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Estimation of Phenolic Substances : Use of Stabilized *p*-Aminodiethylaniline

I REPORTED in a recent communication[1] that, in the presence of periodic acid as oxidant, ketosteroid salicyloylhydrazones couple readily with stabilized *p*-aminodiethylaniline to form intensely coloured blue-green indoanilines[2]. Further investigation has made it clear that this reaction compares favourably with existing phenol reactions in respect of sensitivity, specificity, stability and optical properties.

Estimation of salicyloylhydrazones was carried out in the following way. To 5–50 μgm. of the salicyloylhydrazone dissolved in 1 ml. ethyl acetate was added 1 ml. of 0·1 per cent solution of stabilized *p*-aminodiethylaniline (a sulphur dioxide compound of *p*-aminodiethylaniline marketed by Messrs. May and Baker as 'Genochrome'). 1 ml. of 5 per cent aqueous sodium bicarbonate was added, followed by 2 ml. of a saturated aqueous solution of potassium periodate. The reaction mixture was then shaken thoroughly to ensure intimate mixing of the two phases during the formation of the indoaniline. 3 ml. of water and powdered sodium chloride were then added, followed by the required amount of ethyl acetate. A crystal-clear intensely coloured supernatant organic phase resulted, which was decanted and read in a Spekker colorimeter against a blank determination using a suitable filter.

It was found that potassium ferricyanide was also effective as an oxidant, and the use of this technique is illustrated by the following method for the estimation of phenols. To 1–10 μgm. of phenol dissolved in 1 ml. of water was added 0·4 ml. of 0·1 per cent stabilized *p*-aminodiethylaniline, 0·5 ml. of 0·33 per cent potassium ferricyanide and 0·5 ml. of 5 per cent potassium pyroborate, and the intensity of the colour was read directly. If less than 1 μgm. of phenol is being estimated, the indoaniline should be extracted into *n*-butanol or chloroform. If necessary, the chloroform or *n*-butanol extract may be concentrated by distilling off some of the solvent, the colour remaining stable under these conditions.

As well as periodic acid and potassium ferricyanide, it was found that both silver nitrate and mercuric chloride were effective as oxidants ; these findings are of potential application in histochemistry.

The reaction appears to be quite general for phenolic substances, some colour variation being obtained with different phenols.

The estimation of acid and alkaline serum phosphatases by determination of the liberated phenol as the indoaniline is under investigation. In that the colour is stable for some days and the proteins do not need to be precipitated, this method seems preferable to existing methods. A full report of this work will be presented shortly.

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Destruction of Isoniazid in the Presence of Hæmin

IT has been shown that hæmin strongly antagonizes the antibacterial action of isoniazid (*iso*nicotinoylhydrazine) on strains of *Mycobacterium tuberculosis* sensitive to this drug[1]. These observations have been confirmed and extended[2]. Isoniazid is destroyed in uninoculated media when hæmin is present (see ref. 3, also personal communication from G. P. Youmans). The work to be described was undertaken to find whether the destructive reaction was catalytic or not, to identify the products, and to consider some biological implications.

When isoniazid (10^{-2} *M*) and hæmin (10^{-3} *M*) were shaken with air at 20° (*p*H 7·5, phosphate or 'tris' buffer) only two substances were formed : di*iso*nicotinoylhydrazine (I) and *iso*nicotinic acid (II). Under these conditions, (I) was not formed from the reaction of isoniazid with (II), as has been claimed by Krüger-Thiemer[4], but was an intermediate in the formation of (II). These products were isolated and identified. Quantitative paper chromatography showed that after 24 hr. half the isoniazid was unchanged and the rest was converted to equal amounts of (I) and (II). Without hæmin, the reaction took the same course, but more slowly (cf. Bönicke and Reif[5]), and hence the role of hæmin was catalytic. No reaction occurred under nitrogen, even at 85°.

For following the disappearance of very low initial concentrations of isoniazid (10^{-4} *M* or less) only microbiological methods, using *Myco. tuberculosis* as test organism, were delicate enough. In the presence of hæmin (10^{-4} *M*) the concentration of isoniazid was found to fall below 10^{-7} *M*, and here too the hæmin appeared to be acting catalytically.

With a direct-vision spectroscope it was found that hæmin rapidly formed a hæmochromogen ($\lambda_{max.}$ about 560 and 525 mμ) with an excess of isoniazid, or with less isoniazid when a reducing agent (sodium hyposulphite) was present. The hæmochromogen was purple, and distinct from the orange-red hæmochromogens formed with pyridine, with nicotinic acid hydrazide and with picolinic acid hydrazide. The main band of reduced hæmin as well (580 mμ) was seen when the isoniazid/hæmin molar ratio fell below 2 to 1. Solutions of this hæmochromogen were stable only in the absence of air. Substance (I), but not (II), slowly formed a hæmochromogen. Many hæmochromogens are more efficient oxidation catalysts than hæmin[6], and it is possible that this hæmochromogen is the true catalyst.

To study antagonism of the action of isoniazid, some sets of tubes of asparagine glycerol phosphate medium[7] containing isoniazid (10^{-4} to 10^{-7} *M*) were inoculated with *Myco. tuberculosis H* 37 *Rv*. When hæmin (10^{-4} *M*) was added immediately, the bacteria grew normally ; but if the addition was delayed for one day their growth was slow, and if it was delayed