CYTOTOXIC BRIARANE DITERPENES FROM A GORGONACEAN *BRIAREUM* SP.[1]

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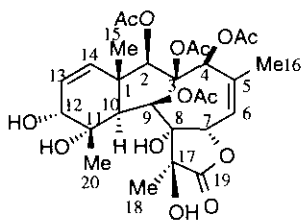
Abstract- Four new briarane diterpenes violides J -M (**1-4**), possessing a 8,17-dihydroxyl group, have been isolated from a gorgonacean *Briareum* sp. Their structures were established by spectral methods and a single crystalline X-Ray analysis. Twelve violides so far isolated from the gorgonian were 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-flammatory, and antiviral activity.[2] The MeOH extract of *Briareum* sp., collected in the area of Bonotsu, Kagoshima prefecture, was partitioned between CH_2Cl_2 and H_2O. The organic extract, exhibiting cytotoxic activity, was subjected to vacuum silica gel chromatography. Fractions eluting with 5-10% MeOH-CH_2Cl_2 were purified by further chromatography and finally by C_{18} 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.[1,3] 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 vilolides (**1-13**).

Compound (**1**) was isolated as prisms, mp 281-283°C. The molecular formula was determined as $C_{28}H_{38}O_{14}$ on the basis of HRFAB MS and NMR spectral data. The ^1H NMR spectrum was similar to that of violide B (**6**),[3] 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 ^{13}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 ^1H 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 (δ 5.81, br d, *J*=10.1 Hz) to H-16 (δ 2.14, 3H, br s) and the coupling constant ($J_{13,14}$=10.3 Hz) between H-13 and H-14 in the ^{1}H NMR spectrum. NOEs from H-2 (δ 4.69, 1H, br s) to H-4 (δ 5.12, 1H, d, *J*=10.3 Hz), H-10 (δ 2.89, 1H, d, *J*=4.0 Hz), and H-16 suggested that H-2 and H-4 were α-oriented, the ring junction was *trans*, and H-6 and H-16 were folded downward. β-Configurations of H-7 (δ 6.09, 1H, d, *J*=10.1 Hz) and H-15 (δ 1.11, 3H, s) and α-configuration of H-3 (δ 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 (δ 1.42, 3H, s) to H-12 (δ 3.75, 1H, d, *J*=6.2 Hz) and H-15 supported the β-orientations of H-12 and H-20. NOEs of H-9 (δ 6.15, 1H, d, *J*=4.0 Hz) to H-20 suggested that H-9 was α-oriented. As the orientation of the dihydroxyl groups at C-8 and C-17 could not be equivocally 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 α- and β-oriented, respectively.



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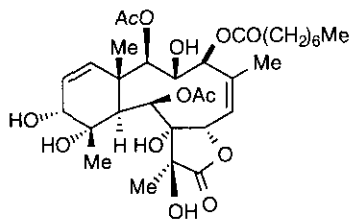

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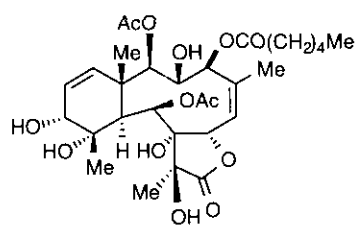

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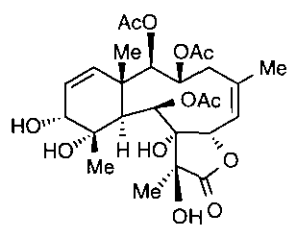

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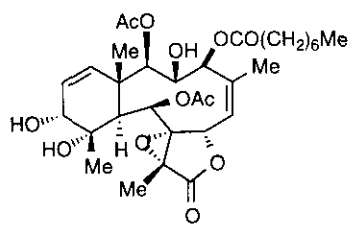

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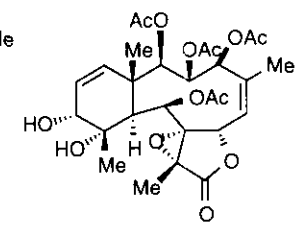

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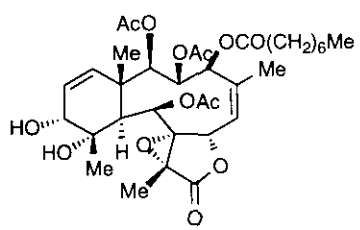

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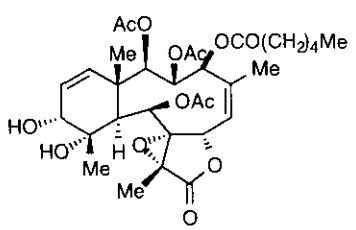

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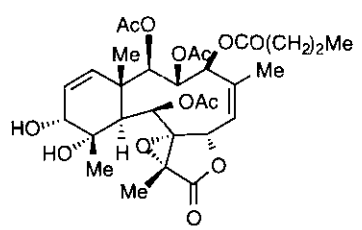

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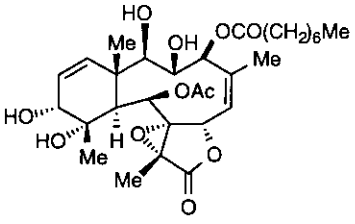

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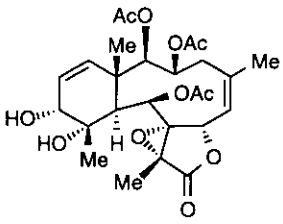

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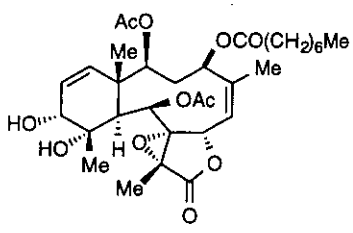

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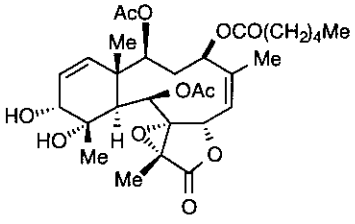

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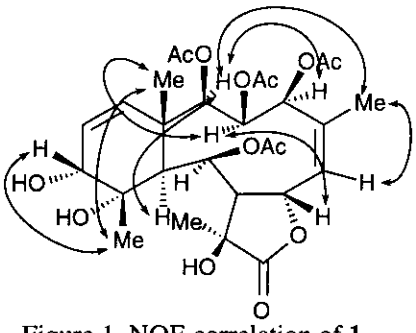

Figure 1. NOE correlation of **1**.

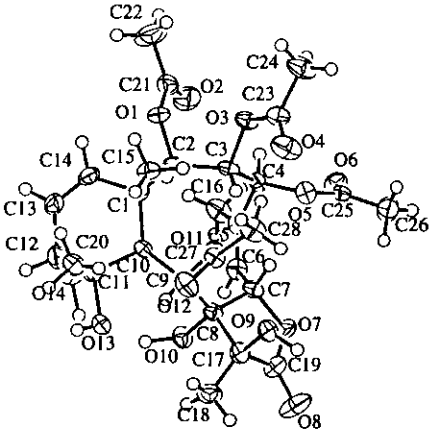

Figure 2. Perspective ORTEP drawing of **1**.

The ^{1}H NMR spectrum of violide K (**2**), $C_{32}H_{48}O_{13}$, 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 (δ 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 (δ 4.83, 1H, d, *J*=9.9 Hz) and C-21 (δ 173.9, s). The relative stereochemisty was deduced from the similar coupling patterns in the ^{1}H NMR spectrum, chemical shifts in the ^{13}C NMR spectrum, and NOE correlations to those of **1**.

The ^{1}H NMR spectrum of violide L (**3**), $C_{30}H_{44}O_{13}$, 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}C NMR spectra of **3**; δ 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 (δ 4.83, 1H, d, *J*=10.6 Hz) and C-21 (δ 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}H NMR spectrum of violide M (**4**), $C_{26}H_{36}O_{12}$, with that of violide G (**11**) indicated that the chemical shifts of H-18 (δ 1.50, 3H, s) and H-20 (δ 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}H NMR Spectral Data of **1-4** in $CDCl_3$.

H	1	2	3	4
2	4.69 (br s)	4.67 (br s)	4.68 (br s)	4.77 (br s)
3	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)
4	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)
6	5.81 (br d, 10.1)	5.69 (br d, 9.7)	5.70 (br d, 9.9)	5.64 (br d, 9.7)
7	6.09(d, 10.1)	5.95 (d, 9.7)	5.95 (d, 9.9)	5.91 (d, 9.7)
9	6.15 (d, 4.0)	6.08 (d, 4.2)	6.08 (d, 4.2)	6.12 (d, 3.7)
10	2.89 (d, 4.0)	2.85 (d, 4.2)	2.83 (d, 4.2)	2.80 (d, 3.7)
12	3.75 (d, 6.2)	3.74 (d, 6.1)	3.74 (d, 6.2)	3.74 (d, 5.9)
13	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)
14	5.50 (d, 10.3)	5.35 (d, 10.3)	5.37 (d, 10.3)	5.44 (d, 10.3)
15	1.11 (s)	1.25 (s)	1.26 (s)	1.12 (s)
16	2.14 (br s)	2.07 (br s)	2.07 (br s)	1.95 (br s)
18	1.49 (s)	1.48 (s)	1.49 (s)	1.50 (s)
20	1.42 (s)	1.41 (s)	1.42 (s)	1.38 (s)
MeCO	2.03, 2.06, 2.16, 2.20	2.14, 2.21	2.14, 2.21	2.01, 2.09, 2.18
***n*-CnHn+1OCO**		0.87 (t, 7.0, H-28)	0.89 (t, 6.8, H-26)	
		ca. 1.25 (overlappted H-	*ca.* 1.33 (m, H-24,25)	
		24, 25, 26, 27)	*ca.* 1.63 (m, H-23)	
		1.63 (m, H-23)	2.40 (t, 7.5, H-22)	
		2.40 (br t, 7.5, H-22)		

The ^{1}H NMR spectrum of violide L (**3**), $C_{30}H_{44}O_{13}$, 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}C NMR spectra of **3**; δ 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 (δ 4.83, 1H, d, *J*=10.6 Hz) and C-21 (δ 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}H NMR spectrum of violide M (**4**), $C_{26}H_{36}O_{12}$, with that of violide G (**11**) indicated that the chemical shifts of H-18 (δ 1.50, 3H, s) and H-20 (δ 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}C NMR Spectral Data of **1-4**.

C	1[a]	2[a]	3[a]	4[b]
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
MeCO	20.7, 20.9	20.9, 22.0	20.9, 22.0	20.5, 20.8
	21.0, 22.1			21.0, 22.6
MeCO	168.9, 170.1	170.0, 170.6	169.9, 170.4	170.6, 171.9
	170.3, 171.9			171.9, 173.8
n-$C_nH_{2n+1}OCO$		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		

[a] Measured in $CDCl_3$. [b] Measured in CD_3OD.

Table 3. Cytotoxic Activity (CC_{50} 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.[6] Compounds (**5**) and (**7-13**) exhibited moderate cytotoxicity against the growth of Vero and MDCK cells with a CC_{50} 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 $CDCl_3$ 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)[3] was chopped into small pieces and extracted with MeOH (30 L) immediately after collection. The MeOH extract (22 g) was suspended in H_2O (1 L) and extracted three times with CH_2Cl_2 (3 x 3 L) for 1 day at rt. The CH_2Cl_2 layer was dried over Na_2SO_4, filtered , and evaporated to dryness (9.6 g) . Portion (5 g) of the CH_2Cl_2 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_2Cl_2-hexane, 4:1), 3-34 (CH_2Cl_2), 5-6 (MeOH-CH_2Cl_2, 1:49), 7-8 (MeOH-CH_2Cl_2, 1:19), 9-10 (MeOH-CH_2Cl_2, 1:9), 11-12 (MeOH-CH_2Cl_2, 1:4), and 13-14 (MeOH). Fractions 8-10 (2.1 g) were chromatographed on silica gel using MeOH and CH_2Cl_2, increasing the proportion of MeOH to elute the fractions from the column. The fractions eluted with MeOH-CH_2Cl_2 (1:49) gave a residue (620 mg), which was applied to HPLC (ODS) with MeOH-H_2O (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_2Cl_2 (1:24) afforded a residue, from which **10** (8.5 mg) was obtained as crystals. The residue was subjected to HPLC with MeOH-H_2O (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_2O, mp 281-283°C, $[\alpha]_D$ +59.8° (*c* 0.12, MeOH); UV (MeOH) λmax (log ε) 206 (3.74) nm; IR (film) νmax 3422, 1746, 1229 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); (-)-FABMS *m/z* 597.2123 $[M - H]^-$ (Calcd for $C_{28}H_{37}O_{14}$ 597.2161).

Violide K (2): Amorphous, $[\alpha]_D$ +29.6° (*c* 0.38, MeOH); UV (MeOH) λmax (log ε) 206 (3.91) nm; IR (film) νmax 3443, 1748, 1227 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); (-)-FABMS *m/z* 639.3007 $[M - H]^-$ (Calcd for $C_{32}H_{47}O_{13}$ 639.3016).

Violide L (3): Amorphous, $[\alpha]_D$ +21.0° (*c* 0.15, MeOH); UV (MeOH) λmax (log ε) 206 (3.85) nm; IR (film) νmax 3382, 1742, 1227 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); (-)-FABMS *m/z* 611.2703 $[M - H]^-$ (Calcd for $C_{30}H_{43}O_{13}$ 611.2704).

Violide M (4): Amorphous, $[\alpha]_D$ +4.83° (*c* 0.29, MeOH); UV (MeOH) λmax (log ε) 206 (3.86) nm; IR (film) νmax 3335, 1741, 1235 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); (-)-FABMS *m/z* 539.2130 $[M - H]^-$ (Calcd for $C_{26}H_{35}O_{12}$ 539.2128).

X-Ray analysis of 1. Crystal data:$C_{28}H_{44}O_{16}$, colorless prisms, monoclinic space group $P2_1$(#4), a=9.199(1)Å, b=20.293(3)Å, c=9.512(1)Å, β=119.43(1)°, V=1546.5(4)$Å^3$, Z=2, Dx 1.367 g/cm^3, F(000)=680.00, μ(MoKα)=1.12 cm^{-1}, Intensity data were collected on a Rigaku RAXIS-IV diffractometer using graphite monochromated MoKα (λ=0.71070 Å) up to 2θ=50.0°. Of the total 2512 unique

reflections, 1990 were observed [I>2.00σ (I)]. The structure was solved by direct methods (SIR92)[4] and expanded using Fourier techniques.[5] 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 Rw=0.077. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at Rigaku Corporation.

ACKNOWLEDGEMENTS

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