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# Synthesis and Biological Activity of *O,O*-Dialkyl *S*-(5-Aryl-1,3,4-oxadiazol-2(3*H*)-on-3-yl)methyl Phosphorothioates and Phosphorodithioates†

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Some phosphorus derivatives of oxadiazoles were synthesized to seek insecticidal lead compounds. The 1,3,4-oxadiazol-2-ones were converted *via* the *N*-methylol derivatives to the corresponding *N*-chloromethyl derivatives. From these derivatives a variety of *O,O*-dimethyl phosphorodithioates **4**, *O,O*-dimethyl phosphorothioates **5** and *O,O*-di-*i*-propyl phosphorothioates **6** were prepared.

These phosphorus derivatives were examined for insecticidal activity towards houseflies and for anti-acetylcholinesterase (anti-AChE) activity using the housefly heads as an enzyme source. Most of the compounds **4** and **5** showed contact toxicity as high as the analogous methidathion insecticides, which appeared to correlate with the strong anti-AChE activity. On the other hand, all the compounds **6** showed a high activity in AChE inhibition but only a poor insecticidal activity.



*O,O*-Dimethyl *S*-(5-methoxy-1,3,4-thiadiazol-2(3*H*)-on-3-yl)methyl phosphorodithioate [methidathion] is known as a commercial insecticide and acaricide, and some insecticidal analogues have been reported.[1,2] They have the characteristic moiety of $-CON\overset{|}{C}H_2S\overset{|}{P}X-$ in the molecule. On the other hand, Jacobson *et al.* have described that, according to their investigations on the three-dimentional X-ray analysis of *O,O*-dimethyl *S*-(benzotriaxin-4(3*H*)-on-3-yl)methyl phosphorodithioate [azinphos-methyl] which has this same moiety, the phosphorus to carbonyl carbon distance in the solid state is very similar to the nitrogen to carbonyl carbon distance (4.83 Å) in acetylcholine.[3] Thus, it is interesting to synthesize the other compounds containing this characteristic moiety and to examine their insecticidal activities.

We take particular interest in oxadiazoles, having synthesized a variety of the derivatives and examined for thier pesticidal activities.[4] This paper deals with the synthesis and biological activities of the *O,O*-dialkyl *S*-(5-aryl-1,3,4-oxadiazol-2(3*H*)-on-3-yl)methyl phosphorodithioates **4**, and the phosphorothioates **5** and **6** as analogues of methidathion.

## *Synthesis*

The syntheses of *O,O*-dialkyl *S*-(5-aryl-1,3,4-oxadiazol-2-(3*H*)-on-3-yl)methyl phosphorodithioates and phosphorothioates (**4**, **5** and **6**) were carried out as shown in the Scheme. The 5-aryl-1,3,4-oxadiazol-2(3*H*)-ones **1** were prepared by the method of Gray *et al.*[5] A mixture of **1** and paraformaldehyde was heated under reflux in water to give the *N*-methylol derivatives **2**. Completion of the reaction was confirmed by the IR spectra as: (a) disappearance of the absorption bands at 3200 $cm^{-1}$ based on the NH stretching vibrations of the NH group at the 3-position in the oxadiazolones **1**, (b) appearance of those at 3340～3400 $cm^{-1}$ based on the OH stretching vibrations of the OH group in the *N*-methylol compounds **2**. These compounds **2** were re-

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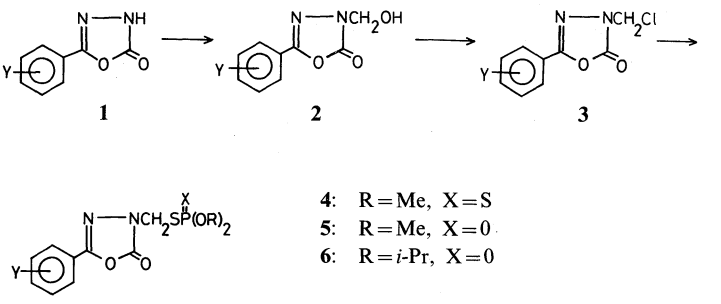

**1** **2** **3**

**4**: R=Me, X=S
**5**: R=Me, X=O
**6**: R=*i*-Pr, X=O

a, Y=H; b, Y=*m*-Cl; c, Y=*p*-$NO_2$; d, Y=*p*-MeO.

SCHEME. Synthesis of Phosphate Derivatives **4**, **5** and **6**.

TABLE I. 5-ARYL-1,3,4-OXADIAZOL-2(3*H*)-ONES **1**, AND THEIR *N*-METHYLOL AND *N*-CHLOROMETHYL DERIVATIVES (**2** AND **3**)

| Compound no. | Yield (%) | mp (°C) | IR $\nu_{max}^{KBr}$ $cm^{-1}$ (NH, OH, CH) | IR $\nu_{max}^{KBr}$ $cm^{-1}$ (C=O) |
|---|---|---|---|---|
| **1a** | 87 | 135~136[a] | 3150, 3200 | 1740 |
| **1b** | 70 | 158~159 | 3150, 3250 | 1780 |
| **1c** | 79 | 107~109 | 3100, 3200 | 1780 |
| **1d** | 96 | 183~184 | 3100, 3200 | 1760 |
| **2a** | 87 | 116~118[b] | 3340 | 1770 |
| **2b** | 83 | 104~106 | 3390 | 1780 |
| **2c** | 92 | — | 3390 | 1785 |
| **2d** | 89 | 166~169 | 3380 | 1780 |
| **3a** | 91 | 102~105 | 3050 | 1780 |
| **3b** | 46 | 98~99 | 3050 | 1775 |
| **3c** | 73 | 134~135 | 3100, 3040 | 1770 |
| **3d** | 84 | 118~119 | 3030 | 1795 |

[a] mp 138°C from ref. 11.
[b] mp 114~115°C from ref. 12.

TABLE II. PHOSPHATE DERIVATIVES **4**, **5** AND **6**

| Compound no. | Yield (%) | mp (°C) | Found (%) (Calcd. %) C | H | N |
|---|---|---|---|---|---|
| **4a** | 54 | 78~79[a] | 40.26 | 3.92 | 8.51 |
| | | | (39.76 | 3.92 | 8.40) |
| **4b** | 77 | 78~80 | 36.27 | 3.30 | 7.79 |
| | | | (36.02 | 3.27 | 7.64) |
| **4c** | 68 | 99~102 | 35.14 | 3.14 | 11.32 |
| | | | (35.01 | 3.18 | 11.14) |
| **5a** | 50 | 118~122[a] | 41.92 | 4.44 | 8.70 |
| | | | (41.77 | 4.11 | 8.86) |
| **5b** | 74 | 76~78 | 37.21 | 3.45 | 8.02 |
| | | | (37.66 | 3.42 | 7.99) |
| **5c** | 71 | 148~149 | 36.14 | 3.40 | 11.67 |
| | | | (36.56 | 3.32 | 11.63) |
| **5d** | 70 | 91~93 | 41.73 | 4.32 | 8.37 |
| | | | (41.62 | 4.33 | 8.09) |
| **6a** | 81 | 74~76 | 48.51 | 5.64 | 7.73 |
| | | | (48.39 | 5.65 | 7.53) |
| **6b** | 73 | 68~69 | 44.65 | 4.60 | 8.28 |
| | | | (44.28 | 4.92 | 7.89) |
| **6c** | 61 | 86~88 | 43.24 | 4.73 | 10.24 |
| | | | (43.17 | 4.80 | 10.07) |
| **6d** | 45 | 59~60 | 39.59 | 4.58 | 8.10 |
| | | | (39.78 | 4.14 | 7.73) |

[a] **4a**, mp 80~81°C; **5a**, mp 123~124°C from ref. 12.

acted with thionyl chloride under reflux in tetrahydrofuran (THF) to afford the chloromethyl derivatives **3**. Similarly, disappearance of the absorption bands due to the OH group supported the structure of **3**. Table I shows the IR spectra data for **1**, **2** and **3**. The coupling reaction of **3** with potassium *O,O*-dimethyl phosphorodithioate, *O,O*-dimethyl phosphorothioate or *O,O*-di-*i*-propyl phosphorothioate proceeded smoothly at room temperature in acetone to give *O,O*-dimethyl *S*-(5-aryl-1,3,4-oxadiazol-2(3*H*)-on-3-yl)methyl phosphorodithioates **4**, *O,O*-dimethyl *S*-(5-aryl-1,3,4-oxadiazol-2(3*H*)-on-3-yl)methyl phosphorothioates **5** or *O,O*-di-*i*-propyl *S*-(5-aryl-1,3,4-oxadiazol-2(3*H*)-on-3-yl)methyl phosphorothioates **6**, respectively. The structures of **4**, **5** and **6** were confirmed by their spectral and elementary analysis data as shown in Tables II and III. In the IR spectra, the phosphorodithioates **4** showed strong absorption bands at about 800 $cm^{-1}$ due to P=S stretching vibrations, and likewise the phosphorothioates **5**

TABLE III. IR AND NMR SPECTRA OF PHOSPHATE DERIVATIVES **4**, **5** AND **6**

| Compound no. | IR $\nu_{max}^{KBr}$ cm$^{-1}$ | | | NMR (acetone-$d_6$) $\delta$ |
|---|---|---|---|---|
| | C=O | P=X | P–O–C | |
| **4a** | 1770 | 832, 818<br>800, 640 | 1000 | 3.93 (6H, d, $J$=16 Hz, $CH_3$) 5.40 (2H, d, $J$=16 Hz, $CH_2$)<br>8.84, 9.13 (3H, 2H, m, phenyl) |
| **4b** | 1780 | 830, 810<br>790, 640 | 1040,<br>1015 | 3.98 (6H, d, $J$=16 Hz, $CH_3$) 5.52 (2H, d, $J$=16 Hz, $CH_2$)<br>7.98, 8.18 (2H, 2H, m, *m*-chlorophenyl) |
| **4c** | 1795,<br>1782 | 825, 648 | 1015,<br>1035 | 3.76 (6H, d, $J$=15 Hz, $CH_3$) 5.25 (2H, d, $J$=14 Hz, $CH_2$)<br>8.07, 8.35 (2H, 2H, d, $J$=8 Hz, *p*-nitrophenyl) |
| **5a** | 1770 | 1261, 1291,<br>1365 | 1010,<br>1049 | 3.88 (6H, d, $J$=13 Hz, $CH_3$) 5.38 (2H, d, $J$=16 Hz, $CH_2$)<br>8.76, 9.04 (3H, 2H, m, phenyl) |
| **5b** | 1775 | 1249, 1290,<br>1360 | 1020,<br>1070 | 4.00 (6H, d, $J$=14 Hz, $CH_3$) 5.56 (2H, d, $J$=17 Hz, $CH_2$)<br>8.04, 8.14 (2H, 2H, m, *m*-chlorophenyl) |
| **5c** | 1780 | 1260, 1290,<br>1360 | 1020,<br>1075 | 4.04 (6H, d, $J$=14 Hz, $CH_3$) 5.64 (2H, d, $J$=17 Hz, $CH_2$)<br>8.58, 8.78 (2H, 2H, d, $J$=8 Hz, *p*-nitrophenyl) |
| **5d** | 1770 | 1260, 1295,<br>1330, 1375 | 1020,<br>1065 | 4.08 (6H, d, $J$=14 Hz, $CH_3$) 4.16 (3H, s, $CH_3O$) 5.62<br>(2H, d, $J$=16 Hz, $CH_2$) 7.58, 8.36 (2H, 2H, d, $J$=10 Hz,<br>*p*-methoxyphenyl) |
| **6a** | 1770 | 1257, 1295,<br>1330, 1375 | 985,<br>1028,<br>1040 | 1.04 (12H, q, $CH_3$) 5.06 (2H, m, CH) 5.54 (2H, d, $J$=12 Hz,<br>$CH_2$) 7.96, 8.20~8.40 (3H, 2H, m, phenyl) |
| **6b** | 1780 | 1255, 1295,<br>1325, 1363,<br>1380 | 1030,<br>1050,<br>1075 | 1.42 (12H, q, $CH_3$) 5.08 (2H, m, CH) 5.58 (2H, d, $J$=12 Hz,<br>$CH_2$) 8.04~8.24 (2H, 2H, m, *m*-chlorophenyl) |
| **6c** | 1780 | 1245, 1283,<br>1300, 1320,<br>1359, 1380 | 980,<br>1010,<br>1030 | 1.40 (12H, q, $CH_3$) 5.00 (2H, m, CH) 5.60 (2H, d, $J$=12 Hz,<br>$CH_2$) 8.56, 8.92 (2H, 2H, d, $J$=9 Hz, *p*-nitrophenyl) |
| **6d** | | | | 1.40 (12H, t, $CH_3$) 4.06 (3H, s, $CH_3O$) 5.04 (2H, m, CH)<br>5.50 (2H, d, $J$=12 Hz, $CH_2$) 7.38, 8.12 (2H, 2H, d,<br>$J$=10 Hz, *p*-methoxyphenyl) |



and **6** between 1100~1400 cm$^{-1}$ due to P=O stretching vibrations. In the NMR spectra, the phosphorus nuclei of **4**, **5** and **6** exhibited coupling with the methyl protons of **4** ($J$=15~16 Hz) and **5** ($J$=13~14 Hz), the methyl and methine protons of **6**, and the *N*-methylene protons of **4** ($J$=15~16 Hz), **5** ($J$=16~17 Hz) and **6** ($J$=12 Hz).

*Contact toxicity and inhibition of AChE activity*

The biological activities of the phosphorus derivatives synthesized were examined against adult houseflies of an organophosphorus-susceptible strain (Lab. em-7-em). Table IV shows the contact toxicity ($LD_{50}$) and the anti-AChE activity ($pI_{50}$) of **4**, **5** and **6**. By combination of the substituents R (*O*-alkyl group; methyl or *i*-propyl group) and X (sulfur or oxygen atom), these compounds are divided into three groups such as methyl phosphorodithioates **4** (R=Me, X=S), methyl phosphorothioates **5** (R=Me, X=O) and *i*-propyl phosphorothioates **6** (R=*i*-Pr, X=O).

By comparing the $LD_{50}$ and $pI_{50}$ values of methyl phosphorodithioates **4** (X=S) with those of methyl phosphorothioates **5** (X=O), it is seen that the oxo derivatives **5a–c** were more potent than the corresponding thiono analogues **4a–c**, respectively.

*i*-Propyl phosphorothioates **6** (R=*i*-Pr) were inactive by topical application, while methyl phosphorothioates **5** (R=Me) showed strong activity. However, compounds **6** had a moderate activity for AChE inhibition. It is considered that the affinities of these inhibitors to AChE can be decreased by the bulky *i*-propyl group.[6] However, the very poor insecticidal activity of *i*-propyl phosphates **6** cannot be accounted for only by their low affinity to the enzyme because the insecticidal

TABLE IV. CONTACT TOXICITY ($LD_{50}$) AND ANTI-AChE ACTIVITY ($pI_{50}$) OF **4**, **5** AND **6**

| Compound no. | R | X | Y | $LD_{50}$[a] (μmol/fly) | $pI_{50}$[b] AChE |
|---|---|---|---|---|---|
| **4a** | Me | S | H | 1.08 | 6.4 |
| **4b** | Me | S | *m*-Cl | 0.95 | 6.3 |
| **4c** | Me | S | *p*-$NO_2$ | 0.56 | 7.3 |
| **5a** | Me | 0 | H | 0.32 | 8.3 |
| **5b** | Me | 0 | *m*-Cl | 0.14 | 8.5 |
| **5c** | Me | 0 | *p*-$NO_2$ | 0.33 | 8.3 |
| **5d** | Me | 0 | *p*-MeO | 7.57 | 7.0 |
| **6a** | *i*-Pr | 0 | H | >80.0 | 6.9 |
| **6b** | *i*-Pr | 0 | *m*-Cl | >100.0 | 7.4 |
| **6c** | *i*-Pr | 0 | *p*-$NO_2$ | >120.0 | 6.5 |
| **6d** | *i*-Pr | 0 | *p*-MeO | >250.0 | 5.8 |
| | Methidathion | | | 0.53 | 6.1 |

[a] Chemical was applied topically on the abdomens of twenty 3-day-old adult houseflies. The $LD_{50}$ values were calculated by probit analysis.[9]

[b] $pI_{50} = -\log I_{50}$; $I_{50}$ means molar concentration required for inhibiting 50% of enzyme activity.

activity of **6a** and **6b** was extremely weak in comparing with that of **5d** ($LD_{50}$ = >80.0, >100.0 and 7.57 μmol/fly, respectively) in spite of their similar inhibition of AChE activity ($pI_{50}$ = 6.9, 7.4 and 7.0, respectively). Therefore, other factors such as poor penetration into the insect and their high susceptibility to metabolic detoxication may be considered.

It was found that the effect of the substituent Y on contact toxicity was small in the methyl phosphorodithioates **4** and phosphorothioates **5**, the $LD_{50}$ values of which were similar excluding **5d** (7.57 μmol/fly). All the *i*-propyl phosphorothioates **6** showed an extremely low insecticidal activity. The effect of Y on the inhibition of AChE activity was also small and the difference of inhibition was found to be 10-fold (**4b** and **4c**), about 32-fold (**5b** and **5d**) and 40-fold (**6b** and **6d**) in compounds **4**, **5** and **6**, respectively. No regularity was found in the effect of Y on enzyme activity.

Compound **6b** was selected and tested as representative to examine the causes of low toxicity when compared with the high inhibition of AChE. The test by topical application was carried out for the synergistic activity of piperonyl butoxide (PB), an inhibitor of mixed function oxydase, and diethyl maleate (DM), an inhibitor of gluthathione *S*-transferase, using **6b** against Lab em-7-em adult housefly. After 24 hr, the mortality of houseflies increased markedly from 10.0% (5μg per insect without a synergist) to 55.0% (+1 μg PB) and to 17.5% (+1 μg DM). Metcalf *et al.* have reported that *i*-propyl parathion was approximately 250 times less toxic to the honey bee than the housefly, while methyl parathion was equally toxic to both insects, and that this lower toxicity of *i*-propyl parathion to the bee was in part due to the slower rate of *i*-propyl paraoxon formation *in vivo* as well as the lower susceptibility of bee AChE to *i*-propyl paraoxon (approximately 1/37).[7] It is thought, however, that the poor insecticidal activity of **6** may be due, in part, to the susceptibility to metabolic detoxication, because **6** are oxo compounds and PB and DM exhibited synergistic effects.

Rüfenacht have reported that some *O,O*-dialkyl *S*-(5-substituted 1,3,4-oxadiazol-2(3*H*)-on-3-yl)methyl phosphoro(di)-thioates were insecticidal.[8] Among the new oxadiazolylmethyl phosphoro(di)thioates synthesized in this paper, all the methyl phosphorus deriv-

atives **4** and **5** exhibited the same degree of insecticidal activity as methidathion, excluding **5d**. In particular, compound **5b** was not only a potent inhibitor against the enzyme ($I_{50} = 3.2 \times 10^{-9}$ M), but also showed about 4-fold stronger insecticidal activity ($LD_{50} = 0.14$ μmol/fly) than methidathion ($LD_{50} = 0.53$ μmol/fly). The *i*-propyl phosphorus derivatives, however, only showed the low insecticidal activity in spite of their moderate inhibition of AChE activity. The synergistic effects of PB and DM suggest that the susceptibility to metabolic detoxication may be in part due to the low insecticidal activity of *i*-propyl esters. Further investigation must be carried out to explain this point.



## EXPERIMENTAL

All melting points are uncorrected. The IR spectra were determined with a Shimadzu IR-27 G Spectrophotometer and the NMR spectra (60 MHz) were measured with a Hitachi R-20 NMR Spectrometer, using tetramethyl silane as an internal standard.

*5-Aryl-1,3,4-oxadiazol-2(3H)-ones.* These compounds were prepared according to the method of Gray *et al.*[5]

*O,O-Dialkyl S-(5-aryl-1,3,4-oxadiazol-2(3H)-on-3-yl)-methyl phosphorodithioates and phosphorothioates.* These compounds were prepared by the method of Rüfenacht.[8]

*5-Phenyl-3-hydroxymethyl-1,3,4-oxadiazol-2(3H)-one (**2a**).* A mixture of 3 g of **1a** and 0.6 g of paraformaldehyde in 50 ml of water was refluxed for 24 hr. After the reaction, the resulting oily substance was extracted three times with ether. The combined ether solution was dried over sodium sulfate and the solvent was evaporated *in vacuo*. The residue was recrystallized from benzene to give **2a** (3.1 g, 87% yield), mp 116~118°C.

The other methylol derivatives **2b–e** were also prepared by the same method.

*5-Phenyl-3-chloromethyl-1,3,4-oxadiazol-2(3H)-one (**3a**).* A mixture of 2.4 g of **2a** and 3 g of thionyl chloride in benzene was refluxed for 24 hr. After the reaction, the solvent was evaporated *in vacuo* and water was added to the residue. The insoluble substance was collected by filtration, washed several times with water and recrystallized from *n*-hexane to give 0.8 g of white crystals, **3a**. From the filtrate, 0.5 g of **3a** was further obtained as benzene eluate after column chromatographic separation. Yield 1.3 g (50%), mp 104~105°C.

When THF was used as the reaction solvent instead of benzene, the yield of **3a** increased remarkably (91%), mp 102~105°C.

The other chloromethyl compounds **3b–d** were similarly prepared by this method.

*O,O-Dimethyl S-(5-phenyl-1,3,4-oxadiazol-2(3H)-on-3-yl)methyl phosphorodithioate (**4a**).* Compound (**3a**) (0.2 g) was added to an acetone solution of 0.16 g of potassium *O,O*-dimethyl phosphorodithioate, and the mixture was stirred at room temperature for 6 hr. On TLC, two orange spots were observed at *Rf* 0.7 and 0.0 (developed with chloroform, sprayed with 5% palladium chloride ethanol solution containing 0.1 N hydrochloric acid). After the reaction, the solvent was evaporated *in vacuo* and water was added to the residue to dissolve the salts. The insoluble substance was extracted with ethyl acetate. The organic layer was further washed with water and then dried over sodium sulfate. The solvent was evaporated *in vacuo* and the residue was recrystallized from *n*-hexane to yield white crystals, **4a** (0.18 g, 54% yield), mp 78~79°C, *Rf* 0.7 (chloroform).

The other phosphorus derivatives **4b** and **4c**, **5a–d** and **6a–d** were similarly prepared.

*Contact toxicity test.* Three-day-old adults houseflies (*Musca domestica vicina*) of an organophosphorus-susceptible strain (Lab em-7-em) were topically treated with 1 μl of a solution of the chemical dissolved in acetone at appropriate concentrations. Replicates of 20 houseflies were treated with each concentration. They kept at 26°C for 24 hr and their mortality was recorded. The $LD_{50}$ value of the chemical was calculated by a probit analysis.[9]

*AChE activity.* The enzyme activity (%) was measured by the Ellmann method.[10] The heads of 3-day-old adult houseflies of a susceptible strain (Lab em-7-em) were homogenized in M/15 phosphate buffer (pH 7.4) in a Potter-Elvehjem glass homogenizer (10 heads/ml) cooled in an ice–water mixture. The homogenate was filtered through a few layers of cheesecloth and the filtrate was centrifuged at 4°C and 900 × *g* for 15 min. The supernatant obtained was used as a crude AChE solution.

The synthesized chemicals were dissolved with acetone at $10^{-3}$, $10^{-5}$, $10^{-7}$ and $10^{-9}$ M and used as the inhibitor solutions. Acetylthiocholine iodide (0.49 g) was dissolved in 28 ml of methanol to prepare a 0.006 M solution which was used as a substrate solution. The color reagent of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB, 0.2 g) was dissolved in 50 ml of distilled water to prepare a 0.01 M solution. Eserine sulfate (5 mg) was dissolved in 10 ml of distilled water and this solution was used to quench the enzyme reaction.

The chemical in acetone (0.38 or 3.8 ml) was put into a 29 ml test tube, and the acetone was allowed to evaporate on a water bath 3.5 ml of M/15 phosphate buffer (pH 7.4), 0.2 ml of DTNB solution and 0.1 ml of a crude AChE

solution were then added to and mixed in this test tube. The mixture was incubated at 27°C for 5 min in a water bath. Acetylthiocholine iodide solution (0.1 ml) was added to this mixture and incubated at 27°C. After 15 min, 0.1 ml of eserine sulfate solution was added to quench the enzyme reaction. The absorption of the reaction mixture at 412 nm was measured with a Shimadzu UV Spectrophotometer (UV-200). These experiments were run in triplicate.

*Synergistic effects.* An acetone solution (1 $\mu$l) of PB or DM (1 $\mu$g) was applied topically to 3-day-old adult houseflies of a susceptible strain (Lab em-7-em). After 15 min, an acetone solution (1 $\mu$l) of **6b** (5 $\mu$g) was applied topically to the same houseflies. Replicates of 20 houseflies were used for each treatment. The houseflies were kept at 26°C and their mortality was counted 24 hr after treatment.

## REFERENCES

1) K. Rüfenacht, U.S. Patent, 3240668 (1966).
2) K. Rüfenacht, *Helv. Chim. Acta*, **56**, 2186 (1973).
3) W. J. Rohrbaugh, E. K. Meyers and R. A. Jacobson, *Agric. Biol. Chem.*, **24**, 713 (1976).
4) H. Kamizono, E. Taniguchi and K. Maekawa, *J. Fac. Agric., Kyushu Univ.*, **24**, 125 (1979).
5) C. J. Gray, J. C. Ireson and R. C. Parker, *Tetrahedron*, **33**, 739 (1977).
6) Y. L. Chiu, A. R. Main and W. C. Dauterman, *Biochem. Pharmacol.*, **18**, 2171 (1969).
7) H. B. Camp, T. R. Fukuto and R. L. Metcalf, *J. Agric. Food Chem.*, **17**, 249 (1969).
8) K. Rüfenacht, *Helv. Chim. Acta*, **55**, 1174 (1972).
9) K. Taturo, *Botyu Kagaku*, **62**, 134 (1951).
10) G. L. Ellmann, K. D. Courty, V. Andres, Jr., and R. M. Featherstone, *Biochem. Pharmacol.*, **7**, 88 (1961).
11) A. Dornow and K. Bruncken, *Chem. Ber.*, **82**, 121 (1949).
12) K. Rüfenacht, U.S. Patent, 3239532 (1966) [*C.A.*, **63**, 1796b (1965)].