Quantum yields were measured by using a "linear optical bench" system described earlier[2] and employing a filter solution combination with three 1-cm compartments containing separately 2.0 M (252.72 g/L) nickel sulfate hexahydrate in 5% sulfuric acid, 0.8 M (224.88 g/L) cobalt sulfate heptahydrate in 5% sulfuric acid, and 0.0012 M (0.378 g/L) bismuth chloride in 2:3 hydrochloric acid/water. The UV transmission of this filter system was 240–310 nm with a maximum at 275 nm. Product analyses for the spirocyclicamine **30** was performed by HPLC (Partisil M-9 10/50). Conversions were in the range of 1.7–1.9%.

Acknowledgment. Support for this research by a grant from the National Institutes of Health (GM-27251) is acknowledged.

Registry No. **8**, 87862-67-7; **9**, 87862-69-9; (*E*)-**10**, 87862-81-5; (*Z*)-**10**, 87862-90-6; **13**, 80348-06-7; **15**, 87862-64-4; **16**, 87862-65-5; **17**, 7743-26-2; **18**, 87862-70-2; **19**, 87862-71-3; **20**, 87862-72-4; **21**, 87862-73-5; **22**, 87862-74-6; **23**, 87862-75-7; **24**, 87862-76-8; **25**, 87862-77-9; **26**, 87862-78-0; **27**, 87862-79-1; **30**, 87862-82-6; **35**, 87862-88-2; (*E*)-**36**, 87862-85-9; (*Z*)-**36**, 87862-92-8; **39**, 87862-86-0; **40**, 87862-83-7; $CH_2{=}C(CH_2SiMe_3)CH_2OSO_2Me$, 74532-54-0; $ClCH_2(CH_2)_2CO_2Et$, 3153-36-4; Ph_3P, 603-35-0; $EtO_2CCH_2NH_3Cl$, 623-33-6; piperonal, 120-57-0.

Structure of Majusculamide C, a Cyclic Depsipeptide from *Lyngbya majuscula*

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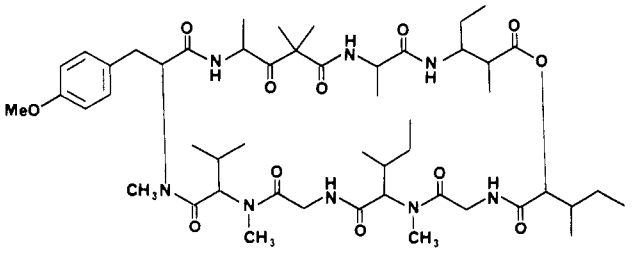
Received July 11, 1983

Majusculamide C, a novel cyclic depsipeptide that inhibits the growth of a number of fungal plant pathogens, has been shown to consist of seven α-amino acid units, one β-amino acid unit, and one hydroxy acid unit. The structures of the units have been determined by acid hydrolysis of the fungicide to glycine, L-alanine, L-*N*-methylvaline, L-*N*,*O*-dimethyltyrosine, L-*N*-methylisoleucine, racemic 2-amino-4-methylpentanone, 3-amino-2-methylpentanoic acid of undefined stereochemistry, and *N*-[2(*S*)-hydroxy-3(*S*)-methylpentanoyl]glycine. Sequencing of the units implied from the structures of the hydrolysis products has been achieved by mass spcetral and proton NOE studies.

Lyngbya majuscula is a toxic blue-green alga which grows abundantly on the pinnacles in the lagoon of Enewetak Atoll in the Marshall Islands.[3] At least two distinct varieties of *L. majuscula* grow in the lagoon, one that is found in shallow water (<6 m) and another which inhabits deep water (7.5–30 m). Two major lipophilic constituents of the shallow water variety are majusculamide A, a lipodipeptide identified as *N*-[(2*R*)-2-methyl-3-oxodecanoyl]-D-*N*,*O*-dimethyltyrosyl-*L*-*N*-methylvalinamide, and majusculamide B, the *N*-[(2*S*)-2-methyl-3-oxodecanoyl] epimer of majusculamide A.[4] A major lipophilic constituent in the deep water variety is majusculamide C (**1**), a novel cyclic depsipeptide that controls



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the growth of a number of fungal plant pathogens such as *Phytophthora infestans*, the causative organism of tomato late blight, and *Plasmopora viticola*, the causative organism of grape downy mildew.[5]

Majusculamide C is not present in the shallow water variety, nor are majusculamides A and B found in the deep water variety.[6] None of the majusculamides are responsible for the toxicity of *L. majuscula*, even though majusculamide C is fairly cytotoxic. The toxicity is attributed to aplysiatoxin, debromoaplysiatoxin, and lyngbyatoxin A, three highly inflammatory agents that have been shown to be powerful tumor promoters.[7,8] In this report we describe the structure determination of majusculamide C.

Isolation. The majusculamide C producing variety of *L. majuscula* grows abundantly on most of the pinnacles in the lagoon of Enewetak. Similar deep water varieties of *L. majuscula* have not been found to date in Hawaii or other areas of the Pacific such as Kwajalein, Johnston, and Fanning Islands. The alga used in this work was collected from Reefer 8 and South Medren Pinnacles at Enewetak. The lipophilic extract of the alga was subjected to absorption chromatography, gel filtration, and reverse-phase chromatography to obtain the depsipeptide as a colorless amorphous solid in 0.05–0.1% yield based on the dry weight of the alga. Attempts to crystallize majusculamide C have so far failed in our hands.

Structure Determination. Majusculamide C was found to have an elemental composition $C_{50}H_{80}N_8O_{12}$ based on a molecular weight of 984 daltons from field-desorption and fast-atom bombardment mass spectrometry and de-

(1) Lilly Research Laboratories, MC-539, Eli Lilly & Co., Indianapolis, IN.
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(3) Mynderse, J. S.; Moore, R. E.; Kashiwagi, M.; Norton, T. R. *Science (Washington, D.C.)* **1977**, *196*, 538.
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(6) In our earlier papers we erroneously referred to the deep water variety of *L. majuscula* from Enewetak as *L. gracilis*. Moore, R. E. *Bioscience* **1977**, *27*, 797.
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0022-3263/84/1949-0236$01.50/0

Table I. Elemental Composition and Preliminary Structural Conclusions for Majusculamide C from ^{13}C and ^{1}H NMR Spectral Data

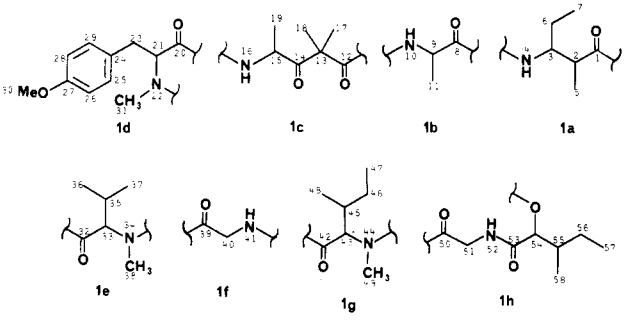
^{13}C chemical shifts, δ	inventory of carbons	nature of carbons and hydrogens	inventory of hydrogens	^{1}H chemical shifts, δ
209.57	1	ketone C=O		
172.25, 172.09, 171.51, 170.52, 169.75, 169.64, 169.44, 168.81, 167.44	5 3 1	secondary amide C=O, tertiary amide C=O, ester C=O[a]	5	7.767, 7.573, 7.355, 7.309, 7.068
158.17, 128.18	2	aromatic =C		
129.94 (2), 113.79 (2)	4	aromatic =CH[b]	4	7.129 (2), 6.834 (2)
54.78	1	$ArOCH_3$	3	3.738 (3)
29.90, 28.85, 28.78	3	amide NCH_3	9	3.201 (3), 2.944 (3), 2.938 (3)
54.41	1	-C-		
77.87, 60.65, 60.56, 57.67, 51.17, 50.78, 47.86, 42.07, 36.91, 32.34, 26.58	11	-CH-	11	
40.34, 40.17, 34.17, 25.57, 24.49, 23.30	6	CH_2	12	
21.72, 21.03, 18.68, 18.02 (2),[c] 15.10, 15.04 (2),[c] 11.24, 10.54, 9.63, 9.33	12	CH_3	36[d]	1.508 (3), 1.462 (3), 1.136 (3), 1.082 (3), 1.042 (3), 1.024 (3), 0.916 (3), 0.873 (3), 0.862 (6), 0.738 (3), 0.393 (3)
atom total[e]	50 carbons		80 hydrogens	

[a] IR spectrum shows ester band. [b] ^{1}H NMR spectrum shows that these four methines are in a para-disubstituted benzene ring. [c] In $CDCl_3$ at 75 MHz; peak doubled in acetone-d_6. [d] Two CH_3-C, seven CH_3-CH, and three CH_3-CH_2. [e] At least 8 nitrogens and 12 oxygens were present.

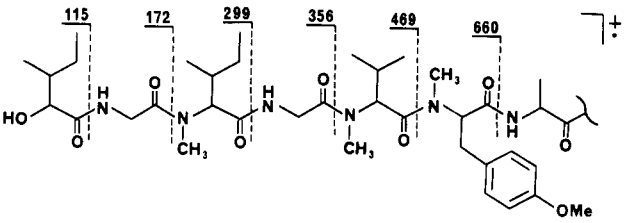
tailed analysis of both the ^{13}C and ^{1}H NMR spectra (Table I). The NMR data established that the eight nitrogens could be accounted for by five secondary amide and three tertiary *N*-methyl amide groups. In addition to a band at 1680 cm^{-1} for amide carbonyl, the IR spectrum showed a band at 1742 cm^{-1}, characteristic of ester carbonyl. The ^{13}C NMR spectrum indicated that a ketone group was also present, but the IR spectrum did not immediately verify this. The twelve oxygens, however, could be accounted for by eight amide carbonyls, an ester group, a ketone carbonyl, and an aromatic methoxy group. The preliminary data suggested that majusculamide C was a cyclic depsipeptide composed of eight amino acid units and one hydroxy acid unit. Spin–spin decoupling experiments, a two-dimensional Jeener spectrum,[9] and a two-dimensional carbon–proton chemical shift correlation spectrum[9] confirmed this proposal and further suggested that six of the amino acid units were two glycyl units, two alanyl units, one *N*-methylvalyl unit, and one *N*,*O*-dimethyltyrosyl unit (Table II). The presence of the *N*,*O*-dimethyltyrosyl unit was supported by the UV spectrum which showed peaks at 230 and 278 nm.

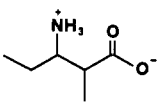
Acid hydrolysis with 6 N hydrochloric acid produced eight degradation products. Five of the compounds were known amino acids, identified as glycine, alanine, *N*-methylvaline,[4] *N*,*O*-dimethyltyrosine,[4] and *N*-methylisoleucine[10] by direct comparison with authentic samples. The sixth compound was a new amino acid, 3-amino-2-methylpentanoic acid (**2**). The seventh product was shown to be 2-hydroxy-3-methylpentanoic acid (isoleucic acid); when acid hydrolysis was carried out with 2 N hydrochloric acid, however, 2-hydroxy-3-methylpentanoylglycine (**3**) was obtained. Finally, the eighth product was found to be 2-amino-4-methylpentanone (**4**, isolated as the hydrochloride) which had to have 4-amino-2,2-dimethyl-3-oxopentanoic acid (**5**), a new amino acid, as its precursor to explain the presence of a quaternary carbon with two methyl groups on it in the intact fungicide; the structure

Chart I



Scheme I

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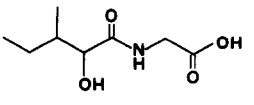
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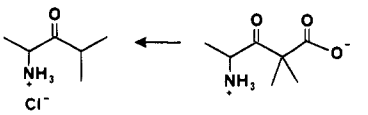
4 5

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(10) *N*-Methylisoleucine was synthesized using the procedure described by: (a) Quitt, P.; Hellerbach, J.; Vogler, K. *Helv. Chim. Acta* **1963**, *46*, 327. (b) Quitt, P.; Studer, R. O.; Vogler, K. *Ibid.* **1963**, *46*, 1715.

Table II. Correlated ^{13}C and ^{1}H NMR Spectral Data and Proton-Proton NOE Data for Majusculamide C[a]

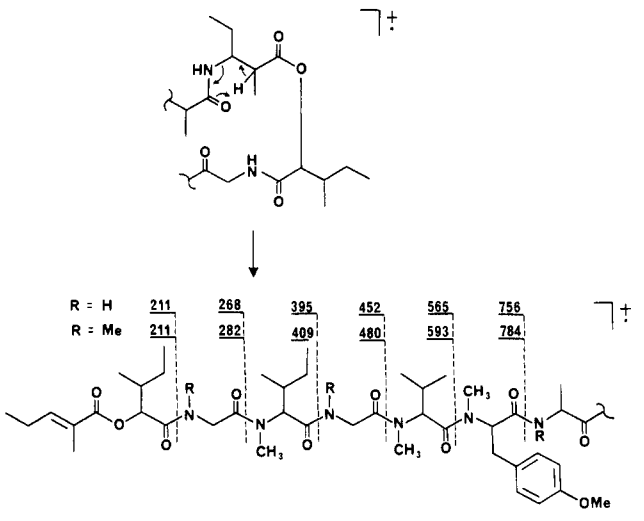
structure	assignment	shift, δ ^{13}C[b] (mult)	shift, δ ^{1}H,[c] (mult; J, Hz)	^{1}H-^{1}H NOE
1a	1	169.44 (s)		
	2	42.07 (d)	2.735 (qd; 7.0, 2.6)	
	3	50.78 (d)	4.509 (dtd; 10.2, 5.7, 2.6)	2, 4, 6, 6′
	4		7.068 (d; 10.2)	9
	5	9.33 (q)	1.082 (d; 7.0)	
	6	25.57 (t)	1.56 (m), 1.41 (m)	3, 5
	7	10.54 (q)	0.916 (t, 7.4)	
1b	8	172.25 (s)		
	9	47.86 (d)	4.42 (m)	4, 10
	10		7.767 (d, 8.1)	9, 18
	11	15.10 (q)	1.042 (d, 6.6)	
1c	12	169.75 (s)		
	13	54.41 (s)		
	14	209.57 (s)		
	15	51.17 (d)	4.893 (p; 6.8)	16, 17, 18, 19
	16		7.309 (d; 6.7)	15, 21
	17	21.03 (q)	1.462 (s)	15
	18	21.72 (q)	1.508 (s)	10
	19	18.68 (q)	1.136 (d; 6.8)	
1d	20	167.44 (s)		
	21	60.56 (d)	5.132 (t; 7.3)	16, 23, 23′, 25, 33
	23	34.17 (t)	3.237 (dd; −14.1, 7.3), 2.792 (dd; −14.1, 7.3)	
	24	128.18 (s)		
	25, 29	113.79 (d)	7.129 (d; 8.5)	
	26, 28	129.94 (d)	6.834 (d; 8.5)	
	27	158.17 (s)		
	30	54.78 (q)	3.738 (s)	
	31	29.90 (q)	3.201 (s)	
1e	32	168.81 (s)		
	33	57.67 (d)	4.777 (d; 10.7)	21, 35, 36,[d] 37,[d] 41[d]
	35	26.58 (d)	2.250 (ds; 10.7, 6.6)	
	36	18.02 (q)	0.738 (d; 6.6)	
	37	18.02 (q)	0.393 (d; 6.6)	
	38	28.85 or 28.78 (q)	2.944 or 2.938 (s)	
1f	39	171.51 (s)		
	40	40.17 (t)	4.606 (dd; −17.6, 7.4), 3.445 (dd; −17.6, 1.0)	33,[d] 40′, 41
	41		7.573 (br d; 7.4, 1.0)	40, 40′, 43
1g	42	170.52 (s)		
	43	60.65 (d)	4.888 (d; 11.1)	41, 45
	45	36.91 (d)	2.07 (m)	
	46	24.49 (t)	1.41 (m), 1.08 (m)	
	47	9.63 (q)	0.862 (t; 7.7)	
	48	15.04 (q)	1.024 (d; 6.2)	
	49	28.85 or 28.78 (q)	2.944 or 2.938 (s)	
1h	50	169.64 (s)		
	51	40.34 (t)	4.437 (dd; −15.9, 5.4), 3.541 (dd; −15.9, 5.4)	51′, 52
	52		7.355 (t; 5.4)	51, 51′, 54
	53	172.09 (s)		
	54	77.87 (d)	5.186 (d; 3.2)	52,[d] 55
	55	32.34 (d)	2.05 (m)	
	56	23.30 (t)	1.44 (m), 1.22 (m)	
	57	11.24 (q)	0.862 (t; 7.7)	
	58	15.04 (q)	0.873 (d; 7.5)	

[a] In $CDCl_3$. [b] $CDCl_3$ as internal reference (δ 76.90). [c] Residual $CHCl_3$ as internal reference (δ 7.25). [d] Weak NOE.

of **4** was verified by synthesis via **5**. The acid hydrolysis products described above implied that the eight structural units **1a–h** (Chart I), which account for all of the atoms in the molecular formula, were present in the majusculamide C molecule.

Sequencing units **1d–h** into an expanded structure was achieved by examining the mass spectrum of saponified majusculamide C (Scheme I). Fragment ions could be seen at m/z 469, 356, 299, and 172, corresponding to consecutive losses of units **1d–g** from the m/z 660 ion to give the m/z 172 ion ($^{+}O{\equiv}CCH_2NHC(O)CH(OH)CH(Me)CH_2CH_3$). Additional support of the **1d–1e–1f–1g–1h** sequence was obtained from the mass spectrum of majusculamide C (Scheme II). Fragment ions were observed at m/z 565, 452, 395, 268, and 211, corresponding to sequential losses of units **1d**, **1e**, **1f**, **1g**, and **1f** from the m/z 756 ion to produce a nonnitrogenous m/z 211 ion. Since the hydroxy acid portion of unit **1h** had to be present in the m/z 211 ion, we rationalized that the m/z 211 ion could only be formed if unit **1h** was connected via an ester linkage to unit **1a**. The mass spectral fragmentation of majusculamide C could then be explained by an initial β

Scheme II



elimination of the amido group on C-3 through a McLafferty rearrangement to give an acyclic molecular ion which could then fragment at various amide linkages along the peptide chain. The sequence of the remaining two units **1b** and **1c**, however, could not be determined from the mass spectral data.

Total sequencing of units **1a–h** into gross structure **1** for majusculamide C was accomplished by proton–proton NOE studies (Table II). Most importantly, one could clearly conclude from these studies that (1) **1a** had to be connected to **1b** since positive NOEs could be seen between H-4 and H-9, (2) **1b** was attached to **1c** since positive NOEs could be observed between 3H-18 and H-10 and between 3H-17 and H-15, and (3) **1c** was joined to **1d** since positive NOEs were shown between H-16 and H-21. The studies provided further support for the **1d–1e–1f–1g–1h** sequence since positive NOEs were seen between H-21 and H-33, H-33 and H-41, H-41 and H-43, and H-52 and H-54.

Relative and Absolute Stereochemistry. The absolute configurations of the alanine, *N*-methylvaline, and *N*-methylisoleucine from acid hydrolysis of majusculamide C were all found to be L. Each of these amino acids in 0.125 N hydrochloric acid showed a positive Cotton effect at about 225 nm in its qualitative ORD curve.[11] The *N*-methylisoleucine exhibited a positive CD curve at 211 nm. L-*N*-Methylvaline and L-*N*-methylisoleucine in 0.5 N hydrochloric acid have been reported to show positive Cotton effects, viz., $[\phi]_{225}$ +3442 and $[\phi]_{225}$ +3553, respectively, in the ORD curves and $[\theta]_{211}$ +5801 and $[\theta]_{212}$ +5146, respectively, in the CD curves.[12] The *N*,*O*-dimethyltyrosine from acid hydrolysis of majusculamide C was found to have a dextrorotatory optical rotation, opposite in sign to that of the levorotatory D-*N*,*O*-dimethyltyrosine obtained from acid hydrolysis of majusculamides A and B.[4] This amino acid was therefore L as synthetic L-*N*,*O*-diemthyltyrosine has an optical rotation of $[\alpha]_D$ +17.5° in 1 N HCl.[4]

The relative and absolute stereochemistry of 3-amino-2-methylpentanoic acid (**2**) is unknown at this writing. A small quantity of a diastereomer of **2** appears to be present in the sample, suggesting that epimerization of C-2 might be occurring during hydrolysis.

The 2-amino-4-methylpentanone obtained from the hydrolyzate appears to be racemic since the ORD and CD curves are flat between 240 and 330 nm.

(11) Jennings, J. P.; Klyne, W.; Scopes, P. M. *J. Chem. Soc.* **1965**, 294.
(12) Shoji, J. *J. Antibiot.* **1973**, *26*, 302.

The ^{1}H NMR spectrum of the 2-hydroxy-3-methylpentanoic acid was compared with spectra of synthetic samples of the two diastereomeric acids to decide the relative stereochemistry. The absolute stereochemistry was therefore 2*S*,3*S* since the CD curve was positive at 209 nm. All 2(*S*)-hydroxyalkanoic acids are reported to exhibit positive Cotton effects around 210 nm in their CD curves.[13] 2(*S*)-Hydroxy-3(*S*)-methylpentanoic acid (L-isoleucic acid)[14] in aqueous acid buffered at pH 2.5 is reported to show a positive Cotton effect, $[\theta]_{208}$ +3500, in its CD curve.[13]

Experimental Section

Spectral Analysis. NMR spectra were determined at 300 MHz for proton NMR and 75 MHz for carbon-13 NMR on a Nicolet NT-300 spectrometer. Proton chemical shifts were referenced in $CDCl_3$ to the residual $CHCl_3$ signal (7.25 ppm) and in D_2O to added *p*-dioxane (3.747 ppm from DSS). Homonuclear ^{1}H connectivities were determined by using the phase-cycled 16-step COSY experiment as described by Bax.[9] Confirmatory information was obtained from difference double-resonance experiments when necessary. ^{1}H–^{13}C connectivities were determined from the phase-cycled 16-step heteronuclear chemical shift correlation experiment.[9] Carbonyl carbon assignments were obtained from a set of selective ^{1}H decoupling experiments where the NH and Hα resonances were irradiated; broad-band decoupling between scans was used to retain the ^{13}C–{^{1}H} Overhauser enhancement. Qualitative homonuclear ^{1}H NOEs were obtained by selective continuous irradiation (decoupler on, hetero mode) for three seconds, followed by data acquisition and a recycling delay (decoupler off) for 3 s; off-resonance experiments were also performed in a similar manner, and the NOE enhancements were observed in difference spectra produced by subtracting on-resonance spectra from off-resonance spectra.

EI mass spectra, including high-resolution mass measurements, were determined on a Varian MAT-311 instrument. ORD curves were recorded on a Cary 60 spectropolarimeter.

Isolation. *Lyngbya majuscula* was collected in the lagoon of Enewetak Atoll in the Marshall Islands. One collection was made from Reefer 8 Pinnacle in Sept 1975 at depths ranging from 35 to 60 ft. The frozen alga (3 kg wet weight) was extracted with chloroform/methanol (1:2). The mixture was filtered, the filtrate was diluted with water, and the chloroform layer was washed repeatedly with water, dried over anhydrous sodium sulfate, and evaporated to give 22 g of crude extract. The extract, dissolved in chloroform, was chromatographed on a 4.7 cm × 40 cm column of Florisil (<200 mesh). The chromatogram was developed with hexane (400 mL), mixtures of hexane/chloroform (200 mL of 9:1, 300 mL of 3:1, 400 mL of 1:1), chloroform (400 mL), and chloroform/methanol mixtures (400 mL of 9:1, 400 mL of 3:1, 200 mL of 1:1). After 1.25 L of eluant had passed through the column, two fractions, A (1250–1500 mL) and B (1500–1725 mL), were collected. Evaporation of fractions A and B, both of which contained comparable amounts of majusculamide C by ^{1}H NMR analysis, provided 2.14 and 1.92 g of material, respectively. Gel filtration of fraction A on a 1.5 cm × 1.15 m column of Sephadex LH-20 with chloroform/methanol (1:1) gave 1.69 g of crude majusculamide C which was eluted immediately after the porphyrins that were in the fraction.

Another collection was made from Reefer 8 and South Medren Pinnacles in early Dec 1981 at depths ranging from 50 to 75 feet. The freeze-dried alga (10.5 kg) was extracted with 2-propanol/dichloromethane (1:1) to give 210 g of extract. Gel filtration of the extract (14 15-g portions) on a 9 cm × 101 cm column of Sephadex LH-20 with 2-propanol/dichloromethane (1:1) yielded in the combined 1900–2500-mL fractions a total of 45 g of material which was further fractionated by reverse-phase chromatography in 3-g portions on a 2.5 cm × 75 cm column of phenyl Porasil B with a 60–100% methanol/water gradient.

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Several fractions were collected and analyzed by ^1H NMR spectroscopy to detect the majusculamide C. Final purification of the majusculamide C was achieved by HPLC with a Whatman Partisil M9 ODS-2 or ODS-3 reverse-phase column by using an acetonitrile/water (7:3) mobile pase (30 mg/injection); 5 g of pure majusculamide C was obtained: $[\alpha]_D$ −96° (c 2.5, CH_2Cl_2); UV (EtOH) λ_{max} 278 nm (ϵ 1420), 230 (5900); IR (KBr) ν_{max} 3336, 1742, 1680, 1643 cm^{-1}; EIMS (probe, 70 eV), m/z (relative intensity) 756 (13), 596 (38), 565 (62), 452 (6), 395 (80), 275 (9), 268 (5), 211 (6), 204 (28), 164 (12), 161 (19), 155 (12), 121 (13), 114 (100); high-resolution EIMS, m/z 756.4348 (calcd for $C_{40}H_{62}N_5O_9$ 756.4548), 565.3570 (calcd for $C_{29}H_{49}N_4O_7$ 565.3601), 452.2730 (calcd for $C_{23}H_{38}N_3O_6$ 452.2761), 395.2529 (calcd for $C_{21}H_{38}N_2O_5$ 395.2546), 211.1327 (calcd for $C_{12}H_{19}O_3$ 211.1334); FDMS, m/z 984 (M^+); SIMS, m/z 985 (M + 1).

Hydrolysis. (A) With 6 N Hydrochloric Acid. A solution of majusculamide C (50 mg) in 6 N HCl was refluxed under nitrogen for 16 h, washed with dichloromethane, and freeze–dried. The residual hydrolyzate was subjected to gel filtration on a 70 × 1.5 cm column of Sephadex LH-20 with water, and four fractions were collected, viz.: a ninhydrin-active, UV-transparent fraction 1 at 100–130 mL; a 280-nm UV-absorbing fraction 2 at 130–150 mL; another 280-nm UV-absorbing fraction 3 at 150–175 mL; a 230-nm UV-absorbing fraction 4 at 175–200 mL.

Separation of fraction 1 on a 27 × 1.5 cm cellulose column by using *n*-butanol/acetic acid/water (80:20:20) gave 2.7 mg of *N*-methylvaline (eluted from 40 to 41 mL) [^1H NMR (D_2O + DCl) δ 3.853 (1 H, d, J = 4.2 Hz), 2.763 (3 H, s), 2.30 (1 H, m), 1.086 (3 H, d, J = 7.0 Hz), 1.037 (3 H, d, J = 7.0 Hz)], 3.2 mg of alanine (eluted from 57 to 65 mL) [^1H NMR (D_2O, + DCl) δ 4.142 (1 H, q, J = 7.3 Hz), 1.564 (3 H, d, J = 7.3 Hz)], and 2.5 mg of glycine (eluted from ~70–80 mL) [^1H NMR (D_2O + DCl) δ 3.888 (s)]. Purification of fraction 2 on cellulose by using *n*-butanol/acetic acid/H_2O (80:20:20) gave 3.2 mg of *N*-methylisoleucine (eluted from 38 to 40 mL) [^1H NMR (D_2O + DCl) δ 3.949 (1 H, d, J = 3.7 Hz), 2.766 (3 H, s), 2.070 (1 H, m), 1.550 (1 H, m, J_{gem} = −13.6), 1.38 (1 H, m, J_{gem} = −13.6), 1.001 (3 H, d, J = 7.0 Hz), 0.956 (3 H, t, J = 7.5 Hz)] and 3.6 mg of 3-amino-2-methylpentanoic acid (**2**, eluted from 36 to 37 mL) (^1H NMR D_2O + DCl) δ 3.515 (1 H, m), 2.940 (1 H, qd, J = 7.4, 4.0 Hz), 1.74 (1 H, d of quintets, J_{gem} = −14.6 Hz), 1.67 (1 H, d of quintets, J_{gem} = −14.6 Hz), 1.242 (3 H, d, J = 7.4 Hz), 0.997 (3 H, t, J = 7.5 Hz)]. Fraction 3 gave 1.4 mg of 2-amino-4-methyl-3-pentanone hydrochloride (**4**): ^1H NMR (D_2O + DCl) δ 4.443 (1 H, q, J = 7.5 Hz), 3.017 (1 H, septet, J = 6.9 Hz), 1.552 (3 H, d, J = 7.4 Hz), 1.142 (3 H, d, J = 6.9 Hz), 1.093 (3 H, d, J = 6.9 Hz). Fraction 4 gave 3.8 mg of *N,O*-dimethyltyrosine.

The CH_2Cl_2 wash above was treated with excess CH_2N_2. Evaporation yielded methyl 2-hydroxy-3-methylpentanoate which was purified by HPLC on a Whatman Partisil M9 column by using ethyl acetate as the eluant: ^1H NMR ($CDCl_3$) δ 4.04 (1 H, d, J = 4 Hz), 3.74 (3 H, s), 1.21 (2 H, m), 0.92 (3 H, d, J = 7 Hz), 0.82 (3 H, t, J = 7 Hz).

In a second experiment 23 mg of hydrolyzate was fractionated into its components by reverse-phase HPLC on a Whatman M9 column of Partisil 10/50 ODS-3 with 0.1% trifluoroacetic acid in H_2O. At a flow rate of 4 mL/min the order of elution and retention times were as follows: 6.0 mg of glycine and alanine (6 min), 2.5 mg of *N*-methylvaline (8–11 min), 4.1 mg of **2** and **4** (15 min), 3.6 mg of *N*-methylisoleucine (29 min), 1.1 mg of *N*-methyltyrosine (42 min). The 90% MeOH–H_2O flush (8.3 mg) was a mixture of *N,O*-dimethyltyrosine and 2(*S*)-hydroxy-3-(*S*)-methylpentanoic acid (L-isoleucic acid); the hydroxy acid was separated from the amino acid by short-path distillation.

(B) With 2 N Hydrochloric Acid. A solution of majusculamide C (25 mg) in 2 N HCl/ethanol (6:1) was refluxed for 24 h. Extraction with dichloromethane yielded an amorphous solid which was chromatographed (HPLC) on a Whatman Partisil M9 column with ethyl acetate to give 2.7 mg of 2-hydroxy-3-methylpentanoylglycine: ^1H NMR $CDCl_3$) δ 6.88 (1 H, br t), 4.3 (2 H, q, J = 7 Hz), 4.16 (3 H, m), 1.84 (1 H, m), 1.5–1.1 (2 H, m), 1.30 (3 H, t, J = 7 Hz), 0.99 (3 H, d, J = 7 Hz), 0.86 (3 H, t, J = 7 Hz).

Permethylation. A solution of majusculamide C (5 mg) in 1 mL anhydrous dimethylformamide was treated with 0.3 g of silver oxide and 0.5 mL of methyl iodide.[15] The mixture was stirred continuously for 3 days at room temperature and filtered. The filtrate was dissolved in CH_2Cl_2, and the resulting mixture was washed with a dilute aqueous KCN solution, followed by water. The organic layer was evaporated in vacuo and analyzed by mass spectrometry: EIMS (probe, 70 eV), m/e (relative intensity) 1055 (15), 1054 (25), 985 (10), 884 (15), 883 (28), 784 (15), 593 (13), 480 (13), 410 (20), 409 (17), 297 (13), 283 (25), 282 (100), 274 (18), 260 (28), 247 (13), 232 (20), 218 (55), 211 (30), 197 (23), 191 (18), 186 (13), 185 (23), 184 (25), 183 (23), 170 (63), 169 (55), 168 (93), 164 (38), 161 (63), 156 (20), 155 (35), 154 (38), 142 (45), 141 (25), 140 (40), 134 (23), 128 (25), 126 (23), 122 (25), 121 (95), 113 (35), 112 (30); high-resolution MS, m/z 784.4695 (calcd for $C_{42}H_{16}N_5O_9$ 784.4860), 593.3825 (calcd for $C_{31}H_{53}N_4O_7$ 593.3914), 480.3054 (calcd for $C_{25}H_{42}N_3O_6$ 480.3074), 409.2669 (calcd for $C_{22}H_{37}N_2O_5$ 409.2703), 282.1699 (calcd for $C_{15}H_{24}O_4N$ 282.1705).

Saponification. A solution of majusculamide C (2 mg) in 2 mL of 0.1 N NaOH and 2 mL of methanol was stirred for 5.5 h. The solution was acidified to litmus with HCl and freeze–dried. The freeze–dried residue was dissolved in ethanol and the solution was filtered and treated with CH_2N_2 to give the methyl ester of the acyclic hydroxy acid resulting from cleavage of the lactone in majusculamide C; EIMS (probe, 70 eV), m/z (relative intensity) 866 (1), 853 (1), 856 (1), 756 (10), 660 (15), 625 (10), 614 (17), 613 (12), 612 (16), 499 (27), 486 (12), 469 (47), 460 (6), 454 (14), 427 (6), 410 (6), 381 (7), 356 (14), 324 (16), 299 (84), 286 (16), 276 (27), 275 (68), 274 (12), 269 (10), 241 (10), 224 (10), 213 (16), 210 (37), 207 (12), 205 (40), 204 (100), 196 (14), 185 (41), 183 (21), 182 (27), 181 (16), 177 (16), 172 (78), 171 (21), 170 (27).

Synthesis of 2-Amino-4-methylpentanone Hydrochloride. A mixture of ethyl carbonate (5 g), methyl ethyl ketone (3 g), and NaH (1 g) was stirred for 7 h at room temperature. Ethanol was carefully added to quench the condensing agent, and the resulting mixture was acidified with acetic acid and partitioned between ethyl acetate and water. The organic layer was concentrated under reduced pressure at room temperature to remove EtOAc and other volatile components. The higher boiling concentrate was distilled under aspirator vacuum at 109–112 °C to give 1 g of ethyl 3-oxopentanoate.

The keto ester was dissolved in 50 mL of 2 M NaOEt in ethanol, and 1.5 mL of methyl iodide was added slowly over a period 30 min at room temperature under a nitrogen atmosphere. The reaction was quenched with acetic acid and the mixture evaporated to dryness in vacuo to give a residue which was triturated with CH_2Cl_2. The CH_2Cl_2 extract was evaporated to give crude ethyl 2,2-dimethyl-3-oxopentanoate which was treated without purification with acetic acid (20 mL) and sodium nitrite (1 g) for 24 h. This reaction mixture was evaporated to dryness in vacuo and the residue distributed between ethyl acetate and H_2O. The ethyl acetate layer was evaporated to give crude keto oxime which was treated with excess zinc, acetic acid (20 mL), and acetic anhydride (20 mL) at 0 °C for 1 h. The reaction mixture was filtered through a small silica gel column and the filtrate evaporated in vacuo. The resulting crude amide was purified by HPLC on a Whatman Partisil M9 column with ethyl acetate to give ethyl 4-acetamido-2,2-dimethyl-3-oxopentanoate: ^1H NMR ($CDCl_3$) δ 6.21 (1 H, br s), 4.98 (1 H, pentuplet, J = 8 Hz), 4.25 (2 H, q, J = 8 Hz), 1.97 (3 H, s), 1.43 (3 H, s), 1.39 (3 H, s), 1.27 (3 H, d, J = 8 Hz), 1.24 (3 H, t, J = 8 Hz).

Hydrolysis of the ester amide and decarboxylation of the intermediate β-keto acid with 6 N HCl for 24 h gave 2-amino-4-methyl-3-pentanone hydrochloride which was identical with the degradation product from majusculamide C.

Synthesis of 2(*S*)-Hydroxy-3(*S*)-methylpentanoic Acid. L-Isoleucine in 4 N HCl was treated with excess $NaNO_2$ at 0 °C. Continuous extraction with ether gave an oil which was subjected to short-path distillation. Separation of the two diastereomeric acids in the distillate was achieved by reverse-phase HPLC on a Whatman Partisil 10/50 ODS-3 column with 4:1 methanol–H_2O. 2(*S*)-Hydroxy-3(*S*)-methylpentanoic acid (L-isoleucic acid) was eluted first: ^1H NMR ($CDCl_3$) δ 4.172 (1 H, d, J = 3.6 Hz), 1.883 (1 H, m), 1.42 (1 H, dqd, J = −13.6, 7.4, 4.6 Hz), 1.30 (1 H, dqd, J = −13.6, 7.4, 9.3 Hz), 1.020 (3 H, d, J = 6.9 Hz), 0.918 (3 H, t,

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J = 7.4 Hz); CD (aqueous HCl, pH 2.5), $[\theta]_{209}$ +2950. 2(*R*)-Hydroxy-3(*S*)-methylpentanoic acid (D-alloisoleucic acid) was eluted second: ^{1}H NMR ($CDCl_3$) δ 4.222 (1 H, d, J = 6.4 Hz), 2.099 (1 H, m), 1.63 (1 H, dqd), 1.35 (1 H, dqd), 1.051 (3 H, d, J = 6.8 Hz), 0.926 (3 H, t, J = 7.5 Hz); negative CD curve at 209 nm in aqueous HCl.

The hydroxy acid from the acid hydrolyzate of majusculamide C had a ^{1}H NMR spectrum that was identical with that of isoleucic acid and a positive CD curve, $[\theta]_{209}$ +2990 (aqueous HCl, pH 2.5).

L-*N*-Methylisoleucine. Sublimed synthetic amino acid[10] showed a positive CD curve, $[\theta]_{211}$ +6880 (0.5N HCl). The literature[12] reports $[\theta]_{212}$ +5146. The crude amino acid from the acid hydrolyzate of majusculamide C showed a positive Cotton effect, $[\theta]_{211}$ +4140 (0.5 N HCl).

Acknowledgment. This research was supported by Grant CHE79-25416 from the Natonal Science Foundation. High-frequency NMR studies at the University of Hawaii were made possible by NSF Grant CHE81-00240. We thank Dr. Kenneth Straub at the University of California Bio-organic, Biomedical Mass Spectrometry Facility (A. L. Burlingame, Director), supported by NIH Grant RR00719, for determining the SIMS and field-desorption mass spectra.

Registry No. 1, 83712-17-8; 4, 79851-67-5; ethyl 4-acetamido-2,2-dimethyl-3-oxopentanoate, 87883-44-1; 2(*S*)-hydroxy-3(*S*)-methylpentanoic acid, 51576-04-6.

Arenarol and Arenarone: Sesquiterpenoids with Rearranged Drimane Skeletons from the Marine Sponge *Dysidea arenaria*

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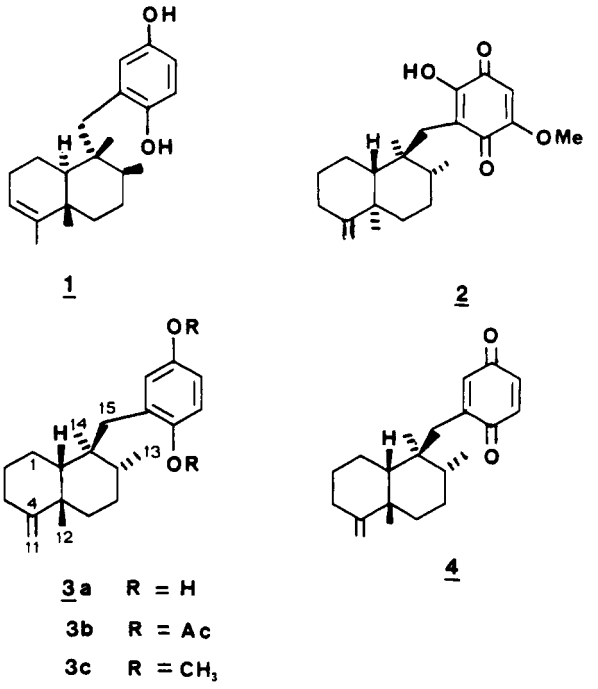
Received May 25, 1983

Two new sesquiterpenoids, arenarol (**3a**) and arenarone (**4**), having identical rearranged drimane skeletons bearing hydroquinol and hydroquinone moieties, respectively, are reported from the Pacific sponge *Dysidea arenaria*. The structure of arenarol was determined by X-ray analysis of its diacetate which crystallizes in the space group $P2_12_12_1$ with unit-cell dimensions (138 K) a = 9.964 (5), b = 6.81 (2), and c = 33.33 (5) Å. The crystal structure was determined from 2268 data. The final R value is 0.065. Arenarol was chemically correlated with arenarone by silver oxide oxidation. Detailed 400-MHz ^{1}H NMR analysis of arenarol assisted by 2-D J-resolved and 2-D homocorrelation spectroscopy is tabulated.

Marine algae and sponges have yielded a variety of compounds having a hydroquinone moiety attached to a terpenoid skeleton, the latter varying from sesqui-, di-, or sesterterpene units and also from acylic propyprenyl chains.[1] Included among these are two sesquiterpenes which have enantiomeric rearranged drimane skeletons, avarol[2] (**1**) from the Mediterranean sponge *Dysidea avara* and illimaquinone[3] (**2**) from the Pacific sponge *Hippiospongia metachromia* (Chart I). In our continuing search[4] for cytotoxic compounds from marine organisms, we have isolated from the Pacific sponge *Dysidea arenaria* a mildly cytotoxic hydroquinone–quinone pair of compounds, arenarol (**3a**) and arenarone (**4**), having the same rearranged sesquiterpene skeleton as avarol and illimaquinone, but with *cis*- rather than *trans*-decalin stereochemistry. In this paper we describe the structures of the new compounds as established by X-ray analysis and chemical interconversion.

Specimens were frozen shortly after collection at -9 M at Truk Island lagoon and kept frozen until the workup. Chloroform–methanol (1:1) extracts of freeze–dried specimens were partitioned between chloroform and water, and

Chart I



the chloroform solubles were chromatographed in chloroform–methanol over Sephadex LH-20. Selected fractions were purified further by column chromatography over silica gel and finally by high-pressure liquid chromatography (HPLC) over a C_{18} bonded-phase column with 15%

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