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A sensitive and specific colour reaction for the detection of free indole in plant material

In a thin-layer chromatographic study of the terpenoid compounds of black tea¹, vanillin-sulphuric acid colour reagent was used for the detection of higher alcohols and ketones. During this study it was observed that a characteristic deep chrome colour was given by a compound which was subsequently identified as free indole.

It is now reported that this colour reaction is a very sensitive and specific test for the detection of free indole. Although the sensitivity of this colour reaction may be considered similar to that of the well known Ehrlich reagent², it possesses the additional advantage of giving a specific colour for free indole. Moreover the vanillin-sulphuric acid-indole colour reaction is a very convenient method of distinguishing free indole from other indole compounds which are usually present in a plant extract, *e.g.* tryptophan and indolylacetic acid. It has also been observed in our laboratories that this colour reaction is more sensitive than the Van Urk and Prochazka reagents which are also used for the detection of indole compounds.

TABLE I

SENSITIVITIES AND COLOURS GIVEN BY FREE INDOLE WITH VARIOUS REAGENTS

Reagent ^a	Colour ^b	Sensitivity (lowest limit)
Vanillin-sulphuric acid	Deep chrome	0.0125 µg
Anisaldehyde-sulphuric acid	Cadmium yellow	0.05 µg
Van Urk	Rose pink	0.05 µg
Ehrlich	Magenta	0.0125 µg
Modified Ehrlich	Magenta	0.0125 µg
Cinnamaldehyde-hydrochloric acid	Venetian red	0.025 µg
Ferric chloride-perchloric acid	Raw sienna	0.1 µg
Sodium nitrite-hydrochloric acid	Magenta	0.025 µg
Prochazka ^c	Pale green	0.025 µg

^a All colour reagents are prepared under similar conditions 0.1 g of the reagent are dissolved in 100 ml of ethanol containing 0.05 ml of concentrated acid.

^b As given in "Derwent", colour chart of the Cumberland Pencil Co. Ltd., Keswick, Great Britain.

^c Fluorescence under ultra violet light 254 mµ.

Indolyl compounds which bear a substituent at the 3-position *e.g.* indolylacetic acid give a general violet coloration with the reagent instead of the characteristic chrome colour of free indole. The use of other compounds in place of vanillin, give various colours with free indole as shown in Table I, but these colours are not as bright as the deep chrome given by the vanillin-sulphuric acid reagent.

The sensitivities and colours given by free indole with the various reagents,

prepared under identical conditions, are tabulated in Table I. The silica gel coated thin-layer plates used in this study are developed in the solvent system, chloroform-ethanol (99:1).

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¹ A. S. L. TIRIMANNA, *Abstract of the Proceedings of the Ceylon Association for the Advancement of Science*, 1968, p. 59.

² I. SMITH (Editor), *Chromatographic and Electrophoretic techniques*, Vol. 1, Heinemann, London, 1960.

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