

My friend, Dr. R. A. M. Case, has for some time employed a method substantially equivalent to the above in his haematological work, and found it to result in a considerable saving of labour.

Full details will be published elsewhere.

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## Adsorption Colorimetry as an Analytical Technique

ADSORPTION of coloured substances on white adsorbents has hitherto been used either to demonstrate qualitatively the existence of the substance in a mixture or as a preliminary to elution in the quantitative assay of the substance.

In devising a simple method for the quantitative estimation of mepacrine (atabrin) in urine, a technique has been adopted which I have called 'adsorption colorimetry'. When a measured quantity of suitable white adsorbent is used, the intensity of colour produced under standard conditions is proportional to the quantity of mepacrine in the urine or in an extract of the urine. The adsorbent may easily, and with sufficient accuracy, be measured with a small scoop made by drilling a hole into a piece of wood and calibrating with a powder of known specific gravity. For mepacrine, the most satisfactory adsorbent is powdered silica gel. This may be used either directly, by adding a measured quantity to a known volume of urine for a given time, or indirectly, by adding it to an ether or chloroform extract of alkalinized urine. In this way, it is possible to estimate concentrations as low as 1 mgm. per litre or less; with the common concentrations of 5 mgm. per litre or more, the error is less than 20 per cent.

This technique may obviously be extended to the estimation or detection of other coloured substances. Again, with silica gel, it has been found possible to detect bile pigments in urine in concentrations lower than those detected by the iodine or Gmelin tests; the technique is much simpler than, and the sensitivity about equal to, the Fouchet or similar adsorption methods.

Other coloured substances not adsorbed by silica gel may be adsorbed by other powders. For example, riboflavin is adsorbed by a white preparation of fuller's earth, and preliminary tests indicate that with this adsorbent an adaptation of the method of adsorption colorimetry should be capable of assaying rapidly and with reasonable accuracy and sensitivity the riboflavin content of substances of biological interest.

With fluorescent substances such as mepacrine, the sensitivity of the method may be increased one hundredfold by 'adsorption fluorimetry', that is, by viewing the adsorbent in ultra-violet light. This should make it possible to evolve a method for the estimation of mepacrine in blood, in which the concentration is much lower than that in the urine.

Details of the technique as applied to the estimation of urinary mepacrine and to the detection of bile pigments in urine will be published elsewhere.

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## Structure of Stipitatic Acid

Birkinshaw, Chambers and Raistrick<sup>1</sup> have described stipitatic acid, a metabolite of the mould *Penicillium stipitatum*, to which, after lengthy examination, they could ascribe no reasonable structure. The evidence they give, however, does seem to indicate a unique structure for the compound. The evidence is as follows:

(1) Stipitatic acid (*A*),  $C_8H_6O_5$ , is a dibasic acid, solutions of the disodium salt of which are deep yellow. It contains three active hydrogen atoms and gives a deep red ferric chloride reaction. It has no ketonic or reducing properties and is optically inactive. It dissolves unchanged in concentrated hydrochloric or nitric acid, being precipitated on dilution. (*A*) itself is cream coloured.

(2) (*A*) is unchanged by bromine in acetic acid; in water it forms a loose addition compound. In 80 per cent acetic acid a monobromostipitatic acid is formed, similar to (*A*) in its properties.

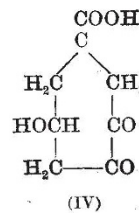
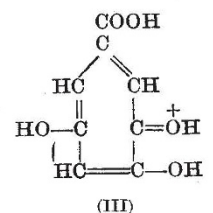
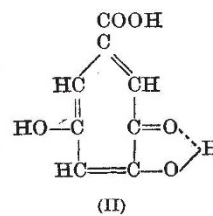
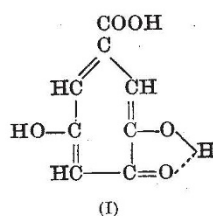
(3) (*A*) is easily converted by alkali fusion to the isomeric 5-hydroxyisophthalic acid, in very good yield.

(4) With diazomethane in ether, (*A*) gives two isomeric, neutral, trimethyl derivatives. With methanolic hydrogen chloride it gives a dimethyl derivative, soluble in sodium hydroxide, but not sodium bicarbonate, solutions. With methyl sulphate and alkali it forms a dibasic monomethyl derivative the disodium salt of which is deep yellow in solution.

(5) With acetic anhydride and sodium acetate, (*A*) forms a monobasic diacetyl derivative; an isomeric but dibasic compound is formed with acetic anhydride and sulphuric acid.

(6) Decarboxylation of (*A*) with copper in quinoline gives a monobasic acid  $C_7H_4O_3$ , solutions of the salts of which are deep yellow; it gives a blood-red ferric chloride reaction and with diazomethane a neutral dimethyl derivative.

(7) Catalytic reduction over platinum oxide gives a crude non-aldehydic product with half the acid equivalent of (*A*); from it tetrahydrostipitatic acid can be isolated as its dinitrophenylhydrazone.



The stability of (*A*), particularly to bromine (2), indicates the presence of an aromatic structure; bridged ring structures are thus eliminated from steric considerations. Benzenoid or oxygen-ring structures do not explain the very facile conversion to hydroxyisophthalic acid (3). There remains a seven-membered ring structure in which an  $\alpha$ -di-