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Structure-Activity Relationships of 1,2,3-Benzothiadiazoles as Synergists for Carbaryl against the House Fly (*Musca domestica*)

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Fifty six 1,2,3-benzothiadiazoles and related compounds were evaluated as carbaryl synergists against the house fly (*Musca domestica*). Many of these were excellent synergists, the most active being those containing various combinations of halogen, alkyl, or alkoxy substituents in the 5- and/or 6-positions of the ring.

Regression analysis on the data from 14 compounds for which substituents constants were available established that synergistic activity can be satisfactorily described by equations in terms of the hydrophobic bonding constant (π) and the homolytic free radical constant ($\sigma\cdot$).

The results with compounds related to the 1,2,3-benzothiadiazoles suggest that synergistic activity is associated primarily with the diazosulfide moiety.

INTRODUCTION

It is now well established that several types of compounds can synergize the biological activity (toxicity, sleeping time, carcinogenicity, etc.) of a variety of drugs and insecticides *in vivo* to both insects and mammals and that this results from the ability of the compounds to inhibit the microsomal enzymes responsible for metabolic detoxication (1–5).

The best known and most thoroughly studied synergists are the methylenedioxyphenyl (1,3-benzodioxole) compounds but only a few compounds of this type have attained any commercial significance. Piperonyl butoxide is the most important commercial synergist and is widely used in aerosol formulations of the pyrethrin insecticides. However, the cost of production of piperonyl butoxide and other methylenedioxyphenyl synergists is likely to increase substantially in the future since the natural product safrole, from which all are produced, is currently in short supply. Consequently, there is a growing need for new types of insecticide synergists which are not based on the methylenedioxyphenyl ring.

In recognition of this, research has been directed towards finding new groups of insecticide synergists and emphasis has been placed on establishing the precise mechanism by which the methylenedioxyphenyl and other compounds inhibit the cytochrome P-450 mediated drug-metabolizing enzymes (5). In the course of these and related studies several other groups of compounds, including the phenyl-2-propynyl ethers (6), oxime ethers (7), substituted imidazoles (8), and 1,2,3-benzothiadiazoles (9) have been discovered to possess properties similar to methylenedioxphenyl derivatives.

In view of the novel structure and broad range of biological activity of the sub-

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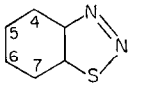
TABLE 1

Physical Properties of 1,2,3-Benzothiadazoles

General structure: 

Compound	Substituents				Synthetic method, yield (%)	mp (°C)	Elemental analysis		Reference
	4	5	6	7			Calc.	Found	
I	Unsubstituted				I, 83	35 (Lit. 35)			(10)
II	Cl				Ic, 32	100 (Lit. 101)			(20)
III		Cl			Ia, 23	103–104 (Lit. 105)			(12)
IV			Cl		Ic, 37	77 (Lit. 77)			(19)
V				Cl	IIIc, 39	76 (Lit. 78)			(25)
VI	Br				IIIc, 30	109 (Lit. 113)			(20)
VII		Br			Ib, 25	105 (Lit. 106)			(12)
VIII			Br		Ic, 21	67 (Lit. 70)			(20)
IX				Br	IIIc, 21	73 (Lit. 77)			(25)
X	I				IIIc, 26	108 (Lit. 111)			(25)
XI		I			Ia, 11	101–102 (Lit. 103)			(12)
XII			I		IIIc, 36	114–115 (Lit. 116)			(25)
XIII				I	IIIc, 35	138–139.5 (Lit. 137)			(25)
XIV			F		II, 8	113–115 (Lit. 105)	C: 46.70 H: 2.00	C: 47.39 H: 2.73	(19)
XV		Cl	Cl		[a]				(13)
XVI	Cl	Cl	Cl	Cl	II, 1.5	168–170 (Lit. 171–173)			
XVII	F	F	F	F	II, 4	104	C: 34.61 H: 0.0	C: 34.00 H: 0.0	
XVIII	Br	NH_2			IIId, 81	183 (Lit. 183)			(18)
XIX	I	NH_2			IIId, 60	163 (Lit. 168)			(18)
XX			NH_2	Br	IIId, 58	165–167 (Lit. 168)			(25)
XXI		F	Cl		II, 5	96 (Lit. 96–98)			(13)
XXII	OH				IIIa, 33	144–146 (Lit. 146)			(18)
XXIII		OH			Ia, 9	157–158 (Lit. 161)			(11)
XXIV			OH		IIIa, 35	213–215 (Lit. 211–213)			(22)
XXV				OH	IIIc, 40	228 (Lit. 230–232)			(22)
XXVI	NO_2				IIIf, 31	117–118 (Lit. 118)			(22)
XXVII		NO_2			Ib, 25	142–143 (Lit. 144)			(15)
XXVIII			NO_2		Ic, 50	134 (Lit. 136)			(20)
XXIX				NO_2	[a]				
XXX			Cl	NO_2	IIIe, 16	99 (Lit. 99–101)			(23)
XXXI			CH_3	NO_2	IIIe, 60	115	C: 43.08 H: 2.56	C: 42.90 H: 2.35	
XXXII	NH_2				IIIb, 70	87–88 (Lit. 88)			(20)
XXXIII		NH_2			IIIb, 60	95–101 (Lit. 95)			(20)
XXXIV			NH_2		IIIb, 53	108 (Lit. 112)			(24)
XXXV				NH_2	IIIb, 70	136 (Lit. 136)			(20)
XXXVI		CN			Ia, 5	195 (Lit. 194–196)			(13)
XXXVII			CN		IIIc, 20	126–127	C: 52.17 H: 1.86	C: 49.87 H: 1.92	
XXXVIII				CN	IIIc, 10	98 (Lit. 116–118)			(13)
XXXIX	CH_3O				IIIa, 33	64 (Lit. 64–66)			(23)
XXXX			CH_3O		Ic, 25	76–77 (Lit. 77)			(18)
XXXXI			C_2H_5O		Ic, 16	102 (Lit. 101)			(18)
XXXXII			C_3H_7O		IIIa, 5	50–51	C: 55.67 H: 5.15	C: 55.20 H: 4.95	
XXXXIII		CH_3O	Cl		II, 5	156 (Lit. 153.5–155.5)			(13)
XXXXIV		CH_3			Ic, 5	29 (Lit. 26)			(10)
XXXXV			CH_3		Ic, 20	38	C: 56.00 H: 4.00	C: 56.01 H: 4.10	
XXXXVI		CH_3	CH_3		Ic, 22	75	C: 58.54 H: 4.88	C: 58.84 H: 4.97	
XXXXVII		CH_3	Cl[a]						
XXXXVIII			CH_3	NH_2	IIIb, 67	134–135	C: 50.90 H: 4.24	C: 50.80 H: 4.21	
XXXXIX			C_4H_9		II, 9	110–111	C: 62.50 H: 6.25	C: 62.10 H: 6.10	

[a] Obtained from Shell Agricultural Research Center, Sittingbourne, U.K.

stituted 1,2,3-benzothiadiazoles comprehensive studies were undertaken to establish structure–activity relationships within this group of compounds. This paper reports the synthesis and evaluation of a large number of substituted 1,2,3-benzothiadiazoles as carbaryl synergists to house flies and the results of regression analyses to investigate possible correlations between biological activity and a variety of physicochemical parameters in the molecule.

MATERIALS AND METHODS

Chemicals. With the exception of compounds XV, XIX, and XXXXVII which were kindly supplied by Dr. Hugh Davies of the Shell Agricultural Research Center,

Sittingbourne, U.K., the 1,2,3-benzothiadiazoles (I to XXXXIX) (Table 1) were synthesized by a variety of routes in the laboratory. Compounds L to LIV were also synthesized and LV and LVI were kindly provided by Dr. L. K. van Beek of the Phillips Research Laboratories, Eindhoven, Netherlands. Analytical grade carbaryl (1-naphthyl *N*-methylcarbamate) was from Union Carbide, N.Y., and organic chemicals used in the syntheses were from Aldrich Chemical Co., Milwaukee, Wisc., and Pfaltz and Bauer, Inc., Flushing, N.Y. All other chemicals and solvents employed were analytical reagent grade.

Synthetic procedures. Several synthetic procedures were employed which will be illustrated with reference to specific examples; the methods of synthesis used as well as the physical properties of the 1,2,3-benzothiadiazoles are shown in Table 1.

Diazotization of the appropriate 2-aminobenzenethiol (Method I) proved the most direct route to the 1,2,3-benzothiadiazoles but its general application was limited by the few commercially available starting materials. The parent 1,2,3-benzothiadiazole (I) was prepared as follows. Technical 2-aminobenzenethiol (223 g, 1.78 mol) was dissolved in 10% HCl, the solution cooled to 0°C and sodium nitrite, (130 g, 1.88 mol), dissolved in the minimum amount of water, added dropwise with stirring below 5°C. The mixture was kept overnight at 4°C and extracted into diethyl ether (1500 ml). The extract was dried over anhydrous sodium sulfate and after evaporation, the black oily residue was distilled under vacuum (88°C, 1.7 mm Hg) to yield a pale yellow oil. On cooling the distillate solidified to give 191 g (83%) 1,2,3-benzothiadiazole, mp 35°C.

In most cases it was necessary to synthesize the 2-aminobenzenethiols although these were not usually purified prior to diazotization. Routes employed included the reduction and diazotization of appropriately substituted diphenyl dinitrodisulfides (Method Ia) (11), thiolation of 2-halogenoanilines or nitrobenzenes (Method Ib) (12, 14), and ring opening of 2-amino- or 2-methyl-benzothiazoles (Method Ic).

Method Ia will be illustrated with reference to the synthesis of 5-chloro-1,2,3-benzothiadiazoles (III) from 2,2′-dinitro-4,4′-dichlorodiphenyldisulfide (II). The latter material (20 g, 0.053 mol) was stirred in 90% acetic acid with 64 g of 20-mesh zinc at room temperature and after the initial evolution of heat had ceased the mixture was refluxed for 30 min. The resulting mixture was filtered hot, and the residue washed with hot 90% acetic acid (250 ml) prior to cooling, suspension in cold H_2SO_4 (D = 1.84, 40 ml), and diazotization with a solution of sodium nitrite. The diazo-solution was poured over ice, extracted into ether, and after clean-up on a column of silica gel was recrystallized from methanol to give white needles mp 103–104°C (yield 23%). Similar reduction and diazotization of 2,2′, 4,4′, tetranitro diphenyldisulfide followed by heating or treatment with the appropriate Sandmeyer reagent (11) yielded compounds XI, XXIII, and XXXVI.

In Method Ib, the 2-aminobenzenethiol is prepared by the reaction between the corresponding 2-halogeno-5-nitroaniline, sodium sulfide, and sodium bicarbonate (12). In the preparation of 5-nitro-1,2,3-benzothiadiazole (XXVII) an aqueous solution (170 ml) containing sodium sulfide nonahydrate (62.3 g, 0.26 mol) and sodium bicarbonate (21.8 g, 0.26 mol) was added over 1 hr to a stirred, vigorously refluxing solution of 2-bromo-5-nitroaniline (37.5 g, 0.173 mol) in ethanol (400 ml). Refluxing was continued for $2\frac{1}{2}$ hr when the mixture was diluted with 100 ml of an aqueous solution of NaOH (6.91 g), poured over ice (1000 g), filtered, and the cold filtrate neutralized with HCl. The mixture was immediately diazotized with sodium nitrite and H_2SO_4 and was steam-distilled to yield 7.67 g (25%) of XXVII obtained as yellow needles (mp 142–143°C) from ethanol.

The ring opening of 2-amino- or 2-methyl-benzothiazoles (Method Ic) with nucleophilic reagents such as hydrazine hydrate (16) or boiling KOH (17) provided a useful synthetic route to the 1,2,3-benzothiadiazoles. Thus in the preparation of 6-ethoxy-1,2,3-benzothiadiazole (XXXXI), 2-amino-6-ethoxy-benzothiazole (62 g, 0.32 mol) was refluxed with KOH (200 g) and water (400 ml) for 15 hr at 160°C. The mixture was cooled, diluted with water (500 ml), the solids filtered off, and the filtrate adjusted to pH 6.5 with HCl (D = 1.2) below 10°C. After collection by filtration and drying *in vacuo* the crude thiol (20 g) was dissolved in H_2SO_4 (D = 1.84, 100 ml), and sodium nitrite (8.5 g in 50 ml H_2SO_4) was slowly added with stirring below 5°C. After cleanup the product was purified by passage through a silica gel column in methylene chloride and yielded 9.4 g (16%) of 6-ethoxy-1,2,3-benzothiadiazole (XXXXI) which recrystallized as white needles (mp 102°C) from methanol–water. A similar procedure was employed in the synthesis of compounds II, IV, VIII, XXVIII, XXXX, XXXXIV, XXXXV, and XXXXVI.

A convenient synthetic route to several substituted 1,2,3-benzothiadiazoles was afforded by the modified Herz reaction on the substituted aniline (19) (Method II). This is typified by the preparation of 6-*n*-butyl-1,2,3-benzothiadiazole (XXXXIX). To a solution of 4-*n*-butylaniline (50 g, 0.335 mol) in glacial acetic acid (50 ml) was added with stirring sulfur monochloride (180 ml) 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 hr and the mixture was left stirring overnight at room temperature. Benzene (300 ml) was added and the thiazolium salt was filtered off and washed several times with benzene until the washings were colorless. When dry, the material was added to 50% H_2SO_4 (500 ml) and heated to 60°C for 30 min prior to cooling to −5°C and slow addition with stirring of sodium nitrite (40 g, 0.58 mol) in water (50 ml). The diazo-mixture was poured over ice and left overnight before extraction into diethyl ether. Purification was effected by elution through a silica gel column in dichloroethylene and yielded 5.7 g (9%) of 6-*n*-butyl-1,2,3-benzothiadiazole (XXXXIX) which crystallized as pale yellow needles (mp 110–111°C) from ethanol. Compounds XIV, XVI, XVII, XXI, and XXXXIII were obtained by this general method (Table I).

Most of the compounds in Table I were synthesized by a variety of methods affecting modifications in existing substituents in the aromatic ring. Among the reactions employed were nucleophilic substitution reactions with 4- or 6-halogeno-1,2,3-benzothiadiazoles (Method IIIa; XXII, XXIV, XXXIX, XXXXII) (21), reduction of nitro-1,2,3-benzothiadiazoles to the corresponding amino derivatives with stannous chloride (Method IIIb; XXXII, XXXV, XXXXVIII) (24) diazotization followed by hydrolysis of Sandmeyer reaction of amino-1,2,3-benzothiadiazoles (Method IIIc; V, VI, IX, X, XII, XIII, XXV, XXXVII, XXXVIII) (25), halogenation of amino-1,2,3-benzothiadiazoles (Method IIId; XVIII, XIX, XX), nitration with potassium nitrate (Method IIIe; XXX, XXXI) (23), and deamination and rearrangement (Method IIIf; XXVI) (23). 6-Nitrobenzothiazole (L) was obtained by the controlled nitration of benzothiazole (26) and the phenyl-1,2,3-thiadiazoles (LI–LIII) and oxadiazole (LIV) were synthesized from ethyl benzoylacetate oxime according to established procedures (27, 28).

All melting points are uncorrected and elemental analyses were provided by Schwarzkoff Microanalytical Laboratory, Woodside, N.Y.

Bioassay. The synergistic activity of the substituted 1,2,3-benzothiadiazoles and related compounds was determined by the extent to which they increased the toxicity

TABLE 2

Synergistic Activity of Substituted 1,2,3-Benzothiadiazoles with Carbaryl against the WHO Standard Insecticide Susceptible Strain of House Flies

Compound	LD_{50} carbaryl (μg/fly)	SR[a]	Compound	LD_{50} carbaryl (μg/fly)	SR
I	1.50	67	XXVI	2.35	43
II	0.90	111	XXVII	0.61	164
III	0.90	111	XXVIII	0.65	154
IV	0.80	125	XXIX	1.30	77
V	0.92	109	XXX	2.20	46
VI	0.44	227	XXXI	6.70	15
VII	0.74	135	XXXII	26.0	3.8
VIII	0.66	151	XXXIII	17.2	5.8
IX	0.85	118	XXXIV	18.2	5.5
X	0.33	303	XXXV	23.0	4.3
XI	0.27	371	XXXVI	—	—
XII	0.50	200	XXXVII	0.48	209
XIII	1.60	62	XXXVIII	1.05	95
XIV	1.10	91	XXXIX	—	—
XV	0.29	345	XXXX	0.61	164
XVI	1.75	57	XXXXI	0.27	371
XVII	N.A.	—	XXXXII	0.48	209
XVIII	20.0	5	XXXXIII	0.22	454
XIX	13.7	7.3	XXXXIV	0.70	149
XX	22.0	4.5	XXXXV	0.65	154
XXI	0.62	161	XXXXVI	0.37	271
XXII	27.5	3.6	XXXXVII	0.26	385
XXIII	18.5	5.4	XXXXVIII	9.0	11
XXIV	8.0	13	XXXXIX	2.15	47
XXV	11.0	9			

[a] SR is synergistic ratio calculated from an LD_{50} for carbaryl alone of 100 μg/fly.

of carbaryl to 3-day-old adult female house flies of the standard insecticide susceptible World Health Organization strain. The required solutions of carbaryl and the test synergists were prepared in acetone at a constant 1:5 ratio and were applied topically in 1-μl aliquots to the dorsal thoraces of the flies by means of an Arnold hand microapplicator (Burkard Manufacturing Co. Ltd, Rickmansworth, U.K.) containing an Agla glass syringe with 27-gauge needle. Twenty flies were used at each concentration, the insects being treated under carbon dioxide anesthesia and subsequently held at 28°C in unwaxed 8-ounce containers with plastic covers provided with dental rolls soaked in 20% sucrose. Mortality was assessed 24 hr after treatment. Tests with at least five concentrations were conducted with different batches of flies on each of three different days and the mean 24-hr mortality at each concentration was plotted on log × probability paper. The log dosage-mortality line was fitted by eye and LD_{50} values obtained directly from these lines. Due to the marked tolerance of the flies to carbaryl alone, the LD_{50} of the insecticide could not be determined directly. Extrapolation showed that it was at least 100 μg/fly and this value was used in calculating the synergistic ratios (SR = LD_{50} of carbaryl alone/LD_{50} of carbaryl plus synergist) shown in Tables 2 and 3. None of the synergists showed any insecticidal activity themselves at concentrations fourfold

greater than that present in combination with the insecticide at the LD_{50} level.

Regression analysis. Correlations were determined by multiple 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 (OLS) including estimates of a variety of related statistics.

RESULTS AND DISCUSSION

Synergistic Activity of 1,2,3-Benzothiadiazoles

The synergistic activity of the series of compounds (I–XXXXIX) is shown in Table 2 from which it is clear that large variations in activity result from relatively small structural changes in the molecule.

In contrast to the reported inactivity of methylenedioxybenzene (29, 30) the unsubstituted 1,2,3-benzothiadiazole (I) itself exhibits appreciable synergistic activity (SR = 67) and substitution with a variety of groups in the aromatic ring leads to several excellent synergists for carbaryl. It appears to make little or no difference, however, whether these are electron withdrawing or donating groups.

Activity of the monohalogenated derivatives of 1,2,3-benzothiadiazole (II–XIV) depends on both the nature and position of the substituent and increases in the order F < Cl < Br < I for those in the 4, 5, and 6 positions of the ring. This suggests that activity is associated in part with the size of the halogen atom as can be seen from Fig. 1 in which SR is plotted against the van der Waals radii of the halogen atoms (31). The effect is most marked with the 4- and 5-substituted compounds, less with the 6-substituted derivatives, and not all with those containing 7-halogeno groups. Indeed the activity of the 7-halogeno-1,2,3-benzothiadiazoles appears to decrease somewhat with increasing size of the group (Fig. 1;

TABLE 3

Synergistic Activity of 1,2,3-Benzothiadiazoles; Observed vs Calculated Data

Compound	Substituent	$\Sigma\pi^a$	$\Sigma\sigma^b$	$\Sigma\sigma'^c$	Observed[d] log SR	Calculated[e] log SR	Δlog SR (obs–calc.)	Calculated[f] log SR	Δlog SR (obs–calc.)
I	Unsubstituted	0.00	0.00	0.00	1.826	1.816	+0.010	1.782	+0.044
III	5-Chloro	0.76	0.373	0.03	2.045	2.126	−0.081	2.150	−0.105
IV	6-Chloro	0.70	0.227	0.03	2.097	2.098	−0.001	2.123	−0.026
VII	5-Bromo	0.94	0.391	0.11	2.130	2.288	−0.158	2.303	−0.173
VIII	6-Bromo	1.02	0.232	0.11	2.179	2.331	−0.152	2.339	−0.160
XXVII	5-Nitro	0.11	0.710	0.47	2.215	2.271	−0.056	2.262	−0.047
XXVIII	6-Nitro	0.24	0.778	0.47	2.188	2.312	−0.124	2.320	−0.132
XV	5,6-Dichloro	1.46	0.600	0.06	2.538	2.554	−0.016	2.491	+0.047
XXXXIII	5-Methoxy-6-chloro	0.82	0.342	0.43	2.657	2.516	+0.141	2.543	+0.114
XXXXVII	5-Methyl-6-chloro	1.21	0.158	0.12	2.586	2.450	+0.136	2.434	+0.152
XXXXIV	5-Methyl	0.51	−0.069	0.09	2.173	2.069	+0.104	2.093	+0.081
XXXXV	6-Methyl	0.52	−0.170	0.09	2.188	2.073	+0.115	2.097	+0.091
XXXXVI	5,6-Dimethyl	1.03	−0.239	0.18	2.433	2.400	+0.033	2.408	+0.025
XXXX	6-Methoxy	−0.04	−0.268	0.40	2.215	2.166	+0.049	2.130	+0.085

[a] Values reported for phenoxyacetic acids (37) (π_m for 5-substituents, π_p for 6-substituents).
[b] Taken from (38) (σ_m for substituent, σ_p for 6-substituents).
[c] Taken from (39).
[d] From Table 2.
[e] From Eq. [9].
[f] From Eq. [6].

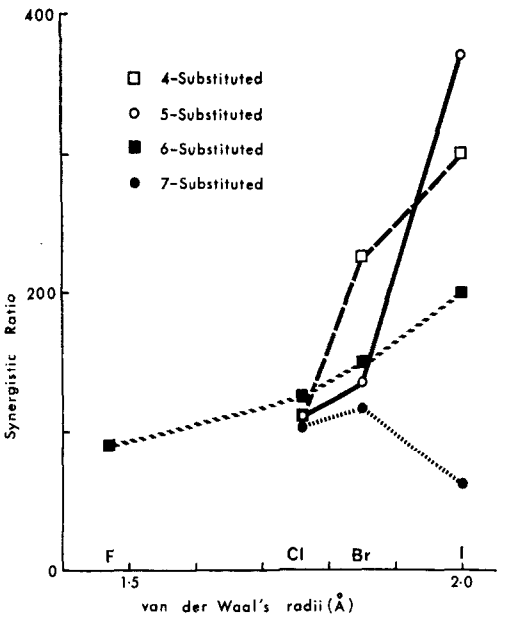


FIG. 1. *Synergistic activity of monohalogenated 1,2,3-benzothiadiazoles in relation to the van der Waal's radii of the halogen atoms.*

Table 2) suggesting the possible importance of steric factors at this position of the ring.

This effect of substituents in the 7-position is also seen with the cyano compounds (compare XXXVII with XXXVIII) and is particularly clear with the nitro-derivatives (XXVI–XXIX) although in this case both the 4- (XXVI) and 7-nitro-1,2,3-benzothiadiazoles (XXIX) have considerably lower activity than the corresponding 5- and 6-compounds. If the nitro group in the 7-position lies in the plane of the aromatic ring (coplanar), a position favored by resonance interactions (32), its effective size will be similar to that of the iodine atom. Under these conditions it is likely to exert a relatively larger steric influence on synergistic activity.

Of the other monosubstituted 1,2,3-benzothiadiazoles evaluated, high synergistic activity was exhibited by compounds containing alkoxy (XXXX–XXXXII), cyano (XXXVII), and methyl (XXXXIV, XXXXV) groups in the 5- or 6-positions of the ring. Rather surprisingly 6-*n*-butyl-1,2,3-benzothiadiazole (XXXXIX) showed little activity. Substitution with amino or hydroxyl groups resulted in compounds with little or no activity. Although no penetration studies were made it seems likely that the presence of these groups will reduce the lipophilicity of the compounds and consequently slow the rate of cuticular penetration (29). Even if they are able to penetrate to the target site it is probable that they will be subject to rapid metabolic conjugation through the amino and hydroxyl groups.

The most active compounds tested were the 5,6-disubstituted 1,2,3-benzothiadiazoles particularly the 5,6-dichloro (XV), 5-methyl-6-chloro (XXXXVII), and 5-methoxy-6-chloro (XXXXIII) derivatives; the latter with a SR of 454 is among the most active carbaryl synergists reported. In contrast, disubstitution in the 6,7-positions usually resulted in compounds of low activity, a fact probably reflecting the steric hindrance associated with substituents in the 7-position. The latter is probably also the cause of the low activity associated with the two tetra-halogenated compounds (XVI, XVII) which were tested.

Regression Analysis

In recent years considerable advances have been made with regard to the quantitative expression of biological activity in terms of equations based on fundamental physicochemical properties such as the lipophilic and electronic character of the molecule (33–36). In view of the valuable information which can often be obtained from by this approach the data in Table 2 were analyzed by regression analysis using various forms of Eq. [1] where SR is the synergistic ratio for the 1:5 carbaryl:synergist combination, π is the hydrophobic

$$\log \mathrm{SR} = a\pi^2 + b\pi + \rho\sigma + k \qquad [1]$$

bonding constant (37), σ the Hammett constant (38), and a, b, ρ, and k are constants obtained from the analysis; homolytic free radical constants ($\sigma\cdot$) were sometimes employed instead of σ (39).

Only the data from compounds with substituents in the 5-, 6-, or 5,6-positions of the 1,2,3-benzothiadiazole ring were analyzed and even here the analysis was somewhat restricted due to the availability of appropriate constants for some substituents. The effect of substituents in the 4- and 7-positions were not included because it was considered that the σ values for these would be affected by the steric factors previously discussed.

As a result of the asymmetry of the 1,2,3-benzothiadiazole ring, substituents at the 5-position are *meta* to the hetero-nitrogen atom of the ring (position 3) and *para* to the hetero sulfur atom (position 1); a similar, though reversed situation exists with respect to 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 values for substituents in the 5- and 6-positions. In agreement with reports that the electronic effects of aromatic substituents in benzothiazoles are transmitted to the heterocylic ring mainly through the hetero-nitrogen atom (40) better correlations with the 1,2,3-benzothiadiazoles were always obtained using σ_m and σ_p values for the 5- and 6-positions, respectively. These are the values which were employed in the regression analyses reported here. This is also true for the π values which are those reported by Fujita *et al.* (37) for phenoxyacetic acids.

Analysis by the method of least squares was conducted with 14 5-, 6-, and 5,6-substituted compounds (Table 3) for which appropriate constants were available. In the following equations n represents the number of compounds employed in the analysis, s is the standard error, and r is the correlation coefficient.

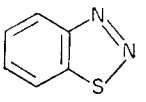
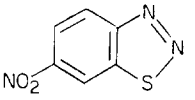
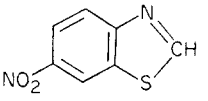
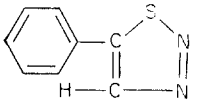
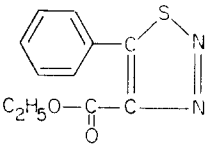
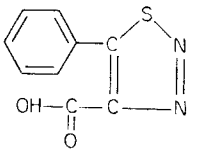
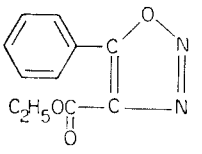
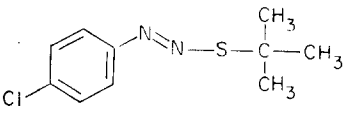
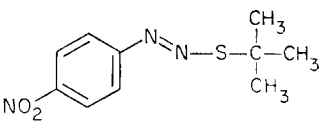
The poor correlation coefficients obtained with Eqs. [2]–[4] establish that the hydrophobic, electronic, and free radical parameters do not by themselves account for synergistic activity. This is not entirely surprising in view of the complex series of events upon which *in vivo* activity depends. Consequently equations containing combinations of more than one parameter were investigated. More satisfactory results were obtained with equations in terms of the hydrophobic bonding constant (π) and either σ [5] or $\sigma\cdot$ [6], the latter giving an r value of 0.882.

	n	r	s	
$\log SR = 0.300\pi + 2.049$	14	0.608	0.188	[2]
$\log SR = 0.070\sigma + 2.232$	14	0.105	0.236	[3]
$\log SR = 0.418\sigma\cdot + 2.171$	14	0.321	0.225	[4]
$\log SR = 0.229\pi + 0.017\sigma + 2.046$	14	0.609	0.917	[5]
$\log SR = 0.448\pi + 0.916\sigma\cdot + 1.782$	14	0.882	0.117	[6]
$\log SR = 0.192\pi^2 + 0.053\pi + 2.091$	14	0.632	0.201	[7]
$\log SR = 0.191\pi^2 + 0.053\pi + 0.005\sigma + 2.090$	14	0.632	0.192	[8]
$\log SR = 0.138\pi^2 + 0.267\pi + 0.903\sigma\cdot + 1.816$	14	0.891	0.118	[9]

Equations [7]–[9], each of which contains a π^2 term, exhibit only slightly higher correlation coefficients than 2, 5, and 6, [9] being the most satisfactory. However, although Eq. [9] is statistically significant at the 99% confidence level, $F(3, 10) = 12.802$, further analysis of the statistical significance of individual pa-

TABLE 4

Synergistic Activity of Compounds Structurally Related to 1,2,3-Benzothiadiazoles

Compound	Structure	LD_{50} carbaryl (μg/fly)	SR[a]
I		1.50	66
XXVIII		0.65	154
L		N.A.*	–
LI		1.06	94
LII		1.50	66
LIII		N.A.*	–
LIV		4.80	21
LV		1.50	66
LVI		2.42	41

[a]SR is synergistic ratio calculated from an LD_{50} for carbaryl alone of 100 μg/fly.

rameters in Eq. [9] (T-values) indicated no justification for the inclusion of π^2. This is further illustrated by the data in Table 3 which show good agreement between the observed values of log SR and those calculated using Eqs. [6] and [9]. That the constant for π^2 is positive indicates that within the range of compounds evaluated, biological activity increases in a manner which is linear and not parabolic with respect to π.

These results suggest that the synergistic activity of the 1,2,3-benzothiadiazoles depends on both their lipophilic character and their ability to form homolytic radicals. The latter is of particular interest since it has also been implicated in the mode of action of the 1,3-benzodioxole synergists (41). Furthermore, Marshall and Wilkinson (42) working with several nonenzymatic model oxidation systems have demonstrated the ability of the 1,3-benzodioxoles, the 1,2,3-benzothiadiazoles, and the phenyl-2-propynyl ethers to interact with a modified Fenton's reagent and other systems considered to generate primarily OH· radicals. In the case of the 1,2,3-benzothiadiazoles this interaction appeared to involve radical formation at the aromatic carbon adjacent to the nitrogen of the heterocyclic ring possibly through a hydrogen abstraction mechanism. However, it is not yet clear precisely how this relates to the inhibitory action at cytochrome P-450 and the *in vitro* activity of the 1,2,3-benzothiadiazoles will be the subject of a subsequent report.

Structural Requirements of the Heterocyclic Ring

Based on the low or nonexistent activity of a variety of heterocyclic ring systems including the 1,2,3-benzothiadiazoles, 1-(H)-benzotriazoles, benzofuranes, and indoles, Felton *et al.* (9) concluded that synergistic activity was specifically associated with the 1,2,3-benzothiadiazole nucleus.

In order to further investigate this, several other compounds with structures closely resembling the 1,2,3-benzothiadiazoles were evaluated as carbaryl synergists. The results are shown in Table 4. As illustrated by 6-nitrobenzothiazole (L) none of several benzothiazoles tested exhibited any observable synergistic activity in combination with carbaryl. However, the 94-fold synergism observed with 5-phenyl-1,2,3-thiadiazole (LI) is approx 1.5-fold higher than that obtained with 1,2,3-benzothiadiazole (I) and clearly shows that fusion of the heterocyclic ring to the aromatic moiety is not an essential prerequisite for activity. The 4-carboxyethyl-5-phenyl-1,2,3-thiadiazole (LII) was also quite effective and the lack of activity of the corresponding 4-carboxy derivative (LIII) is probably due to its high polarity. The slight activity of the oxadiazole (LIV) is also of interest but unfortunately the corresponding 1,2,3-benzoxadiazoles cannot be synthesized. Combined with the data of Felton *et al.* (9) these results indicate that the diazosulfide group plays an important role in synergistic activity a conclusion which is further strengthened by the activity of compounds LV and LVI which are essentially open chain analogs of the 1,2,3-benzothiadiazoles.

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