Tetrahedron Vol 23, pp 4307 to 4315. Pergamon Press Ltd 1967 Printed in Great Britain

KINETICS OF CYCLIZATION OF 3-UREIDOPROPIONIC ACID TO DIHYDROURACIL IN HYDROCHLORIC ACID[1]

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(*Received* 5 *December* 1966; *accepted for publication* 14 *February* 1967)

Abstract—The kinetics of cyclization of 3-ureidopropionic acid (UPA) to dihydrouracil (DHU) in hydrochloric acid solution has been studied. The reaction was found to be reversible with the equilibrium shifting in favour of UPA with increasing acid strength. It was shown that the failure to cyclize 3-ω-N-phenylureidopropionic acid in HCl is due to an equilibrium under which the extent of cyclization is only 30%. The rate of cyclization of UPA is proportional to the fraction of ureido acid protonated at the ureido group. pK_a of DHU in $HClO_4$ was assayed as *ca.* $-4{\cdot}5$. The "r-value", $k_{(D_2O)}/k_{(H_2O)}$ ratio and the entropy of activation indicate a mechanism for the acid hydrolysis of DHU involving nucleophilic attack of water concerted with ring opening as the slow step.

THE cyclization of β-ureido acids has been studied systematically by Rodionow and Vyazkova[2] and by Johnson and Livak[3] and that of β-ureido esters more recently by Schlögl.[4] These authors reported high yields of dihydrouracils on heating the ureido acids or esters in hydrochloric acid solution with the exception in some cases of ω-N-arylsubstituted derivatives where no cyclic product was obtained. However, in all cases (excepting rather bulky ω-substituents as *o*-toluyl or cyclohexyl) cyclization could be achieved by treating the ureido acids with acetic anhydride or acetyl chloride. Kurtev *et al.*[5] have studied the cyclization of diastereomeric β-ureido acids or esters and used the difference in yields or rates of formation of dihydrouracils as a method for establishing relative configurations. The effect of the latter on reactivity could be interpreted by assuming that the reaction passes through a tetrahedric transition state of the type usually considered important in ester hydrolysis. With the view to obtain more data relating to the mechanism of the reaction in HCl, a study of the kinetics of cyclization of the simplest representative—3-ureidopropionic acid, was undertaken the results of which are reported in the present paper. Notwithstanding the amount of work done on hydantoins, the kinetics of cyclization of hydantoic acids has not been studied.

RESULTS*

Reversibility of the reaction

The first interesting observation revealed by the kinetic experiments was that the

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* H_0-values were taken from M. Paul and F. Long, *Chem. Rev.* **57**, 1 (1957).

[1] Taken in part from *Cand. Chem. Sc. Thesis, Bulg. Acad. Sc.* Sofia (1964).

[2] W. Rodionow and S. Vyazkova, *Zh. Obsch. Khim.* **3**, 628 (1933).

[3] T. Johnson and I. Livak, *J. Am. Chem. Soc.* **58**, 299 (1936).

[4] K. Schlögl, *Mh. Chem.* **89**, 61 (1958).

[5] B. Kurtev, M. Lyapova, N. Berova, I. Pojarlieff, A. Orahovatz, N. Mollov and P. erneva in press. Reported in part at the XIXth IUPAC Congress, London (1963).

cyclization of 3-ureidopropionic acid to dihydrouracil proceeds reversibly and even at hydrochloric acid concentrations higher than 5N the state of equilibrium is shifted in favour of the ureido acid (see K'_e = [UPA] st./[DHU] st. in Table 1)

$$\mathrm{UPA} \underset{k_r}{\overset{k_f}{\rightleftarrows}} \mathrm{DHU} + \mathrm{H_2O}$$

Acid hydrolysis of dihydrouracils has been described under rather severe conditions under which it proceeds to amino acids.[6a,b] In our experiments this latter reaction was found to take place at 10 half lives to much less than 1% of the initial substrate concentration. That this is a true equilibrium reaction was demonstrated by rate measurements in both directions—cyclization of UPA, k_f, and acid hydrolysis of DHU, k_r (Exp. 6b and 6c in Table 1). Paper chromatography[7] of infinity reaction mixtures showed the presence of UPA and DHU in approximate amounts corresponding to the results of the titration.

The observed reversibility of the reaction was felt that it may explain the unsuccessful attempts[4] to cyclize 3-ω-N-phenylureidopropionic acid or its ester in HCl as a case of equilibrium strongly shifted in favour of the ureido acid. That this was the case was shown by refluxing for 1 hr 3-phenyldihydrouracil and 3-ω-N-phenylureidopropionic acid in diluted 1:1 by volume HCl. Both reaction mixtures were found to have practically identical compositions: *ca.* 70% of the ureido acid, the rest being PDHU. In Ac_2O or AcCl, where the water freed on cyclization is consumed, the equilibrium is shifted in the opposite direction.

TABLE 1.

Exp. No.	MHCl	t°C	10^5k sec^{-1}	K'_e	10^5k_f sec^{-1}	10^5k_r sec^{-1}
1	0·457	70·0	9·01	0·187	7·57	1·42
2a	0·940	70·0	16·7	0·200	13·9	2·78
b	0·940	65·0	11·7	0·206	9·7	1·99
c	0·940	60·0	7·94	0·194	6·65	1·29
d	0·940	55·0	4·78	0·200	3·98	0·797
3	2·04	70·0	28·6	0·355	21·0	7·61
4	2·97	70·0	38·5	0·396	27·5	11·0
5	5·11	70·0	56·4	0·838	30·7	25·8
6a*	6·48	70·0	75·1	1·37	31·7	43·5
b*	6·50	60·0	31·6	1·31	13·7	17·9
c	6·49	60·0	31·9	1·36	13·5	18·4
7*	7·78	70·0	85·1	1·86	29·8	55·4

* Measured acid hydrolysis of DHU.

Effect of hydrochloric acid concentration

The observed first order rate constant for cyclization, k_f, increases with increasing [HCl] reaching, however, a constant value at Ho of *ca.* −1. This may be explained

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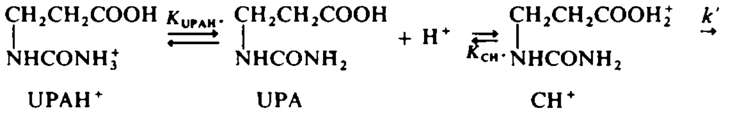
[6] [a] L. Birkofer and I. Storch, *Chem. Ber.* **86**, 529 (1953); [b] D. Keglevic and A. Kornhauser, *Croat. Chem. Acta* **31**, 47 (1959).

[7] R. Fink, R. Cline, C. McGaughey and K. Fink, *Analyt. Chem.* **26**, 4 (1956).

by the considerable basicity of the ureido group which when protonated should lose its nucleophilicity.



$$\underset{\text{UPAH}^+}{\begin{matrix}CH_2CH_2COOH\\ |\\ NHCONH_3^+\end{matrix}} \xrightleftharpoons{K_{UPAH^+}} \underset{\text{UPA}}{\begin{matrix}CH_2CH_2COOH\\ |\\ NHCONH_2\end{matrix}} + H^+ \underset{K_{CH^+}}{\rightleftharpoons} \underset{\text{CH}^+}{\begin{matrix}CH_2CH_2COOH_2^+\\ |\\ NHCONH_2\end{matrix}} \xrightarrow{k'}$$

The rate of cyclization

$$V = k_2[UPA][H^+] \quad (1) \text{ where } k_2 = \frac{k'}{K_{CH^+}}$$

is equal to the observed rate of cyclization

$$\text{Vobs.} = k_f\{[UPA] + [UPAH^+]\};$$

assuming that $K_{UPAH^+} = [UPA]ho/[UPAH^+]$ and that $[H^+]$ in (1) may be presented as ho, the relation is obtained:

$$k_f = k_2 K_{UPAH^+} \frac{ho}{ho + K_{UPAH^+}} \qquad (2)$$

where $ho/ho + K_{UPAH^+}$ is the fraction of molecules protonated at the ureido group. Similar kinetics have also recently been observed in the cyclization of 5-(*o*-aminoarylthio)pyrimidin-4-ones to 1,3-diazaphenothiazines.[8]

The solid line on Fig. 2 is the theoretical curve from (2) plotted with a value for K_{UPAH^+} of 1·8 which was found to fit best the experimental data (pK_a of urea is reported[9] as 0·30).

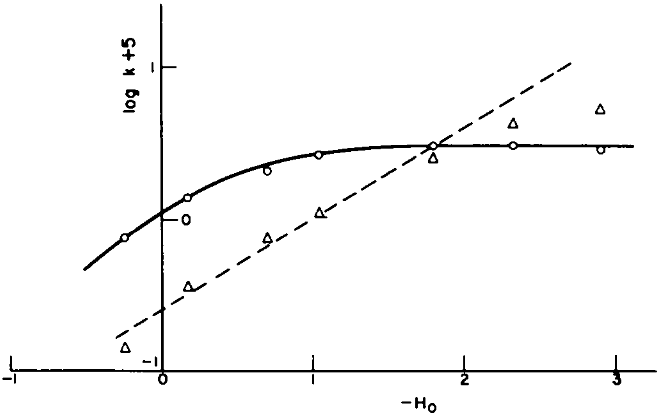


FIG. 2. Effect of acidity on rate of cyclization of 3-ureidopropionic acid (circles) and on hydrolysis of dihydrouracil (triangles).

It may be seen that in the cyclization reaction the use of acidities higher than 2–3N HCl is not necessary as the rate is not further increased.

The rate constant for acid hydrolysis of dihydrouracil, k_r, increases with increasing acid concentration k_r being proportional to [HCl] (aver. slope of a plot of log k_r versus log [HCl] is 1·3) rather than ho (aver. slope 0·5). In the plot log k_r versus Ho a curviture is observed at higher acidities (see dashed line in Fig. 2). In order to

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[8] B. Roth and J. F. Bunnett, *J. Am. Chem. Soc.* **87**, 340 (1965).

[9] A. Albert, E. Sergent, *Ionization Constant of Acids and Bases*, Russ. Transl. p. 137. Khimiya, Moscow (1964).

establish whether this is due to considerable basicity of DHU (considerable basicity leads to maxima in similar plots for rates of acid hydrolysis of amides[10]) attempts were made to determine pK_a of DHU in perchloric acid, although the spectral characteristics of DHUH$^+$ are unfavourable for a more exact pK_a determination. The results obtained are shown in Fig. 3.

Similarly to the protonation of propionic acid amide investigated by Edward and Wang,[11] the protonation of DHU does not follow *Ho*. At lower than pK_a acidities protonation increases more fastly than expected and less so at acidities higher than pK_a. Edward and Wang explain the phenomena as due to differences in hydration of the amide and of the indicator bases used in determining *Ho*. These differences, however, vary with changes of acid concentration so that pK_a is best estimated by the graphical method of Steward and Yates[12] as the mid-point of the straight portion of the plot of versus *Ho*. In our case this is *ca.* $-4{\cdot}7$ and the solid curve is the theoretical one obtained with pK_a of $-4{\cdot}7$ on condition *Ho* is followed by the protonation. This value, however, is too low to account for the observed curviture in the plot log k_r versus *Ho*. The ϕ-value[13] for the protonation of DHU was found from the above results to be 0·55 (r = 0·993) also suggesting that the protonation behaviour of DHU is similar to that of carboxyamides.

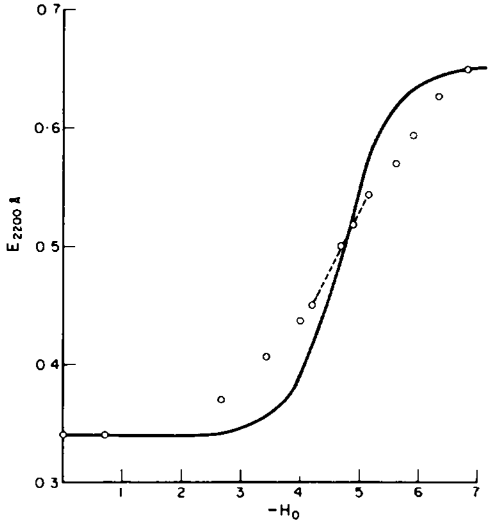

FIG. 3. Protonation of DHU in perchloric acid.

The changes in the observed state of equilibrium K'_e, may be attributed to the protonation of the ureido acid in the range of acidities studied

$$\mathrm{UPAH^+} \underset{K_{\mathrm{UPAH^+}}}{\rightleftharpoons} \mathrm{UPA} + \mathrm{H^+} \underset{K_e}{\rightleftarrows} \mathrm{DHU} + \mathrm{H_2O} + \mathrm{H^+}$$

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[10] J. T. Edward and S. Meacock. *J. Chem. Soc.* 2000 (1957).

[11] J. T. Edward, I. C. Wang, *Canad. J. Chem.* **40**, 966 (1962).

[12] R. Stewart and K. Yates, *J. Am. Chem. Soc.* **80**, 6355 (1958).

[13] J. F. Bunnett and F. P. Olsen, *Chem. Comm.* **1**, 601 (1965).

On the assumption that the protonation of the ureido acid follows *h*o, the following expression is obtained for the apparent equilibrium constant:

$$K'_e = \frac{[\mathrm{UPA}]\,\mathrm{st.}}{[\mathrm{DHU}]\,\mathrm{st.}} = \frac{K_e f_{\mathrm{DHU}}}{K_{\mathrm{UPAH}} + f_{\mathrm{UPA}}} a_{\mathrm{H_2O}}(h_0 + K_{\mathrm{UPAH^+}}) \tag{3}$$

It may be seen that with increasing acidity the state of equilibrium is shifted towards the ureido acid. At higher acidities the decrease of $a_{\mathrm{H_2O}}$ will tend to shift the equilibrium in the opposite direction. The cyclization of β-ureido acids is, however, subject to strong *gem*-dimethyl effect[14] and equilibrium is practically important only with some less heavily substituted ureido acids and apparently with ω-phenylureidopropionic acids.

DISCUSSION

The acid hydrolysis of open-chained amides is known usually to proceed with nucleophilic attack of water on the protonated form as the rate determining step. As regards cyclic amides, which dihydrouracil may be thought to resemble, one example, 2,5-piperazinedione, which has received some attention, has recently shown by Sykes *et al.*[15] to take place via the same mechanism on account of oxygen exchange experiments, although some years ago the rather high w-value for its acid hydrolysis led Bunnett[16] to suggest a mechanism with a general acid catalysed expulsion of the amino group as the slow step. As it has been found that the protonation of amides may be described by a H_A-function, the acidic dependancy of their hydrolysis is better characterized by the r-value of Yates and Stevens which represents a "w-value" calculated using the H_A-function.[17a, b, c] Yates and Riordan found for a range of amides a r-value of (3·1 ± 0·3), thus indicating the participation of three molecules of water in the transition state—the number associated by Bunnett with nucleophilic attack by water. In order to check whether the above controversy regarding 2,5-piperazinedione may be solved by using the H_A we calculated the r-value from the data used by Bunnett.[18] A value of 3·9 was obtained which is not very significantly higher than the range observed by Yates and Riordan.

In our case of dihydrouracil hydrolysis a r-value of 3·1 (r = 0·990) was obtained,[19] which coupled with the fact that DHU behaves similarly to amides on protonation strongly suggests a mechanism similar to that accepted for ordinary acid hydrolysis of amides.

The other evidence obtained also supports this conclusion. The $k_{r(\mathrm{D_2O})}/k_{r(\mathrm{H_2O})}$ ratio[20] for hydrolysis of DHU was found to be 1·75 which is quite close to the range associated with Ac_2 hydrolysis with slow attack of water.[21a, b] In this connection it may be noted that the base hydrolysis of amides, where oxygen exchange is faster

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[14] Unpublished results.

[15] B. Sykes, E. Robertson, H. Dunford and D. Konasewich, *Biochem.* **5**, 697 (1966).

[16] J. F. Bunnett, *J. Am. Chem. Soc.* **83**, 4968 (1961).

[17] [a] K. Yates, J. Stevens and A. R. Katritzky, *Canad. J. Chem.* **42**, 1957 (1964); [b] K. Yates and J. Stevens, *Ibid.* **43**, 529 (1965); [c] K. Yates and J. Riordan, *Ibid.* **43**, 2328 (1965).

[18] From the data of J. Edward and S. Meacock, *J. Chem. Soc.* 2007 (1957).

[19] Plot of log $k_r + H_A$ versus log $a_{\mathrm{H_2O}}$. H_A recalculated on a volume basis.

[20] Calculated assuming proportionality of *k* with [HCl] in the 0·935–0·982 M region.

[21] [a] J. Pritchard and F. Long, *J. Am. Chem. Soc.* **78**, 6008 (1956); [b] F. Long and J. Bigeleisen, *Trans. Faraday Soc.* **55**, 2077 (1959).

than hydrolysis,[22] proceeds a little faster in H_2O.[23] In the case of ring closure a lower $k_{f(D_2O)}/k_{f(H_2O)}$ ratio of 1·16 was observed. This might be in part attributed to the effect of protonation of the ureido group which should be expected to proceed to a greater extent in D_2O and hence reduce the effect of enhanced protonation of the carboxyl group.

The observed ratio for the apparent equilibrium constant $K'_{e(D_2O)}/K'_{e(H_2O)}$ of 1·52 may also be partly explained by effects of protonation. From (3) after omission of activity coefficients and the water term we obtain:

$$\frac{K'_{e(D_2O)}}{K'_{e(H_2O)}} = \frac{K_{e(D_2O)}}{K_{e(H_2O)}} \times \left[\frac{K_{UPAH^\cdot} \cdot (ho + K_{UPAD^\cdot})}{K_{UPAD^\cdot} \cdot (ho + K_{UPAH^\cdot})}\right]$$

Although the K_{BH^+}-ratios depend on acid structure, from the data of Högfeld and Bigeleisen[24] it may be seen that for many different bases with pK_a about 0 the ratio is around 3. Assuming this value, the term in square brackets becomes 2.

The above considerations are further borne out by the observed entropies of activation. For the cyclization reaction $\Delta H^{\neq}$ is 17·9 kcal/mole and $\Delta S^{\neq}$ is $-24·3$ e.u. and for acid hydrolysis of DHU $\Delta H^{\neq} = 18·1$ kcal/mole and $\Delta S^{\neq} = -26·8$ e.u. were found. A value of $-26·8$ e.u. for the ring-opening is compatible with Ac_2 mechanism. Long *et al.*[25] reported $-24·6$ and $-20·9$ e.u. for δ-valerolactone and γ-butyrolactone, respectively, in acid catalysed hydrolysis which is known to take place as Ac_2 in this case. For acid hydrolysis of diketopiperazine for which Sykes *et al.*[17] assume a rate-controlling step of ring-opening concerted with water addition, the value of $\Delta S^{\neq}$ is somewhat lower 14·7 e.u. but also in the usual Ac_2 region.

The above discussion indicates as most probable mechanism of acid hydrolysis of DHU a slow nucleophilic attack of water concerted with ring-opening. The reverse reaction, the cyclization of UPA, necessarily taking place via the same transition state, should have as slow step the attack of the ureido group on the protonated carboxyl group.

EXPERIMENTAL[26]

Materials

Dihydrouracil was prepared according to a modified Fischer and Roeder procedure[27] and the product purified by crystallization alternately from water and EtOH to constant m.p. of 272–274° (reported, 276–278°[27]; 272–275°[28]). (Found: N, 25·61 Calc for $C_4H_6N_2O_2$: N, 24·55%. Equiv wt after hydrolysis with known amount of NaOH. Found: 114·3. Calc 114·1.)

3-*Ureidopropionic acid* was prepared by hydrolysis of DHU with 1N NaOH for 10 hr at room temp. The acid was freed by stirring, after dilution with water, with a 10% excess of Wofatit KPS 200 in H^+-form. After removing the water *in vacuo* and recrystallizing twice from water the ureido acid was obtained 40–50% yield and m.p. 168–169° dec. (reported, 170–171°[29]; 169–170°[28]). (Found: N, 21·48. Calc for $C_4H_8N_2O_3$: N, 21·20% Equiv wt. Found 133·2. Calc 132·1.)

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[22] D. Samuel and B. Silver, *Advances in Physical Organic Chemistry* Vol. 3; p. 159–160. Academic Press, London (1965).

[23] K. Wiberg, *Chem. Rev.* **55**, 719 (1955).

[24] E. Högfeld and J. Bigeleisen, *J. Am. Chem. Soc.* **82**, 15 (1960).

[25] F. Long, J. Pritchard and F. Stafford, *J. Am. Chem. Soc.* **79**, 2362 (1957)

[26] M.ps are in capillary and uncorrected. Analysis were carried out by the Anal. Lab. of the Inst. of Org. Chemistry, Bulg. Ac. Sc. UV-spectra unless otherwise stated on a Zeiss-Jena VSU-1 Universal Spectrophotometer

[27] K. Y. Zee-Cheng, R. K. Robins and C. C. Cheng, *J. Org. Chem.* **26**, 1877 (1961).

[28] R. D. Batt, J. K. Martin, J. McT. Ploeser and J. Murrey, *J Am. Chem. Soc.* **76**, 3663 (1954).

[29] F. Lengfeld and I. Stieglitz, *J. Am. Chem. Soc.* **15**, 504 (1893).

3-ω-N-*Phenylureidopropionic acid* (PUPA) was prepared[4] and had m.p. 169·5–171° dec (reported,[4] 170–171°) (twice from water).

3-*Phenyldihydrouracil* (PDHU) was prepared[30] and crystallized thrice from water, m.p. 226–229° (reported, 231–234°[30]; 230–232°[4]). (Found: N, 14·91. Calc for $C_{10}H_{10}N_2O_2$: N, 14·73%.)

Mineral acid solns were prepared by diluting analytical grade reagents with distilled water.

Kinetic runs

Procedure A. The reaction solns were prepared and mixed at the reaction temp (25 or 50 ml aqueous soln of the substrate, UPA or DHU, and 100 ml appropriately conc HCl, resulting substrate concentration was 0·1M) in a Höppler Ultrathermostate (temp control was ±0·05°). The heat of dilution raised the temp of the reaction mixture by a maximum of 1·5° and for this reason measurements were begun after the soln acquired the bath temp (20 min at most). Samples (5 ml) were taken by means of a thermostatted pipette and quenched on ice. The HCl concentration was determined by titration with 0·1N NaOH using cresol red as the indicator and correcting for the ureido acid present.

The analytical method was based on the ease of alkaline hydrolysis of DHU.[28] The latter permitted the adaption of a method suggested by Salomaa[31] for measuring rates of acid ester hydrolysis. The great excess of mineral acid was crudely neutralized with 50% NaOH and then exactly with 0·1N NaOH using cresol red as the indicator (pH-interval of colour change 7·2–8·8; phenolphthalein could not be used as dihydrouracils are weak acids[32] and moreover DHU itself is highly sensitive to alkali). 0·1N NaOH (10 ml) was then added and the samples, well stoppered, were heated for 1 hr at 65–70°. The excess of NaOH was back titrated with 0·1N HCl (all titrations were carried out under a flow of N_2). $V_{DHU} = 10 - V_{HCl}$; $V_{UPA} = Vo_{UPA} - V_{DHU}$, where V_{oUPA} is the initial concentration of UPA expressed in ml 0·1M soln. It was calculated from the weight of the initial sample, no correction for change of volume on dilution was made.

The reaction was found to be reversible and of first order in each direction giving good straight plots of log ΔV_p versus time (see Fig. 1) ($\Delta V_p = V_{p\infty} - V_{pj}$; V_p is concentration of the product). The apparent equilibrium constant was determined as $K'_e = (V_{UPA}/V_{DHU})$ from 2–4 determinations at ten half lives.

The rate constants were calculated from

$$k = k + k = \frac{2.30 \sum_{j=2}^{n} g_j \cdot \frac{\log\Delta V_1 - \log\Delta V_j}{t_j - t_1}}{} \qquad (g_j = \Delta V_j \Delta t_j)^{33}$$

$$k_f = \frac{k}{1 + K'_e}; \qquad k_r = \frac{k}{1 + \frac{1}{K'_e}}$$

For the calculation of k, 8–12 points were taken spread over more than two half lives conversion. The standard deviation in V, determined separately, was 0·033 ml and the standard error in k_f and k_r did not exceed 3%.

The extent of the side reaction, hydrolysis to amino acid and ammonia, was examined by means of standard series of mock reaction mixtures containing known amounts of NH_4Cl with Nessler's reagent. More exact colometric determinations could not be carried out since Nessler's reagent gives a white ppt with DHU—probably a mercury salt of the anion.

Procedure B. This was applied in the experiments with D_2O. Use was made of the differences of absorption at 2200Å of DHU (ε = 2·38 × 10^3) and of UPA (ε = 0·078 × 10^3) in *ca.* 1M HCl. The reaction was carried out directly in the spectrophotometer in thermostated with a copper block 1 cm square cells supplied with a teflon stopper with closely fitting glass stirrer and thermistor for temp control (±0·05°). 0·05 ml of a 0·0141M stock soln of UPA were added to 2·75 ml of HCl previously brought to the reaction temp. While procedure B gave good results with faster reacting ureido acids at lower temp, in this particular case, namely, cyclization of UPA at *ca.* 60° in *ca.* 1M HCl, the reaction could only be

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[30] E. Fischer and H. Leuchs, *Ber. Dtsch. Chem. Ges.* **35**, 3787 (1902).

[31] P. Salomaa, *Suomen Kemistl.* **B32**, 81 (1959).

[32] C. Janion and D. Shugar, *Acta Biochim. Polon.* **7**, 309 (1960).

[33] L. Batuner and M. Posin, *Matematicheskie metodi v khimicheskoy tehnike* pp. 501–502. Moscow (1962).

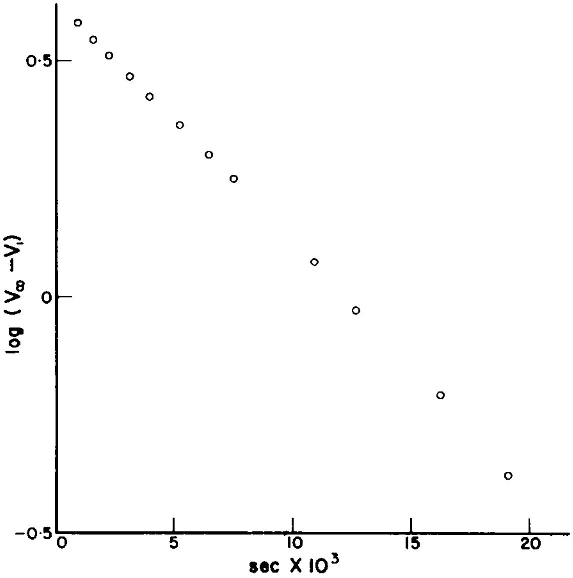

FIG. 1. Cyclization of 3-ureidopropionic acid in 0·940 M HCl at 65·0°.

followed to about 50% conversion since after that evaporation began to affect noticeably the results. K'_e had to be determined in separate experiments in sealed tubes (3 samples). k was obtained by a least squares treatment[34] of the dependance of log (E_∞ − E_j) with time after correction allowing for absorption by UPA. The values of k obtained by this procedure were somewhat higher than those obtained by procedure A. This is partly due to the fact that in 0·94M HCl UPA is about 40% protonated, which should reduce the effective concentration of HCl in case of procedure A with about 4% at the beginning of the reaction.

	t	MHCl	10^5k sec^{-1}	K'_e	10^5k_f sec^{-1}	10^5k_r sec^{-1}
Procedure A	60·0	0·940	7·94	0·194	6·65	1·29
Procedure B	59·96	0·935	8·62	0·196	7·21	1·41
	59·98	0·935	8·34		6·97	1·37

Experiments in D_2O

A soln of DCl was prepared by dripping 99·8% D_2O on freshly distilled PCl_3 and adsorbing the DCl evolved in D_2O. The DCl-gas was passed through a cooled to −20° trap to prevent any contamination with PCl_3. A stock soln of UPA was prepared by dissolving 11·85 mg in 5·00 ml D_2O. The following results were obtained according to procedure B:

t°	MDCl	10^5k sec^{-1}	K'_e	10^5k_f sec^{-1}	10^5k_r sec^{-1}
59·86	0·982	11·52	0·294	8·79	2·73
59·90	0·982	10·96		8·36	2·60

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[34] R. Livingston, *Technique of Organic Chemistry* Vol. VIII; part I; p. 132. Interscience, New York (1961).

Equilibration experiments with* 3-ω-N-*phenylureidopropionic acid* (PUPA) *and with* 3-*phenyldihydrouracil

On a preparative scale 0·507 g of PDHU and 0·566 g PUPA, respectively, were refluxed in 5 ml diluted 1:1 by volume HCl for 2 hr. On cooling from each soln, respectively, separated 0·313 g and 0·322 g of PUPA (m.p. 166–168° and 165–167°, both not giving depression with pure PUPA). The mother liquors gave on evaporation low melting mixtures

The equilibrium composition could be determined by means of UV spectra. PUPA gives in slightly acid and alkaline medium a peak of the same intensity at 2385 Å ($\varepsilon = 1{\cdot}68 \times 10^4$). In neutral and slightly acid medium PDHU gives practically no absorption in this region. In alkaline medium, however, due to the almost instantaneous hydrolysis of 3-substituted dihydrouracils,[32] PDHU shows the absorption of PUPA. In this way PUPA could be determined by directly diluting the reaction mixtures with water, and the concentration of PDHU by diluting an aliquot with 0·1N NaOH from the difference of absorption of both solns. Samples of PDHU and of PUPA refluxed for 1 hr in 1:1 HCl showed a 70·2 and 69·3% content of PUPA, respectively, and total absorption in NaOH equivalent to 101·7 and 100·2%, respectively On longer heating (4 hr) some hydrolysis to aniline was observed.

Protonation of dihydrouracil

In the near UV region to 2050 Å (Leres Automatic Recording Spectrophotometer) in *ca* 70% $HClO_4$ DHU showed only a slope rising towards lower wave lengths similar to that observed in neutral aqueous solution[32] but with about twice as high extinction ($\varepsilon = 4{\cdot}64 \times 10^3$ at 2220 Å). In order to avoid hydrolysis of DHU the solutions were prepared by adding 0·50 ml of a $7{\cdot}00 \times 10^3$M soln of DHU to a cooled in an ice–water bath soln of $HClO_4$ in a 25 ml volumetric flask, the soln immersed for 30 min in 25° bath, brought to the mark and measured. The concentration of $HClO_4$ was determined titrimetrically. For estimation of p*K* see results.

Enthalpy and entropy of activation were calculated by mean squares treatment from the equation $\log k/T = 10{\cdot}319 + (\Delta S^{\neq}/4{\cdot}574) - (\Delta H^{\neq}/4{\cdot}574) \times 1/T$ using the data obtained in 0·94M HCl (Table 1). First order rate constants were used as the solutions are close to 1M HCl.

Acknowledgements—Helpful discussions with Professors B. J. Kurtev and J. F. Bunnett and valuable technical assistance of Miss Zdravka Tosheva are gratefully appreciated.