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A New Phenolic Compound from *Lespedeza tomentosa*

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Abstract – A new phenolic compound and three known flavonoids isolated from the MeOH extracts of *Lespedeza tomentosa*. Based on spectral data, the isolated compounds were identified as methyl 4,5-dihydroxy-3-methoxy-2-(3-methylbut-2-en-1-yl)benzoate (**1**), 1-methoxylespeflorin G_{11} (**2**), farrerol (**3**) and 1-methoxylespeflorin I_2 (**4**). Methyl 4,5-dihydroxy-3-methoxy-2-(3-methylbut-2-en-1-yl)benzoate (**1**) is newly isolated from plant source.
Key words – *Lespedeza tomentosa*, flavanone, coumestan, pterocarpan, phenolic compound

Introduction

The roots and leaves of *Lespedeza tomentosa* (Fabaceae) are used for preparing tonic agent in Korean and Chinese traditional medicine.[1] In previous phytochemical studies, flavonoids have been isolated from the roots and aerial parts of this plant species, and their structures have been elucidated.[2,3]

Recently, as part of ongoing study of the chemical constituents of plants distributed in Gangwon Province, Korea we isolated five pterocarpans and one isoflavanone from the roots of *L. tomentosa* and determined their structures.[4]

The classification of genus *Lespedeza* is very difficult, because this genus has many hybridized species within basic species in Korea.[5-7] The chemotaxonomic classification method is one of useful tools for the classification of genus *Lespedeza*.[8]

This detailed study was performed to clarify chemical constituents and provide key chemotaxonomic markers of residual parts of *L. tomentosa* roots, which led to discovery of one new phenolic compound and three flavonoids. Herein, we described the isolation and structural elucidation of these compounds.

Experimental

General experimental procedures – MS spectra were measured using an API 3200 LC/MS/MS system (AB Sciex, Concord, Canada). HR-EI-MS spectrum was measured using a JMS-700 spectrometer (JEOL, Tokyo, Japan). NMR spectra were recorded using a Bruker AVANCE 600 spectrometer (Bruker, Rheinstetten, Germany) and a JNM-ECZ400S/L1 spectrometer (JEOL, Tokyo, Japan). Column chromatography was carried out using a Kieselgel 60 (63-200 μm and 40-63 μm, Merck, Darmstadt, Germany) and YMC gel ODS-A (150 μm, YMC, Kyoto, Japan). Flash column chromatography was carried out using the CombiFlash®, Retrieve^{TM} system (Teledyne Isco Inc., NE, USA). Medium pressure liquid chromatography was carried out using a Buchi 682 chromatography pump system (Buchi, Flawil, Switzerland). TLC was performed on glass backed Kieselgel 60 F_{254} and RP F_{254s} plates. Distilled extra pure grade solvents (OCI company Ltd, Incheon, Korea) were used for column chromatography. All other chemicals and reagents used were of analytical grade.

Plant Material – In October 2017, the roots of *Lespedeza tomentosa* were collected from the herbal garden of the College of Pharmacy, Kanwon National University. The samples were identified by Professor Yongsoo Kwon (College of Pharmacy, Kangwon National University). A voucher specimen (KNUH-R-1710-1) was deposited in the herbarium of the College of Pharmacy, Kangwon National University, Korea.

Extraction and isolation – Air-dried *L. tomentosa* roots (2.7 kg) were cut into small pieces and extracted three times with 80 °C MeOH (12 L) under reflux conditions for 4 h. All extracts were filtered and concentrated *in vacuo* at 40 °C. The MeOH extract (360 g)

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was suspended in water and successively partitioned with *n*-hexane, $CHCl_3$, and *n*-BuOH, leaving a residual water-soluble fraction (fr.). The obtained *n*-hexane, $CHCl_3$, and *n*-BuOH soluble fractions were evaporated *in vacuo* to yield the residues of *n*-hexane fr. (14 g), $CHCl_3$ fr. (230 g) and *n*-BuOH fr. (23 g), respectively. The $CHCl_3$ fr. (200 g) was subjected to silica gel column chromatography (CC) (63~200 μm, 2 kg, 11 × 100 cm) with stepwise gradient elution using an *n*-hexane/EtOAc system (from 4:1 to 1:1) to give four fractions (LT-1 - LT-4). The LT-1 fraction (50 g) was re-chromatographed over silica gel CC (63-200 μm, 10 × 50 cm, 1.0 kg) with isocratic elution using *n*-hexane: EtOAc (4:1) to obtain nine fractions (LT-1-1 - LT-1-9). The LT-1-2 fraction (2.7 g) was further separated into six fractions (LT-1-2-1 - LT-1-2-6) using medium pressure ODS CC (MeOH - H_2O, 85 -15). The LT-1-2-5 fraction (70 mg) was further fractionated using silica gel column chromatography with isocratic elution of $CHCl_3$: MeOH (100:1) to obtain four sub-fractions (LT-1-2-5-1 to LT-1-2-5-4). Sub-fraction LT-1-2-5-1 (7 mg) was purified by medium pressure ODS CC (150 μm, 70 g, 2 × 45 cm) with isocratic elution using MeOH: H_2O (85:15) to obtain compound **1** (3 mg). LT-1-5 (4.2 g) was further fractionated by MPLC with ODS using 80 % MeOH to obtain six sub-fractions (LT-1-5-1 - LT-1-5-6). The LT-1-5-2 sub-fraction (2.2 g) was purified by ODS CC using a Buchi 682 chromatography pump elution with 75% MeOH to obtain compound **2** (560 mg). The LT-4 fraction (23 g) was further chromatographed over silica gel CC (63-200 μm, 10 × 50 cm, 500 g) with isocratic elution using *n*-hexane: EtOAc (2:1) to afford four sub-fractions (LT-4-1 - LT-4-5). LT-4-2(3.2 g) was further fractionated using flash chromatography with Redi Sep ODS (80 g, MeOH: H_2O (70: 30) to obtain five sub-fractions (LT-4-2-1 - LT-4-2-5). The LT-4-2-2 sub-fraction (0.4 g) was purified by silica gel CC (40-63 μm, 100 g, 3 × 20 cm) with isocratic elution using n-hexane: EtOAc (3: 1) to obtain compound **3** (15 mg). LT-4-3 (8 g) was further chromatographed over silica gel CC (63-200 μm, 10 × 50 cm, 300 g) with stepwise elution using $CHCl_3$: MeOH (49:1; 39:1; 29:1) to obtain six sub-fractions (LT-4-3-1 - LT-s-3-6). Compound **4** (103 mg) was obtained by filtration of the LT-4-3-4 sub-fraction (2 g).

Compound **1** – gum; ^{1}H-NMR(600 MHz, $CDCl_3$) δ: 11.5 (1H, s, OH), 6.27 (1H, s, H-6), 5.19 (1H, t, *J* = 6.7 Hz, H-2′), 3.97 (3H, s, COOC<u>H</u>$_3$), 3.73 (3H, s, OC<u>H</u>$_3$), 3.35 (2H, d, *J* = 6.7 Hz, H-1′), 1.82 (3H, s, 4′-C<u>H</u>$_3$), 1.75 (3H, s, 5′-C<u>H</u>$_3$); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 170.9 (C=O), 163.0 (C-5), 161.4 (C-4), 160.2 (C-3), 135.0 (C-3′), 122.0 (C-2′), 113.6 (C-2), 100.5 (C-1), 100.4 (C-6),

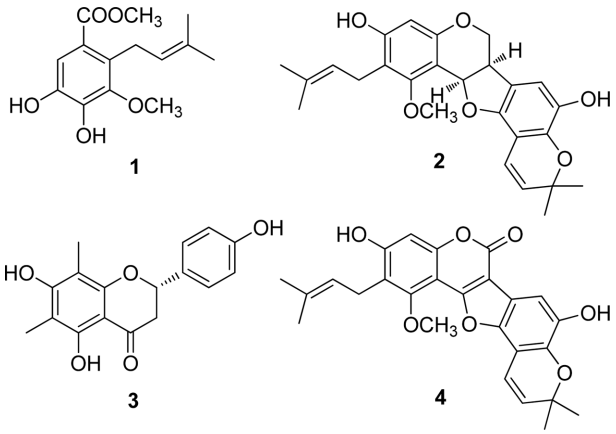

Fig. 1. The structures of compounds **1**-**4**.

62.5 (O<u>C</u>H$_3$), 52.4 (COO<u>C</u>H$_3$), 25.8 (C-4′), 22.5 (C-1′), 17.9 (C-5′); EI-MS *m/z* (rel. int.): 266 [M, 30.7]$^+$, 251 (6.3), 235 (11.5), 234 (37.7), 219 (33.2), 203 (13.7), 191 (13.9), 179 (33.8), 166 (9.2), 151 (5.1), 83 (100); HR-EI-MS *m/z*: 266.1152 (cacld for $C_{14}H_{18}O_5$, 266.1154).

Compound **2** – dark brown powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 6.72 (1H, s, H-7), 6.47 (1H, d, *J* = 9.8 Hz, H-12), 6.27 (1H, s, H-4), 5.58 (2H, m, H-11a, H-13), 5.25 (1H, t, *J* = 6.0 Hz, H-2′), 4.17 (1H, dd, *J* = 11.2, 5.17 Hz, H-6α), 3.97 (3H, s, OCH_3), 3.60 (1H, t, *J* = 11.2 Hz, H-6β), 3.41 (2H, m, H-1′), 3.32 (1H, m, H-6a), 1.84 (3H, s, 5′-CH_3), 1.77 (3H, s, 4′-CH_3), 1.47 (3H, s, 15-CH_3), 1.44 (3H, s, 16-CH_3); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 159.6 (C-1), 157.2 (C-3), 155.3 (C-4a), 148.5 (C-10a), 140.0 (C-9), 138.6 (C-8), 134.9 (C-3′), 129.5 (C-13), 118.2 (C-6a), 117.0 (C-12), 114.1 (C-2), 110.4 (C-7), 107.1 (C-11b), 105.9 (C-10), 100.4 (C-4), 75.6 (C-14), 66.2 (C-6), 39.7 (C-6a), 27.9 (C-15), 27.8 (C-16), 25.8 (C-4′), 23.0 (C-1′), 17.9 (C-5′); ESI-MS *m/z*: 435 [M-H]$^-$.

Compound **3** – pale yellow amorphous powder; ^{1}H-NMR (600 MHz, CD_3OD) δ: 7.32 (2H, d, *J* = 8.5 Hz, H-2, H-6), 6.83 (2H, d, *J* = 8.5 Hz, H-3, H-5), 5.28 (1H, dd, *J* = 12.9, 2.7 Hz, H-2), 3.05 (1H, dd, *J* = 17.0, 12.9 Hz, H-3a), 2.70 (1H, dd, *J* = 17.0, 2.7 Hz, H-3b), 1.99 (3H, s, 8-C<u>H</u>$_3$), 1.98 (3H, s, 6-C<u>H</u>$_3$); ^{13}C-NMR (600 MHz, CD_3OD) δ: 198.4 (C-4), 164.2 (C-7), 160.3 (C-7), 159.3 (C-5), 158.8 (C-4′), 131.5 (C-1′), 128.8 (C-2′, C-6′), 116.3 (C-3′, C-5′), 104.8 (C-6), 104.1 (C-8), 103.2 (C-10), 80.0 (C-2), 44.1 (C-3), 8.2 (8-<u>C</u>H$_3$), 7.4 (6-<u>C</u>H$_3$); ESI-MS *m/z*: 229 [M-H]$^-$.

Compound **4** – white amorphous powder; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.17 (1H, s, H-7), 6.75 (1H, s, H-6), 5.38 (1H, t, *J* = 6.0 Hz, H-2″), 5.18 (1H, t, *J* = 6.8 Hz, H-2′), 3.91 (3H, s, OC<u>H</u>$_3$), 3.59 (2H, d, *J* = 6.8 Hz, H-2′, H-2″), 1.81 (3H, s, 4″-C<u>H</u>$_3$), 1.76 (3H, s, 5′-C<u>H</u>$_3$),

1.64 (6H, s, 5′-C$\underline{H}_3$, 4′-C$\underline{H}_3$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 159.1 (C-3), 157.7 (C-6), 157.2 (C-11a), 153.6 (C-1), 152.6 (C-4a), 148.1 (C-10a), 144.0 (C-8), 142.9 (C-9), 131.4 (C-3″), 130.9 (C-3′), 122.5 (C-2′), 121.7 (C-2″), 119.6 (C-2), 113.1 (C-6b), 112.3 (C-10), 102.6 (C-6a), 101.8 (C-11), 100.1 (C-11b), 99.2 (C-4), 62.2 (O$\underline{C}H_3$), 25.4 (C-4′, C-4″), 22.8 (C-1″), 22.1 (C-1′), 17.7 (C-5′), 17.6 (C-5″); ESI-MS *m/z*: 449 $[M-H]^-$.

Results and Discussion

Compounds **2**, **3** and **4** were identified as 1-methoxylespeflorin G_{11},[9] farrerol[10] and 1-methoxylespeflorin I_2,[9] respectively, by comparing their spectral data with those of literature values. The HR-EI-MS spectrum of compound **1** showed its molecular ion peak at *m/z* 266.1152, which was consistent with that of $C_{14}H_{18}O_5$ (cacld for 266.1154). The ^{1}H-NMR spectrum of compound **1** exhibited an isoprenyl unit at δ_H 3.35 (2H, d, J = 6.7 Hz), 5.19 (1H, t, J = 6.7 Hz), 1.82 (3H, s), and 1.75 (3H, s); a methoxy signal at δ_H 3.73; a methyl ester signal at δ_H 3.97; and an olefinic proton singlet at δ_H 6.27. The ^{13}C-NMR signals at δ_C 170.9 (C=O) and 52.4 (OCH_3) showed that compound **1** is a phenolic methyl ester.[11] The mass fragmentation at *m/z* 235 $[M-OCH_3]^+$ due to *α*-cleavage of compound **1** also showed that this compound is a phenolic methyl ester.[12] In the HMBC spectrum, the methylene signal at δ_H 3.35 (H-1′) was correlated with the signals at δ_C 113.6 (C-2) and 160.2 (C-3); the methoxyl signal at δ_H 3.73 was correlated with the signal at δ_C 160.2 (C-3) which showed that the isoprenyl is located at C-2 position and methoxyl is located at C-3 position of compound **1** (Fig. 2). These data allowed us to establish the structure of compound **1** to be methyl 4,5-dihydroxy-3-methoxy-2-(3-methylbut-2-en-1-yl)benzoate, which was isolated for the first time from plant sources in the present study. Among the isolated compounds, 1-methoxylespflorin G_{11} and 1-methoxylespeflorin I_2 were reported to exhibit cytotoxic effects against blood cancer cells.[9] Farrerol was reported to possess antioxidative,[13] anti-inflammatory,[14, 15] and anti cancer[16] activities.

In conclusion, we isolated four compounds from the roots of *Lespedeza tomentosa*. Based on spectral data, the structures of isolated compounds were determined as methyl 4,5-dihydroxy-3-methoxy-2-(3-methylbut-2-en-1-yl)benzoate (**1**), 1-methoxylespeflorin G_{11} (**2**), farrerol (**3**) and 1-methoxylespeflorin I_2 (**4**). Methyl 4,5-dihydroxy-3-methoxy-2-(3-methylbut-2-en-1-yl)benzoate (**1**) is newly isolated from plant source. These compounds might be useful for bioactivity-related research materials and chemotaxonomic markers.

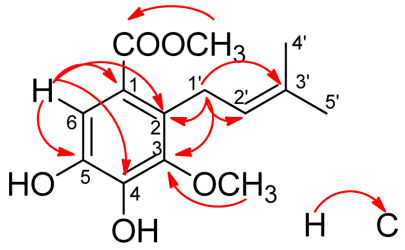

Fig. 2. The Key HMBC correlations of **1**.

Conflict of interest

The authors declare no conflict of interest.

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