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Short Communication

Adsorption chromatography on cellulose

IX. Chiral separations with aqueous solvents and liquid-liquid systems

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ABSTRACT

The enantiomers of substituted tryptophans were examined by thin-layer chromatography on microcrystalline cellulose. Aqueous solvents and liquid-liquid systems yielded essentially the same separations, suggesting that adsorption can play a role in liquid-liquid (partition) systems in some instances.

INTRODUCTION

In previous work [1–3], we had been impressed by the readiness with which tryptophan enantiomers separate with aqueous solvents on microcrystalline cellulose thin layers. The separations and also those of enantiomers of methyl-substituted tryptophans were shown to be insensitive to temperature and to salt concentration in the solvent [1] and also to the salt used [2]. They were, however, sensitive to the type of cellulose used as adsorbent, native cellulose giving poorer separations than microcrystalline cellulose [3].

Enantiomer separations have also been reported with classical partition solvents such as butanol-acetic acid-water by Dalgliesh [4] and with polar solvents such as pyridine–ethanol–water by Yuasa and co-workers [5,6]. In view of the differences in the chiral properties of various celluloses, a comparison of adsorption and partition systems can only be made if the same adsorbent is used in both systems. We report here on comparative results obtained on Merck DC Plastikfolien plates (Art. 5577), which are made of microcrystalline cellulose. We also report on a number of substituted tryptophans that were not available to us in previous work.

EXPERIMENTAL

All chromatograms were prepared by ascending development in glass containers with a tightly closing lid on 20 cm x 20 cm DC Plastikfolien plates (Merck Art. 5577), cellulose layer thickness 0.1 mm. The sample solutions (in water) were applied to the thin layer as fine lines (less

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than 1 mm wide) by means of PTFE paint brushes size 00. With capillaries the porosity of the layers produces much larger zones. After a development of about 100 mm the chromatograms were exposed to iodine vapour (in a desiccator), which produced brown spots that copied well on a photocopier. This proved to be a much better means of documentation than reaction with ninhydrin and subsequent photography.

RESULTS AND DISCUSSION

The results obtained with substituted tryptophans are shown in Table I and Figs. 1 and 2.

TABLE I

R_F VALUES OF SUBSTITUTED TRYPTOPHANS ON MERCK DC PLASTIKFOLIEN CELLULOSE PLATES

Compound	Solvent								
	1 *M* NaCl			Pyridme-ethanol-water (1:1:1)			Butanol-acetic acid-water (4:1:5)		
	D-	L-	A &	D-	L-	A &	D-	L-	ΔR_F
4-Methyltryptophan	0.33	0.25	0.08	0.52	0.42	0.10	0.50	0.45	0.05
5-Methyltryptophan	0.35	0.27	0.08	0.54	0.48	0.06	0.53	0.49	0.04
6-Methyltryptophan	0.33	0.26	0.07	0.55	0.47	0.08	0.52	0.48	0.04
4-Fluorotryptophan	0.47	0.39	0.08	0.60	0.53	0.07	0.54	0.50	0.04
5-Fluorotryptophan	0.48	0.42	0.06	0.64	0.59	0.05		0.52	>0.03
6-Fluorotryptophan	0.47	0.40	0.07	0.65	0.61	0.04	0.54	0.51	0.04
5-Hydroxytryptophan	0.31	0.25	0.06	0.48	0.41	0.07	0.30	0.28	0.02

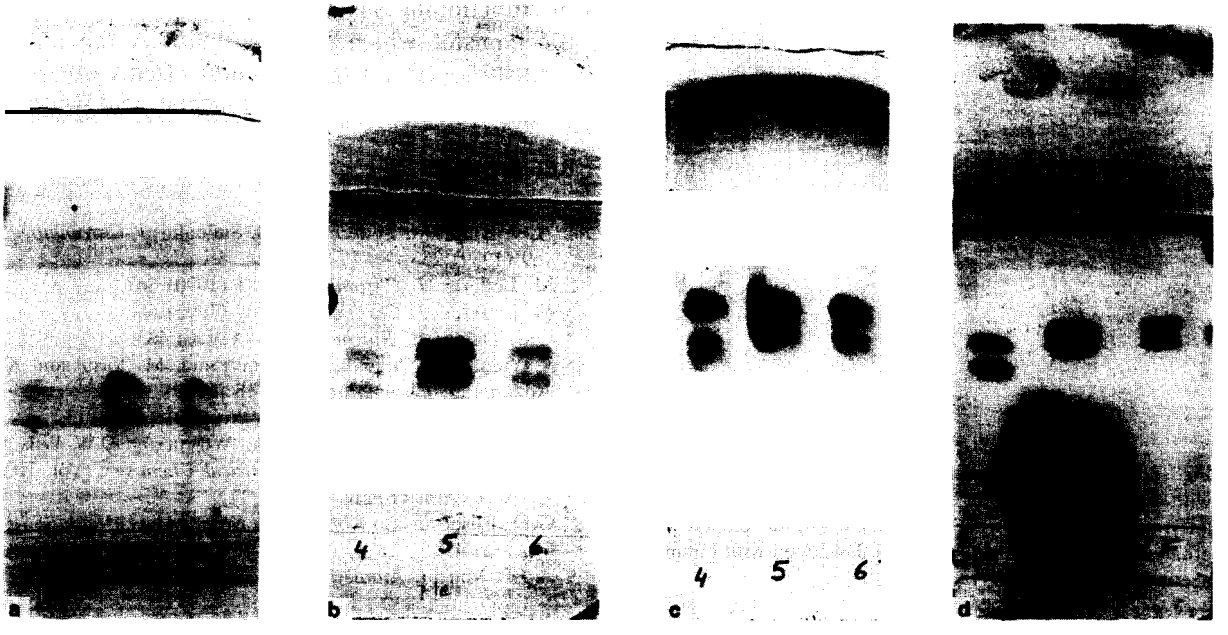

Fig. 1. Thin-layer chromatograms of (a) 4-, 5- and 6-methyltryptophan developed with 1 M NaCl; (b) 4-, 5- and 6-methyltryptophan developed with ethanol-pyridine-water (1:1:1); (c) 4-, 5- and 6-fluorotryptophan developed with 1 M NaCl; and (d) 4-, 5- and 6-fluorotryptophan developed with ethanol-pyridine-water (1:1:1).

There are a few notable features: (a) there is little difference between "adsorption" and "partition" chromatograms as far as the separation of enantiomers is concerned; (b) a substituent in the 5-position increases the R_F value and decreases the separation factor in most instances; (c) the separation factors do not change much with the various substituents; and (d) Yuasa and co-workers' polar solvent [5,6] separates enantiomers as efficiently as an aqueous solvent, whereas Dalgliesh's less polar mixture [4] gives smaller R_F differences.

The results for kynurenine are given in Table II. Several butanol-ethanol-water mixtures were also tried and those rich in ethanol and water gave even larger R_F differences for the enantiomers than an aqueous solvent.

These results pose a problem for the theory of chromatography. It is generally assumed that the cellulose in partition (i.e., liquid-liquid) chromatography plays the role of an inert support. In the treatise by Copius Peereboom [7], comparisons are listed between experimental partition coefficients and those calculated from paper chromatograms by various workers for amino acids, sugars, organic acids and steroids, and in all instances the agreement is excellent. Similar work was also done by Eliseeva [8] with inorganic ions with equally good agreement. Even for water-miscible solvents, Martin [9] postulated a "partition mechanism" if one considers the stationary phase to be like a saturated solution of a sugar.

However, this work clearly presents an exception. For strongly adsorbed compounds, adsorption and hence also chiral discrimination can still be functioning in addition to the liquid-liquid mechanism, which without doubt is the major contributor to most separation effects obtained in solvents such as butanol-acetic acid-water.

TABLE II

R_F VALUES OF D- AND L-KYNURENINE ON MERCK DC PLASTIKFOLIEN CELLULOSE PLATES

Solvent	D-	L-	ΔR_F
1 M NaCl	0.61	0.54	0.07
Ethanol-pyridine-water (1:1:1)	0.51	0.43	0.08
Butanol-acetic acid-water (4:1:5)	0.55	0.50	0.05
Ethanol-butanol-water:			
2:1:2	0.46	0.34	0.12
1:1:1	0.50	0.39	0.11
5:3:7	0.57	0.47	0.10
5:1:9	0.65	0.54	0.11

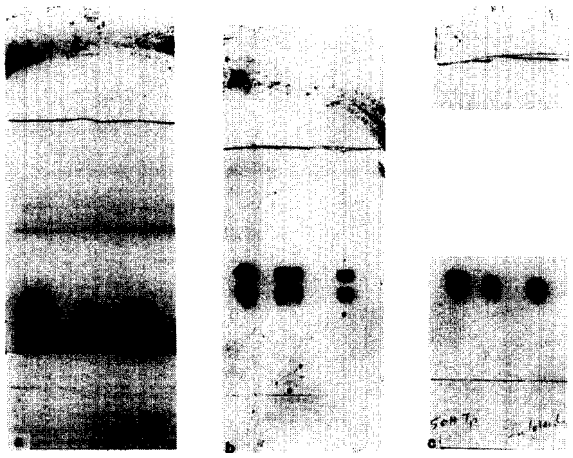

Fig. 2. Thin-layer chromatograms of 5-hydroxytryptophan with different loadings on each layer (most on right, least on left): (a) developed with 1 *M* NaCl; (b) developed with ethanol-pyridine-water (1: 1: 1); (c) developed with butanol–acetic acid-water (4:1:5).

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