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Tandem mass spectrometry fragmentation of the protonated 2-(2-phenylethyl)chromones from Agarwood: radical ions versus non-radical ions

Bing Xia,[a] Jinrong Li,[a] Wenli Mei,[b] Lisheng Ding,[a] Hongxi Xu[c] and Yan Zhou[a]*

Supporting information may be found in the online version of this article.

Dear Sir,

Agarwood, also known as 'chenxiang' in Chinese, is the fragrant resinous heartwood obtained from certain trees of the genus *Aquilaria* (Malvales: Thymelaeaceae). Being a highly valuable non-timber product from Asian tropical forests, agarwood is used for incenses, perfumes and traditional medicines. Studies showed that the chemical constituents of agarwood are sesquiterpenes, 2-(2-phenylethyl)chromones, aromatics, triterpenes, etc.[1,2] The most abundant constituents in agarwood have been found to be 2-(2-phenylethyl)chromones (41%) and sesquiterpenes (52%).[1] Among which, 2-(2-phenylethyl)chromones are considered to be the major group of constituents responsible for the quality of agarwood and were found to possess neuroprotective,[3] cytotoxic,[4,5] antiallergic activities,[6] etc. It is obvious that detection and structure characterization of the main constituent, 2-(2-phenylethyl)chromones are key to the qualification of agarwood.

Recent years, mass spectrometry (MS), especially tandem MS (MS/MS), has been one of the important physicochemical methods that are widely applied in the identification and structure characterization of natural products due to its rapidity, sensitivity and low levels of sample consumption.[7–10] Studying of the MS/MS behavior of 2-(2-phenylethyl)chromones might provide valuable information for the qualification of agarwood, as well as for structural elucidation of 2-(2-phenylethyl)chromone compounds. However, to our knowledge, such studies have rarely been conducted. Herein, to obtain information on the fragmentation characters of 2-(2-phenylethyl)chromones, the fragmentation patterns were studied with electrospray ionization MS/MS (ESI-MS/MS) in positive ion mode. Interestingly, the results illustrated that some of the compounds presented radical ions, while others present only non-radical ions in the MS/MS experiments, which has not yet been reported.

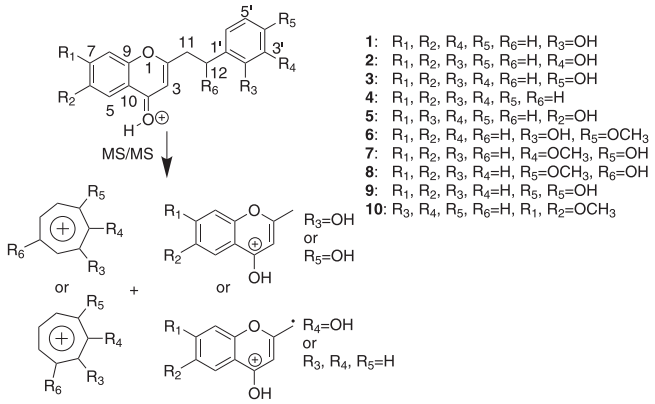
High-performance liquid chromatography (HPLC)-grade methanol was obtained from Fisher Scientific (Fairlawn, NJ, USA). A series of 2-(2-phenylethyl)chromone compounds were isolated in our laboratory, and the purity of each compound was determined to be higher than 98% by HPLC detection. MS experiments were performed on a Waters Xevo TQ (Waters, Manchester, UK) equipped with an ESI source. Argon was used as the collision gas and high-purity nitrogen as the nebulizer and desolvation gas. The ESI source conditions were as follows: capillary voltage, 2 kV; cone voltage, 50 V; source temperature, 150 °C and desolvation gas temperature, 600 °C. The samples with the concentration of 10 ng/ml were introduced into the mass spectrometer with a flow rate of 10 μl/min. Tandem mass spectra were obtained by collision-induced dissociation of selected precursor ion with argon as the collision gas, by applying collision energy of 20 eV. The data acquisition and processing were performed using the software Masslynx 4.1. Theoretical calculations were performed to interpret the fragmentation patterns with the aid of the Gaussian 03 suit of programs[11] using the B3LYP hybrid functional method with the 6-31 G(d) basis set.

2-(2-Phenylethyl)chromone compounds **1–10** (Scheme 1) were analyzed by ESI-MS/MS in positive ion mode. Compounds **1–3**, which share the same elemental composition but differ in molecular structure (the only difference is the substitutions of the hydroxyl group on the benzene ring of the phenylethyl group of compounds **1**, **2** and **3** are *ortho*, *meta* and *para*, respectively), were selected as the representative compounds for the following discussion. As shown in Fig. 1, it is obvious that the peak at *m/z* 267 in each spectrum is the $[M+H]^+$ ion, which was selected as precursor ion for MS/MS analysis. All the three compounds presented ion at *m/z* 107 in ESI-MS/MS corresponds to the elemental composition of C_7H_7O (Table 1), which, according to the structures of compounds **1–3**, is obviously a hydroxyl substituted tropylium ion (Scheme 1). Interestingly, compounds **1** and **3** produced ion at *m/z* 161 with high abundance, yet this ion was not observed in the MS/MS of compound **2**. Instead, an interesting ion at *m/z* 160 was observed in relatively high abundance (30%) in the MS/MS of compound **2**. The accurate mass obtained from the ESI-Orbitrap-MS/MS experiment provides the elemental compositions of the ions at *m/z* 160 and 161 as $C_{10}H_8O_2$ and $C_{10}H_9O_2$, respectively (Table 1). The ion at *m/z* 160 is an odd-electron ion and should be a radical cation. The formation of the radical ion might come from hemolytic β cleavage of C11–C12 bond. The mechanism of the formation of non-radical product ion at *m/z* 161 instead of 160 in the MS/MS of compounds **1** and **3** may be at first sight difficult to explain.

* *Correspondence to: Yan Zhou, Chengdu Institute of Biology, Chinese Academy of Sciences, No. 9 Section 4 Renmin South Road, Chengdu 610041, P. R. China. E-mail: zhouyan@cib.ac.cn*

However, this could be explained by the formation of ion/neutral complex from the precursor ion as reactive intermediate and the following H atom or proton transfer occurred between the two parts of the complex. A detailed mechanism is proposed in Scheme 2. That is, for each of the three compounds, transfer of energy to the $[M+H]^+$ ion gives rise to hemolytic β cleavage of C11–C12 to form ion/neutral complex. In the case of compounds **1** and **3**, the ion/neutral complex can further react in two ways: (1) either an H atom transfers from the hydroxyl group of the hydroxybenzyl radical to the radicalic methylene group on the hydroxychromylium ion, or (2) after intra-complex charge transfer, a proton transfers from the hydroxybenzyl cation to the neutral part of the complex. Both cases would lead to the formation of a stable 4-hydroxy-2-methylchromylium ion (*m/z* 161) along with a quinomethane molecule. In contrast, in the case of compound **2**, H atom or proton cannot transfer from the low reactive *meta*-hydroxybenzyl radical/cation to the counterpart of the ion/neutral complex. Therefore, dissociation takes place without H transfer and thus the radical 4-hydroxy-2-methylchromylium ion at *m/z* 160 is formed.

To understand the proposed formation of the radical and non-radical ions, the isotopic labeling experiments (compounds **1–3** dissolved in D_2O and ACN, and then subjected to ESI-MS/MS) were carried out, and the obtained results agree with the proposed mechanism (see Supporting Information). In addition to isotopic labeling experiments, theoretical calculations were also performed on compounds **1–3** and corresponding transition states and fragments by the hybrid density functional theory (B3LYP/6-31 G(d)) method as implemented in the Gaussian 03



Scheme 1. Chemical structure and proposed fragmentation of protonated compounds **1–10**.

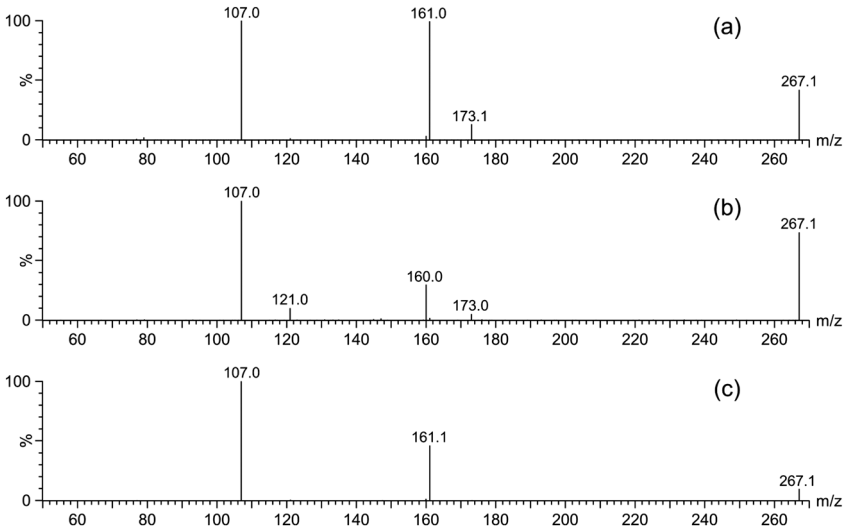
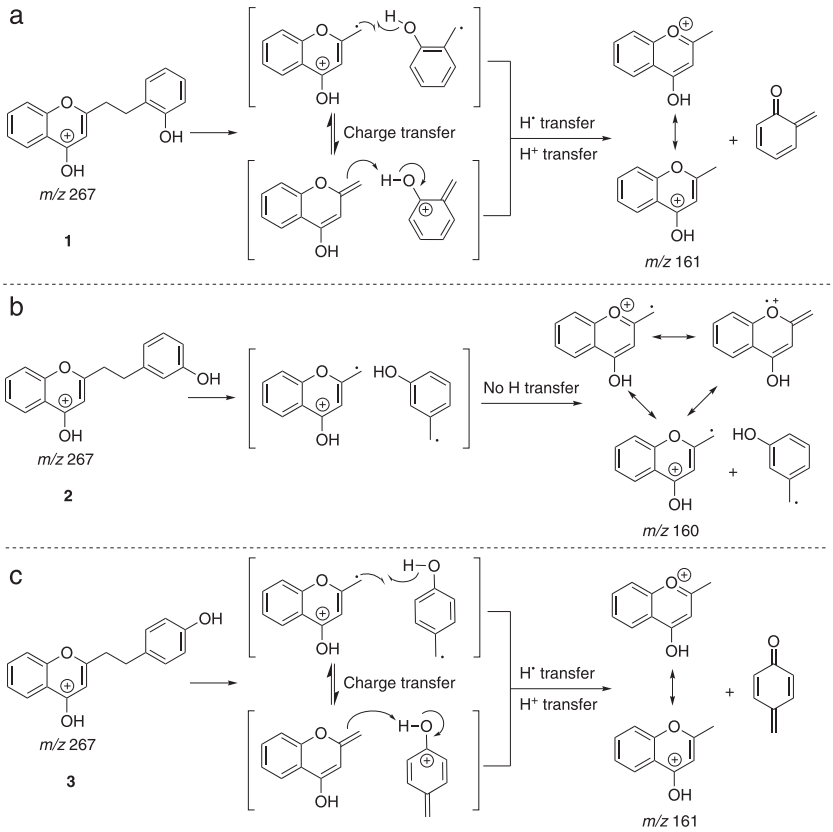


Figure 1. ESI-MS/MS spectra of compounds (a) **1**, (b) **2** and (c) **3** in methanol.

Table 1. Elemental compositions of protonated precursor ion and main product ions for compounds **1–3**

Compound	Observed	Calculated	Elemental composition	Error (ppm)
1	267.1016	267.1016	$C_{17}H_{15}O_3$	0.0
	161.0597	161.0597	$C_{10}H_9O_2$	0.0
	107.0490	107.0491	C_7H_7O	−0.9
2	267.1018	267.1016	$C_{17}H_{15}O_3$	0.7
	160.0519	160.0519	$C_{10}H_8O_2$	0.0
	107.0491	107.0491	C_7H_7O	0.0
3	267.1017	267.1016	$C_{17}H_{15}O_3$	0.4
	161.0597	161.0597	$C_{10}H_9O_2$	0.0
	107.0490	107.0491	C_7H_7O	−0.9



Scheme 2. Proposed pathways of the formation of product ions at *m/z* 160 and 161 for compounds (a) **1**, (b) **2** and (c) **3**.

program. The reaction pathways were traced forward and backward by the intrinsic reaction coordinate (IRC) method for compounds **1–3**. By IRC calculation, the energies and the geometries of the reactants, products and the corresponding transition states involved in the proposed mechanism of compounds **1–3** were acquired. According to the charge distribution of the transition states of compounds **1** and **3**, proton transfer between the two parts of the ion/neutral complex seems to be the predominant reaction in the gas phase (Fig. 2). A schematic potential energy surface of the reactions is given in Scheme 3. The calculations performed proved that the proposed mechanisms are rational.

The ESI-MS/MS experiments of 2-(2-phenylethyl)chromone compounds **4–10** were also performed, and the obtained results are fully in agreement with the proposed mechanism in Scheme 2 (the ESI-MS/MS results of compounds **4–10** can be found in Supporting Information). In summary, those 2-(2-phenylethyl) chromone compounds without hydroxyl group or with a *meta*-substituted hydroxyl group on the benzene ring of the phenylethyl group produce radical product ions in MS/MS, while those with *ortho* or *para*-substituted hydroxyl group produce non-radical product ions. This finding is of great scientific interest and might provide valuable information that might be used in

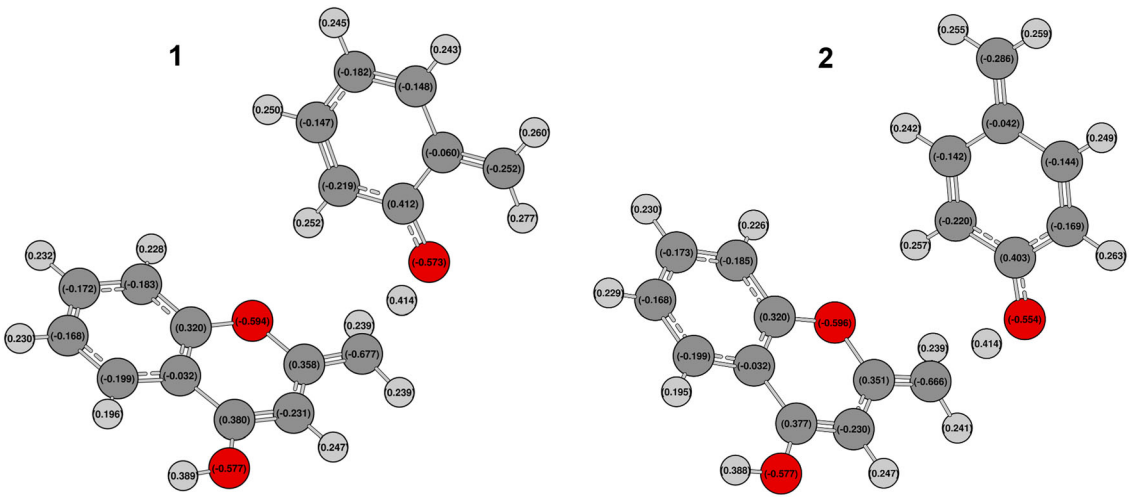
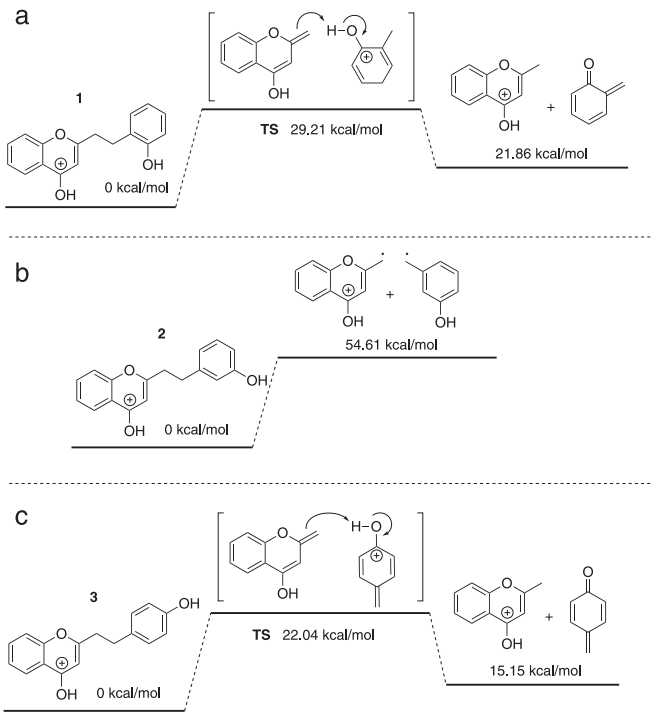


Figure 2. Charge distribution of the transition state of compounds **1** and **3**.



Scheme 3. Schematic potential energy surface of the proposed fragmentation pathways of compounds (a) **1**, (b) **2** and (c) **3**.

the qualification of agarwood, as well as in structure elucidation and identification of 2-(2-phenylethyl)chromones in complex natural matrices.

Yours,

BING XIA,[a] JINRONG LI,[a]
WENLI MEI,[b] LISHENG DING,[a]
HONGXI XU,[c] AND YAN ZHOU[a*]

[a] Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, P.R. China
[b] Key Laboratory of Biology and Genetic Resources of Tropical Crops, Ministry of Agriculture, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agriculture Sciences, Haikou 571101, P.R. China
[c] School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, P.R. China

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Supporting information

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