Kinetic Study of the Nitrosation of *N*-Alkylureas in Dioxane-Acetic Acid Mixtures

GUILLERMO GONZÁLEZ ALATORRE, JAVIER G. ZAPIAIN S., PEDRO A. QUINTANA H., GLORIA M. MARTÍNEZ G.

Departamento de Ingeniería Química, Instituto Tecnológico deCelaya, E-38010, Celaya, Guanajuato, México

Received 23 January 1997; accepted 26 May 1997

ABSTRACT: The rate constants were determined for the nitrosation reactions of the following substrates: Methyl (MU), Ethyl (EU),Propyl (PU)Butyl (BU), and Allylurea (AU). The rate equation found at a constant pH was: $v = k[HNO_2]$ [Urea]. The reactions were carried out in predominantly organic media(dioxane–acetic acid–water) with differing polarities. The proposed reaction mechanism involves the proton transfer from the protonated *N*-alkyl-*N*-nitrosourea to the acetate anion. As the polarity of the medium decreased, an approximation of the rate constants of the nitrosation of the different substrates was observed. This approximation can be interpreted as a function of the impediment generated by the R alkyl radical in the rate controlling step. Accordingly, the substrate reactivity will be associated with the ease in which the protonated *N*-alkyl-*N* nitrosurea can transfer the proton to the acetate anion. The results achieved in this study are in accordance with there activities observed in the nitrosation of these substrates in aqueous media MU$\gg$(EU $\approx$ PU $\approx$ BU) $>$ AU. © 1998 John Wiley & Sons, Inc. Int J Chem Kinet **30:** 145–150, 1998.

INTRODUCTION

Nitrosation reactions have attracted wide interest in recent years due to the fact that most nitrosocompounds are toxic [1], carcinogenic [2,3], teratogenic and mutagenic [4–8]. In the case of nitrosamines, a large number of studies have been carried out to determine both their kinetic characteristics and their reaction mechanisms [9,10]. Nevertheless, as far as nitrosamides, in general, and alkylnitrosoureas, in particular, are concerned, studies carried out are few and far between [11,12], even though the biological [13] and kinetic [14–16] differences between both types of nitrosocompounds are well known. From the biological point of view, it is a well known fact that where as nitrosamides require an enzymatic activation prior to acting as carcenogenic agents [17], nitrosoureas do not need this activation [13]. The above can be explained in terms of the formation of DNA alkylizing diazone ions. Therefore, whereas nitrosamides are stable compounds, needing to be attacked by enzymes such as microsomal mono-oxygenase [17], nitrosamides are unstable in aqueous solutions generating the above mentioned ions as they decompose [13].

In a recent study [12] of the nitrosation of ureas

Correspondence to: G. González Alatorre

Contract grant Sponsor: Consejo del Sistema Nacional de Educación Tecnológica, COSNET

Contract grant Sponsor: Mexican Consejo Nacional de Ciencia y Tecnología, CONACYT

 CCC 0538-8066/98/020145-06

with the general formula $RNHCONH_2$ in aqueous media, the following order in the rate constants was found: MU$\gg$(EU > PU > BU) > AU. There action mechanism involved considers the proton transfer from the *N*-alkyl-*N*-nitrosourea to the solvent (water) as the rate controlling step. On the basis of the above mechanism, the observed reactivity was explained as a function of the impediment generated by the alkyl chains, which when found in apolar medium (water), double back on themselves, partially obstructing exit of the proton to the solvent.

This article describes the results of a kinetic study of the same series of ureas in predominantly organic media (dioxane–acetic acid–water). The polarity of the medium was gradually decreased so as to observe the effect on the rate constants, revealing an approximation of them as the polarity was reduced. This was interpreted as being due to partial unfolding of the aliphatic chains.

Lastly, a relationship is proposed between the structure and the reactivity of the nitrosable substrate with the biological activity of the product (nitrosocompound).

EXPERIMENTAL

Solutions of MU, EU, BU, and AU were made up by weight from p.a. products from Aldrich (St. Louis, Missouri, USA.) with nominal purities of 97–99%. Solutions of PU were made up by weight from a product from Alfa (Karlsruhe, Germany) after prior recrystalization from ethanol/water. $NaNO_2$, 99% from Sigma (St. Louis, Missouri USA), 1–4 dioxane, 99% from Merck (Tlanepantla, Mexico), and CH_3COOH, 99+ % from Sigma, were all used without previous purification.

Nitrosation kinetics were recorded by monitoring absorbance by nitrosocompounds at 260 nm in a PerkinElmer UV/VIS spectrophotometer equipped with a thermoelectric cell holder thermostat maintaining temperature within ± 0.1°C. Under these conditions, the molar absorptivity of the nitrosoureas studied is much greater (e.g., for MNU $\epsilon_{260} = 3405\ M^{-1}\ cm^{-1}$) than that of nitrite ($\epsilon_{260} = 6M^{-1}\ cm^{-1}$), and absorbance by both alkylureas and carboxylic acids is constant. Absorbance time data were analyzed by the integration method following the reactions until 90%. The A∞ was estimated using the Greg [19] programme for the nonlineal data adjustment. All kinetic experiments were carried out with a large excess of alkylurea (henceforth called urea) over nitrite.

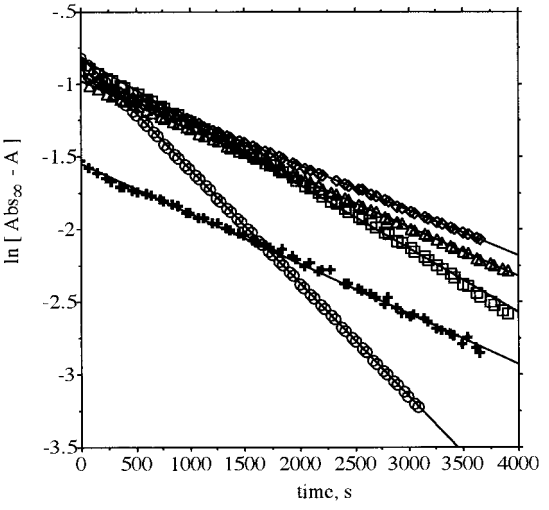


Figure 1 Determination of the order with respect to the nitrite for the series of reactions studied: (◯) MU; (□) EU; (△) PU; (◇) BU; and (+) AU. [urea] = 0.223 M, 25% acetic acid, 70% dioxane, 5% water, and $T = 298$ K.

Given the insolubility of nitrites in organic mixtures (dioxane–acetic acid) it was found to be necessary to add a small percentage (5%) of water to the solutions. Water was omitted in the proposed reaction mechanism, given both its low nucleophic power in relation to the acetate anion and its low concentration in the reactive solutions.

RESULTS AND DISCUSSION

The kinetic studies were begun with the determination of the orders with respect to the nitrite and urea in a predominantly organic medium. The first-order with respect to the nitrite became established on analyzing the adjustment of the experimental data to eq. (1) (See Fig. 1)

$$\ln[A_\infty - A] = -k't + \ln[A_\infty - Ao] \qquad (1)$$

where

$$k' = k[\text{urea}]^\alpha \qquad (2)$$
$$[\text{urea}] >> [\text{nitrite}]$$

When the values of k' vs. [urea] were plotted, the experimental data formed straight lines, indicative of a first-order with respect to the urea (see Fig. 2).

On the basis of the above results, the experimental rate equation was established as follows:

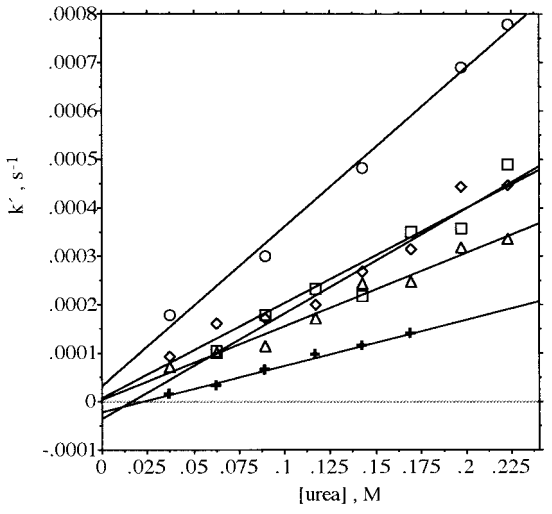


Figure 2 Determination of the order with respect to the urea for the series of reactions studied: (◯) MU; (□) EU; (△) PU; (◇) BU; and (+) AU. [nitrite] = 1×10^{-4} M, 25% acetic acid, 70% dioxane, 5% water, and $T = 298$ K.

$$v = -\frac{d[\text{nit}]}{dt} = k[\text{urea}][\text{nitrite}] \quad (3)$$

$$k' = k[\text{urea}] \quad (4)$$

where the constant $k = f$(pH, [CH_3COOH], I, and T) and I = ionic strength

In addition to the values for k determined with 25% acetic acid Figure 2, values were also determined with 35% and 10%, Figures 3 and 4, respectively.

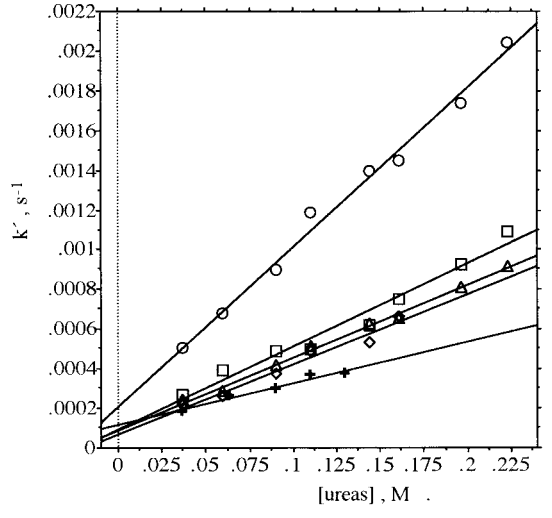


Figure 3 Determination of the rate constant k for the nitrosation of the substrates: (◯) MU; (□) EU; (△) PU; (◇) BU; and (+) AU. [nitrite] = 1×10^{-4} M, 35% acetic acid, 60% dioxane, 5% water, and $T = 298$ K.

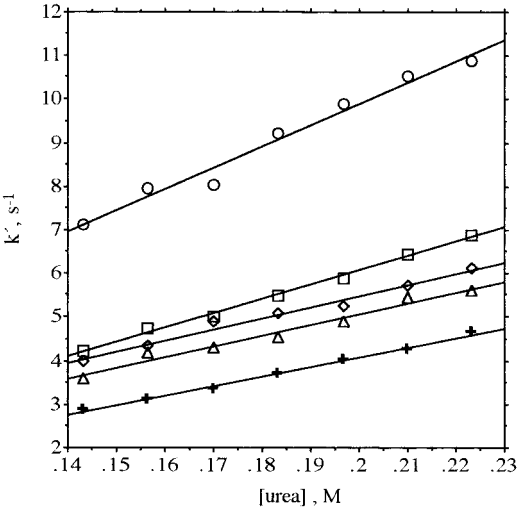
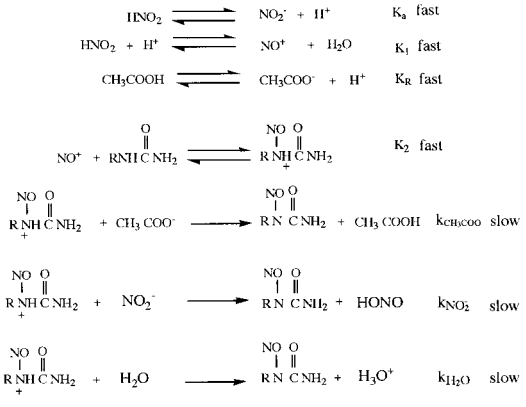


Figure 4 Determination of the rate constant k for the nitrosation of the substrates: (◯) MU; (□) EU; (△) PU; (◇) BU; and (+) AU. [nitrite] = 1×10^{-4} M, 10% acetic acid, 85% dioxane, 5% water, and $T = 298$ K.

The values of k (Table 1) rise in proportion to the increase in the percentage of acetic acid. The above effect on k cannot be attributed exclusively to the change in the [H^+] in the medium, given the known catalytic effect [11,23] of the carboxylate anions on the reaction rate both in aqueous [12,14] and in organic media [20].

To discern between the influence of [CH_3COO^-] and [H^+] on k, and therefore to be able to interpret the results of Table I, the reaction mechanism shown in Scheme I is proposed. This mechanism for the nitrosation reactions in an organic medium, is founded as much on the results of the kinetic study (first-order in nitrite and urea) as on previous studies that consider the nitrosation of the same kind of substrates both in aqueous and an organic media [12,20].



Scheme I Proposed mechanism for the nitrosation of ureas in an organic medium (dioxane–acetic acid–water).

Table I Values of *k* Eq. (4)

Acetic Acid Percentage	MU	EU	PU	BU	AU
			k, $M^{-1}s^{-1}$		
0[a]	10.1 ± 0.01	3.03 ± 0.05	2.77 ± 0.07	3.02 ± 0.15	1.94 ± 0.04
			$k \times 10^4$, $M^{-1}s^{-1}$		
35	80.1 ± 0.32	41.7 ± 0.28	36.6 ± 0.10	35.1 ± 0.29	23.6 ± 0.23
25	32.9 ± 0.18	18.8 ± 0.29	16.5 ± 0.10	18.7 ± 0.08	9.64 ± 0.03
10	4.90 ± 0.32	3.28 ± 0.01	2.59 ± 0.13	2.61 ± 0.10	2.20 ± 0.08

[a] Aqueous media [28].

In order to determine the theoretical equation corresponding to this mechanism the following suppositions were made: (a) Both the concentration of water and of the nitrite ion are insignificant compared to those of acetic acid and dioxane and (b) The nucleophic power of both water and the nitrite ion are equally insignificant in relation to that of the acetate anion acetate anion. On the basis of the above, it is possible to eliminate the final two elemental reactions in Scheme I.

$$v = -\frac{d[\text{nit}]}{dt} = k_{CH_3COO^-}K_1K_2K_RK'_a \times \frac{[H_2O][CH_3COOH][H_3^+O][\text{urea}][\text{sodium nitrite}]}{(K'_a[H_2O]^2)([H_3^+O] + K'_a[H_2O])} \quad (5)$$

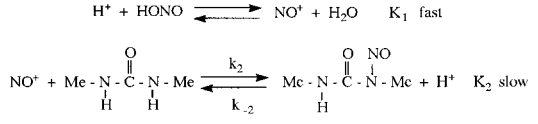
Where K'_a is the dissociation constant of H_2NO_2 in water. If we compare eqs. (3) and (5), we end up with:

$$k = k_{CH_3COO^-}K_1K_2K_RK'_a \times \frac{[H_2O][CH_3COOH][H_3^+O]}{(K'_a[H_2O]^2)([H_3^+O] + K'_a[H_2O])} \quad (6)$$

When the pH, $[H_2O]$, $[CH_3COOH]$, T, and I remain constant throughout the kinetic study, the k in eq. (6) will be the exclusive function of $k_{CH_3COO^-}$ given that K_1, K_2, K_R, and K'_a are constants common to the five substrates (See Scheme I). Given the above, it is possible to express eq. (5) as $\nu = k[\text{urea}][\text{nitrite}]$ eq. (3). Given that $k_{CH_3COO^-}$ corresponds to the limiting step of the mechanism, it will be a measurement of the reaction rate, function of the substrate (see Scheme I) eq. (7).

$$k = f(k_{CH_3COO^-}) = f(\text{substrate characteristics}) = f(R) \quad (7)$$

Before applying eq. (7) in the interpretation of the results, it is vital to take into consideration the kinetic studies carried out by Bravo et al. [20] on the nitrosation of the dimethylurea in dioxane–water media. This study found that for percentages of dioxane greater than 75%, the nitrosation mechanism is no longer that proposed in Scheme I but corresponds to that presented in Scheme II. This effect had already been observed by Crookes and Williams [21,22] when the reaction medium was changed from aqueous to organic during the nitrosation reactions.



Scheme II Proposed mechanism for the nitrosation of ureas in a dioxane–water medium, for a percentage of dioxane greater than 75% [20].

The equation related to this mechanism is:

$$v = -\frac{d[\text{nitrite}]}{dt} = \left(\frac{K_1k_2[\text{DMU}]}{1 + K_1[H^+]} + k_{-2}\right)[\text{nitrite}] + k_{-2}[\text{nitrite}]_o \quad (8)$$

Given the impossibility of equivalence between the theoretical, eq. (8) and the experimental, eq. (3), it can be concluded that the reaction mechanism involved for the three percentages of acetic acid shown in Table II, is that described in Scheme I.

The fact that the mechanism described in Scheme II has still not occurred with a 90% concentration of dioxane can be attributed to the nucleophile nature of the CH_3COO^- anion which enables it to extract protons (in the rate controlling step) even at low concentrations. In the case of the dioxane–water system[20], this situation does not occur because a greater percentage of water (25%) is needed to extract protons at the rate controlling step.

Given that the rate constants (Table II) were determined under different conditions of acidity and ionic intensity, it was necessary to refer the value of the rate

Table II Relative Values of *k* with Respect to *k*(MU)

Acetic Acid Percentage	*k* (MU)/*k*(urea)				
	MU	EU	PU	BU	AU
0[a]	1.00 ± 0.00	3.32 ± 0.12	3.63 ± 0.16	3.33 ± 0.23	5.18 ± 0.21
35	1.00 ± 0.00	1.92 ± 0.20	2.18 ± 0.15	2.27 ± 0.30	3.39 ± 0.42
25	1.00 ± 0.00	1.75 ± 0.40	1.99 ± 0.20	1.76 ± 0.18	3.41 ± 0.31
10	1.00 ± 0.00	1.49 ± 0.10	1.88 ± 0.23	1.87 ± 0.20	2.27 ± 0.23

[a] Aqueous media [28].

constants pertaining to all the substrates *k* (ureas) to the value corresponding to that of the methylurea *k*(MU). The above was carried out using the relationship *k*(MU)/*k*(urea). The above values are shown in Table II.

Table II shows how a reduction in the polarity in the medium is accompanied by an approximation of the *k*(MU)/*k*(urea) values for the different substrates. The highest separation between the values of *k*(MU)/*k*(urea) for different substrates occurs in the case of an aqueous medium (0% of acetic acid). This can be interpreted in terms of the high polarity of the water. Such polarity will cause the folding of the alkylic radical R (see Fig. 5) which partially impedes the departure of the proton from the protonated *N*-alkyl-*N*-nitrosourea to the proton extracter (CH_3COO^-), in the rate controlling step of the reaction mechanism. As the polarity is reduced in the medium, it is expected that the alkylic chains R tend to linealize, allowing for the approximation of the rate constants for the different substrates. In Table II we can observe a certain tendency towards unity in the relationship *k*(MU)/*k*(urea) as the polarity of the medium decreases. At first, sharply as they pass from the aqueous to the organic medium with 35% acetic acid, then gradually as the polarity declines in proportion to the reduction in the concentration of acetic acid. Note how neither the BU nor the AU follow the expected sequence when the acetic acid reduces from a 35 to 25% concentration, probably as a result of the combination of experimental error and the low gradient of the concentration of acetic acid involved.

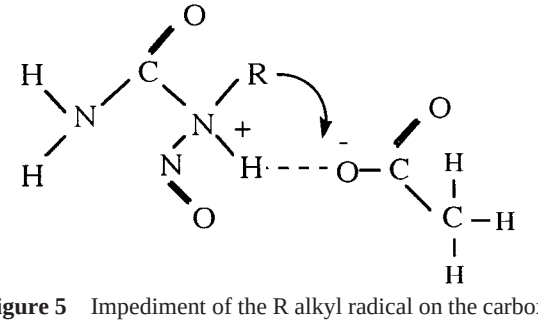


Figure 5 Impediment of the R alkyl radical on the carboxilic anion CH_3COO^- during the rate controlling step of the reaction mechanism.

In order to be certain that the tendency observed in the data obeys a correlation between polarity and the unfolding of the R groups, it would be necessary to carry out experiments at lower polarities until the total unfolding of the alkyl chain was achieved, *k*(MU)/*k*(urea) = 1. However, this would be impossible as it would require the elimination of both the 5% of water used in the solution of the sodium nitrite and the acetic acid used in the extraction of protons in the rate controlling step of the reaction mechanism.

The effect the R alkyl group has on the reactions of the nitrosation of ureas could also have biomedical implications. It is a well known fact that the carcenogenic activity of the nitrosocompounds depends on the facility with which diazone ions are formed, themselves responsible for the alkylation of the DNA molecules [5,24]. Whereas for nitrosamines, a previous enzimatic activation is required for the formation of such ions, in the case of nitrosoureas, only the hydrolysis of such compounds is needed(probably because of their instability) [13]. In function of what has been presented in this study, it is to be expected that the most stable nitrosourea is methylnitrosourea, which, because of the low steric impediment of the R alkylic radical, would be more difficult to hydrolize and therefore hindering the formation of the diazone ions in relation to the other substrates. If this were to occur, the methylnitrosourea would be the least biologically active. In fact there is experimental evidence from various studies that show the low biological activity (in laboratory animals) of the methylnitrosourea, compared to the activity of other alkyl nitrosoureas [25–27].

CONCLUSION

The kinetic studies of the nitrosation of the five ureas in a predominantly organic medium have shown the following results: (a) First-order with respect to both

the nitrite and the urea. (b) Approximation of the rate constants for the different substrates involved in the study as the polarity of the medium is reduced. This has been interpreted in function of the unfolding of the hydrophobic alkyl radical R; and (c) A probable relationship between both the chemical reactivity and structure of the nitrosable substrate with its biological activity has been established.

The authors wish to thank the Consejo del Sistema Nacional de Educación Tecnológica, COSNET, for their financial support which enabled the research for this project to be carried out and the Mexican Consejo Nacional de Ciencia Tecnologiá, CONACYT, for both the financial assistance to Javier G. Zapiain S. and for their financial support. The authors also thank Dr. Julio Casado for is useful comments and suggestions.

BIBLIOGRAPHY

1. P.N. Magee and P.F. Swann, *Brit. Med. Bul.,* **25,** 240 (1969).
2. R. Preussmann and B.W. Stewart, *Am. Chem. Soc.,* **182,** 643 (1984).
3. E. Lijinsky, *Can. Met. Rev.,* **6,** 301 (1987).
4. L.B. Russell and C.S. Montgomery, *Mutat. Res.,* **1,** 193 (1982).
5. W. Lijinsky and A. W. Andrews, *Mutat. Res.,* **1,** 68 (1979).
6. W. Lijinsky, *Oncology,* **37,** 223 (1980).
7. P. Bogovsky and S. Bogovsky, *Int. J. Can.,* **27,** 471 (1981).
8. D. Schmähl and H.R. Sherf, *IARC Sci. Publ.,* **57,** 677 (1985).
9. J.H. Ridd, *Quart. Rev.,* **15,** 418 (1961).
10. J. Casado, A. Castro, and M.A.L. Quintela, *Monatsh. Chem.,* **112,** 1221 (1981).
11. J. Casado, A. Castro, M. Mosquera, M.F. Rodriǵuez Prieto, and J. Vázquez Tato, *Ber. Bunsenges. Phys. Chem.,* **87,** 1211 (1983).
12. J. Casado, G. González-Alatorre, C. Izquierdo, and C. Brunner, *Int. J. Chem. Kinet.,* **28,** 307 (1996).
13. C. Heidelberger, *Ann. Rev. Biochem.,* **44,**79 (1975).
14. G. Hallett and D.L.H. Williams, *J. Chem. Soc., Perkin Trans.,* **2,** 1372 (1980).
15. J. Casado, A. Castro, M. Mosquera, M.F. Rodriguez Prieto, and J. Vázquez Tato, *Ber. Bunsenges. Phys. Chem.,* **87,** 1211 (1983).
16. J. Casado, A. Castro, J.R. Leis, Mosquera, and M.E. Peña, *Monatsh. Chem.,* **115,** 1047 (1984).
17. A.R. Tricker and R. Preussmann, *Mutat. Res.,* **202,** 325 (1988).
18. J.H. Weissburger, in *'N-nitroso Compounds'*, R.A. Scanlan and S.R. Tannenbaum, Eds., American Chemical Society, Washington, DC, 1981, p. 305.
19. E.W. Stewart, M. Caracotsios, J. P. Sorensen, and Subrutine Greg, University of Wisconsin-Madison, version of june 1990.
20. C. Bravo, P. Hervés, E. Iglesias, J.R. Leis, and E. Peña, *J. Chem. Soc. Perkin Trans,* **2,** 1969 (1990).
21. M.J. Crookes and D.L.H. Williams, *J. Chem. Soc. Chem. Comun,* 571 (1988).
22. M.J. Crookes and D.L.H. Williams, *J. Chem. Soc. Perkin Trans,* **2,** 1319 (1989).
23. A. Castro, E. Iglesias, J. R. Leis, E. Peña, and J. Vazquez Tato, *J. Chem. Soc. Perkin Trans II,* 1725 (1986).
24. A.R. Tricker and R. Preussmann, *Mutat. Res.,* **259,** 277 (1991).
25. W. Lijinsky and R.M.J. Kovatch, *Toxicol. Ind. Health,* **5,** 925 (1989).
26. W. Lijinsky, J.E. Saavedra, and R.M.J. Kovatch, *Toxicol. Ind. Health,* **28,** 27 (1989).
27. W.L. Russell, E.M. Kelly, P.R. Hunsucker, J.W. Bangham, and S.C. Maddux, *E.L. Phipps, Proc. Natl. Acad. Sci. U.S.A.,* **76,** 5818 (1979).
28. G. González, *Ph. D. Thesis,* University of Salamanca, Spain 1994.