BRIEF COMMUNICATIONS

PHYTOCHEMICAL STUDY OF Betonica officinalis. I. ISOLATION OF BIOLOGICALLY ACTIVE SUBSTANCES FROM THE EPIGEAL PART OF THE PLANT

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In an investigation of the specific cholagogic and anti-inflammatory activity of various medicinal forms and preparations from the herb common betony Betonica officinalis L., it has been found that the optimum pharmacological action is possessed by freshly prepared aqueous infusions [1].

By chemical and chromatographic methods, the presence of seven flavonoids, four iridoids, four aromatic acids, 15 essential amino acids, and a number of unidentified compounds in an aqueous extract has been established. There are no cardiotonic glycosides, coumarins, or tanning substances. We had previously identified steroid and triterpene compounds in the herb common betony [2].

From the combined biologically active substances by fractionation on a column of polyamide we isolated only one individual compound of unestablished structure exhibiting the properties of quinones in reactions with alkalis (chloroform–ethanol (97.5:2.5) system). A satisfactory separation of polyphenols and iridoids was achieved when the combined material was separated chromatographically on polyamide in two stages: the combined iridoids were eluted with water (fraction 1), and the residue on the column was eluted completely by ethanol in the cold and on heating (fraction 2).

Fraction 1 contained (according to chromatography) iridoids, amino acids, monosaccharides, and a small amount of aromatic acids, while fraction 2 contained aromatic acids and flavonoids.

Two iridoids were isolated from fraction 1 by preparative paper chromatography (thrice-repeated chromatography in BAW (4:1:2)).

Substance 1, $C_{15}H_{24}O_{10}$, R_f 0.41 (BAW (4:1:2)) was identified by the results of chromatography and a comparison of its IR spectrum with that described in the literature [3] as harpagide.

Substance 2, $C_{17}H_{26}O_{11}$, mp 154-156°C, was identified from chromatographic results and the absence of a depression of the melting point with an authentic sample as acetyl harpagide.

Fraction 2, by column chromatography and preparative paper and thin-layer chromatography yielded in the individual state eight substances the structure of which are being studied. On the basis of chemical reactions and chromatographic results, four of them have been assigned to the flavonoids and four to hydroxycinnamic acids.

When the raw material was extracted with a 5% solution of sodium carbonate and the combined aromatic acid so obtained were separated by TLC on silica gel (BAW (4:1:2)), hydroxycinnamic acid was obtained in the form of colorless crystals with mp 210°C, although it was absent from fractions 1 and 2.

Thus, from the epigeal part of common betony we have isolated in the individual state: an unidentified compound exhibiting the properties of a quinone, two iridoids – (harpagide and acetylharpagide), four flavonoids, and five aromatic acids.

LITERATURE CITED

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PHYTOCHEMICAL STUDY OF Beonica officinalis.

II. ACIDS FROM THE EPIGEAL PART OF Betonica

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The composition of the aromatic acids isolated in the individual state from the herb common betony has been studied. Qualitative chemical reactions, the results of UV spectroscopy (Table 1), and chromatographic analysis characterize these substances as hydroxycinnamic acids [1, 2].

Substance 1, $C_9H_8O_4$, mp 192-295°C, R_f 0.82 (BAW (4:1:2) – system 1); 0.20 (0.1 N HCl on paper previously washed with 2% HCl and dried – system 2). This was obtained by eluting fraction 2 [3] with chloroform. The product of acetylation – $C_{13}H_{12}O_6$; mp 197-198°C – corresponded to the diacetate of caffeic acid. A comparison of substance 1 with an authentic sample of caffeic acid (absence of a depression of the melting point) showed their identity.

Substance 2, $C_{16}H_{19}O_9$, mp 201-204°C, R_f 0.62 (system 1); 0.48 (system 2). This was obtained by eluting fraction 2 with chloroform containing 20% of ethanol followed by the silica-gel TLC chromatography of the eluates obtained (system 2). Fusion with KOH gave protocatechic acid. Alkaline hydrolysis led to the formation of caffeic and D-quinic acids, which were identified by paper chromatography (systems 1 and 2, and 15% CH_3COOH – system 3) in the presence of markers [4]. A mixture with an authentic sample of chlorogenic acid gave no depression of the melting point.

Substance 3, $C_{16}H_{18}O_9$, could not be crystallized, R_f 0.56 (system 1); 0.64 (system 2). The products of fusion and alkaline hydrolysis were identical with those described for substance 2, i.e., substances 2 and 3 were isomers. Paper chromatography (systems 1-3) in the presence of markers characterized substance 3 as neochlorogenic acid.

Substances 4, $C_{16}H_{18}O_9$, could not be crystallized. It was obtained by the TLC method on silica gel (system 2) from the same fractions as substances 2 and 3; R_f 0.86 (system 1), 0.35 (system 2). The products of fusion and alkaline hydrolysis were identical with those described for substances 2 and 3. Two-dimensional paper chromatography (systems 1 and 3; two runs in each system) showed three partially overlapping spots, which is possible when isochlorogenic acid is present. A chromatographic comparison of substance 4 with an authentic sample of isochlorogenic acid (systems 1-3) showed their identity.

TABLE 1. Characteristics of the Hydroxycinnamic Acids from the Herb Common Betony

Acid	mp, °C	Color on the spot on the chromatogram		R_f in		Absorption maxima in the UV region of the spectrum			
		UV	UV +KOH	BAW, 4:1:2	15% acetic acid	solution in methanol	+KOH	+NaOAc	+H_3BO_3
Caffeic	193—196	Deep blue	Deep blue	0,82	0,53	325, 295 (sh.), 245	+22	10	+7
Chlorogenic	201—204	Deep blue	Deep blue	0,62	0,62	325, 293 (sh.), 245	+47	+7	+27
Neochlorogenic	—	Light blue	Turquoise	0,56	0,66	325, 293 (sh.), 245	+47	+7	+27
Isochlorogenic	—	"	"	0,80—0,86	0,45—0,55	325, 298 (sh.), 245	+47	+7	+27
p-Coumaric	210	—	Deep blue	0,93	0,58	325, 310, 300	+22	0	0

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