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-quinone[6].

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 smoke[7] 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 susceptible[8].

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[1] Cook, J. W., Hewett, C. L., and Hieger, I., *J. Chem. Soc.*, 396 (1933).
[2] Cook, J. W., *et al.*, *J. Soc. Chem. Indust.*, **64**, 27 (1945).
[3] Kleinenberg, G. E., *Arch. Biol. Sci. U.S.S.R.*, **51**, 127 (1938); Badger, G. M., Cook, J. W., Hewett, C. L., Kennaway, E. L., Kennaway, N. M., Martin, R. H., and Robinson, A. M., *Proc. Roy. Soc.*, B, **129**, 439 (1940).
[4] Lacassagne, A., Zajdela, F., Buu-Hoï, N. P., and Chalvet, H., *C.R. Acad. Sci. Paris*, **245**, 273 (1957).
[5] Schoental, R., Abstracts of Papers, VII Intern. Cancer Congress (London, 1958).
[6] Buu-Hoï, N. P., and Lavit, D., *Rec. trav. chim.*, **75**, 1094 (1956).
[7] Wynder, E. L., and Wright, G., *Cancer*, **10**, 255 (1957).
[8] Lacassagne, A., Buu-Hoï, N. P., and Zajdela, F., *C.R. Acad. Sci. Paris*, **245**, 991 (1957).

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

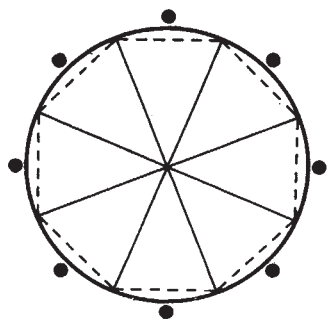

Fig. 1. Diagram showing the cutting and bending of the paper. The solid lines represent the cuts. The broken lines the places where the paper is bent down. The dots represent the spots

Further details of this method will be published elsewhere.

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[1] Bersin, T., and Müller, A., *Helv. Chim. Acta*, **35**, 475 (1952).
[2] Brockman, H., and Patt, P., *Naturwiss.*, **40**, 221 (1953).

Configuration of N-acetylneuraminic Acid

Recently, Comb and Roseman[1] 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-acetylneuramic acid from N-acetyl-*d*-glucosamine as reported by Cornforth, Firth and Gottschalk[2]. 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 (*p*H 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 acid[2]. 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 mμ), infra-red spectrum in compressed potassium bromide and X-ray powder diffraction pattern (copper K_α). Mr. Boschman of this laboratory found that both substances inhibited influenza virus enzyme to the same degree[3].

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 *p*H 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) mixture[4]. Colouring the chromatograms with Ehrlich's reagent after pretreatment 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 Roseman[5] have also reported the

epimerization of N-acetyl-*d*-glucosamine in aqueous solution at *p*H 11.

These results indicate that the opinion of Cornforth, Firth and Gottschalk[2] that their synthesis confirms Gottschalk's[6] proposal of the configuration of N-acetylneuraminic acid is not wholly justified. Further, we conclude that the isolation of N-acetyl-*d*-glucosamine after an alkaline degradation of N-acetylneuraminic acid as reported by Kuhn and Brossmer[7] and by Zilliken and Glick[8] can be ascribed to the rapid epimerization of N-acetyl-*d*-mannosamine formed originally. This means that the isolation of N-acetyl-*d*-glucosamine can no longer be taken as a proof of the configuration at C_5 of N-acetylneuraminic acid[7]. Our experiments lend support to the results of Comb and Roseman[1]. This implies that the configuration at carbon atoms 5–8 of N-acetylneuraminic acid is the same as the configuration at carbon atoms 2–5 of N-acetyl-*d*-mannosamine.

We are indebted to Mr. A. H. Bierens and Mr. J. Schut for their assistance.

Note added in proof. R. Kuhn and R. Brossmer (*Ann.*, **616**, 221; 1958) have re-investigated the degradation of N-acetylneuraminic acid with pyridine and nickel acetate[7]. They now find that N-acetyl-*d*-mannosamine is formed primarily and that this rapidly epimerizes to N-acetyl-*d*-glucosamine.

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[1] Comb, D. G., and Roseman, S., *J. Amer. Chem. Soc.*, **80**, 497 (1958).
[2] Cornforth, J. W., Firth, M. E., and Gottschalk, A., *Biochem. J.*, **68**, 57 (1958).
[3] Walop, J. N., Abstract of communications to 4th Int. Congress of Biochem. Vienna (1958).
[4] Cardini, C. E., and Leloir, L. F., *J. Biol. Chem.*, **225**, 317 (1957).
[5] Comb, D. G., and Roseman, S., *J. Amer. Chem. Soc.*, **80**, 3166 (1958).
[6] Gottschalk, A., *Nature*, **176**, 881 (1955).
[7] Kuhn, R., and Brossmer, R., *Chem. Ber.*, **89**, 2471 (1956).
[8] Zilliken, F., and Glick, M. C., *Naturwiss.*, **43**, 536 (1956).

3-Ethoxy-4-hydroxybenzoic Acid in Human Urine

In a recent communication[1] an increased excretion of vanillic acid during the stress of motor-rally driving was reported. The 1958 Royal Automobile Club rally offered an opportunity to confirm this finding, and three samples of urine were collected from each of two subjects during the third and fourth days. This was after a considerably longer period of stress than had been experienced in the previous experiment and was determined by the accessibility of the subjects. Acid-hydrolysed urines were examined by the method used previously[2] and both hydrolysed and unhydrolysed urines by a modification of the method of Armstrong *et al.*[3]. In all the samples, vanillic acid was detected only in perfectly normal amount.

However, chromatograms of the final sample from one of the subjects showed the presence in comparatively large quantity, particularly after hydrolysis, of an unusual substance which had previously been detected in four of the ten urines in the first experiment, although in comparable amount in only one of them. Further, reference to chromatograms (prepared some years ago by P. S.) of urines from subjects rendered anoxic by decompression for two hours to a pressure equivalent to an altitude of 14,000 ft. showed that a similar substance had been recorded as present in small amount in six of twenty-three such urines. In thirty-eight normal urines collected over a similar period of time (approximately 9.30–11.30 a.m.) from the same subjects (fifteen normals were duplicated) the compound had been detected only twice. Because no particular attention was paid at the time to this then rather obscure substance the numbers quoted above should be regarded with some reservation, since it is possible that trace amounts might have escaped notice on some chromatograms on which its presence had not been recorded.

The new compound was indistinguishable from vanillic acid in its behaviour towards diazotized sulphanilic acid or *p*-nitroaniline, Folin and Ciocalteu's reagent or 2 : 6-dichloroquinone chloroimide[2], but had higher R_F values in all organic solvents tried. These properties suggested a homologue of vanillic acid, and the substance was readily shown to be excreted following ingestion of ethyl vanillin; it has now proved to be chromatographically identical with 3-ethoxy-4-hydroxybenzoic (ethyl vanillic) acid.

Present evidence suggests ethyl vanillic acid may be detected in normal urines, though rarely in more than trace amounts, rather more frequently than is suggested by the anoxia controls mentioned above, particularly in samples collected at different times of the day. One urine collected after lunch contained the substance in large quantity. Inquiry revealed that the subject in question had, during the previous twenty-four hours, consumed a surprising variety of sweets, but no difficulty was encountered in demonstrating the origin of ethyl vanillic acid to be a well-known confection which evidently contains much ethyl vanillin.

From the two driving-stress experiments, urines were collected from two of the subjects, co-drivers, on five occasions, in each case over similar periods of time. On two occasions nothing of interest was found, but the excretions of vanillic and ethyl vanillic acids in the remaining three pairs of urines are compared in Table 1. The average normal excretion-rate for vanillic acid is about 85 μgm./hr. while ethyl vanillic acid is usually present only in trace amounts. Considerable differences between the subjects in the rates of excretion of at least one of the substances are evident on two of the occasions. Since it is known that these two subjects consumed virtually identical meals throughout, and taking into account our present knowledge of dietary sources of the two compounds, we find it difficult to escape the conclusion that both have an endogenous origin responsible for their increase during stress. The alternative possibility that some substance—which

Table 1. Rates of Excretion of Vanillic and Ethyl Vanillic Acids by Two Subjects under Stress

Substances estimated by visual comparisons of chromatograms from acid-hydrolysed urines with known quantities of authentic material. Spots developed with diazotized sulphanilic acid

Subject and occasion	Vanillic acid (μgm./hr.)	Ethyl vanillic acid (μgm./hr.)
J. B. } 1	330	0
A. M. H. B. } 1	420	0
J. B. } 2	30	0
A. M. H. B. } 2	330	20
J. B. } 3	30	150
A. M. H. B. } 3	30	0