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Adsorption chromatography on cellulose

VII. Chiral separations on cellulose with aqueous solvents

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ABSTRACT

The chiral properties of different celluloses were examined in adsorption chromatography from aqeous solvents. Much better separations were obtained on microcrystalline cellulose than on "native" cellulose for D- and L-tryptophan and D- and L-methyltryptophan. The separation of D- and L-5-fluorotryptophan was achieved using long (40 cm) thin layers of microcrystalline cellulose.

INTRODUCTION

In Part VI [1], some variables of the separation of D- and L-5-methyltryptophan on cellulose with aqueous solvents were described. It was observed that the usually good separation obtained on Merck DC Plastikfolien (Art. 5577) could not be obtained on MN Polygram CEL 300 layers.

Wollenweber [2] subsequently confirmed these observations, commenting that the Merck layers contain microcrystalline cellulose (Avicel) whereas the MN Polygram CEL 300 layers are made from native cellulose. If the MN Polygram CEL 400 layers of microcrystalline celulose had been used, a separation equal to that on the Merck layers would have been obtained. We could confirm this as shown in Fig. 1. No explanation can be offered for this difference in behaviour [2].

From the vast literature on partition chromatography on cellulose we had the general impression that different kinds of cellulose behave essentially alike. They could differ in ion-exchange capacity and in the amounts of amorphous regions in the crystalline structure, but were still essentially the same anhydroglucose units joined together in the 1 4 configuration in chains of about 3000 units.

Since we started to investigate this problem, we have found two other examples of large differences in chromatographic behaviour between various celluloses.

Müller [3] separated mixed chloro- and bromoosmiates(IV) and mixed chloro- and bromorhenates (IV) on MN Polygram CEL 300-50 layers 40 cm long with 30% aqueous sulphuric acid. Although the R_F differences between members of the series are only 0.02–0.03, "baseline" separations were obtained (and could be repeated by us). On microcrystalline MN Polygram CEL 400 layers, however, no separation took place. It was also found that not all lots of the CEL 300-50 layers gave good separations [4]. here is another interesting aspect to these separations. It is generally assumed that in thin-layer chromatography (TLC) a maximum plate number is achieved with 10–12 cm development (and thus most ready-made layers are sold in dimensions based on this). However, Müller [3] obtained his separations with 40 cm development (or more). This is consistent with our inability to obtain good

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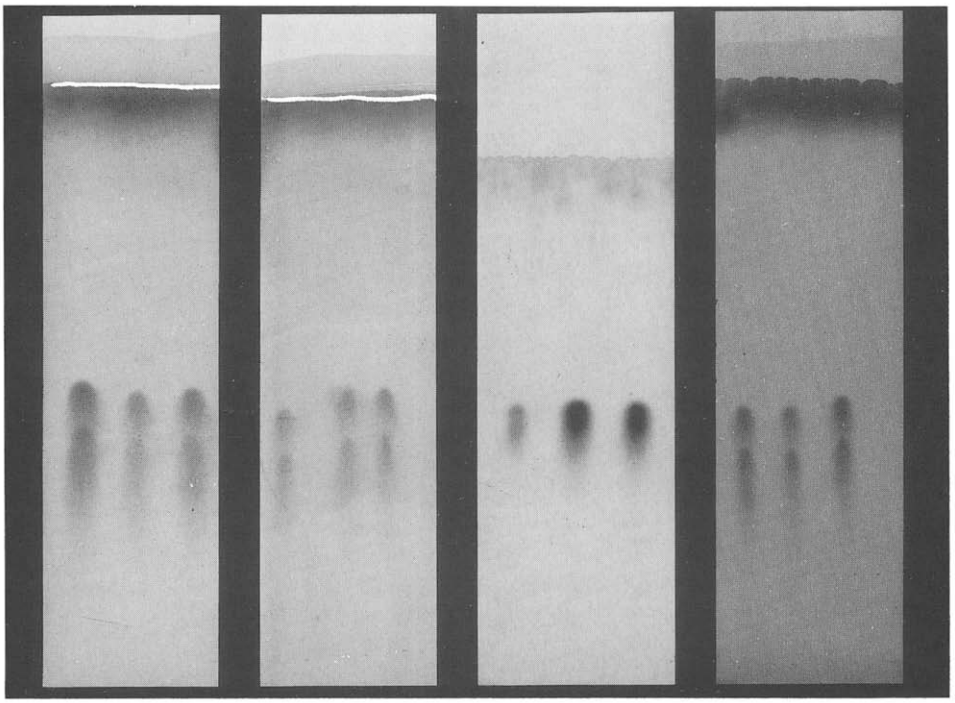

Fig. 1. Chromatograms of DL-5-methyltryptophan developed with 1 *M* NaCl on (from left to right) Merck Art. 5577 Plastikfolien, the same, MN CEL 300 and MN CEL 400 cellulose thin layers.

separations on shorter layers (20 × 20 cm). A re-examination of these generally accepted principles might therefore be justified.

Another example is shown in Fig. 2. In Fig. 2a the chromatograms of some ball-point pen inks are compared for Whatman No. 3MM (*i.e.*, native cellulose), CEL 300 and CEL 400 thin layers. Whatman 3MM and CEL 400 (*i.e.*, microcrystalline cellulose) are similar, whereas CEL 300 ("native" cellulose) shows much stronger adsorption. In Fig. 2b another set of ball-point pen inks (chosen at random) are compared for CEL 300, CEL 400 and Merck 5577 thin layers. Again, CEL 300 shows much stronger adsorption. Ion exchange should play only a minor role in these systems as a high salt concentration was used throughout [5].

In this paper some results from a comparison of thin layers of native cellulose, microcrystalline cellulose and cellulose papers in the separation of optical isomers are presented.

EXPERIMENTAL

All chromatograms were obtained by ascending development in glass containers with a tightly closed glass lid. For Whatman 3MM papers and for the 40-cm thin layers this usually took 7–8 h. The chromatograms were then dried in an oven at 70–80°C. Colour with ninhydrin developed very slowly on the thin layers. The following media were used: Whatman No. 3MM papers extra thick for chromatography; Merck Art. 5577 DC Plastikfolien Cellulose, layer thickness 0.1 mm, 20 cm × 20 cm; Merck Art. 5787 HPTLC-Fertigplatten Cellulose, 10 cm × 10 cm; Macherey–Nagel MN-Polygram CEL 300 precoated plastic sheets for TLC, 40 cm × 80 cm; Macherey–Nagel MN Polygram CEL 300-50 layers, 0.5 mm Cellulose MN 300 on glass, 40 cm × 20 cm; and Macherey–Nagel MN Polygram CEL 400 for TLC, Art. 801114, 0.1 mm microcrystalline cellulose precoated plastic sheets, 40 cm × 20 cm.

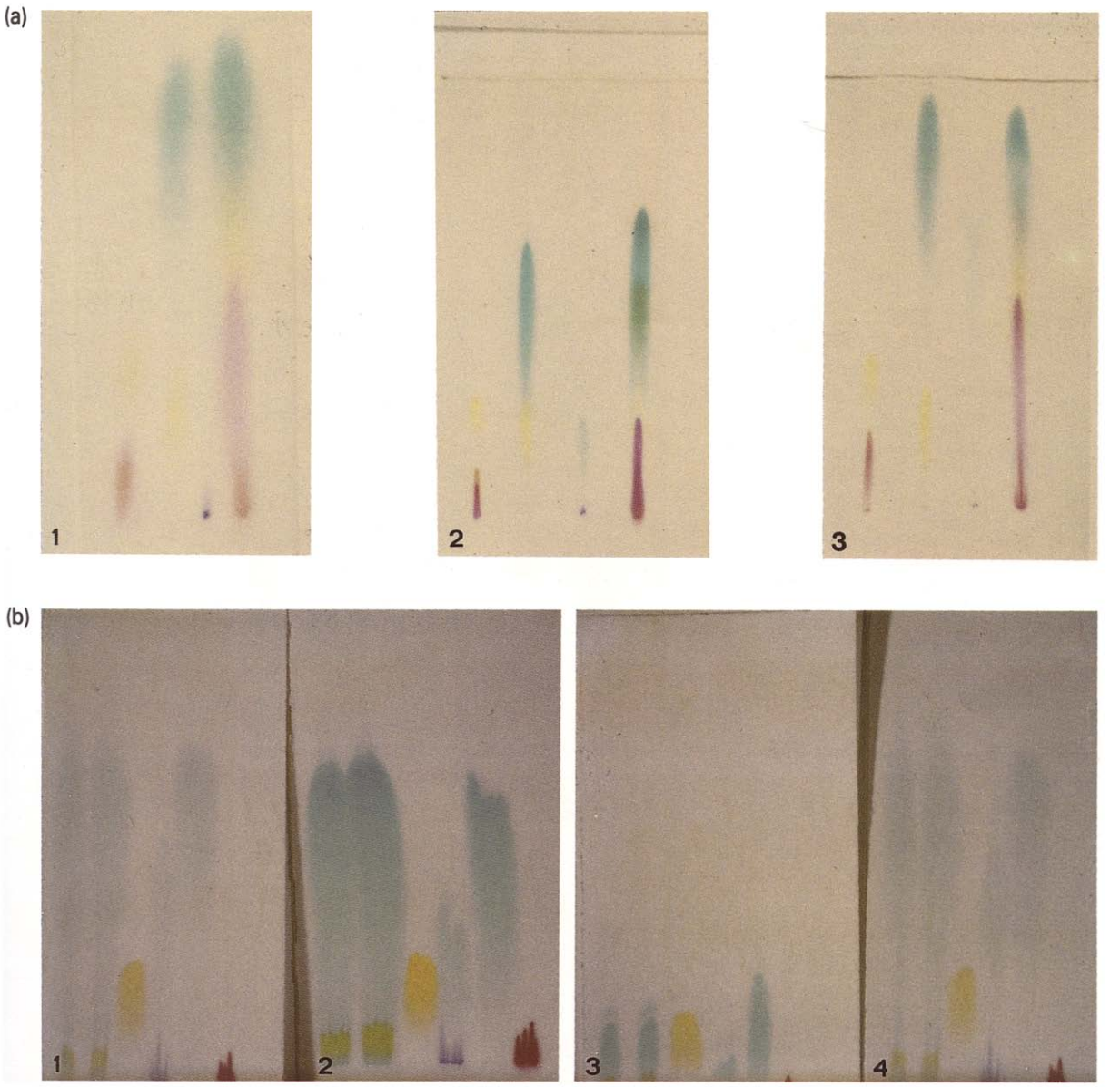

Fig. 2. Chromatograms of ball-point pen inks developed with (a) (1) Whatman 3MM paper, (2) MN CEL 300 cellulose thin layer and (3) MN CEL 400 cellulose thin layer, all developed with 1 *M* NaCl; (b) (1) MN CEL 400 thin layer cellulose, (2) Merck Art. 5577 thin layer cellulose, (3) MN CEL 300 thin layer and (4) MN CEL 400 thin layer cellulose.

RESULTS

Separations of the antipodes of 5-methyltryptophan

Figs. 3 and 4 show the chromatograms obtained on short and long thin layers and on Whatman 3MM paper.

The chromatogram on the long CEL 300 layer shows that there is a separation that cannot be discerned on the short layers. The R_F values on all three media were calculated and the results are given in Table I. Thus, on the CEL layer, made of "native" cellulose, there is merely a smaller separa-

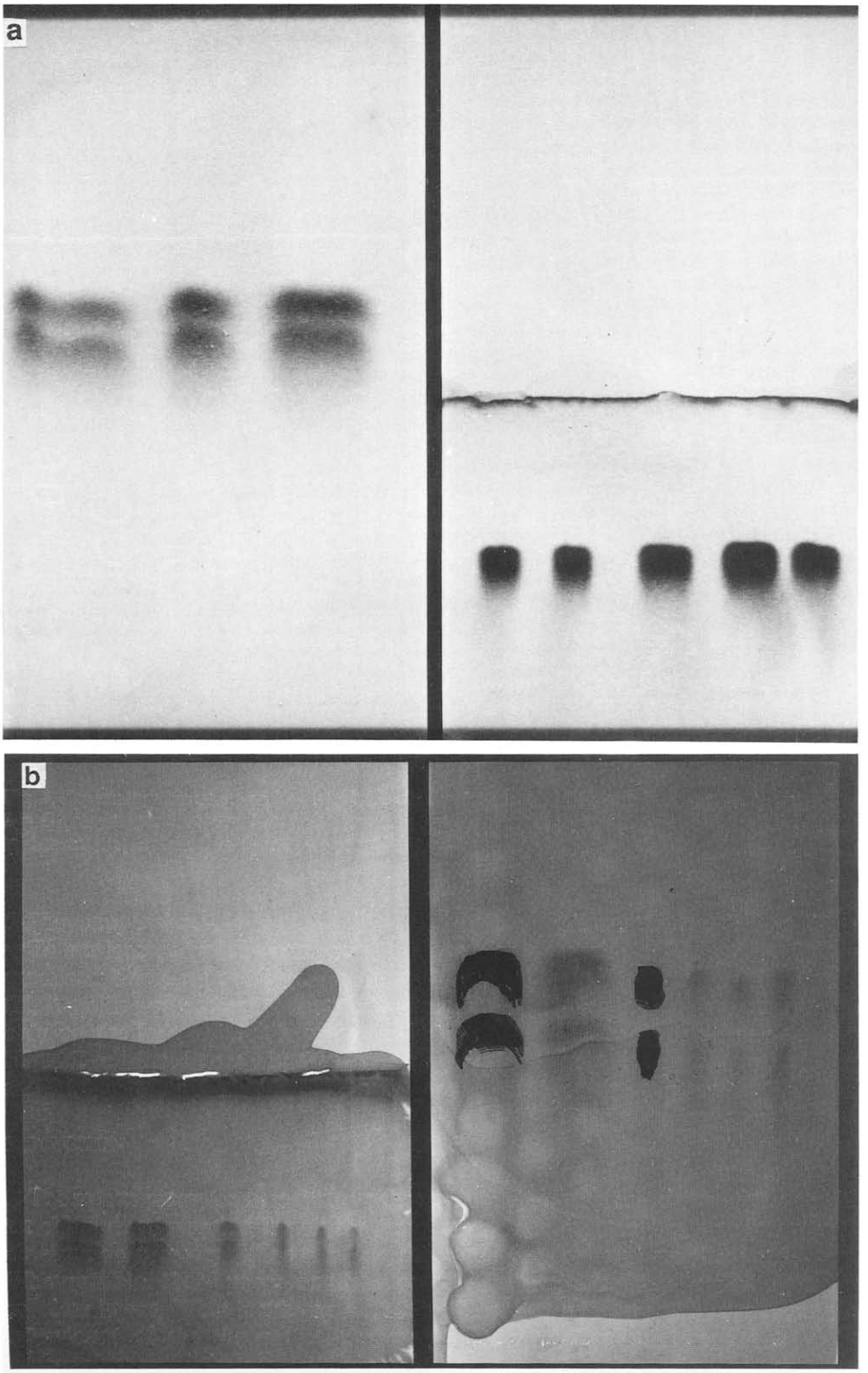

Fig. 3. Chromatogram of 5-methyltryptophan developed with 1 *M* NaCl on (a) 40-cm long MN Polygram CEL 300-50 layer, (left) long development and (right) short development; (b) 40-cm MN Polygram CEL 400 sheets, (left) short development and (right) long development. This chromatogram later developed a spotted background owing to the oven shelf on which it was dried. The actual spots were emphasized with ink.

tion factor, which means that visual detection of the two adjacent zones is difficult on short layers. On the chromatogram in Fig. 4 D- and L-tryptophan are also shown. As was previously recorded by Weichert [6], there is an R_F difference of about 0.03, which, however, is insufficient for a complete separation. It is also interesting that the plate numbers for the three media are not radically different when the same length of development is compared.

Separations of other methyltryptophans

In a previous paper [5], good separations of 1-, 5-, 6- and 7-methyltryptophan on short (20 cm × 20 cm) Merck cellulose layers were reported. We therefore wanted to examine how these would behave on long layers. Fig. 5a is a drawing of a chromatogram obtained on CEL 400 showing baseline separation of all the four methyltryptophans; D- and L-phenylalanine move too fast in this system and do not separate (Fig. 5b). On CEL 300 layers (Fig. 6) only adjacent zones are obtained with long development. On Whatman No. 3MM paper 1-methyltryptophan produces a single spot, whereas the 6- and 7-methyltryptophan each produce two discernible adjacent spots (Fig. 7). α-Methyltryptophan is not separated even with long development or on short Merck plates [7].

Kynurenine

Kynurenine has previously been separated into its antipodes by Dalgleish [8] and Weichert [6] by paper chromatography. The antipodes separated well on all the thin layers used (see Figs. 5 and 6) except for the Whatman 3MM paper. We used this separation to calculate some plate numbers (Table II).

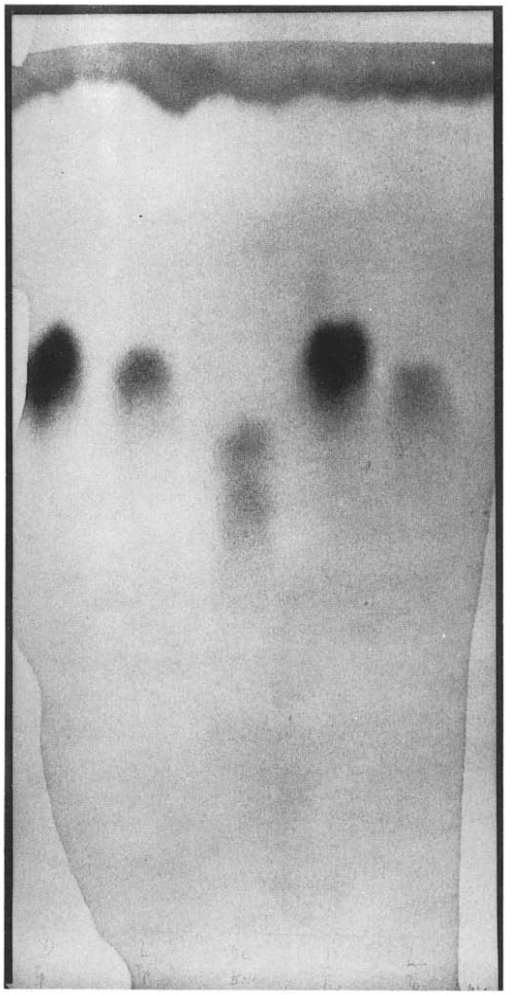

Fig. 4. Chromatogram of (from left to right) D-tryptophan, L-tryptophan, DL-5-methyltryptophan, D-tryptophan and L-tryptophan on Whatman 3MM paper, 40-cm long, with 1 *M* NaCl.

TABLE I

DATA FOR THE SEPARATION OF D- AND L-5-METHYLTRYPTOPHAN

Medium	R_F		Separation factor, α	Plate number of the faster spot, *n*
	D-	L-		
MN CEL 300-50 (40 cm long)	0.50	0.45	1.22	2000
Whatman 3MM paper	0.54	0.47	1.32	1600
CEL 400 (40 cm long)	0.48	0.385	1.46	1800

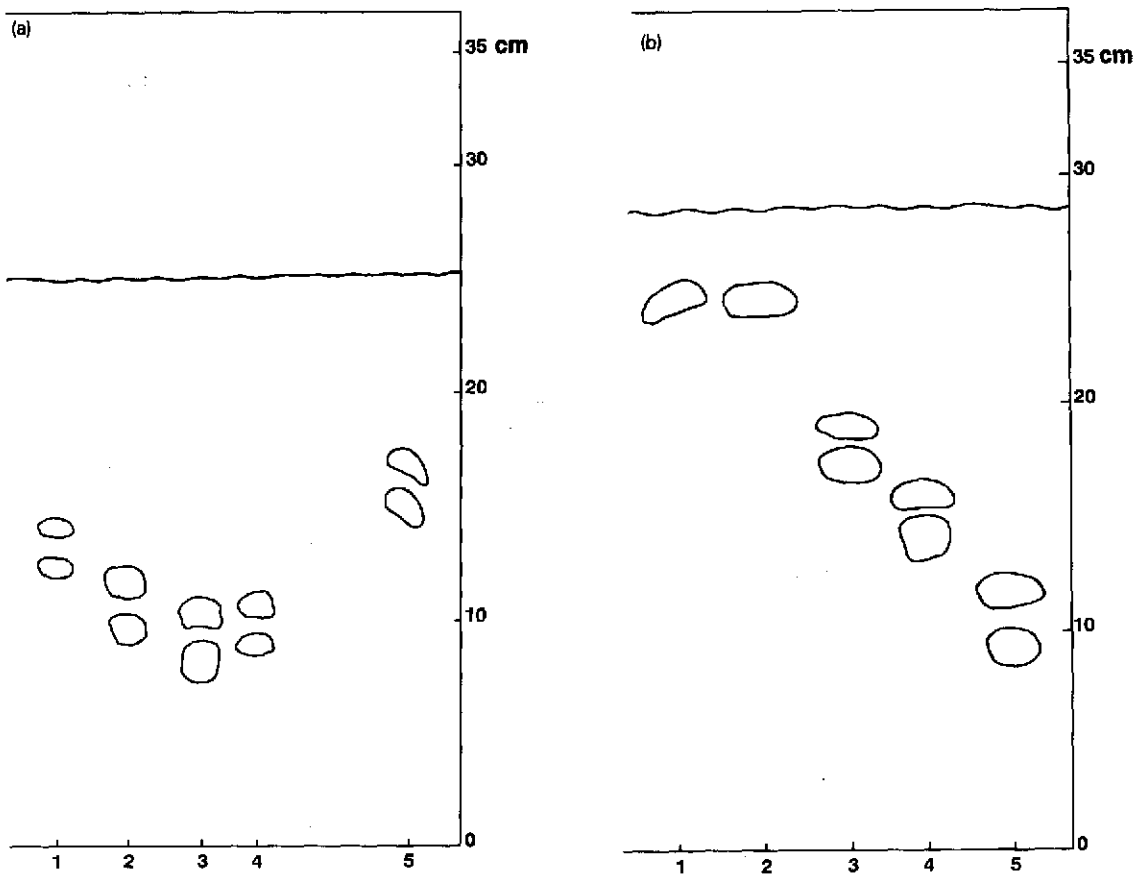

Fig. 5. Chromatograms on 40-cm long MN CEL 400 developed with 1 *M* NaCl: (a) 1 = 1-methyltryptophan; 2 = 5-methyltryptophan; 3 = 6-methyltryptophan; 4 = 7-methyltryptophan; 5 = kynurenine; (b) 1 = D-phenylalanine; 2 = L-phenylalanine; 3 = kynurenine; 4 = 1-methyltryptophan; 5 = 6-methyltryptophan.

5-Fluorotryptophan

To our knowledge, this compound has not been examined before on cellulose. As shown in Table III, it yields well separated spots of the antipodes only on the long CEL 400 layers and on Merck HPTLC cellulose layers with R_F differences of 0.06 and 0.04, respectively.

DISCUSSION

The results presented here indicate that the highest chiral discrimination for all the compounds is obtained on microcrystalline cellulose, followed by Whatman No. 3MM paper, and least on "native" cellulose layers. What is the cause of the different behaviours of the various celluloses? We have shown that the differences are only a matter of degree.

From our results the reasons for the differences are not clear; however, if we assume that microcrystalline cellulose has a greater surface area than "native" cellulose, this could account for some of the results, but not all (*e.g.*, the results of Müller [3]). Obviously, however, there is a close resemblance with the observations made from experiments with cellulose triacetate, in which case the ordered structure, associated with microcrystalline regions of the sorbent, has been found to play a major role in the chiral discrimination process [9].

Against the general opinion in TLC we obtained a better result with long development although the plate numbers calculated by the usual procedure

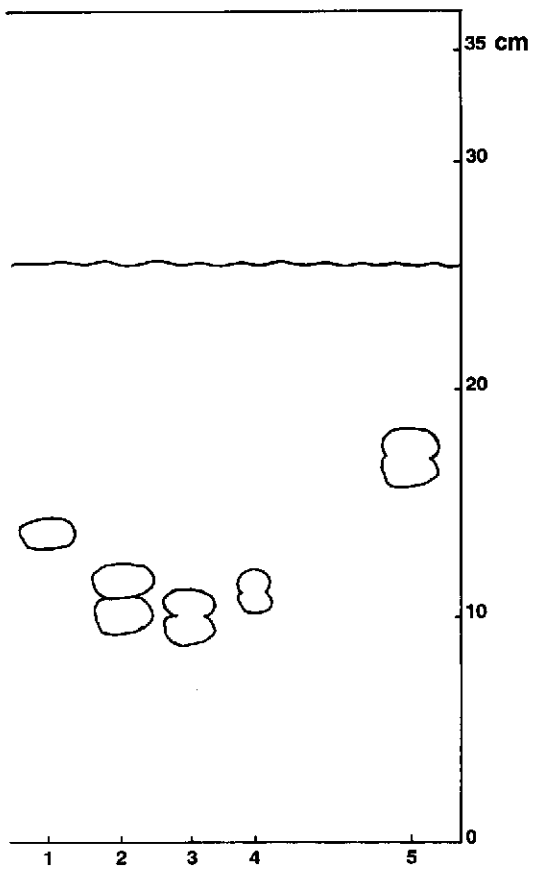

Fig. 6. Chromatogram on 40-cm long MN CEL 300 developed with 1 *M* NaCl. 1 = 1-methyltryptophan; 2 = 5-methyltryptophan; 3 = 6-methyltryptophan; 4 = 7-methyltryptophan; 5 = kynurenine.

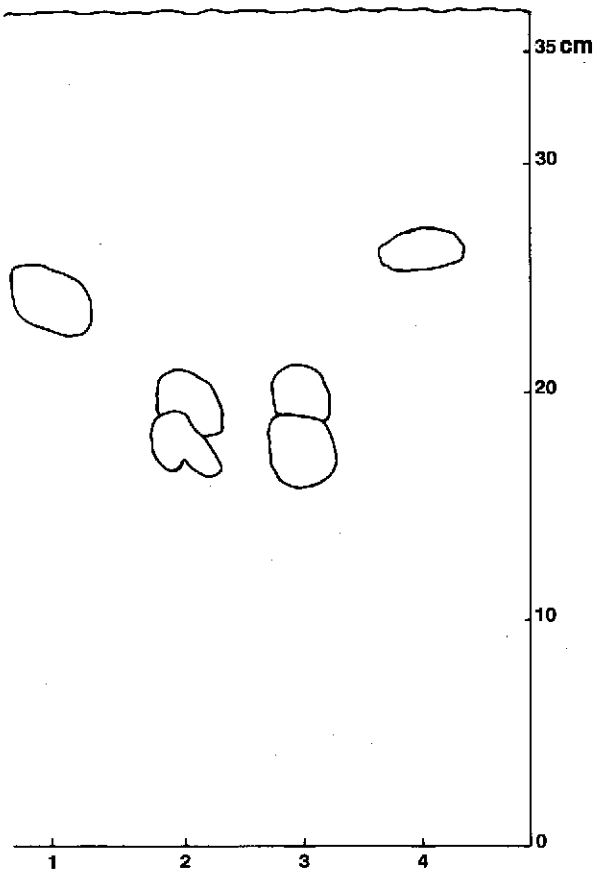

Fig. 7. Chromatogram on 40-cm long Whatman No. 3MM paper developed with 1 *M* NaCl. 1 = 1-Methyltryptophan; 2 = 6-methyltryptophan; 3 = 7-methyltryptophan; 4 = α-methyltryptophan.

TABLE II

DATA FOR THE SEPARATION OF D- AND L-KYNURENINE

Medium	Isomer	Spot front (mm)	Spot rear (mm)	Liquid front (mm)	*n*
Merck Art. 5577 DC	L-	64	61	103	7000
Plastikfolien Cellulose	D-	58.5	53	103	1600
Merck Art. 5787	L-	60	57	86	6100
HPTLC-Fertigplatten Cellulose	D-	55	51	86	2610
MN CEL 400	L-	263	247	373	4000
(40 cm long)	D-	243	225	373	2700
MN CEL 300-50 plates	L-	181	169	254	4050
(40 cm long)	D-	169	157	254	2550

TABLE III

DATA FOR THE SEPARATION OF D- AND L-FLUOROTRYPTOPHAN

Medium	R_F	
	D	L
MN CEL 400 (40 cm long)	0.57	0.51
Merck Art. 5787 HPTLC-Fertigplatten Cellulose	0.51	0.47
Merck Art. 5577 DC Plastikfolien Cellulose	0.54 (single spot)	
MN CEL 300 (40 cm long)	0.54 (single spot)	
Whatman 3MM paper	0.56 (single spot)	

were then lower than with short development.

In all the chromatograms the slower spot is longer and hence has a lower plate number. This could be explained by the Dalgleish "three-point" model: When three interactions are really involved, the adsorption process kinetics are slower than when mainly "two points" are involved.

We calculated the ΔR_M values for the usual functional groups on cellulose [10]. The maximum ΔR_M between two optical isomers observed here was 0.165 (for 5-methyltryptophan on MN CEL 400 layers), which is less than the ΔR_M of the usual functional groups (about half of the ΔR_M of the NH_2 group).

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