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BRIARANE DITERPENES FROM A GORGONIAN *BRIAREUM* SP.

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Abstract – Eleven new briarane diterpenes have been isolated from a gorgonian *Briareum* sp., collected in the area of Bonotsu, Kagoshima Prefecture. Their structural elucidation and cytotoxicity tests toward Vero and MDCK cells were performed.

INTRODUCTION

More than 300 briarane-type diterpenoids containing a □-lactone in a bicyclo [8.4.0] system have been so far isolated from the Alcynonaria class such as gorgonians, soft corals, sea pansy, and sea pens, a nudibranch, and a sponge.[1] Many briarane diterpenes exhibit a range of interesting bioactivities such as cytotoxic, anti-inflammatory, antiviral, insecticidal, and antifouling activity.[2] Our previous investigations of the secondary metabolites of the dichloromethane soluble part of the methanol extract of a *Briareum* sp., collected in the area of Bonotsu, Kagoshima Prefecture, afforded a number of cytotoxic briarane-type diterpenes, and the relationship between the structure and cytotoxicity was elucidated.[3-6] Further careful examination of the extract has yielded a series of briarane-type diterpenes (**1**)-(**11**). We report herein the isolation and structural elucidation of the new compounds as well as the cytotoxicity toward Vero and MDCK cells.

RESULTS AND DISCUSSION

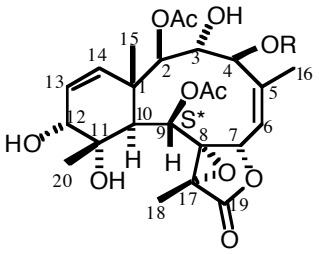
The dichloromethane soluble part of the methanol extract of a *Briareum* sp. was subjected to flash silica gel chromatography. Fractions eluted with 5-20% methanol-dichloromethane were repeatedly subjected to silica gel chromatography and finally by C_{18} reversed phase HPLC to give new compounds (**1**-**11**).

Violides (**1**-**11**) possessed a common briarane skeleton with a 9-acetoxyl group, a 12-hydroxyl group, and 5,6 and 13,14 carbon-carbon double bonds. The gross structures were determined by 1D NMR (Table 1 and 2) and extensive 2D NMR experiments including ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY. Their structures were divided into three classes based on differences in the kinds of 8, 17 moieties. The first class has an 8,17-epoxide and contains **1**-**4**. The second class comprises **5**-**9**, which have an 8,17-diol moiety. The third class contains **10** -**11** possessing an 8-hydorxy-17-acyloxy moiety.

Compound (**1**) was isolated as an amorphous powder and the molecular formula was determined by HRFABMS *m/z* 595.2761 $[M+H]^+$ ($C_{30}H_{43}O_{12}$ Calcd for *m/z* 595.2754). The IR spectrum exhibited absorptions due to a hydroxyl group (ν_{max} 3501 cm^{-1}), γ-lactone carbonyl (ν_{max} 1782 cm^{-1}), and an ester carbonyl (ν_{max} 1742 cm^{-1}). In the ^{1}H NMR spectrum, resonances due to four tertiary methyls (δ 1.16, s; 1.32, s; 1.70, s; 2.08, d, *J*=1.1 Hz, each 3H), two acetyls (δ 2.22, 2.26, 3H, s each), and hexanoate protons (δ 0.89, t, *J*=7.0 Hz; 1.33, 4H, overlapped; 1.64, 2H, overlapped; 2.37, 2H, m) were observed. The ^{13}C NMR spectrum indicated the presence of resonances due to nine oxygenated carbons (δ 64.5-76.7), two acetyls, a hexanoyl, and a γ-lactone carbonyl carbons (δ 168.4-173.5, CO x 4). The ^{1}H NMR spectrum was similar to that of violide A (**12**),[3] except that the octanoyl group was replaced by the hexanoyl group. The hexanoate group was also established to be positioned at C-4 from the HMBC correlation of H-4 (δ 4.86, 1H, overlapped) with C-21 (δ 173.5). The relative stereochemistry was confirmed by the ^{1}H NMR coupling patterns and the NOE experiments: H-2/H-4, H-10, H-3/H-7, H-4/H-16, H-9/H-18, H-20, H-15/H-14, H-20. The structure was, therefore, shown as **1**.

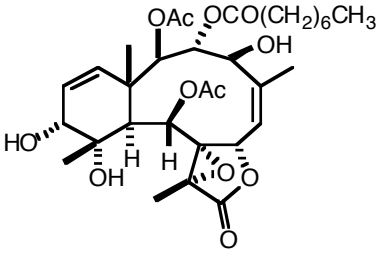
Compound (**2**), designated as violide Q, was isolated as an amorphous powder, and had the same molecular formula, $C_{32}H_{46}O_{12}$, as that of **12**. Compound (**2**) had a similar ^{1}H NMR spectrum as that of **12**, except that H-3 (δ 6.00, 1H, br d, *J*=10.3 Hz) and H-4 (δ 4.15, 1H, br dd, *J*=10.3, 4.0 Hz) shifted downfield by 1.13 ppm and upfield by 0.71 ppm, respectively, in comparison with those of **12**, suggesting that **2** had a 3-acyloxy-4-hydroxy moiety. The octanoate group was presumed to be located at C-3 by the HMBC correlation of H-3 and C-21 (δ 173.3). The stereochemistry was deduced from the similar coupling patterns in the ^{1}H NMR spectrum and NOE correlations to those of **12**. Thus, the structure of violide Q, was determined as **2**.

The HRFABMS established the molecular formula $C_{32}H_{46}O_{11}$ for compoud (**3**), violide R, an amorphous powder. The ^{1}H NMR spectrum of **3** indicated resonances due to four methyl protons (δ 1.23, s; H-15,



1 R=$\overset{21}{C}O(CH_2)_4CH_3$

12 R=$CO(CH_2)_6CH_3$



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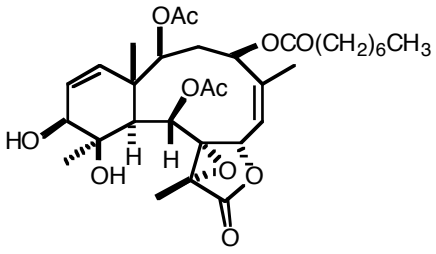
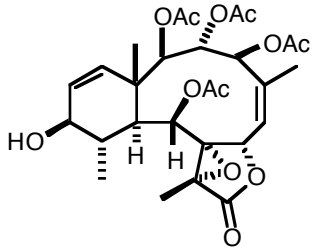


3

13 11,12-epimer



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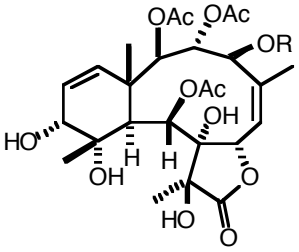
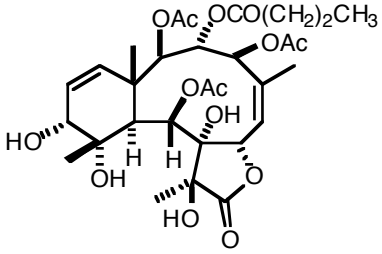
14 11β-OH, 11α-Me, 12β-OH



5 R=$CO(CH_2)_6CH_3$

15 R=Ac



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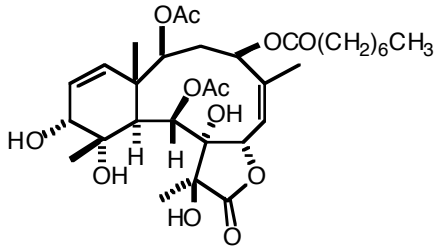

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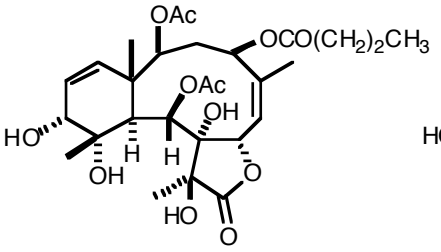

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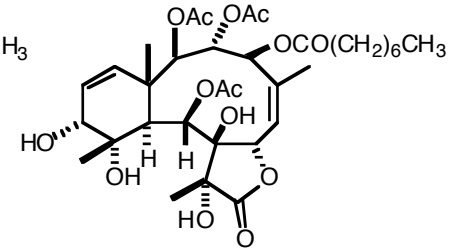

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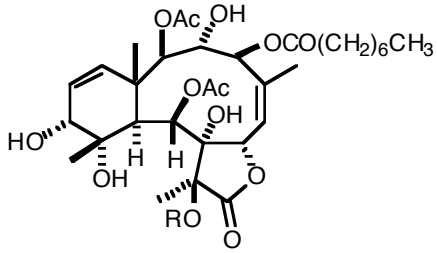
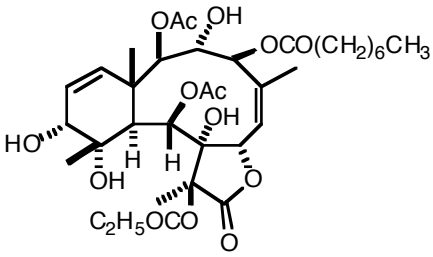


10 R=Ac

16 R=H



11

1.40, s H-20; 1.62, s, H-18; 2.10, d, *J*=1.5 Hz, H-16), two acetyl protons (δ 2.12, 2.24, 3H, s each), and protons (δ 0.88, 3H, t, *J*=7.0 Hz; 1.28, 8H, m, 1.59, 2H, overlapped; 2.31, 2H, t, *J*=7.5 Hz). A broad

triplet proton (δ 2.82, 1H, br t, *J*=13.6 Hz) was assigned as H-3, since it was coupled geminally to the other H-3 proton (δ 2.10, 1H, overlapped) and vicinally to the methine proton (δ 4.62, d, *J*=6.6 Hz, H-2).

Table 1. ^{1}H NMR Spectral Data of **1-11**.[a]

No	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[c]
2	4.70 br s	4.63 br s	4.62 d (6.6)	4.65 d (2.2)	4.68 br s	4.70 br s
3	4.86 ov.	6.00 br d (10.3)	2.10 ov 2.82 br t (13.6)	6.08 dd (10.3, 2.2)	6.22 d (10.4)	6.25 br d (10.1)
4	4.86 ov.	4.15 br dd (10.3, 4.0)	5.06 dd (13.6, 5.5)	5.18 d (10.3)	5.11 d (10.4)	5.11 d (10.1)
6	5.50 br d (9.5)	5.57 br d (9.5)	5.49 br d (9.9)	5.63 br d (10.1)	5.78 br d (9.9)	5.82 ov.
7	5.72 d (9.5)	6.09 d (9.5)	5.84 d (9.9)	5.93 d (10.1)	6.09 d (9.9)	6.12 d (9.9)
9	5.92 d (3.8)	5.95 d (3.8)	5.82 d (4.6)	5.53 d (5.0)	6.15 d (4.0)	6.15 d (4.0)
10	2.58 d (3.8)	2.69 d (3.8)	2.55 d (4.6)	2.64 dd (8.6, 5.0)	2.90 d (4.0)	2.88 d (4.0)
11				1.99 m		
12	3.69 d (6.0)	3.69 m	3.75 d (5.4)	4.01 m	3.74 d (6.2)	3.75 d (6.2)
13	5.82 dd (10.3, 6.0)	5.84 dd (10.3, 6.6)	5.81 dd (10.1, 5.4)	5.78 dd (10.1, 4.8)	5.81 dd (10.3, 6.2)	5.82 ov.
14	5.36 d (10.3)	5.47 d (10.3)	5.52 d (10.1)	5.49 d (10.1)	5.48 d (10.3)	5.50 d (10.3)
15	1.32 s	1.15 s	1.23 s	1.02 s	1.09 s	1.10 s
16	2.08 d (1.1)	2.08 d (1.5)	2.10 d (1.5)	2.14 d (1.5)	2.14 br s	2.15 br s
18	1.70 s	1.68 s	1.62 s	1.57 s	1.48 s	1.50 s
20	1.16 s	1.15 s	1.40 s	1.12 d (7.0)	1.40 s	1.42 br s
MeCO	2.22 s, 2.26 s	2.16 s, 2.28 s	2.12 s, 2.24 s	2.02 s, 2.08 s 2.17 s, 2.27 s	2.05 s, 2.16 s 2.20 s	2.02 s, 2.16 s 2.21 s
n -C_nH_{2n+1}-	0.89 3H t (7.0)	0.89 3H t (6.3)	0.88 3H t (7.0)		0.87 3H t (6.6)	0.96 3H t (7.3)
CO_2	1.33 4H ov. 1.64 2H ov. 2.37 2H m	1.28 8H m 1.67 2H ov. 2.40 2H m	1.28 8H m 1.59 2H ov. 2.31 2H t (7.5)		1.27 8H m 1.57 2H m 2.27 2H m	1.63 2H ov. 2.29 2H t (7.3)

No.	**7**[b]	**8**[b]	**9**[c]	**10**[c]	**11**[c]	**12**[c]
2	4.58 d (7.0)	4.59 d (7.3)	4.67 br s	4.70 br s	4.71 br s	4.70 br s
3	2.00 m 3.17 t (13.2)	2.02 m 3.17 dd (15.2, 13.2)	5.72 br d (11.2)	5.03 br dd (10.4, 8.4)	5.02 br dd (10.8, 9.2)	4.87 ov.
4	5.01 dd (13.2, 5.5)	5.02 dd (13.2, 5.3)	5.04 d (11.2)	4.84 d (10.4)	4.85 d (10.8)	4.86 ov.
6	5.65 br d (9.9)	5.67 br d (9.9)	6.01 br d (9.9)	5.68 br d (9.7)	5.69 br d (9.7)	5.50 br d (9.5)
7	5.98 d (9.9)	5.99 d (9.9)	5.87 d (9.9)	5.91 d (9.7)	5.91 d (9.7)	5.72 d (9.5)
9	6.08 d (4.0)	6.07 d (4.0)	6.33 d (2.9)	6.07 d (4.0)	6.07 d (4.2)	5.92 d (3.8)
10	2.81 d (4.0)	2.80 d (4.0)	3.14 br s	2.97 d (4.0)	2.97 d (4.2)	2.58 d (3.8)
11						
12	3.74 m	3.73 d (6.4)	3.70 d (6.2)	3.74 d (6.2)	3.74 m	3.69 t (6.2)
13	5.79 dd (10.3, 6.2)	5.80 d (10.3, 6.4)	5.77 dd (9.9, 6.2)	5.79 dd (10.3, 6.2)	5.79 dd (10.3, 6.2)	5.82 dd (10.4, 6.2)
14	5.38 d (10.3)	5.39 d (10.3)	5.40 d (9.9)	5.35 d (10.3)	5.37 d (10.3)	5.35 d (10.6)
15	1.10 s	1.11 s	1.18 s	1.26 s	1.27 s	1.32 s
16	2.09 d (1.1)	2.09 d (1.1)	2.13 br s	2.05 d (1.5)	2.05 d (1.5)	2.08 d (1.1)
18	1.49 s	1.50 s	1.58 s	1.67 s	1.68 s	1.70 s
20	1.41 s	1.42 s	1.27 s	1.39 s	1.38 s	1.16 s
MeCO	2.10 s, 2.11 s	2.10 s, 2.12 s	2.12 s, 2.12 s 2.18 s	2.06 s, 2.21 s 2.23 s	2.21 s, 2.22 s	2.22 s, 2.27 s
n -C_nH_{2n+1}-	0.87 3H t (6.8)	0.96 3H t (7.3)	0.88 3H t (6.8)	0.87 3H t (6.8)	0.87 3H t (6.8)	0.86 3H t (7.0)
CO_2	1.28 8H m 1.61 2H m 2.33 2H t (7.3)	1.65 2H m 2.32 2H t (7.3)	1.27 8H ov. 1.63 2H. ov 2.25 2H m	1.29 8H ov. 1.63 2H m 2.39 2H t (7.5)	1.29 8H ov. 1.64 2H ov. 2.38 2H t (7.5)	1.29 8H ov. 1.63 2H m 2.38 2H m
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).

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

These signal patterns were reminiscent of those of violide H (**13**).[4] Furthermore, substituted patterns at the remaining positions were also similar to those of **13**. The position of the octanoyl group was determined from the observation of the correlation from H-4 (δ 5.06, 1H, dd, *J*=13.6, 5.5) to octanoate carbonyl (δ 173.0). The stereochemisty in the ten-membered ring and γ-lactone ring was found to be similar to that of **13** on the basis of the NOE spectrum and values of the coupling constants. However, chemical shifts of C-11, C-12, C-15, and C-20 were shifted downfield 1.6, 1.5, 2.6, and 6.7 ppm, respectively, in the ^{13}C NMR spectrum when compared with those **12**. This suggested that the configurations at C-11 and/or C-12 were (was) reverse to those of **13**. NOEs of H-20 to H-10 (δ 2.55, d, *J*=4.6 Hz) and H-12 (δ 3.75, d, *J*=5.4 Hz) and of H-10 to H-2 suggested that these hydrogens were situated on the same face (β). β-Configuration of the hydroxyl group at C-11 was further supported by the downfield shift of C-15 (δ17.8) due to anisotropy deshielding of the hydroxyl group. Violide R was therefore established as the structure of **3**. This is the first isolation of violides possessing a 11β,12β-dihydroxyl moiety from *Briareum* sp., collected in the area of Bonotsu and Amami Island, Kagoshima Prefecture.

The molecular formula of compound (**4**), violide S, an amorphous powder, was assigned as $C_{28}H_{36}O_{12}$ by the HRFABMS. The ^{1}H NMR spectrum indicated resonances due to three tertiary methyl protons (δ 1.02, s H-15; 1.57, s, H-18; 2.14, d, *J*=1.5 Hz, H-16), secondary methyl protons (δ 1.12, d, *J*=8.1 Hz), four acetyl protons (δ 2.02, 2.08, 2.17, 2.27, each 3H, s). The substituted patterns on the 10-membered ring and γ-lactone ring were concluded to be the same as those of violide B (**14**)[3] on the basis of the ^{1}H NMR spectrum. However, comparison of the coupling patterns due to the six-membered ring protons with those of **14** suggested that the C-11 hydroxyl group was missing. Therefore, C-20 methyl protons appeared as a doublet (δ 1.12, d, *J*=8.1 Hz) due to a coupling with H-11 (δ 1.99, 1H, m). The latter proton was also coupled to H-10 (δ 2.64, 1H, dd, *J*=8.6, 5.0 Hz) and H-12 (δ 4.01, 1H, m). An olefinic proton (δ 5.78, 1H, dd, *J*=10.1, 4.8 Hz, H-13) was coupled to H-12 and H-14 (δ 5.49, 1H, d, *J*=10.1 Hz). Based on the above results, the gross structure was elucidated as **4**. The relative structure was confirmed by NOE experiments. NOEs between H-15 and H-11 indicated that these protons were β-situated. NOEs from H-20 to H-12 and H-10, the latter of which showed NOE correlation with H-2 (δ 4.65, 1H, d, *J*=2.2), suggested β-configuration of H-20 and α-configuration of the hydroxyl group at C-12. Therefore, the structure (**4**) was elucidated for violide S.

Compound (**5**), violide T, was isolated as an amorphous powder with a molecular formula $C_{34}H_{50}O_{14}$. The IR spectrum showed a hydroxyl absorption (ν_{max} 3445 cm^{-1}), γ-lactone carbonyl (ν_{max} 1780 cm^{-1}), and an ester carbonyl (ν_{max} 1748 cm^{-1}). The presence of resonances due to three acetyl protons (δ 2.05, 2.16, 2.20, 3H, s each) and octanoyl protons (δ 0.87, 3H, t, *J*=6.6 Hz; 1.27, 8H, m; 1.57, 2H, m; 2.27, 2H, m),

Table 2. ^{13}C NMR Spectral Data of **1-11**.[a]

C	**1**[D]	**2**[D]	**3**[D]	**4**[D]	**5**[b]	**6**[c]	**7**[D]	**8**[c]	**9**[D]	**10**[D]	**11**[D]	**12**[D]
1	47.1	46.9	45.4	44.2	46.6	48.0	46.2	47.6	47.9	46.5	46.5	47.1
2	76.7	77.6	76.8	75.5	77.2	79.1	78.1	79.7	75.3	76.9	76.9	76.7
3	71.5	73.7	38.8	71.2	71.2	72.5	37.5	39.4	71.3	71.1	71.2	71.5
4	76.6	75.5	72.2	76.5	76.2	78.3	72.1	74.1	74.8	76.5	76.5	76.6
5	141.1	142.9	144.9	140.6	138.5	139.1	142.4	143.2	138.5	139.4	139.3	141.0
6	124.2	124.3	122.6	126.0	127.0	129.2	124.6	126.4	130.0	125.8	125.9	124.2
7	73.6	73.3	73.7	73.6	78.0	79.4	78.1	79.7	84.4	77.9	77.8	73.6
8	71.2	71.5	70.3	70.7	78.9	80.0	78.9	80.1	81.8	80.1	80.0	71.3
9	65.4	65.5	67.1	68.4	66.4	67.7	66.8	67.8	69.3	67.0	67.0	65.4
10	43.8	43.1	43.8	40.3	39.5	39.5	39.7	39.4	39.5	40.4	40.6	43.8
11	73.7	74.0	75.3	38.2	76.2	76.5	75.9	76.2	77.0	75.9	75.8	73.7
12	70.2	70.2	71.8	67.8	70.8	71.9	70.9	72.0	70.9	70.8	70.9	70.2
13	125.0	125.2	124.1	127.7	124.2	126.3	123.4	125.5	124.3	123.6	123.5	125.1
14	138.0	138.6	138.9	136.0	139.1	140.1	139.4	140.3	139.6	138.6	138.7	137.9
15	15.5	15.6	17.8	16.4	15.5	16.5	15.0	15.9	18.0	15.5	15.6	15.5
16	25.5	25.8	25.6	25.5	25.9	26.2	26.2	26.4	25.0	25.9	25.8	25.5
17	64.5	64.7	62.7	62.1	80.2	80.8	80.4	80.8	78.1	84.9	84.8	64.5
18	9.7	9.9	9.7	9.8	16.7	16.2	16.8	16.0	16.6	14.9	14.8	9.7
19	170.5	170.5	171.0	171.2	176.3	178.8	175.8	178.6	175.5	171.2	171.0	170.5
20	21.4	21.3	28.1	17.0	22.8	22.8	23.2	22.9	22.8	23.5	23.5	21.4
MeCO	20.9, 21.4	20.7, 21.3	21.1, 21.7	20.6, 20.8	20.7, 21.0	20.5, 20.9	21.1, 22.2	21.0, 22.4	20.7, 21.1	20.9, 21.6	20.9, 22.3	20.9, 21.4
				20.9, 21.2	22.1	22.5			21.3	22.4		
MeCO	168.4, 169.5	169.7, 170.1	169.4, 170.4	169.0, 169.9	168.8, 170.2	171.9, 171.9	170.2, 170.6	171.9, 172.1	169.9, 170.0	168.3, 169.0	169.0, 169.7	168.4, 169.6
				170.2, 170.4	172.2	173.7			170.2	169.7		
n-C_nH_{2n+1}-	13.9, 22.3	14.1, 22.6,	14.1, 22.6		14.1, 22.6	14.0,19.6	14.0, 22.6	14.0, 19.4	14.1, 22.6	14.1, 22.6	14.0, 22.6	14.1, 22.6
CO_2	24.6, 31.1	25.0, 28.9	24.8, 28.9		24.7, 28.9	37.2, 173.0	24.9, 28.9	37.1, 174.6	24.7, 28.9	24.9, 29.0	24.9, 28.9	26.6, 28.9
	34.3, 173.5	29.1, 31.7	29.0, 31.7		29.0, 31.6		29.0, 31.6		29.0, 31.6	29.0, 31.6	29.1, 31.7	29.0, 31.6
		34.5, 173.3	34.2, 173.0		34.2, 173.1		34.4, 173.2		34.2, 172.6	34.5, 173.8	34.5, 173.8	34.3, 173.5
$C_2H_5CO_2$-											8.9, 27.9, 171.9	

[a]Chemical shift values for **1-6** and **9-12** are in ppm from TMS, **7** from $CDCl_3$ (δ 77.0), and **8** from CD_3OD (δ 49.0). [b]Measured in $CDCl_3$. [c]Measured in CD_3OD.

besides those of four tertiary methyl protons (δ, 1.09, 3H, s, H-15; 2.14, 3H, s, H-16; 1.40, 3H, s, H-20; 1.48, 3H, s, H-18), two latter methyl groups being attached to a carbon carrying a hydroxyl group, was elucidated from the observation of the ^{1}H NMR spectrum. Protons on an acyloxylated carbon were assigned as H-2 (δ 4.68, 1H, br s), H-3 (δ 6.22, 1H, d, *J*=10.4 Hz), H-4 (δ 5.11, 1H, d, *J*=10.4 Hz), H-9 (δ, 6.15, d, *J*=4.0 Hz) by comparing the ^{1}H NMR spectrum with that of related compounds.[3-5] On the above data, the ^{1}H NMR spectrum of **5** had close similarity to that of violide J (**15**),[4] except that one acetyl group was replaced by an octanoyl group. The octanoate group was determined to be positioned at C-4 by HMBC correlation between H-4 and C-21 (δ 173.1). Thus, the structure of violide T was formulated as **5**.

The ^{1}H NMR spectrum of compound (**6**), violide U, $C_{30}H_{42}O_{14}$, was similar to that of **5**; however, resonances due to octanoyl protons in **5** were missing and instead butanoyl protons (δ 0.96, 3H, t, *J*=7.3 Hz; 1.63, 2H, overlapped; 2.29, 2H, t, *J*=7.3 Hz) appeared in **6**. The position of the butanoyl group at C-3 was established by the correlation of H-3 (δ 6.25, 1H, br d, *J*=10.1 Hz) with C-21 (δ 173.0). Therefore, violide U has the structure (**6**) as shown.

Compound (**7**) was obtained as an amorphous powder and had the molecular formula $C_{32}H_{48}O_{12}$. The presence of resonances due to two acetyl protons (δ 2.10, 2.11, 3H, s, each) and octanoyl protons (δ 0.87, 3H, t, *J*=6.8 Hz; 1.28, 8H, m; 1.61, 2H, m; 2.33, 2H, t, *J*=7.3 Hz) was shown in the ^{1}H NMR spectrum. The chemical shifts and coupling patterns of H-6 to H-20 were comparable to those of **5** and **6**. The remaining resonances due to H-2 (δ 4.58, 1H, d, *J*=7.0 Hz), H-3 (δ 2.00, 1H, m, 3.17, t, *J*=13.2), and H-4 (δ 5.01, 1H, dd, *J*=13.2, 5.5 Hz) were similar to those of **3**. The octanoyloxyl group was determined to be positioned at C-4 by the HMBC correlation of H-4 to C-21 (δ 173.2). Therefore, compound (**7**) was concluded to be 3-deacetoxyviolide T.

The molecular formula of compound (**8**), $C_{28}H_{40}O_{12}$ indicated that **8** had four less CH_2 groups than **7**. The ^{1}H NMR spectrum was similar to that of **7**. However, the only difference between **8** and **7** in the ^{1}H NMR spectrum was that resonances due to butanoyl protons (δ 0.96, 3H, t, *J*=7.3 Hz; 1.65, 2H, m; 2.32, 2H, *J*=7.3 Hz) were observed instead of those of octanoyl protons in **7**. The butanoyloxyl group was positioned at C-4 on the basis of the HMBC correlation of H-4 (δ 5.02, 1H, dd, *J*=13.2, 5.3 Hz) to H-21 (δ 174.6). Thus, the structure was elucidated as **8**.

Comopund (**9**), violide V, $C_{34}H_{50}O_{14}$, was isomeric with **5**, and the ^{1}H NMR spectrum was fundamentally similar to that of **5**, though several chemical shifts were different to some degree. The octanoate group was proved to be located at the C-4 position from the HMBC correlation of H-4 (δ 5.04, 1H, d, *J*=11.2 Hz) to C-21 (δ 172.6). Therefore, the stereochemistry must be different from that of **5**. The stereochemistry except for the hydroxyl group at C-8 and H-20 (δ 1.27, 3H, s) was the same with that of **5**

on the basis the NOE analysis; H-2/H-4, H-10, H-3/H-7, H-20/H-15, H-12. H-18 (δ 1.58, 3H, s) indicated an NOE correlation with H-7 (δ 5.87, d, 1H, *J*=9.9 Hz), suggesting the β-orientation. The chemical shifts of H-3 (δ 5.72, 1H, br d, *J*=11.2 Hz) and H-7 were shifted downfield by 1.50 and 0.22 ppm, respectively, when compared to those of **5**, suggesting that the hydroxyl group at C-8 was in the proximity of H-3 and H-7 and hence in a β-situation. This was also supported by the similar chemical shifts of C-7 (δ 84.4), C-8 (δ 81.8), C-17 (δ 78.1), and C-18 (δ 16.6) in the ^{13}C NMR spectrum to those of briaexcavatolide K.[7] Thus, violide U was a 8,17-epimer of violide T.

The ^{1}H NMR spectrum of compound (**10**), $C_{34}H_{50}O_{14}$, indicated the presence of resonances due to three acetyl protons (δ 2.06, 2.21, 2.23, 3H, s, each) and octanoyl protons (δ 0.87, 3H, t, *J*=6.8 Hz; 1.29, 8H, overlapped, 1.63, 2H, m; 2.39, 2H, t, *J*=7.5 Hz). On the basis of ^{1}H-^{1}H COSY and NOE spectra, the substituent patterns and stereochemistry on the six-membered rings and 10-membered ring were readily assigned, which were similar to those of violide K (**16**)[5] except for an extra acetyl group. The octanoate group was elucidated to be located at C-4 on the basis of the HMBC correlation of H-4 (δ 4.84, 1H, d, *J*=10.4 Hz) and C-21 (δ 173.8). The extra acetyl group is, therefore, positioned at C-8 (δ 80.1) or C-17 (δ 84.9). The hydroxyl group at C-17 was assumed to be acetylated, since the chemical shifts of the C-17 and C-19 (δ 171.2) were shifted drastically downfield by 4.6 ppm and upfield by 5.0 ppm, respectively, when compared to those of **16**. The β-configuration of the hydroxyl group at C-8 was confirmed by the fact that the chemical shifts of H-6 in **10** and **5**-**8** containing a C-8 β-hydroxyl group appeared in the range of δ 5.65 to 5.82, whereas the corresponding chemical shift of **9** with a C-8 α-hydroxyl group was observed at δ 6.01. The acetoxyl group at C-17, whose chemical shift was determined by the HMBC correlation of the acetyl protons (δ 2.06, 3H, s) to C-8 and C-17, was proved to be in a β-configuration from the observation of an NOE between the acetyl protons and H-7 (δ 5.91, 1H, d, *J*=9.7 Hz). Therefore, violide Z was concluded to be C-17 acetylviolide K.

The ^{1}H NMR spectrum of compound (**11**), $C_{35}H_{52}O_{14}$, was similar to that of **10**, except that the acetyl group was replaced by a propionate group (δ 1.14, 3H, t, *J*=7.3 Hz; 2.29, 2H, m). The HMBC correlations from H-2 (δ 4.71, 1H, br s) and H-9 (δ 6.07, 1H, d, *J*=4.2 Hz) to acetyl carbonyls (δ 169.7 and 169.0), respectively, and from H-4 (δ 4.85, 1H, d, *J*=10.8 Hz) to C-21 (δ 173.8) suggested that the propionate group was positioned at C-17. Thus, compound (**11**) was C-17 propionylviolide K.

Compounds (**10**) and (**11**) were the first isolation of briaranes possessing an acyloxyl group at C-17, though briaranes with an ester moiety at C-2-C-4, C-9, C-11, C-12, and C-14 had been reproted.[1]

Cytotoxic activity tests of **2**-**8** against the growth for Vero and MDCK cells were performed (Table 3). Compound (**2**) exhibited mild cytotoxicity against both cells and somewhat weaker activity than its 12-*O*-acetyl analogue, briarlide F.[8] The activity of **3** was less than its 11,12-epimer, violide H (**13**).[5]

Compound (**4**) with a 11-β methyl and a 12β-hydroxyl moieties was inactive, while its related analogue with the same substituents on B ring as **4**, violide B (**14**) showed mild activity (CC_{50} 9.83 and 28.8 mg/mL for Vero and MDCK cells, respectively). Compounds (**5**-**8**) possessing a 8,17-dihydroxyl moiety were week or inactive as expected.[5]

Table 3. Cytotoxic activity (CC_{50} μg/mL) of **2**-**8**.

Compd	2	3	4	5	6	7	8
Vero	5.09	2.57	>100	39.5	>100	32.5	>100
DMCK	4.88	3.96	>100	55.3	>100	19.1	>100

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.

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

Extraction and Isolation. The organisms (wet weight: 7.6 kg) was chopped into small pieces and extracted with 30 L of MeOH at rt for a week. The MeOH extract (22 g) was suspended in H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried over Na_2SO_4, filtered, and evaporated to dryness (9.6 g). In the same way as above, the extraction of the organisms with MeOH was done two more times, each MeOH extract (62.5 g and 63.8 g) was suspended in H_2O, extracted with CH_2Cl_2 to give the organic extracts (12.0 g and 37.8 g), respectively. A portion (12 g) of the CH_2Cl_2 extract (54.4 g)[2] was absorbed on silica gel and subjected to chromatography on silica gel packed in hexane, fractions (200 mL) being collected as follows: 1-2 (CH_2Cl_2-hexane, 4:1), 3-4 (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 8-11 (9.5 g) were chromatographed over 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 (2.8 g), which was subjected to HPLC (ODS) with MeCN-H_2O (7:3) and MeCN-H_2O (2:3) yielded **1** (1.0 mg) and **2** (2.6 mg), respectively. From the fractions eluted with MeOH-CH_2Cl_2 (1:24 to 2:43) a residue (3.8 g) was obtained, which was further applied to chromatographed over silica gel MeOH-CH_2Cl_2 (2:43) and HPLC with MeOH-H_2O (11:9) to give **6** (0.8 mg) and **8** (1.0 mg).

A second portion (20.5 g) of the CH_2Cl_2 extract (54.4 g)[2] was treated in the same way as the above method. It was absorbed on silica gel and subjected to chromatography on silica gel packed in hexane,

fractions (150 mL) being collected as follows: 1-3 (CH_2Cl_2), 4-5 (MeOH-CH_2Cl_2, 1:49), 6-7 (MeOH-CH_2Cl_2, 1:19), 8-12 (MeOH-CH_2Cl_2, 1: 9), 13-17 (MeOH-CH_2Cl_2, 1:4), and 18-19 (MeOH). Fractions 9-12 (13.0 g) were chromatographed over silica gel with MeOH-CH_2Cl_2 (1:99) and HPLC with MeCN-H_2O (9:11), giving **3** (5.3 mg). Elution with MeOH-CH_2Cl_2 (1:49) gave a residue, which was purified by HPLC with MeCN-H_2O (3:7) to furnish **4** (0.5 mg). Compounds **5** (6.3 mg), **7** (2.9 mg), **9** (1.5 mg), **10** (3.8 mg), and **11** (2.2 mg) were isolated from the fractions eluted with MeOH-CH_2Cl_2 (1:24) followed by HPLC with MeCN-H_2O (11:9 to 43:57).

$C_{28}H_{41}O_{12}$, 569.2598).

Compound (1). Amorphous powder, $[\alpha]_D$ +26° (*c* 0.13, MeOH); IR (film) ν_{max} 3501, 1782, 1742, 1216 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 595.2761 $[M + H]^+$ (calcd for $C_{30}H_{43}O_{12}$, 595.2754).

Compound (2)(violide Q). Amorphous powder, $[\alpha]_D$ +44° (*c* 0.17, MeOH); IR (film) ν_{max} 3490, 1784, 1742, 1215 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 621.2892 $[M - H]^-$ (calcd for $C_{32}H_{45}O_{12}$, 621.2911).

Compound (3)(violide R). Amorphous powder, $[\alpha]_D$ +14° (*c* 0.08, MeOH); IR (film) ν_{max} 3503, 1780, 1738, 1215 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 607.3120 $[M + H]^+$ (calcd for $C_{32}H_{47}O_{11}$, 607.3118).

Compound (4)(violide S). Amorphous powder, $[\alpha]_D$ +51° (*c* 0.11, MeOH); IR (film) ν_{max} 3355, 1792, 1748, 1221 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 565.2290 $[M + H]^+$ (calcd for $C_{28}H_{37}O_{12}$, 565.2285).

Compound (5)(violide T). Amorphous powder, $[\alpha]_D$ +75° (*c* 0.32, MeOH); IR (film) ν_{max} 3445, 1780, 1748, 1227 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 683.3295 $[M + H]^+$ (calcd for $C_{34}H_{51}O_{14}$, 683.3279).

Compound (6)(violide U). Amorphous powder, $[\alpha]_D$ +76° (*c* 0.09, MeOH); IR (film) ν_{max} 3310, 1775, 1746, 1235 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 649.2485 $[M + Na]^+$ (calcd for $C_{32}H_{42}O_{14}Na$, 649.2472).

Compound (7). Amorphous powder, $[\alpha]_D$ -5° (*c* 0.15, MeOH); IR (film) ν_{max} 3420, 1780, 1740, 1242 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 625.3216 $[M + H]^+$ (calcd for $C_{32}H_{49}O_{12}$, 625.3224).

Compound (8). Amorphous powder, $[\alpha]_D$ -6° (*c* 0.14, MeOH); IR (film) ν_{max} 3517, 1784, 1728, 1258 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 569.2605 $[M + H]^+$ (calcd for $C_{28}H_{41}O_{12}$, 569.2598).

Compound (9)(violide V). Amorphous powder, $[\alpha]_D$ +50° (*c* 0.08, MeOH); IR (film) ν_{max} 3445, 1786, 1746, 1225 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 681.3107 $[M - H]^-$ (calcd for $C_{34}H_{49}O_{14}$, 681.3123).

Compound (10): Amorphous powder, $[\alpha]_D$ +5° (*c* 0.19, MeOH); IR (film) ν_{max} 3482, 1790, 1744, 1233 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 683.3260 $[M + H]^+$ (calcd for $C_{34}H_{51}O_{14}$, 683.3279).

Compound (11): Amorphous powder, $[\alpha]_D$ +4° (*c* 0.11, MeOH); IR (film) ν_{max} 3480, 1788, 1744, 1223 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); HRFABMS *m/z* 695.3288 $[M - H]^-$ (calcd for $C_{35}H_{51}O_{14}$, 695.3279).

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