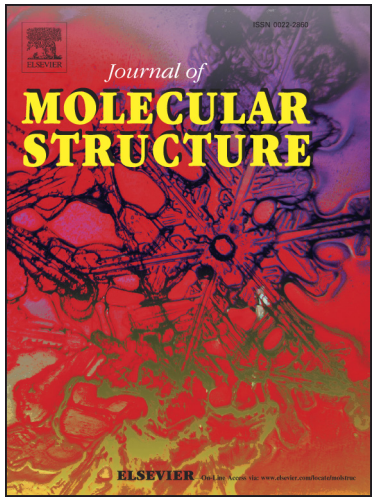

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Mass spectrometry and theoretical calculations about the loss of Methyl radical from methoxilated coumarins.

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Abstract

In this study we have performed CID mass spectrometry measurements and theoretical calculations in a selected series of coumarins. Our theoretical and experimental results indicate that there is room for reasonable doubts about the fragmentation way previously proposed by Shapiro and Djerassi (1965). A complementary explanation about the fragmentation way of the methyl loss from methoxy coumarins has been reported in this work. Our results demonstrated that different theoretical models are very useful to explain the fragmentation occurred in MS, supporting the usual rules of fragmentation. Although the QTAIM analysis gives a good correlation in order to explain the formation of p-quinoid resonance forms; however, the best correlation has been obtained using the NBO approximation as well as from the Wiberg indexes.

Keywords: mass spectrometry, coumarins, DFT calculation, QTAIM, NBO method

1. Introduction

Coumarin is the name given to the basic structural unit present in a large group of heterocyclic oxygen compounds that possess the benzopyran-2-one nucleus [1]. They are found in many plants such as Tonka bean, lavender, sweet clover grass, licorice, strawberries, apricots, cherries, and cinnamon. Coumarin derivatives have been proven to function as anti-coagulants [2], antibacterial agents [3, 4], antifungal agents [5], and biological inhibitors [6], chemotherapeutics [7, 8] and as bio-analytical reagents [9]. They are useful antioxidants and show antitumour activity [10.a, 10.b] and cytotoxicity [11–16]. They also show anti-inflammation effects [17], hepatic drug-metabolizing enzyme-inducing [18], and anti - dermatosis functions [19].

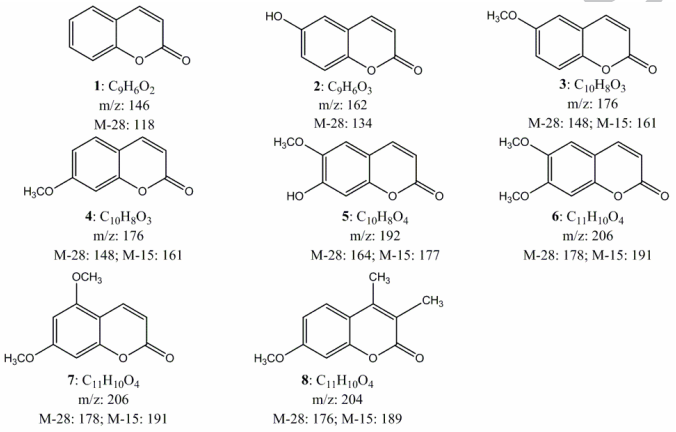
Studies on the spectroscopic behavior of coumarin derivatives have been reported in the literature. Both proton (^{1}H) and carbon-13 (^{13}C) nuclear magnetic resonance (NMR) spectroscopic properties of coumarins have been studied [20 – 22]. Mass spectrometry has been found to be an important tool in the characterization of natural as well as synthetic coumarins. Electronic Impact - Mass Spectrometry (EI-MS) [23, 24, 25, 26, 27], positive and negative chemical ionization (CI) [28, 29], and electron attachment [30] have been employed successfully. Also electrospray ionization mass spectrometry (ESI-MS) [31, 32] have been shown to be useful in structural characterization of coumarins.

Traldi and co-workers [33, 34, 35, 36] have investigated the structures of furanocoumarin isomers, which cannot be distinguished with conventional mass spectrometric techniques. They established a new approach in the investigation of these compounds based on high and low energy collision-activated dissociation

Recently, fragmentation behaviors and pathways of coumarins in electrospray ionization mass spectrometry (ESI-MS) have been studied [37], and coumarins were analyzed with LC–MS in *Radix Angelicae Dahuricae* [38, 39] and other plants and dietary supplements.

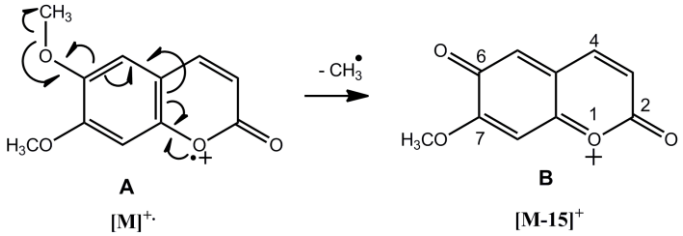
Our main interest is focused in the methyl loss from methoxycoumarins, comparing this process with the characteristic CO loss of these compounds. The molecules selected for our study are shown in Figure 1. It should be noted that all these compounds possess one or two methoxyl groups in their structures (except compounds 1 and 2 which were taken as reference compounds).

Figure 1: Structural features of the coumarins under study



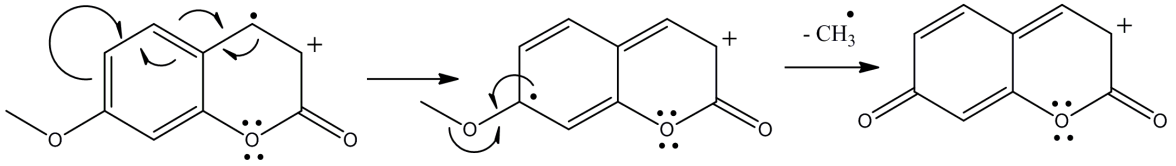
The CO loss from the coumarins and furanocoumarins has been exhaustively studied by using CID mass spectrometry and the fragmentation patterns of metastable ions [40]. Previously Shapiro and Djerassi had compared the methyl and CO loss in 6, 7-dimethoxycoumarin (**6**), postulating that the *para*-quinoid structure formed for the $[M -CH_3]^+$ (when the methyl loss occurs from position 6) is the energetically preferred form [41]. These authors affirm that localization of positive charge is a very useful approach to the rationalization of many mass spectrometric fragmentation processes and, when is applied to 6,7-dimethoxycoumarin, leads to the prediction that it is the methyl radical from the C-6 methoxyl group which is preferentially lost due to the formation of a *para* -quinoid structure. Thus, Shapiro & Djerassi have proposed the formation of $[M-15]^+$ from 6,7-dimethoxycoumarin as is shown in Figure 2.

Figure 2: Formation of $[M-15]^+$ for 6,7-dimethoxycoumarin (6) by Shapiro and Djerassi [41]



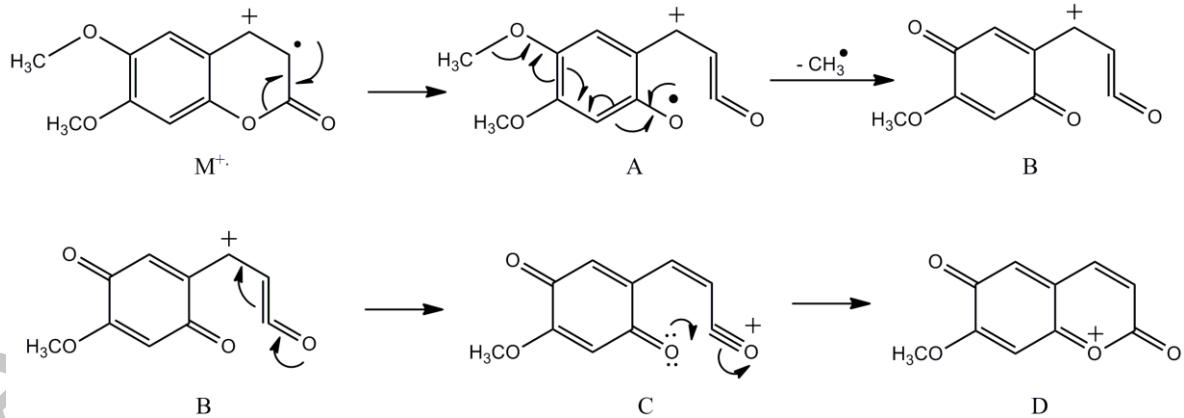
Following the habitual rules of ionization-fragmentation it is possible to test the starting points of ionization. However, in this particular case, although there are some fragments theoretically predicted, they were not experimentally detected. This can be taken as a clear indication about the strength of the bonds that could be broken but they did not. For example, Figure 3 shows a plausible way that predicts the methyl loss from 7-methoxycoumarin (**4**). However, this fragmentation is not experimentally observed neither its electron impact MS nor its CID, via MSn. This implies that the original ionization process hardly occurs at the double bond C3-C4.

Figure 3: a plausible theoretical way for methyl loss from compound **4**



On the other hand there are other experimentally observed fragments for which there is not a clear and unique starting point of ionization. Detectable fragments can be predicted from different starting points of ionization-fragmentation, but the real fragmentation advances and the outlined mechanism must be confirmed by comparing with the real registrations of electronic impact MS and CID via MSn. For example, by using the normal fragmentation rules in MS, it can be observed that the methyl loss in compound **6** proposed by Shapiro and Djerassi it is not the unique possible way. Figure 4 shows a possible alternative path for loss of methyl group for compound **6**, locating the initial positive charge on another atom.

Figure 4: a plausible theoretical way for methyl loss from compound **6**



There are in the MS literature several papers in which theoretical calculations – including semi-empirical methods - are helpful to explain and better understand the ways of fragmentation [for example, see [45], [46], [47], [48], [49], [50]). Therefore in a first step we conducted a preliminary and exploratory study using B3LYP/6-31G(d) calculations in order to compare the neutral compounds with their corresponding molecular ions. The Mulliken's analysis, reasonably, assign the positive charge at C2 instead of O1. But, it should be noted that these results displayed an increased positive charge on O1 which is bigger than

the increase on C2 (except for compounds **7** and **8**) and on C4. This is in agreement with the Shapiro-Djerassi postulation (Table 1). However, this is not enough to explain why compounds **5** and **6** show a greater $[M-15]^+$ peak in their CID spectrum that compound **3**. On the other hand considering only the electrostatic charge, the increase of positive charge at O1 is always greater than the increase at C2 and C4. But compounds **7** and **4** should have a $[M-15]^+$ peak bigger than compound **3**. These preliminary results indicate that there is room for reasonable doubts about the fragmentation way proposed by Shapiro and Djerassi. Thus, in this study we have performed CID mass spectrometry measurements and theoretical calculations in a selected series of coumarins with the aim to investigate another plausible explanation for this fragmentation process.

Table 1: Mulliken and Electrostatic charges on atoms O1, C2 and C4 calculated for significant molecules under study. Calculation Method: B3LYP/6-31G(d)

Mulliken charge									
Cpd.	**M**			**$M^{+\cdot}$**			**Charge difference**		
	O1	C2	C4	O1	C2	C4	**O1**	**C2**	**C4**
3	-0,518	0,595	-0,119	-0,47	0,617	-0,101	0,048	0,022	0,018
4	-0,522	0,594	-0,121	-0,489	0,625	-0,107	0,033	0,031	0,014
5	-0,526	0,594	-0,12	-0,49	0,616	-0,102	0,036	0,022	0,018
6	-0,525	0,593	-0,12	-0,491	0,615	-0,104	0,034	0,022	0,016
7	-0,527	0,593	-0,1	-0,501	0,625	-0,086	0,026	0,032	0,014
8	-0,519	0,565	0,133	-0,487	0,601	0,143	0,032	0,036	0,01
Electrostatic charge									
3	-0,411	0,767	0,008	-0,37	0,776	0,016	0,041	0,009	0,008
4	-0,397	0,728	-0,009	-0,348	0,687	-0,032	0,049	-0,041	-0,023
5	-0,386	0,755	0,007	-0,336	0,774	-0,009	0,05	0,019	-0,016
6	-0,393	0,768	0,021	-0,337	0,735	0,022	0,056	-0,033	0,001
7	-0,42	0,766	0,159	-0,376	0,711	0,11	0,044	-0,055	-0,049
8	-0,361	0,595	0,29	-0,326	0,576	0,198	0,035	-0,019	-0,092

2. Material and Methods

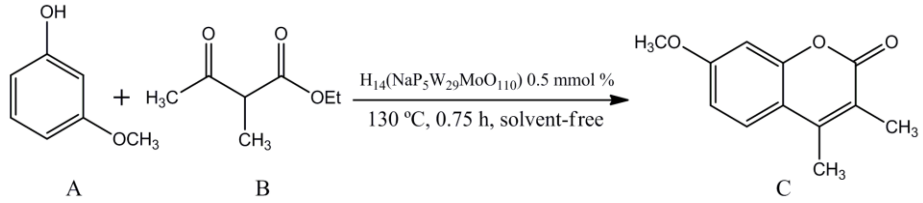
Compound **1 (c**oumarin), *compound* **2 (**6–hydroxycoumarin), *compound* **5 (**7–hydroxyl–6–methoxycoumarin, scopoletin, 6-methoxyumbelliferone), *compound* **6** (6,7-dimethoxycoumarin, scoparone), and *compound* **7** (5,7- dimethoxycoumarin, citropten, limettin)**;** were purchased from Sigma-Aldrich (catalogues: C4261, 642665-1G, S2500, 254886 and , 116238, respectively).

Compounds **3** (6-metoxycoumarin) and **4** (7-methoxycoumarin) were obtained as has been reported in reference [53]. Such compounds were kindly provided by Lic. Celeste Aguirre Pranzoni

Compound **8 (**7-methoxy-3,4-dimethylcoumarin) was synthesized from 3-metoxyphenol and ethyl α-methylacetoacetate by the Pechmann reaction, using a Preyssler heteropolyacid ($H_{14}(NaP_5W_{29}MoO_{110})$) as catalyst by a solvent-free procedure (*Scheme 1*). The procedure was performed following essentially the protocol report in the literature for

the general synthesis of coumarins using other heteropolyacids, with slight modifications [58, 59].

***Scheme 1*:** Synthesis of 7-methoxy-3,4-dimethylcoumarin, by Pechmann reaction



A B C

Chemicals were purchased from Aldrich and Fluka chemical companies and were freshly used after purification by standard procedures (distillation and recrystallization). All the reactions were monitored by TLC on precoated silica gel plates (254 mm). All the yields were calculated from crystallized products. The product was identified by comparison of physical data (mp, TLC and NMR) with those reported or with these of authentic sample prepared by the respective conventional methods using sulfuric acid as catalyst. Melting point of the compound was determined in sealed capillary tube and is uncorrected. The ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a NMR Bruker Advance DPX 400 spectrometer as d_6-DMSO solutions, and the chemical shifts were expressed in δ units with Me_4Si (TMS) as the internal standard. The catalyst $H_{14}(NaP_5W_{29}MoO_{110})$ was synthesized according to a procedure of the literature [60].

Optimized procedure to the synthesis of 7-methoxy-3,4-dimethylcoumarin

The catalyst was dried overnight prior to use. The reaction was performed in a round bottom flask, which was equipped with a condenser and immersed in an oil bath. A mixture of 3-methoxyphenol (10 mmol) and ethyl α-methylacetoacetate 2 (10 mmol) was stirred at 130 °C in the presence of bulk Preyssler acid (0.5% mmol) for 45 minutes. The reaction mixture was extracted with hot toluene and was filtered to separate the catalyst (3 x 20 mL). The solution was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was recrystallized from methanol yielding 92 % of pure 7-methoxy-3,4-dimethylcoumarin.

Characterization data of 7-methoxy-3,4-dimethylcoumarin

Mp: 139-141 °C (methanol) (lit. p.f.: 142-143 °C [61]). ^{13}C NMR (DMSO-d6, 100 MHz): δ 161.1, 161.0, 152.9, 146.3, 125.9, 117.8, 113.4, 111.6, 100.2, 55.6, 14.6, 12.8. ^{1}H NMR (DMSO-d6, 400 MHz): δ 7.61 (1H, d, J: 6,7 hz), 6.88-6.92 (2H, m), 3,85 (3H, s), 2,32 (3H, s), 2,05 (3H, s).

3. Mass spectrometry

We use an ion trap mass spectrometer, which allows isolating an ion, or an ion-radical fragment, and delivering energy enough to fragment it. It can be registered the intensity (referred to total ionic current) of peaks of interest as a function of the applied voltage to the ion trap, which is proportional to the energy applied to the ion.

EI-LRMS was performed at 70 eV using an ion trap (GCQ Plus) with MSn (Finnigan, Thermo-Quest, Austin, TX, USA), operated at a fundamental rf-drive of 1.03 MHz. Helium was used as the damping gas at an uncorrected gauge reading of 6×10^{-5}

Torr.

For the analysis of tandem mass spectrometric (MS/MS) product ions, the precursor ion was selected using a MS/MS standard function, with a peak width of 0.5–1.0 m/z units, and dynamically programmed scans. The supplementary voltage was in the range 0 – 3.5 V, as described previously [52].

4. Calculations

All the calculations reported here were performed using the GAUSSIAN 09 program ([53.a]). DFT calculations were employed to account for the electron correlation effects. The widely employed hybrid method denoted by B3LYP ([53.b], [53.c], [53.d]) was used, along with the double-zeta-split valence basis set 6-31G(d). This method includes a mixture of Hartree–Fock (HF) and DFT exchange terms and the gradient corrected correlation functional of Lee, Yang, and Parr, ([55.a], [55.b]) as proposed and parameterized by Becke. ([56.a], [56.b])

Geometries were optimized at B3LYP/6-31G(d) and B3LYP/6-311G+(2d,p) levels of theory. With any conformational search, it is very important to examine the structures obtained to make sure that they are true minima and not transition structures or other structures with very low or zero forces on the atoms (stationary points). Vibrational frequencies were calculated at both levels of theory. NBO calculations were performed by using the routines incorporated in the Gaussian packages. The wave functions of the coumarins (and their corresponding molecular ions) generated at the B3LYP/6-31G(d) level of theory, were subjected to a QTAIM analysis [57.a] using Multiwfn software [57.b].

5. Results and Discussion

The EI-MS of the compounds studied here are given as supporting material. As expected, it can be observed that when the [M-15] peak appears significantly in these spectra, it appears simultaneously with the [M-28] peak in the MS-MS (CID) experiments.

Figures 5.a and 5.b show the EI-MS spectra of compounds **3** and **4**, which are representative of the entire series.

Figure 5.a: EI-MS obtained for 6-methoxycoumarin (**3**)

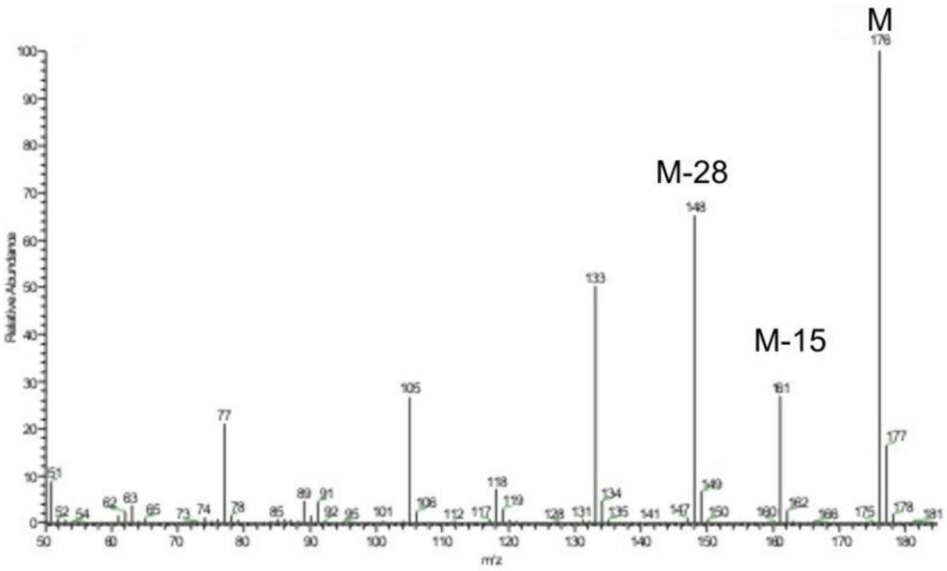
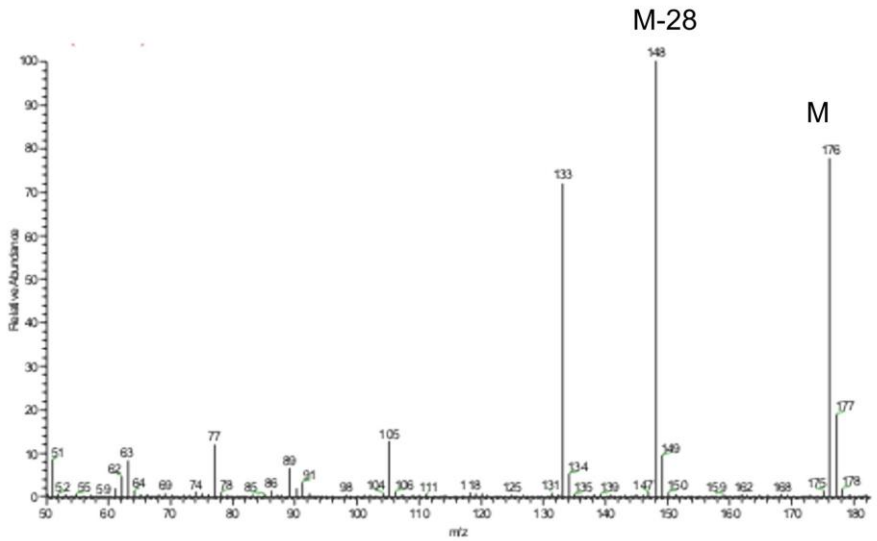


Figure 5.b: EI-MS obtained for 7-methoxycoumarin (**4**)



Figures 6.a and 6.b show a typical graphic representation of CID spectra obtained for the same compounds**.** The same type of results obtained for compounds **5** - **8** are given in figures 5S-8S as supporting material.

Figure **6.a**: Graph showing the %Total Ionic Current vs. Applied potential for 6-methoxycoumarine (**3**): $M^{+\cdot}$; $[M-15]^{+}$ and $[M-28]^{+\cdot}$

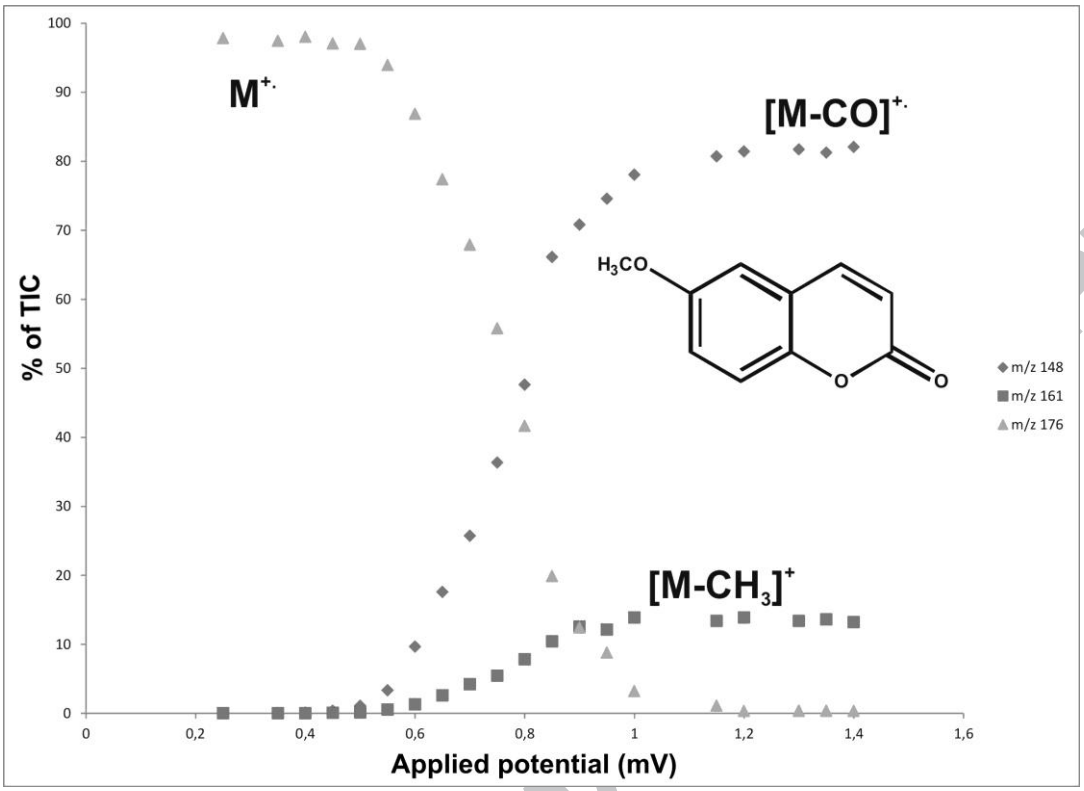
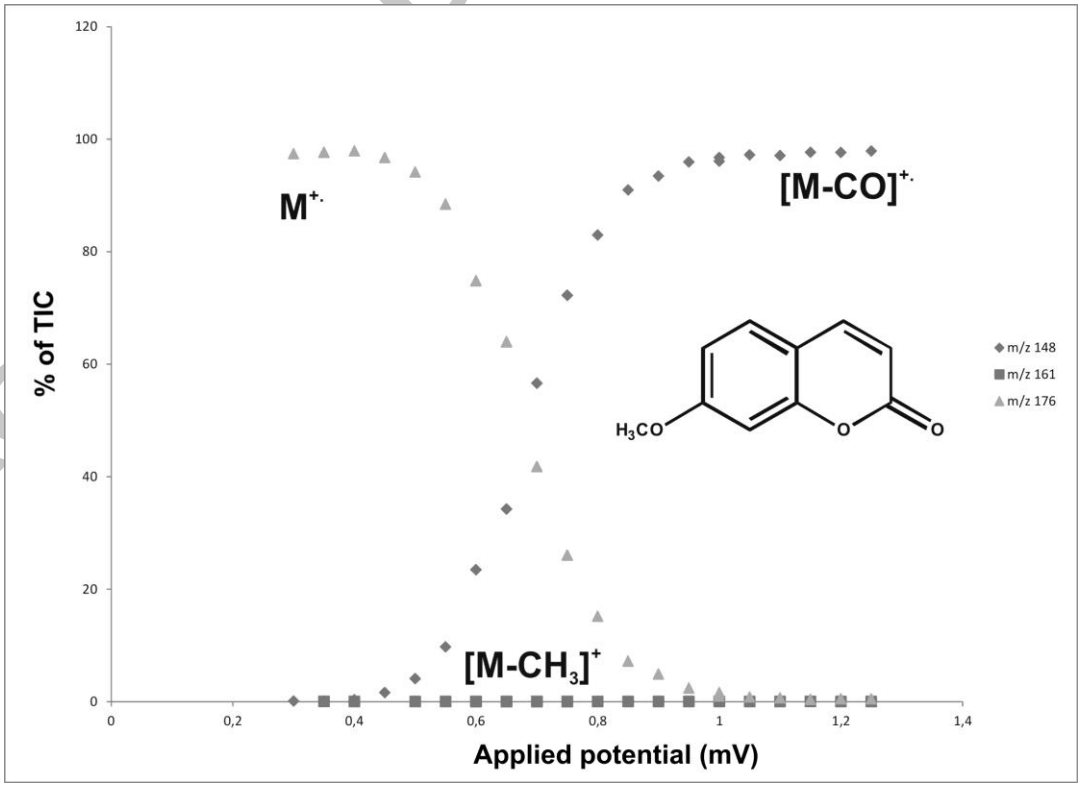


Figure 6.b: Graph showing the %Total Ionic Current vs. Applied potential for 7-methoxycoumarine (**4**): M^{+}; $[M-15]^{+}$ and $[M-28]^{+\cdot}$

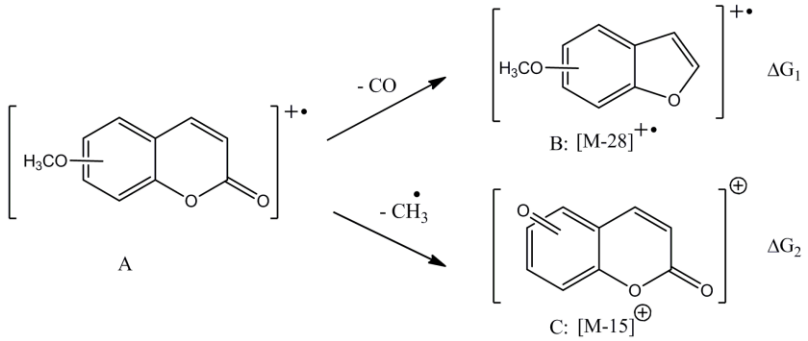


Considering that the CO loss is the predominant fragmentation in these molecules, we took it as a reference to study the methyl loss. We measured the percentage of TIC of the [M-15] peak at the maximum percentage of TIC of the [M-28] peak. These results are shown in the first column of Table 2.

For 6-methoxycoumarin (**3**), it can be see that the loss of the methyl radical occurs at the same time of the loss of CO in the applied potential range selected. As we said, this experimental fact is reflected in the EIMS spectra of this compound where the [M-15] peak has a significant intensity. Instead, for 7-methoxycoumarin (**4**) experimental data indicate that the methyl loss doesn't happen at the same time that the CO loss. Therefore, for this molecule, the [M-15] peak doesn't appear in the EI-MS spectrum.

The reactions under study might be generalized as:

Figure 7: The two fragmentation reactions here considered.



Considering the coincidence with various studies previously reported ([40] – [45]) we assumed that the fragment $[M - CO]^{+\cdot}$ has a benzofuranoid structure (Figure 7.B). Thus, we can calculate the thermodynamic parameters considering the temperature and pressure in the ion trap and then to plot such parameters vs ln $\{[(M\text{-}CH_3)^+]/[(M\text{-}CO)^{+\cdot}]\}$. We assume that $[(M\text{-}CH_3)^+]/[(M\text{-}CO)^{+\cdot}]$ is proportional to the relationship $\%[(M\text{-}CH_3)^+]/\%[(M\text{-}CO)^{+\cdot}]$.

Table 2 also shows the experimental relationship between the % of TIC obtained for $[M\text{-}15]^+$ and $[M\text{-}28]^{+\cdot}$, and ΔG obtained from B3LYP/6-31G(d) calculations, for each reaction (at a temperature of 473 ^oK and a pressure of 0.01 atm). Regarding the results shown in table 2 it can be see that the CO loss is clearly favored with respect to the methyl radical loss, from a thermodynamic point of view.

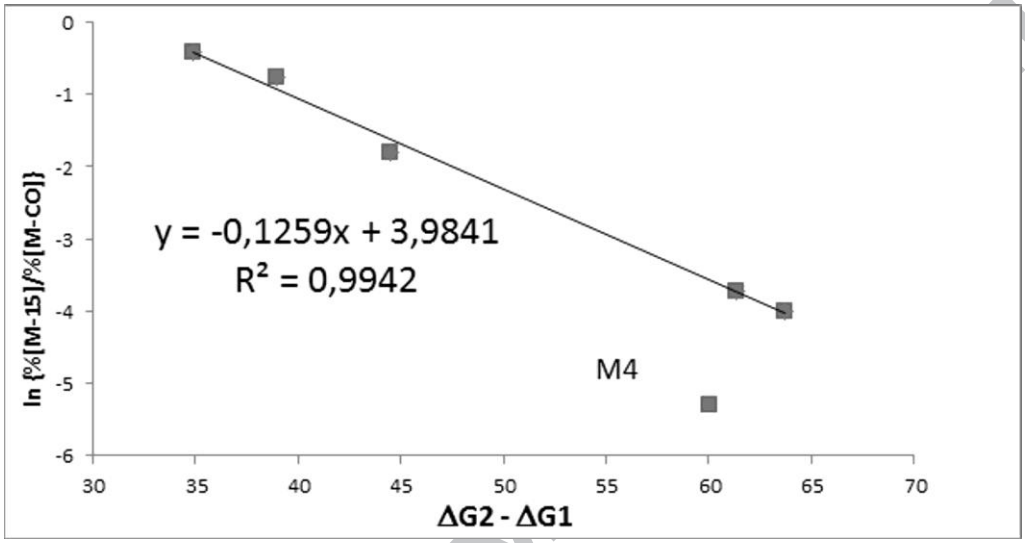
In turn, the plot of $\Delta\Delta G$ vs. $\ln\{[(M\text{-}CH_3)^+]/[(M\text{-}CO)^{+\cdot}]\}$ results in a negative slope straight line. These results are summarized in Table 2 and Figure 8.

Table 2: % [M-15] / % [M-28] and its ln, ΔG_1 and ΔG_2 calculated for fragmentation reactions, for compounds **1** – **8**

Compound	% [M-15] / % [M-28]	Ln {%[M-15]/%[M-28]}	ΔG_1(kcal/mol)	ΔG_2(kcal/mol)
1			-8,117	
2			-3,116	
3	0,163886933	-1,80857852	-3,350	41,130

4	0,005034305	-5,291479758	-8,017	52,043
5	0,466681641	-0,762107964	-6,207	32,746
6	0,659176925	-0,416763305	-3,394	31,524
7	0,024105309	-3,725323167	-9,372	51,935
8	0,018336086	-3,998884268	-15,632	48,075

Figure 8: Ln {%[M-15]/%[M-28]} vs.ΔG_2-ΔG_1 obtained for compounds **3** – **8**



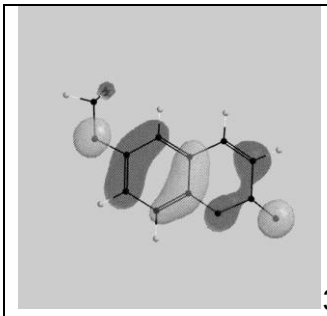
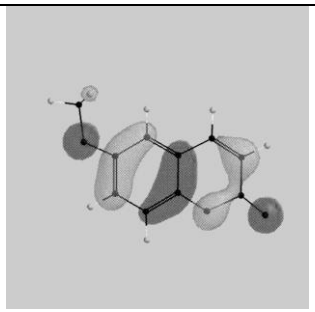
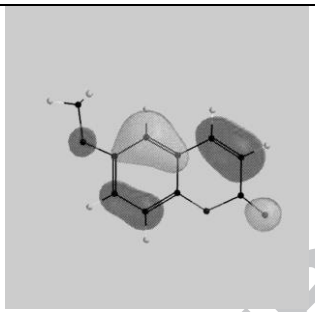
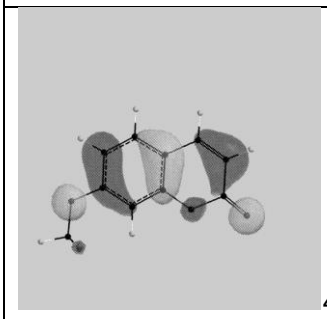
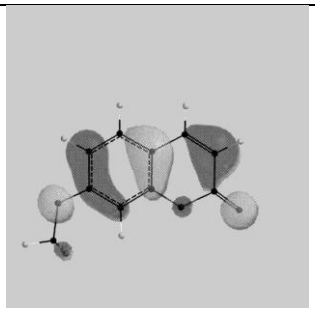
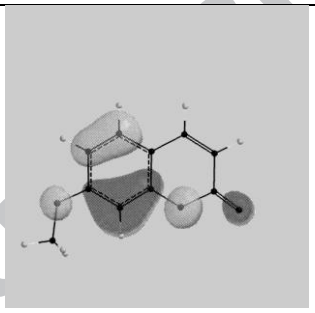
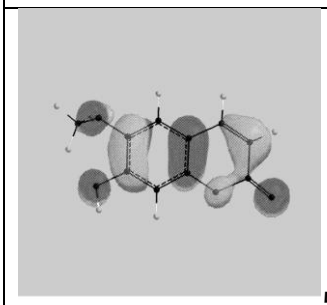
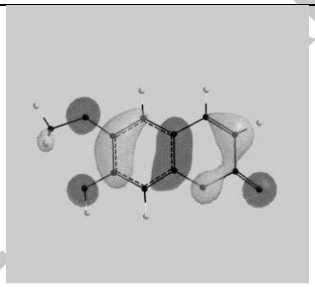
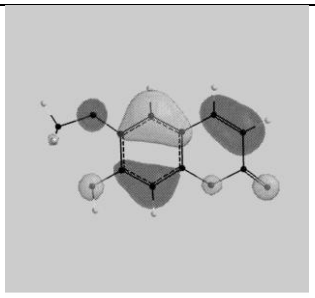
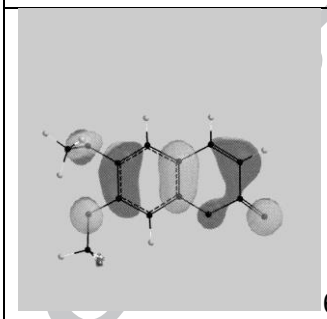
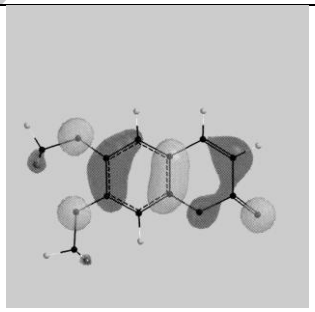
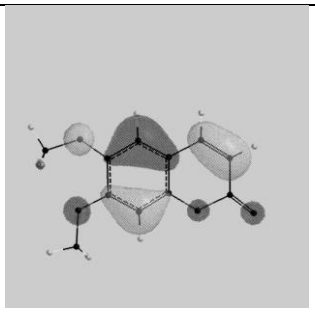
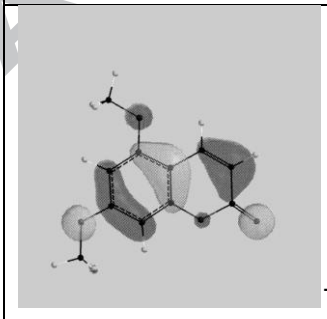
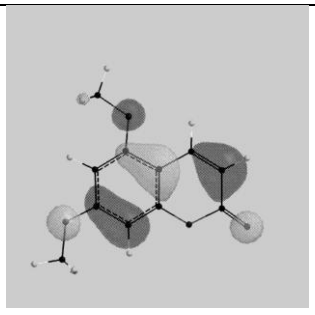
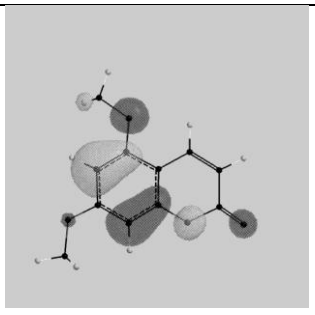
This linearity (except for compound **4**) could be explained through the postulation of reactions which are in equilibrium state in the ion trap, but this would require various additional studies.

In order to search an alternative or complementary explanation to that postulated by Shapiro-and Djerassi, we calculated and analyzed the HOMO orbitals of the compounds when they are as neutral molecules and when they have lost an electron to form the molecular ion. Calculations have been made using unrestricted B3LYP/6-31G(d) computations. Results are shown in Table 3. It can be observed that, in the molecular ions of compounds **3**, **5** and **6**, the still occupied orbital (shown in the right column of Table 3) is covering the bonds that extend the conjugation, promoting a *p*-quinoid structure. In contrast, the molecular ions of compounds **4**, **7** and **8** showed occupied HOMOs that did not extend the conjugation to form the *p*-quinoid structure.

Another qualitative indication about the process under study comes from the calculations and representations of the bond density of the molecular ion. Figure 9 exhibits the corresponding surfaces obtained with an isovalue of 0.3, from B3LYP/6-31G(d) calculations, for the six molecules which could suffer the loss of the methyl radical. As can be see, the conclusions cannot be considered as final.

Table 3: Unrestricted B3LYP/6-31G(d) calculated HOMO

Neutral molecule	molecular ion: α (E=[e.V])	molecular ion: β (E=[e.V])

 3	 E=-11.0	 E=-11.6
 4	 E=-11.2	 E=-11.7
 5	 E=-10.6	 E=-11.2
 6	 E=-10.4	 E= -11.0
 7		

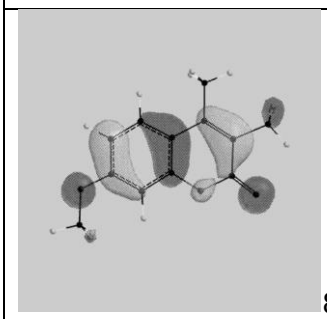
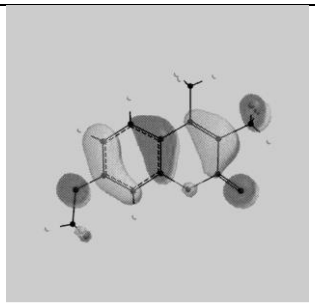
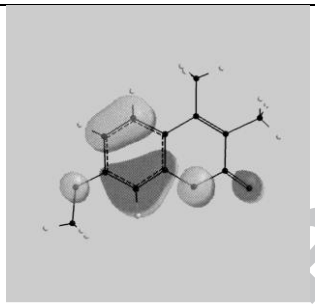
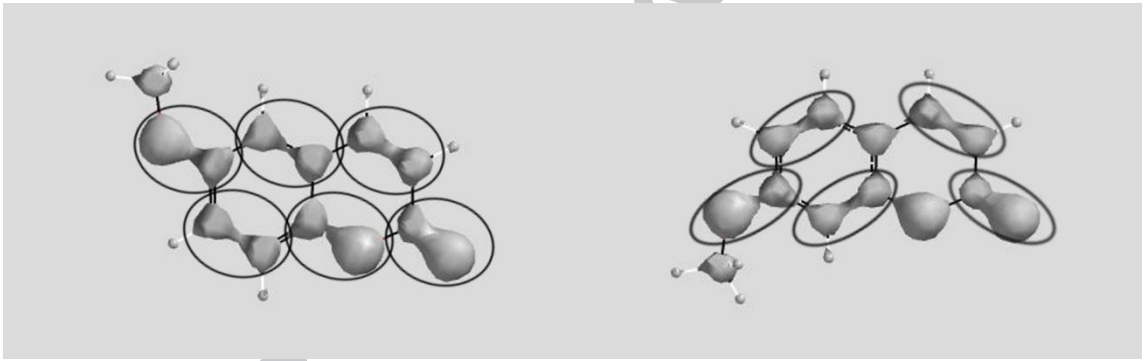
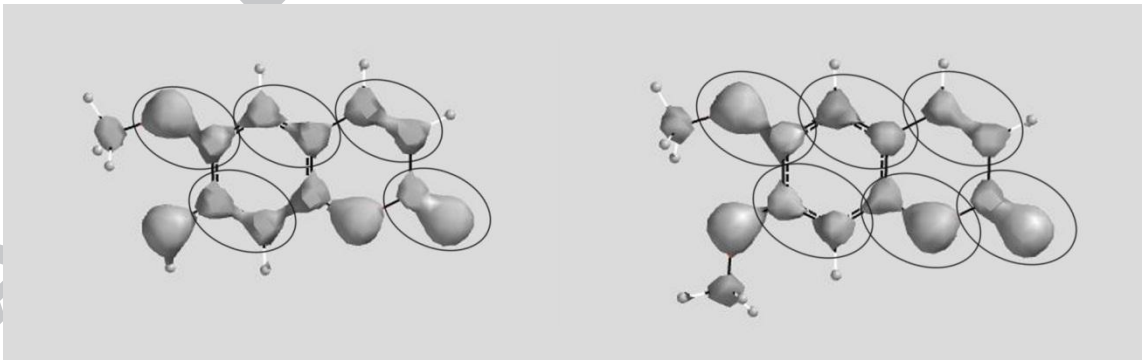
 8	E=-10.9  E==-10.7	E=-10.9  E=-11.3

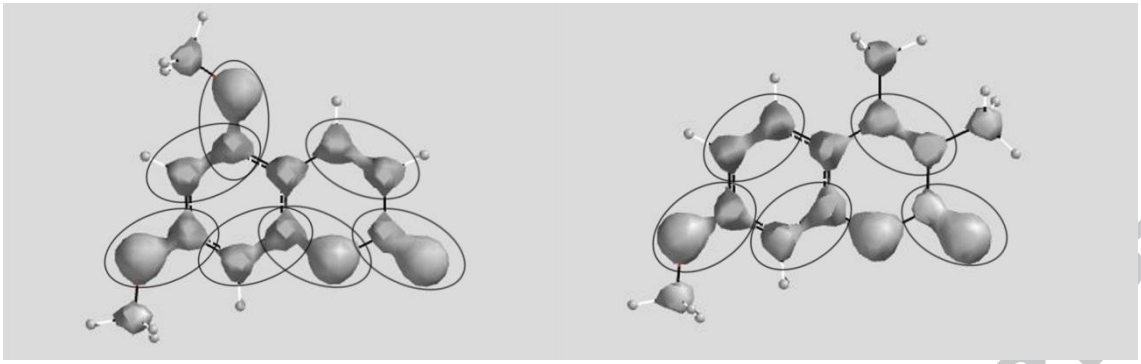
Figure 9: B3LYP/6-31G(d) bond density of molecular ions calculated for compounds **3** – **8**



3 **4**



5 **6**



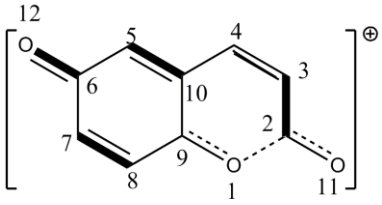
7 **8**

These qualitative indications lead us to attempt to obtain a quantitative approach to the problem. Thus, we also performed a QTAIM analysis, which showed that the extension of conjugation produced with the methyl loss in 6-methoxycoumarin (**3**) to acquire *p*-quinone type conjugation, can also be demonstrated using this theoretical approach. We selected compounds **3** and **4** as representative cases of the entire molecules studied. Table 4 gives the percent change in the ε (ellipticity) and ρ (density at the bond critical point) calculated for the significant bonds of compounds **3** and **4** when their respective molecular ion loses a methyl radical.

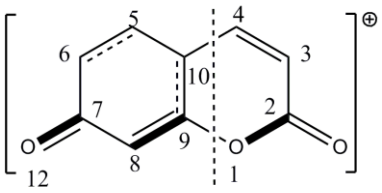
Table 4: Percent variation of ellipticity and density at critical points of the indicated bonds. Values calculated for compounds **3** and **4** by using B3LYP/6-31G(d) wave function, when the molecular ion loss a methyl radical.

6-methoxycoumarine (**3**)			7-methoxycoumarine (**4**)		
Bond	**Δε %**	**Δρ %**	**Bond**	**Δε %**	**Δρ %**
O1 – C2	653.9	-22.5	**O1 – C2**	**125.2**	**5.7**
C2 - C3	-0.4	0.6	C2 - C3	-24.8	-2.33
C2 – O11	-11.6	**3.4**	**C2 – O11**	**4.1**	**0.6**
C3 – C4	**5.6**	**0.5**	**C3 – C4**	3.5	1.7
C4 – C10	-13.2	-1.8	C4 – C10	-18.2	-0.8
C10 – C5	**10.9**	**6.6**	C10 – C5	-26.2	-2.7
C5 – C6	-63.1	-12.0	C5 – C6	-1.9	**2.6**
C6 – C7	-52.1	-8.8	C6 – C7	-49.8	-7.8
C7 – C8	**5.4**	**3.6**	C7 – C8	-59.7	-11.9
C8 – C9	-29.8	-4.8	**C8 – C9**	**2.7**	**4.9**
C9 – C10	-27.5	-3.9	**C9 – C10**	-3.2	**0.7**
C9 – O1	-70.7	10.3	C9 – O1	173.4	-3.7
C6 – O12	**112.3**	**29.5**	**C7 – O12**	**189.9**	**27.2**

Figure 10: % of change in density (ρ) (as double bonds) in the critical point for the different bonds in **3** and **4** when the molecular ion loss a methyl radical



A



B

We assume that an increase in the density at the bond critical point (ρ) implies an increase in the bond strength, particularly when it occurs with a positive change on ellipticity. These results can be well appreciated in figure 10 where the bolds are used to indicate in which bonds take place such situation, taking into account the magnitude of change. In this figure the bonds in which ρ increases but ε diminishes are indicated as dashed lines, as well as that single bonds where ρ diminishes.

From figure 10 it can be observed that the resultant in compound **3** resembles a *p*-quinoid form, while is not in such a way for compound **4,** where the dashed line indicates the conjugation interruption.

There are various studies about parameters to describe the strength of bonds (see, for example,[51]), but we choose to employ parameters of general use. The parameters that show a significant correlation are the Wiberg indexes which were calculated by using the NBO approximation. In these calculations the method employed was B3LYP/6-311G+(2d,p). The increase of the Wiberg indexes at the indicated bonds when the methyl radical is lost from molecular ion, explains the stabilization due to the M-15 fragment loss in the case of compound **3**, while this additional stability due to the extension of conjugation is not observed in the case of compound **4**.

Table 5: Wiberg indexes calculated (B3LYP/6-311G+(2d,p)) using the NBO approximation, for significant bonds in **3** and **4.**

6-methoxycoumarine (**3**)				7-methoxycoumarine (**4**)			
Bond	**$M^{+\cdot}$**	**$[M-15]^{+}$**	**% increase**	**Bond**	**$M^{+\cdot}$**	**$[M-15]^{+}$**	**% increase**
O1 – C2	0,7993	0,6414	-19,75	O1 – C2	0,863	0,9063	**5,02**
C2 - C3	1,0888	1,1105	**1,99**	C2 - C3	1,0824	1,0470	-3,27
C2 – O11	1,8568	1,9734	**6,28**	C2 – O11	1,8149	1,8276	**0,70**
C3 – C4	1,7131	1,7406	**1,61**	C3 – C4	1,5692	1,6119	**2,72**
C4 – C10	1,1529	1,1177	-3,05	C4 – C10	1,2684	1,2572	-0,88
C10 – C5	1,4462	1,6232	**12,24**	C10 – C5	1,2390	1,2058	-2,68
C5 – C6	1,2756	1,0303	-19,23	C5 – C6	1,6084	1,6814	**4,54**
C6 – C7	1,1720	1,0251	-12,53	C6 – C7	1,1497	1,0210	-11,19
C7 – C8	1,6108	1,7338	**7,64**	C7 – C8	1,3080	1,0466	-19,98
C8 – C9	1,2323	1,1564	-6,16	C8 – C9	1,4420	1,5671	**8,68**
C9 – C10	1,1553	1,0958	-5,15	C9 – C10	1,1335	1,1593	**2,28**
C9 – O1	1,1553	1,3416	**16,13**	C9 – O1	1,0722	1,0120	-5,61
C6 – O12	1,1681	1,7994	**54,05**	C7 – O12	1,1789	1,7831	**51,25**

Figure 11: Change (bold) in Wiberg Indexes when forming the molecular ion of **3** and **4**

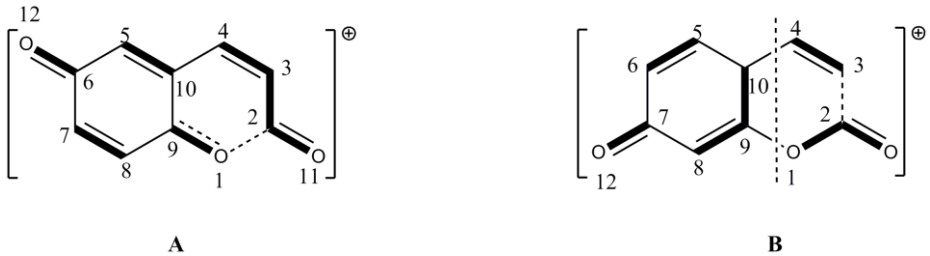


Figure 11 shows the Wiberg indexes obtained before and after the loss of the methyl radical, for compounds **3** and **4**. The bold lines show the bonds with increased indexes. Nevertheless, it is interesting to note that the magnitude of this increase is different for similar bonds of distinct molecules. For example, the Wiberg index of bond C2-O11 increase a 6.27% in the 6-methoxycoumarin but only 0.70% in the 7-methoxycoumarin.

6. Conclusions

A complementary explanation from a quantitative point of view about the fragmentation way for the methyl loss from methoxy coumarins has been reported in this work. These theoretical and experimental results were obtained in a series of selected coumarins.

Our results demonstrated that different theoretical models are very useful to explain the fragmentation occurred in MS, supporting and/or complementing the usual rules of fragmentation.

From a qualitative point of view, the comparative analyses of HOMO of the neutral molecule and the molecular ion, as well as the calculation of the bond density, have given a good explanation of the methyl loss in 6-methoxycoumarin. These analyses also explain the lack or minimal – loss of that group in methylated coumarins substituted in other positions. From a quantitative point of view, the thermodynamics parameters have displayed an interesting correlation with the quantitative parameters of CID mass spectrometry. The QTAIM analysis gives a good correlation in order to explain the formation of *p*-quinoid resonance forms. However, the best correlation has been obtained using the NBO approximation and from the Wiberg indexes.

While our study was limited to coumarins, it is important to note that the methods used here are of general use. Thus, it is reasonable to believe that this methodology can be used to explain fragmentation in other compounds having similar behavior.

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Highlights

We obtained a quantitative explanation about the methyl loss from methoxy coumarins.

Different theoretical models are useful to explain the fragmentation occurred in MS.

The QTAIM analysis explains adequately the formation of p-quinoid resonance forms.

We obtained the best correlation using the NBO approximation and the Wiberg indexes.

This kind of explanation can be used in compounds with similar MS behavior.