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Note

Investigations on the mechanism and selectivity of chromatography on thin layers of polyamide

IV. Chromatography of amino acids and complex phenolic substances

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In the preceding papers on polyamide thin-layer chromatography (TLC)[1–4], the mechanism of chromatography in polyamide systems was investigated by the dilution method, using hydroxy derivatives of benzene and naphthalene as test solutes. In this paper, the results obtained for a number of phenolic acids and flavones and also for strongly hydrophilic amino acids are reported. The developing solvents were chosen based on experience with the chromatographic analysis of phenols and naphthols; as in preceding papers, binary solvent systems suitable for gradient elution were employed.

All of the compounds investigated were strongly retained by the polyamide sorbent as a result of their molecular structure, especially the presence of proton donor groups (OH and COOH) capable of interaction with the peptide groups of the polymer. From the earlier investigations with phenols[1–4] and literature data[5,6], it would be expected that the compounds would require the use of polar solvents with considerable elution strength; the results confirmed these expectations.

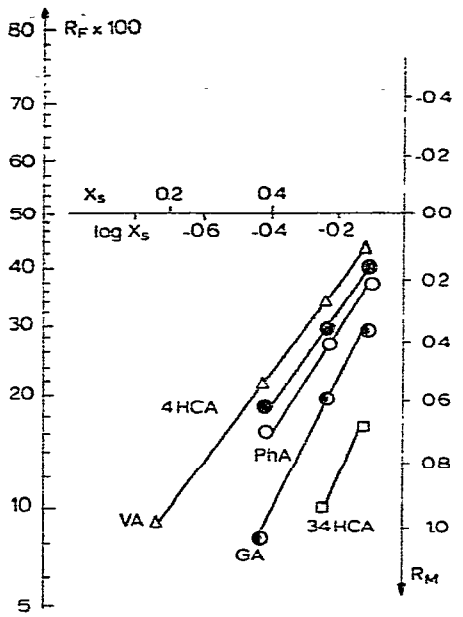
EXPERIMENTAL

The solutes were chromatographed on thin layers of polyamide (Woelm, Eschwege, G.F.R.) supported on glass plates[1].

Flavones and phenolic acids were detected with bis-diazotized benzidine and amino acids with modified ninhydrin reagent[7] (a 0.3% solution of ninhydrin in n-butanol containing 3% of glacial acetic acid); the plates were sprayed with this reagent and then heated in an oven at 110° for 2–3 min. The R_F values are given in Tables I and II.

RESULTS AND DISCUSSION

In the first series of experiments, binary solvents containing one polar component of class B or AB were used, with cyclohexane as diluent. Owing to the strong sorption of phenolic acids and flavones, low R_F values were obtained, except for high



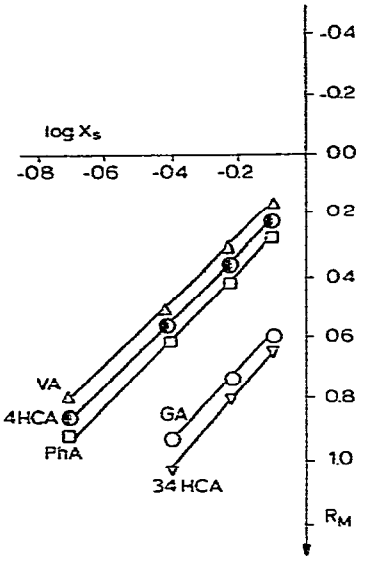


Fig. 1. R_M *versus* log X_S relationships for phenolic acids for developing solvents composed of cyclohexane and ethanol (S).

Fig. 2. R_M *versus* log X_S relationships for phenolic acids for developing solvents composed of cyclohexane and propanol (S).

concentrations of the polar solvents (Figs. 1 and 2). The R_M *versus* log X_S relationships were linear and mostly in the range above $R_M = 0.0$.

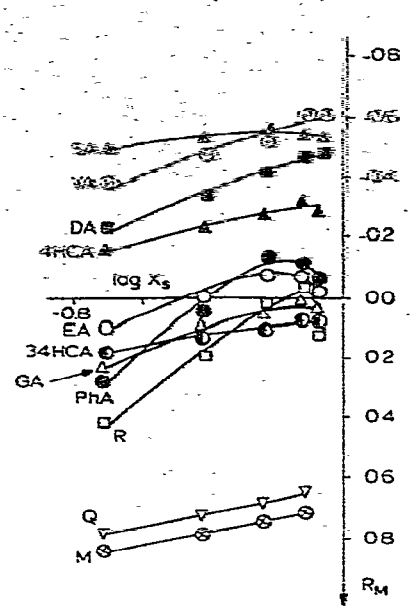
Therefore, in the present experiments binary mobile phases consisting of two polar solvents were employed: solutions of acetic acid in acetone (AB + B)[8], ethanol or propanol (AB + AB). The R_F values obtained for these systems (Figs. 3 and 4) were markedly higher than for polar solvents diluted with cyclohexane. The R_M versus log X_S relationships (where S denotes the more polar component, acetic acid) were approximately linear for solutions of acetic acid in ethanol and propanol. On the other hand, for solutions of acetic acid in acetone the curves passed through maxima at higher concentrations of acetic acid (Fig. 4). The decrease in R_F values at high X_S values is probably caused by increasing dimerization of acetic acid and swelling of the polyamide. The occurence of maxima on the curves suggests that in gradient elution for systems of this type the concentration of acetic acid in the gradient should not exceed *ca.* 40–50 vol.-%. An advantage of these systems is the formation of compact, well defined spots of the solutes (good capacity and high efficiency of the chromatographic system).

Three flavones (rutin, quercetin and morin) were chromatographed in the same solvent systems. For solvent systems of lower elution strength, such as solutions of ethanol or propanol in cyclohexane, low R_F values were obtained even at the highest concentrations of the polar solvent. For solvent systems such as cyclohexane + pyridine, propanol + acetic acid and acetone + acetic acid, the R_F values were in the range 0.1–0.5. It is worth noting the unexpected behaviour of rutin, which in all instances had higher R_F values than those of morin and quercetin. There are

TABLE I

$R_F \times 100$ VALUES OF PHENOLIC ACIDS AND FLAVONES FOR VARIOUS MOLAR FRACTIONS OF THE POLAR SOLVENT (*S*)

Solute	*Abbreviation*	*Cyclohexane + ethanol (S)*					*Cyclohexane + propanol (S)*					*Cyclohexane + pyridine (S)*					*Propanol + acetic acid (S)*					*Acetone + acetic acid (S)*				
		0.1	*0.2*	*0.4*	*0.6*	*0.8*	*0.1*	*0.2*	*0.4*	*0.6*	*0.8*	*0.1*	*0.2*	*0.4*	*0.6*	*0.8*	*0.2*	*0.4*	*0.6*	*0.8*	*0.9*	*0.2*	*0.4*	*0.6*	*0.8*	*0.9*
4-Hydroxycinnamic acid	4HCA	0	2	20	30	42	4	14	21	30	37	6	8	34	68	78	60	60	63	67	65	66	77	76	72	69
3,4-Hydroxycinnamic acid (caffeic acid)	34HCA	0	1	5	10	18	2	4	9	14	18	4	7	28	55	75	40	42	45	46	47	42	53	58	56	51
Gallic acid	GA	0	3	9	20	30	0	3	11	15	20	2	8	29	80	98	36	42	46	49	48	57	65	68	66	61
Chlorogenic acid	ChA	0	0	0	2	8	0	0	1	3	6	0	2	4	15	30	—	—	—	—	—	—	—	—	—	—
Pherulic acid	PhA	2	3	17	28	37	4	11	20	27	34	3	9	36	68	80	35	47	57	55	53	20	40	48	53	46
Divarcatinic acid	DA	0	0	1	5	8	0	1	2	3	5	0	1	4	22	40	62	67	71	74	76	42	53	57	55	51
Evernic acid	EA	0	0	1	5	9	2	5	7	9	10	4	7	10	23	32	45	50	53	52	51	47	56	61	60	56
Sphaerophorinic acid	SA	0	0	1	2	11	1	3	7	9	10	3	6	12	32	45	75	76	77	76	75	75	80	79	77	72
Vanillic acid	VA	2	9	22	34	46	4	14	23	32	39	4	8	32	63	77	70	74	76	80	80	85	85	86	80	79
Quercetin	Q	0	0	2	5	10	0	0	1	3	7	0	0	12	31	53	14	16	17	18	—	9	20	23	22	21
Morin	M	0	0	0	1	2	0	0	0	0	0	0	0	2	15	35	13	14	15	16	—	6	16	20	19	18
Rutin	R	0	0	0	3	8	0	0	1	3	8	0	3	16	57	92	27	39	49	52	42	11	31	45	45	43



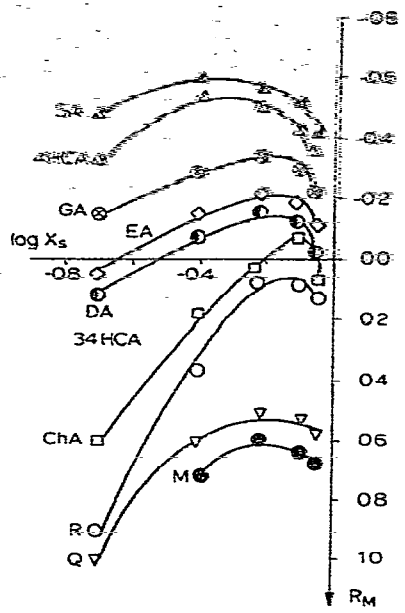


Fig. 3. R_M *versus* log X_S relationships for phenolic acids and flavones for developing solvents composed of propanol and acetic acid (*S*).

Fig. 4. R_M *versus* log X_S relationships for phenolic acids and flavones for developing solvents composed of acetone and acetic acid (*S*).

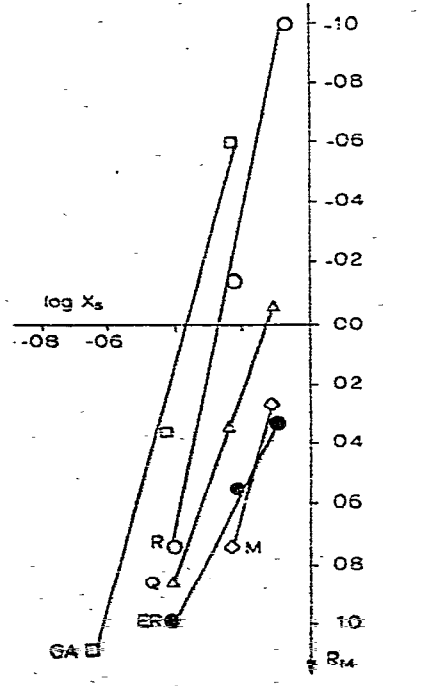
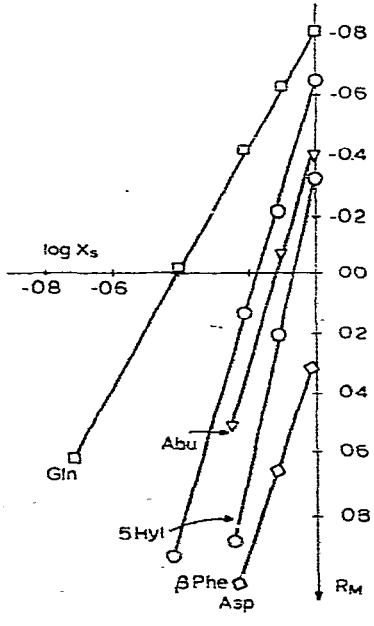


Fig. 5. R_M *versus* log X_S relationships for phenolic acids and flavones for developing solvents composed of cyclohexane and pyridine (*S*).

numerous hydroxyl groups in the molecule of rutin and it would be expected that it would be more strongly sorbed by polyamide than the two other flavones, which have fewer hydroxyl groups. However, the results indicated a relatively weak retention of rutin (Figs. 3–5), which might be caused by a molecular sieve effect that controls the penetration of molecules of different molecular dimensions into the polyamide gel[9–11]. This effect plays a fundamental role in the separation of molecules according to their size in gel permeation chromatography with polyacrylamide, polystyrene and dextran gels, and it might also occur for polyamide gel. If the penetration of the larger molecules of rutin into the sorbent were limited, this would explain its higher R_F values.

Although aliphatic amines (di-*n*-propylamine, diethylamine and triethylamine) are stronger bases than pyridine, it was found that the last solvent is a much better eluent of the solutes in polyamide systems. For cyclohexane solutions of amines, the R_F values of the phenolic acids were equal or close to zero. On the other hand, for developing solvents containing pyridine, the R_F values were much higher, the spots compact and well defined and the R_M *versus* log X_S relationships linear.

In the next series of experiments, strongly polar compounds (amino acids) interacted strongly with the amide groupings of polyamide and their small molecules were able easily to penetrate into the polyamide gel. Strong sorption of amino acids required the application of special solvent systems, which, although relatively simple, could interact competitively with amino acids in the mobile phase. Most solvent sys-



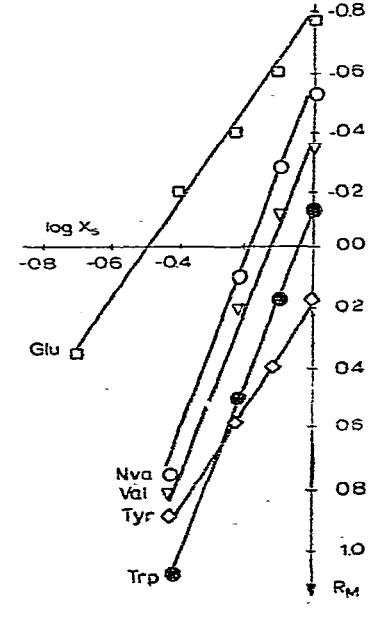


Fig. 6. R_M *versus* log X_S relationships for amino acids for developing solvents composed of cyclohexane and propionic acid (*S*).

Fig. 7. R_M *versus* log X_S relationships for amino acids for developing solvents composed of cyclohexane and propionic acid (*S*).

TABLE II

$R_F \times 100$ VALUES OF AMINO ACIDS FOR VARIOUS MOLAR FRACTIONS OF THE ACTIVE SOLVENT

Solute	*Abbreviation*	*Cyclohexane + propionic acid (S)*					*Benzene + acetic acid (S)*				
		0.2	*0.4*	*0.6*	*0.8*	*1.0*	*0.3*	*0.4*	*0.5*	*0.6*	*0.8*
Acetylglutamic acid	Ac-Glu	2	8	22	54	67	11	25	43	63	79
α-Alanine	αAla	0	6	21	43	64	9	20	35	47	65
β-Phenylalanine	βPhe	2	10	42	62	82	27	37	50	62	74
β-Alanine	βAla	2	11	27	52	64	9	22	32	52	65
α-Aminobutyric acid	Abu	0	3	23	53	71	15	27	45	58	72
Arginine	Arg	0	2	7	20	44	0	2	11	23	57
Aspartic acid	Asp	0	2	8	17	32	0	7	12	28	44
Asparagine		0	8	25	50	70	0	0	9	17	44
Cysteic acid		0	0	0	0	0	0	0	0	0	0
Cysteine		0	5	15	34	48	—	—	—	—	—
Glutamic acid	Glu	30	60	70	78	86	8	15	25	41	50
Glutamine	Gln	18	51	74	80	82	—	—	—	—	—
Glycine		0	6	18	33	48	—	—	—	—	—
Histamine		8	17	35	60	67	9	20	28	46	67
Histidine		0	4	20	46	67	4	10	25	56	80
Creatinine		6	25	56	67	69	35	53	60	75	80
Leucine	Leu	3	13	43	62	67	17	34	47	65	80
Isoleucine		2	10	30	55	68	20	35	55	75	85
Lysine	Lys	0	3	12	51	65	2	7	15	40	75
Hydroxylysine	5Hyl	0	3	11	37	62	0	2	7	15	45
Methionine		0	9	27	43	75	15	22	40	65	85
Ornithine		0	1	5	13	55	2	8	16	42	80
Proline	Pro	4	17	55	63	71	34	50	64	75	85
Hydroxyproline		0	7	20	36	55	2	10	28	50	80
Threonine		0	7	32	50	60	10	20	35	67	85
Tryptophan	Trp	3	7	24	41	57	6	14	27	57	75
Tyrosine	Tyr	4	11	20	27	40	—	—	—	—	—
3,5-diiodotyrosine	Tyr (I_2)	2	7	22	37	52	7	20	27	45	65
Valine	Val	2	12	38	56	67	10	22	35	62	75
Norvaline	Nva	4	15	45	65	76	—	—	—	—	—

tems of the N + A and N + B types gave very low R_F values; good results were obtained with the binary solvent systems benzene + acetic acid and cyclohexane + propionic acid. Typical R_M *versus* log X_S relationships for some of the amino acids investigated are presented in Figs. 6–9. The relationships are linear over wide composition ranges ($-R_M$ = constant + $n$log X_S) and the slopes (n) are mostly high, usually in the range 3–5. Only for tyrosine, glutamic acid, glutamine, β-alanine and proline were the lines less steep (slope *ca.* 2.0); for amino acids with an aromatic ring the slope was *ca.* 3.0.

The use of non-aqueous solvents containing acetic acid for the chromatography of amino acids was found to be advantageous owing to satisfactory selectivity and compact, well defined spots. It seems that acetic and propionic acids give optimal swelling of polyamide and sufficient loosening of its structure, with a resulting high capacity and rapid equilibration of the mobile and stationary phases. Systems of the

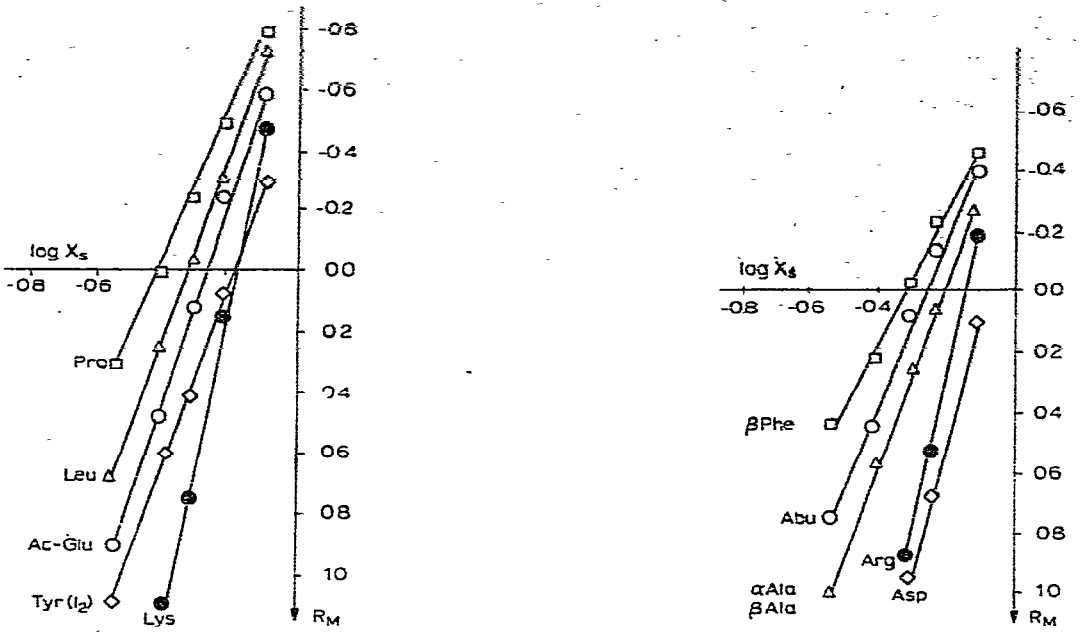


Fig. 8. R_M *versus* log X_S relationships for amino acids for developing solvents composed of benzene and acetic acid (*S*).

Fig. 9. R_M *versus* log X_S relationships for amino acids for developing solvents composed of benzene and acetic acid (*S*).

type polyamide–acetic acid + polar diluent can therefore be suitable for the chromatographic analysis of polar solutes, including column chromatography with gradient elution. Acetic acid can also be replaced with propionic acid (but not by formic acid, which at higher concentrations dissolves polyamide).

REFERENCES

1 E. Soczewiński, W. Gołkiewicz and H. Szumiło, *J. Chromatogr.*, 45 (1969) 1.
2 E. Soczewiński and H. Szumiło, *J. Chromatogr.*, 81 (1973) 99.
3 H. Szumiło and E. Soczewiński, *J. Chromatogr.*, 94 (1974) 219.
4 E. Soczewiński and H. Szumiło, *J. Chromatogr.*, 94 (1974) 229.
5 P. R. Bhandari, *J. Chromatogr.*, 16 (1964) 130.
6 K. Egger, *Planta Med.*, 12 (1964) 265.
7 E. Stahl, *Dünnschichtchromatographie*, Springer, Berlin, 1967.
8 G. C. Pimentel and A. L. McClellan, *The Hydrogen Bond*, Freeman, San Francisco, 1960.
9 H. Determann, *Gelchromatographie*, Springer, Berlin, 1967.
10 J. Popowicz, *Postepy Biochem.*, 9 (1963) 245.
11 M. Stahl, *Postepy Biochem.*, 13 (1967) 407.