Structure and Absolute Configuration of Clerodane Diterpene Glycosides and a Rearranged Cadinane Sesquiterpene Glycoside from the Stems of *Tinospora sinensis*

Wei Li, Kun Wei, Hongwei Fu, and Kazuo Koike*

Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan

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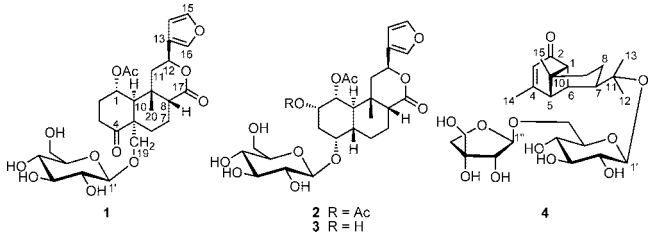
Three new clerodane diterpene glycosides, tinosposinensides A−C (**1**−**3**), and a new rearranged cadinane sesquiterpene glycoside, tinosinenside (**4**), were isolated from the stems of *Tinospora sinensis*. The structure including the relative configuration was elucidated on the basis of spectroscopic analysis. The absolute configuration was determined by application of the modified Mosher's method and chemical transformation. The inhibitory activities of the isolated compounds against α-glucosidase are also described.

Tinospora sinensis is a plant widely distributed in southern China. Its stems, known locally as "Shen-Jin-Teng", are used as a folk medicine in the treatment of rheumatism and muscle pain. Previous phytochemical studies resulted in the isolation of two diterpenes and a phenylpropanoid diglycoside.[1,2] As part of our phytochemical investigations of the medicinal plants of Hainan Island, People's Republic of China, we reported the isolation of two new lignan glucosides, tinosposides A and B, from the EtOH extract of *T. sinensis*.[3] Our continuing phytochemical study on this extract led to the isolation of three new clerodane diterpene glycosides, tinosposinensides A−C (**1**−**3**), and a new rearranged cadinane sesquiterpene glycoside, tinosinenside (**4**). In this paper, we report the isolation, structure elucidation, and absolute configuration of the new diterpene and sesquiterpene glycosides. The inhibitory activities of the isolated compounds against α-glucosidase were also investigated.



Results and Discussion

The stems of *T. sinensis*, collected on Hainan Island, People's Republic of China, were extracted with 95% EtOH. The EtOH extract was partitioned between EtOAc and H_2O. The H_2O layer was subjected to Diaion HP-20 column chromatography (CC) and eluted with 30% MeOH and then MeOH. The MeOH eluate fraction was further purified using ODS CC and reversed-phase HPLC to afford compounds **1**−**4**.

Tinosposinenside A (**1**) was isolated as an amorphous powder, $[\alpha]^{22}_D$ +3.7 (*c* 0.7, MeOH). Its analysis by HRFABMS (*m/z* 575.2114 $[M + Na]^+$, calcd for 575.2104) led to the molecular formula $C_{27}H_{36}O_{12}$, supported by the ^{13}C NMR and DEPT data. The ^1H NMR spectrum (Table 1) showed a set of typical downfield resonances at δ 6.53 (1H, dd, J = 1.8, 0.7 Hz), 7.47 (1H, d, J = 1.8 Hz), and 7.56 (1H, t, J = 0.7 Hz), characteristic of the resonances of the C-12 furano ring of the clerodane-type diterpenoids reported from *Tinospora* sp.[2] It also showed the resonances of the β-glucopyranosyl moiety with the resonance for the anomeric proton at δ 4.23 (1H, d, J = 7.8 Hz). Upon acid hydrolysis, **1** afforded D-glucose, which was identified by GLC analysis of its trimethylsilylthiazolidine derivative.[4] Enzymatic hydrolysis of **1** with naringinase afforded the aglycone **1a**. Alkaline hydrolysis (NaOMe) of **1a** yielded two isomers, **1b** and **1c**. Compound **1b** is the known compound malaborolide B,[5] also isolated from a *Tinospora* sp., with an X-ray structure available, but without its absolute configuration determined. The absolute configuration of **1b** was determined by application of the modified Mosher's method. Compound **1b** was esterified by (−)- or (+)-α-methoxy-α-trifluoromethylphenyl acetyl chloride (MTPACl) in dry pyridine to give the 1-(*S*)- and 1-(*R*)-MTPA esters (**1d** and **1e**), respectively. The distribution of the Δδ values ($\delta_S - \delta_R$) obtained from the ^1H NMR data of **1d** and **1e** indicated the 1*S*-configuration.[6]

Hydrolysis of **1a** with 0.28% NaOMe in MeOH to afford products **1b** and **1c**, epimeric at C-8, was unexpected. Analysis of the ^1H NMR spectra showed a change in the $^3J_{1,10}$ value from 3.6 Hz in **1a** to 8.3 Hz in **1b** and 8.4 Hz in **1c**. The $^3J_{5,10}$ coupling constant (12.4 Hz) in **1b** and **1c** suggested that in **1b** and **1c**, rings A and B were *trans*-fused, as opposed to their *cis*-fusion in **1a**. Furthermore, the ^1H NMR spectra of **1b** and **1c** revealed differences in the $^3J_{7a,8}$ and $^3J_{7b,8}$ values, namely, 1.4 and 3.2 Hz for **1b** and 4.6 and 12.1 Hz for **1c**. The NOESY correlations H-8/H-12, H-8/H-10, H-8/H-6, H-20/H-1, and H-20/H-5, in **1c**, confirmed the *trans*-fusion of rings B and C. The conversion of **1a** into epimers **1b** and **1c** proceeded through a retro-aldol reaction, followed by epimerization at C-8 as shown in Scheme 2.

Further 2D NMR analyses of **1** indicated that in the HMBC spectrum the C-19 oxymethylene protons showed three-bond correlations between H-19a and H-19b (δ_H 4.59 and 3.59) and C-4 (δ_C 215.2) and C-6 (27.1). The attachment of the β-glucopyranosyl moiety at C-19 was determined by the HMBC correlation between H-1′ (δ_H 4.23) and C-19 (δ_C 78.1). The *O*-acetyl group was located at C-1 by the HMBC correlation between H-1 (δ_H 5.31) and 1-*C*OCH_3 (δ_C 172.1). The H-1 proton was observed as a broad doublet (J = 2.8 Hz), suggesting its β-equatorial orientation. NOESY correlations of H-1/H_3-20 and H-8/H_3-20 indicated the β-orientations of H-8 and CH_3-20 (Figure 1). The $^3J_{11\beta,12}$ value (12.4 Hz) and 1,3-diaxial NOESY correlation of H-11β/H-8 suggested the α-orientation of H-12. The α-orientations of H-10 and 19-CH_2-OGlc were deduced by the NOESY correlations of H-3α/H_2-19 and H-10/H_2-19.

Tinosposinenside B (**2**) was isolated as an amorphous powder, $[\alpha]^{22}_D$ −19.4 (*c* 0.6, MeOH). The molecular formula of **2** was established as $C_{28}H_{38}O_{13}$ by the HRFABMS (*m/z* 605.2220 $[M + Na]^+$, calcd 605.2210). The analysis of the ^1H and ^{13}C NMR spectra showed the occurrence of resonances due to a β-substituted furan ring, a δ-lactone ring, a β-glucopyranosyl, and two *O*-acetyl moieties. Comparison of the NMR data of **2** with those of **1**

* To whom correspondence should be addressed. Tel: +81-47-4721396. Fax: +81-47-4721404. E-mail: koike@phar.toho-u.ac.jp.

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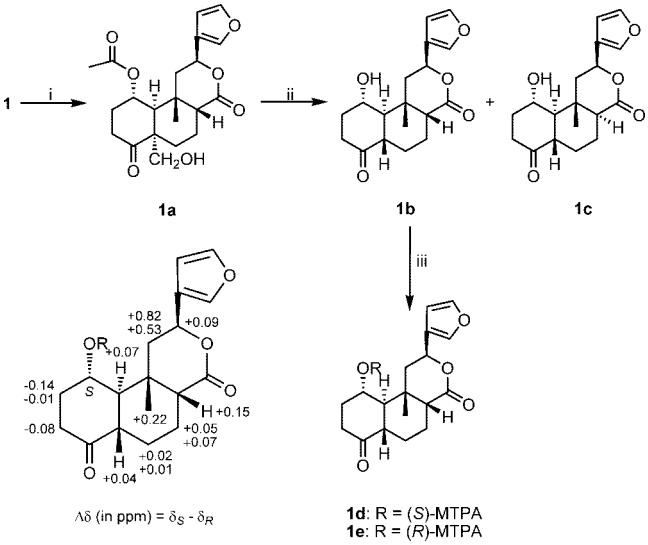
Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Compounds **1**, **2**, and **3** in CD_3OD

	1		**2**		**3**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	5.31 br.d (2.8)	69.6	4.98 dd (11.0, 3.4)	75.1	4.85 dd (11.2, 3.1)	77.8
2	2.26 m	31.3	5.34 q (3.4)	70.2	4.08 q (2.9)	69.4
	2.45 m					
3	2.25 m	34.8	1.77 dt (15.8,3.6)	30.7	1.70 dd (15.6, 2.9)	32.8
	3.07 ddd (15.4, 13.5, 8.0)		2.29 dt (15.8, 3.0)		2.32 dt (15.6, 2.9)	
4		215.2	4.00 q (2.3)	74.0	3.92 q (2.0)	77.2
5		52.8	1.78 m	40.0	1.80 m	40.5
6	1.22 td (14.0, 3.9)	27.1	1.52 br.dd (13.1, 3.0)	26.5	1.51 br.dd (12.8, 2.4)	26.6
	2.44 m		1.70 td (13.1,3.4)		1.70 m	
7	1.82 m	19.7	1.79 m	22.6	1.76 m	22.6
	2.14 m		2.29 dt (15.8, 3.0)		2.28 dt (14.5, 2.4)	
8	2.42 m	51.0	2.45 br.s	52.2	2.45 br.s	52.5
9		36.6		36.2		36.1
10	2.58 br.s	48.5	2.57 t (11.0)	36.0	2.58 t (11.2)	35.4
11	1.82 dd (14.9, 12.6)	42.7	1.87 dd (14.5, 12.8)	44.2	1.88 dd (14.4, 12.6)	44.5
	2.57 dd (14.9, 3.4)		2.43 dd (14.5, 3.2)		2.43 dd (14.4, 3.3)	
12	5.70 dd (12.4, 3.4)	72.1	5.67 dd (12.3, 3.2)	72.6	5.80 dd (12.4, 3.3)	72.7
13		126.9		127.0		127.1
14	6.53 dd (1.8, 0.7)	109.5	6.52 dd (1.7, 0.7)	109.5	6.54 dd (1.6, 0.7)	109.6
15	7.47 t (1.8)	144.8	7.50 t (1.7)	145.2	7.48 t (1.6)	145.2
16	7.56 t (0.7)	141.1	7.60 t (0.7)	141.1	7.63 t (0.7)	141.3
17		174.6		174.9		175.1
19	3.59 d (9.7)	78.1				
	4.59 d (9.7)					
20	1.04 s	26.9	1.12 s	21.9	1.14 s	22.1
Glc-1′	4.23 d (7.8)	104.5	4.29 d (7.8)	100.9	4.37 d (7.7)	101.3
2′	3.12 dd (8.9, 7.8)	75.0	3.21 dd (8.9, 7.8)	75.1	3.20 dd (9.0, 7.7)	75.1
3′	3.31[a]	78.3	3.35 t (8.9)	78.3	3.38 t (9.0)	78.3
4′	3.26 t (8.9)	71.7	3.26 t (8.9)	72.0	3.27[a]	71.9
5′	3.26 m	78.0	3.21 m	78.1	3.26 m	78.2
6′	3.66 dd (11.5, 5.5)	62.9	3.63 dd (11.9, 5.9)	63.1	3.65 dd (11.9, 4.7)	63.0
	3.86 dd (11.5, 1.8)		3.84 dd (11.9, 2.3)		3.85 dd (11.9, 1.9)	
1-$COCH_3$		172.1		171.4		171.7
1-$COCH_3$	2.16 s	21.6	1.90 s	21.4	2.00 s	21.7
2-$COCH_3$				173.1		
2-$COCH_3$			2.11 s	21.3		

[a] Overlapped signals.

Scheme 1[a]



[a] Reagents and conditions: (i) naringinase, acetate buffer (pH = 4.0), 70 h, 40 °C; (ii) NaOMe (0.28% in MeOH), rt, 2 h; (iii) (−)- or (+)-MPTACl, dry pyridine.

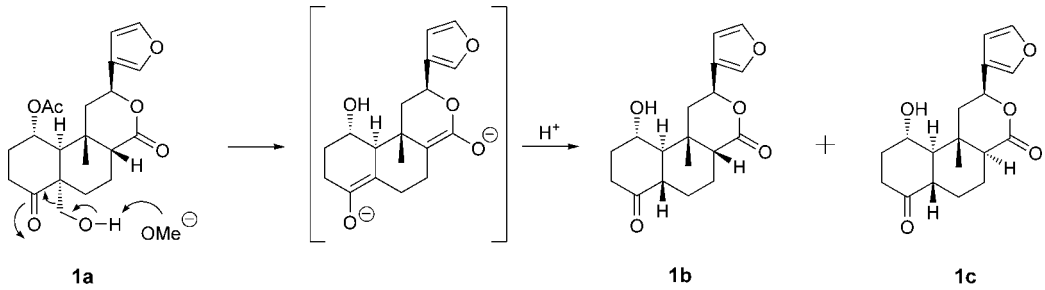
suggested that **2** was also a clerodane diterpene glycoside (Table 1). The most obvious differences between the two compounds were the absence of resonances due to a ketone carbonyl group and an oxymethylene and the presence of resonances due to an additional *O*-acetyl group and two oxymethines in **2**. A further comparison of the NMR data of **2** and **1** revealed that the difference was apparent only in the chemical shifts of the A ring. The DQFCOSY correlations from H-1 to H-8 indicated that the additional two oxymethines were placed at C-2 and C-4. The location of the *O*-acetyl group at C-2 was confirmed by the HMBC correlation between H-2 (δ_H 5.34) and 2-$COCH_3$ (δ_C 173.1) (Figure 3). The attachment of the β-glucopyranosyl moiety at C-4 was established by the HMBC correlation between H-1′ (δ_H 4.29) and C-4 (δ_C 74.0). Further elucidation of the 2D NMR data determined the planar structure of **2**.

The relative configuration of **2** was elucidated on the basis of the coupling constants and NOESY correlations (Figure 4). The $^3J_{1,2}$ and $^3J_{1,10}$ values (3.4 and 11.0 Hz) indicated that H-1, H-2, and H-10 were β-axially, β-equatorially, and α-axially oriented, respectively. The β-axial orientation of H-5 was deduced from the $^3J_{5,6\alpha}$ value (13.1 Hz) and the NOESY correlation of H-1/H-5. The $^3J_{4,3}$ and $^3J_{4,5}$ values (both 2.3 Hz) indicated the β-equatorial orientation of H-4. The 1,3-diaxial NOESY correlations of H-1/H-20 and H-5/H-20 established the β-axial orientations of H-20. The NOESY correlations of H-8/H-20 and H-10/H-12 indicated the β-orientation of H-8 and α-orientations of H-12.

The absolute configuration of **2** was determined by the strategy shown in Scheme 3. Enzymatic hydrolysis of **2** afforded **2a**, which was converted into its (*S*)- or (*R*)-MTPA ester (**2b** and **2c**). The distribution of the $\Delta\delta$ values ($\delta_S - \delta_R$) obtained from the ^{1}H NMR data of **1d** and **1e** indicated the 4*R*-configuration.

Tinosposinenside C (**3**) was isolated as an amorphous powder, $[\alpha]^{22}_D$ +4.0 (*c* 1.3, MeOH). Analysis of the HRFABMS (*m*/*z* 563.2112 $[M + Na]^+$, calcd 563.2105) led to the molecular formula $C_{26}H_{36}O_{12}$. The ESIMS data showed that **3** had 42 mass units less

Scheme 2. Plausible Mechanism for the Production of **1b** and **1c** from **1a**



than **2**. The ^{1}H and ^{13}C NMR resonances of **3** were almost identical to those of **2** (Table 1), except for the absence of resonances due to an *O*-acetyl group. The *O*-acetyl group was located at C-1 by the HMBC correlation between H-1 (δ_H 4.85) and 1-*C*OCH$_3$ (δ_C 171.7). The absolute configuration was determined by the chemical transformation shown in Scheme 3. Compounds **3** and **2** were treated with Ac_2O in dry pyridine, respectively, to afford their peracetylated derivates, which were the same product, **2d**, by comparison of their $[\alpha]_D$, ^{1}H and ^{13}C NMR, and MS data. Thus, **3** and **2** possessed the same absolute configurations.

Tinosinenside (**4**) was isolated as an oil, $[\alpha]^{22}_D$ −49.3 (*c* 0.6, MeOH). The molecular formula was determined to be $C_{26}H_{40}O_{11}$ by the HRFABMS (*m*/*z* 551.2487 [M + Na]$^+$, calcd for 551.2468). Upon acid hydrolysis, **4** afforded equimolar amounts of D-glucose and D-apiose, identified by the GC-MS analysis of their trimethylsilylthiazolidine derivatives. The β-anomeric configuration for the glucopyranosyl moiety was determined from the large $^3J_{H1,H2}$ value (7.8 Hz) and for the apiofuranosyl moiety by the chemical shift of its anomeric carbon (δ 110.9).[7] Enzymatic hydrolysis of **4** with naringinase afforded the known compound **4a**, the aglycone from the glucoside tinocordoside,[8] for which the absolute configuration was not determined. Compound **4a** was reduced with $NaBH_4$ to yield **4b**. The orientation of the hydroxy group in **4b** was determined to be β, on the basis of the NOESY correlation of H-2/H-6 (Figure

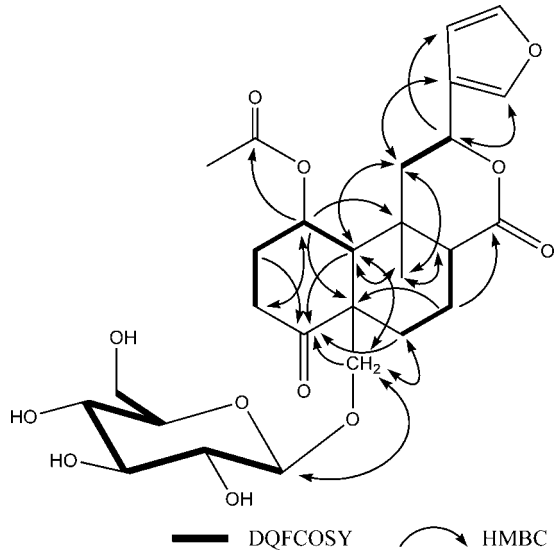


Figure 1. Key DQFCOSY and HMBC correlations for **1**.

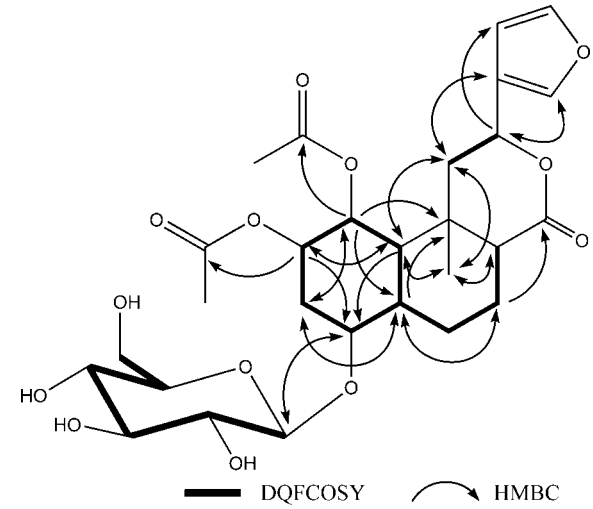


Figure 3. Key DQFCOSY and HMBC correlations for **2**.

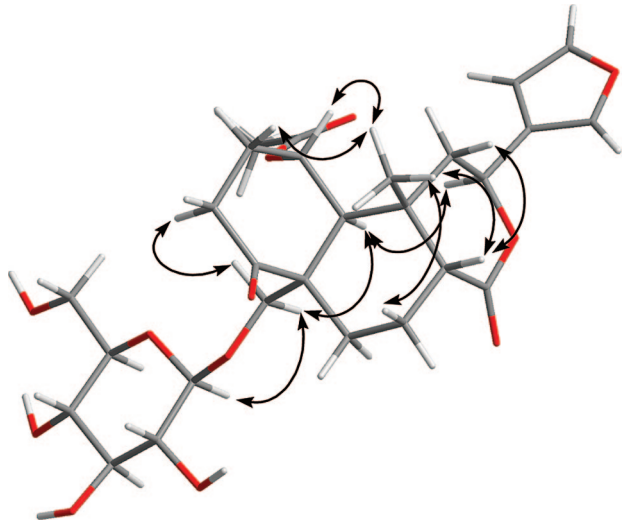


Figure 2. Selected NOESY correlations for **1**.

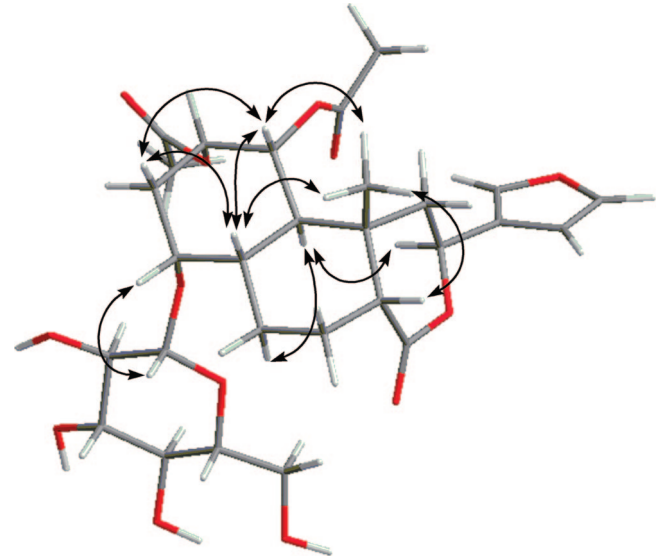
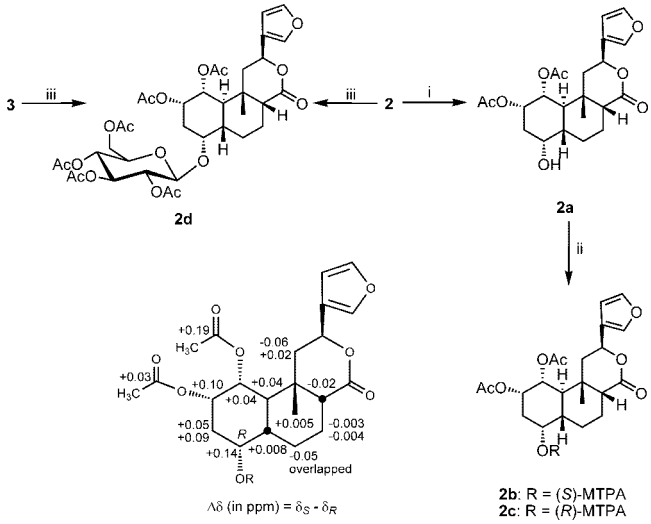


Figure 4. Selected NOESY correlations for **2**.

Scheme 3[a]



[a] Reagents and conditions: (i) naringinase, acetate buffer (pH = 4.0), 115 h, 40 °C; (ii) (−)- or (+)-MPTACl, dry pyridine; (iii) Ac_2O, dry pyridine.

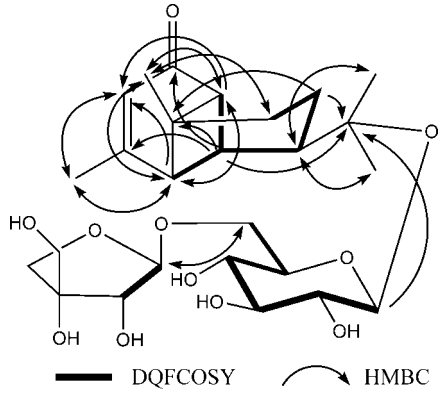


Figure 5. Key DQFCOSY and HMBC correlations for **4**.

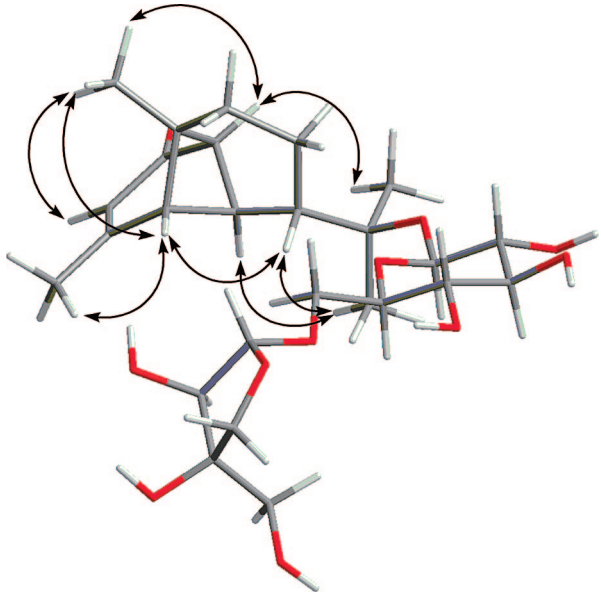


Figure 6. Selected NOESY correlations for **4**.

7). Compound **4b** was converted into its (*S*)- or (*R*)-MTPA esters (**4c** and **4d**). The $\Delta\delta$ values ($\delta_S - \delta_R$) of H-3 and H-14 were positive, while those of H-1, H-8, H-9, and H-15 were negative, suggesting that C-2 possessed an *R*-configuration. Analysis of the

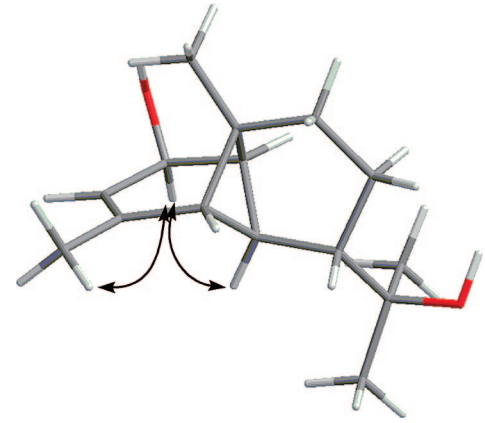


Figure 7. Selected NOESY correlations for **4b**.

2D NMR data explained the relative configuration of **4**. The NOESY correlations of H-5/H-7 and H-5/H_3-14 indicated that these protons were cofacial. In the DQFCOSY spectrum of **4**, characteristic *W*-type long-range coupling (J = 6.7 Hz) was observed between H-1 (δ_H 2.72, dd, J = 6.7, 1.6 Hz) and H-5 (δ_H 2.05, dd, J = 6.7, 1.1 Hz) in a bridgehead cyclobutane system. Small coupling constants between H-1 and H-6 (1.6 Hz) and between H-5 and H-6 (1.1 Hz) indicated dihedral angles, $H_1-C_1-C_6-H_6$ and $H_5-C_5-C_6-H_6$, of ca. 90° (calculated −96.94° and 96.58°). The positions of the β-glucopyranosyl and the β-apiofuranosyl moieties were determined on the basis of the HMBC correlations between Glc-H-1′ (δ_H 4.43) and C-11 (δ_C 81.6) and between Api-H-1″ (δ_H 4.94) and Glc-H-6′ (δ_C 68.7), respectively.

The isolated compounds **1**−**4** were subjected to an α-glucosidase inhibition assay. Compounds **1**, **2**, **3**, and **4** exhibited IC_{50} values to α-glucosidase at 2.9, 3.8, 3.3, and 1.9 mM, respectively. Meanwhile, the positive control, acarbose, demonstrated an IC_{50} value to α-glucosidase of 0.84 mM.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-370 digital polarimeter in a 0.5 dm cell. The IR spectra were measured on a JASCO FT/IR-300E (the KBr disk method) spectrometer. The ^1H and ^{13}C NMR spectra were measured on a JEOL ECP-500 spectrometer with TMS as the internal reference, and the chemical shifts are expressed in δ (ppm). ESIMS was recorded on an LCQ mass analyzer, and HRFABMS on a JEOL Mstation spectrometer. For HPLC, a JASCO PU-1580 HPLC system, equipped with a Shodex RI-71 differential refractometer detector, was used. Reversed-phase column chromatography (RP-CC) was accomplished using RP-C18 silica gel (Chromatotex DM1020T ODS, Fuji Silysia Chemical Ltd.). Si gel CC was carried out using Kieselgel 60 (E. Merck). TLC was conducted using Kieselgel 60 F254 plates (E. Merck). GLC was carried out on a Perkin-Elmer Clarus 500 GCMS instrument.

Extraction and Isolation. The stems of *T. sinensis* were collected on Hainan Island, People's Republic of China. The air-dried stems (3.0 kg) were extracted three times with 95% EtOH under reflux for 2 h each at 80 °C. The EtOH extract was concentrated (171.8 g), suspended in H_2O, and then successively partitioned with EtOAc (1200 mL). The H_2O layer was passed through a Diaion HP-20 column and then washed with H_2O, 30% MeOH, and MeOH. The MeOH fraction (17.2 g) was chromatographed over Si gel, ODS columns, and preparative reversed-phase HPLC to afford four compounds, **1** (7 mg), **2** (80 mg), **3** (11 mg), and **4** (13 mg).

Tinosposinenside A (1): colorless, amorphous solid; $[\alpha]^{22}_D$ +3.7 (*c* 0.7, MeOH); IR (KBr) ν_{max} 3423, 2931, 1738, 1709, 1366, 1246, 1075, 1033, 875 cm^{-1}; ^1H and ^{13}C NMR (Table 1); ESIMS *m/z* 575.2 $[M + Na]^+$; HRFABMS *m/z* 575.2114 $[M + Na]^+$ (calcd for $C_{27}H_{36}O_{12}Na$, 575.2104).

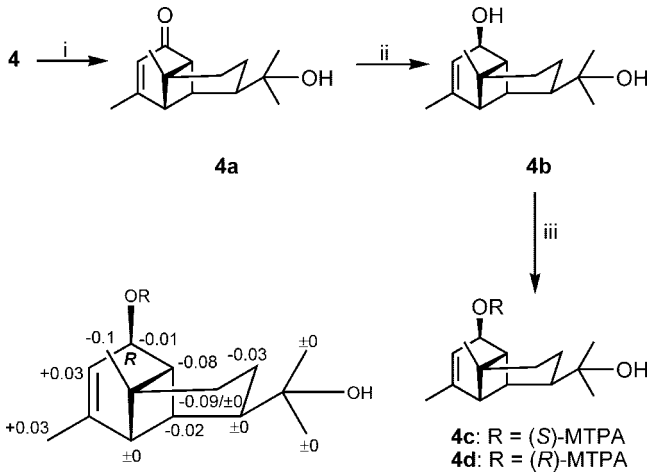
Tinosposinenside B (2): colorless, amorphous solid; $[\alpha]^{22}_D$ −19.4 (*c* 0.6, MeOH); IR (KBr) ν_{max} 3412, 2922, 2875, 1720, 1712, 1375, 1245, 1080, and 1038 cm^{-1}; ^1H and ^{13}C NMR (Table 1); ESIMS *m/z* 605.2 $[M + Na]^+$; HRFABMS *m/z* 605.2220 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_{13}Na$, 605.2210).

Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of **4** in CD_3OD

position	δ_H (*J* in Hz)	δ_C
1	2.72 dd (6.7, 1.6)	56.0
2		207.3
3	5.74 br dd (1.6, 1.4)	121.8
4		174.2
5	2.05 dd (6.7, 1.1)	58.5
6	3.08 s	55.8
7	2.09 dd (12.1, 6.0)	49.8
8	1.67 m ax	21.5
	1.80 m eq	
9	1.95 m ax	37.7
	1.83m eq	
10		58.4
11		81.6
12	1.20 s	24.5
13	1.14 s	22.5
14	2.03 d (1.4)	23.8
15	0.97 s	20.3
Glc-1′	4.43 d (7.8)	98.2
2′	3.10 dd (9.1, 7.8)	75.2
3′	3.33[a]	78.5
4′	3.23 t (8.9)	71.9
5′	3.34 m	76.5
6′	3.53 dd (11.0, 5.2)	68.7
	3.90 dd (11.0, 2.1)	
Api-1″	4.94 d (2.0)	110.9
2″	3.84 d (2.0)	78.1
3″		80.5
4″	3.73 d (9.6)	75.0
	3.91 d (9.6)	
5″	3.56 s	65.9

[a] Overlapped signals.

Scheme 4[a]



[a] Reagents and conditions: (i) naringinase, acetate buffer (pH = 4.0), 96 h, 40 °C; (ii) $NaBH_4$, EtOH; (iii) (−)- or (+)-MPTACl, dry pyridine.

Tinosposinenside C (3): colorless, amorphous solid; $[\alpha]^{22}_D$ 4.0 (*c* 1.3, MeOH); IR (KBr) ν_{max} 3445, 2922, 2874, 1735, 1721, 1372, 1237, 1074, 1027, 875 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); ESIMS *m/z* 563.2 [M + Na]$^+$; HRFABMS *m/z* 563.2112 [M + Na]$^+$ (calcd for $C_{26}H_{36}O_{12}Na$, 563.2105).

Tinosinenside (4): colorless oil; $[\alpha]^{22}_D$ −49.3 (*c* 0.6, MeOH); IR (KBr) ν_{max} 3430, 2931, 1720, 1374, 1236, 1036 cm^{-1}; ^{1}H and ^{13}C NMR (Table 2); ESIMS *m/z* 551.2 [M + Na]$^+$; HRFABMS *m/z* 551.2487 [M + Na]$^+$ (calcd for $C_{26}H_{40}O_{11}Na$, 551.2468).

Acid Hydrolysis and Determination of the Absolute Configuration of Sugars in 1−4. A solution of each of **1**−**4** (each 0.5 mg) in 1 M HCl (dioxane−H_2O, 1:1, 200 μL) was heated at 100 °C for 1 h under an Ar atmosphere. After the dioxane was removed, the solution was extracted with EtOAc (1 mL × 3) to remove the aglycone. The aqueous layer was neutralized by passing it through an ion-exchange resin (Amberlite MB-3, Organo, Tokyo, Japan) column, then concentrated under reduced pressure to dryness, to provide a residue of the sugar fraction. The residue was dissolved in pyridine (0.1 mL), to which 0.08 M L-cysteine methyl ester hydrochloride in pyridine (1.5 mL) was added. The mixture was kept at 60 °C for 1.5 h. After the mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 mL) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (0.3 mL each), and the *n*-hexane extract was analyzed by GC-MS under the following conditions: capillary column, EQUITY-1 (30 m × 0.25 mm × 0.25 μm, Supelco), column temperature, 230 °C; injection temperature, 250 °C; carrier N_2 gas. On the basis of the acid hydrolysate of **1**−**4**, D-glucose and D-apiose were confirmed by comparison of the retention times of their derivatives with those of the D-glucose, L-glucose, and D-apiose derivatives prepared in a similar way, which showed retention times of 11.02, 11.43, and 6.11 min, respectively.

Enzymatic Hydrolysis of Tinosposinenside A (1). A solution of **1** (4.3 mg) in 0.1 M acetate buffer (pH 4.0, 1.0 mL) was treated with naringinase (Sigma Chemical Co., 3.0 mg). The mixture was stirred at 40 °C for 70 h, then partitioned with EtOAc (1 mL × 3). The EtOAc layer was concentrated and further purified by reversed-phase HPLC with CH_3CN-H_2O (40:60) to give the aglycone **1a** (2.5 mg) as a colorless oil: $[\alpha]^{22}_D$ +32.1 (*c* 0.25, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (1H, t, *J* = 0.7 Hz, H-16), 7.41 (1H, t, *J* = 1.8 Hz, H-15), 6.45 (1H, dd, *J* = 1.8, 0.7 Hz, H-14), 5.69 (1H, dd, *J* = 12.4, 3.6 Hz, H-12), 5.26 (1H, br.d, *J* = 3.6 Hz, H-1), 4.06 (1H, dd, *J* = 11.0, 6.7 Hz, H-19a), 3.76 (1H, dd, *J* = 10.6, 5.0 Hz, H-19b), 2.88 (1H, ddd, *J* = 14.2, 11.5, 6.9 Hz, H-3β), 2.56 (1H, dd, *J* = 14.9, 3.7 Hz, H-11α), 2.54 (1H, br.s, H-10), 2.43 (1H, m, H-2β), 2.40 (1H, m, H-6β), 2.38 (1H, m, H-3α), 2.28 (1H, m, H-8), 2.27 (1H, m, H-7α), 2.25 (1H, m, H-2α), 2.14 (3H, s, 1-COC*H*$_3$), 1.81 (1H, m, H-7β), 1.77 (1H, td, *J* = 14.2, 3.9 Hz, H-6α), 1.74 (1H, dd, *J* = 14.9, 12.4 Hz, H-11β), 1.05 (3H, s, H_3-20); ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.2 (C-4), 171.5 (C-17), 170.2 (1-*C*OCH$_3$), 143.6 (C-15), 139.5 (C-16), 125.2 (C-13), 108.2 (C-14), 70.9 (C-12), 70.5 (C-19), 68.1 (C-1), 52.8 (C-5), 50.2 (C-8), 46.1 (C-10), 42.2 (C-11), 35.5 (C-9), 33.6 (C-3), 29.9 (C-2), 27.1 (C-20), 25.3 (C-6), 21.5 (1-CO*C*H$_3$), 18.5 (C-7).

Alkaline Hydrolysis of 1a. A solution of **1a** (2.53 mg) in NaOMe−MeOH (2.8 mg/mL, 200 μL) was stirred at room temperature for 2 h. The mixture was neutralized by an ion-exchange resin (DOWEX 500-8 H$^+$), filtered, then concentrated under reduced pressure to dryness. The residue was purified by RP-HPLC with CH_3CN-H_2O (35:65) to give two isomers, **1b** (1.1 mg) and **1c** (1.0 mg), as two colorless oils. **1b**: $[\alpha]^{20}_D$ +23.2 (*c* 0.11, $CHCl_3$); ^{1}H and ^{13}C NMR data consistent with the literature.[5] **1c**: $[\alpha]^{20}_D$ +26.5 (*c* 0.10, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (1H, t, *J* = 0.7 Hz, H-16), 7.41 (1H, t, *J* = 1.8 Hz, H-15), 6.44 (1H, dd, *J* = 1.8, 0.7 Hz, H-14), 5.30 (1H, *J* = 11.7, 5.7 Hz, H-12), 4.16 (1H, ddd, *J* = 12.4, 8.3, 3.5 Hz, H-1), 2.65 (1H, dd, *J* = 15.2, 5.8 Hz, H-11α), 2.49 (1H, dd, *J* = 12.1, 4.6 Hz, H-8), 2.45 (2H, m, H_2-3), 2.23 (1H, td, *J* = 12.4, 3.4 Hz, H-5), 2.19 (1H, m, H-2β), 2.12 (1H, dd, *J* = 15.2, 11.7 Hz, H-11β), 2.09 (1H, dq, *J* = 14.0, 3.5 Hz, H-6a), 2.04 (1H, dq, *J* = 14.2, 3.2 Hz, H-7a), 1.86 (1H, m, H-2α), 1.70 (1H, dd, *J* = 12.6, 8.5 Hz, H-10), 1.59 (1H, tdd, *J* = 14.0, 12.2, 3.7 Hz, H-7b), 1.39 (1H, tdd, *J* = 14.0, 11.7, 3.9 Hz, H-6b), 1.14 (3H, s, H_3-20); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.2 (C-4), 174.0 (C-17), 143.6 (C-15), 139.6 (C-16), 124.5 (C-13), 108.7 (C-14), 70.4 (C-1 and C-12), 56.4 (C-10), 46.8 (C-8), 46.4 (C-5), 44.8 (C-11), 37.4 (C-3), 36.8 (C-9), 34.3 (C-2), 24.1 (C-6), 20.5 (C-7), 19.3 (C-20).

(*S*)-MTPA Ester 1d and (*R*)-MTPA Ester 1e. To solutions of **1b** (each 0.5 mg) in pyridine-d_5 (0.1 mL) were separately added (*R*)-(−)-MTPACl (10 μL) and (*S*)-(+)-MTPACl (10 μL). The mixtures were stirred at room temperature for 4 h, then directly transferred into an NMR tube to measure the ^{1}H NMR spectra. (*S*)-MTPA ester **1d**: ^{1}H NMR (500 MHz, pyridine-d_5) δ 7.64−7.59 (2H, m, H-15 and H-16), 6.68 (1H, br.s, H-14), 5.72 (1H, td, *J* = 7.6, 3.2 Hz, H-1), 5.65 (1H, dd, *J* = 12.4, 3.5 Hz, H-12), 2.62 (1H, dd, *J* = 14.4, 3.6 Hz, H-11a), 2.60 (1H, td, *J* = 14.4, 3.6 Hz, H-5), 2.57 (1H, m, H-7a), 2.55 (2H, m, H_2-3), 2.51 (1H, br s, H-8) 2.42 (1H, dd, *J* = 12.6, 7.5 Hz, H-10), 2.36 (1H, m, H-2a), 2.03 (1H, m, H-6a), 1.96 (1H, m, H-2b), 1.96 (1H, dd, *J* = 14.6, 12.8 Hz, H-11b), 1.64 (2H, m, H-6b and H-7b), 1.22 (3H, s, H_3-20). (*R*)-MTPA ester **1e**: ^{1}H NMR (500 MHz, pyridine-d_5) δ 5.56 (2H, m, H-1 and H-12), 2.63 (2H, m, H_2-3), 2.56 (1H, td, *J* = 12.2, 3.5 Hz, H-5), 2.52 (1H, m, H-7a), 2.50 (1H, m, H-2a), 2.36 (1H, m, H-8), 2.35 (1H, dd, *J* = 12.4, 9.2 Hz, H-10), 2.01 (1H, m, H-6a), 1.97 (1H, m, H-2b), 1.80 (1H, dd, *J* = 14.7, 3.5 Hz, H-11a), 1.63 (1H, m, H-6b), 1.57 (1H, m, H-7b), 1.43 (1H, dd, *J* = 14.6, 12.6 Hz, H-11b), 1.00 (3H, s, H_3-20).

Enzymatic Hydrolysis of Tinosposinenside B (2). A solution of **2** (8.0 mg) in 0.1 M acetate buffer (pH 4.0, 1.0 mL) was treated with naringinase (6.0 mg). The mixture was stirred at 40 °C for 115 h, then treated with a Sep-Pak cartridge, eluted with H_2O and MeOH, to afford subfractions A1 and A2. Subfraction A2 was chromatographed on Si gel CC with $CHCl_3$−MeOH (98:2) to yield **2a** (5.2 mg) as a colorless oil: $[\alpha]^{22}_D$ −23.2 (*c* 0.52, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (1H, t, J = 0.6 Hz, H-16), 7.43 (1H, t, J = 1.8 Hz, H-15), 6.40 (1H, dd, J = 1.8, 0.6 Hz, H-14), 5.62 (1H, dd, J = 12.5, 3.3 Hz, H-12), 5.49 (1H, q, J = 3.0 Hz, H-2), 4.95 (1H, dd, J = 11.3, 3.2 Hz, H-1), 3.75 (1H, q, J = 2.7 Hz, H-4), 2.44 (1H, t, J = 11.3 Hz, H-10), 2.44 (2H, m, H_2-7), 2.42 (1H, dd, J = 14.4, 3.4 Hz, H-11α), 2.33 (1H, m, H-8), 2.16 (1H, ddd, J = 15.6, 3.2, 2.8 Hz, H-3β), 2.15 (3H, s, 2-COCH_3), 1.95 (3H, s, 1-COCH_3), 1.88 (1H, dt, J = 15.5, 3.4 Hz, H-3α), 1.76 (1H, dd, J = 14.5, 12.5 Hz, H-11β), 1.61 (1H, tt, J = 11.7, 2.7 Hz, H-5), 1.56 (2H, m, H-6), 1.14 (3H, s, H_3-20; ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4 (C-17), 169.4 (1-*C*OCH_3 and 2-*C*OCH_3), 143.9 (C-15), 139.1 (C-16), 125.6 (C-13), 108.2 (C-14), 73.5 (C-1), 70.9 (C-12), 70.5 (C-2), 69.3 (C-4), 51.4 (C-8), 43.8 (C-11), 40.5 (C-5), 35.2 (C-9), 34.5 (C-10), 34.2 (C-3), 25.5 (C-6), 22.1 (C-20), 21.6 (C-7), 21.2 (1-CO*C*H_3 and 2-CO*C*H_3).

(*S*)-MTPA Ester 2b and (*R*)-MTPA Ester 2c. To solutions of **2a** (each 1.0 mg) in dry pyridine (0.1 mL) were separately added (*R*)-(−)-MTPACl (10 μL) and (*S*)-(+)-MTPACl (10 μL). The mixtures were stirred at room temperature for 4 h. After the addition of MeOH and evaporation of the solvent, the residue was purified by RP-HPLC with MeOH−H_2O (70:30) to give the (*S*)-MTPA ester **2b** (1.3 mg) and (*R*)-MTPA ester **2c** (1.2 mg) as two colorless oils. (*S*)-MTPA ester **2b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (2H, m, Ph), 7.42 (5H, m, Ph, H-15 and H-16), 6.36 (1H, t, J = 1.5 Hz, H-14), 5.52 (1H, dd, J = 12.1, 3.2 Hz, H-12), 5.42 (1H, q, J = 3.2 Hz, H-2), 5.35 (1H, q, J = 2.7 Hz, H-4), 4.99 (1H, dd, J = 10.8, 3.2 Hz, H-1), 3.50 (3H, s, OCH_3), 2.58 (1H, t, J = 11.0 Hz, H-10), 2.48 (1H, dd, J = 14.5, 3.5 Hz, H-11α), 2.40 (1H, dq, J = 14.0, 2.7 Hz, H-7α), 2.31 (1H, m, H-8), 2.30 (1H, ddd, J = 16.2, 3.0, 2.3 Hz, H-3β), 2.05 (1H, dt, J = 16.2, 3.6 Hz, H-3α), 1.97 (3H, s, 2-COCH_3), 1.89 (3H, s, 1-COCH_3), 1.85 (1H, m, H-5), 1.75 (1H, m, H-7β), 1.71 (1H, dd, J = 14.7, 12.6 Hz, H-11β), 1.58 (1H, m, H-6β), 1.44 (1H, td, J = 13.8, 3.9 Hz, H-6α), 1.11 (3H, s, H_3-20). (*R*)-MTPA ester **2c**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (2H, m, Ph), 7.42 (5H, m, Ph, H-15 and H-16), 6.37 (1H, t, J = 1.4 Hz, H-14), 5.52 (1H, dd, J = 12.4, 3.5 Hz, H-12), 5.32 (1H, q, J = 3.5 Hz, H-2), 5.21 (1H, m, H-4), 4.96 (1H, dd, J = 10.7, 3.2 Hz, H-1), 3.56 (3H, s, OCH_3), 2.54 (1H, t,, J = 11.0 Hz, H-10), 2.46 (1H, dd, J = 14.4, 3.2 Hz, H-11α), 2.40 (1H, dq, J = 13.5, 3.0 Hz, H-7α), 2.33 (1H, m, H-8), 2.21 (1H, ddd, J = 16.3, 3.5, 2.3 Hz, H-3β), 2.00 (1H, dt, J = 16.3, 3.9 Hz, H-3α), 1.94 (3H, s, 2-COCH_3), 1.70 (3H, s, 1-COCH_3), 1.85 (1H, m, H-5), 1.75 (1H, m, H-7β), 1.72 (1H, dd, J = 14.4, 12.3 Hz, H-11β), 1.58 (1H, m, H-6β), 1.49 (1H, td, J = 12.6, 3.7 Hz, H-6α), 1.11 (3H, s, H_3-20).

Acetylation of Tinosposinensides B (2) and C (3). To solutions of **2** and **3** (each 5.0 mg) in dry pyridine (0.5 mL) was added Ac_2O (each 0.5 mL). The mixtures were stirred at room temperature for 24 h, then chromatographed on Si gel CC with $CHCl_3$−MeOH (98:2) and purified by reversed-phase HPLC with MeOH−H_2O (60:40) to afford the same product, **2d** (each 4.0 mg), as a colorless oil: $[\alpha]^{20}_D$ −18.9 (*c* 0.40, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (1H, br s, H-16), 7.42 (1H, t, J = 1.8 Hz, H-15), 6.38 (1H, dd, J = 1.8, 0.9 Hz, H-14), 5.56 (1H, dd, J = 12.4, 3.2 Hz, H-12), 5.30 (1H, q, J = 3.4 Hz, H-2), 5.17 (1H, t, J = 8.9 Hz, Glc-H-3′), 5.09 (1H, t, J = 9.4 Hz, Glc-H-4′), 4.97 (1H, dd, J = 8.7, 7.6 Hz, Glc-H-2′), 4.93 (1H, dd, J = 10.6, 3.2 Hz, H-1), 4.61 (1H, d, J = 7.4 Hz, Glc-H-1′), 4.20 (1H, dd, J = 12.4, 3.0 Hz, Glc-H-6′a), 4.15 (1H, dd, J = 12.1, 4.8 Hz, Glc-H-6′b), 3.84 (1H, br s, H-4), 3.65 (1H, ddd, J = 10.1, 4.8, 2.8 Hz, Glc-H-5′), 2.50 (1H, t, J = 10.8 Hz, H-10), 2.43 (1H, dd, J = 14.4, 3.4 Hz, H-11a), 2.42 (2H, m, H-7), 2.29 (1H, br s, H-8), 2.18 (1H, ddd, J = 15.8, 3.6, 2.2 Hz, H-3a), 2.10−1.95 (COCH_3 × 6), 1.79 (1H, m, H-6a), 1.77 (1H, dt, J = 15.6, 3.8 Hz, H-3b), 1.71 (1H, dd, J = 14.2, 12.4 Hz, H-11b), 1.65 (1H, tt, J = 11.7, 3.2 Hz, H-5), 1.45 (1H, br d, J = 13.5 Hz, H-6b), 1.09 (3H, s, H_3-20); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1−169.0 (*C*OCH_3 × 6 and C-17), 143.8 (C-15), 139.0 (C-16), 125.7 (C-13), 108.2 (C-14), 99.2 (Glc-C-1′), 75.5 (C-4), 73.6 (C-1), 73.2 (Glc-C-3′), 71.8 (Glc-C-2′ and Glc-C-5′), 70.6 (C-12), 68.5 (C-2 and Glc-C-4′), 61.9 (Glc-C-6′), 51.2 (C-8), 43.6 (C-11), 39.3 (C-5), 35.1 (C-9), 35.0 (C-10), 30.9 (C-3), 25.4 (C-6), 21.9 (C-20), 21.7 (C-7), 21.3−20.6 (CO*C*H_3 × 6).

Enzymatic Hydrolysis of Tinosinenside (4). A solution of **4** (9.0 mg) in 0.1 M acetate buffer (pH 4.0, 1.0 mL) was treated with naringinase (12.0 mg). After stirring at 40 °C for 96 h, the mixture was partitioned with EtOAc (1 mL × 3). The EtOAc fraction was concentrated, and the residue was purified by reversed-phase HPLC with MeOH−H_2O (60:40) to give the aglycone **4a** (2.9 mg) as a colorless oil: $[\alpha]^{22}_D$ +21.0 (*c* 0.29, $CHCl_3$). Compound **4a** was identified by comparison of the spectroscopic data (^{1}H and ^{13}C NMR and MS) with the reported values.[8]

$NaBH_4$ Reduction of 4a. To a solution of **4a** (2.9 mg) in EtOH (1.0 mL) was added $NaBH_4$ (4.0 mg). The mixture was stirred at room temperature for 24 h, then neutralized with an ion-exchange resin (DOWEX 500-8 H^+), filtrated, and concentrated. The residue was isolated by RP-HPLC with MeOH−H_2O (55:45) to yield **4b** (1.8 mg) as a colorless oil: $[\alpha]^{22}_D$ −26.5 (*c* 0.08, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39 (1H, m, H-3), 4.42 (1H, br.s, H-2), 2.38 (1H, m, H-1), 2.06 (1H, s, H-6), 1.88 (1H, m, H-9a), 1.78 (1H, dd, J = 11.2, 5.0 Hz, H-7), 1.72 (3H, t, J = 1.7 Hz, H_3-14), 1.66 (1H, m, H-9b), 1.62 (1H, m, H-8a), 1.58 (1H, m, H-8b), 1.52 (1H, dd, J = 6.2, 1.3 Hz, H-5), 1.13 (3H, s, H_3-12), 1.10 (3H, s, H_3-13), 1.01 (3H, s, H_3-15).

(*S*)-MTPA Ester 4c and (*R*)-MTPA Ester 4d. To solutions of **4b** (each 0.9 mg) in pyridine-d_5 (0.6 mL) were separately added (*R*)-(−)-MTPACl (10 μL) and (*S*)-(+)-MTPACl (10 μL). The mixtures were stirred at room temperature for 4 h and transferred into an NMR tube to measure the ^{1}H NMR spectra. (*S*)-MTPA ester **4c**: ^{1}H NMR (500 MHz, pyridine-d_5) δ 6.04 (1H, br s, H-2), 5.58 (1H, br s, H-3), 2.77 (1H, br s, H-1), 2.64 (1H, s, H-6), 2.01 (1H, t, J = 8.8 Hz, H-7), 1.77 (1H, dd, J = 11.7, 6.9 Hz, H-9a), 1.70 (2H, m, H_2-8), 1.67 (1H, dd, J = 11.4, 6.8, Hz, H-9b), 1.65 (3H, t, J = 1.6 Hz, H_3-14), 1.61 (1H, dd, J = 6.2, 0.9 Hz, H-5), 1.33 (3H, s, H_3-12), 1.30 (3H, s, H_3-13), 0.88 (3H, s, H_3-15). (*R*)-MTPA ester **4d**: ^{1}H NMR (500 MHz, pyridine-d_5) δ 6.05 (1H, br.s, H-2), 5.55 (1H, br.s, H-3), 2.85 (1H, br s, H-1), 2.66 (1H, s, H-6), 2.01 (1H, dd, J = 10.6, 7.1 Hz, H-7), 1.86 (1H, dd, J = 11.7, 6.9 Hz, H-9a), 1.73 (2H, m, H_2-8), 1.67 (1H, dd, J = 11.4, 6.8 Hz, H-9b), 1.62 (3H, s, H_3-14), 1.61 (1H, m, H-5), 1.33 (3H, s, H_3-12), 1.30 (3H, s, H_3-13), 0.98 (3H, s, H_3-15).

α-Glucosidase Inhibition Assay.[9] α-Glucosidase from Baker's yeast was purchased from the Sigma Chemical Co. (St. Louis, MO). α-Glucosidase (25 μL, 0.2 U/mL), 25 μL of various concentrations of samples, and 175 μL of 67 mM phosphate buffer (pH 6.8) were mixed at room temperature for 10 min. The reaction was started by the addition of 25 μL of 23.2 mM *p*-nitrophenyl-α-D-glucopyranoside. The reaction mixture was incubated for 15 min at 37 °C in a final volume of 0.25 mL, then 50 μL of 1 M Na_2CO_3 was added to the incubation solution to stop the reaction. The activities of glucosidase were detected in a 96-well plate, and the absorbance was determined at 405 nm (for *p*-nitrophenol). The negative control was prepared by adding phosphate buffer instead of the sample in the same way as the test. Acarbose was utilized as the positive control. The blank was prepared by adding phosphate buffer instead of the α-glucosidase using the same method. The inhibition rates (%) = $[(OD_{\text{negative control}} - OD_{\text{blank}}) - (OD_{\text{test}} - OD_{\text{test blank}})]/(OD_{\text{negative blank}} - OD_{\text{blank}}) \times 100\%$. IC_{50} values of the sample were calculated using the IC_{50} calculative software.

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