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Communications

Antineoplastic Agents. 190. Isolation and Structure of the Cyclodepsipeptide Dolastatin 14[1]

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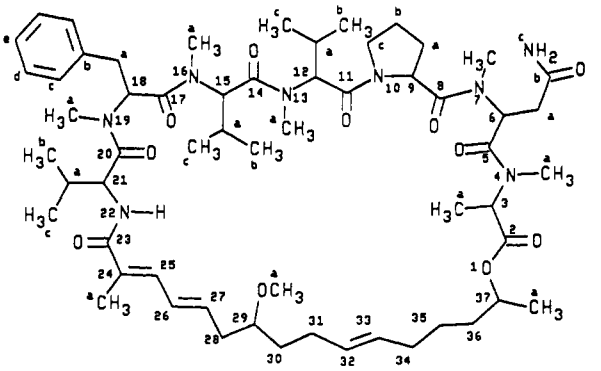
Summary: The Indian Ocean sea hare *Dolabella auricularia* has been found to contain a novel cytostatic (PS ED_{50} 0.022 μg/mL) cyclodepsipeptide designated dolastatin 14. Structure 1 was assigned dolastatin 14 and was deduced primarily by a series of 2D NMR studies at high field (400 MHz).

The auriculate rhinophores (sensory tentacles resembling a hare's ears) prominent as appendages on sea hares (Aplysiidae family) may be used for tasting. If those organs are also responsible for selection of *Dolabella auricularia*'s (Indian Ocean) most impressive array[2] of antineoplastic and cytostatic constituents (and/or their biosynthetic precursors), unraveling the biochemical mechanisms involved would be quite enlightening. Discovery of cyclodepsipeptides dolastatins 11, 12, and 15 and assignment of structures suggested possible dietary sources among the blue-green algae.[2a-d] We have now found *D. auricularia* to contain another new and structurally intriguing cytostatic cyclodepsipeptide designated dolastatin 14 (1) that may also be of blue-green algae origin. The very productive investigations of these little-explored microorganisms by Moore and colleagues have led to the isolation of interesting cyclopeptides with potentially important biological properties, such as the calcium antagonist scytonemin A.[3]

Dolastatin 14 was found to inhibit cell growth of the NCI murine P388 lymphocytic leukemia (PS system) with ED_{50} 0.022 μg/mL. After the isolation of dolastatin 13 ((6×10^{-8})% yield from 1000 kg of wet sea hare), we explored by bioassay (PS) directed separation minor fractions near dolastatin 10 which led to pure dolastatin 14 (1) as an



DOLASTATIN 14

amorphous solid (12.0 mg total, (1.2×10^{-7})% yield) from methanol: mp 123–125 °C; $[\alpha]^{24}_D$ −146° (c = 0.14, CH_3OH); TLC (R_f 0.35 in 90:10:0.8 CH_2Cl_2–CH_3OH–H_2O); UV (CH_3OH) λ_{max} 211 (ε 23 420) and 262 (ε 14 160) nm; IR (NaCl plate) ν_{max} 3320, 2963, 2930, 2875, 1732, 1670, 1640,

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(2) (a) Pettit, G. R.; Kamano, Y.; Dufresne, C.; Cerny, R. L.; Herald, C. L.; Schmidt, J. M. *J. Org. Chem.* **1989**, *54*, 6005–6006. (b) Pettit, G. R.; Kamano, Y.; Herald C. L.; Dufresne, C.; Cerny, R. L.; Herald, D. L.; Schmidt, J. M.; Kizu, H. *J. Am. Chem. Soc.* **1989**, *111*, 5015–5017. (c) Pettit, G. R.; Kamano, Y.; Kizu, H.; Dufresne, C.; Herald, C. L.; Bontems, R.; Schmidt, J. M.; Boettner, F. E.; Nieman, R. A. *Heterocycles* **1989**, *28*, 553–558. (d) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinman, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, K. B.; Bontems, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 6883–6885. (e) Pettit, G. R.; Kamano, Y.; Fujii, Y.; Herald, C. L.; Inoue, M; Brown, P.; Gust, D.; Kitahara, K.; Schmidt, J. M.; Doubek, D. L.; Michel, C. *J. Nat. Prod.* **1981**, *44*, 482–485.

(3) Carter, D. C.; Moore, R. E.; Mynderse, J. S.; Niemczura, W. P.; Todd, J. S. *J. Org. Chem.* **1984**, *49*, 236–241.

0022-3263/90/1955-2989$02.50/0

Table I. Dolastatin 14 Correlated ^{13}C and ^{1}H NMR Assignments in Dichloromethane-d_2 Solution[a]

structure assignment	^{13}C	chemical shift, ppm, ^{1}H mult (J, Hz)	structure assignment	^{13}C	chemical shift, ppm ^{1}H mult (J, Hz)
2	171.0		18b	137.7	
3	55.8	4.82 q (7.0)	18c	129.9 × 2	7.22 m
3a	16.1	1.45 d (7.0)	18d	128.6 × 2	7.21 m
4a	29.9	2.75 s	18e	126.9	7.19 m
5	169.4		19a	31.1	3.11 s
6	49.7	5.77 dd (9.3, 5.3)	20	172.9	
6a	35.5	2.37 dd (15.3, 5.3)	21	55.2	4.65 t (9.9)
		2.99 dd (15.3, 9.3)	21a	30.8	1.95 d heptet (9.9, 6.6)
6b	172.1		21b	18.6	0.90 d (6.7)
6c		5.25 brs, 5.75[b]	21c	19.1	0.64 d (6.5)
7a	30.5	2.93 s	22		6.01 d (9.9)
8	171.6		23	169.3	
9	57.6	4.66 (dd (8.8, 4.0)	24	128.4	
9a	28.7	1.67 m, 2.02 m	24a	12.9	1.88 (2.2)
9b	25.1	1.67 m, 1.87 m	25	134.7	6.70 dq (11.2, 2.2)
9c	47.7	3.52 m, 3.68 m	26	127.8	6.35 dd (14.9, 11.2)
11	168.6		27	138.0	5.99 ddd (14.9, 8.8, 6.1)
12	60.1	4.89 d (10.8)	28	37.1	2.30 m, 2.48 m
12a	27.2	2.08 d heptet (10.8, 6.7)	29	80.0	3.27 m
12b	18.1	0.50 d (6.8)	29a	56.9	3.32 s
12c	19.8	0.88 d (6.5)	30	34.4	1.47 m
13	30.4	2.64 s	31	29.7	2.05 m
14	170.3		32	131.2	5.44 dt (15.1, 6.1)
15	58.9	5.01 d (10.7)	33	130.8	5.38 dt (15.1, 6.1)
15a	27.9	2.15 d heptet (10.7, 6.7)	34	33.1	2.02 m
15b	18.5	0.72 d (6.8)	35	26.6	1.30 m
15c	19.7	0.78 d (6.5)	36	35.6	1.37 m, 1.63 m
16a	29.9	2.67 s	37	73.1	4.79 m
17	169.6		37a	19.8	1.20 d (6.1)
18	55.0	5.74 dd (8.4, 7.0)			
18a	35.6	2.83 dd (13.7, 7.0)			
		3.25 dd (13.7, 8.4)			

[a] Residual $CHDCl_2$ as internal reference (δ 5.32). [b] Overlapping signal.

1510, 1455, 1405, 1360, 1315, 1100, 785, and 700 cm^{-1}; high-resolution SP-SIMS[4] 1089.7009 $[M + H]^+$, $C_{59}H_{93}N_8O_{11}$ requires [M + H] 1089.6964. Amino acid analyses suggested the presence of valine and proline.

In contrast to our prior experience with utilizing collision-activated decomposition (MS/MS) of HREI ions from dolastatin-type peptides to assist in structural elucidation, the mass spectral fragmentation of dolastatin 14 (1) gave primarily obtuse results. However, an unequivocal structure (1) for dolastatin 14 was eventually deduced by combining various high-field (400 MHz) NMR techniques that included $^{1}H,^{1}H$ COSY, $^{1}H,^{13}C$ COSY (consult Table I), and $^{1}H,^{1}H$-relayed COSY.[5] By means of these methods the new hydroxy hexadecenoic acid (herein named dolatrienoic acid) and amino acid units were ascertained. Two of the olefin segments of dolatrienoic acid were clearly E as the respective vinyl protons were coupled with J = 15 Hz. The E configuration for the trisubstituted double bond was derived from the very large $^{1}H-[^{1}H]$ NOE difference experiment value involving the C-24a methyl protons and the proton at C-26.

The sequence of dolastatin 14 components was established primarily by results of COSY, HETCOR, NOE, and heteronuclear multiple bond correlation (HMBC)[6] NMR experiments in methylene-d_2 chloride. The most difficult connectivity problems were solved as follows. Lack of an N-10 to C-11 connection in the HMBC spectra was circumvented by the strong NOE's observed in both directions between the protons at C-9c and C-12. In methylene-d_2 chloride the carbonyl group chemical shifts of C-23 and C-5 fell within 0.1 ppm of one another and did not allow an unambiguous linkage assignment in the ring system. However in acetone-d_6 the difference increased to 0.3 ppm, and their connections were then safely established using HMBC methods. When the C-6a proton absorbing at δ 2.99 was irradiated, strong NOE's were observed for both amide protons at N-6c, thereby locating this otherwise elusive amino group.

Based on our complete structure determinations for dolastatins 3,[7] 10,[1,2d] and 15,[2a] the Phe, Pro, and Val amino acid units of dolastatin 14 may bear the usual S configuration. Conclusive evidence for these assumptions and chiral assignments for the N-Me-Ala, N-Me-Asn, and dolatrienoic acid units will have to await larger scale reisolation and/or synthesis of dolastatin 14. Present biological results for this extraordinary cyclodepsipeptide indicate such future efforts will be quite productive.

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(4) (a) Holzapfel, C. W.; Pettit, G. R.; Cragg, C. M. *J. Nat. Prod.* **1985**, *48*, 513–522. (b) Pettit, G. R.; Holzapfel, C. W.; Cragg, G. M.; Herald, C. L.; Williams, P. *J. Nat. Prod.* **1983**, *46*, 971–922.
(5) Bax, A.; Drobny, G. *J. Magn. Reson.* **1985**, *61*, 306.
(6) Bax, A.; Summers, M. A. *J. Am. Chem. Soc.* **1986**, *108*, 2094.
(7) Pettit, G. R.; Kamano, Y.; Holzapfel, C. W.; van Zyl, W. J.; Tuinman, A. A.; Herald, C. L.; Baczynskyj, L.; Schmidt, J. M. *J. Am. Chem. Soc.* **1987**, *109*, 7581–7582.