

mixture. Without chromatographing, the dried spot could be visualized as a brown spot by spraying with iodide buffered with carbonate-bicarbonate; under these conditions the other oxy-chloro anions are unreactive.

Defensive Research Division,
U.S. Army Chemical Research and Development Laboratories,
Edgewood Arsenal, Md. (U.S.A.)

BERNICE L. HARRISON
 DAVID H. ROSENBLATT

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Use of 2-(*o*-hydroxyphenyl)-benzoxazole in detection of phosphate esters and other ferric-complexing compounds on paper chromatograms

Spots of phosphate esters on paper chromatograms are usually detected by the method of HANES AND ISHERWOOD¹ or one of its modifications, involving hydrolysis to inorganic phosphate, reaction with molybdate and reduction to molybdenum blue. This is sensitive to about 1 nanomole (0.03 μ g) of P but is relatively slow and inconvenient to use. WADE AND MORGAN² introduced a detection based on the masking of the color reaction between ferric ions and sulfosalicylic acid. This method is less specific and is sensitive only to 30 nanomoles of P, but it is fast and does not destroy the compounds being detected. We have found that the fluorescent compound, 2-(*o*-hydroxyphenyl)-benzoxazole, which reacts with many metallic ions³, can be used instead of sulfosalicylic acid. The benzoxazole fluorescence reaction requires only very low reagent concentrations, it is somewhat more sensitive, and works well over a wide pH range (while the sulfosalicylic color requires a pH near 2).

Reagent PD-1A is a 0.54 % solution of $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$ in 80 % (v/v) ethanol and is stable for months. Before use, a 1:50 dilution in acetone (reagent PD-1B, approximately 0.0004 *M* ferric ion) is prepared. It is applied to the paper with a pipet, in quantity just sufficient to saturate it. The paper is allowed to dry for a few minutes, and then reagent PD-1C, a 0.05 % (0.0024 *M*) solution of 2-(*o*-hydroxyphenyl)-benzoxazole and 2 % (v/v) dimethyl sulfoxide in 2-butanone is applied with a pipet. This kind of application is more convenient than spraying or dipping and gives a more uniform deposit of reagents. When the paper is examined in long-wave ultraviolet light, spots of ferric-complexing compounds fluoresce yellow (if the paper is acidic) or

blue (if alkaline) on a dark background. The effect of the dimethyl sulfoxide seems to be to intensify weak spots by preventing adsorption of minute amounts of the benzoxazole on the paper (which quenches the fluorescence); it can be replaced by other slightly volatile solvents such as ethylene glycol but not by formamide. Sensitivity varies for various phosphate esters but is of the order of 10 nanomoles of P. If the concentration of benzoxazole is halved or that of ferric ion is doubled the fluorescence of both background and spots is reduced but there is no striking change in sensitivity.

The reaction also detects many other ferric-complexing compounds (such as citric or malic acids) in the 10–100 nanomole range. Heavy metals (such as copper, nickel and cobalt) and substances that absorb long-wave ultraviolet light strongly or quench the benzoxazole fluorescence (such as dinitrophenyl derivatives of amino acids) may show as black spots on the dark blue background. The lack of specificity makes the reaction useless with complex mixtures, and we have applied it primarily to characterize spots which have been through at least one purification step⁴. It is also useful in identifying traces of phosphate esters labeled with ¹⁴C or ³²P; larger amounts of known phosphate esters are added and spots detected with the benzoxazole reagent, and if radioactivity coincides with one of the known spots in several chromatographic or ionophoretic separations the radioactive material is tentatively identified.

The very low reagent concentration in the benzoxazole reaction facilitates recovery of spots from paper. They can be cut out, washed with acetone to remove reagents, extracted in water and run in other solvent systems or subjected to microcolorimetric analysis for phosphate. It is also possible to apply other reagents, such as ninhydrin, to ferric-benzoxazole-treated chromatograms or ionograms without serious interference.

Division of Entomology and Acarology, University of California, H. T. GORDON
Berkeley, Calif. (U.S.A.) and Biological Research Laboratory, L. N. WERUM
California Packing Corporation, Emeryville, Calif. (U.S.A.) W. W. THORNBURG

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Comportement de peptides de l'acide meso- α,α' -diaminopimélique au cours de leur chromatographie sur papier *

RHULAND *et al.*¹ avaient constaté que l'acide α,α' -diaminopimélique, après chromatographie sur papier dans un mélange méthanol–pyridine–acide chlorhydrique 10*N*–eau (80:10:2.5:17.5, v/v) se différencie des autres acides aminés de deux manières: d'une part, il donne, avec la ninhydrine, une coloration vert-olive qui vire avec le

* 4ème Communication sur les peptides de l'acide α,α' -diaminopimélique; 3ème communication, voir réf. 4. Ce texte a fait l'objet d'une Communication à la Réunion du Groupe d'Étude de la Chromatographie de la Société Chimique de France, le 31 mai 1963.

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