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Kinetics and Mechanism of General Acid-Catalysed Thiolytic Cleavage of 9-Anilinoacridine

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Abstract—The rates of the reactions of 2-mercaptoethanol (2-ME) with 9-anilinoacridine (9-ANA) have been studied in the buffer solutions of 2-ME, hydroxylamine, phosphate and morpholine. Both ionised and non-ionised forms of 2-ME and free hydroxylamine show nucleophilic reactivity toward protonated 9-ANA. The rate constants for general acid-catalysed thiolytic cleavage of protonated 9-ANA reveal a Brønsted plot of slope (α) of 0.93 which indicates that probably the rate-determining step involves proton transfer in a thermodynamically unfavourable direction. A stepwise mechanism for thiolysis has been suggested. General acid catalysis could be detected for thiolysis of non-protonated 9-ANA only in the buffer solutions of phosphate and morpholine. General acid catalysis seems to be unimportant when the nucleophile is non-ionised 2-ME which is attributed to the probable occurrence of intramolecular general acid catalysis.

Introduction

Many acridine derivatives have been tested for antitumor activity.[1] The biological activity of acridines is most likely due to their ability to intercalate strongly with DNA.[2] The kinetic studies on hydrolysis of a few acridine derivatives have been carried out by Skonieczny and coworkers[3,4] as well as by Kalatzis[5] in order to understand their physicochemical properties. More recently, O'Connor *et al.*[6] studied the kinetics of hydrolysis of 4-substituted analogs of 9-[3-(*N*,*N*-dimethylamino)propylamino]-1-nitroacridine with an aim to explore the substituent effects on the rate of hydrolysis. These workers found that the rate of hydrolysis was dependent upon the pH of the reaction medium. The possibility of the occurrence of general acid–base (GA–GB) catalysis was not investigated.[6]

Cain and coworkers[7] have attempted to find a correlation between $t_{1/2}$ and pK_a of acridine derivatives where $t_{1/2}$ represents the half-life periods for thiolytic degradation of acridines at a constant concentration of 2-mercaptoethanol (2-ME) under phosphate buffer of desired pH. Under these conditions, the occurrence of GA catalysis in these reactions was not realized. Khan and Malspeis[8] have studied the thiolytic cleavage of 4'-[(9-acridinyl)amino]methanesulfon-*m*-anisidide (*m*-AMSA) under the buffer solutions of various thiols and the rate of thiolysis was found to be sensitive to GA catalysis. In these studies, the buffer components acted both as nucleophile and GA catalyst. The present study was started with an aim to explore the GA catalysis in the reactions of 2-ME with 9-anilinoacridine under the presence of GA catalysts (hydroxylammonium ion, morpholinium ion and NaH_2PO_4) other than 2-ME. There was not any special reason for choosing 2-ME as the nucleophile except that its use appeared to be easier and expected to generate relatively less complicated rate law for the thiolytic reactions. The results and their probable explanations are described in this paper.

Experimental

Materials

9-Anilinoacridine was synthesised according to the published method.[9] All other chemicals used were of reagent grade and were obtained from BDH and Aldrich. Glass-distilled water was used throughout. The stock solutions of 9-anilinoacridine were prepared in methanol and were always stored at low temperature whenever they were not in use.

Kinetic measurements

The rates of the reactions of 2-ME with 9-anilinoacridine (9-ANA) at several pH levels were studied monitoring the disappearance of 9-ANA at 420 nm by using a Beckman Model 35 UV-visible spectrophotometer. The desired ionic strength of the reaction medium was kept constant with potassium chloride. All the experiments were carried out in tightly stoppered glass-ware and cuvettes prepurged with nitrogen. The concentrations of thiol were determined by weight. All thiol as well as other buffer solutions were freshly prepared in degassed glass-distilled water immediately before the start of a kinetic run to avoid any significant probable oxidation. Four kinetic runs were carried out simultaneously by the use of the manually controlled sample changer of the spectro-

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photometer. The pH values of the reaction mixtures were determined with Philips digital pH meter Model PW 9409 and the pH for each kinetic run was found to be constant during the course of the reaction.

In a typical kinetic run, all the reaction ingredients except 9-ANA required for an appropriate experimental condition were placed in a 50 cm^3 reaction vessel which was in turn placed in a thermostatted water bath. A total volume of ≥ 24.0 cm^3 of reaction solution was allowed to equilibrate for about 10 min at 30 °C. The reaction was then initiated by adding ≤ 1.0 cm^3 of 1.05 × 10^{-3} mol dm^{-3} 9-ANA solution prepared in methanol. This procedure thus ensured ≤ 4% (v/v) methanol content into the aqueous reaction mixture. An aliquot of nearly 2.5 cm^3 was quickly withdrawn from the reaction mixture and transferred to a 3 cm^3 quartz cuvette kept in the thermostatted cell compartment of the spectrophotometer. The decrease in the absorbance at 420 nm with time was monitored by the use of a digital display of the spectrophotometer. The constant temperature (30 °C) of the cell compartment was controlled electronically by the use of the temperature controlled unit of the spectrophotometer equipped with digital display as well as chart recorder Model 24-25 ACC.

All the kinetic runs were carried out under pseudo first-order kinetic conditions. The reactions were carried out for the periods of more than 6 half-lives of the reactions. The details of the data analysis are described elsewhere.[10]

Product characterization

In a typical kinetic run containing 2-ME buffer of pH 9.32 and 9-ANA, the TLC of the reaction mixture was carried out at different time intervals which revealed the formation of aniline as identified by using an authentic sample of it. The thioethers of the type 1 display a characteristic strong absorption peak at 363 nm[11] where the other expected products do not show absorption maxima.

In a kinetic run containing 2-ME buffer of pH 8.79 and 9-ANA, the spectra of the reaction mixture scanned at different time intervals showed the appearance of a peak at 363 nm (Fig. 1). The absorption at 363 nm was found to increase up to nearly 44 min since the start of the reaction and then it started to decrease with increase of reaction time (Fig. 1). At about 19 h, the absorption peak at 363 nm disappeared and another two new absorption peaks appeared at 384 and 402 nm as evident from Figure 1. The spectrum obtained at about 19 h is similar to a typical absorption spectrum of 9-methoxyacridine obtained under similar experimental conditions. These observations thus indicate that the initially formed product 1 underwent Smiles rearrangement to yield 2. It should be noted that both 9-methoxyacridine and 9-acridinylpropylthioether revealed very low molar extinction coefficients at 420 nm.

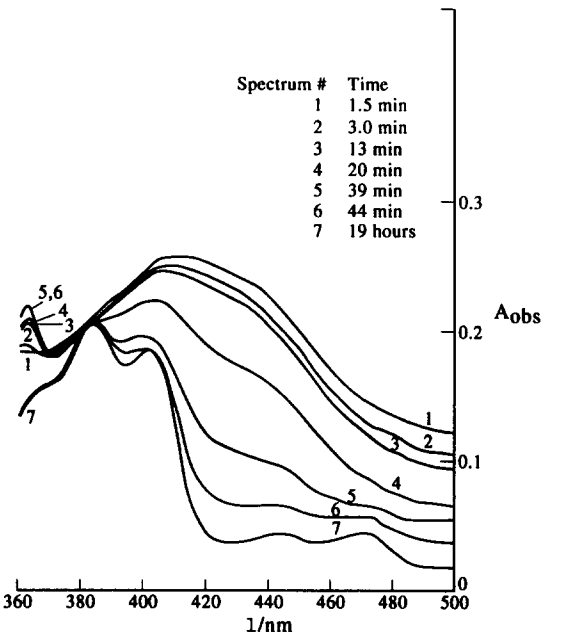
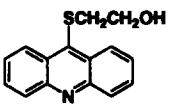


Figure 1. Spectra scanned at different time intervals of the reaction mixture containing 4.2 × 10^{-5} mol dm^{-3} 9-ANA, 0.2 mol dm^{-3} 2-ME buffer of pH 8.79 and 1.0 mol dm^{-3} ionic strength at 37 °C.



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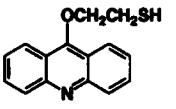

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Results and Discussion

Hydrolysis

The hydrolysis of 9-ANA was studied at 0.6 mol dm^{-3} phosphate buffer of pH 6.45 and 30 °C. The reaction was carried out for several weeks and the observed pseudo first-order rate constant (k_{obs}) turned out to be (4.56 ± 0.42) × 10^{-5} min^{-1}. Similar results were obtained for hydrolysis of a related compound within the pH range of nearly 6–10 at 30 °C.[8] The value of k_{obs} of 4.56 × 10^{-5} min^{-1} is smaller than even the standard deviation of the lowest obtained k_{obs} value in the thiolysis of the present study. Thus, the hydrolytic rate constants (k'_0) are regarded as negligible compared with the rate constants for thiolysis.

Hydroxylaminolysis of 9-ANA

Hydroxylamine, being an α-nucleophile, is considered to be an extremely strong nucleophile for its basicity. In order to check the probable nucleophilic reactivity of hydroxylamine toward 9-ANA, a few kinetic runs were carried out under the total hydroxylamine buffer concentration range of 0.2–0.6 mol dm^{-3} at pH 8.03 and 30 °C. The observed pseudo first-order rate constants (k_{obs}), as summarised in Table 1, were found to follow equation (1):

$$k_{obs} = k_n [Buf]_T \quad (1)$$

Table 1. Effect of hydroxylamine buffer on the cleavage of 9-ANA[a]

$[Buf]_T$[b] /mol dm^{-3}	$10^3 k_{obs}$ /min^{-1}	$10^3 k_{calcd}$[c] /min^{-1}
0.2	31.1 ± 1.0[d]	31.2
0.3	47.5 ± 1.4	46.8
0.4	62.3 ± 1.2	62.4
0.5	75.0 ± 2.4	78.0
0.6	96.9 ± 4.8	93.6

[a]Condition: $[9\text{-ANA}]_0 = 4.07 \times 10^{-1}$ mol dm^{-3}, pH = 8.03 ± 0.00, 30 °C, ionic strength 1.0 mol dm^{-3}. [b]Total concentration of hydroxylamine buffer. [c]Calculated from relationship: $k_{obs} = k_n[Buf]_T$ with $k_n = 0.156$ dm^3 mol^{-1} min^{-1}.

where $[Buf]_T$ represents the total hydroxylamine buffer concentration and k_n is the nucleophilic second-order rate constant. The linear least-squares calculated value of k_n is $(15.6 \pm 0.4) \times 10^{-2}$ dm^3 mol^{-1} min^{-1}. When the observed data were tried to fit to an equation: $k_{obs} = k'_0 + k_n [Buf]_T$, the calculated values of k'_0 and k_n were -1.1×10^{-3} min^{-1} and $(15.9 \pm 0.8) \times 10^{-2}$ dm^3 mol^{-1} min^{-1}, respectively. The negative value of k_0 is physically meaningless and hence k'_0 is not different from zero.

Thiolysis of 9-ANA under the buffer solutions of 2-ME

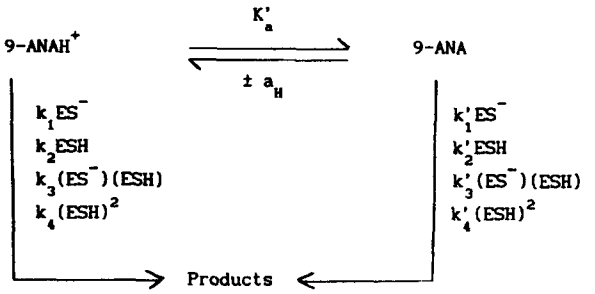
Several kinetic runs were carried out at different total buffer concentrations ($[RSH]_T$) and at a constant pH and ionic strength. The observed pseudo first-order rate constants (k_{obs}) followed equation (2):

$$k_{obs} = k_n [RSH]_T + k_b [RSH]_T^2 \quad (2)$$

where k_n and k_b represent nucleophilic second-order and buffer-catalysed third-order rate constants, respectively, for thiolytic cleavage of 9-ANA. The least-squares method was used to calculate k_n and k_b from equation 2 and these calculated values at different pH are summarised in Table 2.

The rate constants (k_{obs}), obtained at pH 9.93, did not reveal the detectable contribution of $k_b [RSH]_T^2$ term in equation 2. These rate constants were found to fit to equation 1 with $[Buf]_T$ changed to $[RSH]_T$ and the least-squares calculated value of k_n is shown in Table 2. In terms of the conventional definition of the general base (GB) catalysis, one cannot expect the occurrence of GB catalysis in the reaction of ionized 2-mercaptoethanol (ES^-) and 9-ANA. The absence of buffer catalysis at pH 9.93 shows the non-occurrence of GB catalysis in these reactions.

Both the protonated (9-$ANAH^+$) and non-protonated (9-ANA) forms of 9-ANA are expected to be reactive toward both the ionised (ES^-) and non-ionised (ESH) 2-ME nucleophile. The general reaction scheme for the catalysed and uncatalysed cleavage of 9-ANA in the buffer solution of 2-ME may be given as:



Scheme 1.

Table 2. The apparent second- and third-order rate constants for the reaction of 2-ME with 9-ANA[a]

pH	$10^2 k_n$ /dm^3 mol^{-1} min^{-1}	$10^2 k_b$ /dm^6 mol^{-2} min^{-1}	$[Buf]_T$[b]/mol dm^{-3}
8.82 ± 0.01[c]	6.01 ± 1.59[c]	28.8 ± 3.2[c]	0.2 - 0.6
9.03 ± 0.01	8.53 ± 1.40	16.7 ± 2.9	0.2 - 0.6
9.08 ± 0.01	8.16 ± 1.74	19.7 ± 3.5	0.2 - 0.6
9.54 ± 0.02	2.27 ± 0.47	14.4 ± 0.9	0.2 - 0.6
9.77 ± 0.07	1.51 ± 0.30	2.50 ± 0.60	0.2 - 0.6
9.93 ± 0.01	2.10 ± 0.20[d]	0.0	0.2 - 0.6

[a]Conditions: $[9\text{-ANA}]_0 = 4.20 \times 10^{-5}$ mol dm^{-3}, 30 °C, λ = 420 nm, ionic strength 1.0 mol dm^{-3}, the rate constants, k_n and k_b, were calculated from equation (2) and five kinetic runs were carried out at each pH. [b]Total buffer concentraction range. [c]Error limits are standard deviations. [d]Calculated from equation (1).

Based upon the Scheme 1, the general rate law for the reaction of 2-ME with 9-ANA may be given as

$$-d[9\text{-ANA}]_T/dt = (k_1[ES^-] + k_2[ESH])[9\text{-ANAH}^+] + (k'_1[ES^-] + k'_2[ESH])[9\text{-ANA}] + k_3[ES^-][ESH][9\text{-ANAH}^+] + k'_3[ES^-][ESH][9\text{-ANA}] + k_4[ESH]^2[9\text{-ANAH}^+] + k'_4[ESH]^2[9\text{-ANA}] \quad (3)$$

where $[9\text{-ANA}]_T$ represents the total concentration of 9-ANA and $[ES^-]$ and [ESH] represent the concentration of 2-ME in the ionised and non-ionised forms, respectively. Similarly, [9-ANA] and $[9\text{-ANAH}^+]$ represent the concentration of 9-ANA in non-protonated and protonated forms, respectively. The observed rate law ($-d[9\text{-ANA}]_T/dt = k_{obs}\,[9\text{-ANA}]_T$) and equations 2 and 3 may be used to derive equations 4 and 5

$$k_n = \frac{k'_1K_aK + (k_1K + k'_2K_a)a_H + k_2a_H^2}{(a_H + K)(a_H + K_a)} \quad (4)$$

$$k_b = \frac{k'_3K\,K_aa_H + (k_3K + k'_4K_a)a_H^2 + k_4a_H^3}{(a_H + K)^2(a_H + K_a)} \quad (5)$$

where K and K_a represent the ionisation constants of ESH and 9-ANAH^+, respectively. The value of pK_a was considered to be 7.93 at 30 °C.[13]

The thiolytic cleavage of 9-ANA was studied within the pH range 8.82–9.93. The plot of $k_n\,(a_H + K)(a_H + K_a)$ versus a_H appeared to be linear and hence it ruled out any significant contribution of $k_2a_H^2$ term compared with other terms of equation 4. Under such conditions, the equation 4 is reduced to equation 6.

$$k_n\,(a_H + K)(a_H + K_a) = k'_1K_aK + (k_1K + k'_2K_a)a_H \quad (6)$$

The linear least-squares calculated values of k'_1K_aK and $(k_1K + k'_2K_a)$ turned out to be -0.6×10^{-20} mol dm^{-3} min^{-1} and $(11.7 \pm 2.1) \times 10^{-10}$ min^{-1}, respectively. The negative value of k'_1K_aK is physically meaningless and hence it may be considered to be zero The least squares calculated value of $k_1K + k'_2K_a$ with $k'_1 = 0.0$ was found to be $(10.2 \pm 4.0) \times 10^{-10}$ min^{-1}. The insignificant contributions of the k'_1 and k_2 terms and the fact that $k_1/k'_1 = 10^3$ for *m*-AMSA[8] (an analogue of 9-ANA) rule out the k'_2 term compared with the k_1 term. The calculated value of k_1K [$= (10.2 \pm 4.0) \times 10^{-10}$ min^{-1}] was used to calculate k_1 as 2.87 ± 1.13 dm^3 mol^{-1} min^{-1} with $pK = 9.45$. The value of k_1 may be compared with the corresponding value (4.21 dm^3 mol^{-1} min^{-1}) obtained for *m*-AMSA[8] under similar experimental conditions.

The plot of $k_b\,(a_H + K)^2(a_H + K_a)/a_H$ versus a_H turned out to be linear with the least-squares calculated values of intercept and slope as -1.1×10^{-19} min^{-1} and $(5.44 \pm 0.94) \times 10^{-19}$ dm^3 mol^{-1} min^{-1}, respectively. The negative value of intercept is physically meaningless and therefore it may be considered to be zero. These observations showed the insignificant contributions of the k'_3 and k_4 terms compared to other terms in equation 5. The insignificant contribution of the k'_3 term ruled out the k'_4 term compared to the k_3 term in equation 5. Thus, it is apparent that within the pH range 8.82–9.77, equation 5 is reduced to equation 7.

$$k_b(a_H + K)^2(a_H + K_a)/a_H = k_3K\,a_H \quad (7)$$

The value of k_3K [$= (5.30 \pm 2.10) \times 10^{-9}$ dm^3 mol^{-1} min^{-1}] was calculated from equation 7 and the value of k_3 ($= 14.9 \pm 5.9$ dm^6 mol^{-2} min^{-1}) was obtained from the calculated value of k_3K with $pK = 9.45$. The value of k_3 may be compared with the value (64.8 dm^6 mol^{-2} min^{-1}) of the corresponding rate constant for *m*-AMSA.[8]

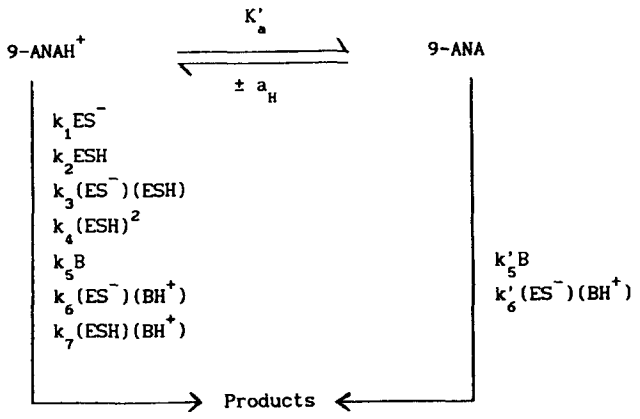
Reaction of 2-ME with 9-ANA under the presence of external buffers

In order to discover the effects of the buffers of hydroxylamine, phosphate and morpholine on the rates of the reactions of 2-ME with 9-ANA, several kinetic runs were carried out at a constant concentration of 2-ME and at different total buffer concentrations ($[Buf]_T$) of hydroxylamine, phosphate and morpholine of the desired pH. The observed pseudo first-order rate constants (k_{obs}) were found to obey equation (8)

$$k_{obs} = k_0 + k_B\,[Buf]_T \quad (8)$$

where k_0 and k_B represent buffer-independent and buffer-dependent apparent first- and second-order rate constants, respectively. The linear least-squares method was used to calculate k_0 and k_B from equation 8 and these results at different pH and total 2-ME concentrations for hydroxylamine, phosphate and morpholine are summarised in Table 3. The fitting of the observed data to equation 8 is evident from the standard deviations associated with k_0 and k_B (Table 3). Most of the k_0 values for highly reactive buffers such as hydroxylamine and phosphate, turned out to be either negative or positive with considerably high standard deviations which showed that the contributions of the k_0 term were insignificant compared to the $k_B[Buf]_T$ term in equation 8. Under such circumstances, the values of k_B were also obtained by setting $k_0 = 0.0$ in equation 8 and these results are also summarised in Table 3.

The cleavage of 9-ANA at a constant concentration of 2-ME in the buffer solutions of hydroxylamine, phosphate and morpholine should involve ESH and BH^+ (acid component of the buffer) as general acid catalysts. The free base component (B) of hydroxylamine buffer is also expected to show nucleophilic reactivity toward both 9-ANAH^+ and 9-ANA. Hence the general reaction scheme for the cleavage of 9-anilinoacridine under such conditions may be given as:



Scheme 2.

Based upon the reaction in Scheme 2, the general rate law for the reaction of 2-ME with 9-ANA in the presence of external buffer (Buf) may be given as

$$-d[9\text{-ANA}]_T/dt = (k_1[ES^-] + k_2[ESH])[9\text{-ANAH}^+] + (k_3[ES^-] + k_4[ESH])[9\text{-ANAH}^+][ESH] + k_5[B][9\text{-ANAH}^+] + k'_5[B][9\text{-ANA}] + (k_6[ES^-] + k_7[ESH])[BH^+][9\text{-ANAH}^+] + k'_6[ES^-][BH^+][9\text{-ANA}] \quad (9)$$

where [B] and [BH^+] represent the respective concentrations of general base and general acid forms of external buffer (Buf). Observed rate law and equations 8 and 9 can be used to derive equations 10 and 11 where $[RSH]_T = [ES^-] + [ESH]$ and K'_a is the ionisation constant of external general acid (BH^+).

The most reliable values of k_0 were obtained with morpholine buffer where the contribution of the $k_B[Buf]_T$ term was negligible compared with the k_0 term (in equation 8) at most of the pH values. These k_0 values were treated with equation 10 using known values of k_1 (= 2.87 $dm^3\ mol^{-1}\ min^{-1}$) and k_3 (= 14.9 $dm^6\ mol^{-2}\ min^{-1}$) obtained under the buffer solutions of 2-ME. The least-squares calculated values of k_2 and k_4 are $(22.7 \pm 7.9) \times 10^{-2}$ $dm^3\ mol^{-1}\ min^{-1}$ and -7.0×10^{-2} $dm^6\ mol^{-2}\ min^{-1}$, respectively. The negative value of k_4 is physically meaningless and hence it may be considered to be zero. The least-squares calculated value of k_2 is $(21.3 \pm 9.6) \times 10^{-2}$ $dm^3\ mol^{-1}\ min^{-1}$ with $k_4 = 0.0$. Although the value of k_2 is associated with high standard deviation, it may be compared with k_2 (= 27.3×10^{-2} $dm^3\ mol^{-1}\ min^{-1}$) obtained for the reaction of *N*-acetylcysteine with *m*-AMSA.[8]

The effect of hydroxylamine buffer on the rate of reaction of 2-ME with 9-ANA was studied at several pH and $[RSH]_T$ values. The values of k_B (Table 3) obtained from equation 8 at different pH and $[RSH]_T$ values were used to calculate rate constants, k_5, k'_5, k_6, k'_6 and k_7 from equation 11 with known values of pK_a, pK'_a and pK. The least-squares calculated values of k_5, k'_5, k_6, k'_6 and k_7 are 0.193 ± 0.040 $dm^3\ mol^{-1}\ min^{-1}$, 0.282 ± 0.151 $dm^3\ mol^{-3}\ min^{-1}$, $(19.1 \pm 1.9) \times 10^3$ $dm^6\ mol^{-2}\ min^{-1}$, -0.16 $dm^6\ mol^{-2}\ min^{-1}$ and -2.0 $dm^6\ mol^{-2}\ min^{-1}$, respectively. The negative values of k'_6 and k_7 are physically meaningless and therefore the contributions of the k'_a and k_7 terms compared to the other terms of equation 11 may be considered to be insignificant. The insignificant contribution of the k_7 term rules out the significance of the k'_7 term which is kinetically indistinguishable from the k_6 term. The observed data were used to calculate k_5, k'_5 and k_6 from equation 11 by setting $k'_6 = k_7 = 0$. The least-squares calculated values of k_5, k'_5 and k_6 are 0.176 ± 0.069 $dm^3\ mol^{-1}\ min^{-1}$, 0.326 ± 0.168 $dm^3\ mol^{-1}\ min^{-1}$ and $(14.3 \pm 2.2) \times 10^3$ $dm^6\ mol^{-2}\ min^{-1}$, respectively. Although the calculated values of k_5 and k'_5 are not very reliable because they are associated with high standard deviations, the derived value of k_n from these values of k_5 and k'_5 using the relationship: $k_n = k_5 f_B f_{9\text{-ANAH}^+} + k'_5 f_B f_{9\text{-ANA}}$ [where $f_B = K'_a/(a_H + K'_a)$, $f_{9\text{-ANAH}^+} = a_H/(a_H + K_a)$ and $f_{9\text{-ANA}} = 1 - f_{9\text{-ANAH}^+}$] at pH 8.0 is 0.25 $dm^3\ mol^{-1}\ min^{-1}$. This value of k_n may be compared with the corresponding value of k_n (= 0.16 $dm^3\ mol^{-1}\ min^{-1}$ obtained from equation 1 at pH 8.03 in the absence of 2-ME.

A series of kinetic runs was carried out at different $[RSH]_T$ under the phosphate buffer solutions of pH 5.75–10.70. The buffer catalysis could be detected only within the pH range 5.75–7.00 while no such catalysis could be detected at pH 10.43 and pH 10.70 (Table 3). The buffer-catalysed apparent second-order rate constants (k_B) were used to calculate the rate constants, k_6, k'_6 and k_7 from equation 11 where $k_5 = k'_5 = 0$. The free base component of phosphate buffer is an extremely weak nucleophile compared to the free base component of hydroxylamine buffer and that is why k_5 and k'_5 could not be detected under the presence of phosphate buffer. The least-squares calculated values of k_6, k'_6 and k_7 are $(12.7 \pm 1.4) \times 10^3$ $dm^6\ mol^{-2}\ min^{-1}$, $(36.9 \pm 17.6) \times 10^3$ $dm^6\ mol^{-2}\ min^{-1}$ and -1.7 $dm^6\ mol^{-2}\ min^{-1}$, respectively. The negative value of k_7 is physically meaningless and hence the values of k_6 and k'_6 were calculated by setting $k_7 = 0$. These values of k_6 and k'_6 are $(9.28 \pm 1.06) \times 10^3$ $dm^6\ mol^{-2}\ min^{-1}$ and $(72.2 \pm 17.9) \times 10^3$ $dm^6\ mol^{-2}\ min^{-1}$, respectively.

$$k_0 = \left\{\frac{k_1K + k_2a_H}{(a_H + K)(a_H + K'_a)}\right\} a_H [RSH]_T + \left\{\frac{k_3K + k_4a_H}{(a_H + K)^2(a_H + K_a)}\right\} a_H^2 [RSH]_T^2 \quad (10)$$

$$k_B = \frac{k'_5K_aK'_a + k_5K'_aa_H}{(a_H + K'_a)(a_H + K_a)} + \left\{\frac{k'_6KK_a + k_6Ka_H + k_7a_H^2}{(a_H + K)(a_H + K'_a)(a_H + K_a)}\right\} a_H[RSH]_T \quad (11)$$

Table 3. Uncatalysed and buffer-catalysed rate constants, k_0 and k_B, obtained from equation (8) for thiolysis of 9-ANA[a]

Buffer	pH	10^3k_0 min^{-1}	10^2k_B ← $dm^3\ mol^{-1}\ min^{-1}$	$10^2k_B^b$ →	$[RSH]_T^c$ ← mol	$[Buf]_T^d$ dm^{-3} →	# of runs
hydroxyl-	5.45±0.01[e]	20.3±1.2[e]	8.02±0.27[e]	17.3[f]	0.12	0.2 - 0.6	7
amine	5.53±0.01	13.0±1.0	7.59±0.23	16.0	0.09	0.2 - 0.6	7
	5.61±0.02	10.2±0.8	6.36±0.19	14.0	0.06	0.2 - 0.6	7
	5.63±0.04	2.04±1.44	5.99±0.33	-	0.02	0.2 - 0.6	7
		0.0	6.56±0.35	8.5			
	5.82±0.04	36.4±4.4	22.2±1.0	30.9	0.12	0.2 - 0.6	7
	5.86±0.01	27.6±4.4	19.5±1.0	26.4	0.09	0.2 - 0.6	7
	5.89±0.03	16.8±5.2	17.3±1.2	20.9	0.06	0.2 - 0.6	7
	6.06±0.01	4.27±2.15	15.7±0.5	-	0.03	0.2 - 0.6	7
		0.0	16.9±0.8	17.6			
	6.27±0.04	55.2±8.8	39.8±1.9	-	0.12	0.2 - 0.7	7
		0.0	54.4±6.1	49.7			
	6.30±0.04	32.0±11.2	39.5±2.6	-	0.09	0.2 - 0.6	7
		0.0	48.1±3.5	41.2			
	6.34±0.03	15.0±3.7	32.8±0.8	-	0.06	0.2 - 0.6	7
		0.0	36.8±1.5	32.5			
	6.44±0.03	12.4±2.4	25.2±0.6	-	0.03	0.2 - 0.6	6
		0.0	28.9±1.8	24.1			
	7.36±0.01	39.2±13.7	72.5±3.1		0.12	0.2 - 0.6	7
		0.0	83.2±4.7	73.6			
	7.33±0.02	8.2±40.9	63.6±8.4		0.09	0.35 - 0.6	5
		0.0	65.4±2.8	59.9			
	7.36±0.02	4.4±9.4	48.5±2.2		0.06	0.2 - 0.6	7
		0.0	49.6±1.5	46.8			
	7.70±0.03	-1.0	31.8±2.0		0.03	0.2 - 0.6	7
		0.0	31.7±1.5	36.2			
	8.03±0.00	-1.1	15.9±0.8	-	0.0	0.2 - 0.6	5
		0.0	15.6±0.4				
phosphate	5.77±0.08	48.8±3.3	11.6±0.7	24.9[g]	0.15	0.2 - 0.6	4
	5.75±0.06	19.9±2.7	10.4±0.6	14.3	0.09	0.2 - 0.6	7
	5.79±0.04	13.2±3.3	6.94±0.76	10.3	0.06	0.2 - 0.6	7
	5.89±0.11	8.30±1.5	3.00±0.01	6.28	0.03	0.2 - 0.6	7
	6.32±0.05	42.1±6.7	31.2±1.7	44.5	0.10	0.1 - 0.6	6
	6.49±0.01	30.8±35.5	64.4±7.9	-	0.12	0.2 - 0.6	4
		0.0	72.9±5.8	69.4			
	6.49±0.01	11.2±15.8	70.0±4.0	-	0.12	0.2 - 0.6	7
		0.0	73.1±2.9	69.4			
	6.49±0.01	39.3±17.7	45.2±3.9	-	0.09	0.2 - 0.6	6
		0.0	55.4±4.5	52.0			
	6.33±0.03	18.2±6.3	30.1±1.5	-	0.06	0.2 - 0.6	7
		0.0	35.1±2.2	27.1			
	6.43±0.03	0.9±8.7	16.3±1.9	-	0.03	0.2 - 0.6	6
		0.0	16.6±1.6	15.9			
	6.74±0.03	-37	118±9	-	0.12	0.2 - 0.6	7
		0.0	108±7	100			
	6.78±0.03	-43	90.9±4.1	-	0.09	0.2 - 0.6	7
		0.0	79.4±4.3	78.4			
	6.98±0.01	-21	69.6±2.9	-	0.06	0.2 - 0.6	6
		0.0	63.8±2.4	68.4			

Table 3. *Continued*

	7.00±0.01	-14	41.8±1.7	-	0.04	0.2 - 0.6	6
		0.0	38.0±1.7	46.8			
	6.96±0.02	-5	18.1±1.3	-	0.02	0.2 - 0.6	7
		0.0	16.9±0.1	22.2			
	10.43±0.03	4.00±1.88	-0.1	-	0.09	0.3 - 0.6	6
		3.42±0.97	0.0				
	10.70±0.05	6.14±0.57	-0.5	-	0.06	0.2 - 0.5	4
		4.34±0.67	0.0				
morpholine	6.74±0.01	12.7±0.9	0.4±0.2	-	0.12	0.2 - 0.6	7
		14.3±0.9	0.0				
	6.75±0.02	6.91±0.34	0.7±0.1	-	0.09	0.2 - 0.6	7
	6.62±0.01	6.04±0.76	0.07±0.18	-	0.06	0.2 - 0.55	6
		5.95±0.26	0.0				
	6.78±0.03	2.00±0.11	0.25±0.03	-	0.03	0.2 - 0.6	7
		3.05±0.37	0.0				
	8.19±0.02	30.7±5.0	9.62±1.16	9.66	0.12	0.2 - 0.6	7
	8.19±0.01	20.9±1.6	7.30±0.40	7.24	0.09	0.2 - 0.6	6
	8.34±0.03	10.6±1.5	5.49±0.35	5.44	0.06	0.2 - 0.6	7
	8.35±0.03	11.8±6.6	2.64±1.52	2.74	0.03	0.2 - 0.6	7
	9.02±0.03	35.8±3.7	0.64±0.79	-	0.12	0.3 - 0.6	6
		38.7±2.0	0.0				
	9.04±0.01	22.4±3.7	1.13±0.87	-	0.09	0.2 - 0.6	6
		26.9±3.2	0.0				
	9.32±0.04	26.3±5.5	-2.0	-	0.06	0.2 - 0.6	7
		16.9±4.4	0.0				
	9.51±0.03	24.6±4.1	-2.0	-	0.03	0.2 - 0.6	6
		15.8±4.0	0.0				
	9.20±0.04	30.5±1.3	-0.8	-	0.12	0.2 - 0.6	6
		27.2±1.5	0.0				

[a][9-ANA]$_0$ = 4.07 × 10^{-5} mol dm^{-3}, ionic strength 1.0 mol dm^{-3}, 30 °C, λ = 420 nm and rate constants, k_0 and k_B were calculated from equation (8). [b]Calculated from equation (11) as described in the text. [c]Total concentration of 2-ME. [d]Total buffer concentration range. [e]Error limits are standard deviations. [f]Calculated from equation (11) with $k'_6 = k_7 = 0.0$. [g]Calculated from equation (11) with $k_5 = k'_5 = k_7 = 0.0$.

Several kinetic runs were carried out at different total morpholine buffer concentrations ([Buf]$_T$), and [RSH]$_T$ within the pH range 6.62–9.51. But the buffer catalysis could be detected only within the pH range 8.19–8.35 (Table 3). The absence of buffer catalysis within the pH range 6.62–6.78 rules out the significance of the k_7 term compared to the other terms of equation 11. Morpholine and ES$^-$ are known as hard and soft nucleophile, respectively, while 9-ANA is a soft electrophile. The reaction between a soft nucleophile and a soft electrophile is energetically much more favorable than the reaction between a hard nucleophile and a soft electrophile. Thus, although the pK_a of ESH is only 0.85 pK units larger than the pK_a of morpholinium ion, the nucleophilic reactivity toward 9-ANA of morpholine (free base) is considered to be negligible compared to that of ES$^-$. The free base component of morpholine buffer is a much weaker nucleophile than the free hydroxylamine nucleophile and hence the k_5 and k'_5 terms are unlikely to contribute significantly compared with the other terms of equation 11. Thus, the values of k_B obtained within the respective pH and [RSH]$_T$ range of 8.19–8.35 and 0.03–0.12 mol dm^{-3} were used to calculate k_6 and k'_6 from equation 11 with $k_5 = k'_5 = k_7 = 0$. The least-squares calculated values of k_6 and k'_6 are listed in Table 4.

Mechanistic Proposal

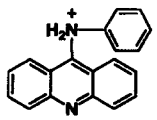
9-Anilinoacridine (9-ANA) apparently contains two basic sites where the first protonation can occur (**3** and **4**). In a related study on *m*-AMSA, we suggested the ring nitrogen as the most basic site for the first protonation, i.e. monoprotonated *m*-AMSA exists in the structural form similar to **4**. But most recently, O'Connor and coworkers[6] considered a structure like **3** for the related monoprotonated acridine derivatives. The greater stability of **4** compared with **3** may be attributed to the presence and absence of an energetically

Table 4.[a] Nucleophilic second-order rate constants, k_n, and general acid-catalysed rate constants, k_{ga}, for the reactions of 2-ME with 9-ANA

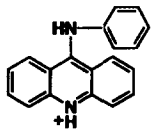
catalyst	pK_a	k_n/dm^3 mol^{-1} min^{-1}	k_{ga}/dm^6 mol^{-2} min^{-1}
2-ME	9.45[b]	k_1 = 2.87 ± 1.13[c] 10^2k_2 = 21.3 ± 9.6	k_3 = 14.9 ± 5.9[c]
morpholine	8.60[d]		k_6 = 38.6 ± 2.9 k'_6 = 12.1 ± 1.5
phosphate	6.42[e]		k_6 = 9279 ± 1065 k'_6 = 72167±17882
hydroxylamine	5.97[f]		k_6 = 14292±2228

[a]The various rate constants summarized in this table represent the same rate constants defined in equations (3) and (9). [b]Ref. 8. [c]Error limits are standard deviations. [d]Ref. 21. [e]Ref. 22. [f]Ref. 23.

favourable electron donating resonance effect in **4** and **3**, respectively. Bagno *et al.*[14] have recently determined the pK_a (= 9.10) of 4-aminopyridine by NMR relaxation rate measurements technique and demonstrated the ring nitrogen as the most basic site in 4-aminopyridine. In a kinetic study on aminolysis of ionised phenyl salicylate, it has been concluded that the most basic site in 2-aminopyridine was the ring nitrogen.[15] These studies support our proposal that the monoprotonated 9-ANA exists as **4**.



3



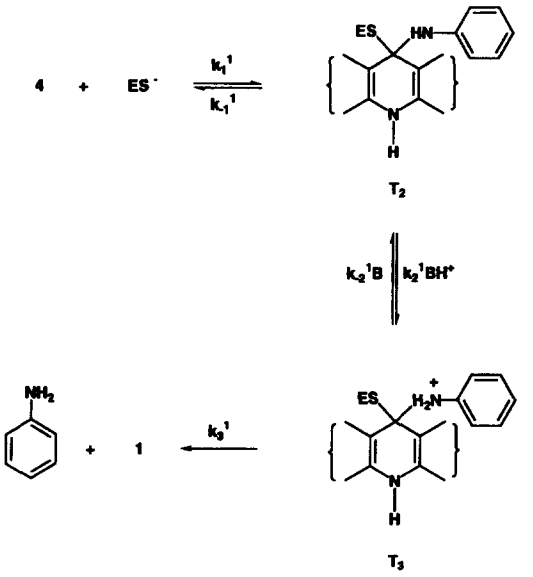
4

The general acid-catalysed third-order rate constants (k_6) for the reactions of 2-ME with 9-ANAH$^+$ were found to obey Brønsted relationship (equation 12). The least-squares

$$\log k_6 = C_1 - \alpha pK_a \qquad (12)$$

calculated values of C_1 and α are 9.766 ± 0.571 dm^6 mol^{-2} min^{-1} and 0.93 ± 0.07, respectively. The fitting of the observed data to equation 12 is evident from the standard deviations associated with the values of C_1 and α. The value of α (= 0.93) is not very much different from 1.0 which indicates that the general acid-catalysed thiolytic cleavage of 9-ANAH$^+$ involves the thermodynamically unfavourable proton transfer as the rate-determining step.[16]

This observation favours a stepwise mechanism shown in Scheme 3 where the k_2^1 step is considered to be the rate-determining step. It has been concluded in a number of studies that the pK_a of the ammonium moiety in an intermediate like $\mathbf{T}_1$ is not very different from that for parent ammonium ion.[17,18] If the k_2^1 step is the rate-determining step then $k_{-1}^1 >> k_2^1$ [BH$^+$] and $k_3^1 >> k_{-2}^1$[B]. The k_{-2}^1 step involves proton transfer in a possibly thermodynamically favourable direction and hence $k_{-2}^1 \simeq 10^{10}$ dm^3 mol^{-1} s^{-1}.[19] Thus, under the buffer systems of this study, k_{-2}^1 [B] $\simeq 10^9$ s^{-1}. This shows that k_3^1 must be greater than 10^9 s^{-1} which may not be unreasonable for the fact that (i) the k_3^1 step involves a very good leaving group (pK_a of protonated aniline is 4.71)[20] and (ii) there is a restoration of resonance for the central ring of the acridine moiety in k_3^1 step.

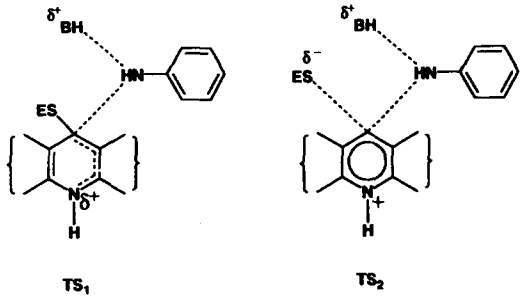


Scheme 3.

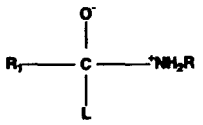
An alternative rate-determining step involving concerted general acid-catalysed expulsion of leaving group from $\mathbf{T}_2$ through transition state $\mathbf{TS}_1$ may be ruled out for the reason that under such conditions, the proton transfer from general acid (BH$^+$) to the leaving group is no longer thermodynamically unfavourable. This is against the observed value of α (0.93). This reasoning may also be used to rule out a concerted reaction mechanism involving transition state $\mathbf{TS}_2$.

The absence of morpholine buffer catalysis at pH 6.62–6.78 and the presence of such catalysis at pH 8.19–8.35 reveals that the general acid catalysis operates only when an anionic thiol nucleophile (ES$^-$) is involved in the reaction of 2-ME with 9-ANAH$^+$. The unimportance of general acid catalysis in the reaction of the neutral nucleophile (ESH) with 9-ANAH$^+$ may be attributed to

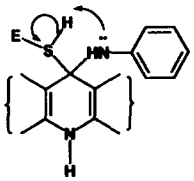
the probable occurrence of intramolecular general acid catalysis through transition state TS_3 or TS_4 to produce T_3.



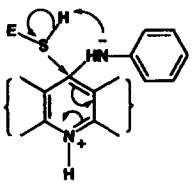
TS_1 TS_2



T_1



TS_3



TS_4

Conclusion

It has been shown by a few investigators[7,24,25] that a major route of *in vivo* breakdown for *m*-AMSA is a nonenzymatically mediated chemical attack by thiols at C-9 of acridine moiety and this provides thiolytic removal of the essential 9-methanesulfonamide function. The present mechanistic study on thiolytic cleavage of 9-ANA indicates that the rate of thiolysis is highly sensitive to the pK_a of both the catalyst and the substrate (9-ANA). Thus, an attempt to correlate the pseudo first-order rate constants for thiolysis of 9-ANA and its derivatives with their corresponding biological activity (such as antitumor activity) may not be considered to be very useful. The present study might be useful to understand the mechanistic aspects of *in vivo* thiolytic degradation of 9-ANA and its derivative drug compounds.

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