

## CYTOTOXIC BRIARANE DITERPENES FROM A GORGONACEAN *BRIAREUM* SP.<sup>1</sup>

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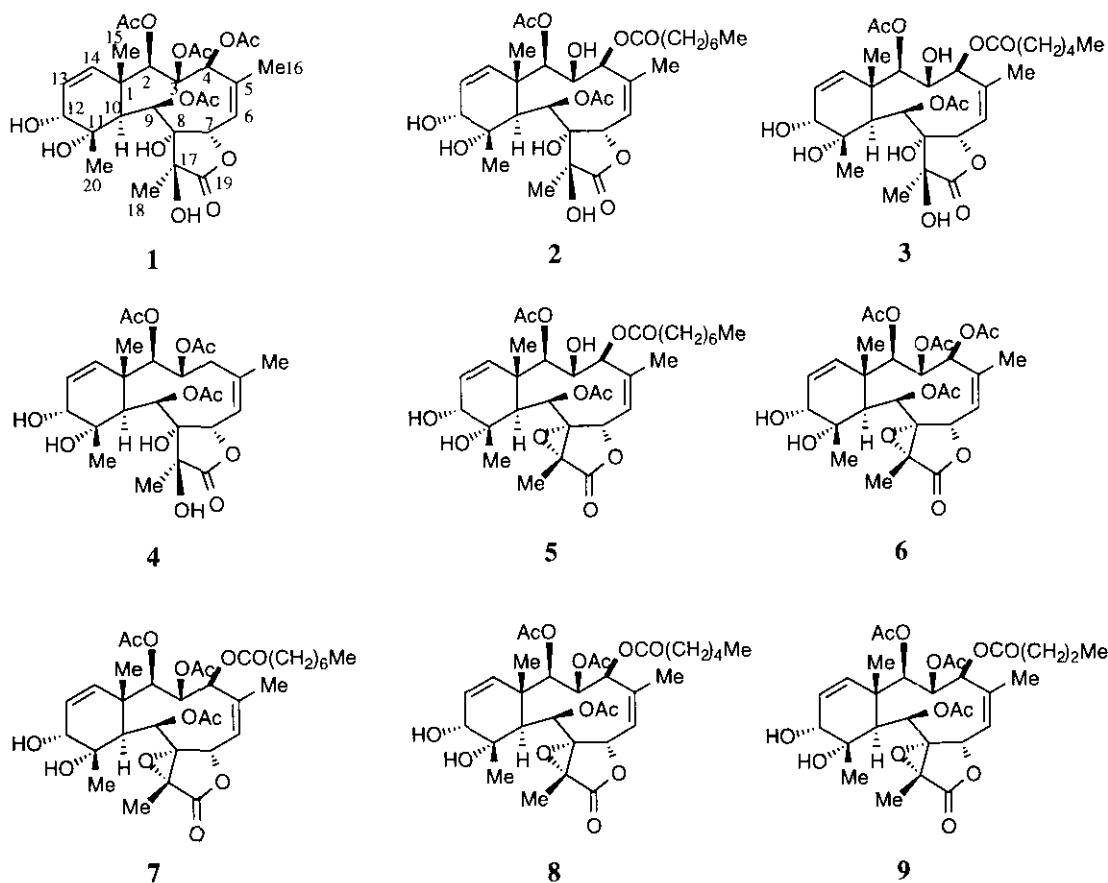
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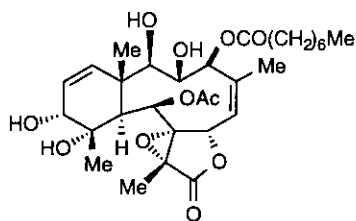
**Abstract-** Four new briarane diterpenes violides J -M (**1-4**), possessing a 8,17-dihydroxyl group, have been isolated from a gorgonacean *Briareum* sp. Their structures were established by spectral methods and a single crystalline X-Ray analysis. Twelve violides so far isolated from the gorgonian were performed on biological activity tests.

The gorgonian octocorals belonging to the genus *Briareum* sp. are a rich source of briarane diterpenes with interesting bioactivities such as cytotoxic, anti-inflammatory, and antiviral activity.<sup>2</sup> The MeOH extract of *Briareum* sp., collected in the area of Bonotsu, Kagoshima prefecture, was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O. The organic extract, exhibiting cytotoxic activity, was subjected to vacuum silica gel chromatography. Fractions eluting with 5-10% MeOH-CH<sub>2</sub>Cl<sub>2</sub> were purified by further chromatography and finally by C<sub>18</sub> reversed phase HPLC to give nine new briaranes, violides A-I (**5-13**), possessing a 2,3,4-, 2,3-, and 2,4-oxygen function and an epoxide between C-8 and C-17.<sup>1,3</sup> Further examination of the same fractions yielded a series of four new briaranes, violides J -M (**1-4**), with a 2,3,4- and 2,4-oxygen and C-8 and C-17-diol functions. In this paper, we describe the isolation and structure elucidation of violides J -M (**1-4**) and biological activity for violides (**1-13**).

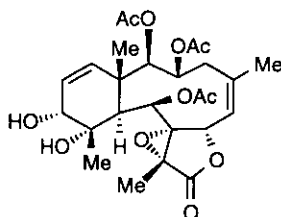
Compound (**1**) was isolated as prisms, mp 281-283°C. The molecular formula was determined as C<sub>28</sub>H<sub>38</sub>O<sub>14</sub> on the basis of HRFAB MS and NMR spectral data. The <sup>1</sup>H NMR spectrum was similar to that of violide B (**6**),<sup>3</sup> the major difference being the upfield shift of H-18 (δ 1.49, 3H, s) by 0.21 ppm and the downfield shift of H-20 (δ 1.42, 3H, s) by 0.26 ppm (Table 1). In the <sup>13</sup>C NMR spectrum (Table 2), the signals of C-8 (δ 78.8, s), C-17 (δ 80.2, s), and C-18 (δ 16.9, q) were shifted downfield by 7.3, 14.7, 6.9 ppm, respectively, compared to those of **6**. This suggested that **1** was a 8,17-dihydroxyl derivative of violide B (**6**) in which the epoxide between C-8 and C-17 in **6** was hydrolyzed. The relative stereochemistry was concluded to be similar to those of violides A-I on the basis of the proton-proton coupling constants and NOE experiments of **1** (Figure 1). Thus, the coupling constants  $J_{2,3}=0$  between H-2 and H-3 and  $J_{3,4}=10.3$  Hz between H-3 and H-4 in the <sup>1</sup>H NMR spectrum indicated that H-2 and H-3 are

orthogonal to each other and H-3 and H-4 were antiparallel as for valiolides A-I. The diaxial relationship between H-6 and H-7 was confirmed with the large coupling constant ( $J_{6,7}=10.1$  Hz) between them. *Z*-Geometries of the olefinic bonds at C-5 and C-14 were evidenced from an NOE of H-6 ( $\delta$  5.81, br d,  $J=10.1$  Hz) to H-16 ( $\delta$  2.14, 3H, br s) and the coupling constant ( $J_{13,14}=10.3$  Hz) between H-13 and H-14 in the  $^1\text{H}$  NMR spectrum. NOEs from H-2 ( $\delta$  4.69, 1H, br s) to H-4 ( $\delta$  5.12, 1H, d,  $J=10.3$  Hz), H-10 ( $\delta$  2.89, 1H, d,  $J=4.0$  Hz), and H-16 suggested that H-2 and H-4 were  $\alpha$ -oriented, the ring junction was *trans*, and H-6 and H-16 were folded downward.  $\beta$ -Configurations of H-7 ( $\delta$  6.09, 1H, d,  $J=10.1$  Hz) and H-15 ( $\delta$  1.11, 3H, s) and  $\alpha$ -configuration of H-3 ( $\delta$  6.22, 1H, br d,  $J=10.3$  Hz) were deduced from NOEs of H-3 to H-7 and H-15. NOEs from H-20 ( $\delta$  1.42, 3H, s) to H-12 ( $\delta$  3.75, 1H, d,  $J=6.2$  Hz) and H-15 supported the  $\beta$ -orientations of H-12 and H-20. NOEs of H-9 ( $\delta$  6.15, 1H, d,  $J=4.0$  Hz) to H-20 suggested that H-9 was  $\alpha$ -oriented. As the orientation of the dihydroxyl groups at C-8 and C-17 could not be unequivocally established by the NOE experiment, an X-Ray diffraction experiment was performed (Figure 2). Thus, it was concluded that the hydroxyl groups at C-8 and C-17 were  $\alpha$ - and  $\beta$ -oriented, respectively.

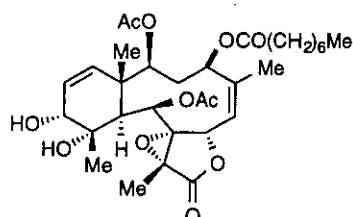




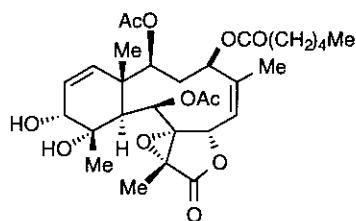
10



11



12



13

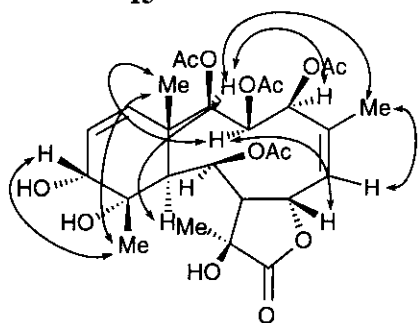


Figure 1. NOE correlation of 1.

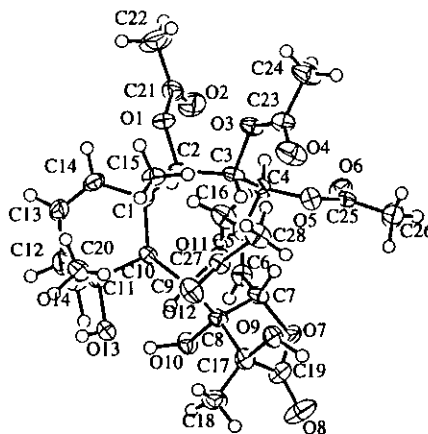


Figure 2. Perspective ORTEP drawing of 1.

The  $^1\text{H}$  NMR spectrum of violide K (**2**),  $\text{C}_{32}\text{H}_{48}\text{O}_{13}$ , was similar to that of violide A (**5**), except for resonances due to H-18 and H-19, as in the case of **1** for **6**. The chemical shifts of H-18 ( $\delta$  1.48, s) and H-20 (1.41, s) were shifted downfield by 0.22 ppm and upfield by 0.25 ppm, respectively, compared to those of **5**, suggesting that **2** was a 8,17-dihydroxyl derivative of **5**. Placement of the *n*-octanoate group at C-4 was determined from a HMBC correlation between H-4 ( $\delta$  4.83, 1H, d,  $J=9.9$  Hz) and C-21 ( $\delta$  173.9, s). The relative stereochemistry was deduced from the similar coupling patterns in the  $^1\text{H}$  NMR spectrum, chemical shifts in the  $^{13}\text{C}$  NMR spectrum, and NOE correlations to those of **1**.

The  $^1\text{H}$  NMR spectrum of violide L (**3**),  $\text{C}_{30}\text{H}_{44}\text{O}_{13}$ , was nearly identical to that of **2**, except for resonances corresponding to aliphatic portion. The presence of a hexanoate group in **3** was confirmed by resonances due to the acyl group in the  $^{13}\text{C}$  NMR spectra of **3**;  $\delta$  13.9 (q), 22.3 (t), 24.6 (t), 31.1 (t), 34.4 (t), 173.8 (s). The acyl group was concluded to be located at C-4 from the observation of a correlation of H-4 ( $\delta$  4.83, 1H, d,  $J=10.6$  Hz) and C-21 ( $\delta$  173.8, s) in the HMBC spectrum. The stereochemistry was determined on the basis of similarity of the coupling patterns and chemical shifts in the NMR spectrum and NOE correlations between **3** and **1**.

Comparison of the  $^1\text{H}$  NMR spectrum of violide M (**4**),  $\text{C}_{26}\text{H}_{36}\text{O}_{12}$ , with that of violide G (**11**) indicated that the chemical shifts of H-18 ( $\delta$  1.50, 3H, s) and H-20 ( $\delta$  1.38, 3H, s) were shifted upfield by 0.19

ppm and downfield by 0.25 ppm, respectively. The rest of the resonances was similar to those of **11**. Thus, violide **M** was a 8,17-dihydroxyl derivative of **11**. On the basis of the signal patterns, chemical shifts in the NMR spectra and NOE correlations, the stereochemistry of violide **M** was determined to have the structure (**4**).

Table 1.  $^1\text{H}$  NMR Spectral Data of **1-4** in  $\text{CDCl}_3$ .

H	1	2	3	4
<b>2</b>	4.69 (br s)	4.67 (br s)	4.68 (br s)	4.77 (br s)
<b>3</b>	6.22 (br d, 10.3)	5.07 (br d, 9.9)	5.09 (br d, 10.6)	5.74 (dd, 5.7, 12.5)
<b>4</b>	5.12 (d, 10.3)	4.83 (br d, 9.9)	4.83 (d, 10.6)	ca. 1.9 (overlapped) 3.00 (br dd, 5.7, 12.5)
<b>6</b>	5.81 (br d, 10.1)	5.69 (br d, 9.7)	5.70 (br d, 9.9)	5.64 (br d, 9.7)
<b>7</b>	6.09(d, 10.1)	5.95 (d, 9.7)	5.95 (d, 9.9)	5.91 (d, 9.7)
<b>9</b>	6.15 (d, 4.0)	6.08 (d, 4.2)	6.08 (d, 4.2)	6.12 (d, 3.7)
<b>10</b>	2.89 (d, 4.0)	2.85 (d, 4.2)	2.83 (d, 4.2)	2.80 (d, 3.7)
<b>12</b>	3.75 (d, 6.2)	3.74 (d, 6.1)	3.74 (d, 6.2)	3.74 (d, 5.9)
<b>13</b>	5.82 (br dd, 6.2, 10.3)	5.78 (dd, 6.1, 10.3)	5.79 (dd, 6.2, 10.3)	5.81 (dd, 5.9, 10.3)
<b>14</b>	5.50 (d, 10.3)	5.35 (d, 10.3)	5.37 (d, 10.3)	5.44 (d, 10.3)
<b>15</b>	1.11 (s)	1.25 (s)	1.26 (s)	1.12 (s)
<b>16</b>	2.14 (br s)	2.07 (br s)	2.07 (br s)	1.95 (br s)
<b>18</b>	1.49 (s)	1.48 (s)	1.49 (s)	1.50 (s)
<b>20</b>	1.42 (s)	1.41 (s)	1.42 (s)	1.38 (s)
<b>MeCO</b>	2.03, 2.06, 2.16, 2.20	2.14, 2.21	2.14, 2.21	2.01, 2.09, 2.18
<b><i>n</i>-C<sub>n</sub>H<sub>n+1</sub>OCO</b>		0.87 (t, 7.0, H-28) ca. 1.25 (overlapped H-24, 25, 26, 27) 1.63 (m, H-23) 2.40 (br t, 7.5, H-22)	0.89 (t, 6.8, H-26) ca. 1.33 (m, H-24,25) ca. 1.63 (m, H-23) 2.40 (t, 7.5, H-22)	

The  $^1\text{H}$  NMR spectrum of violide **L** (**3**),  $\text{C}_{30}\text{H}_{44}\text{O}_{13}$ , was nearly identical to that of **2**, except for resonances corresponding to aliphatic portion. The presence of a hexanoate group in **3** was confirmed by resonances due to the acyl group in the  $^{13}\text{C}$  NMR spectra of **3**;  $\delta$  13.9 (q), 22.3 (t), 24.6 (t), 31.1 (t), 34.4 (t), 173.8 (s). The acyl group was concluded to be located at C-4 from the observation of a correlation of H-4 ( $\delta$  4.83, 1H, d,  $J=10.6$  Hz) and C-21 ( $\delta$  173.8, s) in the HMBC spectrum. The stereochemistry was determined on the basis of similarity of the coupling patterns and chemical shifts in the NMR spectrum and NOE correlations between **3** and **1**.

Comparison of the  $^1\text{H}$  NMR spectrum of violide **M** (**4**),  $\text{C}_{26}\text{H}_{36}\text{O}_{12}$ , with that of violide **G** (**11**) indicated that the chemical shifts of H-18 ( $\delta$  1.50, 3H, s) and H-20 ( $\delta$  1.38, 3H, s) were shifted upfield by 0.19 ppm and downfield by 0.25 ppm, respectively. The rest of the resonances was similar to those of **11**. Thus, violide **M** was a 8,17-dihydroxyl derivative of **11**. On the basis of the signal patterns, chemical shifts in the NMR spectra and NOE correlations, the stereochemistry of violide **M** was determined to have the structure (**4**).

Compounds (**1-4**) were the first example of briaranes with a 8, 17-diol group which seemed to be formed from a 8,17-epoxide by hydrolysis.

Table 2.  $^{13}\text{C}$  NMR Spectral Data of 1-4.

C	1a	2a	3a	4b
1	46.6	46.6	46.6	48.0
2	77.1	77.2	77.2	79.0
3	71.2	70.9	71.0	72.8
4	76.3	75.8	75.9	78.3
5	138.4	139.3	139.3	139.0
6	127.1	125.9	125.9	129.2
7	77.8	78.3	78.1	79.4
8	78.8	79.4	79.3	80.0
9	66.4	66.3	66.3	67.7
10	39.8	39.8	40.1	39.5
11	77.7	75.8	75.9	76.6
12	70.8	70.8	70.8	72.0
13	124.0	123.7	123.5	126.3
14	139.2	138.6	138.7	140.2
15	15.5	15.3	15.3	16.4
16	25.9	26.0	25.9	26.3
17	80.2	80.3	80.3	80.8
18	16.9	16.6	16.8	16.2
19	175.9	176.2	175.8	178.8
20	23.0	23.1	23.3	22.8
<u>MeCO</u>	20.7, 20.9	20.9, 22.0	20.9, 22.0	20.5, 20.8
	21.0, 22.1			21.0, 22.6
<u>MeCO</u>	168.9, 170.1	170.0, 170.6	169.9, 170.4	170.6, 171.9
	170.3, 171.9			171.9, 173.8
<i>n</i> -C <sub>n</sub> H <sub>2n+1</sub> OCO		14.1, 22.6	13.9, 22.3	
		24.9, 28.9	24.6, 31.1	
		29.0, 31.6	34.4, 173.8	
		34.4, 173.9		

<sup>a</sup> Measured in CDCl<sub>3</sub>. <sup>b</sup> Measured in CD<sub>3</sub>OD.

Table 3. Cytotoxic Activity (CC<sub>50</sub> mg/mL) of 1-5 and 7-13.

	1	2	3	4	5	7	8	9	10	11	12	13
Vero	>100	>100	>100	>100	1.90	1.69	2.53	3.65	3.93	9.37	0.85	1.41
MDCK	>100	>100	>100	>100	1.90	1.67	3.57	4.69	4.03	11.7	0.85	1.30

Biological activity tests for 1-5 and 7-13 were performed.<sup>6</sup> Compounds (5) and (7-13) exhibited moderate cytotoxicity against the growth of Vero and MDCK cells with a CC<sub>50</sub> of 0.85 to 9.37 μg/mL and 0.85 to 11.7 μg/mL, respectively (Table 3). In regard to the relationship between the cytotoxicity and the structure, compounds (12 and 13) without a substituent at C-3, showed the strongest cytotoxicity. Compounds (7, 8, and 9), possessing an aliphatic ester at C-4, were stronger than 11 without it. Compounds with a longer aliphatic chain were more active: 7>8>9. When an acetyl group at C-3 is

replaced by an hydroxyl group, the activity decreased: **5**<**7**. Compounds (**1-4**), containing a 8,7-dihydroxyl group, were inactive.

## EXPERIMENTAL

**General Experimental Procedures.** Melting points were uncorrected. Optical rotations were obtained at 22° C on a JASCO DIP-370S spectropolarimeter. UV and IR spectra were recorded on a UV-210 and a MASCO FT/IR 5300. NMR spectra were recorded with a 400 MHz JEOL or VARIAN UNITY-500 NMR instrument using TMS as internal standard and CDCl<sub>3</sub> as solvents. MS were obtained with a JEOL XD-303 instrument. Rigaku RAXIS-IV diffractometer was used in the X-Ray work.

**Extraction and Isolation.** The organisms (wet weight: 7.6 kg)<sup>3</sup> was chopped into small pieces and extracted with MeOH (30 L) immediately after collection. The MeOH extract (22 g) was suspended in H<sub>2</sub>O (1 L) and extracted three times with CH<sub>2</sub>Cl<sub>2</sub> (3 x 3 L) for 1 day at rt. The CH<sub>2</sub>Cl<sub>2</sub> layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated to dryness (9.6 g). Portion (5 g) of the CH<sub>2</sub>Cl<sub>2</sub> extract was absorbed on silica gel (55 g) and subjected to chromatography on silica gel packed in hexane, fractions (100 mL) being collected as follows: 1-2 (CH<sub>2</sub>Cl<sub>2</sub>-hexane, 4:1), 3-34 (CH<sub>2</sub>Cl<sub>2</sub>), 5-6 (MeOH-CH<sub>2</sub>Cl<sub>2</sub>, 1:49), 7-8 (MeOH-CH<sub>2</sub>Cl<sub>2</sub>, 1:19), 9-10 (MeOH-CH<sub>2</sub>Cl<sub>2</sub>, 1:9), 11-12 (MeOH-CH<sub>2</sub>Cl<sub>2</sub>, 1:4), and 13-14 (MeOH). Fractions 8-10 (2.1 g) were chromatographed on silica gel using MeOH and CH<sub>2</sub>Cl<sub>2</sub>, increasing the proportion of MeOH to elute the fractions from the column. The fractions eluted with MeOH-CH<sub>2</sub>Cl<sub>2</sub> (1:49) gave a residue (620 mg), which was applied to HPLC (ODS) with MeOH-H<sub>2</sub>O (1:1), yielding **7** (8.6 mg), **8** (3.0 mg), **11** (15.9 mg), **12** (13.4 mg), and **13** (2.8 mg). Further elution with MeOH-CH<sub>2</sub>Cl<sub>2</sub> (1:24) afforded a residue, from which **10** (8.5 mg) was obtained as crystals. The residue was subjected to HPLC with MeOH-H<sub>2</sub>O (2:3), giving **1** (2.3 mg), **2** (7.6 mg), **3** (3.2 mg), and **4** (1.0 mg).

**Violide J (1):** Colorless prisms from MeOH-H<sub>2</sub>O, mp 281-283°C, [ $\alpha$ ]<sub>D</sub> +59.8° (c 0.12, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 206 (3.74) nm; IR (film)  $\nu_{\max}$  3422, 1746, 1229 cm<sup>-1</sup>; <sup>1</sup>H NMR (see Table 1); <sup>13</sup>C NMR (see Table 2); (-)-FABMS *m/z* 597.2123 [M - H]<sup>-</sup> (Calcd for C<sub>28</sub>H<sub>37</sub>O<sub>14</sub> 597.2161).

**Violide K (2):** Amorphous, [ $\alpha$ ]<sub>D</sub> +29.6° (c 0.38, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 206 (3.91) nm; IR (film)  $\nu_{\max}$  3443, 1748, 1227 cm<sup>-1</sup>; <sup>1</sup>H NMR (see Table 1); <sup>13</sup>C NMR (see Table 2); (-)-FABMS *m/z* 639.3007 [M - H]<sup>-</sup> (Calcd for C<sub>32</sub>H<sub>47</sub>O<sub>13</sub> 639.3016).

**Violide L (3):** Amorphous, [ $\alpha$ ]<sub>D</sub> +21.0° (c 0.15, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 206 (3.85) nm; IR (film)  $\nu_{\max}$  3382, 1742, 1227 cm<sup>-1</sup>; <sup>1</sup>H NMR (see Table 1); <sup>13</sup>C NMR (see Table 2); (-)-FABMS *m/z* 611.2703 [M - H]<sup>-</sup> (Calcd for C<sub>30</sub>H<sub>43</sub>O<sub>13</sub> 611.2704).

**Violide M (4):** Amorphous, [ $\alpha$ ]<sub>D</sub> +4.83° (c 0.29, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 206 (3.86) nm; IR (film)  $\nu_{\max}$  3335, 1741, 1235 cm<sup>-1</sup>; <sup>1</sup>H NMR (see Table 1); <sup>13</sup>C NMR (see Table 2); (-)-FABMS *m/z* 539.2130 [M - H]<sup>-</sup> (Calcd for C<sub>26</sub>H<sub>35</sub>O<sub>12</sub> 539.2128).

**X-Ray analysis of 1.** Crystal data: C<sub>28</sub>H<sub>44</sub>O<sub>16</sub>, colorless prisms, monoclinic space group P2<sub>1</sub>(#4), a=9.199(1)Å, b=20.293(3)Å, c=9.512(1)Å,  $\beta$ =119.43(1)°, V=1546.5(4)Å<sup>3</sup>, Z=2, Dx 1.367 g/cm<sup>3</sup>, F(000)=680.00,  $\mu$ (MoK $\alpha$ )=1.12 cm<sup>-1</sup>, Intensity data were collected on a Rigaku RAXIS-IV diffractometer using graphite monochromated MoK $\alpha$  ( $\lambda$ =0.71070 Å) up to 2 $\theta$ =50.0°. Of the total 2512 unique

reflections, 1990 were observed [ $I > 2.00\sigma(I)$ ]. The structure was solved by direct methods (SIR92)<sup>4</sup> and expanded using Fourier techniques.<sup>5</sup> The non-hydrogen atoms were refined anisotropically. Hydrogen atoms, excluding those of water, were included but not refined. It was refined by full-matrix least-squares and converged with  $R=0.057$  and  $R_w=0.077$ . Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at Rigaku Corporation.

#### ACKNOWLEDGEMENTS

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#### REFERENCES AND NOTES

1. New briarane diterpenes from *Briareum* sp., collected at Bonotsu, Kagoshima prefecture. 3. For part 2. see: T. Iwagawa, K. Takayama, H. Okamura, M. Nakatani, and M. Doye *Heterocycles*, **1999**, *51*, 1653.
2. J.-H. Sheu, P.-J. Sung, L.-H. Huang, S.-F. Lee, T. Wu, B.-Y. Duh, C.-Y. Chang, L.-S. Fang, K. Soong, and T.-J. Lee, *J. Nat. Prod.*, 1996, **59**, 935. and the references cited therein.
3. T. Iwagawa, N. Takenoshita, H. Okamura, M. Nakatani, M. Doye, K. Shibata, M. Shiro, *Heterocycles*, **1998**, *48*, 123.
4. A. Altomare, M. C. Burla, M. Camalli, M. Cascarano, C. Giacovazzo, A. Guagliardi, and G. Polidori, *J. Appl. Cryst.*, 1994, **27**, 435.
5. P. T. Beurskens, G. Admiraal, G. Beurskens, W. P. Bosman, R. de Gelder, R. Israel, and J. M. M. Smits, The DIRDIF-94 program system, Technical Report of the Crystallography Laboratory, University of Nijmegen, The Netherlands.
6. Cytotoxicity of compounds was evaluated by the growth of Vero and MDCK cells, with XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2*H*-tetrazolium-5-carboxanilide; Sigma) method: see, D. A. Scudiero, *Cancer Research*, **1998**, *48*, 4827.

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