METABOLISM OF INSECTICIDES

Permanganate Oxidation Products of Schradan

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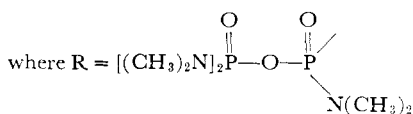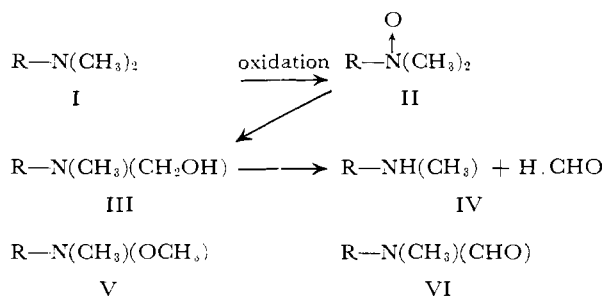
Synthesis of schradan derivatives and Craig countercurrent separation of the components of permanganate-oxidized schradan, kinetic hydrolysis values, and infrared absorption spectra, yield data which give evidence against the existence of a phosphoramide oxide structure, and suggest that the methylol derivative is the active form. Important properties of the active component are that it is a potent anticholinesterase, rather unstable in aqueous solutions, readily forms heptamethylpyrophosphoramide, and has a partition coefficient similar to the biologically produced material.

CONVERSION OF THE SYSTEMIC INSECTICIDE SCHRADAN (octamethyl-pyrophosphoramide) to a more active anticholinesterase has been studied extensively. Hartley first postulated this activation by oxidation with the production of the phosphoramide-N oxide (II)

$$R-N(CH_3)_2 \xrightarrow{\text{oxidation}} R-\overset{\uparrow}{N}(CH_3)_2$$

$$\text{I} \qquad\qquad \text{II}$$

$$R-N(CH_3)(CH_2OH) \longrightarrow R-NH(CH_3) + H.CHO$$

$$\text{III} \qquad\qquad\qquad \text{IV}$$

$$R-N(CH_3)(OCH_3) \qquad\qquad R-N(CH_3)(CHO)$$

$$\text{V} \qquad\qquad\qquad\qquad \text{VI}$$

$$\text{where } R = [(CH_3)_2N]_2P-O-P\overset{\displaystyle O}{\underset{\displaystyle N(CH_3)_2}{\|}}$$

(6), with subsequent rearrangement to the methylol derivative (III). This could then split out formaldehyde, leaving heptamethylpyrophosphoramide (IV). The active anticholinesterase obtained by permanganate oxidation of schradan has been shown to be identical with that produced metabolically by mammals and plants (2, 77), and insects (77). O'Brien and Spencer suggested that the active anticholinesterase might be the amide oxide (II) or the methylol derivative (III) of schradan. Tsuyuki, Stahmann, and Casida, in a recent paper (77), introduced experimental evidence which supports the amide oxide structure as the active anticholinesterase product of schradan oxidation. Spencer (74) reported evidence against this postulation. Casida and his colleagues also presented arguments favoring the methyl ether structure (V) for the less reactive isomerization product of oxidized schradan. More recently Hartley's associates, Heath, Lane, and Park (7) presented evidence supporting the methylol structure (III) as the active component, and arguments against the postulation of Casida, Allen, and Stahmann (7). Fish, Johnson, and Horning (3) from work on dimethylamino-containing alkaloids suggested that the methyl ether isomer (V) is improbable as a product of schradan oxidation. Recently they reported (4) the demethylation of dimethylamine oxides of certain alkaloids and more recently (76) the dealkylation of N,N-dimethylglycine N-oxide via what was presumed to be a methylol derivative followed by loss of formaldehyde.

Methods and Materials

Schradan was prepared from technical material kindly supplied by Monsanto Co., Ltd. (6).

The rates of hydrolysis were determined as reported earlier (75), except that pH change was indicated by means of a Beckman Model G pH meter using a calomel electrode with a sleeve. More recently the pH was maintained with an automatic titrator (Radiometer Model TTT2) and readings were therefore required at less frequent intervals.

Phosphorus was determined essentially according to the method of Rockstein and Herron (72). A digestion for 30 minutes in a boiling water bath prior to the addition of ferrous sulfate converted all the schradan and oxidation products to inorganic orthophosphate. Readings were taken with a red filter on a Klett-Summerson colorimeter. Formaldehyde, dimethylamine, and the anticholinesterase activity were determined as reported earlier (75). The methods of permanganate oxidation of schradan and extraction with chloroform were similar to those reported previously (77) except that when larger quantities were oxidized the temperature was maintained at 25° C. by outside cooling.

The components of the oxidized schradan were partitioned between water and U.S.P. redistilled chloroform in a 60-tube Craig countercurrent extractor. The water was buffered at pH 7.2 with $0.05M$ tris(hydroxymethyl)aminomethane buffer and saturated with chloroform, while the chloroform was saturated with the buffer prior to partitioning. The extractor was shaken mechanically by means of a device described elsewhere (9) and 50 aqueous fractions were collected in a synchronized circular collector; 60 chloroform-water fractions remained in the machine. The first tube in the circular collector was designated number one and contained the most hydrophilic component. Aliquots of the aqueous phase of every fifth tube were taken for phosphorus and formaldehyde assay. The partititioning was indicated by plotting Klett readings against tube number. At the hydrophobic end the partition coefficient chloroform-water is large and therefore the aqueous phases contain only a small part of the total components. The figures show the results for aqueous phases only and therefore the values for the total components per tube would be much greater at the hydrophobic end.

Where definite Craig peaks were indicated from the analyses, the contents of the tubes in each peak were combined and extracted into chloroform. After drying over sodium sulfate, the solvent was removed in vacuo with a rotating evaporator. Infrared absorption spectra were obtained in carbon tetrachloride or chloroform solutions with a Perkin-Elmer Model 21 infrared spectrophotometer equipped with a rock salt prism. The rates of hydrolysis were determined on chloroform extracts of the oxidized schradan after removal of the solvent, as well as on fractions from the Craig extractor. The rate constants and half lives were calculated as before (*15*). Hydrolysis rates were also found by maintaining components derived from the Craig extractor in borate buffer at pH 8 and 25° C. and removing aliquots at increasing time intervals. The extent of hydrolysis was determined by extracting the aliquots with chloroform and then assaying the aqueous residue for phosphorus. When hydrolysis had ceased, the aqueous solution was extracted with chloroform, the solvent removed and the extract dissolved in glycine buffer at pH 10 and maintained at 45° C. Aliquots were removed and analyzed as described above.

Heptamethylpyrophosphoramide was synthesized by the method outlined by Heath, Lane, and Park (*7*) except for the synthesis of the intermediate trimethyl-phosphordiamidic chloride. This was prepared by the addition of two equivalents of dimethylamine in dry ether, to an ether solution of monomethyl-phosphoramidic dichloride, the temperature being maintained between 8° and 9° C. The product was filtered from the amine hydrochloride, the ether flashed off and the desired product distilled at 112° C., 0.4 mm. of mercury pressure, n_{24}^{d} 1.4726. Analysis. Phosphorus, 19.8%; nitrogen, 17.3%; chlorine, 23.7%. Theoretical. Phosphorus, 19.8%; nitrogen, 17.9; chlorine, 22.7. The initial monomethylphos-phoramidic dichloride was prepared by refluxing 68 grams of monomethylamine hydrochloride (1 mole) with 610 grams of redistilled phosphorus oxychloride (4 moles) for 24 hours. The excess phosphorus oxychloride was removed in vacuo and the desired product obtained at 71 to 74° C., 0.4-mm. of mercury pressure; n_{23}^{d} 1.4702. Found, chlorine, 49.3%; theoretical, 48.0%. It was important to use redistilled phosphorus oxychloride and to recover the product with minimum heating. Several earlier attempts to distill hepta-methylpyrophosphoramide resulted in decomposition. This instability was later confirmed by Heath, Lane, and Park (*7*) and Schrader and Kükenthal (*13*).
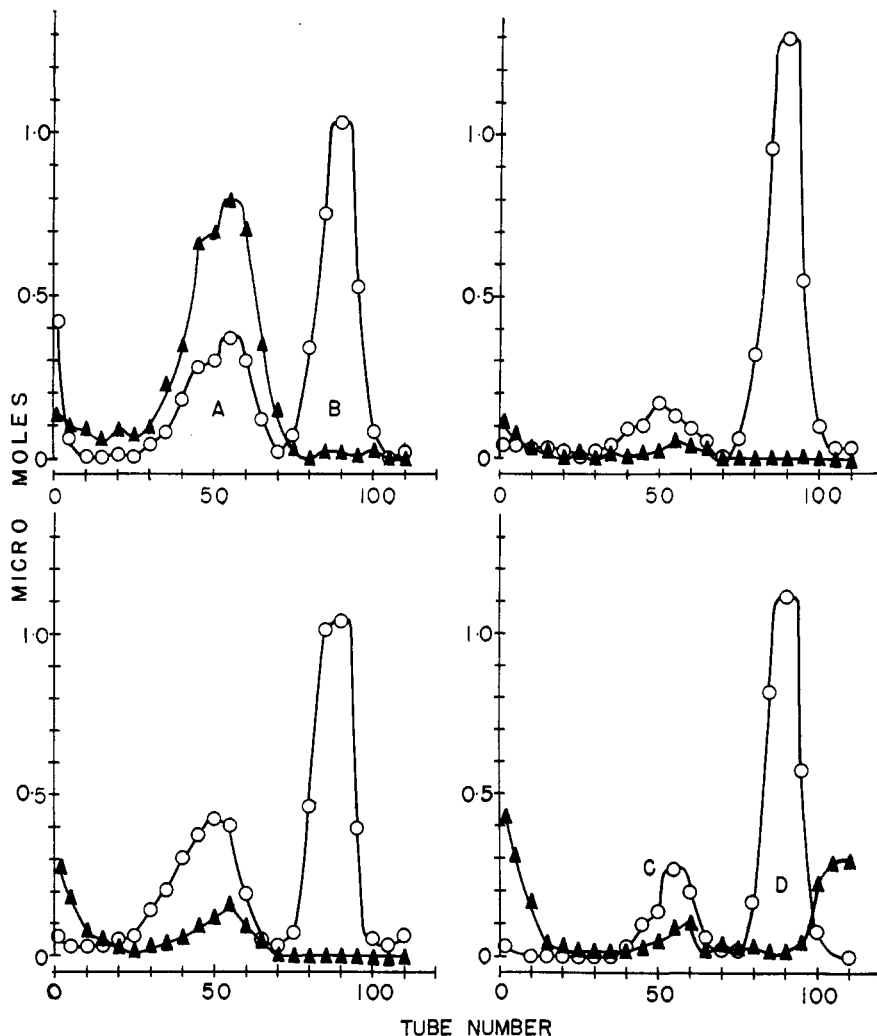

Figure 1. Craig countercurrent fractionation

A. Potassium permanganate—oxidized schradan
B. After hydrolysis at pH 8 and 25° C. for 6 hours
C. After acetic acid treatment
D. After refluxing chloroform solution 4 days
▲ Formaldehyde
○ Phosphorus

Heptamethylpyrophosphoramide *N*-methoxide (V) was synthesized by heating equimolecular quantities of sodium tetramethylphosphordiamidate and *N,N,N'* - trimethyl - *N'* - methoxy-phosphordiamidic chloride at 90° to 100° C. for 7 hours. The product was dissolved in water and extracted with chloroform. After drying over sodium sulfate and removing the solvent, the product distilled at 104° to 110° C., 0.02-mm. of mercury pressure. The forerun contained some unreacted material. Analysis. Found, phosphorus, 20.0%; dimethylamine, 45.3%. Theoretical, phosphorus, 20.5%; dimethyl-amine, 44.8%, n_{24}^{d} 1.4613. Total nitrogen was low, because of the incomplete hydrolysis of *O,N*-dimethylhydroxyl-amine. The Craig extractor indicated one sharp peak at tube 100. An earlier attempted synthesis by heating ethyl tetramethylphosphordiamidate with *N,N,N'* - trimethyl - *N'* - methoxyphos-phordiamidic chloride in boiling xylene was unsuccessful. Sodium tetramethyl-

phosphordiamidate was prepared according to the method of Heath, Lane, and Park (*7*). *N,N,N'*-trimethyl-*N'*-meth-oxyphosphordiamidic chloride was synthesized by the addition of 2 moles of dimethylamine in dry ether to 1 mole of *N*-methyl-*N*-methoxyphosphoramidic dichloride in dry ether at 5° C. The salt was filtered off, the ether was removed, and the desired product was distilled at 43° to 46° C., 0.005-mm. of mercury pressure; n_{23}^{d} 1.4602. Analysis. Found, chlorine, 20.3%; theoretical, 19.1%. The value is slightly high because of slight contamination with hydrochlorides. *N*-methyl-*N*-methoxy-phosphoramidic dichloride was prepared by refluxing *O,N*-dimethylhydroxyl-amine hydrochloride with a fourfold excess of redistilled phosphorus oxy-chloride for 20 hours. The excess was removed in vacuo and the desired product distilled at 80° to 85° C., 0.12-mm. of mercury pressure. Analysis. Found, chlorine, 39.8%; theoretical, 41.4%; n_{23}^{d}, 1.4564. *O,N*-dimethylhy-

droxylamine hydrochloride was prepared essentially according to the method of Hantzsch (5) starting with ethyl chloroformate, reacting with hydroxylamine, then dimethyl sulfate, and finally, after saponification and decarboxylation (8), distilling the O,N-dimethylhydroxylamine into hydrochloric acid.

Results

Countercurrent Distribution. Fractionation of the synthetic sample of heptamethylpyrophosphoramide in the Craig apparatus indicated one main component with a peak at tube 50, but much sharper than that of fraction A, Figure 1, upper left. Heptamethylpyrophosphoramide N-methoxide also showed only one component on countercurrent fractionation with a sharp peak at tube 100, outside the main components (fractions A and B of Figure 1, upper left). Paraformaldehyde and trioxane had sharp maxima at tubes 50 and 80, respectively, whereas formalin showed, in addition to the major portion at the extreme hydrophilic end, a small peak at tube 50 and a larger peak at the hydrophobic end beyond the trioxane peak. Schradan had a sharp peak at tube 90.

Countercurrent fractionation of schradan oxidized with potassium permanganate indicated two main fractions by phosphorus analysis, with maxima at tubes 55 and 90, designated fraction A and B, respectively. Although these present the appearance of two homogeneous components, later evidence demonstrated that each peak contained more than one phosphorus compound. A slight amount of phosphorus appeared in the extremely hydrophilic end, owing to phosphates from hydrolyzed pyrophosphate. Some formaldehyde appeared also in the extreme hydrophilic end because of release from the active component, while the large mass coincided with fraction A and an almost insignificant amount with fraction B. When a chloroform solution of oxidized schradan was refluxed for 4 days and then fractionated in the Craig countercurrent apparatus, two phosphorus peaks, C and D (Figure 1, lower right), were found, corresponding to peaks A and B of Figure 1, upper left. However, the formaldehyde in fraction C was much less than in A and a much larger amount appeared in the extreme hydrophilic end—presumably free formaldehyde produced on refluxing. Another portion appeared in the extreme hydrophobic end, even beyond that found for the cyclic polymer, trioxane. The phosphorus in fraction C was less than that in A and decomposition of heptamethylpyrophosphoramide was suspected. This was confirmed when a sample of heptamethylpyrophosphoramide was refluxed in chloro-

form for 2 days. Craig countercurrent analysis showed the appearance of another component with a maximum at tube 90. In the former experiment this would be in the D peak.

Hydrolysis of permanganate-oxidized schradan at pH 8 and 25° C. for 6 hours followed by Craig countercurrent fractionation of the chloroform-soluble material indicated the presence of a fraction having the same location as heptamethylpyrophosphoramide.

Tsuyuki, Stahmann, and Casida (17) reported isomerization of the perchlorate oxidation product of schradan following treatment of the chloroform solution with acetic acid, followed by washing with bicarbonate. The isomer formed was more hydrophobic than schradan. Acetic acid was therefore added to a 1.5% solution of permanganate oxidized schradan in chloroform to the extent of 3% final concentration. After 1 hour at room temperature, the acetic acid was removed by washing the chloroform solution with a saturated bicarbonate solution. Craig countercurrent extraction indicated two main peaks shown in Figure 1, lower left, similar in location to A and B of Figure 1, upper left. The total phosphorus peak corresponding to peak A was similar in size to A and shifted slightly toward that for heptamethylpyrophosphoramide, but with much less formaldehyde. The hydrophobic formaldehyde-producing material formed by refluxing was not produced by this acid treatment. Evidently under the acid conditions none of this material formed—its hydrophobic nature would preclude the possibility that it was formed only to be lost on washing with bicarbonate.

Hydrolysis kinetics. The kinetics of hydrolysis of freshly oxidized schradan were studied. Hydrolysis rates were followed at pH 7, 8, and 9.5 (25° C.) and were of the first order. The value of $k/[OH]$ was constant at 1.65×10^4 liters mole^{-1} min.$^{-1}$ demonstrating that the hydrolysis was hydroxyl-catalyzed. This gives a half life at pH 8 and 25° C. of 42 minutes. At pH 10 and 45° C. two more components were found with half lives of 46 minutes and 10 hours.

Under these conditions schradan has a half life of approximately 80 hours, heptamethylpyrophosphoramide 10 hours, and heptamethylpyrophosphoramide N-methoxide 18 hours.

Fraction A from the Craig separation was shown to contain the least stable component with a half life of 42 minutes at pH 8 and 25° C. When fraction C (Figure 1, lower right) from oxidized schradan was held at 25° C. and pH 8 for 6 hours, the chloroform-insoluble products in the aqueous phase (derived from the hydrolysis of the least stable component) had a formaldehyde: phosphorus–total amine–dimethylamine ratio of 0.990:2.00:4.11:3.10. (The theoretical ratio expected for the methylol derivative (III) is 1:2:4:3.) The chloroform-soluble fraction when then held at pH 10 and 45° C. for 5 hours yielded a chloroform-insoluble fraction with a ratio of the above-mentioned constituents of 0:2.00:3.90:3.08; theoretical, 0:2:4:3.

Acetic acid–treated oxidized schradan, besides yielding less formaldehyde than the untreated material, also required one third less alkali on hydrolysis at pH 8 and 25° C.

Infrared spectra. A chloroform solution of the permanganate-oxidized schradan showed a characteristic infrared absorption at 1690 cm.$^{-1}$, which was absent in the schradan spectrum. When the least stable component was removed by hydrolysis at pH 8 and 25° C. for 6 hours, the unhydrolyzed fraction still showed this characteristic absorption (Figure 2). Only after hydrolysis at pH 10 and 45° C. did this absorption disappear (Figure 2). Fraction B of Figure 1, upper left contained the component with the infrared absorption at 1690 cm.$^{-1}$ Division of this fraction in two parts indicated that the 1690 cm.$^{-1}$ component was slightly more hydrophobic than schradan. Fraction A, Figure 1, upper left, had a nitrogen-hydrogen absorption at 3300 cm.$^{-1}$, as did heptamethylpyrophosphoramide.

Although trimethylamine oxide dihydrate shows a broad weak band at 1660 cm.$^{-1}$, which is lacking in tri-
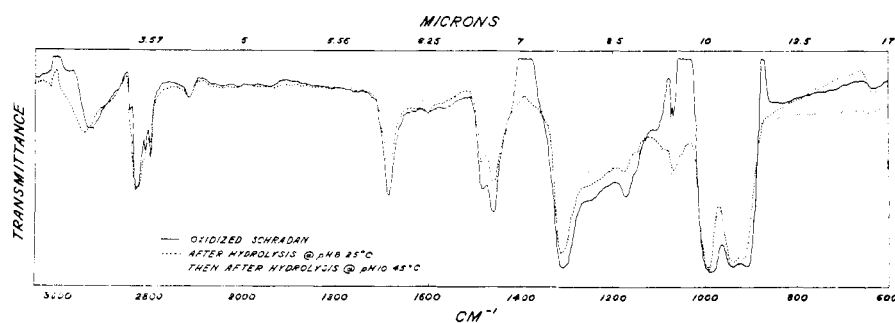

Figure 2. Infrared spectra of chloroform solutions of oxidized schradan before and after selective hydrolysis

methylamine, this absorption disappears on complete dehydration. Anhydrous dimethyltryptamine N-oxide showed no absorption in the range of 1600 to 1700 cm.$^{-1}$ nor did heptamethylpyrophosphoramide N-methoxide.

Toxicity and Enzyme Inhibition. Fraction A of Figure 1, upper left, gave 50% inhibition of serum cholinesterase at $3.2 \times 10^{-7}M$, as molar pyrophosphate, fraction B at $4.0 \times 10^{-4}M$, heptamethylpyrophosphoramide N-methoxide at $3.5 \times 10^{-3}M$, and heptamethylpyrophosphoramide at $1.7 \times 10^{-3}M$. Fraction A had an LD_{50} (γ per gram) to the squash bug of 60 compared to less than 44 for heptamethylpyrophosphoramide. The latter was lethal to the mouse at doses down to 50 γ per gram but was nontoxic to the roach at 250 γ. As fraction A contains heptamethylpyrophosphoramide and the methylol derivative (III), these values suggest that III is relatively nontoxic to the squash bug. This is in accord with the hypothesis concerning the selective action of schradan proposed earlier (10).

Discussion and Conclusions

The only evidence that the most active component of permanganate-oxidized schradan is the N-oxide is a slight similarity in infrared absorption of the crude oxidation mixture and trimethylamide oxide dihydrate at 1690 cm.$^{-1}$ and approximately 1660 cm.$^{-1}$, respectively. The authors have shown that this absorption is not associated with dehydrated trimethylamine oxide but only with the dihydrate; that dimethyltryptamine N-oxide does not show absorption in the infrared in the region of 1690 cm.$^{-1}$, although it is a relatively stable N-oxide; and that this absorption is not shown by the oxidized schradan fraction, isolated by the Craig countercurrent extractor, which has the potent anticholinesterase and most unstable component and a partition coefficient similar to the biologically produced material.

Important properties of the active component are that it is a potent anticholinesterase; that it has a partition coefficient, chloroform-water, of between 1 and 2; that it is rather unstable in aqueous solutions; and that it readily forms heptamethylpyrophosphoramide (IV). The first three properties would be accounted for if the component had a markedly electrophilic substituent (15). The N-oxide (II) and the methylol (III) forms would fit this requirement, and either could split out formaldehyde to give (IV). The choice would seem to be between these two. Infrared data give no indication, as no new peak is found associated with the Craig fraction containing the active component. Heath, Lane, and Park (7) present con-

vincing evidence that the methylol is the more probable form, based upon a study of the ratios of partition coefficients. Wenkert (18) has proposed a mechanism for the oxidation of N-methyl alkaloids by various agents to form a hydroxymethyl structure; the mechanism is equally appropriate for schradan oxidation. The authors assume in later discussion that the active component is the methylol derivative (III), and that the N-oxide is formed transiently or not at all.

The methylol derivative is relatively unstable and under refluxing in chloroform or treatment with acetic acid yields formaldehyde and heptamethylpyrophosphoramide. During the refluxing some of the heptamethylpyrophosphoramide is lost by decomposition, so that the total phosphorus in C (Figure 1, lower right) is less than in the corresponding fraction A (Figure 1, upper left). However, in the presence of a trace of glacial acetic acid in a nonaqueous medium at room temperature there is no loss of phosphorus from the component corresponding to A (Figure 1, upper left), since heptamethylpyrophosphoramide is found in the same fraction. Part of the large formaldehyde peak shown in fraction A (Figure 1, upper left) is probably due to a carryover of paraformaldehyde from the oxidation mixture after chloroform extraction.

Heptamethylpyrophosphoramide N-methoxide was postulated by Tsuyuki, Stahmann, and Casida (17) to be the stable isomerization product of the unstable N-oxide. Heath, Lane, and Park (7) and Fish, Johnson, and Horning (3) on the basis of indirect evidence, disagreed with this. In the present work the compound has been synthesized and the results of countercurrent distribution showed that it did not correspond with any component found in the permanganate oxidation mixture of schradan.

The unfortunate coincidence of the peaks in the Craig fractionation of the methylol isomer, paraformaldehyde, and heptamethylpyrophosphoramide makes it impossible to determine how much of the formaldehyde found in this region is still bound to phosphorus—i.e., in the methylol isomer—and how much is present as paraformaldehyde possibly derived from a breakdown of the methylol isomer to heptamethylpyrophosphoramide. Attempts to resolve these fractions with mixed solvents (carbon tetrachloride–chloroform and benzene-chloroform) merely shifted the peaks. However, the observation (Figure 1, upper right) that after the elimination by hydrolysis of the least stable component about 50% of the phosphorus in the A peak remains, suggests that heptamethylpyrophosphor-

amide was present in the original oxidation mixture.

The question of the identity of the oxidation product with an infrared absorption at 1690 cm.$^{-1}$ still remains. It partitions in the same range as schradan, has a half life of 42 minutes at pH 10 and 45° C., and does not yield formaldehyde. It is not the methoxy isomer (V). Carbonyl (amide I) compounds absorb in the 1660 cm.$^{-1}$ region and tertiary amides in the 1680 cm.$^{-1}$ region. Therefore the formyl derivative (VI) mentioned by Hartley (6) appeared of interest. Its synthesis from N-methyl formamide (which absorbs at 1660 cm.$^{-1}$) was attempted without success.

Small variations in the conditions of permanganate oxidation (time, temperature, buffer concentration, and reagent ratios) produced varying amounts of Craig fractions not described above. One small peak at tube 10 showing phosphorus and formaldehyde may be a further oxidation product such as the methylol derivative of heptamethylpyrophosphoramide. This derivative may be identical with the polar, unstable second inhibitor reported by Heath, Lane, and Park (7) from liver oxidation. That heptamethylpyrophosphoramide may be further oxidized is suggested by its similarity to schradan in toxicity and symptoms in the mouse (symptoms appear 30 minutes after injection) and in its selective toxicity (squash bug and roach).

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INSECTICIDE ANALYSIS

Infrared Determination of Dichlorodiphenyltrichloroethane and Benzene Hexachloride in Insecticides

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An infrared spectrophotometric method has been developed for the determination of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (p,p'-DDT) and the gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane (γ-BHC) in cotton dust insecticides. Direct extraction of the sample with the infrared transparent solvent, carbon disulfide, reduces the time required for analysis as well as errors incurred in sample transfer and solvent evaporation. An average error of ± 0.05% was obtained for single determinations.

DURING AN INSPECTION PROGRAM by the Alabama State Department of Agriculture, the State Chemical Laboratory was called upon to analyze a large number of cotton dust insecticides for 1,1,1 - trichloro - 2,2 - bis(p - chlorophenyl) ethane (DDT, both p,p'- and o,p'-isomers) and the gamma isomer of 1,2,3,4,5,6 - hexachlorocyclohexane (γ-BHC). The partition chromatographic method of Harris (8) was earlier employed in these analyses but was of limited value because in formulations containing sulfur it gave too high results for DDT. Several other methods have been developed for the determination of DDT and BHC in insecticides containing mixtures of the two. A partition chromatographic method of Prat and Colas (19) can be successfully used to determine BHC but cannot always be relied upon in the analysis for DDT because the elution zones of hepta- and octachlorocyclohexanes, sometimes present as impurities in BHC, overlap that of DDT. Chemical methods reported by Perkow (18) and Weber (24) appear to be complicated and are unable to distinguish among the several isomers of BHC found in commercial preparations. A polarographic determination of γ-BHC in the presence of DDT has been described by Wolf (26) and a polarographic determination of both γ-BHC and DDT in mixtures of the two was reported by Tamamushi and Tanaka (22). The latter investigators were unable to dis-

tinguish p,p'-DDT from its isomers by this procedure.

A number of articles dealing with the infrared spectrophotometric analysis of technical DDT for the para-, para'-isomer and of technical BHC for the gamma isomer lead to the conclusion that this method might be adapted to the analysis of formulations containing both of these products in a range of concentrations considerably lower than that found in the technical materials.

The infrared spectrum of p,p'-DDT as well as that of four related compounds in the range of 1 to 13 microns has been reported by Andrews and coworkers (1). Quantitative analyses of samples of technical DDT have been carried out by Downing and associates (5) and Henry, Colas, and Prat (10).

Spectra for the five isomers of BHC, α-, β-, γ-, δ-, and ε-, in the range 2.5 to 14 microns were published by Kauer, DuVall, and Alquist in a paper describing the isolation of the ε-isomer from crude BHC (12). Quantitative procedures for the analysis of technical BHC concentrate have been described by Harris (2, 9), Kuratani, Shimanouchi, and Mizushima (13), Mecke and Mutter (16), Kamada and Tanaka (11), Trenner and coworkers (23), Larnaudie (14), Whiffen and Thompson (25), Daasch (4), and Marrison (15).

This paper deals with a rapid routine infrared spectrophotometric method for the simultaneous determination of p,p'-

DDT and γ-BHC in insecticidal mixtures containing a high percentage of other constituents such as sulfur, talc, and clay. As the latter two ingredients are insoluble in the solvents used in infrared analyses, an extraction was carried out to separate DDT and γ-BHC from the inert constituents prior to measuring the infrared absorption.

The customary extraction procedure uses the Soxhlet extractor with ethyl ether in an overnight extraction. Although with hexane, the extraction period may be shortened to 2 hours, the higher operating temperature of the extractor brings about additional difficulties. If the sample contains traces of ferric ions or other catalysts, thermal decomposition on the hot walls of the extraction flask above the level of the solution becomes a problem (7). Also, contamination with silicone stopcock grease must be avoided since this interferes with DDT determination. Hence, an improved extraction technique was needed to give rapid and reliable results.

Experimental

Preparation of Standards. p,p'-DDT (melting point 108.5–109.0° C.) was recovered from a technical grade by repeated recrystallizations from 95% ethyl alcohol. The infrared spectrum of the purified product was in agreement with that reported by Downing and coworkers (5).