Table III. Recovery by P-A-E Method of Atrazine Added to Water Extracts of Soils

Soil Type	Soil pH	Organic Matter, %	Clay, %	Atrazine, μg. Added	Atrazine, μg. Found	Recovery, %
Poygon sil	6.9	13	26	6.16	6.36	103
Ella ls	4.6	4	5	6.16	6.40	104
Kewaunee c	7.3	2	40	6.16	6.36	103

of atrazine to hydroxyatrazine. Interferences in the ultraviolet method are numerous when applied to determination of atrazine in a microbiological medium. Furthermore, in investigations requiring determination of atrazine and its degradation product, hydroxyatrazine, the preferred method is colorimetric determination of atrazine and ultraviolet determination of hydroxyatrazine after removal of atrazine by chloroform extraction (*1*). No interference in the colorimetric determination of atrazine is encountered from the presence of hydroxyatrazine.

Both the P-A and P-A-E techniques were investigated for the determination of simazine and propazine. The factors affecting the determination of atrazine were similarly important in the determination of the other two *s*-triazine herbicides. The absolute absorbance for each compound was different, and the techniques are probably readily adaptable to the analysis of other 2-chloro-*s*-triazine herbicides.

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STRUCTURE AND SYNERGISM

Some Structural Requirements of Methylenedioxyphenyl Derivatives as Synergists of Carbamate Insecticides

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Sixty-two compounds, many of them new, were evaluated as synergists for 1-naphthyl *N*-methylcarbamate (carbaryl), 3,4-dimethoxyphenyl *N*-methylcarbamate, and 4-dimethylamino-3,5-xylenyl *N*-methylcarbamate against the common housefly. Maximum synergistic activity is associated with the planar methylenedioxyphenyl ring system. However, replacement of oxygen by sulfur causes only a slight decrease in activity. Synergistic activity in esters of piperonyl alcohol is considerably modified by the nature and position of the side chain on the alpha-carbon.

The essentiality of the 3,4-methylenedioxyphenyl moiety (1,3-benzodioxole) in synergism of the pyrethrins with sesamin was established by Haller *et al.* (*26, 27*). This discovery led to the evaluation of thousands of compounds as potential pyrethrin synergists (*8, 28, 32*) and several, such as piperonyl butoxide, piperonyl cyclonene, *n*-propyl isome, sulfoxide, sesoxane, and sesamex, have become commercially important. However, few structure-activity relationships have been developed. Moore and Hewlett (*37*) demonstrated the inactivity of pyrethrin synergists of compounds containing isopropylidenedioxy (2,2-dimethyl-1,3-benzodioxole), carbonyldioxyphenyl (1,3-benzodioxole-2-one), and ethylenedioxyphenyl (1,4-benzodioxane) groups (*21, 45*).

The discovery of Moorefield (*38, 39*) that the established methylenedioxyphenyl synergists could greatly enhance the activity of the carbamate insecticides stimulated renewed interest in the field, chiefly because of their possible potential use in combating carbamate-resistant strains of insects. A considerable amount of work has since been undertaken on a variety of aspects of carbamate synergism (*22, 24, 25, 33, 34*). However, the only study relating synergist structure to carbamate synergism is that of Moorefield and Weiden (*40*), who evaluated various substituted benzyl acetals and related compounds as synergists for carbaryl (Sevin) and concluded that the 1,2-methylenedioxyphenyl structure was important for maximum synergism.

In contrast to the situation with the pyrethrins, where little is known regarding the site or mode of action or the detoxication process, considerably more data are available on these aspects of carbamate toxicology (*13, 14, 19, 20*). Therefore, it appeared of value to study the structure-activity relations for carbamate synergists in order to further the knowledge of the mode of action of these compounds and to clarify the detoxication pathways of carbamates in susceptible and resistant strains of insects. Three major aspects were studied: the effect of the nature of the side chain on activity of the 1,3-benzodioxole nucleus, the effect of nuclear substitution, and the effect of alterations in the 1,3-benzodioxole ring.

Materials and Methods

The physical constants of the compounds synthesized and evaluated as carbamate synergists in this investigation

are shown in Tables I, II, and III. Elemental analyses of new compounds were made by C. F. Geiger, Ontario, Calif.

Carbinols. Other than piperonyl alcohol (I), prepared according to Davidson and Weiss (*18*), and 5-(1-hydroxy-2,2,2 - trichloroethyl) - 1,3 - benzodioxole (XXXI), obtained in a manner similar to that described by Howard (*30*), all carbinols were prepared by two general methods: A, treatment of the corresponding aldehyde with the respective alkyl Grignard reagent, and B, reduction of the corresponding ketone with lithium aluminum hydride (*42*). Most of the carbinols were high boiling oils and were purified by distillation in a falling-film molecular still.

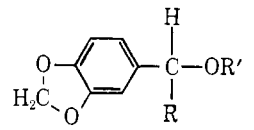
Method A enabled the direct synthesis of the α-substituted piperonyl alcohols (VI to XVIII) from piperonal, and was also used in the synthesis of 6-(1-hydroxyethyl)-1,4-benzodioxane (XXXXIII), 4-(1 - hydroxyethyl) - 1,3 - benzodioxole (XXXXV), 5-(1-hydroxyethyl)-indane (XXXXVII), 4-(1-hydroxyethyl)-1,2-dimethoxybenzene (LVII), and 3-(1-hydroxyethyl)-1,2-dimethoxybenzene (LIX). Method B was employed in the preparation of 5-(1-hydroxyethyl)-2,3 - dihydrobenzofuran (XXXXIX), 5 - (1 - hydroxyethyl) - 2,3 - dihydrobenzothiophene (LI), 5-(1-hydroxyethyl)-1,3-benzoxathiole (LIII), 5-(1-hydroxyethyl) - 2,2 - dimethyl - 1,3-benzodioxole (LV), and 2-(1-hydroxyethyl)-naphthalene (LXI). The various aldehyde and ketone intermediates required for these preparations were either obtained from commercial sources or synthesized according to the procedures outlined below.

Intermediate Aldehydes. 6-Formyl-1,4-benzodioxane (3,4-ethylenedioxybenzaldehyde) (m.p. 48–49° C.) was prepared by the Adams modification of the Gatterman aldehyde synthesis (*1, 2, 23*) using zinc cyanide and hydrogen chloride on 1,4-benzodioxane, and 5-formylindane was prepared according to the method described by Arnold (*4*).

4 - Formyl - 1,3 - benzodioxole (2,3-methylenedioxybenzaldehyde) was prepared by the Rosenmund reduction (*41*) of the acid chloride of 1,3-benzodioxole-4-carboxylic acid (*44*). To a dry 300-ml. three-necked flask was added 9.3 grams of acid chloride of 1,3-benzodioxole-4-carboxylic acid (0.05 mole) in dry xylene. The catalyst (1.3 grams) and 1.3 ml. of regulator (*41*) were added, and hydrogen gas was passed slowly into the mixture, heated at 160° C. with continuous stirring. The exit gases were passed into a 500-ml. Erlenmeyer flask containing 200 ml. of water and 2 drops of phenolphthalein, and the hydrogen chloride evolved was estimated with 5.*N* sodium hydroxide. Following the liberation of 90+% of the theoretical amount of hydrogen chloride, the reaction mixture was cooled and filtration of the mixture after addition of activated carbon yielded a clear solution. The product, 4-formyl-1,3-benzodioxole, was

Table I. Physical Constants of Compounds Synthesized for Investigations on Effect of Side Chain Variation on the Activity of the 1,3-Benzodioxole Nucleus

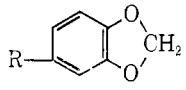
General structure:



	R	R'	B.P. °C.	B.P. Mm.	n_D^{25}	Analysis Calcd.	Analysis Found
I	H	H	49.5–50.5	(m.p.)	...		
II	H	CH_3CO	117–21	1.0	1.5250	C 61.54 H 5.64	C 61.49 H 5.31
III	H	C_2H_5CO	123–25	1.0	1.5181	C 63.46 H 5.80	C 63.97 H 6.05
IV	H	C_3H_7CO	130–35	1.5	1.5119	C 64.86 H 6.31	C 65.07 H 6.33
V	H	C_6H_5CO	61.5–62	(m.p.)	...		
VI	CH_3	H	115	0.5	1.5518	C 65.06 H 6.02	C 65.29 H 6.02
VII	C_2H_5	H	105	0.2	1.5415	C 66.66 H 6.66	C 66.28 H 6.80
VIII	n-C_3H_7	H	140	1.5	1.5375	C 68.04 H 7.21	C 68.48 H 7.17
IX	n-C_4H_9	H	144	1.0	1.5339	C 69.23 H 7.69	C 69.10 H 7.35
X	n-C_5H_{11}	H	156	1.5	1.5219	C 70.27 H 8.11	C 69.88 H 8.49
XI	n-C_6H_{13}	H	168	1.5	1.5135	C 71.18 H 8.47	C 70.93 H 8.62
XII	n-C_7H_{15}	H	170	1.0	1.5095	C 72.00 H 8.80	C 71.67 H 8.66
XIII	n-C_8H_{17}	H	177	1.0	1.5030	C 72.72 H 9.09	C 71.27 H 9.28
XIV	n-C_9H_{19}	H	186 (semisolid)	1.5	1.5035	C 73.38 H 9.35	C 73.47 H 9.56
XV	n-$C_{10}H_{21}$	H	198 (waxy solid)	1.5	...	C 73.93 H 9.59	C 73.50 H 10.56
XVI	Iso-C_3H_7	H	110	0.5	1.5459	C 68.03 H 7.21	C 67.60 H 6.89
XVII	*tert*-C_4H_9	H	140	1.0	1.5352	C 69.23 H 7.69	C 68.65 H 7.41
XVIII	Cyclohexyl	H	145 (waxy solid)	1.5	...	C 71.80 H 7.69	C 71.88 H 8.08
XIX	CH_3	C_6H_5CO	55.0–56.5	(m.p.)	...	C 71.11 H 5.18	C 71.26 H 5.48
XX	C_2H_5	C_6H_5CO	131	0.5	1.5628	C 71.83 H 5.63	C 71.97 H 5.65
XXI	n-C_3H_7	C_6H_5CO	126	0.3	1.5565	C 72.48 H 6.04	C 72.83 H 6.33
XXII	n-C_4H_9	C_6H_5CO	153	1.0	1.5529	C 73.08 H 6.41	C 73.78 H 6.45
XXIII	n-C_5H_{11}	C_6H_5CO	150	0.6	1.5517	C 73.62 H 6.74	C 73.90 H 6.56
XXIV	n-C_6H_{13}	C_6H_5CO	152	0.4	1.5414	C 74.12 H 7.06	C 73.52 H 7.02
XXV	n-C_7H_{15}	C_6H_5CO	168	1.0	1.5335	C 74.57 H 7.34	C 74.67 H 7.74
XXVI	n-C_8H_{17}	C_6H_5CO	162	0.5	1.5285	C 75.00 H 7.61	C 74.98 H 7.90
XXVII	n-C_9H_{19}	C_6H_5CO	173	0.4	1.5283	C 75.39 H 7.85	C 75.11 H 8.01
XXVIII	Iso-C_3H_7	C_6H_5CO	120	0.6	1.5600	C 72.48 H 6.04	C 72.18 H 5.96
XXIX	*tert*-C_4H_9	C_6H_5CO	104.5–105	(m.p.)	...	C 73.08 H 6.41	C 73.23 H 6.73
XXX	Cyclohexy	C_6H_5CO	70–73	(m.p.)	...	C 74.56 H 6.51	C 74.19 H 6.88
XXXI	CCl_3	H	155–60	2.0	...	C 40.07 H 2.60	C 40.22 H 3.08
XXXII	CCl_3	CH_3CO	117–18	(m.p.)	...	C 42.38 H 2.89	C 42.08 H 2.81
XXXIII	CCl_3	C_2H_5CO	50.5–51.5	(m.p.)	...	C 44.24 H 3.38 Cl 32.73	C 45.41 H 3.87 Cl 32.40
XXXIV	CCl_3	C_3H_7CO	51–52	(m.p.)	...	C 45.95 H 3.83 Cl 31.34	C 45.83 H 4.05 Cl 30.95
XXXV	CCl_3	C_6H_5CO	87.5–88.5	(m.p.)	...	C 51.41 H 2.95	C 51.49 H 3.20

Table II. Physical Constants of Compounds Synthesized for Investigations on Effect of Nuclear Substitution on Activity of 1,3-Benzodioxole Nucleus

General structure:



	R	B.P. °C.	B.P. Mm.	n_D^{25}	Analysis Calcd.	Analysis Found
XXXVI	H	45–47	5.0	1.5370		
XXXVII	OH					
XXXVIII	CH_3O	70–73	2.0	1.5390	C 63.16 H 5.26	C 62.77 H 5.53
XXXIX	NO_2	143–144.5	(m.p.)	...	C 50.30 H 2.99	C 51.12 H 3.20
XXXX	$C(CH_3)_3$	65–70	0.6	1.5155	C 74.15 H 7.87	C 74.07 H 7.98
XXXXI	CH_3	39–40	1.5	1.5297	C 70.59 H 5.88	C 70.18 H 6.15
XXXXII	Cl	65–69	2.7	1.5526	C 53.67 H 3.19	C 53.42 H 3.38

collected in yields of 60% by distillation at 90° C. and 3.5 mm., and solidified in the receiver (m.p. 32–33° C.).

Intermediate Ketones. 5-Aceto-2,3-dihydrobenzofuran (m.p. 62–63° C.) was prepared by the Friedel-Crafts acylation of 2,3-dihydrobenzofuran (coumaran) (*15*, *17*) in a manner similar to that described by Arnold and McCool (*5*) for the acylation of 2-methyl-2,3-dihydrobenzofuran, using acetic anhydride, and aluminum chloride in nitrobenzene.

5-Aceto-2,3-dihydrobenzothiophene was obtained in good yield by the Friedel-Crafts acylation (*5*) of 2,3-dihydrobenzothiophene (thiaindane) (*9*). The product was obtained as a solid and recrystallized from ethyl alcohol (m.p. 46–47° C.). Elemental analysis: calculated for $C_{10}H_{10}OS$, C 67.42, H 5.62; found, C 67.19, H 5.36.

5-Aceto-1,3-benzoxathiole was prepared in a yield of 30% by the Friedel-Crafts acylation (*5*) of 1,3-benzoxathiole which was obtained in 62% yield by ring closure of *o*-hydroxybenzenethiol (*30*) with diiodomethane. The product was obtained as a white crystalline solid and was recrystallized from ether (m.p. 129–31° C.). Elemental analysis: calculated for $C_9H_8O_2S$, C 60.00, H 4.44; found, C 59.16, H 5.46.

5-Aceto-2,2-dimethyl-1,3-benzodioxole was prepared by the Friedel-Crafts acylation (*5*) of 2,2-dimethyl-1,3-benzodioxole (*11*). It was collected as a yellow oil (46% yield) by distillation at 110–12° C. and 2.0 mm. Elemental analysis: calculated for $C_{11}H_{12}O_3$, C 68.75, H 6.25; found, C 68.06, H 6.20.

Esters. The benzoate esters (XIX to XXX, V, XXXV, XXXIV, XXXXVI, XXXXVIII, L, LII, LIV, LVI, LVIII, LX, and LXII) were prepared by treating the corresponding carbinol in pyridine with a slight excess of benzoyl chloride. The remaining esters (II to IV, and XXXII to XXXIV) were similarly prepared from the corresponding carbinol and the respective acid anhydride or acyl chloride. The liquids were purified by distillation in a falling-film molecular still, the solids by recrystallization from ethanol.

5-Substituted 1,3-Benzodioxoles. 1,3-Benzodioxole (XXXVI) was prepared by deformylation of piperonal with palladium on charcoal (*17*), and 5-hydroxy-1,3-benzodioxole (sesamol, XXXVII) by the method of Böeseken (*10*). The methyl ether of sesamol (XXXVIII) was prepared by treatment of XXXVII with dimethyl sulfate (*29*). Compounds XXXIX to XXXXII were obtained by ring closure with diiodomethane of the corresponding 4-substituted catechols (*12*).

Evaluation Methods. The synergists were evaluated by the topical application of 5 to 1 (w./w.) combinations in acetone with each of the three carbamates—carbaryl (Sevin or 1-naphthyl *N*-methylcarbamate, Figure 1), 3,4-dimethoxyphenyl *N*-methylcarbamate, and Zectran (4-dimethylamino-3,5-xylenyl *N*-methylcarbamate). One-microliter droplets of the carbamate-synergist combinations were applied to the thoraces of 2- to 3-day-old female houseflies (*Musca domestica* L.) of the susceptible NAIDM strain (*35*). The 5 to 1 ratio of synergist to carbamate was chosen to flood the detoxication system in the fly with synergist and thus to obviate the effects of variations in the molecular weights of the synergists. Thus with synergist XIX the LD_{50} of Sevin in micrograms per gram of fly was 21.0 at 1 to 1, 13.0 at 2 to 1, 10.0 at 3 to 1, and 9.5 at 4 to 1, showing that a maximal response to the synergist was reached.

The activity of the synergists evaluated is indicated in the tables by the synergistic ratio, which is the ratio of the LD_{50} value for carbamate alone to that of the carbamate-synergist combination. Each carbamate-synergist dosage was replicated on three different days and the average per cent mortalities were plotted on log-probit paper and the dosage-mortality regression lines fitted by eye (Figure 1). The LD_{50} values and the synergistic ratios were reproducible with a standard error of less than 10% of the mean for five complete replications. Except where indicated in the tables, the synergists alone were nontoxic at the maximum concentrations employed.

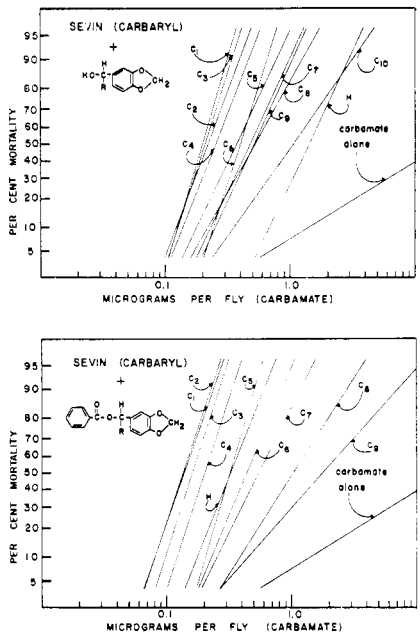


Figure 1. Dosage mortality curves from topical applications to housefly showing synergistic effects of side chain variation in substituted piperonyl alcohols and their benzoates used with carbaryl

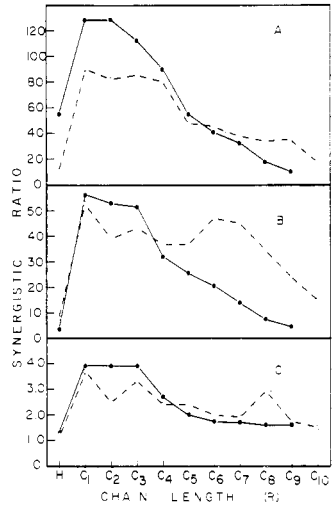


Figure 2. Changes in synergistic ratio with chain length in a series of α-substituted piperonyl alcohols and their benzoate esters

A. Sevin
B. 3,4-Dimethoxyphenyl *N*-methylcarbamate
C. Zectran

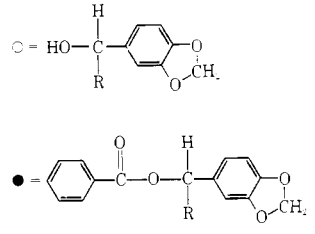
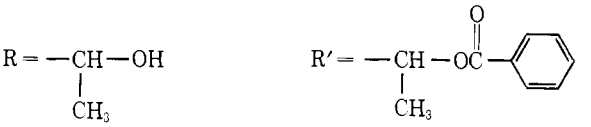
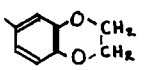
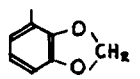
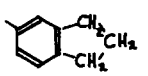
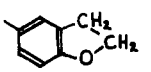
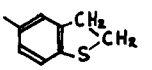
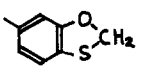
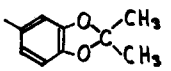
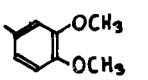
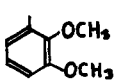
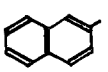


Table III. Physical Constants of Compounds Synthesized for Investigations on Effect of Variations in Bicyclic Ring



No.	Nucleus		B.P. °C.	B.P. Mm.	n_D^{25}	Analysis Calcd.	Analysis Found
XXXXIII		R	115	0.4	1.5652	C 66.67	C 66.73
						H 6.67	H 6.51
XXXXIV		R'	148	0.6	1.5745	C 71.83	C 71.49
						H 5.63	H 5.91
XXXXV		R	105	0.6	1.5465	C 65.06	C 65.34
						H 6.02	H 6.20
XXXXVI		R'	150	0.4	1.5652	C 71.11	C 71.77
						H 5.18	H 5.46
XXXXVII		R	105	2.0	1.5466	C 81.48	C 80.95
						H 8.64	H 8.80
XXXXVIII		R'	120	2.0	1.5683	C 81.20	C 79.66
						H 6.77	H 6.86
XXXXIX		R	122	0.4	1.5634	C 73.17	C 72.87
						H 7.31	H 7.10
L		R'	155	1.0	1.5760	C 76.11	C 75.92
						H 5.97	H 5.88
LI		R	63–67 (m.p.)		...	C 66.67	C 66.84
						H 6.67	H 6.60
LII		R'	68–69 (m.p.)		...	C 71.83	C 72.21
						H 5.63	H 5.72
LIII		R	132	0.2	1.6105	C 59.34	C 59.11
						H 5.49	H 5.47
LIV		R'	[a]		...	C 67.13	C 65.87
						H 4.89	H 5.32
LV		R	118	0.3	1.5289	C 68.04	C 68.30
						H 7.25	H 6.83
LVI		R'	67–70 (m.p.)		...	C 72.48	C 72.46
						H 6.04	H 5.78
LVII		R	95	0.5	1.5415	C 65.93	C 66.46
						H 7.69	H 7.33
LVIII		R'	130	0.5	1.5592	C 71.33	C 71.56
						H 6.29	H 6.21
LIX		R	100	1.0	1.5411	C 65.93	C 65.27
						H 7.69	H 7.23
LX		R'	80–81 (m.p.)		...	C 71.33	C 71.52
						H 6.29	H 6.50
LXI		R	71–72.5 (m.p.)		...	C 83.72	C 83.37
						H 6.97	H 6.78
LXII		R'	53.5–54.5 (m.p.)		...	C 82.61	C 82.59
						H 5.79	H 5.70

[a] Insufficient material for distillation (analysis on crude product).

Discussion of Structure-Activity Relationships

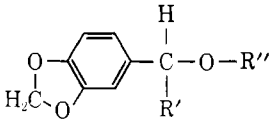
Sevin and 3,4-dimethoxyphenyl *N*-methylcarbamate were chosen because of their low insecticidal activity to the housefly when evaluated alone (LD_{50} values 900 μg. per gram for Sevin and 450 μg. per gram for the 3,4-dimethoxy compound), together with the pronounced synergism which could be obtained with the methylenedioxy synergists such as piperonyl butoxide. Zectran was chosen as an example of a carbamate showing considerable insecticidal activity when used alone (LD_{50} 35 μg. per gram). The LD_{50} values given above for the compounds alone were used throughout the study in evaluating synergistic ratios.

Effects of Side Chain Variation. The results of systematic variations in the side chains of esters of piperonyl alcohol (5-methylol-1,3-benzodioxole) (compounds I to XXXV) are summarized in Table IV and an example is shown in Figure 1. These demonstrate that relatively small and apparently trivial structural modifications result in pronounced differences in the effectiveness of these compounds as carbamate synergists. Piperonyl alcohol (I) is only slightly active, as are its simple esters (II to V), although the benzoate (V) shows considerable activity in combination with Sevin. The replacement of one of the α-hydrogen atoms of piperonyl alcohol by an alkane group, however, results in a remarkable increase in activity (VI to XVII) and this is even more marked in the benzoate esters of these carbinols (XIX to XXX). The results are shown graphically in Figure 2, where it can be seen that a similar pattern exists with each of the three carbamates used. Maximum synergistic activity is shown by the substitution of the short alkane chains, CH_3 (VI, XIX), C_2H_5 (VII, XX), and C_3H_7(VIII, XXI), and any further increase in the chain length is associated with a gradual decline in effectiveness. In each of the three cases, the activity of the benzoate esters, although considerably greater than the corresponding carbinols in the compounds comprising the lower members of the series, tends to decline more rapidly as the series is ascended, so that in the long-chain compounds (C_7 to C_{10}) the carbinols become the most active. The crossover point occurs between C_4 and C_6 in each of the three carbamates studied.

The most active compounds in the series were those containing a trichloromethyl grouping in the α-position (XXXI to XXXV), but in these cases it is possible that the trichloromethyl group itself is endowing the molecule with additional properties not unrelated to those of DDT. Thus, the acetate ester (XXXII) is to some extent toxic, having an LD_{50} of 3 μg. per fly, and is itself slightly synergized (two- to threefold) in combination with piperonyl butoxide. The synergistic activity is nonetheless real, however, as at the concentrations used in combination with the carbamates, it would show little or no inherent toxicity. The remaining esters containing the trichloromethyl grouping (XXXIII to XXXV) are nontoxic at concentrations of 10 μg. per fly, and it is difficult to understand why the acetate of this

Table IV. Effects of Side Chain Variations on Synergistic Activity of Esters of Piperonyl Alcohol with Three Carbamates against Housefly

General structure:



	R′	R″	Synergistic Ratio,[b] Sevin[a]	3,4-Dimethoxyphenyl N-methylcarbamate[c]	Zectran[d]
I	H	H-	12.4	8.7	1.3
II	H	CH_3CO-	25.0	...	1.1
III	H	C_2H_5CO-	20.0	...	1.4
IV	H	C_3H_7CO-	39.1	...	1.2
V	H	C_6H_5CO-	54.5	3.2	1.3
VI	CH_3	H-	90.0	53.0	3.7
VII	C_2H_5	H-	81.8	39.0	2.5
VIII	n-C_3H_7	H-	85.7	43.0	3.3
IX	n-C_4H_9	H-	80.0	37.5	2.4
X	n-C_5H_{11}	H-	47.4	37.5	2.4
XI	n-C_6H_{13}	H-	45.0	47.0	2.0
XII	n-C_7H_{15}	H-	38.3	45.0	1.9
XIII	n-C_8H_{17}	H-	34.6	34.6	2.9
XIV	n-C_9H_{19}	H-	35.3	23.7	1.8
XV	n-$C_{10}H_{21}$	H-	17.8	15.0	1.5
XVI	Iso-C_3H_7	H-	26.8	27.3	1.2
XVII	*tert*-C_4H_9	H-	40.0	30.0	1.8
XVIII	Cyclohexyl	H-	34.6	20.9	2.4
XIX	CH_3	C_6H_5CO-	128.6	56.0	3.9
XX	C_2H_5	C_6H_5CO-	128.6	53.0	3.9
XXI	n-C_3H_7	C_6H_5CO-	112.5	51.4	3.9
XXII	n-C_4H_9	C_6H_5CO-	90.0	32.1	2.7
XXIII	n-C_5H_{11}	C_6H_5CO-	54.5	25.7	2.0
XXIV	n-C_6H_{13}	C_6H_5CO-	40.9	20.4	1.8
XXV	n-C_7H_{15}	C_6H_5CO-	32.7	14.0	1.7
XXVI	n-C_8H_{17}	C_6H_5CO-	17.1	7.7	1.6
XXVII	n-C_9H_{19}	C_6H_5CO-	10.0	4.4	1.6
XXVIII	Iso-C_3H_7	C_6H_5CO-	69.2	53.0	3.6
XXVIX	*tert*-C_4H_9	C_6H_5CO-	40.0	37.5	2.2
XXX	Cyclohexyl	C_6H_5CO-	29.5	20.0	2.4
XXXI	CCl_3	H-	...	...	3.9
XXXII	CCl_3	CH_3CO-[e]	180.0	118.4	11.7
XXXIII	CCl_3	C_2H_5CO-	72.0	56.3	4.4
XXXIV	CCl_3	C_3H_7CO-	54.5	50.0	3.9
XXXV	CCl_3	C_6H_5CO-	62.1	47.4	5.0

[a] LD_{50} = 900 μg./g. fly.
[b] Ratio $\frac{LD_{50}\text{ carbamate alone}}{LD_{50}\text{ carbamate + synergist}}$.
[c] LD_{50} = 450 μg./g. fly.
[d] LD_{50} = 35.0 μg./g. fly.
[e] Slightly toxic (see I-D-2-a).

group should be the only member possessing this activity.

In the writers' view (*37*), the considerable variations observed in the activities of these synergists result from the net effects of the several factors of lipoid solubility as it affects penetration, binding at the surface of the detoxifying enzyme (probably a microsomal oxidase similar to phenolase) (*45*, *47*, *48*), and steric inhibition of binding. Thus the α-substituents might enhance the binding through interaction of van der Waals forces in the case of short chains and yet interfere with this binding in the case of long chains.

The synergized LD_{50} values for each of the carbamates used fall into a remarkably narrow range, despite the considerable variations in the LD_{50} values obtained for the carbamates alone. Thus the synergistic ratios are inversely correlated with the innate toxicity of the carbamate, as discussed by Metcalf *et al.* (*33*) and Georghiou (*24*), those for Sevin and 3,4-dimethoxyphenyl *N*-methylcarbamate being considerably higher than the ones obtained with Zectran, which possesses considerable innate toxicity to the housefly (LD_{50}, 35.0 μg. per gram).

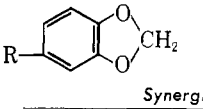
A large number of compounds of a similar nature to the ones discussed here—α-substituted piperonyl alcohols and their esters—have been screened as synergists for pyrethrin and allethrin against the body louse (*Pediculus humanus humanus* L.) and the housefly (*Musca domestica* L.) (*6*, *7*, *16*). Clark and Cole (*16*), in a report on 796 compounds evaluated, included several of the compounds studied here and found that piperonyl alcohol and its simple esters were ineffective as synergists with allethrin and pyrethrin against the body louse. These workers found that in the series of α-substituted carbinols, compounds VI, XVII, XVI, IX, XVIII, and X were active, but for some reason the C_3H_7 derivative (VIII) is reported as inactive. Barthel and Alexander, however, (*6*) report these compounds as ineffective with pyrethrin insecticides against the housefly and body louse, so there appear to be considerable discrepancies in the bioassay techniques used by these authors.

From the evaluation of the carbinol series (VI to XV), it can be seen that the C_2H_5 compound (VII) has a lower activity than either the CH_3 (VI) or C_2H_7 (VIII), so that there appears to be greater activity associated with side chains containing an odd number of carbon atoms (Figure 2). This phenomenon, which is similar to that found in some types of herbicides where it is indicative of the importance of β-oxidation of the alkyl chain, seems to disappear in the longer chain compounds and is not observed in the series of corresponding benzoate esters.

Effects of Nuclear Substitution. In order to define more clearly the minimal molecular requirements for synergistic activity, a number of simple molecules (XXXVI and XXXXII) were synthesized which contained the 1,3-benzodioxole system with a variety of simple groups incorporated into the 5-position of the phenyl ring (Table V). The parent compound, 1,3-benzodioxole (XXXVI), proved to be devoid of any synergistic activity in combination with Sevin or Zectran, and only slight activity was shown by the hydroxy derivative, sesamol (XXXVII). Substitution with CH_3 (XXXXI) gave a considerable in-

Table V. Effects of Nuclear Substitution on Synergistic Activity of 1,3-Benzodioxole with Three Carbamates against Housefly

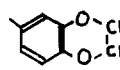
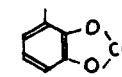
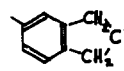
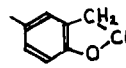
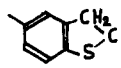
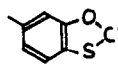
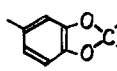
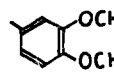
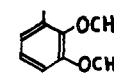
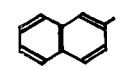
General structure:



	R	Synergistic Ratio, Sevin	3,4-Dimethoxyphenyl N-methylcarbamate	Zectran
XXXVI	H	1.0	...	1.1
XXXVII	OH	4.6	4.1	1.4
XXXVIII	CH_3O	81.8	77.6	4.2
XXXIX	NO_2	90.0	65.7	4.0
XXXX	$C(CH_3)_3$	37.5	49.2	3.4
XXXXI	CH_3	31.5	42.8	2.8
XXXXII	Cl	30.0	32.2	2.3

Table VI. Effects of Variations in Bicyclic Ring System on Synergistic Activity of Benzyl Alcohols and Esters with Three Carbamates against Housefly

General structure: R = —CH(CH$_3$)—OH; R′ = —CH(CH$_3$)—OC(=O)—

	Structure of Ring System	Synergistic Ratio		
		Sevin	3,4-Dimethoxyphenyl N-methylcarbamate	Zectran
XXXXIII		R 3.6	0.6	1.1
XXXXIV		R′ 3.6	0.8	1.03
XXXXV		R 13.5	6.4	1.4
XXXXVI		R′ 72.0	24.0	2.4
XXXXVII		R 1.0	0.8	0.9
XXXXVIII		R′ 1.0	0.8	0.9
XXXXIX		R 19.4	7.0	1.2
L		R′ 30.0	9.0	1.8
LI		R 8.0	2.7	1.3
LII		R′ 10.9	3.6	1.6
LIII		R 66.7	30.0	3.0
LIV		R′ 78.3	42.8	3.9
LV		R 1.0	1.0	1.0
LVI		R′ 1.0	1.0	1.1
LVII		R 2.9	4.3	1.1
LVIII		R′ 4.1	4.5	1.1
LIX		R 4.5	<1.0	1.0
LX		R′ 1.0	<1.0	1.1
LXI		R <1.0	<1.0	<1.0
LXII		R′ <1.0	<1.0	1.1

crease in synergistic activity, showing a synergistic ratio of 30 in the case of Sevin. A similar degree of effectiveness was also demonstrated with the tertiary butyl derivative (XXXX), and to a slightly lesser degree with the corresponding chloro compound (XXXXII), but substitution in the same position with methoxy (XXXVIII) and nitro (XXXIX) groups caused a remarkable increase in synergistic activity. This resulted in synergistic ratios of 81.8 to 77.6 with Sevin and 3,4-dimethoxyphenyl *N*-methylcarbamate, respectively, in the case of the methoxy compound, and ratios of 90.0 and 65.7 with the nitro derivative. Except in the case of Sevin, where the nitro compound was more effective than the methoxy, the same order of effectiveness was found with each of the three carbamates and was $CH_3O > NO_2 > C(CH_3)_3 > CH_3 > Cl > OH > H$. It was observed during the evaluation of these compounds that 5-chloro-1,3-benzodioxole resulted in an extremely rapid knockdown of the treated insects, but many of these recovered during the subsequent 24 hours. The very effective synergist 5-nitro-1,3-benzodioxole, however, showed little evidence of a rapid knockdown, the flies dying much later.

The finding that 5-methoxy-1,3-benzodioxole (XXXVIII) is an effective carbamate synergist is interesting in view of the work of Kerr (*37*), who found that the incorporation of nuclear methoxy groups into a methylene-dioxyphenyl molecule increased the synergistic activity of pyrethrins toward houseflies.

Reports in the literature also indicate the activating effect of a nuclear nitro group. Moore and Hewlett (*37*) thus found that the pinacol acetal of 6-nitropiperonal was active, and a German patent (*3*) protects several other nitro-containing compounds.

Effects of Changes in 1,3-Benzodioxole (Methylenedioxyphenyl) Nucleus. The data in Table VI (compounds XXXIII to LXII) illustrate the results of changes in the nature of the bicyclic nucleus. Because of the activity of 5-(1-hydroxyethyl)-1,3-benzodioxole (VI) and its benzoate ester (XIX), these side chain groups were retained in the compounds considered. Substitution of one of the oxygen atoms in the 1,3-benzodioxole ring (VI and XIX) by a methylene group to give 2,3-dihydrobenzofuran derivatives (XXXXIX and L) causes marked decrease in synergistic activity, about fourfold for Sevin and six- to eightfold for 3,4-dimethoxyphenyl *N*-methylcarbamate. None of the derivatives of 2,3-dihydrobenzofuran (coumaran) seems to have been previously tested as a synergist. Replacement of the remaining oxygen atom of the ring by methylene to give the indane derivatives (XXXXVII and XXXXVIII) resulted in complete loss of activity.

This requirement for oxygen, thus demonstrated, is not absolute, as the sulfur-containing 2,3-dihydrobenzothiophenes (thiaindanes LI and LII) showed some activity and the 1,3-benzoxathiole compounds (LIII and LIV) were about 0.7 as active as VI and XIX. Attempts to synthesize the corresponding 1,3-benzodithiole derivatives failed because of the very unstable nature of this system.

The inactivity of 5-(1-hydroxyethyl)-2,2-dimethyl-1,3-benzodioxole (LV) and its benzoate (LVI) demonstrates that an unsubstituted methylene group in the benzodioxole ring is necessary for synergistic activity, in agreement with the conclusions of Moore and Hewlett (*37*). No 2-methyl-1,3-benzodioxoles were evaluated but a German patent (*43*) suggests, without data, that these possess some synergistic activity.

Two compounds containing the 1,4-benzodioxane (ethylenedioxyphenyl) grouping (XXXXIII and XXXXIV) showed a synergistic ratio of 3.6 with Sevin but no activity with the other two carbamates. Other workers (*21*, *46*) found no synergism of this type of compound with the pyrethrins.

Only slight synergistic activity was shown by the dimethoxy compounds (LVII and LVIII) and the isomers (LIX and LX). This low activity is in accord with results obtained with similar dimethoxyphenyl derivatives as pyrethrin synergists (*16*, *31*).

Comparison of VI and XIX with XXXXV and XXXXVI where the side chain is ortho to the ring shows that the latter condition, which may result in steric hindrance at the site of action, causes a substantial decrease in activity. Two naphthalene derivatives were included as possible competitors for the detoxication system which so efficiently detoxifies Sevin. However, neither the carbinol(LXI) nor its benzoate(LXII) showed any synergistic activity.

Summary and Conclusions

Maximum synergistic activity for the carbamates evaluated is associated with the methylenedioxyphenyl ring system,

compared with the other bicyclic systems studied.

The rigid planar nature of this system, combined with the presence of two oxygen atoms, is considered of major importance in determining the biological activity.

Sulfur can replace oxygen in the system with only a slight decrease in activity.

Synergistic activity of the esters of piperonyl alcohol is considerably modified by the nature and relative position of the side chain attached to the alpha-carbon. This apparently results from such factors as lipoid solubility, steric hindrance, and binding on the active surface of the target enzyme(s).

Minimal requirements for synergistic activity are the presence of the methylenedioxyphenyl nucleus in combination with a simple group such as methyl substituted in the phenyl ring. In such simple compounds, nuclear methoxy and nitro groups greatly enhance synergistic activity.

The best synergist studied, α-methylpiperonyl benzoate (XIX), had synergistic ratios of 128, 56, and 3.9 with Sevin, 3,4-dimethoxyphenyl *N*-methylcarbamate, and Zectran. This compared very favorably with the synergistic ratios at 5 to 1 for piperonyl butoxide of 75, 37.5, and 2.6, respectively.

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STRUCTURE AND ACTIVITY

Effects of Deuteration, Fluorination, and Other Structural Modifications of the Carbamyl Moiety upon the Anticholinesterase and Insecticidal Activities of Phenyl *N*-Methylcarbamates

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The processes of intoxication and detoxication of the aryl *N*-methylcarbamate insecticides are under intensive study in a number of laboratories. There is, however, no general agreement that the ability of these compounds to inhibit cholinesterase (ChE) is either competitive or the result of noncompetitive carbamylation of the esteratic site of the enzyme (*11*) or that detoxication occurs through hydroxylation of the aromatic ring or of the *N*-methyl group, or by hydrolysis (*5, 11*). It appeared that additional evidence regarding both processes might be obtained by investigating the comparative anticholinesterase activity, insecticidal action, and

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