Structural Requirements for Activity of Juvenile Hormone Mimetic Compounds against Various Insects

Tetsuyoshi Hayashi,† Hajime Iwamura,*,† Toshio Fujita,† Nobuo Takakusa,‡ and Tomio Yamada‡

Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan, and Odawara Research Center, Nippon Soda Company, Ltd., Takada, Odawara 250-02, Japan

The activity of a set of insect juvenile hormone (JH) mimetics was examined against the metamorphosis of *Musca domestica*, *Spodoptera litura*, and *Nephotettix cineticeps* larva, the hatching of *S. litura* eggs, and the propagation of *Aphis gossypi* nymphs. The structures of the compounds were varied systematically in their dimensions and the position of a functional group. These 4-alkyl-, 4-alkoxy-, 4-benzyl-, and 4-phenoxyphenyl alkyl ether compounds had functions such as ether, oxime, hydroxylamine, ester, amide, carbamate, benzene, and pyridine in the alkane moiety. The structure vs activity profiles were collated with those previously found for *Culex pipiens*, and the results showed that the information on the mode of action obtained for one species of insects could be transposed to another, by which means the structural factors important for potency could be identified.

We have developed highly potent classes of compounds with insect juvenile hormone (JH) activity, evaluated by their activity against the common mosquito, *Culex pipiens*. The compounds are (4-phenoxyphenoxy)- and (4-benzylphenoxy)alkanaldoxime *O*-ethers (Niwa et al., 1988), their ether (Niwa et al., 1989) and hydroxylamine (Niwa et al., 1990) congeners, (4-alkoxyphenoxy)- and (4-alkylphenoxy)alkanaldoxime *O*-ethers (Hayashi et al., 1989), and their congeners in which the oxime function is replaced by the functions ether, ester, amide, carbamate, urea (Hayashi et al., 1991), or aromatic benzene or pyridine (Hayashi et al., 1990a). Structure vs activity studies have been done for these compounds as well as for some reported by other workers (Bowers, 1969; Cruickshank and Palmere, 1971; Karrer and Farooq, 1981; Röller, et al., 1967), and the results showed that the most important features for activity are the overall length of the chain molecule, the dimensions of the molecular ends, and the position of the functional group incorporated at one end of the molecule (Hayashi et al., 1990b, 1991). For *C. pipiens*, the optimum length is about 21–22 Å, and the position-specific interaction site of the functional groups in the molecules with optimum activity is about 4.6 Å distant from one end of the molecule. Quantum chemical calculations showed that the contours of the electrostatic potentials of these functions have a negative peak in the plane that perpendicularly bisects their skeletal plane. Thus, highly potent compounds against *C. pipiens* could be designed so that they fulfill the dimensional conditions and so that the negative potential peak of the functional group is on the 4.6-Å site (Hayashi et al., 1990b).

The design principle developed for *C. pipiens* could be applied to other insects also if we could find favorable dimensions for activity against the other species and for the site of the electrostatic interaction. In this study, we examined structural profiles for potency against *Musca domestica* (house fly), *Spodoptera litura* (cutworm), *Nephotettix cincticeps* (green rice leafhopper), and *Aphis gossypi* (cotton aphid) of a set of compounds in which the structure and position of the function built in were systematically varied. These results were collated with those for *C. pipiens* previously reported (Hayashi et al., 1989, 1990a,b), showing that the information obtained for one species of insects could be transposed to another and that the favorable features of the molecule could be identified.

† Kyoto University.
‡ Nippon Soda Co. Ltd.

MATERIALS AND METHODS

The compounds reported elsewhere are **12–15**, **21–24**, **26–30** (Hayashi et al., 1989), **55–66**, **88** (Hayashi et al., 1990b), **1–7**, **16–18**, **35–37**, **40–54**, **77**, **79**, **85** (Hayashi et al., 1991), **31**, **32**, **73–75** (Niwa et al., 1988), **9**, **67**, **68**, **70** (Niwa et al., 1989), **33**, **38**, **39**, **82–84**, and **86** (Niwa et al., 1990).

Compounds **8**, **19**, **20**, **25**, **34**, **69**, **71**, **72**, **76**, and **79–81** were prepared as described in the literature (Hayashi et al., 1991), and **87** and **89** were synthesized according to the method of Hayashi et al. (1990a). ^{1}H NMR spectra were obtained with a JEOL PMX-60 spectrometer in $CDCl_3$ with tetramethylsilane as the internal reference.

***N*-[3-[4-(2-Ethylbutyl)phenoxy]propyl]-*N*-isobutylamine (10).** To a dimethylformamide solution (5 mL) of 3-[4-(2-ethylbutyl)phenoxy]propyl chloride (0.37 g, 1.5 mmol) and Na_2CO_3 (0.23 g, 2.2 mmol) was added isobutylamine (0.16 g, 2.2 mmol). The mixture was stirred for 12 h at 50–60 °C, poured into water, and extracted with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate 1/1), giving 0.37 g (87%) of amine **10**: ^{1}H NMR ($CDCl_3$) δ 6.72 (m, 4 H, ArH), 3.97 (t, J = 7 Hz, 2 H, Ar OCH_2), 2.77 (t, J = 7 Hz, 2 H, CH_2CH_2-NH), 2.45 (d, J = 6 Hz, 2 H, Ar CH_2), 2.43 (d, J = 7 Hz, 2 H, NHCH_2CH), 1.92 (m, 3 H, $OCH_2CH_2CH_2NH$, $NHCH_2CH$), 1.25 [m, 5 H, $2CH_2CH_3$, $CH(CH_2)_2$], 0.90 [d, J = 6 Hz, 6H, $CH(CH_3)_2$], 0.85 (t, J = 6 Hz, 6 H, $2CH_3$).

***N*-[3-[4-(2-Ethylbutyl)phenoxy]propyl]-*N*-isobutyl-*N*-methylamine (11).** To a dimethylformamide solution (5 mL) of *N*-[3-[4-(2-ethylbutyl)phenoxy]propyl]-*N*-isobutylamine (**10**) (0.30 g, 1.0 mmol) and Na_2CO_3 (0.16 g, 1.5 mmol) was added methyl iodide (0.30 g, 1.0 mmol). The mixture was stirred for 10 min at room temperature, poured into water, and extracted with diethyl ether. The diethyl ether layer was washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate 1/1), yielding 0.15 g (48%) of amine **11**: ^{1}H NMR ($CDCl_3$) δ 6.72 (m, 4 H, Ar H), 3.97 (t, J = 7 Hz, 2 H, Ar OCH_2), 2.47 (t, J = 7 Hz, 2 H, CH_2CH_2N), 2.45 (d, J = 6 Hz, 2 H, Ar CH_2), 2.15 (s, 3 H, NCH_3), 2.07 (d, J = 6 Hz, 2 H, NCH_2CH), 1.27 (m, 3 H, $OCH_2CH_2CH_2N$, NCH_2CH), 1.25 [m, 5 H, $2CH_2CH_3$, $CH(CH_2)_2$], 0.88 [d, J = 6 Hz, 6 H, $CH(CH_3)_2$], 0.85 (t, J = 6 Hz, 6 H, $2CH_3$).

0021-8561/91/1439-2039$02.50/0

Bioassay Procedures. The potency against *C. pipiens* metamorphosis is cited from earlier papers (Niwa et al., 1988, 1989, 1990; Hayashi et al., 1989, 1990a, 1991).

House Fly (M. domestica) Larvae. Full-grown (wandering) larvae of house flies adapted to a wheat bran diet were collected, placed in Petri dishes, treated topically while still mobile with a test compound in ethanol solution (1 μL/larva, 20 larvae at each dose level), and kept at 25 °C for 10 days (during which period all of the healthy controls emerged as adults). The percentage of flies that did not emerge, corrected for by the number of ethanol-treated controls that did not emerge, was calculated, and the potency was expressed as pI_{50} (M), the logarithm of the reciprocal of the concentration at which 50% inhibition of metamorphosis was observed. The experiments were repeated at least twice, and the mean value is reported.

Cutworm (S. litura) Larvae. Cutworm larvae were reared in mass culture on an artificial diet based on mottled kidney beans, dried yeast, and wheat bran. The eggs were a gift of Ishihara Sangyo Kaisha, Ltd. Mature larvae (within 24 h before pupation) were collected, and 1 μL of ethanol solution of a test compound was applied to the dorsal parts of each larva (20 larvae at each dose level). The treated larvae were allowed to develop at 25 °C for 10 days, by which time all of the healthy controls had emerged as adults. The percentage of moths that had not emerged, corrected for by nonemergence in ethanol-treated controls, was calculated, and the potency was expressed as pI_{50} (M), as above.

Cutworm (S. litura) Eggs. Egg masses (0–1 day old) of the common cutworm were brushed with a small brush to separate individual eggs. Twenty to 30 eggs on a sheet of adhesive tape were dipped for 30 min in a test solution prepared by dissolving a test compound in dimethylformamide containing 1.5% Tween 20 and diluting this with water. Then the eggs were kept at 25 °C and 65% relative humidity. The numbers of hatched and unhatched eggs were counted 7 days after treatment. Egg mortality was calculated with correction for untreated controls and expressed as a percentage.

Cotton Aphid (A. gossypii) Nymphs. Five apterous viviparous females were put onto a primary leaf of a young cucumber planted in a pot, and 2 days later, they were removed to remain nymphs. About 40 of the nymphs produced on the plant were sprayed with a chemical solution prepared as above for the treatment of cutworm eggs and then put in a room at 25 °C and 65% relative humidity. The numbers of live aphids on the plant were counted before the spraying and 7 days after. The efficacy of the control was calculated as $\{1 - [(T_2/T_1)/(C_2/C_1)]\} \times 100$, where C_1 is the number of aphids in an untreated plot at the 0th day, C_2 is the number of aphids 7 days later in an untreated plot, T_1 is the number of aphids before treatment in a plot to be treated, and T_2 is the number of aphids 7 days after treatment in the treated plot.

Green Rice Leafhopper (N. cincticeps) Larvae. The organophosphate-resistant strain of green rice leafhoppers was used in the experiment. A rice seedling was dipped for 30 s in a chemical solution prepared as described above and put in a glass tube after being air-dried. Third instar or last instar larvae were released into the glass tube and placed at 25 °C and 65% relative humidity. Dead, abnormal, and blackened insects were investigated 5 days (if last instar) or 7 days (if third instar) after the treatment, and the potency was expressed as a percentage.

RESULTS

Potency To Prevent Metamorphosis of *M. domestica* and *S. litura*. Table I lists the potency in terms of pI_{50} to prevent metamorphosis of *M. domestica* and *S. litura*, together with that against *C. pipiens* reported previously (Niwa et al., 1988, 1989, 1990; Hayashi et al., 1989, 1990a, 1991). The most active compounds against *M. domestica* were [4-(2-ethylbutyl)phenoxy]propionaldoxime *O*-ethyl (**17**) and *O*-isopropyl (**26**) ethers, (4-isobutylphenoxy)propionaldoxime *O*-isopropyl ether (**22**), isopropyl 4-[4-(2-ethylbutyl)phenoxy-2-methyl]-2(*E*)-butenoate (**42**), and 2-[4-(2-ethylbutyl)phenoxy]-1-methylethyl 2-pyridyl ether (**59**). Those for *S. litura* were [4-(2-ethylbutyl)phenoxy]- and [(4-cyclohexylmethyl)phenoxy]-propionaldoxime *O*-isopropyl ethers (**20** and **27**), [4-(3-methylphenoxy)phenoxy]propionaldoxime *O*-isopropyl ether (**32**), and propionaldoxime *O*-[4-(2-ethylbutyl)phenoxy]ethyl ether (**35**), the I_{50} being less than 10^{-11} M. The potencies of these compounds were far higher than that of JH III and higher than or as high as that of fenoxycarb [ethyl *N*-(4-phenoxyphenoxyethyl)carbamate] and of methoprene [isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate]. The *D* (angstroms) given in the table is the overall length of the molecule in the extended conformation, the definition of which has been reported elsewhere but is shown in Figure 1 for later discussion.

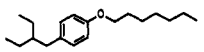
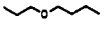
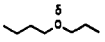
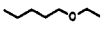
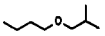
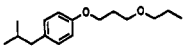
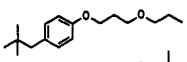
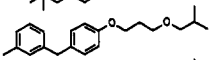
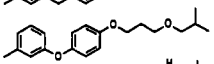
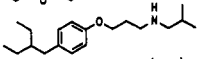
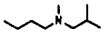
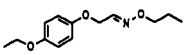
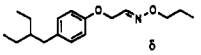
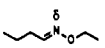
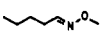
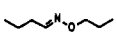
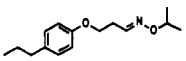
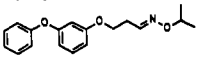
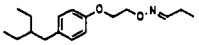
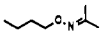
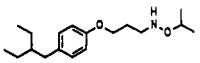
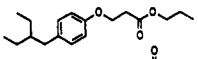
In the ether series of compounds **2–4** in which the length of the molecule was kept constant (about 21 Å) but the position of the oxygen atom was systematically varied, compound **3** had the highest activity for *M. domestica*, *S. litula*, and *C. pipiens*. This suggests that the favorable position of the oxygen atom is at δ from the phenoxy oxygen atom for the potency against these three insect species. This position corresponds to the site about 4.6 Å from the end of the molecule (Niwa et al., 1989). When the oximes **16–18**, were compared, compound **17** was found to be most potent, showing that a nitrogen atom is more favorable for the activity than an oxygen atom at the 4.6 Å (δ) site. The same was found also for the reverse oximes, δ-nitrogenous **35** being more potent than ε-nitrogenous **36**. In amides (**43–45**) and carbamates (**46, 47, 51,** and **52**), the potency against *M. domestica* and *S. litura* was highest when the carbonyl group was at δ from the phenoxy oxygen atom (compounds **44, 46,** and **51**). The situation was the same as that observed for potency against *C. pipiens* (Hayashi et al., 1989, 1991).

The overall length of the molecules was systematically varied in oximes **12–15**, in which 4-*n*-alkoxy substituents of the benzene ring were altered from ethyl to *n*-amyl, and also in the oximes **19, 17,** and **20**, in which the length was varied at the oxime *O*-ether end. Compounds **12–15** were not active against *S. litura* but they had enough activity against *M. domestica* for estimation of the optimum length. This seemed to be 21–22 Å (compounds **13** and **14**). Compounds **17** and **20** were highly potent against both insects, and the favorable dimension was again suggested to be 21–22 Å, which is nearly the same value as that estimated for *C. pipiens*.

The effects of 4-alkyl substituents of the central benzene ring were examined with compounds **21–29**. For *C. pipiens*, β-branching or cyclization at β is favorable for activity (Hayashi et al., 1989). For *M. domestica* and *S. litura*, the same was found; β-methylpropyl **22** was more potent than the corresponding nonbranched **21**. The double branching seen in **23** seemed, however, to be detrimental to *M. domestica*. β-Methylbutyl **25**, β-ethylbutyl **26**, and cyclohexylmethyl **27** were more potent than *n*-butyl **24**, with the same length. The high potency of **29** seemed to be also due to the β-branch effect.

The effects of branching at the other end were different among insects. A methyl branch at distance α from the end of the molecule increases the potency against *C. pipiens* (Niwa et al., 1988, 1989, 1990; Hayashi et al., 1989), but it had little effect or else somewhat decreased potency against *M. domestica* and *S. litura*, as seen by comparison of the PI_{50} values of **5, 26, 48,** and **53** with that of the corresponding compounds **3, 17, 46,** and **51**, respectively. The steric requirements in this region of the receptor seem to be somewhat stricter in the case of these insects than in *C. pipiens*. Of the pyridine and benzene compounds **55–66**, compounds **57** and **59** exhibited high potency against the metamorphosis of the three insect species.

Table I. Activity against Metamorphosis of *C. pipiens*, *M. domestica*, and *S. litura*

No.	Structure	pI_{50} *Culex pipiens* (M)	pI_{50} *Musca domestica* (mol/larva)	pI_{50} *Spodoptera litura* (mol/larva)	D (Å)
1		5.18	7.39	6.71	21.08
2		6.78	7.80	6.94	20.90
3		8.17	10.91	9.98	20.91
4		6.68	9.13	7.94	20.90
5		8.81	10.01	8.52	20.91
6		7.86	9.81		19.63
7		8.01	7.97		19.63
8				9.52	21.88
9		10.49			21.71
10		6.65	8.44	6.96	20.97
11		7.19	7.68		20.97
12		5.27	6.11	na[a]	19.42
13		6.82	6.96	na	20.71
14		6.39	7.53	na	21.93
15		6.21	6.38	na	23.21b
16		8.40	9.65	8.44	20.88
17		9.53	11.22	10.61	20.79
18		6.60	8.92	6.52	20.88
19		7.33	9.54	9.65	19.53
20		8.66	10.56	11.35	22.03
21		7.28	8.15	6.90	19.51
22		10.85	11.51	9.30	19.51
23		10.03	8.67	9.39	19.51
24		7.34	8.39	7.27	20.79
25		9.70	10.22	10.57	20.79
26		10.76	11.07	10.96	20.79
27		9.37	10.93	11.34	20.79
28		8.46	8.56		20.79
29		9.78	10.40	9.41	20.02
30		9.30	9.39		20.62
31		9.76	9.81		20.51
32		10.06	9.71	11.95	21.78
33		10.00	9.04	11.00	21.62
34		7.58	8.82	8.39	
35		9.88	10.42	11.11	20.88
36		8.65	9.45	9.26	20.79
37		8.97	9.73	8.90	20.79
38		9.95	8.25	10.23	21.76
39		10.00	8.68	9.61	21.60
40		na	na		20.84
41		na	na	na	20.84
42		9.08	11.38	8.60	20.81
43		4.81	na		20.92
44		5.87	7.50		20.92
45		5.15	na		20.92
46		7.57	10.25	10.95	20.74
47		6.50	9.01	na	20.74
48		8.62	9.90	8.55	20.74
49		8.59	8.80		20.74
50		7.77	8.73	9.49	20.74
51		7.54	9.51	9.78	20.74
52		6.31	8.56	na	20.74
53		8.12	9.62	8.78	20.74
54		6.97	na	na	20.79
55		7.15	7.23	7.41	19.55
56		7.56	9.69	7.48	20.78
57		10.04	10.85	10.36	19.55
58		8.22	10.50	10.07	20.78
59		9.64	11.45	10.78	19.55
60		6.63	na		17.22
61		8.03	9.64		18.42
62		8.65	10.61		19.71
63		8.07	9.18	8.27	20.93
64		6.42	na		22.21
65		8.80	10.19	8.54	19.71
66		9.04	9.94	8.84	20.76
JH III		6.15	7.56	na	
Fenoxycarb		8.82	10.06	7.43	
Methoprene		9.50	11.78	10.08	

[a] Not active at concentrations of less than 10^{-5} M.

Table II. Activity toward *S. litura* Eggs, *N. cineticeps* Metamorphosis, and *A. gossypi* Propagation

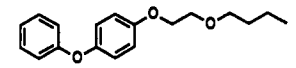
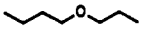
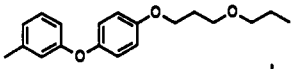
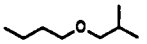
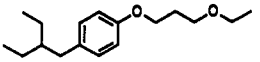
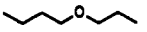
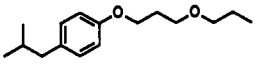
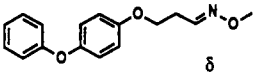
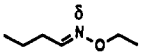
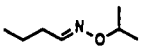
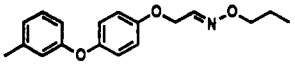
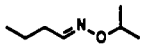
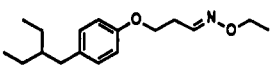
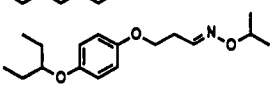
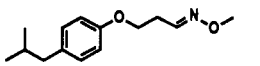
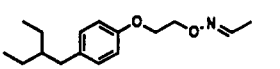
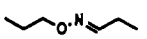
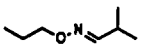
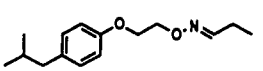
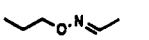
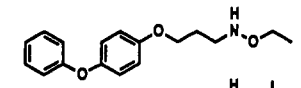
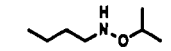
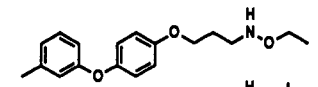
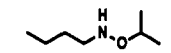
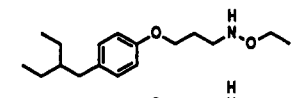
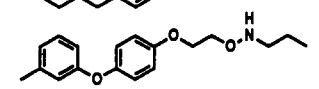
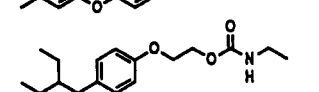
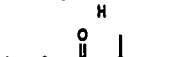
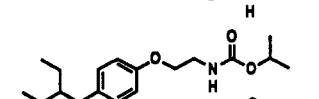
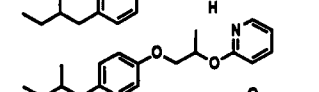
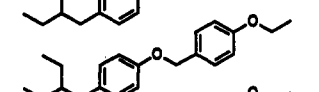
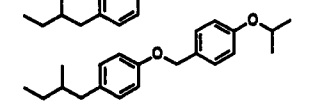
		Spodoptera litura eggs	Naphotettix cineticeps		Aphis gossypi nymphs
		Mortality %	Abnormal metamorphosis %		Inhibition of propagation %
			3rd instar	last instar	
No.	Structure	100 33 10 3.3 (ppm)	100 10 (ppm)	100 10 (ppm)	100 33 10 3.3 1 (ppm)
67		65 33	0	20	93 93
68		76 64 68	40 20	0	97 95
69		64 48	0	0	98 88 69
70		97 89	0	0	0
9		80 69 49	0	0	0
71		3	0	40 22	0
3		56 36	0	60 0	0
6		0	0	40	0
72		79 34	40 0	40 0	91 92 0
73		100 64	100 0	60 0	94 91 70
31		82 71 62	0	0	93 0
74		79 73 65	20	0	0
75		99 87 86 81	40	40 0	46 0
33		93 85 79 55	0	0	0
17		54	0	60 40	92 74
26		40	0	20 0	0
76		0	0	20	0
77		22	0	0	63 0
78		59 52	0	20 20	93 95 0
35		61 39	0	20 20	100 67
79		28	0	0	91
80		57 36	0	0	0
81		59 59	0	20 20	0

Table II (Continued)

No.	Structure	*Spodoptera litura* eggs Mortality % 100 (ppm)	33 (ppm)	10 (ppm)	3.3 (ppm)	*Naphotettix cineticeps* Abnormal metamorphosis % 3rd instar 100 (ppm)	10 (ppm)	last instar 100 (ppm)	10 (ppm)	*Aphis gossypi* nymphs Inhibition of propagation % 100 (ppm)	33 (ppm)	10 (ppm)	3.3 (ppm)	1 (ppm)
82		75	58	51		0		20	0	92		76		
83		69	39	35		0		20	20	60		0		
84		89	87	80		100	20	40	20	0				
39		70	56			0		0		0				
85		85		35		0		20		80		0		
86		98		83		0		0		0				
46		78		51		0		0		94	62	0		
48		34				0		0		0				
53		42		38		0		0		0				
87		46		52		0		0		92		76		
88		51		4			0	0		93	94	49		
89		20					0	20		78				
Methoprene		22		17		30	10	20		0				

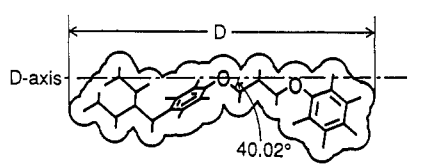


Figure 1. Definition of length parameter *D*. The model compound is 4-(2-ethylbutyl)phenyl phenoxypropyl ether (**56**). The ends of the bars of the structure represent hydrogen atoms.

In consequence, the fundamental conditions for high potency, such as the optimum molecular length and the position of the functional group, were the same for all three insect species. Some other conditions were different, and this explains why the most potent members listed above were not the same for all three insect species.

The α,β-unsaturated ester **42** showed high potency for all three insect species, but the simple esters **40** and **41** had few signs of activity. As mentioned elsewhere (Hayashi et al., 1991), the main reason for this difference is probably the high chemical stability of the unsaturated structure.

Potency for *S. litura* Eggs, *N. cineticeps* Larvae, and *A. gossypi* Nymphs. Table II shows the activity against *S. litura* eggs, *N. cineticeps* larvae, and *A. gossypi* nymphs. The experiments were done under practical conditions, and the results are given as a percentage.

The structure vs ovicidal activity for *S. litura* eggs was in some points the same and in some points different from the results for the metamorphosis of *C. pipiens*, taken as a standard. The favorable length of the molecule seemed to be about 21 Å, although the difference in potency from that of a shorter congener was small (compare the potencies of ethers **68**, **73**, and **35**, about 21 Å long, with those of the shorter compounds **69**, **71**, and **78**, respectively). The electrostatic point interaction site was about the δ site, to judge from the comparison of the potency of γ-oxygenous **67** and δ-**68**. In oximes, the favorable atom seemed to be a nitrogen rather than an oxygen, since δ-nitrogenous oxime **75** was more potent than the corresponding γ-nitrogenous **74**. (*m*-Methylphenoxy)phenoxy derivatives were far more potent than the corresponding unsubstituted compounds (**68** vs **70**, **31** vs **33**, and **73** vs **75**). A similar feature has been observed in studies of (4-phenoxyphenoxy)- and (4-benzylphenoxy)alkanaldoxime *O*-ethers against *C. pipiens* metamorphosis as well (Niwa et al., 1988). In general, these conditions for activity corre-

sponded to those observed for the metamorphosis of larvae of *C. pipiens* and also of *S. litura* (Table I).

Oxime *O*-isopropyl ethers **31** and **33**, isobutyraldoxime **79**, isopropoxylamines **83** and **39**, and *N*-isopropylcarbamate **48** were less potent than the corresponding nonbranched congeners (**73**, **75**, **35**, **82**, **84**, and **46**, respectively). The methyl branch at the functional end seemed to be decelerative, and this feature corresponded to that observed for the metamorphosis of larvae of the same species, *S. litura*, but not of *C. pipiens* larvae (Hayashi et al., 1989). The feature peculiar to activity for *S. litura* eggs was that 4-alkyl- and 4-alkoxyphenoxy congeners were far less active than 4-phenoxyphenoxy derivatives, as seen from the comparison of the potency of phenoxyphenoxy-type ethers **67–70** with that of alkylphenoxy **71**, **3**, and **6**, from the comparison of the potency of oxime compounds **73**, **75**, **31**, **72**, and **33** with that of **17**, **26**, **26**, and **77**, and from the comparison of the potency of hydroxylamines **82** and **84** with that of **85** (Table II). Accordingly, the most potent members were those having this 4-phenoxyphenoxy structure with an *m*-methyl substituent at its terminal, namely compounds **70**, **75**, **33**, **84**, and **86**, their potency being far higher than that of the methoprene measured as a reference. The corresponding 4-(*m*-methyl)phenoxy derivatives of the reverse oximes **78**, **35**, and **79–81**, carbamates **46**, **48**, and **53**, pyridyl **87**, and benzenes **88** and **89** were not prepared and included in the present set of compounds, but they may have potency as high as that of **70**, **75**, **33**, **84**, and **86**.

The effect of causing abnormal metamorphosis of *N. cineticeps* larva was not readily apparent so that little could be deduced from our experiments. To guess from the fact compounds **68**, **72**, **73**, **75**, and **84** having detectable effects on third instar larvae, their phenoxyphenoxy structure may be more favorable than 4-alkyl or 4-alkoxy structure, since compounds with these substituents had little effect in the concentration range tested. This structure–activity relationship was not observed with last inster larvae. Methoprene was not particularly effective. For high potency, alteration of the skeletal structure would be necessary.

Activity against *A. gossypi* nymphs was much stronger. The γ-oxygenous ether **67** was as potent as the δ-oxygenous congener **68**. Moreover, **69**, one methylene unit shorter than **68** (about 21 Å), was only slightly less potent than **68**. The situation was similar in oximes **72** and **73** and in reverse oximes **78** and **35**. The electronic interaction site of the receptor may have a certain kind of extension in this insect, and the diameter of the receptor may be a little less than that of *C. pipiens*. The introduction of a methyl substituent into the meta position of the phenoxyphenoxy compounds greatly reduced the potency, as seen from the results of **70**, **75**, and **84** compared with those of **68**, **73**, and **82**, respectively. This is in contrast to the situation observed for the activity against *S. litura* eggs and against *C. pipiens* larvae. The effects of the α branch at the functional end seemed to be unfavorable for activity as seen from the comparison of the potencies of oxime **31** with **73**, **33** with **75**, **79** with **35**, hydroxylamine **83** with **82**, and carbamate **48** with **46**. Many of the compounds studied here had much higher potency than methoprene.

DISCUSSION

The compounds that prevented *M. domestica* metamorphosis most effectively were **17**, **22**, **26**, **42**, and **59**, with pI_{50} values as high as the pI_{50} of methoprene, and the compounds that prevented *S. litura* metamorphosis most effectively were **20**, **27**, **32**, and **35**, with potency at least dozens of times higher than that of methoprene (Table I).

Structural characteristics that affect activity against the metamorphosis of these insects were deduced with reference to those for *C. pipiens*. For *M. domestica*, the optimum molecular length was 21–22 Å, and the electronic interaction site seemed to be at about 4.6 Å from the receptor edge. 4-Phenoxy- and 4-benzylphenoxy compounds had comparable effects. These features were the same as those observed previously for *C. pipiens* (Niwa et al., 1988, 1989; Hayashi et al., 1989, 1990a,b; and the results in Table I cited from these papers). The branching or cyclization at β in the 4-substituent of the 4-alkylphenoxy end was favorable for activity as is true for *C. pipiens* (Hayashi et al., 1989), but an α-methyl substituent at the other end was rather decelerating. Similarly for *S. litura*, the suitable molecular length, position-specific electrostatic interaction site, and branching or cyclization at β in the 4-alkyl substituent were the same as those for *C. pipiens*, but the α-methyl effect at the functional end was an unfavorable one.

For *C. pipiens*, *M. domestica*, and *S. litura* metamorphosis, the structural profiles were basically similar to those just mentioned, but some were different. A possible design principle for new compounds active against *C. pipiens* is to construct a molecule so that its dimensions satisfy the optimum steric conditions and so that the negative, electrostatic potential peak of the functional group (whatever it is, so long as it has a peak) is on the right at the 4.6 Å site (Hayashi et al., 1990b). The same principle could be applied to design compounds active against *M. domestica* and *S. litura* except that branching or bulkiness at the functional end is unfavorable for activity against these insects.

The structural profiles for ovicidal activity toward *S. litura* eggs had in common with those for prevention of *C. pipiens* metamorphosis the optimum length of the molecule and the site of the electronic interaction. The introduction of a methyl group into the meta position of 4-phenoxy and 4-benzylphenoxy derivatives significantly increased activity, but such a group in the other end tended to have the opposite effect. The 4-alkyl- and 4-alkoxyphenoxy compounds were less potent than the 4-phenoxyphenoxy derivatives. These features are different from those observed for activity against the metamorphosis of the same insect, showing that the features of the JH receptor differ even in the same insect species depending on its function or the growth stage. This information may be useful in physiological and biochemical studies of JH receptors or receptive proteins. The affinity of a ligand may be different depending on what function the proteins the researchers deal with have in vivo. Many of compounds here were far more potent than methoprene. Probably other useful compounds could be made available either by the design of new compounds or by modification of the compounds already reported by us and other workers (Henrick, 1982, and the references cited therein) so as to satisfy the structural conditions described above.

The compounds tested here were not at all effective against third and last larval instars of *N. cineticeps*. The features of the receptive site in this insect may be considerably different from those of the other insect species examined here, and a drastic change in the structure is necessary for potency. Still, compounds **73** and **84** could be a starting point for structural modification, since they had more effect than the other compounds with low activity.

With *A. gossypi* nymphs as the target of activity, structural profiles were different from those against *C. pipiens* metamorphosis used for comparison. The position

specificity of the functional group seemed to be less strict, and the condition for molecular length was shorter. An *m*-methyl substituent in the 4-phenoxyphenoxy compounds decreased activity, and this may be related to the smaller diameter of the receptor site.

Systematic exploration with the present set of compounds of the characteristics of the receptor did not result in the identification of a compound effective against *N. cineticeps*, but the principle used here could be applied to many species of insects in addition to *M. domestica*, *S. litura*, and *A. gossypi*. The results would be of use in increasing understanding of the mode of action of JH mimetic compounds as well as in designing new potent compounds.

ACKNOWLEDGMENT

H.I. is indebted to the Ministry of Education for financial support by Grant-in-Aid for Scientific Research 01560194.

Supplementary Material Available: Table of analytical data about the compounds prepared (1 page). Ordering information is given on any current masthead page.

LITERATURE CITED

Bowers, W. S. Juvenile Hormone: Activity of Aromatic Terpenoid Ethers. *Science* **1969**, *164*, 323–325.

Cruickshank, P. A.; Palmere, R. M. Terpenoid Amides as Insect Juvenile Hormones. *Nature (London)* **1971**, *233*, 488–498.

Hayashi, T.; Iwamura, H.; Nakagawa, Y.; Fujita, T. Development of (4-Alkoxyphenoxy)- and (4-Alkylphenoxy)alkanaldoxime O-Ethers as Potent Insect Juvenile Hormone Mimics and Their Structure-Activity Relationships. *J. Agric. Food Chem.* **1989**, *37*, 467–472.

Hayashi, T.; Iwamura, H.; Fujita, T. Developments of 4-Alkylphenyl Aralkyl Ethers and Related Compounds as Potent Insect Juvenile Hormone Mimetics and Structural Aspects of Their Activity. *J. Agric. Food Chem.* **1990a**, *38*, 1965–1971.

Hayashi, T.; Iwamura, H.; Fujita, T. Electronic and Stereochemical Aspects of Insect Juvenile Hormone Active Compounds: A Clue for High Activity. *J. Agric. Food Chem.* **1990b**, *38*, 1972–1977.

Hayashi, T.; Iwamura, H.; Fujita, T. Insect Juvenile Hormone Mimetic Activity of (4-Substituted)phenoxyalkyl Compounds Having Various Nitrogenous and Oxygenous Functions, and Its Relation to Their Electrostatic and Stereochemical Properties. *J. Agric. Food Chem.* **1991**, preceding paper in this issue.

Henrick, C. A. Juvenile Hormone Analogs: Structure-Activity Relationships. In *Insecticide Mode of Action*; De Coats, J. R., Ed.; Academic Press: New York, 1982; pp 315–402.

Karrer, F.; Farooq, S. Some Insect Growth Regulators with Aromatic Rings: Their Synthesis and Biological Properties. In *Regulation of Insect Development and Behaviour*; Sehnal, F., Zabza, A., Menn, J. J., Cymborosky, B., Eds.; Wroclaw Technical University: Wroclaw, Poland, 1981; pp 289–302.

Niwa, A.; Iwamura, H.; Nakagawa, Y.; Fujita, T. Development of (Phenoxyphenoxy)- and (Benzylphenoxy)alkanaldoxime O-Ethers as Potent Insect Juvenile Hormone Mimics and Their Quantitative Structure-Activity Relationship. *J. Agric. Food Chem.* **1988**, *36*, 378–384.

Niwa, A.; Iwamura, H.; Nakagawa, Y.; Fujita, T. Development of (Phenoxyphenoxy)- and (Benzylphenoxy)propyl Ethers as Potent Insect Juvenile Hormone Mimetics. *J. Agric. Food Chem.* **1989**, *37*, 462–467.

Niwa, A.; Iwamura, H.; Nakagawa, Y.; Fujita, T. Development of N,O-Disubstituted Hydroxylamines and N,N-Disubstituted Amines as Insect Juvenile Hormone Mimetics and the Role of the Nitrogenous Function for Activity. *J. Agric. Food Chem.* **1990**, *38*, 514–520.

Röller, H.; Dahn, K. H.; Sweely, C. C.; Trost, B. M. The Structure of Juvenile Hormone. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 179–180.

Received for review May 13, 1991. Accepted August 12, 1991.

Registry No. **1**, 136588-18-6; **2**, 136588-23-3; **3**, 136588-24-4; **4**, 136588-25-5; **5**, 136588-27-7; **6**, 136588-88-0; **7**, 136588-87-9; **8**, 136589-10-1; **9**, 118358-10-4; **10**, 136589-11-2; **11**, 136589-12-3; **12**, 116799-45-2; **13**, 116799-46-3; **14**, 116799-47-4; **15**, 116799-48-5; **16**, 136588-19-7; **17**, 136588-20-0; **18**, 136588-21-1; **19**, 136589-13-4; **20**, 136589-14-5; **21**, 118358-15-9; **22**, 136589-15-6; **23**, 116799-83-8; **24**, 116799-80-5; **25**, 116799-82-7; **26**, 118358-19-3; **27**, 116799-84-9; **28**, 118358-18-2; **29**, 118358-20-6; **30**, 116815-69-1; **31**, 112597-79-2; **32**, 136589-16-7; **33**, 124224-11-9; **34**, 136589-17-8; **35**, 136588-28-8; **36**, 136588-30-2; **37**, 136588-34-6; **38**, 136589-18-9; **39**, 136589-19-0; **40**, 136589-20-3; **41**, 136588-38-0; **42**, 136589-21-4; **43**, 136588-54-0; **44**, 136588-55-1; **45**, 136588-56-2; **46**, 136588-58-4; **47**, 136589-22-5; **48**, 136588-59-5; **49**, 136588-61-9; **50**, 136589-23-6; **51**, 136588-66-4; **52**, 136588-69-7; **53**, 136588-67-5; **54**, 136589-24-7; **55**, 125796-89-6; **56**, 125796-97-6; **57**, 128550-32-3; **58**, 128550-37-8; **59**, 128631-98-1; **60**, 125796-68-1; **61**, 125796-69-2; **62**, 125796-70-5; **63**, 125796-71-6; **64**, 125796-72-7; **65**, 125796-73-8; **66**, 125796-81-8; **71**, 136589-26-9; **72**, 135701-85-8; **73**, 98116-74-6; **74**, 112597-87-2; **75**, 112597-80-5; **76**, 136589-27-0; **77**, 136588-81-3; **78**, 136589-28-1; **79**, 136588-29-9; **80**, 136589-29-2; **81**, 136589-30-5; **82**, 136589-31-6; **83**, 136589-32-7; **84**, 136589-33-8; **85**, 136588-32-4; **86**, 131985-68-7; **87**, 136589-34-9; **88**, 125796-80-7; **89**, 136589-35-0; 3-[4-(2-ethylbutyl)phenoxy]propyl chloride, 136589-25-8; juvenile hormone 3, 22963-93-5; fenoxycarb, 72490-01-8; methoprene, 40596-69-8.