Trends in Organic Chemistry
Vol. 14, 2010

Original Communication

Mass spectrometric study and theoretical calculations on the tautomerism of nitrocompound-nitronic acid

Danila L. Ruiz[1], Sergio L. Laurella[1], María S. Cortizo[1,2], María M. Schiavoni[1], Eduardo A. Castro[2,*], Jorge J. P. Furlong[1,*,#], and Patricia E. Allegretti[1,*,$]

[1]Laboratorio LADECOR, División Química Orgánica, Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calle 47 y 115,
[2]INIFTA, Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Diagonal 113 y 64, Suc. 4, C. C. 16, (1900) La Plata, Buenos Aires, Argentina

ABSTRACT

The tautomerism of nitrocompounds is investigated by the analysis of their mass spectra and semi-empirical molecular orbital calculations. The influence of substitution is discussed and found to be consistent with the expected electronic and steric effects. Reactivity studies are supporting as well. Acceptable correlation between the experimental data and theoretical results are found only with the neutral molecules although calculations were also done with the corresponding molecular ions. Both methods provide a useful tool to study the tautomerization phenomena.

KEYWORDS: tautomerism, nitro compounds, mass spectrometry, theoretical calculations

INTRODUCTION

Nitronic acids have been the subject of several studies, in part because of their equilibrium with nitroalkanes. Experimental evidences [1-4] and theoretical studies [5, 6] suggest the relative importance of the nitronic acid form. Nitronic acids play an important role in thermal and redox reactions, in photochemical processes, and pyrolysis [7-9]. The toxicity of nitro compounds [8] and their participation in the Nef reaction which converts nitroalkanes in ketones [7], have also been demonstrated.

A kinetic analysis of the tautomeric transformation of phenylnitromethane has confirmed that there are two forms of the anion: *aci-* and *nitro-* forms [10].

A nitronic acid tautomer form has been proposed as intermediate in reactions of natural products, such as carbohydrates, thus showing that these tautomers behave as precursors of glucosinolates [11].

On the other hand, it has been shown that 2-nitropropane is a genotoxic agent and hepato-carcinogene in rodents. Conversion to the tautomeric *aci*-form of 2-nitropropane, plays a fundamental role in the mechanism by which 2-nitropropane causes its toxicity [12].

In this paper the mass spectra of selected nitrocompounds have been studied in order to discuss the predictive power of mass spectrometry in the occurrence of their tautomerism.

It has already been shown that mass spectrometry is a powerful tool for studying fast equilibria. Semiempirical calculations not only confirm the results obtained by mass spectrometry, but also support the fact that ionization does not affect the tautomeric equilibrium established between tautomer neutral species [13-26].

*Corresponding authors
#furlong@quimica.unlp.edu.ar
$pallegre@quimica.unlp.edu.ar

METHODS

Synthesis of nitrocompounds

The commercially unavailable nitrocompounds [28], the anhydride of nitronic acid by reaction of nitroethane with benzoyl chloride [29] and the methyl nitronate ester [30] were synthesized according to literature procedures.

Gas chromatography - mass spectrometry - single quadrupole

These determinations were performed by injection of methanol solutions (1 µl, 100 µg/ml aprox.) in an HP 5890 Chromatograph coupled to an HP 5972 A mass selective detector (unit mass resolution). An HP5-MS capillary column (30 m x 0.25 mm x 5 µm) has been used with Helium as the carrier gas (0.6 ml/min in column, split ratio 1:30). The temperatures set points were: 200 °C in the split injector, 300 °C in the interface, 185 °C in the ion source and the oven ramp started at 40 °C (5 min) and ended at 290 °C with a heat rate of 20 °C/min. The electron energy was 70 eV and the pressure in the mass spectrometer was lower than 10^{-5} torr, thus precluding ion molecule reactions.

The relevance of spectrometric data as a predictive tool in regard to tautomeric equilibria depends mainly on the fact that the contribution due to tautomerization of molecular ions in the gas phase does not take place or can be ignored. The importance of this point comes from the physicochemical properties of ionic and radical species, quite different from the neutral ones. This could be the reason of possible distortion of results and loss of the desirable predictive power of the methodology.

It has been demonstrated in the case of keto-enol tautomerism of a variety of carbonyl and thio-carbonyl compounds [13, 15-26], that there is no significant interconversion of the tautomeric forms in the gas phase following electron impact ionization in the mass spectrometer (molecular ions, $M^{+\cdot}$, do not seem to undergo unimolecular tautomerization) and, even more surprising, for GC/MS experiments, once the solvent is separated after injection in the injection port of the gas chromatograph, tautomerism mechanisms (inter-molecular, unimolecular) would not seem to take place even with no GC separation (under the selected experimental conditions). These conclusions are supported by temperature studies at the ion source (negligible effect) and at the injection port of the gas chromatograph with a shifting effect in agreement with the corresponding heats of tautomerization [21]. In fact, this process would take place very fast under the working conditions in the GC.

Separation of tautomers in the analytical column are often very difficult, consequently the different pathways of fragmentation of the tautomeric forms have to be used for identification of individual tautomers. For this reason and because of the high similarity between MS (commercial databases) and GC/MS spectra, analytical separation has not been considered critical for the present work. Analogously, it is thought that most of the conclusions could be useful to analyze spectra registered with mass spectrometers equipped with direct insertion probes.

Gas chromatography-mass spectrometry - ion trap

These determinations were performed by injection of methanol solutions (1 µl) in a Thermo Quest Trace 2000 coupled to Finnigan Polaris ion trap detector (unit mass resolution) under the same experimental conditions already mentioned for the single quadrupole GC/MS system. This instrumentation was utilized to confirm proposed fragmentation pathways by CID (collision induced dissociation) using Helium as the damping gas, a CID voltage of 5-7 eV and an excitation energy of 0.35-0.45 (values were optimized for each ion transition). These experiments were done by selecting a precursor ion from the full-scan spectrum and carrying out the corresponding MS/MS product ion scan (Schemes 2 and 3).

Magnetic nuclear resonance determinations

^{1}H NMR spectra in DMSO-d_6 were recorded with a Varian Mercury Plus 200 spectrometer operating at 4.5 T. The typical spectral conditions were as follows: spectral width 3201 Hz, acquisition time 4.09 s and 8-16 scans per spectrum. Digital resolution was 0.39 Hz per point. Deuterium from the solvent was used as the lock and TMS as the internal standard. Sample concentration was 0.05M.

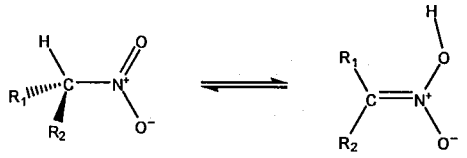
^{13}C proton decoupled and gated decoupled spectra were recorded with the same spectrometer from DMSO-d_6 solutions at 25°C. The spectral conditions were the following: spectral width 10559 Hz, acquisition times 1.303 s and 512-1000 scans per spectrum. The concentration was 30 mg mL^{-1} and digital resolution was 1.29 Hz per point.

Computational procedure

AM1 calculations [31] were performed using the standard Hyperchem package [32]. Since we resorted to heat of formation values in order to rationalize experimental findings and the AM1 technique has been specially parameterized to reproduce this sort of experimental data, we deem this choice is a sensible one for the molecular set under consideration. Besides, previous computations obtained for this sort of studies have given quite sensible results in order to correlate experimental and theoretical data, so that we deem it is not necessary to resort to higher levels of molecular electronic structure sophistication.

RESULTS AND DISCUSSION

Nitrocompound-nitronic acid (*nitro-aci*) tautomerism is shown in Scheme 1.



Scheme 1. Tautomerism of nitrocompound-nitronic acid.

Mass spectrometry studies

Nitrocompounds were analyzed by gas chromatography-mass spectrometry with no chromatographic separation of the tautomers, so that their mass spectra are the result of the overlapping of the spectra of individual tautomers.

The Fig. 1 shows the mass spectrum for nitroethane.

From the analysis of the main fragment peaks it is clear the occurrence of the *aci* form since that there exist fragment ions that can only been explained through that tautomer.

The peaks at m/z 46 $(M\text{-}C_2H_5)^+$ and m/z 15 $(CH_3)^+$ can be justified from both tautomeric forms.

The loss of NO_2H_2 from the molecular ion (m/z 27) and the $(M\text{-}OH)^+$ (m/z 58) could be assigned to the *aci*-form and the loss of NO_2 (m/z 29) to the *nitro*-form (Scheme 2). It should be pointed out that the ion at m/z 27 can also be assigned to the corresponding alkenyl ion (always present along with the alkyl series by H_2 loss).

The Fig. 2 shows the mass spectrum for 1-nitropropane.

The loss of the hydroxyl group, ion at m/z 72, $(M\text{-}OH)^+$ can be rationalized as coming from the *aci* form, (Scheme 2 (c)). The ion at m/z 43 corresponds to the loss of NO_2, $(M\text{-}NO_2)^+$ according to Scheme 2 (a) for the *nitro* tautomer and the peak at m/z 41 can be originated in a similar fragmentation to that one shown in Scheme 2 (b) for the ion at m/z 27 $(M\text{-}NO_2H_2)^+$ and/or loss of a molecule of hydrogen from the corresponding alkyl ion. The ion at m/z 27 could

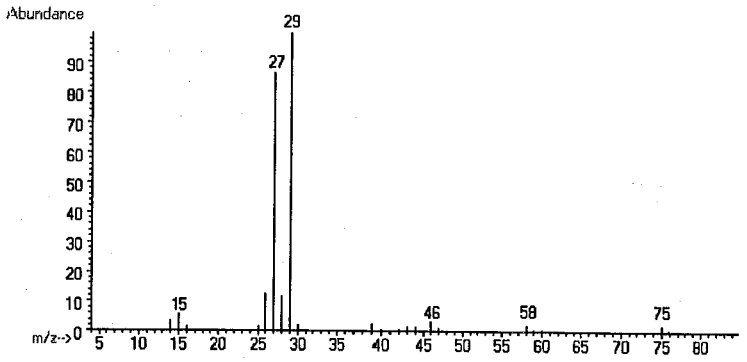
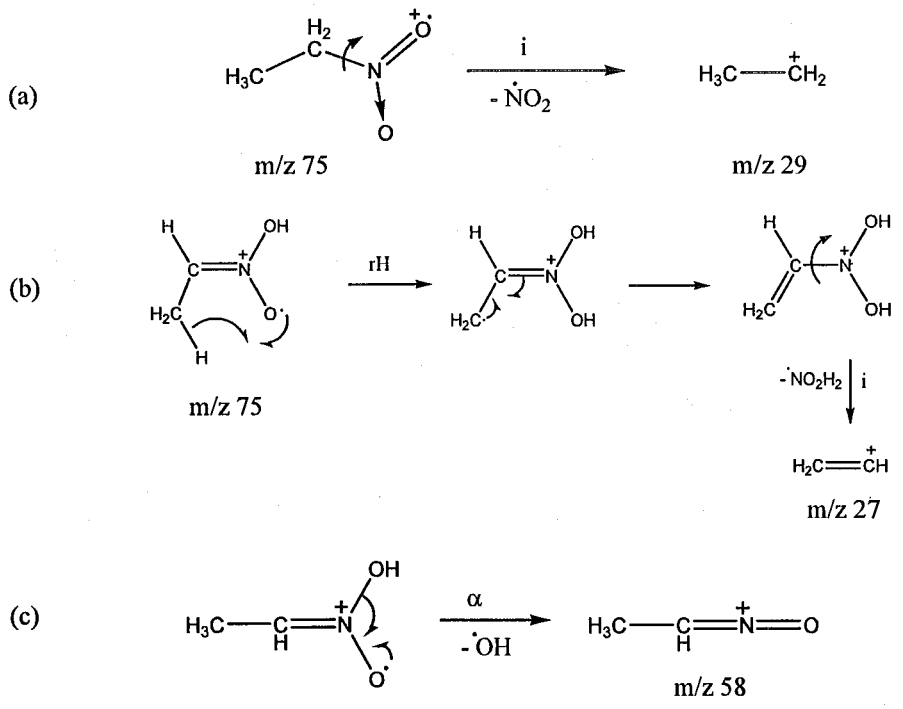


Figure 1. Mass spectrum of nitroethane.



Scheme 2. Possible fragmentation mechanisms for the ions $(M\text{-}NO_2)^+$ (a), $(M\text{-}NO_2H_2)^+$ (b) and $(M\text{-}OH)^+$ (c).

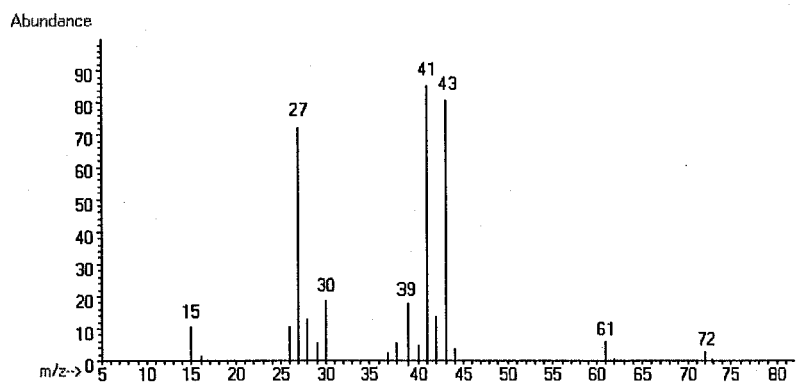


Figure 2. Mass spectrum of 1-nitropropane.

be formed by the molecular ions of the *aci* structure according to Scheme 3.

The Fig. 3 shows the mass spectrum for 2-nitrobutane.

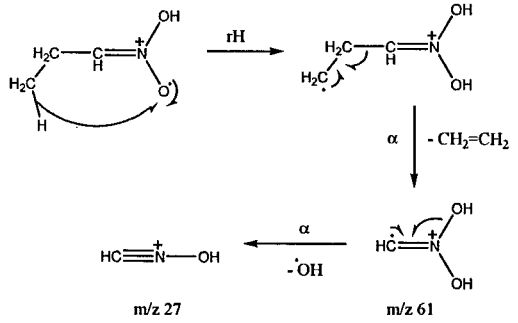
The m/z 29 $(C_2H_5)^+$ and m/z 27 can be can be justified from both tautomeric forms. The ions at m/z 86, $(M\text{-}OH)^+$, m/z 55, $(M\text{-}NO_2H_2)^+$ or $(M\text{-}NO_2\text{-}H_2)^+$, and the m/z 57 $(M\text{-}NO_2)^+$ would keep the already discussed assigments. The m/z 58 and m/z 75 can be rationalized as coming from the *aci* tautomer, according to Scheme 3. The m/z 41 could be assigned to the *aci* structure from loss of OH from m/z 58.

The Fig. 4 shows the mass spectrum 3-nitro-2-methylpropene.

The ions at m/z 29 and m/z 39 can be justified from both tautomeric forms. The ions at m/z 84, $(M\text{-}OH)^+$, m/z 53, $(M\text{-}NO_2H_2)^+$ or $(M\text{-}NO_2\text{-}H_2)^+$, and the fragment ion at m/z 55, $(M\text{-}NO_2)^+$, would correspond to the assigments done for the previous nitro-compounds.

The Fig. 5 shows the mass spectrum nitrocyclopentane.

The loss of NO_2 (m/z 69) can be rationalized as coming to the nitro form (Scheme 2 (a)). The ions at m/z 39 and m/z 41 can be justified from both tautomeric forms. The ion at m/z 98 $(M\text{-}OH)^+$ and the m/z 67 $(M\text{-}NO_2H_2)^+$ could be explained by the Scheme 2 (c) and (b) respectively and would support the *aci* form, although the peak at m/z 67 can also be originated from the alkyl cation (m/z 69) by H_2 loss. The presence of the ion at m/z 97 $(M\text{-}H_2O)^{+\cdot}$ constitutes evidence in favor of the *aci* structure.



Scheme 3. Possible fragmentation mechanisms for the ion at m/z 27.

Table 1 shows the most relevant mass spectal data for selected nitrocompounds.

Despite the fact the analyses have been carried out by GC-MS, no chromatographic separation has been observed so that the mass spectra are the result of the superposition of both tautomers.

In order to weigh the occurrence of both tautomers in equilibrium, suitable fragmentations have been assigned to the *nitro* and *aci* forms.

The loss of NO_2 from the molecular ion can be assigned to the *nitro* form.

A specific assignment to the *aci* form could be the loss of hydroxyl group as well as the water loss from the molecular ions (this last one has often been not detected). The loss of NO_2H_2 could be thought as coming from the *aci*-tautomer, but the mass of this fragment is also the mass of $(M\text{-}NO_2\text{-}H_2)^+$, ion that would be originated from the *nitro* tautomer. This precludes their consideration for correlation purposes.

As it can be observed, the *aci*-tautomer content increases with the inductive effect of the substitution (1-nitropropane *vs.* nitroethane) and whenever steric effects take place (sp^2 carbon atoms accommodate two alkyl groups better than sp^3 carbon atoms, *e.g.*, 2-nitrobutane *vs.* 1-nitropropane). Besides, conjugation of the *aci*-form with the unsaturation of the alkyl moiety also increases the content of this isomer (*e.g.* 3-nitro-2-methylpropene).

For the nitrocyclopentane the comparison with 2-nitrobutane applies by considering its rigid structure that would decrease the chance to form

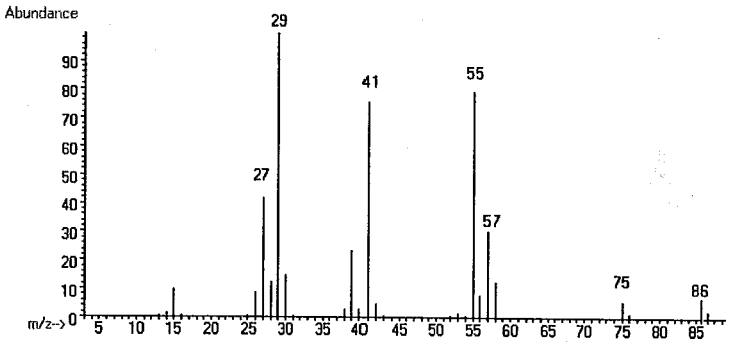


Figure 3. Mass spectrum for 2-nitrobutane.

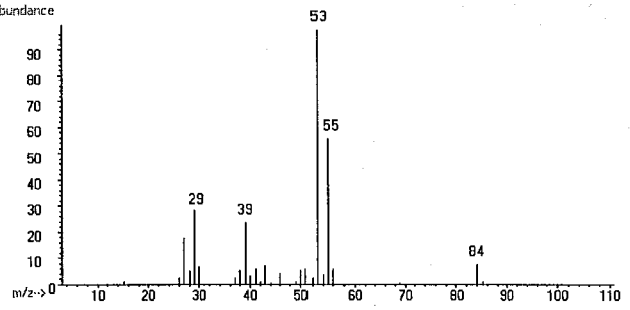


Figure 4. Mass spectrum of 3-nitro-2-methylpropene.

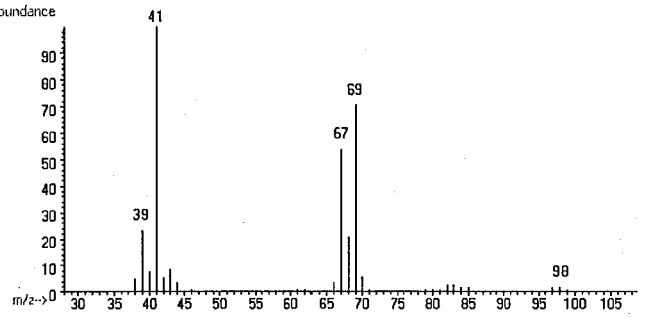


Figure 5. Mass spectrum of nitrocyclopentane.

the corresponding *aci*-tautomer. All these results are reflected in the $[(M\text{-}OH)^+]$ / $[(M\text{-}NO_2)^+]$ ion ratio (Table 1).

Theoretical calculations

Table 2 shows the difference of heats of formation between the *nitro* and *aci* forms considering the neutral molecules and the corresponding radical cations. A reasonable good correlation with the mass spectra observations is achieved only in the case of the neutral molecules.

When considering the radical ions not only there are no correlation with the experimental data but also no logical tendencies are observed (*e.g.* compare 3-nitro-2-methylpropene vs nitroethane or 2-nitrobutane vs nitroethane). Then, these findings are consistent with the tautomerism occurrence for the neutral species, before ionisation. (approximate calculation of the equilibrium constant values for the tautomerisation can be done with this data taking into account that the corresponding enthropy changes are near neglectable or at least very similar).

The correlation between mass spectra observations and the theoretical calculations gives additional support to the predicting value of the proposed ion abundances ratio.

Reactivity studies

Chemical evidences have been found about the occurrence of nitronate anion (Scheme 4) and the nitronic acid in the corresponding anhydride and ester formation reactions.

The IR spectrum of the product exhibits an intense band at 1600 cm^{-1}, which is characteristic of $C{=}N^+ O^-$ stretching [27].

On the other hand, the nitronate anion was derivatized to the corresponding anhydride (Scheme 5) and methyl ester (Scheme 6) of benzoic acid, whose structures were confirmed by ^1H and ^{13}C NMR (Table 3). (Yield: 86% and 79% respectively).

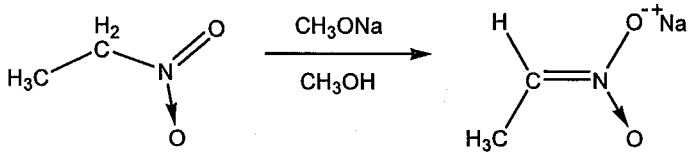
Table 1. Relevant mass spectral data for selected nitrocompounds[a].

Compound	$[M^{+\cdot}]$	$[(M\text{-}NO_2)^+]$	$[(M\text{-}OH)^+]$	$\frac{[(M\text{-}OH)^+]}{[(M\text{-}NO_2)^+]}$
nitroethane	0.1	360.2	10.8	$3.0\text{x}10^{-2}$
1-nitropropane	-	239.3	9.6	$4.0\text{x}10^{-2}$
2-nitrobutane	-	98.5	24.6	$2.5\text{x}10^{-1}$
3-nitro-2-methylpropene	1.1	154.2	20.0	$1.3\text{x}10^{-1}$
nitrocyclopentane	-	167.4	3.3	$2.0\text{x}10^{-2}$

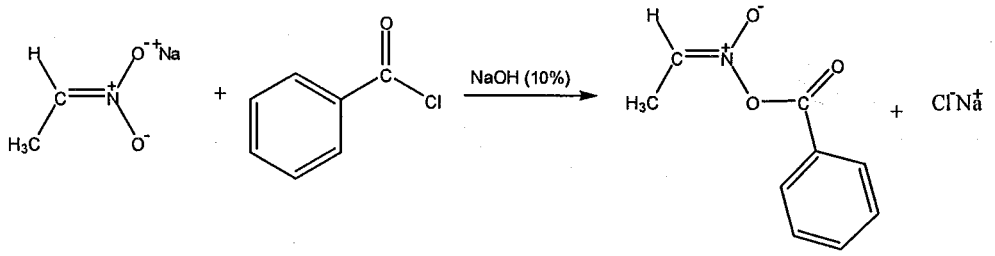
[a]For a better correlation the reported electron impact data are displayed according to the following ratio: Ion abundance x 1000 / Σ abundances.

Table 2. *Nitro-aci* heats of formation difference (kJ/mol) by AM1 calculations and mass spectrometry data correlation.

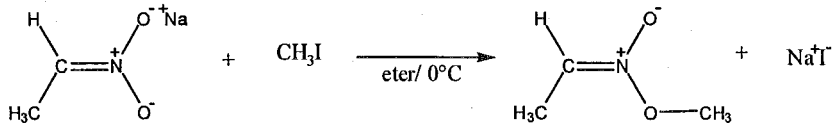
Compound	ΔH *nitro-aci* (Neutral molecules)	ΔH *nitro-aci* (Molecular ions)	Ion abundance ratio
nitroethane	37.62	30.60	$3.0\text{x}10^{-2}$
1-nitropropane	36.03	39.50	$4.0\text{x}10^{-2}$
2-nitrobutane	32.65	34.78	$2.5\text{x}10^{-1}$
3-nitro-2-methylpropene	34.27	35.70	$1.3\text{x}10^{-1}$
nitrocyclopentane	39.17	37.41	$2.0\text{x}10^{-2}$



Scheme 4. Nitronate anion formation reaction.

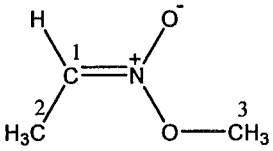
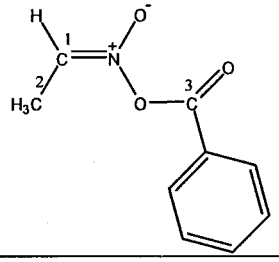


Scheme 5. Anhidride formation reaction from nitroethane and benzoyl-chloride.



Scheme 6. Ester formation reaction from nitroethane and methyl-yodide.

Table 3. NMR spectra (^{1}H and ^{13}C) for the methyl ester and the anhidride of benzoic acid of the nitronate anion (200MHz, $CDCl_3$).

COMPOUND	^{1}H NMR δ (ppm)	^{13}C NMR δ (ppm)
	1.8 (d, 3H (2)); 3.8 (s, 3H (3)); 6,9 (q, 1H (1))	7.1 (2); 55.9 (3); 152.0 (1)
	1.8 (d, 3H (2)); 6,9 (q, 1H (1)); 7.6-8.2 (m, 5 H, aromatics)	6.8 (2); 128.0-133 (aromatics); 151.9 (1); 165.4 (3)

CONCLUSIONS

As shown in previous papers [13-26] the usefulness of mass spectrometry (and GC/MS) to predict tautomeric behavior is demonstrated here along with additional support provided by the reactivity of the studied nitro compounds and their n.m.r. data. The mass spectra of nitro compounds can provide valuable information regarding the *nitro-aci* equilibria taking place in the gas phase (fast tautomerization equilibrium at the injection port of the gas chromatograph). The predictive value of this methodology is supported by the influence of the nature and size of substituents on tautomeric equilibria. Chemical and spectrometric determinations give additional support. Results show that the *nitro-aci* equilibrium can be studied by mass spectrometry and not only ionization in the ion source has a negligible effect on the position of that equilibrium but also the chromatographic conditions (with exception of the injection port temperature) seem to exert no effect.

ACKNOWLEDGMENTS

Financial support from Universidad Nacional de La Plata, is gratefully acknowledged.

REFERENCES

1. Schwarz, H. and Levsen, K. 1982, The Chemistry of Amino, Nitroso, and Nitro Compounds and their Derivatives. Patai E. (Ed.), Wiley, Chichester (West Sussex), New York, 85.
2. Miyashita, M., Awen, B. Z. E., and Yoshikoshi, A. 1990, Tetrahedron, 46, 7569.
3. Shaw, A. J. and Gescher, A. 1990, J. Chromatography A, 505, 402.
4. Kornblum, N. and Brown, R. A. 1992, J. Org. Chem., 57, 1132.
5. Turner A. G. 1986, J. Phys. Chem., 90, 6000.
6. Politzer, P., Seminario, J. M., and Bolduc, P. R. 1989, Chem. Phys. Lett., 158, 463.

7. Noland, W. E. 1955, Chem. Rev., 55, 137.
8. Nielsen, A. T. 1969, Nitronic acids and esters. In: The Chemistry of the Nitro and Nitroso Groups, Feuer, H. (Ed.), Interscience, New York, Part I, 349.
9. Patai, S. 1982, The Chemistry of Amine, Nitroso and Nitrocompounds and Their Derivatives, Wiley, New York, Morrison, H. A., 165, Chow, Y. L., 181, Batt, L. P., 417.
10. Belikov, V. M., Maairanovskii, S. G., Korchemnaya, T. B., Novikov, S. S., and Klimova, V. A. 1960, Russian Chem. Bull., 9, 1553.
11. Matsuo, M., Kirkland, D. F., and Underhill, E. W. 1972, Phytochemistry, 11, 697.
12. Kohl, C. and Gescher, A. 1996, Chem. Biol. Interact., 99, 219.
13. Allegretti, P. E., Castro, E. A., and Furlong, J. J. P. 2000, J. Mol. Structure, (Theochem), 499, 121.
14. Allegretti, P. E., Schiavoni, M. M., Di Loreto, H. E., Furlong, J. J. P., and Della Védova, C. O. 2001, J. Mol. Structure, 560, 327.
15. Allegretti, P. E., Gavernet, L., Castro, E. A., and Furlong J. J. P. 2000, J. Mol. Structure (Theochem), 532, 139.
16. Allegretti, P. E., Cánepa, A. S., Bravo, R. D., Castro, E. A., and Furlong, J. J. P. 2000, Asian Journal of Spectroscopy, 4, 133.
17. Allegretti, P. E., Gavernet, L., Castro, E. A., and Furlong, J. J. P. 2001, Asian Journal of Spectroscopy, 5, 63.
18. Allegretti, P. E., Cortizo, M. S., Guzmán, C., Castro, E. A., and Furlong, J. J. P. 2003, Arkivoc, X, 24.
19. Allegretti, P. E., Peroncini, V., Castro, E. A., and Furlong, J. J. P. 2003, International Journal Chemical Science, 1, 1.
20. Allegretti, P. E., Asens, D., Schiavoni, M. M., Bravo, R. D., Castro, E. A., and Furlong, J. J. P. 2003, Arkivoc, XV, 134.
21. Allegretti, P. E., Milazzo, C. B., Castro, E. A., and Furlong, J. J. P. 2002, J. Mol. Structure Theochem, 589, 161.
Allegretti, P. E., Milazzo, C. B., and Furlong, J. J. P. 2005, Eur. J. Mass Spectrom., 11, 53.
22. Allegretti, P. E., Schiavoni, M. M., Cortizo, M. S., Castro, E. A., and Furlong, J. J. P. 2006, Int. J. Mol. Sci., 5, 294.
23. Allegretti, P. E., Castro, E. A., and Furlong, J. J. P. 2006, Eur. J. Mass Spectrom., 12, 317.
24. Allegretti, P. E., Saraví Cisneros, H., Castro, E. A., and Furlong, J. J. P. 2006, Trends in Heterocyclic Chemistry, 11, 81.
25. Allegretti, P. E., Schiavoni, M. M., Guzmán Sampay, C., Ponzinibbio, A., and Furlong, J. J. P. 2007, European Journal Mass Spectrometry, 3, 291.
26. Allegretti, P. E., Ruiz, D. L., Castro, E. A., and Furlong, J. J. P. 2009, The Open Physical Chemistry Journal, 3, 18.
27. Nielsen, A. T. and Archibald, T. G. 1970, Tetrahedron, 26, 3475.
28. Kornblum, N. 1962, Org. Reactions, 12, 101.
29. Thurston, A. G. and Shriner, R. L. 1937, J. Org. Chem., 2, 183.
30. Kornblum, N. and Brown, R. A. 1964, J. Am. Chem. Soc., 86, 2681.
31. Dewar, M. J. S., Zoebisch, E.G., Healy, E. F., and Stewart, J. J. P. 1985, J. Am. Chem. Soc., 107, 3902.
32. Hyperchem ® 6.03 for Windows Molecular Modeling System, Hypercube Inc., Gainesville, FL. (2000).