

Note

A method for detecting free phenolic groups and indole derivatives in thin-layer or paper chromatography

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Diazonium salts^{1,2}, tetrazotized benzidine^{3,4}, antimony pentachloride⁵ or phosphomolybdic acid² are the most generally used spraying reagents for phenols. Their disadvantage is a low specificity. The color of ceric ammonium nitrate in nitric acid or ammonium hydroxide changes from yellow to orange or red in the presence of alcohols, phenols and aromatic amines⁶⁻¹⁰. We have found that hydroxylamine can participate in this reaction. It stabilizes the color complex of cerium with phenols and increases the specificity so that the color reaction is useful for the quantitative estimation of aromatic hydroxy compounds¹¹. This reaction can be arranged as a detection method for phenolic or indolyl groups in thin-layer or paper chromatography.

MATERIALS AND METHODS

5-, 10-, and 50- μ l samples of each compound (0.1% in water or dioxane) were spotted on silica gel G and cellulose thin-layer chromatographic (TLC) plates and on Whatman No. 1 chromatographic paper. The spots were dried on air and the TLC plates or paper sheets were sprayed at first with a freshly prepared and filtered 5% solution of $(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$ in acetone. The spots were then sprayed with 5% NH_2OH (hydrochloride) in 80% acetone and dried with hot air. Brown spots were observed in daylight. The spots and the colorless background are stable for a long time (at least one year). The heating of plates at 110° for 5 min can increase the sensitivity in some cases.

RESULTS AND DISCUSSION

As is apparent from Table I, the reaction under these conditions is specific for aromatic hydroxy compounds and indole derivatives. Aliphatic alcohols or amines, sugars, saturated cycloalcohols, carboxylic acids, aldehydes, and ketones do not react. The intensity of the spots differs on different materials. The sensitivity of the reaction was estimated on TLC silica gel G plates (Table I). Some aromatic amines in higher concentrations can also give colors, especially with heat, for example, *p*-phenylenediamine, diphenylamine, naphthylamine and carbazole.

Heat increased the intensity of the spots of tyrosine, thymol, safrole, *p*-

TABLE I
 COLOR REACTION OF CERIC NITRATE PLUS HYDROXYLAMINE DETECTION REAGENT WITH
 DIFFERENT COMPOUNDS ON TLC SILICA GEL G PLATES

<i>Compound</i>	<i>Color</i>	<i>Sensitivity</i> (μg)	<i>Compound</i>	<i>Color</i>	<i>Sensitivity</i> (μg)
Tryptophan	brown	1-5	Thymol	brown	5-10
Indole	brown	5-10	Eugenole	brown	1-5
Indoleacetic acid	brown	1-5	Safrrole	brown	5-10
Tyrosine	brown	1-5	Diphenylamine	brown	1-5
Dihydroxyphenylalanine	brown	1-5	<i>p</i> -Nitrophenol	brown	1-5
Tocopherol	brown	1-5	Morphine	pink	1-5
Diiodotyrosine	brown	1-5	Cacotheline	green	5-10
Estrone	brown	1-5	Chromotropic acid	brown	1-5
Estradiol	brown	1-5	H-acid	brown	1-5
<i>p</i> -Hydroxyphenylpyruvic acid	brown	1-5	<i>p</i> -Hydroxyacetophenone	brown	5-10
Phenol	brown	1-5	3-Methylindole	brown	5-10
Resorcinol	brown	1-5	3-Indoleacetone	brown	5-10
Hydroquinone	brown	1-5	Indole-3-aldehyde	brown	5-10
Pyrogallol	brown	1-5	Indole-3-butyric acid	brown	5-10
Pyrocatechine	brown	1-5	Indole-2-carboxylic acid	brown	5-10
<i>p</i> -Aminophenol	brown	1-5	3-Indoleglyoxyamine	brown	5-10
<i>o</i> -Aminophenol	brown	1-5	3-Indoleglyoxylic acid	brown	5-10
<i>m</i> -Aminophenol	brown	1-5	3-Indolepyruvic acid	brown	5-10
α -Naphthol	brown	1-5	Phenolphthalein	brown	5-10
β -Naphthol	brown	1-5	Eosine	brown	5-10
<i>p</i> -Hydroxybenzoic acid	brown	1-5	Vanilline	brown	1-5
Guaiacol	brown	1-5	Tryptophol	brown	1-5
<i>p</i> -Hydroxyanisole	brown	1-5	J-acid	brown	1-5
Orcinol	brown	1-5	<i>p</i> -Cresol	brown	1-5

hydroxyacetophenone, 1,4-benzoquinone, 1,4-naphthoquinone or fluoresceine. But some other compounds such as codeine, inositol or aniline also appeared as slightly colored spots after heat. This detection reagent can be used, for example, for the specific discrimination of tyrosine and tryptophan from other amino acids, progesterone, testosterone or corticosterone from estradiol or estrone, menthol from thymol, benzoic acid from *p*-hydroxybenzoic acid. The following compounds did not produce colored spots (in concentrations up to 50 μg): acetaldehyde, acetic acid, acetoacetic acid, acetone, acetylacetone, acetylcholine, N-acetylcysteine, acetylsalicylic acid, aconitic acid, adenine, adonitol, adrenaline, alanine, albumin, 2-amino-5-chlorobenzoxazole, 4-aminoantipyrine, aminolevulinic acid, aminopyrine, amphetamine, *n*-amyl alcohol, amytal, aniline, *p*-anisaldehyde, *p*-anisic acid, anisole, anthrone, arabinose, arabitol, arachidic acid, arginine, ascorbic acid, asparagine, aspartic acid, ATP, benzaldehyde, benzidine, benzimidazole, benzofuran, benzothiazole, benzphetamine, betaine, biotin, *n*-butanol, cadaverine, caffeine, camphor, cephalin, cetyl alcohol, chloral, cholesterol, cholic acid, cholin, cinnamic acid, citric acid, citruline, corticosterone, cortisone, coumarin, creatine, crotonic acid, 1,3-cyclohexanediol, cyclamic acid, cyclohexane, 1,2-cyclohexanedione, cyclohexanol, cyclohexanone, cyclohexylamine, cysteine, cystine, cytidine, cytochrome *c*, decyl alcohol, dehydroascorbic acid, dehydrofolic acid, diethylphthalate, dihydrosphingosine, dihydroxyacetone, dilantin, N,N-dimethyl aniline, N,N-dimethyl-*p*-phenylene-

diamine, dimethylamine, dithioerythritol, ergosterol, erythritol, erythromycin, ethanolamine, ethanol, ethylene glycol, folic acid, formic acid, formaldehyde, furan, fructose, fucose, fumaric acid, geraniol, globulin, gluconic acid, glycosamine, glucose, glucuronic acid, glutamic acid, glutamine, glutathione, glyceraldehyde, glyceric acid, glycerin, glycine, glycolaldehyde, glyoxal, guanine, hexamethylenediamine, hexobarbital, *n*-hexyl alcohol, histidine, hydroxyproline, indene, inositol, β -ionone, isoamyl alcohol, isoleucine, isoprene, isopropanol, ketoglutaric acid, leucine, limonene, linoleic acid, lysine, malic acid, menthol, methionine, methanol, methyl ethyl ketone, *N*-methylaniline, NADPH, nicotinamide, nitrobenzene, norvaline, *n*-octyl alcohol, oleic acid, ornithine, oxaloacetic acid, oxalic acid, oxytocin, pantothenic acid, perchloric acid, phenylalanine, phenylpyruvic acid, phosphoric acid, phytol, prephenic acid, progesterone, proline, *n*-propanol, purine, pyridine, pyridoxal, pyridoxine, pyrimidine, pyruvic acid, reduced FMN, reduced glutathione, rhamnose, ribose, sarcosine, sedoheptulose, serine, shikimic acid, sphingosine, squalene, stilbene, succinic acid, sulfanilamide, testosterone, tetrahydrofolic acid, thiamine, thioctic acid, thioglycolic acid, thiophene, thiophenol, thiourea, thyroxine, threonine, thymine, tocopherol, trichloroacetic acid, UPDGA, ubiquinone, uracil, urea, uric acid, urotropin, valine and xanthine.

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