Monatshefte für Chemie 115, 1047—1057 (1984)

Monatshefte für Chemie
Chemical Monthly

Kinetic Studies on the Formation of N-Nitroso Compounds X. The Nitrosation of *N*-Methylacetamide and Its Differences with Respect to the Nitrosation of Amines

Julio Casado[a], **Albino Castro**[b], **J. Ramón Leis**[b], **Manuel Mosquera**[b], and **M. Elena Peña**[b,*]

[a] Departamento de Química Física, Facultad de Química, Universidad, Salamanca, España

[b] Departamento de Química Física, Facultad de Química, Universidad, Santiago de Compostela, España

(Received 18 October 1983. Accepted 9 February 1984)

The kinetics of the nitrosation of *N*-methylacetamide have been studied using spectrophotometry. Significant differences with respect to the mechanism of nitrosation of amines were observed: the absence of catalysis by halides, the existence of general basic catalysis by acetate and its chlorated derivatives obeying *Brønsted's* law (with $\beta = 0.49$), and the primary isotopic effect (with a ratio of 7.9 between the rate constants for the elementary process in H_2O and D_2O). All this indicates that the slow step of the mechanism must be the transfer of a proton from the protonated nitrosamide to the reaction medium.

(Keywords: Kinetics of nitrosation; N-Nitrosation of N-methylacetamide; Proton transfer)

Kinetische Untersuchungen zur Bildung von N-Nitroso-Verbindungen, 10. Mitt.: Die Nitrosierung von N-Methylacetamid und die Unterschiede in bezug auf die Nitrosierung von Aminen

Die Kinetik der Nitrosierung von *N*-Methylacetamid wurde mittels Spektrophotometrie untersucht. Es wurden signifikante Unterschiede zum Mechanismus der Nitrosierung von Aminen beobachtet: die Abwesenheit einer Katalyse durch Halogenide, die Existenz einer generellen basischen Katalyse durch Acetat und dessen chlorierten Derivaten unter Übereinstimmung mit dem *Brønsted*schen Gesetz (mit $\beta = 0{,}49$) und einem beobachtbaren Isotopeneffekt (mit einem Verhältnis von 7,9 zwischen den Geschwindigkeitskonstanten in H_2O und D_2O). All das zeigt an, daß der geschwindigkeitsbestimmende Schritt im Mechanismus der Transfer eines Protons vom protonierten Nitrosamid zum Reaktionsmedium sein muß.

Introduction

Although abundant literature exists concerning the mechanisms involved in the nitrosation of amines (see, for example, Refs. [1, 2]), the same cannot be said regarding the nitrosation of amides and ureas, in spite of the unquestionable interdisciplinary interest due to the fact that some of the nitrosation products are potentially carcinogenic[3–5] whereas others have been used against cancer[6, 7]. However, the discovery that, unlike nitrosamines, carcinogenic nitrosamides generally induce tumours at the site of contact[3] has caused greater interest in their reaction mechanisms. It has become increasingly apparent that there are significant differences between nitrosated amines and amides with regard to their effects and their mechanisms of formation.

Until 1974 the commonly accepted reaction mechanism for the nitrosation of amides[8, 9] was that proposed by *Bruylants* et al.[10, 11], in which, by analogy with the amine case, the rate controlling step is the attack on the amide by the nitrosating agent, which can be either a nitrosyl halide[10] or the NO^+ ion itself[12]. However, a number of subsequent discoveries have thrown doubt on the validity of this analogy. During a study on the decomposition of *N-n*-butyl-*N*-nitrosoacetamide in aqueous media, *Berry* and *Challis* found that the denitrosation reaction which takes place simultaneously with deamination is not susceptible to nucleophilic catalysis by Cl^- and involves protonation of nitrogen as its slow step[13]. They concluded that, contrary to the opinion then current[10], the nitrosation of amides would not be susceptible to nucleophilic catalysis either. The same conclusion was reached in 1980 by *Hallett* and *Williams,* who considered that in the nitrosation of methylurea the slow step might be the transfer of a proton to the medium[14].

Complementary results have been reported by *Yamamoto* et al., who found that organic acid buffer solutions, which had no effect on the nitrosation of amines, did catalyse the nitrosation of ureas[15]. This has been checked for methylurea by *Casado* et al., who found buffers composed of acetic acid or its derivatives to exert basic catalysis satisfying *Brønsted*'s equation, and used isotopic effect data to confirm that the slow step is the loss of a proton from the protonated nitrosamide intermediate[16].

With the aim of helping to clarify the differences between the two kinds of mechanism, the present article reports a detailed investigation of the nitrosation of *N*-methylacetamide (*MAC*), a simple amide whose nitrosation product, *N*-methyl-*N*-nitrosoacetamide is carcinogenic[17]. Apart from its biological value (the amide group abounds in living

beings), this study is also of interest since it allows comparison with the mechanism of nitrosation of ureas.

Experimental

All the reagents used were Merck, p.a., except the *N*-methylacetamide and mono- and dichloroacetic acids, which were Merck p.s. The *N*-methylacetamide was double distilled and recrystallized from the molten state (m.p. 30 °C). The other reagents were dried and used without further purification. The concentrations of the carboxylic acid solutions used were determined by titration with NaOH. The 99.77% D_2O heavy water used was supplied by the Spanish Nuclear Energy Board.

Reaction rates were measured using spectrophotometry to follow the appearance of the nitroso compound by recording the increase in absorbance at 249 nm ($\varepsilon = 4410 \pm 40\, M^{-1}\,cm^{-1}$) and 265 nm ($\varepsilon = 1560 \pm 10\, M^{-1}\,cm^{-1}$), which are isosbestic points for nitrous acid. Because the reaction is slow and there is competition from the decomposition of nitrous acid, the initial rate method was used and the reaction was never followed further than 1%. All experiments were duplicated, and the values obtained from the duplicated for the reaction rates never differed by more than 3%.

Absorbance measurements were taken on Coleman 55 and Uvikon 820 spectrophotometers and *pH* measurements using a Radiometer 82 *pH*-meter. All experiments were carried out at 25 °C and at an ionic strength of 0.2 *M* using appropriate solutions of $NaClO_4$.

N-methyl-*N*-nitrosoacetamide was found to be thermally and photochemically stable under the working conditions employed.

Results and Discussion

At a concentration of nitrite (*Nit*) of $4.05 \cdot 10^{-3}\, M$ and *pH* 2.17 the influence of the concentration of *MAC* on the initial rate of nitrosation was studied by varying [*MAC*] between 0.04 and 0.4 *M*. The results, plotted in Fig. 1, show that the order of the reaction with respect to the amide is one:

$$v_0 = a\,[MAC] \tag{1}$$

with $a = (1.25 \pm 0.02) \cdot 10^{-7}\,s^{-1}$.

Next, the influence of the concentration of nitrite was investigated by varying it between $1.52 \cdot 10^{-3}$ and $1.52 \cdot 10^{-2}\, M$ at *pH* 2.23 and $[MAC] = 0.586\, M$. The results are shown in Fig. 2, where the order of the reaction with respect to nitrite can be seen to be one over the whole range studied:

$$v_0 = b\,[Nit] \tag{2}$$

with $b = (1.55 \pm 0.01) \cdot 10^{-5}\,s^{-1}$.

The influence of acidity on the rate of reaction was studied at

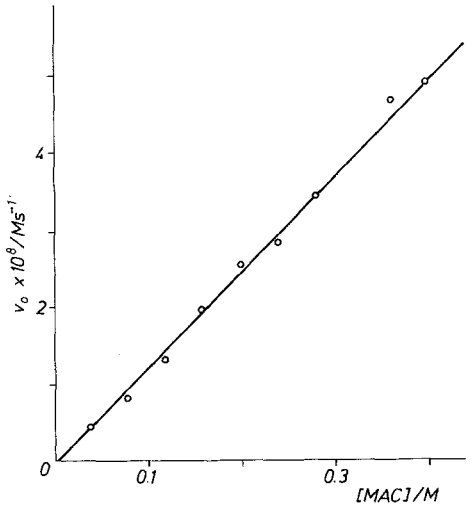

Fig. 1. Dependence of the initial rate of nitrosation of MAC upon the concentration of MAC at pH 2.17, $[Nit] = 4.05 \cdot 10^{-3} M$, $\mu = 0.2 M$

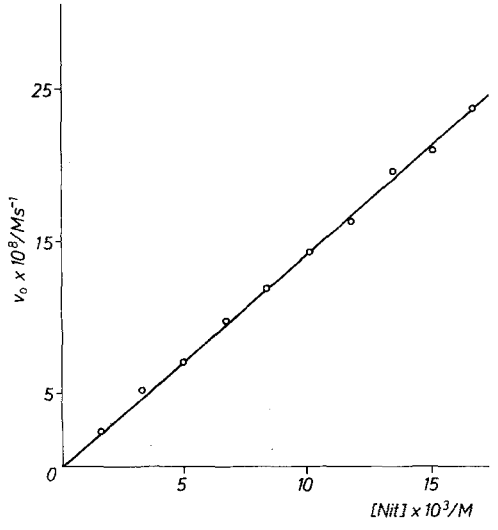

Fig. 2. Dependence of the initial rate of nitrosation of MAC upon the concentration of nitrite at pH 2.23, $[MAC] = 0.586 M$, $\mu = 0.2 M$

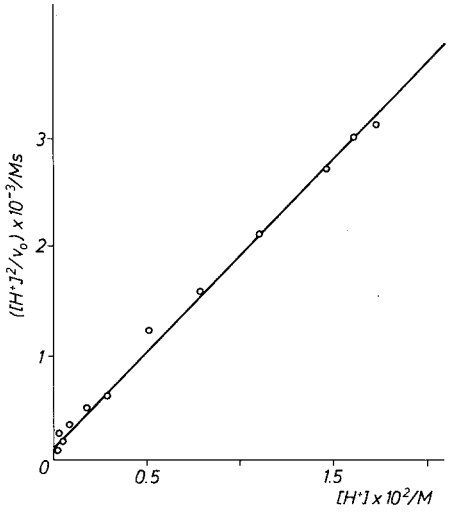

Fig. 3. Influence of acidity on the rate of nitrosation of MAC at $[MAC] = 0.401\,M$, $[Nit] = 2.53 \cdot 10^{-3}\,M$, $\mu = 0.2\,M$

$[MAC] = 0.401\,M$ and $[Nit] = 2.53 \cdot 10^{-3}\,M$ by varying the pH between 1.76 and 3.39. The relationship observed:

$$v_0 = c\,\frac{[\mathrm{H}^+]^2}{d + [\mathrm{H}^+]} \tag{3}$$

is linearized in Fig. 3 as

$$\frac{[\mathrm{H}^+]^2}{v_0} = \frac{d}{c} + \frac{[\mathrm{H}^+]}{c} \tag{4}$$

where $c = (5.9 \pm 0.1) \cdot 10^{-6}\,\mathrm{s}^{-1}$ and $d = (1.3 \pm 0.3) \cdot 10^{-3}\,M$.

When the influence of halides was investigated it was found that, as in the case of methylurea[14,16], the presence of significant quantities (of Br^-, in this case) produced no change whatsoever in the rate of reaction. The absence of nucleophilic catalysis means that the attack on the amide by the nitrosating agent cannot be the rate controlling step; otherwise a catalytic term reflecting the attack by BrNO should be detected. By analogy with the findings for methylurea the step that does control the rate of reaction may be expected to be the loss of a proton from the protonated nitrosamide intermediate. To confirm this, experiments were carried out to prove basic catalysis.

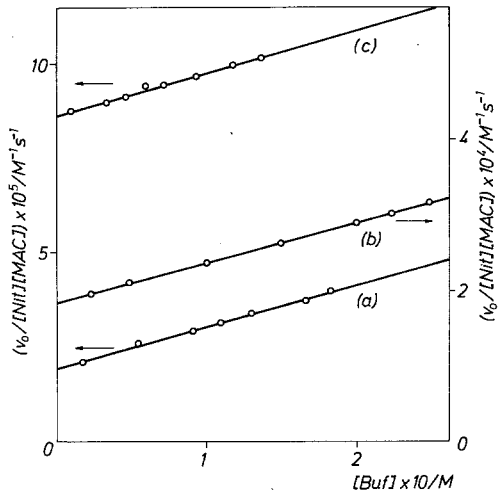

Fig. 4. Dependence of the initial rate of nitrosation of MAC upon the concentration of (a) monochloroacetate buffer at pH 2.31, $[Nit] = 4.11 \cdot 10^{-3} M$, $[MAC] = 0.425 M$, $\mu = 0.2 M$; (b) dichloracetate buffer at pH 1.48, $[MAC] = 0.344 M$, $[Nit] = 7.99 \cdot 10^{-3} M$, $\mu = 0.2 M$; (c) trichloracetate buffer at pH 1.78, $[Nit] = 5.83 \cdot 10^{-3} M$, $[MAC] = 0.132 M$, $\mu = 0.2 M$

The possibility of basic catalysis was investigated by studying the effect of monochloroacetate buffer (*Buf*) on the reaction rate. A linear relationship of the form:

$$\frac{v_0}{[Nit]\,[MAC]} = e + f[Buf] \tag{5}$$

was observed (Fig. 4 a), and the catalytic effect increased with pH suggesting basic catalysis by the monochloroacetate ion. With this assumption (5) may be rewritten in the form

$$\frac{v_0}{[Nit]\,[MAC]} = e\left(1 + g\frac{K}{K + \mathrm{H}^+}[Buf]\right) \tag{6}$$

where K is the acidity constant of monochloroacetic acid. To check this a series of experiments at different values of pH was carried out. The results (Table 1) show that e/f depends linearly on $[\mathrm{H}^+]$, which is consistent with (6). The determined value of $K = 1.8 \cdot 10^{-3} M$ is in agreement with published values. Similar studies were also carried out using acetate, dichloroacetate and trichloroacetate buffers, and in all

Table 1. *Influence of the concentration of monochloroacetate buffer on the rate of nitrosation of MAC at different values of pH [equation (5)]*

pH	$e \cdot 10^5/M^{-1}s^{-1}$	$f \cdot 10^4/M^{-2}s^{-1}$	$(e/f) \cdot 10/M$
2.31	1.93 ± 0.05	1.10 ± 0.04	1.75
2.41	1.65 ± 0.03	1.11 ± 0.03	1.49
2.72	0.69 ± 0.03	0.66 ± 0.03	1.05
2.92	0.33 ± 0.01	0.46 ± 0.01	0.71
2.98	0.28 ± 0.01	0.37 ± 0.01	0.75

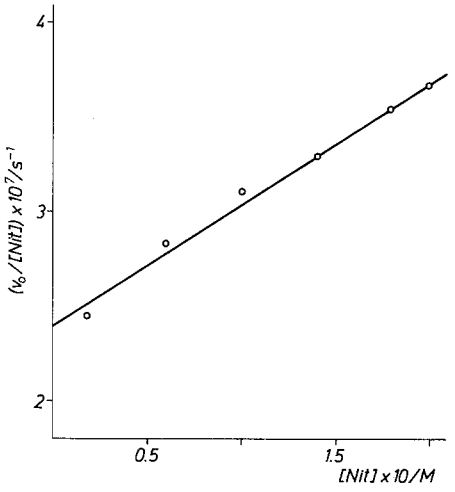

Fig. 5. Dependence of the initial rate of nitrosation of *MAC* upon the concentration of nitrite [equation (7)] at *pH* 3.53, $[MAC] = 0.471$, $\mu = 0.2\,M$

cases basic catalysis was observed (Figs. 4 b and 4 c) and the values of K are in agreement with literature data.

In view of the low concentration of nitrite and the working *pH* used in the experiments described so far, it is not surprising that no second order term reflecting basic catalysis by NO_2^- had been observed. In a series of experiments carried out at *pH* 3.53 [*Nit*] was varied between $2 \cdot 10^{-2}$ and $0.2\,M$. As Fig. 5 shows, the relationship now found was:

$$v_0 = h\,[Nit] + i\,[Nit]^2 \qquad (7)$$

The catalytic effects of the acetic and mono-, di- and trichloracetate buffers comply with *Brønsted*'s equation (Fig. 6), with a slope $\beta = 0.49$

(it is quite common for H_2O to lie off the line[18], and since we are dealing with correlations of free energy the deviation of NO_2^- is also reasonable). The catalysis in question is therefore a general basic catalysis of a reaction whose slow step must be the loss of a proton which, to judge by the value of β, is in the transition state half way between the reagents and products[18]. This is in contrast to the case of methylurea, in which the proton in the transition state is close to the reaction products[16].

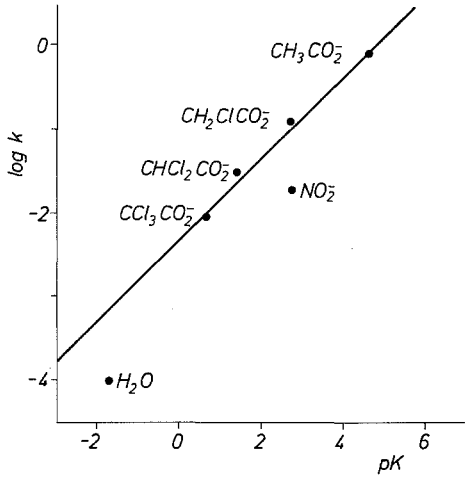

Fig. 6. *Brønsted* plot for the general basic catalysis of the nitrosation of *MAC*

In the light of the results given above, the following reaction mechanism is put forward:

$$\left.\begin{aligned} HNO_2 &\rightleftarrows NO_2^- + H^+ && K_1 \\ HRCO_2 &\rightleftarrows RCO_2^- + H^+ && K_2 \\ HNO_2 + H^+ &\rightleftarrows NO^+ + H_2O && K_3 \\ NO^+ + CH_3NHCOCH_3 &\rightleftarrows CH_3\overset{+}{N}H(NO)COCH_3 && K_4 \end{aligned}\right\} \text{fast}$$

$$\left.\begin{aligned} CH_3\overset{+}{N}H(NO)COCH_3 + H_2O &\rightarrow CH_3N(NO)COCH_3 + H_3O^+ && k_5 \\ CH_3\overset{+}{N}H(NO)COCH_3 + NO_2^- &\rightarrow CH_3N(NO)COCH_3 + HNO_2 && k_6 \\ CH_3\overset{+}{N}H(NO)COCH_3 + RCO_2^- &\rightarrow CH_3N(NO)COCH_3 + HRCO_2 && k_7 \end{aligned}\right\} \text{slow}$$

It should be pointed out that although—for sake of simplicity—the effective nitrosating agent has been assumed to be NO^+, its real identity is actually not known, and cannot be ascertained kinetically because its attack on the amide is not the rate controlling step. Since the various nitrosating agents present are in equilibrium and at concentrations that

are negligible compared with those of NO_2^- and HNO_2, the rate equation deduced from this mechanism is independent of which nitrosating agent is actually involved:

$$v_0 = \frac{[MAC]\,[Nit]\,[H^+]^2}{(K_1 + [H^+])}\left(\alpha + \beta\,\frac{K_1\,[Nit]}{K_1 + [H^+]} + \gamma\,\frac{K_2\,[Buf]}{K_2 + [H^+]}\right) \qquad (8)$$

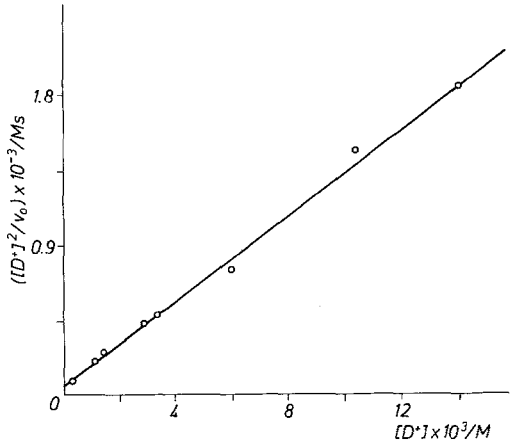

Fig. 7. Influence of pD on the initial rate of nitrosation of MAC at $[MAC] = 0.690\,M$, $[Nit] = 5.79 \cdot 10^{-3}\,M$, $\mu = 0.2\,M$

where $\alpha = K_3 K_4 k_5$, $\beta = K_3 K_4 k_6$ and $\gamma = K_3 K_4 k_7$. This equation agrees with our experimental findings; for low concentrations of nitrite in the absence of buffer it reduces to:

$$v_0 = \alpha\,\frac{[MAC]\,[Nit]\,[H^+]^2}{(K_1 + [H^+])} \qquad (9)$$

The form of this latter equation coincides with the case where the rate controlling step may be as well the attack on the amide by NO^+. This explains the errors of interpretation that have appeared in literature[11, 19, 20].

When equation (9) was fitted to the experimental data using an optimization program[21] based on *Marquardt*'s method[22] the following values were found:

$$\alpha = (5.70 \pm 0.05) \cdot 10^{-3}\,M^{-2}\,s^{-1}$$
$$K_1 = (1.59 \pm 0.09) \cdot 10^{-3}\,M$$

The value of K_1 agrees well with that found by *Tummavuori* and *Lume* by a non-kinetic method[23].

To confirm the mechanism proposed above, the reaction was studied in D_2O to look for the expected primary isotopic effect. The influence of pD on the reaction rate was observed in order to calculate the values of α and K_1 in D_2O ($pD = pH_{\text{measured}} + \Delta pH$, with $\Delta pH = 0.4$[24]). The linearized results are shown in Fig. 7 and the ratios of the values of α and K_1 in H_2O and in D_2O were found to be:

$$\frac{\alpha(H_2O)}{\alpha(D_2O)} = 2.85 \quad \text{and} \quad \frac{K_1(H_2O)}{K_1(D_2O)} = 2.92$$

The latter value agrees with the results of direct measurements[25]. Since $\alpha = K_3 K_4 k_5$, the ratio $\alpha(H_2O)/\alpha(D_2O)$ includes both the effect on k_5 and on the equilibrium constants $K_3 K_4$. When the ratio between the values of $K_3 K_4$ in D_2O and H_2O is taken as 2.79[25] it follows $k_5(H_2O)/k_5(D_2O) = 7.9$ indicating a proton transfer in the rate controlling step. The high value for this ratio also confirms that the transition state is symmetrical[26] as the value of β suggested.

It is now clear that the similarity between the mechanisms of nitrosation of amines and amides is only partial. Although the general reaction scheme is the same for both classes of compound, it is the functional group itself (amino or amido) that determines the rate controlling step.

Acknowledgements

One of the authors (*J. C.*) wishes to thank the Spanish Comisión Asesora de Investigación Científica y Técnica for subsiding the research reported in this article as a part of an investigation into the mechanisms of formation of carcinogenic nitroso-compounds. *J. R. L.* and *M. E. P.* likewise wish to thank the Spanish Ministry of Education and Science for the grants (Formación de Personal Investigador) allowing them to take part in this research.

References

[1] *Williams D. L. H.*, Adv. Phys. Org. Chem. **19**, 381 (1983).
[2] *Ridd J. H.*, Quart. Rev. **15**, 418 (1961).
[3] *Druckrei H., Preussman R., Ivankovic S., Schmahl D.*, Z. Krebsforsch. **69**, 103 (1967).
[4] *Lawley P. D.*, Chemical Carcinogens (*Searle C. E.*, ed.), p. 83. Washington, D.C.: Amer. Chem. Soc. 1976.
[5] *Weisburger J. H.*, N-Nitroso Compounds (*Scanlan R. A., Tannenbaum S. R.*, eds.), p. 305. Washington, D.C.: Amer. Chem. Soc. 1981.
[6] *Hansen H. H., Selawry O. S., Muggia F. M., Walker M. D.*, Cancer Res. **31**, 223 (1971).

[7] *Carter S. K., Schabel F. M., Broder L. E., Johnston T. P.,* Advan. Cancer Res. **16**, 273 (1972).
[8] *Mirvish S. S.,* J. Natl. Cancer Inst. (U.S.) **46**, 483 (1971).
[9] *Mirvish S. S.,* IARC Sci. Publ. **1972**, 104.
[10] *Kezdy F. J., Jaz J., Bruylants A.,* Bull. Soc. Chim. Belg. **67**, 687 (1958).
[11] *Jaz J., Bruylants A.,* Bull. Soc. Chim. Belg. **70**, 99 (1961).
[12] *Bender M. L., Ladenheim M.,* J. Amer. Chem. Soc. **82**, 1895 (1960).
[13] *Berry C. N., Challis B. C.,* J.C.S., Perkin Trans. 2 **1974**, 1638.
[14] *Hallett G., Williams D. L. H.,* J.C.S., Perkin Trans. 2 **1980**, 1372.
[15] *Yamamoto M., Yamada T., Tanimura A.,* J. Food Hyg. Soc. Jap. **17**, 363 (1976).
[16] *Casado J., Castro A., Mosquera M., Rodríguez Prieto M. F., Vázquez Tato J.,* Ber. Bunsenges. Phys. Chem. **87**, 1211 (1983).
[17] *Digenis G. A., Issidorides C. H.,* Bioorg. Chem. **8**, 97 (1979).
[18] *Bell R. P.,* The Proton in Chemistry. London: Chapman and Hall. 1973.
[19] *Mirvish S. S.,* Toxicol. and Appl. Pharmacology **31**, 325 (1975).
[20] *Casado J., Castro A., López Quintela M. A., Cachaza J. M.,* Monatsh. Chem. **110**, 1331 (1979).
[21] *Casado J., Castro A., López Quintela M. A.,* Monatsh. Chem. **112**, 1221 (1981).
[22] *Marquardt D. W.,* J. Soc. Ind. Appl. Math. **11**, 431 (1963).
[23] *Tummavuori J., Lume P.,* Acta Chem. Scand. **22**, 2003 (1968).
[24] *Salomaa P., Schaleger L. L., Long F. A.,* J. Amer. Chem. Soc. **86**, 1 (1964).
[25] *Casado J., Santaballa A.,* to be published.
[26] *Melander L., Saunders W. H., Jr.,* Reaction Rates of Isotopic Molecules. New York: J. Wiley. 1980.