Major Fecal Metabolite of Dieldrin in Rat. Structure and Chemistry

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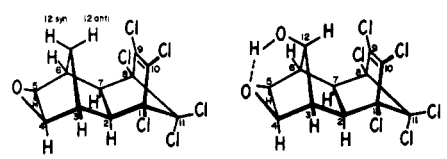
The structure (C-12 *syn*-hydroxydieldrin) of the major fecal metabolite of dieldrin in the rat has been confirmed *via* pmr spectroscopy employing spin decoupling techniques and the new shift reagent, $Eu(DPM)_3$. Detailed studies of the spectral and reaction properties of this metabolite have established the stereochemical structure and have shown that the system is susceptible to degradation. Evidence is presented which indicates that the major fecal metabolite in the rat cannot be converted to the major urinary metabolite but that a common intermediate hybrid ion (or radical) may exist in their formation from dieldrin. It is proposed that an activated dieldrin molecule may mimic certain male steroidal hormones or their precursors in regard to the capability of anchimeric participation of a double bond.

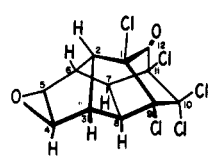
Studies of the metabolism and other degradative processes of dieldrin (I) continue to be complicated by difficulties in the unequivocal identification of the transformation products which generally require stereochemical structure proof. This difficulty in making positive structure assignments seems to be primarily due to the lack of substantial spectral and chemical data on related strained model systems. The only metabolite of dieldrin whose structure is unequivocal, having been confirmed by synthesis, is that of *trans*-4,5-dihydroxy-4,5-dihydroaldrin. [The numbering system used herein for these compounds complies with that suggested by Benson (1969).] Since this metabolite was reported (Korte and Arent, 1965) to possess optical activity, it would be one of the enantiomers of this structure. Recent investigations in our laboratory (McKinney *et al.*, 1971) employing proton magnetic resonance (pmr) spectroscopy including decoupling experiments and sample treatment with the new pmr shift reagent, tris(dipivalomethanato)-europium [$Eu(DPM)_3$], enabled the rapid and accurate stereochemical structure determination of some postulated chlorinated polycyclodiene pesticide metabolites.

The purpose of this work was to test first the applicability of this pmr method for positive structure assignment of the major fecal metabolite of dieldrin in the rat for which most recent investigators (Baldwin *et al.*, 1970a) have assigned structure II. Secondly, structure confirmation of this metabolite would render its conversion reactions more predictable, and since it is the primary metabolite excreted by the rat, its toxicological meaning in relation to the problems of environmental contamination should be carefully assessed. As a working hypothesis in the chemical studies, it was thought that if the assignment of the position of hydroxylation of dieldrin to the ostensibly aliphatic C-12 methylene group was correct and, if this hydroxyl group had a syn relationship to the epoxide group, conjugation or any other similarly activating derivatization would enable a rather facile rearrangement to occur with the formation of a bridged ketone of identical structure (III) to that proposed (Damico *et al.*, 1968b) for the major urinary metabolite of dieldrin in the male rat. Such a rearrangement would be consistent with previous observations on analogous compounds (de Vries and Winstein, 1960) and with the ease of formation of the bridged isomer photodieldrin (IV) on photolysis of dieldrin (Robinson *et al.*, 1966; Rosen *et al.*, 1966; Benson, 1971).

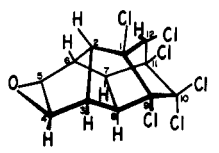


I

II



III



IV

MATERIALS AND METHODS

Purified dieldrin metabolite (II, *ca.* 5 mg) was obtained from male rats according to previously reported methods (Matthews *et al.*, 1971). The pmr studies were made with a Varian HA-100 spectrometer equipped with spin decoupling accessories and using tetramethylsilane (TMS) as an internal reference. The pmr shift reagent, $Eu(DPM)_3$, was used according to previously reported methods (McKinney *et al.*, 1971) to induce paramagnetic shifts in protons and enable superimposed signals to be separated from one another. The high-resolution mass spectra were obtained with a Perkin-Elmer Hitachi Model RMU-7 mass spectrometer *via* the direct probe inlet at 70 eV, and the infrared (ir) spectra were obtained with a Perkin-Elmer Model 621 spectrophotometer. Thin-layer chromatography (tlc) was done on Uniplate, Silica Gel GF plates, 250 μ in thickness. The bands or spots were detected for labeled (^{14}C) materials by exposure to X-ray film or when unlabeled by exposure to uv light or iodine vapors.

Chemical conversions were done on a 0.1 ml of acetone solution of the purified dieldrin-^{14}C metabolite (II) (*ca.* 105,000 cpm), and the products were analyzed by tlc and autoradiography. The following reactions were carried out and analyzed in this manner.

(a) A 0.1-ml solution of II was diluted to 0.7 ml with acetic anhydride (Ac_2O), followed by further dilution to 1.0 ml with triethylamine (Et_3N). The resulting solution was heated at 80°C for 30 min and allowed to stand at room temperature overnight, followed by an additional 30 min heating at 80°C. The reaction, after cooling, was diluted with 6 vol of water and extracted with two 5-ml portions of ether. Combined ether extracts were washed with dilute HCl (20%, v/v) followed by saturated Na_2CO_3, water, and

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then dried over anhydrous sodium sulfate. The ether solution was evaporated to a residue which was redissolved in 0.1 ml of chloroform for tlc.

(b) A 0.1-ml solution of **II** was diluted to 0.7 ml with Et_3N, followed by addition of 10 mg of *p*-toluenesulfonyl chloride (*p*-TsCl). The solution was reacted under the same conditions with the same workup procedure as described for reaction (a) above.

(c) A 0.1-ml solution of **II** was diluted to 0.8 ml with silyl grade pyridine, and 10 mg of anhydrous solid chromium trioxide (CrO_3) was added to this solution. The resulting reaction mixture was heated at 100°C for 1 hr. After cooling, the mixture was cautiously diluted with water and carried through the workup procedure previously described (a).

(d) A 0.1-ml solution of **II** was diluted to 0.25 ml with reagent grade acetone, and the diluted solution was irradiated with 5.6 W of high-intensity ultraviolet light of 2537 A radiation for 1 hr in a quartz tube. The solution was then concentrated to 0.1 ml for tlc.

(e) A 0.1-ml solution of **II** was diluted to 0.7 ml with *N*,*N*-dimethylformamide (DMF) and the resulting solution was heated at 150°C for 22 hr. After cooling the solution was diluted to 15 ml with water and extracted with ether. The ether layers were dried and evaporated as described for (a).

(f) A 0.1-ml solution of **II** was diluted to 0.8 ml with acetic anhydride, followed by addition of 10 mg of *p*-toluenesulfonic acid (*p*-TsOH). The reaction mixture was then heated at 100°C for 2.5 hr. The workup procedure was also as described previously (a).

Dieldrin-^{14}C and metabolites-^{14}C (**II** and **III**) used as standards for tlc comparison were obtained and purified as reported earlier (Matthews *et al.*, 1971).

RESULTS AND DISCUSSION

Synthetic Attempts. Chemical studies of dieldrin have thus far failed to offer substantial evidence for the formation of such oxidative conversion products as depicted by structures **II** and **III**. Since the C-12 methylene group is aliphatic, a certain degree of inertness would be expected. The chlorinated portion of the molecule is, for the most part, considered inert although recent workers (Adams and Mackenzie, 1969) have demonstrated a stereospecific dechlorination with sodium methoxide. However, a study of the Dreiding molecular model of dieldrin suggested that the C-12 methylene group could possibly be activated by a "push and pull" effect of the dichlorovinyl and epoxide moieties on the proton syn to the epoxide group. This phenomenon of anchimeric assistance to ionization likewise suggested a very appealing and direct synthetic route to **II**, since previous investigators (Story, 1961) had demonstrated a similar synthesis of 7-norbornadienol from norbornadiene. Repeating their published procedures utilizing both *tert*-butyl hypochlorite or *tert*-butyl perbenzoate as a radical initiator for abstraction of hydrogen at C-12 in dieldrin failed to produce any detectable **II**. Since hydrogen abstraction at C-12 seemed to be the logical process in the photolysis of dieldrin (**I**) to photodieldrin (**IV**), an attempt was made to trap such a radical by saturating the solution with molecular oxygen during photolysis. Likewise, this was unsuccessful although photodieldrin (**IV**) remained as the major conversion product. This latter result suggested that if hydrogen abstraction occurs at C-12, the intimacy of radical rearrangement and recombination (unimolecular) was much more energetically favorable than any radical dissociation and recombination (bimolecular). However, this does not preclude the possibility that the juxtaposition obtained on an enzyme surface in a biological system during metabolism of **I** could mimic the stereochemistry and hence the energy requirements of the unimolecular process affording either **II**, **III**, or both. Consequently, a different synthetic approach was tried involving 7-norbornadienyl acetate and hexachlorocyclopentadiene under Diels–Alder condition. Although one-to-one adducts were obtained from this reaction, they were shown primarily by pmr analysis to have the wrong stereochemistry (*endo–endo*) at the ring fusion. The importance of confirming the proposed structure of this metabolite was heightened by the apparent failure of these synthetic methods. However, reaction conditions were by no means exhaustively investigated. Nevertheless, metabolism of dieldrin on a large scale was our only source of the proposed metabolite (**II**), and the following spectral studies were run on approximately 5 mg of such purified metabolic material.

Pmr Analysis. Proton magnetic resonance spectroscopy appeared to be the most promising tool for differentiation between alternative structures of the dieldrin metabolites. The major fecal metabolite (**II**) was an ideal test for our recently developed method for the pmr analysis of such systems, even though many difficulties have been encountered by previous workers in obtaining interpretable spectra on small amounts of the isolated metabolite. Our spectra of **I** are essentially identical to those reported by the previous workers (Baldwin *et al.*, 1970a; Feil *et al.*, 1970); however, these workers made no attempt to fully analyze their spectra. The pmr spectrum of **II** in chloroform-*d* contained overlapping signals centered at τ 7.25 and 7.28, along with a broad signal at τ 6.45 and an apparent AB quartet centered at τ 5.92 and τ 6.24, with the latter doublet consisting of relatively broad signals indicative of an hydroxyl group. The upfield portion of the spectrum, where the dieldrin C-12 protons normally fall, was obscured by impurities which remained even after many recrystallizations. This did not present a problem since it was later discovered that the signals from the impurities coalesced and shifted on initial addition of the pmr shift reagent Eu(DPM)$_3$; however, continued addition of the reagent no longer affected this signal while the other signals were increasingly affected. Integration of the large coalesced signal likewise indicated that it was due to impurities.

The spectrum of **II** compares quite well with that obtained for dieldrin (**I**) in the same solvent. The overlapping signals at τ 7.25 and 7.28 can be assigned to the C_2–C_7 and C_3–C_6 protons (in dieldrin, τ 7.30, 7.34), respectively, while the signal at τ 6.45 can be assigned to the C_4–C_5 epoxide protons (in dieldrin, τ 6.89). The remaining AB pattern found in the spectrum of **II** was markedly deshielded in comparison with the normal AB pattern observed for the C-12 protons of dieldrin (τ 8.73 and 9.00). It was conceivable that hydration of the dichlorovinyl group could have deshielded the C-12 protons, since the C_9-C_{10} chlorine atoms would be spatially much closer to the C-12 protons. On the other hand, one could assign this pattern to the vicinal coupling of a C-12 proton and a hydroxyl proton, although this was not appealing since the size of the coupling constant (11.5 Hz) indicated a very strong coupling with a dihedral angle approaching 180°. Such a large coupling would represent one of the largest of its type ever reported and would suggest that the hydroxyl proton is very rigidly held *via* hydrogen bonding with the epoxide group. The following pmr information

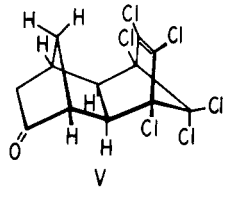
confirms that the latter assignment of a C-12 syn hydroxyl group is indeed correct. As previous workers (Baldwin *et al.*, 1970a) have observed, shaking the chloroform solution of **II** with D_2O did not alter the spectrum; however, on addition of acid, the quartet collapsed to a singlet, indicating exchange of the hydroxyl proton. Furthermore, addition of the shift reagent, $Eu(DPM)_3$, to a chloroform solution of **II** likewise destroyed the quartet multiplicity, since the rigidity of the hydrogen bond is effectively weakened by the competitive Lewis acid properties of the shift reagent for the lone pair of electrons on the epoxide oxygen. $Eu(DPM)_3$ normally does not alter the coupling pattern of protons while inducing paramagnetic shifts *via* association with lone pair bearing functional groups.

The shift reagent enabled the superimposed signals in **II** to be separated from one another and confirmed the other proton assignments, since the equivalent pairs of protons at C_2-C_7, C_3-C_6, and C_4-C_5 appeared as apparent singlets which integrated for two protons each, while the remaining C-12 proton appeared as a broad perturbed signal due to its close proximity to the associating site. The compound (**II**) was recovered by preparative tlc and gave pmr spectra identical to those obtained before treatment with the shift reagent, indicating that no rearrangement had occurred during the complexing process. Pmr spectra of **II** in benzene-d_6 showed solvent shifts which were both qualitatively and quantitatively similar to those found for dieldrin (**I**). Decoupling experiments involving the C-12 proton permitted resolution of the H_3-H_4 and H_5-H_6 ($J_{3,4} = J_{5,6} = 1.5$ Hz) and H_3-H_5 H_4-H_6 ($J_{3,5} = J_{4,6} = 1.0$ Hz) couplings as well as confirming chemical shift assignments of these protons. The smaller magnitude of these couplings as compared to the same couplings in dieldrin ($J_{3,4} = 2.1$ Hz; $J_{3,5} = 1.5$ Hz) reflects steric compression of the C-12 hydroxyl group against the electronic clouds of the epoxide group. Since previous workers (McCullock *et al.*, 1969) have provided evidence that even the *anti*-methylene proton in *endo-exo* fused rings (such as H_{12a} in dieldrin) is sterically compressed against the π-cloud of the ethano-bridge, it was suspected that **II** would react when subjected to external forces (such as heat, ultraviolet light, etc) to relieve one or both steric compressions. In addition, irradiation of the H_3-H_6 proton signals of **II** revealed that the $H_{4,5}$ protons were coupled with the H_{12} anti proton *via* the expected "W effect" coupling (1.8 Hz), thereby confirming that the hydroxyl group has a syn relationship to the epoxide group. The $Eu(DMP)_3$ shift reagent has been utilized (Keith, 1971) to confirm the somewhat disputed structure of photodieldrin (**IV**) and establish the stereochemistry of the migrated proton to be syn to the epoxide, as would be expected if an intramolecular mechanism were operating as previously postulated.

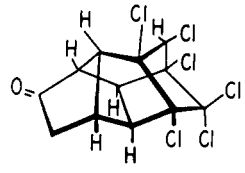
Ir Analysis. The infrared spectrum of **II** is consistent with the proposed structure. Most diagnostic is the occurrence of a strong-sharp band at 2.87 μ which is very indicative of a strong intramolecular hydrogen-bonded hydroxyl group. A band near 6.3 μ can be attributed to the normal dichloroethylene absorption, while a band near 6.9 μ can be ascribed to an abnormal absorption of the epoxide group produced by involvement in the hydrogen bonding.

Ms Analysis. Structural assignments based on mass spectral interpretations for strained ring systems such as these are questionable, since rearrangements would probably be extensive. However, careful studies (Damico *et al.*, 1968a; Benson, 1971) of these systems have revealed that differences in the intensities of certain low mass fragment ions are very helpful for identification purposes. An analysis of the mass spectrum of **II** indicates *m/e* of 95 (100%, $C_6H_7O^+$) and 394 (<0.5%, $C_{12}H_8O_2Cl_6^+$). Continuous mass spectral scans produced spectra in which the parent ion at 394 had completely disappeared with the intensification of an ion at 378, while the base peak of 95 changed to 79. Since this was a temporal change it was attributed to a thermally influenced vapor phase decomposition of the compound in the direct probe and not to a rearrangement in the ion source. The possibility that the mass spectra dominating in the later sequential scans were those of less volatile impurities previously detected in the pmr studies was ruled out since the metabolite was radioactively pure.

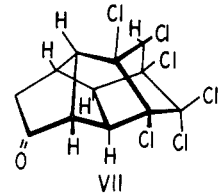
Thermal decomposition is not entirely surprising since loss of an oxygen atom to form the isomer of dieldrin would relieve the steric compression previously noted for **II**. Further analysis of the spectra suggested that the decomposition product was not dieldrin (**I**), photodieldrin (**IV**), nor oxodihydroaldrin (**V**), which are all conceivable products, but was a compound producing fragments indicative of both **IV** and **V** (*m/e* 65, 79, 81, and 105). A plausible structure would be one of the photooxodihydroaldrins **VI** or **VII** which could occur if rearrangement of the epoxide group to a carbonyl group were concomitant with displacement of the hydroxyl group. Previous workers (Skerrett and Baker, 1959, 1960) have shown that dieldrin when heated in the presence of Lewis acids rearranges to **V**; therefore, it is possible that the hydroxyl proton in **II** could act as an internal Lewis acid to effect rearrangement of the epoxide. A mixture of the isomeric compounds **VI** and **VII** was prepared



V



VI



VII

by the reported method (Bieniek and Korte, 1969) involving photolysis of **V** in acetone. Mass spectral analysis of this mixture indicated that all of the suspected fragment ions are present. Reinvestigation of this arrangement–decomposition of **II** will be made with the aid of computer normalized spectra to facilitate their comparisons and confirm our assignment. It is interesting to note that compound **VI** has been proposed (Matsumura *et al.*, 1968) as a degradation product of dieldrin by a soil microorganism.

Chemical and Biological Conversion Attempts (II to III). Since the structure (**II**) of the major fecal metabolite has been rigorously established, it was necessary to test the chemical, thermal, and photolytic lability of the molecule, and at the same time test the feasibility of converting **II** to the bridged ketone **III**, the proposed structure for the major urinary metabolite. Table I lists the results of two-dimensional tlc of product mixtures from the specified reactions of **II**. The values given are relative to dieldrin (Rd, dieldrin = 1.00) in order to compensate for variations in the plates. The standards or reaction products were spotted in the lower left

Table I. Thin-Layer Chromatography of the Major Fecal (II) and Urinary (III) Metabolites of Dieldrin in the Rat and the Specified Reaction Products of II

	Metabolite Rd's[a] in mobile phase[b]	
Standards	Ether:hexane (1:1)	Methylene chloride
II[c]	0.63	0.56
III[c]	0.46	0.82
	Reaction products Rd's	
Reaction conditions	Ether:hexane (1:1)	Methylene chloride
Ac_2O + Et_3N	0.61	0.59
p-TsCl + Et_3N	0.79	0.68
CrO_3 + pyridine	0.45	0.79
	0.64	0.67

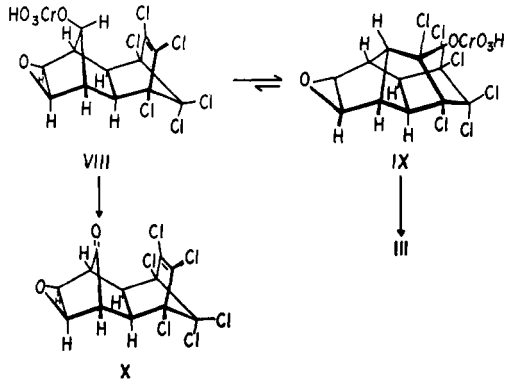
	Major[c]	Minor[d]	Major	Minor
Uv light	0.27	0.42	0.57	0.72
(2537 A)	0.43	0.48	0.62	0.80
DMF, Δ	0.62		0.65	
Ac_2O + *p*-TsOH	0.27		0.29	
	0.42		0.36	
	0.34		0.55	

[a] Rd = R_f std or reaction product/R_f dieldrin. [b] In each case the stationary phase was a precoated 250-μ tlc plate of silica gel GF obtained from Analtech, Inc. Duplicate studies indicated that the values given are reproducible from one batch of plates to another which were used without prior activation. [c] Our samples of labeled II and III cochromatographed with isolated samples of these metabolites that give the reported spectral properties. [d] Major and minor denote the intensity of the black spot in the X-ray film.

hand corner of the plate which was eluted first with ether–hexane (1:1) and then, after drying, rotated (90°) and eluted with methylene chloride.

Solvolytic rearrangement of the acetate or tosylate (*p*-toluene-sulfonate) of **II** represented a possible route to **III**, and attempts were made to generate them in the presence of pyridine or triethylamine. The tlc results indicate that these esters, if formed, failed to rearrange to **III**. Previous workers (Feil *et al.*, 1970) claim to have prepared the acetate using Et_3N. Variations in the reaction conditions designed to facilitate rearrangement as well as generally provide more favorable derivatizing conditions did not produce any detectable **III**; however, it is not certain that ester formation occurred.

CrO_3 is known (Wiberg and Evans, 1960) to form chromate esters with alcohols *via* an equilibrium process followed by slow decomposition of the ester to the carbonyl compound. The chromate ester (**VIII**) of **II** might be expected to equilibrate in favor of a second chromate ester **IX** *via* neighboring double bond participation in order to relieve steric compression. Subsequent decomposition of these esters should lead to two carbonyl compounds (**X** from **VIII**, **III** from **IX**).

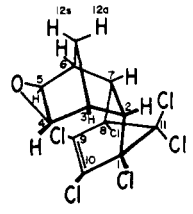


Transannular homoallylic cations have been previously proposed (Winstein *et al.*, 1950) as intermediates in solvolytic displacement reactions on polycyclic systems such as these to account for the formation of rearranged products. The tlc results of the reaction products of **II** with CrO_3 in pyridine show that there are two compounds present, one of which is identical in Rd to **II**, and the other similar in chromatographic behavior to **III**. However, cochromatography of the reaction product with authentic metabolite **III** confirmed that they are not identical. Therefore, structure **X** was assigned to this product, since it is difficult to conceive of a more probable one. Similar chromatographic behavior to **III** might be predicted if one compares their infrared carbonyl absorptions (**X**, 5.60 μ; **III**, 5.55 μ) previously reported (Klein *et al.*, 1970; Baldwin *et al.*, 1970a). The structure of metabolite **III** seems unequivocal since recent work (Klein *et al.*, 1970) has shown it to be a metabolite of photodieldrin (**IV**) of known structure.

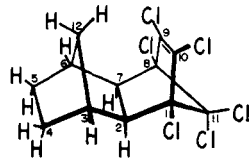
Although chemical conversion of **II** to **III** could not be demonstrated (See NOTE ADDED IN PROOF) under the conditions of extremely low concentrations of **II**, biological conversion of **II** to **III** possibly *via* conjugation cannot be ruled out. Sulfoconjugation ("ethereal sulfate") (Dodgson and Rose, 1970), which is found for phenolic and certain enolic type compounds, is possible and is considered a detoxicating mechanism for such compounds. Conjugation with glucuronic acid is also a common biological process for detoxicating certain hydroxylic substances; and recent work (Matthews *et al.*, 1971) has shown that metabolite **II** is rapidly conjugated *in vitro* by a glucuronyl transferase from the microsomal fraction of male and female rat liver. This glucuronide was unique in that it could not be hydrolyzed by β-glucuronidase or 1 *N* HCl under the usual conditions. Since the structure of the glucuronide was not investigated, it is not certain whether the conjugate is stable to hydrolysis in its unrearranged form or exists in another form, which may possibly be enzyme bound, *via* a concomitant rearrangement during glucuronide formation. Nevertheless one might expect the unrearranged glucuronide of **II** to have a stability comparable with that of enol-glucuronides which have been observed (Layne, 1970) to be unstable. Tlc of ether extracts of the aqueous conjugation solutions in both male and female rats indicated the presence of **II**. Vigorous hydrolysis of these solutions with concentrated HCl and heat afforded a new material with Rd 0.49 in ether–hexane (1 to 1) and 0.14 in methylene chloride, along with small amounts of more polar material, which is probably the result of epoxide ring opening. The new material may be the hydrolysis product of a rearranged conjugate and, in the case of the female rat, was accompanied by **II**, possibly from hydrolysis of the unrearranged conjugate. Although glucuronide conjugation of **II** does not afford any **III**, it does form a conjugate whose properties are difficult to define. It was further shown that incubation of metabolite **II** with whole rat liver homogenates does not yield any detectable **III**.

These results suggest that **III** may not be formed directly from **II** but that a common hybrid ion or radical may exist in their formation from dieldrin. This is further supported by evidence that endrin (**XI**), a stereoisomer of dieldrin (**I**), is metabolized to a hydroxy compound analogous to **II**, but not to a keto compound analogous to **III** (Baldwin *et al.*, 1970b). In addition, 4,5-dihydroaldrin (**XII**) has no metabolites which are analogous to either **II** or **III** (Brooks and Harrison, 1969). These results imply that the epoxide group is a prerequisite for formation of C-12 hydroxy compounds such as **II**, and that keto compounds such as **III** cannot be formed by direct attack on the dichlorovinyl group of **I**, but can only be formed when

this dichlorovinyl group is in close proximity to a suitably activated position to receive its electrons, as would be the case if hydrogen abstraction at C-12 occurred to produce a common hybrid ion.

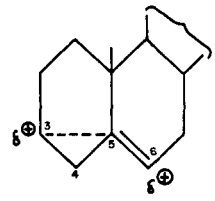


XI

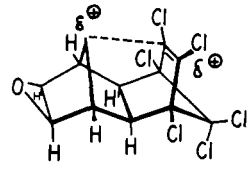


XII

Metabolism studies (Klein *et al.*, 1970; Matthews *et al.*, 1971) of both **I** and **IV** have shown that female rats excrete very little, if any, of compound **III**. If interaction of the dichlorovinyl group with the C-12 methylene group is a necessary process for affording the correct molecular conformation for further metabolism only in the male, then one would expect photodieldrin (**IV**) to be further selectively metabolized by the male, since its molecular topography closely resembles that of the proposed resonance hybrid ion. If one compares the structures of the normal estrogen steroidal hormones of the female with the normal androgens of the male, it is immediately evident that the estrogens are, for the most part, of the phenolic type (A ring), while the androgens are of the normal secondary alcohol type with several possessing C_5-C_6 unsaturation in the B ring which can undergo homoallylic participation with C-3 in the A ring bearing the hydroxyl group. In fact, previous investigators (Winstein and Adams, 1948) have shown that cholesteryl tosylate is solvolyzed at many times the rate of model systems (cyclohexyl tosylate,) providing convincing evidence that rate enhancement is probably achieved by a hybrid ion such as **XIII**. Dreiding stereomodels of the interacting conformer of **XIII** and the proposed dieldrin hybrid ion show a stereochemical resemblance at the atoms involved.



XIII



from II

Therefore, an activated dieldrin molecule (from **I** or **II**) may mimic a steroid hormone or hormone precursor which is further metabolized by male rats but not by females. On the other hand, the glucuronide conjugation of **II** and testosterone by rat liver glucuronyl transferases are in some ways similar (Lucier *et al.*, 1971) but do not vary with sex. Nevertheless, if this system could be shown to mimic certain steroid hormones, it would be of considerable biological interest, not only with reference to the elimination of the metabolites of endogenously produced "steroid like" systems, but also in the context of the use of exogeneous systems, for various forms of therapy. In addition, it could provide useful information on the nature of these hormone receptor sites.

Photolytic, Thermal, and Other Chemical Reactions of II. As would be expected, photolysis of **II** gave a mixture of products, of which four constituted the major portion Of the two minor products, one appeared to behave chromatographically like **III** or **X**, while the other cochromatographed with an authentic sample of the most polar photooxodihydroaldrin assigned structure **VII** by the previous workers (Bieniek and Korte, 1969). Formation of these products suggested that both homolytic cleavage of the hydroxyl group and its recombination, as well as epoxide rearrangement, were occurring. Therefore, the major products could possibly be a mixture of isomeric diketones (the C-4 or C-5 ketones of **III**) which would be highly polar relative to dieldrin. On the other hand, if photooxidation of the C-12 hydroxyl group is occurring to produce **X**, then another pair of isomeric diketones (the C-4 or C-5 ketones of **X**) is possible which would also have similar chromatographic behavior. Previous investigators (Ivie and Casida, 1971) have used rotenone and other related substituted 4-chromanones as photosensitizers to accelerate the photoalteration of the chlorinated polycyclodiene pesticides. These investigators concluded that such pesticide–photosensitizer interactions may be helpful in manipulating persistent pesticides in the environment. In the case of dieldrin (**I**), it appears that its photoalteration products and certain metabolic products are at least as toxic (Harrison *et al.*, 1967) if not more toxic (Rosen and Sutherland, 1967; Khan *et al.*, 1969). Photolysis of dieldrin-^{14}C in our laboratory in acetone serving as solvent and sensitizer gave at least seven products, as indicated by two-dimensional tlc. There were two major products with Rd's 0.65 and 0.80 in ether–hexane (1 to 1) and 0.90 in methylene chloride. These products are in the expected polarity range of the usual products (Benson, 1971) photodieldrin (**IV**) and pentachlorodieldrin, respectively. Therefore, such accelerated photoalterations may be very undesirable for some pesticides.

Since **II** had evidently undergone a thermally influenced vapor phase rearrangement in the mass spectrometer, its thermal instability in solution was investigated. When **II** was heated at 150° C for 22 hr in *N*,*N*-dimethylformamide (DMF), it was apparently unaffected, since the recovered material was similar in chromatographic behavior to starting **II**, with no detectable products in the suspected polarity range. However, the stability of **II** to higher temperatures and in different solvents was not investigated further.

Previous workers (Feil *et al.*, 1970) reported that three products were obtained when metabolite **II** is reacted with acetic anhydride in the presence of methanesulfonic acid with one material disappearing on prolonged heating under these conditions. Reaction of **II** in our laboratory which Ac_2O in the presence of *p*-toluenesulfonic acid also gave three products which are probably identical to those obtained by the previous workers. On the basis of spectral evidence it was proposed that these products were isomeric triacetates resulting from opening of the epoxide ring. The absolute structures of these compounds were not determined. Furthermore, other investigators (Chau and Cochrane, 1971) have shown that dieldrin (**I**) is converted to three products when treated with Ac_2O in the presence of concentrated H_2SO_4. Spectral evidence has indicated that the major product is one in which reaction between the dichlorovinyl and C-12 methylene groups has occurred along with epoxide ring opening. A second product was identified as the *cis*-diol of **I** in which a cis ring opening of the epoxide *via* an intimate ion pair (acetate) is speculated (Chau and Cochrane, 1970). These studies have demonstrated the chemical feasibility of cis opening of the epoxide ring of **I**, as well as chemically induced involvement of the C-12 methylene group with the dichlorovinyl moiety. Although no standards are available for comparative chromatography, it is likely that at least two of our products from Ac_2O and *p*-TsOH treatment are the result of either cis or trans ring opening of the epoxide group (both might be expected under these condi-

tions) and possibly of acetate displacement at C-12 by the dichlorovinyl group followed by recombination and hydrolysis (during workup) at C-9 or C-10. Such triacetates or keto diacetates might be expected to have Rd's in the range found for these products.

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NOTE ADDED IN PROOF. Two-dimensional cochromatography of the six major products (three from each) of the reactions of metabolites **II** or **III** with Ac_2O and *p*-TsOH resulted in four spots, indicating that two products may be common to both metabolites. This is possible if epoxide ring opening (both cis and trans products) occurs in the case of **III** with epoxide ring opening and rearrangement occurring for **II**.

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Metabolism of Atrazine and 2-Hydroxyatrazine by the Rat

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Rats were given either ^{14}C-ring-labeled atrazine (**I**) or 2-hydroxyatrazine (**II**) as a single oral dose. Seventy-two hours after dosing the radioactivity was recovered, mainly in the urine (**I**, 65.5%; **II**, 78%) and feces (**I**, 20.3%; **II**, 5.5%). Less than 0.1% of the dose from either compound was detected in the expired air, and less than 0.1% of the ^{14}C from **II** was detected in the body tissues. The carcasses of the rats given **I** contained 15.8% of the dose, and the distribution of this radioactivity in selected tissues was determined. **II** and its two mono-*N*-dealkylated analogs (**VI** and **VII**) were identified in the urine from rats given **II**. These represented 88.4% of the urinary radioactivity. Nineteen urinary metabolites from **I** were separated by ion-exchange chromatography. Four of these (approximately 47% of the urinary radioactivity) were identified as **II**, **VI**, **VII**, and ammeline. Two additional metabolites of **I**, representing approximately 10% of the urinary radioactivity, were characterized by mass spectrometry.

Corn and sorghum metabolize atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, **I**, Figure 1) to 2-hydroxyatrazine (**II**), to both of the mono-*N*-dealkylated analogs of **II**, and to the glutathione and cysteine conjugates of atrazine (Shimabukuro, 1968; Shimabukuro *et al.*, 1970; Lamoureux *et al.*, 1970). Shimabukuro *et al.* (1970) have also shown that the plant residues of the glutathione conjugate and unchanged **I** decreased with time, while the residues of the 2-hydroxytriazines and the amounts of radioactivity unextractable with methanol increased with time after application of ^{14}C-labeled **I**. These studies suggested that mature plants would contain little, if any, unchanged atrazine, and that the diet of animals feeding on atrazine-treated crops would contain residues of 2-hydroxyatrazines.

Larson *et al.* (1971) compared the metabolism of 2-methoxy-4-ethylamino-6-*sec*-butylamino-*s*-triazine (**III**) with that of its

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