

CHROM. 4335

SYSTEMATIC STUDIES OF CHROMOGENIC REAGENTS FOR DETECTING ORGANIC COMPOUNDS ON THIN-LAYER CHROMATOGRAMS

I. PRIMARY, SECONDARY, AND TERTIARY AMINES

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(Received July 16th, 1969)

SUMMARY

Seven chromogenic reagents for detecting primary, secondary, and tertiary amines on thin-layer chromatograms have been evaluated systematically. The reagents are *p*-dimethylaminobenzaldehyde, 2,3-dichloro-1,4-naphthoquinone, salicylaldehyde, furfural, copper sulfate, 2-thiobarbituric acid, and *o*-tolidine. All were screened for usefulness on at least three amines; the first four were further evaluated on 28 amines and 24 other organic compounds. 2,3-Dichloro-1,4-naphthoquinone is the most generally useful reagent, although it also reacts with classes of compounds other than amines. *p*-Dimethylaminobenzaldehyde will detect most aromatic amines and a few aliphatic amines and gives the best sensitivity of any of the tests. Salicylaldehyde is useful only for detecting primary amines.

INTRODUCTION

Because of the increasingly widespread use of thin-layer chromatography for the separation and identification of organic compounds, many classes of chromogenic reagents for detecting these separated compounds must be systematically studied to permit the elucidation of the most satisfactory reagents. This paper is the first in a series intended to fulfil this objective. Because of widespread interest in detection of primary, secondary, and tertiary amines, chromogenic reagents for their detection were selected for our first report.

Aliphatic and aromatic amines must often be detected or determined at trace levels in complex samples or in micro amounts as impurities in natural waters or in body fluids. If the detection follows separation by paper or thin-layer chromatography, the detection reagent should be capable of producing a visible color with microgram amounts of many different amines.

Many chromogenic reactions have been proposed for the detection of amines

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on paper and thin-layer chromatograms and for spot-test and colorimetric analysis. Among the more widely used reactions are those employing Dragendorff's reagent for tertiary and quaternary amines^{1,2}, diazo salts for aromatic amines³⁻⁶, furfural for aromatic amines⁷⁻¹⁰, ninhydrin^{11,12}, salicylaldehyde for primary arylalkyl amines^{13,14}, and *p*-dimethylaminobenzaldehyde^{5,6,15}.

Other reactions of interest employ 2,4-dinitrofluorobenzene^{16,17}, 2-nitro-1,3-indanedione¹⁸, aconitic anhydride for tertiary and quaternary amines^{19,20}, piperonal for aromatic amines²¹, 2,3-dichloro-1,4-naphthoquinone for *n*-alkylvinylamines^{22,23}, copper sulfate for secondary aliphatic and cyclic amines²⁴, 2-thiobarbituric acid for pyrimidines²⁵, *o*-tolidine for pyridines²⁶, and nitroprusside for secondary amines²⁷. Most other reagents, such as acid-base indicators, will detect too many other functional groups and structures.

Thorough examination of the literature relating to the detection and identification of amines by chromogenic reagents resulted in the selection of seven reagents for systematic study. These were *p*-dimethylaminobenzaldehyde, 2,3-dichloro-1,4-naphthoquinone, salicylaldehyde, furfural, copper sulfate, 2-thiobarbituric acid, and *o*-tolidine. These reagents were selected because (1) they have been reported to selectively detect trace amounts of different types of amines by producing brilliant colors, (2) they are simple to use, and (3) they are relatively specific for amines.

Four of these reagents survived preliminary evaluation and were tested with 28 different amines and compounds containing amino groups, to determine their sensitivity, and with 24 other organic compounds, to determine what other types of compounds might also react and thus interfere with the test under the selected experimental conditions. The ISCC-NBS color name charts²⁸ were used to classify the observed colors. The 2,4-dichloro-1,4-naphthoquinone reagent appears to be the most widely useful reagent. Both *p*-dimethylaminobenzaldehyde and salicylaldehyde also have areas of utility, and the latter reacts with the greatest variety of amines. Both were reasonably free from interferences caused by other classes of organic compounds.

EXPERIMENTAL

In order to evaluate as many chromogenic reagents as possible, the experimental program was divided into three series of tests: preliminary screening, general applicability, and evaluation of interferences. In the first series, all of the reagents selected for study were screened for their potential usefulness by studying their reactions with 5, 10, and/or 50 μg of three amines of the type with which the reagent was reported to react. The compounds selected for the preliminary screening tests are given in Table I. An additional six amines were used to evaluate the copper sulfate reagent because of inconclusive results on the first three.

In the second series, the four reagents that survived the first series were evaluated on twenty-eight amines. These compounds are listed in Table IV. Both simple amines and amines containing other functional groups were included. Heterocyclic nitrogen compounds were omitted unless they also contained an amino group.

In the third series, the same four reagents were evaluated on twenty-four organic compounds representing a variety of functional groups and structures. These compounds are shown later in Table VI. One amino compound, morpholine, was included in this group rather than in the second group because it often undergoes

TABLE I

COMPOUNDS USED IN PRELIMINARY SCREENING TESTS

<i>Chromogenic reagent</i>	<i>Compound</i>
<i>p</i> -Dimethylaminobenzaldehyde	<i>p</i> -Nitrosodiphenylamine, furosemide, benzidine
2,3-Dichloro-1,4-naphthoquinone	Allylamine, benzimidazole, <i>n</i> -butylamine
Salicylaldehyde	Nicotinamide, isonicotinic acid hydrazide, <i>n</i> -butylamine
Furfural	2,4-Toluenediamine, phenylenediamine, 2-amino-naphthalene
Copper sulfate	Morpholine, diphenylamine, 1(—)-proline, dipropylamine, piperidine, adrenaline, caprolactam, diethylamine
2-Thiobarbituric acid	Barbituric acid, folic acid, theobromine
<i>o</i> -Tolidine	Isonicotinic acid hydrazide, nicotinonitrile, nicotinamide

different reactions than do amines. Because of our interest in organometallic and organophosphorus compounds in natural waters, four of these compounds were included in the test group.

All of the tests were conducted on Brinkman Catalog No. 5762 precoated silica gel thin-layer chromatoplates. Ten microliters of the test solutions were evaporated on the plates with the aid of a 50- μ l Hamilton syringe, using a technique to produce a spot about 2 mm in diameter. Most evaporations were accomplished by warming the chromatoplate slightly on a hot plate. When the volatile amines were spotted, the chromatoplate was kept at room temperature. These amines are strongly retained by the silica gel, and the solvent was simply removed by directing air across the plate. All chromogenic reagent solutions were sprayed on the plates with MISCO 10-ml tube sprayers selected to produce a fine spray when compressed air at about 10 p.s.i.g. was used as a carrier.

The three sets of test compounds were originally prepared for use as 5 or 2.5 mg/ml solutions, in the solvents shown in Table II, so that 10 μ l would contain the largest amount (50 or 25 μ g) of the compound that was tested. When smaller amounts of compounds were desired for testing, they were obtained by diluting the 5 or 2.5 mg/ml solution with the appropriate solvent to produce a new solution containing the required amount in 10 μ l. All of these test solutions were stored in 2-oz. glass bottles fitted with Poly-Seal caps, which contain a polyethylene conical seal.

Chromogenic reagents

p-Dimethylaminobenzaldehyde. Spray the plate with a 1% (w/v) solution of *p*-dimethylaminobenzaldehyde in glacial acetic acid.

2,3-Dichloro-1,4-naphthoquinone. Prepare a 1% (w/v) solution of 2,3-dichloro-1,4-naphthoquinone in benzene. Use this solution as the chromogenic reagent.

Salicylaldehyde. Prepare a 1% (w/v) solution of salicylaldehyde in benzene. Use this solution as the chromogenic reagent.

Furfural. Add 500 mg (about ten drops) of furfural to 10 ml of glacial acetic acid and mix the solution thoroughly. Use this solution as the chromogenic reagent.

Copper sulfate. Prepare a 5% (w/v) solution of copper sulfate pentahydrate in distilled water. Prepare a 1:3 (w/v) mixture of carbon disulfide and benzene. Spray

TABLE II

SOLVENTS USED FOR TEST SOLUTIONS

<i>Solvent</i>	<i>Compound</i>	<i>Solvent</i>	<i>Compound</i>
Ethyl alcohol, absolute	Adipic acid	Diethyl ether (cont.)	Benzonitrile
	Benzenearsonic acid		Benzyl alcohol
	Benzimidazole		Benzylamine
	Caprolactam		<i>n</i> -Butylamine
	Carbazole		3-Chloroaniline
	Diphenylamine		<i>p</i> -Chlorobenzenethiol
	Folic acid		Diethylamine
	Isonicotinic acid hydrazide		<i>N,N</i> -Diethylaniline
	Maleic acid hydrazide		Dipropylamine
	<i>N</i> -Methylphenylamine		<i>n</i> -Heptylamine
	Nicotinamide		2-Hydroxyethylhydrazine
Piperidine	Isobutylamine		
Acetone	Furosemide	Methyl benzoate	
	Hexamethylenetetramine	Morpholine	
Benzene	Anthracene	1-Naphthol	
	1,6-Hexanediamine	Nicotinonitrile	
	Lindane	Nitrobenzene	
		<i>N</i> -Nitrosodimethylamine	
Water	Adrenaline	<i>p</i> -Nitrosodiphenylamine	
	Chloramine-T	2,4-Pentanedione	
	1(-)-Proline	<i>o</i> -Phenylenediamine	
	Theobromine	<i>p</i> -Phenylenediamine	
		Phloroglucinol dihydrate	
Diethyl ether	Acetophenone	Propylamine	
	Acridine	Pyrrrole	
	Allylamine	2,4-Toluenediamine	
	1-Aminonaphthalene	α -Toluenethiol	
	2-Aminonaphthalene	Tributylamine	
	Aniline	Triphenylamine	
	Anisole		
	Barbituric acid	Methyl alcohol	
	Benzaldehyde	Dimethylchlorophosphate	
	Benzoic acid	Monoethyl acid ortho-phosphate	
		Phenylphosphonic acid	

the thin layer plate consecutively with the copper sulfate solution, with ammonium hydroxide, and with the carbon disulfide-benzene mixture.

2-Thiobarbituric acid. Dissolve 250 mg of 2-thiobarbituric acid and 0.25 ml of 4 *N* sodium hydroxide in distilled water and dilute the solution to 25 ml with distilled water. Dissolve 1.85 g of sodium citrate dihydrate in 1.6 ml of concentrated hydrochloric acid and dilute this solution to 12.5 ml with distilled water. Mix these two solutions to obtain the chromogenic reagent.

o-Tolidine. Two different sets of reagents were tested; in one the order of addition of the reagents was changed so that three different studies were conducted. (1) Prepare a 1% solution of *o*-tolidine in ethyl alcohol. Add saturated bromine water to a 1% (w/v) aqueous solution of potassium cyanide until a permanent yellow color is obtained. Then decolorize the solution by dropwise addition of more of the potassium cyanide solution. A solution of cyanogen bromide is thus produced. Spray the thin layer plate first with the *o*-tolidine solution, then with the cyanogen bromide solution. (2) Prepare a saturated aqueous suspension of *o*-tolidine, a saturated aqueous solution

of bromine, and a 1% aqueous solution of potassium cyanide. Spray these reagents in the order given. (3) Prepare a saturated aqueous solution of bromine, a 1% aqueous solution of potassium cyanide, and a 1% solution of *o*-tolidine in ethyl alcohol. Spray these reagents in the order given.

The sources of the compounds used in these studies are shown in Table III.

TABLE III

SOURCES OF COMPOUNDS, SOLVENTS, AND REAGENTS

<i>Compound</i>	<i>Source</i>	<i>Grade (if any)</i>
Acetic acid	Matheson Scientific	ACS Reagent
Acetone	Mallinckrodt	Analytical Reagent
Acetophenone	Fisher	Certified
Acridine	Eastman	
Adipic acid	Fisher	Certified
Adrenaline	Eastman	
Allylamine	Eastman	
1-Aminonaphthalene	MCB	
2-Aminonaphthalene	Aldrich	
Ammonium hydroxide	Matheson Scientific	ACS Reagent
Aniline	Eastman	
Anisole	Fisher	Certified
Anthracene	Eastman	
1(+)-Ascorbic acid	MCB	
Barbituric acid	Eastman	
Benzaldehyde	Fisher	Certified
Benzene	Mallinckrodt	Analytical Reagent
Benzeneearsonic acid	Eastman	
Benzidine	E. Merck AG	Reagent grade
Benzimidazole	Eastman	
Benzoic acid	J. T. Baker	Bakers Analyzed
Benzonitrile	Eastman	
Benzyl alcohol	Fisher	Certified
Benzylamine	Eastman	
<i>n</i> -Butylamine	Eastman	
Caprolactam	National Aniline	Spectranalyzed
Carbon disulfide	Fisher	Certified
Carbazole	Fisher	Certified
Chloramine-T	Eastman	
<i>p</i> -Chlorobenzenethiol	Eastman	Practical
Copper sulfate pentahydrate	Mallinckrodt	Analytical Reagent
Chloroaniline	MCB	
<i>N,N</i> -Diethylamine	Eastman	
<i>N,N</i> -Diethylaniline	Eastman	
Diethyl ether	Mallinckrodt	Analytical Reagent
Dimethylchlorophosphate	Aldrich	
<i>p</i> -Dimethylaminobenzaldehyde	E. Merck AG	
Diphenylamine	Fisher	Purified
Dipropylamine	Eastman	
Ethyl alcohol	U.S. Industrial	USP/NF
Folic acid	City Chemical	USP
Furaldehyde (furfural)	MCB	
Furosemide	National Laboratories	
<i>n</i> -Heptylamine	Eastman	
Hexamethylenetetramine	Eastman	
Hexanediamine	Eastman	
Hydrochloric acid	Matheson Scientific	ACS Reagent
2-Hydroxyethylhydrazine	Aldrich	
Isobutylamine	Eastman	

TABLE III (continued)

Isonicotinic acid hydrazide	Eastman	
Maleic acid hydrazide	Eastman	Practical
Methyl alcohol	Mallinckrodt	Analytical Reagent
Methyl benzoate	Fisher	Reagent
N-Methylphenylamine	Eastman	
Monoethyl acid orthophosphate	Victor	
Lindane	Diamond-Shamrock	
Morpholine	Fisher	Purified
1-Naphthol	Eastman	
Nicotinamide	Eastman	
Nicotinonitrile	Eastman	
Nitrobenzene	Fisher	Certified
N-Nitrosodimethylamine	Eastman	
<i>p</i> -Nitrosodiphenylamine	Eastman	
2,4-Pentanedione	Eastman	
<i>o</i> -Phenylenediamine	Eastman	Practical
<i>p</i> -Phenylenediamine	Fisher	Certified
Phenylphosphonic acid	MCB	
Phloroglucinol dihydrate	MCB	
Piperidine	Fisher	Certified
Potassium cyanide	Merck	Reagent grade
1(-)-Proline	Aldrich	
Propylamine	Eastman	
Pyrrole	MCB	Practical
Salicylaldehyde	MCB	Practical
Sodium citrate	Merck	Reagent grade
Sodium hydroxide	Mallinckrodt	Analytical Reagent
Theobromine	Eastman	
α -Toluenethiol	Eastman	
2,4-Toluenediamine	Eastman	Practical
Tributylamine	Eastman	
Triphenylamine	Eastman	

RESULTS AND DISCUSSION

Four reagents—*p*-dimethylaminobenzaldehyde, 2,3-dichloro-1,4-naphthoquinone, salicylaldehyde, and furfural—survived the preliminary evaluation, while the other three tests—copper sulfate, 2-thiobarbituric acid, and *o*-tolidine—failed. The four tests chosen for further study all gave positive tests with a least one amine at the 5- μ g level. The copper sulfate test proved not to be reliable because it produced a color only with two of the nine amines that were tested. 2-Thiobarbituric acid was rejected because it produced no color with 50 μ g of the three amines and produced rapidly fading colors with 5 μ g. *o*-Tolidine suspended in water or dissolved in absolute alcohol was evaluated in several combinations with *in situ* prepared cyanogen bromide. The combination of *o*-tolidine in absolute ethyl alcohol and preprepared cyanogen bromide gave a color with 50 μ g of the three amines but not with smaller amounts, and the other combinations gave no positive tests.

Results that were obtained on the 28 amines with the four chromogenic reagents that survived the preliminary screening tests are shown in Table IV. Results obtained on the 24 other organic compounds with the 2,3-dichloro-1,4-naphthoquinone reagent are shown in Table V. Tests on these 24 compounds with *p*-dimethylaminobenzaldehyde

TABLE IV

COLORS PRODUCED BY FOUR CHROMOGENIC REAGENTS FOR AMINES

	Color observed for amount tested (μg)															
	<i>p</i> -Dimethylaminobenzaldehyde quinone					Salicylaldehyde					Furfural					
	50	5	I	0.1	0.01	50	5	I	0.1	0.01	50	5	I	0.1	0.01	
Propylamine	a ^a	a	a	a	—	4 ^b	52 ^b	a	a	—	83	89	89	a	a	—
<i>n</i> -Butylamine	52	a	a	a	—	52	52 ^b	a	a	—	89	83	89	a	a	—
Isobutylamine	a	a	a	a	—	a	52 ^b	52 ^b	a	—	83	93	89	a	a	—
<i>n</i> -Heptylamine	a	a	a	—	—	4	52 ^b	a	a	—	83	83	89	a	a	—
Allylamine	89	a	a	a	—	52 ^b	73 ^b	a	a	—	82	83	92	a	a	—
Diethylamine	a	a	—	—	—	a	a	—	—	—	a	a	—	—	—	—
Dipropylamine	a	—	—	—	—	a	—	—	—	—	a	—	—	—	—	—
Tributylamine	a	—	—	—	—	a	—	—	—	—	a	—	—	—	—	—
<i>N</i> -Nitrosodimethylamine	a	—	—	—	—	a	—	—	—	—	a	—	—	—	—	—
Chloramine-T	a	a	a	—	—	a	a	a	a	—	86 ^b	89 ^b	a	—	—	—
1,6-Hexanediamine	89	a	a	a	—	52	52 ^b	a	a	—	82	83	89	a	a	—
Hexamethylenetetramine	a	—	—	—	—	a	—	—	—	—	a	—	—	—	—	—
Maleic acid hydrazide	a	—	—	—	—	a	—	—	—	—	a	—	—	—	—	—
Polic acid	a	—	—	—	—	a	—	—	—	—	a	—	—	—	—	—
Diphenylamine	86 ^b	a	a	a	—	223	221	a	a	—	a	a	a	a	a	—
<i>N</i> -Methyldiphenylamine	a	a	a	a	—	223	221	a	a	—	a	a	a	a	a	—
<i>p</i> -Nitrosodiphenylamine	a	—	—	—	—	a	—	—	—	—	a	—	—	—	—	—
Triphenylamine	89	a	a	—	—	221	221	a	—	—	a	a	a	a	a	—
Benzylamine	89	a	a	a	—	52	52 ^b	a	a	—	82	83	89	a	a	—
Aniline	82	82	83	89	92	26	52 ^b	a	a	—	83	89	a	a	a	—
3-Chloroaniline	82	82	83	89	92	52	52 ^b	a	a	—	83	89	a	a	a	—
<i>N,N</i> -Diethylaniline	226 ^b	a	a	—	—	224/	221/	a	—	—	a	a	a	a	a	—
						18 ^d	5 ^d									
2,4-Toluenediamine	67	70	83	86	89	230	224/	231	9	—	82	83	90	a	—	257
						201 ^d										256
<i>p</i> -Phenylenediamine	37	52	27	31	9	213	256	231	a	—	84	83	83	89	92	257
<i>o</i> -Phenylenediamine	82	70	67	73	73	221	52	a	a	—	83	89	92	a	—	256/
																87 ^d
1-Aminonaphthalene	82	83	83	89	92	221	221	9	a	—	83	70	73	a	—	250
2-Aminonaphthalene	50	70	83	73	89	43	52 ^b	9	a	—	83	83	73	a	—	224
2-Hydroxyethylhydrazine	52 ^b	89/	a	a	—	67	52 ^b	a	a	—	a	a	a	a	—	83
						86 ^d										a

^a a = No color observed.^b b Color developed on standing.^c c Fluorescent color.^d d Color changed on standing.

TABLE V

COLORS PRODUCED BY ORGANIC COMPOUNDS WITH 2,3-DICHLORO-1,4-NAPHTHOQUINONE

Compound	Color observed for amount tested (μg)		
	25	5	1
Benzoic acid	a ^a	a	a
Adipic acid	a	a	a
Anisole	a	a	a
Benzaldehyde	a	28	28
Acetophenone	a	28	28
2,4-Pentanedione	a	a	a
Methyl benzoate	a	a	a
Benzonitrile	a	a	a
<i>p</i> -Tolyl disulfide	86	a	31
Morpholine	34	34	34
Carbazole	29	2	4
Acridine	98/97 ^b	98/97 ^b	98/101 ^b
Benzylalcohol	a	a	a
Nitrobenzene	a	a	a
1-Naphthol	15/55 ^b	5/55 ^b	7/54 ^b
Phloroglucinol dihydrate	68	66	76
α -Toluenethiol	a	86	86
Anthracene	221	221	226
Benzenearsonic acid	a	a	a
Lindane	a	a	a
<i>p</i> -Chlorobenzenethiol	83	101	28
Dimethylchlorophosphate	a	a	a
Monoethyl acid ortho-phosphate	a	a	a
Phenyl phosphonic acid	a	a	a

^a a = No color observed.^b First color listed is color observed before spraying.

gave only one positive reaction, color number 84, with acridine at 25, 5 and 1 μg . Tests with salicylaldehyde and furfural gave the colors listed in Table VI.

For convenience in comparing the colors, a list of names for the color numbers, as designated by the National Bureau of Standards, is shown in Table VII.

TABLE VI

COLOURS PRODUCED WITH SALICYLALDEHYDE AND FURFURAL

Compound	Color observed for amount tested (g)					
	Salicylaldehyde			Furfural		
	25	5	1	25	5	1
Acridine	98/104 ^b	98/101 ^b	98/104 ^b	97	97	97
1-Naphthol	42/15 ^b	33/29 ^b	31/32 ^b	9	8	9
Phloroglucinol dihydrate	73	73	a	a	a	a

^a No color observed.^b First color is color before spraying.

TABLE VII

COLOR NUMBERS AND COLOR NAMES

Number	Name	Number	Name
2	Strong pink	68	Strong orange yellow
4	Light pink	70	Light orange yellow
5	Medium pink	73	Pale orange yellow
7	Pale pink	76	Light yellowish brown
8	Grayish pink	79	Light grayish yellowish brown
9	Pinkish white	82	Vivid yellow
15	Medium red	83	Brilliant yellow
18	Light grayish red	84	Strong yellow
26	Strong yellowish pink	86	Light yellow
27	Deep yellowish pink	87	Medium yellow
28	Light yellowish pink	89	Pale yellow
29	Medium yellowish pink	90	Grayish yellow
31	Pale yellowish pink	92	Yellowish white
32	Grayish yellowish pink	97	Vivid greenish yellow
33	Brownish pink	98	Brilliant greenish yellow
34	Vivid reddish orange	101	Light greenish yellow
37	Medium reddish orange	104	Pale greenish yellow
42	Light reddish brown	201	Dark purplish blue
43	Medium reddish brown	213	Vivid pale violet
50	Strong orange	221	Vivid light purple
52	Light orange	223	Moderate purple
54	Brownish orange	224	Dark purple
55	Strong brown	226	Vivid pale purple
66	Vivid orange yellow	230	Blackish purple
67	Brilliant orange yellow	231	Purplish white
		248	Deep purplish pink
		250	Moderate purplish pink
		256	Deep purplish red
		257	Very deep purplish red

p-Dimethylaminobenzaldehyde test

The *p*-dimethylaminobenzaldehyde test used in this study was developed for use in detecting aromatic amines¹⁶. The compound will form Schiff-type bases with primary aromatic amines⁵. It has also been used for detecting carboxylic acids²⁹ and pyrroles³⁰. Table IV shows that *p*-dimethylaminobenzaldehyde gave a color primarily with aromatic amines. It failed to give a color with only three of the ones tested, *viz.* folic acid, *N*-methyldiphenylamine, and *p*-nitrosodiphenylamine. It gave colors with five aliphatic amines, *viz.* *n*-butylamine, allylamine, 1,6-hexanediamine, 2-hydroxyethylhydrazine, and benzylamine.

No clear pattern emerges regarding the classes of amines that did or did not give a color with *p*-dimethylaminobenzaldehyde. The differences in color formation observed for similar compounds, such as *n*-butylamine and isobutylamine, or diphenylamine and *p*-nitrosodiphenylamine, may have arisen because of the arbitrary rules selected for determining if a color formed, *i.e.*, more colors may have been observed if higher levels of some amines were tested or if longer times were allowed for color formation.

As previously noted, *p*-dimethylaminobenzaldehyde gave a color with only one of the other types of compounds tested, *i.e.* acridine. This is not surprising because this reagent is nearly specific for compounds containing the amine group. This ability

of the test to be specific for amines makes it useful for detecting this class of compounds, even though many aliphatic amines are not detected at the 50- μ g level.

2,3-Dichloro-1,4-naphthoquinone test

The 2,3-dichloro-1,4-naphthoquinone test used in this study was devised for the detection of *n*-alkylvinylamines²². The reaction produces purple 2-chloro-3,2'-alkylvinyl-naphthoquinones. The reagent has not been generally used for detection of amines.

No *n*-alkylvinylamines were included in the 28 amines tested, but Table IV shows that this reagent will give colors with a wide variety of amines. Colors produced varied from orange to pink to purple. Aliphatic amines generally gave light orange or pink colors, while aromatic amines gave more intense violet or purple colors. Limits of detection were not as low as with *p*-dimethylaminobenzaldehyde for aromatic amines.

The mechanism that has been proposed²² for the reaction of 2,3-dichloro-1,4-naphthoquinone with *n*-alkylvinylamines—splitting out HCl to form a monochloro-derivative—cannot be the mechanism for reaction with these amines. The wide variety of compounds that will react with the reagent suggests that molecular association complexes may be formed. It is futile to speculate further regarding the mechanism without more information about the reaction, which these studies were not designed to produce. The reaction mechanism should be investigated in detail.

Unfortunately, the usefulness of this chromogenic reagent for detecting amines is limited because, as shown in Table V, it also reacts with other types of compounds, among them disulfides, heterocyclics, phenols, polynuclear aromatics, and thiols. Nevertheless, the compound should receive consideration as a possible chromogenic reagent for amines in systems where other types of compounds are not present.

Salicylaldehyde test

The salicylaldehyde test used in this study was devised for primary arylalkyl-amines¹⁴. It has also been used for detecting phloroglucinol³¹. Table IV shows that this reagent gives colors with primary alkyl and aryl amines. It does not give colors with secondary or tertiary alkyl or aromatic amines under the conditions studied. The proposed reaction is the formulation of aldimines¹⁴ by reaction with primary amines. Evidently other chromogenic reactions cannot take place with secondary and tertiary amines.

As already stated, it reacted only with acridine, 1-naphthol, and phloroglucinol among the other types of compounds tested. Salicylaldehyde is thus a useful chromogenic reagent only for detection of primary amines.

Furfural test

The furfural test selected for this study was devised for detecting aromatic amines^{7,8}. A condensation reaction takes place between furfural and the aromatic imine to give a Schiff base. FEIGL³² reports that furfural will also react with secondary aromatic amines, aliphatic amines, and amino acids. Table IV shows that furfural reacts with all of the primary, none of the secondary, and two of the three tertiary aromatic amines under the reaction conditions employed in these studies. The only other type of amine that gave any color formation with furfural at the levels studied was 2-hydroxyethylhydrazine.

As was previously stated, only acridine and naphthol among the other classes of compounds gave a chromogenic reaction with furfural. Unfortunately, this lack of interference is of little consequence since furfural is not a useful chromogenic reagent for more different types of amines.

Changes in test colors with concentration

Perhaps the most surprising result of these studies is the observation that some of the chromogenic reagents produce different colors for different amounts of the same compound. In several of these instances the intensity of the color merely decreases as the relative amount of the compound to be detected decreases. The apparent change in color is probably produced by the presence of an excess amount of the chromogenic reagent in the area on the plate containing the other compound, or by the effect of the white silica gel. Examples of this type of change are the reactions of salicylaldehyde with allylamine and *p*-phenylenediamine with 2,3-dichloro-1,4-naphthoquinone.

However, in other systems, a definite change in color occurs. For example, the color produced by the reaction between *p*-phenylenediamine and *p*-dimethylaminobenzaldehyde changes from medium reddish orange to light orange to deep yellowish pink to pale yellowish pink to pinkish white as the amount of *p*-phenylenediamine decreases, and the color produced by the reaction between 2-aminonaphthalene and *p*-dimethylaminobenzaldehyde changes from strong orange to bright orange yellow to brilliant yellow to pale orange yellow to pale yellow. Two of the most striking color changes occur in the reactions between 2,4-toluenediamine and furfural, in which the color changes from very deep purplish red to deep purplish red to light orange yellow to pale yellow, and in the reaction between 2-aminonaphthalene and furfural, in which the color changes from dark purple to deep purplish red to pinkish white to pale yellow. The most dramatic color changes occur in reactions with furfural. FEIGL³² reports that transient colors are formed in reactions between furfural and *m*-hydroxybenzenes. However, he also reports that the Schiff bases formed between furfural and aryl amines are purple, whereas our results show that the violet color is produced with large amounts of the amine and a yellow color is produced with smaller amounts.

These remarkable color changes suggest that a different compound is formed as the ratio of the reactants changes. The nature of the compound produced with smaller amounts of chromogenic reagents is not known and could be the subject of a future investigation. The important corollary pertinent to these studies is that colors produced by some chromogenic reagents cannot be simply reported.

Comparison of tests for usefulness

The principal criteria for selection of a general purpose chromogenic reagent for detecting amines are (1) sensitivity, (2) general applicability to a variety of amines, and (3) lack of colors produced by other types of compounds. Critical examination of the results in Tables IV and V shows that the most widely useful reagent is 2,3-dichloro-1,4-naphthoquinone, although it also reacts with classes of compounds other than amines. The *p*-dimethylaminobenzaldehyde test will detect most aromatic amines and a few aliphatic amines; it gives the best sensitivity of any of the tests, but only for aromatic amines. Salicylaldehyde is useful only for detecting primary aliphatic and aromatic amines. Hence, if a variety of amines are to be detected, the 2,3-dichloro-1,4-naphthoquinone test should be used. If maximum sensitivity is desired in the

reagent and only aromatic amines are to be detected, then the *p*-dimethylamino-benzaldehyde test is the best.

Precautions

It should be pointed out that the results presented herein were obtained for the seven chromogenic reagents under the stated experimental conditions. The changes in colors with amount and the known dependence of the reactions involved on pH and relative amounts of reactant indicate that different colors and lower limits of detection may be obtained under different experimental conditions. To obtain the results presented here, the stated experimental conditions should be followed carefully.

ACKNOWLEDGEMENTS

This research was supported by the Advanced Research Project Agency of the Department of Defense and was monitored by the Life Sciences Division of the U.S. Army Research Office under Contract No. DAHC-19-67-C-0043.

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