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THE OPTICAL RESOLUTION OF DL-AMINO ACID MENTHYL ESTER DERIVATIVES BY PAPER CHROMATOGRAPHY

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Several DL-amino acid menthyl ester hydrochlorides were synthesized and attempted to resolve by means of paper chromatography with various buffer solutions. It was found that the three amino acid (Phe, Trp, and Tyr) derivatives were completely resolved with "CM-Cellulose" paper.

A number of methods for the optical resolution of DL-amino acids have been known. Paper chromatography is one of the important methods because of its operational facility and availability with a minute amount of material. Several papers on the optical resolutions of DL-amino acids by paper chromatography have already appeared. For example, Kotake et al.[1] attempted to resolve tyrosine, glutamine, and tyrosine-3-sulphonic acid, and also Berlingozzi et al.[2] resolved α-amino-phenylacetic acid.

Further studies were made for optical resolution of DL-amino acid menthyl ester derivatives by the seeding method[3], by the difference in the rates of formation of D- and L- menthyl esters[4], and by gas chromatography[5]. In the present study, we undertook to resolve several DL-amino acid menthyl ester hydrochlorides by means of paper chromatography.

DL-Amino acid menthyl ester hydrochlorides (abbreviated as DL-amino acid·M·HCl) were synthesized from DL-amino acids (alanine, phenylalanine, tryptophane, and tyrosine) and l-menthol by the use of the azeotropic method[3]. p-Toluenesulfonic acid was used as a catalyst. Similarly, D- and L-amino acid·M·HCl were synthesized and were used as the standard samples. Resolution was attempted by means of one dimensional ascending chromatography on cation-exchange "CM-Cellulose" paper[6] (Whatman CM 82) with various buffer solutions. After drying at 60° C, these chromatograms were developed with ninhydrin. The results of the experiments are shown in Table 1.

The chromatograms of four successful cases are shown in Fig. 1.

DL-Phe·M·HCl was resolved in two cases (Table 1. I and III), their Rf values being different (0.24 and 0.17). Chromatographic resolution of DL-Tyr·M·HCl and DL-Trp·M·HCl are shown in Fig. 1-c and d with Rf values 0.50 of L-isomer and 0,37~0.40 of D-isomer, respectively. And each resolved spot of DL-diastereomeric mixtures was identical with that of D- as well as L-isomer used as the standard.

A trial to resolve DL-Ala·M·HCl with various different buffer solutions was unsuccessful.

Table 1. Rf values of α-amino acid menthyl ester hydrochlorides.

Sample	I	II	III	IV
D-Ala·M·HCl	0.83	0.78	0.57	0.81
DL-Ala·M·HCl	0.81	0.79	0.57	0.81
L-Ala·M·HCl	0.79	0.79	0.57	0.80
D-Phe·M·HCl	0.00	0.00,(0.32)*	0.00	0.65
DL-Phe·M·HCl	0.00, 0.24	0.00, 0.32*	0.00, 0.17	0.65
L-Phe·M·HCl	0.24	(0.00),0.30*	0.17	0.66
D-Trp·M·HCl	0.00	0.00	0.00	0.37
DL-Trp·M·HCl	0.00	0.00	0.00	0.40, 0.00
L-Trp·M·HCl	0.00	0.00	0.00	0.00
DL-Tyr·M·HCl	0.00, 0.48*	0.00, 0.50	0.19	0.59
L-Tyr·M·HCl	(0.00),0.46*	0.50	0.19	0.59

The former of the two Rf values shows always that of D-isomer and the latter of the L-isomer.

solvent: I:acetic acid-sodium acetate (2.0M, pH=5.0) II:acetic acid-sodium acetate (1.5M, pH=5.4) III:sodium citrate-hydrochloric acid (2.0M, pH=3.0) IV:formic acid-ammonium formate (2.0M, pH=2.5) buffer.

( ):traceable extent *:tailing

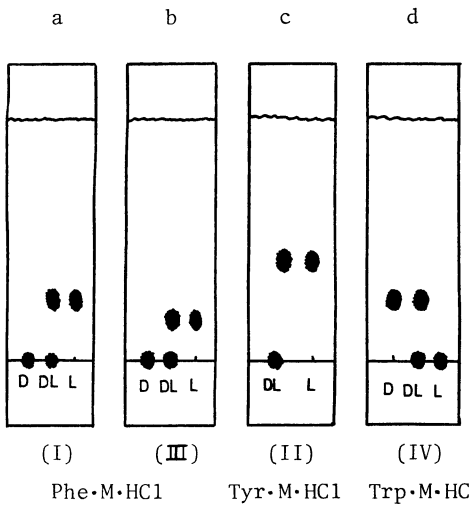

Fig. 1. Chromatograms of α-amino acid menthyl ester hydrochlorides.

Table 2. Rf values of DL-Phe·M·HCl in various pH and salt concentrations of buffer solution.

acetic acid-sodium acetate buffer		Rf value	
		CM-paper	normal-paper
pH=5.0	0.5M	0.48	0.48
	1.0M	0.44	0.36
	2.0M	0.00, 0.24	0.00, 0.24*
	3.0M	0.00, 0.22*	0.00, 0.18*
	5.0M	0.00	0.00
2.0M	pH=4.0	0.69	0.61
	pH=4.5	0.58	0.00, 0.41*
	pH=5.0	0.00, 0.24	0.00, 0.22*
	pH=5.5	0.00, 0.16*	0.00, 0.16*
	pH=6.0	0.00	0.00, 0.09*

The former of the two Rf values shows always that of D-isomer and the latter of the L-isomer. *:tailing

In the case of DL-Phe·M·HCl, we studied the effects of pH (4.0~6.0) and concentration (0.5~5.0M) in an acetic acid-sodium acetate buffer solution on the resolution with both "CM-Cellulose" paper and Toyo Roshi No.51.A paper (abbreviated hereafter as normal paper) in order to discuss the cause of the optical resolution.

The results are shown in Table 2, where it is seen that the Rf values decrease with the increasing salt concentrations of the buffer solution.

This phenomenon would be so-called a salting-out effect. It may, therefore, be presumed that the reason for the optical resolution in the concentration of 2.0 ~3.0M is due to the difference of the salting-out effect[7] and in some degree to the asymmetric adsorptive character[1] of the cellulose for both D- and L-diastereomer. As for the change of pH, the optical resolution was possible with "CM-Cellulose" paper at only pH=5.0~5.5, but in the case with the normal paper it was almost independent of pH change. The difference in both cases seemed to depend on the cation-exchange effect. Therefore, it is concluded that the combined effect of three factors, i.e., the salting-out effect, the asymmetric adsorptive character of the cellulose, and the cation-exchange is important to the complete optical resolution.

Furthermore, we attempted to confirm the optical resolution with column experiment. DL-Phe·M·HCl (0.5g) was passed through a 2×30 cm column of "CM-Cellulose" with a 2.0M acetic acid-sodium acetate buffer solution (pH=5.0). By this operation only L-Phe·M·HCl was eluted. When the eluent was changed to 0.05M acetic acid-sodium acetate buffer solution (pH=5.0), D-Phe·M·HCl was eluted. The L-rich fraction (F-A) and D-rich fraction (F-B) were thus obtain by this method. To each fraction a 50% aqueous solution of potassium carbonate was added and extracted with ether to obtain the free ester. After the ether solutions were dried over anhydrous sodium sulfate, the $[\alpha]_D$ values were determined. An aliquot of the solution was titrated with 0.01N hydrochloric acid, using methyl red as indicator to determine the content of the free ester. The values of $[\alpha]_D^{17}$ of F-A and F-B were found to be -26.9° (c:2.60×10^{-1}, in ether) and -72.0° (c:1.53×10^{-1}, in ether), respectively. As the values of $[\alpha]_D^{12}$ of L-Phe·M and D-Phe·M used as the standards were -22.5° and -72.5° (c:4.00×10^{-1}, in ether), respectively, these results show that the fractions F-A and F-B contain 91% optically pure L-Phe·M·HCl and 99% optically pure D-Phe·M·HCl, respectively.

The optical rotatory dispersion curves are shown in Fig. 2, from which it is apparent that the resolution of these DL-amino acid esters can be achieved with "CM-Cellulose" paper.

Finally mention should be made that paper chromatography may be used advantageously for optical resolution of various racemic compounds, since the salting-out effect and ion-exchange action simultaneously work in this method, so far as a difference in the relentiveness is observed between the enantiomers or diastereomers as in these cases.

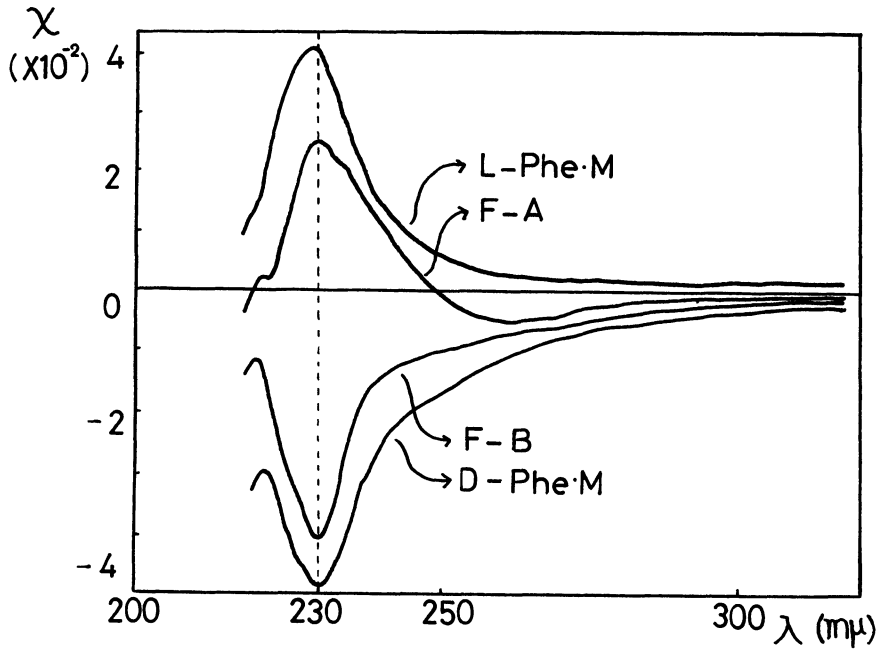

Fig. 2. Confirmation of the resolution of DL-Phe·M·HCl on the basis of ORD curves.
(c:4.00 × 10^{-1}, in ether, cell length: 1 mm)

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