Isolation and Structures of Attenols A and B. Novel Bicyclic Triols from the Chinese Bivalve *Pinna attenuata*

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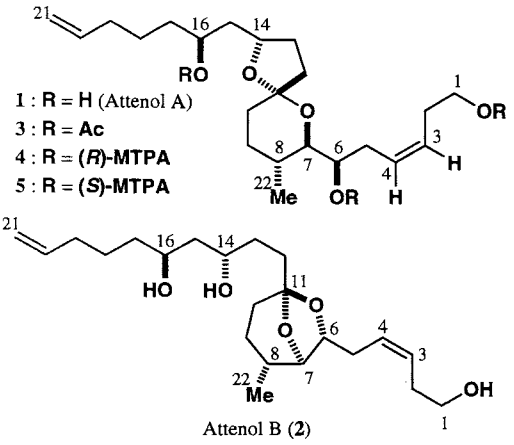
Novel bicyclic triols, attenols A (**1**) and B (**2**), were isolated from the Chinese bivalve *Pinna attenuata*. The absolute stereostructures of **1** and **2** were determined by spectroscopic analysis and the modified Mosher's method. These attenols exhibited moderate cytotoxicity against P388 cells.

Recently, we reported the isolation and structure determination of pinnatoxins A[1] and D[2] from the Okinawan bivalve *Pinna* sp. In a continuation of this work, we have isolated attenols A (**1**) and B (**2**), novel bicyclic triols, from the Chinese bivalve *Pinna attenuata*. In this paper, we report the isolation and structure determination of **1** and **2**.



The CH_2Cl_2-soluble fraction of the aqueous EtOH extract of the Chinese bivalve *Pinna attenuata* was separated by column chromatography (SiO_2 and ODS), and reversed phase HPLC to give attenols A (**1**) and B (**2**).[3] They exhibited moderate cytotoxicity against P388 cells, with IC_{50} values of 24 and 12 μg/mL, respectively.

The molecular formula of **1** was determined to be $C_{22}H_{38}O_5$ by HRFABMS (*m/z* 405.2594, calcd for $C_{22}H_{38}NaO_5$ $[M+Na]^+$ 405.2617). The IR spectrum showed a band at 3460 cm^{-1} that was assigned to a hydroxy functionality. The NMR data for **1** and **2** are summarized in Table 1. The ^1H NMR, ^{13}C NMR, and HSQC spectra of **1** showed the presence of one methyl carbon connected to a methine carbon, eleven sp^3-methylene carbons, five sp^3-methine carbons, one quaternary carbon, and four olefinic carbons (δ_c 114.6, 128.0, 129.6, 138.7). The carbon chemical shifts of **1** suggested that one methylene carbon (δ_c 61.9) and four methine carbons (δ_c 69.6, 70.1, 78.0, 78.0) were connected to an oxygen atom and that the quaternary carbon (δ_c 106.4) was an acetal carbon. Since compound **1** had two carbon-carbon double bonds and no carbonyl carbon, **1** was confirmed to be bicyclic based on its molecular formula and degree of unsaturation.

Table 1. NMR Data for Attenols A (**1**) and B (**2**) in $CDCl_3$

	Attenol A (**1**)		Attenol B (**2**)	
Atom	^1H (ppm)	^{13}C (ppm)	^1H (ppm)	^{13}C (ppm)
1a	3.65 m[a,e]	61.9 t[b,f]	3.63 m[c,e]	61.9 t[d,f]
1b	3.65 m		3.63 m	
2a	2.29 m	30.9 t	2.31 m	31.3 t
2b	2.41 m		2.39 m	
3	5.54 m	128.0 d	5.53 m	128.5 d
4	5.68 m	129.6 d	5.54 m	127.9 d
5a	2.12 m	33.0 t	2.31 m	33.7 t
5b	2.51 br dt (14.8, 8.8)		2.39 m	
6	3.72 m	70.1 d	4.09 t (6.7)	80.1 d
7	3.31 dd (1.2, 10.4)	78.0 d	3.92 s	83.1 d
8	1.74 m	30.4 d	1.67 m	31.2 d
9a	1.50 m	29.0 t	1.34 dd (5.7, 15.2)	23.1 t
9b	1.65 m		2.02 m	
10a	1.64 m	33.9 t	1.51 m	30.3 t
10b	1.75 m		1.68 m	
11		106.4 s		109.6 s
12a	1.70 m	38.5 t	1.85 m	34.5 t
12b	2.02 m		1.90 m	
13a	1.84 m	30.8 t	1.61 m	30.3 t
13b	2.02 m		1.81 m	
14	4.31 m	78.0 d	3.95 m	70.2 d
15a	1.72 m	43.6 t	1.58 m	42.5 t
15b	1.72 m		1.65 m	
16	3.83 m	69.6 d	3.93 m	69.2 d
17a	1.50 m	36.6 t	1.43 m	36.9 t
17b	1.50 m		1.54 m	
18a	1.43 m	25.1 t	1.40 m	25.0 t
18b	1.56 m		1.53 m	
19a	2.09 m	33.7 t	2.08 m	33.7 t
19b	2.09 m		2.08 m	
20	5.81 ddt (10.2, 17.2, 6.8)	138.7 d	5.81 ddt (10.1, 17.2, 6.8)	138.8 d
21a	4.95 br d (10.2)	114.6 t	4.95 br d (10.1)	114.5 t
21b	5.01 br d (17.2)		5.01 br d (17.2)	
22	0.87 d 3H (6.4)	17.3 q	1.12 d 3H (7.0)	16.9 q

[a]Recorded at 400 MHz. [b]Recorded at 100 MHz. [c]Recorded at 600 MHz. [d]Recorded at 150 MHz. [e]Coupling constants (Hz) are in parentheses. Signals of hydroxy groups were not observed. [f]Multiplicity was based on the HSQC spectrum.

A detailed analysis of the phase-sensitive DQF-COSY and HOHAHA spectra of **1** allowed two partial structures, C1-C9 and C12-C21, to be constructed (Figure 1). The HMBC correlations H10/C11, H12/C11, and H13/C11 suggested that C10 and C11 were connected. The remaining connection between C9 and C10 was obvious based on a consideration of their chemical shifts (δ_c 29.0 and 33.9 ppm, respectively) and the molecular formula of **1**. The locations of three hydroxy groups in **1** were determined by the down-field shifts observed for H1 (δ

3.65→4.07), H6 (δ 3.72→5.12), and H16 (δ 3.83→5.00) in the ^{1}H NMR spectrum of triacetate **3**, which was prepared by acetylation of **1**. Therefore, the remaining C7 and C14 oxygens were ethereal oxygens. Furthermore, the stereochemistry of the C3 olefin in **1** was clarified to be 3*Z* by the coupling constant between H3 and H4 (10.4 Hz) and by an NOE experiment. Thus, the gross structure of attenol A (**1**) was determined as shown in Figure 1.

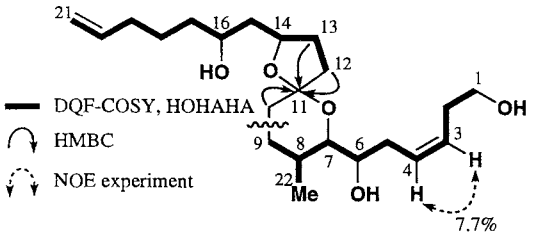


Figure 1. Partial structures of attenol A (1), based on 2D NMR correlations.

The relative stereochemistry of **1** was determined as follows. The value $J_{7,8}$ = 10.4 Hz suggested that H7 and H8 were located in a diaxial arrangement (Figure 2A). The NOESY correlations H5a/H7, H6/H7, H6/H22, H7/H22, and H7/H9a were observed. Although the stereochemistry of spiroacetal moiety (C11) could not be determined by NOE experiments due to overlap of the ^{1}H NMR signals, the relative stereochemistry of C11 was determined based on a consideration of the anomeric effect. This information suggested that the relative stereochemistries at C6, C7, C8, and C11 were 6*R**, 7*R**, 8*R**, and 11*S**. The values $J_{14,15a}$ = 4.8 Hz and $J_{14,15b}$ = 8.0 Hz in triacetate **3** suggested that H14 and H15a were located in a *gauche* arrangement and that H14 and H15b were located in an *anti* arrangement (Figure 2B). Similarly, the values $J_{16,15a}$ = 7.2 Hz and $J_{16,15b}$ = 4.8 Hz in **3** also suggested that H16 and H15a were located in an *anti* arrangement and that H16 and H15b were located in a *gauche* arrangement. Therefore, based on the presumption that the alkyl chain of **3** may have a zigzag conformation, we deduced that H14 and H16 are located as shown in Figure 2B.

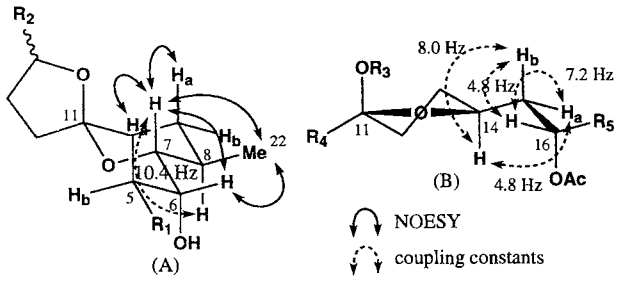


Figure 2. Relative stereochemistry of attenol A (1), based on NOESY correlations and coupling constants.

The absolute stereochemistries of C6 and C16 were determined using the modified Mosher's method.[4] The ^{1}H NMR signals of the two trisMTPA esters, **4** and **5**, were assigned based on the 2D NMR spectra, and the Δδ values (δ_S-δ_R, ppm) were then calculated. The results, shown in Figure 3, established that the absolute stereochemistries of C6 and C16 were 6*R* and 16*S*. Therefore, the absolute stereochemistry of **1** was determined to be 6*R*, 7*R*, 8*R*, 11*S*, 14*S*, and 16*S*.

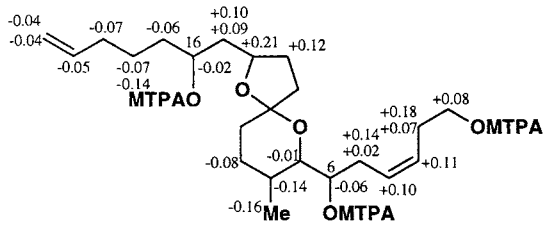


Figure 3. Δδ values (δ_S-δ_R) for the trisMTPA esters **4** and **5** in ppm

Attenol B (**2**) was determined to be an isomeric triol of **1** with a 6,8-dioxabicyclo[3.2.1]octane ring in the same manner as described above for **1**. In dioxabicyclooctane ring, the NOEs H6/H7 (2.1%), H6/H8 (3.9%), and H6/H9b (3.6%) were observed. Furthermore, attenol A (**1**) could be isomerized to attenol B (**2**) (PPTS, 1,2-dichloroethane, 50 °C), the ^{1}H NMR spectrum of which was identical to that of natural **2**. Therefore, the relative stereochemistry of **2** was determined to be 6*R**, 7*R**, 8*R**, 11*S**, 14*S**, and 16*S**. Furthermore, the absolute stereochemistry of **2** was deduced to be 6*R*, 7*R*, 8*R*, 11*S*, 14*S*, and 16*S* in view of biosynthesis.

In conclusion, attenols A and B, novel bicyclic triols, were isolated from the Chinese bivalve *Pinna attenuata*. The structures of these attenols were determined by their 2D NMR spectra and the modified Mosher's method. A synthetic study of attenols is in progress to confirm their stereochemistries. The framework of these attenols resembles those of halichlorine,[5] pinnaic acid,[6] and haterumalides,[7] which have been successfully isolated in our laboratory. This implies that these polyketides may be formed by similar biosynthetic processes.

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References and Notes

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3 Conditions for the isolation of attenol A (1): column, Develosil ODS HG-5 (20×250 mm); solvent, MeCN:MeOH:H_2O (30:30:40 → 40:40:20); flow rate, 5.0 mL/min; detection at 215 nm. Conditions for the isolation of attenol B (**2**): column, Develosil PhA-5 (20×250 mm); solvent, MeCN:H_2O (40:60 → 60:40); flow rate, 5.0 mL/min; detection at 215 nm.
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