CHEMISTRY OF C_4-FUNCTIONALIZED 1,3,2-OXAZAPHOSPHORINANE 2-OXIDES RELATED TO THE ACTIVE METABOLITE OF CYCLOPHOSPHAMIDE

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Current status of synthetic and stereochemical studies of the activated species of cyclophosphamide and related 1,3,2-oxazaphosphorinane antitumor alkylating agents is reviewed.

I. Introduction

Cyclophosphamide (1) was first synthesized by Arnold and Bourseaux[1] in 1958 and is now widely used as a cancer chemotherapeutic agent. This drug is peculiar in that it is ineffective by itself but becomes metabolically activated as a cytostatic-alkylating species after microsomal oxidation in the liver.[2-6] The major metabolic reaction is thought to be the oxidation at the C_4 position of its 1,3,2-oxazaphosphorinane ring because 4-ketocyclophosphamide (4) and carboxycyclophosphamide (5) are produced from cyclophosphamide as

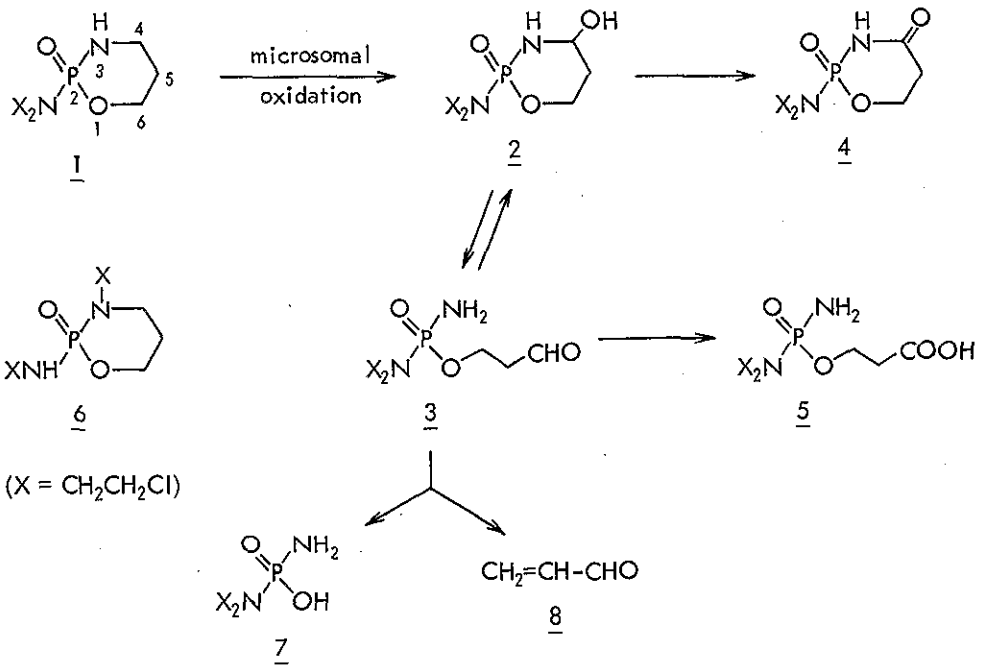


Chart 1

urinary metabolites in animals[7-9] or as enzyme-mediated oxidation products in in vitro experiments.[10,11] The metabolites 4 and 5, however, give no increased cytostatic activity, suggesting that the activation occurs in an earlier phase of the C_4 oxidation. Thus it is now most generally accepted that 4-hydroxycyclophosphamide (2) or aldophosphamide (3) are important metabolites responsible for antitumor effects.[10-13] Isophosphamide (6)[14] is a cyclophosphamide analogue differing only in the position of alkylating functionalities and is currently under clinical investigation.[15,16] The action mechanism of isophosphamide is thought to be similar to

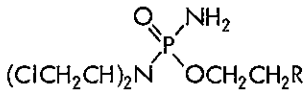
that of cyclophosphamide.[17-19]

Many attempts have been made to identify the active metabolite of these drugs, but its definite characterization was unsuccessful until recently.[20] This was primarily due to great instability of the ring system 4-hydroxy-1,3,2-oxazaphosphorinane 2-oxide which is in equilibrium with the ring-opened aldehyde (3) and spontaneously decomposes giving phosphoramidic acid (7) and acrolein (8). Such fragments were in fact identified as the metabolites of cyclophosphamide.[21,22] Interestingly, both of these fragments are potentially cytotoxic and have been proposed as the ultimate active metabolites which act independently or concertedly to produce antitumor activity when they are intracellularly released from 2 (or 3),[21-23] but some negative evidences against the cytostatic role of acrolein have also been reported.[19] Since a well-documented excellent review[24] is available, we will not discuss further on the metabolic dispositions of cyclophosphamide. The present article gives an account of our current studies on the chemistry of C_4-functionalized 1,3,2-oxazaphosphorinane 2-oxide related to the active metabolite of cyclophosphamide.

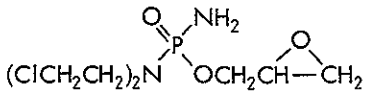
II. The First Successful Synthesis of 4-Hydroxycyclophosphamide

In search of cancer chemotherapeutic agents with greater efficacy than cyclophosphamide, synthesis of the activated species of cyclophosphamide and related antitumor agents is

of significant interest because such activated agents are especially desirable for cancer patients with hepatic lesions, a situation in which cyclophosphamide can not be activated effectively. The first attempt to obtain the active species of cyclophosphamide by a purely chemical method was made by Rauen et al.[25] who reported that the Fenton oxidation of cyclophosphamide gave a mixture of oxidized products having potential alkylating activity, but the products were not fully characterized. $KMnO_4$ oxidation of cyclophosphamide also did not produce 4-hydroxycyclophosphamide, giving 4-ketocyclophosphamide and carboxycyclophosphamide besides N-dechloroethylated by-products.[26,27] Controlled partial reduction of 4-ketocyclophosphamide with $LiAlH_4$ gave a highly cytotoxic, but unstable product which could also not be characterized unambiguously.[28] Some aldehyde-forming reactions were also attempted for the open chain precursors 9-15;[29,30] namely Pfitzner-Moffatt[31] oxidation of 9, reductive hydrolysis of 10 by the Backeberg-Staskun procedure,[32] partial reduction of 11 and 12 with $LiAlH_4$ or $LiAlH(OC_2H_5)_3$, acid hydrolysis of 13, ozonolysis of 14, and rearrangement of an epoxide 15. All of these attempts led to discouraging results suggesting that the possible product 3 readily undergoes the β-elimination reaction under the reaction conditions employed.



9 R = CH_2OH
10 R = CN
11 R = $CON(CH_3)_2$
12 R = $COOC_2H_5$
13 R = $CH(OC_2H_5)_2$
14 R = $CH{=}CH_2$



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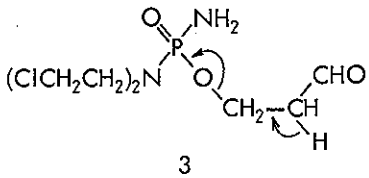


3

Chart 2

In 1973, we first synthesized 4-hydroxycyclophosphamide which was isolated in a pure crystalline state and fully characterized.[20] With careful re-examination of the ozonolysis reaction of 14, we found that 4-hydroperoxycyclophosphamide (16) could be obtained in a low yield after chromatographic purification of the crude ozonolysis mixture. The hydroperoxide 16 could be obtained in more than 50% yield when the ozonized solution was treated with hydrogen peroxide or t-butyl hydroperoxide. 4-Hydroxycyclophosphamide (2) was then produced from 16 by deoxygenation with triphenylphosphine. The synthetic specimen of 2 was confirmed to be potentially cytotoxic, giving definitive evidence that cyclophosphamide is actually activated by the C_4 hydroxylation. Compound 2 is labile in most organic solvents, whereas it is considerably stable in neutral aqueous medium where its equilibrium concentration is greater than that of the ring-opened species 3.[33] Surprisingly, 4-hydroperoxycyclophosphamide (16) is more

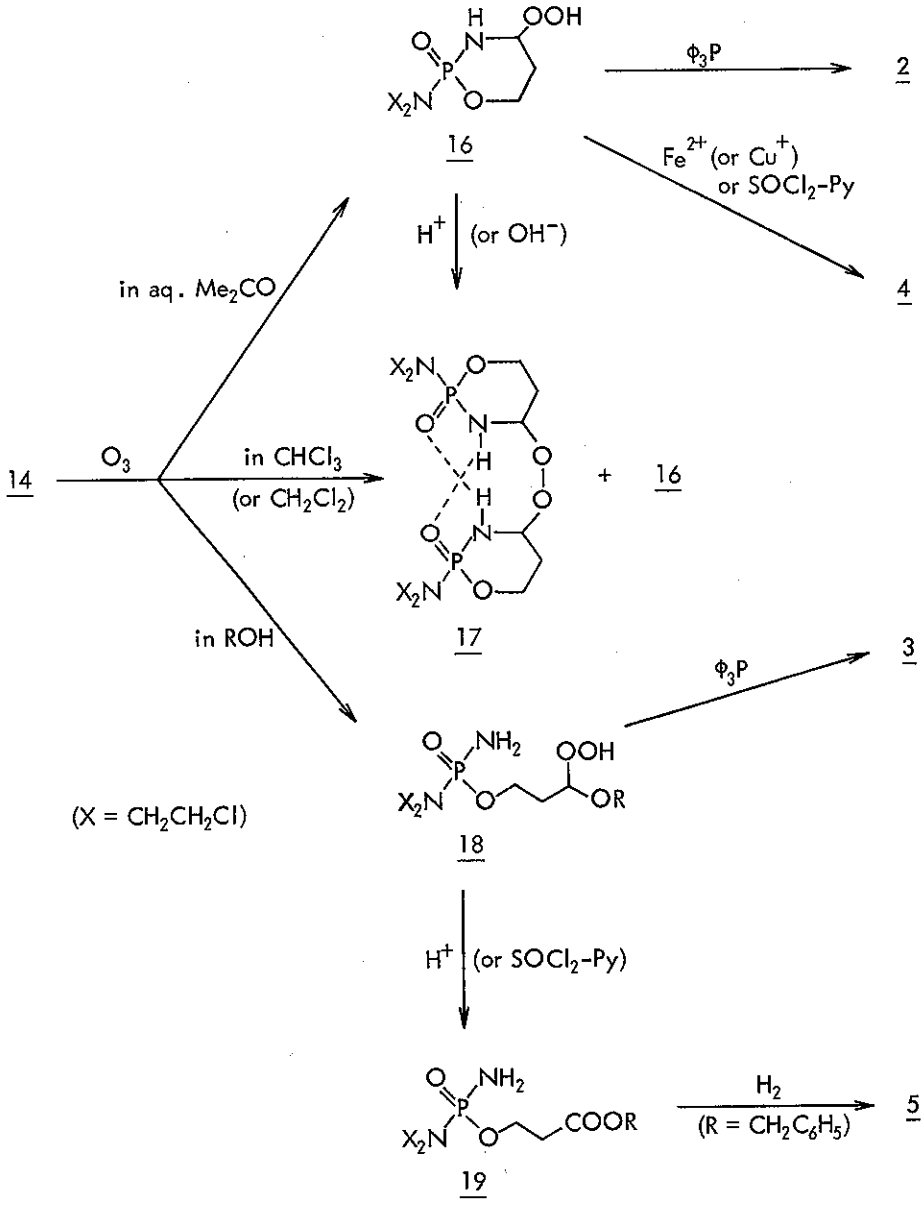

16
ϕ_3P
2
Fe^{2+} (or Cu^+)
or $SOCl_2$-Py
4
H^+ (or OH^-)
in aq. Me_2CO
14
O_3
in $CHCl_3$
(or CH_2Cl_2)
17
+ 16
in ROH
ϕ_3P
3
18
(X = CH_2CH_2Cl)
H^+ (or $SOCl_2$-Py)
H_2
($R = CH_2C_6H_5$)
5
19

Chart 3

stable than 2; after being kept for more than four years at -20°, it showed biologic activities almost equal to those of 2.[34] Compound 16 is quantitatively convertible into 4-keto-cyclophosphamide (4) on treatment with Fe^{2+} or Cu^{+} ions. The lactam formation from 16 is also affected by $SOCl_2$ and pyridine. Interestingly, treatment of 16 with a catalytic amount of p-toluenesulfonic acid (TsOH) in chloroform gives 4-hydroxycyclophosphamide anhydro-dimer[35] (4-peroxycyclo-phosphamide[36]) (17). Conversion of 16 into 17 also occurs due to the action of alkali. X-Ray analysis of this dimer, done by Sternglanz et al.,[37] revealed that it has a sym-metrical structure in which two 1,3,2-oxazaphosphorinane rings are fixed to each other by intramolecular cross-linking hydrogen bonds between the ring NH protons and P=O groups. Compound 17 is more stable than 16 and shows almost equal biologic activity to that of 2 and 16.[35,36] The in vivo occurrence of 17 as a cyclophosphamide metabolite has been suggested.[38]

When ozonolysis of 14 is carried out in the presence of an appropriate alcohol, the corresponding open chain hemi-acetal hydroperoxide 18 is obtained in a good yield after column chromatographic purification.[34] Compound 18 is also very cytotoxic but less stable than 16 and 17. Deoxygenation of 18 with triphenylphosphine under a mild condition yields a spectroscopically characterizable product which is believed to be aldophosphamide (3) but is very labile readily turning to the phosphoramidic acid (7) and acrolein (8). On treatment

with TsOH or $SOCl_2$-pyridine, compound 18 is smoothly converted to the corresponding ester 19 and the catalytic reduction of 19 (R = $CH_2C_6H_5$) gives carboxycyclophosphamide (5). All of the possible cyclophosphamide metabolites 2-5, 7, 8 and 17 could thus be produced from 14.

Ozonolysis of 14 in a solvent of low polarity such as chloroform or methylene chloride gives the dimer 17 as a major product besides 16, although in a low yield. In such a less polar medium, the zwitterion intermediate 21, which is produced from the primary ozonide 20 as a possible precursor for 16 and 18, can not be stabilized as well as in a polar medium such as aqueous acetone, therefore it will readily recombine with another fragment 3 giving the symmetrical ozonide 22 from which dimer 17 will be produced after double cyclization reactions via 23.

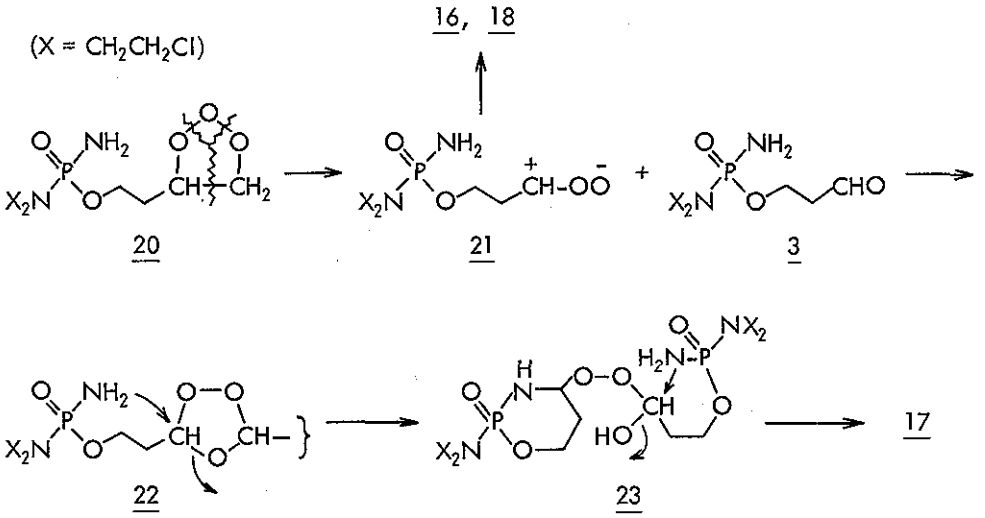


Chart 4

Soon after the synthesis of 4-hydroxy- and 4-hydroperoxycyclophosphamide had been reported, the Fenton oxidation products of cyclophosphamide were identified as 4, 16 and 17.[36,38] Oxidation of cyclophosphamide with $NaOCl-H_2O_2$ also gave 4, 16, and 17 besides N-chlorocyclophosphamide (24), which was a major product.[39] With these direct oxidation reactions of cyclophosphamide, the yield of the oxidized product, particularly that of 16, was very poor. Recently, Hohorst et al.[40] reported that the oxidation of cyclophosphamide and isophosphamide with $O_3-H_2O_2$ gave the corresponding 4-hydroperoxy derivatives in more reasonable yields, and they suggested singlet oxygen to be the oxidizing species in this novel reaction.

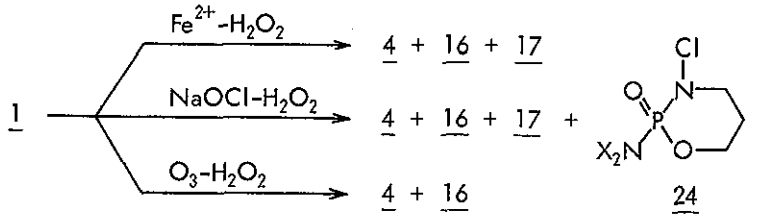


Chart 5

III. Syntheses of 4-Hydroperoxycyclophosphamide Analogues

The high antitumor efficacy, as well as considerable stability, of 4-hydroperoxycyclophosphamide prompted us to synthesize related "active hydroperoxides." Such activated species are thought to be preferable for investigation of the structure-activity relationships. 4-Hydroperoxy-1,3,2-oxaza-

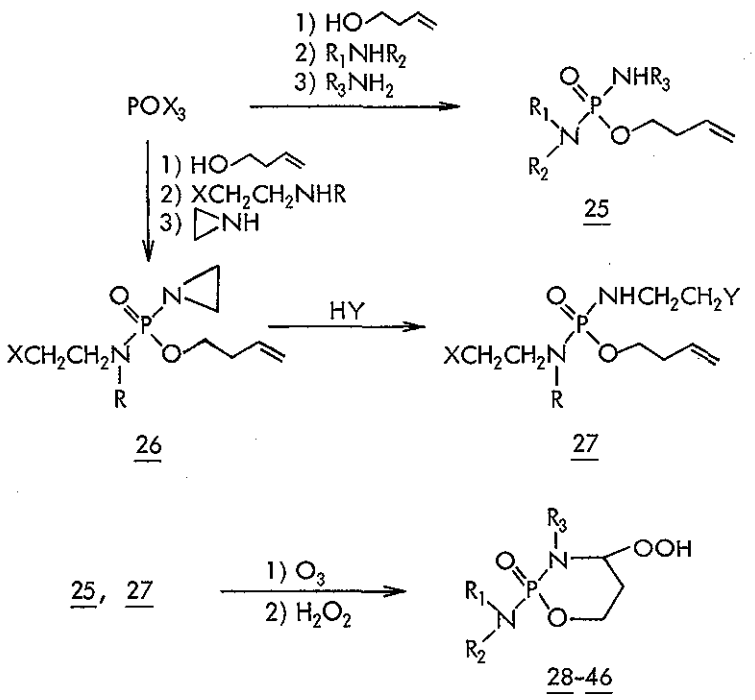


Chart 6

phosphorinane 2-oxides having different kinds of alkylating groups were synthesized by the ozonolytic cyclization reactions. Starting material 25 is simply obtainable by reaction of phosphoryl halide POX_3 (X = Cl or Br) with 3-buten-1-ol, followed by the triethylamine-mediated reactions with appropriate amines (R_1NHR_2 and R_3NH_2). Compounds possessing different alkylating groups in a molecule such as 27 are most conveniently prepared via the phosphoramidoyl aziridine 26 which is smoothly convertible into 27 by treatment with an acid HY (HCl, HBr, HI or sulfonic acids).[42] A number of

cyclic hydroperoxides 28-46 were obtained by the ozonolysis of 25 or 27 (Table I).[34,41,42]

There is a possibility, at least theoretically, that the ozonolytic cyclization reaction gives two epimeric products having cis and trans configurations of the C_4-OOH and P=O groups. In most cases, however, the cis-isomer is isolated exclusively or predominantly. This is perhaps due to greater thermodynamic stability of the cis-isomer (see Section V).

All of the listed compounds are potentially cytotoxic in in vitro experiments. Of particular interest is that compounds possessing different kinds of alkylating groups in a molecule, such as 37-46, are more toxic than those possessing the same alkylating functions, such as 28-36. However, this is not necessarily the case for the in vivo antitumor activity which does not appear to be significantly influenced not only by the kind of alkylating functions but also by their positions on the six-membered ring. Two compounds, 2-(2-chloroethyl)amino-3-(2-chloroethyl)-4-hydroperoxy-1,3,2-oxazaphosphorinane 2-oxide (4-hydroperoxyisophosphamide, NSC-227114) (32)[41] and 2-[N-methyl-N-(2-chloroethyl)]amino-3-(2-methylsulfonyloxyethyl)-4-hydroperoxy-1,3,2-oxazaphosphorinane 2-oxide (NSC-280122 D) (41),[42] were selected as the most promising agents and one of them (NSC-227114) is now under clinical evaluation.

Table I. 4-Hydroperoxy-1,3,2-oxazaphosphorinane 2-oxides (28-46)

Compound	R_1	R_2	R_3	Mp (°C)[a]	Yield[b] (%)
28(= 16)	CH_2CH_2Cl	CH_2CH_2Cl	H	107-108	55
29	CH_2CH_2Cl	CH_2CH_2Cl	CH_3	99-100	25
30	CH_2CH_2Cl	CH_2CH_2Cl	$CH_2CH_2CH_3$	112-114	11
31	CH_2CH_2Cl	CH_2CH_2Cl	CH_2CH_2Cl	115-117	26
32	CH_2CH_2Cl	H	CH_2CH_2Cl	113-114	30
33	CH_2CH_2Cl	CH_3	CH_2CH_2Cl	109.5-110	15
34	CH_2CH_2Cl	C_2H_5	CH_2CH_2Cl	112-113	13
35	CH_2CH_2Br	H	CH_2CH_2Br	98-99	37
36	$CH_2CH_2OSO_2CH_3$	H	$CH_2CH_2OSO_2CH_3$	oil	29
37	CH_2CH_2Br	CH_3	CH_2CH_2Cl	97-98	34
38	CH_2CH_2Cl	CH_3	CH_2CH_2Br	101-102	39
39	CH_2CH_2Cl	CH_3	CH_2CH_2I	96-97	5[c]
40	CH_2CH_2Cl	H	$CH_2CH_2OSO_2CH_3$	oil	29
41	CH_2CH_2Cl	CH_3	$CH_2CH_2OSO_2CH_3$	119-121	42
42	CH_2CH_2Cl	C_2H_5	$CH_2CH_2OSO_2CH_3$	oil	30
43	CH_2CH_2Cl	CH_3	$CH_2CH_2OSO_2C_2H_5$	103-105	30
44	$CH_2CH_2OSO_2CH_3$	CH_3	CH_2CH_2Cl	oil	32
45	$CH_2CH_2OSO_2CH_3$	C_2H_5	CH_2CH_2Cl	oil	28
46	CH_2CH_2Br	CH_3	$CH_2CH_2OSO_2CH_3$	108-109	27

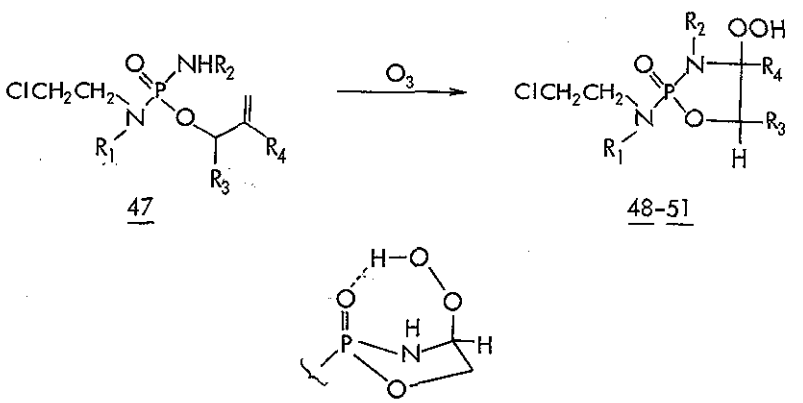
[a] Determined in an open glass capillary and uncorrected. All of the crystalline compounds melted with violent decomposition. [b] Yield of the isolated product after purification by recrystallization or column chromatography. [c] The low yield is due to considerable instability of the product.

IV. Syntheses of the Active Cyclophosphamide Analogues Having Different Ring Systems

The ozonolytic cyclization reaction can further be applied to the syntheses of activated cyclophosphamide analogues having different kinds of phosphorus-containing heterocyclic systems such as the C_4-functionalized 1,3,2-oxazaphospholidine 2-oxide, 1,3,2-diazaphosphorinane 2-oxide, 1,3,4,2-oxadiazaphosphorinane 2-oxide, perhydro-1,3,4,2-oxadiazaphosphepine 2-oxide and some related ring sytems. Unfortunately, no effective compound could be found among these ring-modified cyclophosphamide derivatives.

i) 1,3,2-Oxazaphospholidine 2-oxide

O-(2-Propenyl)phosphorodiamidates (47) can be obtained in a manner similar to the O-butenyl analogues (25) by reaction of phosphoryl chloride with 2-propenols and amines, and the ozonolysis of 47 gives the corresponding five-membered hydroperoxides 48-51 (Table II).[34,44]



47 48-51

52

Chart 7

Table II. 4-Hydroperoxy-1,3,2-oxazaphospholidine 2-oxides (48-51)

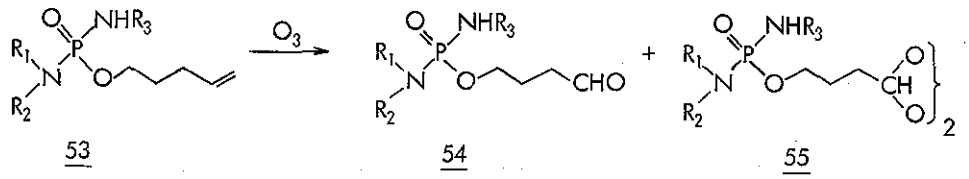
Compound	R_1	R_2	R_3	R_4	Mp (°C)[a]	Yield (%)[b]
48	CH_2CH_2Cl	H	H	H	135-135.5	33
49	CH_2CH_2Cl	H	CH_3	H	124-126	28
50	CH_2CH_2Cl	H	H	CH_3	oil	3
51	H	CH_2CH_2Cl	H	H	oil	25

[a] Determined in an open glass capillary and uncorrected.

[b] Isolated yield of the pure product.

Surprisingly, these five-membered hydroperoxides are more stable than the six-membered ones. For example, compound 48 remained essentially unchanged for over two years on standing at room temperature, whereas 4-hydroperoxycyclophosphamide completely decomposed under such conditions. This contradicts the general concept[43] that five-membered phosphorus-containing heterocycles are more labile than the corresponding six-membered analogues. This is probably because the molecular structure of the five-membered hydroperoxide is more effectively stabilized by the intramolecular hydrogen bond between the C_4-OOH and P=O group as shown by 52 than that of the six-membered hydroperoxide. However, C_4-hydroxy derivative generated by deoxygenation of 48 with triethylphosphite was, in contrast, very unstable, and could not be isolated and characterized.

Attempts to obtain the seven-membered hydroperoxides by ozonolysis of O-(4-pentenyl)phosphorodiamidates (53) resulted



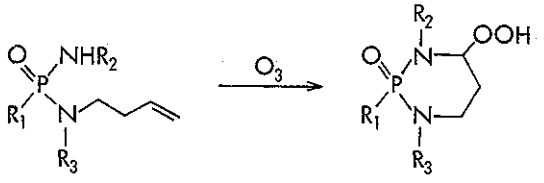
53 54 55

Chart 8

in isolation of stable aldehydes 54 besides dimeric diperoxides 55 which might be produced by dimerization reaction of the zwitterion intermediate.[44]

ii) 1,3,2-Diazaphosphorinane 2-oxide

4-Hydroperoxy-1,3,2-diazaphosphorinane 2-oxides (57-60) are similarly obtainable by the ozonolytic cyclization reaction of N-(3-butenyl)phosphoramidates (56) (Table III).[45] In contrast to the cases of 4-hydroperoxy-1,3,2-oxazaphosphorinane 2-oxides, these products were isolated as an oily mixture of cis- and trans-isomers with predominance of the latter isomer. In the case of 60, the two isomers could be separated. The predominant formation of the trans-isomer is possibly due to unfavorable steric interactions between the phosphorus and the N_1,N_3 substituents in the cis-isomer,



56 57-60

Chart 9

Table III. 4-Hydroperoxy-1,3,2-diazaphosphorinane 2-oxides (57-60)

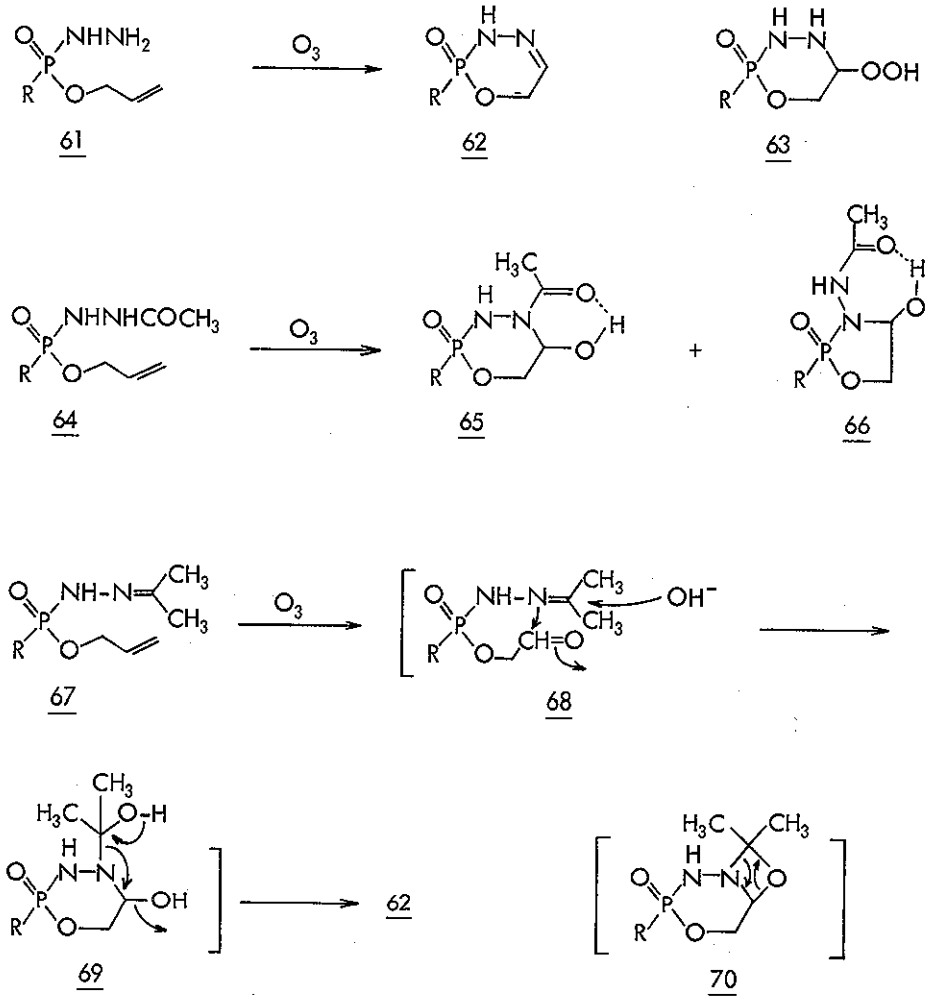
Compound	R_1	R_2	R_3	Yield (%)[a]
57	$(ClCH_2CH_2)_2N$	CH_2CH_2Cl	H	28
58	$(ClCH_2CH_2)_2N$	CH_2CH_2Cl	CH_2CH_2Cl	24
59	$ClCH_2CH_2O$	CH_2CH_2Cl	CH_2CH_2Cl	34
60	C_6H_5O	CH_2CH_2Cl	CH_2CH_2Cl	56[b]

[a] Isolated yield of the pure mixture of cis- and trans-isomers after column chromatography. [b] Cis-isomer: 24%; trans-isomer: 32%.

which might result in decrease of its thermodynamic stability (see Section V-iii).

iii) 1,3,4,2-Oxadiazaphosphorinane 2-oxide, Perhydro-1,3,4,2-oxadiazaphosphepine 2-oxide and Some Related Heterocyclic Systems

In the case of the ozonolysis of O-alkenylphosphoramidoyl hydrazides, the aldehyde fragment produced by cleavage of the primary ozonide undergoes cyclization reaction, giving a stable aldehyde derivative instead of cyclic hydroperoxide. For example, the ozonolysis of O-(2-propenyl)phosphoramidoyl hydrazide (61) gave cyclic hydrazone 62, but another possible product 63 could not be isolated.[46] The ozonolysis of an acetyl hydrazide 64 also gave the aldehyde derivative as a 1:2 mixture of 65 and 66 which are possibly stabilized by the hydrogen bond between OH and C=O groups. Interestingly,

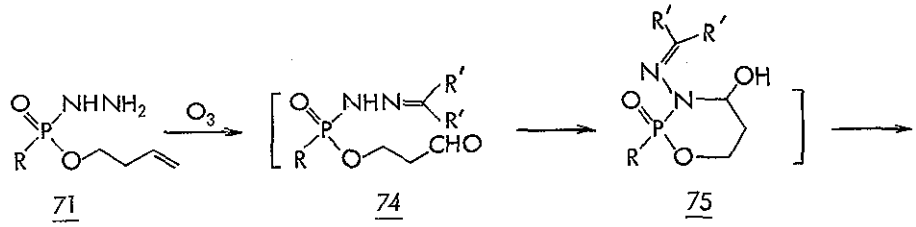


[R = $(ClCH_2CH_2)_2N$]

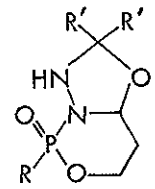
Chart 10

ozonolysis of the acetone hydrazone 67 yielded 62, involving intramolecular "carbonyl-exchange" reaction of the aldehyde intermediate 68.[46] The exchange reaction provably proceeds via an intermediate 70, or more likely via 69.

The ozonolysis behavior of O-(3-butenyl)phosphoramidoyl hydrazide (71) is somewhat different from that of 61, giving a bicyclic product 72 instead of the corresponding cyclic hydrazone. In this reaction, formalin generated in the reaction medium was incorporated into the aldehyde fragment to give a hydrazone intermediate 74 (R′ = H) from which 72 might be produced via 75 (R′ = H). When acetone was present in the

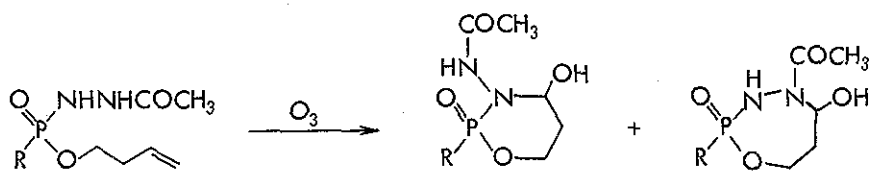


71 74 75



72 R′ = H
73 R′ = CH3

[R = (ClCH2CH2)2N]



76 77 78

Chart 11

ozonolysis medium, another bicyclic product 73 was obtained instead. This product 73 was obtainable in a better yield by the ozonolysis of an acetone hydrazone of 71. Formation of the bicyclic products 72 and 73 from the hydrazone intermediate 74 (R' = H or CH_3) probably competes with the "carbonyl-exchange" reaction as found for 67, but in this case the formation of a seven-membered intermediate corresponding to 69 or 70 is perhaps less favorable than that of the six-membered intermediate 75. In the case of the ozonolysis of an acetyl hydrazide 76, a six-membered product 77 was predominantly produced accompanied by a small amount of a seven-membered phosphepine derivative 78.[46]

V. Stereochemistry of the C_4-Functionalized 1,3,2-Oxazaphosphorinane 2-oxide

Cyclophosphamide is a dissymmetric molecule containing a chiral center at the phosphorus atom and was recently shown to be metabolized with highly stereoselective manner in man. Cox et al.[47] have observed that, following the administration of racemic cyclophosphamide to patients, the drug recovered from the urine was optically active (laevorotatory). The optical purity of the recovered (-)-cyclophosphamide was estimated to be 83 to 91% by comparison of its $[\alpha]_D$ value with that of an optically active synthetic specimen[48] of which absolute configuration was most recently determined by X-ray crystallographic analysis.[49] The preferential urinary excretion of the (-)-isomer implies that the (+)-isomer might

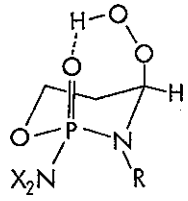
be more efficiently oxidized than the (-)-isomer. Therefore, the stereochemical result of the *in vivo* C_4-hydroxylation which produces another chiral center on the six-membered ring is of particular interest. Although direct studies on the stereochemistry of enzymatically produced active metabolite are difficult because of great instability of the metabolite, investigations of the stereochemical properties, particularly the relative configuration of the C_4-oxygen functionality, of the synthetically produced active species will provide some insights into the stereochemical aspects of the *in vivo* activation of cyclophosphamide.

i) 4-Hydroperoxycyclophosphamide and Related Compounds

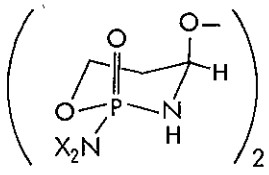
X-Ray crystallographic studies of cyclophosphamide[50,51] and 4-ketocyclophosphamide[52] revealed that the 1,3,2-oxazaphosphorinane ring is in the chair form in which the phosphoryl oxygen is *axial* and the bis(2-chloroethyl)amino group is *equatorial*. The *equatorial* preference of the bis(2-chloroethyl)amino group in cyclophosphamide was also inferred from the solution studies of 2-dialkylamino-1,3,2-dioxaphosphorinane-2-oxides for which the *equatorial* dialkylamino configuration has commonly been proposed as a stable conformation,[53-55] the same phosphorus stereochemistry therefore being also assumed for 4-hydroperoxycyclophosphamide (*16*).

For assignment of the C_4-OOH configuration of *16*, the proton magnetic resonance (pmr) spectroscopy was particularly informative and served to provide strong evidence for its

axial configuration. The 60 MHz pmr spectrum of 16 shows the C_4 proton signals in a well-separated region from those of other protons, allowing unambiguous assignments of its coupling patterns. As given in Table IV, the large vicinal P, H coupling constant [$J(P\text{-}N_3\text{-}C_4\text{-}H)$], as well as the small H, H coupling constant [$\Sigma J(C_4\text{-}H, C_5\text{-}H)$] evidently indicates an equatorial configuration of the C_4 proton. The $J(P\text{-}N_3\text{-}C_4\text{-}H)$ value agrees well with that of an equatorial C_4 proton of some 2-dialkylamino-1,3,2-dioxaphosphorinane 2-oxides (79) with a stable chair conformation,[53,55] suggesting that there might be a similar Karplus-type relationship in the P-N-C-H and P-O-C-H vicinal coupling constants. The cis-diaxial stereochemistry of the C_4-OOH and P=O groups is therefore predictable for 16, which was ultimately verified by X-ray analysis by Camerman et al.[56] It is notable that the

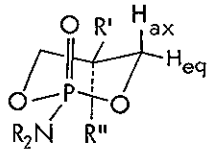


16 R = H
29 R = CH_3
30 R = $CH_2CH_2CH_3$
31 R = CH_2CH_2Cl



17

(X = CH_2CH_2Cl)



79

Chart 12

ozonolytic cyclization reaction, and also the direct oxidations of cyclophosphamide, selectively gives a product having such a stereochemistry. This is possibly due to greater thermodynamic stability of this conformation in which the unfavorable 1,3-diaxial interactions between the C_4-OOH and P=O groups are cancelled by formation of a possible intramolecular hydrogen bond (see Section V-ii). In addition, the axial preference of the C_4-OOH group is attributed to an anomeric effect.

Compared with the $J(P\text{-}N_3\text{-}C_4\text{-}H)$ value of 16, the J value for 4-peroxycyclophosphamide (17) is greater (Table IV). X-Ray analysis of 17 confirmed the same cis-diaxial relation-

Table IV. 60 MHz Pmr Data for the C_4 Proton of 4-Hydroperoxycyclophosphamide Derivatives[a]

Compound	$\delta(C_4\text{-}H)$ ppm	$J(P\text{-}N_3\text{-}C_4\text{-}H)$ Hz	$\Sigma J(C_4\text{-}H, C_5\text{-}H)$[b]
16	4.90	24.5	5.7
17	5.22	28.0	7.0
29	4.82	20.6	6.0
30	4.92	21.4	5.3
31	5.00	19.8	5.0

[a] Determined in d_6-DMSO solution at 38° with TMS as an internal standard. [b] The splitting pattern of the C_4 proton with the C_5 protons is generally observed as a triplet (or double doublet) and the $\Sigma J(C_4\text{-}H, C_5\text{-}H)$ value means the width of the triplet (or double doublet); this is also the case for Tables V, VI.

ship of the C_4-oxygen and P=O groups as in 16[37] and revealed the presence of intramolecular cross-linking hydrogen bonds between the ring NH protons and the P=O groups as shown in Chart 3. Such hydrogen bonds will stabilize its molecular conformation, which will be preferred also in solution and result in increase of the π-bond character of the P-N_3 bond, this being a reason for the greater J(P-N_3-C_4-H) value. In contrast to 17, N-substituted 4-hydroperoxycyclophosphamides (29-31) show a smaller P, H coupling constant than 16. It is likely that this is due to the eclipsed interactions between the ring-nitrogen substituent with the equatorial bis(2-chloroethyl)amino group, which results in an increased population of other conformations free from such unfavored interactions.

ii) 4-Hydroperoxyisophosphamide and Related Compounds

In the ozonolytic cyclization reaction producing 4-hydroperoxyisophosphamide (32), a small amount of stereoisomer 80 was isolated besides the major product 32.[57] Interestingly, 32 and 80 were interconvertible in the presence of a catalytic amount of TsOH in chloroform at room temperature, giving a 1:1 equilibrium mixture without producing any dimeric compound as found for 4-hydroperoxycyclophosphamide (see Section II). On treatment with ferrous sulfate, 32 and 80 quantitatively gave the same lactam 81, while treatment with triethylphosphite converted them into the corresponding 4-hydroxy derivatives 82 and 83. The pmr spectra of 32 and 80 show a J(P-N_3-C_4-H) value similar to those of N-substituted 4-hydroperoxycyclo-

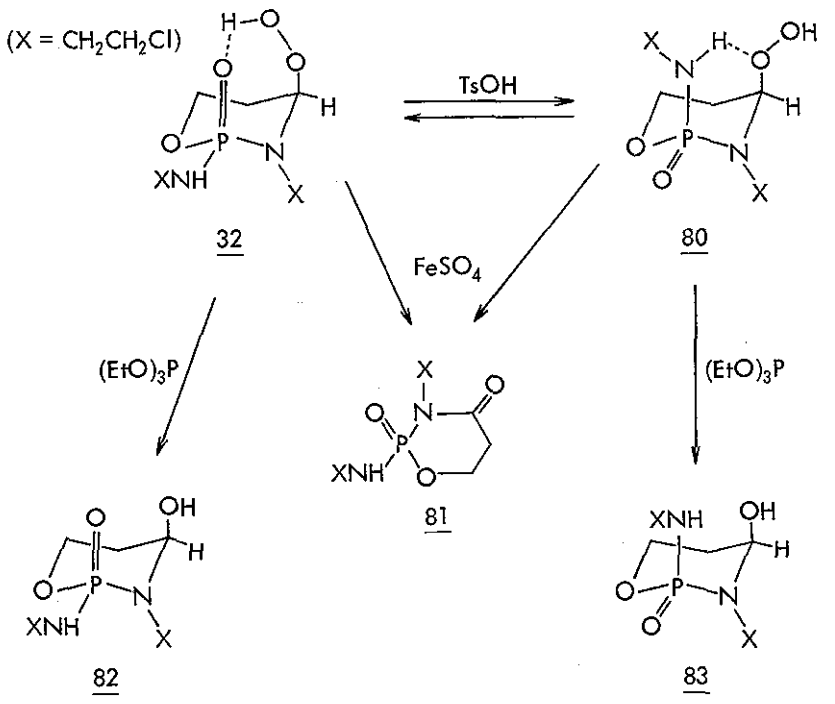


Chart 13

phosphamide derivatives, suggesting again an axial configuration of their C_4-OOH group (Table V). Comparative ir experiments confirmed the presence of intramolecular hydrogen bonds between the P=O and C_4-OOH groups in 32 and their absence in 80.[58] In addition, the ir experiments also suggested that 80 partly existed as a structure in which the exocyclic NH proton was hydrogen bonded with the C_4 oxygen because the absorption intensity due to the free NH band was significantly smaller for 80 than for 32. Molecular weight measurements in chloroform suggested that there was a partial

molecular association in 80 but 32 mainly existed as a monomeric form. Compound 80 is less stable but more soluble in water than 32. These different properties suggest that the C_4-OOH and P=O groups are masked in 32 but free in 80 as shown in Chart 13. The inverted phosphorus configurations of these compounds were substantiated by measurements of the dipole moment which was greater for 80 than for 32,[59] and also by their ^{31}P-nmr chemical shift, determined in d_4-methanol with H_3PO_4 as an external reference, which was shifted to lower field for 32 ($\delta^{31}P$ = 9.75 ppm) than for 80 ($\delta^{31}P$ = 9.46 ppm).[58] The ^{31}P chemical shift difference is similar to that between the cis- and trans-isomers of 2-dimethylamino-5-t-butyl-1,3,2-dioxaphosphorinane 2-oxide.[55] Stereochemistry of 32 and 80 was definitively confirmed by X-ray analyses by Camerman et al.[60] Interestingly, 80 showed biologic activities almost equal to those of 32, indicating that the inverted phosphorus configuration is also effective in promoting the antitumor activity.[57]

These isomeric hydroperoxides 32 and 80 showed different behavior on alkali treatment. On treatment with an aqueous alkali, 32 quantitatively gave a bicyclic product 84 which was in 1:1 equilibrium with an isomer 85 in the presence of TsOH. Interestingly, alkali treatment of 80 gave 85. It is apparent from the pmr data listed in Table V that the configuration of the C_4 proton of 84 is the same as that of 32 but it is inverted to the axial form in 85. 6-Methyl analogue 86 showed behavior similar to 32 on alkali treatment, giving

Table V. 60 MHz Pmr Data for the C_4 Proton of 4-Hydroperoxyisophosphamide Derivatives[a]

Compd	Solvent	$\delta(C_4\text{-H})$ ppm	$J(P\text{-}N_3\text{-}C_4\text{-H})$ Hz	$\Sigma J(C_4\text{-H}, C_5\text{-H})$ Hz
32	d_6-DMSO	4.96	19.5	6.0
80	d_6-DMSO	5.02	18.0	8.1
	$CDCl_3$	5.09	20.3	7.0
84	d_6-DMSO	5.34	18.6	9.3
	$CDCl_3$	5.38	22.5	7.0
85	d_6-DMSO	5.36	5.0	15.5
	$CDCl_3$	5.48	5.7	14.0
86	d_6-DMSO	4.92	20.1	5.2
	$CDCl_3$	4.97	22.5	5.6
87	$CDCl_3$	5.33	23.4	6.2
88	$CDCl_3$	5.44	5.0	14.9
89	$CDCl_3$	5.03	21.0	5.4
90	$CDCl_3$	5.38	14.0	8.3
91	$CDCl_3$	5.35	4.8	15.0

[a] Determined at 38° with TMS as an internal standard.

87 which was in equilibrium with 88 by the action of TsOH. On the contrary, alkali treatment of its stereoisomer 89 gave 90 which was not identical with 88 and gave an equilibrium mixture with another isomer 91 in the presence of TsOH. It seems strange that the alkali treatment of 80 gave a bicyclic product 85 having an axial configuration of the C_4 proton. It is likely that 80 first gives a bicyclic product corresponding to 90 which might be in conformational equilibrium with 85' (=85) and the latter conformation might

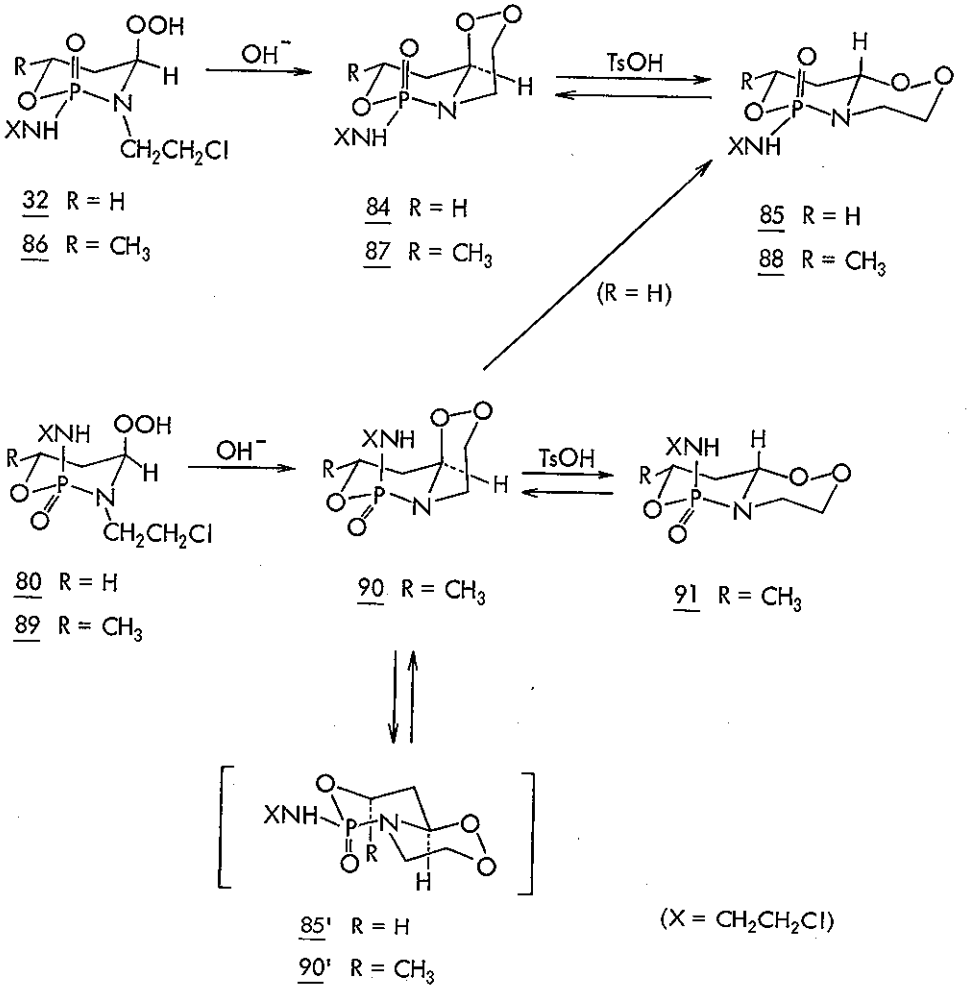


Chart 14

be more preferred because of its stable equatorial configuration of the phosphorus 2-chloroethylamino substituent. In the case of 6-methyl derivative 90, on the other hand, the conformation 90' is less favored than 85' because of axial orientation of its 6-methyl group, therefore

both conformations 90 and 90' exist with comparable populations, a situation which accounts for its J(P-N_3-C_4-H) value intermediate between that of the axial and equatorial C_4 protons (Table V).

In an earlier paper,[58] the conformation of some 4-hydroperoxyisophosphamide derivatives was suggested to be rigid based on the fact that the J(P-N_3-C_4-H) values are virtually unchanged on variable-temperature pmr measurements at -53° to 72°. This conclusion however appears questionable because their ^{13}C-nmr signals became broad below -110°.[61] Egan et al.[62] recently reported that the six-membered ring of cyclophosphamide underwent rapid conformational interconversions at room temperature. Therefore it seems more reasonable to consider that the six-membered ring of 4-hydroperoxyisophosphamide and its derivatives is also under such a rapid conformational equilibrium, giving the time-averaged pmr data. However, the apparent difference in the J(P-N_3-C_4-H) value between 84 and 85, for example, suggests that the populations of the conformations with equatorial and axial C_4-protons are respectively predominant, even though they undergo rapid conformational interconversions. This probably is also the case for 4-hydroperoxycyclophosphamide derivatives (vide supra).

iii) 4-Hydroperoxy-1,3,2-diazaphosphorinane 2-oxides

The ozonolysis synthesis of 4-hydroperoxy-1,3,2-diazaphosphorinane 2-oxides generally resulted in formation of a stereoisomeric mixture which could be separated in the case

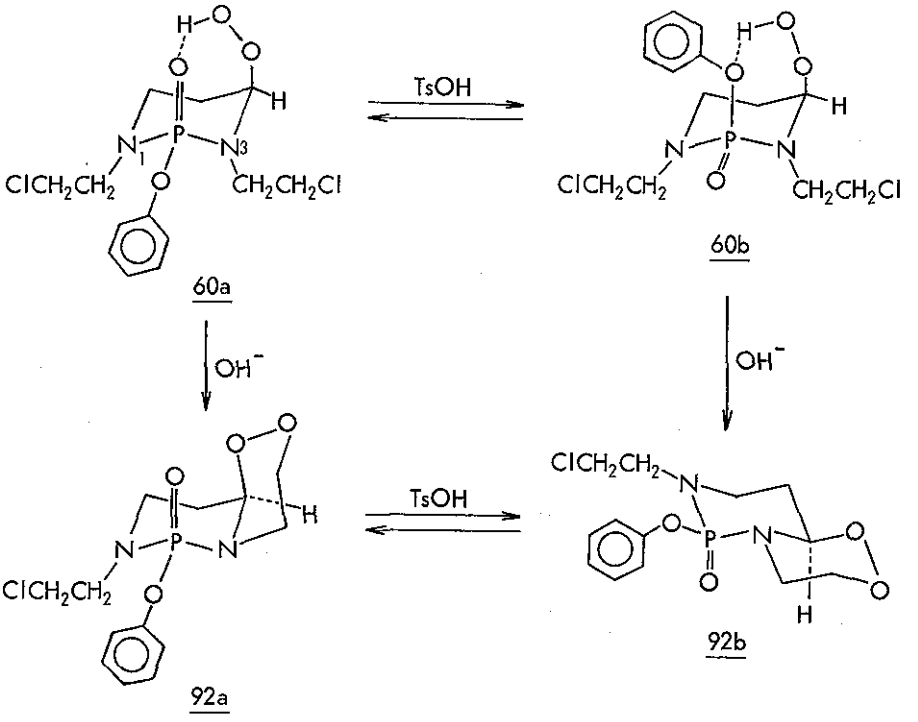


Chart 15

of the 2-phenoxy derivative 60 (see Section IV-ii). Interestingly, in this case, the trans-isomer 60b was more abundantly produced than the cis-isomer 60a. These isomers also gave the TsOH-catalyzed equilibrium mixture with a predominance of the trans-isomer (60a/60b = 1/2). 60a and 60b behaved similarly to 32 and 80, respectively on alkali treatment, giving the corresponding bicyclic peroxides 92a and 92b which were also interconverted by the action of TsOH. Although the cis-diaxial configuration of the C_4-OOH and P=O groups in 60a can be stabilized for the same reasons proposed for 4-hydroperoxycyclophosphamide (see Section V-i), unfavored steric

interactions exist between the equatorial phenoxy group and the N_1 and N_3 substituents and such interactions become significant when the ring nitrogen atoms have the sp^2-like geometry found for isophosphamide[63] and 4-hydroperoxyisophosphamide.[60] The smaller $J(P-N_3-C_4-H)$ value of 60a is probably due to the increased population of other conformations free from the unfavored eclipsed interactions (Table VI). On the other hand, 60b is free from such interactions and, in addition, the cis-diaxial configuration of the C_4-OOH and phenoxy group can be stabilized by the possible hydrogen bond, this being responsible for the abundant formation of this isomer.

Table VI. 60 MHz Pmr Data for the C_4 Proton of 4-Hydroperoxy-1,3,2-diazaphosphorinane 2-oxides[a]

Compd	$\delta(C_4-H)$ ppm	$J(P-N_3-C_4-H)$ Hz	$\Sigma J(C_4-H, C_5-H)$ Hz
60a	5.12	17.7	7.6
60b	5.13	21.9	8.0
92a	5.36	22.8	9.4
92b	5.31	5.2	11.8

[a] Determined at 38° in $CDCl_3$ solution with TMS as an internal standard.

VI. Mechanism of the TsOH-Catalyzed Stereoisomerization Reaction of 4-Hydroperoxyisophosphamide and Related Compounds

The TsOH-catalyzed stereoisomerization reaction of

4-hydroperoxyisophosphamide is of particular interest because it gives an isomer with inverted phosphorus configuration and also because the product has a high antitumor activity. Other potentially active hydroperoxide analogues 33, 41 and 31 could also be equilibrated with the corresponding stereoisomers 93-95 in the presence of TsOH. Although these isomers could not be separated, the equilibrium ratios were estimated by converting into the readily separable bicyclic peroxides corresponding to 84 and 85. As shown in Chart 16, the cis/trans equilibrium ratios suggest that the 1,3-diaxial interactions between the C_4-OOH and the phosphorus substituent in the trans-isomer are more significant than the eclipsed interactions between the N_3 and phosphorus substituents in the cis-isomer when the phosphorus substituent becomes bulky. However, it is uncertain whether the preferred conformation of 93-95 is the same as that of 80 because no distinctive pmr data could be determined for them.

When 32 (or 80) was treated with TsOH in the presence of a nucleophilic reagent, for example, such as methanol or thiophenol, a mixture of the corresponding cis and trans C_4-substituted derivatives 96a, b and 97a, b was obtained in good yield. The pmr spectra of these adducts indicate that the C_4 substituent (R) is axial again for both cis and trans adducts. It is most likely that an immonium ion intermediate 98 is produced in these reactions and the stereoisomerization reaction of 32 can also be interpreted by such an intermediate as follows. The resulting counter anion

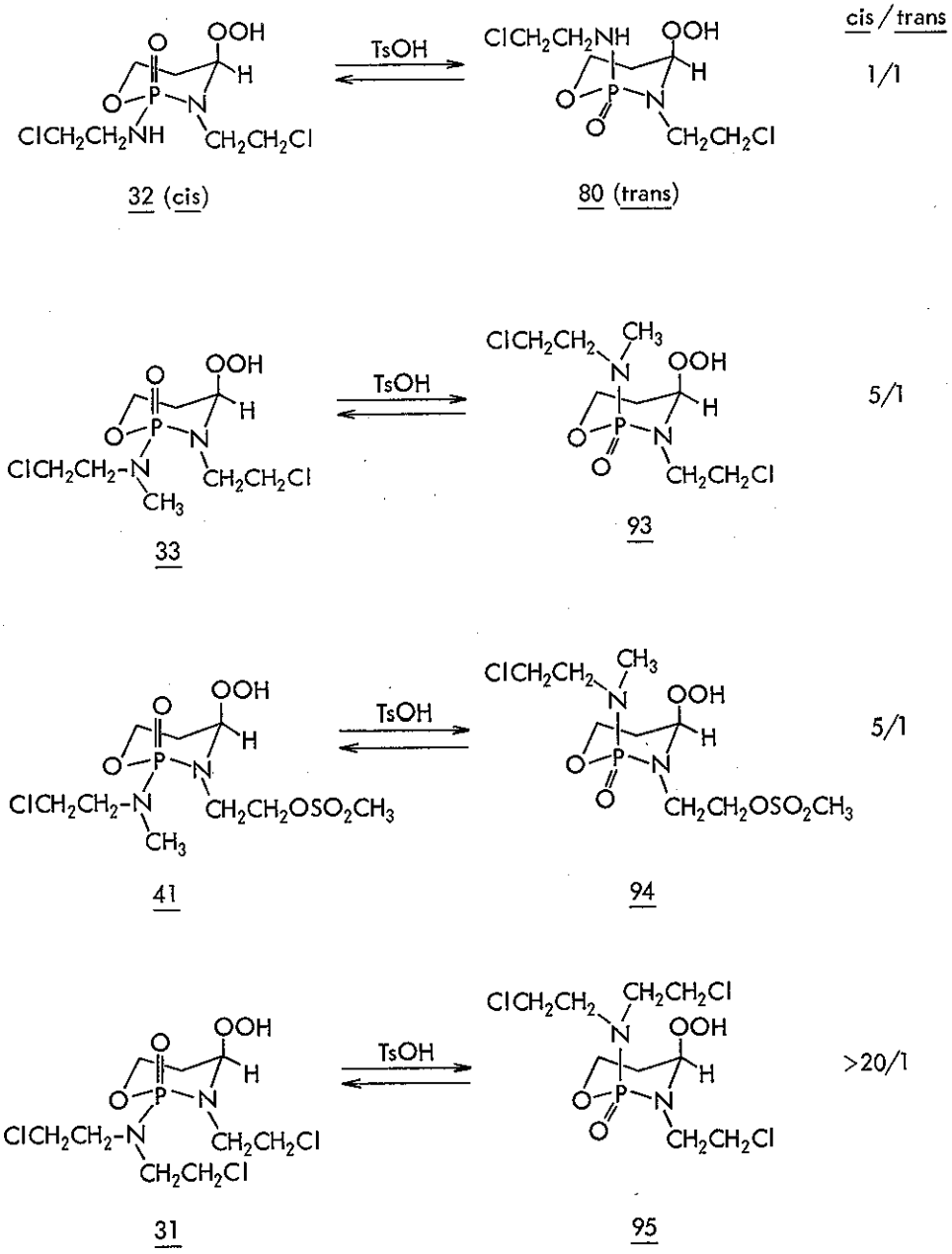

cis / trans
TsOH
1/1
32 (cis)
80 (trans)
TsOH
5/1
33
93
TsOH
5/1
41
94
TsOH
>20/1
31
95

Chart 16

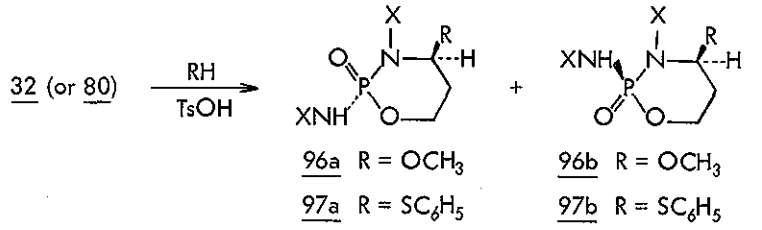


Chart 17

(OOH^-), which is fully separated from 98, will reattack from cis and trans directions to the P=O group. In both cases, the entering hydroperoxy group will preferably be disposed to an axial orientation because of an anomeric effect, the cis attack regenerating 32 and the trans attack giving 99 which is just a mirror image of the trans isomer 80. Therefore by this mechanism, the position actually undergoing inversion of the configuration is not the phosphorus atom but the C_4 position. Although this mechanism remains tentative until experiments using optically active compounds are done, the TsOH-catalyzed isomerization reactions of the bicyclic peroxides shown in Chart 14 can be similarly explained by

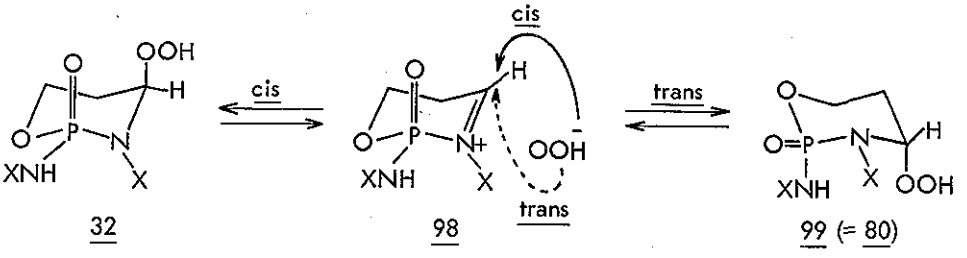


Chart 18

assuming the existence of the corresponding immonium ion intermediate. In the case of the TsOH-catalyzed dimerization reaction of 4-hydroperoxycyclophosphamide, a similar intermediate corresponding to 98 will also be produced, but in this case the reattack of hydroperoxide anion competes with the attack of another molecule of 4-hydroperoxycyclophosphamide and the thermodynamically stabilized dimer 17 is thought to be preferentially obtained from such a competitive equilibrium system. The stereoisomerization reaction of 32 (or 80) would also be in competition with the dimerization reaction, but the presence of the ring N_3 substituent prevents the stabilization of the dimer, a situation which suggests that the cross-linking hydrogen bonds between the P=O and ring NH protons may be important for stabilizing the dimer produced from 4-hydroperoxycyclophosphamide.

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