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THE OXIDATIVE METABOLISM OF ALDRIN AND DIHYDROALDRIN BY HOUSEFLIES, HOUSEFLY MICROSOMES AND PIG LIVER MICROSOMES AND THE EFFECT OF INHIBITORS

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Abstract—Dihydroaldrin undergoes hydroxylation at the C_6-position (or the equivalent C_7-position) in both pig liver and housefly microsomes to give a mixture of 6-exo- and 6-endo- hydroxydihydroaldrin. The oxidative metabolism of aldrin and dihydroaldrin therefore resembles the epoxidation and hydroxylation of certain similarly related steroids. The lower toxicity of dihydroaldrin, compared with aldrin, to normal houseflies is partly correlated with dihydroaldrin hydroxylation. Although there are differences between the two species, the microsomal enzymes responsible for epoxidation and hydroxylation in any one species are similar with regard to pH dependence, stability and response to inhibitors. The competitive inhibition of aldrin epoxidation by dihydroaldrin is consistent with these compounds acting as alternative substrates for the same enzyme, though conclusive evidence for this is lacking.

THE OBSERVATION of Sun and Johnson[1] that sesamex (Table 1), a known inhibitor of microsomal oxidation *in vitro* and *in vivo*, antagonises the poisoning action of aldrin on houseflies is in accord with the facts that (i) the conversion of aldrin into dieldrin (2; Fig. 1) is inhibited *in vivo*[1] and in microsomal systems *in vitro*[2] and (ii) the conversion of aldrin into dieldrin appears to be a toxication reaction, since dieldrin exerts its poisoning effect more rapidly and is apparently more toxic than aldrin to the housefly. The low order synergistic effects obtained by these authors[1] with some related compounds were difficult to explain on the ground of oxidative metabolism, since these compounds appeared to be rather stable in the housefly and it was generally believed that apart from epoxidation reactions[3] this type of insecticide was biologically inert.

With this background we assumed that dihydroaldrin (Fig. 1) would not be attacked *in vivo*, so that the 30-fold lower toxicity, compared with aldrin, of this compound to the housefly[4] would be a true reflection of the effect of the structural change, namely the reduction of the 6,7-double bond, that eliminates the possibility of epoxidation. However, the observation of an appreciable synergistic effect of sesamex with dihydroaldrin in houseflies[4] indicated that this compound also underwent an oxidative conversion, in this case a detoxication, and necessitated a reconsideration of the role of the epoxidation process in toxicity.[5] It became clear that when the detoxication mechanism is suppressed, dihydroaldrin is more toxic than had been supposed. A subsequent report[6] indicated that the detoxicative process involves hydroxylation of dihydroaldrin to give the 6-exo- and 6-endo-hydroxy derivatives (Fig. 1).

TABLE 1. INHIBITORS OF DRUG METABOLISM

Code*	Chemical name	Reference
Sesamex[a]	2-(3,4-methylenedioxyphenoxy)-3,6,9-trioxaundecane	4
SKF 525A[b]	2-diethylaminoethyl 2,2-diphenylvalerate hydrochloride	16, 18
Octachlorodipropyl ether[c] (O.D.E.)	*bis*-(2,3,3,3-tetrachloropropyl)ether	19
Norethynodrel[d]	17α-ethynyl-17-hydroxyestr-5(10)-en-3-one	20
SU 4885[e] (Metopirone®)	1,2-*bis*(3-pyridyl)-2-methyl-1-propanone	21
SU 10603[e]	3-(1,2,3,4-tetrahydro-1-oxo-7-chloro-2-naphthyl)-pyridine	22
SU 9055[e]	3-(1,2,3,4-tetrahydro-1-oxo-2-naphthyl)-pyridine	23
SU 8000[e]	3-(6-chloro-3-methyl-2-indenyl)-pyridine	22

* Supplied by: [a] Shulton Chemicals Inc., New York, U.S.A.
[b] Smith, Kline and French Laboratories Ltd., Welwyn Garden City, England.
[c] Badischen Anilin- & Soda-Fabrik AG, Ludwigschafen, Germany.
[d] G. D. Searle & Co., High Wycombe, England.
[e] Ciba Pharmaceutical Company, Summit, New Jersey, U.S.A.

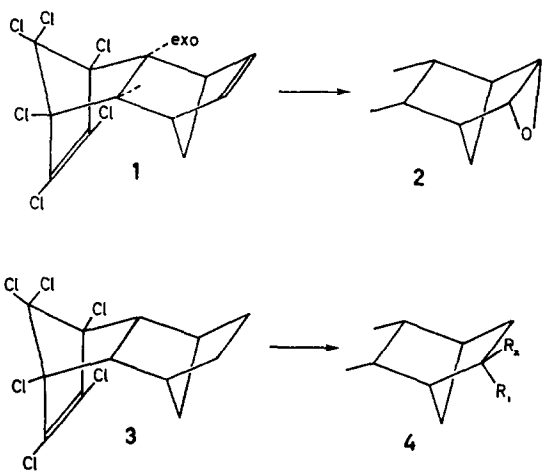

FIG. 1. Aldrin and dihydroaldrin and their metabolites. 1, Aldrin; 2, dieldrin; 3, dihydroaldrin; 4(R_1=OH, R_2=H), 6-exo-hydroxydihydroaldrin; 4(R_1=H, R_2=OH), 6-endo-hydroxydihydroaldrin.

Aldrin epoxidation and dihydroaldrin hydroxylation are of particular interest from a comparative standpoint since the epoxidation produces a rather stable, toxic product, whereas the hydroxylation is a detoxicative process which may be followed *in vivo* by conjugation and elimination. Since the two reactions involve the interaction of virtually isosteric compounds with microsomal enzymes it was of interest to compare the metabolism of these compounds more closely and to explore the possibility that both conversions might be effected by the same enzyme.

EXPERIMENTAL

Materials (Melting points are not corrected)

Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo, exo-5,8-dimethanonaphthalene; 1, Fig. 1), m.p. 103–106°, and its 6,7-exo-epoxide, dieldrin (2, Fig. 1), m.p. 175°, were obtained by recrystallisation of Shell technical samples. 6,7-Dihydroaldrin (3, Fig. 1), m.p. 78° was obtained by catalytic reduction of aldrin.[4] Treatment of

aldrin with acetic acid containing sulphuric acid gave 6-exo-acetoxydihydroaldrin[7] which afforded 6-exo-hydroxydihydroaldrin (Fig. 1; 4, $R_1 = OH$, $R_2 = H$), m.p. 125°, on hydrolysis. Cyclopentadiene condensed with vinyl acetate to give 5-endo-acetoxy-norborn-2-ene which with hexachlorocyclopentadiene afforded mainly 6-endo-acetoxydihydroaldrin[8] together with a little of the exo-epimer. Hydrolysis of the acetate with methanolic hydrogen chloride gave 6-endo-hydroxydihydroaldrin (Fig. 1; 4, $R_1 = H$, $R_2 = OH$), m.p. 131°, which was shown to contain a little of the exo-alcohol by thin-layer chromatography (TLC). γ-Hexachlorocyclohexane (γ-HCH) m.p. 113°, was provided by Imperial Chemical Industries Ltd. The code names, chemical names and sources of other compounds used as inhibitors of metabolic oxidation are listed in Table 1, together with references to their previous use in this connection. These compounds were used as obtained from the supplier, without further purification. Nicotinamide-adenine dinucleotide phosphate (NADP), glucose-6-phosphate (G-6-P) and G-6-P dehydrogenase were purchased from Boehringer, London, and all other chemicals were A.R. grade.

Identification of dihydroaldrin metabolites

Electron-capture (100 mc tritium source) gas–liquid chromatography (GLC) was conducted on a Pye Panchromatograph with a 2 ft, 0·25 in. internal diameter glass column packed with 100–120 mesh celite carrying 2·5% Apiezon L plus 0·25% Epikote 1001. The carrier gas was nitrogen; flow rate 150 ml/min; column temperature 160°.

Dihydroaldrin was incubated with pig liver microsomes as described below for 30 min and the products isolated as before.[6] GLC-analysis under the above conditions indicated, initially, the production of one major metabolite, together with two minor components with shorter retention times. The combined organic extract[6] from ten such incubations, containing about 400 μg of the hydroxylated chlorohydrocarbon, was concentrated and the residue, in petroleum spirit (fraction b.p. 40–60°) was passed through a column (5 × 0·5 cm) of alkaline alumina (Peter Spence, type H). Unchanged dihydroaldrin ran off with the petroleum spirit. The hydroxylated derivatives were then eluted with ether/methanol (4:1 v/v) and the eluate concentrated. Thin-layer chromatography on alkaline alumina (Woelm; no binder), with ether as mobile phase, indicated the presence of two major chlorinated components which were then separated by applying the total material (in ether) to ten channels of a fluted glass plate spread with dry alumina (Peter Spence Type H; 120–150 mesh). The "loose layers" were developed with ether and the separated zones located on the outside channels with ammoniacal silver nitrate reagent.[9] Corresponding zones from all channels were then bulked and the materials eluted from the alumina with ether.

The separated components were further purified by TLC on 0·25 mm layers of alkaline alumina (Woelm) with ether as eluant, outside spots being detected by Mitchell's method[9] and inside spots by exposure to iodine vapour. When purified, the separate components gave faint colours with iodine, whereas at this stage, the slower running component was contaminated with a substance strongly absorbing iodine. Subsequent runs on the same absorbent using benzene as eluant separated this contaminant from the hydroxyl-derivative. The hydroxyl-derivative of higher R_f value was similarly treated but appeared to be less contaminated. Finally, the components were extracted from the alumina with ether, the solvent replaced by spectroscopic

grade carbon disulphide, and the infrared spectra measured with a Perkin–Elmer Infracord spectrophotometer using ultra-micro cavity cells with a beam condenser and reference beam attenuator.

The purified metabolites, A and B, were also compared with authentic 6-exo- and 6-endo-hydroxydihydroaldrin, respectively, under various conditions of TLC- and GLC-analysis. The trimethylsilyl ethers[10] of the unknowns were similarly compared with those of the authentic compounds. The compounds obtained by incubation of dihydroaldrin with housefly microsomes were not isolated but were compared with authentic 6-exo- and 6-endo-hydroxydihydroaldrin by chromatographic techniques.

Investigations of insecticide fate in houseflies and microsomal preparations

Houseflies used were of the normal and dieldrin resistant strains described previously, the resistant strain being that described as RND (no longer exposed to dieldrin).[4] The penetration of dihydroaldrin into houseflies and its disappearance from the insect body were investigated in the presence or absence of sesamex by methods used earlier,[11] the insects being held in closed flasks during the experimental period.

Preparation of microsomes. Housefly microsomes were prepared from mixed sex RND flies by the method of Ray[12] and the microsomal pellet was finally suspended in 1·15% KCl. Pig liver microsomes were prepared as before[6] and usually contained 20–25 mg microsomal protein per ml as measured by the Biuret method.[13]

Incubation procedures. The insecticide or insecticide mixture, in ethanol (50 μl), was added to incubation medium (total volume 5 ml) consisting of the appropriate microsomal suspension (0·5 ml) and (final concentrations) Tris-phosphate buffer (5×10^{-2} M; pH 7·3 for pig liver microsomes, 8·3 for fly microsomes); G-6-P ($2{\cdot}4 \times 10^{-3}$ M); nicotinamide ($2{\cdot}45 \times 10^{-3}$ M); KCl ($1{\cdot}23 \times 10^{-2}$ M); NADP ($5{\cdot}2 \times 10^{-5}$ M) and G-6-P dehydrogenase (1·4 units). The incubation mixture for pig liver microsomes also included EDTA (10^{-3} M)[2] while that for housefly microsomes included KCN (2×10^{-4} M).[12] To examine the effect of pH on metabolism, KH_2PO_4/NaOH buffers (5×10^{-2} M) replaced the Tris-phosphate buffer. Incubations were conducted in open 25 ml conical flasks for 10 min at 30° in air, after which the products were isolated and determined as previously described.[6] With the GLC conditions described, the mixture of hydroxydihydroaldrin isomers was estimated as a single peak by comparison of peak height with a calibration curve obtained for the exo-isomer; the detector response to the two isomers was practically the same under these conditions. Dieldrin formation and the hydroxylation of dihydroaldrin were linear during the incubation period.

Inhibition experiments

In experiments on the effects of inhibitors listed in Table 1, these were added in ethanol (50 μl) immediately prior to addition of the insecticide as above. The I_{50} values (Table 2) were obtained by plotting per cent inhibition against molar concentration of inhibitor on semi-logarithmic paper. Four concentrations of each inhibitor were used and the lines were fitted by eye in each case. To determine the nature of the inhibition, up to 50-fold ranges of substrate (aldrin or dihydroaldrin) concentration were normally used, with two appropriate concentrations of inhibitor.

RESULTS

Identity of metabolites

Housefly and pig liver microsomes metabolised dihydroaldrin in the presence of $NADPH_2$ and air. No reaction occurred in the absence of the cofactor or when microsomes heated at 60° for 1 min were used, indicating the enzymic nature of the reaction. GLC-analysis of the products from incubation of dihydroaldrin with fly microsomes revealed one major peak clearly resolved from the unchanged chlorohydrocarbon. With pig liver microsomes, a major peak was observed having the same

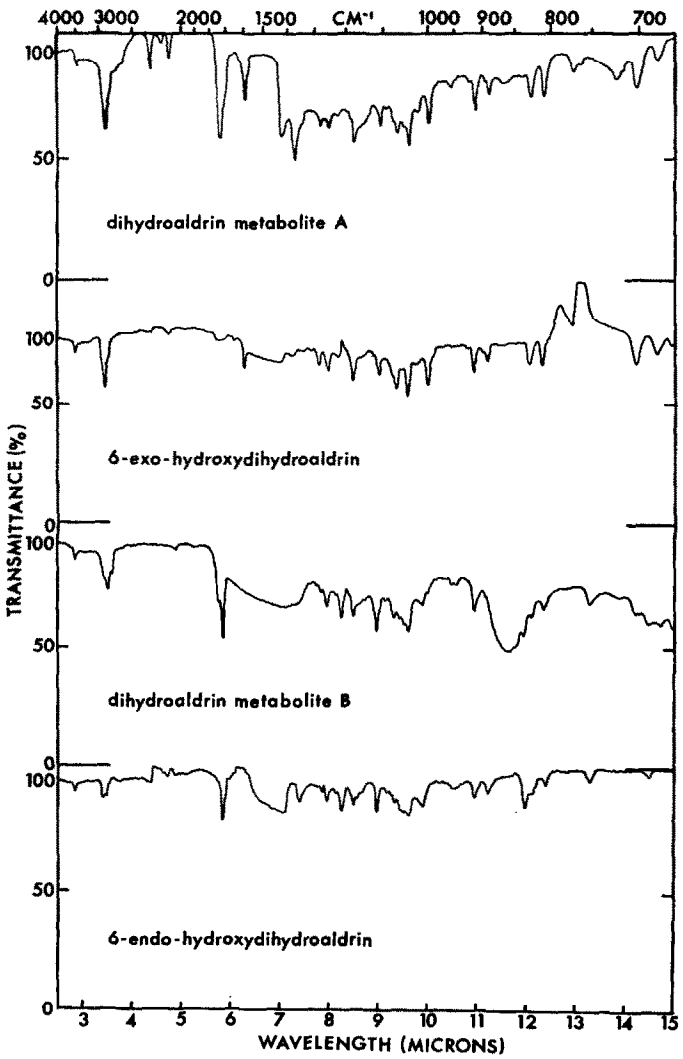

FIG. 2. Infrared spectra of dihydroaldrin metabolites and of authentic 6-exo- and 6-endo-hydroxydihydroaldrin measured in carbon disulphide in NaCl ultramicrocavity cells of light path 0·5 mm mounted in a beam condenser with reference beam attenuation. Cells contained 10–15 μg of the compounds. The greater CH-stretching absorption ($\sim$ 3000 cm^{-1}) in the metabolite spectra indicates the presence of impurities of biological origin, as is also shown by extraneous peaks in other parts of the spectra. The 2500 cm^{-1}–1400 cm^{-1} region of all spectra is complicated by the presence of incompletely compensated CS_2 absorption bands.

retention time (t_R) as the unknown from fly microsomes, together with two minor components of shorter t_R which were not further investigated. The major metabolite produced by either microsomal system was resolved into approximately equal amounts of two compounds, metabolites A and B, by TLC-analysis and the corresponding compounds produced by either preparation behaved identically in different TLC-systems, as did their trimethylsilyl ethers in both TLC[10]- and GLC-systems. Metabolite A and its trimethylsilyl ether were chromatographically indistinguishable from 6-exo-hydroxydihydroaldrin and the corresponding trimethylsilyl ether, respectively, while metabolite B was similarly indicated to be 6-endo-hydroxydihydroaldrin.

Infrared spectra of metabolites A and B are compared with those of 6-exo- and 6-endo-hydroxydihydroaldrin, respectively, in Fig. 2. Although the metabolites were not completely freed from natural contaminants, and each may have been contaminated with a little of the other, the spectral regions from 1400 cm^{-1} to 680 cm^{-1} were sufficiently definitive to confirm the identities.

Behaviour of dihydroaldrin in vivo *in houseflies*

Figures 3 and 4 show the penetration of dihydroaldrin (0·37 μg/fly) into adult houseflies and its disappearance from insect tissue. There was a considerable increase

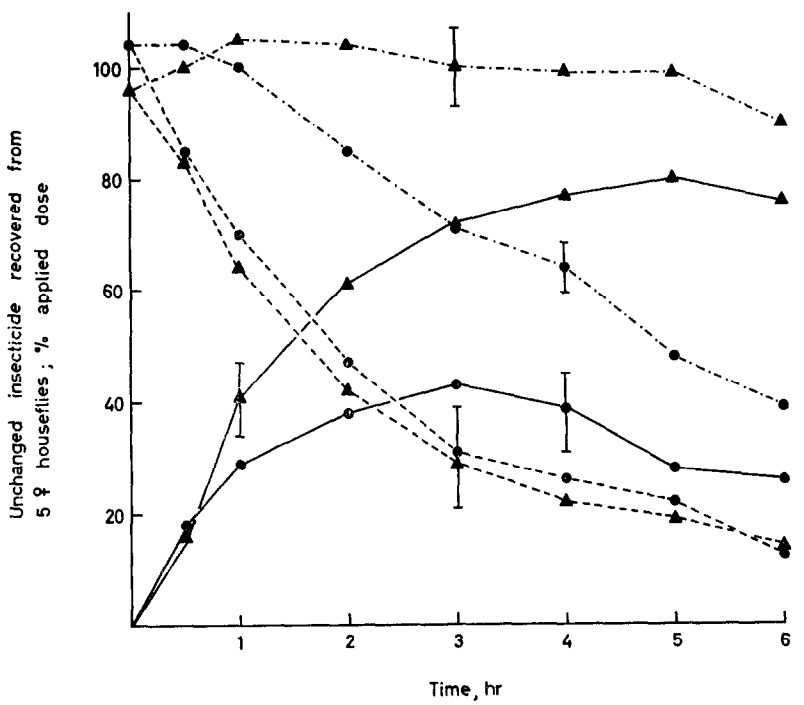

FIG. 3. Effect of sesamex on the recovery of dihydroaldrin (0·37 μg/fly) from normal houseflies. Applied alone, this dose was virtually sub-lethal. With sesamex (5 μg/fly) applied 3 hr before the insecticide, signs of poisoning appeared within 3 hr and irreversible knock-down followed. Dashed curves, cuticle rinses; full lines, tissue extracts; dash-dots, total recoveries. Dihydroaldrin alone, ●; following pre-application of sesamex, ▲. Points averaged from two experiments. Vertical lines show maximum range on each curve.

in the amount of dihydroaldrin remaining in the tissues when sesamex (5 μg/fly) was applied 3 hr before the insecticide and in the case of the normal strain (Fig. 3), this effect was accompanied by synergism resulting in complete knock-down.[4] Because of the possible influence of toxic effects on the tissue levels of toxicant, the same experiment was conducted with the RND strain (Fig. 4), which was resistant to dihydroaldrin

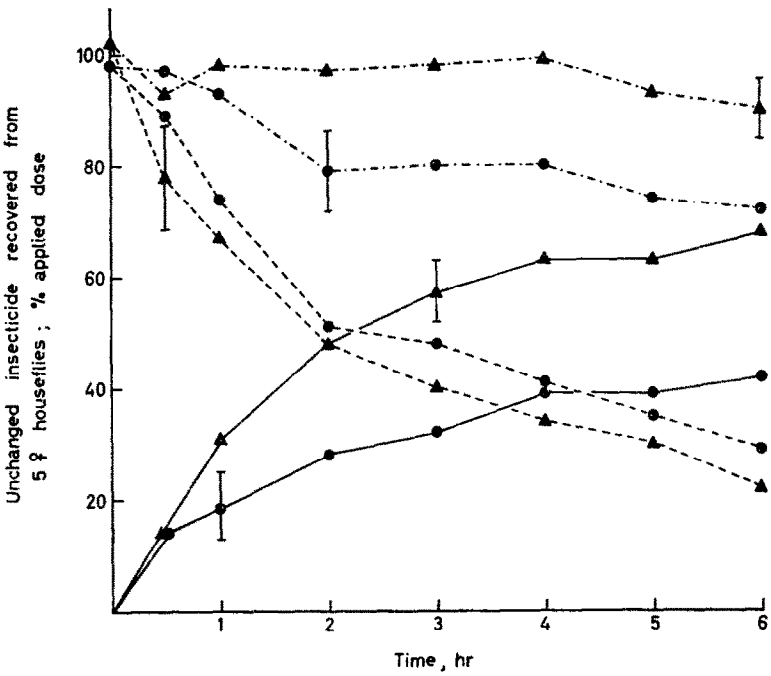

FIG. 4. Effect of sesamex on the recovery of dihydroaldrin (0·37 μg/fly) from houseflies resistant to this toxicant and unaffected by it when sesamex (5 μg/fly) was applied 3 hr previously. Other details as for Fig. 3.

both with and without sesamex,[4] thereby eliminating any effects due to toxicity. Though the effect was less pronounced with this strain, there was nevertheless a clear increase in the amount of toxicant remaining in the tissues following pre-application of sesamex. Metabolites were not measured in these experiments but GLC-analysis of tissue extracts of dihydroaldrin treated flies indicated the presence of the hydroxylation products previously discussed.

pH optima for aldrin epoxidation and dihydroaldrin hydroxylation by microsomes

The effect of pH on these microsomal oxidations in housefly and pig liver microsomes is shown in Fig. 5. For housefly microsomes, the curve for dihydroaldrin hydroxylation resembled that found for aldrin epoxidation by Ray.[12] With pig liver microsomes the curves were again similar but pH optima for the two oxidations were better defined in this case, with activities declining rapidly as the pH increased beyond 7·5. The activities in the KH_2PO_4/NaOH buffer were the same at the pH optima as those in the Tris-phosphate buffer normally used.

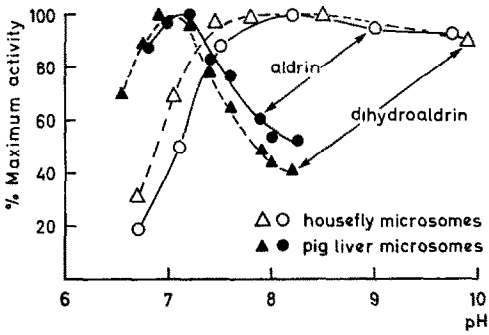

FIG. 5. Effect of pH on the epoxidation of aldrin and hydroxylation of dihydroaldrin by housefly and pig liver microsomes. Incubations at 30° for 10 min. Incubation mixture (total volume 5 ml): KH_2PO_4/NaOH buffer (5×10^{-2} M); G-6-P ($2{\cdot}4 \times 10^{-3}$ M); nicotinamide ($2{\cdot}45 \times 10^{-3}$ M); KCl ($1{\cdot}23 \times 10^{-2}$ M); NADP ($5{\cdot}2 \times 10^{-5}$ M); G-6-P dehydrogenase (1·4 units); KCN (2×10^{-4} M; fly only); EDTA (10^{-3} M; pig only); aldrin or dihydroaldrin (100 μg in 50 μl ethanol); appropriate suspension (0·5 ml) containing microsomal protein (12·8 mg, fly; 10 mg, pig).

Time course of the decline of oxidative activities

The ability of both pig liver microsomes and housefly microsomes to epoxidise aldrin and hydroxylate dihydroaldrin declined with time as shown in Fig. 6. Both

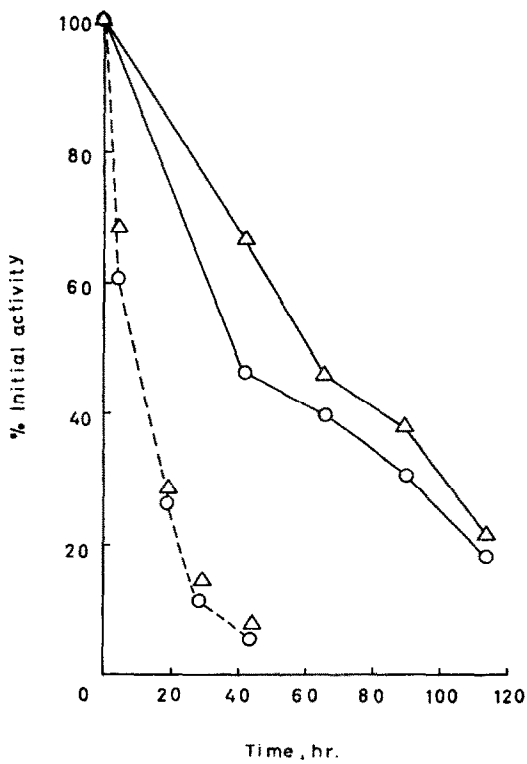

FIG. 6. Decline with time in the ability of housefly and pig liver microsomes to epoxidise aldrin and hydroxylate dihydroaldrin. Incubation conditions as in Table 2. Pig,——; housefly,- - - - -; Aldrin epoxidation, ○; dihydroaldrin hydroxylation △. Initial activities:—○—, 0·052 μg/min/mg microsomal protein; —△— 0·06 μg/min/mg protein;- - -○- - - 0·072 μg/min/mg protein; - - -△- - - 0·067 μg/min/mg protein.

activities persisted for much longer in the pig system, but for either system there was a close parallel in the rate of their decline. The ratio of initial hydroxylation rate to epoxidation rate in freshly prepared pig liver microsomes varied in different preparations and was occasionally nearly 2:1 (mean ratio $\pm$ S.E., eight determinations, $1{\cdot}35 \pm 0{\cdot}16$). Housefly preparations gave a mean ratio (13 determinations) of $1{\cdot}09 \pm 0{\cdot}13$.

Inhibition experiments

The results of inhibition experiments are shown in Table 2. For a particular inhibitor and microsomal system, the I_{50} values determined for the epoxidation of aldrin and the hydroxylation of dihydroaldrin were closely similar, while the variety of inhibitory structures chosen afforded a 40-fold range of values for pig liver microsomes and a 100-fold range for the housefly system. Housefly microsomes appeared, with two exceptions, more susceptible than the pig liver system to the inhibitory effect of the compounds listed.

Reciprocal plots constructed from inhibition data were unsatisfactory since they frequently showed the curvilinear trend described previously.[2, 12] However, the Lineweaver–Burk plots relating to the effect of dihydroaldrin on aldrin epoxidation (Fig. 7) indicated that the inhibition is competitive, although there is a departure from deality with pig liver microsomes at low substrate concentrations.

The oxidative activity of microsomal preparations from both houseflies and pig liver was rather variable and kinetic values obtained differed between preparations, although the variation was reduced when K_m and related quantities were expressed as μg per mg of microsomal protein (as a measure of concentration in the microsomal material). In these circumstances there was no significant difference ($P > 0{\cdot}05$) between K_i for dihydroaldrin (as an epoxidation inhibitor) and K_m for its hydroxylation, with microsomes from either source.

DISCUSSION

Pig liver and housefly microsomes attack aldrin at the C_6- and C_7-positions to give dieldrin. With these microsomal systems there is no evidence for oxidative attack at any other position in the aldrin molecule, so that attack on the sterically similar dihydroaldrin should involve the same positions. The present work confirms that hydroxylation occurs at C_6 (or C_7) with both housefly and pig liver microsomes to give, with either system, a mixture of about equal amounts of the exo- and endo-hydroxy derivatives, which are known to have very low toxicity.[14] It may be noted that 6-exo-hydroxydihydroaldrin and 7-exo-hydroxydihydroaldrin are an enantiomeric pair, as are the corresponding endo-derivatives. In the present investigation the metabolites were not examined for optical activity, which might arise if enzymic hydroxylation occurred preferentially at one of the positions C_6 or C_7 to give predominantly one enantiomeric form. Under the conditions used there was no evidence for the formation of idols by hydroxylation at both C_6 and C_7; the 2 additional minor components formed by pig liver microsomes were less polar than the monohydroxy-compounds and were not identified. The oxidative conversions of aldrin and dihydroaldrin are thus fully analogous to the corresponding conversions of certain unsaturated steroids and their saturated analogues.[15]

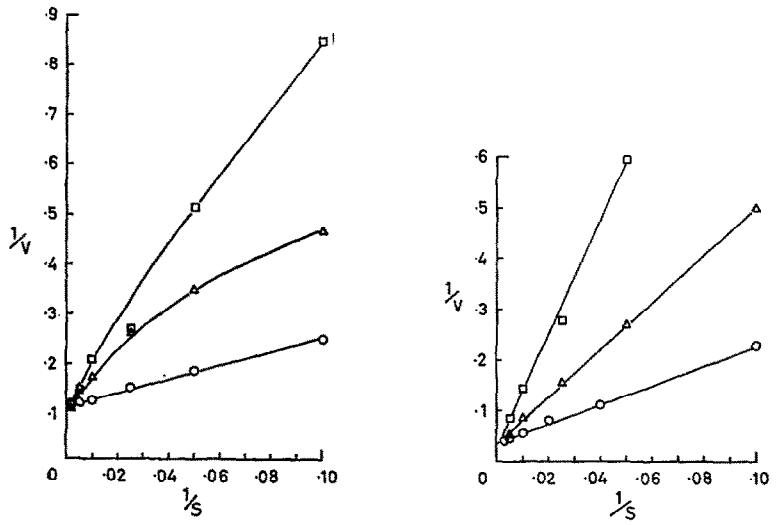

FIG. 7. Reciprocal plots of aldrin epoxidation by (a) pig liver microsomes and (b) housefly microsomes and the effect of added dihydroaldrin. Incubation conditions as described in Table 2. S = aldrin added (μg) in ethanol (50 μl); V = dieldrin produced (μg/10 min). 50 μl ethanol + no inhibitor, ○; 50 μg dihydroaldrin in 50 μl ethanol, △; 100 μg dihydroaldrin in 50 μl ethanol, □.

TABLE 2. THE INHIBITION BY VARIOUS COMPOUNDS OF ALDRIN EPOXIDATION AND DIHYDROALDRIN HYDROXYLATION IN MICROSOMES PREPARED FROM HOUSEFLIES AND PIG LIVER

Compound inhibiting	I_{50} (Molar concentration)			
	Pig liver microsomes		Housefly microsomes	
	E*	H*	E*	H*
SU 10,603	$2{\cdot}6 \times 10^{-5}$	$2{\cdot}5 \times 10^{-5}$	$1{\cdot}7 \times 10^{-6}$	$1{\cdot}4 \times 10^{-6}$
SU 4885	$5{\cdot}8 \times 10^{-5}$	$7{\cdot}4 \times 10^{-5}$	$1{\cdot}1 \times 10^{-6}$	$1{\cdot}4 \times 10^{-6}$
SU 9055	$5{\cdot}4 \times 10^{-5}$	$6{\cdot}0 \times 10^{-5}$	$4{\cdot}5 \times 10^{-6}$	$3{\cdot}0 \times 10^{-6}$
SU 8000	16 % at $1{\cdot}0 \times 10^{-3}$	22 % at $1{\cdot}0 \times 10^{-3}$	$1{\cdot}2 \times 10^{-4}$	$1{\cdot}2 \times 10^{-4}$
SKF 525A	$1{\cdot}1 \times 10^{-3}$	$6{\cdot}6 \times 10^{-4}$	$6{\cdot}0 \times 10^{-4}$	$4{\cdot}0 \times 10^{-4}$
Sesamex	$5{\cdot}3 \times 10^{-4}$	$7{\cdot}0 \times 10^{-4}$	$8{\cdot}0 \times 10^{-4}$	$5{\cdot}0 \times 10^{-4}$
O.D.E.	$6{\cdot}8 \times 10^{-4}$	$8{\cdot}5 \times 10^{-4}$	$1{\cdot}5 \times 10^{-4}$	$1{\cdot}2 \times 10^{-4}$
Norethynodrel	$2{\cdot}0 \times 10^{-4}$	$1{\cdot}8 \times 10^{-4}$	$1{\cdot}7 \times 10^{-6}$	$1{\cdot}7 \times 10^{-6}$
Dieldrin	—	5×10^{-5}	—	$3{\cdot}5 \times 10^{-5}$
γ-HCH†	$6{\cdot}0 \times 10^{-4}$	$5{\cdot}0 \times 10^{-4}$	$3{\cdot}0 \times 10^{-6}$	$2{\cdot}0 \times 10^{-6}$

Incubations at 30° for 10 min. Incubation medium (final concentrations): tris/phosphate (5×10^{-2} M; pH 7·3 for pig liver microsomes, 8·3 for fly microsomes); G-6-P ($2{\cdot}4 \times 10^{-3}$ M); nicotinamide ($2{\cdot}45 \times 10^{-3}$ M); KCl ($1{\cdot}23 \times 10^{-2}$ M); NADP ($5{\cdot}2 \times 10^{-5}$ M) and G-6-P dehydrogenase (1·4 units); 0·5 ml of appropriate microsomal suspension containing approximately 12 mg protein. Aldrin or dihydroaldrin (100 μg) added in ethanol (50 μl) and inhibitors in ethanol (50 μl); total volume 5 ml. EDTA (10^{-3} M) added to pig liver microsomes and KCN (2×10^{-4} M) to fly microsomes.

* E = aldrin epoxidation; H = dihydroaldrin hydroxylation.

† Approx. values estimated from Lineweaver–Burk reciprocal plots.

Figures 3 and 4 show the stabilising effect of sesamex on dihydroaldrin *in vivo* in normal and dieldrin resistant houseflies, respectively. Sesamex stabilises dihydroaldrin in both strains, but the lack of toxic effects with the dieldrin resistant strain indicates that although this particular metabolic route is involved in the natural tolerance of houseflies for dihydroaldrin, it is not involved in the cross tolerance to dihydroaldrin that accompanies acquired dieldrin resistance.[4]

For a particular species the pH requirements for epoxidation and hydroxylation, though not identical, are closely similar. They differ markedly between housefly and pig. The parallel rates of decline of the two activities for either species (Fig. 6) again suggests a relationship between the enzyme systems involved.

Nakatsugawa *et al.*[16] found slightly different pH optima for the epoxidation of aldrin and the related compound heptachlor and showed that each substrate inhibited the epoxidation of the other, while the epoxides produced also inhibited the epoxidation reactions. If different enzymes were involved, they clearly each had an affinity for the substrate (and product) of the other.

The I_{50} values listed in Table 2 show that any particular compound from the variety of structures chosen (Table 1) inhibited the epoxidative and hydroxylative activities similarly, as would be expected if the same enzyme catalysed both reactions. The generally greater susceptibility of the housefly enzymes to inhibition by these compounds resembles the finding for 1,3-benzodioxoles tested as inhibitors of aldrin epoxidation in housefly and pig liver microsomes.[2] For the 1,3-benzodioxoles, the I_{50} values for the housefly system were 10–100 times lower than for the pig liver system. The first four compounds in Table 2 were chosen because they are good inhibitors of steroid hydroxylation[21–23] and extend the types of inhibitory structures tested. It is noteworthy that although these SU compounds are good inhibitors *in vitro* of both aldrin epoxidation and dihydroaldrin hydroxylation they do not synergise dihydroaldrin in houseflies and evidently do not have the requirements for *in vivo* activity possessed by 1,3-benzodioxoles such as sesamex. Nothing is known of the rate of penetration of these compounds through the insect cuticle or of their fate *in vivo* in insects.

The inhibition of aldrin epoxidation by dihydroaldrin appears to be competitive (Fig. 7). The departure from linearity in the reciprocal plots from some experiments at low substrate concentrations is difficult to understand since it implies abnormally high dieldrin production at these concentrations; obvious factors such as depletion of substrate leading to lowered initial velocities would give artificially high reciprocals. The situation is complicated by the low water solubility of these compounds and it is likely that they are taken up rapidly and completely by the lipids associated with the microsomes. In their discussion of aldrin epoxidation, for example, Lewis *et al.*[2], showed that for a given amount of microsomal suspension, dieldrin formation depended not upon the calculated concentration of aldrin in the aqueous medium, but upon the absolute amount of aldrin added; that is, on the concentration of aldrin in the microsomal material. In these circumstances, quantities such as K_m values have doubtful meaning.

In a report by Rubin *et al.*,[17] all the drugs inhibiting the *N*-demethylation of ethylmorphine by rat liver microsomes were themselves oxidised by microsomes, while barbital, which was not metabolised, was not an inhibitor. They indicated that the drugs which were metabolised could compete with each other by acting as alterna-

tive substrates for the same enzyme. However, Nakatsugawa *et al.*,[16] showed that heptachlor epoxide, dieldrin and γ-HCH all inhibited aldrin epoxidation although they were not themselves metabolised by microsomes. In the present study, aldrin epoxidation was inhibited, apparently competitively, by γ-HCH in both housefly and pig liver microsomes. Dieldrin and γ-HCH also competitively inhibited dihydroaldrin hydroxylation in the housefly system, so that the inert compounds evidently have strong inhibitory effects (Table 2). On this basis, dihydroaldrin might be an inert competitive inhibitor for the aldrin epoxidising enzyme, while being itself hydroxylated by a different but similar enzyme, a possibility supported to some extent by the variable ratio of the hydroxylative to the epoxidative activity in different microsomal preparations from either species. For both species, however, the mean ratios were close to unity, emphasising the similar order of the activities and supporting the alternative substrate hypothesis.

Experiments to test the role of dihydroaldrin as an alternative substrate[17] for the aldrin epoxidising enzyme by comparing its K_i as an inhibitor of this enzyme with K_m for the hydroxylation process gave unsatisfactory results arising probably from a combination of solubility difficulties and variations in the activity of successive microsomal preparations. The similarity between K_i and K_m when expressed as concentrations of chlorohydrocarbon in the microsomal material indicates an alternative substrate role for dihydroaldrin but a detailed examination of this hypothesis would be better conducted with a more reproducible microsomal preparation such as that obtainable from rat liver.

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