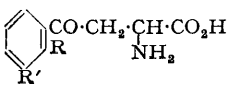
756. *The Optical Resolution of Aromatic Amino-acids on Paper Chromatograms.*

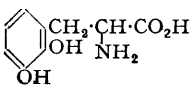
By C. E. DALGLIESH.

It has been found that many racemic aromatic amino-acids can be resolved into their optical enantiomorphs on paper chromatograms. Sufficient cases have been examined to enable a preliminary formulation to be made of the structural features necessary for resolution to occur.

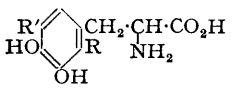
THE optical resolution of amino-acids on paper chromatograms was first reported in 1951 by Kotake, Sakan, Nakamura, and Senoh (*J. Amer. Chem. Soc.*, 1951, **73**, 2973). In a recent paper (Dalgliesh, *J.*, 1942, 137) it was shown that DL-kynurenine (I; R = NH_2, R′ = H) is resolved into its optical enantiomorphs, a result confirmed by Mason and Berg (*J. Biol. Chem.*, 1952, **195**, 515). Similar resolution has also been found to occur with 3-hydroxy-DL-kynurenine (I; R = NH_2, R′ = OH) (Dalgliesh, *Biochem. J.*, 1952, **52**, 3).



(I)



(II)



(III)

While investigating aspects of tyrosine metabolism the resolution of 2 : 5-dihydroxyphenyl-DL-alanine was observed, and other related amino-acids have therefore been investigated.

Resolution has also been found with 2 : 3-dihydroxyphenyl-DL-alanine (II) and 3 : 4-dihydroxy-2-methylphenyl-DL-alanine (III; R = Me, R′ = H), whereas none occurred with 3 : 4-dihydroxyphenyl-DL-alanine (III; R = R′ = H) or its 5-methyl derivative (III; R = H, R′ = Me). Resolution was shown by the appearance of two spots on the chromatogram of a chemically pure racemate, these being of equal size, shade, and intensity by whatever means they were revealed. Where optical isomers were available, these gave only a single spot which corresponded to one of the spots given by the racemate: thus, both D- and L-isomers of 2 : 5-dihydroxyphenylalanine (Neuberger, *Biochem. J.*, 1948, **43**, 599) gave spots corresponding to the two spots given by the racemate, and the ratio of the R_F values of the authentic D- and L-compounds was the same as that for the two spots given by the racemate.

If paper chromatography were to involve purely partition phenomena, as is assumed in, *e.g.*, the initial theory of Consden, Gordon, and Martin (*Biochem. J.*, 1944, **38**, 224) no resolution of a racemate could occur from optically inactive solvents. However, observed R_F values do not always agree with values calculated from partition coefficients, and other factors must therefore be involved. One of the more important of these is adsorption, and a higher degree of adsorption of one optical isomer of a racemic solute on the surface of the optically active cellulose would lead to resolution. Cellulose consists of long flat molecules, packed in sheets and containing large numbers of groups forming hydrogen bonds. Adsorption would therefore be most likely with substances containing flat areas, such as an aromatic ring, and hydrogen-bonding groups, such as the α-amino-acid grouping. That it does occur is well shown in the case of tryptophan which, when run on a paper chromatogram with pure water as solvent, does not move with the solvent front, but shows an R_F value of 0·6—0·7 (Synge and Tiselius, *Acta Chem. Scand.*, 1949, **3**, 231). No evidence has been reported of the resolution on chromatograms of racemic tryptophan, and it must therefore be assumed that the degrees of adsorption of the D- and the L-form on the cellulose surface are similar. It is, however, reasonable to suppose that other racemic substances might fit the molecular architecture of the cellulose surface more closely in one of the optically active forms, and this is considered to be the explanation of the resolution observed in the cases described here.

In all the cases in which resolution has been observed (see above), the ratio of the R_F values of the two optical isomers is about 0·9, and this suggests that a common mechanism is operative in all cases. With 2 : 5-dihydroxyphenylalanine the D-isomer is the faster, so that the L-isomer is more strongly adsorbed. By analogy it can be provisionally assumed that the D-isomer is the faster for the other phenylalanine derivatives which show resolution, though it is hoped to obtain more definite evidence on this point in the future by using D- and L-amino-acid oxidases. In the case of the kynurenines (I; R = NH_2, R′ = H or OH) the faster-running isomer corresponds to the naturally occurring L-isomer (Dalgliesh, *locc. cit.*), and the D-isomers must in these cases be more strongly adsorbed. The side-chain of the kynurenines contains one more carbon atom (in the form of a carbonyl group attached to, and therefore coplanar with, the benzene ring) than that of phenylalanine derivatives and the flat (aromatic) area of the molecule therefore bears a different spatial relation to the asymmetric centre, so that a difference between the phenylalanine and kynurenine series is not unreasonable.

In the present work no indication of resolution was observed with 3 : 4-dihydroxyphenyl-DL-alanine or its 5-methyl derivative. No evidence has been reported for the resolution of racemic phenylalanine or tyrosine, and previous work (Dalgliesh, *loc. cit.*) has shown that no resolution occurs with *o*-nitrophenacylglycine, phenacylglycine, or with kynurenine acylated on either the aliphatic or the aromatic amino-group. It is thus possible to formulate tentatively the structural features necessary for resolution: (1) The α-amino-group (and probably also the carboxyl group) should be intact. If both these groups were unable to become simultaneously attached to the cellulose surface, presumably by hydrogen-bonding, resolution would be unlikely, as a "three-point" attachment of the molecular is required for stereochemical specificity. (2) To give this "three-point" attachment the molecule must contain some other portion, such as an aromatic ring, which is also adsorbed on the cellulose surface. Further work is necessary to show to what extent the distance between the two adsorbing centres (aromatic ring and α-amino-acid grouping) is critical, and what degree of configurational rigidity is necessary in the intervening chain. (3) The ring must carry one or more substituents allowing a closer "fit" with the cellulose surface, and hence greater adsorption, with one of the optical isomers than with the other. It is considered likely from the present work that any phenylalanine containing a small substituent in the *ortho*-position may be resolvable on cellulose chromatograms, and that resolution would be due to steric interference of the *ortho*-substituent with some element in the cellulose surface which prevents the D- from being so strongly adsorbed as the L-isomer. (4) Although the cases showing resolution all contain hydrogen-bonding groups on the benzene ring there is no evidence as yet to show whether these groups are essential for resolution.

If these assumptions are correct and if hydrogen-bonding groups on the aromatic ring are unnecessary, it is possible that *o*-tolyl-DL-alanine and *o*-chlorophenyl-DL-alanine, for example, should be optically resolvable on paper chromatograms, whereas the corresponding *m*- and *p*-isomers, or a substance such as α-naphthylalanine (cf. tryptophan) would not. Optical resolution might also occur with, say, substituted phenyl-lactic acids. It is hoped to extend this investigation to other aromatic amino-acids. The method is obviously of potential value for preparative resolutions on columns of cellulose or other optically active materials.

Experimental

Amino-acids.—2 : 5-Dihydroxy-DL, -D-, and -L-alanine were supplied by Dr. A. Neuberger; racemic 2 : 3-dihydroxy-, 3 : 4-dihydroxy-2-methyl, and 3 : 4-dihydroxy-5-methylphenylalanine were supplied by Mr. J. Harley-Mason (cf. Cromartie and Harley-Mason, *J.*, 1952, 1052); 3 : 4-dihydroxyphenylalanine was commercial material.

Chromatography.—This was carried out by the descending technique on strips, 30″ in length, of Whatman No. 4 paper. Racemates which were resolved showed, after travelling a short distance, an elongaged spot which as it proceeded down the paper became dumb-bell shaped and finally separated into two spots. It was frequently considered desirable to allow substances to travel about 60 cm. This necessitated allowing the solvent to run off the paper, which was serrated at the lower edge to ensure an even flow. All chromatograms were run with the organic phase of butanol–acetic acid–water mixtures. It was found that variation in the composition of the mixture did not alter the order in which substances moved, but did alter the rate of travel. The composition was therefore varied, usually by reduction of the acetic acid component, to give mixtures which caused the amino-acid to travel the required distance during a given time (usually 40 hours). As R_F values could not be directly measured tyrosine was used as a reference substance and was given the R_F value 0·4 which it shows in the standard 4 : 1 : 5 butanol–acetic acid–water system of Partridge (*Nature*, 1946, **158**, 270). The values so obtained, being derived from solvents of varying composition, are not strictly comparable but give an approximate idea of the true R_F. They are therefore recorded in the Table as "apparent" mean R_F values (where resolution has occurred the mean value for the two spots is taken). Of greater significance than the R_F values, in the cases of those amino-acids showing resolution, is the ratio of the R_F values of the two resolved components. This is included in the Table, together with the number of determinations on which the value is based.

R_F values.

Phenylalanine derivative	"Apparent" mean R_F Average	Range	Ratio of R_F values of optical isomers Observed	No. of determinations
2 : 5-Dihydroxy	0·32	0·28—0·35	0·89 *	14
2 : 3- ,,	0·32	0·26—0·35	0·91	7
3 : 4-Dihydroxy-2-methyl	0·29	0·22—0·34	0·89	8
3 : 4-Dihydroxy	0·25	0·18—0·29	—	—
3 : 4-Dihydroxy-5-methyl	0·34	0·26—0·39	—	—

* The mean ratio for 12 determinations with authentic 2 : 5-dihydroxyphenyl-D- and -L-alanine was also 0·89.

Detection.—For routine work ninhydrin was used in the usual way. A wide range of other reagents applicable to dihydroxyphenyl derivatives was also examined with results reported in the succeeding paper.

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National Institute for Medical Research,
Mill Hill, London, N.W.7.

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