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Effects of 2,2-dimethylchromenes against the feeding behavior of *Coptotermes formosanus* Shiraki

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Precocene I and II are naturally occurring 2,2-dimethylchromenes that have a great influence on various insects. Using phenylboronic acid in acidic conditions, precocenes, natural products octandrenolone, clusiaphenone A, and relatives were prepared. In addition, the effects of synthetic chromene derivatives against *Coptotermes formosanus* Shiraki were evaluated in a no-choise test. The results demonstrate that they were able to induce antifeedant activities. It would seem that the precocene skeleton affects termites. © Pesticide Science Society of Japan

Keywords: precocene, phenylboronic acid, antifeedant activity, *Coptotermes formosanus* Shiraki.

Introduction

Chromenes are benzopyran derivatives that represent a class of natural products with interesting biological properties. Several experiments have revealed the insecticidal effect of these compounds, and some of them also have shown acaricidal activity.^{1,2)} Precocenes are plant-derived chromenes (2*H*-1-benzopyran) with insecticidal activities.^{3–5)} Chromene derivatives 7-methoxy-2,2-dimethylchromene (precocene I) and 6,7-dimethoxy-2,2-dimethylchromene (precocene II) from *Ageratum houstonianum* have been shown to be cytotoxic to the insect corpora allata, thus preventing the production of juvenile hor-

mone. These compounds are more effective against heteropteran insects as well as some other insect species such as grasshoppers and cockroaches.^{6–9)} Additionally, they showed antifeedant effects against several insect species, including storage pests the confused flour beetle (*Tribolium confusum* Duv.), the granary weevil beetle (*Sitophilus granarius* L.), and the khapra beetle (*Trogoderma grananum* Ev.) and herbivorous pest insects the Colorado potato beetle (*Leptinotarsa decemlineata* Say) and aphids (*Myzus persicae* Sulz.).¹⁰⁾ Recently, it has been reported that precocene I significantly reduced the termite soldier proportion for 40 days.¹¹⁾

Our recent investigations resulted in identifying of scopoletin isolated from *Protium javanicum* Burm. f. and its derivatives as excellent antitermite agents.^{12,13)} These compounds are structurally similar to precocenes. To identify the effective and environmentally friendly termite control agents, we planned to synthesize the compounds with termiticidal and antifeedant activity against *Coptotermes formosanus* Shiraki. We report here the synthesis of precocene analogues and their antifeedant activity against *C. formosanus*.

Materials and Methods

1. General chemistry methods

All solvents and reagents were purchased from the suppliers and used without further purification. IR spectra were recorded on a JASCO FT/IR-460 Plus spectrophotometer. ¹H (400 MHz) and ¹³C (100 MHz) NMR spectra were recorded with a JEOL ECX 400 spectrometer with tetramethylsilane as an internal standard. MS spectra were obtained using a JEOL JMS-700/GI spectrometer. Silica gel column chromatography (CC) was performed on silica gel N-60 (40–50 μm). Thin-layer chromatography (TLC) spots on plates pre-coated with silica gel 60 F₂₅₄ were detected with a UV lamp (254 nm). Fractionations for all CCs were based on TLC analyses.

2. Preparation of precocenes and the synthetic analogues

The synthetic scheme and chemical structures of precocenes and the synthetic analogues are shown in Fig. 1. Formation of the pyran ring was carried out using phenylboronic acid (PhB(OH)₂) in acidic conditions. Precocene I and II were prepared from suitable phenols with 2.0 eq of 3-methyl-2-butenal using 1.6 eq of PhB(OH)₂ in benzene for 20 hr of refluxing. The cooled reaction mixture was poured into 20% ammonium acetate solution and extracted with EtOAc, washed with brine, and dried over Na₂SO₄. After evaporation, the residue was purified by silica gel CC to yield the corresponding precocene I (1) and II (2). Dipyrans ring derivatives were synthesized from suitable 2',4',6'-trihydroxyacetophenones using 9.6 eq of PhB(OH)₂ with a twofold amount of other materials. After 20 hr of refluxing, the cooled reaction mixtures were worked up following the

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same procedures. Silica gel CC with the residue afforded the dipyrans ring analogues **3–6**. Alkylation of the C5-hydroxy group performed using alkyl iodide and potassium carbonate in acetone for 24 hr of refluxing gave the desired compounds **7** and **8**.

Precocene I (**1**): Pale yellow oil. IR (film): 1617, 1503, 1316, 984, 803 cm^{-1} . EIMS: m/z 190 $[\text{M}]^+$. HREIMS: m/z 190.0966 $[\text{M}]^+$ (calcd. for $\text{C}_{12}\text{H}_{14}\text{O}_2$, 190.0994). ^1H NMR (400 MHz, CDCl_3): δ 6.86 (1H, d, $J=8.2$ Hz, H-5), 6.39 (1H, s, H-8), 6.38 (1H, d, $J=8.2$ Hz, H-6), 6.25 (1H, d, $J=10.1$ Hz, H-4), 5.44 (1H, d, $J=10.1$ Hz, H-3), 3.74 (3H, s, OMe), 1.41 (6H, s, 2Me). ^{13}C NMR (100 MHz, CDCl_3): δ 160.7, 154.3, 127.9, 127.0, 122.0, 114.7, 106.7, 102.1, 76.4, 55.3, 28.1.

Precocene II (**2**): Pale yellow oil. IR (film): 1615, 1506, 1458, 1363, 1011, 904, 856, 754 cm^{-1} . EIMS: m/z 220 $[\text{M}]^+$. HREIMS: m/z 220.1093 $[\text{M}]^+$ (calcd. for $\text{C}_{13}\text{H}_{16}\text{O}_3$, 220.1099). ^1H NMR (400 MHz, CDCl_3): δ 6.52 (1H, s, H-5), 6.41 (1H, s, H-8), 6.22 (1H, d, $J=10.1$ Hz, H-4), 5.46 (1H, d, $J=10.1$ Hz, H-3), 3.82 (3H, s, OMe), 3.80 (3H, s, OMe), 1.40 (6H, s, 2Me). ^{13}C NMR (100 MHz, CDCl_3): δ 149.7, 147.3, 143.1, 128.2, 122.0, 113.1, 109.9, 101.1, 76.0, 56.6, 55.9, 27.7.

Octandrenolone (**3**): Yellow solid. IR (film): 2977, 1643, 1598, 1463, 1139, 1118, 1003, 882, 728, 669 cm^{-1} . EIMS: m/z 300 $[\text{M}]^+$. HREIMS: m/z 300.1367 $[\text{M}]^+$ (calcd. for $\text{C}_{18}\text{H}_{20}\text{O}_4$, 300.1362). ^1H NMR (400 MHz, CDCl_3): δ 6.63 (1H, d, $J=10.1$ Hz, H-4''), 6.57 (1H, d, $J=10.1$ Hz, H-4''), 5.45 (1H, d, $J=10.1$ Hz, H-3''), 5.42 (1H, d, $J=10.1$ Hz, H-3''), 2.64 (3H, d, $J=6.4$ Hz, H-2'), 1.48 (6H, s, 2Me), 1.42 (6H, s, 2Me). ^{13}C NMR (100 MHz, CDCl_3): δ 203.4, 160.6, 156.8, 155.1, 125.5, 124.8, 116.5, 116.2, 105.6, 102.4, 102.3, 78.34, 78.25, 33.4, 28.4, 28.1.

Clusiaphenone A (**4**): Pale yellow oil. IR (film): 2979, 1645, 1589, 1464, 1137, 1113, 923, 877, 697 cm^{-1} . EIMS: m/z 362 $[\text{M}]^+$. HREIMS: m/z 362.1494 $[\text{M}]^+$ (calcd. for $\text{C}_{23}\text{H}_{22}\text{O}_4$, 362.1518). ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$): δ 7.49–7.41 (5H, m, 2'-Ar), 6.62 (1H, d, $J=10.1$ Hz, H-4''), 6.49 (1H, d, $J=10.1$ Hz, H-4''), 5.61 (1H, d, $J=10.1$ Hz, H-3''), 5.40 (1H, d, $J=10.1$ Hz, H-3''), 1.47 (6H, s, 2Me), 1.00 (6H, s, 2Me). ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{CO}$): δ 200.2, 159.4, 155.9, 155.2, 142.8, 130.2, 127.7, 127.2, 125.9, 125.5, 115.7, 115.5, 105.0, 102.1, 78.3, 77.9, 27.8, 27.0.

Methyl 5-hydroxy-2,2,8,8-tetramethyl-2H,8H-benzo[1,2-*b*:3,4-*b'*]dipyrans-6-carboxylate (**5**): Orange solid. IR (film): 2977, 1652, 1611, 1442, 1210, 1139, 999, 884, 814, 713 cm^{-1} . EIMS: m/z 316 $[\text{M}]^+$. HREIMS: m/z 316.1304 $[\text{M}]^+$ (calcd. for $\text{C}_{18}\text{H}_{20}\text{O}_5$, 316.1311). ^1H NMR (400 MHz, CDCl_3): δ 12.04 (1H, s, OH), 6.64 (1H, d, $J=10.1$ Hz, H-4''), 6.56 (1H, d, $J=10.1$ Hz, H-4''), 5.44 (1H, d, $J=10.1$ Hz, H-3''), 5.43 (1H, d, $J=10.1$ Hz, H-3''), 3.88 (3H, s, OMe), 1.46 (6H, s, 2Me), 1.42 (6H, s, 2Me). ^{13}C NMR (100 MHz, CDCl_3): δ 172.0, 158.9, 156.0, 154.0, 125.56, 125.52, 116.51, 116.45, 103.3, 102.4, 96.5, 77.8, 52.0, 28.3, 27.8.

1-(5-Hydroxy-2,8-dimethyl-2H,8H-pyrano[2,3-*f*]chromen-6-yl)ethanone (**6**): Yellow solid. IR (film): 2931, 1598, 1431, 1365, 1262, 1199, 1027, 724, 687 cm^{-1} . EIMS: m/z 272 $[\text{M}]^+$. HREIMS: m/z 272.1026 $[\text{M}]^+$ (calcd. for $\text{C}_{16}\text{H}_{16}\text{O}_4$, 272.1049). ^1H

NMR (400 MHz, CDCl_3): δ 6.68 (1H, d, $J=10.1$ Hz, H-4''), 6.59 (1H, d, $J=10.1$ Hz, H-4''), 5.49–5.46 (2H, m, H-3'' and H-3''), 5.07–4.99 (2H, m, H-2'' and H-2''), 2.65 (3H, s, H-2'), 1.47 (3H, d, $J=6.4$ Hz, Me), 1.43 (3H, d, $J=6.4$ Hz, Me). ^{13}C NMR (100 MHz, CDCl_3): δ 203.5, 160.5, 155.6, 155.5, 121.5, 121.0, 117.7, 117.6, 103.3, 102.8, 72.9, 72.6, 33.2, 21.8, 21.2.

O-Methyloctandrenolone (**7**): Yellow oil. IR (film): 1697, 1638, 1591, 1363, 1300, 1244, 1184, 1001, 883, 744 cm^{-1} . EIMS: m/z 314 $[\text{M}]^+$. HREIMS: m/z 314.1490 $[\text{M}]^+$ (calcd. for $\text{C}_{19}\text{H}_{22}\text{O}_4$, 314.1518). ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$): δ 6.61 (1H, d, $J=10.1$ Hz, H-4''), 6.49 (1H, d, $J=10.1$ Hz, H-4''), 5.67 (1H, d, $J=10.1$ Hz, H-3''), 5.64 (1H, d, $J=10.1$ Hz, H-3''), 3.72 (3H, s, OMe), 2.43 (3H, s, H-2'), 1.44 (6H, s, 2Me), 1.43 (6H, s, 2Me). ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{CO}$): δ 199.1, 153.5, 151.0, 150.2, 128.1, 118.2, 116.5, 115.9, 108.1, 106.4, 77.1, 76.9, 63.1, 31.9, 27.2, 27.1.

O-Ethyloctandrenolone (**8**): Yellow oil. IR (film): 1698, 1638, 1590, 1578, 1362, 1299, 1244, 1186, 1031, 989, 884, 691 cm^{-1} . EIMS: m/z 328 $[\text{M}]^+$. HREIMS: m/z 328.1678 $[\text{M}]^+$ (calcd. for $\text{C}_{20}\text{H}_{24}\text{O}_4$, 328.1675). ^1H NMR (400 MHz, CDCl_3): δ 6.58 (1H, d, $J=10.1$ Hz, H-4''), 6.45 (1H, d, $J=10.1$ Hz, H-4''), 5.50 (1H, d, $J=2.7$ Hz, H-3''), 5.47 (1H, d, $J=2.7$ Hz, H-3''), 3.87 (2H, q, $J=6.9$ Hz, OEt), 2.48 (3H, s, H-2'), 1.403 (6H, d, $J=2.7$ Hz, 2Me), 1.396 (6H, d, $J=2.7$ Hz, 2Me), 1.30 (3H, t, $J=6.9$ Hz, OEt). ^{13}C NMR (100 MHz, CDCl_3): δ 201.1, 152.8, 151.4, 150.5, 127.8, 127.7, 118.0, 117.1, 116.3, 108.6, 106.5, 77.2, 76.9, 72.2, 32.8, 28.0, 27.9, 15.5.

3. Antifeedant test

Workers and soldiers of *C. formosanus* Shiraki were obtained from a laboratory colony maintained at $28^\circ\text{C}\pm 2$ and $>85\%$ Relative Humidity (RH) at the Deterioration Organisms Laboratory of the Research Institute for Sustainable Humansphere (RISH), Kyoto University, Japan. A no-choice test was employed for evaluating antifeedant activity.¹²⁾ A solution of the compounds (5 μmol) was dissolved in 0.05 mL chloroform and applied to paper discs (1.5 mm thick, 8 mm diameter, and 30-mg average mass, Advantec); the paper discs were dried overnight in a vacuum desiccator. Subsequently, each disc was placed on a plastic spacer in an acrylic resin cylinder with a plaster bottom (80-mm diameter $\times$ 60-mm height), and 33 active termites (30 workers and 3 soldiers) were added to each cylinder. The cylinders were then placed on a cotton sheet in a plastic tray and kept in a dark incubator at $28^\circ\text{C}\pm 2$ and $80\%\pm 5$ RH. Paper discs treated only chloroform were used as a control. Three replications were performed for each sample. Paper disc consumption by the termites was measured after 28 days. Mass loss of the paper discs was determined by subtracting the vacuum-dried weight at the end of the test from the weight the night before the start of the test.

Results and Discussion

Recently, we reported that natural coumarins and their synthetic derivatives are potent antifeedant agents.^{12,13)} Both coumarins and precocenes are chromene derivatives, but precocenes have

Fig. 1. Synthetic scheme and chemical structures of precocenes and synthetic analogues 1–8. Percentages (%) represent isolated yields.

dimethyl groups in the C2-position in place of a carbonyl group. Thus, we were interested in the possibility of precocene derivatives as termite-control agents. The previous results sparked our interest to study whether precocenes having more than a 2,2-dimethylpyran ring are able to induce the stronger activity. Although a literature review revealed that while synthetic studies of 2,2-dimethylchromenes that bear one pyran ring have been eagerly reported, there are only a few studies of the direct synthetic route of the dipyrane ring analogues. It has been reported that phenylboronic acid (PhB(OH)_2) is useful for benzopyran synthesis.^{14,15} At first, we prepared precocene I and II by the reaction of methoxyphenols with 3-methyl-2-butenal using PhB(OH)_2 in acidic conditions. In addition, the dipyrane analogues including natural products octandrenolone, clusiaphenone A, and relatives were prepared. The synthetic scheme and chemical structures are shown in Fig. 1.

We evaluated the antifeedant effects of the precocenes and their analogues against *C. formosanus* with the no-choice test for 28 days. Paper disc consumption by the termites after exposure to compounds (1–8) was compared to that of the control, and the average mass losses are shown in Fig. 2. At a dose of 5 μmol , all derivatives showed antifeedant activity. Among the compounds tested, *O*-ethyloctandrenolone **8** elicited the lowest average mass loss (avg. 5.5%), showing a significantly greater reduction in feeding activity as compared to the control (59.1%), followed consecutively by octandrenolone **3** (12.0%), methyl 5-hydroxy-2,2,8,8-tetramethyl-2*H*,8*H*-benzo[1,2-*b*:3,4-*b'*]dipyran-6-carboxylate **5** (6.3%), and *O*-methyloctandrenolone **7** (5.8%). Although two additional derivatives (clusiaphenone A **4** and 1-(5-hydroxy-2,8-dimethyl-2*H*,8*H*-pyrano[2,3-*f*]chromen-6-yl)ethanone **6**) showed relatively weak antifeedant activity in comparison with the compounds noted above, their effects were stronger than those of precocenes I (**1**) and II (**2**). In general, the compounds having two pyran rings tended to exhibit stronger antifeedant activity than did the precocenes having one pyran ring. The hydroxyl or methoxy group's presence in the C6-posi-

Fig. 2. Paper disc consumption of *Coptotermes formosanus* Shiraki after 4 weeks of exposure to precocenes and the synthetic analogues (1–8) in a no-choice test at a dose of 5 μmol . Each experiment was performed in triplicate. 33 termites were used per replication, and the data were averaged ($n=3$). Numbers followed by different letters (a–d) are significantly different at the level of $p<0.05$ according to Tukey's multiple comparison test.

tion of the dipyrane analogues has little or no effect on their ability. However, addition of the phenyl group into the functional group of the C2'-position (**4**) and lack of the methyl groups in the C2''- and C2'''-position (**6**) decreased their antifeedant activity.

Scopoletin (7-hydroxy-6-methoxycoumarin) was found to be a strong antifeedant compound, having a low mass loss value (avg. 2.0%).¹² Compounds **5**, **7**, and **8** showed much the same effect as scopoletin. Antifeedant compounds are substances that, when tasted or initially consumed can result in the cessation of feeding, either temporarily or permanently depending on potency of the compounds. Azambuja *et al.* found that precocene II, added to the diet, acted as a feeding deterrent for *Rhodnius prolixus*, reducing the amount of blood consumed by this insect.¹⁶ Precocene II has shown the strongest antifeedant effect ($\text{ED}_{50}=48 \mu\text{g/mL}$) among precocene I, ethoxy precocene, and chromane of precocene. The precocenes are presumed to undergo biotransformation into reactive epoxides through the action of monooxygenase enzymes within the corpora allata.⁸ The

antifeeding effect of precocene II on *R. prolixus* may be due to a direct cytotoxic action on gut tissues which contain activating monooxygenases. Because two-pyran ring-compounds tend to exhibit stronger activity, these compounds might be converted to bis(epoxide) forms.

Although chemical inhibition of feeding has been studied in detail for a few phytophagous insect species, the mechanisms of action could function by blocking input from receptors that normally respond to phagostimulants or by stimulating specific deterrent cells. The mechanism of the feeding inhibition of precocene analogues on *C. formosanus* requires further investigation. The chromene analogues synthesized have the potential to be developed further as termiticides.

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