as the model mucin system possessed rheological properties considerably removed from normal gastric mucus (Janowitz & Hollander, 1954; Curt & Pringle, 1969). This model mucus system is at present being evaluated although it has been accepted as representative by other workers (Barry & Braybrooks, 1974).

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November 26, 1974

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Chemical aspects of penicillin allergy: imidazole-catalysed penicilloylation

We recently established the quantitative relationship of amine structure-reactivity for the penicillin aminolysis (Yamana, Tsuji & others, 1975). The result led to the general rule that, in solutions of neutral pH, amines with a pKa around 7 are the most reactive toward penicillin, whereas amines having a pKa in the region of 10–11, such as ϵ-aminocaproic acid, catalyse the penicilloylation at a very slow rate.

In the understanding of penicillin allergy, the most important problem is how to explain the rapid penicilloylation with the ϵ-amino group of tissue protein under physiological pH and temperature. The following chemical aspects for the *in vivo* formation of penicillin antigen are more acceptable than other possibilities (*e.g.*, see Schneider, 1970); (1) intramolecular rapid penicilloylation *via* combination of the neighbouring functional groups and the ϵ-amino group of lysine (Schneider & de Weck, 1968; Schwartz, 1968, 1969). (2) imidazole-catalysed penicilloylation *via* the highly reactive intermediates of *N*-penicilloylimidazole (Bundgaard, 1972c; Schneider & de Weck, 1974) or its isomerized product, the penicillenic acid (Bundgaard, 1971, 1972a,b). Although a considerable controversy has developed as to which route is important *in vivo*, the decisive pathways are not sufficiently clear.

To explore some possibilities for rapid penicilloylation under physiological conditions, we have examined extensively the reactions of penicillins with various types of imidazole derivatives because these compounds have their pKa values near 7 and can produce more reactive penicilloylamides than other amines. The penicillins we used were benzylpenicillin, ampicillin, cloxacillin and 6-ethoxycarbonylaminopenicillin (ethoxypenicillin) all of which can isomerize to the corresponding penicil-

lenic acids depending on the nature of their side-chain, and 6-(α-toluenesulphonamido)-penicillanic acid (TSPA) which cannot isomerize to the corresponding penicillenic acid (Yamana, Tsuji & Mizukami, 1974). The imidazole compounds (MI-X) used are summarized in Table 1, in which L-histamine and L-histidine were considered to be a simple protein model with a primary amino-group adjacent to the imidazole.

The pseudo-first-order rate constants, k_{obs}, for the hydrolysis of penicillins (5×10^{-3}M) by IM-X-catalysis were determined iodometrically at 35°, 45° and 60° ($\mu = 0{\cdot}5$) and found to depend on the concentrations of the conjugate acid [IMH^+-X] and base species [IM-X] of imidazole derivatives as in the following equation:

$$k_{obs} = k_{pH} + k_1[IM\text{-}X] + k_2[IM\text{-}X]\,[IMH^+\text{-}X] + k_3[IM\text{-}X]^2 \quad \ldots\ldots (1)$$

where k_{pH} represents the rate constant for the hydrolysis in the absence of imidazoles. All imidazoles exhibited the second-order rate constants, k_1, and the relative importance of the other two terms depend on the basicity and steric requirement of these compounds. Imidazole itself exhibited both general-acid (k_2) and general-base (k_3) catalysis. The reaction with *N*-methylimidazole is represented by the k_2 term. In the reactions with mono- and di-methylimidazoles, catalysis by a second molecule of these imidazoles is negligible or only just detectable due to steric hindrance. L-Histamine and L-histidine are represented only by the k_1 terms and the values exhibit a large positive deviation from the Brönsted plot for k_1 (Yamana & others, 1975) undoubtedly due to the intramolecularly catalysed rate enhancement by the neighbouring primary amine. These rate constants are listed in Table 1.

The products from the reactions with the imidazoles were analysed spectrophotometrically at 310–345 nm in the presence of $HgCl_2$ in the reaction mixtures. All the imidazoles examined catalysed the formation of the corresponding penicillenic acids from penicillins except for TSPA. A similar observation for imidazole itself has already been demonstrated by Bundgaard (1971, 1972a, b). In most imidazole-catalysed reactions of benzylpenicillin, the formation rate constants of benzylpenicillenic acid were in good agreement with the sum of the k_1, k_2 and k_3 terms which were determined from the rates of the cleavage of the β-lactam bonds by iodometric titration. The observed solvent isotope effect on k_1 for 2,4-dimethylimidazole is consistent with a nucleophilic catalysis mechanism [$k_1(H_2O)k_1(D_2O) = 1{\cdot}2$]. The activation enthalpies for the imidazole reaction of benzylpenicillin were determined to be 15, 6 and 5 kcal mole $^{-1}$ (63, 25 and 19 kJ mole^{-1}) for k_1, k_2 and k_3, respectively.

Table 1. *The catalytic rate constants for the reaction of benzylpenicillin with various imidazole compounds at 35° (60°) and ionic strength of 0·5.**

Imidazole compound	pKa**	k_1 $M^{-1}h^{-1}$	k_2 $M^{-2}h^{-1}$	k_3 $M^{-2}h^{-1}$
Imidazole	6·96 (6·65)	0·035 (0·25)	22·2 (52·5)	5·9 (11·0)
N-Methylimidazole	7·11 (6·70)	0·035 (0·30)	7·8 (18·6)	—
2-Methylimidazole	7·89 (7·40)	0·033 (0·46)	0·34	0·62
1,2-Dimethylimidazole	(7·60)	(0·17)	—	—
2,4-Dimethylimidazole	8·24 (7.82)	0·035 (0·33)	—	0·75
L-Histamine	6·25	0·91	—	—
L-Histidine	6·10	0·18	—	—

*All rate constants were determined by iodometric titration method. These specific rate constants are defined in eqn 1. Data in parentheses are those at 60°.

**Dissociation constant for imidazolyl moiety. These values were determined by the method of half-neutralization at 35° (60°) and $\mu = 0{\cdot}5$.

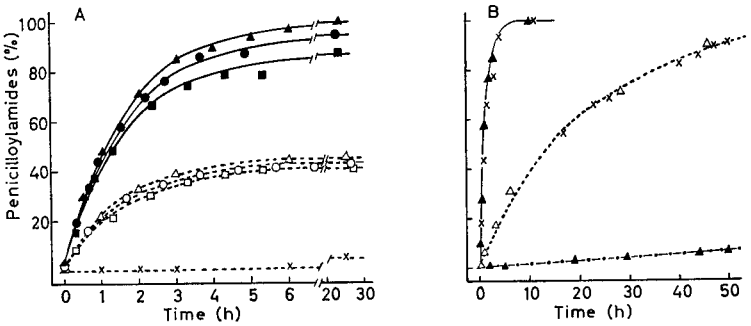


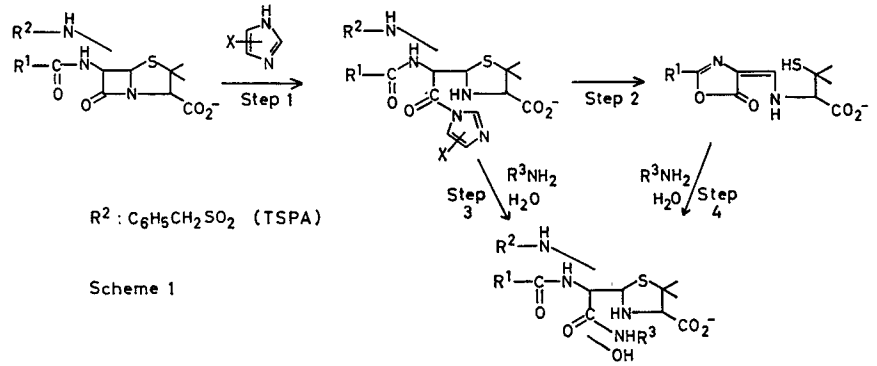
FIG. 1. A. The percentage yield of penicilloylamides produced from the reactions of various types of penicillins (5×10^{-3} M) with imidazole (0·3 M) in the presence of glycylglycine (0·1 M, solid line) and ϵ-aminocaproic acid (0·2 M, dashed line). All reactions were carried out at pH 7·40 and 35° ($\mu = 0{\cdot}5$). Benzylpenicillin (triangles); ampicillin (squares); ethoxypenicillin (circles); 6-(α-toluenesulphonamido)- penicillanic acid (TSPA) (×).

B. The percentage yield of penicilloylamides produced from the reactions of penicillins (5×10^{-3} M) with L-histamine (1 M, —), L-histidine (0·25 M, – –) and ϵ-aminocaproic acid (0·25 M, – · –). All reactions were carried out at pH 7·40 and 35°. Benzylpenicillin (triangles); 6-(α-toluenesulphonamido)-penicillanic acid (TSPA) (×).

When glycylglycine (pKa 8·05) and ϵ-aminocaproic acid (pKa 10·6) are added so that their respective concentrations are 0·1 and 0·2 M in a 0·3 M imidazole solution (pH 7·4, 35°) of benzylpenicillin, ampicillin or ethoxypenicillin (each penicillin concentration is 5×10^{-3} M), the corresponding penicilloylamides were formed with almost the same rates to the extent of about 90% with glycylglycine and 45% with ϵ-aminocaproic acid (determined by penamaldate assay) at the end of the reactions (Fig. 1A).

Bundgaard (1972c) stated that ethoxypenicillin cannot produce the corresponding penicillenic acid and argued that the formation of ethoxypenicilloylamide from the imidazole-catalysed reaction of this penicillin proceeds through the reaction of *N*-(ethoxypenicilloyl)imidazole with amines. His argument, however, was inconsistent with the data of the present work. We found that ethoxypenicillin degrades slowly in the acid-catalysed isomerization reaction but has twice the reactivity of ampicillin (see Table 2) and that imidazole catalyses the hydrolysis of ethoxypenicillin to produce ethoxypenicillenic acid (λ_{max} 320 nm). The rate constants for the penicilloylamide formation are in close agreement with those for the β-lactam cleavage of benzylpenicillin, cloxacillin, ethoxypenicillin and ampicillin (see Table 2). The percentage yield of the penicilloylamides is slightly affected by the penicillin side-chain structure, *i.e.*, the isomerization rates to the corresponding penicillenic acid (see k_H in Table 2), and is largely dependent on the basicity of amines. From an imidazole solution of TSPA, which cannot produce the corresponding penicillenic acid, on the other hand, the formation of the penicilloylamide produced from ϵ-aminocaproic acid was negligible under the same conditions. Bundgaard's discussion about the reactivity of *N*-(penicilloyl)imidazole determined by use of ethoxypenicillin (Bundgaard, 1972c) would seem not to offer a satisfactory explanation of the present results.

The most likely mechanism for imidazoles-catalysed penicilloylation is indicated in Scheme 1. The rate-determining step of the overall reaction is the initial formation of *N*-(penicilloyl)imidazoles (Step 1). For the simple imidazoles-catalysed reaction [X = —H, $-CH_3$, $-(CH_3)_2$], the penicilloylation by amines present in the reaction solution proceeds not through *N*-(penicilloyl)imidazoles (Step 3) but through its isomerized product, penicillenic acid (Step 4). Although *N*-(penicilloyl)imidazoles certainly have strong acylating ability, the intermolecular reaction between *N*-(penicil-



Scheme 1

loyl)imidazoles and nucleophilic agents such as amine and water (Step 3) must be slower than the intramolecularly isomerized reaction (Step 2) however slow the latter rate. The argument for the proposed mechanism was based on the two factors: (1) the formation rates of penicilloylamides are in agreement with those of the corresponding penicillenic acids produced rapidly from the rate-determining reaction between penicillins and imidazoles. (2) the imidazole-catalysed reaction of TSPA strongly indicates that *N*-(penicilloyl)imidazole exhibits negligible reactivity in the intermolecular reaction with highly basic amines such as ϵ-aminocaproic acid at pH 7·4 (see Fig. 1A).

The reactions of benzylpenicillin and TSPA with L-histamine and L-histidine exhibited different behaviour from those with simple imidazole compounds and were facilitated *via* intramolecular general-acid catalysis instead of the intermolecular catalysis involving two amine species. The penicilloylamides resulted from the reaction with the neighbouring primary amine (pKa 9·71 for L-histamine and pKa 9·03 for L-histidine) were formed to the extent of 100% (see Fig. 1B). The formation rate of the penicilloylamide from L-histidine reaction is 15 times faster than that from a similar reaction with ϵ-aminocaproic acid which does not involve catalysis (see Fig. 1B and Table 2), and were in fair agreement with those for β-lactam bond cleavage of benzylpenicillin and TSPA.

Table 2. *Rate constants for acid-, base-and imidazole-catalysed degradation of various penicillins.**

Penicillins	k_H**	k_{OH}**	k_1†	k_2†	k_3†	$k_{obs}(h^{-1})$‡		
						0·3M IM	0·25M L-Hist.	0·25M ϵ-ACA
	$M^{-1}h^{-1}$	$M^{-1}h^{-1}$	$M^{-1}h^{-1}$	$M^{-2}h^{-1}$	$M^{-2}h^{-1}$			
Benzylpenicillin	601	$1·44 \times 10^3$	0·04	22·2	5·9	0·660	0·043	0·003
Cloxacillin	35·6	1·34	0·06	24·5	6·5	0·720	—	—
Ethoxypencillin	4·0	0·92	—	—	—	0·600	—	—
Ampicillin	1·8	2·57	0·10	20·4	9·0	0·693	—	—
6-(α-Toluenesulphonamido)-penicillanic acid (TSPA)	0·9	0·47	0·04	11·7	1·4	0·310	—	—

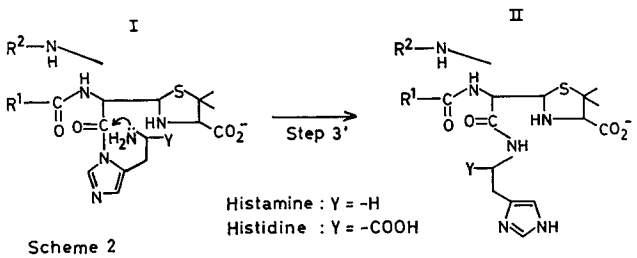
IM=Imidazole L-Hist.=L-Histidine ϵ-ACA=ϵ-Aminocaproic acid

*All rate constants are at 35° and μ=0·5, and were determined by iodometric titration method

**Second-order rate constants for hydronium ion-catalysed hydrolysis of undissociated penicillin (k_H) and hydroxide ion-catalysed hydrolysis of dissociated penicillin (k_{OH}). The data for for ethoxypenicillin were determined in the present work and others are those reported previously (see Yamana & others, 1974).

†Specific catalytic rate constants for imidazole.

‡Pseudo-first-order rate constants at pH 7·4.



Scheme 2

The mechanism of the reaction with L-histamine and L-histidine is explicable by the process that the *N*-(penicilloyl)imidazoles (I) initially produced give the corresponding penicilloylamines (II) by intramolecular attack of the neighbouring primary amine (see Scheme 2). The intramolecular penicilloylation (Step 3′) proceeds more rapidly than the isomerization to the penicillenic acid, because the percentage yield of II is independent of the production of the penicillenic acid as demonstrated in the reaction of TSPA and benzylpenicillin (see Fig. 1B).

These results strongly suggest that the histidine residue of proteins can catalyse penicilloylation with the nucleophilic attack of the ϵ-amino group of the lysine residue adjacent to the imidazolyl moiety. This penicilloylation may proceed predominantly through *N*-(penicilloyl)imidazole produced from all types of penicillins that are structually capable and also those that are incapable of undergoing rearrangement to the penicillenic acids.

We thank Meiji Seika Kaisha, Ltd and Takeda Chemical Ind., Ltd for the gifts of some of the penicillins and 6-aminopenicillanic acid.

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September 10, 1974

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