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The epoxidation of aldrin by a modified Fenton's reagent and its inhibition by substituted 1,3-benzodioxoles

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FENTON'S reagent[1,2] and the Udenfriend system[3,4] have been widely employed as models in investigations of the mechanisms of enzymatic hydroxylation.[5,6] These systems often serve as useful models for the microsomal mixed-function oxidases, and the products resulting from aromatic hydroxylation are similar to those produced enzymatically.[7]

Although the epoxidation of double bonds is a well established property of microsomal enzymes,[8] there are no reports of epoxidation with either Fenton's reagent or the Udenfriend system. It has recently been reported that model systems involving OH-radicals are unable to hydroxylate aliphatic CH-bonds and do not form epoxides at a double bond.[9] Epoxidation is usually effected by peracid oxidation and is thought to occur by means of a mechanism involving the electrophilic OH^+ ion.[10] Thus, the epoxidation of 8- and 12-membered cyclic olefins has recently been reported to occur with hydrogen peroxide in the presence of metallic oxide catalysts and is considered to proceed through a highly active peracid intermediate.[11]

In the course of our continuing investigations of microsomal metabolism and of the mode of action of insecticide synergists, it was of interest to develop a model system capable of converting the cyclodiene insecticide, aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,3-endo-exo-5,8-dimethanonaphthalene), to its 6,7-epoxide, dieldrin. Initial attempts in this laboratory to epoxidize aldrin by means of either the Udenfriend or Fenton's system were unsuccessful. It was, however, established that the incorporation of bovine serum albumin (BSA) into Fenton's reagent resulted in the epoxidation of aldrin to dieldrin (Table 1). No similar initiation of dieldrin production was observed to occur, however, as a result of the inclusion of BSA in the Udenfriend system. In the presence of a similar concentration of egg albumin, aldrin epoxidation by the Fenton's reagent was approximately half that obtained with BSA. Although serum albumin and other sulfhydryl-containing proteins have been found to be necessary for some enzymatically catalyzed hydroxylation reactions,[12]

TABLE 1. EFFECT OF ALBUMIN ON ALDRIN EPOXIDATION BY FREE RADICAL SYSTEMS

System*	Dieldrin produced (μg)
Fe^{2+}/EDTA/H_2O_2 (Fenton)†	0
Fe^{2+}/EDTA/ASC (Udenfriend)‡	0
Fe^{2+}/EDTA/ALB/H_2O_2 (Fenton)	2·25
Fe^{2+}/EDTA/ALB/ASC (Udenfriend)	0
Fe^{2+}/EDTA/Egg ALB/H_2O_2 (Fenton)	1·21

* Incubations at 30° for 30 min. ALB = albumin; ASC = ascorbic acid.

† $FeSO_4$, 15 μM; EDTA, 15 μM; ALB, 0·75 ml, 0·5% aqueous soln. ($1·8 \times 10^{-5}$M); H_2O_2, 0·5 ml, 30%; aldrin, 25 μg (25 μl acetone); final volume, 3·5 ml.

‡ $FeSO_4$, 15 μM; EDTA, 15 μM; ALB, 0·75 ml, 0·5% aqueous soln.; ascorbic acid, 30 μM; aldrin, 25 μg (25 μl acetone); final volume, 3·5 ml.

the role of serum albumin in Fenton's reagent is unclear. It is possible that the protein is supplying a surface upon which the highly lipophilic aldrin could be distributed in the aqueous Fenton's reagent.

As a result of a series of experiments in which the concentration of each of the components of the Fenton's system was independently varied, a satisfactory conversion of aldrin to dieldrin was obtained in incubations containing 0·75 ml of a 0·5% aqueous solution of BSA, 0·5 ml water, 2·0 ml of 15 μM $FeSO_4$. 7 H_2O–EDTA and 0·25 ml of 30% H_2O_2. Aldrin (25 μg) was added in acetone (25 μl) and the 30-min incubations were carried out aerobically in 25-ml Erlenmeyer flasks shaken at 30°. The reaction was terminated by addition of 4 ml acetone and immediate extraction with two 5-ml aliquots of petroleum ether. Dieldrin was determined by gas–liquid chromatography on a Varian Aerograph Hy Fi instrument containing a column of 5% SE 30 on Chromosorb W.[13]

The effect on aldrin epoxidation of varying the concentration of BSA in the Fenton's system is shown in Table 2. This clearly shows that maximal conversion occurs on incorporation of 0·75 ml of a 0·5% solution of BSA (3·75 mg). Concentrations of BSA below or in excess of this value resulted in a reduction in epoxidation.

Dieldrin production was also found to be dependent on the amount of hydrogen peroxide in the system. A yield of 2·29 μg dieldrin was obtained with incorporation of 0·25 ml of 30% H_2O_2, while 0·1, 0·5 and 0·75 ml produced yields of 1·70, 1·75 and 1·03 μg dieldrin respectively.

To determine the effect of aldrin concentration on dieldrin production by Fenton's reagent, the

TABLE 2. EFFECT OF BOVINE SERUM ALBUMIN ON ALDRIN EPOXIDATION BY FENTON'S REAGENT*

Albumin (ml 0·5% soln.)	Dieldrin produced (μg)
0	0
0·25	0·97
0·50	1·65
0·75	1·73
1·00	1·43

* Incubations at 30° for 30 min. Incubation mixture (final concentration): 2·0 ml of 15 μM $FeSO_4$·7 H_2O–EDTA, 0·25 ml of 30% H_2O_2, and water and/or 0·5% solution BSA to give total incubation volume of 3·5 ml. Aldrin, 25 μg, was added in acetone (25 μl).

TABLE 3. EFFECT OF ALDRIN CONCENTRATION ON DIELDRIN PRODUCTION BY MODIFIED FENTON'S REAGENT*

Aldrin (μg)	Dieldrin produced (μg)
12·5	1·15
25	2·20
50	3·40
100	4·35

* Incubation mixture and conditions as described in Table 2.

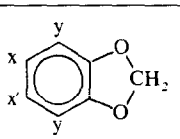
amount of aldrin added to the system was varied from 12·5 to 100 μg. A steady increase in epoxidation was observed to occur with increasing aldrin concentration over this range (Table 3), and the double reciprocal plot of these data shows a straight line relationship similar to that obtained in enzymatic reactions. The significance of this is not entirely clear at the present time, but may indicate that aldrin is binding to the BSA.

The mode of action of insecticide synergists of the 1,3-benzodioxole type has received considerable attention in recent years.[14-16] It is now generally accepted that they are active by virtue of their ability to inhibit the microsomal mixed-function oxidases responsible for insecticide metabolism, although the mechanism of inhibition is not yet clear. Casida *et al.*[17] have suggested that because the 1,3-benzodioxoles are themselves metabolized by the microsomal enzymes they are capable of competitively inhibiting the metabolism of other materials by the alternative substrate mechanism. Support of this mechanism is found in the results of work on the microsomal metabolism of 4,5,6,7-tetrachloro-1,3-benzodioxole.[18] Hennessy,[19] however, suggested that inhibition might occur as a result of hydride ion transfer from the methylene group of the ring and consequent formation of the electrophilic benzodioxolium ion. More recently Hansch[20] has suggested that the synergistic activity of the 1,3-benzodioxoles may be associated with their ability to form homolytic free radicals. He obtained a good correlation between the synergistic activity of a series of ring-substituted 1,3-benzodioxoles and a $\sigma\cdot$ constant derived from the substituent effects on homolytic free radical formation.

In view of Hansch's suggestion and of the ability of the 1,3-benzodioxoles to inhibit aldrin epoxidation in liver microsomes, it was of considerable interest to evaluate the possible inhibitory activity of these materials on aldrin epoxidation by the modified Fenton's system.

It is clear from Table 4 that the substituted 1,3-benzodioxoles are effective inhibitors of aldrin

TABLE 4. INHIBITION OF MODIFIED FENTON'S SYSTEM BY SUBSTITUTED 1,3-BENZODIOXOLES*

General structure 

Compound	x	x′	y	σ·†	ER†	I_{50F}‡	PI_{50}§
I	Cl	Cl	H	0·06	0·20	$1·3 \times 10^{-3}$	2·885
II	Cl	Cl	Cl	0·12	0·40	$1·0 \times 10^{-3}$	3·000
III	Br	Br	H	0·22	0·24	$1·0 \times 10^{-3}$	3·000
IV	H	OCH_3	H	0·40	0·11	$1·7 \times 10^{-3}$	2·769
V	Cl	OCH_3	H	0·43	0·21	$4·6 \times 10^{-4}$	3·337
VI	H	NO_2	H	0·47	0·41	$8·2 \times 10^{-5}$	4·086
VII	Br	OCH_3	H	0·51	0·23	$4·3 \times 10^{-4}$	3·366
VIII	NO_2	OCH_3	H	0·87	0·52	$3·4 \times 10^{-4}$	3·468
IX	NO_2	NO_2	H	0·94	0·82	$5·4 \times 10^{-4}$	3·267

* Incubation mixture and conditions as described in Table 2. Aldrin (25 μg) and the appropriate amounts of each of the synergists were added simultaneously in the same solution of acetone (25 μl).
† Reference 20.
‡ I_{50} Fenton's reagent aldrin epoxidation.
§ $PI_{50} = -\log I_{50F}$.

epoxidation by the modified Fenton's system and I_{50} values range from approximately 2×10^{-3}M for compound IV to $8{\cdot}2 \times 10^{-5}$M for the 5-nitro-1,3-benzodioxole (VI).

A positive correlation exists ($r = 0{\cdot}50$) between the σ· values reported by Hansch for these compounds and the pI_{50} ($-\log I_{50}$) values of aldrin epoxidation in the Fenton's system. When compound IX (5,6-dinitro-1,3-benzodioxole) is omitted, because of Hansch's[20] suggestion that electronic perturbation results from ortho-dinitro functions, the correlation is improved slightly ($r = 0{\cdot}54$).

Upon introduction of a resonance interaction factor, ER,[20] the correlation improves still further to $r = 0{\cdot}59$. These data suggest that inhibition of the entirely nonenzymatic free radical system might result from the free radical character of the 1,3-benzodioxoles.

Work is proceeding in this laboratory to elucidate further the nature of these relationships and to investigate their possible significance in the biological mode of action of insecticide synergists of this type.

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