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PHENYLETHANOID GLYCOSIDES FROM THE BARK OF *Fraxinus mandschurica*

Yu-Juan Chen,[1,2*] Hong-Gui Zhang,[3] and Xin Li[4]

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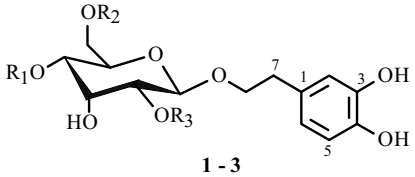
The new phenylethanoid glycoside calceolarioside A-2′-α-L-rhamnopyranoside and calceolarioside A and calceolarioside B were isolated from the bark of Fraxinus mandschurica *Rupr. for the first time by column chromatography.*

Key words: *Fraxinus mandschurica*, phenylethanoid glycosides, calceolarioside A-2′-α-L-rhamnopyranoside.

The dried bark of *Fraxinus mandschurica* Rupr., which is on the market as a substitute for the crude "qinpi" (*Cortex fraxini*), has been used as a diuretic, antifebrile, analgesic, and anti-rheumatic in China, and to treat rheumatism in folk medicine by the Chinese people. It is also used popularly in other Asian countries such as Japan [1].

The plant of *Fraxinus mandschurica* Rupr. contains coumarins, secoiridoids, lignans, flavonoids, and triterpenes [2].

In this paper, we report the results of a study of phenylethanoid glycosides from the bark of *Fraxinus mandschurica* Rupr. Column chromatography of various fractions of the ethanol extract isolated three compounds. Compounds **1** (calceolarioside A) and **2** (calceolarioside B) were isolated from the bark of *Fraxinus mandschurica* Rupr. for the first time, and compound **3** was a new one. The molecular formula of compound **3** is $C_{29}H_{36}O_{15}$, which we called calceolarioside A-2′-α-L-rhamnopyranoside.



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1: R_1 = Caff, R_2 = R_3 = H
2: R_1 = R_3 = H, R_2 = Caff
3: R_1 = Caff, R_2 = H, R_3 = Rha,

The structures of the phenylethanoid glucosides were confirmed through chemical investigation and NMR spectra. The spectra of compounds **1** and **2** were similar to those published (see Experimental).

The ^{13}C NMR spectrum of **3** in DMSO-d_6 showed signals with the presence of glucose, rhamnose, and two aromatic rings. The ^1H NMR spectra of **3** in DMSO-d_6 indicated the presence of *trans*-caffeoyl [an aromatic AMX spin system at δ 6.98, 6.94, and 6.65, a pair of *trans*-olefinic protons at δ 7.41 and 6.16 (each d, J = 15.8 Hz)], and 3,4-dihydroxyphenethyl [an aromatic AMX spin system at δ 6.60, 6.57, and 6.58]. The HMBC spectrum of **3** revealed a correlation of C=O (δ 166.58) with H-4′ (δ 3.65), C-2′ (δ 80.80) with H-1‴ (δ 4.98), and C-7 (δ 69.99) with H-1′ (δ 4.30), suggesting that the *trans*-caffeoyl group was esterified with the 4′-hydroxyl group of the glucose, and rhamnose was esterified with the 2′-hydroxyl group of the glucose by the 1‴-hydroxyl group. The hydrolysate of compound **3** and calceolarioside A have the same R_f value in TLC. Thus, the structure of compound **3** was established as calceolarioside A-2′-α-L-rhamnopyranoside (see Fig. 1).

1) School of Life Science and Technology, Changchun University of Science and Technology, Changchun 130022, China; 2) Pharmaceutical College, Jilin University, Changchun 130021, China, e-mail: jychenzxcv@163.com; 3) College of Traditional Chinese Pharmacy, Beijing University of Traditional of Chinese Medicine, Beijing, 100102, China; 4) Laboratory of Drug Metabolism and Pharmacokinetics, Shenyang Pharmaceutical University, Shenyang 110016, China. Published in Khimiya Prirodnykh Soedinenii, No. 3, pp. 283–284, May–June, 2009. Original article submitted December 27, 2007.

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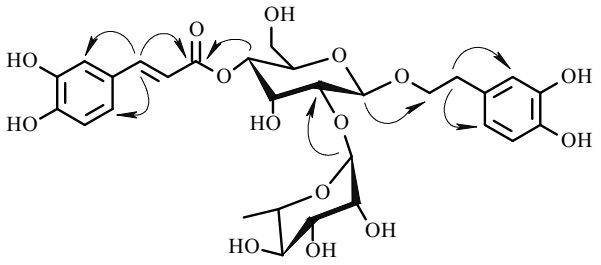

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Fig. 1. Most significant correlations HMBC for compound **3**.

EXPERIMENTAL

Plant Materias. The Bark of *Fraxinus mandschurica* Rupr. was collected from Changbai Mountain in 2002, and identified by Prof. Jing-min Zhang from Jilin University.

Isolation and Identification of Phenylethanoid Glycosides. The Bark of *F. mandschurica* Rupr. was extracted with ethanol (5 × 10 L) by boiling for 3 h, yield 273.04 g (5.46%). The ethanol extract (100 g) was diluted with 1.0 L water, then shaken with ether (3 × 1 L), ethyl acetate (3 × 1 L), and *n*-butanol (3 × 1 L). The solvents were distilled to produce the ether (5.3 g), ethyl acetate (8.9 g), and butanol (46.7 g) fractions.

The solvent system $CHCl_3$–CH_3OH–H_2O (10:3:1, 1; 7:3:1, 2; lower levels) was used. TLC used G_F254 plates (Qingdao Haiyang Chemical Co., Ltd). Column chromatography was performed over silica gel of particle size 100/160 μm.

The butanol fraction (40 g) was subjected to the chromatography over a silica-gel (600 g) column (6.5 × 60 cm) using system 1 and 2. The compositions of fractions were analyzed by TLC. Similar fractions were combined to afford fraction 1, 14.456 g; fraction 2, 7.017 g; fraction 3, 4.864 g, fraction 4, 0.779 g.

Spots of phenylethanoid glycosides on TLC were developed using H_2SO_4–CH_3CH_2OH (1:9), heated at 105°C after spraying with H_2SO_4–CH_3CH_2OH (1:9).

NMR spectra were recorded on Bruker NMR instrument at working frequency 300 MHz.

Fraction 1 did not contain phenylethanoid glycosides according to TLC and gave color with UV (365 nm). It suggested that fraction 1 contained coumarin, which was not reported in this paper.

Fraction 2 was recrystallized from ethanol at 4°C for 24 h, and calceolarioside A was obtained.

Fraction 3 was recrystallized from ethanol at 4°C for 24 h, and calceolarioside B was obtained.

Fraction 4 was purified by column chromatography over silica gel with elution of system 2, and calceolarioside A-2′-α-L-rhamnopyranoside was obtained.

Calceolarioside A (1). Pale yellow amorphous powder, $C_{24}H_{28}O_{11}$. ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.43 (1H, d, J = 15.8, H-β), 6.98 (1H, s, H-2″, Caff), 6.94 (1H, d, J = 7.9, H-6″, Caff), 6.72 (1H, d, J = 7.9, H-5″, Caff), 6.59 (1H, d, J = 7.9, H-5), 6.57 (1H, s, H-2), 6.46 (1H, d, J = 7.9, H-6), 6.22 (1H, d, J = 15.8, H-α), 4.59 (1H, t, J = 9.3, H-4′, Glc), 4.23 (1H, d, J = 10.8, H-1′, Glc). ^{13}C NMR (DMSO-d_6, δ, ppm): 130.11 (C-1), 117.16 (C-2), 145.81 (C-3), 144.35 (C-4), 116.33 (C-5), 120.42 (C-6), 71.03 (C-7), 35.91 (C-8), 103.64 (C-1′, Glc), 74.47 (C-2′, Glc), 75.45 (C-3′, Glc), 72.17 (C-4′, Glc), 74.94 (C-5′, Glc), 61.71 (C-6′, Glc), 126.40 (C-1″, Caff), 115.67 (C-2″, Caff), 146.42 (C-3″, Caff), 149.26 (C-4″, Caff), 116.67 (C-5″, Caff), 122.25 (C-6″, Caff), 146.22 (β, Caff), 114.87 (α, Caff), 166.79 (C=O). ^{1}H NMR and ^{13}C NMR data have been published [3] .

Calceolarioside B (2). Pale yellow amorphous powder, $C_{24}H_{28}O_{11}$. ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.42 (1H, d, J = 15.8, H-β), 7.00 (1H, s, H-2″, Caff), 6.90 (1H, d, J = 8.1, H-6″, Caff), 6.65 (1H, d, J = 8.1, H-5″, Caff), 6.55 (1H, s, H-2), 6.52 (1H, d, J = 8.1, H-5), 6.42 (1H, d, J = 8.1, H-6), 6.24 (1H, d, J = 15.8, H-α), 5.12 (1H, d, J = 4.7, H-6′, Glc), 4.98 (1H, d, J = 4.7, H-6, Glc). ^{13}C NMR (DMSO-d_6, δ, ppm): 130.17 (C-1), 117.11 (C-2), 145.76 (C-3), 144.28 (C-4), 116.32 (C-5), 120.38 (C-6), 71.06 (C-7), 35.99 (C-8), 103.80 (C-1′, Glc), 74.21 (C-2′, Glc), 77.32 (C-3′, Glc), 70.92 (C-4′, Glc), 74.40 (C-5′, Glc), 64.37 (C-6′, Glc), 126.34 (C-1″, Caff), 115.50 (C-2″, Caff), 146.39 (C-3″, Caff), 149.24 (C-4″, Caff), 116.66 (C-5″, Caff), 122.40 (C-6″, Caff), 114.71 (α, Caff), 146.12 (β, Caff), 166.44 (C=O). ^{1}H NMR and ^{13}C NMR data have been published [4].

Calceolarioside A-2′-α-L-rhamnopyranoside (3). Pale yellow amorphous powder, $C_{29}H_{36}O_{15}$. ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, J/Hz): 7.41 (1H, d, J = 15.6, H-β), 6.98 (1H, s, H-2″, Caff), 6.94 (1H, d, J = 8.1, H-6″, Caff), 6.65 (1H, d, J = 8.1, H-5″, Caff), 6.60 (1H, d, J = 8.1, H-5), 6.57 (1H, s, H-2), 6.58 (1H, d, J = 8.1, H-6), 6.16 (1H, d, J = 15.8, H-α), 4.98 (1H, s, H-6‴, Rha), 4.30 (1H, d, J = 7.8, H-1′, Glc), 3.66 (1H, H-2′, Glc), 3.65 (1H, H-4′, Glc), 0.92 (3H, t, J = 6.06, CH_3, Rha). ^{13}C NMR (DMSO-d_6, δ, ppm): 130.07 (C-1), 117.16 (C-2), 146.40 (C-3), 144.35 (C-4), 116.33 (C-5), 120.44 (C-6), 69.99 (C-7), 35.84 (C-8), 103.13 (C-1′, Glc), 80.80 (C-2′, Glc), 75.34 (C-3′, Glc), 69.58 (C-4′, Glc), 75.34 (C-5′, Glc), 61.59 (C-6′, Glc), 126.39 (C-1″, Caff), 115.49 (C-2″, Caff), 146.40 (C-3″, Caff), 149.29 (C-4″, Caff), 116.66 (C-5″, Caff), 122.34 (C-6″, Caff), 114.46 (α, Caff), 145.80 (β, Caff), 166.58 (C=O), 102.06 (C-1‴, Rha), 71.26 (C-2‴, Rha), 71.37 (C-3‴, Rha), 72.54 (C-4‴, Rha), 71.10 (C-5‴, Rha), 18.98 (C-6‴, Rha).

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REFERENCES

1. Hiroki Isukamoto, Sueo Hisada, and Sansei Nishibe, *Chem. Pharm. Bull*., **33**, 4069 (1985).
2. I. Kostova and T. Iossifova, *Fitoterapia*, **78**, 85 (2007).
3. T. Tanahashi, A. Shimada, N. Nagakura, K. Inoue, H. Kuwajima, and K. Takaishi, *Chem. Pharm. Bull*., **41**, 1649 (1993)
4. T. Iossifova, B. Vogler, I. Kostova, and W. Kraus, *Phytochemistry*, **50**, 297 (1999).