DACTYLIN AND LONICERIN FROM THE AERIAL PART OF *Hedysarum setigerum*

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We previously reported the isolation from the aerial part of *Hedysarum setigerum* Turcz. ex Fisch. et Meyer flavonoids, sterols, phenolic acids, lignan, and an alkaloid [1-4]. The present communication reports the isolation and identification of two rarely encountered minor flavonoid glycosides.

Column chromatography over polyamide sorbent of butanol extracts (for general comments, see [1]) using H_2O:MeOH produced two fractions of flavonoids: A (90:10→85:15, v/v) and B (80:20→65:35).

Fraction A (1.5 g) was worked up with diethylether. The solid that was insoluble in ether was repeatedly chromatographed over silica gel ($CHCl_3$:CH_3OH:H_2O, 80:36:7; water-saturated *n*-C_4H_9OH) to produce fractions containing flavonoid **1** (12.3 mg). Then, they were processed by gel filtration over Sephadex (Sephadex G-25 and G-10, gradient, H_2O:C_2H_5OH, 100:0→50:50) and reversed-phase chromatography over Silasorb C-2 (15 μm, gradient, H_2O:C_2H_5OH, 100:0→85:15) to isolate **1** (5.3 mg).

Fraction B (1.0 g) was separated over Sephadex G-10 (gradient, H_2O and CH_3OH:H_2O, 50:50→60:40). Elution by CH_3OH:H_2O gave a fraction (158.3 mg) that was then chromatographed over polyamide sorbent using the same solvent systems to afford a fraction (56.0 mg) containing the total two flavonoids **2** and rhoifolin (5,7,4′-trihydroxyflavone 7-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside) [2]. Treatment with acetone produced an acetone-insoluble fraction (16.5 mg) that was enriched in **2** and was used to establish its structure.

5,7,3,4′-Tetrahydroxy-3′-methoxyflavone 3-O-β-D-glucopyranoside-4′-O-β-D-glucopyranoside (Dactylin) (1), pale yellow crystals, mp 179-180°C (CH_3OH) [5].

Mass spectrum (HR-FAB, *m*/*z*): found $[M - H]^-$ 639.1550, $C_{28}H_{31}O_{17}$, calc. 639.1561.

Mass spectrum (FAB^-, *m*/*z*, I_{rel}, %): 639 (3.8) $[M - H]^-$, 477 (19.8) $[M - H - 162]^-$, 314 (20.3) $[M - H - 324]^-$.

PMR spectrum (250 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.60 (1H, s, OH-5), 10.99 (1H, s, OH-7), 7.96 (1H, d, J = 2.0, H-2′), 7.60 (1H, dd, J = 2.0, 8.7, H-6′), 7.28 (1H, d, J = 8.7, H-5′), 6.47 (1H, d, J = 1.4, H-8), 6.25 (1H, d, J = 1.4, H-6), 5.58 (1H, d, J = 7.5, H-1″, C-3), 5.06 (1H, d, J = 7.3, H-1‴, C-4′), 3.84 (3H, s, OCH_3-3′).

^{13}C NMR spectrum (62.5 MHz, DMSO-d_6, δ, ppm): 155.80 (C-2), 133.4 (C-3), 177.53 (C-4), 161.25 (C5), 98.82 (C-6), 164.30 (C-7), 93.84 (C-8), 156.48 (C-9), 104.16 (C-10), 123.64 (C-1′), 113.46 (C-2′), 148.10 (C-3′), 148.50 (C-4′), 114.53 (C-5′), 121.41 (C-6′), 55.67 (OCH_3-3′), 100.75 (C-1″), 72.80 (C-2″), 76.84 (C-3″), 69.55 (C-4″), 77.00 (C-5″), 60.58 (C-6″), 99.78 (C-1‴), 74.00 (C-2‴), 76.30 (C-3‴), 69.70 (C-4‴), 77.30 (C-5‴), 60.58 (C-6‴).

5,7,3′,4′-Tetrahydroxyflavone 7-O-α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranoside (Lonicerin) (2). Mass spectrum (HR-FAB, *m*/*z*): found $[M - H]^-$ 593.1511, $C_{27}H_{30}O_{15}$, calc. 593.1506.

Mass spectrum (FAB^-, *m*/*z*, I_{rel}, %): 593 (51) $[M - H]^-$, 459 (12) $[M - H - 134]^-$ $^{0,1}X_1$, 315 (67) $[M - H - 278]^-$ $^{1,5}X_0$, 299 (100) $[M - H - 294]^-$ $^{0,1}X_0$. Lonicerin was ionized only under FAB^--MS negative ion conditions so that fragment ions $^{0,1}X_1$, $^{1,5}X_0$, and $^{0,1}X_0$ were formed upon fragmentation of the carbohydrate [6]. The typical ions Y_0 and Y_1 that are due to loss of carbohydrate units and generally occur in FAB^--MS of negative ions and in FAB^+-MS of positive ions of flavonoids [7] were not observed. This may be explained by the presence of a 1→2 bond between the carbohydrate units in lonicerin because in this instance the Y_0 and Y_1 ions are difficult to produce and mainly other fragment ions of the carbohydrate part appear in the spectrum [8].

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PMR spectrum (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.42 (1H, dd, J = 7.3, 2.1, H-6′), 7.40 (1H, d, J = 2.1, H-2′), 6.90 (1H, d, J = 7.3, H-5′), 6.79 (1H, d, J = 2.1, H-8), 6.66 (1H, s, H-3), 6.45 (1H, d, J = 2.1, H-6), 5.20 (1H, d, J = 7.2, H-1″), 3.67 (1H, m, H-2″), 3.65 (1H, m, H-3″), 3.40 (1H, m, H-4″), 3.50 (1H, m, H-5″), 3.71 and 3.90 (2H, m, H-6″), 5.30 (1H, d, J = 1.5, H-1‴), 3.93 (1H, m, H-2‴), 3.41 (1H, m, H-3‴), 3.60 (1H, m, H-4‴), 3.97 (1H, m, H-5‴), 1.43 (3H, d, J = 6.1, H-6‴).

^{13}C NMR spectrum (62.5 MHz, CD_3OD, δ, ppm): 166.82 (C-2), 104.13 (C-3), 183.99 (C-4), 162.93 (C-5), 100.91 (C-6), 164.37 (C-7), 95.90 (C-8), 158.93 (C-9), 107.03 (C-10), 123.43 (C-1′), 114.24 (C-2′), 147.06 (C-3′), 151.19 (C-4′), 116.7 (C-5′), 120.51 (C-6′), 99.76 (C-1″), 79.05 (C-2″), 79.02 (C-3″), 71.36 (C-4″), 78.27 (C-5″), 62.41 (C-6″), 102.54 (C-1‴), 72.19 (C-2‴), 73.95 (C-3‴), 72.19 (C-4‴), 70.00 (C-5‴), 18.26 (C-6‴).

The nature of the substitution, position of substituents, and configuration of glycoside bonds (OH, OCH_3, β-D-Glc, α-L-Rha) in the isolated compounds were determined using 2D NMR COSY, ROESY, and HMBC spectra.

Both compounds were observed in the genus *Hedysarum* for the first time. Lonicerin was previously found only in secondary metabolites of plants from the genus *Lonicera* (honeysuckle) [9, 10]; dactylin, in plants of the two genera *Phleum* (timothy) and *Crocus* (crocus) [11].

REFERENCES

1. O. V. Neretina, A. S. Gromova, V. I. Lutskii, and A. A. Semenov, *Rastit. Resur.*, **38**, No. 1, 82 (2002).
2. O. V. Neretina, A. S. Gromova, V. I. Lutskii, S. V. Fedorov, and Yu. N. El′kin, *Khim. Prir. Soedin.*, 161 (2002).
3. O. V. Neretina, A. S. Gromova, V. I. Lutsky, A. A. Semenov, I. A. Ushakov, T. N. Makar′eva, and N. L. Owen, *Chem. Nat. Comp.*, **40**, 91 (2004).
4. O. V. Neretina, A. S. Gromova, V. I. Lutskii, A. A. Semenov, I. A. Ushakov, P. S. Dmitrenok, and N. L. Owen, *Khim. Prir. Soedin.*, 500 (2004).
5. W. Karrer, *Konstitution und vorkommen der organischen pflanzenstoffe*, Birkhauser Verlag Basel, Stuttgart (1958), 617.
6. R. Franski, P. Bednarek, P. Wojtaszek, and M. Stobiecki, *J. Mass Spectrom.*, **34**, 486 (1999).
7. M. Stobiecki, *Phytochemistry*, **54**, 237 (2000).
8. F. Cuyckens, R. Rozenberg, E. de Hoffmann, and M. Claeys, *J. Mass Spectrom.*, **36**, 1203 (2001).
9. K. H. Son, J. O. Park, K. C. Chung, H. W. Chang, H. P. Kim, J. S. Kim, and S. S. Kang, *Arch. Pharmacal Res.*, **15**, 365 (1992).
10. K. H. Son, J. S. Kim, S. S. Kang, H. P. Kim, and H. W. Chang, *Kor. J. Pharmacogn.*, **25**, 24 (1994).
11. Z. P. Pakudina and A. S. Sadykov, *Distribution in Plants and Physicochemical Properties of Flavones, Flavonols, and Their Glycosides* [in Russian], Fan, Tashkent (1970).