# Flavonoids from *Millettia nitida* var. *hirsutissima*

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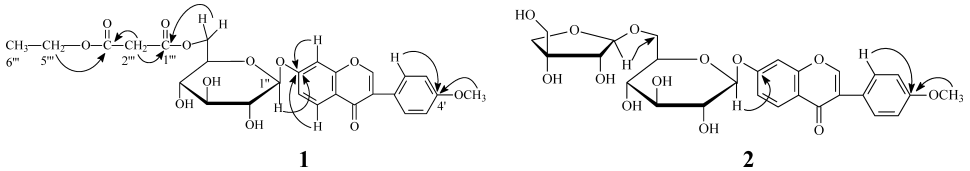
**From the stems of *Millettia nitida* var. *hirsutissima*, three new isoflavone glycosides, formononetin 7-*O*-β-D-(6″-ethylmalonyl)-glucopyranoside (1, hirsutissimiside A), 5-*O*-methyl genistein 7-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (3, hirsutissimiside B), retusin 7,8-di-*O*-β-D-glucopyranoside (4, hirsutissimiside C) and two known isoflavone glycosides (2) and (5) have been isolated. The structures of the compounds were determined by spectroscopic and chemical means.**

**Key words** *Millettia nitida* var. *hirsutissima*; Leguminosae; isoflavone; hirsutissimiside A; hirsutissimiside B; hirsutissimiside C

*Millettia nitida* var. *hirsutissima* (Leguminosae) is an indigenous vine in the Jiang Xi province of China, and has been used as a traditional Chinese medicine named Ji-Xue-Teng for thousands of years. The decoction of stem is useful in promoting blood circulation or relieving stasis. It has been used to treat pain or numbness of the wrists, knees or other joints and to battle irregular menstruation.[1)] However, there has been no report on the constituents from this plant. Investigation of this plant led to the isolation of three new isoflavonoid glycosides named formononetin 7-*O*-β-D-(6″-ethylmalonyl)-glucopyranoside (**1**, hirsutissimiside A), 5-*O*-methylgenistein 7-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**3**, hirsutissimiside B), retusin 7,8-di-*O*-β-D-glucopyranoside (**4**, hirsutissimiside C), along with two known isoflavonoid glycosides formononetin 7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**2**)[2)] and genistein 8-C-apiosyl-(1→6)-glucopyranoside (**5**).[3)] Their structures were determined by the application of spectroscopic and chemical methods.

## Results and Discussion

Compound **1**, a white powder, has the molecular formula $C_{27}H_{28}O_{12}$, as revealed by positive HR-FAB-MS at *m*/*z* 545.1651 $[M+H]^+$. Its IR spectrum revealed the presence of hydroxyl groups (3481 $cm^{-1}$) and carbonyl groups (1745, 1700 $cm^{-1}$). The UV spectrum of **1** showed absorption maxima (log ε) at 206 (3.74), 258 (3.77) nm, and in the $^1$H-NMR, one proton signal at δ 8.43 and seven aromatic protons were observed, indicating **1** is an isoflavone compound. One proton signal at δ 8.43 (1H, s) was assignable to H-2, whereas the $A_2B_2$ patterns at δ 7.01 (2H, d, *J*=8.7 Hz), 7.53 (2H, d *J*=8.7 Hz) were assigned to H-3′,5′ and H-2′,6′ of the B-ring, respectively. A typical ABX spin system at δ 8.08 (1H, d, *J*=9.0 Hz), 7.15 (1H, dd, *J*=9.0, 2.1 Hz) and 7.23 (1H, d, *J*=2.1 Hz) were assigned to H-5, H-6 and H-8 of the A-ring, respectively. Therefore, the structure of **1** was concluded to be a 7,4′-disubstituted isoflavone. In the $^{13}$C-NMR spectrum of **1**, the signals of the sugar moiety were in good agreement with those of the reported $^{13}$C-NMR data for glucose of the compound naringenin 7-*O*-(6″-*p*-coumaroyl)-glucoside.[4)] The $^1$H-NMR spectrum of **1** contained an anomeric signal of the sugar moiety at δ 5.17 (1H, d, *J*=6.9 Hz), showing that glucose had a β-configuration. And a singlet at δ 3.79 (3H) due to a methoxy group was observed. The HMBC experiments gave the following cross-peaks at δ 3.79 ($OCH_3$) and 7.53 (2′,6′-H) with 159.04 (4′-C), and 5.17 (glc 1-H) and 8.08 (5-H) with 161.16 (7-C), which indicated the $OCH_3$ was attached to C-4′ and the sugar moiety was determined to be linked to an aglycone *via* C-7 of **1**. In the $^{13}$C-NMR spectrum, except for the resonance of carbon belonging to aglycone and sugar moieties, two carbonyl signals were visible at δ 166.50, 166.36, and two methylene, and one methyl signal could be seen at δ 60.82, 41.06 and 13.87, respectively. The corresponding proton signals were observed at δ 4.04 (2H, q, *J*=7.2 Hz), 3.51 (2H, s), 1.11 (3H, t, *J*=7.2 Hz) in HMQC, respectively. The $^1$H–$^1$H COSY spectrum showed a correlation between $CH_2$ (δ 4.04, 5‴-H) and $CH_3$ (δ 1.11). The HMBC spectrum showed the following $^1$H and $^{13}$C long-range correlations: 2‴-H (δ 3.51, 2H, s) with two carbonyl carbons at δ 166.50 and 166.36, and 5‴-H (2H, q) with 3‴-C, indicating the presence of an ethylmalonyl moiety. A cross peak in the HMBC spectrum between H-6″ (δ 4.14) of the glucose with a carbonyl signal at δ 166.50 of the ethylmalonic acid ester moiety indicated that the ethylmalonic acid ester was attached to 6-hydroxyl of the glucose residue (Fig. 1). One and two-dimensional NMR techniques (HMQC, HMBC) permitted assignments of all $^1$H and $^{13}$C signals of **1** (Tables 1, 2). Therefore, **1** was characterized as formononetin 7-*O*-β-D-(6″-ethylmalonyl)-glucopyranoside. Formononetin 7-*O*-β-D-(6″-*O*-malonyl)-glucopyranoside was reported to be a constituent of *Trifolium subteeraneum*.[5)] **1** was a new compound named hirsutissimiside A. The powdered *Millettia nitida* var. *hirsutissima* (1 g) was successively extracted with $CH_3CN$ and MeOH for 0.5 h by an ultrasonic



**1** **2**

Fig. 1. The Structure and Key HMBC of **1**, **2**

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method, respectively. The extracts were compared with **1** on HPLC (LUNA 5 $\mu$ C18 250×4.60 mm, flow: 1 ml/min, 40% MeOH), showing approximately the same peak at Rt 29.5. So compound **1** is not an artifact.

Compound **2**, a white powder, showed the molecular formula $C_{27}H_{30}O_{13}$ as revealed by positive HR-FAB-MS at *m/z* 563.1758 $[M+H]^+$. The UV absorption maxima ($\log \varepsilon$) at 209 (3.80), 257.5 (3.83) nm, and the $^1$H- and $^{13}$C-NMR data (Tables 1, 2) of **2** showed close resemblance with those of **1**, except for the signals due to C-6″ substituent in the NMR, indicating it to be an analogue of **1**. Two anomeric proton signals of sugars at $\delta$ 4.82 (1H, $J$=3.0 Hz, api 1-H) and 5.07 (1H, $J$=6.6 Hz, glc 1-H) were observed. All the glycosidic linkages were $\beta$-configuration, as revealed from the coupling constants and $^{13}$C-NMR data of the sugars. The HMBC experiments showed correlations of glc 1-H ($\delta$ 5.07) and api 1-H ($\delta$ 4.82) with the C-7 of aglycone ($\delta$ 161.39) and C-6 of glucose ($\delta$ 67.83), respectively; and the methoxy protons at 3.79 (3H, s) with C-4′ of the B-ring at $\delta$ 159.04 (Fig. 1). Therefore, **2** was identified as formononetin 7-*O*-$\beta$-D-apiofuranosyl-(1→6)-$\beta$-D-glucopyranoside. As the structure of **2** in the reference was confirmed to be an *O*-acetyl derivative,[2] the spectral data of **2** were provided for the first time.

Compound **3** was obtained as a colorless slice crystal. The UV spectrum of **3** showed absorption maxima ($\log \varepsilon$) at 208.5 (3.80), 252.5 (3.12) nm. The UV and its $^1$H-NMR data showed **3** to be an isoflavone. The singlet at $\delta$ 8.13 (1H, s) was characteristic of an isoflavone and assignable to H-2; the $^1$H-NMR spectrum also showed two doublets at $\delta$ 7.30 (2H, d, $J$=8.5 Hz, H-2′, 6′) and $\delta$ 6.79 (2H, d, $J$=8.5 Hz, H-3′, 5′) due to a 1′,4′-disubstituted B-ring, whereas a pair of meta-coupled aromatic protons at $\delta$ 6.74 (1H, d, $J$=2.0 Hz) and $\delta$ 6.54 (1H, d, $J$=2.0 Hz) were attributed to H-8 and H-6 of the A-ring. Two anomeric proton signals of sugars at $\delta$ 4.52 (1H, br s) and $\delta$ 5.03 (1H, d, $J$=7.0 Hz), a methoxy proton signal at $\delta$ 3.83 (3H, s) and hydroxy proton signal at 9.48 (1H, s) were observed. The UV spectrum in band II did not change on the addition of NaOAc and $AlCl_3$, suggesting the absence of 7 and 5 hydroxy groups, and the presence of a free 4′-hydroxy group.[6] Upon acidic hydrolysis with 2.0 M HCl, **3** gave glucose and rhamnose, which were identified by TLC with authentic samples. The $^1$H-NMR spectrum of **3** contained signals of the sugar moiety at $\delta$ 5.03 (1H, d, $J$=7.0 Hz), $\delta$ 4.52 (br s), showing that glucose had the $\beta$-configuration, and rhamnose was an $\alpha$-configuration configuration. D- and L-forms for glucose and rhamnose were based on the fact that all of the glucosyl and rhamnosyl residues in natural glycosides found in higher plants are D- and L-forms, respectively. One and two-dimensional NMR techniques (HMQC, HMBC) permitted assignments of all $^1$H and $^{13}$C signals of **3** (see Tables 1, 2). The HMBC experiments gave the following cross-peaks: a methoxyl proton ($\delta$ 3.83) with C-5 of aglycone ($\delta$ 160.6), H-1″ of glucose ($\delta$ 5.03) with C-7 of aglycone ($\delta$ 161.2), and H-1‴ of rhamnose ($\delta$ 4.52, br s) with C-6 of glucose ($\delta$ 66.5), respectively (see Fig. 2). Thus, the structure of the sugar moiety was elucidated as $\alpha$-L-rhamnopyranosyl-(1→6)-$\beta$-D-glucopyranose, and linkages of sugar and methoxyl were determined to be attached at C-7 and C-5, respectively. NOESY correlations were found between H-1″ of glucose at $\delta$ 5.03 and the H-6 signal at $\delta$ 6.54 and H-8 signal at $\delta$ 6.74; and between protons of methoxyl group at $\delta$ 3.83 and H-6 proton signal at $\delta$ 6.54, further supporting the above conclusion. Consequently, the structure of **3** was identified as 5-*O*-methylgenistein 7-*O*-$\alpha$-L-rhamnopyranosyl-(1→6)-$\beta$-D-glucopyranoside. **3** had the molecular formula $C_{28}H_{32}O_{14}$ as revealed by negative HR-FAB-MS at *m/z* 591.1700 $[M-H]^-$. **3** was a new compound and named hirsutissimiside B.

Compound **4**, was obtained as a white powder. The UV spectrum of **4** showed absorption maxima ($\log \varepsilon$) at 206.5 (3.82), 252.5 (3.12) nm, and in the $^1$H-NMR one proton signal at $\delta$ 8.48, and six aromatic protons were observed, indicating **4** possesses an isoflavone structure. In the $^1$H-NMR

Table 1. $^1$H-NMR (300 MHz) Data for **1**—**4** (DMSO-$d_6$)

| Proton | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 2 | 8.43, s | 8.41, s | 8.13, s | 8.48, s |
| 5 | 8.08, d (9.0) | 8.07, d (8.7) | | 7.82, d (9.0) |
| 6 | 7.15, dd (9.0, 2.1) | 7.16, dd (8.7, 2.1) | 6.54, d (2.0) | 7.40, d (9.0) |
| 8 | 7.23, d (2.1) | 7.25, d (2.1) | 6.74, d (2.0) | |
| 2′, 6′ | 7.53, d (8.7) | 7.53, d (8.7) | 7.30, d (8.5) | 7.52, d (9.0) |
| 3′, 5′ | 7.01, d (8.7) | 7.01, d (8.7) | 6.79, d (8.5) | 6.99, d (9.0) |
| glc 1″ | 5.17, d (6.9) | 5.07, d (6.6) | 5.03, d (7.0) | 5.00, d (7.5) |
| Sugar | | api | rha | glc |
| 1‴ | | 4.82, d (3.0) | 4.52 br s | 5.11, d (8.0) |
| 6‴ | | | 1.09, d (6.0) | |
| $OCH_3$ | 3.79, s | 3.79, s | 3.83, s | 3.78, s |
| OH | | | 9.48 (4′-OH) | |
| $-CH_2-$ | 3.51, s | | | |
| $CH_3\underline{CH_2}$ | 4.04, q (7.2) | | | |
| $\underline{CH_3}CH_2$ | 1.11, t (7.2) | | | |
| C=O | | | | |

Table 2. $^{13}$C-NMR (75 MHz) Data for **1**—**4** (DMSO-$d_6$)

| Proton | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 2 | 153.76 | 153.66 | 151.0 | 153.3 |
| 3 | 123.99 | 124.06 | 124.7 | 122.9 |
| 4 | 174.68 | 174.72 | 173.9 | 174.8 |
| 5 | 127.06 | 127.04 | 160.6 | 120.8 |
| 6 | 115.37 | 115.49 | 97.1 | 114.5 |
| 7 | 161.16 | 161.39 | 161.2 | 153.1 |
| 8 | 103.54 | 103.66 | 95.8 | 134.0 |
| 9 | 157.01 | 157.05 | 158.8 | 150.1 |
| 10 | 118.54 | 118.52 | 109.6 | 119.6 |
| 1′ | 123.37 | 123.33 | 122.6 | 124.0 |
| 2′ | 130.08 | 130.10 | 130.1 | 130.0 |
| 3′ | 113.65 | 113.66 | 114.8 | 113.7 |
| 4′ | 159.04 | 159.04 | 157.0 | 159.0 |
| 5′ | 113.65 | 113.66 | 114.8 | 113.7 |
| 6′ | 130.08 | 130.10 | 130.1 | 130.0 |
| glc | | | | |
| 1″ | 99.61 | 99.97 | 99.9 | 101.5 |
| 2″ | 72.99 | 73.09 | 73.1 | 73.5 |
| 3″ | 76.16 | 75.93 | 76.6 | 76.5 |
| 4″ | 69.57 | 69.97 | 70.1 | 69.7 |
| 5″ | 73.69 | 75.66 | 75.6 | 77.3 |
| 6″ | 64.19 | 67.83 | 66.5 | 60.8 |
| Sugar | | api | rha | glc |
| 1‴ | | 109.48 | 100.6 | 103.5 |
| 2‴ | | 76.46 | 70.3 | 74.2 |
| 3‴ | | 78.71 | 70.7 | 75.9 |
| 4‴ | | 73.30 | 72.1 | 69.6 |
| 5‴ | | 63.10 | 68.4 | 77.4 |
| 6‴ | | | 17.8 | 60.7 |
| $OCH_3$ | 55.19 | 55.15 | 56.1 | 55.1 |
| $-CH_2-$ | 41.06 | | | |
| $CH_3\underline{CH_2}$ | 60.82 | | | |
| $\underline{CH_3}CH_2$ | 13.87 | | | |
| C=O | 166.50, 166.36 | | | |

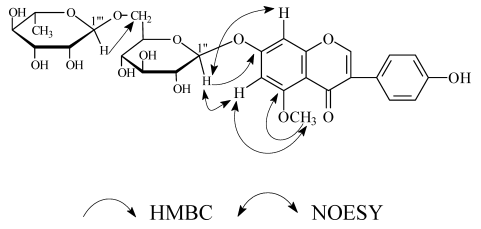


Fig. 2. The Structure, Key HMBC and NOESY of **3**

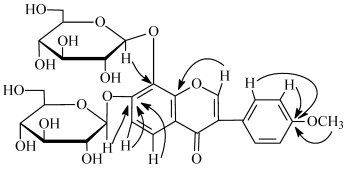

Fig. 3. The Structure and Key HMBC of **4**

spectrum, one proton singlet at $\delta$ 8.48 (1H, s) was characteristic of an isoflavone and assignable to H-2, and showed two doublets at $\delta$ 7.52 (2H, d, $J$=9.0 Hz) and 6.99 (2H, d, $J$=9.0 Hz) due to 1′,4′-disubstitution on the B-ring; signals at $\delta$ 7.82 (1H, d, $J$=9.0 Hz) and 7.40 (1H, d, $J$=9.0 Hz) were attributed to ortho-coupled aromatic protons of the A-ring. The presence of a methoxy group was exhibited by a proton signal at $\delta$ 3.78. Upon acidic hydrolysis with 2.0 M HCl, **4** gave glucose, which was identified by TLC with an authentic sample. The $^1$H-NMR spectrum of **4** contained two anomeric proton signals at $\delta$ 5.00 (1H, d, $J$=7.5 Hz) and $\delta$ 5.11 (1H, d, $J$=8.0 Hz), showing that the glycosidic linkages were both $\beta$-configuration. Two-dimensional NMR techniques (HMQC, HMBC) permitted assignments of all $^1$H- and $^{13}$C-NMR signals of **4** (see Tables 1, 2). The HMBC experiments showed correlation of the methoxyl proton ($\delta$ 3.78), 3′,5′-H ($\delta$ 6.99, d, $J$=9.0 Hz) and 2′,6′-H ($\delta$ 7.52, d, $J$=9.0 Hz) of the B-ring with C-4′ ($\delta$ 159.0); and of glc 1″-H ($\delta$ 5.00, $J$=7.5 Hz), glc 1‴-H ($\delta$ 5.11, $J$=8.0 Hz) with 7-C ($\delta$ 153.1) and C-8 ($\delta$ 134.0), respectively (see Fig. 3). Thus, the structure of **4** was elucidated as retusin-7,8-di-*O*-$\beta$-D-glucopyranoside, which was consistent with the molecular formula $C_{28}H_{32}O_{15}$ as revealed by negative HR-FAB-MS at *m*/*z* 607.1664 [M−H]. **4** was a new compound and named hirsutissimiside C.

## Experimental

IR spectra were measured on a Perkin-Eimer 243B Polarimeter. UV spectra were recorded on Shimadzu UV-260 spectrometer. NMR was taken on a VXR-300 instrument, with TMS as an internal standard. Mass spectra were obtained with AEI-MS-50 (EI-MS), APEX II (HR-FAB) and PE-QSTAR (TOF-MS) mass spectrometers. Silica gel $GF_{254}$ and Silica gel (100—200, 200—300 mesh) were purchased from Marine Chemical Plant, Qingdao. Sephadex LH-20 (Amersham Pharmacia Biotech AB, Sweden) was used for chromatographic separations.

**Plant Material** The dried stems of *Millettia nitida* var. *hirsutissima* were collected in Feng Cheng, Jiang Xi province, in May 2003, and identified by Professor Hu Biao Chen. A voucher specimen was deposited in the Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University.

**Extraction and Isolation** The dried stems of *Millettia nitida* var. *hirsutissima* (6.5 kg) was extracted with 95% EtOH and 50% EtOH at room temp., and the combined extract was concentrated under reduced pressure. The residue was suspended in $H_2O$, and then extracted with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH, successively. After removal of the solvent *in vacuo*, the following residues were sequentially obtained: petroleum ether extract (47 g), $CHCl_3$ extract (20.2 g), EtOAc extract (47.0 g) and *n*-BuOH extract (169.5 g).

The EtOAc extract (46.6 g) was subjected to CC on silica gel, eluted with $CHCl_3$–MeOH (10 : 3), and five fractions (A—E) were obtained.

Fr. C (21.93 g) was chromatographed over silica gel [$CHCl_3$–MeOH (10 : 1)] to give two fractions, 1 and 2. Fr. 2 was separated by HPLC [Kromasil, C18, 5 $\mu$m, 250×10 mm, MeOH–$H_2O$ (1 : 2)] to yield compound **2** (12 mg).

Fr. B (19.40 g) was subjected to CC on silica gel, and eluted with a gradient solvent system $CHCl_3$–MeOH (100 : 2→10 : 1) to afford fractions 1—7. Fraction 6 was purified by CC over Sephadex LH-20 [$CHCl_3$–MeOH (1 : 1)] to give compound **1** (8 mg).

The *n*-BuOH extracts from 95% EtOH and 50% EtOH were combined (160 g), and the mixture was subjected to CC on silica gel, eluted with $CHCl_3$–MeOH–$H_2O$ (65 : 35 : 10), and four fractions (A—D) were obtained. Fr. C was chromatographed on silica gel, and eluted with $CHCl_3$–MeOH–$H_2O$ (65 : 35 : 10) to yield four fractions 1—4.

Fr. 2 was chromatographed on Sephadex LH-20 and eluted with $CHCl_3$–MeOH (1 : 1) to give subfractions 1—3, then subfraction 1 was subjected to reversed-phase silica gel column chromatography and eluted with a gradient solvent system of MeOH–$H_2O$ (1 : 10→10 : 1), followed by HPLC [Kromasil 250×10 mm, C18, 5 $\mu$m, MeOH–$H_2O$ (1 : 2)] to produce **3** (8 mg) and **4** (12 mg).

Fr. 3 was chromatographed on silica gel, eluted with $CHCl_3$–MeOH–$H_2O$ (65 : 35 : 10), and 21 fractions were obtained. Fr. 13—15 was subjected to reversed-phase silica gel column chromatography and eluted with a gradient solvent system of MeOH–$H_2O$ (1 : 10→10 : 1) to obtain subfractions 1—10, then subfractions 7 and 8 were chromatographed on Sephadex LH-20 and eluted with $CHCl_3$–MeOH (1 : 1) to give compound **5** (15 mg).

Hirsutissimiside A (**1**): White powder, $[\alpha]_D^{25}$ −26.3° ($c$=0.13, MeOH : $H_2O$=1 : 0.5); UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 206 (3.74), 258 (3.77); IR (KBr) cm$^{-1}$: 3481, 3275 (OH), 1745, 1700 (ester carbonyl), 1621, 1511, 1444, 1349, 1249, 1184, 1078, 1035; for $^1$H-, $^{13}$C-NMR spectral data, see Tables 1 and 2; HR-FAB-MS *m*/*z* [M+H]$^+$: 545.1651 (Calcd for $C_{27}H_{29}O_{12}$: 545.1653).

Formononetin 7-*O*-$\beta$-D-Apiofuranosyl-(1→6)-$\beta$-D-glucopyranoside (**2**): White powder, $[\alpha]_D^{25}$ 78.2° ($c$=0.25, MeOH : $H_2O$=1 : 0.5); UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 209 (3.80), 257 (3.83); IR (KBr) cm$^{-1}$. 3355 (OH), 2924, 2856, 1625, 1448, 1251, 1068. For $^1$H-, $^{13}$C-NMR spectral data, see Tables 1 and 2. HR-FAB-MS *m*/*z* [M+H]$^+$: 563.1758 (Calcd for $C_{27}H_{31}O_{13}$: 563.1759).

Hirsutissimiside B (**3**): White powder, $[\alpha]_D^{25}$ −73.3° ($c$=0.19, MeOH); UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 208.5 (3.80), 252.5 (3.12); NaOAc: 212 (4.21), 256.5 (3.77); $AlCl_3$: 206.5 (3.71), 252.5 (3.95); for $^1$H-, $^{13}$C-NMR spectral data, see Tables 1 and 2. HR-FAB-MS *m*/*z* [M−H]$^+$: 591.1700 (Calcd for $C_{28}H_{31}O_{14}$: 591.1708).

Hirsutissimiside C (**4**): White powder, $[\alpha]_D^{25}$ −38.0° ($c$=0.14, MeOH : $H_2O$=1 : 1); UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 206.5 (3.82), 252.5 (3.12); NaOAc: 209.5 (4.11), 242.5 (3.63); $AlCl_3$: 208.5 (4.07), 252.5 (3.65); for $^1$H-, $^{13}$C-NMR spectral data, see Tables 1 and 2. HR-FAB-MS *m*/*z* [M−H]$^+$: 607.1664 (Calcd for $C_{28}H_{31}O_{15}$: 607.1657).

**Acid Hydrolysis of 3 and 4** A small amount of **3** and **4** was hydrolyzed by 2 M HCl in 100 °C for 4 h., respectively. After filtration of the reaction mixture, the filtrate was neutralized with $BaCO_3$ to give a residue, which was examined by HP-TLC comparison with authentic samples [$CHCl_3$–MeOH–$H_2O$ (75 : 30 : 10, lower level–HOAc=9 : 1), colour reagent: 0.9% aniline-0.05% mol/l oxalic acid]. **3** gave glucose and rhamnose, **4** gave only glucose.

## References

1) Jiang Su Xin Yi Xue Yuan, "Dictionary of Chinese Medicine (1)," Shanghai People's Press, Shanghai, 1977, p. 1206.
2) Kamara B. I., Brandt E. V., Ferreira D., Joubert E., *J. Agric. Food Chem.*, **51**, 3874—3879 (2003).
3) Kinjo J. E., Furusawa J. I., Baba J., Takeshita T., Yamasake M., Nohara T., *Chem. Pharm. Bull.*, **35**, 4846—4850 (1987).
4) Harborne J. B., Mabry T. J., "The Flavonoids: advances in research," Champman and Hall Press, London, 1982, p. 115.
5) Beck A. B., Knox J. R., *Aust. J. Chem.*, **24**, 1509 (1971).
6) Division of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, "The Handbook of Identification of Flavonoids," Science Press, Beijing, 1981, p. 518.