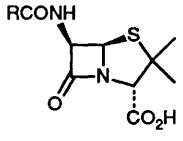
# The Mechanisms of Hydrolysis of the γ-Lactam Isatin and its Derivatives

**Lorraine A. Casey,[a] Ron Galt[b] and Michael I. Page*[,a]**
[a] *Department of Chemical Sciences, The University of Huddersfield, Queensgate, Huddersfield HD1 3DH, UK*
[b] *ICI Pharmaceutical Division, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK*

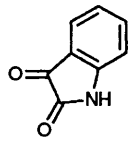
The pH dependences of the rates of hydrolysis of isatin, its *N*-carboxymethyl derivative and its 5-nitro substituted analogues exhibit a complex behaviour, showing a first- and second-order dependence upon hydroxide ion concentration, as well as a pH-independent pathway. The pH dependence is interpreted in terms of the formation of tetrahedral intermediates in different protonic states which may break down to products *via* hydroxide ion, hydronium ion and water catalysed pathways. These γ-lactams are as reactive, or more reactive, than benzylpenicillin.

The antibacterial activity of β-lactam antibiotics arises from a combination of their chemical reactivity and their molecular recognition by target enzymes.[1] One aspect of their chemical reactivity is their acylating power and although, *e.g.*, penicillins, **1**, are not very good acylating agents they are more reactive than simple unsubstituted amides.[2] Since ring strain and reduced amide resonance within the β-lactam has been shown not to contribute significantly to the chemical reactivity of the antibiotics,[3,4] the possibility of non-β-lactam structures showing antibacterial activity has gained credibility.[5]

γ-Lactams are obvious candidates for potentially showing similar antibacterial behaviour to that of the β-lactam antibiotics.[6] Two important aspects of the acylating power of amides are the activation of the carbonyl group towards nucleophilic attack and the stabilisation of the amine leaving group to facilitate C–N bond fission. Both these requirements are met in isatin (1*H*-indole-2,3-dione) **2** with the α-carbonyl group both activating the γ-lactam carbonyl carbon and stabilising the expelled amine anion, **3**.



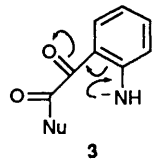

**1**




**2**

The hydrolysis of amides generally proceeds by the formation of unstable tetrahedral intermediates.[7,8] The rate-limiting step of the reaction can involve either formation or breakdown of this intermediate in a variety of protonic states. There appear to be relatively small differences in the energies of the transition states linking these intermediates because changes in the rate-limiting step are often observed.[9] This is one of the reasons why β-lactams are not much more reactive than acyclic amides towards alkaline hydrolysis.[3,4]

The rates of hydrolysis of isatin **2** and its derivatives show a complex dependence upon pH indicative of subtle changes in mechanisms and rate-limiting step. As an interesting mechanistic problem, but also as a background study for our attempts to prepare antibacterial agents based on the isatin structure, the results of this pH dependence are reported here.




**3**

## Experimental

*N-Carboxymethylisatin.*—Isatin (14.7 g, 0.1 mol) was dissolved, with warming, in 2 mol $dm^{-3}$ sodium hydroxide solution (45 $cm^3$). Chloroacetic acid (14.1 g, 0.15 mol) dissolved in 0.1 mol $dm^{-3}$ sodium carbonate solution (50 $cm^3$) was added and the solution refluxed for 4 h. Excess concentrated hydrochloric acid was added to precipitate both product and starting material. The precipitate was filtered and stirred with 0.1 mol $dm^{-3}$ sodium carbonate solution (50 $cm^3$) to extract the product. The solution was filtered and the filtrate was acidified to pH 2 with concentrated hydrochloric acid. The resultant solid was recrystallised from water to give a bright orange powder. Yield = 6.10 g (30%). M.p. 204–206 °C. $\nu_{max}$(Nujol mull)/$cm^{-1}$ 1730, 1620, 1260; $\delta_H$(270 MHz, [$^2H_6$]DMSO) 4.50 (2 H, s, 10-H), 7.17 (1 H, t, *J* 7.4, 5-H),† 7.20 (1 H, d, *J* 7.9, 7-H), 7.60 (1 H, d, *J* 7.3, 4-H) and 7.68 (1 H, t, *J* 6.8, 6-H); *m/z* (relative intensity) 206 ($M^+$ + 1, 0.6), 205 ($M^+$, 4.7), 161 ($M^+$ – $CO_2H$, 0.5) and 147 ($M^+$ – $CH_2CO_2H$, 1.7) (Found: C, 53.75; H, 4.1; N, 6.05. Calc. for $C_{10}H_7NO_4 \cdot H_2O$: C, 53.81; H, 4.04; N, 6.28%).

*N-Carboxymethyl-5-nitroisatin.*—A solution of potassium hydroxide (3.30 g, 0.05 mol assuming 15% water) in methanol (25 $cm^3$) was added to a stirred partial solution of 5-nitroisatin (9.7 g, 0.05 mol) in DMSO (35 $cm^3$). The suspended reactant dissolved and the solution changed from deep yellow to very dark red with precipitation of the potassium salt. *tert*-Butyl bromoacetate (10.25 g, 0.0525 mol, 7.7 $cm^3$) was added and the mixture was stirred for 2 h at room temperature. During this time the potassium salt dissolved and some salts precipitated. TLC in diethyl ether of the red solution showed product and some remaining reactant. More *tert*-butyl bromoacetate (2 $cm^3$) was added and the mixture was heated on a steam bath for 15 min but TLC showed no further reaction. The mixture was poured into water (1 $dm^3$) and extracted with ethyl acetate. The organic phase was washed with water and saturated sodium chloride, dried over anhydrous magnesium sulfate and evaporated under reduced pressure leaving a semi-solid mass. This was triturated with dichloromethane and some reactant was filtered off (1.8 g, 19% recovery). The solution was evaporated again under reduced pressure and triturated with cold diethyl ether to give the crude product (9.1 g). This was recrystallised from isopropanol (300 $cm^3$), cooling to 0 °C to give yellow, fluffy needles. Yield = 8.1 g (53%), m.p. 167–168 °C. $\nu_{max}$/$cm^{-1}$ 1750, 1530 and 1345 (Found: C, 55.0; H, 4.6; N, 9.1. Calc. for

† *J* Values are given in Hz.

$C_{14}H_{14}N_2O_6$: C, 54.9; H, 4.6; N, 9.1%). $\delta_H(CDCl_3)$ 1.49 (s, 9 H, $CMe_3$), 4.45 (s, 2 H, N-$CH_2$–), 6.95 and 8.55 (m, 3 H, ArH).

The *tert*-butyl ester was converted to the acid by the following method. A solution of the ester (2.45 g, 8.0 mmol) in trifluoroacetic acid (5 $cm^3$) was refluxed for 5 min. Some product crystallised. The mixture was cooled, triturated with diethyl ether and the crude product was filtered (1.7 g). A second crop (0.2 g) was obtained on evaporation and triturating with diethyl ether. The combined crops were recrystallised from water (20 $cm^3$) to give golden needles. TLC in 10% dichloromethane–methanol 2% acetic acid showed one spot. Yield = 1.85 g (93%). M.p. 206–207 °C (loss of $H_2O$ at ~100 °C). $\nu_{max}/cm^{-1}$ 1740, 1730, 1525 and 1350 (Found: C, 43.9; H, 3.0; N, 9.6. Calc. for $C_{10}H_6N_2O_6 \cdot H_2O$: C, 44.78; H, 2.99; N, 9.5%). $\delta_H([^2H_6]DMSO)$ 4.60 (s, 2 H, N-$CH_2$–), 7.40 (d, *J* 9, ArH), 8.27 (d, *J* 3, 1 H, *m*-coupled ArH), 8.52 (d of d, *J* 9, *J* 3, *m*-coupled AB).

*Kinetics.*—AnalaR grade chemicals were used exclusively in the preparation of buffers. Freshly boiled glass-distilled water was used throughout and the ionic strength was maintained at 1.0 mol $dm^{-3}$ with potassium chloride except where otherwise indicated.

The pH of buffer solutions was measured with a Philips PW 9409 digital pH meter equipped with a Russel type CEL glass combination electrode, calibrated against standard buffers of known pH at 30 °C. The electrode could be dipped into a reaction cuvette before and after a kinetic run to ensure the pH of the solution had not altered by more than 0.03 of a pH unit.

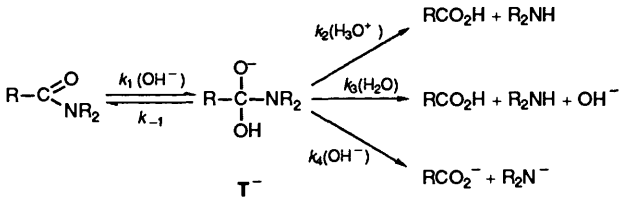
The spectrophotometer used for the majority of reactions was a Gilford model 2600 single-beam instrument which has a four-cell compartment with an automatic cell change. The temperature in the cell compartment was maintained at 30 ± 0.1 °C by water circulated from a Haake water-bath to the cell block. Absorbance–time plots or UV spectra were plotted on a Hewlett–Packard 7225BX-Y plotter. Reactions were initiated by the addition of 25 $mm^3$ of substrate to 2.5 $cm^3$ of a temperature equilibrated solution in a quartz cuvette, and the time-dependent change in absorbance monitored. Data was transferred to an Apple Europlus 2 or an IBM PC for analysis. Hard copies of results were printed on an Epson MX-80 printer and data stored on disk.

For very fast reactions where the half-life of the reaction was less than 2 s, the reactions were followed in a Nortech SF3A Mk. 4 stopped flow spectrometer. Reactants at twice the desired final concentration were placed in two piston-driven syringes. These feed into the reaction cell *via* coiled glass tubes immersed in a water-bath thermostatically maintained at 30 ± 0.1 °C. Absorbance changes after mixing were followed at 280–290 nm. The signal from the photomultiplier was transmitted to a Datalab DL 901 transient recorder which was automatically triggered by the outlet syringe, simultaneously causing a display on a Gould Advance OS 250B oscilloscope. Changes in absorbance *vs.* time were output from the transient recorder to a Servogor 210 chart recorder.

Data transferred from the Gilford 2600 to the Apple Europlus 2 was analysed using a programme which calculates a first-order rate constant using an iterative non-linear least-squares procedure which treats the initial absorbance, final absorbance and rate constant as adjustable parameters. Besides accepting data from the Gilford, data can be entered from a disk file or manually on the keyboard. The experimental data may be compared graphically with the curve derived from the calculated parameters and edited where necessary.

## Results and Discussion

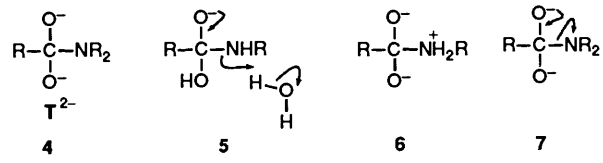
The mechanism of the alkaline hydrolysis of amides and lactams is generally as outlined in Scheme 1,[7,8,9] with the observed pseudo-first-order rate constant given by eqn. (1).




**Scheme 1**

$$k_{obs} = \frac{k_1[OH^-](k_2[H_3O^+] + k_3[H_2O] + k_4[OH^-])}{k_{-1} + k_2[H_3O^+] + k_3[H_2O] + k_4[OH^-]} \quad (1)$$

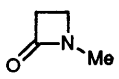
For pathways which are first-order in hydroxide ion either formation or breakdown of the anionic tetrahedral intermediate, $T^-$, may be rate limiting. Reactive amides with electron withdrawing substituents in the amine residue often show a rate term which is second-order in hydroxide ion. This has been observed for the hydrolysis of acetanilides,[10] formanilides,[11] trifluoroacetanilides[12] and acetylpyrroles.[13] The higher order term in base appears to occur only with amides which have weakly basic amines as leaving groups. This pathway is thought to result from proton abstraction of the reversibly formed $T^-$ by hydroxide ion to give a dianionic tetrahedral intermediate $T^{2-}$ (**4**) which subsequently collapses to products by expulsion of the amine anion or by the assistance of general acid catalysis from the solvent water to give the neutral amine.

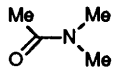


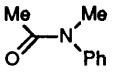

The reason why the second-order base term is seen for reactive amides of weakly basic amines is because the Brønsted $\beta_{lg}$ for this pathway is more negative than that for the process which is first-order in hydroxide. The monoanionic tetrahedral intermediate can only collapse to products by expelling the amine partially protonated by water **5**[14] or by a proton switch to give the anionic zwitterionic intermediate **6** and its subsequent breakdown.[9,10] Protonation of nitrogen and C–N bond fission have opposing electronic demands. By contrast, the rates of all steps in the formation of the dianionic tetrahedral intermediate $T^{2-}$ and its breakdown to products **7** are facilitated by electron withdrawing substituents attached to the amine.

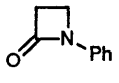
Rate-limiting formation of the tetrahedral intermediate $T^-$ is thought to occur during the alkaline hydrolysis of β-lactams which exhibit a Brønsted $\beta_{lg}$ of −0.44.[4,8] The hydrolyses of other lactams, amides and anilides which are first-order in hydroxide ion have a smaller dependence upon the basicity of the expelled amine with the interesting consequence that the rate of hydrolysis of β-lactams of basic amines is similar to that for analogous acyclic amides.[3,4] For example, the second-order rate constant for the alkaline hydrolysis of the β-lactam **8** is only three times greater than that for the amide **9**. Similarly, there is little difference in reactivity between the amide **9** and the unsubstituted anilide **10** whereas a difference of 100-fold is seen between the rates of alkaline hydrolysis of the *N*-phenyl β-lactam **11** and the acyclic acetanilide **10**. The expected increased reactivity of β-lactams towards alkaline hydrolysis is only seen for β-lactams of weakly basic amines.[3,4]

Amides of primary amines may undergo NH ionisation, and


8


9

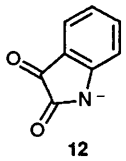

10


11

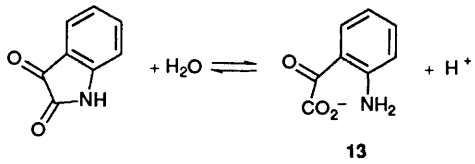
at high pH this process inhibits the rate of alkaline hydrolysis of amides which eventually then becomes zero-order in hydroxide ion.[11,15]

There is no evidence which indicates that the conformational state or mobility of the anionic tetrahedral intermediate, and hence stereoelectronic effects,[16] are dominant factors in determining the relative rates of C–O and C–N bond cleavage. The leaving group ability of an amine is controlled mainly by its basicity, and its steric and solvation effects.

*The Hydrolysis of Isatin.*—Isatin **2** is an orange solid which upon dissolution in water of pH > 11 forms a deep-violet colour which changes to yellow on standing. The violet colour is not formed with *N*-alkyl isatins and is probably due to formation of the highly conjugated anion **12**. The yellow


12

colouration is due to hydrolysis of the isatin to the ring-opened amino acid **13**, Scheme 2. This ring opening is reversible and acidification reforms isatin. Changes in the UV spectrum with

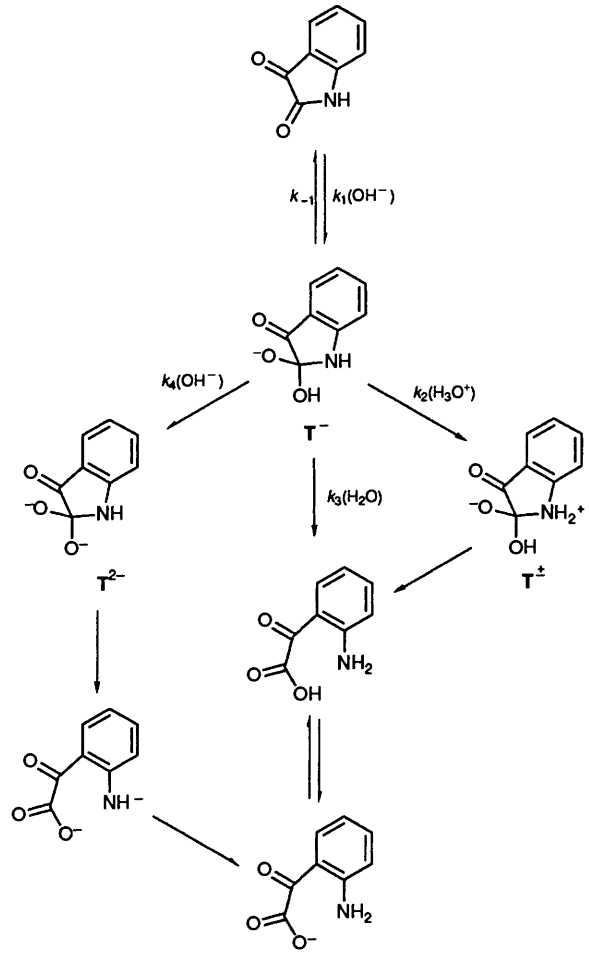

13

**Scheme 2**

pH indicate that above pH 6 the ring-opened form predominates and at pH 4 there are approximately equal amounts of the ring-closed and -opened systems. Below pH 3 isatin is the thermodynamically stable form. The kinetics of hydrolysis reported here refer to the effectively irreversible hydrolysis above pH 5.

The pH–rate profile for the hydrolysis of isatin is shown in Fig. 1. There are several distinct regions of pH dependence and independence. From pH 5 to 6, the rate of hydrolysis is apparently first-order in hydroxide ion, or inversely proportional to the hydronium ion concentration, but from pH 6.5 to 10.5 it is pH independent. As the pH is increased the rate becomes second-order in hydroxide ion but then returns to first-order above pH 12.

This apparently complex behaviour is explicable in terms of the reaction mechanisms given in Scheme 3. There are, however, several possible mechanisms which are compatible with the kinetic observations. That shown in Scheme 3 involves the initial attack by hydroxide ion ($k_1$) to generate reversibly the anionic tetrahedral intermediate $\mathbf{T}^-$. This intermediate can spontaneously revert to the reactants, $k_{-1}$, or be converted to products by hydronium ion catalysed expulsion of the amine, $k_2$. The rate-limiting step below pH 6 is formation of the tetrahedral intermediate because $\mathbf{T}^-$ is converted to product faster than reactants ($k_2[H_3O^+] > k_{-1}$). Between pH 6 and 10.5 the relative rates of decomposition of $\mathbf{T}^-$ change as $k_{-1}$ becomes greater than $k_2[H_3O^+]$. There is thus a change in rate-limiting step to breakdown of the anionic intermediate catalysed by the hydronium ion.



**Scheme 3**

An alternative mechanism for this pH range would involve reversible formation of $\mathbf{T}^-$ by attack of a water molecule on isatin catalysed by a second water molecule acting as a general

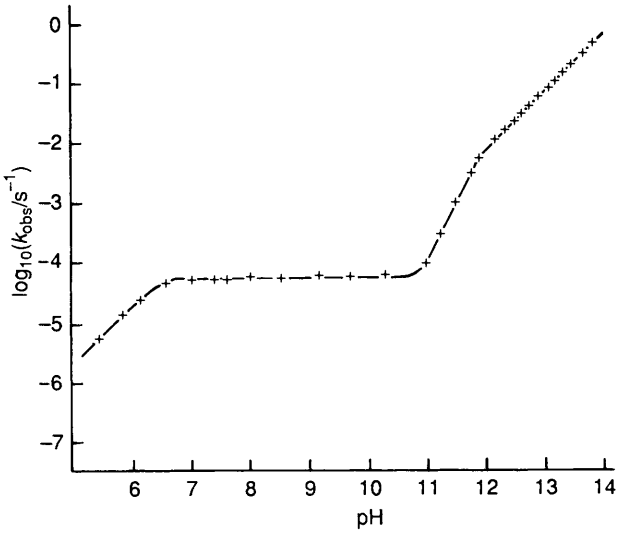
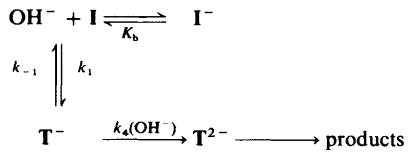


**Fig. 1** A plot of the logarithm of the observed pseudo-first-order rate constants *vs.* pH for the hydrolysis of isatin in water at 30.0 °C; points have been extrapolated to zero buffer concentration




**Scheme 4**

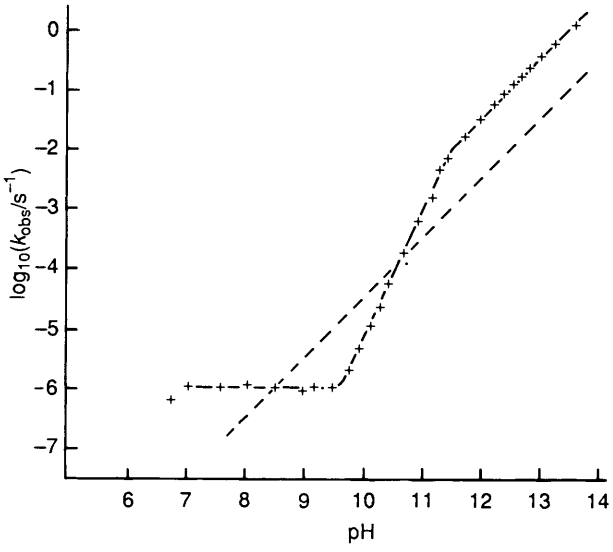



**Fig. 2** A plot of the logarithm of the observed pseudo-first-order rate constants *vs.* pH for the hydrolysis of *N*-carboxymethyl isatin (**14**) in water at 30.0 °C; points have been extrapolated to zero buffer concentration. The dashed line represents the similar plot for the hydrolysis of benzylpenicillin under the same conditions.

base catalyst. Microscopic reversibility then demands that the intermediate collapses back to reactants by acid-catalysed expulsion of the hydroxide ion by the hydronium ion. Below pH 6, this retro step is faster than the spontaneous or water-catalysed decomposition of $T^-$ into products which is thus the rate-limiting step, and so the observed rate is inversely proportional to the hydronium ion concentration. Between pH 6 and 10.5, the reverse step becomes slower as the hydronium ion concentration decreases and the pH-independent rate-limiting step becomes attack of water on isatin. This type of behaviour has been suggested for the hydrolysis of thioesters[17] although a subtlety involves the stepwise attack of water so that breakdown of the intermediate becomes rate limiting at the lowest pH.[18] Although initial attack by water on isatin remains a possible alternative it is difficult to encompass this mechanism with the kinetic observations at higher pH which demand the formation of $T^-$ by attack of hydroxide ion on isatin.

The rate equation for the rate of hydrolysis of isatin between pH 5 and 10.5 is thus given by eqn. (2) with the constants as defined in Scheme 3 and $K_w$ as the dissociation constant for water.

$$k_{obs} = \frac{k_1[OH^-]k_2[H_3O^+]}{k_{-1} + k_2[H_3O^+]} = \frac{k_1k_2K_w}{k_{-1} + k_2[H_3O^+]} \quad (2)$$

The value of $k_1$, the attack of hydroxide ion on isatin, is $1.25 \times 10^3$ dm$^3$ mol$^{-1}$ s$^{-1}$ and the 'spontaneous' hydrolysis rate constant, $k_1k_2K_w/k_{-1}$, is equal to $5.62 \times 10^{-5}$ s$^{-1}$.

From pH 11 to 12 the observed pseudo-first-order rate constant for the hydrolysis of isatin shows a second-order dependence upon the hydroxide ion concentration. This presumably results from the reaction occurring through the formation of the dianionic intermediate $T^{2-}$. The rate of conversion of $T^-$ to $T^{2-}$ must become faster than its rate of breakdown, catalysed by the very low concentration of hydronium ion, $k_2[H_3O^+]$ (Scheme 3). The third-order rate constant $k_{OH}^2$ is then given by $k_1k_4/k_{-1}$ and equals 95.4 dm$^6$ mol$^{-2}$ s$^{-1}$.

At pH 12 the rate of hydrolysis of isatin changes from second-order to first-order dependence upon hydroxide ion. This pH corresponds to the colour change described earlier which is attributed to ionisation of the amide to its *N*-conjugate base **12**. The amide anion is presumably not susceptible to attack by hydroxide ion and consequently increasing hydroxide concentration reduces the second-order dependence to an apparently first-order one as the concentration of the hydrolytically labile undissociated isatin decreases. This reaction sequence is outlined in Scheme 4 where $I^-$ is the amide anion **12**. The observed pseudo-first-order rate constant is given by eqn. (3) where $K_b$,

$$k_{obs} = \frac{k_1k_4[OH^-]^2}{k_{-1} + k_4[OH^-]} \frac{K_b}{K_b + [OH^-]} = \frac{k_1k_4[OH^-]^2}{k_{-1} + k_4[OH^-]} \frac{K_w}{K_w + K_a[OH^-]} \quad (3)$$

$K_a$ and $K_w$ are the base and acid dissociation constants of isatin and water respectively.

If the rate of collapse of $T^-$ back to reactants, $k_{-1}$, is greater than the rate of conversion to the dianion and its subsequent breakdown to products, $k_4[OH^-]$, then the pseudo-first-order rate constant reduces to eqn. (4). At pHs above the

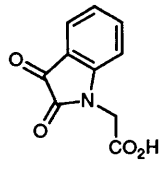
$$k_{obs} = \frac{k_1k_4[OH^-]^2}{k_{-1}} \frac{K_w}{K_w + K_a[OH^-]} \quad (4)$$

p$K_a$ of isatin, $K_a[OH^-] > K_w$ and $k_{obs}$ becomes first-order in hydroxide ion.

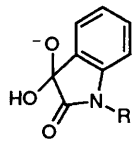
The p$K_a$ of isatin is estimated to be 11.90 ± 0.03 and the apparent first-order rate constant to be 1.12 dm$^3$ mol$^{-1}$ s$^{-1}$.

It is conceivable that the change in kinetic order results not from ionisation but from the addition of hydroxide ion to the α-carbonyl carbon. This in fact occurs with the *N*-alkyl isatin derivative where NH ionisation cannot occur.

*The Hydrolysis of* N-*Carboxymethylisatin.*—The hydrolysis of *N*-carboxymethylisatin (**14**) is of interest both as a potential model for a penicillin analogue (**1**) and as an amide unable to undergo NH ionisation.




**14**

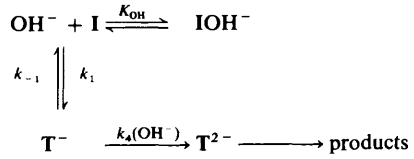



**15**

The pH–rate profile for the hydrolysis of *N*-carboxymethylisatin is given in Fig. 2 and shows at least three distinct regions. From pH 7 to 9.5 there is a pH-independent pathway followed by a second-order dependence upon hydroxide ion from pH 9.5 to 11.5. At even higher pH, the pseudo-first-order rate constant then shows a change to a first-order dependence on hydroxide ion.

For comparison, the pH dependence of the rate of hydrolysis of benzylpenicillin is also shown in Fig. 2. The isatin derivative is slightly more reactive than penicillin over the pH range studied, indicating that, at least on a chemical basis, γ-lactams based on the isatin structure are reactive enough to be effective acylating agents of nucleophilic groups within target enzymes.

The pH-independent pathway between pH 7 and 9.5 may be indicative of rate-limiting acid-catalysed breakdown of the




**Scheme 5**

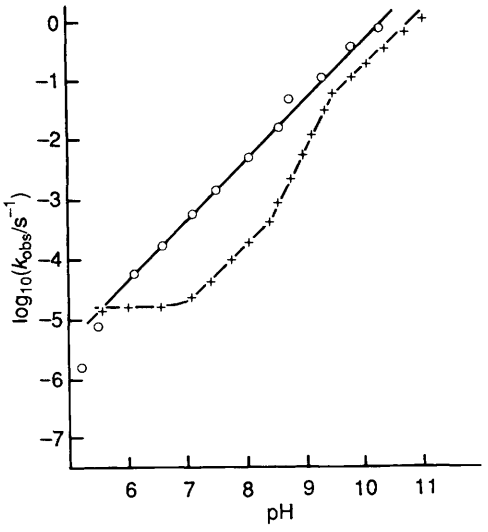
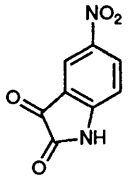
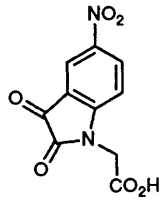



**Fig. 3** A plot of the logarithm of the observed pseudo-first-order rate constants *vs.* pH for the hydrolysis of 5-nitroisatin (**16**) (○) and *N*-carboxylmethyl 5-nitroisatin (**17**) (+) in water at 30.0 °C, points have been extrapolated to zero buffer concentration




**16**




**17**

reversibly formed tetrahedral intermediate $T^-$ ($k_2$ in Scheme 3). As with isatin itself this pathway could also involve initial attack by water. The first-order rate constant for this spontaneous hydrolysis, $9.5 \times 10^{-7}$ $s^{-1}$, is about 50-fold less than that for isatin.

Similar to isatin, the *N*-alkyl derivative (**14**) changes from a pH-independent rate of hydrolysis to a base-dependent one, but at about pH 9.5. This change occurs about 2 pH units lower than that shown by isatin although the dependence of the rate upon base concentration is apparently immediately second-order. The observed first-order rate constant for hydrolysis of the *N*-alkyl isatin **14** thus becomes greater than that for isatin above pH 10.5. The second-order dependence of this rate of hydrolysis of **14** upon hydroxide ion between pH 9.5 and 11.5 is indicative of a mechanism involving the dianion of the tetrahedral intermediate $T^{2-}$ (Scheme 3). The third-order rate constant for this process $k_{OH}^2$ is $6.31 \times 10^2$ $dm^6$ $mol^{-2}$ $s^{-1}$. At pH 11.5 there is another change in the kinetic dependence on hydroxide ion to first-order. Because **14** is a *N*-alkylated isatin this cannot be attributed to amide ionisation. However, it is almost certainly due to hydration of the α-carbonyl group by the addition of hydroxide ion to give the relatively unreactive anion **15**. Consequently, although increasing hydroxide ion concentration increases the observed rate constant this is offset by a depletion of the hydrolytically labile *N*-alkylisatin (**14**). This reaction sequence is outlined in Scheme 5 where I is the isatin derivative (**14**), $IOH^-$ the ionised hydrated adduct (**15**) and the other symbols are as defined in Scheme 3.

The observed pseudo-first-order rate constant is then given by eqn. (5). When $k_{-1} > k_4[OH^-]$ and $K_{OH}[OH^-] > 1$, the

$$k_{obs} = \frac{k_1 k_4 [OH^-]^2}{k_{-1} + k_4[OH^-]} \quad \frac{1}{(1 + K_{OH}[OH^-])} \qquad (5)$$

rate constant $k_{obs}$ reduces to eqn. (6) and shows a first-order

$$k_{obs} = \frac{k_1 k_4 [OH^-]}{k_{-1}} \quad \frac{1}{K_{OH}} \qquad (6)$$

dependence upon hydroxide ion concentration. The second-order rate constant $k_{OH}$ above pH 11.5 is 3.98 $dm^3$ $mol^{-1}$ $s^{-1}$. The ratio of the third-order rate constant to that of the second-order one for hydrolysis $k_{OH}^2/k_{OH}$ gives $K_{OH}$ which is thus calculated to be 159 $dm^3$ $mol^{-1}$. When $K_{OH}[OH^-]$ is equal to unity the observed pseudo-first-order rate constant will be one half of that calculated from the second-order dependence $k_{OH}^2$, eqn. (5), in the absence of hydroxide ion addition to the α-carbonyl group. This occurs at pH 11.8 giving a $K_{OH}$ of 158 $dm^3$ $mol^{-1}$.

*The Hydrolysis of* 5-*Nitroisatin* (**16**) *and its* N-*Carboxymethyl Derivative* (**17**).—The pH–rate profiles for the hydrolysis of 5-nitroisatin (**16**) and its *N*-carboxymethyl derivative (**17**) are given in Fig. 3. The observed first-order rate constants for the hydrolysis of the nitro *N*-alkyl isatin (**16**) show several features with increasing pH, namely pH independent, first-order in hydroxide, second-order in hydroxide ion, and finally back to first-order.

Below pH 7 the 'spontaneous' hydrolysis rate constant $k_0$ is $1.56 \times 10^{-5}$ $s^{-1}$ and is 16-fold greater than that for *N*-carboxymethylisatin (**14**). From pH 7 to 8.5 the rate is first-order in hydroxide with a second-order rate constant equal to 130 $dm^6$ $mol^{-2}$ $s^{-1}$. This presumably represents rate-limiting breakdown of the tetrahedral intermediate $T^-$ catalysed by water, $k_3$ in Scheme 3. From pH 8.5 to 9.5 the rate is second-order in hydroxide ion with a third-order rate constant $k_{OH}^2$ of $5.62 \times 10^7$ $dm^6$ $mol^{-2}$ $s^{-1}$ which is nearly $10^5$-times greater than that for the unsubstituted *N*-carboxymethylisatin (**14**). These two points would indicate a Hammett ρ value of 3.9, compatible with rate-limiting breakdown of the dianionic tetrahedral intermediate $T^{2-}$.

Above pH 9.5, the observed pseudo first-order rate constant changes from a second- to a first-order dependence on hydroxide ion concentration. The second-order rate constant for this process is $1.58 \times 10^3$ $dm^3$ $mol^{-1}$ $s^{-1}$. This change in kinetic dependence is probably also due to addition of hydroxide ion to the α-carbonyl group to give the nitro-substituted analogue of **15** and is illustrated by Scheme 5.

The calculated equilibrium constant for the addition of hydroxide ion to the α-carbonyl group of the nitro *N*-alkyl isatin (**17**) is $3.56 \times 10^4$ $dm^3$ $mol^{-1}$. This corresponds to a change from second- to first-order dependence of the rate of hydrolysis at pH 9.5 in good agreement with the observed value.

The ratio of the equilibrium constants for addition of hydroxide ion for the nitro (**17**) and unsubstituted (**14**) *N*-alkyl isatin is 225, indicative of a Hammett ρ value of 1.8.

By contrast, the rate of hydrolysis of 5-nitroisatin (**16**) shows a simple first-order dependence upon hydroxide ion concentration between pH 6 and 10. The second-order rate constant for this process is $2.52 \times 10^3$ $dm^3$ $mol^{-1}$ $s^{-1}$.

Although the hydrolysis of isatin and its derivatives shows a complex dependence upon pH, the kinetic dependence is explicable in terms of the normal tetrahedral intermediates. The chemical reactivity of the isatin derivatives shows that they are

as chemically reactive as penicillin with respect to their acylating power. This work, therefore, demonstrates that such γ-lactams are potentially capable of forming non-β-lactam antibiotics.

## Acknowledgements

We thank Deborah Thorley and Julian Stone for some of the kinetic measurements.

## References

1 M. I. Page, ed., *The Chemistry of β-Lactams*, Blackie and Son, Glasgow, 1992; J. M. Frère and B. Joris, *CRC Crit. Rev. Microbiol.*, 1985, **11**, 299.
2 A. M. Davis, P. Proctor and M. I. Page, *J. Chem. Soc., Perkin Trans. 2*, 1991, 1213.
3 M. I. Page, *Adv. Phys. Org. Chem.*, 1987, 165.
4 M. I. Page, *Acc. Chem. Res.*, 1984, **17**, 144; P. Proctor, N. P. Gensmantel and M. I. Page, *J. Chem. Soc., Perkin Trans. 2*, 1982, 1185.
5 J. Marchand-Brynaert and L. Ghosez, *Recent Progress in Chemical Synthesis of Antibiotics*, ed. G. Lukacs and M. Ohno, Springer-Verlag, Berlin, 1990, p. 728–794; J. E. Baldwin, G. P. Lynch and J. Pitlik, *J. Antibiotics*, 1991, **44**, 1; L. N. Jungheim and R. J. Ternansky in *The Chemistry of β-Lactams*, ed., M. I. Page, Blackie and Son, Glasgow, 1992, pp. 306–324.
6 J. E. Baldwin, C. Lowe, C. J. Schofield and E. Lee, *Tetrahedron Lett.*, 1986, **27**, 3461; S. Coulton, I. Francois and R. Southgate, *Tetrahedron Lett.*, 1990, **31**, 6923; J. E. Baldwin, R. T. Freeman and C. Schofield, *Tetrahedron Lett.*, 1989, **30**, 4019.
7 L. D. Kershner and R. L. Schowen, *J. Am. Chem. Soc.*, 1971, **93**, 2014; V. Gani and P. Viout, *Tetrahedron*, 1976, **32**, 1669; R. J. E. Talbot in *Comprehensive Chemical Kinetics*, ed. C. H. Bamford and C. F. H. Tipper, Elsevier, Amsterdam, 1972, vol. 10, ch. 3; B. C. Challis and J. A. Challis in *The Chemistry of Amides*, ed. J. Zabicky, Interscience, London 1970; K. Kavalek, F. Krampera and V. Sterba, *Collect. Czech. Chem. Commun.*, 1976, **41**, 1685; J. M. Moreau, M. Annex de Taboada, P. Van Brandt and A. Bruylants, *Tetrahedron Lett.*, 1970, 1255.
8 G. M. Blackburn and J. D. Plackett, *J. Chem. Soc., Perkin Trans. 2*, 1972, 1366; K. Bowden and K. Bromley, *J. Chem. Soc., Perkin Trans. 2*, 1990, 2103.
9 A. J. Bennet, H. Slebocka-Tilk, R. S. Brown, J. P. Guthrie and A. Jodhan, *J. Am. Chem. Soc.*, 1990, **112**, 8497; H. Slebocka-Tilk, A. J. Bennet, J. W. Keillor, R. S. Brown, J. P. Guthrie and A. Jodhan, *J. Am. Chem. Soc.*, 1990, **112**, 8507.
10 M. L. Bender and R. J. Thomas, *J. Am. Chem. Soc.*, 1961, **83**, 4183.
11 R. H. de Wolfe and R. C. Newcombe, *J. Org. Chem.*, 1971, **36**, 3870.
12 J. K. Young, S. Pazharnisamay and R. L. Schowen, *J. Org. Chem.*, 1984, **49**, 4148; S. O. Ericksson, *Acta Chem. Scand.*, 1968, **22**, 892; R. W. Taft and S. Beichler, *J. Am. Chem. Soc.*, 1973, **95**, 4463; R. M. Pollack and T. C. Dumsha, *J. Am. Chem. Soc.*, 1973, **95**, 4463.
13 F. M. Menger and J. A. Donahue, *J. Am. Chem. Soc.*, 1973, **95**, 432; A. Cipiciani, P. Linda and G. Savelli, *J. Heterocycl. Chem.*, 1979, **16**, 677.
14 J. J. Morris and M. I. Page, *J. Chem. Soc., Perkin Trans. 2*, 1980, 685.
15 R. M. Pollack and M. L. Bender, *J. Am. Chem. Soc.*, 1970, **92**, 7190; J. Kavalek and V. Sterba, *Collect. Czech. Chem. Commun.*, 1975, **40**, 1176.
16 P. Deslongchamps, *Stereoelectronic Effects in Organic Chemistry*, Pergamon, Oxford, 1983.
17 L. R. Fedor and T. C. Bruice, *J. Am. Chem. Soc.*, 1965, **87**, 4138.
18 R. J. Zygmunt and R. E. Barnett, *J. Am. Chem. Soc.*, 1972, **94**, 1996.

*Paper* 2/03916H
*Received* 22*nd July* 1992
*Accepted* 10*th September* 1992