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ARTICLE *in* PHYTOCHEMICAL ANALYSIS · JULY 2010

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Received: 23 September 2009, Revised: 31 December 2009, Accepted: 14 January 2010 Published online in Wiley Interscience: 23 March 2010

(www.interscience.wiley.com) DOI 10.1002/pca.1209

Analysis of sesterterpenoids from *Aspergillus terreus* using ESI-QTOF and ESI-IT

Zhi-Jun Wu,[a,b] Dong-Mei Fang,[a] Dan Han,[c] Guo-You Li,[a] Xiao-Zhen Chen,[a] Hua-Yi Qi[a] and Guo-Lin Zhang[a]*

ABSTRACT:

Introduction – Biosynthesis of terretonin was studied due to the interesting skeleton of this series of sesterterpenoids. Very recently, López-Gresa reported two new sesterterpenoids (terretonins E and F) which are inhibitors of the mammalian mitochondrial respiratory chain. Mass spectrometry (MS), especially tandem mass spectrometry, has been one of the most important physicochemical methods for the identification of trace natural products due to it rapidity, sensitivity and low levels of sample consumption. The potential application prospect and unique skeleton prompted us to study structural characterisation using MS.

Objective – To obtain sufficient information for rapid structural elucidation of this class of compounds using MS.

Methodology – The elemental composition of the product ions was confirmed by low-energy ESI-CID-QTOF-MS/MS analyses. The fragmentation pathways were postulated on the basis of ESI-QTOF-MS/MS/MS and ESI-IT-MS^n spectra. Common features and major differences between ESI-QTOF-MS/MS and IT-MS^n spectra were compared. For ESI-QTOF-MS/MS/MS experiments, capillary exit voltage was raised to induce in-source dissociation. Ammonium acetate or acetic acid were added into solutions to improve the intensity of $[M + H]^+$. The collision energy was optimised to achieve sufficient fragmentation. Some fragmentation pathways were unambiguously proposed by the variety of abundance of fragment ions at different collision energies even without MS^n spectra.

Results – Fragmentation pathways of five representative sesterterpenoids were elucidated using ESI-QTOF-MS/MS/MS and ESI-IT-MS^n in both positive- and negative-ion mode. The key group of characterising fragmentation profiles was ring B, and these fragmentation patterns are helpful to identify different types of sestertepenoids.

Conclusion – Complementary information obtained from fragmentation experiments of $[M + H]^+$ (or $[M + NH_4]^+$) and $[M - H]^-$ precursor ions is especially valuable for rapid identification of this kind of sesterterpenoid. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: sesterterpenoids; terretonin; fragmentation; ESI-MS

Introduction

In recent years, sesterterpenoids have been proved to be a potential source of drugs. Some sesterterpenes exhibit biological properties such as anti-inflammatory (Kikuchi *et al.*, 1983), cytotoxic (Charan *et al.*, 2002; Renner *et al.*, 1998; Rueda *et al.*, 1997; Tsuchiya *et al.*, 1998), antifeedant (Terem and Scheuer, 1986), inhibition of platelet aggregation (Kazlauskas *et al.*, 1982) and antimicrobial effects (Bowden *et al.*, 1992). The potential application prospects raise the need for reliable and, if possible, fast and low-cost analysis of this class of compounds.

Because the structural information provided by UV and IR spectroscopy is limited, nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography analysis have become important methods for structural identification of sesterterpenoids. However, NMR and X-ray crystallographic analyses require laborious purification and the production of monocrystals, respectively. This is a challenge to analytical chemists. Mass spectrometry (MS), especially tandem mass spectrometry, has been one of the most important physicochemical methods for identification of trace natural products due to it rapidity, sensitivity and low levels of sample consumption (Wu *et al.*, 2007, 2008, 2009).

Recently, a series of sesterterpenoids (Fig. 1) were isolated from *Aspergillus terreus* in our laboratory (Li *et al.*, 2005). The differences between these compounds mainly focus on ring B. Biosynthesis of terretonin has been studied due to its interesting skeleton (McIntyre and Simpson, 1981; McIntyre *et al.*, 1982, 1989a, b). EI-MS was reported for the analysis of terretonin (**2**) (Fig. 1), but the analysis was very simple (Springer *et al.*, 1979). Very recently, López-Gresa reported two new sesterterpenoids (terretonins E and F) which inhibited the mammalian mitochondrial respiratory chain (López-Gresa *et al.*, 2009). The potential application prospect and unique skeleton prompted us to study the structural characterisation of this series of compounds

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using MS. Electrospray ionisation (ESI) is one of softest ionisation techniques and has been successfully applied in various fields. To our knowledge, ESI-MS has not been applied for characterisation of these compounds except for determination of the molecular weight (Li *et al.*, 2005; Nielsen and Smedsgaard, 2003). To obtain sufficient information for the structural elucidation of this class of compounds, such as their degradation products, metabolites and biosynthesis intermediates, the detailed fragmentation pathways of these sesterterpenoids were studied using ESI-QTOF-MS/MS/MS and ESI-IT-MS^n in both positive- and negative-ion modes.

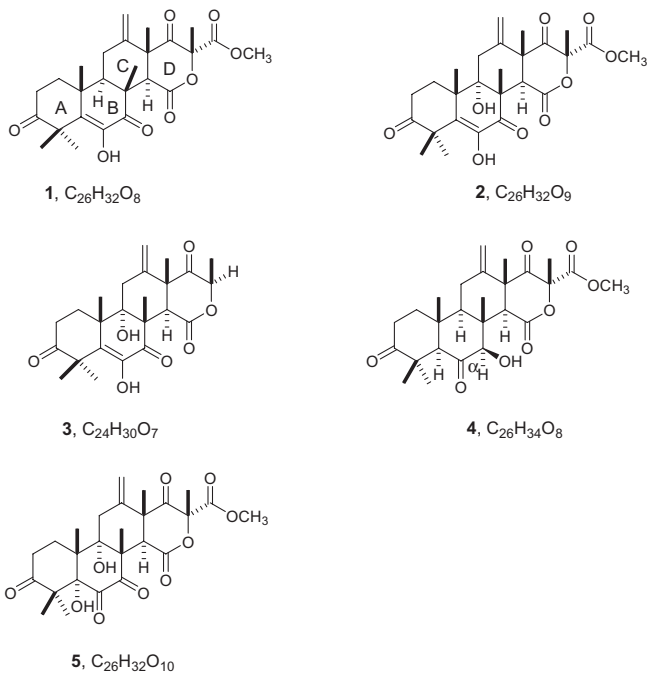


Figure 1. Sesterterpenoids: terretonin A (**1**, *M*, 472.2097); terretonin (**2**, *M*, 488.2046); terretonin C (**3**, *M*, 430.1991); terretonin D (**4**, *M*, 474.2254); terretonin B (**5**, *M*, 504.1956).

Experimental

Chemicals and samples

HPLC-grade methanol was purchased from Fisher Scientific (Pittsburgh, PA, USA), acetic acid from Sigma-Aldrich (Buchs, Switzerland) and ammonium acetate from Guoyao (Shanghai, China). The sesterterpenoids were isolated from ethyl acetate extract of a solid-state fermented culture of *Aspergillus terreus* (Li *et al.*, 2005).

Apparatus

High-resolution experiments were performed on a Bruker BioTOF-Q mass spectrometer (Billerica, MA, USA) in both positive- and negative-ion modes. Accurate masses of fragment ions were determined by external mass calibration using the mass calibrants of 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. Another injector was used to introduce the mass calibrants into MS, shortly after introducing the samples. High-purity nitrogen gas at a pressure of 30 psi was used as collision, nebuliser and auxiliary heated gas. The samples introduction rate was 115 µL/h. The ESI source conditions were as follows: capillary voltage, –4500 V (positive), 4000 V (negative); end plate voltage, –4000 V (positive), 3500 V (negative); capillary exit voltage, 100–160 V; and dry gas temperature, 150°C. For MS/MS/MS experiments, capillary exit voltage was raised to induce in-source dissociation. Ammonium acetate were added into solutions to improve the intensity of $[M + H]^+$ except for terretonin B. The collision energy was optimised to achieve sufficient fragmentation.

ESI-MS^n spectra were acquired using a Finnigan LCQ^{DECA} ion-trap mass spectrometer (San Jose, CA, USA) equipped with an ESI source. The samples were introduced via a syringe pump at a flow rate of 10 µL/min and the spray voltage was –5.0 kV in the positive mode, while the capillary voltage was 4.0 V and the temperature was at 350°C.The ion gauge pressure was 2.4×10^{-5} Torr. Nitrogen was used as a sheath gas at a pressure of 100 psi and helium was used as the buffer gas. Acetic acid were added into solutions to improve the intensity of $[M + H]^+$ except for terretonin B.

Results and Disccussion

Positive-ion ESI-MS

Terretonin A (1). The protonated molecule, $[M + H]^+$ at *m/z* 473, was selected as the precursor ion for the product ion scan. ESI-QTOF-MS/MS spectra were selected for detailed analysis due

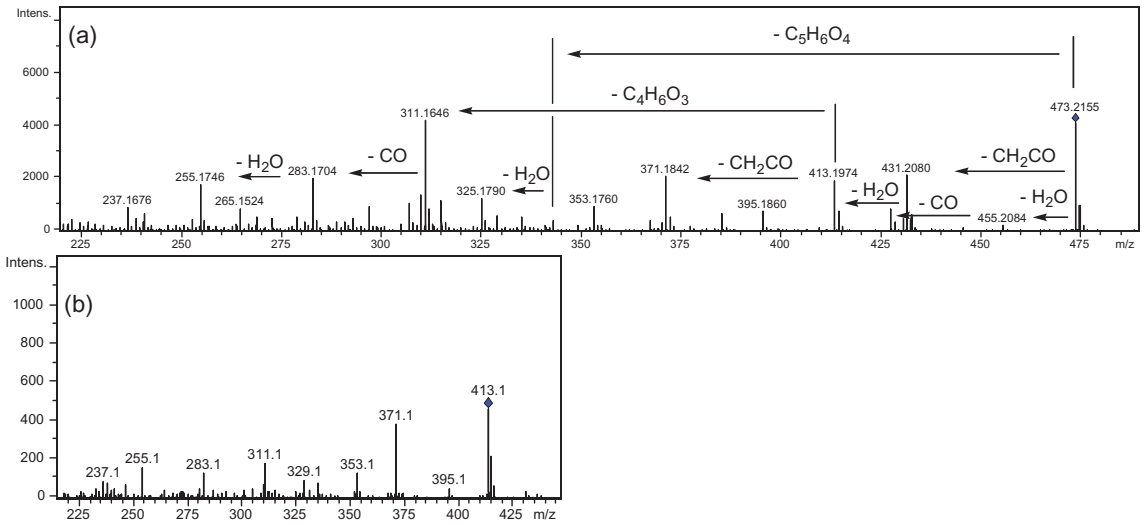
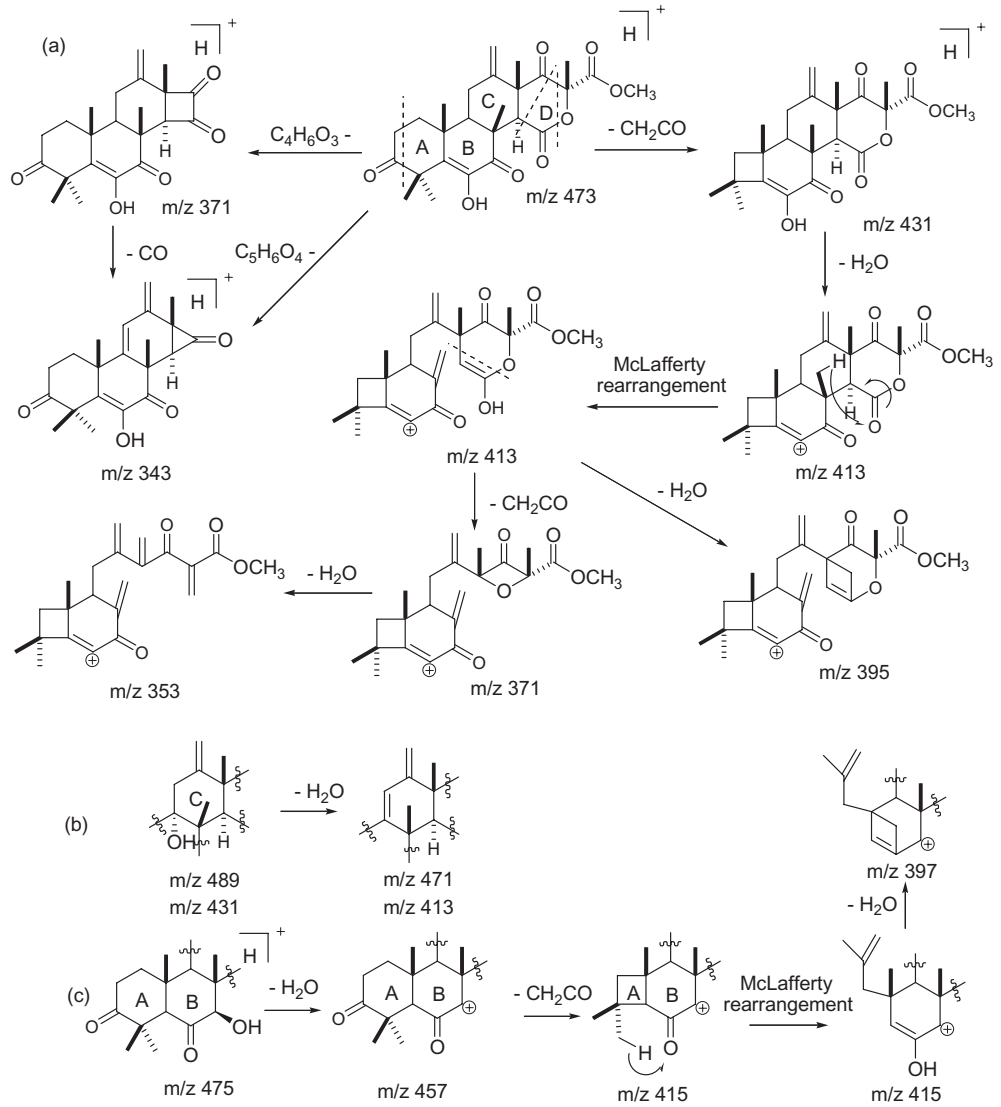


Figure 2. (a) ESI-QTOF-MS/MS spectra of selected $[M + H]^+$ at *m/z* 473 for terretonin A (**1**) (collision energy at 27 eV) and (b) ESI-QTOF-MS/MS/MS spectrum of selected ions at *m/z* 413 from *m/z* 473 (collision energy at 20 eV).



Scheme 1. (a) Major fragmentation patterns of $[M + H]^+$ for terretonin A (**1**); (b) loss of H_2O on ring B for terretonin (**2**) and terretonin C (**3**); and (c) partial fragmentation pathways of $[M + H]^+$ for terretonin D (**4**).

to more abundant fragment information (Fig. 2a). The major fragmentation patterns were shown in Scheme 1(a). The cleavage of ring D occurred by loss of a $C_4H_6O_3$ (102 Da) or $C_5H_6O_4$ (130 Da) molecule. The fragment ion at *m/z* 431 was formed from the precursor ion *m/z* 473 by loss of a CH_2CO (42 Da) molecule from ring A and produced the ion at *m/z* 413 by loss of a H_2O (18 Da) molecule. The ion at *m/z* 413 further yielded the fragment ions at *m/z* 395 and 371, possibly experiencing McLafferty rearrangement followed by loss of a H_2O and a CH_2CO molecule, respectively. The loss of an H_2O molecule from cyclic ether of *m/z* 371 is a relatively favorable process. The loss of a CO (28 Da) molecule occurs readily. Detailed fragmentation pathways of the precursor ion $[M + H]^+$ at *m/z* 473 were postulated on the basis of ESI-QTOF-MS/MS/MS and ESI-IT-MS^n spectra (Figs 2 and 3, Scheme 2). The relative abundance of fragment ions from *pathway b* was high (Figs 2a and 3). On the contrary, low-abundance fragment ions from *pathways a and c* were observed. The exact masses of major fragment ions are shown in Table 1.

Terretonin (2) and terretonin C (3). The protonated molecule ion was selected as the precursor ion for the product ion scan. The loss of a H_2O molecule on ring C occurred first (Scheme 1b). Although the fragmentation pathways of protonated terretonin (**2**), terretonin C (**3**) and terretonin A (**1**) were very similar, the relative abundance of fragment ions from the same fragmentation pathways varied between compounds **2**, **3** and **1**. For terretonin (**2**) and terretonin C (**3**), as well as *pathway b*, the fragment ions from *pathways a and c* showed high relative abundance (Fig. 4a and b). Incidentally, for terretonin C (**3**), the losses of C_2H_4O (44 Da) or $C_3H_4O_2$ (72 Da) resulted from the cleavage of ring B. The major fragment ions except *m/z* 341 in the ESI-IT-MS^n spectra of compounds **2** and **3** (see the Supporting Information) were the same as in the ESI-QTOF-MS/MS spectra, which supports the proposed fragmentation pathways.

Terretonin D (4). Partial fragmentation pathways different from compounds **1**, **2** and **3** are shown in Scheme 1c. The loss of an

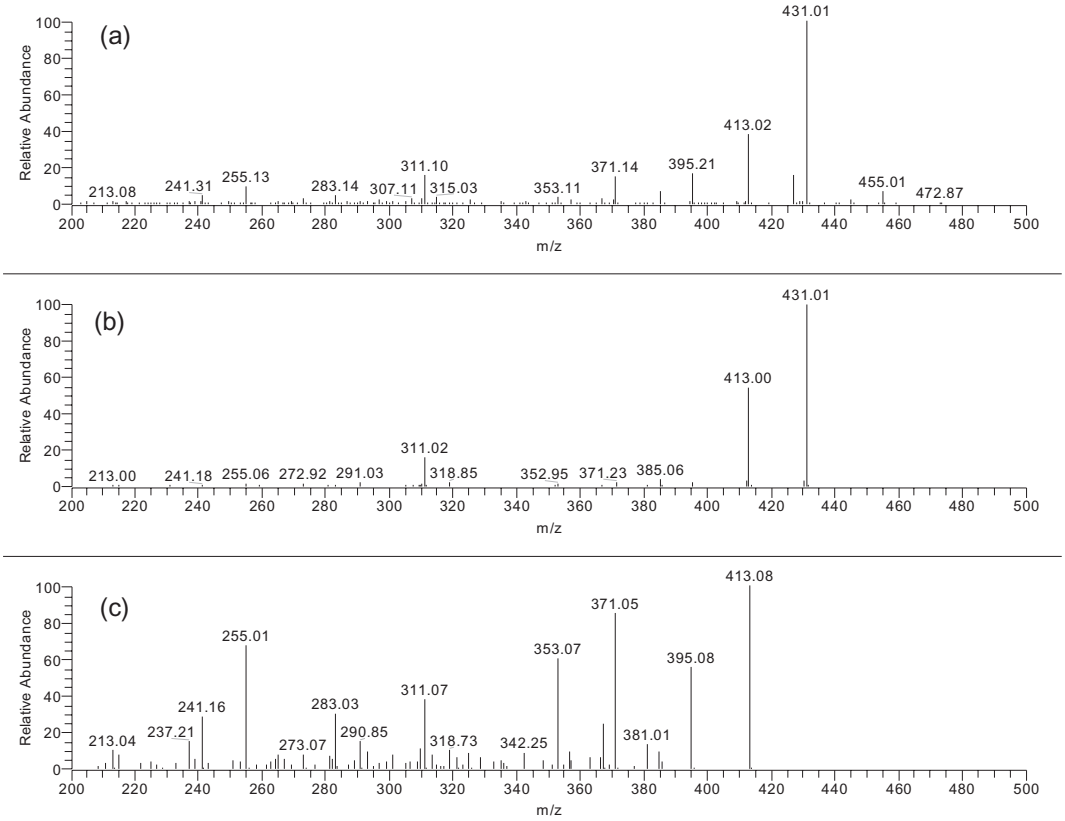
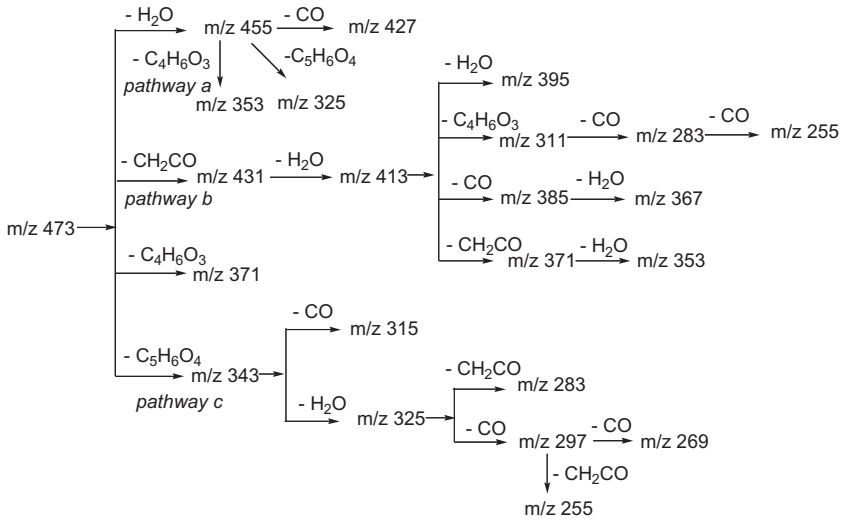


Figure 3. ESI-IT-MS^n spectra of terretonin A (**1**) in positive-ion mode: (a) selected $[M + H]^+$ at *m/z* 473 (collision energy at 25 eV), (b) selected ions at *m/z* 431 from *m/z* 473 (collision energy at 20 eV) and (c) selected ions at *m/z* 413 from *m/z* 473 (collision energy at 24 eV).



Scheme 2. Possible fragmentation pathways of the selected $[M + H]^+$ at *m/z* 473 for terretonin A (**1**). Pathways were postulated from ESI-QTOF-MS/MS/MS and ESI-IT-MS^n spectra.

H_2O molecule from the precursor ion *m/z* 475 on ring B occurred first. The processes of loss of a H_2O molecule from *m/z* 415 (Fig. 4c) might also undergo McLafferty rearrangement. For terretonin D (**4**), high-abundance fragment ions were produced from *pathway c*. The McLafferty rearrangement played an important role in the loss of H_2O in this fragmentation pathway. This might indicate the presence of ketone at C-α (Fig. 1). The same characterising fragmentation profiles were observed in ESI-IT-MS^n spectra (see the Supporting Information).

Terretonin B (5). Although the intensity of $[M + H]^+$ for terretonin A (**1**), terretonin (**2**), terretonin C (**3**) and terretonin D (**4**) could be improved by addition of ammonium acetate, the peak of $[M + H]^+$ for terretonin B (**5**) in ESI-QTOF-MS spectrum was

Table 1. Elemental constituents of major fragment ions from protonated molecule of terretonin A (collision energy at 27 eV)

Fragment ion	Formula	Calculated	Observed	Error (ppm)
$[M + H]^+$	$C_{26}H_{33}O_8$	473.2170	473.2155	+3.2
$[M + H - 18]^+$	$C_{26}H_{31}O_7$	455.2064	455.2084	−4.4
$[M + H - 42]^+$	$C_{24}H_{31}O_7$	431.2064	431.2080	−3.6
$[M + H - 18 - 28]^+$	$C_{25}H_{31}O_6$	427.2115	427.2135	−4.6
$[M + H - 42 - 18]^+$	$C_{24}H_{29}O_6$	413.1959	413.1974	−3.8
$[M + H - 42 - 18 - 18]^+$	$C_{24}H_{27}O_5$	395.1853	395.1860	−1.9
$[M + H - 42 - 18 - 28]^+$	$C_{23}H_{29}O_5$	385.2010	385.1994	+4.1
$[M + H - 42 - 18 - 42]^+$	$C_{22}H_{27}O_5$	371.1853	371.1863	−2.6
$[M + H - 42 - 18 - 28 - 18]^+$	$C_{23}H_{27}O_4$	367.1904	367.1918	−4.0
$[M + H - 18 - 102]^+$	$C_{22}H_{25}O_4$	353.1747	353.1760	−3.7
$[M + H - 130]^+$	$C_{21}H_{27}O_4$	343.1904	343.1898	+1.7
$[M + H - 130 - 18]^+$	$C_{21}H_{25}O_3$	325.1798	325.1790	+2.5
$[M + H - 130 - 28]^+$	$C_{20}H_{27}O_3$	315.1955	315.1945	+3.0
$[M + H - 42 - 18 - 102]^+$	$C_{20}H_{23}O_3$	311.1642	311.1646	−1.4
$[M + H - 130 - 18 - 28]^+$	$C_{20}H_{25}O_2$	297.1849	297.1853	−1.2
$[M + H - 130 - 18 - 42]^+$	$C_{19}H_{23}O_2$	283.1693	283.1704	−4.2
$[M + H - 130 - 18 - 28 - 42]^+$	$C_{18}H_{23}O$	255.1743	255.1746	−1.1

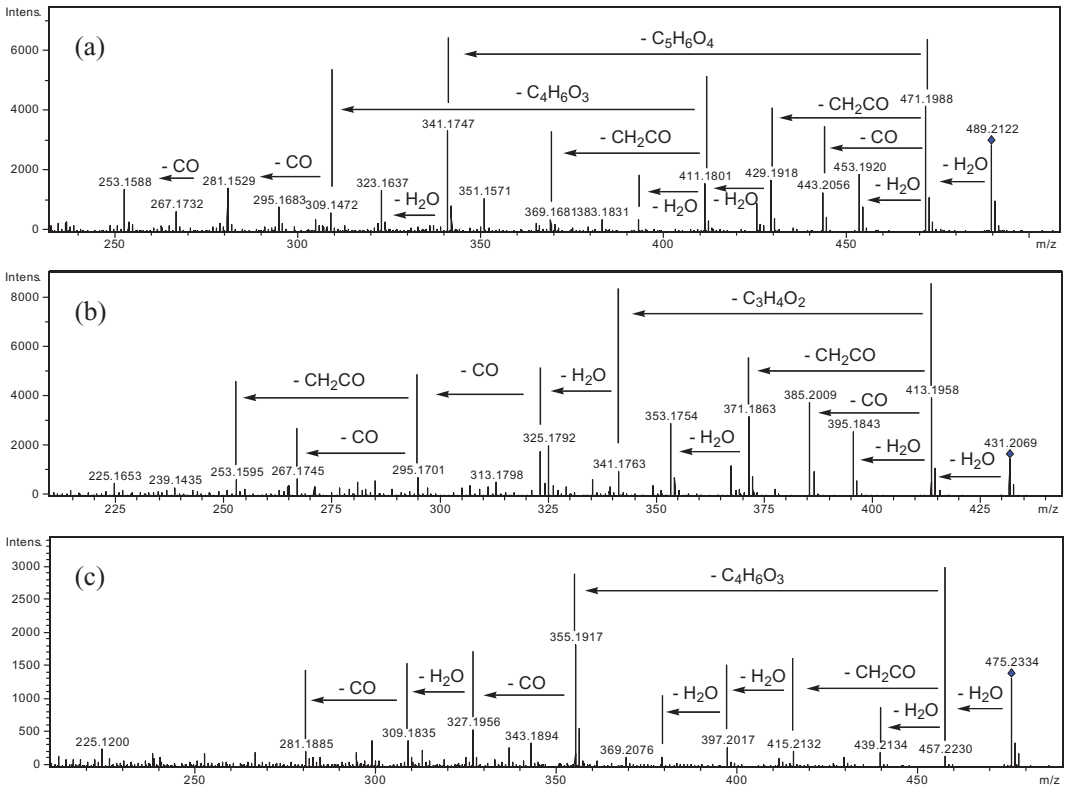


Figure 4. ESI-QTOF-MS/MS spectra of selected $[M + H]^+$: (a) at *m/z* 489 for terretonin (**2**) (collision energy at 17 eV); (b) at *m/z* 431 for terretonin C (**3**) (collision energy at 20 eV); and (c) at *m/z* 475 for terretonin D (**4**) (collision energy at 26 eV).

negligible. The high-abundance precursor ion, $[M + NH_4]^+$ at *m/z* 522, was selected for MS/MS analysis. The abundance of the fragment ion at *m/z* 505 formed from *m/z* 522 by loss of a NH_3 molecule was very low (Fig. 5). Compared with other compounds, more O–H–N hydrogen bonding between OH or O on ring B and NH_4^+ could have formed, which might be the reason for the phenomena mentioned above. Different from compounds **1**–**4**, the loss of a CO_2 (44 Da) molecule from the fragment ion at *m/z* 487 in the MS/MS spectrum of compound **5** was observed. Incidentally, the loss of H_2O from *m/z* 339 in *pathway c* (Scheme 3) might undergo McLafferty rearrangement as shown in Scheme 1c. In a word, for terretonin B (**5**), *pathways a and c* are the major fragmentation pathways and *pathway b* is negligible.

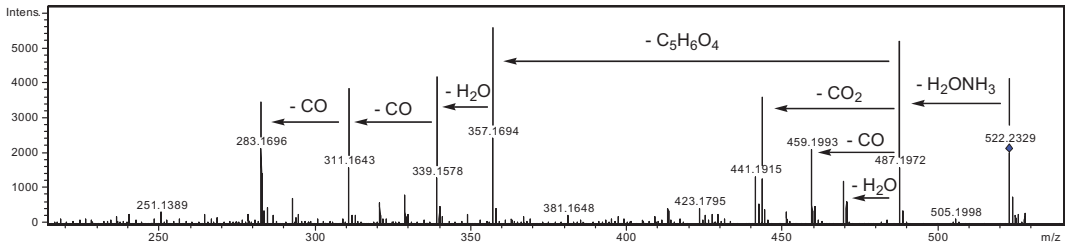
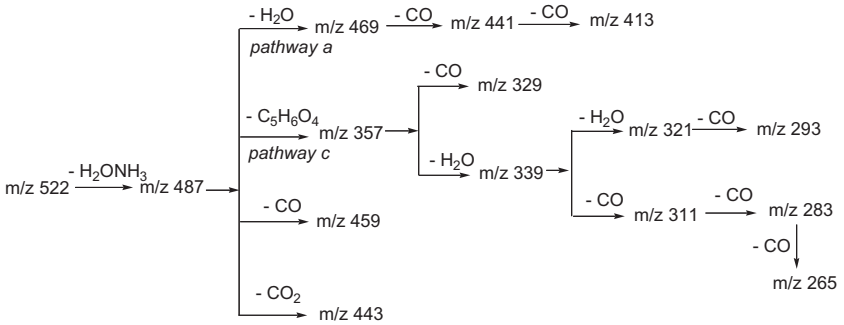
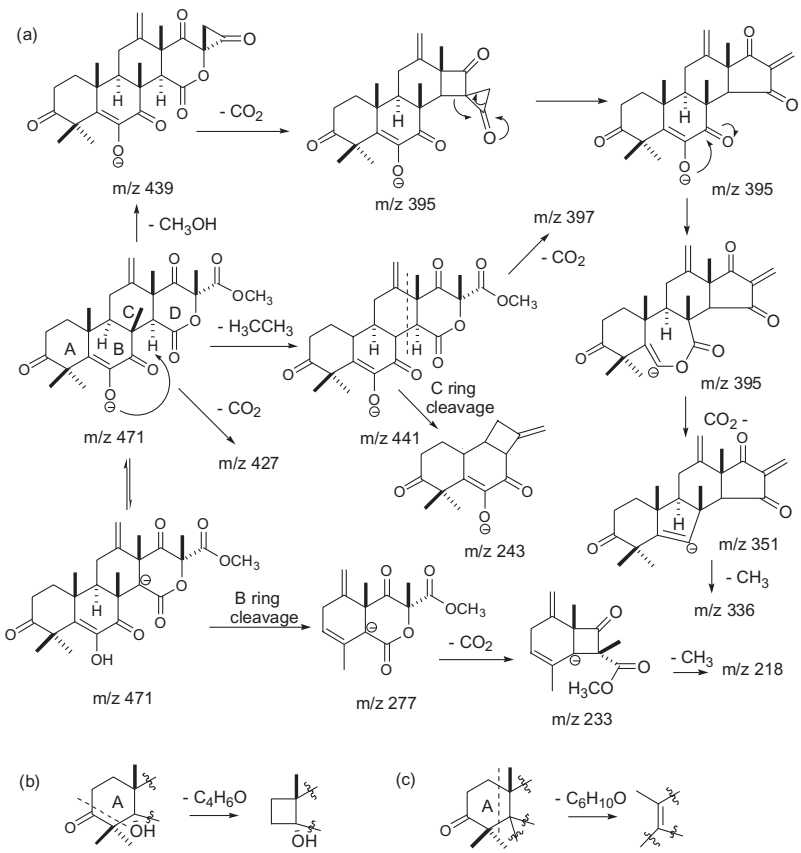


Figure 5. ESI-QTOF-MS/MS spectrum of selected $[M + NH_4]^+$ at *m/z* 522 for terretonin B (**5**) (collision energy at 20 eV).



Scheme 3. Possible fragmentation pathways of the selected $[M + NH_4]^+$ at *m/z* 522 for terretonin B (**5**). Pathways were postulated from ESI-QTOF-MS/MS.



Scheme 4. (a) Major fragmentation patterns of $[M - H]^-$ for terretonin A (**1**). (b) Loss of C_4H_6O from ring A and (c) loss of $C_6H_{10}O$ from ring A.

Negative-ion ESI-MS

Terretonin A (1). For terretonin A (**1**), the cleavage of ring B and the neutral losses of the CH_3OH, CO_2, CH_3 and CO molecules are the main fragmentation patterns for the selected $[M-H]^-$ precursor ion at *m/z* 471 (Scheme 4a). The fragment ion at *m/z* 439 was formed from the precursor ion *m/z* 471 by loss of a CH_3OH (32 Da) molecule and further yielded the ion at *m/z* 395 by loss of a CO_2 molecule. Interestingly, the intermediate of biosynthesis of terretonin included a similar structure to ring D of *m/z* 395 (McIntyre and Simpson, 1981). The loss of CO_2 from *m/z* 395 occured possibly through the formation of a heptacyclic intermediate. The fragment ions at *m/z* 277.1081 and 275 resulting from cleavage of ring B seemed to be the characteristic ions of skeleton fragmentation. Incidentally, the location of negative charge might change between O and C atoms by hexagonal hydrogen rearrangement (Scheme 4a). Similar H rearrangement was reported by Li *et al.* (2008). The high-abundance fragment ion at *m/z* 243 in low mass range was formed by the cleavage of ring C from *m/z* 441 produced by sequential elimination of CH_3 radical from *m/z* 471. Detailed fragmentation pathways of *m/z* 471 are shown in Scheme 5. In order to understand the pathways better, the collision energy was increased gradually for observation of the intensity of product ions (Fig. 6). Interestingly, the relative intensity of the peak at *m/z* 439 vs that at *m/z* 362 and 336 became weaker as the energy increased. This indicates that *m/z* 439 might be the precursor ion of these fragment ions. Different from ESI-QTOF-MS/MS experiments, the fragment ion at *m/z* 439 in ESI-IT-MS^n spectra shows high abundance and further produces the product ions at *m/z* 395, 377, 362 and 336 (see the Supporting

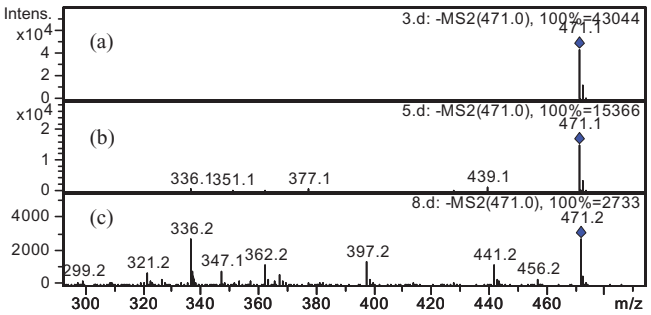


Figure 6. ESI-QTOF-MS/MS spectra of selected $[M-H]^-$ at *m/z* 471 for terretonin A (**1**): (a) collision energy at 0 eV, (b) collision energy at 20 eV and (c) collision energy at 35 eV.

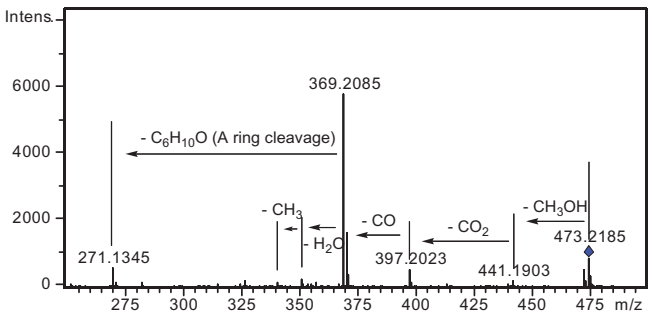


Figure 8. ESI-QTOF-MS/MS spectrum of selected $[M-H]^-$ at *m/z* 473 for terretonin D (**4**) (collision energy at 15 eV).

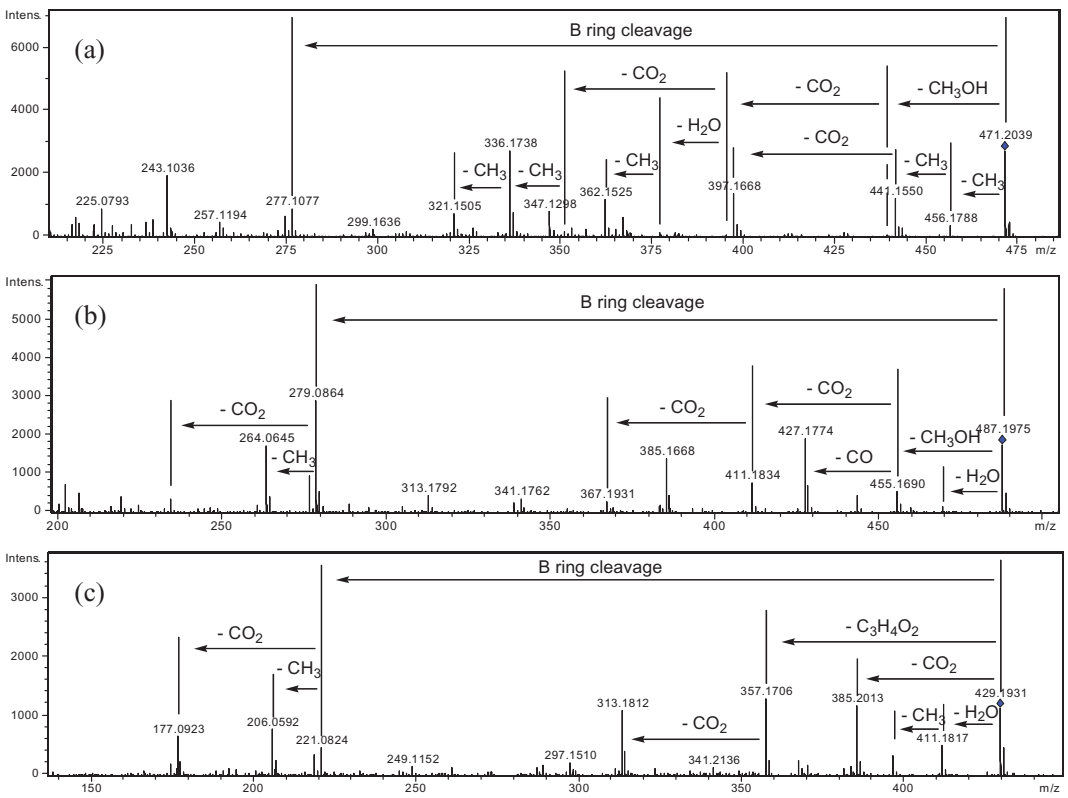
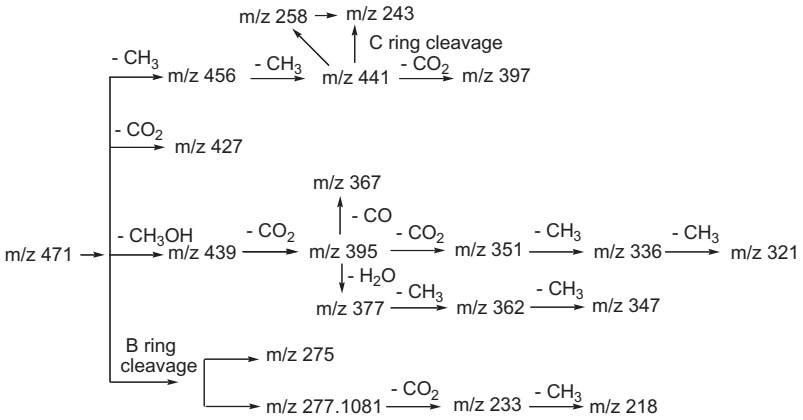


Figure 7. ESI-QTOF-MS/MS spectra of selected $[M-H]^-$: (a) at *m/z* 471 for terretonin A (**1**) (collision energy at 35 eV), (b) at *m/z* 487 for terretonin (**2**) (collision energy at 20 eV) and (c) at *m/z* 429 for terretonin C (**3**) (collision energy at 20 eV).



Scheme 5. Possible fragmentation pathways of the selected $[M - H]^-$ at *m/z* 471 for terretonin A (**1**). Pathways were postulated from ESI-QTOF-MS/MS and ESI-IT-MS^n spectra.

Table 2. Elemental constituents of major fragment ions from deprotonated molecule of terretonin A (collision energy at 35 eV)

Fragment ion	Formula	Calculated	Observed	Error (ppm)
$[M - H]^-$	$C_{26}H_{31}O_8$	471.2024	471.2039	−3.1
$[M - H - 15]^-$	$C_{25}H_{28}O_8$	456.1790	456.1788	+0.3
$[M - H - 15 - 15]^-$	$C_{25}H_{27}O_7$	441.1555	441.1550	+1.2
$[M - H - 32]^-$	$C_{25}H_{27}O_7$	439.1762	439.1750	+2.7
$[M - H - 44]^-$	$C_{25}H_{31}O_6$	427.2126	427.2120	+1.5
$[M - H - 15 - 15 - 44]^-$	$C_{23}H_{25}O_6$	397.1657	397.1668	−2.9
$[M - H - 32 - 44]^-$	$C_{24}H_{27}O_5$	395.1864	395.1842	+5.5
$[M - H - 32 - 44 - 18]^-$	$C_{24}H_{25}O_4$	377.1758	377.1757	+0.4
$[M - H - 32 - 44 - 28]^-$	$C_{23}H_{27}O_4$	367.1915	367.1888	+7.2
$[M - H - 32 - 44 - 18 - 15]^-$	$C_{23}H_{22}O_4$	362.1524	362.1525	−0.5
$[M - H - 32 - 44 - 44]^-$	$C_{23}H_{27}O_3$	351.1966	351.1941	+6.9
$[M - H - 32 - 44 - 18 - 15 - 15]^-$	$C_{22}H_{19}O_4$	347.1289	347.1298	−2.7
$[M - H - 32 - 44 - 44 - 15]^-$	$C_{22}H_{24}O_3$	336.1731	336.1738	−2.2
$[M - H - 32 - 44 - 44 - 15 - 15]^-$	$C_{21}H_{21}O_3$	321.1496	321.1505	−2.7
$[M - H - 194]^-$ (ring B cleavage)	$C_{15}H_{17}O_5$	277.1081	277.1077	+1.4
$[M - H - 196]^-$ (ring B cleavage)	$C_{15}H_{15}O_5$	275.0925	275.0926	−0.4
$[M - H]^-$ (ring C cleavage)	$C_{16}H_{18}O_2$	258.1260	258.1266	−1.7
$[M - H]^-$ (ring C cleavage)	$C_{15}H_{15}O_3$	243.1027	243.1036	−3.8
$[M - H - 194 - 44]^-$	$C_{14}H_{17}O_3$	233.1183	233.1194	−4.8
$[M - H - 194 - 44 - 15]^-$	$C_{13}H_{14}O_3$	218.0948	218.0940	+4.0

Information). This indicates that some fragmentation pathways can be proposed by the variety of abundance of fragment ions at different collision energies even without MS^n spectra. The high-resolution MS/MS spectra and the exact masses of major fragment ions are shown in Fig. 7a and Table 2, respectively.

Terretonin (2),terretonin C (3), terretonin D (4) and terretonin B (5). Unlike positive-ion mode, the fragmentation pathways vary in negative-ion mode for these compounds. The fragmentation patterns of ring B for terretonin (**2**) and terretonin C (**3**) are the same but different from terretonin (**1**). The cleavage of ring B was probably accompanied by H transfer. The fragment ions at *m/z* 279 and 277.1081 on MS/MS spectrum of terretonin (**2**) and ions at *m/z* 221 and 219 on the MS/MS spectrum of terretonin C (**3**) are respective characteristic ions (Fig. 7b and c). The high-abundance fragment ion at *m/z* 219 and no observation of *m/z* 221 in the ESI-QTOF-MS/MS/MS spectrum of *m/z* 411 for terretonin C (**3**) support the structural analysis of these characteristic ions (supporting information). No observation of the loss of a CH_3OH molecule for terretonin C (**3**) indicates that CH_3OH resulted from side chain ($COOCH_3$) on ring D. The cleavage of ring D for terretonin (**2**) and terretonin C (**3**) can be observed. Major fragment ions were also observed in ESI-IT-MS^n spectra for compounds **2** and **3** (see the Supporting Information) except the ion at *m/z* 177 in the low-mass range of compound **3**. For terretonin D (**4**), the product ions resulting from the cleavage of ring B were not observed (Fig. 8), which implies the stability of ring B. This should be in accordance with its relatively saturated structure. The sequential loss of CO_2 from $[M - H]^-$ for compound 4 readily occurred in ESI-IT-MS experiments but was not observed in ESI-QTOF-MS experiments. For terretonin B (**5**), the fragment ion at *m/z* 277.0718 and 275 from the cleavage of ring B might

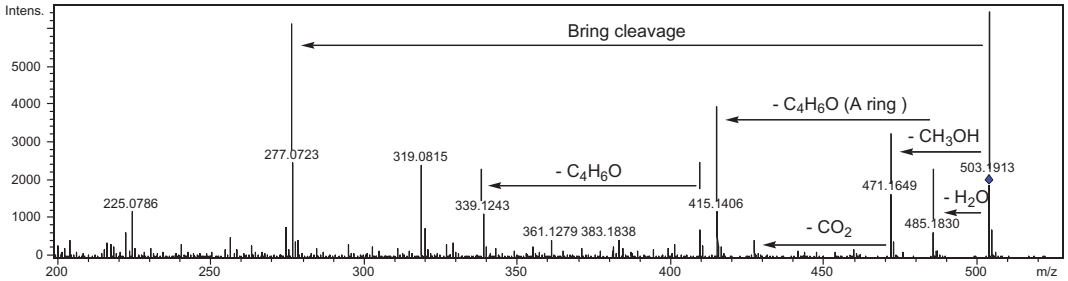


Figure 9. ESI-QTOF-MS/MS spectrum of selected $[M - H]^-$ at *m/z* 503 for terretonin B (**2**) (collision energy at 20 eV).

be characteristic ions (Fig. 9). The loss of a C_4H_6O (70 Da) molecule from ring A occurred readily (Scheme 4b) and could be directly observed in the ESI-QTOF-MS/MS/MS spectrum of *m/z* 471 (see the Supporting Information). In addition, for compounds **4** and **5**, the loss of $C_6H_{10}O$ (98 Da) by cleavage of ring A was observed (Scheme 4c). It seems that the loss of CO_2 occurs readily in ESI-IT-MS^n experiments and loss of CH_3OH readily occurs in ESI-QTOF-MS/MS experiments for compound **5**. For example, the fragment ion at *m/z* 459 resulting from the precursor ion at *m/z* 503 by loss of CO_2 shows high abundance and *m/z* 471 resulting from *m/z* 503 by loss of CH_3OH is a low-abundance ion in ESI-IT-MS^n spectra (see the Supporting Information). Contrary phenomena were observed in ESI-QTOF-MS/MS spectra.

Conclusions

Fragmentation patterns of five representative sesterterpenoids were elucidated using ESI-QTOF-MS/MS/MS and ESI-IT-MS^n in both positive- and negative-ion mode. Major differences between ESI-QTOF-MS/MS and IT-MSn spectra were illustrated. In positive-ion mode, the characteristic fragmentation pathways were similar but the related abundance of product ions from the same pathways varied. It seems that the cleavage of ring D readily occurs. The losses of some H_2O molecules possibly underwent McLafferty rearrangement. This might indicate the presence of ketone at C-a of ring B for compounds **4** and **5**. The extremely low abundance of $[M + H]^+$ for terretonin B (**5**) observed might be related to the structure of ring B. In negative-ion mode, the fragmentation pathways were probably influenced by the structure of ring B and varied obviously. A common fragmentation pathway ($[M - H]^- \rightarrow [M - H - 32]^- \rightarrow [M - H - 32 - 44]^-$) was observed when the side chain on ring D presented. The characteristic ions resulting from the cleavage of ring B were gained. In some cases, the fragmentation pathways can be unambiguously proposed by the variety of abundance of fragment ions at different collision energies even without MS^n spectra. It seems that the loss of CO_2 occurs readily in ESI-IT-MS^n experiments for compounds **4** and **5**. In summary, complementary information obtained from fragmentation experiments of $[M + H]^+$ (or $[M + NH_4]^+$) and $[M - H]^-$ precursor ions is especially valuable for rapid identification of this kind of sesterterpenoid.

Supporting information

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

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