

was identified by direct comparison of melting point and ultra-violet and fluorescence spectra with those of an authentic sample prepared by zinc dust reduction of 3 : 4-8 : 9-dibenzpyrene-5 : 10-quinone⁶.

3 : 4-8 : 9-Dibenzpyrene is a carcinogen of particular interest, not only because it has been detected among the constituents of tar from tobacco smoke⁷ but also because it has been shown to possess considerable sarcoma-producing activity in male mice of strain XVII of this Institute, whereas female animals of the same strain are far less susceptible⁸.

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A Simple, Rapid Method for Circular Paper Chromatography

I HAVE recently developed in our laboratory the following method for the circular development of a large paper disk which has the advantages of (a) rapidity, requiring no more than two and a half hours for a satisfactory separation of sugars and amino-acids, and (b) simplicity, as it does not need the various devices of other workers^{1,2}.

Whatman paper disks 26 cm. in diameter are used. In the centre of the paper a circle 1.5 cm. in diameter is drawn with a pencil. Four to eight cuts, depending upon the number of the samples which will be examined, are made from the periphery up to the centre (Fig. 1). The resulting tips of the triangular areas are bent downward. Each spot is applied on the periphery of the circle, between two adjacent cuts. The solvent is placed in a small vessel which is supported on a glass stand in a glass desiccator 25 cm. in diameter, so that the rim of the vessel is approximately 1 cm. below the rim of the desiccator. Then the tips of the triangular spaces are dipped into the

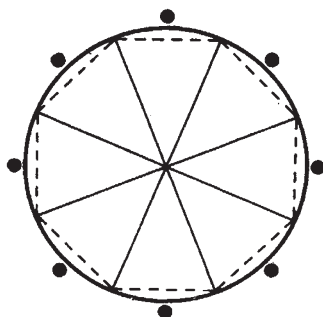


Fig. 1. Diagram showing the cutting and bending of the paper. The solid lines represent the cuts. The broken lines the places where the paper is bent down. The dots represent the spots

solvent and the desiccator is closed. The front develops as a circle, provided that the desiccator is levelled carefully.

Further details of this method will be published elsewhere.

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Configuration of N-acetylneuraminic Acid

RECENTLY, Comb and Roseman¹ observed that N-acetyl-*D*-mannosamine and not N-acetyl-*D*-glucosamine was a substrate for the enzyme synthesizing N-acetylneuraminic acid (nanaldolase) isolated from *Clostridium perfringens*. These results seem to be in conflict with the synthesis of N-acetylneuraminic acid from N-acetyl-*D*-glucosamine as reported by Cornforth, Firth and Gottschalk². The observations of Comb and Roseman suggest that N-acetyl-*D*-mannosamine should be the starting product for the synthesis of N-acetylneuraminic acid.

From the reaction mixture of a condensation between N-acetyl-*D*-mannosamine and oxaloacetic acid in aqueous alkaline solution (*pH* 11) at room temperature we isolated an acidic crystalline substance. This acid proved to be identical with N-acetylneuraminic acid isolated from a similar reaction between N-acetyl-*D*-glucosamine and oxaloacetic acid². In both cases the yield was only 1–2 per cent. Variation of the conditions of the reactions and of the relative amount of the reactants did not improve the yield.

Both acids had the same equivalent weight, optical rotation (in water), R_F value (0.13) on Whatman No. 1 paper (descending; butanol/pyridine/water 6 : 4 : 3), intensity of the colour produced with Ehrlich's reagent (measured at 565 $m\mu$), infra-red spectrum in compressed potassium bromide and X-ray powder diffraction pattern (copper $K\alpha$). Mr. Boschman of this laboratory found that both substances inhibited influenza virus enzyme to the same degree³.

The isolation of identical products from the reaction of N-acetyl-*D*-glucosamine and N-acetyl-*D*-mannosamine with oxaloacetic acid appeared to be due to the epimerization of the two amino-sugars under the conditions of the reaction. Aqueous solutions of both amino-sugars (concentration 75 mgm./ml., that is, the same concentration as used in the condensation reaction) were brought to *pH* 11. After standing some time at room temperature the solutions were neutralized with 'Dowex' 50. Samples were run on borate-treated paper with the butanol/pyridine/water (6 : 4 : 3) mixture⁴. Colouring the chromatograms with Ehrlich's reagent after pre-treatment with alkali revealed that both solutions contained N-acetyl-*D*-mannosamine as well as N-acetyl-*D*-glucosamine. The epimerization attained equilibrium after 10 hr. as was shown by a determination of the amount of amino-sugar in eluates of the individual spots. At equilibrium the ratio of N-acetyl-*D*-glucosamine to N-acetyl-*D*-mannosamine was 2.2 to 1. After 48 hr., the total amount of amino-sugar was about 75 per cent of the original. Recently, Comb and Roseman⁵ have also reported the