CHROMATOPOLAROGRAPHIC DETERMINATION OF THE ALKALOIDS FROM THE SEEDS OF HAPLOPHYLLUM PERFORATUM

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Khimiya Prirodnykh Soedinenii, Vol. 2, No. 5, pp. 333-337, 1966

From the seeds of Haplophyllum perforatum (M.B.) Kar. et Kir., family Rutaceae, S. Yu Yunusov et al. have isolated the alkaloids haplophyllidine [1], perforine [2], haploperine, and haplopine [3], which possess tranquillizing, narcotic, and antispasmodic properties [4].

The present communication gives the methods for the quantitative determination of these alkaloids in pure samples and plant raw materials. Without dwelling in detail on the procedure [5], we give only the characteristics of the capillaries which we used: for capillary No. 1 at h_{Hg} = 55 cm, m = 2.175 mg·sec^{-1}, t = 1.0 sec in 1 N KCl, $m^{2/3}$, $t^{1/6}$ = 1.884 $mg^{2/3}\cdot sec^{-1/2}$; for No. 2 at h_{Hg} = 60 cm, m = 0.19 mg·sec^{-1}, t = 8.0 sec in 1 N HCl without polarization and t = 2.0 sec at a polarization of 2.5 V; $m^{2/3}$, $t^{1/6}$ = 0.482 $mg^{2/3}\cdot sec^{-1/2}$. For this purpose we used an electrolyzer with an internal anode. The mercury on the bottom served as the comparison electrode. The temperature of the determination was 25 ± 0.5° C.

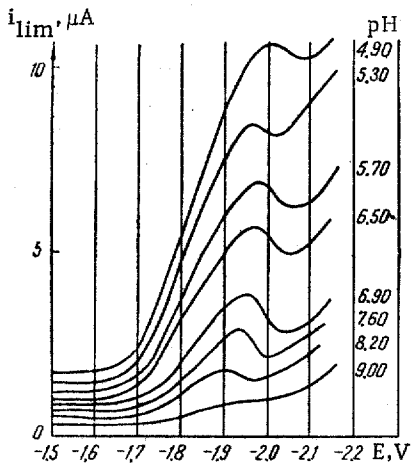

Fig. 1. Influence of pH on the magnitude of the limiting current of haploperine and haplopine in Britton-Robinson buffer solutions (C = 2.50 mM/l in 50% ethanol; S = 1/50).

The polarography was carried out in Britton-Robinson buffer solutions [6] and a 0.1 N solution of $(C_2H_5)_4NOH$ in 80% ethanol. The substances under investigation were dissolved in 96% ethanol and, for polarography, the solutions were mixed with the buffer in a 1:1 ratio. The resulting values of the pH were measured after polarography on a LP-58 pH meter. Under these conditions, no polarographic waves were found for haplophyllidine and perforine in the range of pH values from 2 to 12; haploperine and haplopine formed a wave with a characteristic curved maximum instead of limiting current plateau (Fig. 1).

The shape of the wave, the dependence of the height of the maximum on the pH of the solution, and its rise when the capacity of the buffer was increased at constant pH give grounds for assuming that the polarographic wave of both alkaloids is connected with the catalytic evolution of hydrogen at the cathode. The process is accompanied by the adsorption of the catalyst on the surface of the electrode. This is shown by the nature of the "current intensity versus concentration" curve. The limiting current rises in proportion to the height of the mercury column h_{Hg}. The role of catalysts may be fulfilled by the alkaloids themselves, in particular the N-containing functional groups [7].

Table 1

Polarographic Characteristics of Haplophyllidine and Perforine in 0.1 N $(C_2H_5)_4NOH$ in 80% Ethanol

Haplophyllidine				Perforine			
C, mM/l	i_d, μA	$K=\frac{i_d}{C}$	$E_{1/2}$, V	C, mM/l	i_d, μA	$K=\frac{i_d}{C}$	$E_{1/2}$, V
10.14	20.07	1.98	−2.25	10.12	20.06	1.98	−2.26
5.07	10.31	2.03	−2.23	5.00	10.10	2.02	−2.26
3.17	6.13	1.92	−2.23	3.00	5.72	1.91	−2.25
2.53	5.11	2.02	−2.24	2.53	5.02	1.98	−2.24
1.90	3.87	2.03	−2.22	2.00	3.88	1.94	−2.22
1.27	2.59	2.04	−2.22	1.26	2.51	1.99	−2.21
0.64	1.22	1.90	−2.20	0.63	1.20	1.90	−2.20
0.32	0.58	1.81	−2.19	0.32	0.59	1.84	−2.19
0.16	0.27	1.69	−2.18	0.16	0.26	1.62	−2.17

In view of this, we considered it impossible to use the buffers mentioned for analytical purposes, particularly for the analysis of extracts, and therefore selected 0.1 N $(C_2H_5)_4NOH$ in 80% ethanol as the background. In this medium, all four alkaloids form clear waves whose height is proportional to $h_{Hg}^{1/2}$. The half-wave potentials of the alkaloids are as follows: for haploperine −1.85 V; for haplopine −2.05 V; for haplophyllidine −2.25 V; and for perforine −2.25 V

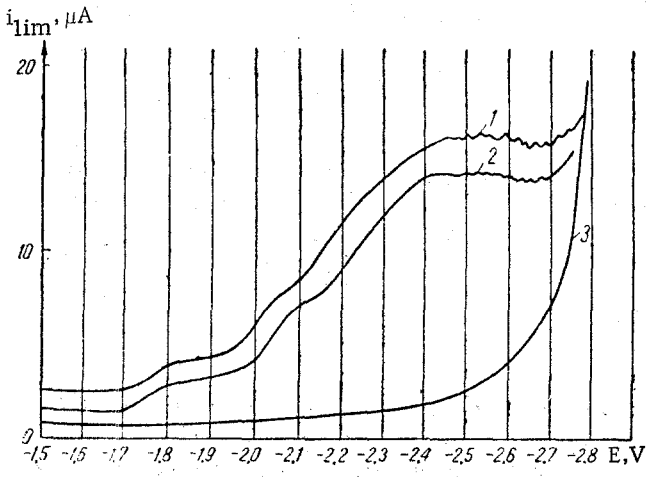

Fig. 2. Polarization curves: 1) Total alkaloids from the seeds of H. perforatum in 0.1 N $(C_2H_5)_4NOH$ in 80% ethanol; 2) model mixture of alkaloids; 3) background.

Table 2

Characteristics of the Degree of Desorption of Haplophyllidine in Elution

Added, mg	Found, mg	Relative error, %
4.03	3.91	−3.00
4.03	3.98	−1.24
4.03	3.81	−5.43
3.99	3.76	−5.80
5.40	5.24	−2.96

To obtain well-defined waves of haplophyllidine and perforine, which are reduced in the region of large negative potentials, we used the slow dropping capillary No. 2 [8]. The polarographic behavior of haplophyllidine and perforine are similar (Table 1), and therefore in mixtures it is possible to determine only their sum. As can be seen from the table, for both alkaloids within the range of concentrations from 0.5 to 10 mM/*l*, the diffusion current is proportional to the concentration; the coefficient of proportionality is 2 μA/mM·*l*.

Under analogous conditions and in the same range of concentration, for haploperine K was found to be 6.88 and for haplopine 6.37 μA/mM/*l*. However, as we have reported previously [5], at an alkaloid concentration greater than 3 mM/*l*, a maximum appears in the polarographic wave. It is easily eliminated by gelatin, but in practical work it is better to use solutions with alkaloid concentrations of less than 3 mM/*l* (0.2–1.0 mg/ml). In this case, all the determinations can be carried out with capillaries of the usual type at t = 2–4 sec.

The total bases were isolated by petroleum ether (or gasoline) extraction of the seeds of H. perforatum which had not been treated with ammonia in order to obtain the bulk of the haplophyllidine [1] and by chloroform extraction of the defatted seeds to obtain the other alkaloids [2]. In the alcoholic solution of the total alkaloids in a background of 0.1 N $(C_2H_5)_4NOH$, the alkaloids haploperine (1st wave), haplopine (2nd wave), and haplophyllidine and perforine combined (3rd wave) were detected and quantitatively estimated by the standard solutions method or the additive method [9] (Fig. 2).

Table 3

Results of Analyses of Model Mixtures of Alkaloids

Mixture No.	Alkaloids	Added, mg/ml	Found, mg/ml	Relative error, %
1	Haploperine	0.22	0.23	+4.5
	Haplopine	0.31	0.32	+3.2
	Haplophyllidine	1.15	1.22	+6.1
	Perforine	0.38	0.40	+5.3
2	Haploperine	0.28	0.27	−3.5
	Haplopine	0.38	0.40	+5.3
	Haplophyllidine	0.56	0.53	−5.3
	Perforine	0.28	0.27	−3.5
3	Haploperine	0.47	0.49	+4.3
	Haplopine	0.22	0.23	+4.5
	Haplophyllidine	0.90	0.85	−5.5
	Perforine	0.56	0.53	−5.3

We used thin-layer chromatography [10] to separate the haplophyllidine and the perforine. 0.8–1.0 ml of an alcoholic solution of the total alkaloids containing 10–15 mg/ml was chromatographed on a nonfixed layer of alumina (Brockmann grade II) about 1 mm thick in the benzene–ethanol (9.5:0.5) system. The dimensions of the plates were 13 × 18 cm. A control sample and a reference sample (pure material) were applied to the same plate. The control plate was treated with iodine vapors after chromatography. We found the following R_f values for haploperine, haplopine, perforine, and haplophyllidine: 0.12, 0.16, 0.19, and 0.52, respectively, and then by elution in a column with ethanol (20–25 ml) the haplophyllidine was separated from the other three alkaloids combined. The eluate containing the haplophyllidine was concentrated, the background solution was added to a total volume of 6–7 ml, and the amount of haplophyllidine was determined polarographically by the additive method. As the total amount of haplophyllidine and perforine was known, the amount of perforine was calculated by difference.

Table 4

Results of the Analyses of the Total Alkaloids with the Addition of Crystalline Samples

Mixture No.	Total alkaloids taken, mg	Crystalline samples	Alkaloids, mg			Relative error, %
			added	total amount	found	
1	4.93	Haloperine	0.88	1.24	1.21	−2.42
		Haplopine	0.54	0.87	0.82	−5.75
		Haplophyllidine + perforine	0.82	2.59	2.48	−4.24
2	6.13	Haloperine	0.92	1.37	1.34	−2.18
		Haplopine	0.35	0.76	0.79	+4.00
		Haplophyllidine + perforine	0.80	4.47	4.37	−2.24
3	5.00	Haloperine	0.26	0.62	0.65	+4.84
		Haplopine	0.24	0.58	0.54	−7.00
		Haplophyllidine + perforine	0.78	3.78	3.70	−2.15

Note: The composition of the total alkaloids was: haloperine 7.28%, haplopine 6.73%, combined haplophyllidine and perforine 59.92%.

The completeness of the extraction of the haplophyllidine in the process of the chromatographic separation of the alkaloids was shown with model preparations (Table 2). The method of the chromatopolarographic analysis of the total alkaloids was checked on model mixtures of them (Table 3) and on the total alkaloids extracted from the seeds with the addition of crystalline samples of the individual alkaloids (Table 4). The results of the analyses of a number of extracts are given in Table 5.

Table 5

Results of the Analyses of the Total Alkaloids from the Seeds of H. perforatum

Extracts	Content, %				
	haloperine	haplopine	haplophyllidine and perforine combined	haplophyllidine	perforine
Petroleum ether	7.28	6.73	59.92	48.40	11.52
Gasoline	6.38	—	66.02	54.25	11.77
Chloroform	6.63	19.27	30.91	9.45	21.46

Summary

1. The polarographic behavior of haloperine, haplopine, haplophyllidine, and perforine in Britton-Robinson buffer solutions and in $(C_2H_5)_4NOH$ solutions has been studied. Optimum conditions for their quantitative determination in solutions within the range of concentrations from 5×10^{-4} to 1×10^{-2} M have been found.

2. A method for the separate quantitative determination of the alkaloids in the seeds of H. perforatum has been developed.

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15 November 1965

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