

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

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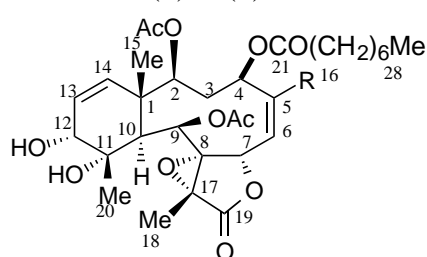
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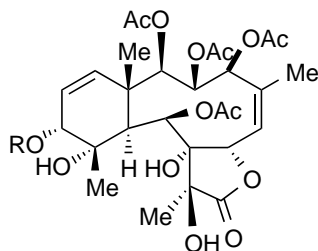
Abstract- Three new briarane diterpenes, violides N-P, have been isolated from a Gorgonacean *Briareum* sp. and the structures elucidated. Violide N exhibited moderate cytotoxicity toward Vero and MDCK cells.

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-inflammatory, 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).



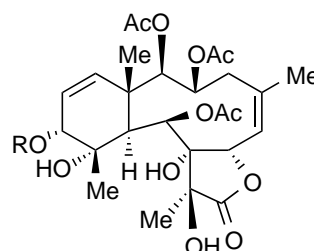
1 R=CH₂OH

4 R=Me



2 R=Ac

5 R=H



3 R=Ac

6 R=H

The molecular formula of violide N (1) was determined as C₃₂H₄₆O₁₂ 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 ¹³C 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. ¹H NMR and ¹³C NMR Spectral Data of **1-3** in CDCl₃.^a

No.	1	2	3
1		46.7	46.8
2	4.62 (br d)	78.0	77.1
3	α 3.01 (dd, 12.8, 14.7)	38.3	71.0
	β ca 2.10 (ov.)		
4	5.01 (dd, 5.1, 12.8)	69.5	76.4
5		146.4	138.0
6	5.78 (d, 9.0)	123.7	127.6
7	5.76 (d, 9.0)	73.4	77.6
8		71.1	78.6
9	5.95 (d, 3.7)	65.8	65.3
10	2.49 (d, 3.7)	43.3	39.2
11		73.6	76.2
12	3.66 (d, 6.1)	70.4	72.0
13	5.82 (br dd, 6.1, 10.2)	124.9	121.2
14	5.36 (d, 10.2)	138.5	142.2
15	1.18 (s)	15.2	15.5
16	4.33 (br t-lke, 7.0)	65.9	25.8
17		64.4	80.1
18	1.70 (s)	9.8	16.9
19		171.0	175.8
20	1.15 (s)	21.4	23.7
<u>MeCO</u>	2.13, 2.24	21.1, 21.6	20.6, 20.9 x 3, 22.0
<u>MeCO</u>		168.1, 170.6	168.8, 169.3, 170.3
<u>n-C₇H₁₅OCO</u>	0.88 (t, 6.5, H-28)	14.1, 22.6	172.0, 172.6
	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	
			46.7
			77.8
			71.4
			34.4
			137.9
			123.2
			78.9
			79.0
			65.7
			39.4
			75.9
			72.2
			120.8
			141.9
			15.4
			27.4
			80.3
			16.9
			175.7
			23.7
			20.8, 21.0
			21.2, 21.9
			170.0, 170.2
			171.1, 172.7

^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 ^1H 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 ^1H NMR spectrum of violide O (**2**), $\text{C}_{30}\text{H}_{40}\text{O}_{15}$, 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 ^1H 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	3a
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

^aThe signals of H-4 and H-16 were overlapped with each other.

The ^1H NMR data of violide P (**3**), $\text{C}_{28}\text{H}_{38}\text{O}_{13}$, 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 ^1H NMR spectra and NOE correlations (Table 2), the stereochemistry 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 cytotoxicity against the growth for Vero and MDCK cells with a CC_{50} of 3.3 $\mu\text{g}/\text{mL}$ and 3.2 $\mu\text{g}/\text{mL}$, respectively. Compounds (**2**) and (**3**) containing 8,7-dihydroxyl groups, were inactive against the both cells at 100 $\mu\text{g}/\text{mL}$ as well as violides J-M.¹ The cytotoxicity 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 CDCl₃ as solvents. MS spectra were obtained with a JEOL XD-303 instrument.

Extraction and Isolation. The procedures were described earlier¹. Portion (5 g) of the CH₂Cl₂ 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 (CH₂Cl₂-hexane, 4:1), 3-4 (CH₂Cl₂), 5-6 (MeOH-CH₂Cl₂, 1:49), 7-8 (MeOH-CH₂Cl₂, 1:19), 9-10 (MeOH-CH₂Cl₂, 1:9), 11-12 (MeOH-CH₂Cl₂, 1:4), and 13-14 (MeOH). Fractions 8-10 (2.1 g) were chromatographed on silica gel using MeOH and CH₂Cl₂, increasing the proportion of MeOH to elute the fractions from the column. The eluate eluted with MeOH-CH₂Cl₂ (1:19 to 1:10) gave a residue (2.1 g), which was again applied to silica gel chromatography MeOH-CH₂Cl₂ (1:24). The elute (68.4 mg) was subjected to HPLC (ODS) with MeOH-H₂O (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, [α]_D -2.3° (*c* 0.1, MeOH); UV λ _{max} (log ϵ) 206 nm (3.85); IR ν _{max} (film) 3428, 1782, 1740, 1213 cm⁻¹; ¹H NMR (see Table 1); ¹³C NMR (see Table 2); (+)-HRFBMS *m/z* 623.3053 [M + H]⁺ (calcd for C₃₂H₄₇O₁₂ 623.3068).

Violide O (2): Amorphous, [α]_D +31.0° (*c* 0.20, MeOH); UV λ _{max} (log ϵ) 206 nm (3.79); IR ν _{max} (film) 3354, 1746, 1229 cm⁻¹; ¹H NMR (see Table 1); ¹³C NMR (see Table 2); (-)-HRFBMS *m/z* 639.2275 [M - H]⁻ (calcd for C₃₀H₃₉O₁₅ 639.2289).

Violide P (3): Amorphous, [α]_D -14.6° (*c* 0.18, MeOH); UV λ _{max} (log ϵ) 206 nm (3.72); IR ν _{max} (film) 3422, 1741, 1236 cm⁻¹; ¹H NMR (see Table 1); ¹³C NMR (see Table 2); (+)-HRFBMS *m/z* 583.2407 [M + H]⁺ (calcd for C₂₈H₃₉O₁₃ 583.2391).

REFERENCES AND NOTES

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