# Alkaline Hydrolysis of Esters possessing Readily Ionisable Amide Groups: Evidence for the 1→4 Migration of an Anilino-group in the Alkaline Reaction of *O*-Aroyl-*N*-arylglycolamides

By **James A. Boudreau** and **Andrew Williams,*** University Chemical Laboratories, Canterbury, Kent CT2 7NH

U.v. scanning experiments indicate the formation of diacylamine intermediates in the alkaline reaction of *O*-4-nitrobenzoyl- and *O*-benzoyl-*N*-nitrophenylglycolamide. The product of these reactions includes the benzanilide derived from a 1→4 migration of the anilino-group. As the electron-withdrawing power of the substituent on the anilino-group decreases the mechanism involving direct hydroxide attack on the acyl group takes the major portion of the reaction flux.

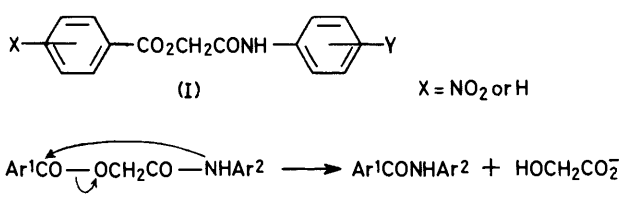
We have been studying the hydrolysis of esters possessing amide groups as analogues of discrete reactions constituting the overall catalytic action of serine proteases.[1] At high alkaline strength the rate constants for hydrolysis of *O*-benzoyl-*N*-phenylglycolamides are no longer proportional to the hydroxide ion concentration and the *O*-4-nitrobenzoyl derivative showed good evidence for the formation and decay of an intermediate as demonstrated by scanning the u.v. spectrum of the solution during reaction. The rate constants for the alkaline hydrolysis of the benzoates except the *N*-4-nitrophenyl derivative were close to those expected for direct attack of hydroxide ion on the ester;[1] we decided to investigate the hydrolysis of esters of type (I) in more detail with a view to establishing the mechanistic paths involved because there was the possibility that these possessed an interesting 1 → 4 shift of an anilino-group.

TABLE 1

Analytical and physical properties of substrates[a]

| Compound | M.p. (°C) | Found (%) C | H | N | Formula | Requires (%) C | H | N |
|---|---|---|---|---|---|---|---|---|
| α-Chloroacetanilides | | | | | | | | |
| 4-Nitro | 185—186 | 44.8 | 3.4 | 13.0 | $C_8H_7ClN_2O_4$ | 44.8 | 3.3 | 13.1 |
| 3-Chloro | 100—102 | 47.2 | 3.5 | 6.8 | $C_8H_7Cl_2NO_2$ | 47.1 | 3.4 | 6.9 |
| 4-Chloro | 172—173 | 47.1 | 3.3 | 6.9 | $C_8H_7Cl_2NO_2$ | 47.1 | 3.4 | 6.9 |
| 4-Methyl | 165—156 | 59.2 | 5.2 | 7.6 | $C_9H_{10}ClNO_2$ | 58.9 | 5.5 | 7.6 |
| 4-Methoxy | 122—122.5 | 54.0 | 5.3 | 6.9 | $C_9H_{10}ClNO_3$ | 54.1 | 5.0 | 7.0 |
| α-Chloro-*N*-methylacetanilide | | | | | | | | |
| | 70—71 | 59.2 | 5.5 | 7.6 | $C_9H_{10}ClNO_2$ | 58.9 | 5.5 | 7.6 |
| *O*-Benzoyl-*N*-(substituted phenyl)glycolamides | | | | | | | | |
| 4-Nitro | 169—170 | 59.8 | 4.2 | 9.3 | $C_{15}H_{12}N_2O_5$ | 60.0 | 4.0 | 9.4 |
| 3-Chloro | 157—159 | 62.2 | 4.1 | 4.8 | $C_{15}H_{12}ClNO_3$ | 62.2 | 4.1 | 4.8 |
| 4-Chloro | 152—154 | 61.9 | 4.1 | 4.7 | $C_{15}H_{12}ClNO_3$ | 62.2 | 4.1 | 4.8 |
| 4-Methyl | 180—181 | 71.7 | 5.8 | 5.0 | $C_{16}H_{15}NO_3$ | 71.4 | 5.6 | 5.2 |
| 4-Methoxy | 135—137 | 67.4 | 5.2 | 4.9 | $C_{16}H_{15}NO_4$ | 67.4 | 5.3 | 4.9 |
| *O*-Benzoyl-*N*-methyl-*N*-phenylglycolamide | | | | | | | | |
| | 86—88 | 71.1 | 5.7 | 5.1 | $C_{16}H_{15}NO_3$ | 71.4 | 5.6 | 5.2 |
| *O*-4′-Nitrobenzoyl-(*N*-substituted phenyl)glycolamides | | | | | | | | |
| 4-Nitro | 181—182 | 52.5 | 3.4 | 12.3 | $C_{15}H_{11}N_3O_7$ | 52.2 | 3.2 | 12.2 |
| 3-Chloro | 169—170 | 53.6 | 3.4 | 8.1 | $C_{15}H_{11}ClN_2O_5$ | 53.8 | 3.3 | 8.4 |
| 4-Chloro | 187—188 | 53.6 | 3.5 | 8.1 | $C_{15}H_{11}ClN_2O_5$ | 53.8 | 3.3 | 8.4 |
| 4-Methyl | 180—181 | 60.9 | 4.7 | 8.7 | $C_{16}H_{14}N_2O_5$ | 61.2 | 4.5 | 8.9 |
| 4-Methoxy | 179—180 | 58.1 | 4.4 | 8.3 | $C_{16}H_{14}N_2O_6$ | 58.2 | 4.2 | 8.5 |
| *O*-4′-Nitrobenzoyl-*N*-phenyl-*N*-methylglycolamide | | | | | | | | |
| | 113—114 | 61.0 | 4.5 | 8.7 | $C_{16}H_{14}N_2O_5$ | 61.2 | 4.5 | 8.9 |

[a] M.p.s were determined with a Kofler hot stage Thermospan instrument; analyses were carried out by Mr. G. Powell of the University of Kent Microanalytical Laboratory using a Hewlett–Packard model 185 CHN analyser.




## EXPERIMENTAL

*Materials.*—α-Chloroacetanilides were prepared by dissolving or suspending the appropriate aniline (0.2 mol) in dichloromethane (300 ml) and cooling the mixture to −10°. A solution of α-chloroacetyl chloride (0.1 mol) in dichloromethane was then slowly added dropwise with efficient stirring. The precipitated aniline hydrochloride was removed by filtration and the solvent evaporated *in vacuo* to yield a residue which was diluted with water. The solid product was isolated by filtration and recrystallised from methanol. An alternative procedure was to evaporate the reaction mixture directly and treat it with water. Table 1 collects the analytical and physical data.

*O*-Aroyl-*N*-arylglycolamides were prepared by mixing the

[1] J. A. Boudreau and A. Williams, *J.C.S. Perkin II*, 1977, 1221.

appropriate acid (50 mmol) with triethylamine (50 mmol) in ethyl acetate. The α-chloroacetanilide (50 mmol), dissolved in a suitable solvent (ethyl acetate, ethanol, or dimethyl sulphoxide), was added dropwise to the solution which was then refluxed gently overnight. The mixture was cooled, excess of water added, and the precipitate removed by filtration. Recrystallisation from methanol–water gave analytically pure material (see Table 1 for analytical and physical data). The substrates were characterised by i.r. and n.m.r. spectroscopy using Perkin-Elmer 237 or 257 and Perkin-Elmer R10 instruments respectively.

*Methods.—Kinetics.* Reactions were followed using either a Unicam SP 800 or Beckman DBG u.v. recording spectrophotometer. The substrate was introduced into buffer (2.5 ml in a silica cell in a thermostatted cell holder) on the flattened tip of a glass rod (typically 25—50 λ). The recorder was activated at the instant of entry and the reaction followed either at a constant wavelength or, in the former instrument, by repetitive spectral scanning. Pseudo-first-order rate constants were obtained by plotting $A_t - A_\infty$ (the absorption difference) *versus* time on semi-logarithmic graph paper.

Where the repetitive scanning of the u.v. spectrum showed the existence of an intermediate [equation (1)] the kinetics for its formation and decay were determined, in general, by

$$A \longrightarrow B \longrightarrow C \qquad (1)$$

following the A ⟶ B reaction at an isosbestic wavelength between B and C and the B ⟶ C reaction at an isosbestic wavelength between A and B.

*Product analysis.* This was carried out using a large volume of buffer with substrate at the same low concentration as in the kinetic experiments. The solution was allowed to stand till reaction was complete as judged from the kinetic experiments and then acidified to pH 1 with dilute HCl. The solution was extracted with chloroform and the chloroform layer dried ($MgSO_4$) and evaporated to a volume of 10 ml. Reference solutions of possible products were made up to the theoretical concentration in chloroform assuming 100% conversion. The solutions were then analysed by t.l.c. on pre-made silica gel plates (Merck) with 4 : 1 ethyl acetate–methanol as eluant (ascending). The $R_F$ value and intensity of the spots, detected by a u.v. lamp, allowed an analysis of the product mixture to be made.

Measurements of pH were carried out using a Pye–Dynacap instrument with a Radiometer B-type electrode.

## RESULTS

*N-Aryl-O-benzoylglycolamides.*—These esters decomposed in alkaline solution with no evidence for an intermediate except in the case of the *N*-4-nitrophenylglycolamide. The hydrolytic data for the former compounds in increasing base concentration are given in Supplementary Publication No SUP 22129 (4 pp.) * and illustrated in Figure 1. The 4-nitrophenyl species exhibited an initial fast reaction followed by a slower one and each reaction possessed its own set of isosbestic wavelengths. The rate data for the individual steps in the hydrolysis of the 4-nitrophenyl species are given in SUP 22129 and are illustrated in Figures 2 and 3.

*N-Aryl-O-4′-nitrobenzoylglycolamides.*—These species reacted, as expected, far more rapidly than the parent *O*-benzoyl derivatives in alkali. Spectral scanning of the u.v. region indicated that the reaction of the *N*-4-nitrophenylglycolamide possessed an intermediate and the rate constants

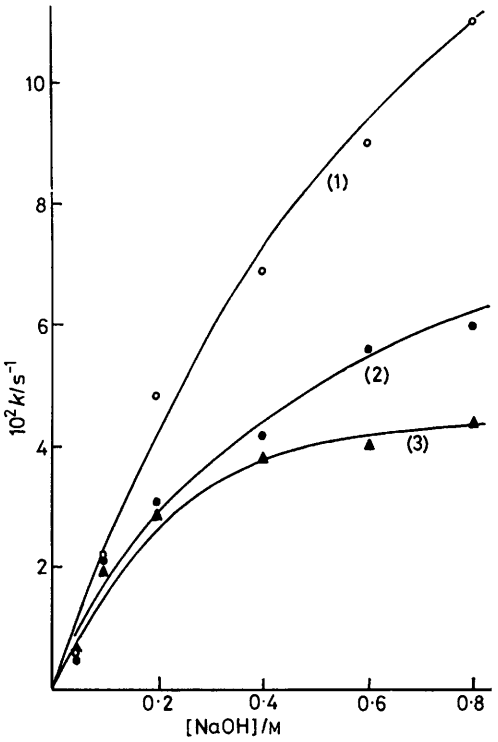



FIGURE 1 Hydrolysis of *O*-benzoyl-*N*-(substituted phenyl)-glycolamides as a function of hydroxide ion concentration: 25°, ionic strength made up to 1M with NaCl, 20% dioxan–water (v/v): (1) 4-methyl; (2) 4-chloro; (3) 3-chloro. Lines are theoretical from parameters in Table 2

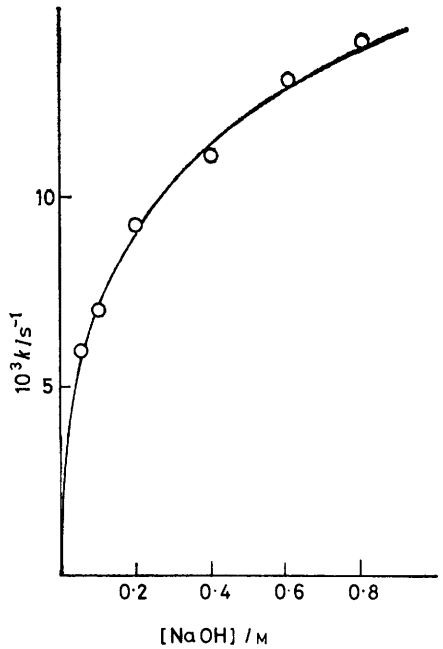



FIGURE 2 Dependence on hydroxide ion concentration of the rate constant for reaction A ⟶ B for decomposition of *O*-benzoyl-*N*-4-nitrophenylglycolamide at 25°, 20% dioxan–water (v/v), ionic strength made up to 1M with NaCl. Line is theoretical from parameters collected in Table 2

* For details of Supplementary Publications see Notice to Authors No. 7 in *J.C.S. Perkin II*, 1976, Index issue. Items less than 10 pp. are supplied as full-size copies.

for the A ⟶ B and B ⟶ C reactions are given in SUP 22129. The rate constants for the other species are recorded in SUP 22129 and u.v. spectral scanning for these compounds

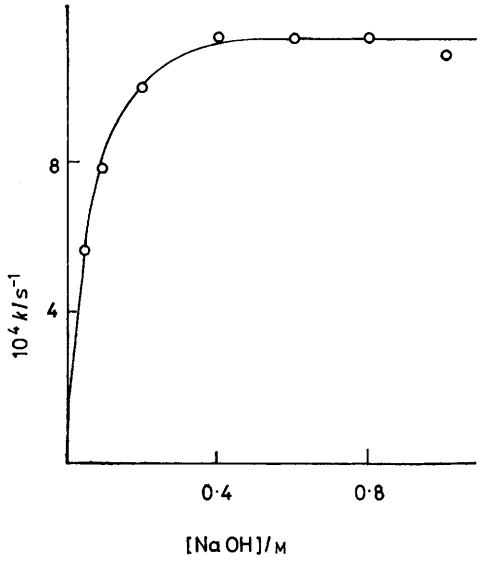



FIGURE 3 Dependence on hydroxide ion concentration of the rate constant for reaction B ⟶ C from alkaline decomposition of *O*-benzoyl-*N*-4-nitrophenylglycolamide at 25°, 20% dioxan–water (v/v) and ionic strength made up to 1M with NaCl. Line is theoretical from parameters in Table 2

indicated no intermediate. Hydrolysis of the *N*-phenyl-*O*-4-nitrobenzoylglycolamide however in water as opposed to 20% dioxan–water buffers gave definite evidence for an intermediate as judged from the u.v. scanning experiments. The formation and decay of this intermediate is illustrated in Figure 4.

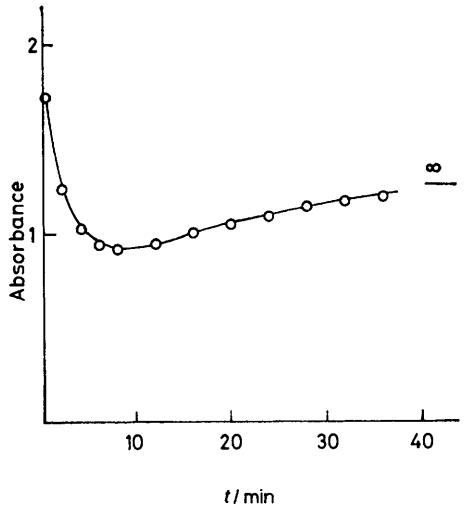



FIGURE 4 Formation and decay of intermediate determined at 250 nm for the hydrolysis of 4-nitrobenzoyl-*N*-phenylglycolamide in pH 9.4 buffer at 25°; ionic strength is made up to 0.1M with NaCl; carbonate buffers

The hydrolysis of *O*-benzoyl-*N*-phenylglycolamide and *O*-4-nitrobenzoyl-*N*-methyl-*N*-phenylglycolamide in alkali exhibited no evidence for intermediates and the rate constants at different pH values are given in SUP 22129.

Table 2 collects the rate data for all the substrates together with apparent p$K_a$ values obtained from plots of reciprocal

TABLE 2

Collection of p$K_a$ and rate constant data for the alkaline reaction of *O*-aroyl-*N*-arylglycolamides [a,g]

| Ester | p$K_a$ [b] | $k_{OH}$[c]/ l mol$^{-1}$ s$^{-1}$ | $k_{OH}^{NMe}$/ l mol$^{-1}$ s$^{-1}$ | $k_{plateau}$[c]/s$^{-1}$ |
|---|---|---|---|---|
| *O*-Benzoyl-*N*-(substituted phenyl)glycolamides [e] | | | | |
| 3-Chloro | 13.8 | 0.33 | | 0.052 |
| 4-Chloro | 14.0 | 0.30 | | 0.076 |
| Unsubstituted [f] | 14.3 | 0.30 | 0.031 | 0.15 |
| 4-Methyl | 14.5 | 0.25 | | 0.20 |
| 4-Methoxy | 14.4 | 0.25 | | 0.16 |
| 4-Nitro A ⟶ B | 13.8 | | | 0.016 |
| B ⟶ C | 13.5 | 0.011 4 | | |
| *O*-4-Nitrobenzoyl-(*N*-substituted phenyl)glycolamide [d] | | | | |
| 3-Chloro | 13.8 | 11 | | |
| 4-Chloro | | 11 | | |
| Unsubstituted [f] | | 9.1 | 0.13 | |
| 4-Methyl | | 9.0 | | |
| 4-Methoxy | | 8.4 | | |
| 4-Nitro A ⟶ B | 12.7 | | | 0.14 |
| B ⟶ C | | 0.010 6 | | |

[a] 25°, 20% dioxan–water (v/v). [b] These were estimated from reciprocal plots of $1/k$ *versus* 1/[NaOH] using the value for $K_w$ in the medium (see footnote *a*) of 2.399 × 10$^{-15}$ mol l$^{-1}$ from H. S. Harned and L. D. Fallon, *J. Amer. Chem. Soc.*, 1939, **61**, 2374. [c] The value of $k_{plateau}$ for reactions with an ionisable group proceeding *via* direct attack of hydroxide on the neutral species is $k_{OH}K_w/K_a$. The value of $k_{OH}$ for acidic species undergoing decomposition of the conjugate base is $k_{plateau}K_a/K_w$. [d] Ionic strength kept at 0.1M with NaCl. [e] Ionic strength kept at 1M with NaCl. [f] This compound is from a previous study.[1] [g] All compounds except the 4-nitro-derivatives are hydrolysed without evidence for an intermediate.

rate constants *versus* reciprocal hydroxide ion concentrations.

*Product Analysis.*—Table 3 collects the t.l.c. results for the analysis of the products of hydrolysis of *O*-4′-nitro-

TABLE 3

T.l.c. analysis of the products of alkaline hydrolysis [a]

| Compound | $R_F$ | |
|---|---|---|
| *O*-Benzoyl-*N*-4-nitrophenylglycolamide | | |
| Benzoic acid | 0.39 | |
| *N*-Benzoyl-4-nitroaniline | 0.54 | |
| Hydrolysis product | 0.36 faint [c] | 0.38 strong [b] |
| | 0.52 strong [c] | 0.52 strong [b] |
| *O*-4′-Nitrobenzoyl-*N*-4-nitrophenylglycolamide | | |
| 4-Nitrobenzoic acid | 0.08 | |
| *N*-4′-Nitrobenzoyl-4-nitroaniline | 0.70 | |
| Hydrolysis product | 0.08 strong [d] | |
| | 0.70 strong [d] | |

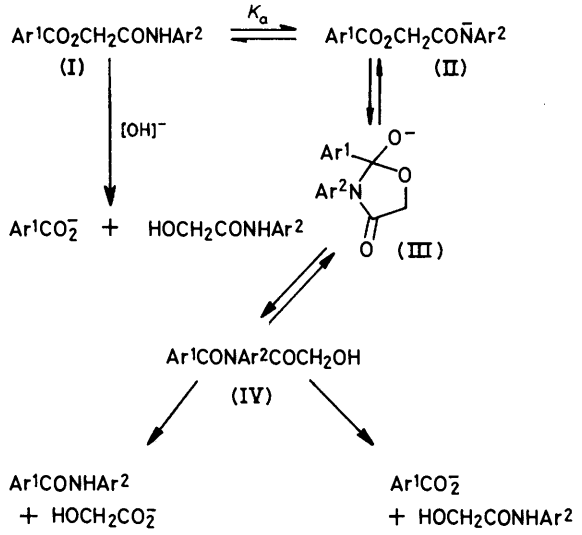
[a] T.l.c. on silica gel (ascending) with eluant ethyl acetate–methanol (4 : 1). [b] Hydrolysis in 1M-NaOH. [c] Hydrolysis in 0.05M-NaOH. [d] Hydrolysis in 0.01M-NaOH.

benzoyl-*N*-4-nitrophenyl- and *O*-benzoyl-*N*-4-nitrophenylglycolamide. Both benzoic acid and 4-nitro-*N*-benzoylaniline were products of the latter reaction in alkali and 4-nitro-*N*-4′-nitrobenzoylaniline and 4-nitrobenzoic acid for the former.

## DISCUSSION

The isolation from the product mixtures of benzanilides as well as benzoic acids (Table 3) clearly indicates that

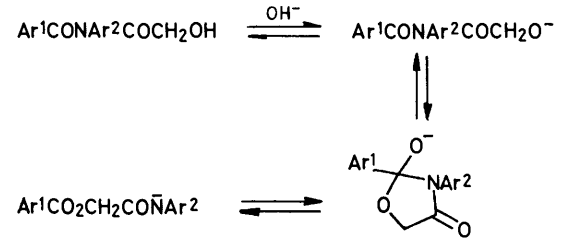
simple hydrolysis is not occurring in all reactions of *O*-aroyl-*N*-arylglycolamides and is strong evidence for an intermediate which can break down to give the transfer products. These results together with the observation of intermediates (see for example Figure 2) suggest that




SCHEME 1

the mechanism for the alkaline reaction involves a diacylamine intermediate as postulated in Scheme 1. Moreover, there is no evidence for the existence of an intermediate in the alkaline hydrolysis of *N*-methyl-*N*-phenylglycolamide esters and these species have rate constants corresponding to hydroxide ion attack at the ester carbonyl (Table 2).

The pH-dependence for the decomposition of the diacylamine intermediate (Figure 3) is consistent with the ionisation of a weakly acidic species (p$K_a$ 13.5). The pH-dependence of the hydrolysis of diacylamines substituted on the nitrogen should not possess an inflection [2] and we ascribe the observed behaviour to the ionisation of the alcohol group; linear free energy estimations of



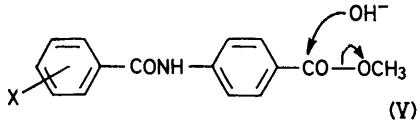

SCHEME 2

the p$K_a$ values for two models for the alcohol (IV), phenacyl alcohol, and hydroxyacetone give values close to the observed figure (12.8 and 13.5 respectively).[3] The oxyanion species is resistant to further attack by hydroxide ion and the only path left open for decomposition of the anion is reversion to reactants (Scheme 2).

The rate constant for formation of the intermediate has a pH-dependence (Figure 2) which is also consistent with the scheme; the inflection corresponds to a p$K_a$ (Table 2) characteristic of an anilide NH.[4]

Hydroxide ion attack on intermediates from the *N*-4-nitrophenylglycolamides has rate constant $1.06 \times 10^{-2}$ and $1.14 \times 10^{-2}$ l mol$^{-1}$ s$^{-1}$ at 25°, 20% dioxan–water for *O*-4-nitrobenzoate and benzoate respectively (Table 2). These figures are lower than those found by Edward and Terry [2b] for the alkaline hydrolysis of diacetylamine, a good model for the intermediates in question. Attack in the case of the present intermediates is presumably on the aliphatic acyl group because attack on the aroyl group would be expected to yield a rate constant much larger for the 4-nitrobenzoate than for the benzoate. There would also be no benzanilide products; some cleavage must of course be occurring at the aroyl group to yield the observed benzoic acid products. We suggest that the lower rate constants for the intermediates compared with the diacetylamine are due to the larger steric requirements for attack of hydroxy at the former species.

*Change in Mechanism.*—Only the *N*-4-nitrophenylglycolamide gives evidence for an intermediate in the alkaline hydrolysis of the *O*-benzoyl series of esters; this is consistent with a changeover in the path taking the major part of the reaction flux from direct attack of hydroxide on the ester link to the path involving the intermediate (for the *N*-4-nitrophenyl derivative). The effect of substituents on the rate constant for hydroxide attack on *O*-benzoyl-*N*-(substituted phenyl)glycolamides is essentially zero as expected (see Table 2); owing to ionisation of the amino-group the observed first-order rate constants at a given low hydroxide ion concentration have a positive $\beta$ (+0.25) for the Brønsted plot *versus* p$K_a$ of the corresponding anilinium species (see SUP 22129 for 0.05M-NaOH). This type of behaviour has been noted before in compounds where there is an ionisable species (V) and the effect can be to make the hydroxide ion reaction *more* efficient in substrates with electron-*donating* substituents.[4a] The Brønsted $\beta$ for the ionisation of the anilide group (*versus* anilinium p$K_a$) is −0.35



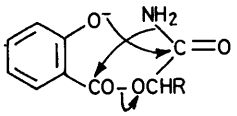

(data in Table 2) and the $\beta_{nuc}$ for intramolecular attack of the anionic nitrogen on the ester is presumably positive but less than +0.35 since the negative charge is not totally neutralised in the transition state. The overall $\beta$ for the intramolecular reaction is probably therefore $\leq 0$;

[2] (*a*) A. H. Lamberton and A. E. Standage, *J. Chem. Soc.*, 1960, 2957; (*b*) J. T. Edward and K. A. Terry, *ibid.*, 1957, 3527.
[3] G. B. Barlin and D. D. Perrin, *Quart. Rev.*, 1966, **20**, 75.
[4] (*a*) A. Williams and K. T. Douglas, *J.C.S. Perkin II*, 1972, 2112; (*b*) K. Bowden, *Chem. Rev.*, 1966, **66**, 119; (*c*) W. P. Jencks and J. Regenstein in ' Handbook of Biochemistry, section J–187,' ed. H. A. Sober, Chemical Rubber Company, Cleveland, 1970, 2nd edn.

as the substituents become more electron withdrawing a changeover in mechanism from direct hydroxide ion attack to the intramolecular mechanism is possible.

The effect of increasing the pH on the hydrolysis of *N*-aryl-*O*-benzoylglycolamides *via* direct attack is to decrease the proportion of neutral ester available for reaction with hydroxide ion thus giving rise to the curved plots of Figure 1. It is unlikely that the $pK_a$ values (see Table 2) refer to ionisation of the proton on the α-carbon as these would be expected to have much higher values.[4c] In the substituent variations discussed above the selectivity differences for direct and intramolecular attack are small so that the overall rates will be similar for both mechanisms and it is therefore likely that both mechanisms will exist on a relatively equal footing over a range of substituent variation. The balance between direct hydroxide attack and the intramolecular mechanism is quite fine and this results in a change in mechanism for the *O*-4-nitrobenzoyl-*N*-phenylglycolamide reaction; in 20% dioxan–water the intermolecular mechanism predominates whereas in water solvent the intramolecular mechanism is favoured.

Recently we reported an alkaline rearrangement of *O*-salicyloylglycolamides (VI) involving intramolecular transfer of the amino-group from the amide to the salicyloyl function.[5] This rearrangement requires the presence of an *o*-hydroxy-group on the benzoyl function (as in the salicylates). The present work describes an intramolecular transfer process giving essentially the same type of

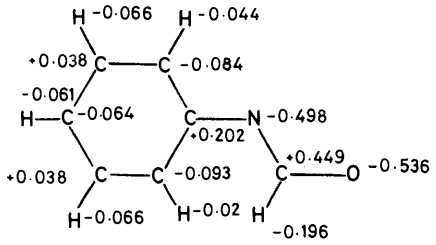



(VI)

transfer products but without the requirement for an *o*-hydroxy-group on the acyl function of the ester. The transfer process in this reaction requires the presence of an anilino-group preferably possessing an electron-withdrawing substituent; this presumably increases the concentration of the nitrogen anion at a given pH sufficiently to enable the intramolecular transfer to compete successfully with the intermolecular hydroxide attack. The amido-function in the *O*-salicyloylglycolamide is not sufficiently acidic for a kinetically significant concentration of the anion to be formed; activation of the amide nitrogen as a nucleophile comes in this case from attack on the amide by the *o*-oxyanion creating a more nucleophilic amino-group.

*Ionisation of the Amino-group of the* N-*Arylglycolamides.*—The selectivity of the ionisation ($pK_a$) of the amino-group in the *N*-arylglycolamides (Table 2) *versus* the $pK_a$ of the corresponding anilinium species is low (+ 0.35) and Steward and O'Donnell [6] found a value of + 0.6 for the ionisation of substituted anilines. The acidity difference between aniline and anilide from this and Steward and O'Donnell's work for the 4-nitro-substituent is *ca.* 6 $pK_a$ units.

Semi-empirical SCF MO calculations using the INDO [7] method indicate that for the anion of a model anilide




(VII)

[formanilide (VII)[6]] there is very little charge on all but the *ipso*-position of the aromatic ring; most of the unit charge resides almost equally on the oxygen and nitrogen. Thus the electronic effect of substituents on *ortho*-, *meta*-, and *para*-positions is predicted to be small, consistent with the observed selectivity. The calculations are probably quite valid for the estimation of excess of charge on the various atoms as Pople and Beveridge [7] show that there is remarkable agreement between observed and calculated dipole moments for a range of compounds. We are of course aware of the serious limitations of the INDO method in calculating absolute energies [7] and of the limitations even in estimating relative energies.

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[7/778 *Received*, 6*th May*, 1977]

[5] J. A. Boudreau, C. R. Farrar, and A. Williams, *J.C.S. Perkin I*, 1977, 1804.

[6] R. Stewart and J. P. O'Donnell, *Canad. J. Chem.*, 1964, **42**, 1694.

[7] The INDO calculations were carried out using the programme from J. A. Pople and D. L. Beveridge, 'Approximate Molecular Orbital Theory,' McGraw-Hill, New York, 1970; no minimisation was attempted and the molecular parameters were from the ' Tables of Interatomic Distances and Configurations in Molecules and Ions,' Chemical Society Special Publications Nos. 11 and 18. The bond lengths and angles for the amido-anion were assumed to be close to those for the conjugate acid. Computations were performed on the London University CDC 7600 machine connected with the University of Kent Computer Centre by land line.