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BRIVIOLIDES, NEW BRIARANE DITERPENES FROM A GORGONIAN *BRIAREUM* SP.[1]

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Abstract – Further investigation of a gorgonian *Briareum* sp., collected in the area of Bonotsu, Kagoshima Prefecture, afforded thirteen new briarane diterpenes. Their structural elucidation and cytotoxicity tests toward Vero and MDCK cells were performed.

INTRODUCTION

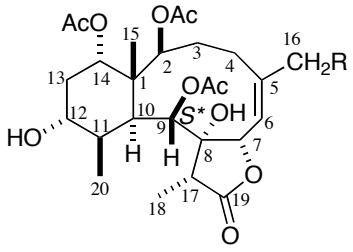
The gorgonian coral *Briareum* genus (Anthozoa, Alcynonaria, Gorgonacea, Briareidae) has a rich source of briarane diterpenes with different biological activities, including cytotoxic, anti-inflammatory, antiviral, insecticidal, and antifouling activity.[2] We have so far isolated twenty-seven kinds of briaranes diterpenes from the dichloromethane soluble part of the methanol extract of *Briareum* sp., collected in the area of Bonotsu, Kagoshima Prefecture.[3-7] Some of them exhibited cytotoxicity against Vero and MDCK cells,[8] and the structure-activity relationship was also discussed.[5] In the course of our continuing investigation of the same dichloromethane, thirteen new briarane diterpenes (**1**)-(**13**) were obtained. We wish to describe the isolation and characterization of these compounds.

RESULTS AND DISCUSSION

Compounds (**1**)-(**13**) possessed a briarane skeleton without an epoxy group at C-8 and C-17 unlike many

briaranes isolated so far from the same extract. Compounds (**1**)-(**3**) have a 2,9,14-triacetoxyl-8-12-dihydroxyl moiety and a γ-lactone.

Compound (**1**), briviolide A, was isolated as an amorphous powder and the molecular formula was determined by HRFABMS *m/z* 527.2503 $[M + H]^+$ ($C_{26}H_{39}O_{11}$ Calcd for *m/z* 527.2492). The IR spectrum indicated absorptions due to hydroxyl group (ν_{max} 3385 cm^{-1}), γ-lactone (ν_{max} 1767 cm^{-1}), and ester carbonyl (ν_{max} 1734 cm^{-1}) functionalities. In the NMR spectrum (Table 1), a tertiary methyl (δ 1.06, s), two secondary methyls (δ 1.08, □, *J*= 7.6 Hz; 1.16, d, *J*= 7.1 Hz), an isolated hydroxymethylene (δ 4.05, d, *J*= 14.8 Hz; 4.29, d, *J*= 14.8 Hz), and three acetyls (δ 1.93, 1.95, 2.14, each s) were observed. The above data, together with the fact that many briaranes have been so far found in the species, implied that briviolide A was a briarane diterpene. The ^1H-^1H COSY spectrum revealed sequences of the correlations from H-2 to H-3, from H-6 to H-7, from H-9 to H-14, from H-12 to H-20, and H-17 to H-18. Lowfield chemical shifts of H-2 (δ 4.92, 1H, br d, *J*= 6.8 Hz), H-9 (δ 5.26, 1H, d, *J*= 2.3 Hz), and H-14 (δ 4.82, 1H, t, *J*= 3.2 Hz) suggested that three acetyl groups were positioned at C-2, C-9, and C-14. The position of the secondary methyl group at δ 1.16 was assigned as C-18 by a correlation of H-17 (δ 2.38, q, *J*= 7.1 Hz) to the C-19 γ-lactone carbonyl (δ 175.9) (Table 2) in the HMBC spectrum. The presence of the tertiary hydroxyl group at C-8 was defined by the correlation of H-7 (δ 5.18, 1H, d, *J*= 9.8 Hz), H-9, and H-17 to C-8 (δ 82.4). Furthermore, the lowfield chemical shifts of H-12 (δ 3.68, 1H, br d, *J*= 2.9 Hz) indicated the presence of a hydroxyl group at C-12. From the above results, the gross structure was readily established. The relative stereochemistry was elucidated by the coupling patterns and the NOESY spectrum (Figure 1). Thus, NOEs from H-2 to H-4 (δ 1.92, 1H, overlapped) and H-10 (δ 2.72, 1H, dd, *J*= 5.2 and 2.3 Hz) supported that these protons were situated on the same ring (α). The *trans* ring junction of the A ring was assigned by the lack of an NOE between H-10 and H-15. One of the methylene protons (δ 2.44, 1H, m, H-4β) showed a correlation with H-7, which in turn was correlated with H-3 (δ 2.79, 1H, br d tt, *J*= 14.6, 14.6, 4.6 Hz), suggesting that both H-7 and H-3 were β-oriented. β-Orientations of H-12, H-13 (δ 1.94, 1H, overlapped), H-14, and H-20 were deduced from the following NOEs data; H-15/H14, H-13 (δ 1.94), H-12/H-13 (δ 1.94), H-20. Acetoxyl protons (δ 2.14, 3H, s) correlated with another acetoxyl protons (δ 1.93, 3H, s) and H-15, suggesting β-configurations of the two acetoxyl protons. The two acetoxyl groups were determined to be located at C-2 and C-9 on the basis of correlations of both H-2 and acetyl protons (δ 1.93) to ester carbon (δ 171.8) and of both H-9 and acetyl protons (δ 2.14) to ester carbon (δ 168.9). The configurations of the hydroxyl group at C-8 and H-17 could not be equivocally established on the basis of NOE correlations. Finally, they were elucidated as depicted by comparing the ^{13}C NMR spectral data with those of the related compounds.[8]



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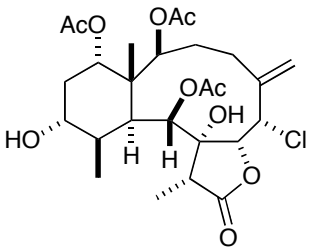

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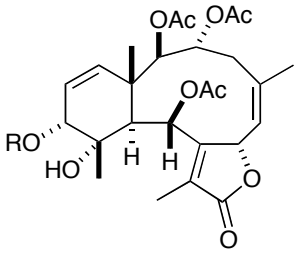

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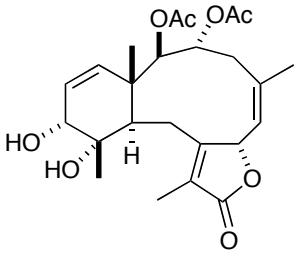

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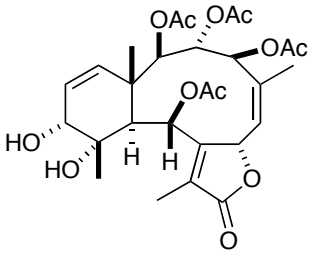

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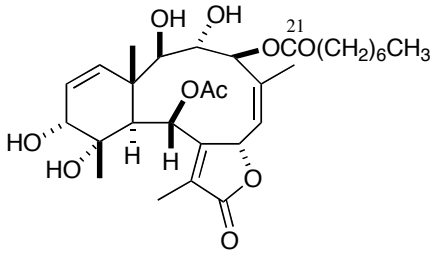

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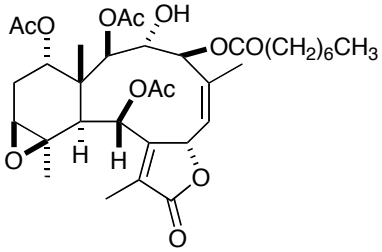

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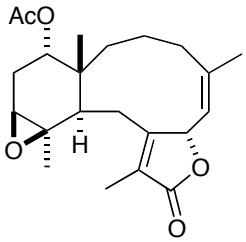

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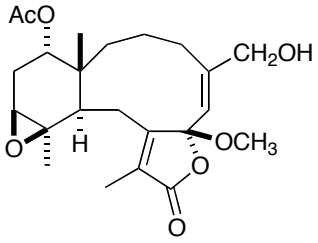

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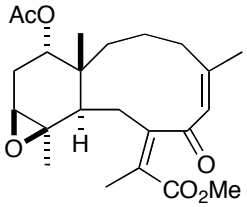

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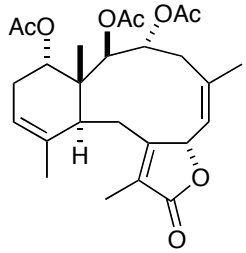

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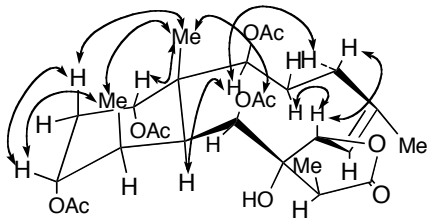

Figure 1. NOE correlations of **1**.

Compound (**2**), briviolide B, was isolated as an powder, and the molecular formula $C_{26}H_{37}O_{10}Cl$ was established by the HRFABMS. The ^{1}H NMR spectrum was similar to that of **1**, the major difference being that the chemical shift of C-16 (δ 50.5) in the ^{13}C NMR spectrum was shifted upfield by 17.6 ppm, when compared to that of **1**. Thus, briviolide B had a chlorine atom at C-16 instead of a hydroxymethyl group.[8] The relative stereochemistry was confirmed to be the same as that of **1** on comparison of the ^{1}H NMR and ^{13}C NMR spectra features, and NOE correlations.

Table 1. ^{1}H NMR Spectral Data of **1**-**13**.[a]

No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
2	4.92 b rd (6.8)	4.98 br d (7.0)	5.63 m	4.51 br s	4.46 br s	4.45 br s	4.48 d (2.8)
3	1.56 m	1.64 ov.	1.63 m	5.66 dd (12.3, 5.5)	5.67 ov.	5.42 br dd	6.19 dd
	2.79 br dt t	2.74 m	1.28 o			(12.4, 5.3)	(10.1, 2.8)
	(14.6, 14.6, 4.6)						
4	1.92 ov.	2.14 m	2.44 m	2.12 t (12.3)	2.21 t (11.4)	2.28 t (12.4)	5.22 d (10.1)
	2.44 m	2.57 m		3.01 dd (12.3, 5.5)	3.03 m	2.67 ov.	
6	5.66 br d (9.8)	5.79 brd (9.7)	4.72 br s	5.12 br d (9.5)	5.11 br d (9.7)	5.17 br d (9.3)	5.19 br d (10.1)
7	5.18 d (9.8)	5.25 d (9.7)	4.59 m	5.95 br d (9.5)	5.96 br d (9.7)	5.68 br d (9.3)	6.24 br d (10.1)
9	5.26 d (2.3)	5.29 d (1.8)	5.35 br s	6.76 d (5.5)	6.73 d (5.7)	2.68 br d (15.8)	6.80 d (6.0)
						3.04 dd (15.8, 7.0)	
10	2.72 dd (5.2, 2.3)	2.80 m	3.08 d (4.4)	2.86 d (5.5)	2.91 d (5.7)	3.04 br d (7.0)	3.06 d (6.0)
11	2.01 m	2.00 ov.	2.04 m				
12	3.68 d (2.9)	3.73 m	3.65 m	3.70 d (6.0)	4.77 d (6.2)	3.71 d (6.2)	3.72 d br d (6.2)
13	1.79 m	1.87 br d (14.7)	1.91 ov.	5.82 dd	5.86 dd	5.87 dd	5.84 dd
	1.94 ov.	2.01 ov.	2.02 ov.	(10.3, 6.0)	(10.3, 6.2)	(10.3, 6.2)	(10.3, 6.2)
14	4.81 t 3.2	4.90 m	4.96 t (2.9)	5.52 d (10.3)	5.63 d (10.3)	5.61 d (10.1)	5.57 d (10.3)
15	1.06 s	1.15 s	1.20 s	1.16 s	1.17 s	1.00 s	1.19 s
16	4.05, 4.29 AB (14.8)	4.35, 4.43 AB (12.6)	5.44, 5.72 br s each	1.97 br s	2.03 br s	1.97 br s	2.15 br s
17	2.38 q (7.1)	2.52 q (7.1)	2.98 q (7.4)				
18	1.16 d (7.1)	1.24 d (7.1)	1.24 d (7.4)	2.02 s	2.00 d (1.1)	1.91 br s	2.01 br s
20	1.08 d (7.6)	1.12 d (7.3)	0.96 d (7.7)	1.25 s	1.37 s	1.18 s	1.30 s
MeCO	1.93, 1.95	1.99, 2.02	2.00, 2.01	2.02, 2.10	2.03, 2.07	2.10, 2.16 each s	2.03, 2.07
	2.14 each s	2.23 each s	2.24 each s	2.18 each s	2.10, 2.17 each s		2.15, 2.22 each s

No.	**8**	**9**	**10**	**11**	**12**	**13**
2	3.15 br d (8.4)	4.85 br s	1.21 ov.	1.60 m	1.19 m	4.86 d (1.9)
			1.49 ov.	1.79 m	1.44 m	
3	4.92 br d (10.8)	4.94 br d (11.2)	1.77 m	1.72 m	1.69 m	5.42 ddd
			1.80 m	1.84 m	1.56 ov.	(12.6, 5.1, 1.9)
4	4.84 d (10.8)	4.98 d (11.2)	2.07 m	2.45	2.03 ov.	2.40 t (12.6)
			2.79 m	2.88 ov.	2.93 ov.	2.68 ov.
6	5.12 br d (9.0)	5.19 br d (9.2)	4.99 br d (8.8)	5.58 br s	6.28 br s	5.24 ov.
7	6.02 br d (9.0)	6.13 br d (9.2)	5.74 br d (8.8)			5.70 br d (9.9)
9	6.78 d (5.1)	6.64 br d (5.5)	2.72 dd (15.2, 9.2)	2.77 dd (13.4, 11.5)	2.50 d (15.8)	2.56 dd (16.7, 7.0)
			2.40 br d (15.2)	2.49 br d (13.4)	2.72dd (15.8, 8.8)	3.01 br d (16.7)
10	2.52 d (5.1)	2.63 d (5.5)	2.60 d (9.2)	2.61 br d (13.4)	2.47 d (8.8)	2.68 ov.
12	3.72 br s	3.09 br d (4.2)	2.94 d (5.9)	2.88 ov.	2.91 br d (6.2)	5.24 ov.
13	5.82 dd (9.9, 6.2)	2.08 -2.22 2H ov.	2.11-2.21 ov.	2.01 ov.	2.03 ov.	2.06 m
				2.13 dd (16.7, 2.2)	2.14 ov.	2.27 br d (18.7)
14	6.00 d (9.9)	4.72 br s	4.71 br s	4.63 br s	4.64 br s	4.96 m
15	1.18 s	1.17 s	0.87 s	0.88 s	0.85 s	0.94 s
16	1.99 br s	2.22 br s	1.73 br s	4.18 AB (14.3)	1.81 br s	2.06 br s
18	2.02 br s	2.10 br s	1.93 br s	1.99 br s	2.02 br s	1.89 br s
20	1.31 s	1.25 ov.	1.17 s	1.19 s		1.61 s
MeCO	2.16 s	1.99, 2.14		2.07 s	2.05 s	1.95, 2.10, 2.10
		2.15 each s				each s
RCOO	0.88 3H m	0.87 3H t (6.6)				
	1.28 8H ov.	1.30 3H ov.				
	1.66 2H m	1.62 2H m				
	2.41 2H t (7.5)	2.38 2H m				
MeO				3.34 s	3.71 s	
CH_3CH_2-				1.14 3H t (7.3)		
				2.29 2H m		

[a]Chemical shift values are in ppm from TMS, and *J* values (in Hz) in presented in parentheses.
[a]Measured in $CDCl_3$. ov.: overlapped.

Table 2. ^{13}C NMR Spectral Data of **1-13**.[a]

C	1	2	3	4	5	6	7	8	9	10	11	12	13
1	45.7	45.7	45.7	46.0	43.3	45.9	46.1	48.1	44.9	39.3	37.7	38.8	41.6
2	77.0	77.2	74.5	77.8[c]	78.4	77.7[c]	77.2[c]	76.3	71.5	35.7	44.9	36.8	72.2
3	31.2	30.9	29.0	71.1	69.4	70.8	70.8	70.7	70.1	23.2	20.7	22.7	68.5
4	26.3	26.2	32.7	35.2	35.7	35.3	77.1[c]	77.7	77.2	31.4	29.8	31.7	36.1
5	147.9	143.7	146.2	138.2	136.8	138.3	138.4	139.1	141.2	140.6	149.0	148.9	137.9
6	120.1	122.4	55.7	126.0	126.3	126.0	130.1	128.5	127.4	122.9	120.4	128.6	125.5
7	77.7	78.2	81.2	78.6[c]	80.4	78.5[c]	76.9	76.9	77.2	79.1	108.0	202.2	80.6
8	82.4	82.4	81.6	157.2	161.0	157.0	157.4	157.2	155.8	163.9	160.0	154.7	159.7
9	75.1	75.4	84.2	66.9	25.2	66.4	66.4	67.1	69.5	24.4	24.2	31.9	29.1
10	33.0	32.7	33.4	41.6	38.7	42.2	40.8	42.6	41.0	37.6	36.7	35.4	38.1
11	45.5	45.7	47.1	74.8	74.7	74.0	75.1	74.8	61.8	61.8	61.0	62.5	136.1
12	71.4	71.7	72.2	70.1	70.5	73.1	70.1	70.5	61.1	59.7	59.9	59.6	116.9
13	27.0	26.8	27.5	124.3	125.8	121.5	124.8	122.7	24.8	27.7	27.3	27.8	25.9
14	76.6	76.6	75.8	140.6	138.4	142.0	140.8	142.9	73.9	77.2	79.4	77.6	72.9
15	15.2[c]	15.1[c]	16.0	16.3	14.1	16.5	16.5	14.4	16.0	14.1	22.2	22.3	14.5
16	68.1	50.5	120.6	26.9	26.8	27.2	25.5	25.9	25.2	22.7	65.9	24.0	26.8
17	43.5	43.5	50.9	128.2	125.3	128.2	127.5	128.7	129.0	122.9	128.1	123.6	125.1
18	6.6	6.6	7.2	9.7	9.2	9.7	9.6	9.5	10.3	9.1	9.3	14.3	9.8
19	175.9	176.0	174.7	174.0	174.4	173.9	174.0	174.0	173.0	174.8	171.1	168	174.0
20	15.7[c]	15.6[c]	15.2	21.4	20.9	21.4[d]	21.5	20.9	23.6	23.0	23.4	22.8	21.5
MeCO	21.4, 21.5	21.3, 21.6	21.3, 21.4	20.7, 20.8	20.6, 20.7	20.6, 21.1	20.4, 20.8	21.5	20.6, 21.0	21.6	21.4	21.6	20.5, 21.0
	21.7	21.7	21.4	21.1		21.1, 21.3[d]	20.8, 20.9		21.4				21.1
MeCO	168.9, 170.0	169.1, 169.9	170.0, 170.0	169.2, 169.9	170.3, 170.6	169.2, 169.8	168.6, 169.9	168.9	167.9, 170.4	170.2	170.3	170.5	70.5, 170.7
	171.8	171.0	171.0	170.6		170.3, 170.5	170.1, 170.2		170.5			52.0	171.0
RCOO								13.9, 22.4	14.1, 22.6				
								24.7, 28.7	24.8, 28.9				
								28.8, 31.5	29.0, 31.6				
								34.1, 174.0	34.2, 173.3				

[a]Chemical shift values for **1-2, 4-9,** and **13** are in ppm from TMS and **3, 10, 11, 12** from $CDCl_3$ (δ 77.0). [b]Measured in $CDCl_3$.

[c, d] These values may be exchangeable.

Compound (**3**), briviolide C, an amorphous powder, had a molecular formula $C_{26}H_{37}O_{10}Cl$, and was isomeric with **2**. The ^{1}H NMR spectrum was fundamentally similar to that of **2**; however, resonances due to H-6, H-7, and H-16 changed drastically. Thus, they were observed as a broad singlet (δ 4.72, 1H, H-6), a multiplet (δ 4.59, 1H, H-7), and two broad singlets (δ 5.44, 1H; 5.72, 1H, H-16). This suggested the presence of an *exo* methylene at C-5 and a chlorine atom at C-6. The configurations of H-6 and H-7 could not be determined by the NOE data, since the ^{1}H NMR signals were not well resolved. So an X-Ray diffraction experiment was performed, indicating that Cl at C-6 and H-7 were α- and β-oriented, respectively (Figure 2). The stereochemistry of the remaining chiral centers was confirmed by the coupling patterns, NOE data, and X-Ray data.

Compounds (**4**)-(**8**) were obtained as amorphous powders, and their molecular formulas were established by HRFAMS spectra. They were closely related briarane diterpenes, and possessed 2, 3, 9, 11, and 12-pentaoxygenated carbons or a more acylated 4-carbon and an 8,17-unsaturated lactone.

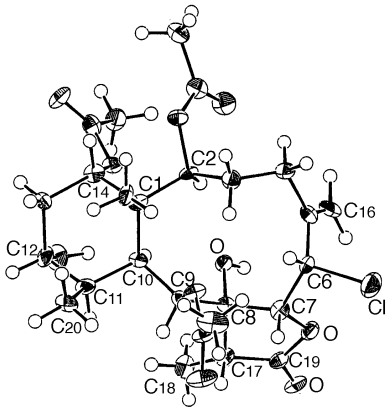

Figure 2. ORTEP repsentation of **3**.

Compound (**4**), briviolide D, $C_{26}H_{34}O_{10}$, indicated absorptions due to hydroxyl group (ν_{max} 3503 cm^{-1}), α,β-unsaturated five-membered lactone, (ν_{max} 1740 cm^{-1}) and acetyl (ν_{max} 1740 and 1231 cm^{-1}) functionalities. The presence of the unsaturated lactone was also supported by UV absorption [λ_{max} 213 nm (ε 10400)]. The ^{1}H NMR spectrum indicated resonances due to three acetyl protons (δ 2.02, 2.10, 2.18, each s), the positions of which were easily determined to be at C-2, C-3, C-9; δ 4.51, 1H, br s, H-2; 5.66, 1H, dd, *J*= 12.3, 5.5 Hz, H-3; 6.76, 1H, d, *J*= 5.5 Hz, H-9. The structure and stereochemistry of the six- and ten-membered rings were elucidated by comparing the ^{1}H NMR spectrum of those of related compounds isolated from the same species.[1-7] Olefinic methyl protons (δ 2.02, 3H, s) were assigned as H-18 on the basis of a correlation of H-18 to C-19 (δ 174.0). The stereochemistry was confirmed by the NOESY spectrum; H-2/H-10, H-16, H-3/H-7, H-9/H-18, H-20, H-13/H-20. Thus, the structure of briviolide D was shown as **4**.

The ^{1}H NMR spectrum of **5**, $C_{28}H_{36}O_{11}$, was similar to that of **4**, except for additional acetyl protons (δ 2.07, 3H, s). The chemical shift of H-12 (δ 4.77, 1H, d, *J*= 6.2 Hz) was shifted downfield by 1.07 ppm when compared with that of **4**, suggesting that the hydroxyl group at C-12 was acetylated. The stereochemistry was determined by comparing the signal patterns in the ^{1}H NMR spectrum and the NOESY data with those of **4**. Thus, compound (**5**) was 12-*O*-acetylbriviolide D.

Comparison of the ^{1}H NMR spectrum of **6**, $C_{24}H_{32}O_8$, with that of **4** indicated that the acetyl group at C-9 was missing and methylene protons (δ 2.68, 1H, br d, *J*= 15.8 Hz; 3.04, 1H, dd, *J*= 15.8, 7.0 Hz) appeared. On the signal patterns in the ^{1}H NMR spectrum and NOE correlations, the stereochemistry was

determined. Therefore, compound (**6**) was assigned as 9-deacetoxybriviolide D.

Compounds (**7**), $C_{28}H_{36}O_{11}$, exhibited similar ^{1}H NMR spectra to that of **4**, except that four acetyl protons (δ 2.03, 2.07, 2.15, 2.22, each s) and the downfield chemical shift of H-4 (δ 5.22, 1H, d, *J*= 10.1 Hz) were observed. Thus, compound (**7**) was a 4-acetoxyl derivative of **4**. The stereochemistry was established on the basis of the signal patterns in the ^{1}H NMR spectrum and the NOESY data with those of **4**. Compound (**7**) was therefore elucidated as 4-acetoxybriviolide D .

The ^{1}H NMR spectrum of **8**, briviolide E, $C_{30}H_{44}O_{10}$, was similar to that of **7**. However, the major difference was that only resonances due to acetyl protons (δ 2.16, 3H, s) and octanoyl protons (δ 0.88, 3H, m; 1.28, 8H, overlapped; 1.66, 2H, m; 2.41, 2H, t, *J*= 7.5 Hz) as acyl protons were observed. The positions were determined by the lowfield chemical shifts of H-4 (δ 4.92, 1H, d, *J*= 10.8 Hz) and H-9 (δ 6.78, 1H, d, *J*= 5.1 Hz) and by a correlation between H-4 and C-21 (δ 174.0) in the HMBC spectrum. Moreover, the higher field chemical shifts of H-2 and H-3 (δ 3.15, 1H, br d, *J*= 8.4 Hz, H-2; 4.92, H-3) suggested that C-2 and C-3 were hydroxylated. The stereochemistry was established by comparing the signal patterns in the ^{1}H NMR spectrum and the NOESY data with those of **7**. Thus, briviolide E was shown to have the structure (**8**).

Compounds (**9**), (**10**), (**11**), and (**12**) contained a double bond between C-5 and C-6, an α,β-unsaturated lactone or ester, a 11,12-β-epoxy moiety, and an α-acetyl group at C-14.

The ^{1}H NMR spectrum of **9**, briviolide F, $C_{34}H_{48}O_{12}$, confirmed resonances due to two olefinic methyl protons (δ 2.10, 3H, br s, H-18; 2.22, 3H, br s, H-16), three acetyl protons (δ 1.99, 2.14, 2.15, s each), and octanoyl protons (δ 0.87, 3H, t, *J*= 6.6 Hz; 1.30, 8H, m; 1.62, 2H, m; 2.38, 2H, m). Chemical shifts and coupling constants of H-2 to H-4 (δ 4.85, 1H, br s, H-2; 4.94, 1H, br d, *J*= 11.2 Hz, H-3; 4.98, 1H, d, *J*= 11.2 Hz), H-9 (δ 6.64, 1H, br d, *J*= 5.5 Hz), and H-14 (δ 4.72, 1H, br s) were reminiscent of those of the related briaranes.[10] This suggested the locations and stereochemistries of the acyl groups at C-2, C-4, and C-14, and the hydroxyl group at C-3. The position of the octanoyloxyl group was deduced as C-4 from a correlation of H-4 to C-21 (δ 173.3). The presence of an epoxide between C-11 and C-12 and its β-orientation were elucidated on the basis of the chemical shifts (δ 61.8, C-11; 61.1, C-12).[11]

The stereochemistry of the chiral centers was established by the coupling patterns in the ^{1}H NMR spectrum and NOESY spectrum; H-2/H-10, H-16, H-3/H-7, H-9/H-18, H-20, H-14/H-15.

The ^{1}H NMR spectrum of **10**, briviolide G, $C_{22}H_{30}O_5$, was similar to that of **9**; however, the 2,9-diacetoxy-4-octanoyloxy-3-hydroxy moiety on the ten-membered ring in **9** was missing. Therefore, the structure of briviolide G was shown as **9**.

Compound (**11**), briviolide H, $C_{23}H_{32}O_7$, showed similar resonances in the ^{1}H NMR spectrum to that of **10**, except for additional methoxyl protons (δ 3.34, 3H, s) and hydroxymethyl protons (δ 4.18, 2H, AB, *J*= 14.3 Hz) instead of methyl protons at C-16. The methoxyl group was determined to be positioned at C-7,

since resonance corresponding to H-7 was lacking and H-6 (δ 5.58, 1H) appeared as a broad singlet. The β-configuration of the methoxyl group was assumed from the NOESY correlations: H-10/H-3, H-6; H-6/H-20; H-15/H-2 (δ 1.60, m), H-9, H-14, H-9/H-2 (δ 1.60), H-18, H-20; H-16/H-4 (δ 2.45, m); -OMe/H-4 (δ 2.88, overlapped) (Figure 3).

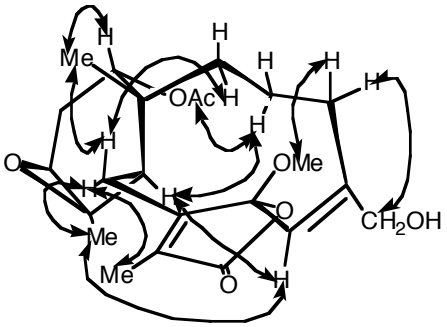

Figure 3. NOE correlations of **11**.

Compound (**12**), briviolide I, $C_{23}H_{32}O_6$, indicated bands due to an ester group (1734 cm^{-1}), an unsaturated carbonyl group (1680, 1640 cm^{-1}) in the IR spectrum. The ^1H NMR spectrum was similar to that of **10**, except that additional protons due to carbomethoxyl protons (δ 3.71, s) appeared and H-7 was missing. In the ^{13}C NMR spectrum, resonances due to an unsaturated carbonyl (δ 202.2) and an unsaturated methyl ester carbon (δ 168.1) were observed. This implied that the unsaturated γ-lactone as seen in **10** was cleaved to a methyl ester and the resulting alcohol at C-7 was oxidized to the carbonyl group. The HMBC spectrum also supported this assumption; H-9 (δ 2.72)/C-7, C-8, C-17, H-18/-COOMe, -OMe/-COOMe. The stereochemistry of the six-membered ring was the same as that of **9**-**11** on the basis of NOESY and NMR spectral data.

Compound (**13**), briviolide J, $C_{26}H_{34}O_8$, had different substituents on the six-membered ring than **5**. Two overlapped olefinic protons (δ 5.24) were coupled to two methyls in the ^1H-^1H COSY spectrum, which were assigned as H-16 (δ 2.06, br s) and H-20 (δ 1.61, br s). One of the olefinic protons was further coupled to methylene protons (δ 2.06, 1H, m; 2.27, 1H, br d, *J*= 18.7 Hz), which in turn were coupled to proton (δ 4.96, 1H, m) at C-14 carrying an acetoxyl carbon. The relative stereochemistry of the chiral center was determined on the basis of the signal patterns in the ^1H NMR spectrum and NOESY spectrum; H-2/H-4, H-16; H-14/H-15. Thus, briviolide **J** had a structure (**13**), as shown.

The cytotoxicity of **1**,**2**, **4**-**7**, **9**, and **10** against the growth of Vero and MDCK cells was examined. Compounds (**1**), (**7**), and (**11**) are not cytotoxic against both cells. Compounds (**2**), (**4**), and (**5**) exhibited weak cytotoxicity toward Vero cells (CC_{50}= 91.5, 87.6, 45.3 μg/mL, respectively), though they were inactive in MDCK cells. However, weak cytotoxicity against Vero cells (CC_{50} 69.0 and 31.9 μg/mL) and MDCK cells (CC_{50} 82.1 and 42.2 μg/mL) was shown for **6** and **9**, respectively. The other compounds were not examined

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured at 22 ˚C on a JASCO DIP-370S polarimeter. IR spectra were recorded on a MASCO FT/IR 5300. NMR spectra were recorded with either 400 MHz JEOL or a VARIAN UNITY-500 NMR instruments using TMS as internal standard and

$CDCl_3$ as solvent. MS spectra were obtained with a JEOL JMS XD-303 instrument. A Rigaku AFC7R diffractometer was used in the X-Ray work.

Animal Material. Specimens of *Briareum* were collected at Bonotsu, Kagoshima Prefecture. The reference sample (collection no. 222) was deposited at the Department of Chemistry and Bioscience.

Extraction and Isolation. The fractions 8-11(9.5 g) obtained by chromatography of the first portion (12.0 g) of the dichloromethane extract (54.4 g)[7] were chromatographed with MeOH-CH_2Cl_2 (2:49) and then subjected to HPLC (ODS) with MeCN-H_2O (3:7) to give (**4**) (1.2 mg), (**6**)(2.3 mg), (**7**)(1.1 mg), and (**2**)(1.6 mg). Slower elution with MeOH-CH_2Cl_2 (2:49) afforded needles (**3**) (11.2 mg). The fractions of 9-12 (13.0 g) obtained from the second portion (20.5 g)[7] of the extract were chromatographed over silica gel with MeOH-CH_2Cl_2 (1:99) and HPLC with MeCN-H_2O (3:2) to give (**9**)(1.4 mg) and with MeCN-H_2O (43:57) to provide (**10**)(1.1 mg), (**12**) (0.7 mg), and (**13**)(1.4 mg). The fraction eluted with MeOH-CH_2Cl_2 (2:49) followed by HPLC with MeCN-H_2O (3: 7) yielded (**11**)(1.0 mg) and (**5**)(0.3 mg). Further elution with MeOH-CH_2Cl_2 (1:14) gave a residue, which was purified by HPLC with MeCN-H_2O (11: 9) to furnish (**8**)(0.8 mg). Compound (**1**) (2.4 mg) was obtained from the eluate obtained by silica gel chromatography with MeOH-CH_2Cl_2 (1:24) followed by HPLC with MeOH-H_2O (2:3).

Compound (1) (briviolide A). Amorphous powder, $[\alpha]_D$ –23° (*c* 0.04, MeOH); IR (film) ν_{max} 3385, 1767, 1734, 1651, 1217 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 527.2503 $[M + H]^+$ (calcd for $C_{26}H_{39}O_{11}$, 57.2492).

Compound (2) (briviolide B). Amorphous powder, $[\alpha]_D$ –32° (*c* 0.34, MeOH); IR (film) ν_{max} 3445, 1773, 1732, 1655, 1215 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 545.2153 $[M + H]^+$ (calcd for $C_{26}H_{38}O_{10}{}^{35}Cl$, 545.2153).

Compound (3) (briviolide C). Needles from MeOH, 171-172 °C (decomp) , $[\alpha]_D$ –36° (*c* 0.19, MeOH); IR (film) ν_{max} 3480, 1786, 1734, 1217 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 545.2153 $[M + H]^+$ (calcd for $C_{26}H_{38}O_{10}{}^{35}Cl$, 545.2154).

Compound (4) (briviolide D). Amorphous powder, $[\alpha]_D$ +9.4° (*c* 0.12, MeOH); UV (MeOH) λ_{max} : 213 nm (ε 10400); IR (film) ν_{max} 3503, 1740, 1680, 1231 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 507.2235 $[M + H]^+$ (calcd for $C_{26}H_{35}O_{10}$, 507.2230).

Compound (5) (12-*O*-acetylbriviolide D). Amorphous powder, $[\alpha]_D$ –21° (*c* 0.06, MeOH); UV (MeOH) λ_{max} : 215 nm (ε 8900); IR (film) ν_{max} 3503, 1744, 1678, 1231 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 549.2325 $[M + H]^+$ (calcd for $C_{28}H_{37}O_{11}$, 549.2326).

Compound (6) (9-deacetoxybriviolide D). Amorphous powder, $[\alpha]_D$ +35° (*c* 0.11, MeOH); UV (MeOH) λ_{max}: 218 nm (ε 13200); IR (film) ν_{max} 3459, 1738, 1669, 1231 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see

Table 2); HRFABMS *m/z* 449.2179 $[M + H]^+$ (calcd for $C_{24}H_{33}O_8$, 449.2175).

Compound (7) (4-acetoxybriviolide D). Amorphous powder, $[\alpha]_D$ +44° (*c* 0.05, MeOH); UV (MeOH) λ_{max}: 217 nm (ε 10100); IR (film) ν_{max} 3484, 1746, 1671, 1223 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 565.2274 $[M + H]^+$ (calcd for $C_{28}H_{37}O_{12}$, 565.2285).

Compound (8) (briviolide E). Amorphous powder, $[\alpha]_D$ -24° (*c* 0.04, MeOH); UV (MeOH) λ_{max}: 216 nm (ε 14300); IR (film) ν_{max} 3466, 1746, 1669, 1221 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 565.3037 $[M + H]^+$ (calcd for $C_{30}H_{45}O_{10}$, 565.3013).

Compound (9) (briviolide F). Amorphous powder, $[\alpha]_D$ +104° (*c* 0.1, MeOH); UV (MeOH) λ_{max}: 213 nm (ε 10700); IR (film) ν_{max} 3503, 1746, 1219 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 649.3231 $[M + H]^+$ (calcd for $C_{34}H_{49}O_{12}$, 649.3224).

Compound (10) (briviolide G). Amorphous powder, $[\alpha]_D$ -42° (*c* 0.06, MeOH); UV (MeOH) λ_{max}: 219 nm (ε 13600); IR (film) ν_{max} 1755, 1676, 1242 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 375.2187 $[M + H]^+$ (calcd for $C_{22}H_{31}O_5$, 375.2171).

Compound (11) (briviolide H). Amorphous powder, $[\alpha]_D$ +183° (*c* 0.14, MeOH); UV (MeOH) λ_{max}: 230 nm (ε 9400); IR (film) ν_{max} 3468、1755, 1738 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 421.2228 $[M + H]^+$ (calcd for $C_{23}H_{33}O_7$, 421.2226).

Compound (12) (briviolide I). Amorphous powder, $[\alpha]_D$ -122° (*c* 0.04, MeOH); UV (MeOH) λ_{max}: 224 nm (ε 9200); IR (film) ν_{max} 1734, 1680, 1640、1242 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 405.2265 $[M + H]^+$ (calcd for $C_{23}H_{33}O_6$, 405.2277).

Compound (13) (briviolide J). Amorphous powder, $[\alpha]_D$ -104° (*c* 0.07, MeOH); UV (MeOH) λ_{max}: 224 nm (ε 9200); IR (film) ν_{max} 1739, 1667, 1242 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 475.2331 $[M + H]^+$ (calcd for $C_{26}H_{35}O_8$, 475.2332).

Crystal data for 4: $C_{26}H_{37}O_{10}Cl$ MW (525.27), space group C2, *a*= 33.931(4) Å, *b*= 9.800(1) Å, β= 128.432(1)°, *c*= 22.115(2) Å, V= 5760(1) $Å^3$, Z= 8, Dc= 1.327 g/cm^3, T= -150 °C, F(000)=2444, μ(MoKα)=2.31 cm^{-1}, Intensity data were collected on a Rigaku RAXIS-IV diffractometer using graphite monochromated MoKα (λ=0.71069 Å) up to 2θ=55°. Of the total 22306 reflections which were collected; equivalent reflections were merged. The structure was solved by direct methods (SIR97)[12] and expanded using Fourier techniques.[13] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. It was refined by full-matrix least-squares and converged with *R*= 0.159 and *Rw*= 0.145. Considerably large *R* values may be due to a poor quality of the intensity data collected by use of very small cryatal. Atomic coordinates, bond lengths and angles,

and thermal parameters have been deposited at Rigaku Corporation.

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