Synthesis and insecticidal activity of new phosphoramidates

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Compounds known as phosphoramidates have been reported in the literature to have insecticidal activity. In the present investigation, four new phosphoramidates were synthesized and biologically evaluated with regard to their potential insecticidal activity. Moreover, another fifteen compounds had their biological activity profile re-evaluated in further detail against three Lepidoptera species of economic importance. The best results were observed with the four new organophosphates against Ascia monuste.©Pesticide Science Society of Japan

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Introduction

Organophosphates inhibit acetylcholinesterase (AChE) as the primary site of action, an enzyme of the central nervous system of insects that degrades the neurotransmitter acetylcholine, triggering the process of paralysis and eventually culminating in the death of the insect.1–4)

Like insects, humans also possess acetylcholine as a neurotransmitter, and irreversible inactivation of acetylcholinesterase can result in severe poisoning. Although they have these disadvantages, organophosphate insecticides account for more than 24% of the insecticide market and several phosphoramidates are commercially available.5–6)

To continue our effort to develop new compounds with insecticidal7–9 and phytotoxic activity, we describe in the present investigation the synthesis of new phosphoramidates and the evaluation of their activity against Lepidoptera species that attack important commercial crops, namely Ascia monuste (Latr.) (Pieridae), Diaphania hyalinata (L.) (Crambidae) and Plutella xylostella (L.) (Plutellidae).12) A. monuste orseis is a key pest of brassica, which may cause losses of up to 100% in production due to severe defoliation.12) D. hyalinata is among the main pests that attack squash (Cucurbita pepo), cucumber (Cucumis sativus), melon (Cucumis melo), strawberry (Fragaria vesca) and watermelon (Citrus vulgaris).13) P. xylostella is a cosmopolitan pest, found in over 80 countries, and causes considerable damage to cruciferous crops such as cabbage, kale, cauliflower, broccoli and mustard.14–15) In addition, other fifteen compounds, previously synthesized, had their insecticidal activity profile re-evaluated in further detail against the same species.

Materials and Methods

1. General procedures

Infrared spectra were recorded on a Perkin Elmer Spectrum 1000 FTIR spectrophotometer scanning from 4000 to 600 cm⁻¹. The 1H and 13C NMR spectra were recorded on a Varian MERCURY 300 instrument at 300 and 75 MHz, respectively, using deuterated chloroform as a solvent and TMS as an internal standard (δ = 0.00). Mass spectra were recorded on a SHIMADZU GCMS-QP5050A instrument by direct insertion or EI mode (70 eV).

2. Synthesis

Furan-2(SH)-one (1), 3-benzylfuran-2-yl N,N,N′,N′'-tetraalkyl-diamidophosphate derivatives (2–11), bearing substituted benzyl groups, and new phosphoramidates (12–15) were synthesized as previously described.13) Structures of compounds 12–15 were supported by the following spectroscopic and spectrometric data.

2.1. Furan-2-yl N,N,N′,N′'-diclohexylamidophosphate (12)

White crystals. mp 133.2–134.3°C. IR νmax (film, KBr) cm⁻¹: 3444, 3294, 3221, 2931, 2850, 1603, 1514, 1455, 1445, 1428, 1385, 1243, 1187, 1150, 1114, 1083, 956, 718. 1H NMR δ [ppm]: 1.05–1.96 (20H, m, –(CH2)5), 2.64 (2H, t, JNH,PC=O=NH=CH=CH=CH=O), 5.66 (1H, dd, J3,4 = 3.3 Hz, H3), 6.27 (1H, ddd, J1,2 = 1.2 Hz, J5,3 = 1.2 Hz, C-5), 7.32 (1H, d, J2,3 = 1.2 Hz, C-2), 7.48 (1H, d, J2,3 = 1.2 Hz, C-3), 8.9 (1H, d, J3,4 = 2.2 Hz, C-3), 13.24 ppm (C-4), 17.31 ppm (C-5). 13C NMR δ [ppm]: 25.26 (C-9/C-9′), 25.58 (C-8/C-8′), 35.99 (d, J5,P = 1.2 Hz, C-5), 66.2 (1H, d, J3,4 = 3.3 Hz, H3), 68.6 (1H, d, J3,4 = 3.3 Hz, H3), 71.2 ppm (C-9/C-9′), 102.6 ppm (C-8/C-8′), 152.0 ppm (C-5), 152.1 ppm (C-5).
2.2. Furan-2-yl dipiperidin-1-ylphosphinate (13)

Yellow oil. IR νmax (film, KBr) cm⁻¹: 2934, 2851, 1603, 1513, 1465, 1451, 1443, 1380, 1341, 1245, 1206, 1165, 1113, 1077, 959. ¹H NMR δH (300 MHz, CDCl₃): 1.50–1.59 (12H, m, –CH₂–(CH₂)₂–CH₂–), 2.26 (3H, s, –CH₃), 5.66 (1H, dd, J₃,4 = 2.1 Hz, J₂,3 = 1.2 Hz, –O–CH₂–CH–CH–), 6.27 (1H, dd, J₃,4 = 3.3 Hz, J₄,5 = 0.6 Hz, –O–CH = CH–), 6.94 (1H, dd, J₃,4 = 2.1 Hz, J₂,3 = 1.2 Hz, –O–CH₂–CH–CH–), 6.94 (1H, d, J₃,4 = 3.3 Hz, J₄,5 = 0.6 Hz, –O–CH = CH–), 7.01 (1H, d, J₃,4 = 2.1 Hz, J₂,3 = 1.2 Hz, –O–CH₂–CH–CH–). ¹³C NMR δC (75 MHz, CDCl₃): 29.31, 29.32, 31.39, 34.50, 50.30 (d, J₃,4 = 3.3 Hz, C-2), 61.58, 65.76, 120.10, 120.11, 126.35, 130.02, 133.41, 147.87, 147.96. MS (EI) m/z (%): 298 ([M⁺]⁺, C₇H₈N₂O₂P), 216, 164, 113, 102, 91, 80, 79 (4), 78 (9), 66 (100), 55 (100), 44 (75), 33 (51), 32 (43), 21 (42), 19 (100). Biological assays

2.3. Difuran-2-yl piperidin-1-ylphosphonate (14)

Yellow oil. IR νmax (film, CsI) cm⁻¹: 2934, 2851, 2760, 1603, 1513, 1453, 1382, 1347, 1288, 1252, 1238, 1168, 1113, 1081, 1010, 967. ¹H NMR δH (300 MHz, CDCl₃): 1.42–1.61 (6H, m, –CH₂–(CH₂)₂–CH₂–), 1.35–1.37 (6H, m, –CH₂–(CH₂)₂–CH₂–), 3.20–3.27 (4H, m, –CH₂–(CH₂)₂–CH₂–), 5.75 (2H, dd, J₃,4 = 3.3 Hz, J₂,3 = 2.1 Hz, J₃,5 = 0.9 Hz, –O–CH₂–CH–CH–), 6.31 (2H, dd, J₃,4 = 3.3 Hz, J₂,3 = 2.1 Hz, J₃,5 = 0.9 Hz, –O–CH₂–CH–CH–), 6.99 (2H, dd, J₃,4 = 2.1 Hz, J₂,3 = 1.2 Hz, J₃,5 = 0.9 Hz, –O–CH₂–CH–CH–). ¹³C NMR δC (75 MHz, CDCl₃): 24.24 (C-8), 25.77 (d, J₃,4 = 3.98 Hz, C-7/C-9), 45.90 (d, J₃,4 = 2.25 Hz, C-6/C-10), 89.95 (d, J₃,4 = 3.38 Hz, C-3/C-5), 111.53 (d, J₃,4 = 1.73 Hz, C-4/C-6), 135.37 (d, J₃,4 = 1.13 Hz, C-5/C-7), 150.50 (d, J₃,4 = 4.6 Hz, C-2/C-4). MS (EI) m/z (%): 297 ([M⁺]⁺, C₁₁H₁₂N₂O₃P), 214 (4), 187 (4), 186 (4), 168 (3), 158 (3), 150 (61), 132 (18), 122 (7), 94 (4), 88 (4), 86 (23), 84 (54), 83 (100), 78 (8), 76 (4), 63 (9), 56 (6), 55 (34), 51 (27), 50 (4)

2.4. Furan-2-yl N,N-diisopropylamidochlorophosphate (15)

White crystals. mp 50.6–51.3°C. IR νmax (film, KBr) cm⁻¹: 2971, 2939, 2834, 2808, 2755, 2737, 2676, 2600, 2490, 2475, 2414, 2080, 1579, 1474, 1433, 1398, 1383, 1170, 1151, 1035, 849, 804. ¹H NMR δH (300 MHz, CDCl₃): 1.31 (12H, dd, J₃,4 = 19.2 Hz, J₂,3 = 6.9 Hz, 4×CH₃), 3.55–3.76 (2H, m, 2×–(CH₂CH₃)₂), 5.85 (1H, d, J₃,4 = 3.3 Hz, J₄,5 = 2.4 Hz, J₅,6 = 0.9 Hz, –O–CH–CH–CH–), 6.33 (1H, dd, J₃,4 = 3.3 Hz, J₄,5 = 2.4 Hz, J₅,6 = 0.9 Hz, –O–CH–CH–CH–). ¹³C NMR δC (75 MHz, CDCl₃): 21.35 (d, J₃,4 = 1.43 Hz, 2×CH₃), 22.50 (d, J₃,4 = 2.93 Hz, 2×CH₂), 48.13 (d, J₃,4 = 4.58 Hz, 2×–(CH₂CH₃)), 91.26 (d, J₃,4 = 3.98 Hz, C-3), 111.61 (d, J₃,4 = 2.33 Hz, C-4), 135.68 (d, J₃,4 = 1.73 Hz, C-5), 149.67 (d, J₃,4 = 6.3 Hz, C-2). MS (EI) m/z (%): 267 ([M⁺⁺]⁺, C₁₅H₁₂ClN₂O₅P), 265 ([M⁺⁺]⁺, C₁₅H₁₂ClN₂O₅P), 178 (12), 184 (13), 182 (39), 146 (8), 142 (19), 140 (56), 124 (9), 110 (10), 104 (12), 98 (11), 86 (46), 85 (5), 84 (29), 83 (20), 82 (7), 70 (4), 58 (36), 56 (13), 55 (27), 51 (100), 50 (14).

3. Biological assays

The insecticidal activities of compounds 1–19 were evaluated against second-instar larvae of A. monuste, D. hyalinata and P. xylostella. The insecticidal activity of chlorpyrifos-methyl (96% purity) purchased from Dow AgroSciences was utilized as a positive control. Groups of 10 insects of each species were transferred to

![Scheme 1](image)

**Scheme 1.** Intermediate 21 obtained in the synthetic route described by Barbosa and co-workers (2006).³⁹

**Fig. 1.** Compounds synthesized by Paula et al. (2008)³⁹ (1–19) and chlorpyrifos-methyl.
glass Petri dishes. The average weight of each insect species was obtained by measuring the mass of ten groups containing 10 insects each. Each individual insect was applied topically, via a Hamilton micro syringe, with 0.5 $\mu$L of a solution of the test compound, dissolved in acetone, corresponding to a dose of 1.0 $\mu$g compound per mg of the insect. After application, the insects were kept in individual Petri dishes and D. hyalinata, A. monuste and P. xylostella were supplied with appropriate food. The Petri dishes were placed in an incubator, at 25±0.5°C, 75±5% relative humidity, with a photoperiod of 12 hr. Mortality was counted after 24, 48

### Table 1. Contact toxicity of synthetic compounds 1–19 at 1 $\mu$g $^{-1}$ insect

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Ascia monuste</th>
<th>Diaphania hyalinata</th>
<th>Plutella xylostella</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>24h</td>
<td>48h</td>
<td>72h</td>
</tr>
<tr>
<td>1</td>
<td>12.50 aD</td>
<td>22.50 aC</td>
<td>25.00 aC</td>
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<td>5.00 aE</td>
<td>15.00 aD</td>
<td>17.50 aD</td>
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<td>7.50 aE</td>
<td>12.50 aD</td>
<td>12.50 aD</td>
</tr>
<tr>
<td>4</td>
<td>22.50 aD</td>
<td>27.50 aC</td>
<td>25.00 aC</td>
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<td>5</td>
<td>2.50 aE</td>
<td>7.50 aD</td>
<td>10.00 aD</td>
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<td>5.00 aE</td>
<td>5.00 aD</td>
<td>5.00 aD</td>
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<tr>
<td>7</td>
<td>5.00 aE</td>
<td>17.50 aC</td>
<td>15.00 aD</td>
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<tr>
<td>8</td>
<td>22.50 aD</td>
<td>27.50 aC</td>
<td>32.50 aC</td>
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<tr>
<td>9</td>
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<td>7.50 aD</td>
<td>12.50 aD</td>
</tr>
<tr>
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<td>2.50 aE</td>
<td>5.00 aD</td>
<td>7.50 aD</td>
</tr>
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<td>11</td>
<td>5.00 aE</td>
<td>15.00 aD</td>
<td>20.00 aC</td>
</tr>
<tr>
<td>12</td>
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<td>70.00 aB</td>
<td>70.00 aB</td>
</tr>
<tr>
<td>13</td>
<td>70.00 aC</td>
<td>75.00 aB</td>
<td>75.00 aB</td>
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<td>87.50 aA</td>
<td>90.00 aA</td>
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<td>95.00 aA</td>
<td>95.00 aA</td>
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<tr>
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<td>2.50 aE</td>
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<td>72.50 aB</td>
<td>77.50 aB</td>
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<td>65.00 aB</td>
<td>67.50 aB</td>
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<tr>
<td>19</td>
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<td>12.50 aD</td>
<td>17.50 aD</td>
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<tr>
<td>Control (acetone)</td>
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<td>7.50 aD</td>
<td>7.50 aD</td>
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<tr>
<td>Commercial insecticide (Chlorpyrifos-methyl)</td>
<td>100.00 aA</td>
<td>100.00 aA</td>
<td>100.00 aA</td>
</tr>
</tbody>
</table>

1 Means followed by the same lower-case letter in a row or by the same upper-case letter in a column are not significantly different by the Scott–Knott group analysis test at P<0.05.
and 72 hr of the experiment. In a control experiment, carried out under the same conditions, 0.5 μL acetone was applied to each insect. All experiments and respective controls were carried out in four replicates and the data were analyzed by the Scott–Knott test at 0.05 probability level. The insects were considered dead when immobile.

Results and Discussion

1. Synthesis

In previous work, Barbosa and co-workers (2006) described the synthesis of compounds similar to natural products called nostocides, starting from furan-2-yl \(N,N,N',N''\)-tetramethyl-diamidophosphate (20). The synthetic methodology employed provided the intermediate 3-benzylfuran-2-yl \(N,N,N',N''\)-tetraalkyl-diamidophosphate (21) after treatment of 20 with \(n\)-butyllithium and benzyl bromide (Scheme 1).

The literature relates the insecticidal activity of compounds containing heterocyclic rings and bonds P-N. In this context, Paula and co-workers (2008)7) synthesized and evaluated the biological activity of several organophosphate analogues of 21. Among the substances evaluated, compounds 16, 17 and 18 (Fig. 1) showed activity comparable to reference commercial chlorpyrifos-methyl, used as a positive control.

Encouraged by these results, we decided to prepare new phosphoramides (12–15) as described in the literature, using 5 mol% 4-dimethylaminopyridine (DMAP) as a catalyst (Scheme 2).7) The structures of compounds 12–15 were confirmed based on detailed, IR, NMR and MS analyses.

The products were obtained in low yield (6–21%), which is in agreement with previous reports in the literature of this type of reaction.16–18 More recently, Dominguez and colleagues synthesized 43 phosphoramide derivatives, with yields ranging from 0.7 to 54.4%.19

2. Biological activity

As shown in Table 1, substances 14 and 15 were the most effective ones against A. monuste causing, respectively, 90.0 and 95.0% of mortality after 72 hr of exposure (similar to the standard of efficiency for chlorpyrifos-methyl), while compound 18 caused the highest mortality to D. hyalinata (70.0% for the same period of time). For P. xylostella, the best results were observed with compounds 3–7 causing mortality ranging from 45.0 to 52.5% after 72 hr. These results demonstrate compound specificity in terms of toxic action.

Among the most effective compounds, only the mortality caused by compounds 14 and 15 to A. monuste did not significantly increase with the evaluation time. This means that the toxic action on this species was faster than compounds 18 and 11–15.

It was observed that P. xylostella was the most tolerant to the compounds evaluated and A. monuste the most sensitive. This trend is similar to that reported by other authors, especially for P. xylostella, which is a species with recognized insecticide resistance.19) The results of the biological assays indicate that some of the evaluated compounds can be useful for selective insect control purposes.

Acknowledgements

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