FLAVONOIDS FROM *Trifolium resupinatum* VAR. *microcephalum*

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The flavonoids, a substance class of the most numerous and widespread natural constituents, are of great importance and interest due to a wide variety of physical and biological activities, including antioxidative, anti-mutagenic, and anti-carcinogenic effects [1]. In the previous studies, flavonoid compounds have been reported from species including *T. medium*, *T. subterraneum*, *T. incarnatum* [2], *T. repens* [2, 3], *T. pratense* [2, 4, 5], and seeds of 57 *Trifolium* species [6]. No previous phytochemical studies have been reported on *T. resupinatum* L. var. *microcephalum* so far. In this report, we describe the isolation and characterization of an isoflavone (**3**) [7, 8], two isoflavone glycosides (**1** [9, 10] and **4** [11]), a flavonol glycoside (**2**) [3, 12], and steroid glycoside (**5**) [13, 14] from *T. resupinatum* L. var. *microcephalum*. This is the first report on the chemistry of *T. resupinatum* L. var. *microcephalum*.

Genistein-7-*O*-β-glucoside (genisitin) (1). UV (MeOH, λ_{max}, nm): 262, 332; +$AlCl_3$: 272, 310, 377;+ NaOAc: 262, 333; IR (KBr, ν_{max}, cm^{-1}): 3420 (OH), 1661 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 5.01(1H, d, J = 7.2, H-1″), 3.13–3.65 (6H, m, sugar protons), 6.41 (1H, br.s, H-8), 6.64 (1H, br.s, H-6), 6.78 (2H, dd, J = 8.4, 2.1, H-3′, H-5′), 7.34 (2H, dd, J = 8.7, 2.1, H-2′, H-6′), 8.34 (1H, s, H-2); ^{13}C NMR (DMSO-d_6, δ, ppm): 155.20 (C-2), 123.28 (C-3), 181.18 (C-4), 162.35 (C-5), 100.30 (C-6), 163.65 (C-7), 95.17 (C-8), 158.20 (C-9), 106.80 (C-10), 121.61 (C-1′), 130.85 (C-2′), 115.83 (C-3′), 157.93 (C-4′), 115.83 (C-5′), 130.85 (C-6′), 100.52 (C-1″), 73.72 (C-2″), 76.96 (C-3″), 70.26 (C-4″), 77.80 (C-5″), 61.27 (C-6″); ^{1}H-^{1}H COSY (DMSO-d_6 , δ, ppm): 7.34/6.78 (H-2′, H-6′/H-3′, H-5′), 6.64/6.41 (H-6/H-8), 5.01/3.13–3.65 (H-1″/H-2″); FAB-MS *m/z* (rel. int.%): 432 $[M]^+$ (5), 339 $[M\text{-}C_6H_5O]^+$ (25), 179 $[M\text{-}C_{15}H_9O_4]^+$ (90), 163 $[M\text{-}C_{15}H_9O_5]^+$ (25), 269 [M-glucose unit]$^+$ (5), 153 $[A_1]^+$ (40).

Kaempferol-3-*O*-(6″acetyl)-β-galactopyranoside (2). UV (MeOH, λ_{max}, nm): 221, 266.5, 351.5; +NaOMe: 225.5, 275, 323, 401.5; +$AlCl_3$: 224.5, 273, 396; + HCl: 224, 271, 392; IR (KBr, ν_{max}, cm^{-1}): 3420 (OH), 1660 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 1.69 (3H, s, CO$\underline{CH_3}$), 3.30–4.03 (6H, m, sugar protons), 6.38 (1H, d, J = 2.1, H-8), 6.15 (1H, d, J = 2.1, H-6), 6.82 (2H, dd, J = 9, 2.1, H-3′, H-5′), 8.01 (2H, dd, J = 8.7, 1.8, H-2′, H-6′), 5.25 (1H, d, J = 7.8, H-1″); ^{13}C NMR (DMSO-d_6, δ, ppm): 157.13 (C-2), 133.85 (C-3), 177.93 (C-4), 161.76 (C-5), 99.64 (C-6), 165.96 (C-7), 94.49 (C-8), 156.85 (C-9), 104.04 (C-10), 121.44 (C-1′), 131.55 (C-2′), 115.71 (C-3′), 160.65 (C-4′), 115.71 (C-5′), 131.55 (C-6′), 102.49 (C-1″), 71.60 (C-2″), 73.41 (C-3″), 68.77 (C-4″), 73.41 (C-5″), 63.78 (C-6″), 20.78 (CO$\underline{CH_3}$), 170.54 (C=O); FAB-MS *m/z* (rel. int.%): 490 $[M]^+$(45), 489 $[M\text{-}1]^+$ (75), 473 $[M\text{-}OH]^+$ (5), 447 $[M\text{-}CH_3CO]^+$ (5), 285 $[M\text{-}C_8H_{13}O_6$(sugar unit)$]^+$ (95), 268 $[M\text{-}C_8H_{13}O_7]^+$ (8), 153 $[A_1]^+$ (10), 60 $[CH_3OCO+1]^+$ (25).

Formononetin (3). UV (MeOH, λ_{max}, nm): 241, 248, 259, 310; +NaOMe: 257, 275, 323, 336.5; +$AlCl_3$: 243, 251, 263, 303; + HCl: 244, 251, 263, 303; +NaOAc: 256, 313, 335; +H_3BO_3: 266, 304; ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 6.70 (1H, br.s, H-8), 6.78 (1H, br.d, J = 8.7, H-6), 6.82 (2H, d, J = 7.8, H-3′, H-5′), 7.34 (2H, d, J = 7.8, H-2′, H-6′), 8.16 (1H, s, H-2), 3.62 (3H, s, OCH_3), 7.81 (1H, d, J = 8.4, H-5); ^{13}C NMR (DMSO-d_6, δ, ppm): 153.78 (C-2), 124.90 (C-3), 175.30 (C-4), 127.95 (C-5), 115.89 (C-6), 163.29 (C-7), 102.79 (C-8), 158.13 (C-9), 117.26 (C-10), 123.84 (C-1′), 130.73 (C-2′), 114.28 (C-3′), 159.63 (C-4′), 114.28 (C-5′), 130.73 (C-6′), 55.81 (OCH_3).

Formononetin-7-*O*-β-glucoside(4). UV (MeOH, λ_{max}, nm): 253, 260.5, 302; +NaOMe: 252, 262, 325; +$AlCl_3$: 253, 262, 303; + HCl: 253, 260.5, 302.5; +NaOAc: 260.5, 303; +H_3BO_3: 261.5, 303; ^{1}H NMR(CD_3OD, δ, ppm, J/Hz): 3.81 (3H, s, OCH_3), 7.23 (1H, d, J = 2.7, H-8), 7.21 (1H, dd, J = 8.7, 2.7, H-6), 6.97 (2H, d, J = 8.1, H-3′, H-5′), 7.46 (2H, d, J = 7.8, H-2′, H-6′), 8.20 (1H, s, H-2), 8.13 (1H, d, J = 9, H-5), 3.40–3.56 (4H, m, sugar protons), 3.71 (1H, dd, J = 6, 12, H-6_a″), 3.92 (1H,

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dd, J = 2.1, 12, H-6_b″), 5.09 (1H, d, J = 7.2, H-1″); FAB-MS *m/z* (rel. int.%): 430 $[M]^+$ (5), 397 $[M\text{-}2\text{-}OCH_3]^+$ (10), 268 [M+1-glucose unit]$^+$ (100), 267 [M-glucose unit]$^+$ (75), 136 $[A_1]^+$ (5), 132 $[B_1]^+$ (40).

Sitosterol-3-*O*-β-glucoside (5). ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 0.61 (3H, s, H-18), 0.91 (3H, s, H-19), 0.86 (3H, d, J = 6.3, H-21), 0.79 (3H, t, J = 6, H-29), 0.77 (3H, d, J = 5.7, H-27), 0.75 (3H, d, J = 6.9, H-26), 3.50 (1H, m, H-3), 5.28 (1H, d, J = 5.1, H-6), 4.18 (1H, d, J = 7.5, H-1″), 4.07–4.83 (6H, m, sugar protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 12.33 (C-18), 12.45 (C-29), 19.28 (C-21), 19.62 (C-26), 19.75 (19), 20.35 (C-27), 21.27 (C-11), 23.31 (C-28), 24.51 (C-15), 26.21 (C-23), 28.44 (C-16), 29.45 (C-25), 32.04 (C-2), 32.12 (C-7, C-8), 34.05 (C-22), 36.13 (C-20), 36.89 (C-10), 37.50 (C-1), 39.01 (C-12), 42.53 (C-4, C-13), 45.85 (C-24), 50.31 (C-9), 56.14 (C-17), 56.86 (C-14), 121.83 (C-6), 141.16 (C-5), 70.85 (C-3), 101.46 (C-1″), 70.85, 77.38, 77.46, 77.69 (C-2″, C-3″, C-4″, C-5″), 61.82 (C-6″).

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