Antisweet Saponins from *Gymnema sylvestre*

Wencai Ye,[†,‡] Xin Liu,[‡] Qingwen Zhang,[‡] Chun-Tao Che,*[,†,§] and Shouxun Zhao[‡]

Department of Chemistry, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, Department of Phytochemistry, China Pharmaceutical University, Nanjing, People's Republic of China, and School of Chinese Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong

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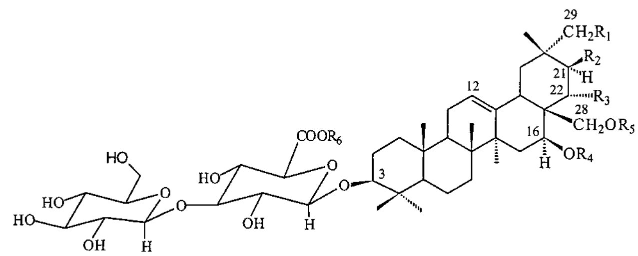
Three new oleanane-type triterpene glycosides (**1**−**3**), along with the sodium salt of alternoside II (**4**), were isolated from an ethanol extract of the leaves of *Gymnema sylvestre*. The structures of these new saponins were identified as 21*β*-*O*-benzoylsitakisogenin 3-*O*-*β*-D-glucopyranosyl(1→3)-*β*-D-glucuronopyranoside (**1**), the potassium salt of longispinogenin 3-*O*-*β*-D-glucopyranosyl(1→3)-*β*-D-glucuronopyranoside (**2**), and the potassium salt of 29-hydroxylongispinogenin 3-*O*-*β*-D-glucopyranosyl(1→3)-*β*-D-glucuronopyranoside (**3**). The aglycon of **3**, gymnemagenol (**3a**), was characterized as 3*β*,16*β*,28,29-tetrahydroxyolean-12-ene. Structure elucidation was accomplished by interpretation of NMR (DQF-COSY, HMQC, and HMBC) results, FABMS, and hydrolysis. Saponin **1** and the sodium salt of alternoside II (**4**) exhibited antisweet activity.

Gymnema sylvestre R. Br. (Asclepiadaceae) is distributed in India and the southwestern region of the People's Republic of China. It has a reputation as a traditional remedy to control diabetes mellitus.[1] In addition, the leaves of this plant, popularly known as "Gur-mar" in India, are used for inhibiting the taste of sweetness.[2] As early as 1887, it was noted that the antisweet ingredients could be precipitated from the water extract by acidification with mineral acid, and the substance thus obtained was named "gymnemic acid".[3,4] Subsequently, a number of oleanane-type triterpene saponins with different sapogenin structures such as gymnemagnenin, 23-hydroxylongispinogenin, and gymnestrogenin, as well as certain dammarane derivatives, have been purified from plants growing in India.[2,5−7] The antisweet properties of the extracts as well as some of the saponin components have also been reported.[2,8−10]

In a continuation of our study on saponin constituents of medicinal plants, we have examined the saponin fraction of *G. sylvestre* leaves collected from the People's Republic of China. In this paper, we report on the isolation of three new triterpene saponins (**1**−**3**), along with a known compound (**4**). The antisweet activity of these substances was tested.

An aqueous ethanol extract of the leaves was chromatographed on a D101 macroporous resin column to afford a saponin-rich fraction. From this fraction, saponins **1**−**4** were obtained following repeated chromatographic procedures.

The FABMS of compound **1** displayed signals of quasi-molecular ions $[M + H]^+$ and $[M + Na]^+$ at *m/z* 917 and 939, respectively. Combining with the ^{13}C NMR spectral data, a molecular formula of $C_{49}H_{72}O_{16}$ was derived for **1**. The NMR spectra suggested the presence of two sugar residues, a benzoyl group, an olefinic group, a hydroxymethyl, three oxymethines, and seven methyl groups. Interpretation of the 2D NMR (DQF-COSY, HMQC, and HMBC) spectra led to the assignment of all proton and carbon resonances (Tables 1 and 2). Further analysis of



1 $R_1 = R_3 = R_4 = R_5 = R_6 = H$, R_2 = O−CO−C$_6H_5$ (7′, 1′–6′)

2 $R_1 = R_2 = R_3 = R_4 = R_5 = H$, $R_6 = K^+$

3 $R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = H$, $R_6 = K^+$

4 $R_1 = R_2 = H$, $R_3 = OH$, $R_4 = Ac$, R_5 = rhamnopyranosyl, $R_6 = Na^+$

NMR data suggested that the aglycon could be a substituted oleanane-type triterpene. The ^1H and ^{13}C NMR resonances of the sugar moiety indicated the presence of glucuronic acid and glucose, displaying anomeric carbon and proton signals at δ_C 106.6 and 105.9 and at δ_H 4.84 (d, $J = 7.2$ Hz) and 5.25 (d, $J = 7.2$ Hz), respectively. Acid hydrolysis of **1** afforded a mixture of D-glucose, D-glucuronic acid, and benzoic acid, all identified by high-performance thin-layer chromatography (HPTLC), and sitakisogenin, which was identified by comparison of NMR data with literature values.[11] The locations of the sugar moieties and the benzoyloxy group on the aglycon were subsequently deduced from the 2D NMR data. Thus, in the HMBC spectrum of **1**, correlation signals were observed between H-1 (δ_H 4.84) of glucuronic acid and C-3 (δ_C 89.0) of the aglycon and between H-21 (δ_H 5.70) of the aglycon and the carboxylic carbon (C-7′, δ_C 166.3) of the benzoyloxy group. This evidence suggested that the glucuronic acid and the benzoyloxy group are attached to the C-3 and C-21 positions, respectively. In addition, long-range coupling was observed between H-1 (δ_H 5.25) of the glucose unit and C-3 (δ_C 87.4) of the glucuronic acid unit. Hence, compound **1** was established to be 21*β*-*O*-benzoylsitakisogenin 3-*O*-*β*-D-glucopyranosyl(1→3)-*β*-D-glucuronopyranoside.

Saponin **2** was obtained as an amorphous powder. The ^{13}C and DEPT NMR spectra displayed 42 signals, of which

* To whom correspondence should be addressed. Tel: (852) 2609-8130. Fax: (852) 2603-7203. E-mail: chect@cuhk.edu.hk.
† Hong Kong University of Science and Technology.
‡ China Pharmaceutical University.
§ The Chinese University of Hong Kong.

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Table 1. ^{13}C NMR Data of the Aglycons of **1**−**4** and **3a**[a]

carbon	1	2	3	4	3a
1	38.7	39.0	39.0	38.8	39.4
2	26.5	26.7	26.7	26.5	28.4
3	89.0	89.3	89.2	88.7	78.3
4	39.5	39.8	39.7	39.5	39.7
5	55.6	55.9	55.8	55.6	56.0
6	18.4	18.7	18.7	18.5	19.1
7	32.9	33.2	33.2	33.1	33.3
8	40.1	40.4	40.4	40.4	40.5
9	47.0	47.4	47.3	47.0	47.5
10	36.7	36.9	37.0	36.8	37.5
11	23.9	24.2	24.1	24.1	24.2
12	123.1	122.9	122.8	123.0	122.8
13	142.6	144.0	144.1	141.8	144.2
14	43.7	44.1	44.1	43.1	44.1
15	36.7	37.0	37.0	33.8	37.0
16	66.4	67.0	67.1	67.9	67.1
17	43.7	41.3	41.7	45.4	41.8
18	44.2	44.7	44.0	44.1	44.2
19	47.2	47.3	41.7	46.7	41.9
20	36.0	31.4	42.1	31.9	42.2
21	75.6	34.6	29.2	43.4	29.3
22	33.3	26.4	25.9	67.5	26.0
23	28.1	28.5	28.4	28.1	29.0
24	16.9	17.2	17.2	17.1	16.9
25	15.7	16.0	16.0	15.8	16.1
26	17.0	17.4	17.3	17.4	17.3
27	27.0	27.5	27.5	27.6	27.4
28	66.7	69.0	69.1	65.1	69.2
29	29.2	33.8	74.2	33.4	74.2
30	18.8	24.4	20.4	25.2	20.4
Ph-CO moiety					
1′	131.5				
2′	129.9				
3′	128.9				
4′	133.2				
5′	128.9				
6′	129.9				
7′	166.3				
Ac moiety					
C=O				170.6	
Me				22.1	

[a] Recorded in pyridine-d_5. Assignments were established by ^{13}C DEPT, HMQC, and HMBC spectra.

12 could be readily assigned to the saccharide portion and the remaining 30 to a triterpene skeleton. Acid hydrolysis of **2** afforded longispinogenin (identified by comparison with literature values),[11] glucuronic acid, and glucose (determined by HPTLC). Further comparison of the ^{13}C NMR data of **2** with those of longispinogenin revealed a glycosylation shift at the C-3 position. The sequence of sugar residues was subsequently determined by long-range NMR studies. Thus, in the HMBC spectrum of **2**, correlations were observed between H-1 (δ_H 5.29) of glucose and C-3 (δ_C 87.2) of glucuronic acid, as well as between H-1 (δ_H 4.82) of glucuronic acid and C-3 (δ_C 89.3) of the aglycon. The above finding led to the conclusion that **2** has the same sugar chain at C-3 of the aglycon as in compound **1**. Interestingly, the carbonyl carbon signal of glucuronic acid in **2** displayed a downfield shift (+4.1 ppm) when compared to that in **1**, consistent with an ionization state of the carboxylic acid group. Indeed, the results of an inductively coupled plasma-mass spectrum (ICPMS) indicated the presence of potassium ion in a sample of **2**. Furthermore, the HRESIMS of **2** showed an $[(M\cdot K) + H]^+$ ion at *m/z* 835.4065, in accordance with the molecular formula $C_{42}H_{67}O_{14}K$ [calcd for $C_{42}H_{68}O_{14}K$, *m/z* 835.4046]. These findings led to the assignment of **2** as the potassium salt of longispinogenin 3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucuronopyranoside.

Saponin **3** displayed NMR properties similar to those of **2**, especially in the ^1H and ^{13}C NMR chemical shifts of the saccharide portion (Tables 1 and 2), indicating the presence of the same sugar chain at the C-3 position of the aglycon. Compound **3** was found to have an additional hydroxyl group in the aglycon, however, when compared to **2**. Acid hydrolysis of **3** afforded D-glucuronic acid and D-glucose and a sapogenin, which has been named gymnemagenol (**3a**). The EIMS of **3a** displayed a molecular ion at *m/z* 474, together with two retro-Diels−Alder fragment ions at *m/z* 207 and 266, suggesting the presence of three hydroxyl groups in the D/E rings and one hydroxyl group in the A/B rings. The ^{13}C NMR and DEPT spectra of gymnemagenol (**3a**) revealed the presence of six methyl groups, an olefinic bond, two oxymethines, and two hydroxymethyl groups. Comparison of the carbon signals with model compounds[12,13] allowed an assignment of a hydroxyl group at the C-29 position. Furthermore, the location of the hydroxylmethyl substitute could be confirmed by a selective INEPT experiment. Thus, irradiation of CH_3-30 (δ_H 1.21) enhanced the signal at δ_C 74.2 (C-29), indicating that C-29 was substituted by a hydroxyl group. The structure of gymnemagenol (**3a**) was therefore defined as 3β,16β,28,29-tetrahydroxyolean-12-ene.

The FABMS of **3** displayed an $[(M\cdot K) + H]^+$ ion peak at *m/z* 851, suggesting a potassium salt structure. Indeed, the carboxylic carbon signal of the glucuronic acid residue in **3** showed a significant downfield shift (+4.6 ppm) in comparison to glucuronic acid in the nonionized state such as this unit in **1**. Results obtained from the ICPMS further supported the presence of potassium in **3**. Compound **3** is thus the potassium salt of 29-hydroxylongispinogenin 3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucuronopyranoside.

Saponin **4** was obtained as a white powder. The ^1H and ^{13}C NMR spectra of **4** indicated the presence of an acetyl group, three sugar residues and a triterpene skeleton possessing an olefinic bond, one hydroxymethyl group, three oxymethines, and seven methyl groups. Acid hydrolysis of **4** afforded D-glucuronic acid, D-glucose, L-rhamnose, and chichipegenin (identified by comparison with literature values).[14] A comparison of ^{13}C NMR data of **4** with those of chichipegenin indicated that the triterpene was glycosylated at C-3 and C-28. The sequences of sugar chains were subsequently deduced from an HMBC experiment. Thus, the HMBC spectrum of **4** exhibited correlations between H-1 (δ_H 5.31) of rhamnose and C-28 (δ_C 65.1) of the aglycon, between H-1 (δ_H 4.81) of glucuronic acid and C-3 (δ_C 88.7) of the aglycon, and between H-1 (δ_H 5.25) of glucose and C-3 (δ_C 87.0) of glucuronic acid. These spectroscopic data led to the conclusion that **4** has the same structure as alternoside II, which was isolated initially from *Gymnema alternifolium*.[14] The ICPMS results indicated the presence of sodium in the sample of **4**; the isolated compound was therefore a sodium salt. This was further supported by the presence of an $[(M\cdot Na) + H]^+$ ion peak at *m/z* 1023 in the FABMS and by comparison of the carboxylic carbon shift of the glucuronic acid residue with that of alternoside II. All available evidence led to the conclusion that **4** was a sodium salt of alternoside II.

The antisweet properties of saponins **1**−**4** were evaluated by using the method of Kurihara and co-workers.[15] A solution (1 mM) of **1** and the sodium salt of alternoside II (**4**) was found to completely suppress the sensation of sweetness induced by sucrose (0.2 M). Such an activity was comparable to that reported for alternoside II.[14] Compounds **2** and **3** were inactive even when tested at a lower concentration of sucrose solution (0.1 M). This finding seems to indicate that the antisweet activity of these type of triterpene saponins is related to the presence of acyl

Table 2. ^{1}H and ^{13}C NMR Data of the Saccharide Portions of **1**−**4**[a,b]

	1		**2**		**3**		**4**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
GlcA 1	4.84 d (7.2)	106.6	4.82 d (7.2)	106.3	4.80 d (7.2)	106.2	4.81 d (7.2)	106.0
2	4.05[c]	74.4	4.03[c]	74.7	4.02[c]	74.5	4.03[c]	74.5
3	4.26[c]	87.4	4.28[c]	87.2	4.27[c]	87.5	4.26[c]	87.0
4	4.27[c]	71.8	4.30[c]	72.2	4.26[c]	72.1	4.29[c]	72.0
5	4.46[c]	77.3	4.41[c]	76.6	4.40[c]	76.3	4.41[c]	76.6
6		172.2		176.3		176.8		175.6
Glc 1	5.25 d (7.2)	105.9	5.29 d (7.2)	105.6	5.24 d (7.2)	105.6	5.25 d (7.2)	105.3
2	4.01[c]	75.6	4.05[c]	75.5	4.01[c]	75.4	4.02[c]	75.6
3	4.21[c]	78.2	4.22[c]	78.3	4.20[c]	78.2	4.21[c]	78.2
4	4.10[c]	71.6	4.12[c]	71.7	4.06[c]	71.6	4.09[c]	71.5
5	4.05[c]	78.7	4.05[c]	78.9	4.01[c]	78.8	4.03[c]	78.7
6	4.27[c]	62.4	4.28[c]	62.6	4.28[c]	62.5	4.28[c]	62.4
	4.57 dd (10.4, 4.0)		4.56 dd (10.4, 4.0)		4.56 dd (10.4, 4.0)		4.57 dd (10.4, 4.0)	
Rha 1							5.31 br s	101.7
2							4.46[c]	72.4
3							4.40[c]	73.1
4							4.32[c]	73.9
5							4.12 m	69.9
6							1.70 d (6.0)	19.0

[a] Recorded in pyridine-d_5. Assignments were established by ^{13}C DEPT, DQF-COSY, HMQC, and HMBC spectra. [b] *J* values (in Hz) in parentheses. [c] Overlapped signals.

groups on D/E rings. This is consistent with the view that ester groups in the genin of "gymnemic acids" can play an important role in the manifestation of antisweet activity.[16]

Experimental Section

General Experimental Procedures. Melting points were uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 16 PC FT-IR. ^{1}H (400 or 500 MHz), ^{13}C (100 or 125 MHz), and 2D NMR spectra were recorded on JEOL JNM-EX 400 and Varian Unity INOVA-500 spectrometers. ICPMS were recorded on a Perkin-Elmer SCIEX ELAN-6000 spectrometer. FAB(and EI)MS and HRESIMS were determined in the positive-ion mode on Finnigan MAT TSQ7000 and Bruker APEX FT-MS instruments, respectively. Column chromatography was carried out using octodecyl Si gel (ODS) (10−40 μm, Merck), Sephadex LH-20, and D101 macroporous resin (40−60 mesh, Tian-Jing Agricultural Chemical Ltd., People's Republic of China) as stationary phases. TLC was conducted on Si gel 60 F_{254} and RP-18 F_{254} S plates (Merck).

Plant Material. The leaves of *Gymnema sylvestre* R. Br. were collected in October 1996, from Beihai City, Guangxi Autonomous Region of China. They were authenticated by Dr. Qin Min-Jang. Voucher specimens (No. 96047) were deposited in the Herbarium of China Pharmaceutical University, Nanjing, People's Republic of China.

Extraction and Isolation. Dried leaves of *G. sylvestre* (2.0 kg) were extracted with 60% EtOH (3 × 2000 mL). The extract was filtered, concentrated, and chromatographed on a D101 resin column (40−60 mesh, 800 g) using EtOH/H_2O mixtures (15:85→75:25) as eluents to yield a crude saponin mixture (6.8 g). This fraction was further separated on a medium-pressure reversed-phase C_{18} column using MeOH/H_2O mixtures (20:80→70:30) as eluents to afford **1** (85 mg), **2** (40 mg), **3** (110 mg), and **4** (80 mg).

Compound 1: amorphous powder; mp 226−228 °C; $[\alpha]_D^{20}$ +15.4° (*c* 0.16, MeOH); IR (KBr) ν_{max} 3422, 2948, 1702, 1636, 1460, 1388, 1282, 1160, 1076, 1026 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) δ_H 0.84 (3H, s, Me-25), 0.97 (3H, s, Me-24), 0.98 (3H, s, Me-26), 1.01 (3H, s, Me-30), 1.30 (3H, s, Me-23), 1.32 (3H, s, Me-29), 1.37 (3H, s, Me-27), 3.17 (1H, dd, *J* = 4.5 and 13.3 Hz, H-3α), 3.37 (1H, d, *J* = 10.0 Hz, H-28a), 4.40 (1H, d, *J* = 10.0 Hz, H-28b), 4.66 (1H, m, H-16α), 5.37 (1H, br s, H-12), 5.70 (1H, dd, *J* = 4.6 and 12.2 Hz, H-21α), 7.49 (3H, overlapped, H-3′, -4′, and -5′), 8.26 (2H, dd, *J* = 1.3 and 7.2 Hz, H-2′ and -6′); ^{1}H NMR data of the saccharide residues, see Table 2; ^{13}C NMR (125 MHz, pyridine-d_5), see Tables 1 and 2; FABMS *m*/*z* 917 $[M + H]^+$, 939 $[M + Na]^+$.

Compound 2: amorphous powder; mp 305−310 °C; $[\alpha]_D^{20}$ +18.1° (*c* 0.08, MeOH); IR (KBr) ν_{max} 3440, 2948, 1636, 1420, 1078, 1028 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5) δ_H 0.80 (3H, s, Me-25), 0.95 (3H, s, Me-29), 0.97 (3H, s, Me-24), 0.98 (3H, s, Me-26), 1.01 (3H, s, Me-30), 1.26 (3H, s, Me-23), 1.38 (3H, s, Me-27), 3.30 (1H, dd, *J* = 4.0 and 12.1 Hz, H-3α), 3.70 (1H, d, *J* = 10.4 Hz, H-28a), 4.56 (1H, d, *J* = 10.4 Hz, H-28b), 4.69 (1H, m, H-16α), 5.30 (1H, br s, H-12); ^{1}H NMR data of the saccharide residues, see Table 2; ^{13}C NMR (100 MHz, pyridine-d_5), see Tables 1 and 2; HRESI-MS *m*/*z* 835.4065 [(M·K) + H]$^+$ (calcd for $C_{42}H_{68}O_{14}K$, 835.4046).

Compound 3: amorphous powder; mp 290−293 °C; $[\alpha]_D^{20}$ +10.3° (*c* 0.12, MeOH); IR (KBr) ν_{max} 3422, 2928, 1618, 1430, 1028 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5) δ_H 0.80 (3H, s, Me-25), 0.96 (3H, s, Me-24), 0.99 (3H, s, Me-26), 1.21 (3H, s, Me-30), 1.24 (3H, s, Me-23), 1.46 (3H, s, Me-27), 3.28 (1H, m, H-3α), 4.01(1H, d, *J* = 7.6 Hz, H-29a), 4.06 (1H, d, *J* = 7.6 Hz, H-29b), 3.73 (1H, d, *J* = 10.4 Hz, H-28a), 4.45 (1H, *J* = 10.4 Hz, H-28b), 4.79 (1H, m, H-16α), 5.30 (1H, br s, H-12); ^{1}H NMR data of the saccharide residues, see Table 2; ^{13}C NMR (100 MHz, pyridine-d_5), see Tables 1 and 2; FABMS *m*/*z* 851 $[(M·K) + H]^+$.

Compound 4: amorphous powder; mp 294−296 °C; $[\alpha]_D^{20}$ +1.5° (*c* 0.19, MeOH); IR (KBr) ν_{max} 3418, 2948, 1738, 1713, 1621, 1430, 1374, 1266, 1076, 1031 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5) δ_H 0.84 (3H, s, Me-25), 0.93 (3H, s, Me-29), 0.94 (3H, s, Me-24), 0.98 (3H, s, Me-26), 1.18 (3H, s, Me-30), 1.25 (3H, s, Me-23), 1.44 (3H, s, Me-27), 2.13 (3H, s, Ac), 3.30 (1H, m, H-3α), 4.28 (1H, d, *J* = 10.0 Hz, H-28a), 4.96 (1H, *J* = 10.0 Hz, H-28b), 4.52 (1H, m, H-16α), 5.31 (1H, br s, H-12); ^{1}H NMR data of the saccharide residues, see Table 2; ^{13}C NMR (100 MHz, pyridine-d_5), see Tables 1 and 2; FABMS *m*/*z* 1023 $[(M·Na) + H]^+$.

Acid Hydrolysis of 1−4. The saponins (each 15−25 mg) were refluxed with 15% HCl in 60% EtOH (5 mL) for 12−18 h. Each reaction mixture was diluted with H_2O, neutralized with Ag_2CO_3, and extracted with EtOAc. The EtOAc extract was purified on a Sephadex LH-20 column using MeOH as eluant to afford the aglycon. The sapogenins of **1**, **2**, and **4** were identified as sitakisogenin,[11] longispinogenin,[11] and chichipegenin,[14] respectively, by comparing their NMR data with the literature values. The structure of sapogenin **3a** was elucidated by NMR and MS techniques. The neutral hydrolysate revealed the presence of glucose, glucuronic acid, rhamnose (only in **4**), and benzoic acid (only in **1**) by HPTLC [$CHCl_3$/CH_3OH/H_2O (6.5:3.5:1), lower layer] when compared with authentic samples.

Gymnemagenol (3a): colorless needles; mp 198−201 °C; $[\alpha]_D^{20}$ +68.5° (*c* 0.10, MeOH); IR (KBr) ν_{max} 3420, 2930, 1622,

1428 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5) δ_H 0.92 (3H, s, Me-25), 1.04 (3H, s, Me-24), 1.06 (3H, s, Me-26), 1.21 (6H, s, Me-23 and 30), 1.35 (3H, s, Me-27), 3.45 (1H, dd, J = 6.0 and 10.0 Hz, H-3α), 3.74 (1H, d, J = 10.8 Hz, H-28a), 4.03 (1H, d, J = 7.6 Hz, H-29a), 4.07 (1H, d, J = 7.6 Hz, H-29b), 4.47 (1H, J = 10.8 Hz, H-28b), 4.76 (1H, dd, J = 4.4 and 11.2 Hz, H-16α), 5.32 (1H, br s, H-12); ^{13}C NMR (100 MHz, pyridine-d_5), see Table 1; EIMS m/z 474 [M]$^+$, 266, 207.

Determination of Antisweet Activity. Solutions of compounds **1**−**4** (5 mL, 1 mM) and alternoside II (positive control) were evaluated by four adult volunteers. Each participant held the solution in the mouth for 5 min, expectorated, and then rinsed the mouth with distilled water. Immediately after this, different concentrations of sucrose solution (0.1−0.5 M) were given to the individuals. The sweetness-inhibiting activity of each compound is expressed as the maximum concentration of the sucrose solution at which its sweetness was completely suppressed.

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