Structural Effects on the *N*-Nitrosation of Amino Acids

RAFAEL GIL, JULIO CASADO, CARMEN IZQUIERDO

Departamento de Química física, Facultad de Química, Universidad de Salamanca, E-37008 Salamanca, Spain

Received 7 August 1996; accepted 18 November 1996

ABSTRACT: The relative importance of three different routes for the N-nitrosation of amino acids (nitrosation by N_2O_3, by $NO^+/NO_2H_2^+$ and by intramolecular migration of the nitroso group from the initially nitrosated carboxylate group) was investigated for methylaminobutyric acid, methylaminoisobutyric acid, azetidine-2-carboxylic acid, azetidine-3-carboxylic acid, indoline carboxylic acid, and phenylaminoacetic acid. Reaction kinetics were determined by the initial rate and Guggenheim methods, by spectrophotometric monitoring of the formation of nitroso amino acid. Kinetic parameters were calculated using a nonlinear optimization algorithm based on Marquardt's method. In the experimental rate equation the dominant term corresponds to nitrosation by dinitrogen trioxide, which experiments at various temperatures show to take place via an ordered transition state. Nitrosation by intramolecular migration is significant for substrates facilitating the formation of a transition state structure with a 5- or 6-membered ring. © 1997 John Wiley and Sons, Inc. Int J Chem Kinet **29:** 495–504, 1997.

INTRODUCTION

N-Nitroso compounds may play a significant role in human carcinogenesis [1–3], and considerable effort has accordingly been focused on estimating the extent of human exposure to them via synthesis of endogenous precursors or exposure to exogenous precursors [4,5]. Studies of the mechanisms of *N*-nitrosation have been numerous, but have paid relatively little attention to the nitrosation of amino acids, in spite of its chemical and biochemical importance. For the *N*-nitrosation of proline, hydroxyproline, and sarcosine, Mirvish *et al.* [6] obtained the rate equation

$$v = k\,[\text{nit}]^2[\text{amino acid}] \qquad (1)$$

where [nit] is the concentration of nitrite. Measured values of k were of the same order of magnitude as for other aliphatic amines [7]. Later, however, the equation

$$v_0 = (a[\text{nit}]_0 + b[\text{nit}]_0^2)[\text{amino acid}]_0 \qquad (2)$$

(where v_0 is the initial rate and $[\text{nit}]_0$ and $[\text{amino acid}]_0$ are the initial concentrations of nitrite and amino acid, respectively) was obtained for the *N*-nitrosation of sarcosine and proline [8], suggesting that both N_2O_3 and $NO^+/NO_2H_2^+$ were acting as nitrosating agents. Furthermore, the influence of the acidity of the medium on the term of first order with respect to nitrite suggested to the authors that $NO^+/NO_2H_2^+$ acted not only directly but also indirectly via a third route: formation of a nitrosyl carboxylate, followed by the intramolecular migration of the nitroso group from the carboxylate group to the secondary amino group [8]. Mirvish *et al.*'s failure to detect a term of first order with respect to nitrite is attributable to their

Correspondence to: J. Casado

Contract grant Sponsor: Commission of the European Communities

Contract grant number: (Contract no. 93CVVF1-610-0)

Contract grant Sponsor: Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT)

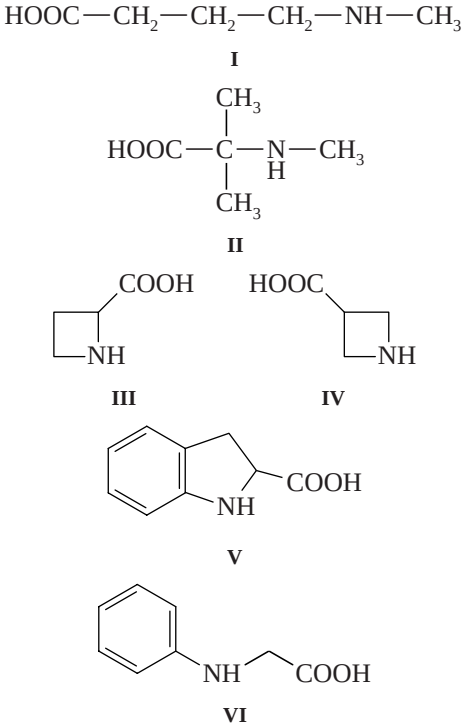
Contract grant number: (Project no. ALI389/90)

Contract grant Sponsor: Spanish Ministerio de Educación y Ciencia

 CCC 0538-8066/97/070495-10

use of discontinuous monitoring, analysis by the integration method even when very small percentages of the reaction were followed, and graphical methods for analysis by the initial rate method.

Differences in nitrosation behavior among nitrosatable substrates can be due to structural differences. For example, the results of a study of the nitrosation of five amino acids (including piperidine 2-, 3-, and 4-carboxylic acids) were explicable in terms of (a) whether the $C_{ring}—C_{carboxyl}$ bond was equatorial or axial and (b) whether the ring was a chair or a boat [9]. In the work reported here, we investigated the relative importance of the three routes mediating the nitrosation of amino acids and examined the effects of both structural and functional factors, particularly as regards the intramolecular NO migration route. To this end we studied the kinetics of the nitrosation of methylaminobutyric acid (Mab, **I**), methylaminoisobutyric acid (Maib, **II**), azetidine-2-carboxylic acid (2-Aza, **III**), azetidine-3-carboxylic acid (3-Aza, **IV**), indoline carboxylic acid (Ica, **V**), and phenylaminoacetic acid (phenylglycine, PhGly, **VI**). These amino acids were chosen for both chemical and biochemical reasons (L-azetidine-2-carboxylic acid, which occurs in nature, has been shown to inhibit the growth of *E. coli* cultures and of certain seedlings [10,11] and to cause abnormalities in growing embryos [12]). Biochemically, cyclic nitrosamines such as nitrosoazetidine are often considerably more potent as mutagens than their acyclic analogues [3], but the mechanisms of neither their toxicity nor their carcinogenicity are close to being understood. Chemically, it is of interest to compare the nitrosation of azetidine carboxylic acids with that of amino acids that are acyclic or have larger rings because of the greater rigidity of the azetidine compounds and because of the differences between the p*K*s of their amino and/or carboxyl groups and those of acyclic and aromatic amino acids [13–15].



EXPERIMENTAL

Reagents

4-Methylaminobutyric acid (nominal purity 99%), methylaminoisobutyric acid (98%), indoline carboxylic acid (99%), and phenylaminoacetic acid (95%) were supplied by Aldrich (Steinheim, Germany); L-azetidine-2-carboxylic acid (97%) by Fluka (Buchs, Switzerland); and azetidine-3-carboxylic acid (98%) by Sigma (St. Louis, U.S.A.). Sodium nitrite (p.a., from Merck, Darmstadt, Germany) was desiccated at 110°C before use. All solutions were made up fresh immediately before the kinetic experiments in which they were used. Ionic strength was adjusted with Merck p.a. $NaClO_4$ or NaOH; $HClO_4$ solutions were prepared by dilution of 60% $HClO_4$ (prs. grade, from Panreac, Barcelona, Spain) and their concentration was determined by conventional methods.

Apparatus

Nitrosation kinetics were monitored spectrophotometrically in a Shimadzu UV-2101 apparatus equipped with a thermoelectric cell holder controlling temperature to within ±0.1°C and linked to a Hewlett–Packard Color Pro printer and a Gridec-386 computer. IR spectra in capillary films were recorded in Beckman IR-33 and Bonnem FTMB-100 spectrographs. ^1H NMR spectra in $CDCl_3$ were recorded in a 200 MHz Bruker WP 200 SY spectrometer with TMS as internal reference. Acidity was measured using a Radiometer M64 digital pH-meter with a GK2401 combined electrode. Melting points were measured in a Büchi apparatus.

Preparation of Pure Samples of the Nitroso Compounds

N-Nitrosomethylaminobutyric acid (*N*Mab) and *N*-nitrosomethylaminoisobutyric acid (*N*Maib) were prepared by the method described by Lijinsky *et al.* [16]

for nitrosoisonipecotic acid. The presence of CO and NO groups was confirmed by the bands at, respectively, 1725 and 1450 cm^{-1} in the *N*Mab spectrum and at 1718 and 1340 cm^{-1} in the *N*Maib spectrum. Since *N*Mab is relatively unstable it was stored in a desiccator at 4°C. *N*-nitrosoazetidine-2-carboxylic acid (*N*-2-Aza) and *N*-nitrosoazetidine-3-carboxylic acid (*N*-3-Aza) were prepared by a slightly modified version of Lijinsky *et al.*'s [16] method (the solvent of the initial amino acid was dilute sulphuric acid, and the nitrosated product was extracted from the final reaction mixture with ethyl ether). Both nitrosamines were recrystallized from ether by addition of CH_2Cl_2 and cooling. *N*-2-Aza with IR and NMR spectra in agreement with published data [17] crystallized as colorless needles of M.P. 108–110°C (Lit. 107°C, [17]).

We were unable to prepare pure samples of nitrosoindoline carboxylic acid (*N*Ica) or nitrosophenylglycine (*N*PhGly).

Spectroscopic Properties and Working Wavelengths

Whereas the nitrosatable substrates had a (slightly pH-dependent) band at 200 nm but no UV bands at wavelengths longer than 230 nm, all the nitrosamines exhibited a strong —NO band centered at 210–240 nm. However, to prevent pH from affecting the total absorbance of the reaction mixture via its effect on the NO_2H/NO_2^- deprotonation equilibrium, the formation of *N*Mab, *N*Maib, *N*-3-Aza, *N*Ica, and *N*PhGly was not monitored at absorbance peaks but at the nearest isosbestic points of that equilibrium: 249 nm for *N*Mab, *N*Maib, and *N*-3-Aza (the measured molar absorption coefficient of nitrite at 249 nm was $50.0 \pm 0.5\ M^{-1}\ cm^{-1}$, in agreement with the value in the literature [17]), 323 nm for *N*Ica and 265 nm for *N*PhGly. At these wavelengths, absorption by the nitrosamines was strong enough for precise monitoring. Absorption by *N*-2-Aza was significantly pH-dependent at 249 nm, and the formation of this nitrosamine was accordingly monitored at 240 nm, where its absorbance was not affected by pH; for this reaction, absorbance by nitrite was taken into account by measuring its absorption coefficient at each working pH. The molar absorption coefficients, ϵ, of *N*Mab, *N*Maib, *N*-2-Aza, *N*-3-Aza, and the corresponding substrates at the working wavelengths are listed in Table I.

Kinetic Measurements

Nitrosation kinetics were monitored by recording UV absorbance by the nitrosamines at the listed wavelengths. To avoid any interference from the decomposition reaction of nitrous acid, the nitrosation kinetics of Mab, Maib, 2-Aza, and 3-Aza were determined by the initial rate method. For each set of conditions, 3–5 replicate experiments were performed, in each of which a straight line was fitted by the least-squares method to the absorbance-time data for at most 2% of the total reaction (the fit was good enough in each case to provide assurance that deviation from the true tangent to the curve at $t = 0$ was negligible [18,19]). The initial reaction rate v_0, in mol $dm^{-3}\ s^{-1}$, was calculated as the mean of the slopes of these lines, divided by $\Delta\epsilon$ [$= \epsilon$(nitrosamine) $- \epsilon$(nitrite) $\approx \epsilon$(nitrosamine)]. Since no data for the ϵ of pure NIca and NPhGly were available, and since the nitrosation of Ica and PhGly were considerably faster than those of the other substrates, the kinetics of these reactions were determined by Guggenheim's method [19,20].

Table I Molar Absorption Coefficients of the Nitrosatable Substrates and the Nitroso Compounds Formed[a]

Compound	ε_{240} $mol^{-1}dm^3cm^{-1}$	ε_{249} $mol^{-1}dm^3cm^{-1}$
NMaib	–	2750 ± 24; 3450 ± 35
NMab	–	3938 ± 38
N-2-Aza	7220 ± 123	–
N-3-Aza	–	3823 ± 35
Maib	–	0.75 ± 0.08
Mab	–	1.2 ± 0.1
2-Aza	2.58 ± 0.2; 2.13 ± 0.2	–
3-Aza	–	10.8 ± 0.5; 12.3 ± 0.6

[a] When two values for ε figure, the first corresponds to pH = 1 and the second to pH = 4. If only one value figures it is the same for both pHs.

RESULTS AND DISCUSSION

Plots of reaction rate against pH were bell-shaped for all the amino acids studied, with negligible reaction at pH > 4.5 (Fig. 1). Experiments to investigate the influence of amino acid concentration showed first-order behavior in all cases:

$$v_0 = a\,[AA]_0 \tag{3}$$

where v_0 is the initial rate and $[AA]_0$ is the total initial concentration of amino acid; Table II lists the values of a obtained for Mab and Maib at various pH values and nitrite concentrations. For all the substrates, plots of $v_0/[nit]_0$ against $[nit]_0$ are linear with non-zero ordinates at the origin, showing the involvement of processes that were first- and second-order with respect to nitrite:

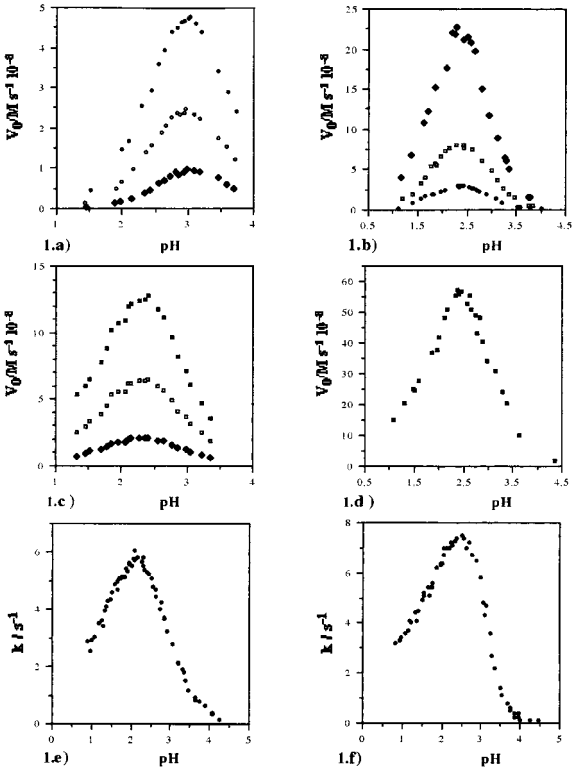


Figure 1 Dependence of the rate of nitrosation of amino acids on the acidity of the medium. I = 0.5 M, T = 298 K: a) $[Mab]_0 = 0.2$ M, $[nit]_0 = 8.0 \times 10^{-3}$ (■), 6.4×10^{-3} (□), and 3.6×10^{-3} (◆); b) $[Maib]_0 = 0.1$ M, $[nit]_0 = 8.0 \times 10^{-3}$ (◆), 5.0×10^{3} (□), and 3.0×10^{-3} (■); c) $[2\text{-Aza}]_0 = 0.1$ M, $[nit]_0 = 4.0 \times 10^{-3}$ (■), 2.8×10^{-3} (□), and 1.6×10^{-3} (◆); d) $[3\text{-Aza}]_0 = 0.1$ M and $[nit]_0 = 5.0 \times 10^{-3}$; e) $[Ica]_0 = 5.0 \times 10^{-5}$ M and $[nit]_0 = 1.0 \times 10^{-3}$ M; and f) $[PhGly]_0 = 5.0 \times 10^{-5}$ M and $[nit]_0 = 1.0 \times 10^{-3}$ M.

$$v_0 = a[AA]_0(b[nit]_0 + c[nit]_0^2) \tag{4}$$

The results are compatible with those of previous studies [8,9] and with the mechanism shown (for Maib) in Scheme I, which, if the right-hand side of step 1 is ignored (in view of the results of Fig. 1), implies the rate equation

$$v_0 = \varphi\frac{[AA]_0[nit]_0^2[H^+]_0^2}{(K_1 + [H^+]_0)(K_a + [H^+]_0)^2} + \eta\frac{[AA]_0[nit]_0[H^+]_0^2}{(K_1 + [H^+]_0)(K_a + [H^+]_0)} + \xi\frac{[AA]_0[nit]_0[H^+]_0}{(K_1 + [H^+]_0)(K_a + [H^+]_0)} \tag{5}$$

where $\varphi = k_b\tilde{K}_I'K_3$, $\eta = k_a\tilde{K}_I'K_2$, and $\xi = k_cK_2K_4K_5(K_I - \tilde{K}_I')$. Table III lists the values of φ, η, ξ, and K_I obtained by fitting eq. (5) to the experimental data using a nonlinear optimization algorithm based on Marquardt's method [21,22]; the value of K_a was taken from the literature [23]. In support of the adequacy of eq. (5) and Scheme I, the pK_I so obtained agree well with the directly measured values also shown in Table III.

The results listed in Table III show that all the substrates are nitrosated predominantly by N_2O_3. Nitrosation by $NO^+/NO_2H_2^+$ is certainly significant for Ica and PhGly, but the large standard deviations of η for Mab, Maib, 2-Aza, and 3-Aza prevent any conclusion as to the importance of this route for these substrates. *N*-Nitrosation via the carboxyl group is significant for both the azetidine carboxylic acids, for one of the acyclic amino acids but not for the other, and for one of the aromatic amino acids but not the other.

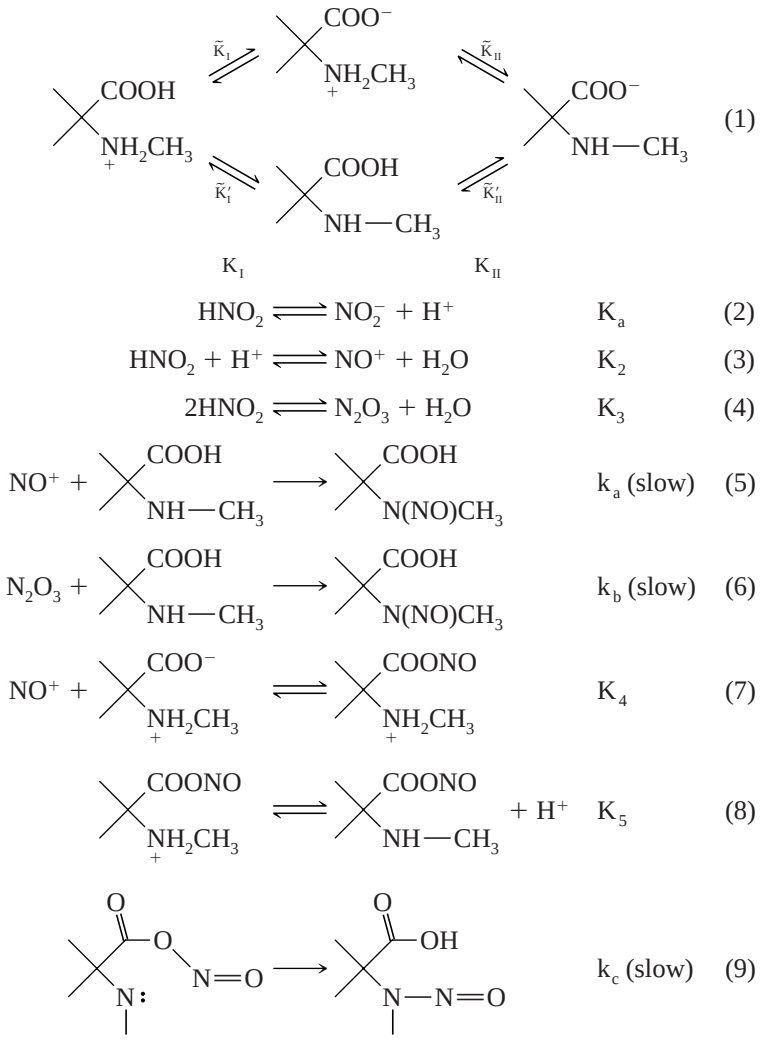
To investigate the influence of temperature on the three reaction paths, the values of φ, η, and ξ for Maib, 2-Aza, and Ica (*i.e.*, for one member of each of the three structural groups considered) were determined for a range of different temperatures (Table IV). The kinetic significance of these parameters, and the validity of the optimization procedure by which they were calculated, are confirmed by their conforming well to Arrhenius' equation (Fig. 2 shows the results for Ica); the path-specific activation parameters so obtained are listed in Table V. The salient feature of this table is that all the entropies of activation are negative, indicating that for all three groups of substrates the transition states of all three routes are highly ordered. Nitrosation by N_2O_3, for example, probably involves the transition state illustrated, for Maib in Scheme II.

If the microscopic constant $\tilde{K}_I'$ is assumed equal to the acidity constant K_e of an ester of the amino acid [26], then we may write $\varphi = k_bK_eK_3$ and $\eta = k_aK_eK_2$.

Table II Values of the Parameter a (Eq. (3)) for Methylaminobutyric Acid, Mab, and Methylaminoisobutyric Acid, Maib

	10^{-7} a, s^{-1}			
Amino Acid	pH	$[nit]_0$ 8×10^{-3} M	pH	$[nit]_0$ 5×10^{-3} M
Mab	2.31	1.40 ± 0.04	1.75	0.136 ± 0.007
	3.38	2.09 ± 0.05	3.20	1.00 ± 0.02
	3.83	1.16 ± 0.01	3.83	0.473 ± 0.011
Maib	2.40	24.6 ± 0.9	2.40	10.5 ± 0.3

For Mab, Maib, 2-Aza, and 3-Aza the unreliability of the value of η prevents calculation of k_a from the second of these expressions, but Table VI lists the values of k_b calculated from φ using published values of K_2 and K_3 [27,28] and the K_e values of their methyl esters, which are also shown. For Ica and PhGly, no experimental values of K_e are available, the methyl ester of PhGly, for example, is too poorly soluble to work with [29], but since the acidity constants of the methyl esters of amino acids are related to those of the parent amino acids by a Taft correlation [30] ($pK_a - pK_e \approx 2$ [6,31,32]), and since the pK_a of



Scheme I

Table III Optimized Values of the Parameters φ, η, and ξ of Eq.(5) Corresponding to Nitrosation by N_2O_3, by $NO^+/NO_2H_2^+$ and by Internal Migration of the NO Group, Respectively (see Scheme I). T = 298 K

Amino Acid	$\varphi \times 10^4$ $dm^3mol^{-1}s^{-1}$	$\eta \times 10^6$ $dm^3mol^{-1}s^{-1}$	$\xi \times 10^8$ s^{-1}	$K_I \times 10^3$ mol dm^{-3}	pK_I	$pK_{I\,exp}$	$pK_{II\,exp}$
Mab	0.126 ± 0.002	[b]	NS	(6.0 ± 0.7)10^{-2}	4.22	4.03[a]	10.78[a]
Maib	7.0 ± 0.3	[b]	12.5 ± 4.3	11.1 ± 0.8	1.96	2.20[a]	10.75[a]
2-Aza	18.3 ± 0.6	[b]	134 ± 40	9.53 ± 0.95	2.02	2.01 [24]	10.21 [24]
3-Aza	6.86 ± 0.30	[b]	110 ± 25	0.640 ± 0.124	3.19	3.20 [14]	10.30 [14]
Amino Acid	φ $dm^3mol^{-1}s^{-1}$	η $dm^3mol^{-1}s^{-1}$	ξ s^{-1}	$K_I \pm 10^3$ mol dm^{-3}	pK_I	$pK_{I\,exp}$	
Ica	3026 ± 99	10.4 ± 2.0	1.17 ± 0.09	54.5 ± 0.21	1.26	–	–
PhGly	1254 ± 303	24.7 ± 4.3	NS	9.3 ± 2.8	2.03	1.83 [14]	4.4 [25]

NS: Not significantly different from zero.

[a] Measured by us.

[b] Vaules of η for Mab, Maib, 2-Aza, and 3-Aza exhibited large standard deviations, and have been considered unreliable.

PhGly is 4.4 [25], then the pK_e's must be about 2.4. This value of pK_e implies values of k_a and k_b of the order of 10^{10} and 10^8 dm^3 mol^{-1} s^{-1}, respectively. Thus k_b for Ica and PhGly is of the same order as for 2-Aza and 3-Aza; these values are in the range for diffusion-controlled reactions [33], and are similar to the corresponding rate constants for sarcosine and proline [8], and to those for pipecolic, nipecotic, and isonipecotic acids (Pip, Npa, and Ipa, respectively) and their esters ethylpipecolate, EPip, ethylnipecotate, ENpa, and ethylisonipecotate, EIpa [34] (Table VII). The values of k_b for Mab and Maib are one or two orders of magnitude smaller. The estimated order of magnitude of k_a for Ica and PhGly, like that of k_b, is the same as for sarcosine and proline [8] and for ENpa and EIpa [34] (Table VII).

Under the same assumption as above, that $\tilde{K}_I'$ is approximately equal to the acidity constant K_e of an

Table IV Optimized Values of the Parameters φ, η, and ξ of Eq.(5) for Methylaminoisobutyric Acid, Azetidine-2-Carboxylic Acid, and Indoline Carboxylic Acid at Various Temperatures

T, K	$10^4 \times \varphi$ $dm^3mol^{-1}s^{-1}$	$10^6 \times \eta$ $dm^3mol^{-1}s^{-1}$	$10^8 \times \xi$ s^{-1}	$10^3 \times K_I$ mol dm^{-3}
Methylaminoisobutyric Acid				
296.0	3.29 ± 0.14	[a]	4.8 ± 3.0	5.18 ± 0.61
298.0	5.41 ± 0.18	[a]	8.35 ± 3.50	8.48 ± 0.54
300.0	5.68 ± 0.38	[a]	[a]	7.37 ± 0.71
302.0	6.65 ± 0.22	[a]	11.46 ± 3.37	6.87 ± 0.38
304.0	7.68 ± 0.32	[a]	15.66 ± 5.72	6.42 ± 0.47
Azetidine-2-Carboxylic Acid				
292.0	6.11 ± 0.25	[a]	7.8 ± 0.8	7.1 ± 0.3
294.0	7.33 ± 0.34	[a]	9.3 ± 1.7	9.4 ± 0.5
295.0	8.22 ± 0.44	[a]	11.1 ± 2.2	9.4 ± 0.5
296.0	8.67 ± 0.33	[a]	12.5 ± 1.7	9.5 ± 0.4
298.0	11.55 ± 0.90	[a]	13.4 ± 4.0	9.53 ± 1.4
299.0	12.54 ± 0.66	[a]	16.3 ± 3.3	11.2 ± 0.7
300.0	13.29 ± 0.33	[a]	17.5 ± 1.7	10.7 ± 0.3
Indoline Carboxylic Acid				
283.0	1480 ± 307	3.71 ± 2.4	0.23 ± 0.07	4.71 ± 1.10
287.0	1755 ± 88	4.48 ± 1.21	0.16 ± 0.05	4.83 ± 0.34
290.5	1962 ± 400	[a]	0.22 ± 0.04	4.39 ± 0.17
294.5	2591 ± 256	8.88 ± 1.52	0.46 ± 0.08	5.34 ± 0.64
298.0	3026 ± 99	10.39 ± 2.00	1.17 ± 0.09	5.45 ± 0.21

[a] Since these values exhibited large standard deviations, they have been considered unreliable.

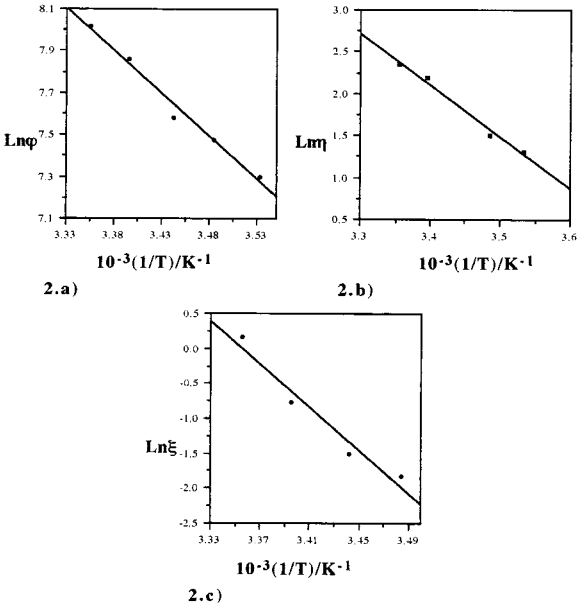


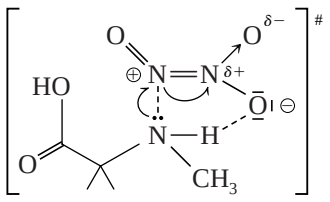
Figure 2 Temperature-dependence of the optimized parameters φ, η, and ξ of eq. (5) corresponding to nitrosation of indoline carboxylic acid by N_2O_3, by $NO^+/NO_2H_2^+$, and by internal migration of the NO group, respectively (see Scheme I).

ester of the amino acid [26], we may also write $\xi = k_c K_2 K_4 K_5 (K_I - K_e)$. For all the amino acids studied except PhGly, the measured or estimated value of K_e is negligible in comparison with K_I, so that $\xi \approx k_c K_2 K_4 K_5 K_I$; in the case of PhGly, the estimated value of K_e is about half that of K_I. In either case the expression for ξ has too many unknowns for unequivocal analysis of the observed variation. Nevertheless, a Taft correlation for K_5 similar to that for K_e suggests that the difference in ξ between Ica and the azetidines and acyclic amino acids is essentially due to differences in K_5. The remaining differences in ξ within and between groups (*i.e.*, ξ(Maib)$-\xi$(Mab), ξ(Ica)$-\xi$(PhGly), and ξ(azetidines)$-\xi$(acyclics)) may be explained, in keeping with the observed negative entropies of activation for this route, in terms of the effect on k_c of differences as regards the facility with which the required highly ordered transition state structures can be attained and maintained, as follows.

Table V Route-Specific Activation Parameter for the Nitrosation of Amino Acids

Nitrosation by	$\Delta H^{\#}$ kJ mol^{-1}	$-\Delta S^{\#}$ J K^{-1} mol^{-1}
Methylaminoisobutyric Acid		
N_2O_3[a]	68 ± 6	78 ± 4
Migration[c]	98 ± 17	53 ± 6
Azetidine-2-Carboxylic Acid		
N_2O_3[a]	72 ± 5	60 ± 3
Migration[c]	71 ± 5	120 ± 7
Indoline Carboxylic Acid		
N_2O_3[a]	32 ± 3	72 ± 4
$NO^+/NO_2H_2^+$[b]	49 ± 5	61 ± 3
Migration[c]	125 ± 20	175 ± 23

[a,b,c] Values obtained from Arrhenius plots of φ, η, and ξ, respectively.



Scheme II Probable transition state structure in the nitrosation of methylaminoisobutyric acid by N_2O_3.

The fact that *N*-nitrosation via the carboxyl group is negligible for Mab may be attributed to the length of the flexible butyric acid chain, which means (a) that there is little probability of a CO-borne nitroso group becoming contiguous with the amino nitrogen and (b) that even if the two nitrogen atoms do approach each other, the transition state involved in NO exchange in this molecule is a 7-membered ring, i.e., a relatively unstable structure [35]. By contrast, contiguity of the nitroso and amino nitrogens in C-nitroso-Maib can be achieved by rotation about a single C—C bond, and the transition state involved in NO exchange is a relatively stable 5-membered ring (Fig. 3). In fact, the value of ξ for Maib is of the same order as the 8.4×10^{-7} s^{-1} found for the structurally similar amino acid sarcosine [9], which

Table VI Values of the Rate Constants k_b Corresponding to Attack on Amino Acids by N_2O_3 (See Scheme I), Together with the K_e Values used to Calculate k_b from φ (See Text)

Amino Acid	K_e[a]	k_b dm^{3}mol^{-1}s^{-1}
Mab	1.66×10^{-9}	2.6×10^6
Maib	4.27×10^{-9}	5.4×10^7
2-Aza	3.16×10^{-9}	1.2×10^8
3-Aza	1.01×10^{-9}	2.3×10^8

[a] Measured by us for the methyl esters.

Table VII Values of the Rate Constants k_b and k_a Corresponding to Attack on Amino Acids with 6-Membered Rings by N_2O_3 and $NO^+/NO_2H_2^+$, Respectively [34]

Amino Acid	k_b $dm^3mol^{-1}s^{-1}$	k_a $dm^3mol^{-1}s^{-1}$
Pip	0.17×10^8	[a]
Npa	1.12×10^8	[a]
Ipa	2.11×10^8	[a]
EPip	1.85×10^8	[a]
ENpa	3.66×10^8	6.18×10^{10}
EIpa	0.23×10^8	4.49×10^9

[a] Since these values showed large standard deviations, they are considered unreliable.

likewise forms a 5-membered ring during NO exchange (Fig. 3).

For the 2-Aza two facts are known: (i) compared with other nitroso amino acids, in this particular case the corresponding nitroso compound crystallizes preferentially in anti conformation and (ii) the energy barrier for the interconversion of the isomers (Fig. 4) is by far smaller for the 2-Aza than for any other amino acid [17].

If we acknowledge for the transition state a structure similar to that of the *N*-2-Aza, the $\Delta H^{\#}$ value corresponding to the intramolecular NO migration will also be substantially smaller in the nitrosation of 2-Aza. Consequently, the ξ value should be higher as, in fact, has been observed (see Table III).

In the nitrosation of 3-Aza, the mechanism will be analogous, probably with greater tension in the transition state (see Fig. 5).

Although the transition state structure for PhGly is, as for Ica, a relatively stable 5-membered ring (Fig. 6), the probability of its formation must be lower than for Ica because of the flexibility of the extracyclic moiety of PhGly. This explains why there is no significant nitrosation by PhGly by this route.

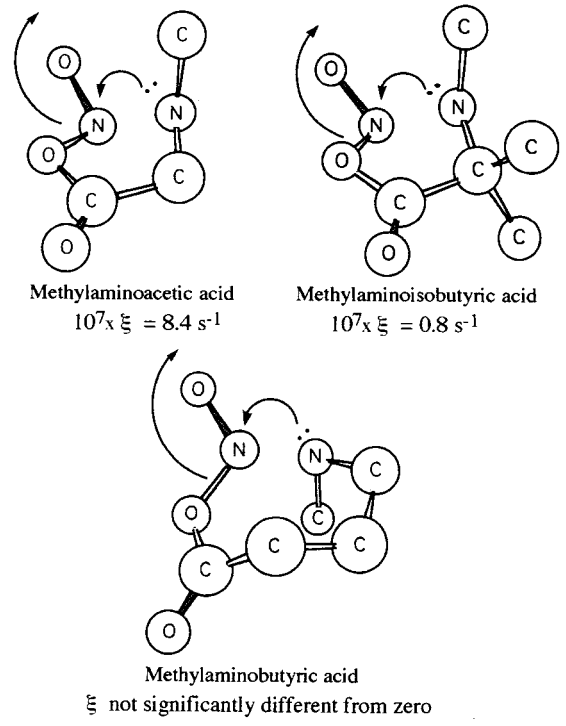


Figure 3 Structure required for intramolecular NO transfer: acyclic amino acids.

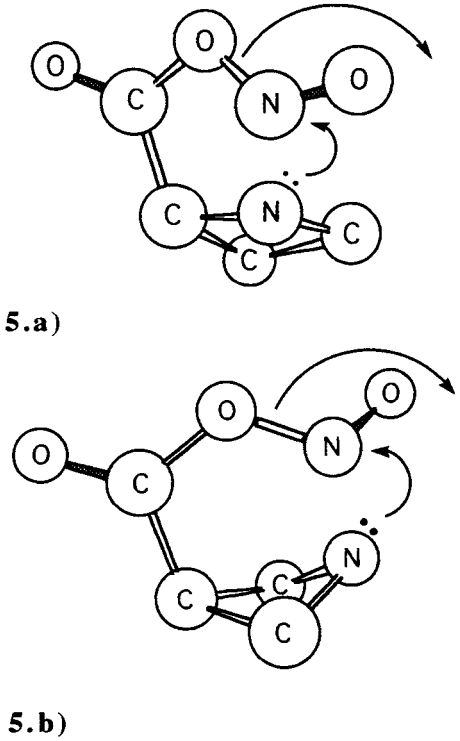


Figure 5 Structures required for intramolecular NO transfer: a) azetidine-2-carboxylic acid and b) azetidine-3-carboxylic acid.

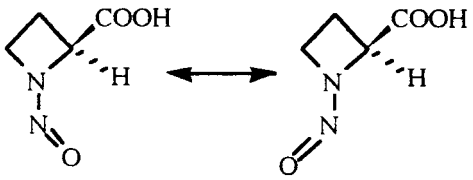


Figure 4 Interconversion syn-anti in the *N*-nitrosoazetidine-2-carboxylic acid.

CONCLUSIONS

The above results on the nitrosation kinetics of six cyclic and acyclic amino acids with their —COOH and amino groups in different relative positions and electronic environments allow the following conclusions.

Amino acids can be nitrosated via three kinetically distinguishable routes: direct *N*-nitrosation by dinitrogen trioxide; direct *N*-nitrosation by nitrosonium or nitrosacidium ions; and indirect *N*-nitrosation by *C*-nitrosation of the carboxyl group by $NO^+/NO_2H_2^+$ followed by intramolecular migration of the NO group to the amino nitrogen atom.

The predominant route is nitrosation by N_2O_3 via a highly ordered transition state.

N-Nitrosation via the carboxyl group is only significant if the size or rigidity of the substrate facilitates formation of a transition state with a 5- or 6-membered ring.

The authors wish to thank the Commission of the European Communities for supporting the research reported in this article as part of a project on nitrosation mechanisms (Contract no. 93CVVF1-610-0). Thanks are also due to the Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT) for its support of research on precursor mechanisms of carcinogenesis by *N*-nitroso compounds (Project no. ALI389/90), and to the Spanish Ministerio de Educación y Ciencia for financial assistance to R. G. during his Ph.D. studies.

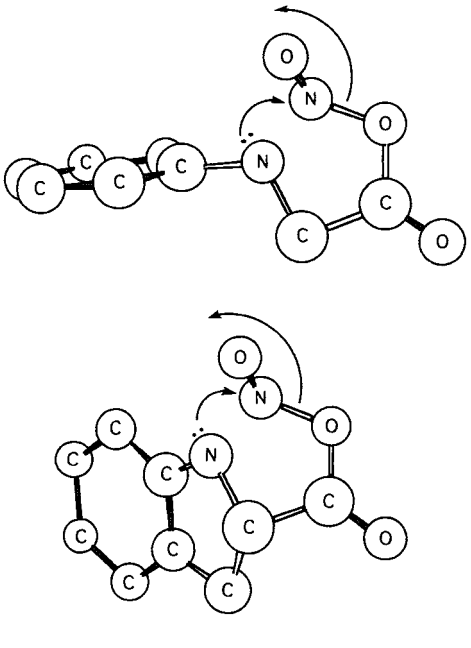


Figure 6 Structures required for intramolecular NO transfer: a) phenylaminoacetic acid and b) indoline carboxylic acid.

BIBLIOGRAPHY

1. S. S. Mirvish, *Cancer Lett.*, **93,** 17 (1995).
2. R. N. Loeppky and C. J. Michejda, Eds., *Nitrosamines and Related N-Nitroso Compounds. Chemistry and Biochemistry,* ACS Symposium Series 553, Am. Chem. Soc., Washington, 1994.
3. W. Lijinsky, *Chemistry and Biology of N-Nitroso Compounds*, Cambridge Monographs on Cancer Research, Cambridge, 1992, p. 16.
4. M. C. Archer, *Catalysis and Inhibition of N-nitrosation reactions* in *N-Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer,* IARC Sci. Publ. No. 57, International Agency for Research on Cancer, Lyon, 1984, pp. 263–274.
5. H. Bartsch, H. Ohshima, and B. Pignatelli, *Mutat. Res.*, **202,** 307 (1988).
6. S. S. Mirvish, J. Sams, T. Y. Fan, and S. R. Tannenbaum, *J. Natl. Cancer,* **51,** 1833 (1973).
7. J. Casado, A. Castro, J. R. Leis, M. A. López Quintela, and M. Mosquera, *Monatsh. Chem.*, **114,** 639 (1983).
8. J. Casado, A. Castro, J. R. Leis, M. Mosquera, and M. E. Peña, *J. Chem. Soc., Perkin Trans. 2,* 1859 (1985).
9. R. Gil, J. Casado, and C. Izquierdo, *Int. J. Chem. Kinet.*, **26,** 1167 (1994).
10. L. Fowden and M. H. Richard, *Biochem. Biophys. Acta*, **71,** 459 (1963).
11. E. J. Hewitt and B. A. Notton, *Phytochemistry*, **6**, 1329 (1967).
12. D. J. Cummings, V. A. Chapman, S. S. Delong, and L. Mondale, *J. Virol.*, **1,** 193 (1967).
13. H. M. Berman, E. L. McCandy, J. W. Burgner, II, and R. L. Van Etten, *J. Am. Chem. Soc.*, **91,** 6177 (1969).
14. A. G. Anderson, Jr., and R. Lok, *J. Org. Chem.,* **37,** 3953 (1972).
15. J. Casado, *Nitrosation Reactions,* Invited Lecture in *FRIS '94, Fast Reactions in Solution,* Royal Society of Chemistry, Univ. de Burgos, 1994.
16. W. Lijinsky and H. W. Taylor, *Z. Kresbsforsch,* **89,** 31 (1977).
17. W. Lijinsky, L. Keefer, and J. Loo, *Tetrahedron,* **26,** 5137 (1970).
18. J. Casado, M. A. López Quintela, and F. M. Lorenzo Barral, *J. Chem. Educ.*, **63,** 450 (1986).
19. K. A. Connors, *Chemical Kinetics*, VCH Publishers, New York, 1990, Chap. 2.
20. E. A. Guggenheim, *Phil. Mag.*, **2,** 538 (1926).
21. D. W. Marquardt, *J. Soc. Ind. Appl. Math.*, **11,** 431 (1963).
22. J. Casado, M. Mosquera, A. Rivas, M. F. Rodríguez Prieto, and J. Santaballa, *Computers Chem.*, **7,** 209 (1983).

23. J. Tummavuori and P. Lumme, *Acta Chem. Scand.,* **22,** 2003 (1968).
24. R-S. Tsai, B. Testa, N. El Tayar, and P-A. Carrupt, *J. Chem Soc., Perkin Trans. 2,* 1797 (1991).
25. *Handbook of Chemistry and Physics,* CPR Press, Boca Ratón, Florida, 52th ed., 1971–72.
26. H. Rossoti, *The Study of Ionic Equilibria*, Longman, New York, 1978, p. 28.
27. G. Y. Markovits, S. E. Schwartz, and L. Newman, *Inorg. Chem.,* **20,** 445, (1981).
28. H. Schmid, *Chem. Ztg./Chem. Apparatur,* **86,** 809 (1962).
29. *The Merck Index,* Merck & Co. Inc., 9th ed., 1976.
30. R. W. Taft, Jr., in *Steric Effects in Organic Chemistry,* M.S. Newman, Ed., Wiley, New York, 1963, Chap. 13.
31. J. P. Greenstein and M. Winitz, *Chemistry of Amino Acids,* Wiley, New York, 1961, pp. 487 and 486.
32. J. T. Edsall and M. H. Blanchard, *J. Am. Chem. Soc.,* **55,** 2337 (1933).
33. J. H. Ridd, *Adv. Phys. Org. Chem.,* **16,** 1 (1978).
34. R. Gil, *Ph.D. Thesis* (Sp), Univ. de Salamanca, 1996, unpublished results.
35. G. Nata and M. Farina, *Stereochimica: Molecole in 3 D,* Mondadori, Milán, 1968, Chap. 1.