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The fragmentation mechanism of five-membered lactones by electrospray ionisation tandem mass spectrometry☆

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Abstract

A series of five-membered lactones (both saturated and unsaturated) and a lactam were analysed by electrospray ionisation tandem (ESI-MS/MS) and sequential mass spectrometry (ESI-MS^n). It was observed that the main fragment ions were derived from neutral losses of CO and/or H_2O (NH_3 for the lactam). The fragmentation pathways followed are rationalised in terms of the resulting carbocation stability and the energy of the possible fragment ions, calculated by the CBS-Q composite method.

Keywords: Lactones; Electrospray mass spectrometry; Fragmentation mechanism; Carbocation stability

1. Introduction

Five-membered lactones, with saturated or unsaturated rings, commonly occur as a structural feature in natural and synthetic compounds. These compounds can exhibit ecological function as anti-feeding agents [1], economic impact as food flavourings [2], and medicinal activities such as antibiotics [3], antifungals [3], anti-cancers [3,4] and analgesics [5].

The correlation between the thermochemistry and gas-phase chemistry of $C_5H_6O_2$ lactones in strong acid solutions was recently demonstrated [6]. This study showed that the protonation of conjugated α,β-unsaturated lactones takes place on the carbonyl oxygen atom to produce a stable species. The ^{13}C NMR studies of the unconjugated lactones in strong acid conditions revealed the formation of the less stable C-protonated species [6]. Ammonia chemical ionisation (CI) showed the same protonation as with ^{13}C NMR. Fragments resulting from the loss of CO or H_2O were observed in all spectra, but for the unconjugated lactones the loss of CO was more significant than H_2O [6]. In both systems, conjugated and unconjugated, ring opening preceded these losses. The 3-methyl-2(5*H*)-furanone **6** (see Fig. 1) CI spectrum showed a more intense ion of *m*/*z* 69 due to the loss of $H_2C{=}O$ than the peaks at *m*/*z* 71 (loss of CO) or *m*/*z* 81 (loss of H_2O), and an aromatic three-membered ring ion was proposed as the structure for this fragment [6].

Electrospray ionisation mass spectrometry (ESI-MS) studies with the above cited lactones are previously unreported. This analytical technique, one of the most important for the analysis of non-volatile and thermally labile compounds, produces highly abundant protonated molecules due to the low residual energy of the ionisation process. The study of fragmentation pathways is required for monitoring the synthesis of organic products and for identification of biological metabolites and novel compounds. Tandem mass spectrometry (MS/MS) [7] coupled to chromatographic techniques (LC or GC) is the method of choice for the analysis of complex mixtures or organic and natu-

☆ Supplementary data, detailing the total and optimised energies of the intermediates and fragment ions proposed in the fragmentation pathways, is available from the corresponding author on request.

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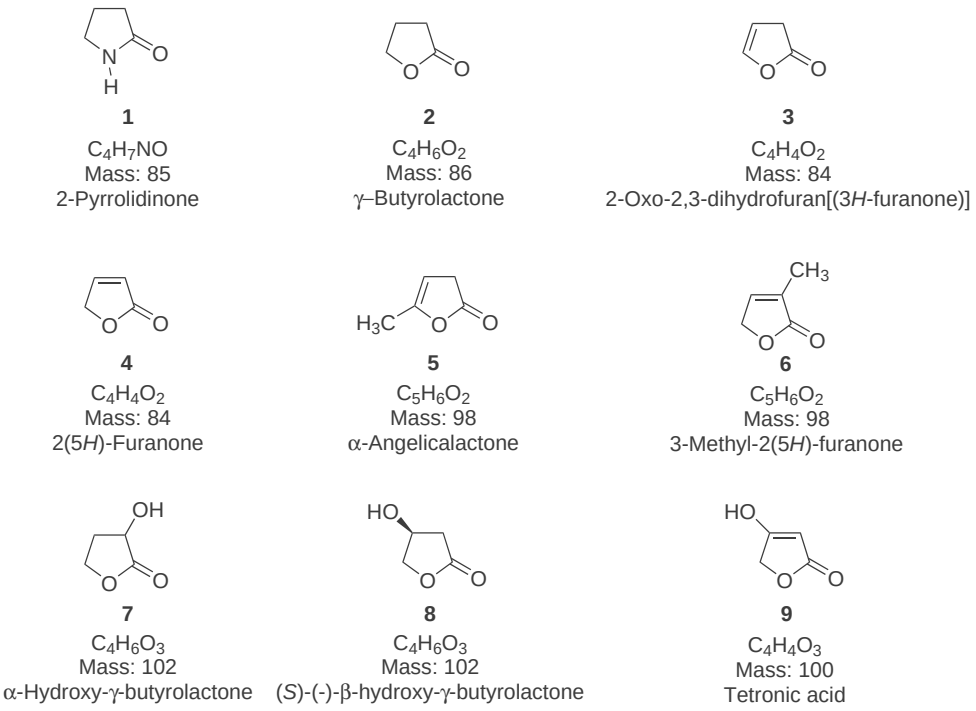


Fig. 1. Structures of compounds **1**–**9**.

ral products present in their natural conditions (metabolite extracts, biosynthetic broths or reaction media). ESI, with collision-induced dissociation (CID), where the parent ions are isolated and allowed to react with a collision gas in the collision cell, is a standard technique for just such studies. An understanding of the fragmentation mechanisms of lactones has significance in that this can be applied to the fragmentation of larger, more complex natural products and compounds of biological significance that may contain lactones as part of their structures.

In the present study, we report the fragmentation of the protonated compounds **1**–**9** (Fig. 1) by ESI-CID-MS/MS. Two alternative open-chain fragmentation mechanisms followed by neutral losses of CO and/or H_2O are proposed. Calculations of the relative stability of the intermediates and fragment ions, performed using the CBS-Q [8–12] method, are used to determine which route is most likely to occur. We also show that their gas-phase chemistry can be rationalised in terms of carbocation stability of the resulting fragment ions.

2. Experimental

Furan-2(3*H*)-one **3** was synthesised according to the method described by Näsman and Pensar [14]. Methanol (HPLC grade), dichloromethane (HPLC grade) and the pure compounds **1**, **2**, **4**–**9** were obtained from Aldrich (Gillingham, Dorset, UK). Samples were made up as 0.25 mg ml^{-1} solutions in methanol/dichloromethane. MS analysis was performed on an LCQ ion-trap mass spectrometer (Thermo-Finnigan, Hemel Hempstead, UK) by ESI. CID fragmentation was performed using helium collision gas on isolated parent ion. The collision energies used were in the range from 22 to 28% of the normalised value.

The CBS-Q [8–12] composite theoretical method as implemented in the Gaussian 98 [13] suite of programs was used for the energy calculations of the proposed intermediate and fragment ions, at 0 K. All optimised structures were shown to be the minima on the potential energy surface via vibrational frequency computations. All the geometries and absolute energies are available as supplementary data directly from the corresponding author.

3. Results and discussion

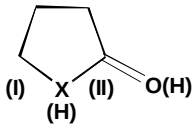
The MS/MS spectrum of the γ-butyrolactone **2** showed only one fragment ion formed by the loss of H_2O (Table 1). MS/MS analysis of the N analogue, 2-pyrrolidinone **1**, also showed only a single fragment ion corresponding to the loss of NH_3. These results are indicative of a common fragmentation mechanism for these two compounds involving loss of the heteroatom from the ring as a neutral species (Scheme 1). Data from theoretical calculations revealed that O- and N-protonated species **1a2** and **2a2**, that are required for this mechanism, exhibit a (H)–X–C=O (X = O or N) bond length longer than normal (1.63 and 1.86 Å, respectively, Table 2). This is indicative of the heterolytic cleavage of this bond in the collision cell, during the CID process, that results in the opening of the five-membered ring and arises in the acylium ions **1b** (*m*/*z*

Table 1
Ratio of the loss of CO to H_2O or NH_3 from the lactones and lactam

Compound	$[MH–CO]^+$	$[MH–H_2O]^+$	$[MH–NH_3]^+$	Ratio
1			$C_4H_5O^+$	0:1
2		$C_4H_5O^+$		0:1
3	$C_3H_5O^+$			1:0
4	$C_3H_5O^+$			1:0
5	$C_4H_7O_2^+$	$C_5H_5O_2^+$		9:1
6	$C_4H_7O_2^+$	$C_5H_5O_2^+$		6:1
7	$C_3H_6O_2^+$	$C_4H_4O_2^+$		1:2
8		$C_4H_4O_2^+$		0:1
9	$C_3H_5O_2^+$	$C_4H_3O_2^+$		4:1

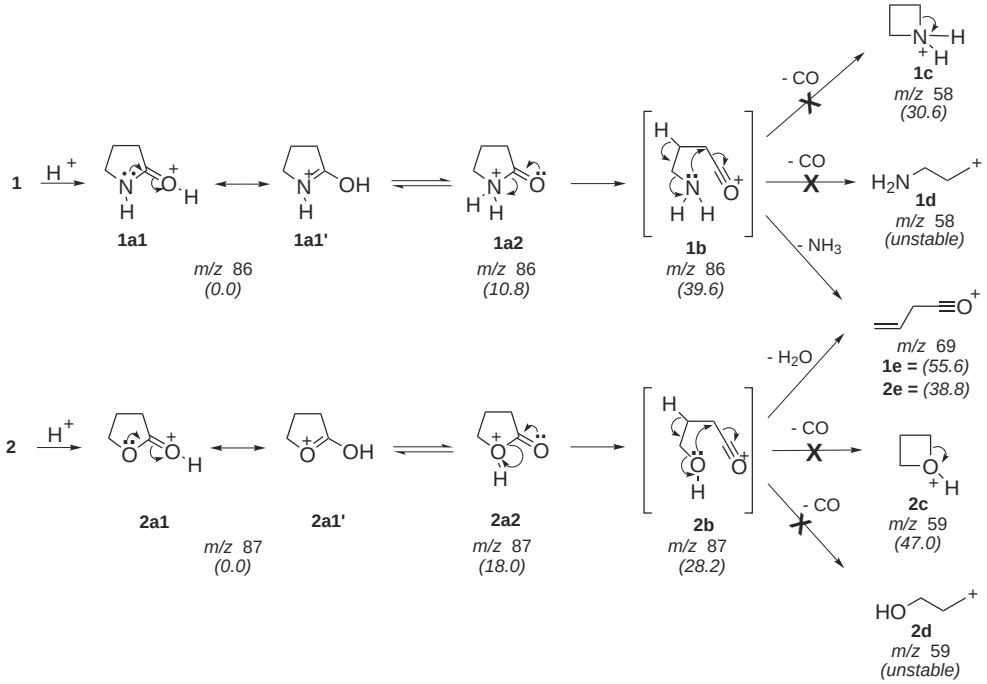
86) and **2b** (*m*/*z* 87), as shown in Scheme 1. In principle, losses of CO or H_2O/NH_3 from these rearrangement ions are possible, but the first was not observed in the experiments. This tendency for the H_2O/NH_3 elimination is probably due to the higher stability of the acylium ion **1e** or **2e** (*m*/*z* 69) in comparison to the primary carbocations **1d** (*m*/*z* 58) and **2d** (*m*/*z* 59), resulting from the possible loss of CO. These later structures also proved to be unstable during the geometry optimisation calculations resulting in fragmentation to produce $[H_2N–CH_2]^+ + H_2C{=}CH_2$ and $[HO–CH_2]^+ + H_2C{=}CH_2$, respectively. The oxetanium (**2c**) and azetidinium (**1c**) ions (Scheme 1), resulting from the lone pair assisted elimination of CO, should also be considered. However, their formation requires a highly organised four-membered transition states that are entropically disfavoured (have a high entropy barrier) even though in the case of **1c**—the enthalpy of formation is reduced.

Table 2
Selected bond lengths for the cyclic cations (Å), (X = N or O)

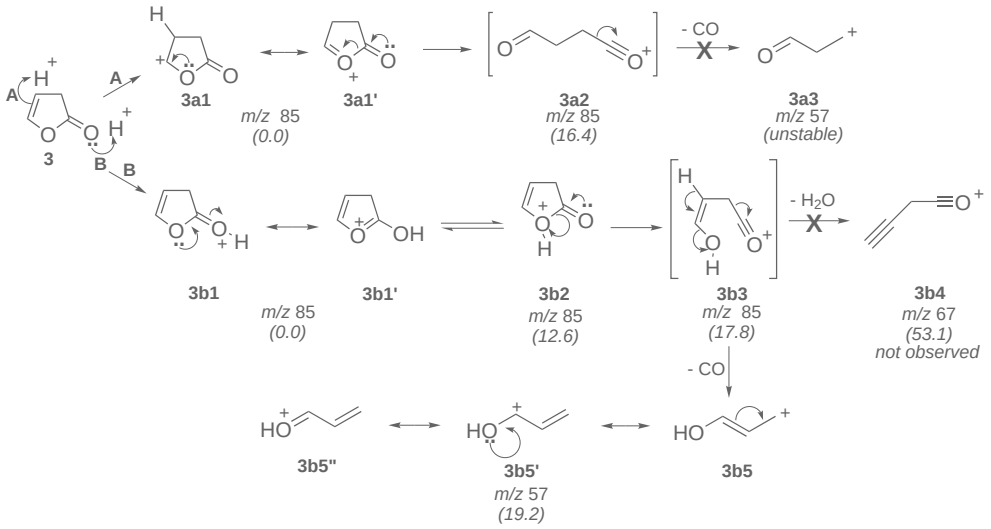


	I	II	X
1a1	1.488	1.308	N
1a2	1.500	1.629	N
2a1	1.507	1.276	O
2a2	1.456	1.862	O
3a1	1.232	1.887	O
3b1	1.449	1.265	O
3b2	1.355	3.667	O
4a1	1.413	2.419	O
4a2	1.468	1.293	O
7a	1.514	1.272	O
7b	1.466	1.773	O

MS/MS spectra of the unconjugated lactones **3** and **5** exhibit the most intense fragment ions resulting from the loss of CO (Table 1). Water elimination is observed only for **5** and produces a fragment ion with a very low relative abundance. It was proposed in the CI studies [6] with the methyl-lactone **5** that the loss of CO occurs via the initial protonation of the double bond and results in an unstable primary carbocation (**3a3**, *m*/*z* 57), as follows in route **A** (Scheme 2). However, the H_2O elimination for the methyl-lactone that is previously unreported in the CI studies indicates that in ESI the protonation takes place on the heteroatom (Scheme 2), as is more



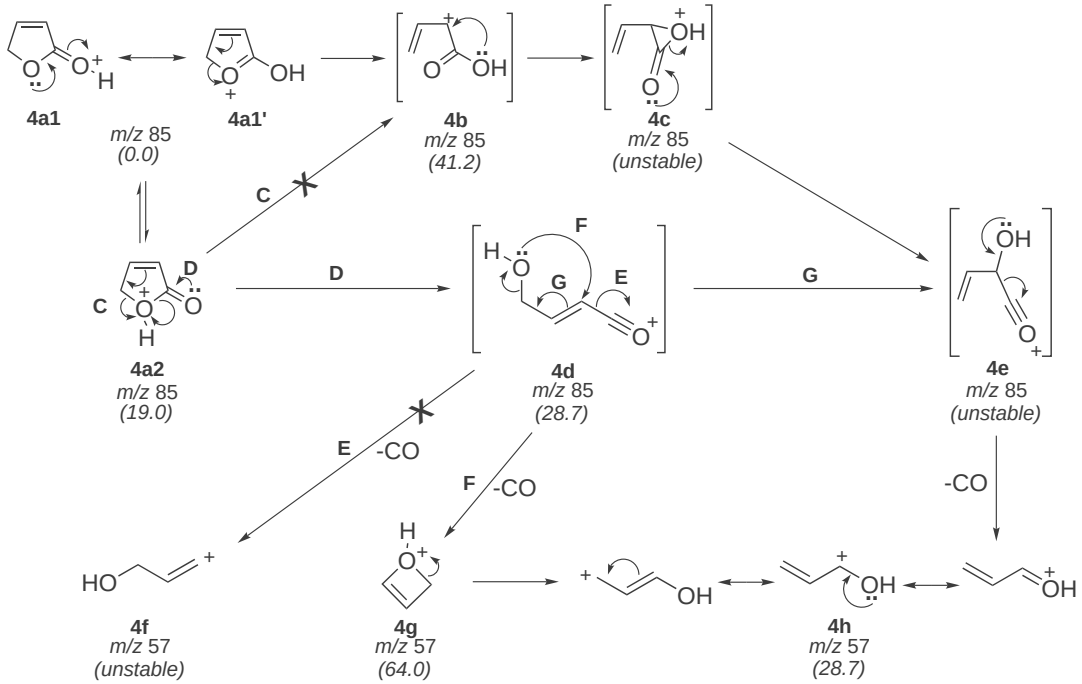
Scheme 1. Proposed opening-chain fragmentation mechanism for compounds **1** and **2** followed by neutral loss of H_2O and NH_3, respectively. The relative energies (kcal mol^{-1}) are shown in parenthesis. (Ions **1e** and **2e** have the same structures but different relative energies due to this have different precursor ions.)



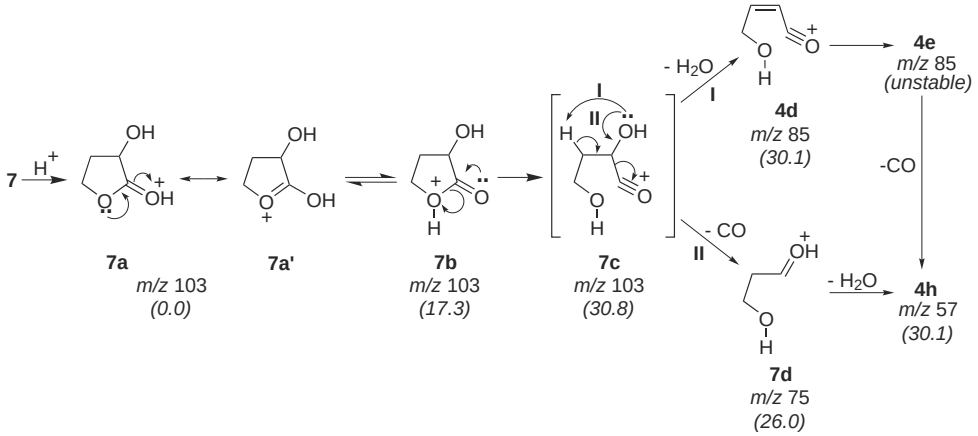
Scheme 2. Proposed opening-chain fragmentation mechanism for compound **3** followed by neutral loss of CO and resonance stabilisation of the resulting carbocation. The relative energies (kcal mol^{-1}) are shown in parenthesis.

usual. Opening of the five-membered ring of compounds **3** and **5** then occurs likewise for **1** and **2**, but in this case the CO elimination, instead of H_2O, is favoured due to the formation of an allylic carbocation **3b5** (*m*/*z* 57) which is much more stable than **3b4** (*m*/*z* 67) due the resonance stabilisation [8]. For compound **5**, the fragment ion analogous to **3b5** (*m*/*z* 71) is additionally stabilised by the electron-releasing inductive effect of the methyl group. The neutral elimination of H_2O, in this case, occurs with participation of one of the hydrogens from this group but, similar to compound **3**, this process is less favoured energetically than elimination of CO. These results demonstrate that the tendency for CO or H_2O elimination is driven by the formation of the most stable carbocation.

The MS/MS spectra of the conjugated lactones **4** and **6** showed only a 14 mass unit difference (due to the methyl group on **6**). Although the H_2O elimination has been observed for the methyl lactone **6**, the loss of CO is the predominant process in both of the spectra (Table 1) and is evidence for the same fragmentation mechanism for both compounds.



Scheme 3. Proposed opening-chain fragmentation mechanism for compound **4** followed by neutral loss of CO and resonance stabilisation of the resulting carbocation. The relative energies (kcal mol^{-1}) are shown in parenthesis.



Scheme 4. Proposed opening-chain fragmentation mechanism for compound **7** followed by neutral loss of CO assisted by the α-OH. The relative energies (kcal mol^{-1}) are shown in parenthesis.

However, the ESI results are not in agreement with the previously reported CI studies [6], where the major fragment was due to the loss of CH_2O. This is most probably due to the different identities of protonated species obtained from ESI and CI ionisation, that result in two different fragmentation mechanisms. The initial protonation of the carbonyl group, as occurs in CI, makes it more susceptible to the nucleophilic attack of the π-cloud with the further elimination of CH_2O resulting in formation of a three-membered aromatic ring (as previously published) [6]. However, the opening of the five-membered ring that is required for the CO elimination in ESI is more likely to occur with the initial protonation on the heteroatom. Heterolytic cleavage of the adjacent C–O bond then follows, as occurs with compounds **2**–**5**. This cleavage can be assisted by the π-cloud or by the lone pair of the carbonyl group, resulting in the intermediates **4b** and **4d**, respectively, as shown in Scheme 3. Comparison between the calculated relative energies of these possible intermediates showed that **4d** is 12.5 kcal mol^{-1} more stable than **4b** and so its formation is energetically more favoured. This difference is probably due to the higher energy required for the cleavage of the C–O bond **I** (Table 2) in comparison with **II** whose length is longer than normal (2.42 Å). Loss of CO from **4d** is the predominant process since the dehydration requires the abstraction of a vinylic hydrogen similar to that proposed for **3b3** (Scheme 2). The CO elimination following route **E** results in the highly unstable vinylic carbocation **4f** (*m*/*z* 57) that fragments to form $[HO–CH_2]^+ + HC{\equiv}CH$ during the geometry optimisation calculations. The lone pair assisted loss of CO (route **F**) results in the cyclic oxonium cation **4g** (*m*/*z* 57) that produces the resonantly stabilised species **4h** (*m*/*z* 57) by further opening of the four-membered ring. These species are formed rather than **4f** due to their increased thermodynamic stability. They could also be the result of the fragmentation of **4e** from **4b** (route **C**) and **4d** (route **G**). However, the thermochemical data analysis, of the proposed intermediate structures, showed that the loss of CO via structures **4d** and **4e** (route G) is the most energetically favoured pathway.

The hydroxy-lactones **7** and **8** were investigated to confirm the significance of the carbocation stability in the fragmentation mechanism. As expected, the MS/MS spectrum of compound **8** showed a single fragment ion at *m*/*z* 85 due to the loss of the β-hydroxy group by dehydration resulting in an ion that is similar to lactone **4**. The MS^3 analysis of $[\mathbf{8}\text{-}H_2O]^+$ results in the fragment ion at *m*/*z* 57 (loss of CO) as occurs with the compound **4** (Scheme 3), confirming the structure of $[\mathbf{8}\text{-}H_2O]^+$ as being analogous to that of **4a2**. The MS/MS spectrum of compound **7** exhibits fragment ions due to the loss of water (*m*/*z* 85) or elimination of CO (*m*/*z* 75). Both of these ions fragment further by loss of CO and H_2O, respectively, resulting in the same product ion at *m*/*z* 57. Protonation takes place on the oxygen of the lactone ring, as in **2** (Scheme 2), and results in the α-hydroxy-acylium intermediate **7c**, as shown in Scheme 4. Loss of CO, in this case, is favoured by the formation of a carbocation stabilised by the lone-pair of the adjacent hydroxy group (Table 1, Scheme 4). This difference in the fragmentation between compounds **7** and **8** can also be explained based on carbocation stability. The β-hydroxy group on compound **8** does not favour CO elimination, whereas the α-hydroxy group on compound **7** can assist in the elimination of CO as proposed in Scheme 4. The occurrence of both the elimination of CO and loss of H_2O can easily be explained by the neutral loss of H_2O prior to ring opening resulting in a fragment ion similar to **4d**. Finally, as expected, the MS/MS of tetronic acid **9** showed loss of CO as the major fragment (Table 1) by the same mechanism proposed for the compounds **4** and **6** (Scheme 3).

4. Conclusions

The theoretical calculations show that initial protonation in ESI-MS occurs on the carbonyl oxygen of the lactones, but to initiate fragmentation the proton has to move to the heteroatom. This movement of the proton from the carbonyl oxygen to the heteroatom results in an increase in relative

energy. It is suggested that this energy is supplied to the structures during the CID process resulting in an increase of the (H)–X–C=O (X = O or N) bond length. This is indicative of the heterolytic cleavage of this bond, leading to the opening of the five-membered ring and formation of the acylium ion. MS/MS analysis of lactam **1** and the saturated lactone **2** showed only the neutral loss of NH_3 and H_2O, respectively. For the unsaturated lactones the MS/MS spectra showed the loss of CO as the major fragment. The analysis of the saturated hydroxy-lactones showed only the loss of H_2O, for the β-hydroxy lactone **8**, and losses of CO and H_2O for the α-hydroxy compound **7**. The unsaturated hydroxy-lactone **9** showed the same fragmentation as the conjugated lactones. These results demonstrate that the fragmentation pathway in ESI-MS/MS analysis can be correlated with the stability of the cations produced by the various potential fragmentation pathways. It should be possible, therefore, in further studies of more complex molecules containing lactone ring systems as part of their structures, to make rational predictions of probable fragmentation pathways.

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