Journal of Medicinal Plants Research Vol. 5(26), pp. 6152-6159, 16 November, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.339

Full Length Research Paper

Identification and characterization of saponins in extract of *Ziziphi spinosae* Semen (ZSS) by ultra-performance liquid chromatography-electrospray ionization-quadrupole time-of-flight tandem mass spectrometry (UPLC-ESI-QTOF-MS^E)

Jin-Jie MA[1,2,3], Li-Ping KANG[2,3], Wen-Bin ZHOU[1,2], He-Shui YU[2,3], Ping LIU[1]* and Bai-Ping MA[2#]

[1]Pharmacy of Traditional Chinese Medicine (TCM), Chinese People's Liberation Army (PLA) General Hospital, Beijing 100853, P. R. China.
[2]Beijing Institute of Radiation Medicine, Beijing 100850, P. R. China.
[3]Tianjin University of Traditional Chinese Medicine, Tianjin 300193, P. R. China.

Accepted 7 October, 2011

This paper was designed to develop a novel method via ultra-performance liquid chromatography-electrospray ionization-quadrupole time-of-flight tandem mass spectrometry (UPLC-ESI-QTOF-MS^E) for the analysis and identification of saponins from extract of *Ziziphi spinosae* Semen (ZSS). The possible fragmentation pathways were proposed on the basis of MS^E data. In results, 13 saponins, including two unknown compounds, were identified and they were divided into two groups on the basis of mass spectral analysis. Compound 1~6, which were of keto-dammarane type triterpene saponin, underwent McLafferty-type (or McLafferty) rearrangement, and compound 7~13, which were of dammarane type triterpene saponin, underwent retro Diels-Alder (RDA) cleavage. The results testified that the developed method was useful to rapidly and simultaneously identify the constituents in ZSS. In this paper, the principles of McLafferty-type (or McLafferty) rearrangement were firstly utilized to elucidate the proposed fragmentation pathways of saponins from ZSS.

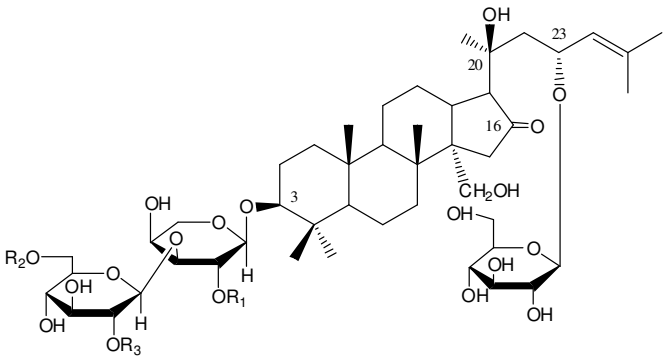
Key words: *Ziziphi spinosae* semen, ultra-performance liquid chromatography (UPLC), quadrupole time-of-flight tandem mass spectrometry (QTOF-MS^E), retro Diels-Alder cleavage, McLafferty-type rearrangement.

INTRODUCTION

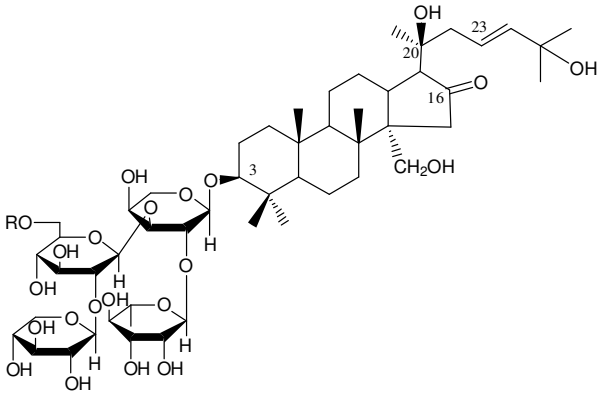
Ziziphi spinosae Semen (ZSS), which has been known as Suanzaoren in traditional Chinese medicines (TCMs), is the dried mature seeds of *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H. F. Chou. In traditional clinical practice, it is mainly utilized for the treatment of insomnia and anxiety (Pharmacopoeia Commission of PRC, 2005). Modern pharmacological studies have demonstrated that flavonoids and saponins are the main bioactive substances responsible for the sedative and hypnotic effects by ZSS (Li and Bi, 2006; You et al., 2010; Wang et al., 2010). In view of various saponins existing in ZSS (Otsuka et al., 1978; Yoshikawa et al., 1997; Matsuda et al., 1999; Wang and Yang, 2008; Wang et al., 2009; Wang et al., 2009; Niu et al., 2010; Bao et al., 2009; Liu et al., 2007), an accurate and sensitive method should be established to analyze saponins from ZSS. In previous experiments about saponins of ZSS (Otsuka et al., 1978; Yoshikawa et al., 1997; Matsuda et al., 1999; Wang and Yang, 2008; Wang et al., 2009; Wang et al., 2009), eleven saponins have been isolated from crude drugs. Though high-performance liquid chromatography (HPLC)

*Corresponding author. E-mail: liuping0707@yahoo.com.cn. Tel(fax): +86-10-66936427.

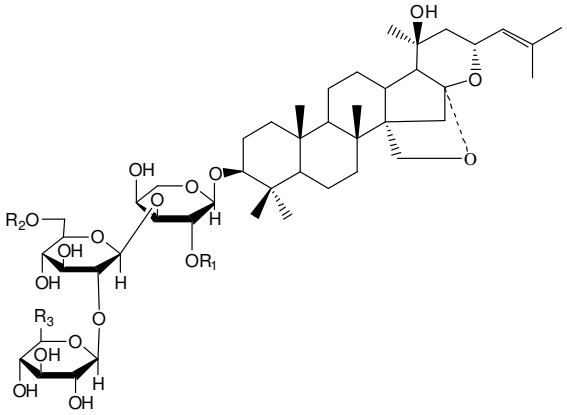
#This author contributed equally to this work.



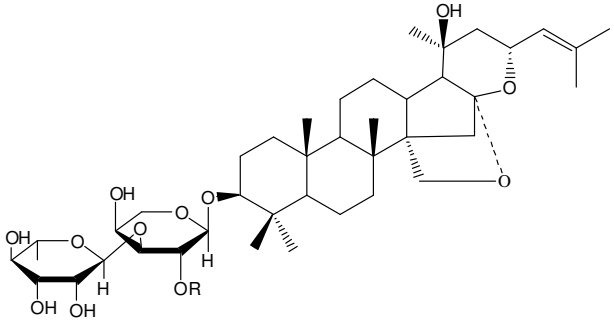
NO.		R1	R2	R3	MW
1	**protojujuboside A**	-*a*-L-Rha	-*β*-D-Glc	-*β*-D-Xyl	1386.6667
2	**protojujuboside B**	-*a*-L-Rha	-H	-*β*-D-Xyl	1224.6139
4	**unknown**	-*a*-L-Rha	-H	-H	1092.5716
5	**protojujuboside B_1**	-*a*-D-Fuc	-H	-*β*-D-Xyl	1224.6139



NO.		R	MW
3	**jujuboside H**	-*β*-D-Glc	1224.6139
6	**jujuboside G**	- H	1062.5611



NO.		R_1	R_2	R_3	MW
7	**jujuboside A**	-*a*-L-Rha	-*β*-D-Glc	-H	1206.6033
8	**jujuboside C**	-*a*-L-Rha	-*β*-D-Glc	$-CH_2OH$	1236.6139
9	**jujuboside A_1**	-*a*-D-Fuc	-*β*-D-Glc	-H	1206.6033
10	**jujuboside B**	-*a*-L-Rha	-H	-H	1044.5505
11	**jujuboside B_1**	-*a*-D-Fuc	-H	-H	1044.5505
13	**acetyljujuboside B**	-*a*-L-Rha	-Ac	-H	1086.5611



NO.		R	MW
12	**unknown**	-*a*-L-Rha or *a*-D-Fuc	896.5133

Figure 1. Chemical structures of 13 saponins from ZSS.

coupled with evaporative light-scattering detector (ELSD) and liquid chromatography–mass spectrometry (LC/MS) have been used to study saponins from ZSS in recent years (Wang et al., 2009; Bao et al., 2009; Liu et al., 2007), there are several compounds which have not been chromatographically identified yet. In our experiment, saponins from ZSS were firstly detected by UPLC-QTOF-MS^E, an effective and sensitive analytical instruments combination, with which accurate molecular weights are detected, characterization of fragments are obtained. Owing to the ultra performance of UPLC-QTOF-MS^E, we obtained a large number of diagnostic fragments data, which were processed by MassLynx version 4.1. In results, thirteen saponins were detected and 11 of them were determined comparing to MS information and literatures, and 2 of them (4 and 12), which have never been reported yet, were tentatively characterized. Mainly focusing on saponins, this paper gave deductions in detail about 13 saponins from ZSS. The structures of 13 saponins are shown in Figure 1.

MATERIALS AND METHODS

Instruments and materials

The LC separation was performed on Waters Acquity UPLC™ system (Waters Corporation, Milford, MA, USA) with an Acquity UPLC HSS T3 column (100×2.1 mm, 1.8 μm). The MS was Synapt

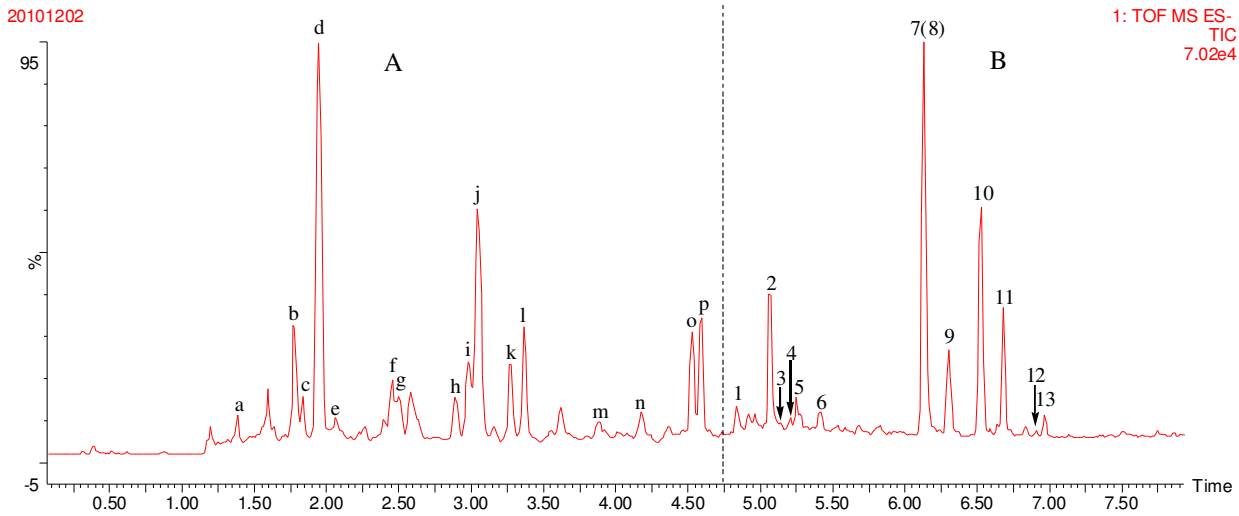


Figure 2. TIC of the ZSS extract analyzed by UPLC-QTOF-MS^E in negative ESI mode. A. flavonoids zone; B. saponins zone.

MS system equipped with electrospray ionization (ESI) source (Waters Corp., Manchester, UK). The instrument was controlled by Masslynx 4.1 software (Waters Corporation, Milford, MA, USA). Acetonitrile and methanol (HPLC grade) were purchased from Fisher Scientific Co. (Loughborough, UK). Formic acid (HPLC grade) was purchased from Acros Co. Ltd. (NJ, USA). Water (18.2 MΩ) was purified on a Milli-Q system (Millipore, Billerica, USA). Other reagents were commercially available of analytical purity. Two authentic compounds of protojujuboside B and jujuboside A (series No. 110734–200509) were purchased from National Institution for control of pharmaceutical and biological product (NICPBP) of P.R. China. The dried ZSS was purchased from Lvye Pharmaceutical Company of Beijing (origin: Hebei Province, P.R. China; series No. 0809604) and was identified as mature seeds of Z. *jujuba* Mill. *var spinosa* (Bunge) Hu ex H. F. chou by director pharmacist Ping Liu who was from Pharmacy of TCM, Chinese People's Liberation Army (PLA) General Hospital.

Sample preparation

The powdered ZSS (1 kg) was degreased with 8 L petroleum ether (60 to 90°C) three times (each time for 120 min) and the residue was refluxed with 8 L 75% ethanol twice (each time for 120 min). The combined extract of 75% ethanol was concentrated under reduced pressure and the residue was suspended in 0.5 L distilled water and extracted three times with n-BuOH saturated with water to obtain the n-BuOH fraction. The n-BuOH fraction was concentrated and lyophilized to total flavonoids and saponins (18.5 g), 20 mg of which was dissolved in 1.5 ml of 30% CH_3CN. The solution was filtrated through a 0.22 µm syringe filter and prepared to be injected into the UPLC/TOF instrument for analysis. The purity of each authentic compound, protojujuboside B (2) and jujuboside A (7), was determined to be higher than 97% by HPLC–ELSD. To prepare standard mixture solution, two authentic compounds were respectively dissolved in 30% CH_3CN at 0.5 mg·ml^{-1} for stock solutions, then 20 µl of each these solutions was combined and diluted to 1 ml as the final standard solution.

UPLC and MS condition

The chromatographic separation was performed on an Acquity UPLC HSS T3 column (100 × 2.1 mm, 1.8 µm) maintained at 45°C. The flow rate was kept at 600 µl·min^{-1}. Mobile phase A was 0.1% formic acid aqueous solution and mobile phase B was acetonitrile. The gradient was programmed as follows: (0~0.5) min, 5% B; (0.5~0.6) min, 5%→18% B; (0.6~4) min, 18%→25% B; (4~10) min, 25%→80% B; (10~11) min, 80%→100% B; (11~13) min, 100% B. then, the column was re-equilibrated with the initial condition before the next injection. The sample injection volume was 2 µl.

The experiment was performed on both positive and negative ESI mode. The source temperature was set at 120°C, and the desolvation temperature was set at 450°C with desolvation gas flow set at 900 L·h^{-1}. The capillary voltage was sat at 3 KV. The cone voltage was sat at 40 V for positive ESI or 50 V for ESI (–). The collision energy was sat as 6 eV (trap) and 4 eV (transfer) for low–energy scan, and 45 to 60 eV ramp (trap) and 12 eV (transfer) for high–energy scan. Mass accuracy was maintained using a lock spray with leucine enkephalin for positive ion mode ($[M + H]^+$ = 556.2771) and negative ion mode ($[M - H]^-$ = 554.2615) at a concentration of 200 pg·µl^{-1} and a flow rate of 5 µl·min^{-1} as reference. The full scan data acquisition range was 100 to 1 500 Da.

RESULTS

The UPLC-QTOF-MS^E chromatogram of the ZSS extract

The total ions chromatogram (TIC) of saponins from the ZSS extract was investigated by UPLC-QTOF-MS^E technique in both positive and negative ESI modes. In each mode, the MS^E data was acquired in two separate functions simultaneously.

The first used low CE so that precursor ion information can be obtained; the second used high CE so that fragmentation data can be acquired. The TIC of the ZSS extract was assumably divided into flavonoids zone (Ⅰ) and saponins zone (Ⅱ) (Figure 2). In Table 1, the MS^E data for 1~13 detected in negative ESI mode was listed,

Table 1. Mass data for the 13 saponins detected in the ZSS extract by UPLC-QTOF-MS^E.

S/N	RT[a] (min)	[M – H] $^-$	Proposed formula	RDB[b]	Calculated Mass ([M – H] $^-$, *m/z*)	Error (ppm)	Possible compound
1	4.84	1385.6653	$C_{64}H_{106}O_{32}$	12	1385.6589	4.6	Protojujuboside A (Matsuda et al., 1999)
2	5.06	1223.6074	$C_{58}H_{96}O_{27}$	11	1223.6061	1.0	Protojujuboside B (Matsuda et al., 1999)
3	5.14	1223.6116	$C_{58}H_{96}O_{27}$	11	1223.6061	4.5	Jujuboside H (Wang et al., 2009)
4	5.21	1091.5657	$C_{53}H_{88}O_{23}$	10	1091.5638	1.6	3-*O*-*β*-D-glucopyranosyl-3*β*, 20S, 23S, 30- tetrahydroxydammar-24-en-16-on-3-*O*-*β*-D- glucopyranosyl-(1→3)-[*α*-L-rhamnopyranosyl- (1→2)]-*α*-L -arabinopyranoside
5	5.25	1223.6145	$C_{58}H_{96}O_{27}$	11	1223.6061	6.9	Protojujuboside B1 (Matsuda et al., 1999)
6	5.41	1061.5537	$C_{52}H_{86}O_{22}$	10	1061.5533	0.4	Jujuboside G (Wang and Yang, 2008)
7	6.13	1205.5944	$C_{58}H_{94}O_{26}$	12	1205.5955	- 0.9	Jujuboside A (OtsukaH et al., 1978)
8	6.15	1235.6172	$C_{59}H_{96}O_{27}$	12	1235.6061	9.0	Jujuboside C (OtsukaH et al., 1978)
9	6.31	1205.6016	$C_{58}H_{94}O_{26}$	12	1205.5955	5.1	Jujuboside A1 (Yoshikawa et al., 1997)
10	6.53	1043.5428	$C_{52}H_{84}O_{21}$	11	1043.5427	0.1	Jujuboside B (Yoshikawa et al., 1997)
11	6.68	1043.5490	$C_{52}H_{84}O_{21}$	11	1043.5427	6.0	Jujuboside B1 (Yoshikawa et al., 1997)
12	6.91	895.5071	$C_{47}H_{76}O_{16}$	10	895.5055	1.8	Jujubogenin-3-*O*-*α*-L-rhamnopyranosyl-(1→3)-[*α*- L-rhamnopyranosyl-(1→2)]-*α*-L-arabinopyranoside or Jujubogenin-3-*O*-α-L-rhamnopyranosyl-(1→3)- [*α*-D-fucopyranosyl-(1→2)]-*α*-L- arabinopyranoside
13	6.97	1085.5590	$C_{54}H_{86}O_{22}$	12	1085.5533	5.3	Acetyljujuboside B (Yoshikawa et al., 1997)

Notes: a RT, retention time. b RDB, ring and double-bond .

in which the proposed molecular formulas and possible compounds were given on the basis of retention time, calculated mass and information from reference (Niu et al., 2010). The dominant fragmentation pathways of authentic saponins were investigated in both positive and negative ESI modes. The MS^E data were obtained by collision-induced dissociation (CID) with low energy and high energy, respectively, and were available for the structural identification of compounds with similar fragmentation patterns. Through analyzing high-resolution mass data and comparing the retention times with those of the authentic standards, peaks 2 and 7 were unambiguously identified as protojujuboside B and jujuboside A, respectively.

UPLC-QTOF-MS^E investigation of peaks 1, 2, 4 and 5

In negative ESI mode with high CE, peak 2 (protojujuboside B) yielded characteristic ions [M – H – $C_{14}H_{24}O_7$ (304 Da)]$^-$ at *m/z* 919.4517 and a series of successive ions, which accounted for an important finding, namely, McLafferty-type even-electron rearrangements in negative ESI mode of TOF-MS. Through detailed analysis and literature review (Grossert et al., 2006), the mechanism of the rearrangement was deduced as seen in the following. With the loss of *γ*-hydrogen at C22, [M – H]$^-$ ions underwent a neutral loss of $C_{14}H_{24}O_7$ that involved *β*-cleavage between C17 and C20 and yielded [M – H – $C_{14}H_{24}O_7$]$^-$ ions. Furthermore, other ions confirmed that the successive fragmentation pathways were based on the initial rearrangement reaction (Figure 3). On the basis of MS^E data, the possible molecular formulas of compounds 1, 4 and 5 were deduced as $C_{64}H_{106}O_{32}$, $C_{53}H_{88}O_{23}$ and $C_{58}H_{96}O_{27}$ respectively and they underwent similar fragmentation pathways as 2.

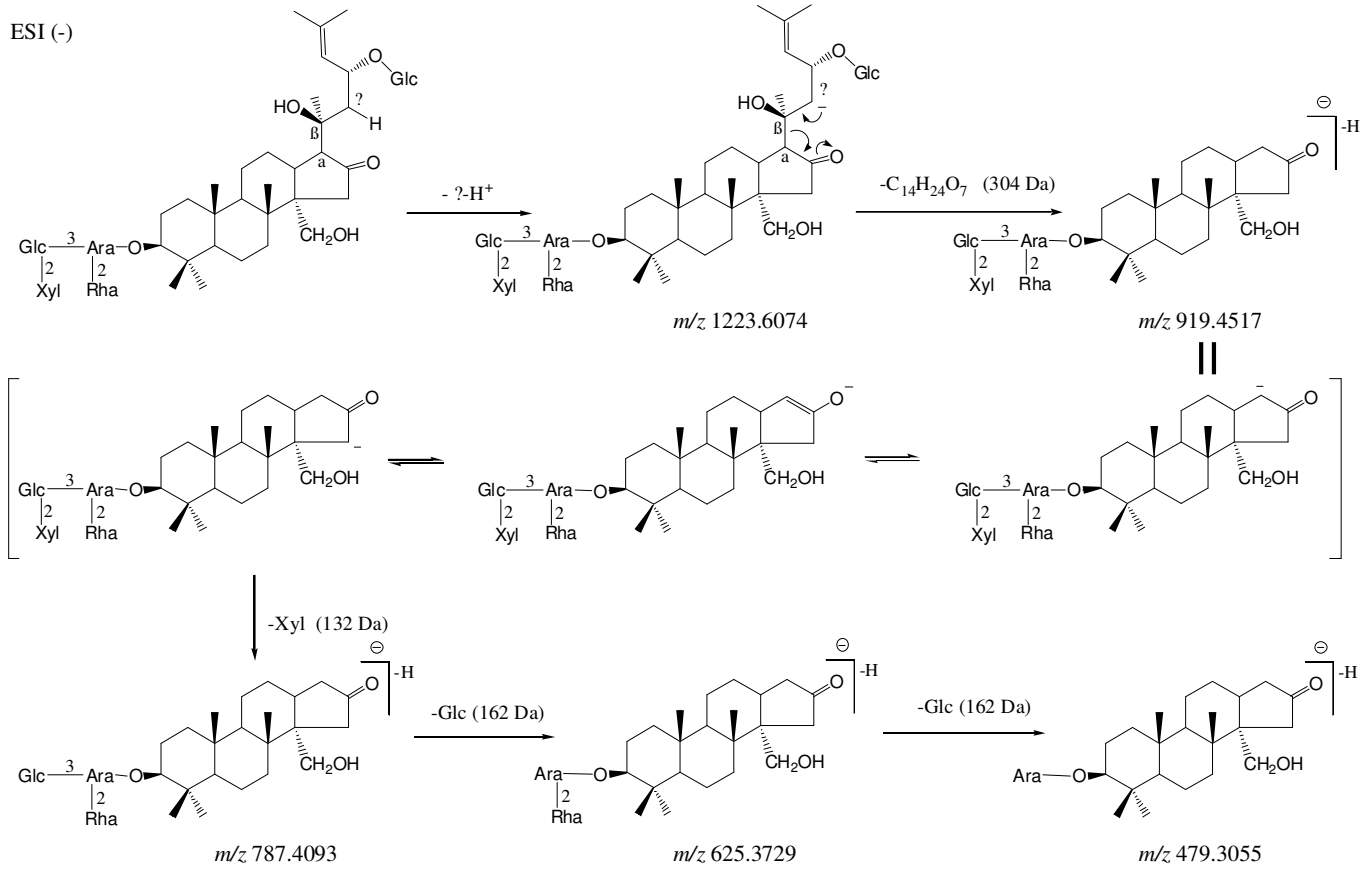


Figure 3. Proposed mechanism of McLafferty-type rearrangement and fragmentation pathways of peak 2 in negative ESI mode.

In positive ESI mode, they produced similar characteristic ions such as $[M + Na - C_{14}H_{24}O_7]^+$ at *m/z* 1105.5493, 943.4489, 811.4044 and 943.4362 respectively, suggesting that four peaks underwent similar fragmentation pathways in which McLafferty rearrangement occurred. After detailed analysis and literature review (Hsu and John, 2008; Yang et al., 2008; Li et al., 2006), the mechanism was proposed as follows. Take peak 2 for example, sodium adduct $[M + Na]^+$ ion at *m/z* 1247.5989 underwent a charge-remove fragmentation ($C_{14}H_{24}O_7$) loss that involved *β*-cleavage (between C17 and C20) with *γ*-hydrogen shift and yielded $[M + Na - C_{14}H_{24}O_7]^+$ ion at *m/z* 943.4489. This fragmentation pathway was considered as fragmentation pathway 1 of peak 2 in positive ESI mode.

While, in fragmentation pathway 2 of peak 2 in positive mode, the reaction started with the loss of 23-glucosyl moiety and one H_2O, yielding the ion at *m/z* 1045.5568. With one or more neutral loss of one H_2O, a conjugated 17, 22, 24-triene-16-one ion at *m/z* 1027.5520 was yielded. And successive ions at *m/z* 895.5219, 733.4604 and 455.3584, which were product ions of eliminations reaction occurred at sugar moiety, were observed.

On the basis of the previous analysis and according to Matsuda et al. (1999) compounds 1 and 5 were deduced as protojujuboside A and protojujuboside B_1. The structure of 4 was tentatively identified as 23-*O*-*β*-D-glucopyranosyl-3*β*, 20S, 23S, 30-tetrahydroxydammar-24- en-16-on-3-*O*-*α*-L-rhamnopyranosyl- (1→2)-*β*-D-glucopyranosyl-(1→3)-*α*-L–arabinopyranoside, uggesting it was an unknown compound. The structure and proposed fragmentation pathway of peak 4 in negative ESI mode was depicted in Figure 4.

UPLC-QTOF-MS^E investigation of peaks 3 and 6

The possible molecular formulas of 3 and 6 were given as $C_{58}H_{96}O_{27}$ and $C_{52}H_{86}O_{22}$ from Table 1. In negative mode with high CE, peaks 3 and 6 produced characteristic ions $[M - H - C_8H_{14}O_2 (142 Da)]^-$ at *m/z* 1081.5524 and *m/z* 919.4199 respectively, suggesting they underwent similar fragmentation pathways in which a McLafferty-type rearrangement occurred. In addition, successive ions elicited that other fragmentation pathways were based on the initial rearrangement reaction. The proposed fragmentation pathways of peak 6 are subsequently explained.

In positive ESI mode, peaks 3 and 6 produced similar characteristic ions such as $[M + Na - C_8H_{14}O_2 (142 Da)]^+$

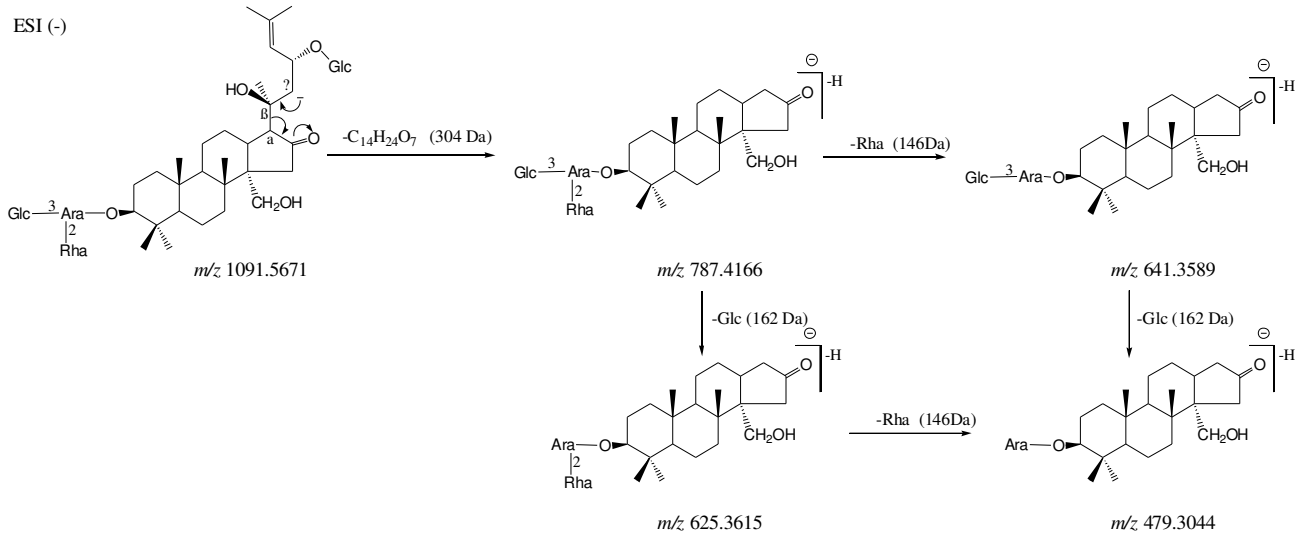


Figure 4. Proposed fragmentation pathway and characteristic ions of peak 4 in negative ESI mode.

at *m/z* 1105.5493 and 943.4489, respectively, suggesting that both peaks 3 and 6 underwent McLafferty rearrangement reaction. Take peak 6 for example, sodium adduct $[M + Na]^+$ ion at *m/z* 1085.5331 underwent a charge-remove fragmentation ($C_8H_{14}O_2$) loss that involved *β*-cleavage (between C17 and C20) with *γ*-hydrogen shift yielding $[M + Na - C_8H_{14}O_2]^+$ ion at *m/z* 943.5589. This fragmentation pathway was considered as fragmentation pathway 1 of peak 6 in positive ESI mode.

In fragmentation pathway 2 of peak 6 in positive ESI mode, there were ions at *m/z* 1063.5331, 1045.5243, 1027.5942, 895.4489, 749.4389, 733.4609, 587.3609 and 455.3529, which were product ions of cleavages without McLafferty rearrangement reaction. From the previous results, peak 6 was identified as jujuboside G.

In view of the chromatography data, it was concluded that there were similar characteristic ions and similar fragmentation pathways between peaks 3 and 6. In addition, peak 3 was isomer of 2 and 5. The characteristic $[M – H – C_8H_{14}O_2$ (142 Da)$]^–$ ion at *m/z* 1081 Da existed in peak 3 but do not existed in peaks 2 and 5, and $[M – H – C_{14}H_{24}O_7$ (304 Da)$]^–$ ion at *m/z* 919 Da existed in peaks 2 and 5 but do not existed in peak 3. Therefore, peak 3 was unambiguously discriminated from peaks 2 and 5. Therefore, peak 3 was identified as jujuboside H which shared the same aglycone skeleton with jujuboside G.

UPLC-QTOF-MS^E investigation of peaks 7, 8, 9, 10, 11 and 13

In positive ESI spectrum, peak 7 (jujuboside A) generated the $[M + H]^+$ ion at *m/z* 1207.6191 and $[M + Na]^+$ ion at *m/z* 1229.5970 in low CE. In high CE, the fragments ions at *m/z* 1121.5938, 587.3647, 455.3513 were observed. From the previous results, it was concluded that the cleavages of jujuboside A conformed to the rules as following. In positive mode, the fragmentation pathways began with a neutral loss of one H_2O (18 Da), producing an unstable transition in which a double bond between C20 and C22 was yielded; successively, with C16 to C17 single bond and C23–O(C16) single bone being broken, the unstable transition was immediately transformed to 17(E)-ebelin lactone derivatives coupled with 17(Z)-ebelin lactone derivatives (ions $[M + H – H_2O]^+$) via RDA cleavage (Kumar et al., 2009) (Figure 5). Eventually, 17(E)-ebelin lactone and 17(Z)-ebelin lactone were yielded and showed an intense peak at *m/z* 455 Da.

The possible molecular formulas of peak 8, 9, 10, 11 and 13 were $C_{59}H_{96}O_{27}$, $C_{58}H_{94}O_{26}$, $C_{52}H_{84}O_{21}$, $C_{52}H_{84}O_{21}$ and $C_{54}H_{86}O_{22}$. Peaks 8, 9, 10, 11 and 13 underwent similar fragmentation pathways to jujuboside A, suggesting they shared the same aglycone skeleton with jujuboside A. According to the literature review and structural analysis (Otsuka et al., 1978; Yoshikawa et al., 1997; Matsuda et al., 1999), it was easily deduced that peaks 8, 9, 10, 11 and 13 were identified as jujuboside C, jujuboside A1, jujuboside B, jujuboside B1 and acetyljujuboside B, respectively.

UPLC-QTOF-MS^E investigation of peaks 12

On the basis of Table 1, the possible molecular formula of 12 was $C_{47}H_{76}O_{16}$. In negative ESI mode, the predominant ions at *m/z* 749.4473 $[M – H – 146$ Da$]^–$, 603.4095 $[M – H – 146$ Da $– 146$ Da$]^–$, 471.3271 $[M – H – 146$ Da $– 146$ Da$–$ Ara$]^–$ appeared (Figure 6).

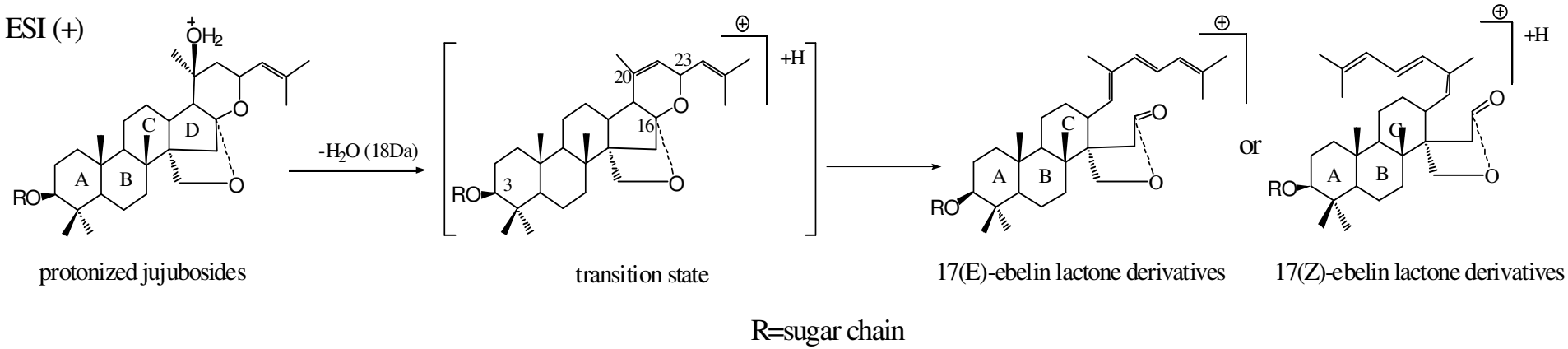


Figure 5. Proposed mechanism of RDA cleavage occurred in fragmentation pathways of jujubosides in positive ESI mode.

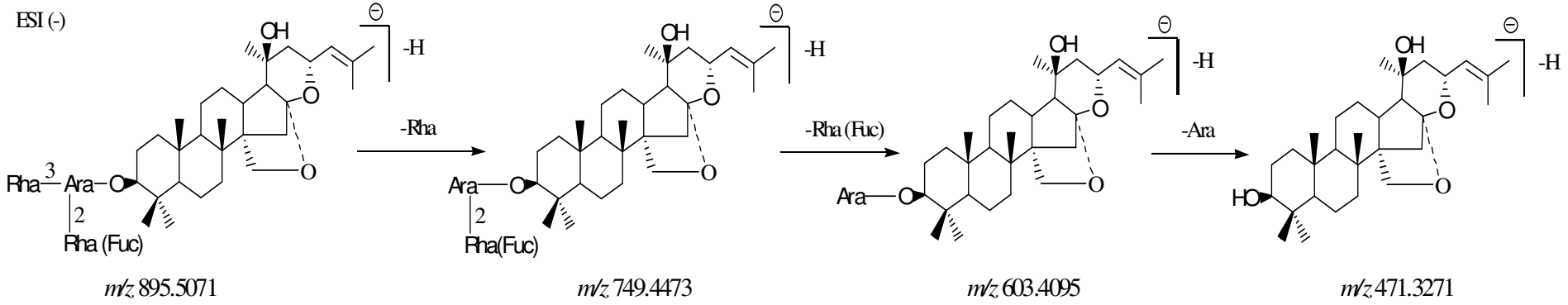


Figure 6. Proposed fragmentation pathways and characteristic ions of peak 12 in negative ESI mode.

In positive high CE mode, a series of product-ions, initiated by RDA cleavage, at *m/z* 879.5165 $[M + H - H_2O]^+$, 733.2313 $[M + H - H_2O - 146$ Da$]^+$, 587.3963 $[M + H - H_2O - 146$ Da $- 146$ Da$]^+$ and 455.3510 $[M + H - H_2O - 146$ Da $- 146$ Da $- 132$ Da$]^+$ were seen. On the basis of above deductions and comparing to reference [6], the chemical structure of peak 12 has been tentatively determined as jujubogenin-3-O-α-L-rhamnopyranosyl (1→3)-[α-L- rhamnopyranosyl-(1→2)]-α-L-arabinopyranoside or jujubogenin-3-*O*-α-L-rhamnopyranosyl- (1 3)-[*α*-D-fucopyranosyl (1→2)]-*α*-L-arabinopyranoside.

DISCUSSION

Above all, eleven known saponins and two

unknown saponin were identified in ZSS utilizing UPLC-ESI-QTOF-MSE in both positive and negative ESI mode. Due to the availabilities of MSE data provided by the advanced instrument of UPLC-ESI-QTOF-MSE, a great deal of fragments information was available to account for the principles of RDA cleavage and McLafferty-type (and McLafferty) rearrangement, which were firstly utilized in the proposed fragmentation pathways of ZSS's saponins. Compounds 1~6 are keto-dammarane type triterpene saponin and 7~13 are dammarane type triterpene saponin. In negative ESI mode, compounds 1~6, which underwent McLafferty-type rearrangement reaction, had common characteristic ions $[M - H - C_{14}H_{24}O_7$ (304 Da)$]^-$ or $[M - H - C_8H_{14}O_2$ (142 Da)$]^-$ as well as successive ions. In positive ESI mode, they had characteristic ions $[M + Na - C_{14}H_{24}O_7]^+$ or $[M + Na - C_8H_{14}O_2]^+$, which triggered by McLafferty rearrangement. In positive ESI mode, compounds 7~13 yielded common characteristic ions $[M + H - H_2O]^+$ and successive ions due to RDA cleavage.

Contrasting to HPLC, UPLC can rapidly separate complex components due to its high efficiencies. UPLC coupled with Acquity UPLC HSS T3 column (100 × 2.1 mm, 1.8 μm) can provide significant improvements in resolution, speed and sensitivity. In addition, QTOF-MS^E can provide accurate molecular weights of multi-stage fragments with single injection. On the basis of the previous merits, the combination of UPLC and QTOF-MS^E dramatically shorten experimental time and enhance sensitivity. In summary, UPLC-ESI-QTOF-MS^E has been proved to be an effective, sensitive, selective, rapid and guided method for compound identification, especially for phytochemical research which is of trace amounts.

Abbreviations: CE, Collision energy; **CID,** collision-induced dissociation; **EIC,** extracted ions chromatogram; **RDA,** retro Diels-Alder; **TCM,** traditional Chinese medicines; **ZSS,** *Ziziphi spinosae* Semen; **TIC,** total ions chromatogram.

ACKNOWLEDGEMENTS

This work was financially supported by the National Natural Science Foundation of China (No. 30472202).

REFERENCES

Bao KD, Li P, Li HJ, Qi LW (2009). Simultaneous determination of flavonoids and saponins in Ziziphi spinosae semen(Suanzaoren) by high performance liquid chromatography with evaporative light scattering detection. Chin. J. Nat. Med., 7: 47-53.

Grossert JS, Cook MC, White RL (2006). The influence of structural features on facile McLafferty-type, even-electron rearrangements in tandem mass spectra of carboxylate anions. Rapid Commun. Mass Spectrom, 20(10): 1511-1516.

Hsu FF, John Turk (2008). Elucidation of the double-bond position of long-chain unsaturated fatty acids by multiple-stage linear ion-trap mass spectrometry with electrospray ionization. J. Am. Soc. Mass Spectrom, 19: 1673-1680.

Kumar N, Bhandari P, Singh B, Bari SS (2009). Antioxidant activity and ultra-performance LC-electrospray ionization-quadrupole time-of-flight mass spectrometry for phenolics-based fingerprinting of Rose species: Rosa damascena, Rosa bourboniana and Rosa brunonii. Food Chem. Toxicol., 47(2): 361-367.

Li R, Zhou Y, Wu ZJ (2006). Ding LS. ESI-QqTOF-MS/MS and APCI-IT-MS/MS analysis of steroid saponins from the rhizomes of Dioscorea panthaica. J. Mass Spectrom, 41: 1-22.

Li YJ, Bi KS (2006). Study on the Therapeutic material basis of traditional Chinese medicinal preparation suanzaoren decoction. Chem. Pharm. Bull., 54: 847-851.

Liu J, Chen B, Yao S (2007). Simultaneous analysis and identification of main bioactive constituents in extract of Zizyphus jujuba var. sapinosa (Ziziphi spinosae semen) by high-performance liquid chromatography-photodiode array detection-electrospray mass spectrometry. Talanta, 71: 668-675.

Matsuda H, Murakami T, Ikebata A, Yamahara J, Yoshikawa M (1999). Bioactive saponins and glycosides. XIV.1) structure elucidation and immunological adjuvant activity of novel protojujubogenin type triterpene bisdesmosides, protojujubosides A, B, and B1, from the seeds of Zizyphus jujuba var. spinosa (*Zizyphi spinosi* semen). Chem. Pharm. Bull., 47: 1744-1748.

Niu CY, Wu CS, S YX, Zhang JL (2010). Identification and characterization of flavonoids from semen Zizyphi spinosae by high–performance liquid chromatography/linear ion trap FTICR hybrid mass spectrometry. J. Asian Nat. Prod. Res., 12: 300-312.

OtsukaH, Akiyama T, Kawai K (1978). The structure of jujubosides A and B, the saponins isolated from the seeds of Zizyphus jujube. Phytochemistry, 17: 1349.

Pharmacopoeia Commission of PRC (Eds.) (2005). Pharmacopoeia of the People's Republic of China (English edition). Chemical Industry Press, P. 254.

Wang JZ, Chen XB, Ye LM (2009). Chemical constituents in seeds of Zizyphus jujubavar spinosa. Chin. Trad. Herb Drugs, 40: 1534-1536.

Wang JZ, Yang JS (2008). Structural elucidation of triterpene saponins from the seeds of Zizyphus jujube Mili. Chin. J. Organ Chem., 28: 69-71.

Wang LE, Cui XY, Cui SY, Cao JX, Zhang J, Zhang YH, Zhang QY, Bai YJ, Zhao XY (2010). Potentiating effect of spinosin, a C–glycoside flavonoid of Ziziphi spinosae semen, on pentobarbital–induced sleep may be related to postsynaptic 5–HT_{1A} receptors. Phytomedicine, 17: 404-409.

Wang WJ, Luo JG, Kong LY (2009). HPLC–ESI–MS^n analysis of chemical constituents in Ziziphi Spinosae Semen. China J. Chin. Mater. Med., 34: 2769-2773.

Yang SZ, Liu XY, Mu BZ (2008). The McLafferty rearrangement in the glu residue in a cyclic lipopeptide determined by Q-TOF MS/MS. J. Mass Spectrom, 43: 1673-1678.

Yoshikawa M, Murakami T, Ikebata A, Wakao S, Murakami N, Matsuda H, Yamahara J (1997). Bioactive saponins and glycosides. X. on the constituents of Zizyphi spinosi semen, the seeds of Zizyphus jujube Mill. var. spinosea Hu (1): structures and histamine release-inhibitory effects of jujubosides A1 and C and acytyljujuboside B. Chem. Pharm. Bull., 45: 1186–1192.

You ZL, Xia Q, Liang FR, Tang YJ, Xu CL, Huang J, Zhao L, Zhang WZ, He JJ (2010). Effects on the expression of $GABA^A$ receptor subunits by jujuboside A treatment in rat hippocampal neurons. J. Ethnopharmacol., 128: 419-423.