Chromatographic separation of amino acid isomers

Several naturally occurring amino acids possess two asymmetric centres and can exist in diastereomeric forms. We have succeeded in separating the *threo* and *erythro* isomers of a number of these compounds by paper chromatography. The solvent systems used also discriminated between the straight-chain and branched-chain forms of some aliphatic amino acids.

The most generally useful solvent investigated was the upper phase of *tert.*-amyl alcohol–acetic acid–water (20:1:20). Table I gives the conditions used for the separation of several groups of isomers together with their $R_{Leucine}$ values.

This method provided a poor alternative to paper electrophoresis[1] for resolving *threo* and *erythro* β-methyl aspartic acid, but was more successful in separating the geometrical forms of β-hydroxy aspartic acid. It also distinguished between the corresponding diastereoisomers of γ-methyl glutamic acid, which were separated by ion exchange chromatography on Dowex 1 X 8, acetate form. A column of this resin,

TABLE I

SEPARATION OF AMINO ACID ISOMERS ON PAPER USING THE UPPER PHASE OF FRESHLY PREPARED *tert.*-AMYL ALCOHOL–ACETIC ACID–WATER (20:1:20)

	$R_{Leucine}$ at 20°	*Time in days taken for separation of each group of isomers on Whatman 3 MM paper**
β-Methyl-L-aspartic acid (*threo* form)***	0.20	
β-Methyl-DL-aspartic acid (*erythro* form)***	0.23	20
β-Hydroxy-DL-aspartic acid (*threo* form)***	0.03	
β-Hydroxy-DL-aspartic acid (*erythro* form)***	0.06	12
γ-Methyl-DL-glutamic acid (natural form)	0.41	
Allo-γ-methyl-DL-glutamic acid	0.48	4
L-Isoleucine (*erythro* form)***	0.88	
D-Alloisoleucine (*threo* form)***	0.81	4
Cis-α-(methylcyclopropyl)glycine	0.56	3
Trans-α-(methylcyclopropyl)glycine	0.67	
4-Hydroxy-L-proline (*erythro* form)***	0.23	
Allo-4-hydroxy-D-proline (*threo* form)***	0.19	4
DL-Valine	0.49	
DL-Norvaline	0.66	1
L-Isoleucine	0.88	
L-Leucine	1.00	2
L-Norleucine	1.14	
Homoisoleucine** (2-Amino-4-methyl hexanoic acid)	1.39	
Homoleucine** (2-Amino-5-methyl hexanoic acid)	1.50	2
Homonorleucine** (2-Amino-heptanoic acid)	1.59	

* Samples (10 γ) of the compounds were applied to the origin of 44 cm long descending chromatograms as streaks 2 cm × 0.3 cm. The amino acids were finally revealed with ninhydrin.

** These identifications are only tentative. The compounds were obtained by catalytic hydrogenation of hypoglycin A[9].

*** The relationship between the two asymmetric centres, where this is known, is expressed by the use of the terms *threo* and *erythro* as proposed by VICKERY[10].

65 cm long and 1.2 cm in diameter almost completely resolved 750 mg of synthetic γ-methyl glutamic acid when eluted with 0.2 *N* acetic acid. The second isomer eluted from the column co-chromatographed with natural γ-methyl glutamic acid present in tulip leaf and peanut seedling extracts.

Separation of isoleucine and alloisoleucine, previously achieved with ion exchange resins[2], was also obtained by paper chromatography in *tert.*-amyl alcohol–acetic acid–water.

Our solvent clearly distinguished between the two geometrical forms of the ring compound α-(methylcyclopropyl)glycine[3], but was less successful when applied to the isomers of certain proline derivatives. It adequately resolved a mixture of 4-hydroxyproline and 4-allohydroxyproline but probably did this no more satisfactorily than the solvents described by FRIEDBERG[4] and BEYERMAN[5]. It did not distinguish at all between the *threo* and *erythro* forms of 4-methylproline. These latter isomers were, however, separated by paper chromatography, using water-saturated butan-1-ol in the presence of 3 % (w/v) ammonia, a system first described by CONSDEN *et al.*[6]. An acceptable result was obtained in 5 days under the conditions given in Table I (see footnote*), providing the solvent was equilibrated at 2–4° and the chromatogram was run at the same temperature. *Cis*-4-methylproline (the *threo* form) ran at $R_{Leucine}$ 0.61 and its diastereoisomer at $R_{Leucine}$ 0.57.

The original amyl alcohol-based system readily distinguished between the isomers of some aliphatic amino acids and has been used routinely in this laboratory to resolve valine and norvaline as well as leucine, isoleucine and norleucine. It may be superior to the other solvents already recommended for this purpose[7,8] partly because chromatograms, after treatment with ninhydrin, show relatively compact spots on a clean background. Finally, it satisfactorily separated the three higher homologues of these leucine isomers. Only a mixture of the relevant compounds was available and identifications given in the last section of Table I are tentative, being based on chromatographic position alone.

We are grateful to Dr. H. J. SALLACH, Dr. H. A. BARKER, Prof. G. W. KENNER and Prof. C. H. HASSALL for kindly providing us with authentic samples of some of the amino acid isomers used in this investigation.

Department of Botany, Westfield College, London (Great Britain) — D. O. GRAY

Department of Botany, University College, London (Great Britain) — JENNET BLAKE, D. H. BROWN, L. FOWDEN

1 H. A. BARKER, R. D. SMYTH, R. M. WILSON AND H. WEISSBACH, *J. Biol. Chem.*, 234 (1959) 320.
2 K. A. PIEZ, *J. Biol. Chem.*, 207 (1954) 77.
3 D. O. GRAY AND L. FOWDEN, *Biochem. J.*, 82 (1962) 385.
4 F. FRIEDBERG, *Naturwiss.*, 41 (1954) 141.
5 H. C. BEYERMAN, *Rec. Trav. Chim.*, 80 (1961) 556.
6 R. CONSDEN, A. H. GORDON AND A. J. P. MARTIN, *Biochem. J.*, 38 (1944) 224.
7 K. HEYNS AND W. WALTER, *Hoppe-Seyler's Z. Physiol. Chem.*, 287 (1951) 15.
8 M. WOLFE, *Biochim. Biophys. Acta*, 23 (1957) 186.
9 C. v. HOLT AND W. LEPPLA, *Angew. Chem.*, 70 (1958) 25.
10 H. B. VICKERY, *J. Org. Chem.*, 28 (1963) 291.

Received July 4th, 1963