Metabolism of Endrin in the Rat

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Three metabolites of endrin have been isolated from the rat. One, found in the tissues and urine, is the ketone produced by replacing the methylene group of endrin by a carbonyl group; the other two occur in the feces. The major one of the fecal metabolites is a secondary alcohol formed by substituting one hydrogen of the methylene group of endrin by a hydroxyl group. Oxidation of this compound gives the tissue metabolite. The other fecal metabolite is also an alcohol, and seems to be a product of substitution of a hydrogen atom by an hydroxyl group on a carbon atom other than carbon 9.

Endrin is the chlorinated insecticide having the structure and conformation shown in Figure 1.

Soloway *et al.* (1960) described many of the chemical properties of endrin, and showed that heat or acid causes molecular rearrangements which give, among other products, an isomeric ketone, Δketo endrin (Figure 1). Brooks (1969) has reviewed most of the published metabolic studies of endrin. Richardson (1965) examined the tissues of rats which had been fed a diet containing 10 ppm of endrin to see if Δ keto endrin occurred as a metabolite, but found none by gas chromatography. Instead, he found an unidentified compound which was shown to be halogenated.

Ludwig (1966) studied the metabolism of endrin in rats using endrin-^{14}C, and found that most of the excreted radioactivity was present in the feces as two metabolites which he did not identify, though he concluded that neither was identical with Δketo endrin.

Recently Klein *et al.* (1968) reported that they had positively identified the major fecal metabolite of endrin in the rat as Δketo endrin. This identification was based on the thin-layer and gas chromatographic characteristics of the metabolite. Since this conclusion did not agree with those of Ludwig, nor could it be substantiated by chromatographic investigation of fecal extracts (Richardson *et al.*, 1970), experiments were begun to identify the metabolites of endrin in the rat to clarify the situation. The identification of the metabolite as Δketo endrin has been subsequently amended (Korte, 1970).

Preliminary experiments using endrin-^{14}C gave results in agreement with those of Ludwig, and investigation of organochlorine residues in tissues of rats fed endrin confirmed the conclusion of Richardson. The concentration of the metabolite found in the tissues of female rats was of the same order as that of the endrin, while in the male rats the concentration of endrin was very low, the metabolite constituting the main stored organochlorine compound. These estimates of concentration were based on gas chromatographic peak areas. Tables I and II give the chromatographic characteristics of the three metabolites. The metabolite from the tissues of rats is termed "Metabolite I" and the two fecal metabolites are termed "Metabolite II" and "Metabolite III," the latter being the more polar of the two.

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APPARATUS

Infrared spectra were obtained using either a Grubb Parsons Spectromaster or a Perkin Elmer Model 257 Infrared Spectrophotometer. Potassium bromide microdiscs or 0.1 mm cavity cells were used for sample preparation, the solvent spectrum in the latter case being compensated by a variable path length cell in the reference beam.

Mass spectra were obtained using an AEI MS9 with a direct insertion sample probe.

Proton magnetic resonance spectra were obtained using a Varian HA 100 Spectrometer using a noise averaging computer (CAT), which was necessary because of the restricted sample sizes available.

Gas chromatography was performed using a Perkin Elmer Fractometer, modified for on-column injection, with an electron capture detector.

Thin-layer plates were used as supplied by Anderman & Co., Ltd. They were not activated before use.

REAGENTS

Hexane was produced by redistilling 60–70° C boiling range petroleum spirit in a glass fractionating column up to a column head temperature of 66° C. Dimethyl formamide was redistilled in a rotary evaporator to give a colorless distillate. Chromium trioxide/pyridine oxidizing solution was prepared by drying pyridine with potassium hydroxide and making a saturated solution of chromium trioxide in it. This reagent ignited on one occasion on contact with organic matter. Silica gel SG 31 was supplied by Whatman.

PROCEDURE

Isolation of Metabolite I from Endrin Rat Fat. 12.4 g of omental fat from male Carworth Farm Strain E rats fed a diet of 4 ppm endrin for a period of several weeks was extracted in a Soxhlet with 2:1 v/v hexane acetone. The extract was evaporated to dryness, taken up in hexane, and partitioned with dimethyl formamide by the method of de Faubert Maunder *et al.* (1964) to give a substantially fat free hexane solution.

Column chromatography was performed using a 1 cm diam-

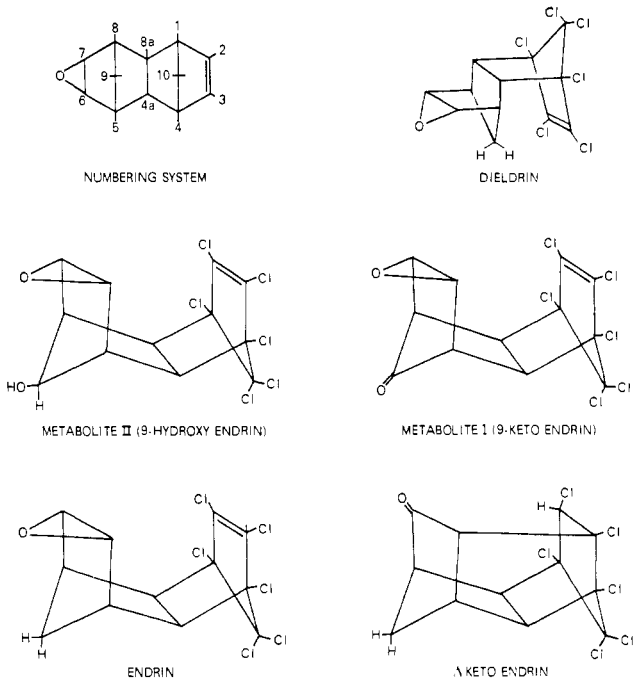

Figure 1. Structures of the Metabolites I and II, endrin, Δketo endrin and dieldrin

eter column containing 8 g Florisil, in which the water content had been adjusted to 3% by weight. The sample was eluted with 25 ml hexane and then with a solution of 1.5% v/v acetone in hexane, the metabolite being eluted between the 45th and 60th ml of the second eluate. The metabolite was further purified by thin-layer chromatography on Merck type E alumina using 10% v/v acetone in hexane as the eluting solvent.

The resulting product, although less than 1 mg, was sufficient to obtain an infrared and a mass spectrum.

Isolation of Metabolites II and III from Endrin Rat Feces. In a typical experiment, 986 g of feces collected from male rats fed diets containing 5 or 10 ppm endrin were extracted by maceration with methanol. After evaporating the extract to dryness in a rotary evaporator, the residue was dissolved in diethyl ether, washed with saturated brine, and dried with anhydrous sodium sulfate. The solution was boiled and hexane was added, this resulting in the gradual removal of the ether to give a hexane solution from which a thick, black material settled out which contained no metabolite II or III shown by gas chromatography, and this was discarded.

The hexane solution was applied to a 3.5 cm diameter column of 100 g silica gel SG 31. This column was eluted with 10% v/v acetone in hexane, 50 ml fractions being collected. Fractions 4 to 8 were bulked and rechromatographed on a similar column.

The extract at this stage was bright green and had a strong odor. It was evaporated to dryness, dissolved in acetone, and an excess of potassium permanganate in acetone was added at room temperature to remove components that were easily oxidized. The excess permanganate was precipitated by addition of hexane, and a straw colored solution was obtained after filtration. Gas chromatography showed that no change had occurred in the two metabolites present in the solution during the treatment.

The filtrate was evaporated to small volume and taken up in hexane, then applied to a 1 cm diameter column of 20 g silica gel SG .31 using 10% v/v acetone in hexane as the eluent.

Table I. Relative Retention Volumes of Endrin Metabolites and Δketo Endrin

Compound	Relative retention volumes for stationary phases: SE30[1]	Oronite[2]	GEXE 60[3]
Endrin	1.00[a]	1.00[b]	1.00[c]
Δketo Endrin	1.80	2.41	5.37
Metabolite I	1.19	1.39	2.27
Metabolite II	2.48	3.03	8.53
Metabolite III	1.615	2.25	6.375
Oxidation product of Metabolite II	1.19	1.39	2.27

(1) Column: 1 meter × 0.4 cm i.d. glass tube. 3.8% SE30 on 80/100 mesh Diataport S. T = 183° C, inlet pressure = 25.5 psi N_2.
(2) Column: 1 meter × 0.4 cm i.d. glass tube. 3% Oronite Polybutene 128 + 0.3% Epikote 1001 on 80/100 mesh Diataport S. T = 183° C, pressure = 20 psi N_2.
(3) Column: 1 meter × 0.4 cm i.d. glass tube. 1.3% XE 60 + 0.13% Epikote 1001 on 80/100 mesh. A.W. DCMS Chromosorb G. T = 183° C pressure = 20.0 psi N_2.

[a] Rt endrin = 1.93 min. [b] Rt endrin = 11.3 min. [c] Rt endrin = 7.98 min.

Table II. R_f Values of Endrin, Its Metabolites, and Δketo Endrin

Compound	Absorbent, Alumina Type E (F254, Merck), Solvent Systems: Benzene/Ethyl Acetate 3:1	Hexane/Acetone 4:1	Hexane/dichloromethane 1:1
Endrin	0.79	0.76	0.71
Δketo Endrin	0.74	0.57	0.55
Metabolite I	0.73	0.69	0.63
Metabolite II	0.40	0.44	0.04
Metabolite III	0.31	0.36	0.02
Oxidation product of Metabolite II	0.73	0.69	0.63

The elution profile of this column is given in Figure 2. The two metabolites were separated by this treatment. After this stage the solutions containing metabolites II or III were treated separately.

METABOLITE II. The extract was rechromatographed on 20 g SG 31 as above. The metabolite fraction was then applied to a Kieselgel F254 preparative tlc plate using ^{14}C labeled metabolite which had been obtained in earlier studies as a marker. The plate was eluted with benzene:ethyl acetate (3:1 v/v). The metabolite was then removed from the plate and chromatographed on a Merck type E alumina plate using the same developing solvent. The material removed from this plate gave about 1 mg of a white crystalline solid on evaporating to dryness. The glc characteristics of the product were identical to those of the metabolite in crude feces extract.

METABOLITE III. This extract was treated in the same way as the Metabolite II extract, except that an extra thin-layer cleanup was used (Kieselgel F254, 20% acetone). The glc characteristics of the product were again identical with those of the material in crude feces extract. Less than 1 mg of Metabolite III was obtained, and this could not be crystallized.

Examination of Metabolites by Physical Methods. Metabolites I, II, and III were examined by mass spectrometry and infrared spectrophotometry. Metabolite II was also examined by proton magnetic resonance spectroscopy and, in the light of the results obtained by these methods, oxidized by chromium trioxide in pyridine. The product of this oxidation

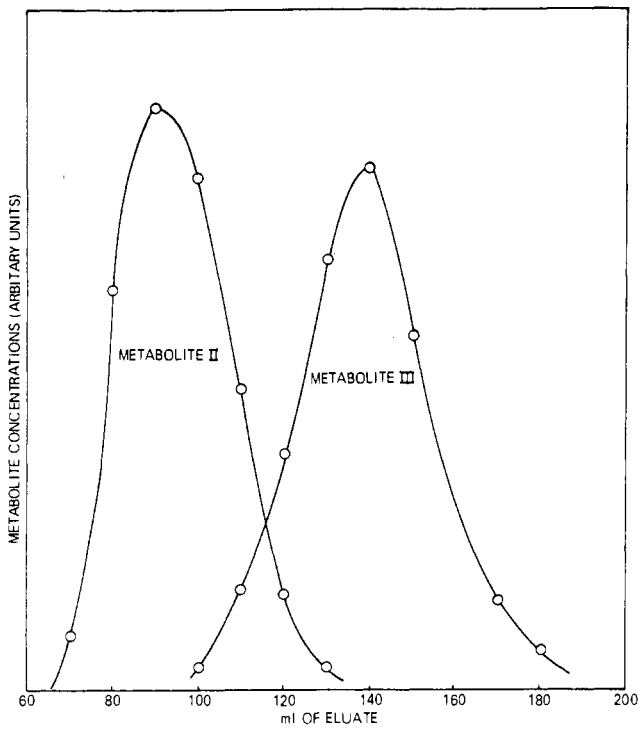

Figure 2. Elution profile of the two feces metabolites from 20 g SG31 using a 1 cm diameter column and 10% v/v acetone in hexane eluting solvent

was also examined by mass spectrometry, infrared spectrophotometry, and proton magnetic resonance spectroscopy.

Examination of Urine from Endrin Fed Rats. A sample of urine from male rats given an oral dose of 1.1 μCi of endrin-^{14}C was extracted with ethyl acetate. The urine and the extract were analyzed by scintillation counting for ^{14}C activity.

Urine from rats fed a diet of 10 ppm endrin was mixed with a sample of endrin-^{14}C urine and then extracted with diethyl ether. The extract was analyzed by gas chromatography using an electron capture detector, and thin-layer chromatography using silver nitrate as a chromogenic spray and also scanning the plate for ^{14}C activity.

RESULTS AND DISCUSSION

Infrared Examination of Metabolite I. The infrared spectrum of Metabolite I is shown in Figure 3. The main features are a strong band at 5.51 μ, assigned to a carbonyl group, and a band at 6.25 μ assigned to an olefinic double bond. The wavelength of this olefin absorption is the same as occurs in the spectrum of endrin. The wavelength of the carbonyl absorption is abnormal, since five-membered ring ketones usually absorb at or near 5.75 μ. There is no strong absorption band at or near 11.8 μ, the presence of which is associated with an epoxy group. However, proton magnetic resonance studies (see below under "Oxidative Studies with Metabolite II") suggest that the epoxy group is present in the molecule but is masked by interaction with the carbonyl group.

Mass Spectral Examination of Metabolite I. The most prominent feature of the mass spectrum of Metabolite I is a group of peaks assigned to the positively charged hexachlorocyclopentadiene ion. This is considered to be a fragment of the original molecule because the infrared spectrum of the metabolite is different from that of hexachlorocyclo-

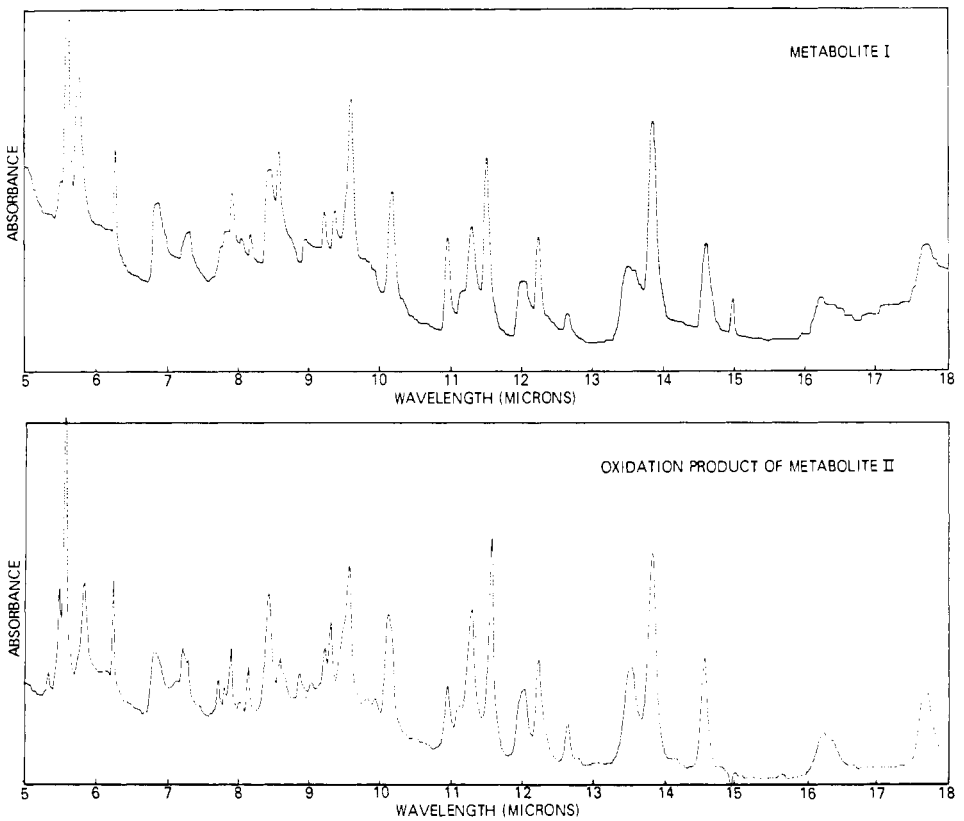

Figure 3. The infrared spectra of Metabolite I and the oxidation product of Metabolite II (KBr disc)

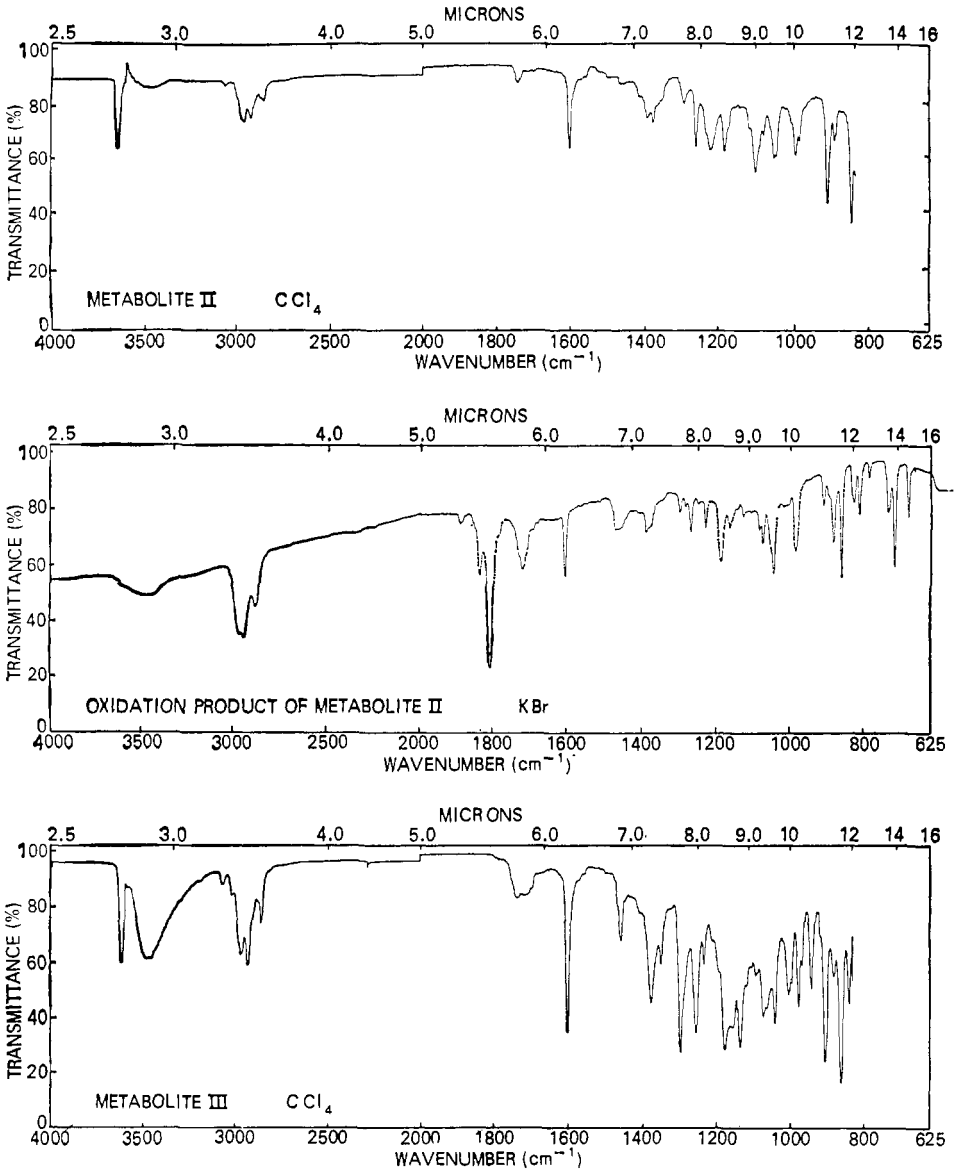

Figure 4. The infrared spectra of the three rat metabolites of endrin

pentadiene itself. The presence of this ion strongly indicates that the parent molecule contains a five-membered carbon ring bearing six chlorines and containing a double bond. A fuller discussion of the mass spectrum of this compound is found in the section entitled "Oxidative Studies with Metabolite II."

Infrared Examination of Metabolite II. The solution spectrum of Metabolite II in carbon tetrachloride is shown in Figure 4. Both solution and disc spectra of Metabolite II show absorption at 6.17 μ which we attribute to an olefinic double bond. There is also strong absorption at 11.83 μ and this is assigned to an epoxide group. These absorption bands are also shown by endrin. In carbon tetrachloride the spectrum of Metabolite II shows absorption at 2.72 μ which is attributed to an hydroxyl group.

Mass Spectral Examination of Metabolite II. The metabolite gives a parent ion corresponding to a molecule containing six chlorine atoms, and which has a mass of 394 based on ^{35}Cl (Figure 5). This, together with the infrared evidence, shows that the molecular formula of Metabolite II is $C_{12}H_8O_2Cl_6$, and since an hydroxyl group is present, it is likely that the structure is that of a monohydroxylated endrin.

Oxidative Studies with Metabolite II. The endrin molecule has a plane of symmetry, so that positions 4a and 8a, 5 and 8, 6 and 7 are, respectively, chemically identical. The two positions on carbon 9 are not identical (See Figure 1 for the numbering system).

If the hydroxyl group in Metabolite II were on carbons 4a, and 8a, 5 or 8, then it would be a tertiary hydroxyl group, and as such would not oxidize without rupturing the molecule.

If the hydroxyl group were on carbons 6 or 7, corresponding to a cyclic hemi-ketal, the oxidation product would be difficult to predict. If the hydroxyl group were on carbon 9, then the product of oxidation, in the absence of rearrangements, would be a ketone.

Oxidation of Metabolite II for $^1/_2$ hr at 100° C with chromium trioxide in pyridine gave complete conversion to a single product. Thin-layer chromatography of the oxidation product showed that the compound which responded to silver nitrate spray (chlorinated) had the same R_f value as that of the component which gave an orange spot with 2,4-dinitrophenyl hydrazine (carbonyl compound), thus indicating the presence of a carbonyl group in the oxidation product.

The mass spectrum of a large sample of oxidized compound

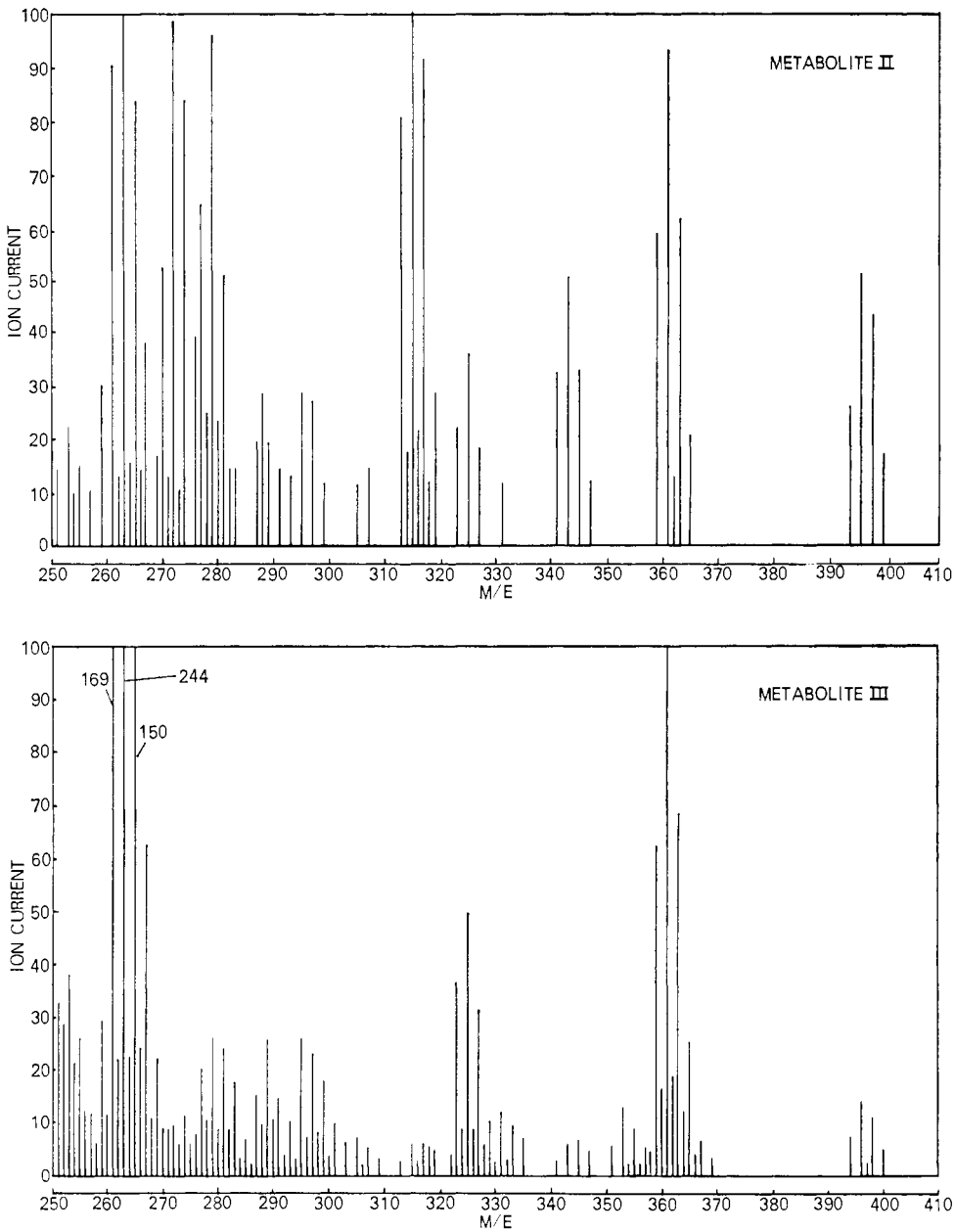

Figure 5. The mass spectra of Metabolites II and III

is similar to that of Metabolite I. There are minor peaks starting at *m/e* 392, corresponding to the molecular ion of 9 keto endrin ^{33}Cl, with fragment ions at *m/e* 357 (parent minus chlorine) and *m/e* 329 (parent minus chlorine and carbon monoxide). The major peaks were, as in the Metabolite I spectrum, due to the hexachlorocyclopentadiene ion. Clearly, the parent ion is very unstable and readily fragments to give the positive hexachlorocyclopentadiene ion.

Proton magnetic resonance studies were also made of the oxidation product using deutero-chloroform as solvent. With this solvent alone small peaks were observed at δ values of 1.8 and 3.6 ppm. In Figure 6 these peaks should be ignored. The region of the spectrum up to a δ value of 1.5 ppm was contaminated by coextracted material, but the rest of the spectrum was free from interference. Three peaks at δ values of 2.925, 3.15, and 3.55 ppm are prominent, and their areas are identical; therefore each corresponds to the same number of protons. 9-Keto endrin would be expected to give a similar pattern.

The infrared spectrum of the oxidation product contains an absorption band at 5.51 μ which, although it is at an unusual wavelength, is ascribed to a carbonyl group. Solution spectra do not show hydroxyl group absorption. The spectrum is very similar to that of Metabolite I (Figure 3) and this, together with glc evidence and the similarity of the mass spectra of these two compounds, shows that they are identical.

Molecular models of 9-keto endrin show that the epoxy group and the carbonyl group are very close together. It is possible that the anomalous infrared absorption bands of the epoxy and carbonyl groups are caused by interaction between these two groups.

Proton Magnetic Resonance Studies of Metabolite II. Several samples of Metabolite II were subjected to pmr analysis using carbon tetrachloride as solvent. All the spectra obtained were subject to some degree of interference because of impurities, but the spectrum above 1.5 ppm was free of interference. The spectrum is shown in Figure 6. There are four prominent absorption bands at δ values of 2.66,

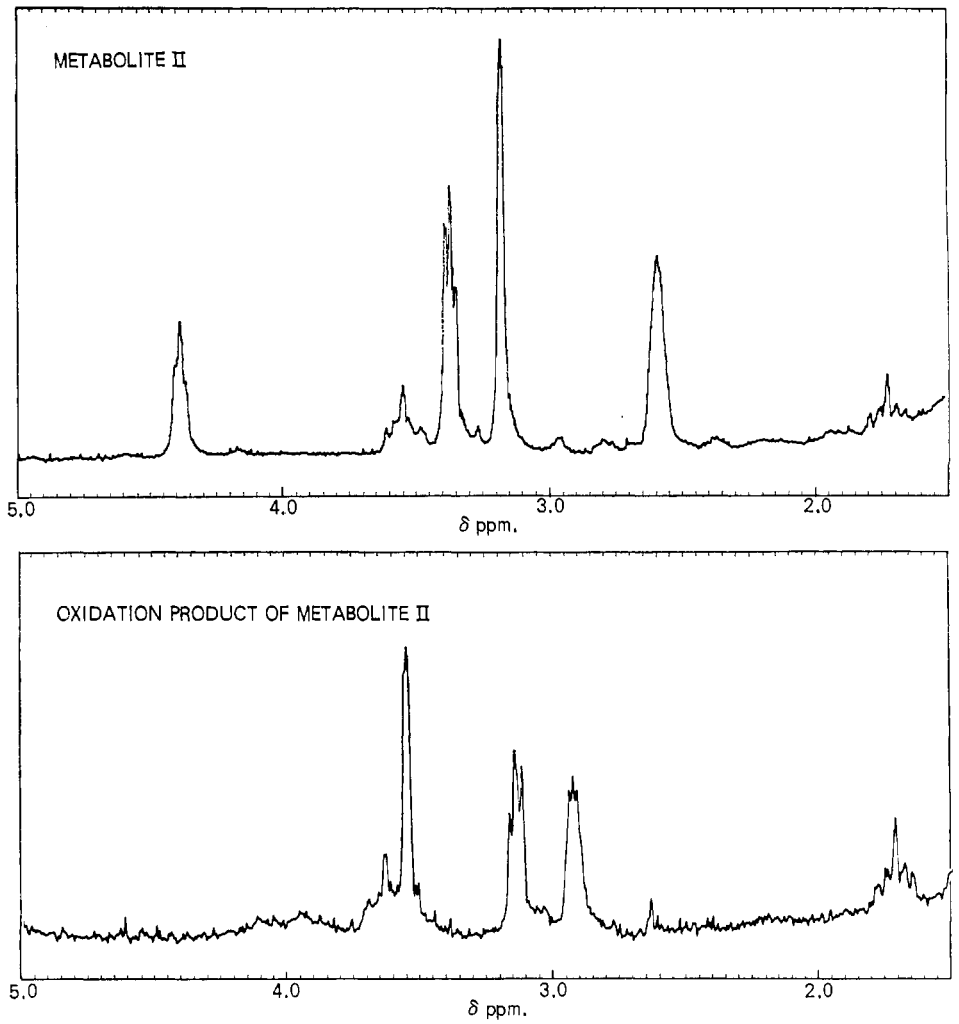

Figure 6. The proton magnetic resonance spectra of Metabolite II and its oxidation product

3.25, 3.44, and 4.45 ppm, with areas in the ratio of 2:2:2:1. The molecular formula of the compound implies the presence of eight protons, while the pmr spectrum shows only seven. The eighth proton, if it were the hydroxylic proton, would not necessarily be observed in the pmr spectrum. None of the seven protons that are observed can be attributed to a hydroxylic proton, since it was shown by deuteration of the metabolite with D_2O that no change occurred in the spectrum.

The pmr spectrum of endrin has a group of peaks forming a pair of doublets centered at 0.95 ppm and 1.80 ppm. This feature is caused by the methylene group on carbon 9. The Metabolite II spectrum does not have this group of peaks, and therefore it is unlikely that the methylene group is present. The single proton absorption at 4.45 ppm in the Metabolite II spectrum is consistent with the nonhydroxylic proton in the H—C—OH group. The presence of the three peaks of two protons each in the spectrum is strong evidence for the symmetry of the molecule, and all of the evidence from the pmr spectrum is consistent with Metabolite II being one of the two isomers of 9-hydroxy endrin.

It is possible, having accepted the structure of Metabolite II as being one of the two isomers of 9-hydroxy endrin, to assign tentatively the configuration of the hydroxyl group by comparison of the pmr spectra of endrin, dieldrin, and Metabolite II.

Dieldrin has the same structural formula as endrin, except that the five-membered ring containing carbons 1, 2, 3, 4, and 10 is so arranged that the double bond of that ring is adjacent to carbon 9; hence, the proton on carbon 9 which is *exo* with respect to the epoxy group is directed towards the double bond and lies near its center (Figure 1). This proton would therefore be expected to be more shielded in dieldrin than in endrin.

Comparison of the pmr spectra of dieldrin and endrin shows a shift in the δ values from 1.25 ppm to 1.80 ppm for a doublet associated with one proton of the methylene group. This proton is considered to be that which is *exo* with respect to the epoxy group. In the endrin spectrum this proton signal shows a fine splitting of about 2 c/s; the other methylene proton does not. One of the endrin absorptions due to two protons shows a similar fine splitting; the rest do not. This can be reasonably interpreted as meaning that the two protons on carbons 5 and 8 split the signal from the proton on carbon 9 *exo* to the epoxy group by about 2 c/s in the endrin molecule.

In the Metabolite II spectrum under high resolution, the single proton absorption and the two proton absorptions at 3.44 ppm show a mutual splitting of 1.8 c/s. This can reasonably be interpreted on the same basis as before; therefore, the proton on carbon 9 *exo* with respect to the epoxy group is present in Metabolite II, and it is concluded that the hydroxy group in this molecule is *endo* with respect to the epoxy group (Figure 1).

Infrared Examination of Metabolite III. The solution spectrum in CCl_4 of Metabolite III shows absorption at 2.70

μ ascribed to an hydroxyl group, 6.25 μ ascribed to an olefinic double bond, and 11.6 μ ascribed to an epoxide group.

Mass Spectral Examination of Metabolite III. The mass spectrum of Metabolite III shows a parent peak which corresponds to a molecule containing six chlorine atoms and which has a mass of 394 based on ^{35}Cl (Figure 5). This, in association with the infrared evidence, shows that Metabolite III is a monohydroxylated endrin.

Proton Magnetic Resonance Studies of Metabolite III. A very weak pmr spectrum was obtained from Metabolite III, and no interpretation of the structure of the metabolite could be obtained from it. However, the general appearance of the spectrum suggests that the symmetry of the endrin molecule has been destroyed. Further, the highest δ value of any peak in the spectrum occurs at 3.7 ppm, and it is unlikely that an H—C—OH group is present in Metabolite III.

Oxidative Studies with Metabolite III. No recognizable oxidation products of Metabolite III were produced. CrO_3 in pyridine does not oxidize it, while acid potassium permanganate destroys it without producing any product which gives a well defined gas chromatographic peak. It is unlikely that Metabolite III is a secondary alcohol, and therefore, since the double bond is still present in the molecule, it must be either a tertiary alcohol or a cyclic hemi-ketal formed by direct insertion of an oxygen into a carbon-hydrogen bond of endrin other than those on carbon 9.

Examination of Urine from Endrin Fed Rats. Approximately 35% of the radioactivity in the urine of the rats was extractable at pH 8 by ethyl acetate. The nature of the water-soluble material was not investigated, but the extractable radioactivity was present as endrin and Metabolite I.

CONCLUSIONS

Endrin is metabolized in the rat to give at least three metabolites. One is 9-keto endrin, and this is found in the tissues and urine.

The other two metabolites are excreted in the feces and have not been found in the tissues. The major one is that isomer of 9-hydroxy endrin having the hydroxyl group *endo* with respect to the epoxy group. The other metabolite is a monohydroxylated endrin, but the hydroxyl group is not on carbon 9.

These conclusions are supported by investigations of the metabolism of dieldrin, a conformational isomer of endrin, which have recently been reported (Feil *et al.*, 1970; Baldwin *et al.*, 1970).

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