ed with a linear gradient of acetonitrile in water (100 percent water to 100 percent CH_3CN, 30-minute gradient at 1.5 ml/min).
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20. Gas-liquid chromatography was performed as described in Bergman *et al.* (*11*).
21. Synthesis of compounds and characterization of metabolites will be described (J. E. Bakke, Å. L. Bergman, G. L. Larsen, in preparation).
22. Heating in 3*M* HCl in methanol for 30 minutes at 100°C followed by heating in acetic anhydride at 100°C for 30 minutes.
23. Fast atom bombardment mass spectral determinations were carried out at the Middle Atlantic Mass Spectrometry Laboratory, a National Science Foundation shared-instrumentation facility.
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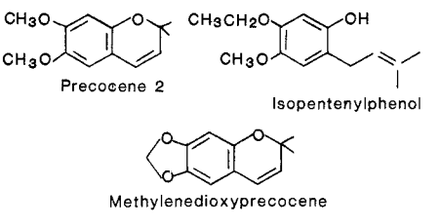
Natural and Synthetic Allatotoxins: Suicide Substrates for Juvenile Hormone Biosynthesis

Abstract. *Cytotoxic agents with antijuvenile hormone activity in insects have been discovered. Their mechanism of action may involve an oxidative bioactivation into a reactive quinone methide.*

The precocenes are simple chromenes derived from plants in the genus *Ageratum* (*1, 2*). Many insect species on contact, feeding, or fumigation with the precocenes undergo physiological responses suggestive of the induced absence of juvenile hormone. These responses include precocious metamorphosis, sterilization, inhibition of sex attractant production, embryogenetic damage, interrupted circadian feeding rhythms, diapause induction, and disruptive actions on caste and morph determination (*3*).

In micromorphological studies, we found that precocene treatment inhibited the normal postimaginal development of the corpus allatum. Similarly, the treatment of mature reproducing females induced permanent sterility and caused a diminution in the size of the corpus allatum (*4*). Although the brain regulates allatal function in many insects, surgical denervation techniques (*5*) and culture in vitro (*6, 7*) have shown that precocenes act directly on the corpus allatum. Histological examination of glands from insects treated with precocenes gave evidence of direct cytotoxic destruction of the parenchymal cells of the corpus allatum (*8–11*). Studies of the relation of their chemical structure to their biological activity and studies of the metabolism of the precocenes (*12–18*) have indicated that these compounds undergo oxidative activation within the corpus allatum and form highly reactive epoxides that alkylate nucleophilic substrates (Fig. 1). It seemed to us that the precocenes were serving as "suicide" substrates (that is, substrates capable of enzymatic destabilization, leading to destructive alkylation of cellular constituents) for oxidative enzymes in the corpus allatum that participate in juvenile hormone biosynthesis. This oxidative bioactivation seemed to parallel the course of activation of certain other plant-derived compounds, such as obtusaquinone (*19, 20*) in which oxidative activation results in the formation of a quinone methide that subsequently reacts with nucleophiles. Since, reactive epoxides and quinone methides result from the action of monooxygenase enzymes on related aromatic substrates (*3*), we synthesized some simple analogous isopentenylphenols (*21*), which might more readily be converted into tautomeric quinone methide intermediates (Fig. 1), and discovered that they displayed physiological activity indistinguishable from that of the precocenes (Table 1). The methylenedioxy analog of precocene inhibits the biological activity of precocene (*15*) through competitive inhibition of the allatal oxidases necessary for its activation (for example, through epoxidation). When we combined methylenedioxyprecocene and 3-ethoxy-4-methoxy-6-*iso*-pentenylphenol, we observed complete inhibition of the antihormonal activity (*22*).



Although the isopentenylphenols could be acting by conversion through ring closure to the related precocene and formation of a reactive epoxide, this seems unlikely because of the complexity of the transformations required. The inhibition of the biological activity of precocene and the isopentenylphenols by methylenedioxyprecocene indicates a similar oxidative activation into allatotoxic agents. The corpora allata of newly emerged milkweed bug females treated with the isopentenylphenols, as when they were treated with precocenes, failed to undergo postimaginal development. Oxidative bioactivation of the isopentenylphenols into quinone methides appears to be the most reasonable explanation for their biological activity.

Although the full spectrum of biologi-

Table 1. Induction of precocious metamorphosis and sterilization in the milkweed bug with 3-ethoxy-4-methoxy-6-*iso*-pentenylphenol and precocene 2.

Allatotoxin	Precocious metamorphosis*		Sterilization†	
	Concentration ($\mu g/cm^2$)	Precocious adults (%)	Concentration ($\mu g/cm^2$)	Sterile females (%)
Precocene 2	1.0	100	8.0	100
Isopentenylphenol	1.0	100	80.0	100

*Twenty second-stage nymphs were confined to a 9-cm petri dish coated with the test compound residue. †Ten newly emerged females were confined to a treated 9-cm petri dish for 48 hours. Ovaries were examined for development after 6 days.

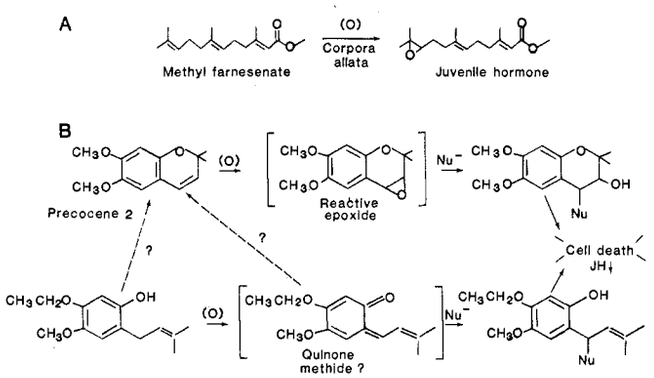


Fig. 1. Oxidative interactions occurring within the insect corpus allatum resulting (A) in juvenile hormone biosynthesis or (B) in the generation of cytotoxic agents from proallatotoxins through the formation of reactive epoxides or quinone methides capable of alkylating nucleophilic (*Nu*) substrates. *JH*, juvenile hormone.

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cal activity for the isopentenylphenols remains to be determined, we have found them to be active on selected Hemiptera and Orthoptera. The development of additional suicide substrates with selective cytotoxic actions on the insect corpus allatum could provide new approaches to insect control.

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21. Molar equivalents of the appropriate phenol and 3-methyl-2-butene-1-ol (for example, prenol or its equivalent, 3-methyl-1-butene-3-ol) were reacted in 20 percent phosphoric acid with stirring at 80°C for 1 hour. Work-up and recrystallization or open-column chromatography over Florisil, yields 40 to 60 percent of the desired isopentenylphenol. Proton nuclear magnetic resonance ($CDCl_3$) for 5-ethoxy-4-methoxy-2-(3′-methylbut-2′-enyl)phenol: 1.45 (3H, triplet), 1.8 (6H, singlet), 3.3 (2H, doublet, J = 7), 3.85 (3H, singlet), 4.05 (2H, quartet), 5.0 (1H, singlet), 5.35 (1H, triplet, J = 7), 6.45 (1H, singlet), 6.65 (1H, singlet).
22. Second-instar nymphs were continuously exposed to residues of the test compounds, as detailed in Table 1. No precocious adults developed on treatment with methylenedioxyprecocene (MDP) alone (1 μg/cm²) or with combined treatments of MDP (2 μg/cm²) and precocene 2 (1 μg/cm²) or MDP (2 μg/cm²) and 3-ethoxy-4-methoxy-6-*iso*-pentenylphenol (1 μg/cm²).
23. We thank the Rockefeller Foundation for their support of these investigations.

4 June 1982

Active Genes Are Sensitive to Deoxyribonuclease I During Metaphase

Abstract. *The active exogenous murine leukemia virus sequences of mouse cells growing in culture are preferentially digested by deoxyribonuclease I in metaphase chromosomes. As determined by nuclear nick translation, all of the gene sequences of these cells active during interphase are in a deoxyribonuclease I–sensitive conformation during metaphase. This method of nick translation can therefore be used to label chromosomes in situ in order to visualize the active regions of the genome.*

When nuclei from many types of organisms are partially digested with deoxyribonuclease I, the active genes are found to be in a special sensitive conformation (*1–3*). The nuclei are usually examined in cells at interphase when these genes are actively being transcribed. It was of interest to investigate the deoxyribonuclease I sensitivity of active genes at different stages in the cell cycle and, in particular, during metaphase when most active genes are usually silent.

To determine whether potentially active genes are sensitive to deoxyribonuclease I during metaphase, we examined the nuclease sensitivity of the exogenous murine leukemia virus (MuLV) sequences in mouse cells growing in monolayer cultures. These genes are known to be active, as determined by the production of viral RNA (*4*) and by the observation that they are three to four times more sensitive to deoxyribonuclease I than the total nuclear DNA is (*5*). Cells in mitosis were isolated from growing cultures, and the nuclei of these cells were treated with deoxyribonuclease I until about 10 percent of the DNA was digested. The undigested DNA was then hybridized to an MuLV-specific probe and compared to DNA from total unsynchronized cultures. Figure 1A shows

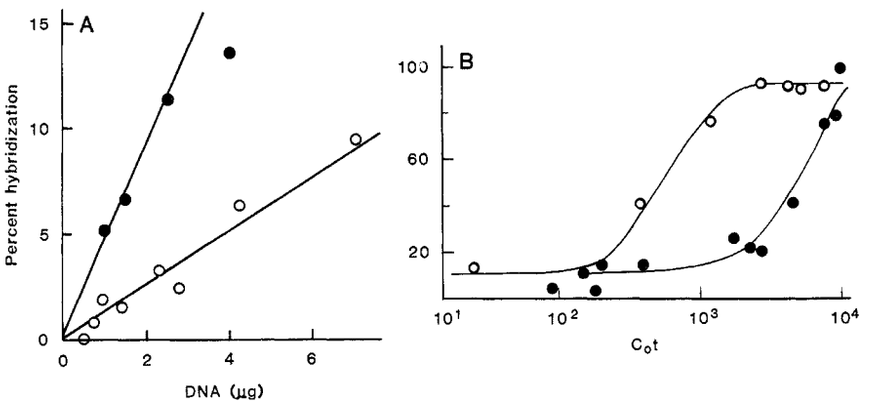

Fig. 1. Deoxyribonuclease I sensitivity of active genes in mitotic chromosomes. (A) NIH Swiss mouse cells were maintained in culture as described (*5*). These cells were synchronized by treatment with thymidine and Colcemid (*13*), and mitotic cells were selected by gentle shaking of the monolayer cultures. Metaphase cells were then purified by differential centrifugation in detergent containing hypotonic medium (*14*). We regularly obtained cells with a mitotic index of 90 percent by this procedure. The resulting metaphase chromosomes were treated with deoxyribonuclease I to obtain 10 percent digestion as determined by acid solubility (*5*). Total DNA (●) and deoxyribonuclease I–treated DNA (○) were purified by phenol and chloroform extractions (*5*) and hybridized for 72 hours at 70°C to a tritium-labeled MuLV complementary DNA (cDNA) probe (10,000 cpm/ng) with an excess of cDNA (*5*). The results are expressed as percent hybridization, determined by S1 nuclease analysis (*1*), after subtraction of 2 percent background hybridization observed in the absence of added DNA. (B) Mitotic chromosomes were nick-translated essentially as described previously for interphase nuclei (*7*). These chromosomes (1 mg/ml) were incubated with deoxyribonuclease I (0.1 μg/ml) in 50 m*M* tris-HCl (*p*H 7.9), 5 m*M* $MgCl_2$, 10 m*M* β-mercaptoethanol, and bovine serum albumin (50 μg/ml) for 15 minutes at 37°C. DNA polymerase (Boehringer Mannheim, 10 unit/ml), 4 μ*M* deoxyadenosine triphosphate, deoxycytidine triphosphate, and deoxyguanosine triphosphate and 0.3 μ*M* 3H-labeled thymidine triphosphate (New England Nuclear, 40 to 50 Ci/mmole) were then added, and the reaction was incubated at 15°C for 5 minutes. The resulting DNA (5000 cpm/μg) was purified by phenol extraction and Sephadex G-50 chromatography and hybridized to itself to remove highly repeated sequences (*7*). This radioactive probe representing the deoxyribonuclease I–sensitive regions of the metaphase chromosomes was then hybridized to excess total DNA (○) or DNA obtained from 10 percent deoxyribonuclease I digestion of interphase nuclei (●) (*5*). The conditions for hybridization and analysis of DNA duplex formation were exactly as described by Levitt *et al.* (*7*). C_0t, concentration of the probe (moles per liter) multiplied by the time of digestion (seconds).

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