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Inhibition of Epoxidation of Methyl Farnesoate to Juvenile Hormone III by Cockroach Corpus Allatum Homogenates[1]

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Radiolabeled methyl farnesoate is epoxidized to juvenile hormone III by an NADPH-dependent reaction occurring in corpus allatum homogenates from the cockroach *Blaberus giganteus* L. Most of the enzymatically produced juvenile hormone has the 10*R* configuration described for previously isolated natural juvenile hormones. The unnatural 2*Z* geometrical isomer of methyl farnesoate is epoxidized by the above system faster than the natural 2*E* isomer. Several series of chemicals known to be inhibitors of mixed-function oxidases were surveyed as inhibitors of methyl farnesoate epoxidation. The anti-juvenile hormone precocene II caused negligible inhibition at $1 \cdot 10^{-4}$ *M*, whereas the best inhibitor was *o*-bromophenoxymethylimidazole with an apparent I_{50} of $4 \cdot 10^{-7}$ *M*. None of the inhibitors tested were potent morphogenetic agents on *Tenebrio molitor* pupae, and they failed to cause precocious development of *Oncopeltus fasciatus* nymphs. The inhibition of *in vitro* juvenile hormone biosynthesis suggests the possibility of finding an anti-hormone which acts by blocking juvenile hormone biosynthesis.

INTRODUCTION

The insect nervous system has been long exploited as a site of action for pesticides. A second regulatory system, the endocrine system, is also critical for insect survival. The tremendous activity of juvenoids [juvenile hormone (JH)[2] or JH-mimics] on insects illustrates how disruption of the endocrine system may result in effective insect control. The recent reports on anti-juvenile hormones (1, 2) demonstrate the feasibility of obtaining effective pest control chemicals acting to disrupt rather than mimic endocrine function. One method of approaching the problem of developing anti-hormones is to attempt the *in vitro* inhibition of a critical step in the biosynthetic pathway leading to the hormone. The expoxidation of farnesoic acid, methyl farnesoate, or their homo or dihomo analogs has been implicated as a vital step in the biosynthesis of the JHs, probably occurring on the smooth endoplasmic reticulum of the corpora allata (3–7). In this study the effect of several anti-oxidants, mixed function oxidase inhibitors, and related compounds were examined on the NADPH-dependent conversion of tritiated methyl farnesoate to JH III (methyl 10,11-epoxyfarnesoate).

MATERIALS AND METHODS

Chemicals. Tritium-labeled methyl farnesoate (~8 Ci/mmol, ^{3}H, α position to 2,3-olefin, >98% 2*E*,6*E* or >99% 2*Z*,6*E*)

[1] The inhibition studies in this report were presented at the 172nd American Chemical Society National Meeting, San Francisco, California, August 31, 1976, as Paper 41 in the Pesticide Chemistry Division.

[2] Abbreviations used: JH(s), juvenile hormone(s); tlc, thin-layer chromatography; MTP, (+)-α-methoxy-α-trifluoromethylphenylacetate; lsc, liquid scintillation counting.

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TABLE 1

Structures and Apparent I_{50} Values of Compounds Tested as Inhibitors of Methyl Farnesoate Epoxidation by Corpora Allata Homogenates[a]

Disubstituted methylenedioxy phenyl compounds

Number	Structure (R^1)	(R^2)	Source[b/]	I_{50}
1	CHO	OCH_3	F	$>1\cdot10^{-4}$
2	CHO	NO_2	F	$>1\cdot10^{-4}$
3	CHO	SCH_3	F	$>1\cdot10^{-4}$
4	CHO	Br	F	$>1\cdot10^{-4}$
5	COOH	Br	F	$>1\cdot10^{-4}$
6	NO_2	Br	F	$1\cdot10^{-4}$
7	Br	Br	F	$1\cdot10^{-4}$
8	SC_6H_5	$NH_3^+Cl^-$	F	$1\cdot10^{-4}$
9	SC_6H_5	$NHCOCH_3$	F	$>1\cdot10^{-4}$
10	SC_6H_5	$NHCOCH_2CH_3$	F	$1\cdot10^{-4}$
11	SC_6H_5	$NHCOC_6H_5$	F	$>1\cdot10^{-4}$
12	p-$SC_6H_4CH_3$	NO_2	F	$>1\cdot10^{-4}$
13	p-SC_6H_4Cl	NO_2	F	$>1\cdot10^{-4}$
14	p-SC_6H_4Cl	$NH_3^+Cl^-$	F	$>1\cdot10^{-4}$
15	p-SC_6H_4Cl	$NHCOCH_3$	F	$>1\cdot10^{-4}$
16	o-$SC_6H_4NH_2$	NO_2	F	$>1\cdot10^{-4}$
17	o-SC_6H_4NHCHO	NO_2	F	$>1\cdot10^{-4}$
18	SOC_6H_5	NO_2	F	$>1\cdot10^{-4}$
19	SOC_6H_5	$NH_3^+Cl^-$	F	$1\cdot10^{-4}$
20	OC_6H_5	NO_2	F	$>1\cdot10^{-4}$
21	OC_6H_5	$NH_3^+Cl^-$	F	$5\cdot10^{-5}$
22	OC_6H_5	$NHCOCH_3$	F	$>1\cdot10^{-4}$
23	p-OC_6H_4Br	NO_2	F	$>1\cdot10^{-4}$
24	H	CH_2OH	F	$>1\cdot10^{-4}$
25	H	$CH_2CH{=}CH_2$	ALH	$>1\cdot10^{-4}$
26	H	$CH{=}CH{-}CH_3$	E	$>1\cdot10^{-4}$
27	H	geranyl	L	$1\cdot10^{-5}$
28	H	6,7-epoxy geranyl	L	$5\cdot10^{-5}$
29	H	dihomo-6,7-epoxy geranyl	S	$5\cdot10^{-5}$
30	H	$CH(OC_2H_4OC_2H_4OC_4H_9)_2$	W	$5\cdot10^{-5}$
31	C_3H_7	$CH_2(OCH_2CH_2)_2OC_4H_9$	W	$1\cdot10^{-5}$
32	3,4,5,6 pentachloro		F	$>1\cdot10^{-4}$
	Polycyclic MDP related			
33			F	$>1\cdot10^{-4}$
34			F	$>1\cdot10^{-4}$
35			F	$>1\cdot10^{-4}$
36	Cl, Cl		F	$>1\cdot10^{-4}$
37			F	$5\cdot10^{-5}$
38			F	$1\cdot10^{-4}$
39	N, Cl		F	$>1\cdot10^{-4}$
	Catechols			
40	t-Bu, OH, OH		F	$>1\cdot10^{-4}$
41	t-Bu, OH, OH, Br		F	$>1\cdot10^{-4}$
42	NO_2, OH, OH		F	$>1\cdot10^{-4}$
43	CH_3CH_2S, NO_2, OH, OH		F	$1\cdot10^{-4}$
44	NO_2, Br, OH, OH		F	$1\cdot10^{-4}$
45	OH, OH		ALH	$>1\cdot10^{-4}$
46	Cl, Cl, OH, OH		F	$>1\cdot10^{-4}$
47	Br, Br, OH, OH		F	$5\cdot10^{-5}$
48			F	$1\cdot10^{-4}$
	Other Compounds			
49	OCH_3, NO_2		ALH	$1\cdot10^{-4}$
50	NO_2, OCH_3, OH		F	$>1\cdot10^{-4}$
51	NO_2, OCH_3, OCH_3		F	$>1\cdot10^{-4}$
52	NO_2, OCH_3, $OCCH_3$		F	$>1\cdot10^{-4}$
53	Br, Br		F	$>1\cdot10^{-4}$
54	OCH_3, OCH_3		F	$1\cdot10^{-4}$
55	SCH_3		F	$1\cdot10^{-5}$
56	S, S		F	$1\cdot10^{-4}$
57			F	$>1\cdot10^{-4}$
58	CH_3O, CH_3O		B	$>1\cdot10^{-4}$
59	FMC 16824 O-(2-methylpropyl)-O-(2-propynyl)phenyl phosphonate)		FMC	$>1\cdot10^{-4}$
60	SKF 525A		SKF	$5\cdot10^{-5}$

Table 1 (Continued)

No.	Compound	Source	Concentration
	Herbicides		
61	CH_3-N$^+$ (bipyridinium) N$^+$-CH_3	ALH	$1 \cdot 10^{-4}$
62	$C_6H_5CH_2$-N$^+$ (bipyridinium) N$^+$-$CH_2C_6H_5$	SM	$5 \cdot 10^{-5}$
63	(bipyridinium, N$^+$–CH_2–CH_2–N$^+$ bridged)	F	$5 \cdot 10^{-5}$
	N-containing Heterocycles		
64	(phenazine, NH_2, NH_2)	F	$5 \cdot 10^{-5}$
65	(phenazine, OH, NH_2)	F	$5 \cdot 10^{-6}$
66	(N-H phenazinone, OH, O)	F	$>1 \cdot 10^{-4}$
67	(N-CH_3 phenazinium, $CH_3SO_4^-$)	ALH	2×10^{-5}
68	(imidazolyl)–C(=O)–(imidazolyl)	F	$5 \cdot 10^{-5}$
69	Br-(phenyl)-O-CH_2-(imidazolium, H Cl^-)	A	1.5×10^{-6}
70	(2-Br-phenyl)-O-CH_2-(imidazolium, H Cl^-)	A	$4 \cdot 10^{-7}$

[a] Incubations were carried out at 30°C for 2 hr with corpus allatum homogenates at 1 corpus allatum equivalent per 100 µl. The 2*E* methyl farnesoate concentration was initially $5 \cdot 10^{-7}$M with $5 \cdot 10^{-5}$M NADPH.

[b] Sources:

A: A. O. Geiszler, Abbott Laboratories, North Chicago, IL.; ALH: Aldrich Chemical Company; B: William S. Bowers, Cornell University, Geneva, NY.; E: Eastman Organic Chemicals; F: T. R. Fukuto, this Division; FMC: FMC Corporation, Middleport, NY.; L: This laboratory; S: SOCAR Ltd., Duebendorf, Switzerland; SKF: C. E. Berkoff, Smith Kline and French Laboratories, Philadelphia, PA.; SM: Schwarz/Mann Biochemicals; W: D. A. Wustner, this division.

was prepared and purified as described earlier (4) as were cold standards of farnesoic acid, methyl farnesoate, JH III, and JH III metabolites (4, 8, 9). Candidate inhibitors provided by other workers (Table 1) were analyzed by thin-layer chromatography (tlc) for purity before use while compounds prepared for this study were identified based on their proton magnetic resonance, infrared, and mass spectral characteristics.

Bioassays. Except for the compounds noted (Nos. 8, 14, 17, 19, 21, 35, 60, 65, 69, and 70), which were tested by applying 1 µl of a $1 \cdot 10^{-3}$ *M* solution, the compounds in Table 1 were assayed for morphogenetic activity by applying 1 µl of a $1 \cdot 10^{-2}$ *M* solution on the ventral surface of the three posterior abdominal segments of the yellow mealworm, *Tenebrio molitor* (10). Responses were scored as positive if the pupa molted to a pupa adult intermediate or a second pupa.

The acute toxicity and developmental effects of the compounds were examined on the large milkweed bug, *Oncopeltus fasciatus* (Dallas), by a slight modification of the methods of Bowers (1, 2, 11). The compound was added in acetone (4 ml) to Whatman No. 1 filter paper (9 cm) in a petri dish (100 × 15 mm) and the solvent was allowed to evaporate. Early second-instar milkweed bugs were added (15–20) and provided with water and sunflower seeds. Mortality was recorded at 48 hr and periodically during development to adulthood, and observations were made on development, specifically examining for precocious adults and extra instars. The reproductive capacity of the treated insects was determined from the number of first-instar larvae produced within 2 weeks of adulthood.

Microchemistry. The structure of the biochemically produced JH III was supported by microchemical tests described earlier (4, 8, 10). The configuration at C-10 of the epoxide was determined by a modification of the method of Judy *et al.* (12) (Fig. 1). Radiolabeled biosynthetic JH III was combined with cold authentic JH III (1 mg), purified by tlc, extracted, and converted to the corresponding diol by incubation for 1 week in 0.1 *M* sodium acetate buffer, pH 4.5. The buffer was saturated with sodium chloride, extracted with peroxide-free ether, dried (Na_2SO_4), and concentrated, and the residue was purified by liquid chromatography (5 µ Spherosorb, 3 mm × 50 cm), eluted with ether:hexane (1:1), and monitored by its uv absorbance at 254 nm. The product diol was derivatized with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTP chloride; from

2E,6E METHYLFARNESOATE
($1.5 \cdot 10^6$ CPM)

CA EPOXIDASE + NADPH

JH III
(41% EPOXIDE BY TLC, 62% EXTRACTED YIELD)

pH 4.0 BUFFER

JH III DIOL
(87% DIOL BY TLC, 50% EXTRACTED YIELD FROM TLC, 35% FROM HRLC)

MTP CHLORIDE

JH III DIOL MTP ESTER
(73% ESTER BY TLC, 65% EXTRACTED YIELD FROM TLC)

HRLC

<25% 10S DIOL MTP ESTER + >75% 10R DIOL MTP ESTER

FIG. 1. *Synthetic and purification steps (and yields) used for establishment of the absolute configuration of enzymatically produced JH III [after Judy et al. (12)].*

the corresponding acid obtained from Aldrich Chemical Co.) by established procedures (13). The resulting esters were purified on tlc in benzene: *n*-propanol (10:1) and extracted, and the diasteromers were separated by liquid chromatography (5μ Spherosorb, 3 mm × 1.5 m slurry packed in 25-cm segments and joined by drilled out Swageloc 0.25 to 0.25-in. unions) and eluted with ether:hexane (1:3).

Enzyme source. Adult female *Blaberus giganteus* L. reared at 27°C on an 18:6 photoperiod with Purina Laboratory Chow and water provided *ad libitum* were lightly chilled (5°C) before surgery. The *B. giganteus* were immersed in chilled sodium phosphate buffer (0.1 *M*, pH 7.4) and the corpora allata were removed through the dorsal neck membrane. Extraneous tissue was removed, and the corpora allata were washed and homogenized in the same buffer containing 1% bovine serum albumin to give a final concentration of 1 corpus allatum Eq/50 μl (9). The presence of bovine serum albumin in the incubation buffer led to more consistent epoxidation of methyl farnesoate. Earlier it was reported that it was possible to inhibit JH esterases by washing corpora allata with $1 \cdot 10^{-4}$ *M* *O*-methyl-*S*-ethylphosphoramidothioate (9). With one exception noted in this study, careful dissection and washing of corpora allata eliminated the need for organophosphorus treatment since no JH esterase activity was detected.

Enzyme incubations. Enzyme incubations were shaken in Carbowax 50M-treated to 6 × 50-mm glass tubes (8) (to block binding of methyl farnesoate to glass) at 30°C for 2 hr unless otherwise noted. The enzyme reaction was initiated by the addition of 50 μl of enzyme (with or without $5 \cdot 10^{-5}$ *M* NADPH) to 50 μl of buffer in the Carbowax-treated tube containing labeled methyl farnesoate to give a $5 \cdot 10^{-7}$ *M* final concentration, and various inhibitors were added in ethanol or water (1 μl). Up to 3% final volume of ethanol causes negligible change in enzyme activity. The reaction was stopped by the addition of ethyl acetate and sodium chloride. The tubes were vortexed and centrifuged, and the organic extract cospotted with the appropriate standards and was analyzed on tlc developed in hexane:ethyl acetate (3:2). The plates were analyzed by tlc radioscanning and liquid scintillation counting (lsc). Time courses were run using both 2*Z* and 2*E* methyl farnesoate as substrate, utilizing the same pooled enzyme for both substrates, whereas inhibitor studies utilized only 2*E* methyl farnesoate. The results were corrected for nonenzymatic epoxidation (<1%) and NADPH-independent epoxidation (<1%), and the inhibitors were evaluated by comparing apparent I_{50} values found by plotting

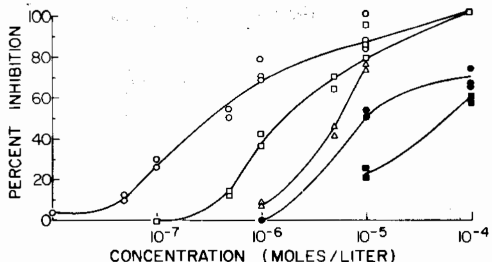

FIG. 2. *Percentage inhibition of methyl farnesoate epoxidation by selected compounds. Compound Nos. 70 (○), 69 (□), 65 (△), 27 (●), and 64 (■).*

the inhibition of methyl farnesoate epoxidation against the logarithm of the inhibitor concentration (Fig. 2). All inhibitors were assayed on at least three separate enzyme preparations with inhibition values obtained both above and below the I_{50} values for all compounds with I_{50} values below $1 \cdot 10^{-4}$ M.

RESULTS AND DISCUSSION

Bioassays

The geranyl phenyl ether juvenoids (Nos. 27–29) gave ED_{50} values on *T. molitor* of 3600, 586, and 470 pmol/pupa as reported earlier (10, 14). No other compound gave an ED_{50} at the highest dose tested, but several compounds did cause some morphogenetic defects when 1 μl of the concentration indicated was applied (Nos. 13: $1 \cdot 10^{-2}$; 25: $1 \cdot 10^{-2}$; 26: $1 \cdot 10^{-2}$; 31: $1 \cdot 10^{-2}$; 58: $1 \cdot 10^{-2}$; and 69: $1 \cdot 10^{-3}$ M).

Five compounds (Nos. 19, 30, 40, 41, and 50) gave 48-hr ED_{50} values to *O. fasciatus* second-instar nymphs of about 1 μmol/cm², whereas several others (Nos. 7, 33, 34, 49, 53, and 68) gave complete kill. A tenfold reduction in concentration led to negligible 48-hr mortality. When tested at doses of 1 μmol/cm² or lower, none of the compounds caused precocious development except precocene II (No. 58), which caused 50% precocious development at 12 nmol/cm² (1, 2, 11). The juvenoids (Nos. 27–29) blocked the final molt to the adult form. SKF 525A (No. 60) and the imidazole compound (No. 70) were also tested by adding them to the drinking water at 2.5×10^{-4} to 2.5×10^{-6} *M*. There was no 48-hr mortality, but both compound Nos. 60 and 70 slowed development at the highest doses, with No. 70 leading to 100% mortality before the imaginal molt and the 2.5×10^{-5} *M* solutions leading to no detectable effects. The free bases or dodecyl sulfate common ions of compound Nos. 69 and 70 caused no effects when tested at >1000 nmol/cm².

Microchemistry

As described earlier (4), microchemical tests indicate that the radioactivity cochromatographing with authentic JH III on tlc is JH III. Acid treatment results in the radioactivity cochromatographing with JH diol, and, after further treatment with lead tetraacetate, acetic anhydride–pyridine, or *n*-butylboronic acid, the radioactivity cochromatographs with the corresponding aldehyde, ester, or diester, respectively.

The 2,3-olefinic JH acid isomers can be easily separated by tlc, while tlc separation of the JH 2,3-olefinic isomers has not been accomplished in this laboratory; thus enzymes were used to help establish the geometry of biosynthetic products. *B. giganteus* and *Trichoplusia ni* JH esterases are similar to those described from *Manduca sexta* in that they appear specific for 2*E* conjugated methyl esters (9, 15). Exposure of JH III formed enzymatically from 2*E* methyl farnesoate to either esterase results in the conversion of the radioactivity to a product cochromatographing with 2*E* JH acid, whereas no acidic product is formed from incubation of JH III enzymatically formed from 2*Z* methyl farnesoate with the above esterases. These results support the retention of the 2,3 geometry of both the 2*Z* and the 2*E* isomers during epoxidation to JH III.

When the procedure of Judy *et al.* (12) was used to establish the absolute configuration of JH III at C-10 (Fig. 1), only about 10% of the total radioactivity from JH III was found to cochromatograph

ultimately with the slower eluting 10*R* MTP diasteromer. However, the yields at each step in conversion of radiolabeled biosynthetic JH III to its diasteromeric derivative were reasonable for the small amount of compound used and were roughly equivalent to the loss of 220-nm absorbance of cold carrier JH III. Of the diasteromeric derivative injected into the final liquid chromatography step, at least 75% of the radioactivity cochromatographed with the faster eluting isomer in a system which resulted in approximately 20% overlap of the diasteromers monitored by absorbance at 254 nm. This study indicates that the *in vitro* epoxidase system produced JH III of the same absolute stereochemistry as has been described from JH isolated from cultured corpora allata and whole insects. This finding supports the validity of the corpora allata homogenates as a model for *in vivo* JH biosynthesis.

Although higher rates of epoxidation can be obtained by using larger amounts of methyl farnesoate, the $5 \cdot 10^{-7}$ *M* concentration was chosen because it is well below the critical micelle concentration of methyl farnesoate ($\sim 10^{-5}$ *M* determined from surface tension measurements), it minimizes the number of corpora allata which must be dissected, and it gives a useful amount of expoxidation for inhibitor studies (Fig. 3). Epoxidation of methyl farnesoate at $5 \cdot 10^{-7}$ *M* seems to be linear (correlation: 0.977) from 1 to 3 hr, so 2-hr incubation periods were utilized (Fig. 3). Inhibition kinetics are difficult when studies are not run near substrate saturation, so the apparent I_{50} values presented should only be taken as a relative indication of inhibitor potency rather than being transformed into kinetic expressions. As reported earlier (4, 9) homogenates of cleanly dissected corpora allata apparently contain no esterases or epoxide hydrases acting on JH III.

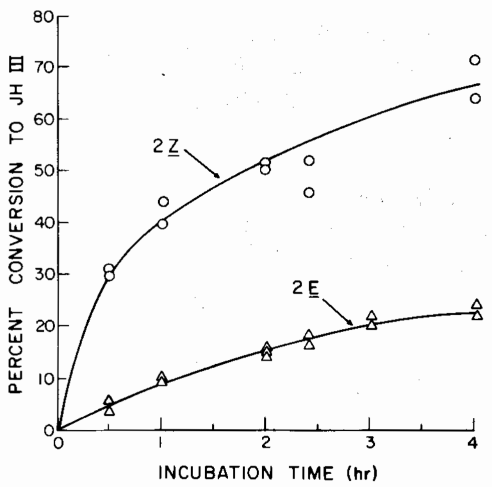

FIG. 3. *Percentage conversion of 2Z (○) and 2E (△) methyl farnesoate ($5 \cdot 10^{-7}$ M) to labeled products cochromatographing with JH III by cockroach corporus allatum homogenates fortified with NADPH at six incubation times.*

Surprisingly, 2*Z* methyl farnesoate, which is not thought to be a natural substrate, is epoxidized faster than 2*E* methyl farnesoate (Fig. 3). One hypothesis was that 2*E* specific esterases were degrading 2*E* JH much faster than 2*Z*; however, when esterase inhibitors (9) were added and/or the epoxy acid and other regions of the tlc plate were examined, the 2*Z* isomer was still found to be epoxidized more quickly. Although 2*Z* JHs have never been reported and synthetic 2*Z* analogs have reduced biological activity compared to 2*E* analogs, 2*E*/*Z* mixtures of farnesol are commonly isolated from plants (16). Possibly the 2*Z* form of farnesol is biosynthesized and eliminated at the stage of the methyl transferase which seems quite specific (3, 6) or is isomerized to the 2*E* isomer as has been observed for a juvenoid (17, 18). As has been suggested by work on *Manduca sexta*, farnesoic acid may be the preferred substrate for epoxidation (3, 6) and the 2*Z* form simply has a geometry which keeps the ester from interfering with the enzyme active site.

Structure–Activity Relationships

Among the substituted methylene dioxyphenyl compounds (Nos. 1–32, Table 1),

most showed very low inhibition of methyl farnesoate epoxidation. Compound No. 21 demonstrated moderate activity with an I_{50} of about $5 \cdot 10^{-5}$ *M*. Piperonyl butoxide and Tropital (Nos. 30 and 31) showed moderate activity, and methylene dioxyphenyl juvenoids (Nos. 27–29) are moderately active as inhibitors as reported earlier (19). The activity of the epoxy derivatives is comparable to that of piperonyl butoxide, and the terpenoid substituent may largely contribute hydrophobicity rather than mimicking JH at the enzyme active site. It is likely that the more active 6,7-unsubstituted geranyl juvenoid (No. 27) is acting as an alternate substrate for epoxidation, whereas the mono- and disubstituted olefins of safrole and isosafrole (Nos. 25 and 26) are apparently poor alternate substrates.

The activity of the three juvenoids (Nos. 27–29) and piperonyl butoxide (No. 30) as inhibitors of the epoxidation of methyl farnesoate illustrate a problem with *in vivo* bioassays. Compounds having some similarity to JH may be good inhibitors of JH biosynthesis, but they may also have enough intrinsic JH activity to overshadow *in vivo* anti-hormone activity. The effects of piperonyl butoxide on *Manduca sexta* larvae may illustrate this point since moderate doses of piperonyl butoxide demonstrate possible anti-JH effects whereas high doses give JH-like effects (20).

Methylenedioxy-substituted anthracene (No. 37) is a more active inhibitor of methyl farnesoate epoxidation than aldrin epoxidation (21), and the anthracene derivative may mimic the hydrophobic backbone of methyl farnesoate. As noted earlier (21), some catechols also show inhibitory activity (Nos. 43, 44, 47, and 48). A commonly used substrate for microsomal *O*-demethylation, *p*-nitroanisole (No. 49), inhibited methyl farnesoate epoxidation only at high levels. Three substituted naphthoquinones (Nos. 54–56) gave poor to moderate inhibition.

A compound interesting for its lack of activity is the anti-juvenile hormone, precocene II (No. 58). Precocene shows negligible inhibition of epoxidation at $1 \cdot 10^{-4}$ *M*, indicating that its anti-hormone activity in cockroaches cannot be accounted for by direct inhibition of the epoxidation step of JH biosynthesis. This observation supports data presented by Bowers (1, 2, 22) that the precocenes act at the level of corpus allatum regulation.

The phosphonate, FMC 16824 (No. 59), was totally inactive at $1 \cdot 10^{-4}$ *M*, whereas SKF 525A (No. 60) showed moderate inhibition as reported earlier (4). The bipiridinium herbicides (Nos. 61–63) were found to be moderate inhibitors as would be expected from the earliest observation that another electron transfer agent, methylene blue, was quite a good inhibitor of methyl farnesoate epoxidation (4). Several nitrogen-containing heterocyclic compounds were moderate to good inhibitors (Nos. 64, 65, 67, and 68). Many of the above compounds are active as mosquito larvicides, and the effects of some of these mixed-function oxidase inhibitors and antioxidants on mosquito larvae suggest endocrine involvement, although a myriad of sites of action are possible (23). None of the above compounds appear active enough *in vitro* to expect anti-hormonal activity *in vivo* based on the inhibition of methyl farnesoate conversion into JH by cockroach corpora allata.

The most active compounds examined were imidazole derivatives (Nos. 69 and 70), giving I_{50} values at 1.5×10^{-6} and $4 \cdot 10^{-7}$ *M*, respectively. Imidazole derivatives have been examined as inhibitors of histidine decarboxylase (24) and are described as potent inhibitors of NADPH-dependent cyclodiene epoxidation and hydroxylation in rat liver microsomes, while causing only slight inhibition of *N*-demethylation (25). The relative and absolute activities of the *ortho*- and *para*-bromophenoxyimidazoles were similar for aldrin epoxidation in mammalian microsomes and methyl farnesoate epoxidation by corpora allata homogenates.

Imidazole derivatives have been suggested as potential insecticide synergists (25, 26) and may offer potential as models for the development of anti-hormones.

CONCLUSIONS

These studies illustrate the possibility of designing insect control agents which act by disrupting juvenile hormone biosynthesis. Continued elucidation and *in vitro* inhibition studies of hormone biosynthetic pathways will provide leads in a search for anti-hormones. The activity of several of the compounds evaluated *in vitro* as inhibitors of JH biosynthesis may warrant a reevaluation of anti-oxidants and mixed-function oxidase inhibitors in *in vivo* bioassays designed to detect anti-hormone effects.

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