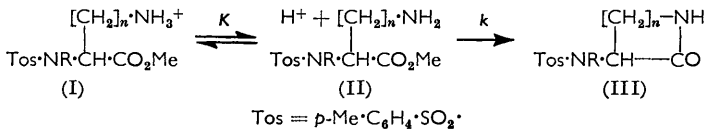
572. *The Kinetics of Lactamization of Methyl Esters of Some α-N-Toluene-p-sulphonyl Derivatives of αω-Diamino-Acids in Aqueous Solution.*

By E. F. Curragh and D. T. Elmore.

The methyl esters of α-*N*-toluene-*p*-sulphonyl-DL-ornithine (II; R = H, *n* = 3), γ-amino-L-α-toluene-*p*-sulphonamido- and γ-amino-L-α-*N*-methyl-toluene-*p*-sulphonamido-butyric acid (II; R = H and Me respectively, *n* = 2) undergo lactamization in aqueous solution at slightly alkaline pH values. Hydrolysis is negligible under these conditions. The kinetics of these reactions are consistent with a mechanism involving hydroxyl-ion catalysis.

For some time we have been interested in kinetic studies of the trypsin-catalysed hydrolysis of esters of α-*N*-toluene-*p*-sulphonyl derivatives of αω-diamino-acids.[1] Since some of these compounds readily lactamize in presence of base,[2] we decided to study the kinetics of this process in aqueous solution at the alkaline pH values likely to be used in later studies with trypsin. The mechanism of the aminolysis of esters, of which lactamization is a particular example, has received considerable attention; much of the literature has been reviewed by Bender.[3]

The present investigation was carried out over a range of pH values, which were maintained constant during a run by a pH-stat;[4] the latter delivered alkali equivalent to the acid liberated in the process (I ⟶ II). That lactamization occurs to the virtual



(I) (II) (III)

Tos = *p*-Me·C_6H_4·SO_2·

exclusion of hydrolysis was proved by two methods. First, all the methyl esters afforded good yields of the corresponding lactams after being kept in aqueous solution at pH values of 7·8—8·5. A second, more rapid proof depended on allowing the reaction to proceed at a constant pH for a given time, then adding formaldehyde, and titrating back to the original pH value with sodium hydroxide. The total alkali uptake in these two steps was identical within experimental limits to the Sørensen titre at zero time. Results were similar for all three substrates and were independent of the extent to which reaction had occurred. The theoretical basis of this proof may be simply established. Let there be at zero time n_1 moles of ester hydrochloride buffered to a given pH by the addition of n_{Na^+} moles of sodium hydroxide so that n_2 moles of ester are unprotonated. After lactamization has proceeded for a time *t*, during which the pH has been kept constant by the addition of n'_{Na^+} moles of alkali, let there be n_3 moles of ester and n_5 moles of amino-acid [*i.e.*, $(n_1 - n_3 - n_5)$ moles of lactam are formed]. Suppose also that n_4 and n_6 moles of ester and amino-acid are unprotonated at time *t*. The condition of electrical neutrality at zero time gives

$$n_1 - n_2 + n_{Na^+} + n_{H^+} = n_{OH^-} + n_{Cl^-},$$

and at time *t*

$$n_3 - n_4 + n_{Na^+} + n'_{Na^+} + n_{H^+} = n_{OH^-} + n_{Cl^-} + n_6.$$

Hence,

$$n'_{Na^+} = n_1 - n_2 - n_3 + n_4 + n_6.$$

[1] Baines and Elmore, *Biochem. J.*, 1960, **76**, 25 P; *Bull. Soc. Chim. biol.*, 1960, **42**, 1305.
[2] (*a*) Barrass and Elmore, *J.*, 1957, 4830; (*b*) Rudinger, *Coll. Czech. Chem. Comm.*, 1954, **19**, 365.
[3] Bender, *Chem. Rev.*, 1960, **60**, 53.
[4] Jacobsen, Léonis, Linderstrøm-Lang, and M. Ottesen, *Methods Biochem. Analysis*, 1957, **4**, 171.

If the Sørensen titrations at zero time and t require n_0 and n_t moles of alkali respectively, then

$$n_0 = n_1 - n_2,$$

and

$$n_t = n_5 - n_6 + n_3 - n_4.$$

Hence,

$$n_t + n'_{Na^+} = n_1 - n_2 + n_5.$$

But, since

$$n_t + n'_{Na^+} \approx n_0 \text{ by experiment, } n_5 \approx 0.$$

The kinetic equations, which govern lactamization, are derived on the assumption that only the unprotonated form of the ester undergoes aminolysis and that hydrolysis may be neglected. Activity corrections are ignored. Let n g.-ions of H^+ be liberated in a volume v in the stage (I $\rightleftharpoons$ II); this is equivalent to the number of moles of sodium hydroxide added by the pH-stat to maintain constant pH. Let c_A and c_B represent the molar concentrations of the protonated ester and its conjugate base, respectively. Then the total substrate concentration, [S], is given by $c_A + c_B$. Now $c_B[H^+] = Kc_A$.

Hence,

$$(c_A + c_B)/c_B = ([H^+] + K)/K = [S]/c_B.$$

But

$$-d[S]/dt = kc_B = kK[S]/([H^+] + K).$$

The pH-stat measures the rate of liberation of H^+ ions and, if the process (I $\rightleftharpoons$ II) is virtually instantaneous, then

$$-\frac{dc_A}{dt} = \frac{1}{v} \cdot \frac{dn}{dt} = u$$

But

$$(c_A + c_B)c_A = ([H^+] + K)/[H^+] = [S]/c_A.$$

Hence

$$\begin{aligned} u &= \{-[H^+]/([H^+] + K)\}\, d[S]/dt \\ &= kK[H^+][S]/([H^+] + K)^2 \end{aligned}$$

At zero time, let $u_0 = u$ and $[S]_0 = [S]$, then by rearrangement,

$$\sqrt{\frac{[H^+][S]_0}{u_0}} = \frac{[H^+]}{\sqrt{kK}} + \sqrt{\frac{K}{k}}$$

and a graph of $\sqrt{([H^+]/u_0)}$ against $[H^+]$ should be linear. In practice, this was found not to be the case. If, however, the reaction is catalysed by hydroxyl ion, $-d[S]/dt = k[OH^-]c_B$, and

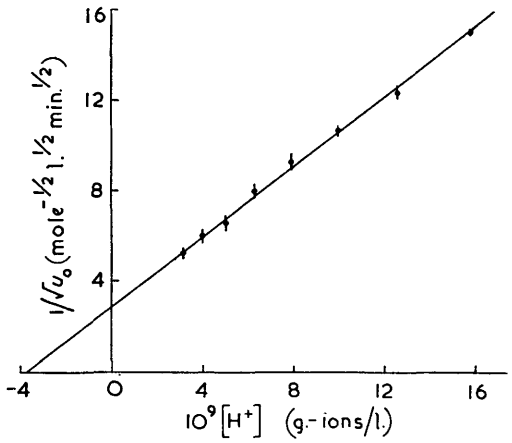
$$u_0 = kK[OH^-][H^+][S]_0/([H^+] + K)^2 = kKK_w[S]_0/([H^+] + K)^2,$$

where K_w is the ionic product of water. Rearrangement gives

$$\frac{1}{\sqrt{u_0}} = \frac{[H^+]}{\sqrt{kKK_w[S]_0}} + \sqrt{\frac{K}{kK_w[S]_0}}$$

and a plot of $1/\sqrt{u_0}$ against $[H^+]$ should be linear with slope $1\sqrt{(kKK_w[S]_0)}$, ordinal intercept $\sqrt{(K/kK_w[S]_0)}$, and abscissal intercept K. Fig. 1, which is typical of a number of experiments, shows that the regression curve, over the pH range tested (7·8 —8·5), was in fact linear. The results are summarized in Tables 1 and 2. The pK'_a values are of the expected magnitude for ω-amino-groups of α-*N*-acylated amino-esters. Moreover, the pK'_a of the methyl ester of α-*N*-toluene-*p*-sulphonyl-DL-ornithine is higher than that of methyl γ-amino-L-α-toluene-*p*-sulphonamidobutyrate, as expected. Although it was impossible to determine accurately the pK'_a values of these compounds by standard methods, an approximate value of 8·63 ± 0·07 was obtained for the latter compound at 25°, in satisfactory agreement with that obtained from kinetic studies. Unfortunately,

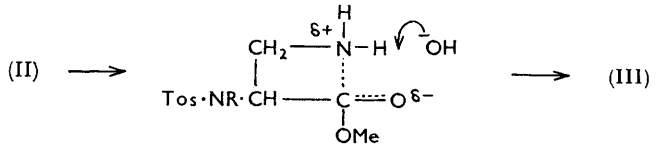
the energies of activation for these reactions and the heats of ionization of the amino-groups of the ω-amino-esters could not be determined satisfactorily because the regression curves of $1/\sqrt{u_0}$ on $[H^+]$ had very small intercepts, particularly at the lower temperatures. Since the intercepts were obtained by extrapolation, the errors (Table 2) were considerable. Nevertheless, the results support the kinetic equation developed above and our postulate that hydroxyl-ion catalysis is involved in the lactamization of these esters. An alternative mechanism, suggested to one of us by Dr. G. Baddeley, in which ionization of the sulphonamido-group assists the reaction, seems unlikely, since the pK_a' would be expected to be in the region 9·8—10·0. The first alternative is evidently correct, since the kinetics of lactamization of methyl γ-amino-L-α-*N*-methyltoluene-*p*-sulphonamidobutyrate, in which ionization of the sulphonamido-group is precluded, are very similar to those of the compounds lacking the *N*-methyl group. It is possible that, for the two substrates (II; R = H, $n = 2$ or 3), ionization of the sulphonamido-group may modify the mechanism



Kinetic test for hydroxyl-ion catalysis. (Vertical lines through points represent standard deviations.)

at pH values higher than those we have used. Although ionization of the toluene-*p*-sulphonamido-group apparently plays no part in the mechanism of lactamization in the pH range which we have used, this group nevertheless favours the reaction, since neither methyl γ-aminobutyrate nor methyl γ-amino-α-benzamidobutyrate underwent lactamization at an appreciable rate under these conditions.

Hydroxyl-ion catalysis, during the aminolysis of esters, has been observed several times. Thus the reactions of n-butylamine with *S*-ethyl thioacetate,[5a] *S*-2-acetamidoethyl thioacetate,[5a] α-naphthyl acetate,[5b] and *S*-ethyl *p*-nitrothiobenzoate [5c] in aqueous solution follow this mechanism. Morpholine and piperidine, but not ammonia, glycylglycine, glycine ethyl ester, or hydroxylamine, react with phenyl acetate with hydroxyl-ion catalysis.[6] The closest analogy to our work, however, is the observation by Bruice and Sturtevant [7] that lactamization of esters of γ-4-imidazolylbutyric acid proceeds with hydroxyl-ion catalysis in aqueous solution. The mechanism of the reactions studied by us possibly involves detachment of a proton from the transition state by hydroxyl ion:



[5] (*a*) Hawkins and Tarbell, *J. Amer. Chem. Soc.*, 1953, **75**, 2982; (*b*) Hawkins and Piscalnikow *ibid.*, 1955, **77**, 2771; (*c*) Connors and Bender, *J. Org. Chem.*, 1961, **26**, 2498.
[6] Jencks and Carriuolo, *J. Amer. Chem. Soc.*, 1960, **82**, 675.
[7] Bruice and Sturtevant, *J. Amer. Chem. Soc.*, 1959, **81**, 2860.

Lactamization of these esters appears not to be subject to general-base catalysis, since neither pyridine nor imidazole affected the velocity of reaction. If the mechanism which we propose is correct, the second-order rate constants are very high and further underline the kinetic facility of intramolecular reactions.[8]

Of the substrates used in this work, the compound (II; R = Me, $n = 2$) deserves mention. It was obtained by methylation of γ-benzyloxycarbonylamido-L-α-toluene-*p*-sulphonamidobutyric acid in a manner similar to that described for the synthesis of sarcosine.[9] Hydrogenation and esterification completed the synthesis. The γ-benzyloxycarbonylamido-group was proved to be unaffected by methylation, since the acid afforded by hydrogenation yielded nitrogen when treated with nitrous acid in a Van Slyke apparatus. In addition, the ultraviolet spectrum of the *N*-methyl-acid, like that of *N*-toluene-*p*-sulphonylsarcosine, was identical in acid and alkaline solution, whereas that of γ-amino-L-toluene-*p*-sulphonamidobutyric acid exhibited a pronounced hyperchromic effect at high pH values resulting from ionization of the sulphonamido-group. This behaviour has been noticed before in these laboratories and has been used for the spectrophotometric determination of the pK'_a values of derivatives of α-toluene-*p*-sulphonamido-acids.

Experimental

Measurement of Reaction Velocity.—The course of lactamizations was followed at constant pH and temperature by using a Radiometer autotitrator TTT1a fitted with electrodes GK 2021 B and temperature-compensator. The volume of standard sodium hydroxide solution, delivered from an "Agla" micrometer syringe, was recorded automatically as a function of time. The reaction mixture was stirred by a brisk stream of nitrogen which had been freed from carbon dioxide and saturated with water vapour at the temperature of the reaction. On average, about five runs were performed on each substrate at a particular temperature and pH. Velocities of alkali uptake at zero time were computed by the empirical curve-fitting procedure described by Booman and Niemann.[10] The linear regression curve of $1/\sqrt{u_0}$ on $[H^+]$ was computed by the method of weighted least squares with the assumption that errors in measurement of $[H^+]$ were negligible. Computations were performed on the DEUCE digital computer.[11]

Sørensen Titrations.—Kinetic runs at constant temperature and pH values, carried out as above, were interrupted at different times by the addition of an excess of neutral aqueous formaldehyde. The solution was titrated automatically with alkali to the original pH value. The total alkali consumed during the two steps was identical with the Sørensen titre at zero time and at the same temperature and pH.

Table 1.

Initial rates ($10^3 u_0$, mole $l.^{-1}$ $min.^{-1}$) of proton release during lactamization of the esters (II) (standard errors of the means in parentheses).

	R = H, $n = 2$			R = Me, $n = 2$	R = H, $n = 3$	
pH	20°	25°	30°	25°	25°	30°
7·80	0·76(0·01)	1·30(0·03)	4·47(0·04)	1·02(0·01)	1·72(0·05)	5·25(0·11)
7·90	1·22(0·06)	2·31(0·08)	6·62(0·17)	1·45(0·07)	2·98(0·07)	6·94(0·12)
8·00	1·86(0·03)	3·30(0·07)	8·80(0·22)	2·19(0·14)	4·32(0·17)	11·08(0·25)
8·10	2·53(0·04)	4·35(0·07)	11·34(0·41)	3·54(0·08)	6·27(0·08)	16·22(0·16)
8·20	3·75(0·07)	5·31(0·12)	15·89(0·69)	4·70(0·17)	9·30(0·40)	23·09(0·40)
8·30	4·89(0·17)	7·58(0·24)	24·45(0·33)	6·18(0·44)	12·07(0·39)	30·35(0·77)
8·40	7·23(0·20)	9·48(0·36)	27·94(0·93)	8·27(0·53)	21·39(0·80)	44·44(1·32)
8·50	9·98(0·49)	12·70(0·19)	36·45(0·77)	11·32(0·28)	30·43(1·07)	59·77(4·19)

Determination of the Approximate pK_a' *of Methyl* γ-*Amino*-L-α-*toluene*-p-*sulphonamidobutyrate.*—Measured volumes of standard sodium hydroxide were added rapidly from a micrometer syringe to 4-ml. portions of methyl γ-amino-L-α-toluene-*p*-sulphonamidobutyrate (0·01M),

[8] Morawetz and Oreskes, *J. Amer. Chem. Soc.*, 1958, **80**, 2591; Bender, Chow, and Chloupek, *ibid.*, p. 5380; Bender, Chloupek, and Neven, *ibid.*, p. 5384; Bender and Neven, *ibid.*, p. 5388; Bruice and Pandit, *ibid.*, 1960, **82**, 5858.

[9] Cocker and Lapworth, *J.*, 1931, 1894; Cocker, *J.*, 1937, 1693.

[10] Booman and Niemann, *J. Amer. Chem. Soc.*, 1956, **78**, 3642.

[11] Elmore, Kingston, and Shields, unpublished work.

TABLE 2.

pK_a' values for the esters (II) and second-order rate constants ($10^{-4}k$, mole^{-1} l. min.$^{-1}$) for their lactamization (standard deviations in parentheses).

	R = H, n = 2			R = Me, n = 2	R = H, n = 3	
	20°	25°	30°	25°	25°	30°
pK_a'	8·72(0·10)	8·47(0·09)	8·42(0·03)	8·66(0·06)	8·97(0·20)	8·69(0·10)
$10^{-4}k$	1·77(0·18)	1·54(0·13)	3·00(0·09)	1·45(0·09)	4·66(0·93)	5·13(0·52)

and the maximum pH reached was determined. The pK_a' (8·63 ± 0·07) was calculated from the Henderson–Hasselbalch equation by the least-squares method.

Values of K_w in 0·1M-sodium chloride were calculated from the data of Harned and Owen.[12]

*γ-Benzyloxycarbonylamido-*L*-α-*N*-methyltoluene-*p*-sulphonamidobutyric Acid.*—γ-Benzyloxycarbonylamido-α-toluene-*p*-sulphonamido-L-butyric acid (2·0 g.) in aqueous sodium hydroxide was stirred with dimethyl sulphate (0·64 g.) at pH 11 and room temperature for 6 hr. Acidification to pH 4 gave an oil, which crystallized from ethyl acetate–light petroleum (b. p. 40—60°); the *acid* (1·7 g.) had m. p. 139—140°, $[\alpha]_D^{18}$ +10·9° (*c* 2·0 in dimethylformamide) (Found: C, 56·9; H, 6·0; N, 7·0. $C_{20}H_{24}N_2O_6S$ requires C, 57·1; H, 5·8; N, 6·7%).

*γ-Amino-*L*-α-*N*-methyltoluene-*p*-sulphonamidobutyric Acid.*—Hydrogenolysis of γ-benzyloxycarbonylamido-L-α-*N*-methyltoluene-*p*-sulphonamidobutyric acid (1·5 g.) in methanol containing a few drops of acetic acid over palladous oxide afforded the *amino-acid* (0·78 g.). After crystallisation from water, it had m. p. 234—235° (Found: C, 48·5; H, 6·2; N, 9·9. $C_{12}H_{18}N_2O_4S,\frac{1}{2}H_2O$ requires C, 48·8; H, 6·5; N, 9·5%). The compound ran as a single spot on a paper chromatogram irrigated with butan-1-ol–acetic acid–water (4 : 1 : 5); the R_F value was considerably greater than that of γ-amino-L-α-toluene-*p*-sulphonamidobutyric acid. In 0·01N-hydrochloric acid it had $\lambda_{max.}$ 274 (ε 360) and 263 mμ (ε 780), unchanged in N-sodium hydroxide. γ-Amino-L-α-toluene-*p*-sulphonamidobutyric acid in 0·01N-hydrochloric acid had $\lambda_{max.}$ 275 (ε 370) and 262 mμ (ε 750), but in N-sodium hydroxide 274 (ε 1200) and 262—263 mμ (ε 2800).

*Methyl γ-Amino-*L*-α-*N*-methyltoluene-*p*-sulphonamidobutyrate Hydrochloride.*—Treatment of γ-amino-L-α-*N*-methyltoluene-*p*-sulphonamidobutyric acid (0·5 g.) with methanolic hydrogen chloride yielded the *ester hydrochloride* (0·45 g.), m. p. 184—185° (Found: C, 44·4; H, 6·3; N, 8·3. $C_{13}H_{21}ClN_2O_4S,H_2O$ requires C, 44·0; H, 6·5; N, 7·9%).

3-N-*Methyltoluene-*p*-sulphonamido-*L*-pyrrolid-2-one.*—This compound (62%) was isolated from the pooled solutions obtained from kinetic runs with methyl γ-amino-L-α-*N*-methyltoluene-*p*-sulphonamidobutyrate. In each case, the reaction was allowed to proceed until alkali uptake ceased. After recrystallization from dioxan–methanol, the *lactam* had m. p. 215—216° (Found: C, 53·9; H, 5·7; N, 10·0. $C_{12}H_{16}N_2O_3S$ requires C, 53·7; H, 6·0; N, 10·4%).

3-Toluene-*p*-sulphonamido-L-pyrrolid-2-one (60%; m. p. 205—206°) and -piperid-2-one (82%; m. p. 185—186°) were similarly obtained. Mixed m. p.s with authentic specimens [2a] were undepressed.

*Methyl γ-Benzyloxycarbonylamido-*L*-α-benzamidobutyrate.*—γ-Benzyloxycarbonylamido-L-α-benzamidobutyric acid [13] (2 g.) was converted into the above *ester* (1·6 g.) by treatment with methanolic hydrogen chloride. Recrystallized from water, it had m. p. 105° (Found: C, 65·2; H, 5·9; N, 7·2. $C_{20}H_{22}N_2O_5$ requires C, 64·8; H, 6·0; N, 7·6%).

*Methyl γ-Amino-*L*-α-benzamidobutyrate Hydrobromide.*—The foregoing ester (1·5 g.) was treated with hydrogen bromide in acetic acid (1·5 ml. of 50% w/w) at 70° for 30 min. Addition of ether gave an oil which crystallized from methanol–ether to give an *octahydrate* of the required *compound* (0·81 g.) having m. p. 184—185° (Found: C, 31·3; H, 7·4; N, 6·2. $C_{12}H_{17}BrN_2O_3,8H_2O$ requires C, 31·3; H, 7·2; N, 6·1%).

We thank Professor D. C. Harrison for his interest and encouragement. We are grateful to Imperial Chemical Industries Limited and the Chemical Society for the loan of apparatus. This work was supported by the U.S. Department of the Army through its European Research Office.

DEPARTMENT OF BIOCHEMISTRY,
QUEEN'S UNIVERSITY, BELFAST. [*Received, January 30th*, 1962.]

[12] Harned and Owen, "The Physical Chemistry of Electrolytic Solutions," Reinhold Publ. Corp., New York, 1943, p. 480.

[13] Takagi, Tsukatani, and Hayashi, *Chem. and Pharm. Bull.* (*Japan*), 1959, **7**, 619.