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The Interaction of Insecticide Synergists with Nonenzymatic Model Oxidation Systems[1]

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Insecticide synergists such as the 1,3-benzodioxoles, the 1,2,3-benzothiadiazoles and the phenyl-2-propynyl ethers inhibit the epoxidation of aldrin to dieldrin by a modified Fenton's reagent (H_2O_2, Fe^{2+}, EDTA and bovine serum albumin). Inhibition appears to result from the ability of the synergists to compete with aldrin for the OH· radicals generated by the system, and as a result of this interaction the synergists are themselves chemically modified. In the case of the 1,3-benzodioxoles the reaction results in the formation of the corresponding catechols and the rate at which this occurs correlates favorably with the ability of the synergist to inhibit aldrin epoxidation in the system. Although a number of nonenzymatic systems generating radical species other than OH· are also capable of aldrin epoxidation, these are not affected by the presence of insecticide synergists and the synergists are not themselves modified by these systems. The possible relevance of these results to the mode of action of synergists is discussed.

INTRODUCTION

Although it is generally accepted that insecticide synergists are active by virtue of their ability to inhibit the microsomal enzymes responsible for insecticide detoxication (1–5) the precise mechanism by which they interact remains a subject of some conjecture.

The fact that the 1,3-benzodioxoles are themselves metabolized to the corresponding catechols by the microsomal enzymes (3, 6–9) has given rise to the suggestion that these compounds are acting competitively as oxidizable alternative substrates for the enzymes. Although this phenomenon is well established with various drug combinations (10) and may play some role in the synergistic mechanism, the often rigid structural requirements for synergism suggest that other more specific interactions may be involved (4, 5).

Hennessy (11, 12) proposed that inhibition by the 1,3-benzodioxoles might result from the formation of an electrophilic benzodioxolium ion produced by hydride ion transfer from the methylene group of the ring and suggested that this ion could, through a mechanism involving ligand displacement or addition, alter the activity of cytochrome P-450. The ability of the 1,3-benzodioxoles to liberate a hydride ion is supported by molecular orbital studies (13) and it has also been pointed out that 2-hydroxy-1,3-benzodioxole, the proposed intermediate in microsomal ring cleavage of the 1,3-benzodioxoles, is the pseudo-base of the benzodioxolium ion (12).

Hansch (14) has suggested that the synergistic activity of the 1,3-benzodioxoles may be associated with their ability to form relatively stable homolytic free radicals

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through a mechanism involving hydrogen abstraction at the methylene group of the ring. He obtained a good correlation between the synergistic activity of a series of ring substituted 1,3-benzodioxoles and a $\Sigma\sigma\cdot$ constant derived from the effect of different substituents on homolytic free radical formation (14).

The possible importance of radical interaction in the mode of action of insecticide synergists is particularly attractive in view of the fact that the mechanism of microsomal hydroxylation is considered to involve the formation of one or more radical oxygen species at cytochrome P-450 (15, 16). In recent years the nature of the oxygen species responsible for microsomal oxidation has been extensively studied by comparing the patterns of the hydroxylated products obtained from microsomes with those formed by known chemical hydroxylation mechanisms in a series of model systems (15,17–19). Numerous model systems have been described in which the reduction of molecular oxygen by various metal ions generates several different active oxygen species which effect the hydroxylation of organic compounds.

It was therefore of considerable interest to investigate the possible interaction of insecticide synergists with model systems mediated by different species of active oxygen. The epoxidation of aldrin to dieldrin was used throughout the investigation as a model reaction to measure the activity of the several systems employed (20).

MATERIALS AND METHODS

Chemicals. Aldrin (1,2,3,4,10,10-hexachloro-1,4,4*a*,5,8,8*a*-hexahydro-1,4-*endo*, *exo*-5,8-dimethanonaphthalene) and its 6,7-epoxide, dieldrin, were supplied by the Shell Development Co., Modesto, CA. Substituted 1,3-benzodioxoles were synthesized as previously described (21) and a sample of methylene dideuterated 5-nitro-1,3-benzodioxole was kindly supplied by D. J. Hennessy, Fordham University, NY. The 1,2,3-benzothiadiazoles were synthesized by D. L. Gil of this department (22), and the substituted phenyl-2-propynyl ethers were supplied by J. Fellig, Hoffman-LaRoche, Nutley, NJ. Peroxyacetic acid (40%) was purchased from Pfaltz and Bauer Inc., Flushing, NY and *p*-nitrobenzylthiocyanate from Aldrich Chemical Co., Milwaukee, WI. Bovine serum albumin (BSA) and gamma globulin (horse fraction II) were obtained from Pentex Biochemicals, Kankakee, IL, and ethylenediaminetetraacetic acid, tetrasodium dihydrate (EDTA) and ascorbic acid from Calbiochem Inc., Los Angeles, CA. All other reagents and solvents employed were analytical reagent grade.

Model systems. Most of the model systems employed were modified somewhat from those described in the literature.

All incubations were carried out in 25-ml Erlenmeyer flasks gently shaken in a constant temperature water bath. Unless otherwise indicated total incubation volume was 3.5 ml, and the various components of each system are listed in order of addition.

The Fenton system (23, 24) finally adopted contained, 0.75 ml of an aqueous solution of 0.5% BSA, aldrin in 25 μl acetone, 0.5 ml water, 15 μmoles $FeSO_4$ and EDTA each added in 1 ml water, and 0.25 ml 30% H_2O_2. Incubations were at 30°C for 30 min.

The Udenfriend system (25) consisted of 0.5 ml water (or 0.5 ml of aqueous 0.75% BSA), 25 μl acetone containing aldrin, and 30 μmoles of $FeSO_4$, EDTA, and ascorbic acid each added in 1 ml of 0.1 *M* acetate buffer (pH 5.8). Incubations were at 30°C for 60 min.

Incubations with peroxyacetic acid were carried out at 22°C for 30 min. and were effected by the addition of aldrin or synergists in 25 μl acetone to 3.5 ml of an aqueous solution containing 30 μmoles peroxyacetic acid.

The titanous chloride system was formu-

lated in two ways to produce either OH· or HO_2· radicals (15). For the former, incubations contained 0.5 ml of aqueous 0.75% BSA, 25 μl acetone containing aldrin and 1 ml each of aqueous solutions containing 15 μmoles $TiCl_3$ and 2.2 mmoles H_2O_2. Incubations were at 30°C for 30 min. To produce HO_2· radicals, oxygen was continuously bubbled through a solution containing 0.5 ml of aqueous 0.75% BSA and 1 ml each of solutions containing 200 μmoles $TiCl_3$ and 150 μmoles EDTA. Incubations were at 20°C for 20 min.

The mercaptobenzoic acid system (26) contained 0.5 ml water, 25 μl acetone containing aldrin or synergist, and 100 μmoles 2-mercaptobenzoic acid, 10 μmoles $FeSO_4$ and 5 μmoles sodium hydroxide each added in 1 ml water. Incubations were at 30°C for 60 min.

Reactions in each of the model systems were terminated by the addition of 4 ml acetone followed by immediate extraction with two 5-ml aliquots of petroleum ether which were combined and dried over anhydrous Na_2SO_4.

Gas chromatographic analyses of dieldrin and various synergists were carried out on a Varian Aerograph Hi-Fi instrument provided with a tritium foil electron capture detector, and a 6-ft column of 5% DC-200 on Chromosorb W; column temperature was 190° and nitrogen was the carrier gas.

The relative inhibitory potency of the various synergists was determined from their I_{50} values (molar concentration required for 50% inhibition of aldrin epoxidation in the model system). These were obtained from plots of percentage inhibition vs logarithm of synergist concentration using the means of duplicate incubations of at least three synergist concentrations exhibiting between 10 and 80% inhibition. Correlation analyses were made by the method of Huntsberger (27).

Synergist conversion studies. In order to obtain sufficient quantities of material for product identification, reactions were usually carried out at room temperature in 500-ml beakers containing 10 times the Fenton's reaction mixture previously described. This contained 1 mg of the appropriate synergist, and 2.5 ml of 30% H_2O_2 was added dropwise over 30 min with constant agitation; stirring was continued throughout the 2–3 hr incubation period. The reaction mixture was subsequently transferred to a 500-ml flask rapidly frozen in a thin shell using a dry ice–acetone bath and lyophilized in a Virtis freeze–dry apparatus. The lyophilized material was extracted with two 10-ml aliquots of acetone which after volume reduction to about 1 ml was applied to Brinkman preparative thin layer chromatography (tlc) plates (5 × 20 cm precoated glass, silica gel F-254 with fluorescence indicator). The plates were eluted with diethyl ether:hexane (2:1) and the spots visualized under uv light. Authentic starting material and possible products were chromatographed on the same plates as the incubation extract.

Major spots or bands were removed from the plates, eluted with acetone and transferred to porcelain probes for mass spectral analysis, on an AEI MS 902 mass spectrometer operating at 10^{-7} mm Hg and a resolution of 1500.

RESULTS

Optimization of incubation conditions with Fenton's system. Initial experiments revealed that in order to achieve aldrin epoxidation with the Fenton's system it was necessary to incorporate BSA into the reaction medium. Dieldrin production increased with the addition of increasing amounts of BSA up to 3.75 mg/incubation but showed no further increase beyond this level. In view of the possibility that BSA was simply increasing the solubility of aldrin in the aqueous incubation medium the effects on dieldrin production of several other materials with potentially similar properties were evaluated. Table 1 shows that gamma globulin, egg

TABLE 1

The Effect of Proteins and Other Materials on Aldrin Epoxidation by Fenton's System[a]

Addition	Dieldrin produced (nmoles)
None	0.0
BSA	5.77
Egg Albumin	3.17
Gamma globulin	4.20
Liver microsomes (rat)	0.024
Triton B 1956	4.46

[a] Incubation system and conditions as described in Methods. Aldrin concn, 68.4 nmoles.

albumin and Triton B 1956 all supported aldrin epoxidation by the Fenton's reagent though none were as effective as BSA. Little or no further increase in dieldrin production could be obtained by increasing the concentration of any of these materials above 3.75 mg/incubation. Rat liver microsomal protein at the same concentration allowed only traces of dieldrin to be produced.

The effect of hydrogen peroxide concentration was investigated at concentrations ranging from 0.88 mmoles (0.10 ml 30% H_2O_2) to 6.16 mmoles/incubation. Dieldrin production was observed to attain a maximal level at 2.20 mmoles (0.25 ml 30% H_2O_2) and showed a steady decline at higher concentrations. The levels of Fe^{2+} and EDTA were not found to be critical although it was established that equimolar concentrations of these two components were required. No epoxidation occurred in incubations in which any of the components were omitted.

During the course of the reaction the pH of the unbuffered reaction medium was consistently observed to fall from about 7.0 to 4.6. All attempts to buffer the reaction mixture and to maintain a constant pH during the reaction resulted in a decrease in epoxidation (Table 2), and even under unbuffered conditions the amount of dieldrin produced was significantly decreased when

TABLE 2

Effect of pH on Aldrin Epoxidation by the Modified Fenton's System[a]

Buffer	pH		Dieldrin produced (nmoles)
	Initial	Final	
0.5 *M* phosphate	8.0	6.6	2.89
	7.8	6.0	3.94
	7.7	5.7	5.17
	7.0	5.5	5.10
0.1 *M* acetate	4.5	4.5	0.00
Unbuffered	7.0	4.5	6.17
	6.5	4.5	5.51
	4.5	4.5	0.00

[a] Incubation mixture and conditions as described in Methods. Aldrin concn, 68.4 nmoles.

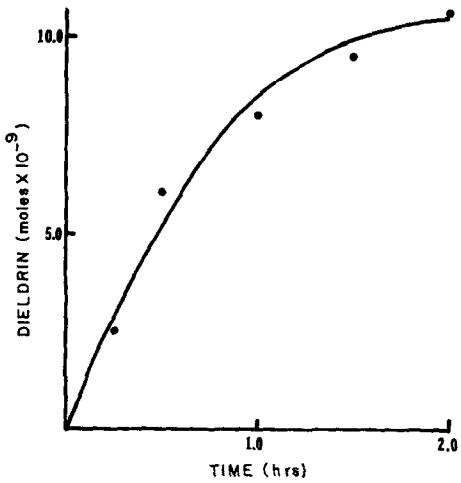


FIG. 1. *Time course of dieldrin production in the modified Fenton system.*

the initial pH of the medium was below 7.0 and no reaction occurred at pH 4.5 (Table 2). As a consequence of these results all incubations were effected under unbuffered conditions.

Characteristics of aldrin epoxidation by Fenton's system. An investigation into the time course of the reaction over a period of two hours established that the amount of dieldrin produced was a linear function of time for about 30 min (Fig. 1). Routine incubations were carried out over this time period.

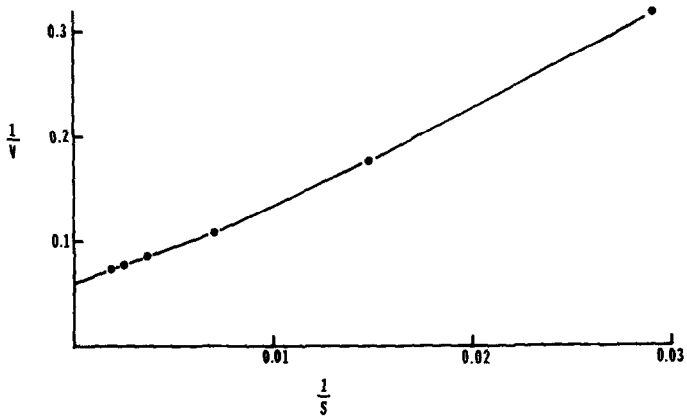


FIG. 2. *Lineweaver-Burk plot of aldrin epoxidation in the modified Fenton system. S = aldrin concentration (nmoles/incubation); V = dieldrin produced (nmoles/min).*

Dieldrin production also increased with increasing concentration of aldrin in the reaction mixture and appeared to approach a limiting value above 2–3 μmoles/incubation. A double reciprocal plot of these data (Fig. 2) is similar to that usually observed with enzymatic reactions and although there is some deviation from linearity the ordinate intercept indicates a "maximum velocity" of about 16.7×10^{-9} moles dieldrin/30 min.

Effect of insecticide synergists on aldrin epoxidation by the modified Fenton's reagent. The possible interaction with the Fenton's reagent of several materials representing several major groups of compounds known to possess synergistic activity was evaluated.

As reported earlier (20) and shown in Table 3 the addition to the Fenton's system of each of a series of substituted 1,3-benzodioxoles resulted in substantial inhibition of aldrin epoxidation; I_{50} values ranged from 8.2×10^{-5} *M* for 6-nitro-1,3-benzodioxole (VII) to 1.7×10^{-3} *M* for the 6-methoxy derivative (VI). The effects of other insecticide synergists are shown in Table 4. This clearly indicates that 6-nitro- and 5,6-dichloro-1,2,3-benzothiadiazole (XII and XIII), 2-nitrophenyl propynyl ether (XIV), 4-nitrobenzylthiocyanate (XV) and 2-diethylamino diphenyl propyl acetate (SKF 525-A) (XVI) also inhibit epoxidation by the modified Fenton's reagent, although no effect was observed with 2,4,5-trichlorophenyl propynyl ether up to concentrations of about 10^{-3} *M*.

The inhibitory activity of the 1,3-benzodioxoles was studied in greater detail. Examination of the data using regression analysis reveals a rather poor correlation ($r = 0.50$) between the pI_{50} values (negative log I_{50}) for aldrin epoxidation and the $\Sigma\sigma\cdot$ values for these compounds reported by Hansch (14). The correlation was only slightly improved when the result for compound IX (5,6-dinitro-1,3-benzodioxole) was omitted due to Hansch's suggestion that *ortho*-dinitro functions cause electronic perturbation. Although these data suggest that the inhibition of aldrin epoxidation might be associated with the ability of the 1,3-benzodioxoles to form homolytic radicals, the correlation is not sufficiently high to indicate that this can adequately define the mechanism.

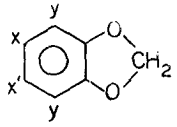
During the course of investigations with the 1,3-benzodioxoles it was observed that the amount of inhibitor initially added to the incubations could not be recovered at the end of the reaction. It was therefore of interest to determine whether the substituted 1,3-benzodioxoles were themselves

TABLE 3

Interaction of Substituted 1,3 Benzodioxoles with Modified Fenton's System[a]

Compound	x	x'	y	$\Sigma\sigma$-[b]	I_{50}	pI_{50}	Rate of disappearance (μmoles/min)
I	H	H	H	0.00	1.0×10^{-3}	3.000	—
II	H	CN	H	—	1.4×10^{-3}	2.852	—
III	Cl	Cl	H	0.06	1.3×10^{-3}	2.885	0.026
IV	Cl	Cl	Cl	0.12	1.0×10^{-3}	3.000	0.027
V	Br	Br	H	0.22	1.0×10^{-3}	3.000	0.016
VI	H	OCH_3	H	0.40	1.7×10^{-3}	2.769	—
VII	Cl	OCH_3	H	0.43	4.6×10^{-4}	3.337	0.251
VIII	H	NO_2	H	0.47	8.2×10^{-5}	4.086	0.557
IX	Br	OCH_3	H	0.51	4.3×10^{-4}	3.366	0.208
X	NO_2	OCH_3	H	0.87	3.4×10^{-4}	3.468	0.255
XI	NO_2	NO_2	H	0.94	5.4×10^{-4}	3.267	0.199

[a] Incubation system and conditions as described in Methods. Aldrin concn, 68.4 nmoles/incubation and initial concentration of synergists for disappearance study, 0.6 μmoles/incubation. General structure:



[b] Ref. (14).

chemically modified during the course of the reaction. It soon became evident that this was the case, and that a strong positive correlation existed (r = 0.98) between the rate of disappearance of the materials and their pI_{50} values for aldrin epoxidation (Table 3). Furthermore, it was established that the 1,2,3-benzothiadiazoles, the phenyl propynyl ethers and 4-nitrobenzylthiocyanate also acted with the modified Fenton system and were converted to other materials during the incubation (Table 4).

The inhibitory effect of 6-nitro-1,3-benzodioxole (VIII) on the epoxidation of different concentrations of aldrin was also evaluated and the data expressed in the form of a double reciprocal plot are shown in Fig. 3. This clearly shows that although the lines deviate somewhat from linearity they exhibit a common intercept on the y axis. Plots of this type are characteristic of competitive inhibition when applied to enzyme kinetics, and in this particular case suggest that the 1,3-benzodioxole is competing with aldrin for one or more radical species generated by the modified Fenton's reagent. Further support for this suggestion is provided by the fact that aldrin competitively inhibits the rate at which 6-nitro-1,3-benzodioxole reacts with the Fenton's system (Fig. 4).

Nature of the reaction between synergists and modified Fenton's system. In view of the clear relationship between the inhibitory activity of the synergists and their chemical interaction with the Fenton's system it was of considerable interest to identify the products to which they were converted.

Large scale incubations were therefore effected with 6-nitro-1,3-benzodioxole and the resulting products subjected to chromatographic separation and analysis. Thin layer chromatography on silica gel showed two major spots with R_f values of 0.75 and 0.17, respectively, in diethyl ether:hexane (2:1). The former of these cochromatographed with starting material and consisted of unchanged 6-nitro-1,3-benzo-

TABLE 4

Interaction of Insecticide Synergists with Modified Fenton's System[a]

	I_{50}	Rate of disappearance (μmoles/min)
6-NO_2-1,2,3-Benzothiadiazole	5.5×10^{-5}	0.215
5,6-Dichloro-1,2,3-benzothiadiazole	4.2×10^{-4}	0.183
2-Nitrophenyl propynyl ether	6.0×10^{-4}	0.312
2,4,5-Trichlorophenyl propynyl ether	1.0×10^{-3}	0.0014
4-Nitrobenzylthiocyanate	4.3×10^{-4}	0.209
SKF 525-A	2.0×10^{-3}	—

[a] Incubation system and conditions as described in Methods. Aldrin concn, 68.4 nmoles/incubation and initial concentration of synergists for disappearance study, 0.6 μmoles/incubation.

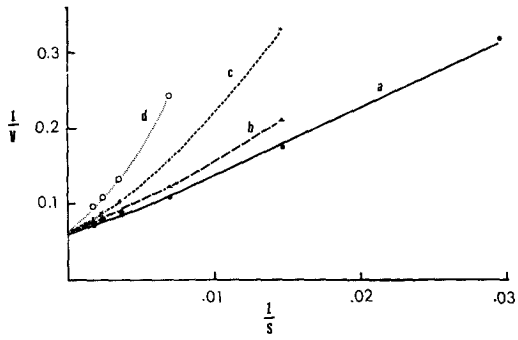


FIG. 3. *Lineweaver-Burk plots of the inhibition by 6-nitro-1,3-benzodioxole of aldrin epoxidation in the modified Fenton system. S = aldrin concentration (nmoles/incubation); V = dieldrin produced (nmoles/min); concentration of 6-nitro-1,3-benzodioxole: 0 (a), 4.25 × 10^{-5} M (b), 8.5 × 10^{-5} M (c), and 1.7 × 10^{-4} M (d).*

dioxole. The more polar material (R_f 0.17) cochromatographed with an authentic sample of 4-nitrocatechol and mass spectral analysis confirmed that the material had a parent ion of mass 155 and a molecular fragmentation pattern consistent with the structure of 4-nitrocatechol. In-

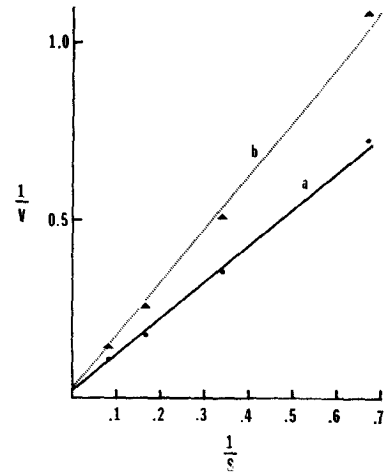


FIG. 4. *The effect of aldrin on the reaction of 6-nitro-1,3-benzodioxole with the modified Fenton's reagent. S = concentration of 6-nitro-1,3-benzodioxole (μmoles); V = rate of disappearance (μmoles/min); concentration of aldrin: 0 (a) and 7.8 × 10^{-5} M (b).*

corporation of this latter material into the incubation mixture at 1.6 × 10^{-4} *M* caused no inhibition of aldrin epoxidation thus obviating the possibility that the inhibition observed with 6-nitro-1,3-benzodioxole resulted from the 4-nitrocatechol generated during the reaction.

A similar examination of the reaction products of 6-nitro-1,2,3-benzothiadiazole revealed three major spots on the thin layer chromatograms, with R_f values of 0.92, 0.76 and 0.16, respectively; the material with R_f 0.76 represented unchanged starting material. Mass spectral analysis of the faster running spot (R_f 0.92), which is apparently less polar than the parent material, indicated a molecular ion of mass 396 and a fragmentation pattern which indicated an intact thiadiazole ring. This suggests that 6-nitro-1,2,3-benzothiadiazole undergoes free radical dimerization and hydroxylation and the structure tentatively assigned to this material is shown in Fig. 5a. The major more polar metabolite (R_f 0.16) had a molecular mass of 229 and a fragmentation pattern which indicated that it possessed both

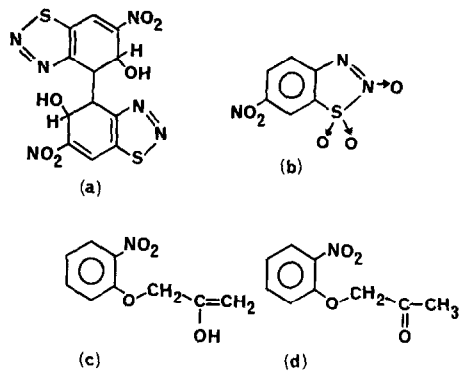


FIG. 5. *Products of the reaction between synergists and the modified Fenton system.*

sulfone and N-oxide groupings; it was assigned the structure shown in Fig. 5b. Small amounts of another product were also detected on the thin layer plates. This had an R_f value of 0.42 and mass spectral analysis showed it to have a molecular ion of mass 256. No further identification of this material was achieved.

Thin layer chromatograms of the reaction products of 2-nitrophenyl propynyl ether showed the presence of two spots (R_f values 0.86 and 0.35) in addition to that resulting from the parent compound (R_f 0.68). The more polar product (R_f 0.35) was established to have a molecular weight of 195 and its structure was tentatively assigned as either Fig. 5c or d, indicating radical attack at carbon-2 of the propynyl ether side chain. As with the 1,2,3-benzothiadiazole a product less polar (R_f 0.86) than the parent compound was also formed but this was not identified.

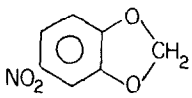
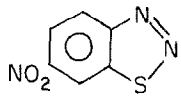
Interaction of synergists with other model systems. In view of the interaction of the synergists with Fenton's reagent it was of interest to investigate whether similar interactions occurred with a variety of other model oxidation systems mediated by different species of active oxygen. It should be emphasized from the outset that the reactions occurring in many of these systems are extremely complex and incompletely understood. Although for the purpose of this discussion distinct active species are associated with particular model systems it is probable that in any one system several different species of active oxygen are generated. Consequently it is not always possible to fully define the species responsible for the reaction observed. Results of the investigations of synergist interactions with several model systems are shown in Table 5.

In contrast with Fenton's reagent which generates mainly the hydroxyl radical ($\cdot$OH) (15, 17–19, 26, 28), the Udenfriend and mercaptobenzoate systems are considered to operate largely by the so-called "oxene mechanism" involving the perhydroxyl radical ($\cdot O_2H$) or the corresponding superoxide anion ($\cdot O_2^-$) (15, 17–19, 26, 28). As with the Fenton system no epoxidation of aldrin was observed in the absence of BSA and even after addition of this material the amounts of dieldrin produced were extremely small in both systems. The addition of either 6-nitro-1,3-benzodioxole (1.7×10^{-4} *M*) or 6-nitro-1,2,3-benzothiadiazole (1.6×10^{-4} *M*) caused no inhibition of epoxidation and the fact that both of these materials could be completely recovered following the incubations indicated that they were not themselves acted upon by the systems.

The titanous chloride system can be formulated to produce either hydroxyl ($\cdot$OH) or perhydroxyl ($\cdot O_2H$) radicals in the presence of hydrogen peroxide and oxygen respectively (15). In the presence of BSA the titanous chloride/H_2O_2 system produced 1.6 nmoles dieldrin in 30 min and the addition of 100 μg 6-nitro-1,3-benzodioxole (1.7×10^{-4} *M*) resulted in approximately 58% inhibition. Furthermore, incubations of the inhibitor alone with this system revealed that the parent compound rapidly disappeared (37 μg/min) from the reaction mixture presumably indicating its conversion to other products. The nature of these were not investigated, although as a result of the similarity of this system with Fenton's

TABLE 5

Effect of Synergists on Different Model Systems[a]

Model system	Radical species generated	Aldrin concn (nmoles)	Dieldrin formed (nmoles/30 min)		
			No inhibitor	 (1.7×10^{-4} *M*)	 (1.6×10^{-4} *M*)
Fenton[b]	OH·	68.4	5.77	1.73	1.44
Udenfriend[b]	O_2H·	136.8	0.60	0.60	0.60
Mercaptobenzoic[b] acid	O_2H·	136.8	0.66	0.66	0.66
Titanous chloride[b]					
with H_2O_2	OH·	68.4	1.60	0.67	—
with O_2	O_2H·	68.4	0	—	—
Peracetic acid	OH^+ (oxirane)	68.4	11.8	11.8	11.8

[a] Incubation conditions as described in Methods.

[b] Formulated with BSA as described in Methods.

reagent it is likely that the catechol was the major product. When the titanous chloride system was formulated with oxygen instead of H_2O_2 no epoxidation could be detected even in the presence of BSA and under these conditions no chemical conversion of the synergists was observed.

The epoxidation of a variety of unsaturated compounds by peracids is well established and although there is still some disagreement regarding the precise mechanism it is usually considered to occur through a concerted action involving the OH^+ ion (17, 19, 28, 29). The results in Table 5 show that although peracetic acid constituted an efficient epoxidation system the inclusion in the reaction mixture of either 6-nitro-1,3-benzodioxole (1.7×10^{-4} *M*) or 6-nitro-1,2,3-benzothiadiazole (1.6×10^{-4} *M*) resulted in no decrease in the amount of dieldrin produced. The complete recovery of both compounds provides further evidence of their failure to interact with the system. A similar experiment using trifluoroperacetic acid yielded identical results (30).

DISCUSSION

Several model oxidation systems have been shown capable of effecting the epoxidation of aldrin to dieldrin and although this is not unexpected with the peracid system, which constitutes a standard laboratory procedure for epoxide synthesis, the reaction has not previously been reported to occur with the Fenton regent. Indeed it is usually considered that model systems which are mediated by OH-radicals are unable to form epoxides at a double bond (18, 26). The established requirement for BSA in the Fenton system is probably a reflection of the extremely hydrophobic nature of aldrin and the small concentration of material consequently available for reaction in aqueous solution. The binding of various drugs to BSA and proteins has been associated with hydrophobic interactions (31, 32) and has been found to increase the solubility of lipophilic substrates in certain enzyme studies (33). The contention that BSA is acting simply as a solubilizing agent for aldrin is supported by the fact that other proteins as well as Triton B 1956 have a similar effect on epoxidation (Table 1). It would seem unlikely that the addition of BSA to the Fenton system could cause any significant change in the nature of the radicals produced although its presence could perhaps modify reactivity to some extent by

stabilizing a particular radical species. The mechanism by which OH-radicals can carry out epoxidation is not immediately obvious.

Epoxidation by the modified Fenton system is markedly inhibited in the presence of a variety of insecticide synergists including 1,3-benzodioxoles, 1,2,3-benzothiadiazoles, phenyl propynyl ethers, benzylthiocyanates and SKF 525-A. In the case of the 1,3-benzodioxoles the inhibition appears to be of a competitive nature and the fact that the materials themselves react with the Fenton system and are converted to the corresponding catechols strongly suggests that they are competing with aldrin for the supply of radicals available. The high correlation (r = 0.98) between the inhibitory potency of the substituted 1,3-benzodioxoles and the relative ease with which these materials react with the Fenton system supports this view and there is evidence to suggest that similar correlations probably exist with some of the other groups of synergists investigated.

The rather poor correlation (r = 0.5) between the inhibitory potency of the 1,3-benzodioxoles and the $\Sigma\sigma\cdot$ constants reported by Hansch (14) need not necessarily obviate the involvement of homolytic radical formation in their interaction with the Fenton system. In view of the fact that C–H bonds adjacent to O and N functions are especially labile to radical attack (34) it is entirely possible that the initial step in the reaction mechanism involves hydrogen abstraction from the methylene group of the 1,3-benzodioxole ring to produce the homolytic radical proposed by Hansch (14). The properties of the electrophilic OH-radical produced in the Fenton system are consistent with this proposal, and presumably the resulting homolytic radical could react with an additional OH-radical to give the 2-hydroxy-1,3-benzodioxole proposed as an intermediate in 1,3-benzodioxole cleavage by the microsomal enzyme system (Fig. 6) (3, 5, 6, 9). Further evidence that the mechanism involves C–H bond cleavage is provided by the fact that the I_{50} value for methylene dideuterated 6-nitro-1,3-benzodioxole (2.3×10^{-4} M) is 2.8-fold higher than the undeuterated compound (8.2×10^{-5} M) and its rate of reaction with the Fenton system is 2.1 times slower. Although the nature of the reaction between other synergists and the Fenton's reagent are not yet clearly defined it is clear that they too are readily converted to several products.

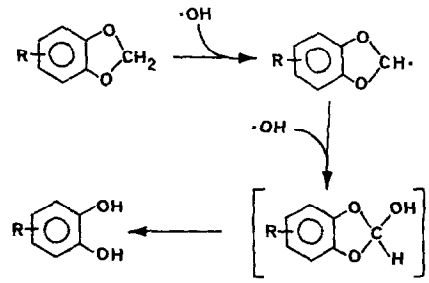


FIG. 6. *Proposed mechanism for interaction of 1,3-benzodioxoles with the modified Fenton system.*

Of the other models investigated only the titanous chloride/H_2O_2 system, which like the Fenton's reagent is considered to produce OH-radicals, showed any ability to interact with insecticide synergists. Epoxidation by this system was inhibited by 58% by 6-nitro-1,3-benzodioxole (1.7×10^{-4} M) and the latter material was itself rapidly converted to other products. The relatively small amounts of dieldrin produced by the Udenfriend and mercaptobenzoate systems, generally thought to generate $\cdot O_2H$ radicals, were unaffected by the presence of either 6-nitro-1,3-benzodioxole or 6-nitro-1,2,3-benzothiadiazole and the synergists failed to undergo any type of reaction with these systems. This was also found to be true with respect to the peracetic acid system despite its effectiveness in epoxidizing aldrin. Consequently it can be concluded from these results that the 1,3-benzodioxole and 1,2,3-benzothiadiazole synergists only interact with those systems which generate OH-radicals. This is apparently at variance with an earlier report that several insecticide

synergists, including 1,3-benzodioxoles are able to inhibit the activation of parathion to paraoxon by the Udenfriend system (35).

The ability of synergists to react with some radical species and not with others is of considerable interest although it must be emphasized that the conditions which exist in aqueous model systems are far removed from those likely to be encountered in the microsomes where the reactions occur at the active site of a largely hydrophobic enzyme protein. Nonetheless it is tempting to speculate that perhaps a part of the inhibitory activity of synergists is due to their ability to interact with radicals generated at cytochrome P-450 and that their stereochemical and physicochemical properties enhance this activity through facilitating a close approach to the active site.

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