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CHROMATOGRAPHIC BEHAVIOUR OF ALKALOIDS ON THIN LAYERS OF CATION EXCHANGERS

II. ALGINIC ACID, REXYN 102, DOWEX 50-X4 AND CMCNa

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SUMMARY

The chromatographic behaviour of 48 alkaloids on cation exchangers with cellulose, paraffin and polystyrene matrices in both the acid and sodium salt forms has been investigated. Water–organic solvent mixtures, aqueous buffer solutions and organic and mineral acid solutions in both water and in aqueous–organic solvents have been used as eluents.

The retention mechanisms of these compounds on alginic acid, Rexyn 102 (H^+) and Dowex 50-X4 (H^+) thin layers are discussed. Interesting separations of the alkaloids were carried out on alginic acid and Rexyn 102 (H^+).

INTRODUCTION

In Part I[1], the chromatographic behaviour of 48 alkaloids on thin layers of some anion exchangers (AG 1-X4 and Cellex D) was described. This part concerns the chromatographic behaviour of the same alkaloids on layers of cation exchangers with cellulose (alginic acid and sodium carboxymethylcellulose), paraffin (Rexyn 102) and polystyrene (Dowex 50-X4) matrices.

This study was carried out in order to give a complete picture of the use of ion exchangers in the separation of alkaloids and, at the same time, to characterize the influence of the matrix on the chromatographic behaviour of these compounds.

EXPERIMENTAL

Preparation of layers and solutions

The solutions of the alkaloids were prepared as described in Part I[1]. The layers (thickness 300 μm) of alginic acid[2], Rexyn 102 (Fisher Scientific, Fair Lawn, N.J., U.S.A.) and Dowex 50-X4 were prepared with 3 g of resin and 9 g of microcrystalline cellulose (E. Merck, Darmstadt, G.F.R.) in 50 ml of water. Before use, the exchangers were rinsed with water and methanol and dried at room temperature.

The sodium carboxymethylcellulose (CMCNa) layers were prepared with 4 g

of exchanger (1.18 mequiv./g, No. 132, Schleicher & Schüll, Dassel, G.F.R.) in 50 ml of water. The chromatographic measurements were carried out at 25 ± 0.5° in a thermostatted developing chamber (Desaga, Heidelberg, G.F.R.). The migration distance was 11 cm unless stated otherwise.

Detection

The alkaloids were detected as described in Part I[1], except that for the exchangers in the acid form it was not necessary to spray the layers with 6 *M* acetic acid solution.

Of the 48 alkaloids, four cannot be detected on alginic acid (veratrine, ephedrine, cevadine and protoveratrine A) and four on Dowex 50-X4 (veratrine, ephedrine, spermine and spermidine) thin layers; on Rexyn 102 (H^+) thin layers, only veratrine and ephedrine cannot be detected.

RESULTS AND DISCUSSION

Cation exchangers in the acid form

Alginic acid. In Table I are reported the R_F values of 44 alkaloids on layers of alginic acid, eluting with solutions of pH between 0.60 and 2.35. Table I also indicates the amounts of each alkaloid deposited on the layer. It should be noted that such amounts are generally smaller than those used with anion exchangers[1].

The different alkaloids were classified in order of increasing R_F values on elution at pH 2.35 because at this pH many interesting deductions on the retention of these compounds by the exchanger can be made. In fact, on the basis of the R_F values, it can be stated that the retention of the alkaloids by alginic acid is correlated with the number of positive charges that are present in the molecule of the different compounds at pH 2.35. In accordance with this assertion, all compounds with two or more positive charges at this pH have $R_F \leq 0.08$; those with a completely protonated amino group and a partly protonated second group have R_F values between 0.12 and 0.19. The alkaloids with only one positive charge have R_F values between 0.21 and 0.39 and uncharged alkaloids between 0.69 and 0.91.

The chromatographic behaviour of the alkaloids at different pH values of the eluent is also correlated with the number of positive charges in the molecule. In fact, as the pH is decreased, a remarkable increase in the R_F values is observed for those compounds with two or more positive charges in the molecule, while the corresponding increase for those compounds, such as theobromine, whose basic groups are not protonated in the pH range studied, is negligible.

Fig. 1 shows the R_M *versus* pH curves for some compounds selected on the basis of the number of positive charges. The trends for most alkaloids fit the types of curves in Fig. 1. In particular, alkaloids with two positive charges have an R_M *versus* pH trend similar to curve (a), those with one positive charge to curve (b) and those with no charge to curve (c).

Curve (d) refers to berberine, which is protonated in the whole range explored, and is similar, although to a lesser extent, to the trends for yohimbine, boldine, reserpine, ergocristine, ergotamine and ergonovine. Such trends can be ascribed to the progressive dominance of the adsorption process over the ion-exchange process as the pH of the eluent is decreased. With curves (a) and (b), on the other hand, the

TABLE I

R_F VALUES OF ALKALOIDS ON ALGINIC ACID THIN LAYERS

Acetic acid + hydrochloric acid solutions were used as eluent. Acetic acid concentration, 1 mole/l. Abbreviations: e.s. = elongated spot; n.d. = not determined.

Alkaloid	*pH of eluent*					*Amount (μg)*
	2.35	*2.00*	*1.38*	*1.15*	*0.60*	
Spermine	0.01	0.01	e.s.	e.s.	n.d.	5.0
Spermidine	0.01	0.02	e.s.	0.61	n.d.	5.0
Quinine	0.02	0.06	e.s.	0.69	0.85	0.3
Quinidine sulphate	0.02	0.06	e.s.	0.69	0.85	0.3
Cinchonine hydrochloride	0.03	0.08	0.49	0.74	0.91	0.7
Cinchonidine	0.03	0.08	0.49	0.74	0.91	0.7
Tubocurarine	0.04	0.10	0.49	0.68	0.83	6.0
Ergocristine	0.04	0.13	0.28	0.32	0.36	0.4
Berberine hydrochloride	0.05	0.09	0.13	0.14	0.13	0.01
Emetine hydrochloride	0.05	0.13	0.54	0.75	0.91	4.0
Reserpine	0.06	0.13	0.23	0.26	0.31	0.5
Ergotamine	0.06	0.16	0.28	0.32	0.36	0.4
Sparteine sulphate	0.08	0.22	0.68	0.86	0.96	5.0
Narceine	0.12	0.12	0.10	0.09	0.07	0.25
Ergonovine	0.12	0.18	0.28	0.29	0.25	0.4
Boldine	0.15	0.23	0.41	0.40	0.39	0.4
Yohimbine hydrochloride	0.15	0.28	0 62	0.63	n.d.	3.0
Strychnine	0.17	0.30	0.58	0.65	0.72	5.0
Brucine	0.18	0.31	0.59	0.67	0.75	5.0
Jbogaine	0.19	0.33	0.55	0.62	0.64	0.4
Papaverine	0.21	0.39	0.65	0.75	0.80	0.4
Hydrastine	0.22	0.41	0.67	0.79	0.88	0.7
Narcotine	0.23	0.42	0.68	0.80	0.88	1.5
Ajmaline	0.27	0.46	0.74	0.80	0.81	1.0
Morphine	0.27	0.49	0.77	0.82	0.88	5.0
Lobeline hydrochloride	0.28	0.45	0.72	0.77	0.80	4.0
Scopoline	0.28	0.48	0.78	0.87	0.94	5.0
Tropine	0.29	0.51	0.80	0.88	0.94	7.0
Ethylmorphine	0.29	0.51	0.80	0.87	0.92	5.0
Cocaine	0.30	0.51	0.80	0.87	0.92	7.0
Scopolamine hydrochloride	0.31	0.52	0.80	0.88	0.91	5.0
Eserine sulphate	0.32	0.56	0.83	0.92	0.96	2.5
Arecoline hydrochloride	0.33	0.56	0.82	0.91	0.94	7.0
Atropine	0.33	0.56	0.82	0.91	0.94	7.0
Hyoscyamine	0.33	0.56	0.82	0.91	0.94	7.0
Homatropine	0.33	0.56	0.82	0.91	0.94	7.0
Eucatropine hydrochloride	0.33	0.56	0.82	0.91	0.94	7.0
Hyoscyne	0.34	0.56	0.86	0.91	0.94	7.0
Prostigmine	0.39	0.61	0.89	0.94	0.96	7.0
Theophylline	0.69	0.70	0.73	0.72	0.76	5.0
Aminophylline	0.69	0.70	0.73	0.72	0.76	5.0
Theobromine	0.76	0.77	0.76	0.76	0.76	5.0
Caffeine	0.81	0.82	0.83	0.81	0.84	5.0
Colchicine	0.91	0.91	0.93	0.91	0.89	3.0

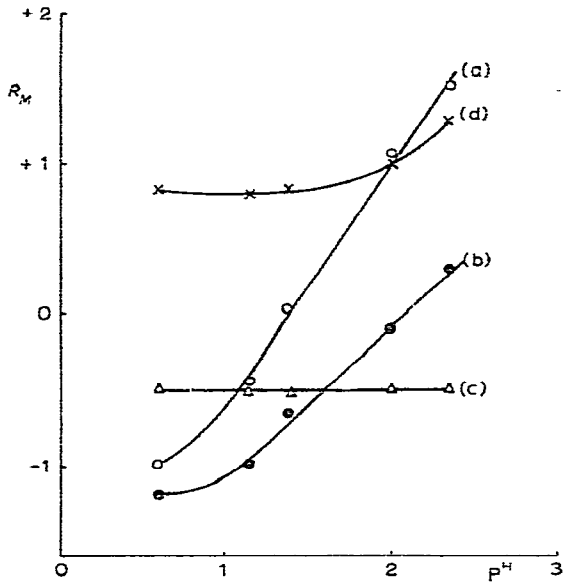

Fig. 1. R_M *versus* pH curves for some alkaloids on alginic acid thin layers. (a) Cinchonine hydrochloride; (b) arecoline hydrochloride; (c) theobromine; (d) berberine hydrochloride.

ion-exchange process prevails over the whole pH range, with the exception of pH 0.60. The slopes of the linear portions of curves (a) and (b) are 1.64 and 1.02, respectively, in good agreement with the presence on the layer of a divalent ion in the first instance and a monovalent ion in the second.

The trend of curve (c), whose slope is zero, indicates that in the pH range studied theobromine and the other alkaloids that show the same chromatographic behaviour are uncharged and therefore their retention mechanism is controlled by the adsorption process. From a comparison of curves (c) and (d), a different dependence of the adsorption process on the pH, depending on the form of alkaloid (protonated or unprotonated), is indicated. Such an occurrence agrees with the results obtained for phenols under comparable experimental conditions[3]. For phenols, in fact, it was shown that the adsorption of the phenate ion is more affected than the corresponding neutral form by the increase in the ionic strength of the eluent.

The use of water–alcohol mixtures usually does not result in improved separations of alkaloids in comparison with the above aqueous eluents. In fact, with 1:1 (v/v) methanol–water mixtures, most alkaloids remain at the application point ($R_F \leq 0.11$), with the exception of aminophylline and theophylline ($R_F = 0.70$), theobromine ($R_F = 0.71$), narceine ($R_F = 0.78$), caffeine ($R_F = 0.79$) and colchicine ($R_F = 0.96$), so that these five can therefore be separated from other alkaloids. With higher concentrations of methanol in the aqueous solvent (70–80%), the weakly basic alkaloids exhibit R_F values above 0.80, while those with more marked basic characteristics (brucine, strychnine, berberine, quinine, cinchonine, etc.) are retained more strongly, but give rise to elongated spots. Similar behaviour is observed when methanol is replaced by ethanol.

Analytical applications of alginic acid layers. Many separations can be effected on the basis of the R_F values listed in Table I. As an example, Fig. 2 shows the separation of nine alkaloids, eluting with a solution of pH 2.00 that was 1 *M* in acetic acid and 0.01 *M* in hydrochloric acid.

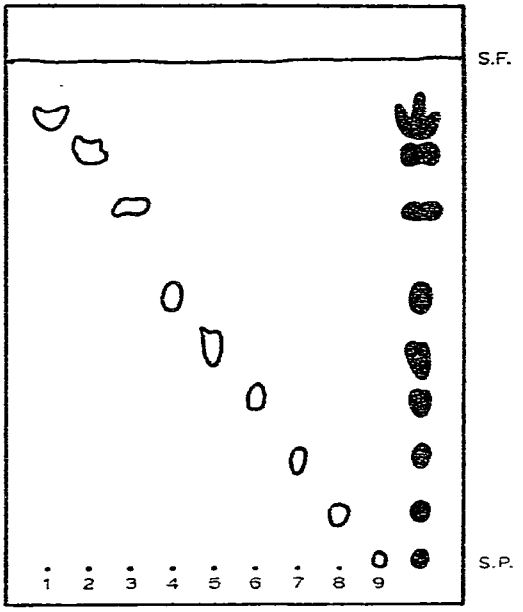

Fig. 2. Thin-layer chromatogram of alkaloids on alginic acid. Eluent: 1 *M* acetic acid + 0.01 *M* hydrochloric acid (pH = 2.00). Migration distance: 13.5 cm. White spots: 1 = colchicine; 2 = caffeine; 3 = theophylline; 4 = tropine; 5 = papaverine; 6 = strychnine; 7 = ergonovine; 8 = berberine hydrochloride; 9 = spermine. Black spots: mixtures. S.P. = starting point; S.F. = solvent front.

Rexyn 102 (H^+). Table II gives the R_F values of 46 alkaloids on Rexyn 102 (H^+) layers, eluting with aqueous solutions at three different pH values. The compounds were classified in order of increasing R_F values on elution at pH 1.00.

On this exchanger the alkaloids are retained more strongly than on alginic acid owing to the stronger adsorption by the paraffin matrix than by the cellulose matrix. Also, the R_F sequence is different from that observed on alginic acid, as adsorption is the prevailing parameter that determines the retention of these compounds on Rexyn 102 (H^+). This effect leads to new separations among the alkaloids, as the degree of adsorption is correlated with the structure of the compound. From the data in Table II, it should also be noted that at pH 0.30 many alkaloids are retained more strongly than at higher pH values, indicating that the retention of these compounds is associated with interactions between the matrix of the exchanger and the individual compound.

The increase in the R_F values when ethanol is added to the eluent is in good agreement with an adsorption process. Table II gives the R_F values obtained on eluting with 1 *M* acetic acid in water–ethanol mixtures (containing 30 and 50% of

TABLE II

R_F VALUES OF ALKALOIDS ON REXYN 102 (H^+) THIN LAYERS

Eluents: (a) 1 *M* acetic acid + hydrochloric acid solutions; (b) 1 *M* acetic acid in water–ethanol mixtures.

Alkaloid	*(a) pH of eluent*			*(b) Ethanol concentration (%)*	
	2.35	*1.00*	*0.30*	*30*	*50*
Berberine hydrochloride	0.00	0.00	0.01	0.02	0.09
Reserpine	0.00	0.02	0.02	0.04	0.20
Ergocristine	0.00	0.02	0.02	0.06	0.30
Narceine	0.03	0.02	0.02	0.46	0.78
Ergotamine	0.01	0.05	0.04	0.08	0.25
Papaverine	0.02	0.06	0.04	0.16	0.29
Tubocurarine	0.01	0.07	0.06	0.11	0.15
Jbogaine	0.01	0.08	0.06	0.12	0.30
Colchicine	0.08	0.10	0.14	0.42	0.65
Lobeline hydrochloride	0.02	0.11	0.07	0.12	0.32
Boldine	0.02	0.11	0.10	0.25	0.41
Ergonovine	0.02	0.12	0.10	0.15	0.27
Hydrastine	0.02	0.15	0.13	0.16	0.27
Strychnine	0.03	0.17	0.14	0.20	0.28
Brucine	0.03	0.17	0.14	0.23	0.30
Yohimbine hydrochloride	0.03	0.18	0.10	0.18	0.23
Cevadine	0.04	0.18	0.12	0.27	0.42
Emetine hydrochloride	0.01	0.22	0.19	0.18	0.25
Narcotine	0.05	0.22	0.19	0.21	0.30
Ajmaline	0.08	0.22	0.28	0.25	0.39
Cocaine	0.07	0.26	0.20	0.26	0.44
Hyoscyne	0.07	0.27	0.24	0.24	0.39
Protoveratrine A	0.06	0.27	0.24	0.24	0.51
Eserine sulphate	0.12	0.36	0.37	0.37	0.47
Caffeine	0.28	0.36	0.42	0.55	0.67
Theophylline	0.35	0.40	0.53	0.58	0.68
Aminophylline	0.35	0.40	0.54	0.58	0.68
Ethylmorphine	0.13	0.42	0.37	0.34	0.46
Theobromine	0.36	0.42	0.46	0.60	0.66
Quinine	0.02	0.43	0.36	0.13	0.19
Atropine	0.14	0.43	0.42	0.36	0.47
Hyoscyamine	0.14	0.43	0.42	0.36	0.46
Eucatropine hydrochloride	0.14	0.43	0.42	0.36	0.46
Quinidine sulphate	0.02	0.45	0.57	0.12	0.21
Prostigmine	0.17	0.47	0.47	0.36	0.45
Homatropine	0.18	0.53	0.53	0.40	0.50
Cinchonine hydrochloride	0.03	0.55	0.46	0.15	0.20
Cinchonidine	0.03	0.55	0.47	0.14	0.22
Scopolamine hydrochloride	0.21	0.59	0.51	0.42	0.51
Morphine	0.23	0.62	0.57	0.43	0.52
Arecoline hydrochloride	0.33	0.76	0.77	0.46	0.54
Sparteine sulphate	0.18	0.85	0.77	0.30	0.34
Scopoline	0.42	0.86	0.82	0.53	0.53
Tropine	0.42	0.86	0.83	0.53	0.52
Spermine	0.06	0.97	0.97	0.12	0.07
Spermidine	0.11	0.97	0.97	0.26	0.18

ethanol). Higher ethanol concentrations cannot be used as the layer disgregates during the elution. On comparing the R_F values in the last two columns in Table II, it should be noted that the R_F values are greater at the higher ethanol concentration, with the exception of spermine and spermidine, whose R_F values are lower at the higher ethanol concentration, and scopoline and tropine, whose R_F values do not change. With spermine and spermidine, the presence in the molecule of three and four protonated amino groups, respectively, decreases their solubility in aqueous–organic mixtures. The behaviour of scopoline and tropine, which is different from that of the other alkaloids of the tropane group, can be ascribed to the presence in their molecules of a free hydroxyl group, which in the other tropane alkaloids is esterified by an aromatic acid. The presence of a free hydroxyl group involves a higher polarity and a smaller size; both such occurrences limit the solubility of scopoline and tropine in water–alcohol mixtures. The addition of a mineral acid to these aqueous–organic mixtures usually involves an increase in the R_F values; this increase is more marked for alkaloids with smaller R_F values and therefore it causes a levelling of the chromatographic behaviour of these compounds.

On eluting with water–alcohol mixtures without mineral or organic acids present, the alkaloids behave in the same manner as on alginic acid under the same experimental conditions. In particular, most compounds remain at the application point, with the exception of colchicine, narceine and the four purine alkaloids. The replacement of ethanol with dimethylformamide does not result in marked differences in the R_F values until a 1:1 water:dimethylformamide ratio is reached. Solutions with higher proportions of dimethylformamide can still be used as eluents, in a different manner to water–ethanol mixtures. With 1:2 water–dimethylformamide, for instance, many alkaloids move to different extents from the application point. With this eluent, however, those alkaloids which react with Dragendorff reagent cannot be detected, which is a serious limitation to the use of such mixtures.

Analytical applications of Rexyn 102 (H^+) layers. Many interesting separations are possible on the basis of the R_F values in Table II, and we carried out the following: tropine/atropine and quinine/cinchonine (1 *M* CH_3COOH + 0.1 *M* HCl, pH 1.00); spermine/spermidine, ergocristine/ergonovine and narceine from papaverine, hydrastine and narcotine (1 *M* CH_3COOH in 7:3 water–ethanol). Fig. 3 shows two separations of four of the more important opium and tropane alkaloids, eluting with a solution of pH 1.00 that was 1 *M* in acetic acid and 0.1 *M* in hydrochloric acid.

Dowex 50-X4 (H^+). On Dowex 50-X4 (H^+) layers, the retention of the alkaloids is more marked than on alginic acid and Rexyn 102 (H^+) even if more concentrated hydrochloric acid solutions are used as eluents. As shown by the data in Table III, on eluting with 1 *M* hydrochloric acid, most alkaloids remain at the application point, except for arecoline, the three alkaloids of the tropane group (scopoline, tropine and scopolamine) and the four purine alkaloids.

If large amounts of ethanol are added to the eluent, many alkaloids move from the application point. It should be noted, however, that even with 80% of ethanol (Table III), the R_F values of most compounds are less than 0.40, except for colchicine ($R_F = 0.58$), cevadine ($R_F = 0.65$) and narceine ($R_F = 0.95$), which can therefore be separated from the other compounds.

On replacing ethanol with dimethylformamide, the alkaloids move from the

TABLE III

R_F VALUES OF ALKALOIDS ON DOWEX 50-X4 (H^+) THIN LAYERS

Abbreviation: e.s. = elongated spot.

Alkaloid	*Eluent*		
	1 M HCl in H_2O	*1 M HCl in H_2O–C_2H_5OH (1:1)*	*1 M HCl in H_2O–C_2H_5OH (1:4)*
Quinine	0.00	0.01	0.01
Quinidine sulphate	0.00	0.01	0.01
Cinchonine hydrochloride	0.00	0.01	0.01
Cinchonidine	0.00	0.01	0.01
Emetine hydrochloride	0.00	0.02	0.05
Sparteine sulphate	0.04	0.03	0.04
Tubocurarine	0.00	0.04	0.04
Berberine hydrochloride	0.00	0.04	0.11
Reserpine	0.00	0.05	0.33
Ergocristine	0.01	0.06	e.s.
Ajmaline	0.01	0.06	0.18
Jbogaine	0.00	0.06	0.27
Strychnine	0.01	0.10	0.23
Eserine sulphate	0.01	0.10	0.30
Ergotamine	0.00	0.11	e.s.
Yohimbine hydrochloride	0.02	0.11	0.26
Papaverine	0.00	0.11	0.32
Ergonovine	0.05	0.12	0.21
Lobeline hydrochloride	0.01	0.13	0.34
Brucine	0.01	0.14	0.25
Hydrastine	0.01	0.16	e.s.
Boldine	0.00	0.17	0.31
Ethylmorphine	0.04	0.19	0.28
Hyoscyne	0.03	0.19	0.30
Narcotine	0.02	0.20	0.36
Cocaine	0.05	0.21	0.25
Protoveratrine A	0.08	n.d.	n.d.
Arecoline hydrochloride	0.17	0.22	0.20
Prostigmine	0.13	0.25	0.24
Morphine	0.13	0.27	0.27
Scopoline	0.32	0.29	0.17
Caffeine	0.20	0.29	0.30
Eucatropine hydrochloride	0.06	0.29	0.39
Tropine	0.28	0.30	0.23
Scopolamine hydrochloride	0.18	0.30	0.37
Atropine	0.07	0.30	0.40
Hyoscyamine	0.08	0.31	0.40
Homatropine	0.10	0.31	0.40
Aminophylline	0.28	0.32	0.31
Theophylline	0.29	0.33	0.33
Theobromine	0.24	0.38	0.25
Cevadine	0.03	0.39	0.65
Colchicine	0.01	0.48	0.58
Narceine	0.01	0.61	0.95

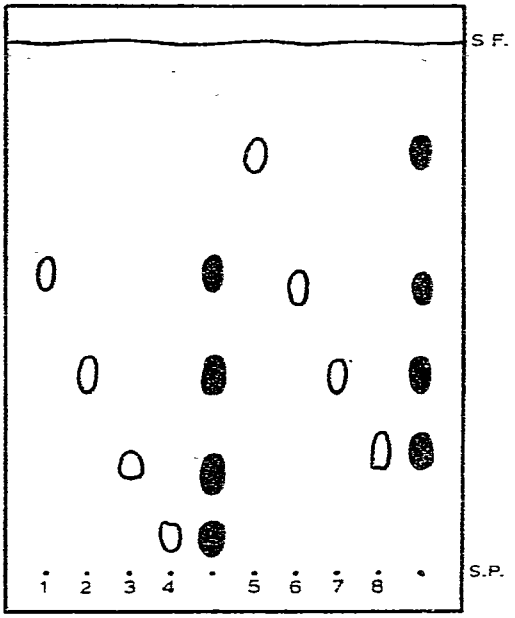

Fig. 3. Thin-layer chromatogram of alkaloids on Rexyn 102 (H^+). Eluent: 1 *M* acetic acid + 0.1 *M* hydrochloric acid (pH = 1.00). Migration distance: 14 cm. White spots: 1 = morphine; 2 = ethylmorphine; 3 = narcotine; 4 = papaverine; 5 = scopoline; 6 = scopolamine hydrochloride; 7 = atropine; 8 = cocaine. Black spots: mixtures. S.P. = starting point; S.F. = solvent front.

application point; however, elongated spots are usually obtained and, as on Rexyn 102 (H^+), Dragendorff reagent cannot be employed.

TABLE IV

R_F VALUES OF ALKALOIDS ON CMCNa THIN LAYERS

Eluent: 0.5 *M* acetate buffer.

Alkaloid	R_F	*Alkaloid*	R_F
Boldine	0.17	Papaverine	0.67
Berberine hydrochloride	0.23	Hydrastine	0.71
Reserpine	0.24	Narcotine	0.72
Ergocristine	0.29	Sparteine sulphate	0.72
Ergotamine	0.29	Ajmaline	0.73
Narceine	0.36	Lobeline hydrochloride	0.75
Ergonovine	0.38	Eserine sulphate	0.87
Quinine	0.53	Ethylmorphine	0.89
Quinidine sulphate	0.53	Scopoline	0.90
Cinchonine hydrochloride	0.54	Tropine	0.91
Cinchonidine	0.54	Theobromine	0.94
Brucine	0.56	Caffeine	0.95
Yohimbine hydrochloride	0.57	Theophylline	0.95
Ibogaine	0.58	Aminophylline	0.95
Strychnine	0.64	Colchicine	0.95

Cation exchangers in the sodium salt form

The results achieved with the cation exchangers in the sodium salt form are worse than those with the corresponding exchangers in the acid form, in terms of both the compactness of the spots and the separation possibilities. Such behaviour was checked on Rexyn 102 (N^+) and Dowex 50-X4 (Na^+), eluting with acetate buffer and water–ethanol mixtures. It was not possible, however, to compare the results on alginic acid and sodium alginate, as the latter gelatinizes in aqueous solutions.

Interesting results were achieved on CMCNa, the matrix of which is similar to that of alginic acid. Table IV gives the R_F values of 30 alkaloids on layers of CMCNa, eluting with 0.5 *M* acetate buffer. Fower alkaloids were studied on this exchanger than on alginic acid, Rexyn 102 (H^+) and Dowex 50-X4 (H^+) layers because only a few compounds react with Dragendorff reagent. The R_F sequence is different from those obtained on alginic acid and paraffin- or polystyrene-based exchangers in the sodium salt form, which suggests new possibilities for the separation of the alkaloids.

The difference between the chromatographic behaviour of the alkaloids on alginic acid and CMCNa can be ascribed to the higher pH of the eluent used with the latter exchanger.

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