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Note

Alkaline chloramine-T reagent for the detection of phenolic compounds on thin-layer plates

K. L. BAJAJ*
Department of Vegetable Crops, Punjab Agricultural University, Ludhiana-141004 (India)
and
Y. K. ARORA*
Department of Biochemistry, Punjab Agricultural University, Ludhiana-141004 (India)
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A number of chromogenic reagents for the detection of phenolic compounds on thin-layer plates have been reported[1–3]. In addition to these, specific reagents such as nitric acid for methylated phenolic compounds[4] and sodium tungstate[5], thiosemicarbazide[6,7], isonicotinic acid hydrazide[8] and chloranil[9] for *o*-dihydroxyphenolic compounds have been reported. This paper describes the use of 5% chloramine-T in 0.5% sodium hydroxide solution for the detection of phenolic compounds on silica gel G thin-layer plates.

In an earlier paper[10] the use of chloramine-T in concentrated sulphuric acid for the detection of carbonyl groups was described, and this reagent was subsequently used for the detection of steroids. Its strongly oxidising action on hydroxysteroids converts them into ketosteroids. It was reported that phenolic compounds did not react with chloramine-T–concentrated sulphuric acid reagent[11]. However, it has been found that an alkaline solution of chloramine-T can be used for the detection of various phenolic compounds such as *o*-dihydroxy, *m*-dihydroxy and vicinal trihydroxy compounds. The advantage of this reagent over the other reagents is the possibility of detecting different classes of phenolic compounds on the same chromatogram with minimal interferences from non-phenolic compounds. Monohydroxyphenolic compounds which are not detected by this reagent can be detected by spraying the thin-layer plates with Folin–Ciocalteu reagent[2]. *o*-Dihydroxyphenolic compounds appear as yellow, *m*-dihydroxyphenolic compounds as purple and vicinal trihydroxyphenolic compounds as brown spots with alkaline chloramine-T reagent.

PROCEDURE

Various phenolic compounds dissolved in methanol were applied to activated silica gel G thin-layer plates. The chromatograms were developed using the solvent systems toluene–chloroform–acetone (40:25:35) (I) or benzene–methanol–acetic

* Present address: Department of Chemistry and Biochemistry, Haryana Agricultural University, Hissar, India.

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TABLE I

COLOUR REACTIONS OF PHENOLIC COMPOUNDS ON SILICA GEL G THIN-LAYER PLATES

Type	*Phenolic compound*	R_F *values*		*Colour with reagent A*	*Sensitivity (μg)*
		Solvent I	*Solvent II*		
Monohydroxy*	4-Hydroxybiphenyl	–	0.98	–	
	m-Hydroxybenzaldehyde	–	0.51	–	
	2,6-Di-*tert.*-butyl-*p*-cresol**	–	0.92	–	
	o-Hydroxyphenylacetic acid	–	0.95	–	
	m-Hydroxybenzoic acid**	–	0.51	–	
	p-Hydroxybenzoic acid	–	0.60	–	
	o-Aminophenol	–	0.90	–	
	α-Nitroso-β-naphthol***		0.92	Red	
	3-Hydroxyanthranilic acid	–	0.90	–	
	Thymol	–	0.98	–	
	2,2′-Dihydroxybiphenyl	–	0.85	–	
	p-Hydroxybenzaldehyde	–	0.51		
	2-Hydroxynapthoic acid	–	0.83	–	
	o-Coumaric acid	0.38	–	–	
	p-Coumaric acid	0.38	–	–	
	8-Hydroxyquinoline	0.56	–	–	
	p-Hydroxyquinoline	0.52	–	–	
	m-Hydroxyphenylacetic acid**	0.44	–	–	
Hydroxymethoxy*	Homovanillic acid	0.80	–	–	
	Vanillin	0.65	–	–	
	Vanillic acid	0.36	–	–	
	Ferulic acid	0.38	–	–	
	Syringic acid	0.37	–	–	
	Sinapic acid	0.35	–	–	
	Isoferulic acid	–	0.66	–	
	Capsaicin	–	0.85	–	
	Guaiacol	–	0.72	–	
	Vanillal		0.66	–	

m-Dihydroxy/trihydroxy	α-Resorcylic acid	—	0.18	Peach	1
	β-Resorcylic acid	—	0.52	Purple	1
	2,4-Dihydroxyresorcylaldehyde	—	0.84	Purple	1
	1,3-Dihydroxynaphthalene	—	0.86	Purple	1
	Resorcinol	—	0.53	Purple	0.5
	Gentisic acid	—	0.40	Purple	1
	Phloroglucinol	0.17	—	Yellowish red	0.5
Flavonoids	Theaflavin	0.00	—	Yellowish red	0.5
	Kaempferol	0.40	—	Yellowish red	0.5
	Quercetin	0.28	—	Yellowish red	0.5
	Myricetin	0.13	—	Yellowish red	0.5
	Epicatechin	0.64	—	Yellowish red	0.2
	Naringenin	0.50	—	Yellowish red	0.5
	D(-)-Catechin	0.32	—	Yellowish red	0.2
	Apigenin	0.52	—	Yellowish red	0.5
	Hesperidine	0.0	—	Yellowish red	0.5
	Rutin	0.0	—	Yellowish red	0.5
o-Dihydroxy	3,4-Dihydroxyphenylacetic acid	0.51	—	Yellow	0.5
	Caffeic acid	0.27	—	Yellow	0.5
	Protocatechuic acid	0.20	—	Yellow	0.5
	Pyrocatechol	0.51	—	Yellow	0.2
	Protocatechuic aldehyde	0.46	—	Yellow	0.5
	Chlorogenic acid	0.05	—	Yellow	1.0
	Ellagic acid	0.06	—	Yellow	1.0
	2,3-Dihydroxybenzoic acid	0.05	—	Yellow	0.5
Vicinal trihydroxy	Pyrogallol	0.28	—	Brown	0.2
	Methyl gallate	0.12	—	Brown	0.2
	Gallic acid	0.07	—	Brown	0.2
	Propyl gallate	0.10	—	Brown	0.2
	Tannic acid	0.0	—	Brown	0.2

* Detected as blue spots using Folin–Ciocalteau reagent. Detection limit = 0.5 μg.
** Spots detected with iodine vapour.
*** Gave a red colour with alkali alone.

acid (45:8:4) (II) under conditions of chamber saturation. A 5% solution of chloramine-T in 0.5% sodium hydroxide solution (reagent A) was used to detect *o*-dihydroxy, *m*-dihydroxy/trihydroxy and vicinal trihydroxy compounds. The colours produced were observed after 5 min at room temperature and the spots detected with this reagent were marked. After drying at room temperature, the thin-layer plate was again sprayed with Folin–Ciocalteu reagent to detect monohydroxy- and hydroxymethoxyphenolic compounds.

RESULTS AND DISCUSSION

The colours observed with different phenolic compounds and the sensitiivty of reagent A are given in Table I. All of the vicinal trihydroxyphenolic compounds were detected as brown spots, while *o*-dihydroxyphenolic compounds appeared as yellow spots. In addition to *o*-dihydroxyphenolic compounds, flavonoid compounds, irrespective of whether they contain *o*-dihydroxyphenolic groups or not, were detected as yellowish red spots. This is due to the well known colour reaction of the flavonoid compounds with alkali. *m*-Dihydroxyphenolic compounds were detected as purple spots, except for α-resorcylic acid, which gave peach colour. Monohydroxy- and vicinal hydroxymethoxyphenolic compounds were not detected with reagent A. This is in contrast with periodate reagent[12], which detects vicinal hydroxymethoxy groups, but does not detect *m*-dihydroxyphenolic compounds. However, vanillin and vanillal, in much larger amounts (100 μg), are detected as yellow spots.

The reaction mechanism of alkaline chloramine-T with *o*-dihydroxy- and vicinal trihydroxyphenolic compounds has not been investigated, but it is likely that chloramine-T[13] oxidizes the *o*-dihydroxyphenolic compounds to quinones and vicinal trihydroxyphenolic compounds to hydroxyquinones. This is supported by a recent report[14] that chloramine-T partially decreases the reactivity of Folin–Ciocalteu reagent towards a few phenolic compounds. However, all monohydroxy and vicinal hydroxymethoxy compounds that are not detected with chloramine-T reagent alone could be detected after subsequent spraying with Folin–Ciocalteu reagent, except for those with a carbonyl group in the *meta*-position, e.g., *m*-hydroxybenzoic acid and *m*-hydroxyphenylacetic acid, or with a sterically hindered phenolic OH group, e.g., 2,6-di-*tert*.-butyl-*p*-cresol. These were detected using iodine vapour.

No colour reaction of the chloramine-T reagent with steroids, reducing substances such as ascorbic acid, aliphatic and aromatic amines, sulphydryl compounds, carbohydrates and fatty acids was observed. This shows that this reagent is better than other chromogenic reagents such as molybdophosphoric acid[2] or periodate[12], which give colour reactions with other readily oxidizable substances.

The reaction of the chloramine-T reagent with various phenolic compounds was not affected when the adsorbent used was silica gel (without binder) or cellulose.

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