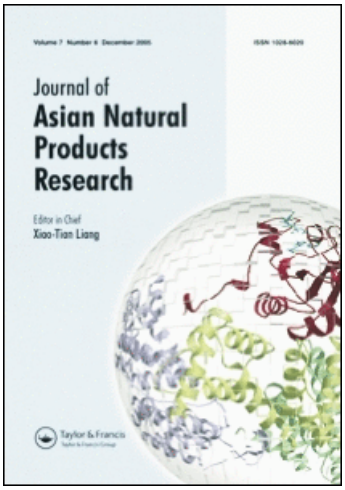

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Four new triterpenoid saponins from Ilex Godajam

Ming-An Ouyang[a]; Dian-Peng Li[b]
[a] Department of Chemistry, Huaqiao University, Quanzhou, Fujian, China [b] Guangxi Institute of Botany, Chinese Academy of Sciences, Guilin, China

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FOUR NEW TRITERPENOID SAPONINS FROM *ILEX GODAJAM*

MING-AN OUYANG[a,*] and DIAN-PENG LI[b]

[a]*Department of Chemistry, Huaqiao University, Quanzhou, Fujian, 362011, China*
[b]*Guangxi Institute of Botany, Chinese Academy of Sciences, Guilin, China*

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Four new triterpenoid saponins, godosides **A**–**D** (**1**–**4**), along with eight known saponins, have been isolated from the fresh aerial parts of *Ilex godajam*. Structure elucidation of **1**–**4** was based on NMR, MS, IR and chemical analyses.

Keywords: *Ilex godajam*; Aquifoliaceae; Triterpenoid saponin; Structure elucidation

INTRODUCTION

The genus *Ilex* consists of many species that are employed in various countries of the world to treat different diseases, especially cardiovascular diseases such as angina pectoris, hypertension, arteriosclerosis and obesity [1–3]. Chemical and pharmacological studies have confirmed that some of these plants produce active principles that show inhibition activity of ACAT (acyl CoA cholesteryl acyl transferase) and anti-allergic activities etc. [4,5]. *Ilex godajam* (Colebr.) Wall. (Aquifoliaceae) is distributed in the tropical and subtropical provinces of south and southwest China such as Guangdong, Hainan, Guangxi and Yunnan. The bark is used to treat stomach ache and ascariasis in Chinese folk medicine [6]. As part of an ongoing phytochemical and pharmacological investigations in the genus *Ilex*, we collected the title plant from Yunnan Province and isolated four new saponins together with eight known saponins: ilexoside XXX (**5**) [7], suavissmoside R1 (**6**) [8], ilexoside XXXIX (**7**) [9], copteroside B (**8**) [10], 3-*O*-β-D-glucopyranosyl(1 → 2)-β-D-glucuronopyranosyl hedezagenin-28-*O*-β-D-glucopyranoside (**9**) [11], 3-*O*-β-D-glucuronopyranosyl oleanolic acid-28-*O*-β-D-glucopyranoside (**10**) [12,13], 3-*O*-β-D-galactopyranosyl(1 → 2)-β-D-glucuronopyranosyl oleanolic acid-28-*O*-β-D-glucopyranoside (**11**) [14] and 3-*O*-β-D-glucopyranosyl(1 → 4)-β-D-glucuronopyranosyl-oleanolic acid-28-*O*-β-D-glucopyranoside (**12**) [15]. The present paper reports the isolation and structural elucidation of the four new saponins, godosides A (**1**)–D (**4**).

*Corresponding author. Tel.: +86-0595-2693976. Fax: +86-0595-2693639. E-mail: maouyang@hqu.edu.cn

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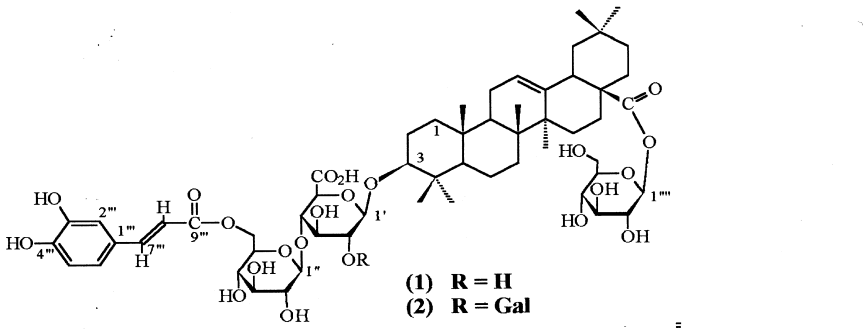
RESULTS AND DISCUSSION

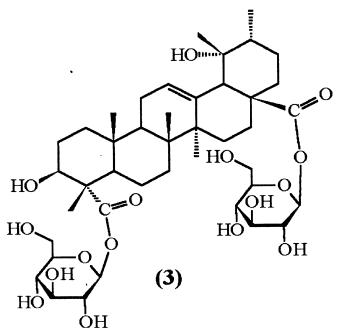
The water soluble fraction from the methanol extract of *I. godajam* was passed through a porous polymer gel D_{101} column and eluted with water and methanol. The methanol eluate was subjected to Sephadex LH-20 (90% EtOH), MCI-gel CHP20P (10–50% MeOH), Si gel using a chloroform–methanol–water system as solvent and RP-8 gel column chromatograph, to yield 12 pure glycosides (**1**–**12**). Their ^{13}C NMR spectra showed that the aglycones of saponins (**3**–**7**) were ursane type and the aglycones of saponins (**1**, **2**, **8**–**12**) were oleanane type.

Godoside A (**1**) showed a quasi-molecular ion peak at *m/z* 1117 $[M\text{-}H]^-$ and main fragment peaks at *m/z* 955 $[M\text{-}H\text{-}162]^-$, 793 $[M\text{-}H\text{-}162\times2]^-$, 631 $[M\text{-}H\text{-}162\times3]^-$ and 455 $[M\text{-}H\text{-}162\times3\text{-}176]^-$ in the negative FABMS. Whereupon, **1** had a molecular formula $C_{57}H_{82}O_{22}$ deduced by its ^{13}C NMR and the negative FABMS. Acid hydrolysis of **1** gave a mixture of glucose, glucuronic acid, caffeic acid and oleanolic acid, where the aglycone structure was determined by ^1H, ^{13}C NMR spectra. The ^{13}C NMR chemical shifts of C-28 at δ 176.6 (esterified state) and C-3 at δ 89.5 (glycosidation), suggested that the sugars were connected to these positions. Three sugar anomeric carbon signals were observed at δ 95.8, 105.5 and 106.5 corresponding to the anomeric protons at δ 6.27 (1H, d, $J = 8.0$ Hz), 5.09 (1H, d, $J = 7.4$ Hz) and 4.87 (1H, d, $J = 7.6$ Hz), respectively, in the HMQC spectrum. The ^{13}C NMR spectrum showed nine downfield carbon signals including conjugated carboxyl, olefinic and aromatic signals (see Table I). The ^1H NMR spectrum showed downfield signals of an ABX system, H-6‴ [7.05 (1H, dd, $J = 7.9$, 1.6 Hz)], H-5‴ [7.14 (1H, d, $J = 7.9$ Hz)] and H-2‴ [7.50 (1H, d, $J = 1.6$ Hz)], which indicated the presence of a 3‴, 4‴ di-substituted benzene ring. Signals for two olefinic protons [6.48 (1H, d, $J = 16.0$ Hz, H-8‴), 7.90 (1H, d, $J = 16.0$ Hz, H-7‴)], whose coupling constant was characteristic for a trans configuration, were also observed. This suggested that the structure of **1** had a caffeoyl unit. The exact sequence of the sugars and its linkage to the aglycone were solved by 2D NMR spectroscopic techniques. The ^1H–^1H COSY and TOCSY experiments allowed the sequential assignment of the proton resonances, the easily distinguished anomeric protons being used as the starting point of analysis. The proton chemical shifts of the glycoside moiety composed of two D-glucopyranoses, one D-glucuronopyranose, and caffeoyl unit, were assigned. The HMQC spectrum correlated all the proton resonances with those of the corresponding one-bond coupled carbons, leading to the unambiguous assignment of the carbon shifts. In the HMBC spectrum (see Fig. 1), the important correlations were displayed: C-28 (δ 176.6) and H-1⁗ of glucose (glc, δ 6.27), C-3 (δ 89.5) and H-1′ of glucuronic acid (GlcA, δ 4.87), C-4′ (δ 84.8 of GlcA) and H-1″ of glucose (Glc, δ 5.09), C-9‴ (δ 167.6 of caffeoyl group) and H-6″ of Glc. Accordingly, the structure of **1** was concluded to be 3-*O*-[6-*O*-caffeoyl-β-D-glucopyranosyl-(1 → 4)]-β-D-glucuronopyranosyl oleanolic acid 28-*O*-β-D-glucopyranoside, named godoside A.

Godoside B (**2**) afforded a molecular ion peak at *m/z* 1279 $[M\text{-}H]^-$, appropriate for a molecular formula of $C_{63}H_{92}O_{27}$, deduced from the ^{13}C NMR and negative FABMS. Acid hydrolysis of **2** gave a mixture of glucose, galactose, glucuronic acid and caffeic acid. The aglycone was determined oleanolic acid by comparing its NMR data [14] with that of **1**. The ^{13}C NMR spectrum gave four anomeric carbon signals at δ 95.8, 104.9, 105.7 and 107.0, corresponding to the anomeric proton signals at 6.32 (1H, d, $J = 8.0$ Hz), 5.08 (1H, d, $J = 7.6$ Hz), 5.02 (1H, d, $J = 7.6$ Hz) and 4.84 (1H, d, $J = 7.2$ Hz), respectively, in the HMQC spectrum. On comparison of the whole ^{13}C NMR spectrum of **2** with that of **1**, a set of six additional signals corresponding to a terminal β-D-galactopyranosyl group appeared at δ 105.7 (CH), 74.7 (CH), 74.9 (CH), 69.7 (CH), 76.8 (CH), and 61.5 (CH_2). The signal due to C-2′ at δ 74.8 of the glucuronosyl moiety in **1** was downfield shifted by 8.1 ppm and appeared



(1) R = H
(2) R = Gal



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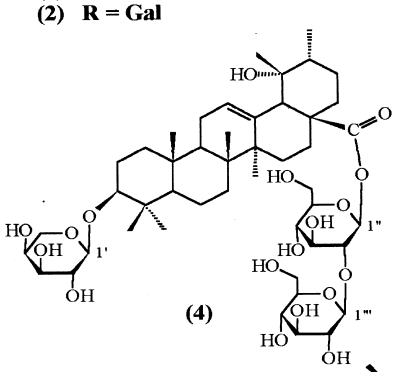

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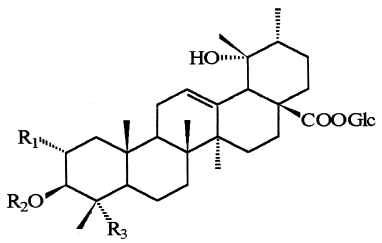
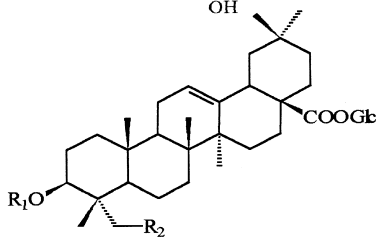


	R_1	R_2	R_3
(5)	H	H	COOH
(6)	OH	H	COOH
(7)	H	GlcA	CH_2OH



	R_1	R_2
(8)	GlcA	OH
(9)	GlcA(2-1)Glc	OH
(10)	GlcA	H
(11)	GlcA(2-1)Gal	H
(12)	GlcA(4-1)Glc	H

at δ 82.9 in **2**, this suggested that the C-2′ hydroxyl group of the glucuronosyl moiety was glycosylated by the D-galactose. This was confirmed by a long-range correlation from the anomeric proton of the galactosyl at δ 5.02 to C-2′ oxymethine carbon of the glucuronose at δ 82.9. Thus, the structure of **2** was formulated as 3-*O*-β-D-galactopyransyl(1 → 2)-[6-*O*-caffeoyl-β-D-glucopyranosyl(1 → 4)]-β-D-glucuronopyranosyl oleanolic acid 28-*O*-β-D-glucopyranoside, named godoside B.

Godoside C (**3**) gave a typical ^1H and ^{13}C NMR spectral feature of triterpenoid saponin. Its negative FAB mass spectrum showed a molecular ion peak at *m/z* 825 $[M\text{-}H]^-$ and main fragment ion peaks at 663 $[M\text{-}H\text{-}162]^-$ and 501 $[M\text{-}H\text{-}162 \times 2]^-$. The 1D NMR spectra exhibited six methyl signals at δ 0.96 (s, CH_3), 1.04 (d, $J = 6.4$ Hz, CH_3), 1.14, 1.37, 1.55, 1.58 (s, $CH_3 \times 4$), two carboxyl signals at δ 177.0, 177.7 in the aglycone, two anomeric proton signals at δ 6.43 (d, $J = 7.6$ Hz, H-1), 6.26 (d, $J = 7.4$ Hz, H-1), and two anomeric carbon signals at δ 96.6 and 95.9 in the sugar moieties. On acid hydrolysis, **3** afforded only glucose as the sugar moieties. By comparing the NMR spectral data of **3** with that of

TABLE I ^{13}C NMR spectral data for compounds **1**–**4** (pyridine-d_5)

	1			**2**					**3**			**4**		
1	38.8		3-*O*-GlcA	38.8		3-*O*-GlcA		28-*O*-glc	39.2		28-*O*-glc	39.0		3-*O*-Ara
2	26.5	1′	106.5	26.6	1′	104.9	1′′′′′	95.8	27.5	1′	95.9	26.8	1′	106.0
3	89.5	2′	74.8	89.4	2′	82.9	2′′′′′	74.2	75.3	2′	74.1	89.1	2′	73.4
4	39.6	3′	76.7	39.5	3′	76.2	3′′′′′	79.0	55.2	3′	78.7	39.6	3′	74.2
5	56.0	4′	84.8	55.9	4′	84.7	4′′′′′	71.3	52.3	4′	71.3	56.0	4′	68.3
6	18.7	5′	75.7	18.6	5′	75.7	5′′′′′	79.3	21.4	5′	79.0	18.8	5′	64.9
7	32.7	6′	175.4	32.7	6′	175.6	6′′′′′	62.4	33.3	6′	62.3	33.6		
8	40.1		(1–4)Glc	40.0		(1–2)Gal			40.9		23-*O*-glc	40.7		28-*O*-glc
9	48.1	1″	105.5	48.1	1″	105.7			48.0	1″	96.6	47.9	1″	95.9
10	37.1	2″	75.0	37.0	2″	74.7			37.0	2″	74.5	37.1	2″	80.9
11	23.6	3″	76.9	23.5	3″	74.9			24.1	3″	79.2	24.2	3″	78.2
12	123.0	4″	71.3	123.4	4″	69.7			128.2	4″	71.9	128.6	4″	71.4
13	144.3	5″	77.3	144.3	5″	76.8			139.4	5″	79.4	139.4	5″	78.3
14	42.3	6″	64.6	42.3	6″	61.5			42.1	6″	62.5	42.2	6″	62.6
15	28.4		Caff.	28.3		(1–4)Glc			29.2			29.4		(1–2)Glc′
16	23.8	1‴	127.0	23.8	1‴	107.0			26.8			26.6	1‴	104.8
17	47.1	2‴	116.0	47.1	2‴	74.6			48.6			48.8	2‴	76.4
18	41.9	3‴	147.6	41.9	3‴	76.8			54.5			54.5	3‴	79.0
19	46.4	4‴	150.4	46.4	4‴	71.7			72.8			72.8	4‴	71.8
20	30.9	5‴	116.8	30.9	5‴	77.3			42.1			42.2	5‴	79.2
21	34.2	6‴	122.2	34.2	6‴	64.9			26.1			26.3	6‴	62.8
22	30.9	7‴	146.4	30.0		Caff.			37.7			37.8		
23	28.4	8‴	114.7	28.2	1′′′′	127.0			177.7			28.4		
24	17.1	9‴	167.6	16.8	2′′′′	115.3			12.0			16.8		
25	15.7		28-*O*-glc	15.7	3′′′′	147.6			16.2			15.7		
26	17.6	1′′′′	95.8	17.6	4′′′′	150.6			17.6			17.5		
27	26.3	2′′′′	74.2	26.2	5′′′′	117.2			24.5			24.7		
28	176.6	3′′′′	78.9	176.6	6′′′′	123.1			177.0			177.1		
29	33.3	4′′′′	71.3	33.3	7′′′′	146.8			27.1			27.2		
30	23.8	5′′′′	79.3	23.7	8′′′′	114.6			16.7			16.9		
		6′′′′	62.4		9′′′′	167.9								

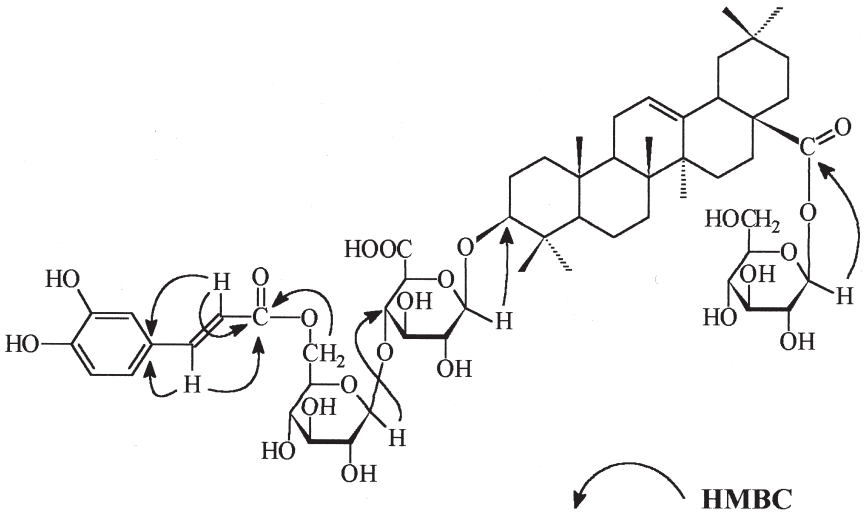


FIGURE 1 HMBC correlations for godoside A.

ilexoside XXX (**5**) (rotundioic acid 28-*O*-β-D-glucopyranoside) [7], **3** had the same aglycone and similar structures as **5** except an additional glucose unit in the sugar moieties. The linkage position of the glucosyl residue was determined by 2D NMR. $^{1}H-^{1}H$ COSY, HMQC and TOCSY established each sugar's spin-coupling network. The signal of C-23 of **3** was significantly shifted to a downfield value of δ 3.9 when compared with **5**. This indicated that the C-23 was esterified and linked to the glucose. This result was confirmed by correlations of HMBC experiment. Thus, compound **3** was determined as 23-*O*-β-D-glucopyranosyl rotundioic acid 28-*O*-β-D-glucopyranoside, named godoside C.

Godoside D (**4**) gave a molecular ion peak at *m/z* 927 $[M-H]^-$ and main fragment peaks at *m/z* 765 $[M-H-162]^-$, 603 $[M-H-162\times 2]^-$ and 471 $[M-H-162\times 2-132]^-$ in negative FABMS. A molecular formula $C_{47}H_{76}O_{18}$ was deduced by its ^{13}C NMR and negative FABMS. On acid hydrolysis, **4** afforded arabinose and glucose identified by HPTLC. The 1D and 2D NMR spectra revealed three anomeric proton and carbon signals at δ 4.93 (d, $J = 6.5$ Hz, H-1′), 106.0 of Ara, δ 5.16 (d, $J = 7.0$ Hz, H-1‴), 104.8 of Glc′, and δ 6.28 (d, $J = 7.6$ Hz, H-1″), 95.9 of glc. By comparing the ^{13}C NMR spectrum data of **4** with that of ziyuglucoside I, 3-*O*-α-L-arabinopyranosyl pomolic acid 28-*O*-β-D-glucopyranoside [16], compound **4** had the same aglycone and similar structure except an additional glucose unit as ziyuglucoside I. The carbon signals at C-3 (δ 89.1) and C-28 (δ 177.1) of **4** indicated that glycosylations occurred at the C-3 and the C-28. Compound **4** was treated with alkaline hydrolysis to afford a prosapogenin, which yielded only arabinose on acidic hydrolysis. Thus only arabinose was linked at the C-3 position of the aglycone. The signal of C-2″ of glucose (glc) in **4** was significantly shifted to a downfield value of δ 6.8 when compared with ziyuglucoside I, indicating the glycosylating position of the glucose (glc). The HMBC spectrum exhibited key correlations of H-1′ of Ara and C-3, H-1‴ of Glc and C-2″ of glc, H-1″ of glc and C-28. Therefore, **4** was deduced as 3-*O*-α-L-arabinopyranosyl pomolic acid 28-*O*-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranoside, named godoside D.

Eight known saponins **5–12** were characterized by comparing their spectral data (^{1}H, ^{13}C NMR, MS and IR) with those in the literature.

EXPERIMENTAL SECTION

General Experimental Procedures

^{1}H and ^{13}C NMR spectra were obtained on a Bruker AM-400, DRX-500 spectrometer. FAB-MS were measured on a VG Autospec 3000 system spectrometer. Optical rotations were taken on a JASCO-20C digital polarimeter. The IR spectrum was recorded as a KBr pellet on a Perkin–Elmer 1750 FTIR spectrometer and gas chromatography (GC) was run on a Hitachi G-3000 gas chromatographer. Chromatographic materials were RP-8 (40–60 μm, Merck), silica gel (160–200 mesh and 10–40 μm), Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical) and MCI-gel CHP20P (75–150 μm, Mitsubishi Chemical Industries). Spot of TLC was detected by spraying with 5% H_2SO_4 followed by heating.

Plant Material

Fresh aerial parts of *I. Godajam* (Colebr.) Wall. were collected at Xishuang Banna, Yunnan, P.R. China in October 1994. A voucher specimen (No. 551327) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences. The plant was identified by Prof. C. R. Yang.

Extraction and Isolation

Fresh aerial parts (1 kg) were extracted (× 3) with 70% MeOH. The methanol extract was evaporated under reduced pressure and then was filtered. The water soluble extract was passed through a D_{101} column and eluted with water and methanol. Evaporation of the methanol eluate yielded 40 g of a brown extract (A). Extract (A) was chromatographed on Sephadex LH-20 (90% EtOH) to give two fractions B (fr. saponin) and C (fr. flavonoid glycoside). Fraction B (15 g) was separated by MCI-gel CHP20P (10–50% MeOH) and yielded seven fractions (I–VII). The fractions were further purified by Si gel column chromatograph using $CHCl_3$–MeOH–H_2O (80:20:3–70:30:5) as solvent and then by RP-8 gel column chromatograph (solvent: MeOH–H_2O, 10–70%) to yield **1** (60 mg), **2** (60 mg), **3** (20 mg), **4** (30 mg), **5** (100 mg), **6** (60 mg), **7** (20 mg), **8** (20 mg), **9** (30 mg), **10** (50 mg), **11** (30 mg), **12** (25 mg).

Godoside A (**1**) $[\alpha]_D^{27.4}$ +98.3° (c 0.019, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3600–3000, 1740, 1686, 1680, 1620, 1585, 1500, 1060; FAB-MS *m/z*: 1117 $[M\text{-}H]^-$, 955 $[M\text{-}H\text{-}162]^-$, 793 $[M\text{-}H\text{-}162 \times 2]^-$, 631 $[M\text{-}H\text{-}162 \times 3]^-$, 455 $[M\text{-}H\text{-}162 \times 3\text{-}176]^-$; ^{1}H NMR (C_5D_5N) δ 0.77, 0.86, 0.89, 0.94, 1.04, 1.24, 1.25 (s, $CH_3 \times 7$), 4.87 (d, $J = 7.6$ Hz), 5.09 (d, $J = 7.4$ Hz), 5.40 (m, H-12), 6.27 (d, $J = 8.0$ Hz), 6.48 (1H, d, $J = 16.0$ Hz, H-8‴), 7.05 (1H, dd, $J = 7.9$, 1.6 Hz, H-6‴), 7.14 (1H, d, $J = 7.9$ Hz, H-5‴), 7.50 (1H, d, $J = 1.6$ Hz H-2‴), 7.90 (1H, d, $J = 16.0$ Hz, H-7‴).

Godoside B (**2**) $[\alpha]_D^{27.2}$ + 17.9° (c 0.014, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3400–3010, 1730, 1685, 1618, 1620, 1522, 1062; FAB-MS *m/z*: 1279 $[M\text{-}H]^-$, 1117 $[M\text{-}H\text{-}162]^-$, 955 $[M\text{-}H\text{-}162 \times 2]^-$, 793 $[M\text{-}H\text{-}162 \times 3]^-$, 631 $[M\text{-}H\text{-}162 \times 4]^-$, 455 $[M\text{-}H\text{-}162 \times 4\text{-}176]^-$; ^{1}H NMR (C_5D_5N) δ 0.78, 0.87, 0.90, 1.01, 1.07, 1.21, 1.25 (s, $CH_3 \times 7$), 4.84 (d, $J = 7.2$ Hz), 5.02 (d, $J = 7.6$ Hz), 5.08 (d, $J = 7.6$ Hz), 5.42 (m, H-12), 6.32 (d, $J = 8.0$ Hz), 6.72 (1H, d, $J = 15.7$ Hz, H-8⁗), 7.12 (1H, dd, $J = 7.6$, 1.4 Hz, H-6⁗), 7.25 (1H, d, $J = 7.6$ Hz, H-5⁗), 7.96 (1H, d, $J = 15.7$ Hz, H-7⁗), 8.06 (d, $J = 1.4$ Hz, H-2⁗).

Godoside C (**3**) $[\alpha]_D^{27.3}$ + 27.0° (c 0.015, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3400, 1722, 1680, 1460, 1060; FAB-MS *m/z*: 825 $[M\text{-}H]^-$, 663 $[M\text{-}H\text{-}162]^-$, 501 $[M\text{-}H\text{-}162 \times 2]^-$; ^{1}H NMR (C_5D_5N) δ 0.96 (s, CH_3), 1.04 (d, $J = 6.4$ Hz, CH_3), 1.14, 1.37, 1.55, 1.58 (s, $CH_3 \times 4$), 6.26 (d, $J = 7.4$ Hz), 6.43 (d, $J = 7.6$ Hz).

Godoside D (**4**) $[\alpha]_D^{27.3}$ +66.7°(c 0.026, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3402, 2930, 1720, 1070, 1030; FAB-MS *m/z*: 927 $[M\text{-}H]^-$, 765 $[M\text{-}H\text{-}162]^-$, 603 $[M\text{-}H\text{-}162 \times 2]^-$, 471 $[M\text{-}H\text{-}162 \times 2\text{-}132]^-$; ^1H NMR (C_5D_5N) δ 0.88 (s, CH_3), 1.03 (s, CH_3), 1.05 (d, $J = 6.5$ Hz, CH_3), 1.13, 1.14, 1.39, 1.69 (s, $CH_3 \times 4$), 6.28 (d, $J = 7.6$ Hz, H-1), 5.16 (d, $J = 7.0$ Hz, H-1) and 4.93 (d, $J = 6.5$ Hz, H-1).

Acid Hydrolysis of Godosides A–D

Each saponin (4 mg) in 1 ml MeOH was refluxed in 10 ml of 4N aq. HCl for 4 h and then extracted with AcOEt. The aqueous layer was adjusted to pH 6 with $NaHCO_3$. After evaporating to dryness, the sugars were extracted with pyridine from the residue. The extraction were analyzed by comparing with authentic caffeic acid and sugars on silica gel ($CHCl_3$:MeOH:H_2O: AcOH [7:3:0.5:1]) using 4% α-naphthol-EtOH-5% H_2SO_4 as spray reagent, in which the presence of arabinose, glucose, galactose and glucuronic acid were detected. The pyridine extract was derivatized with thiazolidine as described previously [17]. Monosaccharides were detected by GC and conditions: column, SupelcoSPB-1 0.25 mm × 27 m; column temperature, 230°C; carrier gas, N_2; t_R, L-arabinose (8.3 min), D-arabinose (8.6 min), L-glucose (13.3 min), D-glucose (13.8 min), L-galactose (14.2 min), D-galactose (14.7 min), L-glucuronic acid (10.6 min), and D-glucuronic acid (10.8 min). D-glucose and D-glucuronic acid were detected in **1**. D-glucose, D-glucuronic acid and D-galactose were detected in **2**. D-glucose was detected in **3**. D-glucose and L-arabinose were detected in **4**.

Alkaline Hydrolysis of Godoside D

Saponin (8 mg) was refluxed in 1 N aq. KOH (2 ml) for 2 h at 70°C. The mixture was adjusted to pH 6 with 1N aq. HCl and then extracted with AcOEt. The AcOEt extract was concentrated to dryness and treated to acidic hydrolysis. After neutralizing and evaporating to dryness, the residue was extracted with pyridine and analyzed by HPTLC to detect sugars.

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