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Penicillin allergy: imidazole-catalysed formation of the penicilloyl determinant

The major antigenic determinant in penicillin allergy is the penicilloyl group (Levine, 1965; de Weck & Blum, 1965), and it has been reported that most of the immunogenicity of the penicillins is due to *in vivo* formation of penicilloyl conjugates (de Weck, Schneider & Gutersohn, 1968; Schneider & de Weck, 1970). Such conjugates may be formed by reaction of penicillin with free amino-groups of protein and/or by reaction via the reactive degradation product, penicillenic acid (see e.g. Schneider, 1970). The claim (Schneider & de Weck, 1968, 1969) that the imidazole group cannot be penicilloylated by penicillin has recently been disproved in this laboratory (Bundgaard, 1971, 1972a, b). The studies have shown that imidazole at neutral pH reacts with several penicillins with the quantitative formation of corresponding penicillenic acids, the initially formed products being *N*-penicilloylimidazoles. Since the rate of this nucleophilic imidazole catalysis is much higher than the rate of aminolysis of penicillins by other amines at physiological pH and temperature, and since the penicilloylating properties of the reaction product, penicillenic acid, in some respects are different from those of penicillins [e.g. the free thiol group can be penicilloylated by penicillenic acid, but not by penicillin (Wagner, Davis & Gorman, 1969)], the imidazole reaction must be considered as a potential pathway in the formation of penicilloyl conjugates. However, the question arises whether imidazole can catalyse penicilloylation of amino- or other functional groups of proteins by penicillins which are structurally incapable of undergoing rearrangement to penicillenic acids. Such penicillins have been demonstrated to be as immunogenic as benzylpenicillin (Schneider & de Weck, 1966). The present report shows that imidazole at pH 7·4 and 37° catalyses the penicilloylation of amino- and hydroxyl groups by 6-ethoxycarbonylaminopenicillanate, a penicillin in which the alkoxy side chain prevents penicillenate formation.

Sodium 6-ethoxycarbonylaminopenicillanate (ethoxypenicillin sodium) was prepared by treatment of 6-aminopenicillanic acid with ethyl chloroformate in aqueous acetone with sodium bicarbonate as acid acceptor. The penicillin was isolated as its sodium salt by treating the free acid in ether with a 30% solution of sodium 2-ethylhexanoate in n-butanol. The infrared spectrum showed characteristic bands at 1770 (β-lactam carbonyl), 1710 (carbamate carbonyl), and 1600 cm^{-1} (carboxylate). Found: C, 40·2; H, 5·9; N, 8·3. Calc. for $C_{11}H_{19}N_2O_5S$ Na,H_2O: C, 40·2; H,5·2; N, 8·5%.

The reactivity of ethoxypenicillin toward the nucleophilic agents imidazole, glycylglycine, and hydroxide ion was found to be similar to that of benzylpenicillin (Table 1).

Table 1. *Rate data for alkaline hydrolysis, aminolysis by glycylglycine, and imidazole-catalysed hydrolysis of ethoxypenicillin and benzylpenicillin.**

	k_{OH^-}† (M^{-1} min^{-1})	k_{obs} (min^{-1})‡ 0·15 M glycylglycine	0·15 M imidazole	0·25 M imidazole
Ethoxypenicillin	27·7	$1·9 \times 10^{-4}$	$2·4 \times 10^{-3}$	$6·5 \times 10^{-3}$
Benzylpenicillin	29·0	$2·5 \times 10^{-4}$	$3·1 \times 10^{-3}$	$8·7 \times 10^{-3}$

* All rate constants are at 37° and a total ionic strength of 0·5 M (with potassium chloride).
† Determined by a pH-stat as previously described (Bundgaard, 1972b).
‡ Pseudo-first-order rate constants at pH 7·40 (37°). The rate constants were obtained by measuring the appearance of products (penicilloylamide or penicilloic acid) by the penamaldate method (Schwartz & Delduce, 1969). The initial concentration of the penicillins was 3×10^{-3} M.

As the spontaneous hydrolysis is negligible compared with the observed rate constants (k_{obs}) these reflect solely the reactions caused by glycylglycine and imidazole. By means of the highly specific penamaldate assay (Schwartz & Delduce, 1969) the product of the reaction with imidazole and hydroxide ion was identified as ethoxypenicilloic acid, while the reaction with glycylglycine produced *N*-(ethoxypenicilloyl)-glycylglycine (95%) and ethoxypenicilloic acid (5%). Obviously, the effect of imidazole is to catalyse hydrolysis, but if even small amounts of glycylglycine are added to the imidazole solution, *N*-(ethoxypenicilloyl)glycylglycine is formed together with the penicilloic acid and so, the effect is also a catalysis of penicilloylation of the amino-group of glycylglycine. Fig. 1A shows the percentage yield of penicilloyl amides as a function of the concentration of glycylglycine and two other amines, glycine ethyl ester and glycine. The apparent pKa values of the amino-groups of these compounds at 37° and $\mu = 0·5$ M are: glycine ethyl ester, 7·74; glycylglycine, 8·05; glycine, 9·50 (determined by the method of half-neutralization). Evidently, the curves show that it is the basic component of the amino-group that is penicilloylated.

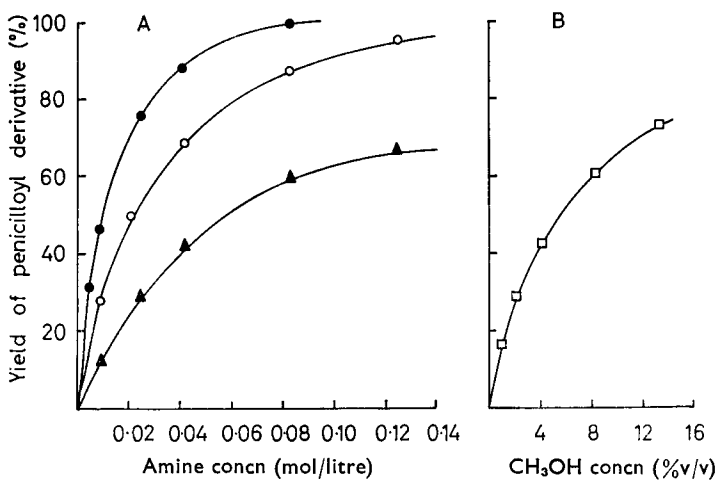
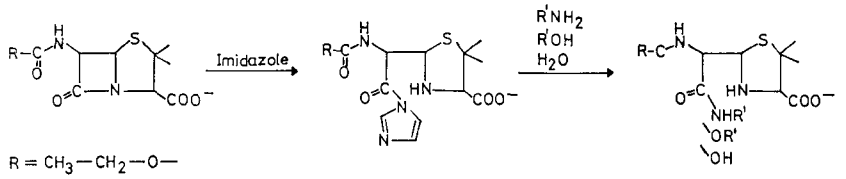


FIG. 1. Effect of amine and methanol concentration on the percentage yield of penicilloyl amides (A) and penicilloyl methyl ester (B), formed by reaction of ethoxypenicillin sodium (10^{-3} M) with imidazole (0·25 M) and glycine ethyl ester (●), glycylglycine (○), glycine (▲), and methanol (□). All reactions were carried out at pH 7·40 and 37°.

When methanol is added in varying quantities to the imidazole solution ethoxypenicilloyl methyl ester (determined by the penamaldate method) is formed as well as the penicilloic acid (Fig. 1B). This penicilloylation of the hydroxyl group of methanol particularly demonstrates the catalytic effect of imidazole as no reaction occurs between penicillin and methanol in the absence of imidazole.

In the concentrations used, the glycine derivatives and the methanol caused no changes in the reaction rate, this being solely dependent on the concentration of imidazole. When the concentration of the acceptor amine or alcohol was held constant, and the concentration of imidazole varied between 0·1 and 0·5 M, no changes in the percentage yield of penicilloyl derivatives were observed.



Scheme 1

The most likely mechanism for the imidazole-catalysed penicilloylation involves as the rate-determining step an initial formation of *N*-(ethoxypenicilloyl)imidazole. Analogous to *N*-acylimidazoles, e.g. *N*-acetylimidazole (Jencks & Carriuolo, 1959a, b; Oakenfull & Jencks, 1971) the *N*-penicilloylimidazole certainly is a strong acylating agent and once formed, it would rapidly transfer the penicilloyl group to the acceptor nucleophiles or water (Scheme 1). Besides being in agreement with the experimental results, this nucleophilic catalysis mechanism is consistent with the mechanism worked out for the imidazole-catalysed transformation of benzylpenicillin into benzylpenicillenic acid (Bundgaard 1972a).

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