Clarification of the 30 Da releases from the [M-H] − and M −• ions of trinitrotoluene by electrospray high resolution mass spectrometry

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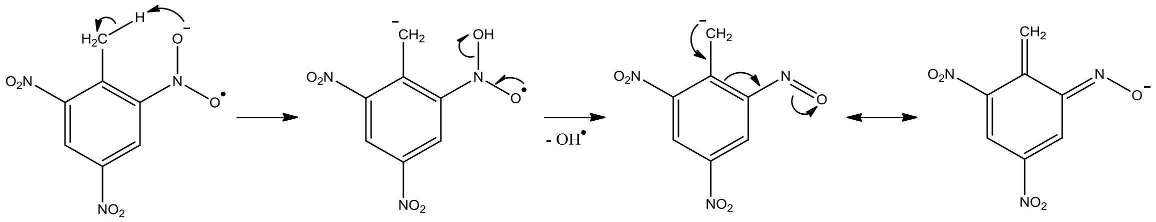
Clarification of the 30 Da Releases from the $[M\text{-}H]^-$ and $M^{-\bullet}$ ions of Trinitrotoluene by Electrospray High Resolution Mass Spectrometry

Dear Sir,

Although some nitroaromatic compounds can naturally occur in the environment, the vast majority of them come from anthropogenic sources. Indeed, nitroaromatic compounds such as 2,4,6-trinitrotoluene (TNT) and related-compounds are widely used as chemicals or synthetic intermediates in industrial manufacturing of explosives, dyes, pharmaceuticals, polyurethane foams and pesticides.[1] Considering the proven toxicity of nitroaromatic compounds on living organisms[2], and their significance in the forensic sciences, much attention has been given to these compounds[3]. Thus, nitroaromatic compounds have been extensively studied by mass spectrometry (MS) coupled with different ionization sources. At first, classical vacuum ionization techniques such as Electron Ionization (EI)[4, 5] and Chemical Ionization (CI)[5-7] were widely used to examine nitroaromatic compounds. Upon the development of atmospheric pressure ionization (API) techniques, atmospheric pressure chemical ionization (APCI)[8] and electrospray ionization (ESI)[9] became established as preferred techniques to analyze nitroaromatic compounds[10]. In particular, ESI of TNT in the negative ion mode can produce competitive processes : (i) deprotonation $[M\text{-}H]^-$ and (ii) electrochemical reduction $M^{\bullet -}$.[11, 12] Afterwards, Collision-Induced-Dissociation (CID) has been extensively used for structural as well as quantitative information[13] TNT samples are commonly analyzed at low resolution by tandem mass spectrometry. Under CID conditions, these negatively charged molecular species dissociate by competitive losses of either $OH^\bullet$ (implicating the "*ortho* effect", Scheme 1), or by loss of $NO^\bullet$ (after NO_2/ONO isomerization). In addition, $NO_2^\bullet$ release was also observed.



Scheme 1. Stepwise $OH^\bullet$ release implicating the "*ortho* effect" promoted by the radical anion $[M]^{\bullet -}$ (*m/z* 227) of TNT.

In this study, we have used a standard solution (1 mg/mL in MeOH:ACN (1:1)) of 2,4,6-trinitrotoluene (TNT), obtained from AccuStandard Europe (Niederbipp, Switzerland). TNT was prepared by dilution at 1 µg mL^{-1} in H_2O/MeOH (1:1), then infused at a flow rate of 5 µL min^{-1} into an LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Courtaboeuf, France) and ionized by ESI in the negative ion mode. The employed spray voltage was -2.5 kV giving mainly the deprotonated molecule $[M\text{-}H]^-$ at *m/z* 226 and the radical anion $[M]^{\bullet -}$ in minor abundance at *m/z* 227. Fragment ions were generated through resonant excitation by CID in the LTQ cell (30 ms activation time, 5 to 30 % normalized collision energies, NCE), and accurate mass measurements at high-resolution were performed using the Orbitrap analyzer operated at 60,000 resolving power (FWHM) at *m/z* 400.

The CID spectrum of the radical anion $[M]^{-\bullet}$ at *m/z* 227 displays the well-known competitive dissociations involving $OH^{\bullet}$ and $NO^{\bullet}$ losses, giving rise to formation of two abundant ions: $[M\text{-}OH]^{-}$ at *m/z* 210 and $[M\text{-}NO]^{-}$ at *m/z* 197 (Figure 1 a). The assigned $OH^{\bullet}$ and $NO^{\bullet}$ losses were confirmed by accurate mass analysis at high resolution displayed in Table 1. The $OH^{\bullet}$ loss has been observed from many *ortho* substituted nitroaromatic radical anions, owing to the "*ortho* effect".[14, 15] This effect involves an intramolecular benzylic proton transfer originating from an H-containing neighboring substituent (such as a methyl or OH group) located in the *ortho* position relative to the nitro group; this is followed by a simple cleavage induced by the radical. In the case of TNT, the fragment $[M\text{-}OH]^{-}$ ion (*m/z* 210) is stabilized by delocalization of the negative charge (Scheme 1). On the other hand, the fragment $[M\text{-}NO]^{-}$ ion (*m/z* 197) must be preceded by NO_2/ONO isomerization, thus allowing $NO^{\bullet}$ release. The sequential MS^3 experiments examining further decompositions of the product $[M\text{-}OH]^{-}$ ion (*m/z* 210) yielded the *m/z* 182 and *m/z* 152 fragment ions due to consecutive losses of CO followed by $NO^{\bullet}$ (CID spectrum not shown). MS^3 of the fragment $[M\text{-}NO]^{-}$ ion (*m/z* 197), isolated and activated by collision, gave the competitive losses of $OH^{\bullet}$ or $NO^{\bullet}$ producing the ions at *m/z* 180 and *m/z* 167, respectively (Figure 1 b).

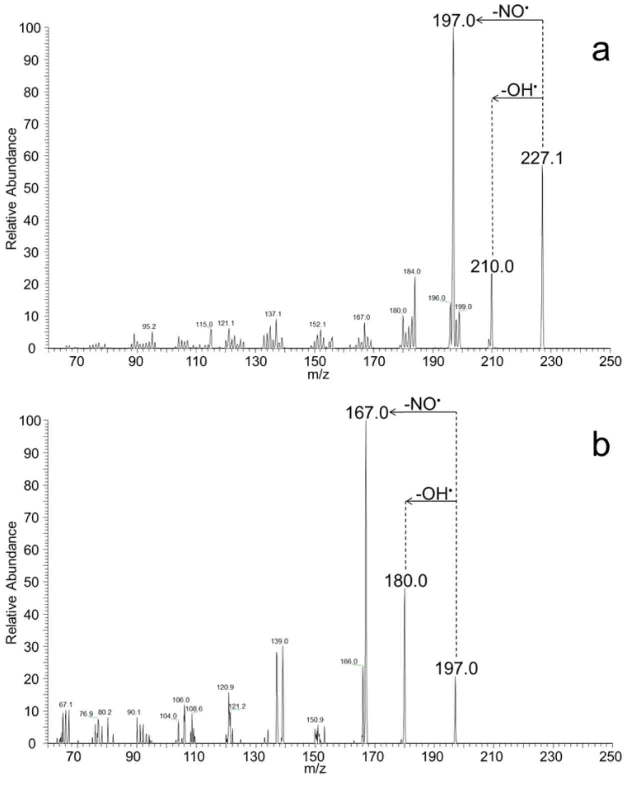


Figure 1. Sequential MS^n experiments in LTQ cell from radical $[M]^{\bullet -}$ anion of TNT: a) MS^2 CID spectrum of *m/z* 227 (25% NCE) and b) MS^3 CID spectrum of the product *m/z* 210 ion (15 % NCE).

On the other hand, fragmentation of $[M-H]^-$ (*m/z* 226) of TNT (**M1**) yielded a fragment ion at *m/z* 196 by 30 Da neutral release, a loss that is commonly attributed to departure of $NO^\bullet$ (Figure 2 a). However, accurate mass measurements at high resolution revealed, surprisingly, this 30 Da loss corresponds to CH_2O instead of $NO^\bullet$ (Table 1). The accurate *m/z* measured for the corresponding product ion at *m/z* 196 differed from the theoretical *m/z* of $[(M-H)-NO]^{-\bullet}$ (**M3**) by approximately 60 ppm whereas it differed by only 0.03 ppm from the theoretical *m/z* of $[(M-H)-CH_2O]^-$ (**M2**) allowing unambiguous assignment of the loss of CH_2O. Note that the loss of $NO^\bullet$ is still observed in low abundance (less than 1 % of the base peak). To our knowledge, this is the first time that the loss of CH_2O is described for deprotonated TNT prepared in API mode (ESI or APCI). Then, this CH_2O loss from $[M-H]^-$ can be explained by an intramolecular cyclisation induced by nucleophilic attack of the nitro group on the neighboring methylene group followed by CH_2O release (Scheme 2). We also noticed that the loss of $OH^\bullet$, expected by the "*ortho* effect", does not take place, probably due to the hindrance produced by the two planar $-NO_2$ groups, each in *ortho* position, impeding the H-transfer.

Table 1. Accurate mass measurements performed at high resolution of negative molecular species from (a) $[M-H]^-$ and (b) $M^{\bullet-}$ precursors of TNT.

Precursor ion	**Assigned ion**	**Elemental composition**	**Theoretical accurate *m/z***	**Measured accurate *m/z***	**Δ ppm**
(a) $[M-H]^-$	$[TNT-H]^-$	$C_7H_4O_6N_3$	226.01055	226.01054	0.08
	$[(TNT-H)-NO]^{\bullet-}$	$C_7H_4O_5N_2$	196.01256	Not observed	------
	$[(TNT-H)-CH_2O]^-$	$C_6H_2O_5N_3$	196.0000	195.9999	0.03
(b) $M^{\bullet-}$	$[TNT]^{\bullet-}$	$C_7H_5O_6N_3$	227.01838	227.01830	0.3
	$[TNT-OH]^{\bullet-}$	$C_7H_4O_5N_3$	210.01564	210.01569	0.2
	$[TNT-NO]^{\bullet-}$	$C_7H_5O_5N_2$	197.02039	197.02036	0.2

Sequential fragmentation of the product $[(M-H)-CH_2O]^-$ ion at *m/z* 196 yielded mainly the *m/z* 179 ion by release of $OH^\bullet$ (Figure 2 b). In addition, the *m/z* 196 product ion gives rise to formation of *m/z* 166 by release of $NO^\bullet$ in low abundance after NO_2/ONO isomerization. This $NO^\bullet$ loss is competitive with $OH^\bullet$ loss, although the latter is favored.

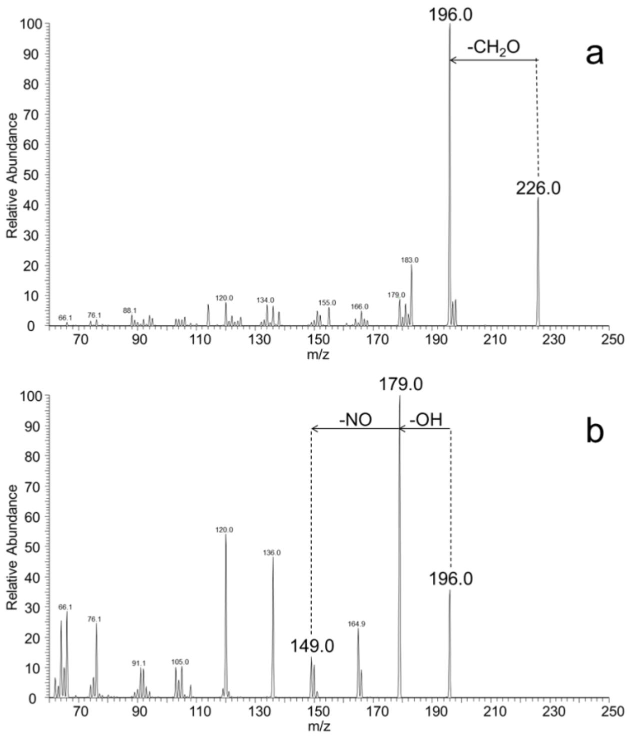
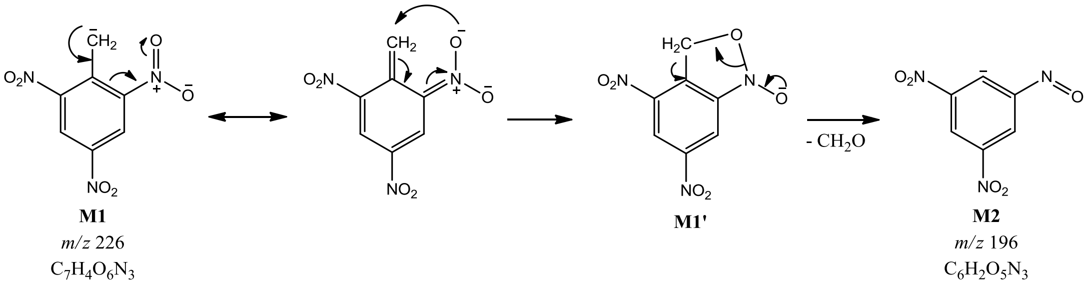


Figure 2. Sequential MS^n experiments in LTQ cell from deprotonated $[M-H]^-$ anion of TNT a) MS^2 CID spectrum of *m/z* 226 (25% NCE), and b) MS^3 CID spectrum of the product ion at *m/z* 196 ion (15 % NCE).



Scheme 2. Proposed fragmentation pathways for the unexpected loss of CH_2O

To support this experimental observation, calculations have been performed using the GAUSSIAN 09[16] suite of program. Geometry optimization and single point energy calculations were carried out with the OPBE functional[17, 18] coupled to the 6-311++G(d,p) basis set[19]. Results are presented in Figure 3 and show that formation of **M2** ion is a barrierless process (ΔG_{298} = 133 kJ/mol) whereas the loss of $NO^\bullet$ from **M1** requires first a NO_2/ONO isomerization with a pretty high barrier (ΔG_{298} = 179 kJ/mol) followed by formation of **M3** ion (ΔG_{298} = -25 kJ/mol). Formation of **M2** appears thus to be the most favorable decomposition way of **M1** from a kinetic and energetic point of view, which is in agreement with experimental study.

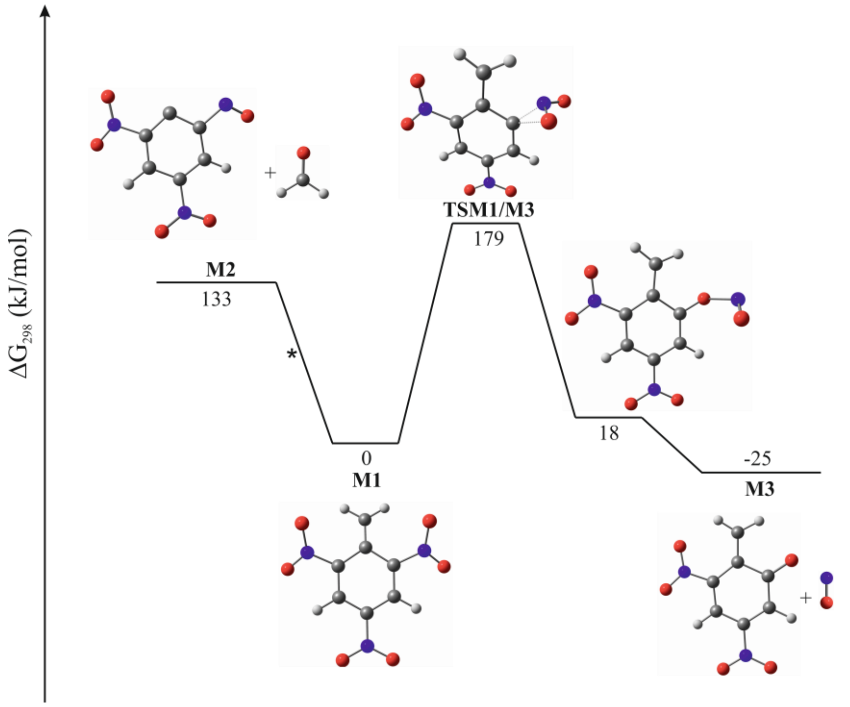


Figure 3. Free energies (ΔG_{298} in kJ/mol) for the dissociation of **M1** ion. *Dissociation of **M1** into **M2** proceeds via the **M1'** structure.

To summarize, we report herein an unexpected CH_2O neutral loss from deprotonated TNT at *m/z* 226, instead of the usually presumed $NO^{\bullet}$ loss, as observed from the radical anion *m/z* 227. This new finding was established by accurate mass measurements at high resolution performed in a LTQ-Orbitrap XL giving a new insight into the dissociation process of deprotonated TNT. It now seems appropriate to probe other multi-substituted nitroaromatic systems for the production of formaldehyde loss rather than $NO^{\bullet}$ radical departure during dissociation of their corresponding anions.

Yours,

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