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Note

New chromogenic reagent for the detection of arylhydroxylamines and arylamines on paper and thin-layer chromatograms

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A number of chromogenic reagents are known to detect primary arylamines on paper and thin-layer chromatograms[1–9]. Specific spray reagents used for detection of amines include nitroaniline, sulfanilic acid and β-naphthol reacting through a diazotization mechanism[1,2,4,6–9], sodium 1,2-naphthoquinone-4-sulfonate[3] and glucose/phosphoric acid[5]. Little or no information is available concerning spray reagents for the detection and identification of arylhydroxylamines. Since arylhydroxylamines readily rearrange to arylamines (*i.e.* aminophenols)[10] in the presence of acid, it is assumed that many of the existing reagents used for visualization of arylamines could be employed for arylhydroxylamines. However, there would be little, if any, selectivity with the use of these reagents in chromatography systems containing both arylamines and arylhydroxylamine components. Color and spot tests[11–13] have been reported for hydroxylamine itself, but no reports have been made concerning applicability of these tests to either hydroxylamine or arylhydroxylamine identification on paper and thin-layer chromatograms. No methods are available for the concomitant detection and differentiation of arylamines and aromatic hydroxylamines.

In this paper, the use of 9-chloroacridine is described as a sensitive spray reagent that not only detects primary arylamines but can be used to identify arylhydroxylamines in the presence of arylamines on paper and thin-layer chromatograms. Primary arylamines and arylhydroxylamines are characterized by the formation of a yellow to orange color. Color detection is based on the reaction between 9-chloroacridine and nucleophilic arylamines or arylhydroxylamines to yield 9-substituted acridines. When the sprayed spots are viewed under long-wavelength UV light, arylhydroxylamines exhibit a blue fluorescence whereas arylamines show quenching. These observations have been utilized in the development of colorimetric[14] and quenching fluorometric methods[15,16] for the quantitative determination of both amines and hydroxylamines in our laboratory. The possibility of using these observations for the detection of primary arylamines and the specific detection of arylhydroxylamines in the presence of arylamines on paper and thin-layer chromatograms has been investigated.

MATERIALS AND METHODS

Silica gel G plates (0.2 mm; Brinkmann, Westbury, N.Y., U.S.A.) and Whatman

No. 1 chromatography paper (Whatman Biochemicals, Maidstone, Great Britain) were utilized in this study.

One to two microlitres of primary arylamines and arylhydroxylamines (1 mg/ml) dissolved in 95% ethanol were spotted on paper and thin-layer plates with a microlitre syringe (Hamilton, Model 701). The paper and thin-layer plates were sprayed with 9-chloroacridine spray reagent in a well ventilated hood, dried for 5 min at 110° and the colors were observed. The sprayed spots were subsequently viewed under long-wavelength UV light (Model C-3F; Spectronics Corp.) to detect blue fluorescence or quenching of fluorescence.

Preparation of chromogenic reagent

A 10^{-3} *M* solution of 9-chloroacridine (Eastman-Kodak, Rochester, N.Y., U.S.A.) was prepared in 95% ethanol. A fresh solution was prepared daily and could be kept in a refrigerator for 8 h without decomposition. Commercially obtained 9-chloroacridine was recrystallized from light petroleum (b.p. 30–60°) before use.

RESULTS AND DISCUSSION

The results in Table I show that a variety of arylhydroxylamines and primary arylamines can be detected with 9-chloroacridine spray reagent. The presence of electron-donating and/or electron-withdrawing substituents on the arylamine

TABLE I

COLOR REACTIONS OF ARYLHYDROXYLAMINES AND PRIMARY ARYLAMINES ON PAPER AND THIN-LAYER CHROMATOGRAMS

Compound	*With chromogenic reagent*	*Detection limits (ng)*	
		*Colorimetric**	*Fluorescence*
N-Phenylhydroxylamine	yellow to orange	1000	0.5
N-(*p*-Tolyl)hydroxylamine	yellow to orange	1000	0.5
N-(*p*-Chlorophenyl) hydroxylamine	yellow to orange	1000	0.5
Aniline	yellow	2000	0.5
o-Aminophenol	yellow	2000	0.5
p-Aminobenzoic acid	yellow	1000	0.5
p-Bromoaniline	yellow	1000	0.5
p-Chloroaniline	yellow	1000	0.5
m-Phenylenediamine	yellow	1000	0.5
p-Phenylenediamine	yellow	1000	0.5
p-Nitroaniline	yellow	1000	0.5
3,4-Dichloroaniline	yellow	1000	0.5
p-Aminophenylmercaptoacetic acid	yellow	1000	0.5
Ethyl *p*-aminobenzoate	yellow	2000	0.5
Sulfisoxazole	yellow	2000	0.5
Sulfamethoxypyridazine	yellow	2000	0.5
Sulfamethizole	yellow	2000	0.5
2-Aminofluorene	yellow	1000	0.5

* Minumum concentration that can be detected visually.

or arylhydroxylamine nucleus did not significantly affect the color reaction. The minumum detectable concentration of colored product obtained from the amine or hydroxylamine was 1–2 μg. Limits of detectability were comparable to reported methods. Only one spray reagent is necessary to detect the arylamines thereby making this procedure more advantageous than existing methods.

The spray reagent allows for the specific detection of arylhydroxylamines even in the presence of corresponding arylamines. When viewed under long-wavelength UV light, the arylhydroxylamines exhibit a blue fluorescence which is distinguishable from the quenching of fluorescence observed with arylamines. Minimum detectable concentration of the amines and hydroxylamines by this fluorescence route was 0.5 ng. Since arylhydroxylamines are most often associated with the corresponding amine in chemical and biochemical systems, detection and differentiation of these functional groups are important. The reagent is essentially inert towards other functional groups.

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