Table IV. 3,4-Methylenedioxyphenyl Urethans

Urethan	Formula	Yield, %	M.P., °C.	Nitrogen, % Calcd.	Nitrogen, % Found
N-Phenyl	$C_{14}H_{11}O_4N$	90	122.0–122.5	C, 65.35 H, 4.32	C, 65.68 H, 4.42
N-*o*-Tolyl	$C_{15}H_{13}O_4N$	87	142.5–143.5	5.16	5.15
N-*m*-Tolyl	$C_{15}H_{13}O_4N$	82	84.5–85.5	5.16	5.12
N-*p*-Tolyl	$C_{15}H_{13}O_4N$	88	152.5–153.5	5.16	5.26
N-1-Naphthyl	$C_{18}H_{13}O_4N$	90	158.0–158.5	4.56	4.56

acetals were strongly synergistic. The carboxylic acid esters showed practically no synergism whereas the sulfonic acid esters were strongly synergistic. Although the urethans were synergistic, the activity was not sufficient to warrant further investigation. Furthermore, the urethans had the disadvantage of being relatively insoluble in kerosine, the most widely used base for insecticides. The compounds that are active are true synergists, as they show no appreciable toxicity in the absence of pyrethrins. Sesamol itself is not appreciably synergistic with pyrethrins (*5*).

The fact that so many of the foregoing compounds having the 3,4-methylenedioxyphenoxy structure exhibited synergism indicates that the premise that this group would give good synergists was correct. The comparison of safrole and the allyl ether of sesamol shows that the latter is much more synergistic. Like sesamin and sesamolin, this is another example where a compound containing the methylenedioxyphenoxy group is superior to the corresponding compound containing a methylenedioxyphenyl group.

Synergism was much greater with the natural pyrethrins than with allethrin. However, the results were generally parallel—that is, a compound synergistic with one was synergistic with the other, although not always to the same degree.

Of all the compounds, the acetals appear to be the most promising candidates for synergists of commercial value. Some of these compounds may be prepared in close to quantitative yield simply by adding sesamol to the vinyl ether plus an acidic catalyst. This reaction is unsatisfactory if the addition is reversed—that is, if the vinyl ether is added to the sesamol plus the catalyst. The acetals are generally soluble in kerosine.

Acknowledgment

The author wishes to express his deep appreciation to the Trubek Laboratories, Inc., East Rutherford, N. J., for supplying much of the sesamol used in this study and for the directions given in this paper for the preparation of sesamol. These directions were used to prepare additional quantities of sesamol needed in this study.

He is also grateful to the Carbide and Carbon Chemicals Co., New York, N. Y., for supplying some of the vinyl ethers used in this study.

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INSECTICIDE SYNERGISTS

Determination of Methylenedioxyphenyl-Containing Synergists Used in Analysis of Fly Sprays

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The chromotropic-sulfuric acid method for the determination of methylenedioxyl groups has been applied to the determination of pyrethrum synergists containing these groups. The utility of the method has been demonstrated by its application to the estimation of piperonyl butoxide, sulfoxide, piperonyl cyclonene, and *n*-propyl isome in fly sprays. The method may also be applicable to the determination of the synergists in aerosol formulations, and means of overcoming interferences encountered with several of the more important constituents are given.

IMPORTANCE OF THE METHYLENEDIOXYPHENYL GROUP in contributing toward synergism with pyrethrins was first recognized by Haller and his coworkers (*6*). Since their fundamental discovery, a number of excellent synergists containing this group (*10–12*) have been produced commercially.

Three methods for the determination of methylenedioxyphenyl-containing synergists have been reported. One is for piperonyl butoxide (*7*), but it is not applicable to other methylenedioxyphenyl synergists. The writer (*1*) has described a general method for the

determination of methylenedioxyl or combined formaldehyde groups, as in sesamin and asarinin. Another method applicable to these synergists, reported by Blum (*2*), is based on their ability to form color complexes in the presence of gallic and sulfuric acids.

The present communication illustrates the application of the writer's method to the determination of synergists for pyrethrum. The method is based on the liberation of formaldehyde from methylenedioxyl or combined formaldehyde groups by strong sulfuric acid and the determination of the formaldehyde with chromotropic acid in the same acid medium (*3–5*, *13*). The method is very sensitive, 1 γ piperonyl butoxide being detectable. It is more specific than that of Blum, in that it does not respond to ethylenedioxy groups. Pyrethrins and allethrin interfere, but this interference is eliminated by treatment with methanolic alkali at room temperature.

The utility of the method is demonstrated by its application to the determination of four commercial synergists in three types of fly sprays. Preliminary tests indicate that it may also be applicable to methylenedioxy-containing synergists in aerosol formulations.

Method

Reagents. Chromotropic Acid Reagent. Prepare daily a solution containing 100 mg. of sodium 1,8-dihydroxynaphthalene-3,6-disulfonate (Eastman Kodak P230) per ml. of distilled water. Filter and keep in the dark except when using.

Sulfuric Acid. 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 tightly glass-stoppered container.

Methanolic Potassium Hydroxide (0.5*N*). To a solution of 1.4 grams of reagent grade potassium hydroxide in 5 ml. of distilled water, add 95 ml. of methanol purified as described below.

Methanol, c.p. Reflux 1 liter with 1 gram of 5,5-dimethyl-1,3-cyclohexanedione (Eastman Kodak 1259) for 1 hour and then distill. This treatment removes formaldehyde and other aldehydes present in the commercial product.

Hexane, Chloroform, Acetone. Redistill.

Test Materials. The commercial synergists analyzed in this study were technical products, except for the piperonyl butoxide, which was a pure sample, kindly supplied by the Fairfield Chemical Division, Food Machinery and Chemical Corp., Baltimore, Md. Sesamin and sesamolin were pure compounds isolated from sesame oil.

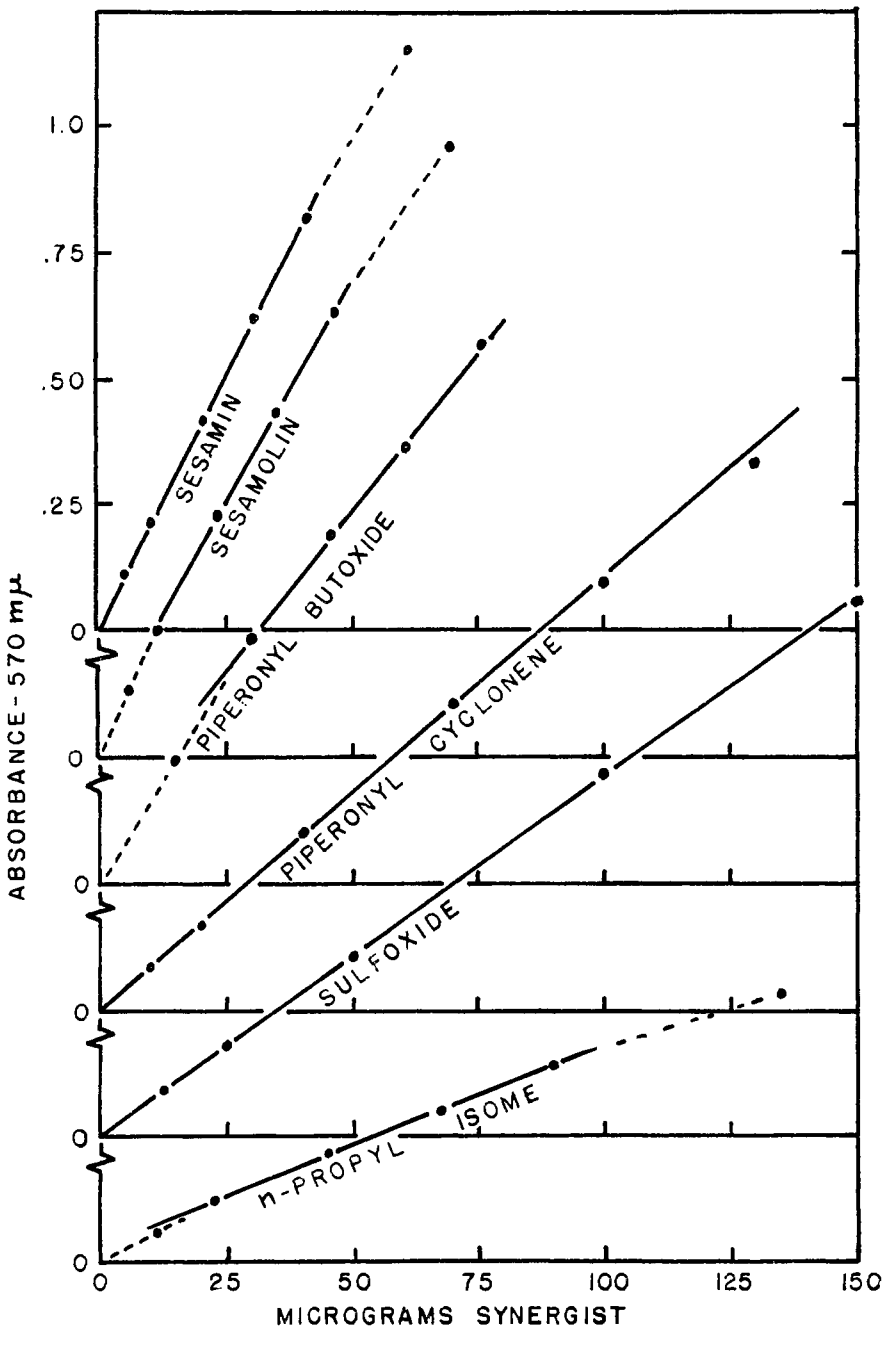


Figure 1. Color development of solutions of synergists in deodorized kerosine

Apparatus. Test tubes, 16 × 150 mm., made of borosilicate glass with a 16/15 female ground joint and glass stopper to fit, such as J-2345, manufactured by Scientific Glass Co., Bloomfield, N. J. The tubes are permanently numbered (etched, ceramic ink, or by any other means).

Adaptor consisting of a 16/15 male ground joint and a stopcock, which is connected by rubber tubing to a water-pump aspirator and a manometer.

Beckman Model DU spectrophotometer. Absorbance measurements were made with cells 1 cm. square. Any colorimeter having a narrow wave band should be satisfactory.

Procedure

Dilute the fly spray with acetone or another volatile solvent, and introduce into the bottom of the test tube an aliquot (usually 1 ml.) containing 160 to 320 γ of synergist. Evaporate the solvent and add 1 ml. of methanolic potassium hydroxide. Swirl vigorously at 10-minute intervals. After 30 minutes add 3 ml. of water and 4 ml. of hexane. Stopper the tube and shake vigorously for 1 minute. Evaporate a 1-ml. aliquot of the hexane layer in another test tube (contains one fourth the original amount of synergist). (The amount of deodorized kerosine in the aliquot is so small that its contribution to the volume of the

hexane may be ignored.) Add 1 ml. of chromotropic acid reagent and follow with 5 ml. of sulfuric acid. Swirl vigorously to be sure the acid has reached all the sample, and place this test tube in a boiling-water bath. Place stoppers in each tube loosely at first and then tighten. After 45 minutes in the bath, remove and cool to room temperature by setting the tubes in a beaker of cold water. Measure the absorbance at 570 mμ against distilled water and subtract the absorbance of a reagent blank similarly treated.

At the same time carry out the same procedure on known amounts of synergist plus the same amount of deodorized kerosine as is present in the fly-spray aliquot, assuming that the fly spray is 100% deodorized kerosine.

When testing piperonyl cyclonene and *n*-propyl isome, add 0.1 ml. of the sulfuric acid reagent after adding the water to the methanolic alkali and substitute chloroform for hexane. Shake vigorously for 1 minute. The upper layer may be aspirated out and an aliquot taken of the lower chloroform layer for analysis.

Evaporations. Attach the test tube to the adaptor with the stopcock open and evaporate the solvent under reduced pressure while swirling the bottom of the tube in a beaker of water at room temperature. Detach the tube as soon as the manometer indicates that all the solvent is evaporated.

Calibration Curves. Prepare calibration curve showing the absorbance against known amounts of synergist plus the deodorized kerosine. From the absorbance of the unknown sample the amount of synergist may be read from this curve. For best results, run the standards and unknown(s) at the same time with the same reagents.

Results

The results obtained with solutions of *n*-propyl isome, sulfoxide, piperonyl cyclonene, piperonyl butoxide, sesamolin, and sesamin are presented in Figure 1. Five milligrams of deodorized kerosine was present in each of the samples except *n*-propyl isome, which contained 10 mg. Pyrethrins were not present, so that the alkali treatment was not included.

Three types of liquid fly sprays were tested. These sprays were prepared according to Federal Specification O-I-551 (GSA-FSS), dated April 1, 1952, except that no odor neutralizer was added.

Ingredient	Composition, % I	II	III
Synergist	0.8	0.8	0.8
Pyrethrin	0.1	0.0	0.1
Allethrin	0.0	0.1	0.0
DDT	1.0	1.0	0.0
Deodorized kerosine	To make 100%		

The analyses of the three types of fly sprays employing four synergists are shown below (figures in per cent):

Synergist	I	II	III
Piperonyl butoxide	0.813, 0.798	0.789, 0.781	0.795, 0.806
Sulfoxide	0.800, 0.785	0.800, 0.773	0.787, 0.795
Piperonyl cyclonene	0.802, 0.800	0.797, 0.800	0.817, 0.797
n-Propyl isome	0.806, 0.802	0.770, 0.797	0.785, 0.785

Three significant figures have been given in the foregoing analyses, because the absorbance was determined to three significant figures. Although the third figure is of doubtful significance, it has been included to give an idea of the reproducibility of the method.

Discussion

The previous method for the determination of methylenedioxyl groups (*1*) was concerned primarily with pure compounds. The application of the method to analyses involving the presence of foreign material showed that certain revisions were required, inasmuch as the high acidity of the reaction mixture caused color to be developed by the pyrethrins, allethrin, DDT, and deodorized kerosine in the absence of any synergist. The use of 3 + 5 aqueous sulfuric acid reagent was found to be the maximum concentration of acid, which sufficiently minimized this effect and still allowed practically maximum color development. The use of this more dilute sulfuric acid made it necessary to extend the heating period from 30 to 45 minutes. The absorbance developed by piperonyl butoxide plus kerosine over a 2-hour heating period was determined. Throughout the entire period the absorbance increased, but the increase was rather slow after the first 45 minutes. No appreciable difference was found between 45 minutes and 1 hour, provided the standards and the unknown were heated for the same time. However, in analyses on some methylenedioxyphenyl-containing compounds—piperonal, for instance—it may be necessary to heat for longer periods to approach maximum color development.

By reducing the sample size from that used in the previous procedure (*1*) and eliminating the dilution step, the sensitivity of the method has been increased about 8 times. One microgram of piperonyl butoxide will give an absorbance of 0.015. As the blank varied ±0.002 for a given set of reagents, this amount of synergist is readily detected.

The sodium salt of chromotropic acid was used, because the acid is no longer supplied commercially. No purification of the commericial product (practical grade) is necessary. Varying the concentration of the chromotropic acid reagent does affect the amount and rate of color development. Good results were obtained with lower concentrations, but 100 mg. per ml. was preferred because previous work had shown that interference was minimized with higher concentrations.

In view of the known sensitivity of chromotropic acid to light (*9*), exposure of the solutions to light was avoided, especially while they were in the boiling-water bath. For this reason the absorbance was measured, not against the blank, but against distilled water. The blank absorbance was then subtracted. The absorbance of the solution after color development is stable for hours.

As shown in Figure 1, three of the six synergists give good agreement with Beer's law to a definite point. The others exhibit a straight-line relationship over a sufficient portion of the curve, so that determinations may be made on the straight-line section. With piperonyl butoxide very good agreement to at least the 50-γ level was obtained when no kerosine was present. However, even with kerosine present, agreement was fair to the 45-γ level. Thus, if the absorbance of the unknown is within 10% of the known, very little error will be involved in assuming Beer's law is followed. With careful technique results within 2 to 3% of the true value have been obtained.

Interference. Pyrethrin and allethrin interference is eliminated by treating the sample with methanolic alkali. This treatment probably causes the pyrethrins or allethrin to form complex dimers (*8*). After dilution of the methanolic alkali with water, the synergist is extracted with hexane or chloroform.

Treatment of *n*-propyl isome and piperonyl cyclonene with alkali causes partial saponification of the ester groups in these compounds, so that the acid product remains in the alkaline layer upon extraction with hexane. Even when the aqueous methanol layer is acidified prior to extraction, the acid product is not completely extracted by hexane, particularly in the case of *n*-propyl isome, which may be degraded partially to a dibasic acid. When chloroform is used instead of hexane, the extraction is practically complete.

In experiments in which each of the four commercial synergists was added in 5 to 1, 8 to 1, and 15 to 1 ratios of synergist to pyrethrins or allethrin, results were usually within 2% of the true value when the alkali treatment was used. The data are shown in Table I.

The analysis is affected to a small degree by the presence of deodorized kerosine, which is therefore included in the analyses of the standards. DDT in amounts up to $1^1/_2$ times the amount of synergist caused practically no interference.

Limitations. It is not possible by the present method to distinguish between a synergist and a closely related material which may or may not be synergistic if both contain groups which liberate formaldehyde in strong sulfuric acid. Consequently this method cannot be used to determine the purity of a synergist. In the analysis of a commercial synergist, it would be best to have a sample of the pure product to serve as a standard. Of course, with an unknown sample, it would be necessary to establish the identity of the synergist in order to use the present analysis.

Table I. Effect of Pyrethrin and Allethrin on Analysis of Synergists

Synergist	Synergist, γ	Pyrethrins, γ	Allethrin, γ	Absorbance
Piperonyl butoxide	40	0	...	0.576
		8	...	0.570
		5	...	0.565
		2.67	...	0.566
		...	0	0.562
		...	8	0.570
		...	5	0.559
		...	2.67	0.562
Sulfoxide	40	0	...	0.285
		8	...	0.287
		5	...	0.283
		2.67	...	0.290
		...	0	0.285
		...	8	0.294
		...	5	0.283
		...	2.67	0.282
Piperonyl cyclonene	40	0	...	0.384
		8	...	0.390
		5	...	0.387
		2.67	...	0.391
		...	0	0.384
		...	8	0.390
		...	5	0.388
		...	2.67	0.395
n-Propyl isome	80	0	...	0.346
		16	...	0.354
		10	...	0.344
		5.33	...	0.350
		...	0	0.349
		...	16	0.358
		...	10	0.347
		...	5.33	0.356

Aerosols. As aerosol formulations contain various materials not discussed above, preliminary studies were carried out to determine the effect of some of these materials on the determination of piperonyl butoxide and sulfoxide by the method described.

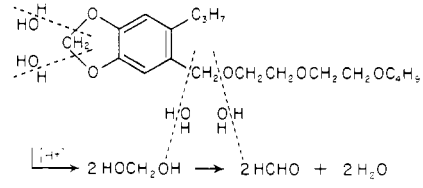
The most serious interference was that encountered with methylated naphthalenes, which cause a marked decrease in absorbance. It was found that this interference could be eliminated by steam distillation. For instance, 25 mg. of piperonyl butoxide or sulfoxide was placed in a 100-ml. long-necked round-bottomed flask with a 24/40 ground joint, and 200 mg. of methylated naphthalenes, 25 ml. of water, and a boiling stone were added. A condenser was attached by means of a two-way 75° connecting tube (such as Corning No. 8920), and 15 ml. of water was distilled over at about 1 ml. per minute. The contents of the flask were cooled, and 50 ml. of hexane was added. The flask was stoppered and shaken for 1 minute. After proper dilution it was found that the absorbance developed by an aliquot of the hexane layer was the same, within experimental error, whether the naphthalenes were present originally or not. Less than 10% of the synergist was removed from both the sample and the standard by the steam distillation. If kerosine is present, it steam-distills over. DDT does not.

Alkyl thiocyanates and Metazene (Kilgore Chemical Co.) offered little if any interference in respective concentrations of 8 and 4 times the amount of synergist. When DDT was added in amounts of from 2 to 8 times the amount of synergist, inhibitions of color development as high as 7% were encountered. Probably the simplest means of overcoming this interference is to add the same amount of DDT to the standard as is present in the sample. When present in large amounts, DDT may be noticed as an insoluble material on the surface of the sulfuric acid solution after color development.

Several typical aerosol formulations were analyzed using the steam-distillation procedure given above. A formulation containing 0.1% pyrethrins, 0.8% sulfoxide, 4.1% kerosine, 6.0% alkylated naphthalenes, and 2% Lethane was analyzed for sulfoxide and was found to contain 0.82%. Another formulation containing 0.2% pyrethrins, 1.0% piperonyl butoxide, 6% kerosine, 6% alkylated naphthalenes, and 2% DDT was analyzed for piperonyl butoxide and was found to contain 1.02%.

Other Applications. The procedure described above is similar to that used by MacFayden (*9*) for the determination of formaldehyde in biological fluids, the main difference being the larger amount of chromotropic acid used. In so far as these biological fluids do not offer serious interference, it appears likely that the method described may be applicable to the determination of methylenedioxyphenyl-containing synergists in such fluids. Of course, in analyses in which no pyrethrins or allethrin are present, the alkali treatment should be omitted.

Reaction Mechanism. On the basis of previous work (*1*, *3–5*, *13*), the formation of formaldehyde by hydrolytic cleavage of the methylenedioxy groups in synergists is expected. However, the absorbance developed by piperonyl butoxide indicates that each mole of this synergist produces approximately 2 moles of formaldehyde. It has been pointed out (*7*) that primary carbinol groups, especially those attached to a substituted phenyl group, may produce formaldehyde even if the hydroxyl is etherified. By analogy it may be concluded that the second mole of formaldehyde must arise from the etherified primary carbinol group by hydrolytic scission as follows:



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