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Reactivity of sulfur nucleophiles with *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide

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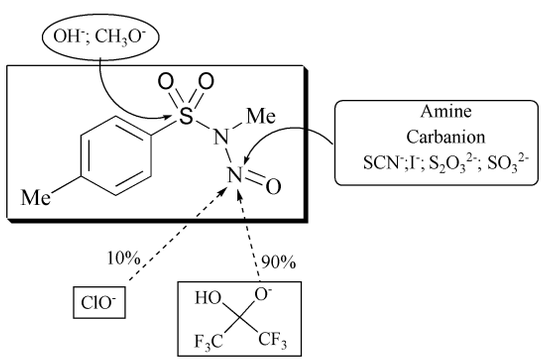
The transfer of the nitroso group from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (MNTS) to cysteine (CYS) and 2-aminoethanethiol (AET) has been studied in a pH range between pH = 7 and pH = 13. Kinetic results clearly indicate that both nucleophiles react through the corresponding thiolate to give the corresponding nitrosothiol. The existence of two (AET) or three (CYS) macroscopic acidity constants has been kinetically evidenced and the nitrosation rates of the corresponding bases have been identified. Nitrosation rate constants of the different species present in the reaction medium have been determined and a Brønsted-type plot has been established giving a β_{nuc} value ≅ 0.08 clearly different from the values of β_{nuc} ≅ 0.7 obtained in the nitrosation of primary and secondary amines by MNTS. The low β_{nuc} value has been attributed to the need for previous desolvation of the nucleophile.

Introduction

Nitroso compounds have attracted considerable interest mainly due to their important biological relevance. A wide variety of structurally related compounds possessing the *N*-nitroso-*N*-alkyl functionality have demonstrated a cancer chemotherapeutic potential.[1] As is well documented, the basic hydrolysis of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (MNTS) yields diazomethane,[2] a powerful alkylating agent[3] of DNA. The possible carcinogenic effect of these *N*-nitroso compounds can be even larger when considering the possibility that they may transfer the nitroso group to other nucleophilic substrates by a transnitrosation process.[4]

The chemical and biological activity of MNTS becomes more complex due to the existence of two reactive centers: the nitrogen atom of the nitroso group and the sulfur atom of the sulfonyl group (Scheme 1). Experimental results obtained in our laboratory show that different types of nucleophiles react at either one or the other electrophilic center. Soft nucleophiles[5] react exclusively at the nitroso group. Amines,[6] carbanions[7] and other nucleophiles such as:[8] SCN^-; I^-; $S_2O_3^{2-}$ and SO_3^{2-} must be included in this category. Hard nucleophiles such as the hydroxyl ion[9] or very basic alkoxides react through the sulfonyl group. There is a continuous gradation between these behaviors, in such a way that when the basicity of the alcohol decreases the percentage of reaction on the nitroso group increases.[10] As an extreme case, the hexafluoroacetone derived diol has been observed to react almost exclusively at the nitroso group of the MNTS. The possibility that a nucleophile may react simultaneously through both electrophilic centers of the MNTS has also been confirmed when studying its reaction with the hypochlorite ion (90% of reaction through the sulfonyl group) or with acetohydroxamic acid.[8]



Scheme 1

Another type of nitroso compounds are the *S*-nitrosothiols[11] (henceforth called RSNO), which have attracted growing interest since *ca.* 1990 due to a spectacular series of discoveries related to the synthesis *in vivo* of nitric oxide and its effects on a wide range of physiological functions.[12] In spite of the fact that the reactivity of the MNTS with different nucleophilic agents has been studied, there is, however, little information on its behavior when the nucleophilic atom is a sulfur atom. We have found in the literature only one mechanistic study of the reactivity of MNTS with cysteine.[13] In this study, the MNTS is found to transfer its nitroso group to the sulfur atom of the cysteine giving the corresponding *S*-nitrosothiol.

The acid–base behavior of the cysteine allows us to establish the existence of two possible nucleophilic species: **Hcys(−)** and **Cys(2−)** whose macroscopic pK_a are 8.21 and 10.4 respectively (see Scheme 2). The existence of these two nucleophilic forms should be apparent from its behavior toward the nitroso group. However, the results obtained when studying its reaction with MNTS[13] indicate that the reaction rate increases as the pH of the medium increases, but ignoring that this should reach a limiting value for values of pH > 11. The absence of the limiting value should be a consequence of the high ethanol concentration in the reaction media (≅ 25%). At high pH values the presence of ethanol yields $CH_3CH_2O^-$ and a new decomposition pathway for MNTS. The existence of this new decomposition pathway (its importance increases on increasing the basicity of the medium) is contrary to the tendency of the cysteine nitrosation reaction rate to reach a limiting value. An anomalous behavior has also been observed when studying the reactivity of cysteine with the nitroso group of alkyl nitrites.[14] The results obtained show that the only reactive species is **Hcys(−)**, noting that the rate constant reaches a limiting value for pH > 10.

These results contrast with the parallelism existing between basicity and nucleophilic character, widely reported in the literature and observed in particular in nitroso group transfer reactions.[15] In order to provide insight into the causes of this anomaly, we have carried out the present study which includes the reaction of MNTS with cysteine (CYS) and 2-amino-

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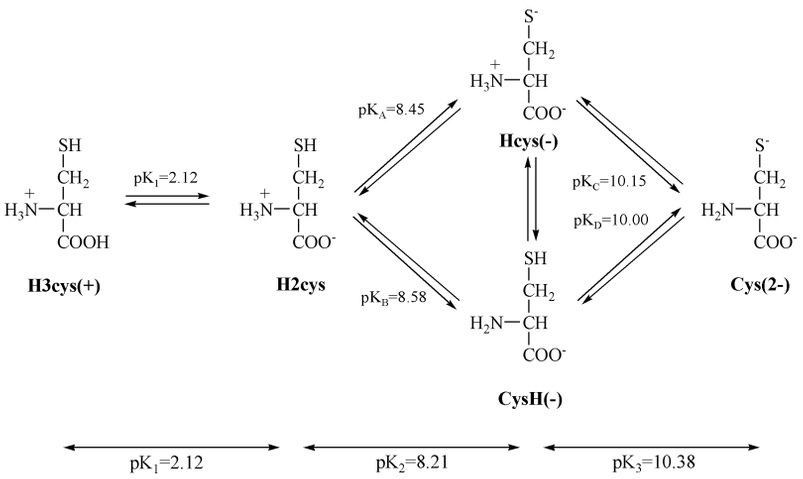


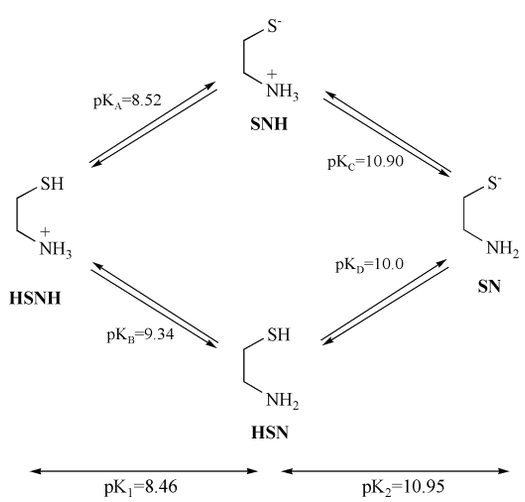
Scheme 2

ethanethiol (AET) in a pH range between pH = 7 and pH = 13. The results obtained show that, unlike those reported in the literature, both the cysteine and the AET react with the MNTS through the two microscopic forms possessing a negative charge on the sulfur atom, with the reaction rate leveling off for high pH values.

Experimental section

All reactants used (MNTS, CYS and AET) were from Aldrich, of the highest purity commercially available and used without further purification. Kinetic experiments were performed with a large excess of nucleophile over MNTS, [MNTS] = 1×10^{-4} M. The concentrations of the nucleophiles used were typically in the range 1.00×10^{-2} to 0.25 M. Due to the low solubility of MNTS in water, its solutions were prepared in an acetonitrile/water mixture, in such a way that the percentage of acetonitrile in the reaction mixture was always < 1% (v/v). The pH was controlled by buffer solutions of the nucleophile itself. The pH was measured with a Radiometer 82 pH-meter equipped with GK2401C or GK2401B (for pH > 11) combined glass electrodes.

Values of cysteine microscopic ionization constants have been obtained from the literature (Scheme 2). The corresponding AET values (Scheme 3) have been experimentally obtained using the method described by Clement and Hartz.[16]



Scheme 3

Kinetic experiments were performed in an Applied Photophysics MX17 stopped-flow spectrophotometer monitoring the increasing absorbance at λ = 320 nm (CYS) and λ = 330 nm (AET) due to the formation of thionitrites. All experiments were conducted in aqueous solution at 25 °C using a number of buffer solutions to cover the pH range 7–12. Good first order behavior was found throughout, following every run to at least 90% reaction. Each run was repeated at least five times and the average value of the pseudo-first-order rate constant, k_{obs}, used in the subsequent analysis was obtained. Standard error of k_{obs} is < 3%.

Results

When MNTS is placed in a CYS or AET buffer solution two kinetic processes are observed. Both kinetic processes take place on very different time scales. A fast process, occurring within a few seconds and characterized by an increase in the absorbance at λ = 330 nm due to the formation of the corresponding thionitrite, is observed. The obtained variation in absorbance is consistent with the quantitative formation of the corresponding S–NO compound and therefore in agreement with an attack of the sulfur nucleophile on the nitroso group of the MNTS. The second kinetic process occurs over a period of hours and results in the disappearance of the absorption band centered at 330 nm corresponding to the thionitrite.[17]

The influence of the cysteine concentration on k_{obs} in its reaction with MNTS has been studied. The results shown in Fig. 1 indicate the existence of a clear linear dependence with a zero intercept between k_{obs} and [CYS]. The absence of intercept is indicative of the fact that the basic hydrolysis of MNTS does not take place on time scales competing with the transfer of the nitroso group to the sulfur atom of the cysteine.[9] The results illustrated in Figs. 1 and 2 (influence of the AET concentration on k_{obs} in its nitrosation by MNTS) satisfy equation 1, where k_2^{app} is an apparent bimolecular rate constant since it includes the different acid–base equilibrium constants of the nucleophile

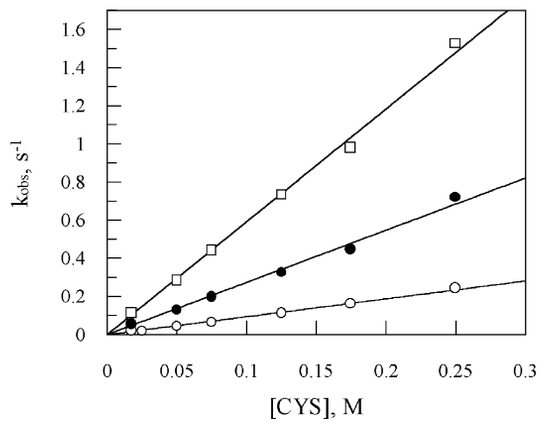


Fig. 1 Influence of the cysteine concentration on k_{obs} in its reaction with MNTS at 25 °C. (○) pH = 7.47; (●) pH = 7.94 and (□) pH = 8.63.

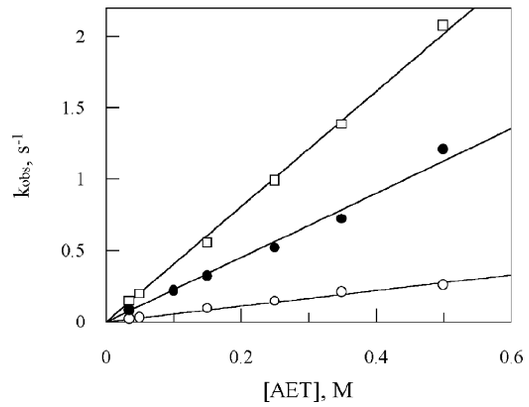


Fig. 2 Influence of the 2-aminoethanethiol (AET) concentration on k_{obs} in its reaction with MNTS at 25 °C. (○) pH = 7.44; (●) pH = 8.19 and (□) pH = 8.56.

and, [*Nucleophile*] represents the total concentration of CYS or AET. As can be seen in Figs. 1 and 2, the slopes of the plots of k_{obs} *versus* the concentration of CYS or AET increase with the pH of the medium or, according to equation 1, k_2^{app} increases with the pH.

$$k_{obs} = k_2^{app}[Nucleophile]_T \qquad (1)$$

Figures 3 and 4 show the influence of the pH on k_2^{app}. As can be observed, the k_2^{app} values increase with decreasing $[H^+]$ reaching a first leveling off for pH values ≅ 9.5. That leveling off is not very clear because of the third and second pK_a values of cysteine and AET respectively. Increasing the pH increases the concentration of **Hcys(−)** in the cysteine solution, with a corresponding increase in k_2^{app}. For pH ≅ 9.5 all the cysteine should be in the form of **Hcys(−)**, and the rate constant should be pH-independent, however because of the third pK_a of cysteine the structure **Hcys(−)** is in equilibrium with **Cys(2−)** and the rate constant will continue increasing with the pH. Such an increase in the rate constant will reach a limiting value when all the cysteine in solution is in the structure **Cys(2−)**, which happens for pH larger than 12. Subsequently, the rate constant k_2^{app} increases again with the pH until it reaches a limiting value for pH > 12.

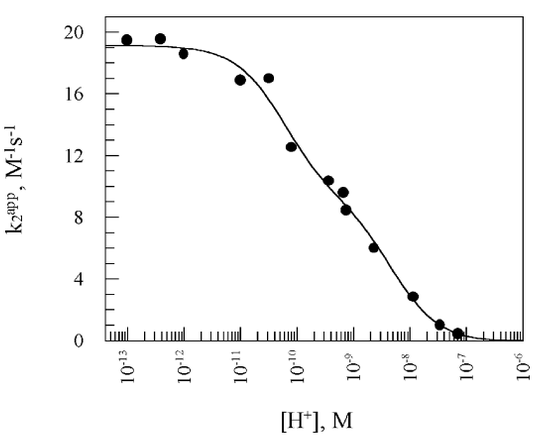


Fig. 3 Influence of pH on k_2^{app} for the transfer of the nitroso group from MNTS to cysteine at 25 °C.

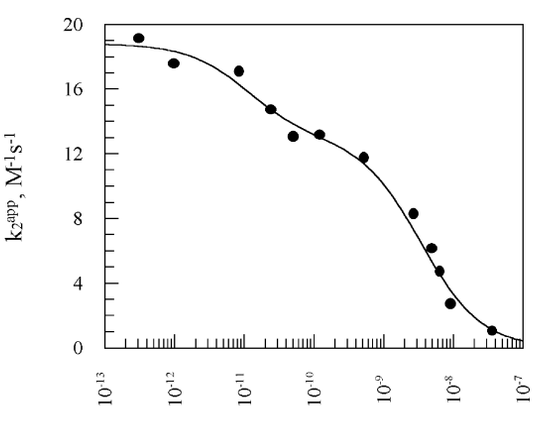


Fig. 4 Influence of pH on k_2^{app} for the transfer of the nitroso group from MNTS to 2-aminoethanethiol (AET) at 25 °C.

Discussion

The experimental results can be quantitatively explained by a reaction mechanism involving nitroso group transfer from MNTS to the forms of the cysteine **Hcys(−)** and **Cys(2−)** (Scheme 4).

From Scheme 4, the following expression (equation 2) for the apparent rate constant k_2^{app} can be obtained:

$$k_2^{app} = \frac{k_{obs}}{[CYS]_T} = \frac{\frac{k_1}{K_C}[H^+] + k_2}{1 + \frac{1}{K_3}[H^+] + \frac{1}{K_2K_3}[H^+]^2 + \frac{1}{K_1K_2K_3}[H^+]^3} \qquad (2)$$

where the equilibrium constants refer to the processes depicted in Scheme 2 and k_1 and k_2 are the rate constants corresponding to the nitrosation of **Hcys(−)** and **Cys(2−)** respectively as shown in Scheme 4.

The experimental results nicely fit equation 2 as is made clear by the curve plotted in Fig. 3. The value corresponding to the first ionization, pK_1 = 1.87, has been kept constant for the fitting process. From the fit of the kinetic results to equation 2, the macroscopic ionization constants can be obtained for the second and third process: pK_2 = 8.33 and pK_3 = 10.24. These values compare well with those reported in the literature (see Scheme 2). From the kinetic analysis, the rate constants k_1 = 11.4 M^{-1} s^{-1} and k_2 = 19.2 M^{-1} s^{-1} have also been obtained. These rate constants indicate that **Cys(2−)** has a higher reactivity toward the nitroso group than **Hcys(−)**, as might be expected from its higher basicity.

The experimental behavior observed for the nitroso group transfer from MNTS to AET can be quantitatively explained on the basis of the existence of two simultaneous reaction pathways: reaction of **SNH** and **SN** species (see Scheme 3). These two reaction pathways allow us to obtain the following expression (equation 3) for k_2^{app}:

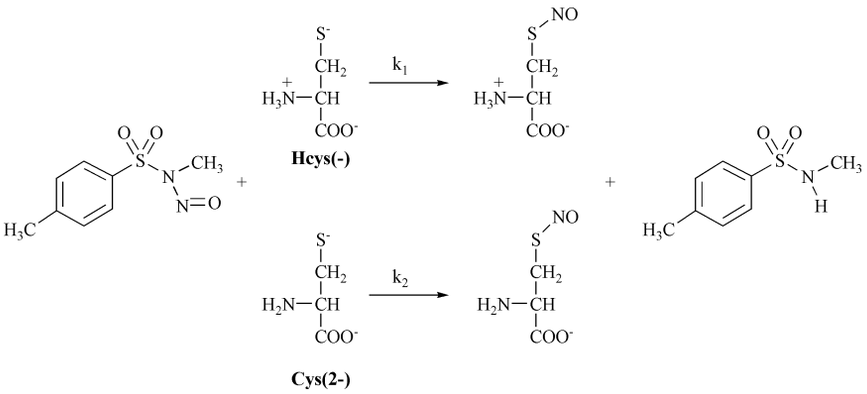
$$k_2^{app} = \frac{k_{obs}}{[AET]_T} = \frac{\frac{k_1}{K_C}[H^+] + k_2}{1 + \frac{1}{K_2}[H^+] + \frac{1}{K_1K_2}[H^+]^2} \qquad (3)$$

where the equilibrium constants refer to the processes shown in Scheme 3 and the rate constants k_1 and k_2 refer to the nitrosation of **SNH** and **SN** respectively.

The analysis of kinetic data yielded values for the macroscopic acidity constants: pK_1 = 8.46 and pK_2 = 10.95, which are in very good agreement with literature values. Likewise, rate constants k_1 = 13.0 M^{-1} s^{-1} and k_2 = 18.8 M^{-1} s^{-1} have been obtained. As had been observed in the cysteine nitrosation, the rate constants increase as the corresponding basicity of the nucleophile increases.

Structure–reactivity correlations

The values obtained for the nitroso group transfer from MNTS to sulfur nucleophiles show that these are more effective than

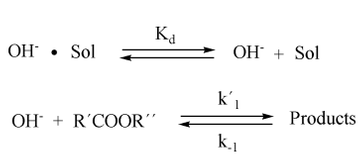


Scheme 4

the corresponding nitrogen or carbon nucleophiles of similar basicity. In particular, the rate of nitrosation of **SNH** and **Hcys(−)** by MNTS is approximately 2000 times higher than that of morpholine, a nitrogen nucleophile of the same basicity. This higher strength of the sulfur nucleophiles is well documented in the literature as evidenced by the different values in Ritchie's N_+ scale.[18]

The variation of the nucleophilic reactivity of **SN/SNH** and **Cys(2−)/Hcys(−)** with the basicity of the nucleophile shows a behavior clearly different from that of the nitrogen or carbon nucleophiles. The rate constant increases slightly by 75% by increasing the basicity of the nucleophile approximately 200 times. The same increase in basicity brings about a rise of 10^4% in the rate constant of the nitrosation of amines or carbanions by MNTS. Such a difference of behavior is made clear when establishing a Brønsted correlation giving values of $\beta_{nuc} \cong 0.7$ for the nitrosation of primary and secondary amines by MNTS,[15] whereas the value obtained for the nitrosation of the sulfur nucleophiles studied is $\beta_{nuc} \cong 0.08$.

This change in the sensitivity of the reaction to the basic strength of the nucleophile is unusual although not without precedent and it has traditionally been regarded as a consequence of the effect of desolvation on the reaction rate or on reactivity–structure correlations. In fact, there are many cases where the rate of certain nucleophilic attacks has been found to decrease as the basicity of the nucleophile is increased, leading to negative Brønsted exponents. This behavior has been observed for some phosphoryl transfer reactions to amines,[19] and for reactions of highly reactive carbocations with amines[20] and for reactions of thiolate ions with Fischer carbene complexes.[21] In the same way, values of the Brønsted exponent close to zero have been found for reactions of diphenylketene with amines.[22] Studies carried out by Jencks[19] indicate that these anomalous Brønsted exponents result from a requirement for partial desolvation of the nucleophile prior to the reaction. The desolvation is usually considered to be a pre-equilibrium that occurs in a separate step, in such a way that a two-step model like that illustrated in Scheme 5 can be adopted for a nucleophilic attack:



Scheme 5

As Scheme 4 shows, the experimental value of the rate constant for the process of nucleophilic attack corresponds to the product $K_d k_1$, where K_d is the equilibrium constant for the partial desolvation of the nucleophile.

Taking into account this approach, we can assume that β_{nuc} is given by equation 4:

$$\beta_{nuc} = \frac{d \log k_1}{d pK_a^{RSH}} = \frac{d \log K_d k_1'}{d pK_a^{RSH}} = \frac{d \log K_d}{d pK_a^{RSH}} + \frac{d \log k_1'}{d pK_a^{RSH}} = \beta_d + \beta_{nuc}' \quad (4)$$

In view of the fact that the higher the basicity of RS^-, the more difficult the desolvation is, $\beta_d < 0$ can be expected. Thus, if β_{nuc} is low, β_{nuc} may be dominated by β_d and be close to zero or even negative. The values reported in the literature where β_{nuc} values are quite low for thiolate ion addition to a variety of electrophiles are very common.[23] These low β_{nuc} values are indicative of a transition state with little bond formation.

Acknowledgements

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References

1 W. A. Skinner, H. F. Gram, M. O. Greene, J. Greenberg and B. R. Baker, *J. Med. Pharm. Chem.*, 1960, **2**, 299.
2 M. Regits, in *The Chemistry of the Diazonium and Diazo Groups*; S. Patai; Ed.; Wiley: New York, 1978.
3 (*a*) S. Rice, M. Y. Cheng, R. E. Cramer, M. Mandel, H. F. Mower and K. Sefff, *J. Am. Chem. Soc.*, 1984, **106**, 239; (*b*) P. D. Lawley, in *Chemical Carcinogens*; C. E. Searle, Ed.; ACS Monograph Series 182; American Chemical Society; Washington, DC, 1984, Vol. 1.
4 D. L. H. Williams, *Nitrosation*; Cambridge University Press: New York, 1988.
5 R. G. Pearson, H. Sobel and J. Songstad, *J. Am. Chem. Soc.*, 1968, **90**, 319.
6 L. García Río, E. Iglesias, J. R. Leis, M. E. Peña and A. Rios, *J. Chem. Soc., Perkin Trans. 2*, 1993, 29.
7 J. R. Leis, M. E. Peña and A. Rios, *J. Chem. Soc., Perkin Trans. 2*, 1993, 1233.
8 J. R. Leis, M. E. Peña and A. Rios, *J. Chem. Soc., Perkin Trans. 2*, 1995, 587.
9 (*a*) M. Pearce, *Helv. Chim. Acta*, 1980, **63**, 887; (*b*) A. Castro, J. R. Leis and M. E. Peña, *J. Chem. Soc., Perkin Trans. 2*, 1989, 1861; (*c*) L. García Río, J. R. Leis, J. A. Moreira and F. Norberto, *J. Phys. Org. Chem.*, 1998, **11**, 756.
10 L. García-Río, J. R. Leis and J. A. Moreira, unpublished results.
11 D. L. H. Williams, *Acc. Chem. Res.*, 1999, **32**, 869.
12 P. L. Feldman, O. W. Griffith and D. J. Stuehr, *Chem. Eng. News*, 1993, **71**, 26.
13 M. N. Y. F. Oh Shirlene and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1989, 755.
14 H. M. S. Patel and D. H. L. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1989, 339.
15 L. García Río, J. A. Leis, J. R. Moreira and F. Norberto, *J. Org. Chem.*, 2001, **66**, 381.
16 G. E. Clement and T. P. Hartz, *J. Chem. Educ.*, 1971, **48**, 395.
17 This second process will be the objective of future research.
18 C. D. Ritchie, *Can. J. Chem.*, 1986, **64**, 2239.
19 W. P. Jencks, M. T. Haber, D. Herschlag and K. L. Nazaretian, *J. Am. Chem. Soc.*, 1986, **108**, 479.

20 (*a*) J. P. Richard, *J. Chem. Soc., Chem. Commun.*, 1987, 1768; (*b*) R. A. McClelland, V. M. Kanagasabapathy, N. S. Banait and S. Steenken, *J. Am. Chem. Soc.*, 1992, **114**, 1816.
21 C. F. Bernasconi, K. W. Kittredge and F. X. Flores, *J. Am. Chem. Soc.*, 1999, **121**, 6630.
22 J. Andraos and A. Kresge, *J. Am. Chem. Soc.*, 1992, **114**, 5643.
23 (*a*) C. F. Bernasconi, J. Fassberg, R. B. Killion, Jr and Z. Rappoport, *J. Am. Chem. Soc.*, 1989, **111**, 6862; (*b*) C. F. Bernasconi, J. Fassberg, R. B. Killion, Jr. and Z. Rappoport, *J. Am. Chem. Soc.*, 1990, **112**, 3169; (*c*) C. F. Bernasconi, R. J. Ketner, X. Chen and Z. Rappoport, *J. Am. Chem. Soc.*, 1998, **120**, 7461; (*d*) C. F. Bernasconi, R. J. Ketner, X. Chen and Z. Rappoport, *Can. J. Chem.*, 1999, **77**, 584; (*e*) D. J. Hupe and W. P. Jencks, *J. Am. Chem. Soc.*, 1977, **99**, 451; (*f*) C. F. Bernasconi and R. B. Killion, Jr., *J. Am. Chem. Soc.*, 1988, **110**, 7506; (*g*) H. F. Gilbert and W. P. Jencks, *J. Am. Chem. Soc.*, 1977, **99**, 7931; (*h*) C. D. Ritchie and J. R. Gandler, *J. Am. Chem. Soc.*, 1979, **101**, 7318; (*i*) C. F. Bernasconi and D. F. Schuck, *J. Org. Chem.*, 1992, **57**, 2365; (*j*) C. F. Bernasconi and R. J. Ketner, *J. Org. Chem.*, 1998, **63**, 6266; (*k*) E. Buncel, C. Cannes, A. P. Chatrousse and F. Terrier, *J. Am. Chem. Soc.*, 2002, **124**, 8766; (*l*) F. Terrier, E. Guével, A. P. Chatrousse, G. Moutiers and E. Buncel, *Chem. Commun.*, 2003, 600.