Aurilol, a Cytotoxic Bromotriterpene Isolated from the Sea Hare *Dolabella auricularia*

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Aurilol (**1**), a novel cytotoxic bromotriterpene, was isolated from the sea hare *Dolabella auricularia*. The structure of **1**, including the absolute stereochemistry of the five stereocenters, was determined by spectroscopic and chemical analyses. Aurilol (**1**) exhibited cytotoxicity against HeLa S_3 cells with an IC_{50} of 4.3 μg/mL.

The Indian Ocean sea hare *Dolabella auricularia* Solander (order Aplysiacea, family Aplysiidae) 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 sea hare *D. auricularia* resulted in the isolation of several cytotoxic and structurally unique organic compounds.[2] We report here the structure determination of aurilol (**1**), a novel cytotoxic bromotriterpene, isolated from Japanese specimens of this animal.

The MeOH extract of the internal organs of the sea hare *D. auricularia*, collected in Mie Prefecture, Japan, was partitioned between EtOAc and H_2O. 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 fractionation using column chromatography (Si gel and ODS Si gel) and reversed-phase HPLC to afford aurilol (**1**, 6.2×10^{-6}% yield based on wet wt) as a colorless oil. Aurilol (**1**) exhibited cytotoxicity against HeLa S_3 cells, with an IC_{50} of 4.3 μg/mL.[3]

The molecular formula of **1** was determined to be $C_{30}H_{53}BrO_7$ by HRFABMS. As its IR and ^{13}C NMR spectra indicated that **1** had no unsaturated bonds, **1** was shown to be tetracyclic. The presence of three hydroxyl groups in **1** was indicated by the IR band at 3450 cm^{-1} and three D_2O exchangeable resonances (δ 4.81, 4.73, and 2.63) in the ^1H NMR spectrum. A detailed analysis of the phase-sensitive DQF−COSY spectrum of **1** allowed five partial structures, C3−C5, C7−C9, C11−C14, C16−C18, and C20−C22 (Figure 1), to be constructed. Furthermore, the HMBC data, summarized in Table 1, allowed the foregoing partial structures, eight methyl groups, and six quaternary carbons to be connected, establishing the connectivities of all the carbon atoms in **1** as shown in Figure 1. The locations of the three hydroxyl groups in **1** were determined by HMBC correlations (C19/19-OH, C23/22-OH, C23/23-OH, and C24/23-OH). The presence of three cyclic ethereal groups (rings A, C, and D), was indicated by the HMBC correlations (C2/H-7, C14/H-11, and C15/H-18). The one additional cyclic ethereal group (ring B) was shown to be present based on the NOESY correlation (H-26/H-27) in C_6D_6, leaving the bromine atom to reside at C3. Thus, the gross structure of aurilol was determined to be that of a bromotriterpene possessing four ethereal rings.

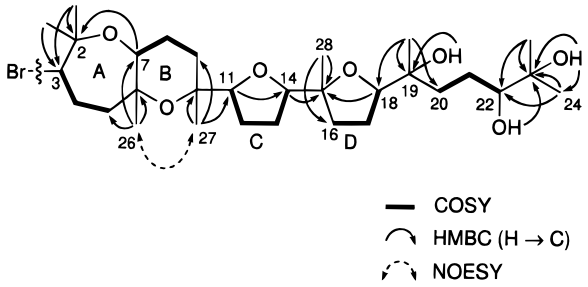

Figure 1. Partial structures of aurilol (**1**), based on the phase-sensitive DQF−COSY spectrum and selected HMBC and NOESY correlations.

A plausible conformation of rings A and B in **1** is shown in Figure 2, and is based on selected NOESY correlations and $^1H-^1H$ coupling constant. The magnitude of $J_{7,8b} = 11.2$ Hz and the NOESY correlations (H-1/H-3, H-1/H-7, H-3/H-7, H-8b/H-26, H-8b/H-27, and H-26/H-27) suggested the relative stereochemistry at C3, C6, C7, and C10 to be 3*R**, 6*R**, 7*S**, and 10*R** (Figure 2). Furthermore, the NOE interaction between H-18 and H_3-28 established the relative stereochemistry of ring D of **1** as cis. To determine the relative stereochemistry of ring C, two model stereoisomers, **4a** and **4b** (Figure 3), were synthesized from (2*S*,3*R*)-1,2-epoxy-3-(methoxymethoxy)-3,7-dimethyl-6-octene.[4] The stereochemistry of ring C in **4a** and **4b** was determined as follows. The NOE was observed between H-11 and H-14 in **4b**, whereas it was not in **4a**. Therefore, the stereochemistry of ring C in **4a** and **4b** is trans and cis, respectively. The patterns and the coupling constants of H-11 and H-14 in the ^1H NMR spectrum of **1** were quite similar to those of **4a**, but were clearly different from those of **4b** (Figure 3), and thus the relative stereochemistry of ring C of **1** was deduced to be trans.

The absolute stereochemistry of rings A and B and of C22 in **1** was determined using a modified Mosher's method.[5] Reduction of **1** with zinc powder afforded tetraol **2**, which was transformed into the (*R*)- and (*S*)-bisMTPA esters, **3a** and **3b**. The ^1H NMR signals of the two esters, **3a** and **3b**, were assigned based on

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Table 1. NMR Data for Aurilol (1) in $CDCl_3$

position	^1H[a]	^{13}C[b]	HMBC[c]
1	1.34 s	25.4 q	H-3, 25
2		77.7 s	H-1, 3, 4ab, 7, 25
3	4.15 d (10.6)	60.1 d	H-1, 4ab, 5ab, 25
4a	2.17 m	31.4 t	H-3, 5a
4b	2.07 m		
5a	1.64 m	44.1 t	H-3, 7, 26
5b	1.60 m		
6		75.9 s	H-5ab, 7, 26
7	3.18 dd (11.7, 3.9)	72.7 d	H-5ab, 26
8a	1.72 m	23.8 t	H-7, 9b
8b	1.52 m		
9a	1.62 m	31.0 t	H-7, 8ab, 11, 27
9b	1.54 m		
10		74.9 s	H-8b, 9ab, 11, 27
11	3.74 dd (9.2, 6.2)	87.6 d	H-9a, 12ab, 13b, 27
12a	1.92 m	27.4 t	H-11, 13ab
12b	1.72 m		
13a	1.92 m	29.4 t	H-11, 12ab, 14
13b	1.40 m		
14	4.06 dd (10.6, 5.1)	85.7 d	H-11, 13ab, 28
15		86.1 s	H-14, 16ab, 18, 28
16a	2.08 m	30.0 t	H-14, 17ab, 18, 28
16b	1.46 m		
17a	2.18 m	26.1 t	H-18
17b	2.00 m		
18	3.86 dd (8.4, 3.3)	85.7 d	H-29
19		74.4 s	H-17ab, 18, 21a, 29, 19-OH
19-OH	4.81 br s		
20a	1.62 m	35.2 t	H-21ab, 22, 29, 19-OH
20b	1.39 m		
21a	1.67 m	25.3 t	H-20a
21b	1.58 m		
22	3.28 br d (9.8)	79.4 d	H-20ab, 21ab, 24, 30, 23-OH
22-OH	4.73 br s		
23		72.7 s	H-24, 30, 22-OH, 23-OH
23-OH	2.63 br s		
24	1.16 s	23.7 q	H-30, 23-OH
25	1.33 s	24.6 q	H-1, 3
26	1.17 s	20.5 q	H-7
27	1.14 s	22.0 q	H-9ab, 11
28	1.12 s	25.0 q	H-14
29	1.27 s	24.1 q	
30	1.19 s	26.2 q	H-24, 23-OH

[a] Recorded at 400 MHz. Coupling constants (Hz) are in parentheses. [b] Recorded at 100 MHz. Multiplicity was based on the $^{13}C-^1H$ COSY spectrum and DEPT experiments. [c] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for $J_{CH} = 6$ and 4 Hz.

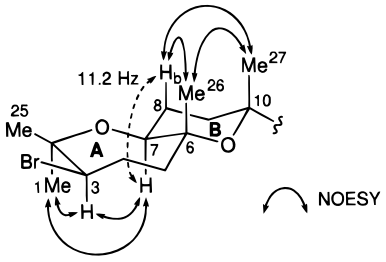


Figure 2. A plausible conformation of rings A and B in **1** with the selected NOESY correlations and $^1H-^1H$ coupling constant in C_6D_6

the 2D NMR spectra, and the $\Delta\delta$ values ($\delta_S - \delta_R$, Hz) were then calculated. The results, shown in Figure 4, established that the absolute stereochemistry of the secondary hydroxyl groups at C7 and C22 in **2** was *S* and *R*, respectively. Thus, the absolute stereochemistry of the five stereocenters in **1** was determined to be 3*R*, 6*R*, 7*S*, 10*R*, and 22*R*.

Aurilol (**1**) is a novel cytotoxic bromotriterpene that possesses a 2,8-dioxabicyclo[5.4.0]undecane structure. Enshuol,[6] isolated from a red alga of the genus *Laurencia*, is a bromotriterpene structurally related to aurilol (**1**) and suggests **1** to be of dietary origin. Bromotriterpenes of the type represented by **1** are currently quite rare, in contrast to thyrsiferol types.[7]

Compounds	H-11	H-14
1	3.62 dd (J = 6.3, 9.3 Hz)	3.96 dd (J = 5.4, 10.4 Hz)
4a	3.49 dd (J = 5.4, 10.3 Hz)	3.91 dd (J = 5.7, 10.3 Hz)
4b	3.49 t (J = 7.3 Hz)	3.79 t (J = 7.3 Hz)

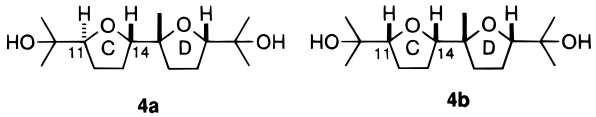


Figure 3. ^1H NMR spectral properties of H-11 and H-14 in **1**, **4a**, and **4b**.

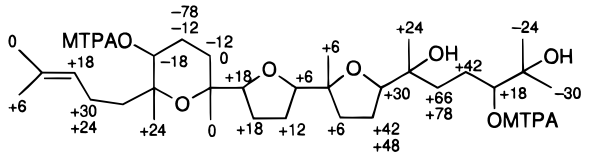
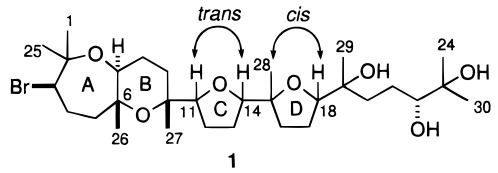
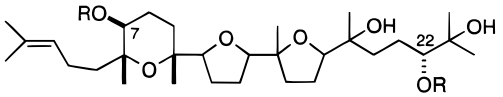


Figure 4. $\Delta\delta$ Values ($\delta_S - \delta_R$) for the bisMTPA esters **3a** and **3b** in Hz (600 MHz).



1



2 R = H
3a R = (*R*)-MTPA
3b R = (*S*)-MTPA

Experimental Section

General Experimental Procedures. IR, NMR, and MS spectra and preparative HPLC were performed on the same instruments as those described in an earlier paper on *D. auricularia*.[8] Unless otherwise stated, materials were obtained from commercial suppliers and used without further purification. Dicyclohexylcarbodiimide was distilled under reduced pressure.

Animal Material. *D. auricularia* animals (45 kg, wet wt) were collected at a depth of 0−1 m off the coast of the Shima Peninsula, Mie Prefecture, Japan, in 1996. *D. auricularia* is very common, and many were observed the following year at the collection location. Our collection thus posed no significant ecological threat to this species.

Extraction and Isolation. The internal organs (23 kg, wet wt) of the specimens were extracted with MeOH (45 L). The MeOH extract was concentrated to ca. 4 L *in vacuo* and extracted with EtOAc (3 × 4 L). After concentration *in vacuo*, the EtOAc portion (120 g) was dissolved in 9:1 MeOH−H_2O (2 L), and the solution was washed with hexane (2 × 2 L). The aqueous MeOH portion (46 g) was chromatographed on Si gel (900 g),

using 1:1 toluene−EtOAc (3.6 L) followed by EtOAc (3.6L) as eluent. The fraction (4.0 g) eluted with EtOAc was then chromatographed on Si gel (200 g, 2:1 hexane−acetone). A material (1.84 g), obtained from the middle fraction (450 mL), was subjected to reversed-phase-MPLC (Develosil ODS 30/60, 75% → 100% MeOH). The fraction (1.17 g) eluted with 96−100% MeOH was further chromatographed on Si gel (20 g, 15:1, 10:1, 5:1 $CHCl_3$−Me_2CO and Me_2CO, successively). The fraction (48 mg) eluted with 5:1 $CHCl_3$−Me_2CO was further separated by reversed-phase-MPLC (Develosil ODS 30/60, 80% → 100% MeOH). The fraction (13.5 mg) eluted with 96−100% MeOH was separated by reversed-phase-HPLC ((a) Develosil ODS−HG-5, 85% MeCN; (b) Develosil Ph-5, 80% MeOH) to afford aurilol (**1**, 2.8 mg, 6.2×10^{-6}% yield, based on wet wt). An additional amount (3.0 mg) of aurilol (**1**) was obtained from the recollection in 1997 (66 kg) by the same procedure.

Aurilol (1): colorless oil; $[\alpha]^{30}_D$ +4.6° (*c* 0.41, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3350 (br), 1450, 1375, 1080 cm^{-1}; ^1H and ^{13}C NMR data in $CDCl_3$, see Table 1; ^1H NMR (C_6D_6, 600 MHz) δ 4.90 (2H, br s, OH), 4.07 (1H, dd, *J* = 5.4, 6.8 Hz, H-3), 3.96 (1H, dd, *J* = 5.4, 10.4 Hz, H-14), 3.73 (1H, dd, *J* = 3.9, 7.8 Hz, H-18), 3.62 (1H, dd, *J* = 6.3, 9.3 Hz, H-11), 3.45 (1H, d, *J* = 8.3 Hz, H-22), 3.04 (1H, dd, *J* = 3.9, 11.2 Hz, H-7), 2.70 (1H, br s, OH), 2.17 (1H, m, H-17a), 2.07 (1H, m, H-4), 2.00 (1H, m, H-16a), 1.75 (1H, m, H-17b), 1.75 (1H, m, H-21a), 1.65 (1H, m, H-8a), 1.65 (1H, m, H-12a), 1.65 (1H, m, H-21b), 1.62 (1H, m, H-20a), 1.57 (1H, m, H-5), 1.50 (1H, m, H-8b), 1.50 (1H, m, H-9a), 1.47 (1H, m, H-12b), 1.47 (1H, m, H-13a), 1.43 (3H, s, H-29), 1.43 (1H, m, H-9b), 1.41 (3H, s, H-25), 1.32 (3H, s, H-30), 1.20 (1H, m, H-16b), 1.20 (1H, m, H-20b), 1.15 (3H, s, H-26), 1.05 (3H, s, H-27), 1.05 (1H, m, H-13b), 0.98 (3H, s, H-28); ^{13}C NMR (C_6D_6, 150 MHz) δ 88.2 (d, C-11), 86.1 (d, C-14), 86.0 (s, C-15), 85.9 (d, C-18), 79.9 (d, C-22), 77.7 (s, C-2), 76.1 (s, C-6), 74.8 (s, C-10), 74.8 (s, C-19), 72.8 (d, C-7), 72.5 (s, C-23), 60.4 (d, C-3), 44.5 (t, C-5), 36.0 (t, C-20), 32.2 (t, C-9), 31.7 (t, C-4), 30.6 (t, C-16), 29.3 (t, C-13), 27.5 (t, C-12), 26.4 (q, C-30), 26.3 (t, C-17), 26.1 (t, C-21), 25.7 (q, C-25), 25.0 (q, C-28), 24.6 (q, C-1), 24.5 (q, C-24), 24.4 (q, C-29), 24.1 (t, C-8), 21.6 (q, C-27), 20.8 (q, C-26); FABMS *m/z* 607/605 (MH^+); HRFABMS *m/z* 627.2888, calcd for $C_{30}H_{53}{}^{79}BrNaO_7$ (MNa^+) 627.2872.

Tetraol 2. A mixture of aurilol (**1**, 2.8 mg, 4.6 μmol), activated zinc powder (11.9 mg), and NH_4Cl (10.2 mg) in EtOH (0.25 mL) was refluxed for 40 min and filtered through a pad of Celite, and the residue was washed with EtOAc (5 mL). The filtrate and the washings were combined and concentrated. The residual oil was purified by column chromatography on Si gel (0.5 g, 2:1 $CHCl_3$−Me_2CO) to give tetraol **2** (2.3 mg, 95%) as a colorless oil: $[\alpha]^{30}_D$ −9.9° (*c* 0.081, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3360 (br), 1450, 1375 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.11 (1H, br t, *J* = 7.0 Hz, H-3), 5.00 (1H, br s, OH), 4.90 (1H, br s, OH), 4.12 (1H, dd, *J* = 4.3, 9.8 Hz), 3.85 (1H, dd, *J* = 3.9, 8.3 Hz), 3.67 (1H, t, *J* = 7.3 Hz), 3.43 (1H, dd, *J* = 5.9, 10.7 Hz), 3.30 (1H, d, *J* = 8.8 Hz, H-22), 2.63 (1H, s, OH), 2.18−1.38 (21H, m), 1.67 (3H, s), 1.60 (3H, s), 1.24 (3H, s), 1.19 (3H, s), 1.18 (3H, s), 1.18 (3H, s), 1.16 (3H, s), 1.12 (3H, s); FABMS *m/z* 549 (MNa^+); HRFABMS *m/z* 549.3739, calcd for $C_{30}H_{54}NaO_7$ (MNa^+) 549.3767.

(*R*)-BisMTPA Ester 3a. A mixture of tetraol **2** (1.2 mg, 2.3 μmol), (*R*)-MTPA (6.9 mg, 0.029 mmol), dicyclohexylcarbodiimide (6.6 mg, 0.032 mmol), and 4-(dimethylamino)pyridine (1.8 mg, 0.015 mmol) in CH_2Cl_2 (0.2 mL) was stirred at room temperature for 3 h, diluted with saturated aqueous $NaHCO_3$ (2 mL), and extracted with EtOAc (3 × 5 mL). The combined extracts were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The residual solid was dissolved in Et_2O (1 mL) and filtered through a small plug of cotton, and the residue was washed with Et_2O (4 mL). The filtrate and the washings were combined and concentrated. The residual oil was purified by column chromatography on Si gel (0.5 g, 50:1 CH_2Cl_2−Me_2CO) to give the (*R*)-MTPA ester **3a** (1.8 mg, 82%) as a colorless oil: $[\alpha]^{29}_D$ +8.5° (*c* 0.054, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3450 (br), 1745, 1600, 1450 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.60 (4H, m), 7.51 (4H, br d, *J* = 8.1 Hz), 7.39 (2H, m), 5.01 (2H, m, H-3, H-22), 4.89 (1H, dd, *J* = 5.1, 11.0 Hz, H-7), 4.05 (1H, dd, *J* = 4.4, 9.5 Hz, H-14), 3.89 (1H, s, OH), 3.75 (1H, dd, *J* = 5.1, 8.1 Hz, H-18), 3.68 (1H, t, *J* = 7.3 Hz, H-11), 3.54 (3H, s, OCH_3), 3.54 (3H, s, OCH_3), 2.10−1.10 (21H, m), 1.67 (3H, s, H-1), 1.57 (3H, s, H-25), 1.22 (3H, s, H-30), 1.19 (3H, s, H-27), 1.17 (3H, s, H-24), 1.16 (3H, s, H-29), 1.12 (3H, s, H-26), 1.10 (3H, s, H-28); FABMS *m/z* 981 (MNa^+); HRFABMS *m/z* 981.4611, calcd for $C_{50}H_{68}F_6NaO_{11}$ (MNa^+) 981.4564.

(*S*)-BisMTPA Ester 3b. The same experimental procedure was followed as described for the (*R*)-bisMTPA ester **3a**. **3b**: $[\alpha]^{29}_D$ −4.1° (*c* 0.048, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3400 (br), 1740, 1600, 1450 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.60 (4H, m), 7.49 (4H, br d, *J* = 7.7 Hz), 7.40 (2H, m), 5.04 (2H, m, H-3, H-22), 4.86 (1H, dd, *J* = 4.4, 11.4 Hz, H-7), 4.06 (1H, dd, *J* = 4.8, 9.9 Hz, H-14), 3.94 (1H, s, OH), 3.80 (1H, dd, *J* = 4.8, 7.7 Hz, H-18), 3.71 (1H, t, *J* = 7.7 Hz, H-11), 3.59 (3H, s, OCH_3), 3.49 (3H, s, OCH_3), 2.10−1.20 (21H, m), 1.67 (3H, s, H-1), 1.58 (3H, s, H-25), 1.20 (3H, s, H-29), 1.19 (3H, s, H-27), 1.17 (3H, s, H-24), 1.16 (3H, s, H-26), 1.13 (3H, s, H-30), 1.11 (3H, s, H-28); FABMS *m/z* 981; HRFABMS *m/z* 981.4543, calcd for $C_{50}H_{68}F_6NaO_{11}$ (MNa^+) 981.4564.

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Supporting Information Available: Experimental data for the two model compounds **4a** and **4b** (4 pages). Ordering information is given on any current masthead page.

References and Notes

(1) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Fujii, Y.; Kizu, H.; Boyd, M. R.; Boettner, F. E.; Doubek, D. L.; Schmidt, J. M.; Chapuis, J.-C.; Michel, C. *Tetrahedron* **1993**, *49*, 9151−9170.

(2) (a) Ishiwata, H.; Nemoto, T.; Ojika, M.; Yamada, K. *J. Org. Chem.* **1994**, *59*, 4710−4711. (b) Sone, H.; Shibata, T.; Fujita, T.; Ojika, M.; Yamada, K. *J. Am. Chem. Soc.* **1996**, *118*, 1874−1880. (c) Suenaga, K.; Mutou, T.; Shibata, T.; Itoh, T.; Kigoshi, H.; Yamada, K. *Tetrahedron Lett.* **1996**, *37*, 6771−6774. (d) Pettit, G. R.; Xu, J.-P.; Hogan, F.; Williams, M. D.; Doubek, D. L.; Schmidt, J. M.; Cerny, R. L.; Boyd, M. R. *J. Nat. Prod.* **1997**, *60*, 752−754. (e) Pettit, G. R.; Xu, J.-P.; Williams, M. D.; Hogan, F.; Schmidt, J. M.; Cerny, R. L. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 827−832.

(3) The cytotoxicity of **1** was weaker than that of the crude extract, from which **1** was isolated. This was due to the presence of more strongly cytotoxic substances (doliculide,[2a] dolastatin H, [2b] and aurilide [2c]) than **1** in the crude extract.

(4) Hashimoto, M.; Harigaya, H.; Yanagiya, M.; Shirahama, H. *J. Org. Chem*. **1991**, *56*, 2299−2311.
(5) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(6) Matsuo, Y.; Suzuki, M.; Masuda, M. *Chem. Lett.* **1995**, 1043−1044.
(7) Blunt. J. W.; Hartshorn, M. P.; McLennan, T. J.; Munro, M. H. G.; Robinson, W. T.; Yorke, S. C. *Tetrahedron Lett.* **1978**, 69−72.
(8) Suenaga, K.; Nagoya, T.; Shibata, T.; Kigoshi, H.; Yamada, K. *J. Nat. Prod.* **1997**, *60*, 155−157.

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