fore suggested that either 'degrading illite' fixes potash or is related to the actual cause of fixation.

A more detailed account will appear shortly.

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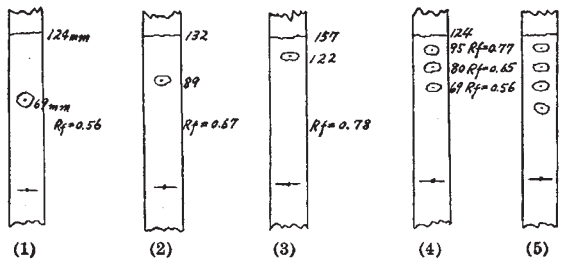
Paper Chromatographic Separation of the Isomeric Pyridinecarboxylic Acids

IN the course of study on the synthesis of the hydrazide of isonicotinic acid, starting with '5⁰-picoline', I realized the desirability of providing a control method to follow the separation of the isomeric pyridinecarboxylic acids. Paper chromatography using *n*-butanol – methanol, a system suggested by the very different solubilities of the acids in methanol and water, has been shown to be very efficient for the separation of the three isomers. The substances used in this experiment were the α-, β- and γ-pyridinecarboxylic acids, a mixture of all three, and the mixture obtained by oxidation of '5⁰-picoline' with potassium permanganate. The solvent used for the development of the substances, as shown in the accompanying figure, was *n*-butanol – methanol containing 35 per cent water by volume at 20° C. The paper used was Tōyō qualitative filter paper No. 3, 2/40 cm., and the usual ascending method was carried out in a thermostat (18°–20° C.).

After the development, the solvent on the paper strips was evaporated at room temperature, and the yellow well-defined spots were revealed on blue background by spraying with bromocresol purple solution (0·2 per cent in *n*-butanol saturated with water). Uni-dimensional chromatograms obtained in these experiments are shown in the figure. It should be stressed that the R_F values of the isomeric pyridinecarboxylic acids have excellent reproducibility under the experimental conditions described above. However, in the case of the developed mixture of the α-, β- and γ-acids, the R_F value of each isomer is slightly lower.

The R_F values of the isomers decrease in the following order: $\beta > \gamma > \alpha$. It may be noted that this is the same sequence as that observed for the dissociation constants[1] of the acids, as determined in aqueous solution.



(1) α-Pyridinecarboxylic acid; (2) γ-pyridinecarboxylic acid; (3) β-pyridinecarboxylic acid; (4) mixture of α-, β-, γ-pyridinecarboxylic acid; (5) oxidative product of '5⁰-picoline' with potassium permanganate

I am very grateful to Prof. N. J. Leonard for advice, and I wish to express my gratitude to Mr. M. Wayaku, director of chemical research, Osaka Gas Co., for supplying '5⁰-picoline', α-picoline and 2,6-lutidine.

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Paper Chromatography for the Separation of Neutral 17-Ketosteroids in Urine

NEUTRAL 17-ketosteroids have been isolated from extracts of urine by methods too elaborate for routine application[1,2]. Using chromatography on columns of alumina or silica, quicker methods have been devised[3–7]; but these are still somewhat tedious. Separations have been achieved by chromatography on paper previously impregnated with the stationary phase[7,8]. A technique is here reported for separating the 17-ketosteroids by paper chromatography without any previous impregnation.

The urinary extracts were prepared by the acid hydrolysis of urine followed by extraction with benzene. The benzene extract was washed once with *N* sodium hydroxide, once with distilled water and then dried over anhydrous sodium sulphate. The extract was then shaken with pellets of anhydrous sodium hydroxide[9] to remove most of the pigment remaining, because it was found that much pigment caused streaking of the chromatograms. Finally, the extract was evaporated to dryness and the residue taken up in a small volume of benzene.

The chromatographic tanks and the solvents used were kept at 34·8° ± 0·5° C. in a thermostatically controlled cabinet provided with an efficient fan. For satisfactory results the atmosphere in the tanks must be in equilibrium with the solvent phases, and the precautions described by Bush[10] were observed. The solvent phases were prepared at 34·8° C. A cyclohexane : 20 per cent (v/v) methanol system was used. The Whatman No. 31 paper employed was washed in the mobile phase and dried before use. The extract of urine was applied as spots of approximately 0·5 cm. diameter along a line 14 cm. from one end of a paper strip 40 cm. × 12 cm. For satisfactory results it was found that the amount of 17-ketosteroid in each spot must not exceed 400 μgm. The paper was equilibrated overnight in the tanks and then run for two hours by the descending technique. The strip was dried, sprayed with a freshly prepared mixture of equal volumes of 14 per cent (w/v) alcoholic potassium hydroxide and 2 per cent (w/v) alcoholic meta-dinitrobenzene, and heated in a stream of warm air for ten minutes.

By this technique six ketosteroid fractions can be detected in extracts of normal male urine. Three of the spots have not yet been identified (approximate R_F values: 0·02, 0·4 and 0·8). One spot (R_F approximately 0·07) appears to be 11-hydroxy-aetiocholan-3α-ol-17-one and another may be 11-oxy-aetiocholan-3α-ol-17-one. The most mobile spot (R_F approximately 0·93) is a mixture of aetiocholan-3α-ol-17-one,