Other papers presented at the 164th National Meeting of the American Chemical Society in the Symposium on the Origin and Fate of Ethylenethiourea Fungicides but not printed in this issue include: "The Determination of Ethylenethiourea in Apples," by W. H. Newsome (this article appeared in *J. Agr. Food Chem.* **20**, 967 (1972)); "Determination of Ethylenethiourea in Food Crops," by John H. Onley, Robert W. Storherr, Randall R. Watts, and Nathan F. Ives; "The Fate of Ethylenethiourea in Taro Culture," by John W. Hylin; and "Determination of Ethylenethiourea Residues in or on Potatoes Using High Speed Liquid Chromatography," by Ronald F. Cook.

# Synthesis of Bioactive Compounds: Juvenile Hormone Mimetics Affecting Insect Diapause

George P. Nilles, Matthew J. Zabik,* Richard V. Connin, and Robert D. Schuetz

A number of juvenile hormone (JH) mimetics based on the farnesyl and geranyl skeleton have been synthesized. They have been bioassayed on the basis of their ability to prevent or terminate diapause in the adult cereal leaf beetle (*Oulema melanopus* L., Chrysomelidae). Oviposition and mortality data for treated insects are described. A theory concerning the molecular requirements of JH mimetics in diapause disruption, based on intramolecular steric and electronic factors, is discussed.

Recent years have shown a truly dramatic increase in the synthesis of juvenile hormone mimetics. This interest may be jointly ascribed to the potential these compounds have as "third generation" pesticides and to the synergistic cooperation between chemists and entomologists. Biological evaluation of these materials has been, almost exclusively, *via* metamorphosis effects on larvae or pupae. To date only limited studies on the disruption of adult insect diapause by external application of juvenile hormone (**1**, R = CH₃) and a few of its mimetics have been conducted (DeWilde, 1968; Slama, 1971). Artificial control of adult diapause could well offer an alternate means of bioassay of candidate hormonomimetic materials. Such substances can be utilized to supply the experimenter with a source of postdiapause insects from field-collected or laboratory-reared specimens. And, finally, disruption of diapause during unfavorable environmental conditions might afford a means of insect control.

Scheme I
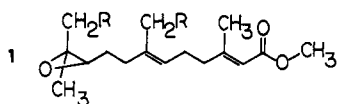
The cereal leaf beetle, *Oulema melanopus* L., is having increasingly significant effects as a pest of small grains in the Northeast and North Central regions of the United States. Laboratory rearing studies (Connin *et al.*, 1968) indicated that the beetles underwent an apparent adult diapause, which required storage of 10-12 weeks to obtain mating and consistent egg production. Connin *et al.* (1967) had reported that diapausing beetles could be put into a postdiapause, sexually active condition by topical application of methyl juvenate (**1**, R = H). Later it was discovered (Connin and Hoopingarner, 1971) that diapause could either be prevented or terminated by treat-

ment with this JH mimetic and that only the female need be treated.

Accordingly, we report an investigation into the feasibility of screening JH mimetics by prevention or termination of diapause in this beetle, the postdiapause state being readily discernible by observation of initiation and extent of oviposition. The compounds selected for this study were from among those shown by previous investigators to have significant juvenilizing effects on various preadult insect species. In addition, several different series of compounds were synthesized as part of an initial structure–activity study based on variations in the juvenile hormone molecule (**1**, R = CH₃).

ANALYTICAL AND PURIFICATION PROCEDURES

Infrared spectra were determined on a Perkin-Elmer 237B grating instrument as films between salt plates. Nuclear magnetic resonance spectra were run in CCl_4 on either a Varian A-56/60 or T60 instrument, using TMS as the internal standard (τ scale). Mass spectra were determined using the direct probe inlet of a Dupont 21-490 spectrometer at ambient temperature, unless otherwise noted, at 20 and 70 eV ionization potential as required.

Gas-liquid chromatographic analyses were performed on a Varian 1400 instrument equipped with a flame ionization detector and using helium (99.997% purity) as the carrier gas. Operating temperatures were: detector and inlet, 270°; column, in the range 160-250°. Columns used were 2 m × 2 mm i.d. glass, packed with 3% XE60 on 60-80 mesh Chromosorb W or 4% EGSP-Z on 80-100 mesh Gas Chrom Q. On-column injection was used. Carrier gas flow rates and column temperatures were adjusted to optimize conditions for each compound. Integration was by triangulation.

Analytical thin-layer chromatography utilized either E. Merck silica gel GF-254 (10-40 μ) on 75-mm plates, 0.1-mm thick, or Analtech Inc. "Uniplates" 0.25-mm silica gel GF on 5 × 20 cm, 0.25-mm thick precoated plates. Solvent systems employed were (for hexane–ether): I, 4:1; II, 3:1; III, 2:1; and IV, 1:1. Preparative tlc was carried out on 20 × 20 cm plates coated with E. Merck silica gel GF-254 (10-40 μ) 0.5-mm thick. Column chromatography utilized E. Merck silica gel, 0.05-0.2 mm, Brockmann activity II-III, with a weight ratio of 20-30:1 (support to

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compound). The solvent systems employed were those optimized by analytical tlc.

Tabulated mass spectra, nmr spectra, and gc retention times for all compounds synthesized by the authors appear in the microfilm edition of this journal on the pages immediately following this article. Elemental analyses were by Galbraith Laboratories, Knoxville, Tenn.

SYNTHETIC PROCEDURES

General. Drying of a reaction solvent refers to equilibration with anhydrous sodium sulfate. Removal of a solvent was always performed *in vacuo* with a rotary evaporator at as low a bath temperature as practicable.

All methyl esters were prepared from the corresponding acids by treatment in ether with a *ca.* 10% excess of ethereal diazomethane for 30 min, followed by solvent removal.

A 5% excess of *m*-chloroperbenzoic acid was used for all epoxidations. The reagent, purified by the method of Schwartz and Blumbergs (1964), was added in one portion at ice bath temperature, with stirring, to the olefin dissolved in 50 times its weight of methylene chloride. After stirring 30 min, the reaction mixture was allowed to warm to room temperature and poured into an excess of 5% aqueous sodium bicarbonate. The organic phase was separated, and the aqueous phase was extracted with an equal volume of methylene chloride. The combined solvent solution was dried and the solvent was removed. Alternatively, the methylene chloride was replaced by an equal volume of 10% aqueous ethylene glycol dimethyl ether. Hexane was then used in the workup extraction. No difference in regioselectivity was noted.

Esterifications (other than methyl) were carried out by mixing an equimolar quantity of acid and alcohol with a 5% excess of dicyclohexylcarbodiimide (DDC) in anhydrous ether at 0° and allowing the reaction to proceed for 48 hr at 0°. After adding excess water, stirring 30 min, and filtering to remove the dicyclohexylurea, the ether solution was dried and the solvent was removed. The residue was purified by column chromatography as noted.

All reagents were purchased from Aldrich Chemical Company, except for geraniol (95% pure) (Chemical Samples Co.), methyl chrysanthemate [methyl 2,2-dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylate, Pfaltz and Bauer], and 3-methylcrotonic acid (J. T. Baker Co.).

Particular. Yields given in these procedures are for the chromatographically purified product, unless otherwise noted.

3,7,11-Trimethyl-10,11-epoxy-2,6-dodecadienoic Acid, Methyl Ester, Methyl Juvenate (2). A solution of 3.0 g of farnesol in 75 ml of dry benzene was stirred and refluxed for 2 hr with 34 g of silver carbonate on Celite (Fetizon and Golfier, 1968). The yield of farnesal, after cooling, filtering, and removing solvent, was 98%. This aldehyde was oxidized to farnesenic acid by the method of Caliezi and Schinz (1949). A typical yield was 100% of theory. Methylation and epoxidation, as described under general procedures, gave 2, which was purified by column chromatography using solvent system I. The yield based on farnesol was 87% (100% purity), of which 38% was the (*E,E*) isomer.

3,7,11-Trimethyl-10,11-epoxy-2,6-dodecadienal (3). Farnesal was epoxidized under the general conditions described above. Purification was by column chromatography using solvent system III. The yield was 48% (98% purity), of which 29% was the (*E,E*) isomer.

3,7,11-Trimethyl-10,11-epoxy-2,6-dodecadienoic Acid (4). Farnesenic acid was epoxidized as usual; however, the reaction mixture was taken to dryness omitting the treatment with aqueous sodium bicarbonate. The residue was taken up in a small amount of hexane and filtered to remove the bulk of the *m*-chlorobenzoic acid. The filtrate

was concentrated and the epoxy acid was purified by tlc using solvent system IV. The yield was 70% (95% purity), of which 28% was the (*E,E*) isomer.

1,1-Diethoxy-3,7,11-trimethyl-10,11-epoxy-2,6-dodecadiene (5). A mixture of 2.0 ml of anhydrous ethanol was stirred 24 hr with 0.1 g of ammonium nitrate. The ethanol was removed and the residue was partitioned between hexane and 5% aqueous sodium bicarbonate. After separating the organic phase, it was dried and the solvent was removed. The acetal was epoxidized as usual. All attempts at chromatographic purification resulted in deacetalization to the epoxyaldehyde. Consequently, bioassays were carried out on the crude material. The crude yield based on farnesal was 95% (88% purity), of which 24% was the (*E,E*) isomer.

13,14-Epoxy-6,10,14-trimethyl-5,9-pentadecadiene-4-one (6). A solution of 1.0 g of farnesal in 20 ml of dry ether was added to a *ca.* 10% excess of *n*-propyllithium in 30 ml of ether at ice bath temperature under nitrogen during 15 min. After stirring 30 min more, hydrolysis with 20 ml of 5% aqueous chloride, separation of the layers, drying, and solvent removal gave the alcohol. A 1.0-g quantity of this alcohol was dissolved in 50 ml of hexane and stirred 1 hr at room temperature with 10 g of activated manganese dioxide (Carpino, 1970; Corey *et al.*, 1968). Filtration and solvent removal gave the crude ketone. The ketone was epoxidized as usual and purified by column chromatography using solvent system I. The overall yield based on farnesal was 62% (94% purity), containing 31% of the (*E,E*) isomer.

10,11-Epoxy-3,7,11-trimethyl-2,6-dodecadienyl-1-acetate (7). A mixture of 5 mmol of farnesol and 10 mmol of pyridine in 20 ml of dry benzene was treated with 5.1 mmol of acetyl chloride in one portion at ice bath temperature. After stirring 3 hr at room temperature, 20 ml of water and 20 ml of ether was added. The phases were separated, the organic layer was washed with water to remove pyridine, separated again, and dried, and the solvent was removed. Epoxidation and purification by column chromatography using solvent system I gave the epoxyacetate in 46% yield (98% purity), containing 37% of the (*E,E*) isomer.

10,11-Epoxy-3,7,11-trimethyl-1,6-dodecadienyl-3-acetate (8). This compound was prepared exactly as described for 7, substituting nerolidol for farnesol. The yield was 32% (90% purity), of which 58% was the (*E*) isomer.

Chrysanthemyl 10,11-Epoxy-3,7,11-trimethyl-2,6-dodecadienoate (9). Epoxyfarnesenic acid (4) was esterified with chrysanthemol (prepared in 63% yield by lithium aluminum hydride reduction of methyl chrysanthemate) using DCC. Purification was by column chromatography using solvent system III. The yield was 42% (84% purity), of which 27% was the (*E,E*) isomer.

Scheme II

Farnesyl-Derived Mimetics with Ester Function Modified
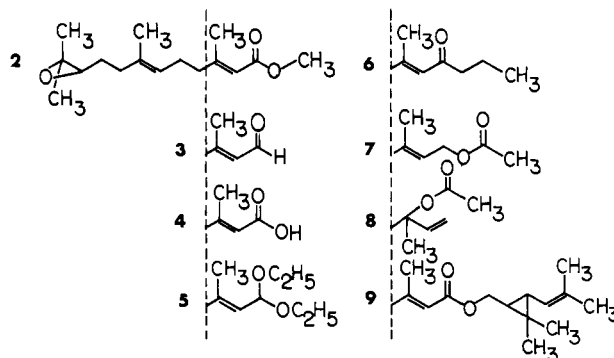
Methyl 6,7-Epoxy-3,7-dimethyl-2-octenoate (10), Chrysanthemyl 6,7-Epoxy-3,7-dimethyl-2-octenoate (11), and Chrysanthemyl 3,7-Dimethyl-2,6-octadienoate (14). Compound 10 was prepared following the same sequence, reaction conditions, and purification procedures as described for 2, except that geraniol was used in place of farnesol. The overall yield based on geraniol was 87% (98% pure), of which 58% was the (*E*) isomer. One of the intermediates, 3,7-dimethyl-(*E*,*Z*)-2,6-octadienoic acid, was esterified with chrysanthemol using DCC to give 14 in 43% yield [100% pure, 62% (*E*) isomer]. Epoxidation of this same acid, followed by esterification with chrysanthemol using DCC, gave 11 in 47% yield [84% pure, 72% (*E*) isomer] based on geranic acid.

Scheme III

Geranyl-Derived Mimetics with Ester Function Modified
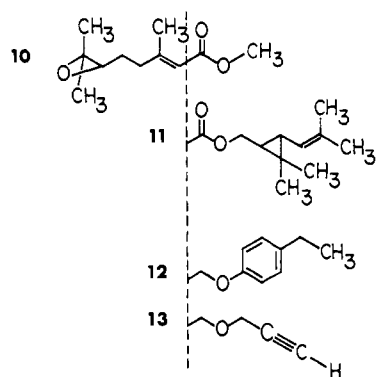
Methyl 2,3-Epoxy-3-methylbutanoate (15). Methyl 3-methylcrotonate in 84% yield (from the corresponding acid with diazomethane) was treated with *m*-chloroperbenzoic acid. Epoxidation of α,β unsaturated carbonyl systems is sluggish, in this case requiring 24 hr at room temperature. Workup as previously described and column chromatography, solvent system I, gave the epoxy ester in 57% yield and 99% purity.

Scheme IV

Miscellaneous Mimetics
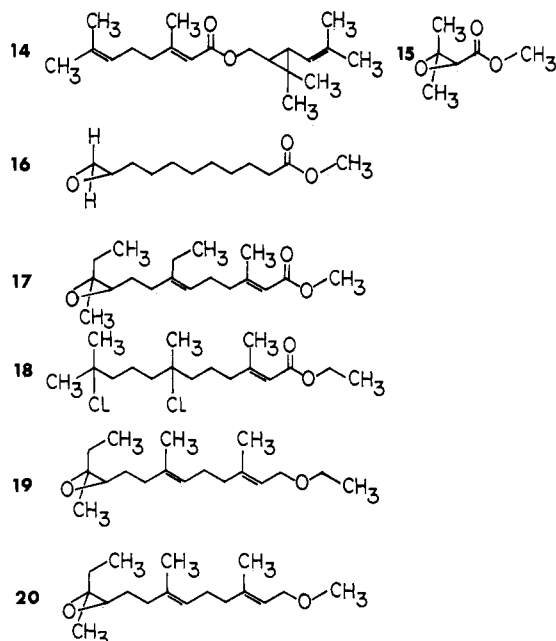
Methyl 10,11-Epoxy-undecanoate (16). Methyl 11-undecenoate was epoxidized as previously described. The crude epoxy ester was better than 98% pure by nmr and gc. The yield was 98%.

Methyl 10,11-Dihydroxy-3,7,11-trimethyl-2,6-dodecadienoate (21). A mixture of 0.5 mmol of methyl juvenate (2), 1.0 ml of tetrahydrofuran, 150 mg of 70% perchloric acid, and 10 ml of water was kept at −8° for 48 hr with occasional shaking. Addition of 0.1 g of sodium carbonate was followed by extraction (3 × 20 ml) with ether. The combined extracts were dried and the solvent was removed. The residue was purified by preparative tlc using solvent system IV; product was at R_f = 0.15. The yield was 45% (90% pure), of which 20% was the (*E*,*E*) isomer.

Methyl 7,11-Dichloro-3,7,11-trimethyl-(E,Z)-2-dodecenoate (22). This compound was prepared following the procedure of Romanuk *et al.* (1967). The crude product was washed with ice cold water until the wash was neutral. After drying, this material was bioassayed without further purification. See text for comment on purity.

Other hormone mimetics [namely, 6,7-epoxy-3,7-dimethyl-(*E*,*Z*)-2-octenyl 2-propynyl ether (13), methyl 10,11-epoxy-3,11-dimethyl-7-ethyl-(*E*,*Z*)-2,(*E*,*Z*)-6-tridecadienoate (17), ethyl 7,11-dichloro-3,7,11-trimethyl-(*E*,*Z*)-2-dodecenoate (18), 10,11-epoxy-3,7,11-trimethyl-(*E*,*Z*)-2,(*E*,*Z*)-6-tridecadienyl ethyl ether (19), 10,11-epoxy-3,7,11-trimethyl-(*E*,*Z*)-2,(*E*,*Z*)-6-tridecadienyl methyl ether (20), and ethyl 10,11-epoxy-3,7,10,11-tetramethyl-(*E*,*Z*)-2,(*E*,*Z*)-6-dodecadienoate (23)] were obtained through the courtesy of Richard Bagley of Hoffman-LaRoche, Inc., Nutley, N J. A sample of 6,7-epoxy-3,7-dimethyl-(*E*,*Z*)2-octenyl 4-ethylphenyl ether (12) was furnished by Julius Menn, Stauffer Chemical Co., Mountain View, Calif. These experimental materials were used as received. Compounds 13, 19, 20, and 23 were stated to be "100%" pure, with no indication of the amount of (*E*,*E*) isomer. Compound 17 (synthetic juvenile hormone) was 97% pure and 30% of the (*E*,*E*) isomer by gc. Compound 12 was 92% pure and 95% of the (*E*) isomer by gc. All hormonomimetics were stored over anhydrous potassium carbonate at −10° until needed.

Scheme V

Farnesyl-Derived Mimetics with Epoxy Function Modified
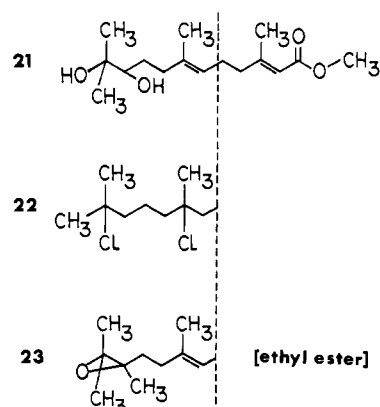
BIOLOGICAL TESTS

Both field-collected and laboratory-reared beetles were used in the tests. The field-collected prediapause beetles were obtained near Kalamazoo, Mich., in early July and caged over seedling barley in a greenhouse until feeding ceased, when they were collected and placed in storage (Wellso *et al.*, 1970) for completion of diapause. Laboratory-reared beetles were obtained from eggs laid by field-collected beetles maintained as described by Connin *et al.* (1968).

Tests to terminate diapause were made using field-collected beetles which had been in diapause 3-6 weeks. Tests to prevent diapause were made against prediapause adults 5-10 days after emergence from the pupal cell. All tests were made in a large rearing room maintained at

24-26° and 50% RH, with a 16-hr daily light period provided by 1000 ft-candles of light delivered at plant height. Seedling "Larker" barley plants *ca.* 12 cm tall growing in 9-cm pots provided food and oviposition sites for the beetles. Treated beetles were placed ten pair each, under lantern globes which are 17 cm tall and have an 8.3-cm bottom diameter. Thus they fitted snugly into the 9-cm pots in which barley seedlings were growing. Masking tape was used to fasten the pot and globe securely, and the top of the globe was covered with nylon cloth. This type cage has been very satisfactory in our oviposition studies with small numbers of beetles (as many as 15 pair). However, some differences in biological effects are apparent because oviposition occurs more quickly in these cages than in larger cages, though the total number of eggs is no greater. Also, beetles which have just entered diapause when they are confined in these cages may become active and deposit small numbers of eggs after as little as 30 days of confinement. However, this was of no concern in these tests since a test period of 14 days was used.

Test materials were applied topically on the venter of the abdomen at dosages of 50 and 100 μg in 1 μl of acetone (Mallinckrodt Chemical Co., Analytical Reagent grade). Each test group of insects consisted of ten males and ten females, with either both sexes treated or females only. Control groups were either treated with acetone or left untreated. Observations were made on feeding and mating activity, number of eggs, and cumulative mortality.

SYNTHETIC DISCUSSION

The stereochemical requirements of the juvenile hormone molecule have been discussed by Wigglesworth (1969) and Trost (1970). Presumably mimetics which are based on the farnesyl skeleton would necessitate the same; *i.e.*, (E,E) configurations at the Δ^2 and Δ^6 olefinic centers. Other geometries seem to result in mimetics requiring larger effective dosages.

JH mimetics whose structures depart from the "farnesyl" type may exert their effect through different biophysiological pathways or by some indirect process. In that case the molecular geometries may require other configurations or perhaps have no particular requirements at all.

Consequently, in the present work, we had two alternatives: either synthesize and bioassay all possible geometric isomers of a given compound or bioassay a given compound as a mixture of isomers. The former requires completely stereoselective and stereoretentive synthetic procedures. The latter only requires an approximate determination of the proportion of different isomers in the product or possibly not even that if there is no geometric requirement on biological activity.

With the exception of **7**, the synthesis of the farnesyl derived mimetics started with farnesol which was oxidized to farnesal, employing silver carbonate on Celite (Fetizon and Golfier, 1968). Silver ion is known to cause geometric isomerization; however, other oxidizing procedures (*e.g.*, manganese dioxide) gave considerably lower yields and also involve some (E,Z) interconversion. Gas chromatography revealed that bioassayed JH mimetics possessed 35 ± 15% of the desired (E,E) isomer. Identification was made by comparison to the nmr and gc work of Burrell *et al.* (1966).

In the same manner, geraniol served as starting material for mimetics **10**, **11**, and **14**, with the (E) isomer being about 60% of the final mixture in each case.

Acetyl chloride treatment of farnesol and nerolidol gave, after epoxidation, mimetics **7** and **8**, respectively. Gas chromatographic analysis of **7** showed two substances, probably the (E)-2 and (Z)-2 isomers resulting from the acid-catalyzed isomerization of the unsaturated ester system. The (E,Z) ratio was about 1.5:1.

Regioselective introduction of the epoxy function was most conveniently performed employing *m*-chloroperbenzoic acid in the presence of water. Of the two nonconjugated olefinic bonds in the farnesyl compounds, the terminal one is selectively attacked, probably due to conformational factors in solution. For example, methyl juvenate (**2**) prepared in this manner was identical in all respects to the same material prepared from methyl farnesate by the *n*-bromosuccinimide–water–sodium isopropoxide route (VanTamelen and Hessler, 1966).

Compounds 18 and 22 show remarkable differences in biological activity despite their quite similar structures. Compound 18 showed two spots on tlc (solvent system I) and 13 substances by gc (EGSP-Z column, 150°). Gc–mass spectra indicated the presence of ethyl farnesate (PP, 264), with other fragments at 249 (P-CH$_3$), 219 (P-OEt), and 191 (P-CO$_2$ET), and the remaining fragmentation pattern was virtually the same as methyl farnesate. This ester is the only, so far, identifiable constituent of the mixture. In addition, mass spectra (direct inlet, ambient temperature) at either 20 or 70 eV failed to reveal any typical chlorine isotope abundance patterns. In contrast to this, elemental analysis gave 20.95% Cl, while 18 demands 21.02% Cl.

Crude mimetic **22** (Romanuk *et al.*, 1967) gave similar mass spectral data. The gas chromatograph revealed at least 19 different materials. One of the principle constituents in the crude reaction mixture was identified as methyl farnesate by gc–ms comparison to an authentic sample. Elemental analysis of the mixture gave 12.88% Cl, which corresponds to approximately 60% of **22**. Again no mass spectral fragments having chlorine isotope abundance patterns could be detected at either 20 or 70 eV. The elemental analyses of either 18 or 22 were not materially affected upon washing the compounds with 10% aqueous sodium carbonate. However, it is known that compounds having chlorines at tertiary positions can give mass spectra with no discernible parent peaks (Budzikiewicz *et al.*, 1967). In addition, loss of chlorine from such positions usually occurs as hydrogen chloride. Thus synchronous or rapid consecutive loss of two HCl molecules upon electron impact could give rise to spectra identical to methyl farnesate (from **22**) or ethyl farnesate (from **18**). It is also conceivable that some of the gc products noted could come from on-column decomposition. For purposes of discussion, we have retained the original proposed structure for these compounds. However, it is possible that the difference in biological activity (notably toxicity) could come from trace amounts of other substances.

All other compounds prepared gave mass spectra, nmr spectra, and other physical parameters completely in accord with the structures shown.

BIOLOGICAL DISCUSSION

In evaluating the data of Table I, the activity of a given mimetic may be compared to either methyl juvenate (**2**) or to the normal postdiapause behavior of the insect. We have interpreted the hormonomimetic activity in terms of the magnitude (total eggs after 14 days) and initiation of oviposition.

Not all insects began oviposition on the same posttreatment day. This results in a higher total egg production for the "early starters." However, oviposition usually occurs at a constant *per diem* rate in this insect (Connin *et al.*, 1967). As a partial correction for comparison purposes, we define the "oviposition index" (O.I.) as the average number of eggs laid by the surviving test colony on the 14th day posttreatment, corrected to 50% mortality and multiplied by the ratio of the molecular weight of the cited mimetic to the molecular weight of methyl juvenate (**2**). This gravimetric factor is to correct to equimolar treatment of the insect. "Correction to 50% mortality" means

that the average number of eggs laid per female on the 14th day posttreatment was multiplied by 5 (50% of the initial test colony population). The gravimetric factor is defined as unity for untreated insects.

It is not intended that the results in the tables be subject to a strictly quantitative interpretation. Thus, arbitrarily, one might define O.I.'s in the range <5 as weak response, 6–19 as fair but below average, 20–50 as normal, and >50 as strong and above average, with all comparison again being made to the normal oviposition behavior of the beetle (O.I. range 20–50, for postdiapause insects). We can now make several observations and generalizations.

An increase in the dose of a given mimetic increased the mortality in 61% of the runs. A decrease was noted in 2 of the runs, and in 15% the mortality was unchanged. In 53% of the runs, administration of the mimetic raised the mortality above that of the acetone blank. Hormone 13 was 100% fatal in 24 hr at 100 μg, while 10 μg caused 50% deaths in 19 days in diapausing beetles.

Mimetics 19 and 20 also exhibited high mortalities when applied to both prediapause and diapausing beetles. This is particularly interesting since 20 is simply the ether bioanalog of the naturally occurring C_{17} cecropia juvenile hormone (Meyer et al., 1970).

It is conceivable that toxic stress alone could induce disruption of diapause in the beetle. However, some

Table I. Hormonal Treatment, Oviposition, and Mortality Data

		Prediapause cereal leaf beetles								Diapausing cereal leaf beetles							
		Days to[a] 1st egg		Total eggs[b] in 14 days		Oviposition[c] Index		Mortality,[d] % dead		Days to[a] 1st egg		Total eggs[b] in 14 days		Oviposition[c] Index		Mortality,[d] % dead	
Compd	Dosage, μg	MF	F	MF	F	MF	F	MF	F	MF	F	MF	F	MF	F	MF	F
2	100	7	7	182	94	40	10	70	55	8	8	192	152	40	15	55	15
	50	7	7	106	45	28	10	60	50	6	8	94	2	20	0	45	45
3	100	10	—	79	0	35	0	45	45	8	—	40	0	4	0	60	65
	50	13	—	12	0	7	0	35	65	14	—	23	0	9	0	50	50
4	100	—	—	0	0	0	0	50	55	14	—	11	0	12	0	50	25
	50	—	—	0	0	0	0	60	45	14	12	5	36	5	21	40	30
5	100	—	—	0	0	0	0	65	45	12	—	38	0	32	0	80	35
	50	8	—	60	0	29	0	30	40	12	12	2	46	6	20	65	35
6	100	10	12	64	50	31	63	40	70								
	50	12	—	48	0	26	0	40	35								
7	100	8	8	113	14	37	0	30	50	8	8	71	114	26	45	45	35
	50	8	0	66	0	30	0	30	40	12	12	43	21	35	15	35	45
8	100	—	—	0	0	0	0	85	80	14	12	24	50	16	26	65	10
	50	—	—	0	0	0	0	50	50	8	12	19	51	11	37	25	25
9	100	—	14	0	7	0	7	30	25								
	50	—	—	0	0	0	0	50	60								
10	100	6	—	88	0	17	0	40	45	8	14	56	33	26	20	55	15
	50	—	—	0	0	0	0	25	65	14	14	1	22	1	10	50	20
11	100	—	—	0	0	0	0	30	25								
	50	—	—	0	0	0	0	50	60								
12	100	10	10	66	101	29	52	70	60								
	50	10	10	53	77	15	42	40	55								
13	100	—		0		0		100		—		0		0		100	
	50																
14	100	—	—	0	0	0	0	45	60	12	8	46	42	23	23	40	30
	50	—	—	0	0	0	0	50	60	12	12	25	69	10	24	15	20
15	100	11	—	56	0	17	0	40	45	12	12	50	17	17	7	50	60
	50	—	11	0	47	0	5	20	15	12	12	30	18	9	5	30	15
16	100	8	—	51	0	26	0	55	55	12	9	52	89	30	42	40	15
	50	10	—	33	0	11	0	40	35	12	14	36	34	34	17	55	15
17	100	7	7	57	25	6	0	70	60	5	7	712	374	44	28	35	40
	50	7	7	145	3	3	0	45	80	5	7	711	273	50	33	35	25
18	100	8		5				40		6		249				30	
	50																
19	100	12		13				90		12		27				85	
	50	12		4				85		12		87				85	
20	100	—		0				100		12		18				80	
	50	7		19				70		12		66				35	
21	100	14	11	2	29	11	25	75	60								
	50	8	11	81	24	27	13	40	45								
22	100	7	7	393	156	73	61	30	40								
	50	7	7	48	172	17	41	60	30								
23	100	6		421				37		8		142				65	
	50																
Acetone	1 μl	—		0		0		40		—	—	0	0	0	0	10	30
Untreated	—	—		0		0		35		—		0		0		20	
Untreated postdiapause	—		5	438		35		23		4		640		42		20	

[a] In columns headed MF, the hormone was applied to both sexes at the indicated dosage; in columns headed F, only the females were treated. [b] Cumulative total. [c] See text. [d] Cumulative for 14 days. Blank entries were not run or not determined; dashes indicate failure of insects to oviposit by 14 days.

mimetics (4, 8, 11, 14) that were quite toxic, as indicated by higher mortality than normal, failed to prevent diapause or had little effect at terminating diapause in the survivors. Other hormones (5, 9, 10) gave low but variable response.

In general, diapausing beetles responded more readily than prediapause beetles to treatment. When both male and female insects were treated, the O.I. was higher, despite the unnecessary treatment of the male (Connin and Hoopingarner, 1971). While an explanation for the former is not immediately obvious, the latter effect might be due to mechanical intromission of the mimetic from the body of the male to the female during mating.

Low response could be due to either transport failure of a given compound through the insect cuticle, as in the case of a polar compound such as 4, or to more rapid systemic inactivation. Alternatively, a poor response could be based on failure of the compound to "fit" a given enzyme receptor cite. For example, response drops as the chain length is shortened (cf. 2, 10, 15). Other compounds such as 3, 6, and 11 are of the farnesyl chain length but response is low or variable.

Mimetic 2 (farnesyl derived) and mimetic 12 (geranyl derived) gave nearly the same mortalities and O.I.'s upon treatment. Molecular models indicate that the distance between the epoxide oxygen and the region of maximum π electron density in both 2 and 12 is nearly the same for those conformations in which hydrogen–hydrogen steric interaction is mimimized (Figure 1).

Thus, 3, 9, and 20, containing only one oxygen at the "ester end," have decreased response, while 6 may be more active due to the contribution by the enol form of the conjugated ketone. Two oxygens, as in 5, are insufficient due to absence of π electrons. The saturated mimetic 16 is moderately active, but lack of the more restrictive carbon skeleton, as in 2, could account for the decrease in activity. The reverse ester of 2, namely 7, is nearly as active since the π electron density center in 7 changes position very little compared to 2. The allylic reverse ester 8 moves the π electron density position too far inward and, as a consequence, suffers reduced activity.

Varying the "epoxy end" of 2 (viz. 17, 21, and 23) has relatively little effect on the O.I. That mimetic 21 is still with moderate activity is of considerable interest since White (1972) has found this diol to be a metabolite of 2 in Schistocerca gregaria and Rhodnius prolixus.

The mechanisms of juvenile hormone activation and inactivation are, as yet, only dimly perceived. It is possible that certain mimetics may be active because they inhibit the system responsible for the metabolism of the insect's juvenile hormone. Alternately, the mimetic could, directly or indirectly, stimulate the corpora allate to produce JH. Such substances need not resemble JH in structure. Thus, in addition to the concept of a critical location for the "π-electron center," one must also consider such things as the correct "fit" to an enzyme receptor site. Mimetics such as 12 may have a critical steric requirement for a group of the size of the aromatic residue in that compound (cf. Jacobsen et al., 1972). In addition, the theory is limited to providing an explanation of activity in our particular bioassay. It is well known that the activity of JH mimetics varies considerably according to the order and morphological development of a given insect. We are presently engaged in testing the validity of these ideas as applied to the cereal leaf beetle.

CONCLUSIONS

We have shown that certain mimetics of juvenile hormone can be used to prevent or terminate diapause in the cereal leaf beetle. Considerable structural departure from the JH molecule can result in still active compounds, although none were more active than the juvenile hormone
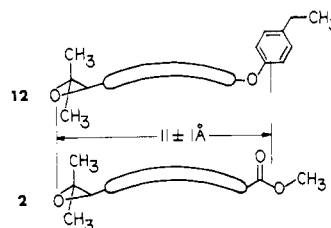

Figure 1. Schematic representation of a critical steric parameter in mimetics 2 and 12.

itself. The mimetics showed considerable variation in mortality to the insects. The dosage per insect used in this study does not seem to augur well for their use in field control. Nonetheless, a change in formulation or methodology such as systemic application (Babu and Slama, 1972) may result in significantly lower effective dosages. At present, they can provide the experimenter with a constant source of postdiapause insects in a relatively short time and introduce an alternate means of bioassay of JH mimetics. By structure–activity studies, we have explored some of the molecular constraints of such compounds in an adult insect. Further investigations of dynamic steric intramolecular relationships on activity will be reported subsequently.

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