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SHORT COMMUNICATIONS

Inhibition of Development in *Caenorhabditis elegans* (Nematoda) by a Reduced Aromatic Schiff Base and Related Compounds

INTRODUCTION

Insect growth regulators with dissimilar chemical structures possess biological activity in parasitic and free-living nematodes (Chitwood, 1987). Certain steroids inhibit growth and reproduction in *Caenorhabditis elegans*, *Nippostrongylus brasiliensis*, and *Heligmosoides polygyrus* (=*Nematospiroides dubius*) (Chitwood et al., 1984; Bottjer et al., 1984; Dennis, 1976). Sesquiterpenoid insect juvenile hormones and their analogs disrupt the development of a wide variety of nematodes (Gibb and Fisher, 1989; Fodor and Tímár, 1989; Glassburg et al., 1983; Jones et al., 1983; Davey et al., 1982; Rogers, 1980; Dropkin et al., 1971; Hansen and Beucher, 1971), whereas nonterpenoid insect juvenile hormone mimics such as benzimidazoles and methoxyphenyl ethers inhibit egg hatch in the pinewood nematode, *Bursaphelenchus xylophilus*, and reduce growth in a goat parasite, *Haemonchus contortus* (Shuto et al., 1989; Boisvenue et al., 1977). The insect anti-juvenile hormone precocene II has biological activity against *Caenorhabditis remanei*, but its role as a nematode antihormone has not been fully clarified (Fodor et al., 1989). The impact of these compounds on the hormonal systems in insects is fairly clear; their role as endocrine-active bioregulators in nematodes has not yet been demonstrated.

Schiff bases and their reduced forms are highly effective insect juvenile hormone mimics (DeMilo and Redfern, 1979). One of these compounds moderately affected the growth of the free-living nematode *C. elegans* in our biological assay. To determine if the biological activity against *C. elegans* could be improved, we made several modifications of the Schiff base structure. We report here the effects of insect juvenile hormone, juvenile hormone mimics, and a reduced Schiff base and its analogs on the development of the free-living nematode *C. elegans*.

MATERIALS AND METHODS

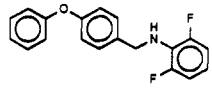
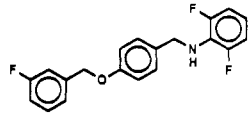
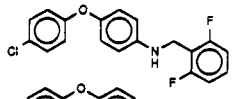
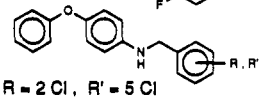
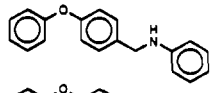
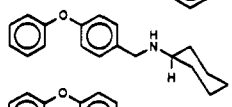
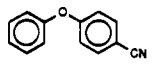
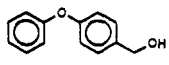
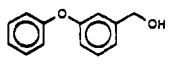
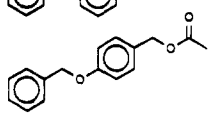
Chemicals. Compounds 1–8 were synthesized according to previously described methods (DeMilo and Redfern, 1979). These methods involved sodium borohydride reduction of Schiff bases that were synthesized from condensation of the appropriate 4-phenoxy(or benzyloxy)benzaldehyde and aniline or from condensation of the appropriate 4-phenoxyaniline and benzaldehyde. Compound **12** [4-(phenylmethoxy)benzenemethanol acetate] was prepared by treatment of 4-(benzyloxy)benzyl alcohol with acetyl chloride in the presence of pyridine. Compound **9** (4-phenoxybenzonitrile) was prepared by reaction of phenol with 4-chloronitrobenzene and K_2CO_3 in DMF, reduction of the resulting 4-phenoxynitrobenzene to 4-phenoxyaniline with Fe/HCl, and conversion to the nitrile via Sandmeyer reaction. Hydrolysis of the nitrile gave 4-phenoxybenzoic acid, and subsequent reduction with $LiAlH_4$ gave 4-phenoxybenzyl alcohol (**10**). The isomer, 3-phenoxybenzyl alcohol (**11**), was available from saponification of a sample of technical permethrin (FMC Corp.).

Biological Tests. Stock cultures of *C. elegans* N2 were maintained axenically in liquid medium [3.0 g of yeast extract (Difco cat. no. 0127-01), 3.0 g of soy peptone (Sigma cat. no. P-0521), and 1.0 g of dextrose per 100 mL of basal medium] (Vanfleteren, 1980). The basal medium (i.e., without hemoglobin or sitosterol) was sterilized in 125-mL flasks in an autoclave, together with 20-mL culture vials and filter syringes. Filter-sterilized hemoglobin and sitosterol solutions were added to cooled medium to make final concentrations of 500 μg/mL hemoglobin and 10 μg/mL sitosterol (Chitwood and Feldlaufer, 1990). The nematodes were subcultured weekly, and inoculum used for biological tests was removed 3 days after subculture.

Compounds tested were insect juvenile hormone (*cis*-10,11-epoxy-3,7,11-trimethyl-*trans*,*trans*-2,6-tridecadienoic acid methyl ester), methoprene (11-methoxy-3,7,11-trimethyl-2,4-dodecadienoic acid 1-methylethyl ester), fenoxycarb [[2-(4-phenoxyphenoxy)ethyl]carbamic acid ethyl ester], a reduced Schiff base [*N*-[4-(phenoxyphenyl)methylene]-2,6-difluorobenzylamine] (**1**) or related compounds, and precocene II [6,7-dimethoxy-2,2-dimethyl-1(2*H*)-benzopyran]. For each experiment, test compounds were emulsified in Tween 80 and water, filter sterilized (0.2 μm), and mixed with the medium to make an inhibitor concentration of 100 ppm and concentration of Tween 80 of 3.0 μL/mL. A serial dilution was made with medium so that the final concentrations for each test compound were 100, 10, 1, 0.1, and 0 ppm, while the concentration of Tween 80 was kept at 3.0 μL/mL. Test and control media were dispensed into 20-mL vials (1 mL/vial). The experiment began when nematodes were inoculated into a total of 23 vials, 5 of each test dose and 3 vials of the control. Subcultures of each vial and the control vials were made after 7 and 14 days.

At 21 days the results of each experiment were evaluated. The medium in each vial was diluted to 100 mL with water; 100-μL aliquots were removed and the nematodes counted under

Table I. Inhibition of *C. elegans* Development by a Reduced Schiff Base and Related Compounds

compound number	structure	biological activity (ED_{50} - ppm)
1		ca. 100
2		> 100
3		> 100
4	 R = 2 Cl, R' = 5 Cl	49
5	R = 3 Cl, R' = 4 Cl	> 100
6	R = 2 Cl, R' = 4 Cl	> 100
7		50
8		30
9		7
10		3
11		90
12		20

a dissection microscope. ED_{50} values are based on the average ED_{50} of two replicates for each experiment. For each experiment, the ED_{50} value was extrapolated from a linear plot of test compound concentrations vs percent inhibition from control for the test doses. The concentration that gave 50% inhibition of reproduction from control was used as the ED_{50}.

RESULTS

Juvenile hormone, the juvenoids methoprene and fenoxycarb, and the juvenile hormone antagonist precocene II had no effect on *C. elegans* at concentrations of 100 ppm or less. Reduced Schiff base 1 (Table I) elicited moderate activity (ED_{50} = 100 ppm).

Structural modification of 1 afforded seven analogs that were more effective than 1 in tests against *C. elegans* (Table I). Lengthening the chain between rings by insertion of a methylene moiety and addition of fluorine to the terminal ring (2) eliminated activity, while substitution at the 2- and 5-positions with chlorine and reversal of the imino and methylene moieties (4) enhanced activity. However, unlike results observed for 4, substitution at positions 3 and 4 with chlorine (5) or at positions 2 and 4 with chlorine (6) eliminated activity entirely. Moreover, activity was also eliminated when chlorine was added to the terminal phenoxy ring (3). Removal of halogens in 1 yielded a compound (7) with activity comparable to that of 4, and replacement of the benzene ring in 7 with a cyclohexyl moiety (8) improved activity.

Surprisingly, simplification of the reduced Schiff bases by replacement of the amino moiety in 1–8 with cyano or hydroxymethyl gave highly active 9 and 10 (ED_{50} values of 7 and 3 ppm, respectively). The 4-phenoxy-substituted benzyl alcohol 10 was considerably more effective than its positional isomer 11. Although ester 12 was severalfold less effective than either of the two most active compounds (9, 10), juveniles were more abundant in vials containing this compound. Diphenyl ether was tested at 100 ppm and was ineffective.

DISCUSSION

Aromatic Schiff bases are potent insect juvenile hormone mimics against the large milkweed bug and mealworm, and secondary amines derived from reduction of these Schiff bases are typically more active (DeMilo and Redfern, 1979). While only slight activity was observed for 1 in our assay, nearly a 100-fold increase in activity was achieved by modifying the skeletal structure of this lead compound. Results from the structure–activity study show that compounds 9 and 10, both highly modified analogs of 1, were the most active compounds against *C. elegans*. Clearly, further studies will be required to elaborate and characterize substituents that will produce optimum compounds in 4-phenoxybenzene-derived growth and developmental regulators affecting nematodes.

Growth and development in nematodes are inhibited by juvenile hormone, methoprene, and fenoxycarb (Fleming, 1988; Chitwood, 1987) and by precocene II (Fodor et al., 1989). The ineffectiveness of these compounds in our tests may be due to nothing more than a dose–response effect; i.e., previously demonstrated activity resulted from higher test concentrations. Alternatively, the ineffectiveness may have resulted from differences in the organism studied or culture conditions.

Our studies were initiated to identify potent compounds that could be used to control nematodes in a novel, safe manner (i.e., through disruption of hormone synthesis, function, or metabolism). The compound suspected of coming closest to eliciting a hormonal effect in our assay was 12. Juvenilization is an effect commonly observed in insects treated with juvenile hormones or mimics and is the principle on which powerful bioassays were created (Bowers, 1971). The effects caused by 12 provide a stimulus to develop an effective and target-specific assay to uncover compounds with similar and more potent activity in nematodes.

In summary, 18 compounds were tested in an in vivo assay to assess their ability to affect growth and development of *C. elegans*. Eight 4-phenoxy(or 4-benzyloxy)benzene-derived compounds were effective in disrupting growth and development of *C. elegans*. Two of these compounds had ED_{50} values of less than 10 ppm. We speculate that the compounds described in this study will serve as a guide in the design of novel control agents for economically important animal and plant parasitic nematodes.

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