METHOD FOR THE QUANTITATIVE DETERMINATION OF NAPELLINE IN Aconitum karakolicum

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The alkaloid napelline, which has been isolated from the epigeal part of Aconitum karakolicum (family Ranunculaceae) [1] is pharmacologically active [2]. We propose a method for the quantitative determination of napelline in plant raw material which consists in obtaining the total alkaloids from the raw material, separating them chromatographically, and determining the napelline in the eluate by a micro method of acid-base titration in a nonaqueous medium [3]. Napelline was separated from the accompanying alkaloids [4] in a fixed layer of type KSK silica gel in the chloroform–benzene–95% ethanol–acetone (10:8:4:1) system. Elution with chloroform–acetone (1:1) gave 98-100% desorption.

The amount of napelline in the raw material was found in the following way: 20 g of comminuted air-dry raw material was covered with 20 ml of 5% sodium carbonate solution, the mixture was stirred, and, after 30 min, the alkaloids were exhaustively extracted with chloroform. The extract was concentrated to 10-15 ml and the combined alkaloids were obtained in the usual way [5] and were dissolved in 5 ml of acetone. On a plate (24 × 18 cm) with a fixed layer of silica gel divided into four equal parts were deposited: in the first band, as marker, 0.2 ml of a 0.1% acetone solution of napelline hydrochloride, and in the second and third bands 0.2 ml each of an acetone solution of the combined alkaloids; the fourth band was left as control. Chromatography was carried out by the ascending method in the system mentioned above, the spots being revealed in the moist state with the Dragendorf reagent only in the first band, the other bands being protected by clean glass during spraying. The sections of the sorbent in the second and third bands with the spots present at the level of the revealed spot of napelline marker, and also the same section of the sorbent from the control band, were quantitatively transferred to flasks the contents of which were then made up to 100 ml with the given mixture. The flasks were shaken for two hours, the mixtures were filtered, the paper filters being washed with the solvent mixture, the filtrates were evaporated to dryness, and the residues were each dissolved in 5 ml of glacial acetic acid and titrated with 0.01 N perchloric acid in the presence of the indicator Crystal Violet until the color changed from violet to blue. The amount of napelline was calculated on the absolutely dry material (x, %) by means of the formula

$$x = \frac{500 \cdot 0{,}00359 \cdot V}{0{,}2 \cdot p\,(100 - h)},$$

where V is the volume of 0.01 N perchloric acid consumed in the titration, ml; p, weight of the sample of raw material, g; and h, loss in weight in the drying of the raw material, %. One ml of 0.01 N perchloric acid corresponds to 0.00359 g of napelline.

The amount of napelline in raw material collected in 1977 in the upper reaches of the R. Tyup (Kirghiz SSR) in the budding period was determined by the method developed. Below we give the characteristics of the statistical treatment of the results of the determination of napelline in this raw material:

f	$\bar{x}$, %	S^2	S	P	$t_{p,f}$	Δx	E, %	E_n, %
4	0,156	$11{,}510^{-6}$	$\pm 3{,}39 \cdot 10^{-3}$	95	2,78	±0,0094	+6,02	±2,68

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GLUCOHAPLOPINE — A NEW GLYCOALKALOID FROM *Haplophyllum perforatum*

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We have previously reported the isolation of new furanoquinoline alkaloids from the epigeal part of *Haplophyllum perforatum* (family Rutaceae) collection on the northern slopes of the Babatag range [1].

Continuing the separation of the combined chloroform-soluble alkaloids, we have treated them with water. The residue obtained from the water-soluble fraction was chromatographed on silica gel. Elution with chloroform–methanol (25:1) gave a crystalline base with the composition $C_{19}H_{21}NO_9$ (I) with mp 217-218°C (ethanol), $[\alpha]_D$ −41° (c 0.516; pyridine), R_f 0.36 [chloroform–methanol (8:1) system]. The substance is readily soluble in dilute acids and sparingly soluble in methanol, ethanol, and water and is insoluble in ether, chloroform, benzene, and ethyl acetate.

The UV spectrum of (I) ($\lambda_{max}^{C_2H_5OH}$ 250, 321, 334, 348 nm) is typical for 7,8-alkoxy-substituted 4-methoxyfuranoquinoline alkaloids [2]. The IR spectrum of (I) resembles that of glycoperine [3]. In the spectrum intense absorption is observed with maxima at 3470, 3340, 3320 cm^{-1} (hydroxy group), and at 3155 cm^{-1} (furan ring).

The acetylation of (I) with acetic anhydride in pyridine formed a tetraacetyl derivative (II), mp 135-137° (ethyl acetate–hexane), R_f 0.18 [chloroform–methanol (8:1)] and 0.24 [benzene–methanol (4:1)]. The mass spectrum of (II) contained, in addition to the peaks with m/e (%) 245 (100), 227 (23), 216 (9), and 202 (7) characteristic for the fragmentation of the molecular ions of haplopine [4], the peak of the molecular ion with m/e 575 (2.8) and the peaks of ions with m/e 331 (38), 271 (6), 169 (27), and 109 (16), which are characteristic for the fragmentation of the (M − 17) ion of 2,3,4,6-tetra-O-acetylglucopyranose [5].

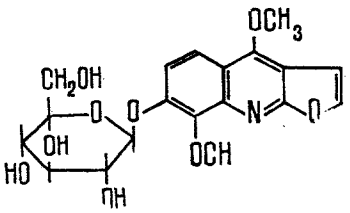
The facts given above permitted the conclusion that base (I) was a glucoalkaloid.

The enzymatic hydrolysis of (I) with snail pancreatic juice led to the formation of a base with mp 204-205°C (methanol), which was identified by TLC and by a mixed melting point with an authentic sample of haplopine [6], and D-glucose, the presence of which was confirmed by a chromatographic (TLC and PC) comparison with an authentic sample.

The results obtained gave grounds for calling the alkaloid isolated glucohaplopine.

The configuration of the glucosidic bond was established with the aid of Klyne's rule. The value of M_D = −225° shows that the D-glucose is attached to the haplopine by a β-glucosidic bond.

Thus, glucohaplopine has the structure of 7-β-D-glucopyranosyloxy-4,8-dimethoxyfuranoquinoline.



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