Colorimetric Method for the Determination of Cyclethrin

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The synthesis of cyclethrin, a pyrethrin-type insecticide, has presented a need for a sensitive and precise method for its determination. Both pyrethrins and cyclethrin, when heated with reagents containing orthophosphoric acid, react to produce red colors having maximum absorbance from 545 to 550 mμ. However, the cyclethrin color reaction differs from that of pyrethrins in respect to the composition of the color-producing reagent, the length of heating time necessary for the production of maximum color, and the stability of the color complex to heat. The cyclethrin color is suppressed by piperonyl butoxide and by sulfoxide. However, these substances can be separated from cyclethrin by chromatography. The method described is applicable to the determination of as little as 10 γ of cyclethrin.

THE STRUCTURE AND SYNTHESIS of cyclethrin, 3-(2-cyclopentenyl)-2-methyl - 4 - oxo - 2 - cyclopentenyl-chrysanthemummonocarboxylate, have been described by Haynes and coworkers (*1*). Cyclethrin is one of the pyrethrin-type insecticides and, like other members of this group, is of interest because it is considered to have a low toxicity to warm-blooded animals.

A simple analytical method, both for the determination of the purity of the product and of its concentration in insecticide formulations, is needed, along with a method suitable for the determination of cyclethrin residues. Such a procedure must be sensitive to microgram quantities of the insecticide. The method of analysis currently used is an adaptation of the procedure developed by Hogsett, Kacy, and Johnson (*2*) for the determination of allethrin. This method is based on a reaction with ethylenediamine to form an equivalent amount of the amine salt of chrysanthemummonocarboxylic acid, which is titrated with sodium methylate in pyridine solution. At least 5 grams of the insecticide are required for a determination by this procedure. The method is lacking in specificity, as the reaction is given by all of the compounds related to allethrin which react with ethylenediamine to give a titratable acid.

A method developed recently by Williams, Dale, and Sweeney (*3*) for the determination of pyrethrins is based on the measurement of the red color produced when pyrethrins are heated with a reagent consisting of ethyl acetate and orthophosphoric acid. In tests of the specificity of this reagent for pyrethrins, cyclethrin also gave a positive reaction. However, the intensity of the cyclethrin color, as developed by the ethyl acetate–phosphoric acid reagent, was considerably less than that produced by pyrethrins. The two insecticides also differed in their behavior when heated with the color-producing reagent. Pyrethrins reacted to produce their maximum color after a heating time of from 1 to 3 minutes at 100° C. If the heating period was prolonged beyond 3 minutes, the result was gradual decomposition of the pyrethrins color complex. Cyclethrin required a much longer heating time for maximum color development.

To develop a satisfactory method for the determination of cyclethrin, a study was made of various modifications of the pyrethrin reagent. As an initial step, small amounts of cyclethrin, 100 γ or less, were heated with orthophosphoric acid and with mixtures of orthophosphoric acid plus other reagents. The factors studied were (*a*) intensity of color produced, (*b*) optimum length of heating time, and (*c*) the observance of Beer's law.

Best results were obtained using a reagent consisting of 85% of orthophosphoric acid and a heating time of 40 minutes at 100° C. The color produced has an absorption maximum from 545 to 550 mμ (Figure 1) and Beer's law is obeyed within the range of 10 and 90 γ (Figure 2). Below 10 γ an approximation may be obtained. Because of its sensitivity, the method should prove useful, not only for the determination of cyclethrin in concentrates, but also for the determination of residues of this insecticide.

Apparatus

Spectrophotometer, Beckman Model B or equivalent.
Test tubes, 50 × 150 mm.
Water bath.
Shaking machine.
Matched test tubes, 15 × 150 mm.
Timing device.
Allihn chromatographic tubes, 20 × 100

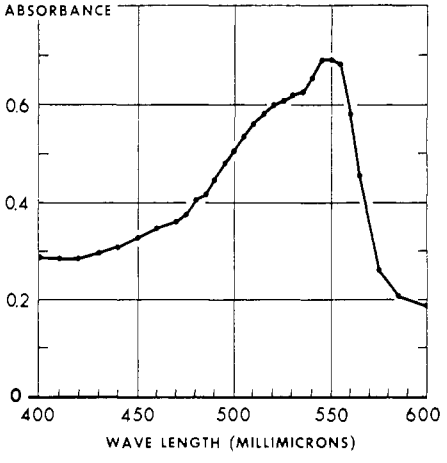

Figure 1. Absorption spectrum of color developed by reaction of cyclethrin with orthophosphoric acid

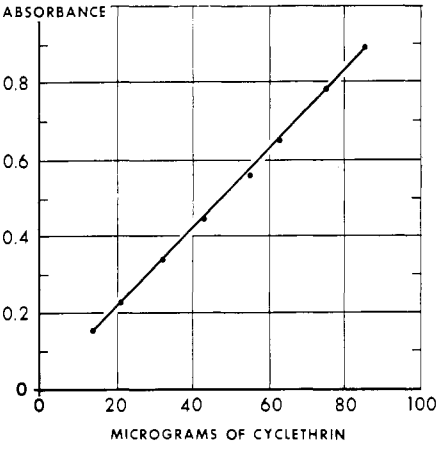

Figure 2. Relation of concentration of cyclethrin to absorbance of color developed by reaction with orthophosphoric acid

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mm., having fritted-glass disks of medium porosity.

Bell glass, micro with side tube and plate.

Suction device.

Reagents

Orthophosphoric acid 85%, reagent grade.

Petroleum ether, reagent grade, boiling point 30° to 60° C.

Celite 545, a diatomaceous earth filter aid manufactured by the Johns-Manville Co.

Silicic acid, Mallinckrodt chromatographic grade.

Sodium sulfate, anhydrous.

Colorimetric Procedure

Preparation of Standard Curve. Cyclethrin concentrate, 131 mg., assayed at 82.3% cyclethrin by the procedure of Hogsett, Kacy, and Johnson was diluted to 1 liter with petroleum ether, giving a primary standard containing approximately 108 γ per ml.

Ten milliliters of the primary standard were diluted to 100 ml. to give a working standard containing 10.8 γ per ml.

Aliquots of the working standard containing 10.8, 21.6, 32.4, 43.2, 54.0, 64.8, and 75.6 γ of cyclethrin were pipetted into 50 × 150 mm. test tubes. The solvent was removed by careful evaporation on a hot water bath, taking care not to overheat the cyclethrin. It is advisable to withdraw the tubes from the water bath before the solutions have reached dryness and to complete the final stages of the evaporation by allowing the last traces of the liquid to make contact with the hot side walls of the tubes.

Table I. Precision and Adherence to Beer's Law Produced by Reaction of Cyclethrin with Orthophosphoric Acid

Cyclethrin, γ	Absorbance	Standard Deviation × 10^3	Absorbance/γ × 10^2
10.8	0.115 0.120 0.125	5.00	1.111
21.6	0.230 2.225 0.225	2.88	1.051
32.4	0.355 0.345 0.345	5.77	1.074
43.2	0.450 0.450 0.460	0.58	1.049
54.0	0.570 0.550 0.560	1.00	1.037
64.8	0.650 0.650 0.670	1.15	1.014
75.6	0.780 0.780 0.800	1.15	1.041

Table II. Recovery of Cyclethrin When Separated from Synergist by Chromatography

Amount of Cyclethrin, γ	Synergist		Cyclethrin Recovered	
	Kind	Amount, γ	γ	%
25	Piperonyl butoxide	250	25	100
50		500	48	96
75		1000	73	97
25	Sulfoxide	250	24	96
50		500	49	98
75		1000	73	97

Five milliliters of 85% orthophosphoric acid were then added, using a pipet with a wide orifice. The tubes were placed in a shaking machine and the contents mixed for 1 minute. They were then placed in a boiling water bath and allowed to remain for exactly 40 minutes. The red color which develops is stable indefinitely. At the end of 40 minutes, the tubes were moved from the boiling water and placed in a bath at 10° C. where they were allowed to remain for 15 minutes. The solutions were then transferred to 15 × 150 mm. tubes. Any air bubbles present were removed by centrifugation. The absorbance of the colors was determined in a spectrophotometer at 550 mμ, using orthophosphoric acid as a blank.

Table I shows results indicating the degree of precision and adherence to Beer's law obtained.

Effect of Cyclethrin Synergists on Color Development. The cyclethrin color was suppressed by compounds frequently employed as synergists for the insecticide. No color was produced if piperonyl butoxide was present in a ratio of 10 parts of piperonyl butoxide to 1 part of cyclethrin. Sulfoxide, when present in a 5 to 1 ratio, also interfered with the color development. When cyclethrin was separated from the synergist compounds, normal color reactions were obtained. These separations were accomplished on a chromatographic column of silicic acid and Celite.

Chromatographic Procedure

Preparation of Column. The absorbent consists of 2 parts by weight of silicic acid and 1 part of Celite 545. These materials are mixed thoroughly and are then activated by heating for 12 hours at 150° C. The chromatographic tube is fitted into the bell glass by means of a one-hole rubber stopper, and the bell glass connected to a suction device, making use of pressure tubing. After starting the suction, the tube is filled to within approximately 1 inch of its top; the absorbent is added in small portions and tamped into position after each addition.

Chromatography. After packing the tube, the absorbent is prewashed by passing 50 ml. of the eluting solution, 10% by volume of ethyl ether in petroleum ether, through the column. The washings are collected in a small Erlenmeyer flask. Just before the last of the prewashing solution passes into the column, the sample to be chromatographed is added. This solution should contain from 25 to 90 γ of cyclethrin and not more than 10 times that amount of synergist in approximately 5 ml. of petroleum ether.

The elution is carried out, with the suction adjusted so that the flow of the eluate is approximately 100 drops per minute, and fresh eluting solution is added, just before the last of that previously added sinks into the absorbent. After the first 50 ml. of the eluate have been discarded, the next 150 ml., which should contain all of the cyclethrin, are collected. The fraction containing the cyclethrin is carefully evaporated to dryness and the color developed in the usual manner.

The synergist, piperonyl butoxide or sulfoxide, will remain on the absorbent.

Table III. Reactions of Various Compounds with Cyclethrin Color Reagent

Compound	Color Obtained
Pyrethrin	Red
Cyclethrin	Red
Pyrethrolone	Red
Furethrin	Yellow turning to green
Ryanodine	Brown
Toxaphene	Turbidity
Allethrin	None
Natural chrysanthemum-monocarboxylic acid	None
Natural chrysanthemum-dicarboxylic acid	None
Synthetic cinerin I	None
Cinerolone	None
Technical allethrolone	None
Technical chrysanthemum-monocarboxylic acid	None
Rotenone	None
Lindane	None
Methoxychlor	None
DDT	None
Chlordan	None
Dieldrin	None
Aldrin	None
Sulfoxide	None
Piperonyl butoxide	None

Continued elution, however, will eventually wash the synergist from the column. The careful following of directions will result in sharp separation and quantitative recovery of cyclethrin from the synergist. Recoveries of cyclethrin obtained, using various combinations of the insecticide with piperonyl butoxide and sulfoxide, are given in Table II.

Discussion

Specificity of Phosphoric Acid Reagent. In order to study the specificity of the cyclethrin reagent, a number of compounds were investigated. As shown in Table III, of the compounds tested, only cyclethrin, pyrethrins, and pyrethrolone reacted to produce a red color. As pyrethrolone gave a positive reaction, the alcohol portion of the cyclethrin molecule might also react positively.

The differences in the behavior of the pyrethrinlike insecticides with the phosphoric acid reagent are of interest. These insecticides are esters of chrysanthemumcarboxylic acids and cyclic ketonic alcohols. They differ only in isomerism and in the structure of a side chain. These differences in side chain structure and in reaction with the phosphoric acid reagent are illustrated in Figure 3.

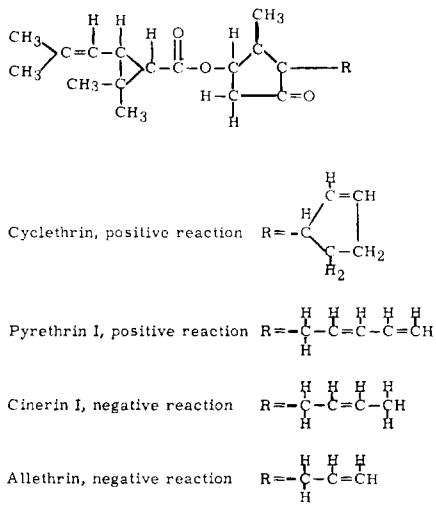

Figure 3. Side chains of pyrethrin-type insecticides and their reaction with orthophosphoric acid

The insecticides giving positive reactions—namely, pyrethrins and cyclethrin—possess side chains, five carbons in length, with at least one double bond. Therefore, a side chain of this type might be necessary for the color reaction.

Because allethrin and the cinerins yield no color when heated with orthophosphoric acid, a means for the differentiation of these insecticides from cyclethrin and from pyrethrins is available.

Also, as detailed above, the pyrethrins and cyclethrin reactions differ in respect to the composition of the color-producing reagent, the length of heating time necessary for the production of maximum color, and the stability of the color complex when heated. On the basis of these differences, a qualitative differentiation between pyrethrins and cyclethrin may be made.

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FUNGICIDAL ACTIVITY AND STRUCTURE

Fungicidal Activity of Trichloromethyl Thiolsulfonates

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A comprehensive series of trichloromethyl thiolsulfonates including several new compounds has been prepared and the effect on the germination of spores of common fungi observed. As a class, the trichloromethyl arenethiolsulfonates were found to be highly effective fungicides, and some generalizations about the effect of structural variance on fungitoxicity have been made. Several o-nitrophenyl arenethiolsulfonates were prepared and were found to lack the high order of fungitoxicity associated with the trichloromethyl analogs.

In recent years, *N*-(trichloromethylthio)tetrahydrophthalimide has been developed as a highly effective and widely used commercial fungicide. It is one of a large group of compounds containing the grouping $NSCCl_3$, which has been shown to have fungicidal properties (*3, 4, 6–9, 12, 16*). Fungicidal properties of compounds containing $CSCCl_3$ and $SSCCl_3$ linkages have also been described (*4, 5, 8*). These linkages were achieved by the addition of trichloromethanesulfenyl chloride to olefins, or by its reaction with amines, mercaptans, amides, imides, xanthates, thiocarboxylic acids, and similar compounds. Reactions such as these suggested the trichloromethyl thiosulfonates as an uninvestigated field of new fungicides. They can be made by metathesis according to the following equation:

$$ArSO_2Na + ClSCCl_3 \longrightarrow ArSO_2SCCl_3 + NaCl$$

During the extended course of synthesizing a representative series of such compounds in this laboratory, two publications appeared, which described the preparation of trichloromethyl arenethiolsulfonates (*2, 10*). Backer and Westerhuis (*2*) demonstrated the fungicidal activity of the *p*-toluenethiolsulfonate by observing its effect on the germination of beet seeds infected with *Phoma betae* Frank.

The trichloromethyl thiolsulfonates, RSO_2SCCl_3, in which R represents a substituted or nonsubstituted aliphatic or aromatic group, were described by Uhlenbroek at the XIVth International Congress of Pure and Applied Chemistry (Zurich, 1955), as a new class of organic fungicides (*15*). It was claimed that the fungitoxicity appeared to be fairly independent of the nature of R. Diminished phytotoxicity was claimed for the only specific structure, trichloromethyl *p*-carboxybenzenethiosulfonate, revealed in an abstract of this paper (*15*).

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