

0.43 (0.02%) of  $\beta$ -sitosterol  $\beta$ -D-glucopyranoside with mp 276-279°C (from methanol),  $[\alpha]_D^{24}$   $-36.5 \pm 2^\circ$  (c 1.04, pyridine) [5]; 5 g (0.23%) of cyclosiversioside E (III) with mp 216-218°C (from methanol)  $[\alpha]_D^{20}$   $+24.5 \pm 2^\circ$  [c 0.81; chloroform-methanol (1:1)] [4, 6]; and 20 g (0.93%) of cyclosiversioside F (IV) with mp 284-286°C (from methanol),  $[\alpha]_D^{20}$   $+38.1 \pm 2^\circ$  (c 0.57; methanol) [3, 7]. The melting point has been corrected from the values given in [3, 7].

All the compounds were also identified from their PMR and IR spectra and their chromatographic behavior on TLC in comparison with authentic samples.

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#### DETERMINATION OF CYTISINE IN THE HERBAGE OF *Thermopsis alterniflora* BY GAS-LIQUID CHROMATOGRAPHY

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Existing methods [1, 2] do not permit the sufficiently rapid and selective determination of the amount of cytosine in various parts of plants of the genus *Thermopsis*. We have investigated the possibility of using gas-liquid chromatography for the quantitative determination of cytosine in the herbage of *Thermopsis alterniflora* Regel et. Schmalh.

We used a Chrom-41 chromatograph with nitrogen as the carrier gas in a glass column 1500 mm long packed with Chromaton N super (fraction with a size of 0.16-0.20 mm) impregnated with 3% of the liquid phase OV-17. The temperature of the evaporator and of the flame-ionization detector was 250°C. The working regime of the thermostat was programmed in the interval from 190 to 230°C at a rate of rise of temperature of 2°C/min. Caffeine corresponding to the demands of GF X (State Pharmacopeia, 10th edn.) was selected as the internal standard.

For the quantitative determination of cytosine, the herbage of *Thermopsis alterniflora* was treated with chloroform in the presence of ammonia for 2 h. An aliquot of the extract was evaporated on the water bath in a current of air, and the dry residue was dissolved in 1 ml of chloroform and 2 ml of an ethanolic solution of the internal standard, 2  $\mu$ l the solution so obtained being injected into the evaporator of the chromatograph. The amount of cytosine was calculated by the internal-standard method. The calibration graph was linear for ratios of the weights of cytosine and caffeine between 1:3 and 3:1. The sensitivity of the method was determined as 60  $\mu$ g/ml. The figures obtained were compared with the results obtained by the NTD [Normative Technical Documentation] method. The error of the determination was  $\pm 2.5\%$ .

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METHOD FOR THE QUANTITATIVE DETERMINATION OF VINCARINE,  
HERBADINE, AND HERBAMINE IN THE HERBAGE OF *Vinca herbacea*

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The alkaloids vincarine, herbadine, and herbamine isolated from the herbage of *Vinca herbacea* Waldstet. et Kit. [1, 2] are pharmacologically active [3]. We propose a method for the quantitative determination in the plant raw material which consists in obtaining the combined alkaloids, separating them by TLC, and determining the alkaloids in the eluates by spectrophotometry on an SF-26 instrument. To separate the herbadine, herbamine, and vincarine from the accompanying bases, the total material was chromatographed on a fixed layer of type KSK No. 2 silica gel in the ethyl acetate-methanol (95:5) system. Elution with chloroform achieved 98-100% desorption of the material from the plate.

The essence of the method was as follows: 25 g of comminuted air-dry raw material was placed in a conical flask with a ground-in stopper, the alkaloids were exhaustively extracted with a 2% aqueous solution of sulfuric acid, the amorphous combined alkaloids were obtained by the usual methods [4], and these were dissolved in chloroform. The solution was transferred quantitatively to a 25-ml measuring flask and it was made up to the mark with chloroform, after which 3 ml of the resulting solution was deposited on a plate (24 × 18 cm) in the form of a continuous line 14 cm long and, beside it at a distance of 1.5 cm, as marker, was deposited 0.1 ml of the same solution in the form of a continuous line 1.5 cm long.

Chromatography was performed by the ascending method in the above-mentioned system. The finished plate was dried in the air, and only the marker band was stained with a solution of cerium ammonium sulfate in 85% orthophosphoric acid. The corresponding sections of the sorbent at the levels of vincarine (orange-red band with  $R_f$  0.16), herbadine (orange-red band with  $R_f$  0.52), and herbamine (carmine-red band with  $R_f$  0.7), were transferred quantitatively to flasks, covered with chloroform, and extracted on a universal shaking machine for 2 h. After separation, the chloroform extract from each zone was evaporated to dryness and the residue was dissolved in 2 ml of methanol. The optical densities were determined after appropriate dilution. The amounts of vincarine, herbadine, and herbamine in the raw material ( $X$ , %), calculated on the absolutely dry weight of the raw material, were calculated from the formula

$$X = \frac{K \cdot 100 \cdot D \cdot V_1 \cdot V_3 \cdot V_5}{a(100-b) \cdot E_{1\text{cm}}^{1\%} \cdot V_2 \cdot V_4}$$

where  $D$  is the optical density of the solution under investigation;  $E_{1\text{cm}}^{1\%}$  is the specific absorption index of vincarine at a wavelength of 243 nm, which is 165; or of herbadine at 292 nm, 90; or of herbamine at 295 nm, 85.5;  $V_1$  is the volume of the chloroform solution, ml;  $V_2$  is the volume of the chloroform solution deposited on the plate, ml;  $V_3$  is the volume of the methanol solution, ml;  $V_4$  is the volume of the methanol solution taken for dilution, ml;  $V_5$  is the volume of the diluted methanol solution, ml;  $a$  is the rate of raw material, g;  $b$  is the loss in weight on the drying of the raw material, %; and  $K$  is a correction factor for the alkaloid being determined on TLC separation.

Below we give the results of a statistical treatment of the figures obtained in the determination of vincarine, herbadine, and herbamine in the raw material. The amount of vincarine in the raw material, calculated on the absolutely dry weight of the plant, was 0.064%; of herbadine, 0.081%; and of herbamine, 0.077%.

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