Research Article

Journal of Physical Organic Chemistry

Received: 23 September 2008, Revised: 16 December 2008, Accepted: 29 December 2008, Published online in Wiley InterScience: 17 February 2009

(www.interscience.wiley.com) DOI 10.1002/poc.1524

Rate limiting electrophilic attack in nitrosation of aminoguanidine

I. Fernández[a], P. Hervés[a]* and M. Parajó[b]

Nitrosation reaction of aminoguanidine (AG) has been studied. The nitrosation rate is first-order with respect to both the AG and acid concentration. The absence of general base catalysis, the existence of catalysis by nucleophilic anions ($X^-$and SCN^-) and the observed inverse deuterium isotope effect lead us to propose a mechanism for AG nitrosation similar to that which operates in the case of amines, in which the electrophilic attack of the nitrosating agents is the rate determining step. From this mechanism, we were able to obtain the values of the rate constants for the nitrosation process. We have found that unlike the other amines, the protonated form of AG is the reactive species. This situation becomes possible for AG because protonation of guanidines occurs mostly on the iminic nitrogen, and this may be the reason for the lower reactivity of this amine. Copyright © 2009 John Wiley & Sons, Ltd.

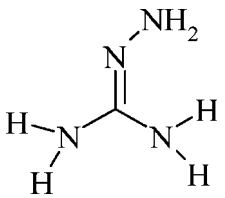
Keywords: nitrosation; guanidines; amines

INTRODUCTION

Nitrosation reactions are of considerable biological significance, because a wide variety of structurally related compounds possess the *N*-nitroso-*N*-alkyl functionality which has demonstrated a cancer chemotherapeutic potential.[1,2] However, the discovery that nitric oxide is produced in the human body[3] has greatly expanded the interest in nitrosation reactions and nitroso transfer, because nitroso compounds are able to deliver nitric oxide, which plays multiple roles in a broad array of physiological processes.[4–10] Due to the widespread significance of nitrosation reactions, knowledge of their mechanisms and kinetics is of much importance.[11]

The mechanisms of nitrosation of amides and ureas in an acidic medium have been exhaustively investigated,[12–17] and a large number of differences have been found between nitrosation of these compounds and amines. Nitrosation of amines occurs with rate limiting attack of the nitrosating agent on the free base form of the substrate, while that of amides involves fast attack of the nitrosating agent followed by slow proton transfer from an intermediary to the reaction medium. The reasons for this different behaviour may be related to the much lower basicity of amides in comparison to amines.

Guanidines can be considered nitrogenated analogues of amides and ureas. However, their peculiar structure makes them the compounds of great basicity, and in this sense, more similar to amines than amides. Previous studies on nitrosation of guanidines of different basicity carried in our group[18,19] and by others[20] showed that their kinetic behaviour differs from that of most amines, whose basicity causes them to be mostly protonated and react with nitrosating agents through the free base, which is more nucleophilic than the neutral guanidines. When the guanidines are in solution in the protonated form the experimental behaviour indicates that protonated guanidine, of very low basicity, is the reactive species. In the present work, we report the results of a kinetic investigation in acidic media of the nitrosation of aminoguanidine (AG), a molecule that combines characteristics of both functional groups (as shown in Chart 1).



(AG)

Chart 1.

EXPERIMENTAL

AG (as aminoguanidine hydrochloride) was provided by Sigma. All the other reagents (from Fluka or Sigma) were of the highest purity available grade and were used without further purification. All experiments were carried out at 25.0 °C, and $NaClO_4$ was used to keep the ionic strength of the medium at 1.0 M. Kinetic runs

* *Correspondence to: P. Hervés, Department of Physical Chemistry, Faculty of Chemistry, University of Vigo, 36310 Vigo, Spain.*
E-mail: jherves@uvigo.es

a *I. Fernández, P. Hervés*
Department of Physical Chemistry, Faculty of Chemistry, University of Vigo, 36310 Vigo, Spain

b *M. Parajó*
Department of Physical Chemistry, Faculty of Chemistry, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

were monitored following the decrease in absorbance ($\lambda = 372$ nm) due to the disappearance of nitrous acid using an Applied Photophysics SX-18MV Stopped-Flow Reaction Analyzer or, for the slower ones, in a UV–Vis spectrophotometer Agilent 8453, both thermostated with an accuracy of 0.2 °C. All kinetic experiments were performed under pseudo-first-order conditions keeping in deficit the nitrite concentration ($[NO_2^-] = 5 \times 10^{-4}$ M). In all the cases, the absorbance–time data fitted the corresponding first-order integrated rate equations accurately. The observed first-order rate constant, k_{obs}, was reproducible within 5%. In the experiments to study the effect of buffers, different amounts of buffer solutions were added to the mixtures that already contained the amount of acid required to achieve the desired pH.

RESULTS

Guanidines are traditionally viewed as strong organic bases with the pK_a for guanidinium $(H_2N)_2C{=}NH_2^+$ being 13.6 in water.[21] However, *N*-substitution can reduce the basicity such that the pK_a value of the corresponding aminoguanidinium ions in water[22] is 11.04, then AG exists mainly in the protonated form under the experimental conditions used in this study ($[H^+] = 0.02$–0.4 M).

The influence of the concentration of AG on the reaction rate was studied at three different constant H^+ concentrations (0.1, 0.2 and 0.3 M) and AG concentrations ranging from 0 to 0.02 M. Figure 1 shows the influence of AG concentration on the observed rate constant. The plots are all good straight lines with slightly negative intercepts. These negative values may be due to the fact that AG is added to the reaction media as AG hydrochloride. We have taken into account this fact to keep the concentration of protons constant, but the $[Cl^-]$ increases as the AG concentration increases and chloride ions catalyse the reaction *vide infra*. The fact that the plots are all good straight lines is indicative of a first-order term with respect to [AG]. Besides, we can also observe in Fig. 1 that the values of the slopes increase with increasing $[H^+]$. This result suggests us that the reaction is catalysed by H^+. To study this effect in more detail, we analysed the influence of acidity on k_{obs} at a constant concentration of AG. We can see in Fig. 2 that the plot is a good straight line that passes through the origin, indicative of a first-order dependence on the concentration of H^+.

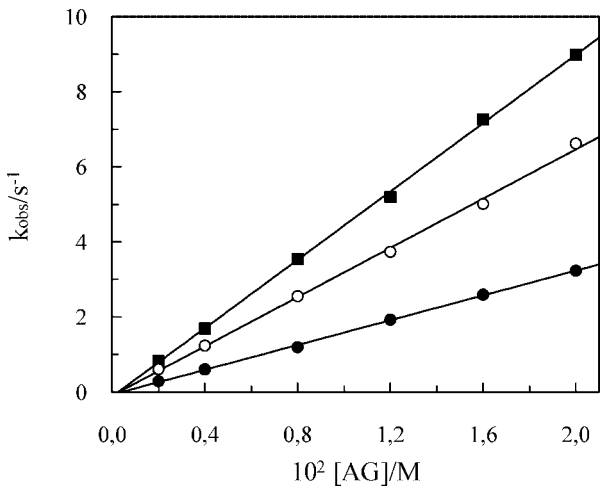


Figure 1. Influence of aminoguanidine concentration upon k_{obs}, (●) $[H^+] = 0.1$ M, (○) $[H^+] = 0.2$ M, (■) $[H^+] = 0.3$ M

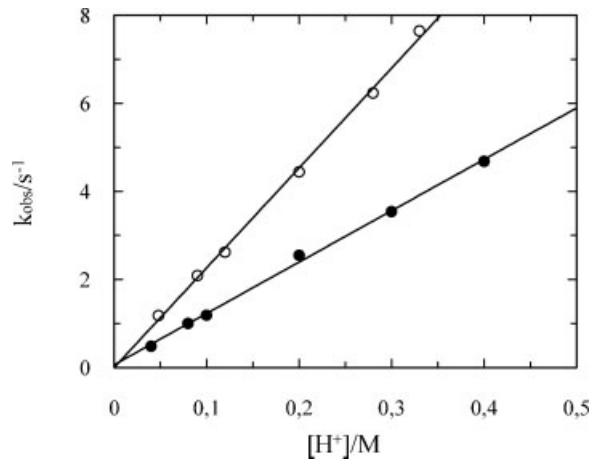


Figure 2. Influence of H^+ concentration upon k_{obs} $[AG] = 8 \times 10^{-3}$ M, (●) in H_2O, (○) in D_2O

Previous studies on nitrosation of guanidines of different basicity[18–20] showed that their kinetic behaviour differs from that of most amines. In order to explore the apparent differences between amines and guanidines, we studied the influence of the usual catalysts of the nitrosation process on the rate of the reaction. These catalysts (halides, thiocyanate, etc.) considerably accelerate the rate at which the amines[11] and amino acids[23] nitrosate by providing important concentrations of new and effective nitrosating agents (ONCl, ONBr, ONSCN, etc.). However, nitrosation of amides and related compounds[11] is not susceptible to this type of catalysis. Figure 3 shows the effect of the addition of X^- and SCN^- to the reaction media, on the rate constant for nitrosation of AG. As can be observed, the plots are all good straight lines with similar positive intercepts (note the different scale in the SCN^- axis) representing the reaction rate for the uncatalysed reaction. The slopes of the plot display a familiar catalytic sequence, $Cl^- < Br^- < SCN^-$, found in nitrosation reactions. This behaviour indicates that, unlike other guanidines,[18–20] nitrosation of AG is subject to nucleophile catalysis,

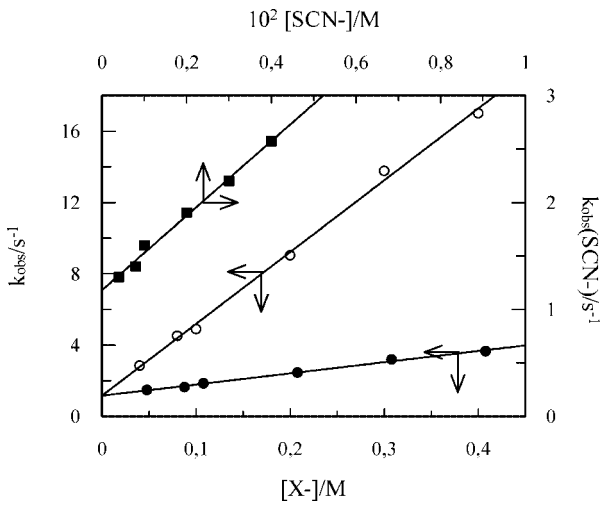


Figure 3. Influence of halides concentration upon k_{obs} $[AG] = 8 \times 10^{-3}$ M, $[H^+] = 0.1$ M, (●) Cl^-, (○) Br^-, (■) SCN^-

Table 1. Influence of buffers concentration on k_{obs} for the nitrosation of aminoguanidine, [AG] = 0.01 M (MCA pH = 2.7, DCA pH = 1.1)

[MCA]/M	k_{obs}/s^{-1}	[DCA]/M	k_{obs}/s^{-1}
0.1	0.031	0.1	1.03
0.2	0.032	0.2	1.06
0.4	0.029	0.4	1.07
0.6	0.037	0.6	1.19
0.8	0.033	0.8	1.09

Table 2. Values of the bimolecular rate constant for the uncatalysed (k_1) and catalysed reaction (k_2) for the nitrosation of aminoguanidine

Nitrosating agent	K_{XNO} (M^{-1})	k_1 or $k_2/M^{-1}\,s^{-1}$
NO^+	3.0×10^{-7}	$(5.0 \pm 0.4) \times 10^9$
ClNO	1.1×10^{-3}[a]	$(7.1 \pm 0.3) \times 10^6$
BrNO	5.1×10^{-2}[a]	$(9.9 \pm 0.4) \times 10^5$
SCNNO	30[a]	$(1.5 \pm 0.1) \times 10^4$

[a] Taken from Reference [11].

and seems to confirm a mechanism for AG nitrosation that is similar to that for amines, i.e. a mechanism whose slow step is the reaction between the nitrosable substrate and the nitrosating agent.

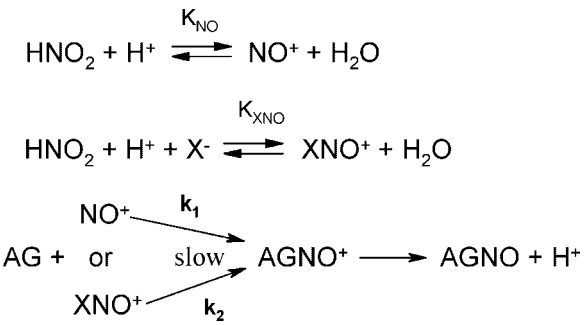
In order to further explore the apparent similarities between amines and AG, the possibility of the existence of general base catalysis, of the type found in the nitrosation of amides and ureas was investigated, even though amines are not susceptible to this type of catalysis. For this, buffers of monochloroacetic (MCA) and dichloroacetic acid (DCA) were employed. Table 1 shows the effect of the addition of buffers to the reaction media, on the rate constant for AG nitrosation. As can be observed there is no trace of catalysis. At these concentrations, general bases produce substantial catalytic effects in the nitrosation of amides. Again, opposite to other guanidines, nitrosation of AG shows the absence of general base catalysis and reassert the conclusion that the mechanism for AG nitrosation is similar to that of amines.

The mechanism for the nitrosation of AG is shown in Scheme 1. The first step, the pre-equilibrium formation of the nitrosating agents (XNO), is followed by the rate limiting attack of the nitroasating agents on the substrate (k_2), leading to the formation of an intermediate ($AGNO^+$). The final step is a fast transfer of a proton from ($AGNO^+$) to the reaction medium to give the nitrosoaminoguanidine (AGNO). This mechanism leads to the next expression for the observed first-order rate constants (Eqn 1), which explains the influence of the concentration of AG and $[H^+]$ on the reaction rate, and also the presence of catalysis by X^-.

$$k_{obs} = k_1 K_{NO}[AG][H^+] + k_2 K_{XNO}[AG][H^+][X^-] \quad (1)$$

The observed rate equation (Eqn 1) is similar to that found in the nitrosation of amines, ketones and aminoacids.[11,23,24] The values of the bimolecular rate constant for the uncatalysed reaction (k_1) were calculated taking a value of $3 \times 10^{-7}\,M^{-1}$ for K_{NO},[25] (although there is a considerable uncertainty over the correct value of this equilibrium constant, with measured values ranging[26,27] from this value to $1.2 \times 10^{-8}\,M^{-1}$). The values obtained for k_1 from different experiments were: $5.3 \times 10^9\,M^{-1}\,s^{-1}$ when studying the influence of AG (as shown in Fig. 1), $4.9 \times 10^9\,M^{-1}\,s^{-1}$ from the influence of H^+ on the reaction rate (as shown in Fig. 2) and $4.85 \times 10^9\,M^{-1}\,s^{-1}$ from the values of the intercepts of the influence nucleophiles on k_{obs} (Fig. 3). Therefore the agreement is very good, and allows a mean value of $(5.0 \pm 0.4) \times 10^9\,M^{-1}\,s^{-1}$ to be established for k_1 (as shown in Table 2). This value is close to the encounter-controlled limit ($7 \times 10^9\,M^{-1}\,s^{-1}$ for neutral substrates),[11] implying that in presence of NO^+ as nitrosating agent, this reaction is indeed a true diffusion-controlled process. The values of the second-order rate constant for the catalysed reaction (k_2) are listed in Table 2, together with the corresponding values of K_{XNO} and the value obtained for the attack by NO^+ in the absence of the catalyst. The values of the bimolecular rate constant show that the reactivities of the nitrosating agents follow the same order as usual in the nitrosation reactions ($NO^+ > ClNO > BrNO > SCNNO$).[11] In the presence of X^-, the reaction is subjected to chemical control; even the most reactive nitrosyl halide reacts with a rate constant 10^3 times less than the encounter limit.

In order to confirm the proposed mechanism, the corresponding isotope effect was measured. The results were obtained and compared with the reaction in presence of H_2O, as shown in Fig. 2. The observed deuterium isotope effect on the nitrosation reaction $k_1(H)/k_1(D)$ was 0.50. This result confirms that AG behaves like an amine and not like amide or urea, which should show primary isotope effect (typically between 3 and 7).[11] Amines show inverse isotope effect, due to the lower concentration[28] of the species derived from the protonation of nitrous acid in H_2O than in D_2O.



Scheme 1.

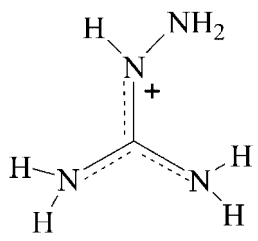


Chart 2.

Finally we would like to highlight that at acidities used in this work, AG reacts with nitrosating agents in its stoichiometric form. This behaviour differs from that of most amines, whose basicity causes them to be mostly protonated. Therefore the reactive species (free base) is only a minor proportion of the stoichiometric concentration, a fact that leads to the appearance of a term with $[H^+]$ in the denominator of the rate equation and then, absence of acid catalysis at the acidities used in this work. In our case, we have observed a first order dependence on the concentration of H^+ (as shown in Fig. 2) and the observed rate equation indicates, therefore, that the protonated AG is the reactive species. This situation becomes possible for AG because protonation of guanidines occurs mostly on the iminic nitrogen[20] (as shown in Chart 2). The reaction via neutral AG cannot be detected because its concentration is so low that the reaction rate of this process is much lower than that resulting from the less basic and less reactive, but more abundant protonated AG. The fact that AG reacts through its protonated form could explain its slight lower reactivity in nitrosations reactions.

Nitrosation reaction of AG shows a different behaviour than other previously studied guanidines, whose nitrosation behaviour is similar to amides. The existence of catalysis by X^- species (halides, SCN^-), the absence of general base catalysis and the fact that the reaction is subjected to an inverse solvent isotope effect led us to conclude that the electrophilic attack of the nitrosating agents on the substrate is, as in the case of amines, the rate limiting step. We have found that unlike other amines, the protonated AG is the reactive species. This situation is possible for AG because protonation of guanidines occurs mostly on the iminic nitrogen, which may be the reason for the lower reactivity of AG.

Acknowledgements

Financial support from the Ministerio de Educación y Ciencia (Project CTQ2007-64758/BQU) is gratefully acknowledged.

REFERENCES

[1] W. A. Skinner, H. F. Gram, M. O. Greene, J. Greenberg, B. R. Baker, *J. Med. Pharm. Chem.* **1960**, *2*, 299–333.
[2] S. Rice, M. Y. Cheng, R. E. Cramer, M. Mandel, H. F. Mower, K. Seff, *J. Am. Chem. Soc.* **1984**, *106*, 239–243.
[3] R. M. Palmer, A. G. Ferrige, S. Moncada, *Nature* **1987**, *327*, 524–526.
[4] J. S. Stamler, D. J. Singel, D. J. Loscalzo, *Science* **1992**, *258*, 1898–1902.
[5] A. R. Butler, D. L. H. Williams, *Chem. Soc. Rev.* **1993**, *22*, 223–241.
[6] F. Murad, *Angew. Chem. Int. Ed.* **1999**, *38*, 1857–1868.
[7] R. F. Furchgott, *Angew. Chem. Int. Ed.* **1999**, *38*, 1870–1880.
[8] L. J. Ignarro, *Angew. Chem. Int. Ed.* **1999**, *38*, 1882–1892.
[9] P. G. Wang, M. Xian, X. Tang, X. Wu, Z. Wen, T. Cai, A. J. Janczuk, *Chem. Rev.* **2002**, *102*, 1091–1134.
[10] D. L. H. Williams, *Org. Biomol. Chem.* **2003**, *1*, 441–449.
[11] D. L. H. Williams, in *Nitrosation*, Cambridge University Press, UK, **1988**.
[12] A. Castro, E. Iglesias, J. R. Leis, M. E. Peña, J. Vázquez-Tato, *J. Chem. Soc. Perkin Trans. 2* **1986**, 1725–1729.
[13] F. Meijide, J. Vázquez-Tato, J. Casado, A. Castro, M. Mosquera, *J. Chem. Soc. Perkin Trans. 2* **1987**, 159–165.
[14] C. Bravo, P. Hervés, J. R. Leis, M. E. Peña, *J. Chem. Soc. Perkin Trans. 2* **1991**, 2091–2095.
[15] P. Hervés, J. R. Leis, *J. Chem. Soc. Perkin Trans. 2* **1995**, 2035–2040.
[16] L. García-Río, J. R. Leis, J. A. Moreira, F. Norberto, *J. Chem. Soc. Perkin Trans. 2* **1998**, 1613–1620.
[17] G. González-Alatorre, S. H. Guzmán-Maldonado, E. M. Escamilla-Silva, G. Lorca-Piña, C. Hernández-Benítez, *Int. J. Chem. Kinet.* **2004**, *36*, 273–279.
[18] P. Hervés, R. G. Button, D. L. H. Williams, *J. Chem. Res. (S)* **1998**, 474–475.
[19] I. Fernández, P. Hervés, M. Parajó, *J. Phys. Org. Chem.* **2008**, *21*, 713–717.
[20] F. Norberto, J. A. Moreira, E. Rosa, J. Iley, J. R. Leis, M. E. Peña, *J. Chem. Soc. Perkin Trans. 2* **1993**, 1561–1566.
[21] S. J. Angyal, W. K. Warburton, *J. Chem. Soc.* **1951**, 2492–2494.
[22] P. J. Taylor, A. R. Wait, *J. Chem. Soc. Perkin Trans. 2* **1986**, 1765–1770.
[23] G. Da-Silva, E. M. Kennedy, B. Z. Dlugogorski, *J. Am. Chem. Soc.* **2005**, *127*, 3664–3665.
[24] E. Iglesias, *J. Org. Chem.* **2003**, *68*, 2689–2697.
[25] J. H. Ridd, *Adv. Phys. Org. Chem.* **1978**, *16*, 1–49.
[26] G. Da-Silva, E. M. Kennedy, B. Z. Dlugogorski, *J. Phys. Org. Chem.* **2007**, *20*, 167–179.
[27] K. H. Becker, J. Kleffmann, R. Kurtenbach, P. Wiesen, *J. Phys. Chem.* **1996**, *100*, 14984–14990.
[28] A. Castro, M. Mosquera, M. F. Rodríguez-Prieto, J. A. Santaballa, J. Vázquez-Tato, *J. Chem Soc. Perkin Trans. 2* **1988**, 1963–1967.