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A COLORIMETRIC METHOD FOR THE ESTIMATION OF PYROGALLOL

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ABSTRACT

Pyrogallol in the 5–20 μg range has been estimated colorimetrically in acetic acid after treatment with paradimethyl amino benzaldehyde and sulphuric acid. Some factors affecting the formation of colour have been studied.

INTRODUCTION

PYROGALLOL which occurs either free or as a constituent of pyrogallol tannins in plants (Hifny, Mahran, and El-Alfy, 1968) has been recognised as a factor responsible for the resistance of the chestnut, *Castanea* species, to the chestnut blight pathogen *Endothia parasitica* (Nienstaedt, 1953). The accumulation of vicinal trihydroxy phenols in soya-bean, *Glycine max*, inoculated with *Helminthosporium carbonum* has also been recorded (Biehn, Kuc, and Williams, 1968).

Preliminary studies indicate a change in the total phenol content of coconut roots with the incidence of root (wilt) disease (Joseph and Jayasankar, 1974). While characterizing these phenolic constituents, it was observed that an alcoholic extract of the roots produced a red colour with Ehrlich's reagent (Ehrlich, 1946), consisting of an acidic solution of paradimethyl aminobenzaldehyde (PDAB). This finding was further investigated since PDAB has been employed under varying conditions for the quantitative estimation of phloroglucinol (Jayasankar and Bhat, 1966) and usnic acid (Jayasankar and Towers, 1968); the result was a rapid method for the estimation of pyrogallol.

MATERIALS AND METHODS

All the chemicals used were reagent grade.

Standard pyrogallol.—50 mg pyrogallol was dissolved in 500 ml glacial acetic acid. Suitable dilutions of this stock solution were made with acetic acid to give working standards of varying concentrations.

PDAB reagent.—A 10% solution of PDAB in 99–100% glacial acetic acid.

Sulphuric acid.—Concentrated.

Solvent for chromatography.—The developing solvent consisted of a mixture of 45 parts of benzene, 8 parts of methanol, and 4 parts of acetic acid.

Intensity of colour was measured on a Klett-Summerson photoelectric colorimeter with filter No. 54 (520–580 mμ). The absorption spectrum of the colour complex was recorded on a Beckmann DU2 spectrophotometer.

To a 5 ml aliquot of sample containing 5–20 μg of pyrogallol was added 1 ml of PDAB solution and the mixture was kept in an ice bath. This was followed by the addition of 1 ml conc. H_2SO_4 and the optical density of the coloured product was measured against a reagent blank. The coloured complex is developed almost instantaneously and should be measured immediately.

The thin layer chromatographic separation of the compounds was achieved on cellulose (20 × 20 cm plates). The spots were located on chromatogram by spraying with diazotised sulphanilic acid. Each band of pyrogallol, after submitting to TLC, was extracted with 15 ml glacial CH_3COOH. After centrifuging, a 5 ml aliquot was withdrawn and colour developed by the method outlined earlier.

RESULTS AND DISCUSSION

The absorption spectrum of the product of pyrogallol and PDAB in the presence

of H_2SO_4 shows absorption maxima at 405 and 485 mμ (Fig. 1). When filter No. 42 (400–450 mμ) was used, the readings were exhorbitantly high and it was not used further.

The main basis of the procedure depends on the use of PDAB under controlled conditions. The intensity of the colour complex increases with increase in the concentration

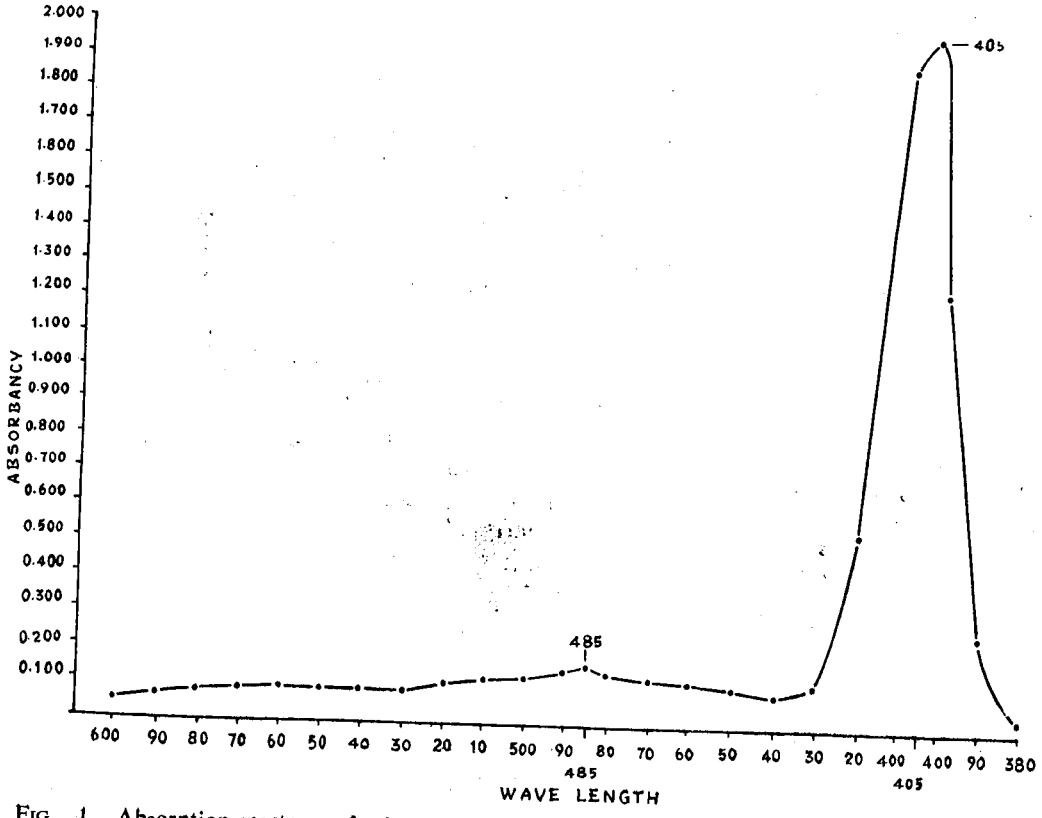

FIG. 1. Absorption spectrum of coloured complex formed by the reaction of pyrogallol with PDAB in the presence of sulphuric acid. Colour was developed by the method described in the text.

of PDAB. Any increase beyond 10% was not significantly beneficial to the colour formation. The time lag subsequent to the addition of H_2SO_4 to the system has a marked influence on the stability of the colour and specificity. PDAB is used for the chromatographic detection of a variety of phenolic compounds (Shaw and Trevarthen, 1958), but has not been so far used for the quantitative estimation of pyrogallol. There is linearity between the concentration of pyrogallol and the intensity of the colour in the range of 5–20 μg (Fig. 2).

Amongst the compounds tested, resorcinol, phloroglucinol, m. cresol, and catechol responded to the test in varying degrees (Table I). Their interference on the estimation of pyrogallol is also indicated in this Table. Aromatic amines are known to interact with PDAB and the results revealed their possible interference on the chromogenic reaction at higher concentrations (Table II). But the interference caused by any of those compounds that are likely to be present in biological systems could be avoided by chromatography, prior to estimation (Fig. 2). The interference by aromatic amines on the chromogenic reaction could not be reversed by the addition of formaldehyde which has an adverse effect on colour formation.

The R_f values of pyrogallol, catechol, phloroglucinol, and resorcinol were 0·23, 0·51, 0·12, and 0·47, respectively. The R_f value of m. cresol was not recorded because of its volatile nature.

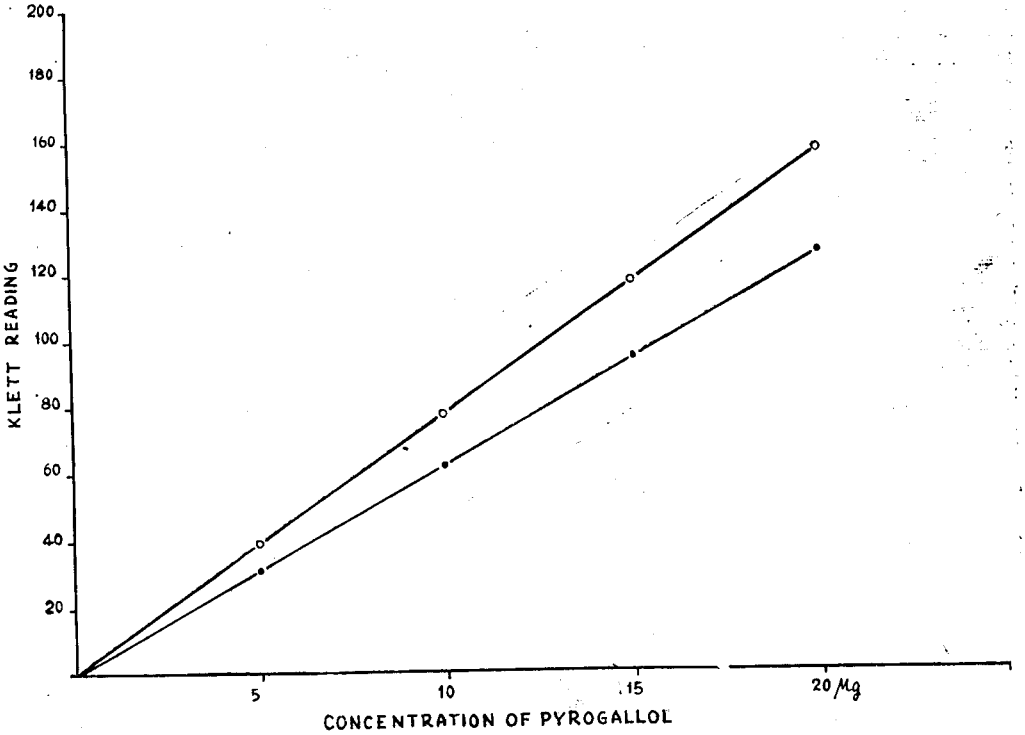

FIG. 2. Comparison of colour intensities obtained for different amounts of pyrogallol with PDAB reagent employing the method described in the text.

(O) values for untreated pyrogallol.

(●) colorimetric estimation of pyrogallol after submitting to thin layer chromatography.

TABLE I

Specificity of the colour reaction and the effect of interfering substances on the development of colour***

Substance added	Klett readings	
	Colour reaction	Interfering substances
Pyrogallol ..	160	160
Catechol ..	15	175
Resorcinol ..	133	220
Phloroglucinol ..	50	210
m. cresol ..	60	185

* A 5 ml solution containing 20 µg of each substance was used in the development of colour. Phenol, hydroquinone, α-naphthol, *p*-cresol, *p*-hydroxybenzoic acid, salicylic acid, β-naphthol, anthranilic acid, aniline, O-aminophenol, gallic acid, DOPA, sulphanilic acid, cinnamic acid, caffeic acid, tyrosine, and chlorogenic acid did not give any readings under the conditions specified.

** A 5 ml solution containing 20 µg of pyrogallol, and 20 µg of each compound was used in the development of colour. Control contained only 20 µg of pyrogallol.

TABLE II

*Effect of aromatic amines on colour reaction**

Substance added	Klett readings
Control ..	160
Anthranilic acid ..	176
Aniline ..	184
Naphthylamine ..	192
Sulfanilic acid ..	194
O-Aminophenol ..	176

* A 5 ml solution containing 20 µg of pyrogallol and 60 µg of each amine was used in the development of colour. Control contained only pyrogallol.

Indole responded positively to the test. But unlike the indole complex, the colour developed by pyrogallol under the conditions specified was unstable on the addition of ether. Varying amounts of pyrogallol added

to the alcoholic extracts of coconut roots could be estimated with over 90% recovery of the added amounts.

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