ISOLATION AND STRUCTURE OF THE CELL GROWTH INHIBITORY DEPSIPEPTIDES DOLASTATINS 11 AND 12.[1a,b]

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Abstract - Two antineoplastic cyclic depsipeptides designated dolastatin 11 (**1**) and dolastatin 12 (**2**) were isolated from the Indian Ocean sea hare *Dolabella auricularia*. Dolastatins 11 and 12 inhibited growth of the PS leukemia with ED_{50} 2.7 x 10^{-3} and 7.5 x 10^{-2} μg/mL respectively. Detailed structural studies employing 400 MHz $^1H,^1H$-COSY, $^1H,^{13}C$-COSY, NOE, and HMBC experiments (in addition to acid and base hydrolysis, followed by GC-MS analysis) led to structure **1** for dolastatin 11 and to structure **2** for dolastatin 12.

Among the aplysiomorphs, the Indo-Pacific *Dolabella* genus of sea hares contains the world's largest (to 2 kg) species. We have found another species in this genus, *D. auricularia*, to produce a series of novel antineoplastic peptides designated dolastatins 1-10.[2] Of these, the remarkable pentapeptide dolastatin 10[2a] appears to be the most potent (low dose) substance presently known against the U.S. National Cancer Institute's (NCI) experimental (*in vivo*) melanomas. Among the many scientific challenges offered by the dolastatins is whether or not some (or all) are derived from dietary sources,[3] or represent true biosynthetic products of this versatile marine animal. We now wish to report the isolation and structural elucidation of two new cyclic depsipeptides, dolastatins 11 and 12, which exhibit cell growth inhibitory activity (ED_{50} 2.7 x 10^{-3} and 7.5 x 10^{-2} μg/mL respectively) against the murine P388 lymphocytic leukemia (PS system)[4] and provide the first evidence for a blue-green algae dietary origin.[5]

A combined ethanol-2-propanol extract prepared from *ca* 1600 kg of wet *D auricularia* (collected in 1982) was concentrated and subjected to an initial separation by solvent partitioning.[6] The 2.75 kg methylene chloride active fraction was separated (PS bioassay guided) by an extensive series of gradient column chromatographic (large scale HPLC on silica gel, size exclusion and partition on Sephadex, small scale HPLC and adsorption on silica gel) procedures. Dolastatin 11 (**1**) was isolated as a colorless amorphous powder (43.6 mg, 3 x 10^{-6} % yield) from hexane - methylene chloride: R_f 0.57 in methylene chloride - methanol - water 90 : 10 : 0.8; mp 134- 137° C; $[\alpha]_D^{26}$ -143.9° (c=0.33, CH_2Cl_2); uv (CH_3OH) λ_{max} (log ε) : 223 (3.28), 277 (3.34), and 283 (3.28) nm ; ir (KBr) : ν_{max} 3334, 1740, 1681, 1644, 1514, 1497, 1410, 1249, 1179, and 1033 cm-1 ; high resolution FAB MS· 985.6070 ($[M+H]^+$ for $C_{50}H_{80}N_8O_{12}$), 660, 549, 535, 469, 356,

299, 160, 100, 86. Dolastatin 12 (2) was isolated as a colorless amorphous powder (10.6 mg, 6 x 10^{-7} % yield) from hexane - methylene chloride: R_f 0.50 in the 90:10:0.8 solvent; mp 130 - 135° C; $[\alpha]_D^{24}$ -98.0° (c=0.01, CH_3OH); uv (CH_3OH) λ_{max} (log ε): 224 (3.82), 275 (shoulder) nm; ir (KBr): ν_{max} 3340, 1740, 1680, 1652, 1636, 1530, 1470, 1408, 1288, 1250, 1176, and 1090 cm^{-1}; and FAB MS: 969 $[M+H]^+$.

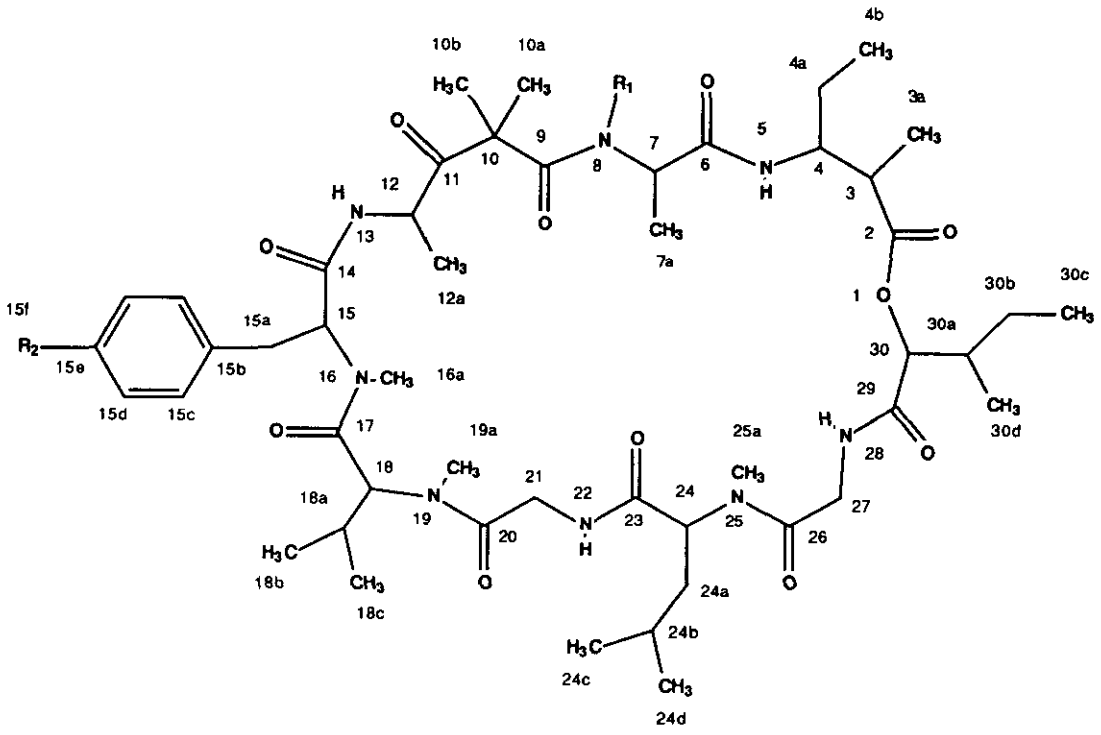
Preliminary examination of the proton and carbon nmr spectra indicated that dolastatin 11 was primarily a peptide. Using a combination of $^1H,^1H$-COSY, $^1H,^{13}C$-COSY, and $^1H,^1H$-relayed COSY,[7] all of the spin systems were derived. The presence of two glycine (Gly), two alanine (Ala), one N-methylleucine (MeLeu), one O,N-dimethyltyrosine (O,N-diMeTyr), and one N-methylvaline (MeVal) units, was thus deduced. Glycine and alanine were also detected by amino acid analysis of the dolastatin 11 acid hydrolysis (6 N HCl; 110 °C; 24 h) product. Three other structural units were found: isobutyric acid (Ibu), 2-hydroxy-3-methylpentanoic acid (Hmp), and the β-amino acid, 2-methyl-3-aminopentanoic acid (Map). The sequence of dolastatin 11 was first realized using nOe experiments and further established by the sensitivity enhanced heteronuclear multiple bond correlation experiment (HMBC).[8]

As recorded in Table 1, the chemical shifts for three of the ten carbonyl groups were unresolvable in the HMBC experiment. Hence, a complete sequence determination by this method was not possible. Nevertheless, the presence of the following three fragments were unambiguously derived : -O,N-diMeTyr-(Ala-Ibu)-Ala-Map-O-Hmp- , -MeLeu-Gly-MeVal- , and -Gly-.

In turn, these segments were assembled to yield two possible sequences forming cyclic depsipeptides. Only one of the sequences was in agreement with the observed nOe's and structure **1** was established for dolastatin 11. Analysis of the collision activated spectra[9] of selected FAB produced ions from dolastatin 11 and from its saponification (0.1 N NaOH; 5.5 h, rt) product afforded the same conclusion. Furthermore, the all L-pentapeptide hydrochloride Gly-MeLeu-Gly-MeVal-O,N-diMeTyr-OMe ($[\alpha]_D^{25}$ +66.7° (c= 0.5, $CHCl_3$), mp 182-184 °C dec) was synthesized and its nmr characteristics were found quite comparable with that segment of dolastatin 11.

Spectral analyses of dolastatins 11 and 12 gave almost identical results. Inspection of the 2D spectra (^1H and ^{13}C) suggested differences in only two units: the N-8 amide proton of the dolastatin 12 Ala unit was found replaced by a methyl group, and the Tyr methoxyl group by a hydrogen to give the phenylalanine analog. These minor structural modifications were in accord with the observed mass of dolastatin 12 being 16 a.m.u. lower than that of dolastatin 11 (**1**) and led to structure **2** for dolastatin 12.

Interestingly, Moore and colleagues[5] isolated, and nicely determined the structure of a cyclic depsipeptide named majusculamide C from a deep water (to 30 m) variety of the toxic blue-green algae *Lyngbya majuscula* (Enewetak Atoll, Marshall Islands) that only differs from dolastatin 11 in having Ile in place of the C-23, 24 Leu unit. Now it seems likely that the Indian Ocean *D.auricularia* has a blue-green algae source for at least dolastatins 11 and 12 or their immediate biosynthetic precursors. The apparent close relationship of dolastatins 11 and 12 to majusculamide C suggests they may share the same chirality. Our synthesis of the all-L pentapeptide segment and the resultant spectral comparison also points in that direction.



1: $R_1 = H$; $R_2 = CH_3O$, Dolastatin 11
2: $R_1 = CH_3$; $R_2 = H$, Dolastatin 12

Even more importantly, except for Ile *vs* Leu the high field (400 MHz) ^1H- and ^{13}C- nmr chemical shifts for dolastatin 11 and majusculamide C were nearly identical. In addition, these observations provide further strong support for the majusculamide C structural proposal.[5] The Ala, Ile, Tyr, and Val units of majusculamide were found to possess the usual L-configuration while the pentanoic acid unit carried 2-*S*-hydroxyl and 3-*S*-methyl groups. The remaining asymmetry was not established and will require appropriate synthetic endeavors.

Table 1. Nmr assignments, selected nOe's, and HMBC correlations for dolastatin 11 (1) in $CDCl_3$ solution .*

	no	^{13}C	^{1}H (mult., J(Hz))		^{1}H-^{1}H NOE's	HMBC (^{1}H to ^{13}C no.)
Map	2	172.61				
	3	42.43	2.79	dq, 7.2, 2.6		2, 3a, 4
	3a	9.86	1 10	d, 7 0, 3H	3, 5	2, 3, 4
	4	51.38	4.47	br	4a, 4b, 7a	
	4a	25.87	1.46		3a, 4b, 5	
			1.56			
	4b	10.93	0.93	t, 7 4, 3H	4	
	5		7.09	d, 10 6	3a, 4a, 4b, 7	4, 6
Ala-1	6	172.78				
	7	48.30	4.44	quint, 6.9	5, 8	
	7a	15.53	1.07	d, 7.0, 3H	7, 8	6, 7
	8		7 78	d, 8.3	7, 7a, 10b	7, 7a, 9
Ibu	9	171 94				
	10	54.93				
	10a	21.63	1 44	s, 3H	12, 13	9, 10, 10b, 11
	10b	21.99	1.49	s, 3H	8	9, 10, 10a, 11
Ala-2	11	209.73				
	12	51.18	4.91	quint, 6 9	10b, 13	11
	12a	19 22	1.13	d, 7.0, 3H	12, 13	11, 12
	13		7.15	d, 9.0	12, 12a, 15, 15a, 16a	11, 12, 14
Me_2Tyr	14	168.00				
	15	61 07	5.11	dd, 8.4, 6.6	12a, 13, 15c, 18	14, 15a, 15b, 16a, (1
	15a	34.68	3.25	dd, 14 1, 6 6	15, 15c, 15d	
			2.82	dd, 14 1, 8 4		14, 15, 15b, 15c
	15b	128.65				
	15c	130.36	7.14	d, 8 7, 2H	15d, 15f	
	15d	114.35	6 81	d, 8.7, 2H		
	15e	158.74				
	15f	55.27	3 75	s, 3H	15c	
	16a	29.35	2 96	s, 3H		15, (17)
MeVal	17	170.12 ‡				
	18	58.23	4.78	d, 10 7	15, 18a, 18b, 18c, 19a	(17), 18a, 19a
	18a	27.05	2.23		18, 19a	
	18b	18 49	0.74	d, 6 7, 3H	18a	
	18c	18 44	0 37	d, 6 6, 3H	18a	18, 18a, 18b
	19a	29.15	2.95	s, 3H	18b, 21	18, 20
Gly-1	20	169.31				
	21	41 09	4.42	dd, 17 9, 2 1	19a	20
			3.60	dd, 17 9, 7 4	19a, 22	20
	22		7 41	dd, 7.4, 2 1	21, 24	20, 23
MeLeu	23	171.71				
	24	54 67	5.37	dd, 11 1, 4.9	22, 24a, 24b, 24c, 25a	23, 24a, 24b, 25a, (2
	24a	38.13	1 87	ddd, 13.3, 11 1, 3.9	24, 24c, 24d, 25a	
			1.62		24, 24c, 24d, 25a	
	24b	24 90	1.56			
	24c	21.43	0.92	d, 6.4, 3H		
	24d	23.15	0 98	d, 6.5, 3H		24a, 24b, 24c
	25a	30.15	3.14	s, 3H	24a, 27	24, (26)
Gly-2	26	169.98 ‡				
	27	40.70	4.46	dd, 16.1, 7 0	25a, 28	
			3.58	dd, 16 1, 4 7	25a, 28	
	28		7.35	dd, 7.2, 4 6	27, 30	
Hmp	29	170.09 ‡				
	30	78 38	5.19	d, 3.3	3, 28, 30a, 30b, 30c, 30d	2, (29), 30a, 30b, 30
	30a	37.39	2 07		30, 30b, 30c	
	30b	23.83	1.47		30, 30c, 30d	
			1 23		30, 30a	
	30c	11.61	0 88	t, 7.5, 3H	30, 30a, 30b	
	30d	15.46	0.89	d, 7.0, 3H	30, 30a, 30b	

* *with overlapping signals, no multiplicity was recorded in Table 1.*

‡ *these signals may be interchanged.*

Biological evaluation and total synthetic approaches to dolastatins 11 and 12 are in progress. So far dolastatin 11 has provided a 25% life extension in the NCI PS leukemia at 300-600 μg/kg.

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