Kinetic Study of the Nitrosation of 1,3-Dialkylureas in Aqueous-Perchloric Acid Medium

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ABSTRACT: The kinetics of the nitrosation of 1,3-dimethyl (DMU), 1,3-diethyl (DEU), 1,3-dipropylurea (DPU), 1,3-dibuthyl (DBU), and 1,3-diallylurea (DAU) were studied in a conventional UV/vis spectrophotometer in aqueous-perchloric acid media. The kinetic study was carried out using the initial rate method. The reaction rate observed was

$$r = \frac{k[\mathrm{H}^+]^2}{[\mathrm{H}^+] + K_\mathrm{a}}[\mathrm{Diurea}][\mathrm{Nitrite}]$$

where K_a is the acidity constant of nitrous acid. The diureas exhibited the reactivity order DMU $\gg$ DEU $>$ DPU $>$ DAU, which can be interpreted as a function of the steric impediment generated by the R alkyl group in the rate controlling step. A probable relationship between both the chemical reactivity and structure of the nitrosable substrate with the biological activity of the N-nitroso compounds generated is proposed. © 2004 Wiley Periodicals, Inc. Int J Chem Kinet 36: 273–279, 2004

INTRODUCTION

For many years, nitrosoalkylureas have served as an example of direct carcinogenic agents, those that do not require enzymatic activation (as do nitrosamines) to reveal their mutagenic and carcinogenic capacity [1–4].

In the case of the biological activity of the nitrosamines, statistically significant correlations have

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been found between carcinogenic activity and water–hexane partition coefficients [5]. As far as the authors are aware, there is no published data regarding a correlation between partition coefficients and the biological activity of nitrosoalkylureas. This lack of data is possibly due to the difference between the carcinogenesis mechanism of nitrosamides and that of nitrosamines. In nitrosamines, which are more stable in aqueous solutions than nitrosoalkylureas [6], the requirements for carcinogenic potency appear dominated by the structure of the unmetabolized precarcinogen [5]. Therefore, the transport of the original molecule to its active site has an important effect on its potency. Nitrosoalkylureas, unstable in an aqueous medium [3], and therefore direct carcinogenic agents, are unlikely to have the transport of molecules to their active sites as the rate limiting step in the carcinogenesis mechanism.

The mechanistic model for the biological activity of nitrosoureas is based on the relative ease with which these compounds decompose, giving way to the diazonium ions responsible for the alkylation of the DNA molecules [4–6]. Diverse studies have been conducted to relate the chemical structure of the nitrosoureas (nitrosomonoalkylureas and nitrosodialkylureas) to their biological activity to form a more solid base for this model [2,3]. The experiments were carried out using different experimental animals and nitrosoureas with different stabilities and chemical structures [7–9]. It has not been possible to establish a simple pattern that indicates that the observed mutagenic power is the sole consequence of the stability of the associated nitroso-compound; rather, it depends on sex and type of experimental animal, application form, dose and functional group linked to the N-nitroso compound, among others.

Studies have been undertaken on the reactions of the nitrosation of ureas [10–15] and as a result, a reaction mechanism for the nitrosation of monoureas with the general formula $RNHCONH_2$ (R = Methyl, ethyl, propyl, buthyl, and allylurea) has been proposed [11].

The results of the kinetic study on a series of diureas with the general formula RNHCONHR (R = Methyl, ethyl, propyl, buthyl, and allylurea), in an aqueous-perchloric acid medium, using the initial rate method, are presented in this work. By using this method it was possible to carry out the kinetic study at slow reaction rates where it would have been impossible to use the integral method [10,11]. This study produced an experimental rate equation consistent with the reaction mechanism proposed in previous works [10–15].

The kinetic study yielded the reactivity order associated with the five nitrosation reactions and was related to the structure of the substrate to be nitrosated and the N-nitroso compound produced.

EXPERIMENTAL

Solutions of 1,3-dimethylurea (DMU), 1,3-diethylurea (DEU), 1,3-dipropylurea (DPU), 1,3-dibuthylurea (DBU), 1,3-diallylurea (DAU), and sodium nitrite ($NaNO_2$) were prepared from analytical reagents (Aldrich Co. Mexico). Ionic strength (I) was maintained with $NaClO_4$ monohydrate (Aldrich Co. Mexico).

It was found that *N*-nitrosodimethylurea, *N*-nitrosodimethylurea, *N*-nitrosodipropylurea, and *N*-nitrosodibuthylurea products showed a maximum absorbance of 249 nm whereas the *N*-nitrosodiallylurea had 238 nm. At these wavelengths, the nitrite molar absorption coefficient is negligible with respect to the nitrosoalkylureas (e.g., for nitrosomethylurea $\varepsilon_{240} = 3500\ M^{-1}\,cm^{-1}$ and $\varepsilon_{240} = 8\ M^{-1}\,cm^{-1}$ for nitrite).

Nitrosation kinetics were recorded using a Perkin-Elmer UV/vis spectrophotometer lambda 25 equipped with a thermoelectric cell holder thermostat Haake DC1 maintaining the temperature within $\pm 0.1^{\circ}C$. Acidity was measured with an Ultra Basic Bench top Denver pH meter.

The initial rate method was applied to the nitrosation of diureas.

$$\underset{\text{(DRU)}}{\text{RNHCONHR}} + \underset{\text{(nitrite)}}{\text{NaNO}_2} \xrightarrow{H_3^+O} \underset{\text{(NDRU)}}{\text{RN(NO)CONHR}} + Na^+ + 2H_2O$$

where R = Methyl, Ethyl, Propyl, Buthyl, and Allyl groups.

The rate expression is

$$r_o = k[\text{DRU}]_o^{\alpha}[\text{nitrite}]_o^{\beta} \tag{1}$$

In order to determine the reaction orders, Eq. (1) became

$$r_o = \left(\frac{1}{\varepsilon_{\text{NDRU}}}\right)\left(\frac{dA}{dt}\right)_{t\to 0} = k[\text{DRU}]_o^{\alpha}[\text{nitrite}]_o^{\beta} \tag{2}$$

where $\varepsilon_{\text{NDRU}}$ is the molar absorption coefficient of the corresponding nitrosodiurea and A is the absorbance of the NDRU at the wavelength of maximum absorption.

In the determination of the influence of acidity on k, all the variables involved in the nitrosation, such as nitrite concentrations and DRU, ionic strength, and temperature, were kept constant whereas the proton concentration was varied in the interval $1 \times 10^{-4} < [H^+] < 1.7 \times 10^{-3}$ M. The following expression,

obtained from Eq. (2), was used to find the functionality between k and $[H^+]$

$$k = \frac{(1/\varepsilon_{NDRU})(dA/dt)_{t\to 0}}{[DRU]_o^{\alpha}[nitrite]_o^{\beta}} = M\left(\frac{dA}{dt}\right)_{t\to 0} = f([H^+]) \quad (3)$$

All the terms that remained constant throughout the experimentation were grouped in parameter M.

RESULTS AND DISCUSSION

The reaction orders for the nitrosation of the five diureas are shown in Table I (Figs. 1 and 2).

The functionality between k and $[H^+]$, Eq. (3), was consistent with that observed in monoureas, Eq. (4), using the integral method [10,11]. See Fig. 3.

$$\frac{[H^+]^2}{(dA/dt)_{t\to 0}} = b + c[H^+] \quad (4)$$

Table I Reaction Orders for the Nitrosation of Diureas (Figs. 1 and 2)

Substrate	α	β
DMU	1.06 ± 0.05	1.01 ± 0.030
DEU	1.03 ± 0.02	0.98 ± 0.010
DPU	1.03 ± 0.00	1.03 ± 0.010
DBU	1.01 ± 0.03	1.00 ± 0.028
DAU	1.03 ± 0.05	1.00 ± 0.048

where b and c are constants resulting from the lineal correlation. Equations (3) and (4) yield

$$k = \frac{M[H^+]^2}{b + c[H^+]} \quad (5)$$

Substituting orders $\alpha = 1$ and $\beta = 1$ in Eq. (1), the experimental rate for the nitrosation of diureas is reached.

$$r = \frac{M[H^+]^2}{b + c[H^+]}[nitrite][DRU] \quad (6)$$

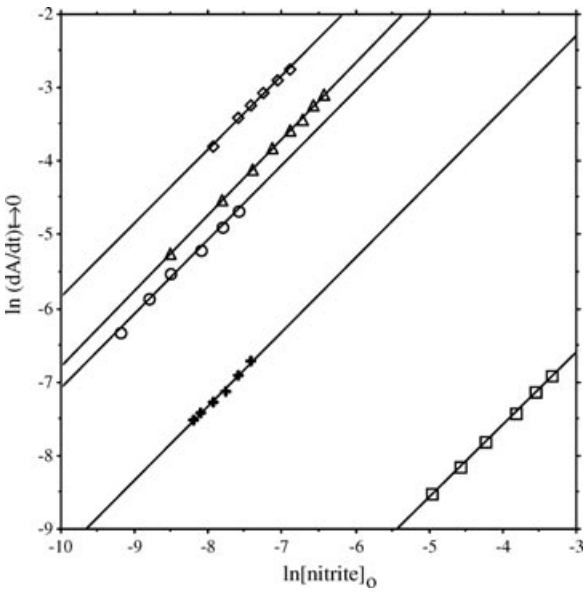


Figure 1 Order reaction with respect to the nitrite [Eq. (3)] in the nitrosation of the five diureas under study, $T = 298$ K and $I = 0.1$ M; ○: $[DMU]_o = 5 \times 10^{-4}$ M, $1 \times 10^{-4} < [nitrite]_o < 5 \times 10^{-4}$ M, $[H^+] = 5 \times 10^{-4}$ M; □: $[DEU]_o = 5 \times 10^{-4}$ M, $7.4 \times 10^{-4} < [nitrite]_o < 3.6 \times 10^{-2}$ M, $[H^+] = 1 \times 10^{-2}$ M; △: $[DPU] = 5 \times 10^{-4}$ M, $2 \times 10^{-4} < [nitrite]_o < 1.5 \times 10^{-3}$ M, $[H^+] = 1 \times 10^{-2}$ M; ◇: $[DBU]_o = 2.5 \times 10^{-4}$ M, $3 \times 10^{-4} < [nitrite]_o < 1 \times 10^{-3}$ M, $[H^+] = 2.5 \times 10^{-2}$ M; +: $[DAU]_o = 2.5 \times 10^{-4}$ M, $2.4 \times 10^{-4} < [nitrite]_o < 1.7 \times 10^{-3}$M, $[H^+] = 2.5 \times 10^{-2}$ M.

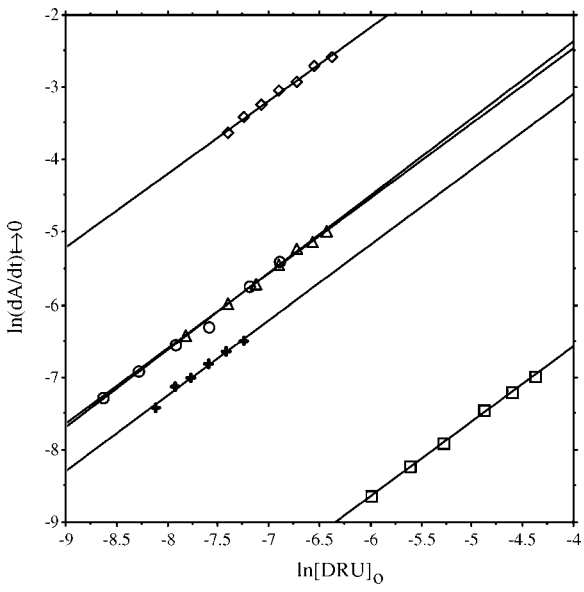


Figure 2 Order with respect to the diureas under study [Eq. (4)], $T = 298$ K and $I = 0.1$ M; ○: $1.7 \times 10^{-4} < [DMU]_o < 1 \times 10^{-3}$ M, $[nitrite]_o = 3 \times 10^{-4}$ M, $[H^+] = 5 \times 10^{-4}$ M; □: $2.5 \times 10^{-3} < [DEU]_o < 1.2 \times 10^{-2}$ M, $[nitrite]_o = 3 \times 10^{-4}$ M, $[H^+] = 1 \times 10^{-2}$ M; △: $4.1 \times 10^{-4} < [DPU]_o < 1.53 \times 10^{-3}$ M, $[nitrite]_o = 5 \times 10^{-5}$ M, $[H^+] = 1.5 \times 10^{-2}$ M; ◇: $6.1 \times 10^{-4} < [DBU]_o < 1 \times 10^{-3}$ M, $[nitrite]_o = 2.5 \times 10^{-4}$ M, $[H^+] = 2.5 \times 10^{-2}$ M; +: $3 \times 10^{-4} < [DAU]_o < 7.1 \times 10^{-4}$ M, $[nitrite]_o = 2.5 \times 10^{-4}$ M, $[H^+] = 2.5 \times 10^{-2}$ M.

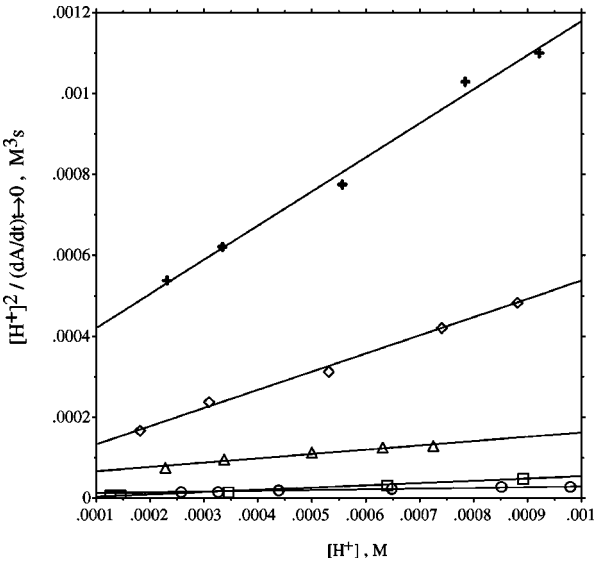


Figure 3 Fitting of experimental data to Eq. (14) [DMU] = [DEU] = [DPU] = [DBU] = [DAU] = 8.2×10^{-2} M, [nitrite] = 1×10^{-4} M, $1 \times 10^{-4} < [H^+] < 9.8 \times 10^{-4}$ M, $T = 298$ K, $I = 0.03$ M. ○: DMU, □: DEU, △: DPU, ◇: DBU, +: DAU.

The mechanism for the nitrosation of the series of diureas under study, Scheme 1, is consistent with the experimental rate equation [compare Eqs. (6) and (7)] and is similar to that proposed for the nitrosation of mono and dialkylureas [10–12,14,15]. The proton transfer from the protonated N-nitroso compound to the water as the rate limiting step in the formation mechanism of nitrosoamides has been confirmed by the following observations:

i. absence of nucleophilic catalysis on the nitrosation of amides [12,14]
ii. presence of primary solvent isotope effects on the nitrosation of mono and dialkylureas [11,15]
iii. general base catalysis in the mono and dialkylureas nitrosation [11,15]

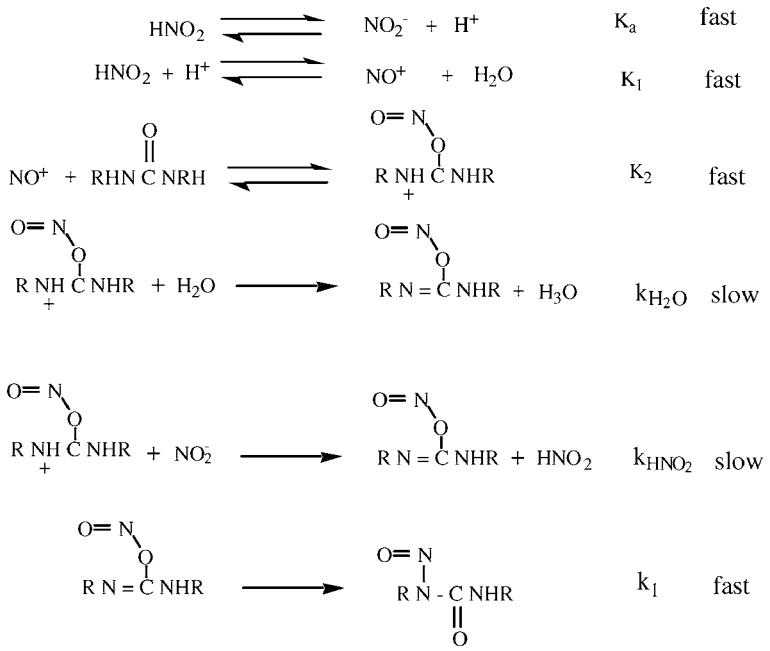
The initial attack of the nitrosating agent (Scheme 1) occurs on the oxygen atom given that it is the most nucleophilic site of the molecule [12,14].

Theoretical rate Eq. (7) is obtained considering that $[NO_2^-] = 1 \times 10^{-4}$ M $\ll [H_2O]$, and that $[NaNO_2] = [HNO_2] + [NO_2^-]$:

$$r = \frac{k_{H_2O} K_1 K_2 [H^+]^2}{[H^+] + K_a} [DRU][NaNO_2]$$
$$= \frac{[H^+]^2}{[H^+]/k_{w\,exp} + K_a/k_{w\,exp}} [DRU][NaNO_2] \quad (7)$$

where $k_{w\,exp} = k_{H_2O} K_1 K_2$.

Parameter $K_a = 7 \times 10^{-4}$ [16], [see Eq. (7)], is obtained from parameters b and c, Eq. (4), for the five diureas under study. The differences between the value of K_a determined by direct methods and the values obtained from the kinetic study (see Table II) can be



Scheme 1 Proposed mechanism for the nitrosation of diureas in an aqueous-perchloric acid medium.

Table II Values of K_a [Eq. (4)]

Substrate	K_a (M)
Dimethylurea	6.7×10^{-4}
Diethylurea	3.6×10^{-4}
Dipropylurea	2.0×10^{-4}
Dibuthylurea	1.9×10^{-4}
Diallylurea	3.9×10^{-4}

attributed to experimental error and/or the presence of other chemical species in the reagent solution.

The reactivity of the substrates was related to the $k_{w\,exp}$ parameter (see Scheme 1), determined by the integral method, considering ($\alpha = 1, \beta = 1$) and using the concentrations of the diureas in excess with respect to the nitrite concentration. To enable the use of the integral method, it was necessary to take $[H^+] > 3.5 \times 10^{-3}$ M in such a way that the value of A_∞ could be obtained in a reaction time of less than 6 h. Under these conditions, the rate equation for the nitrosation of diureas is $r = k'$ [nitrite], where $k' = k$ [DRU]. The corresponding integrated equation in function of the absorbance of the product is

$$\ln(A_\infty - A) = -kt + \ln(A_\infty - A_o) \qquad (8)$$

Applying this equation to a study of the influence of acidity on k values, Fig. 4, experimental data were

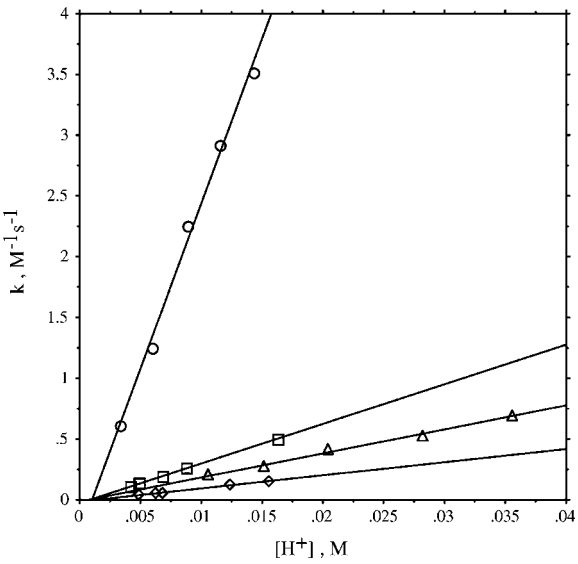


Figure 4 Determination of $k_{w\,exp}$ for the four substrates under study. $I = 1.0$ M, $T = 298$ K. ○: [DMU] $= 1.5 \times 10^{-2}$ M, [nitrite] $= 1 \times 10^{-4}$ M, $3.3 \times 10^{-3} < [H^+] < 1.35 \times 10^{-2}$ M; □: [DEU] $= 1.5 \times 10^{-2}$ M, [nitrite] $= 1 \times 10^{-4}$ M, $4.2 \times 10^{-3} < [H^+] < 1.6 \times 10^{-2}$ M; △: [DPU] $= 6.25 \times 10^{-3}$ M, [nitrite] $= 1 \times 10^{-4}$ M, $1 \times 10^{-3} < [H^+] < 3.6 \times 10^{-2}$ M; +: [DAU] $= 3 \times 10^{-2}$ M, [nitrite] $= 1 \times 10^{-4}$ M, $4.9 \times 10^{-3} < [H^+] < 1.6 \times 10^{-2}$ M.

Table III Values of $k_{w\,exp}$ [Eq. (9)]

Substrate	$k_{w\,exp}$ (s^{-1} M^{-2})
Dimethylurea	258.4 ± 9.1
Diethylurea	32.5 ± 0.7
Dipropylurea	19.8 ± 0.9
Diallylurea	10.5 ± 0.4

Note: The substrate dibuthylurea was discontinued when this experiment was carried out.

obtained that fit Eq. (9).

$$k = m[H^+] \qquad (9)$$

Therefore, under these conditions, the experimental rate equation is

$$r = m[H^+][\text{nitrite}][\text{DRU}] \qquad (10)$$

Equations (10) and (7) are consistent when $[H^+] \gg K_a$, where $m = k_{w\,exp}$ (see Table III).

According to the proposed mechanism, the longitude of the R groups will affect the rate controlling step. This can be justified by bearing in mind that the R alkyl groups are hydrophobic and will therefore tend to fold back on themselves in an aqueous medium, Fig. 5(A) [10]. This affects the $k_{w\,exp}$, and by extension, the chemical reactivity of the substrates giving way to the sequence DMU > DEU > DPU > DAU (Table III). This sequence can also be related to the stability of the resulting nitrosoureas and their biological activity. The instability of the *N*-nitrosoamides in an aqueous medium is attributed to the closeness of the two highly electropositive functional groups (NNO and CO), which in turn gives way to the breaking up of the molecules as shown in Fig. 5(B) [17,18].

The volume of the R (hydrophobic) alkyl group could be another factor that facilitates the breaking up of the nitrosourea by increasing the steric

Table IV Stability of Nitrosoalkylureas Half-Life at pH 7 and pH 8 (22°C), [3,7]

	$t_{1/2}$ (h)	
Compound	pH 7	pH 8
1-Nitroso-		
1,3-dimethylurea	300	43
1,3-diethylurea	87	18
1-methyl-3-ethylurea	230	38
1-ethyl-3-methylurea	84	18

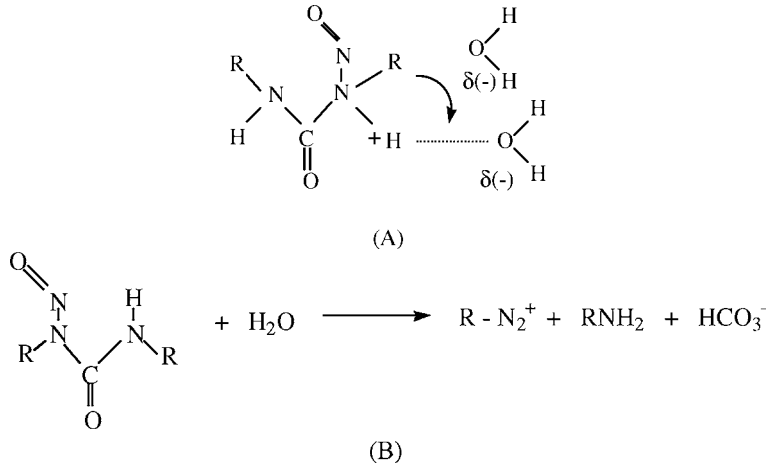


Figure 5 (A) Proposed hydrogen bonding between water and protonated *N*-alkyl-*N*-nitrosodiureas; (B) breaking up of nitrosodiureas molecules to form diazone ions [4].

impediment and weakening the N-C link. This hypothesis is founded on the following arguments:

a. One reaction path to the generation of potent alkylating agents involves the attack of a nucleophile on the C═O group to generate a tetrahedral intermediate which decomposes with the generation of diazotate ion (R─N=N─O─) [4,14,15]. The length of the R alkyl group could favor this path.
b. The reported instability of the nitrosoureas in an aqueous medium (see Table IV) [3,7]. At pH values of 7 and 8, the N-nitroso compounds with methyl groups are markedly more stable than those with ethyl groups. In this same table, the greater stability of the nitrosoalkylureas at low pH values can be observed which agrees with the studies carried out for the decontamination and disposal of nitrosoureas [19].

The results shown in Table IV indicate that the biological activity of the N-nitroso compounds will depend on the ease with which their molecules decompose to form alkylating agents. It is to be expected, therefore, that the sequence in the biological activity of the nitrosodialkylureas be the opposite of that observed in the reactivity DNMU $\ll$ DNEU $<$ DNPU $<$ DNAU. Lijinsky et al. [3] have carried out studies on the biological activities of the first two members of the series, NDMU and NDEU, where the greatest differences in chemical reactivity are observed. In the studies done on rats, Table V, the biological activity of the NDEU is markedly greater than that observed for the DMEU. These results agree with our hypothesis regarding the relationship that exists between the chemical reactivity/reaction mechanism/structure of the substrate to be nitrosated and the biological activity of the N-nitroso compound generated.

As mentioned in the introduction, the differences in the qualitative and quantitative carcinogenic effect between these two nitrosodiureas can only be partly explained by their alkylating properties. Other factors exist, such as receptors in the cells of certain experimental animals that have a specific affinity to the structure of one N-nitroso compound in particular [4].

Table V Carcinogenesis by Nitrosodialkylureas in Rats [4]

	Rats (Gavage)				Rats (Drinking Water)			
	Dose 1.2 mmol		Dose 0.6 mmol		Dose 1.2 mmol		Dose 0.6 mmol	
RN(NO)CONHR R	$t_{1/2}$ M	$t_{1/2}$ F	$t_{1/2}$ M	$t_{1/2}$ F	$t_{1/2}$ M	$t_{1/2}$ F	$t_{1/2}$ M	$t_{1/2}$ F
CH_3	81	40	83	80	86	71	95	100
C_2H_5	33	–	36	29	49	32	58	47

$t_{1/2}$ M = median week of death in a male rat, $t_{1/2}$ F = median week of death in a female rat.

CONCLUSION

The kinetic study of the nitrosation of diureas in an aqueous medium showed the following characteristics: (1) the application of the initial rate method to the study of the effect of the $[H^+]$ on the reaction rate constant and consequently on the establishment of the reaction mechanism, showed several significant advantages over the integral method. (2) The reactivity observed can be justified in function of the rate-limiting step of the mechanism and of the hypothesis of the folding of the hydrophobic alkyl group. (3) A probable relationship between the chemical reactivity and structure of the substrate to be nitrosated and the activity of the nitrosourea generated has been established.

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