2 mL of CH_2Cl_2 and treated with excess diazomethane. No change in TLC behavior was noted. The solvent and excess reagents were removed under a stream of nitrogen and the residue was chromatographed on a Whatman 1 × 50 cm ODS-3 column using a linear gradient of acetonitrile (A) and H_2O (B) (flow rate 2 mL/min, 50% A for 10 min followed by increasing A to 65% over 40 min). Compound **5** (0.9 mg) eluted at 16 min followed by Ac-HyMePro(Ac)-HyMePro(Ac)-Leu-Hse lactone (1 mg) at 18 min.

Combined fractions 7–10 were further chromatographed on a 1 × 13 cm column of cellulose (Baker) by using 4:2:1 *n*-BuOH/H_2O/HOAc as the eluant. After an initial 5 mL had passed, 26 1-mL fractions were collected. TLC analysis showed that fractions 9–12 were UV-absorbing and ^1H NMR indicated the presence of the aromatic amino acids. These fractions were combined, acetylated,[31] and treated with diazomethane as described above. Analysis by TLC showed that only one component of the mixture had undergone a change after the esterification step. Chromatography on a Whatman 1 × 50 cm ODS-3 column using a linear gradient of acetonitrile (A) and H_2O (B) (flow rate 1.5 mL/min; 50% B for 12 min followed by ramping B to 35% over 30 min and then to 15% over 10 min). The dipeptide Ac-Phe-Gly-OMe (0.7 mg) eluted at 17 min followed by compound **7** (1 mg) at 67 min and compound **8** (1.1 mg) at 69 min.

Compound **5**: FABMS (glycerol), *m/z* 781 $(M + H)^+$; IR ($CHCl_3$) ν_{max} 1780, 1738, 1657 cm^{-1}; ^1H NMR ($CDCl_3$) Hse lactone unit δ 8.16 (NH), 4.72 (H2), 4.38 (H4), 4.24 (H4′); Leu unit δ 6.57 (NH); Gly II unit δ 6.58 (NH), 3.96 (H2), 3.95 (H2′); Ser unit δ 6.56 (NH), 4.29 (H3), 4.18 (H3′); acetate Me δ 1.92, 1.89, 1.88, 1.82 (NAc); all other resonances are within ±0.01 ppm of the values listed in Table II.

Compound **7**: FABMS (glycerol), *m/z* 1022 $(M + H)^+$; ^1H NMR ($CDCl_3$) Phe unit δ 6.93 (NH), 4.65 (H2), 3.16 (H3), 2.99 (H3′); Gly I unit δ 6.98 (NH), 4.23 (H2), 3.49 (H2′); HyLeu unit δ 6.42 (NH), 4.82 (H2), 5.02 (H3), 1.89 (H4), 0.96 (Me), 0.92 (Me); MePro unit δ 4.00 (H2), 2.62 (H3), 3.67 (H5), 3.64 (H5′); Ahda unit δ 6.90 (NH), 4.55 (H3), 4.72 (H5); acetate Me δ 2.20, 2.04, 2.00, 1.99 (NAc), 1.92; butyl ester group δ 4.02 (dt, *J* = −11.0 and 6.7 Hz, H1), 3.96 (dt, *J* = −11.0 and 6.8 Hz, H1′), 1.55 (m, 2 H on C2), 1.35 (sextet, *J* = 7.7 Hz, 2 H on C3), 0.89 (t, *J* = 7.4 Hz, Me); all other resonances are within ±0.05 ppm of those listed in Table II.

Compound **8**: FABMS (glycerol), *m/z* 818 $(M + H)^+$; ^1H NMR ($CDCl_3$) HyLeu unit δ 6.48 (NH), 4.94 (H2), 1.00 (Me), 0.91 (Me); Ahda unit δ 6.95 (NH); acetate Me δ 2.04, 2.02, 2.00, 1.98 (2 signals, one of which is the NAc); all other resonances are within ±0.02 ppm of those listed for **7**.

(D) With 1.2 N Methanolic Hydrochloric Acid. A solution of 11.5 mg of scytonemin A in 2 mL of methanol and 0.5 mL of 6 N HCl was stirred at room temperature and hydrolysis was monitored by TLC on silica with 4:2:1 *n*-BuOH/H_2O/HOAc. After 62 h starting material could not be detected, but three major ninhydrin-active spots (R_f 0.36, 0.39, 0.46) were visualized. The mixture was evaporated to dryness and the residue was repeatedly dissolved in water and the solution lyophilized to remove traces of HCl from the hydrolyzate. The hydrolyzate was acetylated[31] and treated with diazomethane as described above and the acetylated hydrolysis product was chromatographed on a Whatman 1 × 50 cm ODS-2 column using a linear gradient of acetonitrile (A) and water (B) (flow rate 1.5 mL/min; 50% B for 12 min followed by ramping B to 35% over 30 min and then to 15% over 10 min). Major fractions eluted at 55 min (2.4 mg) and 61 min (2.2 mg). ^1H NMR analysis showed that the faster moving fraction was a single component, the Hse lactone **9**. The slower moving fraction was a mixture of a methyl ester resulting from methanolysis of the Hse lactone in **9**, as well as several smaller hydrolysis products which appeared to be methyl esters.

Compound **9**: FABMS (glycerol), *m/z* 1757 $(M + H)^+$, 1780 $(M + Na)^+$; ^1H NMR ($CDCl_3$) Phe δ 7.41 (NH), 4.68 (H2), 3.19 (H3), 2.92 (H3′); Gly I δ 6.95 (NH), 4.36 (H2), 4.34 (H2′); Hse lactone δ 8.23 (NH), 4.79 (H2), 2.62 (H3), 2.07 (H3′); 4.38 (H4), 4.32 (H4′); Leu δ 6.94 (NH), 4.47 (H2), 1.50 (H4); acetate Me δ 2.15, 2.12, 2.07, 2.06, 1.98, 1.97, 1.96, 1.91; all other resonances are within ±0.10 ppm of those listed in Table II.

Acetylation of 3 to 4. A solution of 4.2 mg of Ahda (**3**) in 3 mL of MeOH at 0 °C was adjusted to pH 9 with triethylamine. Acetic anhydride (0.25 mL) was added while stirring and the acetylation was monitored by TLC analysis (silica gel; 2:4:1 *n*-BuOH/MeOH/HOAc). No ninhydrin-positive spots were ob-

(29) *threo*-3-Hydroxy-L-leucine shows $[\alpha]_D$ −3.5° (H_2O, *c* 2), $[\alpha]_D$ +15° (5 N HCl, *c* 2). See: Dalby, S.; Kenner, G. W.; Sheppard, R. C. *J. Chem. Soc.* **1960**, 968.

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(31) The two-step acetylation was carried out to prevent the formation of artifacts from the reaction of acetic anhydride and pyridine. [Fleming, I.; Mason, J. B. *J. Chem. Soc. C* **1969**, 2509].

served after 1 min. After an additional 15 min the solvent was evaporated under reduced pressure. To the resulting oil was added 2 mL of acetic anhydride and the mixture was stirred at 40 °C for 20 min. The mixture was cooled to room temperature, 1 mL of pyridine was added, and the mixture was stirred overnight. Standard workup resulted in 6.0 mg of a yellow oil which was chromatographed on a 2 × 0.9 cm silica column (Bond Elut Si, Analytichem International) with 5 mL of CH_2Cl_2 followed by 10 mL of EtOAc. The EtOAc fraction was further purified by HPLC on a Whatman 4.6 mm × 25 cm Partisil 5 column using 60:40 EtOAc/CH_2Cl_2 as eluant. At a flow of 1.2 mL/min the major fraction eluted at 20 min which gave, after evaporation, 3.5 mg of pure lactone 4. TLC analysis on silica gel with 19:1 EtOAc/EtOH showed a single spot, R_f 0.43.

Compound 4 had the following properties: EIMS (70 eV), m/z (relative intensity) 359 (8.7, M - HOAc), 300 (6.4, M - HOAc - CH_3CONH_2), 286 (12.2), 244 (17.7), 240 (6.7, M - HOAc - CH_3CONH_2 - HOAc), 130 (67.5), 91 (46.5, $PhCH_2^+$), 43 (100); ^1H NMR ($CDCl_3$) δ 5.09 (d, J = 11.2 Hz, H2), 4.40 (m, H3), 1.65 (q, J = 12.4 Hz, H_{ax}-4), 2.35 (dt, J = -13.8 and 4.0 Hz, H_{eq}-4), 4.41 (m, H5), 1.31-1.60 (br m, 6 H on C6, C7, and C8), 5.03 (pentet, J = 6.1 Hz, H9), 2.85 (dd, J = -13.8 and 6.5 Hz, H10), 2.77 (dd, J = -13.8 and 6.0 Hz, H10'), 7.16 (dt, J = 7.2 and 1.6 Hz, H2'' and H6''), 7.27 (tt, J = 7.2 and 1.6 Hz, H3'' and H5''), 7.22 (tt, J = 7.2 and 1.6, H4''), 5.58 (br d, J = 7.4 Hz, NH), 2.17 (s, Ac), 1.98 (s, Ac), 1.95 (s, Ac).

Derivatization of Amino Acids with Marfey's Reagent. To a 1-mL vial containing 2 μmol of pure amino acid standard in 40 μL of H_2O was added 2.8 μmol of 2-[(5-fluoro-2,4-dinitrophenyl)amino]propanamide (FDAA)[20] in 80 μL of acetone followed by 20 μL of 1 N $NaHCO_3$. The mixture was heated for 1 h at 40 °C. After cooling to room temperature, 10 μL of 2 N HCl was added and the resulting solution was filtered through a 4.5 μm filter and stored in the dark until HPLC analysis.

To prepare FDAA derivatives of the amino acids in the scytonemin A hydrolyzate, a 40-μL aliquot containing 0.3 mg of the amino acid mixture was reacted with 4.2 μmol of FDAA in 115 μL of acetone as described above. A 5-μL aliquot of the resulting mixture of FDAA derivatives was analyzed by HPLC using a 4.6 mm × 10 cm C-18 column (Brownlee) fitted with a 4.6 mm × 1.5 cm precolumn. A linear gradient of (A) 90% triethylammonium phosphate (50 mM, pH 3.0)/10% MeCN and (B) MeCN with 0% B at the start → 40% B over 40 min (flow rate 2 mL/min) was used to separate the FDAA derivatives which were detected by UV absorption at 340 nm. Each peak in the chromatographic trace was identified by comparing the retention time with that of the FDAA derivative of the pure amino acid standard. One fraction, which contained Ala, Ser, Gly, and Hse, showed peaks at 8.5, 9.6, and 11.2 (broad with shoulder at 11.3); another fraction, which contained mainly Leu, showed a peak at 27.0 min. The amino acid standards gave the following retention times in minutes: Hse, 8.5 for L; Ser, 7.6 for L and 9.6 for D; Ala, 11.2 for L and 15.3 for D; Gly, 11.3; Leu, 21.0 for L and 26.6 for D. In all cases a peak at 16.5 min was observed which was attributed to 2-[(1-hydroxy-2,4-dinitrophenyl)amino]propanamide.

Derivatization of Amino Acids with Fluorescamine for CD Analysis. The procedure described by Toome et al.[22a] was used to make the chiroptically active derivatives in situ. The CD spectra of the derivatives were recorded immediately.

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Supplementary Material Available: Symmetrized 2D homonuclear J-resolved spectrum of compound **2a** and the positive ion FAB mass spectrum of compound **9** (4 pages). Ordering information is given on any current masthead page.

Notes

Easy Approach to *N*-(Aminoacyl)taurine Derivatives

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It has been recently reported[1] that a derivative of taurine, L-(-)-ornityltaurine hydrochloride (**5a**), possesses a salty taste greater than that of sodium chloride.

More recently, Huynh-ba and Philippossian[2] questioned the organoleptic properties of the peptide, suggesting that the previously claimed saltiness resulted from NaCl present as a not easily removable artifact.

These facts prompted us to report a new synthetic pathway suitable for a wide range of *N*-acyl derivatives of taurine. The present method in fact excludes any contamination with inorganic salts and allows the preparation of otherwise hardly accessible compounds.

Though it is well-known that in biological systems[3,4] taurinamido derivatives can form from taurine when specific enzymes are used and that taurine or taurine sodium salt can be acylated (though in low yield) by acetic[5] or butyric[6] anhydrides or lactones,[6] any attempt to condense taurine with anhydrides, mixed anhydrides, chlorides, and activated esters (e.g., succinimide derivative) of N-protected amino acids led to negligible yields of the expected product. This is probably due to the fact that taurine is largely present in betainic form, $^+NH_3CH_2CH_2SO_3^-$, in which the nucleophilic character of the amino group is strongly reduced.

The use of taurine sodium salt did not give better results.

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