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Synthesis and Insect Antifeedant Activity of Precocene Derivatives with Lactone Moiety

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Starting from precocenes I and II, four of their derivatives with a lactone moiety were obtained. The compounds have been assessed as antifeedants against several diverse insect species including the storage pests the confused flour beetle (*Tribolium confusum* Duv., larvae and adults), the granary weevil beetle (*Sitophilus granarius* L., adults), and the khapra beetle (*Trogoderma granarium* Ev., larvae) and against the herbivorous pest insects Colorado potato beetle (*Leptinotarsa decemlineata* Say, adults and larvae) and aphids (*Myzus persicae* Sulz.). Precocenes, especially precocene II, showed a very strong antifeedant effect against all storage pests and aphids. The introduction of a lactone moiety caused a decrease in antifeedant activity against these species. Both precocenes were moderately active against *L. decemlineata* adults. The best antifeedants to this species were precocene derivatives, especially iodolactones. The introduction of iodine into a molecule had a great effect on the antifeedant activity of those compounds.

KEYWORDS: Precocenes; lactones; antifeedants; storage pests; *Tribolium confusum*; *Sitophilus granarius*; *Trogoderma granarium*; *Leptinotarsa decemlineata*; *Myzus persicae*

INTRODUCTION

Precocenes, the methoxy derivatives of 2,2-dimethylchromene also called ageratochromenes, isolated from *Ageratinae* species, are known for their allatocidal activity and for inducing precocious metamorphosis in immature hemipterans (*1*). Those compounds applied topically or orally are biotransformed into highly reactive 3,4-epoxy derivatives through the action of monooxygenase enzymes, causing rapid, irreversible degeneration in the glandular parenchyma cells of the *corpora allata* and cessation of the production of juvenile hormones (*2*). Ageratochromenes reduce the length of larval life in sensitive species and prevent ovarian development in some adult insects. It proved to be impossible to induce precocious metamorphosis in *Holometabola* species. This selective allatotoxic activity of those chemicals may be related to the presence or absence of epoxy hydrases in *corpora allata* or other tissues (*3*). In insensitive *Lepidoptera* and *Coleoptera* those compounds are transformed into less toxic, more easily water-soluble ones and eliminated (*4*, *5*). Thus, in *Holometabola* species, the effective dosage for precocious metamorphosis may be very close to a toxic concentration (*6*).

According to many researchers, precocenes also show antifeedant activity. Neonate corn earworm, *Heliothis zea* larvae, cultured on a diet containing precocene II, did not feed and starved to death (*7*). The presence of precocene II in the diet of *Rhodnius prolixus* acted as a feeding deterrent and reduced the amount of blood consumed by this insect (*8*). Our earlier studies have also demonstrated that precocenes and several of their derivatives are very good feeding deterrents for some storage pests and aphids (*9*). Those properties of precocenes make possible that they or their synthetic analogues can be used for the control of insect pests. On the other hand, precocenes show hepatoxic and nephrotoxic properties toward vertebrates (*10*, *11*), so changing their structure may reduce those undesired properties.

The key objective of the present study was the synthesis of precocene derivatives with a lactone moiety and the comparison of their antifeedant activity with that of precocenes against

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several pest insect species including storage pests, the Colorado potato beetle (CPB) and aphid. The combination of precocenes with a lactone moiety is justified by the fact that among feeding deterrents there are many compounds with a lactone ring (*12*−*15*). The deterrent activity of intermediate products of lactones synthesis, that is, methoxy- and dimethoxycarboxylic acids, has also been studied.

MATERIALS AND METHODS

Reagents. All chemicals except alkyl phosphonates were purchased from POCh, Merck, Aldrich, and Fluka. Precocenes I and II were synthesized from resorcinol (precocene I) and 1,2,4-benzotriol (precocene II) according to methods described by Timar (*16*−*18*).

General Procedures. *Analytical TLC* was performed on silica gel (Kieselgel 60, Merck) with mixtures of hexane, ethyl acetate, and acetone in various ratios as developing systems. Compounds were detected in iodine (plastic plates) or by dipping the plates in an ethanolic solution of $Ce(SO_4)_2$ (1%)/H_3 [$P(Mo_3O_{10})_4$] (2%)/H_2SO_4 (10%) followed by heating to 120 °C.

Column chromatography was carried out on silica gel (Kieselgel 60, 40−63 μm, 230−400 mesh, Merck) with mixtures of hexane, ethyl acetate, acetone, and methylene chloride in various ratios as eluents.

GC analyses were performed with a Varian CP-3380 instrument using the following capillary column: HP-1 (cross-linked methyl siloxane), 25 m × 0.32 mm × 0.52 μm; HP-5 (cross-linked 5% phenyl methyl siloxane), 25 m × 0.32 mm × 0.52 μm (injector temperature, 250 °C; detector temperature, 300 °C; FID, carrier gas, H_2).

^{1}H NMR spectra were recorded in $CDCl_3$ solutions on a Bruker Avance DRX 300 (300 MHz) spectrometer with TMS as internal standard.

IR spectra were recorded for liquid films or KBr tablets on a Specord M80 infrared spectrometer (Carl Zeiss Jena).

Melting points (uncorrected) were determined using a Boetius apparatus.

Synthesis and Separation of Compounds. *Synthesis of the 3-Chromanones (**3a** and **3b**) from Precocenes (**1a** and **1b**).* The dry solution of the *m*-chloroperbenzoic acid (8.63 g, 0.050 mol) in dichloromethane (100 mL) was added dropwise to the cooled (0 °C) and stirred solution of precocene I (**1a**, 9.5 g, 0.05 mol) or precocene II (**1b**, 11.0 g) in dichloromethane (100 mL). Stirring, without cooling, was continued for 2−4 h (TLC). The dichloromethane was evaporated under vacuum at room temperature. The residue was dissolved in acetone (150 mL), and concentrated hydrochloric acid (8 mL) was added in one portion. The solution was stirred at 40 °C for 0.5−1 h. The progress of the reaction was monitored by TLC. When the reaction was completed, acetone was evaporated in vacuo, and the residue was dissolved in chloroform (100 mL), washed with water, and washed several times with a solution of sodium bicarbonate and, finally, brine. Organic solution was dried ($MgSO_4$) and, after evaporation of solvent, crude 3-chromanone (**3a** or **3b**) was purified on silica gel using as eluent a mixture of hexane and ethyl acetate 12:1 (v/v ratio). The yields, physical, and spectral data of 3-chromanones obtained are as follows.

*7-Methoxy-2,2-dimethylchroman-3-one **(3a)**:* 9.54 g, 92% yield, pale yellow oil; n_D^{20} 1.515; ^{1}H NMR ($CDCl_3$), δ 1.38 [s, 6H, −OC(CH_3)$_2$−], 3.50 (s, 2H, −CH_2CO−), 3.76 (s, 3H, −OCH_3), 6.54 (s, 1H, *H*-8), 6.56 (d, J = 8.1 Hz, 1H, *H*-6), 6.94 (d, J = 8.1 Hz, 1H, *H*-5); IR (film), 1728 (s, C═O), 1288 (s, C−O−C), 1200 (s, C−O−C), 1156 (s, C−O−C).

*6,7-Dimethoxy-2,2-dimethylchroman-3-one **(3b)**:* 9.56 g, 81% yield, yellow oil; n_D^{20} 1.509; ^{1}H NMR ($CDCl_3$), δ 1.40 [s, 6H, −OC(CH_3)$_2$−], 3.52 (s, 2H, −CH_2CO−), 3.84 (s, 3H, −OCH_3), 3.86 (s, 3H −OCH_3), 6.58 (s, 2H, *H*-5, *H*-8); IR (film), 1728 (s, C═O), 1232 (s, C−O−C), 1200 (s, C−O−C), 1168 (s, C−O−C).

Horner−Wadsworth−Emmons Olefination of 3-Chromanones (3a,b). A solution of the methyl (6.30 g) or ethyl (6.73 g) diethylphosphonoacetate (0.030 mol) in anhydrous tetrahydrofuran (30 mL) was cooled to −80 °C (dry ice bath) under argon, and a 1.3 M solution of methyllithium in diethyl ether (24 mL, 0.0312 mol) was added dropwise by syringe. The reaction mixture was stirred at −80 °C for 15 min, and a solution of 3-chromanone (5.15 g of **3a** or 5.91 g of **3b**, 0.025 mol) in tetrahydrofuran (25 mL) was added slowly. The dry ice bath was removed, and the stirred mixture was left to reach room temperature for 1 h. Then, the solution was boiled under reflux for 12−24 h (TLC). When the reaction was completed (TLC), the mixture was cooled, diluted with diethyl ether (150 mL), and quenched with brine. The organic layer was separated, and the water phase was extracted with diethyl ether. The organic solution was washed twice with brine and dried ($MgSO_4$). All esters (**4a**,**b** and **5a**,**b**) obtained were purified by column chromatography on silica gel (hexane/ethyl acetate 30:1 or 15:1). The yields, physical, and spectral data of obtained esters are given below.

*Methyl (7-methoxy-2,2-dimethyl-2H-chromen-3-yl)acetate **(4a)**:* 4.58 g, 70% yield, pale yellow oil, n_D^{20} 1.548; ^{1}H NMR ($CDCl_3$), δ 1.40 [s, 6H, −C(CH_3)$_2$−], 3.11 (s, 2H, −CH_2CO_2), 3.70 (s, 3H, −CO_2CH_3), 3.75 (s, 3H, −OCH_3), 6.21 (s, 1H, *H*-4), 6.37 (d, J = 2.2 Hz, 1H, *H*-8), 6.40 (dd, J = 8.2, 2.2 Hz, 1H, *H*-6), 6.87 (d, J = 8.2 Hz, 1H, *H*-5); IR (film), 1724 (s, C═O), 1656 (w, C═C), 1272 (s, C−O−C), 1152 (s, C−O−C).

*Methyl (6,7-dimethoxy-2,2-dimethyl-2H-chromen-3-yl)acetate **(4b)**:* 6.21 g, 85% yield, yellow oil, n_D^{20} 1.546; ^{1}H NMR ($CDCl_3$), δ 1.40 [s, 6H, −C(CH_3)$_2$−], 3.13 (s, 2H, −CH_2CO_2), 3.72 (s, 3H, −CO_2CH_3), 3.81 (s, 3H, −OCH_3), 3.83 (s, 3H, −OCH_3), 6.20 (s, 1H, *H*-4), 6.42 (s, 1H, *H*-8), 6.54 (s, 1H, *H*-5); IR (film), 1736 (s, C═O), 1664 (w, C═C), 1248 (s, C−O−C), 1200 (s, C−O−C), 1136 (s, C−O−C).

*Ethyl (7-methoxy-2,2-dimethyl-2H-chromen-3-yl)acetate **(5a)**:* 4.49 g, 65% yield, pale yellow oil, n_D^{20} 1.543; EI-MS (relative intensity), m/z 276 [M$^+$] (24), 261 (100), 247 (99), 233 (17), 187 (17); ^{1}H NMR ($CDCl_3$), δ 1.26 (t, J = 7.1 Hz, 3H, −$CO_2CH_2CH_3$), 1.40 [s, 6H, −C(CH_3)$_2$−], 3.09 (s, 2H, −CH_2CO_2−), 3.74 (s, 3H, −OCH_3), 4.16 (q, J = 7.1 Hz, 2H, −$CO_2CH_2CH_3$), 6.22 (s, 1H, *H*-4), 6.37 (d, J = 2.4 Hz, 1H, *H*-8), 6.39 (dd, J = 8.2, 2.4 Hz, 1H, *H*-6), 6.86 (d, J = 8.2 Hz, 1H, *H*-5); IR (film), 1732 (s, C═O), 1676 (w, C═C), 1284 (s, C−O−C), 1160 (s, C−O−C).

*Ethyl (6,7-dimethoxy-2,2-dimethyl-2H-chromen-3-yl)acetate **(5b)**:* 5.53 g, 72% yield, yellow oil, n_D^{20} 1.538; EI-MS (relative intensity), m/z 306 [M$^+$] (40), 291 (100), 263 (38), 233 (25); ^{1}H NMR ($CDCl_3$), δ 1.28 (t, J = 7.1 Hz, 3H, −$CO_2CH_2CH_3$), 1.41 [s, 6H, −C(CH_3)$_2$−], 3.11 (s, 2H, −CH_2CO_2−), 3.81 (s, 3H, −OCH_3), 3.83 (s, 3H, −OCH_3), 4.18 (q, J = 7.1 Hz, 2H, −$CO_2CH_2CH_3$), 6.20 (s, 1H, *H*-4), 6.42 (s, 1H, *H*-8), 6.53 (s, 1H, *H*-5); IR (film), 1732 (s, C═O), 1636 (w, C═C), 1248 (s, C−O−C), 1200 (s, C−O−C), 1136 (s, C−O−C).

Alkaline Hydrolysis of Esters 4a,b and 5a,b. Ester (**4a**, 3.93 g; **4b**, 4.38 g; **5a**, 4.14 g; or **5b**, 4.59 g, 0.015 mol) was added to a solution of KOH in methanol (50 mL, 5% w/v), and the mixture was refluxed for 4−6 h. Then, the methanol was evaporated in vacuo, and the residue was diluted with water. Organic impurities were extracted with diethyl ether. The aqueous solution was cooled by adding ice, acidified with 5% HCl, and extracted with diethyl ether. The ethereal extract was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Crude, solid acids were purified on silica gel (hexane/ethyl acetate/dichloromethane 10:1:1) or by crystallization (hexane with a small amount of ethyl acetate). The yields, physical, and spectral data of acids obtained (**6a**,**b**) are given below.

*(7-Methoxy-2,2-dimethyl-2H-chromen-3-yl)acetic acid **(6a)**:* 2.99 g, 80% yield (from **4a**, after crystallization), white crystals, mp 124−125 °C; EI-MS (relative intensity), m/z 248 [M$^+$] (100), 204 (67), 189 (100), 161 (32), 151 (42), 69 (34); ^{1}H NMR ($CDCl_3$), δ 1.43 [s, 6H, −C(CH_3)$_2$−], 3.16 (s, 2H, −CH_2CO_2), 3.76 (s, 3H, OCH_3), 6.28 (s, 1H, *H*-4), 6.38 (s, 1H, *H*-8), 6.41 (d, J = 8.3 Hz, 1H, *H*-6), 6.88 (d, J = 8.3 Hz, 1H, *H*-5); IR (KBr), 2750−3200 (s, b, O−H), 1716 (s, C═O), 1660 (m, C═C), 1240 (s, C−O−C), 1208 (s, C−O−C), 1144 (s, C−O−C), 1092 (s, C−O−C).

*(6,7-Dimethoxy-2,2-dimethyl-2H-chromen-3-yl)acetic acid **(6b)**:* 3.10 g, 74% yield (from **4b**, after crystallization), pale yellow crystals, mp 108−110 °C; EI-MS (relative intensity), m/z 276 [M$^+$] (48), 263 (100), 233 (22), 218 (28), 203 (21), 69 (21); ^{1}H NMR ($CDCl_3$), δ 1.41 [s, 6H, −C(CH_3)$_2$−], 3.15 (s, 2H, −CH_2CO_2), 3.80 (s, 3H, −OCH_3), 3.82 (s, 3H, −OCH_3), 6.24 (s, 1H, *H*-4), 6.41 (s, 1H, *H*-8), 6.52 (s, 1H,

H-5); IR (KBr), 2760−3100 (s, b, O−H), 1712 (s, C═O), 1648 (m, C═C), 1248 (s, C−O−C), 1200 (s, C−O−C), 1136 (s, C−O−C), 1104 (s, C−O−C).

Iodolactonization of Acids 6a,b. A 0.5 M $NaHCO_3$ (20 mL) solution was added to the solution of acid (**6a**, 1.24 g; or **6b**, 1.39 g, 0.005 mol) in diethyl ether (25 mL). The mixture was stirred at room temperature for 30 min, and a solution of KI (0.015 mol) and I_2 (0.01 mol) in water (20 mL) was gradually added. The mixture was stirred for 1 h, diluted with diethyl ether (50 mL), and washed twice with saturated $Na_2S_2O_3$. The ethereal solution was washed with $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. Crude iodolactone was purified by column chromatography on silica gel (hexane/ethyl acetate/dichloromethane 10:1:1) or by crystallization (hexane with a small amount of ethyl acetate). Yields, physical, and spectral data of pure iodolactones **7a**,**b** are as follows.

*(±)-3a-Iodo-7-methoxy-4,4-dimethyl-3a,9b-dihydro-3H,4H-furo[3,2-c]chromen-2-one (**7a**):* 1.57 g, 84% yield (after crystallization), pale yellow crystals, mp 99.5−100 °C; EI-MS (relative intensity), *m*/*z* 374 [M^+] (tr), 246 (81), 231 (100), 203 (43), 189 (31), 151 (21); ^{1}H NMR ($CDCl_3$), δ 1.66 [s, 3H, −C(CH_3)CH_3−], 1.68 [s, 3H, −C(CH_3)CH_3−], 3.08 (d, J = 18.2 Hz, 1H, −$CH_aH_bCO_2$), 3.31 (d, J = 18.2 Hz, 1H, −C$H_a$$H_b$$CO_2$), 3.77 (s, 3H, −OC$H_3$), 5.85 (s, 1H, *H*-9b), 6.34 (s, 1H, *H*-6), 6.59 (d, J = 8.6 Hz, 1H, *H*-8), 7.21 (d, J = 8.6 Hz, 1H, *H*-9); IR (KBr), 1784 (s, C═O), 1240 (s, C−O−C), 1200 (s, C−O−C), 1156 (s, C−O−C), 1112 (s, C−O−C), 552 (w, C−I).

*(±)-3a-Iodo-7,8-dimethoxy-4,4-dimethyl-3a,9b-dihydro-3H,4H-furo-[3,2-c]chromen-2-one (**7b**):* 1.67 g, 83% yield (after crystallization), pale yellow crystals, mp 89−90 °C (dec); EI-MS (relative intensity), *m*/*z* 404 [M^+] (tr), 382 (29), 231 (100), 279 (37), 219 (60), 205 (49), 187 (52) 167 (41), 142 (48); ^{1}H NMR ($CDCl_3$), δ 1.64 [s, 6H, −C(CH_3)$_2$−], 3.06 (d, J = 18.2 Hz, 1H, −$CH_aH_bCO_2$), 3.31 (d, J = 18.2 Hz, 1H, −C$H_a$$H_b$$CO_2$), 3.82 (s, 3H, OC$H_3$), 3.83 (s, 3H, −OC$H_3$), 5.81 (s, 1H, *H*-9b), 6.34 (s, 1H, *H*-6), 6.71 (s, 1H, *H*-9); IR (KBr), 1784 (s, C═O), 1264 (s, C−O−C), 1200 (s, C−O−C), 1160 (s, C−O−C), 1132 (s, C−O−C), 584 (w, C−I).

Dehydrohalogenation of Iodolactones 7a,b. To a solution of β-iodo-γ-lactone (**7a**, 0.748 g; or **7b**, 0.808 g, 2 mmol) in anhydrous diethyl ether (10 mL) was added triethylamine (0.56 mL, 4.02 mmol), and the mixture was stirred under argon at room temperature for 1 h. Then, the reaction mixture was diluted with diethyl ether (20 mL) and washed with 5% HCl and brine and dried ($MgSO_4$). Solvent was evaporated, and the crude product was purified by column chromatography (silica gel, hexane/ethyl acetate, 8:1). Yields, physical, and spectral data are given below.

*7-Methoxy-4,4-dimethyl-3H,4H-furo[3,2-c]chromen-2-one (**8a**):* 0.370 g, 75% yield, pale yellow crystals, mp 117−119 °C' EI-MS (relative intensity), *m*/*z* 246 [M^+] (52), 231 (100), 203 (68), 151 (18), 175 (13), 151 (18); ^{1}H NMR ($CDCl_3$), δ 1.52 [s, 6H, −C(CH_3)$_2$−], 3.32 (s, 2H, −CH_2CO_2), 3.80 (s, 3H, −OCH_3), 6.44 (d, J = 2.1 Hz, 1H, *H*-6), 6.48 (dd, J = 8.4, 2.1 Hz, 1H, *H*-8), 7.11 (d, J = 8.4 Hz, 1H, *H*-9); IR (KBr), 1808 (s, C═O), 1696 (w, C═C), 1232 (s, C−O−C), 1168 (s, C−O−C), 1028 (s, C−O−C).

*7,8-Dimethoxy-4,4-dimethyl-3H,4H-furo[3,2-c]chromen-2-one (**8b**):* 0.400 g, 72% yield, yellow solid, mp 93−94 °C; EI-MS (relative intensity), *m*/*z* 276 [M^+] (40), 261 (100), 233 (80), 181 (10); ^{1}H NMR ($CDCl_3$), δ 1.52 [s, 6H, −C(CH_3)$_2$−], 3.34 (s, 2H, −CH_2CO_2), 3.86 (s, 3H, −OCH_3), 3.87 (s, 3H, −OCH_3), 6.49 (s, 1H, *H*-6), 6.75 (s, 1H, *H*-9); IR (KBr), 1808 (s, C═O), 1696 (w, C═C), 1224 (s, C−O−C), 1144 (s, C−O−C), 1032 (s, C−O−C).

Bioassays. *Insect Culture.* The experiments were conducted with *Tribolium confusum* Duv. (larvae and adults), *Sitophilus granarius* L. (adults), *Trogoderma granarium* Ev. (larvae), *Leptinotarsa decemlineata* Say (larvae and adults), and apterous viviparous females of the peach potato aphid, *Myzus persicae* Sulz. The stored product insect pests were reared in a chamber maintained at 26 ± 1 °C and 60 ± 5% relative humidity on wheat grain or whole-wheat meal diet. The first generation of Colorado potato beetle adults was collected from an unsprayed potato field. Eggs were collected and hatched in the laboratory. Larvae were reared on potato leaves in Petri dishes at 24 ± 1 °C and 60 ± 5% relative humidity under a 16:8 (L/D) photoperiod (climate chamber). *M. persicae* were reared on Chinese cabbage in the laboratory at 20 ± 1 °C, 60 ± 5% relative humidity, and 16:8 L/D photoperiod.

Feeding Deterrent Activity Tests. The choice and no-choice tests for chewing insects were conducted. In the experiments with the storage pests the wheat wafer disk bioassay described earlier (*19*) was used. The wafer disks (1 cm diameter × 1 mm thick) were saturated by dipping either in solvent only (control) or in 1% acetone solution of compounds. After evaporation of the solvent (30 min of air-drying), the wafers were offered to 3 adults of *S. granarius*, 20 adults and 10 larvae of *T. confusum*, and 10 larvae of *T. granarium*. The number of individual insects depended on the intensity of their food consumption. Adults used for experiments were unsexed, 7−10 days old, and the larvae were 5−30 days old. The wafer disks were weighed before offering them to the insects during the following five-day period in choice and no-choice tests.

The experiments with *L. decemlineata* were conducted according to the classical leaf-disk bioassay described earlier by Bellés et al. (*20*) using adults collected every day from unsprayed potato field and newly ecdyzed third-instar larvae obtained from a laboratory colony. For the feeding tests 0.5% alcohol solutions of compounds were prepared. Disks (4.0 cm in diameter) were cut from potato leaves and were dipped in the test solutions or alcohol. After the solvent evaporated, the disks were offered to 10 larvae or 6 adults (3 pairs). Control and treated disks were placed at alternate corners in Petri dishes lined with moistened filter paper (choice test). In the no-choice test only control or only treated disks were placed in the dishes. In each four replicates, the insects were allowed to feed *ad libitum* for 24 h at 24 °C under a 16:8 (L/D) photoperiod.

After the completion of the experiments, the wafer disks were reweighed (storage pests), and the areas of remaining uneaten potato leaf disks (CPB) were measured using a scanner and special software. On the basis of the amount of food consumed in all variants the three deterrency coefficients (relative, *R*; absolute, *A*; and total, *T*) were calculated using the formulas according to Nawrot et al. (*19*):

$$R = C - T/C + T \times 100 \quad \text{(choice test)}$$

$$A = \mathrm{CC} - \mathrm{TT}/\mathrm{CC} + \mathrm{TT} \times 100 \quad \text{(no-choice test)}$$

C and CC are the amount of food consumed from the control disks , and *T* and TT are the amount of food consumed that had been treated with the tested compound.

The measure of the deterrent activity of tested compounds is the total coefficient of deterrence: $T = A + R$.

The total coefficient of deterrence, which ranged from −200 to 200, served as the index activity. The compounds with *T* values ranging from 151 to 200 are very good deterrents, those with coefficients values of 101−150 are good deterrents, and those with *T* values ranging from 51 to 100 were moderately active. Compounds with *T* values lower than 50 are weak deterrents. Negative *T* values point to attractant properties of the compound.

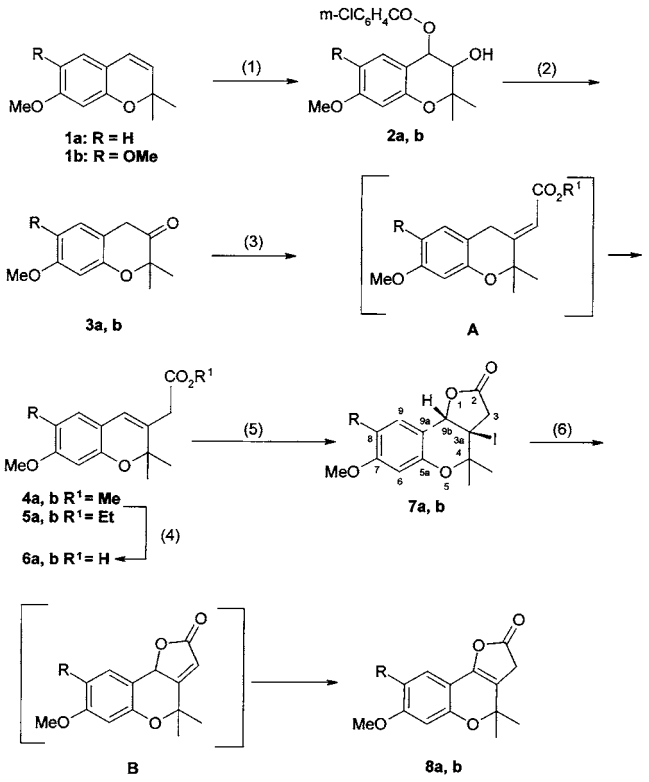
The values of deterrence coefficients were statistically analyzed by means of one-way analysis of variance ANOVA. In the cases where ANOVA results were statistically significant, Tukey's test was performed.

Aphid Settling Tests. These experiments were conducted with 1−3-day-old adult apterous aphids selected from laboratory colonies. The test was carried out according to the procedure described earlier (*21*). The test chemicals were applied as 0.1% ethanol solution painted on the leaf surface at ∼0.01 mL/cm^2 on one side of the upper surface of Chinese cabbage leaves. The other side of the midrib was treated with solvent only. The insects (20 aphids per treatment) had a choice between equal areas of treated and control surfaces. Settled aphids were counted on both sides of the leaf after 15, 30, 60, and 120 min and 24 h. The results were examined using analysis of variance at $P = 0.05$. The activity was expressed as the significance level $P < 0.05$ after comparison of the number of aphids settled on the treated and control halves of the leaf.

RESULTS AND DISCUSSION

Syntheses. Lactones **8a** and **8b** were obtained in a six-step synthesis from precocenes I and II, respectively (**Scheme 1**).

Scheme 1[a]



[a] (1) *m*-CPBA, CH_2Cl_2, room temperature, 2–4 h; (2) HCl/acetone, 40 °C, 0.5–1 h; (3) $(EtO)_2P(O)CH_2CO_2Me$ or $(EtO)_2P(O)CH_2CO_2Et$/MeLi, THF, –80 °C, 1 h, then reflux of 12–24 h; (4) KOH/MeOH, reflux of 4–6 h, then 5% HCl; (5) I_2/KI, $NaHCO_3$, Et_2O/H_2O, room temperature, 1 h; (6) Et_3N, Et_2O, room temperature, 1 h.

Transformation of precocenes into the corresponding 3-chromanones (**3a** and **3b**) was carried out according to the Jennings methodology (*22*) as modified by us. In our modification the hydrolysis and rearrangement of 3,4-diol was done in one step. Hydrochloric acid in acetone was used for this transformation. In this way chromanones **3** (**3a**, **3b**) were obtained in 80% yield. Ketones (**3a** and **3b**) were subjected to Horner−Wadsworth−Emmons reaction with methyl and ethyl diethyl phosphonoacetate. The direct products of olefination of chromanones, esters with exocyclic double bond (A), isomerized in the reaction medium to the more stable products **4a**,**b** and **5a**,**b**.

The structure of these esters was confirmed by their ^{1}H NMR and IR data. The chemical shift of singlet of H-4 (δ 6.20−6.24) is almost the same as for precocenes (δ 6.19−6.20). The absorption bands ($\nu = 1724-1736$ cm^{-1}) found in the IR spectra are characteristic for esters with a carbalkoxy group unconjugated with double bond.

Hydrolyses (KOH in methanol) of esters **4a**/**5a** and **4b**/**5b** afforded acids **6a** and **6b**, respectively, in good yields (68−80%). Iodolactonization of these acids was carried out in basic conditions according to procedure described by Mori (*23*). Iodolactones **7a** and **7b** were obtained as crystals in 84 and 83% yield. The presence of a γ-lactone ring in the iodolactones is proved by the absorption band at 1784 cm^{-1} the in IR spectra of both **7a** and **7b**.

Unsaturated lactones **8a** and **8b** were obtained in the reaction of corresponding iodolactones with triethylamine in diethyl ether at room temperature. The structure of these products was confirmed by their spectral (^{1}H NMR and IR) data. The absorption band characteristic for the γ-lactone ring is present in the IR spectra of both lactones at 1808 cm^{-1}. The presence of a singlet of methylene protons of CH_2-3 at 3.32 and 3.34 ppm in ^{1}H NMR spectra of **8a** and **8b**, respectively, and lack of signals of olefinic proton (H-3) and H-9a undoubtedly proved the position of the tetrasubstituted double bond in the molecule. These lactones are products of isomerization of α,β-unsaturated lactones (B) in the reaction medium.

Table 1. Feeding Deterrent Activity of Precocenes and Their Derivatives against *T. confusum*

	deterrence coefficients[a]					
	larvae			adults		
compd	*A*	*R*	*T*	*A*	*R*	*T*
1a	55.1 b	64.1 ab	119.2 b	55.2 b	83.8 a	139.0 b
1b	68.6 a	97.1 a	165.7 a	100 a	94.2 a	194.2 a
6a	–6.2 d	86.4 ab	80.2 bc	1.8 cd	95.7 a	97.5 bc
6b	40.5 b	73.1 ab	113.6 b	15.0 cd	88.9 a	103.9 bc
7a	16.7 c	77.5 ab	94.2 bc	21.1 c	93.4 a	114.5 bc
7b	54.2 b	93.6 a	147.8 a	3.9 cd	89.5 a	93.4 c
8a	–6.7 d	60.3 b	50.6 c	19.7 c	94.4 a	114.1 b
8b	59.0 b	90.7 ab	149.7 a	14.2 cd	91.6 a	105.8 bc

[a] *A*, absolute coefficient; *R*, relative coefficient; *T*, total coefficient. Values followed by the same letter within a column are not significantly different at the $P < 0.05$ level.

Table 2. Feeding Deterrent Activity of Precocenes and Their Derivatives against *T. granaruim* (Larvae) and *S. granarius* (Adults)

	deterrence coefficients[a]					
	T. granarium larvae			*S. granarius* adults		
compd	*A*	*R*	*T*	*A*	*R*	*T*
1a	41.8 b	38.3 c	80.1 b	55.2 b	83.8 a	139.0 b
1b	98.6 a	100 a	198.6 a	100 a	94.2 a	194.2 a
6a	22.0 b	86.0 b	108.0 b	1.8 cd	95.7 a	97.5 bc
6b	11.9 b	85.5 b	97.4 b	15.0 cd	88.9 a	103.9 bc
7a	31.6 b	79.8 b	111.4 b	21.1 c	93.4 a	114.5 bc
7b	39.6 b	80.5 b	120.1 b	3.9 cd	89.5 a	93.4 c
8a	34.5 b	73.1 b	107.6 b	19.7 c	94.4 a	114.1 b
8b	31.2 b	85.0 b	116.2 b	14.2 cd	91.6 a	105.8 bc

[a] *A*, absolute coefficient; *R*, relative coefficient; *C*, total coefficient. Values followed by the same letter within a column are not significantly different at the $P < 0.05$ level.

Biological Activities. *Storage Pests.* The results of studies of the deterrent activity of the compounds obtained toward storage pests are presented in **Tables 1** and **2**. The deterrence coefficient values point to differences in antifeedant properties related to the structure of the compounds. It can be seen that precocene II (**1b**) showed a very strong antifeedant effect against all storage pests. It exhibited activity comparable to that of the best-known antifeedant, azadirachtin (*9*, *15*). Our data clearly indicate that the high activity of precocene II was caused by the presence of two methoxy groups in the molecule. Similar results were obtained in studies of the antifeedant activity of precocenes toward *Rhodnius prolixus.* A marked inhibiting effect of the methoxy group at C-6 on the feeding activity of this bloodsucking bug was observed by Azambuja et al. (*8*). If one of these substituents was replaced by H, the antifeedant activity was decreased, and the effective antifeedant dose of precocene I was 6-fold higher than that of precocene II. In our studies a particularly conspicuous drop in the activity of precocene I was observed in relation to *T. granarium* larvae (**Table 2**). A reduction was also observed in the activity of precocene I toward both developmental stages of *T. confusum* (**Table 1**). That structural change, however, only slightly affected the feeding of *S. granarius*, and precocene I was also a very good deterrent

Table 3. Feeding Deterrent Activity of Precocenes and Their Derivatives against *L. decemlineata*

	deterrence coefficients[a]					
	larvae			adults		
compd	*A*	*R*	*T*	*A*	*R*	*T*
1a	77.8 b	86.8 d	164.6 d	41.9 ab	73.8 b	115.7 bc
1b	32.9a	91.2 d	124.1bc	44.2 ab	71.8 ab	116.0 bc
6a	35.2 a	63.6 ab	98.8 abc	23.2 ab	70.9 ab	94.1 bc
6b	17.2 a	79.1 bcd	96.3 ab	34.4 ab	63.7 ab	98.1 bc
7a	38.3 a	95.8 d	134.1 bc	61.6 bc	81.4 b	143.0 cd
7b	41.4 a	90.6 cd	132.0 bc	87.1 c	89.1 b	176.2 d
8a	21.7 a	55.0 a	76.7 a	27.6 ab	49.2 ab	76.8 ab
8b	32.7a	62.5 ab	95.2 ab	19.7 a	29.9 a	49.6 a

[a] *A*, absolute coefficient; *R*, relative coefficient; *C*, total coefficient. Values followed by the same letter within a column are not significantly different at the $P < 0.05$ level.

with a deterrence coefficient of >150 for that species. The addition of the carboxyl group at the C-3 considerably reduces the deterrent activity of the derivatives of both precocenes. Only in the case of methoxy carboxylic acid (**6a**) was a slight increase in activity toward *T. granarium* larvae compared with **1a** observed. The differences, hovewer, were insignificant. The conjuction of the structure of precocene with the lactone ring and iodine atom affected the antifeedant properties of those compounds in different ways. Methoxy- and dimethoxyiodolactones (**7a** and **7b**) also showed lower activity than the respective precocenes, but were better deterrents than their precursors **6a** and **6b**, particularly toward *S. granarius* adults and *T. confusum* larvae. A considerable drop in activity of unsaturated methoxylactone **8a** against *S. granarius* adults and *T. confusum* larvae was found when hydrogen iodide was eliminated from methoxyiodolactone **7a**. That structural change, however, did not affect the deterrent properties of **8a** toward *T. confusum* adults and *T. granarium* larvae. Only slight and statistically insignificant changes were observed in the activity of unsaturated dimethoxylactone **8b** compared with **7b** toward all storage pests under study, and the elimination of hydrogen iodide did not significantly affect the activity of the compound obtained.

All derivatives of precocenes with a lactone moiety were weaker feeding deterrents for storage pests compared with precocenes, but some of them can still be included among good antifeedants. Here belong compounds **7b** and **8b**, reducing considerably feeding in larvae of *T. confusum*. Good deterrent properties against adults of *S. granarius* have also been found in iodolactones **7a** and **7b**.

Colorado Potato Beetle. The results of our investigation showed that precocenes were moderate deterrents and did not differ in their activity against CPB adults. As shown in **Table 3**, the values of all deterrence coefficients (*A*, *R*, and *T*) for both of those compounds were very similar. The moderate deterrent activity of precocenes against that beetle species can be related to the lack of toxic effect of those compounds not only on their *corpora allata* (*24*) but also on other tissues, especially the gut and fat body. According to Azambuja et al. (*8*), the antifeeding response induced by precocenes may be due to direct cytotoxic action on the gut tissue, which contains activating monooxygenases. CPB larvae showed greater sensitivity to the antifeedant activity of precocenes. However, a better deterrent for that stage was precocene I. In contrast to many other insect species, the presence of the methoxy group at C-6 caused a decrease in the antifeedant potency, but only in the no-choice test. In the choice test precocene II was a very good

Table 4. Effect of Precocenes and Their Derivatives on the Settling of Aphids on the Leaf[a]

		time after access to the plants				
compd	treatment	15 min	30 min	1 h	2 h	24 h
1a	treated	nt[b]	nt	nt	nt	5
	control					9
	P					*0.0038*
1b	treated	nt	nt	nt	nt	6
	control					10
	P					*0.0010*
6a	treated	9	8	9	9	9
	control	9	9	8	9	10
	P	0.6185	0.6100	0.8569	0.8494	0.444888
6b	treated	8	10	10	11	11
	control	7	7	7	7	7
	P	0.3006	0.1136	0.0592	0.0809	*0.0040*
7a	treated	10	11	9	14	10
	control	7	6	7	7	8
	P	*0.0137*	*0.0073*	*0.0430*	*0.0475*	0.1639
7b	treated	8	6	6	7	7
	control	9	9	9	10	9
	P	0.1347	*0.0165*	0.0787	0.3010	0.2544
8a	treated	6	7	7	8	8
	control	11	10	9	9	9
	P	*0.0011*	*0.0058*	0.2053	0.2782	0.3469
8b	treated	9	9	9	8	9
	control	8	10	8	9	9
	P	0.5885	0.5401	0.5683	0.6159	1.0000

[a] Values represent mean number of aphids on control or treated half of the leaf (8 replications, 20 aphids each). *P* values in italic type indicate statistical significance (analysis of variance, $P = 0.05$). [b] Not tested.

feeding deterrent. The acidic derivatives of both precocenes **6a** and **6b** were moderate feeding deterrents against both developmental stages of *L. decemlineata*. The introduction of the carboxyl group to the structure of precocenes caused, as in the case of storage pests, a decrease in deterrent activity. That drop was particularly noticeable with precocene I and its acidic derivative in relation to larvae, in which case the difference between the deterrence coefficient values of the two compounds is statistically significant. Iodolactones **7a** and **7b** were good antifeedants for larvae and very good for CPB adults. The values of total coefficient (*T*) for those compounds indicate that the addition of the lactone ring with an iodine atom to the structure of precocenes causes a considerable increase in their activity toward CPB adults. Their feeding was particularly strongly inhibited in both tests by dimethoxyiodolactone (**7b**). Only slightly weaker antifeedant properties were shown by methoxyiodolactone (**7a**) in the no-choice test. In choice tests iodolactones were also very good feeding deterrents for CPB larvae, but in no-choice tests they reduced feeding to a lesser degree. Nevertheless, they showed the highest activity toward both developmental stages of *L. decemlineata* among all precocene derivatives studied. The introduction of the iodine atom had a great impact on the antifeedant potency of those compounds. According to Darvas et al. (*6*), chloro-containing analogues of precocenes I and II showed higher toxicity toward CPB than the parent precocenes. Our most recent studies (unpublished data) also indicate that lactones containing a chloro atom in their structure are good deterrents against CPB larvae and adults. The decrease in **8a** and **8b** activity also suggests that the iodo atom may play a significant role in the antifeedant effect against CPB.

Aphids. Both precocenes proved to be very good antifeedants against aphids (**Table 4**). Of their derivatives tested, only **8a**

showed deterrent properties. However, that antifeedant effect was very short-lived and ceased in <1 h. The remaining compounds were either inactive or attractant. Compound **6b** showed attractant properties after 24 h of observation, whereas compound **7a**, during 2 h after aphids had had access to the plants.

To sum up, with regard to the antifeedant activity of precocene derivatives, it was concluded that the structural changes consisting in combining precocene molecules with a lactone ring caused a decrease in the feeding deterrent activity of the compounds thus obtained toward most investigated insect species. Only in the case of adult CPB did the structural change considerably increase the antifeedant activity of **7a** and **7b**. It was, however, the presence of the iodine atom that was essential for that increase in activity. The halogen also improved the feeding deterrent properties of iodolactones against *S. granarius* adults. The effect of the methoxy group on the antifeedant potency was observed only in the case of *T. confusum* larvae. All derivatives of precocene II were more active toward those insects than the derivatives of precocene I. The effect of the compounds studied on insect feeding was species- and developmental stage-specific.

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