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PHENYLPROPANOIDS FROM SUBTERRANEAN ORGANS OF *Inula helenium*

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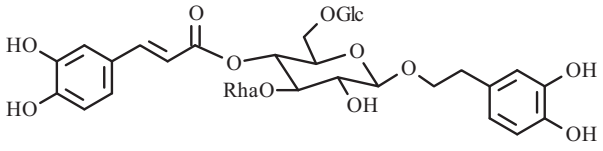
Inula helenium L. is a plant species of the family Asteraceae. Its rhizomes and roots are widely used in medical practice. Glucofructans [1], sesquiterpenes [2], thymol derivatives [3], and dammaradienyl acetate [4] were observed earlier in roots of *I. helenium*. No information is available on phenolic compounds from roots of *I. helenium*. Preliminary studies showed that subterranean organs of *I. helenium* contained phenylpropanoids, the chemical study of which is the focus of the present work.

Subterranean organs of *I. helenium* were collected at the experimental plantation in the vicinity of Goryachinsk (Pribaikal Region, Republic of Buryatiya, 30 Aug., 2009, 52°48′98″ N, 108°29′01″ E). The species was determined by Dr. T. A. Aseeva (IGEB, SB, RAS).

Extraction and Fractionation. Ground raw material (1.5 kg) was extracted (5×) with EtOH (60%, 1:10, 2 h) on a boiling-water bath. The combined EtOH extracts were concentrated to a watery residue that was extracted with C_6H_{14}, $CHCl_3$, EtOAc, and BuOH to produce C_6H_{14} (61.8 g, 4.12% of air-dried raw material mass), $CHCl_3$ (24.2 g, 1.61%), EtOAc (7.8 g, 0.52%), and BuOH fractions (260.9 g, 17.39%) and an aqueous residue (166.2 g, 11.08%). The EtOAc fraction (5 g) was chromatographed over Sephadex LH-20 (3 × 120 cm) using an EtOH:H_2O gradient (96:4→0:100) with subsequent column chromatography (CC) of subfractions over polyamide (5 × 10, H_2O:EtOH eluent, 0:100→96:4) and preparative TLC on cellulose (solvent systems 1 and 2) and SiO_2 (solvent system 3). The separation isolated from the EtOAc fraction nine compounds that were identified by MS, UV, IR and ^{13}C NMR spectroscopy as the methyl ester of caffeic acid (**1**, 12 mg) [5]; caffeic (**2**, 31 mg) [6]; 3,5-di-*O*-caffeoylquinic (**3**, 17 mg); 1,5-di-*O*-caffeoylquinic (**4**, 16 mg) [7]; 2,3-di-*O*-caffeoyltartaric (chicoric, **5**, 11 mg) [8]; 2-*O*-caffeoyltartaric (caftaric, **6**, 37 mg) [9]; 5-*O*-caffeoylquinic (**7**, 12 mg) [6]; and 3-*O*-caffeoylquinic acids (**8**, 46 mg) [10]; and echinacoside (**9**, 11 mg) [11].

Echinacoside (9), $C_{35}H_{46}O_{20}$. UV spectrum (MeOH, λ_{max}, nm): 221, 246 sh, 290 sh, 330. FAB-MS (*m*/*z*): 785 $[M - H]^-$. ^{13}C NMR spectrum (125 MHz, DMSO-d_6): dihydroxyphenylethanol: 146.27 (C-4), 144.31 (C-3), 134.22 (C-1), 121.24 (C-6), 117.10 (C-5), 116.21 (C-2), 72.00 (C-7), 36.21 (C-8); caffeate: 168.92 (C-9), 149.93 (C-4), 148.21 (C-8), 146.63 (C-3), 127.56 (C-1), 123.24 (C-6), 116.11 (C-2), 115.11 (C-7), 114.69 (C-5); glucose I: 104.15 (C-1), 81.50 (C-3), 74.61 (C-2), 73.60 (C-5), 70.43 (C-4), 69.52 (C-6); glucose II: 104.08 (C-1), 77.80 (C-3), 76.16 (C-5), 74.83 (C-2), 71.39 (C-4), 62.60 (C-6); rhamnose: 102.57 (C-1), 73.63 (C-4), 72.17 (C-2, C-3), 70.27 (C-5), 18.27 (C-6).



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All compounds were isolated for the first time from subterranean organs of *I. helenium*. Compounds **7** and **8** in addition to 1-*O*-, 5-*O*-feruloyl, 5-*O*-*p*-coumaroyl, and 3-*O*-caffeoyl-5-*O*-*p*-coumaroylquinic acids were observed earlier in leaves of *I. helenium* [12]. Compound **8** was observed in roots of *I. cappa* [13], *I. britannica*, *I. salicina*, and *I. spiraeifolia* [14].

Six compounds in *I. helenium* (**2**, **4**-**6**, **8**, **9**) were analyzed quantitatively by HPLC (Table 1). The typical qualitative composition of phenylpropanoids from the subterranean organs was established. The distribution of pure compounds among morphological groups was uneven. Thus, adventitious roots had the greatest total contents of phenylpropanoids (32.95 mg/g) in addition to caffeic (**2**), 1,5-di-*O*-caffeoylquinic (**4**), and caftaric acids (**6**) and echinacoside (**9**).

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TABLE 1. Phenylpropanoid Content in Subterranean Organs of *I. helenium*, mg/g

Raw material sample	Compound							TPC*
	2	**4**	**5**	**6**	**8**	**9**	Σ	
Roots	0.74	2.22	0.60	1.24	3.07	1.52	9.39	13.48
Rhizomes	0.66	2.15	0.69	1.20	4.25	1.65	10.60	13.07
Adventitious roots	1.44	4.14	0.38	1.77	3.61	1.68	13.02	32.95

*Total phenylpropanoid content.

Rhizomes characteristically had the highest accumulation of chicoric (**5**) and 3-*O*-caffeoylquinic acids (**8**). The content of this group of compounds was least in the roots. The dominant phenylpropanoids of subterranean organs of *I. helenium* were 3-*O*-caffeoylquinic (**8**) and 1,5-di-*O*-caffeoylquinic acids (**4**).

According to the literature, phenylpropanoids exhibit a broad spectrum of physiological activity. Their presence can determine the nature of the overall biological effect of *I. helenium* preparations in man. In particular, the manifestation of immunomodulating activity can be explained by the presence of this class of compounds, namely caffeoylquinic and caffeoyltartaric acids and echinacoside, which are the active principles of Echinacea preparations.

TLC was carried out on PTSKh-AF Sorbfil silica gel plates (Imid Ltd.) and cellulose CEL 400 (Polygram); CC, over Sephadex LH-20 (Pharmacia) and polyamide (Woelm). The solvent systems were AcOH:H_2O (1, 95:5; 2, 90:10) and toluene:EtOAc:HCOOH (3, 5:4:1). Spectrophotometric studies were carried out on an SF-2000 spectrophotometer (OKB Spektr). IR spectra were recorded as films on ZnSe windows on an IR-Fourier FT-801 spectrometer (Simex) in the range 4000–600 cm^{-1}. MS analysis was performed in a high-resolution MAT 8200 mass spectrometer (Finnigan). ^{13}C NMR spectra were taken on a VXR 500S NMR spectrometer (Varian). HPLC used a Summit liquid chromatograph (Dionex) with a LiChrosorb RP-18 column (Merck, 250 × 4.6 mm, 5 μm) and mobile phase HCOOH (0.1%) (A) and MeCN (B) with a linear gradient B–A 10:90→70:30 0–15 min; flow rate 0.7 mL/min; 40°C; and a UVD 170S UV-detector at 330 nm. The total phenylpropanoid content (TPC) was determined by the Arnow method [15].

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