Fragmentation of 2-aroylbenzofuran derivatives by electrospray ionization tandem mass spectrometry

Herbert J. Dias, Tatiana M. Vieira, Eduardo J. Crevelin, Paulo M. Donate, Ricardo Vessecchi and Antônio E. M. Crotti*

Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900, Monte Alegre, CEP 14040-901. Ribeirão Preto, SP, Brazil.

Correspondence to: Antônio Eduardo Miller Crotti.

E-mail: millercrotti@ffclrp.usp.br

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Abstract

We investigated the gas-phase fragmentation reactions of a series of 2-aroylbenzofuran derivatives by electrospray ionization tandem mass spectrometry (ESI-MS/MS). The most intense fragment ions were the acylium ions *m/z* 105 and $M+H–C_6H_6]^+$, which originated directly from the precursor ion as a result of two competitive hydrogen rearrangements. Eliminations of CO and CO_2 from $[M+H–C_6H_6]^+$ were also common fragmentation processes to all the analyzed compounds. In addition, eliminations of the radicals •Br and •Cl were diagnostic for halogen atoms at aromatic ring A, whereas eliminations of $•CH_3$ and CH_2O were useful to identify the methoxyl group attached to this same ring. We used thermochemical data, obtained at the B3LYP/6-31+G(d) level of theory, to rationalize the fragmentation pathways and to elucidate the formation of **E**, which involved simultaneous elimination of two CO molecules from **B.**

Keywords: 2-aroylbenzofuran, fragmentation mechanisms, computational chemistry, benzofuran compounds

Introduction

Aroylbenzofurans and their derivatives are important scaffolds to develop drugs.[1] More specifically, interest in several natural and synthetic 2-aroylbenzofurans has risen due to their broad spectrum of biological effects, including antifungal,[2, 3] antibacterial,[3-5] antileishmanial,[6] antioxidant,[3] anti-HIV,[7] anti-tumor,[8, 9] anti-inflammatory,[10] antiproliferative, and cytotoxic actions.[11] In addition, some 2-aroylbenzufuran derivatives can be employed as sensors and semiconductors [12, 13] and as probes for β-amyloid plaques in Alzheimer's Disease.[14]

Over the last two decades, the advent of electrospray ionization mass spectrometry (ESI-MS) has enabled analysis of natural and synthetic high-molecular-weight compounds and of thermolabile and non-volatile molecules.[15] Moreover, tandem mass spectrometry (MS/MS) has allowed scientists to study, understand, and control some aspects of gas-phase ion chemistry.[16] Currently, ESI-MS/MS has been used to investigate an increasing number of classes of synthetic and natural products.[17-21] The resulting data have helped to feed the literature and to identify the structure of these compounds and their metabolites in complex mixtures by liquid chromatography tandem mass spectrometry (LC-MS/MS).[15, 16] Despite efforts to build spectral libraries to identify known compounds on the basis of ESI-MS/MS data, the lack of systematic studies on the gas-phase fragmentation reactions of some classes of compounds remains a major barrier.[22, 23]

In spite of the biological activities of 2–aroylbenzofuran derivatives, data on their fragmentation are still scarce. To date, Givens and co-workers have reported elimination of the ring moiety from halogenated benzofuran derivatives ionized by electron ionization (EI) at 70 eV, followed by CO and HCO• eliminations. Moreover, the benzocyclopropene radical has been identified as a marker of this class of compounds. Eliminations of •Br and •F in early stages of fragmentation followed by CO elimination have also been described.[24]

As part of our ongoing project on the fragmentation reactions of natural products and synthetic compounds,[17, 25, 26] and given the scarcity of data about the gas-phase fragmentation reactions of compounds displaying the benzofuran moiety, here we investigate the fragmentation pathways of a series of selected 2-aroybenzofuran derivatives by ESI-MS/MS and multiple-stage mass spectrometry (MS^n).

Experimental

Synthesis of 2-aroylbenzofuran derivatives

Compounds **1-5** were obtained by condensation of 2-bromoacetophenone with an *o*-hydroxyaldehyde or *o*-hydroxyketone, according to the methodology described by Yang and co-workers (Scheme 1).[27] In a two-neck flask (100 mL) connected to a reflux condenser, 2-bromoacetophenone (1.0 g, 5.0 mmol) and K_2CO_3 (2.15 g, 15.6 mmol) were dissolved in DMF (10 mL) for 10 min under inert N_2 atmosphere. Next, the *o*-hydroxyaldehydes **II** (1.0 g, 6.6 mmol) and **IV** (1.0 g, 8.1 mmol) or the *o*-hydroxyketones **I** (1.0 g, 4.7 mmol), **III** (1.0 g, 5.0 mmol), and **V** (1.13 g, 8.3 mmol) were added to 2-bromoacetophenone in DMF, and the mixture was stirred under reflux (120 °C) for 1 h. The reaction mixture was cooled to room temperature, and 30 mL of distilled water was added. The mixture was stirred for 5 min and filtered under reduced pressure. The solids were dried under vacuum, to yield compounds **1** (66.4% yield), **2** (54.7% yield), **3** (70.0% yield), **4** (51.7% yield), and **5** (58.7% yield). All the compounds were identified on the basis of ESI-MS, IR, ^1H, and ^{13}C NMR analyses (see supplementary information).

Mass spectrometry analysis

Electrospray ionization tandem mass spectrometry (ESI-MS/MS) analyses of the aroylbenzofurans **1-5** were performed on a tandem quadrupole mass spectrometer (Acquity H-class Xevo TQS, Waters, Milford, MS, USA) equipped with a Z-spray ionization source operating in the positive ion mode. The samples were dissolved in MeOH/H_2O (80:20 v/v) with 0.1% formic acid; the final concentration of aroylbenzofuran was 1.0 μg mL^{-1}. A trace of 0.1% formic acid was added, and the sample was infused directly into the ionization source at a flow rate of 0.1 mL min^{-1}. In the case of the samples used during the deuterium-labeled experiments, compounds **1-5** were dissolved in MeOH/D_2O (1:1 v/v). To maximize the relative intensity of the peaks corresponding to the protonated aroylbenzofuran compounds, the capillary and cone potentials were optimized to 3.2 kV and 40 V, respectively. The ionization source temperature was 150 °C, and the desolvation gas temperature (N_2) was 250 °C. The injected sample volume was 5 μL.

Multiple-stage ESI mass spectrometry (ESI-MS^n) analyses were conducted on an Ion Trap (IT) mass spectrometer (AmaZon Speed, Bruker Daltonics, Bremen, Germany) operating in the positive ion mode. N_2 was used as nebulizing (6 psi) and drying gas (8 $mL.min^{-1}$, 220 °C). The capillary voltage and the end plate offset were set to 3.5 kV and 500 V, respectively. The analytical mass range was 50-350 *m/z*.

Computational methods

The most stable conformers of the 2-aroylbenzofuran derivatives were obtained by conformational analysis and optimization of geometries with the MM2 force field and molecular dynamics. The most stable conformers were re-optimized on the basis of DFT calculations, and optimized geometries were obtained for each ion at the B3LYP/6-31+G(d) level with the Gaussian 03 software.[28] The stationary point was evaluated through

calculation of vibration frequencies for the same model. To obtain the protonation sites, proton affinity (PA) was employed as a descriptor to suggest the protonation sites of 2-aroylbenzofurans **1-5**, as observed in some literature reports.[29] The enthalpy of the proton for the reaction $M + H^+ \rightarrow MH^+$ was considered to be 1.48 kcal.mol^{-1}.[30] The fragmentation mechanisms were proposed on the basis of relative Gibbs energies and enthalpies at 298 K for all the aroylbenzofuran compounds; the same computational model was used throughout the experiments. However, the Gibbs energies were used with caution because equilibrium was not reached during the collision-induced dissociation (CID) experiments. No transition state was computed because it was assumed that CID supplied the minimal energy for fragmentation.

Results and Discussion

Structure-fragmentation relationships

In previous studies, our research group showed how important structure-fragmentation relationships are to elucidate the fragmentation pathways of a series of compounds that share the same structural core, such as sesquiterpene lactones [31] and alkaloids.[17] Ideally, these relationships are deduced from product ion spectra obtained under an optimum collision energy (E_{lab}), which is expected to decrease the relative intensity of the protonated molecule in the MS/MS spectrum by 50% and to increase the relative intensity of the fragment ions without promoting extensive fragmentation. On the basis of relative intensity (RI, %) versus E_{lab} (eV) plots, the optimum E_{lab} for aroylbenzofurans **1-5**, is 20 eV (Figure 1 for compound **4** and Supplementary material for compounds **1-3** and **5**).

Figure 2 shows the product ion spectra of protonated aroylbenzofurans **1-5** at E_{lab} = 20 eV; Table 1 lists the assignments of the main product ions (relative intensity higher than 2%). Direct elimination of benzene (78 mass units) from the protonated molecule is common to

compounds **1-5**. Product ion **B** is the most intense in the spectra of aroylbenzofuran compounds **1-5**. Moreover, acylium ion **F** (*m/z* 105) and phenyl ion **G** (*m/z* 77), produced by further CO elimination from **F**, are common fragments to compounds **1-5**. Other common product ions include **C**, **D**, and **E**, which originate from **B** by elimination of CO (28 mass units), CO_2 (44 mass units), and two CO molecules (56 mass units), respectively. MS^3 spectra support formation of **C**, **D**, and **E** from **B** (see Supplementary information).

A detailed comparative analysis of the MS/MS spectra of compounds **1-5** allowed us to identify some diagnostic ions that could help to distinguish the nature of the substituents at the benzene ring, as depicted in Scheme 2. Eliminations of a halogen (for **1** and **3**) and a methyl radical (for **2**) from **B** produces **H** (*m/z* 102) and **I** (*m/z* 160), whereas elimination of formaldehyde (30 mass units) from **E** is diagnostic for methoxyl group at the benzene ring of the benzofuran moiety. MS^3 experiments also support formation of **H** and **I** from **B** (see Supplementary information).

Protonation sites and gas-phase reactivity

The relevance of determining the protonation site in gas-phase fragmentation studies under CID conditions has been extensively discussed. In general, two major possibilities have been considered: a) the proton is initially attached to the most basic site of the molecule, but it can migrate to other less basic sites under CID conditions, to trigger fragmentation – this is known as "mobile proton model";[32] b) the proton remains attached at the most basic site of the structure even after CID. In this case, CID only increases the internal energy content.[33] Also, protonation could occur at different sites, with an excess of the population of species having the proton attached to the most basic site of the structure. Here, we will use the proton affinity (PA) values to identify the site that is most susceptible to protonation. We will explain all the fragmentation routes starting from the most stable precursor ion for each

compound, which results from proton attachment to the most basic site. We will only consider the mobile proton model if protonation of less basic sites results in an unstable species, which could trigger the fragmentation process.

Figure 3 gives the PA values. Besides the oxygen atoms of the furan ring and carbonyl, carbons C8 and C9 are potential protonation sites, as discussed in previous studies.[34, 35] PA values indicate that the carbonyl oxygen is the most susceptible to protonation for all the 2-aroylbenzofuran derivatives. Consequently, we will only consider the species protonated at the carbonyl oxygen as precursor ion.

Formation of the product ions B-G

Product ions **B** (**A**–78) and **F** (*m/z* 105) are the most intense in the product ion spectrum of 2-aroylbenzofurans **1-5** (Figure 2, Table 1). These acylium ions originate directly from the precursor ion (i.e., the protonated molecule) by two competitive pathways (I and II, Scheme 2), which result in elimination of benzene (78 u) or $C_8H_3OR_1R_2R_3$, respectively. Acylium ion *m/z* 105 (**F**) is widely reported as a marker of compounds displaying carbonyl at benzylic positions.[36] In the literature, acylium ions originate mostly from charge-induced α–cleavages, which leads to elimination of a positively-charged leaving group as a neutral group.[37] However, compounds **1-5** do not contain a leaving group attached to the aromatic ring of the aroyl moiety that could induce the formation of acylium ion **B**. Therefore, we consider that **B** (**A**–78) and **F** (*m/z* 105) originate from hydrogen rearrangement from the carbonyl oxygen to C1' and C8, respectively (Scheme 3). Data from deuterium-labeled experiments reveal that all the product ions of $[M+D]^+$ of compounds **1-5** have the same *m/z* as the product ions of the protonated molecule (Table 1). Hence, the proton/deuterium attached to the carbonyl oxygen participates in two competitive hydrogen rearrangements that generate **B** and **F** (*m/z* 105), as shown in Scheme 3. Competitive formation of acylium ions **B**

and **F** is similar to the competitive formation reported by Hu and co-workers for α, β-unsaturated aromatic ketones.[38]

We used thermochemical data for fragmentation, estimated by computational chemistry at the B3LYP/6-31+G(d) level of theory, to investigate differences between the relative intensities of **B** (**A**–78) and **F** (*m/z* 105) in the product ion spectra of protonated **1-5**. The ΔG and ΔH values involved in the formation of **B** and **F** are similar; the data demonstrate that differences between the relative intensities of these ions cannot be associated with their relative stabilities.

At E_{lab} = 20 eV, the energy transferred to the mass center (E_{com}) of protonated **1** (*m/z* 315), **2** (*m/z* 253), **3** (*m/z* 271), **4** (*m/z* 223), and **5** (*m/z* 237) by the collision gas (argonium) is estimated to be 51.9, 62.9, 59.2, 70.1, and 66.5 kcal.mol^{-1}, respectively, as previously reported for the equation $E_{com} = E_{lab}\ [m_c/(m_c+m_i)]$).[33, 39] This energy will be enough to promote dissociation of **A** into **B** and **F** (enthalpy between 30 and 40 kcal.mol^{-1}). Additionally, the remaining energy content provided by the CID process when E_{lab} is 20 eV can convert most of fragment ions **F** (*m/z* 105) into **G** (*m/z* 77). On the other hand, this E_{lab} can convert only a small fraction of ions **B** into **C** (ΔH between 74.4 and 84.1 kcal.mol^{-1}), **D** (ΔH between 68.4 and 78.0 kcal.mol^{-1}), and **E** (ΔH between 49.7 and 58.1 kcal.mol^{-1}). At least in principle, this could account for the higher relative intensity of **B** as compared to **F** in the product ion spectra of most of the aroylbenzofurans investigated at E_{lab} = 20 eV. In contrast, product ions **B** and **F** have similar relative intensities in the case of aroylbenzofuran **2**. The estimated ΔH and ΔG involved in the conversion of [**2**+H]$^+$ into **F** is about 4.0–8.6 kcal.mol^{-1} higher as compared to the corresponding processes for **1** and **3-5**. This difference is not associated with the relative stability of **B**, but it results from increased stability of the protonated molecule of **2** due to the electron-releasing mesomeric effect of the methoxyl group attached at ring A of compound **2**. Thereafter, more energy is necessary to produce **F**

from **A** (ΔH = 40.1 kcal.mol^{-1} and ΔG = 26.8 kcal.mol^{-1}) for compound **2** than for compounds **1**, **3**, **4**, and **5** ($\Delta H \cong$ 31.5–36.1 kcal.mol^{-1}; ΔG = 18.4–22.8 kcal.mol^{-1}). Consequently, product ion **F** of compound **2** does not dissociate into **G** (*m/z* 77), and its relative intensity is therefore higher as compared to the intensity of **F** in the product ion spectra of the other compounds.

According to MS^3 experiments, the general product ions **C**, **D**, and **E** originate from **B**, as illustrated in Scheme 3. These ions result from competitive elimination of CO (28 u), CO_2 (44 u), and C_2O_2 (56 u), respectively. Formation of **C** involves a single heterolytic cleavage of the C8–C10 bond, whereas formation of **D** from **B** comprised two steps: (1) formation of intermediate **B'** (ΔH between 36.1 and 62.7 kcal.mol^{-1}), which involves expansion of the furan ring to produce the δ–lactone ring; and (2) CO_2 elimination, due to contraction of the δ–lactone ring to a four-membered ring by a concerted mechanism. CO_2 eliminations have been reported for heterocyclic structures, such as deprotonated flavones, flavonols, and flavanones.[40, 41] On the other hand, the structure of benzocyclopropenium cation **E** has been previously proposed by Givens and co-workers for aroylbenzofuran compounds under electron ionization mass spectrometry (EI-MS) conditions.[24] Here, we first considered that product ion **E** originates from elimination of ethylenedione (C_2O_2) from **B**. Elimination of ethylenedione has also been reported for benzodiazepines investigated by ESI-MS/MS.[42] However, Schroder and co-workers have described ethylenedione as a short-lived molecule that is quickly dissociated in two CO unities.[43] On the basis of theoretical calculations, the enthalpy of **E** relative to precursor ion **A** (H = 0.0) is higher than the enthalpy of **C** and **D** relative to **A**. Nevertheless, the experimental data revealed that product ion **E** is more intense in the product ion spectra of **1**-**5** than **C** and **D**. Thus, we compared the ΔH values for the formation of **C**, **D**, and **E** from **B**. The ΔH value calculated for the formation of **E** by elimination of C_2O_2 is higher than the ΔH value involved in the formation of **C** and **D** by about 25.7-44.0 kcal.mol^{-1} and 31.8-50.0 kcal.mol^{-1}, respectively. In contrast, when we

considered that **E** originates from elimination of two CO molecules, we found that the formation of **E** is energetically favored as compared to the formation of **C** and **D** (lower ΔH), which agrees with its relative intensity in the product ion spectrum of **1-5**. Therefore, product ion **E** results from elimination of two CO molecules formed by dissociation of C_2O_2.

Formation of diagnostic ions

Product ion **H** (*m/z* 102) emerges only for compounds **1** and **3** and is diagnostic for halogen at ring A, as depicted in Scheme 4. This ion originates from **E** by homolytic cleavage of the C–X bond (X = Cl or Br). The estimated ΔH and ΔG values for the conversion of **E** into **H** are 88.5 and 78.3 kcal.mol^{-1} for **1** and 88.5 and 78.8 kcal.mol^{-1} for **3**, respectively. However, this process violates the *even-electron rule*.[36, 44, 45] Other similar cases have been reported for aromatic systems.[46, 47]

Product ions **J** and **I** (Scheme 5) are diagnostic for a methoxyl group at aromatic ring A. Formation of **I** (*m/z* 160) involves elimination of a methyl radical by homolytic cleavage of the C4–O bond (Scheme 5). In contrast to **H**, whose formation from **E** also violates the *even-electron rule*,[36, 44, 45] the unpaired electron participates in the resonance with the aromatic ring, so product ion **I** is a resonantly stabilized radical cation. Elimination of methyl radical has also been reported for other classes of compounds displaying methoxy groups attached to aromatic rings.[45] In addition, elimination of formaldehyde by hydrogen rearrangement yields fragment ion **J** (*m/z* 89), which is also diagnostic for methoxyl group at aromatic ring A.[48] The ΔH and ΔG involved in the formation of **J** from **B** are 75.1 and 39.4 kcal.mol^{-1}, respectively, whereas the ΔH and ΔG involved in the formation of **I** from **B** are 74.8 and 61.8 kcal.mol^{-1}, respectively.

Conclusions

Formation of acylium ions **B** and **F** by two competitive hydrogen rearrangements is the major fragmentation pathway of the 2-aroylbenzofuran derivatives investigated here. The relative intensities of **B** and **F** in the corresponding CID spectrum are due to the different energy required for their decomposition rather than their relative stabilities, estimated by thermochemical data. Moreover, the higher intensity of benzocyclopropene cation **E** as compared to vinylic cations **C** and **D** can only be understood on the basis of the decomposition of C_2O_2 into two CO molecules, as evidenced by Computational Chemistry. To the best of our knowledge, this is the first study on the gas-phase fragmentation of protonated benzofuran compounds by ESI-MS/MS and MS^n. Studies involving other classes of benzofuran compounds (e. g., dihydrobenzofuran neolignans) are underway.

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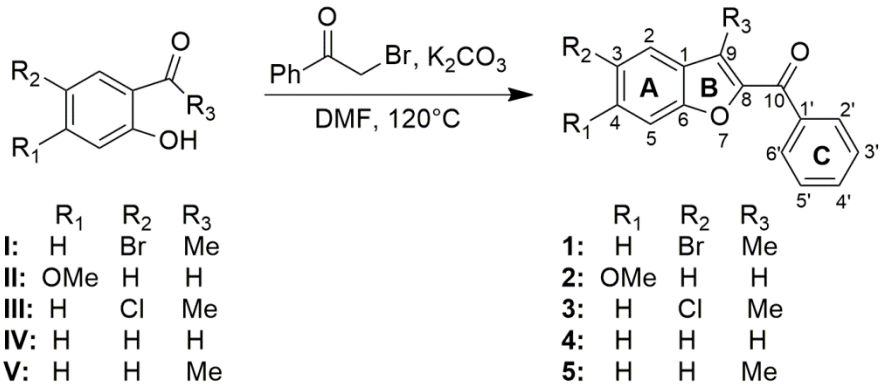
Table 1. Product ions of protonated 2-aroylbenzofurans at collision energy of 20 eV. Relative intensities (%) are in parenthesis. Data from the deuterium-labeled experiments are given in italics.

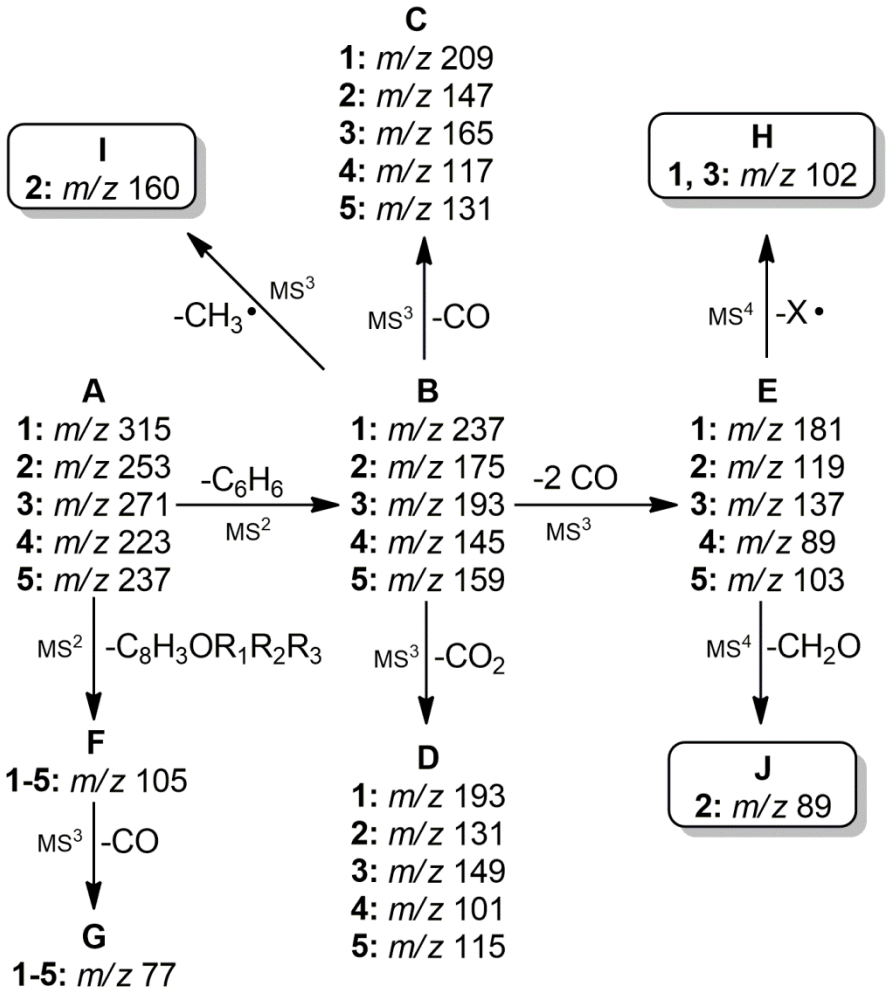
Assignment	**1**	**2**	**3**	**4**	**5**
A ($[M+H]^+$)	315 (19)	253 (23)	271 (11)	223 (8)	237 (8)
	316	*254*	*272*	*224*	*238*
B (**A** – C_6H_6)	237 (100)	175 (100)	193 (100)	145 (100)	159 (100)
	237	*175*	*193*	*145*	*159*
I (**B** – •CH_3)	----	160 (1) / 160	----	----	----
C (**B** – CO)	209 (2)	147 (4)	165 (4)	117 (2)	131 (6)
	209	*147*	*165*	*117*	*131*
D (**B** – CO_2)	193 (2)	131 (2)	149 (5)	101 (2)	115 (4)
	193	*131*	*149*	*101*	*115*
E (**B** – 2 CO)	181 (8)	119 (43)	137 (13)	89 (28)	103 (21)
	181	*119*	*137*	*89*	*103*
F (**A** – $C_8H_3OR_1R_2R_3$)	105 (19)	105 (98)	105 (25)	105 (62)	105 (29)
	105	*105*	*105*	*105*	*105*
H (**E** – •X)	102 (11)	----	102 (11)	----	----
	102		*102*		
J (**E** – CH_2O)	----	89 (19)	----	----	----
		89			
G (**F** – CO)	77 (6)	77 (30)	77 (6)	77 (21)	77 (12)
	77	*77*	*77*	*77*	*77*



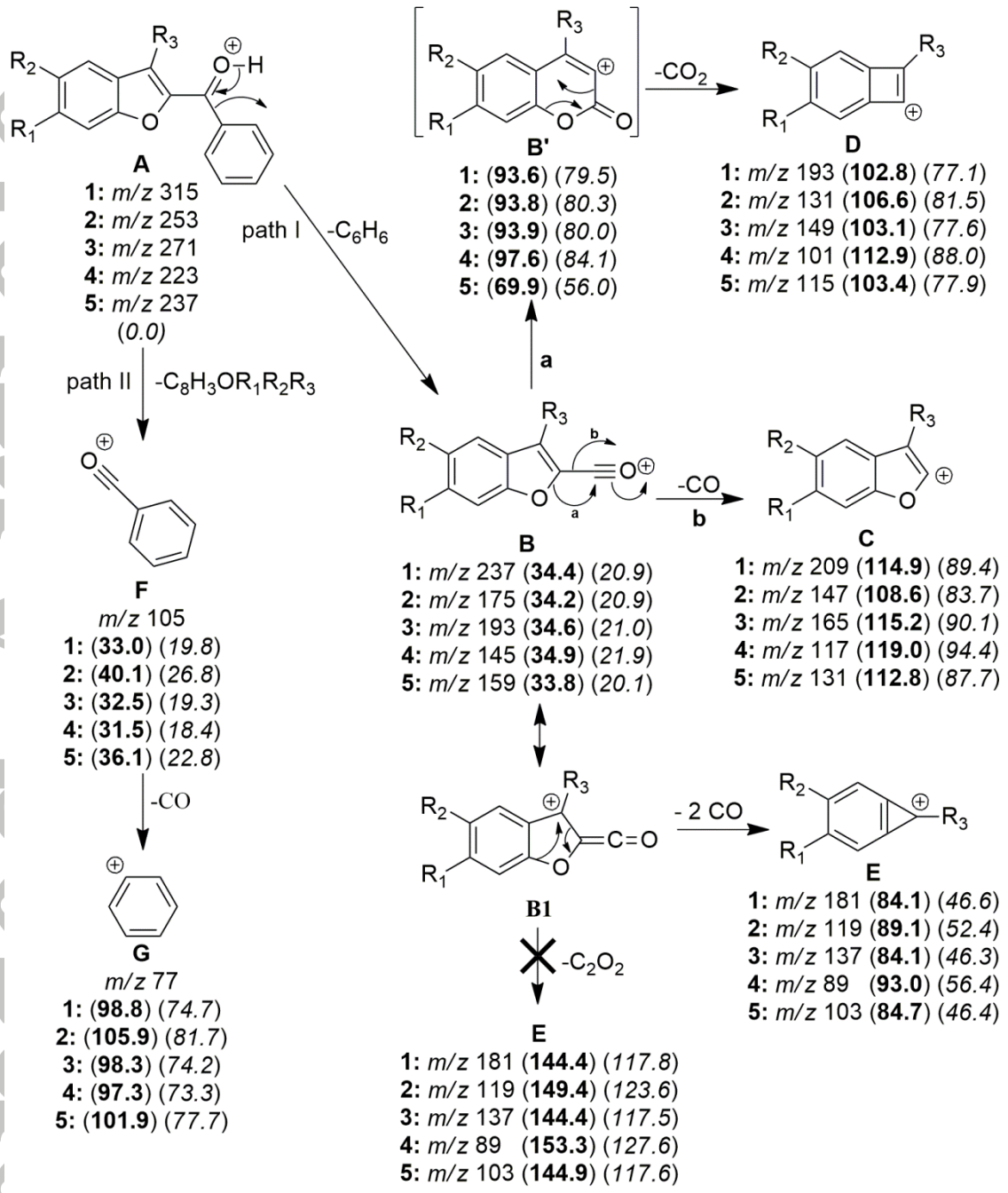
	R_1	R_2	R_3
I:	H	Br	Me
II:	OMe	H	H
III:	H	Cl	Me
IV:	H	H	H
V:	H	H	Me

	R_1	R_2	R_3
1:	H	Br	Me
2:	OMe	H	H
3:	H	Cl	Me
4:	H	H	H
5:	H	H	Me

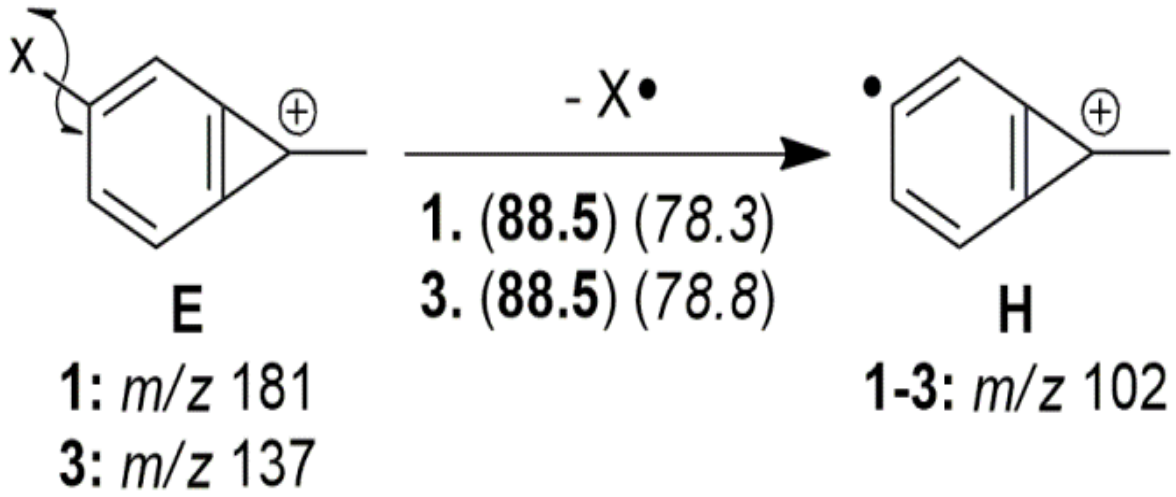
Scheme 1. Synthesis of 2-aroylbenzofuran compounds **1-5**.



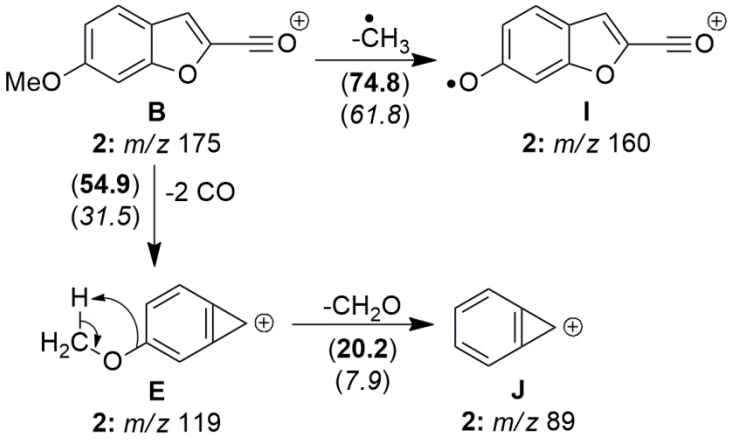
Scheme 2. Structure-fragmentation relationships in protonated aroylbenzofuran compounds 1-5.



Scheme 3. Formation of the common fragment ions observed in the ESI-MS/MS spectra of compounds **1-5**, their **Enthalpies** (H), and *Gibbs energies* (G). The G and H values were calculated at the B3LYP/6-31 + G(d) level and are in kcal.mol^{-1}.



Scheme 4. Formation of diagnostic fragment ion **H** of compounds **1** and **3**. Relative **nthalpies** (ΔH) and *Gibbs energies* (ΔG) values are in kcal.mol^{-1}.



Scheme 5. Formation of diagnostic fragment ions **I** and **J** observed in the product ion spectra of compound **2.** Relative enthalpies, bold values, and Gibbs energies, italic values. All the values are in kcal.mol^{-1}.

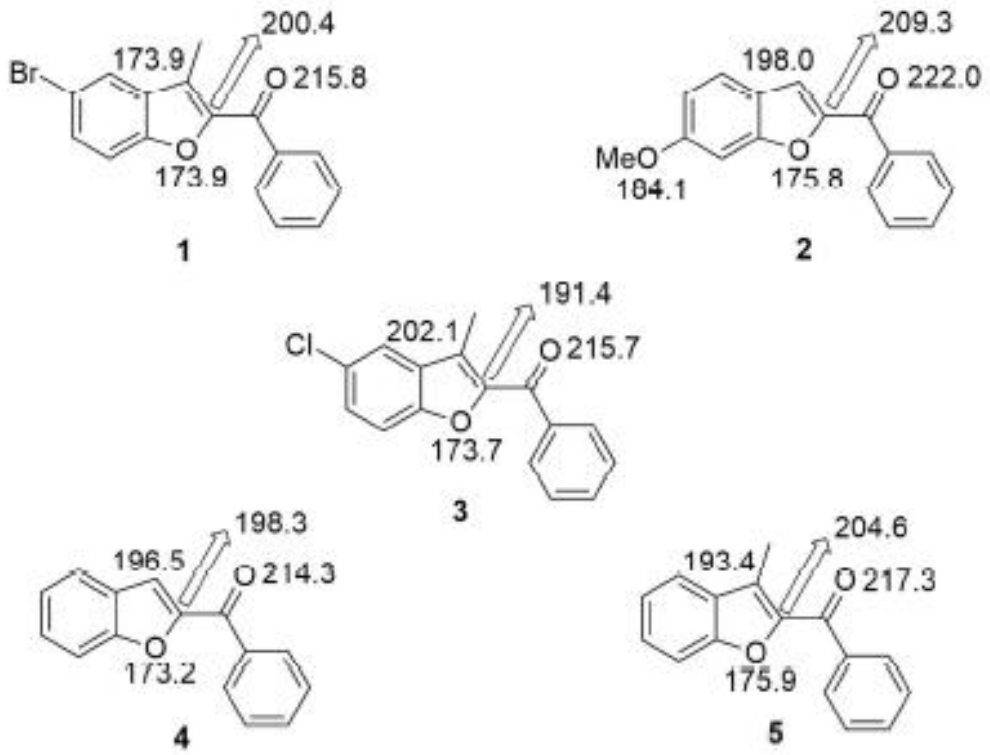


Figure 1. Correlation between relative intensity (%) and collision energy (eV) for compound **4.**

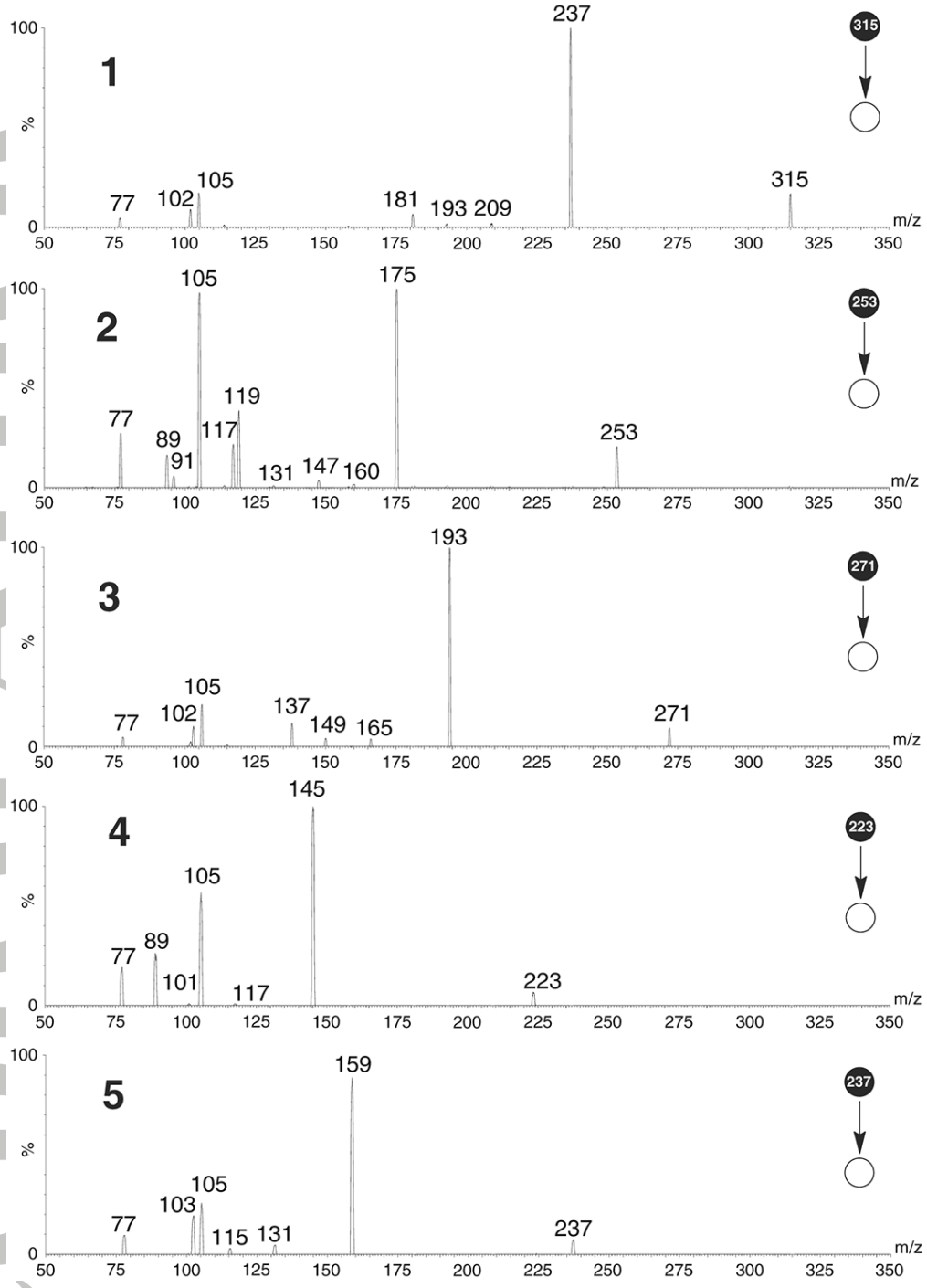


Figure 2. Product ion spectrum of protonated 2-aroylbenzofurans **1-5** (Ar, 20 eV).

Scheme 2. Structure-fragmentation relationships in protonated aroylbenzofuran compounds **1-5**.

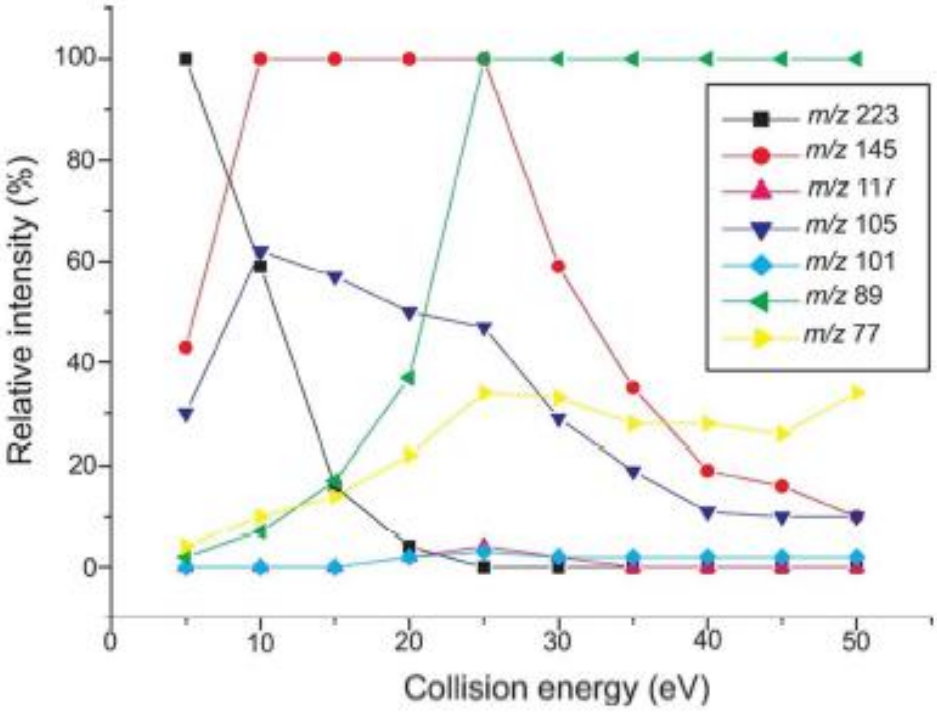


Figure 3. Proton affinities (PA) for aroylbenzofurans 1-5. The PA values were calculated at the B3LYP/6-31 + G(d) level. All the values for PA are in kcal.mol^{-1}.