Fukami, J-I., Yamamoto, I., Casida, J. E., *Science* **155,** 713 (1967).
Haller, H. L., LaForge, F. B., *J. Amer. Chem. Soc.* **52,** 3207 (1930).
Hamada, M., Chubachi, M., *Agr. Biol. Chem.* **33,** 793 (1969).
Ivie, G. W., Casida, J. E., *Science* **167,** 1620 (1970).
Ivie, G. W., Casida, J. E., J. AGR. FOOD CHEM. **19,** 410 (1971).
Jones, H. A., Haller, H. L., *J. Amer. Chem. Soc.* **53,** 2320 (1931).
Kelly, D. P., Pinhey, J. T., *Tetrahedron Lett.* **46,** 3427 (1964).
LaForge, F. B., *J. Amer. Chem. Soc.* **54,** 3377 (1932).
LaForge, F. B., Smith, L. E., *J. Amer. Chem. Soc.* **51,** 2574 (1929).
Nishizawa, Y., Casida, J. E., J. AGR. FOOD CHEM. **13,** 522 (1965).
Rajagopalan, S., Seshadri, T. R., Varadarajan, S., *Proc. Indian Acad. Sci., Sect. A* **30,** 265 (1949).
Shepard, H. H., Campbell, F. L., *J. Econ. Entomol.* **25,** 142 (1932).
Subba Rao, N. V., Pollard, A. G., *J. Sci. Food Agr.* **2,** 462 (1951).
Takei, S., Miyajima, S., Ōno, M., *Ber.* **64,** 248 (1931).
Taub, D., Girotra, N. N., Hoffsommer, R. D., Kuo, C. H., Slates, H. L., Weber, S., Wendler, N. L., *Tetrahedron* **24,** 2443 (1968).
Unai, T., Yamamoto, I., Cheng, H-M., Casida, J. E., unpublished results (1972).
Yamamoto, I., Unai, T., Ohkawa, H., Casida, J. E., *Pestic. Biochem. Physiol.* **1,** 143 (1971).

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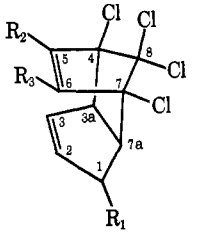
Photochemistry of Bioactive Compounds. Photochemical Reactions of Heptachlor: Kinetics and Mechanisms

Raymond R. McGuire,[1] Matthew J. Zabik,* Robert D. Schuetz, and Richard D. Flotard

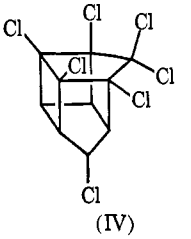
The product formation, reaction kinetics, and mechanisms of the photolysis of heptachlor (1,4,5,-6,7,8,8 - heptachloro - 3a,4,7,7a - tetrahydro - 4,7-methanoindene, I) have been investigated. Photolysis at 253.7 nm in hydrocarbon solvents yields two olefinic monodechlorination isomers (II, III) ($\Phi = 0.025$); at 300 nm in acetone, a 2 + 2 cycloaddition or cage compound (IV) is the exclusive product ($\Phi = 9.35 \times 10^{-5}$ based on total absorption of energy by acetone); and in mixed cyclohexane/acetone solutions at 300 nm, IV and a C-1 cyclohexyl adduct (V) are formed *via* a triplet and allylic free radical, respectively. A cage opening reaction of IV occurs at 200 nm to yield I ($\Phi = 0.195$). Photodechlorination to yield II and III occurs *via* excitation of the 5,6 double bond, most probably through a singlet state; while formation of IV and V occurs *via* a triplet mechanism involving the 2,3 double bond. A kinetic mechanism and specific rate constants are reported.

The products formed by the photolysis of heptachlor (I) (1,4,5,6,7,8,8 - heptachloro - 3a,4,7,7a - tetrahydro - 4,7-methanoindene) depend upon the reaction conditions. Irradiation of heptachlor at wavelengths below 260 nm in a nontriplet sensitizing solvent yields only a mixture of the two monodechlorination isomers 1,4,5,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene (II) and 1,4,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene (III) in equal amounts.



I: R_1 = Cl, R_2 = Cl, R_3 = Cl
II: R_1 = Cl, R_2 = Cl, R_3 = H
III: R_1 = Cl, R_2 = H, R_3 = Cl
V: R_1 = S, R_2 = Cl, R_3 = Cl

If the reaction is carried out at higher wavelengths (300 nm) in a triplet-sensitizing solvent such as acetone, the only product is a cage compound, 1,2,3,6,9,10,10-heptachloropentacyclo(5.3.0.$0^{2,5}$.$0^{3,9}$.$0^{4,8}$) decane (IV). Although this cage compound is also formed upon irradiation (300 nm) of heptachlor solutions in mixtures of acetone and cyclohexane, the principal product formed under these conditions is a substitution product (V) where the allylic chlorine of carbon-1 is replaced by a cyclohexyl (-S) group (McGuire *et al.*, 1970).



(IV)

Anderson *et al.* (1968) have investigated the dependence of photoproduct formation on the reaction conditions for systems analogous to heptachlor and have proposed a singlet transition state for the photodechlorination and a triplet state for cage formation. A study of the kinetics of these heptachlor photolyses should further delineate the mechanisms involved.

EXPERIMENTAL

Materials. A commercial sample of heptachlor, 25% by weight, was dissolved in acetone, filtered, and the solvent evaporated. The resulting solid was redissolved in *n*-hexane

Department of Entomology, Pesticide Research Center, Department of Chemistry, and Institute of Biology and Medicine, Michigan State University, East Lansing, Michigan 48823.
[1] Present address: F. J. Seiler Research Laboratories, U.S.A.F. Academy, Colorado Springs, Colorado.

and chromatographed on an activated alumina column. Aliquots were analyzed by vapor phase chromatography, and the pure heptachlor fractions were pooled and evaporated to dryness. Heptachlor purified in this manner was shown to be identical (when gas chromatographed using an electron capture detector) with a sample from City Chemical Corp. of 99+% purity ("ESA" Pesticide Reference Standard).

The *n*-hexane, cyclohexane, and acetone used in this study were "Distilled in Glass" solvents purchased from Burdick and Jackson Laboratories Inc. and were used without further purification. The benzophenone was purified by vacuum sublimation just prior to use.

Gas Chromatography. All gas chromatograms were obtained using a Beckman Model GC-4 gas chromatograph. An arc discharge electron capture detector was used for kinetic studies. The packing used throughout this study was a 11% DC-11 on 60/80 mesh Gas Chrom Q in a $^1/_8$-in. × 6-ft stainless steel column.

Irradiation Sources. Three sources of ultraviolet radiation were used in the course of this study. For exploratory irradiations and for kinetic determination at 200 nm, a high energy deuterium source for the Beckman DB-G spectrophotometer with an effective band width of 5.5 nm was used. This lamp yields about three times the energy of the normal hydrogen source used in this instrument and was found to be adequate for these irradiations. The irradiations at 300 nm were carried out in a Rayonet Photochemical Reactor manufactured by the Southern N. E. Ultraviolet Co. This reactor was equipped with filtered medium pressure lamps having a peak output of 300 nm. The final source was a NFU-300 low pressure mercury discharge lamp manufactured by the Nester-Faust Co. This lamp yields 96% of its total energy as a single line at 253.7 nm as measured with the DB-G. This lamp was further filtered by K_2CrO_4, K_2CrO_3 solutions as described by Wagner (1967) to give approximately 99% pure 253.7 nm radiation.

Quantum Yield. The quantum yield of each of the products was measured using a potassium trisoxalatoferrate (III) actinometer, according to the method described by Calvert and Pitts (1966).

Kinetics. Kinetic measurements were determined using the gas chromatograph. Under the conditions normally employed, 130°C column temperature and 40 ml/min helium flow, the photodechlorination isomers eluted in 2 min, heptachlor, 3 min, cage compound, 4.2 min, and the cyclohexyl adduct in 5.5 min from the solvent peak. The general procedure used in these rate measurements is as follows.

A sample of heptachlor, 1.0×10^{-4} *M*, dissolved in the solvent to be studied was pipetted into the reaction vessel. For irradiations at 300 nm spectronic "20" borosilicate sample tubes containing 6.0 ml each were used as reaction vessels. All other irradiations were carried out in silica DB cells equipped with septums and containing 3.7 ml of sample. A carrousel was used with the 253.7-mm source to ensure even irradiation of all samples. (The irradiation sources were all operated for an hour prior to exposure of the reaction mixture to allow them to stabilize.) A 0.5-μl sample of the starting material was injected into the gas chromatograph and the suppression voltage adjusted so as to give a peak height of 60 to 80% of full scale. One-half-microliter samples were then removed from the reaction flasks and analyzed by gas chromatography. The areas of the peaks were measured with a planimeter, normalized, and expressed as a percentage of the total peak area. This procedure was deemed to be valid since the reactions were allowed to proceed only to the point of initiating competing reactions. Kinetics were determined from a plot of the least squares line.

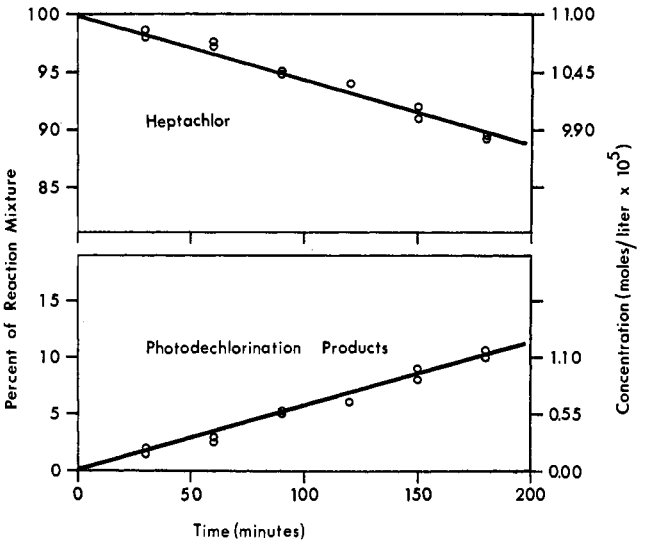
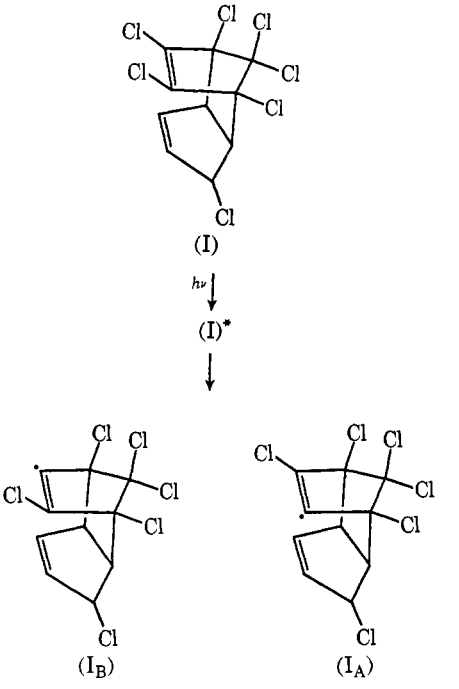


Figure 1. Rate of photodechlorination of heptachlor at 253.7 nm

RESULTS AND DISCUSSION

Photodechlorination. It has been previously reported (McGuire *et al.*, 1970) that the irradiation at 253.7 nm of a dilute solution of heptachlor (I) in hexane or cyclohexane yields, exclusively, a pair of monodechlorination isomers II and III in equal amounts. While these isomers can be separated by gas chromatography, they have been treated as a single product in the course of these kinetic studies. A plot of concentration *vs.* time shows the reaction to be zero order and to proceed with a quantum yield of 0.025 on 2% absorption of the incident light beam (Figure 1). This monodechlorination of heptachlor under the influence of ultraviolet radiation can be viewed as a simple nonchain free radical process. Heptachlor (I) is activated by high energy ultraviolet light (280 nm) to give the activated complex I*. The exact nature of I* is only speculation at this time. Although it is probably a singlet state, a high energy triplet cannot be elim-



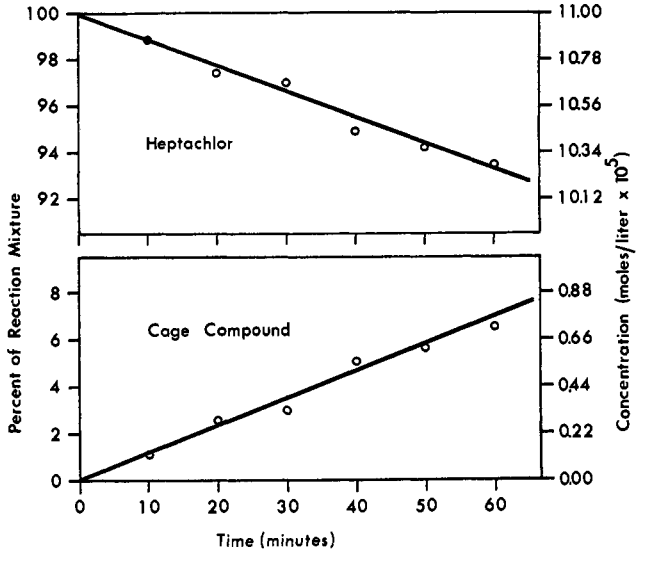


Figure 2. Rate of formation of cage compound in acetone at 300 nm

Table I. Zero-Order Rate Constants as a Function of Selected Acetone/Hexane Concentrations

Mole fraction acetone	Viscosity	"0" order rate constant $k^0 \times 10^{26}$ mol/l.-quantum		
		Cage formation	Adduct formation	Heptachlor decay
0.141	0.756	2.27	50.1	52.8
0.269	0.673	2.86	42.4	45.7
0.387	0.595	2.77	37.7	41.2
0.495	0.536	3.26	29.1	36.9
0.594	0.482	4.57	29.9	35.0
1.000	0.303	2.72	...	2.72

inated; this excited state can then decompose to yield either free radical I_A or I_B and a chlorine radical. Radicals I_A and I_B can then abstract a proton from the solvent cyclohexane (S) to give the monodechlorination isomers II and III, respectively, plus solvent radicals. The chlorine radicals can like-

$$I \xrightarrow{h\nu} (I^*) \longrightarrow I_A + I_B \quad (1)$$

$$I_A + S \longrightarrow II + S\cdot \quad (2)$$

$$I_B + S \longrightarrow III + S\cdot \quad (3)$$

wise abstract a proton from the solvent to give hydrogen chloride and a solvent radical. Finally, two cyclohexyl radicals can

$$Cl\cdot + S \longrightarrow HCl + S\cdot \quad (4)$$

combine to form bicyclohexyl.

$$2\,S\cdot \longrightarrow S-S \quad (5)$$

Hydrogen chloride and bicyclohexyl have been isolated and identified as reaction products. Processes such as those shown in eq 6 through 9

$$S\cdot \longrightarrow \text{cyclohexane} + H\cdot \quad (6)$$

$$Cl\cdot + I \longrightarrow I_A \text{ or } I_B + Cl_2 \quad (7)$$

$$S\cdot + I \longrightarrow I_A \text{ or } I_B + SCl \quad (8)$$

$$H\cdot + I \longrightarrow I_A \text{ or } I_B + HCl \quad (9)$$

are unlikely in that they would lead to a chain process and result in a quantum yield much higher than that observed (Φ = 0.025) if the initiation process was efficient.

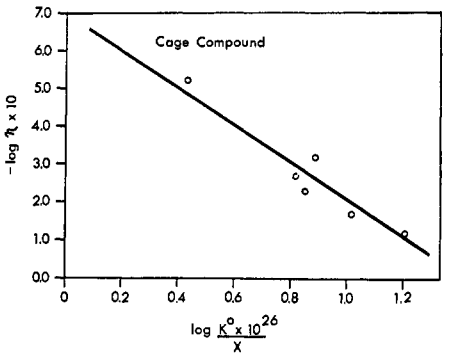


Figure 3. Effect of viscosity (η) on the rate of formation of cage compound

Cage Formation and Solvent Substitution. The irradiation at 300 nm of a 10^{-4} *M* solution of heptachlor in acetone proceeds smoothly, yielding the cage compound IV as the sole product during irradiation periods of up to 60 min (10% reaction). A plot of cage compound concentration *vs.* time gives a straight line, showing "0" order kinetics (Figure 2). The quantum yield for this reaction was determined to be 9.35×10^{-5} based upon the total absorption of the energy by the acetone.

The fact that the two types of photochemical reactions, photodechlorination and cage formation, do not occur together can be explained in either of two ways: that they proceed *via* different and distinct mechanisms and are unrelated, or that they are related by a very inefficient intersystem crossing, with the photodechlorination being the product of the singlet state. The results of the photolysis of heptachlor in mixtures of acetone and cyclohexane show that the reaction mechanisms are different and distinct.

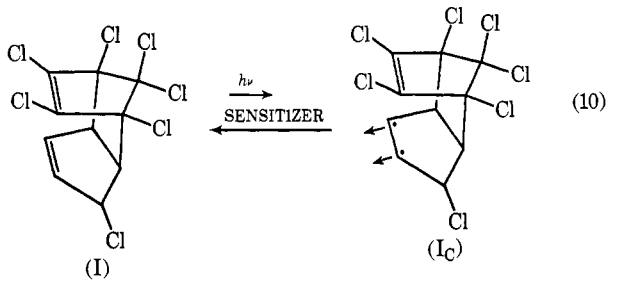
The photodecomposition of heptachlor proceeds rapidly in mixed cyclohexane/acetone solution (10 to 50% by volume acetone solutions) at 300 nm. Unexpectedly, the rate of decay of heptachlor is much greater in the mixed solvents (2.49×10^{-6} mol/l.-min for 10% acetone) than in pure acetone (1.21×10^{-7} mol/l.-min), and the major reaction product is not the cage compound (IV) but a solvent substitution product (V) where a cyclohexyl group replaces the allylic chlorine attached to carbon-1 (McGuire *et al.*, 1970). The rates of formation of cage compounds (IV) and cyclohexyl adduct (V) and the rate of decay of heptachlor (I) are summarized in Table I. Inspection of Table I shows that the values of the "0" order rate constants for the reaction in pure acetone are out of line with the other values.

Wagner and Kochevar (1968) have shown that for solutions of low viscosity ($k_g < k$ diffusion) where the sensitization step is rate-controlling or of the same order of magnitude as the rate-controlling step (this is not unlikely in this case where the sensitization is an inefficient process), the rates of reaction should be related to the amount of sensitizer (X) and the speed with which the reactant (heptachlor) comes in contact with the sensitizer (acetone), which is in turn related to the viscosity (η^n). Thus, if log of the rate constant or quantum efficiency divided by the mole fraction of sensitizer is plotted against the log of the viscosity, a straight line should result of slope n. That this is indeed the case can be seen in Figures 3 through 5. These figures show that the rate of cage formation depends upon $X^{1/2}$, while the rate of formation of the cyclohexyl adduct and the rate of decay of heptachlor depend on, approximately, $X^{1/3}$. Values for the viscosities of the binary mixtures were culated by the method of Kendall and Monroe (1917).

Alternately the effect may arise out of the better hydrogen-donating power cyclohexane relative to acetone. As the amount of cyclohexane goes up, the ability of acetone to produce ketyl radicals will increase and there also will be a better chance for cyclohexane to donate hydrogens to other intermediates. A similar effect has been reported in the literature (Wagner, 1966).

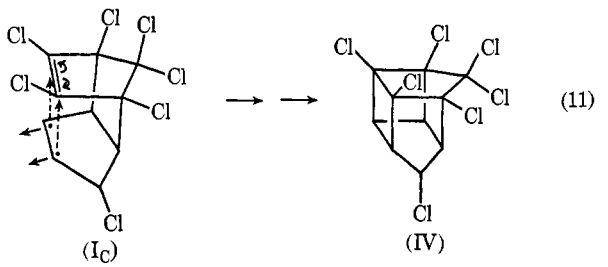
The formation of the solvent adduct at carbon-1 shows that the sensitized triplet reaction, or cage formation, involves excitation of the 2,3 double bond. Since the photodechlorination involves excitation of the 5,6 double bond, the two reactions are not closely related but proceed through two separate and discrete transition states. It is understandable, therefore, that the two reactions have not been found to occur simultaneously.

The sensitized triplet reaction of heptachlor to form cage compound or add solvent radical can be viewed as proceeding by a mechanism such as the following. Heptachlor (I) is activated by ultraviolet radiation (eq 10) through the sensitizer



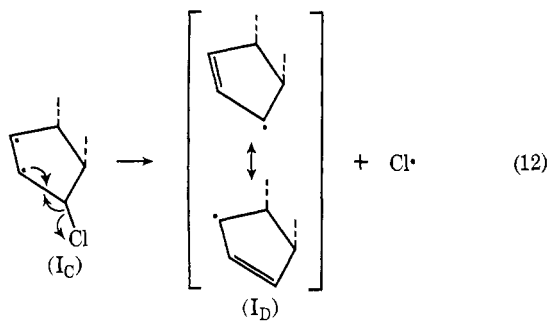
(I) (Ic) (10)

to form the triplet I_c, which has two product-forming pathways open: it can close to form the cage compound IV (eq 11), or



(Ic) (IV) (11)

it can eliminate a chlorine radical to form the stable allyl radical I_D (eq 12). The allyl radical (I_D) can be then react with



(Ic) (I_D) (12)

a solvent radical, formed by the abstraction of a proton by the chlorine radical, to form the solvent adduct V (eq 13, 14).

$$Cl\cdot + SH \longrightarrow HCl + S\cdot \quad (13)$$

$$I + S\cdot \longrightarrow V \quad (14)$$

This mechanism has also been written as a nonchain process because of the low quantum efficiency with which it proceeds.

Kinetic Mechanism For the Triplet Reaction. The above mechanism for the triplet reaction can be rewritten in a little different manner as follows:

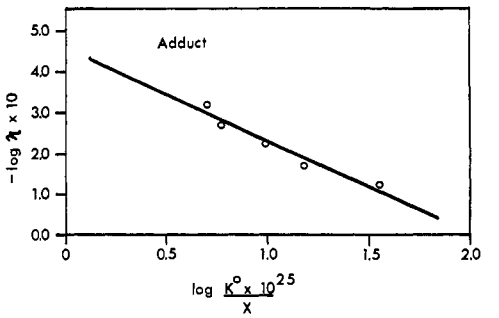


Figure 4. Effect of viscosity (η) on the rate of formation of adduct

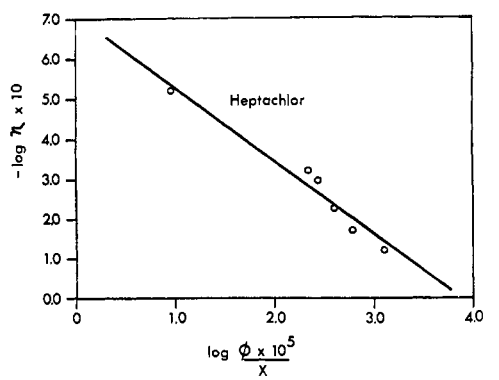


Figure 5. Effect of viscosity (η) on the quantum efficiency of heptachlor decay

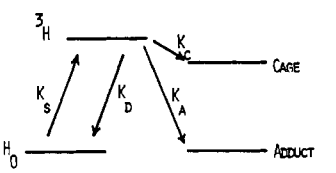


Figure 6. Kinetic diagram

(This diagram is not meant to indicate relative energy levels except in a qualitative and intuitive way.) The equations for each of the separate steps can be written:

$$H_0{}^k \xrightarrow{s} (H^3) \qquad \text{rate} = k_s (H_0) = R_s \quad (15)$$

$$(H^3)^k \xrightarrow{d} H_0 \qquad \text{rate} = k_d (H^3) = R_d \quad (16)$$

$$(H^3)^k \xrightarrow{c} \text{cage} \qquad \text{rate} = k_c (H^3) = R_c \quad (17)$$

$$(H\cdot{}^3)^k \xrightarrow{a} \text{adduct} \qquad \text{rate} = k_a (H^3) = R_a \quad (18)$$

where H_0 refers to the ground state heptachlor and H^3 is the excited triplet. (The rate constant for sensitization is dependent, to some extent, on the amount of sensitizer and the viscosity of the solution, as has been discussed previously.) If a steady state approximation is now applied to the triplet state, the concentration of the triplet state (H^3) can be expressed as

$$d(H^3)/dt = 0 = k_s - k_d (H^3) - k_a(H^3)\cdot \quad (19)$$

$$(H^3) = k_s/(k_a + k_c + k_c) \quad (20)$$

The rate of formation of cage compound (eq 17) can now be written as

$$R_c = k_s k_c/(k_a + k_d) \quad (21)$$

Table II. Specific Rate Constants for the Triplet Sensitized Photodecomposition of Heptachlor

% Acetone	10	20	30	40	50
k_s	1.24×10^{-3}	1.08×10^{-3}	9.70×10^{-4}	8.70×10^{-4}	8.25×10^{-4}
k_a	9.0×10^{-4}				
k_c	7.5×10^{-5}				
k_d	6.46×10^{-1}				

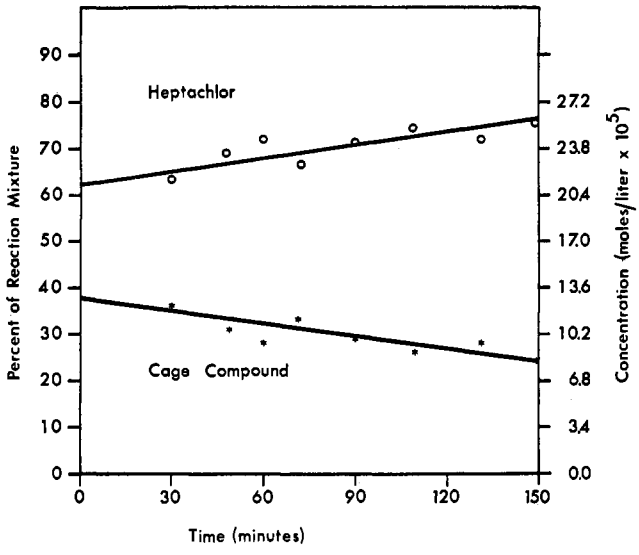


Figure 7. Rate of cage opening at 200 nm

In like manner the rate of adduct formation and the rate of decay of heptachlor can be written as

$$R_a = k_s k_a/(k_a + k_c + k_d) \quad (22)$$

$$R_H = k_s - k_d k_s/(k_a + k_c + k_d) \quad (23)$$

Dividing eq 21 by eq 22 gives eq 24,

$$R_c/R_a = k_c/k_a = 0.045 \quad (24)$$

where the values for the reaction with 10% acetone are used for R_c and R_a. Two additional terms must now be introduced: Φ_c, the quantum yield of cage compound, and Φ_H, the quantum efficiency of decay for heptachlor.

$$\Phi_c = k_c/(k_a + k_c + k_d) \quad (25)$$

$$\Phi_H = k_s/(k_s + k_a + k_c + k_d) \quad (26)$$

If it is now assumed that the rate of decay from the triplet to the ground state heptachlor is much faster than the other rates

$$k_d \gg k_s, k_a, k_c \quad (27)$$

then

$$\Phi_c \cong k_c/k_d \quad (28)$$

and therefore

$$\Phi_H \cong k_s/k_d \quad (29)$$

Now, dividing eq 28 by eq 29 and substituting the values obtained for the 10% acetone solution

$$\Phi_c/\Phi_H \cong \frac{k_c}{k_s} = \frac{8.25 \times 10^{-5}}{1.93 \times 10^{-3}} \cong 0.0427 \quad (30)$$

or

$$k_c \cong 0.0427 k_s \quad (31)$$

Now, since, $k_c = 0.045\, k_a$, from eq 24,

$$k_a = 0.0427/0.045 k_s \cong 0.946 k_s$$

and from eq 29

$$k_d \cong k_s/\phi_H = 518 k_s \quad (32)$$

All of the rate constants can be written in terms of k_s

$$k_d = 518 k_s \quad (33)$$

$$k_c = 0.427 k_s \quad (34)$$

$$k_a = 0.946 k_s \quad (35)$$

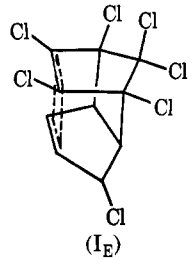
If these values are substituted into eq 23 and the rate determined for the 10% acetone solution is used for k_H, one obtains a value for k_s of

$$k_s = 1.243 \times 10^{-3} \text{ mol/l.-min} \quad (36)$$

The values of these constants for all the reactions carried out in mixed solvents (cyclohexane–acetone) are summarized in Table II.

Reversibility of Cage Formation. The irradiation of a mixture of cage compound and heptachlor in cyclohexane at 200 nm, without the addition of sensitizer, yielded a decrease in the concentration of the cage compound and a corresponding increase in the concentration of the heptachlor. This reaction proceeds at a rate of 3.22×10^{-7} mol/l.-min giving a quantum yield of 0.95 based on absorption of 2.3% of the available energy (Figure 7).

Although this is the first instance of a photolytic cage opening to be reported for a pesticide system, Hammond *et al.* (1964) have noted a similar phenomenon in the photoisomerization [2.2.1]bicycloheptane to [2.2.1.$0^{2,6}$.$0^{3,5}$]tetracycloheptane. In the case of the bicycloheptane system, the reaction seems to be a true equilibrium process and to be sensitized in both directions at the same wavelength. The lack of formation of any solvent adduct during the opening of the heptachlor cage compound and the fact that cage opening occurs at 200 nm while cage formation occurs at 300 nm indicates that this is not an equilibrium process but takes place by way of an excited stage other than I_C. The possibility of an excited state such as I_E cannot be discounted. The forward reaction, cage formation, cannot proceed through I_E since such an intermediate cannot explain the facile formation of the solvent adduct.



(I_E)

CONCLUSION

The two photochemical reactions of heptachlor, photodechlorination and cage formation, take place *via* two different and distinct mechanisms. The formation of cage compound can be reversed at higher energy. This reverse reaction, or cage opening, proceeds through an excited state which is different from that of the forward reaction, or cage formation.

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LITERATURE CITED

Anderson, C. M., Bremner, J. B., McCay, I. W., Warrener, R. N., *Tetrahedron Lett.* 1255 (1968).
Calvert, J. G., Pitts, Jr., J. N., "Photo Chemistry," Wiley, New York, N.Y., 1966, p 783.
Hammond, G. S., Wyatt, P., De Boer, C. D., Turro, N. J., *J. Amer. Chem. Soc.* **86,** 2532 (1964).
Kendall, J., Monroe, K. P., *J. Amer. Chem. Soc.* **39,** 1787 (1917).
McGuire, R. R., Zabik, M. J., Schuetz, R. D., Flotard, R. D., J. AGR. FOOD CHEM. **18,** 319 (1970).
Wagner, P. J., *J. Amer. Chem. Soc.* **88,** 5672 (1966).
Wagner, P. J., *Tetrahedron Lett.* 1752 (1967).
Wagner, P. J., Kochevar, I., *J. Amer. Chem. Soc.* **90,** 2232 (1968).

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Preforan Metabolism by Tobacco Cells in Suspension Culture

Raymond K. Locke* and Ronald L. Baron[1]

Preforan (*p*-nitrophenyl α,α,α-trifluoro-2-nitro-*p*-tolyl ether), labeled with ^{14}C in the C_1 position of the 4-nitrophenyl moiety or in the CF_3 group, was introduced into the medium of tobacco cells in suspension culture. After 15 days of incubation, recovery of added radioactivity varied between 52 and 76%. Of the recovered radioactivity, 60 to 80% was incorporated into the cells, with the remainder appearing in the medium or cell wash. No unchanged parent compound could be detected in any fraction. Radiolabeled Preforan incubated in cell-free medium was recovered unchanged. Metabolites present in cells and culture medium produced from $^{14}C_1$-labeled Preforan were characterized as conjugates of 4-nitrophenol, including probable glucoside and amino acid or protein conjugates, together with unidentified acidic conjugates. $^{14}C_1$-labeled 4-nitrophenol appeared in the medium but not in the cells. Metabolites present in cells and medium produced from $^{14}CF_3$-labeled Preforan probably represent ^{14}C incorporation into natural products, resulting from oxidation and cleavage of the $^{14}CF_3$ group from the parent compound.

Tobacco cells in suspension culture have previously been utilized in studies of DNA replication in higher plant cells (Filner, 1965), in studies of enzyme synthesis, regulation, and activation (DeJong *et al.*, 1967, 1968; Filner, 1966; Filner and Varner, 1967), and in studies of amino acid metabolism (Olson, 1964). Kemp and Sutton (1971) investigated the rates of protein synthesis, accumulation, and degradation in callus cultures of tobacco cells. More recently, tobacco cells in suspension culture have been used in studies of carbaryl (1-naphthyl *N*-methylcarbamate) metabolism (Locke *et al.*, 1971). The same techniques were used to study the metabolism of Preforan [fluorodifen; C-6989; 4-nitrophenyl 2-nitro-4-(trifluoromethyl)phenyl ether; *p*-nitrophenyl α,α,α-trifluoro-2-nitro-*p*-tolyl ether], a relatively new herbicide. Because residues of this herbicide may appear on crops destined for human consumption, a knowledge of the nature of the residue complex is essential.

The β-D-glucoside of 4-nitrophenol has been proposed as a major metabolite of Preforan in soybean and maize seedlings cultured in nutrient solution (Geissbühler *et al.*, 1969). Rogers (1971) reported that the metabolism of Preforan in soybean seedlings grown under similar conditions primarily involved a cleavage of the diphenyl ether linkage, resulting in degradation products yielding 4-nitrophenol upon acid hydrolysis. Cleavage of the diphenyl ether linkage of Preforan to yield unconjugated 4-nitrophenol has been reported in peanut seedlings, although the nature of the conjugated products produced was not investigated (Eastin, 1971).

The purpose of the present study was to determine the metabolites produced from Preforan by an established plant cell line, and to compare the *in vitro* metabolites produced with those reported in whole plant studies.

Plant cell culture techniques possess certain advantages over whole plant studies, especially with regard to the relative ease of growth, treatment, and isolation and purification of metabolites. A rapid *in vitro* system reflecting plant metabolism would greatly aid in indicating those pesticides whose residue complexes might pose a toxicological hazard to man.

MATERIALS AND METHODS

The XD cell line of *Nicotiana tabacum* L. var. Xanthi, the chemically defined M-1D medium, and the procedure used in subculturing have been previously described (Filner, 1965; Locke *et al.*, 1971). This cell line proliferates well in a chemically defined medium; therefore, in metabolism studies,

Division of Pesticide Chemistry and Toxicology, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D.C. 20204.
[1] Present address: Environmental Protection Agency, Washington, D.C. 20460.