A NEW METHOD FOR THE PURIFICATION AND DETERMINATION OF THE TOTAL ALKALOIDS IN A CULTURE OF RAUWOLFIA TISSUE

S. I. Gubar' and E. P. Konstantinova

UDC 547.944/945+581.143.6

The existing methods of determining the total alkaloids in cultivated Rauwolfia cells require the laborious purification of the isolated alkaloids [1-3]. With the aim of shortening the time of performing the analysis, we have developed conditions for the purification of the alkaloid extracts on silica gel and the subsequent determination of the sum of the indole alkaloids with the use of microcolumn chromatography.

The alkaloids were extracted with chloroform (20 ml) for 3 h from the biomass of a tissue culture of Rauwolfia serpentina Benth. that had been dried at 60°C (0.2 g, accurately weighed). The extract was evaporated to dryness, the residue was dissolved in 1 ml of chloroform, and the solution was deposited on silica gel (column: 8 × 20 mm) that had previously been washed with chloroform, chloroform–methanol–20% ammonia (100:25:4), and chloroform (20 ml each). The alkaloids were eluted with chloroform–methanol–25% ammonia (100:25:4). The resulting solution was evaporated to dryness, and the residue was dissolved in chloroform (2 ml, accurately measured). For the quantitative determination of the sum of the alkaloids 1 μl of this solution was used for each measurement. The sum of the alkaloids was determined on a Milikhrom-1A microcolumn chromatograph (Nauchpribor Production Combine, Orel) under chromatographic conditions permitting the alkaloids to issue as a single peak (column: silica gel C_{600} (Czechoslovakia), 2 × 62 mm; eluent: chloroform–methanol–25% ammonia (21:15:8); rate of flow: 0.1 ml/min; detection: UV absorption, λ 250 nm). A calibration graph was plotted by using a purified (according to [3]) mixture of alkaloids from the biomass of cultivated Rauwolfia cells and a model mixture of ajmaline and vomilenine (1.5:1), which showed identical results.

The evaluation of the method was carried out by using a model solution with a known alkaloid content. The metrological characteristics of the method showed a fairly high accuracy (the systematic error of the determinations, δ = 1.3%, is statistically uncertain at the 95% confidence level) and reproducibility of the results of the determination of the total alkaloids (μ is the true value of the concentration of alkaloids in the model solution):

μ, μg/ml	$\bar{x}$, μg/ml	f	S^2	S	P, %	$t(P,f)$	Δx	E, %	$\Delta\bar{x}$	$\bar{E}$, %	$t_{calc.}$	δ
1000	1013	9	518	23	95	2,26	52	5,2	16	1,6	1,79	1,3

The total indole alkaloids in lines of a culture of Rauwolfia tissue differing in the levels of accumulation of indole alkaloids (line 1 – 1.14%; 2 – 0.61%; 3 – 0.90%) were determined by the proposed method. The results of the determination showed a good correlation with the content of indole alkaloids (% on the dry biomass)

Line	n	$\bar{x}$	S	$S\bar{x}$	$\bar{E}_{0,95}$, %
1	5	3,85	0,13	0,06	4,3
2	5	2,56	0,12	0,05	5,4
3	5	3,22	0,09	0,04	3,4

Thus, the method developed permits a rapid quantitative analysis of microsamples of Rauwolfia tissue culture for their indole alkaloid content.

LITERATURE CITED

1. A. G. Vollosovich, L. A. Nikolaeva, S. G. Duksina, and L. Kh. Kartashova, Farmatsiya, 29 (1977).

Institute of Molecular Biology and Genetics, Academy of Sciences of the Ukrainian SSR, Kiev. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 148-149, January-February, 1991. Original article submitted April 18, 1990; revision submitted July 16, 1990.

 0009-3130/91/2701-0132$12.50

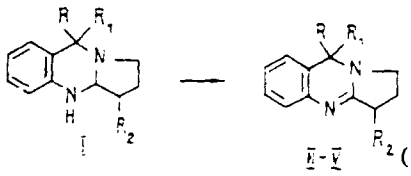
2. L. A. Nikolaeva, A. G. Vollosovich, and N. N. Pozdnyakova, Rast. Res., 144 (1978).
3. L. L. Shimolina, T. V. Astakhova, L. A. Nikolaeva, and S. A. Minina, Rast. Res., 137 (1984).

SPONTANEOUS DEHYDROGENATION OF TETRAHYDRO-2,3-TRIMETHYLENEQUINAZOLINES AND -QUINAZOLONES

M. V. Telezhenetskaya and V. N. Plugar'

UDC 547.944/945

In a study of the mass spectra of dihydro derivatives of quinazoline and quinazalone alkaloids (I) and their deutero analogues [1, 2] we detected traces of dehydrogenation products in all the specimens (II), deoxypeganine; R = R_1 + R_2 = H; (III), peganine: R = R_1 = H, R_2 = OH; (IV), deoxyvasicinone, R_1(R)C=O, R_2=H, (V), vasicinone: R_1(R)C=O, R_2=OH. R_2 = OH. To find whether this process takes place spontaneously or under the action of electron impact, a sample of (I) was heated to the melting point. Heating was also carried out in amyl alcohol and in methanol in a sealed tube at 120°C. In all cases the formation of (II)-(V) was established with the aid of TLC and mass spectrometry. After samples of (I) in the form of bases had been stored for 2-5 years their mass spectra were recorded again.



Dihydrodeoxypeganine R = R_1 = R_2 = H
Dihydropeganine R = R_1 = H, R_2 = OH

Dihydrodeoxyvasicinone R_1(R)C=O, R_2 = H

Dihydrovasicinone R_1(R)C=O, R_2 = OH

(II). Deoxypenine R = R_1 = R_2 = H
(III). Peganine R = R_1 = H, R_2 = OH

(VI). Deoxypeganine R_1(R)C=O, R_2 = H

(V). Vasicinone R_1(R)C=O, R_2 = OH

The spectra showed the conversion of dihydropeganine (M^+ 190 into (III) by approximately 50%; of dihydrodeoxyvasicinone (M^+ 188) into (IV) completely; and of dihydrovasicinone (M^+ 204) into (IV) and (V) in equal proportions.

Among quinazoline derivatives similar transformations have been described previously only with the aid of oxidants (chromium trioxide [3] and potassium ferricyanide [4]) and only in the synthesis of the β-carbolinoquinazoline alkaloid evodiamine has the spontaneous dehydration of the tetrahydroquinazoline moiety of the molecule to the dihydroquinazolone analogue been observed [5].

Institute of Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 149-150, January-February, 1991. Original article submitted April 23, 1990.