Insect Juvenile Hormones: Highly Potent Synthetic Mimics

Abstract. *The substitution of a 7-alkoxy group for the 6,7-epoxy moiety in aryl terpenoid ethers having high juvenile hormone activity has produced several compounds exceptionally active against* Tenebrio molitor. *The most potent compound [7-ethoxy-1-(p-ethylphenoxy)-3,7-dimethyl-2-octene, called JH-25] is active at the level of 10 picograms per insect, or about 100 times more active than other promising juvenile hormone mimics so far reported. Compound JH-25 was also active against* Tribolium confusum.

Insect-controlling chemicals more selective in action than the currently used broad-spectrum insecticides are needed to suppress injurious insect populations without damage to nontarget organisms and the environment. The possibility that juvenile hormones, vital growth-regulating chemicals of insects, might be used to derange normal growth patterns of insects and thereby control injurious species (*1*) has led to the synthesis of a wide variety of juvenile hormone (JH) mimics, that is, compounds with JH activity that differ structurally from the natural hormones (*2*).

We prepared and tested a series of such compounds, some of which proved to be exceptionally potent in bioassays against the yellow mealworm, *Tenebrio molitor* L. The general formulas of the compounds (**1** to **4**) are shown in Fig. 1. The compounds are related to aromatic terpenoid ethers previously reported to be highly effective (*3–6*), but they differ mainly in having a 7-alkoxy group in place of the 6,7-epoxy group (*7*).

Juvenile hormone activity was determined (*8*) by applying 1 μl of an acetone solution of each chemical topically to the venter of the last three abdominal segments of newly molted pupae (2 to 8 hours old) of the yellow mealworm and to last-instar nymphs of the large milkweed bug, *Oncopeltus fasciatus* (Dallas); insects were held at room temperature until the following molt to determine JH activity, which was signaled by the retention of larval characteristics.

Table 1 presents the bioassay data for *T. molitor*. In the first group of compounds (structure **1**), R′ denotes C_2H_5 and the aromatic substituent R is varied. The compounds selected for synthesis and testing were 7-alkoxy analogs of 6,7-epoxides that had previously exhibited high JH activity against *T. molitor* or *O. fasciatus* (*3, 5, 6*). The unusually high potency of the *p*-ethyl analog (R = *p*-C_2H_5) led us to systematically vary the 7-alkoxy group of this compound, and the bioassays of these compounds are grouped next in Table 1. The same compound (structure **1**; R and R′ = C_2H_5), which will be referred to as JH-25, was again the most potent of the group; the propoxy analog (structure **1**; R = *p*-C_2H_5, R′ = C_3H_7) also exhibited excellent activity. Other analogs of the structures **2**, **3**, and **4**, comprising the third group of compounds in Table 1, were considerably less active.

Compound JH-25 is by far the most potent JH mimic we have tested against *T. molitor*, which is probably the most widely used test insect for evaluating JH compounds. In our tests, its activity was about 100 times that reported for related epoxy analogs, such as 6,7-epoxy-1-(*p*-ethylphenoxy)-3,7-dimethyl-2-octene (*9*) and 6,7-epoxy-3,7-dimethyl-1-[(3,4-methylenedioxy)phenoxy]-2-nonene (*5*); both of these epoxides are promising enough to have been extensively evaluated in the field (*10*).

Despite its high potency against *T. molitor*, JH-25 and the various other alkoxy analogs were comparatively ineffective against *O. fasciatus*. This result is in line with observations (*6, 11*) that JH compounds effective against one order are often ineffective against another order (Coleoptera and Hemiptera in this instance). This difference exemplifies the high selectivity of action often mentioned in connection with JH compounds, an action that can conceivably be utilized to combat an injurious insect species while allowing beneficial species of another order to survive unharmed (*11*).

We also tested JH-25 against another coleopteron, the confused flour beetle, *Tribolium confusum* Jacquelin duVal. In one test, last-instar larvae (20 per jar) were placed in jars containing different concentrations of JH-25 in 10 g of 80-mesh bleached flour. With as little as 0.1 part per million (ppm) of JH-25 in the flour, no adults were found after 4 weeks; with 0.05 ppm in the flour, five adults survived after 46 days, but their eggs failed to hatch. In contrast, 13 adults and 75 larvae per jar were present in the control (no JH

Table 1. Activity of juvenile hormone mimics on *T. molitor*. Ten pupae were used for each dose of each compound. The responses, rated as follows, were averaged: 0, perfect adult, no JH activity; 1, retention of gin traps or urogomphi; 2, retention of gin traps and urogomphi; 3, retention of gin traps and urogomphi, and pupal cuticle retained around treatment area; 4, second pupa—retention of all pupal characters. For structures **1** to **4** see Fig. 1.

R	R′	Average response of insect to indicated dose (μg/insect)							
		10	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
				Structure **1**					
p-CH_3	C_2H_5	4.0	4.0	4.0	2.6	1.5			
p-C_2H_5*	C_2H_5*	4.0	4.0	4.0	4.0	3.3	2.1	1.8	1.1
p-C_3H_7	C_2H_5	4.0	4.0	4.0	2.5	2.0			
p-$CH(CH_3)_2$	C_2H_5	4.0	4.0	3.0	1.8				
p-OC_2H_5	C_2H_5	4.0	4.0	4.0	2.3	1.0			
3,4-OCH_2O-	C_2H_5	4.0	4.0	4.0	3.0	0.5			
m-Cl	C_2H_5	3.0	3.0	2.4	0.2				
p-Cl	C_2H_5	4.0	4.0	4.0	2.8	0.0			
3,4-diCl	C_2H_5	4.0	2.8	1.2					
p-NO_2	C_2H_5	4.0	4.0	3.7	1.2				
p-C_2H_5	CH_3	4.0	4.0	2.4	0.0				
p-C_2H_5*†	C_2H_5*†	4.0	4.0	4.0	4.0	3.3	2.1	1.8	1.1
p-C_2H_5‡	C_3H_7‡	4.0	4.0	4.0	4.0	4.0	2.7	1.7	
p-C_2H_5	C_4H_9	4.0	4.0	4.0	2.2	1.2			
p-C_2H_5	$CH_2CH(CH_3)_2$	4.0	3.8	4.0	3.0	1.6			
				Structure **2**					
p-C_2H_5	C_2H_5	4.0	4.0	4.0	3.0	1.6			
3,4-OCH_2O-	C_2H_5	4.0	4.0	4.0	3.0	1.0			
3,4-diCl	C_2H_5	3.4	3.0	1.4					
				Structure **3**					
p-C_2H_5	C_2H_5	4.0	4.0	4.0	2.6	1.3			
				Structure **4**					
p-C_2H_5	C_2H_5	4.0	4.0	2.7	0.4				

* Compound JH-25. The values are averages of responses from four series of tests on two separate preparations of JH-25. † For convenience in comparing compounds, the values for JH-25 (second compound listed) are repeated. ‡ In comparative tests, JH-25 was more active than this compound.

mimic) after 46 days. In another test, eggs of the confused flour beetle (20 per cup) were placed in ⅝-ounce (18.5 ml) jelly cups each containing 5 g of 80-mesh bleached flour. With as little as 0.1 ppm of JH-25 in the flour, no adults were observed; with 0.02 ppm, two adults were noted after 47 days, but they produced no eggs. In similar tests with pupae and adults, 50 ppm of JH-25 did not prevent reproduction. Thus, the chemical will be most effective when administered before the insects reach the pupal stage.

We speculate that the high activity of JH-25 may stem from the inability of the insect to destroy the JH mimic at the appropriate stage of its development; the insect is thus prevented from maturing normally.

The compounds were prepared by alkoxymercuration of the phenyl terpenoid ether in the appropriate alcohol, followed by demercuration with sodium borohydride (*12*). The crude product was purified by chromatography on a Florisil (*13*) column developed first with hexane and then with 2 percent ether in hexane when the desired product emerged. Each fraction from the column was analyzed by gas chromatography (*14*), and those containing the desired compound were combined. Infrared and nuclear magnetic resonance data were consistent with the structures presented (*15*). Compound JH-25 appears to be potentially inexpensive judged by the method of synthesis and the intermediates used. The configuration of the double bond in several preparations of JH-25 was 75 to 80 percent (*E*). Bioassays of the individual isomers prepared by other routes (*16*) showed the (*E*) isomer to be ten times more active than its (*Z*) analog.

The stability of JH-25 was compared with that of its 6,7-epoxy analog (*9*) by exposing 40 mg of each compound coated on glass plates (5 by 20 cm, two per treatment) to sunlight for 8 hours; the chemicals were rinsed off the plates with acetone, and the amounts remaining were determined by gas chromatography (*14*). Losses from treatment were less than 1 percent for JH-25 and 13 percent for the epoxide. Another set of plates coated with JH-25 was left in water for 24 hours; 88 percent of the chemical was recovered, indicating good stability of JH-25 to water.

The exceptionally high activity of JH-25 and some of its analogs, the likelihood that their cost will be low, and the good stability they demonstrate make these JH mimics especially promising as selective pest control agents.

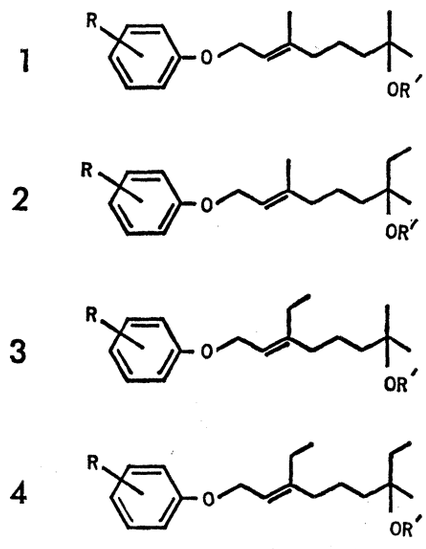

Fig. 1. Structures of aromatic terpenoid ethers.

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References and Notes

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7. A methoxy group in place of an epoxide was reported in a promising JH compound in *Chem. Eng. News* **49** (No. 49), 33 (1971). A similar substitution in methyl juvenate decreased JH activity. [See (*2*, p. 257); the methoxy group in the fourth compound should be in position 11].
8. R. E. Redfern, T. P. McGovern, M. Beroza, *J. Econ. Entomol.* **63**, 540 (1970).
9. This is compound IV in (*3*). When tested concurrently, JH-25 was at least 100 times more active than compound IV.
10. See R. W. Bagley and J. C. Bauerfeind (*2*, pp. 113–151) and F. M. Pallos and J. J. Menn (*2*, pp. 303–316).
11. W. Mordue, *Proc. Brit. Insectic. Fungic. Conf. 5th* **2**, 386 (1969).
12. N. Wakabayashi, *J. Med. Chem.* **12**, 191 (1969).
13. Mention of a proprietary product does not constitute an endorsement of this product by the U.S. Department of Agriculture.
14. A stainless steel column (180 cm long, 0.32 cm in outer diameter) containing 3 percent HI-EFF-8BP (cyclohexanedimethanol succinate) on 100/120 mesh Gas-Chrom Q (Applied Science Laboratory, State College, Pennsylvania) was used with a column temperature of 185°C and a nitrogen flow rate of 30 ml/min. Retention times were 10.2 and 12.2 minutes for the (*Z*) and (*E*) isomers, respectively.
15. For example, the nuclear magnetic resonance data on JH-25 were CCl_4, δ): 6.88 (4H, two doublets, aromatic H); 5.48 (H, triplet, $J \sim 7$ hertz, $C{=}C\underline{H}CH_2$); 4.47 (2H, doublet, $J \sim 6$ hertz, $C{=}C\underline{H}CH_2O$); 3.30 (2H, quartet, $J \sim 7$ hertz, $O{-}CH_2\underline{C}H_3$); 2.58 (2H, quartet, $J \sim 8$ hertz, $\phi C\underline{H}_2CH_3$; 208 (2H, multiplet, $C\underline{H}_2{-}C{=}C$); 1.7 (3H, singlet, $C\underline{H}_3C{=}C$); 1.2 (16H, multiplets of CH_2 and CH_3 on saturated carbon atoms). (The numbers of protons assigned were rounded to the nearest whole numbers.)
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Retrograde Amnesia Gradients: Effects of Direct Cortical Stimulation

Abstract. *Electrical stimulation was delivered bilaterally to either the anterior or posterior cortex in rats from 0.1 second to 4 hours after a single training trial on an inhibitory avoidance task. As indicated by a retention test given 24 hours later, the length of the retrograde amnesia gradients ranged from 5 seconds to 240 minutes, depending on the brain region stimulated and the intensity of the stimulating current. The stimulation intensity that was threshold for amnesia varied directly with the length of the interval between training and treatment.*

Electroconvulsive shock (ECS) administered shortly after a training experience produces a retention deficit, usually taken as evidence for retrograde amnesia (RA). As the interval between training and ECS increases, the degree of amnesia decreases. This phenomenon is termed a RA gradient (*1*). It has generally been assumed that the RA gradient directly reflects the time course of memory consolidation. Estimates of the memory consolidation period have ranged from 10 seconds to several hours (*2*, *3*). Although the fact of the RA gradient has been widely demonstrated, variations in the length of the RA gradients found in different studies have made it difficult to draw