General base-catalyzed hydrolysis and carbonyl-¹⁸O exchange of *N*-(4-nitrobenzoyl)pyrrole

Laurence J. Beach, Raymond J. Batchelor, Frederick W.B. Einstein, and Andrew J. Bennet

Abstract: Base-promoted hydrolysis kinetics for *N*-(4-nitrobenzoyl)pyrrole (1) have been measured as a function of buffer concentration at several pH values at 25°C. In addition carbonyl-¹⁸O exchange kinetics have been determined at a single pH value (9.48) as a function of 1,4-diazobicyclo[2.2.2]octane (DABCO) concentration. At zero buffer concentration the measured ratio of ¹⁸O exchange to hydrolysis (k_{ex}/k_{hyd}) is approximately 0.04, and this value increases and finally levels off at about 0.23 as the DABCO concentration is increased. These observations are consistent with the buffer acting as a general-base to catalyze both the attack of water to generate an anionic tetrahedral intermediate (T_{O^-}) and the breakdown of T_{O^-} to give hydrolysis products.

Key words: amide, hydrolysis, catalysis, general-base, tetrahedral intermediate.

Résumé : Opérant à 25°C, à plusieurs valeurs de pH, on a mesuré la cinétique de l'hydrolyse catalysée par les bases du *N*-(4-nitrobenzoyl)pyrrole (1) en fonction de la concentration du tampon. De plus, opérant à une seule valeur de pH (9,48), on a déterminé la cinétique d'échange du ¹⁸O en fonction de la concentration du 1,4diazobicyclo[2.2.2]octane (DABCO). À une concentration nulle de tampon, le rapport des vitesses d'échange du ¹⁸O et d'hydrolyse (k_{ec}/k_{hyd}) est approximativement égal à 0,04; lorsqu'on augmente la concentration du DABCO, cette valeur augmente pour atteindre une valeur limite d'environ 0,23. Ces observations sont en accord avec l'hypothèse selon laquelle le tampon agit comme une base générale qui catalyse aussi bien l'attaque de l'eau qui génère l'intermédiaire anionique tétraédrique (T_{O^-}) que la rupture de T_{O^-} qui conduit aux produits d'hydrolyse.

Mots clés : amide, hydrolyse, catalyse, basique générale, intermédiaire tétraédrique.

[Traduit par la Rédaction]

Introduction

The amide C—N bond is one of the main structural units in biological chemistry, and as such hydrolysis of this linkage has been widely studied (1). Scheme 1 shows the generally accepted mechanism for hydrolysis of amides in basic nonbuffered solutions. These hydrolysis reactions involve reversible formation of an anionic tetrahedral intermediate (T_{O}) followed by subsequent breakdown to generate amine and carboxylate anion.

Whether these base-promoted hydrolysis reactions exhibit a second-order domain in their pH–rate profiles depends on the basicity of the amine leaving group (2). For example, simple amides such as benzamides (3) and simple aliphatic amides (1*c*, 4) only show first-order terms in [HO⁻] during hydrolysis (i.e., $k_2 >> k_3$ [OH⁻]). In these cases the tetrahedral intermediate T_O- (Scheme 1) is sufficiently reactive to break down to products without the assistance of a second hydroxide ion. In contrast, hydroxide-catalyzed breakdown of T_O- has been reported to occur during the hydrolysis of amides that contain less basic amines, for example acetanilides (5), formanilides (6), trifluoroacetanilides (7), and *N*acyl pyrroles (8). Furthermore, during the base-promoted hydrolysis of trifluoro- and trichloroacetanilide general basecatalysis has been observed for the breakdown of T_{O^-} (9).

Menger and Donahue (8*a*) reported that the hydrolysis of N-(4-nitrobenzoyl)pyrrole (1) was catalyzed by buffers, such as 1,4-diazobicyclo[2.2.2]octane (DABCO) and trimethylamine (TMA). In contrast, Slebocka-Tilk et al. (2*c*) reported that neither hydrolysis nor ¹⁸O-carbonyl exchange of the structurally similar amide *N*-toluoyl pyrrole (2) was catalyzed by added buffers.



Menger and Donahue (8*a*) analyzed the observed effects of added buffer on the rate of hydrolysis of **1** as a bufferassisted attack of water on the carbonyl group to generate the anionic tetrahedral intermediate T_{O^-} , i.e., general basecatalysis. However, given that the structurally similar amide **2** undergoes a hydroxide-catalyzed breakdown of T_{O^-} it is possible that the observed general base-catalyzed hydrolysis of **1** may involve catalysis of the breakdown rather than (or

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Scheme 1.

Ar
$$\overset{O}{\underset{NR_2}{\leftarrow}}$$
 + $OH^- \overset{k_1}{\underset{k_{-1}}{\leftarrow}} Ar \overset{O^-}{\underset{OH}{\leftarrow}} NR_2 \overset{k_2 + k_3 [OH^-]}{\underset{NR_2}{\leftarrow}} Products$

Fig. 1. Plot of k_{obs} versus [TMA] for **1**, $T = 25^{\circ}$ C, $\mu = 1.0$ (KCl). Lines drawn through the points are the best linear fits to the data.



in addition to) the formation of the anionic tetrahedral intermediate.

The present report contains the details of a kinetic study on the rates of *carbonyl*-¹⁸O exchange and hydrolysis for *N*-(4-nitrobenzoyl)pyrrole (1) as a function of buffer concentration with the aim of elucidating the mechanism of general catalysis. In addition, the measured solvent deuterium kinetic isotope effect (SDKIE) on the hydroxide-promoted rate of hydrolysis is included in this report, as is the solid state structure of *N*-(4-nitrobenzoyl)pyrrole (1).

Results

The experimental conditions employed in the present study to elucidate the mechanism of buffer-catalyzed hydrolysis of **1** were modified from those used by Menger and Donahue (8*a*) so that ionic strength was kept constant. Tables S1 and S2 (supplementary material)² list the complete rate constant data (k_{hyd}) measured for the hydrolysis of **1** at various pH values and buffer concentrations ($\mu = 1.0$, KCl; $T = 25^{\circ}$ C). The corresponding plots of k_{hyd} versus buffer concentration are shown in Figs. 1 and 2. To determine the active component of the buffer, second-order rate constants (k_{BT}) for the TMA-catalyzed reaction were calculated using a linear fit of the data from Table S1.³

Figure 3 illustrates the calculated second-order rate constants ($k_{\rm BT}$) versus the fraction of base ($X_{\rm B}$) for the TMAcatalyzed hydrolysis of **1**, also included in the plot is the best linear fit to this data.





Fig. 3. Plot of $k_{\rm BT}$ versus fraction of base (X_B) for DABCOcatalyzed hydrolysis of **1**, $T = 25^{\circ}$ C, $\mu = 1.0$ (KCl). Line drawn through the points is the best linear fit to the data.



Rate constants for the hydroxide-promoted hydrolysis of 1, at low pH values, were determined by extrapolating the measured rate constants in the presence of buffers to zero buffer concentration. The derived values (k_{calc}) are compiled in Table 1. Using the data from Table 1, for pH values <10, the calculated second-order rate constant for the hydroxide-promoted hydrolysis of 1 is 31.5 ± 2.7 M⁻¹ s⁻¹.

At high pH values (12-14) the rate of hydrolysis of **1** at varying $[OH^-]$ and $[OD^-]$ was measured directly, and the obtained pseudo-first-order rate constants are listed in Table 2.

The second-order rate constant for hydroxide- and deuteroxide-promoted hydrolysis of **1** at high pH were calculated to be 35.2 \pm 1.5 and 39.4 \pm 1.4 M⁻¹ s⁻¹, respectively. Thus, the SDKIE ($k_{\rm H_2O}/k_{\rm D_2O}$) for this base-promoted hydrolysis reaction is 0.89 \pm 0.05 (25°C). To determine partitioning of tetrahedral intermediates formed during the hydrolysis of **1**

²Supplementary material may be purchased from: The Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, Canada, K1A 0S2. Tables of atomic coordinates have also been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from: The Director, Cambridge Crystallographic Data Centre, University Chemical Laboratory, 12 Union Road, Cambridge, CB2 1EZ, U.K.

³Only the data with $[TMA] \le 0.138$ M were used in the linear fit.

Table 1. Calculated pseudo-first-order rate constants for the hydrolysis of **1** at zero buffer concentration versus pH; $T = 25^{\circ}$ C, $\mu = 1.00$ (KCl).^{*a*}

Buffer	pН	$k_{\rm calc} \times 10^4 \ ({ m s}^{-1})$
DABCO	9.04	3.78 ± 0.07
TMA	9.11	3.58 ± 0.03
TMA	9.41	8.20 ± 0.02
DABCO	9.42	8.24 ± 0.09
DABCO	9.59	11.4 ± 0.2
TMA	9.76	19.7 ± 0.7
TMA	10.18	51.8 ± 1.4
TMA	10.55	113.7 ± 0.6

 $^{\it a}Extrapolated$ from the data given in Tables S1 and S2 where $[{\rm buffer}]_{\rm total} < 0.14~M.$

Table 2. Observed pseudo-first-order rate constants for the hydrolysis of **1** in the absence of added buffer versus $-\log$ [OL⁻]; $T = 25^{\circ}$ C, $\mu = 1.00$ (KCl).

–log [OH [–]]	$k_{\rm hyd}~({\rm H_2O})~({\rm s^{-1}})^a$	–log [OD [–]]	$k_{\rm hyd}~({\rm D_2O})~({\rm s^{-1}})^a$
0.31	18.2 ± 0.5	0.29	21.2 ± 0.2
0.62	8.5 ± 0.3	0.59	10.0 ± 0.5
1.01	3.38 ± 0.06	0.99	4.1 ± 0.1
1.62	0.85 ± 0.02	1.99	0.39 ± 0.02
2.01	0.33 ± 0.01		

^{*a*}Mean value of seven or eight independent kinetic runs; quoted error = σ_{n-1} .

Fig. 4. Plot of $k_{\rm ex}/k_{\rm hyd}$ versus [DABCO] for reaction of **1** at a pH value of 9.48, $T = 25^{\circ}$ C, $\mu = 1.0$ (KCl). Line drawn through the points is the best nonlinear fit to eq. [6].



the ratio of *carbonyl*-¹⁸O exchange to hydrolysis was measured as a function of buffer concentration at a single pH value. In an initial set of experiments (pH = 9.0) *carbonyl*-¹⁸O labelled amide was subjected to the hydrolysis conditions for one half-time of hydrolysis. After reisolation of the labelled amide remaining and determination of the percentage of ¹⁸O present estimates for the ratio $k_{\rm ex}/k_{\rm hyd}$ were made using eq. [1].

[1]
$$\frac{k_{\text{ex}}}{k_{\text{hvd}}} = \frac{\ln ({}^{18}\text{O}_0 / {}^{18}\text{O}_t)}{\ln (2)}$$

Table 3. Observed pseudo-first-order rate constants for *carbonyl*-¹⁸O exchange and hydrolysis of **1** in the presence of added DABCO, pH = 9.48, $T = 25^{\circ}$ C, $\mu = 1.00$ (KCl).

DABCO (M)	$k_{\mathrm{ex}} \times 10^4$ $(\mathrm{s}^{-1})^a$	$k_{ m hyd} imes 10^4 \ ({ m s}^{-1})^b$	$k_{\rm ex}/k_{\rm hyd}^{c}$
0.50	5.08 ± 0.41	22.4 ± 0.1	0.227 ± 0.018
0.40	4.76 ± 0.31	21.3 ± 0.6	0.223 ± 0.016
0.30	4.44 ± 0.54	19.1 ± 0.6	0.232 ± 0.029
0.20	3.65 ± 0.19	16.0 ± 0.2	0.228 ± 0.012
0.10	2.55 ± 0.15	12.7 ± 0.3	0.201 ± 0.013
0.08	1.98 ± 0.25	12.1 ± 0.8	0.164 ± 0.023
0.06	1.77 ± 0.34	11.6 ± 0.3	0.153 ± 0.037
0.04	1.75 ± 0.25	10.7 ± 0.2	0.164 ± 0.024
0.02	1.58 ± 0.23	10.4 ± 0.4	0.152 ± 0.023
0.00	0.36 ± 0.19^{d}	$9.7 \pm 0.2^{e,f}$	0.037 ± 0.020^{g}

^aExchange rate constant calculated according to ref. 10, p. 150, from the data given in Tables S4–S6.

^{*b*}Mean value of three independent kinetic runs; quoted error = σ_{n-1} . ^{(Errors calculated according to ref. 10.}

 d Additional estimate for the pseudo-first-order rate constant $k_{\rm ex}$ using data from Table S6 is 0.55 \pm 0.25 \times 10⁻⁴ s⁻¹.

^eExtrapolated value from data with [DABCO] ≤ 0.1 M.

^{*f*}Value interpolated from the data given in Table 1 is $9.55 \times 10^{-4} \text{ s}^{-1}$. ^{*s*}Additional estimate using data from Table S6 is $k_{\text{ex}}/k_{\text{hyd}} = 0.057 \pm 0.026$.

Given in Table S3 are the measured values for ¹⁸O_t (percentage ¹⁸O at time = $t_{1/2hyd}$) and the calculated rate constant ratios k_{ex}/k_{hyd} . This tabulated data suggests that the ratio k_{ex}/k_{hyd} varies as a function of buffer concentration however, the reproducibility of the measured ¹⁸O_t values was generally worse at higher buffer concentrations, a possible consequence of the required acid quench. Therefore, it was decided to follow *carbonyl*-¹⁸O exchange and hydrolysis at a higher pH value using a flow system where the rapid acid quenching of large volumes of DABCO buffers could be avoided (see experimental section).

The measured rate constants for hydrolysis, *carbonyl*-¹⁸O exchange and their ratio (k_{ex}/k_{hyd}) at a pH of 9.48 using the flow system are given in Table 3 (10), and the corresponding plot of k_{ex}/k_{hyd} versus buffer concentration is shown in Fig. 4. In addition, the measured ¹⁶O:¹⁸O ratios for reisolated **1** are given in Tables S4, S5, and S6 (supplementary material).²

A single crystal diffraction study was undertaken on 1 to examine possible structural differences between 1 and 2. Crystallographic details are summarized in Table 4. The final fractional atomic coordinates for the non-hydrogen atoms are listed in Table S7 (supplementary material).² The observed bond lengths and those calculated after a rigid body analysis (see experimental section) for 1 are included in Table 5, and the important bond angles are given in Table 6. Figure 5 presents an ORTEP diagram of the so obtained structure for amide 1.

Given in Table 7 is a comparison of the C—N and C—O amide bond lengths for amides **1** and **2** (11). Also included in Table 7 are the calculated Dunitz parameters (χ_C and χ_N) for these two amides (12).

Formula	$C_{11}H_8O_3N_2$	Crystal system	Monoclinic	
fw	216.19 Space group		$P2_{1}/c$	
a (Å) ^a	6.027(3)	$\rho_c (g \text{ cm}^{-3})$	1.456	
<i>b</i> (Å)	21.832(5)	λ (Mo K _{$\alpha 1$}) (Å)	0.7093	
<i>c</i> (Å)	7.580(3)	μ (Mo K _{α}) (cm ⁻¹)	1.0	
<i>b</i> (°)	98.63(3)	min–max 20 (°)	4–48	
V (Å ³)	986.1	Crystal dim. ^b (mm)	$0.18\times0.27\times0.32$	
Ζ	4	Crystal dim. ^c (mm)	$0.21\times0.24\times0.32$	
Temperature (K)	205	GoF^d	2.15	
\mathbf{R}_{F}^{e}	0.034	R_{wF}^{f}	0.041	

^{*a*}Cell dimensions were determined from 22 reflections ($36^{\circ} \le 2\theta \le 41^{\circ}$).

^{*b*}First crystal: measured complete quadrant shell (±*h*, *k*, *l*; 4° ≤ 2θ ≤ 45°) and incomplete quadrant shell (±*h*, *k*, *l*; 45° ≤ 2θ ≤ 48°). ^{*c*}Second crystal: complete quadrant shell (±*h*, *k*, *l*; 45° ≤ 2θ ≤ 48°). $R_{\text{merge}} = 0.027$ for ~100 reflections measured on both crystals. ^{*d*}GoF = [$\Sigma(w(F_o - F_c)^2)$ /degrees of freedom]^{1/2}.

 ${^eR_F} = \Sigma |(|F_{\rm o}| - |F_{\rm c}|)| / \Sigma |F_{\rm o}| \mbox{ for 1142 data } (I_{\rm o} \geq 2.5 \sigma(I_{\rm o})). \label{eq:RF}$

 ${}^{f}\!R_{wF} = [\Sigma(w(|F_{\rm o}| - |F_{\rm c}|)^{2})/\Sigma(wF_{\rm o}{}^{2})]^{1/2} \text{ for 1142 data } (I_{\rm o} \ge 2.5\sigma(I_{\rm o})); w = [\sigma(F_{\rm o})^{2} + 0.0001F_{\rm o}{}^{2}]^{-1}.$

Table 5. Selected bond distances (Å) for 1 at 205 K.

Bond	Distance	Corrected ^a	Bond	Distance	Corrected ^a
O(1)—C(10)	1.216(3)	1.22	C(4)—C(5)	1.334(4)	1.337
O(2)—N(2)	1.220(3)	1.249^{b}	C(10)—C(11)	1.491(3)	1.493
O(3)—N(2)	1.214(3)	1.243^{b}	C(11)—C(12)	1.389(3)	1.392
N(1)—C(2)	1.398(3)	1.402	C(11)—C(16)	1.398(3)	1.401
N(1)—C(5)	1.395(3)	1.398	C(12)—C(13)	1.380(3)	1.382
N(1)—C(10)	1.386(3)	1.388	C(13)—C(14)	1.383(3)	1.387
N(2)—C(14)	1.470(3)	1.472	C(14)—C(15)	1.371(3)	1.374
C(2)—C(3)	1.344(3)	1.346	C(15)—C(16)	1.381(3)	1.383
C(3)—C(4)	1.426(4)	1.429			

^aBond distances corrected for rigid-body thermal motion.

^bCorrection includes a contribution for internal libration of the NO₂ group about the N(2)–C(14) bond.

	Table 6	j.	Selected	bond	angles	(°) for	1	at	205	K
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Bond	Angle	Bond	Angle
C(2)-N(1)-C(5)	107 15(19)	N(1)-C(10)-C(11)	119 36(18)
C(2)-N(1)-C(10)	129.33(18)	C(10)-C(11)-C(12)	117.45(19)
C(5)-N(1)-C(10)	123.51(19)	C(10)-C(11)-C(16)	122.53(19)
O(2)-N(2)-O(3)	122.95(21)	C(12)-C(11)-C(16)	119.75(20)
O(2)-N(2)-C(14)	118.35(21)	C(11)-C(12)-C(13)	120.54(21)
O(3)-N(2)-C(14)	118.70(22)	C(12)-C(13)-C(14)	118.27(21)
N(1)-C(2)-C(3)	108.39(20)	N(2)-C(14)-C(13)	118.31(21)
C(2)-C(3)-C(4)	107.70(22)	N(2)-C(14)-C(15)	119.08(21)
C(3)-C(4)-C(5)	108.05(22)	C(13)-C(14)-C(15)	122.61(21)
N(1)-C(5)-C(4)	108.71(22)	C(14)-C(15)-C(16)	118.86(21)
O(1)-C(10)-N(1)	120.26(19)	C(11)-C(16)-C(15)	119.95(21)
O(1)-C(10)-C(11)	120.38(19)		

Discussion

(i) Hydrolysis of 1 in the absence of buffers

The reported hydrolysis mechanism for *N*-toluoylpyrrole (2) includes a hydroxide-catalyzed term for breakdown of the anionic tetrahedral intermediate (T_{O^-}) to generate carboxylate and pyrrole anions (2*c*). The derived expressions of the rate constants for hydrolysis (k_{hyd}), exchange (k_{ex}), and their ratio (k_{ex}/k_{hyd}) from the mechanism shown in Scheme 1

are given by eqs. [2]–[4] where $k_2' = k_2/k_{-1}$ and $k_3' = k_3/k_{-1}$. The factor of two in eqs. [3] and [4] arises from the necessary assumption that one-half of T_{O^-} reversal to regenerate amide leads to *carbonyl*-¹⁸O exchange, i.e., the two oxygen atoms in the tetrahedral intermediate are protonically equilibrated. This assumption has been shown to be valid for T_{O^-} formed during the hydrolysis of **2** (2*c*).

[2]
$$k_{\text{hyd}} = \frac{k_1[\text{OH}^-](k_2' + k_3'[\text{OH}^-])}{1 + k_2' + k_3'[\text{OH}^-]}$$

Fig. 5. Molecular structure for amide 1, 50% enclosure ellipsoids are shown.



 Table 7. Comparison of the structural parameters for the amides 1 and 2.

Parameter	1^{a}	2
r(C—O) (Å)	1.220	1.211
<i>r</i> (C—N) (Å)	1.388	1.409
χ _C (°)	1.1	0.0
χ _N (°)	1.0	10.1

^{*a*}Bond distances corrected for rigid-body thermal motion (see Table 5).

[3]
$$k_{\text{ex}} = \frac{k_{\text{I}}[\text{OH}^-]}{2(1 + k_2' + k_3'[\text{OH}^-])}$$

[4]
$$\frac{k_{\text{ex}}}{k_{\text{hvd}}} = \frac{1}{2(k_2' + k_3'[\text{OH}^-])}$$

An indication that a similar pathway exists for the hydrolysis of 1 in the absence of buffers is shown by the difference in the calculated second-order rate constants at high and low pH values in the absence of added buffers (2c). In the low pH domain, $k_3'[OH^-] \ll k_2'$, the value for $k_{hyd}/[OH^-]$ of $31.5 \pm 2.7 \text{ M}^{-1} \text{ s}^{-1}$ is equal to $k_1 k_2'/(1 + k_2')$, while in the high pH domain, $k_3'[\text{OH}^-] >> k_2'$, the value for $k_{\text{hyd}}/[\text{OH}^-]$ of $35.2 \pm 1.5 \text{ M}^{-1} \text{ s}^{-1}$ is equal to k_1 . Using these two values an estimate of 0.12 ± 0.11 can be computed for the uncatalyzed partitioning (k_{-1}/k_2) of the anionic tetrahedral intermediate $T_{\Omega^{-}}$ between regeneration (k_{-1}) and hydrolysis (k_2) of the amide. Although, this calculated partitioning ratio contains a large associated error, a second estimate of this quantity can be made from the rate of *carbonyl*-¹⁸O exchange (k_{ex}) relative to hydrolysis (k_{hyd}) . Where in the absence of buffers and at a pH value of 9.48, assuming $k'_3[OH^-] \ll k'_2$, the measured value for $k_{\rm ex}/k_{\rm hyd}$ (Table 3) of 0.037 \pm 0.02 (repeat value 0.06 \pm 0.03) generates an estimated value for k_{-1}/k_2 of 0.07 \pm 0.04. That the observed value for $k_{\rm ex}/k_{\rm hyd}$ (0.04–0.06) for the base-promoted hydrolysis of 1, at low pH measurements, is less than that reported ($k_{ex}/k_{hyd} = 0.24$) for the corresponding reaction of **2** (2*c*) requires that T_{O⁻} partitioning to products is favoured to a greater extent by the electronwithdrawing nitro group (1) than by the electron-donating methyl group (2). This difference corresponds to a relative reduction in the kinetic barrier (k_2) for product formation from amide **1** compared to amide **2** of approximately 3–4 kJ mol⁻¹. The anionic intermediate T_{O^-} formed during the reactions of **1** will be more stable than the corresponding intermediate formed from **2** because of the electron-withdrawing nitro group. Therefore, it can be safely assumed that the two oxygens in the intermediate (T_{O^-}) formed from **1** are protonically equilibrated and that the change in the k_{ex}/k_{hyd} ratio, in the absence of buffer, indicates a greater tendency for the unimolecular ejection of the pyrrole anion, relative to hydroxide ion, from the more stable tetrahedral intermediate. The origin of this effect could be the formation of a hydrogen bond, at the transition state for pyrrole anion ejection, between the anionic tetrahedral intermediate's O–H group and a solvating water molecule.

(ii) Mechanism of buffer catalysis

The intercept with the *y*-axis ($X_B = 0$, Fig. 3) for the second-order rate constants for buffer-catalyzed hydrolysis of **1** is, within experimental error, zero. Therefore, the basic form of the buffer is the active species for the general-catalyzed hydrolysis of **1**. Shown in Scheme 2 is the modified mechanism for hydrolysis of **1**, where the basic form of the buffer can catalyze the formation (k_{BF}) and (or) the breakdown (k_{BP}) of T_{O^-} . The principal of microscopic reversibility requires that if general base-catalysis for the formation of T_{O^-} occurs then reversal to generate starting material must also occur with general acid-catalysis.

The derived expressions of the rate constants for hydrolysis (k_{hyd}) , exchange (k_{ex}) , and their ratio $(k_{\text{ex}}/k_{\text{hyd}})$ from the mechanism shown in Scheme 2 are given by eqs. [5]–[7] where $k_{\text{BF}}' = k_{\text{BF}}/k_{-1}$, $k_{\text{BH}}' = k_{\text{BH}}/k_{-1}$, and $k_{\text{BP}}' = k_{\text{BP}}/k_{-1}$.

[5]
$$k_{\text{hyd}} = \frac{(k_{\text{I}}[\text{OH}^-] + k_{\text{BF}}[\text{B}])(k_2' + k_{\text{BP}}'[\text{B}] + k_3'[\text{OH}^-])}{1 + k_{\text{BH}}'[\text{BH}^+] + k_2' + k_{\text{BP}}'[\text{B}] + k_3'[\text{OH}^-]}$$

[6]
$$k_{\text{ex}} = \frac{(k_1[\text{OH}^-] + k_{\text{BF}}[\text{B}])(1 + k_{\text{BH}}'[\text{BH}^+])}{2(1 + k_{\text{BH}}'[\text{BH}^+] + k_2' + k_{\text{BP}}'[\text{B}] + k_3'[\text{OH}^-])}$$

[7]
$$\frac{k_{\text{ex}}}{k_{\text{hyd}}} = \frac{1 + k_{\text{BH}}'[\text{BH}^+]}{2(k_2' + k_{\text{BP}}'[\text{B}] + k_3'[\text{OH}^-])}$$

Scheme 2.

Scheme 3.



 $Ar \stackrel{O}{\longleftarrow} NR_{2} \xrightarrow{Ar \stackrel{I}{\longleftarrow} NR_{2}} X \xrightarrow{Ar \stackrel{O}{\longleftarrow} NR_{2}} Products$ T_{ZW}

Three immediate conclusions can be drawn from the data shown in Figs. 1 and 2, and given in Table 3, namely, (i) the hydrolysis reactions of **1** are buffer-catalyzed; (*ii*) since $k_{\rm ex}/k_{\rm hvd} \ll 1$, at low pH and in the absence of buffer, the rate-limiting step for the hydroxide-promoted hydrolysis must be formation of T_{O-}; and (iii) buffer accelerates the slow step of the hydrolysis reaction, i.e., T_{O^-} formation. However, if the buffer only catalyzed the formation of the tetrahedral intermediate $(k_{BP}' = 0, eq. [7])$ then at a constant pH value the ratio k_{ex}/k_{hvd} should increase in a linear fashion when the buffer concentration is increased. The observation that the $k_{\rm ex}/k_{\rm hyd}$ ratio increases with buffer concentration and levels off at high concentration provides convincing evidence that both the attack of water on the amide to generate T_{O^-} and the breakdown of T_{O^-} to generate the hydrolysis products are catalyzed by buffer. At high buffer concentration, i.e., when $k_{BP}'[B] >> k_2' + k_3'[OH^-]$ and $k_{BH}'[BH^+] >> 1$, the derived expression for $k_{\rm ex}/k_{\rm hvd}$ (eq. [7]) simplifies to eq. [8].

[8]
$$\frac{k_{\text{ex}}}{k_{\text{hyd}}} = \frac{k_{\text{BH}}'[\text{BH}^+]}{2k_{\text{BP}}'[\text{B}]}$$

In DABCO buffer at a pH value of 9.48 where $(k_{\rm ex}/k_{\rm hyd})_{\rm limit} \approx 0.23$ and [B]/[BH⁺] = 2.13 substitution of these values into eq. [8] gives a ratio for $k_{\rm BH}'/k_{\rm BP}'$ of approximately 1 (0.23 × 2 × 2.13 = 0.98).

Since the kinetic expression for buffer-catalyzed hydrolysis of 1 contains several variables estimation of the two parameters $k_{\rm BF}$ and $k_{\rm BP}'$ for the DABCO-catalyzed formation and breakdown of T_{O-} were made using a constrained nonlinear least-squares fit of all the DABCO-catalyzed hydrolysis data (Tables S2 and 3) to eq. [5]. The constraints used in the nonlinear fit were as follows: $k_{BP}' = k_{BH}'$, $k_1 = 35.2 \text{ M}^{-1} \text{ s}^{-1}$, and $k_3'[OH^-] \approx 0$. The calculated rate constants and standard errors that were derived using the program SYSTAT are $k_{\rm BF}$ = 0.0066 ± 0.0003 M⁻¹ s⁻¹, $\vec{k}_{BP}' = 29 \pm 6 M^{-1} s^{-1}$, and $k_2' =$ 11.2 ± 0.02 . Although, the complexity of eq. [5] means that these derived values are correlated the estimated value for k_2' is consistent with that obtained from the ratio of exchange to hydrolysis at zero buffer concentration (Table 3), i.e., $k_{\rm ex}/k_{\rm hvd} \approx 1/2k_2'$. Implicit in the above arguments is that the buffer acts as a general base rather than as a nucleophile during the catalytic reaction. If the buffer nucleophilically attacked the carbonyl carbon to generate an intermediate such as T_{ZW} then the ratio of k_{ex}/k_{hyd} should drop to zero, since no *carbonyl*-¹⁸O exchange into the starting amide is possible from T_{ZW} . Therefore, if any nucleophilic attack by DABCO occurred the intermediate T_{ZW} must always revert to starting amide (Scheme 3).

From the above analysis it must be concluded that the observed DABCO-catalyzed breakdown of T_{O} -to give hydrolysis products is an example of general base-catalysis. Consequently, the ejection of the pyrrole anion from the anionic tetrahedral intermediate must occur simultaneously with, and not subsequent to, proton removal. This conclusion is in contrast to the proposal of Menger and Donahue (8*a*).

(iii) Solvent deuterium kinetic isotope effect

The measured values for the SDKIE of 0.89 ± 0.05 for the hydroxide attack at the carbonyl carbon is similar to the reported value for 2 (2c) and is consistent with the transition state for this step involving direct attack of a partially desolvated hydroxide ion on the carbonyl carbon (2).

(iv) Structural comparison of amides 1 and 2

The data reported in Table 7 shows that in the solid state both amide linkages in 1 and 2 have similar structural parameters. Consequently, no information is obtained that can explain either the absence of buffer-catalysis during the hydrolysis of 2, or the presence of this type of catalysis in the case of 1.

Conclusions

In conclusion, replacement of the 4-methyl group in pyrrole amide 2 with a 4-nitro group (amide 1) activates the amide linkage sufficiently that general-base-catalyzed attack of water on the carbonyl group becomes a viable mechanism. In addition, although the nitro group unquestionably increases the lifetime of the anionic tetrahedral intermediate (T_{O^-}), via inductive withdrawal, the uncatalyzed breakdown of T_{O^-} favours ejection of the pyrrole anion over hydroxide ion ($k_{ex}/k_{hyd} = 0.04-0.06$) to a greater extent than does the tetrahedral intermediate formed during the hydrolysis of 2 ($k_{ex}/k_{hyd} = 0.24$). This effect might be caused by the differences in acidity of the two respective tetrahedral intermediates. Thus, in compound 1 the electron-withdrawing nitro

group should result in the development of a stronger hydrogen bond at the transition state (k_2) for product formation.

Experimental

The following materials were purchased and used as received unless stated otherwise: pyrrole (Aldrich), 4-nitrobenzoyl chloride (Lancaster), potassium metal and thionyl chloride (Anachemia), H₂¹⁸O (Isotec, 98.5 atom % ¹⁸O, lot no. DU2255), trimethylamine hydrochloride (SIGMA, TMA-HCl), deuterium oxide (Isotec, 99.9 atom % D), and chloride (BDH). DABCO potassium (1,4-diazabicyclo[2.2.2]octane; Aldrich, 98%) was recrystallized from pet ether for the ¹⁸O-oxygen exchange experiments. Reagent grade tetrahydrofuran (BDH, THF) was distilled from its sodium benzophenone ketal, and reagent grade toluene (BDH) was distilled from calcium hydride. NMR spectra were acquired using a Brucker AMX400 NMR spectrophotometer operating at frequencies of 400.1 and 100.6 MHz for ¹H and ¹³C, respectively. Mass spectra were obtained using a direct insertion probe and electron impact ionization (70 eV) on a Hewlett Packard 5985 mass spectrometer. Melting points are uncorrected.

N-(4-Nitrobenzoyl)pyrrole (1)

Under a dry nitrogen atmosphere potassium metal (0.9 g, 23.1 mmol) was added in small portions to a solution containing freshly distilled pyrrole (1.6 mL, 23.1 mmol) in anhydrous THF (20 mL). After the addition was complete the resulting solution was heated to reflux until the potassium had complete reacted. After cooling to ambient temperature anhydrous toluene (70 mL) was added followed by the dropwise addition of a solution containing 4-nitrobenzoyl chloride (4.29 g, 23.1 mmol) in anhydrous toluene (200 mL). The resulting solution was heated to reflux for 24 h, and after cooling to ambient temperature a mixture of 1:1 ice-cold water – diethyl ether (total volume 100 mL) was added. The diethyl ether layer was separated and washed with ice-cold water (50 mL). The two aqueous layers were combined and washed with diethyl ether (50 mL), after the two layers were separated the organic layers were combined and volatile materials were removed under reduced pressure. The resulting black residue was sublimed (100°C; 0.25 mTorr) onto a cold finger, and the resulting product was further purified by recrystallization from hexane:2-butanol to give an analytically pure sample of 1 (1.25 g; 25%): mp 128.5-130.0°C (lit. (8) mp 127-128°C); ir (KBr pellet), v: 3155 (w), 3107 (w), 1692 (s), 1601 (m), 1519 (s), 1474 (s), 1409 (s), 1332 (s), 1256 (w), 1189 (w), 1112 (w), 1091 (m), 1075 (m), 1037 (w), 1012 (m), 972 (m), 884 (m), 865 (m), 844 (s), 747 (s), 730 (s) 712 (s); ¹H NMR (400 MHz; CD₃COCD₃), δ: 6.40 (2H, app dd), 7.29 (2H, app dd), 8.04 (2H, m), 8.43 (2H, m); ¹³C NMR (100 MHz; CD₃COCD₃), δ: 166.6, 150.9, 140.0, 124.6, 122.0, 114.5; EI-MS, m/z: $216(M^+, 65), 150(100), 120(20), 104(25), 92(21), and$ 76(23). Anal. calcd. for C₁₁H₈N₂O₃: C 61.11, H 3.73, N 12.95; found: C 61.30, H 3.69, N 12.97.

N-(4-Nitro-*carbonyl*-¹⁸O-benzoyl)pyrrole

To a solution of freshly distilled 4-nitrobenzoyl chloride (1.33 g, 7.2 mmol) in anhydrous THF (15 mL) was added ¹⁸O-water (129 mL, 6.5 mmol) under a nitrogen atmosphere.

After this solution had been stirred at ambient temperature for 24 h the volatile materials were removed under reduced pressure. To the resulting residue anhydrous toluene (15 mL) was added, followed by the dropwise addition of thionyl chloride (1.04 mL, 14.3 mmol), and the reaction mixture was heated to reflux for 18 h. After the volatile materials were removed under reduced pressure the resulting 4nitro-*carbonyl*-¹⁸O-benzoyl chloride was purified by vacuum distillation (125°C; 5 mTorr). The ¹⁸O-labelled acid chloride was used to prepare N-(4-nitro-carbonyl-18O-benzol)pyrrole in an analogous fashion to the preparation of unlabelled 1. The overall yield for the synthesis of ¹⁸O-labelled 1 from 4nitrobenzoyl chloride was 21%; ir (KBr pellet), v: 3155 (w), 3107 (w), 1692 (s), 1660 (s), 1601 (m), 1519 (s), 1474 (s), 1409 (s), 1332 (s), 1256 (w), 1189 (w), 1112 (w), 1091 (m), 1075 (m), 1037 (w), 1012 (m), 972 (m), 884 (m), 865 (m), 844 (s), 747 (s), 730 (s) 712 (s); EI–MS, m/z: 218(¹⁸O–M⁺, 59), 216(¹⁶O-M⁺, 72), 152(68), 150(100), 122(11), 120(11), 106(21), 104(30), 94(16), 92(17), and 76(44).

Hydrolysis kinetics

Hydrolysis of **1** was monitored by measuring the change in absorbance at either 240 nm (trimethylamine buffers) or 278 nm (OH⁻ solutions or DABCO buffers) using either a Cary-3E UV-vis spectrophotometer equipped with the Cary six cell Peltier constant temperature accessory or a DURRUM 110 stopped-flow apparatus thermostatted with a Lauda RM6 circulating water bath. The slower reactions were initiated by injection of a stock solution of **1** in DME (20–50 mL; 18.5 mM) into a equilibrated solvent mixture (3 mL; 30 min), whereas for the stopped-flow reactions an equal volume of a solution of **1** (0.4 mM) in 10⁻⁴ M HCl ($\mu =$ 1, KCl) was mixed with NaOH. Rate constants in deuterated solvents were measured in an analogous fashion using D₂O solutions.

Exchange kinetics

¹⁸O-Carbonyl exchange of labelled-1 in the presence of DABCO buffers was monitored utilizing a fluid flow system identical to that reported earlier (2c) except that the reaction was quenched by utilizing a pH-stat assembly consisting of Radiometer TTT80 titrator, ABU80 autoburette, a PHM82 standard pH meter, and a Broadley-James combination electrode (silver/silver chloride reference) to maintain the quenching solution at a pH of 4. The rate of ¹⁸O-carbonyl exchange in the absence of buffer was measured using the pH-stat assembly to maintain a constant pH for a time equal to one or two times the half-time for hydrolysis of 1. The labelled-1 remaining in the hydrolysis medium was isolated by extracting the aqueous solution with freshly distilled CH_2Cl_2 (4 × 2 mL), and the combined organic layers were washed with a saturated NaHCO₃ solution $(2 \times 4 \text{ mL})$ and water (2 mL). The dichloromethane layer was dried $(MgSO_4)$, filtered, and evaporated onto a small dry lint-free tissue. The tissue was inserted into a melting point tube and the ¹⁸O-content of the sample was determined by mass spectroscopy. In a typical determination 20 separate scans of the M^+ and the M^+ + 2 ions in the mass spectrum were used to estimate the ¹⁸O-content of the sample and its associated standard error. The rate constant for exchange was calculated using eq. [9] where ${}^{18}O_0$ is the ${}^{18}O$ -content of the starting amide and ${}^{18}O_t$ is the ${}^{18}O$ -content of the amide reisolated from the reaction medium at time t.⁴

[9]
$$k_{\text{ex}} = \frac{-\ln ({}^{18}\text{O}_t / {}^{18}\text{O}_0)}{t}$$

Structure determination

A single colourless needle-shaped crystal of 1, obtained from the slow evaporation of a solution of 1 in 1:1 v/v 2butanol:hexane, was cleaved and fragments of a suitable size and shape were mounted on glass fibres using epoxy adhesive. Data were recorded at 205 K with an Enraf Nonius CAD4F diffractometer equipped with an in-house modified low-temperature attachment and using graphite monochromatized Mo K_{α} radiation. Two fragments of the same crystal, having comparable size and shape, were used because the first fragment became dislodged during an interruption of the cooling caused by ice build-up in the low-temperature apparatus. The same two intensity standard reflections were measured every hour of exposure time for both crystal fragments and, in either case, fluctuated by $\pm 2.5\%$ during the course of the measurements. No corrections for absorption were deemed necessary. Data reduction included corrections for intensity scale variations and for Lorentz and polarization effects.

The structure was solved using direct methods. All hydrogen atoms were located from an electron density difference map. All atomic coordinates, anisotropic thermal parameters for the non-hydrogen atoms and isotropic thermal parameters for the hydrogen atoms were refined.

An extinction parameter was refined (13). A weighting scheme, based on counting statistics, was used such that $\langle w(|F_o| - |F_c|)^2 \rangle$ was near constant as a function of both $|F_o|$ and sin θ/λ . Final full matrix least-squares refinement of 178 parameters for 1142 data ($I_o > 2.5\sigma(I_o)$) converged at R = 0.034 with a maximum |shift/error| of 0.001.

The programs used for data reduction, structure solution, refinement, and plot generation were from the NRCVAX crystal structure system (14). The programs suite CRYS-TALS (15) was employed for analysis of structure factors and thermal parameters. Complex scattering factors for neutral atoms (16) were used in the calculation of structure factors. Computations were carried out on MicroVAX-II and 80486 computers.

Rigid body analysis (17) of the anisotropic thermal parameters of the molecule yielded R = 0.176 for the agreement between observed and calculated U_{ij} and an rms discrepancy of 0.0058 Å². In particular the oxygen atoms of the NO₂group showed excess motion. Analysis of internal motion of the molecule in terms of a segmented rigid body (18) in which the NO₂ group was allowed libration about the C(14)—N(2) bond axis gave R = 0.089 and an rms discrepancy of 0.0023 Å².

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⁴Standard errors were calculated according to ref. 10, p. 62.

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