Isolation and Stereostructure of Doliculide, a Cytotoxic Cyclodepsipeptide from the Japanese Sea Hare *Dolabella auricularia*

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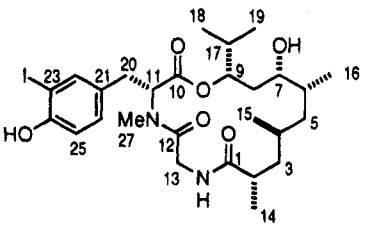
Summary: Doliculide (1), a cytotoxic cyclodepsipeptide of mixed peptide–polyketide biogenesis, has been isolated from the Japanese sea hare *Dolabella auricularia* and its stereostructure determined by spectral and chemical methods.

The sea hare *Dolabella auricularia* (Aplysiidae) is known to be a rich source of antineoplastic and/or cytostatic peptides such as dolastatins 10 and 15.[1] Recently, we have examined the constituents of Japanese specimens of this animal, resulting in the isolation of new cytotoxic depsipeptides[2] and other unique metabolites.[3] We now wish to report the isolation and structural elucidation of a novel cyclodepsipeptide, doliculide (1), from the Japanese sea hare *D. auricularia*. This substance exhibited potent cytotoxic activity against HeLa-S_3 cells with an IC_{50} of 0.001 μg/mL.



doliculide (1)

The MeOH extract of the internal organs (20 kg, wet wt) of the sea hare *D. auricularia* (33 kg, wet wt), collected in Mie Prefecture, Japan, was partitioned between EtOAc and water. The EtOAc-soluble material (91.4 g), which exhibited strong cytotoxicity against HeLa-S_3 cells with an IC_{50} of 1.2 μg/mL, was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion (30.8 g) was subjected to bioassay-guided fractionation using silica gel (i. toluene/EtOAc, EtOAc, and then EtOAc/MeOH, step gradient; ii. hexane/acetone 2:1 and then acetone/MeOH 9:1) and ODS silica gel (70% aqueous MeOH to MeOH, linear gradient), successively, to afford a cytotoxic fraction (91 mg, IC_{50} = 0.37 μg/mL). The fraction was further separated by a combination of reversed-phase HPLC (Develosil ODS 10/20, 50% aqueous MeCN) and silica gel TLC ($CHCl_3$/acetone 2:1) to afford doliculide (1) as an oil (8.2 mg). Recrystallization from hexane/dichloromethane gave pure **1** as colorless fine needles: mp 173–174 °C; $[\alpha]^{23}_D$ −25.5° (*c* 0.670, MeOH); R_f 0.50 on silica gel TLC ($CHCl_3$/acetone 2:1); UV (MeOH) λ_{max} 207 (ε 27 300), 227 (sh, 11 000), and 284 (3100) nm; IR ($CHCl_3$) 3500, 3420, 3200 (br), 1720, 1670 (sh), 1650, 1505, 1285, 1255, 1175, 1030, and 995 cm^{-1}.

Table 1. ^1H and ^{13}C NMR Data and COLOC Correlations for Doliculide (1) in $CDCl_3$

position	^1H[a]	^{13}C[b]	COLOC[c]
1		177.8 s	H-14, 13a
2	2.43 ddq (11.8, 3.4, 6.4)	39.2 d	H-3b, 14
3a	1.05 m	45.0 t	H-14, 15
3b	1.52 br dd (12.7, 11.8)		
4	1.18 m	27.1 d	H-15
5	1.06 m	43.1 t	H-15
6	2.02 m	34.4 d	H-16
7	3.58 br d (11.8)	65.8 d	
8a	1.32 ddd (13.9, 11.8, 2.4)	30.2 t	
8b	1.44 ddd (13.9, 11.8, 1.8)		
9	5.06 ddd (11.8, 5.0, 1.8)	77.4 d	
10		171.6 s	H-11, 20a
11	5.47 dd (12.2, 4.3)	58.2 d	H-20a, 27
12		171.9 s	H-13, 27
13a	3.25 dd (16.8, 1.8)	39.7 t	
13b	4.79 dd (16.8, 9.0)		
14	1.13 d (6.4)	18.4 q	
15	0.97 d (7.0)	17.7 q	H-3b
16	0.84 d (7.0)	14.4 q	
17	1.87 m	32.4 d	H-18, 19
18	0.95 d (7.0)	18.1 q	
19	0.95 d (7.0)	18.8 q	
20a	2.88 dd (15.4, 12.2)	32.9 t	H-11, 22
20b	3.44 dd (15.4, 4.3)		
21		130.4 s	H-20, 25
22	7.49 d (2.1)	138.0 d	H-20, 26
23		85.5 s	H-22, 25
24		154.2 s	H-22, 26
25	6.86 d (8.2)	115.2 d	
26	7.06 dd (8.2, 2.1)	129.7 d	H-20, 22
27	2.95 s	30.8 q	
NH	6.24 br d (9.0)		
7-OH	2.52 br s		
24-OH	6.26 br s		

[a] Recorded at 500 MHz. TMS as internal standard (δ 0.00). Coupling constants (Hz) are in parenthesis. [b] Recorded at 67.8 MHz. $CDCl_3$ as internal standard (δ 77.0). [c] Recorded at 67.8 MHz. Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for J_{CH} = 6 and 8 Hz.

The molecular formula of **1** was established to be $C_{27}H_{41}N_2O_6I$ by combustion analysis (Anal. Calcd: C, 52.60; H, 6.70; N, 4.54. Found: C, 52.50; H, 6.55; N, 4.50) and HREIMS [$(M + H)^+$ *m/z* 616.2022, Δ +1.3 mmu]. The depsipeptide nature of **1** was suggested from the IR [1720, 1670 (sh), and 1650 cm^{-1}] and NMR data (Table 1). The ^{13}C NMR data included three carbonyl signals at δ 177.8, 171.9, and 171.6, and the ^1H NMR data showed the presence of an amide NH group (δ 6.24) and an *N*-methylamide group (δ 2.95), indicating the presence of the following three units in **1**: two amino acids and one hydroxy acid. The presence of the glycine unit in **1** was readily disclosed by ^1H–^1H COSY data. The second amino acid in **1** was deduced to be 3-iodo-*N*-methyltyrosine by comparing the ^1H NMR, ^{13}C NMR, and UV data

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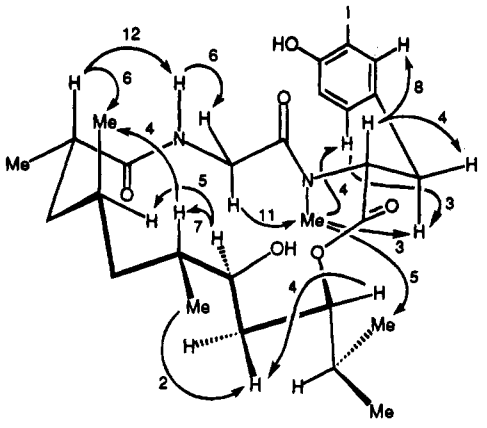

Figure 1. Plausible conformation of doliculide (**1**) in $CDCl_3$ with NOE enhancements in percent.

with those for geodiamolides A and D that contain this amino acid group.[4] The presence of an iodine atom at C23 was substantiated by the characteristic chemical shift of C23 (δ 85.5). The structure of the polyketide unit comprising 15 carbons and two oxymethine functionalities in **1** was determined by the $^1H-^1H$ COSY and $^{13}C-^1H$ COSY (J_{CH} = 140 Hz) experiments. The location of a hydroxyl group in **1** was determined to be at C7 by the ^1H NMR spectrum: addition of D_2O led to sharpening of the signal at H7 (δ 3.58, br d, J = 11.8 Hz → ddd, J = 11.8, 4.2, and 2.4 Hz). The chemical shift at H9 (δ 5.06) in **1** was observed in the rather low-field region, suggesting that an acyloxy group be connected to the C9 position. The degree of unsaturation in **1** indicated that **1** had a cyclic structure. The $^{13}C-^1H$ long-range correlation (COLOC) data shown in Table 1 allowed us to connect the three units described above (two amino acids and a polyketide unit) as well as to assign all the carbonyl carbons. This connectivity was further supported by difference NOE data (Figure 1), establishing the gross structure of doliculide (**1**).

The stereochemistry of doliculide (**1**) was elucidated as follows. Acid hydrolysis of **1** with 9 N HCl at 110 °C provided glycine and *N*-methyl-D-tyrosine (D-MeTyr),[5,6] which were identified by TLC (silica gel, BuOH/AcOH/H_2O 3:1:1) and chiral HPLC (CHRALPAK MA(+), 2 mM $CuSO_4$) analyses, while a product arising from the polyketide portion could not be obtained by the hydrolysis experiments. The conformation of **1** in $CDCl_3$ was elucidated by analysis of vicinal spin–spin coupling constants (Table 1) and extensive difference NOE experiments, revealing the relative stereochemistry of **1** (Figure 1). Further, the absolute stereochemistry of **1** was determined from the known configuration of 3-iodo-*N*-methyl-D-tyrosine to be 2*S*,4*S*,6*R*,7*S*,9*S*,11*R*. Confirmation of the absolute stereochemistry of **1** by its total synthesis is reported in the following paper in this issue.

Doliculide (**1**) is a novel depsipeptide containing a 15-carbon polyketide unit, glycine, and a unique D-amino acid, and regarded as a metabolite of mixed peptide–polyketide biogenesis. Some related depsipeptides, jaspamide (jasplakinolide)[7] and geodiamolides,[4] were isolated from marine sponges. It is noteworthy that doliculide (**1**) possesses a structurally novel polyketide moiety and exhibits potent cytotoxicity.

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Supplementary Material Available: Procedure of the isolation and acid hydrolysis of **1**; 1D and 2D NMR spectra of **1** (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(6) During the course of the acid hydrolysis of **1** reductive deiodination of the 3-iodo-*N*-methyl-D-tyrosine moiety in **1** occurred to yield D-MeTyr.[5]

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