Journal of Chromatography, 79 (1973) 350–352

CHROM. 6591

Note

A new chromogenic reagent for the detection of phenolic compounds on thin-layer plates

A number of chromogenic reagents are known for the detection of phenolic compounds on thin-layer plates[1–2]. Specific reagents[1,3] used for detection of different classes of naturally occurring phenolic compounds, such as vanillin/HCl for catechins, nitric acid for fully methylated phenolic compounds, and *p*-toluenesulphonic acid for the detection of flavonoids, are important as they are of use in the identification of naturally occurring phenolic compounds. The reagents that can specifically be used for the detection of *vic.-ortho*-dihydroxy groups on thin-layer plates are also of use in structure elucidation. *o*-Dihydroxy groups can be detected with Benedict's reagent[4]; this group is responsible for fluorescence quenching, whereas substances that do not contain the group usually fluoresce when sprayed with the reagent. It cannot, however, be used on polyamide layers, and this reagent is only applicable to flavonoids and coumarins.

In this paper a sensitive chromogenic reagent is described that not only detects most of the naturally occurring phenolic compounds, but can also be used to detect specifically the *ortho*-dihydroxy group on both Silica Gel G and polyamide thin layers. Phenolic compounds are detected by using sodium nitrite–sodium tungstate solution containing trichloroacetic acid. When this spray is followed by an alkali solution, the *ortho*-dihydroxy group is characterized by the formation of a red colour. This specific detection of *ortho*-dihydroxyphenolic groups is based on the observation[5] that a red colour is formed when nitrite is added to a solution containing pyrocatechol and sodium tungstate and the solution is subsequently made alkaline. This reaction has been utilized for the development of a colorimetric method[6] for the quantitative determination of pyrocatechol, in which it was observed that the addition of trichloroacetic acid increases the intensity of the colour in the above reaction. The possibility of using this reaction for the detection of phenolic compounds and the specific detection of *ortho*-dihydroxyphenolic groups on thin-layer plates of Silica Gel G and polyamide has been investigated.

Materials and methods

Thin-layer plates. Silica Gel G (E. Merck) and polyamide (E. Merck) were used for the preparation of thin-layer plates of size 5 × 20 cm. With Silica Gel G, a 1:2 slurry in water and with polyamide a 1:2 slurry in methanol was prepared. Silica Gel G thin-layer plates were activated for 1 h at 110°, while polyamide thin-layer plates were dried for 24 h at room temperature before use.

Phenolic compounds (4 μg) dissolved in acetone were spotted on to thin-layer plates with a micropipette. The thin-layer plates were first sprayed with chromogenic reagent I and then, after 3 min, with chromogenic reagent II. With polyamide, after using reagent I, the thin layers were dried at 30° for few minutes in order to remove

TABLE I

COLOUR REACTIONS OF PHENOLIC COMPOUNDS ON THIN-LAYER PLATES

Compound	*With chromogenic reagent I*	*With chromogenic reagent II*
Vanillic acid	Yellow	Yellow
Veratric acid	Yellow	Yellow
Syringaldehyde	Brown	Brown
Vanillin	Yellow	Yellow
Vanillal (bourbonal)	Yellow	Yellow
Syringic acid	Yellow	Yellow
Ferulic acid	Yellow	Yellow
Isoferulic acid	Yellow	Yellow
Guaiacol	Grey	Grey
4,4'-Dihydroxy-3,3'-dimethoxy-stilbene	Pink	Pink
Hesperidin	—	—
Sinapic acid	Pink	Pink
Naringin	—	—
Phenol	—	—
Thymol	Yellow	Yellow
m-Hydroxybenzoic acid	Faint brown	Faint brown
p-Hydroxybenzaldehyde	Yellow	Yellow
p-Hydroxybenzoic acid	Faint yellow	Faint yellow
p-Aminophenol	Yellow	Yellow
α-Naphthol	Faint yellow	Brown
p-Coumaric acid	Yellow	Yellow
o-Coumaric acid	Faint yellow	Faint yellow
3-Hydroxybiphenyl	Faint brown	Faint brown
Salicylic acid	Pink	Pink
3-Hydroxyanthranilic acid	Yellow	Pink
o-Aminophenol	Yellow	Yellow
p-Hydroxyphenylpyruvic acid	Grey	Grey
Tyrosine	Yellow	Yellow
o-Nitrophenol	Yellow	Intense yellow, changing to reddish yellow
Picric acid	Yellow	Reddish yellow
α-Resorcylic acid (3,5-dihydroxy-benzoic acid)	Faint yellow	Faint yellow
Gentisic acid	Reddish yellow	Purple
Homogentisic acid	Grey	Grey
2,4-Dihydroxyresorcylaldehyde	Faint pink	Faint pink
β-Resorcylic acid (2,4-dihydroxy-benzoic acid)	Brownish yellow	Brown
Phloroglucinol	Intense pink–yellow	Red
ortho-Dihydroxyphenolic compounds		
Rutin	Yellow	Red
Caffeic acid	Yellowish brown	Reddish brown
Pyrocatechol	Yellow	Intense red
Taxifolin (dihydroquercetin)	Intense yellow	Yellowish red
D(+)-Catechin	Yellow	Yellowish red
Protocatechuic acid	Yellow	Dark red
Protocatechuic aldehyde	Light yellow	Light red
2,3-Dihydroxybenzoic acid	Yellow	Dark red
Chlorogenic acid	Yellow	Red
Ellagic acid	Grey	Red
Adrenaline	Red	Red
Quercetin	Brown–yellow	Brownish yellow
vic.-Trihydroxyphenolic compounds		
Tannic acid	Yellow	Brownish yellow
Pyrogallol	Pinkish brown	Brown
Gallic acid	Brown	Brown
Methyl gallate	Light brown	Brownish yellow, changing to brown

water and then again lightly sprayed with reagent II, and the colours were observed.

Preparation of the chromogenic reagents. Reagent I: Prepare a mixture containing 6 ml of a 10 % solution of sodium tungstate, 6 ml of a 5 % solution of trichloroacetic acid and 3 ml of 0.5 *N* hydrochloric acid, and to it add 6 ml of freshly prepared 5 % sodium nitrite solution.

Reagent II: A 0.5 *N* sodium hydroxide solution.

Results and discussion

The results in Table I show that most of the naturally occurring phenolic compounds are detected with this reagent, with the exception of phenol, hesperidin and naringin containing a single OH group, which is further deactivated by the presence of an electron repelling group ($-OCH_3$) in hesperidin. Monohydroxyphenolic compounds containing an $-OCH_3$ group can be detected if the aromatic ring is substituted with an electron-withdrawing group, *e.g.* a carboxyl group in ferulic, isoferulic, vanillic, syringic and sinapic acids and an aldehyde group in syringaldehyde, vanillin and vanillal, and these compounds are detected in addition to other monohydroxyphenolic compounds that contain electron-withdrawing groups. All phenolic compounds containing *m*-dihydroxy groups, such as α- and β-resorcylic acids, gentisic and homogentisic acids and 2,4-dihydroxyresorcyaldehyde, are detected.

When the application of chromogenic reagent I is followed by reagent II, all of the *ortho*-dihydroxyphenolic compounds except quercetin gave a red colour, while monohydroxy, *m*-dihydroxy and *vic.*-trihydroxy compounds gave a yellow to brown colour. The red colour formation observed with nitrophenols (which are not naturally occurring compounds) was due to the presence of the nitro group, which is known to give a red colour under alkaline conditions. Phloroglucinol, which lacks an *ortho*-dihydroxy group, also gave an intense red colour. No red colour was observed with quercetin even at high concentrations, but dihydroquercetin gave an intense red colour even at very low concentrations. The anomolous behaviour of quercetin and the mechanism of the colour reaction of the phenolic compounds with this reagent is under investigation.

We thank Dr. P. V. SUBBA RAO, Indian Institute of Science, Bangalore, and Dr. O. P. GUPTA of Haryana Agricultural University, Hissar, for kindly supplying the authentic samples of some of the phenolic compounds used in this work.

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Received December 13th, 1972