The Spontaneous Formation of Amides. The Mechanism of Lactam Formation from 3-(2-Aminophenyl)propionic Acid

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The spontaneous formation of the lactam from the neighbouring amino and carboxy groups of 3-(2-aminophenyl)-propionic acid involves general acid catalysed and spontaneous reactions of the neutral amino-acid. The conditions necessary, and the mechanisms involved, suggest that spontaneous amide formation could be biologically significant.

ONE of the commonest, and most important, biochemical processes is the formation of the amide bond. The reactions concerned are generally of the type used in the chemical synthesis of peptides, involving the formation of an active carboxylic acid derivative which acylates the amine component. Yet the activation of the carboxylic acid is not chemically essential. Reactions are known in which amide formation results apparently from no more than the close proximity of amine and carboxy groups.[1] In this paper we described a mechanistic investigation of one such reaction, the cyclisation of *o*-aminophenylpropionic acids (1) to dihydroquinolones (2).

(1) a; X = H
b; X = MeO
c; X = Me
d; X = Cl

(2)

EXPERIMENTAL

Inorganic chemicals and buffer constituents were of analytical grade. Distilled water was distilled twice more from all-glass apparatus. 5-Substituted 2-aminophenylpropionic acids (1a—d) were prepared as the sodium salts according to Mayer *et al.*[2]

The formation of the dihydroquinolones (2) was followed by monitoring the u.v. absorbance at 250—258 nm, in aqueous buffered solutions maintained at ionic strength 1.0M (KCl) at 39.0 ± 0.1°, in the thermostatted cell compartment of a Zeiss PMQ II (Cambridge) or Unicam SP 800 (Malta) spectrophotometer. The reactions are catalysed by the buffers used, and the points used to construct the pH–rate profiles for the cyclisation reaction (see Figure 1) were obtained by measurements at several buffer concentrations in the range 0.1—0.6M and linear extrapolation to zero buffer concentration.

pK_a *Measurements.*—Since the half-life of the most reactive *o*-aminophenylpropionic acid is little more than 1 min at 39°, the spectrophotometric method of Benesch and Benesch [3] was adapted as follows. A solution of the sodium salt of the amino-acid in 0.01M-NaOH (10 μl) was injected from a calibrated Hamilton syringe into a 10 mm cuvette containing a buffer solution of standard pH, maintained at 39° and ionic strength 1.0M. The injection did not affect the pH of the buffer solution significantly. On injection a Servoscribe recorder was triggered, and the absorbance recorded as a function of time, at a wavelength in the region 290—302 nm where the aniline but not the anilinium forms absorb strongly. The dissociation constants were then calculated [4] from the absorbance extrapolated back to zero time, and the measured pH.

Dixon [5] has drawn attention to the assumptions involved in this method of estimating group dissociation constants. Our results form a self-consistent set and lead to a consistent set of rate constants when used in the analysis of the kinetic data. And the difference in group dissociation constant caused by the difference in the ionic state of the second functional group is no more than *ca.* 0.8 pK units in any instance. So we assume that the aniline chromophore of (1) is not significantly affected by the state of ionisation of the CO_2H group, and have used the group dissociation constants calculated on this basis.

RESULTS

Figure 1 shows the pH–rate profile for the cyclisation of the unsubstituted compound (1a). Derived constants for this, and the other, substituted, compounds used are given in Table 1.

The substrate amino-acids (1a—d) can exist in four ionic forms (Scheme 1). Of these the anion (3) is known to be

TABLE 1

Dissociation and rate constants for the cyclisation of 3-(5-substituted-2-aminophenyl)propionic acids, at 39° and ionic strength 1.0M

Compound	pK_A	pK_B	pK_C	pK_D	k_0/s^{-1} [a]	k_+/dm^3 mol^{-1} s^{-1}	$k_+(D_2O)$/ dm^3 mol^{-1} s^{-1}	k_H/k_D
(1a)	4.01	3.70	4.50	4.81	3.0×10^{-4}	3.55×10^{-3}	1.92×10^{-3}	1.85 ± 0.06
(1b)	4.80	3.68	4.45	5.57	1.8×10^{-4}	1.47×10^{-3}	6.83×10^{-4}	2.15 ± 0.10
(1c)	4.36	3.94	4.72	5.12	2.5×10^{-4}	2.15×10^{-3}	1.05×10^{-3}	2.05 ± 0.10
(1d)	3.66	3.96	4.41	4.11	5.0×10^{-4}	7.87×10^{-3}	4.55×10^{-3}	1.73 ± 0.03
Hammett ρ	2.2			2.8	0.83	1.45	1.63	
Correlation coefficient	0.949			0.983	0.977	0.999	0.999	

[a] Calculated in terms of the combined neutral forms (1) + (4). See text.

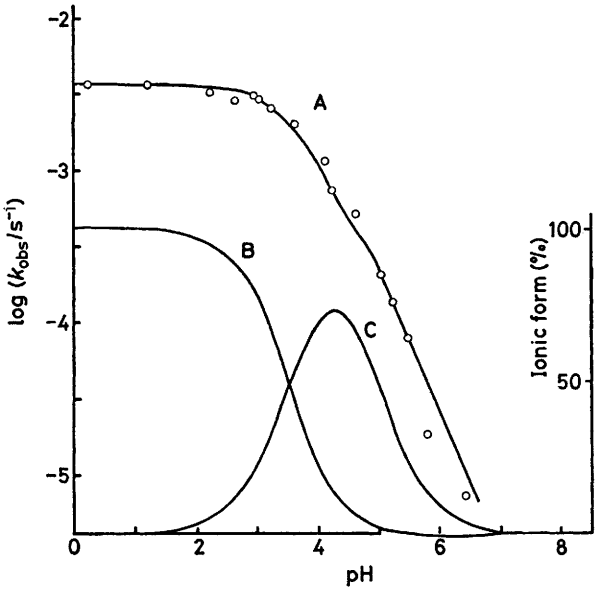


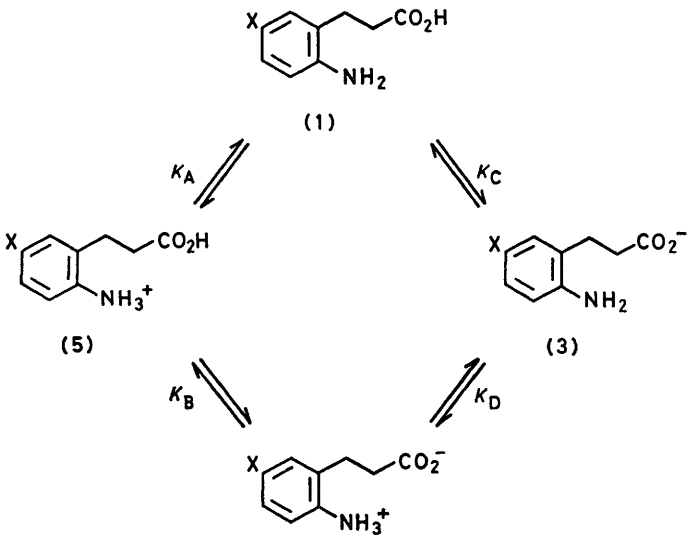
FIGURE 1 A, pH–Rate profile for the cyclisation of 3-(2-aminophenyl)propionic acid (1) to the lactam (2). Curves B and C represent the percentages of protonated and neutral forms as a function of pH, calculated from the pK_a values given in Table 1

unreactive (stock solutions of the sodium salts in 0.01M-NaOH are stable) and the concentrations of the neutral (1) and zwitterionic (4) forms are related by a constant of proportionality which does not depend on pH. So two independent variables suffice to describe the concentrations of the reactive forms as a function of pH.

The cationic form (5) appears to be responsible for at least the major part of the reaction, since cyclisation is fastest below pH 3 where this form predominates. We have therefore analysed the kinetic data in terms of equation (1)

$$k_{obs.} = k_+f_5 + k_0(f_1 + f_4) \qquad (1)$$

where f is the fraction of the starting material present as the specified ionic form at the pH of the measurement, and k_+ is



SCHEME 1

TABLE 2

Second order rate constants for general acid catalysis of the cyclisation of 3-(2-aminophenyl)propionic acid (1a), at 39° and ionic strength 1.0M

General acid	pK_a	k_{HA} [a]/dm^3 mol^{-1} s^{-1}
H_3O^+	−1.74	36 ± 2.4
$ClCH_2CO_2H$	2.86	2.1 ± 0.1 × 10^{-1}
HCO_2H	3.77	9.4 ± 0.3 × 10^{-2}
CH_3CO_2H	4.76	2.2 ± 0.1 × 10^{-2}
$H_2PO_4^-$	7.21	1.9 ± × 10^{-2}

[a] Calculated for the reaction of the conjugate acid of the buffer with the uncharged neutral form (1) of the substrate.

the rate constant measured in the pH-independent region below pH 2 (Figure 1). k_+ was also measured in this region in 1M- and 0.1M-DCl in D_2O.

Second-order rate constants for buffer catalysis of the cyclisation of the unsubstituted compound (1a) are given in Table 2. These are calculated in terms of general acid catalysis by the buffer conjugate acid of the reaction of the

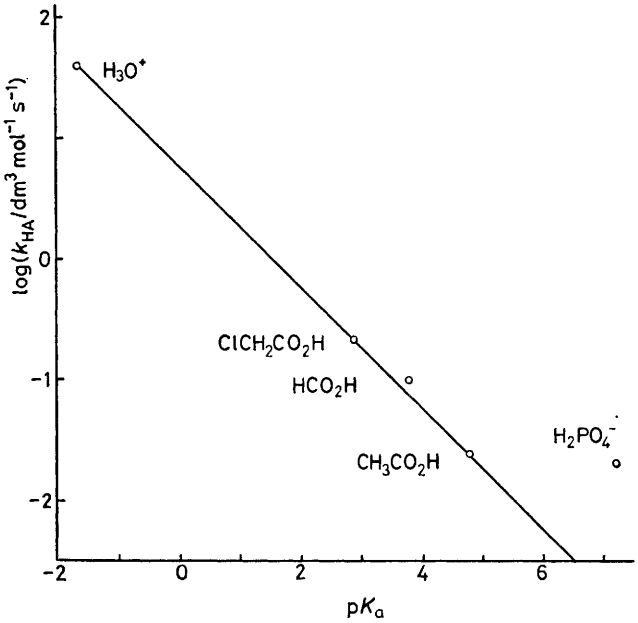


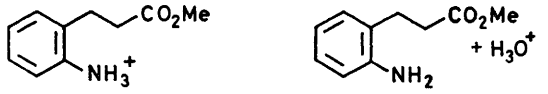
FIGURE 2 Brönsted plot for general acid catalysis of the cyclisation of (1) at 39° and ionic strength 1.0M

neutral form (1) of the substrate amino-acid, rather than the kinetically equivalent general base catalysis of the reaction of the cation (5), for reasons discussed below. The data are correlated by the Brönsted equation (Figure 2) with an exponent $\alpha = 0.49 \pm 0.02$. Similar correlations are found for the less extensive data for buffer catalysis of the cyclisation of the three substituted aminophenylpropionic acids, and the Brönsted exponents α are identical, within experimental error, for the reactions of all four compounds.

DISCUSSION

Mechanism.—The pH–rate profile (Figure 1) for the formation of the dihydroquinolone (2a) from 3-(2-aminophenyl)propionic acid (1a) shows significant reactions of both protonated and neutral (or zwitterionic) forms. The kinetic ambiguities involved are simply resolved in the light of our results with the methyl ester (6) of (1a), described in the preceding paper.[6]

The neutral form of (6) is converted into dihydroquinolone in a reaction which is catalysed by the conjugate acids of buffers with pK_a values below 5; and the

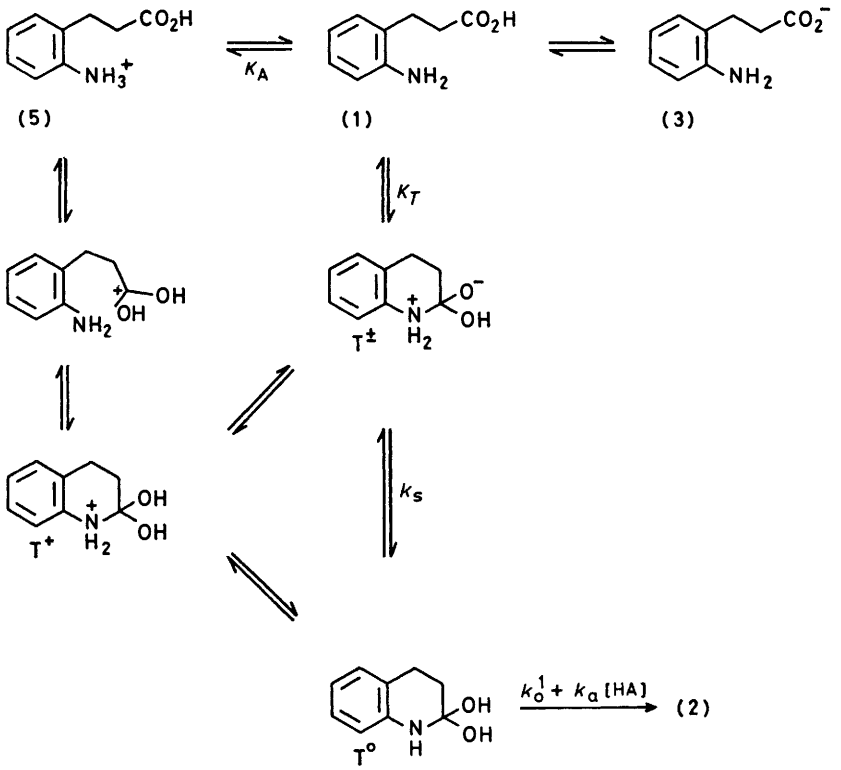


(6)

reaction of the protonated form can be accounted for in terms of general acid catalysis of the same reaction by the hydroxonium ion.[6] The CO_2H and CO_2Me groups should have similar reactivities towards the neighbouring NH_2 group, so we have calculated the second-order rate constants for buffer catalysis of the cyclisation of the acid (1a) on the assumption that this too is a general acid catalysed reaction of the neutral form (1). The rate constants obtained are quantitatively similar to those found for the reaction of (6) under the same conditions, and are correlated by the Brönsted equation with an exponent, α 0.49, similar to that observed for the corresponding reaction of the ester (α 0.41).[6]

We have no reason to doubt, therefore, that similar mechanisms are involved in the two cases. For the cyclisation of the amino-acid (1) the mechanism shown in Scheme 2 applies.[6] This mechanism is the same as that proposed [6] for the cyclisation of the ester (6), except that the pathway from $T^{\pm}$ to T^0 *via* T^-, which accounts for general base catalysis of the ester reaction, has disappeared. General base catalysis of the ester reaction is observed only for buffers with p$K_a > 7$. Of these only phosphate catalyses the cyclisation of the acid (1), and even this acts predominantly as a general acid. The cyclisation of the acid is very slow at pH 7 and above, because it is present almost entirely as the unreactive anion (3), and we have not attempted to confirm that the expected 25—30% of the phosphate catalysed reaction represents general base catalysis.

The reaction of the protonated amino-acid (5) is identified as general acid catalysis by hydroxonium ion of the reaction of the neutral species [(1) $\rightleftharpoons$ T^0], since the rate constant calculated on this basis falls on the Brönsted plot (Figure 2) constructed for catalysis by other general acids. Our evidence on the reaction of the



SCHEME 2

methyl ester,[6] and previous work on ester aminolysis,[7,8] particularly Schmir's study [9] of the hydrolysis of 2-*N*-phenyliminotetrahydrofuran, suggest that the rate-determining step is the general acid catalysed breakdown of the neutral tetrahedral intermediate T^0 (k_a of Scheme 2). This is consistent with the observed value of the Brönsted exponent (α 0.49), midway between zero and unity, and with the solvent deuterium isotope effect [k_H/k_D *ca.* 2 for the H_3O^+ catalysed reactions of (1a—d)] since a proton is being transferred from the general acid to the departing leaving group in the transition state.

The uncatalysed reaction (k_0) of the neutral form is not likely [10] to represent general acid catalysis by the solvent (HA = H_2O in Scheme 2) because this would merely generate hydroxide by a more complex mechanism, and because the second-order rate constant calculated for catalysis by H_2O does not fall on the Brönsted plot of Figure 2, but shows a large positive deviation. We

attribute this to the spontaneous elimination of hydroxide from T^0 (k_0^1 in Scheme 2).[6]

Substituent effects are also consistent, though not uniquely so, with the mechanism of Scheme 2. Both k_+ and k_0 are correlated by the Hammett equation, with moderate positive values of ρ. For the reactions (k_+) of the conjugate acids (5), ρ = 1.45, about half the value for the complete loss of a proton from an aniline [pK_A and pK_D for (1a—d) are correlated by the Hammett equation with ρ values of 2.8 and 2.2 respectively]. Since the ground state (5) has a full positive charge on nitrogen this is consistent with a transition state in which it bears a partial positive charge. For the reaction of the neutral form, where the aniline nitrogen is partially positively charged in the ground state [which is an equilibrium mixture of (1) and (4)] as well as the transition state the observed value of the reaction constant is smaller (ρ 0.83). When the rate constants for this reaction are calculated specifically in terms of the uncharged neutral form (1) the Hammett plot shows the expected negative slope (ρ −0.8).

The Spontaneous Formation of Amides.—It is clear that the ready cyclisation of the 3-(2-aminophenyl)-propionic acids (1) depends on the low basicity of the aniline NH_2 group. Only the neutral amino-group can act as a nucleophile, and only the neutral CO_2H group, or its conjugate acid, is attacked at a significant rate under mild conditions, so that ready lactam formation requires a significant concentration of the neutral form of the amino-acid. This is available in the case of (1) because of the similar pK_a values of anilines and carboxylic acids.

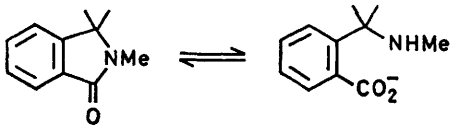
Amide formation by these mechanisms from aliphatic amines, with pK_a values in the region 9—10, will clearly be much less favourable, by factors of up to 10^6 for the reaction through $T^{\pm}$, and by much larger factors for the general acid catalysed reaction, as discussed previously.[6] These figures encourage us to expect that the reaction should be observable in systems activated by ground state strain, and perhaps also in enzyme-catalysed reactions, where the basic requirements of close proximity of amino and carboxy-groups can be combined with control of dissociation constants.

Morawetz and Otaki [11] measured rates of amide formation in aqueous solution from simple aliphatic amines and carboxylic acids, and concluded that the very slow reactions they observed involved the amine and the carboxylate ion [reaction (2)]. Surprisingly, we have

$$R^1COO^- + R^2NH_2 \rightleftharpoons R^1CONHR^2 + HO^- \quad (2)$$

not so far been able to identify a cyclisation involving this mechanism, though the rate constants for the forward reaction should be much more favourable. Neither *o*-aminophenylpropionic acids nor *o*-aminophenylacetic acids appear to cyclize at high pH, nor does *o*-2-aminoethylbenzoic acid under any conditions below 100° in the pH range.

More highly strained compounds are difficult to prepare, largely because of the extreme difficulty of hydrolysing the lactams [*e.g.* (8)] under basic conditions.



(8)

This may well be because the anions recyclise faster than they are formed, and we are actively investigating this possibility.

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