ANALYSIS OF AMINO ACIDS USING PAPER CHROMATOGRAPHY (HORIZONTAL MIGRATION)

By P. S. Rao, F.A.Sc. and R. M. Beri
(Forest Research Institute, Dehra Dun)

Received June 28, 1952

Analysing the amino acids using the horizontal migration method of paper chromatography, Giri has recently reported that "In general the values (Circular R_F values) are found to vary slightly from those reported by other workers by descending and ascending paper chromatographic techniques ".[1] This is however contrary to our experience with the analysis of sugars and uronic acids,[2, 3] wherein the variation between the circular and the straight R_F values has been not slight but considerable. Giri has not given any comparative data in support of his statement but a search into the literature shows that the values reported by Giri are very much different in several cases from the straight R_F values recorded by other workers (comparative data given in Table I), his figures being in some cases as high as 300 per cent. over the straight R_F values. We have, therefore, re-examined the whole problem, but we could not reproduce some of the analytical values reported by Giri nor could we substantiate his conclusion. The values now obtained are quite characteristic and reproducible, and are much different from the straight R_F values, and so this method may offer a convenient, quick and facile way of identifying the amino acids. As in the case of sugars the identification has to be confirmed by carrying out the experiment with two or more solvents.

The method followed is the same as the one already described in our earlier publications.[2, 3] Of the solvents tried, moist phenol, saturated either with water or salt solution, is not at all suitable for the irrigation, since the rings obtained are highly diffused and often leave a trail; further, some of the amino acids like cystine and serine get decomposed on coming into contact with phenol. The other solvents are as a class suitable, though in some cases there is diffusion even with them. It may be worthwhile to note that, as a rule, the values obtained with the three-component solvents are much higher than those obtained with the two-component solvents. In the preparation of these solvents, particularly the three-component systems, it is found necessary to keep the components, taken in measured quantities, together for a long time, preferably overnight, to attain perfect equilibrium conditions. Further, in the case of the three-component solvents, it is

essential to start with fresh amount for every irrigation, because even slight changes in the relative proportion of the components affect the R_f values appreciably.

Experimental.

The experiments were conducted using Whatman No. 1 circular filter-papers (15 cm. in diameter). The provision of a detachable "tail" or "wick" for irrigating the filter-paper with the solvent, as suggested by Giri,[1, 4] was not found to be of any special advantage, and so the fixed tail technique as described by Rao and Beri[2] was followed. The tail was formed by making two parallel cuts perpendicular to the diameter and at equal distance from the centre, cutting off the piece lying between the two parallel cuts, at the ends away from the diameter, and folding it back, so that it is rectangularly perpendicular to the plane of the filter-paper. The length of the tail was cut down to about 1 cm. The amino acid solution (1 per cent. strong) was introduced with the help of a capillary tube as a microdrop at the centre of the filter-paper and air-dried. Taking the solvent into a small dish placed at the centre of a Petri dish (about 14 cm. in diameter), the filter-paper was placed over the Petri dish in the usual way, so that the tail dipped into the solvent below. It was then covered completely by a glass plate and the whole set-up was encaged by an inverted bigger Petri dish. The experiments were conducted in a thermostat maintained at 35° C.

When the irrigation was over (30 to 60 minutes depending on the nature of the solvent), the filter-paper was removed, the position of the solvent front marked and dried in an air-oven at 105° for about 5 minutes. It was then rapidly and evenly sprayed with ninhydrin solution in *n*-butanol (0·1 per cent. in strength) and dried again at 105°. Revealing the positions taken up by the amino acids, characteristically-coloured rings appeared in about two minutes. Their shades varied from bluish to reddish purple, excepting in the case of proline which produced a yellow ring. In a few cases there was a greyish tinge also. It may be noted in this connection that tryptophane was reported to give a blue colour[5] but in our experiments even this acid gave only a bluish purple ring. It should also be noted that the shades produced were to some extent dependent on the nature of the solvent used for the chromatogram. For instance, arginine and norvaline gave a bluish purple ring when moist collidine had been used for the irrigation, while they produced a reddish purple, when *n*-butanol-acetic acid-water was the irrigating medium. In general, the tinges produced with collidine as solvent were bordering on the bluish side. By noting down the distances traversed by the amino acid on the one hand and the solvent on the other, the circular R_f values were calculated in the usual way.

In his article Giri did not mention the temperature at which the R_F values were determined. Presuming that the work might have been done during the months of July, August and September, when the average day temperature of Bangalore would be about 25° C., the circular R_F values were determined at the same temperature using *n*-butanol-acetic acid-water (4:1:5 by volume; upper layer) and the following figures (Table I) were obtained. The values as recorded by Giri[1] and also the straight R_F values as determined by other workers[6,7] are included in the table for the sake of comparison. From the results it would be clear that the circular R_F values differed considerably from the straight R_F values.

TABLE I

Circular R_F Values with Butanol-Acetic Acid-Water as Solvent

	Circular R_F values		Straight R_F values		
			Ascending method		Descending method
	The authors (25° C.)	Giri[1] (presumably 25° C.)	Burma[7]	Govindarajan and Sreenivasaya[6]	Woiwood (cited by Govindarajan & Sreenivasaya[6])
Alanine ..	0·58	0·44	0·25	0·35	0·32
Arginine ..	0·51	0·32	0·14	0·14	0·11
Asparagine ..	0·44	0·31	0·11	..	..
Aspartic acid ..	0·48	0·37	0·16	0·19	0·14
Cystine ..	0·60; 0·41	..	0·07	0·08	0·03
Glutamic acid ..	0·49	0·44	0·21	0·26	0·25
Glycine ..	0·48	0·37	0·18	0·20	0·19
Histidine, HCl* ..	0·52	0·50	0·13	0·11	0·11
Isoleucine ..	0·88; 0·58	0·70	0·61	..	0·65
Leucine ..	0·75	0·73	0·62	0·66	0·85
Lysine, HCl* ..	0·48	0·45	0·11	0·12	0·11
Methionine ..	0·65	0·92	0·43	0·48	0·82
Norleucine ..	0·79	0·75	0·68	..	..
Norvaline ..	0·72	..	0·52	..	..
Ornithine, HBr* ..	0·49	0·25	..	..	..
Phenylalanine ..	0·74	0·70–0·75	0·60	0·60	0·76
Proline ..	0·60	0·49	..	0·40	0·40
Serine ..	0·51	0·40	0·18	0·19	0·19
Threonine ..	0·59	0·44	0·21	..	0·25
Tryptophane ..	0·69	0·70	0·50	0·53	..
Tyrosine ..	0·62	0·58	0·41	..	0·53
Valine ..	0·65	0·62	0·47	0·47	0·62

* The hydrogen halide present in the salt does not interfere with the R_F value of the amino acid.

As reported earlier,[2] since for Indian laboratories 35° C. is a convenient temperature which could be maintained by means of a oven without resorting

TABLE II

Circular R_F Values of the Amino Acids at 35° C.

	Phenol saturated with salt solution		Moist *s*-collidine	Moist *n*-butanol		Moist *ter.* amyl alcohol		Butanol-acetic acid-water (4:1:5 by vol.)		Ethyl acetate-pyridine-water (2:1:2 by vol.)		Ethyl acetate-acetic acid-water (3:1:3 by vol.)	
	R_F values	Remarks	R_F values	R_F values	Remarks	R_F values	Remarks	R_F values	Remarks	R_F values	Remarks	R_F values	Remarks
Alanine ..	0·76	Diffused	0·21	0·27		0·10	Diffusion	0·57		0·21		0·42	
Arginine ..	..	do	0·12	0·18	Diffusion	..	Very little movement	0·53		0·12		0·19	
Asparagine .	0·64	do	0·10	0·15		..	do	0·54	Two over lapping rings, one blue and one purple	0·33		0·28	
Aspartic acid ..	0·18	do	0·11	..	Very little movement	..	do	0·54		0·24	A 2nd ring at 0·18	0·31	
Cystine ..	..	No ring	0·11	0·07		..	No movement	0·60		0·08		0·16	
Glutamic acid ..	0·18	Diffused	0·12	..	Too much diffusion	..	do	0·56		0·21		0·35	
Glycine ..	0·45	do	0·15	0·23		..	do	0·55		0·34	Wide ring	0·35	
Histidine, HCl	..	do	0·18	0·21	Diffusion	..	do	0·53		0·25		0·18	
Isoleucine ..	..	do	0·41	0·54		..	Too much diffusion	0·85	A 2nd ring at 0·61 (yellow)	0·42		0·79	
Leucine ..	0·90	do	0·36	0·48		0·20		0·82		0·64		0·82	Diffusion
Lysine, HCl ..	0·72	do	0·13	.	Very little movement	..	No movement	0·51	Broad	0·16	Wide ring	0·15	
Methionine ..	0·82	do	0·36	0·44		0·22		0·72	A 2nd ring at 0·61 (faint)	0·61		0·67	
Norleucine ..	0·86		0·40	0·60		0·36		0·81		0·62		0·74	Diffusion
Norvaline ..	..	Diffused	0·34	0·52	Diffusion	..	Too much diffusion	0·72	A 2nd ring at 0·61 (faint)	0·36		0·61	
Ornithine, HBr	..	do	0·14	0·13	Diffusion	..	No movement	0·51		0·16	Diffusion	0·19	A 2nd ring at 0·05
Phenylalanine ..	..	do	0·40	0·55		0·32		0·75		0·62		0·69	Diffusion
Proline ..	..	do	0·23	0·31	A 2nd ring at 0·24 (pink)	0·11		0·61		0·25		0·52	
Serine ..	..	No ring	0·18	0·20		..	Very little movement	0·57		0·31	Wide ring	0·29	Diffusion
Threonine ..	0·69		0·19	0·26		0·08		0·57		0·37		0·33	
Tryptophane ..	.	Diffused	0·41	0·48		0·22		0·71		0·62		0·66	Diffusion
Tyrosine ..	..	do	0·34	0·33		0·16		0·62		0·62		0·60	
Valine ..	0·81	do	0·28	0·39		0·16		0·70		0·48	Wide ring	0·61	

to low-temperature incubators, the values were determined at this temperature with the commonly-employed solvents (Table II). It was noticed that in some cases (samples supplied by British Drug House, Ltd., Bombay) more than one ring was obtained. This was probably due to the presence of minute quantities of impurities rather than to any changes taking place during the course of the experiment. In such cases the more prominent ring was taken to represent the substance used. It was also noticed that in some cases, where somewhat broad rings were obtained, the edges were more intensely coloured than the intervening portion and this feature became more pronounced after 2 or 3 days, the central portion fading away more rapidly than the rest. This change was so prominent that in some cases one might mistake the edges to be two different rings. One guiding factor to avoid this mistake was that the edges and the central portion, if they are of the same ring, differed only in intensity. If there were two different rings, the portion in between should be almost colourless.

SUMMARY

Using the horizontal migration method of paper chromatography the separation and identification of naturally-occurring amino acids have been effected.

ACKNOWLEDGMENT

Our thanks are due to Dr. M. Damodaran of the National Chemical Laboratory, Poona, and Dr. D. P. Burma of the Bose Research Institute, Calcutta, for the supply of some amino acids.

REFERENCES

1. Giri, K. V. .. *Curr. Sci.*, 1951, **20**, 295.
2. Rao, P. S. and Beri, R. M. .. *Proc. Ind. Acad. Sci.*, 1951, **33 A**, 368.
3. ———, ——— and Ramachandra Rao, P. *Ibid.*, 1951, **34 A**, 236.
4. Giri, K. V., Krishnamurthy, K. and Venkitasubramanian, T. A. *Curr. Sci.*, 1952, **21**, 11.
5. Consden, R., Gordon, A. H. and Martin, A. J. P. *Biochem. J.*, 1944, **38**, 228.
6. Govindarajan, V. S. and Sreenivasaya, M. *Curr. Sci.*, 1950, **19**, 39.
7. Burma, D. P. .. *J. Ind. Chem. Soc.*, 1951, **28**, 555.