Synthesis and Biological Activities of Substituted 2-Alkoxy-1,3,2-thiazaphospholidine 2-Sulfides

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INTRODUCTION

Finding of L-leucine as an insect neuroactive substance1 led us to synthesize insecticidal five-membered cyclic phosphorothionates such as 4-isobutyl-2-methoxy-1,3,2-oxazaphospholidine 2-sulfide (iBMOS) after numerous modifications of the chemical structure.2) Recently 2-methoxy-5-phenyl-1,3,2-oxazaphospholidine 2-sulfide (5-PMOS) derived from 2-amino-1-phenylethanol (APE), an octopamine analog, was found to be a stronger insecticide than iBMOS,3) iBMOS and 5-PMOS showed an insecticidal activity to the housefly (Musca domestica) and the flour beetle (Tribolium castaneum) by inhibiting acetylcholinesterase (AChE) at lethal concentrations.4) They reduced the larval growth and gut-trehalase activity, and increased larval whole-body cAMP levels of T. castaneum5) and M. domestica6) at sublethal concentrations. 5-PMOS and the related compounds did not activate the adenylate cyclase prepared from ventral nerve cords of Periplaneta americana, but had a phosphodiesterase-inhibitory activity and an antagonistic effect on the adenylate-cyclase-linked octopamine receptor.6) In our preliminary experiments, the effect of heteroatoms (O, S and N) on the insecticidal activity of cyclic phosphorus compounds was examined, and certain thiazaphospholidines (N and S) were found to be more potent insecticides than the corresponding oxazaphospholidines (N and O) and oxathiaphospholanes (O and S) against M. domestica by topical application.7,8) Actually, some thiaza-
phospholines have been reported to have a high insecticidal property against the flour beetle (Triobolium confusum) and the fruit fly (Drosophila melanogaster)." Hence, thiazaphospholide analogs of iBMOS and 5-PMOS, 4-isobutyl-2-methoxy-1,3,2-thiazaphospholidine 2-sulfide (iBMTS) and 2-methoxy-5-phenyl-1,3,2-thiazaphospholidine 2-sulfide (5-PMTS) are expected to be the most potent insecticides in these series. This paper describes the synthesis of 1,3,2-thiazaphospholides and their biological activities, including the insecticidal activity against M. domestica, larval-growth-inhibitory activity against T. castaneum and the effect on adenylate-cyclase activity of P. americana ventral nerve cords. The effect of heteroatoms (S versus O) on the biological activities is also examined: the relative potency of thiazaphospholides versus oxazaphospholines as insecticides and octopaminergic agonists or antagonists is compared.

MATERIALS AND METHODS

1. Apparatus

All melting points (mp) were measured on an MRK (Mitamura Riken Kogyo) apparatus and uncorrected. 1H NMR was measured with a JEOL JNM-FX100 spectrometer at 100 MHz. Tetramethyl silane (TMS) was used as an internal standard, and chemical shift values are expressed in ppm. Mass spectra (MS) were obtained by a JEOL JMS-DX300 spectrometer with a JEOL-JMA3500 data-processing system at an ionizing voltage of 30 eV. Ion peaks are expressed in m/z followed by the percentage of intensity in parentheses.

2. Chemicals

Fenitrothion (dimethyl 3-methyl-4-nitrophenyl phosphorothionate, 96.5% pure) was a gift from Sumitomo Chemical Co., Ltd. (Takarazuka, Japan). Ethylene glycol bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), octopamine [1-(p-hydroxyphenyl)-2-aminoethanol] and theophylline (1,3-dimethyl-xanthine) were purchased from Nacalai Tesque Inc. (Kyoto, Japan), guanosine 5’-triphosphate (GTP) from Sigma Chemical Co., Ltd. (St. Louis, MO), adenosine 5’-triphosphate (ATP) disodium salt from Kohjin Co., Ltd. (Tokyo, Japan), 2-aminoethanethiol (cysteamine) from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) and RIA kit (Code YSI-7701) from Yamasa Shoyu Co., Ltd. (Chiba, Japan), respectively.

3. Synthesis of 4-Isobutyl-2-methoxy-1,3,2-thiazaphospholidine 2-Sulfide (iBMTS)

Conc. sulfuric acid (2.0 g, 20 mmol) was added to l-leucinol (2.3 g, 20 mmol) at 40°C. The mixture was heated at 160°C (1 mmHg) until the viscous syrup crystallized and re-crystallized from MeOH to give a product (0.96 g, 25%), mp 281–282°C. The mixture of 1,2-amino-4-methylpentyl hydrogen sulfate (0.96 g, 5.0 mmol) thus obtained contained carbon disulfide (0.42 g, 6.5 mmol) dissolved in 50% EtOH (10 ml) and a solution of sodium hydroxide (0.50 g, 12.5 mmol) in water (5 ml) was heated under reflux for 40 min. The organic solvent was removed by rotary evaporation and the aq. phase was extracted with ethyl acetate. The combined organic layer was dried over anhyd. sodium sulfate and evaporated to give a crude oil, which was purified by TLC (thin-layer silica-gel plate 60F-254, 20 x 20 cm, 0.5 mm thick, Merck, ethyl acetate–hexane = 1: 3), yielding a desired compound (0.47 g, 57%), mp 58-60°C. [α]D = -50° (0.1, CHCl3). Found: C, 48.12; H, 7.42; N, 7.86. Calcd. for C7H13NS2: C, 47.96; H, 7.47; N, 7.99%. MS: 118 (100), 175 (M+, 98). 1H NMR: 0.9-1.1 (6H, d, J=5.7 Hz, CH3), 1.5-1.9 (2H, m, CCH2C), 3.1-3.7 (2H, m, CH2S), 4.2-4.4 (1H, m, NCH), 7.6-7.8 (1H, s, SH).

2-Amino-1-butyl hydrogen sulfate (ABS) and 2-amino-2-methylpropyl hydrogen sulfate (AMS) were obtained similarly with yields of 39% and 27% from the corresponding β-aminoalcohols, respectively. ABS: mp 221-222°C (255-256°C). Found: C, 28.39; H, 6.40; N, 8.23. AMS: mp 265-267°C. Found: C, 28.39; H, 6.55; N, 8.28%. MS: 265-267°C. Found: C, 28.35; H, 6.47; N, 8.27. 4-Ethyl-2-mercaptothiazoline (ENS) and 4-diethylmercaptothiazoline (DNS) were prepared from ABS and AMS with yields of 84% and 25%, respectively. ENS: mp 47-48°C (48-49°C). Found: C, 40.86; H, 6.16; N,
9.43. Calcd. for C₅H₉NS₂: C, 40.78; H, 6.16; N, 9.51%. ¹H NMR: 1.01 (3H, t, J = 7 Hz, CH₃), 1.80 (2H, m, MeCH₂), 3.5–3.7 (2H, m, SCH₂), 4.21 (1H, m, NCH), 8.7 (1H, s, SH). DNS: mp 117–118°C (118–118.3°C). Found: C, 40.99; H, 6.13; N, 9.51%. ¹H NMR: 1.51 (6H, s, CH₃), 3.35 (2H, s, SCH₂), 8.00 (1H, s, SH).

3.2 L-2-Amino-4-methyl-1-pentanethiol (leucinethiol) hydrochloride

4-Isobutyl-2-mercaptothiazoline (0.44g, 2.5 mmol) in 18% hydrochloric acid (10ml) was heated under reflux for 1 week. Evaporation of hydrolysate gave a thick oil, which was solidified and recrystallized from EtOH-ether to give a desired product (0.22g, 52%), mp 222–224°C. Found: C, 42.51; H, 9.29; N, 8.12. Calcd. for C₆H₁₃CNS: C, 42.46; H, 9.50; N, 8.25%. MS: 86 (100), 133 (M⁺-HCl, 13).

2-Aminobutanethiol hydrochloride (ABT) and 2-amino-2-methyl-1-propanethiol hydrochloride (AMT) were synthesized similarly from ENS and DNS with yields of 76% and 49%, respectively. ABT: mp 126–127°C (136–138°C). Found: C, 33.99; H, 8.38; N, 9.76. Calcd. for C₄H₈CNS: C, 33.91; H, 8.54; N, 9.89%. AMT: mp 185–186°C. Found: C, 34.01; H, 8.15; N, 9.55. Calcd. for C₄H₉CNS: C, 33.91; H, 8.54; N, 9.89%.

3.3 4-Isobutyl-2-methoxy-1,3,2-thiazaphospholidine 2-sulfide (iBMTS)

To a mixture of L-leucinethiol hydrochloride (0.84g, 5.0 mmol) in 10 ml of water and methyl phosphorodichloridothionate (0.85 g, 5.0 mmol) in 10 ml of methylene chloride, sodium hydroxide (0.60g, 15 mmol) in 10 ml of water was added at 0°C. After the mixture was stirred at room temperature for 3 hr, the organic layer was separated. The aqueous layer was extracted with methylene chloride, and the combined organic layers were washed with water and dried over anhyd. sodium sulfate. Evaporation of the organic solvent gave a crude oil, which was purified by column chromatography (hexane-ethyl acetate=9:1), yielding a desired product (0.32 g, 28%). MS: 225 (M⁺, 100). ¹H NMR: 0.9–1.0 (6H, d, J = 5.7 Hz, CH₃), 1.2–1.8 (3H, m, CH₂CH₃), 3.0–3.5 (4H, m, SCH₂CH₃); 3.5–3.9 (3H, d, J = 15.7 Hz, CH₂O).

2-Ethoxy-4-isobutyl-1, 3, 2-thiazaphospholidine 2-sulfide (iBETS) was similarly synthesized from L-leucinethiol hydrochloride and ethyl phosphorodichloridothionate. MS: 162 (100), 239 (M⁺, 79). ¹H NMR: 0.9–1.0 (3H, d, J = 5.7 Hz, CH₂CH₃), 1.2–1.5 (3H, t, CH₂CH₃), 1.5–1.8 (3H, m, CH₂CH₂Me), 2.6–3.2 (2H, m, SCH₂), 3.5–4.0 (2H, m, CH₂O). Other substituted 2-ethoxy-1,3,2-thiazaphospholidine 2-sulfides prepared similarly from ethyl phosphorodichloridothionate with appropriate β-aminothiols showed reasonable ¹H NMR (data not shown) and elemental analytical or MS data (Table 1).

4. Rearing and Bioassay

4.1 Tribolium castaneum

The dietary effect of test compounds on the larval growth of T. castaneum was measured according to the previously published method.4,9-11) Twelve fourth-instar larvae of T. castaneum weighing 1.0±0.1 mg each were introduced into five replicates of test vials (2.5 cm in diameter, 20 ml) along with 1.5-g portions of a diet (47.5% milled wheat bran, 47.5% wheat flour and 5% dry yeast) treated with a test compound, and the vials were held at 30°C for 2 days before weight gain was determined. Assays run with solvent treatments containing no chemicals were used as control, and I₅₀ values were calculated with the PC-9801VM personal computer system using a program designed for log dose-probit larval-growth-inhibitory activity analysis.

4.2 Musca domestica

Three- to five-day-old WHO standard susceptible (SRS) female houseflies (M. domestica L.) fed on 5% sugar solution and maintained at 28°C at a relative humidity of 65–70% with a photoperiod of 12:12 (L:D) were topically treated on the ventral abdominal surface with an acetone solution (1µl/fly) of a test compound. The mortality was determined 24 hr after the treatment, and LD₅₀ values were calculated as mentioned above with a program designed for log dose-probit mortality analysis.

4.3 Periplaneta americana

Ventral nerve cords of adult American cockroaches (P. americana L.) of both sexes
were used for adenylate-cyclase assay. The insects were reared under crowded conditions in this laboratory at 28°C with a photoperiod of 12:12 (L:D) at a relative humidity of 65-70%. They were provided with an artificial mouse diet (Oriental Kobe Co., Ltd., Tokyo, Japan) and water ad libitum.

5. Adenylate Cyclase Assay

Ventral nerve cords of adult American cockroaches (P. americana) were homogenized (15 mg/ml) in 6 mM Tris-maleate buffer (pH 7.4). The homogenate was diluted to a volume of 15 ml in 6 mM Tris-maleate and centrifuged at 120,000×g for 20 min. The supernatant was discarded, and the pellet was resuspended by homogenization in 15 ml of buffer and again centrifuged at 120,000×g for 20 min. The resulting pellet (P2 fraction) was resuspended in a volume of 6 mM Tris-maleate equivalent to the starting amount. Adenylate-cyclase activity was measured by the previously reported method, in test tubes containing 50 μl of 480 mM Tris-maleate (pH 7.4) including 3 mM EGTA and 48 mM MgCl2, 120 ml of 25 mM theophylline, 20 μl of 1.5 mM GTP, 60 μl of P2 fraction and 20 μl of test-compound solution in polyethylene glycol. The enzyme reaction (5 min at 30°C) was initiated by adding 30 μl of 20 mM ATP, stopped by heating at 90°C for 2 min, and the reaction mixture was then centrifuged at 1000×g for 15 min to remove insoluble material. The cAMP level in the supernatant was measured by radioimmunoassay (RIA) with an aid of RIA kit and the protein concentration was determined by the Lowry method.

RESULTS AND DISCUSSION

1. Synthesis (Table 1 and Fig. 1)

iBMTS and iBETS were prepared by reacting phosphorodichloridothionate and L-leucinethiol, a potent inhibitor of leucine aminopeptidase from porcine kidney, which was obtained from L-leucinol via β-aminoalkyl hydrogen sulfate and 4-isobutyl-2-mercaptothiazoline followed by hydrochloric acid-catalyzed hydrolysis. Other substituted 2-ethoxy-1,3,2-thiazaphospholidine 2-sulfides were similarly obtained by reacting ethyl phosphorodichloridothionate with appropriate β-aminothiols. 5-PMTS has not been obtained yet by a similar method. Oxazaphos-

Table 1 Structure and analytical data of substituted 2-alkoxy-1,3,2-oxazaphospholidine and -thiazaphospholidine 2-sulfides.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substituent</th>
<th>Yield (%)</th>
<th>Molecular formula</th>
<th>Found (Calcd.) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R¹ R² X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-PMOS</td>
<td>5-Ph Me O</td>
<td>1.5772</td>
<td>C₆H₁₂NO₂PS</td>
<td>47.08 (47.16)</td>
</tr>
<tr>
<td>iBMOS</td>
<td>4-iBu Me O</td>
<td>1.5129</td>
<td>C₆H₁₄NO₂PS</td>
<td>40.21 (40.19)</td>
</tr>
<tr>
<td>iBMTS</td>
<td>4-iBu Me S</td>
<td>1.5600</td>
<td>C₇H₁₄NOPS₂</td>
<td>225.04 (225.04)</td>
</tr>
<tr>
<td>iBEOS</td>
<td>4-iBu Et O</td>
<td>1.4970</td>
<td>C₆H₁₄NO₂PS</td>
<td>43.04 (43.03)</td>
</tr>
<tr>
<td>iBETS</td>
<td>4-iBu Et S</td>
<td>1.5482</td>
<td>C₇H₁₄NOPS₂</td>
<td>239.057 (239.057)</td>
</tr>
<tr>
<td>EEO5</td>
<td>4-Et Et O</td>
<td>1.4998</td>
<td>C₆H₁₄NO₂PS</td>
<td>36.96 (36.91)</td>
</tr>
<tr>
<td>EETS</td>
<td>4-Et Et S</td>
<td>—</td>
<td>C₇H₁₄NOPS₂</td>
<td>211 (M⁺, 81)</td>
</tr>
<tr>
<td>DEOS</td>
<td>4-Me₂ Et O</td>
<td>1.4982</td>
<td>C₆H₁₄NO₂PS</td>
<td>36.95 (36.91)</td>
</tr>
<tr>
<td>DETS</td>
<td>4-Me₂ Et S</td>
<td>—</td>
<td>C₇H₁₄NOPS₂</td>
<td>211 (M⁺, 79)</td>
</tr>
<tr>
<td>HEOS</td>
<td>H Et O</td>
<td>1.5207</td>
<td>C₆H₁₄NO₂PS</td>
<td>28.86 (28.73)</td>
</tr>
<tr>
<td>HETS</td>
<td>H Et S</td>
<td>—</td>
<td>C₇H₁₄NOPS₂</td>
<td>26.04 (26.22)</td>
</tr>
</tbody>
</table>

a) High-resolution mass spectrum.
b) Ion peaks of mass spectrum are expressed in m/z followed by the percentage of intensity in parentheses.
pholidines prepared\(^2,3\) from phosphorodichloridothionates and the corresponding B-aminoalcohols in the presence of triethylamine showed reasonable \(^1\)H NMR (data not shown) and elemental analytical data (Table 1).

2. Insecticidal and Larval-Growth-Inhibitory Activities (Table 2)

Thiazaphospholidines had a higher insecticidal activity than the corresponding oxazaphospholidines against female housefly \(M. domestica\) by topical application: iBMTS, 4-ethyl-2-methoxy-1,3,2-thiazaphospholidine 2-sulfide (EMTS), iBETS, 2-ethoxy-4-ethyl-1,3,2-thiazaphospholidine 2-sulfide (SETS), 4-dimethyl-2-ethoxy-1,3,2-thiazaphospholidine 2-sulfide (DETS) and 2-ethoxy-1,3,2-thiazaphospholidine 2-sulfide (HETS) were more potent

![Fig. 1 Synthetic routes of substituted 2-alkoxy-1,3,2-oxazaphospholidine and -thiazaphospholidine 2-sulfides.](image)

**Table 2 Biological activity of substituted 2-alkoxy-1,3,2-oxazaphospholidine and -thiazaphospholidine 2-sulfides.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>(M. domestica) (LD_{50}) ((\mu g/)fly)</th>
<th>(T. castaneum) larval weight gain reduction, (I_{50}) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-PMOS</td>
<td>0.035 (0.030–0.040)(^{a,3})</td>
<td>43 (34–54)(^{a,3})</td>
</tr>
<tr>
<td>iBMOS</td>
<td>0.12 (0.10–0.13)</td>
<td>65 (57–74)</td>
</tr>
<tr>
<td>iBMTS</td>
<td>0.073 (0.061–0.088)</td>
<td>—</td>
</tr>
<tr>
<td>iBEOS</td>
<td>0.57 (0.35–0.79)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>iBETS</td>
<td>0.075 (0.060–0.095)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>EMOS</td>
<td>0.43(^{b})</td>
<td>—</td>
</tr>
<tr>
<td>EMTS</td>
<td>0.08(^{b})</td>
<td>—</td>
</tr>
<tr>
<td>EEOS</td>
<td>&gt;1</td>
<td>&gt;100</td>
</tr>
<tr>
<td>EETS</td>
<td>0.17 (0.14–0.21)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>DEOS</td>
<td>&gt;1</td>
<td>&gt;100</td>
</tr>
<tr>
<td>DETS</td>
<td>0.51 (0.34–0.77)</td>
<td>—</td>
</tr>
<tr>
<td>HEOS</td>
<td>&gt;1</td>
<td>&gt;100</td>
</tr>
<tr>
<td>HETS</td>
<td>0.55 (0.49–0.61)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Fenitrothion</td>
<td>0.051 (0.044–0.059)(^{a})</td>
<td>4.7 (4.3–5.2)(^{a})</td>
</tr>
</tbody>
</table>

\(M. domestica\) insecticidal activity was determined 24 hr after topical application and dietary effect of test compounds on the larval growth of \(T. castaneum\) was determined at 30°C 2 days after treatment. Ninety-five percent confidence limits are shown in parentheses.

\(^{a}\) Cited from Ref. 11).

\(^{b}\) Cited from Ref. 3).
(LD₅₀ = 0.073, 0.08, 0.075, 0.17, 0.51 and 0.55 µg/fly) than iBMOS (0.12 µg/fly), 4-ethyl-2-methoxy-1,3,2-oxazaphospholidine 2-sulfide (EMOS, 0.43 µg/fly), 2-ethoxy-4-isobutyl-1,3,2-oxazaphospholidine 2-sulfide (iBEOS, 0.57 µg/fly), 2-ethoxy-4-ethyl-1,3,2-oxazaphospholidine 2-sulfide (EEOS, >1 µg/fly), 4-dimethyl-2-ethoxy-1,3,2-oxazaphospholidine 2-sulfide (DEOS, >1 pg/fly) and 2-ethoxy-1,3,2-oxazaphospholidine 2-sulfide (HEOS, >1 µg/fly), respectively. In both oxazaphospholidines and thiazaphospholidines, methoxy derivatives, iBMOS, EMOS, iBMTS and EMTS, were more potent insecticides than ethoxy derivatives, iBEOS, EEOS, iBETS and EETS, respectively, consistent with the result of T. castaneum assay: iBMOS was a more potent (LD₉₀ = 65 ppm) larval-growth inhibitor than iBEOS (>100 ppm). Oxazaphospholidines with a more bulky hydrophobic substituent (phenyl at C₅ or isobutyl at C₄) were more potent as insecticides against M. domestica than those with a less bulky hydrophobic substituent (ethyl, dimethyl and hydrogen) at C₄. Introduction of a bulky hydrophobic branched-alkyl substituent (isobutyl) to thiazaphospholidines at C₄ was less effective than that to oxazaphospholidines in increasing the insecticidal activity. 5-PMOS (LD₅₀ = 0.035 µg/fly) and iBMOS were 12 and four times as potent as EMOS, respectively, and iBEOS was more than twice as potent as EEOS, whereas iBMTS was not a significantly stronger insecticide than EMTS, and iBETS was only twice as potent as EETS.

In the interaction of oxazaphospholidine with AChE, a serine hydroxy group at the esteratic site may attack the phosphorus atom to form a trigonal bipyramid, in which oxygen more electronegative than nitrogen is supposed to be located at an apical position, maintaining the interaction of substituent R₁ at the binding site (Fig. 2A), followed by P-O bond cleavage leading to inhibit AChE.² The distance between the phosphorus atom and the bulky hydrophobic branched-alkyl center of iBMOS or phenyl of 5-PMOS is about 5 Å, compatible with the hypothetical distance between the esteratic site and the binding site of insect AChE.²,¹¹,²² Furthermore, according to the structure-activity study of 5-PMOS and related compounds, an introduction of any substituents to the phenyl group of 5-PMOS was not favorable for the interaction of 5-PMOS with AChE, deteriorating the insecticidal activity.¹¹ This result was consistent with the hypothesis mentioned above.

The insecticidal activity of phosphorothionates depends on a combination of factors: intrinsic potency to inhibit AChE, bioactivation presumably by S-oxidation (thioether sulfur is more vulnerable to oxidation than phosphorothionate sulfur), detoxification of the parent compound and activated intermediate, aging differences in inhibited AChE.²³ Thiazaphospholidine 2-sulfides have two different types of sulfur atom: thiolate and thionate sulfurs, whereas oxazaphospholidine 2-sulfides have thionate sulfur only, and oxidation of the thiolate sulfur to sulfoxide and further to sulfone may contribute to the toxicity of thiazaphospholidines. Phosphorothiolate sulfoxide and sulfone are too unstable to be isolated or detected. The P-S bond may be
cleaved easily after oxidative bioactivation by an attack of serine hydroxy group at the esteratic site without forming a trigonal bipyramid (Fig. 2B). This may alleviate the interaction of substituent R₁ with AChE at the binding site and reduce the effect of introduction of a bulky hydrophobic substituent R₁ to thiazaphospholidines. Hence, the substituent R₁ of phosphorus moiety may contribute more than substituent R₁ to the insecticidal activity of thiazaphospholidines on the contrary to the case of oxazaphospholidines, where R₁ may do as well as R₂.

3. Effect on Adenylate Cyclase (Table 3)

Oxazaphospholidine 5-PMOS and thiazaphospholidine iBETS, EETS and HETS did not activate adenylate cyclase of P. americana ventral-nerve-cord homogenates but reduced octopamine-stimulated adenylate-cyclase activity. These results were contrary to those with 2-aminooxazolines and 2-aminothiazolines, which were acaricidal by acting as octopaminergic agonists rather than as AChE inhibitors,¹⁶,²⁴ but seem to be consistent with the previous reports on insecticidal salithion enantiomers¹⁴ and 5-substituted oxazaphospholidines,⁶ which significantly inhibited octopamine-stimulated adenylate cyclase in a dose-dependent manner at concentrations of 10–80 μM without affecting basal-enzyme activity. Hence the enzyme inhibition by oxazaphospholidines and thiazaphospholidines, which were partial octopaminergic antagonists lacking any agonist characteristics, was due to the interaction with the octopaminergic receptor. These phenomena, however, do not seem to be specific to cyclic phosphorothionates, since acyclic phosphorothionate fenitrothion showed a similar tendency.

Substituted 2-alkoxy-1,3,2-oxazaphospholidine 2-sulfides and the related compounds seem to be insecticidal by inhibiting AChE at lethal concentrations.⁴ At sublethal concentrations, they may affect various biological systems, e.g., octopamine-linked cAMP production system,⁴,¹⁶,¹⁴ phosphodiesterase-mediated cAMP degradation system⁶ and trehalase-trehalose system.⁴ In addition, there seems to be cross talk between these biological systems.¹⁵,²⁵,²⁶ Meanwhile taurine (2-aminoethanesulfonic acid), a possible oxidatively-metabolized product of HETS, is known as a neuromodulator in the insect central nervous system and has a depressant effect on the spontaneous activity of isolated nervous tissue at concentrations similar to those found in the cockroach and the locust.²⁷ Isoamylamine, which is derived from leucine, stimulates spontaneous activity considerably.

### Table 3 Effect of various compounds on octopamine-stimulated adenylate-cyclase activity in homogenates of American-cockroach nerve cords.

<table>
<thead>
<tr>
<th>Additive (100 μM)</th>
<th>cAMP pmol/min/mg protein</th>
<th>Relative to the control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alone</td>
<td>+ Octopamine (100 μM)</td>
</tr>
<tr>
<td>None</td>
<td>31±6</td>
<td>406±22 (3)</td>
</tr>
<tr>
<td>5-PMOS*</td>
<td>44±3</td>
<td>177±0 (2)</td>
</tr>
<tr>
<td>iBETS</td>
<td>18±1</td>
<td>144±8 (2)</td>
</tr>
<tr>
<td>EETS</td>
<td>22±1</td>
<td>284±13 (2)</td>
</tr>
<tr>
<td>HETS</td>
<td>—</td>
<td>361±9 (4)</td>
</tr>
<tr>
<td>Fenitrothion*</td>
<td>22±2</td>
<td>254±56 (2)</td>
</tr>
</tbody>
</table>

* Adenylate-cyclase activity was measured in the presence of various compounds (100 μM) as described in the text. Values are expressed as mean±range (2) or mean±SE (3–4), the number of replicates being shown in parentheses.

†‡ Difference significantly from each other within the same column at P=0.05 according to Duncan's multiple range test.³⁶

* Compound concentration of 1 mM was used.
when applied at a very low concentration (10^{-6} M), and it has been claimed to be a neurotoxic factor accumulating in the haemolymph of DDT-poisoned silkworm by Tashiro et al. in our laboratory,28,29) which Chang et al. have recently identified as tyramine,30) a precursor in biosynthetic pathway of octopamine. According to Hayakawa et al., increased concentrations of taurine in the cockroach haemolymph diminishes the calcium-dependent release of octopamine from the central nervous system.31) In order to clarify whether thiazaphospholidines and the related compounds possess such actions, more detailed experiments are in progress in our laboratory.

Stereochemistry plays an important role in studies on insecticide mode of action, metabolism and enzyme reactions, etc. Since 4- or 5-substituted oxazaphospholidine and thiazaphospholidine derivatives have asymmetric phosphorus and carbon atoms, there exist four optical isomers. Optical isomers of oxazaphospholidines were synthesized by a chiral two-step phosphorylating method,32) and their absolute configurations were determined by 1H and 31P NMR.33,34) The relationship between the insecticidal activity and the absolute configuration of these optical isomers were studied in detail.34,35) In our study racemates were used for biological assay including iBMOS (cis-isomer: trans-isomer = 58:42)2) and 5-PMOS (trans-isomer: cis-isomer = 54:46),36) and the biological activity and action mechanism of optical isomers of thiazaphospholidines will be reported elsewhere.

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要約

2-アルコキシ-1, 3, 2-チアザホスホリジン 2-スルフィドの合成と生物活性

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賀 紅武、江藤守総

チアサホスホリジン類は、アミノアルコールより硫酸エステルを経て得た 2-メルカプトチアゾリンの塩酸塩水解により合成したアミノチオールと、チオリン酸ジクロリドより合成した、チアサホスホリジン（SP）は、局所麻酔薬としてイエバエに対し、オキササホスホリジン（OP）より高い殺虫活性を示した。OP、SP とともに、メトキシン体はメトキシン体よりも活性が高く、よりかさ高い硫化水素置換基を 4 位に持つ誘導体もより高い殺虫活性を示したが、SP においては、かさ高い硫化水素置換基の導入による殺虫活性の増加は OP の場合よりも著しくなかった。OP、SP とともに、ゴキブリ神経アデニレートシンクラーゼを活性化しなかったが、オクトパミンに対して拮抗作用を示した。