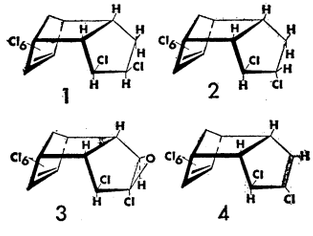
Oxychlordane, Animal Metabolite of Chlordane: Isolation and Synthesis

Abstract. *Oxychlordane ($C_{10}H_4Cl_8O$), a minor heretofore unidentified metabolite, was isolated from fat of pigs on diets heavily dosed with pure isomers of the insecticide, chlordane ($C_{10}H_6Cl_8$). Chemical and spectroscopic evidence provides bases for proposal of structure of the metabolite as 1-*exo*-2-*endo*-4,5,6,7,8,8-octachloro-2,3-*exo*-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene.*

While chlordane (**2**) is largely metabolized to hydrophilic products (*1*), there is evidence that an unidentified nonpolar metabolite is stored in the fat of rats fed massive doses of certain pure chlordane isomers (*2*). We now report isolation, identification, and synthesis of the previously unrecognized compound.

The source was fat of pigs fed 90 days on diets to which pure α-chlordane (**1**) or γ-chlordane (**2**) was added, the final concentration being 300 ppm.



Metabolites from each of the respective isomers in the feed were isolated as follows. Fat (16 kg) was homogenized in a blender and extracted with ether; the ether extract was filtered, and the ether was removed by evaporation. The concentrate was partitioned five times between hexane and acetonitrile (300 g of fat per 500 ml of hexane and 500 ml of acetonitrile). The acetonitrile layer was retained and treated with fresh hexane. Excess acetonitrile was removed by heating under reduced pressure on a steam bath. The resulting concentrate was purified by chromatography on a Davidow column (Celite, H_2SO_4, fuming H_2SO_4) (*3*). The eluate, taken to dryness, was a mixture of the metabolite with a relatively small amount of the administered unchanged chlordane isomer. This concentrate was dissolved in a minimum volume of pentane and chromatographed through a Florisil column. The first eluate contained a large amount of metabolite, which was recovered at about 80 percent purity upon removal of the solvent. Recrystallization from pentane yielded the metabolite of 96 percent purity, melting at 99.0° to 101.0°C (uncorrected).

Isolated products from the feeding of either α-chlordane or γ-chlordane are identical (*4*) with respect to melting point, infrared and nuclear magnetic resonance (NMR) spectra, behavior on gas chromatography and thin-layer chromatography, and *p*-values (*5*). The metabolite is assigned the molecular formula, $C_{10}H_4Cl_8O$, on the basis of elemental analysis and is referred to as oxychlordane (**3**).

The oxychlordane isolated from feeding γ-chlordane was optically active, $[\alpha]_D^{25}$ being +2.7°; no optical activity was observed in either the small available sample of the isolated alpha-form or in the synthesized compound.

Oxychlordane was synthesized independently in three laboratories (*6*) via oxidation of 1-*exo*-2-dichlorochlordene-2 (**4**) (*7*). A 60 percent yield of oxychlordane was obtained by 90-minute treatment of 1 g of **4** in 30 ml of acetic acid with 3 g of CrO_3 and 5 ml of H_2O at 100°C. The mixture was diluted with 300 ml of water, extracted with hexane, washed with distilled water, dried with Na_2SO_4, and freed of solvent by evaporation. Recrystallization from pentane yielded a product identical in properties (melting point, infrared and NMR spectra, gas chromatography, and thin-layer chromatography) with those isolated from metabolism of chlordane.

Other reactions were capable of producing oxychlordane in moderate yields, as indicated by analysis of reaction mixtures. Oxidation of **4** with *m*-chloroperbenzoic acid yielded about 15 percent oxychlordane. Direct oxidation of **1** by treatment with chromic acid, as described above, yielded about 1 percent oxychlordane. Oxidation of **2** in the same manner yielded 10 percent of oxychlordane, confirmed by isolation.

Structure **4** appears to be formed as an intermediate in the direct oxidation of **2** with chromic acid. A substance in the reaction mixture (<0.2 percent) with retention time identical to that of **4** was concentrated (to about 30 percent) and chlorinated in CCl_4 saturated with Cl_2, to prove its identity. The products of reaction gave, on gas chromatography, retention times (two columns) identical to that from the same treatment of authentic **4**. An indication of the 1 : 2 nature of the epoxide group in oxychlordane was obtained when reaction with triphenyl phosphine produced **4** in small yield (~ 12 percent).

On the basis of infrared and NMR spectra and synthetic reactions, the structure of the oxychlordane is considered to be 1-*exo*-2-*endo*-4,5,6,7,8,8-octachloro-2,3-*exo*-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene (**3**). The infrared spectrum showed bands at 1605 cm^{-1} (ClC=CCl); 1250 cm^{-1}, 910 cm^{-1}, 822 cm^{-1} (epoxy group); 3045 cm^{-1} (epoxymethine group); there was no band at 1445 cm^{-1} (CH_2). The NMR spectrum shows a multiplet at 3.20 to 3.65 ppm (2H; H-C-C-H); a singlet, 3.85 ppm (1H; epoxy); and doublet, 4.30 to 4.40 ppm (1H; H-C-Cl). The mass spectrum gave ion peaks at *m/e* 420 (M^+); 385 (M-Cl); 261, 251, 235, and 185 (base peak), together with retrodiene fragments at *m/e* 270 and 150.

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References and Notes

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5. The *p*-value is the fraction of solute in the nonpolar phase when the solute has been distributed between equal volumes of two immiscible solvents [M. Beroza and M. C. Bowman, *Anal. Chem.* **37**, 291 (1965)].
6. This paper reports jointly on the work in two laboratories, Velsicol and Canada Department of Agriculture; results from a third laboratory, U.S. Food and Drug Administration, were published after our manuscript was submitted [J. H. Lawrence, R. P. Barron, J.-Y. T. Chen, P. Lombardo, W. R. Benson, *J. Ass. Offic. Anal. Chem.* **53**, 261 (1970)].
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8. We thank, at the Velsicol Laboratory, C. Paluch and A. Rozek for spectroscopic interpretation, J. Benziger and J. Gausas for confirmatory gas chromatography and thin-layer chromatography, and J. Long for technical assistance; and at the Canada Department of Agriculture, E. C. Martelock for technical assistance. The research was suggested by the Commission on Terminal Pesticide Residues, International Union of Pure and Applied Chemistry.

26 February 1970; revised 31 July 1970