Phytochemistry, Vol. 27, No. 10, pp. 3101–3103, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00

MAJUSCULAMIDE D AND DEOXYMAJUSCULAMIDE D, TWO CYTOTOXINS FROM *LYNGBYA MAJUSCULA*

RICHARD E. MOORE* and MICHAEL ENTZEROTH

Department of Chemistry, University of Hawaii, Honolulu, HI 96822, U.S.A.

(*Received* 21 *January* 1988)

Key Word Index—*Lyngbya majuscula*; Oscillatoriaceae; blue-green alga; lipopentapeptides; cytotoxins.

Abstract—Majusculamide D and deoxymajusculamide D are cytotoxic lipopentapeptides that are minor constituents of a deep-water variety of the marine blue-green alga *Lyngbya majuscula* from Enewetak.

INTRODUCTION

The strongly cytotoxic, antifungal cyclic depsipeptide, majusculamide C, is a major constituent of a variety of *Lyngbya majuscula* that grows abundantly on the pinnacles in the lagoon of Enewetak Atoll in the Marshall Islands [1]. When majusculamide C was isolated from the lipophilic extract of this blue-green alga by gel filtration followed by reverse-phase HPLC, two moderately cytotoxic acyclic lipopentapeptides, majusculamide D (**1**) and deoxymajusculamide D (**2**), were found in the HPLC fraction eluted immediately after majusculamide C.

RESULTS AND DISCUSSION

The molecular compositions of **1** and **2** were determined to be $C_{43}H_{65}N_5O_{10}$ and $C_{43}H_{65}N_5O_9$, respectively. The positive FAB mass spectra of **1** and **2** showed MH^+ ions at *m/z* 812 and 796, respectively, and the secondary ion mass spectra showed $[M+Na]^+$ ions at *m/z* 834 and 818. Detailed ^{1}H NMR analysis of the two cytotoxins (Table 1) suggested the presence of units **1a–1f** in **1** and units **2a–2f** in **2**. This was supported by ^{13}C NMR spectral data for **2**; for example, the chemical shifts associated with units **2b–2e** compared favourably with those of other peptides containing these amino acid residues [1, 2] and the chemical shifts for unit **2f**, viz. 14.0 (C-46), 22.9 (C-45), 28.8 or 28.9 (C-44), 37.0 (C-43), 30.1 (C-42), and 19.3 (C-48), agreed closely with calculated values [3], viz. 13.9, 22.9, 29.7, 36.7, 30.5, and 20.1, respectively.

Total sequencing of units **1a–1f** and **2a–2f** into gross structures **1** and **2** was achieved from the following mass spectral and difference NOE ^{1}H NMR spectral data. The positive FAB and SIMS of **1** and **2** showed prominent fragment ions at *m/z* 602, 542 (loss of HOAc from *m/z* 602 ion), 489, 429 [489 – HOAc], and 346, strongly suggesting the sequences depicted by structures **1** and **2**. Permethylation of **1** and **2** led to dimethylmajusculamide D (Me groups on N-22 and O-10) and methyldeoxymajusculamide D (Me on N-22) which showed MH^+ ions at *m/z* 840 and 810, respectively, in the FAB mass spectra. The FABMS of the permethylated compounds further showed +14 mass units shifts for all the fragment ion peaks mentioned above except the one at *m/z* 346. Further proof that units **1d** and **1e** (or **2d** and **2e**) were attached to each other was indicated by positive NOEs between the protons on N-22 and C-28. Unit **1b** was linked to **1c** (and **2b** to **2c**) since irradiation of H-14 produced a positive NOE on the two hydrogens on C-11.

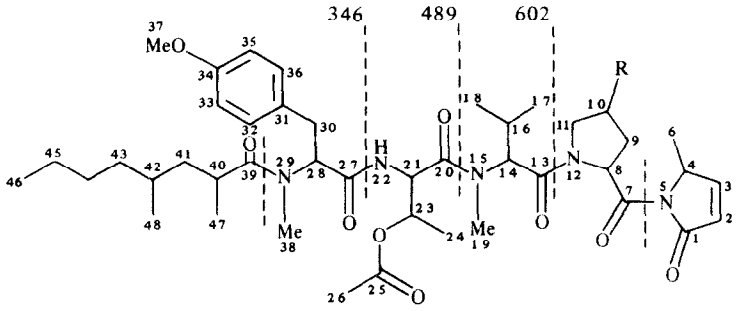
The stereochemistry of **2** was established by chemical degradation. Vigorous acid hydrolysis led to a mixture of amino acids which was separated into threonine, proline, *N*-methylvaline, and *N*,*O*-dimethyltyrosine by reverse-phase HPLC on Whatman Partisil ODS-3 with 0.1% trifluoroacetic acid in H_2O. The threonine was determined to be L by GC analysis [4, 5]. The proline, *N*-methylvaline, and *N*,*O*-dimethyltyrosine were all shown to be L by CD analysis [1]. When **2** was subjected to ozonolysis (oxidative work-up with H_2O_2) prior to vigorous acid hydrolysis, however, alanine was also obtained from the amino acid hydrolysate. GC analysis indicated that the alanine was L and therefore the absolute configuration of C-4 in **2** had to be *S*. Unfortunately we were not able to determine the absolute stereochemistry of C-40 in **2**. The 2,4-dimethyloctanoic acid from the acid hydrolysis of **2** was obtained impure and in relatively poor yield, and an optical rotation or CD spectrum could not be detected. The ^{1}H NMR spectrum of **2** in benzene-d_6 showed couplings of 4.3, 10.2, 3.4, and 9.7 Hz for $J_{40,41}$ (*syn*), $J_{40,41'}$ (*anti*), $J_{41',42}$ (*syn*), and $J_{41,42}$ (*anti*), respectively; however, it was not possible to rigorously establish the relative stereochemistry of C-40 and C-42 from these data.

Hydrolysis studies were not carried out on **1**. Comparison of the proton–proton coupling constants for unit **1b** matched those for *cis*(*allo*)-hydroxyproline and not those for *trans*-hydroxyproline [6].

Both compounds exhibited moderate cytotoxicity in the CCRF-CEM cell culture system at 0.2 μg/ml.

EXPERIMENTAL

^{1}H NMR spectra were obtained at 300 MHz in $CDCl_3$ and ^{13}C NMR spectra at 75 MHz in $CDCl_3$. Chemical shifts are reported in units (ppm) relative to the solvent as internal standard for both ^{1}H (7.25 ppm) and ^{13}C (76.9 ppm). ^{1}H–^{13}C connectivities were determined from a phase cycled 16 step heteronuclear chemical shift correlation map (CSCM) experiment [7].



1 R = OH
2 R = H

Table 1. ^{1}H NMR data for **1** and **2**

	Assignment	Chemical Shift, δ* 1	2		Assignment	Chemical Shift, δ*† 1	2
	2	7.256 *dd*	7.210		28	5.566 *dd*	5.552
a	3	6.069 *dd*	6.039		30	2.959 *dd*	2.939
	4	4.794 *m*	4.740		30′	3.140 *dd*	3.135
	6	1.452 *d*	1.438	e	32, 36	7.081 *d*	7.074
					33, 35	6.758 *d*	6.747
	8	5.649 *dd*	5.442 *dd*		37	3.743 *s*	3.733
	9	2.010 *dt*‡	1.834 *m*		38	3.069 *s*	3.077
b	9′	2.458 *ddd*	2.410 *m*				
	10	4.353 *m*	1.920, 1.990 *m*				
	11	3.803 *dd*‡	3.700 *m*§		40	2.635 *dq d*	2.625 (2.46)
	11′	3.847 *dd*	3.775 *m*§		41	0.87‖ *m*	0.86 (0.832)
					41′	1.624 *dd d*	1.612 (1.936)
	14	4.990 *d*	5.006		42	0.87 *m*	0.86 (0.94)
	16	2.242 *m*	2.237		43	1.11 *br m*	1.15
c	17	0.799 *d*	0.777	f	44	1.2 *br m*	1.2
	18	0.978 *d*	0.974		45	1.21 *br m*	1.23
	19	2.909 *s*	2.902		46	0.869 *br t*	0.859
					47	1.048 *d*	1.037(1.06)
	21	4.954 *dd*	4.965		48	0.490 *d*	0.490 (0.522)
	22	7.041 *br d*	7.035				
d	23	5.243 *qd*	5.230				
	24	1.151 *d*	1.146				
	26	1.969 *s*	1.971				

J(Hz) for **1**: 2,3 = 6.0; 2,4 = 2.1; 3,4 = 1.6; 4,6 = 6.6; 8,9 = 2.4; 8.9′ = 10.2; 9,9′ = −14.4; 9,10 = 2; 9′,10 = 4.4; 10,11 = 4.4; 10,11′ = 1.5; 11,11′ = −11.7; 14,16 = 10.7; 16,17 = 16,18 = 6.6; 21,22 = 9.0; 21,23 = 2.7; 23,24 = 6.6; 28,30 = 11.1; 28,30′ = 5.2; 30,30′ = −14.6; 32,33 = 35,36 = 8.7; 40,41 = 4.4; 40,41′ = 9.5; 40,47 = 6.6; 41,41′ = −13; 41′,42 = 3.5; 42,48 = 6.2; 45,46 = 7.5. *J*(Hz) for **2**: essentially identical with those for 1 except 8,9 = 4.9; 8,9′ = 8.6; 9,9′ = −12.6; 10,10′ = −12.9; 10,11 = 10,11′ = 10′,11 = 10′,11′ = 7.0; 11,11′ = −10.2.

*300 MHz, $CDCl_3$, residual $CHCl_3$ as internal reference = 7.25.

†δ values in parentheses are in benzene-d_6, residual benzene-d_5 as internal reference = 7.15.

‡*cis* to OH on C-10 and *trans* to H on C-8.

§Assignments may be reversed.

‖*syn* to H on C-40 and *anti* to H on C-42.

Isolation. *Lyngbya majuscula* was collected in the lagoon of Enewetak Atoll in the Marshall Islands. Freeze-dried alga (10.5 kg) was processed for majusculamide C as previously described [1]. Majusculamide D (**1**) and deoxymajusculamide D (**2**) were eluted successively from the ODS-3 column immediately after majusculamide C. Repeated reverse-phase HPLC on a Whatman Partisil M9 ODS-3 column yielded pure **1** (5×10^{-3}% of the dried alga) and normal phase, gradient elution HPLC on Whatman Partisil column with 0–2% MeOH/CH_2Cl_2 resulted in pure **2** (5×10^{-4}%).

Majusculamide D had the following spectral properties: FABMS *m/z* 812 $[M]^+$, 602, 542 [602 − HOAc], 489, 429, [489 − HOAc]; high resolution FABMS *m/z* 812.476 (Calcd for $C_{43}H_{66}N_5O_{10}$, 812.481); SIMS *m/z* 834 [M + Na], 602, 542, 489, 429. Deoxymajusculamide D had the following spectral properties: FABMS *m/z* 796 $[MH]^+$, 602, 542, 489, 429; high resolution FABMS *m/z* 796.483 (calcd for $C_{43}H_{66}N_5O_9$, 796.486); SIMS *m/z* 818 $[M+Na]^+$, 602, 542, 489, 429; ^{13}C NMR ($CDCl_3$) δ 177.7 (*s*, C-1), 171.8 (*s*, C=O), 170.4 (*s*, C=O), 169.6 (*s*, C=O), 169.4 (*s*, C=O) 169.3 (*s*, C=O), 168.1 (*s*, C=O), 158.1 (*s*, C-34), 153.6 (*d*, C-2), 129.5 (*d*, C-32 and C-36), 128.6 (*s*, C-31), 125.3 (*d*, C-3), 113.7 (*d*, C-33 and C-35), 68.7 (*d*, C-23), 59.9 (*d*, C-8), 59.1 (*d*, C-14), 57.9 (*d*, C-4), 56.3 (*d*, C-28), 54.9 (*q*, C-37), 51.9 (*d*, C-21), 47.8 (*t*, C-11), 41.5 (*t*, C-41), 37.0 (*t*, C-43), 33.3 (*d*, C-40), 32.3 (*t*, C-30), 30.5 and 30.4 (quartets, C-19 and C-38), 30.1 (*d*, C-42), 28.9 and 28.8 (triplets, C-9 and C-44), 27.2 (*d*, C-16), 24.4 (*d*, C-10), 22.9 (*t*, C-45), 20.8 (*q*, C-26), 19.3 (*q*, C-48), 18.7 (*q*, C-18), 18.3 (*q*, C-17), 18.0 (*q*, C-47), 17.0 (*q*, C-6 and C-24), 14.0 (*q*, C-46).

Acid hydrolysis of deoxymajusculamide D. A soln of **2** (18 mg) in 2 ml of constant boiling HCl was heated at 100–110° in a sealed tube for 20 hr. The mixture was evapd to dryness and the residual acid hydrolysate dissolved in 10 ml of H_2O. The resulting soln was extracted with several portions of CH_2Cl_2 to remove crude 2,4-dimethyloctanoic acid (2.4 mg) and then freeze-dried to give 15 mg of amino acids. Reverse-phase HPLC on a Whatman M9 column of Partisil 10/50 ODS-3 with 0.1% CF_3COOH in H_2O resulted in (order of elution) threonine (3 mg), proline (3 mg), *N*-methylvaline (3 mg) and *N,O*-dimethyltyrosine (2 mg). Each of these amino acids in 0.25 M HCl showed a positive Cotton effect in the CD spectrum, with $[\theta]_{211-212}$ ranging roughly from +3200 to +5600.

In a second experiment, 18 mg of **2** was dissolved in 5 ml of MeOH and cooled to −78°. The soln was treated with excess ozone. When no starting material could be detected by TLC, 0.5 ml of 30% H_2O_2 was added and the soln was allowed to warm up to 0°. After 10 min at 0°, the excess H_2O_2 was destroyed by adding a small amount of Pt catalyst. The Pt was filtered off and the filtrate evapd to dryness. The crude ozonolysis product was then subjected to acid hydrolysis as described above and the mixture of amino acids separated on ODS-3 with 0.1% CF_3COOH in H_2O. In addition to threonine, proline, *N*-methylvaline, and *N,O*-dimethyltyrosine, alanine was found in the fraction (4.2 mg) containing threonine. This mixture of alanine and threonine was converted to *N*-trifluoroacetyl methyl ester derivatives [4]. Separation of these *N*-TFA methyl esters was achieved by GC on a 1.75 m × 2 mm column of 5% SP 300 (*N*-lauroyl-L-valine-*t*-butylamide) on 100/120 Supelcoport with a He flow rate of 20 ml/min and a column temp. of 110°. Retention times of the *N*-TFA methyl esters were 4.32 min (L-alanine) and 8.31 min (L-threonine).

Acknowledgements—This research has been supported by PHS Grant CA12623, awarded by the National Cancer Institute, DHHS. The authors thank Dr Kenneth Straub for determining the FAB and SI mass spectra at the University of California Mass Spectrometry Resource (A. L. Burlingame, director) supported by NIH Grant RR00719.

REFERENCES

1. Carter, D. C., Moore, R. E., Mynderse, J. S., Niemczura, W. P. and Todd, J. S. (1984) *J. Org. Chem.* **49**, 236.
2. Christl, M. and Roberts, J. D. (1972) *J. Am. Chem. Soc.* **94**, 4565.
3. Levy, G. C. and Nelson, G. L. (1972) *Carbon-13 Nuclear Magnetic Resonance for Organic Chemists.* Wiley-Interscience, New York.
4. Cliffe, J., Berridge, N. J. and Westgarth, D. R. (1973) *J. Chrom.* **78**, 333.
5. Charles, R., Beitler, U., Feibush, B. and Gil-Av, E. (1975) *J. Chrom.* **112**, 121.
6. Abraham, R. J. and McLauchlan, K. A. (1962) *Mol. Phys.* **5**, 195, 513.
7. Bax, A. (1982) *Two-Dimensional Nuclear Magnetic Resonance in Liquids.* Delft, Holland.