Proton Ionisation Constants and Kinetics of Base Hydrolysis of Some α-Amino-acid Esters in Aqueous Solution. Part III.[1] Hydrolysis and Intramolecular Aminolysis of αω-Diamino-acid Methyl Esters

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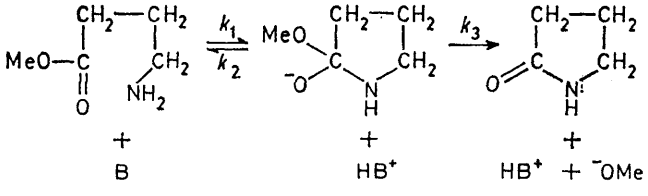
The proton ionisation constants, pK_{a1}^{T} and pK_{a2}^{T}, for the ammonium ionisation of the αω-diamino-acids, $\overset{+}{N}H_3[CH_2]_nCH(\overset{+}{N}H_3)CO_2^-$ have been determined at 25 °C and $I = 0{\cdot}1$ M for 2,3-diaminopropionic acid ($n = 1$), 2,4-diaminobutyric acid ($n = 2$), ornithine ($n = 3$), and lysine ($n = 4$). The corresponding constants for the methyl esters have also been obtained at 25 °C and $I = 0{\cdot}1$ M. The temperature dependence of the ionisation constants has been studied for 2,3-diaminopropionic acid, lysine, and their methyl esters. The thermodynamic parameters ΔH^0 and ΔS^0_{298} for the acids and esters are compared.

The base hydrolysis of the methyl esters of 4-aminobutyric acid, 2,3-diaminopropionic acid, 2,4-diaminobutyric acid, ornithine, and lysine have been studied at 25 °C and $I = 0{\cdot}1$ M. Intramolecular aminolysis (lactamisation) is an important reaction pathway for the methyl esters of 4-aminobutyric acid, 2,4-diaminobutyric acid, and ornithine in basic solution. Simple base hydrolysis occurs with the methyl esters of 2,3-diaminopropionic acid and lysine.

In previous papers [1,2] we have discussed the base hydrolysis of a series of α-amino-acid esters. We have now extended this work to a number of αω-diamino-acid esters. In order to interpret the kinetic data, the ionisation constants of the esters and the corresponding acids have also been determined.

In the case of the diaminoesters and 4-aminobutyric acid, intramolecular aminolysis (lactamisation) may also occur due to the presence of a neighbouring amino-group, and this pathway (Scheme 1) competes with base



SCHEME 1

hydrolysis. Martin *et al.*[3] have studied intramolecular aminolysis in the reactions of the ethyl esters of glutamic acid, glutamine, and 4-aminobutyric acid in basic solution. Similar observations have been made by Pilbrant [4] who found that in basic solution the reactions of asparagine ethyl ester and *N*-(2-aminoethyl)glycine ethyl ester proceed by two pathways, ring closure to aspartimide or piperazin-2-one, respectively, and hydrolysis to asparagine or *N*-(2-aminoethyl)glycine, respectively. Significantly, copper(II) promotes the hydrolysis of both substrates and prevents ring closure. Martin *et al.* suggested the mechanism shown in Scheme 1 for the ring closure of the ethyl ester of 4-aminobutyric acid. The reaction is first order in hydroxide ion when no other base is present. The reaction is also general base catalysed. Thus at constant pH and ionic strength, initial rates increase with buffer concentration, but eventually level off at high buffer concentrations. This effect is probably due to a change in rate-determining step. Martin *et al.* considered the reaction to involve rate-limiting general base catalysed proton removal from the amine nucleophile at low buffer concentrations, with rate-limiting decomposition of the resultant tetrahedral addition intermediate occurring at high buffer concentrations.

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[1] R. W. Hay and L. J. Porter, *J. Chem. Soc.* (*B*), 1967, 1261, is considered as Part II.

[2] R. W. Hay and P. J. Morris, *J. Chem. Soc.* (*B*), 1970, 1577.

[3] R. B. Martin, A. Parcell, and R. I. Hedrick, *J. Amer. Chem. Soc.*, 1964, **86**, 2406.

[4] A. Pilbrant, *Acta Pharm. Suecica*, 1969, **6**, 469.

RESULTS AND DISCUSSION

Proton Ionisation Constants.—The proton ionisation equilibria of a dibasic amino-acid may be represented by processes (1) and (2) and the associated ionisation constants may be defined by equations (3) and (4) where

$$H_3\overset{+}{N}[CH_2]_nCH(\overset{+}{N}H_3)CO_2^- \underset{(AH_2^+)}{\rightleftharpoons} H_3\overset{+}{N}[CH_2]_nCH(NH_2)CO_2^- + H^+ \quad (1)$$

$$H_3\overset{+}{N}[CH_2]_nCH(NH_2)CO_2^- \underset{(AH)}{\rightleftharpoons} H_2N[CH_2]_nCH(NH_2)CO_2^- + H^+ \quad (2)$$

$$K_{a1}^T = [AH]\{H^+\}/[AH_2^+]y_1 \quad (3)$$

$$K_{a2}^T = [A^-]y_1\{H^+\}/[AH] \quad (4)$$

$\{H^+\}$ is the activity of the hydrogen ion as measured by the glass electrode ($pH = -\log_{10}\{H^+\}$) and y_1 is the molar activity coefficient for a univalent ion which may be calculated from the Davies equation;[5] K_{a1}^T and K_{a2}^T are thermodynamic ionisation constants. Values of pK_{a1}^T and pK_{a2}^T are shown in Table 1. The α-$\overset{+}{N}H_3$ is the first ammonium group to deprotonate since it experiences a larger $-I$ effect from the carboxylate group than does the more remote ω-$\overset{+}{N}H_3$. Deprotonation of the α-$\overset{+}{N}H_3$ is subject to the combined $-I$ effects of the carboxylate group (moderate, $\sigma_I = 0.06$)[6] and the ω-$\overset{+}{N}H_3$ (strong, *e.g.* $\sigma_I CH_2\overset{+}{N}H_3 = 0.36$) groups. Consequently the pK_{a1}^T values are considerably lower than those of the α-amino-acid analogues $Me[CH_2]_{n-1}CH(\overset{+}{N}H_3)CO_2^-$ (Table 1) but rise steadily as the terminal amino-group is moved along the carbon chain.

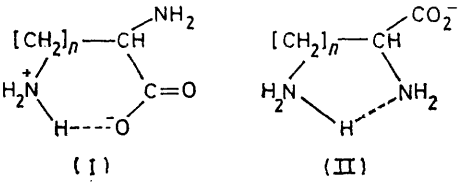
TABLE 1

Proton ionisation constants, pK_{a1}^T and pK_{a2}^T of $\alpha\omega$-diamino-acids, $\overset{+}{N}H_3[CH_2]_nCH(\overset{+}{N}H_3)CO_2^-$, at 25 °C and $I = 0.1$M

Diamino-acid	n	pK_{a1}^T *	pK_{a2}^T
2,3-Diaminopropionic	1	6·674 (9·916)	9·623
2,4-Diaminobutyric	2	8·169 (9·852)	10·424
Ornithine	3	8·739 (9·869)	10·770
Lysine	4	9·116 (9·879)	10·902

* Values of pK_{a1}^T refer to the ionisation of the α-$\overset{+}{N}H_3$ group. Figures in parentheses are the corresponding constants for the α-$\overset{+}{N}H_3$ ionisation in the α-amino-acids $Me[CH_2]_{n-1}CH(\overset{+}{N}H_3)CO_2^-$.

The ω-$\overset{+}{N}H_3$ group is subject to the combined $-I$ effects of the α-NH_2 and CO_2^- groups (both moderate). Thus pK_{a2}^T is much larger than pK_{a1}^T because the weaker electron-withdrawing α-NH_2 replaces the ω-$\overset{+}{N}H_3$ group and because the ω-$\overset{+}{N}H_3$ is more remote from the CO_2^- group than is the α-$\overset{+}{N}H_3$. As the ω-$\overset{+}{N}H_3$ is withdrawn from the β-position, pK_{a2}^T increases, thus reflecting the decreasing $-I$ effect experienced by the terminal ammonium group. When $n = 4$ (lysine), pK_{a2}^T has become 'anomalous' in the sense that the pK is greater than the value expected for a primary aliphatic amine[7] at 25 °C (10·9, *cf.* 10·7). The abnormally low tendency for the ε-$\overset{+}{N}H_3$ of lysine to deprotonate indicates stabilisation of the zwitterionic species. This stabilisation may be due to intramolecular hydrogen bonding [as in (I) and (II), $n = 4$] or to internal 'salt-bridge' formation. Presumably such effects also influence the pK_{a2}^T values of the lower homologues, 2,3-diaminopropionic acid ($n = 1$) and 2,4-diaminobutyric acid ($n = 2$) where the more thermodynamically stable rings are possible.[8]



(I) (II)

$$H_3\overset{+}{N}[CH_2]_nCH(\overset{+}{N}H_3)CO_2Me \underset{(EH_2^{2+})}{\rightleftharpoons} H_3\overset{+}{N}[CH_2]_nCH(NH_2)CO_2Me + H^+ \quad (5)$$

(EH$^+$)

$$H_3\overset{+}{N}[CH_2]_nCH(NH_2)CO_2Me \underset{(EH^+)}{\rightleftharpoons} H_2N[CH_2]_nCH(NH_2)CO_2Me + H^+ \quad (6)$$

(E)

In the case of the methyl esters of the $\alpha\omega$-diamino-acids, the proton ionisation equilibria may be represented by processes (5) and (6) and the associated ionisation constants may be defined by equations (7) and (8),

$$K_{a1}^T = [EH^+]y_1\{H^+\}/[EH_2^{2+}]y_2 \quad (7)$$

$$K_{a2}^T = [E]\{H^+\}/[EH^+]y_1 \quad (8)$$

where y_2 is the molar activity coefficient of a bivalent ion and the other symbols are as previously defined.

TABLE 2

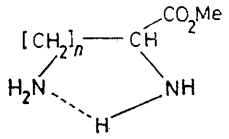
Proton ionisation constants, pK_{a1}^T and pK_{a2}^T, for the methyl esters of $\alpha\omega$-diamino-acids, $\overset{+}{N}H_3[CH_2]_nCH(\overset{+}{N}H_3)CO_2Me$, at 25 °C and $I = 0.1$M

Diamino-ester	n	pK_{a1}^T	pK_{a2}^T
2,3-Diaminopropionic	1	4·412	8·250
2,4-Diaminobutyric	2	5·868	8·655
Ornithine	3	6·501	8·323
Lysine	4	6·964	10·251

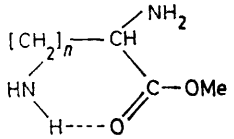
The proton ionisation constants obtained for the methyl esters at 25 °C and $I = 0.1$M are summarised in Table 2.

[5] C. W. Davies, *J. Chem. Soc.*, 1938, 2093.
[6] M. Charton, *J. Org. Chem.*, 1964, **29**, 1222.
[7] J. Clark and D. D. Perrin, *Quart. Rev.*, 1964, **18**, 295.
[8] A. J. Gero, *J. Amer. Chem. Soc.*, 1954, **76**, 5159.

The ionisation equilibria are controlled by the same factors previously discussed for the parent acids. The carboxylate group has now been replaced by the stronger electron-withdrawing methoxycarbonyl group (σ_I = 0·34 compared with σ_I = 0·06 for $CO^-{}_2$) and, as expected, the $pK_{a1}{}^T$ values are lowered by about 2·1—2·3 pK units compared with the corresponding acids. The analogous lowering of the $pK_{a2}{}^T$ values is expected to become smaller as the ω-$\overset{+}{N}H_3$ is withdrawn from the $-I$ influence of the CO_2Me group, *i.e.* as the difference in the inductive effects of the CO_2Me and CO_2^- groups becomes of minor importance. A comparison of the constants for the esters and acids in the 2,3-diaminopropionate and lysinate systems shows the expected trend, but the methyl esters of 2,4-diaminobutyric acid and ornithine have unexpectedly low $pK_{a2}{}^T$ values. If pK_a values are calculated using the inductive effects of the substituents as outlined by Clark and Perrin,[7] the number of α-amino-acids,[1] (*ca.* 10·5 kcal mol^{-1}) but the ΔH^0 values for the ω-$\overset{+}{N}H_3$ groups show a wide variation (9·40 and 13·05 kcal mol^{-1} for 2,3-diaminopropionic acid and lysine, respectively). As ΔH^0 essentially measures the $R\overset{+}{N}H_2$–H bond strength, the β-$\overset{+}{N}$–H bond of 2,3-diaminopropionic acid is similar in strength to that of the α-$\overset{+}{N}$–H bond; however, the ε-$\overset{+}{N}$–H bond of lysine is considerably stronger. As previously noted, this effect may be due to the formation of 8- or 9-membered hydrogen-bonded rings or to salt-bridge formation, which stabilises the zwitterionic molecule.



(III)



(IV)

TABLE 3

Temperature dependence of $pK_{a1}{}^T$ and $pK_{a2}{}^T$ for 2,3-diaminopropionic acid, lysine, and their methyl esters

	25 °C	37 °C	50 °C	ΔH^0/kcal mol^{-1}	ΔS^0_{298}/cal K^{-1} mol^{-1}	ΔG^0/kcal mol^{-1}
Diamino-acids						
2,3-Diaminopropionic						
$pK_{a1}{}^T$ (α-$\overset{+}{N}H_3$)	6·674	6·420	6·132	9·56 ± 0·61	+1·5	9·10
$pK_{a2}{}^T$ (β-$\overset{+}{N}H_3$)	9·623	9·366	9·090	9·40 ± 0·34	−12·5	13·13
Lysine						
$pK_{a1}{}^T$ (α-$\overset{+}{N}H_3$)	9·116	8·801	8·510	10·68 ± 0·42	−5·9	12·43
$pK_{a2}{}^T$ (ε-$\overset{+}{N}H_3$)	10·902	10·546	10·162	13·05 ± 0·50	−6·1	14·87
Methyl esters						
2,3-Diaminopropionic						
$pK_{a1}{}^T$ (α-$\overset{+}{N}H_3$)	4·412	4·176	3·897	9·08 ± 0·76	+10·3	6·02
$pK_{a2}{}^T$ (β-$\overset{+}{N}H_3$)	8·250	7·959	7·632	10·90 ± 0·64	−1·2	11·25
Lysine						
$pK_{a1}{}^T$ (α-$\overset{+}{N}H_3$)	6·964	6·658	6·304	11·64 ± 0·85	+7·2	9·50
$pK_{a2}{}^T$ (ε-$\overset{+}{N}H_3$)	10·251	9·898	9·520	12·89 ± 0·45	−3·7	13·98

following constants are obtained (calculated values followed by observed in parentheses): methyl 2,3-diaminopropionate pK_{a1} 4·2 (4·4), and pK_{a2} 8·7 (8·3); methyl 2,4-diaminobutyrate 6·0 (5·9) and 9·8 (8·7); methyl ornithinate 6·9 (6·5) and 10·3 (8·3); methyl lysinate 7·4 (7·0) and 10·5 (10·3). The low pK values of the ω-$\overset{+}{N}H_3$ group for the methyl esters of 2,4-diaminobutyric acid and ornithine may be due to stabilisation of the neutral ester by intramolecular hydrogen bonding [(III) and (IV), n = 2 and 3], although in the absence of thermodynamic data for the ionisations, no definite conclusions can be drawn.

The temperature dependence of $pK_{a1}{}^T$ and $pK_{a2}{}^T$ were determined for 2,3-diaminopropionic acid, lysine, and their methyl esters (Table 3). For the diamino-acids, the ΔH^0 values of the α-$\overset{+}{N}H_3$ group are fairly constant and similar to those previously determined for a

The ΔS^0_{298} values of both the α- and ω-$\overset{+}{N}H_3$ groups are very different from those of the α-amino-acids (*ca.* −10 cal K^{-1} mol^{-1}) possibly because the introduction of the second amino-group has affected the change in solvent ordering which normally occurs on deprotonation of a monoamino-acid.

Kinetics of Base Hydrolysis.—For base hydrolysis of a diamino-ester there are three potentially hydrolysable species, E, EH^+, and EH_2^{2+}. The rate expression thus takes the form (9) where $[Ester]_T$ is the total concentration of ester and k_{obs} is the observed pseudo-first-order rate constant at constant pH.

$$-d[Ester]_T/dt = k_{EH_2^{2+}}[EH_2^{2+}][OH^-] + k_{EH^+}[EH^+][OH^-] + k_E[E][OH^-] = k_{obs}([E] + [EH^+] + [EH_2^{2+}]) \quad (9)$$

In general, only the hydrolysis of the species E and EH^+ was important as pK_{a1}^T was well below the minimum pH used for the kinetic studies. A reliable value of k_{EH^+} was readily obtained, as pK_{a2}^T was high enough for the net reaction rate to be sufficiently rapid to allow studies at pH values near pK_{a2}^T, *i.e.* where a considerable proportion of the ester is in the monoprotonated form. Such studies are difficult with the monoamino-acid esters [2] and only approximate values of k_{EH^+} can be obtained.

It was immediately apparent from the pH-stat measurements that base hydrolysis of the methyl esters of 4-aminobutyric acid, 2,4-diaminobutyric acid, and ornithine was unusual in the following respects. (*a*) The reactions occurred rapidly at low pH. Values of $k_{obs}/[OH^-]$ were of the order of 10^2—10^3 (methyl 4-aminobutyrate), 10^4 (methyl 2,4-diaminobutyrate) and 10^5 l mol^{-1} min^{-1} (methyl ornithinate), at 25 °C and I = 0·1M. (*b*) The values of $k_{obs}/[OH^-]$ decreased or showed little variation as the pH was lowered, in marked contrast to the behaviour of the 'normal' amino-acid esters. It is apparent that these effects are due to the concurrent lactamisation reaction. The two classes of esters are therefore discussed separately.

Base Hydrolysis of the Methyl Esters of 2,3-Diaminopropionic Acid and Lysine.—The pH-dependence of the k_{obs} values for the two esters are shown in Table 4. Analysis of the results obtained for methyl 2,3-diaminopropionate was carried out as described for the monoamino-acid esters,[1,2] and values of k_E = 43·8 and k_{EH^+} = 3440 l mol^{-1} min^{-1} at 25 °C and I = 0·1M were obtained. The temperature dependence of the base hydrolysis of this ester was also examined at the additional temperatures of 37 and 50 °C. Table 5 shows the resulting thermodynamic parameters. In the case of lysine methyl ester there was a possible contribution to the reaction from EH_2^{2+} (in addition to E and EH^+) in the pH range studied (9·00—11·20 at 25 °C). For this ester the value of pK_{a1}^T = 6·964 at 25 °C is higher than for the other diamino-esters. Plots of $[H^+]$ *versus* $(k_{obs}/[OH^-])(K_{a2}^T + [H^+])$ were linear [1,2] at high pH and of slope k_{EH^+} and intercept $k_E K_{a2}^T$. The plots curved upwards as the pH was lowered below 10·20 due to the contribution of EH_2^{2+}. From the linear portion (Figure) values of k_E = 27·6 and k_{EH^+} = 75·4 l mol^{-1} min^{-1} at I = 0·1M and 25 °C were calculated.

From equation (9), equation (10) is obtained and from

$$k_{EH_2^{2+}}[EH_2^{2+}][OH^-] = k_{obs}([E] + [EH^+] + [EH_2^{2+}]) - k_E[E][OH^-] - k_{EH^+}[EH^+][OH^-] \quad (10)$$

the definitions of K_{a1}^T and K_{a2}^T, equations (11) and (12) are derived. By substituting equations (11) and (12) into equation (10) it is possible to derive an expression

$$[EH_2^{2+}] = (y_1^2[EH^+][H^+])/y_2K_{a1}^T \quad (11)$$

$$[E] = (K_{a2}^T[EH^+])/[H^+] \quad (12)$$

relating $k_{EH_2^{2+}}$ to known parameters. The values of $k_{EH_2^{2+}}$ for lysine methyl ester calculated in this way (in l mol^{-1} min^{-1} at I = 0·1M and 25°) are 3820 (pH 9·40), 4862 (pH 9·20), and 4556 (pH 9·00), the average being 4410 ± 590. The approximate nature of this constant

TABLE 4

Base hydrolysis of methyl 2,3-diaminopropionate and methyl lysinate at I = 0·1M

	pH	10^2k_{obs}/min^{-1}	$k_{obs}/[OH^-]$/l mol^{-1} min^{-1}
Methyl 2,3-diaminopropionate			
25 °C	11·30	12·59	48·30
	11·20	10·23	49·41
	11·10	8·390	51·01
	11·00	6·849	52·45
	10·90	5·527	53·26
	10·80	4·578	55·54
	10·70	3·958	60·44
	10·60	3·351	64·43
	10·50	2·801	67·79
	10·40	2·425	73·89
	10·30	2·106	80·79
	10·20	1·885	91·04
	10·00	1·532	117·3
	9·80	1·319	116·0
	9·60	1·174	225·7
	9·40	1·042	317·5
	9·20	0·9698	468·4
	9·00	0·8807	673·8
	8·80	0·7989	969·2
	8·60	0·6718	1292
	8·40	0·4318	1674
	8·20	0·4318	2085
	8·00	0·3286	2514
37 °C	10·50	11·40	115·8
	10·30	8·061	129·8
	10·10	5·949	151·8
	9·70	3·846	246·4
	9·30	2·909	468·3
	9·10	2·775	770·9
	8·90	2·450	990·7
50 °C	9·508	10·48	452·7
	9·108	7·669	831·9
	8·708	6·308	1719
	8·509	5·776	2495
	8·310	5·129	3494
	8·108	4·484	4864
	7·910	3·249	6417
Methyl lysinate			
25 °C	11·20	7·013	33·86
	11·00	4·887	37·39
	10·80	3·358	40·74
	10·60	2·303	44·28
	10·40	1·665	50·73
	10·20	1·152	55·62
	10·00	0·8146	62·33
	9·80	0·5889	71·44
	9·60	0·4127	79·35
	9·40	0·3199	97·47
	9·20	0·2658	128·3
	9·00	0·2062	157·8
37 °C	10·70	11·69	74·89
	10·50	7·819	79·41
	10·30	5·455	87·81
	10·20	4·470	90·60
	10·10	3·839	97·63
	9·90	2·764	111·8
50 °C	10·109	14·18	153·5
	10·010	11·60	157·7
	9·910	9·221	169·8
	9·809	8·259	178·4
	9·609	5·941	203·3
	9·509	5·053	217·8
	9·409	4·307	233·6
	9·209	3·109	267·3

TABLE 5

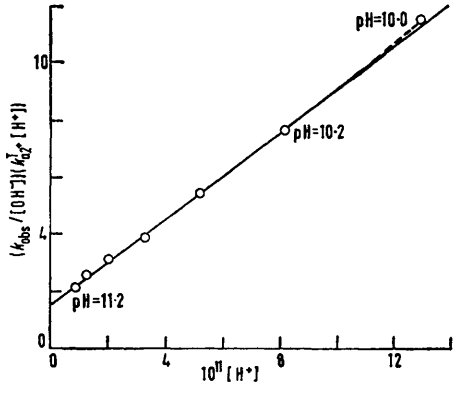
Rate constants and thermodynamic activation parameters for the base hydrolysis of methyl 2,3-diaminopropionate, methyl lysinate, and methyl 4-aminobutyrate at $I = 0{\cdot}1$M

t/°C	k_E *	k_{EH^+} *	$k_{EH_2^{2+}}$ *	$\Delta H^{\ddagger}$/kcal mol^{-1}	$\Delta S^{\ddagger}_{298}$/cal K^{-1} mol^{-1}
Methyl 2,3-diaminopropionate					
25	43·8	3440			
37	93·7	7050		11·6 ± 0·5 (E)	−20·3 ± 1·7 (E)
50	214·3	15,440		10·9 ± 0·5 (EH^+)	−13·9 ± 1·9 (EH^+)
Methyl lysinate					
25	27·6	75·4	4410	11·0 ± 0·3 (E)	−23·1 ± 1·0 (E)
37	60·1	137·5	9800	8·7 ± 0·1 (EH^+)	−29·0 ± 0·1 (EH^+)
50	125·9	251·8	24,000	12·4 ± 0·7 (EH_2^{2+})	−8·4 ± 2·5 (EH_2^{2+})
Methyl 4-aminoburyrate					
25	1758	125·0			
37	2613	216·0		5·8 ± 0·4 (E)	−32·2 ± 1·5 (E)
50	4069	395·8		8·2 ± 0·5 (EH^+)	−29·4 ± 1·5 (EH^+)

* In units of 1 mol^{-1} min^{-1}.

arises because the concentration of EH_2^{2+} is very small, even at pH 9·00. Additional measurements were also carried out at 37 and 50 °C and the derived activation parameters for the species E, EH^+, and EH_2^{2+} are listed in Table 5.

Base Hydrolysis of the Methyl Esters of 4-Aminobutyric Acid, 2,4-Diaminobutyric Acid, and Ornithine.—The pH dependence of the k_{obs} values for the three esters is shown



Analysis of the kinetic data for the base hydrolysis of methyl lysinate at 25 °C and $I = 0{\cdot}1$M

in Table 6. Neglecting methyl ornithinate, these results were analysed by plotting [H$^+$] *versus* $(k_{obs}/[OH^-]) \times (K_{a2}^T + [H^+])$, K_{a2}^T being replaced by K_a^T for the ammonium ionisation in the case of methyl 4-aminobutyrate. Plots of this type were linear indicating that E and EH^+ were probably the only species undergoing reaction. Values of k_E and k_{EH^+} obtained from the plots are summarised in Table 7, while Table 5 gives the temperature dependence of the rate constants for methyl 4-aminobutyrate. That E and EH^+ are both kinetically important is expected for methyl 4-aminobutyrate as it has pK_a^T values [2] of 9·839 (25 °C), 9·461 (37 °C), and 9·091 (50 °C) for the ammonium ionisation, while the hydrolysis was studied over the pH range

TABLE 6

The pH dependence of k_{obs} for the base hydrolysis of the methyl esters of 4-aminobutyric acid, 2,4-diaminobutyric acid, and ornithine at $I = 0{\cdot}1$M

Methyl ester	pH	$10^2 k_{obs}$/min^{-1}	$k_{obs}/[OH^-]$/l mol^{-1} min^{-1}
25 °C			
4-Amino-	10·00	12·90	987·0
butyrate	9·90	9·258	891·9
	9·80	6·656	807·5
	9·70	4·652	710·3
	9·60	3·282	631·0
	9·60	3·282	631·0
	9·50	2·283	552·5
	9·40	1·579	481·1
	9·30	1·102	422·7
	9·20	0·7597	366·7
	9·10	0·5433	330·3
2,4-Diamino-	8·50	10·71	26,550
butyrate	8·40	8·701	26,510
	8·30	6·964	26,710
	8·10	4·415	26,840
	8·00	3·521	26,940
	7·80	2·242	27,200
	7·60	1·415	27,210
	7·40	0·9454	28,810
Ornithine	7·70	9·447	144,300
	7·50	5·833	141,200
	7·30	5·833	138,200
	7·20	2·796	135,000
	7·10	2·156	131,100
	6·90	1·250	120,400
37 °C			
4-Amino-	9·405	9·191	1162
butyrate	9·405	9·191	1165
	9·305	6·610	1052
	9·305	6·711	1068
	9·205	4·650	931·7
	9·105	3·243	817·9
	9·105	3·282	827·7
	9·004	2·268	721·8
	8·905	1·563	624·7
50 °C			
4-Amino-	8·908	9·647	1659
butyrate	8·807	6·589	1430
	8·707	4·629	1264
	8·608	3·305	1134
	8·507	2·276	985·3
	8·407	1·667	908·4

8·4—10·0. The similar result obtained for methyl 2,4-diaminobutyrate ($pK_{a1}^{T} = 5·868$, $pK_{a2}^{T} = 8·655$ at 25 °C) indicates that the pH range used for the kinetics (7·40—8·50) is too high for the concentration of EH_2^{2+} to be kinetically significant.

TABLE 7

Values of k_E and k_{EH^+} for the methyl esters of 4-aminobutyric acid, 2,4-diaminobutyric acid, and ornithine at 25 °C and $I = 0·1$M

Ester	k_E/l mol^{-1} min^{-1}	k_{EH^+}/l mol^{-1} min^{-1}
Methyl 4-aminobutyrate	1758	125·0
Methyl 2,4-diaminoburyrate	2532	2737
Methyl ornithinate *	191,000	138,000

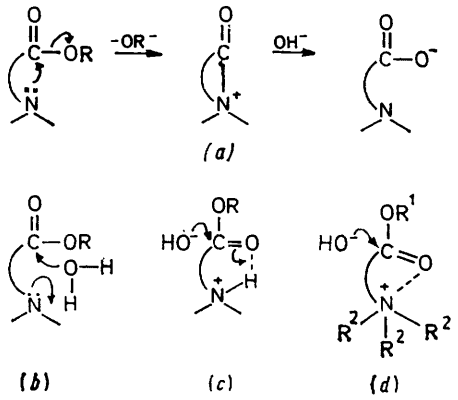
* $k_{EH_2^{2+}} = 125{,}000$ l mol^{-1} min^{-1}.

Methyl ornithinate has pK_a^{T} values of 6·501 and 8·323 (at 25 °C) and the reaction was studied in the pH range 6·90—7·70. Analysis of the results indicated that EH_2^{2+} was undergoing reaction in addition to the species E and EH^+. The equation obtained by substituting equations (11) and (12) in (10) involves only k_E, k_{EH^+}, and $k_{EH_2^{2+}}$ as unknowns. There are 20 possible distinct combinations of the six experimental k_{obs} values taken three at a time without repetition. Solution for k_E, k_{EH^+}, and $k_{EH_2^{2+}}$, neglecting cases involving combinations of low accuracy (*i.e.* where small differences between large numbers occurred), gave $k_E = 1·91 \times 10^5$, $k_{EH^+} = 1·38 \times 10^5$, and $k_{EH_2^{2+}} = 1·25 \times 10^5$ l mol^{-1} min^{-1} at 25 °C. A plot of $[H^+]$ *versus* $k_{obs}/[OH^-](K_{a2}^{T} + [H^+] + y_1^2[H^+]^2/K_{a1}^{T} y_2)$ yielded a curve, approximate 'extrapolation' of which to $[H^+] = 0$ gave $k_E = 2·1 \times 10^5$ l mol^{-1} min^{-1}. A parabolic plot is expected if all three forms of the ester are reacting. A plot of $[H^+]$ *versus* $k_{obs}/[OH^-](K_{a2}^{T} + [H^+])$ showed fair linearity at the higher pH values and gave $k_E = 2·3 \times 10^5$ and $k_{EH^+} = 1·3 \times 10^5$ l mol^{-1} min^{-1}. The results obtained by three methods are in reasonable agreement and Table 7 lists the values obtained by the solution of simultaneous equations.

Table 7 shows that the neutral ester E hydrolyses as fast or faster than the monoprotonated species EH^+. This result is contrary to usual observations [1,2] and suggests that E is reacting by a different pathway. Examination of the products (see Experimental), showed that while base hydrolysis of methyl 2,3-diaminopropionate and methyl lysinate gave solely the respective parent amino-acids, hydrolysis of methyl 4-aminobutyrate, methyl 2,4-diaminobutyrate, and methyl ornithinate gave two products, the parent amino-acid and the corresponding lactam.

The k_{EH^+} values obtained for the αω-diamino-acid esters (Tables 5 and 7) show (with the exception of ornithine methyl ester) the expected regular decrease as the ω-$\overset{+}{N}H_3$ is moved stepwise from the β- to the ε-position. This evidence and the absence of lactamisation for methyl 2,3-diaminopropionate and methyl lysinate, indicates that hydrolysis of the EH^+ form of methyl 2,4-diaminobutyrate occurs 'normally' to give the amino-acid and no lactam. The value of k_{EH^+} for methyl 4-aminobutyrate is also of the expected size,[1,2] again indicating no lactamisation *via* EH^+. Methyl ornithinate has a k_{EH^+} value some 150 times larger than expected, a result discussed in detail later.

The k_E values for the diamino-ester series show that hydrolysis of the neutral forms of methyl 2,4-diaminobutyrate and methyl ornithinate occur, respectively some 10^2 and 10^4 times more rapidly than expected. Such high rate constants suggests facilitation of the reaction by the neighbouring amino-group. A similar argument also applies to methyl 4-aminobutyrate where k_E is some 300 times larger than expected.[1,2] The mechanisms available to the neighbouring amino-group for assisting ester hydrolysis are shown in Scheme 2 and include (*a*) intramolecular nucleophilic catalysis, (*b*) intramolecular general base catalysis, (*c*) intramolecular general acid–specific base catalysis, and (*d*) electrostatic

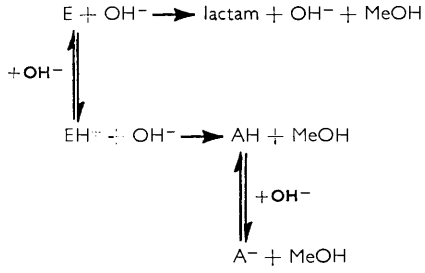


SCHEME 2

facilitation by a quaternary ammonium group. Formation of the lactam, which is stable under the reaction conditions, is consistent with mechanism (*a*) for the hydrolysis of the E forms of methyl 4-aminobutyrate, methyl 2,4-diaminobutyrate, and methyl ornithinate. The large k_{EH^+} value for the latter ester presumably indicates hydrolysis of the EH^+ form is also assisted, perhaps by a mechanism of type (*c*). Such facilitation is unimportant for the other two esters, suggesting a preference for an 8-membered ring involving hydrogen bonding, over a 7-membered one. The k_E values reported for these three esters are the sum of the rate constants for lactamisation and for base hydrolysis, (*i.e.* $k_E = k_{lact} + k_{hyd}$). For the diamino-esters the values of k_{hyd} probably lie within the limits 43·8—27·6 l mol^{-1} min^{-1} (at 25 °C) determined for methyl 2,3-diaminopropionate and methyl lysinate respectively. This estimate assumes that rate-acclerating factors (such as intramolecular hydrogen-bonding), which may vary along the series of diamino-esters, are relatively unimportant. Similarly k_{hyd} for methyl 4-aminobutyrate is probably *ca.* 6 l mol^{-1} min^{-1} at 25 °C (the value for

β-alanine methyl ester is $k_E = 8{\cdot}14$ l mol^{-1} min^{-1} at 25°).[2] Clearly, the prime reaction pathway is lactamisation for which approximate rate constants are (in l mol^{-1} min^{-1} at 25° and $I = 0{\cdot}1$M): $1{\cdot}7 \times 10^3$ (methyl 4-aminobutyrate), $2{\cdot}5 \times 10^3$ (methyl 2,4-diaminobutyrate), and $2{\cdot}3 \times 10^5$ (methyl ornithinate). If ring closure is rate determining, as seems probable (see below), then formation of a 6-membered ring (as in the methyl ornithinate case) occurs *ca.* 100 times more rapidly than formation of a 5-membered ring (the two butyrates); both this order and its reverse have been observed in other reactions of carboxylic acid derivatives, involving nucleophilic participation by amino-groups.[9]

A more detailed mechanism for lactamisation must include the following features. (*a*) The rate law is of the form $k_{lact}[E][OH^-]$. (*b*) Overall, there is no OH^- consumed in forming the lactam. Feature (*b*) arises from the experimentally observed values of the total volume of sodium hydroxide consumed for



SCHEME 3

complete reaction of the ester. Assuming the reaction Scheme (3), equation (13) is obtained, while for

$$\alpha = \left(\frac{B}{B + C}\right)\left(1 + \frac{1/B - [OH^-]}{1/C + [OH^-]} + \frac{[OH^-]}{1/D + [OH^-]}\right) \quad (13)$$

the analogous scheme in which lactam formation and regeneration of OH^- are replaced by the formation of A^- and MeOH, we get equation (14) where α is the

$$\alpha = 1 + 1/(1 - C[OH^-]) - 1/(1 + D[OH^-]) \quad (14)$$

number of mol of OH^- consumed per mol of ester reacted, $B = k_{EH^+}/k_E$, $C = K_a^T(\text{ester})/K_w^c$, [or $K_{a_2}^T(\text{ester})/K_w^c$ for most of the diamino-esters in the studied pH ranges], and $D = K_a^c(\text{amino-acid})/K_w^c$, [or $K_{a_2}^c(\text{amino-acid})/K_w^c$ for the diamino-acids]. For example, with methyl 4-aminobutyrate, Table 8 compares the experimental V_∞ values with those calculated using equations (13) and (14). The agreement between the experimental values and those of equation (13) is good, while the results of equation (14) values bear little relation to the experimental results. The most probable mechanism consistent with all the experimental data, is that essentially proposed by Martin *et al.*[3] where formation of the tetrahedral intermediate is rate determining.

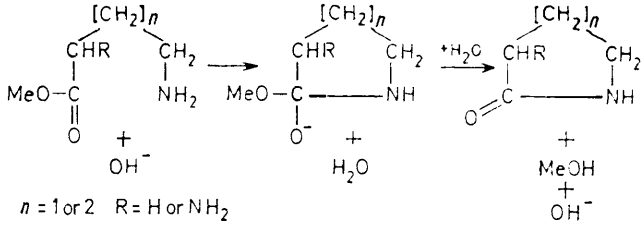
TABLE 8

Experimental and calculated values for V_∞ for the base hydrolysis of methyl 4-aminobutyrate *

	V_∞ ml of 0·1000M-NaOH		
pH	Experimental	Calculated [eqn. (13)]	Calculated [eqn. (14)]
10·00	2·94	3·52	5·50
9·90	3·76	3·97	5·61
9·80	4·18	4·39	5·76
9·70	4·65	4·80	5·93
9·60	5·11	5·33	6·11
9·50	5·50	5·54	5·29
9·40	5·83	5·85	6·46
9·30	6·17	6·13	6·62
9·20	6·39	6·37	6·77
9·10	6·62	6·57	6·89

* For $7{\cdot}5 \times 10^{-4}$ mol of ester, at 25 °C and $I = 0{\cdot}1$M.

Formation of the lactam can also be monitored by u.v. spectroscopy. Saturated aliphatic carboxylic esters have an absorption band near 200 nm (ε 50—80) while pyrrolidones have much more intense absorption bands



in this region. Thus 2-pyrrolidone has $\lambda_{max.}$ 191 nm (ε 6560) and an extinction coefficient of 3320 at 200 nm. The lactamisation of methyl 4-aminobutyrate to give 2-pyrrolidone was followed at 200 nm in borax buffers (buffer anion *ca.* 0·01M) adjusted to $I = 0{\cdot}1$M. Plots of log $(A_\infty - A_t)$ *versus* time were quite linear for at least two half-lives, and values of k_{obs} are listed in Table 9. The rate constants k_{obs} are slightly higher than the pH-state constants probably as a result of general base catalysis by the buffer. For concurrent hydrolysis and lactamisation of the ester, the half-lives are the same for ester disappearance and for the appearance of the lactam and of the acid. The percentage of lactamisation was calculated from the final absorbances at 200 nm, lactamisation increases significantly as the pH is raised reaching *ca.* 95% at pH 9·7. The practical pK_a of the amino-group of methyl 4-aminobutyrate is 9·95 at $I = 0{\cdot}1$M and 25 °C. Thus in the pH range where the unprotonated amino-ester occurs lactamisation predominates. As the pH is decreased the base hydrolysis of the protonated species EH^+ competes effectively with the lactamisation.

In the case of methyl 4-aminobutyrate the rate

[9] B. Capon, *Quart. Rev.*, 1964, **18**, 45.

expression takes the form (15) which leads to the form (16) so that at pH 9·95 (50% ionisation of EH^+) if

$$\text{Rate} = k_{\text{lact}}[E][OH^-] + k_E[E][OH^-] + k_{EH^+}[EH^+][OH^-] \quad (15)$$

$$\frac{k_{\text{obs}}}{[OH^-]}([E] + [EH^+]) = k_{\text{lact}}[E] + k_E[E] + k_{EH^+}[EH]^+ \quad (16)$$

$k_{\text{lact}} = 1752$, $k_E = 6$, and $k_{EH^+} = 125$ l mol^{-1} min^{-1} then $k_{\text{obs}}/OH^- = (876 + 3 + 63) = 942$ l mol^{-1} min^{-1} and lactamisation = 876/942 = 94%.

The temperature dependence study of some of the reaction rates (Table 5) shows that the same relative order of rate constants holds at 37 and 50 as at 25 °C. The studied reactions have similar thermodynamic parameters. Values of the enthalpy and entropy of activation for the E forms of methyl 2,3-diaminopropionate and methyl lysinate are fairly typical, *e.g.* for ethyl propionate, $\Delta H^\ddagger$ 10·1 kcal mol^{-1} and $\Delta S^\ddagger$ −29·5 cal K^{-1} mol^{-1}; for ethyl butyrate, $\Delta H^\ddagger$ 9·7 and $\Delta S^\ddagger$ −32·4.[10] The low $\Delta H^\ddagger$ value for the E form of methyl 4-aminobutyrate reflects the intramolecularly catalysed nature of this reaction. However, $\Delta S^\ddagger$ for this ester is not significantly different from that for 'normal' base hydrolysis of amino-acid esters, which agrees with the proposed rate limiting step in the lactamisation. If a process such as E ⟶ products was rate-limiting, then a much larger $\Delta S^\ddagger$ value would be expected. As discussed previously,[2] the $\Delta H^\ddagger$ value for EH^+ (or EH_2^{2+}) may be slightly larger or smaller than that for the corresponding species E. However, $\Delta S^\ddagger$ is usually larger for the protonated form than for the neutral species. This is the result expected on the basis of simple electrostatic considerations, as two oppositely charged ions form a transition state which is overall neutral in charge, and thus considerable desolvation of the transition state occurs.[11]

The results of the present study show that intramolecular participation by a neighbouring amino-group in the hydrolysis of an ester results in a rate increase of *ca.* 10^4 times in the most favourable case. This effect is some 10 times greater than the highest rate increase observed when copper(II) (the transition metal ion which is the most effective catalyst of amino-acid ester hydrolysis), is co-ordinated to a diamino-ester forming a chelate carrying a 2+ charge.[12]

TABLE 9

Lactamisation of methyl 4-amino-n-butyrate at 25° and I = 0·1M studied spectrophotometrically at 200 nm

pH	$10^2 k_{\text{obs}}$/min^{-1}	$10^{-2} k_{OH}$/l mol^{-1} min^{-1}	Lactamisation (%) *
9·035	0·664	4·67	53
9·278	1·20	4·83	56
9·517	2·58	6·00	71
9·702	5·08	7·72	95

* Calculated from the final absorbances at 200 nm assuming ε(lactam) = 3320.

EXPERIMENTAL

The amino-acids were BDH samples except for L-2,4-diaminobutyric acid dihydrochloride (Mann) and DL-2,3-diaminopropionic acid monohydrochloride which was synthesised using the procedure of Hellman and Haas,[13] m.p. 225 °C (lit.,[14] 225 °C) (Found: C, 26·0; H, 6·73; N, 19·9. Calc for $C_3H_8N_2O_2$,HCl: C, 25·6; H, 6·46; N, 19·9%). Amino-acids and amino-acid hydrochlorides were recrystallised where necessary, from ethanol–water until satisfactory m.p.s and analytical figures were obtained, and the set of pK_a values obtained by potentiometric titration was self-consistent. Esterification of the amino-acids and purification of the ester (di)hydrochlorides has been described previously.[1,2,15] The methyl ester (di)hydrochlorides of 4-aminobutyric acid, 2,4-diaminobutyric acid, and ornithine were hygroscopic which necessitated weighing in a dry-box. Details of the pH equipment, its standardisation and use for determining pK_a values and as a pH-stat have been given.[2,15] The overlapping pK_a values of the diamino-acids and their esters, were separated using the method of Speakman [16] in all cases except for methyl 2,3-diaminopropionate where the large difference in the two pK_a values necessitated use of the method of Noyes.[17] Rate constants were evaluated by the Guggenheim procedure [18] and/or infinity plots. Thermodynamic parameters were calculated as previously outlined.[2]

Chromatograms of the reaction products were run on Whatman No. 2 paper with phenol–water (4 : 1) or n-butanol–acetic acid–water (6·3 : 2·7 : 1) as developers. Spots were identified using ninhydrin spray or iodine vapour. Reaction of methyl 4-aminobutyrate produced two products, with R_F values identical to authentic 4-aminobutyric acid and 2-pyrrolidone. Similar results were obtained with methyl 2,4-diaminobutyrate (formation of 2,4-diaminobutyrate and 3-amino-2-pyrrolidone) and methyl ornithinate (formation of ornithine and 3-amino-2-piperidone). The products of base hydrolysis of these three esters had a strong band in the i.r. spectrum at *ca.* 1700 cm^{-1} [ν(CO) of a cyclic amide]. There was also strong absorption in the region of 1600 cm^{-1} due to ν(CO) of the carboxylate ion. Formation of 2-pyrrolidone from methyl 4-amino-n-butyrate was also followed by u.v. spectrophotometry. An authentic sample of 2-pyrrolidone (Eastman) was purified by vacuum distillation (b.p. 128° at 16 mmHg), $\lambda_{\text{max.}}$ 191 nm (ε 6560). Formation of the lactam was conveniently monitored at 200 nm [ε (lactam) 3320] since the amino-acid and -ester have negligible absorption at this wavelength. The reactions were carried out at 25 °C using the borax buffers

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[17] A. A. Noyes, *Z. Physik*, 1893, **11**, 495.
[18] E. A. Guggenheim, *Phil. Mag.*, 1926, **2**, 538.

described by Perrin [19] adjusted to $I = 0{\cdot}1$M. A known weight of the amino-acid ester hydrochloride was added to the buffer solution (3 ml) and the absorbance monitored as a function of time with a Perkin-Elmer 402 spectrophotometer. Values of k_{obs} were obtained from plots of log $(A_\infty - A_t)$ *versus* time which were linear for at least two half-lives.

[19] D. D. Perrin, *Austral. J. Chem.*, 1963, **16**, 572.

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