The Oxazolinone Intermediate in the Hydrolysis and Aminolysis of *N*-Benzoylglycine Derivatives

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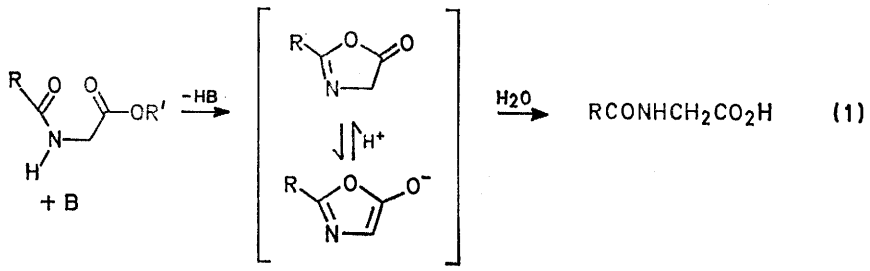
The Brønsted type relationship with the pK_a of the departing hydroxy-group for the alkaline hydrolysis (k_{OH}) of *N*-benzoylglycine esters exhibits a break consistent with a change in mechanism. Substituted phenyl esters hydrolyse *via* an oxazolinone intermediate and the high Brønsted selectivity indicates a rate-determining step with considerable C–OAr bond cleavage in its transition state. The formation of the intermediate occurs *via* a pathway where ionisation of the *N*-benzoylglycine NH, intramolecular attack, and decomposition of the tetrahedral intermediate are discrete steps. Aminolysis proceeds largely through the ester rather than *via* the oxazolinone intermediate.

OXAZOLINONES have been postulated as intermediates in acyl transfer reactions catalysed by α-chymotrypsin [1] and have also been involved in the considerable discussion of the mechanism of racemisation in peptide coupling reactions.[2] Zerner and his co-workers [3] have reported an elegant series of experiments implicating oxazolinones [equation (1)] as intermediates in the hydrolysis of activated esters and derivatives of *N*-benzoylglycine.

We are intrigued by this mechanism on three counts. (i) The hydrolysis of methyl *N*-benzoylglycinate has been shown to involve nucleophilic attack of hydroxide

[1] M. A. Coletti-Previero, C. Axelrud-Cavadore, and A. Previero, *FEBS Letters*, 1973, **11**, 213, 218.
[2] G. T. Young, *Essays in Chemistry*, 1972, **4**, 115.
[3] (*a*) J. de Jersey, P. Willadsen, and B. Zerner, *Biochemistry*, 1969, **8**, 1959; (*b*) J. de Jersey, A. A. Kortt, and B. Zerner, *Biochem. Biophys. Res. Comm.*, 1966, **23**, 745.

ion on the ester carbonyl [4] rather than a mechanism with an oxazolinone intermediate. Where, using the pK_a of the leaving hydroxyl as a standard, does the *mechanism change* from addition–elimination (*A–E*) to the elimination–addition (*E–A*) process involving an oxazolinone? (ii) Is the formation of oxazolinone from the *N*-benzoylglycinate concerted in the sense that proton transfer from the nitrogen is synchronous with attack of carbonyl oxygen on the ester (presumably to form a tetrahedral intermediate)? (iii) Do aminolysis reactions in aqueous solution occur *via* the oxazolinone



or is direct attack on the ester preferred? It is the aim of this paper to investigate these questions.

EXPERIMENTAL

Materials.—Esters of *N*-benzoylglycine were made from the acid and alcohol or phenol using the mixed anhydride method with triethylamine and isobutyl chloroformate. Alternatively the phenol or alcohol was reacted with *N*-benzoylglycyl chloride [5] in the presence of an equimolar amount of triethylamine. *N*-Benzoylglycyl azide was prepared from the hydrazide (from ethyl ester and hydrazine hydrate) using nitrous acid. Isopropyl and cyclohexyl esters were prepared using a Dean and Stark apparatus with Dowex acid resin as a catalyst. Data for the derivatives are collected in Table 1 and structures were confirmed by i.r. and n.m.r. spectroscopy. Reference to previously prepared substrates is made in the footnotes to Table 3.

Buffers were of analytical reagent grade where possible and if not were purified by recrystallisation. Amines were purified by recrystallisation of their hydrochlorides from ethanol or by distillation; deuterium oxide (99.8%) was obtained from Ryvan Co.

Methods.—Rates of hydrolysis were measured either by observing the change in u.v. or visible absorption in buffers using a Beckman DBG spectrophotometer or by titrating the acid released from unbuffered solutions at a constant pH with a Radiometer pH-stat. A Radiometer pH meter 25 or a Pye-Dynacap instrument were used to measure pH and in all cases these machines were calibrated in the pH concerned using E.I.L. standard buffer powders accurate to ±0.01 units. These instruments were employed in pD measurement but the equation of Glasoe and Long [6] could not be used as it refers to a different ionic strength; we repeated their work for 1M ionic strength (made up with sodium chloride) and find pD = meter reading + 0.37.

At high pH the release of phenols was measured with stopped flow equipment consisting of a Unicam SP 500 monochromator, a mixing chamber designed in this laboratory by Dr. P. A. Tregloan, and a photomultiplier (IP 28) detector powered by a Farnell (type E1) stabilised power supply (EHT) and output to a Telequipment DM 64 storage oscilloscope. First-order kinetics with both 'fast' and 'slow' reactions were analysed using infinity readings taken five or six half-lives after initiation of the reaction.

Product Analysis.—The amount of protons neutralised in the hydrolyses carried out using pH stat equipment was taken as an index of stoicheiometry and in the spectroscopic method the extinction coefficient of the product phenol and the total absorption change were used.

TABLE 1

Physical data for new *N*-benzoylglycine derivatives [a]

		Found (%)				Requires (%)		
Ester	M.p. (°C)	C	H	N	Formula	C	H	N
3-Chlorophenyl	124—125	62.1	4.0	4.7	$C_{15}H_{12}ClNO_3$	62.2	4.1	4.8
2,2,2-Trifluoroethyl	134—135	50.7	3.9	5.3	$C_{11}H_{10}F_3NO_3$	50.6	3.8	5.4
Propargyl	94—95	66.2	5.2	6.6	$C_{12}H_{11}NO_3$	66.4	5.1	6.5
2,2-Dichloroethyl	61—62	47.9	3.9	5.2	$C_{11}H_{11}Cl_2NO_3$	47.8	4.0	5.1

[a] M.p.s were recorded with a Kofler Thermospan instrument; analyses were by Mr. G. M. Powell and Miss L. Tidy of this laboratory using a Hewlett–Packard 185 CHN analyser.

Product analysis of the aminolysis reactions with ethyl glycinate buffer and 4-nitrophenyl *N*-benzoylglycinate was carried out by extracting the acidified product with chloroform. The extracted material (none of the buffer is carried through as it is fully protonated in acid solution) was analysed by n.m.r. spectroscopy using [$^2H_6$]dimethyl sulphoxide solvent. One of the 'aromatic' peaks of 4-nitrophenol (equivalent to one proton) was employed as a standard to compute product composition.

RESULTS

Alkaline Hydrolysis of N-Benzoylglycinate Derivatives.—The release of acid in the hydrolysis of alkyl *N*-benzoylglycinates, the release of phenols from the aryl esters and the hydrolysis of the azide obey good first-order kinetics up to *ca.* 90% of the total reaction. The pseudo-first-

[4] R. W. Hay and P. J. Morris, *Chem. Comm.*, 1967, 663.
[5] A. Williams, *Biochemistry*, 1970, **9**, 3383.
[6] P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, 1960, **64**, 188.

order rate constants are proportional to hydroxide ion concentration (Table 2) as calculated from the pH of the solution and the ionic product of water; the bimolecular hydroxide rate constants (k_{OH}; Table 3) are in agreement with Zerner's [3] values where overlap occurs.

TABLE 2

Hydrolysis of 4-nitrophenyl *N*-benzoylglycinate

pH(D)	Buffer	Buffer conc. (M)	k/s^{-1}
Solvent H_2O			
7.06	2,6-Lutidine	0.004 [d]	8.9 (3.5) × 10^{-4} [c]
7.06	2,6-Lutidine	0.04 [d]	9.1 (3.6) × 10^{-4} [c]
6.96	2,6-Lutidine	0.2 [d]	7.1 (3.5) × 10^{-4} [c]
6.88	2,6-Lutidine	0.4 [d]	6.0 (3.5) × 10^{-4} [c]
6.65	2,6-Lutidine	1.0 [d]	3.7 × 10^{-4}
9.20	Borate	0.005	0.12 [a]
9.00	Borate	0.005	8.3 × 10^{-2} [a]
8.50	Tris	0.005	2.6 × 10^{-2}
7.88	Ethyl glycinate	Intercept [d]	0.56 × 10^{-2}
5.50	pH stat	0.000 [d]	2.5 × 10^{-5}
5.50	Acetic acid	1.0 [d]	2.5 × 10^{-5}
8.11	*N*-Ethylmorpholine	1.0 [d]	1.0 × 10^{-2}
8.12	*N*-Ethylmorpholine	0.5 [d]	0.9 × 10^{-2}
8.14	*N*-Ethylmorpholine	0.2 [d]	0.98 × 10^{-2}
10.10	Hydrogen carbonate	0.01	1.1 [a]
10.98	Hydrogen carbonate	0.01	8.3 [a]
11.60	Hydroxide	0.01	29 [a]
12.05	Hydroxide	0.05	69 [a]
12.03	Hydroxide	0.05	76 [a]
Solvent D_2O			
8.66	*N*-Ethylmorpholine	1.0 [d]	7.73 × 10^{-3} [b]
7.14	2,5-Lutidine	1.0 [d]	2.70 × 10^{-4} [c]

[a] Determined using stopped-flow apparatus. [b] Isotope effect at pH 8.11, k_{OH}/k_{OD} = 0.68 (*N*-ethylmorpholine), at pH 7.14 = 0.71 (2,6-lutidine). Values of K_w^D and K_w^H are taken from R. W. Kingerley and V. K. La Mer, *J. Amer. Chem. Soc.*, 1941, **63**, 3256. [c] Value in parentheses is corrected to pH 6.65. [d] Ionic strength 1.0M made up with sodium chloride.

The yields from the hydrolyses measured from proton release for alkyl esters and by visible absorption for the 4-nitrophenyl ester indicate a 1 : 1 stoicheiometry for the reaction. The rate constants for hydrolysis of the 4-nitrophenyl ester are identical (within experimental error) when derived from pH stat or from spectrophotometric measurements using buffers at the same pH. Since the mechanism for 4-nitrophenyl hydrolysis is a two-step one with similar rate constants at a pH in the region of 7 (see ref. 3*a*) the release of protons is complex; above pH 3 the second step releases one proton and above pH 7 the first step releases one proton. We used pH 5.5 where there is no proton from the first, slow, rate-determining, step but one proton from the second, fast, step. Thus, although the proton is derived from oxazolinone hydrolysis, this is a measure of the progress of the first step.

Effect of Buffer on Hydrolysis.—At relatively high buffer concentrations (0.05—1M) of 2,6-lutidine and of *N*-ethylmorpholine (base fr., 0.5) there is no significant change in rate constant for release of 4-nitrophenol from its ester outside the experimental error (Table 2). Since pH values vary slightly as the concentration of the 2,6-lutidine buffer is increased (at the same base fr.) a correction for the hydroxide rate constant has to be applied for comparison at the same pH. The logarithm (base 10) of the rate constants is linear in pH with unit positive slope. Previous work [3] indicates that, at very low concentrations, 2,6-lutidine has a slight catalytic effect.

The above buffer components are not able to yield products, other than from hydrolysis, with the 4-nitrophenyl ester but the use of ethyl glycinate as a buffer can give a peptide; 4-nitrophenol release is linear in buffer concentration (Figure 1) and a bimolecular rate constant (1.34 × 10^{-3} l mol^{-1} s^{-1}) calculated from the slope of the plot *versus* base species. The intercept at zero buffer concentration agrees with that calculated from k_{OH} for the 4-nitrophenyl ester (Tables 2 and 3). The product from this reaction is 97% of the peptide *N*-benzoylglycylglycine ethyl ester as determined by comparing the n.m.r. signal from the methyl protons, τ 8.8 (t), of the ethyl group with that from the aromatic protons of the 4-nitrophenol, two peaks, τ 2.85 and 3.00 (each 1H); the error in this analysis is judged to be no better than 5%. This result refers to 1 molar buffer at base fr. 0.5.

Rate constants for decomposition of *N*-benzoylglycyl azide in ethyl glycinate buffers are linear in buffer concentration yielding a bimolecular rate constant (with the base form) of 1.23 l mol^{-1} s^{-1}; the intercept at zero buffer concentration agrees with that calculated from the pH and k_{OH} for the azide (from Table 3).

TABLE 3

Reaction of hydroxide ion with *N*-benzoylglycine derivatives

Substrate	λ/nm [d]	pH Range [g]	pK_a [f]	k_{OH}/l mol^{-1} s^{-1} [a]	
4-Nitrophenyl [h,r]	400	6—12	7.14	7200	
4-Acetylphenyl [h]	325	7—8	8.05	1780	
3-Nitrophenyl [h]	355	7—8	8.35	1590	
2-Nitrophenyl [l]	420	7—8	7.23	10,100	
Phenyl [h]	290	*c*	9.95	33.8 [c]	
4-Methylphenyl [h]	285	9—10	10.19	10.7	
4-Chlorophenyl [h]	275	9—10	9.38	194	
3-Chlorophenyl	280	9—10	9.02	392	
2,5-Dinitrophenyl			5.22	3.4 × 10^5 [b]	
4-Methoxyphenyl [h]	290	9—10	10.20	39.2	
Benzyl [i]		10—11	15.25 [e]	2.2	(0.25)
Methyl [j]		10—11	15.54	2.7	(0.31)
Ethyl [m]		10—11	16	1.1	(0.13)
2-Chloroethyl [n]		10—11	14.31	4.27	(0.49)
2,2,2-Trifluoroethyl		10—11	12.43	21.5	(2.5)
Propargyl		10—11	13.55	5.38	(0.62)
Azide [p]	250	6—7	4.72	1.47 × 10^4	
Isopropyl [j]		10—11	15.5 [f]	0.39	
Cyclohexyl [q]		10—11	15.5 [f]	0.223	
Cyanomethyl			11.29 [e]	78 [b]	(9.0)
2,2-Dichloromethyl		10—11	12.89	12.3	(1.41)

[a] Aqueous solution, 25°, 0.1M ionic strength made up with sodium chloride; values in parentheses are corrected by dividing true values by 8.7, see Discussion section; errors in the values of k_{OH} are no greater than 5%. [b] Value from Table IV of ref. 3*a*. [c] Stopped-flow kinetics using 290 nm show that the pseudo-first-order rate constant is proportional to hydroxide ion concentration up to 1M. Buffers used were hydrogen carbonate and hydroxide. [d] Wavelength used in kinetic measurements. [e] This value is calculated from the equation for alcohols in G. B. Barlin and D. D. Perrin, *Quart. Rev.*, 1966, **20**, 75; pK_a = 15.9—1.42σ^*; the value for σ^* for CH_2CN is calculated as 3.25 from the pK_a for NH_3CH_2CN and the equation pK_a = 13.23—3.14$\Sigma\sigma^*$ (H. K. Hall, *J. Amer. Chem. Soc.*, 1957, **79**, 5441); σ^* for Ph is taken as 0.46. [f] Except where stated, pK_a values for the leaving alcohols and phenols are taken from the compilation of W. P. Jencks and J. Regenstein in the 'Handbook of Biochemistry,' Section J-187, ed. H. A. Sober, Chemical Rubber Company, Cleveland, 1970, 2nd edn. [g] Buffers were: pH 6—8, phosphate; 9—10, borate; 10—11, hydrogen carbonate. [h] A. Williams, *Biochemistry*, 1970, **9**, 3383. [i] A. Williams, E. C. Lucas, A. Rimmer, and H. C. Hawkins, *J.C.S. Perkin II*, 1972, 627. [j] E. C. Lucas and A. Williams, *Biochemistry*, 1969, **8**, 5125. [l] Ref. 3*a*. [m] T. Curtius, *Ber.*, 1902, **35**, 3226. [n] K.-B. Augustinsson, *Acta Chem. Scand.*, 1955, **9**, 753. [p] T. Curtius, *J. prakt. Chem.*, 1895, **52**, 243. [q] M. Mengelberg, *Chem. Ber.*, 1954, **87**, 1425. [r] The *N*-methyl analogue of this ester [3a] has k_{OH} = 3.34 × 10^2 l mol^{-1} s^{-1} at 25° and 1M ionic strength.

Deuterium Oxide Solvent Isotope Effect.—In 2,6-lutidine and *N*-ethylmorpholine buffers the deuterium oxide solvent

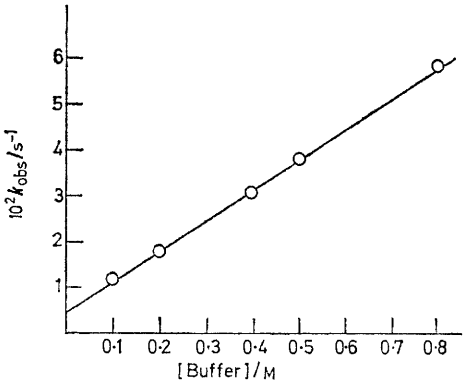
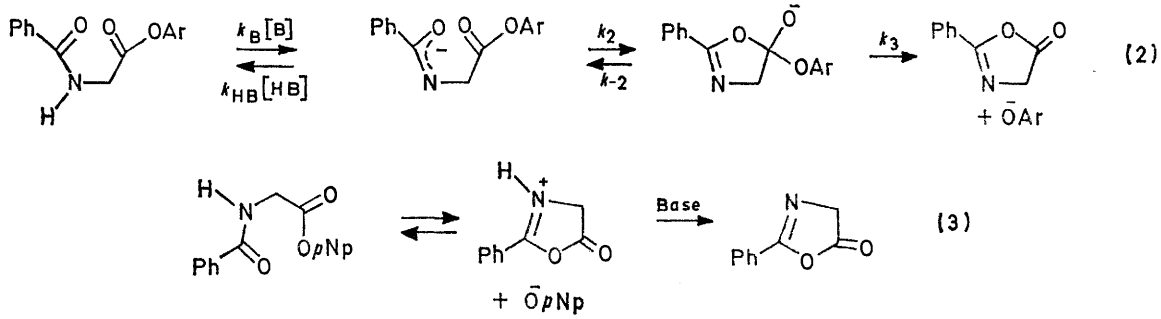


FIGURE 1 Effect of ethyl glycinate buffer concentration on release of 4-nitrophenol from the *N*-benzoylglycinate ester: fraction of buffer as base = 0.5; 25°; ionic strength 1.0M made up with sodium chloride; intercept agrees with that calculated from data of Table 3

isotope effects on the rate of 4-nitrophenol release from the *N*-benzoylglycinate ester are: $k_{OH}(H/D) = 0.71$ and 0.68 respectively (see Table 2).

DISCUSSION

A Brønsted plot of $\log_{10}k_{OH}$ (Table 3; Figure 2) for *N*-benzoylglycinate esters *versus* the pK_a of the leaving hydroxy-group exhibits two distinct linear portions. Alkyl esters fall on a line of low slope ($\beta_{l.g.}$ — 0.35) whilst substituted phenyl esters possess a high Brønsted selectivity ($\beta_{l.g.}$ — 0.8) which is consistent with a stepwise mechanism involving an oxazolinone intermediate [equation (2)] where the cyclisation (k_3 step) is rate



limiting. The rate constant, k_{OH}, for this mechanism is composite and if k_2 were rate limiting should involve an ionisation (relatively insensitive to phenyl substituent, β *ca.* 0) and a k_2 step which by analogy with intermolecular attack of hydroxide ion on substituted phenyl acetates should have $\beta_{l.g.}$ *ca.* — 0.36.[7a] The involvement in k_{OH} of the k_3 step with significant ArO–C cleavage (*i.e.* $k_{-2} > k_3$) is consistent with the high sensitivity to pK_a observed. The data, however, are not unequivocal as regards a σ or σ^- Hammett dependence and we cannot therefore employ this valuable diagnostic tool.

O'Brien and Niemann [7b] showed that *N*-benzoylsarcosine cyclises to yield an oxazolonium ion indicating that the NH of *N*-benzoylglycinates is not necessarily of kinetic significance. De Jersey *et al.*,[3] however, find that 4-nitrophenol is released from the corresponding *N*-benzoylglycinate faster than oxazolinone hydrolysis at pH >7 to yield oxazolinone as an intermediate and the rate constant is *proportional* to hydroxide ion concentration. A scheme involving oxazolonium ion formation [equation (3)] would yield pH-independent rate constants for release of 4-nitrophenol because the deprotonation step would be expected to be diffusion-controlled except to very weak bases and therefore the hydroxide dependence would not be revealed. The apparent pK_a of *ca.* 9 observed by De Jersey *et al.*[3a] for oxazolinone hydrolysis is due to the ionisation of an α-proton.

The 4-nitrophenyl ester of *N*-benzoylsarcosine releases the phenol with a rate constant proportional to hydroxide ion concentration and unlike the glycinate this rate law arises from A*E* attack on the ester by hydroxide. The rate constant is some 20-fold less than that for the non-methylated ester but this difference reflects a change in mechanism (no intermediates are observed spectrophotometrically) and not different steric or electronic requirements; this is confirmed by the good fit of the methyl analogue to the line extrapolated from the Brønsted type line (Figure 2) with low slope.

Reactions involving expulsion of a phenol from an ester by an intramolecular nucleophile appear to fall into groups having a high sensitivity to leaving group pK_a ($\beta_{l.g.}$ *ca.* —0.8) and a low sensitivity ($\beta_{l.g.}$ *ca.* —0.4).[7c-f] The expulsion of a phenol by the ureido-anion possesses a small negative $\beta_{l.g.}$ but the neutral ureido-group has a much higher sensitivity ($\beta_{l.g.}$ —0.87). Hegarty and Bruice [7f] were able to show that attack involved either oxyanion or nitrogen of the ureido-anion and that the

[7] (*a*) T. C. Bruice and M. F. Mayahi, *J. Amer. Chem. Soc.*, 1960, **82**, 3067; (*b*) J. L. O'Brien and C. Niemann, *ibid.*, 1957, **79**, 80; (*c*) E. Gaetjens and H. Morawetz, *ibid.*, 1960, **82**, 5328; (*d*) T. C. Bruice and S. J. Benkovic, *ibid.*, 1963, **85**, 1; (*e*) T. C. Bruice and J. M. Sturtevant, *ibid.*, 1959, **81**, 2860; (*f*) T. C. Bruice and A. F. Hegarty, *ibid.*, 1970, **92**, 6565; (*g*) A. R. Fersht, *ibid.*, 1971, **93**, 3504; (*h*) M. I. Page, *Chem. Soc. Rev.*, 1973, **2**, 295; (*i*) W. P. Jencks, B. Schaffhausen, K. Tornheim, and H. White, *J. Amer. Chem. Soc.*, 1971, **93**, 3917; (*j*) G. M. Blackburn and W. P. Jencks, *ibid.*, 1968, **90**, 2638; (*k*) N. Gravitz and W. P. Jencks, *ibid.*, 1974, **96**, 489, 499, 507.

selectivities of each attack were $\beta_{l.g.}$ −0.74 and −0.28 respectively. Although we are unable to estimate the β for the effect of substituents on the pK_a for the amido

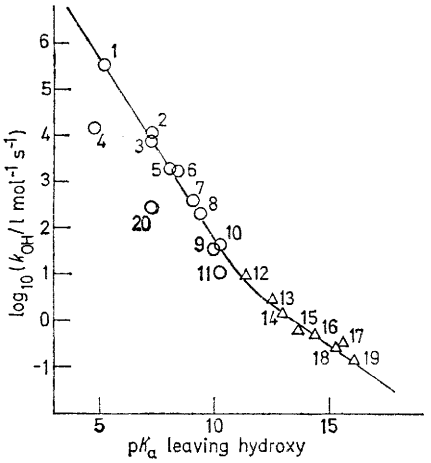
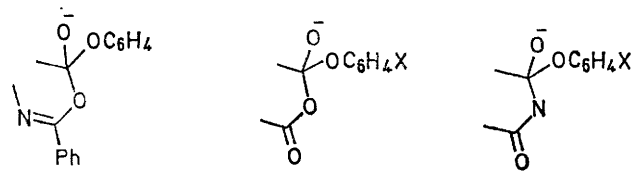


FIGURE 2 Brønsted type plot of $\log_{10} k_{OH}$ and pK_a of the leaving hydroxy: △, 'corrected' values, see Discussion section; lines have slopes 0.8 and 0.35. 1, 2,5-dinitrophenyl; 2, 2-nitrophenyl; 3, 4-nitrophenyl; 4, azide; 5, 4-acetylphenyl, 6, 3-nitrophenyl; 7, 3-chlorophenyl; 8, 4-chlorophenyl; 9, phenyl; 10, 4-methoxyphenyl; 11, 4-methylphenyl; 12, cyanomethyl; 13, 2,2,2-trifluoroethyl; 14, 2,2-dichloroethyl; 15, propargyl; 16, 2-chloroethyl; 17, methyl; 18, benzyl; 19, ethyl; 20, 4-nitrophenyl *N*-benzoylsarcosine

NH in the *N*-benzoylglycinate esters it must be close to zero and the reaction: conjugate base ⟶ oxazolinone would therefore have a $\beta_{l.g.}$ of *ca.* −0.8 in agreement with the ureido-results where oxygen attack yields high selectivities.

We believe that we can rationalise the magnitudes of the selectivities both here and in the other intramolecular reactions in terms of a tetrahedral intermediate. The



(1) (2) (3)

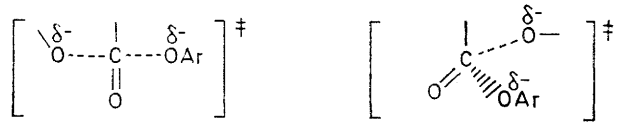
selectivity to leaving group depends on the rate-limiting step: if decomposition is rate limiting then a high $\beta_{l.g.}$ close to unity should result; if formation of the tetrahedral intermediate is rate limiting [$k_{-2} < k_3$ in equation (2)] $\beta_{l.g.}$ will be smaller and should be close to the β for attack of hydroxide ion on acetate esters (*ca.* −0.23).[7g] A fragmentation reaction giving rise to k_3 or k_{-2} in equation (2) as opposed to a bimolecular association with a 'tight' transition-state should not involve entropy terms due to changes in translational or rotational degrees of freedom provided that the transition state is close to reactant.[7h] The decomposition of tetrahedral intermediates probably involves a 'tight' transition state as evidenced by the high $\beta_{nuc.}$ values for aminolysis reactions.[7i-k] Partitioning of the tetrahedral intermediates (1)—(3) should depend on the leaving abilities of the groups and not on entropy factors. The pK_a of the leaving oxygen of an amide anion (1) may be calcu-calculated approximately *via* a thermodynamic cycle from amide pK_a (*ca.* 17) and the equilibrium constant between amide and isoamide (10^{-8}) [7h] and lies in the range for phenols. If we can assume that leaving group ability and pK_a are related (this is a reasonable assumption for the same leaving atom) then phenol departure from (1) is the likely rate-determining step yielding a high $\beta_{l.g.}$; phenol departure is also the likely rate-limiting step in (2). Attack of oxygen in the neutral ureido-group gives rise to a *protonated* nitrogen in (1) and the pK_a of the oxygen is likely to be even less than for phenols yielding a rate-determining k_3 step and a high $\beta_{l.g.}$. Nitrogen attack by the ureido-group yields (3) and the nitrogen pK_a is likely to be >15, well outside the phenol range making k_3 the *fast* step with a low $\beta_{l.g.}$.

The absence of a buffer effect at high buffer concentration eliminates a mechanism where proton transfer from the nitrogen is concerted with attack of the carbonyl oxygen. The effect at low 2,6-lutidine concentrations [3] could result from proton transfer which changes to rate-limiting subsequent steps as the buffer concentration increases; this effect is consistent with the stepwise path formulated in equation (2). Further evidence against the concerted mechanism is the absence of a large primary deuterium isotope effect which ought to be observed on the hydroxide term (k_{OH}). The observation (Table 2 and Results section) of an inverse deuterium oxide solvent isotope effect is evidence for a stepwise mechanism.[8] Proton exchange at the amide nitrogen,[9] more rapid than hydrolysis, ensures that there is complete labelling prior to reaction.

The theoretical rate law derived from application of the steady-state assumption to the kinetic scheme of equation (2) is given in equation (4). Since rate is invariant at high buffer concentration $k_{HB}[HB](k_{-2} + k_3) > k_2k_3$ and the law simplifies to an equation involving no buffer term; if k_3 is rate limiting an even simpler equation holds.

$$d[4\text{-NpO}^-]/dt = \frac{[\text{Ester}][B]k_Bk_2k_3}{k_{HB}[HB](k_{-2} + k_3) + k_2k_3} \quad (4)$$

The 'in-line' mechanism (4) is not warranted because



(4) (5)

it would require large distortion of bond angle and van der Waals radii for the oxygen to expel the

[8] M. L. Bender and R. B. Homer, *J. Org. Chem.*, 1965, **30**, 3975.

[9] (*a*) D. L. Hunston and I. M. Klotz, *J. Phys. Chem.*, 1971, **75**, 2123; (*b*) B. Leichting and I. M. Klotz, *Biochemistry*, 1966, **5**, 4026; (*c*) I. M. Klotz, *J. Colloid Interfacial Sci.*, 1968, **27**, 844.

phenolate. Another mechanism exists where the oxy-anion attacks perpendicularly to the ester plane concerted with departure of the phenolate anion (5) and with realignment of the carbonyl group to yield a planar product.

Changeover in Mechanism.—In order to compare the reactivity of the aryl esters with that of the alkyl esters a correction has to be applied to allow for the steric effect of the *ortho*-hydrogens on the aromatic nucleus of the leaving group. We use the isopropyl and cyclohexyl esters as steric models of the phenyl esters and these are some 8.7-fold less reactive than the primary alkyl esters with the same pK_a for leaving alcohol. The 8.7-fold correction is applied to Figure 2 (see Table 3). The Brønsted exponent for the alkyl esters is close to that expected for hydroxide attack on substituted phenyl esters (*ca.* 0.36).[7] The transition-state energy for the reaction $T^- \rightleftharpoons OX$ (see Figure 3) is

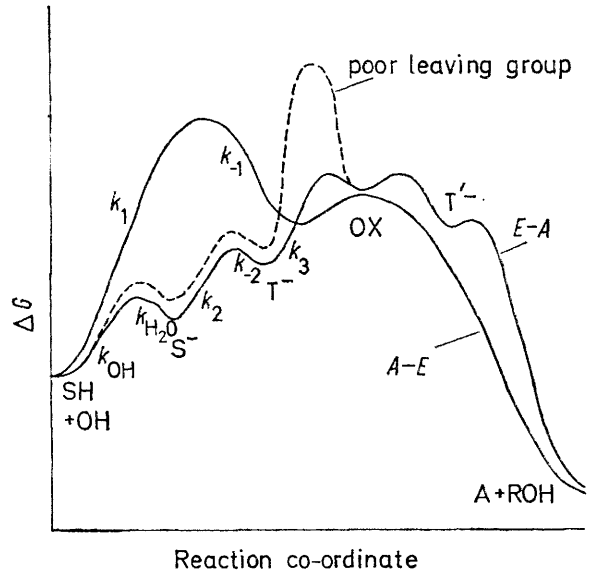


FIGURE 3 Potential energy diagram for the alkaline hydrolysis of *N*-benzoylglycinate esters. The symbols SH, S⁻, T⁻, Ox, and T'⁻ represent substrate, conjugate base, cyclic tetrahedral intermediate, oxazolinone intermediate, and tetrahedral intermediate for oxazolinone hydrolysis. For convenience we have labelled some individual steps with their corresponding rate constants [see equation (2)]. The dotted line is for an ester with a poor leaving group; the line (*A–E*) refers to the ordinary addition–elimination mechanism

very sensitive to leaving group variation and k_3 falls rapidly with increase in pK_a of the hydroxy-group. Eventually the transition-state energy exceeds that of the addition step of the addition–elimination reaction because this is not so sensitive to leaving group variation. The scheme is analogous to that for control of the mechanism in alkaline hydrolysis of carbamate esters [10] where a similar free energy diagram is proposed.[10b]

The changeover in mechanism from *E*1cB to addition–elimination occurs roughly at the phenol–alcohol junction and this is somewhat unfortunate and hints that the change in slope is due to a structural rather than a mechanistic change. A break occurs at the same junction in the hydrolysis of aryl and alkyl acetoacetates [10c] and an oximate ester with a pK_a (of the oxime hydroxy) similar to those of phenols lies on the line for alcohols suggesting some structural influence. In the case of *N*-benzoylglycinates Zerner and his co-workers [3a] detected some 0.35% oxazolinone formation in the hydrolysis of the cyanomethyl ester as would be expected because this ester (no. 12 in Figure 2) is close to the 'break' point on the *A–E* side. That the correction applied to the alkyl esters is a valid one is confirmed by the good fit of k_{OH} for 4-nitrophenyl *N*-benzoylsarcosine (see Figure 2) to the extrapolation of the Brønsted line of low slope.

Aminolysis Reaction.—The proportion of 4-nitrophenyl ester proceeding to oxazolinone, equivalent to the intercept in Figure 1, is some 12 times less than 4-nitrophenol release in 0.8M total ethyl glycinate buffer. This acceleration comes presumably from direct attack of amine at the ester rather than from attack on the oxazolinone because bases are unable to accelerate the formation of the latter intermediate. The 4-nitrophenyl ester itself is unlikely to exchange α-protons and any exchange *via* the oxazolinone (equivalent to racemisation in a chiral analogue) will therefore yield an upper limit of 1/12th of the exchanged final product. By increasing the amine concentration we increase peptide coupling (a bimolecular process) but do not affect oxazolinone formation and hence racemisation; racemisation of the oxazolinone is presumably base catalysed and therefore bimolecular so that the proportion of oxazolinone racemised or exchanged will increase but the upper limit of this reaction cannot exceed the value of 1/12th of the total reaction under the conditions mentioned above. These remarks may not be relevant to peptide couplings in non-aqueous media because the proton transfer step could be rate limiting for oxazolinone formation which could therefore be first order in base concentration.

Various techniques have been employed in peptide coupling reactions to prevent racemisation contributing to the overall product. One of these involves activated esters with leaving groups possessing functions which can catalyse the attack of amine intramolecularly (such as 8-hydroxyquinoline and *N*-hydroxypiperidine esters).[11] We can now see why these are successful: oxazolinone formation (leading to racemisation) is essentially only a specific base catalysed reaction; general bases, and in particular intramolecular general bases will have no effect although they may catalyse the aminolysis reaction.[12]

Our results show that the azide is some 20—30-fold

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[11] (*a*) H.-D. Jakubke, *Z. Naturforsch.*, 1965, **20***b*, 273; (*b*) H.-D. Jakubke, A. Voigt, and S. Burkhardt, *Chem. Ber.*, 1967, **100**, 2367; (*c*) J. H. Jones and G. T. Young, *J. Chem. Soc.* (*C*), 1968, 436; (*d*) B. O. Handford, J. H. Jones, G. T. Young, and T. F. N. Johnson, *J. Chem. Soc.*, 1965, 6814.

[12] T. C. Bruice, A. F. Hegarty, S. M. Fulton, A. Donzel, and N. G. Kundu, *J. Amer. Chem. Soc.*, 1970, **92**, 1370.

less reactive to oxazolinone formation than is the ester derivative with leaving hydroxy of similar pK_a. This lower reactivity is seen in other acyl reactions where azide is compared with phenol leaving groups for example the alkaline hydrolysis of carbamates ($NH_2COX \longrightarrow HNCO + HX$) [13] and methylaminosulphonates ($CH_3NHSO_2X \longrightarrow CH_3NSO_2 + HX$).[14] For these reactions the rate-determining step is the departure of the leaving group (as in oxazolinone formation) but even in a reaction where leaving group departure is fast compared to a prior rate-limiting step the same reactivity difference holds ($PhCH_2SO_2X \longrightarrow PhCHSO_2 + HX$).[15]

It is useful to compare rate constants for aminolysis with ethyl glycinate and for hydrolysis (*via* oxazolinone) for the azide ($k_{RNH_2/OH}$ 8.3×10^{-5}) with those for the 4-nitrophenyl ester ($k_{RNH_2/OH}$ 1.9×10^{-6}). Thus, when reacting with equimolar concentrations of amine or hydroxide ion the azide has *ca.* 50-fold more propensity to yield a peptide than to give oxazolinone compared with the 4-nitrophenyl ester. Of course, at neutral pH with low hydroxide concentration, the balance is heavily loaded in favour of peptide formation in both 4-nitrophenyl and azide cases.

Messrs A. Steltner and R. Orford are thanked for technical assistance. We thank Dr. B. H. Robinson, R. Oliver, W. T. Povey, and P. Smith for help in the construction of the stopped-flow apparatus and the Royal Society for a grant in aid to purchase the necessary equipment.

[4/2293 *Received, 5th November,* 1974]

[13] A. Williams, unpublished observations.

[14] (*a*) A. Williams and K. T. Douglas, *J.C.S. Chem. Comm.*, 1973, 356; (*b*) A. Williams and K. T. Douglas, *J.C.S. Perkin II*, 1974, 1727; (*c*) W. L. Matier, W. T. Comer, and D. Deitchman, *J. Medicin. Chem.*, 1972, **15**, 538.

[15] (*a*) A. Williams, K. T. Douglas, and J. S. Loran, *J.C.S. Chem. Comm.*, 1974, 689; (*b*) A. Williams, unpublished observations.