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RATE AND MODE OF BASIC HYDROLYSIS OF ANHYDROPENICILLIN

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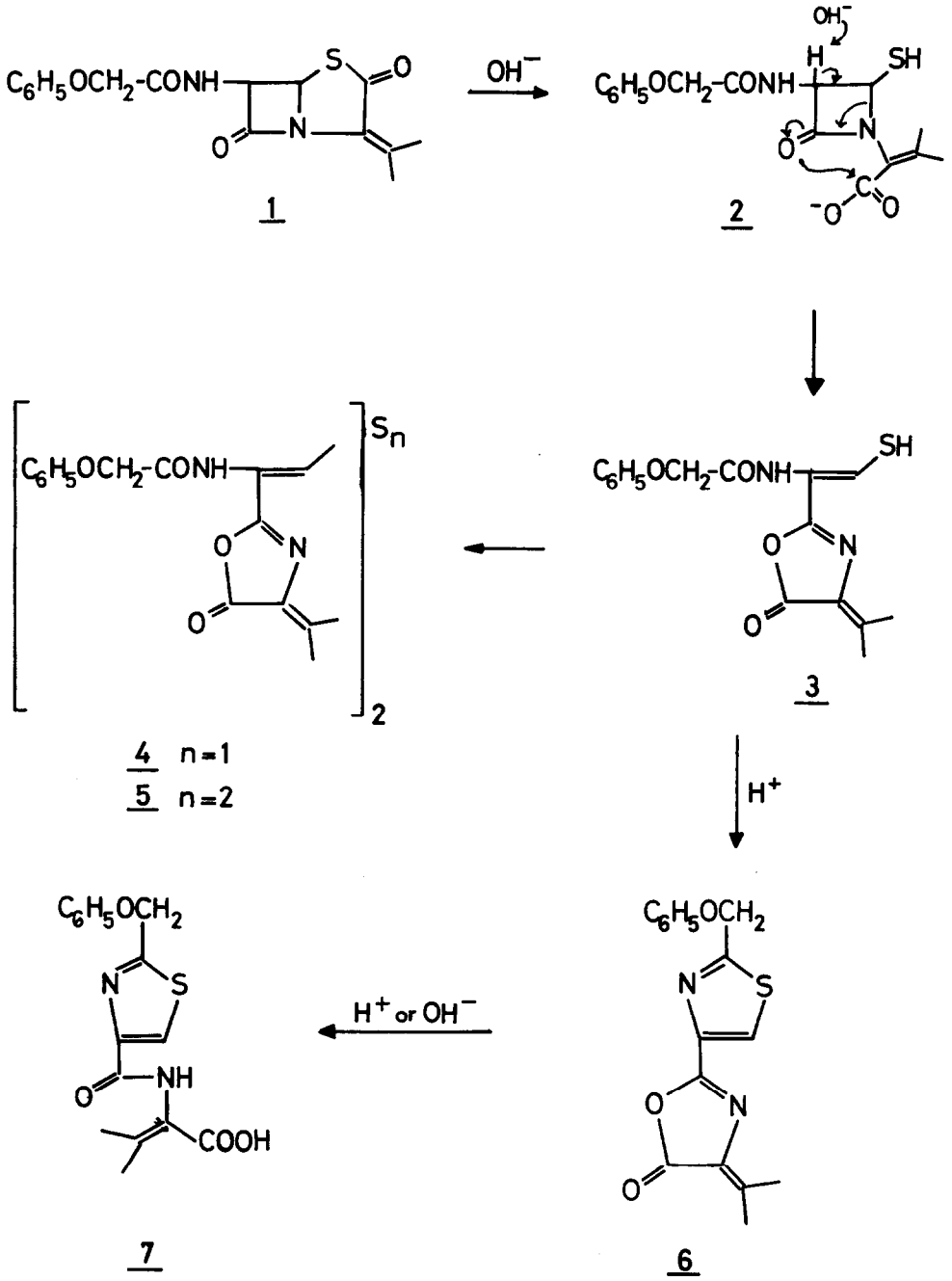
The great sensitivity of the β-lactam moiety of biologically active penicillins and cephalosporins to attack by nucleophiles has been attributed mainly to a suppression of the usual amide resonance resulting from the nonplanarity in the β-lactam nitrogen atom[1,2]. Ease of basic hydrolysis of the β-lactam amide bond can be correlated with the degree of nonplanarity[2] as well as with the β-lactam stretching frequency[3,4]. A notable exception is apparently constituted by anhydropenicillins. In spite of having a molecular conformation similar to that of the penicillins with a nonplanar β-lactam nitrogen atom[5] and of having a carbonyl stretching frequency even greater than that found for normal penicillins[6], these compounds are said to be remarkably stable[6]. Simon et al.[5] have recently shown that the chemical stability of the anhydropenicillins cannot be ascribed to delocalization in the ground electronic state of the electron pair in the nitrogen atom into the adjacent α,β-unsaturated system as previously suggested[6]. The unexpected, and still unexplained, stability of anhydropenicillins led Simon et al. to conclude that the nonplanar character of the β-lactam nitrogen atom is a necessary, but not sufficient condition for chemical activity. Apparently, the characterization of anhydropenicillins as being chemically stable compared with normal penicillins is solely based on the fact that they are recovered unchanged after refluxing in various organic solvents or from a melt[6]. This behaviour does not imply, however, that the compounds are stable toward nucleophilic agents such as hydroxide ion. In fact, we have found and now wish to report that

anhydro-α-phenoxymethylpenicillin (1) in neutral and alkaline aqueous solution degrades with a rate which is about 100 times greater than the rate of hydrolysis of normal penicillins under identical conditions.

The kinetic experiments were carried out in aqueous phosphate and borate buffer solutions (μ = 0.5) at 37°. The rates of degradation of 1 were measured by following the decrease in absorbance at 274 nm (λ_{max} of 1) as a function of time. At constant pH the loss of 1 (initial conc $7 \cdot 10^{-5}$M) obeyed a first-order rate law. After correction for buffer catalysis the observed pseudo-first-order rate constants (k_{obs}) were found to be directly proportional to the hydroxide ion activity over the pH range 7.2 - 10.1, indicating the validity of the equation $k_{obs} = k_{OH} \cdot a_{OH}$. The value of k_{OH} was $3.2 \cdot 10^3$ $M^{-1}min^{-1}$. Under similar reaction conditions the hydroxide ion catalytic rate constant for hydrolysis of α-phenoxymethylpenicillin is 39.1 $M^{-1}min^{-1}$ (ref. 7).

TLC on Merck silica gel 60/kieselguhr F_{254} sheets [developing solvents: acetic acid-methanol-chloroform-cyclohexane (5:5:30:60) and acetic acid-ethyl acetate (3:97)] of reaction solutions consisting of 150 mg of 1, 5 ml of acetonitrile and 5 ml of 0.05 M carbonate buffer pH 9.8 or 10.7 showed the formation of three products during the degradation of 1. After complete disappearence of 1 the yellow reaction solution was acidified to pH 4 with an acetate buffer and then extracted with ethyl acetate. The concentrated extract was subjected to preparative TLC on silica gel G with acetic acid-ethyl acetate-benzene (1:4:20) as developing solvent. The major product, which could not be obtained in a pure state, was assigned the structure 3. It responded positively on treatment with a thiol-specific reagent[8], and on treatment with 0.1 N HCl it cyclized immediately to the thiazole-oxazolone (6)[9], $C_{16}H_{14}N_2O_3S$[10], mp 139-140° (cryst. from EtOH-H_2O); UV (EtOH) λ_{max} 306 nm (ε 27,100) and 240 nm (ε 10,100), sh 296 nm (ε 24,000), sh 319 nm (ε 19,300); IR (KBr) 1780, 1760, 1668, and 1595 cm^{-1}. Compound 6 was further characterized by facile hydrolysis by 2 N HCl or 0.1 N NaOH to thiazole (7)[9], $C_{16}H_{16}N_2O_4S$[10], mp 178-178.5° (cryst. from acetone-H_2O); IR (KBr) 3250, 1700, 1650, and 1600 cm^{-1}. Acid hydrolysis of 6 to 7 has been described previously[9]. Further support of the structure 3 was its facile transformation into 4 and 5 by standing overnight in an ethyl acetate solution.



1 2 3 4 5 6 7

The remaining two compounds were more acid-stable than 3. They had melting points and UV-, IR- and NMR-spectral data similar to those described by Kukolja et al.[9] for the structures 4 and 5. The structures of the products formed (3,4, and 5) by the alkaline degradation of 1 show that these derive from cleavage of the thiolactone bond in 1 and not from attack on the β-lactam moiety. A likely mechanism involves hydroxide ion catalyzed hydrolysis of the thiolactone ring to give 2 which then through a double β-elimination reaction transforms to 3 from which the sulphide 4 and the disulphide 5 finally derive. The high reactivity of the thiolactone moiety of the anhydropenicillin makes a determination or even a rough estimation of the reactivity of the β-lactam moiety very difficult. Even if it is assumed that the β-lactam amide bond in anhydropenicillins is twice as reactive toward hydroxide ion as the bond in normal penicillins it can be calculated that only about 2 per cent of the anhydropenicillin would be degraded through a cleavage of the β-lactam amide bond. Although one cannot, in fact, exclude the possibility of a decreased acylating power of the β-lactam moiety in anhydropenicillins the present study shows that no reasons exist in favour of this and that it is unjustified, on the present state of knowledge, still to consider anhydropenicillins as being an exception to the rule that a strained β-lactam ring and a nonplanar β-lactam nitrogen atom indicate a high chemical reactivity to nucleophiles.

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