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Electrospray ionization mass spectra of phosphacoumarin derivatives

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Abstract

Phosphacoumarins and their 4-*O*-methylsulfonate derivatives, as the analogues of the coumarins with many biological activities, were synthesized, their fragmentation pathways were investigated by electrospray ionization mass spectrometry (ESI-MS) in conjunction with tandem mass spectrometry (MS/MS), and some characteristic fragment ions were observed by losing ethylene, water, CO, HPO_2, HPO_3, CH_3SO_2 or CH_3SO_2H from the precursors.

Keywords: Phosphacoumarin; ESI-MS; Fragmentation pathway

1. Introduction

Coumarins are members of the class of compounds called benzopyrones and have gained considerable synthetic and pharmacological interest for a long time because of their various biological activities, such as anti-tumor [1], anti-HIV [2], anti-oxidation [3], vasorelaxant activity [4], tumor necrosis factor-α (TNF-α) inhibitors [5], antimicrobial activity [6], serine protease inhibitors [7], anticancers [8] and many other biological activities. On the other hand, organophosphorus compounds [9] continue to receive widespread attentions due to their ubiquity in biological systems [10] and their potential to serve as novel pharmaceutical [11], agricultural [12] and chemical agents [13]. Phosphonic acids and their derivatives have often exhibited similar biochemical activity to naturally occurring carboxylic acids and their derivatives [14], so we think that the phosphonic analogues (phosphacoumarin) may have potential biological activities like coumarin derivatives.

2. Experimental

2.1. Synthesis of phosphacoumarin derivatives

Phosphacoumarin derivatives **3**, **4** and **5** were prepared as shown in Scheme 1 in good to excellent yields [15].

2.2. Mass spectrometric conditions

The ESI mass spectra of compounds **3**, **4** and **5** were acquired on a Bruker ESQUIRE-LC^{TM} ESI ion trap spectrometer which was equipped with a gas nebulizer probe. Nitrogen was used as a drying gas at a flow rate of 4 L/min. The nebulizer gas was delivered at a flow pressure of 7 psi. Samples, which were dissolved in methanol at a concentration of about 0.01 mg/mL were continuously pumped into the ESI chamber at a flow rate of 1.4 μL/min by a Cole-Parmer 74 900 syringe pump. The heated capillary temperature was maintained at 300 °C. The scan range of the ions is *m*/*z* 50–500 and a cut-off mass of 50 was used during ion accumulation. The selected ion was isolated and fragmented by collisions with helium to yield MS^n spectra. The fragmentation amplitude values were 0.5–1.0 V and the fragmentation time was 40 ms.

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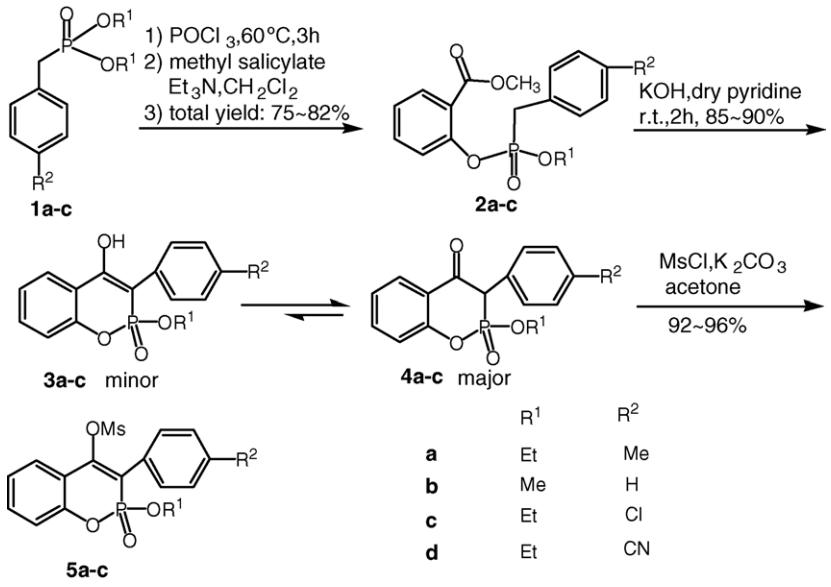


Scheme 1. Synthetic route of phosphacoumarin derivatives (there is an equilibrium balance between **3** and **4**).

Table 1
The analyzed compounds **3**/**4a**–**4d** and **5a**–**5d** by positive ion ESI-MS

Compound	$[M+H]^+$	$[M+Na]^+$
3a/**4a**	317	339
3b/**4b**	289	311
3c/**4c**	337	359
3d/**4d**	328	350
5a	395	417
5b	367	389
5c	415	437
5d	406	428

3. Results and discussion

The compounds **3**/**4** and **5** were analyzed by positive ion electrospray ionization mass spectrometry (ESI-MS) (Table 1). The ESI mass spectral fragmentation pathways of compounds **4c** and **5c** are discussed as typical examples, and the other compounds displayed very similar mass spectral fragmentation patterns to the compounds **4c** and **5c** in ESI-MS.

The positive ion mass spectrum of compound **4c** shows the base peak at *m*/*z* 359 ($[M+Na]^+$) (Fig. 1), and the peaks at *m*/*z* 337, 309 and 288 were also observed, which were corresponding to $[M+H]^+$ and $[M+H\text{-}(CH_2{=}CH_2)]^+$, respectively.

The MS/MS of the ion at *m*/*z* 337 in Fig. 2 produced the daughter ion at *m*/*z* 309 which may be $[MH{-}CO]^+$ or $[MH{-}C_2H_4]^+$, so a control experiment was carried out. We investigated the fragmentation pathway of compound **4b** in which methyl on the phosphonate replaced ethyl in compound **4c**) (Scheme 2), ion at *m*/*z* 261 ($[MH\text{-}28]^+$) was not found, correspondingly, a new ion at *m*/*z* 275 by losing CH_2 from the parent ion ($[MH]^+$ at *m*/*z* 289) was observed (Fig. 3). The result showed that it was easier to lose alkylene on the phosphonate relative to carbonyl, so the daughter ion at *m*/*z* 309 in Fig. 2 is from the loss of ethylene, not CO in the fragmentation pathway.

Fig. 4 shows ESI-MS^3 spectrum of the ion at *m*/*z* 309, and the fragmentation pathway of the precursor ion $[M+H\text{-}(CH_2{=}CH_2)]^+$ at *m*/*z* 309 is summarized in Scheme 3. The ions at *m*/*z* 291, 281 and 263 are from loss of H_2O, CO and

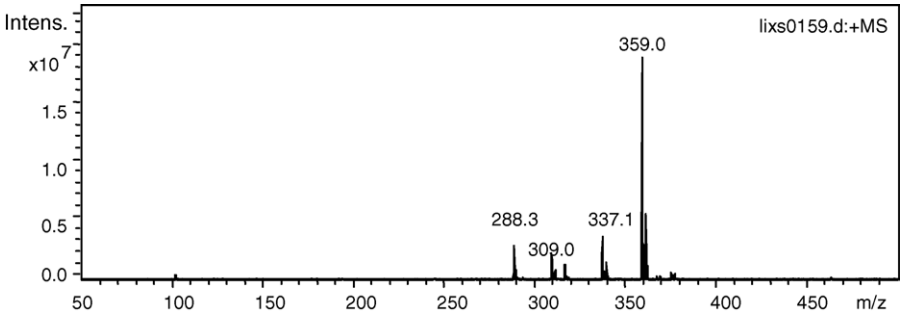


Fig. 1. Positive ion ESI mass spectrum of compound **4c**.

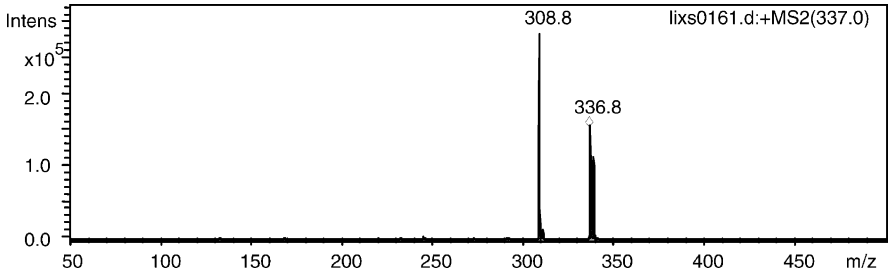
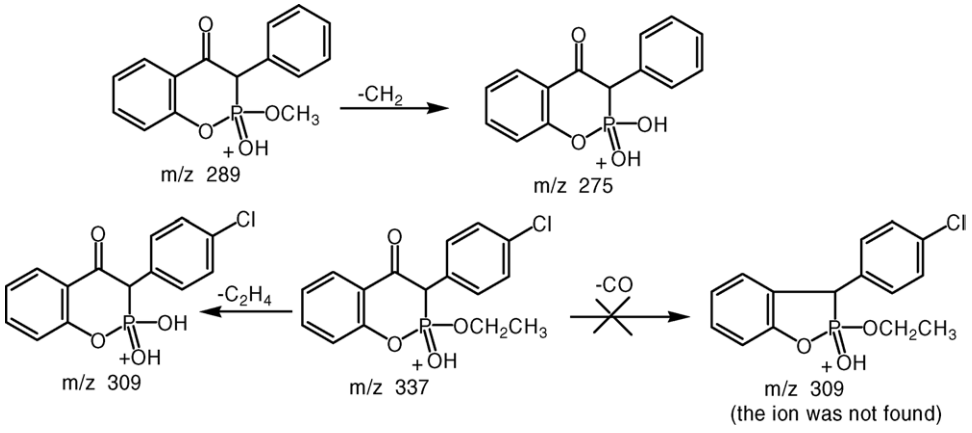


Fig. 2. Positive ion ESI-MS^2 spectrum of the ion at *m/z* 337.



Scheme 2. ESI-MS^2 fragmentation pathway of the ions at *m/z* 289 (**4b**) and 309 (**4c**).

the both of them, respectively. And the other fragment ions [precursor ion-HPO_2]$^+$ at *m/z* 245, [precursor ion-HPO_3]$^+$ at *m/z* 229, [precursor ion-PhCl]$^+$ at *m/z* 169 and [precursor ion-HPO_2–PhCl]$^+$ at *m/z* 133 were also observed.

We also investigated the ESI-MS^4 spectrum of the ion at *m/z* 291 as shown in Scheme 4 (Fig. 5). Interestingly, the parent ions at *m/z* 323, 309 of the ion at *m/z* 291 were observed, and this is probably due to imperfect ejection of ions during the isolation process. At the same time, the daughter ions at *m/z* 273 [precursor ion-H_2O]$^+$, 263 [precursor ion-CO]$^+$, 211 [precursor ion-H_2O–CO], 244 [precursor ion-PO]$^+$, 227 [precursor ion-HPO_2]$^+$,

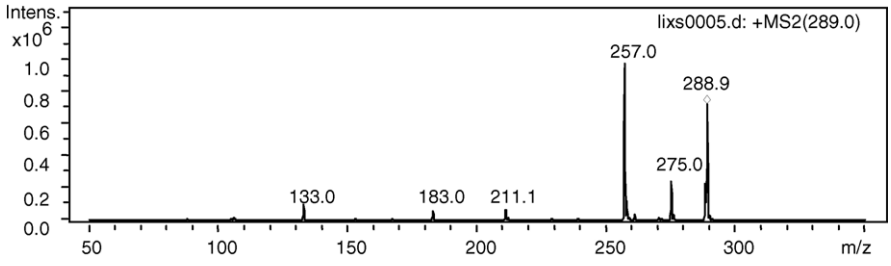


Fig. 3. Positive ion ESI-MS^2 spectrum of the ion at *m/z* 289 (**4b**).

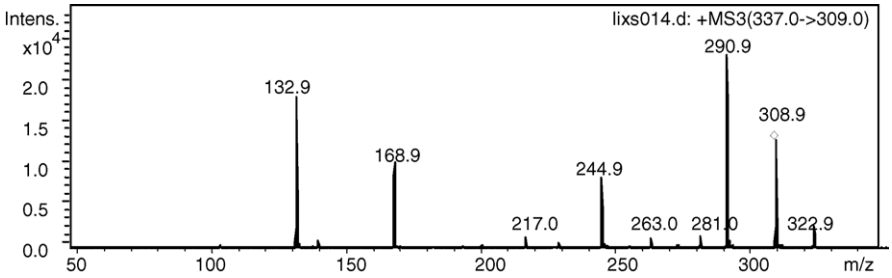
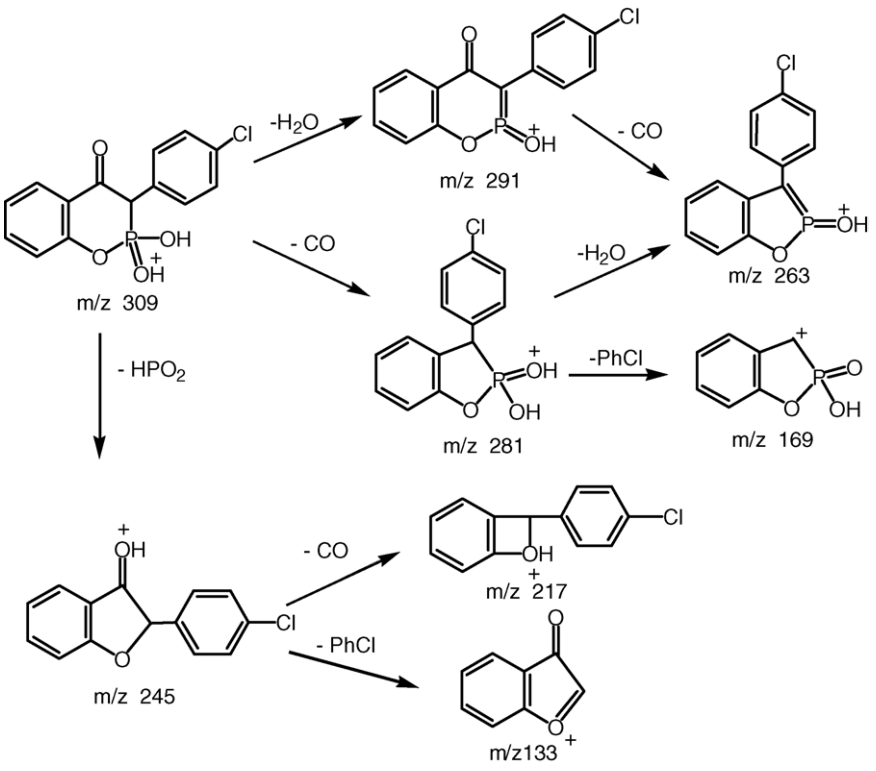


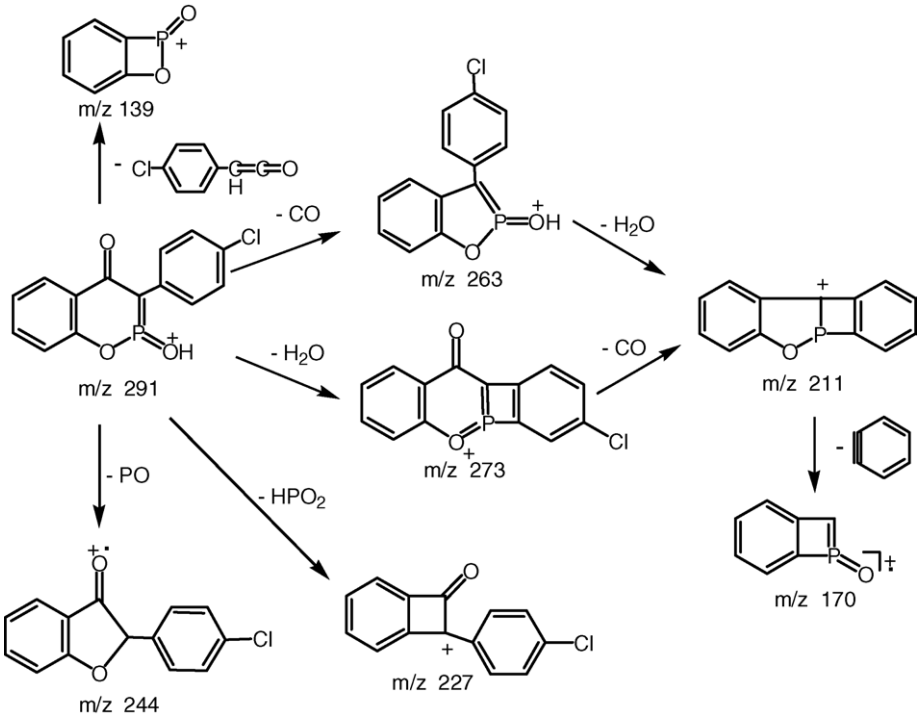
Fig. 4. Positive ion ESI-MS^3 spectrum of the ion at *m/z* 309.



Scheme 3. ESI-MS^3 fragmentation pathway of the ion at *m*/*z* 309 (corresponding to Fig. 4).

139 [precursor ion-(ClPhCH=C=O)]$^+$ were also observed.

We also studied the positive ion ESI mass spectral fragmentation pathway of compound **5c**. The positive ion ESI mass spectrum of compound **5c** shows the base peak at *m*/*z* 437 ([M + Na]$^+$) (Fig. 6) with the peaks at *m*/*z* 415 ([M + H]$^+$) and 453 ([M + K]$^+$) appearing, and the fragment ions at *m*/*z* 387, 358, 336, 316, 289 were also found, which were the



Scheme 4. ESI-MS^4 fragmentation pathway of the ion at *m*/*z* 291 (corresponding to Fig. 5).

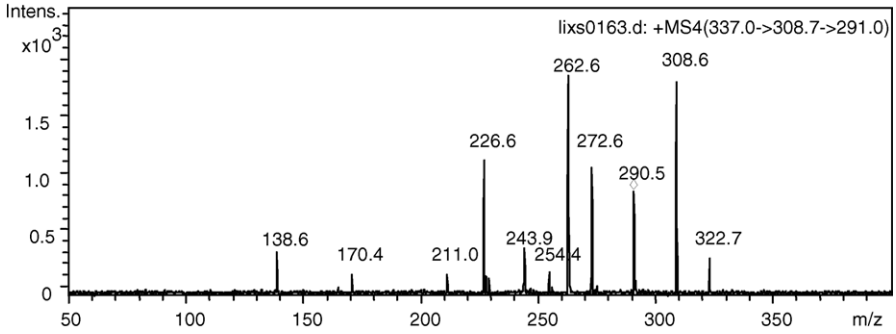


Fig. 5. Positive ion ESI-MS^4 spectrum of the ion at *m*/*z* 291.

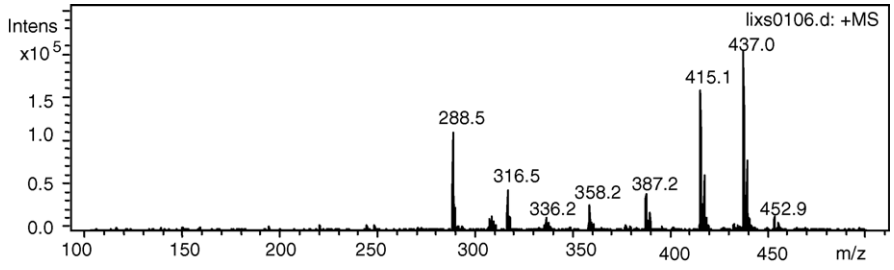


Fig. 6. Positive ion ESI mass spectrum of compound **5c**.

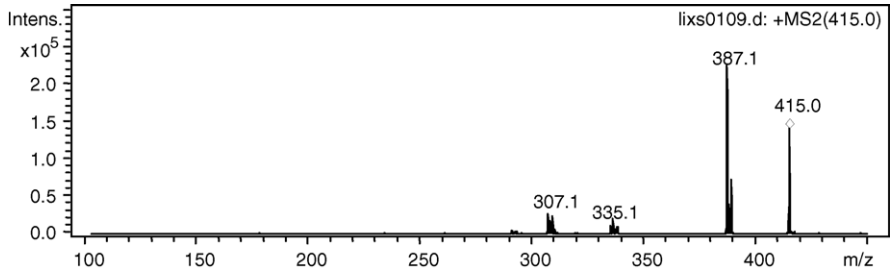
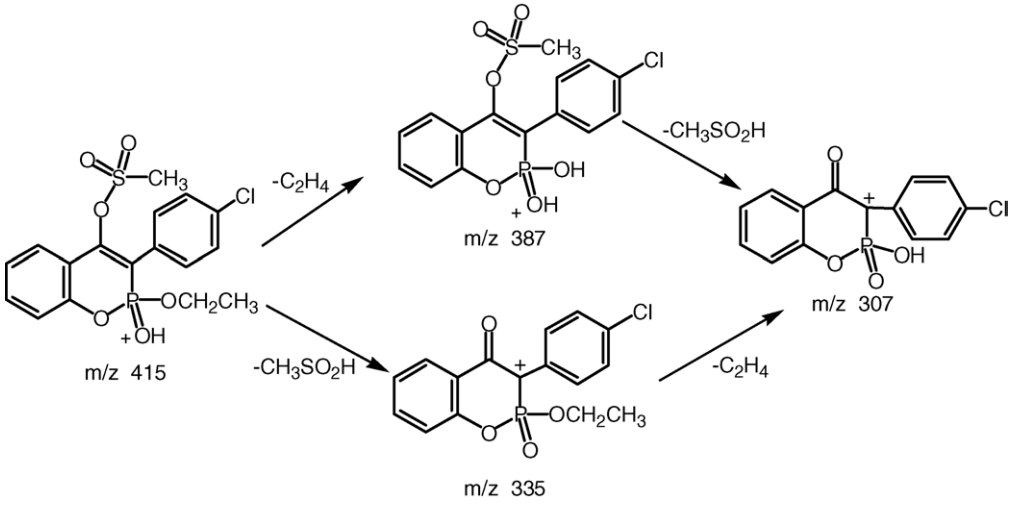


Fig. 7. Positive ion ESI-MS^2 spectrum of the ion at *m*/*z* 415.



Scheme 5. ESI-MS^2 fragmentation pathway of the ion at *m*/*z* 415 (corresponding to Fig. 7).

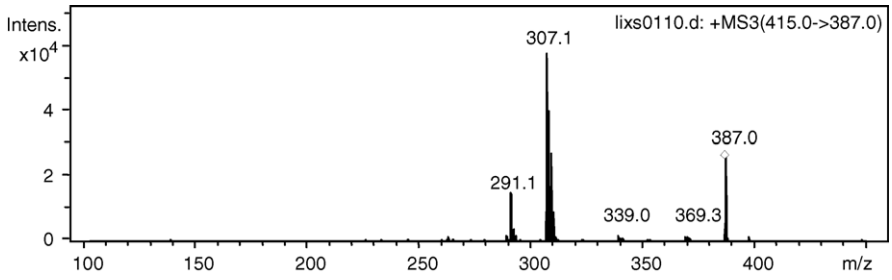
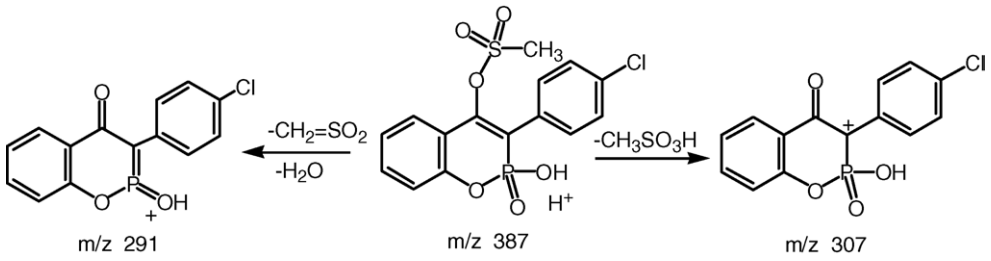


Fig. 8. Positive ion ESI-MS^3 spectrum of the ion at *m*/*z* 387.



Scheme 6. ESI-MS^3 fragmentation pathway of the ion at *m*/*z* 387 (corresponding to Fig. 8).

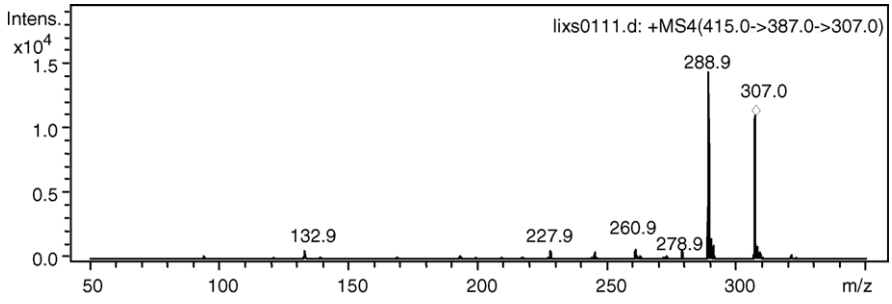
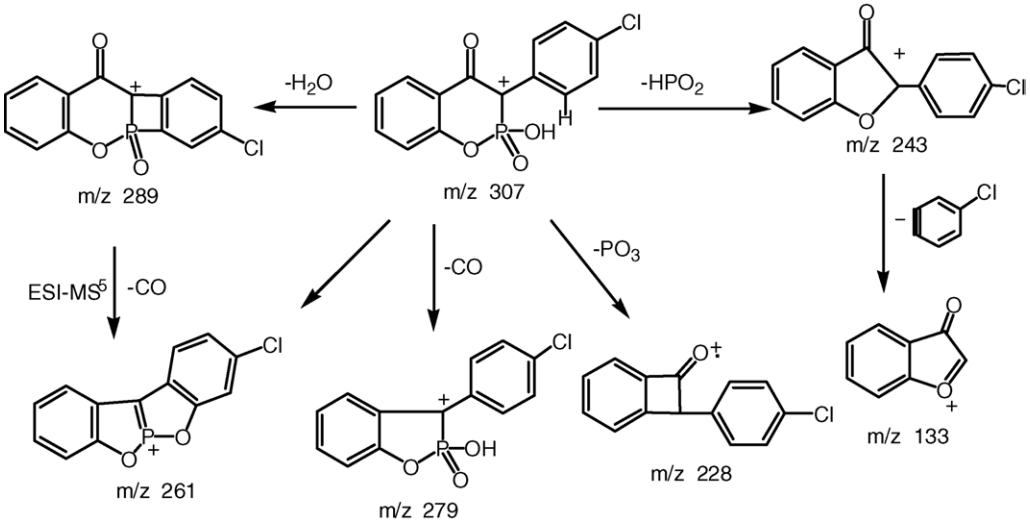


Fig. 9. Positive ion ESI-MS^4 spectrum of the ion at *m*/*z* 307.



Scheme 7. ESI-MS^4 fragmentation pathway of the ion at *m*/*z* 307 and ESI-MS^5 fragmentation pathway of the ion at *m*/*z* 289 (corresponding to Fig. 9).

fragments from the $[M+H]^+$ and $[M+Na]^+$. The ion at *m*/*z* 415 was selected as the precursor to study the fragmentation pathway (Fig. 7), the daughter ions at *m*/*z* 387, 335 and 307 were observed, which were due to the loss of ethylene, CH_3SO_2 and both of them from the precursor ion, respectively (Scheme 5).

The ESI-MS^3 spectrum of the ion at *m*/*z* 387 was also investigated (Fig. 8). The ion at *m*/*z* 369 was from the loss of water, and the loss of methylsulfinic acid led to the fragmentation ion at *m*/*z* 307. In addition, the loss of both water and $CH_2{=}SO_2$ from the precursor ion produced the daughter ion at *m*/*z* 291 (Scheme 6).

The ESI-MS^4 of the precursor ion at *m*/*z* 307 was investigated (Fig. 9); the fragmentation pathway is summarized in Scheme 7. The fragment ion at *m*/*z* 289 is the base peak, which was due to the loss of water. The fragment ions at 279 and 261 were also observed, which were produced by the loss of carbon monoxide, the both carbon monoxide and water, respectively. At the same time, the loss of PO_3 led to the fragment ion at *m*/*z* 228. And the positive ion ESI-MS^5 spectrum of the ion at *m*/*z* 289 yielded the ion at *m*/*z* 261.

4. Conclusions

Positive ion electrospray ionization mass spectral fragmentation pathways of some synthesized phosphacouamarin derivatives were analyzed by multistage mass spectrometry, and the abundant characteristic fragment ions were observed by losing ethylene, water, CO, HPO_2, HPO_3, CH_3SO_2 or CH_3SO_2H from the precursors.

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