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Isolation and Stereostructure of Aurilide, a Novel Cyclodepsipeptide from the Japanese Sea Hare *Dolabella auricularia*

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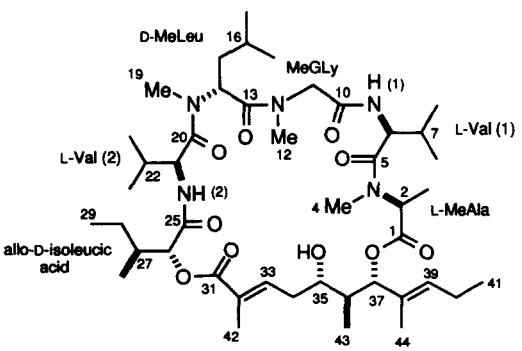
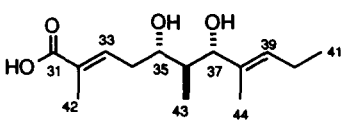
Abstract: Aurilide (**1**), a new 26-membered cyclodepsipeptide, has been isolated from the Japanese sea hare *Dolabella auricularia*. The gross structure of **1** was established by spectroscopic analysis. The absolute stereostructure of **1** was determined by HPLC analysis of amino acid components and isoleucic acid obtained from acidic hydrolysis of **1** and by the enantioselective synthesis of the tris(*p*-bromobenzoate) **3** derived from the dihydroxy acid moiety. Copyright © 1996 Elsevier Science Ltd

The Indian Ocean sea hare *Dolabella auricularia* (Aplysidae) is known to be a rich source of cytotoxic and/or antitumor peptides such as dolastatins 10 and 15.[1] Examination of the constituents of the Japanese sea hare *D. auricularia* resulted in the isolation of several cytotoxic depsipeptides[2] and other unique metabolites.[3] We report here the structure determination of aurilide (**1**), a novel macrocyclic depsipeptide, isolated from the Japanese specimens of this animal.

The MeOH extract of the internal organs of the sea hare *D. auricularia* (262 kg, wet wt), collected in Mie Prefecture, Japan, was partitioned between EtOAc and water. The EtOAc-soluble material, which exhibited 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 was subjected to bioassay-guided fractionation using silica gel (i. toluene/EtOAc, EtOAc, and then EtOAc/MeOH, step gradient; ii. 2:1 hexane/acetone), ODS silica gel (i. 70% aqueous MeOH to MeOH, linear gradient; ii. 80% aqueous MeOH to MeOH, linear gradient), and silica gel (5:1 and then 3:1 $CHCl_3$/acetone), successively, to give a cytotoxic



1



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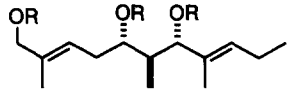


3 R = *p*-BrC_6H_4CO

Table 1. NMR Data for aurilide (**1**) in C_6D_6

Position	$^1H^a$	HMBC^b	Position	$^1H^a$	HMBC^b
1		H-2, 3, 37	23	0.83 d (6.6)	H-24
2	3.10 q (7.0)	H-3, 4	24	0.85 d (6.6)	H-23
3	1.24 d (7.0)	H-2	25		H-26
4	2.57 s		26	4.72 d (7.2)	H-30
5		H-2, 4, 6	27	2.08 m	H-26, 29, 30
6	5.17 dd (7.0, 7.0)	H-8, 9	28a	1.52 m	H-29, 30
7	2.08 m	H-6, 8, 9	28b	1.14 m	
8	1.16 d (7.0)	H-9	29	0.83 t (7.7)	
9	1.33 d (7.0)	H-6, 8	30	1.04 d (7.0)	H-26
10		H-11a, 11b	31		H-42
11a	4.44 d (17.9)	H-12	32		H-42
11b	3.84 d (17.9)		33	7.77 m	H-34, 42
12	3.25 s	H-11a, 11b	34	2.12 m^c	
13		H-11b, 12, 14, 15b	35	4.00 m	H-43
14	5.64 dd (7.0, 7.0)	H-15a, 15b, 19	36	2.00 m	H-37, 43
15a	2.23 ddd		37	5.18 d (11.4)	H-39, 43, 44
	(14.6, 7.0, 7.0)	H-14, 17, 18	38		H-37, 40, 44
15b	1.52 ddd		39	5.63 t (7.6)	H-37, 40, 41, 44
	(14.6, 7.0, 7.0)		40	1.95 dt (7.6, 7.6)	H-41
16	1.85 m	H-17, 18	41	0.89 t (7.6)	H-40
17	1.07 d (7.0)	H-15a, 15b, 18	42	1.90 s	
18	1.07 d (7.0)	H-15a, 15b, 17	43	0.62 d (7.0)	
19	2.91 s	H-14	44	1.55 s	H-37, 39
20		H-19, 21	NH (1)	7.77 br d (7.0)	
21	4.61 dd (8.8, 8.8)	H-23, 24	NH (2)	6.55 br d (8.8)	
22	1.98 m	H-21, 23, 24			

[a] Recorded at 600 MHz. Coupling constants (Hz) are in parentheses. The signal of one proton (OH) was not observed.
[b] Recorded at 600 MHz. Parameters were optimized for J_{CH} = 6 Hz. [c] 2 H.

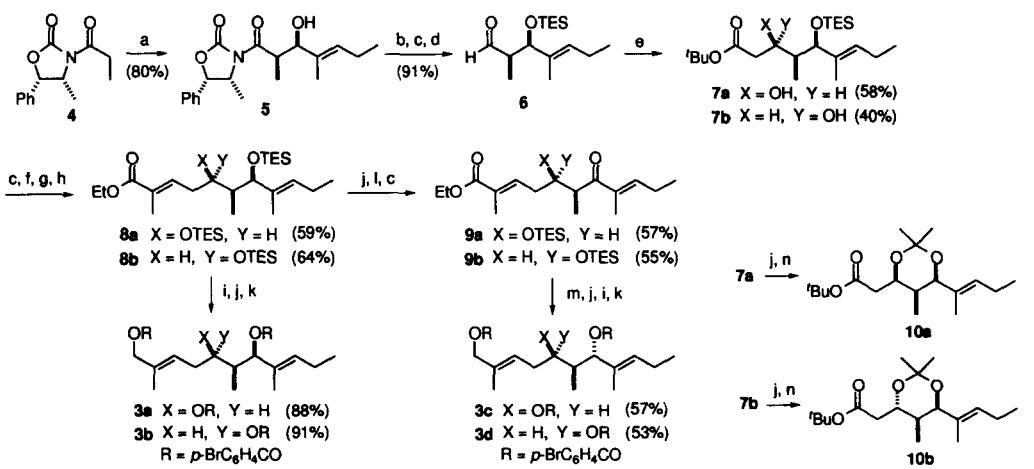
fraction (IC_{50} = 0.28 μg/mL). The fraction was further separated by reversed phase HPLC (Develosil ODS-HG-5, i. MeCN/MeOH/H_2O 75:5:40 to 75:5:0, linear gradient; ii. 80% aqueous MeOH; iii. 70% aqueous MeCN) to afford a cytotoxic fraction (IC_{50} = 0.017 μg/mL) containing aurilide (**1**). The fraction was purified by silica gel TLC (3:1 benzene/acetone) and reversed phase HPLC (Develosil ODS-HG-5, 80% aqueous MeOH) to afford aurilide (**1**) (0.5 mg, 1.9×10^{-7}% yield) as a colorless powder: $[\alpha]^{25}_D$ −17° (*c* 0.058, MeOH).[4]

Aurilide (**1**) has a molecular formula of $C_{44}H_{75}N_5O_{10}$, which was determined by the HRFABMS [*m/z* 856.5432 $(M + Na)^+$, Δ +2.0 mmu] and NMR data (Table 1). The IR spectrum showed bands at 3430, 1735, 1685, 1645, and 1245 cm^{-1} that were assigned to hydroxyl, ester, and amide groups. The ^1H NMR data showed the presence of two amide NH groups (δ 7.77 and 6.55) and three *N*-methylamide groups (δ 3.25, 2.91, and 2.57), suggesting the peptidic nature of **1**. Resonances in the ^1H NMR spectrum were assigned by DQF-COSY, HSQC, and HMBC, as shown in Table 1. Although the ^{13}C NMR spectrum could not be obtained due to the scarcity of the sample, carbon chemical shifts were mostly determined by HSQC and HMBC (J_{C-H} = 6 Hz) experiments. These spectroscopic data suggested the presence of five amino acid residues (two valines, *N*-methylglycine, *N*-methylalanine, and *N*-methylleucine), an isoleucic acid residue, and a dihydroxy acid portion (C31-C44). The low-field chemical shift of H-37 (δ 5.18) suggests that the acyloxy group is attached to C37. Stereochemistry of the two trisubstituted olefins of **1** was determined to be *E* on the basis of the ^{13}C chemical shifts of the respective vinyl methyls (δ_{C42} 12.5 and δ_{C44} 11.0).[5] The degree of unsaturation in **1** suggests the cyclic nature of **1**. The HMBC correlations shown in Table 1 disclosed the two sequences, Val(2)-MeLeu-MeGly and Val(1)-MeAla-**2**. The NOESY correlation of NH(2)/H-26 established the connectivity between

isoleucic acid and Val (2). Further evidence for the connectivities of the partial structures could not be obtained from either HMBC experiments or NOESY data. However, considering the peptidic nature of **1**, the carboxyl carbon (C31) of **2** must be bonded to the hydroxyl oxygen atom of isoleucic acid and the carboxyl carbon (C10) of the MeGly should be connected to the amino nitrogen of Val (1). Thus, the gross structure of aurilide is unequivocally shown as **1**.

The absolute stereostructure of **1** was elucidated as follows. Acidic hydrolysis of **1** (9 M HCl, 110 °C, 72 h) followed by reversed phase HPLC separation afforded four components, MeAla, Val, MeLeu, and isoleucic acid.[6] The absolute configurations of the three components, Val, MeLeu, and isoleucic acid, were determined to be L, D, allo-D, respectively, by the chiral HPLC analysis.[7] The absolute configuration of MeAla was established to be L by derivatization with Marfey's reagent[8] and HPLC analysis of the derivative.[9] The absolute stereochemistry of three contiguous asymmetric carbons (C35, C36, and C37) in **1** was determined by the enantioselective synthesis of tris(*p*-bromobenzoate) **3**[10] that was obtained by reduction of **1** ($LiAlH_4$, ether) followed by acylation (*p*-BrC_6H_4COCl, pyridine). Thus, the four possible diastereomeric tris(*p*-bromobenzoates) **3a**, **3b**, **3c**, and **3d** were synthesized as follows (Scheme 1). The Evans aldol reaction between imide **4** and *trans*-2-methyl-2-pentenal afforded aldol **5**, which was converted into aldehyde **6** in three steps. Treatment of **6** with $LiCH_2COO^tBu$ gave a mixture of diastereomeric alcohols **7a** and **7b**, which was separated by silica gel column chromatography. The stereochemistry of the hydroxyl group in **7a** and **7b** was determined by ^1H and ^{13}C NMR analysis of the derived acetonides **10a** and **10b**, respectively.[11] Conversion of **7a** and **7b** into conjugated esters **8a** and **8b** was effected by a four-step sequence of reactions. The diastereomeric tris(*p*-bromobenzoates) **3a** and **3b** were obtained from **8a** and **8b** in three steps, respectively. Two other diastereomers, **3c** and **3d**, were also prepared from **8a** and **8b** via enones **9a** and **9b** by a seven-step

Scheme 1



Reagents and Reaction Conditions:

(a) Bu_2BOTf, Et_3N, *trans*-2-methyl-2-pentenal, CH_2Cl_2, –78 °C; (b) $Me_2AlN(Me)OMe$, THF, toluene, 0 °C; (c) TESCl, imidazole, DMF, 23 °C; (d) DIBAL, THF, hexane, –78 °C; (e) $LiCH_2COO^tBu$, THF, –78 °C; (f) DIBAL, CH_2Cl_2, hexane, –23 °C; (g) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, –78 °C → 0 °C; (h) $(EtO)_2P(O)CH(Me)COOEt$, NaH, DME, –20 °C; (i) DIBAL, CH_2Cl_2, hexane, –78 °C; (j) HF·pyridine, THF, 23 °C; (k) *p*-BrC_6H_4COCl, pyridine, 23 °C; (l) MnO_2, CH_2Cl_2, 23 °C; (m) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, EtOH, –23 °C; (n) $Me_2C(OMe)_2$, PPTS, acetone, 23 °C.

sequence of reactions, respectively. Of the four synthetic diastereomers, **3a**, **3b**, **3c**, and **3d**, the ^{1}H NMR and the CD spectra of **3d** were identical to those for natural **3**, establishing the absolute stereochemistry of **3**. On the basis of these findings the complete stereostructure of aurilide was determined as shown in **1**.

Aurilide (**1**) is a novel 26-membered cyclodepsipeptide containing a new dihydroxy acid. The structurally related cyclodepsipeptide is dolastatin 14,[12] isolated from the Indian Ocean sea hare *D. auricularia*.

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5. The ^{13}C chemical shifts were determined by HSQC experiments. The ^{13}C chemical shifts for the vinyl methyls in a variety of (*E*)- and (*Z*)-trisubstituted olefins were observed at δ_C 13.8–17.1 and 21.0–24.4, respectively: Carey, L.; Clough, J. M.; Pattenden, G. *J. Chem. Soc. Perkin Trans I* **1983**, 3005–3009.
6. Conditions for the HPLC separation: column, Develosil ODS-HG-5 (4.6 × 250 mm); solvent, MeCN/H_2O/CF_3COOH 1:99:0.05 (20 min), 1:99:0.05 to 10:90:0.05 (20 min, linear gradient), and then 10:90:0.05 (20 min); flow rate, 1.0 mL/min; detection at 205 nm. The retention times (min) of components: MeAla (3), Val (5), MeLeu (21), isoleucic acid (55).
7. Conditions for the chiral HPLC analysis: column, CHIRALPAK MA(+) (4.6 × 50 mm); solvent, 2mM $CuSO_4$ for Val and MeLeu and 2mM $CuSO_4$/MeCN 9:1 for isoleucic acid; flow rate, 1.0 mL/min; detection at 254 nm. The retention times (min) of the authentic samples: L-Val (8.0), D-Val (4.0), L-MeLeu (9.0), D-MeLeu (6.7), L-isoleucic acid (64), D-isoleucic acid (39), allo-L-isoleucic acid (52), and allo-D-isoleucic acid (32).
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9. Conditions for the HPLC analysis: column, Develosil ODS-HG-5 (4.6 × 250 mm); solvent, MeOH/0.02 M AcONa (pH 4.0) 1:1; flow rate 1.0 mL/min; detection at 340 nm. The retention times (min) of the authentic Marfey derivatives of MeAla: L-MeAla (5.3) and D-MeAla (6.3).
10 Colorless oil: CD (MeOH) λ_{ext} 253 nm ($\Delta\varepsilon$ –56), 238 nm ($\Delta\varepsilon$ +94); ^{1}H NMR (600 MHz, C_6D_6) δ 7.90 (d, J = 8.4 Hz, 2 H), 7.78 (d, J = 8.4 Hz, 2 H), 7.61 (d, J = 8.4 Hz, 2 H), 5.63–5.58 (m, 2 H), 5.57 (d, J = 9.9 Hz, 1 H), 5.49 (br t, J = 7.3 Hz, 1 H), 4.48 (d, J = 12.5 Hz, 1 H), 4.42 (d, J = 12.5 Hz, 1 H), 2.58 (ddq, J =9.9, 4.4, 7.3 Hz, 1 H), 2.44–2.40 (m, 2 H), 1.54 (s, 3 H), 1.50 (s, 3 H), 0.86 (d, J = 7.3 Hz, 3 H), 0.83 (t, J = 7.3 Hz, 3 H). The signals due to six protons in **3** were not observed due to the overlap with the solvent signals.
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