Electronic Supplementary Material (ESI) for Analytical Methods

SUPPLEMENTAL INFORMATION:

Pyrrolizidine alkaloid profile in a traditional Chinese herbal medicine *Chuan Zi Wan* (Ligulariae Radix et Rhizoma) by liquid chromatography/electrospray ionization ion trap mass spectrometry

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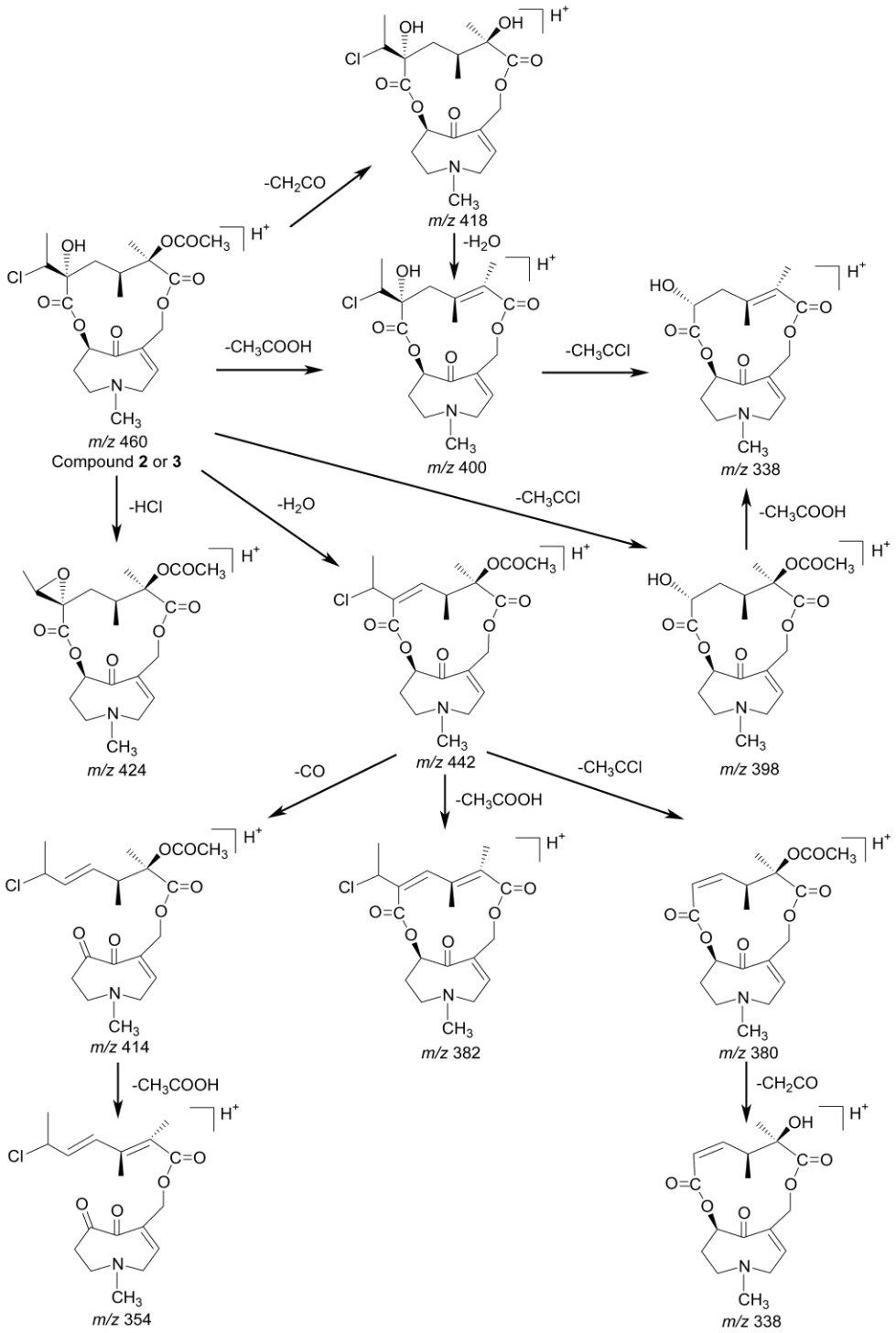
Tentative assignment of compounds 2, 3, 9 and 10.

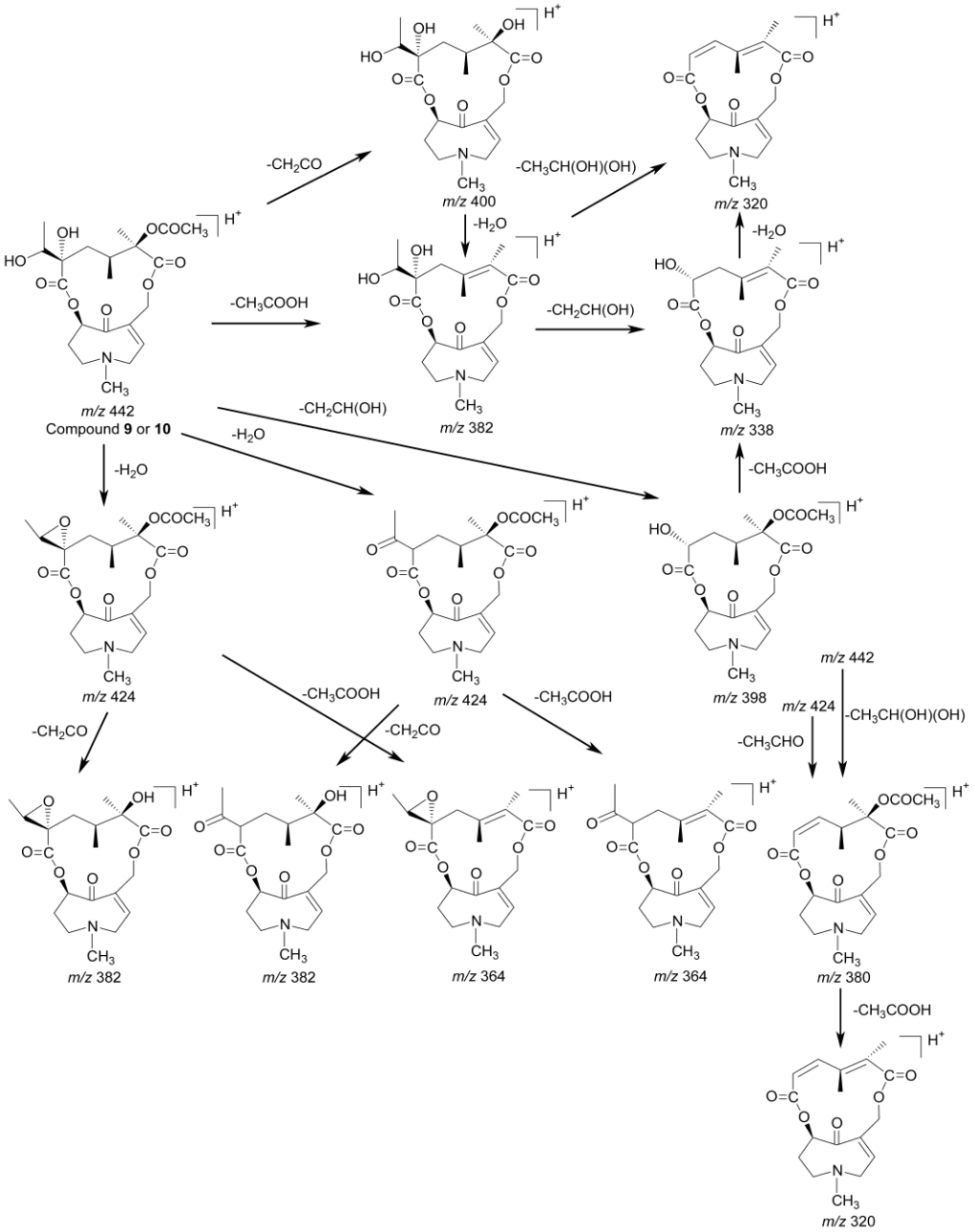
Data acquired from the HPLC-IT-MS^n experiments for compounds **2, 3, 9 and 10** were further analyzed and discussed as follows.

Compounds **2** and **3** had the similar MS/MS profiles and both produced some characteristic fragments, such as at *m/z* 442, 424, 418, 400 and 398, which were originated from the loss of a H_2O ($[M+H-18]^+$), HCl ($[M+H-36]^+$), CH_2CO ($[M+H-42]^+$), CH_3COOH ($[M+H-60]^+$) and CH_3CCl ($[M+H-62]^+$), respectively, and *m/z* 414, 382, 380 and 338 due to the consecutive losses of H_2O and CO ($[M+H-18-28]^+$), H_2O and CH_3COOH ($[M+H-18-60]^+$), H_2O and CH_3CCl ($[M+H-18-62]^+$), and CH_3COOH and CH_3CCl ($[M+H-62-60]^+$), respectively. Among them, the fragment ions at *m/z* 442, 400, 398, 338 were the most intense ones (see main text Table 1). The possible fragmentation pathway for these ions was proposed in **SI Figure 1**. The data suggested that the two PAs may have the same structural motif of CH_3-CHCl-C(OH) and 12-acetyl substituent as those in the necic acid moiety of doronine. However, the isotopic counterparts corresponding to the molecular ion were not clearly shown in the current condition, thus in future studies the high resolution mass spectrometers with enhanced sensitivity and mass accuracy will be exploited. As for the exact structure determination, more investigations involving the phytochemical isolation, structural elucidation by NMR are needed.



SI Figure 1. Scheme for the proposed fragmentation pathway of some major characteristic ions of compound **2** or **3**, which was tentatively assigned as doronine or its isomer.

Compounds **9** and **10** had the MW of 441 Da, both MS/MS spectra showed some identical characteristic fragment ions that can be derived from the vicinal dihydroxyl and acetyl group in structures, such as at *m/z* 424, 400, 398, 382, 380, 364, 338 and 320, corresponding to the loss of a H_2O ($[M+H-18]^+$), CH_2CO ($[M+H-42]^+$), $CH_2CH(OH)$ ($[M+H-44]^+$), CH_3COOH ($[M+H-60]^+$) or the consecutive losses of H_2O and CH_2CO ($[M+H-18-42]^+$), $CH_3CH(OH)(OH)$ ($[M+H-62]^+$) or the consecutive losses of H_2O and CH_3CHO) ($[M+H-18-44]^+$), the consecutive losses of H_2O and CH_3COOH ($[M+H-18-60]^+$), of CH_3COOH and $CH_2CH(OH)$ ($[M+H-60-44]^+$) and of CH_3COOH and $CH_3CH(OH)(OH)$ ($[M+H-60-62]^+$), respectively, **SI Figure 2**. Moreover, some analogous fragment ions especially those following an initial loss of a water molecule, such as at *m/z* 382, 380, 364, and 320, can be detected in the MS/MS spectra of ligularizine, hodgsonine or its isomer with the MW of 423 Da, suggesting that they may share some common structural features. Therefore, the two compounds may be originated from ligularizine or hodgsonine by hydration or have a similar structure to floridanine.



SI Figure 2. Scheme for the proposed fragmentation pathway of some major characteristic ions of compound **9** or **10**, which was tentatively assigned as floridanine or its isomer.