Hydrolysis of 4-imino-imidazolidin-2-ones in acid and the mechanism of cyclization of hydantoic acid amides†

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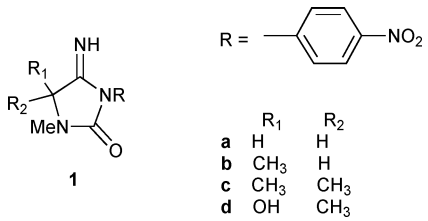
The hydrolysis of iminohydantoins generates the same tetrahedral intermediate **T** as that obtained in the cyclization of hydantoic acid amides to hydantoins. The ratio of the products of imine hydrolysis under kinetic control is determined by the relative height of the barriers of the breakdown of **T** to amide or to hydantoin. Thus the partitioning of products unequivocally proves which is the rate determining step in the cyclization reaction—formation or breakdown of **T**. UV and ^{1}H NMR monitoring of the acid catalyzed hydrolysis of four 5-substituted 4-imino-1-methyl-3-(4-nitrophenyl)imidazolidin-2-ones **1** found hydantoins **3** as the only products. The kinetics of hydrolysis of imines were measured in 0.001–1 M HCl. Contrary to the remaining imines, 1,5-dimethyl-4-imino-3-(4-nitrophenyl)imidazolidin-2-one **1b** is readily oxidized as stock solution in THF containing peroxides to 1,5-dimethyl-5-hydroxy-4-imino-3-(4-nitrophenyl)imidazolidin-2-one **1d**. In all cases, hydrolysis was found to be zero order with respect to [H$^+$]. As imines are fully protonated under the acidity studied, this is evidence of a transition state of a single positive charge. Comparison of imine hydrolysis rates with previous data on rates of cyclization of the corresponding amides of hydantoic acids allowed conditions (acid concentration, substitution pattern—*gem*-dimethyl effect) to be found that guaranteed kinetic control of the products obtained. Thus it was unequivocally proven that formation of the tetrahedral intermediate is rate determining in the cyclization of hydantoic acid amides. The small steric effects upon methyl substitution at 5-C and a solvent kinetic isotope effect k_H/k_D of 1.72 favour a mechanism for imine hydrolysis whereby the rate is limited by water attack on the protonated imine concerted with proton transfer from attacking water to a second water molecule.

Introduction

As an integral part of the Edman degradation procedure for sequencing of peptides, the acid catalysed cyclization of *N*-thiocarbamoyl amino acid amides and peptides has been extensively studied.[1] In the case of the oxygen analogues, the hydantoic acid amides, the mechanism of cyclization under acid catalysis is less well understood[2–4] as opposed to the more extensively studied base catalyzed cyclization.[3–5] In moderately concentrated acids above H_o of −1 (the Hammett acidity function), Kaválek *et al.*[2] observed saturation kinetics due to protonation of the ureido group, which eliminates its nucleophilicity, and also due to considerable protonation of the amide moiety. These authors assume rate determining attack of the ureido group on the protonated amide function to give a tetrahedral intermediate (k_{-2} and **T** of Scheme 2), however, without the support of any experimental proof. Our study of the cyclization of hydantoic acid *N*-methylamides[3] and amides[4] when R = *p*-nitrophenyl showed acid catalysis below pH 2–3. No definite answer could be given as to which is the r.d.s. although according to estimates from fractionation factors[6,7] deuterium solvent kinetic isotope effects fitted better with a rate determining leaving of the amino group from **TH**$^+$. The cyclization of hydantoic acid amides can be compared to acid hydrolysis of amides, which has been shown[8] conclusively to proceed through rate determining attack of water, which is equivalent to attack of the ureido group.

We now report a solution to this problem by studying the hydrolysis of imines **1** shown in Scheme 1.



Scheme 1

As demonstrated in Scheme 2, the first step of the reaction is to generate the tetrahedral intermediate **T** shown in the neutral form because the *O*-protonated form obtained upon addition is a stronger acid than H_3O^+. This same intermediate lies on the reaction path between amide and hydantoins. The products obtained from partitioning of **T** under kinetic control provide an unequivocal answer concerning the relative heights of the energy barriers determining $k2$ and $k3$ and thus, respectively, which is the rate determining step of ureido amide **2** cyclization.

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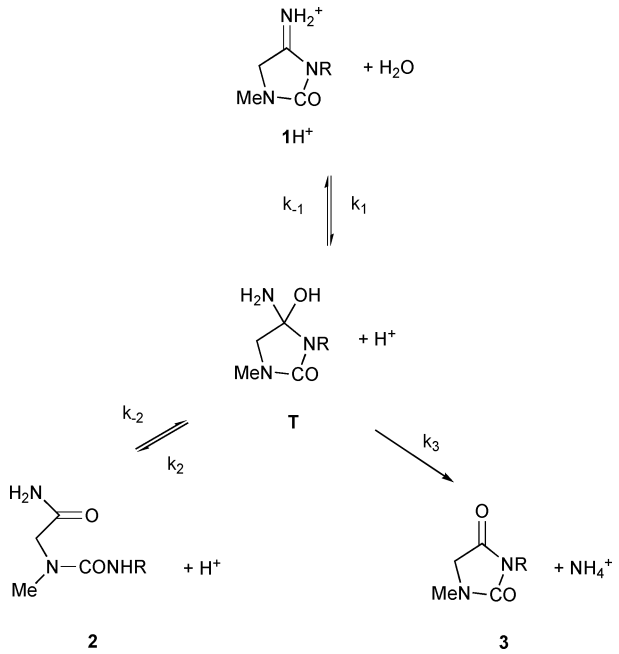
† *In memoriam* to Professor Keith Yates.



Scheme 2

Experimental

UV spectra were measured on a Specord UV–Vis spectrophotometer. NMR spectra were recorded on a Bruker Avance DRX 250 spectrometer, operating at 250.13 MHz for protons. A 5 mm dual ^{1}H–^{13}C probe head was used at room temperature. Chemical shifts are quoted in p.p.m. as δ values against TMS and couplings in Hz.

Materials

Inorganic reagents were of analytical grade and used without further purification. HCl solutions were prepared with CO_2-free distilled water. D_2O, 99 atom % and deuterium chloride, DCl, 22 wt % were from Aldrich. The preparations have been described previously of 4-imino-1-methyl-3-(4-nitrophenyl)imidazolidin-2-one, 1,5-dimethyl-4-imino-3-(4-nitrophenyl)imidazolidin-2-one, 4-imino-3-(4-nitrophenyl)-1,5,5-trimethylimidazolidin-2-one,[9] **1a**, **1b** and **1c** respectively, 1,5-dimethyl-5-hydroxy-4-imino-3-(4-nitrophenyl)imidazolidin-2-one[10] **1d**, 1-methyl-3-(4-nitrophenyl)-imidazolidin-2,4-dione, 1,5-dimethyl-3-(4-nitrophenyl)imidazolidin-2,4-dione and 1,5,5-trimethyl-3-(4-nitrophenyl)imidazolidin-2,4-dione,[3] **3a**, **3b**, **3c** and 1,5-dimethyl-5-hydroxy-3-(4-nitrophenyl)-imidazolidin-2,4-dione[10] **3d**.

Kinetic measurements

The kinetics of hydrolysis of imines **1a-1d** were measured in aqueous HCl (0.001–1 M) by means of UV spectroscopy and in DMSO-d_6–DCl by means of ^{1}H NMR.

The UV experiments were carried out in the thermostatted cell of a Specord UV–Vis spectrophotometer at 25.0 ± 0.1 °C. The rate was followed by monitoring the decrease of absorbance at 250 nm (λ_{max} of the protonated imines; product hydantoins absorb at λ_{max} 280 nm). The reaction was initiated by injecting 30 μl of 0.01 mol dm^{-1} stock solution (THF or acetonitrile, see Results) of the corresponding imine to 2.80 cm^3 of HCl solution. Pseudo-first-order rate constants, k_{obs}, were obtained by non-linear regression curve-fitting to the equation: $A_t = A_o e^{-k_{obs}t} + A\infty$, where A_t, A_o and A are the absorbances at time, t, zero and infinity, respectively. Multiple scan spectra taken during the course of the hydrolysis showed good isosbestic points and the infinity spectra ($10\tau_{1/2}$) were found to be identical with the spectra of the respective 3-(4-nitrophenyl)hydantoins at the same concentration. A "clean" reaction was also supported by well-behaved first order kinetics.

The time dependent NMR studies were recorded at room temperature (22–23 °C) in solutions made up from 2 mg imine, 0.3 ml of DMSO-d_6 and 0.3 ml of 0.11 M DCl yielding *ca.* 0.03 M free DCl in the sample. Series of proton spectra (25–51) were measured at intervals of 5 to 30 min until the hydrolysis of imine was completed (8–16 h). Proton chemical shifts were calibrated according to the signal of DMSO-d_6. The NMR spectra indicated no side reactions.

The Fourier transform spectra were carefully phase corrected and then integrated using the *multiint* au program, which integrates a series of spectra within selected integration regions using the same scaling factor. The obtained integrals for the peaks for methyl groups (in the intervals of 2.87–2.91 and 2.91–2.71 ppm (**1a**), 1.33–1.43 and 1.52–1.66 ppm (**1b**), 1.24–1.49 and 1.50–1.75 ppm (**1c**)) were then fitted simultaneously by the program DynaFit[11] according to the following reaction mechanism of pseudo-first order reaction:

$$1 \xrightarrow{k_{obs}} 3$$

The changes in concentrations of monitored compounds over time were computed by solving an initial value problem described by the following system of differential equations:

$$d[\mathbf{1}]/dt = -k_{obs}\,[\mathbf{1}]$$
$$d[\mathbf{3}]/dt = +k_{obs}\,[\mathbf{3}]$$

Results and discussion

On a preparative scale, iminohydantoins have long been known to give hydantoins in acid.[12] However, for mechanistic conclusions it is important to know whether this is the result of kinetic as opposed to thermodynamic control and further, if any alternate products are formed in significant amounts. The change with time of the UV spectra of iminohydantoins **1** in 0.001–1 M HCl indicates direct conversion into hydantoins **3**: the peaks around 250 nm due to protonated imines deplete with time, accompanied by the formation of a peak at 280 nm due to the respective hydantoins, the process being characterized by a clean isosbestic point. If amides **2** were formed, these would give rise to absorption at 330 nm, characteristic of the *p*-nitrophenylureido group.[3] UV-Spectrophotometry is of course insensitive to small amounts of impurities so product analysis was further carried out by means of ^{1}H NMR. The reaction was followed in 0.03 M DCl in 1 : 1 DMSO-d_6–D_2O; organic solvent was added to overcome poor solubility in water. The scans, taken over periods of time, demonstrate a clean conversion of protonated imine into hydantoins, illustrated for the case of **1b** in Fig. 1.

^{1}H NMR monitoring of the hydrolysis of the remaining imines also showed hydantoin **3** as practically a single product with no significant quantities of amides or other side products. However, crucial for deductions regarding the slow step of hydantoinamide

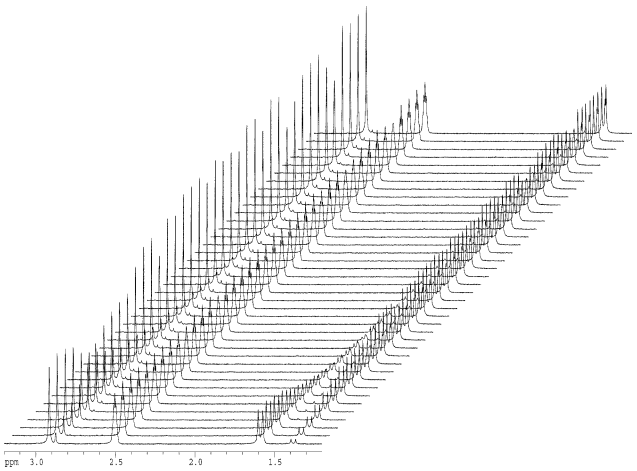


Fig. 1 ^{1}H NMR scans over time intervals showing the conversion of **1b** into **3b** in 0.03 M DCl (1 : 1 DMSO-d_6–D_2O). The *N*-methyl singlet at 2.92 and the *C*-methyl doublet at 1.59 transform into signals at 2.87 and 1.38 ppm, respectively.

cyclizations is selectivity to be kinetically determined. In the present case, if the rate of cyclization of the ureido amides to hydantoins is much faster than the hydrolysis of imine, the amide, if formed, will not accumulate. Thus information on the rates of imine hydrolysis and ureido amides was needed. The kinetics of cyclization of the latter in the case **2b** and **2c** was available from previous work.[4]

We now report the rates of hydrolysis of imines **1** in 0.001–1 M HCl. The rate profiles obtained by measuring the rates by means of UV are presented in Fig. 2

Initially, only the rates of hydrolysis of compounds **1a**,**b**,**c** were measured by means of UV by preparing 1×10^{-2} M stock solutions of the substrates in THF. Surprisingly, imine **1b** with a single methyl group at position 5 of the 4-imino-imidazolidin-2-one ring reacted much faster than the compound without 5-methyl groups **1a** and the compound with two methyl groups **1c**. This did not fit with reactivities observed upon methyl substitution in similar systems, *e.g.*, those observed in alkaline hydrolysis of hydantoins[13] where each methyl group brings about a 5-fold decrease in rate. However, a very different rate of hydrolysis of **1b** in DMSO–DCl was found, which was only slightly greater than that of **1a**. The discrepancy between the rates was solved when it was established by means of NMR that the mono substituted (at 5-C) iminohydantoin **1b** autoxidized in solution into the hydroxy derivative **1d**. In DMSO or AcCN, the process was complete upon standing for about a week at room temperature.[10] On the other hand, **1a** and **1c** were found to be stable under the same conditions.

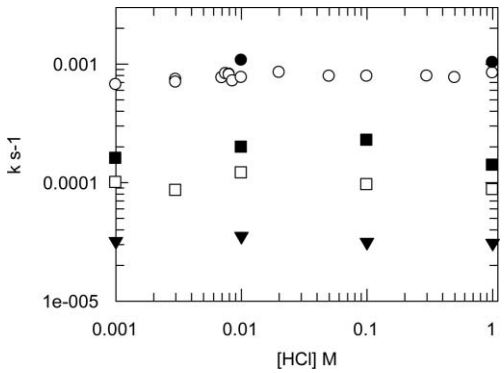


Fig. 2 Log–log plot of the pseudo-first-order rates of hydrolysis of imines **1** against [HCl] at ionic strength of 1 M (KCl): open squares **1a**, full squares **1b** (stock solution in acetonitrile), full triangles **1c**, open circles **1d** (**1b** oxidized as stock solution in THF), full circles **1d** (stock solution from isolated **1d**).

In THF, the batch used was shown to contain peroxides, **1b** oxidized readily and completely, more so because of the relatively small substrate concentration of 0.01 M. When the 5-hydroxy compound **1d** itself was used as a substrate, practically the same rate constant was observed as that of presumably **1b** (Fig. 1). When the stock solution of **1b** was prepared in acetonitrile containing no peroxides, a four times smaller constant was observed, which is listed in Table 1.

The plots in Fig. 1 show zero order of the reaction towards [H$^+$]. Observed rate constants, presented in Table 1, are averaged values of all experiments. Table 1 also contains the rate constants obtained by means of ^{1}H NMR in a DMSO-d_6–D_2O (1 : 1) solution of DCl. According to the pK_{BH^+}-values[9] of the substrates (Table 1), these are completely protonated at the acidities studied. Thus the zero order against [H$^+$] corresponds to a singly positively charged transition state.

Contrarily, in the 0–3 pH range, the rate of cyclization of ureido amides **2** to hydantoin is mainly first order in [H$^+$] and is described by the equation:

$$k_{obs} = k_H a_{H^+} + k_w$$

where k_H is the rate constant for catalysis by H$^+$ and k_w for catalysis by water. Values for amides **2b** and **2c** are available from ref. 4.‡ Thus because of the acid catalysis, product analysis will be least distorted by the conversion of amide into hydantoins at the lowest acidity. For this reason, in Table 1 observed rates of cyclization in 0.001 M are presented. The values listed in Table 1

‡ In these rate comparisons, the difference between rate coefficients based on concentrations and those on activities were ignored.

Table 1 Pseudo-first-order rate constants, 10^4 k_{obs} s^{-1}, of acid catalyzed hydrolysis of 4-iminoimidazolidin-2-one **1** and cyclization rates of hydantoic acid amides **2** at 25 °C and $I = 1$ M (KCl)

	Hydrolysis of imines **1** in H_2O[a]	Hydrolysis of imines **1** in DMSO–D_2O[b]	Cyclization of amides **2** in 0.001 M HCl[c]	p$K_{NH_2^+}$ of imines **1**[d]
a	0.979	1.19		5.38
b	1.81	1.49	0.065	5.02
c	0.324	0.311	0.81	5.13
d	7.77			

[a] Averages from UV data in 0.001–1 M HCl, $I = 1$ M (KCl) 25.0 °C. [b] Data from NMR monitoring in 0.011 M DCl in DMSO–D_2O (1 : 1) at 22–23 °C. [c] Calculated from rate equation and data from ref. 4 (see text). [d] Ref. 9.

demonstrate that in the most dilute acid solution, cyclization of **2b** is almost 30 times more slow than hydrolysis of **1b**; however, with **1c** the ratio is inversed, the cyclization being three times faster. This is a manifestation of the *gem*-dimethyl effect manifested in acceleration of cyclizations or deceleration of ring-opening upon substitution in the chain.[14]

The *gem*-dimethyl effect offers another possibility to manipulate the rate ratio of hydrolysis *versus* cyclization. The former, as already discussed, is generally slowed down by methyl substitution in position 5 while cyclizations are strongly enhanced in hydantoic systems.[14]

The extent of such possible interference is best illustrated by an analysis of the changes in concentration with time in the extreme case of amide as the only kinetic product of imine hydrolysis. Then the following consecutive reactions would be observed:

1 → **2** → **3**

Fig. 3 shows the calculated conversions in 0.01 M HCl—a concentration close to that of the NMR experiments. Even under this more unfavourable HCl concentration, the mol fraction of the 5-methylhydantoin **3b** is less than 10% for a 90% depletion of initial imine. Due to the strong acceleration of cyclization caused by the additional methyl group in **2c**, the picture with compounds **c** is completely different and under these conditions [**2c**] attains a maximum in its concentration, which is less than 10%. Thus the experiments with compound **1c** have no diagnostic power with respect to the r.d.s. in amide cyclization.

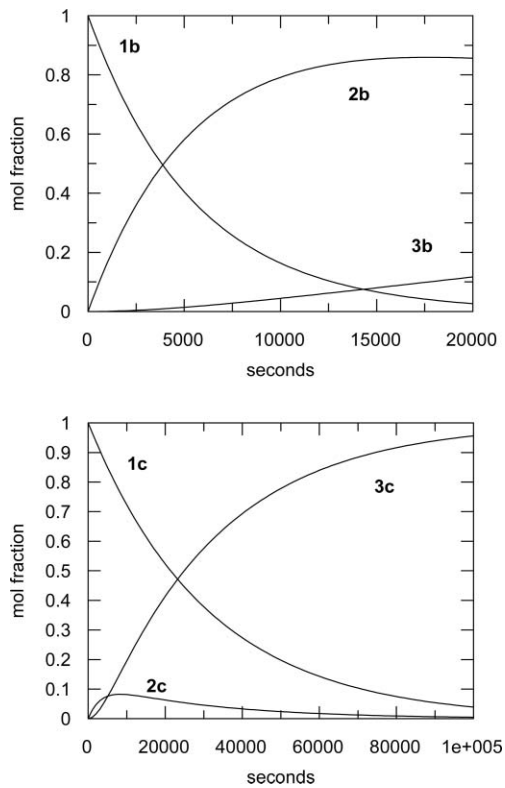


Fig. 3 Calculated plots of the change of concentration in 0.01 M HCl with time for compounds **b** and **c** in the hypothetical case when only the amide forms under kinetic control (see text).

Data on the rate of acid catalyzed cyclization of ureido amide **2a** without methyl groups at 5-C are not available—results on the *N*-methylamides[3] suggest that **2a** should cyclize at least 10 times more slowly than **2b**. Thus contrarily to amide **2c**, the cyclization of **2a** to hydantoin will have practically no effect on the composition of the products of hydrolysis of imine **1a**. As already mentioned, monitoring by means of NMR revealed only hydantoin as the product (Fig. 4) giving fully reliable evidence for the slow stage amide cyclization, *i.e.*, formation of the tetrahedral intermediate.

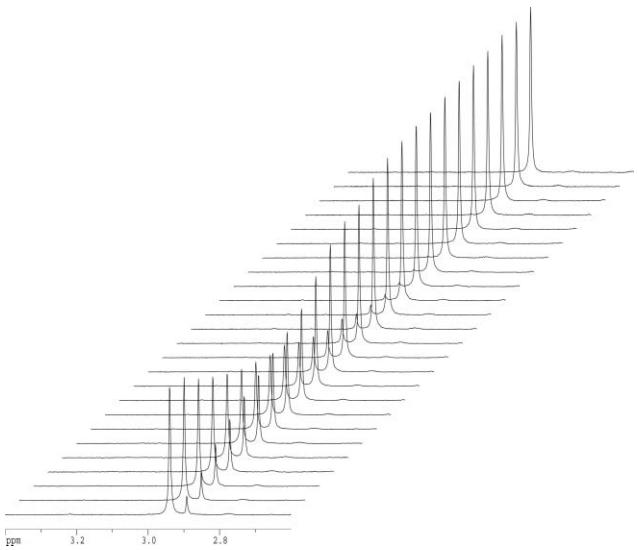


Fig. 4 Change of the *N*-Me signal of **1a** with time in 0.03 M DCl (1 : 1 DMSO-d_6–D_2O).

Since no amide formation could be detected by the methods of analysis used, the barrier to the leaving of the ω-(*p*-nitrophenyl)ureido group from **T** should be much higher than that of the ammonium cation. This finding defines the highest barrier in the amide cyclization reaction; this means, however, that this barrier does not participate in the imine hydrolysis and characteristics of the latter can shed no further mechanistic detail on amide cyclization.

Scheme 2 depicts the first step of imine hydrolysis as addition of a water molecule to protonated imine $\mathbf{1H}^+$ producing $TH^+_{(O)}$ with a protonated OH group which will rapidly shed a proton to give T° because it is a stronger acid than H_3O^+. Acid catalysis by protonation on the *p*-nitroanilino nitrogen leads to amide **2** while protonation of the amino group gives, alternatively, hydantoin **3**. Scheme 2 yields the relationship:

$V = k_{obs}[\mathbf{1H}^+] = k_2[\mathbf{T}][H^+] + k_3[\mathbf{T}][H^+]$

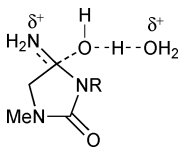
As argued above, $k_2 << k_3$; then application of the steady-state approximation gives

$$V/[1H+] = k_{obs} = \frac{k_1 k_3}{k_{-1} + k_3}$$

Results from acid amide hydrolysis[8] and ester aminolysis[15] favour k_1 as the r.d.s. in acid catalyzed hydrolysis of iminohydantoins because the protonated amino group shows the lower barrier upon leaving from tetrahedral intermediates. In basic media where protons become scarce, even proton transfer to the amino group in **T** becomes rate limiting,[3,4] the splitting off of the amino group being a faster reaction. Recently, the same process was also shown to be a viable reaction pathway in a heterocyclic rearrangement

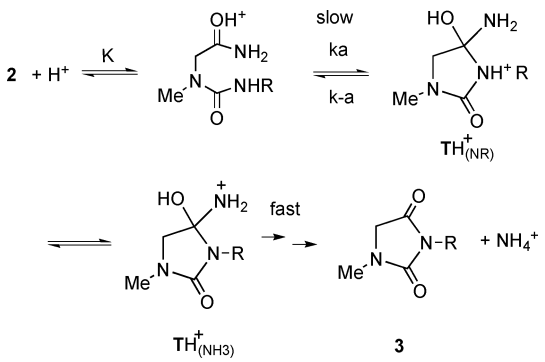
involving attack of the amino group of an isothiourea on an amide function.[16]

The reactivities in the series of imines studied support such an assignment. The small rate decrease of only 3–4 times upon introducing two methyl groups at 5-C in **1c** agrees better with a loose transition state of the addition of water than the second step which involves k_1/k_{-1} equilibrium ([**T**]/[**1**]) where steric strains will be fully expressed. The faster reaction of the hydroxy derivative **1d** is apparently related to destabilization of charge of the ground state dispersing in the transition state. In 0.015 M DCl, a solvent kinetic isotope effect k_H/k_D of 1.72 (experiment with **1a**) was measured, which is evidence of a slow proton transfer. Most likely this is a proton from the attacking molecule being transferred to a second water molecule as depicted in the formula:



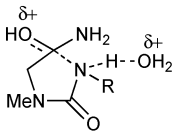
The transition state for the alternative possible r.d.s., k_3, is unlikely to involve a slow proton transfer because the tetrahedral intermediate protonated on the amino group $\mathbf{TH}^+_{(N)}$ is a more stable structure than $\mathbf{TH}^+_{(O)}$ and should allow equilibrium whereby a SKIE < 1 is expected.

Finally, returning to the problem of the rate determining step in acid catalyzed cyclization of amides of hydantoic acids, the evidence presented above, that the imine hydrolysis yields hydantoins as products under kinetic control, is unequivocal proof that the r.d.s. is the formation of the tetrahedral intermediate by attack of the ureido group on the protonated amide (Scheme 3).

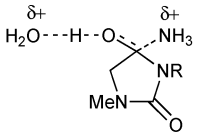


Scheme 3

Previous suggestion[3] that breakdown of the tetrahedral intermediate could be the r.d.s. based only on the size of an inverse solvent kinetic isotope effect can be discarded and shows how insufficient such evidence can be. The suggestion stemmed from comparison with the mechanism of acid catalyzed amide hydrolysis studied extensively by Brown *et al.*[8] who convincingly proved the participation of a water molecule in the proton transfers. Part of his evidence is based on the difference in solvent isotope effects calculated from fractionation factors[7] and reasonable assumptions on the reaction coordinate for the alternative r.d.s., formation and breakdown of the tetrahedral intermediate. In the cyclization of the hydantoic acid amides studied before,[3] the following SKIE were predicted for the formation of **T** transition state $\mathbf{TS}_1$ and breakdown of **T**–$\mathbf{TS}_2$:



$\mathbf{TS}_1$
$SKIE_{calc} = 1.1$



$\mathbf{TS}_2$
$SKIE_{calc} = 0.78$

The cyclization of amide **2c** in 0.95 M and 0.3 M HCl and DCl gave SKIE k_H/k_D equal to 0.67 and 0.79 respectively, substantially lower effects than those observed for amide hydrolysis of slightly above 1 by Brown who calculated for a TS similar to TS_1, SKIE = 1.1. Thus, $\mathbf{TS}_2$ for the cyclization reaction appeared the better alternative, bearing in mind that water and the ureido group do not differ much in their acid–base properties. Since the contradicting evidence above, from partitioning of the tetrahedral intermediate generated from imine hydrolysis, is unequivocal, we can iterate that determination of the rate determining step should be based on solid kinetic evidence.

Conclusions

UV and ^{1}H NMR monitoring of the acid catalyzed hydrolysis of 5-substituted 4-imino-1-methyl-3-(4-nitrophenyl)imidazolidin-2-ones showed hydantoins as the only products. In 0.001–1 M HCl, the reaction is zero order with respect to [H^+]. Comparison with previous data on rates of cyclization of the corresponding amides of hydantoic acids allowed conditions (acid concentration, substitution pattern—*gem*-dimethyl effect) to be found that guaranteed kinetic control of the products obtained. Thus, unequivocal proof was obtained that the formation of the tetrahedral intermediate is rate determining in the cyclization of hydantoic acid amides and the alternative previous suggestion based only on calculated solvent isotope effects was refuted. The small steric effects upon methyl substitution at 5-C and a solvent kinetic isotope effect k_H/k_D of 1.72 favour a mechanism for imine hydrolysis whereby the rate is limited by a water attack on the protonated imine concerted with proton transfer from the attacking water to a second water molecule.

Acknowledgements

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