# STEREOCHEMISTRY OF 4-HYDROPEROXYISOPHOSPHAMIDE, A POTENTIALLY ACTIVE ANTITUMOR ALKYLATING AGENT

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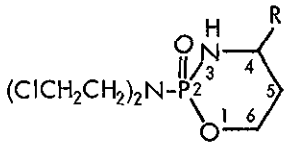
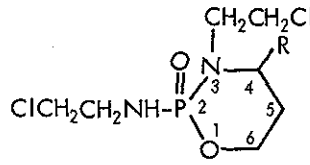
An acid-catalyzed isomerization of 4-hydroperoxyisophosphamide, an antitumor alkylating agent having the 1,3,2-oxazaphosphorinane ring, gave an epimer with inversion of phosphorus configuration. The stereochemistry of the epimer was elucidated and found to be effective in promoting the antitumor activity.

Cyclophosphamide (CP) (1) and isophosphamide (IP) (2) are antitumor alkylating agents having the 1,3,2-oxazaphosphorinane ring and known to be activated in vivo to a cytotoxic species after enzymatic $C_4$-hydroxylation of the ring.[1] Recently, we[2] have synthesized the pre-activated analogues (3-6) of these drugs. The stereochemical aspects of the $C_4$-oxygen functionality and the alkylating group at phosphorus in them are a matter of considerable significance with respect to the structure-activity relationships, but little has been known about their stereochemistry until recent X-ray studies on 5[3] and a Fenton-oxidation product of CP.[4] In the course of studies on the chemistry of $C_4$-functionalized 1,3,2-oxazaphosphorinanes, we found that 4-hydroperoxy IP can

readily be converted into an epimer having an inverted stereochemistry at phosphorus. We now report on the the stereochemistries of these epimeric compounds.



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

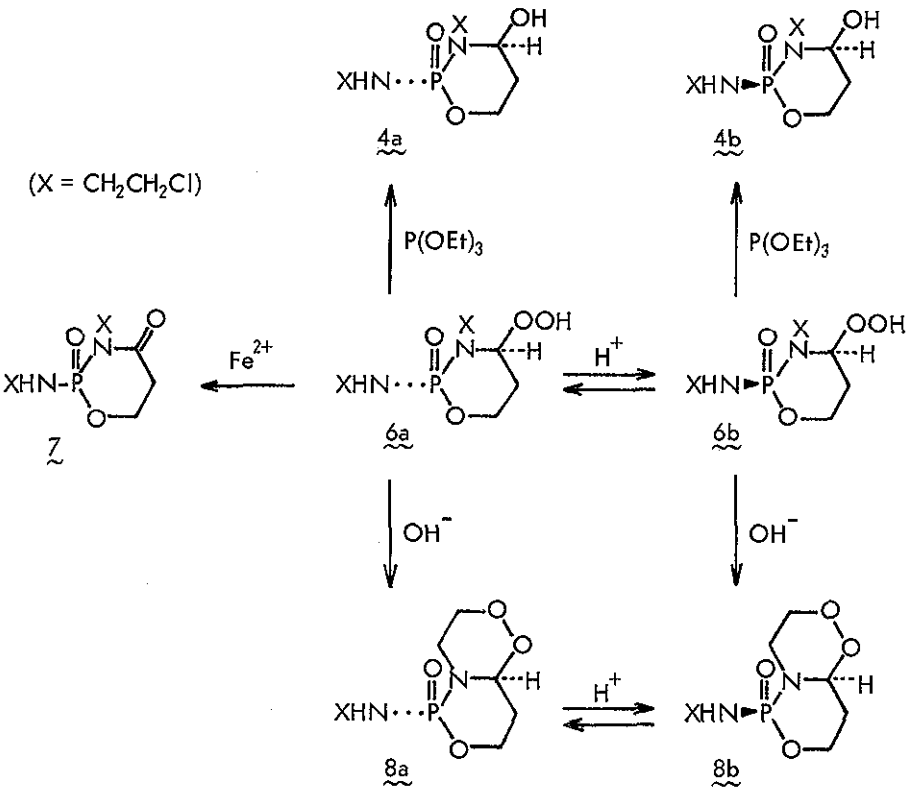


2 R = H
4 R = OH
6 R = OOH

(Chart 1)

In the presence of a catalytic amount of p-toluenesulfonic acid in chloroform at room temperature, 4-hydroperoxy IP (6a) (mp 113-114° (dec)[2b]) gave a 1 : 1 equilibrium mixture with a new isomer 6b (mp 75-76°, dec 112°), which could be separated by column chromatography on silica gel with acetone-chloroform (1 : 2). Chemical properties of the isomer 6b are compared with those of 6a as follows. Both 6a and 6b gave 4-keto IP (7)[5] under the action of ferrous sulfate, while treatment with triethylphosphite converted them into the corresponding 4-hydroxy IP 4a (mp 74-75°[2b]), and 4b (mp 49-50°). Treatment of 6a and 6b with aqueous alkali (1N-KOH) afforded the corresponding bicyclic peroxide 8a (mp 127-129°[2b]), and 8b (mp 103-105°) which were also found to be in equilibrium in the presence of an acid (p-TsOH). All these epimeric compounds were obtained in stereochemically pure state.

In the 60 MHz pmr spectra of these products, signals of $C_4$-proton are well separated from those of other protons and split by couplings with phosphorus and $C_5$-protons (Table I). The large J(P-N-$C_4$-H) values of 6a and 6b, as well as those of other derivatives except 8b, are apparently indicative of an equatorial configuration of the




(Chart 2)

$C_4$-H, which is also predictable from the small couplings between $C_4$-H and $C_5$-H (see $\Sigma J(C_4\text{-H}, C_5\text{-H})$). The pmr data were found to be temperature-independent within the range -53° to 72°, indicating that the phosphorus-containing ring has a stable chair conformation. These pmr results suggest that the isomerization of 6a to 6b proceeds with retention of the $C_4$-configuration, and we believe that it must involve a stereomutation of the P-$NHCH_2CH_2Cl$ group from equatorial to axial for the following reasons. The $^{31}P$ nmr chemical shift, measured in $d_4$-methanol using $H_3PO_4$ as an external reference, is greater for 6a (9.75 ppm) than for 6b (9.46 ppm), which seems to account for the

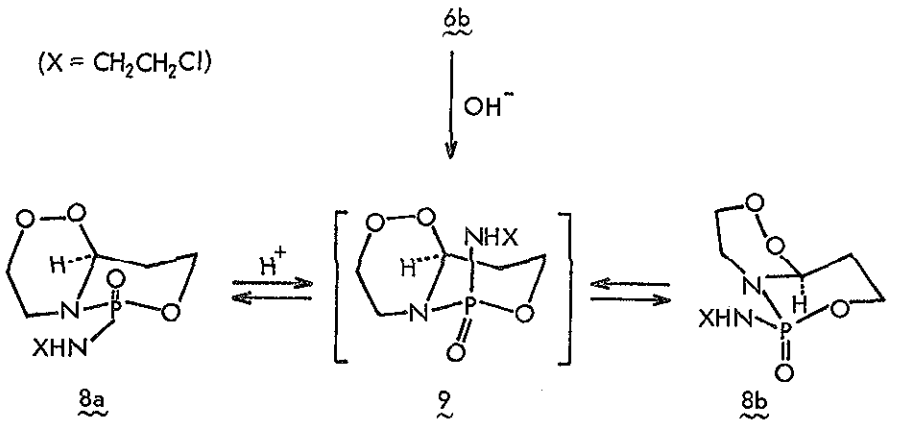
Table I. 60 MHz PMR Data for the $C_4$-Proton of $C_4$-Functionalized Isophosphamide Derivatives

| Compd | Solvent | $\delta(C_4\text{-H})$ (ppm) | Appearance | $J(P\text{-}N\text{-}C_4\text{-H})$ (Hz) | $\Sigma J(C_4\text{-H}, C_5\text{-H})$ (Hz) |
|---|---|---|---|---|---|
| 6a | $d_6$-DMSO | 4.96[a] | d of t[b] | 19.5 | 6.0 |
| 6b | $d_6$-DMSO | 5.02 | d of t[b] | 18.0 | 8.1 |
| 4a | $D_2O$ | 5.05[a] | d of t | 18.0 | 7.0 |
| 4b | $D_2O$ | 5.08 | d of t | 18.4 | 7.2 |
| 8a | $d_6$-DMSO | 5.34[a] | d of t[b] | 18.6 | 9.3 |
| 8b | $d_6$-DMSO | 5.36 | d of dd[b] | 5.0 | 15.5 |

[a] Ref 2b. [b] After addition of $D_2O$.

change in configuration at phosphorus.[6] 6a shows a lower solubility in water than 6b (*6a = 4 mg/ml, 6b = 25 mg/ml at room temperature*), suggesting that the hydrophilic groups (P=O and $C_4$-OOH) in 6a are cis-diaxial and masked by a possible intramolecular hydrogen-bonding. The ir spectrum of 6a in a dilute chloroform solution shows bands at 3539 $cm^{-1}$ (νOH (free), $\epsilon = 25.0$) and 3412 $cm^{-1}$ (νNH, $\epsilon = 81.4$) besides a broad band at 3150 $cm^{-1}$ attributable to a hydrogen-bonded νOH, while 6b shows a νOH (free) band at 3536 $cm^{-1}$ with a greater intensity ($\epsilon = 77.5$) and a νNH band at 3412 $cm^{-1}$ ($\epsilon = 71.0$), clearly supporting the suggested hydrogen-bonding in 6a and its absence in 6b. Thus the stereochemistries of 6a and 6b could be assigned as shown in Chart 2, which have been confirmed by X-ray analyses.[7] As is apparent in Table I, the pmr data of 8b are greatly different from those of other compounds and *both of the $J(P\text{-}N\text{-}C_4\text{-H})$ and $\Sigma J(C_4\text{-H}, C_5\text{-H})$ values are indicative of an axial* configuration of its $C_4$-H. We consider that the formation of 8b is best rationalized by

assuming a common intermediate (9) which turns into a stable conformer 8b, both in the acid-catalyzed isomerization of 8a and the alkali treatment of 6b.




(Chart 3)

In the preliminary bioassay experiments, 2-epi-4-hydroperoxy IP (6b) showed higher cytotoxicity against the cultured L1210 cells than 6a, and its in vivo antitumor activity against some kinds of animal tumors was found to be comparable or slightly superior to that of 6a. This suggests that the inverted stereochemistry of the alkylating group at phosphorus is also effective in promoting the antitumor activity as an active species of IP. Further studies on the stereoisomerization of $C_4$-functionalized 1,3,2-oxazaphosphorinanes including the pre-activated species of CP are in progress.

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