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ISOLATION AND STRUCTURE ELUCIDATION OF SECOIRIDOID GLUCOSIDES FROM *Fraxinus rhynchophylla* LEAVES

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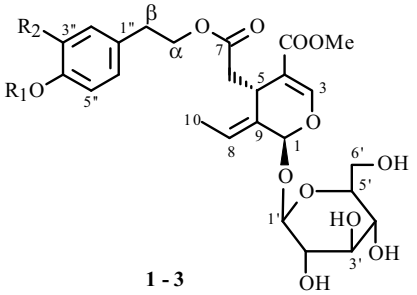
A new secoiridoid glucoside, oleuropein-4″-methyl ether, was isolated, together with two known secoiridoid glucosides, ligstroside and oleuropein, from the leaves of Fraxinus rhynchophylla *Hance by repeated column chromatography over Sephadex LH-20. The structure of the new compound has been characterized on the basis of spectroscopic evidence.*

Key words: *Fraxinus rhynchophylla* Hance, secoiridoid glucosides, leaves, oleuropein-4″-methyl ether.

Fraxinus rhynchophylla Hance (Oleaceae), distributed mainly in China and Korea, is a deciduous tree. The species has been used in traditional medicine to treat chronic bronchitis and bacillary dysentery, to arrest discharges, and to improve eyesight [1, 2]. Previous phytochemical study of this plant barks resulted in the isolation of several coumarins, including ferulaldehyde, scopoletin, fraxidin, fraxetin, aesculetin, aesculin, fraxin, 6,7-dimethoxy-8-hydroxycoumarin, and fraxisecoside [3, 4].

In the present paper, three secoiridoid glucosides were isolated by repeated column chromatography over Sephadex LH-20 from the 70% acetone (v/v) extract of *F. rhynchophylla* leaves collected in September, 2004 in the experimental forest of Kangwon National University, Korea.

Secoiridoid glucosides **1** and **2** were elucidated as the already known ligstroside and oleuropein, respectively, by examination of their ^{1}H and ^{13}C NMR, positive FAB MS, and physicochemical data and comparison with those reported in the literature [5–7]. Compound **3** was obtained as a yellow amorphous powder. The molecular formula was determined to be $C_{26}H_{34}O_{13}$. The positive FAB MS spectrum of **3** revealed $[M+H]^+$ and $[M+Na]^+$ ions at *m/z* 555 and 577, respectively, indicating the molecular weight 554. The melting point was measured at 96–98°C, and $[\alpha]_D^{20}$ was –159° (*c* 0.5, MeOH). The presence of the phenolic hydroxyl group in the molecule of **3** was evident from the gray-green color with 1% $FeCl_3$ (in EtOH) solution on TLC [8], with R_f values of 0.44 and 0.70 in solvents A and B, respectively. The IR spectrum of **3** showed absorption bands for hydroxyls at 3400 cm^{-1}, carbonyl groups at 1700 and 1630 cm^{-1}, and aromatic C=C bonds at 1520 cm^{-1} [9].



1 - 3

1: $R_1 = R_2 = H$; **2:** $R_1 = H$, $R_2 = OH$; **3:** $R_1 = Me$, $R_2 = OH$

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In ^{1}H NMR spectrum of compound **3**, an anomeric proton resonating at δ_H 4.80 (1H, d, J = 7.8 Hz, H-1′) and six protons ranging from δ_H 3.29 to 3.69 (6H, m, H-2′–6′) were attributable to the 1-*O*-acyl-*β*-glucose moiety [6, 7]. The characteristic signals due to an acetal carbinol proton (H-1) appear as a broad singlet at δ_H 5.91, an oxygenated olefinic proton (H-3) appeared at δ_H 7.51 as a singlet, and two methine groups [δ_H 3.96 (1H, m, H-5) and δ_H 6.07 (1H, q, J = 7.1, H-8)], one methylene group [δ_H 2.43 (1H, dd, J = 14.0; 3.9 Hz, H-6_a) and δ_H 2.69 (1H, dd, J = 14.0; 9.6 Hz, H-6_b)], one methyl group [δ_H 1.64 (3H, d, J = 7.1, H-10)], and one methoxyl group [δ_H 3.70 (3H, s, H-12)] were attributed to the secoiridoidic aglycone moiety [10]. A set of aromatic proton signals exhibiting an ABX spin system at δ_H 6.75 (1H, d, J = 1.3 Hz, H-2″), δ_H 7.05 (1H, d, J = 8.5 Hz, H-5″), and δ_H 6.66 (1H, d, J = 1.3; 8.5 Hz, H-6″), together with two methylene groups [δ_H 4.20 (2H, t, J = 7.0 Hz, H-*α*) and δ_H 2.81 (2H, t, J = 7.0 Hz, H-*β*)], revealed the presence of a 1,3,4-trisubstituted phenylethyl group [11]. Cross-linking was found between H-*α* and C-7 in an HMBC experiment, which suggested that in structure **3**, the phenylethyl group and the oleoside 11-methyl ester moiety was linked between C-*α* and C-7.

The above ^{1}H NMR spectral data of **3** were similar to those assignments for oleuropein (**2**) except for a methoxyl group at δ_H 3.82 (3H, s) connecting to the 4″ carbon of compound **3**, which was confirmed by long-range H-C coupling (HMBC) experiments. The ^{13}C NMR spectral data of compound **1** were also identical to those of **2** (oleuropein); moreover, the additional methyl group was typically absorbed at δ_C 55.6 [9].

Based on the foregoing analysis, we conclude that secoiridoid glucoside **3** is oleuropein-4″-methyl ether, which has never been reported in the literature and was isolated and elucidated here for the first time from *F. rhynchophylla* leaves.

EXPERIMENTAL

Instrument. An Electro Thermal 9100 apparatus was used to obtain the melting points (uncorrected). Optical rotations were determined with a JASCO DIP-1000 digital polarimeter in MeOH. IR spectra were measured using a Perkin-Elmer BX FT-IR spectrometer in KBr disks. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in CD_3OD with TMS as an internal standard using a Bruker Avance DPX 400 spectrometer. Positive FAB MS spectroscopy was performed with a Micromass Autospec M363 spectrometer.

For TLC analysis, DC-Plastikfolien Cellulose F (Merck Co.) plates were employed, developed with *t*-BuOH–HOAc–H_2O (3:1:1, v/v/v, solvent A) and HOAc-H_2O (3:47, v/v, solvent B). TLC spots were detected by UV-light (254 and 365 nm) and by spraying with 1% ethanolic $FeCl_3$ solution followed by heating.

Extraction, Fractionation, and Isolation of Secoiridoid Glucosides. The leaves of *F. rhynchophylla* were air dried and ground to a fine powder. A previously weighed amount (1.5 kg) was extracted three times with 70% aqueous acetone at room temperature. After combination, filtration, and concentration *in vacuo*, the aqueous residue was successively fractionated and freeze-dried to yield fractions soluble in *n*-hexane (4.7 g, yield 0.3%), CH_2Cl_2 (9.0 g, yield 0.6%), EtOAc (37.5 g, yield 2.5%), and H_2O (208.6 g, yield 13.9%).

A portion of the resulting EtOAc fraction powder (18.5 g) was applied repeated to Sephadex LH-20 columns using EtOH-hexane (2:1, 1:2 and 1:4, v/v) and MeOH-H_2O (4:1, 2:1, 1:2 and 1:4, v/v) as eluates. With the composition monitored by TLC, purification finally resulted in the isolation of ligstroside (**1**, 184 mg), oleuropein (**2**, 320 mg), and oleuropein-4″-methyl ether (**3**, 32 mg).

Oleuropein-4″-methyl ether (3), $C_{26}H_{34}O_{13}$, mp 96–98°C (dec.), $[\alpha]_D^{20}$ –159° (*c* 0.5, MeOH), TLC: R_f 0.44 (solvent A) and 0.70 (solvent B). IR (ν_{max}, cm^{-1}): 3400 (OH), 1700, 1630 (ketone C=O: two carbonyl groups), 1520 (aromatic C=C). Positive FAB MS: *m/z* $[M+H]^+$ at 555 and $[M+Na]^+$ at 577. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 5.91 (1H, br.s, H-1), 7.51 (1H, s, H-3), 3.96 (1H, m, H-5), 2.43 (1H, dd, J = 14.0; 3.9, H-6_a), 2.69 (1H, dd, J = 14.0; 9.6, H-6_b), 6.07 (1H, q, J = 7.1, H-8), 1.64 (3H, d, J = 7.1, H-10), 3.70 (3H, s, H-12), 4.80 (1H, d, J = 7.8, H-1′), 3.29–3.69 (6H, m, H-2′–6′), 4.20 (2H, t, J = 7.0, H-*α*), 2.81 (2H, t, J = 7.0, H-*β*), 6.75 (1H, d, J = 1.3, H-2″), 7.05 (1H, d, J = 8.5, H-5″), 6.66 (1H, dd, J = 1.3; 8.5, H-6″), 3.82 (3H, s, OMe). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 95.40 (C-1), 155.43 (C-3), 109.64 (C-4), 32.08 (C-5), 41.54 (C-6), 173.49 (C-7), 125.15 (C-8), 130.31 (C-9), 13.84 (C-10), 168.92 (C-11), 52.22 (C-12), 101.01 (C-1′), 75.02 (C-2′), 78.68 (C-3′), 71.72 (C-4′), 78.19 (C-5′), 62.99 (C-6′), 69.98 (C-*α*), 35.66 (C-*β*), 131.15 (C-1″), 111.5 (C-2″), 146.01 (C-3″), 146.20 (C-4″), 115.32 (C-5″), 119.89 (C-6″), 55.6 (OMe).

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