

kan⁴). The values of asymmetry corresponding to the zenith angles have been taken from the asymmetry curve already reported.⁵ A logarithmic plot of $A' = 1 + 0.15 A/\Delta r_0$ against the atmospheric path h (Fig. 1) shows that the points lie nearly on a straight line. The slope of this line gives a value of 0.12 ± 0.015 for δ which is in agreement with that reported by Francis and others¹ for $\lambda = 28^\circ 31'$ while the experiments of Johnson at Peru and Mexico gave a value of 0.16. The probable error is estimated from the scatter of the points in Fig. 1.

The author expresses his grateful thanks to Professor S. Bhagavantam for his valuable guidance.

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ISOBUTYL ALCOHOL-ACETIC ACID-WATER MIXTURE AS A SOLVENT FOR AMINO ACIDS

In the course of an investigation on amino acids using the circular paper chromatographic method, isobutyl alcohol-acetic acid-water mixture was tried as a solvent and found to be quite useful. The R_f values were reproducible to the second decimal place provided the distance of advance of the solvent front was kept constant.

The apparatus and general procedure adopted are based on the method described by Giri and Rao.¹ The amino acid solutions were prepared in concentrations of 0.1 per cent. in 80 per cent. alcohol. 2.5 μ l. of the amino acid solution were spotted at the centre of a Whatman No. 1 filter-paper. For irrigation of the filter-paper, a glass capillary tube of diameter 1-1.5 mm. and length 2 cm. was used in the place of the paper wick. The capillary tube is inserted in a small hole made at the centre of the paper and allowed to dangle into a petri-dish containing the solvent. Irrigation was continued until the solvent boundary had travelled a distance of 5 cm. from the centre and the time taken was about an hour. The paper was then removed, the solvent boundary marked in pencil, dried at room temperature and sprayed with 0.1 per cent. nin-

hydrin in acetone and dried at 55-60° C. for 30 minutes. The R_f values are the average of four determinations. Solvent: 40 c.c. of isobutyl alcohol, 10 c.c. of glacial acetic acid and 50 c.c. of water. This mixture was allowed to stand for sometime and the lower layer discarded.

TABLE I
 R_f values of amino acids

Amino-acid	Isobutyl alcohol-acetic acid-water	N-Butanol-acetic acid-water ²
Arginine	0.28	0.32
Lysine	0.32	0.28
Histidine	0.34	0.28
Asparagine	0.39	0.32
Cystine	0.40	0.20
Aspartic acid	0.44	0.37
Glycine	0.45	0.37
Serine	0.46	0.31
Glutamic acid	0.52	0.44
Threonine	0.53	0.40
Alanine	0.55	0.45
Proline	0.56	0.45
Tyrosine	0.70	0.57
Tryptophane	0.75	0.69
Methionine	0.78	0.75
Valine	0.79	0.72
Phenylalanine	0.80	0.75
Isoleucine	0.90	0.75
Leucine	0.91	0.78

It can be seen from Table I, that the R_f values of the amino acids as separated by isobutyl alcohol-acetic acid-water mixture are higher than those obtained with N-butanol-acetic acid-water mixture. It is further noted from Table I that arginine occupies the lowest position in the chromatogram. Lysine and histidine which are non-separable with butanol solvent, have distinct R_f values and are well separated. The R_f values show a gradual increase from 0.28 for arginine to 0.91 for leucine. Distinct and well-defined bands are obtained, and hence a useful separation of amino acids can be achieved. Although Consden *et al.*,³ reported secondary butyl alcohol as unsatisfactory solvent in that it moved amino acids too fast or unduly broadened the spots, it has been found that isobutyl alcohol-acetic acid-water mixture gave satisfactory results.

My sincerest thanks are due to Dr. S. C. Devadatta, and Dr. K. V. Giri, for their kind interest and continued encouragement.

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