General Acid–Base Catalysed Cyclisation Reactions of a Strained ω-Phenylhydantoic Acid

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In relation to model studies on the carboxylation of biotin, the nucleophilic reactivity of a weakly basic urea towards COOH and COO^- was characterised by means of the ring-closure of (**3**) to (**4**). Owing to the strain introduced by the two methyl groups the reaction was rapid over the whole pH range, being reversible at pH >9. Three general acid–base-catalysed reactions and one uncatalysed reaction were identified, corresponding to a change of the predominant form of catalysis and/or reactive form of the substrate with changing pH. The additional barrier for anionic nucleophilic attack on COO^- *versus* attack on COOH was estimated as *ca.* 9 kcal mol^{-1}. A comparison between the reactivity of the phenylureido and methylureido anion towards COO^- indicated better nucleophilicity by phenylureido anion, despite its lower basicity. The results obtained with the model compound (**3**) illustrated the possibility of a change to a different rate-determining step for intramolecular reactions of compounds activated by ground-state strain.

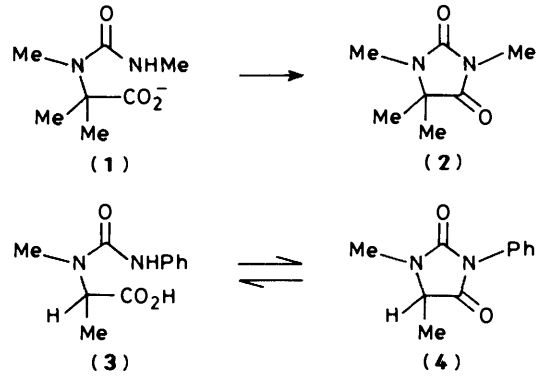
As the carboxylation of biotin by hydrogencarbonate involves reaction of a urea nitrogen with a carboxylate, the cyclisation of the anion of 2,2,3,5-tetramethylhydantoic acid (**1**) to the hydantoin (**2**) has been studied as a model reaction for action of biotin.[1]

Five-membered cyclic acylureas and imides are 4.1 and 3.4 pK units respectively stronger acids than the corresponding acyclic compounds.[2] If a similar effect exists for the NH acidity of ureas, the five-membered urea moiety of biotin should provide a less basic nucleophile than (**1**). *N*-Methylurea has a pK_a value of 18.3.[3]

The nucleophilic reactivity of a less basic urea towards carboxylic centres can be studied by means of the ring-closure of an ω-phenylhydantoic acid, in which a decrease of *ca.* 2 pK units in NH acidity is observed (pK_a values of 16.4 and 16.6 have been measured[4] for 5-phenyl- and 3-methyl-5-phenylhydantoic acid, respectively). The reverse reaction of alkaline hydrolysis of cyclic acylureas has been found to be sensitive to this change in basicity of the leaving group and to proceed by a different rate-determining step.[4,5]

The mechanism of the acid-catalysed reaction is also of interest as the changes in the mechanism of amide bond formation are not well understood for amines with pK_a values <0. Catalysis by methoxyacetic acid has been observed for the ring-closure of 5-methylhydantoic acid,[6] but due to the slowness of the reaction buffer catalysis was not investigated in detail.

We now report on the cyclisation of 2,3-dimethyl-5-phenylhydantoic acid (**3**) to 1,5-dimethyl-3-phenylhydantoin (**4**). The strain introduced by the two methyl groups is considerable as it makes the reaction 10^3—10^4 times faster than the cyclisation of the parent 5-phenylhydantoic acid. This allowed us to study the reaction over the whole pH range and it was found to be reversible above pH 9 and subject to general acid and general base catalysis.



Experimental

Materials.—Inorganic reagents and buffer components were of analytical grade and used without further purification. Potassium hydroxide and buffer solutions were prepared with CO_2-free distilled water.

1,5-Dimethyl-3-phenylhydantoin. Phenyl isocyanate (0.65 ml, 6 mmol) was added dropwise to a solution of *N*-methylalanine (0.56 g, 5 mmol) and potassium hydroxide (6 mmol) in water (5 ml). The ice-cooled mixture was stirred for 1 h and diphenylurea was filtered off. The acidification of the filtrate to pH 3—4 did not give a precipitate, while further acidification led to the separation of (**4**) (0.7 g), m.p. 143—145 °C, which on recrystallisation from ethanol melted at 144—145 °C (lit.,[7] 145—146 °C).

Potassium 2,3-dimethyl-5-phenylhydantoate. The potassium salt of (**3**) was obtained only in solution after alkaline hydrolysis of the hydantoin (**4**). Stock solutions of (**3**) used for the kinetic experiments were 5×10^{-3}M solutions of (**4**) in 0.2M-aqueous potassium hydroxide, which were left overnight at room temperature.

Product Analysis.—The conversion of the phenylhydantoin (**4**) into the potassium phenylhydantoate (**3**) proceeds quantitatively according to u.v.-spectral data and the results obtained for the pK measurements. Solutions of (**4**) in 0.1 or 1.0M-potassium hydroxide have the u.v.-spectral characteristics of an ω-phenylureido acid (see ref. 5), $\lambda_{max.}$ 238 nm (ε 1.5×10^4 dm^3 mol^{-1} cm^{-1}). A repeated cycle of alkaline hydrolysis and cyclisation in 0.1M-hydrochloric acid showed a quantitative recovery of the absorbance in 0.1M-potassium hydroxide, due to (**3**).

An increase of the end-point absorbance in the kinetic cyclisation experiments, when stock solutions of (3) older than 4—5 days were used, indicated the formation of a by-product, most likely diphenylurea. The kinetic results were not affected by this product; however, only fresh stock solutions of the substrate were used.

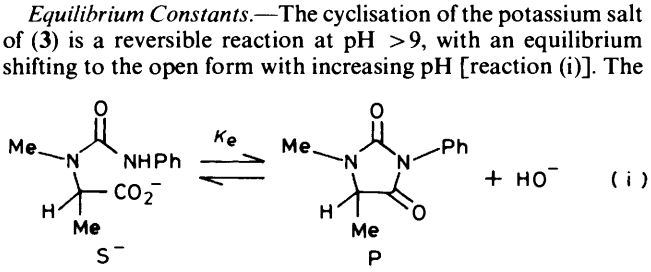
pK_a *Measurements.*—The pK_a value for dissociation of the carboxy group of (3), pK_{SH}, was determined under the conditions of the kinetic experiments [25 °C, ionic strength I 1.0M (KCl)] by potentiometric titration.[8] The pH-meter used was a Radiometer pHM 84 Research pH-meter with a GK2401 C electrode, standardised at pH 7.00 and 4.01. The following procedure was used: the phenylhydantoin (4) (5.0×10^{-4} mol) was dissolved in 1.0M-aqueous potassium hydroxide (5.0 ml). 0.1M-Hydrochloric acid (45 ml) [I 1.0M (KCl)] was added to neutralise the excess of potassium hydroxide and the solution was titrated under nitrogen. The pH readings became stable (±0.001 pH units) 10—15 s after the addition of each portion of titrant and the whole titration was ended in 4 min. The pK values calculated from the data up to 90% neutralisation varied in the interval of ±0.02 units.

Kinetic Measurements.—Rate constants were determined at 25.0 ± 0.1 °C under pseudo-first-order conditions in the thermostatted cell compartment of a Unicam SP-800 or Carl Zeiss Jena VSU 2-P spectrophotometer. The rate of the reaction of cyclisation of (3) or of hydrolysis of (4) was followed by monitoring the change of absorbance at 238 nm due to (3). The cyclisation was initiated by injecting 20—30 μl 5×10^{-3}M-stock solution of (3) to 2.8 ml of preheated aqueous buffer solution. A water solution of (4) with the same concentration was used for the hydrolysis experiments. Pseudo-first-order constants, k_{obs}, were calculated from plots against time of $\log(A_t - A_\infty)$ or $\log(A_\infty - A_t)$, where A_∞ was the absorbance after ten half-lives. The ionic strength was maintained constant (1.0M) with potassium chloride. pH Values were measured directly after each kinetic run, using a Radiometer pHM 84 Research pH-meter, with GK2401 C or B electrodes, standardised at pH 7.00 and 4.01 and 9.18, respectively. All k_{obs} values and derived rate constants were calculated by the least-squares procedure. k_{obs} Values were reproducible to within ±3% and were corrected for the small variations in pH with changing buffer concentrations.

Results

The pK_{SH} value for ionisation of the carboxy group of 2,3-dimethyl-5-phenylhydantoic acid was measured as 3.71 ± 0.02. As the cyclisation of (3) is fast at pH values corresponding to complete neutralisation of its anion ($\tau_{\frac{1}{2}}$ 4 min at pH 1.7; 25 °C), the isolation of the neutral form of (3) was impossible. For this reason in all kinetic measurements of the cyclisation of (3) only stock solutions of its potassium salt were used.

Equilibrium Constants.—The cyclisation of the potassium salt of (3) is a reversible reaction at pH >9, with an equilibrium shifting to the open form with increasing pH [reaction (i)]. The



(i)

attainment of equilibrium was checked by following the reaction in both directions. The pseudo-first-order rate constants for cyclisation (k_f) and for hydrolysis (k_r) were calculated from k_{obs} values by means of the apparent equilibrium constant K_{app}, according to equations (ii) and (iii):

$$k_{obs} = k_f + k_r \quad \text{(ii)}$$

$$K_{app} = [P]/[S^-] = k_f/k_r = K_e/a_{HO^-} \quad \text{(iii)}$$

Owing to errors of mixing small volumes, the experimentally determined values of K_{app} varied more than expected from the variations in pH in a single series of buffer dilutions. For this reason a mean value of the true equilibrium constant K_e = (3.56 ± 0.17) × 10^{-4} mol dm^{-3} was calculated from a set of 24 measurements of pH and K_{app} in glycine and carbonate buffers. The K_{app} values used for the calculation of k_f and k_r were recalculated from the K_e and the pH values.

The data for the cyclisation of (3) are shown on Figure 1. They constitute of two sets of rate constants, k_o for the irreversible reaction up to pH 9, and k_f for the reaction at higher pH.

Irreversible Cyclisation Reaction.—The rate constants k_o were obtained between pH 2 and 9 by extrapolation to zero buffer concentration of k_{obs} values normalised to a single pH value, according to equation (iv). The pH-rate profile is described up to pH 9 by equation (v), with pK_{SH} 3.71 (measured directly),

$$k_{obs} = k_o + k_{cat}[\text{buffer}]_{tot} \quad \text{(iv)}$$

$$k_o = \frac{a_{H^+}}{K_{SH} + a_{H^+}}(k_{H^+}a_{H^+} + k_{H_2O}) + \frac{K_{SH}}{K_{SH} + a_{H^+}}k_p \quad \text{(v)}$$

k_{H^+} 0.135 ± 0.003 dm^3 mol^{-1} s^{-1}, k_{H_2O} (4.8 ± 1.1) × 10^{-5} s^{-1}, and k_p = (6.90 ± 0.20) × 10^{-5} s^{-1}. k_p Represents the pH-independent reaction of the anions of (3), calculated as the mean value of k_o between pH 5.5 and 8.8. k_{H^+} and k_{H_2} are the rate constants for hydronium ion and water-catalysed reactions of the neutral form of (3), obtained as the slope and intercept of the linear plot of the k_o values against a_{H^+}. Data for k_o obtained for the pH interval between 2 and 4.5 were used, corrected appropriately for the ionisation of the substrate and the contribution of the k_p term.

The complete data for buffer catalysis up to pH 8.8 are given in Table 1. In most cases the bimolecular rate constants for general acid (k_{HA}) and general base catalysis (k_{A^-}) were calculated in terms of the neutral form of the substrate by means of equation (vi), with k_{cat} values corrected for the ionisation of

$$k_{cat}^{corr} = \text{f.b.}k_{A^-} + k_{HA}(1 - \text{f.b.}) \quad \text{(vi)}$$

the substrate. The statistically corrected data for catalysis of the ring-closure of the neutral form of (3) give linear Brönsted plots (Figure 2) with respective slopes α 0.60 ± 0.07 and β 0.50 ± 0.03.

Reversible Reaction of Cyclisation and Hydrolysis.—Hydrolysis in potassium hydroxide solutions. The pseudo-first-order constants k_r for hydrolysis of 1,5-dimethyl-3-phenylhydantoin in potassium hydroxide solutions showed higher than unity order in a_{HO^-}. The slope in the pH-rate profile for hydrolysis of (4) (Figure 3) is 1.25 ± 0.01 between pH 11 and 11.6, while at higher pH the slope diminishes, reaching the value of 1.06. This observation indicated that unlike other 3-arylhydantoins[4,5] the hydrolysis of (4) proceeds according to the well established reaction scheme for hydrolysis of *N*-alkyl-substituted acylureas,[2] which includes parallel water- and HO^--catalysed reactions of decomposition of the tetrahedral addition intermediate T^- (Scheme 1). We have also added in Scheme 1 the two theoretically possible general acid–base-catalysed pathways

Table 1. Buffer catalysis data for the irreversible cyclisation of (3) at 25 °C and ionic strength 1.0M

Buffer acid	pK_a[a]	Conc. range (M)	Runs	%A^-	pH	10^5k_{cat}[b] (dm^3 mol^{-1} s^{-1})	10^4k_{HA} (dm^3 mol^{-1} s^{-1})	10^5k_A (dm^3 mol^{-1} s^{-1})
H_3O^+	−1.74						1 370 ± 10[c]	0.0858[d]
H_3PO_4	1.78	0.1—1.0	5	50	1.96	198 ± 18		
		0.1—1.0	5	65	2.18	142 ± 2		
		0.1—1.0	5	80	2.50	78.7 ± 5.7	40.0 ± 0.8[e]	(5.73 ± 4.57)[e,f]
$H_3\overset{+}{N}CH_2COOH$	2.45	0.1—0.7	3	50	2.49	24.7 ± 0.4		
		0.1—1.0	3	70	2.87	15.7 ± 0.9		
		0.1—0.7	3	90	3.46	7.42 ± 0.34	4.39 ± 0.18[e]	7.69 ± 0.84[e]
HCOOH	3.57	0.1—0.3	3	30	3.21	11.0 ± 1.1		
		0.1—0.3	3	50	3.58	11.5 ± 0.8		
		0.1—0.5	3	70	3.95	9.45 ± 0.81	0.60 ± 0.08[e]	34.3 ± 0.1[e]
CH_3COOH	4.62	0.1—0.5	3	30	4.23	4.30 ± 0.26		
		0.1—0.5	3	50	4.62	5.00 ± 0.89		
		0.1—0.5	3	70	5.04	9.90 ± 0.95		220[g,e]
$H_2PO_4^-$	6.48	0.1—0.8	3	10	5.51	2.94 ± 0.29		
		0.1—0.6	3	30	6.09	3.42 ± 0.17		
		0.1—0.5	3	50	6.50	4.23 ± 0.45		
		0.1—0.4	3	70	6.88	4.37 ± 0.17	0.272 ± 0.020[h]	5.27 ± 0.28[h]
							160[i]	2.05 ± 10^8[j,f]
$TrisH^+$	8.42	0.1—1.0	3	30	8.07	1.06 ± 0.12[k]		
		0.1—1.0	3	50	8.42	1.95 ± 0.04[k]		
		0.1—1.0	3	70	8.78	3.06 ± 0.11[k]	(−0.049 ± 0.018)[h]	4.56 ± 0.18[h]
H_2O	15.74							1.33 × 10^{11}[f,l]

[a] pK_a Values calculated according to ref. 8 from pH measurements of the buffers used. [b] Calculated from equation (iv). [c] Calculated from six k_{obs} values with $[H^+]$ in the range 0.1—0.006M. [d] $k_{H_2O}/55.5$. [e] Bimolecular rate constants for reaction of the neutral form of (3), calculated from equation (vi) with k_{cat} values corrected for the ionisation of the substrate. [f] Data not included in the Brönsted correlations. [g] Calculated from the data at 70% A^- (see text). [h] Bimolecular rate constants for reaction of the predominant anionic form of the substrate, calculated from equation (vi) with uncorrected k_{cat} values. [i] $k_{HPO_4^{2-}}$ for reaction of the neutral form of (3) calculated from $k_{H_2PO_4^-}$ given above. [j] $k_{PO_4^{3-}}$ for reaction of the neutral form of (3) calculated from $k_{HPO_4^{2-}}$ given above with a pK_a value for HPO_4^{2-} of 11.3. [k] k_{cat} values corrected for the low degree of reversibility at pH above 8. [l] $k_p/55.5$ Recalculated as k_{HO^-} for reaction of the neutral form of (3).

for decomposition of T^-, to be used later for the treatment of the data obtained in buffer solutions.

The steady-state treatment of Scheme 1 gives the general expressions (vii) and (viii) for k_r and k_f. The decrease of the slope at higher pH in the pH–rate profile for alkaline hydrolysis corresponds to a change of the rate-determining step from decomposition of T^- to formation of T^-, as normally observed with *N*-alkyl-substituted acylureas.[2] This allows the calculation of k_1, according to equation (ix).[9]

With $k_1 = 0.971 \pm 0.008$ dm^3 mol^{-1} s^{-1}, obtained from the data at pH above 11.7, equation (x)[10] was used for the calculation of k_2/k_{-1} and k_3/k_{-1} from the data at pH between 11 and 11.7.

The following results were obtained: $k_2/k_{-1} = 0.203 \pm 0.174$

$$k_r = \frac{k_1 a_{HO^-}\left(\frac{k_2}{k_{-1}} + \frac{k_3}{k_{-1}} a_{HO^-} + \frac{k_B}{k_{-1}}[B] + \frac{k_{BH^+}}{k_{-1}}[BH^+]\right)}{1 + \frac{k_2}{k_{-1}} + \frac{k_3}{k_{-1}} a_{HO^-} + \frac{k_B}{k_{-1}}[B] + \frac{k_{BH^+}}{k_{-1}}[BH^+]} \quad \text{(vii)}$$

$$k_f = \frac{k_{-2} + k_{-3} a_{HO^-} + k_{-B}[B] + k_{-BH^+}[BH^+]}{1 + \frac{k_2}{k_{-1}} + \frac{k_3}{k_{-1}} a_{HO^-} + \frac{k_B}{k_{-1}}[B] + \frac{k_{BH^+}}{k_{-1}}[BH^+]} \quad \text{(viii)}$$

$$a_{HO^-} = k_1 \frac{(a_{HO^-})^2}{k_r} - \frac{k_{-1}}{k_3} \quad \text{(ix)}$$

$$\frac{k_r}{k_1 a_{HO^-} - k_r} = \frac{k_2}{k_{-1}} + \frac{k_3}{k_{-1}} a_{HO^-} + \frac{k_B}{k_{-1}}[B] + \frac{k_{BH^+}}{k_{-1}}[BH^+] \quad \text{(x)}$$

$$k_p = \frac{k_{-2}}{1 + \frac{k_2}{k_{-1}}} = \frac{k_1 \frac{k_2}{k_{-1}} K_e}{1 + \frac{k_2}{k_{-1}}} \quad \text{(xi)}$$

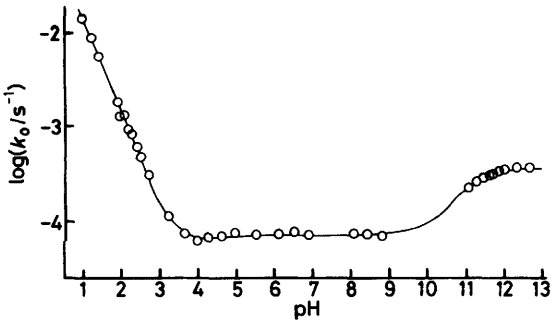


Figure 1. pH–Rate profile for the cyclisation of (**3**), at 25 °C and ionic strength 1.0M. The points are experimental and from pH 2 to 9 represent extrapolations to zero buffer concentration. The curve is calculated from equation (xii) with the values of the respective constants given in the text

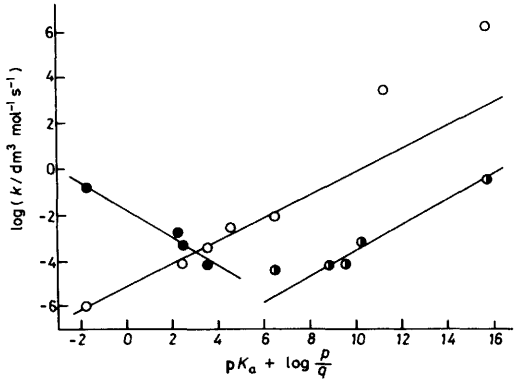


Figure 2. Brönsted plots of the data for general acid and general base catalysis of the cyclisation of (**3**): ●, k_{HA}/p for the reaction of the neutral form of (**3**), data from Table 1; ○, k_A/q for reaction of the neutral form of (**3**), data from Table 1; ◑, k_{-B}/q for the reaction of the anionic form of (**3**), data from Tables 1 and 2 are used, recalculated using the equilibrium constant K_e, see text

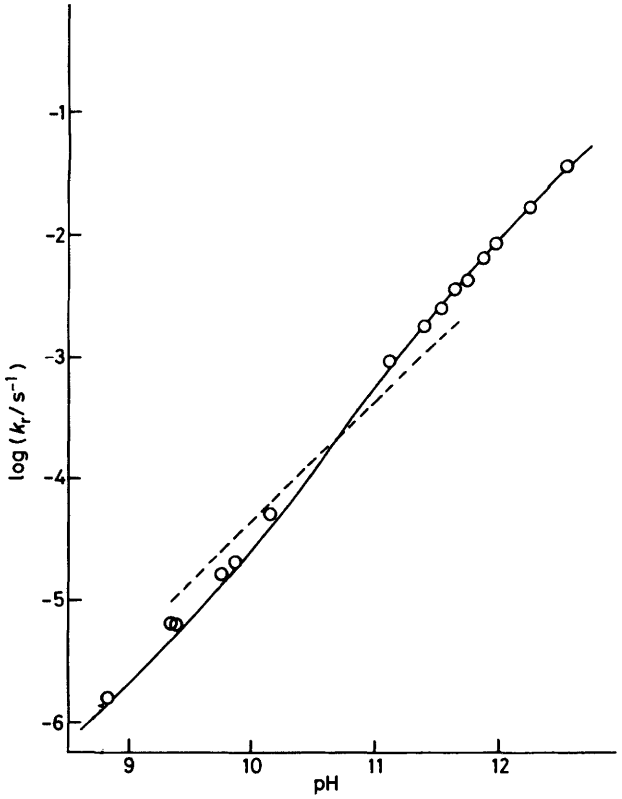


Figure 3. pH–Rate profile for the hydrolysis of (**4**) at 25 °C and ionic strength 1.0M The points are experimental and below pH 11 represent extrapolations to zero buffer concentration. The curve is calculated according to equation (vii) with k_1 0.971 dm^3 mol^{-1} s^{-1}, k_2/k_{-1} 0.25, and k_3/k_{-1} 1 150 dm^3 mol^{-1}, which were obtained without using the extrapolated data at pH below 11 (see text). The dashed line has a slope of +1.

and k_3/k_1 = 1 150 ± 70 dm^3 mol^{-1}. The latter value compares favourably with the value of 1 260 ± 160 obtained by means of equation (ix), but the error for the constant k_2/k_{-1} is very large, as its contribution to the overall rate of hydrolysis is <20% at the lowest concentration of potassium hydroxide used. However, k_2/k_{-1} can be calculated also from the data for the pH-independent cyclisation of the anionic form of (**3**) (k_p) in the pH interval from 5.5 to 8.8 by means of equation (xi), using the equilibrium constant for (i). k_2/k_{-1} = 0.25 and k_{-2} = 8.64 × 10^{-5} s^{-1} were obtained from equation (xi). In a similar way, using the equilibrium constant K_e, the rate constant for the HO^--catalysed cyclisation of the anionic form of (**3**), k_{-3}, was calculated as 0.40 dm^3 mol^{-1} s^{-1}.

Accordingly the entire pH rate profile for cyclisation of (**3**), shown on Figure 1, is described by equation (xii).

transformation (x) is used. As observed for the cyclisation reaction of the anionic form of (**3**) in Tris buffers (Table 1) only general base catalysis is found for the reaction of hydrolysis of (**4**) in carbonate buffers (Table 2). The irregular increase of k_{cat} values for glycine buffers is attributed to the medium effect of the base component of the buffer, as observed [1] for the cyclisation of (**1**), and for this reason only data at the lowest fraction of free base were used.

The values of k_B/k_{-1}, shown in Table 2, demonstrate that the concentration-dependent terms in the denominator of equation (viii) cannot be neglected and, respectively, the data for the buffer-catalysed reaction of cyclisation cannot be treated in a simple way. The value of k_A-, found for catalysis of the cyclisation of the anionic form of (**3**) by Tris base (Table 1), corresponds in a first approximation to $k_{-B}/(1 + k_2/k_{-1})$, and the value of k_B/k_{-1} necessary for the construction of a common Brönsted dependence was calculated using k_{-B}, k_1, and K_e

$$k_o = \frac{a_{H^+}}{K_{SH^+} + a_{H^+}} (k_{H^+} a_{H^+} + k_{H_2O}) + \frac{K_{SH}}{K_{SH} + a_{H^+}} \left(\frac{k_{-2} + k_{-3} a_{HO}}{1 + \frac{k_2}{k_{-1}} + \frac{k_3}{k_{-1}} a_{HO^-}} \right) \quad \text{(xii)}$$

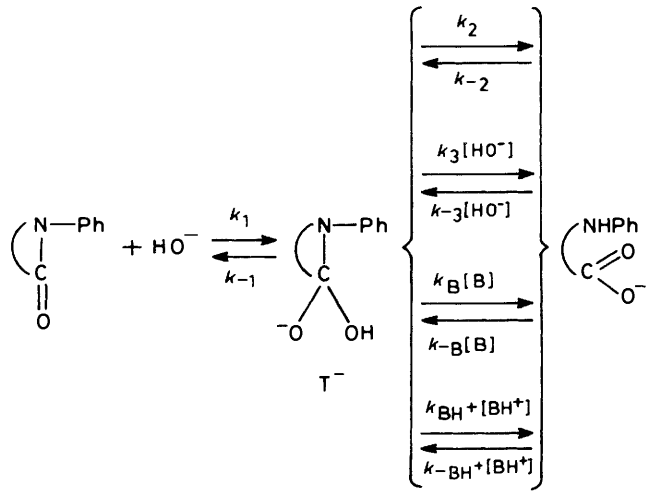
Reaction in Buffer Solutions.—In order to calculate k_o and k_{cat} for the buffer-catalysed reversible reaction above pH 9, equations (vii) and (viii) have to be reduced to equation (iv). This is possible for the reaction of hydrolysis if the

values. The four k_B/k_{-1} values from Table 2 give a linear Brönsted plot with slope β = 0.51 ± 0.06 (r = 0.987), while for the statistically corrected plot, shown on Figure 2, β is 0.55 ± 0.05 (r = 0.992).

Table 2. Buffer catalysis data for the hydrolysis of (**4**) at 25 °C and ionic strength 1.0M

Buffer base	pK_a[a]	Conc. range (M)	Runs	%B	pH	k_{cat}[b] ($dm^3\ mol^{-1}$)	k_B/k_{-1}[c] ($dm^3\ mol^{-1}$)
HO^-	15.74						874 ± 53[d]
CO_3^{2-}	9.84	0.1—0.6	3	10	8.83	0.0518 ± 0.0014	
		0.1—0.6	4	30	9.38	0.113 ± 0.013	
		0.1—0.5	4	50	9.86	0.211 ± 0.030	0.40 ± 0.04[e]
$OOCCH_2NH_2$	9.76	0.1—1.0	4	30	9.39	0.485 ± 0.025	
		0.1--1.0	4	50	9.76	0.724 ± 0.085	
		0.1—1.0	4	70	10.14	0.684 ± 0.048	1.6[f]
Tris	8.42						0.165[g]

[a] From pH measurements at 50% B. [b] Calculated from equation (x) with the total concentration of the buffer. [c] Constants for general base-catalysed decomposition of T^-, calculated from equation (vi). [d] k_3/k_{-1} Multiplied by an activity coefficient of 0.76 for HO^-. [e] The intercept obtained according to (vi) is 0.006 ± 0.018. [f] Calculated from the data at 30% base (see text). [g] Calculated from k_{A^-} (Table 1), see text.



Scheme 1.

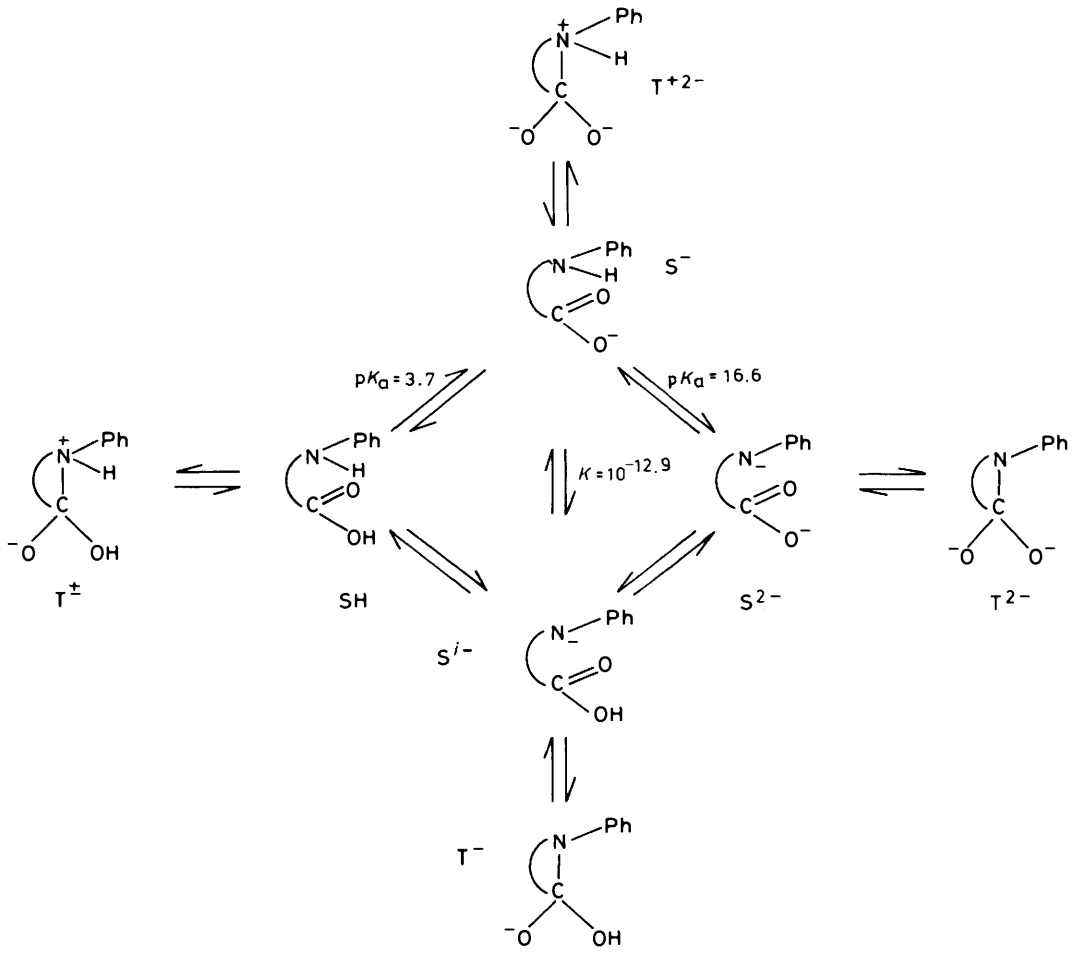
Discussion

Normally, 3-arylhydantoins are hydrolysed irreversibly in base[4,5] while the hydrolysis of the dimethyl-substituted phenylhydantoin (**4**) is reversible at pH > 9. The fast cyclisation of (**3**) over the whole pH range is due to the ground-state strain introduced by the two methyl groups. From the equilibrium constant for cyclisation of the anion K_e and the pK_{SH} value of the acid, the equilibrium constant for reaction of the neutral form of (**3**) is calculated as 6.9×10^6.

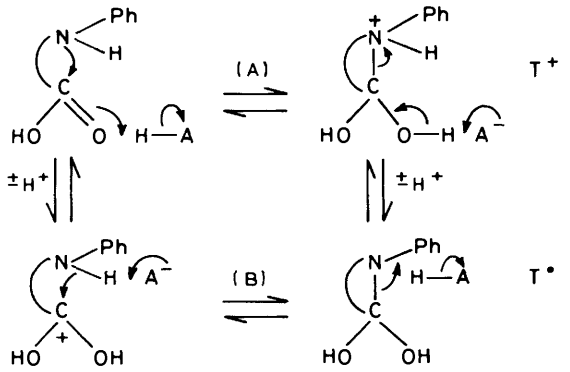
When the ionisation of the substrate is taken into account it is seen that the pH–rate profile in Figure 1 corresponds to cyclisation passing through transition states with the following charges: +1 at pH below 4, −1 at pH between 4 and 9, −2 at pH 9 to 11, and −1 at pH above 11. The NH proton of the phenylureido group does not ionise in the pH range investigated,[4] and the new plateau at higher pH must represent a change in the rate-determining step of the reaction. The analysis of the data for buffer catalysis indicated three general acid–base-catalysed reactions and one uncatalysed (Figure 2). This means that a change of the predominant form of catalysis and/or reactive form of the substrate takes place with changing pH, and this is the main reason for the limited number of points in the Brönsted plots shown on Figure 2. The intersection of the Brönsted plots for cyclisation of the neutral form at a buffer with pK_a *ca.* 3 shows that general base catalysis is effective at relatively low pH, while the reaction predominating at higher pH is general base-catalysed cyclisation of the anionic form of (**3**). All three Brönsted coefficients are close to 0.5 and this is a sound indication for proton transfers concerted with bond-breaking or bond-making of heavy-atom centres.

The four possible reactive forms of the substrate, as well as the respective tetrahedral intermediates which will result from the uncatalysed first step of nucleophilic attack, are shown in Scheme 2. Apart from S^{i-}, all other forms of the substrate will lead to highly charged and presumably unstable intermediates. Although $T^{\pm}$ is a possible intermediate for reactions of basic amines,[11] including the anilino group as shown by the results of Kirby *et al.* for the cyclisation of 3-(2-aminophenyl)propanoic acid[12] and its methyl ester,[13] its existence can be questioned for reactions of more weakly basic amines. Moodie and co-workers have investigated the effect of the basicity of the nucleophile in the cyclisation of 4-arylaminobutanoic acids[14] in comparison with their previous results on the cyclisation of 5-methylhydantoic acid.[6] The most significant information is provided by their results from ^{18}O-exchange during acid hydrolysis,[6,14] which can be summarised as follows: for nucleophiles with $pK_a > 0$ the rate-determining step of the ring-closure reaction is the departure of HO from T^o (as also found by Kirby *et al.*[12,13]), while for less basic nucleophiles like the 2,4-dinitroanilino group (pK_a −4.3[15]) and the *N*-methylureido group (pK_a −3.9 for *N*-protonation[3]) the rate-determining step is catalysis of the formation of the tetrahedral intermediate. In the latter case $T^{\pm}$ is no longer an intermediate that can exist on the reaction co-ordinate. Similar conclusions about the non-existence of T^{2-} were drawn for the base-catalysed cyclisation of the methylhydantoic acid (**1**).[1] We feel that there is sufficient evidence to conclude that the three concerted general acid–base-catalysed reactions of the phenylhydantoic acid (**3**), with a still less basic nucleophile than the methylureido group, will correspond to a case of 'enforced catalysis'[16] of the first step of formation of the tetrahedral intermediate. An additional factor, acting in the same direction as an early rate-determining step of the reaction, is ground-state strain, as discussed later.

General Acid Catalysis of the Cyclisation of the Neutral Form of (**3**).—Concerted general acid-catalysed formation of the tetrahedral intermediate from SH can proceed by one of the kinetically equivalent pathways shown in Scheme 3. At this stage, a choice between the two mechanisms cannot be made, because, although the general acid-catalysed pathway (A) proposed in ref. 6 seems more likely, the specific acid–general base catalysis (B) cannot simply be ruled out. In all the other reaction mechanisms discussed for the ring-closure of (**3**) a general or specific mode of deprotonation of the phenylureido group is involved, as the protonated form of the nucleophile is the most acidic centre in the tetrahedral intermediate. The rate constant calculated from k_{H^+} for attack of the phenylureido group on the protonated carboxy group with an assumed pK_a of



Scheme 2.

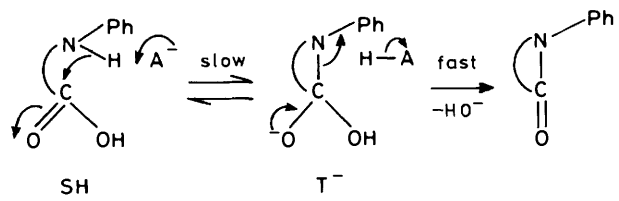


Scheme 3.

-7 is only 1.4×10^6 dm^3 mol^{-1} s^{-1}, well below the diffusion-controlled limit. We expect to obtain more conclusive information by an investigation of the effect of the basicity of the nucleophile on the Brönsted coefficients of the ring-closure reaction.

General Base Catalysis of the Cyclisation of the Neutral Form of (**3**).—As general base catalysis is observed also at low pH, where SH is the predominant form of the substrate, the only plausible mechanism is the simplest one: general base-catalysed formation of the tetrahedral intermediate T^- (Scheme 4).

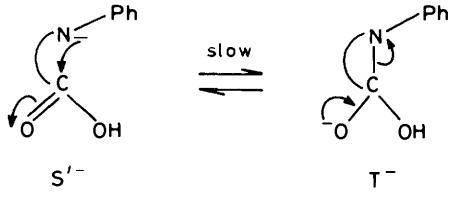
T^- can readily eliminate HO^- and has been shown to be a kinetically important intermediate during alkaline hydrolysis of



Scheme 4.

N-alkyl-substituted acylureas.[2] The formation of T^- during the ring-closure of (**3**) is demonstrated in the present study by the change of the rate-determining step at high pH (Figure 1). In the direction of ring-opening Scheme 4 requires that the proton transfer to the leaving group is concerted with cleavage of the C–N bond. This reaction pathway has not been identified during alkaline hydrolysis of acylureas, probably due to the different pH range for the reactions being compared, but concerted general acid-catalysed decomposition of T^- is the mechanism proposed for hydrolysis of anilides with low basic leaving groups.[10,17] Similarly, for the decomposition of carbamate anions, proton transfer to the leaving group concerted with C–N bond cleavage, was proposed[18] to take place only with the less basic leaving groups like *p*-nitroaniline.

The Brönsted dependence for general base catalysis of the cyclisation of the neutral form of (**3**) (Figure 2) shows that water and HPO_4^{2-} act as normal general bases, while strong positive deviations are observed for catalysis by HO^- and PO_4^{3-}. This is an indication that the latter reactions proceed by different



Scheme 5.

mechanism(s). The alternative treatment of the phosphate reaction as general base catalysis by HPO_4^{2-} of the cyclisation of the anionic form of the substrate will reduce the positive deviation from the Brönsted plot in Figure 2. The unusual reactivity of phosphate ions has often been observed, but in the case of alkaline hydrolysis of anilides[10] it has not been attributed to bifunctional catalysis. This is in agreement with the absence of significant catalysis by HCO_3^- for the reaction of (**4**) at higher pH (see Table 2, footnote *e*).

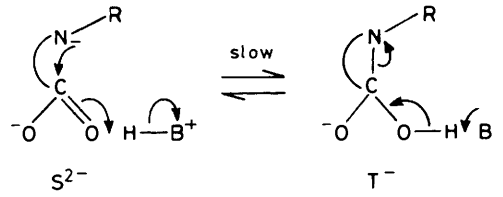
The pH-independent reaction of the anions of (**3**) at pH between 4 and 9 cannot represent general base catalysis by water, because it will mean that both forms of the substrate, SH and S^-, react with practically identical rates, k_{H_2O} 4.6 × 10^{-5} and k_p 6.9 × 10^{-5} s^{-1}. On the other hand, the positive deviation for catalysis by HO^- in the general base-catalysed reaction of SH can be easily explained by a specific catalysed reaction. Proton transfer from the phenylureido group to HO^- is close to the thermodynamically favourable direction, and an uncatalysed addition of the phenylureido anion to the neutral carboxy group will take place, leading again to formation of the stable intermediate T^- (Scheme 5).

This reaction was not detected in the pH–rate profile for cyclisation of (**1**),[1] probably due to the higher pK_a value for ionisation of the methylureido group. The concentration of the inverse anions (S^{i-}) of (**3**) is determined by the ratio of the ionisation constants of the two groups (Scheme 2), and the monomolecular constant for ring-closure of S^{i-}, calculated from k_p 5.6 × 10^8 s^{-1}. The mechanism in Scheme 5 is rather interesting because it shows that for the ring-opening of acylureas with weakly basic leaving groups an uncatalysed expulsion of the leaving group in an anionic form may coexist with the catalytic pathways depicted in Schemes 4 and 6.

General Base Catalysis of the Reversible Cyclisation of the Anionic Form of (**3**).—A general base-catalysed reaction of the anionic form of the substrate with β 0.64 was observed during the cyclisation of the methylhydantoic acid (**1**). The mechanism proposed,[1] shown in Scheme 6, is a nucleophilic attack of the ureido anion on the carboxylate anion, concerted with general acid catalysis.

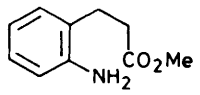
The alternative mechanism of general base catalysis leading to the very unstable intermediate T^{2-} was rejected on consideration of the solvent deuterium isotope effects and the observance of the Brönsted dependence by HO^-. It was argued that the proton transfer to HO^- will be close to the thermodynamically favourable direction and will not meet Jencks' criteria for concerted reactions.[16] The present general base-catalysed cyclisation of the anions of (**3**) with β 0.55 is the reaction depicted by Scheme 6, as HO^- again follows a Brönsted dependence. The main difference is that in the case of (**3**) this reaction takes place in a narrower pH interval, due to the change of the rate-determining step.

The identification of the reaction mechanisms for cyclisation of (**3**) described by Schemes 4 and 5 gives additional evidence in favour of the mechanism of ureido anion attack on the carboxylate anion. First, this time the expected positive deviation for HO^- acting as a base towards the NH proton of the ureido group was found for the ring closure of the neutral

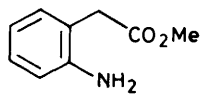


R = Me (**1**)
R = Ph (**3**)

Scheme 6.



(**5**)



(**6**)

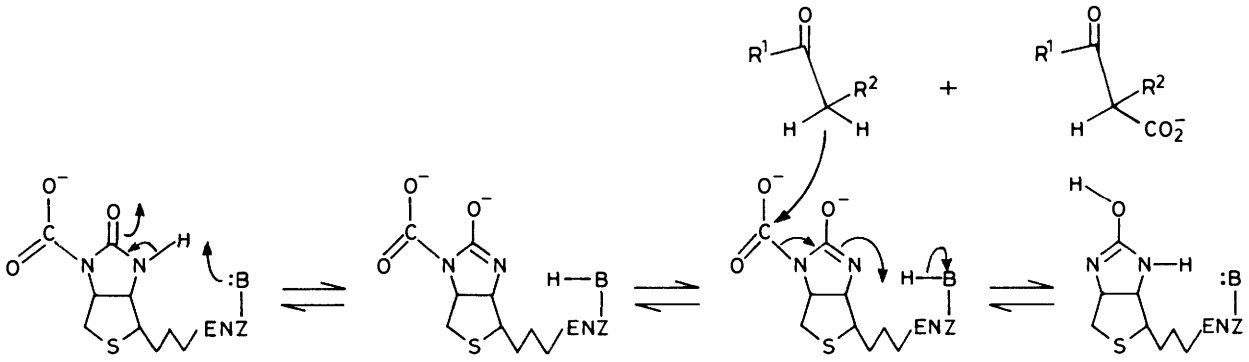
form, and again not for the base-catalysed reaction of the anionic form of the substrate. Second, the anionic form of the nucleophile is proven for the uncatalysed reaction *via* S^{i-}. This is a case involving a more reactive electrophile than the carboxylate anion, and only the reactive ureido anion is expected to be able to add to the deactivated carboxylate.

Influence of the Steric Strain on the Rate-determining Step of the Ring-closure Reaction.—The most interesting point about the base catalysed cyclisation of the anionic form of (**3**) is that it is an entirely unexpected reaction on the basis of existing results on alkaline hydrolysis of 3-aryl-substituted hydantoins.[4,5] We mentioned before that unlike the hydrolysis of 3-alkyl-substituted dihydrouracils and hydantoins,[2] the hydrolysis of 3-arylhydantoins proceeds only *via* rate-determining addition of hydroxide ion. Accordingly, the expected ring-closure reaction was a pH-independent decomposition of the tetrahedral intermediate (Scheme 1). In the present ring-closure of (**3**) this is observed at high pH after the change of the rate-determining step.

Apparently the different kinetic behaviour of the dimethyl-substituted compound (**3**) can be attributed to the steric strain in the acyclic compound. In fact the rate-determining step depends only on the partitioning of the cyclic intermediate T^-, expressed by the terms in the denominators of equations (vii) and (viii). The steric factors which have to be considered are only the factors governing the ease of ring-opening and not those for the ease of ring-closure. A reverse action of the *gem*-dimethyl effect, expressed as a decrease of k_3/k_{-1} values (Scheme 1), was found for alkaline hydrolysis of 1,6-disubstituted dihydrouracils[9] and was attributed to the increase of strain upon ring-opening. This increase of strain is slowing down the departure of the nucleophile in the case of more heavily substituted compounds, and consequently a new, earlier rate-determining step of the ring-closure reaction can result.

Intramolecular reactions of compounds activated by ground-state strain provide some of the most efficient models for the study of reactions of biological interest and it has been claimed[19] that the mechanisms of the most reactive systems are the most relevant to that of enzyme action. Owing to the possibilities for different mechanisms or rate-determining steps depending on the steric situation in the model compounds, a conflicting situation about the best model can sometimes arise, as in the case of the intramolecular aminolysis of esters, which proceeds by different mechanisms[13,20] for (**5**) and (**6**).

Nucleophilic Reactivity of Ureido Groups towards Carboxylic Functions.—The identification of the reaction pathways for ring-closure of (**3**) *via* the discrete ionic forms S^{i-} and S^{2-} allows



Scheme 7.

a comparison of the rates for attack of the phenylureido anion on neutral and ionised carboxy groups. The ratio of the rate constants for the water reactions of S^{i-} and S^{2-} calculated using a pK_a of 16.6 for the phenylureido group is 3.5×10^6, which corresponds to an additional barrier of *ca.* 9 kcal mol^{-1} for the anion–anion reaction.

The same calculation, using a pK_a of 18.3 for *N*-methylurea,[3] can be made for cyclisation *via* S^{2-} of tetramethylhydantoic acid (**1**). The rate constant obtained for (**1**) is only 13 times greater than the corresponding constant for the phenylhydantoic acid reaction *via* S^{2-}. If we account for the fact that (**1**), is a compound activated by a higher ground-state strain (one more methyl group in position 2 of the hydantoic acid), similar or even better nucleophilicity towards COO^- can be deduced for the phenylureido anion. This inverse reactivity of the more weakly basic anion is probably due to a decrease of the electrostatic barrier.

Both ref. 1 and the present study demonstrate that in conditions of an intramolecular reaction the nitrogen atom of the ureido group is an efficient nucleophile towards the carboxylate anion and this is in agreement with the currently predominating opinion about 1-*N*-carboxybiotin as the true intermediate of the reaction.[21a]

Recently a mechanism was proposed[22] in which the transfer of the carboxy group from 1′-*N*-carboxybiotin to acceptor substrates by biotin-containing enzymes occurs by a ureido enolate ion relay mechanism (Scheme 7).

It was pointed out[21b] that concerted mechanisms using the oxygen atom of the ureido group of biotin as proton donor or acceptor are stereochemically unreasonable. It seems to us that the mechanism shown on Scheme 7 is also chemically unreasonable, as the second negative charge on 1′-*N*-carboxybiotin resulting from proton abstraction at 3′-*N*-position will lead to a further decrease of the susceptibility of COO^- to nucleophilic attack. The concerted general acid–base catalysis found for the ring-closure of model compounds (**1**) and (**3**) indicate that a stronger possibility for the biotin-mediated carboxylations would be external catalysis by groups in the active centre of the enzyme, directed to the nucleophilic and electrophilic centres.

Acknowledgements

I am grateful to Drs. I. G. Pojarlieff and A. J. Kirby for discussions and to Mrs. M. Milanova for technical assistance.

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Received 24*th February* 1986; *Paper* 6/383