Competitive endo- and exo-cyclic C–N fission in the hydrolysis of *N*-aroyl β-lactams[1]

Wing Y. Tsang, Naveed Ahmed, Karl Hemming, and Michael I. Page

Abstract: The balance between endo- and exo-cyclic C–N fission in the hydrolysis of *N*-aroyl β-lactams shows that the difference in reactivity between strained β-lactams and their acyclic analogues is minimal. Attack of hydroxide ion occurs preferentially at the exocyclic acyl centre rather than that of the β-lactam during the hydrolysis of *N*-*p*-nitrobenzoyl β-lactam. In general, both endo- and exo-cyclic C–N bond fission occurs in the alkaline hydrolysis of *N*-aroyl β-lactams, the ratio of which varies with the aryl substituent. Hence, the Brønsted β-values differ for the two processes: –0.55 for the ring-opening reaction and –1.54 for the exocyclic C–N bond fission reaction. For the pH-independent and acid-catalysed hydrolysis of *N*-benzoyl β-lactam, less than 3% of products are derived from exocyclic C–N bond fission.

Key words: β-lactams, hydrolysis, linear free energy relationships, strain.

Résumé : La balance entre la fission C–N endo- et exocyclique lors de l'hydrolyse de *N*-aroyl β-lactames montre que la différence de réactivité entre des lactames tendus et leurs analogues acyles est minimale. L'attaque par l'ion hydroxyde se fait préférentiellement au niveau du centre acyle exocyclique plutôt que sur celui du β-lactame lors de l'hydrolyse du *N*-*p*-nitrobenzoyl β-lactame. En général, il se produit des fissions des liaisons C–N tant endo- que exocycliques lors de l'hydrolyse alcaline des *N*-aroyl β-lactames et le rapport des deux varie en fonction de la nature du substituant. En conséquence, les valeurs β de Brønsted diffèrent pour les deux processus étant égales à –0,55 pour la réaction d'ouverture de cycle et de –1,54 pour la réaction de fission de la liaison C–N exocyclique. Les hydrolyses indépendantes du pH et catalysées par les acides du *N*-benzoyl β-lactame fournissent moins que 3 % de produits dérivés de la fission de la liaison C–N exocyclique.

Mots clés : β-lactames, hydrolyse, relations linéaires d'énergie libre, tension.

[Traduit par la Rédaction]

Introduction

β-Lactam antibiotics such as penicillins (**1**) and cephalosporins are often considered unusually reactive when compared with normal amides because most of their biologically important reactions involve the opening of the highly strained four-membered ring (1, 2). The effect of ring strain on the reactivity of β-lactams may be demonstrated by comparing the rates of the reactions of monocyclic β-lactams with their acyclic analogues. For example, and perhaps contrary to expectation, the rate of alkaline hydrolysis of β-lactams is, at most, 100-fold greater than that of an analogous acyclic amide. The second-order rate constant for the hydroxide ion catalysed hydrolysis of *N*-methyl β-lactam is only threefold greater than that for *N*,*N*-dimethyl acetamide (3).

Incorporating a benzamide as a potential leaving group into a simple azetidin-2-one gives the imide *N*-benzoyl β-lactam (**2**), which contains an endocyclic and an exocyclic acyl centre both of which are potential sites for nucleophilic attack. The hydrolysis of **2** could occur by two routes. For example, the hydroxide ion could attack the exocyclic carbonyl to generate benzoic acid and the intact β-lactam (Scheme 1, route a). The β-lactam could then undergo further reaction with the hydroxide ion to give a ring-opened β-amino acid. Alternatively, the hydroxide ion could attack the endocyclic β-lactam carbonyl to give a ring-opened product **3** (Scheme 1, route b). Many would expect this to be the dominant reaction pathway based on the belief that ring strain increases the reactivity of β-lactams. We were interested in exploring the balance between endo- and exo-cyclic C–N fission as a function of substituents in the aroyl group, and herein report further evidence that the difference in reactivity between strained β-lactams and their acyclic analogues is minimal.

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Experimental section

Synthesis

N-Aroyl β-lactams were prepared according to the following general procedure: To a stirred solution of azetidin-2-one (0.5 g, 7.03 mmol) in dry dichloromethane (DCM, 20 mL) was added 4,4-dimethylaminopyridine (0.1 g, 0.82 mmol) at –78 °C, and a solution of aroyl chloride (1.57 g, 8.46 mmol) in dichloromethane (10 mL) dropwise over 5 min. Triethylamine (0.98 mL, 7.02 mmol) was added dropwise

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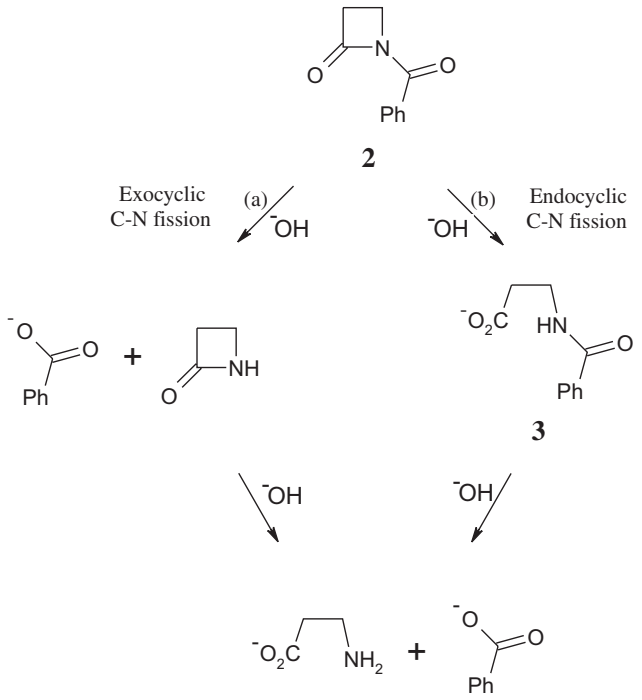
Scheme 1.



over 10 min forming a white precipitate. The reaction mixture was stirred at –78 °C for 1 h and for a further 24 h at ambient temperature. DCM (10 mL) was added to the reaction mixture, and the solution was washed with water (15 mL) and saturated brine (2 × 15 mL). The organic layer was dried over anhydr. Na_2SO_4, and the solvent was removed under reduced pressure by rotary evaporation at 30 °C to yield a pale yellow oil, which was purified by column chromatography.

1-(4′-Methoxybenzoyl)-1-azetidin-2-one

Yield: 0.6 g (42%); mp 125–127 °C. IR υ_{max} (cm^{-1}, $CHCl_3$): 3020, 3009, 2975, 2912, 2842, 1784, 1668, 1606, 1325, 1259, 1195, 1108, 1028. ^1H NMR ($CDCl_3$) δ: 7.99 (2H, d, J = 8.88 Hz), 6.92 (2H, d, J = 8.97 Hz), 3.85 (3H, s, CH_3), 3.77 (2H, t, J = 5.44 Hz, CH_2N), 3.02 (2H, t, J = 5.45 Hz, CH_2CO). ^{13}C NMR ($CDCl_3$) δ: 165.23 (C=O), 163.92 (C=O), 132.57 (quaternary carbon), 131.92 (ArCH), 123.76 (quaternary carbon), 113.948 (ArCH), 55.21 (CH_3), 36.45 (CH_2N), 34.44 (CH_2CO). HR-EI-MS calcd. for $C_{11}H_{11}NO_3$: 206.0812 $[M + H]^+$; found: 206.0812.

1-Benzoyl-1-azetidin-2-one

Yield: 0.91 g (65%). IR υ_{max} (cm^{-1}, $CHCl_3$): 3020, 1786, 1673, 1327, 1298, 1216, 1192. ^1H NMR ($CDCl_3$) δ: 8.00 (2H, d, J = 7.43 Hz), 7.61 (1H, t, J = 7.29 Hz), 7.49 (2H, t, J = 7.83 Hz), 3.81 (2H, t, J = 5.49 Hz), 3.14 (2H, t, J = 5.49 Hz). ^{13}C NMR ($CDCl_3$) δ: 166.26 (C=O), 163.95 (C=O), 133.19 (PhCH), 131.83 (quaternary carbon), 129.69 (PhCH), 128.12 (PhCH), 36.77 (CH_2), 35.05 (CH_2).

1-(4′-Chlorobenzoyl)-1-azetidin-2-one

Yield: 0.85 g (58%). IR υ_{max} (cm^{-1}, $CHCl_3$): 3020, 1788, 1673, 1593, 1404, 1324, 1284, 1217, 1093. ^1H NMR ($CDCl_3$) δ: 7.95 (2H, d, J = 6.68 Hz), 7.44 (2H, d, J = 8.66 Hz), 3.77 (2H, t, J = 5.5 Hz, CH_2N), 3.12 (2H, t, J = 5.61 Hz, CH_2CO). ^{13}C NMR ($CDCl_3$) δ: 165.01 (C=O), 163.84 (C=O), 139.51 (quaternary carbon), 131.14 (CH), 130.1 (quaternary carbon), 128.44 (ArCH), 36.73 (CH_2N), 34.99 (CH_2CO). HR-EI-MS calcd. for $C_{10}H_8NO_2Cl$: 210.0316 $[M + H]^+$; found: 210.0316.

1-(4′-Nitrobenzoyl)-1-azetidin-2-one

Yield: 1.23 g (80%); mp 130 to 131 °C. IR υ_{max} (cm^{-1}, $CHCl_3$): 3020, 1787, 1686, 1599, 1523, 1397, 1309, 1252, 1204, 992. ^1H NMR ($CDCl_3$) δ: 8.33 (2H, d, J = 8.75 Hz), 8.14 (2H, d, J = 8.74 Hz), 3.85 (2H, t, J = 5.58 Hz, CH_2N), 3.2 (2H, t, J = 5.57 Hz, CH_2CO). ^{13}C NMR ($CDCl_3$) δ: 164.16 (C=O), 163.79 (C=O), 150.29 (quaternary carbon), 137.32 (quaternary carbon), 130.76 (CH), 123.28 (CH), 37.0 (CH_2N), 35.5 (CH_2CO).

Kinetic procedures

Standard UV spectroscopy was carried out on a Cary 1E UV–vis spectrophotometer (Varian, Australia) equipped with a 12-compartment cell block. The instrument was used in a double beam mode, allowing six reaction cells to be followed in a single run. The cell block was thermostatted using a Peltier system.

The pH measurements were made with a ϕ40 pH meter (Beckman, Fullerton, California) using a semi-micro calomel electrode (Beckman). A calibration of the pH meter was carried out at 30 °C using pH 6.99 ± 0.01, 4.01 ± 0.02, or 9.95 ± 0.02 calibration buffers. For solution pH ≥ 3 and ≤ 11, the pH was controlled by the use of ≤0.2 mol/L buffer solutions of formate (pK_a 3.75), ethanoate (pK_a 4.72), MES (pK_a 6.1), MOPS (pK_a 7.2), TAPS (pK_a 8.4), CAPSO (pK_a 9.6), and CAPS (pK_a 10.4). Buffer solutions were prepared by partial neutralization of their sodium salts to the required pH. Hydroxide ion concentrations were calculated using pK_w (H_2O) = 13.83 at 30 °C (4).

In all experiments, temperatures were maintained at 30 °C and ionic strength at 1.0 mol/L with AnalaR grade KCl unless otherwise stated. AnalaR grade reagents and deionized water were used throughout. Organic solvents were glass-distilled prior to use and stored under nitrogen.

Reactions studied by UV spectrophotometry were commenced by injections (20 μL) of acetonitrile stock solutions 2×10^{-2} mol/L of the substrate into the cells containing preincubated buffer (2.0 mL). Final reaction cells contained ≤1% acetonitrile (*v*/*v*). The pH of the reaction cells was measured before and after each kinetic run at 30 °C; kinetic runs experiencing a change >0.05 units were rejected. Reactant disappearance or product appearance were followed at absorbance change maxima for individual compounds. The solubility of compounds was ensured by working within the linear range of absorbance in corresponding Beer–Lambert plots. Reaction concentrations were generally within the range of 2×10^{-5} to 2×10^{-4} mol/L. Pseudo-first-order rate constants from exponential plots of absorbance against time or gradients of initial slopes were obtained using the Cary Win UV kinetics application (Version 02.00(26)). The pH–rate profiles were modelled to theoretical equations using the Scientist program (V2.02, Micromath Software Ltd, Saint Louis, Missouri).

HPLC analysis

HPLC analysis was carried out on the Beckman System Gold HPLC with the 127 solvent module running a linear gradient program from solvent A (94% water, 5% acetonitrile, and 1% formic acid) to solvent B (20% water, 79% acetonitrile, and 1% formic acid) over a period of 30 min, using a RP-18 (5 μm) LiChrospher® 100 column, and monitored by the 168 diode array detector at 228 nm.

Results and discussion

The hydrolysis of *N*-benzoyl β-lactam

The rate of hydrolysis of **2** was studied by UV spectrophotometry in buffered solutions of various pH with 1% acetonitrile – water (*v*/*v*) and 1.0 mol/L ionic strength (KCl) at 30 °C. The hydrolysis of **2** shows simple first-order kinetics to give an observed rate constant (k_{obs}). The pseudo-first-order rate constant (k_{int}) for hydrolysis, obtained by extrapolating k_{obs} to zero-buffer concentration, shows acid-catalysed, pH-independent, and alkaline hydrolysis terms, the relative importance of which vary with pH (Fig. 1). The pseudo-first-order rate constant (k_{int}) has a first-order dependence on acid and hydroxide ion concentration, as indicated by the slope of unity on the acid and basic limb, respectively, of the plot of the logarithm of the rate constant (k_{int}) against pH. The pseudo-first-order rate constant can be fitted to eq. [1] using the Scientist program:

$$[1] \qquad \frac{\text{Rate}}{[\beta\text{-lactam}]} = k_{int} = k_o + k_H[H^+] + k_{OH}[OH^-]$$

The rate constants for acid-catalysed hydrolysis (k_H), pH-independent hydrolysis (k_o), and alkaline hydrolysis (k_{OH}) of **2** are $k_H = 3.19 \times 10^{-5}$ (mol/L)$^{-1}$ s^{-1}, $k_o = 8.92 \times 10^{-7}$ s^{-1}, and $k_{OH} = 11.2$ (mol/L)$^{-1}$ s^{-1}. The latter is in the range commonly observed for the alkaline hydrolysis of imides (5).

Alkaline hydrolysis of *N*-benzoyl β-lactam

The site of nucleophilic attack by the hydroxide ion on **2** is not obvious because the rates of alkaline hydrolysis of β-lactams with weakly basic leaving groups are similar to those for analogous acyclic amides (2, 6). The rate of alkaline hydrolysis was also studied by pH-stat to monitor the progress of the reaction by titrating against a NaOH solution. One equivalent of NaOH to the β-lactam concentration was consumed for the overall reaction, and the calculated second-order rate constant (k_{OH}) for the alkaline hydrolysis of **2** from a single pH-stat experiment, carried out at pH 10.50, was very similar to that determined by UV spectrophotometry. At the end of the reaction, the solution was analysed by HPLC with a UV detector. The presence of benzoic acid is clearly demonstrated as a new signal. No benzoic acid would be formed from the hydrolysis of the β-amido acid **3** under these conditions (Scheme 1, route b). Any hydrolysis to give benzoic acid via route b would also consume more than 1 equiv. of hydroxide ion to the β-lactam concentration in contrast to 1 equiv. as seen in the pH-stat experiment. The benzoic acid must then be formed from the alkaline hydrolysis of the exocyclic amide (Scheme 1, route a). Competing exo- and endo-cyclic C–N bond fission was also confirmed by studying the hydrolysis reaction in 1.0 mol/L NaOD–D_2O by ^{1}H NMR. The spectrum, taken immediately after mixing, shows no starting material but two pairs of distinctive triplets in the methylene region. One pair of the triplets belongs to the simple unsubstituted azetidin-2-one, identified by spiking the reaction mixture with the compound. For the other pair of triplets, two-dimensional ^{1}H, ^{13}C HMBC NMR shows a cross peak between the exocyclic carbonyl carbon and one of the triplets, which indicates that the exocyclic carbonyl was still attached to the ring skeleton, i.e., the β-amido acid hydrolysis product **3**.

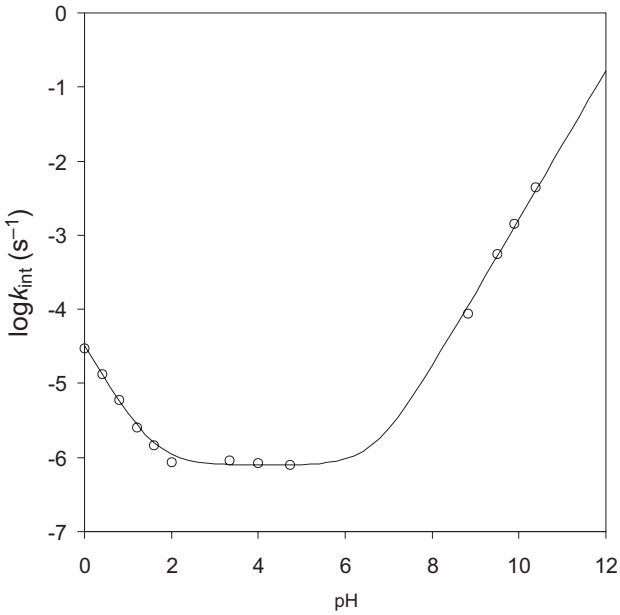


Fig. 1. The pH–rate profile for the hydrolysis of *N*-benzoyl β-lactam (**2**) in 1% acetonitrile – water (*v*/*v*) at 30 °C with 1.0 mol/L ionic strength.

The fractions of the unsubstituted azetidin-2-one and the β-amido acid **3** in H_2O, calculated using the ratio of their NMR signal integrals, are 0.19 and 0.81, respectively. The product ratio was the same when the reaction was carried out at pH 10.50, maintained by pH-stat. This indicates that the rates of hydrolysis of the β-lactam and the exocyclic amide have the same dependence on the hydroxide ion concentration. As the relative rates between two competing pathways in a parallel reaction are proportional to their product distribution (7), the second-order rate constant for the alkaline hydrolysis of the β-lactam of **2** (k_{OH}^{endo}) is 9.07 (mol/L)$^{-1}$ s^{-1} and that for the alkaline hydrolysis of the exocyclic amide (k_{OH}^{exo}) is 2.13 (mol/L)$^{-1}$ s^{-1} (Table 1).

The hydrolysis of *N*-benzoyl β-lactam shows no deuterium incorporation in the hydrolysis product β-amido acid, indicative of a direct addition–elimination mechanism of acyl transfer. The hydrolysis of *N*-acyl β-lactams thus occurs by competitive attack at the endo- and exo-cyclic carbonyl centres. The fact that exocyclic attack is competitive with endocyclic is yet another example of the surprisingly small difference in reactivity between β-lactams and acyclic amides (2, 6). If the rate-limiting step is attack of hydroxide ion on the β-lactam carbonyl carbon to form a tetrahedral intermediate, then the release of strain in the four-membered ring would not be realized in the transition state, although one may have anticipated a preferential conversion of a three-coordinate to a four-coordinate carbon in the four-

Table 1. The second-order rate constants for the hydrolysis at the endo- and exo-cyclic carbonyl centres of *N*-benzoyl β-lactam (**2**) in water and deuterium oxide with 1% acetonitrile (*v*/*v*) and 1.0 mol/L ionic strength (KCl) at 30 °C.

	Observed rate constants	Rate constants for endocyclic C–N fission	Rate constants for exocyclic C–N fission
k_{OH} ((mol/L)$^{-1}$ s^{-1})	11.2	9.07	2.13
k_{OD} ((mol/L)$^{-1}$ s^{-1})	15.1	12.2	2.87
SKIE[a]		0.74	0.74

[a]SKIE (solvent kinetic isotope effect).

membered ring. If the rate-limiting step is the breakdown of the tetrahedral intermediate or if the reaction is concerted, then this appears to be another example of the reluctance of four-membered rings to undergo facile opening (2, 8).

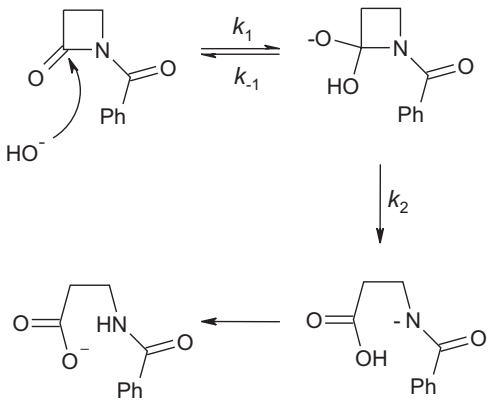
The apparent second-order rate constant (k_{OD}^{app}) for the alkaline hydrolysis of **2** in D_2O, using pK_w^{D2O} = 14.699 (4) and pD = pH meter reading + 0.40 (9), is 15.1 (mol/L)$^{-1}$ s^{-1}. The fraction of the unsubstituted azetidin-2-one and the β-amido acid **3** obtained from the ^{1}H NMR signal integrals of the products of the reaction in 1.0 mol/L NaOD–D_2O is identical to that obtained in 1.0 mol/L NaOH–H_2O. Therefore, the second-order rate constant for the alkaline hydrolysis of the exocyclic amide (k_{OD}^{exo}) is 2.87 (mol/L)$^{-1}$ s^{-1} and that for the alkaline hydrolysis of the β-lactam (k_{OD}^{endo}) is 12.2 (mol/L)$^{-1}$ s^{-1} (Table 1). As the ratio between the alkaline hydrolysis of the exocyclic amide and the β-lactam is the same in water and in deuterium oxide, the two reactions have, within experimental error, the same solvent kinetic isotope effect (k_{OH}/k_{OD}) of 0.74 (Table 1). The inverse solvent isotope effect is compatible with a rate-limiting formation of the tetrahedral intermediate or its breakdown by anion expulsion (Scheme 2).

The rate-limiting step for the alkaline hydrolysis of acyclic amides is normally the breakdown of the tetrahedral intermediate with partial protonation on the leaving group amine nitrogen (6, 10). However, with **2**, protonation on the amide nitrogen is thermodynamically unfavourable. The leaving group may be expelled in the rate-limiting step as an amide anion without N protonation **4** and is compatible with the observed inverse solvent kinetic isotope effect. C–N bond fission expelling an amide anion from the tetrahedral intermediate would be rate limiting if the expulsion of the amide anion is slower than the expulsion of an incoming hydroxide ion in the intermediate, i.e., $k_2 < k_{-1}$ (Scheme 2). The pK_a of amides like benzamide is about 15, so the pK_a of the conjugate acids of the groups expelled, water and amide, are very similar. The relative rate of C–O fission expelling alkoxide anions compared with C–N fission expelling amines of the same basicity is not easy to predict. Some reactions appear to favour amine expulsion (11), whereas others have faster rates for oxygen (2). A large negative charge development on the leaving group nitrogen is expected for expulsion of an amide anion. The hydrolysis was further studied by using linear free energy relationships to elucidate changes in effective charge in the transition state by monitoring the effect of changing the aryl substituents on reactivity.

Structure–activity relationships for the alkaline hydrolysis of *N*-aroyl β-lactams

The rates of alkaline hydrolysis of some aryl substituted

Scheme 2.



N-aroyl β-lactams were studied as a function of pH in 1% acetonitrile – water (*v*/*v*) with 1.0 mol/L ionic strength (KCl) at 30 °C by UV spectrophotometry. The pseudo-first-order rate constants (k_{int}) for the buffer-independent hydrolysis were obtained by extrapolating the observed rate constant to zero-buffer concentration. The apparent second-order rate constant (k_{OH}^{app}) for alkaline hydrolysis was obtained from the slope of the plot of the pseudo-first-order rate constant (k_{int}) against the hydroxide ion concentration. Using the fraction of products obtained, as previously described using ^{1}H NMR, the apparent second-order rate constants (k_{OH}^{app}) are then separated into the second-order rate constants for the alkaline hydrolysis of the exocyclic amide (k_{OH}^{exo}) and for the alkaline hydrolysis of the β-lactam (k_{OH}^{endo}) (Table 2). The product ratios between the unsubstituted azetidin-2-one and the β-amido acid hydrolysis product, and hence the relative rates of endo- and exo-cyclic C–N bond fission, depend on the nature of the para-substituents in the benzamide residue. Electron-withdrawing substituents favour attack at the exocyclic amide, presumably reflecting the relative importance of the electrophilicity of the carbonyl centre compared with the nucleofugality of the leaving group. The more electron-withdrawing is the substituent, the higher the ratio of exocyclic to endocyclic C–N bond fission and the greater the formation of the simple azetidin-2-one relative to the β-amido acid **3** as products. Exocyclic C–N bond fission is the dominant reaction in the alkaline hydrolysis of *N*-*p*-nitrobenzoyl β-lactam in preference to β-lactam hydrolysis (Table 2).

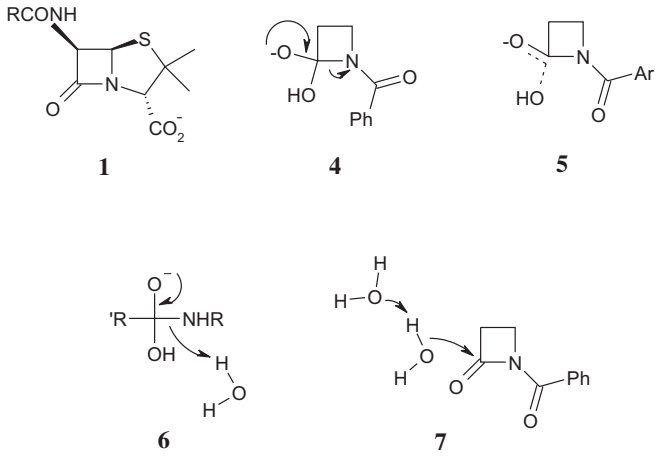
The observation that electron-withdrawing substituents enhance the rate of reaction is compatible with either rate-limiting formation of the tetrahedral intermediate or its breakdown with amide anion expulsion occurring in the

Table 2. The second-order rate constants (SD ± 5%) for the alkaline hydrolysis of *N*-aroyl β-lactams in 1% acetonitrile – water (*v*/*v*) with 1.0 mol/L ionic strength (KCl) at 30 °C.

	pK_a	k_{OH}^{app} ((mol/L)$^{-1}$ s^{-1})	Fraction of endocyclic C–N fission	k_{OH}^{endo} ((mol/L)$^{-1}$ s^{-1})	Fraction of exocyclic C–N fission	k_{OH}^{exo} ((mol/L)$^{-1}$ s^{-1})
p-NO_2	3.44	57.7	0.46	26.5	0.54	31.2
p-Cl	3.99	18.2	0.76	13.8	0.24	4.37
p-H	4.20	11.2	0.81	9.07	0.19	2.13
p-OMe	4.47	8.08	0.90	7.27	0.10	0.81
o-COOH	5.28	2.60	1.00	2.60	0.0	—

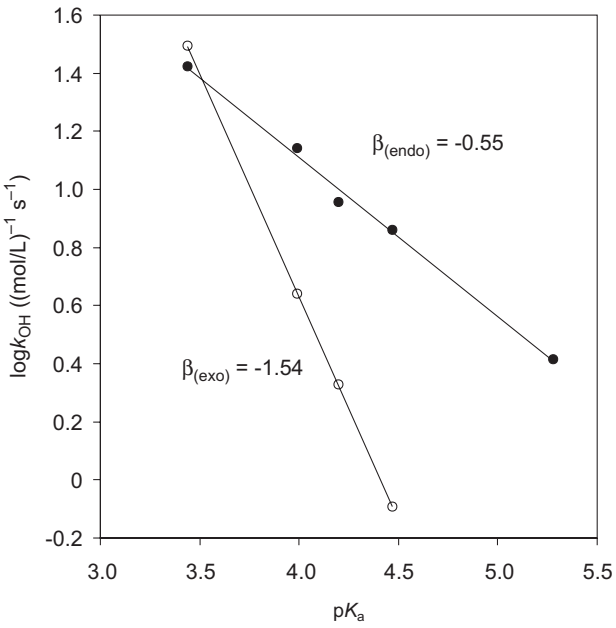
Note: The pK_a of the carboxylic acid corresponding to exocyclic aryl carboxamide is given as an indicator of the inductive effect of the substituent in the aromatic ring. pK_a values are taken from ref. 12.

transition state. The difficulty with correlating the reaction rate to the ionization of the leaving group is the lack of available ionization constants of substituted benzamides. The ionization constants of substituted benzoic acids, as analogues of benzamides, were therefore used for the correlation.



The Brønsted-type plot of the rate constants for the alkaline hydrolysis of the β-lactam ring of *N*-aroyl β-lactams against the pK_a of the corresponding benzoic acids is shown in Fig. 2. This shows a good linear correlation, the slope of which gives the Brønsted β_{lg} value for the alkaline hydrolysis of *N*-aroyl β-lactams as –0.55. This indicates that in the transition state of the rate-limiting step of hydrolysis, the effective charge on the nitrogen atom in the amine leaving group becomes more negative relative to the reactant state by 0.55. The effective charge on the nitrogen of β-lactams is expected to be very similar to that of acyclic amides of +0.7 (13). There is no data on the effective charge on the nitrogen in imides. If there is no levelling of the resonance effect, then presumably the charge could be as high as 1.4. If amide resonance is reduced, then, effectively, imides could be treated as amides to which is attached an electron-withdrawing acyl group not involved in further resonance stabilization. This would make the effective charge on the imide nitrogen greater than 0.7 but less than 1.4. A crude estimation may be obtained from the difference in σ and σ$^-$ values for a *para*-acyl substituent, i.e., 0.50 and 0.84, respectively (13). The additional positive charge on the imide nitrogen due to a second electron-withdrawing but non-resonating acyl substitutent would be (0.5/0.84) × 0.7 = 0.42. This would make the effective charge on the imide nitrogen +1.1. This will be used in subsequent discussions, but a slightly smaller or larger value makes little difference to the conclusions.

Fig. 2. Brønsted plot for the second-order rate constant (k_{OH}), for the alkaline hydrolysis at the endocyclic (●) and exocyclic (○) carbonyl centre of *N*-aroyl β-lactams against the pK_a of the corresponding benzoic acid in 1% acetonitrile – water (*v*/*v*) at 30 °C. I = 1.0 mol/L (KCl).

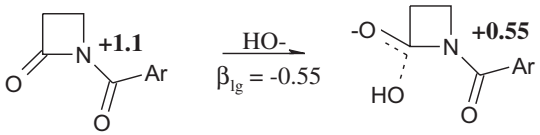


The observed Brønsted value of –0.55 for the alkaline hydrolysis of the β-lactam ring of *N*-aroyl β-lactams is compatible with a late transition state for the formation of the tetrahedral intermediate **5**. The effective charge of +0.55 on N in the transition state structure indicates little or no C–N bond fission and that the nitrogen atom is still involved in normal amide resonance with the aroyl residue (Scheme 3). For comparison, the Brønsted β_{lg} value for the alkaline hydrolysis of N-substituted amides is –0.07 (6), which was interpreted in terms of general-acid-catalysed breakdown of the tetrahedral intermediate owing to partial proton transfer from water **6**. The rates of alkaline hydrolysis of *N*-aryl β-lactams generate a Brønsted β_{lg} of –0.44, which was taken as evidence of rate-limiting formation of the tetrahedral intermediate (2, 14).

Table 3. The apparent rate constants (SD ± 5%) and kinetic parameters for the acid-catalysed hydrolysis, pH-independent hydrolysis, and alkaline hydrolysis of *N*-benzoyl β-lactam (**2**) in 1% acetonitrile – water (*v*/*v*) with 1.0 mol/L ionic strength (KCl) at different temperatures.

	k ((mol/L)$^{-1}$ s^{-1})						
	20.0 °C	30.0 °C	40.0 °C	50.0 °C	60.0 °C	$\Delta S^{\ddagger}$ (J K^{-1} mol^{-1})	$\Delta H^{\ddagger}$ (kJ mol^{-1})
H_2O	3.77×10^{-7}	8.92×10^{-7}	1.34×10^{-6}	2.01×10^{-6}	3.53×10^{-6}	–228 ± 9	40.9 ± 2.8
HO^-	6.72	11.2	22.2	37.7	69.7	–77.3 ± 4.2	44.8 ± 1.3
H^+	9.66×10^{-6}	3.19×10^{-5}		3.78×10^{-4}	1.12×10^{-3}	–19.1 ± 6.7	94.5 ± 0.4

Scheme 3.



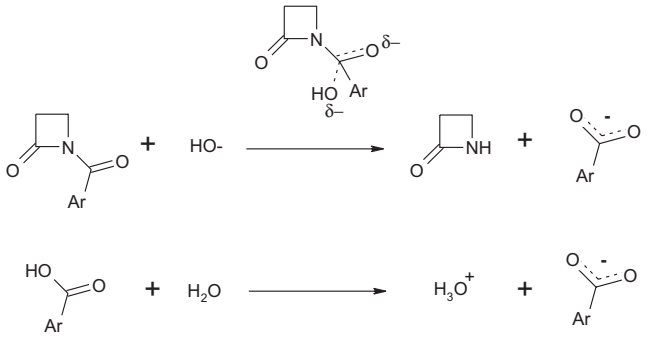
Extrapolating the data for the Brønsted plot for the alkaline hydrolysis of *N*-aryl β-lactams to the rate constant for the alkaline hydrolysis of *N*-benzoyl β-lactam gives an apparent pK_a of –7.5 for the leaving group amine. Formally this corresponds to the N protonation of benzamide, but as protonation of amides corresponds to O rather than N protonation, the predicted pK_a for N protonation of an amide is not unreasonable.

The Brønsted-type plot for the alkaline hydrolysis of the exocyclic amide of *N*-aroyl β-lactams against the pK_a of the corresponding benzoic acids is shown in Fig. 2. The plot is again reasonably linear, but the dependence of the rate on the pK_a is much greater with an apparent Brønsted β of –1.54. The correlation is intriguing, as the product of the exocyclic amide hydrolysis and that of ionization of the carboxylic acid are the same (Scheme 4). The Brønsted value of –1.54 indicates that the effective charge at the reaction centre in the transition state for the amide hydrolysis is more negative than that in the reactant imide compared with the charge in the carboxylate ion relative to that in the undissociated acid. This indicates that there is a relatively greater negative charge density in the transition state for the hydrolysis reaction than that in the carboxylate anion. This may indicate a rate-limiting formation of the tetrahedral intermediate with a late transition state or an early one in a rate-limiting breakdown because in the tetrahedral intermediate the negative charge is more localized. A similar linear free energy relationship was observed for the rates of alkaline hydrolysis of esters against the pK_a of the product carboxylic acid, which gave a Brønsted value of –1.3 (15). The localized negative charge on the oxygen anion in the tetrahedral intermediate is more dependent on the stabilizing effect of electron-withdrawing groups than the more stable delocalized negative charge in the carboxylate anion.

pH-Independent hydrolysis

The rate of hydrolysis of **2** is pH independent from pH 2–6 (Fig. 1). A product analysis using HPLC of the reaction at pH 3.99 (0.1 mol/L acetate buffer), ionic strength 1.0 mol/L (KCl), and at 60 °C was undertaken. The signal for **2** disappeared with time in an exponential manner, but that for benzoic acid, identified by spiking with the compound, was

Scheme 4.



formed in the hydrolysis in barely detectable amounts. Less than 3% of the hydrolysis pathway now occurs by exocyclic C–N fission. Furthermore, the acetate buffer probably affects the relative rates of hydrolysis of the β-lactam and the exocyclic amide. Buffer catalysis accounts for 17% of the observed rate of hydrolysis under the same experimental conditions as determined from kinetic measurements at different acetate concentrations. If, in an extreme case, the buffer-catalysed hydrolysis of **2** occurs exclusively at the exocyclic amide, a minimum of 17% of the product would be benzoic acid. However, as only a maximum of 3% is attributed to the hydrolysis of the exocyclic amide in the acetate buffer at pH 4, the pH-independent hydrolysis of **2** probably occurs exclusively with endocyclic C–N bond fission. This is in contrast to the 4:1 ratio of β-lactam and exocyclic amide hydrolysis seen for the alkaline hydrolysis of **2**. The exocyclic acyl centre must show a greater selectivity (β_{nuc}) towards nucleophilic attack by water and hydroxide ion, than that of the β-lactam.

The rate of hydrolysis of **2** was studied at different temperatures by UV spectrophotometry (Table 3). The entropy of activation ($\Delta S^{\ddagger}$) for the pH-independent hydrolysis is –228 J K^{-1} mol^{-1}, which is threefold more negative compared with –77.3 J K^{-1} mol^{-1} for the alkaline hydrolysis. The more negative entropy of activation for the pH-independent hydrolysis compared to that for the alkaline hydrolysis suggests a more ordered system for the pH-independent hydrolysis. The solvent kinetic isotope effect (k_{H_2O}/k_{D_2O}) for the pH-independent hydrolysis of **2** is 1.25, which indicates the transition state in the rate-limiting step involves a degree of proton transfer (Table 4). The entropy of activation ($\Delta S^{\ddagger}$) and the solvent kinetic isotope effect (k_{H_2O}/k_{D_2O}) is compatible with a hydrolysis mechanism involving nucleophilic attack by water that is general-base-catalysed by another water molecule such as that shown in **7**.

Table 4. The rate constants for the acid-catalysed hydrolysis (k_H), pH-independent hydrolysis (k_o), and alkaline hydrolysis (k_{OH}) of the β-lactam of *N*-aroyl β-lactams in 1% acetonitrile – water (*v*/*v*) with 1.0 mol/L ionic strength (KCl) at 30 °C.

	pK_a	k_o (s^{-1})	k_H ($(mol/L)^{-1}$ s^{-1})	k_{OH}^{endo} ($(mol/L)^{-1}$ s^{-1})
p-NO_2	3.44	3.51×10^{-6}	1.38×10^{-5}	26.5
p-Cl	3.99	1.23×10^{-6}	2.68×10^{-5}	13.8
p-H	4.20	8.92×10^{-7} (H_2O) 7.16×10^{-7} (D_2O)	3.19×10^{-5}	9.07
p-OMe	4.47	6.04×10^{-7}	7.75×10^{-5}	7.27
o-COOH	5.28			2.60

Note: The pK_a of the carboxylic acid corresponding to exocyclic aryl carboxamide is given as an indicator of the inductive effect of the substituent in the aromatic ring. pK_a values are taken from ref. 12.

The first-order rate constants (k_o) for the pH-independent hydrolysis of aryl substituted *N*-aroyl β-lactams were determined (Table 4) and generated a Brønsted β_{lg} value of –0.77. The Brønsted β_{lg} value for the pH-independent hydrolysis is slightly more negative than that for the alkaline hydrolysis (β_{lg} = –0.55) indicating a later transition state probably owing to water being a much weaker nucleophile than hydroxide ion.

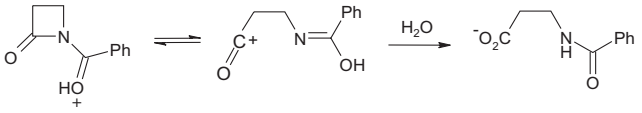
Acid-catalysed hydrolysis

The acid-catalysed hydrolysis of **2** in 1.0 mol/L hydrochloric acid was also followed by HPLC at 30 °C. The signal for **2** disappeared with time in an exponential manner, but that for benzoic acid, identified by spiking with the compound, did not show any significant increase. It is estimated that benzoic acid formation accounted for less than 3% of the hydrolysis pathway.

The second-order rate constants (k_H) for the acid-catalysed hydrolysis of the β-lactam of *N*-aroyl β-lactams increase with electron-donating substituents in the aryl ring of the amide leaving group (Table 4). The rate constants are reasonably well-correlated with the pK_a of the corresponding benzoic acid to give a Brønsted β_{lg} value of 0.68. The change in effective charge of +0.68 indicates a transition state with significant protonation on the amide leaving group. There are three possible sites for protonation of **2**: the β-lactam nitrogen is the least basic compared with the β-lactam and the exocyclic carbonyl oxygens. Protonation on the exocyclic carbonyl oxygen is probably more favourable than protonation on the β-lactam carbonyl oxygen, as the carbonyl oxygen becomes less basic when it is attached to a four-membered ring system (16). Also, protonation on the exocyclic carbonyl oxygen may increase the nucleofugality of the leaving group in β-lactam ring opening.

The second-order rate constants for the acid-catalysed hydrolysis of **2** shows a greater dependence on temperature than that for alkaline hydrolysis (Table 3). The entropy of activation ($\Delta S^{\ddagger}$) for the acid-catalysed hydrolysis is –19.1 J K^{-1} mol^{-1}, which is threefold less negative compared with –77.3 J K^{-1} mol^{-1} for alkaline hydrolysis. The smaller entropy of activation for the acid-catalysed hydrolysis may be indicative of an A1 pathway and a unimolecular breakdown of the conjugate acid of the *N*-aroyl β-lactam. The acid-catalysed hydrolysis of the β-lactam in penicillins **1** and other β-lactams has been suggested to involve an A1 mechanism with protonation on the β-lactam nitrogen leading to intermediate formation of an acylium ion (6, 16). If the acid-catalysed hydrolysis of *N*-benzoyl β-lactam occurs by an A1 mechanism with O protonation on the exocyclic carbonyl, C–N bond fission would form a highly unstable acylium ion and an enol amide (Scheme 5).

Scheme 5.



In conclusion, it has been shown that the alkaline hydrolysis of *N*-aroyl β-lactams occurs at similar rates at the β-lactam and the exocyclic amide centres. Despite the strain energy of the four-membered ring, it is not released in the transition state to lower the activation energy and favour exclusive endocyclic C–N ring fission. The reluctance of four-membered rings to open rapidly has been noted previously (2). The competitive rates of hydrolysis at the endocyclic and exocyclic carbonyl centres provide an opportunity to investigate the relationship between the specificity and reactivity of the acyl centres towards enzymes.

Acknowledgment

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