CHROMATOGRAPHY OF AMINO ACIDS BELONGING TO HOMOLOGOUS SERIES

by

A. POLSON*

Laboratory of Physical Biology, National Institutes of Health, Bethesda, Maryland (U.S.A.)

The positions of the spots produced by various amino acids separated on filter paper according to the chromatographic methods of CONSDON, GORDON, AND MARTIN[1] are determined by their partition coefficients between two phases, one of which is water saturated with organic solvent and the other organic solvent saturated with water. Among the amino acids belonging to a homologous series, these coefficients change in a predictable way with composition and the polarities of their molecules. Addition of OH or COOH groups to the molecule increases polarity and consequently solubility in the water phase, while addition of a CH_2 group to a molecule decreases its polarity and its solubility in water. This note shows the way spot positions, which depend on partition coefficients, vary with composition for amino acids when they are grouped according to composition into the several homologous series to which they can be arranged.

In order to demonstrate the positions and regular distribution of homologues on a chromatogram, mixtures of amino acids falling into different homologous series were analysed on two dimensional chromatograms using collidine saturated with water in one direction and phenol saturated with water containing 0.3 % NH_3 in the direction 90° to the first. This combination of solvents is one used by CONSDON, GORDON, AND MARTIN[1].

Fig. 1 is a tracing of a two-dimensional chromatogram of a synthetic mixture of amino acids which can be identified by the numbers listed in Table I. The basis aliphatic amino acids, though not strictly homologues, are arranged in a group along a line at the top of the paper. Immediately below this are the dicarboxylic amino acids, aspartic and glutamic acids, α-amino adipic acid and α-amino pimilic acid arranged on a smooth curve in order of increasing molecular weight. Close together and below these are the parallel curves formed by the neutral aliphatic branched chain compounds (α-amino iso-butyric acid, valine and isoleucine) and the straight chain aliphatic neutral amino acids (glycine, alanine, α-amino normal butyric acid, norleucine and α-amino heptylic acid). All these appear in order of increasing molecular weight along two smooth curves. The lower curve is identical with that shown before by CONSDON, GORDON, AND MARTIN[1] and by POLSON[2]. The hydroxy amino acids, serine and threonine, fall on a still lower curve that seems to pass through the tyrosine spot. It may be noted that this tyrosine

* Special Fellow, National Institutes of Health, U. S. Public Health Service. Permanent address: Veterinary Research Institute, Onderstepoort, Union of South Africa.

References p. 207.

TABLE I

KEY FOR IDENTIFYING AMINO ACIDS IN THE FIGURES

Amino Acid	Number	Amino Acid	Number
Ornithine	1	Valine	15
Lysine	2	Isoleucine	16
Arginine	3	Serine	17
Citrulline	4	Threonine	18
Aspartic acid	5	Phenyl alanine	19
Glutamic acid	6	Tryptophane	20
α-amino adipic acid	7	Tyrosine	21
α-amino pimilic acid	8	Dihydroxy phenyl alanine	22
Glycine	9	Hydroxy proline	23
Alanine	10	Proline	24
α-amino n-butyric acid	11	Histidine	25
Norleucine	12	Leucine	26
α-amino n-heptylic acid	13	Methionine	27
α-amino iso-butyric acid	14		

spot along with those of phenyl alanine and dihydroxy phenyl analine may also be considered to form a group of three substituted phenyl alanines.

As has already been demonstrated, these regularities in positions of spots due to members of a homologous series make partition chromatography well adapted to the discovery and identification of any new amino acids that may exist.

As an illustration of such a use, one can consider results of the analysis[3] already made of the hydrolysates of *E. coli*. A plot of spot positions observed on a typical two-dimensional chromatogram of digests of these organisms appears in Fig. 2. Here spots belonging to homologous series that have been identified with the help of synthetic mixtures of amino acids run simultaneously with the hydrolysate are connected as in Fig. 1. Five spots were consistently observed which could not be identified with amino acids known to occur in nature. These have been designated by the letters a_1, a_2, b, c

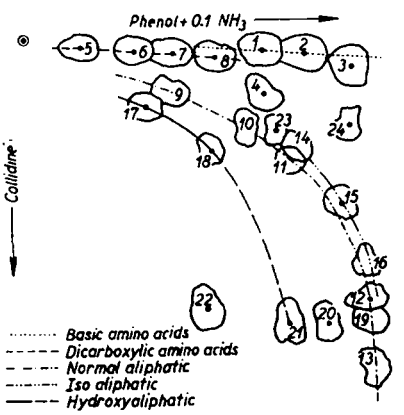

Fig. 1. Tracing of two-dimensional chromatogram of a mixture of amino acids. Spots due to acids of related composition fall on smooth curves.

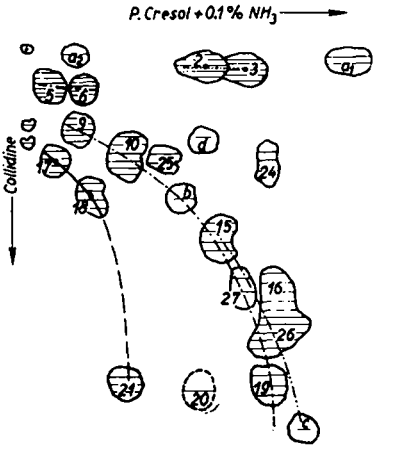

Fig. 2. Tracing of two-dimensional chromatogram of a hydrolysate of *E. coli*. The number of horizontal strips in the spots is indicative of the individual concentrations.

References p. 207.

and d. Assuming that they are indeed amino acids some conclusions regarding their probable nature can be drawn from their positions relative to the homologous series lines of Fig. 1. Thus the compounds responsible for spots a_1 and a_2 are probably basic in character. Spot b falls exactly in the position of α-amino-butyric acid while spot c occupies the position to be expected for a branched chain compound of α-amino heptylic acid. Spot d does not fall on the line of a homologous series; of the two compounds known to produce spots in approximately this position, one is methionine sulphoxide[4], the other is citrulline.

Evidently the mere fact that a spot falls on lines joining members of a homologous series is not sufficient for its identification, but the regularities that exist in the partition coefficients of a series is an important aid in the interpretation of two-dimensional chromatograms.

This work has been carried out in the laboratory of DR. RALPH W. G. WYCKOFF, to whom the writer wishes to express his gratitude for many helpful discussions.

SUMMARY

Because of the regularities that exist in the solubilities and partition coefficients of amino acids, members of homologous series produce spots in two-dimensional chromatograms of their mixtures which fall on smooth curves. This fact can profitably be used for the analysis of chromatograms of protein hydrolysates and especially as a source of suggestions concerning the character of substances producing unusual spots. Results from analyses of hydrolysates of *E. coli* are cited as an example.

RÉSUMÉ

Par suite des relations régulières existant entre la constitution des acides aminés, d'une part, et leur solubilité et leur coefficient de partage, d'autre part, les représentants de séries homologues donnent naissance à des taches, qui, dans des chromatogrammes à deux dimensions, se placent sur des courbes régulières. On peut utiliser ce fait pour l'analyse de chromatogrammes d'hydrolysats de protéines, et en particulier pour avoir une première indication sur la nature d'une substance produisant une tache inattendue. Des résultants obtenus avec des hydrolysats de *E. coli* sont donnés à titre d'exemple.

ZUSAMMENFASSUNG

Die regelmässige Abhängigkeit zwischen der Struktur der Aminosäuren einerseits und ihrer Löslichkeit und ihren Verteilungskoeffizienten andererseits bewirkt, dass die Glieder einer homologen Reihe in einem zwei-dimensionalen Chromatogramm regelmässige Kurven bilden. Man kann diese Regelmässigkeit verwerten, um Eiweisshydrolysate zu analysieren, insbesondere können dadurch auch Hinweise auf die Natur einer Substanz, die einen unerwarteten Fleck ergab, erlangt werden. Als Beispiel werden Analysen von Hydrolysaten von *E. coli* angeführt.

REFERENCES

1 R. CONSDON, A. H. GORDON, AND A. J. P. MARTIN, *Biochem. J.*, 38 (1944) 224.
2 A. POLSON, *Nature*, 161 (1948) 351.
3 A. POLSON, *Biochim. et Biophys. Acta*, 2 (1948) 575.
4 C. E. DENT, private communication.

Received November 11th, 1948