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Syntheses of New Cyclophosphamide Derivatives Having 1,3,4,2-Oxadiazaphosphorinane and Related heterocyclic Systems[1]

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New cyclophosphamide derivatives having the 1,3,4,2-oxadiazaphosphorinane and related heterocyclic systems were prepared by the ozonolysis reactions of *O*-(2-propenyl)- and *O*-(3-butenyl)-*N*,*N*-bis(2-chloroethyl)phosphoramidoyl hydrazides and their derivatives. The newly synthesized heterocyclic systems were dihydro-6*H*-1,3,4,2-oxadiazaphosphorine-2-oxide, 4-acetyl-5-hydroxy-1,3,4,2-oxadiazaphosphorinane-2-oxide, 4-acetyl-5-hydroxyhexahydro-2*H*-1,3,4,2-oxadiazaphosphepine-2-oxide and perhydro-1,3,4-oxadiazolo[3,2-*c*]-1,3,2-oxazaphosphorine-1-oxide. Comparative studies of the *in vivo* antileukemic activity revealed that these cyclophosphamide derivatives were ineffective in promoting the antitumor action.

Keywords——nitrogen mustard derivatives; antitumor agents; phosphorus-containing heterocycles; ozonolytic cyclization; heterocyclic hydroperoxide; vicinal P–H coupling constants; phosphoramidoyl hydrazides

The antitumor agent cyclophosphamide (**1**) is known to show cytostatic activity after enzymatic C_4-hydroxylation *in vivo*.[3] Because of considerable instability of the active metabolite of cyclophosphamide, various efforts for its synthesis have been unsuccessful until our recent studies[4] which demonstrated the first unambiguous synthesis of crystalline 4-hydroxycyclophosphamide (**2**) having pronounced activity against animal tumors both *in vivo* and *in vitro* experiments. The synthesis was simply performed by ozonolysis of *O*-(3-butenyl)-*N*,*N*-bis(2-chloroethyl)phosphorodiamidate (**3**) giving 4-hydroperoxycyclophosphamide (**4**), followed by deoxygenation of the resulting hydroperoxide intermediate under a mild reaction condition. Similarly, several kinds of the pre-activated cyclophosphamide analogues were prepared, establishing a convenient synthetic method leading to the C_4-oxidized 1,3,2-oxazaphosphorinanes and 1,3,2-oxazaphospholidines. More generally, the synthetic pathway is considered to be a promising route for constructing various kinds of phosphorus-containing heterocyclics (**6**) bearing unstable oxygen functionality from the simple open-chain alkenylphosphoramidates (**5**) possessing an appropriate nucleophilic group (Nu) in the molecule. It is of particular interest if the method can be applied to the synthesis of the activated cyclophosphamide analogues having 1,3,4,2-oxadiazaphosphorinane ring (**7**) and related heterocyclic system, because little has been investigated on the chemistry and antitumor activity of such compounds. To our knowledge, Cates[5] was the first reporting on the synthesis of 1,3,4,2-oxadiazaphosphorinane derivative, and no further reports dealing with the synthesis of such ring system have been available until now. We now report the syntheses of new cyclophosphamide derivatives having 1,3,4,2-oxadiazaphosphorinane and related hetero-

1) This paper forms Part IV of Studies of Cyclophosphamide Metabolite and Their Related Compounds. Part III: ref. 6.
2) Location: *Fukushima-ku, Osaka 553, Japan.*
3) N. Brock and H.-J. Hohorst, *Arzneim. Forsch.*, **13**, 1021 (1963).
4) *a*) A. Takamizawa, S. Matsumoto, T. Iwata, K. Katagiri, Y. Tochino, and K. Yamaguchi, *J. Am. 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).
5) L.A. Cates, *J. Heterocyclic Chem.*, **10**, 111 (1973).

cyclic systems, applying the ozonolysis reactions of *O*-alkenylphosphoramidoyl hydrazides and their derivatives.

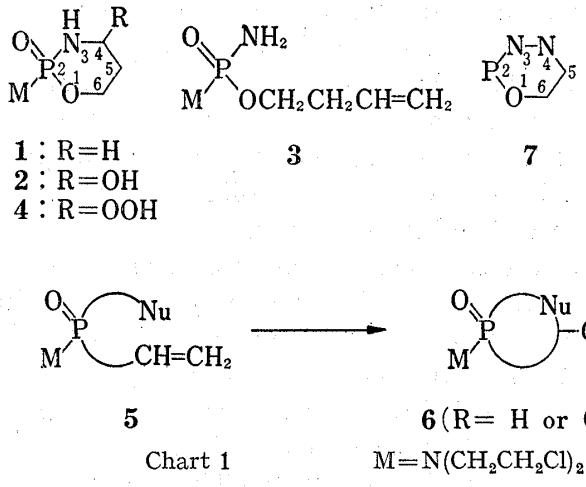

Chart 1

O-(2-Propenyl)-*N*,*N*-bis(2-chloroethyl)phosphoramidoyl hydrazide (**8a**) was prepared by reaction of $POCl_3$ with allyl alcohol, followed by triethylamine-mediated reactions of *N*,*N*-bis(2-chloroethyl)amine (nor mustard) hydrochloride and hydrazine hydrate. A small amount of a side-product (**9a**) was also formed in this reaction, thus the major product (**8a**) could not be obtained in a pure state. An attempted purification of the major product using column chromatography with silica gel-acetone resulted in the formation of a corresponding acetone hydrazone (**10a**) which could be separated from the side-product (**9a**). Likewise, *N*,*N*-dimethyl analogue (**8b**) was obtained as its acetone hydrazone (**10b**), accompanied by a small amount of a side-product (**9b**) (see Experimental). *O*-(2-Propenyl)-*N*,*N*-bis(2-chloroethyl)-2′-acetylphosphoramidoyl hydrazide (**11**) was also prepared by reaction of $POCl_3$ with allyl-alcohol, followed by treatment with nor mustard-HCl and acetyl hydrazide in the presence of triethylamine. Ozonolysis of **8a** in an aqueous acetone (acetone: H_2O=2: 1) and column chromatography of the reaction mixture with silica gel-acetone afforded a crystalline product (**12a**) (mp 114—115°) which was analysed as $C_6H_{12}Cl_2N_3O_2P$. Based on the proton magnetic resonance (PMR) evidences, the structure of the product was unequivocally elucidated to be 2-bis(2-chloroethyl)amino-2,3-dihydro-6*H*-1,3,4,2-oxadiazaphosphorine-2-oxide as follows. The PMR spectrum of **12a** in $CDCl_3$ solution showed a H-D exchangeable broad doublet at δ 7.32 with J=23 Hz, which was obviously attributable to a proton of PO-NH system, and a triplet at δ 6.92 (J=2.6 Hz) corresponding to an olefinic proton of-N=CH-CH_2-

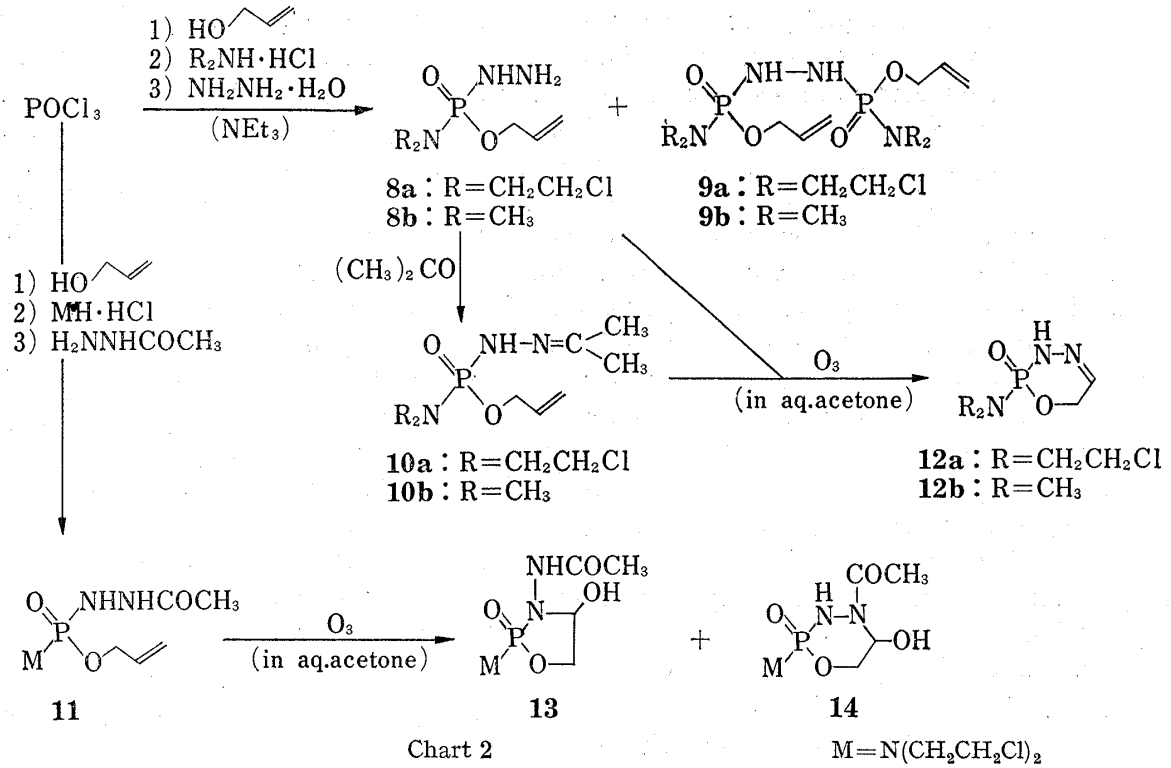

Chart 2

system. The spectrum also showed signals of the C_6-methylene protons as a multiplet composed of fourteen well-separated peaks at δ 4.32—5.28, besides signals of eight protons of two chloroethyl groups as a complex multiplet between δ 3.17 and δ 3.78. Interestingly, compound **12a** was also obtainable in a better yield by the ozonolysis of **10a**. In a similar way, 2-dimethylamino-2,3-dihydro-6*H*-1,3,4,2-oxadiazaphosphorine-2-oxide (**12b**) was obtained by the ozonolysis of **10b**, the structure of the product being confirmed by the PMR properties (see Experimental).

In the case of the ozonolysis of **11**, a crystalline product (mp 127—130°, $C_8H_{16}Cl_2N_3O_4P$) was obtained. The infrared (IR) spectrum (Nujol) of this product showed a carbonyl band at 1680 cm^{-1} and broad bands at 3170—3300 cm^{-1} due to ν_{OH} and ν_{NH}, besides strong bands at 1240, and 1045 cm^{-1} corresponding to $\nu_{P=O}$ and ν_{POC} respectively. Although the homogeneity of this product was suggested by the thin-layer chromatography (TLC), its PMR spectrum in $CDCl_3$ solution indicated that it might be a mixture of two components in the ratio of approximately 2:1. The major component showed an acetyl singlet at δ 1.82, a methine proton signals as a doublet of multiplets at δ 4.20—4.60 splitting by the P-H coupling (J=*ca.* 12 Hz), and a H-D exchangeable singlet at δ 9.47 attributable to amide proton, while the minor component showed an acetyl singlet at δ 2.10, a methine proton signals as a complex multiplet centered at δ 4.40 and a H-D exchangeable broad doublet at δ 9.15 with J=25 Hz. Although various efforts to separate the two products were unsuccessful, these PMR data clearly support the structures of the two components, the major being 2-bis(2-chloroethyl)amino-3-acetamido-4-hydroxy-1,3,2-oxazaphospholidine-2-oxide (**13**) and the minor being 2-bis(2-chloroethyl)amino-4-acetyl-5-hydroxy-1,3,4,2-oxadiazaphosphorinane-2-oxide (**14**), respectively.

The ozonolytic behaviors of *O*-(3-butenyl)-*N*,*N*-bis(2-chloroethyl)phosphoramidoyl hydrazide (**15**) and its acetone hydrazone (**16**), which were prepared in the quite similar ways as employed for the preparation of **8a** and **10a**, were found to be different from those of the *O*-(2-propenyl) analogues (**8a** and **10a**). Both **15** and **16**, when ozonolyzed in aqueous acetone, afforded a product (**17**), mp 126—128° (dec.), which was analyzed as $C_{10}H_{20}Cl_2N_3O_3P$. No product corresponding to the seven-membered analogue of **12a** could be isolated in each experiment. The PMR spectrum of **17** in DMSO-d_6 solution showed two sharp singlets at δ 1.18 (3H) and δ 1.60 (3H), suggesting the presence of two magnetically nonequivalent methyl groups which were possibly originated from an acetone molecule incorporated as hydrazone. The spectrum also showed a complex multiplet between δ 5.10 and δ 5.90 which was integrated to two protons and diminished on D_2O addition to one proton signals splitting by the P-H coupling with J=18.0 Hz, being corresponding to the vicinal P-N-C-H coupling constant reported for the C_4-substituted 1,3,2-oxazaphosphorinane analogues.[4] Thus the bicyclic structure 1-bis(2-chloroethyl)amino-6,6-dimethylperhydro-1,3,4-oxadiazolo[3,2-*c*]-1,3,2-oxazaphosphorine-1-oxide could be assigned for this product. When the ozonolysis of **15** was carried out in an aqueous tetrahydrofuran (THF: H_2O=2:1), a different product **18** [mp 123—126° (dec.), $C_8H_{16}Cl_2N_3O_3P$] was isolated. The compound (**18**) showed the PMR signals in the region δ 5.30—5.90 as a complex multiplet which was integrated to four protons and diminished on D_2O addition to three-proton multiplets composed of an AB quartet (2H) at δ H_A=5.40 and δ H_B=5.78 with J_{AB}=11.0 Hz, and a doublet of multiplet (1H) centered at δ 5.57 with J=22.0 Hz, the latter signals being assignable to a C_4-proton of the 1,3,2-oxazaphosphorinane system, while the AB quartet being assignable to the methylene protons of N-CH_2-O system. Therefore the bicyclic structure **18** could also be assigned for this product. The methylene group was presumably originated from a molecule of formaldehyde which was produced by the ozonolysis of **15** and incorporated as a hydrazone into an intermediate (*vide infra*).

Ozonolysis of *O*-(3-butenyl)-*N*,*N*-bis(2-chloroethyl)-2′-acetylphosphoramidoyl hydrazide (**19**) was found to give a similar result as observed for that of **11**, giving a mixture of

two products. In this case, however, the mixture could be separated into two isomers (**20**) (major, mp 119—121°, $C_9H_{18}Cl_2N_3O_4P$) and (**21**) (minor, mp 131—134°, $C_9H_{18}Cl_2N_3O_4P$) by fractional crystallizations from methanol. The IR spectrum (Nujol) of the major isomer (**20**) showed bands at 3240 and 3200 cm^{-1} corresponding to ν_{OH} and ν_{NH} respectively, besides a carbonyl band at 1680 cm^{-1}, $\nu_{P=O}$ band at 1255 cm^{-1} and ν_{POC} band at 1070 cm^{-1}, while the minor isomer (**21**) showed the corresponding bands at 3360, 3240, 1700, 1225 and 1030 cm^{-1}. The PMR spectrum of the major isomer (**20**) in DMSO-d_6 solution showed a singlet of an acetyl group at δ 1.84, a broad singlet of an amide proton at δ 9.34 and a complex multiplet in the region δ 4.50—5.00 which was integrated to two protons and on D_2O addition turned out to one proton as a doublet of multiplet centered at δ 4.65 with $J_{P,H}$=*ca.* 19 Hz and $J_{H,H}$=*ca.* 3—4 Hz, being assignable again to the C_4-proton of the 1,3,2-oxazaphosphorinane system, thus the structure 3-acetamido-2-bis(2-chloroethyl)amino-4-hydroxy-1,3,2-oxazaphosphorinane-2-oxide could be assigned for **20**. On the other hand, the minor isomer (**21**), showed an acetyl singlet at δ 1.85, a doublet of a phosphoramide proton at δ 9.55 (J=22.5 Hz) and a methine-proton multiplet centered at δ 4.50 which was not so greatly split as found for the C_4-H signals of the major isomer (**20**), thus being agreeable with the structure 4-acetyl-2-bis-(2-chloroethyl)amino-5-hydroxyperhydro-1,3,4,2-oxadiazaphosphepine-2-oxide which is a new seven-membered phosphorus-containing heterocyclic system.

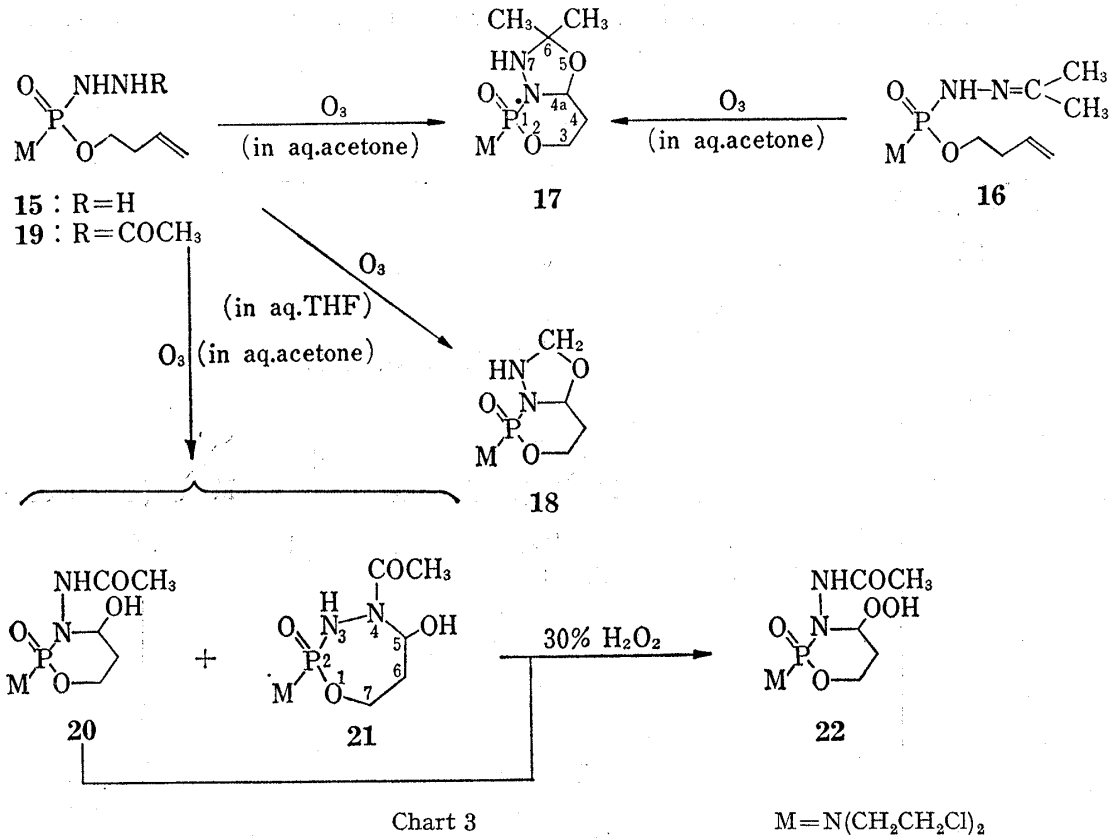

Chart 3

The two products (**20** and **21**) were found to be interconvertible in aqueous acetone giving an equilibrium mixture with predominance of **20** perhaps *via* a ring-opened intermediate (*vide infra*) and addition of 30% hydrogen peroxide to the mixture resulted in the preferential formation of a six-membered hydroperoxide (**22**) [mp 139—140° (decomp.)] whose structure was confirmed by elemental analysis and spectroscopic data (see Experimental).

It is interesting that the ozonolytic behaviors of *O*-alkenylphosphoramidoyl hydrazides are different from those of O-alkenylphosphorodiamidates which always afforded cyclic hydroperoxides as the isolable product.[4] In the present cases, two fragments (**24** and **25**) must also

be formed by cleavage of the primary ozonide (**23**) [Nu=$NHNH_2$, NHN=$C(CH_3)_2$ and NHNH-$COCH_3$],[4,6] but all the isolated products are considered to be produced from the aldehyde fragment (**24**), although it is uncertain that other products resulting from the zwitterion fragment (**25**) might also be present in the reaction mixture. In the case of the synthesis of cyclophosphamide metabolite, the aldehyde fragment (**24**) (Nu=NH_2, n=2) was found to be very unstable because of the facile cleavage of the P-O bond with release of acrolein, therefore the cyclic hydroperoxide (**26**) (Nu=NH, n=2) was obtained as an isolable product. It is also interesting that the reactivity of the aldehyde fragment (**24**) [Nu=NHN=$C(CH_3)_2$] differs by the number of carbon chain bearing aldehyde group. A plausible explanation for this difference is that the intramolecular carbonyl exchange reaction between -CHO and -NHN=C-$(CH_3)_2$ groups proceeds to give cyclic hydrazone (**12a**), possibly *via* an intermediate (**27**) when n=1, while such intermediate is perhaps sterically less favored when n=2, therefore the intramolecular 1,3-dipolar cycloaddition between the two groups is more favored to give bicyclic product **17** *via* an intermediate (**28a**). The formation of **18** by the ozonolysis of **15** in aqueous tetrahydrofuran is also interpreted by assuming a similar intermediate (**28b**) which is produced from the aldehyde fragment (**24**) (Nu=$NHNH_2$, n=2) by reacting with formaldehyde generated in the reaction medium. The ring-size effect upon the cyclization reaction of the intermediate (**24**) (Nu=$NHNHCOCH_3$) was also observed, where the product ratio **29**/**30** was found to be larger for n=1 (2/1) than for n=2 (4/1). Although one must take into consideration the difference in nucleophilic reactivity between the two nitrogen atoms of PO-NH-NH-$COCH_3$

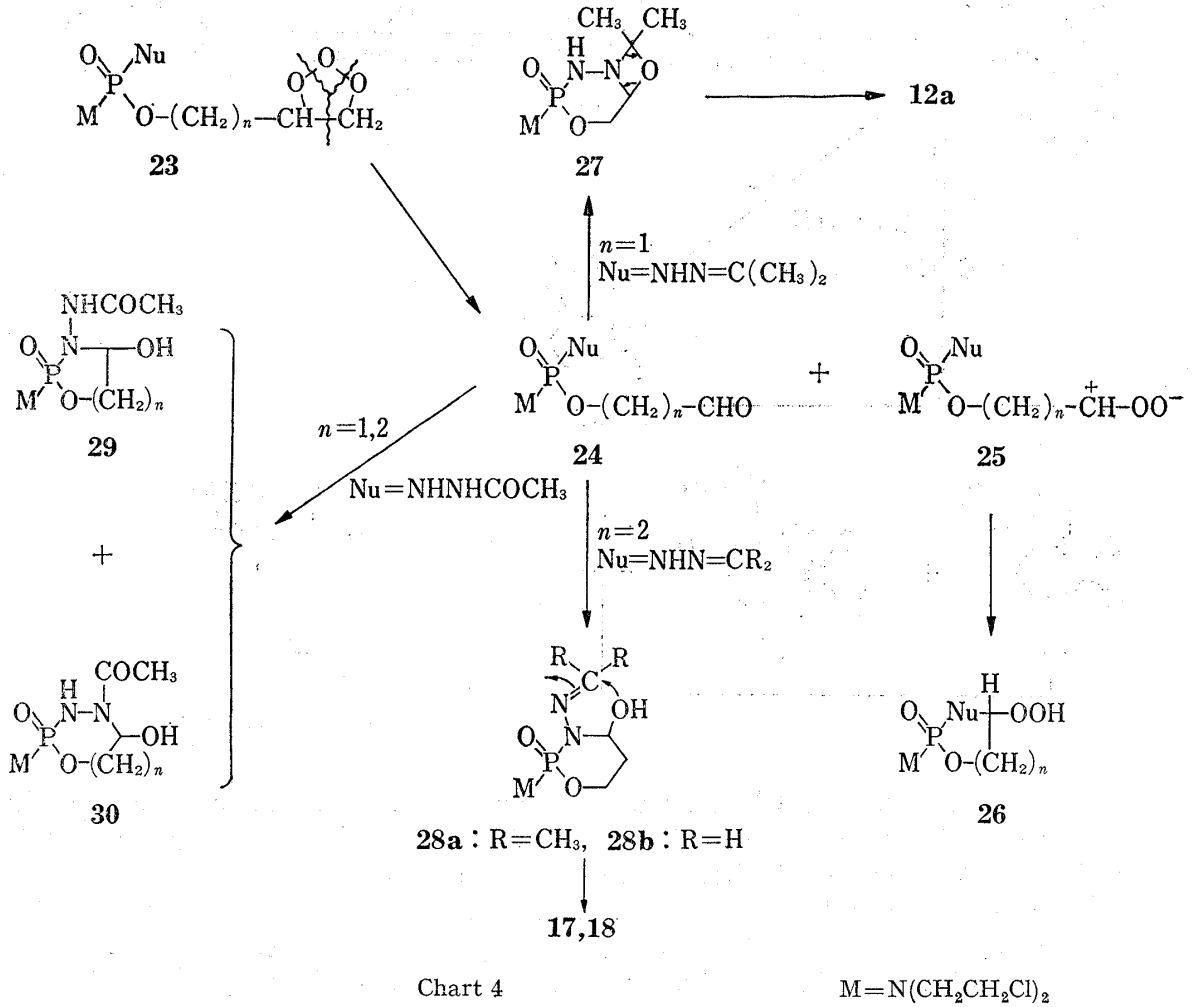

Chart 4

6) A. Takamizawa, S. Matsumoto, and T. Iwata, *Tetrahedron Lett.*, **1974**, 517.

group as one of the factors controlling the distribution of the products **29** and **30**, the difference in thermodynamic stability between them is considered to be more effective for determining the product ratio because they were found to be in equilibrium under the reaction condition.

The antitumor activities of the new cyclophosphamide derivatives **12a**, **20**, **21** and **13** plus **14** as a 2:1 mixture, were tested against L1210 leukemic BDF_1 mice, but the antileukemic activity of these compounds was found to be very low or practically ineffective as compared to cyclophosphamide. This indicates that the 1,3,4,2-oxadiazaphosphorinane ring is ineffective in promoting *in vivo* antitumor activity as a masking moiety of the nitrogen mustard alkylating agents.

Experimental[7)]

***O*-(2-Propenyl)-*N*,*N*-bis(2-chloroethyl)phosphoramidoyl Hydrazide (8a)**——To a stirred solution of $POCl_3$ (15.3 g) in CH_2Cl_2 (100 ml) was added dropwise a solution of allylalcohol (5.81 g) in CH_2Cl_2 (30 ml) at −30——25° for 30 min. After stirring at −25——15° for 2.5 hr, bis(2-chloroethyl)amine (nor mustard) hydrochloride (17.85 g) was added to the mixture, then a solution of triethylamine (30.3 g) in CH_2Cl_2 (30 ml) was added dropwise with stirring at −25——20° for 30 min, and the mixture was stirred at the same temperature for 3 hr. After standing overnight at −20°, the reaction mixture was filtered and concentrated *in vacuo* to give an oily residue. The residue was chromatographed on a column (7.5×40 cm) eluting with ether, and the ether eluate was monitored by TLC and pure fractions were collected and concentrated *in vacuo* to give the crude *O*-(2-propenyl)-*N*,*N*-bis(2-chloroethyl)phosphoramidoyl chloride as a colorless oil (9.4 g, 34%) (This is a general procedure for the preparation of *O*-alkenylphosphoramidoyl chloride in the following experiments). The crude phosphoramidoyl chloride was dissolved in ether (150 ml), and a mixture of triethylamine (15.2 g) and 80% hydrazine hydrate (3 g) was added dropwise to the solution with stirring in an ice-water bath. After standing overnight at 2°, the reaction mixture was filtered, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give a pale yellow oil which was purified on a column (5×30 cm) eluting with AcOEt, giving **8a** (2.9 g, 32%) as a colorless oil, which was used for the ozonolysis reaction without further purification.

***O*-(2-Propenyl)-*N*,*N*-bis(2-chloroethyl)-2′-isopropylidenephosphoramidoyl Hydrazide (10a) and 1,2-Bis[*O*-(2-propenyl)-*N*,*N*-bis(2-chloroethyl)phosphoramidoyl]hydrazide (9a)**——The crude (**8a**), prepared from $POCl_3$ (15.3 g), allyl alcohol (5.8 g), nor mustard hydrochloride (17.85 g) and 80% hydrazine hydrate (3 g) according to the procedure described above, was chromatographed on a column (7.5×40 cm) eluting with acetone. The acetone eluate was concentrated *in vacuo* to give an oily residue which partly crystallized on addition of ether. Recrystallization of the residue from ether gave **9a** (870 mg, 5% from $POCl_3$) as colorless needles, mp 117—118°; IR ν_{max}^{Nujol} cm^{-1}: 3160 (NH), 1650 (-CH=CH_2), 1240 (PO), 1025 (POC). *Anal.* Calcd. for $C_{14}H_{28}Cl_4N_4O_4P_2$: C, 32.33; H, 5.43, Cl, 27.26; N, 10.77; P, 11.91. Found: C, 32.53; H, 5.77; Cl, 27.31; N, 10.66; P, 11.51. The mother liquor of **9a** was concentrated *in vacuo*, and the resulting residue was chromatographed on a column (7.5×30 cm) eluting with AcOEt, giving **10a** (4.4 g, 22% from $POCl_3$) as a faster migrating component and a small amount of the additional **9a** (40 mg) as a later migrating component. **10a** was obtained as a colorless oil; IR ν_{max}^{Film} cm^{-1}: 3184 (NH), 1230 (PO), 1025 (POC). *Anal.* Calcd. for $C_{10}H_{20}Cl_2N_3O_2P$: C, 37.99; H, 6.38; Cl, 22.43; N, 13.29; P, 9.80. Found: C, 38.16; H, 6.55; Cl, 22.61; N, 13.01; P, 9.32.

***O*-(2-Propenyl)-*N*,*N*-dimethylphosphoramidoyl Hydrazide (8b), *O*-(2-Propenyl)-*N*,*N*-bis(2-chloroethyl)-2′-isopropylidenephosphoramidoyl Hydrazide (10b) and 1,2-Bis[*O*-(2-propenyl)-*N*,*N*-dimethylphosphoramidoyl]-hydrazide (9b)**——$POCl_3$ (30.6 g), allyl alcohol (11.6 g) and dimethylamine hydrochloride (16.31 g) were allowed to react in the presence of triethylamine in CH_2Cl_2 according to the general procedure described above, and the product was purified on a column (10×60 cm) eluting with ether to give *O*-(2-propenyl)-*N*,*N*-dimethylphosphoramidic chloride (32.6 g) as an oil which was allowed to react with 80% hydrazine hydrate (11.2 g) in the presence of triethylamine (27 g) in CH_2Cl_2 (150 ml). The resulting product, after similar treatments as described for the preparation of **8a**, was chromatographed on a column (10×50 cm) eluting with AcOEt to give crude **8b** (18 g) as a pale yellow oil containing small amount of **9b**, which was again purified on a column (10×40 cm) with acetone to yield **10b** (8.21 g, 21% from $POCl_3$) as a faster migrating component and **9b** (1.13 g, 7% from $POCl_3$). Both products are obtained as colorless oil; **10b**, IR ν_{max}^{Film} cm^{-1}: 3190 (NH), 1647

7) 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 film. PMR data were determined with a VARIAN Model A-60 spectrometer using tetramethylsilane as an internal reference. Column chromatography was carried out using silica gel (MERCK Kieselgel 60). 3-Buten-1-ol and *N*,*N*-bis(2-chloroethyl)amine hydrochloride were purchased from Chemical Samples Co. (Ohio, U.S.A.) and Aldrich Chemical Co. Inc. (Wisconsin, U.S.A.), respectively.

(CH–CH_2), 1226 (PO). *Anal.* Calcd. for $C_8H_{18}N_3O_2P$: C, 43.83; H, 8.28; P, 14.13; N, 19.19. Found: C, 43.94; H, 8.06; P, 13.74; N, 19.37. **9b**, IR ν_{max}^{Film} cm^{-1}: 3185 (NH), 1645 (CH=CH_2), 1225 (PO). *Anal.* Calcd. for $C_{10}H_{24}N_4O_4P_2$: C, 36.84; H, 7.42; N, 17.19; P, 19.00. Found: C, 36.71; H, 7.38, N, 17.41; P, 18.79.

***O*-(2-Propenyl)-*N*,*N*-bis(2-chloroethyl)-2′-acetylphosphoramidoyl Hydrazide (11)**——$POCl_3$ (15.33 g), allyl alcohol (5.81 g) and nor mustard hydrochloride (17.85 g) were allowed to react according to the general procedure and the resulting *O*-(2-propenyl)-*N*,*N*-bis(2-chloroethyl)phosphoramidic chloride was purified on a column (10×50 cm) with ether to give a colorless oil which was dissolved in CH_2Cl_2 (100 ml) and acetyl hydrazide (13 g) was added to the solution. To the mixture was added dropwise triethylamine (15 g) with stirring at 40—45°, and the reaction mixture was stirred for 3 hr at the same temperature. After filtration of the reaction mixture, the filtrate was concentrated *in vacuo* to give a pale yellow oil which was purified on a column (7.5×40 cm) eluting with AcOEt to give **11** as an oil (7.0 g, 22%). The oil was crystallized by addition of ether, and recrystallized from ether–acetone to give colorless needles, mp 94—95°. IR ν_{max}^{Nujol} cm^{-1}: 3200 (NH), 1670 (CO), 1230 (PO), 1010, 1020 (POC), PMR ($CDCl_3$) δ: 1.94 (3H, singlet, $COCH_3$), 3.13—3.55 [8H, multiplet, $N(CH_2CH_2Cl)_2$], 4.50 (2H, quartet, PO–OCH_2–), 5.83 (1H, double doublet, J=22 Hz, J'=2 Hz, PO–NH), 9.90 (1H, doublet, J=2 Hz, NHCO). *Anal.* Calcd. for $C_9H_{18}Cl_2N_3O_3P$: C, 33.98; H, 5.70; N, 13.21. Found: C, 34.09; H, 5.93; N, 13.33.

2-Bis(2-chloroethyl)amino-2,3-dihydro-6*H*-1,3,4,2-oxadiazaphosphorine-2-oxide (12a)——a) To a stirred solution of **8a** (1.0 g) in aqueous acetone (H_2O: acetone=1: 2) (30 ml) O_3 (350 mg) was bubbled for 10 min at a rate of 35 mg/min, while cooling in an ice-water bath. After standing overnight at 2°, the reaction mixture was concentrated *in vacuo* to give an aqueous residue which was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give **12a** (200 mg, 21%) as crystalline residue which was recrystallized from acetone–ether giving colorless needles, mp 114—115°; IR ν_{max}^{Nujol} cm^{-1}: 3170 (NH), 1230 (PO), 1095 (POC). *Anal.* Calcd. for $C_6H_{12}Cl_2N_3O_2P$: C, 27.71; H, 4.65; Cl, 27.27; N, 16.16; P, 11.91. Found: C, 27.69; H, 4.61; Cl, 27.39; N, 15.91; P, 12.14.

b) **10a** (2.1 g) was ozonized with O_3 (640 mg) in 1: 2 aqueous acetone (30 ml) stirred in an ice-water bath, and after standing overnight at 2° the reaction mixture was concentrated *in vacuo*. The aqueous residue was extracted with $CHCl_3$, and the $CHCl_3$ extract was washed with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oily residue which was chromatographed on a column (4.5×2.0 cm) eluting with AcOEt. The AcOEt eluate afforded crystalline **12a** (518 mg, 30%) which was identified with the specimen obtained by the method a) by IR comparison.

2-Dimethylamino-2,3-dihydro-6*H*-1,3,4,2-oxadiazaphosphorine-2-oxide (12b)——**10b** (2.0 g) was ozonized with O_3 (660 mg) in 1: 2 aqueous acetone (30 ml) with stirring in an ice-water bath, and the resulting reaction mixture was allowed to stand overnight at 2°. After concentration *in vacuo*, the aqueous residue was extracted with $CHCl_3$, and the $CHCl_3$ extract was washed with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oily residue which was purified on a column (3×15 cm) with acetone giving **12b** (123 mg, 8.3%) as a white solid. The solid was recrystallized from acetone–ether to give colorless needles, mp 139.5—140°; IR ν_{max}^{Nujol} cm^{-1}: 3216 (NH), 1205 (PO), 1010 (POC). PMR (d_6-DMSO) δ: 2.57 (6H, doublet, J=10.5 Hz, 2×NCH_3), 6.99 (1H, triplet, J=3 Hz, –N=CH–), 8.92 (1H, broad doublet, J=22 Hz, PO–NH). *Anal.* Calcd. for $C_4H_{10}N_3O_2P$: C, 29.45; H, 6.18; N, 25.76; P, 18.99. Found: C, 29.53; H, 6.28; N, 25.47; P, 18.54.

2: 1 Mixture of 3-Acetamido-2-bis(2-chloroethyl)amino-4-hydroxy-1,3,2-oxazaphospholidine-2-oxide (13) and 4-Acetyl-2-bis(2-chloroethyl)amino-5-hydroxy-1,3,4,2-oxadiazaphosphorinane-2-oxide (14)——*O*-(2-Propenyl)-*N*,*N*-bis(2-chloroethyl)-2′-acetylphosphoramidoyl hydrazide (**11**) (1.5 g) was ozonized in 1: 2 aqueous acetone (25 ml) with O_3 (500 mg) with stirring in an ice-water bath, and the reaction mixture was allowed to stand overnight at 2°. After concentration *in vacuo*, the aqueous residue was extracted with $CHCl_3$, and the $CHCl_3$ extract was washed with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oily residue which was purified on a column (3×15 cm) with AcOEt. The pure AcOEt eluate monitored by TLC was collected and concentrated to give a 2: 1 mixture of **13** and **14** as a crystalline residue (280 mg, 20%) which was recrystallized from acetone–ether giving colorless needles, mp 127—130°. *Anal.* Calcd. for $C_8H_{16}Cl_2N_3O_4P$: C, 30.12; H, 5.04; Cl, 22.15; N, 13.13; P, 9.68. Found: C, 29.73; H, 5.30; Cl, 22.12; N, 13.05; P, 9.50. IR ν_{max}^{Nujol} cm^{-1}: 3300—3170 (OH, NH), 1680 (CO), 1240 (PO), 1045 (POC). PMR ($CDCl_3$) δ: 1.82, 2.10 (singlet, $COCH_3$) 3.15—4.18 [multiplets, $(ClCH_2CH_2)_2N$,P-O–CH_2], 4.26—4.60 (multiplets, –N–CH–), 9.15 (broad doublet, J=25 Hz, PO–NH), 9.47 (singlet, CO–NH).

***O*-(3-Butenyl)-*N*,*N*-bis(2-chloroethyl)phosphoramidoyl Hydrazide (15)**——$POCl_3$ (15.3 g), 3-buten-1-ol (7.2 g), nor mustard-HCl (17.8 g) and 80% hydrazine hydrate (5.2 g) were allowed to react according to the same procedure as described for the preparation of **8a**. The resulting product was purified on a column (5.5×40 cm) with 20: 1 mixture of $CHCl_3$–methanol to give crude **15** (5.1 g, 45%) as a colorless oil which was used for the next experiments without further purification.

***O*-(3-Butenyl)-*N*-*N*,-bis(2-chloroethyl)-2′-isopropylidenephosphoramidoyl Hydrazide (16)**——The crude **15** obtained from $POCl_3$ (15.3 g), 3-buten-1-ol (7.2 g), nor mustard-HCl (17.8 g) and 80% hydrazine hydrate (5.2 g) was dissolved in acetone (30 ml) and the solution was chromatographed on a column (5.5×40 cm) eluting with acetone to give **16** (7.2 g, 22% from $POCl_3$) as a crystalline solid, mp 35—37°; IR ν_{max}^{Nujol} cm^{-1}: 3180 (NH), 1643 (–CH=CH_2), 1225 (PO), PMR ($CDCl_3$) δ: 1.84, 1.97 (each 3H, singlet, 2×CH_3) 2.47 (2H, quartet, J=6 Hz, –$\underline{CH_2}$–CH=CH_2), 3.20—3.80 [8H, multiplet, $(ClCH_2CH_2)_2N$–], 4.07 (2H, quartet, J=7 Hz,

PO–OCH_2–), 4.30—6.20 (3H, multiplet, –CH=CH_2), 6.77 (1H, doublet, J=22 Hz, PO–NH). *Anal.* Calcd. for $C_{11}H_{22}Cl_2N_3O_2P$: C, 40.01; H, 6.72; Cl, 21.47; N, 12.73; P, 9.38. Found: C, 40.21; H, 6.57; Cl, 21.53; N, 12.93; P, 9.18.

***O*-(3-Butenyl)-*N*,*N*-bis(2-chloroethyl)-2′-acetylphosphoramidoyl Hydrazide (19)**——$POCl_3$ (30.6 g), 3-buten-1-ol (14.0 g), nor mustard-HCl (35.6 g) were allowed to react according to the general procedure and the resulting *O*-(3-butenyl)-*N*,*N*-bis(2-chloroethyl)phosphoramidoyl chloride was purified on a column (12×60 cm) eluting with ether to give an oily residue (52 g) which was dissolved in ether (100 ml) and added dropwise to a stirred solution of acetyl hydrazide (40 g) in $CHCl_3$ (200 ml) and triethylamine (70 g) at room temperature, and the reaction mixture was stirred at 50—60° for 3 hr. After standing for 48 hr at room temperature, the reaction mixture was filtered, and the filtrate was concentrated *in vacuo* to give an oily residue which was purified on a column (10×60 cm) eluting with AcOEt. From the AcOEt eluate, after concentration *in vacuo*, **19** was obtained as a crystalline residue (31 g, 47%) which was recrystallized from ether–acetone to give colorless needles, mp 55—59°; IR ν_{max}^{Nujol} cm^{-1}: 3220 (NH), 3060 (NH), 1665 (CO), 1230 (PO), 1050 (POC). PMR ($CDCl_3$) δ: 2.02 (3H, singlet, $COCH_3$), 2.43 (2H, quartet, J=7 Hz, –$\underline{CH_2}$–CH=CH_2), 3.00—3.90 [8H, multiplet, $(ClCH_2CH_2)_2N$], 4.17 (2H, quartet, PO–O–CH_2), 4.90—6.20 (3H, multiplet, –CH=CH_2), 6.25 (1H, double doublet, J=23 Hz, J'=2 Hz, –PO–NH), 9.35 (1H, doublet, J=2 Hz, –NHCO). *Anal.* Calcd. for $C_{10}H_{20}Cl_2N_3O_3P$: C, 36.17; H, 6.07; Cl, 21.36; N, 12.66; P, 9.33. Found: C, 36.45; H, 6.37; Cl, 21.14; N, 12.77; P, 9.32.

1-Bis(2-chloroethyl)amino-6,6-dimethylperhydro-1,3,4-oxadiazolo[3,2-*c*]-1,3,2-oxazaphosphorine-1-oxide (17)——a) *O*-(3-Butenyl)-*N*,*N*-bis(2-chloroethyl)phosphoramidoyl hydrazide (**15**) (2.9 g) was dissolved in 1:2 aqueous acetone (50 ml) and the solution was ozonized by 440 mg of O_3 with stirring in an ice-water bath. After standing overnight at 2°, the reaction mixture was concentrated *in vacuo*, and the resulting aqueous residue was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oily residue which was purified on a column (3×15 cm) with AcOEt. The AcOEt eluate afforded **17** (240 mg, 7.2%) as a crystalline solid which was recrystallized from acetone–ether to give colorless needles, mp 126—128° (dec.); IR ν_{max}^{Nujol} cm^{-1}: 3220 (NH), 1223 (PO), 1010 (POC). PMR (d_6-DMSO) δ: 1.18, 1.60 (each 3H, singlet, 2×CH_3), 1.80—2.40 (2H, multiplet, C_4–H), 3.00—4.70 (2H, multiplet, C_3–H), 5.10—5.90 (2H, multiplet, NH, C_{4a}–H). *Anal.* Calcd. for $C_{10}H_{20}Cl_2N_3O_3P$: C, 36.17; H, 6.07; Cl, 21.36; N, 12.66; P, 9.32. Found: C, 36.12; H, 5.81; Cl, 21.35; N, 12.60; P, 9.73.

b) A solution of *O*-(2-butenyl)-*N*,*N*-bis(2-chloroethyl)-2′-isopropylidenephosphoramidoyl hydrazide (**16**) (3.3 g) in 1:2 aqueous acetone (50 ml) was ozonized by 440 mg of O_3 with stirring in an ice-water bath, then the reaction mixture was allowed to stand overnight at 2°. After quite similar treatments as cited above, the product **17** was purified on a column (3×14 cm) eluting with AcOEt to give 697 mg (21%) of crystalline solid which was identified with the specimen obtained by method a) by IR comparison.

1-Bis(2-chloroethyl)aminoperhydro-1,3,4-oxadiazolo[3,2-*c*]-1,3,2-oxazaphosphorine-2-oxide (18)——*O*-(3-Butenyl)-*N*,*N*-bis(2-chloroethyl)phosphoramidoyl hydrazide (**15**) (9 g) was dissolved in a mixture of THF (100 ml) and H_2O (50 ml), and the solution was ozonized by 2.2 g of O_3. The ozonolyzed solution was allowed to stand overnight at 2°, then THF was evaporated *in vacuo* to give an aqueous residue which was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oily residue which was purified on a column (3.5×20 cm) eluting with AcOEt. The AcOEt eluate afforded **18** as a crude solid (754 mg, 8%) which was recrystallized from acetone–ether to give colorless needles, mp 123—126° (dec.); IR ν_{max}^{Nujol} cm^{-1}: 3199 (NH), 1224 (PO), 1067 (POC). *Anal.* Calcd. for $C_8H_{16}Cl_2N_3O_3P$: C, 31.60; H, 5.30; N, 13.81; P, 10.18. Found: C, 31.36; H, 5.21; N, 13.80; P, 9.55.

3-Acetamido-2-bis(2-chloroethyl)amino-4-hydroxy-1,3,2-oxazaphosphorinane-2-oxide (20) and 4-Acetyl-2-bis(2-chloroethyl)amino-5-hydroxyhexahydro-2*H*-1,3,4,2-oxadiazaphosphepine-2-oxide (21)——*O*-(3-Butenyl)-*N*,*N*-bis(2-chloroethyl)-2′-acetylphosphoramidoyl hydrazide (**19**) (7.5 g) was dissolved in a mixture of acetone (50 ml) and H_2O (20 ml), and the solution was ozonized by 1.5 g of O_3 with stirring in an ice-water bath. After standing overnight at 2°, the reaction mixture was concentrated *in vacuo* and the resulting aqueous residue was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a crystalline residue which was dissolved in MeOH (10 ml) and allowed to stand overnight at 2° giving precipitation of colorless fine needles. The needles were collected to give crude **20** (970 mg, 12.8%) which was recrystallized from MeOH–ether giving colorless needles, mp 119—121°; IR ν_{max}^{Nujol} cm^{-1}: 3240 (OH), 3200 (NH), 1680 (CO), 1225 (PO), 1070 (POC). PMR (d_6-DMSO) δ: 1.84 (3H, singlet, $COCH_3$), 2.00 (2H, multiplet, C_5–H), 3.00—3.90 [8H, multiplet, $(ClCH_2CH_2)_2N$], 3.90—4.50 (2H, multiplet, C_6–H), 4.50—5.00 (2H, multiplet, C_4–H and OH), 9.34 (1H, singlet, NH). *Anal.* Calcd. for $C_9H_{18}Cl_2N_3O_4P$: C, 32.36; H, 5.43; Cl, 21.23; N, 12.58; P, 9.27. Found: C, 32.37; H, 5.56; Cl, 21.20; N, 12.66; P, 8.93. The mother liquor of **20** was concentrated *in vacuo* and the resulting crystalline residue was dissolved again in MeOH (5 ml), then the solution was allowed to stand overnight at 2°, and the precipitated colorless prisms were collected and recrystallized from cold MeOH to give **21** (240 mg, 3.2%) as colorless prisms, mp 131—134° (dec.); IR ν_{max}^{Nujol} cm^{-1}: 3360 (OH), 3240 (NH), 1700 (CO), 1225 (PO), 1030 (POC). PMR (d_6-DMSO) δ: 1.85 (3H, singlet, $COCH_3$), 2.00 (2H, multiplet, C_6–H), 2.80—4.20 [8H, multiplet, $(ClCH_2CH_2)_2N$], 4.20—4.80 (3H, multiplet, OH and C_7–H), 9.55 (1H, doublet, J=22.5 Hz, PO–NH). *Anal.* Calcd. for $C_9H_{18}Cl_2N_3O_4P$: C, 32.36; H, 5.43; Cl, 21.23; N, 12.58; P, 9.27. Found: C, 32.36; H, 5.82; Cl, 20.89; N, 12.41; P, 9.19.

3-Acetamido-2-bis(2-chloroethyl)amino-4-hydroperoxy-1,3,2-oxazaphosphorinane-2-oxide (22)——a) To a stirred solution of **20** (250 mg) in a mixture of acetone (10 ml) and H_2O (5 ml) was added 30% aqueous H_2O_2 (5 ml) while cooling in an ice-water bath, and the reaction mixture was allowed to stand for 72 hr at 2°. After concentration *in vacuo*, the reaction mixture was extracted with $CHCl_3$, and the $CHCl_3$ extract was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a crystalline residue which was recrystallized from acetone to give **22** (130 mg, 50%) as colorless prisms, mp 139—140° (dec.); IR ν_{max}^{Nujol} cm^{-1}: 3280 (OOH), 3180 (NH), 1700 (CO), 1215 (PO), 1065 or 1035 (POC). PMR (d_6-DMSO) δ: 1.87 (3H, singlet $COCH_3$), 2.17 (2H, multiplet, C_5–H), 2.90—4.10 [8H, multiplet, $(ClCH_2CH_2)_2N$], 4.20—4.60 (2H, multiplet, C_6–H), 4.87 (1H, doublet of triplet, $J=17$ Hz, $J'=3$ Hz, C_4–H), 9.74 (1H, singlet, NH), 11.67 (1H, broad, OOH). *Anal.* Calcd. for $C_9H_{18}Cl_2N_3O_5P$: C, 30.87; H, 5.18; Cl, 20.26; N, 12.00; P, 8.85. Found: C, 31.18; H, 5.39; Cl, 19.82; N, 11.86; P, 8.91.

b) To a stirred solution of **21** (250 mg) in a mixture of acetone (10 ml) and H_2O (5 ml) was added 30% aqueous H_2O_2 (5 ml), and the solution was allowed to stand for 72 hr at 2°. After the same treatments as cited above, a crystalline product was obtained and recrystallization of the product from MeOH gave **22** (126 mg, 48%) as colorless prisms which was identified with the specimen prepared by the method a) by IR comparison.