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

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Insect juvenile hormone (JH) active N,O-disubstituted hydroxylamines were developed to examine the role of the nitrogenous function for high activity. They are O-alkyl-N-[(4-phenoxyphenoxy) and (4-benzylphenoxy)alkyl]hydroxylamines and reversely substituted N-alkyl-O-[(4-phenoxyphenoxy)-alkyl]hydroxylamines. The activity against *Culex pipiens* of the most potent member of each class was as high as that of the compounds known so far as the most active of JH mimics. When the overall length of the molecules is kept at the optimum, about 21 Å, suggested in our earlier works, the compounds having a hydroxylamino nitrogen atom, rather than the oxygen atom, at the δ-position from the central phenoxy oxygen atom or at the 4-position (about 4.6 Å) from the alkyl end showed about 10 times higher activity than those having the nitrogen atom at the position one atom missed the point. The corresponding amine compounds also prepared showed this more clearly, but their activity was considerably lower than that of the hydroxylamines. The lower potency of the amines with $pK_a \approx 10$ was attributed to their quarternization at physiological pH, preventing going to or binding with the action site.

In our developmental studies on a series of insect juvenile hormone (JH) mimetic compounds, (4-phenoxyphenoxy)- and (4-benzylphenoxy)alkanaldoximes (Niwa et al., 1988), (4-alkoxyphenoxy)- and (4-alkylphenoxy)alkanaldoximes (Hayashi et al., 1989), and (4-phenoxyphenoxy)- and (4-benzylphenoxy)alkyl alkyl ethers (Niwa et al., 1989), we found that the positions of the oxime and ether functions in a molecule are important for high activity. The positional effect has been clearly specified in the ether series of compounds to be at the δ-position from the common phenoxy oxygen atom; the activity against *Culex pipiens* of (phenoxyphenoxy)- and (benzylphenoxy)propyl propyl ethers was more than 10 times higher than that of the corresponding ethyl butyl and butyl ethyl ethers. This is illustrated in Figure 1, where the shadowing on the structure shows the pertinent δ-position. Comparison of the oximes with these ethers suggests that the activity is highest when a nitrogen atom, rather than oxygen atom, is located at the δ-position in the oxime molecules; in other words, the point of interaction with the receptor is more favorable with nitrogen. This situation is also shown in Figure 1; 3-(4-benzylphenoxy)propionaldoxime O-isopropyl ether, a δ-nitrogen compound for example, is about 10 times more active than the corresponding δ-oxygen compound.

This study was done to examine this in more detail, and the first thing done was to change the sp^2 nitrogen atom in the oximes to the sp^3 type. This led us to develop a new class of highly active JH mimics, O-alkyl-N-[(4-phenoxyphenoxy) and (4-benzylphenoxy)alkyl]hydroxylamines. The optimization of the structure gave O-isopropyl-N-[3-[4-(3-methylphenoxy)phenoxy]propyl]hy-

droxylamine (9); the activity against *C. pipiens* was as high as that of our previous propionaldoxime (Niwa et al., 1988; Hayashi et al., 1989) and propyl ether (Niwa et al., 1989) types of compounds. Again in this series of compounds, those with a nitrogen atom at the δ-position were found to be more potent than the corresponding δ-oxygen compounds. The situation was the same when the hydroxylamine function was built in a molecule in the reverse way, and the optimized member, N-isobutyl-O-[2-[4-(3-methylphenoxy)phenoxy]ethyl]hydroxylamine (16), had activity as high as that of above-mentioned oximes and ethers, constituting another new class of highly active JH mimics.

To examine the nitrogen effect more simply and straightforwardly, we finally prepared N-alkyl-N-(4-phenoxyphenoxy)alkylamines. Their activity was, against expectation, far poorer than that of the corresponding hydroxylamines and also ethers, and this was found to be attributable to the quarternization of the amine function at physiological pH. The generic formulaes of the sets of compounds studied here are shown in Figure 2.

EXPERIMENTAL SECTION

^{1}H NMR spectra were obtained in CDCl$_3$ on a JEOL 60 spectrometer with tetramethylsilane as the internal reference.

4-Methoxy-3'-methylbenzophenone. A solution of 3-methylbenzoyl chloride (5.6 g, 36.2 mmol) in carbon tetrachloride (10 mL) was added dropwise to 15 mL of carbon tetrachloride containing 3.0 g (27.8 mmol) of anisole and 5.0 g (37.5 mmol) of AlCl$_3$ at ice bath temperature. The mixture was stirred for 3 h at room temperature, poured into water, and treated with *n*-hexane. The organic layer was washed with 2 N NaOH and water, dried over MgSO$_4$, and concentrated under reduced pres-
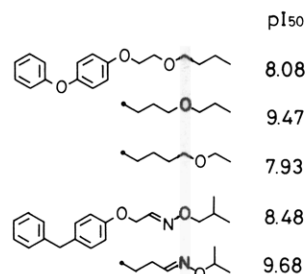
Figure 1. Structures and activities against *C. pipiens* of (4-phenoxyphenoxy)alkyl alkyl ether and (4-phenoxyphenoxy)alkanaldoxime *O*-ether types of compounds. The shadowed portion shows a plausible point interaction site with the receptor.
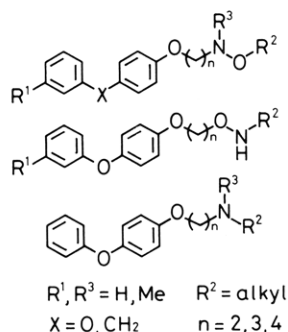

$R^1, R^3 = H, Me$ $R^2 = alkyl$
$X = O, CH_2$ $n = 2, 3, 4$

Figure 2. Generic structures of the hydroxylamines and amines studied.

sure to dryness. The crude product was put on a silica gel column that was eluted with 10% ethyl acetate in *n*-hexane, giving 6.14 g (98%) of the benzophenone as oil.

4-(3-Methylbenzyl)anisole. To a suspension of 0.34 g (9.84 mmol) of LiAlH$_4$ in 5 mL of dry diethyl ether was added a solution of 1.17 g (8.76 mmol) of AlCl$_3$ in 15 mL of dry diethyl ether. A dry diethyl ether solution (5 mL) of 4-methoxy-3'-methylbenzophenone (2.20 g, 9.73 mmol) was added dropwise to the mixture, with the solvent spontaneously refluxing. The mixture was stirred 30 min more at room temperature, and then ethyl acetate was added little by little. After bubbling became weaker, the reaction mixture was made acidic with 6 N HCl, poured into water, and extracted with *n*-hexane. The *n*-hexane layer was washed with water, dried over MgSO$_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography with 3% ethyl acetate in *n*-hexane, yielding 1.90 g (92%) of the anisole as oil: ^{1}H NMR (CDCl$_3$) δ 2.23 (s, 3 H, ArCH$_3$), 3.62 (s, 3 H, ArOCH$_3$), 3.80 (s, 2 H, ArCH$_2$Ar).

4-(3-Methylbenzyl)phenol. A 10-mL portion of concentrated HCl (110 mmol) was added to pyridine (9.0 g, 110 mmol) in an ice bath. Water was removed under reduced pressure, and the residual semisolid was heated at 150 °C to remove water completely. 4-(3-Methylbenzyl)anisole (4.60 g, 21.7 mmol) was added to the solid heated to 120 °C, and the mixture was stirred for 40 min at 200–220 °C. After being cooled to room temperature, the mixture was dissolved in water and then extracted with diethyl ether. The ether layer was washed with water, dried over MgSO$_4$, and concentrated under reduced pressure. The crude product was purified by silica gel column that was eluted with 15% ethyl acetate in *n*-hexane, to give 3.78 g (88%) of the phenol as oil.

3-[4-(3-Methylbenzyl)phenoxy]propionaldehyde Diethyl Acetal. To a solution of 1.30 g (6.57 mmol) of 4-(3-methylbenzyl)phenol in 15 mL of dimethoxyethane (DME) were added 0.85 g (7.59 mmol) of *t*-BuOK and 2.00 g (8.41 mmol, 70% purity) of 3-chloropropionaldehyde diethyl acetal. The mixture was stirred for 2 h at 90 °C, diluted with water, and treated with *n*-hexane. The *n*-hexane layer was washed with 2 N NaOH and water, dried over MgSO$_4$, and concentrated under reduced pressure, giving the oily acetal (2.01 g, 93%).

(4-Phenoxyphenoxy)acetaldoxime *O*-Isobutyl Ether (26). A few drops of 6 N HCl and a suspension of *O*-isobutylhydrox-

ylammonium chloride (0.11 g, 0.88 mmol) in 3 mL of water were added to 0.20 g (0.66 mmol) of (phenoxyphenoxy)acetaldehyde diethyl acetal (Niwa et al., 1988) in 10 mL of ethanol. The mixture was stirred for 4 h at 60 °C, dissolved in water, and extracted with *n*-hexane. The extract was washed with water, dried over MgSO$_4$, and concentrated under reduced pressure. The residue was put on a silica gel column chromatographed with 5% ethyl acetate in *n*-hexane, yielding 0.19 g (96%) of **26** as an oil: ^{1}H NMR (CDCl$_3$) δ 0.88 (d, 6 H, *J* = 7 Hz, CH(CH$_3$)$_2$), 2.0 (m, 1 H, –CH<), 3.78–3.83 (2 d, 2 H, *J* = 7 Hz, *E*- and *Z*-NOCH$_2$), 4.53–4.75 (2 d, 2 H, *J* = 4 Hz, *E*- and *Z*-ArOCH$_2$).

3-[4-(3-Methylbenzyl)phenoxy]propionaldoxime *O*-Ethyl and *O*-Isopropyl Ethers (27, 28). By analogy with the preparation of **26**, oximes **27** and **28** were synthesized from *O*-ethyl- and *O*-isopropylhydroxylammonium chloride and 3-[4-(3-methylbenzyl)phenoxy]propionaldehyde diethyl acetal. **27**: yield 74%; ^{1}H NMR (CDCl$_3$) δ 2.22 (t, 3 H, *J* = 7 Hz, –OCH$_2$CH$_3$), 2.28 (s, 3 H, ArCH$_3$), 3.82 (s, 2 H, ArCH$_2$Ar). **28** yield 73%; ^{1}H NMR (CDCl$_3$) δ 2.19 (d, 6 H, *J* = 6.5 Hz, OCH(CH$_3$)$_2$), 2.27 (s, 3 H, ArCH$_3$), 3.84 (s, 2 H, ArCH$_2$Ar), 4.05 (t, 2 H, *J* = 6.5 Hz, ArOCH$_2$), 4.3 (m, 1 H, –OCH<).

1-Bromo-2-[4-(3-methylphenoxy)phenoxy]ethane. To a dimethoxyethane (DME) solution (10 mL) of 3.00 g (15.0 mmol) of 4-(3-methylphenoxy)phenol (Niwa et al., 1988) were added 7.30 g (38.8 mmol) of 1,2-dibromoethane and 2.10 g (15.2 mmol) of K$_2$CO$_3$. The mixture was stirred for 12 h at 50–60 °C, dissolved in water, dried over MgSO$_4$, and concentrated under reduced pressure, to give 3.12 g (68%) of the bromoethane.

Propionaldoxime *O*-2-[4-(3-Methylphenoxy)phenoxy]-ethyl Ether (32). To a solution of 1-bromo-2-[4-(3-methylphenoxy)phenoxy]ethane (1.50 g, 4.88 mmol) in 20 mL of dioxane were added 0.55 g (7.53 mmol) of propionaldoxime and 0.83 g (7.41 mmol) of *t*-BuOK. The aldoxime had been prepared from propionaldehyde and hydroxylamine. The reaction mixture was refluxed for 1 h, poured into water, dried over MgSO$_4$, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography with 5% ethyl acetate in *n*-hexane, yielding 0.36 g (25%) of **31** as an oil: ^{1}H NMR (CDCl$_3$) δ 1.04 (t, 3 H, *J* = 7.5 Hz, CH$_2$CH$_3$), 2.25 (s, 3 H, ArCH$_3$), 7.35 (t, 1 H, *J* = 6 Hz, N=CH).

Isobutyraldoxime *O*-2-[4-(3-Methylphenoxy)phenoxy]-ethyl Ether (33). Oxime **32** was prepared in 34% yield from the same bromoethane and isobutyraldoxime that had been synthesized from isobutyraldehyde and hydroxylamine, by analogy with **31**: ^{1}H NMR (CDCl$_3$) δ 1.07 (d, 6 H, *J* = 7 Hz), 2.24 (s, 3 H, ArCH$_3$), 7.21 (t, 1 H, *J* = 6.5 Hz, N=CH).

3-(4-Phenoxyphenoxy)propyl Tosylate. 3-(4-Phenoxyphenoxy)-1-propanol (2.0 g, 8.20 mmol) was dissolved in a 20-mL portion of pyridine, and then 1.90 g (9.97 mmol) of tosyl chloride was added to the pyridine solution in an ice bath. The mixture was stirred for 6 h at room temperature, poured into water, and treated with diethyl ether. The ether layer was washed with 2 N HCl and water, dried over MgSO$_4$, and concentrated under reduced pressure. The solid residue was recrystallized from aqueous methanol, giving 2.38 g (73%) of the tosylate.

Acetaldoxime *O*-[3-(4-Phenoxyphenoxy)propyl] Ether (29). By analogy with compound **31**, oxime **29** was prepared in 57% yield from 3-(4-phenoxyphenoxy)propyl tosylate and acetaldoxime: ^{1}H NMR (CDCl$_3$) δ 1.80 (d, 3 H, *J* = 6 Hz, =CHCH$_3$), 4.02 (t, 2 H, *J* = 6.5 Hz, ArOCH$_2$), 4.15 (t, 2 H, *J* = 6.5 Hz, CH$_2$ON=).

Acetoxime *O*-[3-(4-Phenoxyphenoxy)propyl] Ether (30). By analogy with compound **31**, oxime **30** was prepared in 81% yield from 3-(4-phenoxyphenoxy)propyl tosylate and acetoxime: ^{1}H NMR (CDCl$_3$) δ 1.82 (s, 6 H, N=C(CH$_3$)$_2$), 4.00 (t, 2 H, *J* = 6.5 Hz, ArCH$_2$Ar), 4.15 (t, 2 H, *J* = 6 Hz, CH$_2$ON=).

N-[2-(4-Phenoxyphenoxy)ethyl]-*O*-propylhydroxylamine (1). A solution of 0.31 g (1.09 mmol) of (4-phenoxyphenoxy)acetaldoxime *O*-propyl ether (Niwa et al., 1988) was made acidic to Methyl Red with HCl in methanol. To the solution was added 0.12 g (1.90 mmol) of NaBH$_3$CN, and then the mixture was stirred for 1 h at room temperature, with a methanol solution of HCl being added dropwise to keep it acidic. The reaction mixture was diluted with water, neutralized with 2 N Na$_2$CO$_3$, and extracted with diethyl ether. The extract was

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washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was put on a silica gel column that was eluted with 10% ethyl acetate in n-hexane, giving 0.30 g (96%) of 1 as an oil: ^1H NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz, $CH_2CH_2CH_3$), 3.21 (t, 2 H, J = 5.5 Hz, CH_2NH), 3.61 (t, 2 H, J = 6.5 Hz, $NHOCH_2$), 4.03 (t, 2 H, J = 5.5 Hz, $ArOCH_2$), 5.3 (br, 1 H, NHO).

Other Hydroxylamines (2–9, 11–18). By analogy with the preparation of 1, other hydroxylamines was synthesized in 60–99% yields from **26–32** and appropriate oximes (Niwa et al., 1988).

N-Butyl-2-(4-phenoxyphenoxy)ethylamine (19). One milliliter of butylamine was added to a methanol solution (10 mL) of (4-phenoxyphenoxy)acetaldehyde (1.00 g, 4.39 mmol) that had been obtained by hydrolysis of (4-phenoxyphenoxy)acetaldehyde diethyl acetal (Niwa et al., 1988). The solution was made acidic to Bromothymol Blue with methanol solution of HCl. To the solution was added 0.30 g (4.41 mmol) of $NaBH_3CN$, and then the mixture was stirred for 12 h at room temperature, with HCl in methanol being added dropwise to keep it acidic. The reaction mixture was dissolved in water, made strongly basic with 4 N NaOH, and treated with diethyl ether. The ether layer was washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography with 30% ethanol in ethyl acetate, giving 0.80 g (64%) of 19 as an oil: ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 6.5 Hz, $(CH_2)_3CH_3$), 1.15 (s, 1 H, NH), 2.60 (t, 2 H, J = 6.5 Hz, $NHCH_2Pr$), 2.92 (t, 2 H, J = 6.5 Hz, CH_2NH), 3.98 (t, 2 H, J = 6.5 Hz, $ArOCH_2$).

N-Propyl-3-(4-phenoxyphenoxy)propylamine (20). To a solution of 1 mL of propylamine in 20 mL of THF was added 0.20 g (5.00 mL) of 60% NaH. After bubbling was stopped, a THF solution (10 mL) of 1-bromo-3-(4-phenoxyphenoxy)propane (1.00 g, 3.26 mmol), which had been prepared from 4-phenoxyphenol and 1,3-dibromopropane, was added to the solution. The mixture was stirred for 6 h at refluxing temperature, poured into water, and treated with diethyl ether. The ether layer was extracted with 2 N HCl, and then the aqueous layer was made strongly basic with aqueous NaOH and extracted with diethyl ether. The extract was washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was put on a silica gel column that was eluted with 30% ethanol in ethyl acetate, yielding 0.74 g (80%) of 20 as an oil: ^1H NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz, $(CH_2)_2CH_3$), 1.16 (s, 1 H, NH), 2.54 (t, 2 H, J = 6.5 Hz, $NHCH_2Et$), 2.74 (t, 2 H, J = 6.5 Hz, CH_2NH), 3.92 (t, 2 H, J = 6.5 Hz, $ArOCH_2$).

N-Ethyl-4-(4-phenoxyphenoxy)butylamine (21). Amine 21 was prepared in 71% yield from 1-bromo-4-(4-phenoxyphenoxy)butane (Niwa et al., 1988) and ethylamine, by analogy with compound 20: ^1H NMR ($CDCl_3$) δ 1.09 (t, 3 H, J = 7 Hz, CH_2CH_3), 3.86 (t, 2 H, J = 6.5 Hz, $ArOCH_2$).

N-Isobutyl-3-(4-phenoxyphenoxy)propylamine (22). By analogy with compound 20, amine 22 was obtained in 43% yield by alkylating isobutylamine with the same halide: ^1H NMR ($CDCl_3$) δ 0.90 (d, 6 H, J = 6.5 Hz, $CH(CH_3)_2$), 1.19 (s, 1 H, NH), 2.38 (d, 2 H, J = 6.5 Hz, $NHCH_2$), 2.74 (t, 2 H, J = 6.5 Hz, CH_2NH), 3.94 (t, 2 H, J = 6 Hz, $ArOCH_2$).

O-Isopropyl-N-methyl-N-[2-[4-(3-methylphenoxy)phenoxy]ethyl]hydroxylamine (10). A solution of 9 (0.85 g, 2.70 mmol) in 10 mL of methanol was mixed with 1.00 g (7.40 mmol) of methyl iodide and 0.40 g (2.90 mmol) of K_2CO_3. After being stirred for 2 days at room temperature, the mixture was poured into water and extracted with diethyl ether. The organic layer was washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. The crude residue was purified with silica gel column chromatography with 5% ethyl acetate in n-hexane, to give 0.59 g (66%) of 9 as an oil: ^1H NMR ($CDCl_3$) δ 1.12 (d, 6 H, J = 6 Hz, $CH(CH_3)_2$), 2.0 (m, 2 H, $CH_2CH_2CH_2$), 2.25 (s, 3 H, $ArCH_3$), 2.53 (s, 3 H, NCH_3), 2.79 (t, 2 H, J = 6.5 Hz, CH_2NH), 3.99 (t, 2 H, J = 6.5 Hz, $ArOCH_3$), 3.8 (m, 1 H, –OCH<).

N-Butyl-N-methyl-2-(4-phenoxyphenoxy)ethylamine (23). To a DME solution (10 mL) of 0.55 g (1.93 mmol) of secondary amine 19 were added 0.33 g (2.32 mmol) of methyl iodide and 0.30 g (2.17 mmol) of K_2CO_3. After the mixture was stirred for 2 h at room temperature, it was diluted with water and treated

with diethyl ether. The ether layer was washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. The oily residue was put on a silica gel column that was eluted with 5% aqueous ammonia in ethyl acetate, giving 0.19 g (33%) of **23** as an oil: ^1H NMR ($CDCl_3$) δ 0.89 (t, 3 H, J = 7 Hz, $(CH_2)CH_3$), 2.31 (s, 3 H, NCH_3), 2.40 (t, 2 H, J = 6.5 Hz, $NHCH_2Pr$), 2.72 (t, 2 H, J = 6.5 Hz, CH_2NH), 3.92 ($ArOCH_2$).

N-Methyl-N-propyl-3-(4-phenoxyphenoxy)propylamine (24). Tertiary amine 24 was obtained in 46% yield by methylation of secondary amine 20 with MeI, by analogy with the preparation of 23: ^1H NMR ($CDCl_3$) δ 0.86 (t, 3 H, J = 7 Hz, $(CH_2)_2CH_3$), 1.5 (m, 2 H, $CH_2CH_2CH_3$), 2.0 (m, 2 H, $CH_2CH_2CH_2$), 2.20 (s, 3 H, NCH_3), 3.92 (t, 2 H, J = 6 Hz, $ArOCH_2$).

N-Ethyl-N-methyl-4-(4-phenoxyphenoxy)butylamine (25). By analogy with the preparation of **23**, tertiary amine 25 was obtained in 26% yield by methylation of secondary amine 21: ^1H NMR ($CDCl_3$) δ 1.03 (t, 3 H, J = 7 Hz, CH_2CH_3), 1.7 (m, 4 H, $CH_2(CH_2)_2CH_2$), 2.19 (s, 3 H, NCH_3), 3.89 (t, 2 H, J = 6 Hz, $ArOCH_2$).

In the synthesis of oximes, both E and Z isomers were produced, the ratio was estimated to be about 1:1 from ^1H NMR examination (Nakayama et al., 1985). The oximes could not be separated by conventional chromatographic technique, and thus they were bioassayed as mixtures of both isomers (Table IV). Propionaldoxime O-2-(4-phenoxyphenoxy)ethyl ether (31) is a gift of Sumitomo Chemical Industries Co., Ltd. All of the previously unknown compounds were identified by elemental analyses for C, H, and N within ±0.3% error. This information is available as supplementary material.

Bioassay Procedure. Fourth-larval instars of *C. pipiens pallens* Coquillet were selected from colonies maintained at 28 °C in water that contained a feed mixture of mouse food and dry yeast. The eggs were a gift of the Sumitomo Chemical. Three batches of 20 larvae each were transferred to disposable plastic tumblers containing 100 mL of water. An ethanol solution (10 µL) of the test compounds then was added to the tumblers, after which the diet powder was added. The tumblers were covered with transparent plastic cups to prevent the adults from flying away. After 7 days at 28 °C, the results were scored as percentages of unemerged adults, including those that could escape only partly from the pupal cuticles. The experimental results in the bioassays were confirmed mostly by replication at concentrations at which high ratings (usually more than 50% inhibition of metamorphosis) were recorded, but experiments were usually not repeated at concentrations recorded for lower activity. When an abnormal rating was found, repetitions were made at that concentration and those nearby. When more than one abnormal rating was obtained, the experiment was repeated for the entire concentration range. All the data, excluding the abnormal value, were averaged. The nonemergence percentage of the control (no chemicals added except 10 µL of ethanol) was less than 10% through the runs of the experiments.

The activity was expressed in terms of pI_{50} (M), the logarithm of the reciprocal of the concentration at which 50% inhibition of metamorphosis is observed. The data are summarized in Tables I–IV.

RESULTS

O-Alkyl-N-[(4-phenoxyphenoxy) and (4-benzylphenoxy)alkyl]hydroxylamines. The set of compounds in Table I was prepared by simply reducing the corresponding oximes (Niwa et al., 1988) with sodium cyanoborohydride. Since the optimum molecular length for JH-mimetic activity has been suggested to be about 21 Å by quantitative structure–activity relationship analysis of terpenoid compounds (Nakayama et al., 1984) and proved valid through a number of developmental works (Nakayama et al., 1985; Niwa et al., 1988, 1989; Hayashi et al., 1989), we confined compounds to be prepared here to those that satisfy this condition, that is phenoxyphenoxy and benzylphenoxy compounds with a side chain made up with seven atoms.

First prepared were N-(phenoxyphenoxy)ethyl com-

**Table I. JH-Mimetic Activity of
O-Alkyl-*N*-[(4-phenoxyphenoxy) and
(4-benzylphenoxy)alkyl]hydroxylamines**

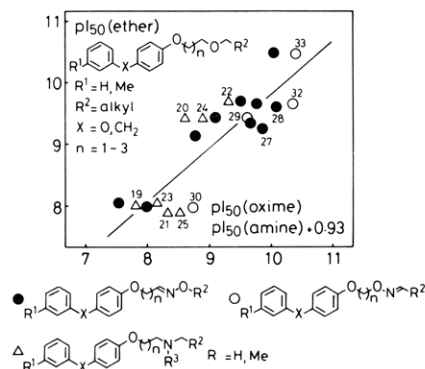| no.	structure	pI_{50}, M
1		8.28
2		8.51
3		8.76
4		8.24
5		8.66
6		9.53
7		9.95
8		9.85
9		10.00
10		8.92
11		9.49
12		9.60
13		9.95
		10.49
		10.00

Figure 3. Plots of activity of ethers against those of oximes and amines. Plotting was made between the types of compounds having the same structural units, R_1, R_2, X, and *n*. For amines, pI_{50} of ethers was plotted against $pI_{50} + 0.93$ to correct the uniform reduction of their activity. The line was drawn according to eq 5. The points made against the oximes and amines prepared in this study were indicated by attaching the compound number. The points with no mark were made between previously reported compounds.

pounds 1–5 to examine the intrinsic potency of the hydroxylamine structure as well as to examine whether or not the structural conditions for activity suggested in our previous reports do work as well. The activity of the simplest member, *O*-(*n*-propyl) compound **1**, was fairly high, and introduction of a methyl branch at the chain end and at the meta position of the terminal benzene moiety gave the somewhat more potent compounds **2** and **3**, respectively. The favorable bulkiness effect at the molecular ends was shown to be operative in the hydroxylamines as was so in the previous series of JH mimics mentioned above. Benzylphenoxy compounds **4** and **5** had an extent of activity similar to that of the congeneric **1** and **2**.

N-(Phenoxyphenoxy)propyl compounds **6–8** showed about 10 times higher potency than the corresponding ethyl compounds **1–3**. The optimization of the structure, i.e., introduction of a methyl branch at both terminals, gave compound **9**, the pI_{50} value at the highest level

known so far aganst *C. pipiens*. N-Methylation of this compound gave compound **10** whose activity was about 10 times less than that of the parent. This may be due to an unfavorable steric interaction between the two methyls at the nitrogen and terminal carbon atoms. Benzylphenoxy derivatives **11–13** corresponded in activity to their phenoxyphenoxy congeners **6–8**.

N-Alkyl-O-[(4-phenoxyphenoxy)alkyl]hydroxylamines. The preparation was again made by simply reducing the corresponding oximes; some of the starting oximes have been reported by Ohsumi et al. (1985). The activities of *O*-(phenoxyphenoxy)ethyl compounds **14** and **15** were more than 10 times higher than that of the corresponding propyl derivatives **17–18** (Table II). The optimization of the structure was thus made for the former series, affording compound **16**. Its activity was as high as that of **9**.

It was noted that through this and the above series of hydroxylamine compounds those with a nitrogen atom δ from the phenoxyphenoxy or benzylphenoxy oxygen atom have higher activity than those with a δ-oxygen atom. This led us to prepare the amine analogues to follow.

N-Alkyl-N-(4-phenoxyphenoxy)alkylamines. Among (phenoxyphenoxy)ethyl-, -propyl-, and -butylamines, **19–21**, where the overall molecular length is kept constant, compound **20** with an amine nitrogen atom at the δ-position had the highest activity, coinciding with the observation made above. The potency itself was, however, about 100 times poorer than that of the corresponding hydroxylamines **6** and **14**. Introduction of a methyl branch to the side chain end of **20** raised the potency several times (compound **22**), but it was still dozens of times less than that of the corresponding **7**. N-Methylation did not alter the potency much (compounds **23–25**).

Relation between Activities. To examine the relation between the action modes of classes of JH mimetics, we made activity vs activity plots between previous ether and oxime compounds and between oximes and these hydroxylamines. The plotting was made between the two types of compounds having the same molecular dimensions or the same structural units, R^1, R^2, X, and *n*, as shown in Figures 3 and 4. The structural correspondence was made on the basis of above interpretation, so that in Figure 3 the nitrogen atom of oximes overlaps
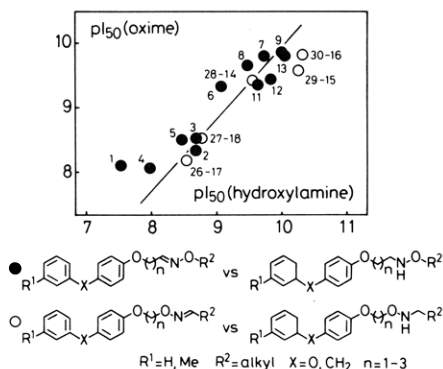
Figure 4. Activity vs activity plots between oximes and hydroxylamines. Plotting was made as described in Figure 3. For the points made between the oximes and hydroxylamines prepared in this study and listed in Tables III and IV, the compound numbers are indicated in pair. The line was drawn according to eq 4.

with the oxygen atom of ethers and both nitrogen atoms of oximes and hydroxylamines overlap each other in Figure 4.

In Figure 3 plots were first made between (4-phenoxyphenoxy)- and (4-benzylphenoxy)alkyl alkyl ethers and the corresponding alkanaldoxime *O*-ethers (closed circles). Most of the data were taken from the literature (Niwa et al., 1988, 1989), but some, compounds **26–28**, were prepared for this study (Table IV). The least-squares line for them was as follows:

$$pI_{50}(\text{ether}) = 0.75 pI_{50}(\text{oxime}) + 2.73 \quad (1)$$
$$\underset{(0.31)}{} \qquad\qquad \underset{(2.91)}{}$$

$$n = 10, r = 0.89, s = 0.36$$

The *n*, *r*, and *s* in this and following equations are numbers of data points, correlation coefficients, and standard deviations, respectively, and the figures in parentheses are the 95% confidence intervals. Since the coefficient of the $pI_{50}(\text{oxime})$ term is about unity within the error range, both series of compounds are considered to have a 1:1 correspondence in the structure–activity relation. Moreover, the points made between ethers and alkanaldoxime *O*-2-(4-phenoxyphenoxy)alkyl ethers **30–33** were about on the line as shown by Figure 3 (open circles). This suggests that ethers also have a 1:1 relation to the compounds where the oxime function is reversely built in. The regression in inclusion of them gave eq 2; it was essentially the same as eq 1. The synthesis and activity test of the oximes **29**, **31**, and **32** have been reported previously (Ohsumi et al., 1983, 1985), but those in this study were made anew in our laboratory together with those of previously unknown **30** and **33**. The data are summarized in Table IV.

$$pI_{50}(\text{ether}) = 0.82 pI_{50}(\text{oxime}) + 1.70 \quad (2)$$
$$\underset{(0.28)}{} \qquad\qquad \underset{(2.65)}{}$$

$$n = 14, r = 0.88, s = 0.40$$

The relation between oximes and hydroxylamines is shown in Figure 4. The regression line with *O*-alkyl-*N*-(4-phenoxyphenoxy and 4-benzylphenoxy)hydroxylamines is given by eq 3, and that in inclusion of reversely substituted *N*-alkyl-*O*-(4-phenoxyphenoxy)hydroxylamines is given by eq 4. Again the slopes of about unity in each line indicate the 1:1 correspondence between the classes of compounds.

Since the number of amines is relatively scarce, the relation of them alone with other classes of compounds is not fully reliable. Thus, we finally incorporated them

Table II. JH-Mimetic Activity of *N*-Alkyl-*O*-[(4-phenoxyphenoxy)alkyl]hydroxylamines

no.	structure	pK_{50}, M
14		9.55
15		9.71
16		10.00
17		8.34
18		8.66

$$pI_{50}(\text{oxime}) = 1.13 pI_{50}(\text{hydroxylamine}) - 1.30 \quad (3)$$
$$\underset{(0.30)}{} \qquad\qquad\qquad \underset{(1.13)}{}$$

$$n = 12, r = 0.94, s = 0.31$$

$$pI_{50}(\text{oxime}) = 1.14 pI_{50}(\text{hydroxylamine}) - 1.26 \quad (4)$$
$$\underset{(0.26)}{} \qquad\qquad\qquad \underset{(1.14)}{}$$

$$n = 17, r = 0.93, s = 0.33$$

into eq 2, since the number of structurally corresponding ethers reported is more than that of oximes. In the resultant eq 5, I_a is the indicator variable that takes 1

$$pI_{50}(\text{ether}) = 0.89 pI_{50}(\text{oxime, amine}) + 0.93 I_a \quad (5)$$
$$\underset{(0.29)}{} \qquad\qquad\qquad\qquad \underset{(0.68)}{}$$

$$n = 21, r = 0.93, s = 0.43$$

for amines and 0 for oximes. Dismemberment of the I_a term into secondary and tertiary amines was not necessary. The results suggest that amines also have the same fashion of interaction with the receptor with ethers. In Figure 3, the pI_{50} value of ethers was plotted against $pI_{50}(\text{amine}) + 0.93$, the figure being the coefficient value of the I_a term and correcting the activity difference due to the quarternization.

DISCUSSION

The potency of the highest active member of the two classes of hydroxylamines, *O*-alkyl-*N*-[(4-phenoxyphenyl) and (4-benzylphenoxy)alkyl]hydroxylamine and *N*-alkyl-*O*-[(4-phenoxyphenoxy)alkyl]hydroxylamine, reached the 10^{-10} M level, the highest level known so far against *C. pipiens* (Tables I and II). This suggests that the hydroxylamine structure is bioisosteric to oxime and ether with respect to JH-mimetic activity.

The positional effect of these functions was made clear through this study. The heteroatom–receptor interaction is most favorable at δ-position from the phenoxyphenoxy or benzylphenoxy oxygen or at the 4-position from the side-chain terminal when a molecule is constructed optimum in its length (about 21 Å). This situation was shown in Tables I and II by shadowing the pertinent atoms or the site. The activity is most potent when a nitrogen atom, rather than an oxygen atom, is at this position in these hydroxylamines and previous oximes.

To examine the effects of the nitrogen atom itself, we prepared a set of amines (Table III). The position specificity was again clear in the dialkylamine series **19–21**, compound **20** with a nitrogen atom at the δ-position being

Table III. JH-Mimetic Activity of
***N*-Alkyl-*N*-(4-phenoxyphenoxy)alkylamines**

no.	structure	pI_{50}, M
19		6.88
20		7.68
21		7.36
22		8.37
23		7.23
24		7.93
25		7.55

Table IV. JH-Mimetic Activity of Alkanaldoxime and Ketoxime *O*-Ethers

no.	structure	pK_{50}, M
26		8.72
27		9.86
28		10.06
29		8.55
30		8.76
31		9.59
32		10.31
33		10.35

Figure 5. Binding model for JH-active compounds. The compound accommodated in A1 and A2 is 3-[4-(3-methylphenoxy)phenoxy]propyl isobutyl ether. The structure was drawn according to the CPK molecular model, and the ends of the bars represent hydrogen atoms. In A1, the terminal benzene and zigzag chain moieties were placed on the page plane, and A2 is the view from its rectangular side. The solid lines show the steric interaction sites or spatial walls. The striped region at the right end in A1 is the wall located in the upward (or downward) direction, shown in A2 as a continuous entity with the right end wall. The striped part of the left end cave shown in A1 is expressed in A2 as a striped region. The shadowed circle indicates the position-specific interaction site with a heteroatom of a JH molecule. The broken circles are the positions of the molecule where an introduction of a substituent or a branch causes depression of the activity. The structure in B is *O*-isopropyl-*N*-{3-[4-(3-methylphenoxy)phenoxy]propyl}hydroxylamine (9).

most active but the other two less potent. The efficacy itself was, however, dozens to one hundred times less than that of corresponding hydroxylamines. N-Methylation affected it little as seen by comparing the activities of **19**, **20**, and **21** with those of **22**, **23**, and **24**, respectively. This suggests that the acidic amino proton bears little responsibility for the weak potency. It could be rather attributable to the basicity difference. The pK_a values of *N,N*-dimethyl- and *N,N,N*-trimethylamines have been reported by Bissot et al. (1957) to be 10.67 and 9.74, respectively, but it is 4.75 in *N,O*-dimethyl- and 3.65 in *N,N,O*-trimethylhydroxylamines. Thus, the amines could be quarternized or protonated at physiological pH, but the others, ethers, oximes, and hydroxylamines are not. The
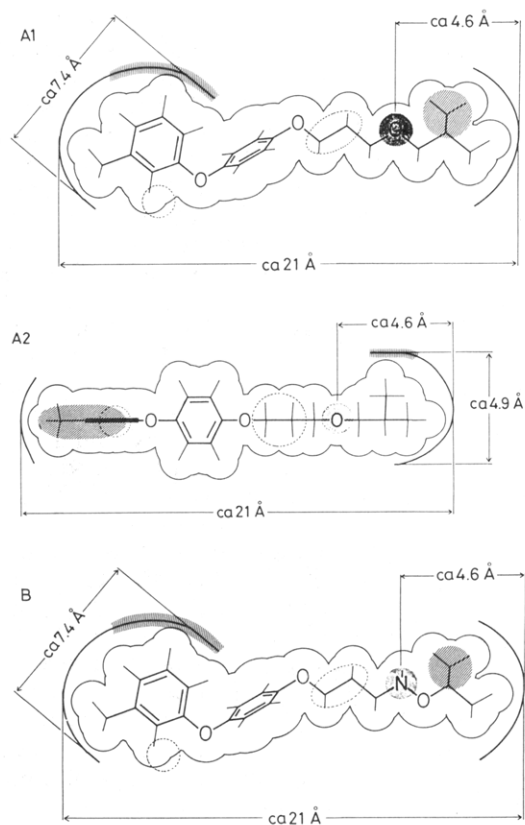
cationic species is thought to be unfavorable in interaction with the receptor or in permeability processes or in both. If the amines had not been protonated, they would show a higher activity than the previous ether congeners.

In each combination of the activity vs activity plots made between structurally corresponding ether, oxime, hydroxylamine, and amine compounds in Figures 3 and 4, the points stand within an error range in a single line with a slope of about unity. The results appear to show that these classes of compounds have a 1:1 relation with respect to the activity expression; in other words, they have a common fashion of interaction with the receptor. This provides the basis for the interpretation made above; their point interaction sites are in common.

Finally, we drew an inclusive mode of action map or receptor map of JH-active molecules in Figure 5. It is based on the map previously drawn for 2,4-dodecadienoate compounds (Nakayama et al., 1984) and on the knowledge obtained through the development and analyses of the alkanaldoxime (Niwa et al., 1988; Hayashi et al., 1989) and ether (Niwa et al., 1989) types of com-

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pounds. The compound accommodated in Figure 5A is the extended form of 3-[4-(3-methylphenoxy)phenoxy]propyl isobutyl ether whose pI_{50} (M) against *C. pipiens* is 10.5 (Niwa et al., 1989). Figure 5A1 is the view when the zigzag plane of the molecule is placed on the page plane, and Figure 5A2 is that from the rectangular direction. The angle between the two benzene rings was set up at 90° so as to minimize the mutual overlap between ortho substituents. Incidentally, recent molecular orbital calculations have shown that the two benzenes are perpendicular to each other in the lowest energy conformation of phenoxyphenoxy- and (benzylphenoxy)alkanaldoximes (Nakayama and Richard, 1987). The solid lines indicate the steric interaction sites or the receptor walls that encompass the molecule of optimum length, about 21 Å. The shadowed region at the alkyl end in A1 shows the site where the receptor walls are located in vertical directions; they are plausibly continuous with the wall at the side chain end and form a cave of about 4.9 Å across as seen in A2. It has been also suggested that there exists an optimum width, about 7.4 Å, for activity for the phenoxy end; it is indicated by a curved line in A1 and by a shadow in A2. The sites enclosed by the broken circle mean that the activity is depressed if a conformational distortion is brought about by introducing there a substituent or a branch (Niwa et al., 1989).

To this summary of previous results, a shadowed circle was added to show the present results, the position-specific interaction site with a heteroatom of JH molecules; it is calculated to be at about 4.6 Å distant from the side chain edge in the optimum molecules. Figure 5B is the accommodation of hydroxylamine compound 9, giving the comparison with the ether compound. In Figure 5A1, the atom that fits the point–interaction site is oxygen, but in Figure 5B it is nitrogen. Examinations like this of the structural correspondence would be of value or help in evaluating structure–function profiles of a number of other JH-mimetic compounds already known (Henrick, 1982) and also those of prospective ones.

The physicochemical basis for the point–interaction remained unclear. It is, however, likely an electronic one, since the hydrophobicity is so different between the functions that it does not explain their bioisosteric nature, and the steric dimensions are the same or at least nearly the same between a δ-compound and its position isomers. On the basis of the present results, the modes of action for species other than *C. pipiens* would be evaluated, if the activities were examined and compared between a common set of compounds. Both are problems to be worked out in future.

Supplementary Material Available: Table of analytical data for hydroxylamines, amines, and oximes (2 pages). Ordering information is given on any current masthead page.

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