120,000 or higher molecular weight, or dissociate into smaller molecular weight forms. The smallest form so far obtained has a molecular weight of approximately 6,000 and retains full biological activity. Studies now in progress are aimed at the complete clarification of the chemical structure of this unit.

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Use of tetracyanoethylene as a thin-layer chromatographic spray reagent

Methods presently used for the detection of aromatic compounds in thin-layer chromatography (TLC) involve irradiation of the plate with ultraviolet (U.V.) light, the use of spray reagents, or transparency methods[1]. The use of U.V. light for the detection of aromatics is restricted to the availability of U.V. lamps, provided, of course, that the compound of interest is U.V. active. Transparency methods do not distinguish between aromatics and aliphatics. Most spray reagents are specific for functional groups, while the corrosive and universal spray reagents do not distinguish between aromatics and aliphatics.

Tetracyanoethylene (TCNE) has been found to undergo a variety of reactions, including addition, replacement and cyclization reactions[2]. Furthermore, TCNE forms 1:1 π-complexes with a variety of aromatic compounds, particularly hydrocarbons, oxy-substituted hydrocarbons, and other electropositively substituted aromatic hydrocarbons[3]. The formation of these characteristically colored complexes makes TCNE highly useful as a rapid means for the detection and identification of aromatic compounds.

We have investigated TCNE as a possible spray reagent for the detection of aromatics in TLC.

A variety of aromatic compounds in solution were applied to a TLC plate, the solvent evaporated and the plate sprayed with a 0.5 percent solution of TCNE in ethyl acetate. At room temperature, some characteristically colored π-complexes were detected visually, but only at concentrations well above the practical loading limits of the TLC plate. MERRIFIELD AND PHILLIPS[4] reported association constants, molar absorptivities and heats of formation of π-complexes of several aromatics in solution and the effect of solvents on these constants. Based on their data we feel that (1) only a limited number of aromatics formed π-complexes on the TLC support at room temperature, and (2) the π-complexes formed were difficult to visualize due to their relatively low molar absorptivities, consequently, only high concentrations could effect visualization. The attempt to form TCNE π-complexes on the TLC plate was therefore not practical.

However, we found that certain aromatics, particularly phenols and amines, slowly developed characteristic colors upon standing at room temperature; furthermore, these colors developed rapidly at 80°. The molar absorptivities of the compounds were considerably higher and the colors different from those observed for their corresponding π-complexes, making possible the visualization of small amounts of material which were well within the loading limits of the plate. HECKERT[5] noted that a relatively slow condensation reaction occurred between both phenol and amines and TCNE at room temperature to give highly colored tricyanovinyl phenols and tricyanovinyl amines with the elimination of hydrogen cyanide. The same or similar reactions were probably occurring when we heated the sprayed TLC plates to 80°.

TABLE I

COLORED COMPLEXES FORMED ON SILICA GEL* WITH TCNE

Compound	*Color*
Phenol	creme
2-Naphthol	brown
m-Cresol	yellow
2-Chlorophenol	pink
2,4,6-Trichlorophenol	yellow (faint)
2,4-Dibromophenol	purple
2,6-Dibromophenol	red
Resorcinol	rust
2-Hydroxypyridine	tan
3-Hydroxypyridine	brown
4-Hydroxypyridine	yellow
2,3-Dihydroxybenzoic acid	blue
2,4-Dihydroxybenzoic acid	tan-brown
2,6-Dihydroxybenzoic acid	purple-brown
3,5-Dihydroxybenzoic acid	tan
Pyrogallol	purple
Phloroglucinol	brown
2-Mercaptobenzothiazole	purple
Aniline	yellow
1,3-Diaminobenzene	purple
Diphenylamine	tan
2,4-Diaminotoluene	green
3,4-Diaminotoluene	orange

* Precoated silica gel plates (E. Merck, A. G., Catalog No. 5715).

The usefulness of TCNE as a TLC spray reagent in preparative chemistry can be realized by the fact that different colors are formed depending on the type of substitution of the aromatic. For example, reaction mixtures containing 2,4- and 2,6-isomers of a particular compound could easily be separated by TLC and semi-quantitatively estimated by spraying with TCNE. Table I shows colored complexes obtained from a number of aromatic amines and phenols including selected isomers of particular compounds. Also included in Table I are random compounds that we found to give characteristic colored complexes with TCNE.

Since colored complexes are extremely sensitive to pH changes and solvents (in the case of TLC, acid or base washed supports and support material, respectively), the effect of the type of support on the color and intensity of the complex was investigated. Polar supports appeared to enhance the intensity of the colored complexes. For example, regarding materials in Table I, TCNE was more sensitive on silica gel containing calcium sulfate binder than on versamide or cellulose acetate. Although colors varied slightly for some compounds on different types of support, differentiation between isomers was an easy task.

We have used TCNE for the detection of certain types of aromatics and feel that TCNE is a valuable spray for detecting aromatic amines and phenols on all types of TLC plates as well as differentiating between isomers in reaction mixtures.

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