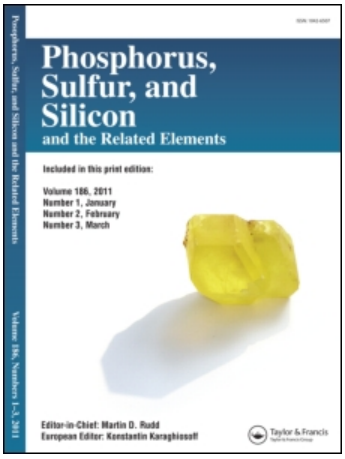

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ACID HYDROLYSIS OF AZIRIDINYL PHOSPHORAMIDES

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ACID HYDROLYSIS OF AZIRIDINYL PHOSPHORAMIDES

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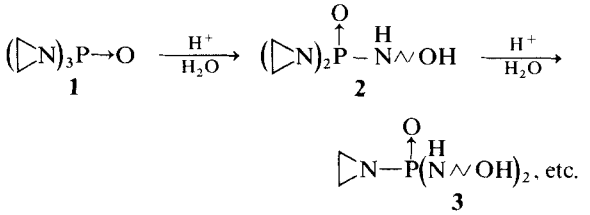
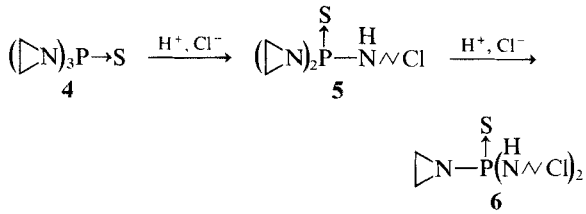
The acid hydrolysis of two model aziridinyl phosphoramides, 1-aziridinylbis(dimethylamino)phosphine oxide (**11**) and bis(1-aziridinyl)(dimethylamino)phosphine oxide (**16**), in aqueous acetic acid proceeded by an initial aziridine ring opening to give 2-hydroxyethyl phosphoramides **12** and **17**, respectively. These intermediates rapidly cyclized to an oxazaphospholidine ring structure **13** or **18**, with the subsequent loss of dimethylamine or aziridine. Analysis of ongoing hydrolysis showed that **13** underwent cleavage of the ring P—N bond to give **14**, which hydrolyzed further to yield **15** (2-aminoethyl dihydrogen phosphate) as a final product. Nuclear magnetic resonance (NMR), high-pressure liquid chromatography (HPLC), thin-layer chromatography (TLC), and infrared data to support this hydrolytic pathway for **11** and **16** are presented and discussed. NMR and HPLC data for the observed hydrolysis of tepa (tris(1-aziridinyl)phosphine oxide, **1**) are also presented and suggest that an analogous reaction mechanism occurs in the acid hydrolysis of tepa and related compounds.

INTRODUCTION

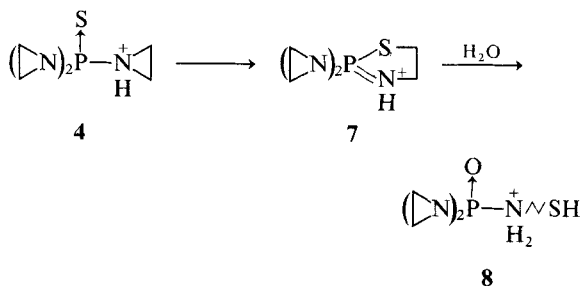
Numerous aziridinyl derivatives of phosphoric and phosphorothioic acids have been investigated as potential cancer chemotherapeutic agents[1] and insect chemosterilants.[2] Because their biological activity depends primarily on their capacity to function as alkylating agents, and because this activity is invariably exerted in aqueous media, the hydrolytic stability of the aziridine moiety and of its attachment to the PO or PS group deserves special attention. The best known representatives of these two classes of compounds are tepa (tris(1-aziridinyl)phosphine oxide, (**1**) and thiotepa (tris(1-aziridinyl)phosphine sulfide, **4**). Beroza and Borkovec[3] investigated the degradation of tepa in acidic and neutral phosphate-buffered solutions and showed that free aziridine (ethylenimine) was generated as a result of the P—N bond cleavage. However, other degradation products were also proposed, and the authors suggested the following generalized progression



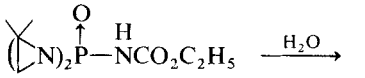
that ultimately would lead to phosphoric acid and ethanolamine; but no specific compounds were isolated and identified. Similar ring-opening reactions were claimed for thiotepa[4] although, because the hydrolysis was conducted with HCl in a saline solution, the products were 2-chloroethyl rather than 2-hydroxyethyl amides.



Thin layer chromatographic data were presented as evidence for the existence of **5**, but **6** was only suggested as a final product. An alternative pathway for the acid hydrolysis of thiotepa was proposed,[5]



but neither the thiazaphospholidine **7** nor the mercapto compound **8** was isolated. Subsequent study of the thiotepa reaction by NMR analysis[6] gave no support for either alternative, but the NMR data did indicate the presence of a hydrolysis product which still contained one intact aziridinyl group bonded to the phosphorus. Also, the NMR data confirmed the formation of aziridine, whose release during the hydrolysis of tepa had been reported.[3] A different mechanism of hydrolysis was proposed[7] for the substituted aziridinyl compound **9**.



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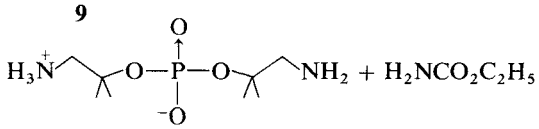

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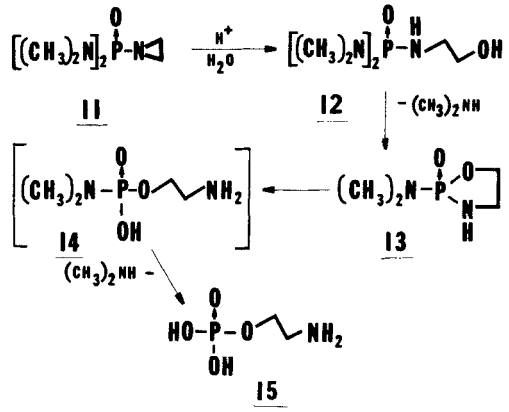
Because the products of this reaction were isolated in nearly quantitative yields, the suggestion that the amides rearranged to esters via a postulated oxazaphospholidine intermediate analogous to **7** appeared reasonable. However, with regard to this analogy, evidence was also presented[7] that the difference between the hydrolytic behaviour of aziridinyl and 2,2-dimethylaziridinyl derivatives is considerable. Therefore, in our study of the hydrolysis of aziridinylphosphine oxides we employed 1-aziridinylbis(dimethylamino)phosphine oxide (**11**) and bis(1-aziridinyl)(dimethylamino) phosphine oxide (**16**) as model compounds, examined their hydrolytic products, and suggested a reaction mechanism that may be applicable to the degradation of tepa and related compounds.

RESULTS AND DISCUSSION

Hydrolysis of 1-Aziridinylbis(dimethylamino) phosphine Oxide (**11**)

When the process was monitored by NMR in a deuterium oxide-acetic acid $-d_4$ mixture, the dimethylamino doublet (δ2.72, J = 10 cps) and the aziridinyl doublet (δ2.19, J = 14 cps) disappeared rapidly, and free dimethylamino (δ2.75) was generated. Furthermore, a new doublet (δ2.76, J = 10 cps) appeared, indicating the formation of an intermediate product with a dimethylamino group still attached to the phosphorus moiety.

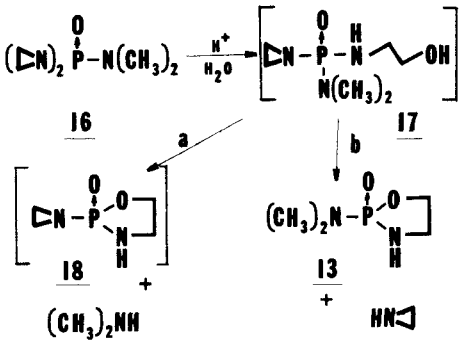


Scheme I

However, as the hydrolysis continued, this new doublet gradually diminished, and the peak for free dimethylamine intensified. When the hydrolysis mixture was analyzed by HPLC and TLC, various products were separated in proportions varying with the duration of hydrolysis. Scheme I shows the likely course of the reaction. Compounds **12** and **13** were identified in the reaction mixture by HPLC, and their identities were confirmed by comparing their retention times with those of synthesized authentic materials. Similarly, the TLC R_f values were in agreement. However, in attempts to isolate **12** and **13** by HPLC fraction collection and subsequent evaporation of the solvent, only **13** was isolated; apparently, **12** either converted to **13** or decomposed during the isolation. Identical isolation techniques with a small synthetic sample of **12** also gave only **13** as a product. The NMR spectrum of **13** showed that it had been responsible for the new doublet (δ2.76, J = 10 cps) observed during the monitoring procedure mentioned earlier. The intermediate **14** could not be isolated or synthesized, but **15** was isolated and identified by comparing its retention time (HPLC) and its infrared spectrum with those of a commercial sample.

Hydrolysis of Bis(1-aziridinyl)(dimethylamino) phosphine Oxide (**16**)

The NMR monitoring of this process indicated that the two alternative pathways **a** and **b** shown in Scheme II occurred simultaneously, but that pathway **a** predominated because more dimethylamine (δ2.75) was formed than aziridine (δ2.77). With the gradual decrease of the doublets for dimethylamino (δ2.80, J = 10 cps) and aziridinyl

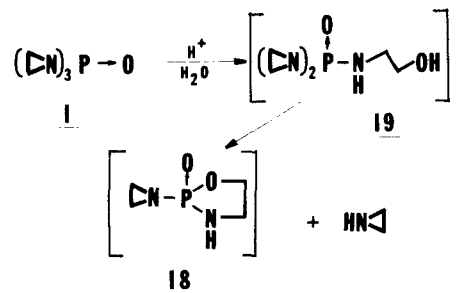


Scheme II

(δ2.21, J = 14 cps) groups pertaining to **16**, a new upfield doublet (δ2.13, J = 14 cps) suggested the presence of **17** or possibly **18**. Because the dimethylamino doublets for **13** and **16** coincide, the reaction mixture was analysed by HPLC, and small amounts of **13** were isolated. Although the relative concentrations of dimethylamine and aziridine indicated that pathway **a** was preferred, **18** could not be isolated and identified directly. However, HPLC analyses of the reaction mixture produced fractions that contained materials different from **17** and aziridine but that possessed strong alkylating ability when tested with the 4-(*p*-nitrobenzyl)pyridine reagent.[8] Probably, these fractions contained **18** or its degradation products.

Hydrolysis of Tepa (**1**)

When tepa was analyzed under the same reaction conditions and with the same techniques used for the previous compounds, the initial sequence of events (Scheme III) was similar to that in the pathways mentioned earlier. The decrease of the aziridinyl doublet (δ2.42, J = 14 cps) was accompanied by the appearance and rise of the aziridine singlet (δ2.77) and a doublet (δ2.30, J = 14 cps) pertaining to a new aziridinyl or bisaziridinylphosphorus compound, possibly **18** or **19**. Although none of these materials could be isolated, a polar fraction different from that containing aziridine was collected by HPLC and did produce a positive alkylation reaction with 4-(*p*-nitrobenzyl)pyridine. Polymerization and mutual interactions of these very reactive intermediates of tepa hydrolysis became apparent in attempts to concentrate and isolate individual compounds, and the progress of the hydrolysis could not be followed in detail. Nevertheless, the initial steps shown in Scheme III are supported by our observations, by analogy with the hydrolytic pathways indicated for the dimethylamino model compounds, and by previous reports of Beroza and Borkovec[3] and Zon *et al.*[6]



Scheme III

EXPERIMENTAL

NMR spectra (deuterium oxide; acetic acid-d_4) were recorded on a Varian Model T-60 spectrometer, with tetramethylsilane as an external standard. Field strength of the various proton resonances are expressed in δ (parts per million); and coupling constants, in cycles per second (cps). Infrared spectra were obtained on a Perkin–Elmer Model 299B grating spectrophotometer. High-pressure liquid chromatography was performed on a Waters Model 202/401 liquid chromatograph equipped with U6K injector and a Waters C_{18} μBondapak column (3.9-mm ID × 30 cm, 10 μ). Samples were eluted with 10% aqueous acetonitrile or 5 and 30% aqueous methanol, and monitored with a Varian Model 631 UV spectrophotometer equipped with 7-μl flow cells at 220 nm and with a Waters Model R-401 Differential Refractometer. Thin-layer chromatography was performed on Quantum Q5 silica gel with methylene chloride: methanol (9:1). Absorbances were measured on a Spectronic 20 at 575 nm. All solvents (including water) were HPLC-grade, and the acetic acid-d_4 was 99.5 atom %D. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. 2-Aminoethyl dihydrogen phosphate (**15**) was purchased from Sigma Chemical. Samples of 1-aziridinylbis(dimethylamino)phosphine oxide (**11**), bis(1-aziridinyl)(dimethylamino)phosphine oxide (**16**), and tepa (**1**) were prepared in our laboratory by standard methods.[9]

Conditions of hydrolysis and NMR monitoring

A sample of an aziridinyl phosphoramide (100 mg) was dissolved in 0.5 ml D_2O and ^{1}H-NMR was recorded. Acetic acid-d_4 (10 or 25 μl) was added, and spectra were recorded at various time intervals during the hydrolysis.

Isolation of 2-aminoethyl dihydrogen phosphate

The hydrolyzed NMR sample of the aziridinyl phosphoramide was lyophilized, and the gummy residue triturated with a small amount of acetonitrile. The solvent was decanted, the insoluble residue was treated with methanol, and the mixture was stirred mechanically for 1 hr. The insoluble solid was then collected, washed with methanol, and dried in vacuo. A small sample was combined with dry KBr, and an infrared spectrum was recorded.

Measurement of chemical alkylating ability

A 1-ml aliquot of various HPLC fractions from ongoing hydrolytic reactions of **1** and **16** was added to 2 ml of 10% 4-(*p*-nitrobenzyl) pyridine in acetone and heated for 15 min in a water bath at 95°C. The mixture was cooled to room temperature, and 4 ml of 3% cyclohexylamine in dimethylformamide was added. The absorbance was measured at 575 nm.

Bis(dimethylamino)[2-hydroxyethyl)amino]phosphine oxide (**12**)

Ethanolamine (122 mg; 2 mmol) in 1 ml of dry dimethoxyethane (DME) was added dropwise to a chilled (−30°C) solution of chlorobis-(dimethylamino)phosphine oxide[10] (171 mg; 1 mmol) in 2 ml of DME. After complete addition, the mixture was allowed to warm to room temperature, then, the DME layer was decanted from the oily ethanolamine HCl and passed through a Sep-pak silica cartridge (Waters Associates). The product was eluted with 5 ml of acetone. Evaporation of the solvent on a rotary evaporator gave compound **12** (100 mg; 51%) as a clear oil. Further purification was not possible, as the material readily rearranged with loss of dimethylamine. NMR [$CDCl_3$] δ 3.66 (*t*, 2, $-CH_2O-$), 3.05 (*m*, 2, $-CH_2N-$), 2.65 (d, 12, $-N(CH_3)_2$), 3.60 (br, 1, —OH) and 2.95 (br, 1, NH). Anal. Calcd for $C_6H_{18}O_2N_3P$: C, 36.92; H, 9.29. Found: C, 36.35; H, 9.30.

2-(Dimethylamino)-3 H-1,3,2-oxazaphospholidine-2-oxide (**13**)

Dichloro(dimethylamino)phosphine oxide[10] (126 mg; 0.85 mmol) in 1 ml of dry DME was added dropwise to a chilled (−30°) solution of ethanolamine (183 mg; 3 mmol) in 2 ml DME. After complete addition, the reaction was allowed to warm to room temperature, and the DME layer was decanted from the oily ethanol-amine-HCl and passed through a Sep-pak silica cartridge with 5 ml acetone. The solvent was removed on a rotary evaporator to yield compound **13** (50 mg; 39%) as a clear oil that crystallized at −20°C; mp 80–82°C. NMR [$CDCl_3$] δ 4.25 (m, 2, $-CH_2O-$), 3.45 (*m*, 2, $-CH_2N-$), 2.70 (*d*, 6, $-N(CH_3)_2$), 3.55 (br, 1, —NH). Anal. Calcd for $C_4H_{11}O_2N_2P$: C, 32.00; H, 7.39. Found: C, 31.76; H, 7.47.

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