Biselides A and B, Novel Macrolides from the Okinawan Ascidian Didemnidae sp.

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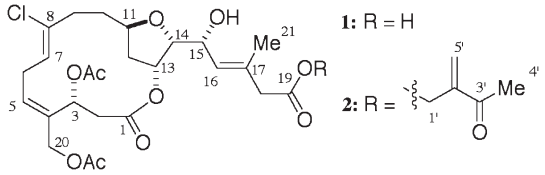
Two new macrolides, biselides A (**1**) and B (**2**), were isolated from the Okinawan ascidian Didemnidae sp. Their structures were determined by spectroscopic analysis.

As part of our continuing chemical studies of marine organisms,[1] we examined the constituents of the Okinawan ascidian Didemnidae sp., whose crude organic extract showed toxicity against brine shrimp. Bioassay-guided fractionation of the extract led to the isolation of toxic haterumalide NA[2] and two new congeners, biselides A (**1**) and B (**2**). We report here the isolation and structural determination of **1** and **2**.



The Okinawan ascidian Didemnidae sp. (1.7 kg), collected at Bise, Okinawa Prefecture, was extracted with methanol (3 L) for 5 days. The extract was filtered, concentrated, and partitioned between EtOAc and H_2O. The EtOAc-soluble material was further partitioned between 90% aq. MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to fractionation guided by toxicity against brine shrimp with column chromatography (silica gel, $CHCl_3$–MeOH; ODS silica gel, MeOH–H_2O) and reversed-phase HPLC (Develosil ODS-HG-5, MeOH–H_2O–TFA) to give biselides A (**1**) [1.2 mg], B (**2**) [160 μg] and haterumalide NA as colorless oils. Interestingly, haterumalide NA showed toxicity against brine shrimp with an LD_{50} of 0.6 μg/mL, while **1** showed no toxicity against brine shrimp even in 50 μg/mL. Owing to lack of samples, toxicity against brine shrimp of **2** could not be examined.

The molecular formula of **1** was found to be $C_{25}H_{33}ClO_{10}$ by ESIMS (*m*/*z* 551.1654, calcd for $C_{25}H_{33}ClO_{10}Na$ $[M + Na]^+$ 551.1660). The NMR data for **1** are summarized in Table 1. The ^1H NMR spectrum of **1** showed the presence of three methyl groups connected to quaternary sp^2 carbons (δ 1.82, 2.02, and 2.07). In the ^{13}C NMR spectrum, 25 carbon signals were observed, including four carbonyl carbons (δ 169.4, 171.1, 172.5, and 175.4), six olefinic carbons (δ 125.8, 130.7, 133.6, 133.9, 135.0, and 135.9), and three methyl carbons (δ 17.3, 20.9, and 21.0). The remaining carbon signals were assigned to seven methylenes and five methines, based on an HMQC experiment. A detailed analysis of the COSY spectra of **1** allowed three partial structures, C2–C3, C20–C5–C7, and C9–C16, to be constructed, as shown in Figure 1. The connectivities of these partial structures were clarified by HMBC correlations: H2/C1, H20/C3, H20/C4, H20/C5, H6/C8, H7/C8, and H9/C8. Furthermore, the HMBC correlations H21/C16, H21/C17, H21/C18, and H18/C19 suggested the connectivities of C16, 17, 18, 19, and 21. Consequently, the entire carbon chain was assembled as shown in Figure 1, and all proton and carbons were assigned as shown in Table 1. The presence of a tetrahydrofuran ring was suggested by the characteristic chemical shifts of C11 (δ 78.1) and C14 (δ 84.5).[3] The HMBC correlations H3/C22, H23/C22, H20/C24, and H25/C24 suggested the existence of two acetoxy groups at C3 and C20. Although no additional connectivities were obtained from the NMR analysis, **1** contains a lactone ring based on its molecular formula and degree of unsaturation. Considering the chemical shift of H13, the lactone ring might be formed between C1 and C13 or C19 and C13. A detailed analysis of the chemical shift and coupling constants of ^1H NMR of **1** suggested that **1** could be an analogue of haterumalide NA,[2] i.e., **1** could be a 14-membered macrolide and the chlorine atom could be at C8. The geometry of the three olefins in **1** were clarified to be 4*Z*, 7*Z*, and 16*E* by NOESY experiments (Figure 1). Thus, the gross structure of biselide A (**1**) was determined to be as shown in Figure 1.

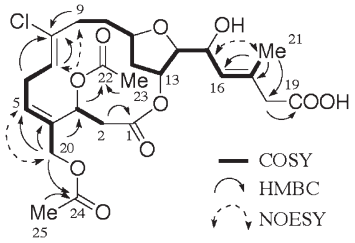


Figure 1. Partial structures of **1**, based on 2D NMR correlations.

The relative stereochemistry of **1** was determined as follows. A plausible conformation of **1** with the important NOESY correlations is shown in Figure 2. In the tetrahydrofuran ring, NOESY correlations H11/H12b, H12a/H13, and H13/H14 suggested that the relative stereochemistries at C11, C13, and C14 were 11*R**, 13*R**, and 14*R**. The magnitude of $^3J_{H2b\text{-}H3} = 5.2$ Hz and $^3J_{H2a\text{-}H3} = 11.1$ Hz suggested that H-2b and H-3 were located in a *gauche* arrangement while H-2a and H-3 were located in an *anti* arrangement. In addition, NOESY correlations H2a/H20, H2b/H3, H3/H6b, H5/H6a, H5/H7, H6a/H7, H7/H12b, H11/H12b, H12a/H13, and H13/H14 suggested the plausible conformation of the macro ring in **1** as shown in Figure 2. Therefore, the stereochemistry of C3 was determined to be 3*R**. The relative stereochemistry at C15 was determined as follows. The magnitude of $^3J_{H14\text{-}H15} = 8.6$ Hz suggested that H-14 and H-15 were located in an *anti* arrangement. Therefore, based on the presumption that the alkyl chain of **1** may have a zigzag conformation, we deduced that the relative stereochemistry at C15 was 15*R**. Thus, the stereochemistries of **1** and haterumalide NA could be superimposed on each other. This result was also supported by the fact that the chemical shifts and coupling constants of NMR in **1** closely resembled those of haterumalide NA.

The molecular formula of biselide B (**2**) was found to be

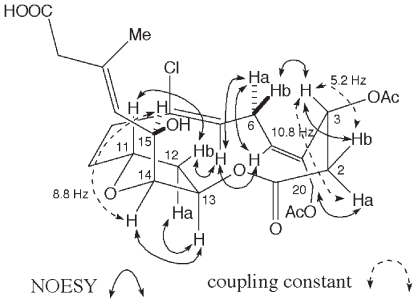


Figure 2. Relative stereochemistry of **1**, based on NOESY correlations.

Table 1. NMR data for **1** and **2** in CD_3OD

Biselide A (**1**)			Biselide B (**2**)	
No.	^1H [a,c]	^{13}C [b]	No.	^1H [c,d]
1		169.4	1	
2a	2.83 dd (12.0, 11.1)	38.7	2a	2.83 dd (12.5, 11.2)
2b	2.88 dd (12.0, 5.2)		2b	2.88 dd (12.5, 5.0)
3	5.84 dd (11.1, 5.2)	67.8	3	5.83 dd (11.2, 5.0)
4		135.0	4	
5	6.08 dd (11.1, 7.1)	133.9	5	6.08 dd (10.9, 7.3)
6a	2.61 m	27.7	6a	2.61 m
6b	3.55 m		6b	3.54 m
7	5.32 m	125.8	7	5.32 m
8		133.6	8	
9a	2.31 m	35.5	9a	2.30 m
9b	2.45 m		9b	2.45 m
10a	1.39 m	29.0	10a	1.39 m
10b	2.28 m		10b	2.27 m
11	3.94 m	78.1	11	3.92 m
12a	1.53 m	38.8	12a	1.53 m
12b	2.09 m		12b	2.07 m
13	5.30 m	76.7	13	5.30 m
14	3.91 dd (8.6, 3.7)	84.5	14	3.90 dd (8.7, 3.7)
15	4.53 t (8.6)	66.5	15	4.53 t (8.7)
16	5.38 d (8.6)	130.7	16	5.39 d (8.7)
17		135.9	17	
18	3.04 br s 2H	45.7	18	3.12 br s 2H
19		175.4	19	
20a	4.70 d (12.9)	65.5	20a	4.70 d (13.0)
20b	4.96 d (12.9)		20b	4.96 d (13.0)
21	1.82 br s 3H	17.3	21	1.81 br s 3H
22		171.1	22	
23	2.02 s 3H	21.0	23	2.02 s 3H
24		172.5	24	
25	2.07 s 3H	20.9	25	2.06 s 3H
			1a′	4.76 d (13.9)
			1b′	4.82 d (13.9)
			2′	
			3′	
			4′	2.36 s 3H
			5a′	6.10 s
			5b′	6.31 s

[a]Recorded at 500 MHz. [b]Recorded at 125 MHz. [c]Coupling constants (Hz) are in parentheses. [d]Recorded at 600 MHz. Signal of hydroxy group was not observed.

$C_{30}H_{39}ClO_{11}$ by ESIMS (m/z 633.2071, calcd for $C_{30}H_{39}ClO_{11}$-Na $[M + Na]^+$ 633.2079). In normal and reversed-phase chromatography, **2** was much less polar than **1**. The ^1H NMR data for **2** are summarized in Table 1. A detailed analysis of the COSY spectra of **2** led to three partial structures, C2–C3, C5–C7, and C9–C16, the same as in **1**. The chemical shifts and coupling constants of protons of **2** closely resembled those of **1**, except for H1′, H4′, and H5′. This result and its chromatographic behavior suggested that **2** has the same macrolide structure and that **2** has an alkyl group at the C19 carboxyl. In the alkyl group in **2**, the chemical shifts and coupling constants of the protons closely resembled those of haterumalide B.[4] This result suggested that **2** has the same alkyl group as haterumalide B. Therefore, the relative stereostructure of biselide B (**2**) was determined to be as shown in formula **2**.

The absolute stereochemistries of biselides A (**1**) and B (**2**) were deduced as shown in formulas **1** and **2**, respectively, based on the similarity of their NMR and CD data[5] to those of haterumalide NA.[2] Thus, the absolute stereochemistries of the five stereocenters in **1** and **2** were determined to be 3*R*, 11*R*, 13*R*, 14*R*, and 15*R*.

In conclusion, the novel macrolides biselides A (**1**) and B (**2**) were isolated from the Okinawan ascidian Didemnidae sp. The structures of these biselides were determined based on their 2D NMR spectra. Interestingly, the structurally related haterumalide NA,[2] haterumalide B,[4] and oocydin A[6] were isolated from an Okinawan sponge, an Okinawan ascidian, and a South American epiphyte, respectively. Further investigations of their biological activities and biosynthetic pathways are in progress.

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References and Notes

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