Nature and Toxicity of Two Oxychlordane Photoisomers

Oxychlordane is converted to two major photoproducts when exposed to sunlight as deposits on silica gel chromatoplates. These derivatives are methylene-bridged isomers, one containing a keto group generated by photochemical cleavage of the oxychlordane oxirane ring and the other with an intact epoxide moiety. Their formation is greatly accelerated by xanthone photosensitizer. Oxychlordane and its keto photoisomer are quite toxic to white mice, but the second isomer is of very low toxicity.

Several chlorinated cyclodiene insecticides undergo rearrangement to methylene-bridged, birdcage isomers when exposed to sunlight or artificial light. In some cases the conversions are to derivatives of enhanced toxicity, and thus the possible occurrence of the photoisomers in the environment must be carefully considered in any evaluation of the potential hazards of these toxicants. Cyclodienes reported to undergo photochemical isomerizations include aldrin (Rosen and Sutherland, 1967), dieldrin (Robinson *et al.*, 1966; Rosen *et al.*, 1966), heptachlor, isodrin (Rosen *et al.*, 1969), endrin (Rosen *et al.*, 1966), heptachlor epoxide (Benson *et al.*, 1971; Fischler and Korte, 1969; Ivie *et al.*, 1972), *cis*-chlordane (Benson *et al.*, 1971; Fischler and Korte, 1969), *trans*-chlordane, and *trans*-nonachlor (Ivie *et al.*, 1972). For the most part, these conversions proceed slowly, but they are accelerated by certain chemicals which act as photosensitizers (Ivie and Casida, 1971a; Rosen and Carey, 1968; Rosen and Siewierski, 1970; Rosen *et al.*, 1969).

Oxychlordane (IV, Figure 1) is a metabolite of *cis*-chlordane (I) and *trans*-chlordane (II) in some animal systems (Schwemmer *et al.*, 1970), and it is a major product excreted in the milk of cows following dietary exposure to technical chlordane (Lawrence *et al.*, 1970) or its purified isomers (Dorough, 1973). Each of the two major chlordane isomers is metabolized to oxychlordane, and a dichlorochlordene intermediate (III, Figure 1) is apparently involved in these transformations. Oxychlordane has also been found as a metabolite in alfalfa, but it is apparently not generated in a number of other crop species (Oloffs, 1973).

Oxychlordane is considerably more toxic to mammals than the parent *cis*- and *trans*-chlordanes (Whitacre, 1973) and thus its occurrence in the environment is of toxicological significance. The environmental degradation of oxychlordane may be of more limited importance due to the mechanisms by which it is generated, but its photochemistry is also of interest because potential photoisomerization reactions involving a dichlorocyclopentene oxide ring structure present in oxychlordane have not been reported for this series of compounds.

MATERIALS AND METHODS

Chemicals and Analytical Procedures. Oxychlordane (>98% purity) and certain other cyclodienes and derivatives used in these studies were supplied by Velsicol Chemical Corp., Chicago, Ill.

Thin-layer chromatography (tlc) was accomplished using silica gel F-254 precoated chromatoplates (0.25 mm gel thickness, Merck AG, Darmstadt, Germany); chlorinated compounds on the developed plates were visualized by diphenylamine reagent as previously described (Ivie and Casida, 1971b). Infrared (ir) spectra were taken as 2% potassium bromide pellets on a Beckman IR-16A spectrophotometer. Mass spectra (ms) were recorded on a Hitachi Perkin-Elmer RMU-6D spectrometer, and proton magnetic resonance (pmr) studies were conducted in deuterated chloroform on a JEOL Model JNM-MH 100 instrument, using tetramethylsilane (TMS) as an internal reference. Chemical shifts are reported as ppm downfield from TMS. Melting points (uncorrected) were taken in open capillaries with a Tottoli-type melting point apparatus.

Preparation and Isolation of Photoproducts. A mixture of 2.5 mmol each of oxychlordane and xanthone in 50 ml of chloroform was streaked over the surface of several 0.25-mm silica gel chromatoplates to a concentration of about 0.2 mg of oxychlordane/cm^2. The plates were exposed to direct sunlight for 24 hr over a period of 3 days; then the gel was scraped and the products were extracted with chloroform. Tlc revealed two major photoproducts (V and VI) in addition to the parent compound. R_f values (hexane–chloroform, 8:1) were as follows: oxychlordane, 0.48; product V, 0.36; product VI, 0.26. Tlc of oxychlordane samples exposed to sunlight in an identical manner as above but without xanthone sensitizer showed very minor conversion to products V and VI, indicating that the reaction rates are greatly accelerated by the presence of photosensitizer.

The photoproducts from xanthone sensitization were isolated by Florisil column chromatography (elution with hexane) to yield 8% V, 45% VI, and 12% recovery as unreacted oxychlordane. The material not accounted for (35%) probably reflects volatility loss of oxychlordane and/or its two major photoproducts from the gel because tlc did not indicate the presence of appreciable quantities of other derivatives. The two photoproducts were crystallized once from boiling hexane to give tlc-pure photoproducts V (mp 167.0–168.5°) and VI (mp 202.0–203.5°).

Toxicity Tests. The mammalian toxicity of compounds was evaluated by stomach tube delivery in 0.1 ml of corn oil solution to lightly etherized male white mice (Swiss-Webster strain, weight approximately 20 g, Camm Research Institute, Wayne, N. J.). Mortality determinations were made 72 hr after treatment.

RESULTS AND DISCUSSION

Nature of Oxychlordane Photoproducts. The ms of product V exhibits a small molecular ion at m/e 420 (Cl = 35), and the fragmentation patterns of the product and oxychlordane are very similar, indicating the two compounds to be isomeric. Elemental analysis is consistent with the assignment of product V as an oxychlordane isomer. Calculated for $C_{10}H_4Cl_8O$: C, 28.34; H, 0.95; Cl, 66.93. Found for product V: C, 28.22; H, 1.05; Cl, 66.09. Pmr of the product integrates four protons, doublets centered at 2.78 and 3.98 ppm, a multiplet at 4.30 ppm, and a low-field singlet at 5.90 ppm, which is suggestive of methylene-bridge formation at the expense of the dichloroethylene moiety. Ir of the product confirms loss of unsaturation, as evidenced by lack of absorption at 1600 cm^{-1}, and the absence of carbonyl or hydroxyl absorption indicates that the epoxide remains intact. Two isomeric structures are proposed for V which seem most probable based on the available data (Va or Vb, Figure 1). Structure Va is preferred for the product because bridging as in Vb would require a highly strained epoxide ring. The structural assignment of Va is also supported by similar photoisomerizations of *trans*-chlordane and *trans*-nonachlor which involve bridging at equivalent chloromethy-

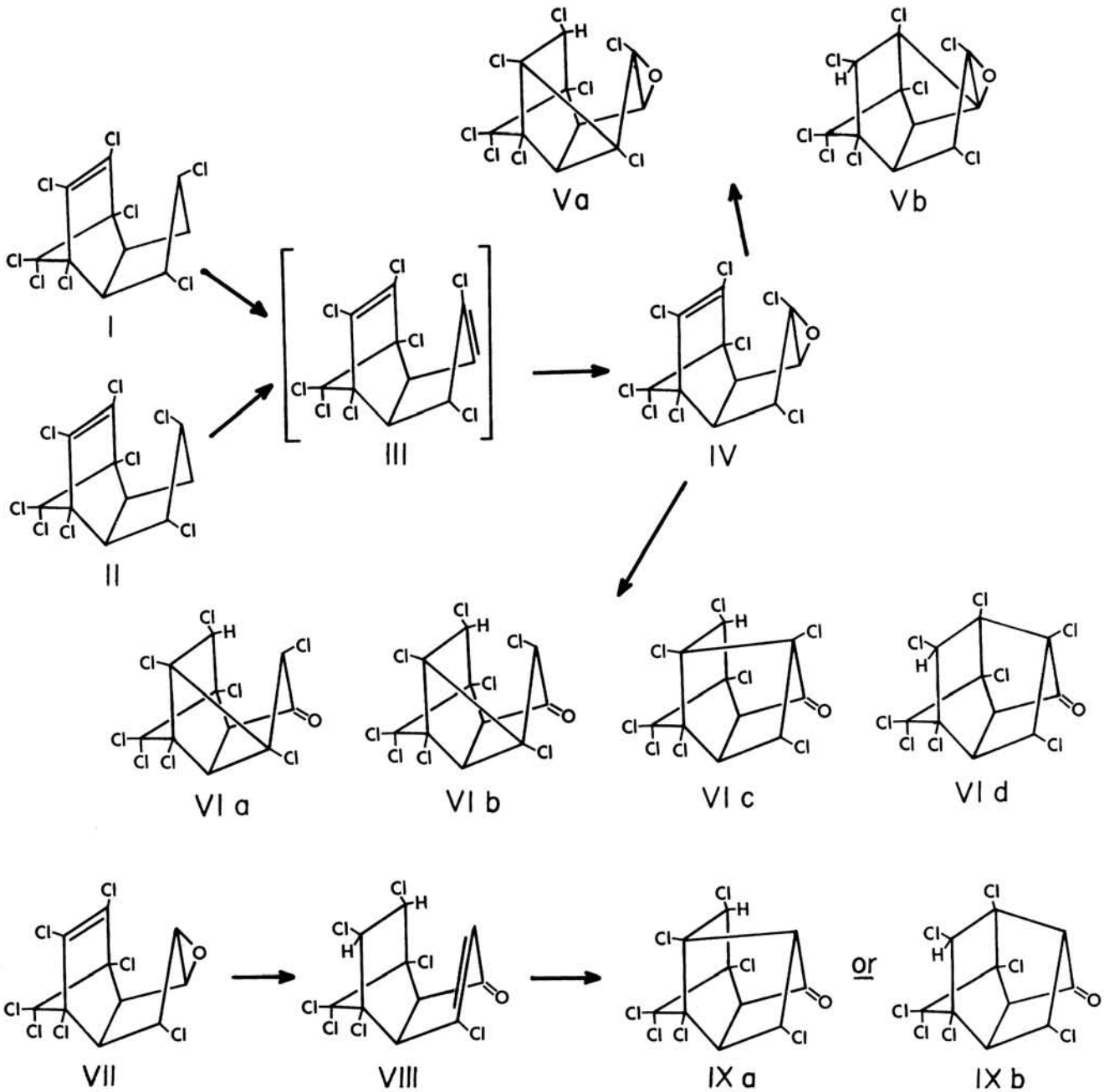

Figure 1. Structures of cyclodienes and their possible photoisomers. I, 1α,2α,4β,5,6,7β,8,8-Octachloro-3aα,4,7,7aα-tetrahydro-4,7-methanoindan; II, 1α,2β,4β,5,6,7β,8,8-octachloro-3aα,4,7,7aα-tetrahydro-4,7-methanoindan; III, 1α,2,4β,5,6,7β,8,8-octachloro-3aα,-4,7,7aα-tetrahydro-4,7-methanoindene; IV, 1α,2β,4β,5,6,7β,8,8-octachloro-2,3α-epoxy-3aα,4,7,7aα-tetrahydro-4,7-methanoindan; Va, 1α,1aα,2,2,3α,5β,5aα,6*R**-octachloro-4α,5-epoxy-1a,2,3,3aα,4,5,5a,5bα-octahydro-1,3-methano-1*H*-cyclobuta[*cd*]pentalene; Vb, 1α,1aα,2,2,3α,4α,5β,6*S**-octachloro-5,5aα-epoxy-1a,2,3,3aα,4,5,5a,5bα-octahydro-1,3-methano-1*H*-cyclobuta[*cd*]pentalene; VIa, 1α,1aα,2,2,3α,5α,5aα,6*R**-octachloro-1,1a,2,3,3aα,5,5a,5bα-octahydro-1,3-methano-4*H*-cyclobuta[*cd*]pentalen-4-one; VIb, 1α,1aα,-2,2,3α,5β,5aα,6*R**-octachloro-1,1a,2,3,3aα,5,5a,5bα-octahydro-1,3-methano-4*H*-cyclobuta[*cd*]pentalen-4-one; VIc, 2β,3,3,3aβ,4β,-5β,6α,8*S**-octachloro-1,2,3,3a,4,5,6,6aβ-octahydro-1α,5:2,4-dimethanopentalen-7-one; VId, 1α,2α,5α,6,6,6aα,7*R**,8*R**-octachloro-1,3aα,4,5,6,6a-hexahydro-1,5:2,4β-dimethanopentalen-3(2*H*)-one; VII, 1α,4β,5,6,7β,8,8-heptachloro-2α,3α-epoxy-3aα,4,7,7aα-tetrahydro-4,7-methanoindan; VIII, 3,4β,5α,6α,7β,8,8-heptachloro-3aα,4,5,6,7,7aα-hexahydro-4,7-methanoinden-1-one; IXa, 2β,3,3,3aβ,-4β,6α,8*S**-heptachloro-1,2,3,3a-4,5,6,6aβ-octahydro-1α,5α:2,4-dimethanopentalen-7-one; IXb, 1α,5α,6,6,6aα,7*S**,8*S**-heptachloro-1,3aα,4,5,6,6a-hexahydro-1,5:2β,4β-dimethanopentalen-3(2*H*)-one.

lene carbons (Ivie *et al.*, 1972). The relatively low yields of product V obtained in these studies may be attributed both to the endo configuration of the chlorine on the center carbon of the cyclopentene oxide ring, which hinders bridge formation at the adjacent carbon, and to the reactivity of the oxirane ring to generate the major photoproduct (VI).

Product VI is also isomeric with oxychlordane, based on ms (molecular ion at m/e 420), pmr (4 protons at 3.35, 3.70, 4.43, and 4.55 ppm), and elemental analysis (found: C, 28.22; H, 1.05; Cl, 66.08). The absence of ir absorption at 1600 cm^{-1} indicated the loss of the dichloroethylene moiety, while a strong absorption at 1795 cm^{-1}, characteristic of a ketone, was observed. Ketone formation, by cleavage of the epoxide, would most likely permit reversal in orientation of the *endo*-chlorine α to the carbonyl, and thus four structures can be considered for VI (Figure 1). Structures VIa and VIb seem least probable since they involve formation of four-membered rings, as compared to structures VIc and VId, which involve formation of more sterically favored five-membered rings.

Heptachlor epoxide (VII) undergoes photoisomerization

Table I. Acute Oral Toxicity of Cyclodienes and Photoisomers to Male Swiss-Webster Mice

Compound	LD_{50}, mg/kg
cis-Chlordane	125
trans-Chlordane	275
Oxychlordane	40
Photooxychlordane (V)	>500
Photooxychlordane (VI)	50

to a methylene-bridged ketone (IX) similar in spectral properties and structure to the major oxychlordane photoisomer VI (Ivie *et al.*, 1972). These two products are apparently not generated by similar reaction mechanisms, however, because a stable intermediate photoisomer (VIII) is produced in the conversion of heptachlor epoxide to IX, while no evidence was obtained of the existence of a similar intermediate in the conversion of oxychlordane to VI. Thus, if oxychlordane photoisomerization to the ketone VI involves an intermediate, it is of such short-lived nature as to be undetectable under the exposure conditions employed.

The heptachlor epoxide photoisomer isolated in previous studies was assigned IXa as the preferred structure (Ivie *et al.*, 1972), but this assignment is currently under reconsideration (Knox *et al.*, 1973). However, it seems very likely that VI and IX have the same bridging pattern because VI and its alcohol derivative obtained by lithium aluminum hydride reduction give pmr chemical shifts and coupling patterns very similar to IX and its alcohol, indicating that the compounds probably have identical carbon skeletons. [After the current studies were underway, it was learned that an isomeric derivative of oxychlordane had earlier been prepared in the laboratories of Velsicol Chemical Corp. by irradiation of oxychlordane in acetone with short wavelength ultraviolet light (Schwemmer, 1973). A sample of this compound exhibited identical spectral behavior to product VI reported here.]

The different modes of photoisomerization observed with oxychlordane and the closely related heptachlor epoxide must be attributed to the *endo*-chlorine of the oxychlordane cyclopentene oxide ring. Oxychlordane shows a degree of resistance to photochemical cleavage of the oxirane ring not observed with heptachlor epoxide, as evidenced by the isolation of a sterically unfavorable methylene-bridged isomer (V) containing the intact epoxide. The oxirane ring of heptachlor epoxide is apparently more labile, because no photoisomeric derivative has been observed which contains this moiety intact.

Toxicology. Oxychlordane and its major photoisomer (VI) are quite toxic to mice and are considerably more potent than either *cis*- or *trans*-chlordane (Table I). The minor oxychlordane photoisomer (V) is essentially nontoxic, which is somewhat surprising in view of the fact that epoxidation in the chlorinated cyclodiene series is generally accompanied by high mammalian toxicity.

Oxychlordane is a relatively minor environmental derivative of chlordane and the current study indicates that it isomerizes slowly in the absence of photosensitizer. Although one of the oxychlordane photoisomers is a toxic compound, it seems doubtful that these products will be generated in sufficient quantity in the environment to necessitate critical toxicological studies.

ACKNOWLEDGMENT

The author thanks Darcy Rushing of this laboratory for technical assistance and Robert Stipanovic, National Cotton Pathology Research Laboratory, USDA, ARS, College Station, Tex., for assisting in the spectral studies. Aid in nomenclature of these compounds was provided by Kurt Loening, Chemical Abstracts Service, Ohio State University, Columbus, Ohio. The cooperation of David Whitacre, Velsicol Chemical Corp., Chicago, Ill., is gratefully acknowledged.

LITERATURE CITED

Benson, W. R., Lombardo, P., Egry, I. J., Ross, R. D., Barron, R. P., Mastbrook, D. W., Hansen, E. A., *J. Agr. Food Chem.* **19**, 857 (1971).
Dorough, H. W., University of Kentucky, Lexington, Ky., private communication, 1973.
Fischler, H. M., Korte, F., *Tetrahedron Lett.* **32**, 2793 (1969).
Ivie, G. W., Casida, J. E., *J. Agr. Food Chem.* **19**, 405 (1971a).
Ivie, G. W., Casida, J. E., *J. Agr. Food Chem.* **19**, 410 (1971b).
Ivie, G. W., Knox, J. R., Khalifa, S., Yamomoto, I., Casida, J. E., *Bull. Environ. Contam. Toxicol.* **7**, 376 (1972).
Knox, J. R., Khalifa, S., Ivie, G. W., Casida, J. E., University of California, Berkeley, Calif., unpublished data, 1973.
Lawrence, J. H., Barron, R. P., Chen, J.-Y. T., Lombardo, P., Benson, W. R., *J. Ass. Offic. Anal. Chem.* **53**, 261 (1970).
Oloffs, P. C., Simon Fraser University, Burnaby, B. C., Canada, private communication, 1973.
Robinson, J., Richardson, A., Bush, B., Elgar, K. E., *Bull. Environ. Contam. Toxicol.* **1**, 127 (1966).
Rosen, J. D., Carey, W. F., *J. Agr. Food Chem.* **16**, 536 (1968).
Rosen, J. D., Siewierski, M., *J. Agr. Food Chem.* **18**, 943 (1970).
Rosen, J. D., Sutherland, D. J., *Bull. Environ. Contam. Toxicol.* **2**, 1 (1967).
Rosen, J. D., Sutherland, D. J., Khan, M. A. Q., *J. Agr. Food Chem.* **17**, 404 (1969).
Rosen, J. D., Sutherland, D. J., Lipton, G. R., *Bull. Environ. Contam. Toxicol.* **1**, 133 (1966).
Schwemmer, B., Velsicol Chemical Corp., Chicago, Ill., private communication, 1973.
Schwemmer, B., Cochrane, W. P., Polen, P. B., *Science* **169**, 1087 (1970).
Whitacre, D. L., Velsicol Chemical Corp., Chicago, Ill., private communication, 1973.

G. Wayne Ivie

Veterinary Toxicology and Entomology Research Laboratory
U. S. Department of Agriculture
Agricultural Research Service
College Station, Texas 77840

Received for review April 9, 1973. Accepted August 1, 1973.