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WONDOSTEROLS A-C, THREE STEROIDAL GLYCOSIDES FROM A KOREAN MARINE TWO-SPONGE ASSOCIATION

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Abstracts: Three steroidal glycosides, wondosterols A-C (**1-3**) have been isolated from a marine two-sponge association. Their stereostructures were elucidated on the basis of the spectroscopic analysis of natural wondosterol and its derivatives.

Key words: steroidal glycoside; cytotoxic and antibacterial; NMR spectroscopy; stereochemistry

To our best knowledge, a few steroidal gycosides from marine sponges have been reported, e.g., eryloside A from the marine sponge *Erylus lendenfeldi*,[1] and pachastelloside A from a marine sponge *pachastrella* sp.[2] In our ongoing research for antitumor compounds from Korean marine sponges, we found activity in the metanolic extract of a two-sponge association collected in Cheju Island, Korea. From this sponge we have isolated three new steroidal glycosides, wondosterols A-C (**1-3**). The MeOH extract of the frozen sponge (1.0 kg) was partitioned between Et_2O and H_2O, and the aqueous phase was further extracted with *n*-BuOH. The *n*-BuOH fraction was subjected to ODS flash chromatography with aqueous MeOH. The 80% MeOH fraction was fractionated by gel filtration on Sephadex LH-20 with MeOH to afford a cytotoxic fraction. This was finally purified by reversed-phase HPLC with 76% MeOH to yield **1-3** (**1**, 34 mg, 3.4 x 10^{-5}% yield; **2**, 22 mg, 2.2 x 10^{-5}%; **3**, 13 mg, 1.3 x 10^{-5}%).

The structure of wondosterol A (**1**) was readily determined by spectroscopic analysis. Compound **1** was obtained as a colorless amorphorous solid: $[\alpha]_D^{23}$ +38.4 (*c* 1.2, MeOH). The IR spectrum of **1** showed absorption bands due to hydroxyl group (3400 cm^{-1}) and double bond (1640). The FABMS data of **1** exhibited a quasimolecular $(M+Na)^+$ ion peak at *m/z* 763 and the molecular formula was determined as $C_{39}H_{64}O_{13}$ by HRFABMS [*m/z* 763.4248 $(M+Na)^+$, Δ+0.2 mmu] in conjunction with ^{13}C

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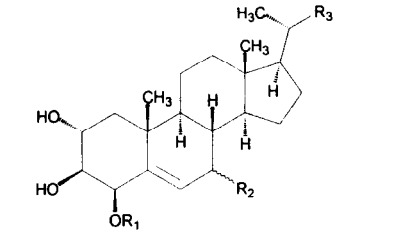


1 R_1=D-xyl-D-gal, R_2=β-OH, R_3=

2 R_1=D-xyl-D-gal, R_2=β-OH, R_3=

3 R_1=D-xyl-D-gal, R_2=β-OH, R_3=

4 R_1=D-gal, R_2=α-O-D-xyl, R_3=

NMR data. ^{1}H and ^{13}C NMR spectra of **1** were similar to those of pachastelloside A (**4**):[2] The ^{1}H NMR spectrum of **1** contained two methyl singlets (δ 0.54 and 1.43) and three methyl doublets (0.82, 0.90, and 0.92) together with four oxygenated methines (4.99, 4.42, 4.12, 3.65) and three olefinic protons (6.45, 4.71, 4.65) associated with steroidal skeletons. In addition, two oxygenated methylenes and nine methines including two anomeric protons (4.83, 4.98) were observed. The ^{13}C NMR spectrum of **1** exhibited five methyls, nine methylenes containing two oxymethylenes (δ 62.6, 67.5) and a exo-methylene (106.9), nineteen methines including eight oxygenated methines and a olefinic methine, two anomeric carbons, two quaternary sp^3 carbons and two sp^2 carbons.

Interpretation of the COSY spectrum of **1** revealed the presence of three partial units ***a***, ***b***, and ***c*** (Fig. 1). The connectivities between the partial unit ***a***, three quaternary carbons (C-5, C-10, C-13), and two tertiary methyls (C-18 and C-19) have been figured out on the basis of the HMBC[3] correlations: CH_3-19 with C-1, C-10, and C-5, and CH_3-18 with C-12, C-13, C-14, and C-17 (Fig. 1). The ^{13}C resonances and HMBC correlations (H-1'/C-5' and H-1"/C-5") data of the partial units ***b*** and ***c*** led to the identification of the monosacharide units as D-xylose and D-galactose, respectively, which were

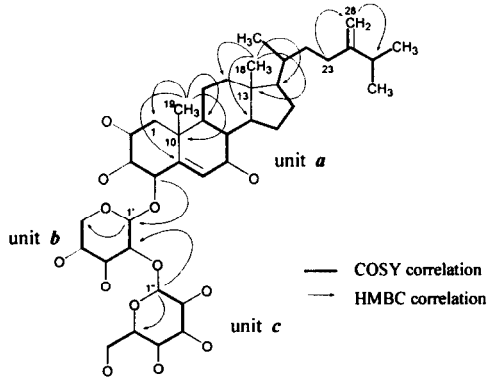


Fig. 1 Correlations obtained from the H-H COSY and HMBC data of wondosterol A.

Table 1. ^{1}H NMR Data of Wondosterols A-C at 400 MHz in Pyridine-d_5.

	1 δ_H (mult., J Hz)	2 δ_H (mult., J Hz)	3 δ_H (mult., J Hz)
1 α	1.15 (H)	1.22 (H)	1.20 (H, m)
β	2.20 (H)	2.22 (H)	2.24 (H)
2	4.42 (H, ddd, 11.2, 9.6, 4.2)	4.40 (H)	4.42 (H)
3	3.65 (H, dd, 11.2, 3.8)	3.68 (H, m)	3.67 (H, m)
4	4.99 (H)	5.02 (H, d, 3.6)	5.01 (H)
6	6.45 (H, br s)	6.43 (H, s)	6.46 (H, s)
7	4.12 (H, br d, 6.1)	4.16 (H)	4.17 (H)
8	1.57 (H)	1.63 (H)	1.62 (H)
9	1.55 (H)	1.62 (H)	1.63 (H)
11 a	1.42 (H, m)	1.45 (H, m)	1.46 (H, m)
b	1.28 (H)	1.30 (H, m)	1.29 (H, m)
12 a	1.81 (H)	1.79 (H)	1.81 (H, m)
b	1.01 (H)	1.02 (H)	1.00 (H)
14	1.72 (H)	1.74 (H)	1.77 (H)
15 a	1.74 (H)	1.76 (H)	1.74 (H)
b	0.91 (H, m)	1.19 (H)	1.18 (H)
16 a	2.23 (H)	2.24 (H)	2.24 (H)
b	1.13 (H)	1.03 (H)	1.06 (H, m)
17	0.95 (H, m)	1.01 (H)	0.98 (H)
18	0.54 (3H, s)	0.53 (3H, s)	0.53 (3H, s)
19	1.43 (3H, s)	1.46 (3H, s)	1.44 (3H, s)
20	1.28 (H)	1.92 (H, m)	1.92 (H, m)
21	0.82 (3H, d, 6.7)	0.92 (3H, d, 6.6)	0.91 (3H, d, 6.7)
22 a	1.44 (H, m)	5.17 (H, dd, 15.7, 6.8)	1.61 (H, m)
b	1.02 (H)		1.27 (H)
23 a	1.98 (H, m)	5.21 (H, m)	5.19 (H, m)
b	1.79 (H)		
24 a		1.74 (H)	5.17 (H, dd, 15.2, 4.9)
b		1.79 (H)	
25	2.09 (H, m)	1.19 (H)	1.74 (H)
26	0.90 (3H, d, 6.8)	0.73 (3H, d, 6.6)	0.84 (3H, d, 6.7)
27	0.92 (3H, d, 6.8)	0.73 (3H, d, 6.6)	0.73 (3H, d, 6.6)
28	4.71 and 4.65 (each 1H, br s)		
1'	4.83 (H, d, 7.9)	4.90 (H, d, 7.7)	4.83 (H, d, 7.8)
2'	3.78 (H, dd, 9.0, 7.9)	3.79 (H, dd, 8.9, 7.8)	3.76 (H, dd, 9.0, 7.8)
3'	3.93 (H)	4.03 (H)	3.95 (H)
4'	4.03 (H, ddd, 9.2, 4.2, 0.2)	4.10 (H)	4.02 (H, m)
5'	4.19 (H)	4.18 (H)	4.19 (H)
	3.56 (H, dd, 11.4, 9.2)	3.54 (H, dd, 11.4, 9.2)	3.56 (H, dd, 11.4, 9.2)
1"	4.98 (H)	4.83 (H, d, 7.4)	4.87 (H, d, 7.5)
2"	4.36 (H, dd, 8.4, 7.2)	4.37 (H)[c]	4.39 (H)
3"	3.95 (H)	4.02 (H)	3.93 (H)
4"	4.09 (H, dd, 9.0, 8.5)	4.22 (H)	4.09 (H, dd, 9.0, 8.5)
5"	4.18 (H)	4.10 (H)	4.18 (H)
6"	4.25 (2H, d, 8.6)	4.32 (2H, d, 8.6)	4.25 (2H)

further confirmed by the GLC analysis of the acid hydroxylate of **1** (see Experimental section). Due to the overlapping nature of the proton resonances, NOEs could not be used as the sole source of evidence for the inter-sugar linkage. Analysis of the HMBC data of **1**, however, revealed differences of **1** from **4**. Sequence of two monosacharides in **1** was established from an HMBC correlation between H-1" and C-

2.' Linkage of the sugar moiety to C-4 of the aglycone was based on the HMBC correlation between H-4 of aglycone and C-1'of xylose. The locations of hydroxyl groups in the aglycone were determined by a deuterium-induced ^{13}C NMR isotope shift experiments[4] of **1** taken in CD_3OD and CD_3OH. Of the carbons bearing heteroatoms, relatively larger upfield shifts (~0.1 ppm) resulting from deuterium replacements with H were observed on 2>7>3 listed in order of magnitude, indicative of location of hydroxyl groups on C-2, C-3, and C-7 of **1**. Also, a series of acetylation and acidic hydrolysis of **1** gave a triacetyldeglycosylated derivative **5**, of which chemical shift values of H-2, H-3, and H-7 were shifted to downfield of 0.8~1.2 ppm, further supporting the location of hydroxyl groups in **1**.

Next, the relative configuration of the aglycone part in **1** was elaborated on the basis of the NOESY[5] correlations and the $^3J_{HH}$ coupling constants to be identical with those of cholesterol's (Fig. 2). H-2 was coupled to H-1α, H-1β, and H-3 with J=9.6 Hz, 4.2 Hz, and 11.2 Hz, respectively, and H-3 to H-2 and H-4 with 11.2 Hz and 3.8 Hz, respectively, which suggested that both H-2 and H-3 were axial, while H-4 was assigned as equatorial. H-7 exhibited a broad doublet peak due to couple to H-8 and H-6 with 6.1 Hz and a small coupling constant (J<1 Hz, ~90°), respectively. Also, NOESY cross peaks

Table 2. ^{13}C NMR Data of Wondosterols A-C at 100 MHz in Pyridine-d_5.

No	1	2	3	No	1	2	3
1	46.8	46.6	46.7	21	19.4	21.7	21.8
2	71.1	70.8	71.0	22	35.5	139.2	37.8
3	77.4	78.0	78.3	23	31.7	126.7	128.8
4	84.4	83.3	83.7	24	157.1	42.6	132.3
5	141.8	141.4	141.3	25	34.5	29.2	45.3
6	128.9	128.8	128.7	26	22.4	22.8	22.4
7	72.1	72.2	72.2	27	22.5	23.0	23.3
8	37.8	38.0	37.9	28	106.9		
9	42.7	43.4	43.7	1'	102.3	102.5	102.5
10	39.6	39.3	39.5	2'	75.3	75.6	75.5
11	20.8	20.6	20.9	3'	78.4	78.8	78.7
12	39.2	39.2	39.2	4'	71.4	71.6	71.5
13	43.3	43.3	42.9	5'	67.5	67.7	67.5
14	49.2	49.4	49.4	1"	101.8	101.6	101.5
15	28.9	29.6	28.8	2"	72.8	72.9	72.9
16	24.4	24.5	24.6	3"	75.6	77.7	76.6
17	56.4	56.3	56.7	4"	70.0	70.0	70.1
18	12.1	12.3	12.2	5"	76.8	76.0	78.6
19	20.7	20.8	20.9	6"	62.6	62.8	62.6
20	36.4	41.0	36.6				

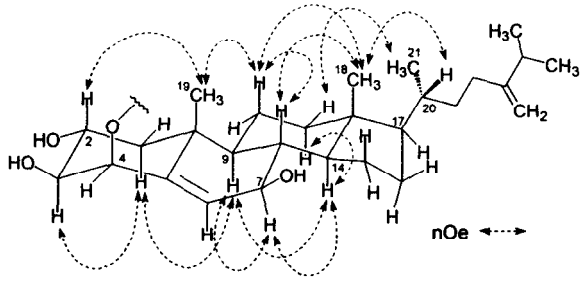
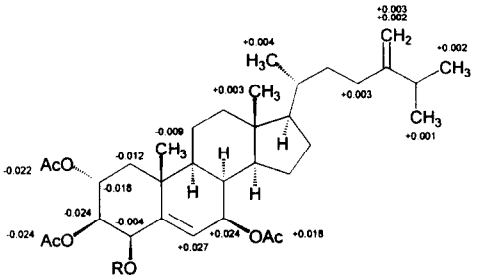


Fig. 2 NOESY correlations of wondosterol A

between H-7 and H-9 and H-14 were observed, indicative of α–orientation of H–7.[6] Irradiation of H-12_{eq} caused an NOE enhancement of the CH_3-21 and saturation of the CH_3-18 caused an NOE enhancement at H-20, indicating that the CH_3-21 occupied an α-position near H-12_{eq} and H-20 occupied a β-position. Furthermore, β-anomeric configurations for the xylose and galactose were determined on the basis of their $^3J_{H1,H2}$ coupling constants (7~8 Hz). The absolute stereochemistry of wondosterol A was determined by the modified Mosher's method.[7-9] Compound **5** was treated with (+)-(*R*)- or (-)-(*S*)-2-methoxy-2-phenyl-2-trifluoromethylacetic acid (MTPA), dicyclohexylcarbodiimide (DCC), *N,N*-dimethylaminopyridine (DMAP) in CH_2Cl_2 to furnish 4-*O*-(+)-(*R*)-MTPA ester **6a**, 4-*O*-(-)-(*S*)- MTPA ester **6b**, respectively. All proton signals of **6a** and **6b** were assigned and the absolute configuration at C4 was determined as *S* by analysis of Δδ (δ_S-δ_R) values. These results strongly suggested **1** to be the 4-*O*-[β-D-galactopyranosyl-(1→2)-β-D-xylopyranosyl]-24-methylenecholest-5-ene-2α,3β,7β-triol.



5 R=H
6a R=(+)-(*R*)-MTPA
6b R=(-)-(*S*)-MTPA

Another member of this series, wondosterol B (**2**) was characterized next. The HRFABMS indicated its molecular formula of $C_{38}H_{62}O_{13}$ [*m/z* 749.4085 $(M+Na)^+$ Δ-0.4 mmu)]. The ^1H and ^{13}C NMR spectral profiles for **2** showed it was a new member of the wondosterol family. The ^1H NMR spectrum of **2** in comparison to **1** included additional resonances: olefinic protons [δ 5.17 (dd, *J*=15.7, 6.8 Hz) and 5.21 (m)] and an HMBC cross peak between CH_3-21 (0.92) and C-22 (139.2) were observed. These data along with the absence of terminal double bond signals led to the proposal of structure **2** for wondosterol B. ^{13}C NMR chemical shifts and data from an NOE experiment indicated the stereochemistry of **2** was the same as that of **1**. Geometry of double bond in the side chain revealed as *E*- form from coupling constant ($J_{22\text{-}23}$=15.7 Hz). Thereby, wondosterol B was established as the 4-*O*-[β-D-galactopyranosyl-(1→2)-β-D-xylopyranosyl]-cholest-5, 22-diene-2α,3β,7β-triol.

Wondosterol C (**3**) showed the same molecular formula and both ^1H and ^{13}C NMR spectra of **3** were very similar to those of **2**. Compound **3** was only different in position of double bond in the side chain from **2**. HMBC correlations between two terminal methyls (0.73 and 0.84) and two olefinic carbons (132.3, 128.8) were observed. Wondosterol C was elucidated to be 4-*O*-[β-D-galactopyranosyl-(1→2)-β-D-xylopyranosyl]-cholest-5, 24-diene-2α,3β,7β-triol.

Wondosterols A-C were moderately cytotoxic against P388 murine leukemia cells (IC_{50}, 46 μg/mL) and wondosterols A and C showed antibacterial activities against *Pseudomonas aeruginosa* and *Escherichia coli* at a concentration of 10 μg/disk, but only wondosterol B exhibited no antibacterial activity.

Experimental

General. Silica gel 60 (0.04-0.063 mm) and ODS (55-105 μm) were used for flash column chromatography. Optical rotations were measured on a JASCO DIP-371 disital polarimeter set on the sodium D line. IR spectra were recorded employing JASCO FT/IR-5300 infrared spectrometer. ^{1}H and ^{13}C NMR spectra were measured on Brucker ARX-400 NMR spectrometers in pyridine-d_5 (δ_H 7.00 and δ_C 124.0). FABMS data were obtained on JEOL JMX-SX 102 mass spectrometer and high resolution FABMS spectra were determined using a glycerol matrix, respectively. Analytical HPLC work was performed on a Varian 9012 HPLC equipped with a Varian Variable wavelength UV-visible detector set at 210 nm and ODS column (4.6 x 250 mm). Prepative HPLC work was performed on a HPLC equipped with fixed wavelength UV detector (210 nm) and a reverse phase column (C18-Cosmosil MS, 20 x 250 mm). HPLC grade MeOH (Tedia Co. Inc.) was filtered through Millipore filters before use. Water for HPLC work was obtained from a Millipore water filtration system.

Collection, Extraction, and Isolation. The sponge was collected at a depth of 15 m off Cheju Island, Korea in 1996. The voucher specimen CM96026 was deposited at Laboratory of Bioorganic Chemistry, Taejon National University of Technology. This two-sponge association was composed of *Poecillastra wondoensis* and *Jaspis wondoensis*. Two sponges were fused to each other like one sponge, and it was not easy to separate them: the upper part is *Poecillastra* and the underpart is *Jsapis*. The thickly encrusting *Poecillastra* sponge covered all the upper part of the sponge *Jaspis*. This sponge was considered to be a symbiotic association of two different sponges. Both sponges were attached tightly to each other, resembling one sponge. A cross section of the sample showed two layers of morphologically distinct sponges. Lyophilized animals (1.0 kg) were sequentially extracted with 70% MeOH (3 x 1 L) and filtered. The MeOH extract concentrated was partitioned between Et_2O (3 x 500 mL) and H_2O (500 mL), and the aqueous phase was further extracted with *n*-BuOH. The *n*-BuOH fraction was subjected to ODS flash chromatography with H_2O, 20%, 40%, 60%, 80%, and 100% MeOH. The 80% MeOH fraction was fractionated by gel filtration on Sephadex LH-20 with MeOH to afford a cytotoxic fraction. This fraction was finally purified by reversed-phase HPLC chromatography eluting with 76% MeOH to afford **1-3** (**1**, 34 mg, 3.4 x 10^{-5}% wet weight; **2**, 22 mg, 2.2 x 10^{-5}%; **3**, 13 mg, 1.3 x 10^{-5}%).

Wondosterol A (1): amorphous solid; $[\alpha]_D^{23}$ +38.4° (*c* 1.2, MeOH); IR (KBr) ν_{max} 3400, 2930, 1640, 1240 cm^{-1}; ^{1}H and ^{13}C NMR data (see Table 1 and 2); FABMS *m/z* 763 $[M+Na]^+$; HRFABMS *m/z* 763.4248 $[M+Na]^+$, calcd for $C_{39}H_{64}O_{13}Na$, 763.4246.

Wondosterol B (2): amorphous solid; $[\alpha]_D^{23}$ +46.2° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3450, 2950, 1640, 1230 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1 and 2); FABMS *m/z* 749 $[M+H]^+$; HRFABMS *m/z* 749.4085 $[M+Na]^+$, calcd for $C_{38}H_{62}O_{13}Na$, 749.4089.

Wondosterol C (3): amorphous solid; $[\alpha]_D^{23}$ +62.7° (*c* 0.8, MeOH); IR (KBr) ν_{max} 3420, 2950, 1630, 1240 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1 and 2); FABMS *m/z* 749 $[M+Na]^+$; HRFABMS *m/z* 749.4086 $[M+Na]^+$, calcd for $C_{38}H_{62}O_{13}Na$, 749.4089.

Acetylation and acid hydrolysis of wondosterols A-C. A mixture of **1** (14 mg), Ac_2O (500 μL), and distilled pyridine (500 μL) was stirred at room temperature overnight. The reagents were evaporated at reduced pressure, and dried by stream of nitrogen for complete removal of pyridine. The polyacetylated derivative was heated in 1 mL of 5% HCl-EtOH at 80°C for 2 h in a water bath. After ethanol was removed, the solution was extracted with EtOAc (2 mL x 3). The organic layer was

washed with H_2O and then concentrated to give an amorphous powder (9.2 mg), which was subjected to ODS HPLC [90% MeOH, 1 ml/min, UV 210 nm] to give **5** (6.6 mg). The monosaccharide portion was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column, concentrated (dried overnight), then treated with 1-(trimethylsilyl)imidazole at room temperature for 2 h. After the excess reagent was decomposed with H_2O, the reaction product was extracted with *n*-hexane (2 mL x 2). The TMSi derivatives of the monosaccharide were identified to be D-xylose and D-galactose (1:1) by co-GLC analyses with standard monosaccharides. By the same method, the monosaccharide of **2** and **3** were shown to be identified as those of **1**.

Triacetyldeglycosylated aglycone (5). amorphous solid; FABMS *m/z* 573 $[M+H]^+$; ^1H NMR (pyridine-d_5, 400 MHz) δ 6.42 (H, s, H-6), 4.69 and 4.62 (each 1H, br s, H-28), 5.26 (H, ddd, 11.3, 9.4, 4.2, H-2), 5.06 (H, br d, 6.0, H-7), 4.78 (H, 3.9, H-4), 4.62 (H, dd, 11.3, 3.9, H-3), 2.22-2.25 (2H, m, H-1β and H-16a), 2.12 (3H, s, 7-OAc), 2.09 (3H, s, 3-OAc), 2.06 (H, m, H-25), 2.04 (3H, s, 2-OAc), 1.94 (H, m, H-23a), 1.80-1.72 (4H, m, H-12a, H-14, H-15a, H-23b), 1.58-1.55 (2H, m, H-8 and H-9), 1.41-1.44 (2H, m, H-11a and H-22a), 1.24 (3H, s, H-19), 1.22-1.25 (2H, m, H-11b and H-20), 1.15-1.12 (2H, m, H-1α, and H-16b), 1.04-1.02 (2H, m, H-12b and H-22b), 0.94-0.92 (2H, m, H-15b and H-17), 0.90 (3H, d, H-27), 0.88 (3H, d, H-26), 0.81 (3H, d, 6.7, H-21), 0.57 (3H, s, H-18); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 170.6 (7-OAc), 170.3 (3-OAc), 169.8 (2-OAc), 156.8 (C-24), 141.1 (C-5), 127.5 (C-6), 106.2 (C-28), 74.6 (C-7), 79.8 (C-3), 76.3 (C-4), 73.4 (C-2), 56.5 (C-17), 48.8 (C-14), 45.2 (C-1), 43.6 (C-13), 43.1 (C-9), 39.4 (C-10), 38.8 (C-12), 38.2 (C-8), 36.4 (C-20), 35.3 (C-22), 34.7 (C-25), 31.6 (C-23), 28.6 (C-15), 24.2 (C-16), 22.6 (C-26), 22.8 (C-27), 21.87 (7-OAc), 21.82 (3-OAc), 20.91 (2-OAc), 20.8 (C-19), 20.5 (C-11), 19.6 (C-21), 12.2 (C-18).

MTPA esterification of 5. To solution of 3.3 mg of **5** in 100 μL of dry pyridine was added 20 μL of (+)-(*R*)-MTPA chloride and 6.0 mg of 4-dimethylaminopyridine (DMAP). The mixture was allowed to stand under N_2 at room temperature for 6 h. After the consumption of starting material was confirmed by TLC, 50 μL of H_2O, 100 μL of CH_2Cl_2, and 200 μL of MeOH were added. The solvents were removed under vacuum, and the residue was separated on prepative TLC [$CHCl_3$/MeOH (95:5)] to give 2.1 mg of (*R*)-MTPA ester **6a**. According to the same experimental procedure, 20 mL of (-)-(*S*)-MTPA chloride and 3.3 mg of **5** were reacted to obtain 1.8 mg of (-)-(*S*)-MTPA ester **6b**. The concentrations of the esters were adjusted to same concentration in pyridine-d_5, and ^1H NMR spectra were measured at 400 MHz.

Ester 6a: FABMS; *m/z* 811 $(M+Na)^+$, 789 $(M+H)^+$; ^1H NMR (pyridine-d_5, 400 MHz) δ 7.441–7.022 (5H, m, ph-MTPA's), 6.393 (H, s, H-6), 4.638 and 4.602 (each 1H, s, H-28), 4.340 (3H, s, CH_3O-MTPA's), 4.926 (H, br d, 6.1, H-7), 5.214 (H, m, H-2), 4.698 (H, d, 4.0, H-4), 4.667 (H, dd, 11.3, 4.0, H-3), 2.123 (3H, s, 7-OAc), 2.096 (3H, s, 3-OAc), 2.038 (3H, s, 2-OAc), 1.934 (H, m, H-23a), 1.243 (3H, s, H-19), 0.961 (3H, d, H-21), 0.903 (3H, d, H-26), 0.878 (3H, d, H-27), 0.572 (3H, s, H-18) and other protons were not assignable due to be overlapped.

Ester 6b: FABMS; *m/z* 811 $(M+Na)^+$, 789 $(M+H)^+$; ^1H NMR (pyridine-d_5) δ 7.453–7.043 (5H, m, ph-MTPA's), 6.420 (H, s, H-6), 4.640 and 4.605 (each 1H, s, H-28), 4.346 (3H, s, CH_3O-MTPA's), 4.950 (H, br d, 6.1, H-7), 5.196 (H, m, H-2), 4.694 (H, d, 4.0, H-4), 4.643 (H, dd, 11.3, 4.0, H-3), 2.141 (3H, s, 7-OAc), 2.072 (3H, s, 3-OAc), 2.016 (3H, s, 2-OAc), 1.937 (H, m, H-23a), 1.234 (3H, s, H-19), 0.965 (3H, d, H-21), 0.905 (3H, d, H-26), 0.879 (3H, d, H-27), 0.575 (3H, s, H-18) and other protons were not assignable due to be overlapped.

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References and Notes

[1] Carmely S, Roll M, Loya Y, and Kashman Y, *J. Nat. Prod.* **1989**; 52; 167-169.
[2] Hirota H, Takayama S, Miyashiro S, Ozaki Y, and Ikegami S, *Tetrahedron Lett.* **1990**; 31; 3321-3324.
[3] Bax A, Aszalos A, Dinya Z, Sudo K, *J. Am. Chem. Soc.* **1986**; 108; 8056-8063.
[4] Jeremic D, Milosavljevic S, Mihalovic M. L, *Tetrahedron* **1982**; 38; 3325-3328.
[5] Bodenhausen G, Kogeler H, Ernst R. R, *J. Magn. Reson.* **1984**; 58; 370-388.
[6] Kobayashi M, Kanda F, Damarla S. R, Rao D. V, and Rao C. B, *Chem. Pharm. Bull.* **1990**; 38; 2400-2403.
[7] Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**; 95; 512-519.
[8] Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Amer. Chem. Soc.* **1991**; 73; 4092-4096.
[9] Kusumi, T.; Fukushima, T.; Ohtami, I.; Kakisawa, H. *Tetrahedron Lett.* **1991**; 32; 2939-2942.