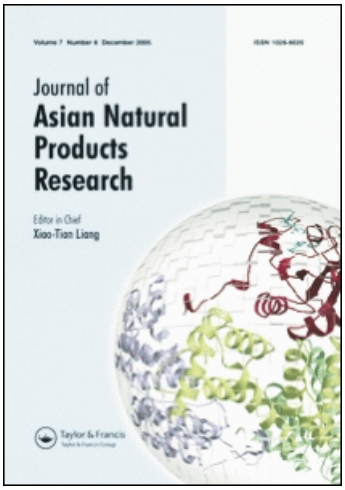

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New flavonoids and other constituents from *Lespedeza cuneata*

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New flavonoids and other constituents from *Lespedeza cuneata*

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Two new flavonoids, 6,8,3′,4′-tetrahydroxy-2′-methoxy-7-methylisoflavanone (**1**) and 6,8,3′,4′-tetrahydroxy-2′-methoxy-6′-(1,1-dimethylallyl)-isoflavone (**2**), were isolated from the 95% ethanolic extract of the dried roots of *Lespedeza cuneata* together with betulinic acid (**3**), β-sitosterol (**4**), hexacosanoic acid 2,3-dihydroxy-propyl ester (**5**), which were isolated from this plant for the first time. Their structures were elucidated by spectral analysis.

Keywords: *Lespedeza cuneata*; Flavonoids; 6,8,3′,4′-Tetrahydroxy-2′-methoxy-7-methylisoflavanone; 6,8,3′,4′-Tetrahydroxy-2′-methoxy-6′-(1,1-dimethylallyl)-isoflavone

1. Introduction

The roots and leaves of *Lespedeza cuneata* have long been used as a traditional folk medicine for the treatment of dysentery and hepatitis [1]. Previously, flavonoids, D-fructose, myo-inositol, D-pinitol and organic acid salts were isolated from the extract of this species [2–7]. As a part of our continued studies on the bioactive constituents of Chinese medicinal plants, the constituents of *Lespedeza cuneata* were investigated. Here we report the isolation and structural elucidation of two new flavonoids, 6,8,3′,4′-tetrahydroxy-2′-methoxy-7-methylisoflavanone (**1**) and 6,8,3′,4′-tetrahydroxy-2′-methoxy-6′-(1,1-dimethylallyl)-isoflavone (**2**) (figure 1). together with the known compounds betulinic acid (**3**), β-sitosterol (**4**) and hexacosanoic acid 2,3-dihydroxy-propyl ester (**5**), which were isolated for the first time from the 95% ethanolic extract of the dried roots of *Lespedeza cuneata*. Anti-tumour tests of compounds **1** and **2** on P388, HL-60, A-549 and BEL-7402 have been performed, but the activities were low

2. Results and discussion

Compound **1** was obtained as yellow amorphous powder, displaying a molecular ion at *m/z* 355.0780 ($[M + Na]^+$, HRESI-MS) corresponding to the formula $C_{17}H_{16}O_7$. The IR spectrum showed the presence of hydroxyl group (3330 cm^{-1}) and carbonyl group

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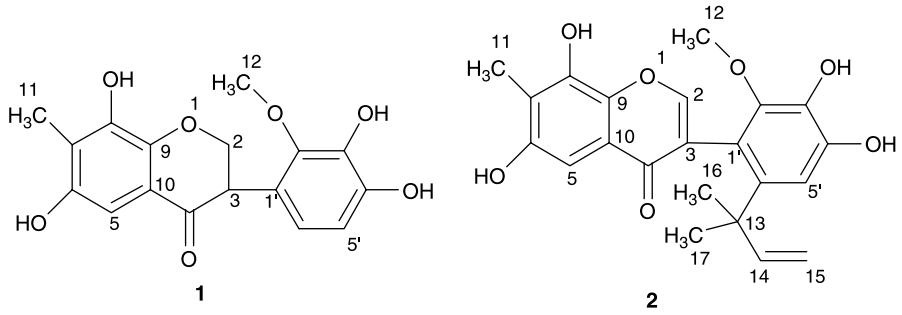

Figure 1. The structures of compounds **1** and **2**.

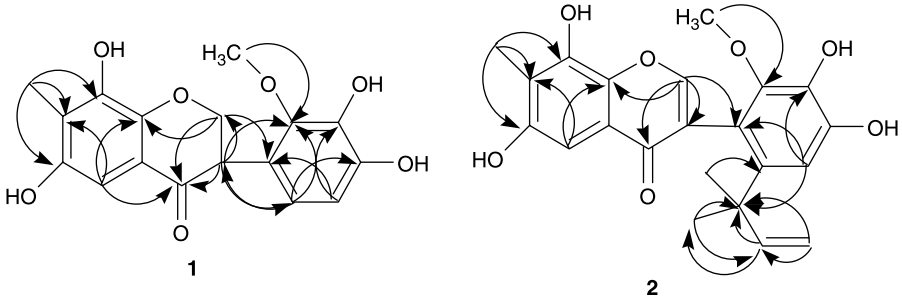

Figure 2. HMBC correlations of compounds **1** and **2**.

(1637 cm^{-1}). The UV absorptions at 202, 293 nm were characteristic of dihydroflavone [8]. The ^{1}H NMR spectrum exhibited *ortho*-coupled signals at δ 6.52 (1H, d, $J = 8.6$ Hz, H-5′) and 6.46 (1H, d, $J = 8.6$ Hz, H-6′) due to ring B, and a proton singlet at δ 5.92 (1H, s, H-5) for ring A. The aliphatic proton signals at δ 4.38 (2H, m, H-2) and 4.14 (1H, dd, $J = 5.5$, 11.2 Hz, H-3) contributed to a CH—CH_2 system of ring C. Furthermore, in the HMBC experiment (figure 2) the proton signal at δ 4.38 (H-2) correlated with carbon signal at δ 163.1 (C-9) which confirmed an iso-flavonone skeleton. The proton signal at δ 5.92 (H-5) correlated with carbon signal at δ 199.8 (C-4) and the proton signal at δ 1.95 (3H, s, H-11) correlated with carbon signals at δ 166.4 (C-6), 105.8 (C-7) and 163.4 (C-8), indicating that the methyl group was linked to C-7 and the two hydroxyl groups were connected with C-6 and C-8, respectively. Both proton signals at δ 4.38 (H-3) and 3.97 (3H, s, H-12) correlated with carbon signal at δ 148.6 (C-2′), suggesting the methoxyl group was connected to C-2′. Thus compound **1** was determined as 6,8,3′,4′-tetrahydroxy-2′-methoxy-7-methylisoflavanone.

Compound **2** was obtained as yellow amorphous powder, displaying a molecular ion at *m/z* 421.1267 ($[M + Na]^+$, HRESI-MS) corresponding to the formula $C_{22}H_{22}O_7$. The IR spectrum showed the presence of hydroxyl group (3430 cm^{-1}) and carbonyl group (1648 cm^{-1}). The UV absorption maximum at 263 nm suggested the presence of either an isoflavonoid or flavonone skeleton [9]. In the ^{1}H NMR spectrum, one singlet observed at δ 7.96 was characteristic of an isoflavone skeleton. Two proton singlets at δ 6.65 and 6.40 were due to ring B and ring A, respectively. A 1,1-dimethylallyl substitution was assigned on the basis of characteristic ^{1}H NMR signals [δ 1.47 (6H, s, H-16, 17), 6.26 (1H, dd, $J = 10.3$, 17.6 Hz, H-14), 5.0 (1H, d, $J = 17.6$ Hz, H-15 trans) and 4.95 (1H, d, $J = 10.3$ Hz, H-15 *cis*)]

which were correlated with corresponding carbon signals [δ 24.8 (C-16, 17), 146.6 (C-14) and 108.3 (C-15)] in the HMQC spectrum. Furthermore, in the HMBC experiment (figure 2), the proton signal at δ 2.06 (3H, s, H-11) correlated with carbon signals at δ 161.6 (C-6), 106.6 (C-7) and 158.0 (C-8), indicating the methyl group was linked to C-7 and the two hydroxyl groups connected with C-6 and C-8, respectively. The proton signal at δ 3.61 (3H, s, H-12) correlated with carbon signal at δ 144.6 (C-2′), suggesting the methoxyl group was linked to C-2′. The proton signal at δ 1.47 (6H, s, H-16, 17) correlated with carbon signal at δ 128.6 (C-6′) indicating the 1,1-dimethylallyl group was connected to C-6′. Based on these spectral data, compound **2** was deduced to be 6,8,3′,4′-tetrahydroxy-2′-methoxy-6′-(1,1-dimethylallyl)-isoflavone.

In addition, the other constituents isolated from *Lespedeza cuneata* were identified as betulinic acid (**3**) [10], β-sitosterol (**4**) [11], and hexacosanoic acid 2,3-dihydroxy-propyl ester (**5**).

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained on a Perkin–Elmer 241 automatic digital polarimeter. UV spectral data were measured on a Shimadzu UV-260 instrument. IR spectral data were measured on a Perkin–Elmer 599B instrument with KBr disks. ^{1}H NMR, ^{13}C NMR, ^{1}H—^{1}H COSY, HMQC, and HMBC spectra were recorded on a Bruker DRX-400 spectrometer (^{1}H 400 MHz and ^{13}C 100 MHz) using TMS as internal standard. The carbon multiplicities were obtained by DEPT experiment. ESI-MS and HRESI-MS data were measured on a Quattro instrument. Silica gel (200–300 mesh) was used for column chromatography. Reversed-phase chromatography column: TSK gel Toyopearl HW-40F (30–60 μm, Toso Co. Ltd.), and Cosmosil 75 C_{18}—OPN (42(105 μm, Nacalai Tesque Inc.) columns. TLC: precoated silica gel 60 F_{254} plates (0.2 mm, Merck).

3.2 Plant material

The dried roots of *Lespedeza cuneata* were collected from Yingtan city of Jiangxi Province, China in August 2003 and identified by Professor Deng Shui Sheng. A voucher specimen (SIMMP 03517) has been deposited in the Herbarium of Shanghai Institute of Materia Medica.

3.3 Extraction and isolation

Dried roots of *Lespedeza cuneata* (6 kg) were extracted with 95% ethanol for 6 days at room temperature. The solvent was removed *in vacuo* to yield 160 g of a gummy residue. The residue was suspended in water and extracted with ethyl acetate. The extract was subjected to silica gel chromatography eluting with cyclohexane/EtOAc (10:1 → 1:2) as gradient eluent to yield fractions A–D. Fraction B (cyclohexane/EtOAc = 5:1) was repeatedly chromatographed on silica gel to give compounds **3** (5.6 g) and **4** (70 mg). Fraction C (cyclohexane/EtOAc = 2:1) was repeatedly chromatographed on silica gel, Cosmosil 75 C_{18}—OPN (eluted with 75% MeOH), and Toyopearl HW-40F (eluted with EtOH) to give **1** (80 mg) and **5** (20 mg), respectively. Fraction D (cyclohexane/EtOAc = 1:1) was repeatedly chromatographed on

silica gel, Cosmosil 75 C_{18}—OPN (eluted with 70% MeOH), and Toyopearl HW-40F (eluted with EtOH) to give **2** (11 mg).

3.3.1 6,8,3′,4′-Tetrahydroxy-2′-methoxy-7-methylisoflavanone (1). A yellow amorphous powder, $C_{17}H_{16}O_7$; $[\alpha]_D^{20}$ −7.9 (*c* 0.49, MeOH); UV (MeOH) λ_{max} (nm): 202, 293; IR (KBr) (cm^{-1}): 3330, 2937, 1637, 1600, 1511, 1471, 1384, 1288, 1161, 1110, 1043, 831; ESI-MS *m/z*: 333 $[M + H]^+$, HRESI-MS *m/z*: 355.0780 $[M + Na]^+$ (calcd for $C_{17}H_{16}O_7Na$, 355.0794); 331 $[M—H]^-$; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.52 (1H, d, $J = 8.6$ Hz, H-5′), 6.46 (1H, d, $J = 8.6$ Hz, H-6′), 5.92 (1H, s, H-5), 4.38 (2H, m, H-2), 4.14 (1H, dd, $J = 5.5$, 11.2 Hz, H-3), 3.79 (3H, s, H-2′), 1.95 (3H, s, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 199.8 (C-4), 166.4 (C-6), 163.4 (C-8), 163.1 (C-9), 148.6 (C-2′), 148.2 (C-3′), 140.1 (C-4′), 121.5 (C-1′), 121.4 (C-6′), 112.2 (C-5′), 105.8 (C-7), 103.9 (C-10), 95.6 (C-5), 72.6 (C-2), 61.2 (C-OMe), 48.8 (C-3), 7.5 (C-Me).

3.3.2 6,8,3′,4′-Tetrahydroxy-2′-methoxy-6′-(1,1-dimethylallyl)-isoflavone (2). A yellow amorphous powder, $C_{22}H_{22}O_7$; UV (MeOH) λ_{max} (nm): 263; IR (KBr) (cm^{-1}): 3430, 2968, 1648, 1577, 1457, 1359, 1301, 1197, 1120, 1076, 819; ESI-MS *m/z*: 399 $[M + H]^+$, 397 $[M—H]^-$; HRESI-MS *m/z*: 421.1267 $[M + Na]^+$ (calcd for $C_{22}H_{22}O_7Na$, 421.1263); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): δ 7.96 (1H, s, H-2), 6.65 (1H, s, H-5′), 6.40 (1H, s, H-5), 6.26 (1H, dd, $J = 10.3$, 17.6 Hz, H-14), 5.10 (1H, d, $J = 17.6$ Hz, H-15 *trans*), 4.95 (1H, d, $J = 10.3$ Hz, H-15 *cis*), 3.61 (3H, s, H-12), 2.06 (3H, s, H-11), 1.47 (6H, s, H-16, 17); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 179.9 (C-4), 161.6 (C-6), 158.0 (C-8), 155.1 (C-9), 153.6 (C-2), 146.6 (C-14), 144.6 (C-2′), 144.3 (C-3′), 136.7 (C-4′), 128.6 (C-6′), 120.1 (C-1′), 118.6 (C-5′), 113.1 (C-3), 108.3 (C-15), 106.6 (C-7), 103.4 (C-10), 91.4 (C-5), 58.6 (C-12), 38.9 (C-13), 24.8 (2C) (C-16, 17), 5.0 (C-11).

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