

berberine². The final oxidation to berberine is easily accomplished. It is of interest to recall that an assumed structural relation between the congeneric alkaloids, hydrastine and berberine led to a revision of the formula for berberine³.

R. MIRZA
R. ROBINSON

Dyson Perrins Laboratory,
University, Oxford.
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² Perkin, *J. Chem. Soc.*, 118, 737 (1918).

³ Perkin and Robinson, *J. Chem. Soc.*, 97, 305 (1910).

Fluorescence of Amino-Acids, Peptides and Amines on Filter Paper

THE fluorescence of amino-acids and peptides on dry filter paper¹ is of great value for locating these substances after paper chromatography, and has been found useful in quantitative work² since it avoids destructive treatment with reagents such as ninhydrin. It also enables substances to be eluted for further investigations from sheets of chromatograms where gross irregularity of solvent flow renders the use of marker strips³ inaccurate as guides for cutting. Unfortunately, marked variations in fluorescence are often observed when identical chromatograms are run on sheets of No. 4 Whatman paper from different packets of papers or even on adjacent sheets from the same packet⁴, and batches of papers are occasionally encountered on which no fluorescence at all can be observed at normal nitrogen-levels. As Phillips¹, quoting De Ment⁵, considered fluorescence of amino-acids on paper to be due to excitation of the acids themselves by ultra-violet light, it seemed possible that some impurity present in 'bad' fluorescent papers might therefore be quenching fluorescence. Sheets from a particularly bad batch of No. 4 Whatman paper could not be improved, however, by washing with water, dilute sodium hydroxide, hydrochloric acid or with solutions of 8-hydroxyquinoline or dithizone.

When crystals of some twenty amino-acids were examined in ultra-violet light from a lamp known to excite fluorescence on chromatograms, only tryptophane, histidine and citrulline were markedly fluorescent, the feeble fluorescence of the remainder suggesting that their intrinsic activity was unlikely to account for their fluorescence on dry chromatograms. When, however, aqueous solutions of these amino-acids were mixed with an experimental cellulose powder (intended for the preparation of cellulose columns) and the mixtures dried at 80° C., the resulting powders, when examined by ultra-violet light, showed an intense blue or purple fluorescence. A control of cellulose powder dried with water instead of amino-acid solution remained a dull purple. Similar results were obtained with a number of simple peptides and amines. The results with amines showed that the amino-group alone was necessary for reaction. Of several cellulose powders tested, only one failed to produce fluorescence with amino-compounds. This powder had not been modified by any acid treatment in the course of its preparation, whereas all the reactive powders had been treated either with nitric or hydrochloric acid, and gave a strong yellow colour when heated with dilute sodium hydroxide solution, a reaction typical of modified cellulose⁶.

Johansen and Nickerson⁷ have suggested that a reaction analogous to the 'browning reaction' between amino-acids and reducing sugars interferes with quantitative paper chromatography of amino-acids, while Graham, Hsu and McGinnis⁸ have recently shown that some fifteen amino-acids react with glucose on autoclaving to produce fluorescent substances, the glucose acting as a source of aldehydic groups. It is suggested, therefore, that the fluorescence of amino-acids, peptides and amines on filter paper arises through reaction with aldehydic groups derived from modified or short-chain celluloses present in the paper. This would account for the failure to recover a 'bad' paper by washing techniques, such a paper, in fact, being more pure than one on which fluorescence was strong. Since this work was done, a note by Patton, Foreman and Wilson⁹ has directed attention to the essential part played by cellulose in the production of fluorescence on paper chromatograms. The reaction between cellulose powders and amino-compounds may be of use in detecting these substances after partition chromatography on cellulose columns as, by heating the column, it should be possible to locate the separated amino-compounds as fluorescent bands. The reaction may also have some bearing on the apparent loss of amino-nitrogen occurring during paper chromatography^{10,11}.

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A. J. WOIWOD

Wellcome Research Laboratories,
Langley Court,
Beckenham, Kent.
April 19.

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³ Dent, C. E., *Biochem. J.*, 41, 240 (1947).

⁴ Jones, T. S. G., Discussions of the Faraday Soc., "Chromatographic Analysis", No. 7, 235 (1949).

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⁹ Patton, A. R., Foreman, E., and Wilson, P. C., *Science*, 110, 593 (1949).

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A Reversed-Phase Partition Chromatogram using Chlorinated Rubber

UNTIL quite recently, the substances used for supporting the stationary phase in partition chromatography have held the more polar solvent preferentially. Chromatograms of this type have been very satisfactory for the separation of substances with predominantly water-soluble character; but several workers have pointed out that full advantage cannot be taken of small differences in partition coefficients if these are in the range which gives *R* values¹ much above 0.5. In the case of compounds such as the higher fatty acids, where the partition coefficients greatly favour the non-aqueous phase, these ideal conditions cannot be realized, and it has been suggested that a system in which the phases are reversed