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IMIDAZOLE-CATALYZED ISOMERIZATION OF PENICILLINS INTO PENICILLENIC ACIDS

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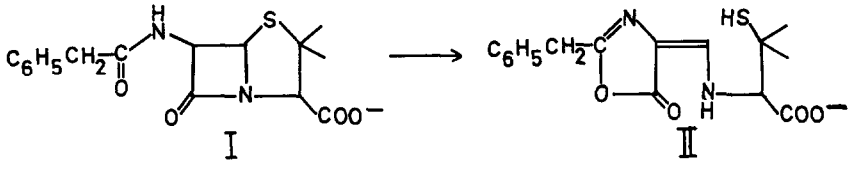
The principal antigenic determinant in penicillin allergy is the penicilloyl group bound by amide linkage to amino groups on proteins[1]. It has been demonstrated that penicilloyl compounds may be formed by a direct aminolysis of penicillins[2,3] and/or by aminolysis via the highly reactive degradation product, penicillenic acid[4]. However, as the rates of both the direct aminolysis[5] and of the isomerization of penicillins to the corresponding penicillenic acids[6,7] at pH 7.2 - 7.4 are quite slow, the chemical mechanism of antigenicity of penicillins is not clearly understood. The present paper describes a strong imidazole-catalyzed isomerization of benzylpenicillin I into benzylpenicillenic acid II[8]. This is the first reported catalysis of the reaction I $\longrightarrow$ II.

The reactions were carried out under conditions in which imidazole was in great excess over penicillin so that pseudo-first-order kinetics would be obtained (3-4 x 10^{-5} M benzylpenicillin, 0.04 - 0.3 M imidazole, μ = 0.5 M with KCl, solvent water, pH 6.44 - 8.18, 37°). $HgCl_2$ was added to the imidazole buffers in a concentration of 5 x 10^{-5} M to stabilize the labile II by blocking the



SH-group. It was unambiguously determined that the mercuric chloride which produces a complex with imidazole[9] has no effect upon the rate or mechanism of degradation of I (cf. Ref.[7,8]). The rates of formation of II were measured by following the increase in absorbance at 325 nm as a function of time; the rate constants (k_{obs}) were determined by the method of Guggenheim. It was found that the production of II goes through a short lag period and thereafter obeys a pseudo-first-order rate law. In all cases the penicillenate formed corresponded to 100% conversion of I (II, λ_{max} 325 nm, log ϵ 4.42, lit.[10] log ϵ 4.42). After completion of a run, treatment of the reaction solution with an excess of 2 M NaOH yielded a product having λ_{max} 298 nm, log ϵ 4.24, in agreement with 2-benzyl-4-hydroxymethyleneoxazol-5(4H)-one (λ_{max} 298 nm, log ϵ 4.25)[10]. This reaction has been described for benzylpenicillenic acid[10] and benzylpenicillenic acid disulphide[11].

Plots of k_{obs} at constant pH as a function of imidazole concentration showed a definite upward curvature with increasing imidazole, indicating an order higher than unity in imidazole. As shown in Fig. 1 plots of $k_{obs}/(Im)$ vs. (Im) are linear and without measurable intercepts. (Im) represents the concentration of imidazole free base (pK'_a = 7.00 ± 0.02, μ = 0.5 M, 37°). For several pH values, plots of the slopes of these straight lines vs. a_H/K'_a provide a linear relationship of slope k_1 and intercept k_2 (Fig. 2). Thus, the overall kinetic expression is given by eq. 1 and 2.

$$-d(I)/dt = d(II)/dt = k_1(Im)(ImH^+)(I) + k_2(Im)^2(I) \quad (1)$$

$$k_{obs} = k_1(Im)(ImH^+) + k_2(Im)^2 \quad (2)$$

The values of the third-order rate constants k_1 and k_2 were 0.38 l^2 $mole^{-2}min^{-1}$ and 0.12 l^2 $mole^{-2}min^{-1}$, respectively. At any fixed totale imidazole concentration maximum values of k_{obs} occur at pH 7.4. The rate equation strongly suggests that the imidazole-catalyzed isomerization of I into II proceeds by a general acid-catalyzed and a general base-catalyzed assistance to nucleophilic attack of imidazole on the penicillin, i.e., at the β-lactam carbonyl carbon. The observed initial induction period in the formation of II indicates the existence of an intermediate, in accordance with the proposal of a nucleophilic catalysis. A support for a mechanism implying nucleophilic catalysis has further come from similar

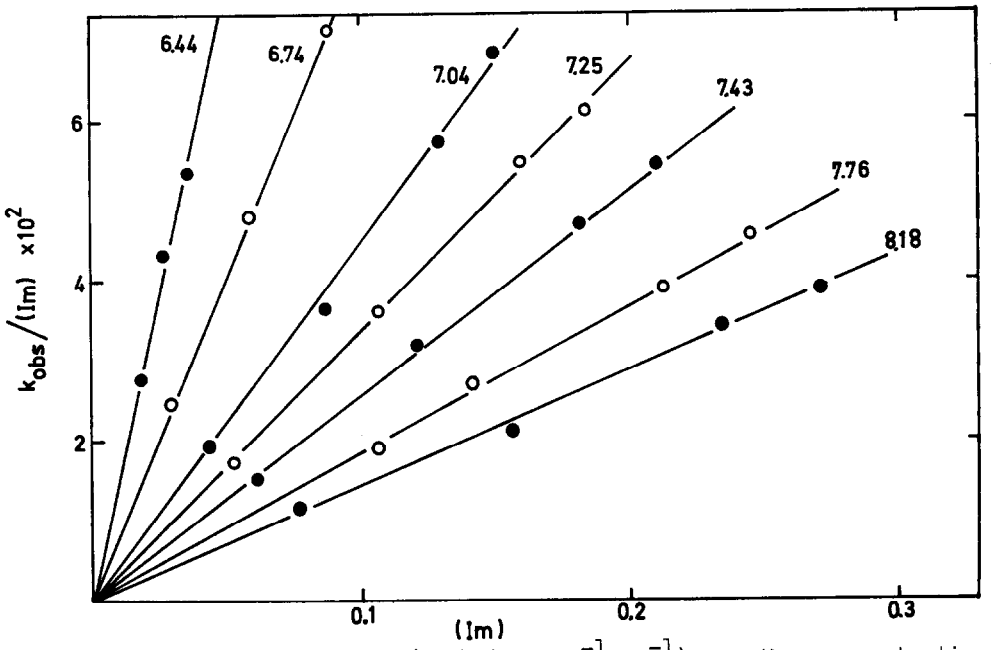


Figure 1. -Plot of $k_{obs}/(Im)$ (l $mole^{-1}min^{-1}$) vs. the concentration of imidazole free base (Im) as a function of pH.

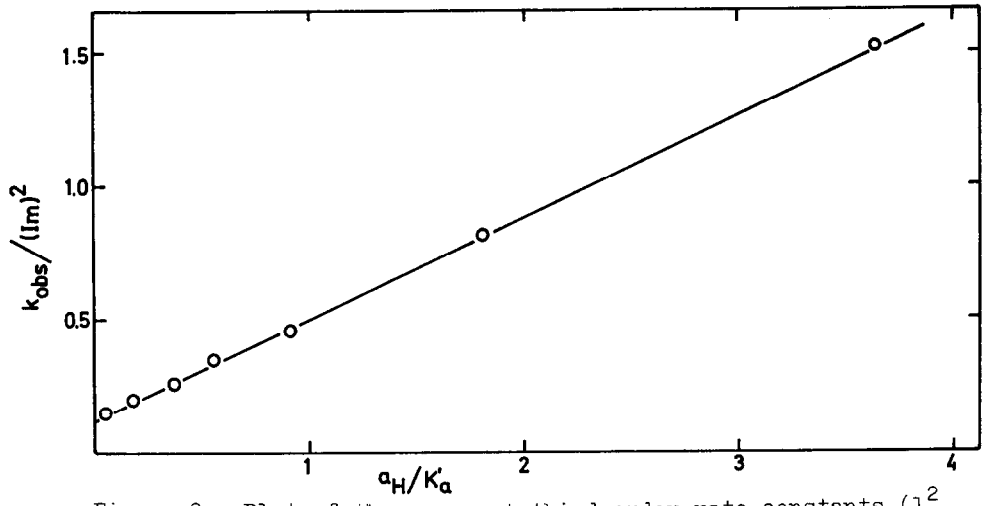


Figure 2. -Plot of the apparent third-order rate constants (l^2 $mole^{-2}min^{-1}$), as a function of a_H/K'_a.

experiments with phenoxymethylpenicillin. The specific H^+-catalyzed isomerization[8] of this penicillin into the corresponding penicillenic acid was determined to be about 28 times slower than that of I. The imidazole-catalyzed reaction, however, proceeded about 1.5 times faster. A determination of the alkaline hydrolysis rates of the two penicillins as a measure of the susceptibility to nucleophilic attack revealed a similar greater (x 1.5) reactivity of phenoxymethylpenicillin.

Presumably, the isomerization involves an initial formation of N-penicilloylimidazole which could then undergo a rapid intramolecular conversion to II. While further studies are in progress to elucidate the imidazole-catalyzed reactions in more detail, it is interesting to note the extremely rapid formation of penicillenic acids under physiological conditions of pH and temperature. In absence of imidazole, the half-life for formation of II at pH 7.4 and 37° was determined to be 9×10^3 hours; imidazole in a concentration of 0.5 M reduces the half-life to 20 min. For phenoxymethylpenicillin the rate enhancement is even greater, $t_{\frac{1}{2}}$ is reduced from 25×10^4 hours to about 13 min.

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