Preparation and activity study of new organophosphate insecticide candidates

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(Received July 21, 2010; Accepted August 24, 2010)

Two new organophosphorus pesticides, analog compounds of chlorpyrifos and phoxim, were prepared and evaluated in terms of acute oral toxicity to SD rats and insecticidal activity against different insects in the laboratory. The two compounds showed toxicity similar to corresponding pesticides but with higher insecticidal activity. © Pesticide Science Society of Japan

Keywords: analogs of chlorpyrifos and phoxim, synthesis, insecticidal activity, acute oral toxicity.

Introduction

Synthetic insecticides have played an essential role not only in modern agricultural pest management but also in the control of infectious diseases transmitted by insect vectors and microorganisms.1,2) They have been widely used in the world for many years, for example, more than 50000 kg/year chlorpyrifos (Fig. 1) are used in Europe,3) while in the U.S.A., more than 5 million kg/year chlorpyrifos are used in agriculture and 4 million kg/year are used for nonagricultural purposes.4) Furthermore, the preparation of an inclusion complex of chlorpyrifos in cyclodextrins was explored recently to maintain its efficiency for long periods and to prevent overdosing.5) In recent years, in order to reduce the toxicity of chlorpyrifos and phoxim, both of them have been used as components to form synergistic insecticidal compounds.5–8)

Due to the ability of insects to rapidly develop resistance and the desire to have compounds with less mammalian and environmental toxicity, the discovery of novel active molecules with ideal properties has been the focus of intense research for decades and continues to be an active area of research today. It is critically necessary to discover new insecticidal candidates with high activity and novel modes of action.

The introduction of one or more chiral centers or asymmetric factor into the molecule usually changes the pesticidal activity because enantiomers of the same chiral compound can degrade at significantly different rates and have very different toxicological characteristics in the environment.10,11,14–23) Occasionally, chiral organophosphate pesticides have not only high selectivity and bioactivity, but also limited potential to cause the cross-resistance of pesticides compared with common or traditional pesticides; therefore, it is important to develop chiral organophosphate pesticides, particularly those based on currently used pesticides, since this strategy is very efficient.

As two important organophosphate pesticides, many tons of chlorpyrifos and phoxim (Fig. 1) are used each year. As a result of their long use, resistance is becoming more and more common. To avoid such problems, we introduced an asymmetric phosphorus center into these molecules by utilizing an important intermediate, O-methyl-O-ethyl-thiophosphoryl chloride 1

which was prepared by an improved method based on a process developed in our laboratory,24) firstly by preparing the racemic O-methyl-O-ethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate 25) and the racemic O-methyl-O-ethyl-O-(α-cyanobenzylidene-amino) phosphorothioate 3 (Fig. 2).26) These are compounds with expired patents;27,28) however, their preparation and activity have not been studied previously. Herein, we present our work on their preparation and bioactivity as new organophosphate pesticide candidates. Toxicity assays were evaluated using chlorpyrifos and phoxim as comparisons, respectively, and laboratory evaluations of the above compounds against different insects were performed. It was found that the two target compounds exhibited toxicity similar to chlorpyrifos and phoxim, respectively; however, compounds 2 and 3 had higher insecticidal activity. In particular, compound 2 showed more than 10 times the insecticidal activity of chlorpyrifos. This result indicates that these molecular might be good pesticide candidates.

Materials and Methods

Melting points were determined with an electrothermal digital melting point apparatus, and were uncorrected. 1H NMR and 31PNMR spectra were run either on a Bruker-200 and Bruker-300 or on a Varian-400; 13C NMR was given by Bruker-200. All raw materials were purchased from commercial sources.

Fig. 1. Structure of chlorpyrifos and phoxim.
1. General procedure of intermediate 1
Anhydrous ethanol (68 g, 1.476 mol) was added dropwise to thio-phosphoryl trichloride (50 g, 0.295 mol) at 0–5°C over 30 min. The solution was kept stirring for 4–5 hr, the reaction was monitored by GC. The reaction solution was washed quickly with cold water to give an organic layer as intermediate A, which was used in the next step directly. To A was added dropwise a slurry of sodium hydroxide (17.7 g, 0.443 mol) in methanol (28.4 g, 0.885 mol) under 5°C over one hour. Then the reaction was stirred at the same temperature for one hour and washed with 1% HCl solution to give product 1, 49.3 g, yield 89%, GC purity 93%.

2. Preparation of O-methyl-O-ethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate 2
To a mixture of sodium 3,5,6-trichloropyridine-2-olate (24.4 g, 0.111 mol), 4-dimethylaminopyridine (4.82 g, 0.0395 mol), benzyltrimethyl-ammonium chloride (4.72 g, 0.0254 mol) and sodium hydroxide (0.72 g, 0.018 mol) in water (250 ml) was added dropwise O-methyl-O-ethyl-thiophosphoryl chloride (20.88 g, 0.120 mol), and then the reaction was stirred at 55–60°C for 3.5 hr. The reaction was cooled to room temperature and the organic layer was separated and concentrated under a vacuum to give product 2, 20.62 g, yield 95%, and purity 97% by HPLC. 1H NMR (CDCl3, main isomer): 1.40 (3H, m, CH3), 3.99 (3H, s, CH3O), 4.43 (2H, m, CH2O), 7.87 (s, 1H, ArH). 13C NMR (CDCl3): 150.6, 143.9, 141.2, 126.8, 120.4, 66.1, 55.8, 15.9. 31P NMR (CDCl3): −28.1.

3. Preparation of O-methyl-O-ethyl-O-(α-cyanobenzylideneamino) Phosphorothioate 3
To a solution of benzene acetonitrile α-(hydroximino) sodium (46 g, 38%, 0.104 mol) was added dropwise O-methyl-O-ethyl-thiophosphoryl chloride (17.4 g, 0.0997 mol), and then the solution was stirred at 40–45°C for 60–70 min. The organic layer was separated and concentrated under a vacuum to give product 3, 26.8 g, yield 82%, content 91% (determined by the standard curve using HPLC). 1H NMR δH (CDCl3, main isomer): 1.41 (3H, m, CH3), 3.94 (3H, s, CH3O), 4.35 (2H, m, CH2O), 7.48–7.91 (5H, m, ArH). 13C NMR δC (CDCl3): 140.9, 127.6, 128.0, 128.2, 129.4, 129.5, 133.2, 108.5, 66.5, 56.0, 16.20. 31P NMR δp (CDCl3): −18.1.

4. Biological Assays
4.1. The SD (Sprague Dawley) rat acute oral toxicity.
The toxicity estimation test was conducted in the Center of Pesticide Research and Development of South China (Zhejiang, China), and the tested Sprague Dawley rats were from The Shanghai Laboratory Animal Center (Shanghai, China), based on Good Laboratory Practice (GLP). This experiment was conducted in accordance with the authorized guideline for care and use of laboratory animals of the institute. The acute oral toxicity value (median lethal dose, LD50) was obtained by orally force-feeding the compound, 2, 3, chlorpyrifos, or phoxim dissolved in salad oil, and by observing the rat mortality after 24 hr. The value of LD50 was the average of three repetitions of each treatment.

4.2. Evaluation of insecticidal activity of chlorpyrifos and compound 2 in the laboratory
All bioassays were performed on representative test organisms reared in the laboratory. The bioassay was repeated at 25°C according to statistical requirements. The test compounds were dissolved in DMF (AP; Shanghai Chemical Reagent Co., Ltd., Shanghai, China) and diluted in distilled water containing Triton X-100 (0.1 mg/L) to obtain series concentrations. For comparative purposes, chlorpyrifos and compound 2 were tested under the same conditions at the same time.

### Table 1. Toxicity of compounds 2, 3 to SD rat compared with chlorpyrifos and phoxim

<table>
<thead>
<tr>
<th>Compd</th>
<th>Chlorpyrifos</th>
<th>2</th>
<th>Phoxim</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD50</td>
<td>163 mg/kg</td>
<td>147 mg/kg</td>
<td>1200 mg/kg</td>
<td>1260 mg/kg</td>
</tr>
</tbody>
</table>

Fig. 2. Synthesis of intermediate 1 and title compounds 2 and 3.
1. The SD rat acute oral toxicity

The activities of insecticidal compounds for *Cnaphalocrocis medialis*, *Chilo suppressalis* and corn earworm were tested by the leaf dipping method. Young leaves of the plant were dipped in diluted solutions of the chemicals for 15 s, and excess liquid was removed with filter paper and left to dry. Corn leaf was used for *Cnaphalocrocis medialis*, rice leaf for *Chilo suppressalis* and cabbage leaf for corn earworm. The leaves were placed in Petri dishes in a conditioned room and then the test pests were added. The mortality was evaluated for different periods after treatment to give the median lethal concentration (LC50). Each treatment had three repetitions. The revised death rate was calculated by the Abbott formula.

4.3. Evaluation of insecticidal activity of phoxim and compound 3 in the laboratory

The test compounds were dissolved in DMF to make a solution and diluted with distilled water containing Triton X-100 (0.1 mg/L) to obtain series concentrations before use. For comparative purposes, phoxim and compound 3 were tested under the same conditions at the same time.

The insecticidal activity against *Pieris rapae*, *Dichocrocis punctiferalis*, *Lygocoris lucoum* and *Ostrinia furnacalis* was tested by the larvae dipping method. The pests were infested for 5 sec with the test compound solution and excess liquid was removed with filter paper. The pests were placed in a conditioned room and treated with a clean cabbage leaf. The mortality was evaluated from 24 hr after treatment to give the median lethal concentration (LC50). The revised death rate was calculated by the Abbott formula.

### Table 2. Insecticidal activities of chlorpyrifos and compound 2

<table>
<thead>
<tr>
<th>Pest species</th>
<th>LC50 (mg/L) of Chlorpyrifos</th>
<th>LC50 (mg/L) of 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cnaphalocrocis medialis</em></td>
<td>13.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Chilo suppressalis</em></td>
<td>2.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.270&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Corn earworm</td>
<td>390&lt;sup&gt;b&lt;/sup&gt;</td>
<td>94.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>63.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mortality was evaluated 3 d after treatment. <sup>b</sup> Mortality was evaluated 24 hr after treatment. <sup>c</sup> Mortality was evaluated 48 hr after treatment.

### Table 3. Insecticidal activities of phoxim and compound 3

<table>
<thead>
<tr>
<th>Pest species</th>
<th>LC50 (mg/L) of phoxim</th>
<th>LC50 (mg/L) of 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pieris rapae</em></td>
<td>0.00880</td>
<td>0.00734</td>
</tr>
<tr>
<td><em>Lygocoris lucoum</em></td>
<td>0.00789</td>
<td>0.00568</td>
</tr>
<tr>
<td><em>Ostrinia furnacalis</em></td>
<td>0.0853</td>
<td>0.0698</td>
</tr>
</tbody>
</table>

3. Comparison of insecticidal activity of phoxim and compound 3

The results presented in Table 3 revealed that compound 3 displayed 20–30% more insecticidal activity than phoxim against the three used test pests. As it is reported in the literature that enantiomers usually display very different bioactivity and degradation, this could explain the above higher bioactivity between compound 2 and chlorpyrifos, and compound 3 and phoxim, since asymmetry phosphorus center were introduced into 2 and 3. These results are interesting, and the further preparation of enantiomers of compound 2 and 3, and studies of their toxicology in environment and insecticidal characteristics are underway, which will be reported in due course.

### Acknowledgements

We gratefully acknowledge financial support (from 2008-2009) of this work by Weihai Bureau of Science and Technology, China. We are indebted to Professor Dr. Jie Chen from the center of pesticide research and development of South China at Zhejiang and Dr. Xingyuan Men from the Plant Protection Institute of Shandong Academy of Agricultural Sciences for their hard work in biological screening in the laboratory. We also wish to thank Jiangsu Provincial Center for Disease Prevention and Control, Ministry of Agriculture of P. R. China for the toxicity study.

### References