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Note

Adsorption chromatography on cellulose

VI. Further studies on the separation of D-and L-tryptophan on cellulose with aqueous solvents

M. LEDERER
Institut de Chimie Minerale et Analytique, Université de Lausanne, Place du Château 3, CH-1005 Lausanne (Switzerland)

In a previous paper[1] we described some of the interesting aspects of the separation of the optical isomers of tryptophan on cellulose using aqueous solvents. The separation factors were almost independent of temperature and of the salt concentration. Further, the addition of a methyl group to various ring positions did not produce great changes in the separation of the optical isomers.

In this paper a number of further aspects of this separation are reported: the effect of various cations and anions in the eluent, the effect of changing the polarity of the eluent by addition of methanol and the effect of the kind of cellulose used as the stationary phase.

EXPERIMENTAL

All developments were performed in small, well stoppered glass jars [for thin-layer chromatography (TLC)] or in 40-cm tall cylinders (for paper chromatography) at room temperature (18–20°C). The development of thin layers took between 10 and 25 min, depending on the length of development. The developed chromatograms were dried for a few minutes in an air oven at 90°C, dipped in a solution of ninhydrin in acetone and heated again for several minutes. Full colour was usually produced within 24 h.

RESULTS AND DISCUSSION

Effect of cations and anions in the aqueous developing solvent

Previously we had observed[1] that the separation of optical isomers of tryptophan and substituted tryptophans is essentially the same in aqueous LiCl and aqueous $(NH_4)_2SO_4$ on Merck Art. 5577 DC Plastikfolien cellulose, and also that the temperature and the concentration of the salt had little influence on the separation.

We have now extended the work to the following salts:

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(a) 0.1 *M* NaCl, 1 *M* NaCl, 0.1 *M* KBr and 1 *M* KI. The separations obtained were identical, hence the size of the anion has no measurable effect.

(b) We also developed chromatograms with 1 *M* NaCl, 0.25 *M* $MgSO_4$, 0.25 *M* $CaCl_2$, 0.25 *M* $BaCl_2$ and 0.033 *M* $Al_2(SO_4)_3$. As shown in Fig. 1, there is no visible difference between the various chromatograms.

(c) When various salts of transition metals were used as eluents, only Cu(II) changed the chromatogram. Fig. 2 shows that 0.25 *M* $CdSO_4$, 0.05 *M* $MnSO_4$, 0.05 *M* $NiSO_4$, 0.05 *M* $Co(NO_3)_2$ and 0.05 *M* $ZnSO_4$ all yielded a separation of the two optical isomers of 5-methyltryptophan. With 0.05 *M* $CuSO_4$, on the other hand, only one spot was observed. Hence it seems complexation with Cu(II) interferes with the chiral distinction between the two optical isomers. We consider it remarkable that none of the other transition elements has an influence here, as they are well known to form complexes.

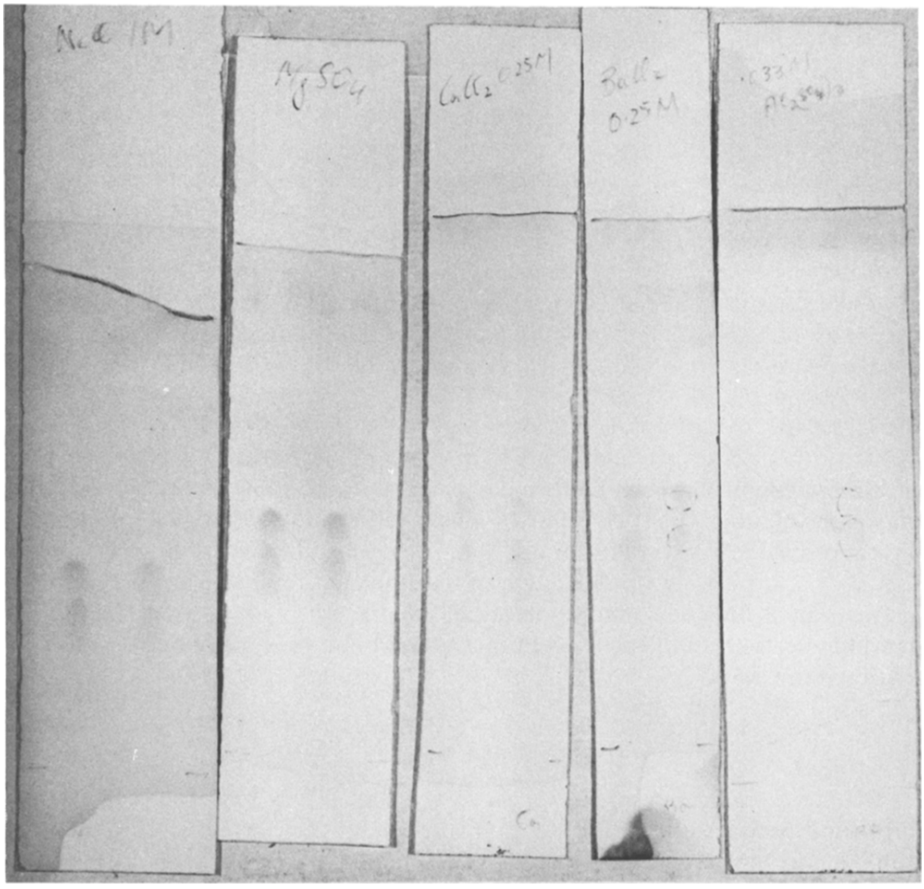

Fig. 1. Thin-layer chromatograms of DL-5-methyltryptophan on Merck Art. 5577 DC Plastikfolien cellulose. Eluents (from left to right): 1 *M* NaCl, 0.25 *M* $MgSO_4$, 0.25 *M* $CaCl_2$, 0.25 *M* $BaCl_2$ and 0.033 *M* $Al_2(SO_4)_3$.

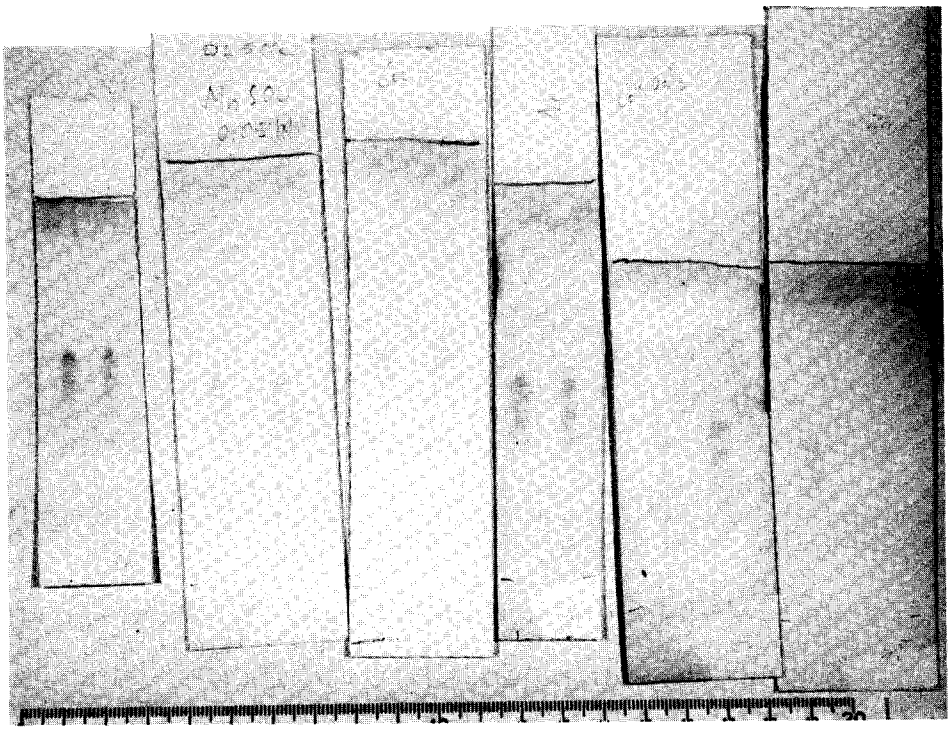

Fig. 2. Thin-layer chromatograms of DL-5-methyltryptophan on Merck Art. 5577 DC Plastikfolien celullose. Eluents (from left to right): 0.25 *M* $CdSO_4$, 0.05 *M* $MnSO_4$, 0.05 *M* $CuSO_4$, 0.05 *M* $NiSO_4$, 0.05 *M* $Co(NO_3)_2$, 0.05 *M* $ZnSO_4$.

Effect of methanol in the eluent

Our previous work on adsorption on cellulose from aqueous solvents showed that the main adsorption mechanism is "hydrophobic" and the only amino acid that is strongly adsorbed is the heterocyclic tryptophan[2]. Yuasa *et al.*[3] separated optical isomers on cellulose with solvent mixtures where a liquid–liquid partition mechanism would be expected. We therefore wanted to investigate the effect that a change in polarity would have on our kind of separation. This is shown in Fig. 3.

Mixtures of 1 *M* NaCl and methanol yield essentially the same separation of the isomers of 5-methyltryptophan between 0 and 75% methanol on Merck Art. 5577 DC Plastikfolien cellulose. Only with 80% methanol is the separation altered with lower R_F values and worse, or no, resolution.

We conclude that the chiral distinction mechanism is not disturbed by minor changes in polarity.

Effect of the structure of the cellulose

All experiments reported above, and also those in the previous study[1] were carried out with Merck cellulose thin layers. Essentially the same separations were also obtained on Merck Art. 5787 Fertigplatten cellulose HPTLC plates (10 cm × 10 cm), as is shown in Fig. 4.

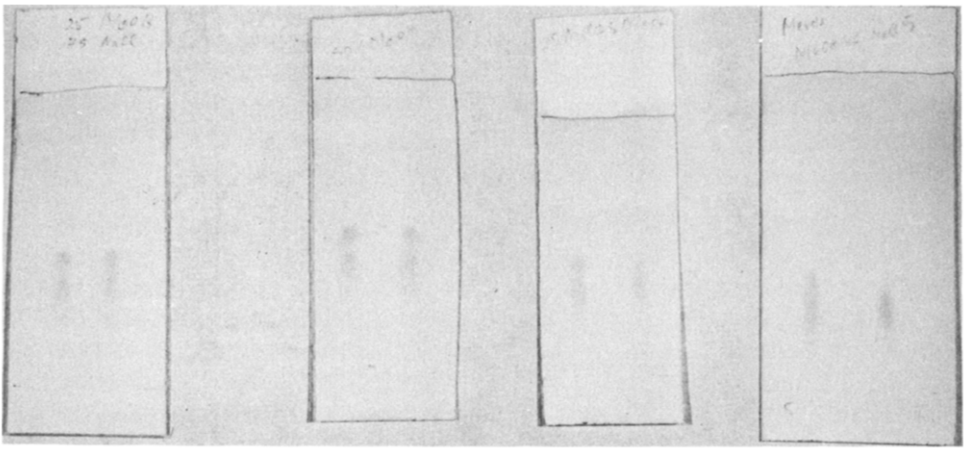

Fig. 3. Thin-layer chromatograms of DL-5-methyltryptophan Merck Art. 5577 DC Plastikfolien cellulose. Eluents (from left to right): 1 *M* NaCl mixed with methanol in the proportions 75:25, 50:50, 25:75, 10:80. On the last chromatogram the spot on the right is DL-tryptophan.

Several types of Macherey–Nagel cellulose layers, MN Polygram Cel 300 UV_{254}, MN Polygram Cell 300 (40 mm × 80 mm) and MN Polygram Cel 300 (20 cm × 20 cm), were tried but yielded no separations of the optical isomers. Typical results are shown in Fig. 5. The lack of separation is certainly not due to the short development (*cf.*, Fig. 4), as longer thin layers also did not yield separations.

On Whatman 1 and 3MM filter-papers, no distinct separation of D- and L-5-methyltryptophan could be obtained, but the DL-mixture usually gave an

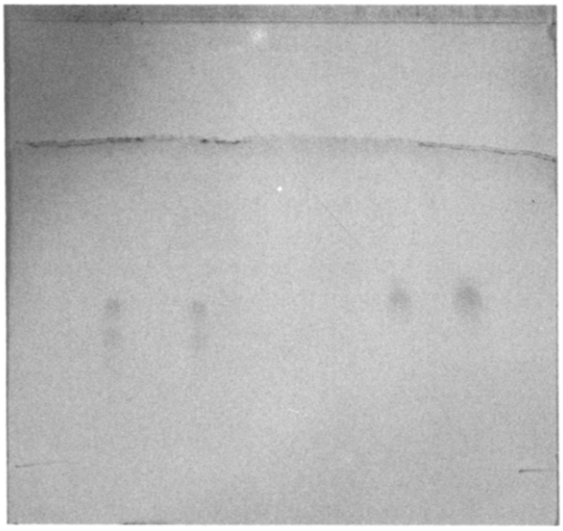

Fig. 4. Thin-layer chromatogram on a Merck Art. 5787 Fertigplatten cellulose HPTLC plate (10 cm × 10 cm) developed with 0.5 *M* NaCl. Spots (from left to right): DL-5-methyltryptophan (two spots); DL-tryptophan (two spots).

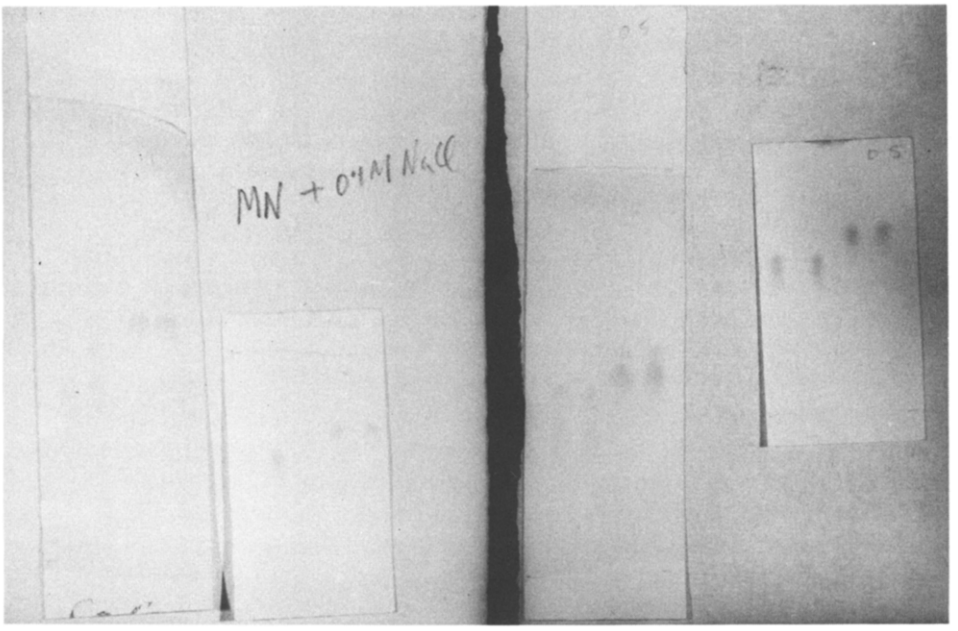

Fig. 5. Thin-layer chromatograms of DL-5-methyltryptophan (two spots on the right) and DL-tryptophan (two spots on the left) developed with 0.1 *M* NaCl (left) and 0.5 *M* NaCl (right) on Merck Art. 5577 DC Plastikfolien cellulose (right) and MN Polygram Cel 300 (40 mm × 80 mm) (left).The latter layers have the same dimensions as the chromatograms shown in Fig. 4.

elongated spot, as previously observed by Weichert[4], indicating a partial separation.

The structure of cellulose was discussed in a recent review by Kremer and Tubb[5], and their account suggests that the differences between the various cellulose supports are due to the content of "amorphous regions", a characteristic that may be enhanced by the mechanical "beating" action during the pulping process. They described these regions as a "two-dimensional colloidal system in which the surface fibrillae have two dimensions in the colloidal range but are anchored to the fibre in the third dimension".

It would be interesting to establish whether this chiral effect of cellulose can be altered at will by a suitable manufacturing process.

REFERENCES

1 A. O. Kuhn, M. Lederer and M. Sinibaldi, *J. Chromatogr.*, 469 (1989) 253.
2 A. O. Kuhn and M. Lederer, *J. Chromatogr.*, 440 (1988) 165.
3 S. Yuasa, A. Shimada, M. Isoyama, T. Fukuhara and M. Itoh, *Chromatographia*, 21 (1986) 79.
4 R. Weichert, *Acta Chem. Scand.*, 8 (1954) 1542.
5 R. D. Kremer and D. Tubb, *Int. Lab.*, July/August (1989) 40.