Chemistry of Natural Compounds, Vol. 49, No. 3, July, 2013 [Russian original No. 3, May–June, 2013]

FLAVONOID CONSTITUENTS OF *Euonymus fortunei*

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UDC 547.972

*Six flavonoid glycosides (**1**–**6**), together with three known compounds (**7**–**9**), were isolated from the 70% ethanol extract of* Euonymus fortunei *and identified on the basis of nuclear magnetic resonance (NMR) data, mass spectroscopy (MS) data, and comparison with literature data. Compound **1** was obtained as a new compound. Compounds **1**–**5**, **7**, and **9** were isolated from this plant for the first time.*

Keywords: *Euonymus fortunei*, chemical constituents, structure identification.

Previous studies showed that the genus *Euonymus* possessed antifeedant, narcotic, and insecticidal properties as well as antitumor properties, mainly due to β-dihydroagarofuran sesquiterpene polyol esters and pyridine alkaloids [1–3]. The current taxonomic classification of *Euonymus* according to chemical constituents is based on the skeleton of β-dihydroagarofuran. However, recent research has reported that the extracts of *Euonymus* contain several flavonoids with antidiabetic and radical scavenging properties [4, 5]. These suggest flavonoids may be one of the most important components in *Euonymus* plants. *Euonymus fortunei*, belonging to the genus *Euonymus*, is a folk medicine and famous ornamental plant in China. Several sesquiterpenoid, triterpenoids, alkaloids, organic acids, and β-sitosterol have been isolated from this plant [2, 6–8]. To the best of our knowledge, flavonoid constituents of *E. fortunei* are rare.

In this paper, six flavonoid glycosides (**1**–**6**) and three other compounds (**7**–**9**) were isolated from the 70% ethanol extract of *E. fortunei*. Identification of compounds **2**–**9** was performed by comparison of the physical constants and spectral data with those of known compounds.

Compound **1**, obtained as a yellow amorphous powder, was assigned the molecular formula $C_{39}H_{50}O_{24}$ by analyses of EI-MS at *m*/*z* 903.38 $[M + H]^+$ and from ^{13}C NMR spectrum. The UV spectrum at 265 and 343 nm (MeOH) showed characteristic bands of a flavonoid. The ^1H NMR spectrum exhibited an AB system at δ 7.80 and 6.94 (J = 8.4 Hz) typical of the four protons H-2′, H-6′ and H-3′, H-5′ of the *p*-substituted B ring of the flavonoid, and a 2H singlet at δ 6.79 and 6.46 attributed to H-8 and H-6 of the A ring, indicating the presence of a kaempferol skeleton. This spectrum also exhibited four anomeric protons (δ 5.51, 5.15, 4.39, and 4.26) and two 3H singlets at δ 1.18 (3H, d, J = 6.0 Hz) and 0.88 (3H, d, J = 6.0 Hz) relative to two methyl groups of rhamnose, suggesting this is a flavonol with four glycosides. The ^{13}C NMR data further supported the presence of a kaempferol skeleton and four sugar moieties, which are indicated by two rhamnose (δ 18.8 and 18.4) and two glucose (δ 62.3 and 62.1) moieties. In comparison with kaempferol [9] the ^{13}C NMR spectrum of **1** exhibited an upfield shift for C-3 and C-7 by 0.8 and 1.0 ppm, respectively, indicating glycosylation at both positions. In addition, in the HMBC spectrum the anomeric proton of one rhamnopyranosyl unit (H-1″, δ 5.51) showed a correlation with C-7 (δ 162.7), and the second rhamnopyranosyl unit (H-1‴, δ 5.15) showed a correlation with C-3 (δ 135.8). The anomeric proton of one glucopyranosyl unit (δ 4.39) showed a correlation with C-4‴ (δ 83.0), and the second glucopyranosyl unit (δ 4.26) also showed a correlation with C-4⁗ (δ 82.4). The rhamnose and glucose units were found to be linked (1→4), as the signal at δ 83.0/82.4 is characteristic for C-4″/4⁗ in a 4-substituted rhamnose unit. Other two-dimensional NMR techniques

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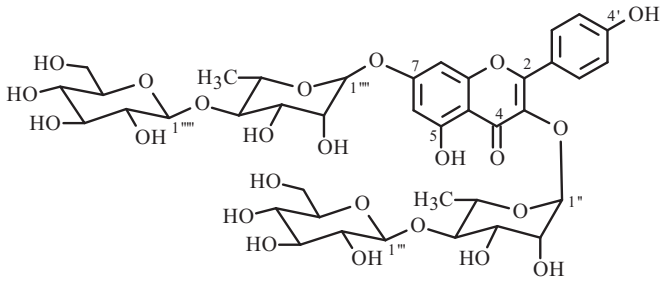
 0009-3130/13/4903-0428

(^{1}H–^{1}H COSY, ROSEY, NOESY, and HSQC) verified the orientation of the complex conjugate as a rhamnosyl-glucosyl-*O* ether as opposed to a glucosyl-rhamnosyl-*O* ether. Furthermore, acid hydrolysis also confirmed that compound **1** contains kaempferol, D-glucose, and L-rhamnose. From the spectroscopic data and also from comparison with [9], compound **1** is identified as kaempferol-3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-7-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside, a new flavonoid glycoside not reported before.



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EXPERIMENTAL

General Procedures. The NMR spectra were run on a DRX-500 MHz spectrometer using TMS as an internal standard. The EI-MS spectra were measured with a Bruker Esquire 3000 spectrometer. The melting point was measured using an X-4 micromelting point apparatus (Beijing Second Optical Instrument Factory, China) and was uncorrected.

Fungus Material. The aerial parts of *E. fortunei* were collected from Yongfu County of Guangxi on October 2008. A voucher specimen was deposited at the Key Laboratory of Medicinal Chemical Resources and Molecular Engineering, Guangxi Normal University, Guilin, Guangxi.

Extraction and Isolation. The powdered aerial parts of *E. fortunei* (1.2 kg) were repeatedly extracted with aqueous ethanol (70%, v/v) by heating under reflux. The extract was then concentrated under reduced pressure to give a residue (194.5 g), which was suspended in deionized water and then successively partitioned with petroleum ether, EtOAc, and finally with *n*-BuOH, affording a known weight of each respective fraction. The *n*-BuOH fraction (49.3 g) was passed through an ODS-C_{18} column and successively eluted with 0% MeOH, 30% MeOH, 60% MeOH, and 95% MeOH. The 60% MeOH eluate portion was subjected to silica gel column chromatography, eluting with $CHCl_3$–MeOH (10:1–1:1), to give compound **7**. A portion of the 30% MeOH eluate was subjected to Sephadex LH-20 column chromatography and eluted with MeOH to obtain compound **1**. The EtOAc fraction (25.5 g) was applied to a silica gel column (200–300 mesh) and eluted with a stepwise gradient mixture of CH_2Cl_2–MeOH (40:1→1:1) to obtain 11 fractions. Fraction 4 was applied to a silica gel column and eluted with CH_2Cl_2–MeOH (20:1, v/v) to obtain **6**. Fraction 5 was repeatedly chromatographed on Sephadex LH-20 (60% MeOH, v/v) to obtain **3**. Fraction 6 was applied to a silica gel column (300–400 mesh) and eluted with CH_2Cl_2–MeOH (8:1, v/v) to obtain **4** and **9**. Fraction 8 was recrystallized twice from MeOH–H_2O to give the pure compounds **2** and **5**. The petroleum ether fraction was subjected to a series of chromatographic techniques using a silica gel column (200–300 mesh) to give compound **8**.

Compound 1. Yellow amorphous powder, mp 231–232°C. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.80 (2H, d, J = 8.4, H-2′, 6′), 6.94 (2H, d, J = 8.4, H-3′, 5′), 6.79 (1H, s, H-8), 6.46 (1H, s, H-6), 5.51 (1H, br.s, H-1″), 5.15 (1H, br.s, H-1‴), 4.39 (1H, d, J = 1.2, H-1⁗), 4.26 (1H, d, J = 1.2, H-1⁗′), 1.18 (3H, d, J = 6.0, H-6″), 0.88 (3H, d, J = 6.0, H-6‴). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 158.9 (s, C-2), 135.8 (s, C-3), 179.0 (s, C-4), 162.0 (s, C-5), 100.6 (d, C-6), 162.7 (s, C-7), 95.7 (d, C-8), 157.2 (s, C-9), 107.0 (s, C-10), 121.4 (s, C-1′), 131.8 (d, C-2′, 6′), 116.6 (d, C-3′, 5′), 161.3 (s, C-4′), Rha1: 103.1 (d, C-1″), 71.0 (d, C-2″), 70.8 (d, C-3″), 83.0 (d, C-4″), 71.3 (d, C-5″), 18.4 (q, C-6″), Glc1: 105.8 (d, C-1‴), 75.6 (d, C-2‴), 78.1 (d, C-3‴), 71.4 (d, C-4‴), 77.7 (d, C-5‴), 62.3 (t, C-6‴), Rha2: 99.2 (d, C-1⁗), 70.5 (d, C-2⁗), 70.5 (d, C-3⁗), 82.4 (d, C-4⁗), 70.1 (d, C-5⁗), 18.8 (q, C-6⁗), Glc2: 105.6 (d, C-1⁗′), 75.6 (d, C-2⁗′), 78.1 (d, C-3⁗′), 71.2 (d, C-4⁗′), 77.7 (d, C-5⁗′), 62.1 (t, C-6⁗′).

Kaempferol-3,7-*O*-α-dirhamnopyranoside (2). Pale-yellow needles (MeOH), mp 185–187°C. EI-MS *m/z* 589 $[M + H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.5 (1H, s, 5-OH), 10.12 (1H, s, 4′-OH), 7.80 (2H, d, J = 8.7, H-2′, 6′), 6.93 (2H, d, J = 8.7, H-3′, 5′), 6.80 (1H, d, J = 1.7, H-8), 6.46 (1H, d, J = 1.7, H-6), 5.56 (1H, br.s, H-1″), 5.02 (1H, s, H-1‴), 1.10 (3H, d, J = 6.4, H-6″), 0.81 (3H, d, J = 6.4, H-6‴). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 158.3 (s, C-2), 135.0 (s, C-3), 178.4 (s, C-4), 161.4 (s, C-5), 99.9 (d, C-6), 162.2 (s, C-7), 95.1 (d, C-8), 156.6 (s, C-9), 106.3 (s, C-10), 120.8 (s,

C-1′), 131.2 (d, C-2′, 6′), 115.9 (d, C-3′, C-5′), 160.6 (s, C-4′), 102.3 (d, C-1″), 70.6 (d, C-2″), 71.2 (d, C-3″), 72.1 (d, C-4″), 70.7 (d, C-5″), 18.4 (q, C-6″), 98.9 (d, C-1‴), 70.6 (d, C-2‴), 70.8 (d, C-3‴), 71.6 (d, C-4‴), 70.3 (d, C-5‴), 17.9 (q, C-6‴) [10].

Kaempferol-3-(4″-*O*-acetyl)-*O*-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (3). Yellow solid, mp 206–208°C. EI-MS *m/z* 619.26 $[M-H]^-$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.08 (2H, d, J = 8.8, H-2′, 6′), 6.90 (2H, d, J = 8.8, H-3′, 5′), 6.81 (1H, s, H-8), 6.44 (1H, s, H-6), 5.54 (1H, br.s, H-1″), 5.47 (1H, d, J = 7.2, H-1‴), 2.01 (3H, s, H-6″), 1.10 (3H, d, J = 6.4, H-6‴). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 158.8 (C-2), 135.5 (C-3), 179.1 (C-4), 162.5 (C-5), 100.1 (C-6), 162.9 (C-7), 95.1 (C-8), 157.3 (C-9), 106.9 (C-10), 122.0 (C-1′), 131.5 (C-2′, 6′), 116.1 (C-3′, 5′), 160.9 (C-4′), 102.1 (C-1″), 70.5 (C-2″), 71.8 (C-3″), 74.2 (C-4″), 69.5 (C-5″), 17.9 (C-6″), 99.2 (C-1‴), 71.1 (C-2‴), 71.2 (C-3‴), 73.1 (C-4‴), 68.8 (C-5‴), 17.3 (C-6‴), 170.5 (-C=O), 20.7 (CH_3) [11].

Apigenin-7-*O*-β-D-glucopyranoside (4). Pale-yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.95 (1H, s, 5-OH), 10.39 (1H, s, 4′-OH), 7.95 (2H, d, J = 8.8, H-2′, 6′), 6.93 (2H, d, J = 8.8, H-3′, 5′), 6.85 (1H, s, H-3), 6.81 (1H, d, J = 2.0, H-8), 6.42 (1H, d, J = 2.0, H-6), 5.06 (1H, d, J = 7.3, H-1″), 3.6–3.7 (1H, m), 3.4–3.5 (2H, m), 3.1–3.2 (1H, m). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 164.7 (C-2), 103.4 (C-3), 182.2 (C-4), 161.7 (C-5), 99.9 (C-6), 163.0 (C-7), 94.9 (C-8), 157.2 (C-9), 106.0 (C-10), 121.5 (C-1′), 128.1 (C-2′), 116.2 (C-3′), 161.4 (C-4′), 116.2 (C-5′), 128.1 (C-6′), 100.5 (C-1″), 73.2 (C-2″), 76.8 (C-3″), 76.9 (C-4″), 70.0 (C-5″), 61.5 (C-6″) [12].

Kaempferol-3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside (5). Pale-yellow solid. EI-MS *m/z* 593 $[M-H]^-$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.08 (2H, d, J = 8.0, H-3′, 5′), 6.87 (2H, d, J = 8.0, H-2′, 6′), 6.82 (1H, s, H-8), 6.44 (1H, s, H-6), 5.54 (1H, d, J = 2.0, H-1″), 5.46 (1H, d, J = 6.4, H-1‴), 1.10 (3H, d, J = 6.4, H-6‴). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 156.7 (C-2), 133.5 (C-3), 177.6 (C-4), 160.9 (C-5), 98.4 (C-6), 161.6 (C-7), 94.5 (C-8), 156.0 (C-9), 105.7 (C-10), 120.8 (C-1′), 130.0 (C-2′, C-6′), 115.1 (C-3′, C-5′), 160.1 (C-4′), 100.8 (C-1″), 74.2 (C-2″), 76.4 (C-3″), 70.2 (C-4″), 77.6 (C-5″), 60.8 (C-6″), 99.4 (C-1‴), 70.1 (C-2‴), 70.2 (C-3‴), 71.6 (C-4‴), 69.8 (C-5‴), 17.8 (C-6‴) [13].

Kaempferol-7-*O*-α-L-rhamnopyranoside (6). Yellow needles (MeOH). EI-MS *m/z* 431 $[M-H]^-$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.13 (2H, d, J = 9.0, H-3′, 5′), 6.92 (2H, d, J = 9.0, H-2″, 6″), 6.76 (1H, d, J = 2.0, H-8), 6.44 (1H, d, J = 2.0, H-6), 5.58 (1H, d, J = 2.0, H-1″), 1.28 (3H, d, J = 6.5, H-6″). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 149.7 (C-2), 138.4 (C-3), 178.4 (C-4), 163.2 (C-5), 100.9 (C-6), 164.2 (C-7), 96.3 (C-8), 158.7 (C-9), 107.1 (C-10), 124.5 (C-1′), 131.7 (C-2′), 117.2 (C-3′), 161.6 (C-4′), 117.2 (C-5′), 131.7 (C-6′), 100.8 (C-1″), 74.6 (C-2″), 73.0 (C-3″), 72.7 (C-4″), 72.1 (C-5″), 19.0 (C-6″) [14].

Syringin (7). White powder, mp 188–190°C. EI-MS *m/z* 395.07 $[M+Na]^+$. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.74 (2H, s, H-3, 5), 6.49 (1H, d, J = 15.8, H-7), 6.37 (1H, dt, J = 15.8, 5.5, H-8), 4.95 (2H, d, J = 2.1, H-9), 4.90 (1H, d, J = 5.5, H-1′), 3.79 (6H, s, 2 × OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 134.0 (C-1), 152.6 (C-2, 6), 104.5 (C-3, 5), 132.6 (C-4), 130.1 (C-7), 128.3 (C-8), 77.1 (C-9), 102.6 (C-1′), 69.9 (C-2′), 76.5 (C-3′), 61.4 (C-4′), 74.1 (C-5′), 60.9 (C-6′), 56.3 (2 × OCH_3) [15].

Friedelin (8). White powder, mp 260–261°C. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.72 (3H, s, H-24), 0.87 (3H, d, J = 7.0, H-23), 0.95 (3H, s, H-29), 0.98 (3H, s, H-30), 1.00 (3H, s, H-26), 1.01 (3H, s, H-27), 1.05 (3H, s, H-25), 1.18 (3H, s, H-28), 2.38–2.39 (1H, m, H-4), 2.23–2.29 (2H, m, H-2). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 22.3 (C-1), 41.4 (C-2), 213.2 (C-3), 58.1 (C-4), 42.1 (C-5), 41.2 (C-6), 18.1 (C-7), 53.0 (C-8), 37.4 (C-9), 59.4 (C-10), 35.5 (C-11), 30.5 (C-12), 39.6 (C-13), 38.2 (C-14), 32.3 (C-15), 36.0 (C-16), 30.0 (C-17), 42.7 (C-18), 35.2 (C-19), 28.1 (C-20), 32.7 (C-21), 39.2 (C-22), 6.7 (C-23), 14.6 (C-24), 17.8 (C-25), 20.3 (C-26), 18.6 (C-27), 32.0 (C-28), 34.9 (C-29), 31.7 (C-30) [16].

Acetamide (9). Colorless needles, mp 81–82°C. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.3 (2H, br.s, NH_2), 1.9 (3H, s, H-2). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 173.8 (C-1), 22.6 (C-2).

ACKNOWLEDGMENT

This study is supported by the 973 project (No. 2011CB512005), Key Technology R&D program (No. 2006BAI06A18-14), and the Natural Science Foundation of Guangxi Province (Nos. 20090106-4, 2010GXNSFF013001, 2011GXNSFD018010, 2012GXNSFAA053027, 0832024, and 2007228).

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