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Short Communication

Adsorption chromatography on cellulose

X. Adsorption of tryptophan and derivatives from $CuSO_4$-containing eluents

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ABSTRACT

The adsorption of tryptophan and substituted tryptophans on cellulose thin layers developed with $CuSO_4$ solutions is reported. The presence of $CuSO_4$ decreases the chiral discrimination of cellulose for tryptophan and substituted tryptophans. The results are compared with those from previously reported work where chiral separations were obtained.

INTRODUCTION

In a study of the effect of various metal salts on the separation of optical isomers of tryptophan on cellulose [1] with aqueous solvents, it was noted that only Cu^{2+} had an effect on this separation, resulting in a diminution of the separation effect. Copper (II) yields the most stable complexes with amino acids with stability constants of the order of 10^{15}, so that an effect was to be expected. What was of interest was that the tryptophan was still adsorbed and did not travel with the liquid front although both the amino and the carboxylic groups were not free. In the eluent used, namely 0.05 *M* $CuSO_4$, there would be a large excess of Cu^{2+} ions and in view of the high stability constants one can assume that the tryptophan would be present as a 1:1 complex with the two free coordination groups of the copper occupied by water molecules. We felt that this observation merited more attention.

EXPERIMENTAL

Merck cellulose (Art. No. 5577) (consisting of microcrystalline cellulose) was used throughout. The chromatograms were developed at room temperature in small, well stoppered glass jars. After drying, the spots were revealed by exposure to iodine vapour, which gave good contrast when copied with a photocopier.

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RESULTS

A number of fluoro-, hydroxy- and methyltryptophans was examined, in addition to tyrosine, tyrosine peptides, tryptophan peptides, kynurenine and a series of glycine peptides. The results are given in Tables I and II, where a comparison is made between copper sulphate and non-complexing eluents such as sodium chloride and sodium sulphate.

DISCUSSION

The separation of enantiomers is diminished or non-existent with $CuSO_4$ as eluent in all instances (Table I). The fact that there is a slight

TABLE I

R_F VALUES OF AMINO ACIDS AND PEPTIDES ON CELLULOSE THIN LAYERS WITH 0.1 *M* SODIUM CHLORIDE AND 0.05 *M* COPPER(II) SULPHATE SOLUTIONS AS ELUENTS

Compound[a]	0.1 *M* NaCl		0.05 *M* $CuSO_4$	
	R_F	ΔR_F	R_F	ΔR_F
L-Tyr	0.75	0.06	0.78	0.04
D-Tyr	0.81		0.82	
L-$(Tyr)_2$	0.75		0.77	
L-$(Tyr)_3$	0.71		0.73	
DL-Kynurenine	0.48	0.07	0.71	One spot only
	0.55			
L-Trp	0.57	0.05	0.70	0.04
D-Trp	0.62		0.74	
L-$(Trp)_2$	0.48		0.38	
DL-4-Methyl-Trp	0.29	0.07	0.48	One spot only
	0.36			
DL-5-Methyl-Trp	0.37	0.09	0.50	0.03
	0.46		0.53	
DL-6-Methyl-Trp	0.32	0.07	0.52	One spot only
	0.39			
DL-7-Methyl-Trp	0.34	0.07	0.54	One spot only
	0.41			
DL-4-Fluoro-Trp	0.38	0.06	0.55	One spot only
	0.44			
DL-5-Fluoro-Trp	0.39	0.06	0.575	One spot only
	0.45			
DL-6-Fluoro-Trp	0.41	0.05	0.565	One spot only
	0.46			
DL-5-hydroxy-Trp	0.31	0.05	0.45	One spot only
	0.36			
Trp–Tyr	0.595		0.545	
Gly–Trp	0.68		0.74	
Gly, $(Gly)_2$, $(Gly)_3$, $(Gly)_4$, $(Gly)_5$, $(Gly)_6$	Move with the solvent front		Move with the solvent front	

[a] Trp = Tryptophan; Tyr = tyrosine; Gly = glycine.

TABLE II

R_F VALUES OF SUBSTITUTED TRYPTOPHANS ON CELLULOSE THIN LAYERS WITH VARIOUS CONCENTRATIONS OF COPPER(II) SULPHATE SOLUTION AS ELUENTS

Compound [a]	0.1 *M* NaCl	0.5 *M* Na_2SO_4	1 *M* Na_2SO_4	0.05 *M* $CuSO_4$	0.5 *M* $CuSO_4$	1 *M* $CuSO_4$
DL-4-Fluoro-Trp	0.38 0.44	0.33 0.37	0.24 0.28	0.55	0.61	0.51
DL-5-Fluoro-Trp	0.39 0.45	0.38 0.43	0.28 0.33	0.57	0.64	0.56
DL-6-Fluoro-Trp	0.41 0.46	0.35 0.40	0.28 0.31	0.58	0.63	0.55
DL-5-Hydroxy-Trp	0.31 0.46	0.25 0.30	0.22 0.26	0.45	0.53	0.46
DL-4-Methyl-Trp	0.29 0.36	0.23 0.24	0.14 0.20	0.48	0.50	0.44
DL-5-Methyl-Trp	0.37 0.46	0.27 0.34	0.18 0.225	0.50 0.53	0.55 0.60	0.47 0.48
DL-6-Methyl-Trp	0.32 0.39	0.26 0.33	0.15 0.21	0.52	0.55	0.37
DL-7-Methyl-Trp	0.34 0.41	0.26 0.31	0.16 0.20	0.54	0.58	0.45
DL-Kynurenine	0.48 0.55			0.71	0.77	0.74

[a] For abbreviations see footnote to Table I.

separation between D- and L-tryptophan and between D- and L-5-methyltryptophan indicates that also other pairs could have small chiral effects, which are too small to be detected in thin-layer chromatography, however.

In $CuSO_4$ solutions the R_F values of the tryptophan derivatives are always higher than in non-complexing solvents by about 0.10–0.15 units, except for tryptophan peptides, which either adsorb more strongly (as in the case of Trp–Trp and Trp–Tyr) or increase only by 0.06 (in the case of Gly–Trp). Tyrosine and tyrosine peptides hardly differ in the presence and absence of Cu^{2+} ions. Kynurenine is desorbed with an R_F increase of 0.2 with a simultaneous loss of enantiomer separation.

Copper ions are not retained on cellulose from aqueous solutions and there is no retardation of the "copper front" in these chromatograms. A size-exclusion effect due to the larger size of the copper complexes is unlikely as much larger molecules such as peptides adsorb well. Except for Dalgliesh [2], no specific discussion of the mechanism of chiral separations on cellulose seems to have been published, but it seems likely from the extensive work on the very similar cyclodextrins (see, *e.g.*, ref. 3) that hydrophobic adsorption and hydrogen bonding are the important contributors to it.

A loss of chiral resolution and an increase in R_F value was also observed when the amino group of tryptophan is methylated. Although an additional methyl group usually increases hydrophobicity, DL-α methyltryptophan moves as a single spot with an R_F value about 0.1–0.15 higher than that for tryptophan [4].

Both results seem to agree with Dalgliesh's theory [2] that a "three-point" adsorption is necessary for chiral separations. As soon as the NH_2 (in the case of the α-methyltryptophan) or both the NH_2 and the COOH groups (in the case of the Cu^{2+} complexes) are unable to participate in the adsorption, the R_F value increases and the chiral separation diminishes.

Several concentrations of $CuSO_4$ were examined (see Table II) and we attempted to correlate the results with R_F values obtained with NaCl and Na_2SO_4. In $CuSO_4$ the R_F values

increase with increase in concentration from 0.05 to 0.5 *M* and then decrease from 0.5 to 1 *M*. There is no similar behaviour with sodium or ammonium salts (see ref. 4). It could be that with increase in the Cu^{2+} concentration from 0.05 to 0.5 *M* there is also an increase in complex formation, which is unlikely in view of the high stability constants, or that there is a considerable concentration gradient along the chromatogram in 0.05 *M* $CuSO_4$. Both are rather unlikely from previous experience. Hence this behaviour cannot be explained at present. The decrease in R_F values with increase in concentration from 0.5 to 1 *M* is consistent with the usual salting-out effect on increasing the electrolyte concentration.

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