Thiol-catalysed hydrolysis of benzylpenicillin

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Thiolysis of benzylpenicillin has been investigated by HPLC and ^{1}H-NMR techniques. Thiols catalyse the hydrolysis of benzylpenicillin through the formation of a thioester intermediate. The catalytically reactive form of the thiol has been demonstrated to be the thiolate anion. Variation of reactivity with changing basicity of the thiolate anion generates a Brønsted β_{nuc} value of 0.96, indicating that the breakdown of the tetrahedral intermediate is the rate-limiting step, as occurs in aminolysis and alcoholysis. Solvent kinetic isotope effects of 2.2–2.4 indicate that the solvent, water, probably acts as a general acid catalyst in the breakdown of the tetrahedral intermediate. PM3 theoretical calculations support the proposal that breakdown of the tetrahedral intermediate is rate-limiting. The experimental activation energies for the thiolysis of benzylpenicillin vary from 6.9 to 10.4 kcal mol^{-1}.†

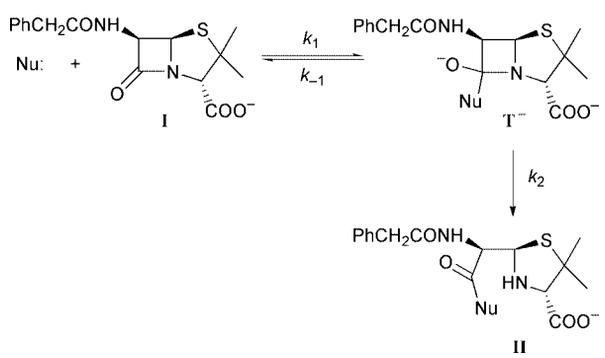
Introduction

β-Lactam antibiotics interfere with bacterial cell-wall synthesis by acylating enzymes—penicillin binding proteins (PBPs)—a process critical to the production of the cell wall. These antibiotics share a common structural similarity, the four-membered β-lactam ring, which in most cases is fused to a secondary ring structure. A thiazolidine ring is present in penicillins, and a dihydrothiazine ring in cephalosporins. In addition to the traditional families of penicillins and cephalosporins there are many other related structures with high antibacterial activity and low toxicity.[1–3]

The reactions of β-lactam antibiotics and their derivatives have been extensively studied.[4–6] The β-lactam ring of β-lactam antibiotics shows susceptibility towards attack by nucleophilic reagents in water, such as amines and alcohols, in competition with that by hydroxide ion. Nucleophilic substitution at the carbonyl centre of β-lactams is an acyl transfer process involving covalent bond formation between the carbonyl carbon and the nucleophile and C–N bond fission of the β-lactam. Previous studies with nitrogen and oxygen nucleophiles have shown that covalent bond formation to the incoming nucleophile occurs before C–N bond fission, resulting in the reversible formation of a tetrahedral intermediate (Scheme 1).[4–6] The rate-limiting step in these reactions is the breakdown of the tetrahedral intermediate (or ring-opening).[5–7]

The rates of alkaline hydrolysis of β-lactams exhibit a first-order dependence on hydroxide ion concentration, but in this case the rate-limiting step appears to be formation of the tetrahedral intermediate.[7,8]

Oxygen anions can catalyse the hydrolysis of penicillins by either acting as general bases or as nucleophiles. Weak oxygen bases act as general bases, whereas basic oxygen anions of $pK_a > 9$ act as nucleophilic catalysts for hydrolysis with the intermediate formation of a penicilloyl ester.[6] The reaction of various phenolate anions, carbohydrates—such as glucose and fructose—and other hydroxy-containing compounds with penicillins and/or cephalosporins has been shown to proceed through the intermediate formation of an unstable ester.[9–13] The phosphate catalysed hydrolysis of benzylpenicillin (**I**) is thought to involve the intermediate formation of a penicilloyl phosphate ester.[14]



Scheme 1

The reaction of amines with penicillins gives the corresponding penicilloyl amide.[15] The aminolysis of penicillins is an amide exchange or a substitution reaction in which an acyl group is transferred from one amino group to another, involving the C–N bond fission of the β-lactam. This reaction requires at least two proton transfers, proton removal from the attacking amine and proton addition to the leaving amino group. There is kinetic evidence for a stepwise process for the general base catalysed aminolysis of penicillins and cephalosporins, involving the reversible formation of a tetrahedral intermediate. For the uncatalysed aminolysis of β-lactam antibiotics the rate-limiting step is thought to be the β-lactam C–N bond fission.[11,15,16]

Despite the large amount of work undertaken on the hydrolysis of β-lactam compounds by nucleophilic reagents there are no studies of the thiol-catalysed hydrolysis of the β-lactam ring. The tendency of thiols to add to the carbonyl group of aldehydes is considerably greater than that of alcohols or most amines. Acyl transfer to thiols has been observed [17] and, for example, in the reaction of thiols with esters and thioesters there is a change in the rate-determining step with changing pK_a of the nucleophile and leaving group. The rate-determining step

† 1 kcal = 4.184 kJ.

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for basic thiols and good leaving groups is formation of the tetrahedral intermediate, which changes to breakdown for weakly basic thiols and/or poor and basic leaving groups.[17]

The present study is an investigation of the reaction of thiols with benzylpenicillin, which indicates that the rate-limiting step is the breakdown of the tetrahedral intermediate rather than its formation. This suggestion is supported by theoretical calculations. This reaction is also of interest because of the potential use of β-lactams to inhibit cysteine proteases.

Experimental

Materials

Thiols (methyl 3-mercaptopropionate, 3-mercaptopropane-1,2-diol, methyl 2-mercaptoacetate, 3-mercaptopropionic acid, 2,2,2-trifluoroethanethiol, 2-mercaptoethanol) were purchased from Sigma. Benzylpenicillin (potassium salt) was a gift from SmithKline Beecham and other materials were of AnalaR grade. Freshly boiled deionised water was used throughout and the ionic strength maintained at 0.5 mol dm^{-3} with potassium chloride. Deuterium oxide (99.9% D) was obtained from Sigma. The thiol buffers were prepared by partial neutralisation just prior to the kinetic run. Due to the high volatility and the oxidation of some of the thiols used in this study (particularly when alkaline solutions of thiols are used) the concentration of thiol in solution was measured just before its use in a procedure described by Ellmann.[18] In order to minimize the disulfide formation the experiments were carried out under argon.

HPLC

A Shimadzu-LC-9A chromatograph with a Rheodyne 7125 universal injector and a Shimadzu-SPD-M6A UV/VIS photodiode array detector were used for the HPLC experiments. The column was a Spherisorb ODS-25 × 0.46 cm × 5 μm; eluent 0.1 mol dm^{-3} NH_4OAc–MeCN 88 : 12; flow rate 1.2 ml min^{-1}.

The kinetics of the reaction were studied by following the decrease in HPLC peak areas for benzylpenicillin at 225–228 nm and at 30 °C. The reactions were initiated by the addition of 10 μl of 6.7×10^{-2} mol dm^{-3} of benzylpenicillin to 2.5 cm^3 of 0.01–0.05 mol dm^{-3} thiol buffer prepared at a constant ionic strength of 0.5 mol dm^{-3}, preincubated at 30.0 ± 0.1 °C.

For some thiols, 3-mercaptopropane-1,2-diol, 2-mercaptoethanol and methyl 2-mercaptoacetate, the rate constants were determined at 20, 25, 30 and 35.0 ± 0.1 °C as previously described[15] and the corresponding activation energies calculated according to the Arrhenius equation.

Deuterium solvent isotope effect

The second-order rate constants of thiolysis of benzylpenicillin with 2-mercaptoethanol and 3-mercaptopropane-1,2-diol were also determined in D_2O solution.

NMR Spectroscopy

The NMR spectra were obtained on a Bruker AMX-300 spectrometer. A sample tube of diameter 5 mm containing 3-(trimethylsilyl)propane-1-sulfonic acid (DSS) was used as internal reference. Chemical shift values (δ) are given in ppm and coupling constants are in Hz.

Product analysis

By analogy with the known substitution reactions of thiols with esters and amides, it was expected that the first product of the reaction of benzylpenicillin with thiols may be a thioester. In order to detect this product, the reaction of benzylpenicillin with 3-mercaptopropane-1,2-diol was followed by ^{1}H-NMR and it was indeed shown that the initial degradation product was the corresponding (5*R*,6*R*)-benzylpenicilloate thioester. ^{1}H-NMR chemical shifts of this compound were determined from the spectrum of the reaction solution as follows.

(5*R*,6*R*)-Benzylpenicilloate thioester of 3-mercaptopropane-1,2-diol. δ_H(300 MHz; D_2O; DSS) 1.22 (3 H, s, 2-α-CH_3), 1.53 (3 H, s, 2-β-CH_3), 3.46 (1 H, s, 3-H), 3.70 (2 H, s, Ph-CH_2), 4.61 (1 H, d, 5-H, $J_{5,6}$ 6.7), 5.14 (1 H, d, 6-H, $J_{6,5}$ 6.7), 7.4 (5 H, m, Ph). Other signals (HOCH_2–C*H*OH–CH_2–) are buried under thiol-buffer signals.

Determination of free thiol content

Aliquots of the reaction mixture ([thiol] = 0.5 mol dm^{-3}, [benzylpenicillin] = 1.1×10^{-2} mol dm^{-3}), were diluted 500 times and then assayed for free thiol content by using the method of Ellmann.[18] A stock solution of 5,5′-dithiobis(2-nitrobenzoic acid), DTNB, (41.3 mg in 10 ml of 0.1 mol dm^{-3} phosphate buffer pH = 7.0) was prepared and stored in the dark. Samples of the diluted reaction (100 μl) were withdrawn at different times, mixed with 2.5 ml of phosphate buffer pH = 8.0 and 40 μl of stock DTNB solution were added. After 2 min the absorbance at 412 nm was measured against a blank solution lacking thiol. A new blank was prepared for every new measurement.

Theoretical calculations

Theoretical calculations were carried out in order to test the interpretation of the experimental results. The *N*-methylazetidin-2-one ring was used as a model β-lactam and methylthiolate as the nucleophile and the system was surrounded by twenty water molecules as a simple estimation of solvation effects. The mechanism considered involves the nucleophilic addition of methylthiolate to the *N*-methylazetidin-2-one ring forming a tetrahedral intermediate and the subsequent cleavage of the C–N bond. All the structures generated in the thiolysis reaction have been optimised using the PM3[19] semiempirical methodology implanted in the AMPAC 6.0 software package,[20] running on a Silicon Graphics Origin 200 computer.

The characterisation of these structures should show no imaginary frequencies (minimum) or one imaginary frequency (maximum). Because of the flat potential surface, some imaginary frequencies (lower than 150 *i*) related to water molecules have been localised. Further refinements would be necessary to eliminate these frequencies, but the time involved would be extremely large with little additional benefit to the overall picture.

Results and discussion

Acyl transfer reactions of penicillins in aqueous solution with oxygen and nitrogen nucleophiles can be competitive with hydrolysis *i.e.* attack by hydroxide ion. This is relevant to the attack on β-lactams by amino and hydroxy groups in proteins on penicillins. There is much current interest in using β-lactams as acylating agents of a wide variety of enzymes and these may include cysteine proteases. This work demonstrates that thiolate anions do react with penicillins by nucleophilic attack on the β-lactam carbonyl carbon to displace the thiazolidine amine and generate a thioester. The kinetics of the reaction were studied in aqueous solutions of the thiol, which was used as both the buffer and reagent.

The reaction of thiols with benzylpenicillin (**I**) in water is outlined in Scheme 2. The presence of the penicilloyl thioester intermediate in the reaction of thiols with penicillin has been identified by ^{1}H-NMR corresponding to the structure (**III**) of Scheme 2, which is the first detectable product in the reaction which then undergoes base-catalysed hydrolysis to give bencilpenicilloic acid (**IV**). Evidence for a thioester intermediate has been directly observed in some other reactions, *e.g.* in the hydrolysis of methyl thiohippurate[21] and (*E*)-*N*-

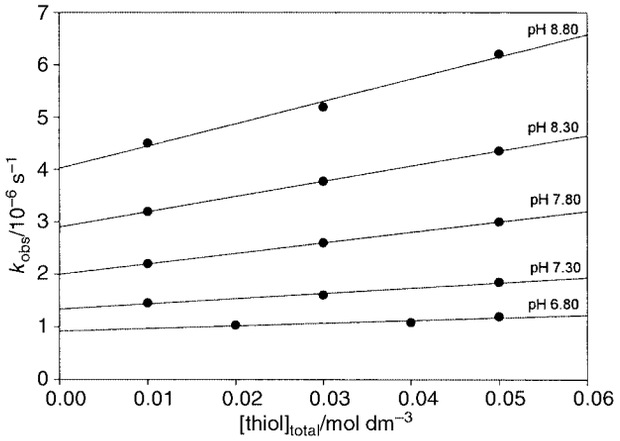
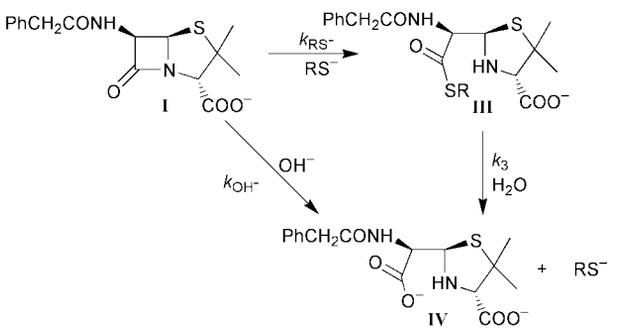


Fig. 1 Plot of the observed pseudo-first-order rate constant, k_{obs}, against the total thiol concentration of methyl 2-mercaptoacetate at the pH's indicated. Temperature 30.0 °C and ionic strength 0.5 mol dm^{-3}.



Scheme 2

cinnamoylimidazole,[22] as well as being deduced from kinetic observations.[23,24]

The hydrolysis of benzylpenicillin is catalysed by thiols as shown in Fig. 1 for methyl 2-mercaptoacetate. The observed pseudo-first-order rate constants, k_{obs}, increase linearly with total thiol concentration, $[RSH]_{tot}$, which indicates that there is no term in the rate law which is second-order in thiol. The intercept of the plot of k_{obs} against total thiol concentration corresponds to the calculated first-order rate constant for the hydroxide-ion-catalysed hydrolysis. The rate law for the hydrolysis of benzylpenicillin in aqueous buffers of thiol is therefore given by eqns. (1) and (2), where $k_{cat} = (k_{RSH}(1\text{-}a) + k_{RS^-} - a)[RSH]_{tot}$ and $a = [RS^-]/[RSH]_{tot}$.

$$k_{obs} = k_{OH^-}[OH^-] + k_{RSH}[RSH] + k_{RS^-}[RS^-] \quad (1)$$

$$k_{obs} = k_{OH^-}[OH^-] + k_{cat}[RSH]_{tot} \quad (2)$$

The slopes of these plots, designated k_{cat}, increase with increasing pH, and plots of k_{cat} against the fraction of thiol present as the free base in the buffer solution, a, give a positive intercept at $a = 1$ and an intercept at $a = 0$ which is indistinguishable from zero (Fig. 2). This indicates that the catalytically reactive form of the thiol is the thiolate anion, and that there is no reaction by the neutral, undissociated thiol. The second-order rate constant, k_{RS^-}, is given by the value of the intercept at $a = 1$. According to these results, the rate law for the hydrolysis of benzylpenicillin in aqueous buffers of thiol is effectively reduced to eqn. (3).

$$k_{obs} = k_{OH^-}[OH^-] + k_{RS^-}[RS^-] \quad (3)$$

The second-order rate constants for thiolysis of the β-lactam carbonyl group increases with increasing basicity of the thiol (Table 1) and a plot of log k_{RS^-} against the pK_a of a series of alkyl thiols (Fig. 3) has a least-squares slope of $\beta_{nuc} = 0.96$,

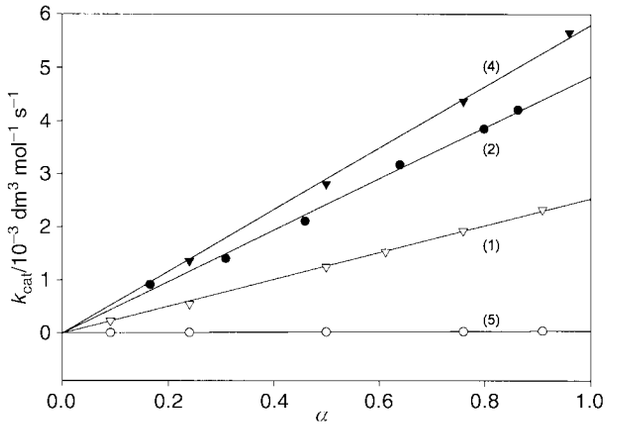


Fig. 2 Plots of k_{cat} against the fraction of thiol present as a free base, a, in the buffer solution, for different thiols. Thiols are: (1) 3-mercaptopropionic acid, (2) 2-mercaptoethanol, (4) 3-mercaptopropane-1,2-diol, (5) methyl 2-mercaptoacetate. Temperature 30.0 °C and ionic strength 0.5 mol dm^{-3}.

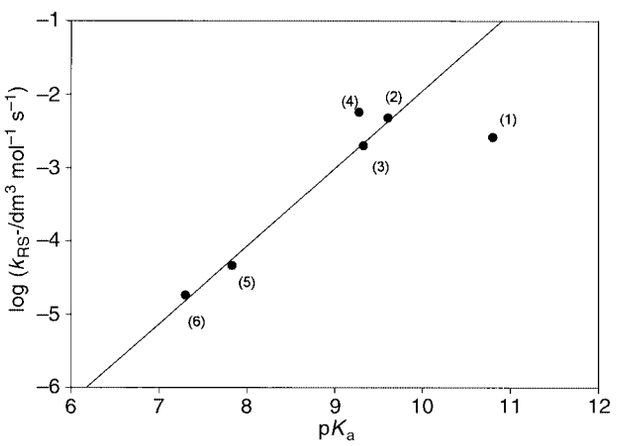


Fig. 3 Plot of the log of the second-order rate constant, k_{RS^-}, against the pK_a of the corresponding thiol: (1) 3-mercaptopropionic acid, (2) 2-mercaptoethanol, (3) methyl 3-mercaptopropionate, (4) 3-mercaptopropane-1,2-diol, (5) methyl 2-mercaptoacetate, (6) 2,2,2-trifluoroethanethiol. Temperature 30.0 °C and ionic strength 0.5 mol dm^{-3}.

which is also indicative of nucleophilic rather than general-base catalysis. The second-order rate constant calculated for 3-mercaptopropane-1,2-diol has a slight positive deviation from the line, whereas a negative deviation is observed in the case of 3-mercaptopropionic acid. This positive deviation of the α-monothioglycerol may be due to the Thorpe–Ingold effect of alkyl substituents favouring an intramolecular *S*- to *O*-acyl-transfer by ring closure. The mechanism of this process is being studied in more detail. The negative deviation of the mercaptopropionic acid is probably attributable to electrostatic destabilisation of the transition states, leading to formation and breakdown of the anionic tetrahedral intermediate.

The Brønsted β_{nuc} of 0.96 indicates that all the negative charge on the thiolate anion is effectively removed in the transition state. The Brønsted β_{nuc} values for the thiolysis of other acyl groups are generally 0.2–0.3 when the rate-determining step is attack on the carbonyl group and is often observed with basic thiols and good leaving groups expelled from the acyl centre. Much larger values of about 0.9 are observed when breakdown of the tetrahedral intermediate is rate-limiting, as seen with weakly basic thiols and poor leaving groups.[25] The Brønsted β_{nuc} value of *ca.* 1 obtained for the thiolysis of benzylpenicillin therefore suggests rate-limiting breakdown of the tetrahedral intermediate $\mathbf{T}^-$ (Scheme 1). This means that regeneration of reactants is faster than ring opening of the β-lactam ($k_{-1} \gg k_2$) (Scheme 1).

Table 1 The second-order rate constants for the reaction of benzylpenicillin with thiols at 30 °C in water and ionic strength 0.5 mol dm^{-3}; unless stated otherwise

Thiol	pK_a	k_{RS^-}/dm^3 mol^{-1} s^{-1} (in H_2O)	k_{RS^-}/dm^3 mol^{-1} s^{-1} (in D_2O)	SKIE
3-Mercaptopropionic acid (1)	10.80[a]	2.60×10^{-3}	—	—
2-Mercaptoethanol (2)	9.61[b]	4.80×10^{-3}	1.47×10^{-3}[c]	2.4[c]
Methyl 3-mercaptopropionate (3)	9.33[d]	2.00×10^{-3}	—	—
3-Mercaptopropane-1,2-diol (4)	9.28[e]	5.70×10^{-3}	2.63×10^{-3}	2.2
Methyl 2-mercaptoacetate (5)	7.83[f]	4.57×10^{-5}		
2,2,2-Trifluoroethanethiol (6)	7.30[d]	1.82×10^{-5}	—	—

[a] R. J. Irving, L. Nelander and I. Wadso, *Acta Chem. Scand.*, 1964, **18**, 769. [b] W. P. Jencks and K. Salvesen, *J. Am. Chem. Soc.*, 1971, **93**, 4433. [c] Determined at 25 °C. [d] Ref. 17. [e] H. F. DeBrabander, L. C. VanPoucke and Z. Eeckhaut, *Inorg. Chim. Acta*, 1971, **5**, 473. [f] G. E. Lienhard and W. P. Jencks, *J. Am. Chem. Soc.*, 1966, **88**, 3982.

Table 2 Second-order rate constants for the reaction of benzylpenicillin with amines and alkoxides

Nucleophile	pK_a	k/dm^3 mol^{-1} s^{-1}
Amine		
n-Propylamine[a]	10.68	8.33×10^{-3}
2-Methoxyethylamine[b]	9.66	1.40×10^{-3}
Glycine[a]	9.49	1.00×10^{-3}
Ammonia[a]	9.15	5.00×10^{-4}
Glycylglycine[a]	8.06	6.60×10^{-5}
2,2,2-Trifluoroethylamine[b]	5.81	$<6.90 \times 10^{-6}$
Alcohol		
2,2,2-Trifluoroethanol[b]	12.43	1.95×10^{-1}
p-Methoxyphenol[a]	10.07	1.17×10^{-3}
p-Chlorophenol[a]	9.24	1.58×10^{-4}
m-Nitrophenol[a]	8.23	2.67×10^{-5}
1,1,1,3,3,3-Hexafluoropropan-2-ol[b]	6.76	8.98×10^{-6}

[a] Ref. 11. Experimental conditions: 35 °C and ionic strength 1.0 mol dm^{-3}. [b] Ref. 6. Experimental conditions: 30 °C and ionic strength 1.0 mol dm^{-3}.

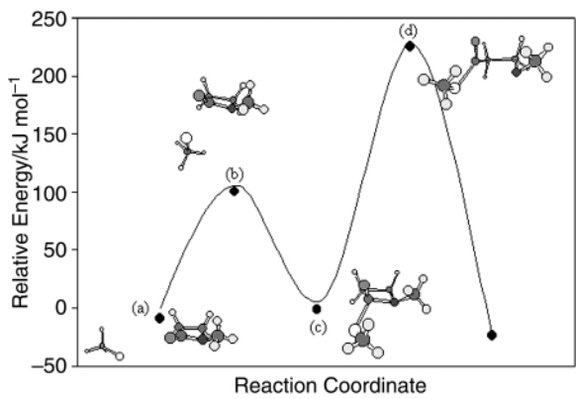


Fig. 4 Plot of the reaction energy pathway for the thiolysis of *N*-methylazetidin-2-one by methylthiolate. Water molecules have been removed from the figures in order to show a better view. Figures represent: (**a**) reagents, (**b**) transition state prior to formation of the tetrahedral intermediate, (**c**) tetrahedral intermediate, (**d**) transition state of the tetrahedral breakdown prior to products formation.

Tables 1 and 2 show that for a same pK_a of the nucleophile, the second-order rate constant for the reaction of benzylpenicillin with thiols is very similar to that for amines, which is slightly greater than that for alcohols but the difference is less than an order of magnitude. All three O, N, and S nucleophiles show similar β_{nuc} values of *ca.* 1 indicative of rate-limiting breakdown of the tetrahedral intermediate. By contrast, the rate of attack on the C=C group of aryl vinylsulfones and thiocyanates, ArN=C=S, by thiol anions is about 10^4 greater than for oxyanions of corresponding structure.[26]

Fig. 4 shows the energy reaction profile for the thiolysis reaction of *N*-methylazetidin-2-one by methylthiolate, as determined by PM3 semiempirical calculations. Figures inserted in the graph represent the four more important structures found in the energy reaction pathway. Water molecules are not included in these representations in order to clarify the graph. Point (**a**) corresponds to individual solvated reagents (S · · · C=O distance of 5.43 Å). As the reaction develops and both reagents get closer together a transition state is found (**b**), in which the sulfur atom is 2.61 Å from the β-lactam carbonyl carbon. Once this transition state is overcome the reaction proceeds to the formation of the tetrahedral intermediate (**c**), where the covalent bond is fully formed (S–C distance of 1.94 Å). This tetrahedral intermediate evolves to products through a new transition state (**d**) characterised by an enlargement of the C–N distance (2.68 Å). The major energy barrier corresponds to breakdown of the tetrahedral intermediate, *ca.* 50 kcal mol^{-1}, compared with the barrier for nucleophilic attack, which is about 30 kcal mol^{-1} lower.

The theoretical energy barriers clearly indicate that the rate-limiting step of the thiolysis reaction corresponds to the C–N bond fission of the tetrahedral intermediate, which is in agreement with the experimental data reported above.

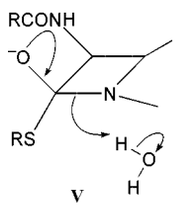
Although the theoretical calculations corroborate the experimental results the energy values obtained are surprisingly high. The calculated activation energy for the breakdown of the tetrahedral intermediate is *ca.* 50 kcal mol^{-1} whilst the experimental activation energy for the same process is *ca.* 10 kcal mol^{-1}. Nevertheless, similarly over-estimated theoretical energy values (~50 kcal mol^{-1}) have been recently reported for the aminolysis[27] of azetidin-2-one, neutral hydrolysis of azetidin-2-one[28] and *N*-methylazetidin-2-one.[29] The experimental activation energies for aminolysis and hydrolysis of benzylpenicillin are *ca.* 15 and 10 kcal mol^{-1}, respectively, and a value of ~15 kcal mol^{-1} has been reported for the hydrolysis of *N*-(3-substituted phenyl) lactams.[7] Therefore, although the energy differences between the formation and breakdown of the tetrahedral intermediate estimated using this level of theoretical calculation are relatively correct, the numerical values of the activation parameters derived from these calculations are over-estimated, probably due to solvation effects.

The thiolyses of benzylpenicillin with 3-mercaptopropane-1,2-diol and 2-mercaptoethanol show solvent kinetic isotope effects (SKIE), $k_{RS^-}(H_2O)/k_{RS^-}(D_2O)$, of 2.2 and 2.4 respectively. Nucleophilic additions in H_2O and D_2O normally show an inverse kinetic isotope effect because the nucleophile is a stronger base in D_2O compared with H_2O.[30] The value seen in this case is therefore indicative of a proton transfer step which is sensitive to isotopic substitution and is incompatible with rate-limiting attack by the thiolate anion. The SKIE is indicative of rate-limiting breakdown of the tetrahedral intermediate, with water acting as a general acid catalyst donating a proton to the departing nitrogen, facilitating C–N fission (**V**). A similar

Table 3 Activation energy for the reaction of benzylpenicillin with hydroxide ion, amines and thiols

Nucleophile	E_a/kcal mol^{-1}
OH^-	10.4[a]
2-Mercaptoethanol	8.2[b]
2-Mercaptoacetate	10.4[b]
3-Mercaptopropane-1,2-diol	6.9[b]
Imidazole	15.7[c]
Ammonia	15.0[c]
Glycine	14.6[c]

[a] T. Yamana, A. Tsuji and Y. Mizukami, *Chem. Pharm. Bull.*, 1974, **22**, 1186. [b] Present work. [c] Ref. 16.



V

situation was observed with the alcoholysis of penicillin.[6] This thiolysis reaction is then yet another example of nucleophilic substitution at the β-lactam carbonyl of penicillins occurring with rate-limiting C–N fission and ring opening.[3] This implies that it is easier to expel the thiolate anion from the tetrahedral intermediate $\mathbf{T}^-$ (Scheme 1) (k_{-1}) than it is to open the strained four membered ring (k_2).

Table 3 shows the experimental activation energies for the reaction of benzylpenicillin with hydroxide ion, amines and thiols. Hydrolysis and thiolysis have approximately similar activation energies, about 10 kcal mol^{-1}, whilst the activation energy for aminolysis is the highest, around 15 kcal mol^{-1}.

A thiolate anion is a "soft base" using Pearson's hard–soft acid–base terminology. For thiolate anions and alkoxide ions with the same substituent pattern, *e.g.* EtS^-/EtO^-, $CF_3CH_2S^-/CF_3CH_2O^-$, the sulfur analogues are much less basic, *e.g.* pK_a EtOH > EtSH. However, in terms of nucleophilicity, sulfur anions are 10–100 times more nucleophilic than amines or oxygen anions of comparable pK_a, particularly when the electrophilic centre undergoing attack is "soft". The higher nucleophilicity of the thiolate anion, in comparison with that of the corresponding alkoxide anion of the same basicity, can be explained by the higher polarizability of sulfur and by higher product stability, if this is reflected in the energy of the transition state. In addition to these polarisability factors, there may also be differential solvation effects which are seen in the transition state for thiolate anions acting as nucleophiles but not in the acid–base equilibrium reaction for protonation. The difference in rate-limiting step for thiolysis, alcoholysis and aminolysis compared with hydrolysis reactions could be due to the lower degree of solvation of thiolate and alkoxide anions compared with hydroxide ions.[31] This lower degree of solvation of thiolate anions is clearly seen in our theoretical calculations. The methylthiolate is surrounded by only two water molecules with an average hydrogen bond distance of 1.89 Å whilst the hydroxide anion is surrounded by six water molecules with an average hydrogen bond distance of 1.76 Å.[32] Furthermore, the thiolate and alkoxide anions are better leaving groups than the hydroxide anion, which makes k_{-1} greater for RO^- and RS^- than for OH^-. This could explain the change in the rate-limiting step of thiolysis and alcoholysis compared with hydrolysis by hydroxide-ion, for which $k_{-1} \ll k_2$.

Acknowledgement

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