Identification of 3,4-Methylenedioxyphenyl Synergists by Thin-Layer Chromatography

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Thin-layer chromatography has been used to identify rapidly all of the methylenedioxyphenyl synergists used commercially as synergists for pyrethroids. Best results were obtained on silicic acid plates with 2.5% acetone in benzene as the developing solvent. From 0.1 to several μg. may be made visible by spraying with a chromotropic–sulfuric acid reagent, but as much as 50 to 100 μg. may be chromatographed as a single spot. Distinctive colors obtained in spraying aid in identification.

THE USE of methylenedioxyphenyl synergists (*17*, *20–22*) with insecticide formulations containing pyrethrins and allethrin is now accepted practice, and a number of procedures have been devised for their quantitative estimation. Blum (*9*) and Beroza (*7*) advanced methods that are applicable to the analysis of methylenedioxyphenyl synergists; however, each synergist develops a different amount of color so that the analyst must know with which synergist he is dealing before employing these procedures. A method proposed by Jones *et al.* (*15*) is applicable only to the analysis of piperonyl butoxide. Budowski described a colorimetric and an ultraviolet spectrophotometric determination for sesamolin and sesamin (*11*, *12*), procedures that were later modified by Suarez *et al.* (*19*) to accommodate the analysis of sesame oil synergist concentrates. Failure of the latter method in the presence of appreciable foreign ultraviolet absorption led the author to advance a highly specific procedure for determining sesamin by chromatography on silicic acid (*3*). Bowman *et al.* published a method for determining sesamex (Sesoxane) (*10*), a pyrethroid synergist that was found to also synergize carbamate insecticides (*13*).

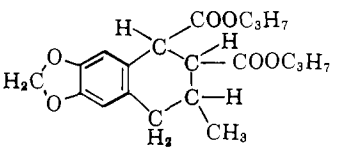
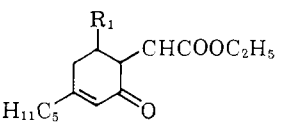
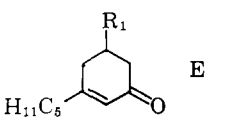
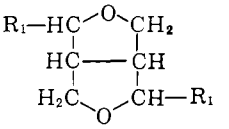
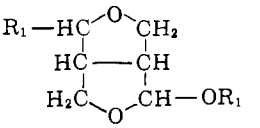
Clearly, a positive means of identifying the synergists is necessary to apply properly most of the foregoing procedures. The author devised a reversed-phase, paper chromatographic method (*8*) which identified synergists by their positions on a paper strip developed with 30% aqueous acetic acid. The difference in ultraviolet absorption at two wave lengths (corresponding to a high and low absorbance value of the synergist) was employed to eliminate fluctuations in absorbance of the paper, while permitting passage of the large absorbance differences of the synergists. The procedure differentiated between piperonyl butoxide, sulfoxide, *n*-propyl isome, sesamin, sesamolin, and piperonyl cyclonene.

The availability of equipment for thin-layer chromatography [developed by Kirchner and Miller (*16*) and Stahl (*18*)] prompted the investigation of this technique for the identification of synergists. A method has been devised that is simpler and much more rapid than the reversed-phase paper technique. In addition, the new procedure permits detection of sesamex (*6*, *14*) and Bucarpolate (*17*), two newer synergists.

Apparatus

The thin-layer apparatus devised by Stahl (*18*) and available from Brinkmann

Table I. Synergist Formulas

Synergist	Formula[a]
Piperonyl butoxide	$C_4H_9O(CH_2CH_2O)_2CH_2—R_2$
Sulfoxide	$C_8H_{17}S(=O)—CH(CH_3)—CH_2—R_1$
Sesamex (Sesoxane)	$C_2H_5O(CH_2CH_2O)_2—CH(CH_3)—R_1$
Bucarpolate	$C_4H_9O(CH_2CH_2O)_2—C(=O)—R_1$
n-Propyl isome	
Piperonyl cyclonene	Mixture of  and  E
Sesamin and asarinin	
Sesamolin	

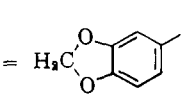
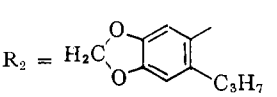
[a] R_1 =  R_2 = 

Table II. R_f Values of Synergists with Different Solvent Systems[a]

Synergist	2.5% Acet. in Benz.	1.5% Prop. in Benz.	3% Prop. in Benz.	5% Prop. in Benz.	10% Ether in Benz.	15% Eth. Ac. in Benz.	3% Ac. Acid in Benz.
Sulfoxide	0.02	0.03	0.14 0.20	0.34 0.43	0.05	0.05	0.05
Sesamex	0.16	0.10	0.44	0.63	0.21	0.23	0.17[b]
Bucarpolate	0.25	0.25	0.61	0.71	0.38	0.36	0.14
Piperonyl butoxide	0.30	0.17	0.60	0.70	0.41	0.43	0.14
Sesamin	0.36	0.28	0.65	0.76	0.47	0.44	0.16
Sesamolin	0.52	0.51	0.75	0.80	0.67	0.53	0.28
Asarinin	0.52	0.51	0.75	0.80	0.67	0.55	0.29
Piperonyl cyclonene	0.39	0.36	0.71	0.73	0.58	0.55	0.24
	0.47	0.60	0.76	0.79	0.76	0.61	0.28
	0.58	0.72	0.81	0.85	0.89		0.42
	0.70						
n-Propyl isome	0.00	0.00	0.01	0.01	0.01	0.01	0.00
	0.36	0.35				0.57	0.23
	0.52	0.55	0.77	0.82	0.77	0.65	0.44
	0.58						
	0.77						

Synergist	50% Chlor. in Benz.	10% Ether in Hex.	50% Ether in Hex.	15% Eth. Ac. in Hex.	10% Eth. Ac. 15% Benz. 75% Chlor.	10% Eth. Ac. 45% Benz. 45% Chlor.	80% Eth. Ac. in Chlor.
Sulfoxide	0.03	0.02	0.03	0.02	0.37	0.12	0.28
					0.29		0.34
Sesamex	0.02	0.03	0.26	0.12	0.41	0.17	0.42
Bucarpolate	0.07	0.08	0.33	0.19	0.56	0.33	0.58
Piperonyl butoxide	0.06	0.09	0.42	0.27	0.64	0.38	0.64
Sesamin	0.09	0.04	0.30	0.15	0.61	0.38	0.60
Sesamolin	0.16	0.07	0.40	0.23	0.70	0.52	0.71
Asarinin	0.15	0.10	0.40	0.23	0.71	0.53	0.72
Piperonyl cyclonene	0.12	0.02	0.35	0.25	0.74	0.50	0.35
	0.36	0.08			0.79	0.63	0.72
		0.28	0.40	0.46	0.83		0.80
							0.86
n-Propyl isome	0.01	0.01	0.01	0.01	0.00	0.00	0.00
	0.22	0.23	0.55	0.26	0.76	0.64	0.81
	0.31	0.52		0.51			

[a] Ac. Acid = acetic acid, Acet. = acetone, Benz. = benzene, Chlor. = chloroform, Eth. Ac. = ethyl acetate, Hex. = hexane, Prop. = propanol.
[b] Spread 0.07 to 0.28.

Instruments, Inc., Great Neck, N. Y. was used. A similar apparatus is available from Microchemical Specialties Co., Berkeley 3, Calif. Wollish *et al.* (*23*) have described recent developments in thin-layer chromatographic apparatus.

Materials

Silica Gel G. Available from Brinkmann Instruments, Inc., Microchemical Specialties Co., Terra Chemicals Inc., and Merck & Co., Darmstadt, Germany.

Synergists. 1% solutions (w./v.) in acetone were prepared for spotting. Sulfoxide, piperonyl cyclonene, *n*-propyl isome, Bucarpolate, and sesamex were commercial products. The piperonyl butoxide was a distilled commercial sample. Sesamin and sesamolin (*2*, *5*) were pure compounds isolated from sesame oil. Asarinin, a diastereoisomer of sesamin, was isolated from the bark of *Zanthoxylum clava-herculis* L. Formulas of the synergists are given in Table I.

Solvents, C.P. redistilled.

Sulfuric Acid Reagent. Add carefully with swirling 5 volumes of concentrated, reagent-grade sulfuric acid to 3 volumes of distilled water. Cool to room temperature and store in a glass-stoppered container.

Chromotropic–Sulfuric Acid Spray. Prepare weekly 10% w./v. aqueous solution of sodium 1,8-dihydroxynaphthalene-3,6-disulfonate (Eastman Kodak P230). Dissolve one volume of this solution in five volumes of the sulfuric acid reagent daily.

Furfural–Sulfuric Acid Spray. Prepare daily a solution of one volume of freshly distilled furfural in 50 volumes of the sulfuric acid reagent.

Procedure

Layers of silica gel G, 250 microns thick, were deposited on glass plates 200 mm. square in accordance with the directions of Stahl (*18*). After 5 minutes of preliminary drying, the plates were activated for one-half hour in an oven set at 105° to 110° C. From 1 to 5 μl. of the 1% synergist solutions (equivalent to 10 to 50 μg. of synergist) were applied from a micropipet at a line about 2.5 cm. from the end of the plate. A plastic template aided in spacing the samples at 1-cm. intervals. As soon as the acetone evaporated, the plate was inserted in a chamber at room temperature (23–25° C.) containing a 1-cm. depth of developing solvent and a paper lining saturated with the same solvent. (Most of the solvent systems, being highly volatile, were renewed several times a day to forestall composition change by evaporation.) The solvent front was allowed to travel about 11 to 13 cm. past the starting line, the front was marked, and the plate removed. After a few minutes, the solvent evaporated, and the plate was sprayed with one of the chromogenic reagents. R_f values and colors of spots were recorded, and the plate was then heated for one-half hour in an oven set at 105° to 110° C. Again, R_f values and colors of spots were recorded.

Results

The synergist R_f values using a variety of solvent systems are given in Table II. The best solvent system for the identification of the synergists was 2.5% acetone in benzene. With this system, all of the synergists were identified.

The earlier paper chromatographic method (*8*) clearly showed that the technical-grade synergists were mixtures rather than pure compounds. By the thin-layer procedure, separations were better than those obtained with paper and at least seven ingredients were identifiable from a chromatogram of a single, technical-grade synergist. Ingredients present in very small amounts were presumed to be impurities, and their R_f values are not included in Table II.

Discussion

Past attempts to find a reagent that would make methylenedioxyphenyl compounds visible on paper chromatograms were unsuccessful (*8*). The methylenedioxyphenyl group is very stable, and reagents needed to hydrolyze it were so corrosive that they destroyed the paper. For this reason, direct photometry was used to detect the synergists on paper chromatograms (*8*). This procedure requires an ultraviolet spectrophotometer equipped with a special adapter and is unduly time-consuming.

Thin-layer chromatography, permitting the application of corrosive chromogenic reagents, made it possible to use a spray solution of chromotropic–sulfuric acid. This solution, which has been shown to produce colors with minute amounts of a wide variety of methylenedioxyphenyl compounds (*4*), readily made visible all of the methylenedioxyphenyl synergists that have been used commercially or proposed for such use. Another corrosive reagent, furfural in sulfuric acid, has been used to estimate sesamolin colorimetrically (*11*). Sesamolin, an acetal, is hydrolyzed by strong acids, liberating sesamol (methylenedioxyphenol), which produces a red

Table III. Lowest Limit of Identification in Micrograms of Synergists with Two Sprays

Synergist	Chromotropic–Sulfuric Acid, Heated 30 Min. at 105° C.	Furfural–Sulfuric Acid, Unheated
Sulfoxide	1	...
Asarinin	0.2	...
Sesamolin	0.1	0.2
Sesamin	0.1	...
Bucarpolate	1	...
Sesamex	0.5	0.1
Piperonyl butoxide	0.5	...
Piperonyl cyclonene	2	...
n-Propyl isome	5	...

Table IV. Colors of Synergist Spots with Two Spray Reagents Before and After Heating 30 Minutes at 105° to 110° C.

Synergist	Chromotropic–Sulfuric Acid		Furfural–Sulfuric Acid	
	Unheated	Heated	Unheated	Heated
Sulfoxides	...	Purple with blue rim	...	Gray
Asarinin	...	Purple	...	Black
Sesamolin	Sea green[a]	Purple with brown rim	Wine red	Black
Sesamin	...	Purple with brown rim	...	Black
Bucarpolate	...	Purple with blue rim	...	Gray
Sesamex	Sea green[a]	Orange	Wine red	Black
Piperonyl butoxide	Orange[a]	Purple with reddish rim	Purple	Black
Piperonyl cyclonene	Yellow	(0.39) Purple[b]	Yellow	(0.39) Black[b]
		(0.47) Yellowish green		(0.47) Black
		(0.58) Purple		(0.58) Brown
		(0.70) Reddish pink		(0.70) Brown
n-Propyl isome	...	(0) Pink[b]	...	(0) Dark brown[b]
		(0.36) Purple		(0.36) Brown
		(0.52) Pink		(0.52) Brown
		(0.58) Dark purple		(0.58) Brown
		(0.77) Pink		(0.77) Brown

[a] Colors are weak and appear gradually if enough compound is present.
[b] Figures in parentheses are R_f values of spots with 2.5% acetone-in-benzene system.

color in the presence of furfural and acid (*7*). Other aldehydes also give colors (*11*). Because sesamex is also a sesamol acetal, it likewise gives a red spot with the furfural–sulfuric acid spray. Upon heating the plates sprayed with the furfural reagent, all of the synergists become visible. The chromotropic–sulfuric acid spray will usually detect smaller amounts of synergist than the furfural spray. Lower limits of detection of synergists, which are given in Table III, range from 0.1 to several micrograms. The method is less sensitive for piperonyl cyclonene and propyl isome because they are mixtures of several ingredients.

The solvent systems used were volatile and consisted of a background solvent (e.g., hexane, benzene, chloroform, ether, or mixtures of these) plus an eluting agent of different classes (e.g., ether, ester, ketone, alcohol, acid, amine) selected to give the best possible resolution. Many of the systems gave good results except that the R_f values were too close together. The 2.5% acetone in benzene gave the widest separation of R_f values. The separation of synergists being adequate, there was no need to resort to two-dimensional chromatography to accomplish identification.

When R_f values reverse their order in different solvent systems, this reversal may be employed as a further aid in identification. Thus, piperonyl butoxide, which follows sesamin and sesamolin and just precedes Bucarpolate in the 2.5% acetone-in-benzene system, precedes sesamin and sesamolin and is well ahead of Bucarpolate in the 50% ether-in-hexane system.

Although as little as a fraction of a microgram could be chromatographed, the plates have the capacity to handle at least 100 μg. of a synergist on a single spot. In general, the spots were well-rounded and compact, making it easy to identify the different synergists by R_f values only (except for distinguishing between sesamolin and asarinin discussed below). However, reproducibility of R_f values, as has been reported by many previous investigators working with thin-layer chromatography, was not as good as with paper. It has not been found practical to try to control precisely the many variables in the thin-layer technique; instead, variability of R_f values was easily resolved by running the unknown alongside known synergists that had approximately the same R_f values and making appropriate corrections. In no case have synergist R_f values with a given system changed their order.

The chromotropic–sulfuric acid spray developed no appreciable color for DDT, pyrethrum extract, aromatic petroleum derivative solvent, or deodorized kerosine, and the R_f values of synergists in typical fly sprays containing these ingredients were not altered appreciably. If it is suspected that foreign ingredients may alter the R_f value and confuse identification, a small amount of known synergist may be added to the unknown mixture to see if the known and unknown will coincide in R_f value. In general, it is worth while to establish final identification by this means and by a side-by-side run. Beroza (*8*) suggests means of overcoming interferences in formulations.

Colors formed by the sprays are an invaluable aid in identifying synergists. Only piperonyl cyclonene gave an intense yellow color on an unheated plate after applying either of the sprays. Only sesamolin and sesamex produced a bright red color on applying the furfural–sulfuric acid spray. This color reaction was most helpful in differentiating sesamolin from asarinin, which had almost the same R_f value as sesamolin in all systems tried. The distinctive colors formed by the action of the sprays with and without heating are given in Table IV. The chromotropic–sulfuric acid spray produces a much greater variety of colors than the furfural–sulfuric acid spray and, therefore, yields much more information.

To obtain further verification of a synergist's identity, if enough of the compound is at hand, milligram amounts can be chromatographed on a single plate, the appropriate zone scraped off and eluted with methanol, and the ultraviolet and infrared spectra compared with those of the synergist it is suspected to be.

Other investigators using thin-layer chromatography have referred to the rapidity of analyses (about 30 minutes for an actual run of as many as 20 samples) with this technique and its applicability to semiquantitative estimations. These advantages apply to the present analysis.

Acknowledgment

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INSECTICIDE PERSISTENCE

The Disappearance of Endrin Residues on Cabbage

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An endrin formulation was sprayed on cabbage heads at rates of 0.8, 0.5, and 0.25 pound per acre. Samples were harvested and analyzed at intervals up to 21 days after spraying. A Barber Colman Model 10 gas chromatographic instrument was modified to analyze the samples by electron affinity detection. Endrin residue was 0.13 p.p.m. or less 21 days after spraying for all levels of application.

CURRENT METHODS for determination of endrin residues are the phenylazide (*1*), total chlorine (*7*), and infrared method (*3*). These methods are time consuming and require rigorous cleanup. They generally require 10 μg. or more of endrin, and, therefore, it is necessary to analyze large crop samples containing of the order of 0.1 p.p.m.

Recently, Lovelock and Lipsky (*5*) described a detector for gas chromatographic systems which is exceptionally sensitive to chlorine-containing compounds. Goodwin *et al.* (*4*) showed that this election affinity detector can be employed with pesticide residues, particularly chlorinated hydrocarbons. Clark (*2*) has also applied this technique to detection of chlorinated pesticides.

Endrin is widely used to control cabbage looper. No published data are available on the persistence or disappearance of this residue on cabbage.

Materials and Methods

Spraying, Sampling, and Extraction of Cabbage. An emulsified formulation of 1.6 pounds of endrin per gallon diluted to apply at rates of 0.8, 0.5, and 0.25 pound of actual pesticide per acre, was sprayed on maturing cabbage on August 29, 1961. A two-row, tractor-mounted sprayer was employed in the application of this pesticide. After being sprayed, cabbage heads were harvested from each plot at intervals of 0, 1, 3, 5, 7, 10, 14, and 21 days.

Upon arrival at the laboratory, the cabbage heads were finely chopped in a Hobart food chopper. A sample of 500 grams of chopped cabbage, 250 grams of ethanol, and 500 ml. of redistilled *n*-hexane was placed in a Waring Blendor, and the mixture macerated for 5 minutes. The mixture was then transferred to 250-ml. centrifuge bottles and centrifuged at 1500 r.p.m. for 10 minutes in a size D International centrifuge. The resulting supernatant hexane solution was decanted and dried over anhydrous sodium sulfate.

Gas Chromatographic Analysis of Endrin. A Barber-Colman Model 10 gas chromatographic instrument was employed in the analysis. The electrometer range switch was modified by the addition of 9×10^{10}-ohm Victoreen resistor to change the sensitivity from 10^{-9} to 3×10^{-10}-amp. full scale.

The normal high voltage power supply was disconnected from the cell, and a 67.5-volt dry cell battery with a wire-wound, 10,000-ohm potentiometer to vary the voltage between 0 and 67.5 volts was substituted. The battery was connected positive to ground. The polarity switch of the electrometer was used in the positive rather than the usual negative position as employed in β-ray detection.

A U-shaped column of heavy-walled, borosilicate glass tubing 5 mm. I.D. and 6 feet long was used. The partitioning medium employed was the ethyl acetate soluble fraction of Dow Corning high vacuum stopcock grease. The packing was prepared by dissolving 20 grams of the liquid partitioning agent to 250 ml. of chloroform. A slurry was made by adding this solution to 100 grams of Chromosorb W 80–100 mesh in a large evaporating dish. The chloroform was evaporated with constant stirring of the slurry. When the Chromosorb-partitioning agent appeared to be dry, the evaporating dish and contents was placed in an oven at 110° C. for 3 hours. The contents were cooled, and an aliquot portion was packed into the column with constant vibration. After being packed, the column was preconditioned by baking at 230° C. and a flow rate of 60 ml. of argon per minute. The progress of the baking was followed by employing the β-ray detection system (*9*). The baking procedure usually took 3 days.

Operating parameters employed were: column temperature, 200° C., cell temperature, 235° C.; flash heater, 265° C.; nitrogen pressure, 18 p.s.i.; flow rate of nitrogen, 60 ml. per minute. The detector employed was the Barber-Colman Model No. A-4071 detector containing 56 microcuries of Radium 226.

Optimum Voltage to Detector. A 1-μl. sample of a hexane solution of endrin containing 1 μg. per ml. was injected into the gas chromatographic