and including calcium phosphate gel treatment could be preserved at 0° in 0.02*M* phosphate buffer pH 8.2 with no loss in activity. Variation in the pH over the range 8.0 to 8.6 had little effect on enzyme stability. Outside this range the rate of inactivation increased. Preparations after the acetone step in purification were less stable than those of the crude extract. For example, a sample lost 20% of its initial activity after standing several days at 2° C. The crude extract and preparations up to and including calcium phosphate gel are relatively heat stable for there is only slight destruction of enzyme activity after heating 15 minutes at 65° C. Heat treatment enabled the use of larger amounts of acetone without objectionable loss of activity in the acetone purification step.

Effect of Metals and Sulfhydryl Compounds. Attempts were made to stimulate enzyme activity by the addition of metal ions. At $10^{-3}M$, Mg^{+2}, Ni^{+2}, Pb^{+2}, Mn^{+2}, Fe^{+2}, and Co^{+2} were without effect. The enzyme fractions were inhibited by $10^{-2}M$ *p*-hydroxymercuribenzoate and iodoacetate. This evidence clearly indicates that both enzyme fractions are sulfhydryl-dependent enzymes. In the presence of either inhibitor, approximately 20% of the enzyme activity remained. Also, under anaerobic conditions and in the absence of glutathione, 13% of the enzyme activity was present. In keeping with these observations, glutathione, cysteine, and thioglycollic acid stimulated the enzyme activity.

Discussion

In the housefly, the only product obtained from the attack of DDT-dehydrochlorinase on DDT is DDE. The human body louse has enzymes which produce a number of DDT-degradation products. The present results indicate that these native enzymes are a mixture of several types. The partial separation of these types was effected by the use of *t*-amyl alcohol. Each type apparently produces a different metabolite. Enzyme fraction *A* gave a 6:1 ratio of neutral to acidic metabolite. The neutral metabolite was predominantly DDE. Similarly, enzyme fraction *B* gave a 1:1 ratio of neutral to acidic metabolite. The neutral metabolite in this case was predominantly 4,4′-dichlorobenzophenone. The acidic metabolite produced by both enzyme fractions appears to be DDA, since the metabolite has the same R_f value and similar infrared spectrum as DDA.

Additional evidence on identification of this metabolite awaits further purification. The results of the identification of metabolites have been significant and seem to indicate that the multiplicity of enzyme types is the consequence of the actual occurrence of various DDT-degrading enzymes in the human body louse. Each enzyme fraction was solubilized by the addition of trypsin. This treatment made it possible to use organic solvents at higher concentration than usual thus effecting a greater degree of purification. No appreciable purification resulted from the use of acetone before trypsin treatment in spite of the great stability of the enzymes in the presence of this solvent. Like most enzymes, DDT-degrading enzymes have a narrow pH range through which they exert their maximum effect. This is shown in Figures 1 and 2. The crude extract is heat stable, although upon purification with acetone it loses this property. The cause for the change in heat stability is not known. The influence of enzyme concentration and substrate concentration was like that of most enzymes, the rate of activity being proportional to enzyme concentration and to the substrate concentration up to a limiting value (*9*). Study of the enzyme substrate relationships in greater detail must await further separation of enzyme types (*9*).

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INSECTICIDE DECOMPOSITION

Nuclear Magnetic Resonance in the Examination of the Thermal Decomposition of *O,O*-Dimethyl *O*-[4-(Methylthio)-3-tolyl] Phosphorothioate

T. R. FUKUTO, E. O. HORNIG, and R. L. METCALF

Department of Entomology, University of California, Riverside, Calif.

During investigations on the metabolic fate of *O,O*-dimethyl *O*-[4-(methylthio)-3-tolyl] phosphorothioate (Baytex) in plants and its breakdown on plant surfaces upon exposure to sunlight and air, it became necessary to identify some of the products which occur under these conditions. Because heat applied to some phosphorothioic acid esters often gives the same products as does irradiation, the effects of heat on Baytex were examined.

Finegold (*2*) showed that the NMR (nuclear magnetic resonance) chemical shift values of P^{31} nuclei are unique for each type of phosphorus compound and vary markedly, depending upon the nature of the group on the phosphorus atom. Therefore, P^{31} NMR spectra can be used to detect and identify the presence of various types of phosphorus compounds in a mixture, provided their concentration exceeds 3 to 10%—the limit of the sensitivity of the instrument. However, P^{31} chemical shift values will differ only when there is a change in the molecular environment just adjacent to the phosphorus atom—e.g., the isomerization of a phosphorothionate to a phosphorothiolate, and no significant variation in the P^{31} signal is found when the change is two bonds removed from the phosphorus atom. Changes in substituents farther removed from phosphorus, however, can be detected by proton (H^1) magnetic resonance spectra, and both P^{31} and H^1 spectra of Baytex were examined after various intervals of heating at different temperatures.

The effect of heat on O,O-dimethyl O-[4-(methylthio)-3-tolyl] phosphorothioate (Baytex) was examined by P^{31} and proton magnetic resonance spectra. Proton spectra showed an initial formation of a sulfonium ion intermediate which then reacted further to give as the primary product the *S*-methyl isomer, O-methyl *S*-methyl [O-4-(methylthio)-3-tolyl] phosphorothioate. The formation of the *S*-methyl isomer from heated Baytex was supported by P^{31} resonance spectra.

Experimental Procedure

Baytex of 98.5% purity was obtained through the courtesy of Gerhard Schrader of Farbenfabriken Bayer, Leverkusen, Germany.

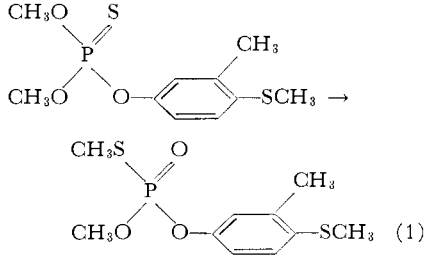
NMR spectra of Baytex were obtained on undiluted samples with a Varian V-4302 high resolution NMR spectrometer with associated V-4102-SM magnet system equipped with a VK-3506 flux stabilizer. Proton spectra were obtained at 56.4 mc. with 5% tetramethylsilane (TMS) as an internal standard. Phosphorus spectra were obtained at 24.3 mc. with phosphoric acid (85%) as an external reference in the annulus of a precision coaxial cell. All spectra were recorded with spinning samples, and the chemical shifts reported are the average values of at least four determinations, reproducible to ±0.5 c.p.s.

The esters were heated in 5-mm. o.d. borosilicate glass tubes, suitable for spinning in the spectrometer, in an air bath of ±1° C. The heating was interrupted at regular intervals, and NMR spectra were recorded.

Results

P^{31} Magnetic Resonance. The P^{31} spectrum of unheated Baytex shows a single peak at −65.8 p.p.m. from phosphoric acid. By spinning the sample, higher resolution was obtained, and this peak was split sevenfold because of spin-spin interaction with the six protons on the two methoxyl groups. This finding agrees with earlier work in which sevenfold splitting of the phosphorus peak was found with *cis*- and *trans*-isomers of methyl 3-(dimethoxyphosphinyloxy)crotonate (*3*). The tabulated value for compounds of the type $(RO)_3PS$ is −68.1 ± 1.2 p.p.m. in compounds where R is greater than methyl and is −73.0 p.p.m. for *O,O,O*-trimethyl phosphorothioate (*2*). The value for Baytex in which one of the R groups is 4-(methylthio)-2-tolyl is, then, consistent with these values.

Heating for 5 hours at 140° C. resulted in two P^{31} peaks—the original peak at −65.8 p.p.m. and a new one at a higher field of −25.9 p.p.m. The area of the −65.8 peak decreased during the heating period until it became about one half of the area of the new −25.9 peak, indicating that a marked change in the compound had occurred. Compounds of the type $(RO)_2(R'S)PO$, where R and R′ are greater than methyl, are reported to have chemical shifts in the region of −26.5 ± 0.3 (*2*). Apparently considerable isomerization of the phosphorothionate to the phosphorothiolate had occurred.



In another experiment, Baytex was exposed for several days in April sunlight, but there was no evidence of another phosphorus peak, indicating that the compound had not isomerized to the *S*-methyl isomer. This finding agrees with concurrent work using P^{32}-Baytex in which 2 to 3 days' exposure to April sunlight of a thin film gave no indication of the *S*-methyl isomer (*7*). The P^{32} work showed, however, that a considerable amount of oxidation had occurred, including the oxidation of the thionate sulfur to a phosphate ester. This change was not evident in the NMR spectrum and may be due to the considerably greater thickness of the film exposed for NMR work necessary because of the larger sample needed.

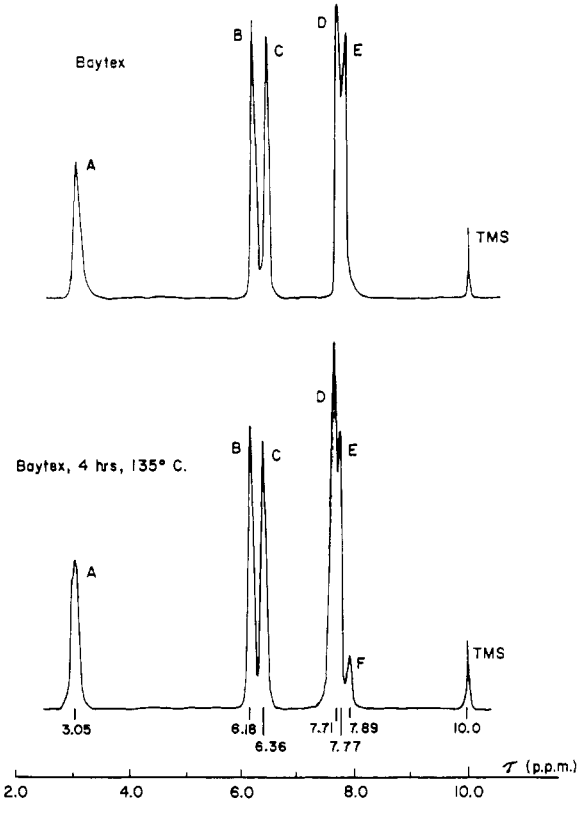


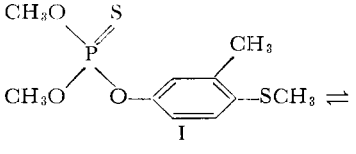
Figure 1. Proton spectra of Baytex and Baytex heated as indicated

Proton Magnetic Resonance. The proton spectrum of unheated Baytex shows five peaks—*A* to *E* in Figure 1. The three aromatic protons resonate at the same frequency at 3.05 p.p.m. from TMS (peak *A*). The six methoxy protons, spin coupled with the phosphorus nucleus, appear as the doublet *B-C* with a separation of 13.0 c.p.s. The H^1-P^{31} spin coupling constant in trimethyl phosphate has been reported as 11.19 ± 0.2 c.p.s. (*1*). The remaining two peaks, *D* and *E*, are assigned to

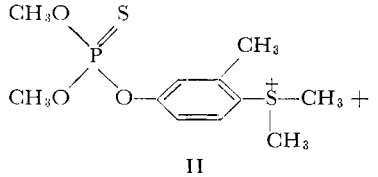
CH_3 and SCH_3 on the aromatic ring. Since in the heating experiments peak *E* remained constant and peak *D* increased in size relative to the other peaks, the more labile SCH_3 moiety is assigned peak *D*, and more stable CH_3, assigned peak *E*.

At 60° C., Baytex appears to be quite stable, and no change in the proton spectrum was observed even after 164 hours. Heating for 1 hour at 100° C. resulted in a slight change in the high field peaks (*D* and *E*)—peak *D* becoming slightly higher than *E;* no further change was noted after 2.5 hours. At 135° C., the first change, observable after 15 minutes, was also a slight increase in peak *D*. *D* continued to grow for another 30 minutes, then remained steady until the total heating period was 4.5 hours. At 45 minutes, the origin of a new peak (*F*) could be detected at low attenuation. *F* increased in size until it was about $^1/_6$ the height of the *B-C* doublet after $1^3/_4$ hours and remained at this size until after $4^1/_2$ hours, when it began to increase further.

The H^1 spectrum of Baytex after being heated for $4^1/_2$ hours at 135° C. is given in Figure 1. The increase in peak *D* and the formation of the new peak *F* is clearly evident. The spectrum is surprisingly "clean" at this point and remained so even after $5^1/_2$ hours, except for slight increases in peaks *D* and *F*. Because of the heightening in peak *D*, this resonance was assigned the $—SCH_3$ moiety, and the increase is probably due to an intermolecular reaction in which the sulfonium ion is formed.



I



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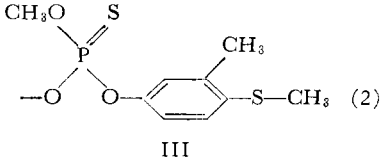
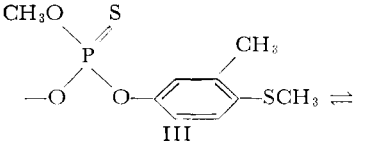


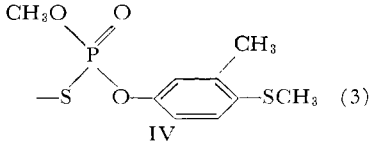
III (2)

An intramolecular mechanism is possible but unlikely because of the low probability of a cyclic transition state. Intermolecular alkylations of thioethers of type depicted in equation 2 are well known and have been described by Heath (*4*) for *O,O*-dimethyl *O*-(2-ethylthio)ethyl phosphorothioate.

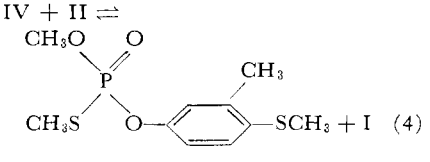
Because of the nearness of peak *F* to the phenyl-SCH_3 resonance (*D-F* separation about equal to the *B-C* doublet), *F* is probably due to the formation of the $PSCH_3$ moiety. Support for this belief is found in the P^{31} spectrum where a considerable quantity of the *S*-methyl isomer was indicated. The initial increase in peak *D* and subsequent formation of peak *F* would indicate that the isomerization may be proceeding through the sulfonium ion. The establishment of an equilibrium between the thionate and thiolate forms of the intermediate anion in equation 2 and subsequent methylation of the thiolate form by the sulfonium ion would lead to the *S*-methyl isomer.



III



IV (3)



(4)

This reaction is similar to that between metal salts of *O,O*-diethyl phosphorothioic acid and an alkyl halide which invariably results in the *S*-alkyl ester. The mechanism suggested here is analogous to that proposed by Hilgetag *et al.* (*5*) for the catalytic isomerization of *O,O,O*-trimethyl phosphorothioate by dimethyl sulfide. Evidence of decomposition appeared in the H^1 spectrum of $5^1/_2$ hours at 135° C. The aryl proton peak *A* became a doublet, probably due to a fission of the P—O—aryl linkage. A slight shoulder appeared on the low field side of peak *C* and became equal to *C* in $5^1/_2$ hours and larger than *C* in $6^1/_2$ hours. The splitting of *C* might be caused by a slight change in the chemical shift in the $POCH_3$ resonance due to gross changes in the rest of the molecule such as fission of the P—O—aryl bond. Furthermore, the *B-C* doublet began to decrease until it was smaller than the aryl proton peak *A* at $6^1/_2$ hours. A concurrent increase in the viscosity of the liquid sample caused decided loss in the resolution of the spectrum, with complete loss at $7^1/_2$ hours. The increase in viscosity plus the loss of methoxy proton resonance would indicate that polymerization was taking place, probably in the same manner as that suggested for methyl parathion (*6*) and *O,O,O*-trimethyl phosphorothioate (*5*).

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Correction

The Metabolism of Dimethoate by Vertebrate Tissues

In this article by Tetsuo Uchida, W. C. Dauterman, and R. D. O'Brien [J. AGR. FOOD CHEM. **12,** 48 (1964)], the guinea pigs were incorrectly identified. They should be described as male guinea pigs.