ISOLARICIRESINOL GLUCOSIDE, BERBERINE, AND PHENOLIC ACIDS FROM THE AERIAL PART OF *Hedysarum setigerum*

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We previously reported the isolation of flavonoids and sterols from the aerial part of *Hedysarum setigerum* [1-3]. In continuation of the study of the chemical composition of the aerial part of this plant, we isolated and identified four minor components.

Chromatography of the butanol fraction [1] over polyamide using H_2O:MeOH produced three fractions: *A* (95:5, vol%), B (90:10), and *C* (60:40 → 40:60). Fraction *A* was worked up successively by flash chromatography over silica gel ($CHCl_3$:CH_3OH:H_2O, 100:12:1) and column chromatography over Sephadex LH-20-100 and G-10 (H_2O) and polyamide (gradient mixture of CH_3OH and $CHCl_3$). Fractions were collected using 10 and 20% CH_3OH. These were chromatographed over silica gel using a hexane:acetone gradient. Elution by 20% acetone isolated **3** (36.8 mg); 30% acetone, **4** (69.8 mg). Fraction *B* was chromatographed successively over Sephadex LH-20-100 ($CHCl_3$:CH_3OH, 10:1) and G-10 (H_2O:CH_3OH, 95:5) to produce white crystalline compound **1** (5 mg). Fraction *C* was worked up by flash chromatography over silica gel ($CHCl_3$:CH_3OH:H_2O, 70:12:1) to isolate bright yellow compound **2** (5.9 mg). The compound was purified by column chromatography over silica gel with elution by CH_3OH (20%) in $CHCl_3$ to produce crystalline **2** (3 mg).

4,9,4′,9′-Tetrahydroxy-3,3′-dimethoxy-β8,α8′,β7′-cyclolignan 9′-O-β-D-glucopyranoside [(+)-isolariciresinyl-9′-O-β-D-glucopyranoside] (1): mp 137-138°C (CH_3OH), $[\alpha]_D^{21}$ +61.5° (*c* 0.45, CH_3OH) [4]. UV spectrum (CH_3OH, λ_{max}, nm): 230, 283 (log ε 4.28, 3.94).

IR spectrum (KBr, ν, cm^{-1}): 3600, 3440, 3000, 2922, 2856, 1649, 1625, 1516, 1454, 1366, 1274, 1120, 1077, 1022, 622. Mass spectrum (FAB^+) *m/z* (I_{rel}, %): 589 (7) $[M - 2H + 3Na]^+$, 567 (42) $[M - H + 2Na]^+$, 545 (100) $[M + Na]^+$, 385 (36) $[M + Na - 136 - Na]^+$ or $[M - H + 2Na - 136 - Na]^+$, 371 (30) $[M + Na + 2H - 153 - Na]^+$, 357 (18) $[M - 2H + 3Na - Glc - 3Na]^+$ or $[M + Na + 2H - 153 - Na - CH_2]^+$, 342 (12) $[M + Na - 180 - Na]^+$ or $[M + Na - Glc - Na - H_2O]^+$, 327 (15) $[342 - CH_3]^+$, 319 (17) $[M + Na - 205 - Na]^+$, 301 (44) $[M + Na - 122 - 123 - Na]^+$, 259 (39) $[M + Na - 122 - 136 - Na]^+$. Mass spectrum (HR-FAB) *m/z*: found $[M + Na]^+$, 545.2003 (calc. for $C_{26}H_{34}O_{11}Na$, 545.2000).

PMR spectrum (250 MHz, CD_3OD, δ, ppm, J/Hz): 6.65 (1H, d, J = 0.7, H-2), 6.18 (1H, d, J = 0.7, H-5), 2.83 (2H, m, H-7), 2.03 (1H, m, H-8), 3.70 (2H, m, H-9), 6.78 (1H, d, J = 1.9, H-2′), 6.73 (1H, d, J = 8.1, H-5′), 6.62 (1H, dd, J = 8.1, 1.9, H-6′), 4.07 (1H, m, H-7′), 1.86 (1H, br.t, H-8′), 4.06 and 3.29 (2H, m, H-9′), 4.13 (1H, d, J = 7.7, H-1″), 3.21 (1H, m, H-2″), 3.47 (1H, m, H-3″), 3.30 (1H, m, H-4″), 3.66 (1H, m, H-5″), 3.63 and 3.81 (2H, m, H-6″), 3.80 (s, OCH_3-3′), 3.81 (s, OCH_3-3).

The fragmentation of the molecular ion in the FAB^+ mass spectrum is determined mainly by the glycosylation site. For the 9′-O-glucoside, we found that the fragmentation involves mainly rupture of C–C bonds in ring B (Fig. 1). For the 4′-O-glucoside, according to the literature [5], fragmentation occurs with loss of glucose, ring C, and CH_2OH from the molecular ion.

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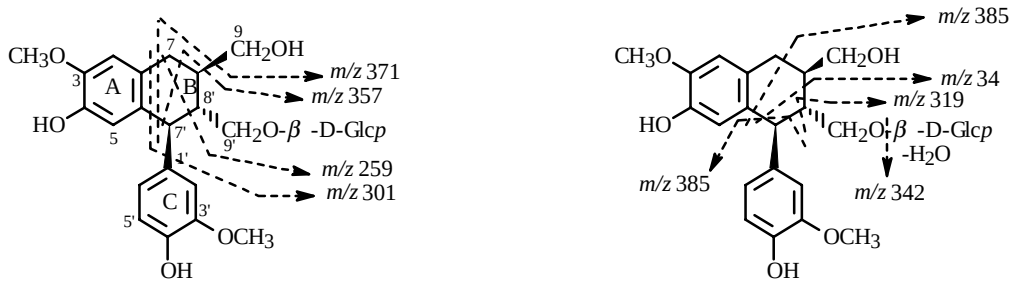

Fig. 1. Formation pathways for fragment ions in the FAB mass spectrum of **1**.

Berberine (2): mp 205°C (CH_3OH) [6]. UV spectrum (CH_3OH, λ_{max}, nm): 229, 266, 350, 429. Mass spectrum (EI^+, 70 eV) *m*/*z* (I_{rel}, %): 337 (76) $[M]^+$, 336 (54) $[M - H]^+$, 322 (36) $[M + H - CH_3]^+$, 307 (24) $[M + H - 2CH_3]^+$, 306 (12) $[M + H - OCH_3]^+$, 292 (9) $[M + H - OCH_2O]^+$, 45 (100) $[OCH_2O]^+$.

Mass spectrum (HR-EI) *m*/*z*: found $[M]^+$ 337.1240 (calc. for $C_{20}H_{18}NO_4$, 337.1314).

***p*-Hydroxybenzoic acid (3)**: mp 209-210°C [$(CH_3)_2CO$] [7].

Protocatechoic acid (4): mp 194-195°C [$(CH_3)_2CO$] [8].

^{13}C NMR spectra of **1** [9], **2** [10], and **3** and **4** [11] agreed with those previously reported.

The identification in *H. setigerum* of carbocyclic lignan and alkaloid of the tetrahydronaphthalene (**1**) and protoberberine (**2**) types, respectively, is the first instance of the isolation of representatives of these chemical classes from plants of the *Hedysarum* genus.

REFERENCES

1. O. V. Neretina, A. S. Gromova, V. I. Lutskii, and A. A. Semenov, *Rastit. Resur.*, 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**, No. 1, 91 (2004).
4. H. Thieme and R. Benecke, *Pharmazie*, **24**, No. 9, 567 (1969).
5. V. Marinos, M. E. Tate, and P. J. Williams, *Phytochemistry*, **31**, No. 12, 4307 (1992).
6. M. P. Dobhal, V. K. Goel, and B. C. Joshi, *Pharmazie*, **43**, No. 9, 659 (1988).
7. E. I. Sal′nikova, N. F. Komissarenko, A. I. Derkach, S. E. Dmitruk, and G. I. Kalinkina, *Khim. Prir. Soedin.*, 759 (1993).
8. B. A. Kazanskii, *Syntheses of Organic Preparations*, Vol. 4 [in Russian], Izd. Inostr. Lit., Moscow (1953).
9. H. Achenbach, M. Lowel, H. Wathel, M. Cupta, and P. Solta, *Planta Med.*, **58**, 270 (1992).
10. G. Blasko, G. A. Cordell, S. Bhamarapravati, and C. W. W. Beecher, *Heterocycles*, **27**, No. 4, 911 (1988).
11. J. Ralph, *NMR Database of Lignin and Cell Wall Compounds*, www.acdlabs.com.