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AMIDE GROUP CATALYSIS OF ESTER HYDROLYSIS

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Abstract—Hydrolysis of phenyl-*N*-acetylanthranilate **1** (Ar = Ph) to *N*-acetyl anthranilic acid **3** is base catalysed and occurs through the intermediacy of 2-methyl-3,1-benzoxazin-4-one **4**. Above pH 6 the formation of **4** from **1** is faster than the base (or acid) catalysed ring opening of **4** to **3**. Electron-withdrawing substituents in the ester moiety (e.g. **1**, Ar = *p*-$NO_2C_6H_4$) aid cyclization to **4** relative to direct hydroxide catalyzed hydrolysis (to **3**). Concomitantly, neutral amide participation is observed so that cyclization of **1** (Ar = *p*-NO_2, *m*-NO_2, *m*-Cl, *p*-ClC_6H_4) occurs even in acidic solution; the Hammett ρ values for neutral and base catalysed cyclizations are compared. Hydrolysis of methyl-*N*-acetylanthranilate **6** to **3** occurs slowly in base, possibly without the formation of the intermediate **4**.

Nucleophilic participation by an amide group in ester,[1,2] carbamate,[3] phosphate ester,[4] and amide group[5] hydrolysis has been widely studied, mainly as an analogue for biologically mediated reactions involving the widely distributed peptide (or amide) linkage. In general, attack by the ambident amide anion occurs through nitrogen; for example β-benzyl esters of N-benzyloxycarbonyl-l-aspartyl amide cyclize in base to give the corresponding imides,[6] while O-acylsalicylamides are also converted to imides in basic solution.[7] The role of the amide (involving nitrogen participation) may not always be regarded as catalytic for overall ester hydrolysis since the intermediate imides formed may be hydrolysed at a rate which is slower than direct hydrolysis of the original ester;[6] The factors which govern imide stability have been summarised by Topping *et al.*[7]

The neutral amide group reacts via oxygen independent of whether the site undergoing reaction is acyl[1] or tetrahedral[8] carbon. When the ester linkage is attached (via a carbon skeleton) to the acyl carbon of the amide group then ring size is unimportant in determining the nucleophilic site (since the same size ring is formed in either case). This is not true when the ester is linked through the amide nitrogen. We have used a series of anthranilate esters **1** of this type; the position of the amide nitrogen in **1** ensures that attack via nitrogen does not occur so that neutral and anionic participation by the oxygen of the amide group can be studied directly.

RESULTS AND DISCUSSION

Repetitive scans of the ultraviolet spectrum of **1** (Ar = Ph) measured in water at 25° at pH 7.5 (maintained by 0.003M phosphate buffer) are shown in Fig. 1. Initially the absorbance increases at 300 nm and good isosbestic points are formed at 322 and 289 nm. After *ca.* 1 h the absorbance at 300 nm had reached a maximum and then decreased slowly. The original isosbestic points are then lost and a new one is formed for the subsequent reaction at 296 nm. The faster initial reaction (disappearance of **1** (Ar = Ph)) was followed by measuring the change in optical density at 333 nm where the subsequent reaction had a negligible spectral change. The results are summarised in Table 1; in all cases a buffer (acetate, phosphate or borate) was present at 0.003M to maintain pH

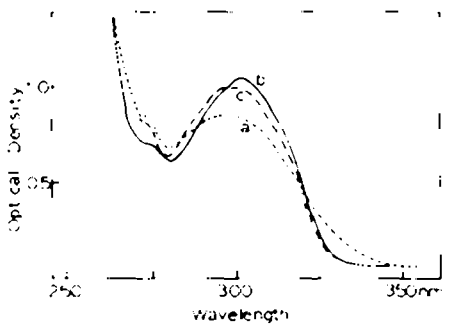


Fig. 1. Repetitive scans of the ultraviolet spectrum of phenyl *N*-acetylanthranilate (**1**, Ar = Ph) at pH 7.5 in water. Scan a is largely unreacted **1** (Ar = Ph); scan b was taken after 1 h, when the benzoxazinone **4** is the major species present; scan c was taken after a further 24 h when **4** is converted to **3**.

and separate experiments showed that at this concentration no buffer catalysis of the reaction was apparent. Clearly the rate of the first reaction is directly proportioned to {HO$^-$} over the range studied.

That the second reaction observed (Fig. 1) represents the ring opening of an initially formed 2-methyl-3,1-benzoxazin-4-one **4** is consistent with the following evidence. (a) The position of the isosbestic point for the subsequent reaction at 296 nm is the same as that ob-

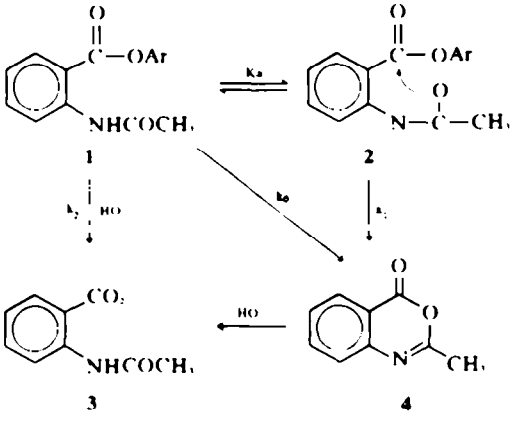


Table 1. Rate constants for the hydrolysis of Phenyl-*N*-acetyl anthranilate at 25° in water (μ = 0.10 M KCl)

pH	5.3	6.5	7.5	8.0	8.5
$10^4 k_{obs}$, s^{-1}	0.39	4.0	36	101	372

served for the hydrolysis of an authentic sample of **4** measured under the same conditions (see also Ref. 9). (b) When the initial reaction of **1** was essentially complete at pH 8.5 (after 95 sec) the pH of the solution was rapidly adjusted to the region 3–6 and maintained constant by the presence of 0.01M formate or acetate buffer. The absorbance change at 310 nm was then measured as a function of time. In this pH region any residual **1** (Ar = Ph) is essentially inert but the benzoxazinone **4** is known to undergo acid catalysed ring opening to give **3**. The rate constants calculated from these data were the same (within experimental error) as those measured for an authentic sample of **4** measured under the same conditions (e.g. at pH 3.95, $k_{obs} = 3.11 \times 10^{-3} s^{-1}$ ($3.16 \times 10^{-3} s^{-1}$ for **4**); at pH 5.25, $k_{obs} = 1.11 \times 10^{-4} s^{-1}$ ($1.16 \times 10^{-4} s^{-1}$ for **4**)). (c) At pH 11.0, when the hydrolysis of **1** (Ar = Ph) was investigated, the absorbance at 310 nm was found to continuously decrease and a good first order plot with $k_{obs} = 3.06 \times 10^{-2} s^{-1}$ was obtained. From the data in Table 1 it can be calculated that the initial hydrolysis of **1** (Ar = Ph) would be very rapid under these conditions (with $k \sim 10 s^{-1}$). However, the observed rate constant is close to that measured independently for the ring opening of **4** at this pH ($k = 2.98 \times 10^{-2} s^{-1}$); clearly under these conditions the ring opening (**4**→**3**) is the reaction observed spectrophotometrically.

Thus we conclude that the hydrolysis of **1** to **3** is stepwise at all pH's, occurring via the intermediacy of the benzoxazinone **4**. Both the initial cyclization and ring opening of **4** are base catalysed but when Ar = Ph the cyclization in basic solution is *ca.* 300-fold faster than the conversion of **4** to **3**. Below pH *ca.* 6 however the ring opening is acid catalysed so that as the pH is decreased this step becomes faster than the initial cyclization.

Since the pKa of the amide group in **1** is >13, the pH dependence observed for the rate of cyclization of **1** (Table 1) is consistent with reaction occurring via the conjugate base **2**, steric restrictions ensuring that only O attack occurs in the present instance. Of the two competing reaction pathways for base catalysed reaction of **1**, (viz. **2**→**4** or **1** + HO → **3**) the overall rate of conversion to **4** via the anion is greatly favoured (*ca.* 15,000-fold) relative to direct HO attack, which is of the order of magnitude ascribable to unambiguous neighbouring group participation.

At pH 12 the rate of hydrolysis of phenyl benzoate (25°, 4:1 water–dioxan, μ = 0.1 KCl) is $6.4 \times 10^{-3} s^{-1}$. At this pH the slow step for the overall conversion of **1** (Ar = Ph) to **3** is the final step (**4**→**3**). Therefore the overall rate enhancement provided by the stepwise route via **4** relative to direct HO catalysed ester hydrolysis (**1**→**3**) is *ca.* 50-fold. This ignores any electronic effect that the amide group might have in promoting HO attack in **1**, but this is expected to be small since the σ value for a p-$NHCOCH_3$ group is quoted[10] as 0.0.

Substituent effects. The rates of hydrolysis of *p*-nitrophenyl *N*-acetylanthranilate (**1**, Ar = p-$NO_2C_6H_4$) are summarised in Table 2. It is seen that at high pH the rate of disappearance of **1** (Ar = p-$NO_2C_6H_4$) is again base

Table 2. Rate constants for the hydrolysis of *p*-nitrophenyl-*N*-acetylanthranilate at 25° in water (μ = 0.10M KCl)

pH	7.0	6.15	5.0	3.0	2.0	1.0	0.0*
$10^4 k_{obs}$, s^{-1}	71	11	1.7	0.83	0.95	0.82	0.83

*μ = 1.0.

catalysed, but enhanced relative to **1** (Ar = Ph). Also at low pH a new reaction becomes apparent which (as measured by the decrease in absorbance at 340 nm) is pH independent. Again at pH 5 there is evidence of the presence of the benzoxazinone **4** since the initial isosbestic point at 294 nm drifts to longer wavelengths as the reaction proceeds. Below pH *ca.* 3, since the pH independent cyclization of **1** (Ar = p-$NO_2C_6H_4$) to **4** is slower than acid catalysed ring opening of **4**, good kinetics are obtained when the reaction is followed at any wavelength. However, under these conditions since **4** does not build up in solution it is difficult to unequivocally confirm its presence. However, the rate of conversion of **1** (p-$NO_2C_6H_4$) to **3** at low pH is several orders of magnitude greater than that expected for ester hydrolysis without amide group participation and if the amide group were merely acting as a general base to aid water attack on the ester linkage in **1** then a smaller rate enhancement (usually to a maximum of 20-fold the unassisted rate)[11] would be expected.

Because of solubility difficulties with other aryl *N*-acetyl-anthranilates, (**1**, Ar = m-$NO_2C_6H_4$, p-ClC_6H_4, m-ClC_6H_4) these were investigated in 4:1 water–dioxan (μ = 0.1, KCl) and at 55° in order to determine the pH independent rate. Repetitive scans in each case in the pH region 6–7 established the presence of the benzoxazinone **4** as initial reaction product. In each case the base catalysed rate of cyclization (expressed as k_1Ka) and the neutral rate (k_0) was determined from several runs at high (6.0–8.0) and at low (0.5–2.0) pH (Table 3).

From Table 3 it is seen that the rates of cyclization by base catalysed and neutral amide group participation are both sensitive to substituents in the leaving group. The Hammett ρ values of 1.86 and 2.56 calculated from these data show that a higher sensitivity is observed when a neutral amide group is the attacking nucleophile; this is shown as a plot of log k_0 vs log k_1Ka (Fig. 2) which has a slope of 1.36. A σ value for the *p*-nitro group of −1.0 was used to correlate the rate constants; this is intermediate between the ordinary σ and σ^- values for this group and has previously been found[12,13] to be appropriate in the correlation of *p*-nitrophenyl ester and carbamate hydrolyses.

Although the base catalysed term is composite (since Ka is not in the measurable range), it is reasonable to expect that a change in the Ar group will have little effect on the equilibrium between **1** and **2**. Consequently the

Table 3. Summary of rate constants for the hydrolysis of aryl-*N*-acetylanthranilates in 4:1 water–dioxan (μ 0.1 KCl)

Substrate (**1**) (Ar =)	$10^{10} k_1Ka$, $M^{-1} s^{-1}$	$10^4 k_0$, s^{-1}
p-$NO_2C_6H_4$	25	89
m-$NO_2C_6H_4$	11	16
m-ClC_6H_4	1.6	2.0
p-ClC_6H_4	1.0	0.89

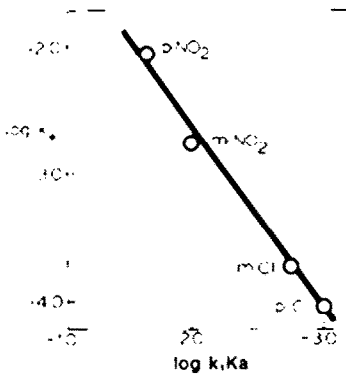
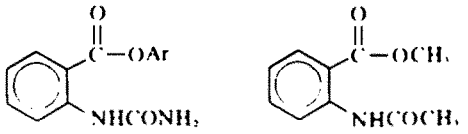


Fig. 2. Log–log plot showing the greater sensitivity of the neutral rate of cyclization (k_0) than the base catalysed rate (k_1 or k_1Ka_1) of cyclization to substituent effects

variation in k_1Ka with substituent should be due almost entirely to the effect on k_1.

The ρ value of 2.56 for substituent variation in Ar when neutral oxygen is the nucleophile is almost identical with the value quoted for cyclization of the *o*-ureido esters **5** to 2-amino benzoxazinones,[3] where kinetic studies indicate the same nucleophilic site, i.e. the ureido oxygen. The ρ values for base catalysed cyclization are however distinctly different (1.86 for **1** and 1.15 for **5**).



5 **6**

The ρ value for **5** is however composite since both O and N attack can yield unstrained 6-membered rings in this case. In fact N attack was dominant in most cases studied and was characterised by a lower sensitivity to the nature of the leaving group. Using the data which refers to O alone for the ureido compounds **5**, we have calculated a ρ value of 2.0, which is again of the same magnitude as we report for **1** (ρ = 1.86 for anionic attack), and similar to that recently reported for the cyclization of *N*-benzoylglycine esters with good leaving groups.[2]

Because of the high sensitivity of both neutral and base catalysed pathways for the cyclization of **1** to **4** to the nature of the leaving group, it is predictable that esters with poor leaving groups would hydrolyse to **3** without the formation of **4**. Consistent with this, methyl *N*-acetylanthranilate **6** is hydrolysed by a base catalysed mechanism (k_{obs} is proportional to {HO$^-$} over the region 12.0–13.3) with an observed rate constant of 2.2×10^{-4} s^{-1} at pH 12. The calculated rate of cyclization of **1** (Ar = Ph) at this pH is 110 s^{-1}; on this basis, cyclization of **6** to **4** should have a rate of 3.7×10^{-4} s^{-1}, i.e. 6-fold slower than the observed rate of hydrolysis of **6** at pH 12. Moreover the rate of hydrolysis of **6** is similar to that for methylbenzoate itself. However, the presence or absence of the benzoxazinone **4** on the reaction pathway is difficult to establish for **6** since the overall observed rate of hydrolysis of **6** is 200-fold slower than the subsequent ring opening of **4** in the basic region (the rate differential is even greater at pH < 7).

In conclusion, the amide group in **1** proves an efficient catalyst for the hydrolysis of the ester linkage in basic solution. With good leaving groups, the rate of cyclization of **1** to **4** is increased and neutral amide group participation is observed so that catalysed ester hydrolysis then also occurs in acidic solution.

EXPERIMENTAL

2-Methyl-3,1-benzoxazin-4-one was prepared as previously described.[9]

N-Acetyl methylanthranilate. Acetic anhydride (10.2 g, 0.1 mol) was added to methylanthranilate (15.12 g, 0.1 mol) and the mixture was refluxed for 30 min. On cooling the *N*-acetyl derivative crystallised out m.p. 95–98° (ex ethanol) (lit.,[14] m.p. 99–100°) IR: 3265 (N–H), 1702 (ester C = O), 1691 cm^{-1} (amide C = O).

N-Acetyl phenylanthranilate. To a stirred solution of isatoic anhydride (8.15 g, 0.05 mol) in dioxan (40 ml), phenol (4.70 g, 0.05 mol) was added, together with a finely ground pellet of sodium hydroxide. The solution was gently heated until effervescence of CO_2 ceased, cooled and diluted with three times its volume of ice-water. The precipitated phenylanthranilate had m.p. 63–65° (lit.,[15] m.p. 70°); IR: 3460, 3352 (NH_2), 1683 cm^{-1} (ester C = O). Similarly prepared were the following aryl anthranilates: *m*-chlorophenyl, m.p. 63–65°. (Found: C, 63.2; H, 4.2; N, 5.8. $C_{13}H_{10}ClNO_2$ requires: C, 63.0; H, 4.0; N, 5.65%); *p*-chlorophenyl, m.p. 77–79° (Found: C, 62.8; H, 3.9; N, 5.4. $C_{13}H_{10}ClNO_2$ requires: C, 63.0; H, 4.0; N, 5.65%); *m*-nitrophenyl, m.p. 118–120° (Found: C, 60.5; H, 4.1; N, 11.0. $C_{13}H_{10}N_2O_4$ requires: C, 60.5; H, 3.8; N, 10.85%); *p*-nitrophenyl, m.p. 123–125° (lit.,[16] m.p. 109° (Found: C, 60.2; H, 4.2; N, 11.0. Calc. for $C_{13}H_{10}N_2O_4$: C, 60.5; H, 3.8; N, 10.85). Acetic anhydride (1.02 g, 0.01 mol) was added to phenylanthranilate (2.13 g, 0.01 mol) and the mixture was refluxed for 15 min. On cooling the precipitated *N*-acetyl phenylanthranilate was crystallised from benzene, m.p. 172–174°; IR: 3280(NH), 1690 (amide C = O), 1709 cm^{-1} (ester C = O) (Found: C, 70.45; H, 5.3; N, 5.4. $C_{15}H_{13}NO_3$ requires: C, 70.6; H, 5.1; N, 5.8%). The other *N*-acetyl arylanthranilates were similarly prepared except that dry benzene was used as solvent and a 2:1 ratio of anthranilate to acetyl chloride was employed (the precipitated hydrochloride of the starting anthranilate was filtered off before evaporation of the solvent): *m*-chlorophenyl, m.p. 98–100° (Found: C, 61.8; H, 4.3; N, 5.0. $C_{15}H_{12}ClNO_3$ requires: C, 62.2, H, 4.1; N, 4.8%); *p*-chlorophenyl, m.p. 108–112° (Found: C, 61.9; H, 3.8; N, 4.8. $C_{15}H_{12}ClNO_3$ requires: C, 62.2; H, 4.1; N, 4.8%); *m*-nitrophenyl, m.p. 150–153° (Found: C, 59.55; H, 4.1; N, 9.3. $C_{15}H_{12}N_2O_5$ requires: C, 60.0; H, 4.0; N, 9.3%); *p*-nitrophenyl, m.p. 97–109° (decomp.) (Found: C, 59.7; H, 4.3; N, 9.55. $C_{15}H_{12}N_2O_5$ requires: C, 60.0; H, 4.0; N, 9.3%).

Kinetic measurements. All kinetic experiments (except where stated) were carried out in water at 25°. Ionic strength was maintained at 0.10M by the addition of KCl (except where pH's less than 1.0 were employed). The water used was deionized and then twice distilled from alkaline permanganate. Where the solubility of the substrates in pure water was too low (see text), a 1:4 dioxan–water (vol/vol) solvent mixture was used. The dioxan was BDH Analar grade used without further purification. The progress of the reactions were followed spectrophotometrically, using a Unicam SP800 ultraviolet spectrophotometer fitted with thermostatable cell block and external AR 25 recorder. Initial repetitive scans of the UV established suitable wavelengths at which an appreciable optical density (O.D.) change occurred during reaction (see text); the first order rate constants were calculated from the slopes of plots of $\log(O.D._t - O.D._\infty)$ vs time (t). The substrate was made up (usually 10^{-2}M) in pure dioxan and reaction was initiated by adding 1 drop of this solution to the cell (which contained *ca.* 2.5 ml of the reaction solution under study). The pH was maintained constant by the addition of small amounts (<0.005M) of buffer (acetate, phosphate or carbonate) where necessary. The pH was measured before and after each experiment and runs showing a pH drift of >0.05 were discarded. A Radiometer PHM 26 pH meter (with expanded scale) and Metrohm 125U glass electrode were used for pH-measurements; the electrode was standardised at 25° using Radiometer standard

buffers. Separate experiments at varying buffer concentration (at constant pH) or, in selected cases, in the absence of pH (using a pH-stat combined with a Cary 14 spectrophotometer) established that buffer catalysis (at the low concentrations used) was unimportant.

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