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CALORIMETRIC INVESTIGATIONS OF THE HYDROLYTIC DEGRADATION FOR A SERIES OF β-SULTAMS

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The hydrolytic degradation of four β-sultams was investigated using isothermal microcalorimetry to determine kinetic and enthalpic data. Firstly, all four compounds were analysed in the solid-state at 310 K, with a significant substituent-based stabilising/destabilising effect being observed. Secondly, the four compounds were analysed in the presence of pH 4 acetate buffer, at three temperatures (298, 310 and 323 K). Under these conditions, the substituent choice affected the rate of hydrolysis and the associated change in enthalpy for each compound. Based on the calorimetric data presented in this work, no change in reaction mechanism for the hydrolytic degradation was observed over the temperature range considered.

Keywords: *antibiotics, β-sultams, degradation, hydrolysis, isothermal calorimetry*

Introduction

β-sultams are four-membered cyclic sulfonamides, primarily developed in recent years in an attempt to inhibit bacterial enzymes that result in the loss of β-lactam antibiotic efficacy. The susceptibility of such antibiotics to the hydrolytic activity of certain enzymes is the most common and growing form of bacterial resistance [1]. Resistance to all clinically used antibiotics has been identified in several hospitals around the world [2], and thus the development of compounds that inhibit these hydrolytic enzymes, such as the β-sultams, is fundamental to maintaining the long-term efficacy of antibiotics.

β-sultams are known to undergo both acid and base-catalysed hydrolysis to give the corresponding amino sulfonic acid [3–5] (Fig. 1).

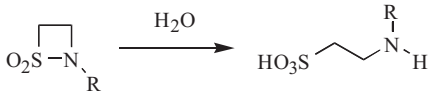

Fig. 1 S–N bond fission of a sultam based compound

The same S–N bond fission has also been observed with serine protease enzymes [6]. One class of serine protease enzyme is the β-lactamases, key enzymes in the development of bacterial resistance [7]. This reaction is of interest as it is the process by which β-sultams have been shown to be β-lactamase/serine protease inhibitors, thus giving them the potential to increase antibiotic efficacy. Therefore, understanding the hydrolytic reactions of β-sultams is of significance to the development of a successful solution to the problem of antibiotic resistance. For a series of both N-alkyl and N-aroyl β-sultams, pH-rate profiles for the hydrolysis in aqueous solution at 303 K have been established [8] using spectrophotometric techniques. From this work, an unexpectedly high reactivity towards acid and base hydrolysis was observed with a lack of an apparent pH-independent hydrolytic pathway [9]. The much more well known β-lactams have also been studied in aqueous solution [10].

In contrast, solid-state stability testing has been limited to β-lactam based compounds [11, 12], with no published data regarding the solid-state stability of the β-sultams. This is believed to be mainly because of the analytical techniques employed to date requiring an aqueous media for assaying the sample, removing the possibility of solid-state analysis. In addition, no calorimetric studies regarding the hydrolysis of β-sultams, either in the solid-state or aqueous state, have been published to date.

Solid-state thermal stability data for many other compounds is widely reported, including the stability of pharmaceutical compounds [13, 14]. The accompanying kinetics can be represented by a general differential form (Eq. (1)) in which α, t and k are respectively the fractional decomposition, time and rate constant; while p and q are parameters lying between zero and unity inclusively [15].

$$\frac{d\alpha}{dt} = k\alpha^{1-p}(1-\alpha)^{1-q} \quad (1)$$

Equation (1) can also be applied to solution-based work as a method for the determination of thermodynamic and kinetic parameters using isothermal microcalorimetry. Established equations [16] can

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be utilised which incorporate calorimetrically accessible data (Φ, the power, and q, the heat output) which lead to determination of the rate constant, k, the change in enthalpy of the reaction, ΔH, and the order of the reaction, n. For a first order reaction which goes to completion, i.e. q is the total heat output for the reaction, A is the number of moles of starting material and n=1, then Eq. (2) can be applied to permit deduction of both k and ΔH.

$$\frac{dq}{dt} = \Phi = k(A\Delta H - q) \quad (2)$$

Thermochemical investigations of the hydrolysis of a variety of compounds have been studied using calorimetry [17–19] including integration of spectroscopic and calorimetric techniques [20, 21]. However, to date no calorimetric studies have been reported for any β-sultam based compounds. In this paper we report the first calorimetric study of the hydrolytic degradation of these compounds.

Experimental

Synthesis of the β*-sultams*

Four β-sultams were chosen for this study (Fig. 2); compound A: 1,2-thiazetidine 1,1-dioxide, compound B: 2-benzoyl-1,2-thiazetidine 1,1-dioxide, compound C: 2-(4'-chlorobenzoyl)-1,2-thiazetidine 1,1-dioxide and compound D: 2-(4'-methoxybenzoyl)-1,2-thiazetidine 1,1-dioxide.

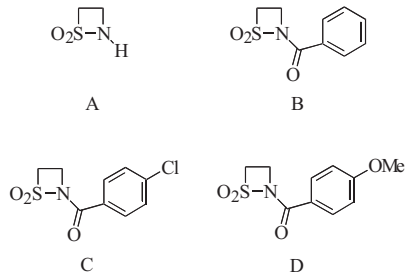

Fig. 2 Chemical structures of the four β-sultams (compounds)

Compound A was prepared using commercially available reagents following literature protocol [22]. Compounds B–D were synthesised from compound A using established protocol [5]. Structures and purities of compounds were confirmed by proton and carbon NMR spectroscopy as reported previously [5].

Calorimetric methodology

Each of the four compounds (A–D) were synthesised as described above, and stored under nitrogen at 253 K until required. Experiments were performed using an isothermal calorimeter (TAM, Thermometric, Sweden), operated under conditions as described in the manufacturer's manual. For all solid-state experiments, 20 mg of each compound (A–D) were separately weighed in glass ampoules (supplied by Thermometric) and loaded into the instrument. Data was continuously recorded using the dedicated instrument software for a total of 50 h with an initial 40 min thermal equilibrium period discounted from that later used for analysis. The data was then processed using Origin software (and manipulation of Eq. (2)) to provide the associated change in enthalpy and rate constant for the reaction. Each experiment was conducted at three temperatures; 298, 310 and 323 K. For solution based experiments, ampoules were filled with 2 mL of pH 4 0.1 M acetate buffer and 1 mL of 0.008 M β-sultam (A, B, C or D). Again, for each experiment three temperatures were considered; 298, 310 and 323 K. As with the solid-state experiments, data was processed using Origin software to provide the associated change in enthalpy and rate constant for the reaction at each temperature. All results reported are an average of five experimental results to confirm the reproducibility of the protocol.

Results and discussion

Hydrolytic degradation of four β-sultams was investigated using isothermal microcalorimetry in both the solid-state and in solution in the presence of pH 4 acetate buffer. It has previously been reported that the hydrolysis of these compounds occurs with exclusive S–N fission rather than by C–N fission (compounds B–D) [9], a fact that was confirmed regularly throughout these experiments using NMR analysis (data not shown).

Solid-state hydrolysis

All four compounds were analysed for hydrolytic degradation in the solid-state as described in the Experimental section using the isothermal microcalorimeter operated at 310 K. A typical calorimetric output for the hydrolytic process is shown in Fig. 3.

The experimental data recorded for each β-sultam were fitted to Eq. (2) to derive values for the rate constant and change in enthalpy associated with the reaction (summarised in Table 1). It can be seen from the data in Table 1 that the nature of the substituents present affects both the rate constant and, more significantly, the change in enthalpy for the hydrolytic process. Compared with the unsubstituted β-sultam (compound A), the rate constants display a ten-fold increase for both the benzoyl and chlorobenzoyl compounds (B and C). For the

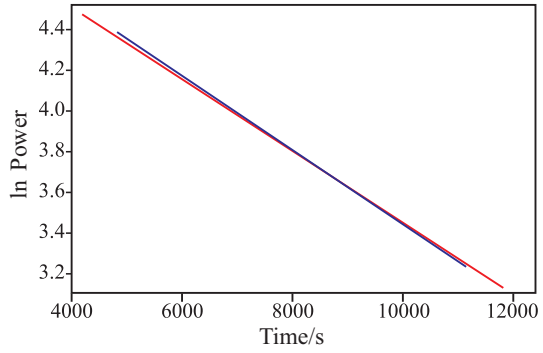

Fig. 3 Calorimetric output for the solid-state hydrolysis of β-sultam B at 310 K

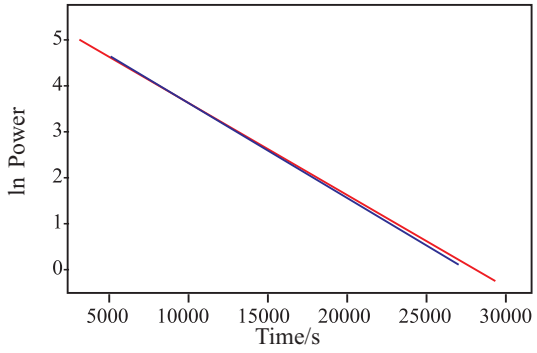

Fig. 4 Calorimetric output for the solution state hydrolysis of β-sultam A at 310 K

Table 1 Calculated kinetic and enthalpic data associated with the hydrolysis of each of the four β-sultams at 310 K in the solid-state

Compound	Rate constant/ s^{-1}	Enthalpy change/ kJ mol^{-1}
A	$1.7 \cdot 10^{-5}$ ($\pm 4.0 \cdot 10^{-9}$)	–2.5 (±0.7)
B	$1.6 \cdot 10^{-4}$ ($\pm 1.0 \cdot 10^{-7}$)	–12.8 (±1.3)
C	$1.3 \cdot 10^{-4}$ ($\pm 5.7 \cdot 10^{-8}$)	–5.0 (±0.7)
D	See text	See text

methoxybenzoyl substituent (D), it was not possible to establish reproducible data. With respect to the associated change in enthalpy, compound A provided the smallest enthalpy change, followed by a two-fold increase for compound C and a more significant increase of approximately 10 kJ mol^{-1} for compound B. Again, compound D did not provide reproducible data thus no enthalpic results can be reported.

Solution state hydrolysis: pH 4 acetate buffer

All four compounds were analysed for hydrolytic degradation in the solution phase as described in 'Experimental' using the isothermal microcalorimeter operated at three temperatures (298, 310 and 323 K) in the presence of pH 4 acetate buffer. A typical calorimetric output for the hydrolytic process observed is shown in Fig. 4.

The experimental data recorded for each β-sultam were fitted to Eq. (2) to derive values for the rate constant and change in enthalpy associated with the reaction (summarised in Table 2).

As with the solid-state results, it can be seen from the data in Table 2 that the nature of the substituents present affects both the rate constant and the change in enthalpy for the hydrolytic process. As expected, there is a steady increase in rate constant for all four compounds with an increase in temperature.

Enthalpic data presented in Table 2 confirms that for each of the four compounds individually there is no significant change in enthalpy over the temperature range, as would be expected, confirming the belief that no change in reaction mechanism was observed with a change in temperature. However, compared with the unsubstituted compound (A), all three substituted compounds (B–D) displayed more negative changes in enthalpy at all temperatures. This is believed to be a reflection of the stabilising effect exerted by the substituents on the S-N bond thus resulting in a more negative change in enthalpy associated with the hydrolytic process.

Conclusions

In conclusion, the application of calorimetric techniques to the study of the hydrolysis of β-sultams has been investigated. This allowed the first report of rate constants and changes in enthalpy for the hydrolytic degradation of β-sultams in the solid-state. In addition, this paper has reported the calorimetric determination of rate constants and changes in enthalpy for the solution phase reaction

Table 2 Calculated kinetic and enthalpic data associated with the hydrolysis of each of the four β-sultams at three temperatures in pH 4 acetate buffer

T/ K	Compd.	Rate constant/ s^{-1}	Enthalpy change/ kJ mol^{-1}
298	A	$8.1 \cdot 10^{-5}$ ($\pm 7.2 \cdot 10^{-9}$)	–14.6 (±0.6)
	B	$1.1 \cdot 10^{-5}$ ($\pm 1.4 \cdot 10^{-9}$)	–21.8 (±1.1)
	C	$3.3 \cdot 10^{-5}$ ($\pm 4.7 \cdot 10^{-9}$)	–28.9 (±0.7)
	D	$5.9 \cdot 10^{-6}$ ($\pm 9.8 \cdot 10^{-10}$)	–31.8 (±1.4)
310	A	$2.0 \cdot 10^{-4}$ ($\pm 1.9 \cdot 10^{-8}$)	–16.1 (±1.1)
	B	$2.0 \cdot 10^{-5}$ ($\pm 3.6 \cdot 10^{-9}$)	–22.0 (±1.3)
	C	$4.9 \cdot 10^{-5}$ ($\pm 1.5 \cdot 10^{-8}$)	–29.5 (±1.7)
	D	$1.0 \cdot 10^{-5}$ ($\pm 1.9 \cdot 10^{-9}$)	–27.0 (±1.1)
323	A	$3.8 \cdot 10^{-4}$ ($\pm 6.7 \cdot 10^{-8}$)	–19.1 (±1.1)
	B	$4.8 \cdot 10^{-5}$ ($\pm 1.3 \cdot 10^{-8}$)	–21.7 (±0.8)
	C	$1.3 \cdot 10^{-4}$ ($\pm 5.4 \cdot 10^{-8}$)	–30.5 (±0.5)
	D	$4.2 \cdot 10^{-5}$ ($\pm 1.1 \cdot 10^{-8}$)	–32.3 (±1.3)

in the presence of pH 4 acetate buffer at three temperatures. Thus, it has been shown that calorimetry can be used to study the hydrolytic degradation of such compounds over a range of temperatures in both the solid and solution states.

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