Phenolic and Triterpene Glycosides from the Stems of *Ilex litseaefolia*

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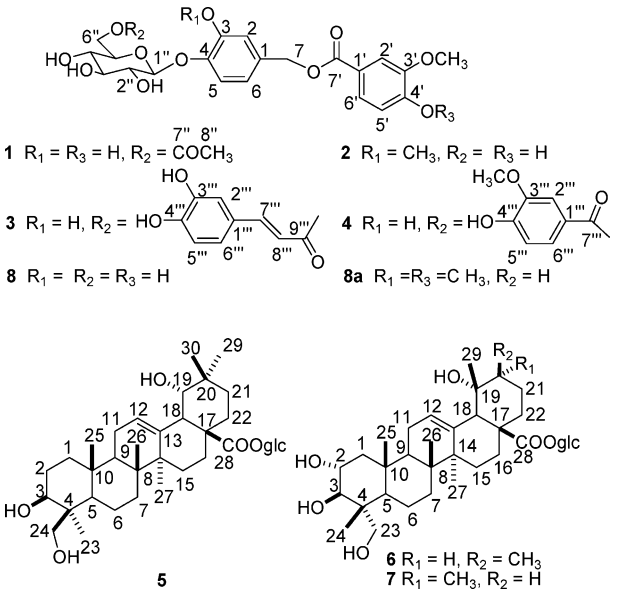
Chemical investigation on the stems of *Ilex litseaefolia* afforded four new phenolic glycosides, litseaefolosides A−D (**1**−**4**), and two new triterpene glycosides, spathodic acid 28-*O*-*β*-D-glucopyranoside (**5**) and (20*S*)-niga-ichigoside F1 (**6**), along with 28 known compounds. The structures of **1**−**6** were determined on the basis of chemical and spectroscopic evidence. Litseaefoloside C (**3**) showed inhibitory activities in vitro for α-glucosidase and lipase with IC_{50} values of 34.0 and 0.31 μg/mL, respectively.

Ilex (Aquifoliaceae) species are distributed widely in the People's Republic of China, and some are used extensively in folk medicine. For example, *I. rotunda* is an antipyretic and antidote and used for the treatment of the common cold, tonsillitis, and stomach and intestinal ulcers.[1] *I. pubescens* is used for the treatment of coronary disease, myocardial infarction, dysentery, and erysipelas. *I. cornuta* and *I. latifolia* are used for the treatment of headache, toothache, bloodshot eyes, and tinnitus.[2]

Previous studies on the *Ilex* genus have led to the isolation of triterpenes, triterpene glycosides,[3−7] hemiterpene glycosides,[8] and phenolic compounds.[9,10] In the present study on the chemical components of the stems of *I. litseaefolia* Hu et Tang, six new (**1**−**6**) and 28 known compounds were isolated. On the basis of chemical and spectroscopic evidence, the new compounds were determined as 3-hydroxy-4-*O*-*β*-D-(6-*O*-acetylglucopyranosyl)-benzyl vanilloate (litseaefoloside A, **1**), 3-methoxy-4-*O*-*β*-D-glucopyranosylbenzyl vanilloate (litseaefoloside B, **2**), 3-hydroxy-4-*O*-*β*-D-(6-*O*-caffeoylglucopyranosyl)benzyl vanilloate (litseaefoloside C, **3**), 3-hydroxy-4-*O*-*β*-D-(6-*O*-vanilloylglucopyranosyl)benzyl vanilloate (litseaefoloside D, **4**), spathodic acid 28-*O*-*β*-D-glucopyranoside (**5**), and (20*S*)-niga-ichigoside F1 (**6**). The known substances were identified as α-amyrin,[11] 3*β*-[(α-L-arabinopyranosyl)oxy]-19α-hydroxyolean-12-en-28-oic acid 28-*O*-*β*-D-glucopyranoside,[5] asiatic acid,[12] 3,5-dimethoxy-4-hydroxybenzacraldehyde, 4-*O*-*β*-D-glucopyranosylbenzyl vanilloate,[13] 5-hydroxy-3-methoxybenzyl alcohol, kaji-ichigoside F1,[14,15] 4-methylphenol, niga-ichigoside F1 (**7**),[14,15] 4-*epi*-niga-ichigoside F1,[16] niga-ichigoside F2,[14,15] pedunculoside,[17,18] protocatechualdehyde,[19] protocatechuic acid,[20] rotundic acid, rotungenic acid, rotungenoside,[17,18] sericoside,[21] sinapaldehyde glucoside,[22] syringaldehyde, syringin,[23,24] 2α,3*β*,23-trihydroxyurs-12-en-28-oic acid 28-*O*-*β*-D-glucopyranoside,[24] ursolic acid,[25,26] uvaol,[27,28] vanillic acid,[29] vanilloylcalleryanin (**8**),[30] 4-*O*-*β*-D-(6-*O*-vanilloylglucopyranosyl)vanillic acid,[31] and ziyuglucoside I.[32,33]



Results and Discussion

Compound **1** was obtained as a white powder. When treated with 3% $FeCl_3$(aq), its acetone solution turned blue. The molecular formula $C_{23}H_{26}O_{12}$ was determined from the quasi-molecular ion peaks at *m/z* 517.1321 $[M + Na]^+$ and *m/z* 533.1060 $[M + K]^+$ in the HRESIMS. The IR absorptions at ν_{max} 1708 and 1689 cm^{-1} revealed the presence of carbonyl groups. The ^1H NMR spectrum (Table 1) exhibited two 1,2,4-trisubstituted phenyl rings, one oxygenated methene (*δ* 5.20, 2H, s, H-7) moiety, and one methoxyl group (*δ* 3.87, 3H, s, OCH_3-3′). One acetyl group was postulated from the ^1H NMR signal at *δ* 2.05 (3H, s, H-8″) and the ^{13}C NMR signal at *δ* 171.3 (C-7″), together with the HMBC correlation between H-8″ and C-7″. Acid hydrolysis of **1** afforded vanillic acid and D-glucose. A vanilloyl moiety was confirmed by the HMBC correlations from H-2′ (*δ* 7.54, 1H, br s) and H-6′ (*δ* 7.57, 1H, dd, *J* = 8.2, 1.7 Hz) to C-7′ (*δ* 166.5) and from H-2′ and H-5′ (*δ* 6.83, 1H, d, *J* = 8.4 Hz) to C-3′ (*δ* 147.4) and C-4′ (*δ* 151.6), together with the NOESY correlation between OCH_3-3′ and H-2′. The *β*-D-glucopyranosyl moiety was thus determined from the ^1H NMR signal at *δ* 4.78 (1H, d, *J* = 7.4 Hz, H-1″) and the ^{13}C NMR signal at *δ* 102.6 (C-1″). The HMBC correlations from H-2 (*δ* 6.96, 1H, d, *J* = 1.7 Hz) and H-6 (*δ* 6.87, 1H, dd, *J* = 8.2, 1.7 Hz) to C-7 (*δ* 65.7) and from H-2 and H-5 (*δ* 7.12, 1H, d, *J* = 8.2 Hz) to C-3 (*δ* 147.1) and C-4 (*δ* 145.1) showed the presence of a 3,4-dioxygenated benzyl group. C-1″ was connected with C-4 via an ether bond in view of a HMBC correlation (H-1″/C-4) and a NOESY cross-peak (H-1″/H-5). The *O*-acetyl unit was located at C-6″ from the HMBC correlation between H-6″ (*δ* 4.41, 1H, dd, *J* = 11.8, 1.9 Hz; 4.25, 1H, dd, *J* = 11.8, 6.6 Hz) and C-7″. The vanilloyl moiety was placed at C-7 on the basis of a HMBC

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Table 1. NMR Data of Compounds **1** and **2** in CD_3OD (^1H: 600 MHz, ^{13}C: 150 MHz)[a]

	1		**2**	
position	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C
1		132.2		131.3
2	6.96 (1H, d, 1.7)	115.8	7.11 (1H, d, 1.6)	112.6
3		147.1		149.5
4		145.1		146.6
5	7.12 (1H, d, 8.2)	117.3	7.18 (1H, d, 8.3)	116.6
6	6.87 (1H, dd, 8.2, 1.7)	119.4	7.01 (1H, dd, 8.3, 1.6)	120.9
7	5.20 (2H, s)	65.7	5.26 (2H, s)	65.9
OCH_3-3			3.88 (3H, s)	55.3
1′		121.2		121.0
2′	7.54 (1H, br s)	112.2	7.55 (1H, br s)	112.1
3′		147.4		147.4
4′		151.6		151.8
5′	6.83 (1H, d, 8.3)	114.6	6.83 (1H, d, 8.3)	114.6
6′	7.57 (1H, dd, 8.3, 1.7)	123.7	7.57 (1H, dd, 8.3, 1.7)	123.7
7′		166.5		166.6
OCH_3-3′	3.87 (3H, s)	55.0	3.88 (3H, s)	55.0
1″	4.78 (1H, d, 7.4)	102.6	4.92 (1H, d, 7.5)	101.3
2″	3.50 (1H, dd, 9.3, 7.4)	73.4	3.50 (1H, dd, 9.1, 7.5)	73.5
3″	3.47(1H, dd, 9.3, 8.1)	76.0	3.46 (1H, dd, 9.1, 8.2)	76.5
4″	3.38 (1H, m)	70.2	3.40 (1H, m)	70.0
5″	3.63 (1H, ddd, 9.7, 6.6, 1.9)	74.1	3.40 (1H, m)	76.8
6″	4.41 (1H, dd, 11.8, 1.9)	63.3	3.68 (1H, dd, 12.1, 5.0)	61.1
	4.25 (1H, dd, 11.8, 6.6)		3.86 (1H, dd, 12.1, 1.7)	
7″		171.3		
8″	2.05 (3H, s)	19.3		

[a] Assignments based on HSQC, HMBC, and NOESY spectroscopic measurements.

correlation (H-7/C-7′). From all of this evidence, compound **1** (litseaefoloside A) was determined as 3-hydroxy-4-*O*-*β*-D-(6-*O*-acetylglucopyranosyl)benzyl vanilloate.

Compound **2** was obtained as colorless needles (CH_3OH). Acid hydrolysis of **2** afforded vanillic acid and glucose. The methylation of **2** and 3-hydroxy-4-*O*-*β*-D-glucopyranosylbenzyl vanilloate (vanilloylcalleryanin, **8**) afforded the same product, odontoside trimethyl ether (**8a**).[34] Thus, the linkages among the D-glucose, 3,4-dioxygenated benzyl, and 3,4-dioxygenated benzoyl units in **2** were the same as those in **8**. The ^1H NMR signals at δ 3.88 (6H, br s, OCH_3-3, -3′) showed that compound **2** possesses one more methoxyl group than compound **8**, which was confirmed by its molecular formula, $C_{22}H_{26}O_{11}$, from the quasi-molecular ion peaks at *m/z* 489.1346 $[M + Na]^+$ and *m/z* 505.1100 $[M + K]^+$ in the HRESIMS. The methoxy groups were assigned on the basis of NOESY correlations between OCH_3-3 and H-2 (δ 7.11, 1H, d, J = 1.6 Hz) and between OCH_3-3′ and H-2′ (δ 7.55, 1H, br s). Thus, compound **2** (litseaefoloside B) was assigned as 3-methoxy-4-*O*-*β*-D-glucopyranosylbenzyl vanilloate.

Compound **3** was obtained as yellow needles (CH_3OH). The molecular formula $C_{30}H_{30}O_{14}$ was provided from the quasi-molecular ion peaks at *m/z* 637.1549 $[M + Na]^+$ and *m/z* 653.1286 $[M + K]^+$ in the HRESIMS. Acid hydrolysis of **3** afforded caffeic acid, vanillic acid, and D-glucose. The presence of caffeoyl and vanilloyl moieties was confirmed by HMBC and NOESY experiments (see Supporting Information). The HMBC correlations from H-2 (δ 6.93, 1H, d, J = 1.9 Hz) and H-6 (δ 6.77, 1H, dd, J = 8.3, 2.0 Hz) to C-7 (δ 65.7) and from H-2 and H-5 (δ 7.14, 1H, d, J = 8.4 Hz) to C-3 (δ 147.0) and C-4 (δ 145.1) showed the presence of a 3,4-dioxygenated benzyl moiety. The 6″-*O*-caffeoyl unit was located according to the HMBC correlation between H-6″ (δ 4.56, 1H, dd, J = 11.9, 2.1 Hz; 4.36, 1H, dd, J = 11.9, 6.9 Hz) and C-9‴ (δ 167.5). The linkages of the vanilloyl, 3,4-dioxygenated benzyl, and *β*-D-glucopyranosyl units were determined by HMBC correlations between H-7 (δ 5.12, 2H, s) and C-7′ (δ 166.5) and between H-1″ (δ 4.79, 1H, d, J = 7.5 Hz) and C-4, together with the NOESY correlation between H-1″ and H-5. Thus, compound **3** (litseaefoloside C) was determined to be 3-hydroxy-4-*O*-*β*-D-(6-*O*-caffeoylglucopyranosyl)benzyl vanilloate.

Compound **4** was obtained as a white powder. The quasi-molecular ion peaks at *m/z* 625.1537 $[M + Na]^+$ and 641.1269 $[M + K]^+$ in the HRESIMS suggested a molecular formula of $C_{29}H_{30}O_{14}$. Acid hydrolysis of **4** afforded vanillic acid and D-glucose. Two vanilloyl substituents and one 3,4-dioxygenated benzyl group were present in **4** on the basis of HMBC and NOESY experiments (see Supporting Information). Of these, the 6″- and 7-*O*-vanilloyl groups could be located from the HMBC correlation from H-6″ (δ 4.71, 1H, dd, J = 11.8, 1.9 Hz; 4.40, 1H, dd, J = 11.8, 7.5 Hz) to C-7‴ (δ 166.47) and from H-7 (δ 5.16, 2H, s) to C-7′ (δ 166.53). The *β*-D-glucopyranosyl moiety was connected to C-4 with C-1″ via an ether bond, in view of the HMBC correlation between H-1″ (δ 4.81, 1H, d, J = 7.3 Hz) and C-4 (δ 145.1) and the NOESY correlation between H-1″ and H-5 (δ 7.09, 1H, d, J = 8.4 Hz). Thus, compound **4** (litseaefoloside D) was determined as 3-hydroxy-4-*O*-*β*-D-(6-*O*-vanilloylglucopyranosyl)benzyl vanilloate.

Compound **5** was obtained as colorless needles (CH_3OH). It gave a positive red coloration in the Liebermann–Burchard reaction. The HRESIMS revealed quasi-molecular ion peaks at *m/z* 673.3950 $[M + Na]^+$ and 689.3605 $[M + K]^+$, corresponding to the molecular formula $C_{36}H_{58}O_{10}$. The ^{13}C NMR spectrum exhibited 36 carbon signals. Acid hydrolysis of **5** afforded D-glucose. The IR spectrum (ν_{max} 1727 cm^{-1}) and the ^{13}C NMR signal at δ 178.2 (C-28) revealed the presence of a carbonyl group. The ^1H NMR spectrum exhibited signals for six tertiary methyl groups (δ 0.90, 0.97, 1.13, 1.14, 1.53, and 1.61, each 3H, s) and one olefinic proton (δ 5.50, 1H, br s). The above evidence revealed an oleanolic acid skeleton. The HMBC correlation between H-18 (δ 3.53, br s) and C-28 confirmed the position of the carbonyl group. The ^1H NMR signal at δ 6.40 (1H, d, J = 8.4 Hz, H-1′) and the HMBC correlation between H-1′ and C-28 suggested a 28-*O*-*β*-D-glucopyranoside unit. The ^1H and ^{13}C NMR spectra showed evidence for two oxymethine groups (δ_H 3.60, H-3; 3.58, H-19; δ_C 81.1, C-3; 82.0, C-19) and one hydroxymethylene group (δ_H 3.68 and 4.49, H-24; δ_C 65.5, C-24). The OH-3*β*, -19α, and -24 groups were determined by the following NOESY correlations: H-3/H-23 (δ 1.53, 3H), H-25 (δ 0.90, 3H)/H-24 and H-26 (δ 1.13, 3H), H-18/H-26, and H-18 and H-19/H-30 (δ 0.97, 3H). Accordingly, compound **5** was determined to be spathodic acid 28-*O*-*β*-D-glucopyranoside.

Compound **6** was obtained as colorless needles (CH_3OH). The molecular formula, $C_{36}H_{58}O_{11}$, was determined from the quasi-molecular ion peaks at *m/z* 689.3889 $[M + Na]^+$ and 705.3643 $[M + K]^+$ in the HRESIMS. The optical rotation and NMR spectra[15] of **6** were closely comparable to those of niga-ichigoside F1 (**7**). However, the H-18 and H-30 signals in **6** resonated at δ 3.15 (1H, s) and 0.96 (3H, d, J = 7.1 Hz), compared with δ 2.92 and 1.06, respectively, in **7**. The ^{13}C NMR signals at δ 47.0 and 31.6 for C-18 and C-22 in **6** shifted respectively to δ 54.4 and 37.7 in **7** suggested that **6** is a 20*S* stereoisomer of **7**. The NOESY correlations [H-18/H-29 (δ 1.35, 3H, s) and H-30, H-29/H-30, and OH-19 (δ 5.21, 1H, s)/H-20 (δ 1.91, 1H, m)]

confirmed this conclusion. Thus, compound **6** was assigned as (20*S*)-niga-ichigoside F1.

Very few 19α-hydroxyursolic acid derivatives with a 20*S*-configuration have been isolated, most of them from *Ilex*.[35−38]

The in vitro α-glucosidase and lipase inhibitory activities of compounds **1**, **3**, and vanilloylcalleryanin (**8**) were evaluated. Compounds **1** and **8** exhibited no inhibitory activity against α-glucosidase and lipase (IC_{50} > 100 μg/mL), whereas compound **3** showed inhibitory activities fpr α-glucosidase and lipase with IC_{50} values of 34.0 and 0.31 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were determined on an X-6 melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 automatic polarimeter. UV and IR spectra were measured on a Lambda 35 spectrometer and a Perkin-Elmer FT-IR spectrometer, respectively. NMR spectra were recorded on a Bruker Advance 600 spectrometer with TMS as internal standard. Electrospray-ionization mass spectra (ESIMS) were acquired on a Finnigan LCQ^{DECA} mass spectrometer. HRESIMS were obtained on a BioTOF-Q mass spectrometer. HPLC isolations were performed using a Perkin-Elmer Series 200 LC pump and a Perkin-Elmer Series 200 UV/vis detector. A Kromasil 100-10 C_{18} column (10 mm × 250 mm, 5 μm) was used for semipreparative HPLC with MeOH−H_2O at a flow rate of 1 mL/min. Silica gel (200−300 mesh, Qingdao Haiyang Chemical Co. Ltd., People's Republic of China), RP-C_{18} (40−63 μm, Merck KGaA, Darmstadt, Germany), and polyamide (100−200 mesh, Jiangsu Linjiang Reagent Chemical Plant, People's Republic of China) were used for column chromatography. Precoated plates (silica gel GF_{254}, 0−40 μm) activated at 110 °C for 2 h were used for TLC. All solvents including petroleum ether (60−90 °C) were distilled prior to use.

Plant Material. The stems of *Ilex litseaefilia* Hu et Tang were collected from Gaoqiao Village, Zemulong Countyside, Yanbian County in Sichuan Province of the People's Republic of China in September, 2002. It was authenticated by Fa-Ding Fu at Chengdu Institute of Biology, Chinese Academy of Sciences (CAS). A voucher specimen (No. 3350) was deposited in Chengdu Institute of Biology, CAS.

Extraction and Isolation. The air-dried and powdered stems of *I. litseaefolia* (6.5 kg) were soaked with 95% EtOH (25 L × 3, 7 days each) at room temperature. The solvents were evaporated under reduced pressure to give 1450 g of residue, which was suspended in 90% CH_3OH (2.5 L) and extracted with petroleum ether (2.5 L × 5) to give a petroleum ether fraction (fraction A: 150 g). The CH_3OH fraction (1300 g) was dissolved in H_2O (5 L) and then partioned successively with EtOAc (5 L × 5) and *n*-BuOH (5 L × 5) to afford the corresponding EtOAc (B: 560 g), *n*-BuOH (C: 390 g), and H_2O fractions (D: 350 g).

An aliquot (50 g) of fraction A (150 g) was subjected to a silica gel column eluted with petroleum ether−ethyl acetate (100:1 → 5:1) to yield subfractions AA−AE. Recrystallization of subfractions AB (5 g) and AC (3 g) from $CHCl_3$ afforded α-amyrin (4 g) and uvaol (1 g), respectively. Ursolic acid (1 g) was obtained by recrystallizing AD (5 g) from CH_3OH.

Fraction B (560 g) was subjected to a silica gel column eluted with $CHCl_3$−CH_3OH (40:1 → 5:1), to yield subfractions BA−BF. Part (1 g) of subfraction BA (35 g) was separated by silica gel column chromatography with $CHCl_3$−CH_3OH (30:1) to give asiatic acid (35 mg), rotungenic acid (65 mg), and rotundic acid (45 mg). The $CHCl_3$-soluble portion (BB′: 2.4 g) of subfraction BB (20 g) was passaged over silica gel with $CHCl_3$−CH_3COCH_3 (100:1) as solvent to give syringaldehyde (60 mg) and 3,5-dimethoxy-4-hydroxybenzacraldehyde (190 mg). Part (10 g) of subfraction BC (110 g) was separated on a silica gel column using $CHCl_3$−CH_3COCH_3 (20:1) as solvent to give vanillic acid (5 g). Subfraction BE (240 g) was distributed between EtOAc (2 L × 5) and 5% Na_2CO_3 aqueous solution (2 L) to give an EtOAc-soluble fraction (BEA: 120 g). The 5% Na_2CO_3 aqueous solution was adjusted to pH 5 with HCl(aq) and extracted with EtOAc (2 L × 5) to give a further EtOAc fraction (BEB: 100 g). Syringaldehyde (130 mg), kaji-ichigoside F1 (9 mg), pedunculoside (10 g), and subfractions BEAB (3 g) and BEAD (6 g) were obtained from part (40 g) of BEA (120 g) by silica gel column chromatography eluted with $CHCl_3$−CH_3COCH_3−H_2O (1:1:0.006). Part (400 mg) of subfraction BEAB (3 g) was separated by HPLC several times to give pedunculoside (244 mg), rotungenoside (86 mg), and **5** (42 mg). Subfraction BEAD (6 g) was subjected to passage over a C_{18} column eluted with CH_3OH−H_2O (1:2 → 1.5:1) to give syringin (84 mg), vanilloylcalleryanin (300 mg), niga-ichigoside F2 (62 mg), and subfractions Fr.2 (200 mg), Fr.4 (1.1 g), and Fr.5 (2.6 g). By separation on HPLC several times, niga-ichigoside F1 (**7**, 120 mg) and **6** (8 mg) were isolated from Fr.2 (200 mg), sericoside (17 mg) and 4-*epi*-niga-ichigoside F1 (59 mg) from part (100 mg) of Fr.4 (1.1 g), and 2α,3β,23-trihydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranoside (10 mg), 3β-[(α-L-arabinopyranosyl)oxy]-19α-hydroxyolean-12-en-28-oic acid 28-*O*-β-D-glucopyranoside (12 mg), and ziyuglucoside I (16 mg) from part (100 mg) of Fr.5 (2.6 g). Subfraction BEB (100 g) was divided into aliquots BEBA−BEBG by polyamide column chromatography using $CHCl_3$−CH_3OH (20:1) as solvent. 5-Hydroxy-3-methoxybenzyl alcohol (8 mg) and sinapaldehyde glucoside (22 mg) were obtained from subfraction BEBA (1 g) by silica gel column chromatography eluted with $CHCl_3$−CH_3OH (15:1). Part (370 mg) of subfraction BEBC (4 g) was separated by HPLC several times to give **2** (5 mg), **1** (250 mg), and vanilloylcalleryanin (**8**, 80 mg). Part (220 mg) of subfraction BEBD (10 g) was separated by HPLC several times to give **4** (20 mg) and 4-*O*-β-D-glucopyranosylbenzyl vanilloate (100 mg). Subfraction BEBF (5 g) was separated over a C_{18} column eluted with CH_3OH−H_2O (1:2 → 1:1) to yield protocatechuic acid (50 mg), 4-methylphenol (15 mg), protocatechualdehyde (150 mg), pedunculoside (1.5 g), 4-*O*-β-D-(6-*O*-vanilloylglucopyranosyl)-vanillic acid (60 mg), and subfraction BEBF′ (250 mg). Subfraction BEBF′ was separated by HPLC several times to give compound **3** (140 mg).

Litseaefoloside A (1): white powder; $[\alpha]^{21}_D$ −54.5° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.43), 264 (4.12), 285 (sh) (3.93), 298 (sh) (3.82) nm; IR (KBr) ν_{max} 3429, 1708, 1689, 1598, 1514, 1456, 1087, 1036 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS (positive mode) *m/z* 517 $[M + Na]^+$ (32), 1011 $[2M + Na]^+$ (86), 1027 $[2M + K]^+$ (100); ESIMS (negative mode) *m/z* 493 $[M - H]^-$ (25), 529 $[M + Cl]^-$ (100), 1023 $[2M + Cl]^-$ (86); HRESIMS (positive mode) *m/z* 517.1321 $[M + Na]^+$ (calcd for $C_{23}H_{26}O_{12}Na$, 517.1316), 533.1060 $[M + K]^+$ (calcd for $C_{23}H_{26}O_{12}K$, 533.1056).

Litseaefoloside B (2): colorless needles (CH_3OH); mp 106.5−109.5 °C; $[\alpha]^{21}_D$ −48.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.58), 264 (4.25), 285 (sh) (4.07), 298 (sh) (3.96) nm; IR (KBr) ν_{max} 3429, 1713, 1597, 1515, 1075, 1032 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS (positive mode) *m/z* 489 $[M + Na]^+$ (51), 955 $[2M + Na]^+$ (100); ESIMS (negative mode) *m/z* 465 $[M - H]^-$ (100), 501 $[M + Cl]^-$ (45); HRESIMS (positive mode) *m/z* 489.1346 $[M + Na]^+$ (calcd for $C_{22}H_{26}O_{11}Na$, 489.1367), 505.1100 $[M + K]^+$ (calcd for $C_{22}H_{26}O_{11}K$, 505.1107).

Litseaefoloside C (3): yellow needles (CH_3OH); mp 122.4−124.5 °C; $[\alpha]^{20}_D$ −43.3°, $[\alpha]^{20}_{436}$ −100.7°, $[\alpha]^{20}_{546}$ −52.2°, $[\alpha]^{20}_{578}$ −45.6° (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.65), 256 (4.29), 289 (sh) (4.30), 297 (4.32), 332 (4.27) nm; UV (MeOH + $AlCl_3$) λ_{max} 220, 264, 298, 361 nm; UV (MeOH + $AlCl_3$ + HCl) λ_{max} 219, 255, 287 (sh) nm, 297, 331; IR (KBr) ν_{max} 3424, 1690, 1679, 1603, 1515, 1448, 1283, 1213, 1081 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; ESIMS (negative mode) *m/z* 613 $[M - H]^-$ (100), 1227 $[2M - H]^-$ (36); HRESIMS (positive mode) *m/z* 637.1549 $[M + Na]^+$ (calcd for $C_{30}H_{30}O_{14}Na$, 637.1528), 653.1286 $[M + K]^+$ (calcd for $C_{30}H_{30}O_{14}K$, 653.1267).

Litseaefoloside D (4): white powder; $[\alpha]^{21}_D$ −32.5° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.62), 264 (4.30), 288 (sh) (4.07), 298 (sh) (4.02) nm; IR (KBr) ν_{max} 3418, 1699, 1598, 1515, 1284, 1220, 1071, 1031 cm^{-1}; ^1H and ^{13}C NMR data, see Table

Table 2. NMR Data of Compounds **3** and **4** in CD_3OD (^1H: 600 MHz, ^{13}C: 150 MHz)[a]

	3		**4**	
position	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C
1		132.0		131.9
2	6.93 (1H, d, 1.9)	115.7	6.92 (1H, d, 1.9)	115.7
3		147.0		146.9
4		145.1		145.1
5	7.14 (1H, d, 8.3)	117.2	7.09 (1H, d, 8.3)	117.1
6	6.77 (1H, dd, 8.3, 1.9)	119.6	6.62 (1H, dd, 8.3, 1.9)	119.3
7	5.12 (2H, s)	65.7	5.16 (2H, s)	65.7
1′		121.1		121.1[b]
2′	7.51 (1H, d, 1.8)	112.1	7.54 (1H, br s)	112.1
3′		147.3		147.4
4′		151.5		151.7
5′	6.82 (1H, d, 8.2)	114.5	6.84 (1H, d, 8.3)	114.6
6′	7.53 (1H, dd, 8.2, 1.8)	123.7	7.55 (1H, dd, 8.3, 1.9)	123.7
7′		166.5		166.53
OCH_3-3′	3.85 (3H, s)	55.0	3.87 (3H, s)	55.0
1″	4.79 (1H, d, 7.5)	102.5	4.81 (1H, d, 7.4)	102.5
2″	3.54 (1H, dd, 9.4, 7.5)	73.4	3.54 (1H, dd, 9.3, 7.4)	73.4
3″	3.50 (1H, dd, 9.4, 8.6)	76.1	3.52 (1H, dd, 9.3, 8.4)	76.1
4″	3.42 (1H, dd, 9.4, 8.6)	70.4	3.44 (1H, dd, 9.5, 8.4)	70.6
5″	3.71 (1H, ddd, 9.4, 6.9, 2.1)	74.4	3.78 (1H, ddd, 9.5, 7.5, 1.9)	74.4
6″	4.56 (1H, dd, 11.9, 2.1)	63.2	4.71 (1H, dd, 11.8, 1.9)	63.6
	4.36 (1H, dd, 11.9, 6.9)		4.40 (1H, dd, 11.8, 7.5)	
1‴		126.3		121.0[b]
2‴	7.06 (1H, d, 1.9)	113.7	7.56 (1H, br s)	112.4
3‴		148.3		147.4
4‴		145.5		151.5
5‴	6.78 (1H, d, 8.1)	115.1	6.87 (1H, 8.3)	114.6
6‴	6.95 (1H, dd, 8.1, 1.9)	121.7	7.60 (1H, dd, 8.3, 1.8)	123.9
7‴	7.58 (1H, d, 15.9)	145.9		166.47
8‴	6.30 (1H, d, 15.9)	113.5		
9‴		167.5		
OCH_3-3‴			3.85 (3H, s)	55.1

[a] Assignments based on HSQC, HMBC, and NOESY spectroscopic measurements. [b] Interchangeable.

2; ESIMS (positive mode) *m/z* 625 $[M + H]^+$ (38), 1227 $[2M + Na]^+$ (100); ESIMS (negative mode) *m/z* 601 $[M - H]^-$ (11), 637 $[M + Cl]^-$ (30), 1203 $[2M - H]^-$ (30), 1239 $[2M + Cl]^-$ (100); HRESIMS (positive mode) *m/z* 625.1537 $[M + Na]^+$ (calcd for $C_{29}H_{30}O_{14}Na$, 625.1527), 641.1269 $[M + K]^+$ (calcd for $C_{29}H_{30}O_{14}K$, 641.1267).

Spathodic acid 28-*O*-*β*-D-glucopyranoside (5): colorless needles (CH_3OH); mp 216.5−218.3 °C; $[\alpha]^{20}_D$ +21.6° (*c* 0.15, MeOH); IR (KBr) ν_{max} 3429, 1727, 1631, 1457, 1073, 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; ESIMS (positive mode) *m/z* 673 $[M + Na]^+$ (23), 1323 $[2M + Na]^+$ (100); ESIMS (negative mode) *m/z* 685 $[M + Cl]^-$ (59), 1335 $[2M + Cl]^-$ (100); HRESIMS (positive mode) *m/z* 673.3950 $[M + Na]^+$ (calcd for $C_{36}H_{58}O_{10}Na$, 673.3922), 689.3605 $[M + K]^+$ (calcd for $C_{36}H_{58}O_{10}K$, 689.3662).

(20*S*)-Niga-ichigoside F1 (6): colorless needles (CH_3OH); mp 227 °C (dec); $[\alpha]^{20}_D$ +14.8°, $[\alpha]^{20}_{365}$ +38.0°, $[\alpha]^{20}_{436}$ +29.2°, $[\alpha]^{20}_{546}$ +18.1°, $[\alpha]^{20}_{578}$ +19.0° (*c* 0.10, pyridine); IR (KBr) ν_{max} 3429, 1717, 1631, 1455, 1072, 982 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; ESIMS (positive mode) *m/z* 689 $[M + Na]^+$ (53), 1355 $[2M + Na]^+$ (100); HRESIMS (positive mode) *m/z* 689.3889 $[M + Na]^+$ (calcd for $C_{36}H_{58}O_{11}Na$, 689.3871), 705.3643 $[M + K]^+$ (calcd for $C_{36}H_{58}O_{11}K$, 705.3611).

Niga-ichigoside F1 (7): white powder; $[\alpha]^{20}_D$ +15.0°, $[\alpha]^{20}_{365}$ +38.5°, $[\alpha]^{20}_{436}$ +26.3°, $[\alpha]^{20}_{546}$ +17.9°, $[\alpha]^{20}_{578}$ +15.6° (*c* 0.10, pyridine); $[\alpha]^{20}_D$ +13.8° (*c* 0.40, MeOH); ESIMS (negative mode) *m/z* 701 $[M + Cl]^-$ (43). Its optical rotation (in MeOH)[14] and NMR data[15] were consistent with literature values.

Acid Hydrolysis. Compounds **1**−**6** and **8** were hydrolyzed on TLC plates as described by Kartnig and Wegschaider.[39] The

Table 3. NMR Data of Compounds **5** and **6** in Pyridine-d_5 (^1H: 600 MHz, ^{13}C: 150 MHz)[a]

	5		**6**	
position	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C
1	0.90 (m), 1.48 (m)	39.5	2.31 (dd, 12.4, 4.3), 2.04 (m)	47.7
2	1.86 (br d, 10.7), 2.00 (m)	29.3	4.27 (td, 9.7, 4.3)	68.7
3	3.60 (m)	81.1	4.20 (m)	78.1
4		44.0		43.4
5	0.95 (m)	57.4	1.83 (m)	47.8
6	1.40 (d, 11.4), 1.64 (m)	20.2	1.43 (m), 1.68 (br d, 10.4)	18.5
7	1.43 (d, 13.7), 1.51 (m)	34.4	1.45 (m), 1.74 (br t, 13.4)	32.9
8		41.1		40.3
9	1.81 (dd, 9.4, 8.6)	49.4	1.39 (t, 11.7)	47.7
10		38.2		38.2
11	1.99 (m)	25.3	2.11 (m)	23.9
12	5.50 (br s)	124.0	5.47 (br s)	127.3
13		145.3		138.6
14		43.0		42.0
15	1.24 (d, 13.4), 2.38 (td, 13.4, 1.9)	30.0	2.47 (td, 13.8, 4.1), 1.17 (m)	28.9
16	2.13 (d, 12.2), 2.85 (br t, 12.2)	28.9	3.18 (td, 13.4, 3.8), 2.03 (m)	26.5
17		47.4		48.1
18	3.53 (br s)	45.5	3.15 (s)	47.0
19	3.58 (m)	82.0		73.1
OH-19	6.13 (d, 5.9)		5.21 (s)	
20		36.5	1.91 (m)	42.7
21	1.02 (m), 2.04 (m)	29.8	1.17 (m), 2.57 (tt, 13.4, 4.3)	24.4
22	1.99 (m), 2.04 (m)	33.9	2.04 (m), 1.85 (m)	31.6
23	1.53 (s)	24.5	4.20 (m), 3.70 (d, 10.4)	66.4
24	3.68 (d, 10.8), 4.49 (d, 10.8)	65.5	1.07 (s)	14.1
25	0.90 (s)	16.9	1.12 (s)	17.3
26	1.13 (s)	18.4	1.22 (s)	17.4
27	1.61 (s)	25.8	1.60 (s)	24.1
28		178.2		176.8
29	1.14 (s)	29.7	1.35 (s)	29.4
30	0.97 (s)	25.6	0.96 (d, 7.1)	15.8
1′	6.40 (d, 8.4)	96.8	6.34 (d, 8.2)	95.6
2′	4.23 (t, 8.6)	75.1	4.22 (m)	73.8
3′	4.31 (dd, 9.2, 8.2)	79.9	4.32 (t, 9.0)	78.8
4′	4.39 (m)	72.0	4.39 (m)	70.8
5′	4.04 (br d, 9.1)	80.3	4.05 (dt, 9.4, 3.0)	79.0
6′	4.43−4.47 (m)	63.1	4.45 (td, 12.2, 2.4), 4.42 (m)	61.9

[a] Assignments based on HSQC, HMBC, and NOESY spectroscopic measurements.

hydrolysis of all compounds gave glucose ($CHCl_3$−CH_3OH−H_2O = 8:4:1, R_f = 0.21; *n*-BuOH−CH_3COCH_3−H_2O = 4:1:1, R_f = 0.53), identified by comparing with an authentic sample. The hydrolysis of compounds **1**−**4** gave vanillic acid ($CHCl_3$−CH_3COCH_3−HOAc = 10:1:0.08, R_f = 0.35; $CHCl_3$−CH_3OH−HOAc = 20:1:0.2, R_f = 0.37), while hydrolysis of compound **4** also afforded caffeic acid ($CHCl_3$−CH_3COCH_3−HOAc = 8:1:0.08, R_f = 0.28; $CHCl_3$−CH_3OH−HOAc = 3:1:0.04, R_f = 0.23). Compounds **1**, **3**, **4**, **5**, and **8** were also hydrolyzed with HCl (10%, 2 mL) at 90 °C for 3 h. After 2 mL of H_2O was added, the mixture was extracted with EtOAc (3 mL × 3). The aqueous phase was left to stand for 1 day. After filtrating the precipitate, the solution was evaporated in vacuo to yield D-glucose with the optical rotation of $[\alpha]^{20}_D$ +50.2° (*c* 0.30, H_2O) from **1**, $[\alpha]^{20}_D$ +52.6° (*c* 0.24, H_2O) from **3**, $[\alpha]^{20}_D$ +52.2° (*c* 0.13, H_2O) from **4**, $[\alpha]^{22}_D$ +53.6° (*c* 0.30, H_2O) from **5**, and $[\alpha]^{22}_D$ +53.8° (*c* 0.32, H_2O) from **8**.

Methylation of 2 and 8. Compounds **2** and **8** (each 2.0 mg) were methylated with CH_2N_2 in anhydrous acetone at 0 °C for 4 h to yield odontoside trimethyl ether (**8a**, each 2.1 mg).

Bioassays. The α-glucosidase and lipase inhibitory activities assays were performed using the methods described by

Kim et al.[40] and Sovik and Rustad,[41] respectively. α-Glucosidase and lipase were purchased from Sigma Chemical Co.

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Supporting Information Available: Major HMBC and NOESY correlations, HRESIMS, ESIMS, ^{1}H NMR and ^{13}C NMR spectra, and HSQC, HMBC, and NOESY diagrams of compounds **1**−**6** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Chinese Ministry of Public Health, Institute of Drug Control. *The Colour Atlas of Chinese Herbs*; Science Press: Beijing, 1987; p 245.
(2) Jangsu Medical College. *Dictionary of Chinese Medicine*; Shanghai People's Press: Shanghai, 1975; pp 441−443, 1288, 1521−1522.
(3) Ouyang, M. A.; Zhang, X.; Li, D. P. *Nat. Prod. Lett.* **2002**, *16*, 137−141.
(4) Pires, V. S.; Gosmann, G.; Guillaume, D.; Schenkel, E. P. *Nat. Prod. Lett.* **2002**, *16*, 401−406.
(5) Mimaki, Y.; Fukushima, M.; Yokosuka, A.; Sashida, Y.; Furuya, S.; Sakagami, H. *Phytochemistry* **2001**, *57*, 773−779.
(6) Haraguchi, H.; Kataoka, S.; Okamoto, S.; Hanafi, M.; Shibata, K. *Phytother. Res.* **1999**, *13*, 151−156.
(7) Nishimura, K.; Miyase, T.; Noguchi, H. *J. Nat. Prod.* **1999**, *62*, 1128−1133.
(8) Fuchino, H.; Tachibana, H.; Tanaka, N. *Chem. Pharm. Bull.* **1997**, *45*, 1533−1535.
(9) Filip, R.; López, P.; Giberti, G.; Coussio, J.; Ferraro, G. *Fitoterapia* **2001**, *72*, 774−778.
(10) Xie, J. B.; Li, P. *Zhongguo Yaoke Daxue Xuebao* **2002**, *33*, 76−77.
(11) Lee, S.; Kim, K. S.; Shim, S. H.; Park, Y. M.; Kim, B. K. *Arch. Pharm. Res.* **2003**, *26*, 902−905.
(12) Zhang, G. L.; Zhou, Z. Z.; Li, B. G. *Tianran Chanwu Yanjiu Yu Kaifa* **1997**, *9*, 10−13.
(13) Bravo B, J. A.; Sauvain, M.; Gimenez T., A.; Muñoz O., V.; Callapa, J.; Le Men-Olivier, L.; Massiot, G.; Lavaud, C. *Phytochemistry* **1999**, *50*, 71−74.
(14) Seto, T.; Tanaka, T.; Tanaka, O. Naruhashi, N. *Phytochemistry* **1984**, *23*, 2829−2834.
(15) Um, B. H.; Pouplin, T.; Lobstein, A.; Weniger, B.; Litaudon, M.; Anton, R. *Fitoterapia* **2001**, *72*, 591−593.
(16) Zhou, X. H.; Kasai, R.; Ohtani, K.; Tanaka, O.; Nie, R. L.; Yang, C. R.; Zhou, J.; Yamasaki, K. *Phytochemistry* **1992**, *31*, 3642−3644.
(17) Nakatani, M.; Hatanaka, S.; Komura, H.; Kubota, T.; Hase, T. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 469−473.
(18) Amimoto, K.; Yoshikawa, K.; Arihara, S. *Phytochemistry* **1993**, *33*, 1475−1480.
(19) Wu, Z. J.; Ou-Yang, M. A.; Yang, C. R. *Yunnan Zhiwu Yanjiu* **1999**, *21*, 393−398.
(20) Wang, X. S.; Mou, Q. M.; Li, Y. M.; He, Y. Q. *Zhongguo Zhongyao Zazhi* **1999**, *24*, 739−740.
(21) Zhang, Y. J.; Yang, C. R. *Yunnan Zhiwu Yanjiu* **1995**, *17*, 468−472.
(22) Wen, D. X.; Chen Z. L. *Zhongcaoyao* **1991**, *22*, 246−248.
(23) Kitajima, J.; Ishikawa, T.; Tanaka, Y.; Ono, M.; Ito, Y.; Nohara, T. *Chem. Pharm. Bull.* **1998**, *46*, 1587−1590.
(24) Adnyana, I. K.; Tezuka, Y.; Banskota, A. H.; Xiong, Q.; Tran, K. Q.; Kadota, S. *J. Nat. Prod.* **2000**, *63*, 496−500.
(25) Guo, H. L.; Zhou, J. Y. *Zhongguo Yaoxue Zazhi* **2003**, *38*, 497−499.
(26) Durham, D. G.; Liu, X. J.; Richards, R. M. E. *Phytochemistry* **1994**, *36*, 1469−1472.
(27) Zhou, J. Y.; Ning, B. M.; Gao, Y. L.; Fang, Q. C. *Zhongguo Yaoxue Zazhi* **2002**, *37*, 574−577.
(28) Cui, S. Y.; Cheng, D. L.; Tian, J.; Wu, F. E. *Tianran Chanwu Yanjiu Yu Kaifa* **2001**, *13*, 11−13.
(29) Guo, D. A.; Liu, Z. A.; Lou, Z. C. *J. Chin. Pharm. Sci.* **1992**, *1*, 81−83.
(30) Challice, J. S.; Williams, A. H. *Phytochemistry* **1968**, *7*, 119−130.
(31) Cui, C. B.; Tezuka, Y.; Yamashita, H.; Kikuchi, T.; Nakano, H.; Tamaoki, T.; Park, J. H. *Chem. Pharm. Bull.* **1993**, *41*, 1491−1497.
(32) Cheng, D. L.; Cao, X. P. *Phytochemistry* **1992**, *31*, 1317−1320.
(33) Kakuno, T.; Yoshikawa, K.; Arihara, S. *Phytochemistry* **1992**, *31*, 2809−2812.
(34) Hori, K.; Satake, T.; Yamaguchi, H.; Saiki, Y.; Murakami, T.; Chen, C. M. *Yakugaku Zasshi* **1987**, *107*, 774−779.
(35) Taketa, A. T. C.; Schmittmann-Schlager, T.; Guillaume, D.; Gosmann, G.; Schenkel, E. P. *Phytochemistry* **2000**, *53*, 901−904.
(36) Athayde, M. L.; Schenkel, E. P.; Gnoatto, S. B.; Gosmann, G.; Giberti, G. C.; Guillaume, D. *Acta Farm. Bonaerense* **2001**, *20*, 13−16.
(37) Taketa, A. T. C.; Gnoatto, S. C. B.; Gosmann, G.; Pires, V. S.; Schenkel, E. P.; Guillaume, D. *J. Nat. Prod.* **2004**, *67*, 1697−1700.
(38) Taketa, A. T. C.; Gondorf, M.; Breitmaier, E.; Schenkel, E. P. *Rev. Bras. Cienc. Farm.* **2002**, *38*, 155−161.
(39) Kartnig, T.; Wegschaider, O. *Planta Med.* **1972**, *21*, 144−149.
(40) Kim, Y. M.; Jeong, Y. K.; Wang M. H.; Lee, W. Y.; Rhee, H. I. *Nutrition* **2005**, *21*, 756−761.
(41) Sovik, S. L.; Rustad, T. *Food Sci. Technol.* **2005**, *38*, 867−876.

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