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FLAVONOID GLYCOSIDES FROM FLOWERS OF *Sisymbrium officinale* AND *Diplotaxis muralis* GROWING IN GEORGIA

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Sisymbrium officinale Scop. (Brassicaceae, Cruciferae), which is indigenous to Georgia, flowers profusely and bears fruit. The flowers are rich in flavonoids.

Yellow needle-like crystals of **1** (2.1%) formed in the aqueous layer after the appropriate work up [1] of the alcohol (80%) extract of the plant flowers and extraction by EtOAc.

The mother liquor remaining after removal of the crystals was fractionated over a polyamide column. The resulting enriched fraction (6.9% of the starting material) was separated by column chromatography over silica gel (2 × 65 cm) with elution by $CHCl_3$:EtOH with increasing concentration of the latter. This eluted another two compounds of flavonoid nature, **2** and **3**.

Compound 1, $C_{27}H_{30}O_{16}$, MW 610.33 (mass spectrometry), mp 189–192°C, soluble in aqueous EtOH, slightly soluble in concentrated, insoluble in H_2O, acetone, $CHCl_3$. The Bryant reaction [2] was negative. UV spectrum (EtOH, λ_{max}, nm): 360, 340 sh, 250; + CH_3COONa: 360, 260; + $AlCl_3$: 430, 360 sh, 270; + $AlCl_3$ + HCl: 400, 270. IR spectrum (KBr, ν_{max}, cm^{-1}): 3425 (OH), 1658, 1604 (γ-pyrone >C=O), 1496 (–CH–).

PMR spectrum (400 MHz, $CDCl_3$–CD_3OD, δ, ppm, J/Hz): 6.48 (1H, d, J = 2.2, H-6), 6.74 (1H, d, J = 2.05, H-8), 7.83 (1H, d, J = 2.1, H-2′), 7.65 (1H, d, J = 8.2, H-6′), 6.91 (1H, s, H-5′), 5.04 (1H, d, J = 7.6, H-1″), 3.82 (1H, br.d, J = 9.4, H-2″), 3.56 (1H, dd, J = 9.4, 3.8, H-3″), 3.80 (1H, dd, J = 10.0, 9.0, H-4″), 3.70 (1H, m, H-5″), 3.54 (1H, dd, J = 12.1, 5.2, H-6″), 3.72 (1H, dd, J = 12.1, 2.2, H-6″), 5.56 (1H, d, J = 2.0, H-1‴), 4.15 (1H, dd, J = 2.0, 3.0, H-2‴), 3.98 (1H dd, J = 3.1, 10.0, H-3‴), 3.8–3.5 (2H, m, H-4‴, H-5‴), 1.22 (3H, d, J = 6, L-rhamnose CH_3).

^{13}C NMR spectrum [100 MHz, $CDCl_3$–CD_3OD (1:1), δ, ppm]: 156.60 (C-2), 133.20 (C-3), 177.30 (C-4), 161.20 (C-5), 97.55 (C-6), 162.79 (C-7), 93.01 (C-8), 157.30 (C-9), 105.67 (C-10), 121.10 (C-1′), 115.20 (C-2′), 144.70 (C-3′), 148.65 (C-4′), 116.45 (C-5′), 121.62 (C-6′), 104.6 (C-1″), 73.60 (C-2″), 76.12 (C-3″), 69.35 (C-4″), 65.80 (C-5″), 60.82 (C-6″), 99.6 (C-1‴), 71.80 (C-2‴), 72.10 (C-3‴), 73.62 (C-4‴), 68.70 (C-5‴), 17.86 (C-6‴).

Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 609 (100) $[M]^-$, 446 (8.6), 463 (2), 301 (9) [3].

Glycoside **1** was hydrolyzed by H_2SO_4 (2%) to an aglycon of formula $C_{15}H_{10}O_7$, mp 316–318°C, MW 302, quercetin [4]. D-Glucose and L-rhamnose were detected in the carbohydrate part of the hydrolysate.

Alkaline hydrolysis of **1** produced L-rhamnose and a monoside with mp 221–223°C; acid hydrolysis cleaved D-glucose and quercetin. The monoside was identified as quercetin-3-*O*-β-D-glucopyranoside (isoquercitrin) [5, 6].

Enzymatic hydrolysis of **1** by rhamnodiastase gave D-glucose and a monoside with mp 263–266°C that was characterized as quercetin-7-*O*-α-L-rhamnopyranoside [7].

The results of the studies indicated that **1** was identical to quercetin-3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside [8].

Compound 2 was yellow crystals, MW 756 (mass spectrometry), $C_{33}H_{40}O_{20}$, mp 219–222°C. The Bryant reaction was negative. UV spectrum (EtOH, λ_{max}, nm): 365, 309 sh, 255; + CH_3COONa: 360, 285 sh, 260; + CH_3COONa + H_3BO_3: 360, 295, 260; + $AlCl_3$, 400, 303 sh, 260; + $AlCl_3$ + HCl: 360, 305 sh, 250; + CH_3ONa: 410, 310 sh, 275.

PMR spectrum (400 MHz, CD_3OD, δ, ppm, J/Hz): 6.0 (1H, d, J = 2.2, H-6), 6.42 (1H, d, J = 2.1, H-8), 7.90 (1H, d, J = 2.3, H-2′), 6.91 (1H, d, J = 8.3, H-5′), 7.63 (1H, dd, J = 2.1, 8.4, H-6′), 3.81 (3H, s, OCH_3), 4.90 (1H, d, J = 7.6, D-galactose H-1″), 5.62 (1H, d, J = 2.0, L-rhamnose H-1‴), 4.62 (1H, d, J = 7.0, D-xylose H-1⁗), 4.0-3.0 (sugar protons), 1.24 (3H, d, J = 6.0, L-rhamnose CH_3).

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^{13}C NMR spectrum [100 MHz, $CDCl_3$–CD_3OD (1:1), δ, ppm]: 157.72 (C-2), 133.10 (C-3), 177.13 (C-4), 161.08 (C-5), 97.0 (C-6), 161.45 (C-7), 93.02 (C-8), 156.07 (C-9), 105.68 (C-10), 121.10 (C-1′), 114.02 (C-2′), 149.40 (C-3′), 149.55 (C-4′), 115.08 (C-5′), 122.30 (C-6′), 55.60 (OCH_3), 102.9 (D-galactose C-1″), 73.72 (C-2″), 74.75 (C-3″), 66.92 (C-4″), 75.80 (C-5″), 66.80 (C-6″); 100.2 (L-rhamnose C-1‴), 79.32 (C-2‴), 76.0 (C-3‴), 69.95 (C-4‴), 67.90 (C-5‴), 15.35 (C-6‴); 105.60 (D-xylose C-1⁗), 75.50 (C-2⁗), 77.62 (C-3⁗), 72.60 (C-4⁗), 65.72 (C-5⁗).

Flavonoid **2** was hydrolyzed by H_2SO_4 (2%) to an aglycon with mp 300–304°C, D-xylose, D-galactose, and L-rhamnose. The algycon was identified as 3,5,7,4′-tetrahydroxy-3′-methoxyflavone or isorhamnetin [1].

A comparative analysis of UV spectra of the glycoside and the aglycon taken with added diagnostic reagents showed that the carbohydrates were located on C-3 and C-7.

Enzymatic hydrolysis of **2** by rhamnodiastase gave D-galactose and a bioside; acid hydrolysis cleaved isorhamnetin, D-xylose, and L-rhamnose. Therefore, the bioside was isorhamnetin-7-*O*-xylosidorhamnoside. The resonance for α-L-rhamnose H-1 appeared in the PMR spectrum at 5.60 ppm, which indicated it was located on C-7 of the aglycon. The chemical shift of D-xylose H-1 was 4.68 ppm (J = 7.0), which indicated it was bonded to rhamnose at C-2 because L-rhamnose C-2 experienced a shift effect (δ 79.32) in the ^{13}C NMR spectrum. The resonance for D-galactose H-1 at 4.90 ppm (d, J = 7.6) and the result of the enzymatic hydrolysis indicated that it was bonded to C-3 of the aglycon.

It was concluded from the results that flavonoid **2** was isorhamnetin-3-*O*-β-D-galactopyranosyl-7-*O*-α-L-rhamnopyranosyl-(2→1)-β-D-xylopyranoside.

Compound 3 was yellow needle-like crystals, $C_{32}H_{38}O_{20}$, MW 742 (mass spectrometry). UV spectrum (EtOH, λ_{max}, nm): 350, 300 sh, 260; + $AlCl_3$: 420, 270; + $AlCl_3$ + HCl: 400, 350 sh, 270; + CH_3COONa: 350, 260; + CH_3COONa + H_3BO_3: 370, 260; + CH_3ONa: 390. IR spectrum (KBr, ν_{max}, cm^{-1}): 3435 (OH), 1660, 1610 (γ-pyrone >C=O), 1493 (–CH–).

PMR spectrum [400 MHz, $CDCl_3$–CD_3OD (1:1), δ, ppm, J/Hz]: 7.80 (1H, d, J = 2.1, H-2′), 7.65 (1H, d, J = 8.2, H-6′), 6.79 (1H, d, J = 8.2, H-5′), 6.49 (1H, d, J = 2, H-6), 6.28 (1H, d, J = 2.2, H-8), 5.20 (1H, d, J = 8, D-galactose H-1″), 5.52 (1H, d, J = 2.2, L-rhamnose H-1‴), 4.60 (1H, d, J = 7.0, D-xylose H-1⁗), 4.0-3.0 (sugar protons), 1.25 (3H, d, J = 6.1, L-rhamnose CH_3).

^{13}C NMR spectrum [100 MHz, $CDCl_3$–CD_3OD (1:1), δ, ppm]: 156.60 (C-2), 133.20 (C-3), 177.30 (C-4), 161.20 (C-5), 97.55 (C-6), 162.79 (C-7), 93.01 (C-8), 157.30 (C-9), 105.67 (C-10), 121.10 (C-1′), 115.20 (C-2′), 144.70 (C-3′), 148.65 (C-4′), 116.45 (C-5′), 121.62 (C-6′), 103.5 (C-1″), 73.60 (C-2″), 74.72 (C-3″), 66.86 (C-4″), 75.80 (C-5″), 66.79 (C-6″); 100.4 (C-1‴), 79.32 (C-2‴), 75.70 (C-3‴), 69.90 (C-4‴), 67.90 (C-5‴), 15.30 (C-6‴); 105.62 (C-4⁗), 75.49 (C-2⁗), 77.63 (C-3⁗), 72.55 (C-4⁗), 65.56 (C-5⁗).

Acid hydrolysis of **3** produced quercetin, D-galactose, D-xylose, and L-rhamnose. A comparison of UV spectra of **3** and its aglycon indicated that the carbohydrates were situated on C-3 and C-7. The products of enzymatic hydrolysis of **3** were D-galactose and a bioside that was cleaved by acid hydrolysis into quercetin, D-xylose, and L-rhamnose. A comparison of PMR and ^{13}C NMR spectra of the carbohydrate parts of **3** and **2** indicated that they were identical. Therefore, the product of enzymatic hydrolysis was quercetin-7-*O*-α-L-rhamnopyranosyl-(2→1)-*O*-β-D-xylopyranoside.

The results suggested that **3** had the structure quercetin-3-*O*-β-D-galactopyranosyl-7-*O*-α-L-rhamnopyranosyl-(2→1)-*O*-β-D-xylopyranoside.

A yellow crystalline precipitate consisting of two compounds that were identical to **2** and **3** after separation was isolated from flowers of *Diplotaxis muralis* (L.) DC. by the method described above.

Isorhamnetin-3-*O*-β-D-galactopyranosyl-7-*O*-α-L-rhamnopyranosyl-(2→1)-β-D-xylopyranoside (**2**) and quercetin-3-*O*-β-D-galactopyranosyl-7-*O*-α-L-rhamnopyranosyl-(2→1)-*O*-β-D-xylopyranoside (**3**) were not previously reported.

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