HETEROCYCLES, Vol. 53, No. 8, 2000, pp. 1789 - 1792, Received, 14th April, 2000

VIOLIDES N-P, NEW BRIARANE DITERPENES FROM A GORGONACEAN *BRIAREUM* SP.[1]

Tetsuo Iwagawa,*[a] Tetsushi Hirose,[a] Keita Takayama,[a] Hiroaki Okamura,[a] Munehiro Nakatani,[a] Matsumi Doe[b], and Kaoru Takemura[c]

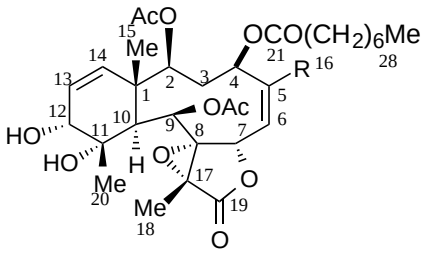
[a] Faculty of Science, Kagoshima University, Kagoshima 890-0065, Japan

[b] Faculty of Science, Osaka City University, Osaka, 558-0022, Japan

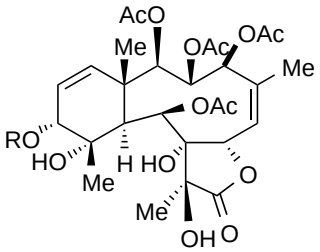
[c] Sankei Kagaku Co. Ltd., Kagoshima 890-0112, Japan

Abstract- Three new briarane diterpenes, violides N-P, have been isolated from a Gorgonacean *Briareum* sp. and the structures elucidated. Violide N exhibited moderate cytotoxicity toward Vero and MDCK cells.

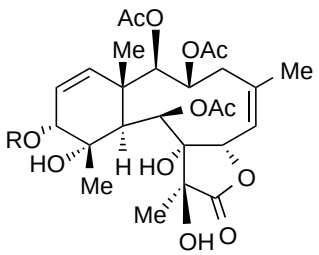
Gorgonacean *Briareum* sp. have proved to be a rich source of highly oxidized diterpenes, possessing the briarane skeleton. Many of them exhibited interesting bioactivities such as cytotoxic, anti-flammatory, and antiviral activity.[2] In a previous investigation of bioactive metabolites from *Briareum* sp., [1,3-4] collected in the area of Bonotsu, Kagoshima Prefecture, we isolated 13 new briarane diterpenes, violides A-M. Some of them exhibited cytotoxic activity against the growth of Vero and MDCK cells.[1] Our continuing examination of the dichloromethane extract has led to the isolation of three new briarane diterpenes, violides N (**1**)-P (**3**).



1 R=CH_2OH



2 R=Ac



3 R=Ac

4 R=Me

5 R=H

6 R=H

The molecular formula of violide N (**1**) was determined as $C_{32}H_{46}O_{12}$ by the HREIMS [m/z 623.3053 $(M + H)^+$, Δ -1.5 mmu]. The IR spectrum indicated absorption bands for a hydroxyl group (3428 cm^{-1}), γ-lactone (1782 cm^{-1}), and ester carbonyl (1740 cm^{-1}). The ^1H NMR spectrum of **1** (Table 1) was similar to that of violide H (**4**)[1], except that oxymethylene protons appeared at δ 4.33 (br t-like, *J*=7.0 Hz) instead of methyl protons at C-5, and the chemical shift of H-6 (δ 5.78, 1H, d, *J*=9.0 Hz) was shifted downfield by 0.33 ppm. This suggested that the methyl group at C-5 in **4** was oxidized to the hydroxymethylene group. In the ^{13}C NMR spectrum (Table 1), the presence of a *n*-octanoate group was confirmed by resonances due to a methyl carbon (δ 14.1, q), six methylene carbons (δ 22.6-34.3, t), and an ester carbonyl carbon (δ 173.2, s). This was also deduced from the molecular formula and the signals in the ^1H NMR spectrum.

Table 1. ^{1}H NMR and ^{13}C NMR Spectral Data of **1-3** in $CDCl_3$.[a]

No.	**1**		**2**		**3**	
1		46.7		46.8		46.7
2	4.62 (br d)	78.0	4.62 (d, 1.8)	77.1	4.72 (br s)	77.8
3	α 3.01 (dd, 12.8, 14.7)	38.3	6.23 (dd, 1.8, 10.3)	71.0	5.75 (br dd, 4.9, 13.0)	71.4
	β ca 2.10 (ov.)					
4	5.01 (dd, 5.1, 12.8)	69.5	5.18 (d, 10.3)	76.4	α 1.96 (dd, 12.8, 13.0)	34.4
					β 3.01 (br dd, 4.9, 13.0)	
5		146.4		138.0		137.9
6	5.78 (d, 9.0)	123.7	5.85 (br d, 9.9)	127.6	5.69 (br d, 9.9)	123.2
7	5.76 (d, 9.0)	73.4	6.08 (d, 9.9)	77.6	5.92 (d, 9.9)	78.9
8		71.1		78.6		79.0
9	5.95 (d, 3.7)	65.8	6.15 (d, 4.4)	65.3	6.10 (d, 4.4)	65.7
10	2.49 (d, 3.7)	43.3	2.99 (d, 4.4)	39.2	2.97 (d, 4.4)	39.4
11		73.6		76.2		75.9
12	3.66 (d, 6.1)	70.4	5.03 (d, 6.2)	72.0	5.03 (d, 6.2)	72.2
13	5.82 (br dd, 6.1, 10.2)	124.9	5.73 (dd, 6.2, 10.1)	121.2	5.70 (dd, 6.2, 10.1)	120.8
14	5.36 (d, 10.2)	138.5	5.68 (d, 10.1)	142.2	5.61 (d, 10.1)	141.9
15	1.18 (s)	15.2	1.13 (s)	15.5	1.11 (s)	15.4
16	4.33 (br t-lke, 7.0)	65.9	2.21 (br s)	25.8	2.02 (br s)	27.4
17		64.4		80.1		80.3
18	1.70 (s)	9.8	1.45 (s)	16.9	1.44 (s)	16.9
19		171.0		175.8		175.7
20	1.15 (s)	21.4	1.49 (s)	23.7	1.47 (s)	23.7
MeCO	2.13, 2.24	21.1, 21.6	2.03, 2.07, 2.10, 2.15, 2.21	20.6, 20.9 x 3, 22.0	2.01, 2.10 x 2, 2.18	20.8, 21.0
						21.2, 21.9
MeCO		168.1, 170.6		168.8, 169.3, 170.3		170.0, 170.2
				172.0, 172.6		171.1, 172.7
n-C_7H_{15}OCO	0.88 (t, 6.5, H-28)	14.1, 22.6				
	1.28 (m, H-24 - 27)	24.8, 28.9				
	1.60 (m, H-23)	29.0, 31.6				
	2.30 (t, 7.5, H-22)	34.3, 173.2				

[a] Chemical shift values are in ppm from TMS, and *J* values (in Hz) are presented in parentheses.

The *n*-octanoate group was established to be located at C-4 on the basis of the correlation of H-4 (δ 5.01, 1H, dd, *J*=5.1, 12.8 Hz) and C-21 (δ 173.2) in the HMBC experiments. The relative stereochemistry of the chiral center was determined by the similar coupling patterns in the ^{1}H NMR spectrum and NOE correlations to those of **4** (Table 2). This is a rare example of briarane diterpene possessing an oxidized moiety at C-16.[5-6]

The ^{1}H NMR spectrum of violide O (**2**), $C_{30}H_{40}O_{15}$, was similar to that of violide J (**5**),[1] except that resonances due to an additional acetyl protons were observed (δ 2.10, 3H, s) and the chemical shift of H-12 (δ 5.03, 1H, d, *J*=6.2 Hz) was shifted downfield by 1.18 ppm, compared to that of **5**. The stereochemistry was determined on the basis of similarity of the coupling patterns and chemical shifts in the ^{1}H NMR spectrum and the NOE correlations between **2** and **5** (Table 2). Therefore, violide O was assigned as 12-*O*-acetylviolide J.

Table 2. NOE Spectral Data for **1-3**.

Proton No.	**1**	**2**	**3**a
2	H-10, H-16	H-4, H-10, H-16	H-10, H-16
3	H-7, H-15	H-7, H-15	H-7, H-15
4	H-16	H-16	H-16?
6		H-16	H-16
7	H-3	H-3	H-3
9	H-18, H-20	H-15, H-18, H-20	H-18, H-20
10	H-2	H-2	H-2
12	H-20	H-20	H-20
14	H-15	H-15	H-15
15	H-3, H-9, H-14, H-20	H-3, H-9, H-14, H-20	H-3, H-14, H-20
16	H-2, H-4	H-2, H-4, H-6	H-2, H-4, H-6?
18	H-9	H-9	H-9
20	H-9, H-12, H-15	H-9, H-12, H-15	H-9, H-12, H-15

[a]The signals of H-4 and H-16 were overlapped with each other.

The ^{1}H NMR data of violide P (**3**), $C_{28}H_{38}O_{13}$, indicated that resonances due to additional acetyl protons (δ 2.10, 3H, s) appeared and H-12 (δ 5.03, 1H, d, *J*=6.2 Hz) was shifted downfield by 1.19 ppm, compared to that of violide M (**6**).[1] Thus, the acetyl group was concluded to be located at C-12. On the basis of the signal patterns, chemical shifts in the ^{1}H NMR spectra and NOE correlations (Table 2), the stereochemisty of violide P was determined to have the structure (**3**). Therefore, violide P was assigned as 12-*O*-acetylviolide M.

Violides O and P are another example of briaranes with 8, 17-dihydroxyl groups which were isolated for the first time from the same animals, collected in the area of Bonotsu, Kagoshima Prefecture.[1]

Violide N exhibited cytotoxity against the growth for Vero and MDCK cells with a CC_{50} of 3.3 μg/mL and 3.2 μg/mL, respectively. Compounds (**2**) and (**3**) containing 8,7-dihydroxyl groups, were inactive against the both cells at 100 μg/mL as well as violides J-M.[1] The cytotoxity of **1** showed less active than that of **4**, indicating that oxidation of the methyl group at C-16 to the hydroxymethylene group reduced the activity.

EXPERIMENTAL

General Experimental Procedures. Melting points were uncorrected. Optical rotations were obtained at 22° C on a JASCO DIP-370S polarimeter. UV and IR spectra were recorded on UV-210 and MASCO FT/IR 5300 spectrometers, respectively. NMR spectra were recorded with either a 400 MHz JEOL or a VARIAN UNITY-500 NMR instrument using TMS as internal standard and $CDCl_3$ as solvents. MS spectra were obtained with a JEOL XD-303 instrument.

Extraction and Isolation. The procedures were described earlier[1]. 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), 3-4 (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 8-10 (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 eluate eluted with MeOH-CH_2Cl_2 (1:19 to 1:10) gave a residue (2.1 g), which was again applied to silica gel chromatography MeOH-CH_2Cl_2 (1:24). The elute (68.4 mg) was subjected to HPLC (ODS) with MeOH-H_2O (3:2 to 33:67) to give **1** (2.0 mg), **2** (2.9 mg), and **3** (3.6 mg) in order of polarity.

Violide N (1): Amorphous, $[\alpha]_D$ -2.3° (*c* 0.1, MeOH); UV λmax (log ε) 206 nm (3.85); IR νmax (film) 3428, 1782, 1740, 1213 cm^{-1}); ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); (+)-HRFBMS *m/z* 623.3053 $[M + H]^+$ (calcd for $C_{32}H_{47}O_{12}$ 623.3068).

Violide O (2): Amorphous, $[\alpha]_D$ +31.0° (*c* 0.20, MeOH); UV λmax (log ε) 206 nm (3.79); IR νmax (film) 3354, 1746, 1229 cm^{-1}); ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); (-)-HRFBMS *m/z* 639.2275 $[M - H]^-$ (calcd for $C_{30}H_{39}O_{15}$ 639.2289).

Violide P (3): Amorphous, $[\alpha]_D$ -14.6° (*c* 0.18, MeOH); UV λmax (log ε) 206 nm (3.72); IR νmax (film) 3422, 1741, 1236 cm^{-1}); ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); (+)-HRFBMS *m/z* 583.2407 $[M + H]^+$ (calcd for $C_{28}H_{39}O_{13}$ 583.2391).

REFERENCES AND NOTES

1. New briarane diterpenes from *Briareum* sp., collected at Bonotsu, Kagoshima Prefecture. 4. For part 3. see: T. Iwagawa, K. Takayama, H. Okamura, M. Nakatani, M. Doe, K. Takemura, and M. Shiro *Heterocycles*, **1999**, *51*, 2619.
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. Doe, K. Shibata, M. Shiro, Heterocycles, **1998**, *48*, 123.
4. T. Iwagawa, K. Takayama H. Okamura, M. Nakatani, and M. Doe *Heterocycles*, **1999**, *51*, 1653.
5. E. O. Pordesimo, F. J. Schimitz, L. S. Ciereszko, M. B. Hossain, and D. van der Helm *J. Org. Chem*., **1991**, *56*, 2344.
6. D. Maharaj, K. O. Pascoe, and W. F. Tinto *J. Nat. Prod*., **1999**, *62*, 313.