

by ammonia printing or dry printing method which is economical as well as very simple in its technique.

The chromatograms after development with ninhydrin are kept over the ammonia process paper and both together are placed over a glass plate, with the chromatogram facing against the glass surface. Then they are tightly fitted into a frame and exposed to solar light, the time of exposure being fixed depending on the intensity of solar light and intensity of the bands. Immediately after exposure the ammonia process paper is placed in a chamber full of ammonia vapour. In about 10 to 15 minutes the dry printing paper is ready. It will be an exact duplicate of the chromatogram except for the colour. Here all the bands are blue in colour on a white background.

The advantages of this method are: (i) it gives an exact replica of the chromatogram in its original size; (ii) it gives a greater intensity of the colour of the band (Figs. 2 and 3) on a white background and (iii) a more interesting point is that proline which cannot be photographed (in black and white) can be recorded by this process (Fig. 2, band 6; and Fig. 3, spot 7).

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INVESTIGATION OF AMINO-ACIDS IN THE BERRIES OF *WITHANIA* *SOMNIFERA* DUNAL

DURING the course of an investigation¹ on the phytochemistry of *Withania somnifera* Dunal, it was observed that the fruits of this plant contained a high proportion of free amino-acids as evidenced by a strongly positive ninhydrin colour reaction. Therefore, an investigation was undertaken to establish the identity of the amino-acids present therein.

TABLE I

Amino-acids identified by the first wash liquid

R _f found	R _f reported	Colour with ninhydrin	Probable amino-acid
0.925	0.920	Yellow	Proline
0.810	0.800	Purple	Valine
0.650	0.640	do.	Tyrosine or alanine
0.520	0.500	do.	Asparagine or glycine

One gram of the dried berries was macerated with 2 ml. of 80% ethanol for one hour and 50 λ of the clear filtered solution was subjected to paper chromatographic separation. The method of circular paper chromatography of Oreskes and Saifer² was employed. The first wash liquid used was composed of phenol : isopropyl alcohol : water, in the proportion of 70 : 5 : 25. The data are recorded in Table I.

The second solvent system was composed of butanol : acetic acid : water in the proportion of 40 : 10 : 50 and the R_f values obtained are recorded in Table II.

TABLE II

Amino-acids identified by the second wash liquid

R _f found	R _f reported	Colour with ninhydrin	Probable amino-acid
0.710	0.710	Purple	Valine
0.470	0.450	do.	Tyrosine
0.450	0.440	Yellow	Proline
0.410	0.400	Purple	Alanine
0.320	0.310	Brown	Glycine

In order to further confirm the above findings, another solvent system was used as final check. This wash liquid was composed of methyl ethyl kytone : propionic acid : water in the proportion of 75 : 25 : 30. With this system the R_f values varied considerably yet the order of migration of the various amino-acids remained the same. Using known amino-acids for comparison, the presence of valine, tyrosine, proline, alanine and glycine was confirmed.

Similar spots could also be detected in the fresh berries of the plant indicating that these amino-acids are present as such in the plant and are not formed by enzymatic hydrolysis during the drying process. The significance of the occurrence of large amounts of free amino-acids may be explained by the fact that the proteolytic enzyme chymase is present in the berries of this plant.

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2. Oreskes, I. and Saifer, A., *Anal. Chem.*, 1955, 27, 854.
3. —, *Ibid.*, 1956, 28, 501.