ISOLATION AND STRUCTURE OF DOLASTATIN 17[1a,b]

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Abstract - The unusual cyclodepsipeptide dolastatin 17 (1) was isolated from the Papua New Guinea sea hare *Dolabella auricularia* and found to contain a new acetylenic β-amino acid designated dolayne (Doy). Dolastatin 17 exhibited significant human cancer cell growth inhibitory activity (GI_{50} 0.45-0.74 μg/mL range).

Marine invertebrates, especially sea hares,[2,3] sponges[4,5] and tunicates,[6] continue to offer a most promising[7] array of potentially useful antineoplastic agents such as the dolastatins,[1-3] criamides,[4] hemiasterlins,[4] arenastatin A[5] (a cryptophycin[8]), and patellamides.[6] Herein we report that an extensive investigation of the shell-less mollusk *Dolabella auricularia* (Aplysiidae) collected (1983) in the Bismarck archipelago of Papua New Guinea has led to isolation of the novel cyclodepsipeptide, dolastatin 17 (1), bearing a new acetylenic β-amino acid designated dolayne (Doy).

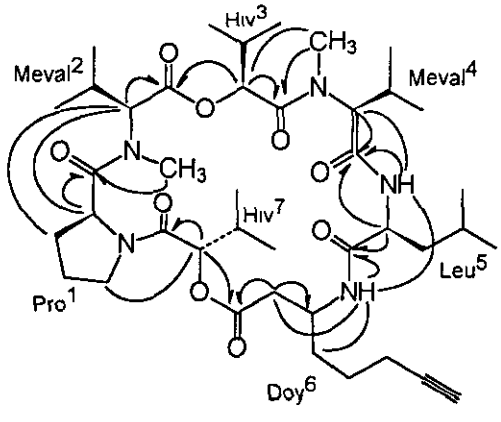
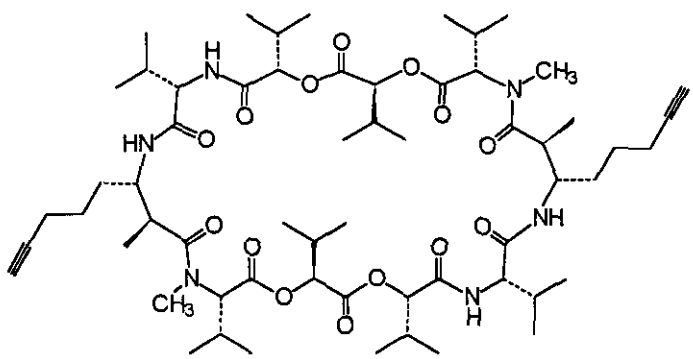


Figure 1: Dolastatin 17 (1) key HMBC (↷) and nOe (⌒) correlations.

A murine P388 lymphocytic leukemia cell-line-active fraction prepared from 1000 kg. (wet. wt.) of *D. auricularia* was separated as briefly described for the isolation of

dolastatin 16.[1] Final purification and isolation of dolastatin 17 (1, 2.3 mg., 2.3 x 10^{-7}% yield) was realized by employment of reversed-phase (C8) HPLC to afford cyclodepsipeptide (1) as a colorless amorphous powder: $[\alpha]_D$ -145° (c = 0.14, CH_3OH) and FTIR ν (NaCl): 3350, 2900, 2116, 1740, 1643, and 1520 cm^{-1}. The IR spectral data (ν 2116 cm^{-1}) suggested that dolastatin 17 contained an alkyne group. The HRFAB mass spectrum afforded a quasi-molecular ion at *m/z* 774.500914 $[M+H]^+$ which corresponded to the molecular formula $C_{41}H_{67}N_5O_9$. All 1D-and 2D-NMR spectra of 1 were measured in two solvents ($CDCl_3$ and DMSO-d_6). Analysis of the two series of ^{1}H-NMR, APT HCOSY, TOCSY and HMQC (500 MHz) spectra combined with the HMBC, NOESY and ROESY NMR spectral results established the presence of seven independent spin systems: one Pro, one Leu, two MeVal, two 2-hydroxyisovaleric acid (Hiv) units and a new β-amino acid (Doy) unit with a terminal alkyne group. The partial molecular formula of the new Doy unit was found to be $C_8H_{11}NO$ by HRFAB mass spectral analysis. Interpretation of cross peaks (Figure 1) observed in the 2D-NMR spectra led to the assignment of 3-amino-7-octynoic acid (Doy) for the new and unique β-amino acid unit. Such a β-amino acid having an alkyne group is rarely encountered in marine invertebrates. Interestingly, the mollusk *Onchidium sp.* collected off New Caledonia has been found to contain the dimeric cyclic depsipeptide onchidin (2) that contains a methyl side



Onchidin (**2**)

chain derivative of Doy.[9] The key HMBC correlations: CH_3N[MeVal^2]/CO[Pro^1], αH[Hiv^3]/CO[MeVal^2], CH_3N[MeVal^4]/CO[Hiv^3], NH[Leu^5]/CO[MeVal^4], αH[Leu^5]/CO[MeVal^4],

Table 1 : The ^{1}H- and ^{13}C-NMR Spectral Data Assignments of Dolastatin 17 (in $CDCl_3$)

No.	*^{13}C* *ppm*	*^{1}H* *ppm*	*J* *Hz*	*HMBC* *(^{1}H to ^{13}C)*	*No.*	*^{13}C* *ppm*	*^{1}H* *ppm*	*J* *Hz*	*HMBC* *(^{1}H to ^{13}C)*
Pro^1 CO	171.81 s				γ '	19 83 q	0.95 d (7.0)		α,β,γ
α	57.27 d	5.12 dd (9.0,5.0)		CO,β,γ	CH_3N	29.81 q	2.92 s		Hiv^{3}CO,CO
β	29.68 t	1.80 m		CO	*Leu^5* CO	172.08 s			
		2.38 m		CO	α	51.64 d	4.60 m		MeVal^{4}CO,CO,β,γ
γ	24.81 t	2.00 m		α,β,δ	β	38.39 t	1.68 m		γ,δ,δ'
δ	47.06 t	3.55 dd (9.0,7.5)		α,β	γ	24.65 d	1.61 m		β,δ,δ'
		3.69 m		α,β	δ	23.59 q	0.86 q (7.0)		β,γ,δ'
MeVal^2					δ'	20.87 q	0.84 q (7.5)		β,γ,δ
CO	171.27 s				NH		7 25 d (7.8)		MeVal^{4}CO,α
α	64.51 d	4.23 d (8.5)		Pro^{1}CO,CO,β	*Doy^6* CO	170.17 s			
				γ,γ ',CH_3N	α	39 51 t	2.27 m		CO,β,γ
β	29 47 d	2.35 m		α,γ,γ '			2.79 dd (16,6)		CO,β,γ
γ	20.43 q	1.20 d (6 5)		α,β,γ '	β	45.32 d	4.32 m		
γ '	21.40 q	1.07 d (7.0)		α,β,γ	γ	33.99 t	1.52 m		α,β,ϵ
CH_3N	30.49 q	2.92 s		Pro^{1}CO,CO,α			1.64 m		α,β,ϵ
Hiv^3 CO	169.54 s				δ	24.48 t	1.52 m		
α	76.60 d	5.21 d (3.0)		MeVal^{2}CO,CO	ϵ	18.18 t	2.16 m		γ,ζ
				β,γ,γ '	ζ	68.43 s			
β	28.92 d	2.14 m		γ,γ '	ξ	84.20 d	1.91 s		ϵ
γ	20.07 q	1 10 d (7.0)		α,β,γ '	NH		6.10 d (7.8)		Leu^{5}CO,α,β
γ '	16.31 q	0.99 d (7.0)		α,β,γ	*Hiv^7* CO	167.20 s			
MeVal^4					α	76.17 d	5.03 d (3.0)		Doy^{6}CO,CO,β,γ,γ '
CO	168.98 s				β	29.47 d	2.35 m		γ,γ '
α	62.15 d	4.68 d (10)		Hiv^{3}CO,CO,β,γ,γ '	γ	16.21 q	0.98 d (7.0)		α,β,γ '
β	25.58 d	2.30 m		α,γ, γ '	γ'	20.02 q	1.02 d (7.0)		α,β,γ
γ	17.91 q	0 78 d (6 5)		α,β,γ '					

NH[Doy^6]/CO[Leu^5] and αH[Hiv^7]/CO[Doy^6] led to the dolastatin 17 (1) structural assignment: Pro^1-MeVal^2-O-Hiv^3-MeVal^4-Leu^5-Doy^6-O-Hiv^7. The sequence of peptide bonds identified by the HMBC correlations was further confirmed by NOESY and ROESY experiments. An nOe relationship from δ 3.69 (δH_1-Pro^1) and δ 3.55 (δH_2-Pro^1) to δ 5.03 (αH-Hiv^7) revealed that the carbonyl carbon of Hiv^7 was linked through the nitrogen of Pro^1. That established dolastatin 17 (1) as a cyclic depsipeptide.

Evidence supporting a cyclic depsipeptide structure was also obtained by consideration of the molecular formula unsaturation numbers.

An hydrolysis procedure (6N HCl) and chiral analyses (CHIREX phase 3126) established the Leu, Pro, MeVal and Hiv amino acid and hydroxy acid units as all L(*S*).[10,11] The difference in chemical shifts of the β-and γ-carbons of Pro^1 ($\Delta\delta_{\beta\gamma}$ = 4.87 ppm) pointed to the presence of a *trans*-Hiv^7-Pro^1 configuration.[12]

Dolastatin 17 (1) was found to display significant human cancer cell growth inhibitory activity against ovary OVCAR-3 (GI_{50} 0.67 μg/mL), brain SF-295 (GI_{50} 0.55 μg/mL), lung HCI-H460 (GI_{50} 0.74 μg/mL) and melanoma KM20L2 (GI_{50} 0.45 μg/mL). Eventual total synthesis of dolastatin 17 (1) will allow further biological studies and assignment of the Doy (presumably *S* configuration as in dolastatin 15)[2] absolute configurations.

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