HYDROLYSIS OF FURAN AND 5-NITROFURAN PENICILLINS BY PENICILLINASE Bacillus licheniformis

G. A. Veinberg, Ya. A. Kastron, and S. A. Giller

UDC 615.779.932'547.722.5.577.15.022

The enzymatic hydrolysis of various furan and 5-nitrofuran penicillins by penicillinase Bacillus licheniformis 749/c was investigated. The effect of various side-chain structural groupings on the rate of inactivation of these compounds was studied. The introduction of bulky substituents in the ortho or α positions to the amide group and lengthening the side chain or increasing its degree of unsaturation sharply decrease the catalytic action of penicillinase and, consequently, the rate of cleavage of the β-lactam ring of the antibiotic. The majority of the furan and 5-nitrofuran penicillins surpass benzylpenicillin in resistance to the enzyme but are inferior to methicillin and oxacillin.

The continuously increasing resistance to penicillin is an important factor that limits its use for the treatment of infectious diseases. The reason and the mechanism for this phenomenon consist in the fact that antibiotic-resistant microorganisms liberate the enzyme penicillinase which promotes the hydrolytic cleavage of the β-lactam ring of penicillin [1]. Natural penicillins are readily decomposed by the action of penicillinase. Attempts were therefore made to eliminate this inadequacy by changing the structure of the side chain of the antibiotic molecule. The successful solution of this problem became possible only after the discovery of 6-aminopenicillanic acid in 1959 [2]. In a short time several penicillinase-resistant, semisynthetic penicillins [3-8] were synthesized from it and introduced into medical practice. A study of the rate of enzymatic hydrolysis of various natural and semisynthetic penicillins made it possible to establish that bulky substituents in the ortho or α positions to the amide bond create steric hindrance to the formation of an intermediate substrate–enzyme complex. As a result the catalytic action of pencillinase decreases sharply [9, 10].

We were influenced by these considerations in the synthesis of penicillins which contain furan and 5-nitrofuran groups in the side chain [11-14]. The majority of the compounds obtained manifested activity toward resistant staphylococcus strains. It was of interest to study the effect of the side-chain structure of the new penicillins on their resistance to penicillinase.

According to the Michaelis–Menten theory, the reaction between penicillin and penicillinase can be expressed by the equation

$$E+S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} P+E, \quad (1)$$

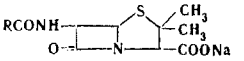
where E is the enzyme (penicillinase), S is the substrate (penicillin), ES is the intermediate complex, P is the reaction product, k_1 is the rate constant for the formation of intermediate complex ES, k_2 is the rate constant for decomposition of ES to the starting compounds, and k_3 is the rate constant for the formation of the reaction products.

The experimentally measurable reaction rate (V)

$$V=\frac{k_3[E][S]}{K_M+[S]} \quad (2)$$

Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 3, pp. 295-298, March, 1971. Original article submitted February 5, 1970.

TABLE 1. Comparative Characteristics of the Resistance of Semisynthetic Penicillins and Benzylpenicillin to the Action of Penicillinase 749/c



Compound	R	$k_{3\,rel}$	Literature*
I	2-Furyl	124	19
II	Benzyl	100	†
III	5-Nitro-2-furyl	68	12
IV	2-(5-Nitro-2-furyl)vinyl	36	12
V	1-(2-Furyl)-2-propenyl	35	14
VI	1-(2-Furyl)-2-butenyl	35	14
VII	2-(2-Furyl)ethyl	35	14
VIII	1-(5-Nitro-2-furyl)-2-propenyl	33	12
IX	1-(5-Nitro-2-furyl)-2-butenyl	33	12
X	2-(2-Furyl)vinyl	25	14
XI	1-(p-Nitrophenyl)-2-(5-nitro-2-furyl)vinyl	16	12
XII	2-(5-Nitro-2-furyl)-1-phenylvinyl	13	12
XIII	2-Furylethynyl	10	14
XIV	2-Bromo-2-(5-nitro-2-furyl)vinyl	9	12
XV	5-Nitro-2-furylethynyl	8	14
XVI	1-(p-Nitrophenyl)-2-(2-furyl)vinyl	8	14
XVII	1-Phenyl-2-(2-furyl)vinyl	7	14
XVIII	5-Methoxy-2-[1-carboxy-2-(5-nitro-2-furyl)vinyl]phenyl	6	13
XIX	2,4-Dimethyl-3-furyl	5	11
XX	2,4-Dimethyl-5-nitro-3-furyl	5	11
XXI	2-[1-Carboxy-2-(5-nitro-2-furyl)vinyl]phenyl	4	13
XXII	5-Chloro-2-[1-carboxy-2-(2-furyl)vinyl]phenyl	4	13
XXIII	5-Methoxy-2-[1-carboxy-2-(2-furyl)vinyl]-phenyl	4	13
XXIV	2-[1-Carboxy-4-(2-furyl)-1,3-butadien-1-yl]phenyl	2	13
XXV	2-[1-Carboxy-4-(5-nitro-2-furyl)-1,3-butadien-1-yl]phenyl	2	13
XXVI	2,6-Dimethoxyphenyl	0.6	3
XXVII	5-Methyl-3-phenyl-4-isoxazolyl	0.4	4

*Method of preparation.

†Krasnoyarsk Medicinal Preparations Plant.

depends on the substrate and enzyme concentrations and the Michaelis constant (K_M). Different quantitative ratios of E and S can change the reaction order from zero to one. If the substrate concentration is so high that it ensures complete saturation of the enzyme, i.e., $K_M \ll S$, the reaction is zero order with respect to the substrate concentration, and its rate reaches a maximum value

$$V = V_{max} = k_3[E]. \quad (3)$$

The relative k_3 values ($k_{3\,rel}$), taking k_3 of benzylpenicillin as 100, are usually calculated for a comparative estimate of the resistance of various penicillins to penicillinase [15, 16].

The $k_{3\,rel}$ values for the enzymatic hydrolysis of semisynthetic penicillins and benzylpenicillin by the penicillinase Bacillus licheniformis 749/c are presented in Table 1. It is apparent from Table 1 that furan and 5-nitrofuran penicillins are inferior to methicillin (XXVI) and oxacillin (XXVII) with respect to resistance to the enzyme but considerably surpass benzylpenicillin (except for I). The rate of cleavage of the β-lactam ring of the penicillins is directly proportional to the structure of their side chains. The data obtained make it possible to ascertain the inhibiting action of various structural groupings of the side chain of the penicillins on their rate of inactivation. As would be expected, the introduction of bulky substituents in the ortho position to the amide group in the benzene and furan rings led to a sharp decrease in $k_{3\,rel}$ for

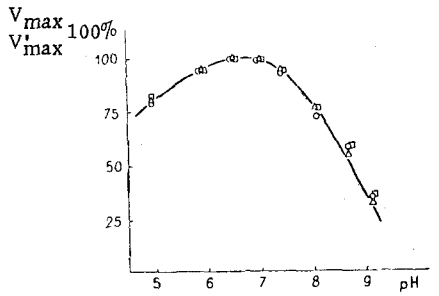


Fig. 1

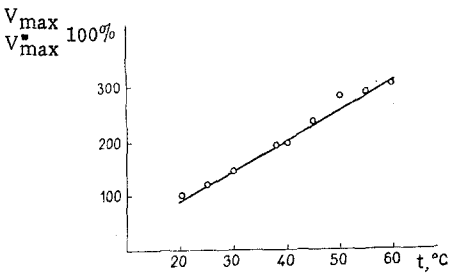


Fig. 2

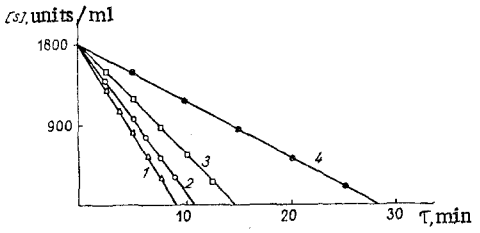


Fig. 3

Fig. 1. Dependence of the activity of penicillinase Bacillus licheniformis 749/c on the pH of the medium with II (Δ), IV (○), and X (□) as the substrate (at 37°; V'_{max} is the maximum rate of hydrolysis at pH 7.0).

Fig. 2. Dependence of the activity of penicillinase Bacillus licheniformis 749/c on the temperature with II as the substrate (pH 7.0; V''_{max} is the maximum rate of hydrolysis at 20°).

Fig. 3. Kinetics of the enzymatic hydrolysis of penicillins by penicillinase Bacillus licheniformis 749/c ([E] = 171 units/ml, 37°, pH 6.8-7.0): 1) I; 2) II; 3) III; 4) IV.

XIX-XXV (a factor of 20-50 in comparison with benzylpenicillin). The same effect, although to a lesser degree, is observed in the aliphatic chain of XI, XII, XIV, XVI, and XVII on replacement of the hydrogen atom in the α position to the amide group by a phenyl or p-nitrophenyl radical or of a hydrogen in the β position by a bromine atom.

The resistance of the new penicillins to the enzyme is provided not only by steric factors but also by the system itself of 5-nitrofuran and furan rings conjugated with the vinylene and ethynylene groups. Due to this property, X and XIII, which do not have bulky substituents in the α position, surpass I in resistance to the enzyme by factors of 5 and 12.5, and a decrease in $k_{3\,rel}$ by a factor of two is also observed for XXV, as compared with XXI, on lengthening the side chain by one vinylene group.

The investigated penicillins can be divided into three groups with respect to the character of the effect of the nitro group in the furan ring on the rate of the enzymatic reaction. The first group contains compounds which contain furan-2-carboxylic, propiolic, and α-alkylacrylic acid residues in the side chain (I, III, XIII, XV, V, VI, VIII, and IX). A higher resistance of the 5-nitrofuran penicillins is observed in this group, as compared with their furan analogs, with respect to the inactivating action of the enzyme. The reverse phenomenon is observed in the second group, which includes penicillins of the acrylic, α-phenylacrylic, and homophthalic series (IV, X, XI, XII, XVI, XVII, XVIII, and XXIII). The third group is made up of penicillins obtained from furan-3-carboxylic and furylallylidenehomophthalic acids (XIX, XX, XXIV, and XXV) for which the presence or absence of a nitro group is not reflected in their enzyme resistance.

EXPERIMENTAL

Penicillins with 90-100% purity and penicillinase Bacillus licheniformis 749/c were used to study the kinetics of the enzyme reaction. The minimum amount of penicillinase which was able to inactivate 10^{-7} mole of benzylpenicillin in 1 h at 37° and pH 6.8-7.0 [17] was taken as its unit activity. A study of the effect of the pH and temperature on the enzyme activity made it possible to select the optimum reaction conditions for

which the enzyme action is most effective (see Figs. 1 and 2). The penicillins were inactivated at 37° in 0.1 M phosphate buffer at pH 6.8-7.0. The amount of undecomposed antibiotic was determined by the hydroxylamine method [18]. The initial penicillin concentration in all cases was constant at 1800 units/ml. For the enzyme this value ranged from 85 to 26,000 units/ml, depending on the properties of each antibiotic and calculated in such a way that the reaction was zero order and was complete in about 30 min. The reaction rate was determined graphically (Fig. 3). The average results of three parallel experiments were used for the calculations, and the relative deviation between them did not exceed 3%.

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