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JMS Letters

Dear Sir,

Electrospray tandem mass spectrometry of longipedlactone triterpenoids

Triterpenoids are a class of important natural product and are widely found in Chinese herb. Some of triterpenoids exhibit biological properties such as antiandrogenic,[1] antihepatitis B,[2] antitumor,[3,4] antioxidant,[5] anticomplement,[6] antimicrobial,[7] anti-HIV[8,9] and angiotensin converting enzyme-inhibitory activities.[10] The potential application brings the need for reliable, fast and low-cost analysis of this class of compounds.

Mass spectrometry (MS), especially tandem MS, has been one of the important physicochemical methods for the identification of trace natural products due to it rapidity, sensitivity, and low levels of sample consumption.[11–14] Many triterpenoids have been rapidly analyzed using electrospray ionization (ESI)-MS or high-performance liquid chromatography (HPLC)-ESI-MS.[15–18]

In our laboratory, a series of triterpenoids possessing a unique skeleton, that is longipedlactone (Fig. 1), were isolated from the stems and leaves *Kadsura longipedunculata*.[9,19,20] Some of them showed significant cytotoxicity and anti-HIV activity.[9,19] The potential application prospect and unique skeleton prompted us to study the structural characterization of this series of compounds using MS. To our knowledge, these compounds have not been studied by electrospray tandem MS. To obtain sufficient information on the structure elucidation of this class of compounds, such as their degradation products, metabolites and biosynthesis intermediates, the detailed fragmentation patterns of longipedlactone triterpenoids were studied using ESI-quadrupole time-of-flight (QTOF) QTOF-MS/MS in both positive- and negative-ion modes.

HPLC-grade methanol was purchased from Fisher Scientific (Pittsburgh, PA). The deuteration of hydrogen on hydroxyl of compound **3** was carried out by incubation of 0.01-mg compound **3** in 2 ml CD_3OD for 24 h. MS experiments were performed on a Bruker micrOTOF Q mass spectrometer in both positive- and negative-ion modes. Accurate masses of product ions were determined by external mass calibration using the mass calibrants of molecular weight (MW) 322.0481, 622.0290 and 922.0098 in the positive mode and of MW 431.9823, 601.9790 and 1033.9870 in the negative-ion mode. Helium gas was used as collision gas and high-purity nitrogen gas as nebulizer and dry gas at a pressure of 30 psi. The sample introduction rate was 115 μl/h. The ESI source conditions were as follows: Capillary V, −4500 V (positive), 4000 V (negative); End Plate voltage, −4000 V (positive), 3500 V (negative); Capillary Exit Voltage, 120 V; and the dry gas temperature, 150 °C. The Collision Energy was optimized to achieve sufficient fragmentation.

For the low-energy collision MS/MS analysis, the precursor sodiated molecular ions, $[M+Na]^+$, were selected and the product ions were recorded by ESI-QTOF-MS/MS. Major fragmentation pathways of $[M+Na]^+$ at *m/z* 517 for longipedlactone F (**1**) are shown in Scheme 1(a) and (b). The accurate masses of product ions are shown in Table 1. A tandem McLafferty-type rearrangement plays a significant role in the skeleton fragmentation. The McLafferty-type rearrangement might involve even-electron ions.[21,22] The product ion at *m/z* 365 (Fig. 2(a)) from *m/z* 517 was formed possibly by the fragmentation pathway. In the MS/MS spectrum of longipedlactone H (**5**) (Supporting Information) possessing the same structure of ring B as that of compound **1**, the product ion at *m/z* 383 was generated from the precursor ion at *m/z* 535 likely by the tandem McLafferty-type rearrangement. A labeling experiment involving H/D exchange of compound **1** was carried out and corresponding MS/MS spectrum was shown in Fig. 2(b). The observation of product ion at *m/z* 365 from *m/z* 519 for deuterated longipedlactone F (**1**) indicated the rationality of proposed fragmentation mechanisms. Interestingly, the product ion at *m/z* 365 with high abundance was observed in the MS/MS spectrum of the precursor $[M+Na]^+$ at *m/z* 533 for longipedlactone G (**3**) (Fig. 3), although the structure of ring B is different from that of compounds **1** and **5**. The process of forming the product ion at *m/z* 365 from the precursor ion at *m/z* 533 might include hexagonal H rearrangement and McLafferty-type rearrangement (Scheme 1(c)). The product ion at *m/z* 395 was produced possibly by sequential hexagonal H rearrangement from *m/z* 533 (Scheme 1(c)). This might indicate the structural characteristic of ring B. The neutral losses of H_2O and CO_2 molecules are important fragmentation patterns for longipedlactone triterpenoids. The product ions in the high mass range are formed by these neutral losses or their combinations. However, the related abundance of product ions from the same fragmentation pathways varied for different longipedlactone triterpenoids. It seemed that the product ions resulting from loss of H_2O show higher related abundance than that resulting from loss of CO_2, if the new double bond generated from loss of H_2O can form conjugation system with original double bond. On the contrary, high-abundance fragment ions produced by loss of CO_2 were observed. Incidentally, the loss of a H_2O molecule from the precursor $[M+Na]^+$ for compounds **1**, **3** and **5** might experience two different pathways (Scheme 1(b)), which indicates the structural characteristic of ring B. The product ion at *m/z* 499 resulting from *m/z* 519 by loss of D_2O in the MS/MS spectrum of deuterated longipedlactone F (**1**) (Fig. 2(b)) supports the fragmentation pathways. The product ion at *m/z* 267 was yielded by the cleavage of ring C for compound **1** (Scheme 1(a)). The product ion resulting from the fragmentation pattern can be observed for compounds **1**–**4**. However, it is neglectable for compounds **5**–**7**. The reason might be that the product ion from the precursor $[M+Na]^+$ for compounds **1**–**4** possesses more conjugation units. The product ion at *m/z* 133 observed in the MS/MS spectrum of Longipedlactone F (**1**) should indicate the structure of ring E (Fig. 1(a)). It seemed that the hydroxyl linked with C-a influence the fragmentation pathways. The product ion at *m/z* 133 can be observed for compounds **1**–**4** and becomes neglectable for compounds **5**–**7**. In addition, the cleavage of ring E is read to occur for compounds **1** and **2**. For example, the product ion at *m/z* 397 was formed from *m/z* 481 by the cleavage of ring E (Scheme 1(a)). However, the fragmentation can not occur for compounds **3**–**7**. Major fragmentation mechanisms mentioned above were supported by D-labeling experiments (Fig. 2(b)).

In the negative-ion mode, the deprotonated molecular ions, $[M-H]^-$, were selected as the precursor ions for the product ion scan. The loss of CO_2 is the main fragmentation pattern. The product ion at *m/z* 139 shows high abundance in the MS/MS spectra of the precursor $[M-H]^-$ for compounds **5**–**7** (Fig. 4 and Supporting Information). The process might go through octagonal H rearrangement (Scheme 2). The H atom should be active H on hydroxyl linked with C-a. A D-labeling experiment of compound **6** was carried out and corresponding MS/MS spectrum was shown in Fig. 3 in the insert. The product ion at *m/z* 140 in the MS/MS spectrum of deuterated compound **6** supports the proposed fragmentation mechanisms. For compounds **4** and **7**, the product ion at *m/z* 151 was observed and corresponding fragmentation mechanisms were proposed (Supporting Information). The product ion indicates the characteristic of ring B.

Notably, these longipedlactone triterpenoids studied include two pairs of isomers, that is, compounds **1**, **4** and compounds **5, 7**. Each pair of isomers can be unambiguously differentiated based on MS/MS spectra. For compounds **1** and **4**, in positive-ion mode, tandem McLafferty-type rearrangement, the cleavage of ring E and loss of H_2O are the main fragmentation patterns for compounds **1**. However, the product ion resulting from loss of a CO_2 molecule shows very high abundance and tandem McLafferty-type rearrangement can not occur for compound **4**. In negative-ion mode, the loss of CO_2 is the main fragmentation pattern

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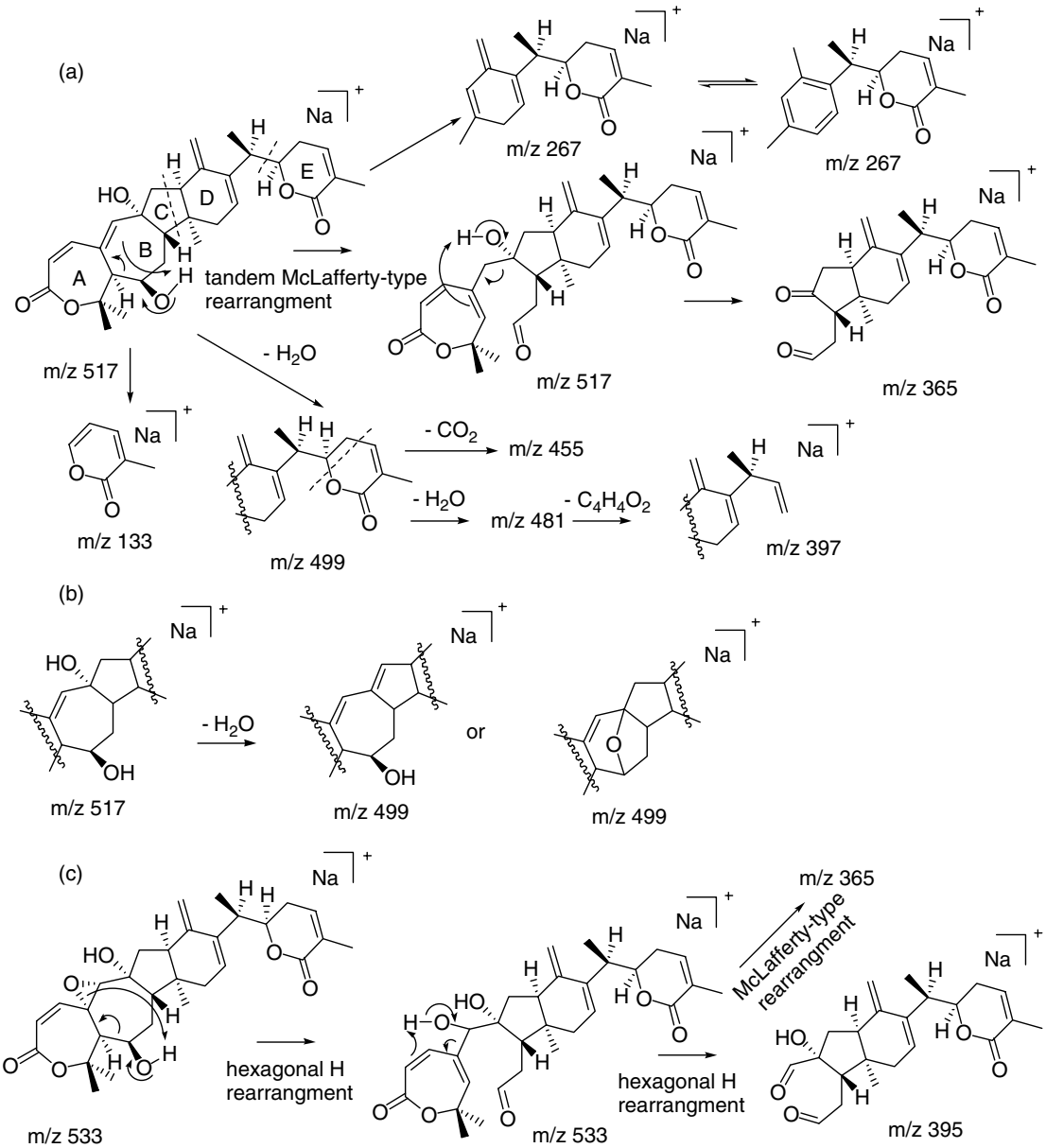
Table 1. Elemental constituents of major product ions from $[M+Na]^+$ for Longipedlactone F (**1**) (collision energy: 35 eV)

Fragment ion	Formula	Calculated	Observed	Error (ppm)
$[M+Na]^+$	$C_{30}H_{38}O_6Na$	517.2561	517.2559	0.4
$[M+Na-H_2O]^+$	$C_{30}H_{36}O_5Na$	499.2455	499.2456	0.2
$[M+Na-H_2O-H_2O]^+$	$C_{30}H_{34}O_4Na$	481.2349	481.2334	3.1
$[M+Na-H_2O-CO_2]^+$	$C_{29}H_{36}O_3Na$	455.2557	455.2542	3.3
$[M+Na-H_2O-H_2O-CO_2]^+$	$C_{29}H_{34}ONa$	437.2451	437.2452	0.2
$[M+Na-H_2O-H_2O-C_4H_4O_2]^+$	$C_{26}H_{30}O_2Na$	397.2138	397.2116	5.5
$[342+Na]^+$ (tandem Mclafferty-type rearrangement)	$C_{21}H_{26}O_4Na$	365.1723	365.1718	1.3
$[244+Na]^+$ (cleavage of ring C)	$C_{16}H_{20}O_2Na$	267.1355	267.1349	2.2
$[110+Na]^+$	$C_6H_6O_2Na$	133.0260	133.0268	6.0



Scheme 1. (a) Major fragmentation patterns of $[M+Na]^+$ for Longipedlactone F (**1**); (b)Possible fragmentation patterns of loss of H_2O from *m/z* 517 for compound **1**; (c) Partial fragmentation patterns of $[M+Na]^+$ for Longipedlactone G (**3**).

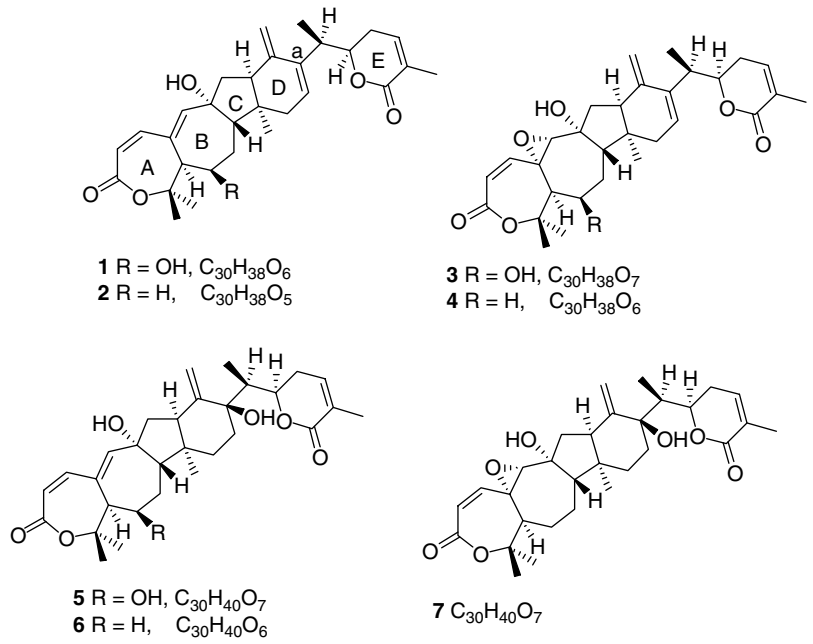


Figure 1. Longipedlactones: Longipedlactone F (**1**, M_r 494.2668); Longipedlactone A (**2**, M_r 478.2719); Longipedlactone G (**3**, M_r 510.2618); Longipedlactone D (**4**, M_r 494.2668); Longipedlactone H (**5**, M_r 512.2774); Longipedlactone C (**6**, M_r 496.2825); Longipedlactone I (**7**, M_r 512.2774).

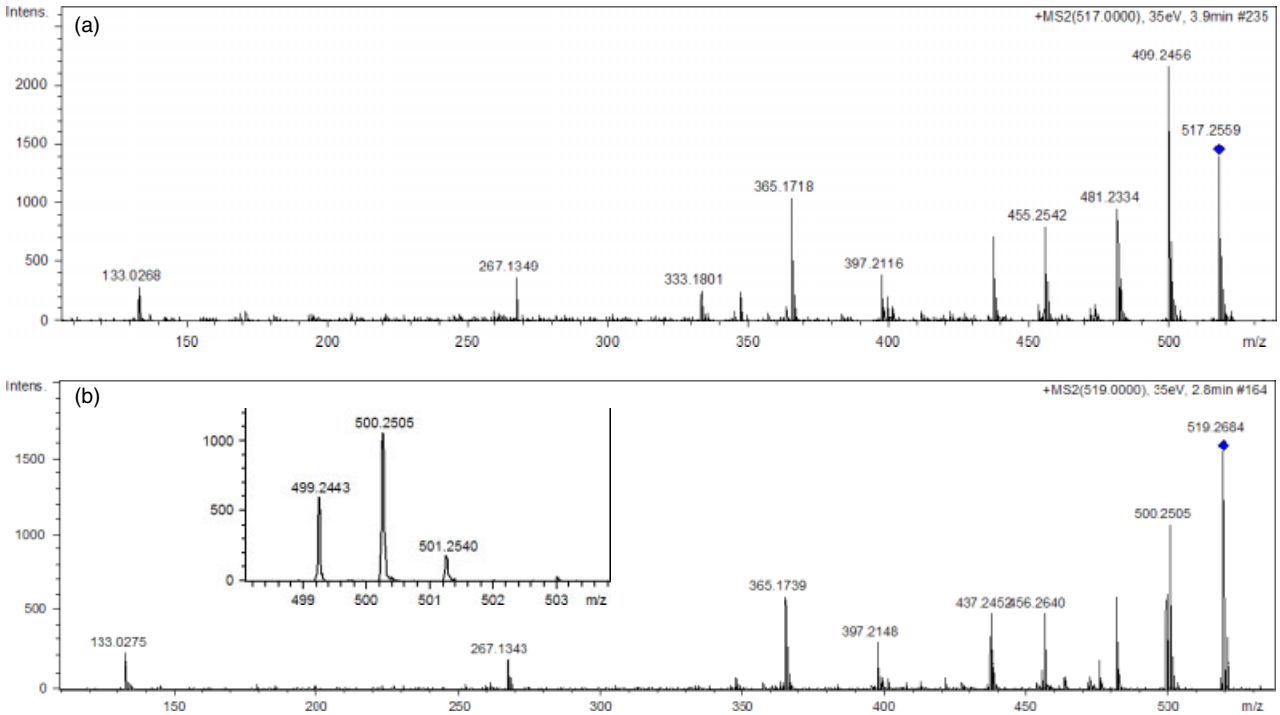


Figure 2. Product ion scan of the selected precursor $[M+Na]^+$ at *m/z* 517 for (a) Longipedlactone F (**1**) (collision energy: 35 eV) and (b) deuterated Longipedlactone F (**1**) (collision energy: 35 eV).

for compound **1**. In addition to loss of CO_2, the high-abundance product ion at *m/z* 151 is observed for compound **4**. For compounds **5** and **7**, in positive-ion mode, tandem McLafferty-type rearrangement is read to occur for compound **5** and not for compound **7**. In negative-ion mode, fragment ion at *m/z* 151 is observed in the MS/MS spectrum of compound **7** and not in that of compound **5**.

CID-fragmentation pathways of seven representative longipedlactone triterpenoids were elucidated using ESI-QTOF-MS/MS in both positive- and negative-ion modes. Tandem McLafferty-type rearrangement in positive-ion mode and octagonal H rearrangement in negative-ion mode are of great scientific interest. Interestingly, hexagonal H rearrangement occurs readily, even if double bond is substituted by epoxy. The characteristic product ions and fragmentation patterns indicate the structural characteristic of rings B, D and E. For example, tandem McLafferty-type rearrangement and loss of H_2O in positive-ion mode and the product ion at *m/z* 151 in negative-ion mode indicate the characteristic of ring B. The cleavage of ring C in positive-ion mode indicates the characteristic of ring D. The product ion at *m/z* 133 in positive-ion mode and octagonal H

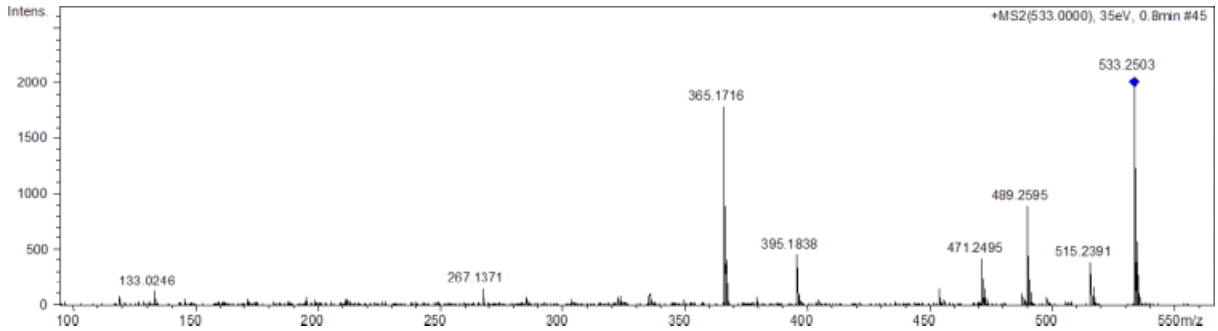


Figure 3. Product ion scan of the selected precursor $[M+Na]^+$ at *m/z* 533 for Longipedlactone G (**3**) (collision energy: 35 eV).

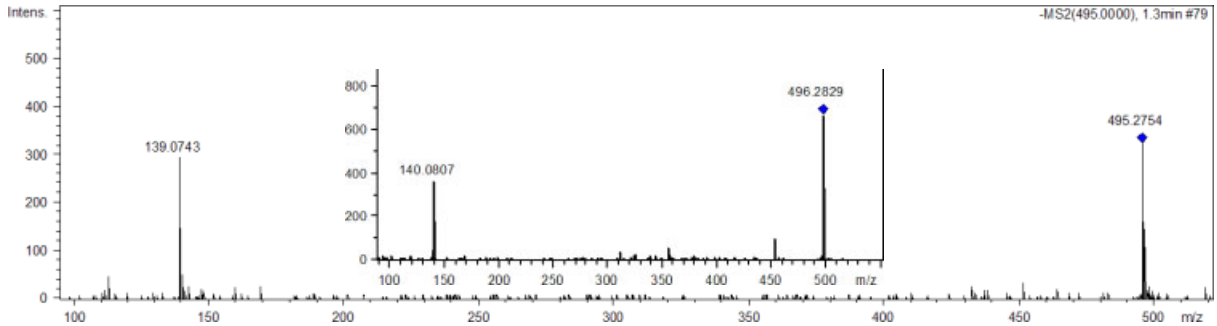
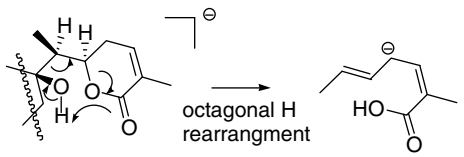


Figure 4. Product ion scan of the selected precursor $[M-H]^-$ at *m/z* 495 for Longipedlactone C (**6**) (collision energy: 20 eV). Product ion scan for deuterated Longipedlactone C (**6**) is shown in the insert (collision energy: 20 eV).



5 m/z 511
6 m/z 495
7 m/z 511

5, 6, 7 m/z 139

Scheme 2. Major fragmentation patterns of $[M-H]^-$ for compounds **5**–**7**.

rearrangement in negative-ion indicate the characteristic of rings D and E. In addition, two pairs of isomers were unambiguously differentiated based on MS/MS spectra. In summary, complementary information obtained from fragmentation experiments of $[M+Na]^+$ and $[M-H]^-$ precursor ions is especially valuable for rapid identification of this kind of triterpenoids.

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Supporting information

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

Yours,

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