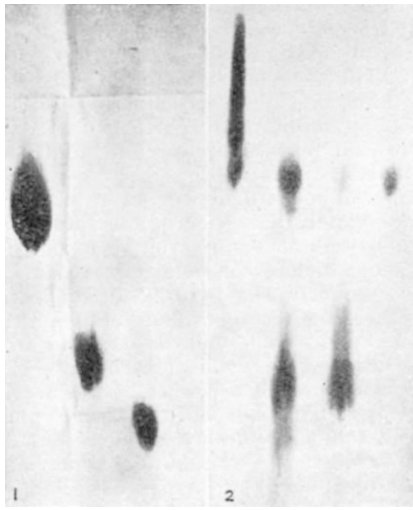

(1) Phenol/trichloracetic acid/water. Left to right: digalloyl glucose, gallic acid, split acid.

(2) Phenol/acetic acid/water. Left to right: gallotannic acid, synthetic pentagalloyl glucose, synthetic pentagalloyl glucose after passage through column of 'Amberlite *IR4b*', gallic acid

with water at room temperature; (2) phenol-trichloracetic acid/water, prepared by adding 35 gm. trichloracetic acid to approximately 500 ml. liquid phenol and just saturating with water at room temperature.

Spots containing approximately 20 μgm. of each substance are eluted down Whatman No. 4 paper and the chromatograms are dried thoroughly and sprayed with ammoniacal silver nitrate solution (0·1 *N*), when the separate substances appear as black spots in the cold. The R_F values of the different tannins vary considerably with temperature, and for identification purposes it is more reliable to run control spots than to use the mathematical technique.

Employing this method, it has been possible to separate widely different types of compounds, such as chebulinic acid[3] and its hydrolysis products, digalloyl glucose, split acid and gallic acid (Fig. 1). The method has been used to check the purity of synthetic and naturally-occurring amorphous pyrogallol tannins. Thus synthetic amorphous pentagalloyl glucose, prepared via the acetyl derivative, contains at least four compounds (Fig. 2) and is not, as assumed, a single substance. Similarly gallotannic acid, when free from gallic acid, contains more than one compound, none of which is present in impure synthetic pentagalloyl glucose. Hydrolysis of gallotannic acid and similar complex materials by acid, or water in sealed tubes at 100° C., gives pyrogallol as well as gallic acid, indicating that some decomposition occurs.

The pyrogallol tannins of *Terminalia chebula* (Myrobalans) have been separated using the two solvents. A more detailed account of this work will be reported later.

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[1] Evans *et al.*, *Nature*, **164**, 674 (1949).
[2] Lugg and Overell, *Nature*, **160**, 87 (1947).
[3] Fischer and Freudenburg, *Ber.*, **52**, 1238 (1919).

Paper Partition Chromatography of Some Organic Bases by the Method of N-Methylation

The precipitate formed between organic basic substances and Dragendorff's reagent is influenced by the valency of the basic nitrogen atom and by hydrophilic groups, such as carboxyl, hydroxyl, etc.[1]. Difficulties in the use of this reagent can often be overcome by a preliminary N-methylation.

The procedure recommended is as follows. Place a small quantity of an aqueous or methanolic solution of the basic substance not reacting readily with Dragendorff's reagent, such as ephedrine, the sulpha drugs, marfanil, benzedrine, etc., on a paper strip and develop it for about twenty hours in butanol acidified with acetic acid (butanol : water : acetic acid = 5 : 4 : 1) by the ordinary procedure of paper partition chromatography (ascending method). After drying, a 10 per cent aqueous solution of potassium carbonate is sprayed on the paper strip and the drying repeated. The paper strip is then immersed in dimethyl sulphate, pressed between layers of filter papers using a rubber roller in order to remove excess reagent, and warmed to 90° for ten minutes in an air bath to complete the N-methylation process.

On spraying with Dragendorff's reagent (diluted five times with 70 per cent acetic acid), a red or reddish-orange colour is revealed at the positions occupied by the basic substances with quaternary nitrogen atoms. The method is also applicable to the extracts of crude drugs.

Examples of R_F values of basic substances obtained by this method are as follows:

(±)-Ephedrine hydrochloride	0·78	Nicotinic acid	0·75
(−)-Ephedrine hydrochloride	0·82	Pyridoxine	0·61
(+)-Ephedrine hydrochloride	0·79	Sulphapyridine	0·82
Marfanil	0·43	Theobromine	0·56

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[1] See *J. Pharm. Soc. Japan*, **70**, 721 (1950); *Jap. J. Pharm. Chem.*, **22**, 468 (1950); cf. also Sakaguchi, T., *J. Pharm. Soc. Japan*, **65** (1945).

Detection of Enzymes by the Agar-Plate Method and its Application to Paper Chromatography

In a communication under the above title, Giri[1] describes the use of a starch agar gel for the location of amylases on the paper chromatogram. The technique seems to be essentially the same as that used in this Department, and previously described in *Nature*[2].

The following comments may be made on the technique. Giri[1] obtains marked movement of certain amylases with 50 per cent acetone, whereas, in this laboratory, 50 per cent acetone produces little, although 40 and 30 per cent acetone show marked movement; this may be due to the higher ambient room temperature in Giri's experiments. Most enzymes examined show a marked tailing effect even