Dolabelides C and D, Cytotoxic Macrolides Isolated from the Sea Hare *Dolabella auricularia*

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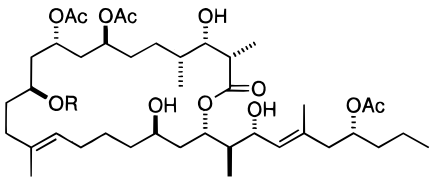
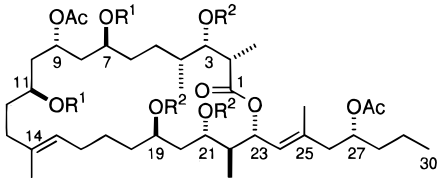
Two new cytotoxic 24-membered macrolides, dolabelides C and D, were isolated from the Japanese sea hare *Dolabella auricularia*. Their gross structures were deduced by spectroscopic analysis including the 2D NMR technique, and their absolute stereochemistry was determined by means of chemical correlation with the known dolabelide A. Dolabelides C and D exhibited cytotoxicities against HeLa S_3 cells with IC_{50} values of 1.9 and 1.5 μg/mL, respectively.

The sea hare *Dolabella auricularia* Solander (family Aplysiidae) is known to be a rich source of bioactive and structurally unique organic compounds.[1] Recently, we isolated cytotoxic 22-membered macrolides, dolabelides A (**1**) and B (**2**), from Japanese specimens of this animal.[2] Further investigation of the extracts of *D. auricularia* resulted in the isolation of dolabelides C (**3**) and D (**4**), 24-membered macrolide analogues of the dolabelides A (**1**) and B (**2**). We describe here the structural elucidation of these compounds.



dolabelide A (**1**) R = Ac
dolabelide B (**2**) R = H



dolabelide C (**3**) R^1 = Ac, R^2 = H
dolabelide D (**4**) R^1 = R^2 = H
5 R^1 = R^2 = Ac

The MeOH extract of the internal organs of the sea hare *D. auricularia*, collected in Mie Prefecture, Japan, was partitioned between EtOAc and water. The EtOAc-soluble material was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to bioassay-guided fractionation using column chromatography (silica gel, ODS silica gel, and alumina) and reversed-phase HPLC to afford dolabelides C (**3**, 7.2×10^{-5}% yield, based on wet weight) and D (**4**, 1.5×10^{-6}% yield). Dolabelides C (**3**) and D (**4**) exhibited cytotoxicities against HeLa S_3 cells with IC_{50} values of 1.9 and 1.5 μg/mL, respectively.

The molecular formula of **3**, which was determined to be $C_{43}H_{72}O_{13}$ by the HRFABMS data, was identical with that of **1**. The IR spectrum of **3** showed bands at 3480, 1730, and 1260 cm^{-1} that were assigned to hydroxyl and ester groups. The NMR data of **3** (Table 1) were similar to those of **1**. These findings indicated that **3** was an isomer of **1**. Detailed analysis of the phase-sensitive DQF-COSY spectrum of **3** allowed us to construct six partial structures, C2−C8 (**a**), C9 (**b**), C10−C13 (**c**), C14−C17 (**d**), C18−C25 (**e**), and C26−C30 (**f**) (Figure 1). The locations of three hydroxyl groups in **3** were determined by the acetylation shifts observed for H-3 (δ 4.02 → 5.41), H-19 (δ 4.33 → 5.38), and H-21 (δ 4.83 → 5.59) in the ^{1}H NMR spectrum of heptaacetate **5** that was prepared by acetylation of **3**. All of the carbons in **3** were assigned by ^{1}H−^{13}C COSY and HMBC spectra (Table 1), and HMBC correlations indicated the connectivities between C9 and C10, between C13 and C14, between C17 and C18, and between C25 and C26 and also the ester linkage between C2 and C23. Although it was difficult to establish the connectivity between C8 and C9 by 2D NMR data because of the overlap of the ^{1}H NMR signals (δ *ca.* 2.0), the connectivity (C8−C9) was obvious by consideration of the foregoing partial structures (Figure 1) and the molecular formula. The *E* geometry of the two trisubstituted olefins (C14 and C24) in **3** was established by the chemical shifts of the vinyl methyl carbons (δ_{14-Me} 15.2, δ_{25-Me} 17.6)[3] and by the NOESY correlation of H-23/25-Me. The low-field chemical shifts at H-7, H-9, H-11, and H-27 suggested that the four acetoxyl groups in **3** were attached to C7, C9, C11, and C27. Thus, the gross structure of dolabelide C (**3**) was deduced to be the 24-membered analogue of dolabelide A (**1**).

The absolute stereostructure of dolabelide C (**3**) was determined by chemical transformation of dolabelide A (**1**) to **3**. Treatment of dolabelide A (**1**) with NaOMe in methanol afforded a 24-membered lactone in 22% yield, which was found to be identical with dolabelide C (**3**) in all respects (IR, ^{1}H NMR, and mass spectra) including the specific rotation. Thus, the absolute stereochemistry of dolabelide C was proved to be identical with that of dolabelide A (**1**), as depicted in the formula **3**.

Dolabelide D (**4**) was deduced to be a bisdeacetyl derivative of dolabelide C (**3**) on the basis of its NMR data and the molecular formula of $C_{39}H_{68}O_{11}$, which was determined by HRFABMS. The chemical shifts of two oxymethine protons (δ_{H-7} 4.04, δ_{H-11} 4.07) in **4** revealed that it was the 7,11-bisdeacetyl derivative of **3**. Because acetylation of **4** gave an acetate that was identical with heptaacetate **5** obtained from dolabelide C (**3**), the absolute stereochemistry of dolabelide D (**4**) was identi-

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Table 1. ^{1}H and ^{13}C NMR Data and HMBC Correlations for Dolabelide C (**3**) in Pyridine-d_5

position	^{1}H[a]	^{13}C[b]	HMBC[c]
1		173.9 s	H-2, 23, 2-Me
2	2.89 dq (9.9, 7.0)	46.4 d	2-Me
2-Me	1.16 d (7.0)	13.8 q	H-2, 3
3	4.02 m	74.3 d	H-2, 2-Me, 3-OH, 4-Me
4	1.68 m	34.1 d	H-2, 4-Me
4-Me	0.90 d (6.6)	12.6 q	
5a	1.53 m	29.3 t	4-Me
5b	1.76 m		
6a	1.81 m	31.8 t	
6b	1.92 m		
7	5.34 m	69.9 d	H-8b
8a	2.02 m	37.2 t	H-6b
8b	2.18 m		
9	5.33 m	68.0 d	H-10a
10a	1.93 m	38.5 t	H-12b
10b	2.05 m		
11	5.12 m	70.0 d	H-12a,b
12a	1.61 m	31.7 t	H-11
12b	1.89 m		
13a	2.03 m	35.2 t	H-12b, 15, 14-Me
13b	2.09 m		
14		132.6 s	14-Me
14-Me	1.59 s	15.2 q	H-15
15	5.27 m	127.3 d	14-Me
16	2.01 m[d]	28.0 t	H-15
17a	1.60 m	26.9 t	H-15, 19
17b	1.73 m		
18	1.61 m[d]	37.9 t	H-17a,b, 20
19	4.33 m	67.9 d	19-OH
20	1.85 m[d]	38.8 t	H-22
21	4.83 m	67.4 d	H-22, 23, 22-Me
22	2.49 m	43.7 d	H-20, 23, 24, 22-Me
22-Me	1.19 d (7.0)	11.0 q	H-22, 23
23	5.70 t (9.5)	73.5 d	H-22, 22-Me
24	5.39 br d (9.5)	127.2 d	H-22, 23, 26a,b, 25-Me
25		136.7 s	H-23, 26a,b, 27, 25-Me
25-Me	1.98 s	17.6 q	H-24, 26a,b
26a	2.28 dd (13.9, 5.5)	44.5 t	H-24, 27, 25-Me
26b	2.32 dd (13.9, 7.7)		
27	5.25 m	71.8 d	H-26a,b, 28, 29
28	1.51 m[d]	36.3 t	H-26a,b, 27, 29, 30
29	1.31 m[d]	18.8 t	H-27, 30
30	0.84 t (7.3)	14.0 q	H-29
3-OH	6.35 br d (7.7)		
19-OH	5.98 br d (6.6)		
21-OH	6.01 br s		
Ac	2.04, 2.04, 2.05, 2.08 s	20.9, 20.9, 21.0, 21.1 q 170.2, 170.4, 170.4, 170.6 s	

[a] Recorded at 600 MHz. Coupling constants (Hz) are in parentheses. [b] Recorded at 100 MHz. Multiplicity was based on the ^{1}H−^{13}C COSY spectrum and DEPT experiments. [c] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for J_{CH} = 6 Hz. [d] 2 H.

cal with that of **3**. Thus, the structure of dolabelide D including the absolute stereochemistry was established as shown in formula **4**.

Dolabelides A (**1**), B (**2**), C (**3**), and D (**4**) constitute a new class of cytotoxic polyketide macrolides. Macrolides from sea hares are quite rare: aplyronine A, a potent antitumor macrolide, and related compounds were isolated from *Aplysia kurodai*.[4]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-1000 polarimeter. UV and IR spectra were recorded on a JASCO UVIDEC-510 spectrophotometer and a JASCO FT/IR-230 spectrophotometer, respectively. NMR spectra were recorded on a JEOL ALPHA600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) or a JEOL ALPHA400 (100 MHz for ^{13}C). NMR chemical shifts were referenced to solvent peaks: δ_H 8.71 (residual pyridine-d_4) and δ_C 149.8 (pyridine-d_5). Mass spectra were determined on a JEOL JMS LG2000 spectrometer operating in the FAB mode (*m*-nitrobenzyl alcohol as a matrix). Fuji Silysia silica gel BW-820 MH was used for column chromatography unless otherwise noted. Preparative HPLC and medium-pressure liquid chromatography (MPLC) were performed using a JASCO TRI ROTAR pump and a JASCO 880 pump, respectively. Unless otherwise stated, materials were obtained from commercial suppliers and used without further purification. Pyridine was distilled from CaH_2.

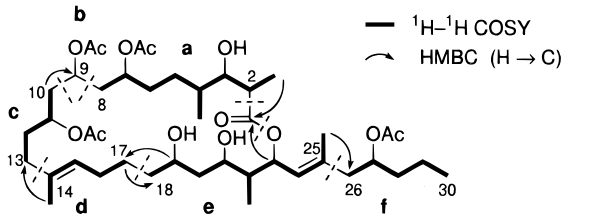

Figure 1. Partial structures (**a**−**f**) of dolabelide C (**3**) based on the ^{1}H−^{1}H COSY spectrum and the selected HMBC correlations.

Isolation of Dolabelide C. *D. auricularia*[5] (138 kg, wet weight) were collected at a depth of 0−1 m off the coast of the Shima Peninsula, Mie Prefecture, Japan, in 1995. The internal organs (71 kg, wet weight) of the specimens were extracted with MeOH (143 L). The methanolic extract was concentrated to ca. 16 L in vacuo and extracted with EtOAc (3 × 16 L). After concentration in vacuo, the EtOAc portion (281 g) was dissolved in 9:1 MeOH/H_2O (5 L), and the solution was washed with hexane (2 × 5 L). The aqueous MeOH portion (86 g) was chromatographed on silica gel (1.7 kg), eluting with 1:1 toluene/EtOAc and EtOAc, successively. The fraction (6.7 g) eluted with EtOAc was chromatographed on silica gel (340 g, 2:1 hexane/acetone). The middle fraction (3.54 g) was subjected to reversed-phase MPLC (Develosil ODS 30/60, 75% → 100% MeOH). The fraction (447 mg) eluted with 89−94% MeOH was further separated by reversed-phase MPLC (Develosil ODS 30/60, 80% → 100% MeOH). The fraction (341 mg) eluted with 86−88% MeOH was chromatographed on silica gel (20 g, 15:1, 10:1, 5:1, and 3:1 $CHCl_3$/acetone, successively). The fraction (181 mg) eluted with 5:1 to 3:1 $CHCl_3$/acetone was separated by preparative HPLC (Develosil ODS-HG-5, 63:4:33 → 94:6:0 MeCN/MeOH/H_2O) to afford dolabelide C (**3**, 99 mg, 7.2 × 10^{-5}% yield, based on wet weight) as a colorless oil: $[\alpha]^{26}_D$ +10° (*c* 0.16, $CHCl_3$); IR ($CHCl_3$) ν max 3480 (br), 1730, 1375, 1260 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; FABMS *m*/*z* 819 (MNa^+); HRFABMS *m*/*z* 819.4863, calcd for $C_{43}H_{72}NaO_{13}$ (MNa^+) 819.4870.

Isolation of Dolabelide D. The aqueous MeOH portion (211 g) was obtained from *D. auricularia*[5] (175 kg, wet weight, collected in 1993) by the same procedure described above and was chromatographed on silica gel (3.9 kg), eluting with 1:1 toluene/EtOAc, EtOAc, 19:1 EtOAc/MeOH, and 9:1 EtOAc/MeOH, successively. The fraction (9.7 g) eluted with 9:1 EtOAc/MeOH was subjected to MPLC (Micro Bead silica gel 4B, 80:16:4

→ 0:80:20 benzene/EtOAc/MeOH). The fraction (2.2 g) eluted with 55:36:9−35:52:13 benzene/EtOAc/MeOH was further separated by MPLC (Develosil ODS 30/60, 70:30 → 100:0 MeOH/0.02 M NH_4OAc). The fraction (926 mg) eluted with 84:16−100:0 MeOH/0.02 M NH_4-OAc was chromatographed on alumina (E. Merck, Aluminum oxide 90, activity II-III), eluting with 14:1, 8:1, and 3:1 $CHCl_3$/MeOH, successively. The fraction (35.6 mg) eluted with 3:1 $CHCl_3$/MeOH was separated by preparative HPLC (Develosil ODS-HG-5, 75:25 MeCN/H_2O) to afford dolabelide D (**4**, 2.7 mg, 1.5×10^{-6}% yield, based on wet weight) as a colorless oil: $[\alpha]^{29}_D$ +2.6° (*c* 0.28, $CHCl_3$); IR ($CHCl_3$) ν max 3600−3100 (br), 1725, 1460, 1375, 1255, 1025 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) δ 6.30−5.90 (5 H, m, OH), 6.07 (1 H, m, H-9), 5.71 (1 H, t, J = 9.5 Hz, H-23), 5.40 (1 H, d, J = 9.5 Hz, H-24), 5.32 (1 H, br t, J = 6.2 Hz, H-15), 5.26 (1 H, m, H-27), 4.80 (1 H, m, H-21), 4.38 (1 H, m, H-19), 4.15 (1 H, br d, J = 9.5 Hz, H-3), 4.10−4.02 (2 H, m, H-7, -11), 2.93 (1 H, dq, J = 9.5, 7.0 Hz, H-2), 2.50 (1 H, m, H-22), 2.38 (1 H, m, H-13b), 2.32 (1 H, dd, J = 13.9, 7.7 Hz, H-26b), 2.27 (1 H, dd, J = 13.9, 5.5 Hz, H-26a), 2.23−2.16 (3 H, m, H-8b, -10b, -13a), 2.11−1.88 (7 H, m, H-5b, -6b, -8a, -10a, -12b, -16ab), 2.06 (3 H, s, Ac), 2.02 (3 H, s, Ac), 1.98 (3 H, s, 25-Me), 1.87−1.83 (2 H, m, H-20ab), 1.80−1.63 (5 H, m, H-4, -6a, -12a, -17b, -18b), 1.63−1.54 (3 H, m, H-5a, -17a, -18a), 1.57 (3 H, s, 14-Me), 1.54−1.46 (2 H, m, H-28ab), 1.37−1.20 (2 H, m, H-29ab), 1.19 (3 H, d, J = 7.0 Hz, 2-Me), 1.18 (3 H, d, J = 7.0 Hz, 22-Me), 0.97 (3 H, d, J = 6.6 Hz, 4-Me), 0.84 (3 H, t, J = 7.3 Hz, H-30); ^{13}C NMR (pyridine-d_5, 150 MHz) δ 174.1 (s, C1), 170.8 (s, *C*OCH$_3$), 170.5 (s, *C*OCH$_3$), 136.7 (s, C25), 133.9 (s, C14), 127.2 (d, C24), 126.4 (d, C15), 74.1 (d, C3), 73.5 (d, C23), 71.9 (d, C27), 71.2 (d, C9), 67.9 (d, C19), 67.8 (d, C21), 67.6 (d, C7), 66.2 (d, C11), 46.4 (d, C2), 44.6 (t, C26), 43.9 (d, C22), 43.0 (t, C8), 42.1 (t, C10), 38.8 (t, C20), 38.0 (t, C18), 36.4 (t, C28), 35.9 (t, C6), 35.7 (t, C12), 35.6 (t, C13), 34.4 (d, C4), 30.4 (t, C5), 27.8 (t, C16), 26.7 (t, C17), 21.4 (q, CO*C*H$_3$), 21.1 (q, CO*C*H$_3$), 18.9 (t, C29), 17.7 (q, 25-Me), 15.7 (q, 14-Me), 14.1 (q, C30), 13.8 (q, 2-Me), 13.3 (q, 4-Me), 11.2 (q, 22-Me); FABMS m/z 735 (MNa^+); HRFABMS m/z 735.4634, calcd for $C_{39}H_{68}NaO_{11}$ (MNa^+) 735.4659.

Isomerization of Dolabelide A to Dolabelide C. To a solution of dolabelide A (**1**, 10.9 mg, 0.0134 mmol) in MeOH (3 mL) was added a 0.53 M solution of NaOMe in MeOH (0.04 mL, 0.021 mmol). After the mixture was stirred at 23 °C for 3 h, ion-exchange resin Amberlite IRC-50 (H^+, 60 mg) was added. The mixture was stirred at 23 °C for 1 h and filtered through a plug of cotton, and the resin was washed with MeOH (5 mL). The filtrate and washings were combined and concentrated. The residual oil was chromatographed on silica gel (6 g, 3:1, 2:1, and 3:2 $CHCl_3$/acetone, successively) to afford dolabelide C (**3**, 2.4 mg, 22%) as a colorless oil along with dolabelide A (**1**, 4.0 mg).

Acetylation of Dolabelide C. A mixture of dolabelide C (**3**, 8.0 mg, 0.010 mmol), acetic anhydride (2.0 mL), 4-(dimethylamino)pyridine (4.0 mg, 0.033 mmol), and pyridine (4.0 mL) was stirred at 23 °C for 3 h and concentrated. The residual oil was chromatographed on silica gel (4 g, 1:1 and 2:3 hexane/ether) to afford heptaacetate **5** (8.3 mg, 90%) as a colorless oil: $[\alpha]^{32}_D$ −5.2° (*c* 0.12, $CHCl_3$); IR ($CHCl_3$) ν max 1730, 1375, 1255, 1020 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) δ 5.59 (1 H, dt, J = 10.1, 4.3 Hz, H-21), 5.54 (1 H, t, J = 8.8 Hz, H-23), 5.41 (1 H, dd, J = 9.0, 3.0 Hz, H-3), 5.38 (1 H, m, H-19), 5.35−5.30 (2 H, m, H-7, -24), 5.28 (1 H, m, H-9), 5.23−5.20 (2 H, m, H-15, -27), 5.11 (1 H, m, H-11), 2.95 (1 H, dq, J = 9.0, 7.3 Hz, H-2), 2.41 (1 H, m, H-22), 2.35 (1 H, dd, J = 13.7, 7.3 Hz, H-26b), 2.27 (1 H, dd, J = 13.7, 5.6 Hz, H-26a), 2.15−2.01 (8 H, m, H-8b, -10b, -13ab, -16ab, -20ab), 2.00−1.92 (3 H, m, H-4, -8a, -10a), 2.14 (3 H, s, Ac), 2.12 (3 H, s, Ac), 2.11 (3 H, s, Ac), 2.09 (3 H, s, Ac), 2.08 (3 H, s, Ac), 2.07 (3 H, s, Ac), 2.06 (3 H, s, Ac), 1.90−1.68 (6 H, m, H-6ab, -12ab, -18ab), 1.88 (3 H, d, J = 1.2 Hz, 25-Me), 1.61 (3 H, s, 14-Me), 1.58−1.49 (5 H, m, H-5a, -17ab, -28ab), 1.38−1.26 (3 H, m, H-5a, -29ab), 1.36 (3 H, d, J = 7.3 Hz, 2-Me), 1.04 (3 H, d, J = 7.0 Hz, 22-Me), 0.94 (3 H, d, J = 6.7 Hz, 4-Me), 0.87 (3 H, t, J = 7.3 Hz, H-30); FABMS m/z 945 (MNa^+); HRFABMS m/z 945.5200, calcd for $C_{49}H_{78}NaO_{16}$ (MNa^+) 945.5187.

Acetylation of Dolabelide D. By the same procedure described for the acetylation of dolabelide C (**3**), dolabelide D (**4**, 1.3 mg, 0.0018 mmol) was acetylated to afford heptaacetate **5** (1.5 mg, 89%) as a colorless oil: $[\alpha]^{30}_D$ −5.0° (*c* 0.083, $CHCl_3$).

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Supporting Information Available: Table of ^1H and ^{13}C NMR assignments of **4** (2 pages). Ordering information is given on any current masthead page.

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(5) Although a large number of sea hares had to be collected for this work, *D. auricularia* is a very common animal, and plenty of them were observed the following year at the collection location. Our collection thus posed no significant ecological threat to this species.

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