384. *Hydrolysis of Amides and Related Compounds. Part II.* Acetylglycine, Piperazine-2 : 5-dione, and Gelatin in Concentrated Hydrochloric Acid.*

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At 61° the rate of hydrolysis of acetylglycine is maximal in about 5M-hydrochloric acid. No maximum was found in the rate of hydrolysis of piperazine-2 : 5-dione or of gelatin as the concentration of hydrochloric acid was increased to 10M.

MANY studies have been made of the kinetics of the hydrolysis of peptides and proteins in aqueous acids,[1] but there appear to have been no attempts to discover whether the rates pass through a maximum as the concentration of acid is increased (cf. Part I). We have accordingly studied the hydrolysis at 61° of acetylglycine, piperazine-2 : 5-dione, and gelatin in hydrochloric acid of varying concentrations, using the " formol " titration [2] to estimate the amino-groups liberated during the hydrolysis.

The rate of hydrolysis of acetylglycine (see Table) was maximal in about 5N-acid at this temperature. This is likely [3] to be the concentration for maximal rate at 25° also, and hence indicates a pK_a of the peptide linkage of about −2.[4] This agrees with a recent spectrophotometric result (pK_a −1·92)[5] and is about the value to be expected from a pK_a of −1·4 for acetamide when the inductive effect of the ·CH_2·CO_2H group [6] is considered.

The rate of hydrolysis of piperazine-2 : 5-dione was a roughly linear function of acid concentration up to the highest concentration investigated (10·18N). This rate refers to its hydrolysis to glycylglycine; the hydrolysis of the latter to glycine goes fifty times more slowly in 2N-hydrochloric acid [7] and (as is shown below) probably in the other concentrations employed, and hence can be ignored. The absence of a maximum rate in this range of acid concentrations indicates a pK_a of the peptide linkage below −4.[4]

As expected,[1] the hydrolysis of gelatin, involving many different types of peptide linkages, did not obey first-order kinetics. Accordingly the quarter-life was chosen as a

* Part I, preceding paper.

[1] Leach, *Rev. Pure Appl. Chem.*, 1953, **3**, 25, and references therein.
[2] Northrop, *J. Gen. Physiol*, 1926, **9**, 767.
[3] Edward, Hutchison, and Meacock, *J.*, 1955, 2520.
[4] Edward and Meacock, preceding paper.
[5] Goldfarb, Mele, and Gutstein, *J. Amer. Chem. Soc.*, 1955, **77**, 6194.
[6] Cohn and Edsall, " Proteins, Amino Acids and Peptides," Reinhold, New York, 1943, p. 116 *et seq.*
[7] Hammel and Glasstone, *J. Amer. Chem. Soc.*, 1954, **76**, 3741.

Rates of hydrolysis in hydrochloric acid at 61°.

k_e = first-order velocity constant in min.$^{-1}$. $t_{1/4}$ = time for 25% hydrolysis in hr. Acid concentrations are in moles/l.

			Acetylglycine					
[HCl]	1·00	2·00	3·00	4·00	5·00		6·00	8·21
10^3k_e	0·86	1·62	2·26	2·51	{2·69, 2·67}		2·35	1·34
			Piperazine-2 : 5-dione					
[HCl]	1·00	2·00	3·00	4·00	5·00	7·52	8·36	10·18
10^3k_e	0·82	1·95	2·67	3·09	4·00	5·18	5·12	6·24
10^3k_e/[HCl]	8·2	9·7	8·9	7·7	8·0	6·9	6·1	6·1
			Gelatin					
[HCl]	3·00	4·94	5·25	7·00	10·4			
$t_{1/4}$	5·1	2·9	2·6	2·5	1·6			
[HCl] × $t_{1/4}$/10	1·5	1·4	1·4	1·7	1·7			

convenient measure of the stabilities in the different acid concentrations; [8] its reciprocal will be proportional to the relative rate of hydrolysis. For this material also, the rate is roughly proportional to the acid concentration up to 10·4N, so that the average pK_a of the peptide linkages must be below −4.[4] A pK_a value of about −4 has been reported from spectrophotometric studies.[9] With both gelatin and piperazine-2 : 5-dione it is evident that the neighbouring peptide linkages strongly depress each other's basicity. Base-weakening effects of proximal peptide linkages on other groups have been observed in many simple compounds.[6]

The differing basicities of amides enable one to choose conditions for obtaining some degree of specificity in their hydrolysis. Thus acetamide [3] is hydrolysed about 50% more rapidly than piperazine-2 : 5-dione in 1N-hydrochloric acid, but about ten times more slowly in 8N-acid. However, there is likely to be no important change in the relative stabilities of the different peptide linkages of proteins (apart from those involving serine and threonine [10]) over the range 1N—10N-hydrochloric acid. The pK_a's of the peptide linkages of proteins are about −4; in the smaller peptides they will be less basic because of the proximity of the charged amino-group.[6] Consequently, in the range of acidities being considered (up to H_0 of −3·6) the rates of hydrolysis should be proportional to the concentration of hydroxonium ion,[4] and hence the order of stabilities should be the same in different strengths of acid. This is in agreement with the limited evidence at present available.[11]

Experimental

Acetylglycine (m. p. 207—208°) and piperazine-2 : 5-dione (m. p. 309—310°) were purified by recrystallization from water to the m. p.s indicated. Nelson No. 3 gelatin was used without further treatment. Its amine titre (0·400 g. of gelatin ≡ 30·8 c.c. of 0·1N-sodium hydroxide in the " formol " titration) was constant after 24 hours' hydrolysis in 7N-hydrochloric acid at 100°, and was taken to represent 100% hydrolysis. Rate measurements were carried out as described previously [3] on 0·3—0·4M-solutions of acetylglycine or piperazine-2 : 5-dione or 4% solutions of gelatin in acid. Results for two typical runs are shown below :

Acetylglycine. 0·339M; *with* 5·00N-*hydrochloric acid*

Time (min.)	31	54	140	177	220	271	340
Hydrolysis (%)	9·2	13·9	32·2	38·1	44·9	51·8	56·7

$k = 2{\cdot}67 \times 10^{-3}$ min.$^{-1}$.

Piperazine-2 : 5-dione. 0·339M; *with* 2·00N-*hydrochloric acid.*

Time (min.)	8	22	30	40	53	74	91
Hydrolysis (%)	20·5	39·5	49·5	57·1	67·0	76·4	78·5

$k = 1{\cdot}95 \times 10^{-2}$ min.$^{-1}$.

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[8] Cf. Sanger, *Adv. Protein Chem.*, 1952, **7**, 1.
[9] Goldfarb and Gutstein, Abs. Papers 126th Meeting Amer. Chem. Soc., 1954, 61c.
[10] Elliott, *Biochem. J.*, 1952, **50**, 542.
[11] Martin, *Nature*, 1955, **175**, 771; Hirohata, Kanda, Nakamura, Izumiya, Nagamatsu, Ono, Fujii, and Kimitsuki, *Z. physiol. Chem.*, 1953, **295**, 368; Harris, Cole, and Pon, *Biochem. J.*, 1956, **62**, 154.