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Note

Detection of N-aryl-N-nitrosamines on thin-layer chromatographic plates with 2,4-dinitrophenylhydrazine and phosphomolybdic acid*

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Detection of N-nitrosamines (NAs) on thin-layer chromatographic (TLC) plates has been accomplished by two general methods. The first involves cleavage with ultraviolet (UV) light or HBr to give an amine, which is detected with ninhydrin¹⁻³ or fluorescamine⁴, and nitrous acid, which is detected with Preussmann's reagent (palladium chloride-diphenylamine)^{5,6}, Griess' reagent (1-naphthylamine-sulfanilic acid)⁶, or NEDSA reagent (N-1-naphthylenediamine-sulfanilic acid)⁷. The second involves reduction of NAs with lithium aluminum hydride to asymmetric hydrazines, which are detected by treatment with appropriate aldehydes to give coloured^{8,9} or fluorescent¹⁰ spots.

In the course of investigating the chemistry of photolytic decomposition of NAs on TLC plates, formation of characteristically coloured spots was observed when certain NAs were sprayed sequentially with 2,4-dinitrophenylhydrazine (DNPH) and phosphomolybdic acid (PMA) reagents. This communication reports the use of the DNPH-PMA spray system for the detection of N-aryl NAs on TLC plates.

EXPERIMENTAL

Materials

The 24 NAs investigated were those reported in a previous paper⁴. Amines and carbonyl compounds were obtained from Aldrich (Milwaukee, Wisc., U.S.A.) or Fisher Scientific (Pittsburgh, Pa., U.S.A.). Standard solutions in methylene chloride were freshly prepared before each determination and stored in a refrigerator. Eastman No. 13179 0.1 mm silica gel Chromagram sheets without fluorescent indicator were stored in a dry atmosphere and used as received for TLC.

Spray reagents

Solutions of 0.5% (w/v) 2,4-dinitrophenylhydrazine (DNPH) (Matheson, Coleman and Bell, East Rutherford, N.J., U.S.A.), 3-nitrobenzhydrazide (NBH) (Eastman-Kodak, Rochester, N.Y., U.S.A.), phenylhydrazine hydrochloride (PH) (BDH, Toronto, Canada), and methylhydrazine sulfate (MH) (Eastman-Kodak) in 2 *N* hydrochloric acid, and 10% (w/v) phosphomolybdic acid (PMA) (Merck, Elmsford, N.Y., U.S.A.) in 95% ethanol were prepared for use as spray reagents.

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Thin-layer chromatography and detection of N-aryl-N-nitrosamines and amines

Standard solutions of NAs and amines (*ca.* 10 μg each) were spotted, developed in hexane-diethyl ether-methylene chloride (10:3:2) and the compounds visualized by being sprayed with one of the hydrazine reagents immediately followed by PMA and then heated at 100° for several minutes. The colour reactions of NAs and amines with PMA-DNPH and with these reagents singly were also investigated.

Detection of carbonyl compounds

Standard solutions of carbonyl compounds (*ca.* 20 μg each) were spotted and the compounds sprayed with DNPH, PMA, and DNPH-PMA, and then heated at 100° for about 10 sec. The colour reactions with NBH-, PH-, and MH-PMA were also investigated.

Visual detection limits

Detection limits were determined by spotting decreasing volumes of standard solutions of each NA, developing, spraying with DNPH-PMA, and heating.

Safety precautions

Many NAs are known to be potent carcinogens. Thus safety precautions to prevent skin contact and inhalation must be exercised at all times.

RESULTS AND DISCUSSION

When the 24 NAs were developed and sprayed sequentially with the DNPH and PMA reagents, only the N-phenyl and carbazoyl compounds gave strong colour reactions after heating. The other NAs showed only very weak or no colour reactions. Table I shows that the nature of the substituent groups on the NA influences both the colour reaction and the visual detection limits. The diaryl NAs gave dark spots and lower detection levels whereas the alkylphenyl NAs gave light spots and higher

TABLE I

TLC VISUAL DETECTION LIMITS OF N-ARYL-N-NITROSAMINES AND COLOURS OBTAINED WITH DNPH AND PMA SPRAY REAGENTS

Detection limits were determined on silica gel by spotting N-nitrosamine, developing in hexane-ether-methylene chloride (10:3:2), spraying sequentially with DNPH and PMA reagents, and heating at 100° for several minutes. Alkylphenyl-N-nitrosamines appeared as yellow spots on a green background at the submicrogram level. Colours, at the 5-10 μg level: bl = blue, br = brown, gn = green, p = purple, v = violet y-or = yellow ring with dark orange centre.

<i>N-Nitrosamine</i>	<i>Detection limits</i>		<i>Colour</i>			
	<i>ng</i>	<i>nmole</i>	<i>DNPH*</i>	<i>DNPH-PMA**</i>	<i>PMA*</i>	<i>PMA-DNPH**</i>
Methylphenyl	300	2.2	gn	y-or	bl	y-or
Propylphenyl	400	2.5	gn	y-or	v	y-or
Benzylphenyl	500	2.4	gn	y-or	br	y-or
Diphenyl	10	0.05	v	p	gn	p
Carbazoyl	50	0.25	gn	bl	gn	bl

* Pale yellow background.

** Green background.

(poorer) detection levels. With these reagents one can detect *N*-nitrosodiphenylamine and *N*-nitrosocarbazole in the pmole range and thus complement the fluorescamine method⁴, which is insensitive to these NAs. However, the latter method is superior for detecting the alkylphenyl NAs. All of the other available TLC methods require that a product derived from an NA be detected. In the DNPH-PMA method, the NA is detected directly.

DNPH is normally used to detect carbonyl compounds (or their precursors)¹¹ and PMA is a general reagent that reacts with a variety of functionalities¹². The nature of the coloured complexes formed with the *N*-aryl NAs is not known. Since spraying with DNPH-PMA or PMA-DNPH gives the same colours, the resulting complexes are likely the same but different from those formed when only the individual reagents are used. There are instances, however, where the order of spraying these reagents does influence the production of coloured spots. For example, Table II shows that of the amines related to the NAs in Table I, only the diaryl amines give a colour reaction with DNPH-PMA, whereas all the aryl amines give colours with PMA-DNPH. This can be important, for one may thus suppress the colour reaction of PMA with some compounds that might otherwise interfere.

TABLE II

COLOUR REACTIONS OF SOME AROMATIC AMINES WITH DNPH AND PMA ON TLC PLATES

Colours were determined on silica gel by spotting amine, developing in hexane-ether-methylene chloride (10:3:2), spraying with DNPH and/or PMA reagents, and heating at 100° for several minutes. Abbreviations: bl = blue, br = brown, gr = green, m = mauve, nc = no colour observed, ol = olive, p = purple, v = violet.

Amine	<i>R_F</i> value	Colour			
		DNPH*	DNPH-PMA**	PMA*	PMA-DNPH**
Phenyl	0.32	nc	nc	v	br
Benzyl	0.05	nc	nc	nc	nc
Methylphenyl	0.52	nc	nc	bl	bl-gr
Propylphenyl	0.61	nc	nc	bl	bl-gr
Benzylphenyl	0.59	nc	nc	v	ol
Diphenyl	0.57	nc	p	bl-gr	m
Carbazole	0.36	nc	ol	bl-gr	bl-gr

* Pale yellow background.

** Green background.

The formation of coloured complexes by aryl NAs with hydrazines and PMA appears to be general. Table III shows the colours obtained with NBH-, PH-, and MH-PMA combinations.

Carbonyl compounds can be detected with a variety of reagents. DNPH is commonly used but it gives yellow to red spots on a yellow background; at low levels, the lack of good colour contrast for the lighter spots makes detection difficult. Although this contrast can be heightened somewhat with a sodium ethoxide overspray¹⁴, a significant improvement in contrast is observed when PMA is used instead. The yellow-orange spots are clearly visible against the green background. Table IV gives

TABLE III

COLOUR REACTIONS OF N-ARYL-N-NITROSAMINES WITH NBH, PH, OR MH AND PMA ON TLC PLATES

Colour abbreviations: bl = blue, br = brown, f = faint, gr = green, gy = gray, ol = olive, or = orange, p-wh = purple ring with white centre, sm = salmon.

<i>N-Nitrosamine</i>	<i>Colour</i>		
	<i>NBH-PMA*</i>	<i>PH-PMA**</i>	<i>MH-PMA***</i>
Methylphenyl	or-br	p-wh	or
Propylphenyl	or-br	p-wh	or
Benzylphenyl	sm	p	or
Diphenyl	bl-gr	bl	ol
Carbazolyl	bl-gr	f-gy	f-gy

* Pale yellow background.

** Gray background.

*** Blue background

TABLE IV

COLOUR REACTIONS OF CARBONYL COMPOUNDS WITH DNPH AND PMA ON TLC PLATES

Colour abbreviations: bl = blue, f = faint, gr = green, nc = no colour observed, or = orange, rd = red, y = yellow.

<i>Carbonyl compound</i>	<i>Colour</i>		
	<i>DNPH*</i>	<i>PMA*</i>	<i>DNPH-PMA**</i>
Nonanal	y	f-gr	y
Citral	y-or	bl	gr
Citronellal	y	nc	y-gr
Benzaldehyde	y-or	nc	y
3-Phenylpropanal	y	nc	y
Cinnamaldehyde	or-rd	nc	or
3-Pentanone	y	nc	nc
2-Methyl-3-heptanone	y	nc	f-y-gr
2-Octanone	y	nc	f-y-gr
Cyclohexanone	y	nc	f-y-gr
Carvone	y-or	nc	y
Acetophenone	y-or	nc	y
1-Phenyl-2-propanone	y	f-gr	y-gr
Benzophenone	or	nc	or
9-Fluorenone	or	nc	or

* Pale yellow background

** Green background. Spraying with PMA-DNPH gave essentially the same results.

the colour reactions of various carbonyl compounds to DNPH, PMA, and DNPH-PMA reagents. Use of NBH-, PH-, and MH-PMA sprays for the detection of carbonyl compounds was also investigated; coloured spots, if formed, were generally faint or of poor contrast with the background. Instead of altering the colour of the background, the colour of the 2,4-dinitrophenylhydrazines of saturated carbonyl compounds can be changed with a potassium ferricyanide-ferric chloride overspray¹³.

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