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THE MECHANISM OF INACTIVATION OF PENICILLIN BY CYSTEINE AND OTHER MERCAPTOAMINES

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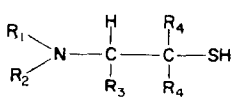
Abstract—The kinetics of penicillin inactivation by various mercaptoamines have been followed by polarimetry. In the case of primary mercaptoamines the reactions are of second order with respect to time and concentration of the reactants. By determining the appropriate rate constants under different conditions of pH and ionic strength the conclusion is reached that the rate limiting step is a bimolecular reaction involving the mercaptide ion. The mechanism probably involves the formation of a semimercaptol. Several possible mechanisms for the role of the amino group in the opening of the β-lactam ring are discussed. The inactivation of penicillin by the tertiary mercaptoamine N:N-dimethylcysteamine nearly follows first order kinetics and gives as end product a partially racemized penicilloic acid.

THE early discovery that penicillin is rapidly inactivated by cysteine and related mercaptoamines with opening of its β-lactam ring[1] focused interest on the reactivity of this structure, and it has been widely assumed that the specific binding of penicillin to sensitive bacteria and its antibiotic action involves a reaction of the β-lactam ring.[2]

The details of the interaction of mercaptoamines and amines with the β-lactam ring of penicillin are still inadequately understood. In the early inactivation studies which have been thoroughly reviewed by Wintersteiner *et al.*[3] only qualitative evaluations were carried out. This work permitted a classification of the compounds tested into three groups: "Fast inactivators", "slow inactivators", and compounds exerting no measurable effect. The 1:2-mercaptoamines were found to be "fast penicillin inactivators". It was established that the activity of the fast inactivators is dependent on the presence of a free sulphydryl group and a basic amine group on the adjacent carbon atom. The influence of substituents on the inactivating ability of 1:2-mercaptoamines may be summarized as follows:



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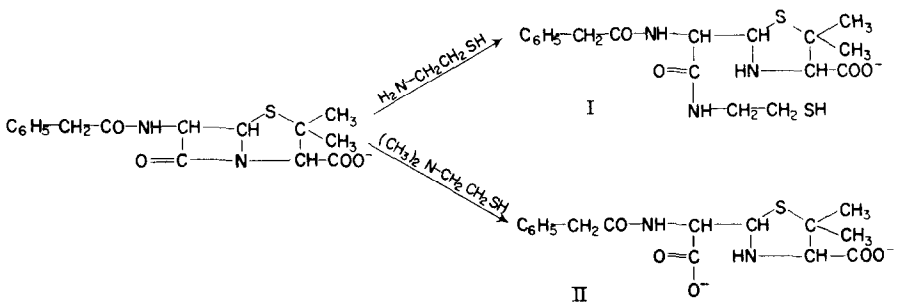
Inactive	*Active*
R_1 = Acyl	R_1 = H or Alkyl
R_3 = Alkyl	R_2 = H or Alkyl
R_4 = Alkyl	R_3 = H, COO^- or COOR
	R_4 = H

* Fellow of Landsforeningen mot Kreft (The Norwegian Cancer Society).

With primary mercaptoamines the overall reaction product was found to be the N-penicilloyl amides (I), whereas with tertiary amines such as N:N-dimethylcysteamine, the corresponding reaction product was the free penicilloic acid (II).



It is thus quite clear that these reactions involve an attack of the inactivator on the carbonyl carbon atom of the β-lactam ring with a rupture of the amide linkage.

In the present paper kinetic data are presented on the inactivation of penicillin by various mercaptoamines and amines. Use has been made of the fact that the opening of the β-lactam ring can be followed by measuring the decline of the optical rotation of the penicillin salt.[3] A mechanism is proposed for the reaction of penicillin with primary and secondary β-mercaptoamines, a mechanism which may have a bearing on the antibiotic activity of penicillin.

EXPERIMENTAL

Materials. The sodium benzylpenicillinate used was a gift from Apothekernes Laboratorium, Oslo. The specific rotation $[\alpha]_D^{23}$ of a 10^{-2} M solution in 0·5 M phosphate buffer at pH 7·4 was +288°. N:N-Dimethylcysteamine and N:N-diethylcysteamine were generously supplied by Deutsche Gold- und Silber-Scheideanstalt. Frankfurt a.M.; L(−)Cysteine hydrochloride was obtained from Hoffmann-La Roche & Co., Basel; D(+)cysteine from Fluka AG. Chemische Fabrik Buchs/SG; L(−) cysteine ethyl ester from Nutritional Biochemicals Corp., Cleveland; L(−)cysteinylglycine from Schwarz Laboratories, Mount Vernon; D(−)penicillamine·HCl·1/2 H_2O from California Foundation for Biochemical Research, Los Angeles; and hydroxylamine hydrochloride from Hopkin & Williams, Ltd., London. Penicillinase was purchased from Schenley Laboratories Inc., New York.

Cysteamine was prepared according to Gabriel *et al.*[4] and aminoethyl*iso*thiouroniumbromide hydrobromide (AET) was synthesized according to the method of Clinton *et al.*[5]

Polarimetric measurements. The reactions were carried out in strong (0·5 M) oxygen-free phosphate buffer in order to counteract the fall in pH during the course of the reaction. The buffer contained 0·02 M disodium ethylenediaminetetra-acetate (EDTA) in order to prevent oxidation of the thiols. The buffer was prepared from doubly distilled water and the solid components were dissolved under a stream of oxygen-free nitrogen. The reactions were started by the addition of penicillin. The polarimeter

tube (20 cm length) was filled under a stream of nitrogen. Readings were taken every minute. The fraction of penicillin which had reacted at any time was estimated from the formula

$$f = \frac{\alpha_0 - \alpha_t}{\alpha_0 - \alpha_\infty}$$

where α_0 is equal to the initial rotation, α_t is the rotation observed at the time t, and α_∞ is the final rotation observed in the reaction mixture. The initial rotation of the reaction mixture was calculated from the specific rotation of penicillin. When D(+)- or L(−)cysteine was used as inactivators, allowance was made for the optical rotation of these compounds. The necessary correction factors varied from 0·98 to 1·02.

Hydroxamic acid was measured according to Lipmann and Tuttle.[6]

RESULTS

The present kinetic measurements are based on the fact that the opening of the β-lactam ring of penicillin is associated with a change in optical rotation. The data here presented will therefore reflect the overall rate of the reaction sequence leading to the rupture of the amide linkage. In Fig. 1 typical data are presented.

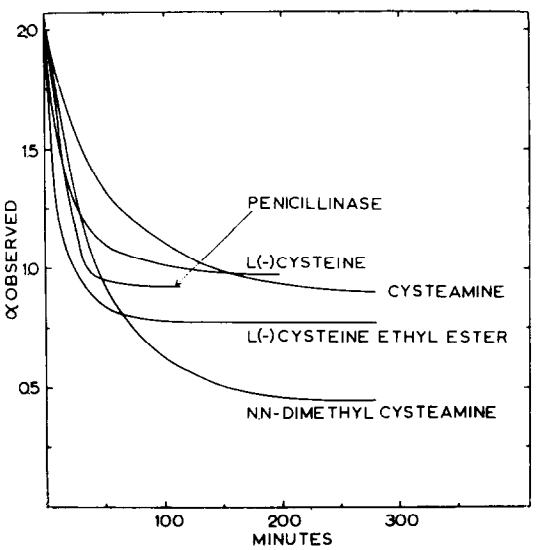
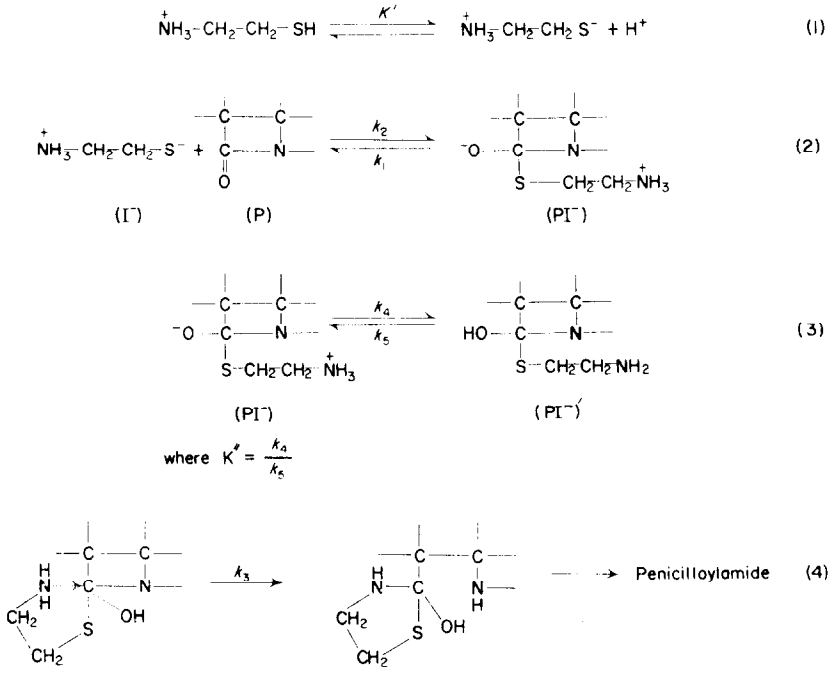


FIG. 1. The inactivation of penicillin followed by polarimetry. The concentration of penicillin was 10^{-2} M and that of the mercaptoamines $1{\cdot}5 \times 10^{-2}$ M, except for N-dimethylcysteamine, which was 3×10^{-2} M. Penicillinase (1000 Schenley units) was incubated with $2{\cdot}5 \times 10^{-3}$ M penicillin. The α plotted in this case is equal to observed $\alpha \times 4$.

The assumed reaction mechanism

In order to facilitate the subsequent discussion it is proposed to present at this time the reaction mechanism which in our view is considered the most likely one. The reactions are believed to proceed by way of the semimercaptol and the C_2-hydroxythiazolidine to give the penicilloylamide (reactions 1–4). This reaction mechanism has features in common with the thiazolidine mechanism proposed by Woodward *et al.* (see ref. 3). On the basis of previous work in this laboratory on the interaction of

cysteamine with aldehydes[8] to form thiazolidines, it is believed that the semimercaptol formation (reaction 2) is the rate limiting step.



(1) (2) (3) (4)

Whereas reactions 1–3 are reasonably well established by our data, reaction 4 is open to discussion.

In the above scheme reactions 1 and 3 undoubtedly are almost instantaneous.

If it is assumed that the semimercaptol is present in small and constant amounts during the reaction (steady state), the rate constant k for the overall reaction can be shown to be:

$$k = \frac{k_2 \cdot k_3 \cdot K''}{k_1 + k_3\left(\frac{k_1}{k_5} + K''\right)} = \frac{k_2}{\frac{k_1}{k_3 K''} + \frac{k_1}{k_4} + 1} \tag{5}$$

If $k_3 \cdot K'' \gg k_1$ and if $k_4 \gg k_1$, k equals k_2. The reaction will then obey the following equation:

$$\frac{dP}{dt} = k_2(I^-)(P) \tag{6}$$

It follows from equation 1 that:

$$(I^-) = \frac{(I_{\text{total}})}{1 + 10^{pK - p\text{H}}} \tag{7}$$

If I_{total} is substituted for I^- in equation 6, equation 8 is obtained:

$$\frac{dP}{dt} = k_p(I_{\text{total}})(P) \tag{8}$$

where k_p is the practical rate constant. Clearly,

$$k = k_p(1 + 10^{pK-pH}) \tag{9}$$

The determination of rate constants

It follows from equation 6 and 8 that the assumed reaction mechanism requires that the inactivation of penicillin by cysteamine be second order both with respect to time and the concentration of the reactants. When the inactivation of penicillin by primary mercaptoamines was plotted as second order reactions with respect to time, straight lines were obtained up to the point where about 40 to 50 per cent of the inactivator had reacted (Fig. 2). This was the case irrespective of the concentration of the react-

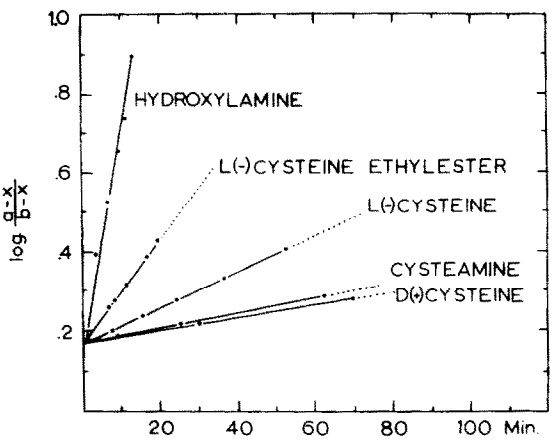


FIG. 2. Second order rate plots of penicillin inactivation by various mercaptoamines and amines. Conditions: pH 7·05, temperature 23 °C, penicillin concentration 10^{-2} M, inactivator concentration $1{\cdot}5 \times 10^{-2}$ M.

ants when L(−)- and D(+) cysteine, cysteamine, L(−)cysteine ethyl ester, L(−)-cysteinylglycine, and penicillamine were used as inactivators. The deviation from second order kinetics in the last part of the reaction, may be attributed to a small but unavoidable drop in the pH of the solution. It was concluded that the reactions were indeed of second order and the rate constants were calculated from the equation:

$$k_p = \frac{2{\cdot}303}{(a-b)t} \log \frac{b(a-x)}{a(b-x)} \tag{10}$$

where a and b are the initial concentration of the inactivator and of penicillin, respectively, and x is the number of moles of penicillin which had reacted at any time. The practical rate constants k_p as well as the corresponding k values are presented in Table 1.

In the case of the tertiary amine N:N-dimethylcysteamine the reaction was found to be nearly first order in any one run. The slight deviation from first order kinetics (Fig. 3) suggests that some intermediate product does accumulate. The most probable intermediate is S-penicilloyl-N:N-dimethylcysteamine, a compound which would be

TABLE 1. THE RATE OF INACTIVATION OF PENICILLIN BY VARIOUS MERCAPTOAMINES AND AMINES

Inactivator	pH	Conc. of reactants		k_p*	pK of inactivator	k†
		Penicillin mM	Inactivator mM			
L(−)Cysteine	6·76	15	10	1·06	$pK_2 = 8·37$	44·2
L(−)Cysteine	6·76	10	10	0·97	$pK_2 = 8·37$	40·4
L(−)Cysteine	6·75	15	20	1·01	$pK_2 = 8·37$	43·1
L(−)Cysteine	7·05	10	15	2·00	$pK_2 = 8·37$	43·8
L(−)Cysteine	7·03	15	10	1·98	$pK_2 = 8·37$	45·3
L(−)Cysteine	7·00	20	15	1·64	$pK_2 = 8·37$	39·8
L(−)Cysteine	7·03	15	20	1·77	$pK_2 = 8·37$	40·5
L(−)Cysteine	7·50	15	10	4·87	$pK_2 = 8·37$	40·7
L(−)Cysteine	7·57	10	10	5·50	$pK_2 = 8·37$	40·2
L(−)Cysteine	7·61	7·5	10	6·08	$pK_2 = 8·37$	41·1
D(+)Cysteine	7·05	15	10	0·78	$pK_2 = 8·37$	17·0
D(+)Cysteine	7·05	15	10	0·77	$pK_2 = 8·37$	16·5
D(+)Cysteine	7·27	15	10	1·25	$pK_2 = 8·37$	17·0
D(+)Cysteine	7·27‡	15	10	1·25	$pK_2 = 8·37$	17·0
Cysteamine	7·07	10	7·5	1·02	$pK_1 = 8·35$	20·5
Cysteamine	7·05	10	15	0·87	$pK_1 = 8·35$	18·2
Cysteamine	7·05	20	15	0·98	$pK_1 = 8·35$	20·6
Cysteamine	7·47	10	7·5	2·28	$pK_1 = 8·35$	19·6
L(−)Cysteine ethyl ester	6·28	15	10	2·22	$pK_1 = 6·69$	7·9
L(−)Cysteine ethyl ester	6·27	10	15	2·24	$pK_1 = 6·69$	7·9
L(−)Cysteine ethyl ester	7·04	10	15	6·15	$pK_1 = 6·69$	8·9
L(−)Cysteine ethyl ester	7·04	15	10	6·12	$pK_1 = 6·69$	8·9
L(−)Cysteine ethyl ester	7·29	10	15	8·58	$pK_1 = 6·69$	10·7
N:N-Dimethyl cysteamine§	7·05	10	10	0·41	$pK_1 = 7·70$	2·2
N:N-Dimethyl cysteamine§	7·05	10	20	0·39	$pK_1 = 7·70$	2·1
N:N-Dimethyl cysteamine§	7·06	20	10	0·39	$pK_1 = 7·70$	2·1
N:N-Dimethyl cysteamine§	7·44	10	30	0·75	$pK_1 = 7·70$	2·1
N:N-Dimethyl cysteamine§	7·49	10	10	0·82	$pK_1 = 7·70$	2·2
Hydroxylamine	6·37	10	15	12·5	$pK_1 = 6·03$	18·2
Hydroxylamine	7·05	10	7·5	18·0	$pK_1 = 6·03$	19·7
Hydrazine	6·95	15	15	0·45	$pK_1 = 7·93$ $pK_1 = 8·2$	4·8 8·5
Hydrazine	6·95	20	15	0·42	$pK_1 = 7·93$ $pK_1 = 8·2$	4·4 7·8
Hydrazine	7·28	10	15	0·68	$pK_1 = 7·93$ $pK_1 = 8·2$	3·7 6·4
Hydrazine	7·31	20	15	0·66	$pK_1 = 7·93$ $pK_1 = 8·2$	3·4 5·8

* k_p (in l. × moles^{-1} × min^{-1}) is the practical rate constant calculated from equation 10.

† k (in l. × moles^{-1} × min^{-1}) is the rate constant for the ionized form of the inactivator (equation 9).

‡ 0·05 M NaCl added.

§ The rate constants are calculated from the initial rates observed.

The second order rate constants were determined at 23 °C in 0·5 M phosphate buffer. Ionic strength 1·37–1·40.

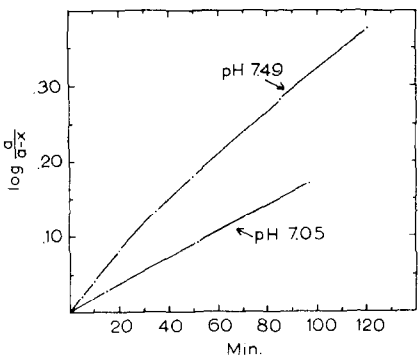


FIG. 3. First order rate plot of penicillin inactivation by N:N-dimethylcysteamine. Concentration of reactants 10^{-2} M. Temperature 23 °C.

expected to form hydroxamic acid with hydroxylamine. In experiments where the optical rotation and the amount of hydroxamic acid forming substance in solution were simultaneously measured (Fig. 4) evidence in favour of this hypothesis was obtained. It is clear, however, that the amount of intermediate formed is slight. It was found that the *initial* reaction rates were first order both with respect to penicillin and the inactivator. The second order rate constants thus determined, are presented in Table 1.

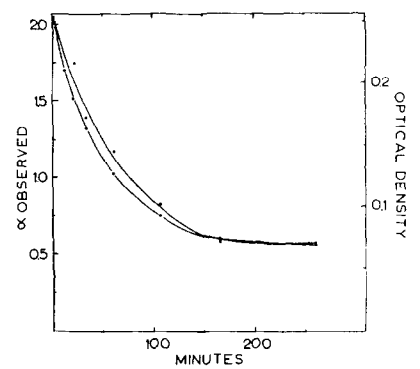


FIG. 4. The inactivation of penicillin by dimethylcysteamine. The concentration of penicillin was 10^{-2} M and that of dimethylcysteamine 3×10^{-2} M. pH 7·9, temperature 23 °C, M/15 phosphate buffer. Lower curve: The reaction followed by polarimetry. Upper curve: The hydroxamic acid formation measured spectro-photometrically in samples taken at different time intervals.

The effect of ionic strength

It can be seen from Table 1 that, whereas the practical rate constants k_p as expected increased considerably with increasing pH, the rate constants k (see equation 7) were, with one exception, independent of pH in the range studied. This finding demonstrates that the reactive form of the inactivator has released one proton. Thus, cysteamine and N:N-dimethylcysteamine will in their reactive form possess a zero net charge. whereas cysteine will have a net charge of minus one. Since penicillin possesses one

negative charge, an increase in the ionic strength would be expected to increase the reaction rate in the case of cysteine, but to have no effect in the case of cysteamine derivatives.[9] These expectations are borne out by the findings in Table 2. Although the Brönsted equation is not quantitatively obeyed because of the very high ionic strength in these experiments, the data seem to demonstrate that a bimolecular reaction (*i.e.*, the first step) is rate limiting.

TABLE 2. THE EFFECT OF SALTS ON THE RATE OF INACTIVATION OF PENICILLIN

Inactivator	pH	Salt added	k_p l mole^{-1} min^{-1}
L(−)Cysteine	6·96	None	1·94
L(−)Cysteine	6·96	3M NaCl	3·25
L(−)Cysteine	6·83	None	1·17*
L(−)Cysteine	6·83	3M Guanidine·HCl	2·20
D(+)Cysteine	6·84	None	0·48*
D(+)Cysteine	6·84	3M Guanidine·HCl	0·95
Cysteamine	6·93	None	0·73*
Cysteamine	6·93	3M Guanidine·HCl	0·74
N:N-Dimethyl-cysteamine	6·95	None	0·33*
N:N-Dimethyl-cysteamine	6·95	3M Guanidine·HCl	0·34

Concentration of penicillin 0·01 M, conc. of inactivator 0·015. The temperature was 23 °C. Otherwise, conditions as in Table 1.

* Calculated on the basis of the data in Table 1.

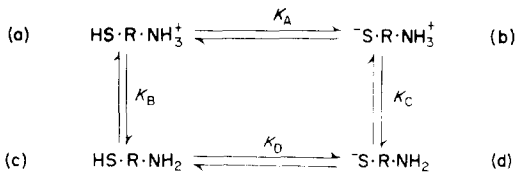
The effect of pH

In equation 7, pK is the titratable pK referring to the dissociation of one proton in the pH interval studied (*e.g.* the pK_1 in the case of cysteamine, and the pK_2 in the case of cysteine). It thus refers to the release of a proton from either the thiol or the amino group. If equation 9 is satisfied with a pK value which unequivocally refers to the thiol or the amino group, this finding constitutes evidence that the group in question is involved in the rate limiting step. For mathematical reasons, such an agreement will carry weight only when the experiments are performed in a pH interval close to the pK of the group in question. It appears from Table 1 that in most experiments this condition is fulfilled.

In the case of cysteamine, the dissociation constants of the thiol and the amino groups are so widely separated that in the pH range studied the pK_1 reflects almost exclusively the dissociation of the thiol group. The data of Table 1 therefore strongly indicate that the rate limiting step involves the ionized thiol. The same holds true for N:N-dimethylcysteamine, since its tertiary amino group undoubtedly is still more basic than the primary amine in cysteamine, and the pK_1 therefore refers unequivocally to the sulphydryl group.

The interpretation of some of the pH data of Table 1 is rendered difficult by the fact that a complicated relationship exists between the pK, as determined by titration, and the relative extent of dissociation of the two ionizable groups.

According to Benesch and Benesch[10] the mercaptoamines dissociate according to the following diagram:



It can be shown that the concentration of b relative to c is independent of pH. In the case of cysteine, cysteine ethyl ester, and cysteinylglycine, the dissociation constants for the sulphydryl and the amino group are so close that significant amounts of c will be present together with b. It can be calculated from the equilibrium constants given by Benesch and Benesch that for these compounds the ratio of b to c is 2·1, 0·21, and 0·19, respectively. In our experiments pH (6·3–7·3) the concentration of d is negligible for the mercaptoamines of Table 1, except for cysteine ethyl ester. For this compound appreciable amounts of the molecular species d will appear with increasing pH in the range studied. This fact may explain the finding (Table 1) that k rises with increasing pH, since the mercaptide group as well as the amino group of the molecular species d will be expected to possess a very high nucleophilic reactivity.

Since I^- of equation 6 equals b + c, the kinetic data fail to reveal which chemical group participates in the rate limiting step in the case of cysteine, cysteine ethyl ester and cysteinylglycine. Certain conclusions can, however, be drawn with the aid of data from other systems[11,12] (Table 3). The comparisons here carried out support the conclusion that the rate determining step involves the thiol group. Thus, it can be seen that when the rate constants are calculated on the basis of the concentration of the mercaptide

TABLE 3. THE RELATIVE RATES OF INTERACTION OF VARIOUS MERCAPTOAMINES AND AMINES WITH PENICILLIN, CYSTINE AND CYSTAMINE

Compound	pK	Penicillin			Cystine	Cystamine
		k	k_{S^-}	k_N	k_{S^-}	k_{S^-}
L(−)Cysteine	8·37*	42	63	126	25,000	19,500
Cysteamine	8·35*	20	20	>5000	12,500	9300
D(+)Cysteine	8·37*	17	25	53	—	—
L(−)Cysteinylglycine	7·07*	4–6	30–35	5–6	14,200	—
L(−)Cysteine ethyl ester	6·69*	8–12	50–90	11–18	9700	7800
N-Dimethylcysteamine	7·70†	2·1	2·1	2100	4600	—
N-Diethylcysteamine	7·80†	1·1	1·1	1100	5900	—
D(+)Penicillamine	7·90†	0·0004	—	—	4400	1400
AET	—	0·0	—	0	—	—
Hydroxylamine	6·03†	19	—	19	—	—
Hydrazine	~8†	4–8	—	4–8	—	—
D,L-Serine	9·15†	0·0	—	0·000	—	—
D,L-Serine methyl ester	7·05‡	0·0	—	0·000	—	—
Ethylenediamine	7·35	0·3	—	0·3	—	—

In the experiments with penicillin the k values are the same as those given in Table 1 whereas k_{S^-} and k_N are the corresponding rate constants obtained on the assumption that the ionized thiol group or the nucleophilic amine group, respectively, were responsible for the rate determining step. The rate constants k_{S^-} given in the reactions with cystine and cystamine refer to the interaction (at pH 7·4 and 37°C) of the ionized thiol with one of the sulphur atoms of the disulphides[11,12].

* Calculated from the data of Benesch and Benesch.[10]
† See Wintersteiner *et al.*[3]
‡ Own determination.

ions, the relative rate of interaction of cysteine, cysteamine, cysteinylglycine, N:N-dimethylcysteamine and N:N-diethylcysteamine with penicillin corresponds fairly well with their relative rates of reaction with disulphides. Cysteine ethyl ester, however, inactivates penicillin at a somewhat faster rate than could be expected from its nucleophilic reactivity observed in the other systems. As pointed out above, for this compound c/d = 0·21, *i.e.* the concentration of nucleophilic amine is far larger than that of the mercaptide ion. Although a direct aminolysis does occur in the case of hydroxylamine, hydrazine and ethylenediamine (Tables 1 and 3), such a mechanism is considered unlikely in the case of cysteine ethyl ester in view of the finding that serine methyl ester with a pK_N of 7·05 did not inactivate penicillin.

Altogether, the results from the pH-experiments strongly indicate that in the interaction of penicillin with mercaptoamines the rate limiting step involves the S^- ion.

Racemization

The final specific rotation in the case of the primary mercaptoamines (*i.e.* that of the penicilloylamides) falls in the same range as that of the free penicilloic acid formed upon incubation with penicillinase (Fig. 1). It is therefore likely that in the case of the primary mercaptoamines the opening of the β-lactam ring proceeds without racemization of any of the three optical carbon atoms.

Despite the fact that the present kinetic data confirm that the final reaction product in the case of the N:N-dialkylcysteamines is free penicilloic acid, it was found that the final specific rotation deviates considerably from that of the enzymatically prepared penicilloic acid. Apparently, in these cases the reaction mechanism involves a racemization of at least one of the three optical carbon atoms.

DISCUSSION

The data here presented seem to establish that a bimolecular reaction is rate limiting and, furthermore, that the rate limiting step involves the mercaptide ion. Since the optical measurements reflect the opening of the β-lactam ring, either the rate limiting step itself results in the opening of the ring, or it is effected by a fast subsequent step.

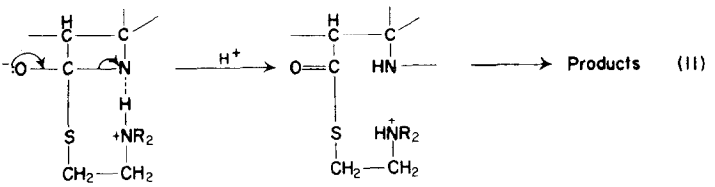
The fact that some amines react almost as rapidly as the aminothiols (Table 2) leads to an element of doubt in the proposed scheme. The data demonstrate that hydroxylamine and hydrazine react at a rapid rate, ethylenediamine and dimethylamine react at a moderate rate, whereas the amino groups of larger molecules such as serine and serine methyl ester are unable to react, in spite of the fact that the pK_N of serine methyl ester is 7·05. These findings indicate that the aminolysis is strongly influenced by steric factors, and it appears unlikely that the scission of the ring by mercaptoamines can be accounted for to any significant extent by a direct aminolysis.

A direct thiolysis is unlikely in view of the previously established facts (Wintersteiner *et al.*[3]) that a number of thiols and N-acetylated mercaptoamines are unable to inactive penicillin.

In our opinion the first step probably involves the formation of a semimercaptol, in accordance with equations 1–3. This view is supported by the fact pointed out by Woodward *et al.*[7] that the amide linkage of the strained β-lactam ring of penicillin is only to a limited extent stabilized by resonance, a circumstance which tends to confer ketonic properties on the amide carbonyl group.

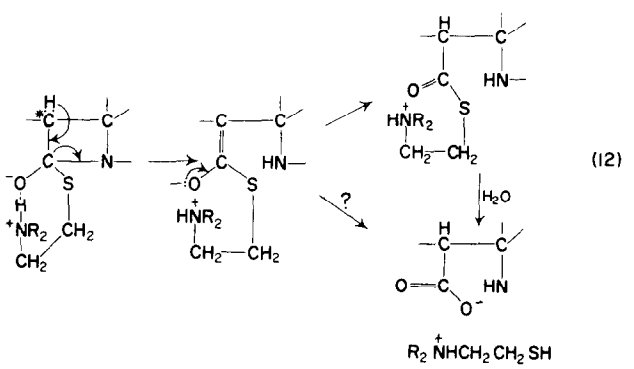
The breaking of the C–N bond of the β-lactam ring is obviously facilitated by the presence of the amino group. This effect can conceivably be brought about in several ways.

One possibility is that the amino group catalyzes the reaction by an electrostatic effect on the nitrogen atom as depicted in equation 11:



This possibility seems unlikely, however, as such a mechanism can not account for the fact that N:N-dimethylcysteamine, in contrast to the primary mercaptoamines, causes a reacemization of the penicillin. Also it is known that the tertiary nitrogen group of the β-lactam ring is not titratable, which renders it unlikely that this nitrogen atom can participate in hydrogen bond formation.

An alternative possibility is that the amino group of the mercaptoamine forms a hydrogen bond with the hydroxyl group of the semimercaptol. Such an interaction will be expected to facilitate the formation of a double bond (equation 12) with concomitant racemization of carbon atom no. 3. This mechanism might explain the observed racemization in the case of N:N-dimethylcysteamine. The product will presumably rearrange to the thioester which is subsequently hydrolyzed.



Thioesters in general are known to be fairly stable at pH 7. Recent data suggest, however, that an amine group in position β to the thiol strongly influences the rate of hydrolysis. Thus, Hansen[11] has found that 2-N:N-dimethylaminoethyl-thiolpropionate is hydrolyzed 240 times faster than the corresponding N-trimethyl derivative and that the Me_2HN^+ form reacts very much faster than the uncharged form of the amino group.

The small racemization, if any, which was observed in the case of primary mercaptoamines, can be satisfactorily explained by assuming reaction 4 to take place more rapidly than reaction 12. The initial semimercaptol formation will abolish the resonance

between a carbonyl group and a nitrogen atom and thereby lower the stability of the ring. Furthermore, a semimercaptol formation may be expected to lower the p*K*-value of the amine by way of an inductive effect along the carbon chain and thereby increase the concentration of the active nucleophilic amine. Obviously, the attack of the amino group is strongly favoured by its steric position.

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