Decomposition of *N′*-Benzoyl-*N*-nitrosoureas in Aqueous Media

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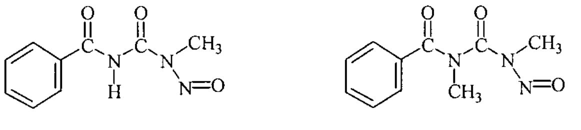
The decomposition of *N′*-benzoyl-*N*-methyl-*N*-nitrosourea (BMNU) in aqueous media over the 0–14 pH range has been studied. In basic and neutral media ($6 < \mathrm{pH} < 14$) the reaction proceeds through abstraction of the acidic proton of BMNU ($\mathrm{p}K_a = 7.8$) and subsequent decomposition of the conjugate base of the thus formed nitrosourea, via an intermediate benzoyl isocyanate. Support for this mechanism is provided by the presence of *N,N′*-dibenzoylurea in the final reaction mixtures, as the result of the trapping of benzoyl isocyanate with benzamide generated from hydrolysis of the former. The hydrolysis of BMNU takes place through three competitive pathways: spontaneous decomposition of the conjugate base of BMNU, and buffer-catalyzed and hydroxide ion catalyzed water addition to the carbonyl group of the deprotonated nitrosourea. *N′*-Benzoyl-*N,N′*-dimethyl-*N*-nitrosourea (BDMNU), a benzoyl nitrosourea lacking the acidic proton of BMNU, is hydrolyzed in basic media by attack of hydroxide ion on the carbonyl group of the urea. In acid media ($0 < \mathrm{pH} < 6$), BMNU gives only deamination products, differing from the reported behavior of other *N*-nitroso compounds and of the isoster nitrosoguanidine, in which denitrosation is almost quantitative. The reaction is acid-catalyzed in the 0–2.5 pH range and pH-independent in the 3–5 pH range. The presence of general acid catalysis ($\alpha = 0.60$), the absence of nucleophilic catalysis, and the thermodynamic activation parameters for the reaction support the mechanism proposed in the literature for the deamination of *N*-nitrosoureas in acidic media.

Introduction

The reactivity of nitrosamides and nitrosoureas is a topic of relevant biomedical interest due to the potential cytotoxicity of the resulting decomposition products. Some nitrosoureas, such as carmustine and lomustine, have found application as antineoplasic drugs,[1] while *N*-methyl-*N*-nitrosourea (MNU), the simplest member of the series, is a powerful carcinogen and mutagen. The biological action of these compounds has been attributed to their capacity to undergo spontaneous non-enzymatic decomposition under physiological conditions, generating strong electrophilic species able to alkylate biological molecules,[2−4] such as the nucleic acid purines and pyrimidines.

In this paper we describe a kinetic study of the stability in aqueous media of *N′*-benzoyl-*N*-methyl-*N*-nitrosourea (**1**, BMNU) and *N′*-benzoyl-*N,N′*-dimethyl-*N*-nitrosourea (**2**, BDMNU), derivatives of MNU each containing a strongly electron-withdrawing substituent in the form of the benzoyl group (Scheme 1). The presence of the second carbonyl group increases the number of alternative pathways for the decomposition reaction.



1, BMNU **2, BDMNU**

Scheme 1

The literature offers a wide range of possible mechanisms for decomposition of nitrosoureas. Three main reaction mechanisms to explain the decomposition of this class of compounds in neutral or basic media have been put forward: (*i*) addition of hydroxide ion to the urea carbonyl group to form a tetrahedral intermediate, which then undergoes decomposition,[5−7] (*ii*) alternatively, the hydrolysis of nitrosoureas with acidic hydrogen atoms ($\mathrm{p}K_\mathrm{a} < 13$) might occur through the abstraction of an acidic hydrogen atom, giving rise to an unstable anion, the conjugate base of the nitrosourea,[8−10] and (*iii*) nucleophilic attack of OH^- on the nitroso group,[11] forming nitrite ion and regenerating the urea.

Decomposition of nitrosoureas in acid media shows two competitive pathways:[5,12−14] deamination and reversible denitrosation, generating the parent urea and nitrous acid. The magnitude of each pathway depends both on the acidity of the medium and on the structure of the nitrosourea.

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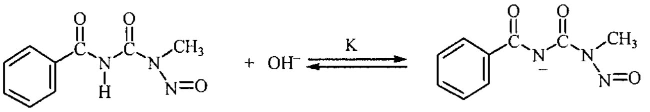
Deamination is not catalyzed by nucleophiles and shows an isotopic effect of 2, both suggesting a process involving an intermediate different from that involved in denitrosation.

Our study shows that acid hydrolysis of *N*′-benzoyl-*N*-methyl-*N*-nitrosourea (BMNU) occurs exclusively through deamination. The absence of denitrosation should be related to the easy formation of the hydrate of BMNU. Moreover, we were able to show that decomposition of BMNU in neutral and basic media involves the formation of the corresponding anion. The presence of the benzoyl group as a substituent enhances the acidic character of the α-hydrogen with respect to the carbonyl group up to pK_a·= 7. The formed anion decomposes in three competitive ways: spontaneous decomposition, base-catalyzed water addition to the carbonyl, and OH^- addition to the carbonyl group or OH^--catalyzed water addition. The overall picture shows quite different behavior for BMNU than reported for other *N*-nitrosoureas.

Results and Discussion

1. Acidity Constants of BMNU

The pK_a of BMNU was determined from the change in absorbance at 240 nm as a function of pH. It was not possible to measure the extinction coefficient accurately at the high-pH end point because of the very high rate of decomposition. From the deprotonation equilibrium of BMNU (Scheme 2) it is possible to derive Equation (1) relating absorbance and OH^- concentration.



Scheme 2

$$Absorbance = \frac{\varepsilon_{AH} + \varepsilon_{A^-} K\left[OH^-\right]}{1 + K\left[OH^-\right]}\left[AH\right]_{tot} \qquad (1)$$

By fitting Equation (1) to the experimentally acquired data (Figure 1) we can obtain a value for BMNU of $pK_a^{BMNU} = 6.3 \pm 0.3$. Figure 1 also shows the absorbance (data obtained at 270 nm) against acidity for the non-nitrosated benzoyl urea (BMU). The calculated pK_a ($pK_a^{BMNU} = 11.9 \pm 0.2$) is in good agreement with the values of pK_a = 9.5 for succinimide and pK_a = 15.1 for acetamide. The difference between the acidity constants of BMNU and BMU ($\Delta pK_a = 5.6$) is a consequence of the presence of the nitroso group and is compatible with pK_a values of pK_a = 12.9 for dimethylnitrosourea[10] and pK_a = 11.45 for *N*-nitroso-2-imidazolidone.[19]

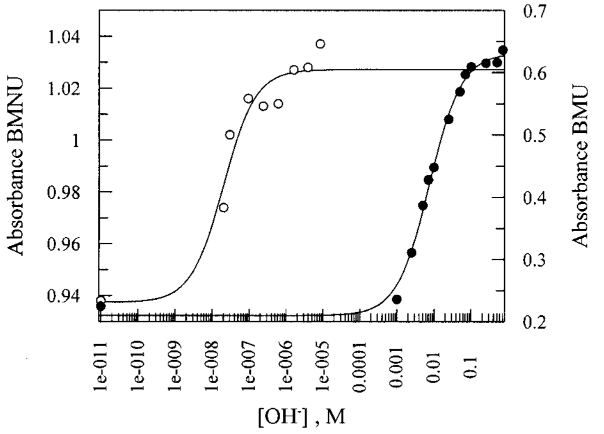


Figure 1. Influence of pH on BMNU and BMU absorbance at 240 and 270 nm, respectively, at 25 °C and ionic strength 1 M ($NaClO_4$); the solid line is calculated from Equation (1) and constants given in the text

2. Hydrolysis of BMNU and BDMNU in Neutral and in Basic Media

The first-order rate constants for the decomposition of BMNU were measured at 25 °C and 1 M ionic strength ($NaClO_4$) in a number of aqueous buffers from pH = 6 to pH = 11 and in the presence of different NaOH concentrations from pH = 11 to pH = 14. Values of the buffer-independent rate constant (k_{ind}) for decomposition of BMNU are equal to k_{obs} measured for reactions in the absence of buffer (pH ranging from 11 to 14). In the presence of buffers the values of k_{ind} were extrapolated at zero buffer concentration according to Equation (2); k_{obs} varies linearly with buffer concentration over the interval [buffer] = (5.00×10^{-2} to 0.500) M for HCO_3^-/CO_3^{2-} and $H_3BO_3/H_2BO_3^-$ buffers.

$$k_{obs} = k_{ind}[OH^-] + k_{buffer}[buffer] \qquad (2)$$

When $H_2PO_4^-/HPO_4^{2-}$ buffers were used, no catalysis was observed. Figure 2 shows the influence of the buffer concentration (HCO_3^-/CO_3^{2-}) on k_{obs} for the decompo-

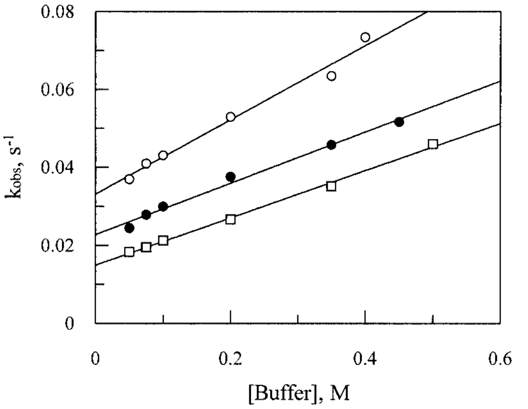


Figure 2. Influence of total buffer concentration upon the pseudo-first-order rate constant (k_{obs}) for decomposition of BMNU in HCO_3^-/CO_3^{2-} buffers; pH = 10.83 (correlation coefficient *r* = 0.992) (open circles), pH = 10.56 (correlation coefficient *r* = 0.995) (filled circles), and pH = 10.30 (correlation coefficient *r* = 0.998) (open squares)

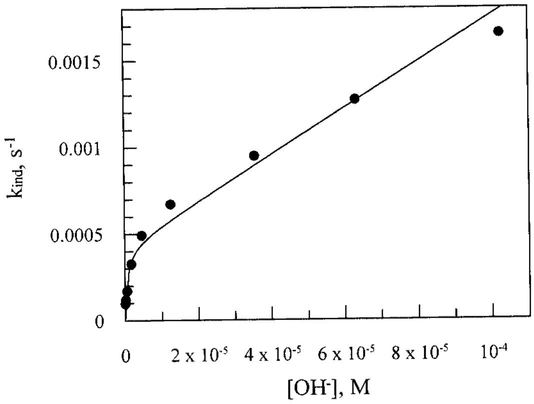


Figure 3. Influence of hydroxide ion concentration on k_{obs} in the basic hydrolysis of BMNU for $6 < pH < 9$

sition of BMNU. As can be seen in Figure 3, the buffer-independent rate constant show a nonlinear dependence on OH^- concentration for $[OH^-] < 1 \times 10^{-5}$ M. Such nonlinear dependence should be related to the acidity constant of BMNU.

In the pH range from pH = 11 to 14, a plot of the pseudo-first-order rate constants (k_{obs}) against hydroxide ion concentration showed first-order behavior with respect to OH^-, yielding a slope of 91.6 M^{-1} s^{-1}. The same behavior was observed for BDMNU, in which the acidic proton atom of BMNU is not present and which therefore cannot undergo hydrolysis through formation of the conjugate base. A plot of k_{obs} against $[HO^-]$ for BDMNU yielded a slope of 11.2 M^{-1} s^{-1}. The similar reactivities of the two benzoyl nitrosoureas at high pH values, with $k_{ind}(BMNU) \approx 10k_{ind}(BDMNU)$, is consistent with attack of OH^- on the carbonyl groups of the nitrosoureas. The slower rate for BDMNU is attributed to steric hindrance due to the additional methyl group.

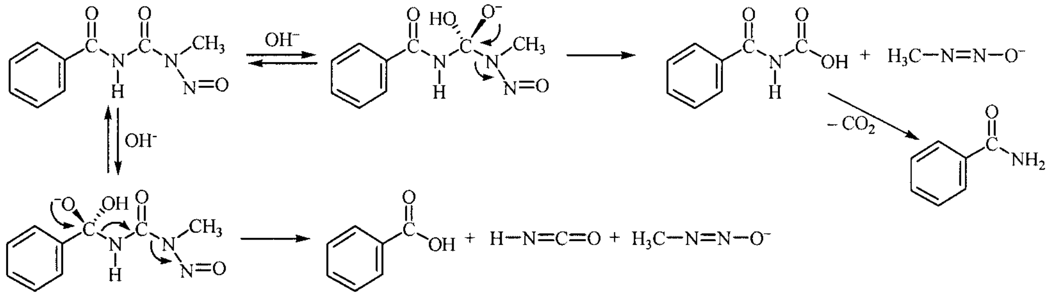
These results can be interpreted by any of the three mechanisms already described: (*i*) classical bimolecular OH^- attack on the carbonyl group of the nitrosourea, (*ii*) pre-equilibrium proton abstraction followed by decomposition of the resulting anion, and (*iii*) nucleophilic attack at the nitroso moiety. However, the absence of NO_2^- in the final reaction mixtures, shown by Shinn's method,[17] rules out nucleophilic attack on the nitroso group for both benzoyl nitrosoureas.

The fact that *N*-methylbenzamide was the only product observed by TLC after decomposition of BDMNU in sodium hydroxide solutions supports the mechanism proposed in the literature[5−10] for the basic hydrolysis of nitrosoureas lacking acidic protons [nucleophilic attack of OH^- on the urea carbonyl group − pathway (*i*)].

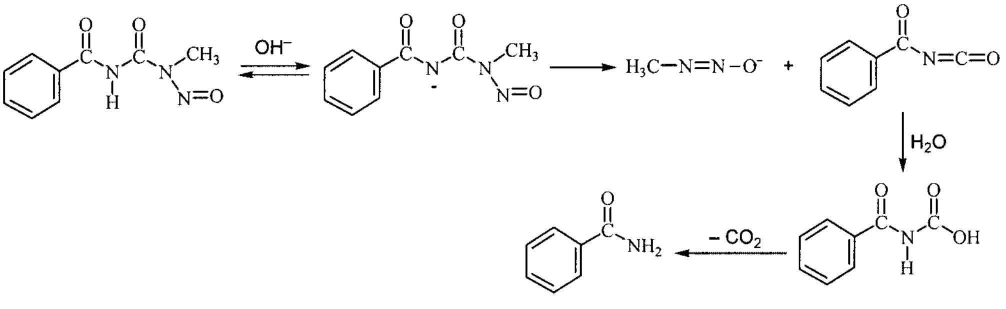
The kinetic results obtained for decomposition of BMNU are compatible with pathways (*i*) (Scheme 3) and (*ii*) (Scheme 4).

The final products from BMNU hydrolysis by pathway (*i*) should be benzamide and benzoic acid. The main product from pathway (*ii*) should also be benzamide. However, the main feature that distinguishes between mechanisms (*i*) (Scheme 3) and (*ii*) (Scheme 4) is the presence of the intermediate benzoyl isocyanate in the latter.

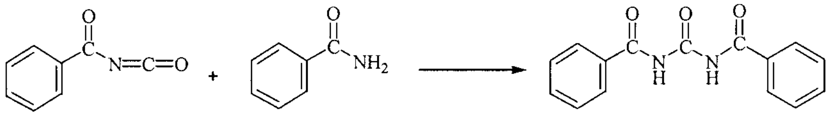
The presence of *N*,*N*′-dibenzoylurea (identified by TLC by comparison with an independently synthesized sample) among the final reaction products can only be explained by allowing the efficient trapping of benzoyl isocyanate by the benzamide generated from its hydrolysis (Scheme 5). This is a striking evidence for the occurrence of pathway (*ii*).[18] Yoshida and Yano[9] also found the presence of *N*,*N*′-di-



Scheme 3



Scheme 4



Scheme 5

phenylurea, originating from the trapping of phenyl isocyanate with aniline in the basic hydrolysis of N'-phenyl-N-methyl-N-nitrosourea.

The experimental results show a nonlinear dependence of k_{ind} on $[OH^-]$ for BMNU hydrolysis at low OH^- concentrations (pH range 6–9), presumably due to ionization of nitrosourea[10,19] at this pH. In order to explain this behavior we propose three parallel decomposition pathways for the deprotonated nitrosourea (Scheme 6): spontaneous decomposition, nucleophilic attack of a second hydroxide ion on the carbonyl group, and base-catalyzed water attack on the carbonyl group.

From this mechanistic scheme the rate Equation (3) can be derived. In the absence of buffer or for rate constants extrapolated to zero buffer concentration Equation (3) can be simplified to Equation (4).

$$k_{obs} = \frac{k_1K[OH^-] + k_2K[OH^-]^2 + k_3K[OH^-]\dfrac{K_a^{Buffer}[OH^-]}{K_w + K_a^{Buffer}[OH^-]}[Buffer]_T}{1 + K[OH^-]} \tag{3}$$

$$k_{obs} = \frac{k_1K[OH^-] + k_2K[OH^-]^2}{1 + K[OH^-]} \tag{4}$$

Equation (4) was fitted to the experimentally acquired data in the 6–10 pH range (solid line in Figure 3), with a K value of $(1.52 \pm 0.3) \times 10^6\ \text{M}^{-1}$ being obtained, and since $K = K_a/K_w$, this allows an estimated pK_a of 7.8 for BMNU, a typical value for an imide-type proton. This value is different from that obtained previously – $pK_a^{BMNU} = 6.3 \pm 0.3$ – probably because of the inaccuracy of absorbance measurements due to the instability of the anion of BMNU. A value for $k_1 = (1.7 \pm 0.4) \times 10^{-4}\ \text{M}^{-1}\text{s}^{-1}$ is also obtained from the fit.

For high hydroxide ion concentrations, $1 << K[OH^-]$ and $k_1 << k_2[OH^-]$, so BMNU exists mainly in its ionized form. In this case Equation (4) simplifies and k_{obs} now depends linearly on $[HO^-]$, according to Equation (5), which is experimentally observed for pH > 9.

$$k_{obs} = k_2[OH^-] \tag{5}$$

A k_2 value of $90.4 \pm 0.9\ \text{M}^{-1}\text{s}^{-1}$ was obtained. Above pH = 9, and according to the proposed mechanism (Scheme 6), the nucleophilic attack of hydroxide ion on the carbonyl group of the deprotonated nitrosourea would be expected to be the predominating pathway for decomposition. This pathway could also account for the similar reaction rates of BMNU and BDMNU observed in sodium hydroxide solutions, a medium in which both nitrosoureas undergo nucleophilic attack of hydroxide ion on the urea carbonyl group, although for BMNU this attack occurs on its deprotonated form (Scheme 6) while for BDMNU it is on the neutral form.

From Equation (3) we can obtain Equation (6) relating the slopes of plots such as those shown in Figure 2 and the rate constant of buffer-catalyzed water addition to carbonyl groups. For HCO_3^-/CO_3^{2-} and $H_3BO_3/H_2BO_3^-$ buffers ($9.10 < pH < 10.83$), we can assume that $1 << K[OH^-]$, so Equation (6) can be simplified to Equation (7).

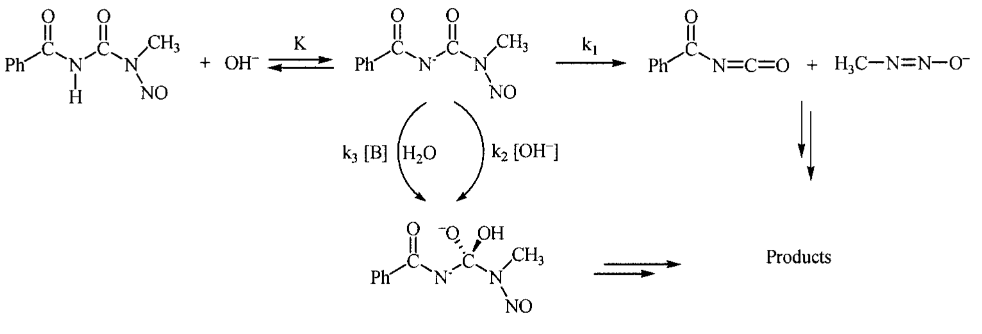
$$Slope = \frac{k_3KK_a^{Buffer}[OH^-]^2}{(K_w + K_a^{Buffer}[OH^-])(1 + K[OH^-])} \tag{6}$$

$$Slope = \frac{k_3K_a^{Buffer}[OH^-]}{(K_w + K_a^{Buffer}[OH^-])} \tag{7}$$

From plots of $(slope[K_W + K_a^{buffer}[OH^-]])/pK_a^{buffer}$ against $[OH^-]$ (not shown) we can obtain the catalytic rate constant $k_3 = (7.3 \pm 0.6) \times 10^{-3}\ \text{M}^{-1}\text{s}^{-1}$ and $k_3 = 0.121 \pm 0.006\ \text{M}^{-1}\text{s}^{-1}$ for water addition catalyzed by $H_3BO_3/H_2BO_3^-$ and by HCO_3^-/CO_3^{2-} buffers, respectively (Table 1). From these values, and from the rate constant for

Table 1. Bimolecular rate constants for base-catalyzed water addition to BMNU anion

Buffer	pK_a	k_B [M^{-1} s^{-1}]
OH^-	15.75	90.4 ± 0.9
HCO_3^-	10.33	0.121 ± 0.006
$H_2BO_3^-$	9.23	$(7.31 \pm 0.6) \times 10^{-3}$



Scheme 6

water addition catalyzed by OH^- (k_2 = 90.4 $M^{-1}s^{-1}$), a Brønsted plot with a slope of $\beta = 0.60$ can be drawn. This value indicates that bond making has made significant progress in the transition state.

3. Acid Hydrolysis of BMNU

In order to complete the pH/rate profile for BMNU, the stability of the nitrosourea was studied in acidic media: in hydrochloric acid solutions for pH < 3 and in acetate buffer in the 3.5−5.5 pH range. No buffer catalysis was observed when CH_3COOH/CH_3COONa buffers were used. A plot of k_{obs} against $[H^+]$ for pH < 2.5 (not shown, correlation coefficient $r = 0.999$) shows the reaction to be first order in $[H^+]$, according to Equation (8), which yields a value of $(2.35 \pm 0.09) \times 10^{-4}$ M^{-1} s^{-1} for k_H.

$$k_{obs} = k_H[H^+] \qquad (8)$$

In the 2.5−5.5 pH range, k_{obs} does not depend on pH and a mean value of 9.8×10^{-6} s^{-1} for the spontaneous decomposition (water-catalyzed) reaction is obtained, very similar to that of $(9.4 \pm 0.4) \times 10^{-6}$ s^{-1} obtained from the intercept of the k_{obs} against $[H^+]$ correlation. Figure 4 shows a plot of k_{ind} against $[OH^-]$ for decomposition of BMNU in aqueous media.

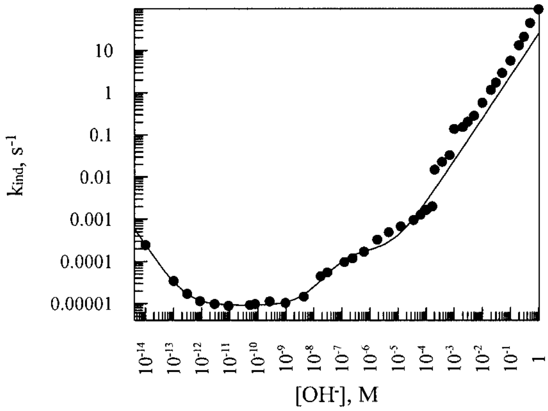


Figure 4. pH/rate profile for the hydrolysis of BMNU in aqueous media (plus 1% dioxane) at 25.0 °C and ionic strength 1.0 M ($NaClO_4$); $[BMNU]_0 = 1.0 \times 10^{-4}$ M

The absence of nitrite in the final reaction mixtures and the fact that only benzamide was detected by TLC suggest deamination as the main pathway for the acid hydrolysis of BMNU (Scheme 7). This behavior is different from that observed for other nitrosoureas[12−14] and for the isoster nitrosoguanidine,[20] in which denitrosation is almost quantitative over the same pH range.

The decomposition kinetics of BMNU in 1.0 M hydrochloric acid solution were reexamined in D_2O, with DCl instead of HCl, producing a value of 1.17×10^{-4} s^{-1} for k_{obs}. Comparison with the value obtained in aqueous 1.0 M hydrochloric acid solution provides a solvent kinetic isotopic effect of k_{H2O}/k_{D2O} = 2:1, similar to that obtained by Snyder and Stock[5] for the deamination of methyl nitrosoureas in acidic media, suggesting slow protonation in the rate-limiting step.

The influence of thiocyanate ion concentration on the reaction rate in 1.0 M hydrochloric acid solutions was studied, and it was found not to affect k_{obs} in a significant way (Table 2). The absence of nucleophilic catalysis is not unexpected, since in our case deamination and not denitrosation is the major pathway for BMNU decomposition in acidic media.

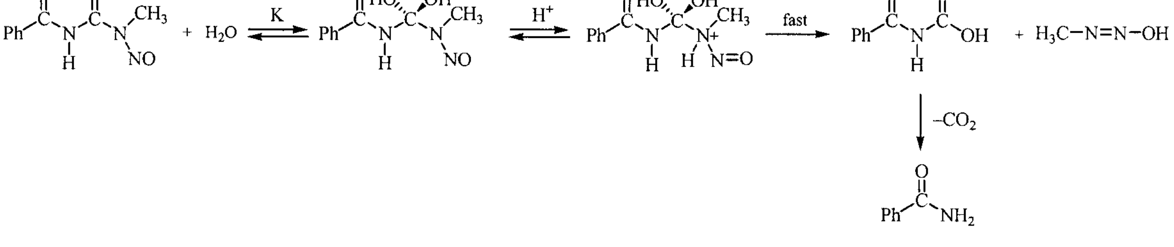
Table 2. Influence of SCN^- concentration on the pseudo-first-order rate constants for decomposition of BMNU in 1.0 M hydrochloric acid solutions

10^3 $[SCN^-]$ [M]	10^4 k_{obs} $[s^{-1}]$
0.0	2.58
2.0	2.21
4.0	2.30
6.0	2.47
8.0	2.73
10	2.98

Decomposition of BMNU in haloacetic acid buffers was also studied at buffer ratios close to unity, thus ensuring the presence of ca 50% of the acid form. At constant pH, k_{obs} was found to depend linearly on total buffer concentration (Figure 5). In this case Equation (9) applies, where k_H and k_{HA} are the bimolecular rate constants for the H^+-catalyzed and the buffer-catalyzed reactions, respectively.

$$k_{obs} = k_H\left[H^+\right] + \frac{k_{HA}\left[H^+\right]\left[Buffer\right]_T}{K_a^{Buffer} + \left[H^+\right]} \qquad (9)$$

The rate constants k_{HA} for the general acid-catalyzed reaction, shown in Table 3, were obtained from the slopes of k_{obs} against $[buffer]_T$ by application of Equation (9). The more acidic the media, the higher k_{HA}, consistently with the increase in the fraction of free haloacid in the reaction



Scheme 7

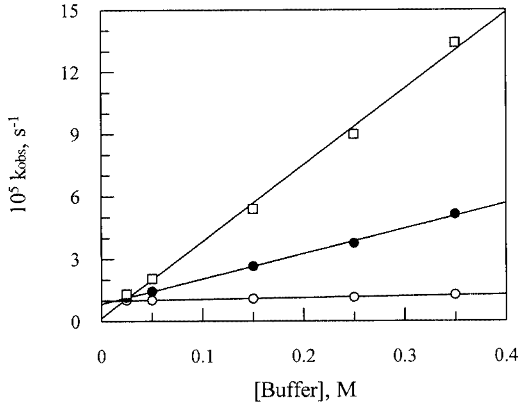


Figure 5. Influence of total buffer concentration upon the pseudo-first-order rate constant for decomposition of BMNU in haloacetic acid buffers: pH = 2.74 (MCA buffer, correlation coefficient r = 0.991) (open circles), pH = 0.64 (TCA buffer, correlation coefficient r = 0.999) (filled circles), and pH = 0.12 (TFA buffer, correlation coefficient r = 0.999) (open squares)

Table 3. Bimolecular rate constants for H^+-catalyzed and buffer-catalyzed decomposition of BMNU in haloacetic acid buffers: MCA = monochloroacetic acid, TCA = trichloroacetic acid, and TFA = trifluoroacetic acid

Buffer	pK_a	k_{HA} [M^{-1} s^{-1}]
MCA	2.86	$(1.7 \pm 0.2) \times 10^{-5}$
TCA	0.66	$(2.48 \pm 0.06) \times 10^{-4}$
TFA	0.23	$(8.4 \pm 0.2) \times 10^{-4}$
H_3O^+	−1.75	$(2.35 \pm 0.09) \times 10^{-4}$

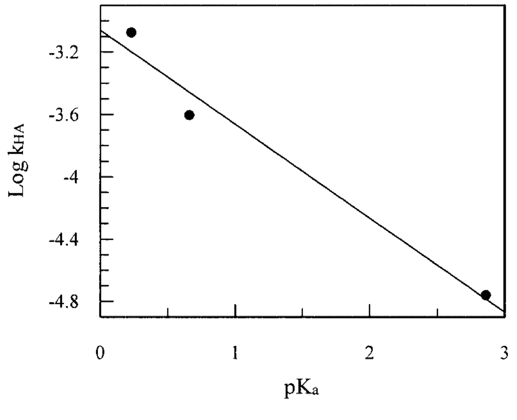


Figure 6. Brønsted plot for the decomposition of BMNU in haloacetic acid buffers: MCA (pK_a = 2.86); TCA (pK_a = 0.66) and TFA (pK_a = 0.23)

medium. The Brønsted plot (Figure 6) shows that the reactivity of the haloacetic acids correlates quite well with their pK_a^{buffer} values (r = 0.99). The value of 0.60 obtained for the Brønsted α parameter is very similar to those observed for N-nitroso-2-pyrrolidone[21] (a N-nitrosamide hydrolyzed via its N-conjugate acid) and N-methyl-N'-cyclohexyl-N-nitrosothiourea.[14] The k_H value was not included in the Brønsted plot. It is clear that this plot is essentially straight, although a downward curvature is observed if the point for H_3O^+ is included. However, the switch from a neutral to a cationic acid (for which temperature and medium effects would be very different) as well as the uncertain statistical correction for H_3O^+ could account for these differences.

The decomposition of BMNU in 1.0 M hydrochloric acid solutions was studied at five different temperatures. Fitting of the Eyring equation to the experimentally acquired data yields a linear correlation (r = 0.996) from which the thermodynamic activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$ can be estimated. The values obtained − $\Delta H^{\neq} = 84 \pm 4$ kJ·mol^{-1} and $\Delta S^{\neq} = -34 \pm 3$ J·mol^{-1}K^{-1} − agree with those referred to in the literature for the acid hydrolysis of N-nitrosamides[17] and N-nitrosoamidines.[22] The negative $\Delta S^{\neq}$ value suggests a bimolecular rate-limiting step in accordance with the mechanism proposed in the literature for the deamination of N-nitrosoureas.[5,12–14]

Conclusion

In basic and neutral aqueous media, BMNU is hydrolyzed by abstraction of its acidic proton and subsequent spontaneous decomposition of the conjugate base of the nitrosourea, via an intermediate benzoyl isocyanate. At high hydroxide ion concentrations (pH > 9), two alternative decomposition pathways predominate: attack of a second hydroxide ion at the carbonyl group (or hydroxide-catalyzed water addition) of the deprotonated nitrosourea and base-catalyzed water addition. This becomes evident from the pH/rate profile of the compound (Figure 4), which in the basic pH range corresponds to two lines of different slopes that intersect at pH ≈ 9. The kinetic behavior of BMNU in basic media was found to be very similar to that of CCNU.[10] BDMNU is hydrolyzed in basic media by nucleophilic attack of hydroxide ion on the urea carbonyl group. In acidic media, BMNU is hydrolyzed according to the mechanism described in the literature for the deamination of N-nitrosoureas (protonation in the r.l.s.) of a benzoylnitrosourea hydrate present at low concentrations.[5,12–14]

Experimental Section

Synthesis: All reagents used for synthetic purposes were commercial products of the highest available purity. NMR spectra were taken in $CDCl_3$ solutions with a Bruker AMX 300 (300 MHz) instrument, and chemical shifts are given in δ values from Me_4Si as an internal standard. IR spectra were recorded with a Nicolet Impact 400 FTIR spectrophotometer. IR spectra of the nitrosoureas were obtained from 5×10^{-3} M solutions of the substrates in chloroform. Melting points were measured in an Electrothermal capillary melting point apparatus and are uncorrected.

Benzoyl Nitrosoureas: The benzoyl nitrosoureas **1** and **2** were obtained by nitrosation of the parent benzoyl ureas.

***N'*-Benzoyl-*N*-methyl-*N*-nitrosourea (1):** Sodium nitrite (1.93 g, 28 mmol) dissolved in water (10 mL) was added to N-benzoyl-N'-methylurea (0.50 g, 2.80 mmol) dissolved in dichloromethane (20 mL). HCl solution (2.0 M, 14.0 mL, 28 mmol) was added dropwise at 0 °C, with stirring, to this biphasic system. The aqueous phase developed a deep blue color indicating the formation of the nitrosating agent N_2O_3, while the organic layer became yellow due to the formation of the nitroso compound. This method has the advantage of preventing the hydrolysis of the nitroso derivative by withdrawing it in the organic phase as soon as it is formed. The reaction was monitored by TLC with diethyl ether/hexane (8:2) as the eluent. After 2 h, the reaction mixture was carefully neutralized with 5% sodium bisulfite solution. The organic layer was separated, washed thoroughly with water, and dried with anhydrous sodium sulfate. After concentration to dryness, the residue was purified by flash chromatography with hexane/diethyl ether (8:2) as the eluent, and BMNU (**1**) was obtained as a yellow oil (yield 2.6%). ^{1}H NMR ($CDCl_3$): $\delta = 3.32$ (s, 3 H, N(NO)C*H*$_3$], 7.49 (t, 2 H, 3,5-phenyl-*H*), 7.59 (t, 1 H, 4-phenyl-*H*), 7.78 (d, 2 H, 2,6-phenyl-*H*) ppm. MS (EI): $m/z = 207.1$ [M$^+$], 206.0 [M$^+$ − H], 177.1 [M$^+$ − NO]. IR ($CHCl_3$): $\tilde{\nu}_{max} = 1708.5$ (urea CO), 1680.6 (benzoyl CO), 1458.7 (NO) cm^{-1}.

***N′*-Benzoyl-*N*,*N′*-dimethyl-*N*-nitrosourea (2):** Nitrosation of *N*-benzoyl-*N*,*N′*-dimethylurea by the above method afforded BDMNU (**2**) as a yellow oil (yield 4.7%). ^{1}H NMR ($CDCl_3$): $\delta = 2.83$ (s, 3 H, NC*H*$_3$), 3.58 [s, 3 H, N(NO)C*H*$_3$], 7.35 (t, 2 H, 3,5-phenyl-*H*), 7.42 (t, 1 H, 4-phenyl-*H*), 7.51 (d, 2 H, 2,6-phenyl-*H*) ppm. MS (EI): $m/z = 205.9$ [M$^+$ − CH_3], 190.9 [M$^+$ − NO]. IR ($CHCl_3$): $\tilde{\nu}_{max} = 1720.1$ (urea CO), 1683.0 (benzoyl CO), 1453.6 (NO) cm^{-1}.

Benzoylureas: *N*-Benzoyl-*N′*-methylurea was obtained by condensation of methylamine and benzoyl isocyanate, while *N*-benzoyl-*N*,*N′*-dimethylurea was prepared by acylation of *N*,*N′*-dimethylurea with benzoyl chloride, by standard literature procedures.[15,16]

***N*-Benzoyl-*N′*-methylurea:** Benzoyl isocyanate (1.50 mL, 11 mmol) was slowly added at 0 °C, with stirring, to a solution of methylamine in THF (2.0 M, 10 mL, 20 mmol). After 2 h, the reaction solvents were evaporated to dryness and the crude product was recrystallized from chloroform/*n*-hexane to afford *N*-benzoyl-*N′*-methylurea as colorless crystals (yield 68.0%), m.p. 168.7−169.5 °C, in good agreement with literature values.[15] ^{1}H NMR ($CDCl_3$): $\delta = 2.95$ (d, 3 H, NHC*H*$_3$), 7.49 (t, 2 H, 3,5-phenyl-*H*), 7.59 (t, 1 H, 4-phenyl-*H*), 7.98 (d, 2 H, 2,6-phenyl-*H*), 8.67 (br. s, 1 H, N*H*CH$_3$), 9.57 (br. s, 1 H, PhCON*H*) ppm. MS (EI): $m/z = 178.2$ [M$^+$], 104.9 [PhCO$^+$]. IR (KBr): $\tilde{\nu}_{max} = 1707.0$ (urea CO), 1683.0 (benzoyl CO) cm^{-1}.

***N*-Benzoyl-*N*,*N′*-dimethylurea:** Benzoyl chloride (1.2 mL, 10 mmol) was added to *N*,*N′*-dimethylurea (0.88 g, 10 mmol) dissolved in anhydrous pyridine (10 mL). The reaction mixture was heated at reflux for 3 h and was then poured into cold HCl solution (1.0 M, 100mL). The precipitate was recrystallized from chloroform/hexane to afford *N*-benzoyl-*N*,*N′*-dimethylurea as colorless crystals (yield 69.3%), m.p. 97.4−99.3 °C, again in good agreement with literature values.[15] ^{1}H NMR ($CDCl_3$): $\delta = 2.93$ (s, 3 H, PhCONC*H*$_3$), 3.18 (d, 3 H, NHC*H*$_3$), 7.46 (m, 5 H, phenyl-*H*), 9.04 (br. s, 1 H, N*H*CH$_3$). MS (EI): $m/z = 191.9$ [M$^+$], 105.1 [PhCO$^+$]. IR (KBr): $\tilde{\nu}_{max} = 1707.0$ (urea CO), 1629.0 (benzoyl CO) cm^{-1}.

Kinetics: Most kinetics were recorded with Milton Roy Spectronic 3000 Diode Array and Shimadzu UV-2100 spectrophotometers fitted with multiple thermostatted cell carriers. Kinetic runs were triggered by injection of a small aliquot of a stock solution of the nitrosourea in dioxane into the reaction medium, ensuring an in situ nitrosourea concentration of 1.0×10^{-4} M and a final dioxane concentration of 1% (v/v). The faster reactions (pH ≥ 11) were monitored with an Applied Photophysics DX17MV sequential stopped-flow spectrophotometer. All experiments were carried out at 25 °C under pseudo-first order conditions, with a large deficit of the nitroso substrate. Ionic strength was kept constant at 1.0 M by addition of $NaClO_4$. Reactions were monitored by recording the change in absorbance at 260−270 nm due to the disappearance of the nitroso compound. Absorbance/time data always fitted the first-order integrated rate equation, thus affording the observed pseudo-first-order rate constants, k_{obs}. The pH in the 3−11 range was controlled by use of buffers of the desired pH, while for pH < 3 and pH > 11 hydrochloric acid or sodium hydroxide solutions of the appropriate concentration were used. The pH values of buffer solutions and of the final reaction mixtures were measured with a Radiometer M82 pH-meter fitted with GK2401C combined glass electrodes. For pH < 3 and pH > 11 titration procedures were employed. All reagents and solvents used in the preparation of solutions for kinetic studies were of the highest purity grade commercially available. Sodium hydroxide and hydrochloric acid solutions were prepared by dilution of commercial Merck Titrisol solutions. The identities of reaction products were confirmed by the UV spectra of the reaction mixtures on completion and in some cases also by TLC and comparison with authentic samples, with diethyl ether/hexane (8:2) and ethyl acetate/methanol (9:1) as eluents. The presence of nitrite in the final reaction mixtures was detected by a modification of Shinn's method.[17]

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[18] Amado et al.[10] discriminated between mechanisms (*i*) and (*ii*) by treating the nitroso substrate with the nucleophile HOO^- ($pK_a = 11.64$), which is a much stronger nucleophile than HO^- but a less strong base. In our case we found that treatment of either BMNU or BDMNU in 0.05−0.5 M hydroperoxide solutions of pH = 10.78 (kept constant by an external carbonate buffer) were too fast to measure. This was expected for BDMNU but not for BMNU unless the latter decomposes by nucleophilic attack on the carbonyl group. However, the possibility of general base catalysis by HOO^- cannot be excluded, as for BMNU a catalytic action was exerted by other bases above pH ≈ 9. Nucleophilic attack of HOO^- on the nitroso groups of both benzoyl nitrosoureas was ruled out by the non-formation of the peroxynitrite ion ($\varepsilon_{302} = 1670 \pm 50\ \text{M}^{-1}\cdot\text{cm}^{-1}$) which is stable under the experimental conditions employed.

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