Design of Novel Insect Anti Juvenile Hormones: Allylic Alcohol Derivatives

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Three analogues of 3,3-dimethyl-2-propenol (dimethylallyl alcohol) were synthesized and found to possess anti juvenile hormone activity when assayed on lepidopterous insect species (particularly the tobacco hornworm, *Manduca sexta*). The most active compound of those described, 3,3-dichloro-2-propenyl hexanoate, caused precocious metamorphosis, inhibited juvenile hormone (JH) biosynthesis in vitro, and reduced JH titers in vivo.

The biosynthesis of insect juvenile hormones (JHs) has been an active research area for more than a decade [for recent reviews, see Kramer and Law (1980), Law (1983), Tobe and Feyereisen (1983), and Schooley and Baker (1984)]. Knowledge of the chemical structures of the biosynthetic intermediates needed to produce JHs has stimulated the synthesis of chemical analogues that would selectively inhibit formation of these terpenoid compounds (Poulter et al., 1981; Quistad et al., 1981, 1982). The design of anti juvenile hormone (AJH) agents as potential insecticides is an appealing alternative to the traditional methods of random screening that are often used to discover new classes of compounds with insecticidal activity.

The precocenes were the first compounds reported to possess AJH activity in insects (Bowers et al., 1976). These natural products were isolated and identified from *Ageratum houstonianum* after a crude extract of this plant was found to have AJH activity. Subsequently, the precocenes were found to destroy the source of juvenile hormone biosynthesis, the corpora allata, probably by formation of highly reactive epoxides within the gland (Soderlund et al., 1980; Brooks et al., 1979; Pratt et al., 1980). A major limitation of the precocenes is their selectivity for only a few species of certain insect orders (Hemiptera and Orthoptera) and their inherent lack of biological activity toward more economically important insect pests.

The order Lepidoptera (moths and butterflies) contains a multitude of agricultural pests, and AJH agents with a commercially useful level of activity on these insects would be of great utility. Relatively few compounds have been reported to exhibit AJH activity when tested on Lepidoptera. Certain compounds such as ethyl 4-[2-[(*tert*-butylcarbonyl)oxy]butoxy]benzoate (ETB), ethyl (*E*)-3-methyl-2-dodecanoate (EMD), piperonyl butoxide, and thiolcarbamates induce black pigmentation (a symptom of JH deficiency) in the larval tobacco hornworm, *Manduca sexta* (Staal, 1982; Kramer et al., 1982, 1983). These same compounds also appear to inhibit JH biosynthesis when incubated with corpora allata in vitro, but treatment of hornworms in vivo results in only limited symptoms of accelerated metamorphosis. Application of ETB to *M. sexta* at certain levels does result in larval–pupal intermediates, but this effect is not seen at higher doses because of the inherent JH agonist properties of the compound (Staal, 1982).

Fluoromevalonate (FMev) was the first substance found not only to affect pigmentation but also to cause precocious metamorphosis in numerous Lepidoptera (Quistad et al., 1981). The AJH symptoms observed include precocious burrowing behavior and formation of larval–pupal intermediates or premature pupae. FMev is devoid of any agonist properties, unlike ETB.

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In subsequent studies, precocious metamorphosis has been observed in certain Lepidoptera following treatment with the hypocholesterolemic agent, compactin (applied to cabbage armyworms, *Mamestra brassicae*; Hiruma et al., 1983) and with several terpenoid imidazoles (applied to silkworms, *Bombyx mori*; Kuwano et al., 1983). AJH effects have been reported also when certain chemosterilants (benzyl-1,3-benzodioxoles and benzylphenols) are assayed by using the *Galleria mellonella* wax test (Van Mellaert et al., 1983), but these compounds seem generally less active on Lepidoptera than FMev.

We now report the discovery of a new class of lepidopteran AJH agents, which are allylic alcohols, and their derivatives. We herein detail the biological properties of these compounds and the rationale used in their design.

EXPERIMENTAL SECTION

^{1}H NMR spectra were obtained on a Varian T-60 spectrometer. Chemical shifts are reported in parts per million relative to Me_4Si as an internal standard. Gas–liquid chromatography coupled with mass spectrometry was performed with a Hewlett-Packard Model 5985A instrument in the electron impact mode at 70 eV. Silica gel 60 (Merck) was used for column chromatography, and precoated silica gel GF plates (Analtech) were used for thin-layer chromatography.

Synthesis. *3,3-Dichloro-2-propenyl Hexanoate* (1). A mixture of 3-bromo-1,1-dichloro-1-propene (682 mg, 3.6 mmol), hexanoic acid (306 mg, 2.6 mmol), and K_2CO_3 (582 mg, 4.2 mmol) was heated at 55 °C for 3 h in hexamethylphosphoramide (840 μL). The crude product was purified with a silica gel column eluted with hexane–ether (20:1): 99% purity; 93% yield; R_f = 0.46 on TLC (hexane–ether, 10:1); mass spectrum *m/z* (rel intensity) 226 (4, M^+ for ^{37}Cl), 224 (6, M^+ for ^{35}Cl), 111 (52), 109 (83), 99 (100), 97 (54); NMR ($CDCl_3$) δ 4.66 (d, 2, *J* = 7 Hz), 6.09 (t, 1, *J* = 7 Hz, C=CH).

(*Z*)-*3-Methyl-3-(trifluoromethyl)-2-propenyl Hexanoate* (2). (β-Trifluoromethyl)crotonic acid (5.0 g, 33 mmol; PCR Research Chemicals, Inc.) was methylated (*p*-toluenesulfonic acid–methanol), and the resultant ester was reduced with diisobutylaluminum hydride (55 mL of a 28% solution in heptane, 98 mmol) in ether (50 mL). A portion of the crude 3-(trifluoromethyl)-2-butenol (520 mg, 3.7 mmol) was esterified with hexanoic acid (860 mg, 7.4 mmol) by using dicyclohexylcarbodiimide (1.7 g, 8.2 mmol) and 4-(dimethylamino)pyridine (91 mg, 0.7 mmol) in CH_2Cl_2 (10 mL). The crude ester was purified with a silica gel column eluted with pentane–ether (30:1): 25% yield; 95% purity; R_f = 0.28 on TLC (pentane–ether, 30:1); mass spectrum *m/z* (rel intensity) 238 (2, M^+), 182 (12), 123 (16), 99 (100), 71 (49), 43 (22); NMR ($CDCl_3$) δ 1.83 (s, 3, CH_3C=C), 4.68 (d, 2, *J* = 7 Hz, CH_2O), 6.13 (t, 1, *J* = 7 Hz, C=CH).

3-(4-Chlorophenyl)-3-methyl-2-propenyl Acetate (3). Trimethylphosphonoacetate (2.4 g, 13 mmol) in ether (10

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mL) was added dropwise to NaH (310 mg, 13 mmol) in ether (10 mL). After this mixture was stirred for 0.5 h, 4-chloroacetophenone (2.0 g, 13 mmol) was added in ether (10 mL) and the reactants were refluxed for 16 h. The products were a mixture of E/Z isomers of methyl 3-(4-chlorophenyl)-2-butenoate that were separated by using a silica gel column eluted with hexane–ether (10:1): 16% yield of (Z)-methyl 3-(4-chlorophenyl)-2-butenoate and 38% yield of the E isomer; R_f = 0.48 and 0.40 for the Z and E isomers, respectively, on silica gel TLC (hexane–ether, 3:1).

(Z)-Methyl 3-(4-chlorophenyl)-2-butenoate (433 mg, 2.1 mmol) was reduced with diisobutylaluminum hydride (6.2 mmol) in ether (10 mL) to give (Z)-3-(4-chlorophenyl)-2-butenol (79% yield), which was acetylated (acetyl chloride) to produce (Z)-3-(4-chlorophenyl)-3-methyl-2-propenyl acetate: 86% yield; 95% purity; R_f = 0.34 on silica gel TLC (hexane–ether, 3:1); mass spectrum m/z (rel intensity) 226 (3, M^+ for ^{37}Cl), 224 (9, M^+ for ^{35}Cl), 181 (13), 164 (24), 129 (100), 128 (48); NMR ($CDCl_3$) δ 2.06 (s, 3, $CH_3C{=}C$), 4.73 (d, 2, J = 7 Hz, CH_2O), 5.83 (t, 1, J = 7 Hz, C=CH), 7.25 (s, 4, ar).

Bioassays. We tested for AJH activity using larval tobacco hornworms (*M. sexta*) and larval tobacco budworms (*Heliothis virescens*). Candidate AJH agents were assayed both by topical application and by incorporation into the synthetic diets on which both species are reared (Quistad et al., 1982). Developmental abnormalities were scored after each larval molt to determine the ED_{50} (effective dose causing 50% of the treated larvae to demonstrate prococious metamorphosis or premature prepupal behavior; Quistad et al., 1981).

The inhibition of JH biosynthesis in vitro was determined by using corpora allata from larval *M. sexta* (0–16 h, fifth stadium) incubated in TC-199 medium (4 h) in the presence of L-[*methyl*-^{14}C]methionine (Kramer and Staal, 1981). The effects of AJH agents on JH titers in vivo were assessed after exposure of synchronized larvae to test chemicals via topical or dietary exposure. After fixed intervals, larvae were homogenized in acetonitrile, worked up, and analyzed for JH titer by using the GC/mass spectral assay of Bergot et al. (1981).

RESULTS AND DISCUSSION

Earlier we found that substitution of a fluorine for hydrogen in the methyl moiety of mevalonate transformed it from an intermediate into an AJH agent, a presumed competitive inhibitor of one of the enzymes responsible for processing mevalonate (Quistad et al., 1981). It seemed reasonable that similar modifications of other intermediates in JH biosynthesis would create inhibitors of other enzymes that could then elicit the same net result in vivo. We chose as the targeted intermediate dimethylallyl pyrophosphate, the chain-initiating unit in the formation of farnesyl pyrophosphate. The condensation of dimethylallyl pyrophosphate with 2 equiv of isopentenyl pyrophosphate is catalyzed by the enzyme prenyltransferase (EC 2.5.1.1). Farnesyl pyrophosphate leads to the skeleton of JH III, which is only a minor JH in *M. sexta* larvae, but the same enzyme probably catalyzes formation of the backbones of JH II and JH I (Schooley and Baker, 1984). Terpenoid pyrophosphates are hydrophilic, chemically labile, and generally regarded to be excluded by cell membranes. Accordingly, we decided to synthesize potential antimetabolites of the free allylic alcohol (**4**, Figure 1) with the hope that if a phosphorylated species were necessary to inhibit prenyltransferase, then the allylic alcohol would be phosphorylated in vivo by the target insect. While there appears to be no published precedent for enzymatic phosphorylation of dimethylallyl alcohol, insect enzymes are known to phosphorylate dolichol, which is also an allylic prenol (Fliesler and Schroepfer, 1983; Quesada Allue, 1979). We hoped to obtain an enzyme inhibitor by substitution of various electron-withdrawing groups (chloro, trifluoromethyl, and 4-chlorophenyl) for the methyl groups in dimethylallyl alcohol. This strategy was influenced by the work of Poulter et al. (1976), who showed profound effects on the rates of condensation between isopentenyl pyrophosphate and trifluoromethyl-substituted allylic pyrophosphates catalyzed by porcine liver prenyltransferase. Since Ogura's group (Nishino et al., 1973) has shown that trans-substituted allylic pyrophosphates are preferred chain-initiating substrates for prenyltransferases, trifluoromethyl and 4-chlorophenyl moieties were constructed trans to the allylic double bond (giving the alcohol portion of **2** and **3**, respectively). A third analogue, **1**, resulted from replacement of both methyl groups with isosteric chlorines, followed by acylation. Acylation renders the allylic alcohol analogues more lipophilic, a presumably desirable attribute for penetration through insect cuticle. Furthermore, acylation of the dichloro and trifluoromethyl analogues significantly reduces volatility as compared to the free allylic alcohols. However, the free alcohols (perhaps as their pyrophosphates) were envisioned as the ultimate AJH agents so we hoped for the assistance of the insect in liberating the allylic alcohols by ester hydrolysis.

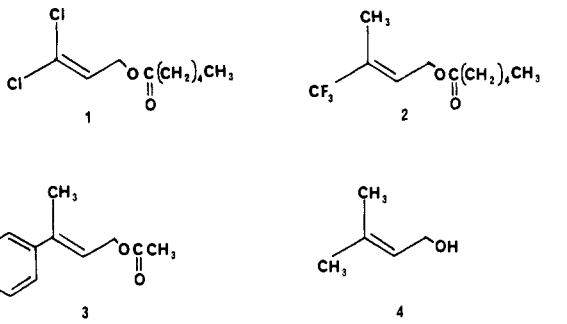

Figure 1. Structures of dimethylallyl alcohol (**4**) and analogues with lepidopteran anti juvenile hormone activity.

Table I. Anti Juvenile Hormone Activity of Compounds on Third-Stadium *M. sexta* and *H. virescens* after Topical Application

compd	ED_{50},[a] mg/g of insect	
	M. sexta	*H. virescens*
1 (dichloro)	1.9	11
2 (CF_3)	7.1	>50
3 (4-chlorophenyl)	>7.1[b]	>50
FMev	0.46	>200[c]

[a] Effective dose causing 50% of the treated larvae to demonstrate precocious metamorphosis or prepupal behavior. [b] 28% AJH response at 7.1 mg/g. [c] 10% AJH response at 200 mg/g.

Table II. Effects of 1 (Dichloro Analogue) on Corpus Allatum Activity in Vitro Using Glands from Fifth-Stadium (0–16 h Old) *M. sexta* Larvae

	JH synthesis	
	pmol h^{-1} (gland pair)$^{-1}$	% inhibition
control	2.1 ± 0.2[a] (9)[b]	0
1 (dichloro) (10^{-4} M)	0.30 ± 0.05 (5)	86
farnesoic acid (2×10^{-5} M)	4.6 ± 1.6 (8)	c
1 (dichloro) (10^{-4} M) + farnesoic acid (2×10^{-5} M)	3.4 ± 1.2 (12)	c

[a] Standard deviation. [b] Number of incubations. Four pairs of corpora allata were used per incubation. [c] No effect or stimulation.

Table III. Effects of Anti Juvenile Hormone Compounds on the Titer of Juvenile Hormones I and II in Larval *M. sexta*[a]

	time of treatment				time of analysis		% of normal titer	
compd	type	rate	larval stadium	age, h	larval stadium	age, h	JH I	JH II
1 (dichloro)	topical	100 μg	III	0–24	IV	0–17	53	42
	topical	250 μg	III	0–24	IV	0–17	6	3
	topical	100 μg	III	40–48	IV	0–16	70	76
	topical	250 μg	III	40–48	IV	0–16	65	74
	topical	250 μg	IV	0–16	IV	24–40	50	71
	diet	10 ppm	I–III	all	IV	0–10	37	57
	diet	100 ppm	I–III	all	IV	0–10	37	44
2 (CF_3)	topical	100 μg	III	24–32	IV	0–16	73	87
	diet	10 ppm	I–III	all	IV	0–17	43	58
	diet	100 ppm	I–III	all	IV	0–17	43	47
FMev	topical	25 μg[b]	III	24–32	IV	0–16	12	5
	topical	100 μg	III	24–32	IV	0–16	5	1
	diet	1 ppm	I–III	all	IV	0–16	85	62
	diet	10 ppm	I–III	all	IV	0–16	5	12

[a] JH I titer was 0.32 ± 0.13 ng/g (six replicates) for untreated fourth-stadium larvae (0–17 h) and 0.08 ng/g (single sample) at an age of 24–40 h. JH II titer was 1.18 ± 0.20 ng/g (six replicates) for untreated fourth-stadium larvae (0–17 h) and 0.14 ng/g (single sample) at an age of 24–40 h. [b] Data from Edwards et al. (1983).

Biological Activity. *M. sexta* larvae were used as the primary targets to screen for AJH activity. Of the three allylic alcohol analogues synthesized, the dichloro derivative (1) was the most active [ca. 4-fold more potent than the trifluoromethyl compound (2)]. The 4-chlorophenyl compound (3) evoked the weakest AJH activity (Table I). Sensitivity to the AJH effects of 1 varied with the age within the stadium. Very young (0–12 h) third-stadium larvae were most sensitive, showing precocious metamorphosis after the next molt. Treatment of older (24–36 h) third-stadium larvae gave AJH effects only after two subsequent molts, which indicates both a sensitivity window for biological activity and also residual effectiveness of 1.

These allylic alcohol derivatives demonstrate several characteristics consistent with the expected properties of AJH agents. Precocious metamorphosis is manifest by formation of larval–pupal intermediates and premature, miniature pupae. These morphological deformities are related to JH deficiency since AJH effects were relieved when 1 was applied to third-stadium larvae in the presence of a JH analogue (10 ppm of hydroprene administered in diet). Large doses of 1 (≥5 mg/virgin female) also reduced the reproductive capacity of adult *M. sexta* females, an expected effect of an AJH agent.

Although most of our bioassay data were obtained by using *M. sexta*, the activity on the tobacco budworm, *H. virescens*, is noteworthy (Table I). While 1 is about 4-fold less active than FMev on *M. sexta*, it is at least 20-fold more active than FMev on *H. virescens*. Even though both 1 and FMev have only a low level of activity against *H. virescens*, the enhanced AJH activity of allylic alcohol derivatives on this species (as compared to FMev) bodes well for further structural modification in this class of compounds to produce efficacious insecticides.

Reduction of Juvenile Hormone Levels. When 1 (at a nominal concentration of 100 μM) was tested on corpora allata which were actively synthesizing JH in vitro, we observed an average 86% inhibition of synthesis (monitored by incorporation of L-[*methyl*-^{14}C]methionine into JH, Table II). Addition of farnesoic acid to the culture medium stimulates the biosynthesis of JH III in control incubations, and levels of biosynthesis in media containing both farnesoic acid and 1 are nearly as high (74%) as in farnesoic acid containing media lacking 1. This result suggests that 1 inhibits JH biosynthesis prior to the final steps (i.e., methylation of the carboxylate and epoxidation of the terminal olefin) and that 1 may not be cytotoxic to the glands, unlike the precocenes. While limited, these data are consistent with 1, or a metabolite of 1, serving as an inhibitor of an intermediate biosynthetic step.

Treatment of *M. sexta* larvae in vivo also reduces the titer of the juvenile hormones which predominate in this stage (JH II and JH I; Table III). The effects on JH titer of 1 and 2 are consistent with the observed morphological effects in the different bioassays: (1) topical treatment is more effective than incorporation of the AJH agent into the diet, (2) 1 (dichloro analogue) is more potent than 2 (trifluoromethyl analogue), and (3) treatment of young (0–24 h) larvae is considerably more effective than treatment of older (40–48 h) larvae. FMev appears to be substantially more efficacious in lowering JH titers in *M. sexta* than 1 whether applied topically or in the diet (Table III). These data are consistent with the results from scoring bioassays based strictly on morphological observations. However, it should be noted that treatment of insects with JH agonists also results in a lowering of JH titer, presumably by a negative feedback process (Edwards et al., 1983).

Conclusions. This project originated as an attempt to discover insect anti juvenile hormone activity by applying knowledge of biosynthetic pathways to designed organic synthesis. Our finding of AJH activity in analogues of dimethylallyl alcohol is unequivocal, but the biochemical basis for this activity is still a matter of conjecture. We assume that the bioactive entity is actually the free allylic alcohol since 3,3-dichloro-2-propenol (hydrolytic product of 1) has inherent (but lower) AJH activity. The possible role of phosphorylation (or pyrophosphorylation) in activation of the free allylic alcohol awaits further investigation.

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Registry No. 1, 93404-30-9; 2, 93404-31-0; 3, 93404-32-1; (*Z*)-3, 93404-37-6; 3-bromo-1,1-dichloro-1-propane, 36469-73-5; hexanoic acid, 142-62-1; (*Z*)-β-(trifluoromethyl)crotonic acid, 93404-33-2; (*Z*)-3-(trifluoromethyl)-2-butenol, 64750-90-9; trimethyl phosphonoacetate, 5927-18-4; 4-chloroacetophenone, 99-91-2; (*Z*)-methyl 3-(4-chlorophenyl)-2-butenoate, 93404-34-3; (*E*)-methyl 3-(4-chlorophenyl)-2-butenoate, 93404-35-4; (*Z*)-3-(4-chlorophenyl)-2-butenol, 93404-36-5.

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Chemistry of Toxic Range Plants. Variation in Pyrrolizidine Alkaloid Content of *Senecio*, *Amsinckia*, and *Crotalaria* Species

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The free base and *N*-oxide pyrrolizidine alkaloid contents of a number of *Senecio*, *Amsinckia*, and *Crotalaria* species, which occur as range plants in the United States, have been measured in order to study variations with season, location, and species. Sampling on a monthly basis during the growing season, carried out for a 3-year period, showed considerable inter- and intraspecies variation in alkaloid content. In most species the total alkaloid content reached a maximum at the preflower or early bud stage.

Range plants that contain hepatotoxic pyrrolizidine alkaloids (PAs) cause considerable economic loss to the livestock industry (Mathews, 1933; Snyder, 1972). Cattle and horses are most often affected but other animals are susceptible (Bull et al., 1968). Sheep, however, appear to be quite resistant to the toxic effects, and their use as a control for these plants by heavily grazing infested ranges has been proposed. The liver is the primary target of the alkaloids and is usually affected chronically, causing a cirrhosis-like condition. Lungs and other organs and tissues may also be insulted (McLean, 1970). The insidious nature of the toxicosis, whereby signs of poisoning and subsequent death of the animal may not be seen for many months after the plant is ingested (Johnson, 1978), results in a large proportion of deaths and illnesses being undiagnosed. The problem is therefore of much greater magnitude than is generally recognized.

Although sporadic episodes of human poisoning and loss of life from PA-contaminated grains have occurred in the past, recent deaths in the United States caused by using *Senecio* species as medicinals (Stillman et al., 1977) and the detection of PAs in certain herbal teas (Culvenor et al., 1980; Roitman, 1981) have focused attention on this problem. Such occurrences, coupled with the detection of PAs in honey (Deinzer et al., 1977; Culvenor et al., 1981) and the potential for PA contamination of milk (Dickinson et al., 1976; Deinzer et al., 1982) have caused increased concern in the PA content of plants and renewed interest in the mechanisms of action of these toxins.

PAs occur in numerous plant species and many genera, but they are found primarily in the families Compositae, Leguminosae, and Boraginaceae (Smith and Culvenor, 1981). The majority of PA-containing plants in the United States are *Senecio* species, and only a few of these are widespread in locations where they can present a problem to either livestock or man. Nevertheless, any plant producing toxic PAs is a potential hazard to animals that may graze it, to unsuspecting but well-meaning individuals who

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