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# Structural Characterization of Anhydroicaritin Glycosides Using ESI-FT-ICR Mass Spectrometry

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**Abstract:** Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) was used to determine the structures of anhydroicaritin glycosides by the MS/MS experiments of anhydroicaritin glycosides and their methylated derivatives. With high accuracy FT-ICR-MS provides much information about the structures of compounds, FT-ICR-MS shows the great potential application in the structural characterization of unknown compounds.

**Keywords:** Mass characterization, anhydroicaritin glycosides, FT-ICR-MS.

Since FT-ICR-MS possesses good sensitivity with high resolution and also unmatchable mass measurement accuracy, the combination of electrospray ionization, a soft ionization technique, with FT-ICR-MS provides a powerful tool for the structure elucidation of bioactive constituents[1-3]. The structural identification of the molecule can be obtained from the masses of the dissociated fragments with ion cyclotron resonance mass spectrometry (tandem MS)[4,5]. In this paper the ESI-FT-ICR-MS and tandem MS was used for the structural characterization of anhydroicaritin glycosides.

Anhydroicaritin glycosides are a series of compounds with the same basic structure as shown in **Figure 1**. There are three indefinable groups ($R_1$, $R_2$ and $R_3$) linking to the body of anhydroicaritin glycosides. The NMR spectra of anhydroicaritin glycosides indicated that $R_2$ group was a hydrogen atom and $R_1$ and $R_3$ were sugar residues. By the use of MS the kinds of the sugars and their linking ways can be determined.

## Experimental

All measurements were performed on an APEX II FT-ICR mass spectrometer (Bruker Daltonics, Inc. USA) with 4.7 tesla superconducting magnet. All the calculated elemental compositions were acquired by the software Xmass of FT-ICR-MS. The solvent for ESI was $CH_3OH$ : $H_2O$ = 1 : 1 (V/V) containing 2 % acetic acid and its flow rate was 30 μ L/h. The length of the electrospray capillary (50 μ m i.d., 150 μ m o.d.) was 30 cm. The potential of the electrospray was set at 3 kV. During tandem MS experiments, the parent ions were isolated and then collided with argon gas, thus

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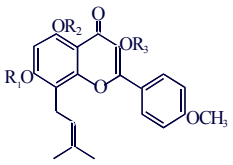
producing the fragment ions.

**Figure 1** The basic structure of anhydroicaritin glycosides




The samples of anhydroicaritin glycosides were extracted from plants, then separated and purified by HPLC. Each of the four samples was divided into halves. One part was directly for FT-ICR-MS analysis, the rest were first methylated[6] and then for FT-ICR-MS analysis. During the ESI-MS experiments, anhydroicaritin glycosides and methylated anhydroicaritin glycosides were dissolved in $CH_3OH$ with the concentration of 10 pmol / μ L.

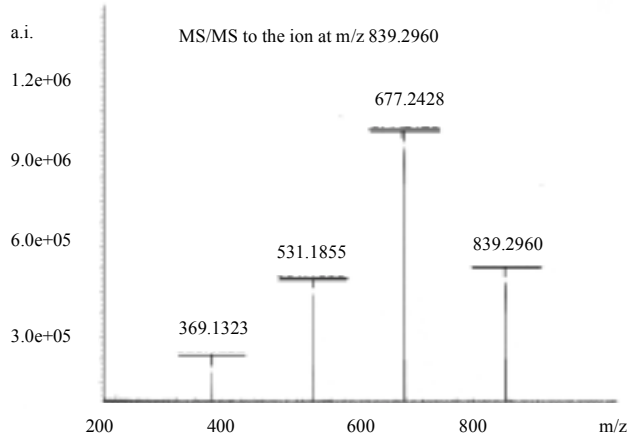
## Results and Discussion

The experimental results of four samples of anhydroicaritin glycosides were listed in **Table 1**. The first ion peak of each sample in the table was the protonated molecule, and the others were their fragmental ions. Due to the high accuracy of high resolution measurement data of FT-ICR-MS (relative error < 4 ppm), the elemental compositions of

**Table 1** The experimental results of four anhydroicaritin glycosides

| Sample | Ion peaks | Mass $_{(cal)}$ | Mass$_{(exp.)}$ | Error | Composition |
|---|---|---|---|---|---|
| **1** | 1 | 839.2968 | 839.2960 | 9.258e-07 | $C_{39}H_{51}O_{20}$ |
| | 2 | 677.2440 | 677.2428 | 1.758e-06 | $C_{33}H_{41}O_{15}$ |
| | 3 | 531.1861 | 531.1855 | 1.145e-06 | $C_{27}H_{31}O_{11}$ |
| | 4 | 369.1333 | 369.1323 | 2.592e-06 | $C_{21}H_{21}O_6$ |
| **2** | 1 | 839.2968 | 839.2971 | 3.300e-07 | $C_{39}H_{51}O_{20}$ |
| | 2 | 677.2440 | 677.2431 | 1.297e-06 | $C_{33}H_{41}O_{15}$ |
| | 3 | 531.1861 | 531.1867 | 1.259e-06 | $C_{27}H_{31}O_{11}$ |
| | 4 | 369.1333 | 369.1332 | 1.155e-07 | $C_{21}H_{21}O_6$ |
| **3** | 1 | 809.2862 | 809.2867 | 5.292e7-0 | $C_{38}H_{49}O_{19}$ |
| | 2 | 677.2440 | 677.2435 | 6.770e-07 | $C_{33}H_{41}O_{15}$ |
| | 3 | 531.1861 | 531.1860 | 1.207e-07 | $C_{27}H_{31}O_{11}$ |
| | 4 | 369.1333 | 369.1330 | 7.819e-07 | $C_{21}H_{21}O_6$ |
| **4** | 1 | 823.3019 | 823.3011 | 9.012e-07 | $C_{39}H_{51}O_{19}$ |
| | 2 | 677.2440 | 677.2436 | 5.929e-07 | $C_{33}H_{41}O_{15}$ |
| | 3 | 531.1861 | 531.1858 | 5.631e-07 | $C_{27}H_{31}O_{11}$ |
| | 4 | 369.1333 | 369.1328 | 1.148e-06 | $C_{21}H_{21}O_6$ |

**Figure 2** Tandem mass spectrum of sample **1**




all the ions could be easily deduced, and the kind of lost sugar residues could be obtained based on the mass differences between parent ions and daughter ions.

From the tandem MS spectrum of sample **1** shown in **Figure 2**, it could be known that the protonated molecular ion at *m/z* 839.2960 produced three fragment ions at *m/z* 677.2428, *m/z* 531.1855 and *m/z* 369.1323, respectively. The mass difference of 162.0532 between the parent ion and the fragment ion at *m/z* 677.2428 is exactly in agreement with the molecular weight of a hexose residue. Therefore, it could be concluded that the fragment ion at *m/z* 677.2428 came from the parent ion by losing a hexose residue. Likewise, the fragment ion at *m/z* 531.1855 (839.2428 - 162.0532 - 146.0573) was produced by losing a deoxyhexose residue and a hexose residue and the fragment ion at *m/z* 369.1323 (839.2428 - 162.0532 - 146.0573 - 162.0532) formed by losing two hexose residues and one deoxyhexose residue.

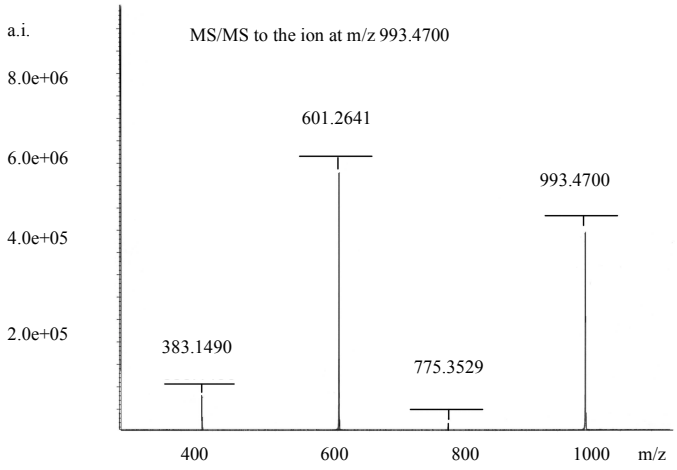
The remaining three samples have been analyzed in the same way, we can clearly see the cleavages of samples by losing hexose residues and deoxyhexose residues. From the experimental results listed in **Table 1**, it could be known that there were two hexose residues and one deoxyhexose residue linking to the body of sample **1** and **2**; One pentose residue, one deoxyhexose residue and one hexose residue in sample **3**; Two deoxyhexose residues and one hexose residue in sample **4**.

In order to identify the linking ways of these sugar residues, the glycoside samples were methylated and the derivatives were analyzed by FT-ICR-MS.

The MS/MS spectrum of the methylated derivative of sample **1** was demonstrated in **Figure 3**. The mathylated sample **1** ( *m/z* 993.4700) can produce three fragment ions at *m/z* 775.3529, 601.2641 and 383.1490, respectively. The mass difference between *m/z* 993.4700 and *m/z* 775.3529 was exactly agreed with the molecular weight of a tetra-methylated hexose residue. Obviously, the fragment ion at *m/z* 775.3529 (993.4700 − 218.1171) came from the parent ion by losing one tetra-methylated hexose residue. Likewise, it could be concluded that the fragment ion at *m/z* 601.2641 (993.4700 - 218.1171 - 174.0888) was produced by losing one tetra-methylated hexose residue and one dimethylated deoxyhexose residue, the fragment ion at *m/z* 383.1490 (993.4700 - 218.1171 - 174.0888 - 218.1151) was produced by losing two

tetra-methylated hexose residues and one dimethylated deoxyhexose residue. By comparison **Figure 3** with **Figure 2**, one can see that for sample **1** after methylation both hexose residues were added four methyls and the deoxyhexose residue was added two

**Figure 3** Tandem mass spectrum of methylated derivative of sample **1**




methyls. So it could be known that there were one linked hydroxyl and four free hydroxyls in both hexose residues and two linked hydroxyls and two free hydroxyls in deoxyhexose residue. Accordingly, it could be known that for sample **1** the deoxyhexose residue and one of the hexose residues linked directly to the body at $R_1$ and $R_3$ sites, and the other hexose residue linked to the deoxyhexose residue.

Combination with the naturally-occurring compound database search results and the gas chromatographic results of the partially methylated alditol acetate derivatives, the $R_1$ and $R_3$ groups in four samples could be completely determined. Sample **1** was 4′ Me ether, 3-O-[glucopyranosyl (1→3) - rhamnopyranoside], 7-O-glucopyranoside; sample **2** was 4′ Me ether, 3-O-[glucopyranosyl (1→2) - rhamnopyranoside], 7-O-glucopyranoside; sample **3** was 4′ Me ether, 3-O-[Arabinofuranosyl (1→2) - rhamnopyranoside], 7-O- glucopyranoside; sample **4** was 4′ Me ether, 3-O-[rhamnopyranosyl (1→2) - rhamnopyranoside], 7-O- glucopyranoside.

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