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Gas-phase dissociation of 1,4-naphthoquinone derivative anions by electrospray ionization tandem mass spectrometry

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Gas-phase dissociation pathways of deprotonated 1,4-naphthoquinone (NQ) derivatives have been investigated by electrospray ionization tandem mass spectrometry (ESI-MS/MS). The major decomposition routes have been elucidated on the basis of quantum chemical calculations at the B3LYP/6-31+G(d,p) level. Deprotonation sites have been indicated by analysis of natural charges and gas-phase acidity. NQ anions underwent an interesting reaction under collision-induced dissociation conditions, which resulted in the radical elimination of the lateral chain, in contrast with the even-electron rule. Possible pathways have been suggested, and their mechanisms have been elucidated on the basis of Gibbs energy and enthalpy values for the anions previously described at each pathway. Copyright © 2009 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Keywords: fragmentation; theoretical calculations; quinone; gas-phase acidity

Introduction

Quinones are ubiquitous in nature and they display several biological activities that have been related to their redox potential.[1,2] Their cytostatic and antimicrobial activities are due to their ability to act as potent inhibitors of electron transport.[2] Numerous quinones are obtained from natural sources[3,4] and many can be synthesized.[5] Therefore, techniques for their characterization and structural elucidation have become mandatory from the perspective of clinical and preclinical trials.

Electrospray ionization mass spectrometry (ESI-MS) is an important technique for the analysis of organic and inorganic compounds from biological matrices.[6] The versatility of this technique can be associated with the generation of protonated,[7,8] deprotonated,[9] metal complex,[10] and radical species[11] in the gas phase. These species can be further analyzed by tandem mass spectrometry.[12,13] There are a few sources available for the structural elucidation of most classes of compounds based on dissociation of their protonated or deprotonated ions. Only small libraries of product ion mass spectra of $[M + H]^+$ and $[M - H]^-$ ions have been reported for some 400 drugs.[14] On the other hand, structural analysis based on the collision-induced dissociation (CID) spectra of peptide $[M + H]^+$ and $[M - H]^-$ ions and related biomolecules is straightforward and well documented, and large libraries concerning the mass spectra of these compounds are available.[15] Therefore, the MS/MS study of several different classes of substances is necessary to furnish information about their fragmentation pathways. The fragmentation pathways of $[M - H]^-$ are usually applied to molecules with acid groups, such as carboxylic acids and phenols.[16,17] Recently, Levsen and coworkers[18] have applied CID to protonated and deprotonated molecules generated by ESI or atmospheric pressure chemical ionization (APCI). The $[M + H]^+$ and $[M - H]^-$ ions have been analyzed and the experimental results have been compared with those obtained by the CBS-QB3 model,[18] which has furnished important data for MS/MS users.

Several ionization techniques have been employed in the analysis of quinonoid compounds, including electron impact mass spectrometry (EI-MS),[19] matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF),[20] APCI-MS,[21] two-step laser desorption/post-photoionization mass spectrometry (L2MS/PIMS),[22] and photoionization mass spectrometry (LIMS).[23] Electrospray ionization tandem mass spectrometry (ESI-MS/MS) through CID has been employed to characterize some protonated derivatives of 1,4-naphthoquinone, where the presence of a hydrogen atom in the lateral chain characterizes the fragmentation pathways.[24] For such studies, however, previous knowledge of the fragmentation pathways of a series of compounds exhibiting a conserved structural core is often necessary.[25–27]

The dynamic transformation intrinsic to MS/MS experiments may lead to different fragmentation pathways, thus generating different ions. This process may depend on the internal stability,[28] activation energy, and kinetic phenomena[29] of each intermediate species during the fragmentation process. In this sense, the study

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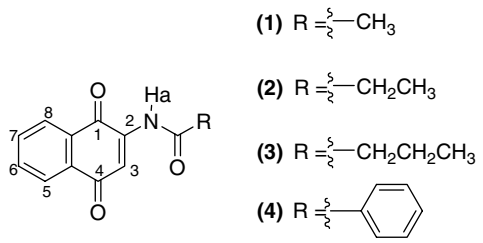

Figure 1. Structure of the 1,4-naphthoquinone derivatives. 2-acetylamino- **(1)**; 2-propyonilamino- **(2)**; 2-butyrilamino-, **(3)** and 2-benzoylamino-1,4-naphthoquinone **(4)**. Ha indicates the most acid hydrogen in molecules. Hb are the α-carbonyl hydrogen.

of this intermediate species in the gas phase is not an easy task, but the information that can be obtained from such investigation may be useful for further MS analysis as well as for organic synthesis and structural elucidation of natural compounds.[25–27,30] For all these reasons, the theoretical approach can be useful for the understanding of the dissociation process and stability of each species during the fragmentation.[18,24,25,27,30] Mass spectrometry fragmentation processes are routinely analyzed using quantum mechanics, in order to postulate plausible structures for unstable intermediate species, such as radicals, carbocations, carbanions, and other species, whose lifetime is often limited to within mass spectrometers.[31,32]

As part of our ongoing project on the use of ESI-MS/MS in the structural elucidation of natural products and new products from organic synthesis, we report here the gas-phase dissociation of a set of 2-acylamino-1,4-naphthoquinone derivatives (Fig. 1) previously synthesized from 1-naphthol.[33] These molecules exhibit biological activities against *Biomphalaria glabrata* and *Aedes aegypti* larvae.[33] Recently, anticancer studies have suggested that these molecules act as important antitumor agents.[34]

In order to rationalize the dissociation pathways, the relative Gibbs energy and enthalpy values of the intermediate and product ions have been calculated using the B3LYP/6-31+G(d,p) model.[35–37] The deprotonation sites have been indicated through analysis of natural charges and gas-phase acidity for each compound analyzed in this study.

Experimental

Chemicals

The 2-acetylamino- **(1)**, 2-propyonilamino- **(2)**, 2-butyrilamino- **(3)**, and 2-benzoylamino-1,4-naphthoquinones **(4)** were obtained from 1-naphthol as previously reported (Fig. 1).[33] Acetonitrile (HPLC grade) was obtained from Aldrich. Deionized water was used throughout the study.

Mass spectrometry

For mass spectrometry analysis, stock solutions of each 2-acylamino-1,4-naphthoquinone derivatives (Fig. 1) were prepared from 0.1 mg ml^{-1} solutions in acetonitrile/water (2 : 1 v/v).

High-resolution ESI-MS analyses were performed on an Ultro-TOF-Q Bruker Daltonics instrument fitted with an electrospray ion source operating in the negative ion mode. Samples were diluted more than 10 times and, directly infused into the ionization source at a flow rate of 10 µl min^{-1}.

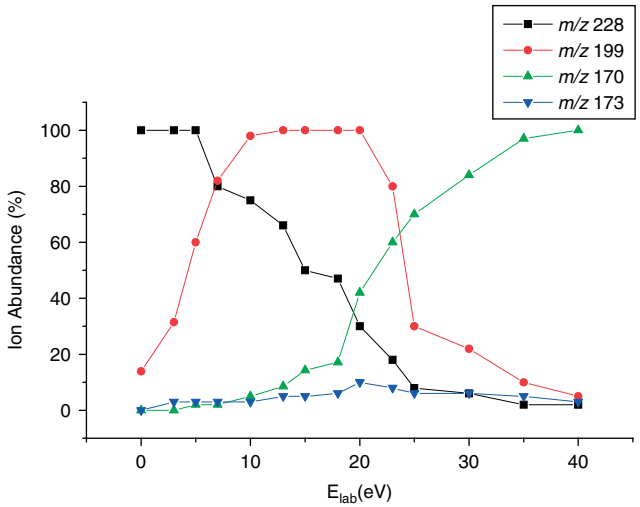

Figure 2. Energy-resolved plot for 2-propyonilamino-1,4-naphtoquinone.

The accuracy masses were obtained by using of TFA-Na$^+$ (sodiated trifluoroacetic acid) as internal standard. The source block and desolvation temperature was 150 °C. The optimum energy applied at the capillary emitter was tested so that a higher intensity of deprotonated molecule [M − H]$^-$ would be obtained. The deprotonated molecule (precursor ion) was selected and fragmented by CID, using N_2 as the collision gas. The variation at E_{lab} was performed in order to obtain the energy-resolved curves. We carry out the MS/MS experiments at different E_{lab} values, and obtain energy-resolved fragmentation curves by varying the E_{lab} (0–40 eV). The optimum energy (E_{lab}) to obtain the main fragment (*m/z* 199) was obtained as 20–25 eV. For compounds **(1)**, **(2),** and **(3)** the energy-resolved plots are similar to those of Fig. 2.

We conclude that multiple collisions can occur during the MS/MS analyses and the formation of *m/z* 199 from [M − H]$^-$ occurs by in-source dissociation (see Fig. 2).

Theoretical calculations

All molecules were optimized by the B3LYP/6-31+G(d,p) model[35–37] using Gaussian98 suite of programs.[38] The vibrational frequencies indicated that all the structures were the minima in the potential energy surface. The natural population analysis (NPA)[39] was obtained by the NBO 5.0 software[40,41] interfaced with Gaussian98. Deprotonation sites were calculated by comparing the natural charge of the hydrogen atoms and the use of ΔG_{acid}. The ionic and covalent characters of the bonds were calculated by natural resonance theory (NRT) analyses.[42]

The ΔG_{acid} value for each molecule was obtained by subtracting the thermally corrected Gibbs energy of the acid from the sum of the thermally corrected Gibbs energies of the anion and proton. The ΔG_{acid} values were obtained following the equation QH → Q$^-$ + H$^+$, where the Gibbs energy of the proton was considered to be −6.28 kcal mol^{-1}.[43] Thus, the most stable anions were proposed, as well as the fragmentation pathways suggested by Gibbs energy calculations at 298.15 K. For each potential energy profile, the relative Gibbs energies at 298 K (ΔG_{298}) and relative enthalpies at 298 K (ΔH_{298}) are reported. It is noteworthy that the Gibbs energy values should be interpreted with caution since the thermodynamic equilibrium might not be reached under CID conditions.

Table 1. Natural charges for the Ha and Hb atoms, (Fig. 1) in a. u., ΔG_{acid}, in kcal mol^{-1}, and ionic character of the bonds N–H and C_α–H for the 2-acylamino-1,4-naphthoquinones using the B3LYP/6-31+G(d,p) model

Molecule	Ha	Hb	$\Delta G_{acid\ (N-H)}$	$\Delta G_{acid\ (C\alpha-H)}$	N–H	C_α–H
(1)	0.45973	0.27589	328.13	344.78	0.48	0.27
(2)	0.45841	0.26212	329.41	342.64	0.48	0.26
(3)	0.45907	0.26885	329.20	341.69	0.48	0.25
(4)	0.45637	–	324.50	–	0.48	–

The breakdown product and the corresponding transition state (TS) energies were obtained and are reported relative to their respective precursors. A standard approach for TS characterization was used. To this end, the synchronous transit guided quasi-Newton method developed by Schlegel and coworkers was employed,[44] and a TS with only one imaginary vibrational frequency was found. The connectivity between the TS, precursor, and product ions was obtained by performing intrinsic reaction coordinate (IRC) calculations.[45]

Results and Discussion

Structure of the [M − H]$^-$ ions

The first step toward the elucidation of a possible fragmentation pathway is obtained from the knowledge of the reaction site.[30,46,47] In some cases, the deprotonation site is easily evidenced on the basis of organic chemistry concepts alone. However, when the molecule exhibits several functional groups that are not single proton donors, the search for a specific reactive group must be accomplished.[30,47,48] Thus, in order to find out the main deprotonation site for molecules **(1)**–**(4)**, two different criteria were applied: atomic charge analysis and gas-phase acidity (ΔG_{acid}). Except for **(4)**, all exhibited two possible deprotonation sites: the amide hydrogen (Ha) and the α-carbonyl hydrogen in the lateral chain (Hb) (Fig. 2).

The hydrogen natural charges indicated that the hydrogen atom bound to the nitrogen is more acid than the one bound to C_α (Table 1). In agreement with the natural charges, the natural bond order showed that the N–H bond has a larger ionic character than C_α–H. These values, in combination with the gas-phase acidity, suggest that the most stable ion should be formed by proton abstraction at the N–H bond (see Table 1). Theoretical calculations also indicated that the most stable ions are obtained by hydrogen abstraction from the nitrogen atom as evidenced by ΔG_{acid} (Table 1). The lower ΔG_{acid} values indicate that the N–H is the most acidic site and, this bond undergoes heterolytic cleavage more easily. By considering the large difference between the energy of the isomers ($\Delta\Delta G > 12$ kcal mol^{-1}), deprotonation at C_α is thermodynamically unfavorable. Thus, our results show that heterolytic cleavage preferentially occurs at the N–H bond, in agreement with the work of Da Silva *et al.*[49] These authors reported that in N-heterocyclic compounds the N–H bonds are weaker than C–H bonds, thus supporting the idea that the hydrogen atoms at the N atom are more acidic.

ESI-MS/MS analysis

The CID spectra of the [M − H]$^-$ ions for compounds **(1)**–**(4)** have common interesting features. All spectral data obtained by fragmentation of the [M − H]$^-$ ions are summarized in Table 2. The most intense fragment ion in the ESI-MS/MS spectra of all the derivatives is *m/z* 199, **B** (Fig. 3(A) and (B)). By analyzing the mass differences between [M − H]$^-$ and *m/z* 199, we can conclude that it is formed via R• radical elimination from the [M − H]$^-$ ion (Fig. 1).

The main difference in the ESI-MS/MS spectra of all the compounds lies in the spectrum of 2-benzoylamino-1,4-naphthoquinone **(4)**, which exhibits the fragment ion **C** (*m/z* 173) with 94% intensity (the second most intense in the spectrum (Fig. 3(B) and Table 2). The spectral data for **(1)**, **(2)**, and **(3)** show that the intensity of the **E** ion is higher than that of the **C** ion. In contrast, the intensities of these ions are interchanged in the case of **(4)**. The formation of the **C** ion occurs by neutral loss of RCN from [M − H]$^-$.

In order to understand these fragmentation processes, the **B** ion (*m/z* 199) was generated by in-source dissociation and fragmented by CID (Fig. 3(C)). The main fragments reported in the MS/MS spectra of **B** were **E** (*m/z* 170) and *m/z* 160. Elimination of 29 mass units (•CHO) from **B** led to the formation of **E** (*m/z* 170). It is worth noting that the **C** ion does not form via dissociation of the **B** ion.

Analysis of the tandem mass spectra obtained for [M − H]$^-$ gives evidence that another dissociation pathway is triggered in the case of these ions, by the formation of the **D** (*m/z* 172) and **F** (*m/z* 145) ions (Fig. 3(A), (B) and Table 2). The **D** ion is observed in the spectra of **(1)**–**(3)**, and it can be considered an indication of α-carbonyl hydrogen in the 2-acylamino moiety. In studies with protonated compounds, our research group has shown that tandem mass spectrometry analyses for the same protonated molecules generate the *m/z* 174 and *m/z* 146 ions, which may also serve as diagnostic ions for the quinonoid moiety.[24] In the

Table 2. Ionic Masses, *u*, and intensities (in parentheses) obtained by ESI-MS/MS analyses

		Compounds			
Ion	Nominal mass molecular formulae	**(1)**	**(2)**	**(3)**	**(4)**
A	[M−H]$^-$	214.0486 (12)	228.0659 (30)	242.0800 (28)	276.0657 (56)
B	199.02749 $C_{11}H_5NO_3^{-\bullet}$	199.0267 (100)	199.0254 (100)	199.0251 (100)	199.0252 (100)
C	173.0244 $C_{10}H_5O_3^-$	173.0213 (5)	173.0252 (10)	173.0252 (2)	173.0217 (94)
D	172.0404 $C_{10}H_6NO_2^-$	172.0404 (8)	172.0374 (11)	172.0382 (7)	*
E	170.02475 $C_{10}H_4NO_2^-$	170.0227 (28)	170.0211 (42)	170.0207 (37)	170.0209 (5)
F	145.0295 $C_9H_5O_2^-$	145.0254 (7)	145.0252 (7)	145.0252 (7)	145.0260 (23)

* Not observed

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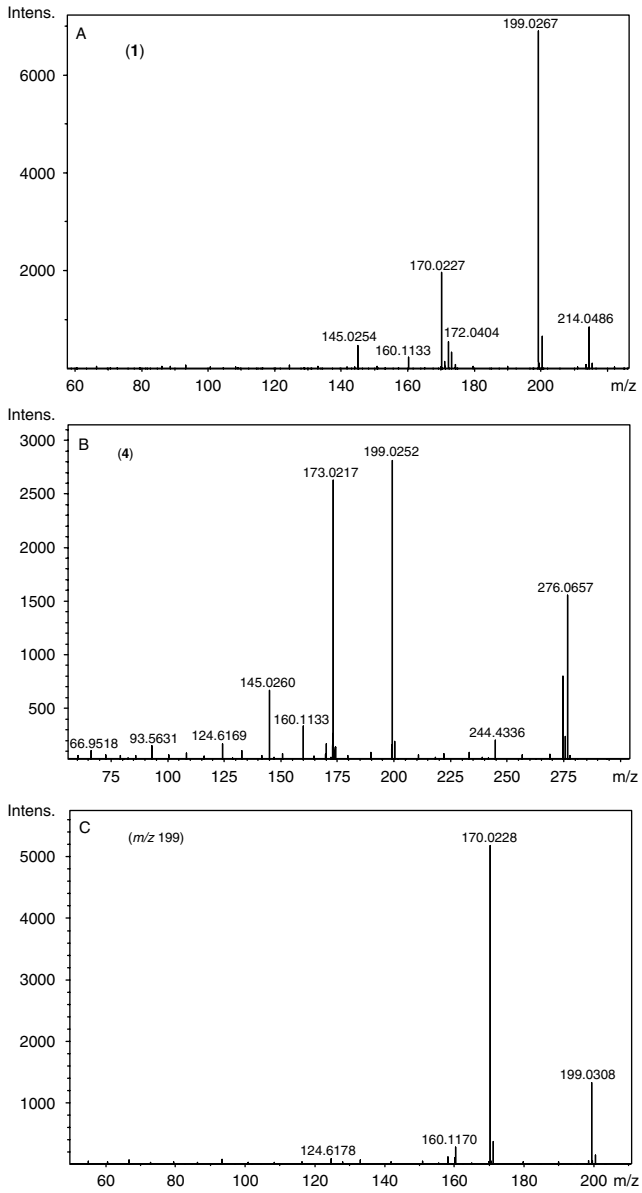


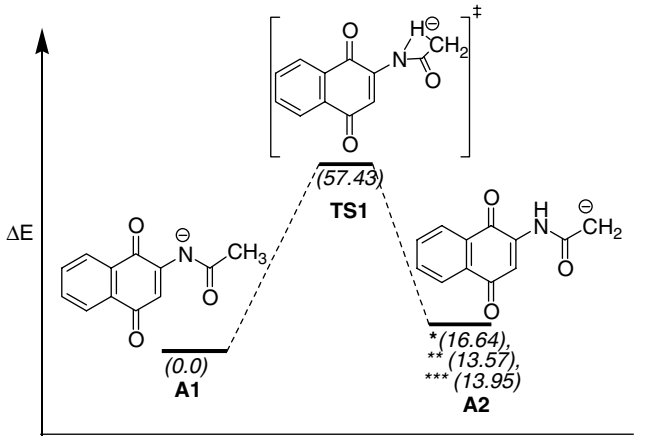
Figure 3. ESI-MS/MS spectra. **(1)** ESI-MS/MS spectrum for [**(1)** − H]$^-$ of 2-acetylamino-1,4-naphthoquinone. **(4)** ESI-MS/MS spectrum for [**(4)** − H]$^-$ of 2-benzoylamino-1,4-naphthoquinone. **(*m/z* 199)** ESI-MS/MS spectrum for *m/z* 199 of 2-butyrilamino-1,4-naphtoquinone.

positive mode, the *m/z* 174 ion does not occur for molecule **(4)** as in the case of the *m/z* 172 ion in the negative mode of [M − H]$^-$. For this reason, the *m/z* 172 peak can be attributed to the presence of α-carbonyl hydrogen atom within the lateral chain.

On the basis of the results described above, we propose two different fragmentation pathways for the [M − H]$^-$ ions. The first pathway consists in the formation of the **D** and **F** ions, while the second leads to generation of the **B, C**, and **E** ions.

Fragmentation pathways

The [M − H]$^-$ ions of NQ can be formed by either C_α or N deprotonation, although the most energetically favored isomer occurs by deprotonation at N, as previously discussed. The transition state (TS1) for hydrogen rearrangement from C_α to the nitrogen of [M − H]$^-$ was obtained (Scheme 1). However, the



Scheme 1. Energy profile for deprotonated 2-acetylamino-1,4-naphthoquinone. All values are in kcal mol^{-1}. Bold numbers indicate the studied compound. Values between parentheses are: * relative Gibbs energies at 298 K, ** relative enthalpies at 298 K and ***relative (E + ZPE) in kcal mol^{-1}. Absolute energies for **A1**: G_{298} = −742.510472; H_{298} = −742.451452; E + ZPE = −742.465952, in Hartree. **TS1** is a transition state which connects **A1** and **A2**.

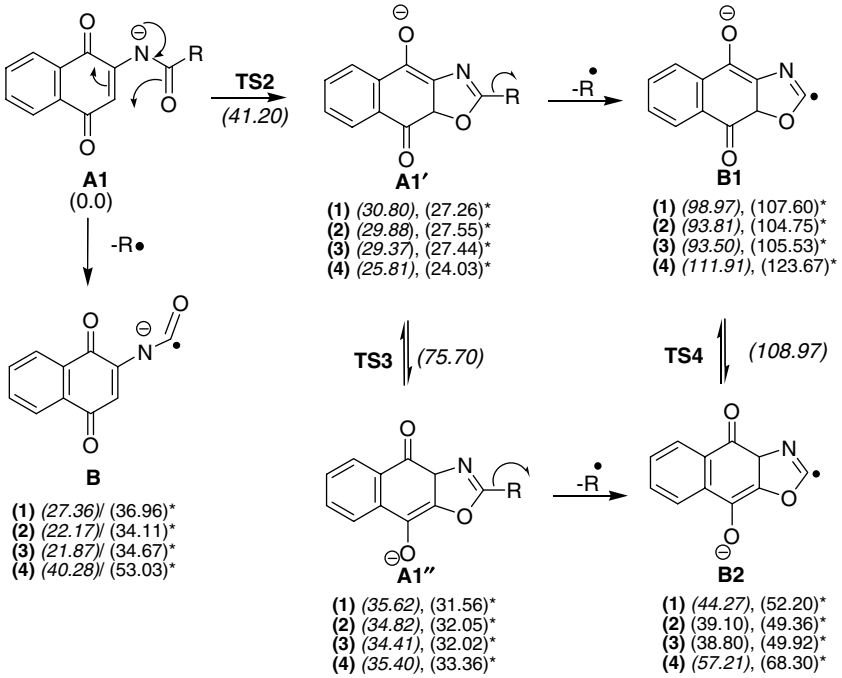
critical energy necessary for conversion of **A1** into **A2** is 57.43 kcal mol^{-1} (2.49 eV) in the case of 2-acetylamino-1,4-naphthoquinone, which is greater than the maximum available energy, which is 2.31 eV. This value decreases 2 kcal mol^{-1} for compounds **(2)** and **(3)**.

The CID process occurs with high energy, where the internal energy necessary for the fragmentation can be supplied by multiple collision occurring during the MS/MS experiments, and the energy required for the TS to be reached shows that the fragmentation process can be triggered by any of the [M − H]$^-$ isomers **A1** or **A2**, since these isomeric species can coexist during the ionization process.[50] For this reason, two alternative fragmentation pathways were analyzed for each [M − H]$^-$ species, **A1** and **A2**.

Formation of the *m/z* 199, *m/z* 173, and *m/z* 170 ions

The fragmentation pathways of **A1** produce common ions that are observed for all the analyzed NQ derivatives. It is expected that the deprotonated ions (closed shell) dissociate to an even-electron product ion and a neutral molecule rather than an odd-electron ion and a radical. Although the even-electron rule holds true for most dissociations, several exceptions to this rule have been reported. Some exceptions have been observed[51,52] for the high-energy CID of [M + H]$^+$ and [M − H]$^-$, such as in the case of methyl elimination from protonated mycosporine[53] and deprotonated metoclopramide[54] or hydroxyl elimination from triazines.[48] Some examples of radical anions produced by CID of molecules ionized by ESI have been described for nitro compounds,[55] amines,[56] amides,[56] and flavonoids.[57] In a review article, Karni and Mandelbaum have pointed out that the dissociation of primary even-electron products generated by electron impact also brings exceptions to the even-electron rule.[58]

We suggest that the formation of product ion **B** (*m/z* 199), which is the most intense fragment in the MS/MS spectra of all the compounds (Table 1), can occur by two different ways: one by direct cleavage of **A1,** and another after cyclization and subsequent R• elimination (Scheme 2). The elimination of R• occurs by direct cleavage of the C(O)–R bond, and the enthalpy



Scheme 2. Proposed mechanisms for formation of the *m/z* 199 ion. Values in italic are relative G_{298} and * values are relative H_{298}, in kcal mol^{-1}. Values below the arrow are critical energies for the reactions. Absolute Gibbs energies at 298K for **A1** are **(1)** −742.510472, **(2)** −781.799969, **(3)** −821.090698, and **(4)** −934.214416, in Hartree. **TS2, TS3,** and **TS4** are the transition states which show only one negative vibrational frequency value.

for this reaction was computed as being approximately 40 kcal mol^{-1} for **(4)** and this values decreases for the other derivatives.

By cyclization, a five-membered ring is formed by the nucleophilic attack of the oxygen carbonyl on the acylamino group to the β-carbon C(3) of the α,β-unsaturated carbonyl quinonoid moiety **A1′**. The critical energy for formation of TS2 was calculated as 41.20 kcal mol^{-1}. The relative enthalpy required for cyclization of **(1)** was calculated as 27.26 kcal mol^{-1} for formation of **A1′** from $[M - H]^-$. This variation in value is very small for the other compounds (Scheme 2). The relative Gibbs energy values behave in the similar way.

The cyclic enolate ion **A1′** may isomerize to **A1″**, which is more unstable. In order to connect **A1′** and **A1″**, a transition state (**TS3**) was proposed for the conversion of these two enolate ions (Schemes 2 and 3). Our calculations indicated that the energy barrier for this step is 44.90 kcal mol^{-1}. The relative energy between these two enolates is less than 10 kcal mol^{-1} for all the compounds. Thus, formation of *m/z* 199 was suggested through fragmentation of **A1′** and **A1″**. The dissociation reaction **A1′** → **B1** is energetically unfavorable when compared to the decomposition **A1″** → **B2**. After formation of the **A1″** ion, R• is eliminated, and this step needs 8.65 kcal mol^{-1} in the case of 2-acetylamino-1,4-naphthoquinone **(1)**, but decreases with increasing number of carbon atoms in the lateral chain. As for 2-benzoylamino-1,4-naphthoquinone **(4)**, the Gibbs energy required for this step is 21.81 kcal mol^{-1} (Scheme 2).

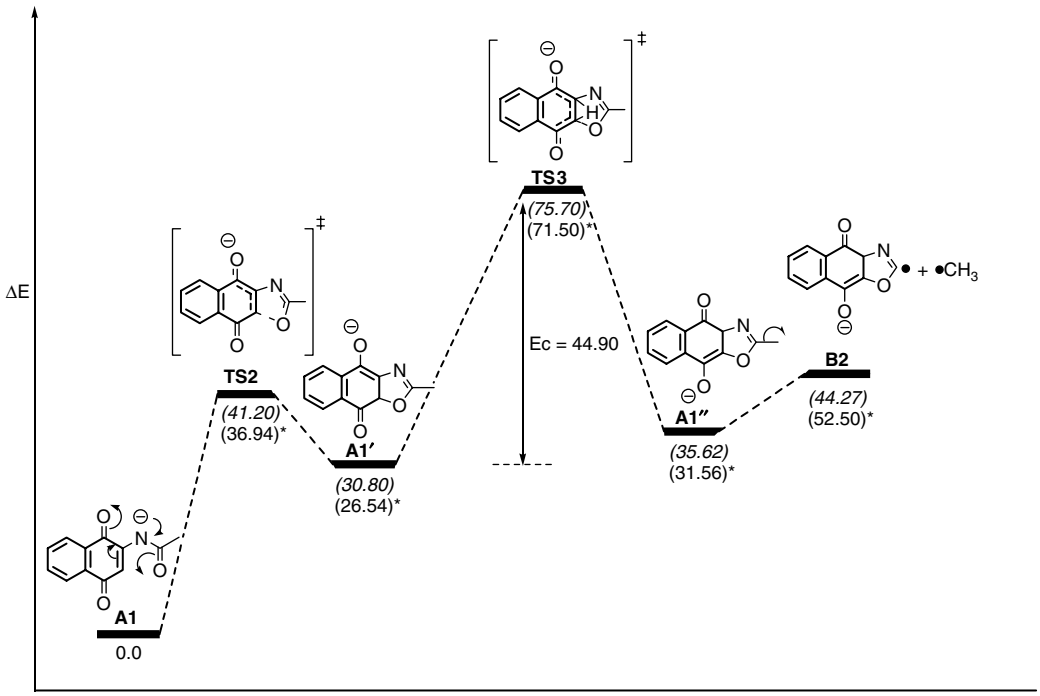
The fragmentation pathway from **A1′** to *m/z* 199 (**B1**) occurs with 68.17 kcal mol^{-1} (difference between relative energies of **A1′** and **B1**) for molecule **(2)** and this value increases with increasing lateral chain (Scheme 2). This characterizes the pathway **A1″** → **B2** as being the most thermodynamically favored. The relative enthalpies of **B1** are higher than 104 kcal mol^{-1}, which shows that the formation of these ions is an endothermic process. The **B2** ion is thermodynamically more stable than **B1,** and the critical energy to convert the **B1** to **B2** was computed as 10.71 kcal mol^{-1}. Thus, the formation of *m/z* 199 should occur through the **A1″** → **B2** pathway, since the dissociation **A1′** → **B1** is a more energetic process (enthalpies for this reaction are more than 80 kcal mol^{-1}).

Scheme 2 can be understood by analyzing the surface energy profiles Scheme 3, where the most favorable pathway is suggested.

We propose that the *m/z* 199 ion is distonic[59] and can be stabilized through its several isomers, as indicated in Scheme 4. The Mulliken spin densities[60] have been calculated for all the *m/z* 199 ions at the B3LYP/6-31+G(d,p) level to identify the charge and spin sites (see Supporting Information). The highest intensity of this ion in tandem mass spectra is explained in terms of the forms that this ion can acquire (Scheme 4). The hydrogen shift can occur between **B1** and **B2** via a transition state (**TS4**), where formation of **B2** is an exothermic process ($\Delta H_{298} = -55.40$ kcal mol^{-1}). The formation of **B3** from **B2** occurs by hydrogen shift, leading to the conjugated species.

Dissociation of **B2** and **B3** by radical elimination of •CN (26 mass units) or •CHO (29 mass units) may result in the product ions **C** (*m/z* 173) and **E** (*m/z* 170), respectively. However, the **B** ion (*m/z* 199) does not dissociate into **C** (Fig. 3(C)), and the rearrangement of **B2** to **B3** is the most favorable process (Scheme 4). The formation of **E** from *m/z* 199 requires 153 kcal mol^{-1}, which characterizes the intensity of the **C** ion in the MS/MS spectra of $[M - H]^-$ ions (Table 2). We suggest that the formation of *m/z* 170 should occur by restoration of the quinonoid moiety and elimination of •CHO. Thus, the highest abundance of *m/z* 199 in the MS/MS spectra can be interpreted not only in terms of the several forms that this ion acquires but also in terms of the energies necessary for their decomposition (Scheme 4).

It is worth mentioning that the formation of **C** (*m/z* 173) can occur by two different processes (Scheme 5). The first is triggered through the formation of **B2** by a concerted mechanism, which should occur for all compounds in the same ratio, by the



Scheme 3. Energy profile for the fragmentation of the 2-acetylamino-1,4-naftoquinone derivatives. Values indicate the relative Gibbs energies at 298K; *values indicate the relative enthapies at 298K. All values are in kcal mol^{-1}. Absolute energies for **A1**: $G_{298} = -742.510472$ and $H_{298} = -742.451452$, in Hartree. E_c is critical energy for formation of **A1**.

elimination of •CN radical (Scheme 5). This step should take place with $\Delta G_{298} = 12.35$ kcal mol^{-1}.

The second process should occur from **A1**″ by elimination of R–CN, which should depend on the nature of the lateral chain (Scheme 5b). For compound **(4)**, this process should occur more easily when compared to compounds **(1)**, **(2),** and **(3)**, because the stability of the neutral molecule eliminated (benzenonitrile) is the driving force of the process. This therefore explains the high relative intensity of C (*m/z* 173) in the MS/MS spectrum of **(4)** (Fig. 3(C)). The enthalpy value for the **A1**″ → **C** process is negative.

The formation of *m/z* 145 from **(4)** occurs by the elimination of 28 mass units (–CO) from the **C** ion through an exothermic process with −2.16 kcal mol^{-1}. The intensity of *m/z* 145 is related to the facile formation of *m/z* 173 in the case of this compound (Table 2 and Scheme 5). For compounds **(1)**, **(2)**, and **(3)**, formation of *m/z* 145 should occur by the mechanisms discussed below.

Formation of the *m/z* 172 and *m/z* 145 ions

Regarding the coexistence of two $[M - H]^-$ isomers **A1** and **A2**, we proposed two main fragmentation pathways for the formation of **D** (*m/z* 172) and **F** (*m/z* 145) from these ions. These two processes can be understood by analysis of Scheme 6.

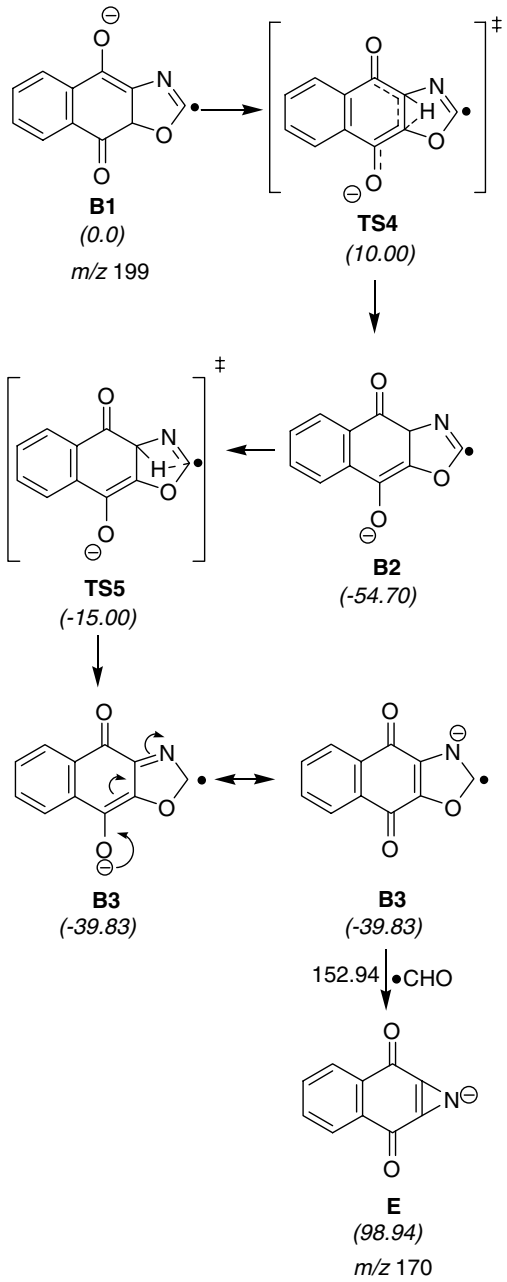
The product ion **D3** (*m/z* 172), which serves as diagnostic for an α-carbonyl hydrogen at the acylamino moiety, is formed by ketene elimination (R═C═O) from **A3**. This step occurs by means of a pericyclic reaction[61] that involves the participation of the α-carbonyl hydrogen, leading to the formation of **A4**. This process is preceded by a 180° rotation of the lateral chain (**A3**), which enables the spatial proximity between C(3) and the α-carbonyl hydrogen in a six-membered arrangement (Scheme 6, **TS_A3**). The Gibbs energy necessary for this process should be approximately 55.0 kcal mol^{-1}. Elimination of ketene (**A4** → **D3**) occurs through an endothermic process with 38.0 kcal mol^{-1} for **(1),** and this value decreases for **(2)** and **(3)** (Schemes 6 and 7).

Through the assistance of an electron pair at the N atom, a nitrile is formed by the opening of the six-membered ring and the subsequent formation of the five-membered ring (**D4**) via a concerted rearrangement of *m/z* 172. This step goes through a transition state **TS_D3_D4**, with a critical energy of 5.60 kcal mol^{-1}. From a thermodynamic viewpoint, it is spontaneous, with $\Delta G_{298} = -16.61$ kcal mol^{-1} and $\Delta H_{298} = -7.23$ kcal mol^{-1}. Elimination of HCN occurs in the next step with $\Delta G_{298} = -15.70$ kcal mol^{-1} and $\Delta H_{298} = -5.24$ kcal mol^{-1}, leading to a stable ion **F** with *m/z* 145 (Scheme 7).

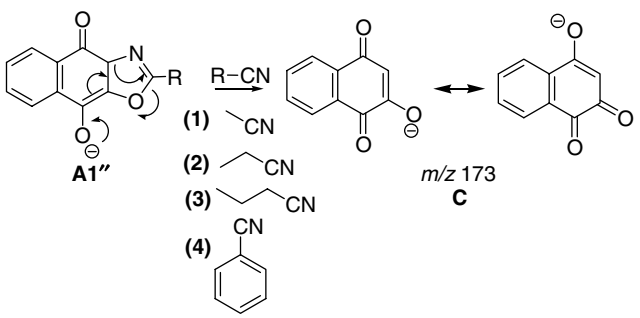
Formation of **D1, D3, D4,** and **F** can also occur by fragmentation of **A2**, as shown in Scheme 8, with the aid of a lone pair from the carbanion, the lateral chain being eliminated as a neutral molecule and the charge stabilized at the N atom (Scheme 8, ion **D1**). This process requires 10 kcal mol^{-1}. The formation of **D3** by further rearrangement of **D1** is an endothermic process ($\Delta H_{298} > 0$) with a critical energy of 67.20 kcal mol^{-1}. After formation of *m/z* 172, the formation of *m/z* 145, i.e. the **C1** ion, should occur by the same spontaneous reactions previously described for **D3** (Scheme 8).

Conclusions

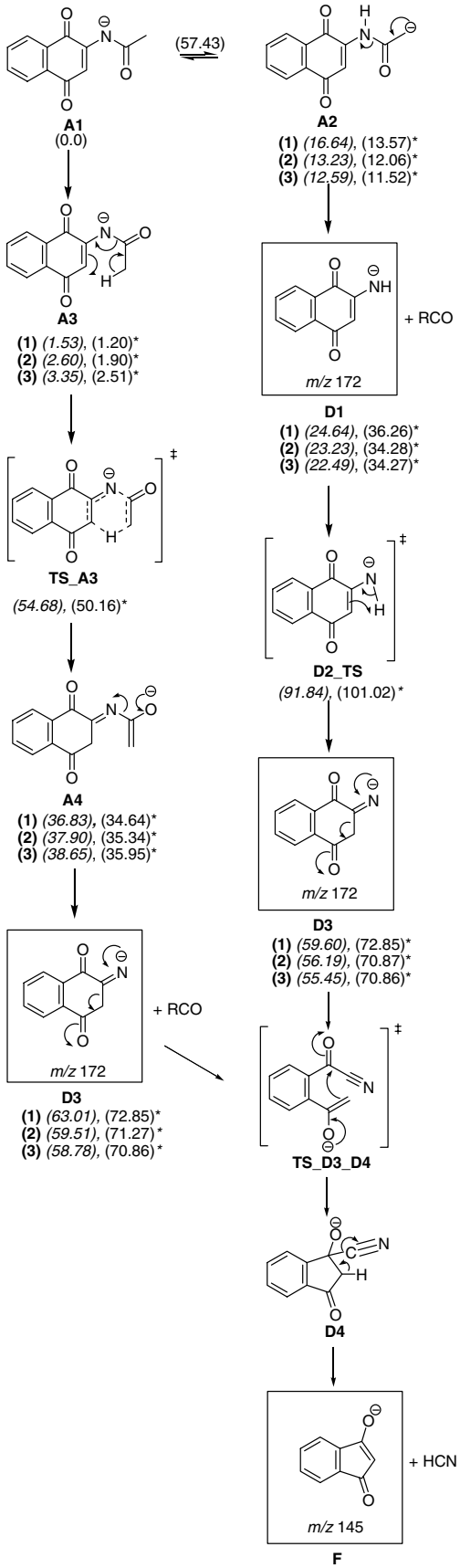
This study has allowed us to investigate the gas-phase anion chemistry of a new series of 2-acylamino-1,4-naphthoquinones. The fragmentation pathways of the deprotonated molecule, which took place via collision-induced dissociation in a Q-TOF mass spectrometer, have shown several features that can be related to the precursor ion. The use of MS/MS analyses in combination with theoretical calculations allowed us to define the precursor and product ions formed during the fragmentation processes.



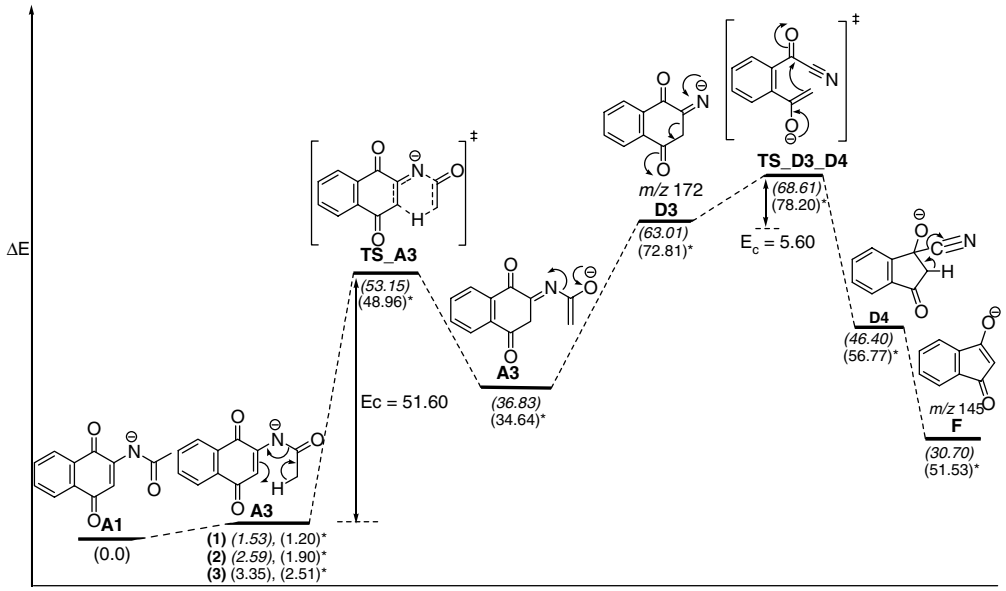
Scheme 4. Fragmentation pathway for the formation of *m/z* 170 from *m/z* 199. All values are relative Gibbs energies regarding the **B1** ion, in kcal mol^{-1}. **TS4** and **TS5** are transition states which connect **B1, B2,** and **B3**. Absolute energies for **B1**: $G_{298} = -702.515394$, in Hartree.



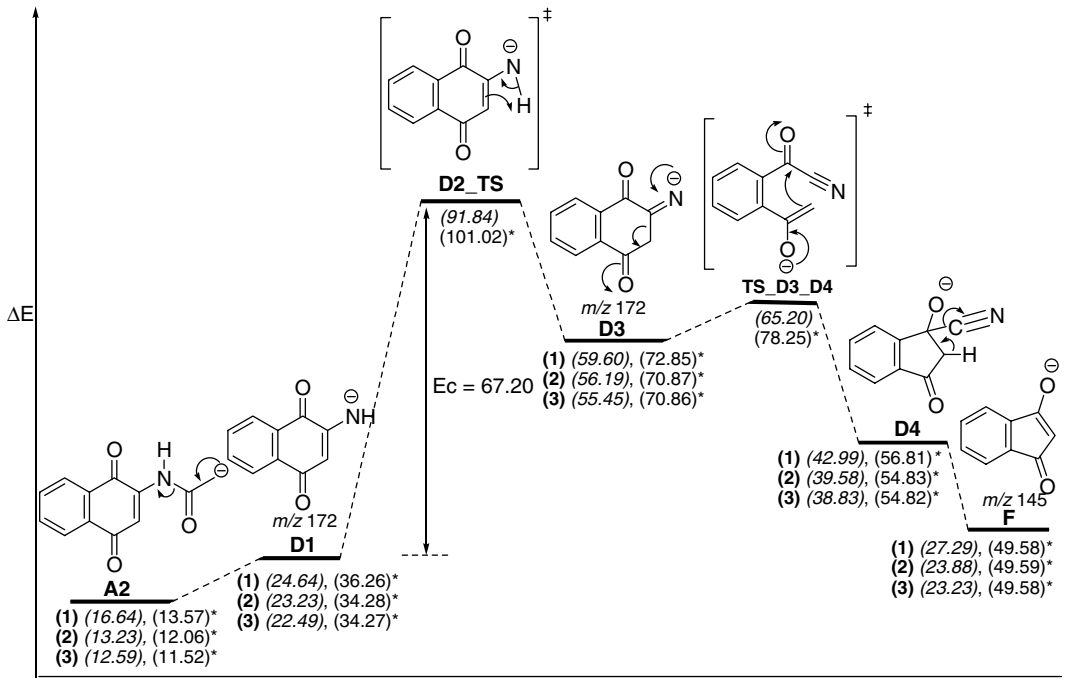
Scheme 5. Proposed mechanism for *m/z* 173 formation from **A1**″.



Scheme 6. Two possible fragmentation pathways proposed for the [M − H]$^-$ ions; the square indicates diagnostic ions. Numbers in italic are the relative Gibbs energies at 298K. * values are relative enthalpies at 298 K. All values are relative to the **A1** ion.



Scheme 7. Energy profile, in kcal mol^{-1}, for the gas-phase dissociation of the **A1** ion in the case of 2-acetylamino-1,4-naphthoquinone. Values in italic are relative Gibbs energies at 298K and * values are relative enthalpies at 298K.



Scheme 8. Energy profile, in kcal mol^{-1}, for dissociation of the deprotonated molecule of the **A2** ion. Values in italic are relative Gibbs energies ΔG_{298}, and * values are ΔH_{298}. Absolute Gibbs energies for **A2** are: **(1)** −742.483949, **(2)** −781.778879, and **(3)** −821.070792. Absolute enthalpies for **A2** are: **(1)** −742.429831, **(2)** −781.720073, and **(3)** −821.008589. All absolute values are in Hartree.

For all the molecules, the N–H bond has been suggested as the deprotonation site, on the basis of natural charges and gas-phase acidity. The potential energy surface for the fragmentation pathways suggests the formation of each ion expected in the ESI-MS/MS spectra. The elimination of R• indicates the formation of *m/z* 199, which is the most intense ion in the MS/MS spectra. The occurrence of the *m/z* 172 ions is due to the cleavage of the amide bond. It is diagnostic for an α-carbonyl hydrogen in the acylamino moiety. This ion fragment spontaneously rearranges ($\Delta G_{298} < 0$) and leads to the formation of *m/z* 145 via HCN elimination.

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Supporting information

Supporting information may be found in the online version of this article.

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