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Isolation, Structure, and Synthesis of Dolastatin D, a Cytotoxic Cyclic Depsipeptide from the Sea Hare *Dolabella auricularia*

Hiroki Sone, Takayuki Nemoto, Hiroyuki Ishiwata, Makoto Ojika,* and Kiyoyuki Yamada*

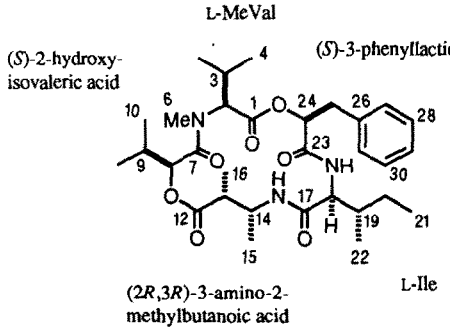
Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464, Japan

Abstract: Dolastatin D (**1**), a cytotoxic cyclic depsipeptide possessing the novel β-amino acid (2*R*,3*R*)-3-amino-2-methylbutanoic acid [(2*R*,3*R*)-**3**] as a component, has been isolated from the Japanese sea hare *Dolabella auricularia*. The absolute stereostructure of **1** was elucidated on the basis of spectroscopic analysis and chemical degradation and was further confirmed by synthesis.

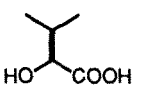
Many antineoplastic and/or cytostatic peptidic compounds such as dolastatin 10[1] have been discovered from the Indian Ocean sea hare *Dolabella auricularia*.[2] Recently, we have described isolation and structure of a new linear depsipeptide dolastatin C from the Japanese sea hare *D. auricularia*.[3] Continued investigation on bioactive constituents of this animal has now resulted in isolation of a new cytotoxic cyclic depsipeptide, dolastatin D (**1**).[4] We report herein the isolation, structure elucidation, and synthesis of this compound.

According to the procedure described in the preceding paper,[3] the material of 90% MeOH portion (36 g) was obtained from the sea hare (32 kg, wet wt) collected on the Pacific coast of the Shima Peninsula, Mie Prefecture, Japan. The material was subjected to bioassay-guided fractionation on silica gel and ODS silica gel[5] to afford the fraction (53 mg) exhibiting cytotoxicity against HeLa-S_3 cells (IC_{50} 0.25 μg/mL). The fraction was further chromatographed on silica gel and ODS silica gel[6] to give pure dolastatin D (**1**) (1.7 mg, 5.2 x 10^{-6}% yield based on wet weight) as fine needles: mp 200–201 °C (hexane/CH_2Cl_2), $[\alpha]^{25}_D$ –73° (*c* 0.13, MeOH).[7] Dolastatin D (**1**) showed cytotoxicity against the same cells with IC_{50} 2.2 μg/mL.[8]

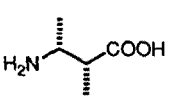
Dolastatin D (**1**) has a molecular formula of $C_{31}H_{47}N_3O_7$ [high-resolution EIMS: *m/z* 573.3407 (M^+), Δ–0.7 mmu]. The peptidic nature of **1** was suggested from its ^1H and ^{13}C NMR data (Table 1). The latter



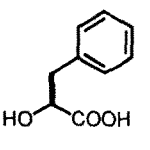
1



(*S*)-**2**



(2*R*,3*R*)-**3**



(*S*)-**4**

Table 1. NMR Data for **1**.[a]

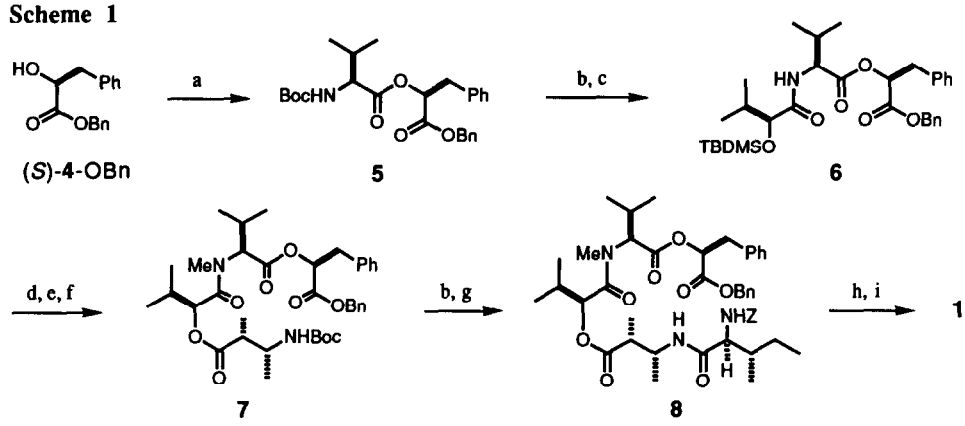
Position	^1H[b]	^{13}C	HMBC[c]	Position	^1H[b]	^{13}C	HMBC[c]
(MeVal)				(Ile)			
1	–	169.3 s	H-2	17	–	171.0 s	14-NH, H-18, 19
2	3.15 d (7.9)	72.1 d	H-3, 6	18	4.32 dd (9.0, 6.1)	59.5 d	
3	2.54 m	28.1 d	H-2, 4, 5	19	1.98 m	36.2 d	H-18, 21, 22
4	0.91 d (7.0)	19.4 q	H-2, 3	20a	1.05 m	25.4 t	H-18, 21, 22
5	0.96 d (6.4)	21.9 q	H-2, 3	20b	1.42 ddq (14.0, 3.6, 7.5)		
6	3.34 s	40.4 q	H-2	21	0.82 t (7.5)	11.6 q	
(2-hydroxyisovaleric acid)				22	0.86 d (6.7)	15.8 q	H-18
7	–	170.6 s	H-2, 6, 8, 9	18-NH	7.51 brd (9.0)		
8	5.21 d (5.5)	73.2 d	H-9	(3-phenyllactic acid)			
9	2.10 m	30.0 d	H-8, 10, 11	23	–	169.1 s	18-NH, H-25b
10	0.97 d (7.0)	17.0 q	H-8, 9	24	5.52 dd (7.9, 3.7)	75.8 d	H-25a
11	1.02 d (7.0)	19.1 q	H-8, 9,10	25a	3.18 dd (14.6, 7.9)	38.0 t	H-24
(3-amino-2-methylbutanoic acid)				25b	3.36 dd (14.6, 3.7)		
12	–	172.1 s	H-13, 14, 16	26	–	136.3 s	H-24, 25a
13	2.57 dq (2.8, 7.0)	45.9 d		27, 31	7.19–7.28 m	129.4 d	H-25a
14	4.26 ddq (9.8, 2.8, 7.0)	48.2 d	H-13	28, 30	7.19–7.28 m	128.4 d	
15	1.20 d (7.0)	20.5 q	H-13	29	7.19–7.28 m	126.8 d	
16	1.22 d (7.0)	14.8 q					
14-NH	6.14 brd (9.8)						

[a] Spectra were recorded at 500 MHz for ^1H and at 67.8 MHz for ^{13}C using $CDCl_3$ as solvent and TMS as internal standard. Chemical shifts are in δ values. [b] Coupling constants in Hz are in parenthesis. [c] Parameters were optimized for J_{CH} = 8 and 6 Hz. Underlined are correlations obtained from the COLOC experiments.

included five carbonyl signals at δ 169.1–172.1, whereas the former showed the presence of only two amide NH groups (δ 6.14 and 7.51) and one *N*-methyl amide group (δ 3.34). Since no signal due to proline unit was observed, two of the five carbonyl groups were presumed to be ascribed to ester functionalities, which were also supported from the IR spectra.[7] A combination of ^1H-^1H and ^{13}C-^1H COSY experiments indicated the presence of the following five units: two α-amino acid units [*N*-methylvaline (MeVal) and isoleucine (Ile)], two α-hydroxy acid units [2-hydroxyisovaleric acid (**2**) and 3-phenyllactic acid (**4**)], and a novel β-amino acid unit [3-amino-2-methylbutanoic acid (**3**)]. The degree of unsaturation in **1** indicated that **1** had a cyclic structure. The ^{13}C-^1H long-range correlation (HMBC and COLOC) data summarized in Table 1 allowed us to assign all the carbonyl carbons of the five units: MeVal-CO (C1: δ 169.3), **2**-CO (C7: δ 170.6), **3**-CO (C12: δ 172.1), Ile-CO (C17: δ 171.0), and **4**-CO (C23: δ 169.1). Further, the sequence of the five units was determined from the ^{13}C-^1H long-range correlation data, disclosing the linear sequence MeVal-**2**-**3**-Ile-**4**. With the linear sequence MeVal-**2**-**3**-Ile-**4** in hand, the gross structure of dolastatin D was established considering the cyclic nature of this compound.

The absolute stereochemistry of the five components of dolastatin D (**1**) was determined as follows. Acid hydrolysis of **1** (6 N HCl, 110 °C, 24 h) followed by reversed-phase HPLC separation afforded the five components.[9] Two α-amino acids (MeVal and Ile) and two α-hydroxy acids (**2** and **4**) were further subjected to chiral HPLC analysis, respectively,[10] establishing the configurations of the four components to be all L (*S*). The absolute stereochemistry of 3-amino-2-methylbutanoic acid (**3**) was determined to be 2*R*,3*R* by derivatization with Marfey's reagent[11] and HPLC analysis of the derivatives.[12] The authentic samples for this analysis[12] were obtained by partial epimerization (6 N HCl, 130 °C, 3 days) of the two enantiomerically pure β-amino acids (2*R*,3*R*)-and (2*S*,3*S*)-**3** which were synthesized according to the procedure of Seebach.[13a,b] We concluded, therefore, that the absolute stereostructure of dolastatin D was represented by the formula **1**.

Scheme 1



a. Boc-L-Val, DCC, DMAP, CH_2Cl_2, 0 °C then rt, 88%. b. CF_3COOH, CH_2Cl_2, 0 °C. c. TBDMS-(*S*)-**2**, DEPC, Et_3N, DMF, 0 °C then rt, 89% from **5**. d. NaH, MeI, DMF, 0 °C, 75%. e. 12% HF, H_2O, MeCN, rt, 98%. f. Boc-(2*R*,3*R*)-**3**, DCC, DMAP, 10-camphorsulfonic acid, CH_2Cl_2, 0 °C then rt, 98%. g. Z-L-Ile, DEPC, Et_3N, DMF, 0 °C then rt, 82% from **7**. h. H_2, 10% Pd-C, MeOH, H_2O, AcOH, rt, 91%. i. condition A: Bop-Cl, Et_3N, CH_2Cl_2, 0–4 °C, 13%; condition B: HOSu, DCC, DMF, CH_2Cl_2, 41%; condition C: DPPA, Et_3N, DMF, 0 °C then rt, 47%; condition D: BOP, $NaHCO_3$, DMF, rt, 66%.

The structure of dolastatin D (**1**) was further confirmed by synthesis as shown in Scheme 1. Esterification of (*S*)-3-phenyllactic acid benzyl ester [(*S*)-**4**-OBn] and Boc-L-Val using dicyclohexylcarbodiimide (DCC) provided ester **5** (oil, $[\alpha]^{25}_D$ –16.4° (*c* 1.04, $CHCl_3$)). Deprotection followed by coupling with (*S*)-2-hydroxyisovaleric acid silyl ether [TBDMS-(*S*)-**2**][14] using diethyl phosphorocyanidate (DEPC)[15] gave tridepsipeptide **6** (oil, $[\alpha]^{24}_D$ –34.6° (*c* 1.11, $CHCl_3$)). *N*-Methylation and desilylation provided an alcohol, which was esterified with the protected β-amino acid Boc-(2*R*,3*R*)-**3**[16] using the Keck method[17] to afford tetradepsipeptide **7** (oil, $[\alpha]^{27}_D$ –20.5° (*c* 0.90, $CHCl_3$)). Subsequent deprotection and coupling with Z-L-Ile using the DEPC method gave pentadepsipeptide **8** (oil, $[\alpha]^{24}_D$ –6.9° (*c* 0.28, $CHCl_3$)). After deprotection of both benzyl and Z groups of **8**, the final cyclization to dolastatin D (**1**) was achieved using the four reagents: bis(2-oxo-3-oxazolidinyl)phosphinic chloride (Bop-Cl)[18] (13%), *N*-hydroxysuccinimide (HOSu)[19] (41%), diphenylphosphoryl azide (DPPA)[20] (47%), and benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP)[21] (66%). Therefore the BOP procedure was the method of choice in the present case. Dolastatin D (**1**) (mp 200–201 °C, $[\alpha]^{25}_D$ –71° (*c* 0.13, MeOH)) thus obtained was identical with the natural compound in all respects ($[\alpha]_D$, IR, 500 MHz ^1H NMR, MS, TLC), establishing the absolute stereostructure of **1** unambiguously.

Dolastatin D (**1**) contains the novel β-amino acid (2*R*,3*R*)-3-amino-2-methylbutanoic acid as a component, which is known as a synthetic compound[13b,c] but not as a natural product.[22] The related β-amino acid 3-amino-2-methylpentanoic acid is known as a component of the cytostatic depsipeptides dolastatins 11 and 12,[23] and the antifungal depsipeptides majusculamide C[24] and normajusculamide C,[25] and synthetic studies revealed the 2*S*,3*R* configurations in all cases.[24b,26]

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3. Sone, H.; Nemoto, T.; Ojika, M.; Yamada, K. *Tetrahedron Lett.* preceding paper in this issue.
4. The name dolastatin D for our compound is used by agreement with Professor G. R. Pettit. See ref. 3.
5. Conditions for the chromatographic separation: 1) silica gel, C_6H_6/EtOAc then EtOAc/MeOH, step gradient; 2) silica gel, hexane/acetone 2:1 then acetone/MeOH 9:1; 3) ODS, 70→100% MeOH, linear gradient.
6. Conditions for the chromatographic separation: 1) reversed-phase HPLC [column, Develosil ODS-5 (10 x 250 mm) (Nomura Chemical Co., Ltd.); solvent, 65% MeCN; flow rate, 2.0 mL/min; detection at 215 nm], retention time 40.6 min; 2) silica gel PLC, EtOAc/$CHCl_3$ 3:2, R_f 0.4; 3) silica gel PLC, $CHCl_3$/MeOH 20:1, R_f 0.4.
7. **1**: UV (MeOH) λmax 206 nm (ε 16300), IR ($CHCl_3$) 3380, 1735, 1675, 1645, 1515, 1230 cm^{-1}, EIMS *m/z* 573 (M^+), 517, 357, 217, 189, 86.
8. Though minor constituents that were more cytotoxic than **1** have also been isolated from the active fraction, scarcity of the samples prevented their chemical characterization.
9. Conditions for the HPLC separation: column, Develosil ODS-HG-5 (4.6 x 250 mm) (Nomura Chemical Co., Ltd.); solvent, H_2O/MeCN/CF_3COOH 100:0:0.05 (20 min), 100:0:0.05→50:50:0.05 (30 min, linear gradient), and then 50:50:0.05 (10 min); flow rate, 0.5 mL/min; detection at 205 nm. The retention times (min) of components: MeVal (16.6), **2** (42.7), **3** (18.3), Ile (38.6), and **4** (51.8).
10. Conditions for the chiral HPLC analysis: column, CHIRALPAK MA(+) (4.6 x 50 mm) (Daicel Chemical Ind., Ltd.); solvent, 2 mM $CuSO_4$ for the α-amino acids (MeVal and Ile) and 2mM $CuSO_4$/MeCN 90:10 for the α-hydroxy acids (**2** and **4**); flow rate, 1.0 mL/min except for MeVal (0.5 mL/min); detection at 254 nm. The retention times (min) of the authentic amino and hydroxy acids: L-MeVal (16.6), D-MeVal (9.5), (*S*)-**2** (19.4), (*R*)-**2** (12.0), L-Ile (25.5), D-Ile (11.6), allo-L-Ile (18.0), allo-D-Ile (9.0), (*S*)-**4** (93.2), (*R*)-**4** (63.0).
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12. Conditions for the HPLC analysis: column, Develosil ODS-HG-5 (4.6 x 250 mm); solvent, MeOH/0.02 M AcONa (pH 4.0) 50:50; flow rate, 1.0 mL/min; detection at 340 nm. The retention times (min) of the authentic β-amino acids: (2*S*,3*S*)-**2** (21.3), (2*R*,3*S*)-**2** (22.5), (2*R*,3*R*)-**2** (27.6), and (2*S*,3*R*)-**2** (30.1).
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16. Boc-(2*R*,3*R*)-**3** (mp 95–96 °C (hexane-$CHCl_3$), $[\alpha]^{25}_D$ +8.3° (*c* 0.50, $CHCl_3$)) was prepared from methyl (3*R*,1'*S*)-3-[(1'-phenylethyl)amino]butanoate by the reported procedure (ref. 13b) with a slight modification: a) H_2, 20% $Pd(OH)_2$-C, MeOH/H_2O/AcOH 40:4:1, rt; b) Boc_2O, Et_3N, CH_2Cl_2, 0 °C then rt, 88% in 2 steps; c) LDA, MeI, THF, –40 °C to rt, 92% (50% de), pure Boc-(2*R*,3*R*)-**3**-OMe after chromatographic separation and recrystalization (hexane), 35%; d) NaOH, MeOH/H_2O, rt, quant.
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