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Note

Detection of aminophenols and related compounds by a modification of the ferric ferricyanide spray reagent

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It has been known for a considerable time that the reaction between iron salts and iron hexacyano compounds would give rise to dark blue pigments (Turnbull's Blue; Prussian Blue) provided that the metal ions were in different oxidation states^{1–3}. This reaction has been employed in chromatography as a detection reagent for phenols; one of the ferric ions in an aqueous solution of ferric ferricyanide (ferric chloride and potassium ferricyanide) sprayed onto the chromatogram is reduced to the ferrous form by the phenol to give a blue spot on a yellow background⁴. Unfortunately, both the background and the dark brown spray reagent itself also rapidly become reduced in daylight (not yellow wavelengths) to give a blue colouration, making the chromatospots indistinguishable and the original spray useless⁵. This problem can be countered in part by overspraying the chromatograms with dilute hydrochloric acid. However, the substitution of zinc chloride for ferric chloride in the above systems gives a spray reagent and chromatograms that remain relatively stable.

EXPERIMENTAL

Thin-layer chromatography (TLC) of compounds was carried out on plates coated with silica gel G (0.2 mm thick; Merck, Darmstadt, F.R.G.), 0.5–25 μ l of 0.1% (w/v) solutions being applied. A 5% (w/v) aqueous solution of zinc chloride (0.37 M) was mixed with an equal volume of 5% (w/v) aqueous potassium ferricyanide (0.15 M) to give a yellow-orange suspension which precipitates out⁶ (eqn. 1).



The precipitate, if filtered, dried and stored in the dark, remains useful for at least ten years. Spraying a chromatogram with either a fresh suspension or the resuspended precipitate [5% (w/v) aqueous], gives rise to coloured spots on a yellow ground which becomes pale green after four days.

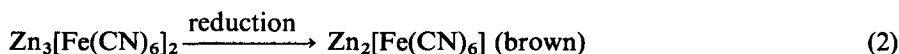
TABLE I
COMPOUNDS INVESTIGATED AND OBSERVED COLOUR REACTIONS

<i>Compound</i>	<i>Initial colour</i>	<i>After spray</i>
2-Aminophenol	Pale yellow	Dark brown
3-Aminophenol	—	Mauve
4-Aminophenol	—	Blue
2-Methoxyaniline (<i>o</i> -anisidine)	—	Purple
3-Methoxyaniline (<i>m</i> -anisidine)	—	Mauve
4-Methoxyaniline (<i>p</i> -anisidine)	—	Yellow-brown
2,4-Dimethoxyaniline	—	Indigo
3-Bromoaniline	—	Purple
4-Bromoaniline	—	Grey
2,5-Dichloroaniline	—	Grey-purple*
2,4,6-Trichloroaniline	—	Mauve
2-Amino-3-hydroxybenzoic acid	—	Mauve
3-Amino-4-hydroxybenzoic acid	Yellow	Brown
4-Amino-1,2-dimethylbenzene	—	Pink-brown
4-Aminotoluene (<i>p</i> -toluidine)	—	Cream-pink
1,2-Diaminobenzene (<i>o</i> -phenylenediamine)	Yellow	Yellow-green
1,3-Diaminobenzene (<i>m</i> -phenylenediamine)	—	Purple
1,4-Diaminobenzene (<i>p</i> -phenylenediamine)	Pink	Indigo
1,2-Dihydroxybenzene (catechol)	Buff	Pink
1,3-Dihydroxybenzene (resorcinol)	—	Pink
5-Methyl-1,3-dihydroxybenzene (orcinol)	—	Pink
1,2,4-Trihydroxybenzene	—	Mauve
Phenol	—	Light brown
4-Bromophenol	—	Grey
4-Methoxyphenol	—	Pink
2,5-Dimethylphenol (<i>p</i> -xylenol)	—	Pale green
4-(Phenylmethyl)phenol (<i>p</i> -benzylphenol)	—	White
Menthol	—	Mauve
β -Naphthol	Beige	Pale blue

* Colour develops after 10 min.

RESULTS

Phenol itself appears as a brown spot owing to the reduction of the ferric ion in the zinc-iron cyanide complex (eqn. 2). Substituted compounds give rise to a variety of different colours owing to reaction of the additional chemical groupings (Table I). The limits of detection after development were 2–5 μg for all compounds examined.



DISCUSSION

The spray reagent described has the advantage over the established ferric ferrocyanide reagent in that the chromatospots are permanent and remain distinguish-

able for many days. In addition, unlike the ferric ferricyanide reagent, a variety of different colours are produced, permitting preliminary identification of isomeric compounds which may be difficult to resolve by other means. Although modern trends are moving away from TLC in favour of other methods, TLC still provides a rapid and useful visual display of the components of a mixture and it is hoped that this modified reagent will prove useful in studies on the chemical synthesis and metabolic conversions of aromatic amines, aminophenols and related compounds.

REFERENCES

- 1 J. Brown, *Phil. Trans. Roy. Soc.*, 23 (1724) 17–24.
- 2 T. Graham, *Phil. Trans. Roy. Soc.*, 151 (1861) 211.
- 3 U. Antony and G. Giglio, *Gazz. Chim. Ital.*, 25 (1895) 2, 8.
- 4 G. M. Barton, R. S. Evans and J. A. F. Gardener, *Nature (London)*, 170 (1952) 249–250.
- 5 H. Vogel, *Ber.*, 4 (1871) 90.
- 6 H. B. Weiser, W. O. Milligan and J. B. Bates, *J. Phys. Chem.*, 46 (1942) 99–111.