THE CONNECTION OF THE R_f VALUES OF ALKALOIDS WITH THEIR $pH_{1/2}$ VALUES

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Information on the connection between the structure of the molecule of a substance and its R_f value and also on the connection between its pK_a and R_f values is given in many papers [1-5].

We were interested in finding whether there is a definite correlation between the $pH_{1/2}$ value of an alkaloid (the pH of the buffer solution extracting half the amount of alkaloids from an organic solvent phase) and its R_f value when the same organic solvent was used to determine the R_f and $pH_{1/2}$ values. To check this hypothesis, we investigated the main alkaloids of *Vinca erecta*, and also a number of opium and isoquinoline bases investigated previously [6]. From the numerical values of $pH_{1/2}$ we have constructed the following sequence of alkaloids: salsoline > morphine > salsolidine > tombosine > codeine > vincanidine > akuammidine > vincarine > thebaine > vincanine > vincamine > papaverine > narcotine. It was found that with respect to their R_f values these bases are arranged in the reverse order and sequence (Table 1). The R_f values were determined chromatographically on type LS 5/40 μ silica gel—gypsum (9:1) and on "Silufol" plates. The solvent system was chloroform—methanol (9:1).

Some discrepancy is observed in the R_f values, which is apparently connected with the different states of the sorption materials (on the Silufol plates there is a mixture of silica gel and starch) and with the method of preparation.

Table 1 shows that for all the alkaloids the R_f rises with a fall in $pH_{1/2}$; however, it does not appear possible to derive a mathematical relation $pH_{1/2} = f(R_f)$. In order to study the nature of the influence of the solvent on the corresponding sequences of alkaloids with respect to their R_f and $pH_{1/2}$ values, we determined the R_f values in the benzene—methanol (9:1) system; these values were then compared with the $pH_{1/2}$ values in benzene.

As can be seen from Table 2, the sequence given above is preserved, although under these conditions the $pH_{1/2}$ and R_f values of the alkaloids are different (see Table 1).

On the basis of the figures of Tables 1 and 2 it may be assumed that the spot of an alkaloid on a plate and its position relative to the spots of the other bases (with known R_f and $pH_{1/2}$ values) may be useful in the choice of a suitable heterogeneous distribution system and the pH of a buffer solution permitting it to be concentrated in a definite fraction.

Example. The amount of kopsinine in the combined alkaloids of *V. erecta* does not exceed 10% [7]. It does not precipitate in the form of a hydrochloride from a solution of the combined alkaloids. When it is obtained in the form of the nitrate, it separates together with pseudokopsinine, which has a similar structure (and which is also present in an amount of about 10%) and other alkaloids. The purification of the kopsinine from the mixture is associated with high losses. Consequently, it was necessary to concentrate the kopsinine in an individual fraction. In relation to its R_f value (0.48), kopsinine comes between vincarine (R_f 0.46, $pH_{1/2}$ 4.9) and vincanine (R_f 0.5, $pH_{1/2}$ 3.6). Its $pH_{1/2}$ value is presumably 4-5. In order to concentrate kopsinine we selected the pH range from 3.0 to 6.0

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TABLE 1. Dependence of $pH_{1/2}$ of Alkaloids on Their R_f Values in the Chloroform–Methanol (9:1) System

No.	Base	$pH_{1/2}$ from chloroform	R_f	
			LS 5/40 μ	Silufol
1	Salsoline	8,45	0,06	0,05
2	Morphine	<9,0	0,07	0,11
3	Salsolidine	6,45	0,13	0,16
4	Tombosine	6,35	0,16	0,19
5	Vincanidine	5,80	0,20	0,26
6	Codeine	5,75	0,23	0,26
7	Akuammidine	5,35	0,40	0,35
8	Vincarine	4,90	0,46	0,37
9	Thebaine	3,70	0,49	0,39
10	Vincamine	3,60	0,50	0,43
11	Vincamine	3,30	0,53	0,76
12	Papaverine	<2,0	0,83	0,96
13	Narcotine	<2,0	0,88	0,96

TABLE 2. Dependence of the $pH_{1/2}$ Values of the Alkaloids on Their R_f Values in the Benzene–Methanol (9:1) System

No.	Base	$pH_{1/2}$ from benzene	R_f	
			LS 5/40 μ	Silufol (Czechoslovakia)
1	Tombosine	7,5	0,06	0,03
2	Vincanidine	7,1	0,07	0,06
3	Akuammidine	6,3	0,16	0,11
4	Vincarine	6,3	0,16	0,16
5	Vincamine	5,7	0,17	0,18
6	Vincamine	4,6	0,24	0,31

(the course of the subsequent experiments is given in the Experimental part). Kopsinine was present in the fraction obtained in an amount of between 40 and 45% and it was isolated in the form of the hydrochloride; its $pH_{1/2}$ value is 4.5. A relationship between the optimum pH of a buffer solution and the R_f value in thin-layer chromatography has also been given by Brutko et al. [8].

EXPERIMENTAL

The alkaloids were chromatographed in a thin layer of type LS 5/40 μ–gypsum (9:1) and on "Silufol" plates (Czechoslovakia) in the following solvent systems: chloroform–methanol (9:1) and benzene–methanol (9:1).

On the "Silufol" plates, the alkaloids were revealed by treatment with I_2 vapor, and on the LS 5/40 μ–gypsum (9:1) plates with cerium ammonium sulfate.

The combined alkaloids (350 g) were dissolved in three liters of chloroform, and the solution was filtered. Then it was treated with a buffer solution having pH 6 (3 × 3 liters) to eliminate strongly basic alkaloids. After this, it was extracted with a pH 3 buffer (3 × 3 liters). The pH 3 buffer solution was washed with chloroform (3 × 1 liter) and made alkaline with ammonia to pH 6. The alkaloids were extracted with chloroform (3 × 3 liters). The chloroform solution was concentrated to three liters and was treated with the pH 6 buffer and then the pH 3 buffer. The pH 3 buffer was washed with chloroform, and so on. The whole cycle was repeated three times. The weight of the fraction obtained was 66 g. Kopsinine hydrochloride was isolated by treatment with an ethanolic solution of hydrogen chloride - 29 g. On a thin-layer chromatogram a single spot with R_f 0.48 was found.

SUMMARY

1. A correlation between the $pH_{1/2}$ and R_f values (on TLC) has been found for the 13 alkaloids studied.

2. For the case of kopsinine, as an example, methods of using the correlation for the separation and isolation of alkaloids from mixtures of them have been shown.

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