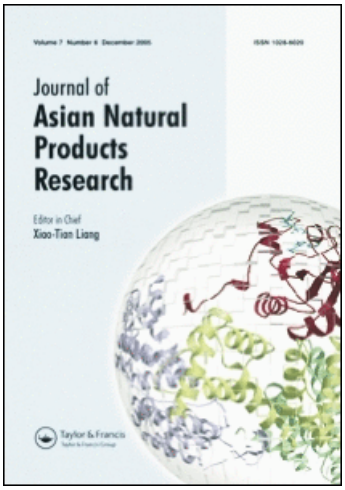

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ORIGINAL ARTICLE

Mass spectral fragmentation analysis of triterpene saponins from *Ardisia crenata* Sims by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry

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We used the electrospray ionization (ESI) Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) technique to study the characteristic mass fragmentation patterns of eight triterpene saponins from *Ardisia crenata* Sims. Eight triterpene saponins were analyzed using parent mass list-triggered data-dependent multiple-stage accurate mass analysis at a resolving power of 100,000 in the external calibration mode. The chemical formula with unsaturation numbers was calculated from accurate *m/z* values of precursor, and product ions were obtained and used to assign the structures of eight triterpene saponins and two trace unknown compounds. The mass accuracies obtained for all full-scan MS and MS^n spectra were within 7 ppm (<5 ppm in most cases) in the ESI negative-ion mode. On FTICR-MS and FTICR-MS/MS, the eight triterpene saponins showed characteristic mass fragmentation patterns that facilitated the identification of their structural types, including the individual monosaccharide types, the monosaccharide numbers, and the sequences of the substituted saccharide groups. We proposed their fragmentation mechanisms. Based on their characteristic mass fragmentation patterns and fragmentation mechanisms, two unknown trace triterpene saponins were identified in the mixture.

Keywords: Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS); triterpene saponins; mass fragmentation; *Ardisia crenata* Sims

1. Introduction

The roots of *Ardisia crenata* Sims (Myrsinaceae), one of the medicinal herbs of the genus *Ardisia*, are used in traditional Chinese medicine to treat respiratory tract infections and menstrual disorders [1]. Pharmacological studies have shown that extracts and constituents of *Ardisia* have antitumor, antihuman immunodeficiency virus, antiviral, and anti-oxidative effects [2–7]. The main constituents of *Ardisia* herbs are triterpene saponins, which are derivatives of oleanane.

In this chemical study, eight triterpene saponins were separated and purified from the roots of *A. crenata* Sims. Their structures (Figure 1) were determined using spectral methods such as IR, NMR, and MS. The eight triterpene saponins belong to two structural types, A and B.

In this paper, we report the use of the Fourier transform ion cyclotron resonance

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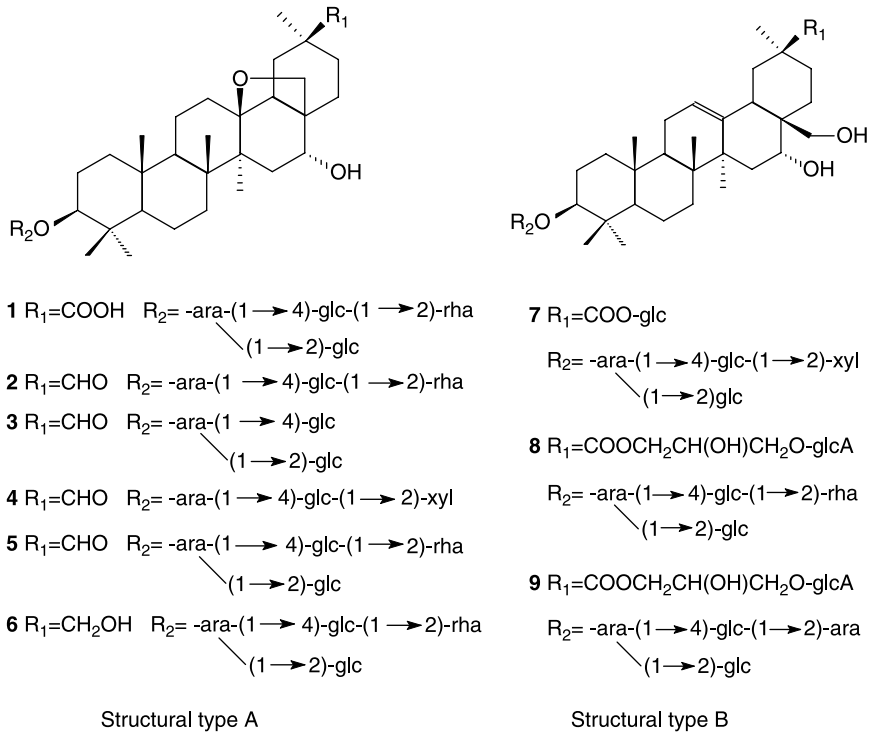

Figure 1. Structures of eight triterpene saponins **1**–**9**.

mass spectrometry (FTICR-MS) technique to monitor the parent triterpene saponins and their mass fragmentation patterns in MS and MS^n experiments. The high-resolution accurate mass of parent compounds and their fragments were obtained. The eight triterpene saponins showed characteristic mass fragmentation patterns that facilitated the identification of their structural types, including the individual monosaccharide types, the monosaccharide numbers, and the sequences of the substituted saccharide groups. We proposed their fragmentation mechanisms. Based on their characteristic mass fragmentation patterns and fragmentation mechanisms, two unknown trace triterpene saponins were identified in the mixture. In the meantime, the high-resolution accurate mass of parent compounds and their fragments increase the confidence level of proposed structures of unknown compounds.

2. Results and discussion

The characteristic electrospray ionization ESI-MS and MS^n data for the eight triterpene saponins are shown in Tables 1–4 with high resolution and accuracy. The mass spectra of compounds **1** and **8** are representative of the two structural types A and B, respectively. Both compounds **1** and **8** showed pseudo-molecule ions $[M-H]^-$ and/or $[M+Cl]^-$. However, they exhibited different characteristic fragmentations in the MS^2 and MS^3 experiments.

Compound **1** showed the pseudo-molecule ion $[M-H]^-$ at *m/z* 1089.5509 ($C_{53}H_{85}O_{23}^-$, calculated $[M-H]^-$ 1089.5476, error + 3.015 ppm). The MS^2 experiment with compound **1** showed that the pseudo-molecule ion at *m/z* 1089.5508 gave rise to fragments at *m/z* 943.4913 and 781.4401. The intensity of the former fragment was greater than that of the latter. The former fragment corresponded to the

Table 1. Mass data from 10 saponin compounds.

Compounds	$[M-H]^-$	Proposed formula	RDB	Calculated mass (*m/z*)	Error (ppm)
1	1089.5509	$C_{53}H_{85}O_{23}^-$	11.5	1089.5476	3.015
2	911.5063	$C_{47}H_{75}O_{17}^-$	10.5	911.5017	7.047
3	927.4954	$C_{47}H_{75}O_{18}^-$	10.5	927.4948	0.656
4	897.4831	$C_{46}H_{73}O_{17}^-$	10.5	897.4842	−1.256
5	1073.5579	$C_{53}H_{85}O_{22}^-$	11.5	1073.5527	4.843
6	1075.5703	$C_{53}H_{87}O_{22}^-$	10.5	1075.5684	1.813
7	1237.5906	$C_{58}H_{93}O_{28}^-$	12.5	1237.5848	4.696
8	1339.6194	$C_{62}H_{99}O_{31}^-$	13.5	1339.6165	2.178
9	1325.6111	$C_{53}H_{85}O_{22}^-$	13.5	1325.6008	7.746
10	1355.6156	$C_{53}H_{85}O_{22}^-$	13.5	1355.6114	3.101

Note: RDB, values for the ring and double bonds equivalents, a measure of the number of unsaturated bonds in a compound.

loss of the terminal rhamnopyranosyl group, originating from the substituted saccharide chain at the C-3 position, whereas the latter fragment was attributable to the loss of the rhamnopyranosyl and glucopyranosyl groups from the substituted saccharide chain at the C-3 position. The MS^3 experiment showed that the fragment ion at *m/z* 943.49 gave rise to fragments at *m/z* 781.4398, 619.3858, and 487.3430, resulting from the further loss of a glucopyranosyl group, two glucopyranosyl groups, and two glucopyranosyl groups and one arabinopyranosyl group, respectively, from the substituted saccharide chain at the C-3 position. The fragment at *m/z* 487.3430 was an aglycone fragment. The MS^4 experiment showed that the ion at *m/z* 781.44 gave rise to the fragment at *m/z* 619.3862, corresponding to the further loss of a glucopyranosyl group from the substituted saccharide chain at the C-3 position. The substituted

Table 2. MS^2 data from 10 saponin compounds.

Compounds	Precursor ion	Observed mass (*m/z*)	Relative intensity (%)	Proposed formula	RDB	Calculated mass (*m/z*)	Error (ppm)
1	1089.57	943.4913	100	$C_{47}H_{75}O_{19}^-$	10.5	943.4897	1.689
		781.4401	22	$C_{41}H_{65}O_{14}^-$	9.5	781.4369	3.349
2	911.51	765.4444	100	$C_{41}H_{65}O_{13}^-$	9.5	765.4420	3.177
		603.3912	18	$C_{35}H_{55}O_{8}^-$	8.5	603.3891	3.406
3	927.49	765.4434	100	$C_{41}H_{65}O_{13}^-$	9.5	765.4420	1.870
		603.3909	17	$C_{35}H_{55}O_{8}^-$	8.5	603.3891	2.908
4	897.48	765.4442	100	$C_{41}H_{65}O_{13}^-$	9.5	765.4420	2.915
		603.3912	9	$C_{35}H_{55}O_{8}^-$	8.5	603.3891	2.908
5	1073.55	927.4983	100	$C_{47}H_{75}O_{18}^-$	10.5	927.4948	3.783
		765.4448	30	$C_{41}H_{65}O_{13}^-$	9.5	765.4420	3.699
6	1075.57	929.5143	100	$C_{47}H_{77}O_{18}^-$	9.5	929.5104	4.151
		767.4604	41	$C_{41}H_{67}O_{13}^-$	8.5	767.4576	3.885
7	1237.59	1075.5359	100	$C_{53}H_{87}O_{22}^-$	11.5	1075.5684	3.659
8	1339.62	1321.6022	100	$C_{62}H_{97}O_{30}^-$	14.5	1321.6059	−2.283
		943.4892	20	$C_{47}H_{75}O_{19}^-$	10.5	943.4897	−0.537
		781.4376	24	$C_{41}H_{65}O_{14}^-$	14.5	781.4369	0.717
9	1325.61	1307.5986	31	$C_{61}H_{95}O_{30}^-$	14.5	1307.5903	6.372
		943.4942	70	$C_{47}H_{75}O_{19}^-$	10.5	943.4897	4.763
		781.4395	100	$C_{47}H_{65}O_{14}^-$	14.5	781.4369	0.948
10	1355.62	1337.6071	23	$C_{62}H_{97}O_{31}^-$	13.5	1337.6008	3.101
		959.4894	83	$C_{47}H_{75}O_{20}^-$	10.5	959.4846	4.981
		797.4339	100	$C_{41}H_{65}O_{15}^-$	9.5	797.4318	2.637

Table 3. MS^3 data from 10 saponin compounds.

Compounds	Precursor ion	Observed mass (*m/z*)	Relative intensity (%)	Proposed formula	RDB	Calculated mass (*m/z*)	Error (ppm)
1	943.49	781.4398	100	$C_{41}H_{65}O_{14}^-$	9.5	781.4369	3.733
		619.3858	34	$C_{35}H_{55}O_9^-$	8.5	619.3841	2.810
2	765.44	603.3912	100	$C_{35}H_{55}O_8^-$	8.5	603.3891	3.406
3	765.44	603.3909	100	$C_{35}H_{55}O_8^-$	8.5	603.3891	2.908
4	765.44	603.3909	100	$C_{35}H_{55}O_8^-$	8.5	603.3891	2.908
5	927.50	765.4448	100	$C_{41}H_{65}O_{13}^-$	9.5	765.4420	3.699
6	929.51	767.4604	100	$C_{41}H_{67}O_{13}^-$	8.5	767.4576	3.885
7	1075.54	943.4921	100	$C_{47}H_{75}O_{19}^-$	10.5	943.4897	2.537
8	1321.60	1131.5618	32	$C_{55}H_{87}O_{24}^-$	12.5	1131.5582	5.409
		943.4935	65	$C_{47}H_{75}O_{19}^-$	10.5	943.4897	4.021
		781.4376	100	$C_{41}H_{65}O_{14}^-$	9.5	781.4369	2.965
		619.3854	46	$C_{35}H_{55}O_9^-$	8.5	619.3841	3.133
9	781.44	619.3861	100	$C_{35}H_{55}O_9^-$	8.5	619.3841	3.294
10	797.43	635.3817	100	$C_{35}H_{55}O_{10}^-$	8.5	635.3790	4.290

Table 4. MS^4 data from six saponin compounds.

Compounds	Precursor ion	Observed mass (*m/z*)	Relative intensity (%)	Proposed formula	RDB	Calculated mass (*m/z*)	Error (ppm)
1	781.44	619.3862	100	$C_{35}H_{55}O_9^-$	8.5	619.3841	3.456
5	765.44	603.3910	100	$C_{35}H_{55}O_8^-$	8.5	603.3891	3.074
6	767.46	605.4069	100	$C_{35}H_{57}O_8^-$	8.5	605.4048	3.477
7	943.50	781.4401	100	$C_{41}H_{65}O_{14}^-$	9.5	781.4369	4.117
		619.3861	9	$C_{35}H_{55}O_9^-$	8.5	619.3841	3.294
8	781.44	619.3860	100	$C_{35}H_{55}O_9^-$	8.5	619.3841	3.778
9	619.39	571.3646	100	$C_{34}H_{51}O_7^-$	9.5	571.3629	2.927

monosaccharide type and the conjugated sequence could be confirmed by the lost fragment masses of 146, 162, 162, and 132. It is interesting to note that the compounds belonging to structural type A had a tendency to lose a monosaccharide from the substituted saccharide chain at the C-3 position to produce a series of triterpene saponin derivative ions in the MS^n experiments. This tendency allowed us to identify the monosaccharide type and the sequence of the substituted saccharide chain at the C-3 position.

Compound **8** showed the pseudomolecule ion $[M-H]^-$ at *m/z* 1339.6194 ($C_{62}H_{99}O_{31}^-$, calculated $[M-H]^-$ 1339.6165, error + 2.178 ppm). The MS^2 experiment demonstrated that the pseudomolecule ion at *m/z* 1339.62 gave rise to abundant fragment at *m/z* 1321.6022, resulting from the loss of H_2O from the glucuronyl glycerol side chain at the C-30 position (mass loss of 18). Fragment ions at *m/z* 1017.5250, 943.4892, and 781.4376 were also obtained in the MS^2 experiment, corresponding to the loss of, respectively, a glucuronyl group from the glucuronyl glycerol side chain at the C-30 position and a rhamnopyranosyl group from the substituted saccharide chain at the C-3 position (mass loss of 322), the glucuronyl glycerol side chain at the C-30 position and the rhamnopyranosyl group at the C-3 position (mass loss of 396), and the further loss of a glucopyranosyl group at the C-3 position based on the fragment ion at *m/z* 943.4892 (mass loss of 558). In the MS^3 experiment, the fragment ion at *m/z* 1321.61

produced fragments at *m/z* 943.4935, 781.4376, and 619.3854, with the same fragmentation tendency as that observed in the MS^2 experiment. The MS^4 experiment showed that the fragment ion at *m/z* 781.44 gave rise to a fragment at *m/z* 619.3860.

It is worth noting that compound **8** showed a strong tendency to lose water from the glucuronyl glycerol side chain at the C-30 position, which is the characteristic substituted group of compound **8**, and then to simultaneously lose the glucuronyl group or glucuronyl glycerol group at the C-30 position and the terminal rhamnopyranosyl group from the substituted saccharide chain at the C-3 position. Thereafter, the monosaccharides of the substituted saccharide chain at the C-3 position underwent fragmentation along the same pathway as that observed for compound **1**.

A mixture containing compound **8** and two unknown trace compounds **9** and **10** was tested based on the fragmentation mechanism proposed previously for the two structural types of triterpene saponins. Both MS and MS^n data were obtained (see Tables 1–4). The pseudo-molecule ion $[M-H]^-$ of compound **9** was at *m/z* 1325.6111 ($C_{53}H_{85}O_{22}^-$, calculated $[M-H]^-$ 1325.6008, error + 7.746 ppm), and the MS^2 experiment showed that the pseudo-molecule ion at *m/z* 1325.61 gave rise to fragments at *m/z* 1307.5986, 1017.5314, 943.4942, and 781.4395. The same fragmentation pathway as that observed for compound **8** was detected in the MS^2 experiment, indicating that compound **9** has a structural skeleton similar to that of compound **8** and also a glucuronyl–glycerol-substituted group at the C-30 position. A slight difference between the two compounds is that pentose, rather than rhamnose, is the terminal monosaccharide of the substituted saccharide chain at the C-3 position. Based on the biogenesis and structures of the triterpene saponins isolated from the genus *Ardisia*, pentose should be xylopyranose [4,5]. The proposed structure of compound **9** is shown in Figure 1.

Compound **10** showed the pseudo-molecule ion at *m/z* 1355.6156 ($C_{53}H_{85}O_{22}^-$, calculated $[M-H]^-$ 1355.6114, error +3.101 ppm). In the MS^2 experiment, the pseudo-molecule ion at *m/z* 1355.62 produced fragment ions at *m/z* 1337.6071, 1033.5260, 959.4894, and 797.4339. It also revealed that fragment mass losses of 18, 322, 396, and 558 are similar to those of compound **8**. This finding indicated that compound **10** has the same glucuronyl–glycerol-substituted group at the C-30 position as compound **8**, and the same substituted saccharide chain at the C-3 position. However, it has a different aglycone skeleton and may have one more hydroxyl group than compound **8**.

3. Conclusion

The FTICR-MS technique was used to monitor the parent triterpene saponins and their mass fragmentation patterns in the MS and MS^n experiments. The high-resolution accurate mass of parent compounds and their fragments were obtained. The mass accuracies obtained for all full-scan MS and MS^n spectra were within 7 ppm (< 5 ppm in most cases).

The triterpene saponins of structural type A underwent a sequential loss of sugar from the substituted saccharide chain at the C-3 position in the FTICR-MS^n experiment. The triterpene saponins of structural type B readily lost the substituted group at the C-30 position, with the subsequent loss of the terminal monosaccharide group from the substituted saccharide chain at the C-3 position. The tendency to lose the monosaccharides from the substituted saccharide chain at the C-3 position was the same as that observed in the triterpene saponins of structural type A.

The mass fragment ions in the MS^2 and MS^3 experiments provided information that facilitated the identification of the structural types of the triterpene saponins. On FTICR-MS, the eight triterpene saponins showed characteristic mass fragmentation patterns

that were useful in determining their structural types, including the individual monosaccharide types, the monosaccharide numbers, and the sequences of the substituted saccharide groups.

4. Experimental

Methanol of ultra resi-analyzed grade was purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). In-house Milli-Q water was used. The ESI-MS and ESI-MS^n analyses were conducted with a Thermo Finnigan LTQ Fourier transform (FT) ion cyclotron resonance mass spectrometer controlled by an Xcalibar workstation. The samples were dissolved in methanol, and then filtered through a 0.25 μm film before analysis. The experiments were performed in the ESI negative-ion mode, with the capillary temperature set at 280°C, capillary voltage at 40 V, ion spray voltage at 4.1 V, and tube lens at 170 V. The direct injection flow rates were 3.0–10.0 μl/min. Compounds were detected by full-scan mass analysis from *m/z* 100 to 2000 at a resolving power of 100,000 with data-dependent MS/MS analysis triggered by the most abundant ions from the parent mass list, followed by MS^3 and MS^4 analyses on the most abundant product ions. The resolving power used for multiple-stage mass analysis was the same as that employed for the full-scan mass analysis. Collision-induced dissociation (CID) was conducted with an isolation width of 1 Da and the collision energy set at 35 V. After their collision with He atoms inside the linear ion trap, they were transferred to the ICR cell and the mass spectra were obtained with high resolution and accuracy. Data acquisition and reduction were performed with the Xcalibur version 1.4 software (Thermo Fisher, Waltham, MA, USA).

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