THESIS

STRUCTURE ACTIVITY RELATIONSHIPS OF 1,2,3-BENZOTHIADIAZOLE INSECTICIDE SYNERGISTS

DIEGO LIONEL GIL

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STRUCTURE ACTIVITY RELATIONSHIPS OF 1,2,3-BENZOTHIADIAZOLE INSECTICIDE SYNERGISTS

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BIOGRAPHICAL SKETCH

Diego Lionel Gil Hormazabal was born on August 4, 1939, in Santiago, Chile. He attended public elementary school (Escuela Brasil) and high school (Instituto Nacional) in Santiago. He studied chemistry and pharmacy in the University of Chile, receiving his degree in 1963. In 1964 he was appointed Assistant Professor in the Department of Chemistry, School of Medicine, University of Chile, obtaining his tenure in 1966. He was responsible for teaching organic chemistry and undertook research in the field of pesticide biochemistry.

In 1969 he was accepted as a graduate student at Cornell University.

He is married to the former Carmen Cubillos, from Talca, Chile. They have one son, Rodrigo.

DEDICATION

To My Wife Carmen

and

Son Rodrigo

ACKNOWLEDGMENTS

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SECTION I

INTRODUCTION AND DEFINITION OF THE PROBLEM

During the last three decades the use of synthetic organic insecticides has been one of the major reasons for the vast improvement in the quality and quantity of agricultural products and for the control of several important insect borne diseases. Recent reports by the World Health Organization, for example, indicate that the control of many of the important insect vectors of human disease is almost entirely dependent on the continued use of insecticides and that no effective or economically feasible alternatives are available. Despite these benefits great concern has arisen in recent years regarding the effect of insecticides in the environment and on wildlife. As a consequence of recent restrictions on the use of several insecticides, particularly the chlorinated hydrocarbons, there is currently an urgent need to find new materials with more acceptable properties, and to improve the effectiveness of existing materials.

Insecticide synergists are compounds which although non toxic *per se* at the dosage employed are able to enhance the toxicity of an insecticide with which they are combined (Wilkinson, 1968). It is generally agreed that their activity results from an inhibition of the enzymes which detoxify insecticides so that they protect the insecticide from metabolic breakdown. Only few synergists are actually employed in insect control, most of these being the methylenedioxyphenyl containing compounds. These materials are expensive and their use is limited by the fact that all are

prepared from the natural product, safrole, which is available in the amount of only about 1,400,000 lbs per year (Hennessy, 1970). It is possible that if new, cheap and effective synergists could be developed they could be of considerable use in future pest control programs.

By effectively blocking the detoxification mechanisms in insects, the synergists could 1) enable the use of biodegradable compounds by selectively stabilizing them in insects; 2) reduce the problem associated with insect resistance to insecticides; and 3) minimize the amount of insecticide required for insect control and therefore minimize environmental contamination. In order to develop new types of synergists it is important to fully understand the mechanism of action of existing materials.

Structure-activity studies are often extremely useful in improving our understanding of the physical and chemical properties that determine the biological activity of a series of compounds, and in addition they often provide an insight into the mechanism of their biological action. Such information is usually very important in predicting and designing new and more active compounds.

The 1,2,3-benzothiadiazoles constitute a relatively new group of compounds with synergistic activity. Since their structure is quite different from that of existing materials it was decided to undertake an investigation of structure-activity relationships with these compounds and to study their mode of action.

The general objectives of the study were as follows:

(1) Since the 1,2,3-benzothiadiazoles required are not commercially available the first step was to synthesize a large series of compounds with simple substituents in the phenyl portion of the aromatic ring.

(2) To study the biological activity of these materials both as insecticide synergists *in vivo* and as potential inhibitors of microsomal oxidation *in vitro*.

(3) To conduct comparative studies on their biological activity in insects and mammals.

(4) To use regression analysis to investigate possible correlations between biological activity and a variety of physicochemical parameters of the molecule, and to investigate possible relationships between *in vitro* and *in vivo* activity.

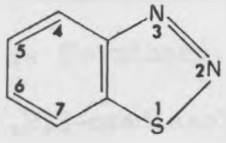
SECTION II

REVIEW OF LITERATURE

A. The Chemistry of 1,2,3-benzothiadiazoles

1. History

Most of the early work on the 1,2,3-benzothiadiazoles was carried out in Germany towards the end of the last century at which time they were described as internal diazosulphides. The nomenclature and numbering of the ring system have subsequently changed. Presently, the parent compound appears in the Ring Index under the number 693 and its numbering is as follows (in early references numbering started from the 3-N-atom):



The first description in the literature of a compound with this structure appeared in 1879 when Beilstein and Kurbatow reported the synthesis of 5-chloro-1,2,3-benzothiadiazole. Jacobson (1888) subsequently prepared the parent compound and described its toxicological effects on the central nervous system of the rabbit. Other important early papers were published in 1889 and 1893 by Jacobson and his group and by Bernthsen 1888 and 1889.

In spite of the fact that these compounds have been known for almost a century and are relatively easy to synthesize, the literature

contains only few references to them; this is particularly true with regard to reports of their biological activity which appear to be almost completely lacking between 1888 and 1964. It is only in recent years that the 1,2,3-benzothiadiazoles have been shown to exhibit biocidal activity, particularly: herbicidal, fungicidal and insecticide synergistic activity.

Important contributions to our understanding of the chemistry of the 1,2,3-benzothiadiazoles have been made by Fries, Hodgson, and Dodgson, Ward and Heard, Kirby and Haddock and their respective groups. Three major review articles have been published (Jacobson et al. 1893, Hodgson and Dodgson, 1948d and Bambas, 1952).

2. Synthesis

The synthesis of 1,2,3-benzothiadiazoles by diazotisation of o-aminobenzene sulfonic acids (Bernthsen, 1889; Green and Perkin, 1903) and by treatment of 1,2,3-oxadiazoles with phosphorous pentasulfide (Bamberger et al., 1923b, 1923a) have been reported but have not been investigated intensively and are only rarely used. From the practical point of view the most convenient methods are: by diazotisation of the substituted benzothiazathiolium salt, a modification of the Herz reaction (Kirby et al., 1970a) by diazotisation of o-aminobenzenethiols, and by diazotisation and decomposition of amino benzothiazoles (Davis and Kirby, 1967) or 7-amino-benzoisothiazoles (Haddock et al., 1971 a,b). These reactions will therefore be discussed in greater detail.

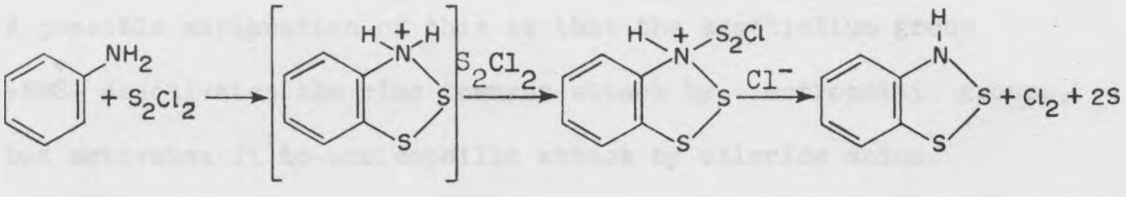
a) Modified Herz Reaction

The starting materials, the benzothiazathiolium salts, are prepared by the Herz reaction on substituted anilines.

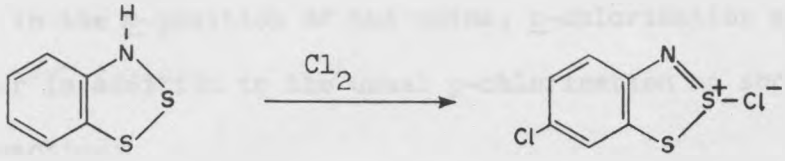
In German patent 360,690 granted in 1914 and published in 1922 in the name of Casella & Co., Richard Herz described a method of preparing o-aminobenzenethiols. Allowing the appropriate aniline hydrochloride to react with five equivalents of sulphur monochloride at moderate temperature he was able to obtain the corresponding benzothiazathiolium chlorides, which on alkaline hydrolysis yielded o-aminobenzenethiols (Herz, 1922a). In several other patents, Herz pointed out that the reaction was not limited to aniline but was applicable to most aromatic amines containing a free o-position.

The Herz reaction has been reviewed by Warbuton (1957) and a mechanism was initially discussed by Grompper et al., 1964. More recently Hope and Wiles (1967) have proposed a mechanism which is now generally accepted and have provided experimental evidence to support each stage. The following steps are considered to occur:

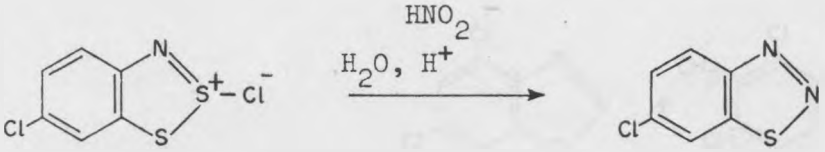
1) Formation of the heterocyclic ring:



2) If the p-position of the amine is unsubstituted, chlorination usually takes place here in addition to oxidation of the sulfur atom in the 2 position.



Kirby et al., 1970a treated the benzothiazathiolium salts with a excess of nitrous acid to obtain the 1,2,3-benzothiadiazoles.



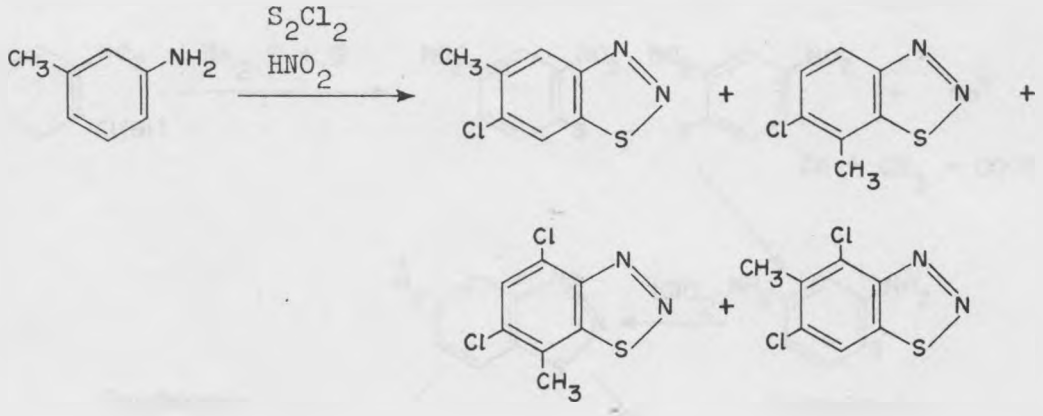
The overall reaction often proceeds in low yield and sometimes may produce complex mixtures of products depending upon a combination of the following factors:

1) Deactivating atoms or groups such as nitro, carboxylic acid, sulphonic acid, or bromide, in the p-position of the amine, are displaced by chloride ion during the reaction (Herz, 1922). A possible explanation of this is that the azathiolium group $-N=\overset{+}{S}-$ deactivates the ring towards attack by electrophilic groups, but activates it to nucleophilic attack by chloride anion.

2) Deactivating groups in a o-position of the aromatic amine may also be displaced by chloride ion (Warbuton, 1957).

3) If the amine is m-substituted with F, Cl, or Br, cyclisation may occur through either of the two available o-positions and as a

result of the usual p-chlorination reaction, mixtures of 5,6-dihalogeno-1,2,3-benzothiadiazoles and 6,7-dihalogeno-1,2,3-benzothiadiazoles are produced (Kirby et al., 1970a). If an o-p directing group is the substituent in the m-position of the amine, o-chlorination may or may not occur in addition to the usual p-chlorination as shown in the following reaction:



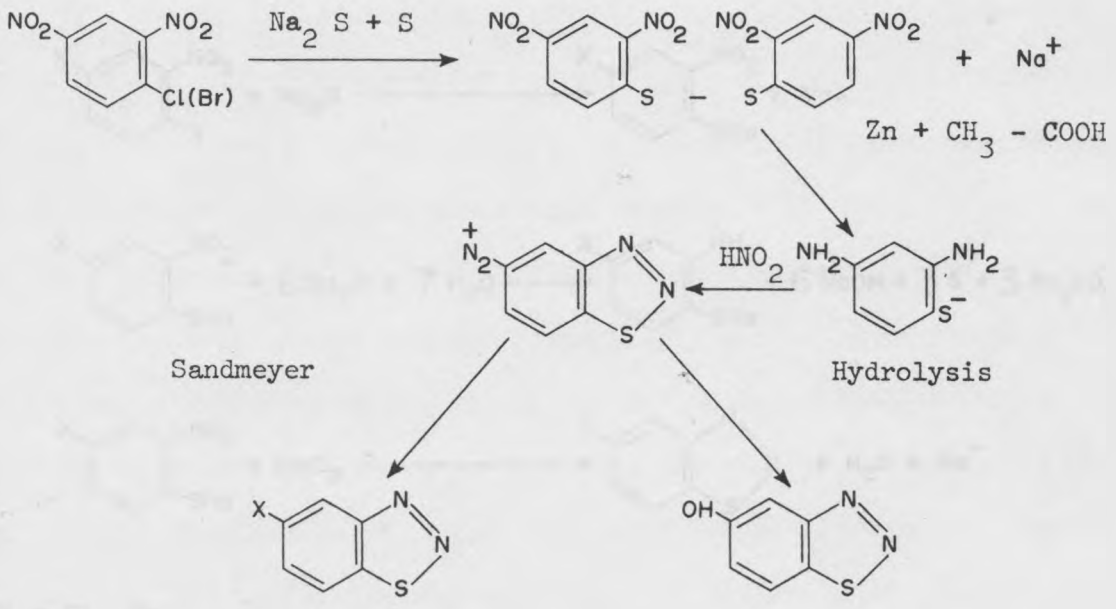
Although the procedure involving the modified Herz reaction is of general application it has failed in the synthesis of 6-alkyl, 6-nitro and 6-unsubstituted compounds.

b) Diazotisation of o-aminobenzenethiols

This is the most widely used procedure and is the most general application, however, the major problem in this procedure is the synthesis of the desired o-aminobenzenethiols, since only few of these are commercially available. The following have been described in the literature:

i. - Synthesis of a disulphide and reduction. The halogen atom in 1-bromo or 1-chloro-2,4-dinitrobenzene is exceedingly reactive and is very easily attacked by sodium disulphide (prepared by the

reaction between sodium sulphide and sulfur), to give the 2,2', 4,4'-tetranitrodiphenyl disulphide (Hodgson and Dodgson, 1948c). Reduction of the disulphide with Zn and acetic acid yields the 2,4-diaminobenzenethiol which is readily diazotised and as shown by the following reactions provides a very useful direct route for the synthesis of 5-halogen, 5-cyano and 5-hydroxy-1,2,3-benzothiadiazoles.

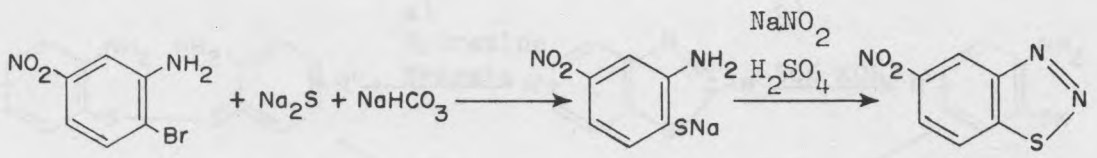


X = Br, I, CN

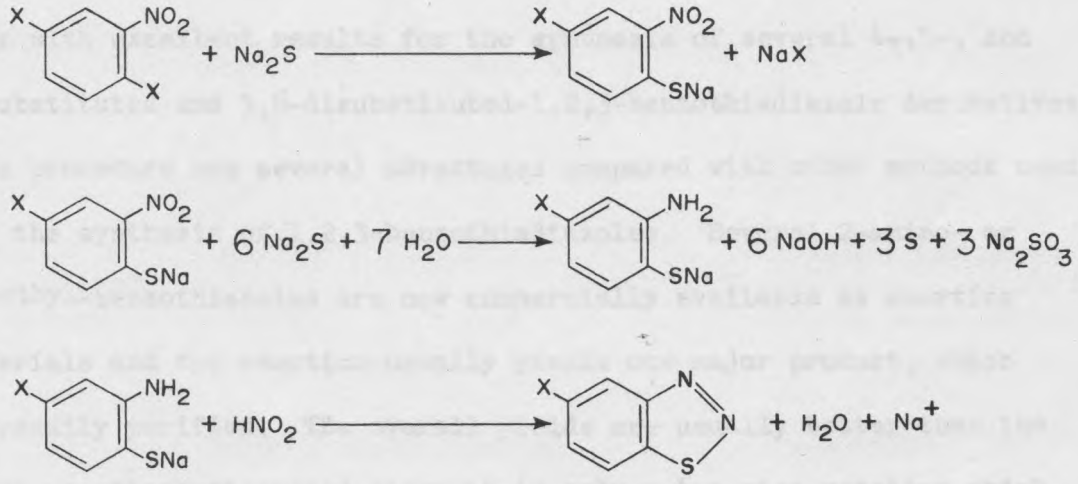
ii. - Thiolation of 2-halogeno-anilines or nitrobenzenes.

Hodgson and Dodgson (1948b) reported the preparation of 4-nitro-2-aminobenzenethiol in 97% yield by the reaction between 2-chloro-5-nitroaniline, sodium sulphide and sodium bicarbonate. This reaction could not be repeated in this investigation but when 2-bromo-5-nitroaniline was employed instead of the 2-chloro-derivative, the corresponding 4-nitro-2-aminobenzenethiol was readily formed and

diazotisation gave 5-nitro-1,2,3-benzothiadiazoles.

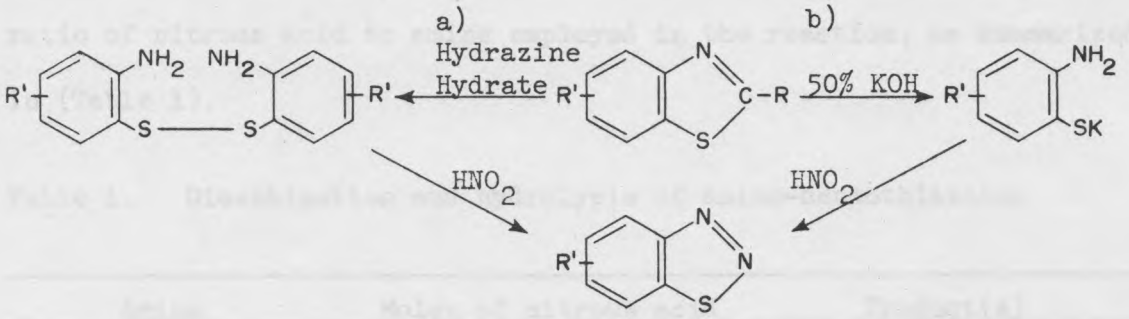


A very similar reaction is the thiolation and reduction of 2,5-dihalogen nitrobenzene with sodium sulphide reported by Jain et al. (1969).



X = Cl, Br

iii. - Ring opening of 2-substituted benzothiazoles. The heterocyclic ring of 2-amino- or 2-methyl-benzothiazoles may be opened by reaction with nucleophilic reagents such as hydrazine hydrate (reaction a, Boggust and Cocker, 1949); boiling dilute sodium hydroxide (Gardner, 1944) or boiling 50% aqueous potassium hydroxide (reaction b, Fridman and Golub, 1961).



The method of Fridman and Golub has been used in this investigation with excellent results for the synthesis of several 4-,5-, and 6-substituted and 5,6-disubstituted-1,2,3-benzothiadiazole derivatives. This procedure has several advantages compared with other methods used for the synthesis of 1,2,3-benzothiadiazoles. Several 2-amino- or 2-methyl-benzothiazoles are now commercially available as starting materials and the reaction usually yields one major product, which is readily purified. The overall yields are usually better than the other reactions discussed since it is only a two step reaction which consists of first opening the benzothiazole ring to give the corresponding thiol and subsequent diazotisation of this product. The route for the synthesis of 7-substituted-1,2,3-benzothiadiazole derivatives by this method has not yet been investigated.

c) Diazotisation and decomposition of o-amino-benzothiazoles or 7-amino-benzoisothiazoles

This method includes two types of reactions:

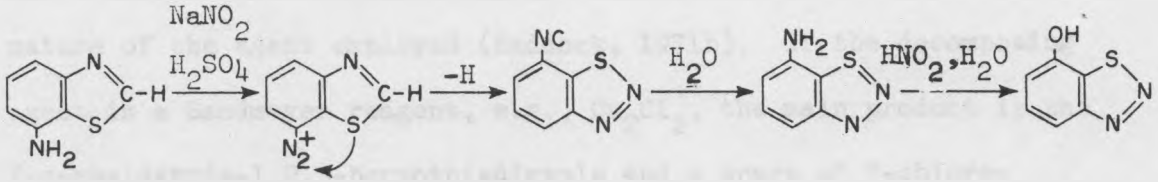
i. - Diazotisation and hydrolysis of amino-benzothiazoles. Davies and Kirby (1967) reported a very interesting route for the synthesis of hydroxy or amino-benzothiadiazoles by diazotisation and hydrolisis

of amino-benzothiazoles. The product(s) of the reaction depend on the ratio of nitrous acid to amine employed in the reaction, as summarized in (Table 1).

Table 1. Diazotisation and hydrolysis of amino-benzothiazoles

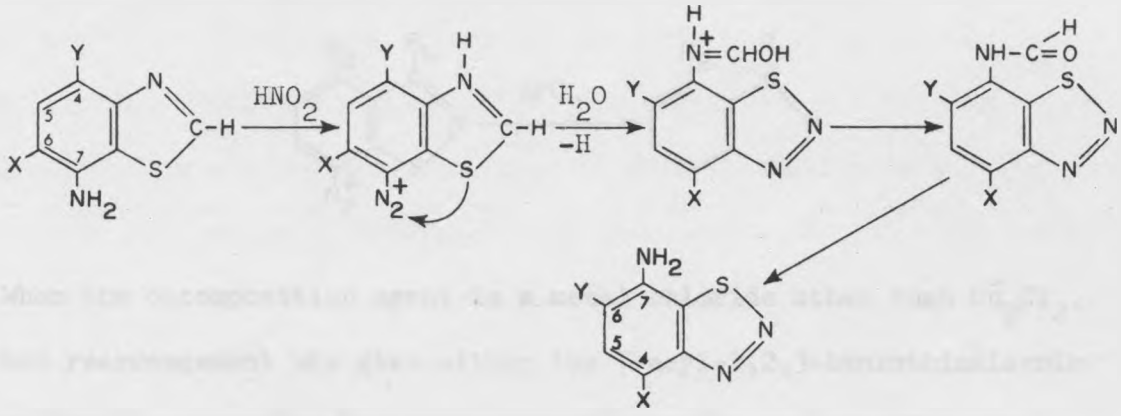
Amine	Moles of nitrous acid	Product(s)
4-NH_2-benzothiazole	2.0	4-OH-benzothiazole
5-NH_2-benzothiazole	1.0	5-OH-benzothiazole
5-NH_2-benzothiazole	2.5	5-OH-benzothiadiazole 5-OH-benzothiazole
6-NH_2-benzothiazole	1.0	6-OH-benzothiazole
6-NH_2-benzothiazole	2.0	6-OH-benzothiadiazole
7-NH_2-benzothiazole	2.0	7-OH-benzothiadiazole
7-NH_2-benzothiazole	1.0	7-NH_2-benzothiadiazole
7-NH_2-benzothiadiazole	1.0	7-OH-benzothiadiazole

The formation of 7-amino-benzothiadiazole by the treatment of 7-amino-benzothiazole with nitrous acid was considered by Davies and Kirby (1967) to proceed by the following mechanism:



This indicates an initial electrophilic attack by the diazonium group on the ring sulphur atom to yield a 7-isocyano-benzothiadiazole, which is subsequently hydrolized to the 7-amino-1,2,3-benzothiadiazole. In the presence of an excess of nitrous acid this will yield the corresponding 7-hydroxy-1,2,3-benzothiadiazole.

More recently Haddock et al. (1971b) have observed the formation of a 7-acylamino-1,2,3-benzothiadiazole intermediate and as a result have proposed the following mechanism for this reaction:

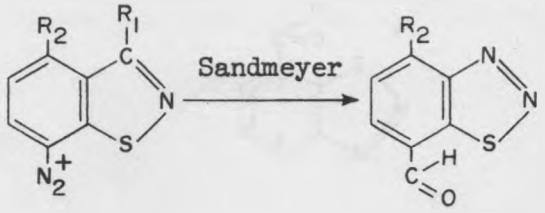


It is interesting to note that during the course of the reaction the positions of the substituents X and Y are reversed.

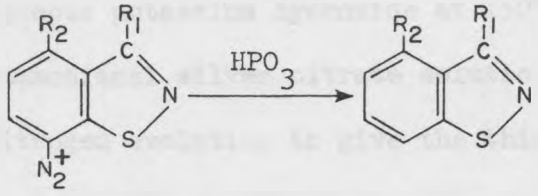
ii. - Diazotisation and decomposition of 7-aminobenzoisothiazole. Haddock et al. (1971a,b) have described the rearrangement of the diazonium salts derived from 7-aminobenzoisothiazoles to give 1,2,3-benzothiadiazoles. Thus, when the diazonium salt of 7-amino-benzoisothiazole is subjected to decomposition, the following reactions can occur depending on the nature of the agent employed (Haddock, 1971b). If the decomposing agent is a Sandmeyer reagent, e.g., Cu_2Cl_2, the main product is the 7-carbaldehyde-1,2,3-benzothiadiazole and a trace of 7-chloro-benzoisothiazole.

R_1 = H, CH_3

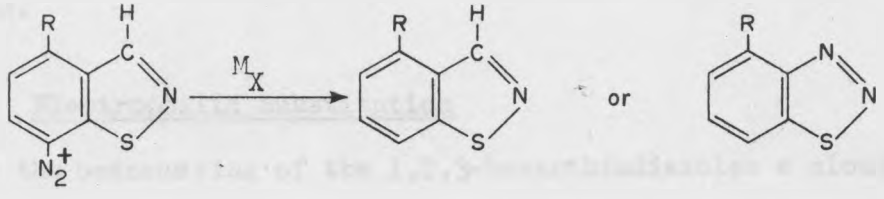
R_2 = OCH_3, CH_3S, Cl



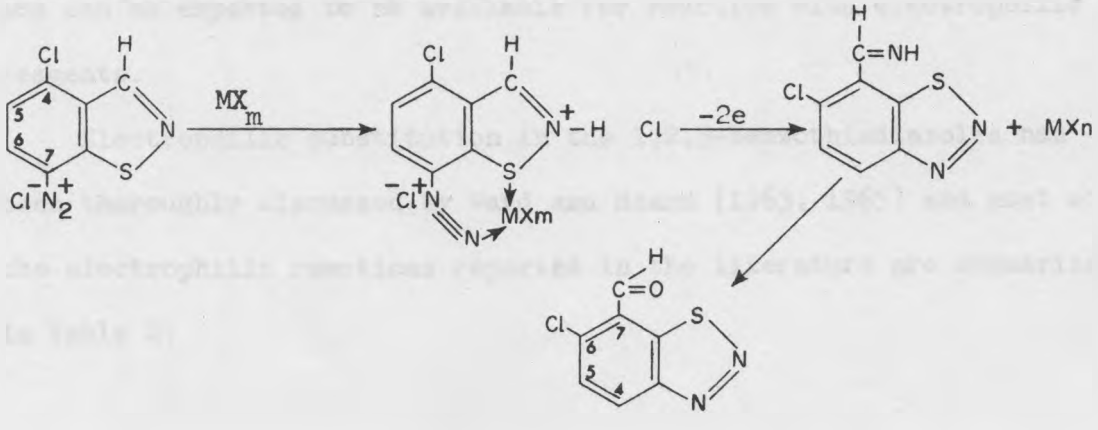
If the decomposing agent is hypophosphorous acid, the diazonium group is removed without rearrangement of the ring system to yield mainly the corresponding substituted benzoisothiazoles.



When the decomposition agent is a metal chloride other than Cu_2Cl_2, the rearrangement may give either the 7-acyl-1,2,3-benzothiadiazole or the corresponding benzoisothiazole depending on the oxidation potential of the metal salt.



The following mechanism was suggested for this reaction where MXm is the metal salt in its lower oxidation state and MXn is the metal salt in its higher oxidation state.



3. Chemical and Physical Properties

The 1,2,3-benzothiadiazole nucleous is extremely stable. The parent compound is stable to treatment with aqueous sulphuric acid at 200°C, to 20% aqueous potassium hydroxide at 150°C, and to boiling alcoholic ammoniacal silver nitrate solution. Heating at 200-250°C causes nitrogen evolution to give the thianthrene. The ring is not destroyed by either oxidizing agents, or reducing agents although tin and HCl can cleave the ring to the corresponding 2-aminobenzenethiol (Jacobson et al., 1893). The 1,2,3-benzothiadiazoles are weakly basic and are therefore soluble in concentrated acids. Most are solids readily crystallized from alcohol and many are volatile in steam.

a) Electrophilic Substitution

In the benzene ring of the 1,2,3-benzothiadiazoles a cloud of π electrons will exist above and below the plane of the ring. Since these electrons are less involved than the σ electrons in holding together the carbons in the aromatic ring, they are held less tightly and can be expected to be available for reaction with electrophilic reagents.

Electrophilic substitution in the 1,2,3-benzothiadiazoles has been thoroughly discussed by Ward and Heard (1963, 1965) and most of the electrophilic reactions reported in the literature are summarized in Table 2.

Table 2. Electrophilic substitution in the 1,2,3-benzothiadiazoles

Substituent	Reaction	Position(s) Attacked)	Reference
Unsubstituted	Br_2, hot CH_3COOH	None	Fries and Reitz (1936)
	H_2SO_4, KNO_3 100°C	5 - 7	Ward *et al.* (1962)
	H_2SO_4, KNO_3 100°C	4 - 7	Hodgson and Dodgson (1948a)
	H_2SO_4, HNO_3	4 -5- 7	Davies and Kirby (1967)
4-NH_2	Br_2, 1 mol.	5,7	Ward and Heard (1963)
4-NHAC	Br_2, 1 mol.	7 - 5,7	" "
4-NH_2-7-Br	Br_2, 20 mol.	5	" "
4-NH_2	I_2	5 - 7	" "
4-NH_2	PhN_2 HSO_4	7	" "
4-NH_2	p-Cl $C_6H_4N_2$ HSO_4	7	" "
4-NH-Tos	HNO_3	5-7; 5,7	" "
5-NH_2	diazocoupling	4	Fries *et al.* (1927)
5-NH_2-4-Cl	diazocoupling	None	" "
5-NH_2 HCl	Cl_2	Addition	" "

- Continued -

Table 2. Continued

Substituent	Reaction	Position(s) Attacked	Reference
5-OH	Br_2	4	Fries et al. (1927)
5-OH	HNO_3	4	" "
5-NH_2	Br_2 1 mol.	4	Ward and Heard (1965)
5-NH-Tos	Br_2 6 mol.	4	" "
5-NH_2	I_2	4	" "
5-NH-Tos	HNO_3	4	" "
6-OH	Br_2	7	Fries et al. (1927)
6-OH	Br_2 (excess)	5,7	" "
6-OH	HNO_3	7	" "
6-OH	Cl_2	7, addition and descomp.	" "
6-OH-7-Cl	HNO_3 (large excess)	5	" "
6-NH_2	Br_2 1 mol.	7	Ward and Heard (1963)
6-NH_2	Br_2 10 mol.	7 and 5,7	" "
6-NH-Tos	Br_2 2 mol.	7	" "
6-NH-Tos	Br_2 10 mol.	5,7	" "
6-NH_2-7-NO_2	Br_2 1 mol.	None	" "

- Continued -

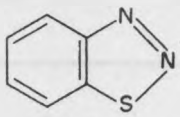
Table 2. Continued

Substituent	Reaction	Position(s) Attacked	Reference
6-NH_2	I_2	7	Ward and Heard (1963)
6-NH_2	p-Cl C_6H_4 N_2 HSO_4	7	" "
6-NH_2	PhN_2 HSO_4	7	" "
6-NH_2	p-NO_2 C_6H_4 N_2 HSO_4	7	" "
6-NH-Tos	HNO_3	7	" "
6-Cl	H_2SO_4 KNO_3	7	Haddock et al. (1970)
6-OC_2H_5	H_2SO_4 KNO_3	7	Gil*
6-CH_3	H_2SO_4 KNO_3	7	" *
7-NH_2	Br_2 2 mol.	4	Ward and Heard (1965)
7-NH-Ac	Br_2 1 mol.	4	" "
7-NH_2	I_2 1.1 mol.	4	" "
7-NH_2	p-NO_2 C_6H_4 N HSO_4	4	" "
7-NH-Tos	HNO_3	4 - 6	" "

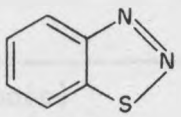
*This thesis, III. B. 11. c. vi.

Fries and co-workers, Hodgson and Dodgson (1948d) and Bambas (1952) suggested that the 1,2,3-benzothiadiazole ring closely resembles that of naphthalene; indeed the 1,2,3-benzothiadiazoles were initially described as "naphthalene-like" compounds. This theory has not been accepted by Ward and Heard (1963, 1965), however, as a result of the differences in the reactivity of the two ring systems towards a variety of electrophilic reagents. Compared with naphthalene, the 1,2,3-benzothiadiazoles are extremely unreactive in this respect. Thus, naphthalene is rapidly trinitrated even at 0°C, whereas 1,2,3-benzothiadiazoles is mononitrated only under extreme conditions.

Based on some of the results presented in Table 2, Ward and Heard (1963, 1965) suggested that in 4-substituted 1,2,3-benzothiadiazoles, electrophilic substitution occurred most readily at positions 5 and/or 7 of the ring, the 7-position being the most favored. In derivatives substituted in the 5-position, electrophilic substitution occurred primarily at position 4- and less readily at position 6-. When substituents are present in either the 6- or 7-position of the ring, electrophilic substitution is associated with the 7- and 5- or 6- and 4-positions respectively. The low reactivity of the 1,2,3-benzothiadiazoles to electrophilic reagents and the positions of the ring at which attack most readily occurs can be explained on the basis of a powerful deactivating effect (electron withdrawal) by the heterocyclic ring. This appears to be most active at the 4- and 6-positions of the ring and is also favored by the tendency towards bond fixation in the benzene ring with structure (I) being predominant over structure (II).



(I)



(II)

Although this hypothesis is in general supported by the results in Table 2 it should be pointed out that it tends to ignore the possible steric hindrance at positions 4- and 7-. Furthermore it is possible that intramolecular interactions might occur between substituents at the 4- and 7- positions of the phenyl ring and the N and S atoms in the hetero portion of the molecule.

b) Nucleophilic Substitution

A typical nucleophilic substitution in a 1,2,3-benzothiadiazole can be represented by the following general equation, where R is the 1,2,3-benzothiadiazole nucleus, X is a halogen (e.g., Cl, F) and Y is a nucleophilic reagent such as OH, OR, or SR.

$$R{:}X + {:}Y \longrightarrow R{:}Y + {:}X^-$$

Table 3 lists most of the nucleophilic reactions described in the literature. From these results it can be seen that fluorine is attacked in preference to chlorine in every position, even in the most unfavourable 5- and 7-positions of the ring. This has been explained by Davies *et al.* (1971) in terms of the soft and hard acid-base concept initially postulated by Pearson and Songstad (1967), which states that hard nucleophiles such as alkoxide and hydroxide ions displace fluorine (a harder leaving group) much more easily than chlorine.

Table 3. Nucleophilic substitution in the 1,2,3-benzothiadiazoles

Substituent	Reaction	Position(s) Attacked	Reference
Unsubstituted	KOH, aqueous 150°C	None	Hodgson and Dodgson (1948d)
4-Cl	KOH, DMSO	4	*Gil
6-Cl	KOH, DMSO	6	*Gil
4-F, 6-Cl	KOH, DMSO	4	Davies et al. (1971)
5-F, 6-Cl	KOH, DMSO	5	" "
7-F, 6-Cl	KOH, DMSO	7	" "
4-Cl, 6-Cl	KOH, DMSO	4	" "
6-Cl, 7-Cl	KOH, DMSO	6	" "
5-Cl, 6-Cl	KOH, DMSO	6	" "
5-F, 6-Cl	CH_3OH, CH_3ONa	5	" "
5-Cl, 6-Cl	CH_3OH, CH_3ONa	6	" "
6-Cl, 7-NO_2	CH_3OH, CH_3ONa	6	" "
6-Cl	C_3H_7OH, C_3H_7ONa	6	*Gil
5-Cl, 6-Cl	pCH_3-C_6H_4-S	6	Davies et al. (1971)
5-F, 6-Cl	CH_3S	6	" "
4-F, 6-Cl	pCH_3-C_6H_4S	4	" "
4-Cl, 6-Cl	pCH_3-C_6H_4S	4	" "
5-Cl, 7-Br	pCH_3-C_6H_4S	6	" "
6-Cl, 7-Cl	pCH_3-C_6H_4S	6	" "
6-Cl	n-butyl cellosolve	6	" "
6-Cl	KOH and 2-ethoxyethanol	6	" "
6-NO_2	NH_2OH at 0°C	7	" "
4-NO_2	NH_2OH at 0°C	4,5 and 4,7	Haddock et al. (1970)

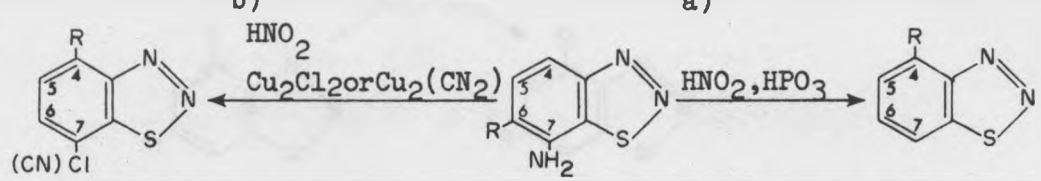
* This thesis, III.B.11.c.i.

Since the positions most deactivated to electrophilic substitutions are usually those most activated towards nucleophilic attack, compounds substituted with halogen at either positions 4- or 6- of the ring would be expected to be most reactive towards hard nucleophilic reagents. Several examples which support this hypothesis are provided in Table 3. However, Davies et al. (1971) pointed out that no predictions can be made in reactions involving a soft nucleophilic reagent such as RS.

As might be expected 5-chloro-1,2,3-benzothiadiazole does not undergo nucleophilic substitution when it is treated with alkoxide ion, but instead yields p-chloro-phenyl disulphide. Davies et al. (1971) have postulated that this degradation might be explained as an attack by the alkoxide ion on the 2-nitrogen atom of the benzothiadiazole ring followed by decomposition of the resulting intermediate. The behaviour of the 7-halogeno-1,2,3-benzothiadiazoles towards nucleophilic reagents has not yet been investigated.

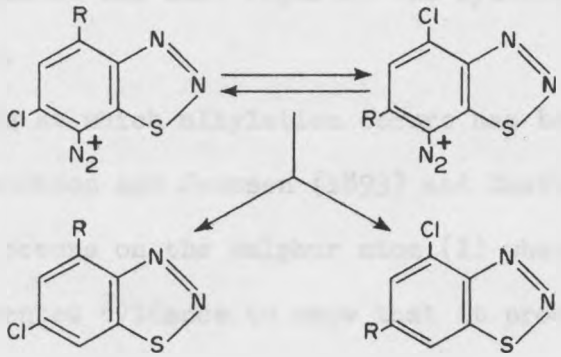
c) Miscellaneous Reactions

i. - Rearrangement of diazonium salts derived from 7-amino-1,2,3-benzothiadiazole. When a substituted 7-amino-1,2,3-benzothiadiazole is diazotised, and the diazo group removed with either hypophosphorous acid or reaction with a Sandmeyer reagent, the following interesting rearrangements may occur (Haddock et al., 1970), where R=F, Cl, OCH_3, or NO_2.

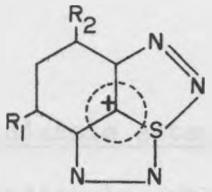


A 6-substituted 7-amino-1,2,3-benzothiadiazole treated with nitrous acid and hypophosphorous acid may lead to the formation of a 4-substituted-1,2,3-benzothiadiazole (reaction a). Alternatively when it is treated with nitrous acid and a Sandmeyer reagent a 4,7-disubstituted-1,2,3-benzothiadiazole may be formed (reaction b). The type of rearrangement which occurs depends on the nature and position of the 4- and 7-substituents in the 7-amino-1,2,3-benzothiadiazole. Thus, when the 6-position is substituted and the 4- is not, the rearrangement occurs. Since substituents such as nitro and methoxy groups which have opposite electronic effects on the ring both favor the rearrangement, it was suggested that the effect must be due largely to steric factors. However, the ability of 6-fluoro-7-amino-1,2,3-benzothiadiazole to also undergo this rearrangement suggests that it cannot be fully explained by steric factors. When the 6-position is unsubstituted and a nitro or halogen (Cl, Br) is the substituent at the 4-position the rearrangement does not occur.

Haddock et al. (1970) have also studied the rearrangements which occur when an alkyl group is present in the 4-position of the ring. The products formed are as follows:



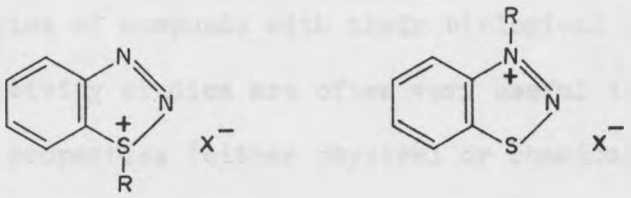
These investigators found that when a group such as ethyl or t-butyl which is more bulky than methyl was placed at the 4-position it produced less hindrance with the heterocyclic ring and, consequently, tended to oppose to the rearrangement. These workers also postulated that the intermediate for these rearrangement reactions might be a material with a delocalized action, although conclusive evidence to support this was not provided.



ii. - Formation of quaternary salts. The 1,2,3-benzothiadiazoles combine with quaternizing agents to give the corresponding quaternary salts. Methyl and ethyl-1,2,3-benzothiadiazolium iodides were first reported by Jacobson and Janssen (1893), and other quaternary salts were reported by Jacobson and Ney (1893). The method of synthesis was prolonged heating (1-3 days at 100°C) of the 1,2,3-benzothiadiazole with a ten fold excess of the appropriate alkyl iodide. Nunn et al. (1964) quaternized 1,2,3-benzothiadiazole by heating at 100°C with dimethyl and diethyl sulphates and also reported the synthesis of several other quaternary salts.

The position at which alkylation occurs has been a matter of some controversy. Jacobson and Janssen (1893) and Hantzsch (1909) suggested that alkylation occurs on the sulphur atom (I) whereas Nunn et al. (1964) have presented evidence to show that it probably occurs at

nitrogen -3 (II). The quaternary salts have been found to be potent *in vitro* inhibitors of monoamine-oxidase, but this activity was greatly diminished *in vivo* (Nunn *et al.*, 1964).



iii. - *1,2,3-benzothiadiazole esters of phosphorus and carbamic acids*. The 1,2,3-benzothiadiazole esters of phosphorus acids have been reported by Hackmann and Kirby (1967). These compounds exhibited a broad spectrum of insecticidal activity against mosquito larvae, aphids, flies, moth larvae and also showed miticidal activity; they possessed a very low acute toxicity to mammals. These compounds have been prepared by the reaction between the acid chlorides of appropriate thiophosphoric, thiophosphonic and thiophosphinic acids and the desired hydroxy or mercapto-1,2,3-benzothiadiazole in the presence of a base (pyridine or triethylamine).

N-methyl carbamates containing the 1,2,3-benzothiadiazole ring have been prepared by refluxing the appropriate hydroxy-1,2,3-benzothiadiazole with methyl isocyanate and triethylamine using dichloromethane as solvent. (Kirby *et al.*, 1970b), but no details about biological activity were reported.

B. The Correlation of Biological Activity and Chemical Structure

Ever since the early work of Meyer (1899) and Overton (1899), scientists have attempted to correlate the physical and/or chemical properties of series of compunds with their biological activity.

Structure activity studies are often very useful in obtaining information on the properties (either physical or chemical) that determine the biological activity of series of congeneric compounds and often provide information on the mechanism of their biological action. They also enable certain predictions to be made concerning the synthesis of more active compounds in a given series and make possible the storage of large amounts of data in a highly condensed form (equations).

In order to fully understand the changes in biological activity which occur within any one series of compounds, several factors need to be considered. These include purely geometrical factors (size, shape); physical properties (hydrophobic interactions and lipid solubility), electronic properties, and biotransformation (metabolism).

Two major models have been used for the mathematical treatment of biological data; the additive model of Free and Wilson (1964) and the linear free-energy related model of Hansch and Fujita (1964). More recently Camarata (1972) has shown how these two models are interrelated.

In the Free and Wilson model it is assumed that the activity contribution of each molecular substituent is additive and constant regardless of the nature of the substituents in other positions of the molecule. In other words in a series of closely related analogs at least some of the substituent groups contribute to the total biological

activity of the molecule in an additive way. Smithfield and Purcell (1967) have pointed out that the method requires additive parameters and a series of closely related analogs that give a gradual change in biological response. Several investigators however, have used this method successfully (Ban and Fujita, 1969; Purcell, 1965; Purcell and Clayton, 1968).

The main advantage of this method over the free energy related methods is that it is completely empirical and requires no physicochemical data. This in itself, however, creates several problems as it means that the steric, electronic and hydrophobic effects of various substituents are all included in each constant (Hansch, 1967) and the method does not compensate for physical properties such as pH and pka (Free and Wilson, 1964). Constants derived for one series of compounds are of no use for another series causing a different biological response (Hansch, 1967) and the constant additive group contribution does not apply when there is a parabolic relationship between the biological response (BR) and the partition coefficient (Singer and Purcell, 1967).

The hypothesis on which the Hansch model is based depends on two complex processes, first the movement of the drug from its point of application in the biological system to the site(s) of action and subsequently a rate limiting physical or chemical reaction which occurs at the receptor site(s).

When the drug interacts at the receptor site(s), a critical physical or chemical reaction occurs and results in a change in free energy

($\Delta F^{\circ}BR$) of the system. This change in free energy is made up of contributions of hydrophobic (ΔF°_{H}), electronic (ΔF°_{E}) and steric (ΔF°_{S}) factors and can be represented by Eq. 1.

$$\Delta F^{\circ}BR = \Delta F^{\circ}_{H} + \Delta F^{\circ}_{E} + \Delta F^{\circ}_{S} + \ldots\ldots\ldots\ldots \qquad \text{Eq.(1)}$$

Hydrogen bonding and charge transfer complex formation can also be considered as having additive and independent contributions to $\Delta F^{\circ}BR$.

Since $\Delta F^{\circ} = -RT \ln K$

then

$$\Delta F^{\circ}BR = -RT \ln K_{BR} \qquad \text{Eq.(2)}$$

where K_{BR} is the equilibrium constant of the rate limiting reaction which produces the biological response. The biological response is usually reported in terms of ED_{50}, LD_{50}, I_{50}, K_{m}, etc.

Considering a substituent effect(s) and for a true equilibrium condition Eqs. 1 and 2 can be combined to give Eq. 3.

$$\delta_X \Delta F^{\circ}BR = \delta_X \Delta F^{\circ}_{H} + \delta_X \Delta F^{\circ}_{E} + \delta_X \Delta F^{\circ}_{S} = -RT \delta_X \ln K_{BR} \qquad \text{Eq.(3)}$$

where the various free energy terms can be associated with appropriate physicochemical constants of the type shown in Table 4. For example:

$\delta_X F^{\circ}_{H}$ = f(logP, π, parachor, chromatographic constants derived from Rf)

$\Delta F^{\circ}_{S} = f(E_s, E_s^{c})$

ΔF°_{E} = f(σ, σ^{+}, σ_I, etc., quantum mechanically calculated electron densities, chemical shifts, etc.)

Table 4. Parameters most often used in free energy correlations

Symbol	Parameter	Reference
E_s	Taft Steric parameter	(Hansch and Deutsch, 1966) (Fukuto, 1969) (Kutter and Hansch, 1969)
E_s^c	Hancock's corrected steric parameter	Hansch et al., 1965a)
π	Hydrophobic bonding constant	(Hansch and Glave, 1972) (Topliss and Yudis, 1972)
log P	Log partition coefficient (water/octanol)	(Martin and Hansch, 1971)
σ	Hammett linear free energy constant	(Jones et al., 1969)
σ_I	Inductive parameter	(Hansch, 1968b)
σ^+	Electrophilic radical constant	(Hansch, 1968b)
$\sigma^\cdot$	Homolytic radical constant	(Hansch, 1968b)
σ^*	Taft's aliphatic constant	(Hansch and Lien, 1968)
R_M	Chromatographic constant	(Boyce and Milborrow, 1965)
P	Parachor	(Mc.Gowan, et al., 1966)
F	Molar attraction constant	(Ostrenga, 1969)
Δk	Hydrogen bonding parameter	(Purcell et al., 1966)
ΔpKa	Dissociation constant difference between parent and derivative	(Fujita, 1966)
M	Molecular weight	(Leo et al., 1969)
Mol. Vol.	Molecular volume	(Craig, 1971)
HOMO	Energy of highest occupied molecular orbital	(Neely et al., 1968)
R	Resonant constant	(Swain and Lupton, 1968)

A general equation containing the parameters most commonly used in structure-activity relationships is shown in Eq. 4, where ρ, k', k", and k"', are reaction constants derived by regression analysis, and C is the molar concentration of material required to produce a given biological response.

$$\log BR = \log \frac{1}{C} = k'\pi + \rho\sigma + k'' E_s + k''' \qquad \text{Eq.(4)}$$

Lipophilicity is very important in determining the penetration, transport and binding of drugs in biological tissues and is associated mainly with hydrophobic bonding. In addition conformational changes occurring in enzymes or at receptor sites might be closely related to hydrophobic character (Hansch, 1970). Fujita *et al.* (1964) have employed a hydrophobic bonding constant (π) derived from partition coefficients between 1-octanol and water and defined as:

$$\pi = \log P_x - \log P_H$$

where P_H is the partition coefficient of a parent molecule in 1-octanol-water and P_x is that of a derivative. Several examples of good correlation between biological activity and hydrophobicity have been reported by Hansch (1967), and because of its overall importance it is by far the most useful parameter in structure-activity studies. One of the most valuable properties of π is its additive character, so that with only a few experimental values for various substituent groups or atoms it is possible to calculate the relative partition coefficients for many other compounds. A comprehensive list of partition coefficients has recently been published by Leo *et al.* (1971).

The Single Parameter Approach

There are only a few examples in which biological activity can be correlated with a single linear physicochemical parameter, but this can occur. Thus, for example, Hansch and Fujita (1964) obtained an excellent correlation between the toxicity of mono and poly substituted benzoic acids to mosquito larvae and their partition coefficients (Eq. 5) and Hansch and Deutsch (1966) found a good correlation between the electronic effects of the p-substituents in a series of substituted diethyl phenyl phosphates and the ability of these compounds to inhibit fly head cholinesterase (Eq. 6).

$$\log\frac{1}{C} = 0.519\pi + 1.540 \quad n = 14 \quad r = 0.977 \quad s = 0.130 \quad \text{Eq.(5)}$$

$$\log\frac{1}{C} = 3.451\sigma + 4.461 \quad n = 6 \quad r = 0.954 \quad s = 0.507 \quad \text{Eq.(6)}$$

Hansch (1968c) has also been able to correlate the inhibition of cholinesterase by a series of alkylphosphonic acids esters with the steric substituent constants (E_s) of the alkylmoiety (Eq. 8).

$$\log K = 3.74\ E_s + 7.54 \quad n = 13 \quad r = 0.901 \quad s = 0.749 \quad \text{Eq.(7)}$$

The Multiple Parameter Approach

Considering the complexity of the electronic, hydrophobic, and steric contributions to drug receptor interactions and the additional factors associated with metabolism and distribution, it is not surprising that only few correlations with a single linear parameter have been reported. In attempts to account for all these factors equations containing several parameters have been widely employed. Eq. 8 illustrates one of these where A_x is the relative rate of hydrolysis

of a series of p-nitrophenyl esters by human serum (Hansch et al., 1965b) and σ* (the Taft constant) is a measure of the electronic effects of the substituents in aliphatic systems.

$$\log A_x = -7.614\sigma^* + 0.389\pi + 3.808E_s + 1.552 \qquad \text{Eq.(8)}$$

$n = 6 \quad r = 0.991 \quad s = 0.381$

In some cases the association of a particular physicochemical parameter to a biological response is not linear over a very wide range but exhibits a parabolic relationship with a distinct optimum value. To avoid the difficulties arising from this type of relationship it has become common practice to include square terms in the equations. This is clearly illustrated by Eq. 9 which expresses the activity of a series of 1,3-benzodioxoles in synergizing carbaryl to houseflies (Hansch, 1968b). It is often possible to calculate the optimum value for a given parameter and this may prove useful in the design and synthesis of more active analogs. Thus, for example, in a particular series of compounds those possessing the ideal lipophilic value (π_o) or the ideal partition coefficient (P_o) may have the highest probability of reaching the site of action.

$$\log \text{S.R.} = -0.195\pi^2 + 0.670\pi + 1.316\sigma^* + 1.612 \qquad \text{Eq.(9)}$$

$n = 13 \quad r = 0.929 \quad s = 0.171$

The Hansch approach of evaluating structure-activity relationships has clearly succeeded over others which have been proposed since it allows the qualitative and quantitative contributions of several different factors to be described in physicochemical terms. It should

be pointed out, however, that the most reliable equations are those containing the fewest terms. One of the main criticisms of the Hansch approach is that sometimes too many terms are included in the equations. Thus, Eq. 10, which contains six terms (Hansch, 1968b), appears as though it has been obtained merely for the sake of improving correlation. Since it is very difficult to explain the meaning of terms, such as σ^2, $\pi\sigma'$ their significance is dubious.

$$\log S.R. = -0.123\pi^2 + 0.633\pi - 1.823\sigma'^2 + 3.162\sigma' - 0.796\pi\sigma' + 0.639E_s + 1.450 \quad n = 16 \quad r = 0.991 \quad s = 0.074 \qquad \text{Eq.(10)}$$

Furthermore, the results are meaningful only when the selected physicochemical constants are tested over a wide range of values and several points are used in the regression equations. Consequently, the high correlation obtained in Eq. 11 (Hansch, 1972) has little or no meaning since it describes the activity of only three compounds.

$$\log BR = 0.63 \log P + 0.60 \quad n = 3 \quad r = 0.999 \quad s = 0.026 \qquad \text{Eq.(11)}$$

C. The Mode of Action of Insecticide Synergists

Synergists are compounds which although having no direct toxic effect *per se* at the dosage employed enhance the pharmacological action (toxicity, sleeping time, carcinogenecity, etc.) of a drug or an insecticide with which they are combined. It is now clear that synergists are inhibitors of the enzymes responsible for the detoxification of foreign compounds (xenobiotics) in insects as well as in mammals (Casida, 1970). Excellent and comprehensive reviews on the subject

have been published (Wilkinson, 1971a,b; Casida, 1970; Brooks, 1968; and Metcalf, 1967).

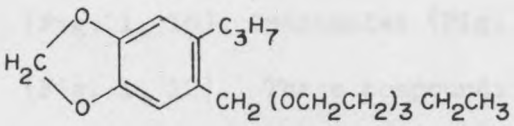
Synergists can be of practical importance in controlling insect pests of agricultural and public health importance since they may: a) increase the spectrum of activity of an insecticide with which they are combined, b) restore the activity of insecticides against some strains of insects which have developed resistance to insecticides (Wilkinson, 1968) and c) reduce the amount of insecticide required for insect control with all the consequent economic and environmental benefits. In addition, basic studies on synergism and synergists have led to a better insight into the mechanisms of detoxification in insects and mammals, the mode of action of insecticides, and the basic biochemical processes involved in insecticide resistance.

Only very few synergists are actually used commercially. These include piperonyl butoxide, tropital, propyl isome and sulfoxide, and as these materials are very expensive they are used mainly in combination with the pyrethroid insecticides in household aerosol formulations. The potential use of synergists as pest control agents, has been extensively discussed by Hennessy (1970) and Wilkinson (1973).

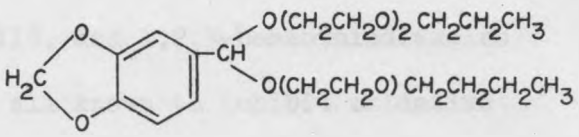
The structural formulas for various groups of insecticide synergists are given in Fig. 1.

The most important and widely investigated are the methylendioxyphenyl compounds (1,3-benzodioxoles) (Fig. 1, 1 to 4), although more recently several groups of compounds have been discovered with similar activity. These include N-alkyl compounds such as Lilly 18947 (Fig. 1, 5) and

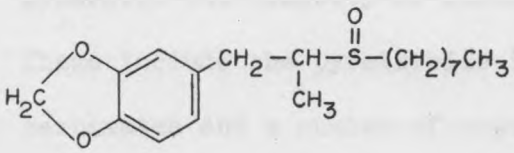
Fig. 1 Structure of several insecticide synergists



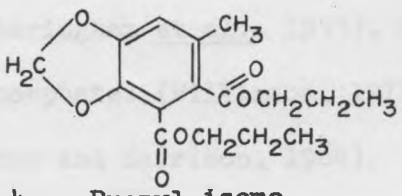
1. - Piperonyl butoxide



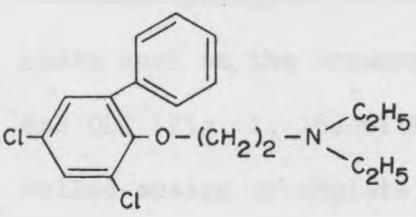
2. - Tropital



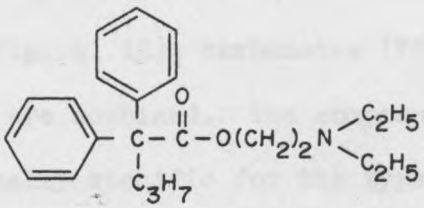
3. - Sulfoxide



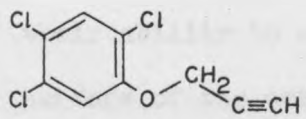
4. - Propyl isome



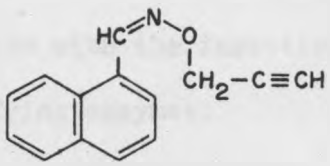
5. - Lilly 18947



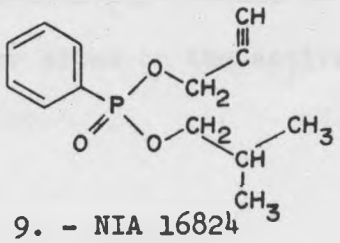
6. - SKF 525-A



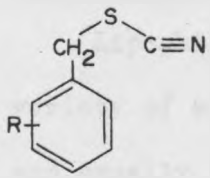
7. - RO-8019



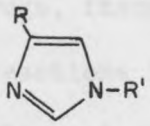
8. - Naphthaldoximino propynyl ether



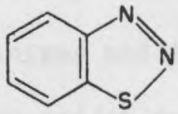
9. - NIA 16824



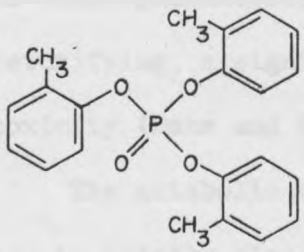
10. - Benzylthiocyanates



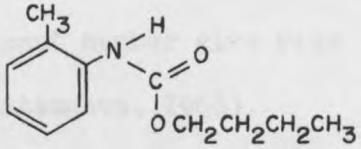
11. - Imidazoles



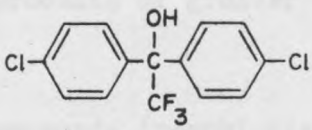
12. - 1,2,3-benzothiadiazoles



13. - TOCP



14. - butyl o-methyl-carbonilate



15. - F.-DMC

SKF-525 (Fig. 1, 6), O-(2-propynyl) ethers (Fig. 1, 7) and oxime ethers (Fig. 1, 8), phosphonate esters (Fig. 1, 9), organo-thiocyanates (Fig. 1, 10), imidazoles (Fig. 1, 11), and 1,2,3-benzothiadiazoles (Fig. 1, 12). These compounds are all known to inhibit oxidative metabolism (Casida, 1970), and consequently have the potential to synergize the toxicity of insecticides detoxified by this mechanism. These include the pyrethroids (Winteringham et al., 1955), the carbamates and a number of organophosphates (Wilkinson, 1971a) and some chlorinated hydrocarbons (Brooks and Harrison, 1964). A variety of other synergists have a close structural similarity to the insecticides such as the organophosphates (Fig. 1, 13), carbamates (Fig. 1, 14) and DDT (Fig. 1, 15) with which they are combined. The compounds are called analog synergists, and are usually specific for the types of insecticide with which they are employed. Their activity depends on their ability to compete with the insecticide for sites on the active surface of the detoxifying enzymes.

Metabolism of Lipophilic Compounds

Lipophilic compounds (drugs, insecticides, etc.) may undergo a variety of enzyme catalyzed reactions in living organisms and these are usually associated with alterations in the degree and/or duration of their pharmacological effect. Although most of the reactions are detoxifying, a significant number give rise to products of greater toxicity (Dahm and Nakatsugawa, 1968).

The metabolic transformations of foreign compounds (xenobiotics) may be broadly classified as either microsomal or nonmicrosomal

(Parke, 1968). The term microsomal means that the reactions are associated with the high speed sediment prepared by centrifugation at 100,000 g of the post-mitochondrial supernatant of a homogenate of a plant or animal tissue (Siekevitz, 1963). The microsomes are small membrane vesicles derived from the endoplasmic reticulum of the intact cells of mammalian liver (Gram and Fouts, 1968) and various insect tissues (Hodgson and Plapp, 1970).

The microsomal enzymes require NADPH and molecular oxygen for activity and are also commonly known as mixed-function oxidases (m.f.o.) (Mason, 1965), since one atom of oxygen is reduced to water and the other incorporated into the drug or insecticide substrate. The m.f.o. system usually initiates the metabolic attack on a foreign compound (Parke, 1968) tending to increase the polarity and water solubility of these lipophidic materials in such a way that they can be more readily removed by the normal excretory system.

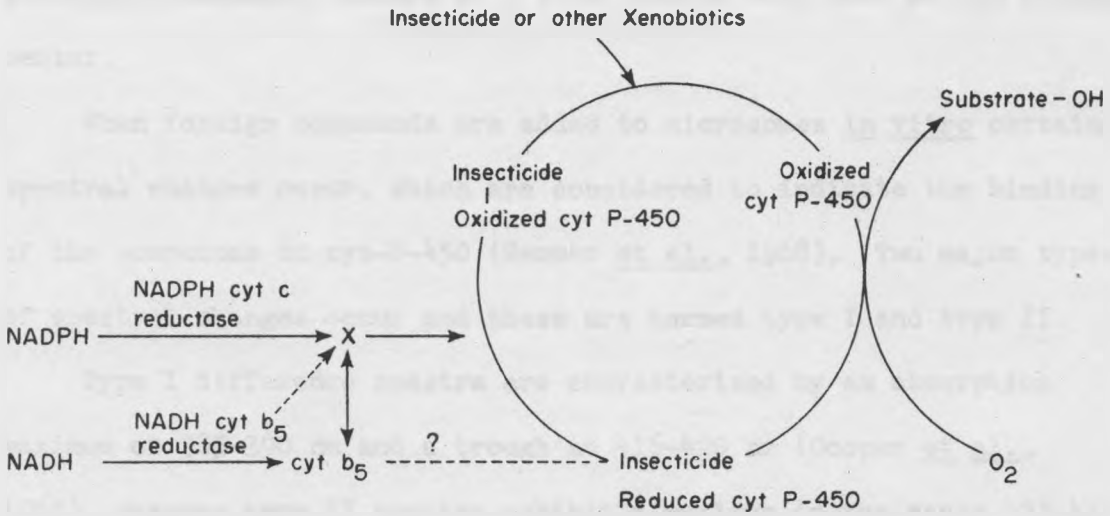
Reactions catalyzed by the m.f.o. system include the hydroxylation of aliphatic compounds or of aromatic and alicyclic rings, the epoxidation of double bonds, the oxidation of thioethers and phosphorothionates, the dealkylation of aromatic ethers, cyclic ethers and of substituted amines (Wilkinson and Brattsten, 1973).

Nonmicrosomal reactions are mediated by various types of enzymes which catalyze reactions such as conjugation, some ester hydrolyses (Parke, 1968) dehydrochlorination, etc. The substrates for these enzymes are often the products of microsomal metabolism. It is generally accepted that the liver m.f.o. system contains an organized electron

transport chain which appears to be involved in the hydroxylation or oxidative demethylation of drugs (Kamin and Masters, 1968). Because of the many difficulties encountered in the solubilization and purification of the components of this system many details are still missing.

The simple scheme shown in Fig. 2, summarizes the current concept of electron transport in liver microsomes. The major components presently thought to be part of this system include two hemoproteins cyt P-450 and cyt b_5, and two flavoproteins NADPH-cyt-c-reductase and NADH cyt b_5 reductase. An unknown carrier (X) possibly exists between cyt b_5, NADPH-cyt-c-reductase and the terminal oxidase cyt P-450.

Fig. 2 Electron transport chain components for liver microsomal hydroxylations



A microsomal electron transport pathway similar to this is present in insect microsomes (Hodgson and Plapp, 1970; Wilkinson and Brattsten, 1973).

The terminal oxidase, cyt P-450 is a hemoprotein complex, detectable spectrophotometrically only in its reduced-CO derivative form which has an absorption maximum at 450 nm (Remmer et al., 1968). Together with the other components of the oxidase complex it is associated with the microsomal membranes and is extremely resistant to solubilization. At the heme moiety of cyt P-450 molecular oxygen is activated, and subsequently incorporated into the drug or insecticide substrate as shown in Fig. 2. The binding and activation of the oxygen molecule occurs at the ferrous ion of the heme which has been defined as the "catalytic site" of the enzyme. The binding of the substrate presumably occurs at a site located very near to the catalytic center.

When foreign compounds are added to microsomes in vitro certain spectral changes occur, which are considered to indicate the binding of the compounds to cyt-P-450 (Remmer et al., 1968). Two major types of spectral changes occur and these are termed type I and type II.

Type I difference spectra are characterized by an absorption maximum at 385-390 nm and a trough at 416-420 nm (Cooper et al., 1965), whereas type II spectra exhibit a maximum in the range 423-435 nm and a minimum at 390-419 nm (Imai and Sato, 1966). Most microsomal enzyme substrates produce either a type I or type II difference spectrum and are therefore termed type I or type II substrates. A

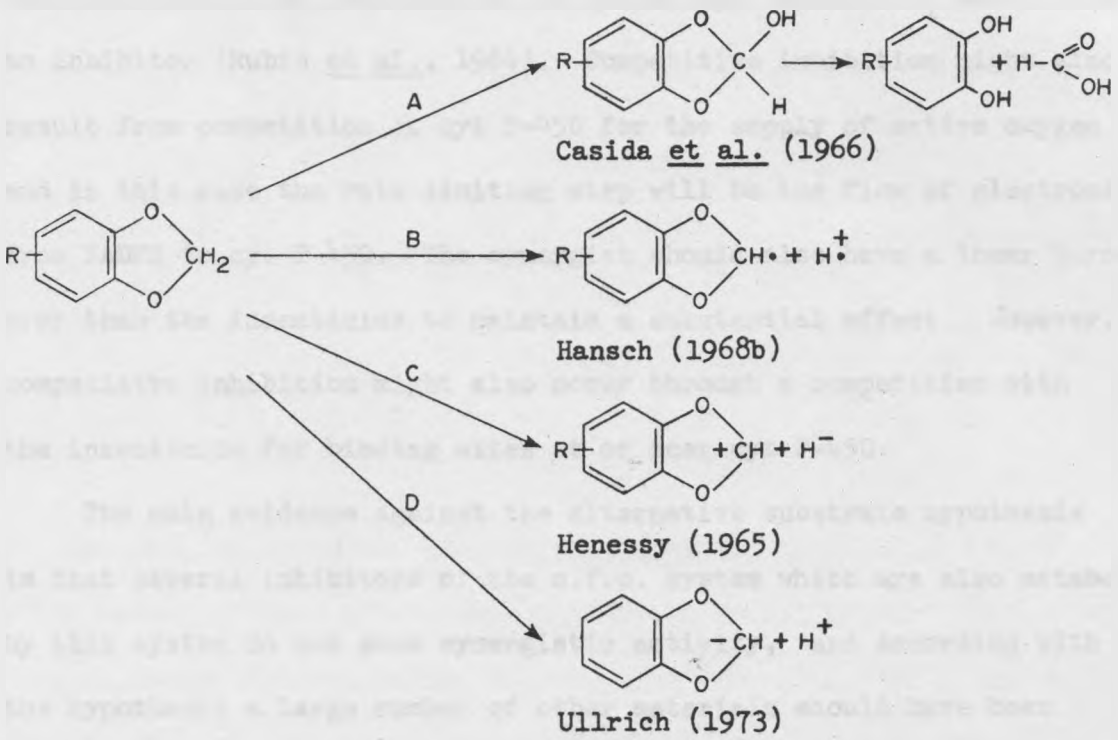
new type III spectral interaction has recently been reported by Philpot and Hodgson (1971) for the binding of some 1,3-benzodioxole synergists to NADPH-reduced cyt P-450. This spectrum is similar to that produced by ethyl isocyanide (Omura and Sato, 1964b) and is characterized by having double Soret peaks at 430 nm 455 nm which are in pH dependent equilibrium.

Mechanism of Inhibition of m.f.o. Activity by Synergists

Since it is well established that synergists inhibit microsomal enzyme activity, considerable attention has been focused on the mechanism by which this occurs. There are of course several ways in which a synergist might interact with the microsomal complex to cause inhibition. It might interact with some component of the electron transport chain to prevent the reduction of cytochrome P-450 or it might bind directly to the hemochrome of cyt P-450 to interfere with the oxygen activating process. Alternatively it might compete with the insecticide or drug substrate for their protein binding sites or for the availability of active oxygen at cyt P-450.

To explain the inhibitory activity of the 1,3-benzodioxoles which to date are the only group of compounds which have been extensively studied, four major theories have been proposed (Fig. 3).

Fig. 3. Theories to explain the mode of action of synergist



1) Casida et al. (1966) working with a series of synergists radiolabelled with C^{14} in the methylene group established that in houseflies the label was liberated as CO_2^{14} in vivo in houseflies and mice, and in vitro as C^{14} formate. This led them to propose that the 1,3-benzodioxoles were themselves metabolized (Fig. 3, A) and as a consequence their inhibitory activity might be explained as metabolic competition with the substrate for the enzyme(s) (alternative substrate). Supporting this hypothesis Wilkinson and Hicks (1969) found that 4,5,6,7-tetrachloro-1,3-benzodioxole was metabolized to the corresponding 3,4,5,6-tetrachloro-catechol by microsomal preparations from both mammalian liver and insect tissues. In order to be

an alternative substrate the synergist should competitively inhibit the enzyme and the Michaelis constant (Km) of the synergist as a substrate should be the same as its inhibition constant K_i when it is an inhibitor (Rubin et al., 1964). Competitive inhibition might also result from competition at cyt P-450 for the supply of active oxygen and in this case the rate limiting step will be the flow of electrons from NADPH to cyt P-450. The synergist should also have a lower turnover than the insecticide to maintain a substantial effect. However, competitive inhibition might also occur through a competition with the insecticide for binding sites at or near cyt P-450.

The main evidence against the alternative substrate hypothesis is that several inhibitors of the m.f.o. system which are also metabolized by this system do not show synergistic activity, and according with the hypothesis a large number of other materials should have been available as insecticide synergists.

2) Hansch (1968b) applying substituent constants and regression analysis to data reported by Wilkinson et al. (1966) for the in vivo synergism of carbaryl by a series of 1,3-benzodioxoles, proposed that the synergists might interact with the m.f.o. system by a homolytic cleavage of a hydrogen atom from the methylene group of the ring to generate a relatively stable free radical that acts as the inhibitor. It has been suggested by Staudinger et al. (1965) that the electrophilic radical (OH·) might be one possible form of active oxygen at P-450 and consequently this could be the hydrogen abstractor.

3) To account for the inhibitory activity of 1,3-benzodioxoles, Hennessy (1965) suggested the formation of an enzyme-attacking electrophilic benzodioxolium ion by loss of hydride ion (H^-) (Fig. 3, C). It was considered that this ion could act as an acylating agent, and could form a π-bonded complex with iron or copper in the terminal oxidase. Hennessy (1970) has pointed out that the same benzodioxolim cation could also be formed from 2-hydroxy-1,3-benzodioxole which is the propoposed intermediate in the microsomal hydroxylation of the 1,3-benzodioxoles (Fig. 3, A). It could also be formed by the reaction with Fe^{3+} of the homolytic radical proposed by Hansch.

4) Although it seems unlikely that protons might be present in the strongly lipophilic microsomal environment, Ullrich (1973) (Fig. 3, D) has recently suggested that the interaction of piperonyl butoxide with cyt P-450 might occur through a mechanism involving the liberation of a proton from the methylene group of the ring and that the subsequent formation of a stable carbanion might block the oxidative metabolism of other substrates.

The fact that all these theories have been proposed to explain the mechanism by which synergists inhibit the m.f.o. system clearly demonstrates that the exact nature of the phenomenon has not yet been elucidated.

SECTION III

MATERIALS AND METHODS

A. Biological Materials and Chemicals

1. Insects

Houseflies

A susceptible strain of *Musca domestica* L., the W.H.O. standard reference strain was used for the toxicological tests. The strain was developed by Professor Milani at the Institute of Zoology, University of Pavia, Italy, and kindly provided to our laboratory through the W.H.O. in Geneva.

Data concerning the origin of this strain and its susceptibility to insecticides have been summarized by Wright and Pal (1967) and by Franco (1964), and additional information is given in two W.H.O. communications. Some of the most advantageous characteristics of the strain are high fertility, uniformly large size, good longevity, extremely low genetic variability, and an absence of known genes causing resistance to insecticides or mutant phenotypes.

Larvae were reared at a temperature of 27 $\pm$ 2°C under constant lighting in glass jars containing a medium prepared with the following ingredients: CSMA fly medium (300g), corn syrup (12g), Fleischmann's yeast (6g), and water (450ml). The larvae were allowed to pupate in the jars and on emerging the adults were transferred to gauze covered cardboard containers provided with water, sucrose and powdered milk.

Madagascar Cockroaches (Gromphadorhina Portentosa, Schaum).

The rearing and maintenance of the roaches was carried out as described by Benke (1971) and by Rose (1972).

Southern Armyworm (Prodenia eridania)

Eggs from the southern armyworm were provided weekly, by the Niagara Chemical Division of the FMC Corporation, Middleport, New York. The rearing conditions have been described by Krieger (1970).

2. Mammals

Sprague-Dawley rats and white mice were purchased from Blue Spruce Farms, Inc., Altamont, New York.

3. Chemicals

2-Amino-2-hydroxymethyl-1,3 propanediol (Tris), cytochrome c, glucose-6-phosphate (G6-P), glucose-6-phosphate dehydrogenase (G6-P dh), reduced nicotinamide adenine dinucleotide phosphate (NADPH), reduced nicotinamide adenine dinucleotide (NADH) were purchased from Calbiochem. Inc., Los Angeles, California.

6,7-Dihydro-isodrin (DHI), 6-exo-hydroxy-6,7-dihydroisodrin, aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo,exo-5,8-dimethanonaphthalene),its epoxide dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene were kindly supplied by the Shell Development Co., Modesto, California.

7-Nitro-1,2,3-benzothiadiazole, 5-nitro-1,2,3-benzothiadiazole, 4-chloro-1,2,3-benzothiadiazole, 5,6-dichloro-1,2,3-benzothiadiazole, and 5-methyl,6-chloro-1,2,3-benzothiadiazole, were kindly provided

by Dr. Hugh Davies of the Shell Agricultural Research Center, Sittingbourne, Kent, England.

Samples of 4-nitro-benzenediazo-*tert*-butyl-sulphide (*trans*) and the 4-chloro-benzenediazo-*tert*-butyl-sulphide (*trans*) were donated by Dr. L. K. H. van Beek of Phillips Research Laboratories, Eindhoven, Netherlands.

Bioallethrin ((±)-allethronyl (+)-trans-chrysanthemate) and bioresmethrin (5-benzyl-3-furylmethyl(+)-trans-chrysanthemate) were a gift from Dr. John E. Casida, Division of Entomology, University of California, Berkeley, California.

Analytical grade carbaryl (1-naphthyl *N*-methylcarbamate) 99.7% (mp. 142°C), was supplied by Union Carbide Company, New York, and piperonyl butoxide (α-[2-(2-butoxyethoxy)ethoxy] 4,5-methylenedioxy-2-propyltoluene by the McLaughlin Gormley King Company, Minneapolis, Minnesota. Pesticide quality petroleum ether (b.p. 40-60°C), acetone, and hexane, and diethylether, carbon monoxide C.P. and hydrazine hydrate, were obtained from the Matheson Coleman and Bell Company, East Rutherford, New Jersey.

Linde nitrogen was purchased from Ames Welding and Supply Company, Ithaca, New York. Organic chemicals used in the syntheses were purchased from Aldrich Chemical Co., Inc., Milwaukee, Wisconsin, Pfaltz and Bauer, Inc., Flushing, New York and Eastman Organic Chemicals, Rochester, New York.

Powdered postassium bromide of infra red quality was obtained from The Harshaw Chemical Co. All other inorganic reagents and solvents were analytical reagent grade.

B. Experimental Methods

1. Preparation of Microsomes

Enzyme preparations were obtained from the midguts of armyworm larvae and Madagascar cockroaches by the methods described by Krieger (1970) and Benke *et al.* (1972), respectively; mammalian enzymes were prepared from rat or mouse liver. The tissues were homogenized in ice-cold 0.15M KCl (10-20%w/v) in a hand operated Ten Broeck tissue grinder with a teflon pestle. The insect homogenates were centrifuged in 15 ml glass tubes at 2000 x g for 2 minutes in an International Equipment Co. (IEC) clinical centrifuge to remove cell debris and the resulting supernatant was employed as the enzyme source in I_{50} determinations. To prepare microsomes, the homogenate was centrifuged in an IEC B-60 preparative ultracentrifuge in an A-211 anglehead rotor at 12,000 x g max for 15 minutes to remove mitochondria and other cell debris (occasionally a second 12,000 x g centrifugation was made to ensure removal of mitochondria), and the postmitochondrial supernatant was centrifuged at 100,000 x g max for 60 minutes. The microsomal pellet was suspended in either 0.15 M KCl or in 0.15 M potassium phosphate 0.05 M sucrose buffered at pH 7.8 and 7.4 in preparations from insects and mammals respectively.

When the microsomal preparation was used for cyt-P-450 determinations, the 100,000 x g max pellet was resuspended in the appropriately buffered 0.15 M potassium phosphate- 0.05 M sucrose buffer and recentrifuged at 20,000 x g, max for 10 minutes to minimize turbidity.

2. Protein Determination

All protein determinations were made by the modified Lowry method (Chaykin, 1966). A 0.5 ml aliquot of the enzyme suspension (40-100 µg of protein) was transferred to a 28 ml tube, and 5 ml of a solution prepared with one part of 2% sodium carbonate in 0.1 N sodium hydroxide and 50 parts of 0.5% copper sulfate pentahydrate in 1% potassium tartrate, was added. After 10 minutes of 0.5 ml of a solution containing 1 part of 2 N phenol reagent (Folin-Ciocalteau) and 2 parts of water was added and mixed immediately. The mixture was allowed to stand for a further 10 minutes at room temperature before the absorbance at 600 mµ was measured against a reagent blank in a Bausch and Lomb Spectronic 20. Protein concentration was obtained by reference to a standard curve obtained with bovine serum albumin.

3. Determination of Aldrin Epoxidase and Dihydroisodrin Hydroxylase Activities

Assays were performed in open 25 ml Erlenmeyer flasks, gently shaken in a water bath at 30°C. The flasks contained in a 5 ml total volume: glucose-6-phosphate dehydrogenase (2 units), NADP (51 uM), Tris-HCl buffer (50 mM, pH 7.4 for liver microsomes and pH 7.8 for insect preparations), KCl (27 mM), aldrin (100 µg) or dihydroisodrin (50 µg) in 20 µl of ethanol and 0.5 ml of enzyme suspension, containing 1.0 to 2.5 mg of protein. A similar system was first described by Lewis *et al.* (1967).

The reaction was initiated by the addition of the enzyme suspension and was terminated 15 minutes later by the addition of 3 ml of

acetone. The content of each flask and a subsequent 3 ml acetone rinse were then transferred to a 45 ml glass-stoppered tube. Ten ml of a hexane-diethyl ether mixture (1 : 1 v/v) and approximately 0.5 g of anhydrous sodium sulfate were added to each tube and the contents were mixed for 60 seconds in a Vortex Genie Mixer (American Hospital Supply Corporation, Evanston, Illinois). An appropriate aliquot (1-5 ml) of the organic layer was transferred to a glass-stoppered 10 ml tube and dilute with the hexane-diethyl ether mixture to 10 ml. The solutions were dried over sodium sulfate and 2-5 µl aliquots were analyzed by electron capture gas chromatography.

Gas chromatographic (GC) determinations of dieldrin and 6-exo-hydroxy 6,7-dihydroisodrin were effected using a Research Specialties instrument provided with a Sr^{90} ionization detector operated at 3 v and connected for electron capture. The 2 foot all-glass column (i.d. 5 mm) was packed with 5% (w/w) SE 30 on Gas-chrom Q and the working temperature was 190°C. Nitrogen was used as the gas carrier.

The metabolites, dieldrin for aldrin epoxidation, and the 6-exo-hydroxy-6,7-dihydroisodrin, for DHI hydroxylation, were quantified by the peak height method, using standard curves obtained each day under identical gas chromatographic conditions. The retention times were aldrin 1.56 minutes, dieldrin 2.68 minutes, DHI 2.14 minutes and DHI-OH 3.55 minutes.

4. Inhibition of Aldrin Epoxidase and DHI Hydroxylase

The molar concentration which inhibits 50% of the enzymatic reaction (I_{50}) was determined from the means of duplicate incubations with each of at least 5 different inhibitor concentrations and in all cases incubations contained a similar protein concentration. The incubation system was the same as that previously described (III. B. 3) except that it contained 10-50 µl of an ethanol solution of the inhibitor. The results are expressed in terms of per cent inhibition of control activity.

5. Measurements of Cytochrome P-450 and Difference Spectra

Cytochrome P-450 was measured by the method of Omura and Sato (1964a,b) using a Unicam SP-800 spectrophotometer, equipped with a scale expansion accessory and auxiliary recorder. All measurements were made at room temperature (20°C) using 1 ml or 3 ml fused silica cells with a 1 cm light path. The microsomal fraction prepared as described in section (III. B. 1) was resuspended in 0.15 M potassium phosphate-0.05 M sucrose buffer to give a protein concentration of 2-10 mg per ml. The suspension was placed in a 15 ml glass tube and nitrogen was gently bubbled for 1 minute prior to addition of 10 µl of a 0.15 M potassium phosphte-0.05 M sucrose buffer containing 2 mg of sodium dithionite. After recording the base line between 500 and 400 nm, CO was bubbled through the sample cuvette for 30-60 seconds and the spectrum from 490-390 nm was recorded. To obtain a measure of cyt P-450, the extinction coefficient of 91 m M^{-1} cm^{-1} reported by

Omura and Sato (1964a) was applied to the absorbance increment between 490 nm and 450 nm.

Difference spectra resulting from the in vitro addition, of 1,2,3-benzothiadiazoles to microsomal suspensions were determined under both oxidized and reduced conditions. The difference spectra were measured by adding the appropriate benzothiadiazole in 5-20 µl ethanol to the sample cuvette containing a suspension of 2-10 mg protein per ml and scanning the preparation against a mixture containing all components except the synergist.

Difference spectra were determined under oxidized conditions or immediately after addition of 2 mg of sodium dithionite to each cuvette.

6. Determination of Cytochrome c-reductase Activities

NADPH and NADH cyt c-reductases were assayed as described by Dallner (1963). The assay system contained 0.1 mM NADPH or NADH, 0.05 mM cyt c, 0.05 M potassium phosphate buffer, pH 7.5, 0.33 mM KCN and microsomal enzyme equivalent to 0.1-0.3 mg of protein in a final volume of 3 ml. For inhibition studies the 1,2,3-benzothiadiazoles were added in 10 µl of ethanol and cyt c reductase activities compared with those in the uninhibited system. The reduction of cyt c was followed by measuring the increase in absorbance at 550 nm in a Unicam SP-800 spectrophotometer at room temperature (20°C), and was initiated by the addition of either NADPH or NADH.

The amount of enzyme which produced a change in optical density of 1.0 in one minute at 550 nm was defined as one unit of NADPH or NADH cyt c reductase.

7. *In vivo* Treatment of Madagascar Cockroaches with 1,2,3-benzothiadiazoles

Groups of four adult female cockroaches of about 2 weeks of age were injected with a single dose of either 80 µg of 4-chloro-1,2,3-benzothiadiazole or 200 µg of 6-ethoxy-1,2,3-benzothiadiazole in 10 µl of acetone. Two control groups were also included, one in which the insects were not injected and the other in which they were injected with 10 µl of acetone. Food and water were provided *ad libitum* and the cockroaches were sacrificed on the fifth day after treatment. The *in vitro* epoxidase activity in each group was then determined as indicated in section (III. B. 3).

8. *In vivo* Treatment of Mice with 6-chloro-1,2,3-benzothiadiazole

Female mice, weighing 22-28 g were given a single intraperitoneal injection of 6-chloro-1,2,3-benzothiadiazole (208 mg/kg) in 0.1 ml of corn oil; control mice received 0.1 ml of corn oil. Three mice from each group were sacrificed at 3,24,48, and 96 hours. The livers were removed, cut into small pieces and washed several times with an ice-cold solution of isotonic 0.15 M KCl to remove hemoglobin. Microsomes from control and treated mice, were prepared as indicated in section (III. B. 1).

The microsomal pellets were suspended and assayed for aldrin epoxidase, cyt P-450 content, and NADPH cyt c reductase activity.

9. Bioassay

Flies were initially immobilized with carbon dioxide and were subsequently transferred to a petri dish held on ice. Insecticide-synergist combinations were topically applied in acetone (1 µl) to the dorsal thoraces of 3-day-old adult female houseflies (W.H.O. strain) by means of a hand-operated Burkard microapplicator containing an "Agla" micrometer syringe with a no. 27 gauge hypodermic needle bent at an angle of 90°. Groups of twenty treated flies were confined at 28°C in unwaxed 8 ounce containers with plastic covers and were provided with dental rolls soaked in 20% sucrose. Mortalities were observed 24 hours after treatment. All tests were made in triplicate using different batches of flies on three different days and employing at least five insecticide concentrations in each case. Control experiments showed no mortality when flies were treated by this method in the absence of insecticide. The median lethal doses (LD_{50} values) were estimated by plotting the average per cent mortalities on log-probit paper; the dosage-mortality regression lines were fitted by eye. The LD_{50} values were reproducible with a standard error less than 10% of the mean.

Solutions of insecticide-synergist combinations were usually prepared in acetone at a 1 : 5 (w/w) ratio although occasionally other ratios were employed. The fixed 1 : 5 insecticide: synergist

ratio was chosen on the basis of preliminary experiments and for practical convenience. It was considered to provide sufficient synergist to flood the detoxification system of the fly with synergist, but neglects to take into account the variations in the molecular weights of the synergists. The required concentrations were prepared in 10 ml glass vials provided with tightly fitting corks and were stored at 0°C in the refrigerator to avoid evaporation of the solvent.

Synergistic activity is described in terms of synergistic ratio which is the ratio of the LD_{50} of the insecticide alone to the LD_{50} of the insecticide-synergist combination. Due to the marked tolerance of the flies the LD_{50} for carbaryl alone could not be determined directly although extrapolation showed it to exceed 100 µg/fly. None of the synergists themselves had any toxicity when applied alone to flies at concentrations of at least two-fold that present in the insecticide-synergist combination required to produce 50% mortality.

10. Regression Analysis

Correlations were determined by regression analysis using the IBM 360/65 computer at Cornell University. Estimates of the parameters of single equation models were obtained by the method of least squares (ϕ LS) including estimates of a variety of related statistics.

11. Synthesis of 1,2,3-benzothiadiazoles

The 1,2,3-benzothiadiazoles were synthesized by the general procedures previously discussed for ring closure (II. A. 2) or by

chemical reactions occurring in the intact 1,2,3-benzothiadiazole nucleus. The methods employed were:

a) Diazotisation of o-aminobenzenethiols (II. A. 2. b)

b) Modified Herz reaction (II. A. 2. a)

c) Modification of aromatic ring substituents in 1,2,3-benzothiadiazoles.

Only one detailed example of each synthetic procedure employed will be provided. A complete list of the 1,2,3-benzothiadiazoles prepared is shown in Table 5, and each of these is assigned a roman numeral (I - XXXXVIII) for ease of reference in the text.

All melting points are uncorrected and were measured on an Electro-thermal ® melting point apparatus. Infrared spectra were measured in KBr pellets using a Perkin Elmer 137 B Infracord spectrophotometer.

a) Diazotisation of o-aminobenzenethiols (II. A. 2. b)

When the o-aminobenzenethiols are commercially available their direct diazotisation to the corresponding 1,2,3-benzothiadiazoles provides a convenient synthetic procedure. This is illustrated by the synthesis of the parent compound 1,2,3-benzothiadiazole (I).

1,2,3-Benzothiadiazole (I). Technical 2-amino-benzenethiol (233g, 1.68 mole) was dissolved in 10% hydrochloric acid, the solution cooled to 0°C, and sodium nitrite (130.2g, 1.86 mole) dissolved in the minimum amount of water, was added dropwise with continuous stirring below 5°C. The mixture was kept over night at 4°C and the next day was extracted into diethyl ether (1500 ml) and the extract dried over

sodium sulfate. Evaporation of the ether yielded a black oil, which was distilled under vacuum at 88°C and 1.7 mm of Hg to give a pale yellow oil. On cooling the distillate solidified to give (19lg) light yellow crystals of 1,2,3-benzothiadiazoles m.p. 35°C (yield 83%). Jacobson et al. (1983) give m.p. 35°C; Hodgson and Dodgson (1948a) give m.p. 35°C.

I.R. spectrum, γ cm^{-1} : 1580w, 1540m, 1445m, 1330m, 1280s, 1215m, 1160w, 1120w, 1075w, 1010m, 945w, 890s, 850w, 770s, 745s, 725s.

More usually, however, it was necessary to synthesize the o-aminobenzenethiols from a variety of available starting materials though in most cases it was not necessary to isolate and purify these materials prior to diazotisation. The procedures employed were:

i) Reduction and diazotisation of disulfides (II. A. 2. b. i)

ii) Thiolation of 2-halogeno anilines or nitrobenzenes (II. A. 2. b. ii)

iii) Ring opening of 2-amino or 2-methyl-benzothiazoles (II. A. 2. b. iii)

i) Reduction and diazotisation of disulfides. This provides a useful synthetic route and is illustrated by the synthesis of 5-chloro-1,2,3-benzothiadiazole (III).

5-Chloro-1,2,3-benzothiadiazole (III). The 2,2',-dinitro-4,4'-dichloro-diphenyldisulfide (20g, 0.053 mole) and 20-mesh Zinc (64g) was stirred into 90% acetic acid (500 ml). After the initial evolution of heat had ceased, the mixture was carefully raised to the boil and refluxed for 30 minutes. It was filtered hot and the residue

was washed with hot 90% acetic acid (250 ml). The residue was suspended in H_2SO_4 (D = 1.84, 40 ml) diazotised with sodium nitrite and the diazo-solution poured onto ice (1000g). The resulting mixture was extracted with diethyl ether (3 x 50 ml) dried over sodium sulfate, and the ether evaporated. The solids were crystallized from methanol to give white needles m.p. 103-104°C. Yield 23%. Beilstein and Kurbatow (1879) reported 103.5°C, Hodgson and Dodgson (1948b) give 105°C.

I.R. γ cm^{-1} : 1590w, 1540m, 1440m, 1410w, 1330w, 1280s, 1250w, 1200m, 1165w, 1075w, 1060m, 918m, 865s, 830s, 817s, 725w

The reduction and diazotisation of 2,2', 4,4'-tetranitrodiphenyl-disulfide represents a special case since it allows the direct synthesis of several 1,2,3-benzothiadiazole derivatives.

2,2', 4,4'-Tetranitrodiphenyl disulfide. To a stirred solution of 40.5g (0.20 mole) of 1-chloro-2,4-dinitro-benzene in 300 ml ethanol maintained below 30°C was added slowly a solution of sodium sulfide nonahydrate 24g (0.1 mole) and sulfur 3.2g (0.1 mole). Following each addition, the reaction mixture became transiently red and a yellow brown precipitate was formed. On complete addition of the sodium sulfide solution, the mixture was heated on a water-bath until most of the red colour had disappeared and the yellow precipitate of the crude disulfide was obtained by filtration. The material was extracted with hot glacial acetic acid (200 ml) and washed with water (200 ml), hot 5% aqueous HCl (200 ml), water (200 ml), hot 5% aqueous

NaOH (200 ml) and hot water (400 ml) to yield 34.7g (87%) of the disulfide which was reduced without further purification.

Reduction of 2,2', 4,4'-tetranitrodiphenyl disulfide and diazotisation. A mixture of 38.4g of 20-mesh zinc and 12g (0.03 moles) of the disulfide was stirred into 90% aqueous glacial acetic acid (300 ml) and after the initial evolution of heat the temperature was carefully raised to boiling. The mixture was refluxed for 45 minutes, filtered whilst hot, and the residue washed with additional hot acetic acid (250 ml). Sulfuric acid, 18 ml (D = 1.84) was added to the filtrate and the mixture was cooled to 9°C and stirred into a solution of sodium nitrite (9g) in 42 ml sulfuric acid (D = 1.84) below 10°C.

The resulting diazo-solution was treated directly with an appropriate Sandmeyer reagent or heated to yield the following compounds:

5-Bromo-1,2,3-benzothiadiazole (VII). The diazo-solution was added to a solution of cuprous bromide (51g) in hydrobromic acid (D = 1.70, 360 ml). The reaction mixture was extracted into diethyl ether (3 x 200 ml) and after drying over anhydrous Na_2SO_4 the solvent was removed by evaporation. The residue was dissolved in methylene chloride and passed through a silica gel column. Individual fractions were evaporated to dryness and the solids recrystallized from methanol to yield 2.2g (17%) white needles m.p. 105°C. Hodgson and Dodgson (1948b) give m.p. 106°C.

I.R. γ cm^{-1} : 1540w, 1430w, 1400w, 1330w, 1290s, 1180s, 1170w, 1070w, 1050w, 914m, 870s, 820s

5-Iodo-1,2,3-benzothiadiazole (XI). The diazo-solution was diluted with iced water and excess nitrous acid was removed by addition of urea (4.5g) prior to addition of potassium idodide (198g). The 5-iodo-1,2,3-benzothiadiazole was extracted and purified as described for compound VII and was obtained as yellow needles m.p. 101-102°C from methanol. Yield 1.67g (11%). Hodgson and Dodgson (1948b) reported m.p. 103°C.

I.R. γ cm^{-1} : 1570w, 1540m, 1440w, 1400w, 1340w, 1270s, 1250w, 1240w, 1200s, 1070m, 910m, 870s, 805s

5-Cyano-1,2,3-benzothiadiazole (XVIII). The diazo-solution was added to an aqueous solution (225 ml) containing cuprous cyanide (34g) and sodium cyanide (45g). The 5-cyano-1,2,3-benzothiadiazole was extracted and purified as described for compound VII and was obtained as white needles crystallized from methanol m.p. 195°C. Yield less than 5%. Kirby et al. (1970b) give 194-196°C.

I.R. γ cm^{-1} : 2250s, 1610m, 1540m, 1450w, 1430s, 1405s, 1330w, 1330s, 1270s, 1235m, 1200s, 1065w, 1070w, 1060m, 920w, 905w, 865w, 855s, 852s, 735w.

5-Hydroxy-1,2,3-benzothiadiazole (XVI). The product of reduction and diazotisation of 2,2', 4,4' tetranitro-diphenyl disulfide was diluted with ice water until all the salts had dissolved, heated to boiling for 60 minutes, cooled and extracted with diethyl ether (3 x 200 ml). The extract was washed with a solution of sodium bicarbonate (60 ml) to remove excess acid, shaken with a 10% solution of sodium

hydroxide (30 ml), and acidified with 10% HCl to give a yellow precipitate of the 5-hydroxy-benzothiadiazole. The material recrystallized from hot water as yellow needles m.p. 157-158°C. Yield 0.82g (9%). Hodgson and Dodgson (1948c) reported 161°C.

I.R. γ cm^{-1} : 3420w, 3150s, 2400w, 1650s, 1580s, 1550s, 1470s, 1420w, 1370m, 1300m, 1205w, 1180w, 1075s, 940m, 815m, 780m, 715w, 685w

ii) Thiolation of 2-halogeno-anilines or nitrobenzenes.

The commercial availability of several 2-halogenated-5-substituted anilines or nitrobenzenes proved a useful synthetic route to some of the 5-substituted, 1,2,3-benzothiadiazoles (II. A. 2. b. ii). It was employed for the synthesis of 5-NO_2-(XXIV), 5-Br-(VII) and 5-Cl-(III)-1,2,3-benzothiadiazoles.

5-Nitro-1,2,3-benzothiadiazole (XXIV). A solution containing 62.3g (0.26 mole) of sodium sulfide nonahydrate and 21.8g (0.26 mole) of sodium bicarbonate in 170 ml of water, was added over 1 hour to a stirred, vigorously refluxing, solution of 2-bromo-5-nitroaniline 37.5g (0.173 mole), in 400 ml of ethanol. Stirring and refluxing were continuing for 150 minutes when the mixture was diluted with an aqueous solution of NaOH (6.91g) in 100 ml of water, poured onto ice (1000g), filtered, and the filtrate neutralized carefully with HCl. Sodium nitrite (14g) was dissolved in the filtrate which was slowly added with stirring to an ice-cold mixture of 43 ml of H_2SO_4 (D = 1.84), ice (1000g) and alcohol (400 ml). The temperature was

maintained below 5°C throughout. After two hours, a brown precipitate of crude 5-nitro-1,2,3-benzothiadiazole, was removed by filtration and purified by steam distillation. It was obtained as pale yellow needles m.p. 142-143°C. Yield 7.67g (25%). Fries et al. (1927) reported m.p. 144°C.

I.R. γ cm^{-1} : 1605m, 1560w, 1515s, 1350s, 1305m, 1210w, 1160w, 1060s, 937m, 900s, 867m, 830s, 795s, 735s.

The material obtained by this procedure was identical to an authentic sample of 5-nitro-1,2,3-benzothiadiazole provided by the Shell Co. (mixed m.p. and I.R. spectrum). Several attempts to prepare this material from 2-chloro-5-nitroaniline as described by Hodgson and Dodgson (1948b) were unsuccessful.

5-Bromo-1,2,3-benzothiadiazole (VII). 2,5-Dibromo-nitrobenzene 21.84g (0.078 moles) was heated at reflux for 24 hours in a solution of 171g (0.71 moles) of sodium sulfide nonahydrate in 300 ml of water. The mixture was cooled, filtered and acidified to pH 5 with HCl at a temperature below 5°C. The crystals which separated were filtered, washed several times with water, and dried in vacuum to yield 5.0g (31%) of the thiol. To a solution of the thiol (5g) in 40 ml of H_2SO_4 (D = 1.84) at 0°C was added dropwise a cooled solution of sodium nitrite (4g) in 50 ml H_2SO_4 (D = 1.84), the temperature not being allowed to rise above 5°C. After two hours stirring, the mixture was poured onto ice (300g) and steam distilled to yield a solid material. This was filtered from the distillate and on recrystallization from methanol gave white needles m.p. 105°C. Yield 1.34g (25%).

The material was identical (mixed m.p. and I.R.) with a sample prepared by the reduction, diazotisation and subsequent Sandmeyer reaction with Cu_2Br_2 of 2,2', 4,4'-tetranitrodiphenyl disulfide.

iii) Ring opening of 2-amino or 2-methyl-benzothiazoles. Compounds (II, IV, VIII, XXXI, XXXII, XXXV, XXVI) were prepared by cleavage of the corresponding 2-amino-benzothiazole and subsequent diazotisation of the resulting aminobenzenethiol.

6-Ethoxy-1,2,3-benzothiadiazole (XXXII). Sixty two grams (0.32 mole) of 2-amino-6-ethoxy-benzothiazole were refluxed at 160°C with potassium hydroxide (200g) and water (400 ml). After 15 hours, the mixture was cooled, diluted with 500 ml of water, and the solids filtered off. Keeping the temperature below 10°C, the pH of the filtrate was adjusted to 6.5 with HCl (D = 1.2) and the precipitated thiol was filtered off, washed several times with water, and dried under vacuum.

The crude 2-amino-5-ethoxy-benzenethiol (20g) was dissolved in 100 ml H_2SO_4 (D = 1.84) and 8.5g of sodium nitrite dissolved in 50 ml H_2SO_4 (D = 1.84) was slowly added with stirring below 5°C. After 60 minutes, the mixture was poured on ice (1000g), extracted with diethyl ether (3 x 200 ml) and the extract dried over sodium sulfate. The solvent was evaporated and the residue purified by chromatography on a silica gel column, using methylene chloride as the solvent.

Eluant fractions were evaporated and the solid residues recrystallized from methanol-water to give white needles m.p. 102°C. Yield 9.4g (16%). Ward and Heard (1965) reported 101°C.

I.R. γ cm^{-1} : 3100w, 3000w, 1605s, 1555w, 1465s, 1400m, 1350w, 1305w, 1275w, 1248s, 1175w, 1160w, 1125m, 1205m, 1060m, 1030m, 950m, 910s, 855m, 827m, 818s, 755s, 710m.

The following compounds were prepared using the same procedure from the appropriate starting materials.

6-Methoxy-1,2,3-benzothiadiazole (XXXI). White needles from methanol-water m.p. 76-77°C. Yield 25%. Ward and Heard (1965) give 77°C, Fries and Engelbertz (1915) reported 72°C.

I.R. γ cm^{-1} : 3120w, 1605s, 1555m, 1480s, 1430s, 1350m, 1300m, 1280m, 1245s, 1200w, 1175w, 1130m, 1120m, 1050s, 1020s, 923s, 860m, 850m, 840s, 825s, 815m, 755s, 690m.

6-Chloro-1,2,3-benzothiadiazole (IV). White needles from methanol m.p. 77°C. Yield 37%. Kirby et al. (1970) give 77°C. Ward et al. (1962) reported 80°C.

I.R. γ cm^{-1} : 1600m, 1550m, 1440s, 1390m, 1340w, 1280s, 1220m, 1120w, 1100s, 1045w, 905s, 875w, 860w, 830m, 820m, 800s, 740m.

6-Bromo-1,2,3-benzothiadiazole (VIII). White needles from methanol m.p. 67°C. Yield 21%. Ward et al. (1962) give 70°C.

I.R. γ cm^{-1} : 1590m, 1540w, 1430s, 1380w, 1340w, 1270s, 1220w, 1125w, 1080m, 1040m, 905m, 865m, 858m, 820s, 805s, 795s, 738m.

6-Methyl-1,2,3-benzothiadiazole (XXXV). Pale yellow needles from methanol m.p. 38°C. Yield 20%. Jacobson et al. (1893) give 42°C.

I.R. γ cm^{-1} : 1605m, 1550m, 1450m, 1410w, 1375w, 1360m, 1290s, 1230m, 1210m, 1125m, 1040w, 1010w, 920s, 983m, 845m, 810s, 750m, 693w.

5,6-Dimethyl-1,2,3-benzothiadiazole (XXXVI). Light brown needles from methanol m.p. 75°C. Yield 22%. Elemental analysis $C_8H_8N_2S$, found (%): C = 58.84, H = 4.97 requires (%): C = 58.54, H = 4.88.

I.R. γ cm^{-1} : 2900w, 2350w, 1650w, 1530m, 1450s, 1370m, 1340w, 1290s, 1260w, 1230s, 1130w, 1025w, 1005w, 870s, 860s, 795m, 722m.

4-Chloro-1,2,3-benzothiadiazole (II). Yellow needles from ethanol m.p. 100°C. Yield 32%. Ward et al. (1962) give 101°C, Haddock et al. (1970) reported 96-98°C.

I.R. γ cm^{-1} : 1580w, 1530w, 1445w, 1390w, 1335w, 1275s, 1200m, 1165w, 1105w, 900s, 842m, 785s, 775m, 739m.

Compound XXXIV was synthesized by cleavage of the corresponding 2-methyl-benzothiazole.

5-Methyl-1,2,3-benzothiadiazole (XXXIV). Yellow needles from methanol-water m.p. 29°C. Yield 5%. Hodgson and Dodgson (1948a) reported 26°C. Elemental analysis $C_7H_6N_2S$, found (%): C = 56.25, H = 3.73 requires (%): C = 56.00, H = 4.00.

I.R. γ cm^{-1} : 1600w, 1550w, 1450m, 1340w, 1295s, 1270m, 1230s, 1160w, 1075w, 875w, 855s, 807s, 742s.

The benzothiazole ring can also be cleaved with hydrazine hydrate (Ward and Poesche, 1961) and thus was used successfully to prepare the 6-nitro-1,2,3-benzothiadiazole (XXV) from the 6-nitro-benzothiazole.

6-Nitro-benzothiazole (LII). This material was synthesized as described by Ward and Poesche (1961). Benzothiazole (267g, 1.97 mole) was added dropwise with vigorous stirring, below 20°C, to H_2SO_4 (D = 1.84, 424 ml) until dissolved. Keeping the temperature at 10°C nitric acid (D = 1.60, 212 ml) was added dropwise to the mixture which was maintained at 10°C for a further 2 hours before being poured onto ice (2500g) with stirring. Aqueous ammonia (D = 0.88) was added below 20°C until the solids became slightly orange (pH = 2.0). The solids were filtered off, washed with dilute aqueous ammonia, water and dried in the oven at 60°C. This procedure yielded 300g of a crude isomeric mixture of mononitro-benzothiazoles. The isomeric mixture (150g) was dissolved in hot ethanol (2100 ml) and set aside to crystallize slowly. The solids obtained were again recrystallized from ethanol (1800 ml) to give 69g yellow crystals of 6-nitro-benzothiazole m.p. 174°C. Ward and Poesche (1961) reported m.p. 177°C.

6-Nitro-1,2,3-benzothiadiazole (XXV). 6-Nitro-1,2,3-benzothiazole (62g, 0.34 mole) was refluxed for 3 hours with ethanol (1250 ml) and 100% hydrazine hydrate (125 ml). The resulting mixture was treated at 35°C with 20 volume hydrogen peroxide (100 ml) until the red colour faded. After 2 hours the 2,2'-diamino-5,5'-dinitro-diphenyldisulfide was separated by filtration and washed with water, to give

50g (85%) of the yellow product which was not purified further, m.p. 238°C. The disulfide (50g) was dissolved in H_2SO_4 (D = 1.84, 185 ml), cooled to 3°C and treated dropwise below 5°C with sodium nitrite (24g) in H_2SO_4. The mixture left overnight at room temperature, the next day the solids were collected by filtration, washed with water and dried in the oven at 50°C. Recrystallization from ethanol gave pale yellow needles m.p. 134°C. Yield 50%. Jacobson et al. (1893) reported m.p. 136-137°C, Ward et al. (1962) give m.p. 136°C.

I.R. γ cm^{-1} : 1605w, 1565m, 1530s, 1435w, 1415m, 1345s, 1290s, 1285s, 1250w, 1225m, 1125m, 1050m, 1045m, 925m, 905m, 860s, 840m, 835m, 785s, 755s, 745s, 720m.

b) Modified Herz reaction (II. A. 2. a)

The modified Herz reaction (Kirby et al., 1970a) on substituted aniline (II. A. 2. a) provides a convenient synthetic route to several substituted 1,2,3-benzothiadiazoles. It was employed for the synthesis of compounds XIV, XXXVII, XXXIX, XXXX, XXXXVI, and XXXXVIII (Table 5).

6-n-Butyl-1,2,3-benzothiadiazole (XXXVII). To a solution of p-n-butylaniline (50g, 0.335 moles) in glacial acetic acid (50 ml) was added with stirring 180 ml of sulfur monochloride at such a rate that the temperature was maintained at about 15°C. On complete addition the temperature was slowly increased to about 65°C for 4 hours and the mixture was left stirring overnight at room temperature. Benzene (300 ml) was added and the thiazathiolium salt was filtered

off and washed several times with benzene until the washings were colourless. When dry, the material was added to 50% H_2SO_4 (500 ml) and heated at 65°C for 30 minutes. The mixture was then cooled to -5°C and sodium nitrite (40g, 0.58 mole) in water (50 ml) was slowly added with stirring. The diazotised solution was poured onto ice (1500g) and allowed to stand overnight. The mixture was extracted with diethyl ether (3 x 200 ml) and after drying over sodium sulfate the solvent was evaporated to yield a yellow solid. This was dissolved in dichloroethylene, and eluted through a silica gel column with the same solvent. Fractions (100 ml) were collected. Fractions 1-4 yielded unidentified materials (1.0g) and fractions 5-12 gave a total of 5.7g of 6-n-butyl-1,2,3-benzothiadiazole. Recrystallization from ethanol give pale yellow needles m.p. 110-111°C. Yield 9%. Elemental analysis $C_{10}H_{12}N_2S$, found (%): C = 62.10, H = 6.10 requires (%): C = 62.50, H = 6.25.

5-Fluoro-6-chloro-1,2,3-benzothiadiazole (XXXIX). Obtained as white crystals from methanol starting from m-fluoro-aniline m.p. 95.5-96.5°C Yield 1%. Kirby et al. (1970b) reported 96-98°C. An unknown product consisting of yellow crystals m.p. 112°C was also obtained.

I.R. γ cm^{-1} : 2400w, 1590w, 1540w, 1450s, 1400m, 1340w, 1300s, 1270w, 1250s, 1160m, 1150m, 1090s, 1000m, 880s, 860s, 850s, 750s, 710s.

5-Methoxy-6-chloro-1,2,3-benzothiadiazole (XXXX). Obtained as white crystals from methanol-water starting from m-methoxy-aniline m.p. 156°C. Yield 0.75%. Kirby et al. (1970b) give 153.5-155.5°C.

6-Fluoro-1,2,3-benzothiadiazole (XIV). Obtained as light brown crystals from methanol starting from p-fluoro-aniline m.p. 113-115°C Yield 8%. Kirby et al. (1970a) reported 105°C. Elemental analysis $C_6H_3N_2SF$, found (%): C = 47.39, H = 2.73, requires (%): C = 46.70, H = 2.00.

I.R. γ cm^{-1} : 2400w, 1620m, 1565m, 1460s, 1420m, 1360s, 1340m, 1240s, 1205s, 1120w, 1045w, 935s, 870s, 865s, 825s, 755s, 695m.

4,5,6,7-Tetrafluoro-1,2,3-benzothiadiazole (XXXXVI). Obtained as grey crystals from methanol starting from 2,3,4,5-tetrafluoro-aniline m.p. 104°C. Yield 4%. Elemental analysis $C_6N_2SF_4$, found (%): C = 34.00, H = 0.00, requires (%): C = 34.61, H = 0.00.

I.R. γ cm^{-1} : 2420w, 1700s, 1675s, 1575s, 1540s, 1500s, 1440m, 1420m, 1390m, 1300m, 1250s, 1195m, 1075s, 1040w, 1025m, 975s, 935m, 875m, 860m, 750m, 710m.

2,3,4,5-Tetrachloro-aniline. To a warm solution of stannous chloride dihydrate (154g) was added 2,3,4,5-tetrachloronitrobenzene (40g, 0.053 mole) at such a rate that the temperature was maintained at 55°C. When addition was complete the mixture was heated at 65°C for 4 hours and kept at 5°C overnight. The solids were filtered off, dissolved in hot water (300 ml) and added dropwise to 40% w/v aqueous sodium hydroxide (600 ml) below 25°C. The mixture was kept at 5°C for 6 hours and the solids filtered off. Recrystallization from petroleum ether give white crystals m.p. 117-118°C. Yield 60%.

4,5,6,7-Tetrachloro-1,2,3-benzothiadiazole (XXXXVIII).
Crystallization from methanol gave white crystals m.p. 168-170°C. Yield 1.5%. Kirby et al. (1970b) reported m.p. 171-173°C. Starting material 4,5,6,7-tetrachloroaniline.

I.R. γ cm^{-1} : 2390w, 1680s, 1580s, 1510m, 1415m, 1360m, 1290w, 1250w, 1175w, 1100w, 1025w, 985w, 870m, 835m, 780m.

c) Modification of aromatic ring substituents in the 1,2,3-benzothiadiazoles

A variety of reactions were employed to modify the nature of the substituents in the aromatic portion of the 1,2,3-benzothiadiazole nucleus. These include:

i) Alkaline hydrolysis of halogeno-1,2,3-benzothiadiazole.
ii) Alkylation of hydroxy-1,2,3-benzothiadiazole.
iii) Reduction of nitro-1,2,3-benzothiadiazole.
iv) Sandmeyer reaction or hydrolysis of amino-1,2,3-benzothiadiazole.
v) Halogenation of 1,2,3-benzothiadiazole.
vi) Nitration of substituted 1,2,3-benzothiadiazole.
vii) Amination of substituted nitro-1,2,3-benzothiadiazole.
viii) Deamination and rearrangement of amino-substituted 1,2,3-benzothiadiazole.

i) Alkaline hydrolysis of halogeno-1,2,3-benzothiadiazole.
Compounds substituted at the 4- and 6-positions with halogen atoms readily undergo hydrolysis with either alkali or metal alkoxides to give the corresponding hydroxy or alkoxy derivatives. Compounds (XV, XVII, XXXIII) were synthesized by this procedure.

4-Hydroxy-1,2,3-benzothiadiazole (XV). To a solution of 4-chloro-1,2,3-benzothiadiazole (6.5g, 0.038 mole) in dimethyl sulfoxide (100 ml) was added a solution of potassium hydroxide (14g, 0.25 mole) in 20% aqueous dimethyl sulfoxide (100 ml). The mixture was refluxed for 6 hours, cooled, and poured into cold water (500 ml). After extraction with diethyl ether (3 x 100 ml) to remove unchanged starting material the solution was acidified and re-extracted with ether (3 x 100 ml). The extract was dried over sodium sulfate and evaporation of the ether yield 1.8g of crude 4-hydroxy-1,2,3-benzothiadiazole. Recrystallization from chlorobenzene give cream needles m.p. 144-146°C. Yield 33%. Ward and Heard (1965) m.p. 146°C.

I.R. γ cm^{-1} : 3400w, 3150s, 1630w, 1570s, 1475m, 1425s, 1340w, 1290w, 1270s, 1220s, 1170m, 1050m, 960s, 900w, 875m, 785s, 750w, 725s.

6-Hydroxy-1,2,3-benzothiadiazole (XVII). Prepared by a similar procedure from 6-chloro-1,2,3-benzothiadiazole (VI) and obtained as cream crystals from chlorobenzene m.p. 213-215°C. Yield 35%. Davies and Kirby (1967) reported m.p. 211-213°C.

I.R. γ cm^{-1} : 3450w, 3150m, 1600s, 1470s, 1390m, 1310w, 1240s, 1135m, 1050w, 935m, 880m, 845m, 825w, 755m, 725w, 700w.

6-Propoxy-1,2,3-benzothiadiazole (XXXIII). Obtained from 6-chloro-1,2,3-benzothiadiazole (IV) and potassim propoxide as white crystals m.p. 50-51°C. Elemental analysis $C_9H_{10}N_2SO$ found (%): C = 55.20, H = 4.95, requires (%): C = 55.67, H = 5.15.

ii) Alkylation of hydroxy-1,2,3-benzothiadiazole. Dimethyl sulfate was used as alkylating agent to synthesise compound (XXX).

4-Methoxy-1,2,3-benzothiadiazole (XXX). Dimethyl sulfate (315mg, 0.0025 mole) was added dropwise to an aqueous solution containing 4-hydroxy-1,2,3-benzothiadiazole (250mg, 0.0016 mole) and a slight excess of sodium hydroxide. After refluxing for 1 hour the mixture was cooled, extracted with diethyl ether (3 x 50 ml) and the extract dried over sodium sulfate. The solvent was evaporated and crystallization of the residue from methanol-water gave 80 mg of 4-methoxy-1,2,3-benzothiadiazole as white needles m.p. 64°C. Yield 29%. Haddock et al. (1970) reported 64-66°C.

I.R. γ cm^{-1} : 1590w, 1560s, 1455s, 1430m, 1340s, 1275s, 1265s, 1255s, 1180m, 1160w, 1020s, 915m, 875w, 780s, 770m, 740m.

iii) Reduction of nitro-1,2,3-benzothiadiazole. The amino-1,2,3-benzothiadiazoles were synthesized by reduction of the corresponding nitro-1,2,3-benzothiadiazole with stannous chloride dihydrate, as described by Ward et al. (1962). This method was employed to prepare compounds XIX, XX, XXI, XXII, XXXXIV (Table 5).

6-Amino-1,2,3-benzothiadiazole (XXI). To a solution of stannous chloride dihydrate (280g) in HCl (500 ml, D = 1.2) at 50°C was added slowly 6-nitro-1,2,3-benzothiadiazole (49.6g). The mixture was heated at 65°C for 30 minutes and was stored in the refrigerator overnight. The next day the solids were collected, dissolved in

hot water (300 ml), and added dropwise to a solution of sodium hydroxide (40% w/v, 800 ml) below 20°C. The mixture was stored in the refrigerator for 6 hours, filtered, and the solid material dried and crystallized from hot benzene (1000 ml) to give green needles 22g m.p. 108°C. Yield 53%. Ward et al. (1962) reported 110°C; Fries and Reitz (1936) give 112°C.

IR. γ cm^{-1} : 3350m, 3200m, 1660m, 1615s, 1550m, 1470m, 1440m, 1360w, 1310w, 1275s, 1260s, 1140m, 928m, 875m, 825s, 755w.

4-Amino-1,2,3-benzothiadiazole (XIX). Yellow needles from petroleum ether m.p. 87-88°C. Yield 70%. Ward et al. (1962) reported m.p. 88°C.

I.R. γ cm^{-1} : 3350m, 3300m, 1640s, 1590s, 1480s, 1420w, 1350m, 1340m, 1320s, 1275s, 1210w, 1175w, 1060w, 955m, 895m, 855m, 780s, 770s, 740m.

5-Amino-1,2,3-benzothiadiazole (XX). Yellow needles from benzene m.p. 90-91°C. Yield 60%. Hodgson and Dodgson (1948b) reported m.p. 95°C.

I.R. γ cm^{-1} : 3350m, 3220w, 1630s, 1600s, 1545w, 1470s, 1430m, 1360m, 1320s, 1270s, 1230s, 1170w, 1160w, 1065w, 945w, 870m, 840m, 820s, 812s, 738m, 693w.

7-Amino-1,2,3-benzothiadiazole (XXII). Yellow needles from benzene m.p. 136°C. Yield 70%. Davies and Kirby (1967) give 130-132°C. Ward et al. (1962) reported m.p. 136°C.

I.R. γ cm^{-1} : 3340m, 3210m, 1650w, 1575s, 1560w, 1480s, 1420m, 1360m, 1270s, 1060w, 865m, 780s, 718w, 710w.

6-Methyl-7-amino-1,2,3-benzothiadiazole (XXXXIV). Was synthesized from 6-methyl-7-nitro-1,2,3-benzothiadiazole (XXXIV) and obtained as cream needles from benzene m.p. 134-135°C. Yield 67%. Elemental analysis $C_7H_7N_3S$, found (%): C = 50.80, H = 4.21 requires (%): C = 50.90, H = 4.24.

I.R. γ cm^{-1} : 3350s, 3250w, 1640s, 1560m, 1475s, 1410w, 1350s, 1290w, 1260m, 1060w, 965w, 945w, 780s, 725w.

iv) Sandmeyer reaction or hydrolysis of diazo-1,2,3-benzothiadiazoles. Amino-1,2,3-benzothiadiazoles readily undergo Sandmeyer reaction to give the corresponding halogeno- or cyano-1,2,3-benzothiadiazole. Compounds V, VI, IX, X, XII, XIII, XXVIII, XXIX (Table 5) were prepared by this procedure. The diazo-1,2,3-benzothiadiazoles are hydrolyzed to give the corresponding hydroxy-1,2,3-benzothiadiazole and this was used to prepared compound XVII.

6-Iodo-1,2,3-benzothiadiazole (XII). 6-amino-1,2,3-benzothiadiazole (750mg, 0.005 mole) was dissolved in H_2SO_4 (10 ml, D = 1.84) and sodium nitrite (0.49g, 0.007 mole) in water, was added dropwise below 5°C. After 60 minutes the mixture was poured on ice (60g). The diazo solution was then added with stirring to a solution of potassium iodide (1.67g) in water. The reaction mixture was allowed to stand for 6 hours, was made alkaline with 10% w/v aqueous sodium hydroxide and 10% w/v aqueous sodium thiosulfate was added. The crude 6-iodo-1,2,3-benzothiadiazole was purified by steam distillation, and was

obtained as white crystals (461mg, yield 36%) after recrystallization from aqueous methanol m.p. 114-115°C. Ward and Heard (1963) give 116°C.

I.R. γ cm^{-1} : 1580w, 1530w, 1430w, 1370w, 1320w, 1275s, 1250m, 1220w, 1025m, 1070m, 1080w, 905s, 890w, 870w, 855w, 815s, 810s, 790s, 735m.

4-Iodo-1,2,3-benzothiadiazole (X). Prepared as described for compound (XII). Obtained as yellow needles from methanol-water m.p. 108°C. Yield 26%. Ward and Heard (1963) give m.p. 111°C.

I.R. γ cm^{-1} : 1575w., 1545w, 1450w, 1380w, 1335w, 1290s, 1240w, 1190m, 1170w, 1080w, 900s, 790m, 775s, 740m.

7-Iodo-1,2,3-benzothiadiazole (XIII). Synthesized as described for compound (XII). Obtained as yellow needles from methanol m.p. 138-139.5°C. Yield 35%. Ward and Heard (1963) give m.p. 137°C.

I.R. γ cm^{-1} : 1540w, 1445w, 1380m, 1345w, 1275m, 1240m, 1200w, 1130w, 1080w, 925s, 805m, 785s, 740w, 720s.

4-Bromo-1,2,3-benzothiadiazole (VI). The diazo-solution prepared by diazotisation of 4-amino-1,2,3-benzothiadiazole (760mg; 0.005 mole) was added to a solution of cuprous bromide (800mg) in hydrobromic acid (45% w/v; 10 ml) below 5°C. Steam distillation, filtration and recrystallization from methanol gave white needles m.p. 109°C. Yield 30%. Ward *et al.* (1962) give m.p. 113°C.

I.R. γ cm^{-1} : 1570w, 1540w, 1450m, 1390w, 1330w, 1300s, 1240w, 1190m, 1170w, 1090w, 900s, 835w, 805m, 780s, 775s, 740m.

7-Bromo-1,2,3-benzothiadiazole (IX). Synthesized as described for compound (VI). Obtained as white needles from methanol m.p. 73°C. Yield 21%. Ward and Heard (1963) give m.p. 77°C.

I.R. γ cm^{-1} : 1570w, 1540w, 1450m, 1380m, 1270m, 1250m, 1230m, 1200w, 1120w, 1080m, 1040m, 935s, 805s, 785s, 745w, 719s.

7-Chloro-1,2,3-benzothiadiazole (V). To the diazo-solution prepared from 7-amino-1,2,3-benzothiadiazole (760mg, 0.005 mole) was added a solution containing cuprous chloride (800 mg) in hydrochloric acid (D = 1.2; 10 ml) below 5°C. Steam distillation, filtration and recrystallization from methanol-water gave white needles (330mg) m.p. 76°C. Yield 39%. Ward and Heard (1963) reported 78°C.

I.R. γ cm^{-1} : 1550w, 1440m, 1390w, 1340w, 1280s, 1240w, 1200w, 1140w, 905s, 815m, 790s, 750w, 720s.

7-Cyano-1,2,3-benzothiadiazole (XXIX). Cuprous cyanide (0.80g) was dissolved in 40% potassium cyanide solution and heated on a water bath to about 60°C. The 7-amino-1,2,3-benzothiadiazole (750mg) was dissolved in 50% H_2SO_4 (10 ml) and diazotised below 5°C. The cold diazonium salt solution was added in small quantities to the warm cuprous cyanide solution, shaking vigorously after each addition, keeping the temperature of the mixture at 60-70°C, and maintaining neutrality with sodium carbonate solution. After complete addition, the mixture was heated at 80°C for 30 minutes. Steam distillation filtration and recrystallization from methanol gave 81mg (10%) yellow needles m.p. 98°C. Kirby *et al.* (1970b) reported 116-118°C.

I.R. γ cm^{-1} : 2400m, 2240m, 1550w, 1460w, 1450w, 1395m, 1300s, 1240m, 1200w, 1160w, 1040w, 950m, 840s, 815w, 805s, 800m, 760m, 720s.

6-Cyano-1,2,3-benzothiadiazole (XXVIII). Synthesized as described for compound (XXIX). Obtained as white crystals from methanol m.p. 126-127°C. Yield 20%. Elemental analysis $C_7H_3N_3S$ found (%): C = 49.87, H = 1.92; requires (%): C = 52.0, H = 1.9.

I.R. γ cm^{-1} : 2410w, 2260m, 1600w, 1550w, 1440m, 1400s, 1350w, 1300s, 1240s, 1130w, 1100m, 1050w, 930m, 910w, 900w, 875w, 830s, 815m, 810m, 750s.

7-Hydroxy-1,2,3-benzothiadiazole (XVIII). Was synthesized by diazotisation and hydrolysis of 7-amino-1,2,3-benzothiadiazole and obtained as a light yellow product m.p. 228°C. Yield 40%. Davies and Kirby (1967) reported m.p. 230-232°C.

I.R. γ cm^{-1} : 3480w, 3150m, 1690m, 1580s, 1510w, 1480w, 1440m, 1360w, 1330w, 1260s, 1240w, 1175w, 1140w, 1075s, 1040w, 870w, 785s, 760m, 717m.

v) Halogenation of 1,2,3-benzothiadiazole. Bromine in chloroform or iodine in 50% ethyl ether were used to halogenate several amino-1,2,3-benzothiadiazole. Compounds XXXVIII, XXXXI, XXXXII (Table 5) were prepared by this method.

6-Amino-7-bromo-1,2,3-benzothiadiazole (XXXVIII). Bromine (1.06 ml, 0.02 mole) was added dropwise to a solution of 6-amino-1,2,3-benzothiadiazole (3g, 0.02 mole) in chloroform (90 ml). The precipitated solids

were separated by filtration, dried, suspended in hot water, made basic with ammonium hydroxide (D = 0.899) and filtered. Crystallization from benzene gave 2.64g (58%) white needles m.p. 165-167°C. Ward and Heard (1963) give 168°C.

I.R. γ cm^{-1} : 3450w, 3350m, 3240m, 1650s, 1610s, 1540w, 1475m, 1430s, 1360w, 1320w, 1280s, 1255s, 1130m, 1070w, 955m, 900m, 820s, 805s.

4-Bromo-5-amino-1,2,3-benzothiadiazole (XXXXI). Synthesized as described for compound (XXXVIII). Obtained as yellow needles from benzene petroleum ether m.p. 178°C. Yield 81%. Ward and Heard (1965) give m.p. 183°C.

I.R. γ cm^{-1} : 3400m, 3330w, 3220w, 1650m, 1600w, 1530w, 1470m, 1400m, 1350w, 1340w, 1275s, 1195s, 1100w, 875s, 810s.

4-Iodo-5-amino-1,2,3-benzothiadiazole (XXXXII). The 5-amino-1,2,3-benzothiadiazole was dissolved in 50% aqueous diethyl ether and refluxed for 8 hours with iodine. Obtained as yellow needles from ethanol-water m.p. 163°C. Yield 60%. Ward and Heard (1965) give 168°C.

I.R. γ cm^{-1} : 3430m, 3320m, 3200w, 1610s, 1580s, 1530w, 1460s, 1400s, 1340m, 1320w, 1270s, 1190s, 1070m, 970s, 815m, 795m.

vi) Nitration of 1,2,3-benzothiadiazoles. Potassium nitrate was used as nitrating agent to synthesize compounds XXVI, XXXXIII, XXXXIV.

6-Ethoxy-7-nitro-1,2,3-benzothiadiazole (XXXXIII). Potassium nitrate (2.5g, 0.025 mole) was added slowly at room temperature to a solution of 6-ethoxy-1,2,3-benzothiadiazole (2.7g, 0.015 mole) in H_2SO_4 (D = 1.84, 15 ml). The mixture was heated at 90°C, for 8 hours and poured onto ice (500g). The solid material was filtered off and crystallized from methanol to give 1.73g (52%) pale yellow needles m.p. 183-184°C.

I.R. γ cm^{-1} : 1610s, 1545w, 1515m, 1450m, 1435w, 1280s, 1240w, 1170s, 1140s, 1070s, 1010m, 860m, 852m, 785m, 750m, 735m.

6-Methyl-7-nitro-1,2,3-benzothiadiazole (XXXXIV). Synthesized as described for compound XXXXIII. Obtained as yellow needles from petroleum ether m.p. 115°C. Yield 60%. Elemental analysis $C_7H_5N_3SO_2$ found (%): C = 42.90, H = 2.35; requires (%): C = 43.08, H = 2.56.

I.R. γ cm^{-1} : 1600m, 1560w, 1515s, 1460m, 1370w, 1310s, 1280s, 1255w, 1205w, 1140w, 1115w, 1025w, 892w, 857s, 836m, 825w, 790m, 765m, 715m.

6-Chloro-7-nitro-1,2,3-benzothiadiazole (XXVI). Synthesized as described for compound XXXXIII. Obtained as yellow needles from ethanol m.p. 99°C. Yield 16%. Haddock et al. (1970) reported 99-101°C.

I.R. γ cm^{-1} : 1600s, 1535s, 1430w, 1330m, 1320m, 1290s, 1240w, 1160m, 1100m, 1000w, 875s, 835s, 790m, 768m, 720w, 685m.

vii) Amination of nitro-1,2,3-benzothiadiazole. Hydroxylamine hydrochloride was used as aminating agent to prepare compound XXXXVII.

6-Nitro-7-amino-1,2,3-benzothiadiazole (XXXXVII). To a solution of 6-nitro-1,2,3-benzothiadiazole (9.1g, 0.05mole) and hydroxylamine hydrochloride (17.4g, 0.25 mole) in ethanol (150 ml) was added dropwise, at 0°C with stirring, a solution of potassium hydroxide (40g, 0.72 mole) in ethanol (150 ml). The mixture was stirred for 3 hours poured onto ice (1000g) and was extracted with methylene chloride. The extract was dried over sodium sulfate and the solvent evaporated to give 5.7g (63%) of a crude yellow product which was not purified further m.p. 228°C. Haddock et al. (1970) reported m.p. 248-249°C.

I.R. γ cm^{-1} : 3510s, 3330s, 1620s, 1570s, 1480s, 1460m, 1400m, 1320s, 1310s, 1300s, 1250s, 1245s, 1160w, 1100m, 1040w, 858m, 815m, 768s, 725m, 685w.

viii) Deamination and rearrangement of 1,2,3-benzothiadiazoles. The deamination and rearrangement of 6-substituted-7-amino-1,2,3-benzothiadiazole discussed in section (II. A. 3. c) was used to synthesized compound XXIII.

4-Nitro-1,2,3-benzothiadiazole (XXIII). Sodium nitrite (2.0g, 0.029 mole) was dissolved in the minimum amount of water and added below 5°C to a suspension of 6-nitro-7-amino-1,2,3-benzothiadiazole (5.0g, 0.025 mole) in hydrochloride acid (D = 1.20, 60 ml). The diazonium salt was poured onto cold 50% hypophosphorous acid (80 ml) and left for 24 hours when the mixture was extracted with diethyl ether

(3 x 100 ml), and the extract washed with 20% solution of sodium hydroxide and with water. The ether layer was separated, dried over sodium sulfate and evaporated to give a yellow solid material (2g). The crude product was passed through a silica gel column in 10% v/v ether-benzene to give a yellow material which was recrystallized from methanol to yield 1.4g (31%) yellow needles m.p. 117-118°C. Davies and Kirby (1967) give m.p. 118-118.5°C, Ward et al. (1962) reported m.p. 122.5°C.

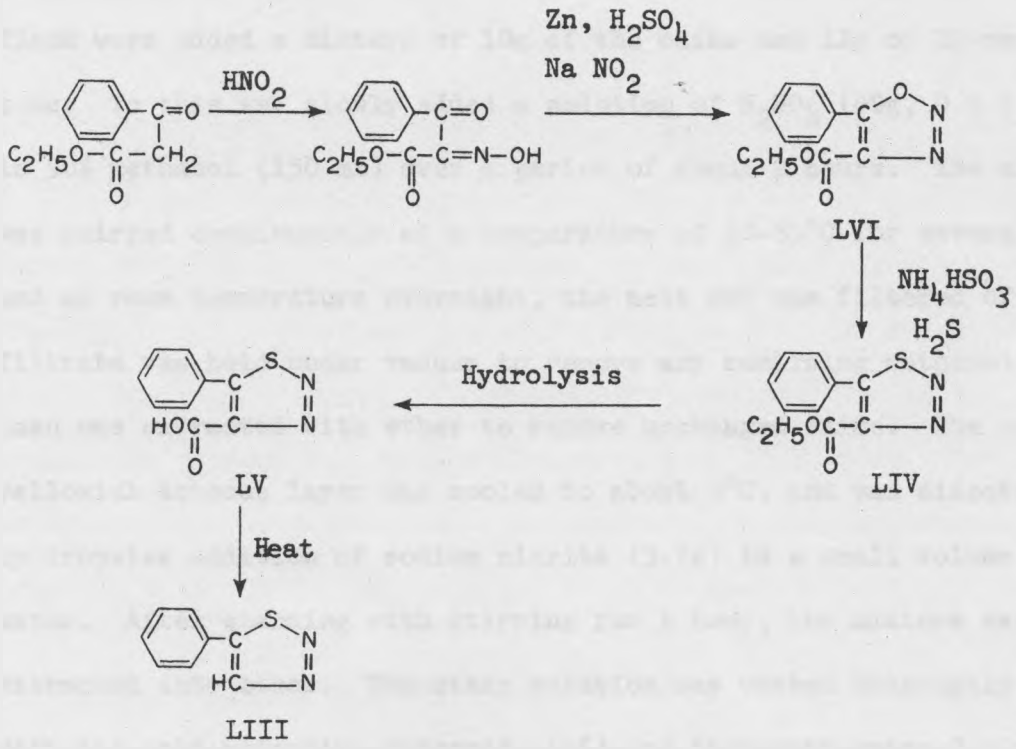
I.R. γ cm^{-1} : 1525s, 1345s, 1295m, 1240m, 1210w, 1180w, 1130w, 930w, 880s, 815m, 780s, 740s, 720w.

Earlier reports (Bernthsen, 1888; Jacobson, 1888; Fries and Reitz, 1936) give a m.p. of 95°C for 4-nitro-1,2,3-benzothiadiazole which they claimed to have synthesized by nitration of 1,2,3-benzothiadiazole with sulfuric acid and potassium nitrate at 100°C. Another product of this reaction, however, was a compound of m.p. 104°C which was later confirmed by Hodgson and Dodgson (1948a) to be the 7-nitro-1,2,3-benzothiadiazole. Ward et al. (1962) have re-examined this reaction, reporting the presence of 5- and 7-nitro-1,2,3-benzothiadiazole and the absence of the 4-isomer. Davies and Kirby (1967) synthesized the 4-nitro-1,2,3-benzothiadiazole by nitration of 1,2,3-benzothiadiazole with nitric acid. They reported a m.p. 118-118.5°C. Bernthsen (1888) reduced the supposed 4-nitro-1,2,3-benzothiadiazole to give a compound of m.p. 136.5°C which he claimed was the 4-amino-1,2,3-benzothiadiazole. Ward et al. (1962) have subsequently synthesized the 4-amino-1,2,3-benzothiadiazole (m.p. 85-87°C) by another route and

all evidence suggests that the supposed 4-nitro-1,2,3-benzothiadiazole reported by Bernthsen, Jacobson, Fries and collaborators, was in fact a mixture of isomers. Consequently the 4-hydroxy-1,2,3-benzothiadiazole (m.p. 235°C) prepared by diazotisation and hydrolysis of 4-amino-1,2,3-benzothiadiazole reported by Bernthsen (1888) was also a mixture.

12. Synthesis of Additional Miscellaneous Compounds

Several compounds closely related to the 1,2,3-benzothiadiazole structure were synthesized by the methods reported by Wolff and Hall (1903) and Wolff et al. (1904). The sequence of reactions is the following:



Ethyl-benzoyl acetate oxime.

Was synthesized as described by Wolff and Hall (1903). To a cooled solution (5-8°C) of ethyl-benzoylacetate (50g) in glacial acetic acid (125g) was added dropwise sodium nitrite (185g) dissolved in a small volume of water. A portion of the oxime crystallized during the reaction, the remaining oxime precipitated after addition of ice water, and the solution was kept at 5-20°C for a further 2-3 hours for complete precipitation. The solids were filtered off and crystallized from hot ethanol to give white crystals (52g) m.p. 120-121°C.

5-Phenyl-4-carboxyethyl-1,2,3-oxadiazole (LVI).

Synthesized as described by Wolff and Hall (1903). To a 2 liter flask were added a mixture of 10g of the oxime and 11g of 20-mesh zinc. To this was slowly added a solution of H_2SO_4 (40g, D = 1.84) in 50% methanol (150 ml) over a period of about 2 hours. The mixture was stirred continuously at a temperature of 30-35°C for several hours, and at room temperature overnight, the next day was filtered off. The filtrate was held under vacuum to remove any remaining methanol and then was extracted with ether to remove unchanged oxime. The remaining yellowish aqueous layer was cooled to about 3°C, and was diazotized by dropwise addition of sodium nitrite (3.7g) in a small volume of water. After standing with stirring for 1 hour, the mixture was extracted into ether. The ether solution was washed thoroughly once with ice cold potassium hydroxide (4%) and then with water 2 times, the ethereal solution was dried over sodium sulfate anhydrous, and the

ether removed under vacuum to give a yellowish oil (6g), this was not purified further. Yield 57%.

5-Phenyl-4-carboxyethyl-1,2,3-thiadiazole (LIV).

Was prepared by the method described by Wolff et al. (1904). To a cooled solution (0-5°C) containing 5-phenyl-4-carboxyethyl-1,2,3-oxadiazole (4g) in ethanol (20 ml) was added ammonium bisulfide (5g, 4%) and hydrogen sulfide was bubbled through the mixture for 24 hours. The precipitated sulfur was filtered off and the solution quickly extracted with diethyl-ether. Evaporation of the ether gave a yellow oil which solidified on cooling and which was crystallized from ethanol to give colorless needles m.p. 142°C. Wolff et al. (1904) reported 142°C.

5-Phenyl-4-carboxy-1,2,3-thiadiazole(LV).

5-Phenyl-4-carboxyethyl-1,2,3-thiadiazole was treated with an excess of 10% (w/v) sodium hydroxide and after saponification was completed, H_2SO_4 was added dropwise. The solution was filtered to remove any oily material prior to complete precipitation of the acid. The product was recrystallized from boiling water after decolorization with charcoal. The acid separated as colorless small leaflets m.p. 157°C. Wolff et al. (1904) reported 158°C.

5-Phenyl-1,2,3-thiadiazole (LIII)

5-Phenyl-4-carboxy-1,2,3-thiadiazole (2g) was heated at 160-170°C until CO_2 evolution ceased. After cooling, the brown product in the flask, was steam distilled. Separation of the solid and crystallization (ethanol, 70%) give white crystals (1.5g) m.p. 46-47°C. Wolff et al. (1904) reported 53-53.5°C.

Table 5. Synthesis of 1,2,3-benzothiadiazoles

Number	Compound	Formula	Recryst. solv.	m.p. °C	Procedure*	Yield %
I	Unsubstituted	$C_6H_4N_2S$	hexane	35	a	83
II	4-Chloro	$C_6H_3ClN_2S$	ethanol	100	a.iii	32
III	5-Chloro	$C_6H_3ClN_2S$	methanol	104	a.i	26
IV	6-Chloro	$C_6H_3ClN_2S$	methanol	77	a.iii	37
V	7-Chloro	$C_6H_3ClN_2S$	meth/water	76	c.iv	39
VI	4-Bromo	$C_6H_3BrN_2S$	methanol	109	c.iv	30
VII	5-Bromo	$C_6H_3BrN_2S$	methanol	105	a.i	25
VIII	6-Bromo	$C_6H_3BrN_2S$	methanol	67	a.iii	21
IX	7-Bromo	$C_6H_3BrN_2S$	methanol	73	c.iv	21
X	4-Iodo	$C_6H_3IN_2S$	meth/water	108	c.iv	26
XI	5-Iodo	$C_6H_3IN_2S$	methanol	101-102	a.i	11
XII	6-Iodo	$C_6H_3IN_2S$	meth/water	114-115.5	c.iv	36
XIII	7-Iodo	$C_6H_3IN_2S$	methanol	138-139.5	c.iv	35
XIV	6-Fluoro	$C_6H_3FN_2S$	meth/water	113-115	b.	8
XV	4-Hydroxy	$C_6H_4ON_2S$	chlorobenzene	144-146	c.i	33
XVI	5-Hydroxy	$C_6H_4ON_2S$	hot water	157-158	a.i	9
XVII	6-Hydroxy	$C_6H_4ON_2S$	chlorobenzene	213-215	c.i	35

- Continued -

Table 5. Continued

Number	Compound	Formula	Recryst. solv.	m.p. °C	Procedure*	Yield %
XVIII	7-Hydroxy	$C_6H_4ON_2S$	chlorobenzene	228	c.iv	40
XIX	4-Amino	$C_6H_5N_3S$	pet. ether	87-88	c.iii	70
XX	5-Amino	$C_6H_5N_3S$	benzene	90-91	c.iii	60
XXI	6-Amino	$C_6H_5N_3S$	benzene	108	c.iii	53
XXII	7-Amino	$C_6H_5N_3S$	benzene	136	c.iii	70
XXIII	4-Nitro	$C_6H_3O_2N_3S$	methanol	117-118	c.viii	31
XXIV	5-Nitro	$C_6H_3O_2N_3S$	methanol	142-143	a.ii	25
XXV	6-Nitro	$C_6H_3O_2N_3S$	ethanol	134	a.iii	50
XXVI	6-Chloro, 7-Nitro	$C_6H_2ClO_2N_3S$	pet. ether	99	c.vi	16
XXVII	5-Cyano	$C_7H_3N_3S$	methanol	195	a.i	>5
XXVIII	6-Cyano	$C_7H_3N_3S$	methanol	126-127	c.iv	20
XXIX	7-Cyano	$C_7H_3N_3S$	methanol	98	c.iv	10
XXX	4-Methoxy	$C_7H_6ON_2S$	meth/water	64	c.ii	29
XXXI	6-Methoxy	$C_7H_6ON_2S$	meth/water	76-77	a.ii	25
XXXII	6-Ethoxy	$C_8H_8ON_2S$	meth/water	102	a.iii	16
XXXIII	6-Propoxy	$C_9H_{10}ON_2S$	methanol	50-51	c.i	>5
XXXIV	5-Methyl	$C_7H_6N_2S$	meth/water	29	a.iii	5
XXXV	6-Methyl	$C_7H_6N_2S$	methanol	38	a.iii	20

- Continued -

Table 5. Continued

Number	Compound	Formula	Recryst. solv.	m.p. °C	Procedure*	Yield %
XXXVI	5,6-Dimethyl	$C_8H_8N_2S$	methanol	75	a.iii	22
XXXVII	6-Butyl	$C_{10}H_{12}N_2S$	ethanol	101-111	b.	9
XXXVIII	6-Amino,7-Bromo	$C_6H_4BrN_3S$	benzene	165-167	c.v	58
XXXIX	5-Fluoro,6-Chloro	$C_6H_2FClN_2S$	methanol	95.5-96.5	b.	1
XXXX	5-Methoxy,6-Chloro	$C_7H_5ClON_2S$	meth/water	156	b.	0.75
XXXXI	4-Bromo,5-Amino	$C_6H_4BrN_3S$	benzene-pet.ether	178	c.v	81
XXXXII	4-Iodo,5-Amino	$C_6H_4IN_3S$	ethanol/water	163	c.v	60
XXXXIII	6-Ethoxy,7-Nitro	$C_8H_7O_3N_3S$	methanol	183-184	c.vi	52
XXXXIV	6-Methyl,7-Nitro	$C_7H_5O_2N_3S$	pet. ether	115	c.vi	60
XXXXV	6-Methyl,7-Amino	$C_7H_7N_3S$	benzene	134-135	c.iii	67
XXXXVI	4,5,6,7-Tetrafluoro	$C_6F_4N_2S$	methanol	104	b.	4
XXXXVII	6-Nitro,7-Amino	$C_6H_4O_2N_4S$	----	228	c.vii	63
XXXXVIII	4,5,6,7-Tetrachloro	$C_6Cl_4N_2S$	methanol	168-170	b.	1.5

* Described in materials and methods III. B. 11.

SECTION IV

RESULTS AND DISCUSSION

A. Synergistic Activity of 1,2,3-Benzothiadiazoles

Since synergists sometimes have an effect on insecticide penetration, preliminary experiments were conducted to study the effect on activity of applying the synergist and the insecticide separately to different parts of the fly. Thus the insecticide was topically applied to the dorsal surface of the thorax and the synergist to the abdomen. No significant difference in synergistic activity was observed whether the materials were applied in this way or whether they were applied together as a solution containing a fixed ratio of insecticide: synergist to the dorsal surface of the thorax. The effect of synergists in the toxicity of different insecticides is often studied by two different methods: 1) insecticide and synergists are applied in a certain fixed ratio, 2) an insecticide-synergist combination containing a fixed amount of synergist (usually that produces the blockage of the detoxification) is used. Both methods were investigated and after considering the advantages and disadvantages it was concluded that from the point of view of practical convenience the most suitable method of evaluating a large series of compounds was to use a fixed insecticide: synergist ratio, and preliminary results indicate that the most satisfactory ratio was 1:5 insecticide:synergist.

1. The Activity of the 1,2,3-benzothiadiazoles as Synergists for Pyrethroids Against the Housefly (Musca domestica L.)

Low synergistic ratios were reported by Felton et al. (1970) when 1,2,3-benzothiadiazoles were tested as synergists for natural pyrethrins. Based on the observation that the degree of synergism in houseflies was higher for chrysanthemic esters than for other pyrethroid esters (Sawicki, 1962a,b) it was decided to use two synthetic chrysanthemic esters, bioresmethrin and bioallethrin, to study the synergistic effects of the 1,2,3-benzothiadiazoles on pyrethroids.

The activity of three 1,2,3-benzothiadiazoles as bioallethrin and bioresmethrin synergists in Musca domestica is shown on Table 6. In spite of the high innate insecticidal activities of bioallethrin (LD_{50} = 0.52µg/fly) and bioresmethrin (LD_{50} = 0.023µg/fly), the 1,2,3-benzothiadiazoles showed moderate synergistic activity. The activities of the 1,2,3-benzothiadiazoles as bioallethrin synergists were slightly lower than that for piperonyl butoxide (SR = 5.77) but when tested as bioresmethrin synergists they were between 1.7 and 2.3-fold more active than piperonyl butoxide. Felton et al. (1970) suggested that the introduction of poly-ether side chains into the 1,2,3-benzothiadiazoles increased their activity with natural pyrethrins. The results in Table 6 are in agreement with this since the best compound tested was 6-ethoxy-1,2,3-benzothiadiazole with SR values of 4.33 and 2.55 for bioallethrin and bioresmethrin respectively. It is possible that the degree of synergism might increase with an increase in the ratio of synergist to insecticide, but this possibility was not investigated.

Table 6. Synergistic activity[a] of 1,2,3-benzothiadiazoles as bioallethrin[b] and bioresmethrin[c] synergists in Musca domestica[d,e]

Synergist	Bioallethrin LD_{50} (µg/fly)	Bioallethrin Syn. Ratio	Bioresmethrin LD_{50} (µg/fly)	Bioresmethrin Syn. Ratio
5,6-Dimethyl-1,2,3-benzothiadiazole	0.16	3.25	0.011	2.09
5-Iodo-1,2,3-benzothiadiazole	0.13	4.00	0.012	1.92
6-Ethoxy-1,2,3-benzothiadiazole	0.12	4.33	0.009	2.55
Piperonyl butoxide	0.09	5.77	0.021	1.10

[a]Ratio 1:5 (insecticide:synergist).

[b]LD_{50} = 0.52 µg/fly.

[c]LD_{50} = 0.023 µg/fly.

[d]Strain W.H.O.

[e]Average weight 21 mg/fly.

Studies on the metabolism of pyrethroids in insects (Yamamoto et al., 1969) and mammals (Casida et al., 1971) have led to the conclusion that ester hydrolysis plays only a very minor role in detoxification compared with microsomal oxidation. It is unlikely therefore, that the low activity of the 1,2,3-benzothiadiazoles as pyrethroids synergists indicates the unimportance of oxidative detoxification. It is also difficult to explain in terms of an inefficient blockage of microsomal enzyme activity. More likely it results from the extremely high innate insecticidal activity of bioresmethrin and biallethrin. Metcalf et al. (1967) made a similar suggestion based on the observation that with the carbamate insecticides the degree of synergism by piperonyl butoxide was inversely correlated with the innate insecticidal activity of the carbamate.

2. The Activity of the 1,2,3-benzothiadiazoles as Synergists for Carbaryl Against the Housefly (Musca domestica L.)

Most strains of flies, even those classified as susceptible to insecticides show a marked natural tolerance to carbaryl. In this investigation the World Health Organization (W.H.O.) susceptible strain of Musca domestica was used and the LD_{50} of carbaryl for this strain was found to be greater than 100 µg/fly. Since the degree of synergism of the carbamates is inversely related to their innate toxicity (Fukuto et al., 1962; Metcalf, et al., 1967), synergistic ratios with carbaryl are often dramatic. Consequently carbaryl is commonly used in synergism studies (Sacher et al., 1968; Bakry et al., 1968; Fellig et al., 1970; Brattsten and Metcalf, 1970; and Wilkinson et al.,

1966) and it was considered that the synergistic ratios with carbaryl would provide a useful indicate for comparing the synergistic activity of the 1,2,3-benzothiadiazoles.

Some examples of the dosage mortality lines resulting from topical application of carbaryl-1,2,3-benzothiadiazole combinations to houseflies are shown in Fig. 4, and the LD_{50} values and synergistic ratios for (1:5) combinations of carbaryl with fifty one derivatives of 1,2,3-benzothiadiazoles are shown in Table 7.

These results clearly demonstrate that several derivatives of 1,2,3-benzothiadiazole are excellent synergists for carbaryl. Even the unsubstituted 1,2,3-benzothiadiazole (I) itself exhibits appreciable synergistic activity (SR = 67) with carbaryl. This contrasts with the observed inactivity of 1,3-benzodiaoxole (Wilkinson et al., 1966) although the unsubstituted 1,2-d and 2,3-d-naphtho-1,3-dioxole do show substantial activity (Sacher et al., 1971).

Synergistic activity with carbaryl was considerably enhanced by the presence of a variety of substituents in the aromatic ring of the 1,2,3-benzothiadiazole and it appears to make little or no difference whether these are electron withdrawing or donating groups. Thus substitutes such as methyl, ethoxy, propoxy, (electron releasing groups) or cyano, fluorine, chlorine, bromine, iodine, (except XII) and nitro (except XXIII) (electron withdrawing groups) all increased synergistic activity relative to the unsubstituted compound.

FIGURE 4. DOSAGE MORTALITY LINES FOR CARBARYL-1,2,3-BENZOTHIADIAZOLES (1:5) TOPICAL APPLICATION TO HOUSEFLIES

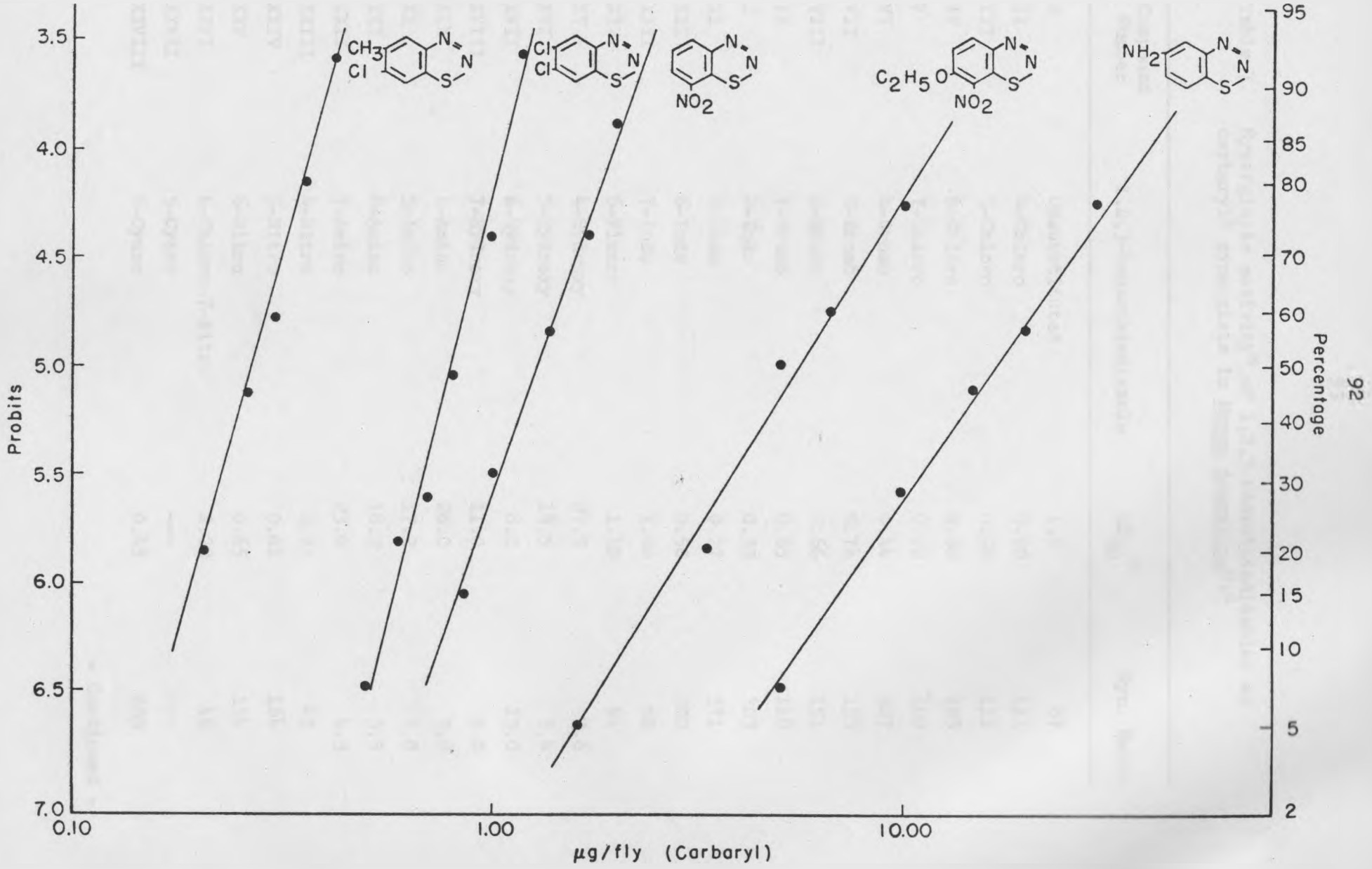


Table 7. Synergistic activity[a] of 1,2,3-benzothiadiazoles as carbaryl[b] synergists in Musca domestica[c,d]

Compound Number	1,2,3-benzothiadiazole	LD_{50}[e]	Syn. Ratio
I	Unsubstituted	1.5	67
II	4-Chloro	0.90	111
III	5-Chloro	0.90	111
IV	6-Chloro	0.80	125
V	7-Chloro	0.92	109
VI	4-Bromo	0.44	227
VII	5-Bromo	0.74	135
VIII	6-Bromo	0.66	151
IX	7-Bromo	0.85	118
X	4-Iodo	0.33	303
XI	5-Iodo	0.27	371
XII	6-Iodo	0.50	200
XIII	7-Iodo	1.60	62
XIV	6-Fluoro	1.10	91
XV	4-Hydroxy	27.5	3.6
XVI	5-Hydroxy	18.5	5.4
XVII	6-Hydroxy	8.0	13.0
XVIII	7-Hydroxy	11.0	9.0
XIX	4-Amino	26.0	3.8
XX	5-Amino	17.2	5.8
XXI	6-Amino	18.2	5.5
XXII	7-Amino	23.0	4.3
XXIII	4-Nitro	2.35	43
XXIV	5-Nitro	0.61	164
XXV	6-Nitro	0.65	154
XXVI	6-Chloro-7-Nitro	2.20	46
XXVII	5-Cyano	---	---
XXVIII	6-Cyano	0.48	209

- Continued -

Table 7. Continued

Compound Number	1,2,3-benzothiadiazole	LD_{50} [e]	Syn. Ratio
XXIX	7-Cyano	1.05	95
XXX	4-Methoxy	---	---
XXXI	6-Methoxy	0.61	164
XXXII	6-Ethoxy	0.27	371
XXXIII	6-Propoxy	0.48	209
XXXIV	5-Methyl	0.70	149
XXXV	6-Methyl	0.65	154
XXXVI	5,6-Dimethyl	0.37	271
XXXVII	6-Butyl	2.15	47
XXXVIII	6-Amino,7-Bromo	22.0	4.5
XXXIX	5-Fluoro,6-Chloro	0.62	161
XXXX	5-Methoxy,6-Chloro	0.22	454
XXXXI	4-Bromo,5-Amino	20.0	5
XXXXII	4-Iodo,5-Amino	13.7	7.3
XXXXIII	6-Ethoxy,7-Nitro	5.6	18
XXXXIV	6-Methyl,7-Nitro	6.7	15
XXXXV	6-Methyl,7-Amino	9.0	11
XXXXVI	4,5,6,7-Tetrafluoro	N.A.	--
XXXXVII	6-Nitro,7-Amino	N.A.	--
XXXXVIII	4,5,6,7-Tetrachloro	1.75	57
XXXXIX	7-Nitro	1.30	77
L	5-Methyl,6-Chloro	0.26	385
LI	5,5-Dichloro	0.29	345

[a] Ratio 1:5 (insecticide:synergist)

[b] LD_{50} 100 µg/fly

[c] Strain W.H.O.

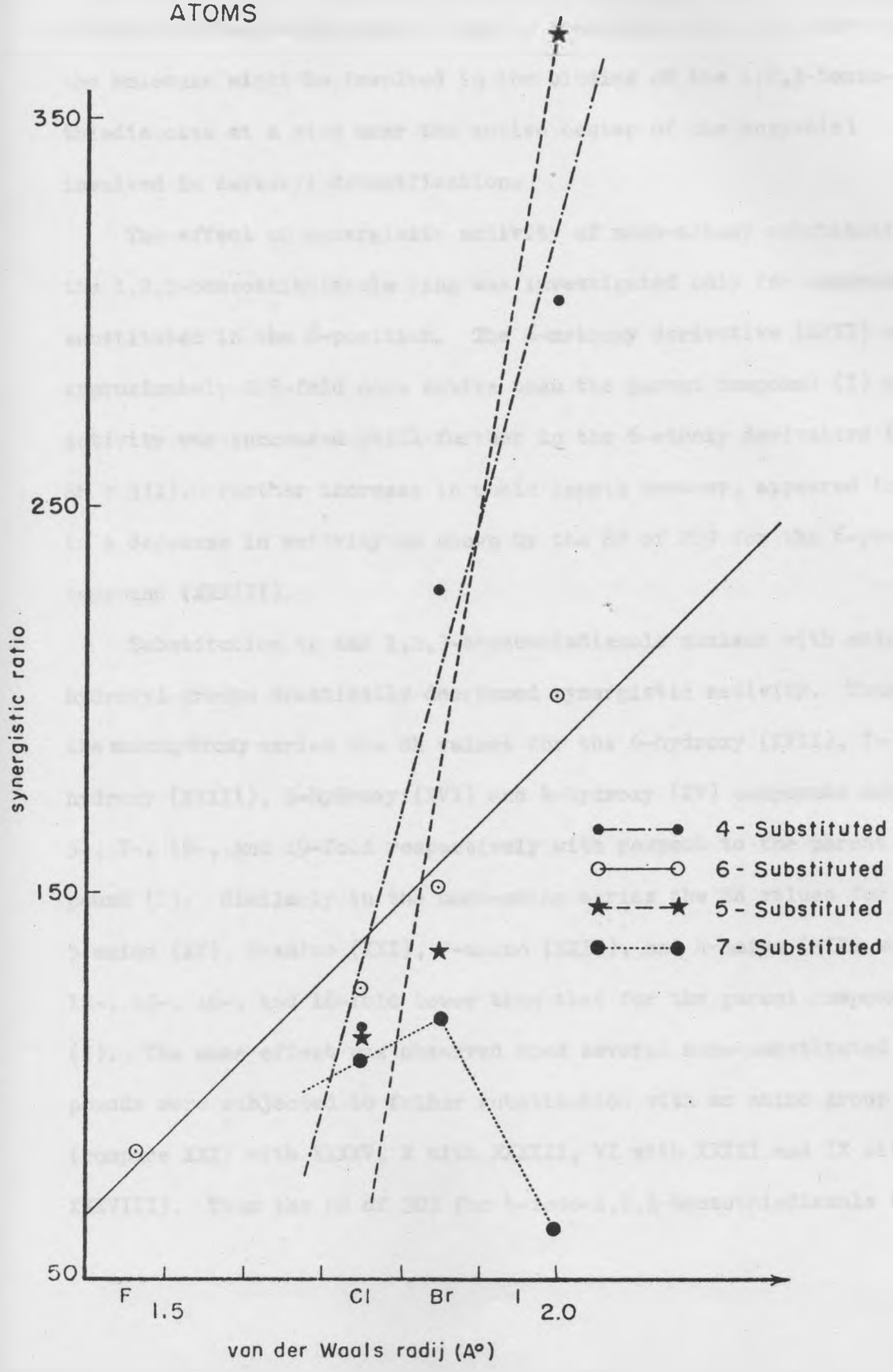
[d] µg of carbaryl/fly.

[e] Average weight 21 mg/fly.

N.A. = no activity.

Monohalogeno-substitution in the 1,2,3-benzothiadiazole ring was observed to increase synergistic activity depending on the nature and position of the substituent. Thus the activity of 4-, 5-, and 6-monohalogeno-derivatives increased in the order F < Cl < Br < I and in the 6-substituted series, compounds XIV, IV, VIII, and XII, SR values were 91, 125, 151, and 200 respectively. These results suggest that synergistic activity in monohalogenated 1,2,3-benzothiadiazoles might be associated with an increase in size of the halogen atom. This is clearly demonstrated by Fig. 5 where the synergistic ratios of monohalogenated 1,2,3-benzothiadiazoles are plotted against the van der Waals radii (Kutter and Hansch, 1969) of the halogen atoms. The increase in activity associated with increasing size of the halogen atom is more marked in compounds substituted in 4- or 5-positions than in those substituted in the 6-position as indicated by the slope of the lines. The effect is even less marked in compounds substituted in the 7-position of the ring and in this series the iodo (XIII) derivative is considerably less active than the corresponding bromo (IX) and chloro (V) compounds. No obvious relationship appears to exist between synergistic activity and the position of the halogen substituent in the ring. In each series, however, synergistic activity is lower when the halogen substituent is in the 7-position of the ring and this effect is most obvious with the 7-iodo-drivative (XIII). A similar effect is also observed in the mono-cyano compounds where the SR of 209 for the 6-cyano-1,2,3-benzothiadiazole (XXVIII) decreases to 95 in the 7-cyano compound (XXIX). These data might indicate steric

FIGURE 5. RELATIONSHIP BETWEEN THE S.R OF MONOHALOGENS 1,2,3-BENZOTHIADIAZOLES AS CARBARYL SYNERGIST IN MUSCA DOMESTICA AND THE VAN DER WAALS RADII OF HALOGEN ATOMS



hindrance between the ring substituents and the hetero-sulfur atom of the thiadiazole ring and it can be speculated that this portion of the molecule might be involved in the binding of the 1,2,3-benzothiadiazoles at a site near the active center of the enzyme(s) involved in carbaryl detoxification.

The effect on synergistic activity of mono-alkoxy substitution in the 1,2,3-benzothiadiazole ring was investigated only for compounds substituted in the 6-position. The 6-methoxy derivative (XXXI) was approximately 2.5-fold more active than the parent compound (I) and activity was increased still further in the 6-ethoxy derivative (XXXII, SR = 371). Further increase in chain length however, appeared to lead to a decrease in activity as shown by the SR of 209 for the 6-propoxy compound (XXXIII).

Substitution in the 1,2,3-benzothiadiazole nucleus with amino or hydroxyl groups drastically decreased synergistic activity. Thus in the monohydroxy series the SR values for the 6-hydroxy (XVII), 7-hydroxy (XVIII), 5-hydroxy (XVI) and 4-hydroxy (XV) compounds decreased 5-, 7-, 12-, and 19-fold respectively with respect to the parent compound (I). Similarly in the mono-amino series the SR values for the 5-amino (XX), 6-amino (XXI), 7-amino (XXII), and 4-amino (XIX) were 12-, 12-, 16-, and 18-fold lower than that for the parent compound (I). The same effect was observed when several mono-substituted compounds were subjected to futher substitution with an amino group (compare XXXV with XXXXV, X with XXXXII, VI with XXXXI and IX with XXXVIII). Thus the SR of 303 for 4-iodo-1,2,3-benzothiadiazole (X)

is decreased to 7.3 following amino-substitution in position 5 (XXXXII). Several factors might be involved in the decrease of synergistic activity observed when amino or hydroxy functions are introduced into the 1,2,3-benzothiadiazole molecule. Although in this investigation no penetration studies have been made, it is possible that substitution with these groups will reduce the lipophilicity of the compound and subsequently slow the rate of cuticular penetration as has been discussed by Wilkinson, (1967) for 1,3-benzodioxoles. The lower SR values with these compounds might also be partially explained by rapid metabolism by the insect, through direct conjugation of the amino or hydroxy groups.

Disubstitution in positions 5- and 6- of the ring produces extremely active compounds (XXXVI, XXXIX, XXXX, L, and LI). Thus the SR of 345 for 5,6-dichloro-1,2,3-benzothiadiazole (LI) is 3.1- and 2.8-fold greater than those observed for the 5-chloro (III) and 6-chloro (IV) compounds respectively. Similarly the 5-fluoro-6-chloro derivative (XXXIX) is 1.3-fold more active than the 6-chloro compound (IV). Synergistic activities of the mono-methoxy (XXXI) or mono-methyl (XXXIV) derivatives are also greatly enhanced by further halogen substitution. Thus the SR for 5-methyl-1,2,3-benzothiadiazole (XXXIV) increases from 149 to 385 following further substitution with a chloro group in the 6-position (L). The SR of 454 for the 5-methoxy-6-chloro derivative (XXXX) is one of the highest reported for any carbaryl synergist.

The high SR values observed in compounds disubstituted in the 5- and 6-positions is not limited to those containing halogen as illustrated by the activities of compounds in the alkyl series. Thus the 5-methyl (XXXIV) and 6-methyl (XXXV) compounds exhibit SR values of 149 and 154 respectively compared with the SR of 271 for the 5,6-dimethyl derivative (XXXVI).

In contrast to the high activity of the 5,6-disubstituted-1,2,3-benzothiadiazoles the synergistic activity of 6,7-disubstituted compounds (XXVI, XXXVIII, XXXXIII, XXXXIV, XXXXV, and XXXXVII) was extremely low. The presence of a nitro group in position-7 seems to be particularly critical. This can be clearly seen by comparing the synergistic activity of 6-ethoxy-1,2,3-benzothiadiazole (XXXII, SR = 371) with that of the 6-ethoxy-7-nitro derivative (XXXXII, SR = 18). A similar effect is observed when the 6-methyl derivative (XXXV; SR = 154) is compared with the 6-methyl-7-nitro compound (XXXXIV, SR = 15) and the 6-chloro (IV, SR = 125) with the 6-chloro, 7-nitro (XXVI, SR = 46). If the nitro group in the 7-position lies in the plane of the benzene ring (coplanar), a position that is favored by resonance interactions (Taft, 1956), its effective size will be as large or larger than the iodine atom. Under these conditions it is likely to exert a large steric influence on synergistic activity in a manner similar to that previously discussed.

Since the effect of polysubstitution was evaluated only with very few compounds it is not possible to make general conclusions. It does seem, however, that polysubstitution tends to decrease synergistic

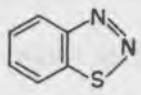
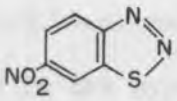
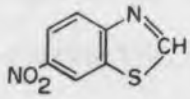
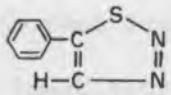
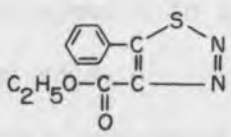
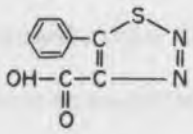
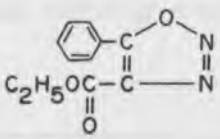
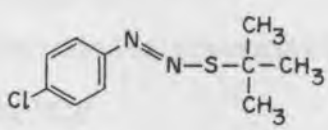
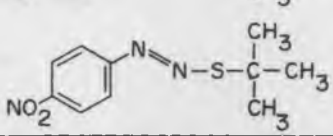
activity and it is possible that this is related to steric hindrance effects associated with the 7-substituent. Thus, the synergistic activity of the tetrachloro compound (XXXXVIII) is 6-fold lower than that of the 5,6-dichloro (LI).

3. The Synergistic Activity of Compounds Closely Related to the 1,2,3-benzothiadiazoles

Felton et al., (1970) compared the synergistic activity of the 1,2,3-benzothiadiazoles with a variety of other bicyclic compounds with five membered nitrogen containing heterocyclic rings. Based on the low or complete absence of activity of the 2,1,3-benzothiadiazoles, 1-(H)-benzotriazoles, benzofurazanes and indoles these workers concluded that synergistic activity was associated with the 1,2,3-benzothiadiazole structure.

This conclusion is supported by the observed inactivity of compounds containing the benzothiazole ring. Thus as it can be seen from Table 8 that 6-nitrobenzothiazole (LII) showed no activity compared with the SR of 154 exhibited by the corresponding 6-nitro-1,2,3-benzothiadiazole (XXV). The synergistic activity of several other compounds closely related to the 1,2,3-benzothiadiazoles were evaluated as carbaryl synergist against Musca domestica and the data are shown in Table 8.

Table 8. Synergistic activity[a] of compounds closely related to the 1,2,3-benzothiadiazole structure as carbaryl[b] synergists in Musca domestica[c,d]

Compound Number	Structure	LD_{50}[e]	Syn. Ratio
I		1.50	66
XXV		0.65	154
LII		N.A.*	--
LIII		1.06	94
LIV		1.50	66
LV		N.A.*	--
LVI		4.80	21
LVII		1.50	66
LVIII		2.42	41

(Footnotes - next page.)

In view of the high synergistic activity of many of the 1,2,3-benzothiadiazoles it was decided to investigate whether compounds containing the thiadiazole ring itself showed similar activity. As shown in Table 8, 5-phenyl-1,2,3-thiadiazole (LIII) exhibited a SR of 94 which is approximately 1.5-fold higher than that shown by 1,2,3-benzothiadiazole (I). The 4-carboxyethyl-5-phenyl-1,2,3-thiadiazole (LIV) was also quite effective (SR = 66) though the corresponding 4-carboxy derivative (LV) was inactive probably due to its high polarity. Since 4-carboxyethyl,5-phenyl-1,2,3-oxadiazole (LVI) was an intermediate in the synthesis of LIII and LIV, this was also tested and it was found to be approximately 3-fold less active than the corresponding thio-derivative. These data strongly suggest the importance of the 1,2,3-thiadiazole grouping in determining synergistic activity, and indicate that fusion to an aromatic ring is not of critical importance. The inactivity of the 2,1,3-benzothiadiazole (Felton et al., 1970) also appears to indicate that the diazosulfide group plays an important role.

In view of this, compounds which are essentially open chain analogs of the 1,2,3-benzothiadiazole (LVII, LVIII) were tested as carbaryl synergists. The moderate activity of these compounds SR values of 66 and 41 respectively clearly establish the importance of the diazosulfide linkage.

Footnotes to Table 8:

[a] Ratio 1:5 (insecticide:synergist)

[b] LD_{50} >> 100 µg/fly.

[c] Strain W.H.O.

[d] average weight 21 mg/fly.

[e] µg of carbaryl/fly.

[*] No activity.

In summary, synergistic activity does not seem to be a property unique to compounds containing the 1,2,3-benzothiadiazole ring as was initially suggested by Felton et al. (1970) but appears to be associated more generally with the 1,2,3-thiadiazole moiety. The appreciable activity of compounds containing the diazosulfide group even in open chain structure might indicate that the target site interaction is associated with the presence of this group.

B. Theoretical Considerations for Regressional Analysis of 1,2,3-Benzothiadiazoles

Equation 12 provides a useful point of departure for studies of structure activity relationships in biological systems.

$$\log BR = -k'\pi^2 + k''\pi + \rho\sigma + k''' \qquad \text{Eq.(12)}$$

The major problem in this investigation was to evaluate how the various physicochemical constants could be applied to a heterocyclic ring system such as that presented by 1,2,3-benzothiadiazole.

The Hammett equation has been widely discussed and applied in structure activity studies with organic compounds and has been reviewed by Jaffe (1953). The equation can be written as follows, where k is the rate or equilibrium constant for a compound with a substituent X and k_o

$$\log \frac{k}{k_o} = \rho\sigma$$

is the corresponding constant for the unsubstituted parent compound.

The electronic character of the substituent as defined by σ, and ρ is a reaction constant which defines the nature of the reaction under

consideration. The application of this equation to heterocyclic compounds is extremely complicated. Among the problems which need to be considered are the relative reactivities of heterocyclies compared with benzene rings, and special reactions which might be associated with the hetero atom(s). The nature of the transmission of the electronic effects of the aromatic substituents to the hetero atoms of the ring creates an additional problem. Very few examples exist where the Hammett equation has been applied to heterocyclic compounds though fortunately an excellent review has been published by Jaffe and Jones (1964). One of the first examples of the application of this equation to heterocyclic compounds was reported by Hammett (1940) for the 1,3-benzodioxoles. In the case of the 1,2,3-benzothiadiazoles, however, the heterocyclic ring is not symetrical as it is in the 1,3-benzodioxoles. More useful examples have been reported by Todesco and Vivarelli (1962). These investigators successfully applied the Hammett equation in a study of methoxydechlorination of a series of 2-chlorobenzothiazoles and also applied it to the oxidation of 2-methylmercaptobenzothiazoles to the corresponding sulfoxides. By comparing the ρ values of these reactions they found that the electronic effects of ring substituents were transmitted to the heterocyclic ring mainly through the hetero nitrogen atom (higher ρ value) and that transmission through the sulfur atom of the ring was almost negligible (ρ near to zero).

These examples clearly demonstrated the feasibility of applying this equation to the 1,2,3-benzothiadiazoles. In this investigation

the effect on synergistic activity with carbaryl of substituents in the 5-, 6-, and 5,6-positions of the 1,2,3-benzothiadiazole ring were investigated. The effects of substituents in the 4- and 7-positions of the ring were not studied as, because of steric considerations, it was considered probable that σ values for substituents at these positions were unlikely to be very meaningful.

Because of the asymmetry of the 1,2,3-benzothiadiazole ring substituents at the 5-position are meta to the hetero-nitrogen (3) atom and para to the hetero sulfur (1) atom. A similar though reversed situation is true for substituents in the 6-position of the ring. In an attempt to consider all possibilities, regression analyses were made using both σ_m and σ_p for substituents in the 5- and 6-positions.

In agreement with the suggestion that electronic effects are transmitted mainly through the hetero nitrogen atom (3) better correlations were always obtained when the σ_m values were used for substituents in the 5-position and σ_p values for those in the 6-position. Similar results have also been reported by Byrson (1960) in relating the basicities of quinolines and isoquinolines with substituents in the 3- and 4-positions of the ring respectively. Thus in the quinoline series basicity was related with the σ_m values of the 3-substituent whereas in the isoquinoline series it was associated with those of the 4-substituent.

Several alternatives have also been investigated with regard to the π constants employed in the regression analyses. Two sets of theoretical values for π have been studied: those for the phenoxyacetic

acids reported by Fujita et al. (1964), and those for a series of 2H-1,2,4-benzothiadiazine 1, 1-dioxide recently reported by Toppliss and Yudis (1972). When π values derived from phenoxyacetic acids were used in Eq. 12 the best correlations were obtained when π_m values were used for substituents in 5-position and π_p values for substituents in the 6-position of the 1,2,3-benzothiadiazole molecule.

C. Regression Analysis of the Activity of 1,2,3-Benzothiadiazole as Carbaryl Synergists

As previously explained, regression analysis was applied only to 1,2,3-benzothiaidazoles substituted in the 5-, 6-, and 5,6-positions. Unfortunately not all compounds tested could be included in the analysis because of the lack of appropriate constants for some substituents groups.

Equation 12 was employed in the regression analysis where SR

$$\log SR = k'\pi^2 + k''\pi + \rho\sigma + k''' \qquad \text{Eq.(12)}$$

represents the synergistic ratio for 1:5: carbaryl:synergist (w/w) combinations. The data could be also be expressed on a molar basis, but it was considered that differences between the w/w ratio and mole/mole ratio are probably small compared with variations in the biological testing.

Constants k', k'', k''' and ρ were obtained in the regression analysis. The hydrophobic bonding constants (π) were derived from either the phenoxyacetic acids (Fujita et al., 1964) or from the 2H-1,2,4-benzothiadiazine 1,1-dioxide system (π_B) (Toppliss and Yudis,

1972). The homolytic free radical constants ($\sigma^{\cdot}$) was sometimes included in Eq. 12 instead of σ. The values employed are those reported by Hansch (1968b) and are derived from the relative rates of phenylation of compounds C_6H_5X (Williams, 1960). The Hammett electronic constant (σ) was taken from the values reported by Jaffe (1953).

Table 9 shows the data for 14 compounds for which substituent constants are available and from these, equations 13-16 were derived by the method of least squares. In these equations n represents the number of compounds employed in the regression analysis, s is the standard error, and r is the correlation coefficient.

	n	r	s	Eq.
$\log SR = 0.300\pi + 2.049$	14	0.608	0.188	(13)
$\log SR = 0.281\pi_B + 2.030$	14	0.656	0.179	(14)
$\log SR = 0.070\sigma + 2.232$	14	0.105	0.236	(15)
$\log SR = 0.418\sigma^{\cdot} + 2.171$	14	0.321	0.225	(16)

As can be seen Eq. 13-16 give only poor correlation coefficients indicating that the hydrphobic, electronic, or free radical parameters do not by themselves account for synergistic activity. This is not entirely surprising considering the complexity of the steps involved from the time that the insecticide-synergist solution is first applied to the cuticle to the time it reaches and interacts with the target site. Because of this equations containing more than one parameter were investigated. Those employing combinations of π and either σ or $\sigma^{\cdot}$ are shown in Eq. 17-20.

Table 9. Observed and calculated activities of 1,2,3-benzothiadiazoles as carbaryl synergists

Compound number	Substituent	a $\Sigma\pi$	b $\Sigma\pi_B$	c $\Sigma\sigma$	d $\Sigma\sigma^{\cdot}$	Obsd.[e] log SR	Calcd.[f] log SR	Δ log S.R.
I	None	0.00	0.00	0.00	0.00	1.826	1.816	0.010
III	5-Cl-	0.76	0.89	0.373	0.03	2.045	1.966	0.079
IV	6-Cl-	0.70	0.91	0.227	0.03	2.097	1.962	0.135
VII	5-Br-	0.94	1.08	0.391	0.11	2.130	2.044	0.086
VIII	6-Br-	1.02	1.08	0.232	0.11	2.179	2.043	0.136
XXIV	5-NO_2-	0.11	0.22	0.710	0.47	2.215	2.254	0.039
XXV	6-NO_2-	0.24	0.36	0.778	0.47	2.188	2.246	0.058
LI	5,6-diCl-	1.46	1.80	0.600	0.06	2.538	1.965	0.573
XXXX	5-OCH_3,6-Cl-	0.82	1.18	0.342	0.43	2.657	2.330	0.327
L	5-CH_3,6-Cl-	1.21	1.36	0.158	0.12	2.586	2.153	0.433
XXXIV	5-CH_3-	0.51	0.45	-0.069	0.09	2.173	1.997	0.176
XXXV	6-CH_3-	0.52	0.52[g]	-0.170	0.09	2.188	1.998	0.190
XXXVI	5,6-diCH_3-	1.03	0.97	-0.239	0.18	2.433	2.106	0.327
XXXI	6-OCH_3-	-0.04	0.27[g]	-0.268	0.40	2.215	2.166	0.049

(Footnotes on next page.)

Footnotes - Table 9

[a]Derived from the phenoxyacetic acid system (Fujita et al., 1964). π_m for 5-position and π_p for 6-position.

[b]Derived from the 2H-1,2,4-benzothiadiazine 1,1-dioxide system (Toppliss and Yudis, 1972), the 6-position in this system was considered equivalent to the 5-position in the 1,2,3-benzothiadiazole.

[c]From Jaffe, H (1953).

[d]From Hansch (1968b).

[e]From Table 7.

[f]Calculated using Eq. 25.

[g]Value for the 6-position of the 2H-1,2,4-benzothiadiazine 1,1-dioxide system.

	n	r	s	Eq.
$\log SR = 0.299\pi + 0.017\sigma + 2.046$	14	0.609	0.197	(17)
$\log SR = 0.291\pi_B + 0.051\sigma + 2.035$	14	0.660	0.186	(18)
$\log SR = 0.448\pi + 0.916\sigma' + 1.782$	14	0.882	0.117	(19)
$\log SR = 0.366\pi_B + 0.781\sigma' + 1.820$	14	0.867	0.123	(20)

The best correlation coefficients were obtained with equations expressed in terms of the free radical constants (σ') and the hydrophobic bonding constants (π). Thus Eq. 19 exhibits a high correlation coefficient ($r = 0.882$) between synergistic activity and hydrophobic bonding constant (π) (derived from phenoxyacetic acids) and the free radical constant (σ'). As shown by Eq. 20, a similar though slightly lower correlation ($r = 0.867$) was observed when the hydrophobic bonding constant π_B (derived from the 2 H-1,2,4-benzothidiazine 1,1-dioxides) was employed instead of π. This indicates the usefulness of the method since hydrophobic bonding constants obtained from two different systems give similar results.

In order to investigate whether the relationship between a particular physicochemical parameter and biological response is parabolic rather than linear (II. B.) several higher order equations (21-26) were investigated.

	n	r	s	Eq.
$\log SR = -0.192\pi^2 + 0.053\pi + 2.091$	14	0.632	0.192	(21)
$\log SR = -0.096\pi_B^2 + 0.127\pi_B + 2.067$	14	0.668	0.184	(22)
$\log SR = -0.191\pi^2 + 0.053\pi + 0.005\sigma + 2.090$	14	0.632	0.201	(23)
$\log SR = -0.103\pi_B^2 + 0.126\pi_B - 0.061\sigma + 2.075$	14	0.673	0.192	(24)
$\log SR = -0.138\pi^2 + 0.267\pi + 0.903\sigma' + 1.816$	14	0.891	0.118	(25)
$\log SR = -0.078\pi_B^2 + 0.240\pi_B + 0.775\sigma' + 1.852$	14	0.873	0.127	(26)

Equation 25 containing terms for π^2, π, and σ' gave the best correlation and accounted for almost 90% of the variance of the data. This represents a small improvement in the correlation coefficients obtained from Eq. 19. From Eq. 25 the ideal lipophilic value (π_0) was calculated (II. B) by taking the partial derivative $\frac{\partial \log SR}{\partial \pi}$ and setting this equal to zero. This indicates that the optimum value of π_0 for this series of compounds is 0.970. It is interesting to note that the σ' coefficient in Eq. 25 (0.903) is relatively high and indicates the importance of this parameter in relation to synergistic activity. Calculations from Eq. 25 indicated good agreement between the observed and calculated log SR values (Table 9, Δlog SR) although for a few compounds particularly (L) and (LI) the observed values were considerably higher than those calculated.

The possible involvement of free radicals in the mechanism of action of some synergists has been previously suggested by Hansch (1968b) and Wilkinson (1971b). Hansch (1968b) using regression analysis and substituent constants reported Eq. 27 for the synergistic activity of a series of 1,3-benzodioxoles as carbaryl synergist against Musca domestica. He postulated that the 1,3-benzodioxoles might interact

	n	r	s	Eq.
$\log SR = -0.195\pi^2 + 0.670\pi + 1.316\sigma' + 1.612$	13	0.929	0.171	(27)

with the microsomal complex to generate a relatively stable homolytic free radical by hydrogen abstraction from the methylene group of the ring, and suggested that this might bind tightly to the enzyme inhibiting its detoxifying action on insecticides. More recently, Marshall and Wilkinson (1970) working with various model oxidation systems

demonstrated that the epoxidation of aldrin by a modified Fenton system (H_2O_2, EDTA, Fe^{2+} and bovine serum albumin) was considerably reduced in the presence of several 1,3-benzodioxoles synergists. It was suggested that the radicals generated by the Fenton system (·OH) might interact with the 1,3-benzodioxole molecule to generate a stable homolytical radical and consequently the rate of aldrin epoxidation will be decreased by the competition between aldrin and 1,3-benzodioxole for the availability of radicals (Wilkinson, 1971b).

In view of the apparent relationship between synergistic activity and σ^+ it is possible that the activity of the 1,2,3-benzothiadiazoles as carbaryl synergists might also be explained on the basis of a homolytic free radical mechanism. Since the 1,2,3-benzothiadiazole ring has several sites which might be available for radical interaction, it is possible that they might interact with the microsomal enzymes by a mechanism similar to that proposed by Hansch for the 1,3-benzodioxoles. This is supported by the observation of Marshall and Wilkinson (1973) that like the 1,3-benzodioxoles the 1,2,3-benzothiadiazoles inhibit the epoxidation of aldrin by the modified Fenton system and that 6-nitro-1,2,3-benzothiadiazole is attacked by this reagent to give two products the formation of which can be explained by a homolytic radical mechanism.

D. In vitro Inhibition of Mixed Function Oxidases by 1,2,3-Benzothiadiazoles

It is well accepted that the effect of synergists on insecticide toxicity is due mainly to their ability to inhibit the m.f.o. system

responsible for oxidative detoxification (Wilkinson, 1971b; Casida, 1970).

This can be demonstrated *in vivo* by comparing the rate of metabolism of an insecticide alone with that following treatment with a synergist or it can be shown *in vitro* by direct addition of the synergist to a microsomal enzyme reaction mixture.

Information concerning the relationship between chemical structure and *in vitro* inhibitory activity of the 1,2,3-benzothiadiazoles might be useful in understanding the mechanism of action of these compounds. Furthermore, comparative studies on the inhibitory activity of the 1,2,3-benzothiadiazoles in both insects and mammals might yield important information regarding differences between the two systems, which might then be used in designing more selective compounds.

The m.f.o. system in the southern armyworm (*Prodenia eridania*) has been thoroughly investigated in this laboratory. The effects on aldrin epoxidase and DHI-hydroxylase of factors such as the presence of endogenous inhibitors, age, tissue distribution of the oxidase activity, incubation conditions and cofactor requirements have been reported by Krieger (1970); and Krieger and Wilkinson (1971). The high oxidase activity of the midgut armyworm preparation suggested that this would provide an excellent enzyme source to study the *in vitro* effects of the 1,2,3-benzothiadiazoles on the insect m.f.o. system. The *in vitro* effect of the 1,2,3-benzothiadiazoles on the mammalian m.f.o. systems was investigated in rat liver microsomes.

Based on the information that a large number of 1,3-benzodioxoles are *in vitro* inhibitors of a variety of reactions catalyzed by the m.f.o. system (Philleo *et al.*, 1965; Wilkinson and Hicks, 1969; Anders, 1968) and that the degree of inhibition often varies considerably with the reaction being studied and the preparation employed (Hansen and Hodgson, 1971), the inhibitory activity of the 1,2,3-benzothiadiazoles was studied on both aldrin epoxidation and DHI-hydroxylation (Fig. 6).

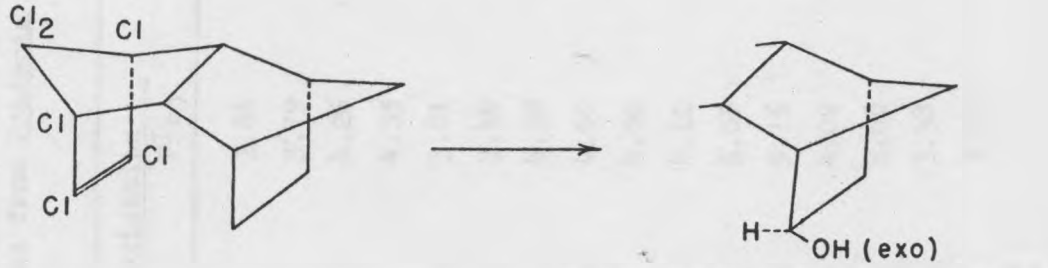
1. Inhibitory Activity of 1,2,3-benzothiadiazoles on Aldrin Epoxidation and DHI-hydroxylation by Midgut Preparations from Prodenia eridania

The concentrations of several 1,2,3-benzothiadiazoles that inhibit 50% (I_{50}M) of the epoxidation of aldrin and the hydroxylation of DHI by midgut preparations of *Prodenia eridania* are shown in Table 10. In general, the degree of inhibition seems to be higher for the DHI-hydroxylation reaction than for aldrin epoxidation.

Although the unsubstituted 1,2,3-benzothiadiazole (I) inhibits both enzymatic reactions several of the other compounds evaluated were more potent *in vitro* inhibitors depending on the nature of the substituent(s) and their position in the phenyl portion of the heterocyclic ring.

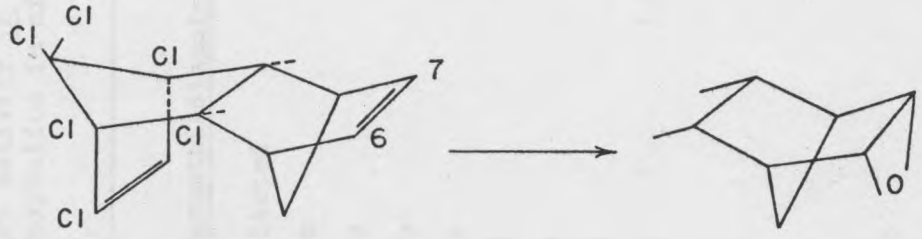
Monohalogeno substitution in the 1,2,3-benzothiadiazole ring affects inhibitory activity in a manner depending on the nature of the halogen and of its position in the ring. The inhibitory potency of the 5- and 6-monohalogenated 1,2,3-benzothiadiazoles was considerably greater than that of the unsubstituted compound (I) in both enzymatic

FIGURE 6. DIHYDROISODRIN HYDROXYLATION AND ALDRIN EPOXIDATION



Dihydroisodrin

6-exo-hydroxy-6,7-dihydroisodrin



Aldrin

Dieldrin

Table 10. Inhibitory activity *in vitro* of 1,2,3-benzothiadiazoles on aldrin epoxidation and DHI hydroxylation in midgut preparations from *Prodenia eridania*[a]

Compound Number	1,2,3-benzothiadiazole	Aldrin Epoxidation		DHI Hydroxylation	
		$I_{50}M$	pI_{50}	$I_{50}M$	pI_{50}
I	Unsubstituted	1.44×10^{-4}	3.84	6.10×10^{-5}	4.22
II	4-Chloro	1.63×10^{-4}	3.79	2.05×10^{-4}	3.69
III	5-Chloro	5.50×10^{-5}	4.26	3.45×10^{-5}	4.46
IV	6-Chloro	4.45×10^{-5}	4.35	5.80×10^{-6}	5.24
V	7-Chloro	1.55×10^{-4}	3.81	4.20×10^{-5}	4.38
VI	4-Bromo	3.25×10^{-4}	3.49	1.64×10^{-4}	3.79
VII	5-Bromo	2.65×10^{-5}	4.58	4.95×10^{-6}	5.31
VIII	6-Bromo	2.50×10^{-5}	4.60	2.85×10^{-6}	5.55
IX	7-Bromo	9.90×10^{-4}	4.00	2.10×10^{-5}	4.68
X	4-Iodo	8.00×10^{-5}	4.10	7.00×10^{-5}	4.16
XI	5-Iodo	5.30×10^{-6}	5.08	1.62×10^{-6}	5.79
XII	6-Iodo	6.85×10^{-6}	5.16	9.30×10^{-7}	6.03
XIII	7-Iodo	9.50×10^{-5}	4.02	3.45×10^{-5}	4.46
XIV	6-Fluoro	2.10×10^{-4}	3.68	1.00×10^{-4}	4.00
XV	4-Hydroxy	4.20×10^{-4}	3.38	2.85×10^{-4}	3.55
XVI	5-Hydroxy	5.80×10^{-4}	3.24	4.50×10^{-4}	3.35

- Continued -

Table 10. Continued

Compound Number	1,2,3-benzothiadiazole	Aldrin Epoxidation $I_{50}M$	Aldrin Epoxidation pI_{50}	DHI Hydroxylation $I_{50}M$	DHI Hydroxylation pI_{50}
XVII	6-Hydroxy	1.90×10^{-4}	3.72	1.26×10^{-4}	3.90
XVIII	7-Hydroxy	1.68×10^{-4}	3.78	3.10×10^{-4}	3.51
XIX	4-Amino	1.29×10^{-3}	2.89	2.30×10^{-3}	2.64
XX	5-Amino	4.35×10^{-4}	3.36	3.90×10^{-4}	3.41
XXI	6-Amino	1.55×10^{-4}	3.81	1.60×10^{-4}	3.80
XXII	7-Amino	3.70×10^{-4}	3.43	8.00×10^{-4}	3.10
XXIII	4-Nitro	2.10×10^{-4}	3.68	1.25×10^{-4}	3.90
XXIV	5-Nitro	2.19×10^{-4}	3.66	5.20×10^{-5}	4.28
XXV	6-Nitro	1.75×10^{-4}	3.76	4.80×10^{-5}	4.32
XXVI	6-Chloro,7-Nitro	4.50×10^{-5}	4.35	2.60×10^{-5}	4.58
XXVII	5-Cyano	8.70×10^{-5}	4.06	8.00×10^{-6}	5.10
XXVIII	6-Cyano	2.67×10^{-5}	4.57	2.10×10^{-5}	4.68
XXIX	7-Cyano	1.20×10^{-4}	3.92	6.00×10^{-5}	4.22
XXX	4-Methoxy	1.00×10^{-3}	3.00	7.70×10^{-4}	3.12
XXXI	6-Methoxy	6.50×10^{-5}	4.19	1.72×10^{-5}	4.77
XXXII	6-Ethoxy	5.10×10^{-6}	5.29	1.86×10^{-6}	5.73
XXXIII	6-Propoxy	7.00×10^{-7}	6.16	N.T.	---

- Continued -

Table 10. Continued

Compound Number	1,2,3-benzothiadiazole	Aldrin Epoxidation $I_{50}M$	Aldrin Epoxidation pI_{50}	DHI Hydroxylation $I_{50}M$	DHI Hydroxylation pI_{50}
XXXIV	5-Methyl	4.10×10^{-5}	4.39	1.76×10^{-5}	4.75
XXXV	6-Methyl	3.25×10^{-5}	4.49	1.67×10^{-5}	4.78
XXXVI	5,6-Dimethyl	2.27×10^{-5}	4.64	8.15×10^{-6}	5.09
XXXVII	6-Butyl	4.90×10^{-7}	6.31	N.T.	---
XXXVIII	6-Amino,7-Bromo	N.T.	---	9.60×10^{-5}	4.02
XXXIX	5-Fluoro,6-Chloro	8.90×10^{-5}	4.05	1.77×10^{-5}	4.75
XXXX	5-Methoxy,6-Chloro	1.32×10^{-5}	4.88	5.90×10^{-6}	5.23
XXXXI	4-Bromo,5-Amino	N.T.	---	N.T.	---
XXXXII	4-Iodo,5-Amino	N.T.	---	N.T.	---
XXXXIII	6-ethoxy,7-Nitro	1.00×10^{-3}	3.00	6.00×10^{-4}	3.22
XXXXIV	6-Methyl,7-Nitro	1.64×10^{-4}	3.78	1.13×10^{-4}	3.95
XXXXV	6-Methyl,7-Amino	N.T.	---	1.25×10^{-4}	3.90
XXXXVI	4,5,6,7-Tetrafluoro	$>>1 \times 10^{-3}$	---	$>>1 \times 10^{-3}$	---
XXXXVII	6-Nitro,7-Amino	N.T.	---	5.20×10^{-5}	4.28

- Continued -

Table 10. Continued

Compound Number	1,2,3-benzothiadiazole	Aldrin Epoxidation		DHI Hydroxylation	
		$I_{50}M$	pI_{50}	$I_{50}M$	pI_{50}
XXXXVIII	4,5,6,7-Tetrachloro	2.60×10^{-5}	4.58	1.75×10^{-5}	4.76
XXXXIX	7-Nitro	1.90×10^{-4}	3.68	1.00×10^{-4}	4.00
L	5-Methyl,6-Chloro	7.30×10^{-6}	5.14	8.60×10^{-7}	6.07
LI	5,6-Dichloro	5.70×10^{-6}	5.24	1.40×10^{-6}	5.85

[a]Source of enzyme midgut Prodenia eridania. Prepared as described in (III. B. 1). Enzyme activity and inhibition estimated as explained in (III. B. 3,4).

N.T. - not tested.

systems. Thus I_{50} values for aldrin epoxidation of the 5- chloro (III), 5-bromo (VII) and 5-iodo (XI) derivatives were 5.50×10^{-5}, 2.65×10^{-5}, and 8.30×10^{-6} respectively and corresponding values for the inhibition of DHI hydroxylation were 3.45×10^{-5}, 4.95×10^{-6}, and 1.62×10^{-6}. Similar values were observed for the 6-substituted compounds (Table 10).

In contrast, the inhibitory potency of the 4- and 7-monohalogenated-1,2,3-benzothiadiazoles was similar or lower than that of the parent compound and considerably lower than that of the 5- or 6-halogenated compounds. Thus the $I_{50}M$ values for aldrin epoxidation by the 4-chloro (II), 4-bromo (VI) and 4-iodo (X) compounds were 1.63×10^{-4}, 3.25×10^{-4} and 8.00×10^{-5} respectively compared with 1.44×10^{-4} for the unsubstituted 1,2,3-benzothiadiazole. Similar values were also obtained with respect to the inhibition of DHI-hydroxylation with both the 4- and 7-substituted compounds.

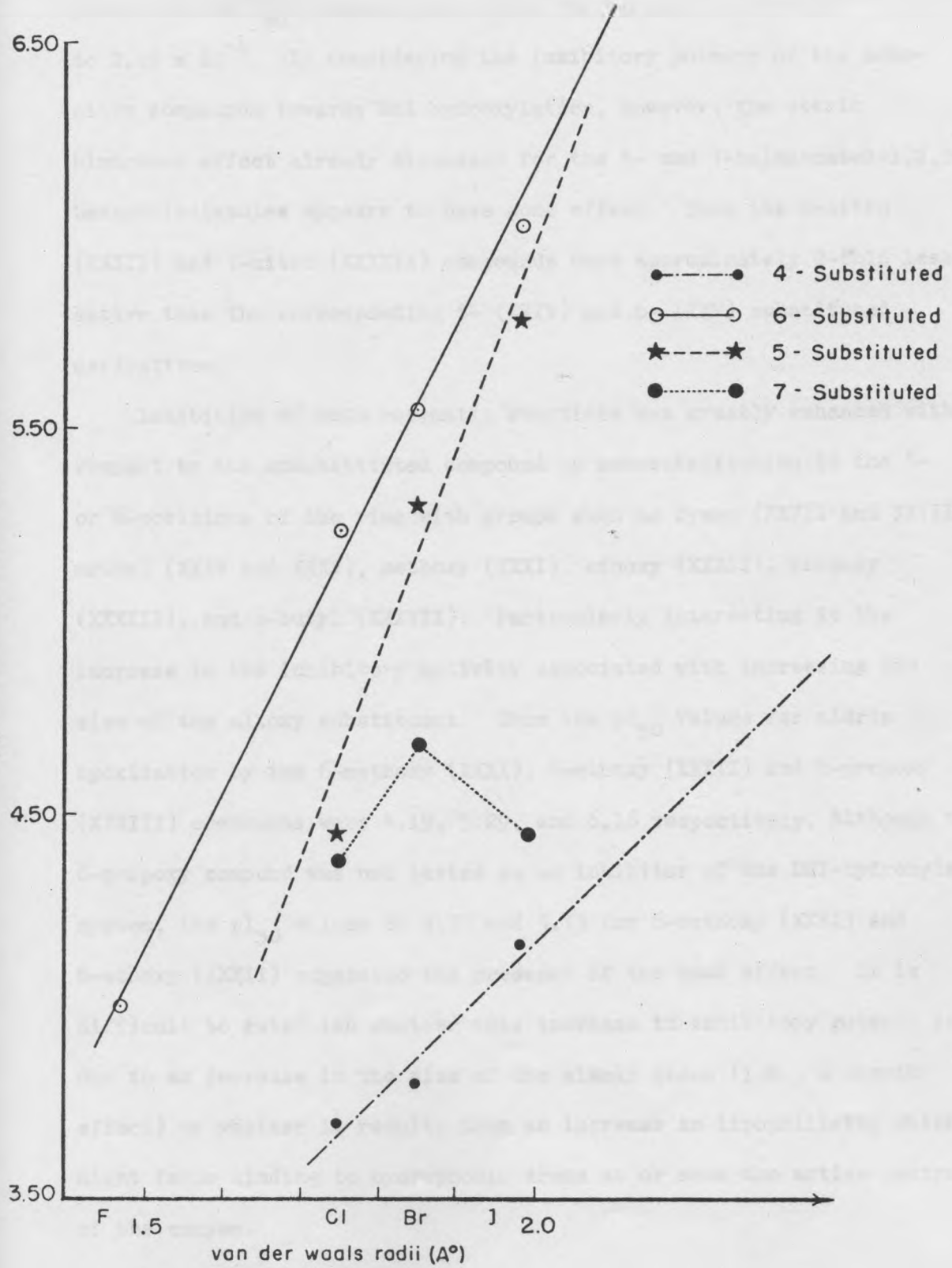
The inhibitory activity of 4-, 5-, 6-, and 7-monohalogenated 1,2,3-benzothiadiazoles increased in the order F < Cl < Br < I as is evident from the pI_{50} values (-log of the I_{50}) shown in Table 10. Thus, in the 6-substituted series the pI_{50} values for aldrin epoxidation are 3.68, 4.35, 4.60, and 5.16 for the 6-fluoro (XIV), 6-chloro (IV), 6-bromo (VIII), and 6-iodo (XII) derivatives respectively and a similar pattern can be seen for DHI-hydroxylation. These results indicate that the *in vitro* potency of the monohalogenated-1,2,3-benzothiadiazoles as inhibitors of aldrin epoxidation and DHI-hydroxylation by enzyme preparations from *Prodenia* is associated with an increase in size of the

halogen atom, an effect similar to that observed with respect to the *in vivo* synergistic activity of these compounds with carbaryl (IV. A. 2). This can be clearly seen by plotting the pI_{50} (DHI hydroxylation) for the monohalogenated-1,2,3-benzothiadiazoles against the van der Waals radii (Kutter and Hansch, 1969), (Fig. 7). As indicated by the slopes of the lines, the increase in inhibitory activity with increasing size of the halogen atom is more marked for the 5- and 6-substituted compounds than for the 4- or the 7-substituted derivatives. The same effect is also observed for aldrin epoxidation.

The degree of inhibition by the monohalogenated-1,2,3-benzothiadiazoles also varies according to the position of the halogen in the phenyl ring. In general with any one halogen substituent, the most active compounds are those substituted in 5- or 6-positions. This is most clearly seen for the inhibition of DHI-hydroxylation by the iodo compounds (Table 10) where the inhibitory potency of the 4-iodo (X) and 7-iodo (XIII) derivatives were 75- and 37-fold less than the 6-iodo (XII) and 43- and 21-fold less than the 5-iodo (XI) respectively. These results suggest that steric hindrance may occur between substituents in the 4- and 7-positions, and the hetero-atoms of the thiadiazole ring and that this might interfere with the binding of the molecule to the active center of the microsomal complex.

The aldrin epoxidase and the DHI hydroxylase in midgut preparations from *Prodenia* appear to behave differently with respect to inhibition by mononitro-1,2,3-benzothiadiazoles. The $I_{50}M$ values of the mononitro compounds (XXIII, XXIV, XXV, and XXXXIX) in the epoxidation reaction were similar to or slightly lower than that of the

FIGURE 7. INHIBITORY ACTIVITY OF 1,2,3-BENZOTHIADIAZOLES. RELALATIONSHIP BETWEEN pI_{50} FOR DHJ HYDROXYLATION AND THE VAN DER WAALS RADII OF HALOGEN ATOMS



unsubstituted compound. With aldrin epoxidation the position of the nitro group in the phenyl rings appears to be of little or no importance since all the $I_{50}M$ values were within the range of 1.75×10^{-4} to 2.19×10^{-4}. In considering the inhibitory potency of the mono-nitro compounds towards DHI hydroxylation, however, the steric hindrance effect already discussed for the 4- and 7-halogenated-1,2,3-benzothiadiazoles appears to have some effect. Thus the 4-nitro (XXIII) and 7-nitro (XXXXIX) compounds were approximately 2-fold less active than the corresponding 5- (XXIV) and 6- (XXV) substituted derivatives.

Inhibition of both enzymatic reactions was greatly enhanced with respect to the unsubstituted compound by monosubstitution in the 5- or 6-positions of the ring with groups such as cyano (XXVII and XXVIII), methyl (XXIV and XXXV), methoxy (XXXI), ethoxy (XXXII), propoxy (XXXIII), and n-butyl (XXXVII). Particularly interesting is the increase in the inhibitory activity associated with increasing the size of the alkoxy substituent. Thus the pI_{50} values for aldrin epoxidation by the 6-methoxy (XXXI), 6-ethoxy (XXXII) and 6-propoxy (XXXIII) compounds were 4.19, 5.29, and 6.16 respectively. Although the 6-propoxy compund was not tested as an inhibitor of the DHI-hydroxylase system, the pI_{50} values of 4.77 and 5.73 for 6-methoxy (XXXI) and 6-ethoxy (XXXII) suggested the presence of the same effect. It is difficult to establish whether this increase in inhibitory potency is due to an increase in the size of the alkoxy group (i.e., a steric effect) or whether it results from an increase in lipophilicity which might favor binding to hydrophobic areas at or near the active centre of the enzyme.

The degree of inhibition of aldrin epoxidation and DHI-hydroxylation by the methoxy and cyano compounds was also affected by the position of the substituents in the ring. Thus with respect to the inhibition of aldrin epoxidation the 7-cyano (XXIX) compound was 5-fold less active than the 6-cyano-1,2,3-benzothiadiazole (XXVIII) and in the DHI-hydroxylation system it was 3- and 8-fold less potent than the 6-cyano (XXVIII) and the 5-cyano (XXVII) derivatives respectively. A similar effect can be seen by comparing the inhibitory activity of the 6-methoxy (XXXI) and 4-methoxy-1,2,3-benzothiadiazole (XXX) with each enzyme system.

As was observed in the in vivo synergism study the inhibitory potency of each of the amino and hydroxy 1,2,3-benzothiadiazoles is lower than that of the parent compound (Table 10). It is probable that the low level of inhibition associated with these substituents is directly related to their low lipophilicity and this is supported by regression analysis (IV. E.).

Some of the most potent inhibitors in the series were those disubstituted in the 5,6-positions of the ring with combinations of chloro, methyl or alkoxy groups. Compounds (XXXVI, XXXX, L, LI) (Table 10). Thus against both enzyme systems the 5,6-dichloro compound (LI) is a considerably better inhibitor than either the 5-chloro (III) or 6-chloro (IV) compounds, the factors being 10- and 8-fold respectively for aldrin epoxidation. Similarly against aldrin epoxidase the 5-methyl-6-chloro (L) compound was 6-fold more active than either the 5-methyl (XXXIV) or the 6-chloro (IV) compounds and the difference was even larger in the hydroxylation reaction.

The effect is only very small (about 2-fold) in the 5-methyl (XXXIV), 6-methyl (XXXV) and 5,6-dimethyl (XXXVI) series and in some cases such as the 5-fluoro-6-chloro(XXXIX), 5,6-disubstitution results in less potent inhibitors than the respective monosubstituted derivatives.

Disubstitution in the 6- and 7-positions of the ring provides another example of the steric hindrance effect previously described, as found in the in vivo studies and is particularly obvious when a nitro group is present in the 7-position. Thus the inhibitory potency of the 6-methyl-7-nitro (XXXXIV) compound is 5- to 7-fold lower than the 6-methyl (XXXV) derivative towards both enzymatic reactions and an even more spectacular decrease (up to 300-fold) is observed when the activity of the 6-ethoxy (XXXII) compound is compared with that of 6-ethoxy-7-nitro-1,2,3-benzothiadiazole (XXXXIII). However, the steric effect of the 7-nitro group seems to be dependent to some extent on the nature of the 6-substituent since it is not obvious in the 6-chloro-7-nitro-(XXVI) compound the activity of which is only slightly lower than that of 6-chlor-1,2,3-benzothiadiazole (IV). It is possible that in the presence of a chlorine atom in the 6-position of the ring the nitro group assumes a position more nearly perpendicular to the plane of the ring and consequently exerts a weaker steric effect in this portion of the molecule.

The inhibitory activity of tetrasubstituted compounds was tested only with compounds (XXXXVI) and (XXXXVIII). The $I_{50}M$ value for the 4,5,6,7-tetrafluoro (XXXXVI) compound could not be obtained even at concentrations as high as $10^{-3}M$. In view of the steric effect associated

with substitution at the 4- and 7-position of the ring it was not surprising to find that the 4,5,6,7-tetrachloro-1,2,3-benzothiadiazole (XXXXVIII) was 5-fold less active than the 5,6-dichloro (LI) compound, but with an I_{50} of about 2×10^{-5}M this was still quite an active compound.

2. Inhibitory Activity of 1,2,3-Benzothiadiazoles on Aldrin Epoxidation and DHI Hydroxylation in Rat Liver Microsomes

As can be seen from the data in Table 11 rat liver microsomes are considerably less susceptible to inhibition by the 1,2,3-benzothiadiazoles than preparations from the southern armyworm. In a few cases the difference in the I_{50} values between the mammalian and insect enzymes are quite small but with other compounds such as 6-iodo (XII), 6-ethoxy (XXXII), 5-methoxy,6-chloro (XXXX), 5-methyl, 6-chloro(L) and 5,6-dichloro (LI) it is substantial and seems to be generally greater with the hydroxylation reaction than with epoxidation.

In addition to the generally lower susceptibility of rat liver microsomes to inhibition some interesting differences in structure-activity relationships are evident when the insect and mammalian data are compared.

Thus with the exception of the 4-iodo (X) compound the I_{50}M values of the monohalogenated-1,2,3-benzothiadiazoles (II-XIII) towards aldrin epoxidation all lie between 1.10×10^{-4} and 4.25×10^{-4} and there seems to be no obvious relationship between inhibitory potency and the size and position of the halogen atom. The

Table 11. *In vitro* inhibitory activity of 1,2,3-benzothiadiazoles on aldrin epoxidation and DHI hydroxylation by rat liver microsomes

Compound Number	1,2,3-benzothiadiazole	$I_{50}M$ Aldrin epoxidation	$I_{50}M$ DHI hydroxylation
I	Unsubstituted	8.50×10^{-3}	1.26×10^{-3}
II	4-chloro	4.12×10^{-4}	3.90×10^{-4}
III	5-chloro	3.62×10^{-4}	2.25×10^{-4}
IV	6-chloro	2.52×10^{-4}	1.72×10^{-4}
V	7-chloro	3.60×10^{-4}	2.30×10^{-4}
VI	4-bromo	1.65×10^{-4}	2.00×10^{-4}
VII	5-bromo	1.34×10^{-4}	1.57×10^{-4}
VIII	6-bromo	1.85×10^{-4}	1.62×10^{-4}
IX	7-bromo	2.10×10^{-4}	1.75×10^{-4}
X	4-iodo	6.00×10^{-5}	2.43×10^{-5}
XI	5-iodo	1.10×10^{-4}	6.00×10^{-5}
XII	6-iodo	3.70×10^{-4}	1.09×10^{-4}
XIII	7-iodo	4.25×10^{-4}	4.50×10^{-5}
XIV	6-fluoro	8.50×10^{-4}	7.20×10^{-4}
XXIII	4-nitro	5.75×10^{-5}	8.10×10^{-5}
XXIV	5-nitro	3.95×10^{-4}	4.05×10^{-4}

- Continued -

Table 11. Continued

Compound Number	1,2,3-benzothiadiazole	$I_{50}M$ Aldrin epoxidation	$I_{50}M$ DHI hydroxylation
XXV	6-nitro	1.67×10^{-4}	1.20×10^{-4}
XXXXIX	7-nitro	7.40×10^{-5}	6.35×10^{-5}
XXVIII	6-cyano	1.00×10^{-4}	4.00×10^{-4}
XXXI	6-methoxy	4.15×10^{-4}	4.85×10^{-4}
XXXII	6-ethoxy	1.00×10^{-4}	1.46×10^{-4}
XXXIV	5-methyl	5.85×10^{-4}	5.85×10^{-4}
XXXV	6-methyl	4.30×10^{-4}	4.70×10^{-4}
XXXIX	5-fluoro-6-chloro	2.52×10^{-4}	3.05×10^{-4}
XXXX	5-methoxy-6-chloro	2.10×10^{-3}	6.20×10^{-4}
L	5-methyl-6-chloro	4.55×10^{-4}	6.05×10^{-5}
LI	5,6-dichloro	1.58×10^{-4}	2.23×10^{-4}
XXXXVIII	4,5,6,7-tetrachloro	2.55×10^{-5}	1.50×10^{-5}

Rat liver microsomes prepared as described in III. B. 1.

Enzyme activity and inhibition estimated as explained in III. B. 3,4.

4-iodo (X) compound with an $I_{50}M$ for aldrin epoxidation of 6.00 x 10^{-5} appears to be the best inhibitor of this series with respect to both the epoxidase and hydroxylase systems. Consequently the steric effects which in the insect preparation were associated with substituents in 4- and 7-position do not appear obvious in the inhibition of rat liver microsomes.

This is particularly clear with the mononitro compounds (XXIII, XXIV, XXV, and XXXXIX) where the 4-nitro (XXIII) and 7-nitro (XXXXIX)-1,2,3-benzothiadiazoles are better inhibitors of both enzymes than the corresponding 5- (XXIV) and 6- (XXV) nitro derivatives.

In contrast to the high inhibitory potency of the 5,6-disubstituted-1,2,3-benzothiadiazoles towards the insect preparation, these compounds were not particularly effective in inhibiting rat liver microsomes. Thus compounds L, LI and XXXX, which were some of the most active inhibitors of the insect enzymes, all had I_{50} values greater than 10^{-4} for rat liver epoxidase and were therefore in the same range as the corresponding monosubstituted derivatives. Thus in rat liver microsomes the 5-methoxy, 6-chloro (XXXX) compound is actually 4- to 8-fold less active than the 6-chloro (VI) derivative whereas in the insect preparation it exhibited a 3-fold greater activity. With respect to inhibition of epoxidation and hydroxylation the 5-methoxy,6-chloro (XXXX) compound is 159 and 105-fold more potent towards the Prodenia gut preparation than to rat liver microsomes. It is possible that in rat liver microsomes the 5-methoxy group is more rapidly metabolized than in the insect preparation although in view

of the other data a real difference between the two species might be indicated. Of the compounds evaluated this material would probably have the best chance of exhibiting some degree of selectivity. This same effect is observed with the 4,5,6,7-tetrachloro-1,2,3-benzothiadiazole(XXXXVIII) which, with an $I_{50}M$ of about 2.00×10^{-5}, is the best compound evaluated against the rat liver enzyme system and in contrast to the insect data is 6- to 15-fold more active than the 5,6-chloro (LI) derivative.

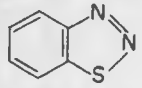
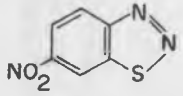
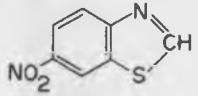
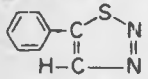
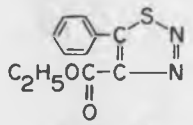
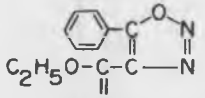
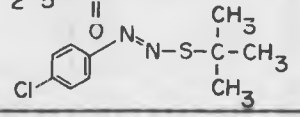
3. *In vitro* Activity of Compounds With Structure Closely Related to the 1,2,3-Benzothiadiazoles

The synergistic activity of several compounds closely related to the 1,2,3-benzothiadiazoles was discussed in section IV. A. 3 and it was concluded that the diazosulfide group played an important role in determining the activity of these compounds as carbaryl synergists with houseflies.

An investigation on the effects of these compounds on epoxidase and hydroxylase activities in both rat liver microsomes and *Prodenia* gut preparations shows that several are effective inhibitors, particularly towards the insect preparation (Table 12).

As shown in Table 12 the 6-nitro-benzothiazole (LII) exhibited no inhibitory activity towards the enzymes in either rat liver or *Prodenia* gut.

Table 12. *In vitro* inhibition[a] of aldrin epoxidation and DHI hydroxylation by compounds closely related to the 1,2,3-benzothiadiazole structure in rat liver microsomes and midgut *Prodenia eridania*[b]

Compound Number	Structure	$I_{50}M$ Aldrin epoxidation		$I_{50}M$ DHI hydroxylation	
		Rat liver	Midgut Prodenia	Rat liver	Midgut Prodenia
I		8.50×10^{-3}	1.44×10^{-4}	1.26×10^{-3}	6.10×10^{-5}
XXV		1.67×10^{-4}	1.75×10^{-4}	1.20×10^{-4}	4.80×10^{-5}
LII		$>>10^{-2}$	$>>10^{-3}$	$>>10^{-2}$	$>>10^{-3}$
LIII		4.20×10^{-4}	3.30×10^{-5}	7.40×10^{-4}	2.15×10^{-5}
LIV		5.80×10^{-4}	1.60×10^{-5}	4.45×10^{-4}	1.12×10^{-5}
LVI		1.59×10^{-4}	1.10×10^{-5}	1.15×10^{-4}	6.00×10^{-6}
LVII		---	1.70×10^{-4}	---	---

[a]Determined as described in III.B. 3,4.

[b]Prepared as explained in III.B. 1.

*For comparative purposes some 1,2,3-benzothiadiazoles are also included.

The two phenyl thiadiazoles (LIII and LIV), however, were approxiately 2- to 20-fold more active than 1,2,3-benzothiadiazole (I) depending on the source of the enzyme and the enzymatic reaction studied.

This clearly demonstrates the importance of the 1,2,3-thiadiazole ring in inhibition of the m.f.o. system and also indicates that its fusion to the aromatic ring is not of critical importance. It is of interest that 4-carboxyethyl-5-phenyl-1,2,3-oxadiazole (LVI) is slightly more active than the corresponding thiadiazole (LIV) thus indicating that the sulfur atom of the ring is not an absolute requirement for activity. Compound LVII which contains the diazosulfide group in an open chain form also showed similar inhibitory activity to 1,2,3-benzothiadiazole (Table 12) and is in agreement with the activity of this compound as a carbaryl synergist *in vivo* (IV. A. 3). This establishes that activity is not dependent on the thiadiazole ring *per se* and might indicate the - N = N- grouping as that mainly responsible for inhibitory and synergistic activity. Additional experiments will have to be carried out to prove more clearly this suggestion.

E. Regression Analysis of the *in vitro* Inhibitiory Activity of the 1,2,3-Benzothiadiazoles

The mixed-function oxidases (m.f.o.) metabolize lipid soluble foreign compounds and their selectivity appears to be related more to the hydrophobic character of their substrates than to electronic

or steric factors. Although Mazel and Henderson (1965) and Ichikawa *et al.* (1969) observed no relationship between the lipophilic character (measured by the partition coefficients) of several drugs and their rates of demethylation or hydroxylation, Gaudette and Brodie (1959), McMahon (1961), Martin and Hansch (1971) and Hansch (1972) have all provided evidence to show that lipophilic or hydrophobic character plays an important role in the oxidation of substrates by the m.f.o. system. Such reports concerning the influence of several physicochemical parameters on the interactions of foreign compounds with the m.f.o. system are not common. Since most have been concerned with mammalian liver microsomes, it was of interest to extend such studies to insects and to investigate possible relationships between various physicochemical parameters and the inhibitory activity of several 1,2,3-benzothiadiazoles towards aldrin epoxidation and DHI hydroxylation by the armyworm midgut preparation.

The theoretical considerations for regression analysis with the 1,2,3-benzothiadiazoles have already been discussed with respect to their *in vivo* synergistic activity (IV. C). These considerations also apply to the analysis of their *in vitro* activity. The general equation on which the analysis was based is shown below, where the pI_{50} is the negative log of the $I_{50}M$, k', k'', k''', k^{iv} and ρ are constants obtained by the method of least squares and E_s is the Taft's steric parameter (Taft, 1956).

$$pI_{50} = -k'\pi^2 + k''\pi + \rho\sigma + k''' E_s + k^{iv}$$

Due to the lack of appropriate substituent constants not all compounds tested as inhibitors were included in the analysis and others which will be mentioned throughout the discussion, were omitted because for one reason or another they were considered to be anomalous. These compounds were not omitted merely for the sake of improving the correlation through using fewer data points, but because they deviated substantially from other members of a given series, a phenomenon which is quite usual in most correlation studies. It was considered important to investigated these compounds carefully since they might be acting by a different mechanism or be producing some change not caused by other members of the series.

In the regression analysis several equations containing various parameters were tested. In order to compare the relative contributions of any one parameter for the same series of compounds, the F Test (Wonnacott and Wonnacott, 1970) for each equation, the t value (Wonnacott and Wonnacott, 1970) for each coefficient, and the correlation coefficient of the equations were analyzed.

The data in Table 10 clearly show that the inhibition of aldrin epoxidation and DHI hydroxylation by the 1,2,3-benzothiadiazoles in Prodenia midgut preparations was dependent upon the position of the substituent in the 1,2,3-benzothiadiazole molecule. It was suggested earlier (IV. D. 1) that the lower inhibitory activity of compounds containing substituents in the 4-, or 7-positions was due to a steric effect between the substituent and the heteroatom of the thiadiazole ring.

The involvement of a steric parameter in structure-activity relationships has been discussed by Hansch (1968a,b); Kutter and Hansch (1969), Coats et al. (1970) and Hansch and Glave (1972), and this parameter seems to be particularly important in some enzyme inhibition studies. Consequently it was decided to investigate the contribution of the steric parameter (E_s) on the inhibitory activity of 4-, 5- and 6-monosubstituted 1,2,3-benzothiadiazoles towards both the epoxidation and hydroxylation reactions. Unfortunately the 7-substituted compounds could not be included in the analysis due to the lack of appropriate constants.

The influence of the steric parameter on the in vitro inhibitory activity of 4- substituted-1,2,3-benzothiadiazoles is clearly illustrated by Equation 30 which is derived from the data in Table 13 for the inhibition of the hydroxylation reaction. The F value for each equation is indicated in parenthesis beneath each equation.

pI_{50}	n	r	s	Eq.
$pI_{50}D = -0.326\ E_s + 3.706$ $F(1,3) = 1.94$	5	0.628	0.346	(28)
$pI_{50}D = 0.429\pi + 3.586$ $F(1,3) = 2.16$	5	0.647	0.339	(29)
$pI_{50}D = 0.509\pi - 0.390E_s + 3.528$ $F(2,2) = 28.34$	5	0.983	0.100	(30)
$pI_{50}D = 0.433\pi - 0.688\sigma + 3.426$ $F(2,2) = 6.12$	5	0.927	0.204	(31)

Table 13. Physical constants used in the regression analysis for 4-substituted-1,2,3-benzothiadiazoles

Compound Number	Substituent	π[a]	σ[b]	$\sigma^{\cdot}$[c]	E_s[d]	$pI_{50}A$[e]	$pI_{50}D$[f]
II	4-chloro	0.59	0.227	0.03	0.27	3.79	3.69
VI	4-bromo	0.75	0.232	0.11	0.08	3.49	3.79
X	4-iodo	0.92	0.180	----	-0.16	4.10	4.16
XXIII	4-nitro	-0.23	0.778	0.47	-1.28	3.68	3.90
XXXI	4-methoxy	-0.33	-0.268	0.40	0.69	3.00	3.12

[a]From Fujita *et al.* (1964). π values for 2-substituted phenoxyacetic acid.

[b]From Jaffe (1953) values for σ_p.

[c]From Hansch (1968b).

[d]Values derived from Kutter and Hansch (1969).

[e,f]From Table 10.

As can be seen from Equation 28 the steric parameter (E_s) by itself accounts for about 63% of the variance, and according to Equation 29 the hydrophobic parameter (π) accounts for 65%. Thus neither of these parameters alone are able to satisfactorily account for the activity of the 4-substituted-1,2,3-benzothiadiazoles. When both parameters are combined, however, as in Equation 30, 98% of the variance of the data is accounted for. Combinations of π and σ, or $\sigma^{\cdot}$ and E_s did not result in any significant improvement over Equation 30, thus establishing that electronic effects play only a minor role in determining the inhibitory activity.

The positive sign for π in Equation 30 indicates that increasing the lipophilic character of the substituent increases inhibitory potency, although this is also dependent on the steric parameter. The negative constant associated with the steric factor E_s, suggests that inhibitory activity is enhanced by increasing steric bulk. Similar results were also obtained following analysis of the data for the inhibition of aldrin epoxidation although in this case the best equation (32) accounts for only 90% of the variance. The F test, however, was highly significant for this equation. In considering the inhibition of both enzymatic reactions by 5- or 6-monosubstituted-

	n	r	s	Eq.
$pI_{50}^{A} = 0.550\pi - 0.320\ E_s + 3.400$	5	0.896	0.257	(32)
$F(2,2) = 4.04$				

1,2,3-benzothiadiazoles, the steric factor E_s seems to be less important than for the 4-substituted compounds. This is clearly shown in equations 33 to 35 which demonstrate that the inhibition of

	n	r	s	Eq.
$pI_{50}^{D} = -2.061\ E_s + 5.223$	5	0.363	0.672	(33)
$F(1,3) = 0.45$				

DHI hydroxylation by the 6-substituted-1,2,3-benzothiadiazoles, is dependent mainly on π. Similar results were obtained for the

	n	r	s	Eq.
$pI_{50}^{D} = 1.393\pi + 3.996$	5	0.943	0.313	(34)
$F(1,3) = 15.10$				
$pI_{50}^{D} = 1.287\pi - 1.425\ E_s + 4.236$	5	0.898	0.388	(35)
$F(2,2) = 4.17$				

inhibition of the epoxidation reaction by the 5-substituted-1,2,3-benzothiadiazoles.

As has been previously discussed (IV. B) regression studies on the biological activity of the 1,2,3-benzothiadiazoles have been designed mainly to clarify the role of the various physicochemical parameters associated with substituents at the 5-, 6- or 5,6-positions of the ring. In order to do this it was first necessary to investigate whether the correlations for 5-substituted compounds were similar to those for the 6-substituted compounds, since it is possible that the biological activity of these two groups might depend on different physicochemical parameters. Few examples of this have been reported in the literature, although Coats *et al.* (1970) found that for the inhibition of thymidine phosphorylase by a series of 1- and 6-substituted uracils the hydrophobic parameter π accounted for most of the variance with the former and the steric constant (E_s) played a major role in determining the activity of the latter. Similarly Hansch and Glave (1972) have shown that for the inhibition of the enzyme phenethanolamine *N*-methyl transferase by several ring-substituted amphetamines, the activity of the *m*-isomers was best correlated by an equation containing π and σ, whereas with the *p*-isomers σ alone accounted for most of the variance.

Several equations containing all possible combinations of π, E_s, σ, and σ^- were tested to investigate the effect of various physicochemical parameters on the inhibition of aldrin epoxidase and DHI hydroxylase by 5-substituted-1,2,3-benzothiadiazoles. The same

equations were also employed for analysis of the inhibition of both enzymatic reactions by the 6-substituted-1,2,3-benzothiadiazoles. For the 5- and 6-substituted compounds respectively the best correlations for the inhibition of aldrin epoxidation were obtained with equations 36 and 37. This clearly establishes that the inhibitory

	n	r	s	Eq.
$pI_{50}A = -0.198\pi^2 + 0.952\pi - 0.040\sigma + 3.774$	6	0.984	0.183	(36)
$F(3,2) = 20.99$				
$pI_{50}A = -0.214\pi^2 + 0.710\pi - 0.048\sigma + 3.910$	6	0.986	0.143	(37)
$F(3,2) = 23.26$				

activities of both 5- and 6-substituted compounds depend on the same physicochemical parameters. The similarities of the constants and the coefficients for π^2, π, and σ in each equation indicate that 5-, 6- and 5,6-derivatives can be analyzed by a single equation.

As in the analysis of the *in vivo* synergistic activity of the 1,2,3-benzothiadiazoles two series of hydrophobic constants were employed in analyzing the inhibitory activity of the 1,2,3-benzothiadiazoles towards the epoxidase and hydroxylase. Thus π derived from phenoxyacetic acid (Fujita *et al.*, 1964) and π_B obtained from 2H-1,2,4-benzothiadiazine 1,1-dioxide system (Topliss and Yudis, 1972) were tested for 16 compounds (Table 14 and 15) for which both sets of constants were available. Several equations were tested including those containing π and π_B alone and in combination with other parameters.

Table 14. Observed and calculated activity of 1,2,3-benzothiadiazoles as inhibitors of midgut Prodenia eridania aldrin epoxidase

Compound Number	Substituent	$\Sigma\pi$[a]	$\Sigma\pi_B$[b]	$\Sigma\sigma$[c]	$\Sigma\sigma^\cdot$[d]	ΣE_s[e]	Aldrin epoxidase Obser.[f] pI_{50}	Aldrin epoxidase Calcd.[g] pI_{50}	ΔpI_{50}
I	None	0.00	0.00	0.00	0.00	----	3.84	3.83	0.01
III	5-chloro	0.76	0.89	0.373	0.03	0.27	4.26	4.39	0.13
IV	6-chloro	0.70	0.91	0.227	0.03	0.27	4.35	4.38	0.03
VII	5-bromo	0.94	1.08	0.391	0.11	0.08	4.58	4.59	0.01
VIII	6-bromo	1.02	1.08	0.232	0.11	0.08	4.60	4.76	0.16
XI	5-iodo	1.15	----	0.352	----	-0.16	5.08	4.88	0.20
XII	6-iodo	1.26	1.32	0.180	----	-0.16	5.16	5.11	0.05
XIV	6-fluoro	0.15	0.33	0.337	----	0.78	3.68	3.92	0.24
XVI	5-hydroxy	-0.49	----	0.121	----	----	3.24	3.46	0.22
XVII	5-hydroxy	-0.61	----	-0.370	----	----	3.72	3.63	0.09
XXIV	5-nitro	0.11	0.22	0.710	0.47	0.23	3.66	3.60	0.06
XXV	6-nitro	0.24	0.36	0.778	0.47	0.23	3.76	3.68	0.08
XXXIV	5-methyl	0.51	0.45	-0.069	0.09	-0.144	4.39	4.32	0.07
XXXV	6-methyl	0.52	0.52[h]	-0.170	0.09	-0.144	4.49	4.37	0.12
XXXVI	5,6-dimethyl	1.03	0.97	-0.239	0.18	----	4.64	4.99	0.35

- Continued -

Table 14. Continued

Compound Number	Substituent	$\Sigma\pi^a$	$\Sigma\pi_B{}^b$	$\Sigma\sigma^c$	$\Sigma\sigma^d$	$\Sigma E_s{}^e$	Aldrin epoxidase Obser.[f] pI_{50}	Aldrin epoxidase Calcd.[g] pI_{50}	ΔpI_{50}
XXXI	6-methoxy	-0.04	0.27	-0.268	0.40	0.69	4.19	3.93	0.26
XXXX	5-methoxy-6-chloro	0.82	----	0.342	0.43	----	4.88	4.47	0.41
XXXIX	5-fluoro-6-chloro	0.83	----	0.564	----	----	4.05	4.38	0.33
L	5-methyl-6-chloro	1.21	----	0.158	0.12	----	5.14	5.05	0.09
LI	5,6-dichloro	1.46	----	0.454	0.06	----	5.24	5.20	0.04

[a]Derived from the phenoxyacetic acid system (Fujita et al., 1964). π_m for 5-position and π_p for 6-position.

[b]Derived from 2H-1,2,4-benzothiadiazine 1,1-dioxide system (Topliss and Yudis, 1972). The 6- and 7-position in this system was considered equivalent to the 5- and 6-position respectively in the 1,2,3-benzothiadiazole.

[c]From Jaffe, H. (1953).

[d]From Hansch (1968b).

[e]From Kutter and Hansch (1969).

[f]From Table 10.

[g]Calculated using Equation 63.

[h]Value for the 6-position of the 2 H-1,2,4-benzothiadiazine 1,1-dioxide system.

Table 15. Observed and calculated activity of 1,2,3-benzothiadiazoles as inhibitors of midgut *Prodenia eridania* DHI hydroxylase

Compound Number	Substituent	$\Sigma\pi$[a]	$\Sigma\pi_B$[b]	$\Sigma\sigma$[c]	$\Sigma\sigma^\bullet$[d]	ΣE_s[e]	DHI hydroxylase Obser.[f] pI_{50}	DHI hydroxylase Calcd.[g] pI_{50}	ΔpI_{50}
I	None	0.00	0.00	0.00	0.00	----	4.22	4.20	0.02
III	5-chloro	0.76	0.89	0.373	0.03	0.27	4.46	4.99	0.53
IV	6-chloro	0.70	0.91	0.227	0.03	0.27	5.24	4.96	0.28
VII	5-bromo	0.94	1.08	0.391	0.11	0.08	5.31	5.25	0.06
VIII	6-bromo	1.02	1.08	0.232	0.11	0.08	5.55	5.43	0.12
XI	5-iodo	1.15	----	0.352	----	-0.16	5.79	5.59	0.20
XXII	6-iodo	1.26	1.32	0.180	----	-0.16	6.03	5.83	0.20
XIV	6-fluoro	0.15	0.33	0.337	----	0.78	4.00	4.33	0.33
XVI	5-hydroxy	-0.49	----	0.121	----	----	3.35	3.72	0.37
XVII	5-hydroxy	-0.61	----	-0.370	----	----	3.90	3.79	0.11
XXIV	5-nitro	0.11	0.22	0.710	0.47	0.23	4.28	4.07	0.21
XXV	6-nitro	0.24	0.36	0.778	0.47	0.23	4.32	4.19	0.13
XXXIV	5-methyl	0.51	0.45	-0.069	0.09	-0.144	4.75	4.81	0.06
XXXV	6-methyl	0.52	0.52[h]	-0.170	0.09	-0.144	4.78	4.85	0.07
XXXVI	5,6-dimethyl	1.03	0.97	-0.239	0.18	----	5.09	5.60	0.51

- Continued -

Table 15. Continued

Compound Number	Substitutent	$\Sigma\pi$[a]	$\Sigma\pi_B$[b]	$\Sigma\sigma$[c]	$\Sigma\sigma'$[d]	ΣE_s[e]	DHI hydroxylase Obser.[f] pI_{50}	DHI hydroxylase Calcd.[g] pI_{50}	ΔpI_{50}
XXXI	6-methoxy	-0.04	0.27	-0.268	0.40	0.69	4.77	4.25	0.52
XXXX	5-methoxy-6-chloro	0.82	----	0.342	0.43	----	5.23	5.09	0.14
XXXIX	5-fluoro-6-chloro	0.83	----	0.564	----	----	4.75	5.03	0.28
L	5-methyl-6-chloro	1.21	----	0.158	0.12	----	6.10	5.75	0.35
LI	5,6-dichloro	1.46	----	0.454	0.06	----	5.85	6.03	0.18

[a] Derived from the phenoxyacetic acid system (Fujita et al., 1964). π_m for 5-position and π_p for 6-position.

[b] Derived from 2H-1,2,4-benzothiadiazine 1,1-dioxide system (Topliss and Yudis, 1972). The 6- and 7-position in this system was considered equivalent to the 5- and 6-position respectively in the 1,2,3-benzothiadiazole.

[c] From Jaffe, H. (1953).

[d] From Hansch (1968b).

[e] From Kutter and Hansch (1969).

[f] From Table 10.

[g] Calculated using Equation 68.

[h] Value for the 6-position of the 2 H-1,2,4-benzothiadiazine 1,1-dioxide system.

The best correlations with respect to the inhibition of aldrin epoxidation were obtained with equations 38 and 39. Equations 40 and 41 were best for DHI hydroxylation. As was demonstrated in the *in vivo*

	n	r	s	Eq.
$pI_{50}A = -0.263\pi^2 + 0.732\pi - 0.027\sigma + 3.803$	16	0.960	0.181	(38)
$F(3,12) = 47.56$				
$pI_{50}A = -0.103\pi_B^2 + 0.848\pi_B - 0.493\sigma + 3.760$	16	0.964	0.172	(39)
$F(3,12) = 53.11$				
$pI_{50}D = -0.301\pi^2 + 0.969\pi - 0.072\sigma + 4.127$	16	0.946	0.279	(40)
$F(3,12) = 34.01$				
$pI_{50}D = -0.023\pi_B^2 + 1.213\pi_B - 0.327\sigma + 4.061$	16	0.932	0.810	(41)
$F(3,12) = 26.84$				

studies these equations clearly show that either π or π_B can be used in correlation studies with the 1,2,3-benzothiadiazoles. Since there are more values of π available in the literature these were used for the remainder of this investigation.

Regression analysis of the *in vivo* activity of the 1,2,3-benzothiadiazoles as carbaryl synergists (IV. C) indicated the importance of the free radical constant $\sigma^{\cdot}$. It was therefore considered likely that this parameter might be of importance in determining the *in vitro* inhibitory activity of the 1,2,3-benzothiadiazoles in aldrin epoxidation and DHI hydroxylation. This possibility was investigated with 14 compounds (Table 14 and 15) for which $\sigma^{\cdot}$ constants were available and which included 1,2,3-benzothiadiazoles substituted in the 5-, 6- and 5,6-positions of the ring. The results are shown in equations 42 and

43, which clearly indicate that for the inhibition of epoxidation and hydroxylation respectively the correlations in terms of π^2, π and σ^*

	n	r	s	Eq.
$pI_{50}{}^{A} = -0.281\pi^2 + 0.561\pi + 0.059\sigma^* + 3.863$	14	0.894	0.243	(42)
$t = (0.851) \quad (1.221) \quad (0.139) \quad (20.56)$				
$F(3,10) = 13.34$				
$pI_{50}{}^{D} = -0.509\pi^2 + 0.467\pi + 0.212\sigma^* + 4.243$	14	0.877	0.322	(43)
$t = (1.165) \quad (0.767) \quad (0.377) \quad (17.32)$				
$F(3,10) = 11.12$				

are not as satisfactory as those based on π^2, π and σ which are shown in equations 44 and 45. A comparison between equations 42 and 44 clearly

	n	r	s	Eq.
$pI_{50}{}^{A} = -0.347\pi^2 + 0.502\pi - 0.385\sigma + 3.960$	14	0.934	0.193	(44)
$t = (1.323) \quad (1.407) \quad (2.414) \quad (35.07)$				
$F(3,10) = 23.00$				
$pI_{50}{}^{D} = -0.255\pi^2 + 1.021\pi - 0.338\sigma + 4.197$	14	0.925	0.314	(45)
$t = (1.364) \quad (0.680) \quad (1.108) \quad (24.82)$				
$F(3,10) = 31.68$				

shows that the inhibitory activity of 1,2,3-benzothiadiazoles on aldrin epoxidation is depending on the hydrophobic character (π) and the electronic factor (σ). The F tests for both equations 42 and 44 were satisfactory at the 95% confidence level (critical value $F_{3,10} = 3.71$), so it was necessary to consider the t values and the correlation coefficient to demonstrate that equation 44 was more significant than 42. The t value for the σ^* coefficient in equation 42 was only 0.139 which is much lower than the critical value (2.228) and means that according to the null hypothesis the coefficient is not statistically different from zero at the 95% confidence level. In contrast the t value for the σ coefficient (2.41 eq. 44) is statistically acceptable

at the 95% confidence level. Additional arguments for accepting equation 44 over 42 were found by comparing the correlation coefficients which for equations 42 and 44 were 0.894 and 0.934 respectively.

The same approach was applied to analyzing equations 43 and 45 but in this case equation 45 proved to be better than 43 only on the basis of the correlation coefficients which were 0.925 and 0.877 respectively. Thus the significance of σ over σ' was statistically demonstrated.

Equations 38, 39, 40 and 41 demonstrated that the inhibitory activity of the 1,2,3-benzothiadiazoles towards both enzymatic systems is highly correlated with hydrophobic and electronic parameters. Since equations 36 and 37 also established that this was true for both 5- and 6-substituted derivatives, a single regression analysis was carried out with 23 compounds for which substituent constants were available. These compounds were the 5-cyano (XXVII), the 6-cyano (XXVIII) and the 6-ethoxy (XXXII) 1,2,3-benzothiadiazoles plus the other 20 compounds listed in Tables 14 and 15. Equations 46 to 49 were obtained for the inhibition of aldrin epoxidation by these compounds, and although the equations for DHI hydroxylation showed a similar pattern the best of these is shown by equation 51.

	n	r	s	Eq.
$pI_{50}A = -0.733\pi + 4.021$	23	0.758	0.386	(46)
$F(1,21) = 28.44$				
$pI_{50}A = -0.255\pi^2 + 0.522\pi + 3.975$	23	0.733	0.396	(47)
$F(2,20) = 14.79$				
$pI_{50}A = -0.069\sigma + 4.400$	23	0.141	0.592	(48)
$F(1,21) = 0.035$				
$pI_{50}A = 0.752\pi - 0.250\sigma + 4.065$	23	0.772	0.385	(49)
$F(2,20) = 14.77$				

	n	r	s	Eq.
$pI_{50}A = -0.260\pi^2 + 0.537\pi - 0.254\sigma + 4.019$	23	0.786	0.384	(50)
$F(3,19) = 10.27$				
$pI_{50}D = -0.294\pi^2 + 0.725\pi - 0.069\sigma + 4.405$	23	0.803	0.466	(51)
$F(3,19) = 11.56$				

A considerable improvement in the correlation was achieved by exclusion of the 5-cyano (XXVII) and 6-cyano (XXVIII) compounds as shown by equations 52 to 56 for the epoxidation reaction. For DHI hydroxylation the best correlations were achieved using equations 57 and 58. Since compounds containing the cyano group appear to deviate from the other members of the series, it is possible that another effect, perhaps one involving resonance, might explain the inhibitory properties of these compounds.

	n	r	s	Eq.
$pI_{50}A = 0.877\pi + 3.889$	21	0.828	0.345	(52)
$F(1,19) = 41.66$				
$pI_{50}A = -0.157\pi^2 + 0.739\pi + 3.867$	21	0.833	0.350	(53)
$F(2,18) = 20.48$				
$pI_{50}A = -0.064\sigma + 4.404$	21	0.156	0.617	(54)
$F(1,19) = 0.02$				
$pI_{50}A = 1.012\pi - 0.667\sigma + 3.929$	21	0.898	0.280	(55)
$F(2,18) = 37.29$				
$pI_{50}A = -0.110\pi^2 + 0.914\pi - 0.658\sigma + 3.914$	21	0.900	0.285	(56)
$F(3,17) = 24.07$				
$pI_{50}D = 1.276\pi - 0.543\sigma + 4.292$	21	0.898	0.352	(57)
$F(2,18) = 37.75$				
$pI_{50}D = -0.120\pi^2 + 1.169\pi - 0.533\sigma + 4.275$	21	0.900	0.360	(58)
$F(3,17) = 24.21$				

It was clear from these results that the 6-ethoxy (XXXII) compound was not well correlated since its activity was considerably higher than expected. Equations 59-63 and 64-67 show the regression analysis for the 20 compounds (compound XXII omitted) listed in Tables 14 and 15 as <u>in vitro</u> inhibitors of aldrin expoxidation and DHI hydroxylation respectively.

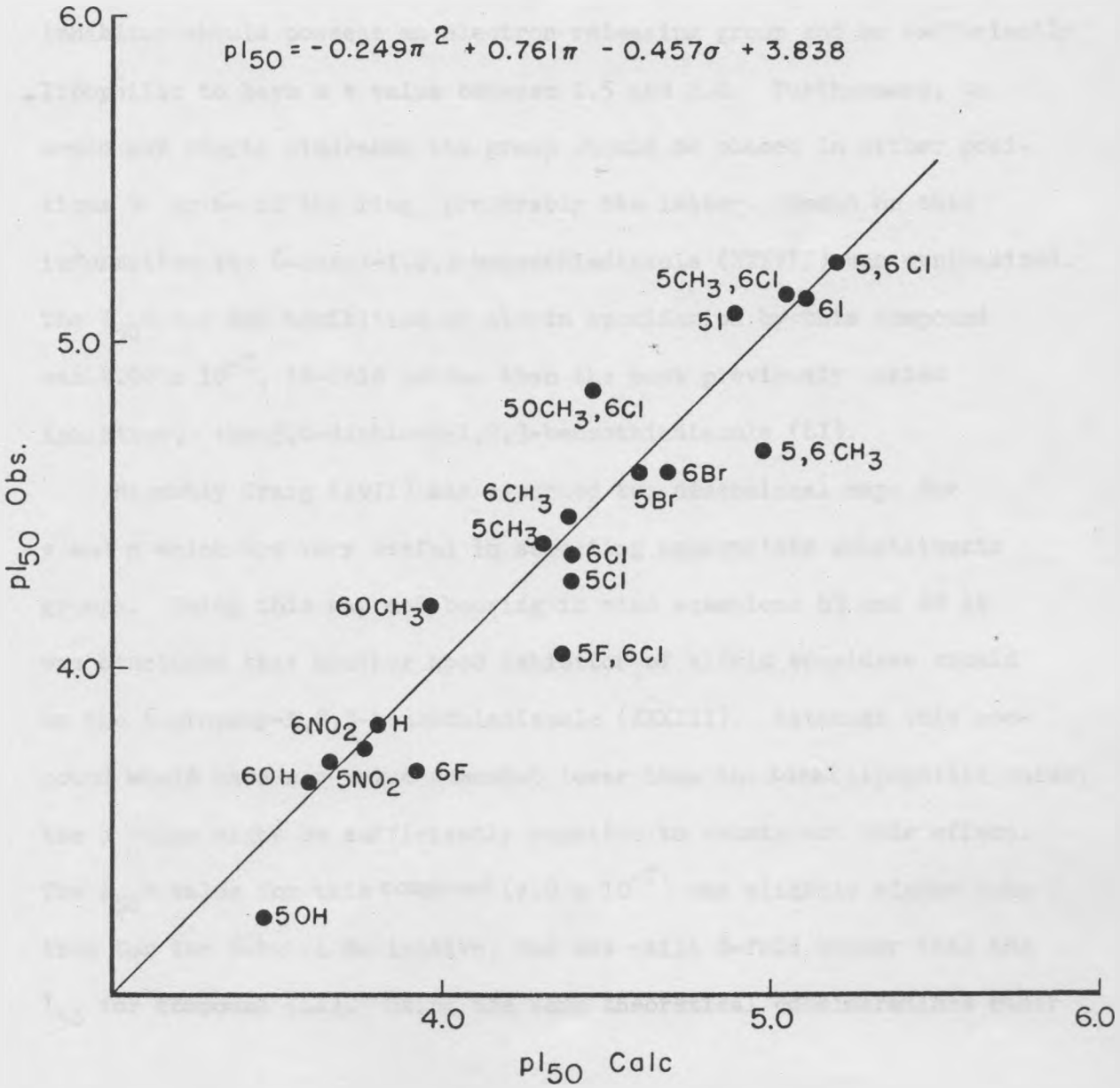
	n	r	s	Eq.
$pI_{50}A = 0.888\pi + 3.834$	20	0.893	0.268	(59)
$F(1,18) = 71.03$				
$pI_{50}A = +0.312\pi^2 + 0.617\pi + 3.786$	20	0.912	0.252	(60)
$F(2,17) = 42.00$				
$pI_{50}A = 0.126\sigma + 4.322$	20	0.222	0.594	(61)
$F(1,18) = 0.090$				
$pI_{50}A = 0.987\pi - 0.501\sigma + 3.876$	20	0.931	0.223	(62)
$F(2,17) = 55.30$				
$pI_{50}A = +0.249\pi^2 + 0.761\pi - 0.457\sigma + 3.838$	20	0.942	0.212	(63)
$F(3,16) = 42.25$				
$pI_{50}D = 1.177\pi + 4.208$	20	0.904	0.331	(64)
$F(1,18) = 81.68$				
$pI_{50}D = +0.301\pi^2 + 0.914\pi + 4.161$	20	0.915	0.323	(65)
$F(2,17) = 43.94$				
$pI_{50}D = 0.413\sigma + 4.806$	20	0.173	0.767	(66)
$F(1,18) = 0.58$				
$pI_{50}D = 1.252\pi - 0.382\sigma + 4.240$	20	0.918	0.318	(67)
$F(2,17) = 45.62$				
$pI_{50}D = +0.255\pi^2 + 1.021\pi - 0.338\sigma + 4.197$	20	0.925	0.314	(68)
$F(3,16) = 31.68$				

Tables 14 and 15 show the pI_{50} values calculated from equations 63 and 68 for aldrin epoxidation and DHI hydroxylation respectively. It can be seen that there is excellent agreement between the observed and calculated values and this is clearly shown in Fig. 8, where the pI_{50} values observed for aldrin epoxidation are plotted against those calculated from equation 63.

Equations 63 and 67 include the π^2 term. Although several investigators have argued that the meaning of this parameter is dubious, the F test for the equations and the t values for the π^2 coefficients in equations 63 and 68 all clearly demonstrate that the π^2 term is statistically significant at better than the 95% level. It was anticipated that a parabolic relationship might exist with these compounds and that since some might be more lipophilic than the ideal value they would tend to be restricted in their movement to the active site due to lipid-lipid interactions. Similarly other less lipophilic compounds will be restricted by interactions with water. It is also possible that certain substituent groups might be attacked by the mixed-function oxidases and this would further affect the linearity of the relationship. As seen by equations 63 and 68 a negative coefficient is associated with the square term (π^2). This is logical since a positive coefficient would indicate that an infinitely lipophilic compound would have infinitely high activity and would therefore be meaningless. The negative coefficient for σ suggests that inhibition is increased by electron releasing groups.

The ideal hydrophobic charater (π_o) with respect to the *in vitro* inhibition of epoxidation and hydroxylation by the 1,2,3-benzothiadiazoles

FIGURE 8. RELATIONSHIP BETWEEN pI_{50} OBSERVED AND CALCULATED FOR THE INHIBITION OF ALDRIN EPOXIDATION BY 1,2,3-BENZOTHIADIAZOLES



can be readily calculated from equations 63 and 68 by taking the partial derivative $\frac{\partial pI_{50}}{\partial \pi}$ and setting it equal to zero. The values calculated in this way were 1.52 and 2.01 for aldrin epoxidation and DHI hydroxylation respectively.

To test the validity of this kind of analysis it was decided to synthesize a compound which could be predicted as a good inhibitor of aldrin epoxidase. From equation 63 it was concluded that the ideal inhibitor should possess an electron releasing group and be sufficiently lipophilic to have a π value between 1.5 and 2.0. Furthermore, to avoid any steric hindrance the group should be placed in either positions 5- or 6- of the ring, preferably the latter. Based on this information the 6-butyl-1,2,3-benzothiadiazole (XXXVII) was synthesized. The $I_{50}M$ for the inhibition of aldrin epoxidation by this compound was 4.90×10^{-7}, 12-fold better than the best previously tested inhibitor, the 5,6-dichloro-1,2,3-benzothiadiazole (LI).

Recently Craig (1971) has reported two dimensional maps for π and σ which are very useful in selecting appropriate substituents groups. Using this map and bearing in mind equations 63 and 68 it was concluded that another good inhibitor of aldrin epoxidase should be the 6-propoxy-1,2,3-benzothiadiazole (XXXIII). Although this compound would have a π value somewhat lower than the ideal lipophilic value, the σ value might be sufficiently negative to counteract this effect. The $I_{50}M$ value for this compound (7.0×10^{-7}) was slightly higher than that for the 6-butyl derivative, but was still 8-fold better than the I_{50} for compound (LI). Using the same theoretical considerations other

good inhibitors should be those containing groups such as ethyl, propyl, allyl, etc., in positions 5-, 6- or 5,6-. The success achieved in predicting the inhibitory activity of these materials clearly shows the usefulness of this approach. This can only be achieved, however, after a careful analysis of the substituents and substituent constants has been done. Thus one should try to avoid premature assumptions resulting from the use of data obtained from series of compounds with inadequate ranges of constants, or from comparisons between constants which are closely related to one another (e.g., π and molecular volume or parachor, or σ constants and dipole moments).

The results reported here also demonstrate that structure-activity analysis using free energy related constants might provide information concerning the mechanism of action of insecticide synergists and can allow the convenient storage of large amounts of information (equations). In certain cases the method also permits predictions to be made regarding the activity of some compounds not yet synthesized.

F. Relationship Between *in vivo* and *in vitro* Activity of the 1,2,3-Benzothiadiazoles

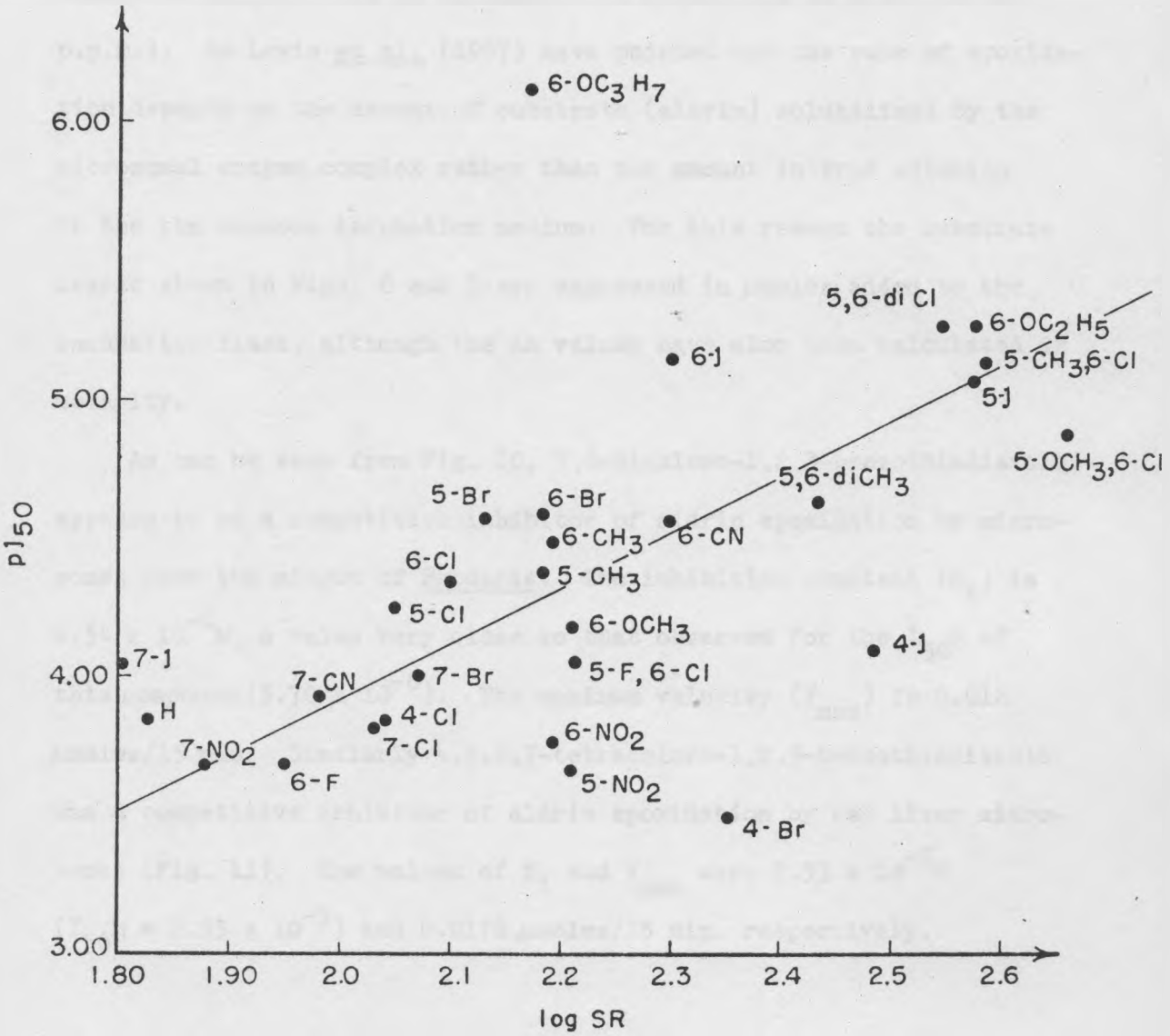
The activity of most synergists, including the 1,2,3-benzothiadizoles, in enhancing the toxicity of carbaryl to houseflies has been explained on the basis of their inhibition of the microsomal enzymes which are primarily concerned with carbaryl detoxification.

If the microsomal enzymes in the midgut of Prodenia eridania are similar to those in the housefly, and if aldrin epoxidase can be considered a model for the microsomal enzymes responsible for carbaryl metabolism some relationship might be expected between the in vivo synergistic activity of the 1,2,3-benzothiadiazoles with carbaryl (Table 7) and their in vitro inhibition of microsomal epoxidation (Table 10).

Thus for most of the compounds investigated this relationship exists and is shown by Fig. 9 where the synergistic ratios of the 1,2,3-benzothiadiazoles with carbaryl in houseflies is plotted against their pI_{50} values for aldrin epoxidase from Prodenia midgut. Because many of the compounds exhibiting synergistic ratios of less than about 60 appear to deviate from this correlation they have been omitted from Fig. 9. The reasons for this deviation are not clear but are probably associated with such factors as penetration and/or metabolism. This is particularly marked in the compounds containing amino and hydroxy groups.

These data demonstrate for the first time a direct relationship between the in vivo and in vitro activity of a series of insecticide synergists and suggest that the potential activity of the 1,2,3-benzothiadiazoles is not modified greatly by such factors as penetration, distribution or metabolism in vivo.

FIGURE 9. RELATIONSHIP BETWEEN IN VIVO AND IN VITRO ACTIVITY OF THE 1,2,3-BENZOTHIADIAZOLES

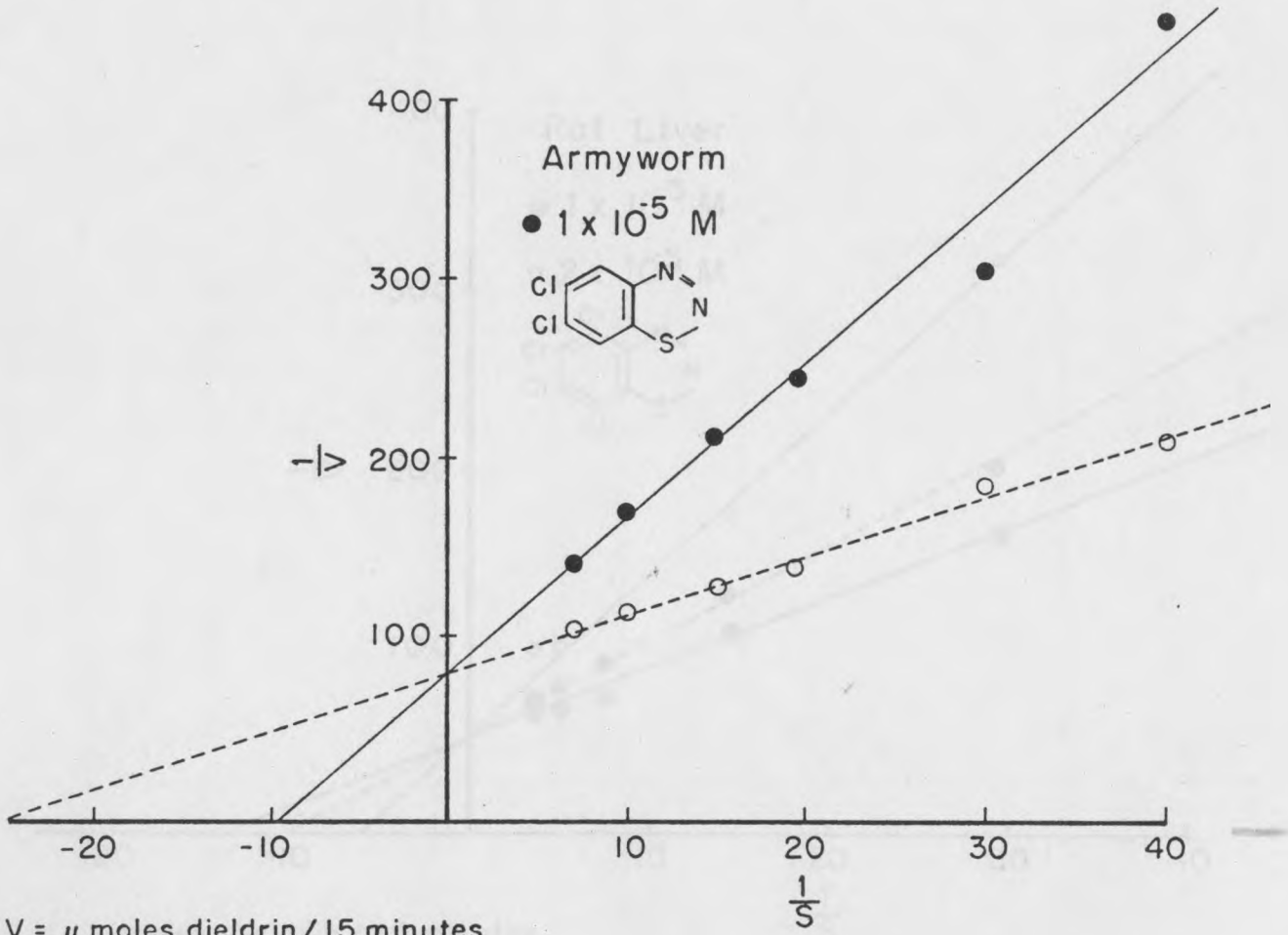


G. Kinetics of Inhibition of Aldrin Epoxidation by 1,2,3-Benzothiadiazoles

Interpretation of the results of kinetic studies with liposoluble substrates or inhibitors and membrane bound enzymes is difficult because of the complex nature of the partitioning and binding processes occurring between the substrate, inhibitor and the enzyme. This is particularly true with the m.f.o. system and in this investigation, the substrate (aldrin) has an extremely low solubility in water (0.001 p.p.m.). As Lewis *et al.* (1967) have pointed out the rate of epoxidation depends on the amount of substrate (aldrin) solubilized by the microsomal enzyme complex rather than the amount in true solution in the the aqueous incubation medium. For this reason the substrate levels shown in Figs. 6 and 7 are expressed in μmoles added to the incubation flask, although the Km values have also been calculated in molarity.

As can be seen from Fig. 10, 5,6-dichloro-1,2,3-benzothiadiazole appears to be a competitive inhibitor of aldrin epoxidation by microsomes from the midgut of *Prodenia*. The inhibition constant (K_i) is 6.54×10^{-6}M, a value very close to that observed for the I_{50}M of this compound (5.70×10^{-6}). The maximum velocity (V_{max}) is 0.012 μmoles/15 min. Similarly 4,5,6,7-tetrachloro-1,2,3-benzothiadiazole was a competitive inhibitor of aldrin epoxidation by rat liver microsomes (Fig. 11). The values of K_i and V_{max} were 2.53×10^{-5}M ($I_{50}M = 2.55 \times 10^{-5}$) and 0.0178 μmoles/15 min. respectively.

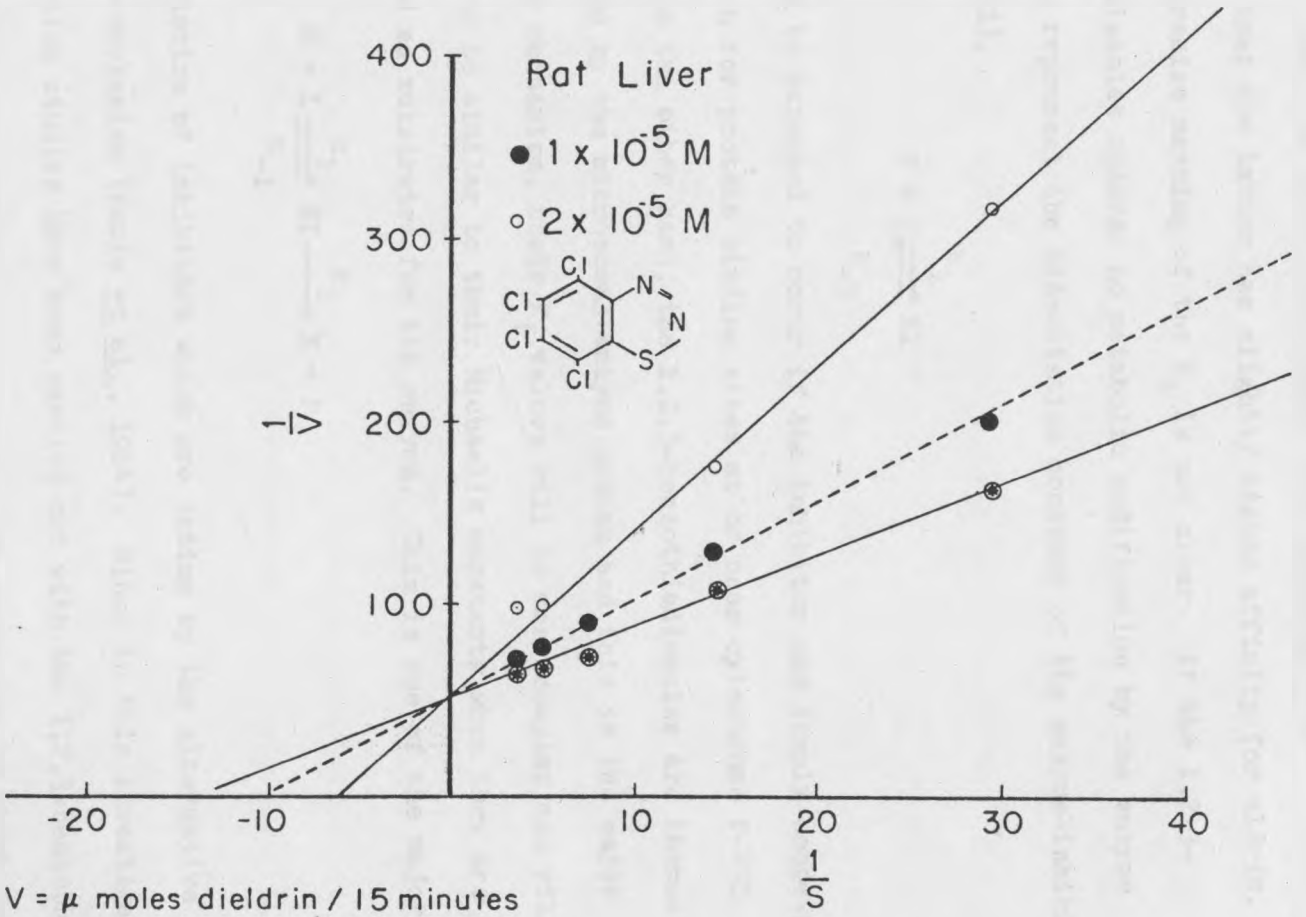
FIGURE 10. KINETICS OF INHIBITION OF ALDRIN EPOXIDATION BY 5,6-DICHLORO-1,2,3-BENZOTHIADIAZOLE IN MIDGUT MICROSOMES FROM PRODENIA ERIDANIA



V = μ moles dieldrin / 15 minutes

S = μ moles aldrin per incubation

FIGURE 11. KINETICS OF INHIBITION OF ALDRIN EPOXIDATION BY 4,5,6,7-TETRACHLORO-1,2,3-BENZOTHIADIAZOLE IN RAT LIVER MICROSOMES



V = μ moles dieldrin / 15 minutes

S = μ moles aldrin per incubation

The Michaelis constant for the aldrin epoxidase of rat liver microsomes is 0.066 μmoles or 1.40×10^{-5}M compared with values of 0.038 μmoles or 9.10×10^{-6}M for the enzyme from Prodenia midgut. This indicates that the latter has slightly higher affinity for aldrin.

The precise meaning of the K_i is not clear. If the 1,2,3-benzothiadiazoles undergo no metabolic modification by the enzyme the K_i may represent the dissociation constant of the enzyme-inhibitor complex (EI).

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EI$$

This would be expected to occur if the inhibitor was simply competing with aldrin for protein binding sites at or near cytochrome P-450.

If, on the other hand, the 1,2,3-benzothiadiazoles are themselves metabolized by the microsomal enzyme system and this is the major inhibitory mechanism, their K_i values will be more complex and will be expected to be similar to their Michaelis constants when they are considered as substrates for the enzyme. This is one of the major

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EI \xrightarrow{k_2} E + P$$

characteristics of inhibitors which are acting by the alternative substrate mechanism (Rubin et al., 1964). Since in this investigation no metabolism studies have been carried out with the 1,2,3-benzothiadiazoles it is not possible to determine the mechanism by which inhibition occurs.

To be effective alternative substrate inhibitors, the 1,2,3-benzothiadiazoles should have a higher affinity (lower K_m) than aldrin for the enzyme. These results would indicate (assuming $K_i = K_m$) that the insect enzyme has a higher affinity for 5,6-dichloro-1,2,3-benzothiadiazole ($K_i = 6.54 \times 10^{-6}$M) than it does for aldrin ($K_m = 9.10 \times 10^{-6}$M). The rat liver enzyme, however, has a slightly higher affinity for aldrin ($K_m = 1.40 \times 10^{-5}$M) than for the inhibitor 4,5,6,7-tetrachloro-1,2,3-benzothiadiazole ($K_i = K_m = 2.53 \times 10^{-5}$M). A further enhancement of inhibitory potency will occur if the 1,2,3-benzothiadizoles have a lower metabolic turnover than aldrin since under these conditions they will remain at the active site for a longer period of time.

H. Interaction of 1,2,3-Benzothiadiazoles With Components of the Microsomal Electron Transport Chain

As was discussed earlier (II. C) the microsomal electron transport chain transfers electrons or reducing equivalents from NADPH to cyt P-450 and although many details of this pathway remain unkown, it is considered by some that NADPH cyt P-450 reductase is rate limiting in transferring reducing equivalents from NADPH to cyt P-450 (Gillette and Gram, 1969). Although not yet fully established it has been suggested that NADPH cytochrome P-450 reductase is identical to (Remmer, 1971) or similar to (Mannering, 1971) the flavoprotein NADPH cyt c reductase. This is supported by the results of Masters *et al.* (1971) who have shown that antibodies prepared from porcine NADPH-cyt c

reductase inhibits both microsomal NADPH cyt c reductase and NADPH-cyt P-450 reductase as well as drug metabolism.

To investigate the effect of the 1,2,3-benzothiadiazoles on the NADPH cyt c reductase from armyworm midgut microsomes some of the best in vitro inhibitors of the m.f.o. (IV. D. 1,2) were selected. The results are presented in Table 16. Although all the 1,2,3-benzothiadiazoles tested have I_{50}M values for aldrin epoxidation and DHI hydroxylation in the range of 10^{-6}M, they were all poor inhibitors of the NADPH cyt-c reductase from armyworm midgut at concentrations as high as 10^{-4}M. The best inhibitor, 6-ethoxy-1,2,3-benzothiadiazole (XXXII) caused only 17% inhibition at this concentration clearly establishing that the inhibitory effect of the 1,2,3-benzothiadiazoles is not explained by their interaction with NADPH cyt-c reductase.

The 1,2,3-benzothiadiazoles are even less potent inhibitors of the NADH cyt-c reductase (Table 17) from Prodenia gut microsomes since the best m.f.o. inhibitor, 6-iodo-1,2,3-benzothiadiazole, inhibits the enzyme by only 11% at 10^{-4}M. If this enzyme reflects in some way the activity of NADH cyt b_5 reductase, these results would indicate that the 1,2,3-benzothiadiazoles are unable to block electron transport from NADH to cyt b_5.

Cyt P-450 is the oxygen-activating enzyme and the site of substrate interaction in the oxidative metabolism of foreign compounds by insect and mammalian microsomes. The oxidation of a foreign compound by the m.f.o. system involves an initial binding of the substrate to the oxidized form of cyt P-450. As competitive inhibitors of mixed-function oxidation the 1,2,3-benzothiadiazoles might be expected to

Table 16. Effect of 1,2,3-benzothiadiazoles on microsomal Prodenia NADPH Cyt c reductase[a]

Compound Number	1,2,3-benzothiadiazole	Concentration	*ΔOD min/mg protein Mean ± S.D.	% Control	% Inhibition
L	5-methyl-6-chloro	1×10^{-4}	0.483 ± 0.028	87.66	12.34
XXII	6-ethoxy	1×10^{-4}	0.455 ± 0.029	82.58	17.42
XXII	6-iodo	1×10^{-4}	0.469 ± 0.014	85.12	14.88

*Control activity 0.551 + 0.011 ΔOD min/mg protein.

[a]NADPH cyt c reductase was determined as described in III. B. 6.

Table 17. Effect of 1,2,3-benzothiadiazoles on microsomal Prodenia NADH Cyt c reductase[a]

Compound Number	1,2,3-benzothiadiazole	Concentration	*ΔOD min/mg protein Mean ± S.D.	% Control	% Inhibition
L	5-methyl-6-chloro	1×10^{-4}	0.787 ± 0.050	95.40	4.60
XXXII	6-ethoxy	1×10^{-4}	0.798 ± 0.042	96.77	3.23
XXII	6-iodo	1×10^{-4}	0.731 ± 0.031	88.60	11.40

*Control activity 0.825 ± 0.014 ΔOD min/mg protein.

[a]NADH cyt c reductase was determined as described in III. B. 6.

compete with the substrate for binding sites at or close to cyt P-450. Consequently a knowledge of the binding characteristics of the 1,2,3-benzothiadiazoles might be of considerable importance to our understanding of their mechanism of action. Since 6-ethoxy-1,2,3-benzothiadiazole (XXXII) was found to be one of the best *in vitro* inhibitors of the m.f.o. system it was decided to study the binding characteristics of this compound to both the oxidized and reduced form of cyt P-450 in microsomes from *Prodenia eridania* gut, Madagascar cockroach gut and rat liver.

As can be seen from Table 18, 6-ethoxy-1,2,3-benzothiadiazole binds to the oxidized form of cyt P-450 in preparations from each of the three species employed to give a difference spectrum with a peak at 422 nm and trough at 390 nm. This is characteristic of a type II difference spectrum (II. C). The magnitude of the spectral change was dependent on the amount of cyt P-450 and the concentration of the synergist. Furthermore, 6-ethoxy-1,2,3-benzothiadiazole was also able to interact with the reduced form of cyt P-450 to produce a different difference spectrum with a peak at 444 nm. This latter peak disappeared completely when CO was bubbled through the sample cuvette, suggesting that CO and 6-ethoxy-1,2,3-benzothiadiazole might bind to the same heme ligand. Alternatively it is possible that 6-ethoxy-1,2,3-benzothiadiazole might bind at a different locus to that of CO and that displacement of the 444 nm peak by CO might result from a conformational change of the heme group in the presence of CO. The interaction of 6-ethoxy-1,2,3-benzothiadiazole with the reduced form

Table 18. The binding of 6-ethoxy-1,2,3-benzothiadiazoles to the oxidized and reduced forms of cytochrome P-450

Source of Enzyme (Microsomes)	mM Conc.	Cyt. P-450 Difference Spectra ΔOD x 1000	
		Oxidized P-450 422 - 390	Reduced P-450 444 - 490
Rat	0.20	146	154
Madagascar Cockroaches	6.67	80	92
Prodenia eridania	4.22	76	79

The spectra were measured at a protein concentration of 1.48 mg/ml for rat liver microsomes and 4.61 and 4.96 respectively for the microsomal preparations from Madagascar cockroaches and Prodenia eridania guts as described in III. B. 5.

of cyt P-450 is very similar to that produced by Fluorene (Ullrich, 1973) and metyrapone (Peterson et al., 1971) and it is likely that it results from ligand binding of one of the nitrogens of the 1,2,3-benzothiadiazole ring to the ferrous form of cyt P-450.

Although the difference spectra were qualitatively similar in both the insect and mammalian liver preparations, the affinity of 6-ethoxy-1,2,3-benzothiadiazole for the oxidized and reduced forms of cyt P-450 appeared to be higher in mammalian liver microsomes than in those from insects. It is possible, however, that the insect preparations used in these experiments had a lower cyt P-450 content despite having a higher protein concentration than the liver microsomes.

These results are in agreement with those reported by Matthews et al. (1970) for the interaction of 5,6-dicholoro-1,2,3-benzothiadiazoles with oxidized and reduced cyt P-450 from mouse liver microsomes. With the exception of the imidazoles, the 1,2,3-benzothiadiazoles are the only group of synergists which exhibit a type II difference spectrum with oxidized cyt P-450 (Mailman and Hodgson, 1972). The fact that the 1,2,3-benzothiadiazoles exhibit a quite different difference spectrum with reduced cyt P-450 suggests that they undergo a special type of interaction under these conditions.

Although the interpretation of these difference spectra is still a matter of controversy, it is possible to speculate how they might relate to the interaction of the 1,2,3-benzothiadiazoles with cyt P-450.

1) Since it has been shown (IV. F.) that the 1,2,3-benzothiadizoles are competitive inhibitors of m.f.o. activity it is probable that they interact with protein binding sites and may therefore compete with the substrate for the formation of an enzyme-substrate complex. As a result of this binding they may or may not be metabolized.

2) Another possibility is that they could bind directly to one of the ligands in the catalytic site of cyt P-450 (Fe^{2+}). In this case the inhibition would result from reduction in the oxygen binding capacity of cyt P-450, although it is not clear what type of kinetics would result.

3) Finally, it is possible that the 1,2,3-benzothiadiazoles might act through interaction with both the catalytic and the protein binding sites of cyt P-450, since they are considered to be very near to one another. The 1,2,3-benzothiadiazoles are excellently suited

to both types of interaction since they have sufficient lipophilic character to interact with the protein binding site and good electron donor properties for ligand binding to the ferrous ion (catalytic site).

I. The Influence of the *in vivo* Administration of 1,2,3-Benzothiadiazoles on the *in vitro* Epoxidation of Aldrin by Insect and Mammalian m.f.o.

The *in vitro* inhibitory activity of the 1,2,3-benzothiadiazoles on aldrin epoxidase has been discussed (IV. D. 1,2). To investigate the effect of the *in vivo* administration of 1,2,3-benzothiadiazoles on the *in vitro* epoxidation of aldrin, female Madagascar cockroaches were injected with 4-chloro (84 µg per insect) and 6-ethoxy-1,2,3-benzothiadiazole (200 µg per insect). After five days the insects were sacrificed and the epoxidase activity in their gut tissues measured *in vitro*. The results in Table 19 show that under these conditions 4-chloro-1,2,3-benzothiadiazole causes a 52% reduction and 6-ethoxy-1,2,3-benzothiadiazole an 80% reduction in aldrin epoxidase activity after five days. These results indicate that the activity of the 1,2,3-benzothiadiazoles is long lasting *in vivo* and suggests that perhaps these compounds are not substantially metabolized by the Madagascar cockroaches.

If on the other hand they are metabolized, the persistent inhibition might be due to the formation of a metabolite which binds very tightly to the enzyme. It is, however, difficult to establish whether

Table 19. Effect on aldrin epoxidase activity of *in vivo* treatment of Madagascar cockroaches with 1,2,3-benzothiadiazoles

Compound	Dose (µg/insect)	Dieldrin n moles/min x 10^3 mg protein	% of control	% of inhibition
		Mean ± S.D.		
Acetone	None	151 ± 18	---	---
	10	126 ± 10	100.00	0
4-Chloro-1,2,3-benzothiadiazole	84	65 ± 7	48.42	51.58
6-ethoxy-1,2,3-benzothiadiazole	200	26 ± 1	20.63	79.37

1,2,3-benzothiadiazoles were injected as a single dose in 10 µl of acetone. Aldrin epoxidase activity measured in preparations from gut tissues 5 days after treatment.

the *in vitro* inhibition observed reflects the state of the enzyme *in vivo* or whether it results from tissue residues of the inhibitor released during enzyme preparation. Whatever the case the results clearly establish the stability of the 1,2,3-benzothiadiazoles *in vivo*. The administration of 6-chloro-1,2,3-benzothiadiazole (208 mg/Kg) to mice, results in a different pattern of enzyme activity, although the results reported are from a single experiment. Three hours after i.p. injection of the synergist, the *in vitro* epoxidation of aldrin by the liver microsomes was inhibited by 84% and the enzyme was still inhibited by almost 40% twenty four hours after treatment. At forty eight hours, however, aldrin epoxidase was 120% that of the control activity, and even at ninety six hours after treatment it was still induced by 111%.

These experiments indicate a significant difference in the *in vivo* effects of the 1,2,3-benzothiadiazoles in insects and mammals. The fact that after an initial inhibition phase the 1,2,3-benzothiadiazoles induce the m.f.o. system in mice suggests that perhaps they are able to enhance RNA and protein synthesis in particular perhaps *de novo* synthesis of δ-aminolevulinic acid synthetase, which is considered to be the rate limiting enzyme in the synthesis of heme and porphyrins. That induction does not appear to take place, following the *in vivo* treatment of Madagascar cockroaches with the 1,2,3-benzothiadiazoles strongly suggests a basic difference in the interactions between insects and mammals.

SECTION V

CONCLUSIONS

Structure activity-relationships and mode of action of 1,2,3-benzothiadiazole insecticide synergists have been investigated. The following conclusions have been reached as a result of this study.

1. In addition to the methods reported in the literature for the synthesis of 1,2,3-benzothiadiazoles, several compounds were prepared by ring opening of the corresponding 2-amino- or 2-methyl-benzothiazoles with boiling potassium hydroxide. This method provides a simple direct route for the synthesis of 4-, 5-, and 5,6-substituted-1,2,3-benzothiadiazoles.
2. The 1,2,3-benzothiadiazoles have been shown to be moderate synergists of pyrethroids in houseflies.
3. Several 1,2,3-benzothiadiazoles are excellent synergists of carbaryl in houseflies. Regression analysis has established that synergistic activity is best correlated with the hydrophobic parameter (π) and the homolytic free radical constant ($\sigma^{\cdot}$), suggesting that their *in vivo* action might be partly explained on the basis of a homolytic free radical mechanism.
4. The 1,2,3-benzothiadiazoles are effective *in vitro* inhibitors of both insect and mammalian m.f.o. systems, although the insect enzymes are inhibited to a greater extent. Regression analysis of the *in vitro* inhibition of the insect m.f.o.

system by the 1,2,3-benzothiadiazoles has clearly indicated the importance of the hydrophobic character of the molecule (π), although steric (E_s) and electronic factors (σ) are also involved to a smaller extent. Regression analysis allowed the prediction of the *in vitro* inhibitory activity of some compounds.

5. A good correlation was observed between the activity of the 1,2,3-benzothiadiazoles as carbaryl synergists in houseflies and the pI_{50} values for the *in vitro* inhibition of aldrin epoxidation preparations from the midgut of *Prodenia*. This suggests that synergistic activity is directly related to the inhibition of m.f.o. system.
6. The 1,2,3-benzothiadiazoles are competitive inhibitors of aldrin epoxidase in the microsomal enzymes from *Prodenia* midgut and rat liver.
7. NADPH-cyt-c-reductase and NADH-cyt-c-reductase are not inhibited by the 1,2,3-benzothiadiazoles suggesting that the interaction is at the level of cyt P-450.
8. In both insect and mammalian microsomes the 1,2,3-benzothiadiazoles bind to the oxidized form of cyt P-450 to give a typical type II spectrum. They also bind to the reduced form of cyt P-450 to give a peak at 444 mu suggesting a special type of interaction.
9. A single injection of 1,2,3-benzothiadiazoles into Madagascar cockroaches *in vivo* inhibited aldrin epoxidation in preparations

from the gut tissues for at least five days after administration. In contrast a similar injection into mice produced first inhibition and subsequently induction of aldrin epoxidase activity in the liver microsomes. These experiments suggest that either the 1,2,3-benzothiadiazoles are not metabolized *in vivo* or that a very stable metabolite is produced.

BIBLIOGRAPHY

Anders, M. W. (1968). Biochem. Pharmacol. 17, 2367.

Bakry, N., Metcalf, R. L. and Fukuto, T. R. (1968). J. Econ. Entomol. 61, 1303.

Bambas, L. (1952). "The Chemistry of Heterocyclic Compounds", p.10, Interscience Publ. New York.

Bamberger, E. Baum, M. and Schlein, L. (1923 b). J. pr. Chem. 105, 266.

Bamberger, E., Bocking, O. and Kraus, E. (1923 a). J. pr. Chem. 105, 251.

Ban, T. and Fujita, T. (1969). J. Med. Chem. 12, 353.

Beilstein, F., and Kubatow, A. (1879). Annalen, 197, 81.

Benke, G. M. (1971). "Microsomal oxidases in selected orthopteran insects, with special reference to the house cricket, Acheta domesticus and the Madagascar cockroach, Gromphadorhina portentosa", M.S. Thesis, Cornell University.

_____, Wilkinson, C. F. and Telford, J. N. (1972). J. Econ. Entomol. 65, 1221.

Bernthsen, A. (1888). Chemiker-Zeitung. 12, 1318.

_____, (1889). Annalen, 251, 1.

Boggust, W. A. and Cocker, W. (1949). J. Chem. Soc., 355.

Boyce, C. B. C. and Milborrow, B. V. (1965). Nature 208, 537.

Brattsten, L. B. and Metcalf, R. L. (1970). J. Econ. Entomol. 63, 101.

Brooks, G. T. (1968). Meded. Rijksfac Landbouwetensch. Gent. 33, 629.

_____, and Harrison, A. (1964). Biochem. Pharmacol. 13, 827.

Bryson, A. (1960). J. Amer. Chem. Soc. 82, 4871.

Camarata, A. (1972). J. Med. Chem. 15, 573.

Casida, J. E. (1969). "Microsomes and Drug Oxidations", Gillette, J. R., Conney, A. H., Cosmides, G. J., Estabrook, R. W., Fouts, J. R. and Mannering, G. J. Eds., p. 517, Academic Press, New York.

_____, (1970). J. Agr. Food Chem. 18, 753.

_____, Kimmel, E. C., Elliot, M. and Janes, N. (1971). Nature (London) 230, (5292), 326.

Casida, J. E., Engel, J. L., Essac, E. G., Kamienski, F. X., Kuwatsuka, S. (1966). Science, 153, 1130.

Chaykin, S. (1966). "Biochemistry Laboratory Techniques". John Wiley and sons, Inc. New York.

Coats, E., Glave, W. R. and Hansch, C. (1970). J. Med. Chem. 13, 913.

Cooper, D. Y., Narashimhulu, S., Slade, A., Raich, W., Foroff, O. and Rosenthal, O., (1965). Life Sci. 4, 2109.

Craig, P. N. (1971). J. Med. Chem. 14, 680.

Dahm, P. A. and Nakatsugawa, T. (1968). "Enzymatic Oxidations of Toxicants", Hodgson, E., Ed., p. 89, North Carolina State University Press, Raleigh, N.C.

Dallner, G. (1963). Acta. Path. Microbiol. Scan. suppl 166, 7.

Davies, J. H. and Kirby, P. (1967). J. Chem. Soc. (C) 321.

_____, Haddock, E., Kirby, P. and Webb, S. B. (1971). J. Chem. Soc. (C) 2843.

Fellig, J., Barnes, J. R., Rachlin, A. J., O'Brien, J. P. and Focella, A. (1970). J. Agr. Food Chem. 18, 78.

Felton, J. C., Jenner, D. W. and Kirby, P. (1968). XIIIth. Inter. Entomol. Congress, August, Moscow.

_____, Jenner, D. W. and Kirby, P. (1970). J. Agr. Food Chem. 18, 671.

Franco, M. (1964). Bolletino di Zoologia XXXI, 651.

Free, S. M. and Wilson, J. W. (1964). J. Med. Chem. 7, 395.

Fridman, S. and Golub, D. (1961). J. Gen. Chem. U.S.S.R. 31, 3162.

Fries, K. and Engelbertz, E. (1915). Annalen 407, 208.

_____, and Reitz, H. (1936). Annalen 527, 38.

_____, Vorbrodt, M. and Siebert, G. (1927). Annalen 454, 172.

Fujita, T. (1966). J. Med. Chem. 9, 797.

_____, Iwasa, J. and Hansch, C. (1964). J. Amer. Chem. Soc. 86, 5175.

Fukuto, T. R. (1969). Res. Rev. 25, 327.

_____, Metcalf, R. L., Winton, M. Y. and Roberts, P. A. (1962). J. Econ. Entomol. 55, 341.

Gardner, A. (1944). Brit. Patent 558, 877.

Gaudette, L. E. and Brodie, B. B. (1959). Biochem. Pharmacol. 2, 89.

Gillette, J. R. and Gram, T. E. (1969). "Microsomes and Drug Oxidations" Gillette, J. R., Conney, A. H., Cosmides, G. J., Estabrook, P. W., Fouts, J. R. and Mannering, G. J. Ed., p. 133, Academic Press, New York.

Gram, T. E. and Fouts, J R. (1968). "Enzymatic Oxidation of Toxicants", Hodgson, E. Ed., p. 81, North Carolina State University Press, Raleigh, N. C.

Green, A and Perkin, A. (1903). J. Chem. Soc. 83, 1201.

Grompper, R., Euchner, H. and Kast, H. (1964). Annalen 675, 151.

Hackmann, J. T. and Kirby, P. (1967). Brit. Patent. 1,059,799.

Haddock, E., Kirby, P. and Johnson, A. W. (1970). J. Chem. Soc. (C) 2514.

_____, Kirby, P., and Johnson, A. W. (1971 a) J. Chem. Soc. (C) 3994.

_____, Kirby, P., and Johnson, A. W. (1971 b) J. Chem. Soc. (C) 3642.

Hammett, L. P. (1940) "Physical Organic Chemistry" Chapter VII. McGraw Hill, N. Y.

Hantzsch, A. (1909) Berichte 42, 81.

Hansch, C. (1967) "Annual Reports in Medicinal Chemistry" p. 347, Academic Press, Inc. New York.

_____, (1968 a). Il Farmaco, Sci. Ed. 23, 293.

_____, (1968 b). J. Med. Chem. 11, 920.

_____, (1968 c). "Proceedings of Third International Pharmacological Meeting" 7, 141.

_____, (1970) "Biochemical Toxicology of Insecticides", O'Brien, R. D. and Yamamoto, I. Eds. p. 33, Academic Press, New York.

_____, (1972) Drug. Met. Rev. 1, 1.

_____, and Fujita, T. (1964) J. Amer. Chem. Soc. 86, 1616.

_____, and Deutsch, E. W. (1966) Biochem. Biophys. Acta. 126, 117.

_____, and Lien, E. J. (1968) Biochem. Pharmacol. 17, 709.

_____, and Glave, W. R. (1972) J. Med. Chem. 15, 112.

Hansch, C., Steward, A. R. and Iwasa, J. (1965 a) J. Med. Chem. 8, 868,

_____, Deutsch, E. W. and Smith, R. N. (1965 b) J. Amer. Chem. Soc. 87,2738.

_____, Lien, E. J. and Helmer, F. (1968). Arch. Biochem. Biophys. 128, 319.

Hansen, L. G. and Hodgson, E. (1971). Pest. Biochem. Physiol. 1, 109.

Hennessy, D. J. (1965) J. Agr. Food Chem. 13, 218.

_____, (1970) "Biochemical Toxicology of Insecticides"., O'Brien, R. D. and Yamamoto, I. Eds., p. 105, Academic Press, New York.

Herz, R. (1922) German Patent 360, 690; Frdl 14, 908.

_____, (1922 a) German Patent 367, 346; Frdl 14, 918.

Hodgson, H. H. and Dodgson, D. P. (1948 d) J. Soc. Dyers and Colourists 64, 65.

_____, and Dodgson, P. (1948 a) J. Chem. Soc. 1006.

_____, and Dodgson, P. (1948 b) J. Chem. Soc. 870.

_____, and Dodgson, P. (1948 c) J. Chem. Soc. 1002.

Hodgson, E. and Plapp, F. W. (1970) J. Agr. Food Chem. 18, 1048.

Hope, P. and Wiles, L. A. (1967) J. Chem. Soc. (C) 1642.

Ichikawa, Y., Yamano, T. and Fujishima, H. (1969) Biochem. Biophys. Acta, 171, 32.

Imai, Y. and Sato, R. (1966) Biochem. Biophys. Res. Commun. 22, 620.

Jacobson, P. (1888) Berichte. 21, 3104 - 3106.

Jacobson, P. and Ney, E. (1889) Berichte 22, 909.

_____, and Janssen, H. (1893) Annalen 277, 218.

_____, and Ney, E. (1893) Annalen 277, 232.

_____, Kwayser, A., Ney, E., Schwarz, C. and Janssen, H. (1893) Annalen 277, 209

Jaffe, H. H. (1953) Chem. Rev. 53, 191.

_____, and Jones, L. J. (1964) "Advances in Heterocyclic Chemistry", R. Katritzby, Ed. Vol. 3, p. 209, Academic Press, New York.

Jain, S. K., Chandra, D., and Mital, R. L. (1969) Chemistry and Industry, 989.

Jones, R. M., Metcalf, R. L. and Fukuto, T. R. (1969) J. Econ. Entomol. 62, 801.

Kamin, H. and Masters, B. S. S. (1968). "Enzymatic Oxidations of Toxicants", Hodgson, E. Ed., p. 5, North Carolina State University Press, Raleigh, N. C.

Kirby, P., Soloway, S. B. and Haddock, E. (1970 b). Brit. Patent. 1,176,799.

_____, Soloway, S. B., Davies, J. H, and Webb, S. B. (1970 a) J. Chem. Soc. (C) 2250.

Krieger, R. I. (1970) "Microsomal oxidases in selected lepidopterous larvae, primarily, the Southern armyworm, Prodenia eridania", Ph. D. Thesis, Cornell University.

_____, and Wilkinson, C. F. (1971) Pesticide Biochem. and Physiol. 1, 92.

Kutter, E. and Hansch, C. (1969) J. Med. Chem. 12, 647.

Leo, A., Hansch, C. and Church, C. (1969). J. Med. Chem. 12, 766.

_____, Hansch, C. and Elkins, D. (1971) Chem. Rev. 71, 525.

Lewis, S. E., Wilkinson, C. F. and Ray, J. W. (1967) Biochem. Pharmacol. 16, 1195.

Mc Gowan, J. C., Atkinson, P. N. and Ruddle, L. H. (1966) J. Appl. Chem. 16, 99.

Mc Mahon, R. E. (1961) J. Med. Chem. 4, 67.

Mailman, R. B. and Hodgson, E. (1972) Bulletin of Environmental Contamination and Toxicology 8, 186.

Mannering, G. J. (1971) Metabolism 20, 228.

Marshall, R. S. and Wilkinson, C. (1973) In Press.

Marshall, R. S. and Wilkinson, C. F. (1970) Biochem. Pharmacol. 19, 2665.

Martin, Y. C. and Hansch, C. (1971) J. Med. Chem. 14, 777.

Mason, H. S. (1965) Annu. Rev. Biochem. 34, 595.

Masters, B. S. S., Baron, J., Taylor, W. E. Isaacson, E. L. and Lo Spalluto, J. (1971) J. Biol. Chem. 246, 4141.

Matthews, H. B., Škrinjarić-Špoljar, M. and Casida, J. E. (1970) Life Sci. 9, 1039.

Mayer, H. (1899) Arch. Exp. Pathol. Pharmakol. 42, 109.

Mazel, P. and Henderson, J. (1965) Biochem. Pharmacol. 14, 92.

Metcalf, R. L. (1967) Annu. Rev. Entomol. 12, 229.

_____, Osman, M. F., Fukuto, T. R. (1967) J. Econ. Entomol. 60, 445.

Neely, W. B., White, H. C. and Rudzik, A. (1968) J. Pharm. Sci. 57, 1176.

Nunn, A. J., Chadbourne, D. J. and Ralph, J. T. (1964) J. Chem. Soc. 6001.

Omura, T. and Sato, R. (1964 a) J. Biol. Chem. 239, 2379.

_____, and Sato, R. (1964 b) J. Biol. Chem. 239, 2370.

Ostrenga, J. A. (1969) J. Med. Chem. 12, 349.

Overton, E. (1899) Vierteljahrsschr. Naturforsch. Ges. Zurich, 44, 88.

Parke, D. V. (1968) "The Biochemistry of Foreign Compounds", Pergamon, Oxford.

Pearson, R. G. and Songstad, J. (1967) J. Amer. Chem. Soc. 89, 1827.

Peterson, J. A., Ullrich, V. and Hildebrandt, A. G. (1971) Arch. Biochem. and Biophys. 145, 531.

Philleo, W. W., Schonbrod, R. and Terriere, L. C. (1965) J. Agr. Food Chem. 13, 113.

Philpot, R. M. and Hodgson, E. (1971) Life Sci. 10, 503.

Purcell, W. P. (1965) Biochem. Biophys. Acta 105, 201.

_____, and Clayton, J. M. (1968) J. Med. Chem. 11, 199.

_____, Beasley, J. G., Quintana, R. P. and Singer, J. A. (1966) J. Med. Chem. 9, 297.

Remmer, H. (1971) "Pesticide Chemistry Proc. Second Intern. IUPAC Congress Tahori, A. Ed., Vol. II, p. 167, Gordon and Breach, New York.

_____, Estabrook, R. W., Schenkman, J. and Greim, H. (1968) "Enzymatic Oxidations of Toxicants", Hodgson, E., Ed., p. 65. North Carolina State University Press, Raleigh, N. C.

Rose, H. A. (1972) "Nitroreductases in the Madagascar cockroach, Gromphadorhina portentosa and the housefly, Musca domestica" Ph. D. Thesis. Cornell University.

Rubin, A., Tephly, T. R., Mannering, G. J. (1964) Biochem. Pharmacol. 13, 1007.

Sacher, R. M., Metcalf, R. L. and Fukuto, T. R. (1968) J. Agr. Food Chem. 16, 779.

_____, Metcalf, R. L., Metcalf, E. R. and Fukuto, T. R. (1971) J. Econ. Entomol. 64, 1011.

Sawicki, R. M. (1962 a) J. Sci. Food Agric. 13, 260.

_____, (1962 b) J. Sci. Food Agric. 13, 591.

Siekevitz, P. (1963). Annu. Rev. Physiol. 25, 15.

Singer, J. A. and Purcell, W. P. (1967) J. Med. Chem. 10, 1000.

Smithfield, W. R. and Purcell, W. P. (1967) J. Pharm. Sci. 56, 577.

Staudinger, H., Kerekjarto, B., Ullrich, V. Zubrzycki, P. (1965) "Oxidases and Related Redox Systems", King, T., Mason, H., Morrison, M. Eds., Vol. 2, p. 815. John Wiley and Sons, Inc. New York.

Swain, C. G. and Lupton, E. C. (1968) J. Amer. Chem. Soc. 90, 4328.

Taft, R. W. Jr. (1956) "Steric Effects in Organic Chemistry", Newman, M. Ed., p. 556, John Wiley and Sons, New York.

Todesco, P. E. and Vivarelli, P. (1962) Gazz. Chim. Ital. 92, 1221.

Topliss, J. G. and Yudis, M. D. (1972) J. Med. Chem. 15, 400.

Ullrich, V. and Schnabel, K. (1973) Personal Communication.

W.H.O. (1965) "Scientific Group on Standard Strains of Insects of Public Health Importance", Working papers 6.3 and 6.13 Geneva.

W.H.O. / Vector Control 113-65.

Warbuton, W. K. (1957) Chem. Rev. 57, 1011.

Ward, E. R., and Poesche, W. H. (1961) J. Chem. Soc. 2825.

_____, and Heard, D. D. (1963) J. Chem. Soc. 4794.

_____, and Heard, D. D. (1965) J. Chem. Soc. 1023.

_____, Poesche, W. H., Higgins, D., and Heard, D. D. (1962) J. Chem. Soc. 2374.

Wilkinson, C. F. (1967) J. Agr. Food Chem. 15, 139.

_____, (1968) World Rev. Pest. Control 7, 155.

_____, (1971 a) Bull. Wld. Hlth. Org. 44, 171.

Wilkinson, C. F. (1971 b) "Pesticide Chemistry Proc. Second Intern. IUPAC Congress Tahori, A. Ed., Vol. II, p. 117, Gordon and Breach, New York.

_____, (1973) Chemtech, In Press.

_____, and Hicks, L. J. (1969) J. Agr. Food Chem. 17, 829.

_____, and Brattsten, L. B. (1973) Drug Metabolism Reviews 1, In Press.

_____, Metcalf, R. L. and Fukuto, T. R. (1966) J. Agr. Food Chem. 14, 73.

Williams, G. H. (1960) "Homolytic Aromatic Substitution" p. 57, Pergamon Press, New York, N. Y.

Winteringham, F. P. W., Harrison, A. and Bridges, P. M. (1955) Biochem. J. 61, 359.

Wolff, L. (1904) Annalen 333. 1.

_____, and Hall, A. (1903) Berichte 36, 3612.

_____, Kopitzsch and Hall, A. (1904) Annalen 333, 4.

Wonnacott, R. J. and Wonnacott, T. H. (1970) "Econometrics," p. 65, 276, J. Wiley & Sons, Inc.

Wright, J. W. and Pal, R. (1967) "Genetics of Insect Vectors Disease," Elsevier Ed., p. 745.

Yamamoto, I., Kimmel, E. C. and Casida, J. E. (1969) J. Agr. Food Chem. 17, 1227.