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HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY OF NITROGEN COMPOUNDS ON LAYERS OF RP-18 AND SIL C_{18}-50 UNTREATED OR IMPREGNATED WITH DODECYLBENZENESULPHONIC ACID AND ON AMMONIUM TUNGSTOPHOSPHATE

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SUMMARY

The chromatographic characteristics of amino acids, peptides, amino sugars, aliphatic mono- and polyamines on ready-for-use plates of RP-18 and SIL C_{18}-50 untreated or impregnated with dodecylbenzenesulphonic acid and on layers of ammonium and tetramethylammonium tungstophosphate have been investigated using water–alcohol mixtures at different pH and ionic strengths or aqueous solutions of nitric acid, ammonium nitrate and tetramethylammonium bromide as eluents. On plates of silanized silica gel impregnated with the anionic detergent, the retention of peptides is governed chiefly by an ion-exchange mechanism. On ammonium tungstophosphate (AWP) layers, the retention of aliphatic diamines and peptides increases with increasing distance between the two $-NH_3^+$ groups or the $-NH_3^+$ group and the carboxylic group. For these reasons the AWP layers allow the separation of several nitrogen-containing compounds which exhibit similar chromatographic behaviour on RP-18 and SIL C_{18}-50 plates.

INTRODUCTION

Home-made layers of silanized silica gel (C_2) untreated or impregnated with anionic and cationic detergents[1–7], and also layers of ammonium tungstophosphate (AWP)[8–10], have been employed for the separation of nitrogen-containing organic compounds. The use of AWP layers is particularly promising since on this exchanger different affinity sequences in comparison with silanized silica gel were found.

Recently, ready-for-use plates of silanized silica gel untreated or impregnated with detergents[11–16] were reported for the separation of amino acids and peptides but, in spite of their attractive features, reversed-phase high-performance thin-layer chromatography (HPTLC) is still little used.

It was therefore of interest to investigate a greater number of nitrogen-containing compounds on RP-18 and SIL C_{18}-50 plates impregnated with dodecylbenzenesulphonic acid (HDBS) in order to obtain a more complete picture of the separations of closely related compounds, to compare the results with those obtained on home-made layers and to understand the retention mechanism on the different materials.

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Such nitrogen compounds are amino acids, di- and polypeptides, amino sugars (including two antibiotics with aminoglycosidic structures), aliphatic mono- and polyamines. Particular attention was directed to the separation of the compounds formed in the enzymatic fragmentation of the model peptide Leu-Trp-Met-Arg-Phe-Ala.

EXPERIMENTAL

The standard solutions (1–2 mg/ml and 4 mg/ml in the case of 2-aminoisobutyric acid) were prepared by dissolving the different compounds (Serva, Heidelberg, G.F.R.) in water–methanol (40:60).

The sample volume used was 0.2 μl in the case of RP-18 (E. Merck, Darmstadt, G.F.R.) and SIL C_{18}-50 (Macherey, Nagel & Co., Düren, G.F.R.) plates and 0.5 μl for the ammonium tungstophosphate layers. The spots were visualized by spraying the layers with an 1 % ninhydrin solution in pyridine–acetic acid (5:1, v/v) and heating the plates for 5 min at 100°C. The RP-18 and SIL C_{18}-50 plates were impregnated as described previously[11,12], and the preparation of AWP and its layers was performed as described previously[9].

Tetramethylammonium tungstophosphate (TMAWP) was prepared in the same way as AWP using a tetramethylammonium nitrate solution as precipitant.

The migration distance was 6 cm in the case of the ready-for-use plates and 10 cm in the case of the inorganic exchanger, unless otherwise stated. The chromatographic measurements were carried out at 25°C.

RESULTS AND DISCUSSION

Model peptide, its fragments and amino sugars

Plates of RP-18 untreated or impregnated with 4% HDBS

Table I lists the chromatographic characteristics of the hexapeptide and its twelve most important fragments, of the three main amino sugars and of two antibiotics with aminoglycosidic structures on layers of silanized silica gel untreated or impregnated with 4% HDBS. Aqueous–organic eluents at different ionic strengths and hydrogen-ion concentrations were used.

On RP-18 plates, eluting with 1 *M* acetic acid in 60 % methanol, the two amino acids and the dipeptides Met-Arg and Phe-Ala are slightly retained and may be separated from all the other peptides. Among these compounds a peculiar behaviour is shown by the hexapeptide which is less strongly retained than the pentapeptide. The three amino sugars run with the solvent front, while the two antibiotics are very strongly retained. When potassium chloride is added to the eluent a general increase of the R_F values and a change in the affinity sequence of many peptides are observed. Under these elution conditions, the two antibiotics can be detected only with difficulty, since they give rise to elongated spots.

In order to demonstrate the influence of the polarity of the amino acids and peptides on their chromatographic behaviour, Table I also lists the values of the Hansch hydrophobicity parameter, π, obtained by Pliška *et al.*[17] from TLC measurements. In the case of peptides, this parameter was calculated by adding up the hydrophobicity constants of the constituent amino acid side-chains. The π values of tryptophan and arginine refer to the charged species.

TABLE I

R_F VALUES OF AMINO SUGARS, OF THE MODEL PEPTIDE AND ITS FRAGMENTS ON PLATES OF RP-18 UNTREATED OR IMPREGNATED WITH 4% HDBS

Eluents: a, 1 *M* acetic acid in 60% methanol; b, 1 *M* acetic acid + 3% KCl in 60% methanol; c, 1 *M* acetic acid + 0.5 *M* HCl in 60% methanol; d, 1 *M* acetic acid + 0.5 *M* HCl + 3% KCl in 60% methanol; e, 0.5 *M* NH_3 + 0.5 *M* NH_4Cl in 60% methanol. π = Hansch hydrophobic parameter (see text); n.d. = not determined.

Compound	*RP-18*		π	*RP-18 + 4% HDBS*				
	a	*b*		*a*	*b*	*c*	*d*	*e*
Ala	0.95	0.95	0.40	0.27	0.71	0.70	0.75	0.84
Leu	0.88	0.90	1.64	0.11	0.54	0.46	0.57	0.72
Met-Arg	0.92	0.95	0.52	0.00	0.50	0.28	0.62	0.74
Arg-Phe	0.58	0.89	0.73	0.00	0.30	0.16	0.43	0.67
Phe-Ala	0.92	0.89	2.03	0.13	0.60	0.53	0.66	0.86
Leu-Trp	0.72	0.74	2.47	0.08	0.44	0.40	0.50	0.72
Arg-Phe-Ala	0.63	0.91	1.13	0.00	0.30	0.19	0.43	0.69
Met-Arg-Phe	0.58	0.79	2.15	0.00	0.19	0.15	0.31	0.62
Leu-Trp-Met	0.61	0.63	3.89	0.05	0.34	0.35	0.41	0.75
Met-Arg-Phe-Ala	0.65	0.84	2.55	0.00	0.26	0.18	0.38	0.61
Leu-Trp-Met-Arg	0.60	0.84	2.99	0.00	0.25	0.15	0.38	0.42
Leu-Trp-Met-Arg-Phe	0.34	0.51	4.62	0.00	0.10	0.06	0.16	0.32
Leu-Trp-Met-Arg-Phe-Ala	0.43	0.57	5.02	0.00	0.10	0.06	0.19	0.26
Glucosamine	0.94	0.97	–	0.41	0.75	0.78	0.83	0.79
Galactosamine	0.95	0.97	–	0.37	0.75	0.76	0.81	0.71
Mannosamine	0.95	0.97	–	0.40	0.76	0.77	0.83	0.76
Kanamycin	0.06	n.d.	–	0.00	0.13	0.06	0.33	0.36
Gentamycin	0.02	n.d.	–	0.00	0.06	0.02	0.18	0.21

From a comparison of the R_F values of the model peptide and its fragments with the π values, some differences in the two sequences can be found. These differences are observed with both eluents (a and b, Table I); in particular, the pentapeptide is more strongly retained than the hexapeptide while Leu-Trp and Met-Arg-Phe are more strongly retained than the two tetrapeptides notwithstanding their lower π values. This demonstrates that the hydrophobic part of the molecule is not the sole factor determining the retention of the polypeptides; their retention, in fact, is correlated also with the charge and its position on the polypeptide, besides the conformation of the macromolecule in the solution, as already shown in the case of angiotensins[13].

On eluting with the solution containing potassium chloride, there is a good agreement between the sequences of the R_F values and the π values within each group (*i.e.*, amino acids, dipeptides, tripeptides, etc.). The different behaviour which can be observed in the absence of the salt must be ascribed to electrostatic interactions with the silanol groups of the stationary phase.

On RP-18 plates impregnated with dodecylbenzenesulphonic acid, under the above elution conditions, a remarkable decrease of the R_F values is observed for all the compounds.

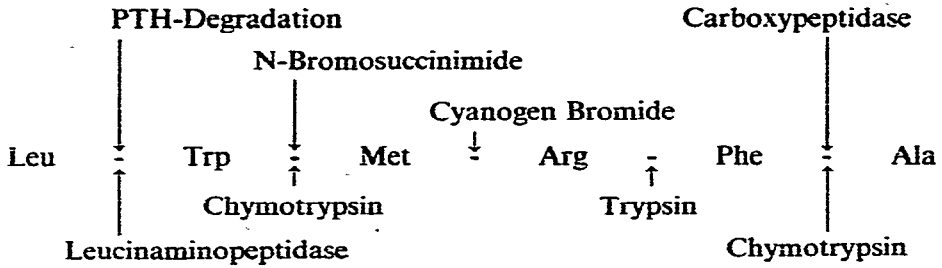
In the absence of potassium chloride in the eluent, the most strongly retained peptides are those containing arginine owing to their marked ionic interactions with the functional group of the detergent adsorbed on the stationary phase. As the acid

concentration in the eluent is increased, an increase of the R_F values and a marked differentiation among the different fragments are observed. The two antibiotics are well separated on eluting with 1 *M* acetic acid + 0.5 *M* hydrochloric acid + 3% potassium chloride in 60% methanol.

An ion-exchange process between the compounds and the detergent sorbed on the layer is indicated by the $R_M/-\log[H^+]$ trends in the 0.3–1 *M* hydrochloric acid concentration range. Straight lines are obtained with slopes between 1.20 and 1.24 in the case of peptides containing arginine, and between 0.64 and 0.71 in the other cases. Such values show that two positive charges are involved in the exchange process of the arginylpeptides and only one positive charge in the other fragments[1–7].

The behaviour of these compounds with an alkaline eluent is also of interest. In this case the two tetrapeptides are well separated and the hexapeptide is more strongly retained than the pentapeptide. Even the amino sugars are separated and galactosamine can be separated from glucosamine.

Separation of the model peptide fragments. The main fragments which are obtained by enzymatic degradation of the hexapeptide are illustrated in the following scheme:



The fragments formed by the different enzymes were separated on RP-18 plates impregnated with 4% HDBS on eluting with 1 *M* acetic acid + 0.5 *M* hydrochloric acid + 3% potassium chloride in 60% methanol (Fig. 1). All the six separations of

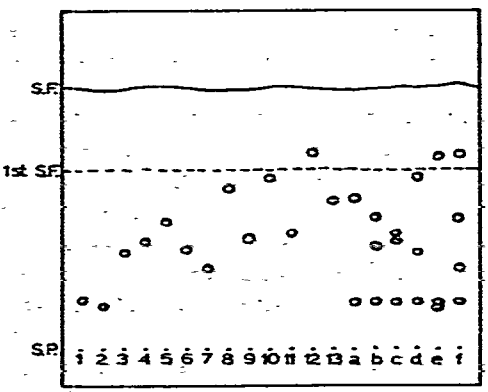


Fig. 1. Thin-layer chromatogram of model peptide and its fragments on RP-18 impregnated with 4% HDBS solution. Migration distance = 7 cm. Eluent: 0.5 *M* hydrochloric acid + 3% potassium chloride in water–methanol (60%)–acetic acid (5.7%). 1 = Leu-Trp-Met-Arg-Phe-Ala; 2 = Leu-Trp-Met-Arg-Phe; 3 = Leu-Trp-Met-Arg; 4 = Leu-Trp-Met; 5 = Leu-Trp; 6 = Met-Arg-Phe-Ala; 7 = Met-Arg-Phe; 8 = Met-Arg; 9 = Arg-Phe; 10 = Phe-Ala; 11 = Arg-Phe-Ala; 12 = Ala; 13 = Leu. a = Mixture of 1,13; b = mixture of 1,5,6; c = mixture of 1,4,11; d = mixture of 1,3,10; e = mixture of 1,2,12; f = mixture of 1,5,7,12. S.P. = Starting point; 1st S.F. = first solvent front; S.F. = solvent front.

the different fragments include also the hexapeptide. On the basis of these data, the specific activity of the enzymes can easily be elucidated.

An increase of the percentage of potassium chloride in the eluent to 4% did not result in a change in the R_F sequence but did cause a more marked separation between the hexa- and the pentapeptide.

Layers of AWP and TMAWP

Table II lists the chromatographic data for the hexapeptide and its fragments on layers of AWP + $CaSO_4 \cdot \frac{1}{2}H_2O$ (2:2 and 4:2, w/w) and of TMAWP + $CaSO_4 \cdot \frac{1}{2}H_2O$ (4:2) on eluting with aqueous salt solutions. Inorganic exchanger calcium sulphate hemihydrate ratios smaller than 2:2 are not advisable since the resulting spots are diffuse even when spherical.

In the case of AWP layers, elution with 4 *M* ammonium nitrate yields the highest R_F values together with the best separations of the fragments. The retention of the amino acids and peptides on this support increases with the number of amino acid residues. This behaviour, already observed in the case of glycine and alanine oligomers[10], seems to be of general interest since it determines also the retention of peptides formed by different amino acids. It must be ascribed to the greater distance between the negative charge of the carboxylic group and the positive charge (or charges) of the amino group (or groups) as the peptidic chain increases.

The amino sugars are very weakly retained ($R_F > 0.8$), while the two antibiotics cannot be detected under these elution conditions. With 1 *M* ammonium nitrate as eluent, mannosamine ($R_F = 0.53$) can be separated from the other two amino sugars ($R_F = 0.60$).

On layers of TMAWP, elution with 1 *M* tetramethylammonium bromide gen-

TABLE II

R_F VALUES OF MODEL PEPTIDE AND ITS FRAGMENTS ON THIN LAYERS OF AWP + $CaSO_4 \cdot \frac{1}{2}H_2O$ AND TMAWP + $CaSO_4 \cdot \frac{1}{2}H_2O$ IN DIFFERENT RATIOS

Eluents: a, 4 *M* ammonium nitrate; b, 1 *M* tetramethylammonium bromide.

Compound	*AWP + $CaSO_4 \cdot \frac{1}{2}H_2O$*		*TMAWP + $CaSO_4 \cdot \frac{1}{2}H_2O$*
	2:2 *a*	*4:2* *a*	*4:2* *b*
Ala	0.92	0.92	0.90
Leu	0.82	0.82	0.87
Met-Arg	0.33	0.25	0.66
Arg-Phe	0.23	0.19	0.48
Phe-Ala	0.65	0.61	0.82
Leu-Trp	0.33	0.27	0.38
Arg-Phe-Ala	0.12	0.08	0.50
Met-Arg-Phe	0.08	0.05	0.22
Leu-Trp-Met	0.14	0.11	0.21
Met-Arg-Phe-Ala	0.06	0.04	0.22
Leu-Trp-Met-Arg	0.03	0.02	0.11
Leu-Trp-Met-Arg-Phe	0.00	0.00	0.04
Leu-Trp-Met-Arg-Phe-Ala	0.00	0.00	0.00

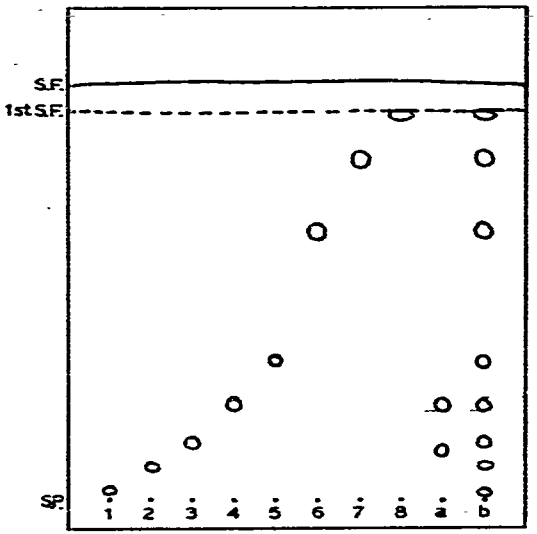


Fig. 2. Thin-layer chromatogram of model peptide fragments on AWP + $CaSO_4 \cdot \frac{1}{2}H_2O$ (2:2 w/w). Migration distance = 11 cm. Eluent: 4 *M* NH_4NO_3. 1 = Leu-Trp-Met-Arg; 2 = Met-Arg-Phe; 3 = Leu-Trp-Met; 4 = Arg-Phe; 5 = Leu-Trp; 6 = Phe-Ala; 7 = Leu; 8 = Ala. a = Mixture of Arg-Phe and Arg-Phe-Ala; b = mixture of 1–8. Other details as in Fig. 1.

erally yields less compact spots and a lower retention with respect to AWP. The R_F sequence on the two exchangers is different and must be ascribed to the tetramethylammonium ion which exhibits a higher affinity than the ammonium ion towards the tungstophosphate anion and confers upon the layer hydrophobic characteristics owing to its methyl groups. The latter property accounts for the reversal in sequence between Leu-Trp (more hydrophobic) and Arg-Phe and, overall, for the higher retention of Leu-Trp-Met with respect to the other tripeptides containing arginine (more polar).

On this exchanger, neither the amino sugars nor the antibiotics can be detected under these elution conditions.

Separations on layers of AWP + $CaSO_4 \cdot \frac{1}{2}H_2O$. Besides the above separations of the amino sugars, on this exchanger the simultaneous separation of eight fragments of the model peptide has been carried out (see Fig. 2). It should be noted that the marked separation between Arg-Phe and Arg-Phe-Ala cannot be obtained on RP-18 plates untreated or impregnated with HDBS.

Aliphatic mono- and polyamines and closely related amino acids

Plates of RP-18 and SIL C_{18}-50 untreated or impregnated with 4% HDBS

In Table III are reported the chromatographic characteristics of fourteen primary aliphatic amines, ten polyamines and nine amino acids on plates of RP-18 and SIL C_{18}-50 untreated or impregnated with 4% HDBS, eluting with aqueous–organic eluents at different ionic strengths and hydrogen-ion concentrations. On untreated RP-18, eluting with 1 *M* acetic acid in 60% methanol, most compounds are very

TABLE III

R_F VALUES OF PRIMARY MONO- AND DIAMINES AND AMINO ACIDS ON PLATES OF RP-18 AND SIL C_{18}-50 UNTREATED OR IMPREGNATED WITH 4% HDBS AND ON THIN LAYERS OF AWP + $CaSO_4 \cdot \frac{1}{2}H_2O$ (4:2)

Eluents: a, 1 *M* acetic acid in 60% methanol; b, 1 *M* acetic acid + 1 *M* HCl in 60% methanol; c, 1 *M* acetic acid + 1 *M* HCl in 30% methanol; d, 1 *M* HNO_3; e, 2 *M* NH_4NO_3. e.s. = Elongated spot.

Compound	*RP-18*	*RP-18 + 4% HDBS*		*SIL C_{18}-50 + 4% HDBS*	*AWP + $CaSO_4 \cdot \frac{1}{2}H_2O$*	
	a	*a*	*b*	*c*	*d*	*e*
Methylamine	0.75	0.18	0.68	0.92	0.30	0.69
Ethylamine	0.76	0.16	0.66	0.85	0.42	0.75
n-Butylamine	0.75	0.10	0.50	0.55	0.23	0.65
Isobutylamine	0.76	0.11	0.53	0.62	0.34	0.70
n-Amylamine	0.70	0.07	0.39	0.34	0.20	0.60
Isoamylamine	0.70	0.08	0.41	0.45	0.19	0.62
n-Hexylamine	0.67	0.05	0.30	0.24	0.16	0.53
n-Heptylamine	0.65	0.04	0.23	0.14	0.02	e.s.
n-Octylamine	0.59	0.03	0.17	0.07	0.00	0.03
n-Decylamine	e.s.	0.03	0.09	0.01	0.00	0.00
n-Dodecylamine	e.s.	0.01	0.03	0.00	0.00	0.00
n-Tetradecylamine	e.s.	0.00	0.00	0.00	0.00	0.00
1-Phenylethylamine	0.75	0.11	0.41	0.37	0.07	0.41
2-Phenylethylamine	0.75	0.09	0.37	0.28	0.06	0.36
1,2-Diaminoethane	0.50	0.02	0.51	0.82	0.05	0.49
1,2-Diaminopropane	0.54	0.02	0.50	0.81	0.07	0.58
1,3-Diaminopropane	0.54	0.02	0.51	0.84	0.02	0.36
1,4-Diaminobutane	0.57	0.01	0.51	0.84	0.02	0.32
1,5-Diaminopentane	0.59	0.02	0.52	0.85	0.01	0.26
1,6-Diaminohexane	0.62	0.03	0.51	0.79	0.01	0.19
1,7-Diaminoheptane	0.63	0.04	0.47	0.72	0.00	0.17
1,8-Diaminooctane	0.62	0.04	0.42	0.56	0.00	0.16
Spermidine	0.29	0.02	0.34	0.81	0.00	0.10
Spermine	0.12	0.00	0.23	0.72	0.00	0.02
2-Aminoisobutyric acid	0.92	0.27	0.76	0.81	0.63	0.84
2-Aminobutyric acid	0.92	0.23	0.75	0.82	0.50	0.82
3-Aminoisobutyric acid	0.87	0.20	0.75	0.84	0.29	0.68
4-Aminobutyric acid	0.88	0.24	0.79	0.89	0.20	0.60
2,4-Diaminobutyric acid	e.s.	0.03	0.61	0.91	0.04	0.55
n-Leucine	0.84	0.09	0.54	0.49	0.39	0.74
ε-Amino-*n*-caproic acid	0.85	0.20	0.73	0.77	0.14	0.52
Aminomethanesulphonic acid	0.94	0.24	0.77	0.82	0.70	0.80
2-Aminoethanesulphonic acid	0.94	0.86	0.92	0.95	0.56	0.71

weakly retained and elongated spots are obtained in the case of aliphatic amines having high numbers of carbon atoms in the side-chain. From an analytical point of view this layer is not useful, except for the case of spermine and spermidine; the latter compounds are very strongly retained and can be separated from each other and from the diamines.

A similar behaviour is observed on SIL C_{18}-50 plates.

On layers of RP-18 impregnated with the detergent all the compounds are strongly retained with the exception of taurine. The addition of potassium chloride to

the eluent results in a remarkable increase of the R_F values. The polyamines and the amino acids exhibit very similar R_F values, while the aliphatic monoamines can be differentiated according to the number of carbon atoms in their side-chain.

A remarkable increase of the R_F values is also observed with increase of the hydrogen-ion concentration in the eluent. This increase, caused by the addition of both potassium chloride and hydrochloric acid to the eluent, confirms the prominent rôle of ion exchange in the retention of these compounds.

The use of alkaline eluents, in contrast to what is observed in the case of peptides, does not involve any advantage with respect to acidic eluents apart from the separation of the first three members of the diamine series (1,2-diaminoethane, R_F = 0.36; 1,2-diaminopropane, R_F = 0.43; 1,3-diaminopropane, R_F = 0.20) and that of 1,2-diaminoethane and 1,2-diaminopropane from all the other diamines (R_F between 0.16 and 0.27).

On layers of SIL C_{18}-50 + 4% HDBS, where eluents with methanol percentages smaller than 60% can be used, a lower retention is observed for diamines and amino acids with respect to the RP-18 + 4% HDBS plates, while the chromatographic behaviour of the aliphatic monoamines is more complex. A decrease of the methanol percentage in the eluent results in an increase particularly of the resolution of the layer towards the early members of the monoamine series (see C_1–C_7); also the two isomers of phenylethylamine are well separated.

It should be noted that the layers of SIL C_{18}-50 + 4% HDBS exhibit a resolution towards these compounds higher than that of the home-made layers of silanized silica gel (C_2) impregnated with the detergent, with the same methanol

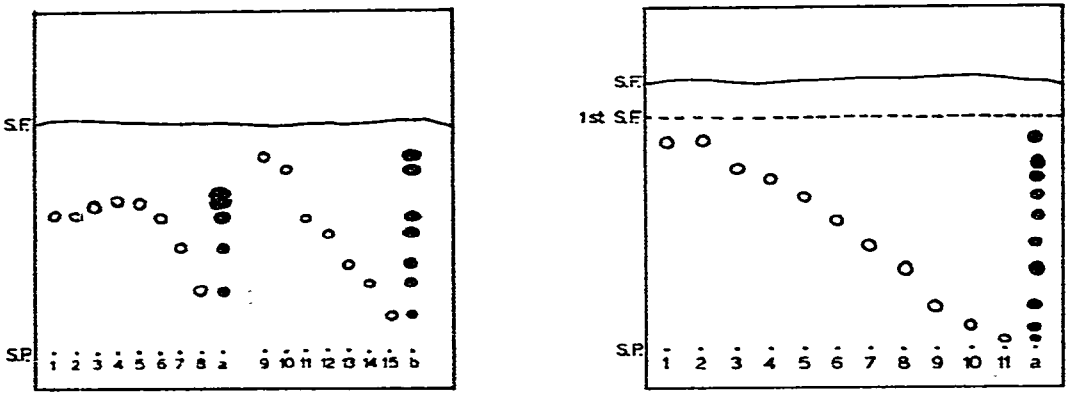


Fig. 3. Thin-layer chromatogram of aliphatic mono- and diamines on SIL C_{18}-50 impregnated with 4% HDBS solution. Elution: 0.5 *M* hydrochloric acid in water–methanol(30%)–acetic acid (5.7%). 1 = 1,2-Diaminoethane; 2 = 1,2-diaminopropane; 3 = 1,3-diaminopropane; 4 = 1,4-diaminobutane; 5 = 1,5-diaminopentane; 6 = 1,6-diaminohexane; 7 = 1,7-diaminoheptane; 8 = 1,8-diaminooctane; 9 = methylamine; 10 = ethylamine; 11 = isobutylamine; 12 = *n*-butylamine; 13 = isoamylamine; 14 = *n*-amylamine; 15 = *n*-hexylamine. a = Mixture of 1–8; b = mixture of 9–15. Other details as in Fig. 1.

Fig. 4. Thin-layer chromatogram of aliphatic monoamines on RP-18 impregnated with 4% HDBS solution. Migration distance = 7 cm. Eluent: 2 *M* HCl in water–methanol(65%)–acetic acid(5.7%). 1 = Methylamine; 2 = ethylamine; 3 = *n*-butylamine; 4 = isoamylamine; 5 = *n*-amylamine; 6 = *n*-hexylamine; 7 = *n*-heptylamine; 8 = *n*-octylamine; 9 = *n*-decylamine; 10 = *n*-dodecylamine; 11 = *n*-tetradecylamine. a = Mixture of 1–11. Other details as in Fig. 1.

percentage in the eluent. The sequence of the affinities of the aliphatic monoamines and diamines is the same in both cases[1–7]. In a single elution (1 *M* acetic acid + 1 *M* hydrochloric acid in 30% methanol) on plates of SIL C_{18}-50 + 4% HDBS the first ten members of the *n*-alkylmonoamine series were separated, although the first two members tend to merge.

If the hydrochloric acid concentration is decreased, the monoamines with low molecular weights (particularly methylamine and ethylamine) and the diamines with high molecular weights can be separated (Fig. 3). On RP-18 plates impregnated with 4% HDBS, eluting with 1 *M* acetic acid + 2 *M* hydrochloric acid in 65% methanol, the monoamines C_2–C_{14} can be separated (see Fig. 4). The high methanol percentage accounts for the separation of the C_{12} and C_{14} members.

From the data of Table III and from the chromatograms in Figs. 3 and 4, some conclusions can be drawn on the usefulness of the reversed-phase technique and of chromatography on sorbed ionic sites[18] in the separation of these nitrogen-containing compounds. The aliphatic monoamines, including the two isomers of phenylethylamine, can be well separated and with better results than on home-made silanized silica gel layers or on ionic exchangers with cellulosic, polystyrolic and paraffinic matrices[8]. As regards the diamines, good results have been achieved only for the members with high molecular weights (C_5–C_8). Finally, the use of these plates for the analysis of the amino acids is somewhat limited, even though some closely related compounds can be separated (see the separation of aminomethane sulphonic acid from 2-aminoethane sulphonic acid and that of the two isomers of amino-*n*-caproic acid).

Layers of AWP

Table III lists the R_F values of the aliphatic mono- and polyamines and of the amino acids on layers of AWP + $CaSO_4 \cdot \frac{1}{2}H_2O$ (4:2, w/w) eluting with 1 *M* nitric acid and 2 *M* ammonium nitrate. With 1 *M* nitric acid all the polyamines, the last members of the monoamines and the two isomers of phenylethylamine are strongly retained. The C_1–C_6 members of the monoamine series are less strongly retained and quite differentiated so that the separations between C_1 and C_2 and between the two C_4 isomers can be achieved.

On this exchanger the behaviour of the amino acids is very interesting since their migration rates are based on

– the distance between the carboxylic or sulphonic group and the $-NH_3^+$ group involved in the cation exchange process (see the R_F sequence of the *n*-butyric and *n*-caproic acids and that of the two sulphonic acids)

– the steric hindrance of the methyl group (see the higher R_F value of 2-aminoisobutyric acid compared with that of 2-amino-*n*-butyric acid)

Such behaviour is in accord with the R_F sequence of some amino acids studied previously[8]; in fact, alanine is less strongly retained than glycine and β-alanine.

As the nitric acid concentration in the eluent is increased (*i.e.*, to 2 *M*), no remarkable differences are observed in the R_F values; the most important change concerns the first two members of the diamines (1,2-diaminoethane, $R_F = 0.13$; 1,2-diaminopropane, $R_F = 0.17$).

The use of 2 *M* ammonium nitrate results in an increase of the R_F values of most compounds with respect to the elution with 2 *M* nitric acid. Under these elution

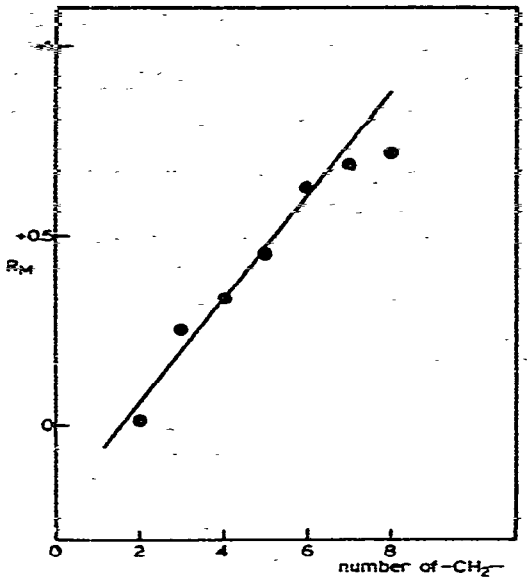


Fig. 5. R_M values plotted against the number of $-CH_2-$groups in aliphatic diamines. Layer: AWP + $CaSO_4 \cdot \frac{1}{2}H_2O$ (4:2). Eluent: 2 *M* NH_4NO_3.

conditions the amino acids are less differentiated and some monoamines give rise to elongated spots. The behaviour of the polyamines, on the contrary, is of interest, since it seems to be correlated to the distance between the protonated amino groups involved in the ion-exchange process and, in the case of 1,2-diaminoethane and 1,2-diaminopropane, to the steric hindrance of the methyl group. As is seen from Fig. 5,

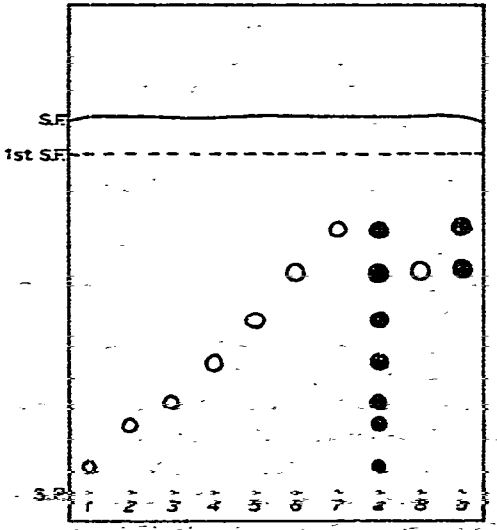


Fig. 6. Thin-layer chromatogram of amino acids on AWP + $CaSO_4 \cdot \frac{1}{2}H_2O$ (4:2). Eluent: 2 *M* HNO_3. 1 = 2,4-Diaminobutyric acid; 2 = ε-amino-*n*-caproic acid; 3 = 4-aminobutyric acid; 4 = 3-aminoisobutyric acid; 5 = *n*-leucine; 6 = 2-aminobutyric acid; 7 = 2-aminoisobutyric acid; 8 = 2-aminoethanesulphonic acid; a = Mixture of 1–7; b = mixture of aminomethanesulphonic acid and 2-aminoethanesulphonic acid. Other details as in Fig. 1.

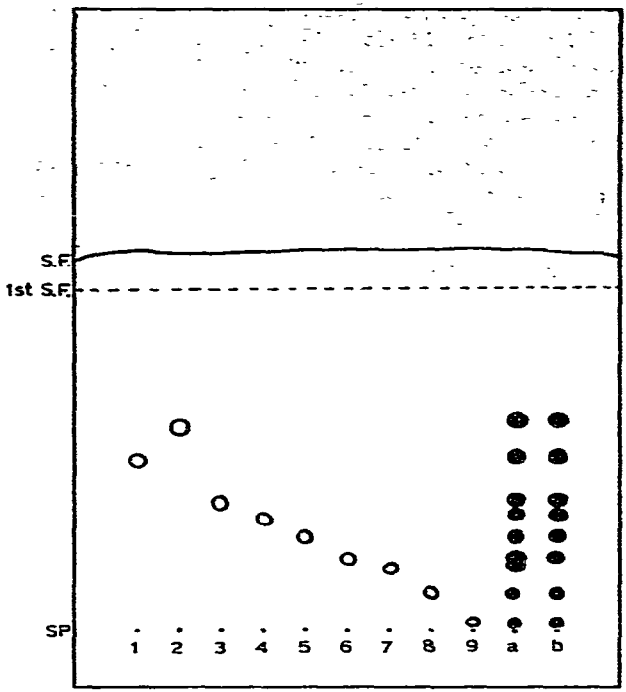


Fig. 7. Thin-layer chromatogram of aliphatic polyamines on AWP + $CaSO_4 \cdot \frac{1}{2}H_2O$ (4:2). Eluent: 2 *M* NH_4NO_3. 1 = 1,2-Diaminoethane; 2 = 1,2-diaminopropane; 3 = 1,3-diaminopropane; 4 = 1,4-diaminobutane; 5 = 1,5-diaminopentane; 6 = 1,6-diaminohexane; 7 = 1,8-diaminooctane; 8 = spermidine; 9 = spermine. a = Mixture of 1–9; b = mixture of 1,2,3,4,5,6,8,9. Other details as in Fig. 1.

where the R_M values are plotted as a function of the number of $-CH_2-$ groups in the molecule, a straight line can be drawn for the first six members of the series, while the last two compounds (C_7 and C_8) clearly deviate. This behaviour can be ascribed to the progressive disappearance, with increasing distance, of the interactions between the two protonated amino groups.

On AWP layers the closely related amino acids can be separated by eluting with 1 *M* and 2 *M* nitric acid (see Fig. 6). With 2 *M* ammonium nitrate as eluent the best results are achieved in the case of the aliphatic polyamines (see Fig. 7).

The above separations cannot be carried out on RP-18 and SIL C_{18}-50 plates untreated or impregnated with detergents or on weak and strong ion exchangers with polystyrolic, cellulosic or paraffinic matrices[19].

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