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COMPONENTS OF *Haplophyllum pedicellatum*

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Haplophyllum pedicellatum Bunge (Rutaceae) is a perennial herbaceous plant indigenous to Central Asia from which alkaloids [1, 2a], flavonoids [3], and coumarins [4] have been previously isolated.

We investigated the aerial part of this plant collected during flowering and fruiting near Aktash, Samarkand District, Republic of Uzbekistan.

Air-dried raw material (6.31 kg) was extracted with methanol. The methanol extract was condensed. The dry solid was distributed between water and $CHCl_3$. The aqueous part was extracted exhaustively with ethylacetate. The ethylacetate solution was condensed, forming a precipitate that was separated (7.35 g). The $CHCl_3$ solution produced by the usual method a mixture of bases (11.21 g, 0.18% of dry wt.) that was separated over a column of silica gel (1:60) with gradient elution by hexane, ethylacetate, and methanol. The ethylacetate effluents produced the known alkaloids skimmianine (0.52 g) [2b] γ-fagarine (0.15 g) [2c], acetylevoxine (20 mg) [2d], haplopine (0.11 g) [2e], and evoxine (80 mg) [2f]; ethylacetate:methanol (4:1), glycoperine (50 mg) [2g] and glucohaplopine (15 mg) [2g].

The usual workup of the $CHCl_3$ solution (after separation of the total fraction of alkaloids) produced a dry solid, half of which (30 g) was separated by column chromatography over silica gel (1:30) analogously to the separation of the alkaloid mixture. The ethylacetate effluents isolated the known lignans suchilactone (20 mg) [5], justicidin B (15 mg) [5], and diphyllin (50 mg) [6]. Alkaloids and lignans were identified by direct comparison with authentic samples (TLC, mixed mp).

The precipitate obtained upon condensing the ethylacetate extract was treated with methanol. The part insoluble in methanol was crystallized from aqueous methanol to give yellow crystals (0.17 g), mp 266-268°C of the known flavonoid limocitrin 7-*O*-β-D-glucopyranoside [7], which was identified based on UV, IR, 1D and 2D PMR, and ^{13}C NMR spectra and acid hydrolysis.

UV spectrum (EtOH, λ_{max}, nm): 259, 272 (sh), 333 (sh), 384.

IR spectrum (KBr, ν_{max}, cm^{-1}): 3371 (OH), 1656 (γ-pyrone C=O), 1607, 1568, 1516 (aromatic C=C), 1101, 1069, 1043, 1029 (glycoside C–O).

PMR spectrum (500 MHz, Py-d_5, δ, ppm, J/Hz): 3.93 and 4.15 (3H each, s, 2 × OCH_3), 4.13 (H-5″), 4.22-4.23 (H-2″, H-3″, H-4″), 4.40 (H-6″b), 4.52 (H-6″a), 5.80 (1H, d, J = 7, H-1″), 7.23 (1H, s, H-6), 7.40 (1H, d, J = 8.4, H-5′), 8.36 (1H, d, J = 1.8, H-2′), 8.39 (1H, dd, J = 8.4, 1.8, H-6′).

^{13}C NMR spectrum (125 MHz, Py-d_5, δ, ppm): 150.5 (C-1a), 147.9 (C-2), 138.1 (C-3), 177.5 (C-4), 105.8 (C-4a), 156.7 (C-5), 98.9 (C-6), 156.8 (C-7), 130.1 (C-8), 61.6 (OCH_3-8), 55.7 (OCH_3-3′), 148.9 (C-1′), 112.2 (C-2′), 148.4 (C-3′), 150.5 (C-4′), 116.7 (C-5′), 122.9 (C-6′), 102.4 (C-1″), 74.7 (C-2″), 78.5 (C-3″), 70.9 (C-4″), 79.1 (C-5″), 62.1 (C-6″).

Resonances of protonated C atoms in the ^{13}C NMR spectrum were assigned using HSQC data; quaternary, HMBC and the ACD/CNMR program, ver. 1.1.

The *O*-methyl groups located on C-8 and C-3′ and the glycosyl substituent on C-7 were found by two independent methods, $^1H,^1H$ ROESY and $^1H,^{13}C$ HMBC spectra.

The acid hydrolysate of the flavonoid contained D-glucose (PC).

Thus, 11 compounds, of which 7 components including the alkaloids acetylevoxine, glycoperine, and glucohaplopine, all three isolated lignans, and the flavonoid glycoside were observed for the first time in the aerial part of *H. pedicellatum*.

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It was shown earlier that the extract of the aerial part of *H. pedicellatum* [4], the total fraction of alkaloids [1], skimmianine [8], γ-fagarine [9], haplopine [9], and diphyllin [10] exhibit cytotoxic activity.

We evaluated the cytotoxicity of glycoperine and limocitrin 7-*O*-β-D-glucopyranoside on two lines of cancer cells HeLa and HCT-116. We used solutions of the compounds in DMSO for the test. The cytotoxicity was determined using WST-1 reagent (450 nm, Elisa). Limocitrin 7-*O*-β-D-glucopyranoside exhibited moderate cytotoxic activity with IC_{50} 50 μM and 58.5 μM for HeLa and HCT-116 cells, respectively; glycoperine, very low cytotoxicity for both lines of cancer cells with $IC_{50} > 100$ μM.

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