Electrospray tandem mass spectrometry of *2H*-chromenes

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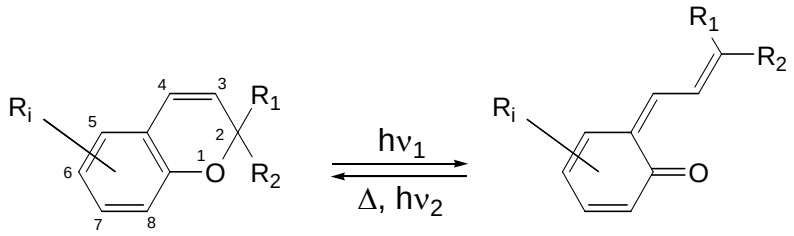
Abstract

Several 2*H*-chromenes, derived from carbazoles, were analyzed by electrospray tandem mass spectrometry. The 2*H*-chromenes constitute an important class of compounds that exhibit photochromic activity. The fragmentation pathways of the protonated molecular species $[M+H]^+$ were studied and main fragmentation pathways of these compounds were identified. Fragmentation pathways of $[M+D]^+$ ions were also studied in order to obtain information about the ionizing proton or deuteron. It was found that proton is not preferentially located on the nitrogen atom. The charge is preferentially located as a tertiary carbocation, resulting from the uptake of the proton (or deuteron) by the zwitterionic open structure of the chromenes. The major fragmentation occurred by cleavage of the γ-bond relative to the carbocation center, leading to a fragment at *m/z* 191 ($C_5H_{11}^+$ or $C_{14}H_9N^{+\bullet}$), which are the most abundant fragment ions for almost all compounds. The presence of substituents in the chromene ring does not change this behavior. Other observed common fragmentation pathways included loss of CH_3 (15 Da), loss of CO (28 Da), combined loss of CO and CH_3 (43 Da), and loss of phenyl ring via combined loss of C_6H_4 and CH_3 (-91Da) and combined loss of C_6H_6 and CO (-106 Da).

The 2*H*-chromenes (benzo- and naphthopyrans) are an important class of compounds that exhibit photochromic activity in polymeric matrices and solutions at room temperature. Such compounds undergo a pyran-ring opening under irradiation due to the breaking of C($sp3$)-O bond ring that leads to an equilibrium between the closed form (CF) and a set of stereoisomers of the open form (OF) also referred to as photomerocyanines (Scheme 1). These open forms have different stabilities and their absorption spectra are in the visible range. The phenomenom is photochemically (with visible light) and thermally reversible.[1,2]

This process can be repeated many times, but the fatigue occurs as a result of irreversible side reactions which limit the commercial application of these systems.



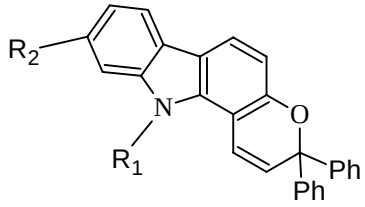
Colorless closed form Colored s-trans open form

Scheme 1 . Equilibrium between the closed and open forms of chromenes.

The photochromic parameters can be significantly improved through heteroatomic annellation, although the effects are very dependent on the nature of the annellated moiety and on the relative positions of the heteroannelation at the 2*H*-1-benzopyran skeleton. In previous work, we prepared and described new 2*H*-1-benzopyrans containing a carbazole moiety,[3,4] and here we describe their mass spectral characteristics. These *2H*-chromenes are represented in Scheme 2.

To our best knowledge, there is only one reference in the literature dealing with mass spectrometric fragmentation studies of chromenes, and this study used electron impact ionization (EI) mass spectrometry; the typical EI

fragmentation of these compounds was found to be the loss of one of the substituents on C-2, giving a stable benzopyrilium ion. [5]

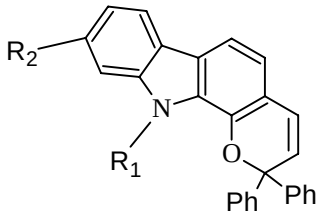


1A R_1=R_2=H

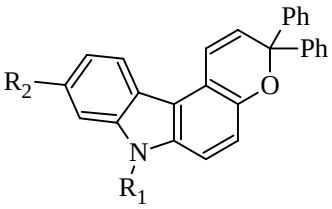
1B R_1=CH_3, R_2=H

6A R_1=H, R_2=Br

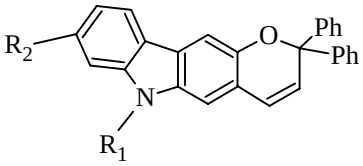
6B R_1=CH_3, R_2=Br



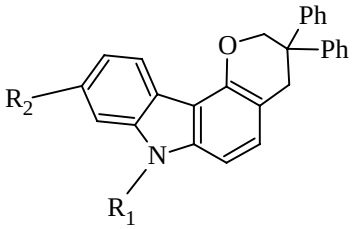
2B R_1=CH_3, R_2=H



3A R_1= R_2=H

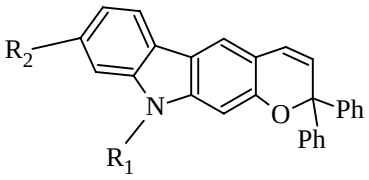


3B R_1=CH_3, R_2=H



4A R_1=R_2=H

4B R_1=CH_3, R_2=H



5B R_1=CH_3, R_2=H

7B R_1=CH_3, R_2=CN

8B R_1=CH_3, R_2=Thienyl

Scheme 2 . *2H*-Chromenes derived from carbazoles studied by ES-MS and ES-MS/MS. Chromenes labelled with **A** have a hydrogen atom linked to the nitrogen atom, and labelled with **B** have a methyl group linked to the nitrogen.

EXPERIMENTAL

Spectra from positive ion mode electrospray mass spectrometry (ESI-MS) and tandem mass spectrometry (ESI-MS/MS) spectra were acquired using a Q-TOF2 instrument (Micromass, Manchester, UK), the needle voltage was set at 3000V with the ion source at 80ºC and sample cone voltage at 35V. Nitrogen was used as nebulizer gas and argon as collision gas. Tandem mass spectra (MS/MS) of $[M+H]^+$ ions were obtained by collision-induced decomposition (CID) using argon as the collision gas and with collision energies between 22-27eV. Data acquisition was accomplished using a Micromass Masslynx 4.0 data system. In addition, some experiments were performed that were intended to obtain accurate mass measurements for some key fragment ions, as well as MS/MS/MS spectra exploiting in-source CID; however, no additional useful information could be obtained by these experiments, and they are not reported here.

Samples were prepared for electrospray analysis by dissolving in chloroform and then diluting 1 μL in 200 μL of MeOH for a final concentration of approximately 10 pmol/μL. Samples were introduced into the mass spectrometer using a flow rate of 10 μL/min. Methanol and chloroform were HPLC grade (Riedel-de-Haen). Methanol-*d*1 (CH_3OD) (Riedel-de-Haen) and chloroform-*d*1 (CCl_3D) (Riedel-de-Haen) were used in the solution preparation to obtain the deuterated molecular species $[M+D]^+$. The chromenes were synthesized by a previously described methodology.[3,4]

RESULTS AND DISCUSSION

Electrospray mass spectra of all compounds show the protonated molecule $[M+H]^+$, that can, as discussed below, be present in either the open or closed form. Table 1 indicates the *m/z* values for the protonated molecules of the compounds studied. Although several of the studied compounds are isomeric, we found that few differences are observed in their MS/MS spectra. In this section the fragmentation pathways of each group of isomers are presented

and discussed, describing the main fragmentation pathways, and the differences among them.

Table 1. Main fragment ions observed in the ES-MS/MS spectra of the $[M+H]^+$ ions of compounds studied.

Chromenes	*m/z* $[M+H]^+$	*m/z (*relative abundance)
1A	374	373 (3); 359(2); 346(2); 331(1); 296(7); 283 (4); 268(5); 196(3); 191(**100**); 182(18)
3A	374	373 (3); 359(4); 346(3); 331(2); 296(34); 283 (15); 268(9); 196(7); 191(80); 182(**100**)
4A	374	373 (3), 359(12); 346(3); 331(2); 296(18); 283 (9); 268(6); 196(6); 191(**100**); 182(14)
2B	388	373 (20); 360(3); 345(2); 310(9); 297(8); 282(2); 210(5); 197(20); 196(39); 191(100)
1B	388	373 (10); 360(3); 345(2); 310(10); 297(6); 296(9) 284(8); 282(6); 220(7); 210(5); 197(15); 196(30); 191(**100**)
5B	388	373 (8); 360(4); 345(4); 310(16); 297(12); 282(5); 210(8); 197(18); 196(20); 191(**100**)
3B	388	373 (20); 360(5; 345(5); 310(20); 297(17); 296(20); 282(5); 210(8); 197(30); 196(**100**); 191(90)
4B	388	373 (9); 360(2); 345(2); 310(18); 297(14); 282(5); 220(<1); 210(7); 197(20); 196(17); 191(**100**)
6A	452	437 (12), 375(<1), 373(50), 358(7), 346(2), 296(7), 295(10), 260(22), 191(**100**)
6B	466	451(5); 388(5); 387(70); 372(5); 379(5); 362(6); 310 (4); 274(32); 246(10); 196(5); 191(**100**)
7B	413	397(15), 385(2), 370(<1), 336(12), 335(40), 322(15), 307(15), 221(15), 191(**100**)
8B	470	455(20), 442(8), 427(7), 392(20), 379(10), 464(5), 279(60), 270 (8),191(**100**)

Table 1 presents the main fragment ions observed in the MS/MS spectra of all compounds. As examples of these MS/MS spectra, the ESI-MS/MS spectra obtained for compounds **1A** (Fig. 1(A)), **1B** (Fig. 1(B)), and for the corresponding bromo derivatives **6A** (Fig. 1(C)), and **6B** (Fig. 1(D)), are shown in Fig. 1. All of these chromenes yield common fragment ions. The fragment ion at *m/z* 191 is observed in all the spectra and corresponds to the base peak for all chromenes except for **3A** and **3B**.

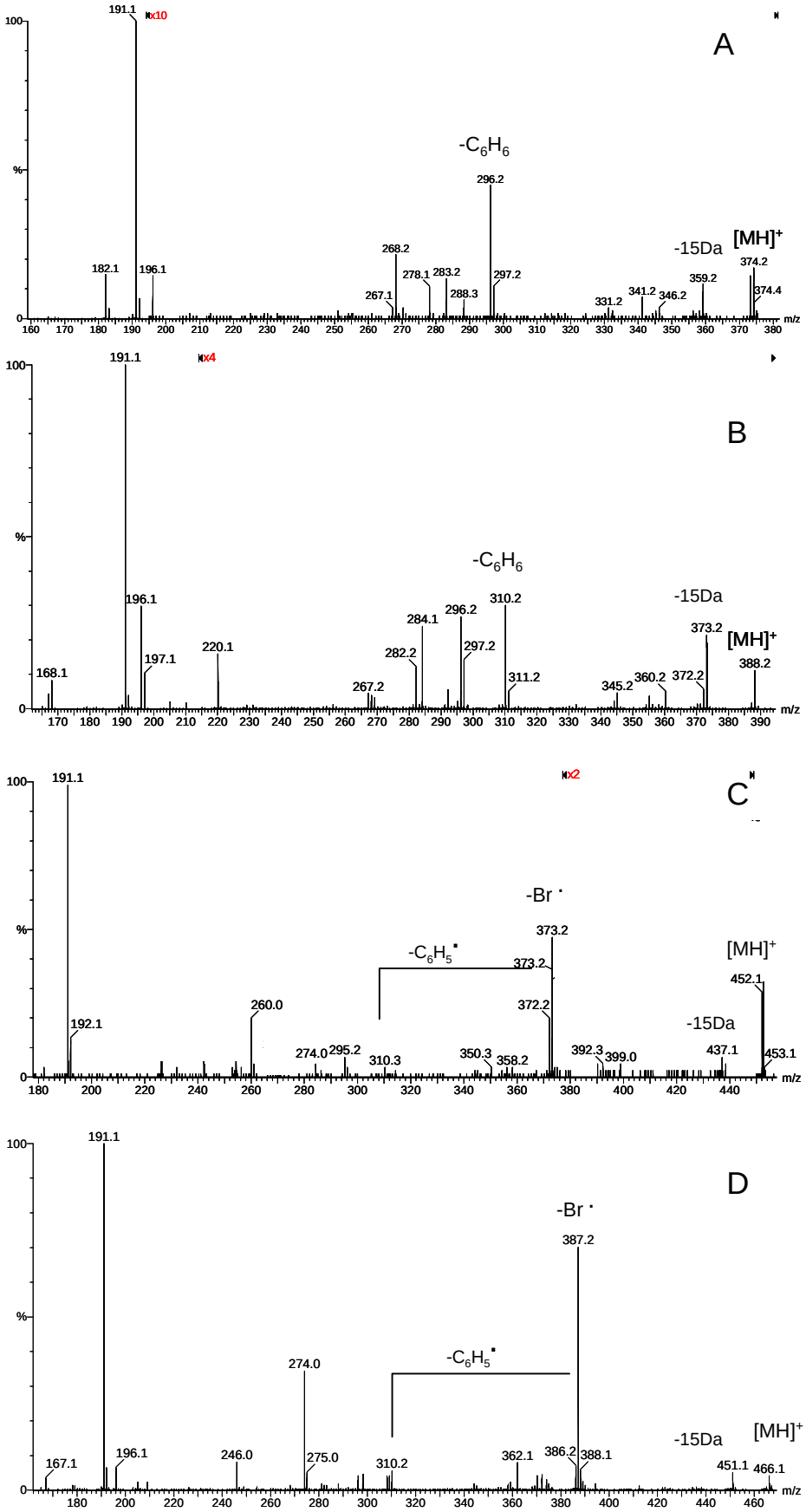
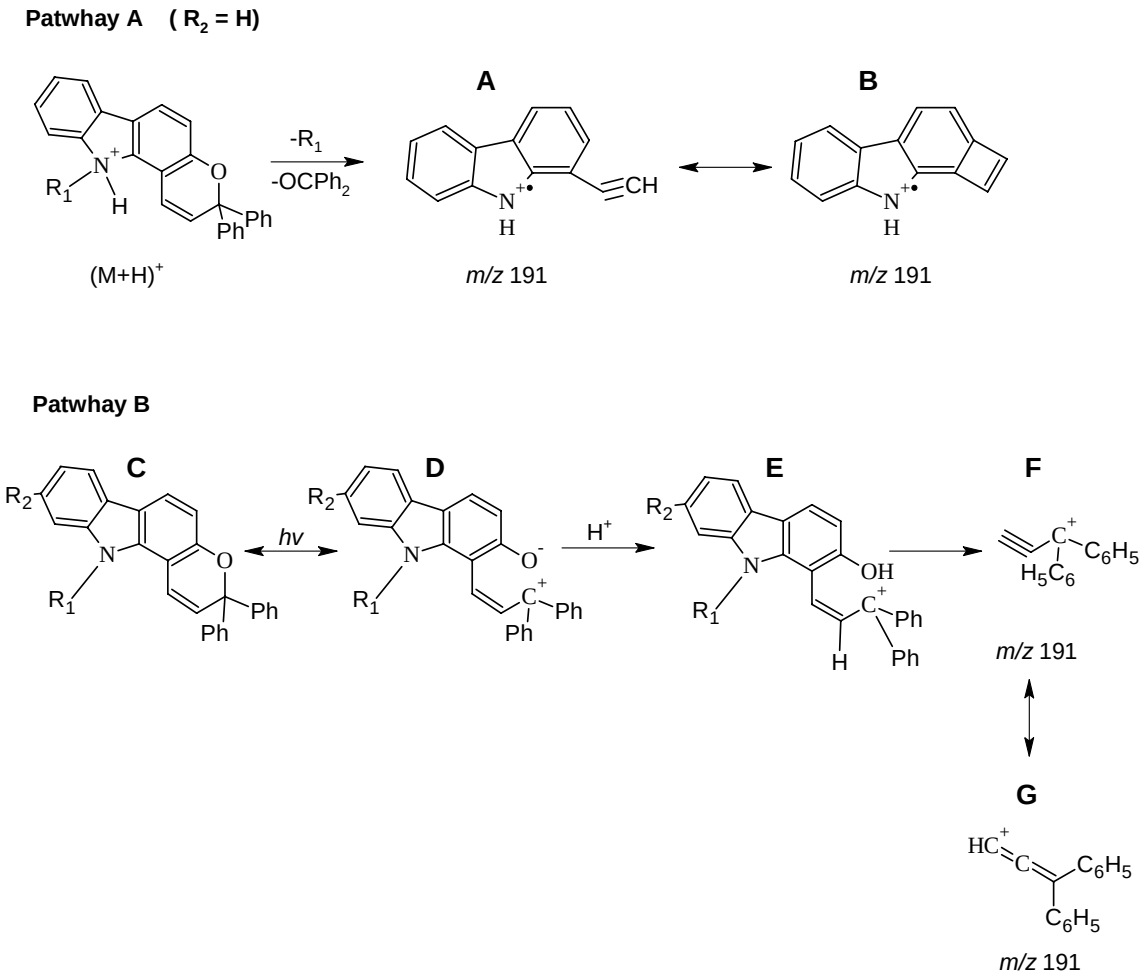


Figure 1 - ES-MS/MS spectra of $[M+H]^+$ ion of chromenes **1A**(A), **1B**(B), **6A**(C) and **6B**(D).

Since the precursor ions are protonated molecules and the proton is reasonably expected to be attached to the nitrogen in each molecule, formation of the ion at *m/z* 191, for compounds with R_2=H, could be due to loss of R_1 and cleavage of chromene ring with loss of $OCPh_2$ (Scheme 3, pathway A, structure **B**).



Scheme 3. Proposed mechanisms for the formation of the ion at *m/z* 191.

Fragmentation of the chromene ring was already observed as a result of electron impact ionization of carboxychromones. [5] In the case of bromo chromenes (R_2=Br), the *m/z* 191 fragment cannot be explained by this fragmentation pathway, because R_2 does not contain a hydrogen atom that can be transferred to the phenyl ring. In order to better understand the

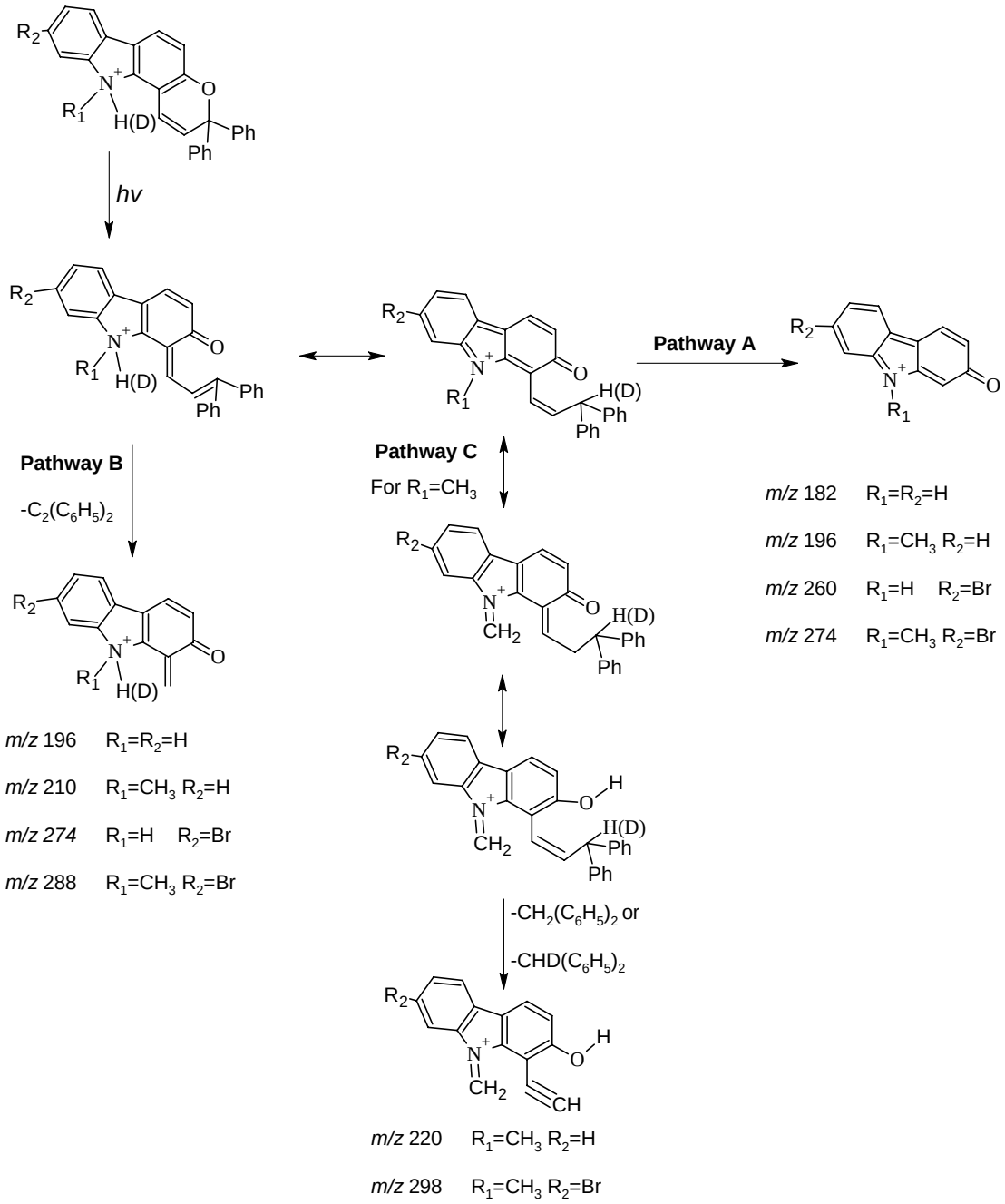
fragmentation mechanism, MS/MS spectra of deuteronated molecules were also studied. It was found that, under those conditions, the ion at *m/z* 191 was still the base peak of the MS/MS spectra of the deuteronated molecules (Fig. 2)

This was unexpected since the expected fragment ion was *m/z* 192, corresponding to the proposed structures **A** and **B** of Scheme 3, with the ionizing proton substituted by a deuteron linked to the nitrogen atom. This ion at *m/z* 192 was indeed formed but with lower relative abundance than the ion at *m/z* 191. This led us to suggest that another precursor ion with the same *m/z* was formed, with the charge located at a position in the molecule different from that initially proposed (the nitrogen atom).

Chromenes display photochromic activity and, when subjected to light, can generate open structures due to the opening of the pyran ring, as show in Scheme 3, pathway B (structures **C** and **D**). This intermediate open structure (zwitterion structure **D**) can be formed in solution, and the negatively charged oxygen can acquire a proton, to generate a stable tertiary carbocation (structure **E**). This molecular ion, with the charge localized on a carbon (pathway B, structure **E**), can fragment by cleavage of the bond adjacent to the charge location to generate a different fragment with *m/z* 191 (pathway B, structure **F**), that is in resonance with an allenic carbocation (pathway B, structure **G**). As a result of its proposed origins, this fragment ion is predicted to have the same *m/z* value irrespective of whether the original ionizing entity was a proton or a deuteron, as observed.

Other common fragmentation pathways with formation of product ions at *m/z* 182 were observed for chromenes containing secondary amine moieties (N-H groups, i.e., compounds **1A**, **3A**, and **4A**, with R_1=R_2=H), for chromenes with N-CH_3 (R_1= CH_3, R_2=H, i.e., compounds **1B**, **2B**, **3B**, **4B**), that form product ions at m/z 196, and for bromochromenes (R_2=Br, compounds **6A** and **6B**) that yield ions at *m/z* 260 and 274. These fragmentations pathways are proposed to be initiated by a hydrogen rearrangement of the hydrogen atom linked to the nitrogen, followed by loss of $HCCCH(Ph)_2$, as shown in Pathway A of Scheme 4. This proposed mechanism is corroborated by absence of the ions at m/z 182 (R_1=R_2=H) and 196 (R_1=CH_3, R_2=H) for the correspondent brominated derivatives, **6A** and **6B**, and the formation of other ions at m/z 260 (R_1=CH_3,

R_2=Br) and 274 (R_1=CH_3, R_2=Br) corresponding to the same fragment ion with the additional bromine atom in its composition, as indicated in Scheme 4. In the MS/MS mass spectra of the deuteronated molecules these ions are still observed, consistent with the proposal that the ionizing proton or deuteron migrates preferentially to the carbon atom to which the two Ph are linked, and is then eliminated in the neutral fragment in this fragmentation pathway.



Scheme 4. Main fragmentation pathways leading to lower *m/z* ions, represented for the **1A**, **1B**, **6A** and **6B**.

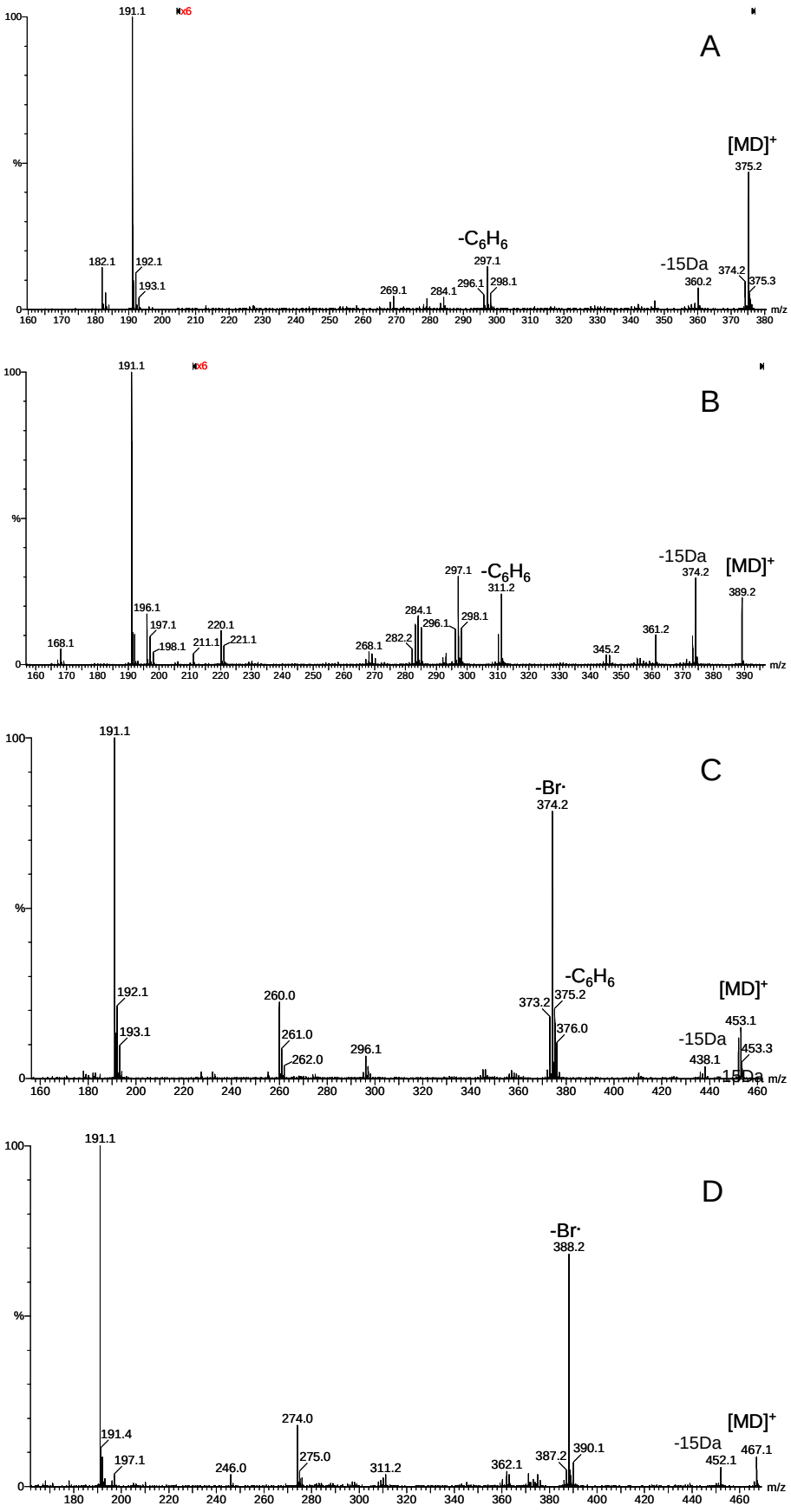


Figure 2 - ES-MS/MS spectra of $[M+D]^+$ ion of chromenes 1A(A), 1B(B), 6A(C) and 6B(D)

As shown in Table 1, the fragment ion at *m/z* 182 corresponds to the most abundant ion of the MS/MS spectrum of compound **3A** and the ion of m/z 196 corresponds to the most abundant ion of the MS/MS mass spectrum of compound **3B**, although the ion at m/z 191 is still observed with high relative abundance. The higher abundances of the reported fragments (*m/z* 182 and 196) are likely related to the position of the oxygen atom since these chromenes have different annellations but the same *N*-position, N-C(6).

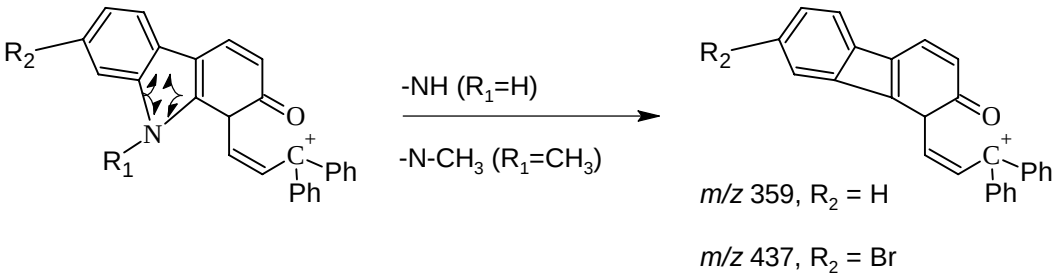
Previous kinetics studies using UV-visible spectroscopy have already indicated that the open forms of both compounds (**3A**, **3B**, Schemes 1 and 2) were more stable when compared with compounds studied with same annellation.[4] This is consistent with our observation that both fragments (*m/z* 182 and 196) appear to be formed from cleavage of the open form, similarly as was described in Scheme 4, pathway A.

The present data suggest that there are other fragmentation pathways that lead to the formation of the ions at m/z 196, 210, 274 and 288 respectively for chromenes with R_1=R_2=H (**1A**, **3A** and **4A**) and R_1= CH_3, R_2=H (**1B**, **2B**, **3B**, **4B** and **5B**) and the corresponding bromo derivatives (**6A** and **6B**). These ions can be rationalized as being also formed from the initial open structure, but with the charge retained at the nitrogen, as represented in pathway B of Scheme 4. The proposed charge location is supported by the observation of an increase of 1 Da in the masses of each of these ions in the MS/MS spectra of the corresponding $[M+D]^+$ ions of the compounds with phenyl substituents. Interestingly, the fragment ions at *m/z* 220, observed for chromene **1B**, and at *m/z* 298 for chromene **6B** (both compounds have R_1=CH_3) do not yield fragment ions corresponding to those in spectra of chromenes with R_1=H, as exemplified in the MS/MS spectra of chromenes **1A** and **6A** (*m/z* 206 and 284, respectively). This observation can be rationalized by the involvement of the R_1 methyl group in this fragmentation pathway. As proposed in Scheme 4- pathway C, the resonant structure, which involves the migration of one hydrogen of the methyl group to one carbon of the side chain, with rearrangement of the aromatic double bonds, allows the elimination of diphenylmethane, $CH_2(C_6H_5)_2$. This proposal is consistent with the observation that this fragmentation pathway is accessible only to the *N*-methylchromenes. The fragmentation pathway A

predicts that the ionizing proton should be absent from the fragment ions (*m/z* 182 and 196) and this is confirmed by the observation of the same fragments in the MS/MS spectra of the $[M+D]^+$ molecular ion.

Several other common fragmentation pathways have been identified for all the studied chromenes such as losses of 15, 28, 77, 78, 91, and 106 Da. In chromenes with R_1=CH_3, loss of 15 Da is due to loss of the methyl group by homolytic cleavage (-CH_3·). Although NH is considered a high-energy fragment, in chromenes containing a N-H (R_1=H), loss of 15 Da is also observed probably due to loss of NH, as proposed in Scheme 5. This suggests that, if this fragmentation pathway is to occur, it is essential that the charge is not located on the nitrogen. Loss of N-CH_3, for compounds with R_1=CH_3 although observed, is much less abundant. Generalizing, loss of N-R_1 was observed for all compounds, as represented in the fragmentation pathway proposed in Scheme 5.

Other fragmentation pathways are also common, such as loss of a phenyl group as a radical (-C_6H_5·, 77Da) or, more frequently, as a neutral (-C_6H_6, -78Da), losses of 91 and 106 Da are observed for all compounds. These losses were also observed in the MS/MS spectra of the $[M+D]^+$ molecular ion, suggesting that the ionizing proton (or deuteron) was not involved in the expelled neutrals and remains attached to the fragment ion formed. Loss of 91 Da corresponds to combined loss of 15 Da with loss of C_6H_4 (loss of a phenyl group with migration of a hydrogen from the phenyl ring to the fragment ion formed). The loss of 106 Da occurred by combined elimination of phenyl ring and CO.



Scheme 5 – Proposed mechanism for the loss of N-R_1.

Loss of CO can also occur from the protonated molecule. This loss of CO is also indicative of the presence of the open structure of chromenes containing a cyclic ketone moiety that is susceptible to elimination of CO; this is a typical fragmentation of cyclic ketones.[6]

For the chromenes with a bromine substituent, loss of Br occurs with formation of an abundant fragment ion. Loss of Br occurs also in combination of loss of $^{\cdot}C_6H_5$, with formation of the fragment ions at *m/z* 296 (or, together with loss of C_6H_6, an ion at *m/z* 295) and 310, for **6A** and **6B**, respectively.

Fragmentation of the protonated molecules of compounds **7B** and **8B** (Scheme 1), with CN and thienyl substituents, and R_1=CH_3, followed fragmentation pathways reported for the other chromenes, such as loss of $^{\cdot}CH_3$ (15Da), loss of CO (28Da), combined loss of CO and $^{\cdot}CH_3$ (43Da), and loss of a phenyl ring (losses of 91 and 106 Da). Formation of the ions at *m/z* 221 (**7B**) and 279 (**8B**) occurred by loss of diphenyl-alkyl chain of the open structure.

CONCLUSIONS

Positive mode electrospray tandem mass spectrometry has shown to be very useful for structural characterization of chromenes, allowing identification of the main common fragmentations of this class of compounds. The charge of the protonated molecule is preferentially located at a stable carbocation, in contrast with the expected location on the nitrogen of the heterocycle. The main fragmentations occurred by cleavage of the charged open form structure rather than the closed form. Common fragmentation pathways included loss of $^{\cdot}CH_3$ (-15 Da), loss of CO (-28Da), combined loss of CO and CH_3 (-43 Da), and loss of a phenyl ring (combined loss of C_6H_4 and $^{\cdot}CH_3$ (-91 Da) and combined loss of C_6H_6 and CO (-106 Da).

REFERENCES

1. Becker RS, Michl J. *J. Am. Chem. Soc*. 1966, **88**: 5931.

2. Van Gemert B., In *Organic Photochromic and Thermochromic compounds*; Vol. 1, Crano JC, Guglielmetti RJ (eds.). Plenum Press: New-York, 1999; 111-140.

3. Oliveira-Campos AMF, Oliveira MM, Carvalho LM, Moustrou C, Samat A, Guglielmetti R, Seita J. *Colour Science 98 Proceedings*, vol.1*, "Dyes and Pigments Chemistry"*. John Griffiths: Leeds, 1999: 26-35.

4. Oliveira MM, Carvalho LM, Moustrou C, Samat A, Guglielmetti R, Oliveira-Campos AMF, *Helv. Chim. Acta* 2001, **84** : 1163.

5. Merlini L. *Adv. Heterocycl. Chem.* 1979, **16** : 159.

6. *Interpretation of Mass Spectra*; McLafferty FW and Turecek F, (eds). University Science Books: Mill Valley, 1993; 247-252.