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Chemical reactions involved in penicillin allergy: kinetics and mechanism of penicillin aminolysis

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In view of the fundamental importance of the reaction of penicillins with amino groups of proteins to the penicillin allergy, the aminolysis of benzylpenicillin by various amines was kinetically investigated. The formation rate constants, k_{amide}, of benzylpenicilloylamides were determined at 35°, 45° and 60° ($\mu = 0{\cdot}5$), and found to obey the general rate law: $k_{amide} = k_1[\text{amine}] + k_2[\text{amine } H^+][\text{amine}] + k_3[\text{amine}]^2 + k_4[\text{amine}]a_{OH}$. All of the amines exhibited the unassisted nucleophilic rate constant, k_1. The relative importance of the other kinetic terms depends on the basicity and the chemical structure of amines. The reaction mechanism of penicillin aminolysis was discussed. Brønsted relations for k_1, k_2 and k_3, except for hydrazines, were satisfactory.

Since a direct reaction of penicillins with free amino groups of proteins *in vivo* is thought to be responsible for the bulk of the immunogenicity of penicillins (for reviews, see Schwartz, 1969; Schneider, 1970), a knowledge of aminolysis reactions of penicillins is required. Detailed kinetic studies have been carried out by Schwartz & Wu (1966) on the aminolysis of penicillins by glycine, and by Bundgaard (1971, 1972a,b,c) on reactions with imidazole. Schneider & de Weck (1968) have described reactions of benzylpenicillin with several amino compounds at pH 7·5. The present work describes the kinetics of aminolysis of benzylpenicillin by a number of amines and attempts to generalize the amine structure-reactivity relation. Related work has been published (Yamana, Tsuji & others, 1975 a,b).

MATERIALS AND METHODS

Materials

Potassium benzylpenicillin (BPC) was kindly supplied by Meiji Seika Kaisha, Ltd. *N*-(Benzylpenicilloyl)amines were prepared by a similar method to that previously reported by Levine (1962): *N*-(benzylpenicilloyl)benzylamine (BPBA), m.p. 118° (from acetone-water), lit. m.p. 119—121° (Mazingo & Folkers, 1949). Anal. calcd for $C_{23}H_{27}N_3O_4S$: C, 62·6; H, 6·2; N, 9·4. Found: C, 62·3; H, 6·2; N, 9·4. *N*-(Benzylpenicilloyl)methoxyethylamine (BPMA), Anal. calcd for $C_{19}H_{27}N_3O_5S$: C, 55·7; H, 6·6; N, 10·3. Found: C, 55·5; H, 6·6; N, 10·0%. Benzylpenicilloic acid (BPO) aqueous solution was prepared just before use (Schwartz & Delduce, 1969). All other chemicals were of reagent grade quality and used without further purification.

Apparatus

Spectrophotometric measurements were made with Shimadzu QV–50 spectrophotometer or with Shimadzu multipurpose recording spectrophotometer, model

MPS-5000, having cell temperature controlled by water circulated from a Haake thermostat. The pH of a kinetic solution was measured both before and at the end of the reaction, with a Radiometer model PHM 26 pH meter. Where the buffer capacity was too low, constant pH was maintained by a Radiometer TTT-2 pH stat. The temperature of the reaction solution was kept constant to $\pm 0{\cdot}1°$ with a Lauda thermostated controlled bath.

Kinetic measurements

Aminolysis. To maintain a constant pH, the amines were used both as buffer and nucleophile. The amine buffers were prepared just before use, and the ionic strength was adjusted to 0·5 by addition of potassium chloride. The total amine concentration, $[\text{amine}]_T$, used was in large excess over the concentration of BPC to obtain pseudo-first-order kinetics. The concentrations of the free (unprotonated) amine, [amine], and its conjugate acid, [amine H^+], were determined from the relation: $Ka = [\text{amine}]a_H/[\text{amine } H^+]$ and $[\text{amine}]_T = [\text{amine}] + [\text{amine } H^+]$. The values of a_H and a_{OH} at $\mu = 0{\cdot}5$ were obtained from the equations: $\log a_H = -\text{pH}$ and $\log a_{OH} = \text{pH} - C$ with $C = 13{\cdot}68$ and 13·02 at 35° and 60°, respectively (Harned & Hamer, 1933). The pKa values of amines were determined under the experimental conditions by the method of half neutralization.

The reaction was initiated by addition of a known weight of BPC to the amine buffer solution preheated to a desired temperature (35°, 45° and 60°). Aliquots of the reaction mixture were withdrawn at appropriate intervals. Unless otherwise stated, the samples were assayed for intact BPC by the iodometric titration procedure (Finholt, Jurgensen & Kristiansen, 1965). Reactions involving hydrazines were followed by the hydroxamic acid assay (Ford, 1947) as modified in this laboratory. The pseudo-first-order rate constants, k_{obs}, were calculated by a least squares method. In most cases, the initial concentration of BPC was 5×10^{-3} M.

Hydrolysis. The pseudo-first-order rate constants of BPC hydrolysis in the absence of amines, k_{pH}, were determined by the following two techniques: (a) External buffers such as phosphate, borate and carbonate were employed to maintain a constant pH, and the values of k_{pH} were obtained by the extrapolation of k_{obs} to zero buffer concentration. The iodometric assay was used for the determination. (b) The k_{pH}'s were determined by a pH-stat alkalimetric titration method. In these cases, approximately 5×10^{-4} M disodium ethylenediamine tetraacetic acid was added to avoid occasional erratic results apparently due to a heavy-metal catalysed decomposition of BPC.

Deuterium solvent isotope effect

The rate constants of aminolysis of BPC with 2,4-dimethylimidazole and glycine and those (k_{pD}) in absence of amines were determined at 60° by the same procedure used for the reaction in water. To determine pD, the glass electrode correction equation of Fife & Bruice (1961) was employed: pD = pH meter reading + 0·25 at 60°.

Product analysis

The penamaldate method for the assay of BPBA, BPME and BPO produced from the reactions of BPC with benzylamine and 2-methoxyethylamine was adapted from the literature procedure (Schneider & de Weck, 1968; Schwartz & Delduce, 1969):

Two ml of the reaction mixture withdrawn at appropriate intervals (BPC, 5×10^{-3} M and amines, 0·05 — 0·3 M) and 10 ml of 0·1 M phosphate buffer of pH 7·0 were diluted to 50 ml with distilled water. Five ml of this solution and 5 ml of 2×10^{-4} M $HgCl_2$ aqueous solution were diluted to 20 ml with distilled water. The solution was allowed to stand for 20 min at room temperature and the absorbance at 285 nm was determined against non-penicillin solution.

Assuming penicilloylamides (BPA) and BPO to be the only products formed during the reactions, the mole fraction yield of BPA and BPO at time t can be calculated from the following equations:

$$[\text{BPA}]/I_0 = \frac{A_t - \epsilon_{\text{BPO}}(I_0 - [\text{BPC}])}{(\epsilon_{\text{BPA}} - \epsilon_{\text{BPO}})I_0} \quad \ldots \quad (1)$$

$$[\text{BPO}]/I_0 = \frac{A_t - \epsilon_{\text{BPA}}(I_0 - [\text{BPC}])}{(\epsilon_{\text{BPA}} - \epsilon_{\text{BPO}})I_0} \quad \ldots \quad (2)$$

where I_0 is the initial concentration of BPC and A_t is the absorbance at time t. ϵ_{BPA} and ϵ_{BPO} represent the molar extinction coefficient. The ϵ values at 285 nm after 20 min for BPA, BPME and BPO were found to be $2{\cdot}55 \times 10^4$, $2{\cdot}34 \times 10^4$ and $1{\cdot}97 \times 10^3$, respectively. The concentrations of BPC at time t, [BPC], were determined simultaneously by the iodometric titration method.

A typical assay result of products of reaction at pH 8·36 of BPC with 0·09 M benzylamine at 60° is shown in Fig. 1.

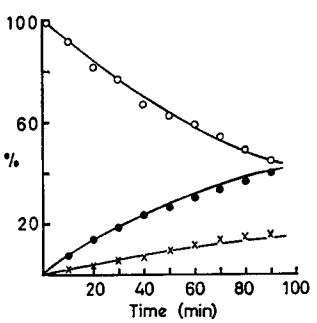


Fig. 1.

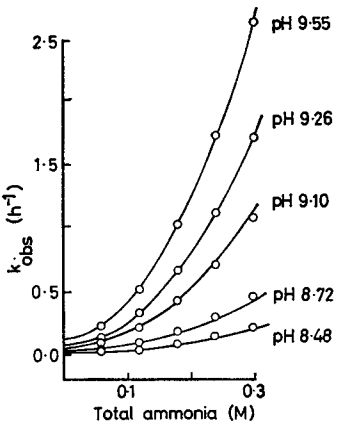


Fig. 2.

FIG. 1. Time courses for benzylpenicillin (BPC, ○), *N*-(benzylpenicilloyl) benzylamine (BPBA, ●) and benzylpenicilloic acid (BPO, ×) during the reaction of BPC (5×10^{-3} M) with benzylamine (0·09 M) at pH 8·36, 60° and $\mu = 0{\cdot}5$. The curves were calculated from the following equations by use of the kinetic parameters in Table 1:

$$[\text{BPC}] = I_0\, e^{-kt};\ [\text{BPBA}] = I_0\, \frac{k_1[\text{amine}] + k_3[\text{amine}]^2}{k}(1 - e^{-kt});$$

$$[\text{BPO}] = I_0\, \frac{k_{pH}}{k}(1 - e^{-kt})$$

where $k = k_{pH} + k_1[\text{amine}] + k_3[\text{amine}]^2$ and $k_{pH} = 0{\cdot}110\ h^{-1}$

FIG. 2. Plots of the pseudo-first order rate constants *vs* total amine concentration for the reaction of benzylpenicillin with ammonia at 35° and $\mu = 0{\cdot}5$. The lines are calculated from equation (3) and the rate constants in Table 1.

In the reaction subjected to the product analysis by the penamaldate assay, the pseudo-first-order rate constants determined from the slope of a plot of log $(A_\infty - A_t)$ *vs* time were in fairly good agreement with the values of k_{obs} determined iodometrically, where A_∞ is the absorbance at infinity. In some cases, the values of A_∞ were estimated by the modified method of Guggenheim (Swinbourne, 1960).

RESULTS AND DISCUSSION

Rate expression of aminolysis

The observed pseudo-first-order rate constants, k_{obs}, for aminolysis of BPC were found to follow the general relation:

$$k_{obs} - k_{pH} = k_1[\text{amine}] + k_2[\text{amine H}^+][\text{amine}] + k_3[\text{amine}]^2 + k_4[\text{amine}]a_{OH} \quad (3)$$

where k_1 represents second-order rate constant. k_2, k_3 and k_4 are third-order rate constants for general acid-catalysed, general base-catalysed and hydroxide ion-catalysed reactions of amine, respectively.

The observed first-order rate constants show, in most cases, a sharp upward curvature in plots against $[\text{amine}]_T$ because of catalysis by a second molecule of amine. The typical experimental data for the reaction of BPC and ammonia are illustrated in Fig. 2.

Equation (3) can be rearranged to equation (4), separating the terms which are first- and second-order for amine.

$$(k_{obs} - k_{pH})/[\text{amine}] = (k_1 + k_4a_{OH}) + (k_3 + k_2a_H/Ka)\,[\text{amine}] \quad (4)$$

Plots of $(k_{obs} - k_{pH})/[\text{amine}]$ *vs* [amine] at a single pH give $(k_3 + k_2a_H/Ka)$ as slope and $(k_1 + k_4a_{OH})$ as intercept. From secondary plots of these slopes *vs* a_H/Ka and the intercepts *vs* a_{OH} obtained at several pH values, the various rate constants can be determined.

The second-order rate constants, k_1, were generally derived for all amines by the plotting technique described above, but not all the reactions exhibit detectable k_2, k_3 and k_4 terms.

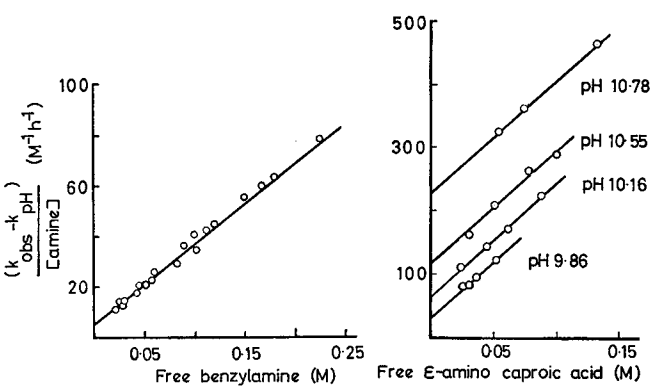


FIG. 3. Plots of the apparent second-order rate constants *vs* free amine concentration for the reaction of benzylpenicillin with benzylamine at 60° and $\mu = 0{\cdot}5$.

FIG. 4. Plots of the apparent second-order rate constants *vs* free amine concentration for the reaction of benzylpenicillin with ε-amino-caproic acid at 35° and $\mu = 0{\cdot}5$.

Table 1. *Rate constants for the reactions of benzylpenicillin with amines at ionic strength of 0·5.*

Amine	Temp. °C	pKa	No. of k_{obs}	k_1 $M^{-1}h^{-1}$	k_2 $M^{-2}h^{-1}$	k_3 $M^{-2}h^{-1}$	$10^{-4}k_4$ $M^{-2}h^{-1}$
Imidazole	35	6·96	20	0·035	22·2	5·9	—
	45	6·84	23	0·080	29·9	7·8	—
	60	6·65	16	0·25	52·5	11·0	—
N-Methylimidazole	35	7·11	16	0·035	7·8	—	—
	60	6·70	17	0·30	18·6	—	—
2-Methylimidazole	35	7·89	15	0·033	(0·34)[a]	(0·62)[a]	—
	60	7·40	16	0·46	—	—	—
2,4-Dimethylimidazole	60	7·82	12	0·33	—	—	—
in D_2O	60	8·25	3	0·28	—	—	—
Glycylglycine	60	7·45	15	0·25	2·6	11·2	—
Morpholine	60	8·17	16	3·0	2·0	46·0	—
Benzylamine	60	8·64	19	5·0	—	320	—
2-Methoxyethylamine	60	8·72	20	2·6	—	186	—
Ammonia	35	9·15	25	0·30	—	53·0	—
	45	8·85	25	0·70	—	65·5	—
	60	8·38	12	2·0	—	92·5	—
Glycine	35	9·41	24	0·40	—	90·0	—
	45	9·11	15	0·80	—	106	—
	60	8·87	11	2·5	—	148	—
in D_2O	60	9·38	3	2·5	—	57·0	—
β-Alanine	35	9·98	16	1·0	—	213	4·2
	60	9·36	16	4·5	—	320	3·6
n-Butylamine	35	10·56	16	6·0	—	2720	9·5
	60	9·78	15	10·0	—	1360	28·5
ε-Aminocaproic acid	35	10·55	15	5·3	—	1770	17·4
	60	9·97	6	16·0	—	1100	10·4
Hydrazine	60	7·50	17	10·0	1430	6950	—
N-Methylhydrazine	60	7·46	16	6·0	370	1450	—

(a) Experimentally detectable, but an uncertain constant.

Some representative data are shown in Figs 3–5. The various rate constants for the reaction of BPC with a number of amines are shown in Table 1. Each reaction was studied at least three different concentrations of total amine (in the range of 0·05–0·3 M) and at three or more different pH values.

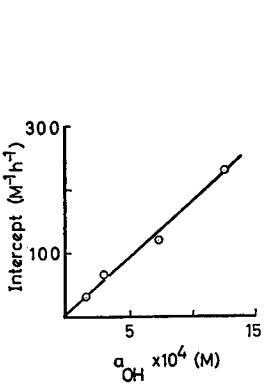


Fig. 5.

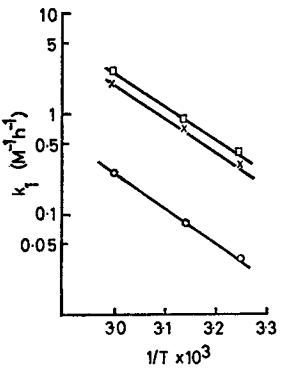


Fig. 6.

FIG. 5. Plots of the intercepts of Fig. 4 *vs* hydroxide-ion activity for the reaction of benzylpenicillin with ε-aminocaproic acid at 35° and $\mu = 0{\cdot}5$.

FIG. 6. Arrhenius plots of k_1 for the reaction of benzylpenicillin with glycine (□), ammonia (×) and imidazole (○) at $\mu = 0{\cdot}5$.

Arrhenius plots of k_1, k_2 and k_3 for the reactions with imidazole, ammonia and glycine are shown in Figs 6 and 7. Also included in Fig. 7 are the data reported by Bundgaard (1972a) for imidazole (37°) and by Schwartz & Wu (1966) for glycine (50°). The activation enthalpies (ΔH^*) determined for these reactions are presented in Table 2. The ΔH^* values for both assisted general acid [6 kcal mol^{-1} (25 kJ mol^{-1})] and general base [*ca* 4 kcal mol^{-1} (17 kJ mol^{-1})] are significantly less than those for unassisted catalysis [*ca* 14 kcal mol^{-1} (59 kJ mol^{-1})].

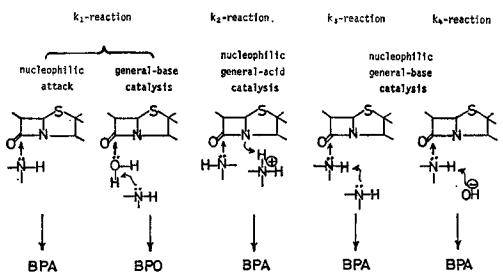
Table 2. *Activation enthalpies† for the aminolysis of benzylpenicillin at* $\mu = 0{\cdot}5$.

	ΔH^* kcal mol^{-1}(kJ mol^{-1})		
Amine	k_1	k_2	k_3
Imidazole	15·1 (64·0)	6·3 (26·4)	3·8 (15·9)
Ammonia	14·4 (60·2)	—	3·8 (15·9)
Glycine	14·0 (58·6)	—	3·6 (15·1)

† Calculated from $\Delta H^* = Ea - RT$ where the values of Ea were obtained from the slopes of the Arrhenius plots, and T = 308°K.

Reaction mechanism

The pseudo-first-order rate constants for aminolysis of benzylpenicillin obey the general rate law (eqn 3) which is known to be a common rate expression for aminolysis of esters (see review by Johnson, 1967) and amides (Oakenfull & Jencks, 1971) including simple β-lactams (Blackburn & Plackett, 1973). Plausible mechanisms which would explain the kinetics for these terms are shown in Scheme 1. Among these kinetic terms, the general acid-(k_2) and general base-catalysed (k_3) reactions with assistance by a second molecule of amine represent the nucleophilic displacement reaction by amine on the penicillin β-lactam bond to yield the corresponding penicilloyl-amides. The dominance of these reactions reflects the fact that expulsion of an amine anion from an amide (in this case, β-lactam) in aqueous solution is difficult or impossible without assistance by general acid-base catalysis (Oakenfull & Jencks, 1971).



Scheme 1

That the reactions connected with the k_1-terms represent nucleophilic displacement reactions to give penicilloyl amide and not general base-catalysed reactions to give penicilloic acid (see Scheme 1) is shown by the following observations:

(1) For the reaction of BPC with 0·09 M benzylamine at pH 8·36 and 60° (see Fig. 1), 75% of the total rate is due to the benzylamine reactions and 35% of these proceed by unassisted amine-reaction. Since the mole fraction yield of BPBA was found to be 75%, the unassisted amine-reaction must represent a nucleophilic reaction.

(2) The reaction with 2,4-dimethylimidazole, which represents only the k_1 term, shows a small deuterium isotope effect with a $k_1(H_2O)/k_1(D_2O)$ ratio of 1·2 (Table 1). The deuterium isotope effect found in the reaction is consistent with nucleophilic attack. Nucleophilic reaction does not lead to a deuterium solvent isotope effect appreciably greater than unity, whereas general base catalysis generally gives rise to a deuterium solvent isotope effect of 2–3 (Johnson, 1967). The other pertinent example in differentiating between two catalysis paths is shown in the reaction of BPC with glycine (Fig. 8). The deuterium isotope effects on these catalytic rate constants show $k_1(H_2O)/k_1(D_2O) = 1·0$ and $k_3(H_2O)/k_3(D_2O) = 2·6$, indicating the nucleophilic attack and general base catalysis, respectively.

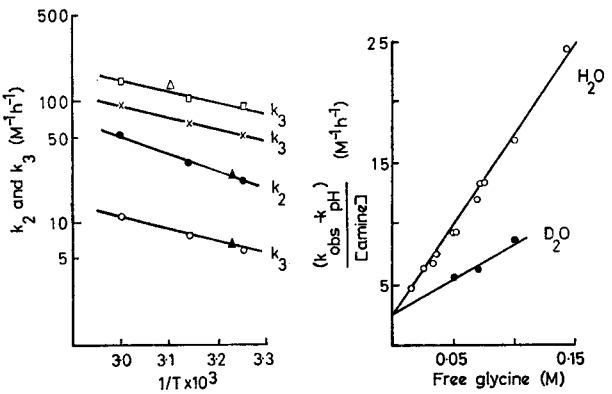


FIG. 7. Arrhenius plots of k_2 and k_3 for the reactions of benzylpenicillin with glycine (□), ammonia (×) and imidazole (○, ●) at $\mu = 0·5$. The triangles refer to the values reported for glycine (open symbol) by Schwartz & Wu (1966) and for imidazole (closed symbols) by Bundgaard (1972a).

FIG. 8. Plots of the apparent second-order rate constants *vs* free amine concentration for the reaction of benzylpenicillin with glycine in H_2O (○) and in D_2O (●) at 60° and $\mu = 0·5$.

In conclusion, the reactions of BPC with almost all of the amines were believed to represent a nucleophilic displacement reaction to produce the corresponding BPA, and can be enhanced by assistance of general acid-base catalysis.

It should be added that the enhanced reactivity of hydrazines toward BPC was observed and may be the result of the α-effect (Johnson, 1967).

Structure-reactivity relationship

Brønsted plots of k_1, k_2 and k_3 of the aminolysis reactions studied at 35° and 60° ($\mu = 0·5$) are presented in Figs 9 and 10 except for hydrazines. The experimental values have been plotted without correction for statistical effects. A least squares fit of the points leads to the following equations:

$$\log k_1\ (M^{-1}h^{-1}) = 0·64(0·56)pKa - 6·17(-4·41) \quad (5)$$

$$\log k_2\ (M^{-2}h^{-1}) = -0·87(-0·87)pKa + 7·21(7·21) \quad (6)$$

$$\log k_3\ (M^{-2}h^{-1}) = 0·68(0·68)pKa - 4·23(-3·76) \quad (7)$$

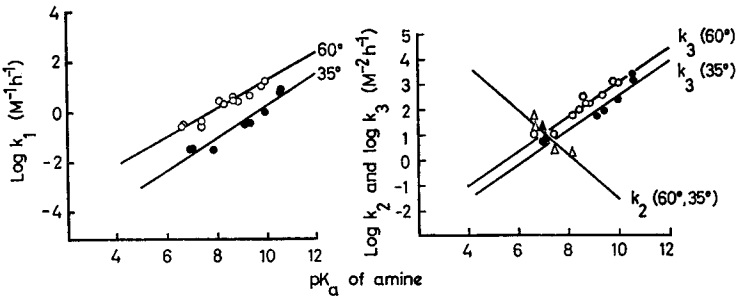


FIG. 9. Brønsted plots of log k_1 *vs* pKa of amine for the aminolysis of benzylpenicillin at 35° (●), 60° (○) and $\mu = 0{\cdot}5$. No statistical corrections have been made.

FIG. 10. Brønsted plots of log k_2 (triangles) and log k_3 (circles) *vs* pKa of amine for the aminolysis of benzylpenicillin at 35° (closed symbols), 60° (open symbols) and $\mu = 0{\cdot}5$. No statistical corrections have been made.

where the parameters correspond to those at 35° and 60° (in parentheses). Although there is a general correspondence between amine basicity and the aminolysis rate constants of carboxylic acid derivatives (Johnson, 1967), equations (5)–(7) give the first quantitative account of the rate constants of penicillin aminolysis, and provide a more complete background for the consideration of possible chemical reactions involved in penicillin allergy.

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