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Original Article

Synthesis of pyripyropene derivatives and their pest-control efficacy

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Pyripyropene A (PP-A), a secondary metabolite produced by filamentous fungi, shows insecticidal activity against agricultural insect pests. Synthesized PP derivatives also show a narrow insecticidal spectrum but high insecticidal activities against such sucking pests. PP-A has a low eco-toxicological impact and satisfies a prerequisite for next-generation insecticides. We investigated the effects of conversion of the 3-pyridyl and α-pyrone rings to other rings, as well as the effects of esterification, dehydration, and oxidization at the C-13 position in natural PP analogues, on the insecticidal activity and spectrum. The conversions of the 3-pyridyl and α-pyrone rings markedly reduced the insecticidal activity with a minimal impact on the spectrum, indicative of an important role for these rings in insecticidal activity. Some derivatives with modified structures at the C-13 position showed a higher inhibitory effect on the motility of canine heartworms and mosquito vectors than did PP-A, suggesting their utility as filaria control drugs.

Keywords: pyripyropene, chemical conversion, insecticidal, microfilaria, mosquito.

Introduction

Pyripyropene A (PP-A; Fig. 1) exhibits high insecticidal activity against sucking pests, such as aphids and whiteflies, which seriously damage a variety of crops.[1)] Although the efficacy was insufficient for practical applications and not comparable with that of commercial insecticides, PP-A could be a new tool to control hemiptera pests because its chemical structure is novel for an insecticide. The structure provides advantageous properties, such as a high efficacy against pest populations resistant to existing insecticides and good pharmacokinetics for high efficacy on crops. PP-A has shown moderate insecticidal and growth-inhibition activities against other agricultural pests, including members of lepidoptera and coleoptera.[2)] However, its efficacy also appeared to be less than suitable for practical applications. Although some natural analogues and synthetic PP derivatives have also been evaluated against agricultural pests, their insecticidal spectra were similar to that of PP-A[1,3,4)] and tended to be limited to hemiptera pests, including aphids and whiteflies.

The mode of action[4–6)] for PP appeared to be different from that of existing respiratory inhibitors, central nervous system drugs, and insect growth regulators. PP showed unique symptoms, such as excessive wandering and abnormality of moving or flying.[2)] The inhibiting activities of natural analogues and the synthetic derivatives of acyl-CoA:cholesterol *O*-acyltransferase have been reported by Ōmura *et al.* at the Kitasato Institute[7–11)]; however, their insecticidal symptoms revealed a neurotoxin-like effect. The acyl-CoA:cholesterol *O*-acyltransferase–inhibiting activity should be independent of the insecticidal activity because strong inhibiting derivatives do not necessarily have strong insecticidal activities. The PP chemistry could form a novel class of insecticides that do not merely show cross-resistance with existing insecticides. In this study, we report the insecticidal activities of new derivatives and determine whether this chemistry can be used in the management of agricultural and veterinary pests besides aphids and whiteflies.

Materials and Methods

1. Chemicals

Imidacloprid, chlorfenapyr, ivermectin and RPMI1640 for the culture medium were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka).

Natural analogues were obtained from the Meiji natural compounds library,[1)] PP derivatives were synthesized from PP-A or PP-I following a previously reported method,[3,12–17)] and a synthetic strategy was applied to obtain the derivatives described in this report, with minor modifications if necessary.

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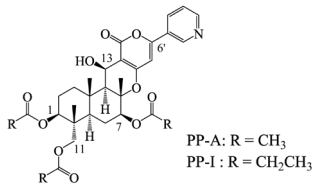


Fig. 1. Structure of natural pyripyropene (PP) analogues

Reagents were obtained from commercial suppliers and were used without purification. ^{1}H NMR spectra were measured on JEOL Lambda 400 MHz, BRUKER Ascend 400 MHz, and 500 MHz spectrometers in $CDCl_3$. Mass spectra were obtained on a JEOL JMS-FABmate, JEOL JMS-700, or Agilent Technologies 6530-Q-TOF LC/MS mass spectrometer. Column chromatography was carried out on silica gel (Varian: Mega Bond Elute) or preparative thin-layer chromatography (Merck: Silica Gel 60 F_{254} 0.5 mm). Melting points were measured with a Shimadzu DSC-60 melting point apparatus.

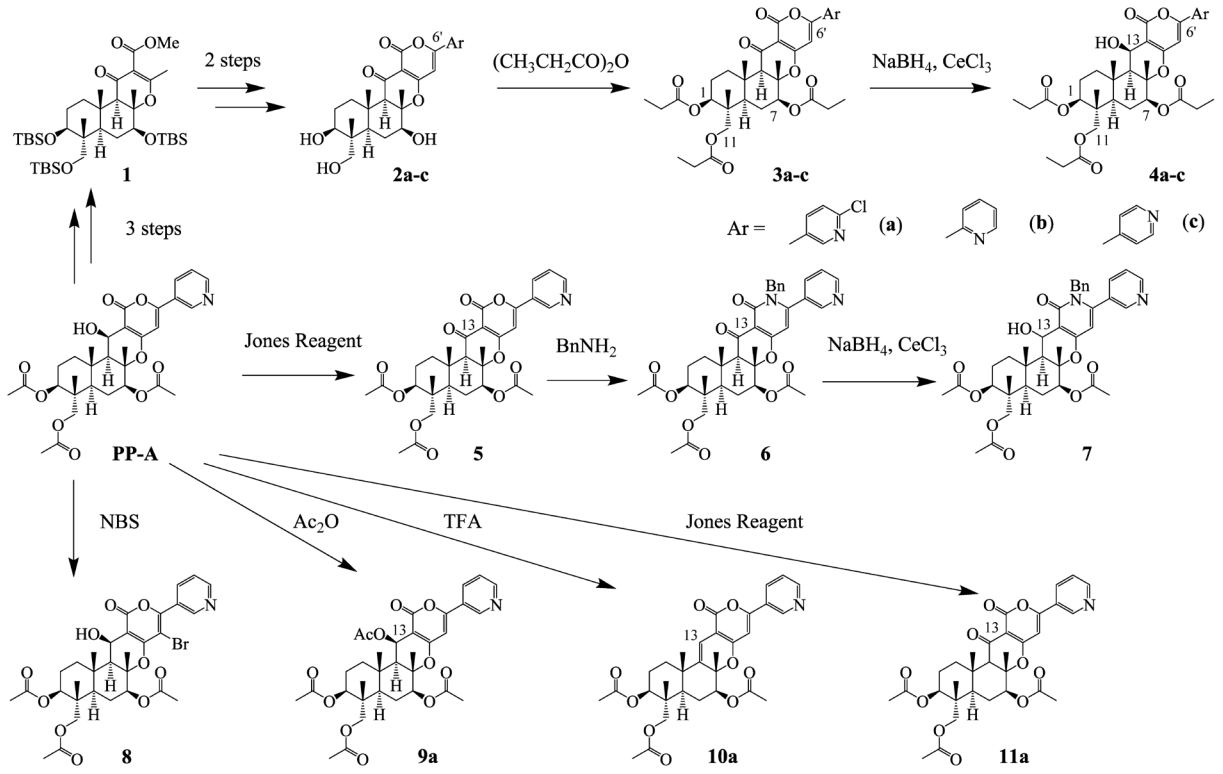
The derivatives **4a** (Ar=6-chloro-3-pyridyl), **4b** (Ar=2-pyridyl), and **4c** (Ar=4-pyridyl) were obtained in accordance with the synthetic procedure described in previous literature,[16] as shown in Scheme 1. A synthesis procedure was applied to the synthesis of **4a**–**4c**, changing the reagent for acylation to propionic anhydride instead of acetic anhydride and the protecting group to *tert*-butyldimethylsilyl (TBS) group instead of trimethylsilyl (TMS) group.

3a

To a solution of **2a** (10 mg, 0.0204 mmol), which was synthesized using the method previously reported, in anhydrous *N*,*N*-dimethylformamide (DMF) (1 mL) were added triethylamine (Et_3N) (24 mg, 0.184 mmol) and 4-dimethylaminopyridine (DMAP) (0.25 mg, 0.00204 mmol) and propionic anhydride (8.0 mg, 0.0612 mmol) and the mixture was stirred at room temperature for 5.5 hr. The reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting residue was purified *via* preparative thin-layer chromatography (PTLC) (acetone : hexane = 1 : 1) to afford **3a** (9.8 mg, 0.0149 mmol) as a solid in 73% yield: ^{1}H NMR ($CDCl_3$) δ 8.83 (d, J=2.7 Hz, 1H), 8.12 (dd, J=8.5, 2.7 Hz, 1H), 7.47 (d, J=8.5 Hz, 1H), 6.45 (s, 1H), 5.24 (dd, J=11.4, 4.9 Hz, 1H), 4.79 (dd, J=11.4, 4.9 Hz, 1H), 3.79 (d, J=11.9 Hz, 1H), 3.69 (d, J=11.9 Hz, 1H), 2.79 (dt, J=13.6, 3.4 Hz, 1H), 2.44 (dq, J=7.5, 2.0 Hz, 2H), 2.42 (dq, J=7.5, 1.7 Hz, 2H), 2.31 (dq, J=7.8, 1.2 Hz, 2H), 1.75–1.84 (m, 2H), 1.55–1.64 (m, 3H), 1.56 (s, 3H), 1.50–1.55 (m, 1H), 1.26 (s, 1H), 1.24 (s, 3H), 1.22 (t, J=7.6 Hz, 3H), 1.19 (t, J=7.5 Hz, 3H), 1.13 (t, J=7.6 Hz, 3H), 0.89 (s, 3H); MS (FAB) m/z 658 $(M+H)^+$.

4a

To a solution of **3a** (10 mg, 0.0152 mmol) in MeOH (1 mL) was added cerium (III) chloride heptahydrate ($CeCl_3 \cdot 7H_2O$, 57 mg, 0.152 mmol), and the mixture was stirred at room temperature for 10 min, then cooled to 0°C. To a cold mixture was



Scheme 1. Synthesis of PP derivatives from PP-A

added sodium borohydride ($NaBH_4$, 6.0 mg, 0.152 mmol) and the reaction mixture was stirred for 6.5 hr. The reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting residue was purified *via* PTLC (acetone : hexane = 1 : 1) to afford **4a** (8.5 mg, 0.0129 mmol) as a solid in 85% yield: ^{1}H NMR ($CDCl_3$) δ 8.78 (d, J=2.4 Hz, 1H), 8.05 (dd, J=8.4, 2.4 Hz, 1H), 7.44 (d, J=8.4 Hz, 1H), 6.41 (s, 1H), 4.92–5.10 (m, 2H), 4.80 (dd, J=11.3, 5.4 Hz, 1H), 3.80 (d, J=11.9 Hz, 1H), 3.69 (d, J=11.9 Hz, 1H), 2.85 (s, 1H), 2.26–2.64 (m, 2H), 2.44 (dq, J=7.6, 1.6 Hz, 2H), 2.31 (dq, J=7.6, 2.7 Hz, 2H), 2.08–2.18 (m, 1H), 1.72–1.92 (m, 2H), 1.69 (s, 3H), 1.61–1.67 (m, 2H), 1.53 (d, J=3.8 Hz, 1H), 1.44 (s, 3H), 1.31–1.39 (m, 1H), 1.26 (s, 1H), 1.10–1.24 (m, 3H), 1.19 (t, J=7.6 Hz, 3H), 1.13 (t, J=7.6 Hz, 3H), 0.89 (s, 3H); MS (FAB) m/z 660 $(M+H)^+$.

3b

Reaction of **2b** (19 mg, 0.0405 mmol), which was synthesized using the method previously reported, with propionic anhydride (16 mg, 0.122 mmol) gave **3b** (7.0 mg, 0.0112 mmol) as a solid in 28% yield *via* a procedure similar to that for **3a**: ^{1}H NMR ($CDCl_3$) δ 8.67–8.69 (m, 1H), 8.09 (d, J=7.9 Hz, 1H), 7.86 (dt, J=7.8, 1.8 Hz, 1H), 7.43 (ddd, J=7.7, 4.7, 1.2 Hz, 1H), 7.14 (s, 1H), 5.26 (dd, J=11.1, 4.8 Hz, 1H), 4.80 (dd, J=11.2, 5.6 Hz, 1H), 3.80 (d, J=12.0 Hz, 1H), 3.68 (d, J=12.0 Hz, 1H), 2.79–2.84 (m, 1H), 2.64 (s, 1H), 2.27–2.47 (m, 6H), 1.73–1.82 (m, 2H), 1.55 (s, 3H), 1.50–1.67 (m, 3H), 1.25–1.28 (m, 1H), 1.24 (s, 3H), 1.22 (t, J=7.5 Hz, 3H), 1.18 (t, J=7.5 Hz, 3H), 1.12 (t, J=7.5 Hz, 3H), 0.88 (s, 3H); MS (FAB) m/z 624 $(M+H)^+$.

4b

Reaction of **3b** (7.0 mg, 0.0112 mmol) with $NaBH_4$ (4.0 mg, 0.112 mmol) gave **4b** (5.2 mg, 0.00832 mmol) as a solid in 74% yield *via* a procedure similar to that for **4a**: ^{1}H NMR ($CDCl_3$) δ 8.64 (d, J=4.6 Hz, 1H), 7.99 (d, J=7.9 Hz, 1H), 7.82 (dt, J=7.8, 1.6 Hz, 1H), 7.36 (dd, J=7.4, 4.8 Hz, 1H), 7.08 (s, 1H), 5.00–5.06 (m, 2H), 4.80 (dd, J=11.2, 4.9 Hz, 1H), 3.81 (d, J=12.0 Hz, 1H), 3.67 (d, J=12.0 Hz, 1H), 2.90 (s, 1H), 2.42 (q, J=8.1 Hz, 2H), 2.38 (q, J=8.1 Hz, 2H), 2.32 (q, J=8.1 Hz, 2H), 2.15–2.20 (m, 1H), 1.72–1.95 (m, 2H), 1.68 (s, 3H), 1.55–1.64 (m, 3H), 1.44 (s, 3H), 1.34–1.39 (m, 1H), 1.24–1.28 (m, 1H), 1.22 (t, J=7.5 Hz, 3H), 1.16 (t, J=7.5 Hz, 3H), 1.13 (t, J=7.5 Hz, 3H), 0.90 (s, 3H); MS (FAB) m/z 626 $(M+H)^+$.

3c

Reaction of **2c** (28 mg, 0.0610 mmol), which synthesized using the method previously reported, with propionic anhydride (24 mg, 0.183 mmol) gave **3c** (6.9 mg, 0.0112 mmol) as a solid in 18% yield *via* a procedure similar to that for **3a**: ^{1}H NMR ($CDCl_3$) δ 8.79 (d, J=4.6 Hz, 2H), 7.68–7.70 (m, 2H), 6.54 (s, 1H), 5.25 (dd, J=10.9, 4.9 Hz, 1H), 4.80 (dd, J=11.2, 5.3 Hz, 1H), 3.79 (d, J=11.9 Hz, 1H), 3.69 (d, J=11.9 Hz, 1H), 2.76–2.81 (m, 1H), 2.63 (s, 1H), 2.27–2.49 (m, 6H), 1.73–1.83 (m, 3H), 1.52–1.65 (m, 2H), 1.57 (s, 3H), 1.22–1.27 (m, 4H), 1.24 (s, 3H), 1.19 (t, J=7.5 Hz, 3H), 1.13 (t, J=7.5 Hz, 3H), 0.88 (s, 3H); MS (FAB) m/z 624 $(M+H)^+$.

4c

Reaction of **3c** (6.9 mg, 0.0112 mmol) with $NaBH_4$ (4.0 mg, 0.112 mmol) gave **4c** (1.0 mg, 0.00160 mmol) as a solid in 14% yield *via* a procedure similar to that for **4a**: ^{1}H NMR ($CDCl_3$) δ 8.77 (m, 2H), 7.65 (m, 2H), 6.53 (s, 1H), 4.99–5.03 (m, 2H), 4.78–4.84 (m, 1H), 3.80 (d, J=11.9 Hz, 1H), 3.69 (d, J=11.9 Hz, 1H), 2.89 (s, 1H), 2.31–2.46 (m, 6H), 2.13–2.18 (m, 2H), 1.72–1.95 (m, 2H), 1.69 (s, 3H), 1.50–1.64 (m, 3H), 1.44 (s, 3H), 1.22–1.33 (m, 4H), 1.18 (t, J=7.5 Hz, 3H), 1.13 (t, J=7.5 Hz, 3H), 0.87 (s, 3H); MS (FAB) m/z 626 $(M+H)^+$.

The derivative **7** was synthesized in accordance with the known synthetic procedure described in the literature[16] by using benzylamine.

6

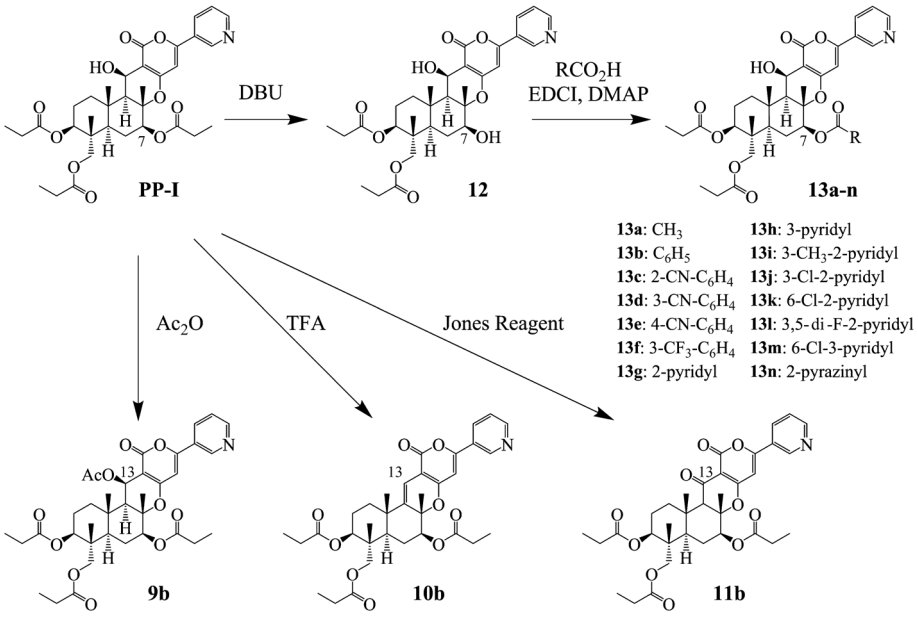
To a solution of **5** (20 mg, 0.0344 mmol), which was synthesized using the method previously reported, in EtOH–H_2O (10 : 1, 2 mL) was added benzylamine (184 mg, 1.72 mmol), and the mixture was stirred at room temperature for 38 hr. The reaction mixture was concentrated, and the residue was dissolved in $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting residue was purified *via* PTLC (acetone : hexane = 1 : 1) to afford **6** (15 mg, 0.0221 mmol) as a solid in 64% yield: ^{1}H NMR ($CDCl_3$) δ 8.66 (dd, J=4.8, 1.8 Hz, 1H), 8.37 (d, J=2.0 Hz, 1H), 7.17–7.34 (m, 5H), 6.83 (dd, J=6.6, 2.6 Hz, 2H), 5.78 (s, 1H), 5.00–5.23 (m, 3H), 4.80 (dd, J=10.9, 5.6 Hz, 1H), 3.69–3.80 (m, 2H), 2.83–2.88 (m, 1H), 2.64 (d, J=3.6 Hz, 1H), 2.18 (d, J=1.6 Hz, 1H), 2.11 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 1.69–1.82 (m, 3H), 1.56 (s, 3H), 1.50–1.59 (m, 2H), 1.26 (s, 3H), 0.87 (s, 3H); MS (FAB) m/z 671 $(M+H)^+$.

7

Reaction of **6** (36 mg, 0.0537 mmol) with $NaBH_4$ (20 mg, 0.537 mmol) gave **7** (3.3 mg, 0.00491 mmol) as a solid in 9% yield *via* a procedure similar to that for **4a**: ^{1}H NMR ($CDCl_3$) δ 8.63 (d, J=3.6 Hz, 1H), 8.39 (s, 1H), 7.20–7.35 (m, 5H), 6.82 (dd, J=6.6, 2.6 Hz, 2H), 5.84 (s, 1H), 5.16–5.27 (m, 1H), 5.12 (d, J=4.3 Hz, 1H), 4.93–5.06 (m, 2H), 4.82 (dd, J=11.2, 5.3 Hz, 1H), 4.44 (d, J=5.6 Hz, 1H), 2.22–2.27 (m, 1H), 2.12–2.18 (m, 1H), 2.10 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.76–1.96 (m, 2H), 1.69 (s, 3H), 1.59–1.64 (m, 3H), 1.47 (s, 3H), 1.23–1.42 (m, 2H), 0.89 (s, 3H); MS (FAB) m/z 673 $(M+H)^+$.

The derivative **8** was obtained *via* the novel synthetic method shown in Scheme 1, and the detailed procedure is described below.

To a solution of **1** (PP-A) (30 mg, 0.0514 mmol) in anhydrous DMF (2 mL) was added *N*-bromosuccinimide (NBS) (18 mg, 0.103 mmol) and the mixture was stirred at room temperature for 14 hr. The reaction mixture was then poured into water and extracted with AcOEt. The organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The resulting residue was purified *via* PTLC (acetone : hexane = 1 : 1) to afford **8** (19 mg, 0.0280 mmol) as a solid in 54% yield: ^{1}H NMR ($CDCl_3$) δ 9.05 (s, 1H), 8.72 (s, 1H), 8.07 (dt, J=8.0, 2.0 Hz, 1H), 7.44 (m, 1H), 5.15 (dd, J=10.6, 5.3 Hz,



Scheme 2. Synthesis of PP derivatives from PP-I

1H), 5.00–5.03 (m, 1H), 4.77–4.83 (m, 1H), 3.81 (d, J=12.0 Hz, 1H), 3.70 (d, J=12.0 Hz, 1H), 3.08 (m, 1H), 2.16–2.18 (m, 1H), 2.16 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.76–1.91 (m, 5H), 1.73 (s, 3H), 1.56–1.63 (m, 2H), 1.45 (s, 3H), 0.90 (s, 3H); MS (ESI) m/z 662 $(M+H)^+$.

As shown in Scheme 1, the derivatives **9a**, **10a**, and **11a** were obtained in accordance with the synthetic route described in previous literature.[13,14]

In addition, as shown in Scheme 2, the derivatives **9b**, **10b**, and **11b** were synthesized using the same methods as above, with PP-I as the starting material.

9b

Reaction of PP-I (104 mg, 0.167 mmol), which was synthesized using the method previously reported, with acetic anhydride (170 mg, 1.67 mmol) gave **9b** (69 mg, 0.0112 mmol) as a solid in 62% yield *via* a procedure similar to that for **3a**: ^{1}H NMR ($CDCl_3$) δ 9.00 (d, J=1.6 Hz, 1H), 8.68 (dd, J=4.8, 1.6 Hz, 1H), 8.09 (dt, J=8.0, 2.0 Hz, 1H), 7.40 (dd, J=8.0, 4.8 Hz, 1H), 6.38 (s, 1H), 6.37 (s, 1H), 5.00–5.04 (m, 1H), 4.81 (dd, J=12.0, 4.0 Hz, 1H), 3.77 (d, J=12.0 Hz, 1H), 3.69 (d, J=12.0 Hz, 1H), 3.27–3.40 (m, 1H), 2.38–2.51 (m, 5H), 2.31 (q, J=8.0 Hz, 2H), 2.10 (s, 3H), 1.85–1.89 (m, 1H), 1.74–1.80 (m, 2H), 1.70 (s, 3H), 1.52–1.68 (m, 2H), 1.59 (s, 3H), 1.29–1.33 (m, 1H), 1.22 (t, J=7.6 Hz, 3H), 1.17 (t, J=7.6 Hz, 3H), 1.12 (t, J=7.6 Hz, 3H), 0.86 (s, 3H); MS (ESI) m/z 668 $(M+H)^+$. Melting point: apparent melting peak was not detected.

10b

To a solution of PP-I (208 mg, 0.330 mmol) in anhydrous THF (2 mL) was added *p*-toluenesulfonic acid monohydrate (317 mg, 1.67 mmol), and the mixture was stirred at room temperature for 47 hr. The reaction mixture was diluted with AcOEt and washed with aqueous $NaHCO_3$. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting residue was purified *via* PTLC (acetone : hexane = 1 : 1) to afford **10b** (121 mg, 0.199 mmol) as a solid in 60% yield: ^{1}H NMR ($CDCl_3$) δ 9.01 (d, J=1.6 Hz, 1H), 8.67 (dd, J=4.8, 1.6 Hz, 1H), 8.11 (dt, J=8.0, 2.0 Hz, 1H), 7.39 (dd, J=8.1, 4.8 Hz, 1H), 6.48 (s, 1H), 6.36 (s, 1H), 5.23 (dd, J=11.9, 5.0 Hz, 1H), 4.80 (dd, J=11.6, 4.6 Hz, 1H), 3.81 (d, J=12.0 Hz, 1H), 3.72 (d, J=11.9 Hz, 1H), 2.42–2.47 (m, 2H), 2.39 (dq, J=8.0, 2.8 Hz, 2H), 2.31 (dq, J=8.0, 1.2 Hz, 2H), 2.06–2.10 (m, 1H), 1.96–2.00 (m, 1H), 1.70–1.85 (m, 2H), 1.59–1.66 (m, 3H), 1.58 (s, 3H), 1.26 (s, 3H), 1.22 (t, J=7.6 Hz, 3H), 1.16 (t, J=7.6 Hz, 3H), 1.13 (t, J=7.6 Hz, 3H), 0.88 (s, 3H); MS (ESI) m/z 608 $(M+H)^+$. Melting point: apparent melting peak was not detected.

11b

To a cold (0 °C) solution of PP-I (117 mg, 0.200 mmol) in $CHCl_3$ (2 mL) was added Dess–Martin periodinane (339 mg, 0.800 mmol), and the mixture was stirred at 0 °C for 2 hr. The reaction was quenched with 10% aqueous Na_2SO_3, and $CHCl_3$ was added to the mixture. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting residue was purified *via* PTLC (acetone : hexane = 1 : 1) to afford **11b** (77 mg, 0.132 mmol) as a solid in 66% yield: ^{1}H NMR ($CDCl_3$) δ 9.05 (d, J=1.6 Hz, 1H), 8.75 (dd, J=4.8, 1.6 Hz, 1H), 8.17 (dt, J=8.0, 2.4 Hz, 1H), 7.44 (dd, J=8.0, 4.0 Hz, 1H), 6.47 (s, 1H), 5.24 (dd, J=11.4, 4.8 Hz, 1H), 4.80 (dd, J=11.4, 4.9 Hz, 1H), 3.79 (d, J=11.9 Hz, 1H), 3.70 (d, J=11.9 Hz, 1H), 2.80 (m, 1H), 2.62 (s, 1H), 2.40–2.45 (m, 4H), 2.31 (dq, J=8.0, 1.2 Hz, 2H), 1.71–1.86 (m, 3H), 1.48–1.64 (m, 2H), 1.57 (s, 3H), 1.26 (m, 1H), 1.24 (s, 3H), 1.22 (t, J=7.6 Hz, 3H), 1.19 (t, J=7.6 Hz, 3H), 1.12 (t, J=7.6 Hz, 3H), 0.88 (s, 3H); MS (ESI) m/z 624 $(M+H)^+$. Melting point: apparent melting

peak was not detected.

As shown in Scheme 2, the derivatives **12**, **13a**, **13b**, **13g**, **13h**, and **13m** were obtained in accordance with the synthetic route described in previous literature.[3)]

The derivatives **13c**, **13d**, **13e**, **13f**, **13i**, **13j**, **13k**, **13l**, and **13n** were synthesized using the same methods[3,13,14)] but using corresponding carboxylic acids.

13c

To a solution of **12** (20 mg, 0.0351 mmol) and 2-cyanobenzoic acid (31 mg, 0.210 mmol) in anhydrous DMF (1 mL) were added 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (28 mg, 0.140 mmol) and DMAP (8 mg, 0.0702 mmol), and the mixture was stirred at room temperature for 14 hr. The reaction mixture was poured into water, then extracted with AcOEt. The organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting residue was purified *via* PTLC (acetone : hexane = 1 : 1) to give **13c** (7 mg, 0.00945 mmol) as a solid in 27% yield: ^{1}H NMR ($CDCl_3$) δ 8.98 (d, J=2.2 Hz, 1H), 8.67 (dd, J=4.9, 1.6 Hz, 1H), 8.19–8.23 (m, 1H), 8.07 (dt, J=8.1, 1.9 Hz, 1H), 7.83–7.88 (m, 1H), 7.68–7.78 (m, 2H), 7.38 (dd, J=7.6, 5.4 Hz, 1H), 6.46 (s, 1H), 5.36 (dd, J=11.3, 4.9 Hz, 1H), 5.04 (m, 1H), 4.83 (dd, J=5.4, 1.6 Hz, 1H), 3.83 (d, J=11.9 Hz, 1H), 3.72 (d, J=11.9 Hz, 1H), 2.96 (s, 1H), 2.42 (dq, J=7.6, 2.4 Hz, 2H), 2.33 (q, J=7.6 Hz, 2H), 2.14–2.23 (m, 1H), 1.93–1.96 (m, 2H), 1.84 (s, 3H), 1.68–1.75 (m, 2H), 1.62 (m, 1H), 1.50 (s, 3H), 1.39–1.44 (m, 1H), 1.26 (s, 1H), 1.20 (t, J=7.6 Hz, 3H), 1.14 (t, J=7.6 Hz, 3H), 0.93 (s, 3H); MS (ESI) m/z 699 $(M+H)^+$.

13d

Reaction of **12** (20 mg, 0.0351 mmol) with 3-cyanobenzoic acid (31 mg, 0.210 mmol) gave **13d** (17 mg, 0.0286 mmol) as a solid in 69% yield *via* a procedure similar to that for **13c**: ^{1}H NMR ($CDCl_3$) δ 8.96 (d, J=2.4 Hz, 1H), 8.67 (dd, J=4.9, 1.5 Hz, 1H), 8.38 (t, J=1.5 Hz, 1H), 8.34 (dt, J=7.8, 1.5 Hz, 1H), 8.07 (dt, J=8.0, 2.2 Hz, 1H), 7.90 (dt, J=7.8, 1.5 Hz, 1H), 7.65 (m, 1H), 7.38 (dd, J=8.0, 4.1 Hz, 1H), 6.41 (s, 1H), 5.26 (dd, J=11.5, 5.1 Hz, 1H), 5.05 (m, 1H), 4.84 (dd, J=11.7, 4.9 Hz, 1H), 3.80 (d, J=11.9 Hz, 1H), 3.73 (d, J=11.9 Hz, 1H), 2.97 (m, 1H), 2.42 (dq, J=7.5, 2.4 Hz, 2H), 2.32 (dq, J=7.6, 1.0 Hz, 2H), 2.19–2.23 (m, 1H), 1.93–2.01 (m, 2H), 1.86 (s, 3H), 1.68–1.82 (m, 2H), 1.62 (d, J=2.4 Hz, 1H), 1.51 (s, 3H), 1.39–1.47 (m, 1H), 1.26 (s, 1H), 1.20 (t, J=7.5 Hz, 3H), 1.14 (t, J=7.5 Hz, 3H), 0.92 (s, 3H); MS (ESI) m/z 699 $(M+H)^+$.

13e

Reaction of **12** (20 mg, 0.0351 mmol) with 4-cyanobenzoic acid (31 mg, 0.210 mmol) gave **13e** (2 mg, 0.00200 mmol) as a solid in 6% yield *via* a procedure similar to that for **13c**: ^{1}H NMR ($CDCl_3$) δ 8.96 (d, J=1.7 Hz, 1H), 8.67 (dd, J=4.9, 1.5 Hz, 1H), 8.21 (d, J=8.8 Hz, 2H), 8.06 (dt, J=8.0, 1.7 Hz, 1H), 7.80 (d, J=8.8 Hz, 2H), 7.38 (dd, J=8.0, 4.9 Hz, 1H), 6.40 (s, 1H), 5.26 (dd, J=11.5, 5.1 Hz, 1H), 5.05 (m, 1H), 4.84 (dd, J=11.7, 4.9 Hz, 1H), 3.81 (d, J=11.9 Hz, 1H), 3.73 (d, J=11.6 Hz, 1H), 2.98 (m, 1H), 2.42 (dq, J=7.6, 2.4 Hz, 2H), 2.33 (dq, J=7.6, 0.9 Hz, 2H), 2.18–2.22 (m, 1H), 1.75–1.97 (m, 2H), 1.85 (s, 3H), 1.69–1.71 (m, 2H), 1.62 (d, J=2.4 Hz, 1H), 1.50 (s, 3H), 1.30–1.47 (m, 1H), 1.26 (s, 1H), 1.20 (t, J=7.5 Hz, 3H), 1.12 (t, J=7.5 Hz, 3H), 0.92 (s, 3H); MS (ESI) m/z 699 $(M+H)^+$.

13f

Reaction of **12** (20 mg, 0.0351 mmol) with 3-(trifluoromethyl)-benzoic acid (40 mg, 0.210 mmol) gave **13f** (14 mg, 0.00200 mmol) as a solid in 55% yield *via* a procedure similar to that for **13c**: ^{1}H NMR ($CDCl_3$) δ 8.97 (d, J=2.2 Hz, 1H), 8.67 (dd, J=4.9, 1.5 Hz, 1H), 8.36 (s, 1H), 8.30 (d, J=8.1 Hz, 1H), 8.06 (dt, J=8.0, 1.8 Hz, 1H), 7.88 (d, J=7.8 Hz, 1H), 7.65 (t, J=7.8 Hz, 1H), 7.38 (dd, J=8.0, 4.9 Hz, 1H), 6.42 (s, 1H), 5.28 (dd, J=11.5, 5.1 Hz, 1H), 5.05 (d, J=4.1 Hz, 1H), 4.84 (dd, J=11.4, 4.9 Hz, 1H), 3.82 (d, J=12.0 Hz, 1H), 3.72 (d, J=11.9 Hz, 1H), 2.97 (m, 1H), 2.43 (dq, J=7.6, 2.5 Hz, 2H), 2.33 (q, J=7.5 Hz, 2H), 2.18–2.23 (m, 1H), 1.90–1.98 (m, 2H), 1.86 (s, 3H), 1.63–1.83 (m, 2H), 1.63 (d, J=2.7 Hz, 1H), 1.51 (s, 3H), 1.39–1.48 (m, 1H), 1.26 (s, 1H), 1.21 (t, J=7.5 Hz, 3H), 1.14 (t, J=7.5 Hz, 3H), 0.92 (s, 3H); MS (ESI) m/z 742 $(M+H)^+$.

13i

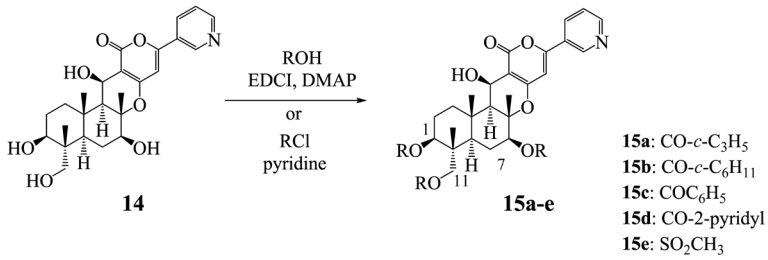
Reaction of **12** (20 mg, 0.0351 mmol) with 3-methylpicolinic acid (14 mg, 0.105 mmol) gave **13i** (17 mg, 0.0243 mmol) as a solid in 69% yield *via* a procedure similar to that for **13c**: ^{1}H NMR ($CDCl_3$) δ 8.98 (d, J=2.4 Hz, 1H), 8.68 (dd, J=4.9, 1.6 Hz, 1H), 8.60 (d, J=4.1 Hz, 1H), 8.08 (dt, J=7.8, 1.9 Hz, 1H), 7.66 (d, J=7.8 Hz, 1H), 7.35–7.42 (m, 2H), 6.42 (s, 1H), 5.36 (dd, J=10.8, 5.4 Hz, 1H), 5.04 (m, 1H), 4.84 (dd, J=11.3, 5.4 Hz, 1H), 3.84 (d, J=11.9 Hz, 1H), 3.72 (d, J=11.9 Hz, 1H), 2.96 (m, 1H), 2.64 (s, 3H), 2.42 (dq, J=7.6, 2.2 Hz, 2H), 2.33 (q, J=7.6 Hz, 2H), 2.14–2.22 (m, 1H), 1.88–2.01 (m, 2H), 1.83 (s, 3H), 1.71–1.77 (m, 2H), 1.62 (m, 1H), 1.49 (s, 3H), 1.34–1.45 (m, 1H), 1.26 (s, 1H), 1.19 (t, J=6.5 Hz, 3H), 1.14 (t, J=7.6 Hz, 3H), 0.92 (s, 3H); MS (ESI) m/z 689 $(M+H)^+$.

13j

Reaction of **12** (20 mg, 0.0351 mmol) with 3-chloropicolinic acid (33 mg, 0.210 mmol) gave **13j** (15 mg, 0.0206 mmol) as a solid in 59% yield *via* a procedure similar to that for **13c**: ^{1}H NMR ($CDCl_3$) δ 8.97 (d, J=2.2 Hz, 1H), 8.69 (d, J=4.9 Hz, 1H), 8.64 (dd, J=4.6, 1.2 Hz, 1H), 8.08 (dt, J=8.3, 1.5 Hz, 1H), 7.87 (dd, J=8.3, 1.5 Hz, 1H), 7.39–7.45 (m, 2H), 6.46 (s, 1H), 5.37 (dd, J=11.7, 4.9 Hz, 1H), 5.05 (m, 1H), 4.83 (dd, J=11.5, 4.8 Hz, 1H), 3.87 (d, J=11.9 Hz, 1H), 3.70 (d, J=12.0 Hz, 1H), 2.96 (m, 1H), 2.41 (dq, J=7.5, 3.4 Hz, 2H), 2.32 (dq, J=7.6, 1.7 Hz, 2H), 2.18–2.22 (m, 1H), 2.02–2.06 (m, 1H), 1.83–1.95 (m, 1H), 1.80 (s, 3H), 1.71–1.74 (m, 2H), 1.63 (d, J=3.0 Hz, 1H), 1.48 (s, 3H), 1.40–1.46 (m, 1H), 1.26 (s, 1H), 1.19 (t, J=7.5 Hz, 3H), 1.14 (t, J=7.5 Hz, 3H), 0.93 (s, 3H); MS (ESI) m/z 709 $(M+H)^+$.

13k

Reaction of **12** (20 mg, 0.0351 mmol) with 6-chloropicolinic acid (33 mg, 0.210 mmol) gave **13k** (13 mg, 0.0179 mmol) as a solid in 51% yield *via* a procedure similar to that for **13c**: ^{1}H NMR ($CDCl_3$) δ 8.98 (d, J=2.0 Hz, 1H), 8.67 (dd, J=4.9, 1.7 Hz, 1H), 8.07 (m, 2H), 7.85 (t, J=7.8 Hz, 1H), 7.56 (d, J=8.1 Hz, 1H), 7.39 (dd, J=8.0, 4.9 Hz, 1H), 6.43 (s, 1H), 5.32 (dd, J=11.7, 5.3 Hz, 1H), 5.05 (m, 1H), 4.83 (dd, J=11.7, 4.9 Hz,



Scheme 3. Synthesis of PP derivatives **15a–e**

1H), 3.84 (d, *J*=12.0 Hz, 1H), 3.68 (d, *J*=11.9 Hz, 1H), 2.96 (d, *J*=1.9 Hz, 1H), 2.41 (dq, *J*=7.7, 2.2 Hz, 2H), 2.32 (dq, *J*=7.7, 1.5 Hz, 2H), 2.18–2.22 (m, 1H), 1.83–1.98 (m, 2H), 1.86 (s, 3H), 1.70–1.73 (m, 2H), 1.63 (d, *J*=2.4 Hz, 1H), 1.50 (s, 3H), 1.38–1.46 (m, 1H), 1.26 (s, 1H), 1.19 (t, *J*=7.5 Hz, 3H), 1.14 (t, *J*=7.5 Hz, 3H), 0.91 (s, 3H); MS (ESI) *m*/*z* 709 $(M+H)^+$.

13l

Reaction of **12** (20 mg, 0.0351 mmol) with 3,5-difluoropicolinic acid (33 mg, 0.210 mmol) gave **13l** (11 mg, 0.0153 mmol) as a solid in 44% yield *via* a procedure similar to that for **13c**: ^{1}H NMR ($CDCl_3$) δ 8.98 (d, *J*=2.6 Hz, 1H), 8.68 (dd, *J*=4.9, 0.7 Hz, 1H), 8.53 (d, *J*=2.0 Hz, 1H), 8.08 (dd, *J*=8.0, 1.7 Hz, 1H), 7.36–7.41 (m, 2H), 6.44 (s, 1H), 5.37 (dd, *J*=11.7, 4.8 Hz, 1H), 5.04 (m, 1H), 4.82 (dd, *J*=11.7, 4.9 Hz, 1H), 3.85 (d, *J*=11.9 Hz, 1H), 3.68 (d, *J*=11.9 Hz, 1H), 2.96 (m, 1H), 2.41 (dq, *J*=7.5, 2.5 Hz, 2H), 2.32 (dq, *J*=7.5, 1.5 Hz, 2H), 2.18–2.22 (m, 1H), 1.84–2.00 (m, 2H), 1.82 (s, 3H), 1.62–1.73 (m, 3H), 1.49 (s, 3H), 1.42–1.45 (m, 1H), 1.26 (s, 1H), 1.19 (t, *J*=7.5 Hz, 3H), 1.14 (t, *J*=7.5 Hz, 3H), 0.92 (s, 3H); MS (ESI) *m*/*z* 711 $(M+H)^+$.

13n

Reaction of **12** (20 mg, 0.0351 mmol) with pyrazine-6-carboxylic acid (26 mg, 0.210 mmol) gave **13n** (11 mg, 0.00200 mmol) as a solid in 46% yield *via* a procedure similar to that for **13c**: ^{1}H NMR ($CDCl_3$) δ 9.38 (m, 1H), 8.97 (m, 1H), 8.80–8.83 (m, 1H), 8.68 (d, *J*=4.4 Hz, 1H), 8.07 (m, 1H), 8.02 (s, 1H), 7.39 (dd, *J*=8.1, 4.9 Hz, 1H), 6.42 (s, 1H), 5.39 (dd, *J*=11.6, 5.2 Hz, 1H), 5.05 (m, 1H), 4.84 (dd, *J*=11.7, 4.9 Hz, 1H), 3.83 (d, *J*=11.9 Hz, 1H), 3.71 (d, *J*=12.0 Hz, 1H), 2.96 (m, 1H), 2.42 (dq, *J*=7.6, 1.5 Hz, 2H), 2.32 (q, *J*=7.6 Hz, 2H), 2.18–2.23 (m, 1H), 1.85–2.00 (m, 2H), 1.87 (s, 3H), 1.73 (m, 2H), 1.64 (d, *J*=2.4 Hz, 1H), 1.51 (s, 3H), 1.40–1.47 (m, 1H), 1.26 (s, 1H), 1.20 (t, *J*=7.5 Hz, 3H), 1.14 (t, *J*=7.5 Hz, 3H), 0.92 (s, 3H); MS (ESI) *m*/*z* 676 $(M+H)^+$.

As shown in Scheme 3, the derivatives **15a**, **15b**, **15c**, **15d**, and **15e** were obtained from **14** in accordance with the synthetic route described in previous literature.[3)]

2. Insecticidal evaluation by foliar application

The insecticidal assays of PP derivatives against the green peach aphid (*Myzus persicae*), cotton aphid (*Aphis gossypii*), small brown planthopper (*Laodelphax striatella*), brown planthopper (*Nilaparvata lugens*), greenhouse whitefly (*Trialeurodes vaporariorum*), western flower thrips (*Frankliniella occidentalis*) and two-spotted spider mite (*Tetranychus urticae*) were conducted by foliar application to leaf disks removed from cabbage (*Brassica oleracea* var. capitate cv. Kinkei 201), cucumber (*Cucumis sativus* cv. Suyo) or kidney bean (*Phaseolus vulgaris* cv. Celina) plants following previously reported methods.[4,18)] The activities were calculated as 50% lethal concentration (LC_{50}) values *via* probit analyses.

3. Insecticidal evaluation against the canine heartworm (Dirofilaria immitis)

The activities of PP derivatives were evaluated based on changes in the motility of microfilariae associated with the canine heartworm following the method described in a previous patent.[18)]

Table 1. Insecticidal activities of PP derivatives **4a–c**, **7**, and **8**

Compound	LC_{90} (ppm)	Mortality at 200 ppm		
	M. persicae Odawara (2002)[a)]	*L. striatella* Odawara (2001)[a)]	*F. occidentalis* Purchased[a)]	*T. urticae* Purchased[a)]
4a	19	5	NT[b)]	NT[b)]
4b	>100	7	30	0
4c	>100	5	25	0
7	>100	0	33	0
8	>100	NT[b)]	NT[b)]	NT[b)]
PP-A	0.56	70	0	0
PP-I	0.043	15	53	0

[a)] The insects collected in Japan respectively or purchased were used in insecticide tests. [b)] Not tested

Table 2. Insecticidal activities of PP derivatives **9a–b**, **10a–b**, and **11a–b**

Compound	LC_{90} (ppm)	Mortality at 5 ppm	Mortality at 200 ppm		
	M. persicae Odawara (2002)[a)]	*T. vaporariorum* Odawara (2001)[a)]	*F. occidentalis* Purchased[a)]	*N. lugens* Kagoshima (1970s)[a)]	*T. urticae* Purchased[a)]
9a	>1.3	57	55	0	0
9b	>1.3	8	15	0	0
10a	>1.3	NT[b)]	84	0	0
10b	>1.3	9	19	90	0
11a	>1.3	0	0	14	0
11b	>1.3	15	13	0	0
PP-A	0.56	80	65	50	0
PP-I	0.043	18.3	53	50	0

[a)] The insects collected in Japan respectively or purchased were used in insecticide tests. [b)] Not tested

Each derivative was dissolved in an RPMI1640-based liquid culture medium to a determined concentration in a 96-well plate. Subsequently, ~20 *D. immitis* microfilariae were placed in each culture fluid and cultured at 37°C. The motility levels of the *D. immitis* microfilariae were observed 48 hr after the start of culturing, and the activities of the compounds were rated based on the mortality level.

4. Tickicidal evaluation against Haemaphysalis longicornis

To determine the tickicidal properties, 30 μL of an acetone solution containing 200 ppm or 10 ppm of each compound was poured into 4 mL glass vials. These vials were placed on a shaker and air-dried while being spun, resulting in the compound forming a dry film on the inner wall of the vial. At 24 hr after drying, 10 1st-instar larvae were released into each vial. Subsequently, the vials were capped and left in an incubation chamber at 25°C and 80% humidity for 24 hr in the dark. One

Table 3. Insecticidal activities of PP derivatives **12** and **13a–n** against insect pests affecting animal health

Compound	Mortality						
	H. longicornis	*D. immitis*[a)]					
	200 ppm	100 ppm	50 ppm	25 ppm	12.5 ppm	6.25 ppm	3.13 ppm
12	4	−					
13a	0	+					
13b	0	−					
13c	10	−					
13d	14	++	+	+	+	−	−
13e	30	−					
13f	20	−					
13g	4	+					
13h	0	−					
13i	35	−					
13j	45	+					
13k	50	++	++	++	++	++	+
13l	0	−					
13m	0	−					
13n	5	−					
PP-I	0	−					
Commercial standard	fipronil 10 ppm	ivermectin 5 ppm					
	100	+++					

[a)] The activities against the microfilariae of *D. immitis* were evaluated using the index; +++: at least two-thirds of the microfilariae died, ++: almost all the microfilariae were affected, or at least one-third died, +: less than one-third of the microfilariae died, −: no influence.

Table 4. Insecticidal activities of PP derivatives **15a**–**e** against insect pests affecting animal health

Compound	Mortality				
	H. longicornis	*D. immitis*[a)]			
	% Mortality at 200 ppm	100 ppm	50 ppm	25 ppm	12.5 ppm
15a	0	+			
15b	24	−			
15c	0	−			
15d	27	++	+	+	−
15e	23	−			
PP-A	0	−	−	−	−
PP-I	0	−			
Commercial standard	fipronil 10 ppm	ivermectin 5 ppm			
	100	+++			

[a)] The activities against the microfilariae of *D. immitis* were evaluated using the index; +++: at least two-thirds of the microfilariae died, ++: almost all the microfilariae were affected, or at least one-third died, +: less than one-third of the microfilariae died, −: no influence.

day after release, the live and dead larvae were counted, and the mortality rate was calculated using the following formula: % mortality=(number of dead larvae/number of live+dead larvae)×100. This test was conducted in duplicate.

5. *Effect against the mosquito* (Aedes albopictus) *by direct spraying onto the insect body*

A solution of natural analogues or PP derivatives dissolved at a concentration of 100 ppm in acetone was directly sprayed onto 10 adult mosquitos in a metal cage using an airbrush. The adults were released into a plastic cup with cotton wool soaked with 10% sucrose/deionized water, and the cup was placed in a temperature-controlled room (light period, 16 hr; dark period, 8 hr; 25°C). Ten minutes and 2 days after treatment, symptoms in the adults were observed.

Results

Among the derivatives with modifications on the 3-pyridyl ring, **4a** was the only compound that showed moderate activity against *M. persicae*, but the level was more than 34 times lower than that of PP-A. Other derivatives did not show activity against other sucking pests, including the aphid.

As a result of the α-pyrone moiety conversion, derivatives **7** and **8** had remarkably lower activities against aphids when compared with a natural analogue with same substituents at the C-1, C-7 and C-11 positions, PP-A or PP-I, respectively, and we did not observe any elevated insecticidal activities against *F. occidentalis*, *L. striatella*, or *Tetranychus urticae* (Table 1).

Next, we investigated the effects of the substituent group at the C-13 position on the insecticidal activity. All of the derivatives tested had low insecticidal activities against *M. persicae* at 1.25 ppm, and the activity levels were less than half that of the natural analogue having a hydroxyl group at the C-13 position, PP-I (Table 2). However, **10b** had a higher activity level against *N. lugens* as compared with PP-A and PP-I in the planthopper test. Furthermore, **9a** exhibited moderate activity against *F. occidentalis* that was a little lower than that of PP-A. Furthermore, the activity of **10a** was slightly higher against *F. occidentalis* than that of PP-A.

We did not find any increase or expansion of the insecticidal activities against sucking pests, including the aphid, of the derivatives with modifications at the C-13 position. Next, we evaluated the use of derivatives as veterinary drugs. The evaluations against *D. immitis* and ticks revealed that some derivatives with modifications at the C-7 position showed insecticidal activities against the microfilariae of *D. immitis* (Table 3), while PP-A and PP-I showed no such activities. Specifically, **13d** and **13k** had higher activity levels against microfilariae. Among the derivatives having the same substitute groups at the C-1, C-7 and C-11 positions, **15d** showed moderate activity (Table 4). However, highly active derivatives, such as **13h** and **15a**, against the green peach aphid did not show high insecticidal activities against microfilariae. Furthermore, although the level was still low, **13k** showed moderate tickicidal activity through contact application. The structure and activity relationship (SAR) appeared to be different from that for insecticidal activity. Therefore, we evaluated whether highly insecticidal derivatives controlled chicken roundworm (*Ascaridia galli*) in *in vivo* tests. However, none of the derivatives showed high endoparacidal activity at 10 mg/kg *via* oral application, while the positive control, moxidectin, exhibited 100% control at 0.1 mg/kg.

As an abnormality of adult houseflies treated with PP-A was observed in our previous paper,[4)] we also investigated effects of PP derivatives on dipteran pests. In the test with adult mosquitos, we found that some PP derivatives affected their flight abilities, and within a few hours, the adults were not able to fly. Notably, **4a** showed longer-lasting efficacy than PP-A (Table 5).

Discussion

Some PP derivatives with modifications at the C-13 position, such as **10a**, exhibited moderate activity against *F. occidentalis* and **10b** exhibited moderate activity against *N. lugens*; these

Table 5. Insecticidal activities of PP derivatives **4a**, **9a**, **10a**, and **13d** against insect pests affecting public health

Compound	Abnormality at 100 ppm *A. albopictus* (Purchased)[a)]	
	30 min AT[b)]	24 hr AT[b)]
4a	100	100
9a	20	50
10a	100	40
13d	100	0
PP-A	100	0

[a)] The insects purchased were used in insecticide tests. [b)] AT: after treatment

were higher than those of their natural analogues, PP-A and PP-I, respectively. However, no derivative was superior to the natural analogues in the aphid test. The 3-pyridyl and α-pyrone moieties were found to be essential for the exhibition of insecticidal activity against any kind of insect. Although some derivatives were also evaluated against veterinary pests, no derivative exhibited high activity. The increased insecticidal activities of some derivatives against the thrips and planthopper might result from the unsaturated bond at the C-13 position, which appears to be more lipophilic, thereby allowing the compound to penetrate insects and crop leaves. At present, planthoppers are a key pest, causing huge amounts of damage to rice in Asian countries, as are thrips, which cause cosmetic damage to the fruits and leaves of a variety of crops globally, diminishing their value. Both pests have developed resistance to many kinds of insecticides.[19–21] Although the PP derivatives in this study were not highly active against the planthopper and thrips, the results of this study provide information on altering the insecticidal spectrum.

While there were no promising candidates among the derivatives with modified pyridine rings or α-pyrone moieties, some with chemical modifications at the C-7 position, such as **13d**, **13k** and **15d**, showed high insecticidal activity against *D. immitis* microfilariae, while PP-A showed no activity. Regarding the SAR, the derivatives with aromatic or hetero rings at the C-7 position exhibited remarkable activity, although it appeared to depend on the specific substitution position and ring structure. Relatively bulky structures at the C-7 position appear to affect the activity level. Although none of the active derivatives in this study showed any anthelmintic activities in *in vivo* trials, this study suggested that they may have applications as veterinary drugs. Furthermore, in the test against mosquitos, which are a public health pest, some PP derivatives affected mosquito's flying ability *via* direct spraying. This action might result from the action on vanilloid-type transient receptor potential (TRPV) channels, which target is reported to be a mode of action for the pyropene insecticide afidopyropen.[6] The channels are expressed in insect chordotonal stretch receptor neurons and normally are responsible for perceiving stimuli from the surroundings.[22] Afidopyropen causes behavior abnormalities in sucking pests such as aphids and whiteflies and, ultimately, their death. Although the action of PP derivatives on mosquitos was not fatal, the possibility of their utility in public health areas was suggested, as there are few substitutes for synthetic pyrethroids to control this destructive human pest. Further research on the SAR in animal and public health areas may help improve the efficacy of PP derivatives, thereby increasing their practical applications.

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