JOC Article

Mechanism for Basic Hydrolysis of *N*-Nitrosoguanidines in Aqueous Solution

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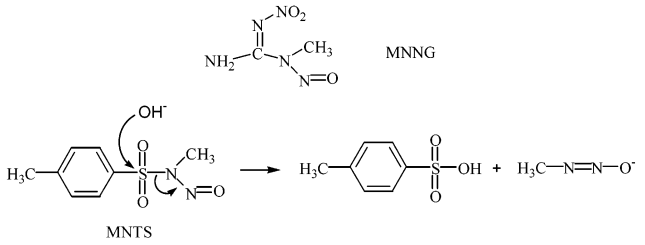
A kinetic study was carried out on the hydrolysis of two *N*-nitrosoguanidines, 1-nitroso-1-methyl-3-tolylsulfonylguanidine (TSGNO) and 1-nitroso-1-methyl-3-benzoylguanidine (BCGNO). We observed an absence of buffer catalysis using $H_2PO_4^-/HPO_4^{2-}$, $H_3BO_3/H_2BO_3^-$, and HCO_3^-/CO_3^{2-} regulators and a complex dependency of the rate constant on the pH. We discovered the existence of three simultaneous reaction paths: spontaneous decomposition of the neutral form of the *N*-nitrosoguanidine, decomposition of the monoanion, and decomposition through the form of the dianion. The analysis of the kinetic data has allowed us to obtain the acidity constant for the formation of the monoanion of the *N*-nitrosoguanidine, with values of $pK_a^I = 11.5$. The reaction rate for the process through the monoanion, k_2, decreases as the acidity increases. The application of the principle of nonperfect synchronization shows that the basicity and reactivity do not correlate when there exists a possibility of stabilization of the negative charge by resonance. This behavior is consistent with the mechanism E1cB whereby the stabler the negative charge, the slower the elimination reaction. When dealing with the case of the elimination through the neutral form we observe that the reaction rate increases together with the capacity of stabilization of the positive charge on the nitrogen atom adjacent to the imino group. For the reaction through the dianion we used a maximum value of $k_3 = 10^{10}$ s^{-1} to estimate the value of pK_a^{II} for the formation of the dianion of the *N*-nitrosoguanidine, obtaining values of $pK_a^{II} < 24$.

Introduction

A wide variety of structurally related compounds possessing the *N*-nitroso-*N*-alkyl functionality have demonstrated a cancer chemotherapeutic potential.[1] As is well-documented, the basic decomposition of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (MNTS) and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) yield diazomethane,[2] a powerful alkylating agent[3,4] of DNA. MNNG is also routinely used in the study of alkylating patterns of DNA.[5] The alkaline cleavage of MNNG is strongly reminiscent of the corresponding reaction of nitrosourethanes. *N*-Nitrosoguanidines have the advantage of greater thermal stability but are skin irritants, as are nitrosourethanes. Other *N*-nitroso compounds, such as *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (MNTS),[6−8] are also used as alkylating agents as a result of the formation of diazomethane during their decomposition, a consequence of decomposition by nucleophilic attack on the sulfonyl group. Both MNNG and MNTS have the same leaving group, CH_3N^-NO, in their hydrolysis processes (Scheme 1).

SCHEME 1



The first systematic study on the basic hydrolysis of MNNG was carried out by Lawley and Tatcher,[10] who established an elimination mechanism similar to that proposed for the nitrosourethanes[11] (Scheme 2).

In 1992 Fishbein[12] approached the problem by studying the hydrolysis of *N*-methyl (MNNG; $pK_a = 7.73$) and

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§ Universidade de Lisboa.

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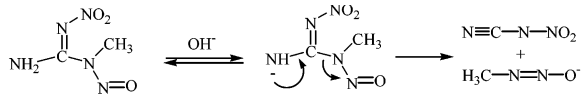
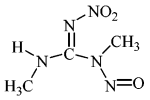
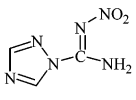
SCHEME 2



N,*N*″-dimethyl (DMNNG; pK_a = 5.72) *N*′-nitro-*N*-nitrosoguanidine, as well as *N*-(*N*′-nitroamidino)triazol (TRNG; pK_a = 6.47).



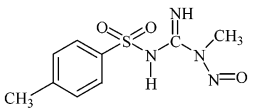
DMNNG



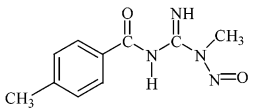
TRNG

The results obtained show a dependency of the rate constant on the pH for values of pH > 8.5 in the case of the *N*-methyl derivative and for values of pH > 6.5 in the case of the triazol. The hydrolysis of the *N*,*N*″-dimethyl derivative is independent of the pH in the range pH = 6−13, this compound being $10^{7.6}$ times more stable than MNNG. These results are incompatible with the mechanism proposed by Lawley and Tatcher, since in accordance with this mechanism the rate constant should present a first-order dependency on the hydroxyl concentration for values of pH < pK_a and be independent for values of pH > pK_a. This behavior led Fishbein to propose a mechanism that involves the transfer of the second proton of the substrate in accordance with Scheme 3.

Recently we have observed in our laboratory that two *N*-nitrosoguanidines, 1-nitroso-1-methyl-3-tolylsulfonylguanidine (TSGNO) and 1-nitroso-1-methyl-3-benzoylguanidine (BCGNO), are efficient transnitrosating agents for nitrogen and sulfur nucleophiles.[13] This behavior contrasts with that observed for MNNG and *N*-nitrosourethanes where the reaction with nucleophiles takes place through nucleophilic attack both on the imino carbon and on the nitroso nitrogen atom of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.[14−16]



TSGNO



BCGNO

This different behavior has given rise to the investigation of the hydrolysis mechanism of TSGNO and BCGNO in a pH interval ranging from 6 to 13. The observed behavior shows certain similarities to that proposed by Fishbein for the hydrolysis of MNNG. Nevertheless, a new decomposition path should be included. Fishbein showed that the pH−rate profile for the decomposition of MNNG has slope 1 above the pK_a, which suggests a rate law that is first order in hydroxide ion and first order in the anion of MNNG (or equally, a term that is second order in hydroxide ion and first order in the neutral form of MNNG). This mode of decomposition is dominant below the pK_a of MNNG as well. At pH 6 and below, a pH-independent reaction becomes dominant. Hence, in his mechanism Fishbein does not consider the possibility that the anion of MNNG may evolve while decomposing by a decomposition path similar to that proposed by Lawley and Tatcher. The results obtained in this study allow us to propose three reaction paths for the decomposition of TSGNO and BCGNO: spontaneous decomposition of the neutral species, of the monoanion, and of the dianion.

It is important to point out that the leaving group of the hydrolysis of TSGNO and BCGNO will be the same as in the hydrolysis of MNNG or MNTS. This characteristic suggests that TSGNO and BCGNO can used as alkylating agents as a result of the fact that diazomethane is generated in their composition.

Experimental Section

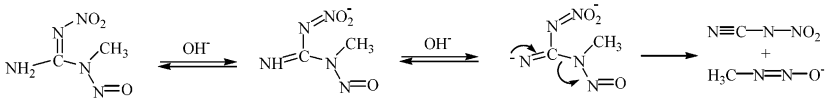
Materials. All chemicals and solvents used in the synthesis were of reagent grade. All reagents used for kinetic studies were of analytical grade or purified prior to use, and deionized water was used throughout this study. Benzoylguanidine was prepared as described previously for tolylsulfonylguanidine[17] and nitrosation was accomplished as already described.[18] Spectroscopic data for the *N*-nitrosoguanidines are as follows. TSGNO: δ_H (300 MHz, $CDCl_3$) 2.44 (3H, s, CH_3-Ar-), 3.18 (3H, s, N(NO)CH_3), 7.32 (2H, d, Ph), 7.87 (2H, d, Ph); *m*/*z* 256 (M^+), 226 (M^+ − NO); mp 168−170 °C. BCGNO: δ_H (300 MHz, $CDCl_3$) 3.47 (3H, s, N(NO)CH_3), 7.24 (3H, m, Ph), 8.15 (2H, m, Ph); *m*/*z* 220 (M^+), 190 (M^+ − NO); mp 99−105 °C.

In most reactions pH was controlled by using buffer solutions that were made up with HCO_3^-/CO_3^{2-} for pH 10.93−10.04, $H_3BO_3/H_2BO_3^-$ for pH 10.41−8.19, and $H_2PO_4^-/HPO_4^{2-}$ for pH 8.36−5.61. pH values were obtained with a pH meter equipped with (for pH > 11) combined glass electrodes.

BCGNO was found to decompose to benzoic acid anion in sodium hydroxide solution. The corresponding sulfonic acid was found in the decomposition of TSGNO.

Kinetics. Decomposition reactions of TSGNO and BCGNO at 25 °C were monitored at 250 and 275 nm, respectively, using a stopped-flow for faster reactions and a UV−vis spectrophotometer for slow ones. Both stopped-flow and spectrophotometer were attached to thermostated water baths. Kinetic runs were triggered by injecting a small aliquot of an acetonitrile solution of the appropriate concentration of the nitroso substrate into the reaction medium. The final percentage of acetonitrile in the reaction mixture was always 3.33% (v/v) and the substrate concentration was (7−8) × 10^{-5} M. The ionic strength was kept constant at 1 M by addition of $NaClO_4$. All experiments were carried out under pseudo-first-order conditions with a large nitrosocompound deficit. The first-order rate constant for disappearance of the starting material was obtained by one of two methods. Analysis of the exponential decay of absorbance for between 4 and 5 half-lives of reaction was generally used for runs with halftimes of 10 h or less. Absorbance−time data always fit the first-order integrated rate equation, and the corresponding observed pseudo-first-order rate constants, k_{obs}, were reproducible within 3% in all cases. The second method for determining k_{obs}, used in slower reactions, was that of initial rates. In this method reactions were monitored for 24−48 h, after which enough 5 M NaOH was added to neutralize the acidic component of the buffer and increase the pH of the solution to ∼12. Reactions were then

SCHEME 3



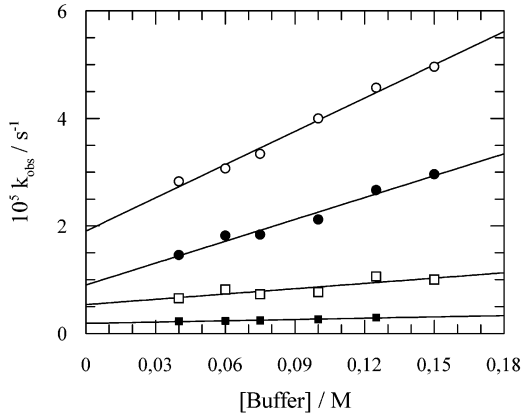


FIGURE 1. Influence of [buffer] on k_{obs} for decomposition of TSGNO in the presence of $H_3BO_3/H_2BO_3^-$ buffer at 25 °C and (○) pH 10.41, (●) pH 10.16, (□) pH 9.84, and (■) pH 9.71.

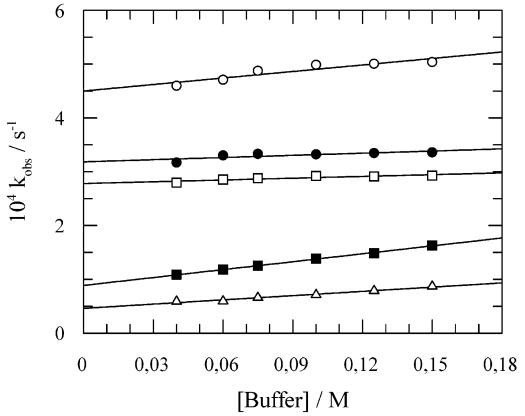


FIGURE 2. Influence of [buffer] on k_{obs} for decomposition of BCGNO in the presence of HCO_3^-/CO_3^{2-} buffer at 25 °C and (○) pH 10.93, (●) pH 10.76, (□) pH 10.67, (■) pH 10.43, and (△) pH 10.14.

further monitored until the infinity value (OD_∞) was reached. Pseudo-first-order rate constants were calculated according to the following equation, where S is the slope of the plot of OD vs time and $\Delta OD_o = OD_\infty - OD_o$: $k_{obs} = S/\Delta OD_o$.

Results

Rate−pH Profile. The first-order rate constants for the decomposition of TSGNO and BCGNO were measured at 25 °C, 1 M ionic strength ($NaClO_4$), in a number of aqueous buffer systems from pH 5.61 to pH 10.93 and in the presence of different NaOH concentrations from pH 11 to pH 13.40.

Values of the buffer-independent rate constant, k_{ind}, for decomposition of TSGNO and BCGNO are equal to k_{obs} measured for reactions in the absence of buffer (pH ranging from 11 to 13.40). In the presence of buffers the values of k_{ind} were extrapolated at zero buffer concentration according to

$$k_{obs} = k_{ind}[OH^-] + k_{Buffer}[Buffer] \qquad (1)$$

k_{obs} depends linearly with buffer concentration in an interval [Buffer] = 0.04−0.15 M, but in general the magnitude of the catalysis is very small and in most cases studied k_{obs} is buffer concentration independent. For the decomposition of TSGNO the catalytic efficiency in the interval of concentration [Buffer] = 0.04−0.15 M is always lower than 100%, with values generally around 30% for $H_3BO_3/H_2BO_3^-$ buffer. For $H_2PO_4^-/HPO_4^{2-}$ or HCO_3^-/CO_3^{2-} systems, no buffer catalysis has been observed. Figure 1 shows the influence of the buffer concentration on k_{obs} for the decomposition of TSGNO using $H_3BO_3/H_2BO_3^-$ buffers.

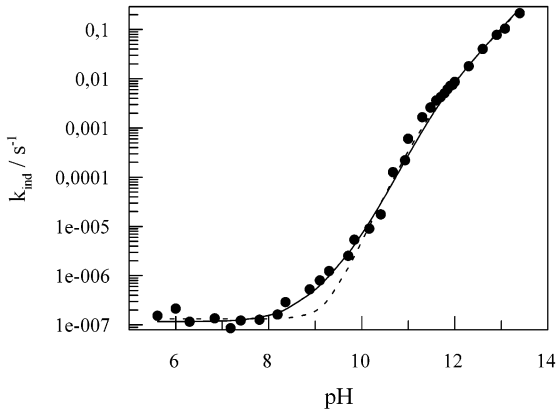


FIGURE 3. Plot of the buffer-independent first-order rate constant for decomposition of TSGNO, k_{ind}, against pH at 25 °C, 1 M ionic strength, 3.3 vol % acetonitrile. The solid line represents values calculated using the rate equation and constants in the text. The dashed line represents values calculated without taking into account the possibility of spontaneous decomposition of the anion of BCGNO (see text).

The buffer effect on the decomposition of BCGNO is similar: catalysis lower than 40% using $H_3BO_3/H_2BO_3^-$ buffers and no buffer catalysis for HCO_3^-/CO_3^{2-} (see Figure 2) and $H_2PO_4^-/HPO_4^{2-}$ buffers. In most cases studied buffer catalysis is very slight or negligible, so that it is not possible to obtain a proper rate constant for the buffer-catalyzed process.

The slight observed catalytic effects allow us to extrapolate the rate constant at zero concentration of regulator, k_{ind}. Figures 3 and 4 show the pH influence on k_{ind} for the decomposition of TSGNO and BCGNO, respectively.

pK_a Values. The pK_a^I of TSGNO was determined from the change in the absorbance at 260 nm as a function of pH. It was not possible to accurately measure the extinction coefficient at the high-pH end point because of the very high rate of decomposition. From the deprotonation equilibrium of TSGNO (Scheme 4) we can obtain the following equation:

$$\text{Absorbance} = \frac{\epsilon_{AH} + \epsilon_{A^-}K[OH^-]}{1 + K[OH^-]}[AH]_{tot} \qquad (2)$$

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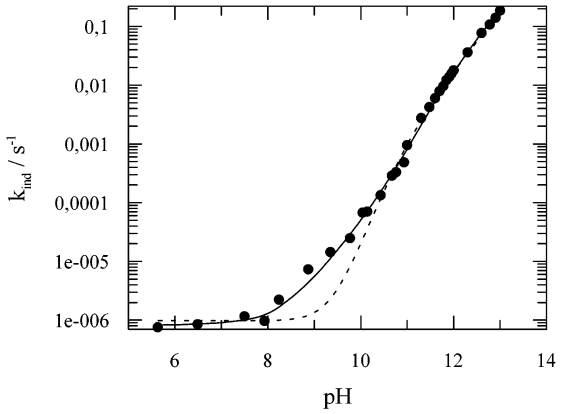


FIGURE 4. Plot of the buffer-independent first-order rate constant for decomposition of BCGNO, k_{ind}, against pH at 25 °C, 1 M ionic strength, 3.3 vol % acetonitrile. The solid line represents values calculated using the rate equation and constants in the text. The dashed line represents values calculated without taking into account the possibility of spontaneous decomposition of the anion of BCGNO (see text).

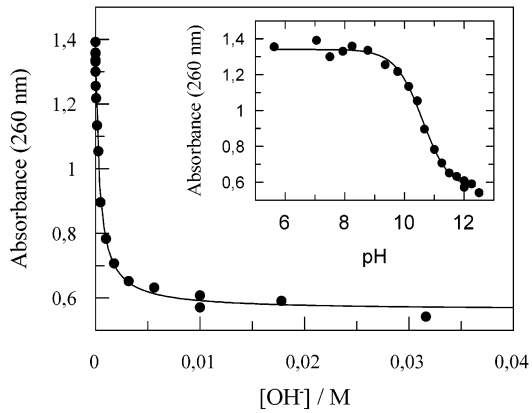
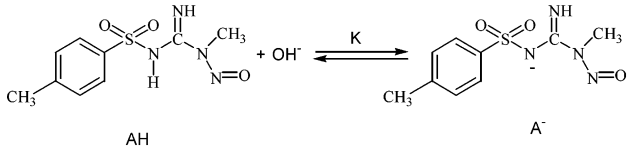


FIGURE 5. Influence of [NaOH] on TSGNO absorbance at 260 nm at 25 °C and ionic strength 1 M ($NaClO_4$). The solid line is calculated using eq 2 and constants given in the text.

SCHEME 4



Fitting eq 2 to the experimental data, curve in Figure 5, we can obtain a value of $pK_a^I = 10.6 \pm 0.1$ for TSGNO.

We have detected variations in the absorption spectrum of BCGNO with the pH. However its higher instability compared with that of TSGNO has prevented us from determining its acidity constant.

Discussion

Hydrolysis Mechanism. Our results show that the Lawley and Tatcher mechanism, Scheme 2, can be ruled out in agreement with Fishbein's previous discussion.[12] Following Fishbein for our compounds the Lowley and Tatcher mechanism requires a plot of log k_{ind}, the first-order rate constant for decomposition of TSGNO or BCGNO, against pH to have a slope of 1 below the pK_a of the *N*-nitrosoguanidine and level off to a slope of zero above the pK_a. Note that pK_a^I for TSGNO has been obtained as $pK_a^I = 10.6 \pm 0.1$.

Taking into account the mechanism proposed by Fishbein for MNNG (Scheme 3) one can obtain the following rate equation:

$$k_{int} = \frac{k_1[H^+]^2 + k_3K_a^IK_a^{II}}{[H^+]^2 + K_a^I[H^+]} \qquad (3)$$

where k_1 and k_3 are the rate constants of spontaneous evolution of the neutral form of the *N*-nitrosoguanidine and the dianion. The equilibrium constants K_a^I and K_a^{II} are the acidity constants for the formation of the mono- and dianion of the *N*-nitrosoguanidine. This rate equation does not verify the experimental behavior observed (see dashed line in Figures 3 and 4 for TSGNO and BCGNO respectively) in the interval of pH 8−10.

The failure of eq 3 to justify the experimental behavior is due to the fact that it does not consider the possibility that the anion derived from the deprotonation of the *N*-nitrosoguanidine can evolve spontaneously for the formation of products. The inclusion of a third reaction path, k_2, for the spontaneous decomposition of the monoanion of the *N*-nitrosoguanidine (see Scheme 5) allows us to obtain the following rate equation:

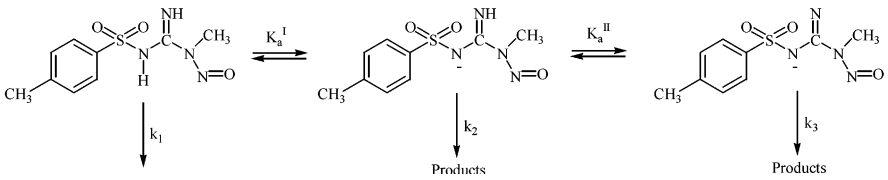
$$k_{int} = \frac{k_1[H^+]^2 + k_2K_a^I[H^+] + k_3K_a^IK_a^{II}}{K_a^IK_a^{II} + K_a^I[H^+] + [H^+]^2} \qquad (4)$$

This rate equation can be simplified bearing in mind that the term $K_a^IK_a^{II}$ of the denominator must always be $\ll K_a^I[H^+]$ (see below). In this way the eq 4 can be rewritten as

$$k_{int} = \frac{k_1[H^+]^2 + k_2K_a^I[H^+] + k_3K_a^IK_a^{II}}{K_a^I[H^+] + [H^+]^2} \qquad (5)$$

This rate equation explains satisfactorily the observed experimental behavior for the decomposition of both TSGNO (Figure 3) and BCGNO (Figure 4). Fitting eq 4 to the experimental results for the decomposition of TSGNO, we can obtain the values of $k_1 = (1.2 \pm 0.1) \times 10^{-7}$ s^{-1}; $k_2 = (1.2 \pm 0.5) \times 10^{-4}$ s^{-1}; $k_3K_a^{II} = (1.1 \pm 0.1) \times 10^{-14}$ M^{-1} s^{-1} and $pK_a^I = 11.5 \pm 0.2$. There is a slight discrepancy between pK_a^I values determined from kinetic parameters and those from absorbance measurements, but taking into account the rapid decomposition of TSGNO in alkaline media it seems more reasonable to consider the pK_a^I values calculated from rate constants as more precise. Using the same procedure for the results of BCGNO decomposition we can obtain values of $k_1 = (8.2 \pm 0.8) \times 10^{-7}$ s^{-1}; $k_2 = (1.5 \pm 0.6) \times 10^{-3}$ s^{-1}; $k_3K_a^{II} = (2.1 \pm 0.2) \times 10^{-14}$ M^{-1} s^{-1} and $pK_a^I = 11.5 \pm 0.2$. It is important to point out that the rate constants for the decomposition of the neutral and ionic forms and the product $k_3K_a^{II}$ are higher for BCGNO than for TSGNO, although the value of pK_a^I is practically identical.

SCHEME 5



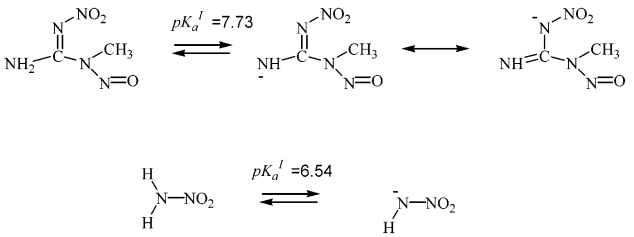
The absence of basic catalysis[19] for the decomposition of TSGNO and BCGNO (see Figures 1 and 2) for moderately weak bases implies that k_2 and k_3 are rate-determining, since it disregards the possibility that a concerted mechanism may exist where proton transfer and the formation of products are concurrent. The obtained results for different buffers show the absence of buffer catalysis both for pH $\ll$ pK_a^I (regulators of $H_2PO_4^-/HPO_4^{2-}$) and for values of pH in the vicinity of pK_a^I (regulators of HCO_3^-/CO_3^{2-} or $H_3BO_3/H_2BO_3^-$).

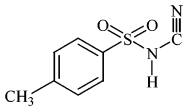
There is no detectable general base catalysis for the elimination reaction. An E2 or irreversible E1cB elimination reaction would be expected to occur with general base catalysis because proton transfer takes place in the rate-determining step. The absence of general base catalysis also means that there is no detectable general acid catalysis for the expulsion of the leaving group from the intermediate anion, which is kinetically equivalent to general base catalysis.

From the mechanism shown in Scheme 5 and rate eq 5 we can estimate an upper limit for pK_a^{II} considering a limiting value for $k_3 = 10^{10}$ s^{-1} taking into account the absence of buffer catalysis. If k_3 were greater than 10^{10} s^{-1}, the dianion would decompose faster than a buffer base could get rid of it by diffusion. This would enforce measurable buffer catalysis by a hydrogen-bonding preassociation mechanism,[20] which is not observed. Hence we can estimate a value of p$K_a^{II} \cong 23.9$ for the dianion of TSGNO and p$K_a^{II} = 23.7$ for BCGNO. These values of pK_a^{II} are compatible with that estimated previously by Fishbein[12] for MNNG (p$K_a^{II} = 20.6$) and with those estimated by Jencks[21] for the hydroxide ion catalyzed nitrile forming elimination reactions of imido esters (pK_a < 23).

The rate constants of 10^{10} s^{-1} for breakdown of the dianion are small enough for a concerted catalysis mechanism not to be enforced by the absence of a significant lifetime for this intermediate. However, the fact that no leveling of the observed rate constants is detected with increasing hydroxide ion concentrations means that proton transfer from the imidate to hydroxide ion and buffer bases is thermodynamically unfavorable. The sulfonamide **I** that would be formed by elimination without proton transfer is strongly acidic, with an estimated pK_a smaller than 4, so that there is a change in pK of the NH proton of some 20 units in this reaction and hence proton transfer to hydroxide ion or buffer bases from this product is strongly favored thermodynamically. Therefore, a concerted reaction mechanism is not excluded by the requirement that a proton-transfer step should change from unfavorable to favorable during the course of the reaction in order to provide a driving force for concerted catalysis.[22]

SCHEME 6





I

This reaction chooses the common course of proceeding through a stepwise rather than a concerted mechanism when the intermediate is stable enough for a stepwise mechanism to be possible; i.e., when a concerted mechanism is not enforced by the nonexistence of the intermediate. Similarly, general acid and base catalysis of the cleavage of formaldehyde hemiacetals is enforced, at least in some cases, by the negligible lifetime of the dipolar intermediate, $T^{\pm}$, that would be required for the stepwise reaction mechanism, but the anionic intermediate, T^-, has a significant lifetime and the hydroxide ion catalyzed reaction proceeds through this intermediate in a stepwise mechanism.[23] It should be emphasized that the requirement for a proton-transfer step to change from unfavorable to favorable is a necessary condition for a concerted reaction mechanism but insufficient on its own.

Variation in Acidity. It is interesting to compare the structural influence on the acidity of various *N*-nitrosoguanidines. MNNG presents a relatively high acidity level, reminiscent of the behavior of the nitramide.[24] The monoanion derived from MNNG presents a predominantly resonant form with the negative charge on the nitro group (Scheme 6).

The behavior observed for TSGNO and BCGNO is substantially different. In the case of TSGNO the value of the acidity constant observed shows a clear parallelism with the acidity of the sulfonamides.[26] Likewise we can

(19) Most experiments with buffers showed clearly noncatalytic behavior. Only a couple of experiments (see Figure 1 for pH = 10.16 and pH = 10.41) showed a dependence of the rate constant with buffer concentration. In these cases the buffer pH is well over its pK_a and we think that we are looking at a medium effect more than buffer catalysis.

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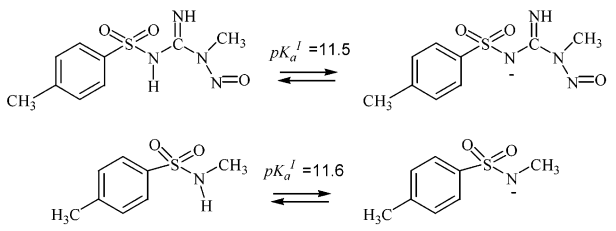
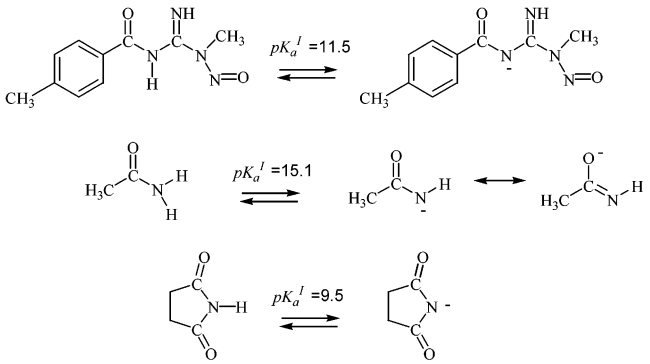
SCHEME 7



SCHEME 8



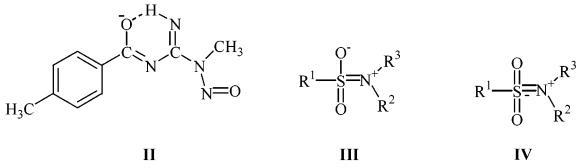
observe that the difference in acidity between the *N*-methyl-*p*-toluenesulfonamide and the nitramide is practically the same as the difference existing between TSGNO and MNNG.

The behavior observed in BCGNO is different, since it presents a much higher acidity level than that of an amide like the acetamide (pK_a^I = 15.1). Its acidity is intermediate between that presented by the acetamide and the succinimide (pK_a^I = 9.5).[27]

The results show that the acidity of the amides is lower than that of the sulfonamides because of the strong inductive effect that the sulfonyl group causes. In the case of BCGNO and on the basis of the behavior observed for MNNG and TSGNO, a value of pK_a^I near to 15 would be expected; however, the value of pK_a^I obtained shows a great stabilization, which suggests the existence of additional paths of the negative charge. On the basis of the behavior observed for the acetamide and for the succinimide we could suggest that the stabilization of the negative charge in BCGNO would be due to the delocalization between the carbonyl group and the imino group (Scheme 9).

However, this type of stabilization can be ruled out because of the great instability of the negative charge on a nitrogen atom. In addition, this type of stabilization should also be present in the case of TSGNO and, consequently, should cause its pK_a^I to be lower than the value obtained. If this type of stabilization was possible the pK_a^I of TSGNO would be expected to have a value of at least 10. The existence of a resonant form with a negative charge on the imino group would cause an increase of the pK_a^I and not a reduction, as occurs in the case of MNNG. In the case of MNNG we observe that the value of pK_a^I is greater than that of the nitramide, in such a way as to be able to conclude that the presence of the amine group tends to destabilize the formation of the anion.[28]

We should consider therefore the existence of an alternative stabilization path of the anion of BCGNO, which cannot manifest itself in the case of TSGNO or in MNNG. This extra stabilization can stem from the establishment of the intramolecular hydrogen bond **II**.



II **III** **IV**

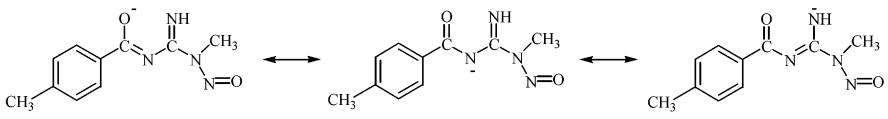
This type of stabilization by intramolecular hydrogen bond is not possible in the case of MNNG because a highly unstable cycle of four members would be formed. In the case of TSGNO it is not possible because various studies carried out on sulfonamides indicate that the structure **III** has an insignificant contribution in its stabilization.[29−31]

The absence of an important resonance stabilization is probably due to the fact that the d orbitals of the sulfur act like a "stopper" for the electrons p-π donated by the nitrogen. The effect of the sulfonyl group should be, fundamentally, inductive. This behavior has also been shown in the study of the stabilization of carbanions α to the sulfonyl group.[32] In any case we cannot disregard an influence of the resonant form **IV**, resulting from the negative charge in the 3d orbitals of the sulfur (not involved in the sulfur−oxygen bond).[33,34] However, this type of resonance could not benefit from the establishment of intramolecular hydrogen bonds as occurs in BCGNO.

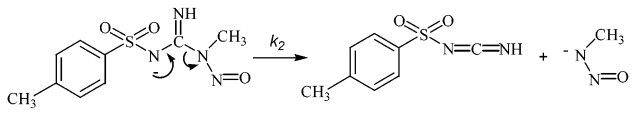
Structure−Reactivity Correlations. There are very few values available in the bibliography to compare with the rate constants obtained in this study for the hydrolysis of TSGNO and BCGNO. The results obtained by Fishbein[12] for MNNG show that the rate constant k_2 is minimal compared with the processes of spontaneous decomposition of the neutral (k_1) and dianion forms (k_3) of *N*-nitrosoguanidine. For the rate constant k_3 we have only been able to obtain a maximum value derived from the fact of the absence of the buffer catalysis. On the basis of the data obtained by Fishbein and the value of pK_a^I = 5.71 we can estimate a value of $k_2 \cong 2 \times 10^{-5}$ s^{-1} for DMNNG. This value is lower than that obtained in this study for TSGNO, $k_2 = (1.2 \pm 0.5) \times 10^{-4}$ s^{-1}, and for BCGNO, $k_2 = (1.5 \pm 0.6) \times 10^{-3}$ s^{-1}.

The results show that the rate constant k_2 decreases as the acidity of the *N*-nitrosoguanidine increases. This behavior is consistent with a mechanism in stages where initially the formation of an anionic form takes place and then the process of elimination occurs. The larger the

SCHEME 9



SCHEME 10



stabilization of the negative charge in the *N*-nitrosoguanidine, the lower the rate constant k_2, corresponding to the process of elimination. That is to say, the rate of the reaction will decrease as the pK_a^I does, as shown by comparing BCGNO, TSGNO, and DMNNG (Scheme 10).

The predicted behavior shows an apparent abnormality when we compare k_2 for TSGNO and BCGNO. Both compounds have the same value of pK_a^I but BCGNO reacts approximately 10 times more quickly than TSGNO. In the case of BCGNO the negative charge of the anion was localized fundamentally on the oxygen atom of the carbonyl group and stabilized by an intramolecular hydrogen bond. The resonant form with the negative charge on the nitrogen atom will be less stabilized than in **II**. In fact the alkyl benzimidates[21] have a $pK_a < 23$ in comparison with a pK_a of the order of 15 for acetamide.

This behavior of BCGNO and TSGNO can be rationalized easily on the basis of the principle of nonperfect synchronization.[35−37] For the elimination reaction of the anion of BCGNO there should be a reorganization of the negative charge, localized predominantly on the oxygen atom (Scheme 11).

On determining the pK_a^I of BCGNO the negative charge of the anionic form resides mainly on the oxygen atom of the carbonyl group. However, for the reaction of elimination the negative charge should be localized on the nitrogen atom. The main repercussion of this delocalization by resonance is that the transition state of the elimination reaction, with a partial negative charge on the nitrogen atom, does not benefit from those factors that establish the negative charge in the anionic form of BCGNO. This difference in stabilization between the reactives and the transition state means that the elimination reaction will be quicker than expected on the basis of the pK_a^I.

A similar situation prevails in the case of DMNNG, of which the acidity is 6×10^5 times greater than that of TSGNO. This difference in acidity should cause a difference in reactivity of at least 10^3 times, due to the greater stabilization of the negative charge in the anion of DMNNG. However k_2 for DMNNG is scarcely 10 times less than k_2 for TSGNO. As in the case of BCGNO this difference in reactivity can be explained by the asynchrony between the formation and breakage of bonds and the stabilization of charge by resonance. In the anion of DMNNG the negative charge will be stabilized by resonance on the oxygen atoms of the nitro group. However, in the transition state of the elimination reaction there will exist a certain development of the negative charge on the nitrogen atom. In this way the transition state of the elimination reaction will not benefit from the factors that stabilize the negative charge on the anion of DMNNG, and consequently, the reactivity will not be reflected in the values of pK_a^I.

We can compare the values of k_1, the rate constant for the spontaneous decomposition of the neutral form for different guanidines: TRNG, $pK_a^I = 6.74$; MNNG, $pK_a^I = 7.73$; TSGNO, $pK_a^I = 11.5$; and BCGNO, $pK_a^I = 11.5$. The values obtained from k_1 increase together with the acidity of the guanidine: $k_1 = 3.1 \times 10^{-5}$ s^{-1} (TRNG); $k_1 = 5.2 \times 10^{-6}$ s^{-1} (MNNG); $k_1 = 8.2 \times 10^{-7}$ s^{-1} (BCGNO); and $k_1 = 1.2 \times 10^{-7}$ s^{-1} (TSGNO). In the sequence of behavior we should differentiate between the TRNG and the other guanidines since the leaving groups are different. The leaving group of the TRNG, the triazol anion, has a pK_a of 10.1, whereas the pK_a of the other guanidines is much greater.[38] This difference in the leaving group can be responsible for the greater value of k_1. The value of k_1 for the hydrolysis of MNNG has been obtained at 40 °C, whereas those of BCGNO and TSGNO have been obtained at 25 °C. In any case the value of k_1 for MNNG is slightly greater than what would be expected. For BCGNO and TSGNO we find that the reactivity of BCGNO is greater, which can be explained as a consequence of the carbonyl group producing a lower destabilization of the reaction product in comparison with the sulfonyl group (Scheme 12).

The carbonyl group has a lesser capacity to withdraw charge, in such a way that the pK_a of the urethanes are close to zero. The capacity to withdraw charge from the sulfonyl group is much greater and is shown in the values of pK_a, which are near to −4. This lesser capacity of the sulfonyl group to establish the positive charge gives rise to a greater destabilization of the partial positive charge that is developed on the nitrogen atom in the transition state and consequently produces an increase in the energy barrier that brings about a decrease in the rate constant k_1.

Conclusions

The results obtained in this study for the hydrolysis of *N*-nitrosoguanidines, namely, absence of buffer catalysis and complex dependence of the rate constant on the pH, allow us to reach a series of conclusions:

(1) The hydrolysis of *N*-nitrosoguanidines takes place by three simultaneous reaction paths: hydrolysis by the neutral form of the guanidine, by the monoanion, and by the dianion. We can conclude that the elimination

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(28) The behavior of the *N*-nitrosoguanidinas is not so simple, since in this discussion we are not taking account of the effect due to the presence of the nitroso group. Its electron-attracting effect stabilizes the negative charges α to the carbonyl, in such a way that the *N*-nitrosourethanes are more acid than the urethanes (see ref 11 and references therein).
(29) Chardin, A.; Laurence, C.; Berthelot, M.; Morris, D. G. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1047.
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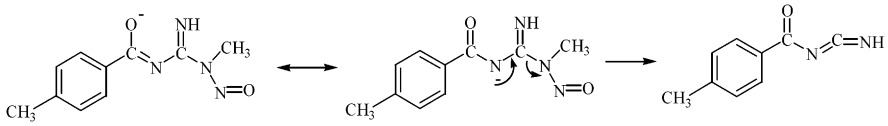
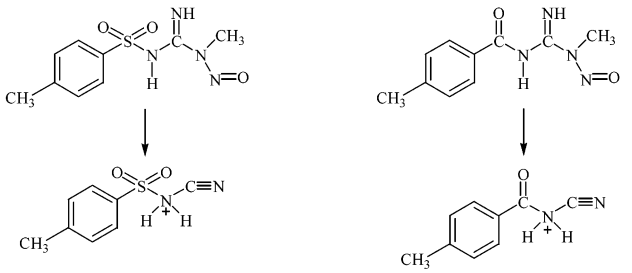
(38) We can estimate a value of pK_a for the anion of the *N*-methyl-*N*-nitrosoamine of approximately 20 considering that the pK_a of formation of the anion of the *N*-methylamine must be in the vicinity of 30 and that the introduction of a nitroso group decreases the pK_a by about 10 units.

SCHEME 11



SCHEME 12



proceeds through an E1cB mechanism with reversible formation of a nitrogen anion ($pK_a^I < 15.5$) followed by expulsion of the primary *N*-nitrosamine.

(2) On the basis of the kinetic data we have been able to determine the values of pK_a^I for the *N*-nitrosamines. These values are compatible with those published previously for *N′*-nitro-*N*-nitrosoguanidines, indicating that the value of pK_a^I is determined by the presence of the sulfonyl groups (TSGNO) and carbonyl groups (BCGNO). In this latter case the value of pK_a^I is three times lower than expected given the presence of the carbonyl groups and has been attributed to the stabilizing effect of the intramolecular hydrogen bond between the carboxylate group and the imino group.

(3) The rate constant for the elimination of the primary *N*-nitrosamine from the anion of the *N*-nitrosoguanidine, k_2, decreases as the stability of the negative charge in the guanidine increases. This behavior is compatible with a mechanism of elimination in stages E1cB. To carry out a quantitative interpretation of the results it is necessary to consider that the transition states of the elimination reactions by the anion, k_2, do not benefit from the factors that stabilize the negative charge of the anions. The application of the principle of nonperfect synchronization shows that the basicity and reactivity do not correlate when there exists a possibility of stabilization of the negative charge by resonance. For the reaction path through the dianion we have established an upper limit for k_3 on the basis of the absence of buffer catalysis. From this value of $k_3 = 10^{10}$ s^{-1} we have been able to estimate the values of $pK_a^{II} < 24$, which are compatible with the existing results in the bibliography for alkyl benzimidates.

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