The detection of amino compounds on paper chromatograms with diazotized *p*-nitroaniline

Diazotized aromatic amines have long been used to detect phenols on paper chromatograms[1]. However, they are not specific for phenols since sulphanilic acid and *p*-nitroaniline give colours with indoles[1], and *p*-nitroaniline with the ammonium ion[2]. These observations together with others arising in the course of studies on cocoa bean phenols prompted this investigation into the specificity of diazotized *p*-nitroaniline, sulphanilic acid and *o*-dianisidine, all of which are used for the detection of phenols[3]. The first was prepared by WHITEFIELD's method[2] and the remaining two by LINDSTEDT's method[4].

The compounds to be tested were made up in aqueous or aqueous alcoholic solution, spotted on filter paper and silica gel thin layers, and sprayed first with the diazotised amine and then with 20% aqueous sodium carbonate solution. Sulphanilic acid and *o*-dianisidine gave brown colours only with aromatic amines and histidine. *p*-Nitroaniline, on the other hand, gave colours with a wide range of amino compounds. On paper, thirteen common amino acids, putrescine and methylammonium chloride gave mauve or purple spots, histidine a purple-brown spot, tyrosine a grey spot, hydroxyproline and pyrrolidine yellow spots, creatinine and *o*-phenylenediamine brown spots, and *p*-aminobenzoic acid a deep red spot. There was no colour with urea, hydrazine hydrochloride, betaine hydrochloride, hydroxylamine hydrochloride and nicotinamide, and only a faint yellow colour with creatine. All the compounds giving mauve or purple colours gave yellow spots on silica gel and yellow spots on paper (sometimes very faint) when the carbonate spray was omitted. The purple colours with amino acids are more stable than those of ninhydrin but fade on prolonged exposure to light. The sensitivity of the reagent for amino acids is similar to that of ninhydrin.

It is clear that diazotised *p*-nitroaniline reacts readily with many amino compounds, both aliphatic and aromatic, and it has now been shown by the usual chromatographic techniques that a number of spots appearing on chromatograms of cacao extracts (spots 17–26, Fig. 4, ref. 5) are not due to phenols but to amino acids.

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Received March 9th, 1966