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Note

Structure-specific reagents for the detection of phenolic compounds

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Clifford and Wight[1] reported the use of metaperiodate as a structure-specific reagent for locating phenolic compounds on paper chromatograms. Some of the reagents that are used for the detection of phenolic compounds also react with aromatic amines[2]. Keeping this fact in view, in the present investigation the reaction of this reagent with various amines was studied and a new specific reagent for the detection of *o*-dihydroxy and vicinal trihydroxy phenolic compounds is reported.

EXPERIMENTAL AND RESULTS

Amino compounds (5 μg) dissolved in acetone were spotted on Whatman No. 1 chromatography paper and 0.5% aqueous potassium metaperiodate solution was used as a chromogenic reagent. The results are given in Table I, and show that metaperiodate is also a sensitive reagent for the detection of aromatic amines, although the colour produced is not specifically yellow-orange as has been observed with *o*-dihydroxy phenolic compounds[1]. Unsubstituted and mono- and dialkylanilines gave the colour instantaneously, as these compounds were readily oxidized by periodate, the rate of oxidation decreasing with substitution in the order *ortho* > *meta* > *para*[3]. With N,N′-dimethylaniline, the reaction was very fast owing to the free *para*-position. *p*-Toluidine and benzidine gave colours instantaneously owing to the presence of electron-withdrawing groups in the *para*-position. The exceptionally fast reaction with *p*-dimethylaminobenzaldehyde (which also has an electron-withdrawing group) was due to the presence of a free aldehyde group which is readily oxidized. Aliphatic amines such as ethylenediamine, methylamine hydrochloride and ethanolamine gave no colour reaction with this reagent. The colours produced are reported for an amount of 5 μg although the reagent is highly sensitive amounts down to 0.5 μg for unsubstituted aromatic amines.

Isoniazid (isonicotinic acid hydrazide), a drug used in treating tuberculosis, in acetic acid solution has been used to detect Δ^4-3-keto steroids, which fluoresce yellow in longwave UV light[4]. Recently, the detection of isoniazid using 0.5% aqueous catechol to give a pinkish orange colour has been reported[5,6]. This encouraged us to

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TABLE I
COLOUR REACTION OF AROMATIC AND ALIPHATIC AMINES WITH POTASSIUM METAPERIODATE

Compound	*Colour developed*	*Approx. time taken (min)*
Aniline	Reddish brown	Instantaneous
N,N-dimethylaniline	Intense violet	Instantaneous
o-Aminophenol	Brown	Instantaneous
o-Aminothiophenol	Purple	Instantaneous
o-Phenylenediamine	Reddish	Instantaneous
m-Phenylenediamine	Reddish	Instantaneous
Dimethylaminobenzaldehyde	Yellow	Instantaneous
β-Naphthylamine	Dark brown	Instantaneous
α-Naphthylamine	Dark brown	Instantaneous
Benzidine	Blue	Instantaneous
p-Aminobenzoic acid	Light violet	Instantaneous
p-Toluidine	Reddish violet	10
Diphenylamine	Grey	30
p-Nitroaniline	Faint yellow	30
Ethylenediamine	No colour	—
Methylamine hydrochloride	No colour	—
Ethanolamine	No colour	—

investigate further the reaction of isoniazid with other phenolic compounds. The phenolic compounds (5 μg) dissolved in acetone were spotted on Whatman No. 1 chromatography paper and were first sprayed with 0.5% aqueous isoniazid, then partly dried for about 10 min, followed by a light spray with 0.5 *N* aqueous sodium hydroxide so as to prevent spreading of the spots. The colour was noted after 10 min. The results are given in Table II, and show that all *o*-dihydroxyphenolic compounds gave a pinkish orange or red colour against a clear background. The colour intensity of the spots was found to increase on keeping the chromatograms in air for a longer period. Monohydroxy, *m*-dihydroxy, *m*-trihydroxy, vicinal hydroxymethoxy and methylated compounds did not respond to this colour reaction.

The advantage of this reagent over the earlier reagent[1] is that no colour reaction is observed with amino compounds. All of the amino compounds mentioned in Table I gave no colour reaction with isoniazid reagent even at a much higher concentration (in milligram amounts); hence this reagent can be of value in the specific detection of *o*-dihydroxy phenolic compounds. It is very sensitive and has been successfully applied in locating naturally occurring phenolic compounds of various plant extracts in our laboratories.

TABLE II

RESPONSE OF PHENOLIC COMPOUNDS TO THE ISONIAZID REAGENT

Response is indicated as negative if no pinkish orange or red colour was detected after 10 min.

Phenolic compound	*Response*
Monohydroxy	
Naringin	Negative
Phenol	
Thymol	
m-Hydroxybenzoic acid	
p-Aminophenol	
α-Naphthol	
o-Coumaric acid	
m-Coumaric acid	
p-Coumaric acid	
2-Hydroxybiphenyl	
Salicylic acid	
3-Hydroxyanthranilic acid	
o-Aminophenol	
p-Hydroxyphenylpyruvic acid	
Tyrosine	
p-Hydroxybenzoic acid	
p-Hydroxybenzaldehyde	
m-Dihydroxy and trihydroxy	
3,5-Dihydroxybenzoic acid	Negative
Gentisic acid	
Homogentisic acid	
2,4-Dihydroxyresorcylaldehyde	
2,4-Dihydroxybenzoic acid	
Phloroglucinol	
o-Dihydroxy	
Rutin	Positive
Caffeic acid	
Pyrocatechol	
Taxifolin (dihydroxyquercetin)	
Quercetin	
Quercitrin	
D(+)-Catechin	
(−)Epicatechin	
Protocatechuic acid	
Protocatechuic aldehyde	
2,3-Dihydroxybenzoic acid	
Chlorogenic acid*	
Ellagic acid	
DOPA	
Adrenaline	
Vicinal trihydroxy	
Myricitrin	Positive
Myricetin	
Tannic acid	
Pyrogallol	
Gallic acid	
Methyl gallate	

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TABLE II *(continued)*

Phenolic compound	*Response*
Purpullogallin carboxylic acid**	
Purpullogallin**	
(−)-Epigallocatechin	
(−)-Epigallocatechin gallate	
(−)-Gallocatechin gallate	
(+)-Gallocatechin	
(−)-Epicatechin gallate	
Vicinal hydroxymethoxy	
Vanillic acid	Negative
Syringaldehyde	
Guaiacol	
Vanillal	
Syringic acid	
Ferulic acid	
Isoferulic acid	
Sinapic acid	
Others	
Anisole	Negative
Veratric acid	
Veratrole	
Veratraldehyde	

* Poor response.
** Gave a violet-coloured spot with alkali only.

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REFERENCES

1 M. N. Clifford and J. Wight, *J. Chromatogr.*, 86 (1973) 222.
2 E. Stahl, *Thin-Layer chromatography; A Laboratory Handbook*, Springer, New York, 1969, p. 705.
3 H. Tanabe, *Yakugaku Zasshi*, 77 (1957) 161.
4 B. P. Lisboa, *J. Chromatogr.*, 16 (1964) 136.
5 M. H. Hashmi, A. S. Adil, F. R. Malik and A. I. Ajmal, *Mikrochim. Acta*, 4 (1969) 772.
6 M. C. Inamdar, S. K. Padlia and V. N. Shah, *Indian J. Pharm.*, 32 (1970) 62.