Table III. Residues[a] of Rotenone and Rotenolone on Tomato Fruit at Various Intervals after Treatment with Rotenone Dust (DU) or Wettable Powder (WP) Formulations

time after treatment, days	residue found, ppm			
	rotenone		rotenolone	
	DU	WP	DU	WP
0	0.82 ± 0.05	0.53 ± 0.13	0.05 ± 0	0.05 ± 0.01
1	0.70 ± 0.03	0.25 ± 0.02	0.06 ± 0.01	Tr
2	0.79 ± 0.37	0.09 ± 0.02	0.06 ± 0.03	0
3	0.22 ± 0.04	0.06 ± 0.01	Tr	0
6	0.21 ± 0.03	Tr[b]	Tr	0
control	0		0	

[a] Values are the means of determinations on four samples ± SE. [b] Trace.

Table IV. Effect of Boiling on the Stability of 5.0 ppm Rotenone and 5.0 ppm Rotenolone in Tomato Homogenate

boiling time, min	residue found,[a] ppm	
	rotenone	rotenolone
5	4.3	4.6
10	3.7	3.7
20	3.9	3.9
40	4.0	4.0

[a] Values are the means of duplicate determinations.

to form and accumulate as a result of storage (Bowman et al., 1978). However, when exposed to light on glass plates in the laboratory, rotenolone (especially the trans isomers) appears to form and then degrade relatively rapidly (Cheng et al., 1972). The results of the present study in the field are consistent with the latter finding.

The data of Table III show that compared to lettuce, tomatoes contained much lower levels of both rotenone and rotenolone, as would be expected from the lower surface area. As with lettuce, the dust preparation resulted in higher residue levels. Significant levels of rotenone from the dust preparation remained after 6 days, while with the wettable powder only trace amounts remained at this time. The half-life of rotenone was calculated to be 2.7 days for dust and 0.9 days for wettable powder formulations.

The stability of rotenone and rotenolone to the effects of cooking was examined by boiling under reflux tomato homogenate fortified with 5.0 ppm of each for several time intervals. After each interval, the mixture was cooled, and samples (5 g) were taken for analysis. As shown by the data in Table IV, little if any decomposition of either compound occurred within 40 min.

ACKNOWLEDGMENT

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Precocene II Metabolism in Insects: Synthesis of Potential Metabolites and Identification of Initial in Vitro Biotransformation Products

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Syntheses are described for the following derivatives of the insect antijuvenile hormone precocene II (6,7-dimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran), all of which are possible metabolites in insects: 6-hydroxy, 7-hydroxy, 3,4-dihydro-3-hydroxy, *cis*- and *trans*-3,4-dihydro-3,4-dihydroxy, 2-hydroxymethyl, 3,4-dihydro-4-hydroxy, and 3,4-epoxy. Of these, the first five are identified by cochromatography as metabolites of [^{14}C]precocene II formed in vitro on incubation with NADPH-dependent monooxygenases in fat body homogenates of cabbage looper (*Trichoplusia ni* Hübner) larvae. The 3,4-epoxide is inferred as a metabolic intermediate from the presence of the isomeric dihydrodiols, but could not be isolated directly. The extreme reactivity of the synthesized epoxide suggests this compound as a possible bioactivation product involved in the allatotoxic action of precocene II. One metabolite, which is apparently nonphenolic, has properties different from the synthesized standards and remains to be identified.

Precocene II (6,7-dimethoxy-2,2-dimethyl-2*H*-benzo-[*b*]pyran, 1) is selectively cytotoxic to the secretory cells of the insect corpus allatum (CA), thereby eliminating the production of juvenile hormone (Bowers and Martinez-Pardo, 1977; Unnithan et al., 1977; Pener et al., 1978; Schooneveld, 1979). There is considerable specificity in the action of 1 and related compounds. Thus, the interruption of normal nymphal development to produce precocious adults and the sterilization of adult females has been reported in several hemimetabolous species, whereas in holometabolous species only a few cases of sterilization, but no induction of precocious metamorphosis, have been reported (Bowers, 1976; Bowers et al., 1976; Pener et al., 1978). Even in generally sensitive groups, large differences

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are seen between species in the observed potency of 1 and analogues.

Qualitative or quantitative differences in the metabolism of 1 between the CA and other insect tissues and between insect species may be responsible for the patterns of selectivity observed. Previous studies (Ohta et al., 1977; Burt et al., 1978) established that large differences exist in the rates of metabolism of 1 in several insect species in vivo and that initial biotransformation steps are catalyzed by NADPH-dependent monooxygenases. However, precocene sensitivity does not appear to depend solely on the rate of metabolism, and metabolic pathways for 1 and the structures of primary metabolites remain poorly characterized.

In this paper we report the synthesis of several potential oxidative metabolites of 1 and the use of these synthesized standards to identify most of the primary metabolites isolated from the incubation of ^{14}C-labeled 1 with highly active fat body monooxygenases of the cabbage looper, *Trichoplusia ni* (Hübner).

MATERIALS AND METHODS

Labeled and Unlabeled Precocene II. Precocene II (6,7-dimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran, 1) labeled with ^{14}C at C-4 was prepared from 3-methylbut-2-enoic acid-*1*-^{14}C (0.67 mCi/mmol) and 3,4-dimethoxyphenol as described by Burt et al. (1978). Unlabeled 1 was synthesized previously in this laboratory (Bowers, 1976).

Chromatography. Precoated 20 × 20 cm chromatoplates were used for thin-layer chromatography (TLC) as follows: silica gel 60 F-254 with 0.25-mm gel thickness (EM Laboratories, Elmsford, NY) for analytical separations; silica gel GF with 0.5- or 1.0-mm gel thickness (Analtech, Inc., Newark, DE) for preparative isolations. Seven TLC solvent systems were used: (A) chloroform–methylene chloride–ethyl acetate–methanol (10:10:1:1), (B) hexane–chloroform–methanol (7:2:1), (C) hexane–chloroform–methanol (4:2:1), (D) ether–hexane (5:1), (E) methylene chloride–ethyl acetate (5:1), (F) benzene (saturated with formic acid)–methanol (8:1), (G) benzene–methanol–acetic acid (40:2:1). References to solvent systems used for metabolite identification are as follows: (A, B) two-dimensional chromatography, eluting with solvent A in the first direction and with solvent B in the second direction; (A×2) one-dimensional chromatography, eluting twice with solvent A. Compounds were detected by exposing chromatoplates to X-ray film (radiolabeled metabolites) or by their quenching of gel fluorescence at 254 nm (synthesized standards). High-pressure liquid chromatography (LC) analyses were performed by using either a Waters Model M-6000 or an Altex Model 110A solvent pump and a Chromatronix Model 230 UV detector at 254 nm. Separations involving adsorption chromatography were achieved by using a 4.6 × 250 mm column packed with Lichrosorb Si 100 (5 μm; Merck) eluted with chloroform–propanol–ethyl acetate (18:1:1) at 2 mL/min. Reversed phase separations were performed by using a 4.6 × 250 mm column packed with octadecylsilane-coated Bio-Sil A (2–10 μm; Bio-Rad Laboratories, Richmond, CA) eluted with water–methanol (1:1) at 1 mL/min after preelution with the same solvent containing 0.01 M NaOH. Gas–liquid chromatographic (GLC) analyses were performed by using a Packard Model 7400 chromatograph equipped with a flame ionization detector. Glass columns (2 mm × 1.8 m) packed with two different coated supports were used: 3% OV-101 on Gas-Chrom Q, 100–120 mesh; 3% QF-1 on Chromosorb W, 100–120 mesh. Gas flow rates were 400, 40, and 25 mL/min for air, H_2, and N_2 (carrier), respectively.

Spectroscopy and Radiocarbon Analysis. Electron impact mass spectra [EI–MS; spectra reported as fragment mass (percent relative intensity)] were obtained by using a Hewlett Packard Model 5985 gas–liquid chromatograph–mass spectrometer and data system at ionization voltages of 20 or 70 eV. Chromatography involved the use of two column systems: a glass column (2 mm × 1.8 m) packed with 2% OV-101 on Chromosorb W (100–120 mesh), operating with a helium flow rate of 10 mL/min, or a glass open tubular column (0.31 mm × 50 m) wall-coated with OV-101, operating at a helium flow rate of 23 cm/s. Column oven temperatures were programmed from 30 to 200 °C at rates from 10 °C/min to 30 °C/min, depending on the compounds analyzed. Under these conditions the relative chromatographic behavior of analyzed compounds was the same as for OV-101 GLC analysis. Infrared spectra (IR; reported in cm^{-1} for major absorption frequencies) were recorded by using a Perkin Elmer Model 257 grating spectrometer and either liquid films or KBr pellets. Nuclear magnetic resonance (NMR) spectra (^1H) were determined for dilute solutions in carbon tetrachloride, chloroform-*d*, or acetone-d_6, using tetramethylsilane as the internal standard (δ 0.00) with a Varian HA-100D spectrometer at 100 MHz. Radioactivity measurements were made by liquid scintillation counting (LSC) by using a Packard 3380 liquid scintillation spectrometer. All reported melting points are uncorrected.

Incubation and Extraction Procedures. Fifth instar larvae of the cabbage looper (*Trichoplusia ni* Hübner) were used as a tissue source in all studies. Two strains derived from larvae field-collected near Geneva, NY, in 1975 or 1976 (designated by year of origin) and reared in the laboratory on broccoli leaves were used for comparative studies. Fat body homogenates were prepared as described by Kuhr (1970). Final incubation mixtures included 0.5 mL of homogenate (0.5–2 tissue equivalents, 2.75–9.85 mg of protein by the Biuret assay) and an NADPH-generating system (Ohta et al., 1977) in 2 mL of 0.05 M phosphate buffer, pH 7.4. ^{14}C-Labeled 1 was introduced in 20 μL of ethanol to a final concentration of 2×10^{-5} M, and the mixtures were incubated aerobically with shaking at 25 °C for 5–60 min. Reactions were terminated by extraction with ether (3 × 5 mL) and the combined extracts were dried (Na_2SO_4) and concentrated under a stream of N_2 for TLC analysis.

Metabolite Characterization. Initial metabolite separation was achieved by one-dimensional TLC (A) of the combined ether extracts of incubation mixtures. Radioactive gel regions were eluted individually with ether or methanol for cochromatography with authentic standards in appropriate solvents with or without prior derivatization. Tentative metabolite identifications in this report are based on at least two independent cochromatographic confirmations as follows: two-dimensional TLC in two systems (four solvents) without derivatization, two-dimensional TLC with and without derivatization, or two-dimensional TLC and LC. Metabolites were derivatized by treatment with acetic anhydride and pyridine (3 h, 50 °C) for cochromatographic comparison with acetylated standards. Amounts of each ether-soluble metabolite formed were determined by LSC of gel regions after TLC separation (A). Metabolite regions not adequately resolved in this system were separated by a second chromatographic step (TLC or LC) prior to counting. Total water-soluble radiocarbon was determined by LSC of an aliquot of the aqueous phase after ether extraction, but metabolites in this fraction were not identified. Results reported are means of two experiments with duplicate determinations

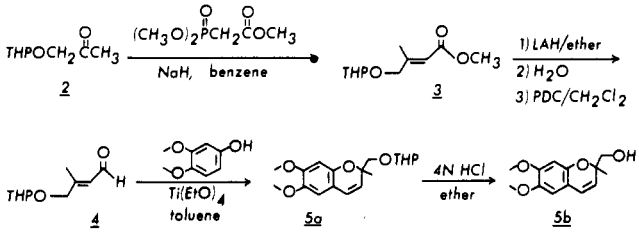

Figure 1. Synthesis of the 2-hydroxymethyl derivative (**5b**) of precocene II (1).

at each incubation time in each experiment.

SYNTHESIS

2-Hydroxymethyl-6,7-dimethoxy-2-methyl-2*H*-benzo[*b*]pyran (5b, Figure 1). A solution of the tetrahydropyranyl ether of acetol (**2**; 3.16 g, 20 mmol) in benzene (40 mL) was added to a stirred suspension of sodium dimethyl (methoxycarbonyl)methylphosphonate [from NaH (1.92 g, 80 mmol) and dimethyl (methoxycarbonyl)methylphosphonate (14.6 g, 80 mmol)] in benzene (150 mL) and held with stirring at 40 °C for 15 h. After treatment with H_2O (30 mL) the aqueous layer was separated and extracted with benzene. The combined organic fractions were washed with saturated NaCl and dried over $MgSO_4$. After solvent removal the residue was chromatographed on silica (50 g, hexane–ether, 95:5) to give 3.48 g of a mixture of the *Z* and *E* isomers of the α,β-unsaturated methyl ester **3** (81%; *Z*:*E* isomer ratio 32:68 by GLC). Analytical data for the isomers of **3** were as follows. *Z* isomer: IR (neat) 1720, 1660; NMR (CCl_4) 2.10 (s, 3 H, $CH_3C{=}C$), 5.63 (br s, 1 H, HC=C); EI–MS 130 (33), 113 (51), 98 (36), 85 (100). *E* isomer: IR as above; NMR (CCl_4) 1.99 (s, 3 H, $CH_3C{=}C$), 5.86 (br s, 1 H, HC=C); EI–MS 130 (11), 113 (17), 85 (100). A solution of **3** (*Z*/*E* mixture, 1.07 g, 5 mmol) in diethyl ether (50 mL) was treated with $LiAlH_4$ (0.19 g, 5 mmol) at room temperature and held for 2 h (GLC monitoring). After the careful addition of H_2O, the mixture was filtered and the recovered organic phase dried over $MgSO_4$. After solvent removal, the residue was oxidized by using a 40% molar excess of pyridinium dichromate [PDC; Corey and Schmidt (1979)] in CH_2Cl_2 (50 mL; 3 h at room temperature) to give a crude product containing as major components the *Z* and *E* isomers of the tetrahydropyranyl ether of 4-hydroxy-3-methylbut-2-enal (**4**; *Z*:*E* ratio similar to that for starting ester mixture): IR (neat) 1680; EI–MS 101 (10), 99 (8), 85 (100).

The crude aldehyde mixture **4** in toluene (5 mL) was added dropwise under N_2 to a solution of the Ti(IV) salt of 3,4-dimethoxyphenol [from 0.385 g (2.5 mmol) of the phenol and 0.160 g (0.7 mmol) of $Ti(EtO)_4$ in toluene (5 mL); Sartori et al. (1979)]. After heating the mixture under reflux with stirring for 10 h, the reaction was quenched by the addition of saturated aqueous NH_4Cl (30 mL) and extracted with diethyl ether. After drying ($MgSO_4$) and solvent removal, the crude product was purified by chromatography on Florisil (15 g, CH_2Cl_2) and preparative TLC (silica, $CHCl_3$; R_f 0.5) to give 0.204 g of a mixture containing as major components the diastereomers of the chromene **5a**: NMR (CCl_4) 1.44 (s, 3 H), 1.47 (s, 3 H), 5.47 (d, 1 H, J = 10 Hz) and 5.49 (d, 1 H, J = 10 Hz) corresponding to the C-3 proton in each diastereomer, 6.27 (br d, 2 H, J = 10 Hz, H-4), 6.34 (s, 2 H), 6.47 (s, 2 H); EI–MS 320 (M^+) (2), 205 (100), 161 (6), 85 (5). The free alcohol **5b** was obtained from treatment of the THP ether **5a** with 4 N HCl in diethyl ether (10 mL). Preparative TLC (silica, $CHCl_3$; R_f 0.2) gave 0.058 g of pure **5b** (10% overall from 3,4-dimethoxyphenol): IR (neat) 3450; NMR ($CDCl_3$) 1.37 (s, 3 H), 1.57 (br s, 1 H, OH), 3.60–3.80 (m, 2 H, CH_2OH), 3.82 (s, 3 H), 3.84 (s, 3 H), 5.44 (d, 1 H, J = 10 Hz, H-3), 6.38 (d, 1 H, J = 10 Hz, H-4), 6.43 (s, 1 H), 6.53 (s, 1 H); EI–MS 236 (M^+) (6), 205 (100).

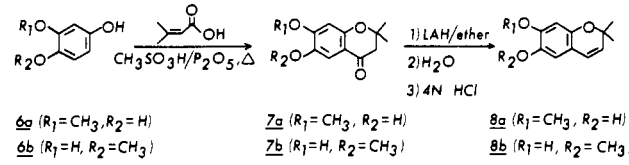

Figure 2. Syntheses of the 6-hydroxy (**8a**) and 7-hydroxy (**8b**) derivatives of precocene II (1).

6-Hydroxy-7-methoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran (8a; Figure 2). Methoxyhydroquinone [**6a**, 0.56 g, 4 mmol; Birch et al. (1975)] and 3-methylbut-2-enoic acid (0.40 g, 4 mmol) were added simultaneously under N_2 with vigorous stirring to a flask containing CH_3SO_3H saturated with P_2O_5 (8 mL; preheated to 70 °C). The reaction mixture was stirred for 30 min at 70 °C, cooled, poured into ice water (100 g), and extracted with diethyl ether. The combined organic fractions were washed (H_2O, saturated NaCl) and dried ($MgSO_4$); the residue obtained after solvent removal was distilled bulb to bulb (155–170 °C/0.25–0.30 Torr) and the distillate crystallized from diethyl ether to give 0.81 g (91%) of chromanone **7a**: mp 98–100 °C; IR (neat) 3400, 1660; NMR ($CDCl_3$) 1.41 (s, 6 H), 2.63 (s, 2 H), 3.88 (s, 3 H), 5.52 (br s, 1 H, OH), 6.34 (s, 1 H), 7.31 (s, 1 H); EI–MS 222 (M^+) (38), 207 (100), 167 (90). A solution of **7a** (0.185 g, 0.8 mmol) in diethyl ether (10 mL) was treated with $LiAlH_4$ (0.057 g, 1.5 mmol) and stirred for 1 h at room temperature. After the careful addition of H_2O, the mixture was filtered; the filtrate, containing the 4-chromanol, was vigorously stirred with 4 N HCl at room temperature for 60 min. The organic fraction was washed (saturated $NaHCO_3$, saturated NaCl) and dried ($MgSO_4$); after solvent removal, the residue was purified by chromatography on silica (15 g; CH_2Cl_2) to give 0.128 g (78%) of the chromene **8a**: mp 65–66 °C; IR (neat) 3450; NMR ($CDCl_3$) 1.41 (s, 6 H), 3.83 (s, 3 H), 5.19 (s, 1 H, OH), 5.48 (d, 1 H, J = 10 Hz), 6.22 (d, 1 H, J = 10 Hz), 6.39 (s, 1 H), 6.57 (d, 1 H); EI–MS 206 (M^+) (22), 191 (100), 176 (21).

7-Hydroxy-6-methoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran (8b; Figure 2). Compound **8b** was prepared by the procedure described for **8a** from 3-hydroxy-4-methoxyphenol (**6b**; 2.10 g, 15 mmol) and 3-methylbut-2-enoic acid (1.50 g, 15 mmol) in CH_3SO_3H (40 mL) saturated with P_2O_5 (30 min, 70 °C). After workup as described above, the crude residue was distilled bulb to bulb (145–160 °C/0.3–0.4 Torr) to give 3.18 g (89%) of the chromanone **7b**: mp 107 °C [lit. 81 °C; Bhat and Venkataraman (1963)]; IR (KBr) 3520, 3450, 1660; NMR ($CDCl_3$) 1.43 (s, 6 H), 2.64 (s, 2 H), 3.88 (s, 3 H), 6.44 (s, 1 H), 6.45 (br s, 1 H, OH), 7.27 (s, 1 H); EI–MS 222 (M^+) (39), 207 (100), 167 (93). Chromene **8b** was obtained as a yellow oil in 76% yield from $LiAlH_4$ reduction of **7b**, followed by dehydration with 4 N HCl as described above: IR (neat) 3450; NMR ($CDCl_3$) 1.39 (s, 6 H), 3.78 (s, 3 H), 5.44 (d, 1 H, J = 10 Hz), 5.68 (s, 1 H, OH), 6.20 (d, 1 H, J = 10 Hz), 6.42 (s, 1 H), 6.48 (s, 1 H); EI–MS 206 (M^+) (21), 191 (100), 176 (25).

3,4-Dihydro-3-hydroxy-6,7-dimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran (9) and 3,4-Dihydro-4-hydroxy-6,7-dimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran (10) (Figure 3). Hydroboration of precocene II (1) (0.550 g, 2.5 mmol) with BH_3/THF followed by H_2O_2 oxidation gave a mixture of alcohols **9** and **10**. The crude mixture (0.400 g) was separated by preparative LC (20 × 1.25 cm Bio-Sil A column eluted with hexane–ethyl acetate

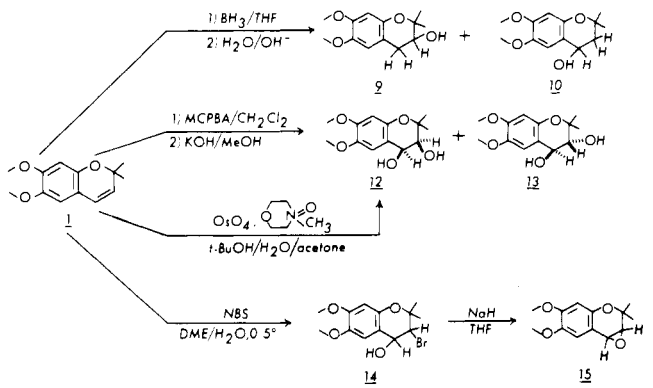

Figure 3. Syntheses of the 3-chromanol (**9**), 4-chromanol (**10**), *cis*-dihydrodiol (**12**), *trans*-dihydrodiol (**13**), and 3,4-epoxy (**15**) derivatives from precocene II (**1**).

(7:3) at 3.2 mL/min) to yield **9** (retention volume 83 mL; 0.162 g, mp 112–113 °C from hexane, 41%) and **10** (retention volume 138 mL; 0.095 g, 24%). Analytical data for **9** were as follows: IR (KBr) 3540; NMR ($CDCl_3$) 1.32 (s, 3 H), 1.38 (s, 3 H), 1.90 (br s, 1 H, OH), 2.71 (dd, 1 H, J_{AB} = 17 Hz, J_{AX} = 5 Hz), 3.00 (dd, 1 H, J_{AB} = 17 Hz, J_{BX} = 4 Hz), 3.70–3.80 (m, 1 H, C*H*OH), 3.83 (s, 6 H), 6.42 (s, 1 H), 6.54 (s, 1 H); EI–MS 238 (M^+) (27), 207 (10), 167 (100). Compound **10** has been described previously (Ohta et al., 1977). Treatment of **9** with acetic anhydride in pyridine gave the corresponding acetate **9a** (mp 96–97 °C): IR (KBr) 1730; NMR (CCl_4) 1.22 (s, 3 H), 1.25 (s, 3 H), 2.00 (s, 3 H, CH_3COO), 2.55 (dd, 1 H, J_{AB} = 17 Hz, J_{AX} = 5 Hz), 2.95 (dd, 1 H, J_{AB} = 17 Hz, J_{BX} = 5 Hz), 4.84 (t, 1 H, J_{AX} = J_{BX} = 5 Hz), 6.22 (s, 1 H), 6.36 (s, 1 H); EI–MS 280 (M^+) (17), 220 (14), 205 (100).

6,7-Dimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran-4-one (11). Compound **11** was obtained is an intermediate from the published synthesis of **1** (Ohta and Bowers, 1977).

***cis*-3,4-Dihydro-3,4-dihydroxy-6,7-dimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran (12) and *trans*-3,4-Dihydro-3,4-dihydroxy-6,7-dimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran (13) (Figure 3).** Compound **12** was prepared by using the procedure described by Van Rheenen et al. (1976). **1** (0.660 g, 3 mmol) in acetone (1 mL) was added under N_2 to a mixture of *N*-methylmorpholine *N*-oxide (0.550 g, 4 mmol) and OsO_4 (3 mg) in H_2O (1.6 mL) and *t*-BuOH (2.4 mL). The reaction mixture was stirred at room temperature for 16 h; after workup, the residue (0.600 g) was purified by chromatography on silica gel (30 g; CH_2Cl_2–ethyl acetate, 9:1 to 5:1), yielding 0.370 g of *cis*-diol **12** (49%): mp 126.5–127.5 °C [lit. 129.5 °C; Alertsen (1961)]; IR (KBr) 3450; NMR ($CDCl_3$) 1.24 (s, 3 H), 1.40 (s, 3 H), 2.68 (s, 2 H, OH), 3.60 (d, 1 H, J = 4 Hz), 3.80 (s, 6 H), 4.68 (d, 1 H, J = 4 Hz), 6.35 (s, 1 H), 6.97 (s, 1 H); EI–MS 254 (M^+) (54), 236 (13), 193 (12), 183 (52), 182 (51), 167 (100). Treatment of the *cis*-diol **12** with acetic anhydride and pyridine gave the corresponding *cis*-diacetate **12a**, mp 131 °C [lit. 130 °C; Alertsen (1961)]: IR (KBr) 1740; NMR ($CDCl_3$) 1.40 (s, 6 H), 2.09 (s, 3 H), 2.12 (s, 3 H), 3.81 (s, 3 H), 3.84 (s, 3 H), 5.26 (d, 1 H, J = 5 Hz), 6.12 (d, 1 H, J = 5 Hz), 6.41 (s, 1 H), 6.62 (s, 1 H); EI–MS 338 (M^+) (19), 278 (14), 263 (20), 221 (100).

A mixture of the *cis*- and *trans*-diols (**12, 13**) was prepared by reacting **1** (0.500 g, 2.7 mmol) with *m*-chloroperbenzoic acid (MCPBA; 0.600 g, 3.2 mmol) in CH_2Cl_2 (75 mL) with stirring at room temperature for 3 h. The reaction mixture, containing the *m*-chlorobenzoate half-esters of the *cis*- and *trans*-diols, was washed (10% Na_2SO_3, 5% Na_2CO_3) and dried ($MgSO_4$). The residue after solvent removal was hydrolyzed by using 10% KOH in 80% aqueous MeOH with stirring at room temperature for 24 h. After removal of MeOH under vacuum, the residue was treated with saturated NaCl and extracted with ethyl acetate to give a crude mixture of the isomeric diols **12** and **13**. A portion of this mixture (0.225 g) was purified by preparative TLC (silica; solvent A×2), yielding the *cis*-diol **12** (R_f 0.35; 0.054 g, 23%) and *trans*-diol **13** (R_f 0.24; 0.110 g, 48%). Treatment of the diols with acetic anhydride in pyridine gave the corresponding *cis*- and *trans*-diacetates (**12a, 13a**). Analytical data for the *cis*-diacetate **12a** were as above; for the *trans*-diacetate **13a**: IR (neat) 1745; NMR ($CDCl_3$) 1.34 (s, 3 H), 1.41 (s, 3 H), 2.10 (s, 3 H), 2.12 (s, 3 H), 3.80 (s, 3 H), 3.83 (s, 3 H), 5.17 (d, 1 H, J = 5 Hz), 5.90 (d, 1 H, J = 5 Hz), 6.40 (s, 1 H), 6.65 (s, 1 H); EI–MS 338 (M^+) (25), 278 (9), 263 (17), 221 (100), 183 (30). Stereochemical assignments for these products are based on comparisons of the chromatographic behavior of the diols and spectral properties of the diacetates with data for the *cis*-diol **12** and *cis*-diacetate **12a** from the stereospecific synthesis described above.

3,4-Epoxy-6,7-dimethoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran (15) (Figure 3). *N*-Bromosuccinimide (0.178 g, 1 mmol) in dimethoxyethane (DME; 5 mL) was added to a cooled (0–5 °C) solution of **1** (0.220 g, 1 mmol) in DME (25 mL) and H_2O (10 mL). The mixture was stirred at 0–5 °C until the starting chromene had reacted completely (10 min, GLC monitoring). DME was removed under vacuum, and the residue was extracted with diethyl ether, washed with 1 N HCl, saturated $NaHCO_3$, saturated NaCl, and dried ($MgSO_4$). In one experiment, the residue after solvent removal was purified for confirmation of structure by chromatography on Florisil (10 g; benzene–hexane, 1:1, to benzene); recrystallization from hexane yielded the pure bromohydrin **14** (mp 94–95 °C): IR (KBr) 3400; NMR (CCl_4) 1.44 (s, 3 H), 1.62 (s, 3 H), 2.71 (br s, 1 H, OH), 3.80 (s, 3 H), 3.82 (s, 3 H), 4.06 (d, 1 H, J = 9 Hz, CHBr), 4.77 (br d, 1 H, C*H*OH), 6.26 (s, 1 H), 6.87 (s, 1 H); EI–MS 300 (34), 298 (31), 285 (92), 283 (100), 219 (56). This compound decomposed on standing to give the stable 3-bromochromenic derivative as the major product.

To prepare the epoxide **15**, crude bromohydrin **14** from the procedure described above was dissolved in THF (5 mL) and added dropwise, under N_2, to a suspension of NaH (0.036 g, 1.5 mmol) in THF (20 mL). The reaction was stirred for 30 min at 50 °C and 16 h at room temperature, with GLC monitoring to assure complete reaction of **14** (retention times for **15**, **14**, and the 3-bromochromene were 2.5, 9.5, and 5 min, respectively, when analyzed on a 2 mm × 1.8 m glass column packed with 3% OV-101 at 180 °C with N_2 at 25 mL/min as the carrier). Filtration of the insoluble salts gave a solution of the epoxide in satisfactory purity (>90%) as determined by GLC. Overall yield, determined by GLC using the chromanone **11** of the same molecular weight as the internal standard, was approximately 55–60%. Because of the lability of the epoxide moiety, **15** was detectable without decomposition only by GLC. Column chromatography, TLC, LC, and vacuum distillation all resulted in significant degradation. Exposure of **15** to acidic or basic aqueous conditions rapidly gave the diols **12** and **13**; however, solutions of **15** in THF, benzene, or hexane were stable for several months at 0 °C. Analytical data for **15** were as follows: NMR ($CDCl_3$) 1.21 (s, 3 H), 1.52 (s, 3 H), 3.39 (d, 1 H, J = 4 Hz), 3.76–3.82 (1 H), 3.77 (s, 3 H), 3.80 (s, 3 H), 6.36 (s, 1 H), 6.79 (s, 1 H) [the doublet corresponding to H-4 (3.76–3.82) was obscured by the methoxy signals; spectra obtained in

Table I. Chromatographic Properties of Precocene II, Possible Metabolites, and Their Derivatives

compound (no.)	TLC R_f in indicated solvent system[a] A	B	other	LC retention vol, mL
precocene II (1)	0.69	0.61		
potential metabolites				
2-hydroxymethyl (**5b**)	0.32	0.18	D (0.18), E (0.28)	
3-chromanol (9)	0.37	0.23	D (0.21), E (0.25)	
4-chromanol (10)	0.33	0.21	D (0.16), E (0.23)	
4-chromanone (11)	0.56	0.45		
cis-diol (12)	0.22	0.18	A×2 (0.35), C (0.32)	7.0[b]
trans-diol (13)	0.15	0.17	A×2 (0.24), C (0.30)	11.6[b]
6-hydroxy (**8a**)	0.56	0.36	F (0.59), G×2 (0.86)	16.4[c]
7-hydroxy (**8b**)	0.63	0.34	F (0.56), G×2 (0.84)	11.4[c]
derivatives				
3-chromanyl acetate (**9a**)	0.70	0.50	D (0.35), E (0.56)	
cis-diacetate (**12a**)	0.58	0.38	C (0.59)	
trans-diacetate (**13a**)	0.58	0.40	C (0.57)	

[a] See the Materials and Methods section for compositions of TLC solvents. [b] Adsorption LC; see the Materials and Methods section for conditions. [c] Reversed phase LC; see the Materials and Methods section for conditions.

benzene–CCl_4 showed the doublets for C-3 and C-4 at 3.30 and 3.68]; EI–MS 236 (M^+) (73), 208 (9), 193 (65), 187 (100).

METABOLISM

TLC in several combinations of seven solvent systems (Table I) afforded adequate resolution of all of the potential metabolites of 1 synthesized in this study with the exception of the 6-hydroxy and 7-hydroxy derivatives (**8a**, **8b**), which were separable by reversed phase LC. After initial separation (A), the *cis*- and *trans*-diols (**12**, **13**) were identified by cochromatography A×2, C). Identification was confirmed by cochromatography of these metabolites after acetylation with the synthesized diacetates **12a** and **13a** (A, C). Similarly, the 3-chromanol **9** was identified after preliminary separation by direct cochromatography (D, E) and by cochromatography after acetylation with the synthesized acetate (**9a**; D, E). The assignment of this structure was confirmed further by lack of cochromatography (D, E) of the ^{14}C metabolite with the synthesized 4-chromanol (**10**) and 2-hydroxymethyl (**5b**) derivatives, both of which have similar but not identical chromatographic properties. Direct two-dimensional cochromatography (A, B) of ether extracts with the synthesized 6- and 7-hydroxy derivatives (**8a**, **8b**) revealed the presence of a least one of these materials as a metabolite, but resolution was inadequate to determine whether only one or both were formed; significant degradation occurred during attempted rechromatography. These compounds were qualitatively resolved by chromatography in F, G×2, which demonstrated that both compounds were formed and that the 6-hydroxy derivative (**8a**) was the major metabolite. Further evidence for the formation of both phenolic metabolites was obtained by cochromatography with synthesized standards, using reversed phase LC. No ^{14}C metabolites were found with chromatographic properties corresponding to those measured for the 2-hydroxymethyl (**5b**), 4-chromanol (11), or 4-chromanone (12) derivatives. In addition, one metabolite [unknown A; R_f values 0.42 (A), 0.16 (D), 0.35 (E)] did not cochromatograph with any of the available synthetic standards and remains to be identified. Since this material does not partition into base, it is not likely to be either the 5-hydroxy or 8-hydroxy derivative of 1 formed by aromatic hydroxylation; however, synthesized standards of these compounds were not available for cochromatographic comparison with unknown A. Very minor metabolite bands occasionally appeared in other R_f regions, but their variable behavior and the small amounts obtained precluded their identification.

Table II. Radiocarbon Balance and Amounts of ^{14}C-Labeled Compounds in Ether Extracts for Incubations of [^{14}C]Precocene II with Fat Body Oxidase Preparations from Larvae of Two Cabbage Looper Strains

compound (no.)	initial radiocarbon recovered, %: 1975 strain[a] 5 min	1975 strain[a] 40 min	1976 strain[b] 10 min	1976 strain[b] 60 min
ether-soluble radiocarbon				
precocene II (1)	44.4	2.0	63.1	8.9
identified metabolites				
trans-diol (13)	7.5	18.4	2.2	6.2
cis-diol (12)	3.0	8.7	1.0	2.6
3-chromanol (9)	2.0	5.1	1.2	3.1
6-hydroxy (**8a**)	4.3	1.6	3.0	4.0
7-hydroxy (**8b**)	0.6	0.2	0.1	0.1
unknowns				
unknown A	3.8	3.4	2.9	3.7
origin	2.2	3.2	2.0	8.5
other[c]	5.8	5.6	3.7	3.0
water-soluble radiocarbon	13.0	46.9	7.0	49.6
total recovery	86.6	95.1	86.2	91.7
loss[d]	13.4	4.9	13.8	8.3

[a] 2.75 mg of protein/reaction; metabolism rate = 0.895 μg of 1 metabolized min^{-1} (mg of protein)$^{-1}$. [b] 9.85 mg of protein/reaction; metabolism rate = 0.016 μg of 1 metabolized min^{-1} (mg of protein)$^{-1}$. [c] Sum of all other TLC regions between origin and solvent front. [d] Predominantly unmetabolized 1 lost by volatilization during analysis; see text.

Incubation with 1975 strain fat body homogenates for 5 min converted more than half of the initial amount of 1 to more polar metabolites; of this, 13% was found in the aqueous fraction (Table II). Quantitative analysis of identified metabolites at 5 min revealed that the *trans*-diol **13** was the major product, but significant amounts of the isomeric *cis*-diol **12** were also found. O-Demethylation at the 6 position to give **8a** and formation of the 3-chromanol **9** were also important pathways, but very little of the 7-hydroxy derivative **8b** resulting from O-demethylation at the 7 position was found. Because of the lability of the O-demethylated derivatives, particularly **8b**, these compounds were isolated and analyzed in the presence of synthesized standards as carriers and corrections for loss during workup were made on the basis of carrier recovery during LC analysis. After 40 min of incubation the metabolism of 1 was virtually complete and nearly half of the initial radiocarbon had been converted to water-soluble

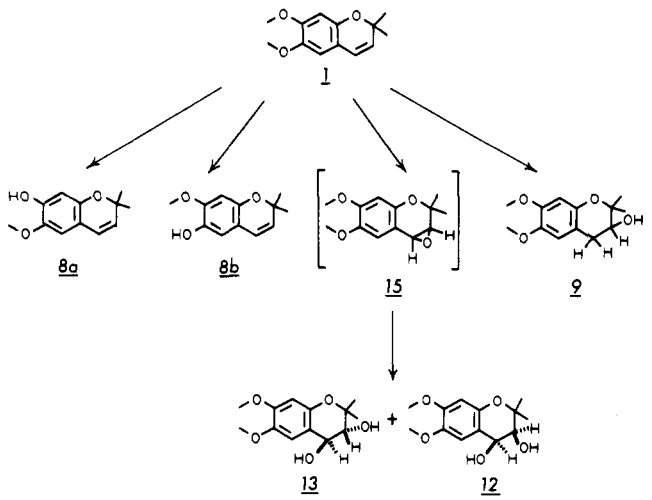

Figure 4. Proposed pathways for the initial oxidative biotransformation of precocene II (1) based on identified metabolites.

secondary metabolites. At this time, the major organosoluble metabolites were the *cis*- and *trans*-diols (**12**, **13**) with the trans isomer predominating the trans/cis ratio similar to that seen at 5 min. These metabolites and the 3-chromanol **9** accumulated in the incubation mixture over time, but the levels of the O-demethylation products were lower than those seen at 5 min.

Fat body preparations from the 1976 strain metabolized 1 at a much slower rate than those from the 1975 strain, but the same metabolites were formed (Table II). At 10 min, diol formation and O-demethylation, again with preference for attack at the 6 position, were of equal importance. After 60 min, the *trans*-diol **13** was the major metabolite, but some increase in the total amount of O-demethylation products was also seen. In this strain, formation of **9** is relatively less important than in the 1975 strain.

In addition to the identified metabolites, both strains produced significant amounts of unknown A; this pathway was particularly important relative to the pathways to identified metabolites in the less active 1976 strain. The remaining ether-soluble radiocarbon recovered from TLC was either at the origin or associated with all TLC regions other than those specifically identified above. Since the radiocarbon in the latter areas could not be resolved into distinct metabolites on rechromatography, it is assumed that this labeled material results either from a variety of very minor metabolites or from the degradation of the parent material or one or more of its metabolites under these analytical conditions. The discrepancy between the sum of radiocarbon recovered from TLC and measured in the aqueous phase and that initially introduced into the incubation mixture (recorded as "loss") is due to volatilization of ^{14}C-labeled 1 from the organic extracts during concentration and analysis; this loss was always highest in extracts containing large amounts of unmetabolized 1.

Figure 4 shows a proposed pathway for the initial oxidative biotransformation of 1 based on the metabolites identified in this study. Additional support for this pathway was obtained through experiments on the formation and interconversion of individual metabolites. Quantitation of *cis*- and *trans*-diol formation from metabolism experiments and from incubations of **15** in either weak acid or buffer (Table III) showed that the resulting diol isomer ratio was virtually constant. Isolation of each diol and reincubation with active enzyme preparations with or without the added cofactor did not reveal any enzymatic isomerization. Thus, the diols **12** and **13** found as metabolites probably arise from nonenzymatic hydration of the epoxide **15**, which could not be isolated due to its extreme lability under most analytical conditions. The 3-chromanol **9** is shown in the pathway as a direct oxidative product of 1; formation of this compound is dependent on an active oxidase system, and isolation and reincubation studies did not show it to be a precursor or a product of any other metabolite.

Table III. Chemical and Biological Formation of the Trans and Cis Isomers of the 3,4-Dihydrodiol Analogue of Precocene II

compound	treatment	isomer formed, %[a] trans	cis
epoxide (15)	hydration (0.05 N HCl, aq THF)	75	25
epoxide (15)	hydration (phosphate buffer, pH 7.4)	72	28
precocene II (1)	fat body oxidases, 40 min (1975 cabbage loopers)	68	32
precocene II (1)	fat body oxidases, 60 min (1976 cabbage loopers)	70	30

[a] Isomer ratios determined by LC (chemical hydration) or radiochemical analysis of TLC gel regions (metabolic oxidation).

DISCUSSION

Synthesis of Metabolite Standards. An earlier paper (Ohta et al., 1977) reported the synthesis of some of the potential metabolites of 1, including the 3-chromanol (**9**), the 3,4-epoxide (**15**), and the dihydrodiols (**12**, **13**). However, these compounds were incompletely characterized, and several other potential oxidative metabolites were not prepared. The syntheses reported in this paper provide the basis for a more comprehensive study of the initial steps of precocene biotransformation.

Attempts to prepare the hydroxymethyl derivative **5b** by conventional routes involving the formation of chromanones (Bowers et al., 1976), chromanes (Camps et al., 1979), or chromenes (Bandaranayake et al., 1971) were unsuccessful. Synthesis of this compound was achieved, although in poor yield, by the method recently described by Sartori et al. (1979), using Ti(IV) phenolates in the formation of chromenes.

Syntheses of the hydroxychromenes **8a** and **8b** were achieved by condensation of the appropriate phenols with 3-methylbut-2-enoic acid in anhydrous methanesulfonic acid to give the corresponding chromanones (**7a**, **7b**), followed by $LiAlH_4$ reduction and dehydration to the chromenes. The use of methanesulfonic acid in these syntheses gives improved yields for **7a** and **7b** when compared to the method using polyphosphoric acid described for the synthesis of precocene II and analogues (Bowers, 1976; Ohta and Bowers, 1977); a full account of the general application of this procedure will be published elsewhere.

Although Ohta et al. (1977) reported the synthesis of the epoxide **15** from MCPBA oxidation of 1, our attempts to repeat this synthesis using a variety of reaction conditions gave in all cases a mixture of *m*-chlorobenzoate half-esters of the dihydrodiols **12** and **13**. Hydrolysis of the half-ester mixture and chromatographic separation gave each diol in good overall yield. Stereochemical assignments for **12** and **13** were confirmed by the stereospecific synthesis of **12** from 1, using OsO_4 and *N*-methylmorpholine *N*-oxide, according to the method of Van Rheenen et al. (1976).

Because of the problems encountered in repeating the previously reported synthesis and the potential significance of the epoxide **15** as a key intermediate in both bioacti-

vation and detoxification, the successful preparation and characterization of this compound became an important synthetic goal. Since MCPBA oxidation proved unsuccessful, several other routes to the epoxide were examined, including oxidation of 1 with H_2O_2, using either acetonitrile (Payne et al., 1961) or *p*-chlorophenyl isocyanate (Matsumura et al., 1970) as the coreactant, photooxidation sensitized with α-diketones (Shimizu and Bartlett, 1976) or with rose bengal (Jefford and Boschung, 1977), and formation of the bromohydrin, followed by dehydrobromination with base. None of these techniques gave the epoxide, but evidence for the existence and thermal stability of this compound was obtained in the GC–MS analysis of chromatographically pure *cis*-diol (**12**), which showed a second component having chromatographic and mass spectral properties consistent with the epoxide structure. Reevaluation of the bromohydrin route led to the first successful synthesis of **15**, dehydrobrominating with NaH in THF to avoid protic reactants and solvents. Subsequently, this procedure has been used to prepare the epoxide of the related natural product, precocene I [7-methoxy-2,2-dimethyl-2*H*-benzo[*b*]pyran; Jennings and Ottridge (1979)]. The epoxide moiety of **15**, activated by the 7-alkoxy substituent, is highly susceptible to nucleophilic and electrophilic attack, so that the compound degrades rapidly during most chromatographic procedures and in aqueous media. Our synthesis results demonstrate that **15** is formed and survives only under carefully controlled conditions; thus, it is unlikely that the product of the MCPBA oxidation of 1 described by Ohta et al. (1977) was in fact the epoxide.

Ohta et al. (1977) also described the synthesis of the 3-chromanol **9** from $NaBH_4$ reduction of **15**. In light of the uncertainty of epoxide synthesis by their route, it was important to achieve the synthesis of this compound (also identified as a metabolite by these authors) by another route. Compound **9**, together with the isomeric 4-chromanol **10**, was prepared by the hydroboration and oxidation of **1**; the structure of **9** was confirmed by comparison of spectral data for the hydroboration products with those for **10** obtained as an intermediate in the synthesis of **1** (Ohta and Bowers, 1976).

In Vitro Metabolism Studies. Although the cabbage looper has not been demonstrated to be sensitive to precocene, fat body preparations from this insect provide a highly active source of insect monooxygenases that rapidly metabolize **1** in vitro, producing the greatest variety of metabolic products of nine species examined (Ohta et al., 1977). Thus, the use of this system allows the identification of the several pathways of oxidative metabolism for **1** potentially involved in key bioactivation or detoxification reactions.

The metabolite identifications described here are in general agreement with the preliminary studies reported by Ohta et al. (1977). These authors found a dihydrodiol as the major biotransformation product of **1** in cabbage lopper fat body oxidase preparations, but the stereochemistry of this product was not determined. We found both the *cis*- and *trans*-diols in a constant isomer ratio; degradation studies with the synthesized epoxide **15** suggest that the diols are formed by chemical hydration rather than by the action of an epoxide hydrase. This result is also consistent with the findings of Burt et al. (1978), who were unable to trap the epoxide as a metabolic intermediate, using epoxide hydrase inhibitors. Ohta et al. (1977) also tentatively identified the epoxide itself as a minor metabolite, but we were unable to confirm this finding; our studies on the degradation of synthetic **15** suggest that it will not survive under in vitro incubation conditions or during TLC analysis. The *cis*-diol **12** has also been observed in extracts of whole cabbage loopers and milkweed bugs (*Oncopeltus fasciatus* Dallas) after injection with ^{14}C-labeled **1** (Soderlund, 1979); these results suggest that a similar mechanism of diol formation operates in vivo as well.

Ohta et al. (1977) also reported the 3-chromanol **9** as an important metabolite by direct GLC–MS analysis of material collected from multiple incubations but the actual indentity of their synthetic standard for this compound is unclear (see above). We are able to confirm this identification by cochromatography with authentic material from hydroboration, and our data suggest that **9** is formed directly from **1** by fat body monooxygenases rather than via an epoxide intermediate, as suggested by Burt et al. (1978). However, the chromanol is not an obvious product of chromene hydroxylation and the mechanism of formation of this compound remains obscure.

The phenolic metabolites **8a** and **8b**, products of O-demethylation, were not previously identified as in vitro metabolites. The importance of this pathway in vivo has recently been demonstrated by the identification of the glucoside conjugates of **8a** and **8b** in the hemolymph of insects treated with **1** (Bergot et al., 1980). Cabbage looper oxidases show a strong preference for attack at the 6-alkoxy substituent, but it is not known whether this preference is a consistent feature of oxidases from other insect species. The higher levels of **8a** and **8b** found early in the incubation period compared to those found later suggest that these compounds, unlike the diols and the 3-chromanol, are susceptible to significant secondary metabolism in this in vitro system. Since sustained conjugation in vitro without added cofactors is not likely to be a major reaction, the phenolic metabolites probably undergo further oxidation in this system. However, this has not been confirmed by synthesis and identification of metabolites formed by oxidative attack at more than one site.

The differences between cabbage looper strains in the relative importance of the major oxidative pathways exemplify the potential for selective metabolism of **1**. Epoxidation is clearly the dominant pathway in the 1975 strain, but in the 1976 strain O-demethylation and formation of unknown A are significant pathways as well. The apparent independent variation in amounts of oxidase attack at different sites is consistent with the existence of several terminal oxidases, each with its own substrate specificity (Wilkinson, 1980). The ability of certain insects to perform only a limited number of these reactions efficiently may be the basis of the observed selectivity of precocene action.

The irreversible degeneration of the CA caused by **1** suggests that this compound exerts its effects through a long-lived, possibly covalent, interaction rather than reversible competitive inhibition of a step in the juvenile hormone biosynthesis pathway. The ease with which epoxidation of **1** occurs and the reactivity of the epoxide **15** once formed suggest that the latter compound, rather than the chromene itself, may be the active principle. Bioactivation, if required, must occur in the target tissue, since the epoxide formed elsewhere is probably not stable enough to undergo transport within the insect. Since **1** inactivates CA in tissue culture (Pratt and Bowers, 1977; Müller et al., 1979) and epoxidation (detected as diol formation) is observed with milkweed bug corpora allata incubated with ^{3}H-labeled **1** in vitro (Aldrich and Soderlund, 1979), it appears that if **1** in fact is bioactivated, such reactions occur in the CA itself. The ability of the 6,7-

methylenedioxyphenyl-substituted analogue of 1 to prevent the antihormonal action of 1 in vivo (Brooks et al., 1979) provides further evidence for an oxidative activation step. If the action of 1 in fact depends on metabolic activation within the CA then the sensitivity of a given species to 1 may reflect the balance between activating and detoxifying metabolic events in target and nontarget tissues.

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Toxicity of *O,O*-Diethyl *O*-Carboalkoxyphenyl Phosphorothionates to the Imported Fire Ant

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A series of diethyl carboalkoxyphenyl phosphorothionates was prepared where R = Me, Et, Pr, Bu, Am, Oct, *i*-Pr, and *i*-Bu for the para series and R = Me and Et for both the ortho and meta series. The toxicities of these phosphorothionates to the imported fire ant were determined for 1.0, 0.1, and 0.01% solutions of the toxicant in soybean oil. All of the toxicant baits were accepted by the ants at the concentrations studied. The I_{50} value of diethyl *p*-carboisopropoxyphenyl phosphate to ant-brain AChE was found to be a power of ten larger than that of paraoxon as expected from electronic considerations. A structure–activity study of the phosphorothionates with *p*-CO_2–R groups showed that the log percent kill was nicely correlated with the hydrophobic constant π. The best fire ant toxicants found here were the ones with para esters of low hydrophobic character.

For years, mirex was the important insecticide used to control the imported fire ants, *Solenopsis richteri* and *Solenopsis invicta*, in the southeastern United States. Problems associated with the use of mirex were surveyed by Alley (1973) and are related to the persistence of this chlorocarbon and to its toxicity to organisms other than the ant. All mirex bait formulations have been cancelled as of 1978 and no viable replacement has been found for general use over large infested areas.

Because of their low persistence, organophosphorus pesticides have often been used as replacements for banned chlorinated hydrocarbon pesticides. The most common

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