



Chromatograms developed in benzyl alcohol (3 vol.), tert. butyl alcohol (1 vol.), iso propyl alcohol (1 vol.), water (1 vol.) and formic acid 2 per cent (see ref. 8) and run at laboratory temperature. Papers sprayed with 0.08 per cent bromo cresol green in 95 per cent aq. ethanol.

1, Standard mixture of malic, quinic, succinic, lactic and citric acids; 2, untreated cider (S.G. 1010); 3, cider-acid extract using 'IRA 400 (OH)'; 4, cider-acid extract using 'IRA 400 (CO<sub>2</sub>)'; 5, sugar solution (fructose, glucose and sucrose) after 'IRA 400 (OH)' treatment; 6, sugar solution (fructose, glucose and sucrose) after 'IRA 400 (CO<sub>2</sub>)' treatment; 7, untreated fructose solution; 8, fructose solution after 'IRA 400 (OH)' treatment; 9, untreated glucose solution; 10, glucose solution after 'IRA 400 (OH)' treatment; 11, untreated sucrose solution; 12, sucrose solution after 'IRA 400 (OH)' treatment

left overnight (17 hr.) before elution. An increase in acidity of 0.76 per cent was recorded, and the chromatogram showed that this increase was represented by the additional acid spots previously found in treated juices (chromatogram 5).

To determine the differential effect of the treatment on these three sugars, corresponding concentrations of each sugar were submitted separately to the resin treatment, the columns being left overnight before elution. The results are summarized in the accompanying table, sugars being estimated by the method of Somogyi<sup>6</sup>.

From the table it can be seen that (1) there is no retention of any of the three sugars by 'Amberlite IR 120', (2) all three sugars are retained by 'IRA 400 (OH)', (3) there is considerable degradation of fructose and glucose with the production of organic acids, (4) sucrose, a non-reducing sugar, is relatively little

	Weight of sugar (gm.)		
	Fructose	Sucrose	Glucose
(a) Entering 'IRA 400 (OH)' column (50 ml. of solution)	4.04	0.85	0.61
(b) Present in effluent and water washings	2.8	0.005	nil
(c) Retained on 'IRA 400 (OH)'	1.24	0.845	0.61
(d) Eluted by ammonium carbonate	0.53	0.80	0.32
(e) Present after 'IR 120' treatment (stage iv)	0.52	0.80	0.32
(f) Percentage of retained sugar (c) degraded in 17 hr.	57.3	0.05	47.6
(g) Acidity of final solution (50 ml.) as per cent malic	1.055	0.08	0.60

attacked by the resin, (5) sugars which have not been degraded can be removed from the column by elution with *N* ammonium carbonate. Chromatograms 7-8, 9-10 and 11-12 show the acids produced by degradation of the sugars.

By using 'Amberlite IRA 400' resin in the CO<sub>2</sub> form, as described by Bryant and Overell<sup>7</sup>, it is possible to obtain practically sugar-free extracts of apple juice or cider showing no increases in acidity (chromatogram 4). Very little sugar is retained by the resin and there is no degradation even after a considerable period of contact (chromatogram 6).

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<sup>1</sup> Roseman, S., Abels, R. H., and Dorfman, A., *Arch. Biochem.*, **36** (1), 232 (1952).

<sup>2</sup> Lugg, W. H., and Overell, B. T., *Aust. J. Sci. Res.*, **A, 1**, 98 (1948).

<sup>3</sup> Hulme, A. C., *J. Exp. Bot.*, **2** (6), 298 (1951).

<sup>4</sup> Bradfield, A. E., Flood, A. E., Hulme, A. C., and Williams, A. H., *Nature* [**170**, 168 (1952)].

<sup>5</sup> Williams, A. H. (unpublished data).

<sup>6</sup> Somogyi, M., *J. Biol. Chem.*, **160**, 61 (1945).

<sup>7</sup> Bryant, F., and Overell, B. T., *Nature*, **167**, 331 (1951).

<sup>8</sup> Stark, J. B., Goodban, A. E., and Owens, H. S., *Anal. Chem.*, **23**, 413 (1951).

### Filter-Paper Disk Chromatography

As a corollary to our comparative studies of the amino-acid metabolism of bacteria<sup>1</sup>, it was necessary to develop a simple chromatographic technique suitable for routine use in the laboratory. Such a technique using filter-paper disks was developed and fully described<sup>2</sup>. Recent publications in *Nature*<sup>3,4</sup> and elsewhere<sup>5</sup> describing almost identical methods would suggest that our publication has escaped notice.

Our experience with paper disk chromatography in the past three years shows that, although the method is admirable for the separation of two or three substances, it is not a satisfactory substitute for large-scale paper chromatography in the separation of more complex mixtures such as protein hydrolysates.

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<sup>1</sup> Proom, H., and Woiwod, A. J., *J. Gen. Microbiol.*, **3**, 319 (1949).

<sup>2</sup> Proom, H., and Woiwod, A. J., *J. Gen. Microbiol.*, **5**, 681 (1951).

<sup>3</sup> Giri, K. V., and Rao, N. A. N., *Nature*, **169**, 923 (1952).

<sup>4</sup> Kariyone, T., Shimizu, S., and Hashimoto, Y., *Nature*, **170**, 422 (1952).

<sup>5</sup> Giri, K. V., Krishnamurthy, K., and Venkatasubramanian, T. A., *Lancet*, **263**, 562 (1952).

### Paper Partition Chromatography of Natural Oestrogens

THE paper chromatography of steroids, for example, cortical steroids, has already been investigated by Zaffaroni *et al.*<sup>1</sup> and by Bush<sup>2</sup>. Although the separation on paper of the natural oestrogens free or in compounds has already been investigated by Bush<sup>3</sup>, Heftmann<sup>4</sup>, Nye *et al.*<sup>5</sup> and Boscott<sup>6</sup>, a satisfactory separation of the three natural steroids on non-treated paper has, to our knowledge, not yet