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METHOD FOR THE QUANTITATIVE DETERMINATION OF CNICIN IN Centaurea squarrosa

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The sesquiterpene lactone cnicin has been isolated from the epigeal part of Centaurea squarrosa (family Compositae) [1].

In the present paper we propose a method for the quantitative determination of cnicin consisting in the extraction of the combined lactones from the plant raw material by the method described [1], its chromatographic separation, and the determination of the lactone in the eluate by a micromethod of volumetric analysis. The completeness of extraction was checked chromatographically on "Silufol" plates in the solvent system chloroform–ethanol (9:1). The cnicin was separated from the accompanying substances in a thin fixed layer of silica gel in the same system. On elution with chloroform–ethanol (9:1) 97-99% desorption was achieved. The amount cnicin in the eluate was determined by an acidimetric method after saponification of the lactone with alkali.

The optimum conditions (concentration of alkali, solvent, time of hydrolysis) in which, in addition to the opening of the lactone ring, hydrolysis of the ester group of cnicin takes place were determined by special experiments. Then a gram-equivalent of cnicin will be equal to half a gram-molecule. Below we give the results of a statistical treatment of the determination of microamounts of cnicin (0.5-2.0 mg):

n	$\overline{x}$	S^2	S	$S_{\overline{x}}$	a	$t_{a,k}$	ε_a	E_{rel}
5	97,0%	0,598	0,773	0,334	0,95	2,776	0,955	0,98%

The amount of cnicin in the raw material was found in the following way: 10 g (weighed with an accuracy of 0.01 g) of the comminuted air-dry raw material was exhaustively extracted with 95% ethanol, and the extract was evaporated to give the total lactones [1], which were dissolved in 20 ml of ethanol. On a plate (13 × 18 cm) with a fixed layer of silica gel (type KSK, particle size 0.16-0.10 mm; pH of a 10% aqueous solution 8.2), 0.3 ml of the ethanolic solution was deposited and chromatography was carried out in the system given above. On the same plates was placed a "marker" – 0.05 ml of a 0.25% ethanolic solution of cnicin, which was revealed with a 1% solution of vanillin in concentrated sulfuric acid (Rf 0.40). The lactone was eluted with 50 ml of the mixture mentioned in a Schott No. 4 funnel, the eluate was evaporated to dryness, the residue was dissolved in 1 ml of ethanol, and 2 ml of 0.01 N caustic soda solution was added and saponification was performed for 5 min. The excess of alkali was back-titrated in the hot state [2] with a 0.01 N solution of hydrochloric acid until the blue color of the solution had disappeared (indicator: thymolphthalein).

The amount of cnicin in the raw material (x, %) on the absolutely dry weight was calculated from the formula

$$x = \frac{10 \cdot \left(V_1 - \frac{N_{\text{acid}} \cdot V_{\text{acid}}}{N_{\text{alk}}} \right) N_{\text{alk}} \mathrm{E} \cdot V_2}{p \cdot V_3 (100 - h)},$$

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where p is the weight of the raw material, g; h is the moisture content of the raw material, %; E is a gram-equivalent of cnicin (190.22); V_1 is the volume of alkali taken for saponification, ml; V_2 is the volume of the ethanolic solution in which the total lactones were dissolved, ml; V_3 is the volume of the solution deposited on the chromatogram, ml; N_{acid} is the normality of the acid solution; and N_{alk} is the normality of the solution of alkali.

A control titration and elution was performed in parallel. The amount of cnicin in plant raw material (1975 harvest) was determined by the method developed and was found to be 0.8% on the weight of the raw material. The maximum deviation on the mean value for six determinations did not exceed ± 3%.

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THE STRUCTURE OF THE SOLUBLE FRACTIONATION OF AMBER

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Information on the chemical structure of amber is given in the literature [1-4], and Rottländer et al. [5, 6] have shown that amber consists predominantly of a polyester the basis of which is hydroxydiabietic acid and they consider a suggested mechanism of the formation of succinite.

Our aim was to refine the structure of the fraction of amber soluble in ethanol or acetone. By exhaustive extraction of succinite in a Soxhlet apparatus with ethanol we isolated a soluble fraction of amber (I) – a resinous dark-colored substance–with a yield of about 25%. Resin (I) was hydrolyzed with 10% KOH in a mixture of ethanol and tetrahydrofuran (1 : 1) in the cold for several hours. Treatment of the hydrolyzate with HCl gave the light yellow acid form of the product (II), which was dried at room temperature in vacuum. The acid numbers of (I) and (II) were determined by titration with 0.5 N ethanolic KOH and their molecular weights by the isopiestic method on a Hitachi instrument (Table 1). The IR spectra were taken on a UR-20 spectrophotometer in the 400-4000 cm^{-1} region using the substances in the form of pastes with paraffin oil (see Table 1).

The molecular weight of the hydrolyzed resin (II) (590) gives grounds for assigning the bulk of it to diabietinic acid and its derivatives (molecular weight of diabietic acid 604). The presence of an acid is also shown by the acid numbers and by absorption bands at 1690 and 1710 cm^{-1} in the IR spectra (see Table 1).

TABLE 1. Physicochemical Properties of the Resins (I) and (II)

Resin	Acid No.	mol. wt. (in dimethylformamide)	Characteristic absorption bands in the IR spectrum and their most probable assignments			
			ν C–O	ν C=C	ν C=O (in COOR)	ν C=O (in COOH)
I	95	100	1055 m 1030 m 1065 sh	1645 sh 1640 m	1690, 1710 s	1735 s
II	145	590			1690, 1705 s	—

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