Dieldrin-[14] C Metabolism in Sheep

Identification of *trans*-6,7-Dihydroxydihydroaldrin and 9-(*syn*-epoxy)Hydroxy-1,2,3,4,10,10-Hexachloro-6,7-Epoxy-1,4, 4*a*,5,6,7,8,8*a*-Octahydro-1,4-*Endo*-5,8-*Exo*-Dimethanonaphthalene

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Two metabolites extracted from sheep urine with hexane have been identified. One metabolite, *trans*-6,7-dihydroxydihydroaldrin, was identified by spectral comparison with an authentic sample, while 9-(*syn*-epoxy)hydroxy-1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo*-5,8-*exo*-dimethanonaphthalene was identified by spectral and chemical considerations. The latter compound had previously been identified as either 4*a*- or 5-hydroxy-1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo*-5,8-*exo*-dimethanonaphthalene.

Dieldrin has been the subject of numerous metabolic studies, but only a few of these studies identified the metabolic products. Korte and Arent (1965) identified *trans*-6,7-dihydroxydihydroaldrin (trans diol), as well as its conjugates from rat urine. Tomlin (1968) identified the trans diol as a metabolite of larvae of *Culex pipiens quinquefasciatus* Say; Wedemeyer (1968) identified it from a culture of *A. aerogenes* containing dieldrin. Richardson *et al.* (1968), and Matthews and Matsumura (1969) isolated an "oxidized dieldrin" from rat feces. Klein *et al.* (1968), Damico *et al.* (1968), Richardson *et al.* (1968), and Matthews and Matsumura (1969) isolated and identified an oxidized, dechlorinated metabolite that no longer had an intact dieldrin ring system. Hedde *et al.* (1969) reported four hexane-soluble metabolites in sheep urine. The identification of two of these is presented in this report.

EXPERIMENTAL

Apparatus. Gas chromatographic separations were done with either a Barber-Colman Series 5000 or a Perkin-Elmer 801 gas chromatograph equipped with effluent splitters so that simultaneous flame ionization detection and trapping could be done; the Barber-Colman chromatograph was also equipped with a radioactive monitor. Infrared spectra were taken with a Perkin-Elmer 337 infrared spectrometer by the micro KBr disk technique. Nuclear magnetic resonance spectra were taken with a Varian A-60A spectrometer in conjunction with a Fabri-Tek 1062 computer of average transients. Mass spectra were obtained with a Varian M-66 mass spectrometer by inserting a capillary into the solid sample inlet system. Column chromatography effluents were continuously monitored with a Picker Nuclear Scinti/Flow using cerium activated silicate glass beads with 2.5% natural lithium (Picker Nuclear) as a scintillator.

Metabolite I. The material designated as metabolite I (Hedde *et al.*, 1969) was purified further by gas chromatography on a 6-ft. × 1/8-in. (i.d.) column of 5% DC 200 on Chromosorb W, temperature programmed from 150° to 225° C. at 8° per minute, or on a 4-ft. × 4-mm. (i.d.) column of 2% SE-30 on Chromosorb W, temperature programmed from 150° to 225° C. at 7.5° per minute.

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Metabolite III. The material designated as metabolite III (Hedde *et al.*, 1969) was purified further by rechromatographing on Sephadex LH-20 (1 cm. × 50 cm.) with redistilled reagent grade acetone as the eluent. This fraction (*ca.* 22 mg.) was recrystallized from a small amount of carbon tetrachloride to yield about 7 mg. of crystalline material.

Acetylation of Metabolite I. A solution of 100 μg. of metabolite I, 200 μl. of acetic anhydride, and 4 μl. of triethyl amine, was allowed to stand at room temperature for 2 days, then poured onto ice and extracted with methylene chloride. The methylene chloride layer was dried over magnesium sulfate and the solvent removed at reduced pressure. The product was purified by gas chromatography on a 4-ft. × 4-mm. (i.d.) column of 2% SE-30 on Chromosorb W, temperature programmed from 150° to 225° C. at 7.5° per minute.

Acetylation of Metabolite III, Method A. A solution of 6.5 mg. of metabolite III (4×10^6 d.p.m.), 2 ml. of acetic anhydride, and 0.3 ml. of triethyl amine was allowed to react at room temperature for 3 days and then heated on a steam bath for 30 minutes. The excess acetic anhydride was removed at reduced pressure. Water and ether were added to the residue; the ether layer was removed, washed with water, and dried over magnesium sulfate. The ether was removed, and the crude product was purified on a column of alumina, using methylene chloride as the eluting solvent. Further purification was achieved on a Sephadex LH-20 column (1 cm. × 50 cm.) eluted with acetone. Final purification was by gas chromatography on a 4-ft. × 4-mm. (i.d.) column of 2% SE-30 on Chromosorb W, temperature programmed from 150° to 225° C. at 7.5° per minute.

Method B. A solution of 100 μg. of metabolite III (60,000 d.p.m.), 100 μl. of acetic anhydride, and 3 μl. of methanesulfonic acid was heated on a steam bath for 1.5 hours. The solution was poured onto ice, extracted with methylene chloride, and the extract dried over magnesium sulfate. The methylene chloride was removed; the residue was dissolved in acetone and gas chromatographed on a 4-ft. × 4-mm. (i.d.) column of 2% SE-30 on Chromosorb W, temperature programmed from 150° to 225° C. at 7.5° per minute. Three radioactive peaks were seen on a radioactive monitor in a ratio of 1:3:2. Infrared and mass spectral data indicated that peak 1 was identical to that obtained by method A. Peaks 2 and 3 were characterized by infrared and mass spectra as isomeric triacetates, but their absolute structures were not determined. When the reactants were heated for 2.5 hours, only the two triacetates were obtained.

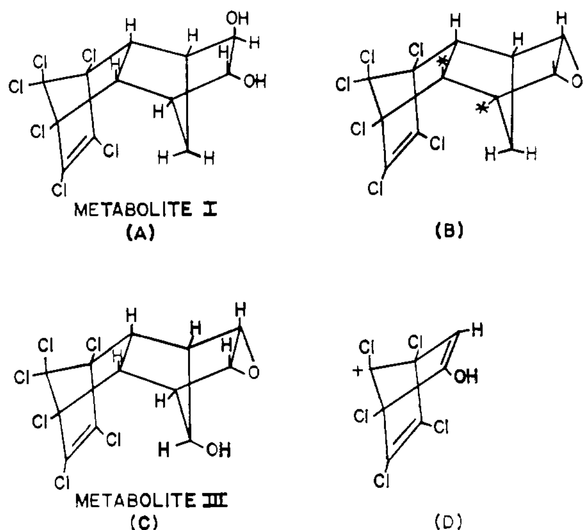

Figure 1. (*A*) *trans*-6,7-Dihydroxydihydroaldrin (metabolite I)
(*B*) Probable structures of metabolite proposed by Richardson *et al.* (* Possible position of hydroxyl group)
(*C*) 9-(*syn*-epoxy)Hydroxy-1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo*-5,8-*exo*-dimethanonaphthalene (metabolite III)
(*D*) Possible mass spectral fragment

Oxidation of Metabolite III, Method A. Half of a solution prepared by mixing 1.0 gram of chromium trioxide, 5 ml. of water, and 0.75 ml. of sulfuric acid was added to a solution of 1.4 mg. (889,000 d.p.m.) of metabolite III in 25 ml. of ether, and the reaction mixture was stirred at room temperature for 8 hours. The remaining portion of the chromium trioxide solution was then added and the mixture stirred for an additional 16 hours. Additional ether was added; the ether layer was removed and washed with water. The ether layer was concentrated and chromatographed on a column of alumina with methylene chloride. After chromatography on Sephadex LH-20 (1 cm. × 50 cm.) with acetone, final purification was accomplished by gas chromatography on a 4-ft. × 4-mm. (i.d.) column of 2% SE-30 on Chromosorb W.

Method B. Chromium trioxide (0.1 gram) was added to 1.5 ml. of pyridine with stirring. A solution of 37 μg. (23,000 d.p.m.) of metabolite III in 2 ml. of pyridine was added to this suspension. The reaction mixture was stirred at room temperature for 16 hours. Water and ether were added; the ether layer was removed, washed with water, and dried over magnesium sulfate. The ether and pyridine were removed at reduced pressure and the crude product purified as described in method A.

Conversion of Metabolite III Acetate to Metabolite III. A solution of 30 μg. (19,000 d.p.m.) of the monoacetate of metabolite III, 0.25 gram of sodium borohydride, and 15 ml. of ethanol was stirred at room temperature for 3 hours. Water and ether were added; the ether layer was removed, washed with water, and dried over magnesium sulfate. A 60% conversion to metabolite III was indicated by gas chromatography.

RESULTS AND DISCUSSION

The infrared and mass spectra of metabolite I are identical to those obtained from an authentic sample of racemic dihydroxydihydroaldrin (trans diol) (Figure 1*A*). Optical activity measurements were not made on metabolite I, but Korte (1965) demonstrated optical activity in a sample of the trans diol isolated from rat urine. Metabolite I was converted to a diacetate which was identical to the diacetate prepared from authentic trans diol (authentic samples of cis and trans diol were obtained from Shell Development Corporation Modesto, Calif.), but which was different from the diacetate prepared from authentic cis diol.

Metabolite III appears to be identical to a metabolite isolated from rat feces by Richardson *et al.* (1968), as indicated by identical infrared spectra and very similar mass spectra (the spectra were run on different instruments). The infrared spectrum of metabolite III is shown in Figure 2. The peak at 3480 cm.$^{-1}$ suggests an internally hydrogen bonded hydroxyl group. A dilution study in carbon tetrachloride provided additional evidence for this. The peak at 1595 cm.$^{-1}$ indicates a Cl—C═C—Cl group, while the peaks at 1260, 1180, 910, and 830 cm.$^{-1}$ provide evidence for an epoxide. Richardson *et al.* (1968) assigned the peak at 1445 cm.$^{-1}$ to a $—CH_2—$ deformation and, along with nmr evidence for the presence of the bridge methylene, arrived at the two possible structures represented by Figure 1*B* (4a- or 5-hydroxy - 1,2,3,4,10,10 - hexachloro - 6,7 - epoxy - 1,4,4*a*,5,6,7,-8,8*a* - octahydro - 1,4 - *endo* - 5,8 - *exo* - dimethanonaphtha-

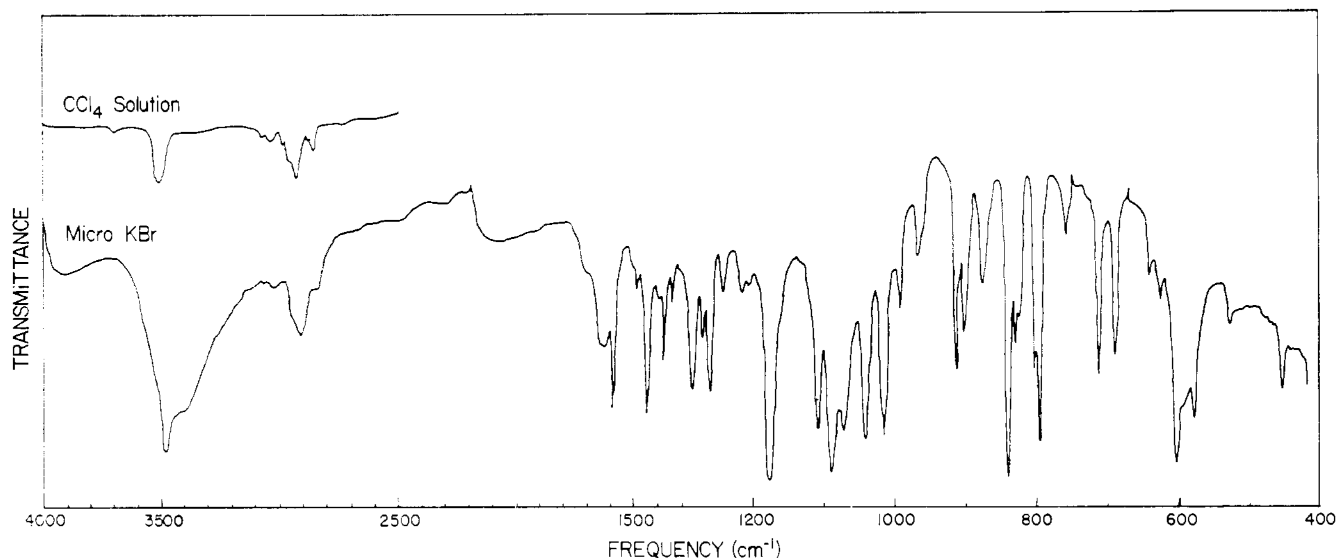

Figure 2. Infrared spectrum of metabolite III

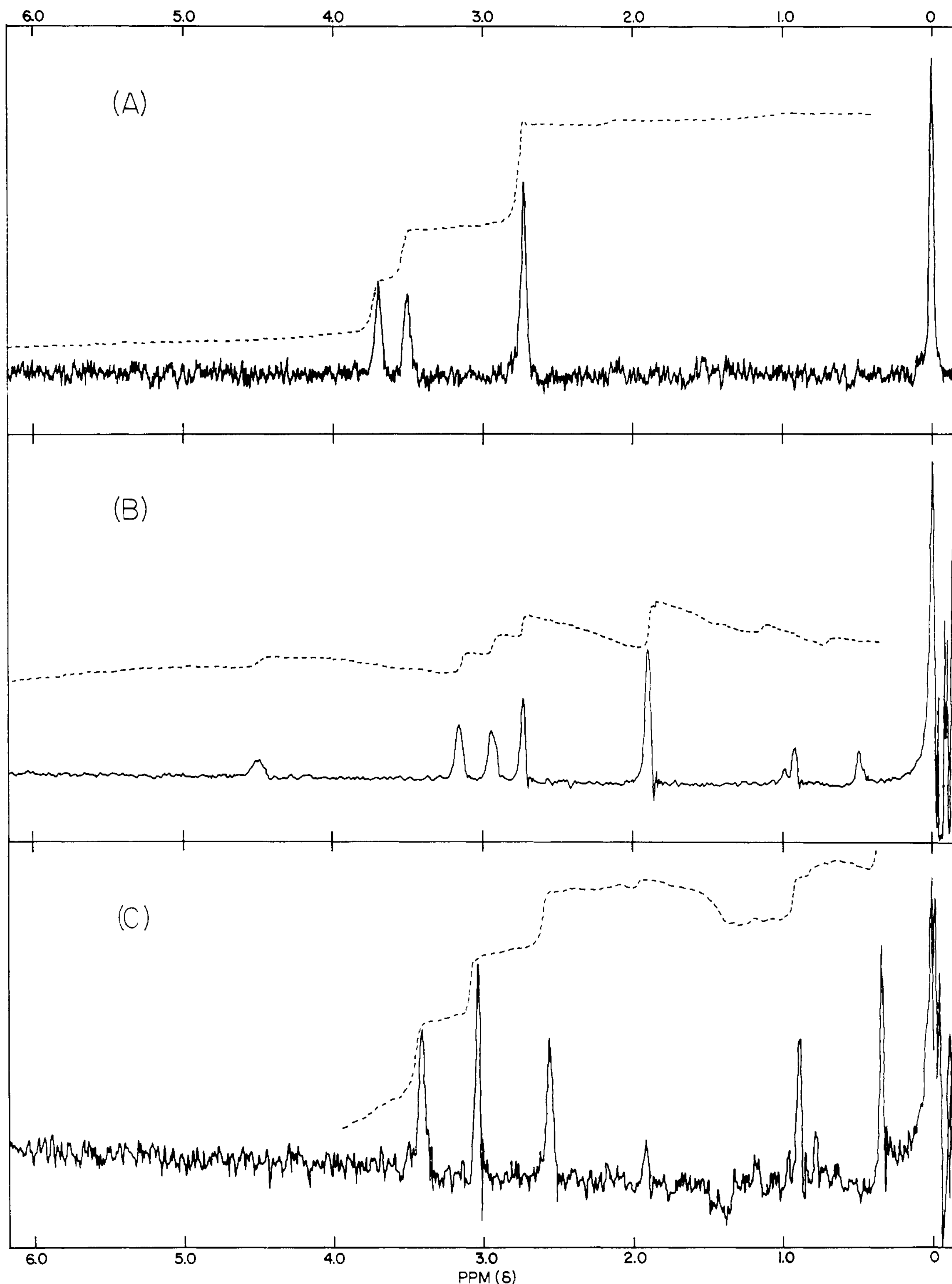

Figure 3. Nuclear magnetic resonance spectra

(*A*) Metabolite III in carbon tetrachloride (slow-scan spectrum, time-averaged integral)
(*B*) Metabolite III acetate, time-averaged in carbon tetrachloride
(*C*) Metabolite III ketone, time-averaged in carbon tetrachloride

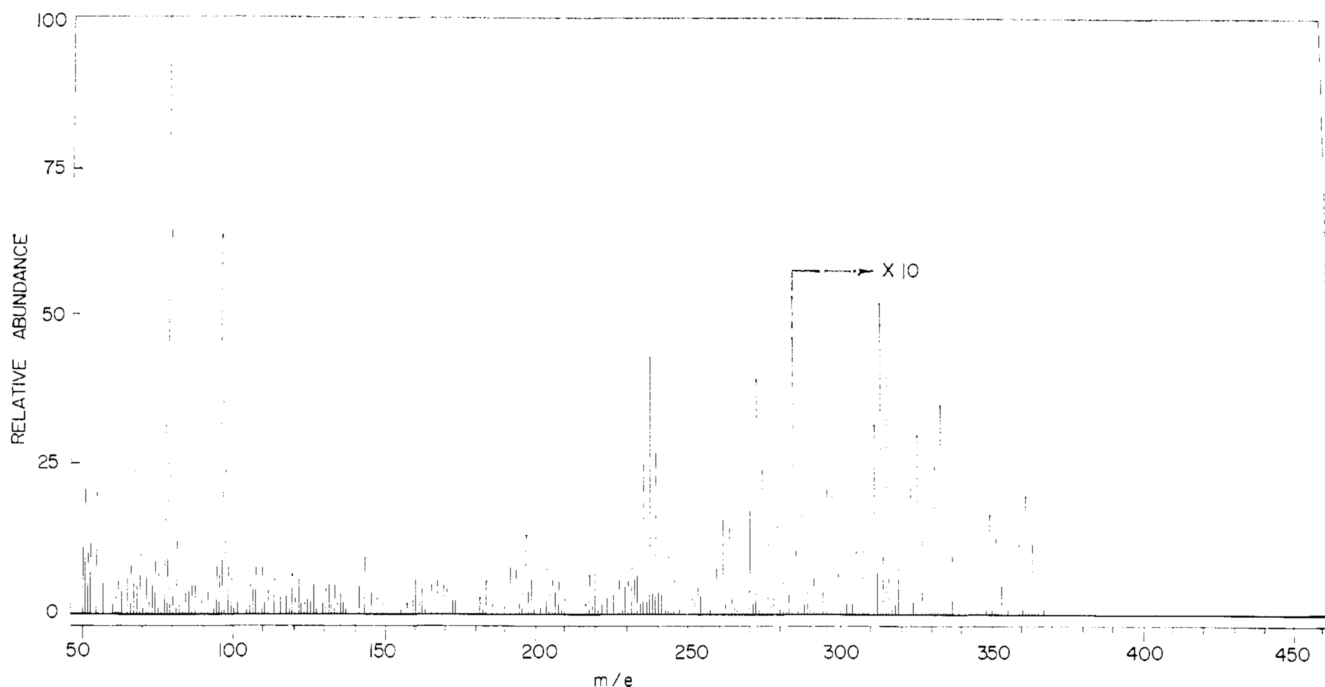

Figure 4. Mass spectrum of metabolite III

lene). Matthews and Matsumura (1969) also isolated this metabolite from rat feces and accepted the structure proposed by Richardson *et al.* (1968). A bridge methylene was indicated in some samples of metabolite III by nmr absorption near 1 δ (dieldrin shows a pair of doublets typical of geminally coupled protons at 1.1 δ, Henderson and Crosby, 1967); however, the integration for this absorption was generally not in proportion to other peaks in the spectrum, and the peaks did not show clean geminal coupling, indicating the presence of impurities.

A sample of metabolite III obtained by recrystallization yielded the nmr spectrum shown in Figure 3*A*. The absence of significant absorption around 1 δ provides evidence that the bridge methylene is no longer intact; thus, the peak at 1455 cm.$^{-1}$ cannot be assigned to the bridge —CH_2— group, but is possibly due to a —CHOH— group (the peak was retained on acetylation but was lost on oxidation). The mass spectrum (Figure 4) was of little value for structural assignment of metabolite III because of rearrangements. For example, an isotope cluster at *m/e* 277 containing five chlorines was initially thought to be due to the fragment shown in Figure 1*D*, in agreement with one of the structures proposed by Richardson *et al.* (1968). However, the mass spectrum of *trans*-diacetoxydihydroaldrin also yielded this peak, and if it had the composition $C_7H_2Cl_5O$, it must have been formed by rearrangement. Extensive rearrangements probably take place because of the strained ring system, making structural assignment by mass spectroscopy questionable.

The simple nmr spectrum of metabolite III in carbon tetrachloride is strong evidence for an intact dieldrin ring system, since almost any other conceivable structure would show extensive coupling among some of the protons. The intact ring system and the absence of methylene protons restrict the location of the hydroxyl group to one of the positions on the methylene bridge. Although the remaining chemical shift assignments were not made with absolute certainty, the methine and hydroxyl protons both absorb at 3.7 δ in carbon tetrachloride. The integration for the spectrum in carbon tetrachloride is 2:2:4. The chemical shifts of the methine and hydroxyl protons are no longer identical in deuterochloroform, and the integration is 1:1:2:4. Additional changes in chemical shift are apparent in deuteropyridine, with an integration of 1:1:2:2:2.

The reactions of metabolite III shown in Figure 5 provide additional evidence for its structure. Oxidation with either chromium trioxide in sulfuric acid or in pyridine yields a compound which gives the simple nmr spectrum shown in Figure 3*C*; both methods result in high yields of ketone. The lack of coupling and an integration of 1:1:1 can be explained by assuming an intact dieldrin ring system with a bridge ketone. The infrared spectrum provided further evidence for the proposed structure, with peaks at 1170, 910, and 827 cm.$^{-1}$ (epoxide), 1770 cm.$^{-1}$ (strained ketone), and 1590 cm.$^{-1}$ (Cl—C=C—Cl), and a lack of absorption at 1445 cm.$^{-1}$ (—CH_2— or —CHOH—). The mass spectrum did not show a molecular ion at *m/e* 392, but indicated an

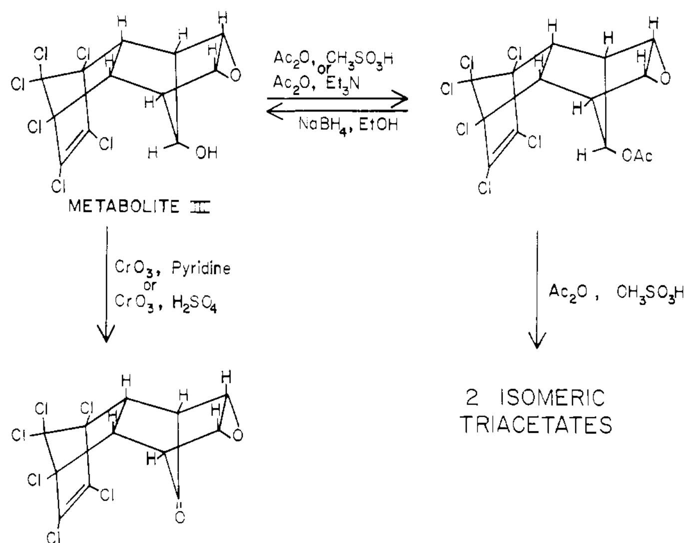

Figure 5. Reactions of metabolite III

isotopic cluster containing five chlorines at 357 (M − Cl) and 329 (M − Cl and CO). The formation of a ketone with an intact dieldrin ring system can only be accounted for by a structure having a bridge hydroxyl group.

Acetylation of metabolite III takes place readily under mild conditions, suggesting an unhindered secondary alcohol. The compound in which the hydroxyl group is syn to the epoxide ring should undergo esterification readily, as it is relatively unhindered, but the compound in which the hydroxyl group is anti to the epoxide ring should not esterify readily because of steric hindrance due to the dichlorovinyl group. However, without an authentic sample of either of the alcohols, the spacial assignment remains speculative. The conversion of the mono acetate to metabolite III with sodium borohydride shows that no degradative reactions took place during the acetylation reaction, and its conversion to isomeric triacetates provides evidence for the presence of an epoxide group. Spectral data on the acetyl derivative are in agreement with the proposed structure. The infrared spectrum shows peaks at 1770 cm.$^{-1}$ (ester) and 1590 cm.$^{-1}$ (Cl—C═C—Cl). The mass spectrum shows an isotopic cluster containing six chlorines at m/e 436 (molecular ion) and successive clusters at 401 (five chlorines, M − Cl), 394 (six chlorines, M − ketene), 393 (six chlorines, M − acetyl), and 359 (five chlorines, M − Cl and ketene). The nmr spectrum is shown in Figure 3*B*. The peaks at 4.6 and 1.9 δ are characteristic of esterified secondary alcohol protons and acetoxy protons, respectively, while the peaks between 2.8 and 3.4 δ are at chemical shifts typical of those in dieldrin. The integration is the expected 1:2:2:2:3. Some TMS side bands and impurities are apparent at 0.5 and 1 δ.

The trans diol appears to be a common route of dieldrin metabolism in animals and bacteria, with its isolation in several metabolic studies. Oxidation may also prove to be a major metabolic route, since Richardson *et al.* (1968) and Hedde *et al.* (1969) have isolated an oxidized dieldrin metabolite from rat and sheep excreta. The chemical and spectral data presented in this paper identify the point of oxidation of this metabolite as the bridge methylene position.

Since this paper was submitted, Baldwin (1969) has stated that additional studies on the rat fecal metabolite (Richardson *et al.*, 1968) have confirmed this structural assignment.

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