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IRIDOIDS OF *Ajuga turkestanica* AND THEIR QUANTITATIVE DETERMINATION

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Substances of iridoid nature identified as harpagide and 8-O-acetylharpigide have been isolated from the herb Ajuga turkestanica. *A procedure for the quantitative chromatographic determination of iridoids in the raw material has been developed.*

The isolation from the epigeal part of *Ajuga turkestanica* (Rgl.) Brig. (family Labiatae) of phytoecdysteroids — cyasterone and ecdysterone — has been reported previously [1]. Continuing a study of this plant, we have obtained iridoids - harpagide and 8-acetylharpagide and have determined them quantitatively. The quantitative determination was carried out by a chromato-photocolorometric method based on the capacity of 8-O-acetylharpagide and harpagide to interact with vanillin and orthophosphoric acid to form colored products having an absorption maximum at a wavelength of 530 nm. The absorption of a solution of iridoids in the range of working concentrations of from 2.0 to 7.0 μg/ml obeys the basic law of light absorption. The relative error of a single determination with a confidence limit of 95%, is ±4.52%. The absence of a systematic error was checked by the method of additives with an extract of 8-O-acetylharpagide.

Several samples of *A. turkestanica* raw material have been analyzed by means of the procedure developed.

EXPERIMENTAL

Iridoids of the Herb *A. turkestanica*. The air-dry comminuted epigeal organs (5 kg) were extracted with alcohol (35 liters). The combined alcoholic extracts were concentrated and diluted with water to 2 liters. The aqueous solution was extracted first with chloroform and ethyl acetate and then with butanol. The butanolic extract was evaporated to dryness, and 5 g of the dry residue was chromatographed on a column of KSK silica gel. The column was eluted with the chloroform–methanol (4:1) system, which yielded 3.25 g of a substance identified as 8-O-acetylharpagide — $C_{17}H_{26}O_{11}$, mp 154-156°C; $[\alpha]_D^{20}$ — 131 ± 2° (methanol). The constants and spectral characteristics of the 8-O-acetylharpagide corresponded to those given in the literature [2-4].

On further elution of the column with the same solvent system, we obtained 1.5 0 g of a substance of iridoid nature, $C_{15}H_{24}O_{10}$, amorphous, $[\alpha]_D^{20}$ — 154 ± 2° (methanol). The agreement of the constants and spectral characteristics with those given in the literature [2, 5] permitted the compound to be identified as harpagide.

Quantitative Determination of the Iridoids. About 2.5 g of the comminuted herb (particle size 3 mm) was extracted with 20 ml of 80% alcohol in the boiling water bath for 30 min. Extraction was repeated three times, and the combined extracts were concentrated to minimum volume, transferred quantitatively to a 25-ml measuring flask, and made up to the mark with the same solvent.

On each of the first three bands of a Silufol plate divided into five bands was deposited 0.05 ml of the extract, on the fourth 0.05 ml of a solution of 8-O-acetylharpagide, and the fifth band was left for a control experiment. The plates were chromatographed in the chloroform–methanol–water (40:10:1) system. The first band was cut out and the iridoid zones were detected by spraying with a 0.1% alcoholic solution of vanillin containing 0.5% of concentrated sulfuric acid and heating to 100-105°C for 1-2 min. The iridoid zones were colored crimson (R_f of harpagide 0.16; R_f of 8-O-acetylharpagide 0.34).

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TABLE 1

Number of the sample	Year of collection (May)	Amount of O-acetylharpagide and harpagide, %
1	1987	4.02
2	1987	3.87
3	1988	4.92
4	1988	4.56
5	1989	5.13

From the spots revealed, the zones of the iridoids on the other bands were marked and they were cut out together with the corresponding zone of the control experiment and each was eluted with 15 ml of 95% alcohol with heating in the boiling water bath for 1 h. To 1.5 ml of each filtered eluate were added 1.5 ml of a 2% alcoholic solution of vanillin and 4.0 ml of concentrated orthophosphoric acid. After 20 min, the optical density of the solution was measured in a photoelectric colorimeter at a wavelength of about 530 mm in a cell with a layer thickness of 10 mm against the background of the control experiment.

The percentage of total iridoids in the raw material (X) calculated to 8-O-acetylharpagide was found from the formula

$$X = \frac{m_0 \cdot D \cdot 100 \cdot 100}{m \cdot D_0 \cdot (100 - W)},$$

where m_0 is the weight of 8-O-acetylharpagide, g; m is the weight of raw material, g; D_0 is the optical density of the solution of 8-O-acetylharpagide; D is the optical density of the solution under investigation; and W is the moisture content of the raw material, %.

Preparation of a Solution of 8-O-Acetylharpagide. About 0.10 g (accurately weighed) of 8-O-acetylharpagide was dissolved in 95% ethanol in a 25-ml measuring flask, the volume of the solution was made up to the mark with the same alcohol, and the contents of the flasks were mixed. The results of an analysis of *A. turkestanica* raw material gathered in the Surkhandar'ya province (village of Derbent), Uzbekistan, are given in Table 1.

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