

Brianodins A–D, Briarane-Type Diterpenoids from Soft Coral *Pachyclavularia* sp.

Haruaki Ishiyama,[†] Taro Okubo,[†] Tetsuro Yasuda,[†] Yohei Takahashi,[†] Kazuo Iguchi,[‡] and Jun'ichi Kobayashi^{*,*}

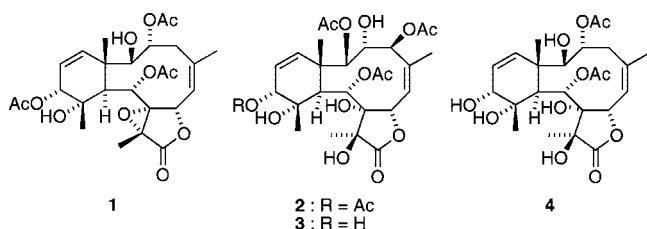
Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, and School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, Horinouchi, Hachioji, Tokyo 192-0392, Japan

Received November 29, 2007

Four new briarane-type diterpenoids, brianodins A–D (**1–4**), were isolated from a soft coral, *Pachyclavularia* sp., and the structures and relative stereochemistry of **1–4** were elucidated on the basis of spectroscopic data. The absolute configurations of **3** and **4** were assigned by the MTPA method. Brianodin A (**1**) showed a modest cytotoxicity.

A characteristic feature of briarane-type diterpenoids is the presence of a highly substituted bicyclo[8.4.0]tetradecane skeleton, and most briarane diterpenoids possess a γ -lactone moiety.¹ More than 300 briarane diterpenoids have been isolated from soft corals so far,² and some of them show interesting biological properties such as cytotoxic,³ anti-inflammatory,^{4–6} antiviral,^{6,7} insecticidal,⁸ immunomodulation,⁹ and multidrug resistance reversing activities.¹⁰

Chemical modifications of natural products such as taxane diterpenoids¹¹ have led to many unprecedented compounds useful for studies of structure–activity relationships. In order to obtain briarane diterpenoids for such a study, the terpenoid fractions of a soft coral *Pachyclavularia* sp. were purified. As a result, four new briarane-type diterpenoids, brianodins A–D (**1–4**), were isolated together with 10 known briarane diterpenoids. Herein, we describe the isolation and structure elucidation of **1–4**.



Results and Discussion

The soft coral *Pachyclavularia* sp. (SC-114) collected in Okinawa was extracted with MeOH, and the extract was partitioned between EtOAc and water. The EtOAc-soluble materials were subjected to passage over a silica gel column (CHCl₃/MeOH, 95:5, and then *n*-hexane/EtOAc, 1:3) followed by C₁₈ HPLC (MeOH/H₂O, 40:60 → 70:30) to afford brianodins A–D (**1–4**) (**1**, 0.0032%, wet wt; **2**, 0.0006%; **3**, 0.0007%; **4**, 0.0013%) together with 10 known briarane-type diterpenoids, briaralides A,¹² G,¹² H,¹² and J¹³ and violides B,¹⁴ G,¹⁵ J,¹⁶ M,¹⁶ O,¹⁷ and P.¹⁷

Brianodin A (**1**) was obtained as a colorless solid, and the molecular formula, C₂₆H₃₄O₁₁, was established by HRFABMS (*m/z* 523.2178 [M + H]⁺, Δ –0.1 mmu). The IR spectrum of **1** implied the presence of an ester (1740 cm^{–1}) functionality. The ¹H NMR spectrum of **1** revealed signals due to three acetyl methyls (δ_{H} 2.09, 2.14, and 2.21), an olefinic methyl (δ_{H} 1.94), and three tertiary methyls (δ_{H} 1.08, 1.24, and 1.72), and the ¹³C NMR spectrum of **1** disclosed the presence of four carbonyl carbons (δ_{C} 168.4, 168.8, 169.3, and 170.1) and four olefinic carbons (δ_{C} 120.7, 121.1, 138.9, and 141.5) (Tables 1 and 2). The gross structure of **1** was elucidated by analysis of 2D NMR data (¹H–¹H COSY, HMQC, and HMBC

(Figure 1). The presence of an 8,17-epoxide was indicated by the molecular formula and ¹³C NMR chemical shifts of C-8 (δ_{C} 71.5) and C-17 (δ_{C} 64.8). Geometry of the trisubstituted olefin at C-5 and C-6 was assigned as *Z* from NOESY correlations of H₃-16 to H-6.

The relative stereochemistry of **1** was elucidated by ¹H coupling constants and NOESY correlations. NOESY correlations of H₃-20 to H-9 (δ_{H} 5.96, d, *J* = 3.9 Hz), H-12, and H₃-15, H-9 to H₃-18, and H₃-15 to H-3 indicated that H-3, H-12, Me-15, Me-18, and Me-20 had β -orientations and H-9 possessed an α -orientation. NOESY correlations of H-2 (δ_{H} 3.31, s) to H-10 and H₃-16, H₃-16 to H-4a and H-6, and H-4b to H-7 suggested that H-2, H-10, and 8,17-epoxide had α -orientations and H-7 and H-9 possessed β -orientations (Figure 2). Thus, the relative stereochemistry of brianodin A was elucidated to be **1**.

Brianodin B (**2**) was revealed to have the molecular formula C₂₈H₃₈O₁₄ by HRFABMS (*m/z* 599.2336 [M + H]⁺, Δ –0.3 mmu). The IR spectrum of **2** suggested the presence of an ester (1740 cm^{–1}) functionality. From the ¹H and ¹³C NMR analyses, **2** was indicated to possess four acetoxy groups, a γ -lactone moiety [δ_{H} 2.10 (3H, s), 2.15 (3H, s), 2.15 (3H, s), 2.21 (3H, s), δ_{C} 169.9, 170.9, 171.3, 172.7, 176.4] and two olefins [δ_{H} 5.55 (1H, d, *J* = 4.5 Hz), 5.68 (1H, m), 5.75 (1H, br d, *J* = 10.0 Hz), δ_{C} 121.1, 126.5, 139.4, 141.7] (Tables 1 and 2). The gross structure of **2** was elucidated from ¹H–¹H COSY and HMBC correlations (Figure 3). The relative stereochemistry of **2** was assigned by ¹H coupling constants and NOESY correlations. NOESY correlations of H₃-20 to H₃-15, H-12 (δ_{H} 5.04, d, *J* = 3.3 Hz) and H-9 (δ_{H} 6.07, d, *J* = 4.8 Hz) indicated that H-12, Me-15, and Me-20 had β -orientations and H-9 possessed an α -orientation. NOESY correlations of H-2 (δ_{H} 4.66, br s) to H-10 (δ_{H} 2.96, d, *J* = 4.8 Hz) and H₃-16, H₃-16 to H-4 and H-6, and H-3 to H-7 suggested that H-2, H-4, and H-10 were α -orientated and H-3, H-7, and H-9 were β -orientated (Figure 4). The relative configuration at C-8 and C-17 was elucidated by comparison of ¹³C NMR chemical shifts of brianodin B (**2**) with those of violide J,¹⁶ whose structure was determined by X-ray analysis. Thus, the relative stereochemistry of brianodin B was elucidated to be **2**.

The molecular formula, C₂₆H₃₆O₁₃, of brianodin C (**3**) was established by HRFABMS (*m/z* 557.2247 [M + H]⁺, Δ +1.3 mmu). The IR spectrum of **3** indicated the presence of ester (1730 cm^{–1}) and γ -lactone (1650 cm^{–1}) functionalities. The ¹H NMR spectrum of **3** was similar to that of brianodin B (**2**), except that H-12 (δ_{H} 3.71, d, *J* = 6.2 Hz) was shifted upfield by 1.33 ppm (Table 1) as compared with that of **2**, indicating that an acetyl group at C-12 in **2** was absent for **3**. The relative stereochemistry of **3** was elucidated by ¹H coupling constants and NOESY correlations. The relative configuration at C-8 and C-17 was elucidated by comparison of ¹³C NMR chemical shifts of brianodin C (**3**) with those of violide J.¹⁶ The absolute configuration of **3** was elucidated by a modified Mosher's method¹⁸ for the 2-methoxy-2-trifluoromethylphenylacetic

* To whom correspondence should be addressed. Tel: +81-11-706-3239. Fax: +81-11-706-4989. E-mail: jkobay@pharm.hokudai.ac.jp.

[†] Hokkaido University.

[‡] Tokyo University of Pharmacy and Life Sciences.

Table 1. ^1H NMR Data of Brianodins A–D (1–4) (J in Hz)

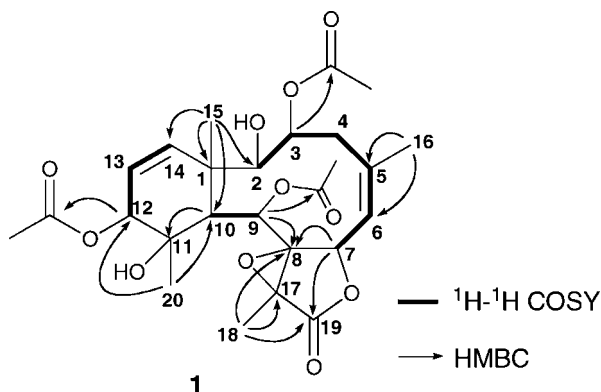
position	1 ^a	2 ^a	3 ^b	4 ^b
2	3.31 br s	4.66 br s	4.57 br s	3.26 br s
3	5.59 dd (12.2, 5.8)	5.07 d (11.2)	4.90 d (10.6)	5.61 dd (12.2, 5.7)
4a	2.11 m	4.86 br dd (11.0, 0.8)	5.17 d (11.3)	1.88 m
4b	3.04 br dd (13.8, 3.9)			2.92 br dd (13.8, 5.3)
6	5.46 br d (9.3)	5.75 br d (10.0)	5.72 br d (10.0)	5.45 br d (9.6)
7	5.70 d (9.6)	5.93 d (10.0)	6.04 d (9.9)	5.81 d (9.8)
9	5.96 d (3.9) ^c	6.07 d (4.8)	6.20 d (4.7)	6.07 d (4.3)
10	2.52 d (3.9)	2.96 d (4.8)	3.01 d (4.7)	2.67 d (4.3)
12	4.79 d (5.9)	5.04 d (3.3)	3.71 d (6.2)	3.58 d (6.0)
13	5.95 dd (10.3, 5.9) ^c	5.68 m	5.79 dd (10.4, 6.3)	5.65 br dd (10.3, 6.0)
14	6.05 d (10.3)	5.55 d (4.5)	5.56 d (10.6)	5.78 d (10.3)
15	1.08 s	1.29 s	1.32 s	0.92 s
16	1.94 s	2.13 br d (1.6)	2.17 br d (1.1)	1.78 br s
18	1.72 s	1.45 s	1.41 s	1.25 s
20	1.24 s	1.49 s	1.43 s	1.27 s
MeCO	2.09 (s), 2.14 (s), 2.21 (s)	2.10 (s), 2.15 (s), 2.15 (s), 2.21 (s)	2.14, 2.18, 2.19	1.85 (s), 2.00 (s)

^a In CDCl_3 . ^b In CD_3OD . ^c Overlapping with other signals in the same column.

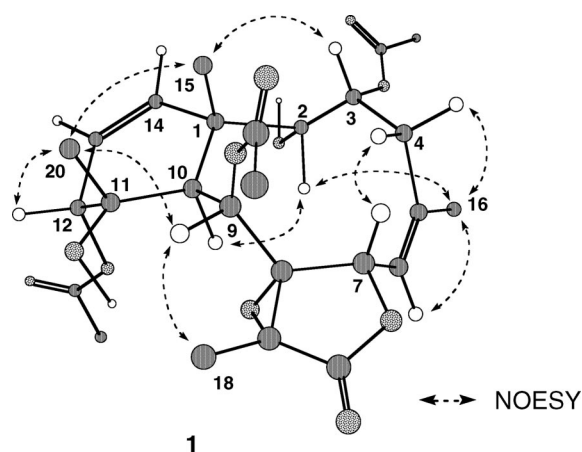
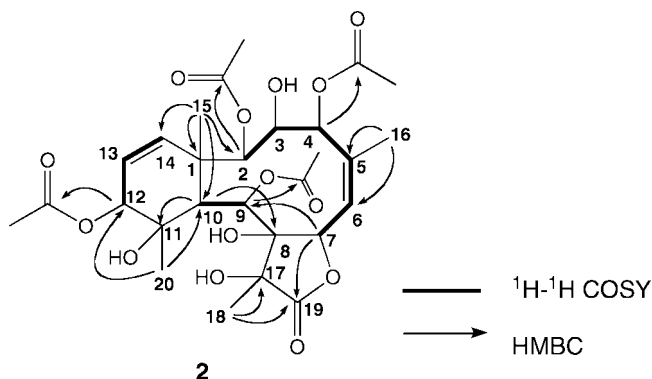
Table 2. ^{13}C NMR Data of Brianodins A–D (1–4)

position	1 ^a	2 ^a	3 ^b	4 ^b
1	48.0	47.0	49.0	49.9
2	77.7	78.4	79.8	79.6
3	71.7	70.8	72.8	75.0
4	33.9	77.1	80.5	35.6
5	138.9	139.4	141.8	139.8
6	120.7	126.5	128.2	124.9 ^c
7	74.0	79.3 ^d	80.1	81.8
8	71.5	79.3 ^d	81.2	81.7 ^d
9	65.0	65.6	68.4	68.8
10	44.1	39.6	40.8	41.4
11	72.0	75.8	77.1	77.0
12	73.0	72.2	72.3	73.2
13	121.1	121.1	126.4	124.6 ^c
14	141.5	141.7	140.9	142.7
15	13.4	16.8	17.1	15.8
16	27.4	25.9	26.9	35.6
17	64.8	80.2	81.7	81.7 ^d
18	9.5	15.6	16.8	17.0
19	170.1	176.4	179.4	179.6
20	21.0	23.6	23.8	23.6
MeCO	20.6, 20.8, 20.8	21.0, 21.2, 21.4, 22.3	21.5, 21.8, 23.1	23.1, 22.0
MeCO	168.4, 168.8, 169.3	169.9, 170.9, 171.3, 172.7	173.1, 173.1, 173.4	173.0, 173.5

^a In CDCl_3 . ^b In CD_3OD . ^c Data interchangeable. ^d Overlapping with other signals in the same column.

**Figure 1.** Selected 2D NMR correlations for brianodin A (1).

acid (MTPA) esters at C-12 of **3**, due to steric hindrance at C-2. The values of $\Delta\delta$ [$\delta(S\text{-MTPA ester}) - \delta(R\text{-MTPA ester})$] for H-2, H-3, H-4, H-13, and H-14 were positive, while the values of $\Delta\delta$ for H-6, H-7, H-9, and H-10 were negative, suggesting that the absolute configuration at C-12 was *R*. Thus, the absolute configuration of **3** was assigned as shown in Figure 5.

**Figure 2.** Selected NOESY correlations for brianodin A (1).**Figure 3.** Selected 2D NMR correlations for brianodin B (2).

Brianodin D (**4**) had the molecular formula $\text{C}_{24}\text{H}_{34}\text{O}_{11}$ by HRFABMS (m/z 499.2187 [$\text{M} + \text{H}$]⁺, $\Delta +0.6$ mmu). The IR spectrum of **4** suggested the presence of an ester (1730 cm^{-1}) functionality. The ^1H NMR spectrum of **4** showed signals due to two acetyl methyls (δ_{H} 1.85, 2.00), an olefinic methyl (δ_{H} 1.78), and three tertiary methyls (δ_{H} 0.92, 1.25, and 1.27). The ^{13}C NMR spectrum of **4** indicated the presence of three carbonyl carbons (δ_{C} 173.0, 173.5, and 179.6) and four olefinic carbons (δ_{C} 124.6, 124.9, 139.8, and 142.7) (Table 2). The gross structure of **4** was elucidated from $^1\text{H}-^1\text{H}$ COSY and HMBC correlations (Figure 6). The relative stereochemistry of **4** was elucidated by ^1H coupling constants and NOESY correlations. NOESY correlations from H₃-20 to H₃-15, H-12 (δ_{H} 3.58, d, $J = 6.0$ Hz) and H-9 (δ_{H} 6.07, d, $J = 4.3$ Hz) indicated β -orientations for H-12, Me-15, and Me-20 and an α -orientation for H-9. NOESY correlations of H-2 (δ_{H} 3.26, s) to

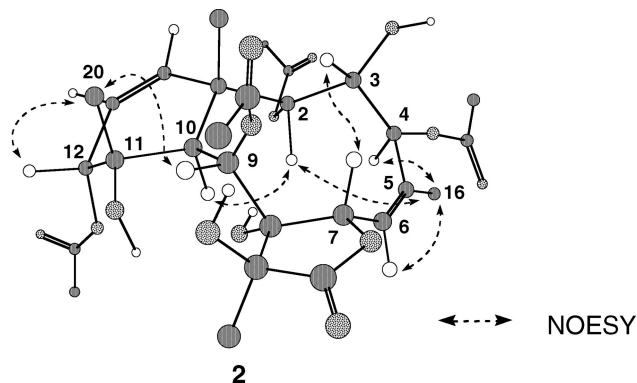


Figure 4. Selected NOESY correlations for briarodin B (2).

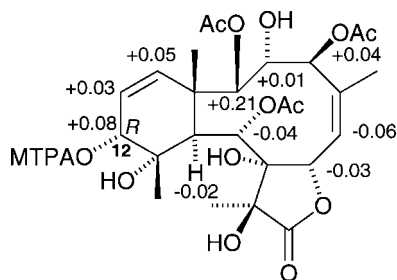


Figure 5. $\Delta\delta$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters at C-12 of briarodin C (3).

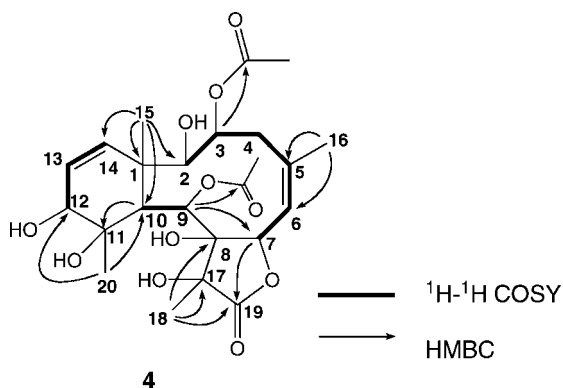


Figure 6. Selected 2D NMR correlations for briarodin D (4).

H-10 and H₃-16, H₃-16 to H-6, and H-4a to H-3 and H-7 suggested that H-2 and H-10 were α -orientated and H-3, H-7 and H-9 were β -orientated (Figure 7). The relative configuration at C-8 and C-17 was elucidated by comparison of ¹³C NMR chemical shifts of briarodin D (4) with those of violide J.¹⁶ Thus, the relative stereochemistry of briarodin D was elucidated to be 4. The absolute stereochemistry of 4 was assigned as follows. Compound 4 was converted into its (*S*)- and (*R*)-MTPA esters of a hydroxy group at C-12, due to steric hindrance at C-2. The $\Delta\delta$ [$\delta(S\text{-MTPA ester}) - \delta(R\text{-MTPA ester})$] values obtained from the ¹H NMR spectra of the MTPA esters suggested that the absolute configuration at C-12 in 4 was *R* (Figure 8).

In this study, four new briarane diterpenoids, briarodins A–D (1–4), were isolated from a soft coral *Pachyclavularia* sp., in which compounds 2–4 are rare examples¹⁶ of briarane diterpenoids with a 1,2-diol moiety at C-8 and C-17. The absolute configurations for briarodins C (3) and D (4) were assigned, although absolute configurations of many briarane diterpenoids remain to be defined. Briarodin A (1) showed cytotoxicity¹⁹ against L1210 murine leukemia (IC₅₀, 1.8 $\mu\text{g/mL}$) and KB human epidermoid carcinoma cells (IC₅₀, 4.3 $\mu\text{g/mL}$) *in vitro*, while briarodins B–D (2–4) did

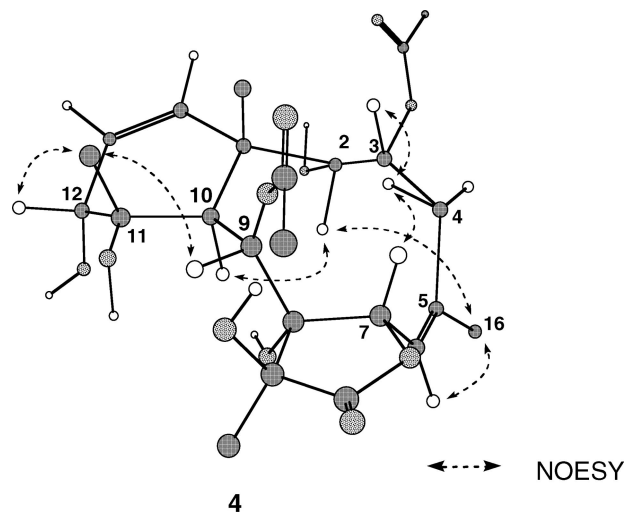


Figure 7. Selected NOESY correlations for briarodin D (4).

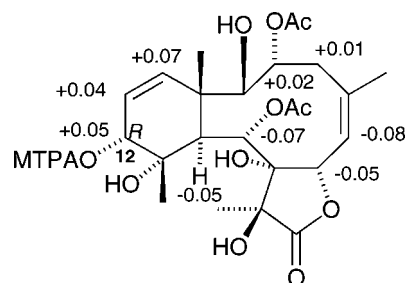


Figure 8. $\Delta\delta$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters at C-12 of briarodin D (4).

not show such activity (IC₅₀, >10 $\mu\text{g/mL}$). Chemical modifications of briarane diterpenoids and SAR studies⁵ are currently underway.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR spectra were taken on a JASCO FT/IR-5300 IR spectrometer. ¹H and ¹³C NMR spectra were recorded on a Bruker AMX-600 NMR spectrometer and a JEOL ECA500 NMR spectrometer. The 7.26 and 77.0 ppm resonances of residual CDCl₃ were used as internal references for ¹H and ¹³C NMR spectra, respectively, while the 3.35 and 49.8 ppm resonances of residual MeOH-*d*₄ were used as internal references for ¹H and ¹³C NMR spectra, respectively. FAB mass spectra were obtained on a JEOL HX110 spectrometer. ESI mass spectra were obtained on a JEOL JMS-T100LP spectrometer.

Animal Material. The soft coral *Pachyclavularia* sp. (SC-114) was collected from Okinawa, Japan, and kept frozen until used. A voucher specimen was deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The soft coral (0.8 kg, wet weight) was extracted with methanol (1.1 L \times 1 and 0.8 L \times 1). The extract (44.3 g) was partitioned between EtOAc (500 mL \times 3) and water (500 mL). A part (1.0 g) of the EtOAc-soluble materials (12.6 g) was subjected to passage over a silica gel column (CHCl₃/MeOH, 95:5) to give fractions I (59.9 mg) and II (56.8 mg). Fraction I was separated by silica gel column chromatography (*n*-hexane/EtOAc, 1:3) to afford briarodin A (1, 26.0 mg, 0.0032%, wet wt). Fraction II in the first silica gel column was chromatographed on C18 HPLC (Luna 5u C18(2), Phenomenax Co., Ltd., 10 \times 300 mm; flow rate, 2.5 mL/min; eluent, MeOH/H₂O, 40:60 to 70:30; UV detection at 220 nm) to yield briarodins B (2, *t*_R 38.0 min, 4.4 mg, 0.0006%), C (3, *t*_R 28.0 min, 5.7 mg, 0.0007%), and D (4, *t*_R 25.5 min, 10.4 mg, 0.0013%).

Briarodin A (1): colorless solid; mp 255–258 °C; [α]_D²⁵ –129 (c 1.0, CHCl₃); IR (NaCl) ν_{max} 3550, 1780, and 1740 cm⁻¹; ¹H and ¹³C NMR (Tables 1 and 2); FABMS (3-nitrobenzylalcohol) *m/z* 523 [M + H]⁺; HRFABMS *m/z* 523.2178 [M + H]⁺, calcd for C₂₆H₃₅O₁₁, 523.2179.

Brianodin B (2): colorless solid; mp 170–172 °C; $[\alpha]_D^{25} -6$ (c 1.0, CHCl₃); IR (NaCl) ν_{\max} 3270, and 1740 cm⁻¹; ¹H and ¹³C NMR (Tables 1 and 2); FABMS (glycerol) m/z 599 [M + H]⁺; HRFABMS m/z 599.2336 [M + H]⁺, calcd for C₂₈H₃₉O₁₄, 599.2339.

Brianodin C (3): colorless solid; mp 286–288 °C; $[\alpha]_D^{25} +20$ (c 0.5, CHCl₃); IR (NaCl) ν_{\max} 3420, 1730, and 1650 cm⁻¹; ¹H and ¹³C NMR (Tables 1 and 2); FABMS (glycerol) m/z 557 [M + H]⁺; HRFABMS m/z 557.2247 [M + H]⁺, calcd for C₂₆H₃₇O₁₃, 557.2234.

Brianodin D (4): colorless solid; mp 181–183 °C; $[\alpha]_D^{25} -79$ (c 0.5, CHCl₃); IR (NaCl) ν_{\max} 3270, and 1730 cm⁻¹; ¹H and ¹³C NMR (Tables 1 and 2); FABMS (glycerol) m/z 499 [M + H]⁺; HRFABMS m/z 499.2187 [M + H]⁺, calcd for C₂₄H₃₅O₁₁, 499.2181.

(S)- and (R)-MTPA Esters of Brianodin C (3). To a solution of **3** (0.3 mg) in pyridine (50 μ L) were added *N,N*-dimethylaminopyridine (50 μ g) and (*R*)-MTPACl (6 μ L). The mixture was allowed to stand at room temperature for 30 min. After addition of *N,N*-dimethyl-1,3-propanedioamine (6 μ L), the residue was concentrated and applied to a silica gel column to give the (*S*)-MTPA ester of **3**. The (*R*)-MTPA ester of **3** was prepared according to the same procedure as described above.

(*S*)-MTPA ester of **3**: ¹H NMR (CD₃OD) δ 6.13 (1H, H-9), 5.93 (1H, H-7), 5.90 (1H, H-13), 5.77 (1H, H-14), 5.43 (1H, H-6), 5.21 (1H, H-12), 5.09 (1H, H-4), 4.79 (1H, H-3), 4.62 (1H, H-2), 2.85 (1H, H-10); ESIMS m/z 795 [M + Na]⁺; HRESIMS m/z 795.2445 [M + Na]⁺, calcd for C₃₆H₄₃F₃NaO₁₅, 795.2452.

(*R*)-MTPA ester of **3**: ¹H NMR (CD₃OD) δ 6.17 (1H, H-9), 5.96 (1H, H-7), 5.87 (1H, H-13), 5.72 (1H, H-14), 5.49 (1H, H-6), 5.13 (1H, H-12), 5.05 (1H, H-4), 4.78 (1H, H-3), 4.41 (1H, H-2), 2.87 (1H, H-10); ESIMS m/z 795 [M + Na]⁺; HRESIMS m/z 795.2431 [M + Na]⁺, calcd for C₃₆H₄₃F₃NaO₁₅, 795.2452.

(S)- and (R)-MTPA Esters of Brianodin D (4). The (*S*)- and (*R*)-MTPA esters of **4** were prepared according to the same procedure as described above.

(*S*)-MTPA ester of **4**: ¹H NMR (CD₃OD) δ 6.20 (1H, H-14), 6.15 (1H, H-9), 5.92 (1H, H-13), 5.87 (1H, H-7), 5.69 (1H, H-3), 5.35 (1H, H-6), 5.27 (1H, H-12), 3.02 (1H, H-4), 2.71 (1H, H-10); ESIMS m/z 737 [M + Na]⁺; HRESIMS m/z 737.2388 [M + Na]⁺, calcd for C₃₄H₄₁F₃NaO₁₃, 737.2397.

(*R*)-MTPA ester of **4**: ¹H NMR (CD₃OD) δ 6.22 (1H, H-9), 6.13 (1H, H-14), 5.92 (1H, H-7), 5.88 (1H, H-13), 5.67 (1H, H-3), 5.43 (1H, H-6), 5.22 (1H, H-12), 3.01 (1H, H-4), 2.76 (1H, H-10); ESIMS m/z 737 [M + Na]⁺; HRESIMS m/z 737.2369 [M + Na]⁺, calcd for C₃₄H₄₁F₃NaO₁₃, 737.2397.

Acknowledgment. We thank S. Oka, Center for Instrumental Analysis, Hokkaido University, for FABMS and ESIMS measurements, and Z. Nagahama for his help with the soft coral collection. This work

was partly supported by a Grant-in-Aid from the Uehara Memorial Foundation and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References and Notes

- (1) Burks, J. E.; van der Helm, D.; Chang, C. Y.; Ciereszko, L. S. *Acta Crystallogr.* **1977**, *B33*, 704–709.
- (2) (a) Zhang, W.; Gao, Y.-W.; Mollo, E.; Cimino, G. *Helv. Chim. Acta* **2004**, *87*, 2341–2345. (b) Blunt, J. W.; Copp, B. R.; Hu, W.-P.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2007**, *24*, 31–86., and references therein.
- (3) (a) Hoshino, A.; Mitome, H.; Tamai, S.; Takayama, S.; Takiyama, H.; Miyaoka, H. *J. Nat. Prod.* **2005**, *68*, 1328–1335. (b) Wu, S.-L.; Sung, P.-J.; Chiang, M. Y.; Wu, J.-Y.; Sheu, J.-H. *J. Nat. Prod.* **2001**, *64*, 1415–1420. (c) Sheu, J.-H.; Sung, P.-J.; Su, J.-H.; Wang, G.-H.; Duh, C.-Y.; Shen, Y.-C.; Chiang, M. Y.; Chen, I.-T. *J. Nat. Prod.* **1999**, *62*, 1415–1420.
- (4) Pordesimo, E. O.; Schmitz, F. J.; Ciereszko, L. S.; Hossain, M. B.; van der Helm, D. *J. Org. Chem.* **1991**, *56*, 2344–2357.
- (5) Kobayashi, J.; Cheng, J.-F.; Nakamura, H.; Ohizumi, Y.; Tomotake, Y.; Matsuzaki, T.; Grace, K. J. S.; Jacobs, R. S.; Kato, Y.; Brien, L. S.; Clardy, J. *Experientia* **1991**, *47*, 501–502.
- (6) Shin, J.; Park, M.; Fenical, W. *Tetrahedron* **1989**, *45*, 1633–1638.
- (7) Coval, S. J.; Cross, S.; Bernardinelli, G.; Jefford, C. W. *J. Nat. Prod.* **1988**, *51*, 981–984.
- (8) Hendrickson, R. L.; Cardellina, J. H., II *Tetrahedron* **1986**, *42*, 6565–6570.
- (9) Hamann, M. T.; Harrison, K. N.; Carroll, A. R.; Scheuer, P. J. *Heterocycles* **1996**, *42*, 325–331.
- (10) Aoki, S.; Okano, M.; Matsui, K.; Itoh, T.; Satari, R.; Akiyama, S.; Kobayashi, M. *Tetrahedron* **2001**, *57*, 8951–8957.
- (11) (a) Hosoyama, H.; Shigemori, H.; Kobayashi, J. *Tetrahedron Lett.* **1999**, *40*, 2149–2152. (b) Komatsu, K.; Tsuda, M.; Tanaka, Y.; Mikami, Y.; Kobayashi, J. *Bioorg. Med. Chem.* **2005**, *13*, 1507–1513.
- (12) Iwagawa, T.; Nishitani, N.; Kurosaki, S.; Okamura, H.; Nakatani, M.; Doe, M.; Takemura, K. *J. Nat. Prod.* **2003**, *66*, 1412–1415.
- (13) Iwagawa, T.; Nishitani, N.; Nakatani, M.; Doe, M.; Morimoto, Y.; Takemura, K. *J. Nat. Prod.* **2005**, *68*, 31–35.
- (14) Iwagawa, T.; Takenoshita, N.; Okamura, H.; Nakatani, M.; Doye, M.; Shibata, K.; Shiro, M. *Heterocycles* **1998**, *48*, 123–128.
- (15) Iwagawa, T.; Takayama, K.; Okamura, H.; Nakatani, M.; Doe, M. *Heterocycles* **1999**, *51*, 1653–1659.
- (16) Iwagawa, T.; Takayama, K.; Okamura, H.; Nakatani, M.; Doe, M.; Takemura, K.; Shiro, M. *Heterocycles* **1999**, *51*, 2619–2625.
- (17) Iwagawa, T.; Hirose, T.; Takayama, K.; Okamura, H.; Nakatani, M.; Doe, M.; Takemura, K. *Heterocycles* **2000**, *53*, 1789–1792.
- (18) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
- (19) Komatsu, K.; Tsuda, M.; Shiro, M.; Tanaka, Y.; Mikami, Y.; Kobayashi, J. *Bioorg. Med. Chem.* **2004**, *12*, 5545–5551.

NP070684P