[Chem. Pharm. Bull. 25(8)1877—1891(1977)]

UDC 547.233'546.185.04.09 : 615.277.3.011.5.015.11

Synthesis, Stereochemistry and Antitumor Activity of 4-Hydroperoxyisophosphamide (NSC-227114) and Related Compounds[1,2]

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(Received November 19, 1976)

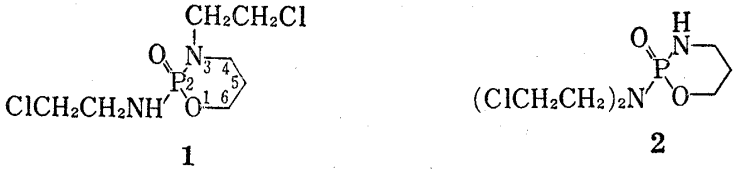
4-Hydroperoxyisophosphamide and its analogues were simply synthesized by ozonolysis reactions of *O*-(3-butenyl)-*N*,*N*′-bis(2-chloroethyl)phosphorodiamidate and related *O*-(3-butenyl)phosphoramidates. An acid-catalyzed isomerization of 4-hydroperoxyisophosphamide proceeded with inversion of its phosphorus configuration giving 2-*epi*-4-hydroperoxyisophosphamide. Both isomers readily afforded C_4-substituted isophosphamide derivatives by reactions with nucleophilic agents and acid. L1210 antileukemic activities were tested for the isomers and some analogues revealing that the C_4-hydroperoxylation of isophosphamide resulted in a marked enhancement of its activity and that the inverted stereochemistry of an alkylating functionality at the phosphorus atom is also effective in promoting the antitumor action as an alternative activated species of isophosphamide.

Keywords——cyclophosphamide analogues; 1,3,2-oxazaphosphorinanes; ozonolytic cyclization; heterocyclic peroxides; inversion of phosphorus configuration; anomeric effect

The antitumor agent isophosphamide (**1**)[4] [2-(2-chloroethyl)amino-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorine-2-oxide (NSC–109724)] is a structural isomer of cyclophosphamide (**2**) differing only in the position of one of the two alkylating functionalities, and is currently undergoing clinical trials.[5] Although the antitumor activity of this drug, like that of cyclophosphamide, has been thought to be effected after enzymatic C_4-hydroxylation of its 1,3,2-oxazaphosphorinane ring,[6] it offers no useful increased antitumor effect in man.[5b,c] One can infer from the molecular structure of isophosphamide that 2-chloroethyl group located at the ring nitrogen atom would have a significant steric effect upon the enzymatic C_4-hydroxylation. In fact, recent studies on the metabolic behaviors of isophosphamide in rabbits[2a] and also in man[7] have indicated that the *in vivo* C_4-oxidation is in competition with side-chain oxidation giving *N*-dechloroethylated metabolites which are ineffective in

1) This paper forms Part V of Studies on Cyclophosphamide Metabolite and Their Related Compounds. Part IV: A. Takamizawa, S. Matsumoto, T. Iwata, and I. Makino, *Chem. Pharm. Bull.* (Tokyo), **25**, 1582 (1977).
2) Preliminary accounts of this work were reported in *a*) A. Takamizawa, S. Matsumoto, T. Iwata, Y. Tochino, K. Katagiri, K. Yamaguchi, and O. Shiratori, *J. Med. Chem.*, **17**, 1237 (1974); *b*) A. Takamizawa, S. Matsumoto, T. Iwata, and I. Makino, *Heterocycles*, **3**, 787 (1975). A part of this work was presented at the Symposium on Metabolism and Mechanism of Action of Cyclophosphamide (London, July 1975) of which proceedings appeared in *Cancer Treatment Rept.*, **60**, 361 (1976).
3) Location: *Fukushima-ku, Osaka, 553, Japan.*
4) N. Brock, *Laval Med.*, **39**, 696 (1968).
5) *a*) J.J. Van Dyk, H.C. Falkson, A.M. Van Der Merwe, and G. Falkson, *Cancer Res.*, **32**, 921 (1972); *b*) D.L. Ahmann, H.F. Bisel, and R.G. Hahn, *Cancer Chemother. Rept.*, **58**, 861 (1974); *c*) D.N. Bremner, J. St. C. McCormick, and J.W.W. Thomson, *Cancer Chemother. Rept.*, **58**, 889 (1974); *d*) H.M. Cohen, P.J. Creaven, F. Tejada, H.H. Hansen, F. Muggia, A. Mittelman, and O.S. Selawry, *Cancer Chemother. Rept.*, **59**, 751 (1975); *e*) J. Schnitker, N. Brock, H. Burkert, and E. Fichtner, *Arzneim. Forsch.*, **26**, 1783 (1976).
6) *a*) L.M. Allen and P.J. Creaven, *Cancer Chemother. Rept.*, **56**, 603 (1972); *b*) R.A. Alarcon, J. Meienhofer, and E. Atherton, *Cancer Res.*, **32**, 2519 (1972); *c*) D.L. Hill, W.R. Laster, Jr., M.C. Kirk, S. El Dareer, and R.F. Struck, *Cancer Res.*, **33**, 1016 (1973).
7) K. Norpoth, G. Müller, and H. Raidt, *Arzneim. Forsch.*, **26**, 1376 (1976).

promoting the antitumor activity, this being presumably responsible for the less efficacy of the drug *in vivo*. Therefore, it is of particular interest to synthesize the pre-oxidized isophosphamide derivative which could be expected to exert increased antitumor activity. In this paper we wish to report studies on the synthesis, stereochemistry and antitumor activity of C_4-oxidized isophosphamide and related compounds.



1 2

Chart 1

Syntheses of 4-Hydroperoxyisophosphamide (NSC-227114) and Related Compounds

The previously reported synthetic method[8] used for the preparation of C_4-oxidized cyclophosphamide derivatives was applied to the present syntheses with slight modifications. *O*-(3-Butenyl)-*N*,*N*′-bis(2-chloroethyl)phosphorodiamidate (**3**) was simply prepared in 70% yield by a one-pot reaction of phosphoryl chloride with 3-buten-1-ol, followed by treatment with 2-chloroethylamine hydrochloride and triethylamine in methylene chloride. Compound **3** was also obtained in almost the same yield by reacting phosphoryl chloride with 3-buten-1-ol and ethyleneimine. Ozonolysis of **3** in aqueous acetone, followed by treatment of the ozonolyzed solution with an excess amount of 30% hydrogen peroxide, afforded an oily mixture consisting of two major products and a trace amount of two side-products. One of the major products could easily be separated from the mixture by crystallization from acetone–ether (1: 10), giving 2-(2-chloroethyl)amino-3-(2-chloroethyl)-4-hydroperoxytetrahydro-2*H*-1,-3,2-oxazaphosphorine-2-oxide (**4a**) (4-hydroperoxyisophosphamide, NSC-227114) in 30% yield. After repeated purifications of the mother liquor of **4a** by column chromatography with silica gel and acetone–chloroform mixture, another major product **4b** (2-*epi*-4-hydroperoxyisophosphamide) was isolated in a crystalline state in 5% yield, accompanied by a trace quantity of 4-ketoisophosphamide (**5**) and a new compound 2-(2-chloroethyl)amino-3-(2-chloroethyl)-4-(1-hydroperoxyisopropyl)peroxytetrahydro-2*H*-1,3,2-oxazaphosphorine-2-oxide (**6**). The stereostructures of the major products **4a** and **4b** were elucidated based on the spectroscopic investigations (*vide infra*). The structure of the minor product **5** was confirmed by identifying with an authentic specimen prepared according to a literature,[6c] while that of the latter minor product **6** was tentatively assigned based on the elemental analysis and NMR data (see Experimental). The formation of the major products **4a** and **4b** is rationalized by cyclization reaction of a zwitterion intermediate **7** which is produced by the ozonolysis of **3**, while the formation of the product **6** is explainable by assuming a 1,2,4,5-tetroxan intermediate **8** which is possibly produced by reaction of the zwitterion **7** with acetone and hydrogen peroxide.

Similarly, *N*-substituted analogues of 4-hydroperoxyisophosphamide **12**, **13** and **14** (NSC 260608) could readily be prepared by the ozonolysis reactions of the corresponding *N*-substituted *O*-(3-butenyl)-*N*,*N*′-bis(2-chloroethyl)phosphorodiamidates **9**, **10** and **11** which were obtained by reaction of phosphoryl chloride with 3-buten-1-ol, followed by treatment with *N*-substituted 2-chloroethylamine hydrochloride and finally with 2-chloroethylamine hydrochloride in the presence of triethylamine. The structures of these products, including their stereochemistry, were assigned by comparing their chemical properties and NMR data with those of **4a** (see Experimental). Isolation of the corresponding stereoisomer and other side-

8) *a*) A. Takamizawa, S. Matsumoto, T. Iwata, K. Katagiri, Y. Tochino, and K. Yamaguchi, *J. Amer. Chem. Soc.*, **95**, 985 (1973); *b*) A. Takamizawa, S. Matsumoto, T. Iwata, Y. Tochino, K. Katagiri, K. Yamaguchi, and O. Shiratori, *J. Med. Chem.*, **18**, 376 (1975).

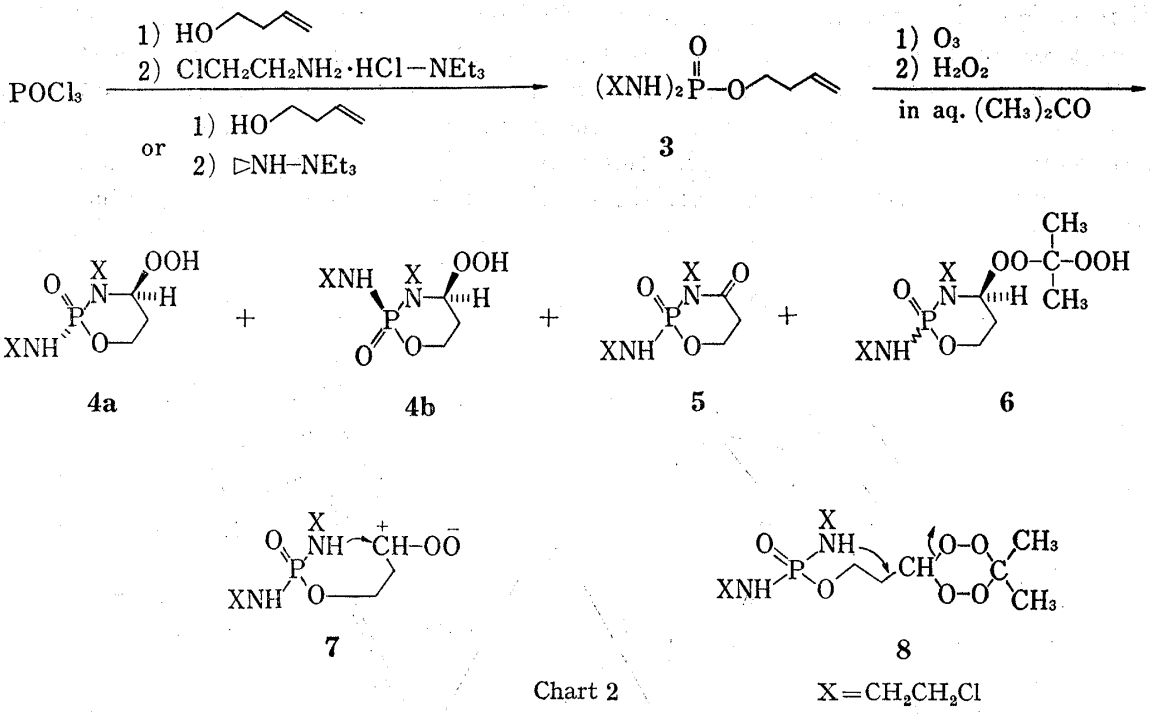


Chart 2

products was not attempted in these ozonolysis reactions. Recently, Hohorst, *et al.*[9] have reported a method for the preparation of **4**-hydroperoxyisophosphamide by direct oxidation of isophosphamide using ozone and hydrogen peroxide as a possible singlet-oxygen source, and the method has been claimed to be advantageous because of its simplicity and its use of commercially available starting material.[9b] From the practical standpoint, however, their method appears to be less economic than ours because of lower yield of the desired product and greater yield of the undesired 4-ketoisophosphamide at the cost of rather expensive starting material, while our method utilizes the readily available intermediate **3** which is simply obtained in a high yield by a one-pot reaction of phosphoryl chloride, 3-buten-1-ol and 2-chloroethylamine and the desired product can be isolated in higher yield from the ozonolysis mixture of **3**. Our method has also been conveniently applied for the preparation of ^{14}C-labelled 4-hydroperoxyisophosphamide.[10]

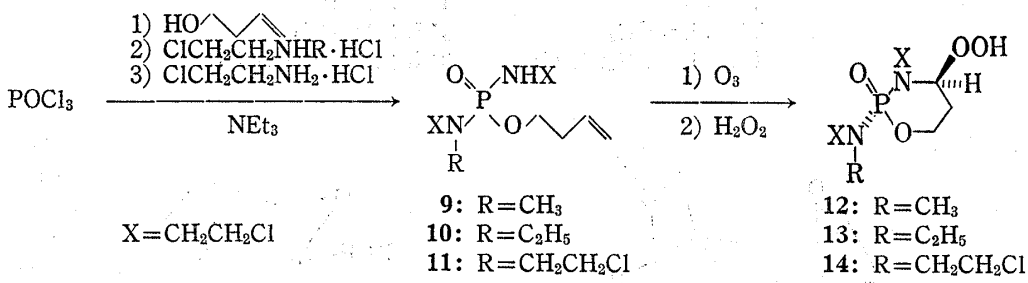


Chart 3

Stereochemistry of 4-Hydroperoxyisophosphamide and Related Compounds

The stereochemical aspects of the C_4-oxygen functionality and the alkylating group at the phosphorus atom in the activated cyclophosphamide derivatives are a matter of particular interest with respect to the structure-activity relationships, but little has been investigated

9) *a*) G. Peter, T. Wagner, and H.-J. Hohorst, *Cancer Treatment Rept.*, **60**, 429 (1976); *b*) H.-J. Hohorst, G. Peter, and R.F. Struck, *Cancer Res.*, **36**, 2278 (1976).
10) T. Nagasaki, Y. Katsuyama, and H. Minato, *J. Labelled Compounds and Radiopharmaceuticals*, **12**, 7 (1976).

until recently. Camerman, *et al.*[11] have reported on the X-ray crystallographic studies on 4-hydroperoxycyclophosphamide which was prepared according to our method,[8] revealing that the 1,3,2-oxazaphosphorinane ring has a chair form with *cis-diaxial* configuration of the P=O and C_4–OOH groups. The same stereochemistry has also been found for the molecular structure of 4-hydroxycyclophosphamide anhydro-dimer(4-peroxycyclophosphamide),[12] a dimeric compound obtained by the partial reduction of 4-hydroperoxycyclophosphamide[13] or by the Fenton oxidation of cyclophosphamide.[14] In the syntheses of these cyclophosph-

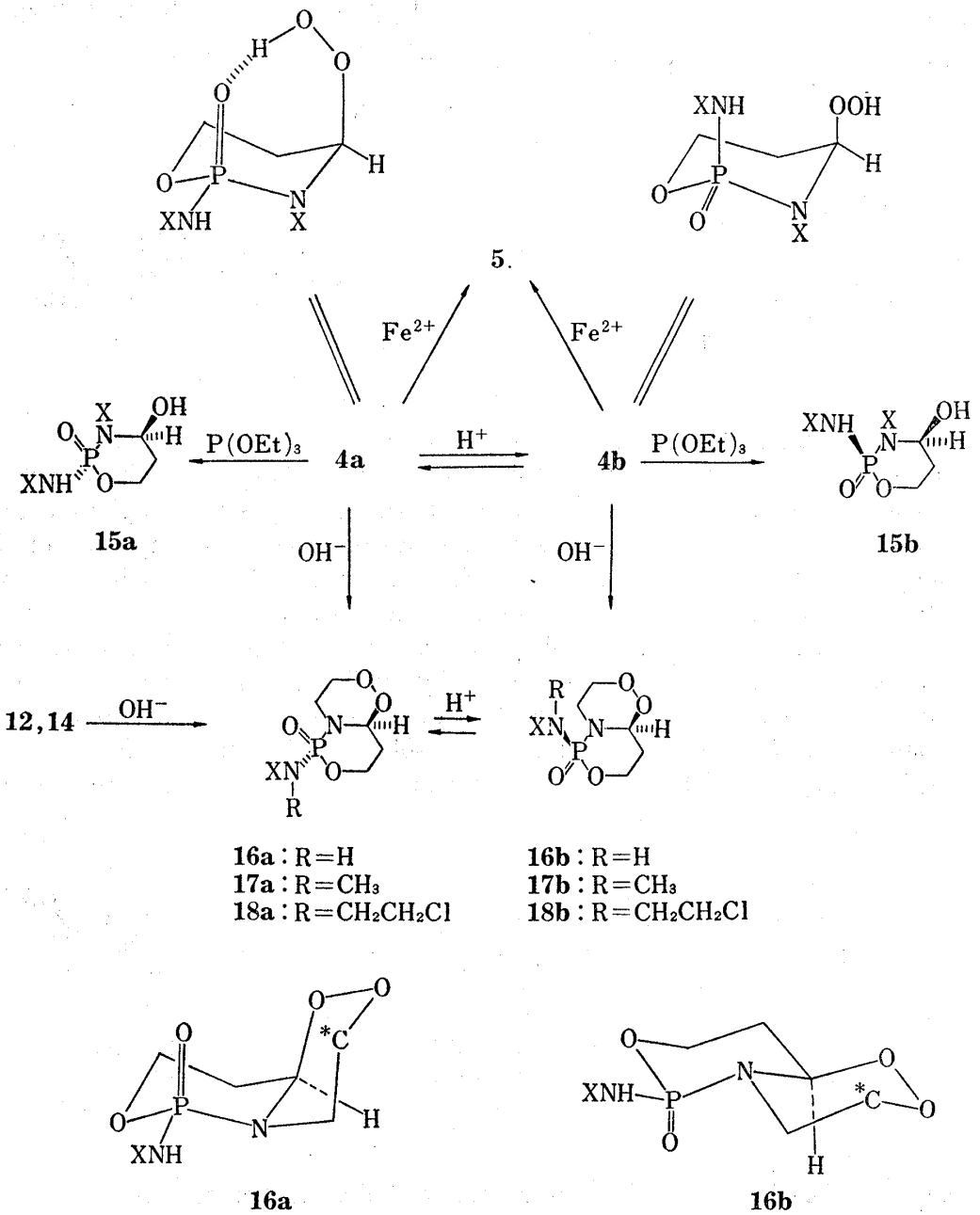


Chart 4

11) A. Camerman, H.W. Smith, and N. Camerman, *Biochem. Biophys. Res. Commun.*, **65**, 828 (1975).
12) H. Sternglanz, H.M. Einspahr, and C.E. Bugg, *J. Amer. Chem. Soc.*, **96**, 4014 (1974).
13) A. Takamizawa, S. Matsumoto, and T. Iwata, *Tetrahedron Letters*, **1974**, 517.
14) *a*) J. Van Der Steen, E.C. Timmer, J.G. Westra, and C. Benckhuysen, *J. Amer. Chem. Soc.*, **95**, 7535 (1973); *b*) R.F. Struck, M.C. Thorpe, W.C. Coburn, Jr., and W.R. Laster, Jr., *J. Amer. Chem. Soc.*, **96**, 313 (1974).

amide derivatives, a possible stereoisomer having *trans* configuration of the P=O and C_4-oxygen atom has not been isolated, therefore the present paper reports the first example of the isolation of such stereoisomer of the activated isophosphamide derivatives.

The ozonolysis products **4a** and **4b** were found to be readily interconvertible by the action of a catalytic amount of *p*-toluenesulfonic acid (TsOH) in chloroform at room temperature, giving approximately 1:1 equilibrium mixture. Both isomers afforded 4-ketoisophosphamide (**5**) quantitatively on treatment with aqueous ferrous sulfate in chloroform, while they yielded the corresponding 4-hydroxyisophosphamides **15a** and **15b** in good yields by treating with triethylphosphite. Action of aqueous alkali (Na_2CO_3 or KOH) upon **4a** and **4b** afforded quantitatively the corresponding bicyclic peroxides **16a** and **16b** which were also found to give the TsOH-catalyzed equilibrium mixture with slight predominance of the latter isomer (**16a**/**16b**=4/5). In the cases of the TsOH-catalyzed isomerization reactions of *N*-substituted 4-hydroperoxyisophosphamides **12**—**14**, the corresponding isomers could not be isolated but their formation was verified by converting into the cyclic peroxide as follows. The alkali treatment of **12** resulted in a quantitative formation of a bicyclic product **17a**, while treatment of **12** with TsOH, followed by the alkali treatment of the resulting mixture, afforded two bicyclic products **17a** and **17b** in the ratio of approximately **17a**/**17b**=5/1. Likewise, the alkali treatment of **14** gave a quantitative yield of **18a**, while a treace amount of **18b** was isolated besides **18a** when **14** was treated with TsOH and subsequently with alkali. The isomeric bicyclic peroxides **17a** and **17b**, as well as **18a** and

Table I. The 60 MHz ^{1}H-NMR Parameters of the C_4-Proton of 4-Hydroperoxyisophosphamide and Related Compounds

Compd.	Solvent	δ (C_4–H) ppm	Appearance[a]	J(P–N–C_4–H) Hz	$\sum J$(C_4–H, C_5–H)[b] Hz
4a	d_6-DMSO	4.96	d of t	19.5	6.0
4b	d_6-DMSO	5.02	d of t	18.9	8.1
	$CDCl_3$	5.09	d of t	20.3	7.0
6	d_6-DMSO	5.33	d of t	20.0	6.0
	$CDCl_3$	5.18	d of t	21.0	7.4
12	d_6-DMSO	4.98	d of t	19.5	5.2
13	d_6-DMSO	4.99	d of t	19.7	5.8
14	d_6-DMSO	5.00	d of t	19.8	5.0
15a	D_2O	5.05	d of t	18.0	7.0
15b	D_2O	5.08	d of t	18.4	7.2
	$CDCl_3$	4.97	d of t	21.6	6.8
16a	$CDCl_3$	5.40	d of dd	21.9	7.3
	d_6-DMSO	5.34	d of t	18.9	9.0
16b	$CDCl_3$	5.46	d of dd	5.8	15.5
	d_6-DMSO	5.36	d of dd	5.0	15.5
17a	$CDCl_3$	5.40	d of dd	21.8	7.0
17b	$CDCl_3$	5.48	d of t	8.9	11.2
18a	$CDCl_3$	5.38	d of dd	22.5	7.0
18b	$CDCl_3$	5.48	d of dd	5.7	14.0
25a	$CDCl_3$	4.53	d of t	21.6	6.0
25b	$CDCl_3$	4.53	d of t	21.0	6.4
26a	$CDCl_3$	5.12	d of t	20.9	7.6
	d_6-DMSO	5.15	d of t	18.2	8.6
27a	d_6-DMSO	5.00	d of t	21.8	7.0
27b	d_6-DMSO	5.10	d of t	15.0	9.0
	$CDCl_3$	4.96	d of t	17.3	8.4

a) Peak assignments were made based on visual inspection of the spectrum after erasing the NH signal by D_2O addition; d: doublet, t: triplet, dd: double doublet.
b) Determined from the width of triplet (or double doublet) part of the C_4-H signals splitting by couplings with two C_5-protons.

18b, also produced the TsOH-catalyzed equilibrium mixture with predominance of the latter isomer (**17a**/**17b**=3/4, **18a**/**18b**=2/3). The stereostructures of these epimeric compounds were elucidated based on the following line of evidences. In the 60 MHz ^{1}H–NMR spectra, the signals of C_4-proton are well separated from those of other protons and split by couplings with phosphorus and C_5-protons. In Table I, the NMR parameters of the C_4-proton of these compounds are listed. As shown in the Table, the large vicinal P, H coupling constants were determined for all compounds except **16b**, **17b** and **18b**. The J(P–N–C_4–H) values are comparable to those of the vicinal P–O–C–H (*equatorial*) coupling constants of 2-dialkylamino-1,3,2-dioxaphosphorinane-2-oxides with a rigid chair conformation,[15] suggesting that the C_4–H is *equatorial* which is also predictable from small couplings between C_4–H and and C_5–H [see $\sum J$(C_4–H, C_5–H) values in Table I]. This suggests that the acid-catalyzed isomerizations of **4a** and **4b**, and also their deoxygenation reactions by triethylphosphite, proceed with retention of the C_4-configuration. Therefore it is proposed that the isomerization between **4a** and **4b** involves inversion of phosphorus configuration, which is supported by their ^{31}P–NMR spectra. The ^{31}P–NMR chemical shift of **4a** (9.75 ppm), measured in d_4-methanol using H_3PO_4 as an external reference, was found to be essentially equal to that of 4-hydroperoxycyclophosphamide (9.73 ppm) having the *cis-diaxial* configuration of P=O and C_4–OOH, while that of the isomer **4b** was observed at a higher field (9.46 ppm), which seems to account for the inversion of the P=O configuration from *axial* (**4a**) to *equatorial* (**4b**) since the ^{31}P–NMR chemical sfift of a related pentavalent six-membered phosphorus heterocycle has been reported to be greater for P=O *axial* configuration than for *equatorial* configuration.[16] The IR spectrum of **4a** in a dilute chloroform solution showed bands at 3539 cm^{-1} (ε=25.0) due to ν_{OH} (free) and 3412 cm^{-1} (ε=81.4) due to ν_{NH}, besides a broad band at 3150 cm^{-1} which could be attributed to a hydrogen-bonded ν_{OH}, while **4b** showed a ν_{OH} (free) band at 3536 cm^{-1} with a greater intensity (ε=77.5) and a ν_{NH} band at 3412 cm^{-1} (ε=71.0). These IR data clearly indicate the presence of an intramolecular hydrogen-bonding of the C_4–OOH group in **4a** and its absence in **4b**, providing a further evidence that **4a** has the *cis-diaxial* configurations of the P=O and C_4–OOH groups and that the two groups are disposed to the *trans* orientation in **4b**. The assigned stereostructures of the epimeric 4-hydroperoxyisophosphamides **4a** and **4b** could account for their different chemical properties. **4b** shows a greater solubility in water than **4a** (**4a**=4 mg/ml, **4b**=25 mg/ml at 25°), suggesting that the polar groups (P=O and C_4–OOH) in **4a** are masked by the intramolecular interaction, while they are free from such interaction in **4b**. The molecular weight measurement of **4b** in a solvent of low polarity such as chloroform gave values intermediate between those for monomer and dimer possibly due to a partial molecular association, while a correct value corresponding to a monomeric form

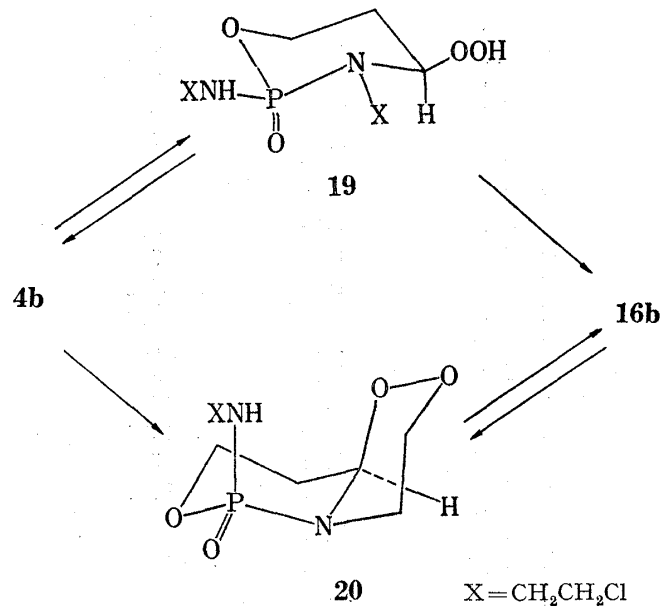
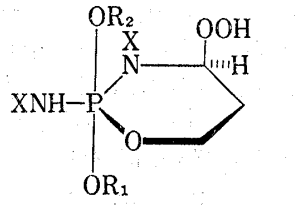


Chart 5

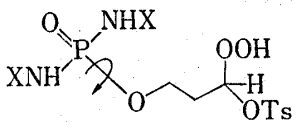
15) R.S. Edmundson, *J. Chem. Soc.* (*C*), **1972**, 1660.
16) *a*) J.A. Mosbo and J.G. Verkade, *J. Amer. Chem. Soc.*, **95**, 4659 (1973); *b*) W.G. Bentrude and H.-W. Tan, *J. Amer. Chem. Soc.*, **95**, 4666 (1973).

was obtained for **4a**. The results of the dipole moment measurements[17] of **4a** and **4b** in dioxane solution have also supported their stereostructures depicted in Chart 4, which were ultimately verified by the X-ray studies by Camerman, *et al.*[18]

As listed in Table I, the ^{1}H–NMR data of the isomer **b** of **16**—**18** are greatly different from those of the corresponding isomer **a** and are apparently indicative of the *axial* configuration of their C_4–H. The formation of the isomer **16b** with different C_4-stereochemistry from **4b** can be interpreted if one supposes that the cyclization of **4b** would proceed *via* a conformer **19**, or more likely that the conformational alteration takes place after cyclization to an intermediate **20** which turns into a more stable conformer **16b**. It is of interest to note here that the ring nitrogen atom of **4a** and **4b**, as well as that of isophosphamide,[19] was shown to have an sp^2-like planar geometry by the X-ray studies.[20] Such a planar structure of the ring nitrogen atom, however, is sterically unfavored for the bicyclic peroxides (**16a,b**—**18a,b**), and it is suggested by inspecting molecular model that the P–N–C_4–H dihedral angle must come into nearly 90° for the both isomers **a** and **b** when their ring nitrogen atom has a planar geometry, this being inconsistent with the fact that the marked differences in the P–N–C_4–H vicinal coupling constants are observed between the two isomers (see Table I). There is also a marked difference between the isomers **16a** and **16b** in their ^{13}C–NMR spectra



21a : R_1=Ts, R_2=H
21b : R_1=H , R_2=Ts
21c : R_1=R_2=H



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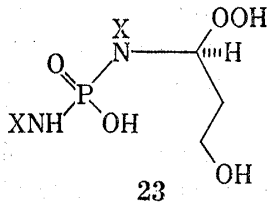

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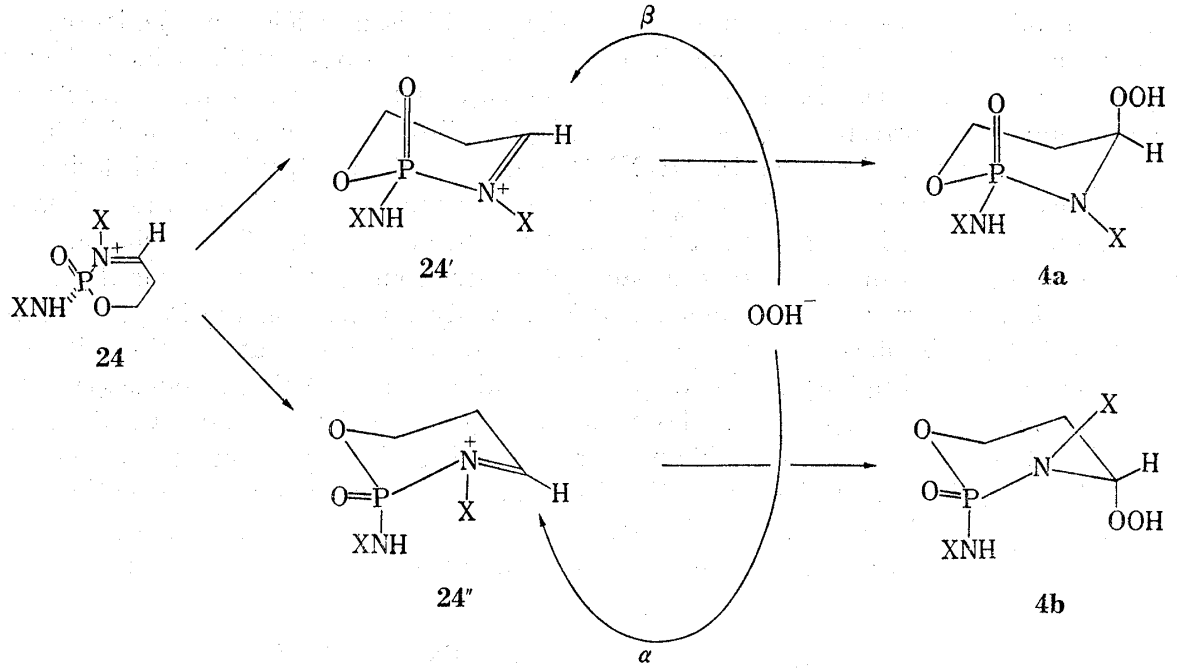
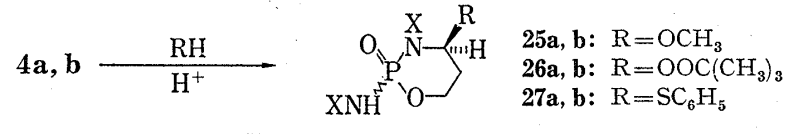


Chart 6 X=CH_2CH_2Cl

17) K. Kuriyama, unpublished result.
18) A. Camerman, H.W. Smith, and N. Camerman, *Cancer Treatment Rept.*, **60**, 517 (1976).
19) H.A. Brassfield, R.A. Jacobson, and J.G. Verkade, *J. Amer. Chem. Soc.*, **97**, 4143 (1975).
20) A. Camerman, Personal communication.

in which the signal of the carbon atom which is marked by an asterisk as shown in Chart 4 was split in **16b**(δ 71.79 ppm) by the vicinal ^{31}P–N–C–^{13}C* coupling with J=6.0 Hz, while the corresponding signal of **16a**(δ 71.92 ppm) was not split by such coupling. This supports a *syn* arrangement of P–N–C–C* in **16a** and an *anti*-coplanar arrangement in **16b**. Both the ^1H- and the ^{13}C-NMR data therefore are interpreted by the structure with an sp^3-like tetragonal geometry of the ring nitrogen atom for the both isomers **a** and **b**.

The TsOH-catalyzed isomerization reactions of **4a** (and **4b**) is of particular interest because it proceed with retention of the C_4-configuration. Some hypothetical mechanisms appear to be possible for the isomerization reactions by assuming a bipyramidal intermediate **21a** from which TsOH is removed after pseudo-rotation *via* **21b**, or a ring-opened intermediate **22** which recyclizes with elimination of TsO^- after rotating the P–O bond. Mechanisms involving a bipyramidal intermediate such as **21c** or a ring-opened intermediate **23** have also been proposed.[18] These mechanisms, however, do not necessarily give a clear-cut explanation why the isomerizations proceed with retention of the C_4–OOH configuration. A more plausible explanation which is experimentally supported is that it takes place involving an immonium ion **24** to which hydroperoxide anion re-attacks from two directions α(**24**″) and β(**24**′), giving isomers having *cis*- and *trans*-configurations of the P=O and C_4–OOH groups, but the electronegative OOH group is preferred to be axially disposed because of favored dipole interactions between the C_4–O bond and unshared electrons of the ring nitrogen atom(anomeric effect),[21] thus the six-membered ring is flipped in **4b**. This mechanistic consideration led us to study the acid-catalyzed C_4-substitution reactions of both **4a** and **4b** with nucleophilic reagents. The reactions of **4a** (and **4b**) with methanol in the presence of TsOH were found to give a 1:1 isomeric mixture of C_4-methoxyisophosphamides **25a** and **25b** which could be separated by column chromatography on silica gel and chloroform–acetone (3:1). The TsOH-catalyzed reaction of **4a** with *tert*-butyl hydroperoxide also afforded an oily mixture consisting of the isomeric C_4-substituted products from which an isomer **26a** could be isolated by crystallization from ether–hexane (2:1). Although the 60 MHz ^1H-NMR spectrum of this oily mixture could not differentiate the possible isomer **26b** from **26a**, its presence was confirmed by the ^{13}C-NMR spectrum in which all signals were observed in pairs in the ratio of approximately 2:1 (see Experimental). In the case of the TsOH-catalyzed reaction of **4a** with thiophenol, corresponding isomeric 4-thiophenylisophosphamides **27a** and **27b** were isolated in a 1:1 ratio. The 60 MHz ^1H-NMR spectra of all the C_4-substituted derivatives thus obtained also showed great P–N–C_4–H coupling constants comparable to those of **4a** and **4b** (see Table I), indicating again the retention stereochemistry at the C_4-position, although unambigous assignment of the phosphorus stereochemistry could not be made for these compounds. It is noteworthy that Connors, *et al.*[22] recently reported on the isolation of two epimers of 4-ethoxyisophosphamide having different stereochemistry at the C_4-position by treating the activated isophosphamide metabolites with ethanol. Although our experiments for isolating the 4-ethoxyisophosphamides by the TsOH-catalyzed reaction of **4a** with ethanol resulted in the formation of an unstable mixture consisting of two products which could not be separated into the expected isomers, the C_4-retention stereochemistry of the substitution reactions of **4a** (and **4b**) suggest that the products obtained by Connors, *et al.* perhaps have an inverted stereochemistry at the phosphorus atom rather than at the C_4-position.



25a, b: R=OCH_3
26a, b: R=$OOC(CH_3)_3$
27a, b: R=SC_6H_5
X=CH_2CH_2Cl

Chart 7

21) For example, see R.U. Lemieux, *Pure Appl. Chem.*, **25**, 527 (1971).
22) T.A. Connors, P.J. Cox, P.B. Farmer, A.B. Foster, and M. Jarman, *Biochem. Pharmacol.*, **23**, 115 (1974).

In Vivo Antitumor Activity of 4-Hydroperoxyisophosphamide and Related Compounds

In Table II, the comparative *in vivo* antileukemic activities of isophosphamide, 4-hydroperoxyisophosphamide and related analogues are given. As expected, 4-hydroperoxyisophosphamide **4a** exhibited remarkably higher activity than the parent isophosphamide, and it is notable that the pre-oxidation of isophosphamide results a greater enhancement in its activity than that of cyclophosphamide.[8] This clearly indicates that the *in vivo* activation of isophosphamide is less efficient than that of cyclophosphamide. As cited in earlier part of this paper, studies on the metabolic behaviors have revealed that the *in vivo* C_4-oxidation of isophosphamide is in competition with oxidation of its side-chain carbons leading to *N*-dealkylated metabolites, a situation which would account for the lower *in vivo* efficacy of the drug. The fact that 2-*epi*-4-hydroperoxyisophosphamide **4b** showed essentially the same activity as that of **4a** is also notable because it suggests that the phosphorus stereochemistry has no significant effect in promoting the antitumor action, although at present it is uncertain that the *in vivo* produced activated metabolite of isophosphamide has a stereochemistry such as **4a** or **4b**. As is apparent from the Table II, the effect of a substituent such as CH_3, C_2H_5 or CH_2CH_2Cl group locating at the exocyclic nitrogen atom of 4-hydroperoxyisophos-

Table II. Comparative *in Vivo* Antitumor Activity of Isophosphamide, 4-Hydroperoxyisophosphamide and Related Compounds against L 1210 Leukemic BDF_1 Mice[a]

Compd.	Dose[b] (mg/kg)	ILS %[c]	Survivors over 30 days / Number of mice tested
Isophosphamide (1)	50(*i.v.*)	45	0/10
	100(*i.v.*)	180	1/10
	500(*i.v.*)	200	4/10
4a	50(*i.v.*)	200	4/10
	100(*i.v.*)	>270	10/10
	200(*i.v.*)	−20	0/10
	25(*i.p.*)	228	6/8
	50(*i.p.*)	>270	8/8
	100(*i.p.*)	>270	8/8
4b	50(*i.v.*)	74	0/8
	100(*i.v.*)	221	7/8
	200(*i.v.*)	>245	7/7
	25(*i.p.*)	231	6/8
	50(*i.p.*)	>270	8/8
	100(*i.p.*)	>270	8/8
6	25(*i.v.*)	34	0/8
	50(*i.v.*)	78	0/8
	100(*i.v.*)	212	7/8
12	25(*i.p.*)	209	6/8
	50(*i.p.*)	246	7/8
	100(*i.p.*)	117	2/8
13	25(*i.p.*)	150	4/9
	50(*i.p.*)	227	6/8
	100(*i.p.*)	201	7/9
14	25(*i.p.*)	200	0/8
	50(*i.p.*)	250	6/8
	100(*i.p.*)	140	3/8

a) The BDF_1 mice were inoculated (*i.p.*) with 10^5 cells of L1210 leukemia suspended in saline.
b) The drugs were administered at 24 hr after inoculation of the cells.
c) Increase of life span in dying animals over control.

phamide was found to be rather slight for the activity, which is somewhat interesting in contrast to the fact that *N*-alkylation of 4-hydroperoxycyclophosphamide resulted in a marked drop in its *in vivo* activity.[23]

Experimental

Melting points were determined in open glass capillary tubes using a Yamato MP-1 apparatus and were uncorrected. IR data were determined with a JASCO IRA-1 spectrometer in Nujol mull or in KBr disk. ^{1}H- and ^{13}C-NMR data were determined with Varian Model A-60 and NV-14 spectrometers using tetramethylsilane as an internal standard unless otherwise indicated. ^{31}P-NMR data were determined in methanol solution with a Varian Model XL-100 spectrometer using 80% H_3PO_4 as an external standard.[24] Column chromatography was carried out using silica gel (Merck Kieselgel 60). TLC chromatography was carried out using pre-coated silica gel plate (Merck, F_{254}, 0.25 mm). 3-Buten-1-ol was purchased from Chemical Samples Co., Ltd. (U.S.A.), and 2-chloroethylamine hydrochloride and *N*,*N*-bis(2-chloroethyl)amine hydrochloride were purchased from Aldrich Chemical Company, Inc. (U.S.A.). *N*-Methyl-N-(2-chloroethyl)amine and *N*-ethyl-*N*-(2-chloroethyl)amine hydrochlorides were prepared by chlorination of the corresponding ethanolamines with $SOCl_2$ according to the usual procedure.

***O*-(3-Butenyl)-*N*,*N*′-bis(2-chloroethyl)phosphorodiamidate (3)**——a) To a mechanically stirred solution of $POCl_3$ (15.3 g, 100 mmol) in CH_2Cl_2 (100 ml) was added dropwise a solution of 3-buten-1-ol (7.2 g, 100 mmol) in CH_2Cl_2 (20 ml) for 30 min at $-10\pm1°$, then the mixture was stirred for 3 hr at $-5\pm1°$. After cooling the reaction mixture to -25——$-30°$, CH_2Cl_2 (100 ml) and 2-chloroethylamine hydrochloride (23.2 g, 200 mmol) were added, then triethylamine (50.5 g, 500 mmol) was added dropwise to the mixture for 1 hr at $-25\pm1°$ with vigorous stirring. After further stirring for 2 hr at -10——$5°$, the reaction mixture was filtered and the filtrate was washed three times with water (each 200 ml), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give crude **3** (19.2 g, 70%) as an yellow-brown oil which was used for the ozonolysis reaction without further purification. Purification of the crude product by chromatography on a column (10×50 cm) eluting with AcOEt gave pure **3** (12.4 g, 45%) as a colorless oil: NMR ($CDCl_3$) δ: 2.47 (2H, quartet, $-\underline{CH_2}-CH=CH_2$), 2.96—3.80 (10H, multiplet, $2\times ClCH_2CH_2NH$), 4.09 (2H, quartet, $PO-O-\underline{CH_2}-CH_2-$), 4.98—6.30 (3H, multiplet, $-CH=CH_2$). *Anal.* Calcd. for $C_8H_{17}Cl_2N_2O_2P$: C, 34.93; H, 6.23; Cl, 25.77; N, 10.18; P, 11.26: Found: C, 34.98; H, 6.06; Cl, 25.48; N, 10.12; P, 10.98.

b) After reacting $POCl_3$ (15.3 g, 100 mmol) with 3-buten-1-ol (7.2 g, 100 mmol) in the same way as described for the method a), an excess amount of ethyleneimine (30 g, 800 mmol) was dropwise added to the stirred reaction mixture for 1 hr at $-20\pm3°$, and the mixture was stirred further for 2 hr at $-5\pm1°$. After standing overnight at 2°, the reaction mixture was washed with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give **3** (18.7 g, 68%) which gave satisfactory NMR data and identified with the specimen prepared by method a).

Ozonolysis of *O*-(3-Butenyl)-*N*,*N*′-bis(2-chloroethyl)phosphorodiamidate (3)——To a magnetically stirred solution of **3** (27.5 g, 100 mmol) in 1:1 aqueous acetone (100 ml), O_3 was bubbled at a rate of *ca.* 90 mg/min for 80 min (total amount of O_3, 7.2 g; 150 mmol) in an ice-water bath, then 30% H_2O_2 (25 ml) was added to the ozonized solution. After standing 72 hr at 2°, the reaction mixture was concentrated to *ca.* 50 ml by evaporation of acetone *in vacuo* below 40° and the resulting aqueous residue was extracted with $CHCl_3$ (3×50 ml). The $CHCl_3$ extracts were combined, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* below 35° to give an oily residue which crystallized partly by addition of ether (50 ml) and acetone (5 ml). After standing overnight at 2°, the crystals were collected by filtration to give crude 4-hydroperoxyisophosphamide (**4a**) as a white solid (3.86 g, 13.2%). The filtrate was concentrated *in vacuo* below 35° and the resulting oily residue was dissolved again in 1:1 aqueous acetone (100 ml), then 30% H_2O_2 (20 ml) was added to the mixture. After standing 48 hr at 2°, the mixture was concentrated to *ca.* 50 ml *in vacuo* below 40°, and the resulting aqueous residue was extracted with $CHCl_3$ from which crude **4a** (2.96 g, 10.1%) was isolated after similar treatments as carried out for the isolation of **4a** from the first fraction. Quite similar treatments were further repeated for the second mother liquor to give **4a** (1.82 g, 6.5%). Total amount of the crude **4a** (8.64 g, 29.8%). Recrystallization of **4a** from methanol gave colorless prisms, mp 113—114° (dec.). IR ν_{max}^{KBr} cm^{-1}: 3268, 3193, 2995, 2963, 2949, 2927, 2858, 2837, 1435, 1322, 1239, 1193, 1160, 1117, 1059, 1040, 990, 934, 879, 826, 800, 770, 744. NMR (d_6-DMSO) δ: 2.09 (2H, multiplet, C_5-H), 2.81—4.10 (8H, multiplet, $2\times CH_2CH_2Cl$), 4.30 (2H, multiplet, C_6-H), 4.96 [1H, doublet of triplet, J (P, C_4-H) = 19.0 Hz, J (C_4-H, C_5-H) = 3.0 Hz, C_4-H], 4.98 [1H, doublet of triplet, J (P, NH) = 19.0 Hz, J (NH, CH_2) = 5.6 Hz, NH], 11.65 (1H, singlet, OOH). *Anal.* Calcd. for $C_7H_{15}Cl_2N_2O_4P$: C, 28.69; H, 5.16; Cl, 24.20; N, 9.56; P, 10.57. Found: C, 28.86; H, 5.14; Cl, 24.02; N, 9.65; P, 10.37. The final mother liquor of **4a** was concentrated *in vacuo* to give a colorless oil which was chromatographed on a column (10×60 cm) eluting with ether. After removal of a

23) Unpublished results.

24) The ^{31}P-NMR spectra were determined by Dr. S. Sato (Japan Electronic Varian Corp., Tokyo) to whom the authors acknowledge their indebtedness.

considerable amount of unidentified peroxidic materials by eluting with ether, the column was then eluted with acetone–$CHCl_3$ (1: 1), fractionating into two parts A, which is mainly consisting of a faster migrating component, and B, which is consisting of two major products besides one minor products as the later migrating components. After concentration of the fraction A *in vacuo*, the resulting oily residue (1.2 g) was chromatographed again on a column (3.5×10 cm) eluting with acetone–$CHCl_3$ (1: 2) to give an almost pure oil which on standing overnight at −20° turned into a crystalline solid (700 mg). Recrystallization of the crystalline solid from ether gave the minor product **6** (450 mg, 1.3%) as colorless prisms, mp 98—99°. IR ν_{max}^{Nujol} cm^{-1}: 3240, 3170, 1330, 1255, 1205, 1173, 1140, 1120, 1097, 1070, 1050, 990, 965, 940. NMR ($CDCl_3$) δ: 1.45 [6H, singlet, $C(CH_3)_2$], 2.20 (2H, multiplet, C_5–H), 3.00—3.90 (8H, multiplet, 2×CH_2CH_2Cl), 4.40 (2H, multiplet, C_6–H), 5.18 [1H, doublet of triplet, J (P, C_4–H)=21.0 Hz, J (C_4–H, C_5–H)=3.7 Hz, C_4–H], 9.17 (1H, broad, OOH). *Anal.* Calcd. for $C_{10}H_{21}Cl_2N_2O_6P$: C, 32.72; H, 5.77; Cl, 19.31; N, 7.63; P, 8.44. Found: C, 32.49; H, 6.00; Cl, 19.53; N, 7.77; P, 8.39. The fraction B was concentrated *in vacuo* to give an oily mixture (7.5 g) which was chromatographed again on a column (7×35 cm) with acetone–$CHCl_3$ (1: 3). Monitoring by TLC with acetone–$CHCl_3$ (1: 1), the fraction of the product 4-ketoisophosphamide **5** (270 mg, 1%) was collected as a faster migrating component. Recrystallization from $CHCl_3$ gave **5** as a colorless fine needles, mp 118—119° (lit.[6c] mp 110°), which was identified with an authentic specimen by IR comparison. After elution of **5**, the column was eluted with acetone–$CHCl_3$ (2: 3) to give 2-*epi*-4-hydroperoxyisophosphamide (**4b**) as an oil which on standing for 72 hr at −20° crystallized giving white solid (1.47 g, 5%). Recrystallization of the solid from ether–acetone (5: 1) gave colorless prisms, mp 75—76° (dec. 112°). IR ν_{max}^{KBr} cm^{-1}: 3162, 2970, 2915, 2900, 2865, 1477, 1440, 1420, 1390, 1374, 1360, 1349, 1328, 1290, 1254, 1217, 1175, 1160, 1140, 1124, 1103, 1083, 1055, 1028, 994, 962, 940, 935, 917, 904, 873, 856, 824, 803, 790, 740, 658. NMR (d_6-DMSO) δ: 1.93—2.20 (2H, multiplet, C_5–H), 2.80—4.38 (10H, multiplet, 2×CH_2CH_2Cl, C_6–H), 4.62 [1H, doublet of triplet, J (P, NH)=12.5 Hz, J (NH, CH_2)=6.3 Hz, NH], 5.02 [1H, doublet of triplet, J (P, C_4–H)=18.9 Hz, J (C_4–H, C_5–H)=4.05 Hz, C_4–H], 11.73 (1H, singlet, OOH), ($CDCl_3$) δ: 2.08—2.35 (2H, multiplet, C_5–H), 3.03—4.67 (11H, multiplet, 2×CH_2CH_2Cl, C_6–H, NH), 5.09 [1H, doublet of triplet, J (P, C_4–H)=20.3 Hz, J (C_4–H, C_5–H)=3.5 Hz, C_4–H], 11.50 (1H, broad, OOH). *Anal.* Calcd. for $C_7H_{15}Cl_2N_2O_4P$. C, 28.69; H, 5.16; Cl, 24.20; N, 9.56; P, 10.57. Found: C, 28.76; H, 5.32; Cl, 24.40; N, 9.69; P, 10.51. After elution of the pure fractions of **4b**, **4a** was eluted as a mixture containing small amount of **4b**, from which **4a** was isolated (1.18 g, 4%) after concentration and crystallization of the resulting residue.

2-[*N*-Methyl-*N*-(2-chloroethyl)]amino-3-(2-chloroethyl)-4-hydroperoxytetrahydro-2*H*-1,3,2-oxazaphosphorine-2-oxide (12)——After reacting $POCl_3$ (15.3 g, 100 mmol) with 3-buten-1-ol (7.2 g, 100 mmol) in a similar way as described for the preparation of **3**, *N*-methyl-2-(chloroethyl)amine hydrochloride (13.0 g, 100 mmol) and CH_2Cl_2 (100 ml) were added to the reaction mixture. To the mixture was added dropwise triethylamine (30 g, 300 mmol) with vigorous stirring at −35——−30° for 30 min, then stirring was continued for 1 hr at −30——25°. Then, 2-chloroethylamine hydrochloride (11.6 g, 100 mmol) was added to the mixture and triethylamine (20 g, 200 mmol) was added dropwise at −20——15° under vigorous stirring. After additional stirring for 1 hr at −15——10°, the mixture was filtered and the filtrate was washed three times with water (each 200 ml), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oily residue which was purified by chromatography on a column (10×50 cm) eluting with AcOEt giving *O*-(3-butenyl)-*N*-methyl-*N*-(2-chloroethyl)-*N'*-(2-chloroethyl)phosphorodiamidate (**9**) (12.14 g, 42%) as a colorless sticky oil; NMR ($CDCl_3$) δ: 2.14 (2H, quartet, J=7 Hz, $-\underline{CH_2}-CH=CH_2$), 2.79 (3H, doublet, J=9 Hz, N–CH_3), 3.19—3.74 (9H, multiplet, 2×CH_2CH_2Cl, NH), 4.07 (2H, quartet, J=7 Hz, PO–O–CH_2–), 5.04—6.27 (3H, multiplet, $-CH=CH_2$). *Anal.* Calcd. for $C_9H_{19}Cl_2N_2O_2P$: C, 37.39; H, 6.62; Cl, 24.52; N, 9.68; P, 10.71. Found: C, 37.16; H, 6.70; Cl, 24.78; N, 9.53; P, 10.98. The compound **9** (2.89 g, 10 mmol) was dissolved in 1: 1 aqueous acetone (20 ml) and the solution was ozonized with excess of O_3 (960 mg, 20 mmol) with stirring in an ice-water bath. After addition of 30% H_2O_2 (3 ml), the ozonized solution was allowed to stand for 72 hr at 2°. The mixture was concentrated *in vacuo*, and the resulting aqueous residue was extracted with $CHCl_3$ (3×20 ml). The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oily residue (3.3 g) which was chromatographed on a column (3.5×20 cm) eluting with AcOEt giving **12** as a crystalline residue (460 mg, 15%). Recrystallization from acetone–ether (1: 5) gave colorless prisms, mp 109.5—110° (dec.); NMR (d_6-DMSO) δ: 2.20 (2H, multiplet, C_5–H), 2.63 (3H, d, J=10 Hz, NCH_3), 3.22—3.65 (8H, multiplet, 2×CH_2CH_2Cl), 4.98 [1H, doublet of triplet, J (P, C_4–H)=19.5 Hz, J (C_4–H, C_5–H)=2.6 Hz, C_4–H], 11.37 (1H, singlet, OOH). *Anal.* Calcd. for $C_8H_{17}Cl_2N_2O_4P$: C, 31.29; H, 5.58; Cl, 23.09; N, 9.12; P, 10.09. Found: C, 31.32; H, 5.63; Cl, 23.01; N, 9.31; P, 9.93.

2-[*N*-Ethyl-*N*-(2-chloroethyl)]amino-3-(2-chloroethyl)-4-hydroperoxytetrahydro-2*H*-1,3,2-oxazaphosphorine-2-oxide (13)——By reacting $POCl_3$ (15.3 g, 100 mmol) with 3-buten-1-ol (7.2 g, 100 mmol), *N*-ethyl-*N*-(2-chloroethyl)amine hydrochloride (14.4 g, 100 mmol) and finally with 2-chloroethylamine hydrochloride (11.6 g, 100 mmol) in a quite similar way as described above, *O*-(3-butenyl)-*N*-ethyl-*N*-(2-chloroethyl-N'-(2-chloroethyl)phosphorodiamidate (**10**) was obtained after column chromatography with AcOEt as a colorless sticky oil (11.5 g, 38%); NMR ($CDCl_3$) δ: 1.20 (3H, triplet, J=6 Hz, $-CH_3$), 2.32—2.64 (2H, multiplet, $-\underline{CH_2}-CH=CH_2$), 2.94—3.73 (11H, multiplet, 2×CH_2CH_2Cl, $-\underline{CH_2}CH_3$, NH), 4.07 (2H, quartet, J=7 Hz, PO–O–CH_2–), 5.00—6.22 (3H, multiplet, $-CH=CH_2$). *Anal.* Calcd. for $C_{10}H_{21}Cl_2N_2O_2P$: C, 39.62; H, 6.98; Cl, 23.29; N, 9.24; P, 10.22. Found: C, 40.01; H, 7.21; Cl, 23.21; N, 9.39; P, 9.97. Ozonolysis of

10 (3.03 g, 10 mmol) was carried out in 1: 1 aqueous acetone (20 ml) with excess of O_3 while cooling in an ice-water bath, and after addition of 30% H_2O_2 (3 ml) to the ozonized solution the mixture was allowed to stand for 72 hr at 2°. After treatments of the reaction mixture in a similar manner cited above and purification of the crude product by column chromatography (3.5×20 cm) with AcOEt, **13** was isolated as a colorless oil (417 mg, 13%) which was crystallized by addition of ether. Recrystallization from ether–acetone (5: 1) gave colorless prisms, mp 112—113° (dec.). NMR (d_6-DMSO) δ: 2.20 (3H, triplet, J=6 Hz, CH_3), 2.33 (2H, multiplet, C_5–H), 2.90—3.90 (10H, multiplet, 2×CH_2CH_2Cl, –N$\underline{CH_2}CH_3$), 4.10—4.92 (2H, multiplet, C_6–H), 4.99 [1H, doublet of triplet, J (P, C_4–H)=19.7 Hz, J (C_4–H, C_5–H)=2.9 Hz, C_4–H], 11.56 (1H, singlet, OOH). *Anal.* Calcd. for $C_9H_{19}Cl_2N_2O_4P$: C, 33.66; H, 5.96; Cl, 22.08; N, 8.72; P, 9.65. Found: C, 33.45; H, 5.93; Cl, 21.91; N, 8.51; P, 9.48.

2-[*N*,*N*-Bis(2-chloroethyl)]amino-3-(2-chloroethyl)-4-hydroperoxytetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide (14) (4-Hydroperoxytrophosphamide, NSC 260608)——By reacting $POCl_3$ (15.3 g, 100 mmol) with 3-buten-1-ol (7.2 g, 100 mmol), *N*,*N*-bis(2-chloroethyl)amine hydrochloride (17.8 g, 100 mmol) and finally with 2-chloroethylamine hydrochloride (11.6 g, 100 mmol) in a manner described above and after column chromatography with AcOEt, *O*-(3-butenyl)-*N*,*N*-bis(2-chloroethyl)-*N'*-(2-chloroethyl)phosphorodiamidate (**11**) was obtained as a colorless oil (13.5 g, 40%). NMR ($CDCl_3$) δ: 2.43 (2H, quartet, J=6.3 Hz, –$\underline{CH_2}$–CH=CH_2), 3.00—3.80 (13H, multiplet, 3×CH_2CH_2Cl, NH), 4.05 (2H, quartet, J=6.7 Hz, PO–O–CH_2–), 5.00—6.20 (3H, multiplet, –CH=CH_2). *Anal.* Calcd. for $C_{10}H_{20}Cl_3N_2O_2P$: C, 35.69; H, 5.99; Cl, 31.61; N, 8.33; P, 9.21. Found: C, 36.55; H, 6.24; Cl, 31.48; N, 8.03; P, 8.83. Ozonolysis of **11** (3.36 g, 10 mmol) was carried out in 1: 1 aqueous acetone (30 ml) with excess of O_3, and the resulting reaction mixture was allowed to stand for 72 hr at 2° after addition of 30% H_2O_2 (3 ml). After the usual treatments of the reaction mixture, the crude product was purified by column chromatography (3.5×20 cm) with AcOEt to give **14** as a colorless oil (996 mg, 26%) which turned into a crystalline solid on standing for one week at −20°. Recrystallization from ether–acetone (1: 3) gave colorless prisms, mp 115—117° (dec.). NMR (d_6-DMSO) δ: 2.10 (2H, multiplet, C_5–H), 2.99—4.10 (12H, multiplet, 3×CH_2CH_2Cl), 4.17—4.56 (2H, multiplet, C_6–H), 5.00 [1H, doublet of triplet, J (P, C_4–H)=19.8 Hz, J (C_4–H, C_5–H)=2.5 Hz, C_4–H], 11.78 (1H, singlet, OOH). *Anal.* Calcd. for $C_9H_{18}Cl_3N_2O_4P$: C, 30.40; H, 5.11; Cl, 29.92; N, 7.88; P, 8.72. Found: C, 30.21; H, 5.16; Cl, 29.85; N, 7.74; P, 8.70.

TsOH-Catalyzed Isomerization of 4-Hydroperoxyisophosphamides 4a and 4b——To a stirred suspension of **4a** (2.93 g, 10 mmol) in $CHCl_3$ (100 ml) was added *p*-toluenesulfonic acid (*ca.* 100 mg), and the mixture was stirred for 4 hr at room temperature to give a clear solution. The solution was washed with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oily residue (2.50 g) which was chromatographed on a column (5×25 cm) with $CHCl_3$–acetone (1: 1). After elution of a small quantity of unidentified oily substance (25 mg), **4b** was eluted as a faster migrating isomer giving colorless sticky oil (920 mg, 31%) which crystallized on standing overnight at −20° and was identified with an authentic specimen by comparison of their IR spectra. After elution of **4b**, **4a** (890 mg, 30%) was eluted as a later migrating isomer and was identified with an authentic specimen by IR comparisons. Similarly, the TsOH treatment of **4b** in $CHCl_3$ afforded a mixture whose TLC pattern ($CHCl_3$: acetone=1: 1) was found to be identical with that of a mixture of an equimolar amount of **4a** and **4b**, and gave **4a** and **4b** in an approximately the same ratio after column chromatography with $CHCl_3$–acetone (1: 1).

4-Hydroxyisophosphamide (15a)——To a stirred suspension of 4-hydroperoxyisophosphamide (**4a**) (293 mg, 1 mmol) in CH_2Cl_2 (20 ml) in an ice-water bath, a slightly excess amount of triethylphosphite (*ca.* 200 mg) was added. After stirring for 10 min, the suspension turned into a clear solution from which colorless fine needles were gradually precipitated, and after 30 min the reaction mixture turned entirely into a white solid. After washing with ether, the solid was collected by filtration to give **15a** (210 mg, 76%) as a white cake. Recrystallization from acetone–ether (1: 2) gave colorless needles, mp 74—75° (dec.). IR ν_{max}^{KBr} cm^{-1}: 3313, 3285, 2990, 2950, 2931, 2888, 2860, 1444, 1313, 1262, 1234, 1215, 1195, 1109, 1063, 1044, 975, 921, 887, 810, 772, 743, 715. NMR (D_2O) δ (from DSS): 1.99 (2H, multiplet, C_5–H), 2.75—3.90 (8H, multiplet, 2×CH_2CH_2Cl), 4.00—4.90 (2H, multiplet, C_6–H), 5.05 [1H, doublet of triplet, J (P, C_4–H)=18.0 Hz, J (C_4–H, C_5–H)=3.5 Hz, C_4–H]. *Anal.* Calcd. for $C_7H_{15}Cl_2N_2O_3P$: C, 30.34; H, 5.46; Cl, 25.59; N, 10.11; P, 11.18. Found: C, 30.49; H, 5.60; Cl, 25.37; N, 9.78; P, 11.01. The purified crystals of **15a** were found to be unstable at room temperature, being decomposed into brown oily material on standing overnight.

2-*epi*-4-Hydroxyisophosphamide (15b)——To a stirred solution of 2-*epi*-4-hydroperoxyisophosphamide (**4b**) (293 mg, 1 mmol) in CH_2Cl_2 (20 ml) was added triethylphosphite (*ca.* 200 mg) while cooling in an ice-water bath, and after stirring for 30 min at 0—5° the mixture was concentrated *in vacuo* to give a crystalline residue which was washed with ether and collected by suction giving **15b** as a white cake (220 mg, 80%). Recrystallization from ether–acetone (1: 10) gave colorless needles, mp 49—50°. IR ν_{max}^{Nujol} cm^{-1}: 3280, 3230, 1247, 1200, 1183, 1178, 1144, 1120, 1103, 1080, 1072, 1042, 991, 973. NMR (D_2O) δ (from DSS): 1.86—2.36 (2H, multiplet, C_5–H), 2.80—4.61 (10H, multiplet, 2×CH_2CH_2Cl, C_6–H), 5.08 [1H, doublet of triplet, J (P, C_4–H)=18.4 Hz, J (C_4–H, C_5–H)=3.6 Hz, C_4–H]; ($CDCl_3$) δ: 1.87—2.37 (2H, multiplet, C_5–H), 2.83—4.60 (11H, multiplet, 2×CH_2CH_2Cl, C_6–H, NH), 4.97 [1H, doublet of triplet, J (P, C_4–H)=21.6 Hz, J (C_4–H, C_5–H)=3.4 Hz, C_4–H], 5.80 (1H, broad, OH). *Anal.* Calcd. for $C_7H_{15}Cl_2N_2O_3P$: C, 30.34; H, 5.46; Cl, 25.59; N, 10.11; P, 11.18. Found: C, 30.51; H, 5.26; Cl, 25.81; N, 9.89; P, 11.43. **15b** was also found to be very

unstable, and it was decomposed into brown oily material on standing overnight at room temperature.

Formation of 4-Ketoisophosphamide (5) from 4a and 4b——To a stirred suspension of **4a** (293 mg, 1 mmol) in $CHCl_3$ (50 ml) was added a solution of $FeSO_4 \cdot 7H_2O$ (556 mg, 2 mmol) in H_2O (50 ml) and the mixture was stirred vigorously for 1 hr at room temperature. The $CHCl_3$ layer, after separating, washing with H_2O, drying over anhydrous Na_2SO_4 and concentration *in vacuo*, gave 4-ketoisophosphamide (**5**) as a white cake (262 mg, 95%) which was identified with an authentic specimen by IR comparison. The similar treatments of **4b** (293 mg, 1 mmole) with an aqueous solution of $FeSO_4 \cdot 7H_2O$ (556 mg) in $CHCl_3$ afforded **5** (258 mg, 94%) which was also identified with an authentic specimen by IR comparison.

6-(2-Chloroethylamino)perhydro[1,2,4]dioxazino[4,3-*c*][1,3,2]oxazaphosphorine-6-oxide (Cyclic Peroxide, 16a)——To a suspension of 4-hydroperoxyisophosphamide (**4a**) (293 mg, 1 mmol) in $CHCl_3$ 50 ml was added a saturated aqueous Na_2CO_3 solution (20 ml) [use of aqueous 1 N KOH solution (20 ml) gave essentially the same result as obtained for Na_2CO_3], and the mixture was vigorously stirred for 1 hr at room temperature. The $CHCl_3$ layer was separated, washed with H_2O, dried over anhydrous Na_2SO_4 and finally concentrated *in vacuo* to give **16a** as a crystalline solid (246 mg, 96%). Recrystallization from Me_2CO gave colorless prisms, mp 127—129° (dec.). IR ν_{max}^{KBr} cm^{-1}: 3200, 2975, 2928, 2920, 2867, 1467, 1428, 1300, 1255, 1215, 1162, 1136, 1080, 1062, 1026, 985, 917, 880, 861, 792. NMR (d_6-DMSO) δ: 1.84 (2H, multiplet, C_5–H), 2.70—3.80 (7H, multiplet, $NHCH_2CH_2Cl$, NCH_2), 3.80—4.80 (4H, multiplet, $2\times$–OCH_2), 5.34 [1H, doublet of triplet, J (P, C_4–H) = 18.9 Hz, J (C_4–H, C_5–H) = 4.5 Hz, C_4–H], ($CDCl_3$) δ: 2.02 (2H, multiplet, –CH–$C\underline{H}_2$–CH_2–), 2.83—4.20 (9H, multiplet, $NHCH_2CH_2Cl$, PO–O–CH_2–, –N–CH_2–), 4.27—5.02 (2H, multiplet, –CH_2–O–O), 5.40 [1H, doublet of double doublet, J (P, H) = 21.9 Hz, J (H, H) = 4.9 Hz, J' (H, H) = 2.4 Hz, –CH–]. ^{13}C-NMR ($CDCl_3$) ppm [J (P, C) Hz]: 28.94 [4.5] (C_5), 43.18 and 44.84 [4.9] ($ClCH_2$ and NCH_2), 61.86 [6.0] (C_6), 71.92 ($OOCH_2$), 89.93 [0.7] (C_4). *Anal.* Calcd. for $C_7H_{14}ClN_2O_4P$: C, 32.76; H, 5.50; Cl, 13.81: N, 10.92; P, 12.07: Found: C, 33.01; H, 5.67; Cl, 14.17; N, 11.05; P, 11.98.

6-*epi*-6-(2-Chloroethylamino)perhydro[1,2,4]dioxazino[4,3-*c*][1,3,2]oxazaphosphorine-6-oxide (Cyclic Peroxide 16b)——2-*epi*-4-Hydroperoxyisophosphamide (**4b**) (293 mg, 1 mmol) was similarly treated with saturated aqueous Na_2CO_3 (or 1N KOH) solution in $CHCl_3$ as cited above, giving **16b** as a crystalline solid (232 mg, 90%) which on recrystallization from acetone gave colorless prisms, mp 103—105°. IR ν_{max}^{KBr} cm^{-1}: 3162, 2970, 2915, 2900, 2865, 1477, 1440, 1420, 1390, 1374, 1360, 1349, 1328, 1290, 1254, 1217, 1175, 1160, 1140, 1124, 1103, 1080, 1055, 1028, 994, 962, 940, 935, 917, 904, 873, 856, 824, 803, 790, 740, 658, 634, 618. NMR ($CDCl_3$) δ: 2.07 (2H, multiplet, –CH–$\underline{CH}_2$–CH_2–), 3.00—4.02 (7H, multiplet, $NHCH_2CH_2Cl$, –N–CH_2–), 4.08—4.77 (4H, multiplet, $2\times$–O–CH_2–), 5.46 [1H, doublet of double doublet, J (P, H) = 5.8 Hz, J (H, H) = 8.2 Hz, J' (H, H) = 7.3 Hz, –CH–]. ^{13}C-NMR ($CDCl_3$) ppm [J (P, C) Hz]: 28.58 [3.5] (C_5), 41.66 (NCH_2), 43.18 and 44.97 [5.3] ($NHCH_2CH_2Cl$), 61.72 [5.8] (C_6), 71.79 [6.0] ($OOCH_2$), 89.67 [1.5] (C_4). *Anal.* Calcd. for $C_7H_{14}ClN_2O_4P$: C, 32.76; H, 5.50; Cl, 13.82; N, 10.92; P, 12.07. Found: C, 32.70; H, 5.62; Cl, 13.88; N, 11.07; P, 12.12.

TsOH-Catalyzed Isomerization of 16a and 16b——To a stirred solution of **16a** (or **16b**) (257 mg, 1 mmol) in $CHCl_3$ (20 ml) was added TsOH (*ca.* 20 mg) at room temperature. After stirring for 30 min, the reaction mixture was allowed to sfand for 48 hr at room temperature. The mixture was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a colorless oily residue (247 mg) of which TLC ($CHCl_3$: acetone = 1: 1) indicated the presence of two components in a ratio of *ca.* 4: 5 and was identical with that of a mixture of **16a** and **16b** in the same ratio (**16a**/**16b** = 4/5). By column chromatography of the residue on a column (3×15 cm) with $CHCl_3$–acetone (1: 1), **16a** (85 mg, 33%) was isolated as a faster migrating component which was identified with an authentic specimen by IR comparison, and **16b** (95 mg, 37%) was eluted as a later migrating component which was also identified with an authentic specimen by IR comparison.

6-[*N*-Methyl-*N*-(2-chloroethyl)amino]perhydro[1,2,4]dioxazino[4,3-*c*][1,3,2]oxazaphosphorine-6-oxide (Cyclic Peroxide 17a)——To a stirred solution of *N*-methyl-4-hydroperoxyisophosphamide (**12**) (307 mg, 1 mmol) in $CHCl_3$ (40 ml) was added a saturated aqueous Na_2CO_3 solution (*ca.* 20 ml), and the mixture was vigorously stirred for 1 hr at room temperautre. The $CHCl_3$ layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oily residue which was crystallized by addition of ether, and the resulting crystals were collected to give **17a** (243 mg, 90%). Recrystallization of **17a** from acetone–ether (1: 2) gave colorless prisms, mp 71—72°. IR ν_{max}^{Nujol} cm^{-1}: 1338, 1298, 1260, 1247, 1240, 1219, 1157, 1140, 1117, 1085, 1064, 1025, 1004, 964, 945, 906, 883, 858, 815, 793, 755. NMR ($CDCl_3$) δ: 1.67—2.57 (2H, multiplet, –CH–$\underline{CH}_2$–CH_2–), 2.72 (3H, doublet, J = 9.7 Hz, NCH_3), 2.98—3.62 (6H, multiplet, CH_2CH_2Cl, –N–CH_2), 3.67—4.05 (2H, multiplet, PO–O–CH_2–), 4.25—5.06 (2H, multiplet, –O–O–CH_2–), 5.40 [1H, doublet of double doublet, J (P, H) = 21.8 Hz, J (H, H) = 4.1 Hz, J' (H, H) = 2.9 Hz, –CH). *Anal.* Calcd. for $C_8H_{16}ClN_2O_4P$: C, 35.50; H, 5.96; Cl, 13.10; N, 10.35; P, 11.45. Found: C, 35.54; H, 6.04; C, 13.17; N, 10.48; P, 11.20.

6-*epi*-6-[*N*-Methyl-*N*-(2-chloroethyl)amino]perhydro[1,2,4]dioxazino[4,3-*c*][1,3,2]oxazaphosphorine-6-oxide (Cyclic Peroxide 17b)——To a stirred solution of **17a** (271 mg, 1 mmol) in $CHCl_3$ (20 ml) was added TsOH (*ca.* 20 mg) and the mixture was allowed to stand for 48 hr at room temperature. The reaction mixture was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oily residue (265 mg) which was chromatographed on a column (2.5×12 cm) with AcOEt. From the first eluate, **17a** (81 mg, 30%) was recovered, and the second eluate gave **17b** (111 mg, 41%) as an oil. NMR ($CDCl_3$) δ: 1.57—2.30 (2H, multiplet, –CH–$\underline{CH}_2CH_2$–), 2.79 (3H, doublet, J = 10.0 Hz, NCH_3), 3.22—3.82 (6H, multiplet, –NCH_2CH_2–

Cl, –N–CH_2), 3.98—4.73 (4H, multiplet, PO–O–CH_2–, –O–O–CH_2–), 5.48 [1H, doublet of triplet, J (P, H) = 8.9 Hz, J (H, H) = 5.6 Hz, –CH–]. *Anal.* Calcd. for $C_8H_{16}ClN_2O_4P$: C, 35.50; H, 5.96; Cl, 13.10; N, 10.35; P, 11.45. Found: C, 35.54; H, 5.81; Cl, 12.88; N, 10.11; P, 11.62.

TsOH-Catalyzed Isomerization Experiment for *N*-Methyl-4-hydroperoxyisophosphamide (12)——*N*-Methyl-4-hydroperoxyisophosphamide **12** (307 mg, 1 mmol) was dissolved in $CHCl_3$ (50 ml), then TsOH (*ca.* 50 mg) was added to the solution and the mixture was allowed to stand for 48 hr at room temperature. To the mixture was added a saturated aqueous Na_2CO_3 solution (20 ml) and the mixture was vigorously stirred for 1 hr at room temperature. The $CHCl_3$ layer was separated, washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oil (210 mg) which was chromatographed on a column (2.5 × 10 cm) with AcOEt giving **17a** (105 mg) and **17b** (25 mg), respectively.

6-[*N*,*N*-Bis(2-chloroethyl)amino]perhydro[1,2,4]dioxazino[4,3-*c*][1,3,2]oxazaphosphorine-6-oxide (Cyclic Peroxide 18a)——To a stirred solution of 4-hydroperoxytrophosphamide (**14**) (355 mg, 1 mmol) in $CHCl_3$ (10 ml) was added an aqueous solution of 1 N-KOH (4 ml) and the mixture was vigorously stirred for 1 hr at room temperature. The $CHCl_3$ layer was separated, washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oily residue which crystallized on addition of ether and the resulting crystals were collected to give **18a** (240 mg, 75%). Recrystallization of **18a** from ether gave colorless prisms, mp 84—87° (dec.). IR ν_{max}^{Nujol} cm^{-1}: 1345, 1341, 1335, 1303, 1280, 1261, 1240, 1223, 1160, 1139, 1117, 1085, 1068, 1030, 1002, 998, 964, 950, 940, 911, 896, 863, 820, 803, 780, 768, 740. NMR ($CDCl_3$) δ: 1.75—2.55 (2H, multiplet, –CH–$\underline{CH_2}$–CH_2–), 2.98—3.62 [10H, multiplet, $N(CH_2CH_2Cl)_2$, –N–CH_2–], 3.68—4.07 (2H, multiplet, PO–O–CH_2–), 4.27—5.05 (2H, multiplet, –O–O–CH_2–), 5.38 [1H, doublet of double doublet, J (P, H) = 22.5 Hz, J (H, H) = 4.1 Hz, J' (H, H) = 2.9 Hz, –CH–]. *Anal.* Calcd. for $C_9H_{17}Cl_2N_2O_4P$: C, 33.87; H, 5.35; Cl, 22.22; N, 8.78; P, 9.71. Found: C, 34.06; H, 5.51; Cl, 22.01; N, 8.81; P, 9.90.

6-*epi*-6-[*N*,*N*-Bis(2-chloroethyl)amino]perhydro[1,2,4]dioxazino[4,3-*c*][1,3,2]oxazaphosphorine-6-oxide (Cyclic Peroxide 18b)——Compound **18a** (320 mg, 1 mmol) was isomerized using TsOH (*ca.* 20 mg) in a quite similar manner as described for the isomerization reaction of **17a** and the resulting mixture was purified by column chromatography (2.5 × 10 cm) with $CHCl_3$–acetone (1: 1). As a faster migrating component, **18a** (90 mg, 28%) was recovered, then **18b** was eluted as a colorless oil (134 mg, 42%) which was crystallized by addition of ether. Recrystallization of **18b** from ether–acetone (3: 1) gave colorless prisms, mp 102—105°. IR ν_{max}^{Nujol} cm^{-1}: 1351, 1323, 1293, 1266, 1255, 1235, 1166, 1159, 1138, 1099, 1092, 1062, 1030, 1019, 999, 950, 930, 923, 905, 878, 811, 804, 780, 743. NMR ($CDCl_3$) δ: 1.62—2.10 (2H, multiplet, –CH–$\underline{CH_2}$–CH_2–), 3.23—3.77 [6H, multiplet, $N(CH_2CH_2Cl)_2$, –N–CH_2–], 3.82—4.23 (2H, multiplet, PO–O–CH_2), 4.32—4.77 (2H, multiplet, –O–O–CH_2), 5.48 [1H, doublet of double doublet, J (P, H) = 5.7 Hz, J (H, H) = 9.1 Hz, J' (H, H) = 4.9 Hz, –CH]. *Anal.* Calcd. for $C_9H_{17}Cl_2N_2O_4P$: C, 33.87; H, 5.35; Cl, 22.22; N, 8.78; P, 9.71. Found: C, 34.05; H, 5.53; Cl, 22.48; N, 8.80; P, 9.52.

4-Methoxyisophosphamides (25a and 25b)——4-Hydroperoxyisophosphamide (**4a**) (or 2-*epi*-4-hydroperoxyisophosphamide (**4b**)) (2.93 g, 10 mmol) was added to a stirred mixture of $CHCl_3$ (30 ml) and MeOH (10 ml) containing small amount of TsOH (*ca.* 50 mg), and the mixture was stirred for 1 hr at room temperature. The solvents were removed by evaporation *in vacuo* to give a slightly colored (brown) oil which was dissolved in $CHCl_3$ and washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on a column (4 × 20 cm) with $CHCl_3$–acetone (3: 1) giving firstly **25b** (670 mg, 23%), and secondly **25a** (610 mg, 21%). **25a** was crystallized by addition of ether and recrystallized from ether–acetone (4: 1) to give colorless prisms, mp 77—77.5° (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3140, 1260, 1220, 1203, 1190, 1160, 1150, 1130, 1078, 1054, 923, 806. NMR ($CDCl_3$) δ: 2.00 (2H, multiplet, C_5–H), 3.03—4.00 (11H, multiplet, 2 × CH_2CH_2Cl, PO–O–CH_2–, NH), 3.33 (3H, singlet, OCH_3), 4.53 [1H, doublet of triplet, J (P, C_4–H) = 21.6 Hz, J (C_4–H, C_5–H) = 3.0 Hz, C_4–H]. *Anal.* Calcd. for $C_8H_{17}Cl_2N_2O_3P$: C, 33.01; H, 5.89; Cl, 24.36; N, 9.62. Found: C, 32.82; H, 6.06; Cl, 24.57; N, 9.72. **25a** was found to be somewhat unstable and decomposed into brown resin on standing overnight at room temperature. **25b** was obtained as an unstable pale yellow oil which also decomposed into brown resinous substance on standing overnight at room temperature. NMR ($CDCl_3$) δ: 1.92—2.30 (2H, multiplet, C_5–H), 3.05—3.92 (9H, multiplet, 2 × CH_2CH_2Cl, NH), 3.36 (3H, singlet, OCH_3), 4.03—4.60 (2H, multiplet, C_6–H) 4.53 [1H, doublet of triplet, J (P, C_4–H) = 21.0 Hz, J (C_4–H, C_5–H) = 3.2 Hz, C_4–H]. *Anal.* Calcd. for $C_8H_{17}Cl_2N_2O_3P$: C, 33.01; H, 5.89; Cl, 24.36; N, 9.62. Found: C, 33.40; H, 5.62; Cl, 24.00; N, 9.31. Further elution of the column with acetone–$CHCl_3$ (1:1) gave 2-*epi*-4-hydroperoxyisophosphamide (**4b**) (120 mg) and subsequently 4-hydroperoxyisophosphamide **4a** (85 mg), which were identified with authentic specimen by IR comparisons.

4-(*tert*-Butyl)peroxyisophosphamides (26a and 26b)——To a stirred suspension of 4-hydroperoxyisophosphamide (**4a**) (2.93 g, 10 mmol) in $CHCl_3$ (50 ml) was added TsOH (*ca.* 50 mg) and *tert*-butylhydroperoxide (2 ml), and the mixture was stirred for 1 hr at room temperature. The reaction mixture was washed with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oily residue (4.7 g) which was chromatographed on a column (4 × 20 cm) with $CHCl_3$–acetone (3: 1) giving a colorless oil (3.1 g). Further elution of the column with $CHCl_3$–acetone (1: 1) gave small amounts of **4b** (75 mg) and **4a** (45 mg). The oily product eluted as a faster migrating component showed a homogeneous TLC spot for the solvents AcOEt, $CHCl_3$–acetone (1: 1 and 3: 1) acetone–ether (2: 1) and ether. The ^{13}C–NMR spectrum ($CDCl_3$) of this product showed peaks corresponding to a 2: 1 mixture of two isomeric products. Major product **26a**: δ: 26.40

($3\times CH_3$), 29.36 [J (P, C) = 4.9 Hz, C_5], 42.68 (CH_2Cl), 44.02 (CH_2Cl), 46.04 [J (P, C) = 4.5 Hz, $NHCH_2$], 50.01 [J (P, C) = 4.2 Hz, $-\dot{N}-CH_2$], 62.92 [J (P, C) = 6.8 Hz, C_6], 80.91 ($-OO-\dot{C}-$), 91.51 (C_4), minor product **26b**: δ: 26.55 ($3\times CH_3$), 28.77 (C_5), 42.37 [J (P, C) = 1.4 Hz, CH_2Cl], 43.33 (CH_2Cl), 44.99 [J (P, C) = 5.2 Hz, $NHCH_2$], 49.04 [J (P, C) = 3.7 Hz, $-\dot{N}-CH_2$), 62.17 [J (P, C) = 6.4 Hz, C_6], 80.56 ($-OO-\dot{C}-$), 90.53 (C_4). The oil partly crystallized on standing for 48 hr at −20° and the resulting crystals were recrystallized from ether–hexane (2: 1) to give **26a** as colorless needles (1.34 g, 38%), mp 49—50°. IR ν_{max}^{Nujol} cm^{-1}: 3235, 1330, 1260, 1243, 1239, 1170, 1110, 1079, 1037, 910, 900, 794. NMR ($CDCl_3$) δ: 1.28 (9H, singlet, $3\times CH_3$), 2.03—2.37 (2H, multiplet, C_5–H), 3.20—3.95 (9H, multiplet, $2\times CH_2CH_2Cl$, NH), 4.03—4.53 (2H, multiplet, C_6–H), 5.12 [1H, doublet of triplet, J (P, C_4–H) = 20.9 Hz, J (C_4–H, C_5–H) = 3.8 Hz, C_4–H], (d_6-DMSO) δ: 1.23 (9H, singlet, $3\times CH_3$), 1.97—2.25 (2H, multiplet, C_5–H), 2.83—3.77 (8H, multiplet, $2\times CH_2CH_2Cl$), 3.82—4.36 (2H, multiplet, C_6–H), 4.65 [1H, doublet of triplet, J (P, NH) = 12.0 Hz, J (NH, CH_2) = 6.1 Hz, NH], 5.15 [1H, doublet of triplet, J (P, C_4–H) = 18.2 Hz, J (C_4–H, C_5–H) = 4.3 Hz, C_4–H]. *Anal.* Calcd. for $C_{11}H_{23}Cl_2N_2O_4P$: C, 37.84; H, 6.64; Cl, 20.31; N, 8.02; P, 8.87. Found: C, 37.86; H, 6.75; Cl, 20.41; N, 8.09; P, 8.68. The crystalline **26a** showed homogeneous ^{13}C-NMR signals corresponding to the major peaks cited above.

4-Phenylthiosophosphamides (27a and 27b)——To a stirred suspension of 4-hydroperoxyisophosphamide (**4a**) (2.93 g, 10 mmol) in $CHCl_3$ (50 ml) was added TsOH (*ca.* 50 mg) and thiophenol (2.2 g, 20 mmol). After stirring for 2 hr at room temperature, the reaction mixture was allowed to stand overnight at 2°. The mixture was washed with H_2O, dried over anhydrous Na_2SO_4 and chromatographed on a column (5 × 25 cm) with AcOEt. After eluting the unreacted thiophenol, **29a** was first eluted and crystallized on concentration *in vacuo* to give a white solid (1.44 g, 39%) which on recrystallization from acetone–ether (1: 1) gave colorless prisms, mp 135—135.5°. IR ν_{max}^{Nujol} cm^{-1}: 3185, 1310, 1276, 1225, 1213, 1203, 1190, 1153, 1143, 1120, 1082, 1080, 1050, 1044, 1035, 979, 940, 925, 898, 800, 760, 759, 725. NMR (d_6-DMSO + D_2O) δ: 1.63—2.38 (2H, multiplet, C_5–H), 2.88—3.97 (8H, multiplet, $2\times CH_2CH_2Cl$), 4.13—4.78 (2H, multiplet, C_6–H), 5.00 [1H, doublet of triplet, J (P, C_4–H) = 21.8 Hz, J (C_4–H, C_5–H) = 3.5 Hz, C_4–H], 7.23—7.62 (5H, multiplet, C_6H_5). *Anal.* Calcd. for $C_{13}H_{19}Cl_2N_2O_2PS$: C, 42.29; H, 5.19; Cl, 19.20; N, 7.59; P, 8.39. Found: C, 42.44; H, 5.39; Cl, 19.42; N, 7.67; P, 8.19. Further elution with the same solvent gave an isomer **27b** as an oil (1.70 g, 46%) which crystallized on standing for 72 hr at −20°. Recrystallization of **27b** with hexane–ether (3: 2) gave colorless needles, mp 61.5—62°. IR ν_{max}^{Nujol} cm^{-1}: 3180, 1317, 1240, 1212, 1180, 1147, 1123, 1087, 1059, 1050, 1030, 980, 941, 918, 895, 805, 796, 758, 727, 700, 695. NMR ($CDCl_3$) δ: 1.82—2.83 (2H, multiplet, C_5–H), 3.03—3.88 (8H, multiplet, $2\times CH_2CH_2Cl$), 3.97—4.78 (2H, multiplet, C_6–H), 4.96 [1H, doublet of triplet, J (P, C_4–H) = 17.3 Hz, J (C_4–H, C_5–H) = 4.2 Hz, C_4–H], 7.21—7.60 (5H, multiplet, C_6H_5), (d_6-DMSO + D_2O) δ: 1.75—2.75 (2H, multiplet, C_5–H), 2.92—4.05 (8H, multiplet, $2\times CH_2CH_2Cl$), 4.15—4.66 (2H, multiplet, C_6–H), 5.10 [1H, doublet of triplet, J (P, C_4–H) = 15.0 Hz, J (C_4–H, C_5–H) = 4.5 Hz, C_4–H], 7.27—7.67 (5H, multiplet, C_6H_5). *Anal.* Calcd. for $C_{13}H_{19}Cl_2N_2O_2PS$: C, 42.29; H, 5.19; Cl, 19.20; N, 7.59; P, 8.39. Found: C, 42.23; H, 5.39; Cl, 19.42; N, 7.77; P, 7.98.

Acknowledgement We wish to thank Drs. K. Tori, S. Matsui, K. Kuriyama, and their coworkers for determining the spectral data and their valuable discussions on the stereochemical problems. We also thank Dr. K. Yamaguchi and his coworkers for evaluations and bioassay experiments on the antileukemic activity.