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

Cécile Hubert, Adrián Schwarzenberg, Richard Cole, Héloïse Dossmann,
Xavier Machuron-Mandard, Jean-Claude Tabet

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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-H]⁻ and (ii) electrochemical reduction M⁻•.[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• (implicating the "*ortho* effect*", Scheme 1), or by loss of NO• (after NO_2/ONO isomerization). In addition, NO_2• release was also observed.
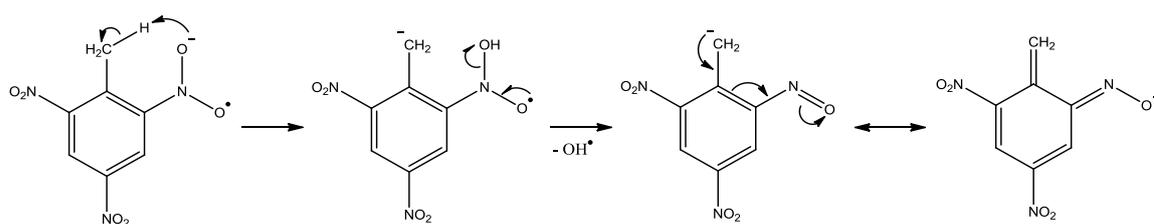
Scheme 1. Stepwise OH• release implicating the "*ortho* effect" promoted by the radical anion [M]⁻• (*m/z* 227) of TNT.

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-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: $[M-OH]^{-}$ at *m/z* 210 and $[M-NO]^{-}$ 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 $[M-OH]^{-}$ ion (*m/z* 210) is stabilized by delocalization of the negative charge (Scheme 1). On the other hand, the fragment $[M-NO]^{-}$ 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 $[M-OH]^{-}$ 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 $[M-NO]^{-}$ 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).
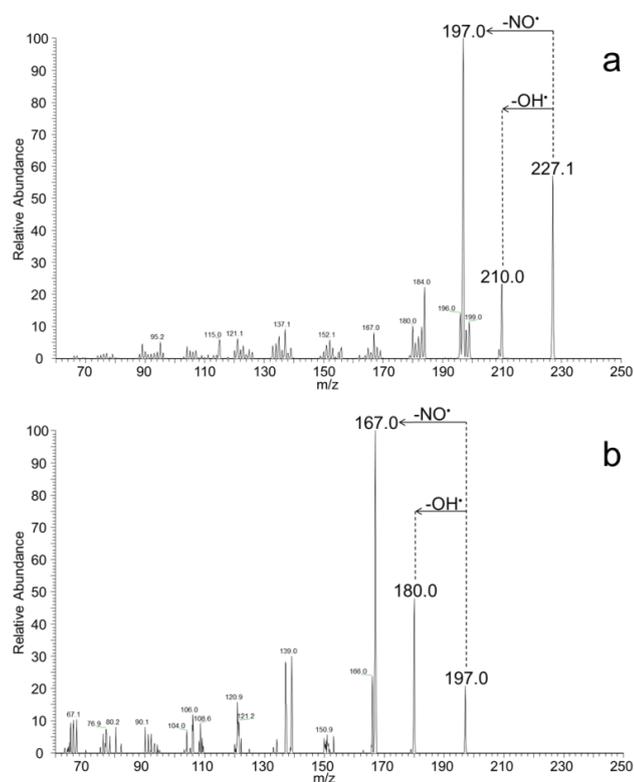

Figure 1. Sequential MS^n experiments in LTQ cell from radical $[M]^{\bullet-}$ anion of TNT: a) MS^2 CID spectrum of *m/z* 227 (25% NCE) and b) MS^3 CID spectrum of the product *m/z* 210 ion (15 % NCE).

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$_2$O 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$_2$O]$^-$ (**M2**) allowing unambiguous assignment of the loss of CH$_2$O. 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$_2$O is described for deprotonated TNT prepared in API mode (ESI or APCI). Then, this CH$_2$O 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$_2$O 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$_7$H$_4$O$_6$N$_3$	226.01055	226.01054	0.08
	[(TNT-H)-NO]$^{\bullet-}$	C$_7$H$_4$O$_5$N$_2$	196.01256	Not observed	------
	[(TNT-H)-CH$_2$O]$^-$	C$_6$H$_2$O$_5$N$_3$	196.0000	195.9999	0.03
(b) M$^{\bullet-}$	[TNT]$^{\bullet-}$	C$_7$H$_5$O$_6$N$_3$	227.01838	227.01830	0.3
	[TNT-OH]$^{\bullet-}$	C$_7$H$_4$O$_5$N$_3$	210.01564	210.01569	0.2
	[TNT-NO]$^{\bullet-}$	C$_7$H$_5$O$_5$N$_2$	197.02039	197.02036	0.2

Sequential fragmentation of the product [(M-H)-CH$_2$O]$^-$ 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.
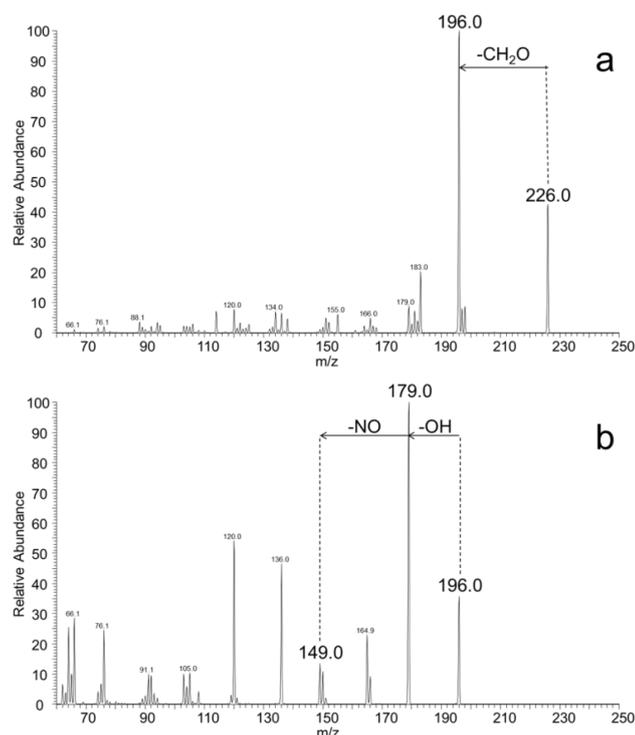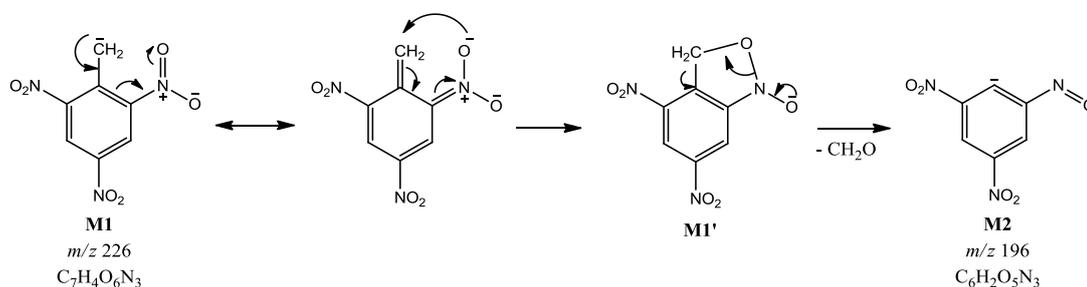
Figure 2. Sequential MS^n experiments in LTQ cell from deprotonated [M-H]$^-$ anion of TNT a) MS^2 CID spectrum of *m/z* 226 (25% NCE), and b) MS^3 CID spectrum of the product ion at *m/z* 196 ion (15 % NCE).

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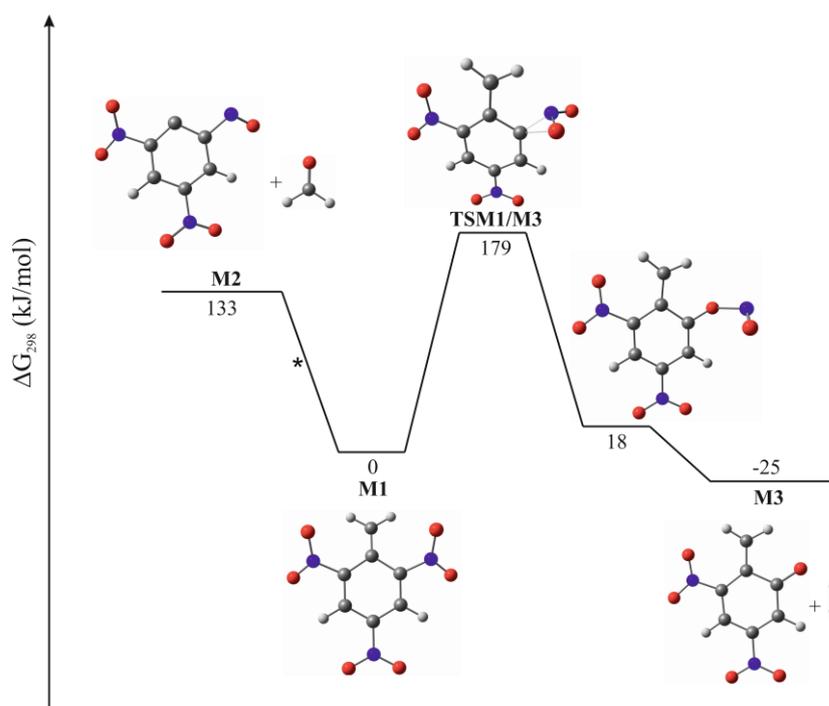
Figure 3. Free energies (ΔG_{298} in kJ/mol) for the dissociation of **M1** ion. *Dissociation of **M1** into **M2** proceeds via the **M1'** structure.

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,

Cécile Hubert[2]*, Adrián Schwarzenberg[1]*, Richard B. Cole[1], Héloïse Dossmann[1], Xavier Machuron-Mandard[2], Jean-Claude Tabet[1]

Authors* have contributed equally to the work as first author

[1]UPMC, IPCM/CSOB, UMR 7201, 4 place Jussieu, 75252 Paris Cedex, France
[2]CEA, DAM, DIF, F-91297, Arpajon, France

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