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Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:

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Ulf Niemeyer, Bernhard Kutscher, Jürgen Engel, Ion Neda, Axel Fischer, Reinhard Schmutzler, Peter G. Jones, Marie-C. Malet-Martino, Véronique Gilard & Robert Martino

^a ASTA Medica AG, D-60314 Frankfurt, Germany

^b ASTA Medica AG, D-60314 Frankfurt, Germany

^c ASTA Medica AG, D-60314 Frankfurt, Germany

^d Institut für Anorganische und Analytische Chemie der Technischen Universität, Postfach 3329, D-38023 Braunschweig, Germany

^e Institut für Anorganische und Analytische Chemie der Technischen Universität, Postfach 3329, D-38023 Braunschweig, Germany

^f Institut für Anorganische und Analytische Chemie der Technischen Universität, Postfach 3329, D-38023 Braunschweig, Germany

^g Institut für Anorganische und Analytische Chemie der Technischen Universität, Postfach 3329, D-38023 Braunschweig, Germany

^h Université Paul Sabatier, 31062 Toulouse, France

ⁱ Université Paul Sabatier, 31062 Toulouse, France

^j Université Paul Sabatier, 31062 Toulouse, France Published online: 06 Aug 2008.

To cite this article: UIf Niemeyer , Bernhard Kutscher , Jrgen Engel , Ion Neda , Axel Fischer , Reinhard Schmutzler , Peter G. Jones , Marie-C. Malet-Martino , Vronique Gilard & Robert Martino (1996) Degradation Products of Cyclophosphamide Synthesis and Structural studies, Phosphorus, Sulfur, and Silicon and the Related Elements, 109:1-4, 473-476, DOI: 10.1080/10426509608545193

To link to this article: http://dx.doi.org/10.1080/10426509608545193

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DEGRADATION PRODUCTS OF CYCLOPHOSPHAMIDE SYNTHESIS AND STRUCTURAL STUDIES

ULF NIEMEYER¹, BERNHARD KUTSCHER¹, JÜRGEN ENGEL¹, ION NEDA², AXEL FISCHER², REINHARD SCHMUTZLER², PETER G. JONES², MARIE-C. MALET-MARTINO³, VÉRONIQUE GILARD³, ROBERT MARTINO³

¹ASTA Medica AG, D-60314 Frankfurt, Germany, ²Institut für Anorganische und Analytische Chemie der Technischen Universität, Postfach 3329, D-38023 Braunschweig, Germany, ³Université Paul Sabatier, 31062 Toulouse, France

<u>Abstract</u> The degradation of cyclophosphamide in neutral or slightly acidic aqueous solution starts with an intramolecular alkylation leading to an intermediary bicyclic compound which hydrolyses immediately and exclusively to a nine-membered heterocycle. Subsequent acid-catalyzed hydrolysis of the P-Nbond leads to a phosphoric acid monoester. In strongly acidic solutions (1 N HCl) cyclophosphamide decomposes exclusively to bis(2-chloroethyl)amine and to the corresponding phosphoric acid monoester H₂N(CH₂)₃OP(O)(OH)₂. In solid samples of cyclophosphamide, heated up to its melting point, the first pathway predominates over the second one. The structures of the phosphorus compounds were established by ¹H and ³¹P NMR spectroscopy, and synthesis. The structure of the bicyclic compound <u>2</u> is confirmed by a single crystal X-ray diffraction study which allows an explanation for the selective and immediate hydrolysis with formation of a nine-membered heterocycle and for the absence of the isomer with a six-membered ring.

Key Words: Cyclophosphamide, degradation pathways, ³¹P NMR spectroscopy, X-ray crystal structure analysis

INTRODUCTION

Cyclophosphamide <u>1</u>, clinically introduced in 1958, is a standard drug in tumor therapy. This alkylating substance is not active itself but requires in the first step an enzymatic activation in the liver to 4-hydroxycyclophosphamide. This first metabolite releases spontaneously via aldophosphamide the alkylating form, phosphoramide mustard.¹ Beside the metabolism of <u>1</u>, its degradation in aqueous solution was also intensively studied.² These results were summarized and completed, using ³¹P NMR spectroscopy and X-ray crystal structure analysis.

DEGRADATION PATHWAYS

Two pathways of degradation are known for $\underline{1}$ in aqueous solution. Scheme 1 shows the degradation products. In neutral or slightly acidic aqueous solutions (at pH values between 5.4 and 8.6) the first pathway starts with an intramolecular alkylation of $\underline{1}$ to the intermediate, not detectable bicyclic compound $\underline{2}$, which hydrolyses immediately and exclusively to form the nine-membered heterocycle $\underline{3}$. The possible isomer of $\underline{3}$, i.e. the six-membered ring compound $\underline{7}$, is not formed. Subsequent acid-catalyzed hydrolysis in aqueous solvents leads to the phosphoric acid monoester $\underline{4}$.

In strongly acidic solutions the second pathway of the decomposition of <u>1</u> leads exclusively to the hydrochloride salt of bis(2-chloroethyl)amine (nor-nitrogen mustard) <u>5</u> and of phosphoric acid monoester <u>6</u> ($t_{1/2} = 1.4$ days at pH 1.2 and 37 °C) by the breakdown of the two P-N bonds. In the range of pH 2.2 - 3.4, both degradation pathways coexist.²

In solid samples of cyclophosphamide monohydrate, heated up to its melting point, the first pathway predominates over the second. All the above mentioned degradation products are found and, additionally, further compounds, which are formed by inter- or intramolecular alkylation reactions. Traces of compound 2 were detected by ³¹P NMR spectroscopy in highly decomposed samples, dissolved in anhydrous solvents.



SYNTHESIS

The pure compound 2 can be synthesized by treament of anhydrous 1 with sodium hydride in tetrahydrofuran. After addition of water compound 2 hydrolyses spontaneously and exo-thermally to compound 3. Further hydrolysis in hydrochloric acid transforms compound 3 to 4. Compound 6 is obtained from phosphoric acid phenyl ester dichloride by treatment with 3-amino-1-propanol, acid-catalyzed hydrolysis of the P-N bond, and removal of the phenylester group by hydrogenation with PtO₂/H₂.

STRUCTURAL STUDIES

³¹P NMR spectra are a useful tool for the analysis of the hydrolysis mixtures of 1. The spectrum after 17 days at pH 3.4 at 37 °C shows the following well separated signals: diamidoester <u>1</u> at 15.58 ppm, monoamidoester <u>3</u> at 8.10 ppm, monoesters <u>4</u> at 1.49 and monoesters <u>6</u> at 1.28 ppm. In heated solid samples of <u>1</u> the ³¹P signal (CDCl₃) of <u>2</u> is found at 24.80 ppm and that of <u>1</u> at 12.94 ppm. The nine-membered ring compound <u>3</u> is characterized by the lack of H-P and C-P couplings between CH₂(7) and P and , especially, by the observation of couplings between CH₂(10) and phosphorus in both the ¹H and ¹³C NMR spectra which rule out the sixmembered ring compound <u>7</u>.



Figure 1 The crystal structure of the bicyclic compound 2

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In order to rationalize the selective cleavage of the P-N6 bond of compound 2, instead of the P-N3 bond, the strain energy between these bonds was calculated by means of the force field program PIMM. The difference in strain energy is between 0.3 - 0.4 kcal/mol, which could be sufficient to explain the experimentally observed cleavage of the P-N6 bond.

The crystal structure of compound 2 shows that the six-membered ring exhibits almost a chair conformation with an axial phosphoryl oxygen (Figure 1). The phosphorus atom is displaced by 65.6 pm and the carbon atom 2 by -67.4 pm outside the plane formed by C9, C7, O1 and N6. The average deviation from this plane is 3.5 pm. The five-membered ring exists in an envelope conformation with P, N6, C4 and N3 in a plane, from which C5 is displaced by -47.7 pm (average deviation 1.4 pm). Both ring planes form an interplanar angle of 41.9°.

The phosphorus atom is oberved in a slightly distorted tetrahedral coordination geometry. The largest angles at phosphorus were found for the doubly-bonded O2, with 117.89(7)° O2-P-N3, 117.98(7)° O-2-P-N6 and 110.66(7)° O2-P-O1. The smallest angle with 97.36(7)° is found between the nitrogen atoms N3 and N6.

Significant differences are observed for the bond angle and bond length of both N-atoms. The angle sum at N6 shows with 339.11° a more pronouncedly pyramidal conformation than at N3 with 350.36°, and, moreover, the P-N3 bond length (162.99(14) pm) indicates a stronger π -bond character than the P-N6- bond length with 165.93 (14) pm.

In summary, the theoretical calculations of the strain energies of the P-N bonds of compound $\underline{2}$ as well as the P-N bond lengths determined in the crystal structure provide a plausible explanation for the observed selective hydrolysis of the P-N6 bond leading to compound $\underline{3}$.

REFERENCES

- 1. N. BROCK, Cancer Res., 49, 1 (1989)
- V. GILARD, R. MARTINO, M. C. MALET-MARTINO, B. KUTSCHER, A. MÜLLER, U. NIEMEYER, J. POHL, and E. E. POLYMEROPOULOS, J. Med. Chem., 37, 3986 (1994).