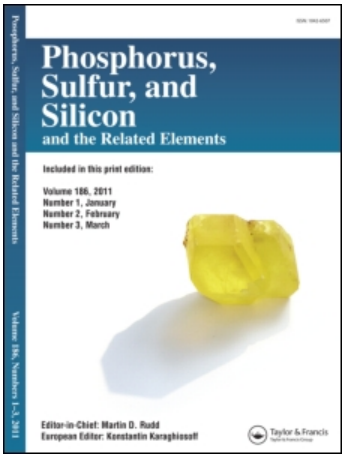

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THE CHEMISTRY OF 4-SUBSTITUTED OXAZAPHOSPHORINANES (OAP) (TOOLS FOR CANCER RESEARCH AND THERAPY)

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Abstract Novel chemical approaches resulted in 4-substituted OAP derivatives, linked by a sulfur, orygen or nitrogen atom to C-4. The cis- and trans-epimers were stereoselectively synthesized from the key intermediate 4-hydroxycyclophosphamide. An alternative route will be presented. The stereochemistry of 4-substituted OAP are confirmed by single crystal X-ray analysis and by NMR-spectra for more than 50 compounds. The connectivities of the nuclei observed in the homonuclear and heteronuclear COSY plots are summarized with data. In aqueous solution 4-substituted OAP are hydrolyzed with a half-life at physiological conditions of a few minutes for S-derivatives and of about 20 hours for the more stable N-derivatives. The pH-dependance of the hydrolysis rates including epimerisation and transthioacetalisation are submitted. Pharmacokinetical and biological activity data are presented.

INTRODUCTION

Clinical and toxicological observations on mustard-gas initiated the development of DNA-alkylating drugs against cancer. Cyclophosphamide (CP, Endoxan^{R}), the most widely used drug of today, was synthesized in 1956[1]. In this compound the original sulfur atom of mustard-gas was exchanged by one nitrogen of a cyclic phosphorodiamidic ester of the OAP type. Physiologically, the first metabolic event is the oxidation of CP by hepatic enzymes to 4-hydroxy-CP. Former approaches to utilize free 4-hydroxy-CP for clinical trials involved the synthesis of 4-substituted OAP[2,3].

SYNTHESIS

The initial step is ozonisation of O-3-butenyl-N,N-bis-(2-chloroethyl)-phosphorodiamidate or of CP yielding in 4-hydroperoxy-CP, which is stable at -20° C. Reduction under mild conditions by triphenyl-phosphine or by aqueous sodium thiosulfate leads to 4-hydroxy-CP. 4-Hydroxy-CP is a cyclic half-animale which reacts with nucleophiles according to the equation:

$$4\text{-OH-CP} + \text{H-XR} \rightleftharpoons 4\text{-XR-CP} + H_2O$$

where X might be oxygen, sulfur or nitrogen.

Up to 1977 only 4-hydroperoxy-CP and some thio-derivatives (X = S) were characterized by us and others[2,3]. Recently an acid-catalysed reaction of 4-hydroxy-CP with hydroxyurea yielded in 4-hydroxyureido-CP. It was the first compound in which a radical is linked at the OAP-ring in the 4-position by a nitrogen. As both the carbon 4 and the phosphorus are chiral, we synthesized stereoselectively the two racemic diastereomeres. Following the general strategy of Fig. 1, more than 50 of 4-N-derivatives were synthesized.

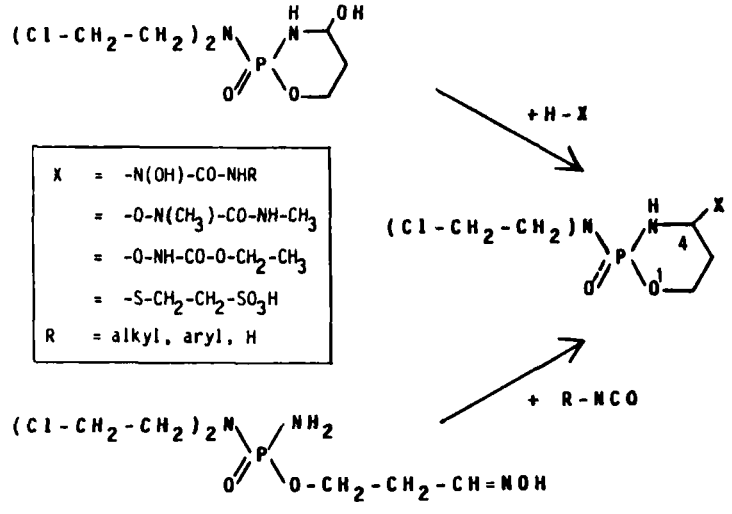

FIGURE 1 Synthesis of 4-substituted OAP

Novel O-connected derivatives were synthesized by reaction of 4-hydroxy-CP with N-hydroxy-N,N'-dimethylurea and N-hydroxy-urethane. Urotoxic 4-hydroxy-CP is detoxified in the urine by a reaction with mesna to 4-sulfonato-ethylthio-CP[4]. Synthetically this reaction in aqueous aceton leads stereoselectively to the crystalline cis-isomer (mafosfamide) as a cyclohexylammonium salt in more than 80 % yield[5]. For clinical studies the less toxic mafosfamide L-lysine salt was prepared on a strong acid resin column.

STEREOCHEMISTRY

The stereochemistry of mafosfamide was confirmed by single-crystal X-ray diffraction. The phosphoryl oxygen and the sulfonatoethylthio side-chain are approximately cis-diaxially corresponding to the cis-isomer of 4-hydroxy-CP. The axial preference of the sulfonato-ethylthio side-chain is attributed to an anomeric effect. Only one antipode was found in the crystal lattice. This separation of anti-podes by crystallization is a specific phenomenon in the OAP field. The X-ray analysis of 4-hydroxyureido-CP established the relative configuration as trans.

The stereochemistry in solution cannot be elucidated by a single NMR-spectrum. However, based on two X-ray analyses semi-empirical rules could be derived, which proved to be conclusive for more than 50 CP-compounds. By high-field NMR using 2D experiments including the phosphorus-decoupled H,H-COSY-technique all questions concerning spin-assignment and molecular structure in solution state could be answered[6]. Low and high temperature NMR showed clearly that only one conformation is preferred in solution.

BEHAVIOUR IN SOLUTION

In aqueous solution, 4-substituted OAP are hydrolyzed, leaving

the corresponding thiol, hydroxyurea or hydroxyurethane and 4-hydroxy-CP. The half-life in solution of the S- and O-derivatives are a few minutes and of the N-derivatives about 20 hours at pH 7, 37° C, with a pH optimum of minimal hydrolysis. For S-derivatives, i.e. mafosfamide, the greatest stability is observed around pH 4. Out of this range the stability decreases exponentially. The N-derivatives are most stable at pH 7.

BIOLOGICAL ACTIVITY

All new OAP are highly cytotoxic agents. The different behaviour in solution, however, affects the pharmacokinetics and the biological availability. S- and O-derivatives are more toxic (LD 50: 150 - 350 mg/kg) in rats and mice than N-derivatives (LD 50: >1,000 mg/kg). In vitro the S- and O-compounds are found to be more active. The curative effectivity in vivo, however, against rat-tumors is nearly equal for all derivatives.

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