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Synthesis of 4-Hydroperoxy-1,3,2-diazaphosphorinane-2-oxides related to the Activated Cyclophosphamide[1]

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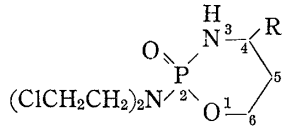
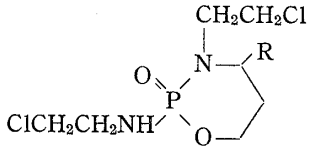
Activated cyclophosphamide analogues having a 4-hydroperoxy-1,3,2-diazaphosphorinane-2-oxide ring were synthesized by ozonolytic cyclization reaction of N-3-butenylphosphorodiamidates. Stereochemistries of the 1,3,2-diazaphosphorinane derivatives were investigated by proton magnetic resonance (PMR) spectroscopy. Both the chemical and PMR properties of these derivatives were similar to those of the corresponding 1,3,2-oxazaphosphorinane derivatives. The 1,3,2-diazaphosphorinane derivatives practically had no antileukemic activity against L1210-BDF_1 mice, providing a further evidence that the 1,3,2-oxazaphosphorinane ring of cyclophosphamide-related compounds is not replaceable by other ring systems for promoting antitumor activity.

Keywords——antitumor agent; ozonolytic cyclization; heterocyclic peroxide; stereoisomerization; phosphorus stereochemistry; PMR; J(P–N–C–H)

Hepatic microsomal oxidation of the antitumor agents cyclophosphamide (**1**) and isophosphamide (**2**) gives hydroxylated species **3** and **4** which are thought to be the active metabolite exerting antitumor effects.[3] Recent synthetic studies[4–6] revealed that C_4 hydroperoxylation was as effective as C_4 hydroxylation for activating these drugs. Surprisingly, C_4-hydroperoxy derivatives **5** and **6** were more stable than the corresponding alcohols **3** and **4**. A number of 4-hydroperoxycyclophosphamide analogues have been prepared,[5–9] and among the hitherto synthesized compounds, 4-hydroperoxyisophosphamide (**6**) (NSC 227114) has exhibited the most increased antitumor activity in pre-clinical experiments.[6,9]



1: R=H
3: R=OH
5: R=OOH



2: R=H
4: R=OH
6: R=OOH

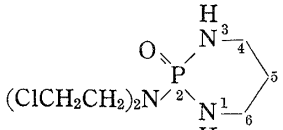


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Chart 1

1) This paper forms Part VII of Studies on Cyclophosphamide Metabolites and Their Related Compounds. Part VI. A. Takamizawa, T. Iwata, and S. Matsumoto, *Chem. Pharm. Bull.* (Tokyo), **25**, 2900 (1977).
2) Location: *Fukushima-ku, Osaka 553, Japan.*
3) A.R. Torkelson, J.A. LaBudde, and J.H. Weikel, Jr., *Drug Metabolism Reviews*, 3, 131 (1974) and references cited therein.
4) A. Takamizawa, S. Matsumoto, T. Iwata, K. Katagiri, Y. Tochino, and K. Yamaguchi, *J. Am. Chem. Soc.*, **95**, 985 (1973).
5) A. Takamizawa, S. Matsumoto, T. Iwata, Y. Tochino, K. Katagiri, K. Yamaguchi, and O. Shiratori, *J. Med. Chem.*, **18**, 376 (1975).
6) *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, *Chem. Pharm. Bull.* (Tokyo), **25**, 1877 (1977).
7) A. Takamizawa, S. Matsumoto, T. Iwata, and S. Sakai, *Chem. Pharm. Bull.* (Tokyo), **25**, 1582 (1977).
8) A. Takamizawa, S. Matsumoto, and T. Iwata, *Tetrahedron Lett.*, **1974**, 517.
9) J.A. Montgomery, and R.F. Struck, *Cancer Treatment Rept.*, **60**, 381 (1976).

In an elaborate paper by Arnold and his coworkers[10] describing antitumor activity of numerous cyclophosphamide-related compounds, a cyclophosphamide analogue (**7**) having the 1,3,2-diazaphosphorinane ring was reported to be practically inactive. We are currently interested in whether the C_4-hydroperoxylated derivative of such compound would exhibit increased activity. This paper describes synthetic and stereochemical investigations of 4-hydroperoxy-1,3,2-diazaphosphorinane-2-oxides related to 4-hydroperoxyisophosphamide.

Starting from 1-tosyloxy-3-butene (**8**),[11] 3-butenylamine hydrochlorides **11** (R=H) and **12** (R=CH_2CH_2Cl) were easily prepared *via* the phthalimide **9** and the aziridine derivative **10**, respectively (Chart 2). The triethylamine-mediated reaction of N,N-bis(2-chloroethyl)-

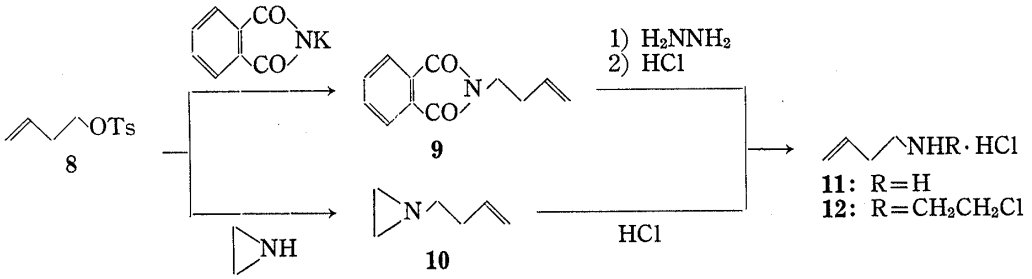
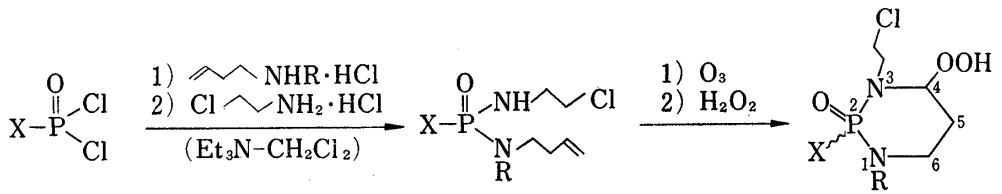


Chart 2

aminophosphorodichloridate (**13**)[12] with **11** or **12** in methylene chloride at −20 to −25°, followed by treatment with 2-chloroethylamine hydrochloride, gave the N-(3-butenyl)phosphorotriamidates **16** and **18** in good yields. Similarly, phenylphosphorodichloridate (**14**) and 2-chloroethylphosphorodichloridate (**15**) were allowed to react with **12** and subsequently treated



13 X=$(ClCH_2CH_2)_2N$
14 X=C_6H_5O
15 X=$ClCH_2CH_2O$

16 R=H, X=$(ClCH_2CH_2)_2N$
17 R=CH_2CH_2Cl, X=C_6H_5O
18 R=CH_2CH_2Cl, X=$(ClCH_2CH_2)_2N$
19 R=CH_2CH_2Cl, X=$ClCH_2CH_2O$

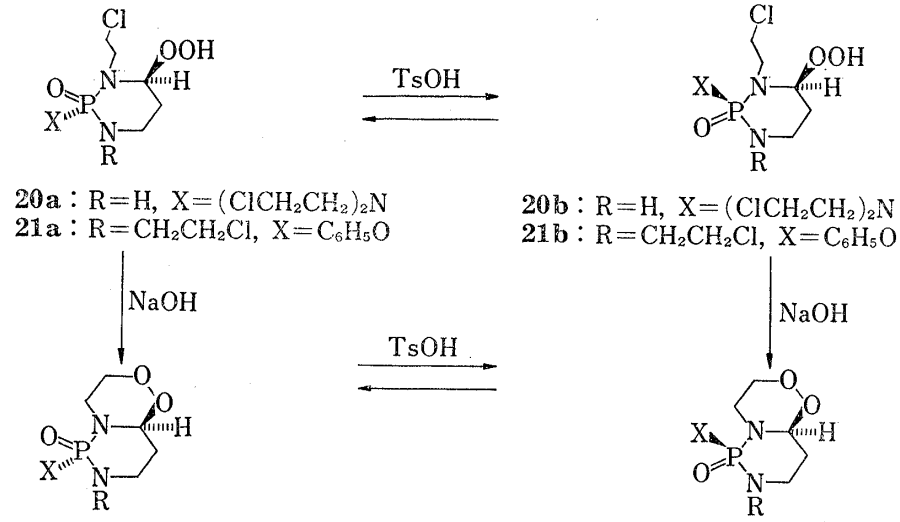
20 R=H, X=$(ClCH_2CH_2)_2N$
21 R=CH_2CH_2Cl, X=C_6H_5O
22 R=CH_2CH_2Cl, X=$(ClCH_2CH_2)_2N$
23 R=CH_2CH_2Cl, X=$ClCH_2CH_2O$

Chart 3

with 2-chloroethylamine hydrochloride in the presence of triethylamine to obtain the corresponding N-(3-butenyl)phosphorodiamidates **17** and **19**. According to the procedures described in an earlier paper,[5] ozonolytic cyclization reaction of the N-(3-butenyl)phosphorotriamidate **16** was carried out in aqueous acetone and the ozonized solution was treated with hydrogen peroxide to obtain 2-[N,N-bis(2-chloroethyl)]amino-3-(2-chloroethyl)-4-hydroperoxy-1,3,2-diazaphosphorinane-2-oxide (**20**) as a stereoisomeric mixture. Although attempts to separate the stereoisomers were unsuccessful, alkali treatment of the product **20** afforded an approximately 1:1 mixture of the bicyclic peroxides which could be separated by column chromatography on silica gel in acetone–chloroform (1:1), giving **24a** (mp 119—121°) as the faster migrating isomer and **24b** (mp 100—101°) as the slower one. As shown in Fig. 1, the 60

10) H. Arnold, F. Bourseaux, and N. Brock, *Arzneim. Forsch.*, **11**, 143 (1961).
11) K.L. Servis and J.D. Roberts, *J. Am. Chem. Soc.*, **86**, 3773 (1964).
12) O.M. Friedman and A.M. Seligman, *J. Am. Chem. Soc.*, **76**, 655 (1954).

MHz proton magnetic resonance (PMR) spectrum of the isomer **24a** showed C_4-proton signals at δ 5.43 as a doublet of a triplet splitting by the P,H and H,H couplings [J(P,H)=20.3 Hz, J(H,H)=4.3 Hz]. The P,H coupling constant corresponds to that of a bicyclic peroxide



20a : R=H, X=$(ClCH_2CH_2)_2N$
21a : R=CH_2CH_2Cl, X=C_6H_5O

20b : R=H, X=$(ClCH_2CH_2)_2N$
21b : R=CH_2CH_2Cl, X=C_6H_5O

24a : R=H, X=$(ClCH_2CH_2)_2N$
25a : R=CH_2CH_2Cl, X=C_6H_5O

24b : R=H, X=$(ClCH_2CH_2)_2N$
25b : R=CH_2CH_2Cl, X=C_6H_5O

Chart 4

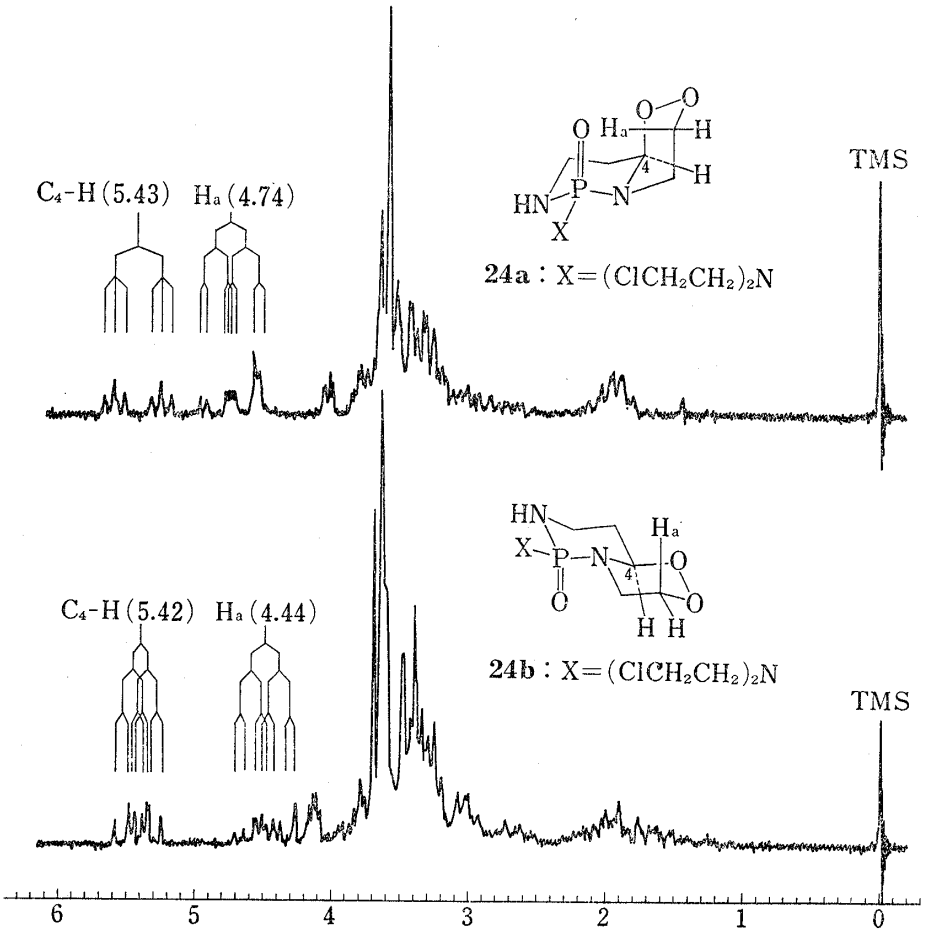
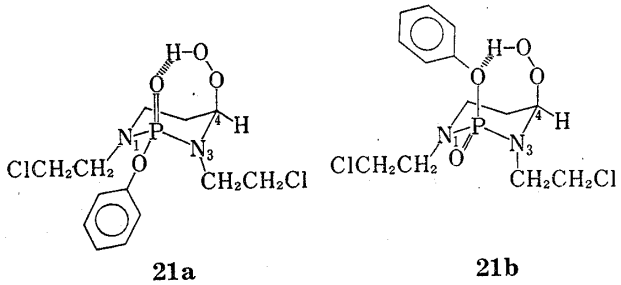


Fig. 1. 60 MHz PMR Spectra of the Bicyclic Peroxides **24a** and **24b** in $CDCl_3$ Solution

produced from 4-hydroperoxyisophosphamide,[6] indicating an *equatorial* configuration of the C_4–H. The PMR spectrum of **24b** in contrast showed the C_4-proton signals at δ 5.42 as a doublet of a double doublet and the P,H coupling constant was estimated to be 5.5 Hz based on visual inspection of the spectrum, which is indicative of an *axial* configuration of the C_4–H as has been assigned for an isomeric bicyclic peroxide produced from 2-*epi*-4-hydroperoxyisophosphamide.[13,14] Signals corresponding to an *axial* proton (Ha) of the $-OOCH_2-$ system were observed at lower field for **24a** (δ 4.74) than for **24b** (δ 4.44), this being attributable to the stereostructure in which Ha might suffer a deshielding effect from an axial P=O group (Fig. 1). As found for the bicyclic isomers produced from 4-hydroperoxyisophosphamide and its stereoisomer, **24a** and **24b** were also interconvertible by the action of *p*-toluenesulfonic acid (TsOH) in chloroform, giving an approximately 1: 1 equilibrium mixture after standing for 72 hr at room temperature. The observed resemblance in the chemical and PMR properties between **24a, b** and the corresponding 4-hydroperoxyisophosphamide derivatives suggests that the stereochemical behavior of the 1,3,2-diazaphosphorinane-2-oxide ring is not significantly different from that of the 1,3,2-oxazaphosphorinane-2-oxide ring. However, there was a difference between the two ring systems in the distribution of the isomeric hydroperoxides produced by the ozonolytic cyclization reaction. As suggested above, ozonolysis of 16 presumably gave a 1: 1 mixture of **20a** and **20b**, whereas similar synthesis of the 1-oxa analogue of **20**, as reported previously,[6b] predominantly gave an isomer with a *cis* configuration of the P=O and C_4–OOH groups. These results appear to be consistent with a recent communication by Mosbo[15] who also reported that 2-substituted 4-methyl-1,3,2-diazaphosphorinanes gave an approximately 1: 1 stereoisomeric equilibrium mixture while the corresponding 1,3-dioxa analogues predominantly existed as a single isomer.

In the case of ozonolysis of O-phenyl-N,N-bis(2-chloroethyl)-N′-(3-butenyl)phosphorodiamidate (**17**), the isomeric products could be separated after repeated column chromatography on silica gel in chloroform–acetone, giving **21a** (mp 110—112°, 24%) and **21b** (mp 94—96°, 32%). These products were interconvertible by the action of TsOH, producing an equilibrium mixture with a predominance of the latter isomer (**21a**/**21b**=1/2). The PMR spectra of **21a** and **21b** showed the C_4–H signals as a typical doublet of a triplet with a great vicinal P,H coupling constant suggestive of the *equatorial* configuration of their C_4–H, although the J(P,H) value of **21a** [J(P,H)=17.7 Hz] was somewhat smaller than that of **21b** [J(P,H) =21.9 Hz]. On alkali treatment, **21a** and **21b** were quantitatively converted into the corresponding bicyclic peroxides **25a** and **25b** both of which also gave the TsOH-catalyzed equilibrium mixture in the ratio of **25a**/**25b**=3/4. The PMR spectra of **25a** and **25b** respectively showed the C_4–H signals in a pattern quite similar to those of **24a** and **24b** as shown in Fig. 1, suggesting that the C_4–H might be *equatorial* and *trans* to the P=O group in **25a** but *axial* and *cis* in **25b**. Therefore it could be proposed that the C_4–H configuration of **21a** and **21b** might also be *trans* and *cis* to the P=O group, respectively, as depicted in Chart 5. Although the *cis-diaxial* conformation of the C_4–OOH and P=O groups in **21a** could be stabilized by the formation of a possible hydrogen bonding as found for 4-hydroperoxyisophosphamide,[6b] there might be un-



21a **21b**

Chart 5

13) A. Takamizawa, S. Matsumoto, T. Iwata, and I. Makino, *Heterocycles*, **3**, 787 (1975).
14) A. Takamizawa, T. Iwata, K. Yamaguchi, O. Shiratori, M. Harada, Y. Tochino, and S. Matsumoto, *Cancer Treatment Rept.*, **60**, 361 (1976).
15) J.A. Mosbo, *Tetrahedron Lett.*, **1976**, 4789.

favorable steric interactions between the *equatorial* phenoxy group and the N_1 and N_3 substituents, and such interactions might become significant when the two ring-nitrogen atoms have an sp^2-like planar geometry as proposed for that of isophosphamide[16] and 4-hydroperoxyisophosphamide.[17] The smaller J(P,H) value of the C_4–H of **21a** than that of **21b** and other 4-hydroperoxyisophosphamide derivatives[6b] having *equatorial* C_4–H might be attributed to those unfavorable interactions which would be responsible for increased population of other conformations free from such eclipsed interactions. On the other hand, the phenoxy group in **21b**, whose molecular structure could also be stabilized by a hydrogen bonding between the C_4–OOH and phenoxy groups, is free from such interactions and the *cis-diaxial* conformation of the two groups might be the most preferred one, which would account for its great J(P,H) value.

Other 4-hydroperoxy-1,3,2-diazaphosphorinane-2-oxides **22** and **23** were similarly prepared as an oily mixture of two possible stereoisomers by the ozonolysis reaction of **18** and **19**. One of the isomers could be separated in a pure state for both cases by repeated chromatographic purification (see Experimental). The PMR spectrum of the purified product of **22** showed the C_4–H signals as a doublet of a triplet at δ 5.14 with J(P,H) =16Hz which was still smaller than that of **21a**, suggesting that it might have a stereochemistry in which the eclipsed interactions between the phosphorus substituent and the 2-chloroethyl groups at N_1 and N_3 would be more significant than in **21a**, and therefore that the P=O and C_4–OOH groups would be in the *cis-diaxial* relationship. On the other hand, the purified product of **23** gave the C_4–H PMR signals as a doublet of a triplet at δ 5.07 with J(P,H)=21.0 Hz which corresponds to that of **21b**, supporting the *trans* configuration of the P=O and C_4–OOH groups. However, these stereochemical assignments are tentative as no comparative PMR data of the corresponding stereoisomers of **22** and **23** are available.

The antitumor activity of 4-hydroperoxy-1,3,2-diazaphosphorinane-2-oxides **20**, **21a**, **b** **22** and **23** was evaluated against L1210 leukemic BDF_1 mice, but they were practically inactive. This suggests that the ineffectiveness of the 1,3,2-diazaphosphorinane cyclophosphamide analogue is not due to inefficient biological C_4 oxidation and also provides an additional evidence that the 1,3,2-oxazaphosphorinane ring is not replaceable by other rings for exerting antitumor effects of cyclophosphamide-related compounds.

Experimental

Melting points were determined in open glass capillary tubes with 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 standard unless otherwise indicated. Column chromatography was carried out on silica gel (Merck Kieselgel 60). Thin-layer chromatography (TLC) was carried out using pre-coated silica gel plate (Merck, F-254, 0.25 mm). 1-Tosyloxy-3-butene was prepared according to literature[11] from 3-buten-1-ol which was purchased from Chemical Samples Co., Ohio, U.S.A. N,N-Bis(2-chloroethyl)aminophosphorodichloridate was prepared according to the procedure described by Friedman *et al.*[12] Phenyl- and 2-chloroethylphosphorodichlorides were purchased from Tokyo Kasei Co., Ltd., Tokyo, Japan. 2-Chloroethylamine hydrochloride was purchased from Aldrich Chemical Co., Ltd., Wisconsin, U.S.A.

N-(3-Butenyl)phthalimide (**9**)——To a magnetically stirred solution of 1-tosyloxy-3-butene[11] (23.6 g, 100 mmol) in dry dimethylformamide (DMF) (100 ml) was added potassium phthalimide (18.5 g, 100 mmol) at room temperature and the mixture was stirred for 6 hr at 100—110°. After the reaction mixture had stood overnight at room temperature, it was concentrated *in vacuo* and the resulting residue was extracted with $CHCl_3$ (200 ml)–H_2O (200 ml). The $CHCl_3$ layer was dried over Na_2SO_4 and concentrated *in vacuo* giving crude **9** as a pale yellow oily residue (17.1 g, 85%) which crystallized gradually on standing overnight at −20°. Recrystallization of **9** from hexane gave pale yellow needles, mp 49—50°. IR ν_{max}^{Nujol} cm^{-1}: 1770, 1720, 1700 (CO). PMR ($CDCl_3$) δ: 2.44 (2H, quartet, J=7 Hz, $-CH_2-CH=CH_2$), 3.77 (2H, triplet, J=7 Hz,

16) H.A. Brassfield, R.A. Jacobson, and J.G. Verkade, *J. Am. Chem. Soc.*, **97**, 4143 (1975).
17) A. Camerman, H.W. Smith, and N. Camerman, *Cancer Treatment Rept.*, **60**, 517 (1976).

$>NCH_2-$), 4.85—6.15 (3H, multiplet, $-CH=CH_2$), 7.58—7.92 (4H, multiplet, C_6H_4). *Anal.* Calcd. for $C_{12}H_{11}NO_2$: C, 71.63; H, 5.51; N, 6.90. Found: C, 71.55; H, 5.48; N, 6.79.

N-(3-Butenyl)aziridine (10)——To a mechanically stirred solution of ethyleneimine (25 ml) in ether (100 ml) was added dropwise a solution of 1-tosyloxy-3-butene (23.6 g, 100 mmol) in ether (100 ml) at −20 to −15° over 30 min, then the mixture was stirred for 4 hr at −15 to −10°. After it had stood overnight at room temperature, the reaction mixture was washed with cold water (50 ml × 2). The ether layer was dried over K_2CO_3 and ether was removed by evaporation *in vacuo* below 20° giving **10** as an oily residue (2.9 g, 30%). PMR ($CDCl_3$) δ: 1.75 (4H, singlet, $-N\begin{smallmatrix}CH_2\\|\\CH_2\end{smallmatrix}$), 2.35 (2H, multiplet, $-\underline{CH_2}-CH=CH_2$), 3.15 (2H, triplet, $-CH_2-N<$), 4.90—6.50 (3H, multiplet, $-CH=CH_2$). To prepare N-(2-chloroethyl)-3-butenylamine hydrochloride (**12**), it was profitable to treat the crude ether solution of **10** with hydrochloric acid without concentration, because much product was lost during evaporation of ether even at low temperature below 20°.

3-Butenylamine Hydrochloride (11)——To a solution of **9** (10 g, 50 mmol) in EtOH (50 ml) was added 100% hydrazine (1.6 g, 50 mmol) and the mixture was stirred for 3 hr at 50—55°. After it had stood overnight at room temperature, the resulting crystalline mass of phthalazine was washed with EtOH (100 ml) and filtered by suction. The filtrate was distilled at room temperature and the fraction boiling at 79—88° was collected (*ca.* 120 ml). A 20% HCl–EtOH solution (10 ml) was added to the distillate and EtOH was removed by evaporation *in vacuo* giving a crystalline residue which when recrystallized from EtOH–ether gave **11** (2.5 g, 47%) as hygroscopic white leaflets, mp 155—159°. PMR (D_2O, DSS[18]) δ: 2.42 (2H, quartet, J= 7 Hz, $-\underline{CH_2}-CH=CH_2$), 3.90 (2H, triplet, J=7 Hz, $-\underline{CH_2}-\overset{+}{N}H_3$), 5.05—6.20 (3H, multiplet, $-CH=CH_2$).

N-(2-Chloroethyl)-3-butenylamine Hydrochloride (12)——1-Tosyloxy-3-butene (11.8 g, 50 mmlo) and ethyleneimine were allowed to react in ether (100 ml) according to the procedure described above, then the reaction mixture was washed with water (25 ml × 2). The ether layer was dried over K_2CO_3, then 20% HCl–EtOH (20 ml) was added to the ether solution with stirring in an ice-water bath. Ether and EtOH were removed by evaporation *in vacuo* and the resulting crystalline mass was recrystallized from EtOH–ether giving **12** (3.94 g, 46.5%) as a hygroscopic white cake, mp 200—205°. PMR (D_2O, DSS) δ: 2.44—3.60 (6H, multiplet, $-\underline{CH_2}-CH=CH_2$, NCH_2CH_2Cl), 3.97 (2H, triplet, $-\underline{CH_2}-\overset{+}{N}H_2-$), 5.07—6.23 (3H, multiplet, $-CH=CH_2$).

N-(3-Butenyl)-N′,N′,N″-tris(2-chloroethyl)phosphorotriamidate (16)——To a mechanically stirred solution of N,N-bis(2-chloroethyl)aminophosphorodichloridate[12] (2.59 g, 10 mmol) in CH_2Cl_2 (20 ml) was added 3-butenylamine hydrochloride (1.07 g, 10 mmol), then a solution of triethylamine (Et_3N) (2.02 g, 20 mmol) in CH_2Cl_2 (10 ml) was added dropwise over 30 min at −20 to −25°. The mixture was stirred for 3 hr at −20 to −25°, 2-chloroethylamine hydrochloride (1.16 g, 10 mmol) was added then a solution of Et_3N (2.02 g, 20 mmol) in CH_2Cl_2 (10 ml) was added dropwise again to the stirred mixture over 30 min at −10 to 0°. After the reaction mixture had stood overnight at room temperature, it was filtered by suction and the filtrate was washed with H_2O (20 ml × 3), dried over Na_2SO_4 and concentrated *in vacuo* giving **16** as an oil (3.2 g, 95%) which could be used for the next run without further purification. An analytical sample of **16** was obtained by purification of the crude oil by column chromatography in Me_2CO–$CHCl_3$ (1 : 1), giving a colorless oil. PMR ($CDCl_3$) δ: 2.22 (2H, quartet, J=5.2 Hz, $-\underline{CH_2}-CH=CH_2$), 2.83—3.77 (16H, multiplet, $3\times CH_2-CH_2Cl$, P–N–CH_2, $2\times NH$), 4.93—6.17 (3H, multiplet, $-CH=CH_2$). *Anal.* Calcd. for $C_{10}H_{21}Cl_3N_3OP$: C, 35.68; H, 6.29; Cl, 31.60; N, 12.48; P, 9.20. Found: C, 35.40; H, 6.31; Cl, 31.51; N, 12.59; P, 8.99.

O-Phenyl-N,N′-bis(2-chloroethyl)-N-(3-butenyl)-phosphorodiamidate (17)——Phenylphosphorodichloride (2.11 g, 10 mmol), N-(2-chloroethyl)-3-butenylamine hydrochloride (1.70 g, 10 mmol) and 2-chloroethylamine hydrochloride (1.16 g, 10 mmol) were allowed to react as described for the preparation of **16** in the presence of Et_3N. The crude product **17** was obtained as a pale yellow oil (2.28 g, 63%) which was used for the next run without further purification. An analytical sample of **17** was also obtained on purification of the crude oil by column chromatography in Me_2CO–$CHCl_3$ (1 : 1), which gave a colorless oil. PMR ($CDCl_3$) δ: 2.27 (2H, quartet, J=5.1 Hz, $-\underline{CH_2}-CH=CH_2$), 2.87—3.73 (11H, multiplet, $2\times NCH_2CH_2Cl$, NCH_2, NH), 4.97—6.18 (3H, multiplet, $-CH=CH_2$), 7.30 (5H, singlet, C_6H_5). *Anal.* Calcd. for $C_{14}H_{21}Cl_2N_2O_2P$: C, 47.88; H, 6.03; Cl, 20.19; N, 7.98; P, 8.82. Found: C, 47.59; H, 5.89; Cl, 20.18; N, 8.12; P, 8.71.

N-(3-Butenyl)-N,N′,N′,N″-tetra(2-chloroethyl)phosphorotriamidate (18)——In a manner similar to the preparation procedure for **16**, N,N-bis(2-chloroethyl)aminophosphorodichloridate (2.59 g, 10 mmol), N-(2-chloroethyl)-3-butenylamine hydrochloride (1.70 g, 10 mmol) and 2-chloroethylamine hydrochloride (1.16 g, 10 mmol) were allowed to react in the presence of Et_3N. The crude product of **18** was obtained as a pale yellow oil which was purified by column chromatography in ether giving purified **18** as a colorless oil (2.35 g, 59%). PMR ($CDCl_3$) δ: 2.67 (2H, quartet, J=5.1 Hz, $-\underline{CH_2}CH=CH_2$), 2.80—4.00 (17H, multiplet, $4\times NCH_2CH_2Cl$, NH), 4.30 (2H, multiplet, P–NCH_2–), 4.97—6.14 (3H, multiplet, $-CH=CH_2$). *Anal.* Calcd. for $C_{12}H_{24}Cl_4N_3OP$: C, 36.11; H, 6.06; Cl, 35.53; N, 10.53; P, 7.76. Found: C, 36.12; H, 6.13; Cl, 35.41; N,

18) DSS: 2,2-Dimethyl-2-silapentane-5-sulfonate.

10.51; P, 7.68.

O-(2-Chloroethyl)-N-(3-butenyl)-N,N′-bis(2-chloroethyl)phosphorodiamidate (19)——2-Chloroethylphosphorodichloridate (1.97 g, 10 mmol), N-(2-chloroethyl)-3-butenylamine hydrochloride (1.70 g, 10 mmol) and 2-chloroethylamine hydrochloride (1.16 g, 10 mmol) were also allowed to react according to the procedure described above, and the resulting crude product of **19** was purified by column chromatography in EtOAc giving a colorless oil (1.89 g, 56%). PMR ($CDCl_3$) δ: 2.37 (2H, multiplet, $-\underline{CH_2}CH=CH_2$), 2.94—3.82 (13H, multiplet, $2\times NCH_2CH_2Cl$, $OCH_2\underline{CH_2}Cl$, P–N–$\underline{CH_2}$–, NH), 4.20 (2H, multiplet, P–O–$\underline{CH_2}$–), 4.92—6.17 (3H, multiplet, $-CH=CH_2$). *Anal.* Calcd. for $C_{10}H_{20}Cl_3N_2O_2P$: C, 35.58; H, 5.97; Cl, 31.51; N, 8.30; P, 9.17. Found: C, 35.59; H, 6.15; Cl, 31.88; N, 8.25; P, 9.03.

2-[N,N-Bis(2-chloroethyl)]amino-3-(2-chloroethyl)-4-hydroperoxy-1,3,2-diazaphosphorinane-2-oxide (20) To a magnetically stirred solution of **16** (3.37 g, 10 mmol) in a mixture of Me_2CO (30 ml) and H_2O (20 ml), O_3 (960 mg, 20 mmol) was bubbled at a rate of approximately 80 mg/min for 12 min with cooling in an ice-water bath. After the bubbling of O_3 had been completed, 30% hydrogen peroxide (3 ml) was added to the ozonized solution and the mixture was allowed to stand for five days at 3°. Me_2CO was removed from the reaction mixture by evaporation *in vacuo* and the remaining turbid aqueous layer was extracted with $CHCl_3$ (30 ml×3). The combined $CHCl_3$ extract was washed with H_2O (20 ml×2), dried over Na_2SO_4 and concentrated *in vacuo* giving a colorless oily residue which was chromatographed on a column (5×12 cm) eluted with Me_2CO–$CHCl_3$ (1: 1). After elution of an unidentified peroxidic oily substance (750 mg), pure fractions containing **20** were collected and concentrated *in vacuo* giving a colorless oil (985 mg, 27.8%). PMR ($CDCl_3$) δ: 2.20 (2H, multiplet, C_5–H), 3.10—3.80 (15H, multiplet, $3\times NCH_2CH_2Cl$, P–$NHCH_2$–), 4.93—5.45 (1H, multiplet, C_4–H). *Anal.* Calcd. for $C_9H_{19}Cl_3N_3O_3P$: C, 30.48; H, 5.40; Cl, 30.00; N, 11.85; P, 8.73. Found: C, 30.31; H, 5.43; Cl, 29.81; N, 11.67; P, 8.77.

1,3-Bis(2-chloroethyl)-2-phenoxy-4-hydroperoxy-1,3,2-diazaphosphorinane-2-oxides 21a and 21b——Compound **17** (3.5 g, 10 mmol) was ozonized in aqueous Me_2CO as described above then treated with 30% hydrogen peroxide (3 ml). The crude ozonolysis product was first chromatographed on a column (4.5×11 cm) eluted with MeCO–$CHCl_3$ (1: 1), giving a mixture of **21a** and **21b** as a colorless oil (2.4 g, 65%). The oily mixture was further chromatographed on a column (5×12 cm) eluted with Me_2CO–$CHCl_3$ (1: 2). Pure fractions of **21b** were eluted first (1.2 g, 32%), then a small amount of a mixture of **21a** and **21b** (70 mg), and finally pure fractions of **21a** (0.92 g, 24%). The products solidified on standing overnight at −20° and were recrystallized from hexane giving colorless prisms: **21a**, mp 110—112°. PMR ($CDCl_3$) δ: 2.12—2.40 (2H, multiplet, C_5–H), 2.84—3.88 (10H, multiplet, $2\times NCH_2CH_2Cl$, C_6–H), 5.12 [1H, doublet of triplet, J(P,H)=17.7 Hz, J(H,H)=3.8 Hz, C_4–H], 7.02—7.52 (5H, broad singlet, C_6H_5). *Anal.* Calcd. for $C_{13}H_{19}Cl_2N_2O_4P$: C, 42.29; H, 5.19; Cl, 19.21; N, 7.59; P, 8.39. Found: C, 42.31; H, 5.38; Cl, 19.47; N, 7.29; P, 8.13: **21b**, mp 94—96°. PMR ($CDCl_3$) δ: 2.00—2.40 (2H, multiplet, C_5–H), 3.07—3.73 (10H, multiplet, $2\times NCH_2CH_2Cl$, C_6–H), 5.13 [1H, doublet of triplet, J(P,H)=21.9 Hz, J(H,H)=4.0 Hz, C_4–H], 7.27 (5H, singlet, C_6H_5). *Anal.* Calcd. for $C_{13}H_{19}Cl_2N_2O_4P$: C, 42.29; H, 5.19; Cl, 19.21; N, 7.59; P, 8.39. Found: C, 42.53; H, 5.37; Cl, 19.38; N, 7.54; P, 8.31.

1,3-Bis(2-chloroethyl)-2-[N,N-bis(2-chloroethyl)]amino-4-hydroperoxy-1,3,2-diazaphosphorinane-2-oxide (22)——Ozonolysis of **18** (4.0 g, 10 mmol) was carried out as described above. After treatment of the ozonized solution with 30% hydrogen peroxide, a crude oily product was obtained as a colorless oil (3.8 g). The oily product was chromatographed on a column (4.5×12 cm) eluted with EtOAc giving crude **22** as an oil, which was further chromatographed on a column (5×11 cm) eluted with Me_2CO–$CHCl_3$ (1: 1). The first eluate of the second chromatography gave an unidentified peroxidic oily substance (130 mg) and the second eluate gave the pure product (1.00 g, 24%). PMR ($CDCl_3$) δ: 2.00 (2H, multiplet, C_5–H), 2.80—4.70 (18H, multiplet, $4\times CH_2CH_2Cl$, P–NCH_2–), 5.14 [1H, doublet of broad triplet, J(P,H)=16.0 Hz, J(H,H)=*ca.* 4 Hz, C_4–H]. *Anal.* Calcd. for $C_{11}H_{22}Cl_4N_3O_3P$: C, 31.68; H, 5.32; Cl, 34.00; N, 10.07; P, 7.43. Found: C, 31.91; H, 5.60; Cl, 34.18; N, 10.16; P, 7.48.

1,3-Bis(2-chloroethyl)-2-(2-chloroethoxy)-4-hydroperoxy-1,3,2-diazaphosphorinane-2-oxide (23)——Ozonolysis of **19** (3.38 g, 10 mmol) was carried out as described above and the ozonized solution was treated with 30% hydrogen peroxide. The resulting ozonolysis product was first chromatographed on a column (4.5×11 cm) eluted with EtOAc, giving first a small amount of peroxidic oily substance (*ca.* 120 mg) then a colorless oil (1.7 g, 48%). The oily eluate was further chromatographed on a column (5×12 cm) eluted with ether giving a pure product **23** as a colorless oil (1.2 g, 34%). PMR ($CDCl_3$) δ: 2.13 (2H, multiplet, C_5–H), 2.67—3.83 (12H, multiplet, $2\times CH_2CH_2Cl$, $OCH_2\underline{CH_2}Cl$, P–N–CH_2–), 4.22 (2H, multiplet, P–O–CH_2–), 5.07 [1H, doublet of triplet, J(P,H)=21.0 Hz, J(H,H)=4.0 Hz, C_4–H]. *Anal.* Calcd. for $C_9H_{18}Cl_3N_2O_4P$: C, 30.40; H, 5.10; Cl, 29.91; N, 7.88; P, 8.71. Found: C, 29.95; H, 5.23; Cl, 30.08; N, 7.63; P, 8.96.

Bicyclic Peroxides 24a and 24b——To a magnetically stirred solution of **20** (710 mg, 2 mmol) in $CHCl_3$ (20 ml) was added an aqueous 10% NaOH solution (10 ml) and the mixture was vigorously stirred for 30 min at room temperature. The $CHCl_3$ layer was washed with H_2O (20 ml×2), dried over Na_2SO_4 and concentrated *in vacuo* giving an oily residue (550 mg) which was chromatographed on a column (4.5×10 cm) eluted with Me_2CO–$CHCl_3$ (1: 1). The first eluate gave **24a** as an oil (140 mg, 22%) which solidified on standing overnight at −20°. Recrystallization of **24a** from Me_2CO–ether gave colorless prisms, mp 119—121°. IR ν_{max}^{Nujol} cm^{-1}: 3085 (NH), 1288, 1249, 1230, 1190 (PO), 1070, 1037 (POC). PMR ($CDCl_3$) δ: 1.78—2.12 (2H, multiplet

C_5–H), 2.63—3.74 (13H, multiplet, $3\times CH_2CH_2Cl$, NH), 3.90 [1H, doublet of double doublet, J(H,H)=12.0 Hz, J'(H,H)=3.0 Hz, J''(H,H)=1.0 Hz, –OO–CH_{eq}–], 4.74 [1H, doublet of double doublet, J(H,H)=12.0 Hz, J'(H,H)=10.8 Hz, J''(H,H)=2.9 Hz, –OO–CH_{ax}–], 5.43 [1H, doublet of triplet, J(P,H)=20.3 Hz, J(H,H)=4.3 Hz, C_4–H]. *Anal.* Calcd. for $C_9H_{18}Cl_2N_3O_3P$: C, 33.98; H, 5.70; Cl, 22.29; N, 13.21; P, 9.76. Found: C, 34.15; H, 5.86; Cl, 22.39; N, 13.16; P, 9.76. The second eluate gave a mixture of **24a** and **24b** as an oil (150 mg, *ca.* 1: 1) and the pure fractions of **24b** were collected from the third eluate as an oil (130 mg, 20%) which was crystallized by trituration in ether–Me_2CO. Recrystallization of **24b** from Me_2CO–ether gave colorless prisms, mp 100—101°. IR ν_{max}^{Nujol} cm^{-1}: 3044, 1280, 1275, 1249, 1230, 1190 (PO), 1080, 1040, 1020 (POC). PMR ($CDCl_3$) δ: 1.52—2.16 (2H, multiplet, C_5–H), 2.98—3.98 (13H, multiplet, $3\times CH_2CH_2Cl$, NH), 4.08—4.72 (2H, multiplet, –$OOCH_2$–), 5.42 [1H, doublet of double doublet, J(P,H)=5.5 Hz, J(H,H)=8.4 Hz, J'(H,H)=6.4 Hz, C_4–H]. *Anal.* Calcd. for $C_9H_{18}Cl_2N_3O_3P$: C, 33.98; H, 5.70; Cl, 22.29; N, 13.21; P, 9.74. Found: C, 34.03; H, 5.84; Cl, 22.58; N, 13.09; P, 9.96.

TsOH-catalyzed Equilibrium between 24a and 24b——To a magnetically stirred solution of **24a** (or **24b**) (32 mg, 0.1 mmol) in $CHCl_3$ (5 ml) was added *p*-toluenesulfonic acid (TsOH) (*ca.* 5 mg) and the solution was stirred at room temperature. The reaction mixture was monitored by TLC in Me_2CO–$CHCl_3$ (1: 1) and after *ca.* 72 hr the spots of **24a** and **24b** appeared with almost equal intensity on the Epstein test.[19] The equilibrium mixture was chromatographed on a column (1.5×5 cm) eluted with Me_2CO–$CHCl_3$ (1: 1) giving **24a** (12 mg) and **24b** (10 mg) which were identified with authentic specimens by IR comparison.

TsOH-catalyzed Equilibrium between 21a and 21b——To a magnetically stirred solution of **21a** (or **21b**) (74 mg, 0.2 mmol) in $CHCl_3$ (20 ml) was added TsOH (*ca.* 10 mg) and the mixture was stirred for 24 hr at room temperature. The TLC pattern in Me_2CO–$CHCl_3$ (1: 1) was identical to that of a mixture of **21a** and **21b** in the ratio of **21a**/**21b**=1/2. The equilibrium mixture was chromatographed on a column (2×7 cm) eluted with Me_2CO–$CHCl_3$ (1: 2) giving **21a** (15 mg, 20%) and **21b** (40 mg, 54%), which were identified with authentic specimens by IR comparison.

Bicyclic Peroxides 25a and 25b——To a solution of **21a** (370 mg, 1 mmol) in $CHCl_3$ (10 ml) was added an aqueous 10% NaOH solution (5 ml) and the mixture was vigorously stirred for 30 min at room temperature. The $CHCl_3$ layer was washed with H_2O (5 ml×2), dried over Na_2SO_4 and concentrated *in vacuo* giving **25a** as a colorless oil (305 mg, 92%). PMR ($CDCl_3$) δ: 1.83—2.38 (2H, multiplet, C_5–H), 2.77—3.60 (8H, multiplet, CH_2CH_2Cl, $2\times NCH_2$), 3.82 [1H, doublet of multiplet, J(H,H)=12.6 Hz, –OOCH_{eq}–], 4.55 [1H, doublet of double doublet, J(H,H)=12.6 Hz, J'(H,H)=11.5 Hz, J''(H,H)=3.2 Hz, –OOCH_{ax}–], 5.36 [1H, doublet of triplet, J(P,H)=22.8 Hz, J(H,H)=4.7 Hz, C_4–H], 7.27 (5H, singlet, C_6H_5). *Anal.* Calcd. for $C_{13}H_{18}ClN_2O_4P$: C, 46.93; H, 5.45; Cl, 10.66; N, 8.42; P, 9.31. Found: C, 46.83; H, 5.31; Cl, 10.76; N, 8.77; P, 9.04. Similarly, **21b** (370 mg, 1 mmol) was treated with 10% NaOH (5 ml) in $CHCl_3$ (10 ml) giving **25b** as a colorless oil (263 mg, 79%). PMR ($CDCl_3$) δ: 1.68—2.12 (2H, multiplet, C_5–H), 2.63—3.78 (8H, multiplet, CH_2CH_2Cl, $2\times NCH_2$), 4.00 [1H, doublet of multiplet, J(H,H)=12.2 Hz, –OOCH_{eq}–], 4.55 [1H, doublet of double doublet, J(H,H)=12.2 Hz, J'(H,H)=11.5 Hz, J''(H,H)=3.0 Hz, –OOCH_{ax}–], 5.31 [1H, doublet of double doublet, J(P,H)=5.2 Hz, J(H,H)=6.8 Hz, J'(H,H)=5.0 Hz, C_4–H], 7.00—7.50 (5H, broad singlet, C_6H_5). *Anal.* Calcd. for $C_{13}H_{18}ClN_2O_4P$: C, 46.93; H, 5.45; Cl, 10.66; N, 8.42; P, 9.31. Found: C, 46.73; H, 5.31; Cl, 10.61; N, 8.39; P, 9.64.

TsOH-catalyzed Equilibrium between 25a and 25b——To a magnetically stirred solution of **25a** (or **25b**) (33 mg, 0.1 mmol) in $CHCl_3$ (10 ml) was added TsOH (*ca.* 5 mg) and the mixture was stirred at room temperature. After 24 hr, TLC of the mixture in Me_2CO–$CHCl_3$ (1: 3) indicated the presence of two components with *Rf* 0.44 (**25a**) and *Rf* 0.55 (**25b**), which was identical with the TLC pattern of a mixture of **25a** and **25b** in the ratio of **25a**/**25b**=3/4. Chromatography of the equilibrium mixture on a column (1.5×4.5 cm) eluted with Me_2CO–$CHCl_3$ (1: 2) gave **25b** (14 mg, 42%) first, then **25a** (9 mg, 27%), which were identified with authentic specimens by IR comparison.

19) J. Epstein, R.W. Rosenthal, and R.J. Ess, *Anal. Chem.*, **27**, 1435 (1955).