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ADSORPTION CHROMATOGRAPHY ON CELLULOSE

IV. SEPARATION OF D- AND L-TRYPTOPHAN AND D- AND L-METHYLTRYPTOPHAN ON CELLULOSE WITH AQUEOUS SOLVENTS[a]

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SUMMARY

Differences in R_F values between D- and L-tryptophan on cellulose paper, developed with water, were observed already in 1954 and now several variables of this separation, such as modifications to the tryptophan molecule, the temperature and salting-out have been examined. Differences were found in the mechanism of the process compared with the systems described by Yuasa *et al.* [*Chromatographia*, 21 (1986) 79], who separated amino acid enantiomers on cellulose with pyridine–ethanol–water mixtures.

INTRODUCTION

The chiral properties of cellulose were first described in 1952 by Dalgleish[1], who obtained separations of the D- and L-forms of kynurenine, hydroxykynurenines and hydroxyphenylalanines with a "partition" solvent, namely butanol–acetic acid–water. Soon afterwards Weichert[2] showed that D- and L-tryptophans had different R_F values when developed on cellulose paper with water (L-form, R_F = 0.53; D-form, R_F = 0.55). This was also reported by Yuasa *et al.*[3]. Later, cellulose derivatives were preferred for chiral resolutions, especially cellulose triacetate and cellulose tribenzoate (for a review, see ref. 4). Relatively few workers, *e.g.*, Yuasa and co-workers[3,5], used underivatized cellulose as a chiral stationary phase with partition solvents. They also pointed out that "native" cellulose had advantages for the preparative separation of amino acid pairs.

In a survey of the adsorptive properties of cellulose from aqueous solutions[6,7], we obtained "baseline" separations with substituted tryptophans on cellulose thin-layers which would be impossible on cellulose paper, as shown by Weichert[2], and we report our findings here.

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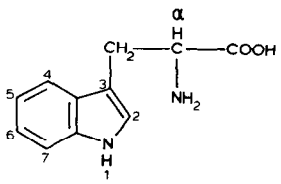

Tryptophan

Salting-out with ammonium sulphate in the eluent

When water is used as the eluent, the difference in R_F values in thin-layer chromatography (TLC) between the D- and L-forms is between 0.06 and 0.08. These values have at best a precision of ± 0.01, but usually 0.02. Hence exact measurements would be illusory. However, it is evident from Table I that the separation decreases with increasing ammonium sulphate concentration. The general decrease in R_F values is probably due to two effects: the higher polarity of the eluent and the dehydration of the cellulose, owing to the presence of ammonium sulphate. We suggest that dehydration affects the lower chiral discrimination.

When lithium chloride solutions are used as eluents, we observe the same tendency with increasing concentration, but the effect is less than with ammonium

TABLE I

hR_F VALUES OF OPTICALLY ACTIVE TRYPTOPHANS AT ROOM TEMPERATURE WITH DIFFERENT ELUENTS

Compound	*Eluent*						
	Water	*Acetic acetic (2%)*	*Ammonium sulphate solution*				
			0.66 M	*1.0 M*	*2.0 M*	*2.65 M*	*4.0 M*
DL-Tryptophan:							
D-Isomer	58	–	57	52	47	35	25
L-Isomer	52(s)[a]	–	52(s)	45(s)	42(s)	31(s)	
DL-1-Methyltryptophan:							
Spot 1	58	57	55	50[b]	37[b]	28	13
Spot 2	52		50	46[b]	34[b]		
DL-5-Methyltryptophan:							
Spot 1	50	46	42	39	29	26	10
Spot 2	42(s)	40(s)	34	32(s)	24(s)	21(s)	
DL-6-Methyltryptophan:							
Spot 1	47	42	43	38	27	22	08
Spot 2	39(s)	38(s)	35(s)	32(s)	23(s)	19(s)	
DL-7-Methyltryptophan:							
Spot 1	52	46	45	41[b]	30[b]	26[b]	10
Spot 2	46(s)		39(s)	37[b](s)	26[b](s)	21[b](s)	
DL-α-Methyltryptophan	72	67	67	64	55	51	–

[a] s = Slight streaking.
[b] Critical separations.

TABLE II

COMPARISON OF LITHIUM CHLORIDE AND AMMONIUM SULPHATE SOLUTIONS AS ELUENTS FOR D- AND L-TRYPTOPHAN AT ROOM TEMPERATURE

Eluent	*Concentration (M)*	hR_F	
		D-*Tryptophan*	L-*Tryptophan*
Lithium chloride	0.5	55	48
	2.4	48	41
	4.7	37	33
Ammonium sulphate	0.66	57	52
	2.0	47	42
	4.0	25	25

sulphate. To illustrate this, the two salts are compared in Table II.

Phosphate buffers of pH 8.8 and 3.0 were also examined. Table III shows that the separations are very similar to those obtained in ammonium sulphate or in lithium chloride. A good separation is shown in Fig. 1.

EXPERIMENTAL

Cellulose thin layers (Merck Cellulose, Art. No. 5577) were developed by the ascending technique in small jars. The development took 10–30 min, depending on the salt concentration in the eluent. Development took place either at room temperature or in thermostatically controlled ovens or cooling cabinets.

The spots were revealed either with iodine vapour or by dipping into an acetone solution of ninhydrin, with subsequent heating with a hair dryer to full colour formation.

TABLE III

R_F VALUES OF SUBSTITUTED TRYPTOPHANS IN PHOSPHATE BUFFERS

Buffers: 0.7 *M* orthophosphoric acid in 100 ml of water adjusted with potassium hydroxide to pH 8.8 or 3.0. Temperature, 40°C.

Compound	*pH 8.8*		*pH 3.0*	
	D-*form*	L-*form*	D-*form*	L-*form*
D-Tryptophan	0.66		0.66	
DL-Tryptophan	0.66	0.60	0.65	0.59
DL-1-Methyltryptophan	0.66	0.59	0.65	0.59
DL-5-Methyltryptophan	0.54	0.47	0.54	0.47
DL-6-Methyltryptophan	0.53	0.47	0.54	0.47
DL-α-Methyltryptophan	0.75		0.75	

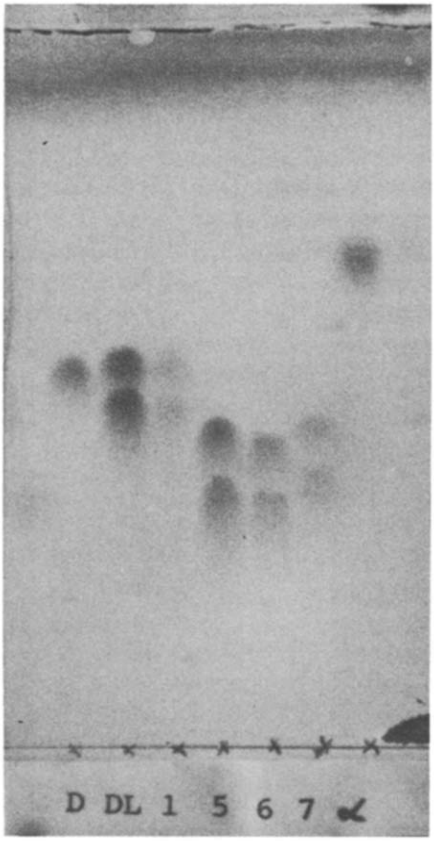

Fig. 1. Chromatogram on a Merck cellulose thin layer developed at room temperature with 0.5 *M* lithium chloride solution. The spots were revealed with iodine vapour. D = D-tryptophan; DL = DL-tryptophan; 1 = DL-1-methyltryptophan; 5 = DL-5-methyltryptophan; 6 = DL-6-methyltryptophan; 7 = DL-7-methyltryptophan; α = DL-α-methyltryptophan.

RESULTS AND DISCUSSION

Development with pure water

The R_F values of tryptophan and some substituted tryptophans are given in Table I. The difference between the D- and L-forms of tryptophan is greater than that reported by Weichert[2] on cellulose, as thin layers offer both a larger surface area owing to the smaller particle size and a more favourable sorbent to solvent ratio. Thus we obtained two distinct spots on the thin layer, whereas this does not occur on the usual filter-papers, such as Whatman No. 1 and 3MM.

Compounds with a methyl group on the benzene ring, *i.e.*, 5-, 6- and 7-methyltryptophan, are all more strongly adsorbed than the parent compound, but the differences in R_F values between the D- and L-forms are of the same order as for tryptophan.

A methyl group on the nitrogen atom of the indole ring does not lower the R_F

TABLE IV

EFFECT OF TEMPERATURE ON THE hR_F VALUES OF OPTICALLY ACTIVE TRYPTOPHANS WITH WATER AS ELUENT

Compound	hR_F			
	1°C	*7°C*	*40°C*	*63°C*
D-Tryptophan	46	49	62	75
L-Tryptophan	40(ss)	43(ss)	57	71
D-1-Methyltryptophan	46	49	64	76
L-1-Methyltryptophan	41(ss)	43(ss)	60	71
D-5-Methyltryptophan	35	39	54	63
L-5-Methyltryptophan	28(ss)	32(ss)	46	55
D-6-Methyltryptophan	31	37	52	63
L-6-Methyltryptophan	23	29(s)	44	56
D-7-Methyltryptophan	–	–	56	66
L-7-Methyltryptophan	–	69	49	61
α-Methyltryptophan	62		76	82

[a] s = Streaking; ss = strong streaking.

values of the two optical isomers, but a methyl group in the α-position decreases the adsorption and seems to hinder the separation of the two isomers.

Development with acetic acid

At a lower pH with 2% acetic acid, most separations are either reduced or do not occur (Table I).

Effect of temperature

Tables IV–VIII show the R_F values obtained at different temperatures, between the lowest possible (between 0 and −10°C, depending on the salt and its concentra-

TABLE V

EFFECT OF TEMPERATURE ON THE hR_F VALUES OF OPTICALLY ACTIVE TRYPTOPHANS IN 0.5 *M* LITHIUM CHLORIDE AS ELUENT

Compound	hR_F			
	−5°C	*22°C*	*40°C*	*63°C*
D-Tryptophan	46	55	66	71
L-Tryptophan	37(s)	48(s)	60(s)	67
D-1-Methyltryptophan	49	55	67	71
L-1-Methyltryptophan	40(s)	48(s)	61(s)	67
D-5-Methyltryptophan	32	41	54	61
L-5-Methyltryptophan	21	31	44	53
D-6-Methyltryptophan	32	41	53	61
L-6-Methyltryptophan	21	32	44	54
D-7-Methyltryptophan	33	44	57	65
L-7-Methyltryptophan	25	37	49	60
α-Methyltryptophan	68	69	77	81

[a] s = Streaking.

TABLE VI

EFFECT OF TEMPERATURE ON THE hR_F VALUES OF OPTICALLY ACTIVE TRYPTOPHANS IN 2.4 *M* LITHIUM CHLORIDE AS ELUENT

Compound	hR_F[a]						
	−10°C	*−5°C*	*1°C*	*7°C*	*22°C*	*40°C*	*63°C*
D-Tryptophan	32	32	35	36	48	58	67
L-Tryptophan	25(s)	24(s)	29(s)	31(s)	41(s)	51	61
D-1-Methyltryptophan	33	33	34	35	48	57	65
L-1-Methyltryptophan	25	26	29	29	41	51	60
D-5-Methyltryptophan	21	22	23	25	36	45	53
L-5-Methyltryptophan	13(s)	15(s)	16(s)	18(s)	28(s)	36(s)	45
D-6-Methyltryptophan	20	21	21	23	33	43	55
L-6-Methyltryptophan	13(s)	15(s)	15	16	25	35	48
D-7-Methyltryptophan	21	22	–	–	38	46	58
L-7-Methyltryptophan	15	17	–	–	31	39	52
α-Methyltryptophan	50	53	53	56	64	70	78

[a] s = Streaking.

tion) and 63°C. There is little difference in the separation of the optical isomers at low and higher temperatures up to 40°C. At 63°C some chromatograms show a decrease in the separation of the entiomers, and this is most pronounced at high lithium chloride concentrations.

However, the separations are essentially the same over a wide temperature range. There is certainly no sign of inverted sequences, as observed in many other systems. From the point of view of obtaining good separations, there is an improvement in the compactness of the spots at 40°C. We suggest that this is due to a faster establishment of equilibrium during development.

TABLE VII

EFFECT OF TEMPERATURE ON THE hR_F VALUES OF OPTICALLY ACTIVE TRYPTOPHANS IN 4.7 *M* LITHIUM CHLORIDE AS ELUENT

Compound	hR_F		
	22°C	*40°C*	*61°C*
D-Tryptophan	37	50	59
L-Tryptophan	33	45	59
D-1-Methyltryptophan	35	47	57
L-1-Methyltryptophan	31	44	57
D-5-Methyltryptophan	22	32	42
L-5-Methyltryptophan	17	28	38
D-6-Methyltryptophan	24	33	46
L-6-Methyltryptophan	20	29	46
D-7-Methyltryptophan	26	37	50
L-7-Methyltryptophan	22	34	50
α-Methyltryptophan	51	63	72

TABLE VIII

EFFECT OF TEMPERATURE ON hR_F VALUES OF OPTICALLY ACTIVE TRYPTOPHANS IN 0.76 *M* AMMONIUM SULPHATE AS ELUENT

Compound	hR_F				
	−5°C	*1°C*	*22°C*	*40°C*	*63°C*
D-Tryptophan	39	40	50	63	75
L-Tryptophan	33(s)	35(s)	44(s)	57	71
D-1-Methyltryptophan	37	40	50	59	69
L-1-Methyltryptophan	33(s)	35	44	54	66
D-5-Methyltryptophan	27	28	38	49	61
L-5-Methyltryptophan	20(s)	21(s)	31(s)	42	54
D-6-Methyltryptophan	23	25	39	46	62
L-6-Methyltryptophan	17	19	32	39	56
D-7-Methyltryptophan	25	–	–	49	–
L-7-Methyltryptophan	21	–	–	44	–
α-Methyltryptophan	59	57	65	70	83

[a] s = Streaking.

DISCUSSION

Separations of the enantiomeric tryptophans can be achieved on cellulose with an R_F difference of about 0.06, over a wide range of salt concentrations and temperatures. Substitution of methyl groups on the benzene ring (positions 5, 6 and 7) or on the indole ring (position 1) does not alter this separation appreciably. There is no separation when the α-carbon is substituted with a methyl group. We therefore have a very simple and cheap chromatographic system, which produces "baseline" separations over a wide range of conditions.

It is interesting to compare these separations with some similar ones reported recently. Yuasa and co-workers[3,5] used cellulose as a chiral support with a "partition solvent" consisting of pyridine–ethanol–water, both in columns and on thin layers. The R_F differences on thin-layer plates were 0.05 for several pairs of aromatic amino acids, *i.e.*, of the same order as we observed with aqueous solvents. However, they found that separation is possible only in a relatively narrow range of solvent mixtures, where the ratios of pyridine + water to water were 3–4. They also found that the separation "was clearly enhanced with a decrease in the temperature and with an increase in the hydrophobicity". Hence their system had very different parameters to ours.

Another interesting comparison can be made with the results of Büyüktimkin and Buschauer[8], who separated by TLC the enantiomers of amino acids as their (*S*)-(+)-naproxen derivatives on silica gel plates (non-chiral). They obtained well separated spots with ten different pairs of amino acid enantiomers. The R_F differences were in the range 0.03–0.10, most being between 0.05 and 0.07. Further, Günther[9] has reported numerous chiral separations on Chiralplates (Macherey, Nagel & Co., Düren, F.R.G.). Most separations showed differences in R_F values of 0.09–0.11. Hence the separations that we obtained seem comparable to those with other systems as far as separation ability is concerned, but offer the advantages of simplicity and robustness.

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