Investigations of the fragmentation behavior of 11 isoflavones with ESI-IT-TOF-MSn = ESI-IT-TOF-MSn方法对11个异黄酮的裂解规律研究

Yazhou Zhang  
*Hong Kong Baptist University*

Feng Xu  
*Peking University*

Jianye Zhang  
*Hong Kong Baptist University*

Tao Yi  
*Hong Kong Baptist University, yitao@hkbu.edu.hk*

Yina Tang  
*Hong Kong Baptist University*

*See next page for additional authors*

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Authors
Yazhou Zhang, Feng Xu, Jianye Zhang, Tao Yi, Yina Tang, Jun Xu, Wanling Peng, and Hubiao Chen
Investigations of the fragmentation behavior of 11 isoflavones with ESI-IT-TOF-MS

Yazhou Zhang¹,², Feng Xu³, Jianye Zhang¹, Tao Yi¹, Yina Tang¹, Jun Xu¹, Wanling Peng¹, Hubiao Chen¹*

1. School of Chinese Medicine, Hong Kong Baptist University, Hong Kong Special Administrative Region 999077, China
2. Guizhou College of Technology, Guiyang 550003, China
3. State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University Health Sciences Center, Beijing 100191, China

Abstract: The fragmentation behavior of isoflavones was studied using electrospray ionization-ion trap-time of flight mass spectrometry (ESI-IT-TOF-MS²). It was found that the isoflavone glycoside bond was easily broken. The fragmentation occurred mostly on the C-ring, and the fragment ions of A¹,³⁺ produced by the RDA cracking will predict the hydroxylation replacement on A-ring or B-ring. In addition, four carbonyl groups on the C-ring were fragmented through neutral loss of 28 (-CO). A and B-rings primarily lose substituents which including a neutral losses of 32 (-CH₃OH), 16 (-CH₄), or 16 (-O), and 18 (-H₂O). A-ring in the presence of adjacent hydroxylation, also easily made to be a neutral losses of 28 (-CO) or 18 (-H₂O). It is likewise common to see methoxy replaced with a neutral loss of 16 (-CH₄) or 32 (-CH₃OH) in B-ring, also the hydroxylation on benzene ring can occasionally results with the neutral loss of 28 (-CO).

Keywords: Isoflavone, Fragmentation behavior, ESI-IT-TOF-MS²


1. Introduction

It is well-known that isoflavonoids are biologically active plant constituents that occur mainly in legumes and act as phytosterogens. A diet rich in isoflavonoids can help to lower the risk of getting certain diseases including breast and prostate cancers, osteoporosis and various cardiovascular diseases. Researches also show that isoflavonoid possess anti-carcinogenic, hormone-altering, and estrogenic and anti-estrogenic properties¹,².

Mass spectrometry is an analytic technique based on determination of m/z, or mass-charge ratios, of ions in the gas phase. Ion trap (IT) technology is currently one of the more mature small mass spectrometer. The technology is convenient for achievement of the measurement of multistage tandem (MSⁿ)²–⁵. The time of flight of the mass spectrometer (TOF) is the space quality analyzer, which can quickly and at high resolution capture positive and negative ion pieces from the ion source to ensure that a majority of the ions can reach the detector²–⁵. IT-TOF-MS² greatly improves the sensitivity and resolution of the mass spectrum by taking advantage of the simultaneous multistage mass spectrometry analysis. The Retro Diels-Alder (RDA) cleavage can be used to predict the structure of the compounds after the ion source boom the C-ring into dienes and dienes fragments with a double-bond. The RDA occurs in structures containing a cyclohexene unit⁶.

It was reported that the cleavage of the isoflavones mostly happened on the C₄ position in C-ring with a neutral loss of 28 (-CO), and formed the fragment ions of A¹,³⁺ by the RDA cracking in mass²–⁶. In the present study, we use high resolution mass spectra to predict the exactly formulas of the fragment ions, and then with the relative ion abundance to indicate which bond broke firstly. At the same time we compared the fragmentation pathway of isoflavones with the similar structure to observe how the substitution location influences the fragmentation behavior. Eleven isoflavonoids were chosen and their fragment ions were generated by ESI-IT-TOF-MS², with both positive
ions (PI) and negative ions (NI) mode. Finally, the analysis was conducted in PI mode because fragment ions of isoflavonoids in MS provide more information than in NI mode. This study aims to study the fragmentation behavior of isoflavonoids, which will be helpful to understand the fragmentation rules of isoflavones. These investigations could likewise provide significant support for the further identification and analysis of isoflavones in plant extracts by ESI-IT-TOF-MS. 

2. Experimental

2.1. Chemicals and materials

The following selective compounds (Fig. 1), including formononetin (M1), 6,7,4′-trihydroxy-isoflavonoid (M2), 7,4′-dihydroxy-isoflavonoid (M3), 7,8-dihydroxy-4′-methoxy-isoflavonoid (M4), 6,7-dihydroxy-4′-methoxy-isoflavonoid (M5), formononetin-7-O-β-D-glucoside (onion) (M6), 6′-O-acetyl-onion (M7), calycosin (M8), 8-hydroxycalycosin (M9), 6-hydroxy-calycosin (M10), calycosin-7-O-β-D-glucoside (M11), were isolated by the authors and their structures were confirmed based on UV, MS, and NMR data. Methanol (Merck Co., Darmstadt, Germany) was of HPLC grade.

2.2. Sample solutions preparation

Weighed amount (1.0 mg) that is carefully transferred into a 50 mL volumetric flask with methanol, respectively, and a portion of the solution (1 μL) is injected into an ESI-IT-TOF-MS system for analysis.

2.3. Instrument and conditions

High resolution mass spectra were recorded on an IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan). Both NI and PI mode were utilized by the ESI source in operation. The full-scan mass spectra covered the range from m/z 100–1000 Da (MS1), m/z 50–1000 Da (MS2 and MS3). The trifluoroacetic acid sodium solution (2.5 mM) was used to calibrate the mass range from 50 to 1000 Da. Additional parameters were configured as below: heat block and curved desolvation line temperature, 200 °C; nebulizing nitrogen gas flow, 1.5 L/min; interface voltage: (PI), 4.5 kV; (NI), −3.5 kV; detector voltage, 1.70 kV; relative collision-induced dissociation energy, 50%.

All data were recorded and processed by Shimadzu software LCMS solution version 3.60, Formula Predictor version 1.2 and Accurate Mass Calculator (Shimadzu, Kyoto, Japan).

Figure 1. Chemical structures of the 11 isoflavonoids.
3. Results and discussion

3.1. The selection of MS conditions

The fragmentation pathways of 11 isoflavones were analyzed in order to facilitate structural identification. Both PI and NI modes were tested through the experiment with the goal to obtain desirable mass spectrometry chromatograms. The analysis was conducted in PI mode owing to the fact that fragment ions give more information in PI than in NI mode.

3.2. The fragment ions analysis of the 11 isoflavones

As illustrated in Figure 2, ions of isoflavones generated by the breaking of C ring in PI mode were named as $A^{0,3+}$, $B^{0,3+[3,4]}$.

The fragment ions and their abundance are shown in Table 1 and mass spectra are shown in Figure 3 (all the fragment ions were originated from precursor ions [M+H]$^+$).

![Figure 2. Nomenclature adopted for isoflavone (illustrated with formononetin).](image)

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<th>MW</th>
<th>Molecule formula</th>
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### Table 1. Continued

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**Figure 3.** The mass spectra chromatograms of the 11 isoflavones.
3.2.1. Isoflavones and their related isoflavone glycosides: compounds M1, M3, M6, M7

The [M+H]+ ion of M1 at m/z 269 was generated as the base peak. And ions at m/z 237 [M-CH$_3$OH+H]+, m/z 253 [M-CH$_2$H]+, m/z 213 [M-CO*2+H]+ were observed. Ion at m/z 163 formed m/z 137 A$_{1,3}^{1,3}$, and B$_{1,3}$–16 (CH$_4$) after RDA cracking.

M3 formed m/z 137 A$_{1,3}^{1,3}$ after RDA cracking as the base peak, and other major fragment ions, such as m/z 227 [M-CO+H]+, m/z 255 [M+H]+ were also observed, and fragment ion m/z 145 was generated from B$_{1}^{1}$-H$_2$O. M6 and M7 are isoflavone glycosides or the isoflavone glycoside acetylated in the glucoside from M1. They predominantly yielded ions [M-162+H]+ or [M-204+H]+ as base peak ion, corresponding to the cleavage of glycosidic bond. The neutral loss of the substituents on M1, such as –16 (CH$_4$), –32 (CH$_3$OH), –28 (CO), and –56 (CO$_2$) were apparent (The main fragment ions of M1, M3, M6, M7 are illustrated in the Fig. 4).
Figure 4. The main proposed fragment ions of M1, M3, M6, M7.
3.2.2. One hydroxylation or two hydroxylation with different site in A ring and with the same substituents in B ring: compounds M4, M5

The base peak of M4 is at \( m/z \) 270, and other major fragment ions are mainly at \( m/z \) 253 \([M-CH_3OH+H]^+\), \( m/z \) 225 \([M-CH_2OH-CO+H]^+\), \( m/z \) 197 \([M-CH_2OH-CO*2+H]^+\), and \( m/z \) 181 \([M-CH_2OH-CO*2-O+H]^+\).

The base peak ion of M5 is at \( m/z \) 285 \([M+H]^+\), and other major fragment ions include \( m/z \) 229 \([M-CO*2+H]^+\), \( m/z \) 253 \([M-CH_2OH+H]^+\), \( m/z \) 239 \([M-CO-H_2O+H]^+\), \( m/z \) 211 \([M-CO-CH_2OH-H_2O+H]^+\), and \( m/z \) 183 \([M-CH_2OH-CO*2-H_2O+H]^+\) (The main fragment ions of M4, M5 are shown in the Fig. 5).

3.2.3. One hydroxylation or two hydroxylation with different site in A ring and with the same substituents in B ring: M8, M9, M10, M11

The base peak of M8 is at \( m/z \) 285 \([M+H]^+\), the primary fragment ions are \( m/z \) 270 \([M-CH_3+H]^+\), \( m/z \) 253 \([M-CH_2OH+H]^+\), \( m/z \) 225 \([M-CH_2OH-CO+H]^+\), and \( m/z \) 137 \(A_{1,3}^{1,3}\) of RDA cracking in the C-ring.

M9 produced \( m/z \) 286 \([M-CH_3+H]^+\) as the base peak, together with the other primary fragment ions at \( m/z \) 269 \([M-CH_2OH+H]^+\), \( m/z \) 241 \([M-CH_2OH-CO+H]^+\), and \( m/z \) 153 \(A_{1,3}^{1,3}\) by RDA cracking in C-ring.

M10 yielded \( m/z \) 286 \([M-CH_3+H]^+\) as the base peak. In addition, ions at \( m/z \) 301 \([M+H]^+\), \( m/z \) 269 \([M-CH_2OH+H]^+\),
m/z 241 [M-CH₃OH-CO+H]⁺, m/z 153 A¹⁺ all were the primary fragment ions.

**M11** is the isoflavone glycoside of **M8**, and its mass spectrum showed that m/z 285 [M-162+H]⁺ as the base peak. In addition, the m/z 270 [M-162-CH₃+H]⁺, m/z 253 [M-162-CH₂OH+H]⁺, and m/z 225 [M-162-CH₂OH-CO+H]⁺ were the primary fragment ions, not exhibiting the fragment ion by RDA cracking or with a low ion abundance (The main fragment ions of **M8**, 9, 10, 11 are shown in the Fig. 6).

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**Figure 6.** The main proposed fragment ions of **M8**, **M9**, **M10**, **M11**.
3.2.4. The different hydroxylation and methylation in B ring with the same substituents in A ring: M2, M5

The base peak ion of M2 was at m/z 197 [M-CO2H2O-H]+, and other major fragment ions were mainly at m/z 253 [M-H2O-H]+, m/z 271 [M+H]+, m/z 225 [M-CO2H2O+H]+, m/z 215 [M-CO2H+H]+, and m/z 153 A1+ of RDA cracking in the C-ring (The main fragment ions of M2, M5 are shown in the Fig. 7).

4. Conclusions

In the present study, the ESI-IT-TOF-MS<sup>®</sup> technique was applied to analyze the fragmentation behavior of isoflavones. The characteristic fragment ions are integral to determining a structure skeleton and substitution patterns for isoflavones. In general, the fragment ions with high ion intensity proved to be the most stable and preferred cleavage.
The neutral loss of 28 u (-CO) from C_4 in the C-ring of isoflavones is the preferred cleavage. The B-ring first underwent the loss of CH_3OH (Δm = 32 u) or H_2O (Δm = 18 u), and fewer cases have a neutral loss of CO (Δm = 28 u) when substituted with hydroxylation. There also occurs a neutral loss of CO, or a loss of H_2O when connected to the adjacent hydroxylation in A-ring.

Based on the fragmentation ions of A_1,3+ originating from the RDA cracking of isoflavones, we could presume the varying hydroxylate location in A-ring or B-ring (m/z 137 A_1,3+ for one hydroxylation be replaced in A-ring, and m/z 153 for A-ring with two replaced hydroxylation). Then the other following fragments in mass spectra are the same as the relating aglycones, with the C_7 glycosidic-bond of isoflavone glycosides in A-ring cleavage in priority. The above fragmentation rules could help to identify the different types of isoflavones.

Acknowledgments

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References