Isolation and Structure of the Cytostatic Linear Depsipeptide Dolastatin 15[1a]

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Summary: The Indian Ocean sea hare *Dolabella auricularia* has been found to contain a strongly cytostatic depsipeptide constituent designated dolastatin 15. The unusual depsipeptide was found to markedly inhibit growth of the P388 lymphocytic leukemia cell line with ED_{50} = 0.0024 μg/mL. The structure was elucidated by employing primarily high-field (400-MHz) 2D NMR and high-resolution mass spectrometry.

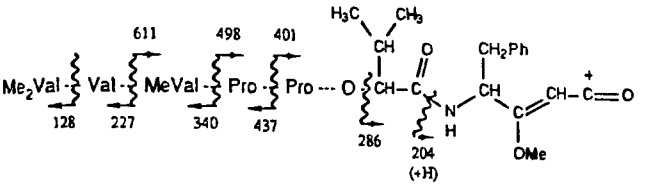
Sir: The marine mollusca phylum has proved to be a very productive source of in vitro cytostatic[2] and in vivo antineoplastic[3] substances of unprecedented molecular types. Analogous structural advances are occurring for other areas of biological activity[4] and more generally in Mollusca natural products chemistry.[5] We now report that the Western Indian Ocean shell-less mollusc *Dolabella auricularia* has provided another unusual and strongly cytostatic depsipeptide constituent, herein named dolastatin 15, active against the U.S. National Cancer Institute's P388 lymphocytic leukemia (PS system)[6] cell line with ED_{50} = 0.0024 μg/mL.

Dolastatin 15 was located (PS bioassay) in a trace fraction (6.2 mg, (4×10^{-7})% yield, from 1600 kg of wet sea hare),[3b] moving slightly faster than dolastatin 10 in a methylene chloride–methanol solvent system on silica gel. Final purification by HPLC on RP8 silica gel (gradient elution with 1:1 methanol–water, 100% methanol) and precipitation from methanol afforded a pure specimen of dolastatin 15 (1) as an amorphous powder: mp 143–148 °C; $[\alpha]^{26}$ −26° (*c* 0.01, CH_3OH); R_f 0.60 in 90:10:0.8 methylene chloride–methanol–water; high-resolution SP-SIMS[7] $[M + H]^+$ 837.5135, calcd 837.5136 for $C_{45}H_{68}N_6O_9$; UV (CH_3OH) λ_{max} (log ε) 220 (3.11), 242 (2.90) nm; IR (NaCl plate): ν_{max} 2960, 2930, 2860, 1730, 1690, 1665, 1630, 1440, 1305, 1245, and 1180 cm^{-1}. The presence of valine (Val), *N*-methylvaline (MeVal), and dolavaline (Dov)[3b] was ascertained by high-field (400-MHz) NMR using ^1H–^1H COSY, ^1H–^{13}C COSY and ^1H–^1H-relayed COSY.[8] Application of the preceding 2D NMR techniques followed by a homonuclear Hartman–Hahn 2D experiment (HOHAHA, recorded with a 33-ms MLEV-17 mixing scheme)[9] unambiguously established the presence of two proline (Pro) units and a 2-hydroxyisovaleric acid (Hiva) unit. By subtracting the elemental composition of the six units thereby derived from the molecular formula of dolastatin 15, the seventh unit was found to correspond to $C_{12}H_{12}NO_2$. By NMR this segment was found to contain a phenyl ring, an isolated olefinic methine, a methoxyl group, and an isolated $-CH_2CH-$ as recognizable spin systems. Further structural deductions required NOE and heteronuclear multiple bond correlation (HMBC)[10] NMR procedures. A series of ^1H–[^1H] NOE difference experiments indicated the phenyl ring was attached to the methylene group forming part of a phenylalanine system and that the methoxyl group was located near the olefinic methine proton. After examining results of the HMBC experiment (Figure 1), final assembly of the subunits became possible and revealed a hitherto unknown phenylalanine biosynthetic product designated dolapyrrolidone (Dpy). Presumably, Dpy might originate biosynthetically from *N*-acetylphenylalanine methyl ester by intramolecular condensation. Related pyrrolidone C-terminal units have previously been found in the *Streptomyces* antibiotic althiomycin,[11] the sponge and blue-green algae component dysidin,[12,13]

Table I. Dolastatin 15 (1) NOE and HMBC Correlations

^1H-position	NOE	HMBC
2	3b	1, 4
3b	2	3
4		2, 3
4a	4c	3
7		6, 9
10		9
13	10, 12, 16	
16	13, 18, 19	15
18	16, 19	
19	22	
22	19	21, 24
22b	26	
23a	22a, 22b, 25	22, 24
25		24
26	28, 29ab	27
28	25, 26	27
29ab		28

Scheme I. Mass Spectral Fragmentation of Dolastatin 15



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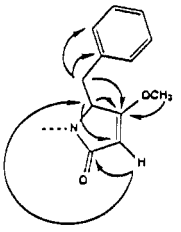
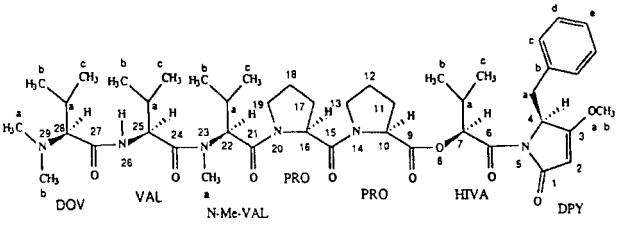


Figure 1. Selected HMBC correlations for the dolapyrrolidone unit.

and the blue-green algae constituents of the malyngamide[13] and pukeleimide[14] types.

Assignment of the ^1H and ^{13}C NMR chemical shifts for dolastatin 15 has been entered in ref 15. Sequence determination of dolastatin 15 (1) was achieved using ^1H-[^1H] NOE and long-range proton carbonyl coupling information from the HMBC experiment (Figure 1 and Table I). The assigned structure (1) was further supported by collision-activated decomposition (MS/MS)[3b] of the SP-SIMS ions combined with HREI mass spectral results



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(see Scheme I). The absolute configuration was established by a combination of X-ray crystal analysis and synthetic procedures that will be summarized in a future report.[16]

Appearance of the pyrrolidone methyl vinyl ether group in dolastatin 15 and in constituents of the *Lyngbya* genus of blue-green algae provides further circumstantial evidence[3a] that *Dolabella auricularia* may be obtaining and/or structurally modifying constituents of such cyanobacteria. Should this exogenous procurement of potent cell growth inhibitory and/or antineoplastic substances eventually be proven correct, the ability of *D. auricularia* to store and/or produce potent cell growth inhibitory and antineoplastic substances of unusual structure is extraordinary.[2a,3] Indeed if these biosynthetic products are eventually shown to be obtained by exogenous procurement, the sensing and selection mechanisms of this sea hare must be magnificent. Further studies concerned with biological evaluations and structural modifications of dolastatin 15 are in progress.

Acknowledgment. With pleasure we thank for financial support Grants CA-16049-05-12 awarded by the NCI, DHHS, the Fannie E. Rippel Foundation, the Robert B. Dalton Endowment, Eleanor W. Libby, The Waddell Foundation (Donald Ware), the Arizona Disease Control Research Commission, Herbert K. and Dianne Cummings (The Nathan Cummings Foundation, Inc.), Polly J. Trautman, and Lotte Flugel. For other assistance we thank Drs. F. E. Boettner, R. A. Nieman, Messrs. P. L. Daschner and L. Williams, NSF Grant CHE-8409644, and the NSF Regional Instrumentation Facility in Nebraska (Grant CHE-8620177).

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(15) Dolastatin 15 (1) 400-MHz ^1H and ^{13}C NMR (deuteriomethylene chloride, in ppm with respect to TMS). Dolapyrrolidone unit: ^1H 4.71 (s, H-2), 3.74 (s, 3 H, H-3b), 4.77 (dd, 4.8, 2.9, H-4), 3.52 (dd, 14.0, 4.5, H-4a), 3.04 (dd, 13.8, 3.5, H-4a'), 7.14 (m, H-4c), 7.20 (m, H-4d), 7.18 (m, H-4e); ^{13}C 169.26 (C-1), 94.73 (C-2), 178.16 (C-3), 59.86 (C-4), 58.29 (C-3b), 34.87 (C-4a), 134.16 (C-4b), 129.97 (C-4c), 128.11 (C-4d), 126.97 (C-4e). 2-Hydroxyisovaleric acid unit: ^1H 5.89 (d, 2.7, H-7), 2.19 (dsept, 2.7, 6.6, H-7a), 0.91 (d, 6.6, 3 H, H-7b), 1.07 (d, 6.6, 3 H, H-7c); ^{13}C 169.47 (C-6), 77.83 (C-7), 28.86 (C-7a), 15.78 (C-7b), 19.82 (C-7c). Proline-1 unit: ^1H 4.83 (dd, 8.9, 2.7, H-10), 2.38 (m, H-11), 2.22 (m, H-11'), 2.25 (m, H-12), 2.03 (m, H-12'), 3.75 (dd, 14.8, 8.9, H-13), 3.60 (dd, 14.7, 6.9, H-13'); ^{13}C 171.44 (C-9), 58.25 (C-10), 28.53 (C-11), 24.61 (C-12), 46.37 (C-13). Proline-2 unit: ^1H 4.63 (dd, 8.1, 4.8, H-16), 2.13 (m, H-17), 2.11 (m, H-17'), 2.15 (m, H-18), 1.85 (m, H-18'), 3.90 (dd, 16.7, 7.1, H-19), 3.78 (dd, 16.5, 7.0, H-19'); ^{13}C 170.79 (C-15), 58.04 (C-16), 28.36 (C-17), 24.68 (C-18), 47.80 (C-19). *N*-Methylvaline unit: ^1H 5.13 (d, 11.1, H-22), 2.27 (dsept, 11.2, 6.6, H-22a), 0.77 (d, 6.6, 3 H, H-22b), 1.03 (d, 6.6, 3 H, H-22c), 3.17 (s, 3 H, H-23a); ^{13}C 169.12 (C-21), 59.18 (C-22), 27.29 (C-22a), 18.52 (C-22b), 19.10 (C-22c), 30.68 (C-23a). Valine unit: ^1H 4.79 (dd, 9.3, 6.8, H-25), 1.98 (oct, 6.7, H-25a), 0.93 (d, 6.6, 6 H, H-25b and H-25c), 6.87 (d, 9.2, H-26); ^{13}C 172.97 (C-24), 53.61 (C-25), 31.10 (C-25a), 18.04 (C-25b), 19.59 (C-25c). *N,N*-Dimethylvaline unit: ^1H 2.44 (d, 6.4, H-28), 2.07 (oct, 6.4, H-28a), 0.92 (d, 6.6, 3 H, H-28b), 0.99 (d, 6.6, 3 H, H-28c), 2.24 (s, 6 H, H-29a and H-29b); ^{13}C 171.80 (C-27), 76.54 (C-28), 27.66 (C-28a), 17.64 (C-28b), 20.17 (C-28c), 42.95 (C-29a and C-29b).
(16) Experiments by Pettit, G. R.; Singh, S. B.; Herald, D. L.

Theoretical Predictions of Torquoselectivity in Pentadienyl Cation Electrocyclizations

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Summary: The electrocyclization of the pentadienyl cation was investigated with ab initio theory. Computations were performed at the RHF/3-21G and MP2/6-31G* levels of theory. The activation energy was predicted to be 1–10 kcal/mol. The heat of reaction was predicted to be −27 to −34 kcal/mol. Hydroxy, formyl, boryl, fluoro, and amino substituent effects were examined at the 3-21G level. The resonance donors (OH, F, NH_2) were found to stabilize the cyclization transition state when substituted on the outside, while the resonance acceptors (BH_2, CHO) were found to prefer cyclization when placed inside, as found earlier for cyclobutenes.

Sir: Since the first report of electronic factors controlling the outward or inward rotation of substituents in the conrotatory electrocyclizations of substituted cyclobutenes,[1] many examples have been observed experimen-

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