J. Assoc. Offic. Agr. Chemists **42,** 725 (1959).
(21) Major, A., Barry, H. C., *Ibid.*, **44,** 202 (1961).
(22) Mattick, L. R., Barry, D. L., Antenucci, F. M., Avens, A. W., J. Agr. Food Chem. **11,** 54 (1963).
(23) Mills, P. A., *J. Assoc. Offic. Agr. Chemists* **42,** 734 (1959).
(24) Minyard, J. P., Jackson, E. R., *Ibid.*, **46,** 843 (1963).
(25) Phillips, D. D., Pollard, G. E., Soloway, S. B., J. Agr. Food Chem. **10,** 217 (1962).
(26) Ralls, J. W., *Anal. Chem.* **32,** 332 (1960).
(27) Robinson, J., Richardson, A., *Chem. Ind. London* **42,** 1460 (1963).
(28) Schafer, M. L., Bush, K. A., Campbell, J. E., *J. Dairy Sci.* **46,** 1025 (1963).
(29) Segal, H. S., Sutherland, M. L., Division of Agricultural and Food Chemistry, Paper 7, 144th Meeting, ACS, Los Angeles, Calif., April 1963.
(30) Shuman, H., Collie, J. R., *J. Assoc. Offic. Agr. Chemists* **46,** 992 (1963).
(31) Walker, K. C., Beroza, M., *Ibid.*, **46,** 250 (1963).

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INSECTICIDE IDENTIFICATION

Hydriodic Acid as a New Selective Reagent for Detection of Rotenone in Chromatography

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Hydriodic acid (1 volume of 5*N* potassium iodide solution in 15 volumes of 85% phosphoric acid) produces a characteristic light blue color with rotenone useful in spot tests and in paper chromatography. After application of the reagent, the color develops within 15 minutes at room temperature. On filter paper the lower limit of detection is 4 μg. per sq. cm. Elliptone gives a pink or violet color: sumatrol, isorotenol, deguelin, dihydrodeguelin, dehydrodeguelin, tephrosin, and toxicarol are faintly visible only after several hours. Except for rotenone and elliptone, none of the materials present in crude extracts of *Derris elliptica* (Wall.) Benth. roots or *Tephrosia vogelii* Hook. f. leaves gave a color with the new reagent.

In recent years there has been renewed interest in rotenone as an insecticide because of its low toxicity (*1*) to warm-blooded animals. Although rotenone has been investigated for many years, no selective reagents have been developed for its detection on paper chromatograms. Both alkaline permanganate and alkaline fluorescein followed by bromine vapor were used by Chen and Tsai (*3*) to locate rotenone on paper chromatograms, but these reagents also react with compounds other than rotenone and the rotenoids.

The chemical procedures for the determination of rotenone include extraction and crystallization (*2*), ultraviolet (*14*) or infrared (*11*) absorption, titration of the mercuric acetate adduct (*8*) or the dichloroacetic acid solvate (*9*), or reaction with sulfuric acid and sodium nitrite to produce a red color (*5, 13*), with vanillin and sulfuric acid to produce a blue color (*6*), with heavy metal oxides in sulfuric acid followed by ammonia to produce various colors (*15, 17*), or with nitric acid followed by ammonia to produce a green or a blue color (*10*). None of these procedures appears to be readily adaptable as a spray reagent for paper, and none is specific for rotenone.

During a study of the reaction of rotenone with various reagents which split ether linkages in an attempt to produce a characteristic chromophore, it was found that rotenone gives a blue precipitate with hydriodic acid (orthophosphoric acid plus potassium iodide) at room temperature. This reagent is a sensitive and selective means of detecting rotenone on paper chromatograms or in evaporated eluates from chromatographic columns.

Materials and Methods

Spray Reagent. One volume of 5*N* potassium iodide is mixed with 15 volumes of 85% orthophosphoric acid just before use. The iodide solution is stable several weeks in a refrigerator and is discarded upon turning yellow. The ratio of the two reagents is not critical; however, reducing the amount of potassium iodide solution to less than 1 to 20 makes the rotenone spots develop more slowly and fade more quickly, whereas increasing the amount to more than 1 to 10 produces a yellow discoloration of the paper.

The spray is applied to the paper until the paper appears slightly damp (approximately 1 ml. per 100 sq. cm. with Whatman No. 1 paper). The color is developed at room temperature; heating the chromatogram discolors the paper.

Purification of Rotenoid Standards. Commercial rotenone (K and K Laboratories, Jamaica, N. Y.) was recrystallized twice from carbon tetrachloride and twice from 95% ethyl alcohol. The other rotenoids were recrystallized once from each of these solvents. All compounds were dried 2 hours at 70° C. in a vacuum oven and stored over silica gel. Their purity and identity were checked by their melting points with a Fisher-Johns apparatus and noncorrosive glass cover slips.

Extraction of Plant Material. Leaves of *Tephrosia vogelii* Hook f. were collected from approximately 5-month-old flowering plants, and roots of *Derris elliptica* (Wall.) Benth. were collected from 5-year-old plants. Both materials were dried at 70° to 80° C. and ground to pass a 60-mesh screen. The rotenoids were extracted for 16 hours with 25 ml. of acetone per gram of dry plant powder. The extracts were filtered and used for paper chromatography without further purification. The amount of aliquot per chromatogram was equivalent to 4 mg. of dried plant material.

Paper Chromatography. Strips of Whatman No. 1 filter paper 5 cm. wide by 45 cm. long were cut with a bridge 0.5 cm. wide toward the bottom, according to the method of Matthias (*12*). The material to be chromatographed was spotted on the bridge and 5:1:1 methanol–benzene–acetic acid (*3*) was used for ascending development.

Results and Discussion

The hydriodic acid reagent is a sensitive indicator of the presence of rotenone on paper chromatograms, giving a characteristic blue color which increases in intensity for 15 to 30 minutes

Table I. Color Reaction of Rotenone and Related Compounds with Hydriodic Acid Reagent

Compound[a]	Color Produced Within 0.5 hr.	After 6 hr.
Rotenone	Blue	Blue
Elliptone	Pink to violet	Violet to purple
Isorotenol	None	Light tan
Deguelin	None	Light tan
Dihydrodeguelin	None	Light tan
Dehydrodeguelin	None	Light tan
Tephrosin	None	Light tan
Toxicarol	None	Light tan
Sumatrol	None	Faint blue

[a] Tested at 50 μg. per sq. cm. on Whatman No. 1 filter paper.

after spraying and persists for several days to several weeks under humid conditions. Should a more permanent record be desired, the chromatogram may be stored in a vacuum desiccator over sodium hydroxide pellets. As little as 4 μg. per sq. cm. can be detected.

The rotenoids tested are listed in Table I. Only elliptone gave an immediate reaction with the spray reagent. Unlike rotenone, it gave a pink or violet color which slowly changed in hue with time. Rotenone and elliptone were readily separated by the chromatographic technique described. Rotenone gave an R_f of 0.88 and elliptone an R_f of 0.80 (measuring to the front of the band). Even when the two zones partially overlapped, the initial colors were sufficiently distinctive to permit the boundaries of the zones to be determined.

As a further test of specificity, the reagent was sprayed on paper chromatograms of the unpurified acetone extracts from *D. elliptica* roots and *T. vogelii* leaves. Both chromatograms showed a single blue band, which was in the position of rotenone. A pink band due to elliptone was found with *D. elliptica* but was absent with *T. vogelii*. Elliptone has been found in *D. elliptica* (*7*) but not in *T. vogelii* (*4*, *16*). Except for rotenone and elliptone, none of the other materials present in the total extracts of these two plants produced color with spray reagent.

Although the search for interfering compounds has not been exhaustive, rotenone is the only compound which has been found to give a characteristic blue color with hydriodic acid.

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Literature Cited

(1) Ambrose, A. M., Haag, H. B., *Ind. Eng. Chem.* **29,** 429 (1937).
(2) Association of Official Agricultural Chemists, Washington, D. C., "Official Methods of Analysis," 8th ed., p. 67, 1955.
(3) Chen, Y. L., Tsai, C. S., *J. Taiwan Pharm. Assoc.* **7,** 31 (1955).
(4) Clark, E. P., *J. Am. Chem. Soc.* **53,** 729 (1931).
(5) Goodhue, L. D., Haller, H. L., *Ind. Eng. Chem., Anal. Ed.* **12,** 652 (1940).
(6) Haeussler, E. P., *Mitt. Gebiete Lebensm. Hyg.* **37,** 178 (1946).
(7) Harper, S. H., *Chem. Ind.* (*London*) **1939,** p. 292.
(8) Hornstein, I., *Anal. Chem.* **23,** 1329 (1951).
(9) Jones, H. A., *Ind. Eng. Chem., Anal. Ed.* **10,** 684 (1938).
(10) Jones, H. A., Smith, C. M., *Ibid.*, **5,** 75 (1933).
(11) Knoerlein, R. G., *J. Assoc. Offic. Agr. Chemists* **44,** 577 (1961).
(12) Matthias, W., *Naturwissenschaften* **41,** 17 (1954).
(13) Meyer, T. M., *Rec. Trav. Chim.* **55,** 954 (1936).
(14) Payfer, R., *J. Assoc. Offic. Agr. Chemists* **37,** 630 (1954).
(15) Pozzi-Escot, E., *Rev. Cienc.* (*Perú*) **38,** 21 (1936).
(16) Rangaswami, S., Rama Sastry, B. V., *Indian J. Pharm.* **18,** 339 (1956).
(17) Tapia Freses, A., *Bol. Soc. Quim. Perú* **3,** 219 (1937).

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INSECTICIDE PERSISTENCE AND TRANSLOCATION

Residues of Aldrin and Heptachlor in Soils and Their Translocation into Various Crops

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The application of pesticides directly to soil and the contamination of soils through "fallout" after crop treatments cause concern over the potential accumulation of toxic residues in soils. It is essential, therefore, to determine at what rate and frequency a given chemical can be applied to soils without gradual accumulation of pesticidal residues over a period of years. Under certain conditions residues of some insecticides can be translocated from soils into crops (*2*, *3*, *5*, *6*). This makes it necessary to determine at what concentrations of pesticidal residues in soils and under what conditions no residues would be absorbed by plants grown therein. It should also be known at what concentration of pesticides in soils translocated residues would still be within the limits of tolerances set by the Food and Drug Administration.

To obtain some insight into these problems, a study was initiated in 1958 at the University of Wisconsin whereby soils were treated at abnormally high rates of aldrin and heptachlor. Two years later this study was expanded to include application rates comparable with those normally used in agriculture. To investigate possible translocation of insecticidal residues, various crops were grown on the contaminated soils.

Procedure

Soil Treatments at Abnormally High Dosages and Soil Sampling. In May 1958, duplicated 30- × 40-foot Carrington silt loam plots were treated (*3*) with emulsions of aldrin and heptachlor at 5 or 25 pounds per acre. The soils were rototilled to a depth of 4 to 5 inches. After that a 30- × 16-foot portion of each plot was seeded with alfalfa.

Only soils to which the insecticides were applied at 5 pounds per 5-inch acre were re-treated at the same rate each May from 1959 through 1962. Since the dense alfalfa cover was maintained throughout the experiment, the re-treated areas were reduced to 30 × 24 feet. At the end of the 5 years, all plots had been treated with a total of 25 pounds of insecticide per 5-inch acre (5 × 5 or 25 pounds).

For soil residue studies, the intention was to determine if and to what extent the insecticides would accumulate in the soil following yearly applications of 5 pounds per 5-inch acre; how fast they would disappear after being applied at one massive dosage; what the residue levels would be at the end of 5 years in the differently treated plots; and finally, what influence a dense cover crop would have on the persistence of these residues in soils.