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CHROMATOPHOTOCOLORIMETRIC DETERMINATION OF INDOLE ALKALOIDS IN A CULTURE OF Rauwolfia serpentina TISSUE

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The known chromatophotocolorimetric method of determining ajmaline (I) in the biomass of a Rauwolfia tissue culture [1] includes a lengthy and laborious process of separating the alkaloids on previously impregnated paper. The disadvantages of the method are eliminated by replacing paper chromatography by thin layer chromatography (Silufol UV-254). The clear separation of derivatives of ajmaline (I) and of 17-O-acetylajmaline (II) makes it possible to determine them when they are present simultaneously. Standard samples of (I) (batch 10183, KhPKhFO "Zdorov'e") and of (II) obtained by acylating (I) [2] (followed by the separation of the mixture of di- and monoacyl derivatives by preparative chromatography) was used as control. In view of the retention in the (II) molecule of the indoline chromophore that is responsible for the bright red coloration on interaction with concentrated nitric acid, its photocolorimetric determination was carried out by the use of the graph plotted for (I) [3]. The mixture of alkaloids from the biomass isolated from 5 g of raw material [4] was dissolved in 5 ml of 95% ethanol. A 0.02-ml sample of this solution and 0.06 ml each of 0.5% ethanolic solutions of (I) and (II) (control) were deposited as bands on the starting line of a chromatogram. Chromatography was carried out by the ascending method in the chloroform–methanol–ammonia (90:10:0.2) solvent system. After the chromatogram had been examined in UV light, λ 254 nm, the sections containing the alkaloids being analyzed (R_f (I) 0.26; R_f (II) 0.50) were cut out and each was eluted with 10 ml of 95% ethanol by steeping with constant shaking for 2 h (which ensures the 95-97% elution of the (I) and (II)). The filtered eluate (6 ml in each case) was subjected to photocolorimetric analysis [3] (FÉK [photoelectric colorimeter] 56 M). The amounts of (I) and (II) in percentages of the absolutely dry raw material were calculated from the formula

$$X = \frac{a \cdot 0.5 \cdot 0.06 \cdot 100}{a_0 \cdot 0.02 \cdot 10^6 \cdot (100 - B)},$$

where a is the amount of (I) or (II) in 1 ml of colored solution (experiment) determined from the calibration curve, μg; a_0 is the amount of (I) or (II) in 1 ml of colored solution (control) determined from the calibration curve, μg; and B is the loss in weight in drying (%).

The metrological characteristics of the method show that it is not inferior in accuracy to the known method [1].

Batch No. of the raw material	I			II		
	$\bar{X}$	$S_{\bar{X}}$	$\varepsilon_{0,95\%}$	$\bar{X}$	$S_{\bar{X}}$	$\varepsilon_{0,95\%}$
1	0,83	0,01	3,61	0,105	0,03	7,54
2	0,77	0,02	6,49	0,082	0,002	4,88
3	1,06	0,02	4,72	0,048	0,002	8,33

The time of analysis is considerably shortened.

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ALKALOIDS OF Buxus sempervirens

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Continuing a study of the alkaloids of Buxus sempervirens L. we have investigated various organs, collected in 1986, of box cultivated in the environs of Tashkent, for the presence of alkaloids:

Plant organ	Total alkaloids, % Date of collection	
	3/25	7/21
1st-year shoots	2.14	1.87
Young roots	1.96	1.65
Flowers	1.85	–
Roots	1.72	1.51
Leaves and small branches	1.51	1.14
Branches of many years	0.94	0.81
Fruit	–	1.75

The ether-extracted fraction of the total alkaloids obtained from 2.7 kg of leaves and small branches collected on March 25 was dissolved in benzene and separated according to basicities with citrate-phosphate buffer solutions at pH 8.0-2.0 (with a pH interval of 0.2). Combined fractions (pH 8.0-7.6, 7.4-6.8, and 6.6-6.0) were separately chromatographed on a column of alumina (Brockman activity grade II). Elution was performed with ether–ethanol mixtures having increasing concentrations of ethanol of 5, 10, 15, 20, 25, 30, 35, and 40%, and from individual fractions were isolated cyclobuxine-D, cyclovirobuxine-D, and cycloprotobuxine-D [1, 2].

The mother liquor from the cyclovirobuxine-D and the cycloprotobuxine-D was chromatographed on a column of silica gel. Elution was performed with hexane–chloroform–ammonia (10:8:0.15). Rechromatography on a column of alumina with elution by ether–ethanol (5:2) and (5:3) yielded cycloprotobuxine-A [3] on trituration with acetone.

The combined alkaloids from the fraction with pH 4.8-4.0 were treated with acetone. The part of this material that was soluble in acetone was chromatographed on a column of alumina with elution by benzene–ethanol (5:1) and (5:2). The product was rechromatographed on column of silica gel. Benzene–hexane–ammonia (5:4:0.25) fractions yielded a base with mp 187-189°C (acetone), $C_{29}H_{48}N_2$, $[\alpha]_D$ +50.11° (c 0.761; chloroform), M^+ 424. This alkaloid was identified as buxamine-E (from a mixed melting point and also by the IR, UV, PMR, and mass spectrum of the base) [4, 6].

The combined alkaloids of the fraction with pH 6.0-5.8 were separated into acetone-soluble and acetone-insoluble groups. From the insoluble group a base was isolated with the composition $C_{28}H_{50}N_2O_2$ mp 215-217°C (ethanol), $[\alpha]_D$ −64.27° (c 0.527; chloroform). The

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