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

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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.

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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.

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 [10a,10b] 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) [23–27], 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.

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Traldi and co-workers [33–36] 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 Fig. 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).

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 and Djerassi have proposed the formation of $[M-15]^+$ from 6,7-dimethoxycoumarin as is shown in Fig. 2.

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, Fig. 3 shows a plausible way that predicts the methyl loss from 7-methoxycoumarin

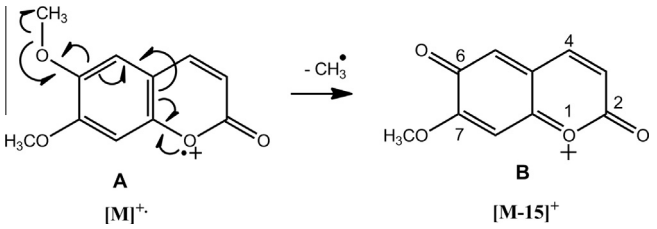

Fig. 2. Formation of $[M-15]^+$ for 6,7-dimethoxycoumarin (**6**) by Shapiro and Djerassi [41].

(**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.

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. Fig. 4 shows a possible alternative path for loss of methyl group for compound **6**, locating the initial positive charge on another atom.

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 [45–50]). 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**)

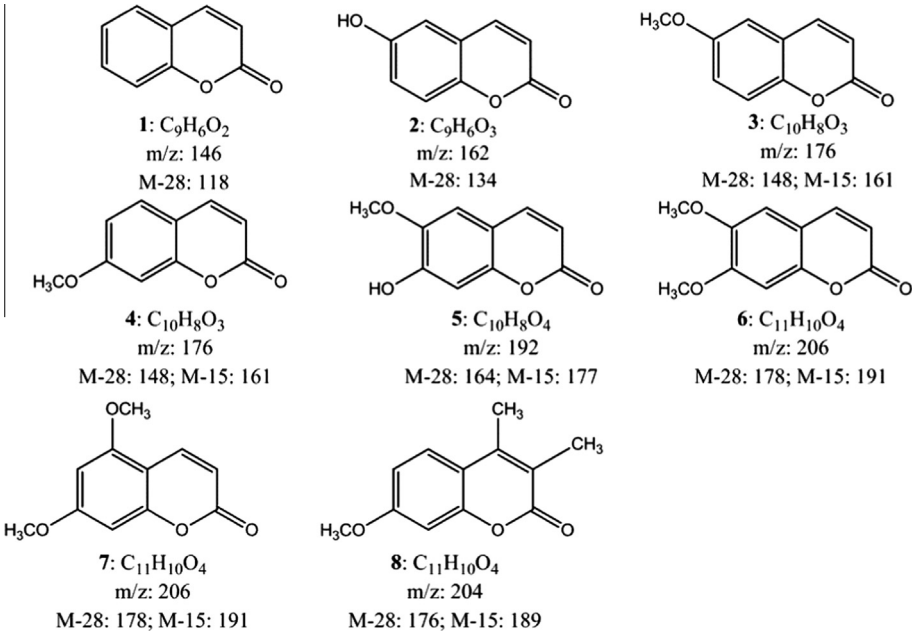

Fig. 1. Structural features of the coumarins under study.