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A new reagent for detecting tryptophan, indole, and indole-3-acetic acid in thin-layer chromatography*

In thin-layer chromatography (TLC), tryptophan and most indole derivatives are usually detected by reagents containing an aldehyde group, most often by *p*-dimethylaminobenzaldehyde (DMAB)¹. Phosphoric acid is now suggested as a new reagent for detecting tryptophan, indole, and indole-3-acetic acid.

Material and methods

Tryptophan and the other eighteen amino acids** (Nutritional Biochemical Corp.), indole and indole-3-acetic acid (Sigma Chemical Co.), phosphoric acid 90% (Mallinckrodt), silica gel (Merck), alcohol, and the other solvents were ACS specification.

Fresh preparations were made of 20% v/v phosphoric acid in 95% alcohol. Indole and indole-3-acetic were dissolved in 2 ml of ethanol, water was added, and the standard amino acids were dissolved in 0.1 *N* HCl. The amino acids, indole, and indole-3-acetic acid had a concentration of 100 mg/100 ml.

0.5, 1, 2, 5, 10 μ l of each component were spotted on a 250- μ m silica gel thin-layer plate. After the butanol-acetic acid-water (65:13:22) solvent¹ had travelled 10 cm, the plate was left to dry, sprayed with phosphoric acid reagent, and heated for 40 min at 110°.

TABLE I

COLOURS OF INDOLE DERIVATIVES WITH PHOSPHORIC ACID REAGENT

<i>Indole derivative</i>	<i>Colour</i>	<i>R_F × 100</i>
Tryptophan	purple	50
Indole	orange	93
Indole-3-acetic acid	brownish orange	90

Results and discussion

The eighteen standard amino acids, except tryptophan, gave no colour with the reagent. Tryptophan, indole, and indole-3-acetic acid gave different colours (Table I). The sensitivity of the reagent was of the same order as that of DMAB. After chromatographic separation, 0.5 μ g could be detected but before separation 0.05 μ g gave the colours.

This method depends on a reaction between phosphoric acid and tryptophan, indole, and indole-3-acetic acid. The nature and mechanism of this reaction is

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** Alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine.

unknown, but ROMIEU² observed a red colour when tryptophan-rich tissues were treated with syrup phosphoric acid. The reaction is not simple; the solutions only react under special conditions, which are being studied. On cooling, the treated plates absorb water from the atmosphere and the colour fades, but on reheating the colour becomes stable for more than two weeks.

This reagent has the advantage of being more convenient and equally sensitive as DMAB and other reagents.

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¹ H. KALDEWEY, in E. STAHL (Editor), *Thin-Layer Chromatography—a Laboratory Handbook*, Springer, New York, 1969, p. 1040.

² M. ROMIEU, *C. R. Acad. Sci. Paris*, 180 (1935) 875.

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