Hydration of HEOD (Dieldrin) and the Heptachlor Epoxides by Microsomes from the Livers of Pigs and Rabbits

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The excretion of metabolic conversion products of dieldrin by mammals is now well established (1,2,3). The products so far identified appear to result from oxidative attack on the carbon skeleton (1,2) or from hydrative cleavage of the epoxide ring (3). For the oxidation processes there are circumstantial links with microsomal mixed function oxidase activity (4,5,6), but there is little direct evidence for metabolic oxidation by liver preparations (7,8) other than that presented by Matthews and Matsumura (9) for the NADPH stimulated metabolism of dieldrin by rat liver microsomes.

Microsomes from the livers of pigs and rabbits contain epoxide hydrases that convert a number of cyclodiene epoxides into the corresponding diols (10,11) and, since trans-dihydroaldrin-diol (DAD) was the main metabolite isolated from the urine of dieldrin-treated rabbits (3), the apparent inertness of dieldrin in early experiments with these preparations was surprising. Experiments undertaken to examine this problem more closely are now reported.

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Materials and Methods

HEOD (1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a, 5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene) was obtained by recrystallisation of a Shell technical sample of dieldrin. trans-Dihydroaldrin-diol (1,2,3,4,10,10-hexachloro-trans-6,7-dihydroxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene) was the gift of Shell Research Ltd. HE 160[1] and the epimeric epoxide HE 90[2] were analytical reference standards provided by the Velsicol Chemical Corporation, Chicago, Illinois. Benzylpenicillin B.P. and streptomycin sulphate B.P. were from Glaxo Laboratories, Ltd. and other chemicals were analytical reagent grade.

Analysis: Electron capture (100 mC tritium source) gas-liquid chromatography (GC) was conducted on a Pye Panchromatograph as in Table 1 or at 180° on 12 in., 0.25 in. i.d. glass columns of 80-100 mesh acid washed DMCS-treated chromosorb G (Perkin-Elmer Ltd.) carrying 2.5% SE.30 plus 0.5% Epikote 1001; carrier gas nitrogen at flow rate 170 ml/min.

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[1] Heptachlor epoxide m.p. 160°

[2] Heptachlor epoxide m.p. 89°

TABLE 1

Gas Chromatography (GC) of Dieldrin and the Heptachlor Epoxides, their Hydration products and the corresponding Trimethylsilyl (TMS) ethers

Compound	Retention time (t_R; min)	
	Underivatised	TMS-ether
Dieldrin	5.7	-
HE 160	3.7	-
HE 90	3.7	-
DAD	62.7	9.1
HE 160-diol	25.6	10.3
HE 90-diol *	25.6	10.3

GC was at 170° on 12 in., 0.25 in i.d. glass columns packed with 80-100 mesh acid washed, DMCS treated chromosorb G carrying 2.5% SE 30 plus 0.5% Epon 1001; carrier gas N_2 at flow rate 120 ml/min (electron capture detection).
* Mixture of two compounds according to TC.

Small amounts of DAD formed by the enzymic hydration of dieldrin were difficult to detect by GC in the presence of large amounts of unchanged dieldrin. Ether extracts of microsomal incubation mixtures were therefore evaporated to dryness in nitrogen, and the residues, redissolved in petroleum, b.p.40-60°, were chromatographed on small columns of alkaline alumina (Woelm, deactivated with 10% water; 12 x 5 mm) pre-washed with diethyl ether and petroleum, b.p.40-60°. Elution with petroleum continued

until the eluate (30-35 ml) contained only traces of dieldrin, when the columns were further eluted with diethyl ether/methanol (9:1; 2 x 10 ml portions). DAD was usually entirely eluted in the first portion and was readily detected by GC following concentration of the solvent to 1-2 ml. To exclude the possibility that other substances simulating DAD might be present in the final eluate, this procedure was followed through with microsomes or heat killed microsomes incubated alone, heat killed microsomes incubated with dieldrin, and microsomes plus dieldrin extracted without incubation. The final eluates were compared with those from the incubations with live microsomes. To test the recovery of DAD from incubation mixtures, dieldrin (500 μg) and DAD (2 μg) were added to pig liver microsomes and the DAD was recovered and estimated as usual (Expt 18, Table 2).

For conversion of the metabolite into its trimethylsilyl (TMS) ether, the final eluates were evaporated to dryness in nitrogen and the residues treated at ambient temperature (1 hr) with 0.4 ml of a mixture of dry pyridine (2.5 ml), hexamethyldisilazane (0.75 ml) and trimethylchlorosilane (0.5 ml). The mixtures, suitably diluted with petroleum b.p.40-60°, were usually sampled directly for GC-analysis.

The enzymic hydration products of HE 160 and HE 90 could be detected by GC without chromatographic removal of the unchanged epoxides, and the corresponding TMS-ethers could usually be detected in the presence of the epoxides following direct derivatisation of the ether extracts of incubation

mixtures.

Preparation of microsomes: Pig liver microsomes were prepared as described previously (12). The livers of freshly killed female New Zealand White rabbits were homogenised at 4° in 1.15% KCl (1:4 w/v) in a Waring Blender. Initial centrifugation at 20,000 *g* max ($\frac{1}{2}$ hr) to remove heavier particles was followed by further centrifugation at 104,000 *g* max (1 hr) to separate the microsomal pellet, which was resuspended in KCl and resedimented in the same manner. The washed microsomes were finally suspended in cold 1.15% KCl.

Incubation procedures: The appropriate epoxide (250 or 500 μg) in ethanol (50 μl), was normally added to the microsomal suspension (0.2 or 0.5 ml) in 0.1M Trisphosphate buffer pH 8.4 (4.5 ml), with 1.15% KCl added to give a total volume of 5 ml. Incubations were conducted in closed tubes at 37° or 45° for various times and parallel incubations were included in which water (1.25 ml) containing benzylpenicillin (3 mg; 5000 units) and streptomycin sulphate (0.54 mg; 400 units) was added to each 5 ml incubation mixture. Control experiments were also conducted with heat killed microsomes. Reactions were stopped by addition of acetone (7 ml) and the products recovered by three extractions with diethyl ether (4 ml portions). The combined organic phase was dried (Na_2SO_4) and adjusted to a volume suitable for GC-determination of diols and unchanged epoxides, or otherwise treated as indicated above.

TABLE 2

Hydration of Dieldrin by Liver Preparations

Expt.	Incubation time (hr) (any additions)	diol recovered (μg)	Expt.	Incubation time (hr) (any additions)	diol recovered (μg)
1	15	0.5	10	72 (antibiotics)	1.7
2	48	0.7	11	72 (water)	None
3	48	None	12	39	0.8
4	50 (water)	0.6	13	39	0.6
5	50 (antibiotics)	0.5	14	21	0.3
6	48	1.4	15	21	None
7	48	1.2	16	48	1.4
8	72 (water)	1.8	17	48	None
9	72 (antibiotics)	2.1	18*	0	1.8

Dieldrin (500 μg) incubated with 0.5 ml pig (Expt. 1-5) or rabbit liver (6-13) microsomal suspension or 3 ml rabbit liver homogenate (14-17) plus 0.1M tris-phosphate pH 8.4 (4.5 ml with microsomes, 7 ml with homogenate) with water (1.25 ml) or antibiotics (in 1.25 ml water; see text) added when shown. Microsomal protein 30 mg/ml. Rabbit liver homogenate equivalent to 0.7 g liver or 0.25 ml microsomal suspension. Incubations at 45° except 12-15 at 37°. 3,11,15 and 17 used heat killed preparations. In 10,11 and 12,13, dieldrin recovered from 10,8 and 9,11, respectively, was re-incubated as indicated. *Dieldrin (500 μg) and DAD (2 μg) added to microsomes to test recovery of DAD.

Results and Discussion

In early experiments with microsomes and liver slices at 30° or 37° for relatively short incubation periods (1-3 hr), hydration of dieldrin was not detected. Later experiments with rabbit and pig liver preparations (Table 2) showed low but consistent DAD formation that was not found with heat killed microsomes, and microorganisms were not responsible for the conversions since the inclusion of antibiotics in the incubation medium did not affect metabolite formation. Moreover, recovered dieldrin, chromatographically separated from the DAD produced by prolonged incubation with microsomes, underwent hydration in the same way on re-incubation, indicating that the product was not an artefact produced by the metabolism of an impurity present initially. Its conversion into a derivative having the GC-characteristics of DAD-trimethylsilyl ether, which was not produced in similarly treated extracts of controls, confirmed the formation of DAD. It is clear that epoxide hydrase activity toward dieldrin is present in these microsomal preparations but is quantitatively very different from the high activity toward other cyclodiene epoxides investigated (10,11). Nevertheless, this low activity toward dieldrin may provide the missing link between detoxication in the liver and the observed excretion of the hydration product by the dieldrin-treated rabbit (3). Rabbit liver microsomes appeared more active toward dieldrin than pig liver microsomes, although the latter had generally higher epoxide hydrase activity toward other compounds examined. Unfortunately,

the metabolic fate of dieldrin in the pig is unknown.

When HE 160 was incubated with these microsomal preparations, as described for dieldrin (Table 2), its rate of hydration was similar. Again, there was no metabolism with heat killed microsomes but the conversion was not affected by the presence of antibiotics, when the HE 160 was further recrystallised, or when recovered HE 160 freed from metabolite was re-incubated with fresh microsomal preparation. The product behaved as a diol and gave a TMS-derivative (Table 1) appearing as a single sharp peak on GC.

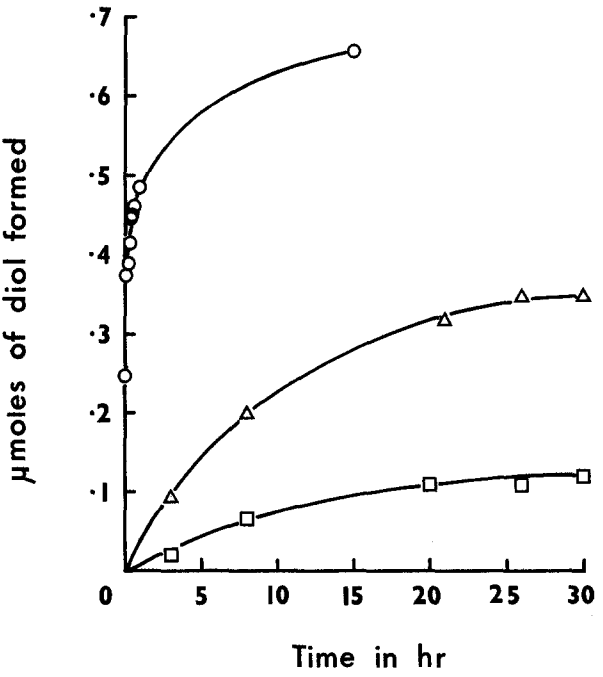

Figure 1. Hydration by pig liver microsomes of racemic chlordene anti-epoxide (ACE, -O-), racemic HE 90 (-△-) and laevorotatory HE 90 (-□-). Epoxides (250 μg) added in ethanol (50 μl) to microsomes (0.2 ml; 6 mg protein) in 0.1M tris-phosphate pH 8.4 (4.5 ml) and incubated at 45°.

In contrast, HE 90 was metabolised relatively rapidly by both preparations, although, as in a case previously reported (11), the hydration was selective toward one enantiomer; the recovered HE 90 was laevorotatory and was hydrated much more slowly than the racemate when re-incubated with pig liver microsomes (Fig. 1). This figure also shows the marked effect on hydration rate of removal of the chlorine atom adjacent to the epoxide ring; the resulting compound (chlordene anti-epoxide; ACE) is hydrated very rapidly. Thin layer chromatography showed the GC-peak called HE 90-diol (Table 1) to consist of two compounds which gave an unresolved (GC) mixture of TMS-ethers, and are presumably alternative products of trans-hydration of the epoxide ring, a conclusion supported by mass spectral data. Also, the preponderant diol appeared to be identical with the hydration product from HE 160.

HE 90 is evidently intermediate, in its susceptibility to epoxide hydrase attack, between the resistant epoxides such as dieldrin and HE 160 on the one hand, and the simpler, readily hydrated ACE on the other.

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