Synthesis and biological activity of some 1,3,2-diheteraphosphorinanes and their acyclic analogs

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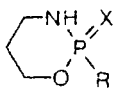
Methods were developed for the synthesis of 2-butylthio-2-oxo-1,3,2-oxazaphosphorinane, 2-butylthio-2-thioxo-1,3,2-dioxaphosphorinane, and 2-butylthio-2-thioxo-1,3,2-diazaphosphorinane, as well as of acyclic *S*-butyl *O*-ethyl (diethylamido)phosphorothioates and -dithioates and *S*-butyl bis(diethylamido)phosphorodithioate. These compounds can serve as models of possible metabolites of cyclic compounds. Based on the data obtained in studies of the antiesterase activity of the resulting compounds and their synergistic activity in mixtures with permethrine, a possible mechanism of *in vitro* and *in vivo* biological action of diheteraphosphorinanes was proposed.

Key words: 2-butylthio-2-thioxo-1,3,2-dioxaphosphorinane, 2-butylthio-2-thioxo-1,3,2-diazaphosphorinane, 2-butylthio-2-oxo-1,3,2-oxazaphopshorinane, *S*-butyl *O*-ethyl (diethylamido)phosphorothioates and -dithioates, *S*-butyl bis(diethylamido)phosphorodithioate, inhibition of esterases, synergists for permethrine, mechanism of action.

Previously,[1] we have reported the synthesis and physiological activity of 1,3,2-oxazaphosphorinane derivatives (**1** and **2**), among which active nematocides and synergists for insecticides of the pyrethroid group have been found.



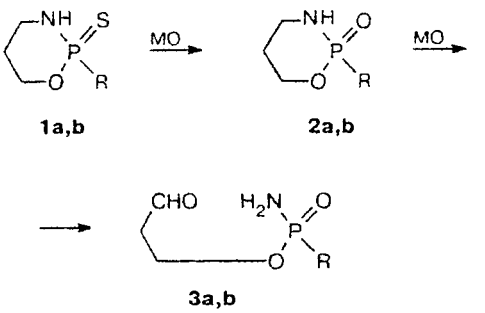
1a–c; 2a,b

1: X = S, **2**: X = O

a: R = $OC_6H_4NO_2$-3, **b**: R = SBu, **c**: R = OBu

Investigation of the mechanism of action of compounds **1** (R = OAr)[2] demonstrated that these compounds actively inhibit monooxygenases (MO), whereas their oxo analogs **2** (R = OAr) are very weak inhibitors of both human erythrocyte acetylcholinesterase (AChE) and cholinesterase from nerve tissues of American cockroaches (the bimolecular inhibition constants k_{II} are ~10^1–10^2 L mol^{-1} min^{-1}). Compounds **2** are somewhat more active with respect to less specific horse blood butyrylcholinesterase (BuChE) (k_{II} ~10^3 L mol^{-1} min^{-1}) and actively inhibit carboxyesterases (CE) from nerve tissues of American cockroaches (k_{II} ~10^4 L mol^{-1} min^{-1}). The weak ability of analogous inhibitors to suppress AChE results from steric hindrances to the nucleophilic attack at the OH group of serine (phosphorylation).[3] It was also demonstrated[2] that compounds **2** (R = OAr) can be further activated under the action of MO accompanied by ring opening at the C—N bond, resulting in the removal of steric hindrances to form a substantially more active inhibitor of esterases (for example, **3a** in the case of **2a**), which is unstable due to spontaneous β-elimination of acrolein (Scheme 1).

Scheme 1



1a,b **2a,b** **3a,b**

a: R = $OC_6H_4NO_2$-3; **b**: R = SBu

Actually, *O*-ethyl *O*-(3-nitrophenyl)amidophosphate (**4**), which is structurally similar to metabolite **3a** and yet

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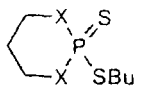
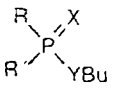
stable, inhibits esterases by three orders of magnitude more actively than oxo analog **2a**, which confirms the suggested scheme of metabolism of compounds of general formula **1**.[2] The ability of these compounds and their metabolites to suppress MO and CE (both enzymes detoxify pyrethroids in insects) is responsible for the synergistic activity.

Among compounds **1**, dithio derivative **1b** is the most active synergist for permethrine with respect to German cockroaches.[1] However, the data obtained in studies of the antiesterase activity of this compound and its oxo analog **2b** in kinetic experiments *in vitro* and in experiments on electrophoresis in polyacrylamide gel (the ability to suppress zones of CE activity) suggest that a somewhat different mechanism of action (compared to that described above) occurs. To elucidate this question, we synthesized a number of cyclic structural analogs of compound **1b** (**1c**, **5**, and **6**) and acyclic compounds (**7a—d** and **8a,b**) which are models of possible products of metabolism of compound **1b** and its analogs in insects.



5, 6



7a—d; 8a,b

5: X = O, **6**: X = NH

7: X = S, **8**: X = O

	a	b	c	d
R	Et_2N	EtO	EtO	Et_2N
R'	EtO	EtO	EtO	Et_2N
Y	S	S	O	S

Compounds **5** and **6** were prepared by the reactions of butyl dichlorophosphorodithioate (**9**)[4] with propane-1,3-diol or propane-1,3-diamine, respectively, in the presence of Et_3N (Eq. (1), Table 1).

$$\underset{\mathbf{9}}{BuSP(S)Cl_2} + Z(CH_2)_3Z \xrightarrow{Et_3N} \mathbf{5\ (6)} \qquad (1)$$

Z = OH, NH_2

Compound **2b** was synthesized analogously from *S*-butyl dichlorophosphorothioate (**10**) and 3-aminopropan-1-ol. The initial acid chloride **10** was prepared by the reaction of ethyl dichlorophosphite with butylsulfenyl chloride (Eq. (2), see Table 1).

$$EtOPCl_2 + ClSBu \longrightarrow \underset{\mathbf{10}}{BuSP(O)Cl_2} \qquad (2)$$

We failed to prepare amidophosphorodithioate **7a** from acid chloride **9** (the reaction gave rise to a mixture of products which were difficult to separate). Hence, compound **7a** was synthesized by the reaction of amidochlorophosphite with sodium butanethiolate followed (without isolation of intermediate amidothiophosphite) by the reaction with sulfur (Eq. (3), see Table 1).

$$Et_2N(EtO)PCl \xrightarrow{BuSNa} Et_2N(EtO)PSBu \xrightarrow{S} \mathbf{7a} \qquad (3)$$

The reaction of acid chloride **10** with ethanol in the presence of Et_3N afforded *S*-butyl *O*-ethyl chloro-

Table 1. Characteristics of the synthesized compounds

Compound	Yield (%)	B.p. /°C (*p*/Torr)	Found / Calculated (%) C	H	P	S	Molecular formula
2d	57	—[a]	39.91 / 40.18	7.48 / 7.71	14.74 / 14.80	14.40 / 15.32	$C_7H_{16}NO_2PS$
5	62	—[a]	37.45 / 37.15	6.50 / 6.68	13.27 / 13.69	—	$C_7H_{15}O_2PS_2$
6	65	—[a]	38.16 / 37.48	7.37 / 7.64	13.07 / 13.81	—[b]	$C_7H_{17}N_2PS_2$
7a	50	95—97 (1)[c]	44.49 / 44.58	9.18 / 8.98	11.29 / 11.50	24.11 / 23.80	$C_{10}H_{24}NOPS_2$
7d	55	—[a]	48.38 / 48.61	9.96 / 9.86	10.76 / 10.45	21.01 / 21.63	$C_{12}H_{29}N_2PS_2$
8a	72	89—90 (1)[d]	47.74 / 47.41	9.34 / 9.55	11.95 / 12.23	—	$C_{10}H_{24}NO_2PS$
10	90	80—81 (1)[e]	23.32 / 23.20	4.35 / 4.38	15.03 / 14.96	—	$C_4H_9Cl_2OPS$
11	70	84—85 (1)[f]	33.52 / 33.26	6.33 / 6.51	14.12 / 14.30	—	$C_6H_{14}ClO_2PS$

[a] Viscous oil, purified by chromatography.
[b] Found (%): N, 11.82. Calculated (%): N, 12.49.
[c] n_D^{20} 1.5082, d_4^{20} 1.0346.
[d] n_D^{20} 1.4741. [e] n_D^{20} 1.5010. [f] n_D^{20} 1.4885.

phosphorothioate (**11**) (see Table 1), whose reaction with Et_2NH gave rise to compound **8a** (Eq. (4), see Table 1).

$$\mathbf{10} \xrightarrow[Et_3N]{EtOH} \underset{\mathbf{11}}{BuS(EtO)P(O)Cl} \xrightarrow{Et_2NH} \mathbf{8a} \qquad (4)$$

We also failed to synthesize compound **7d** from acid chloride **9**. Hence, **7d** was prepared by the reaction of diamidochlorophosphite with BuSNa followed by the addition of sulfur without isolation of intermediate amidothiophosphite (Eq. (5), see Table 1).

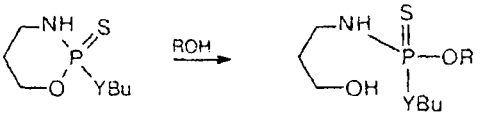
$$(Et_2N)_2PCl \xrightarrow{BuSNa} (Et_2N)_2PSBu \xrightarrow{S} \mathbf{7d} \qquad (5)$$

Unfortunately, attempts to prepare nitrogen-unsubstituted amides (analogs of amides **7a,d** and **8a**) in the pure form according to the above-described methods were unsuccessful due to the formation of large amounts of by-products which were difficult to separate. Compounds **1b,c**,[1] **7b**,[5] **7c**,[6] and **8b**[7] were synthesized according to procedures reported previously.

All compounds are non-toxic to houseflies (LD_{50} > 555 μg g^{-1}) and German cockroaches (*Blattella germanica* L., LD_{50} > 200 μg g^{-1}) but exhibit different synergistic actions (the joint action coefficients (JAC) were calculated) in mixtures with permethrine (the compound : permethrine ratio was 10 : 1). The antiesterase activities of the compounds with respect to AChE, BuChE, and cholinesterases from homogenates of nerve tissues of American cockroaches (*Periplaneta americana* L.) and housefly heads (*Musca domestica* L.) of the Cooper race (ChE_M) were determined by disk electrophoresis in polyacrylamide gel[8] and by Ellman's method.[9] Most of compounds exhibit a combined type of inhibition of esterases, and the irreversible component was characterized by true bimolecular inhibition constants (k_a).[10]

Cyclic compound **1b** proved to be a more active inhibitor of BuChE ($k_a = 6.9 \cdot 10^4$ L mol^{-1} min^{-1}) than its oxo analog **2b** ($k_a = 1.6 \cdot 10^4$ L mol^{-1} min^{-1}). An analogous situation was observed in the case of ChE_M ($k_a = 5.0 \cdot 10^3$ and $7.1 \cdot 10^2$ L mol^{-1} min^{-1}, respectively). In the case of AChE, thione **1c** is also a more active inhibitor than its isomer **2b** ($k_a = 1.7 \cdot 10^3$ and $4.0 \cdot 10^2$ L mol^{-1} min^{-1}, respectively), and acyclic amidophosphate **7c** does not inhibit esterases at all. Experiments on electrophoresis also demonstrated that thiones **1b,c** are stronger inhibitors of CE than oxo compound **2b**. The ability to inhibit the most mobile fraction of CE changes in the following series: **7d** > **7a** ≥ **8a** > **1c** ≥ **8b** > **1b** ≥ **7b** > **2b** ≥ **7c** ≥ **5** > **6**. These data suggest that in experiments *in vitro*, thione compounds **1b,c**, unlike acyclic compounds, which are not inhibitors, can phosphorylate esterases with ring opening at the P—O bond, bypassing steric hindrances (as was observed for 2-alkoxy-2-thioxo-1,3,2-oxazaphospholanes[11]), rather than with elimination of a leaving group (Scheme 2).

Scheme 2



Y = S or O; R is the residue of serine hydrolase

The weak ability of oxo compound **2b** to inhibit esterases agrees well with the concepts of steric hindrances.[3] However, the major distinction between compound **2b** and oxo compound **2a** is that the former does not contain a good leaving group providing the phosphorylating ability. It is known that thioalkyl groups are "activated" (*i.e.*, become good leaving groups) only *in vivo* under the action of MO (according to the literature data,[12] the corresponding sulfoxides were formed). Therefore, one should not expect that the difference in the antiesterase activity of compounds **2b** and **8a** in experiments *in vitro* is as large as that observed in the case of compounds **2a** and **4** (two—three orders of magnitude). In addition, it is known[13] that *N*-substituted amidophosphates are substantially weaker inhibitors of esterases than unsubstituted amidophosphates. However, acyclic oxo compound **8a** is twice as active as cyclic compound **2b** with respect to AChE ($k_a = 7.9 \cdot 10^2$ and $4.0 \cdot 10^2$ L mol^{-1} min^{-1}, respectively) and is five times more active with respect to ChE_M ($k_a = 3.6 \cdot 10^3$ and $7.1 \cdot 10^2$ L mol^{-1} min^{-1}, respectively). In experiments on electrophoresis, compound **8a** is also a more active inhibitor of CE than **2b** (see above). Acyclic compounds **7a** and **8a** exhibit high synergistic activity against cockroaches (JAC are 3.6 and 2.2, respectively), although their activities are lower than that of cyclic compound **1b** (JAC is 5.2). Apparently, the higher activity of compound **1b** is associated with its ability (like **7a**) to inhibit MO as well as with the fact that metabolite **3b** (see Scheme 1) should be a stronger inhibitor of CE than *S*-butyl amidophosphorothioate **8a**. A comparison of the above-considered data suggests that the scheme of *in vivo* metabolism of compound **1b** (see Scheme 1) is analogous to that of compound **1a** studied previously.

Apparently, the metabolism of compounds **1c** and **5** also follows an analogous scheme. However, metabolism of thione **1c**, which does not exhibit the synergistic activity against flies and cockroaches (in both cases, JAC is 1.0), affords a product which unquestionably cannot inhibit esterases. In the case of compound **5**, the low synergistic activity only with respect to flies (JAC is 1.4) is apparently associated with inhibition of MO (the major enzyme which detoxifies pyrethroids in flies, whereas CE is the analogous enzyme for cockroaches[14]) because oxidative ring opening should lead only to detoxification. Model compounds **7b** and **8b** are active synergists only against flies (JAC are 3.2 and 2.2, respectively); compound **8b** inhibits only ChE_M ($k_a = 5.2 \cdot 10^3$ L mol^{-1} min^{-1}). Evidently, the synergistic activity of compound **6** against flies and cockroaches (JAC

are 1.8 and 1.3, respectively) is determined only by inhibition of MO, whereas oxidative ring opening does not occur because model compound **7d** (JAC are 1.20 and 1.36, respectively) is the strongest inhibitor of CE, while compound **6** is the weakest inhibitor (see above). The biochemical data will be published in detail elsewhere.

Therefore, *in vivo* biological activity of the compounds under study is determined by factors identical to those in the case of compounds **1** (R = OAr), and the observed ability to inhibit esterases *in vitro* (see Scheme 2) is, apparently, of little importance in living organisms.

Experimental

The $^{31}P\{^1H\}$ NMR spectra were recorded in acetone solutions (unless otherwise indicated) on Bruker WP 200-SY and Bruker CXP-200 instruments operating at 81.02 MHz for ^{31}P with 85% H_3PO_4 as the external standard. The ^1H NMR spectra were measured on a Bruker AMX-400 instrument (400 MHz) with the use of $CDCl_3$ as the solvent and the internal standard.

The compounds were purified by chromatography on a column with anhydrous silica gel (Aldrich, 130–270 mesh); the compound : SiO_2 weight ratio was 1 : 15; a mixture of petroleum ether (b.p. <70 °C) and acetone was used as the eluent (the gradient from 100 : 2 to 100 : 10).

2-Butylthio-2-oxo-1,3,2-oxazaphosphorinane (2b). A mixture of 3-aminopropan-1-ol (1.50 g, 0.02 mol) and Et_3N (4.04 g, 0.04 mol) in CH_2Cl_2 (15 mL) was added dropwise with stirring to a solution of acid chloride **10** (4.14 g, 0.02 mol) in anhydrous CH_2Cl_2 (30 mL) at 0 °C over 30 min. The mixture was stirred at 20 °C for 3 h and then kept for ~10 h. The precipitate was filtered off. The filtrate was washed with water (2×20 mL) and dried with Na_2SO_4. Then CH_2Cl_2 was distilled off *in vacuo* and the residue was purified by chromatography. Compound **2d** was isolated as a viscous colorless oil in a yield of 2.38 g (57%). ^{31}P NMR, δ: 26.29.

2-Butylthio-2-thioxo-1,3,2-dioxaphosphorinane (5) was prepared under the same conditions from acid chloride **9** (4.46 g, 0.02 mol), 1,3-propylene glycol (1.52 g), and Et_3N (4.04 g, 0.04 mol). After purification, compound **5** was obtained in a yield of 2.80 g (62%). ^{31}P NMR, δ: 91.60.

2-Butylthio-2-thioxo-1,3,2-diazaphosphorinane (6) was prepared analogously from acid chloride **9** (4.46 g, 0.02 mol), 1,3-propylenediamine (1.48 g, 0.02 mol), and Et_3N (4.04 g, 0.04 mol). After purification, compound **6** was obtained as a viscous colorless oil in a yield of 2.90 g (65%). ^{31}P NMR, δ: 73.35.

***S*-Butyl *O*-ethyl (diethylamido)phosphorodithioate (7a).** Sodium (0.46 g, 0.02 g-at.) was dissolved in anhydrous alcohol (30 mL) and then BuSH (1.80 g, 0.02 mol) was added. The alcohol was distilled off *in vacuo*. Anhydrous toluene (10 mL) was added to the residue, the mixture was thoroughly stirred, and the toluene was distilled off *in vacuo*. Then toluene (10 mL) was added to the residue and was distilled off *in vacuo* once again. *O*-Ethyl (diethylamido)chlorophosphite (2.67 g, 0.02 mol) was added dropwise with stirring to a suspension of the resulting BuSNa in anhydrous C_6H_6 (30 mL) and the reaction mixture was stirred at 20 °C for 5 h. After 24 h, finely dispersed sulfur (0.64 g, 0.02 g-at.) was added portionwise with stirring (evolution of heat). The reaction mixture was washed with water (2×20 mL) and dried with Na_2SO_4. After the removal of the solvent, compound **7a** was isolated by distillation *in vacuo* in a yield of 2.69 g (50%). ^{31}P NMR, δ: 95.11. ^1H NMR ($CDCl_3$), δ: 0.88 (t, 3 H, $\underline{CH_3}$–$(CH_2)_3$, $^3J_{HH}$ = 7.2 Hz); 1.11 (t, 6 H, $\underline{CH_3}$–CH_2N, $^3J_{HH}$ = 7.2 Hz); 1.28 (t, 3 H, $\underline{CH_3}$–CH_2O, $^3J_{HH}$ = 7.2 Hz); 1.37 (sext, 2 H, CH_3–$\underline{CH_2}$–CH_2CH_2, $^3J_{HH}$ = 7.2 Hz); 1.61 (quint, 2 H, CH_3CH_2–$\underline{CH_2}$–CH_2, $^3J_{HH}$ = 8.0 Hz); 2.78 (m, 2 H, $CH_3CH_2CH_2$–$\underline{CH_2}$); 3.26 (m, 4 H, CH_3–$\underline{CH_2}$–N); 4.03 (m, 2 H, CH_3–$\underline{CH_2}$–O).

***S*-Butyl bis(diethylamido)phosphorodithioate (7d)** was prepared under the same conditions from Na (0.46 g, 0.02 g-at.), BuSH (1.80 g, 0.02 mol), bis(diethylamido)chlorophosphite (4.21 g, 0.02 mol), and sulfur (0.64 g, 0.02 g-at.). After purification by chromatography on a column, compound **7d** was isolated as a viscous colorless oil in a yield of 3.26 g (55%). ^{31}P NMR, δ: 95.21. ^1H NMR ($CDCl_3$), δ: 0.88 (t, 3 H, $\underline{CH_3}$–$(CH_2)_3$, $^3J_{HH}$ = 7.2 Hz); 1.10 (t, 12 H, $\underline{CH_3}$–CH_2N, $^3J_{HH}$ = 7.2 Hz); 1.38 (sext, 2 H, CH_3–$\underline{CH_2}$–CH_2CH_2, $^3J_{HH}$ = 7.2 Hz); 1.59 (quint, 2 H, CH_3CH_2–$\underline{CH_2}$–CH_2, $^3J_{HH}$ = 7.2 Hz); 2.79 (dt, 2 H, SCH_2, $^3J_{PH}$ = 13.6 Hz, $^3J_{HH}$ = 7.2 Hz); 3.16 (m, 8 H, NCH_2).

***S*-Butyl *O*-ethyl (diethylamido)phosphorothioate (8a).** Et_2NH (2.92 g, 0.04 mol) was added dropwise with stirring to a solution of acid chloride **11** (4.33 g, 0.02 mol) in anhydrous THF (20 mL) at 20 °C and the reaction mixture was kept for 24 h. The precipitate was filtered off and THF was distilled off *in vacuo*. Compound **8a** was isolated by distillation *in vacuo* in a yield of 3.64 g (72%). ^{31}P NMR, δ: 35.38. ^1H NMR ($CDCl_3$), δ: 0.86 (t, 3 H, $\underline{CH_3}$–$(CH_2)_3$, $^3J_{HH}$ = 7.2 Hz); 1.07 (t, 6 H, $\underline{CH_3}$–CH_2N, $^3J_{HH}$ = 7.2 Hz); 1.27 (t, 3 H, $\underline{CH_3}$–CH_2O, $^3J_{HH}$ = 7.2 Hz); 1.35 (sext, 2 H, CH_3–$\underline{CH_2}$–CH_2CH_2, $^3J_{HH}$ = 7.3 Hz); 1.61 (quint, 2 H, CH_3CH_2–$\underline{CH_2}$–CH_2, $^3J_{HH}$ = 8.0 Hz); 2.72 (m, 2 H, $CH_3CH_2CH_2$–$\underline{CH_2}$); 3.11 (m, 4 H, CH_3–$\underline{CH_2}$–N); 4.03 (m, 2 H, CH_3–$\underline{CH_2}$–O).

***S*-Butyl dichlorophosphorothioate (10).** Freshly prepared BuSCl (12.45 g, 0.1 mol) was added dropwise with intense stirring to freshly prepared $EtOPCl_2$ (14.70 g, 0.1 mol) at −15 °C (vigorous evolution of EtCl). After stirring at 20 °C for 1 h, the yellow reaction mixture turned colorless. Compound **10** was isolated by distillation *in vacuo* in a yield of 18.60 g (90%). ^{31}P NMR (THF), δ: 34.0.

***S*-Butyl *O*-ethyl chlorophosphorothioate (11).** A solution of a mixture of anhydrous alcohol (0.92 g, 0.02 mol) and Et_3N (2.02 g, 0.02 mol) in THF (5 mL) was added dropwise with stirring to a solution of dichloride **10** (4.14 g, 0.02 mol) in anhydrous THF (20 mL) at 0 °C. The reaction mixture was stirred at 20 °C for 5 h, anhydrous heptane (20 mL) was added, the precipitate was filtered off, and the solvents were removed *in vacuo*. Compound **11** was isolated by distillation *in vacuo* in a yield of 3.0 g (70%). ^{31}P NMR (THF), δ: 34.82.

True bimolecular constants of inhibition of esterases (k_a) were determined and calculated according to known procedures.[8,9]

Examination of the inhibiting activity of compounds by disk electrophoresis in polyacrylamide gel was carried out according to procedures reported previously.[10,14]

Determination of the toxicity with respect to insects and calculations of the coefficients of joint action with permethrine (JAC) were performed as described previously.[14]

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