Synthesis and Bioassay of Carbamate Inhibitors of the Juvenile Hormone Hydrolyzing Esterases from the Housefly, *Musca domestica*

Susanne M. Mumby, Bruce D. Hammock,* Thomas C. Sparks, and Kenji Ota

Twenty N-alkyl carbamate derivatives of geranyl phenyl ethers were synthesized and tested for juvenile hormone and anti-juvenile hormone activity against *Oncopeltus fasciatus*, *Lygus lineolaris*, *Trichoplusia ni*, *Tenebrio molitor*, *Culex pipens quinquefasciatus*, and *Musca domestica*. Low juvenile hormone-like activity was found on only the Diptera. The compounds were also assayed as inhibitors of the esterases which hydrolyze juvenile hormone in *Blaberus giganteus*, *T. ni*, and *M. domestica*. Negligible inhibition of *B. giganteus* and *T. ni* esterases was found, but the meta-substituted carbamates proved to be very potent inhibitors of *M. domestica* juvenile hormone esterases, as were some N-alkyl carbamates of 1-naphthol.

In the search for new insect control agents, the endocrine system holds great promise as a target site. At least the epithelial endocrine system appears to have evolved late enough that there are large differences in the endocrine biochemistry of insects and mammals. Juvenoids (mimics of insect juvenile hormones) illustrate the exploitation of these differences in the development of safe, highly effective insect control agents. Limiting discussion only to juvenile hormone (JH) physiology, there exist many potential sites for selective disruption of endocrine function. Juvenoids probably act by mimicking the insects' own hormone at inappropriate stages in development or by differential action on tissues. Pathways leading to the biosynthesis of JH such as terpene synthesis (Hammock et al., 1978), epoxidation (Hammock and Mumby, 1978), or methylation (Pratt and Bowers, 1977) as well as degradative pathways such as epoxide hydration, ester cleavage (Pratt, 1975; Ajami, 1975; Hammock et al., 1977a; Sparks and Hammock, unpublished information), and possibly oxidation and conjugation may be selectively inhibited. Agents which compete with insect hormones for carrier or receptor sites may be effective control agents as well as compounds which affect the regulation of any of the above systems (Bowers, 1976; Slâma, 1978).

Several series of juvenoid-like carbamates have been previously reported to have biological activity, although none of these series were apparently designed as carbamylating agents (Schwarz et al., 1970; Sonnet et al., 1971; Jacobson et al., 1972, Sonnet et al., 1972; Slâma et al., 1974; Pallos et al., 1976), while long chain alkyl carbamates of 1-naphthol were previously shown to inhibit the pyrethroid hydrolyzing esterases in *O. fasciatus* (Jao and Casida, 1974). Geranyl and farnesyl phosphonates were found to have marginal in vivo activity on houseflies and mosquitoes (Berteau et al., unpublished information).

It was thought possible that JH-like compounds with a reactive center might act by covalently binding to a JH carrier, receptor, or esterase. In order to test this hypothesis, this laboratory synthesized some new terpenoid carbamates. These compounds were assayed in vivo for acute toxicity, JH, and anti-JH effects in several insects as well as for their activity as inhibitors of JH esterase activity in vitro. The compounds synthesized demonstrated little activity in in vivo bioassays, but several of them were potent inhibitors of juvenile hormone esterase activity from the housefly.

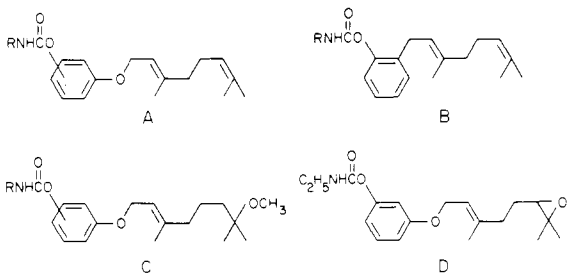
MATERIALS AND METHODS

Synthesis. The geranyl phenyl ethers (Table I) were prepared by a Williamson ether synthesis, reacting the corresponding monoacetate of catechol, resorcinol, or hydroquinone with geranyl bromide (Table I, 1–9) or

Division of Toxicology and Physiology, Department of Entomology, University of California, Riverside, California 92521.

Table I. Inhibition of JH Esterases from the Housefly by Carbamates

carbamate structure



compd no.	structure	substitution	R	I_{50}, M[a] microsomal fraction	I_{50}, M[a] soluble fraction
1[b]	A	para	CH_3	$>10^{-4}$	$>10^{-4}$
2	A	para	C_2H_5	$>10^{-4}$	1×10^{-4}
3	A	para	i-C_3H_7	$>10^{-4}$	1×10^{-4}
4	A	meta	CH_3	$\sim 10^{-4}$	4×10^{-5}
5	A	meta	C_2H_5	$\sim 10^{-4}$	1×10^{-5}
6	A	meta	i-C_3H_7	$\sim 10^{-4}$	5×10^{-5}
7	A	ortho	CH_3	$\sim 10^{-4}$	1×10^{-5}
8	A	ortho	C_2H_5	$\sim 10^{-4}$	5×10^{-5}
9	A	ortho	i-C_3H_7	$\sim 10^{-4}$	$>10^{-4}$
10	B	ortho	CH_3	$\sim 10^{-4}$	2×10^{-5}
11	B	ortho	C_2H_5	$\sim 10^{-4}$	1×10^{-5}
12	B	ortho	i-C_3H_7	$>10^{-4}$	$>10^{-4}$
13	C	meta	CH_3	1×10^{-6}	2×10^{-6}
14[c]	C	meta	C_2H_5	3×10^{-7}	4×10^{-9}
15	C	meta	i-C_3H_7	$\sim 10^{-4}$	1×10^{-5}
16	C	meta	n-C_3H_7	5×10^{-5}	1×10^{-5}
17	C	meta	n-C_4H_9	1×10^{-5}	2×10^{-7}
18	C	para	CH_3	4×10^{-5}	6×10^{-6}
19	C	para	C_2H_5	1×10^{-5}	1×10^{-6}
20	D	meta	C_2H_5	2×10^{-6}	1×10^{-6}
21	1-naphthyl		CH_3	5×10^{-6}	$\sim 10^{-4}$
22	1-naphthyl		C_2H_5	3×10^{-7}	$>10^{-4}$
23	1-naphthyl		i-C_3H_7	1×10^{-5}	$\sim 10^{-4}$
24	1-naphthyl		n-C_3H_7	3×10^{-7}	2×10^{-7}
25	1-naphthyl		n-C_4H_9	2×10^{-6}	1×10^{-6}

[a] Incubated 30 min (linear up to 40 min) with substrate (JH III) after 10-min preincubation with inhibitor. Enzyme concentration = five fly equivalents/mL of sodium phosphate buffer (I = 0.2, pH 7.4 with phenyl thiourea 0.01% w/v), substrate concentration 5×10^{-6} M, compounds assayed on two–four separate enzyme preparations and each concentration run in triplicate on each preparation. [b] Anal. Calcd for $C_{18}H_{23}NO_3$: C, 71.26; H, 8.31. Found C, 70.28; H, 7.96; mp 42 °C. [c] Anal. Calcd for $C_{20}H_{31}NO_4$: C, 68.74; H, 8.94. Found, C, 68.66; H, 8.70.

0021-8561/79/1427-0763$01.00/0

7-methoxygeranyl bromide (**13–19**) (Hammock et al., 1974; Henrick et al., 1978; Mumby and Hammock, 1978). Reaction of geranyl bromide with phenol in anhydrous benzene led to the C-alkylated product ortho to the phenol (**10–12**) (Daves et al., 1967). The carbamates were then prepared from the terpenoid phenols and from 1-naphthol by reaction with the appropriate isocyanate under basic conditions (Skraba and Young, 1959). Equimolar *m*-chloroperoxybenzoic acid selectively oxidized the 6,7-olefin of the carbamate (**5**) to its epoxide (**20**). Compounds were purified by Florisil (60–100 mesh) column chromatography with an ether–hexane step gradient, by preparative thin-layer chromatography, and/or by crystallization. Compounds were judged pure by analytical thin-layer chromatography (250 μm silica gel F-254 plates, EM Laboratories) in several solvents including benzene/propanol (10:1) and detected by their quenching of gel fluorescence and reaction with various spray reagents (Hammock et al., 1974). Proton magnetic resonance (^{1}H NMR) spectra were obtained on either a Varian T-60 or EM-390 in dilute deuteriochloroform/tetramethylsilane solutions; infrared spectra (IR) were run as thin films on silver chloride plates or as potassium bromide pellets on a Beckman Model 4240; mass spectra (MS) were run by direct insertion into a Finnigan Model 1015 mass spectrometer; and elemental analyses were performed by C. F. Geiger (Ontario, CA).

Bioassays. Acute toxicity (48 h) and developmental effects of the carbamates listed in Table I on the large milkweed bug (*Oncopeltus fasciatus*) and the lygus bug (*Lygus lineolaris*) were determined by rearing second instar nymphs to adulthood in a petri plate on Whatman No. 1 filter paper treated with an acetone solution of the test compound as described earlier (Bowers, 1976; Hammock et al., 1978). Third and fifth instar larvae of the cabbage looper (*Trichoplusia ni*) were treated topically with the test compound and examined for physical and behavioral changes until they reached adulthood on their standard diet (Shorey and Hale, 1965); and morphogenic activity was determined on 0–24-h-old pupae of the yellow mealworm (*Tenebrio molitor*) (Hammock et al., 1974). Toxicity to third instar mosquitoes (*Culex pipiens quinquefasciatus*) was found by adding the test compound in acetone to the medium and monitoring adult emergence. Ten white larvae (larvae which had more than a third but not all of their gut cleared) of the susceptible (NAIDM) strain of housefly (*Musca domestica*) were collected at 2:00 p.m. (8 h after lights on) and placed on filter paper in a petri plate (9 cm diameter × 15 mm). A second water-filled plate (3 cm diameter × 10 mm) was added to maintain high humidity. The larvae were treated topically with the candidate compound in acetone (0.5 μL) and held at 24 °C, 18 h light, 6 h dark. After 24 h, larvae which died or failed to pupate were removed, and after 10 days the number of adults to successfully emerge were scored as normal. This procedure results in minimal control mortality.

Esterase Assays. The source, preparation, and assay of JH esterases has been described in more detail elsewhere (Hammock et al., 1977a; Sparks et al., 1978; Sparks and Hammock, unpublished information). *B. giganteus* esterases were from the hemolymph of last instar nymphs, *T. ni* esterases from the first peak of hemolymph JH esterase activity occurring before the wandering stage from day 2 last instar larvae, and *M. domestica* esterases from both the 100000*g* soluble fraction and 100000*g* pellet or "microsomal" fraction of white larvae. The inhibitor was added to the enzyme (100 μL) in ethanol (1 μL), preincubated (10 min), and the substrate (^{3}H *O*-methyl-*E,E*-JH III) added in ethanol to give a concentration of 5×10^{-6} M (Hammock et al., 1977a). After incubation at 30 °C, the reaction was quenched with an acetic acid–charcoal suspension (600 mg of Norit A, 800 mg of Dextran T-40, 0.5 mL of glacial acetic acid, and distilled water to a final volume of 10 mL) and centrifuged to remove the JH bound to the charcoal, and the radiolabeled methanol remaining in solution was quantitated by liquid scintillation counting. Although reactions other than ester hydrolysis may occur, studies with chain-labeled JH indicated that ester hydrolysis was the major reaction and methanol production following ester hydrolysis of JH or JH diol is the only reaction that can be observed using the above techniques. The respective enzymes were diluted before use so that conditions of substrate saturation were approached and the uninhibited reactions showed a linear release of methanol throughout the time used for analysis. Semilog plots of inhibition vs. inhibitor concentrations provided the lines from which the reported I_{50}'s (inhibitor concentration at which the rate of methanol release is half that of control) were taken.

RESULTS

Biological Activity. Low mortality (48 h) on *O. fasciatus* was found with the isopropyl carbamates in the ortho and meta positions (LD_{50}'s 250–300 μg/cm^2) and a similar level of activity was found only for the methyl carbamates in the meta position on *L. lineolaris*. The other compounds demonstrated LD_{50}'s of >400 μg/cm^2 and no disruption of feeding or development was noted. No JH or anti-JH activity was found on *T. ni*, although occasional patches of precocious tanned cuticle were noted in fourth instar larvae. No morphogenic activity was detected on *T. molitor* pupae at doses of at least 30 μg/pupa. Juvenile hormone-like activity was observed when these compounds were applied to *C. pipiens quinquefasciatus*, but the LC_{50}'s of over 10 ppm indicated marginal biological activity, except for **14** which demonstrated an LC_{50} of 0.1 ppm. The LC_{50}'s for the naphthyl carbamates (**21–25**) are 0.1, 0.5, 0.5, 1.0, and 0.05 ppm, respectively. The LD_{50}'s of compounds **15** and **20** are approximately 25 μg/*M. domestica* larva. Pupal–adult intermediates (Srivastava and Gilbert, 1968) were found in dissected puparia of larvae treated with these carbamates. The other carbamates had LD_{50}'s higher than 30 μg/larva.

Esterase Assays. Only the meta-substituted carbamates showed weak ($I_{50} \simeq 1 \times 10^{-5}$ M) inhibition of hemolymph JH esterase activity in *B. giganteus*. The other compounds resulted in 50% inhibition at $\sim 1 \times 10^{-4}$ M. In contrast, none of the compounds in Table I gave detectable inhibition of *T. ni* JH esterase at 1×10^{-4} M. Some of the carbamates were potent inhibitors of both soluble and microsomal esterases from *M. domestica* larvae, but the patterns of inhibition were, in some cases, different. The geranyl dienes demonstrated poor inhibitory activity with both fractions (Table I, **1–12**). With the *O*-alkyl compounds the meta-substituted *N*-methyl and *N*-ethyl carbamates (**13–17**) were clearly better than the corresponding para-substituted carbamates (**18**, **19**). Variation of the *N*-alkyl group indicated that the *N*-ethyl carbamate (**14**) was the best inhibitor of both fractions although the soluble fraction esterase activity was inhibited more strongly than the microsomal esterase fraction. Replacement of the C-7 methoxide with a 6,7-epoxide (**20**) resulted in a decrease in inhibitory activity.

Some of the 1-naphthyl carbamates (**21–25**) were active inhibitors of both esterase fractions from *M. domestica*.

The isopropyl carbamate (**23**) was a poor inhibitor of either esterase fraction, but good inhibition of the microsomal fraction was obtained with the *N*-ethyl and *N*-*n*-propyl carbamates (**22**, **24**) and activity decreased slightly with the *n*-butyl substituent (**25**). In contrast, the *N*-ethyl carbamate demonstrated poor inhibitory activity on the soluble fraction, but the *N*-*n*-propyl and *N*-*n*-butyl carbamates appeared to be of equal activity.

DISCUSSION

Although the poor in vivo biological activity of these compounds clearly indicates their lack of promise for insect control, the concept that reactive hormone-like molecules may covalently bind with and/or block a biopolymer important in hormone action is enticing. Previously synthesized terpenoid phosphonates (Berteau et al., unpublished results) and geranyl phenyl sulfonyl fluorides (Li and Hammock, unpublished results) also showed marginal biological activity.

In the Lepidoptera examined, hemolymph JH esterases are very important in the inactivation of JH (Slade and Zibitt, 1972; Hammock and Quistad, 1976). However, JH esterases apparently exist in such excess, or the insects' development is so plastic, that almost total inhibition of the first JH esterase peak during the last larval instar is required to even delay pupation, much less to cause morphological changes (Sparks and Hammock, unpublished information). In light of the above knowledge and the poor in vitro inhibition of *T. ni* JH esterases, the lack of in vivo biological activity of the carbamates is not surprising.

It should be noted that the carbamates were assayed at a time during the development of *C. pipiens* when either the compounds' ability to inhibit JH metabolism or its intrinsic JH-like activity will interfere with pupation. However, the white larvae of the housefly are already committed to pupation, and JH titers should be low. Therefore, the results of bioassays on *M. domestica* are probably indicative of the carbamates' JH-like activity in disrupting adult emergence. Although the inactivation of JH by epoxide hydrases is probably more important in the Diptera than ester cleavage (Slade and Zibbit, 1972; Hammock and Quistad, 1976; Hammock et al., 1977b; Wilson and Gilbert, 1978), the most active in vitro JH esterase inhibitor (**14**) was also the most active mosquito larvicide. This correlation is consistent with the hypothesis that the larvicidal activity of compound **14** is due, in part, to the inhibition of the JH esterases.

Previous surveys of inhibitors of JH esterases (Hammock et al., 1977a; Sparks and Hammock, unpublished data) have demonstrated that JH esterases from *B. giganteus* and *T. ni* are resistant to inhibition by diisopropyl phosphorofluoridate and the carbamates assayed, but that they are inhibited by phosphoroamidothiolates. Not only are the JH esterases from these insects quite unlike any other carboxylesterases described, they are quite different in their inhibition pattern from *M. domestica* JH esterases. The inhibition of *M. domestica* JH esterases by the compounds described in this paper further serves to illustrate that dipteran esterases which hydrolyze JH have some properties which are different from the JH esterases of other insects examined, as well as housefly head acetylcholinesterase (Yu et al., 1972). Although tremendous selectivity was observed among the terpenoid phenyl carbamates for the inhibition of housefly JH esterases, the naphthyl carbamates were also potent inhibitors. These carbamates may prove to be useful tools for insect biochemists wishing to selectively inhibit dipteran JH esterases.

LITERATURE CITED

Ajami, A. M., *J. Insect Physiol.* **21**, 1017 (1975).

Berteau, P., Fukuto, T. R., Slade, M., Wilkinson, C. F. (Departments of Entomology, University of California, Riverside, CA, and Cornell University, Ithaca, NY), unpublished data, 1973.

Bowers, W. S., in "The Juvenile Hormones," Gilbert, L. I., Ed., Plenum Press, New York, NY, 1976, pp 394–408.

Daves, G. D., Jr., Moore, H. W., Schwab, D. E., Olsen, R. K., Wilczynski, J. J., Folkers, K., *J. Org. Chem.* **32**, 1414 (1967).

Hammock, B. D., Quistad, G. B., in "The Juvenile Hormones," Gilbert, L. I., Ed., Plenum Press, New York, NY, 1976, pp 374–393.

Hammock, B. D., Mumby, S. M., *Pestic. Biochem. Physiol.* **9**, 39 (1978).

Hammock, B. D., Gill, S. S., Casida, J. E., *J. Agric. Food Chem.* **22**, 379 (1974).

Hammock, B. D., Sparks, T. C., Mumby, S. M., *Pestic. Biochem. Physiol.* **7**, 517 (1977a).

Hammock, B. D., Mumby, S. M., Lee, P. W., *Pestic. Biochem. Physiol.* **7**, 261 (1977b).

Hammock, B. D., Kuwano, E., Ketterman, A., Scheffrahn, R. H., Thompson, S. N., Sallume, D., *J. Agric. Food Chem.* **26**, 166 (1978).

Henrick, C. A., Anderson, R. J., Staal, G. B., Ludvik, G. F., *J. Agric. Food Chem.* **26**, 542 (1978).

Jacobson, M., Beroza, M., Bull, D. L., Bullock, H. R., Chamberlain, W. F., McGovern, T. P., Redfern, R. E., Sarmiento, R., Schwarz, M., Sonnet, P. E., Wakabayashi, N., Waters, R. M., Wright, J. E., in "Insect Juvenile Hormones: Chemistry and Action," Menn, J. J., Beroza, M., Ed., Academic Press, New York, NY, 1972, pp 249–302.

Jao, L. T., Casida, J. E., *Pestic. Biochem. Physiol.* **4**, 456 (1974).

Li, M., Hammock, B., unpublished data, 1975.

Mumby, S. M., Hammock, B. D., *J. Agric. Food Chem.*, submitted for publication (1978).

Pallos, F. M., Letchworth, P. E., Menn, J. J., *J. Agric. Food Chem.* **24**, 218 (1976).

Pratt, G. E., *Insect Biochem.* **5**, 595 (1975).

Pratt, G. E., Bowers, W. S., *Nature (London)* **265**, 548 (1977).

Schwarz, M., Wakabayashi, N., Sonnet, P. E., Redfern, R. E., *J. Econ. Entomol.* **63**, 1858 (1970).

Shorey, H. H., Hale, R. L., *J. Econ. Entomol.* **58**, 522 (1965).

Skraba, W. J., Young, F. G., *J. Agric. Food Chem.* **7**, 612 (1959).

Slade, M., Zibitt, C. H., in "Insect Juvenile Hormones: Chemistry and Action," Menn, J. J., Beroza, M., Ed., Academic Press, New York, NY, 1972, pp 155–176.

Slâma, K., *Acta Ent. Bohemoslov.* **75**, 65 (1978).

Slâma, K., Romanuk, M., Sorm, F., "Insect Hormones and Bioanalogues," Springer-Verlag, New York, NY, 1974, pp 394–457.

Sonnet, P. E., Redfern, R. E., Schwarz, M., Wakabayashi, N., Waters, R. M., *J. Econ. Entomol.* **64**, 1378 (1971).

Sonnet, P. E., Waters, R. M., Redfern, R. E., Schwarz, M., Wakabayashi, N., *J. Agric. Food Chem.* **20**, 65 (1972).

Sparks, T. C., Hammock, B. D., *Pestic. Biochem. Physiol.*, unpublished information, 1978.

Sparks, T. C., Willis, W. S., Shorey, H. H., Hammock, B. D., *J. Insect Physiol.*, **25**, 125 (1979).

Srivastava, U. S., Gilbert, L. I., *Science* **161**, 61 (1968).

Wilson, T. G., Gilbert, L. I., *Comp. Biochem. Physiol.* **60A**, 85 (1978).

Yu, C.-C., Kearns, C. W., Metcalf, R. L., *J. Agric. Food Chem.* **20**, 537 (1972).

Received for review November 20, 1978. Accepted February 21, 1979. This work was supported by Grant No. R501260-01 from the National Institutes of Health.