THE SEPARATION OF ALKALOIDS ON PAPER IMPREGNATED WITH ZIRCONIUM PHOSPHATE

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Chromatography on ion-exchange resins has been used comparatively rarely for the separation of alkaloids. A number of separations, concentrations and purifications have been carried out (for a review see ref. 1), but in most cases the adsorption of the alkaloids is so strong that organic solvents are necessary for eluting them from the resin.

It was shown recently by CATELLI[2] that amino acids may be separated on paper impregnated with zirconium phosphate in a manner analogous to separations effected on cellulose phosphate paper. Since zirconium phosphate has exchangeable phosphoric acid groups like an organic resin but lacks the organic network of the resin, we thought that novel separation effects might be possible on zirconium phosphate, *i.e.* separations which cannot be obtained on organic resins where adsorption is a contributing factor or on inorganic exchangers, such as Florisil, which are unstable in acid solutions.

As shown in the experimental part below, the study of 35 alkaloids revealed numerous possibilities of separation, and it was found that paper impregnated with zirconium phosphate may also be used as a novel technique for the chromatographic identification of a number of alkaloids.

EXPERIMENTAL

Preparation of paper impregnated with zirconium phosphate

The method of preparation proposed by ALBERTI AND GRASSINI[3] was used with two modifications. In order to obtain a very uniform loading with zirconium oxychloride the solution was allowed to enter the paper by ascending chromatographic development instead of dipping the paper into a solution. The second modification was brought about by the desirability of examining papers of different exchange capacities. Thus not only a 30% zirconyl chloride solution in 4 N HCl was used to impregnate the paper, but also 5%, 10%, 15%, 20% and 25% solutions, giving papers with relative capacities varying as to the amount of zirconyl phosphate in the solution employed. Subsequent drying, dipping into phosphoric acid, washing and drying were then performed as described by ALBERTI AND GRASSINI[3].

Chromatography of alkaloids

Table I shows the R_F values of some alkaloids on papers of different capacities and using aqueous acetic acid and hydrochloric acid as solvents. The R_F values on pure cellulose paper (Whatman No. 1) are also given for comparison. Development was carried out by the ascending method after equilibration for 24 hours.

The alkaloids were spotted on the paper as bases or chlorides in ethanol or aqueous solution. After development the papers were sprayed with Dragendorff reagent yielding orange spots on a yellow background.

TABLE I

R_F VALUES OF ALKALOIDS ON PAPER IMPREGNATED WITH ZIRCONIUM PHOSPHATE

Conditions: ascending development in containers equilibrated for 24 h at 28° ± 1°. The alkaloids were spotted on the paper as 1 % solutions in ethanol (when a salt was employed, the type is indicated in the table). Reagent: the papers were dipped into Dragendorff's reagent. Paper: T = Whatman No. 1 paper run as control. 5 %, 10 % refers to the degree of impregnation. For details see text.

Alkaloid	*R_F values*										
	10 % Acetic acid							*1 N Hydrochloric acid*			
	T	*5 %*	*10 %*	*15 %*	*20 %*	*25 %*	*30 %*	*T*	*5 %*	*15 %*	*30 %*
Morphine	0.90	0.36	0.24	0.22	0.20	0.12	0.13	0.81	0.80	0.73	0.58
Apomorphine	0.64	0.12	0.06	0.10	0.10	0.04	0.03	0.36	0.32	0.30	0.26
Heroine	0.95	0.33	0.22	0.20	0.22	0.11	0.15	0.86	0.82	0.73	0.62
Papaverine	0.87	0.27	0.16	0.13	0.09	0.04	0.08	0.71	0.60	0.44	0.30
Hydrastine	0.88	0.15	0.10	0.06	0.07	0.00	0.00	0.81	0.62	0.58	0.43
Quinine	0.88	0.03	0.00	0.00	0.00	0.00	0.00	0.88	0.73	0.56	0.38
Cinchonine	0.90	0.03	0.00	0.00	0.00	0.00	0.00	0.89	0.73	0.54	0.37
Cinchonidine	0.91	0.04	0.00	0.00	0.00	0.00	0.00	0.90	0.73	0.58	0.37
Tropine	0.90	0.55	0.40	0.22	0.19	0.18	0.17	0.92	0.91	0.80	0.64
Atropine	0.92	0.42	0.25	0.22	0.19	0.18	0.16	0.87	0.88	0.79	0.59
Homatropine	0.91	0.42	0.25	0.24	0.19	0.18	0.16	0.90	0.87	0.83	0.64
Cocaine	0.88	0.58	0.34	0.33	0.29	0.26	0.20	0.84	0.83	0.76	0.65
Tropacocaine HCl	0.89	0.36	0.27	0.18	0.16	0.17	0.13	0.83	0.78	0.73	0.68
Hyoscyamine	0.90	0.42	0.30	0.25	0.19	0.25	0.14	0.89	0.88	0.84	0.66
Veratrine	0.87	0.80	0.61	0.68	0.58	0.49	0.48	0.79	0.80	0.76	0.70
Protoveratrine	0.94	0.89	(1) 0.71 (2) 0.90	(1) 0.78 (2) 0.92	(1) 0.64 (2) 0.94	(1) 0.58 (2) 0.78	(1) 0.60 (2) 0.92	0.84	0.91	0.92	0.79
Mescaline HCl	0.92	0.55		0.41			0.22	0.58	0.90	0.81	0.80
Ephedrine	0.77	0.63		0.47			0.30	0.60	0.91	0.80	0.72
Eserine	0.93	0.41		0.27			0.13	0.59	0.93	0.77	0.51
Ergotamine tartrate	0.64	0.03		0.02			0.00	0.31	0.27	0.16	0.17
Yohimbine HCl	0.74	0.40		0.26			0.15	0.60	0.62	0.55	0.53
Colchicine	0.83	0.83		0.72			0.67	0.80	0.71	0.73	0.58
Harmine	0.62	0.04		0.03			0.02	0.28	0.21	0.19	0.11
β-Erythroidine HCl	0.90	0.21		0.16			0.06	0.92	0.81	0.75	0.55
Caffeine	0.84	0.73		0.69			0.51	0.83	0.67	0.64	0.48
Theophylline	0.77	0.71		0.70			0.50	0.71	0.69	0.61	0.55
Emetine	0.90	0.05		0.02			0.00	0.83	0.77	0.80	0.64
Solanine	0.88	0.84		0.69			0.53	0.81	0.82	0.70	
Arecoline	0.92	0.45		0.26			0.16	0.92	0.92	0.86	0.76
Aspidospermine	0.88	0.59		0.28			0.14	0.84	0.80	0.75	0.72
Diaboline	0.86	0.38		0.17			0.15	0.85	0.84	0.74	0.66
Brucine	0.81	0.17		0.07			0.05	0.69	0.68	0.54	0.49
Vomicine	0.76	0.52		0.20			0.19	0.63	0.65	0.57	0.53
Strychnine nitrate	0.80	0.13		0.08			0.04	0.74	0.66	0.57	0.53
Jervine	0.76	0.28		0.18			0.12	0.96	0.95	0.87	

DISCUSSION

Most of the alkaloids show a negligible adsorption on cellulose (especially from acetic acid), thus the adsorption can in most cases be credited to the effect of the zirconium phosphate. Depending on the capacity of the paper, the R_F values may be varied over a wide range, thus indicating excellent possibilities both for column and thin-layer separations with zirconium phosphate–cellulose mixtures.

The method permits separation of several alkaloids belonging to the same group, *e.g.* atropine, cocaine, tropacocaine, hyoscyamine (especially with 5 % paper) the separations depending on the pK values of the substances.

In the group of strychnine alkaloids the separation of strychnine and its dimethyl-derivative brucine is rather poor because the two alkaloids have the same fundamental structure of the non-indolic nitrogen group, whereas a good separation of vomicine and diaboline is obtained. The latter, which are separated only with difficulty by paper chromatography with the common solvents, possess different structures as reported by MARINI-BETTÒLO[4] and CAGGIANO AND MARINI-BETTÒLO[5].

Better results are generally obtained with the 5 % paper than with other papers, and acetic acid generally gives better resolution than N HCl.

In the case of quinine, cinchonine and cinchonidine no displacement was observed on zirconium phosphate in acetic acid. This fact can be attributed to the tendency of quinine salts to form insoluble complexes in acetic acid with several metal ions whereas these are unstable in HCl. The same effect is also obtained in acetic acid with harmine, emetine and ergotamine.

SUMMARY

The chromatographic separation has been studied of 35 alkaloids on paper impregnated with zirconium phosphate. It has been shown that in comparison to ordinary Whatman paper the R_F values of the various alkaloids can be varied over a wide range, thus enabling the identification of several closely related alkaloids.

REFERENCES

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