Neighbouring Group Participation by the Carboxy-group in the Solvolysis of the Acylamino-acids in Acetic Acid at Low Water Concentrations

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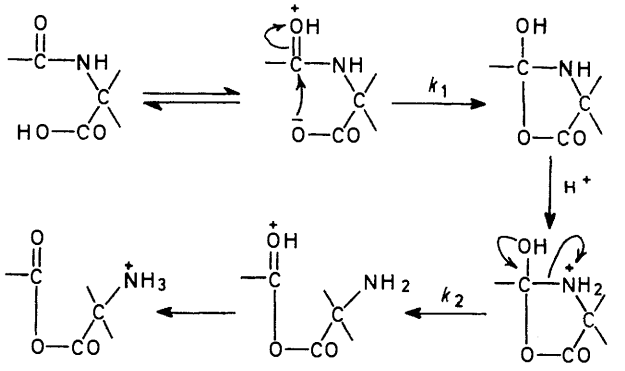
The kinetics of solvolysis of some acylamino-acids have been studied in acetic acid solution containing 0·25M-water. For the alkyl-substituted compounds the rates pass through a maximum at relatively low acid concentrations of *ca.* 0·05M. Increasing methylation at the carbon atoms adjacent to the amide bond produces increasing rates, which is a behaviour characteristic of ring closure reactions. The interpretation of the data is consistent with the rate-controlling step at the higher acid concentrations being a participation by the terminal carboxy-group to form an azlactone or its solvate which is followed by a rapid acid catalysed ring fission to form the products. When account is taken of the acid–base equilibria in the system, it is shown that the ring closure consists of simultaneous reactions by the protonated amide and the non-protonated amide through its zwitterion. The acid–base equilibrium constants which have been estimated from the kinetic data vary with structure in a similar manner to those of the corresponding acetamides which previously had been determined spectrophotometrically. Formylglycine has a faster rate than acetylglycine and its homologous acylglycines which is inconsistent with its lack of methyl groups to aid a ring closure reaction. These slower rates of the homologous acylglycines are due to the inductive effect of the α-carbon atom which is located at the point of ring closure. Methyl groups, therefore, are ambiguous in their behaviour and their position in the molecule determines whether they cause a rate increase or decrease. Strongly electronegative groups such as trifluoroacetyl and the positive terminal amino-group of a dipeptide have a reaction order of one with respect to the acid concentration and show no rate maximum even at the very high acid concentrations. It is suggested in these cases that the rate-controlling step is the acid catalysed ring fission for which the strongly electronegative groups will cause a decrease in the rate of ring fission and an acceleration in the ring closure.

NEIGHBOURING group participation by the carboxy-group in the solvolysis of the amides has been reported for the aminoacylasparagines,[1] succinanilic,[2] phthalamic,[3,4] and maleamic acids.[5-7] All these cases were demonstrated in aqueous solution and it was shown that the reactive forms were the free acids and not their anions. At high acid concentrations, the mechanism by which phthalamic acid [3] reacts changes to that usually observed for the simple amides, namely, a bimolecular reaction between water and the protonated amide.[8]

In contrast to this latter behaviour of phthalamic acid in water, protonated acetylglycine is inactive in acetic acid solution of low water content.[9] Under these conditions the non-protonated acetylglycine is the reactive species and undergoes a neighbouring group participation by the carboxy-group as in Scheme 1. The rate-controlling stages were suggested to be a ring closure at the high acid concentrations and ring fission at the lower acid concentrations.



SCHEME 1

The results reported here are an extension of this investigation and a detailed study has been made of the effects of substituents, mainly alkyl groups at the high

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acid concentrations where it has been suggested that ring closure is the rate-controlling step.

EXPERIMENTAL

Pivaloyl-α-aminoisobutyric acid (Piv-Aibu) was prepared by the Schotten–Baumann reaction [10] and crystallised from water or methanol–ethyl acetate, m.p. 177—178° (Found: C, 57·9; H, 9·05; N, 7·5. $C_9H_{17}O_3N$ requires C, 57·75; H, 9·15; N, 7·5%). Trichloroacetyl- (TClA), pivaloyl- (Piv), isobutyryl- (Ibu), and propionyl- (Prop) glycine were also prepared by the Schotten–Baumann method. Formylglycine [11,12] (Form-Gly), trifluoroacetylglycine [13] (TFA-Gly), acetylalanine,[14] acetylvaline,[15] and acetyl-α-aminoisobutyric acid [16] (Acet-Aibu) were prepared as reported.

The experimental technique has been described previously,[9,17,18] except that the accuracy of the analytical method has been improved. Owing to the large coefficient of expansion of acetic acid, no bulb pipettes were used except for rough measurements and all solutions and aliquot portions were prepared by weighing. Reaction mixtures were analysed by a pH-stat titration with sodium acetate, using a Radiometer automatic titrater. The electrodes used were a glass electrode and a specially designed calomel electrode with 0·25M-sodium perchlorate in acetic acid as the electrolyte. The maximum accuracy was achieved by using a 25 ml burette assembly. Throughout the study, the method of initial rates was used in order to relate the rate with concentration, because it was found that the simple integrated rate equations did not apply to these systems on account of the extensive protonation of both the amide and water. The analytical data were fitted to the empirical equation $y = B - t/(m + nt)$ by the method of least squares where y is the titre of sodium acetate for time t and B, m, and n are constants. The calculations were performed with an IME 86S electronic desk calculater with Digicorder. The rate at any time t is $-(dy/dt) = m/(m + nt)^2$. The accuracy of the analytical data and the applicability of the empirical equation to the rate data are both reflected in the sample set of data in Table 1. Unfortunately the earlier data, some of which are reported in this paper, do not conform to this degree of accuracy.

TABLE 1

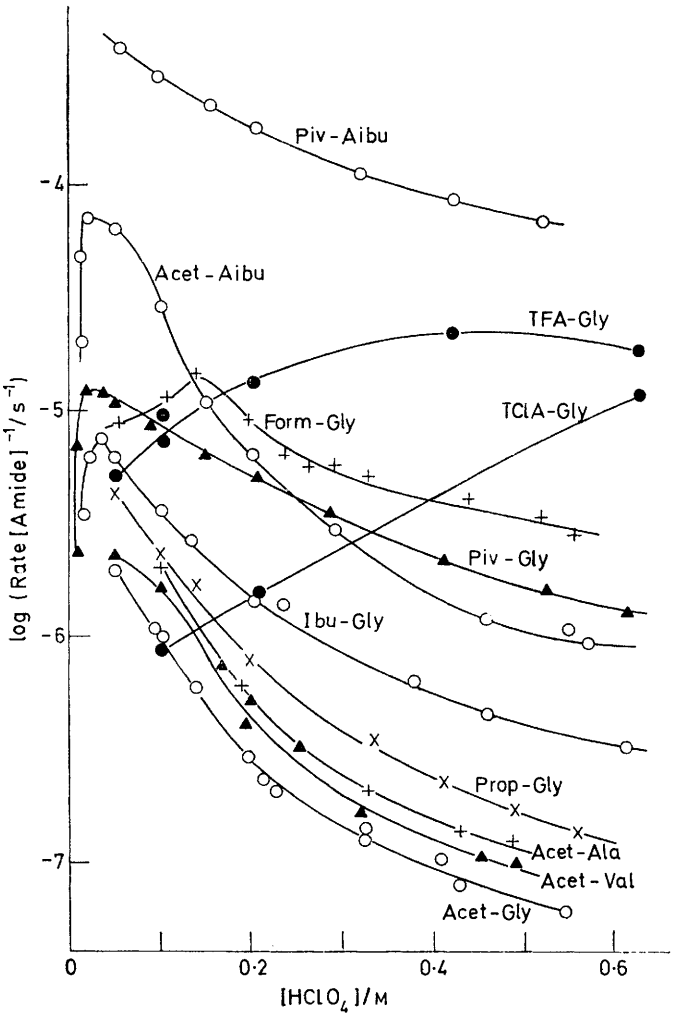
Sample data demonstrating the applicability of the empirical rate equation

t/h	0	0	0·25	0·50	0·75	1·00
y_{obs}/ml	19·388	19·390	19·076	18·782	18·515	18·305
y_{calc}/ml	19·388	19·388	19·072	18·787	18·528	18·291
t/h	1·25	1·50	1·75	2·00	2·25	
y_{obs}/ml	18·065	17·879	17·691	17·524	17·356	
y_{calc}/ml	18·074	17·874	17·690	17·519	17·361	

RESULTS AND DISCUSSION

The results plotted in the Figure are for the stoicheiometric concentrations of amide and perchloric acid and no corrections have been made for the protonation of the amide and the water. It will be noticed that except for the trifluoro- and trichloro-compounds, all the rates pass through a maximum at *ca.* 0·05M-perchloric acid, then decrease with an increasing acid concentration, indicating that the reactive species is the nonprotonated amide and that the ring closure reaction is being observed as the rate-controlling step.

The slowest reacting compound is acetylglycine and apart from formyl, trifluoro-, and trichloro-compounds, increasing methylation in the acyl or amino-acid part of the molecule gives increasing rates of reaction. Methylation in the amino-acid part of the molecule appears to be more effective for a rate increase than methylation in the acyl part. The fastest reacting compound of the series is the completely methylated compound pivaloyl-α-aminoisobutyric acid. This behaviour of increasing rates which are produced by increasing methylation is a characteristic of ring closure reactions and has been observed in the formation of epoxides from chloro-



Effect of substituents and the acid concentration on the rate of solvolysis of the acylamino-acids in acetic acid

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[11] R. S. Tipson and B. A. Pawson, *J. Org. Chem.*, 1961, **26**, 4698.
[12] J. P. Greenstein and M. Winitz, 'Chemistry of the Amino Acids,' Wiley, New York, 1961, p. 921.
[13] Ref. 12, p. 914.
[14] Ref. 12, p. 1831.
[15] Ref. 12, p. 2375.
[16] P. A. Levene and R. E. Steiger, *J. Biol. Chem.*, 1931, **93**, 581.
[17] R. J. L. Martin, *Austral. J. Chem.*, 1957, **10**, 268.
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hydrins,[19] cyclic amines from bromoamines,[20] and cyclic anhydrides from the half esters of succinic [21] and glutaric acids [21,22] and the half amides of succinic [2] and maleic acids.[7] The subject has been summarised recently by Kirby and Lancaster [7] and it has been suggested that methylation produces a more favourable conformation for ring closure.[21,22]

For a fuller interpretation of this system it is necessary to take into account the acid–base equilibria in which both water and amides are protonated.[9] It is difficult to determine the acid–base equilibrium constant of these amides spectrophotometrically [23,24] at the temperature at which the kinetic measurements have been made because of the ready reaction which occurs even with anhydrous conditions. At the best, determinations may be made at lower temperatures where the amount of reaction is negligible, but extrapolation to the temperature required is no doubt done with some degree of uncertainty. It was therefore decided to evaluate the acid–base equilibria constants from the kinetic data.

The relevant equilibria in the system are (1) and (2)

$$\text{Amide} + \Sigma H^+ \rightleftharpoons \Sigma\text{AmideH}^+$$
$$K_1 = [\text{Amide}][\Sigma H^+]/[\Sigma\text{AmideH}^+] \quad (1)$$

$$H_2O + \Sigma H^+ \rightleftharpoons \Sigma H_3O^+$$
$$K_2 = [H_2O][\Sigma H^+]/[\Sigma H_3O^+] \quad (2)$$

where H^+ refers to the acetic acidium ion and Σ represents the total concentration of a particular species as its ions, ion pairs, and triple ions. It has been shown spectrophotometrically that the equilibrium constants so defined do not vary for a wide range of amide salt concentrations [25] but deviations begin to appear at low salt concentrations of 10^{-4}M.

Equation (3) where a is the total concentration of amide, b perchloric acid, and c water, is derived from

$$[\Sigma H^+]\{1 + a/(K_1 + [\Sigma H^+]) + c/(K_2 + [\Sigma H^+])\} = b \quad (3)$$

equations (1) and (2). It was solved for $[\Sigma H^+]$ using various values for K_1 by means of a programmed method of trial and error with the IME 86S electronic desk calculator. K_2 Was interpolated from the known data for water using the van't Hoff isochore and has a value of 0·0634 mol l^{-1} at 65 °C.[23]

The results given in Table 2 show that there are no salt effects for the ring closure reaction and the mathematical treatment of the results is therefore simplified. Previously [9] it had been shown for acetylglycine that the ring closure reaction involves the non-protonated amide, where Rate = k_1[Amide]. However, on detailed mathematical analysis of the data covering a wider range of acid concentrations than those previously used,[9] it was found that the data were more closely represented by equation (4).

$$\text{Rate}/[\text{Amide}] = k_1 + k_1^1[\Sigma H^+] \quad (4)$$

A straight line was fitted by the method of least squares to the calculated values of Rate/[Amide] and $[\Sigma H^+]$ for a particular value of K_1 and that value of K_1 was chosen where 100 × standard deviation/mean (Rate/[Amide]) was a minimum. This was a relatively simple operation because the graph of Rate/[Amide] against $[\Sigma H^+]$ changes from concave downwards to convex as K_1 is decreased in value. The results obtained for K_1, k_1, and k_1^1 are given in Table 3.

TABLE 2

Salt effects on ring closure of pivaloylglycine at 65° in acetic acid

[Amide] = 0·094M	$[H_2O]$ = 0·25M		$[HClO_4]$ = 0·0451M		
$[NaClO_4]$/M	0·000	0·109	0·262	0·396	0·580
10^6Rate[Amide]$^{-1}$/s^{-1}	10·98	10·94	11·23	10·87	10·84

TABLE 3

Data derived from kinetic measurements at 65 °C [Water] = 0·25M

Compound	K_1/mol l^{-1}	10^6k_1/s^{-1}	$10^6k_1^1$/mol^{-1} l s^{-1}
Formylglycine	0·0090	33·6	208·2
Acetylglycine	0·0020	3·67	14·48
Propionylglycine	0·0028	7·49	23·84
Isobutyrylglycine	0·0050	10·07	38·46
Pivaloylglycine	0·0185	13·97	36·98
Acetylvaline	0·0018	6·40	18·76
Acetylalanine	0·0018	7·67	23·22
Acetyl-α-aminoisobutyric acid	0·0013	122·8	229·3
Pivaloyl-α-aminoisobutyric acid	0·024	481·5	862·5

The equilibrium constant of 0·0020 calculated from the kinetic data for acetylglycine agrees very well with that of 0·0018 which was extrapolated from the spectrophotometric determination [9] assuming that the temperature coefficient is the same as that for acetamide.[23,24] Methylation in the acyl part of the molecule gives increasing values of K_1 which has already been observed for the corresponding acetamides.[24] Increasing methylation in the amino-acid part of the molecule gives decreasing values which is the same relative order observed for the corresponding *N*-substituted acetamides.[24] Furthermore, the magnitude of the changes in K_1 with methylation are of the same order with both series of compounds and it would therefore appear that the mathematical analysis of the kinetic data is reasonably correct. The previous interpretation [24] of the effects of these substituents is still valid and the basicity of the amides is decreased by a hyperconjugative effect for alkylation in the acyl group and increased by an inductive effect for alkylation in the amino-acid part of the molecule.

The k_1 values (Table 3) which represent the rate-controlling stage as a ring closure involving the non-protonated amide increase with increasing methylation

[19] H. Nilsson and L. Smith, *Z. physik. Chem.*, 1933, **166A**, 136.
[20] R. F. Brown and N. M. van Gulick, *J. Org. Chem.*, 1956, **21**, 1046.
[21] T. C. Bruice and U. K. Pandit, *J. Amer. Chem. Soc.*, 1960, **82**, 5858.
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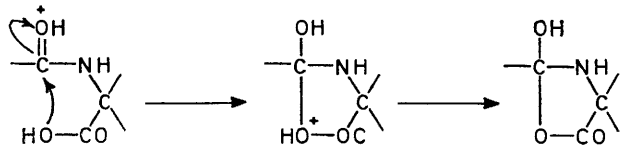
[23] R. J. L. Martin and I. H. Reece, *Austral. J. Chem.*, 1959, **12**, 524.
[24] R. J. L. Martin and I. H. Reece, *J. Chem. Soc.*, 1960, 4697.
[25] R. J. L. Martin, unpublished work.

in both the acyl and amino-acid parts of the molecule. This is consistent with the accepted behaviour for a ring closure reaction.

The k_1^1 values are the specific rate constants for an acid catalysed reaction and it would appear that there may be a contribution to the total reaction by the normal mode of amide hydrolysis involving a bimolecular reaction between water and the protonated amide.[8] During the mathematical analysis it is a simple matter to calculate $[\Sigma H_3O^+]$ and it was found that there was no correlation between the values for $[\Sigma H_3O^+]$ and Rate/[Amide]. Furthermore, the values of k_1^1 increase with increasing methylation indicating that a ring closure reaction is proceeding *via* the protonated amide as in Scheme 2. Such a reaction is to be expected since the

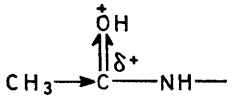


SCHEME 2

positive charge arising from protonation would assist the reaction.

The k_1^1 values do not strictly increase with increasing methylation in the acyl group and an exception appears with pivaloylglycine. This may be due to the fact that the value of k_1^1 is particularly sensitive to small changes in K_1 and the minimum percentage standard deviation is not so well defined for the higher values of K_1 for which there is a wider range of values of K_1 where the deviation is within experimental error.

In all cases acetylglycine and its homologous acylglycines have k_1 and k_1^1 values which are less than the values for formylglycine. This was surprising because formylglycine has no methyl groups which would assist any ring closure reaction. The slower rates of the homologous acylglycines as compared with formylglycine are due to the inductive effect of the alkyl group or the α-carbon atom which is directly attached to the carbonyl carbon atom at which ring closure occurs. This inductive effect will tend to reduce the positive



charge associated with the O–C–N system so that rate of attack by the carboxy-group or the carboxylate ion will be reduced.

The effect of a methyl group on the rate as compared with hydrogen is therefore ambiguous. Whether there is an increase in rate arising from the accelerating effect of methylation associated with ring closure reactions or a decrease in rate due to an inductive effect, depends upon the position of the methyl groups in the molecule. With methylation at carbon atoms other than that at the seat of ring closure, the inductive effects of these methyl groups are minimal and are not relayed with any intensity to the seat of ring closure. Under these circumstances the accelerating effects of increasing methylation which are associated with ring closure now operate. For example, increased rates are observed for increasing α-methylation in the acylglycines but these rates are still less than for formylglycine on account of the inductive effect arising from the α-carbon atom.

Previero *et al.*[26] have also reported that increasing methylation in the acyl group produced increasing rates for the reactions of the acyldi- and acyltri-peptides in trifluoroacetic acid solution and suggested that the ring closure involved participation by the acyl carbonyl group. For the results reported here this explanation is unsatisfactory and does not account for the substituent effects observed.

The much higher reactivity of the non-protonated as compared with the protonated amide is due to the carboxy-group being able to participate in an intramolecular proton transfer to form a highly reactive zwitterion. In this solvent of low dielectric constant, the zwitterion will be stabilised by the formation of intramolecular ion pairs. A further increase in stability will occur with the formation of intimate intramolecular ion pairs where the solvent sheath associated with the charges will be reduced. It is envisaged that this is the situation which exists prior to the act of ring closure.

From a consideration of the data on the dipeptides [17] it is apparent that the ring closure mode of solvolysis is dependent upon the polarity of the solvent. With an increasing water concentration, the relative rate sequence for the methylated dipeptides is reversed and becomes identical with that expected for the normal mode of solvolysis involving a bimolecular reaction between water and the protonated amide. The ring closure mode of solvolysis therefore appears to require a solvent of low dielectric constant where ion pair formation is appreciable. Indeed it is found that the addition of small amounts of a less polar substance such as pivalic acid causes a large increase in the reaction rate (Table 4).

TABLE 4

The effect of pivalic acid on the rate of solvolysis of acetyl-α-aminoisobutyric acid at 65 °C

$[\text{Amide}] = [HClO_4] = 0{\cdot}1\text{M}$ $[H_2O] = 0{\cdot}25\text{M}$

	% Pivalic acid by weight		
	0	5·4	10·8
$10^6\text{Rate}[\text{Amide}]^{-1}/\text{s}^{-1}$	29·0	44·9	85·4

The very strongly electronegative groups such as trifluoro and trichloro in trihalogenoacetylglycine and the positive terminal amino-group of the dipeptides [25] behave differently to the alkylated acylamino-acids because they exhibit no maximum in the rate curve even for acid concentrations greatly in excess of those used for the alkylacylamino-acids. These compounds would appear to react by a similar mechanism since the dipeptides solvolyse by faster rates with increasing methylation. These strongly electronegative groups

[26] A. Previero, M. A. C. Previero, and L. G. Barry, *Biochim. Biophys. Acta*, 1969, **181**, 361.

will tend to withdraw electrons so that the positive charge is enhanced at the point of ring closure thereby greatly accelerating this reaction. At the same time these groups will reduce the acid catalysed ring fission and an examination of the Figure shows that the rate of reaction of the trihalogenoacetylglycines is much less than that of acid catalysed reactions of the alkylated acylamino-acids in the low acid region. Since electronegative groups speed ring closure and reduce ring fission, it can be understood why the former reaction has not been observed as a rate-controlling step with compounds containing these groups because it is not possible to achieve a sufficiently fast acid catalysed reaction to produce this result.

The basicity of the amide is another factor which will determine whether ring closure will be the rate-controlling stage. The amides with strongly electronegative groups will be weakly basic and an increasing acid concentration will alter only to a small extent the relative amounts of non-protonated to protonated amide. On the other hand, the alkyl-substituted acylamino-acids are more strongly basic and an increasing acid concentration sharply reduces the concentration of non-protonated amide so that it becomes possible to observe the ring closure reaction as the rate-controlling step.

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