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PRELIMINARY COMMUNICATIONS

OBSERVATIONS OF 1,1',1"-PHOSPHINOTHIOYLIDYNETRISAZIRIDINE (THIOTEPA) IN ACIDIC AND SALINE MEDIA. A ^{1}H-NMR STUDY

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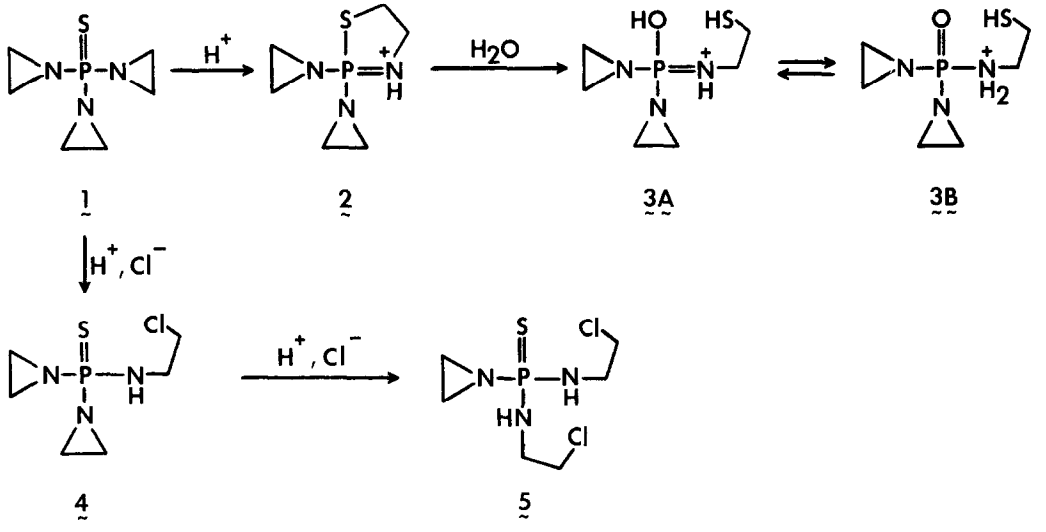
Much of the widespread interest in 1,1',1"-phosphinothioylidynetrisaziridine (thiotepa, 1) derives from its utility as an anticancer agent[1,2] and chemosterilant.[3] Following earlier studies on the acid sensitivity[4] of thiotepa and possible pharmacological effects[5] thereof, Benckhuijsen[6] investigated the behavior of thiotepa under strongly acidic conditions (pH 1.1). It was thus claimed,[6] on the basis of colorimetric assay data for sulfhydryl[7] and alkylating[8,9] groups, that protonation of thiotepa results in rapid (< 5 min) rearrangement to 2, which in turn undergoes relatively slow hydrolysis to 3A or 3B (Fig. 1). Thin-layer



1 2 3A 3B

4 5

Fig. 1.

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chromatographic evidence was offered[6] as proof that aziridine was not formed. In contrast to these conclusions, Maxwell et al.[10] have recently suggested that reaction of thiotepa in acidic saline leads to production of 4 and 5, with 4 being the sole product in saline itself. These proposals[6,10] seemed especially intriguing to us when considered in view of ^{1}H-NMR results obtained with tepa,[11] which clearly demonstrated the vulnerability of phosphorus-bound aziridinyl rings toward acid-catalyzed P-N cleavage and ring-opening. Consequently, we have initiated studies in vitro of thiotepa in various media using sensitive high-resolution ^{1}H-NMR methods to directly elucidate product structures, and now wish to present preliminary findings which disprove the aforementioned claims.[6,10]

In accordance with the reaction conditions specified by Benckhuijsen,[6] a freshly prepared solution of purified thiotepa (m.p. 51.5 to 53°; 6.5 mM) in DCl-D_2O (pD 1.2)* was immediately analyzed by ^{1}H-NMR at 220 MHz (20°) using Fourier transform techniques.[12] The observed spectrum revealed the absence of thiotepa, which would appear in ~ 6 mM D_2O as a doublet ($^3J_{PH} \simeq 17$ Hz) at δ 2.26, as well as the absence of detectable aziridinyl ring proton absorptions attributable to intermediate 2 or final product 3 in the δ 2.0 to 2.5 region.† Instead, a sharp singlet at δ 2.67 was accompanied by complex absorption patterns at δ 3.22 to 3.50 and δ 3.62 to 3.91, with the relative integrated intensities of these signals being 1.0 : 2.2 : 1.4. A similar product spectrum obtains for tepa in D_2O buffered at pH 4.0.[11] The singlet was identified as N-d_2-aziridinium ion by noting an increase in its relative intensity upon addition of aziridine to the NMR sample. From the observed relative signal intensities, it may be calculated that ~ 20 per cent of the original aziridinyl functionalities in thiotepa were converted to aziridinium ion. While characterization of the remaining reaction products formed under these conditions ([thiotepa] : [$^2H^+$] = 1 : 10) is still in progress, it has been found (60 MHz) that an analogous product distribution is formed at higher concentrations of thiotepa (~ 60 mM) in the presence of only one molar equivalent of $^2H^+$. This observation implies that the aziridinyl rings in thiotepa and products subsequently derived from it are subject to acid-catalyzed P-N cleavage and/or ring-opening. However, with 0.5 molar equivalent of $^2H^+$, relative signal integrations showed that ~ 25 per cent of the thiotepa remained; among the usual array of products was a small amount (~ 5-10 per cent) of a new component indicated by a somewhat broadened doublet ($J \simeq 17$ Hz) at δ 2.16. We are pursuing the isolation and characterization of this latter product, which presumably contains at least one intact aziridinyl ring bonded to phosphorus. The doublet at δ 2.16 was not due

* Correction of a precalibrated standard glass electrode reading of "pH" 0.8 to give an actual value for pD of 1.2 was based on studies by Lumry et al.[13]

† All chemical shift values refer to the water-soluble sodium salt of 3-(trimethylsilyl) propionic acid (TSP).

to tepa, since addition of authentic tepa to the reaction mixture gave rise to a relatively low-field doublet ($J \simeq 14.5$ Hz) at δ 2.34. It is also noteworthy that sample preparation involving addition of room temperature acid solutions to crystals of thiotepa yields essentially the same spectrum as that obtained by slow mixing of solutions of thiotepa and acid at ice-bath temperatures.

Reaction of thiotepa with one molar equivalent of $^2H^+$ in a 4-fold molar excess of sodium chloride gave a product mixture indistinguishable (60 MHz) from that obtained in the absence of added chloride ion. In as much as no high-field absorptions expected for the aziridinyl rings in either 4 or 5 were detected, we are compelled to discard the reaction scheme pertaining to thiotepa in acidic saline proposed by Maxwell et al.[10] The additional claim[10] that thiotepa yields 4 in saline was not substantiated by an NMR experiment with 6 mM thiotepa in 1 M sodium chloride: after 2 days no reaction was observed at room temperature; however, at 55° thiotepa did undergo gradual reaction and ultimately afforded (> 3 days) a product spectrum void of an aziridinyl moiety bonded to phosphorus. A control study with thiotepa in pure D_2O at 55° led to similar decomposition, albeit at a somewhat slower rate, thus revealing the competitive incursion of normal hydrolysis. The mechanistic complexity of thiotepa hydrolysis was revealed by ^1H-NMR kinetic measurements in D_2O at 100°, which yielded for the disappearance of thiotepa (0.2 M) an induction period ($\sim$ 20 min) followed by adherence to a first-order rate law ($k \simeq 3 \times 10^{-4}$ sec^{-1}). By way of comparison, substitution of anhydrous dimethylsulfoxide-d_6 (DMSO-d_6) for D_2O led to regular first-order decomposition of thiotepa ($k \simeq 2 \times 10^{-5}$ sec^{-1}) at 100°, even in the presence of approximately one equivalent of sodium chloride (0.2 M). This thermal instability and resistance to nucleophilic attack of thiotepa in DMSO apparently foiled our attempt to prepare authentic 2 by reaction of thiotepa with sodium iodide[14] in DMSO at 80°, which instead gave insoluble dark colored tarry material. Detailed kinetic and product studies for these reactions of thiotepa will be reported at a later date.

While complete definition of the actual reaction pathways available to thiotepa in acidic media awaits further experimentation, it is none the less apparent that conclusions regarding product structures and mechanisms based on indirect chemical evidence (e.g. functional group tests) must now be considered tenuous. A direct and more reliable investigative methodology may be found in the various NMR spectroscopic techniques.

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