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Note

Detection of aminophenols, aromatic amines and related compounds on thin-layer plates

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Aminophenols are frequently formed when compounds of industrial or pharmacological importance, such as aniline[1] or prilocaine[2], are metabolised in mammals by the liver microsomal mixed function oxidase systems. Although these metabolites are important, and may exert physiological effects such as methaemoglobin formation[2], their identification is often difficult, since *o*-, *m*- and *p*-isomers often have similar R_F values. We have therefore developed colour reactions to distinguish between them and also to give characteristic colours with related compounds.

EXPERIMENTAL

Thin layer chromatography (TLC) of compounds was carried out on plates coated with silica gel G (0.3 mm thick; Merck, Darmstadt, G.F.R.)[2], 0.5–25 μl of 0.1% solutions being applied.

(a) A freshly prepared 3% aqueous solution (w/v) of selenium dioxide (I) is sprayed onto a dried TLC plate which is then heated at 120° for 15–20 min.

(b) A freshly prepared 1% solution of phenol in 20% Na_2CO_3 (II) is sprayed and the TLC plate heated for 20–25 min at 120°. Overspraying with concentrated HCl may give a colour change immediately or up to 48 h later.

Both systems give coloured spots on a white ground.

RESULTS

The colour reactions found are listed in Table I. Amino acids gave negative results with both sprays. The limits of detection after development were 1–2 μg for all compounds tested. The chromatospots from spray I turned brown-black after a few days, possibly due to decomposition with release of elemental selenium.

DISCUSSION

Selenious acid in sulphuric acid was introduced by Mecke[3] for the detection of opium alkaloids. Since then, it has found little use as a detecting agent, apart from some steroids[4] and phenothiazines[5]. It is known that phenyl selenoxides can be formed[6,7] and that selenium dioxide can react with certain amino group derivatives

TABLE I

COMPOUNDS INVESTIGATED AND OBSERVED COLOUR REACTIONS WITH THE SPRAYS TESTED

Compound	*Spray I*		*Spray II*	
	Initial colour	*After heat*	*After heat*	*HCl overspray*
o-Aminophenol	pale yellow	yellow	dark yellow	peach
m-Aminophenol	—	dark brown	rust-brown	brown
p-Aminophenol	—	purple	blue	red
o-Anisidine	—	purple	—	lilac
m-Anisidine	—	purple-brown	—	peach
p-Anisidine	—	light brown	—	red
o-Aminobenzoic acid	—	rust-brown	—	—
m-Aminobenzoic acid	—	light brown	—	—
p-Aminobenzoic acid	—	lime-green	—	—
3,5-Diaminobenzoic acid	buff	dark brown-purple	light pink	buff
2,4-Dimethoxyaniline	—	maroon	—	purple
2,5-Dimethoxyaniline	—	black-brown	—	green-brown
3,5-Dimethoxyaniline	—	dark brown, yellow halo	—	yellow
2,4-Dichloroaniline	—	blue-purple	—	buff
2,5-Dichloroaniline	—	cream-yellow	—	mustard
3,4-Dichloroaniline	—	mauve	light blue	buff
2-Amino-5-hydroxybenzoic acid	—	light brown-purple	light blue	dark brown
3-Amino-2-hydroxybenzoic acid	—	gold	—	cream
3-Amino-4-hydroxybenzoic acid	yellow	yellow-black	green	brown
2-Amino-1,3-dimethylbenzene	—	purple	—	light red
2-Amino-1,4-dimethylbenzene	—	green, purple halo	—	light green
3-Amino-1,2-dimethylbenzene	—	rust-brown	—	light green
4-Amino-1,2-dimethylbenzene	—	mustard green	—	yellow
4-Amino-1,3-dimethylbenzene	light brown	mustard, purple halo	—	mauve
2-Amino-5-nitrotoluene	bright yellow	bright yellow	bright yellow	bright yellow
4-Amino-2-nitrotoluene	yellow	lime-green	yellow	cream-yellow
4-Amino-3-nitrotoluene	dark yellow	dark yellow	orange-yellow	deep yellow
Aniline	—	brown-green	—	—
Phenol	—	—	—	—
Eugenol	—	orange-peach	buff	buff
Orcinol	—	rust-brown	pink	cream
Resorcinol	—	brown	khaki	grey-buff
Metol	light brown	rust	blue-grey	brown
Pyrocatechol	buff	dark brown	dark grey	dark brown
Pyrogallol	light brown	brown	brown-yellow	brown-yellow
α-Naphthol	—	green-grey	grey	brown
β-Naphthol	beige	yellow-grey	brown	brown
Cinchonine	—	orange	—	pink
Brucine	—	orange-yellow	—	—
Hydrazine	—	tangerine	—	—
Phenylhydrazine	light yellow	mustard	light yellow	beige

such as hydrazines and phenylhydrazines[8]. Under these conditions, hydrazine[9] and phenylhydrazine[10] are oxidised, as is ascorbic acid, which easily reduces selenious acid[11,12]; the colours found using our spray presumably indicate formation of red elemental selenium, since these compounds gave characteristic tangerine or orange spots. Reducing sugars, such as glucose, were not found to react, in agreement with earlier work[13]. Phenol similarly gave no visible reaction, which was not unexpected, since early work[14] showed that a colourless product was obtained when phenol was treated with acidic selenium dioxide. Nitrophenols also show no colour change[6], although polyphenols, such as resorcinol, do give coloured products.

The range of colours found by reacting aqueous selenium dioxide with aminophenols, some aromatic amines and related compounds has not previously been reported. A similarly extensive range of colours was found using spray II, which is based on the colorimetric test for *p*-aminophenol described by Brodie and Axelrod[15], and later modified by other workers[2,16]. In this reaction, *p*-aminophenol gives a blue coloration in the presence of phenol and carbonate ions, due to the formation of indophenol. The *o*- and *m*-aminophenols give yellow and buff coloured solutions respectively. It is interesting to note that few compounds react with the alkaline phenol reagent even after heating, although on overspraying with concentrated HCl many aromatic amines, phenols and derivatives give a variety of colours. Both sprays clearly differentiate between *o*-, *m*- and *p*-isomers, and also allow distinction between such similar compounds as 2-amino-1,3-dimethylbenzene and 2-amino-1,4-dimethylbenzene. It is hoped that this will prove useful in studies on the metabolism of aromatic amines, aminophenols and related compounds.

REFERENCES

1 D. V. Parke, *Biochemistry of Foreign Compounds*, Pergamon, Oxford, 1968.
2 M. Hjelm, B. Ragnarsson and P. Wistrand, *Biochem. Pharmacol.*, 21 (1972) 2825.
3 P. Mecke, *Z. Oeff. Chem.*, 5 (1899) 351.
4 K. Savard, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 11 (1952) 281.
5 G. H. Lucas and C. Fabierkiewicz, *J. Forensic Sci.*, 8 (1963) 462.
6 V. E. Levine, *Science*, 52 (1920) 207.
7 F. Krafft and R. E. Lyons, *Ber.*, 29 (1896) 424.
8 J. W. Mellor, *A Comprehensive Treatise on Inorganic and Theoretical Chemistry*, Vol. 10, Longmans, London, 1930, pp. 694, 811, 818.
9 F. W. de Coninck, *C.R. Acad. Sci.*, 142 (1906) 571.
10 O. Hinsberg, *Justus Liebigs Ann. Chem.*, 260 (1890) 40.
11 V. E. Levine, *Proc. Soc. Exp. Biol. Med.*, 31 (1934) 1092.
12 V. E Levine, *Proc. Soc. Exp. Biol. Med.*, 35 (1936) 231.
13 H. L. Riley, J. P. Morley and N. A. C. Friend, *J. Chem. Soc. London*, (1932) 1875.
14 Ger. Pat. 299,510 (1917); *C.A.*, 13 (1919) 323.
15 B. B. Brodie and J. Axelrod, *J. Pharmacol. Exp. Ther.*, 94 (1948) 29.
16 Y. Imai and R. Sato, *33rd Ann. Meeting Japanese Biochem. Soc., 1960*, reported by T. Otani, K. Akayi and Y. Sakamoto, *J. Biochem. (Tokyo)*, 52 (1962) 428.