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Structural characterization and identification of five triterpenoid saponins isolated from *Momordica cochinchinensis* extracts by liquid chromatography/tandem mass spectrometry

Zhi-Yan Lin[a,b], Xiaoyun Liu[a], Fan Yang[a], Yun-Qiu Yu[a,*]

[a] Department of Pharmaceutical Analysis, School of Pharmacy, Fudan University, Shanghai 201203, China
[b] Department of Pharmacy, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200092, China

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ABSTRACT

In this work, we reported the use of high-performance liquid chromatography (HPLC) with electrospray ionization multi-stage tandem mass spectrometry (ESI-MS^n) to study the characterization of five triterpenoid saponins from *Momordica cochinchinensis* extracts in positive ion mode and negative ion mode, revealed the fragmentation behavior of these five *M. cochinchinensis* saponins in different ion modes. Results showed that in negative ion mode, fragmentation on the *M. cochinchinensis* triterpenoid saponins at positions C28 and O bond were the primary pathways. In positive ion mode, the predominant diagnostic ions were a series of ions resulting from the rupture of the saccharic chain on C28. Comparison of different fragmentation patterns showed that the results from positive and negative ion ESI MS^n were complementary. Both modes can yield structurally different information for the characterization of triterpenoid saponins. This study provides a powerful method for the online structural identification of five triterpenoid saponins from *M. cochinchinensis*, and some of which have not been reported.

1. Introduction

As we know saponins were widely distributed in Traditional Chinese Medicine and had various biological activities, such as anticancer, antioxidant, and cardiovascular effects. They were abundantly found in many medicinal herbs and had attracted great attention. *Momordica cochinchinensis*, a plant that belongs to the Cucurbitaceae family. The fruit pulp was widely used in Vietnam to color rice, and the seeds of the fruit were used in traditional Chinese medicine (Mubiezi in Chinese), which can treat many common disease such as boils, pyodermatitis, mastitis, tuberculous cervical lymphadenitis, ringworm infections, freckles, sebaceous, hemorrhoids and hemangiomas [1,2]. Recently, *M. cochinchinensis* was used in anticancer compound recipe and as the critical component in prescriptions, and saponins were considered to be the major bioactive ingredients in it. Many researches had revealed that *M. cochinchinensis* saponins had antitumor activity [3–5]. *M. cochinchinensis* saponins were the oleanane type triterpene saponins, which had two saccharic chains. Iwamoto first time isolated three saponins from the seeds and roots of *M. cochinchinensis* which named as momordicasaimin I, momordicasaimin II and momordicasaimin III [6,7]. As we know saponins from plant extracts had many oligosaccharic chains which made the separation and structural characterization more difficult. Positive ion ESI-MS method was used for triterpenoids analysis in many studies [8–14]. But studies on the use of multi-stage mass spectrometry for characterization of triterpenoids were very limited [15]. Now liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS^n) has become the most popular method for online identification of the bioactive ingredients in natural products for its competitive advantages of superior sensitivity, short analysis time and considerable structural information [16]. In this work, preparative HPLC method was used to separate the total saponins from *M. cochinchinensis* extracts. The further isolation and structural characterization of *M. cochinchinensis* saponins were performed by LC/MS^n. Five *M. cochinchinensis* saponins were isolated and some of them have not been reported before. Both positive and negative electrospray ionization modes were assessed for characterizing these five *M. cochinchinensis* saponins. Results showed that the fragmentation patterns of *M. cochinchinensis* saponins in positive and negative ion ESI-MS^n were complementary. Both modes can yield structurally different information for the characterization of triterpenoid saponins. The recent success with the use of LC/MS^n for the analysis of constituents in complex plants extracts suggests that this method may be effective for the rapid determination of triterpenoid saponins in botanical extracts.

* Corresponding author.
E-mail address: yqyu@shmu.edu.cn (Y.-Q. Yu).

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2. Experimental

2.1. Materials and chemicals

M. cochinchinensis were purchased from Shanghai Chemical Reagent (Shanghai, P.R. China). HPLC grade acetonitrile was purchased from Tedia (United States of America). Water used for HPLC was purified with a Milli-Q plus system (Millipore, Bedford, MA, USA).

2.2. Sample extraction and purification

M. cochinchinensis powder was extracted in ice-cold sodium acetate (20 mM, pH 5.0) overnight at 4 °C. The extract solution was freeze-dried as crude extract. The crude extract was further purified using preparative HPLC on YMC C_{18} column (150 mm × 10 mm, 5 μm) at the low rate of 2 mL/min. The separation was achieved using a Waters system (Waters, USA) consisting of a Waters-1525 EF Binary HPLC pump and a Waters-1996 photodiode array detector. The mobile phase consisted of solvent A (0.05%, v/v, aqueous formic acid) and B (90%, v/v, acetonitrile in 0.045%, v/v, aqueous formic acid), and a gradient from 5% B to 50% B in 40 min was run. The eluate was monitored at 254 nm. The peaks eluting at retention times of 6–16 min, 16–26 min, 26–40 min were collected, freeze-dried to powder, and labeled as "F1", "F2" and "F3" (see in Fig. 1.). "F3" was dissolved in water, passed through a 0.45 μm filter and directly injected into the LC–MS system by further analysis.

2.3. Mass spectrometry analysis

Electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (MS/MS) experiments were performed on a LXQ linear ion trap system (Thermo, USA) interfaced to a surveyor HPLC system (Thermo, USA). MBZC was injected onto an Agilent Extend C-18 reversed-phase column (3.1 mm × 100 mm, 3 μm). Chromatographic separation of F3 was achieved using a gradient solvent system of solvent A (0.05%, v/v, aqueous formic acid) and B (90%, v/v, acetonitrile in 0.045%, v/v, aqueous formic acid). A step gradient from 20% to 50% B was applied for 30 min at the rate of 0.3 mL/min. The column temperature was maintained at 25 °C. The full scan mass spectra were recorded in the positive and negative ion modes over an m/z range 300–2000. The electrospray voltage was 4 kV. The capillary temperature was 300 °C, and the sheath gas flow was 35 units.

3. Results and discussion

3.1. Separation of M. cochinchinensis saponins with LC/MS^n

Using an Agilent Extend C-18 column (3.1 mm × 100 mm, 3 μm) and mobile phase of A (0.05%, v/v, aqueous formic acid) and B (90%, v/v, acetonitrile in 0.045%, v/v, aqueous formic acid) provided symmetrical and sharp chromatographic peaks for *M. cochinchinensis* saponins in both positive and negative electrospray ionization modes. A faster baseline separation of five *M. cochinchinensis* saponins was investigated in F3 within a runtime of 20 min. The total ion chromatograms acquired in the positive and negative ionization modes from the LC/MS^n analysis of F3 was shown in Fig. 2 and labeled the five *M. cochinchinensis* saponins as "A", "B", "C", "D" and "E".

3.2. Elucidation of fragmentation rules of M. cochinchinensis saponins in positive and negative ion modes

Both positive and negative electrospray ionization modes were assessed for characterizing five *M. cochinchinensis* saponins in "F3". Positive ion mode showed predominantly the $[M+H+H_2O]^+$ ions and very low abundance of $[M+H]^+$ ions. Compared with positive mode, the $[M-H]^-$ ion was the base peak in the negative ESI ion mode. The MS^2 and MS^3 spectrums of base peak were significant to infer the instructure of saponins. Figs. 3–7 showed the MS^n spectras of *M. cochinchinensis* saponins A, B, C, D and E in positive ion mode and negative ion mode. Figs. 8–12 showed the proposed fragmentation pathways of *M. cochinchinensis* saponin A, B, C, D and E in positive ion mode and negative ion mode. The result showed that the fragmentation pathways of *M. cochinchinensis* saponins were distinct indifferent ion modes. In negative ion mode, the bond between C28 and O was priority to cleave and generate high abundance of fragment ions at m/z 953 or m/z 969. MS^n information of the ions at m/z 953 or m/z 969 were helpful to presume the structure of saccharic chain on C3 bond. In contrast, the fragmentation information of product ions in positive ion mode were conduced to infer the structure of saccharic chain on C28 bond. Both ion modes can yield structurally different information for the characterization of triterpenoid saponins and the information was complementary.

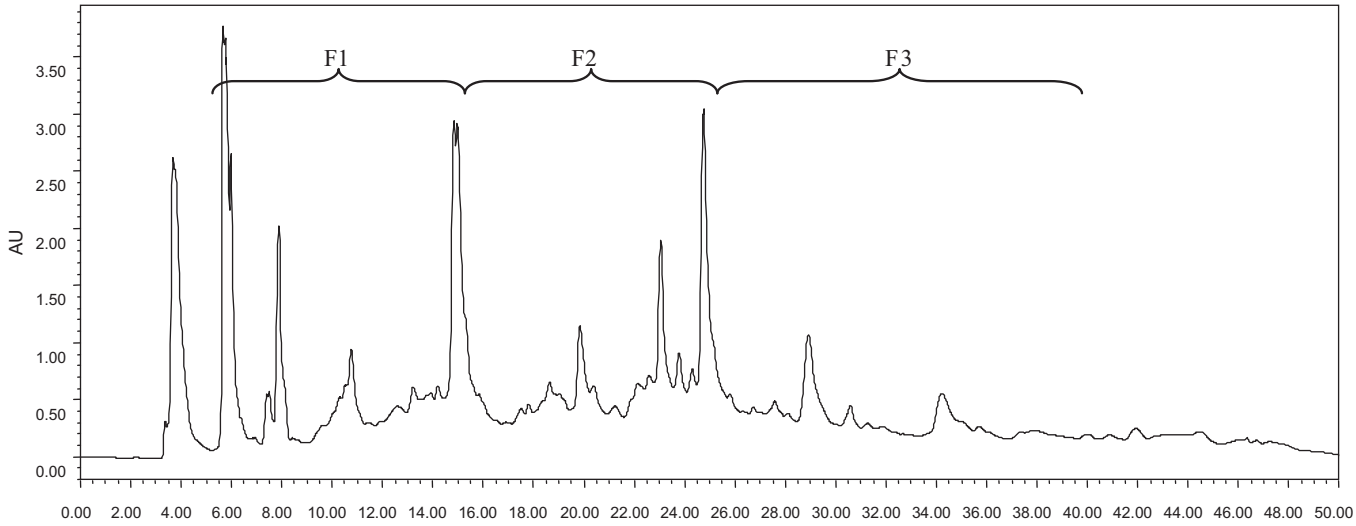


Fig. 1. Typical preparative HPLC elution profile of *Momordica cochinchinensis* extract. The peaks eluting at retention times of 6–16 min, 16–26 min, 26–40 min were collected, freeze-dried to powder, and labeled as "F1", "F2" and "F3".

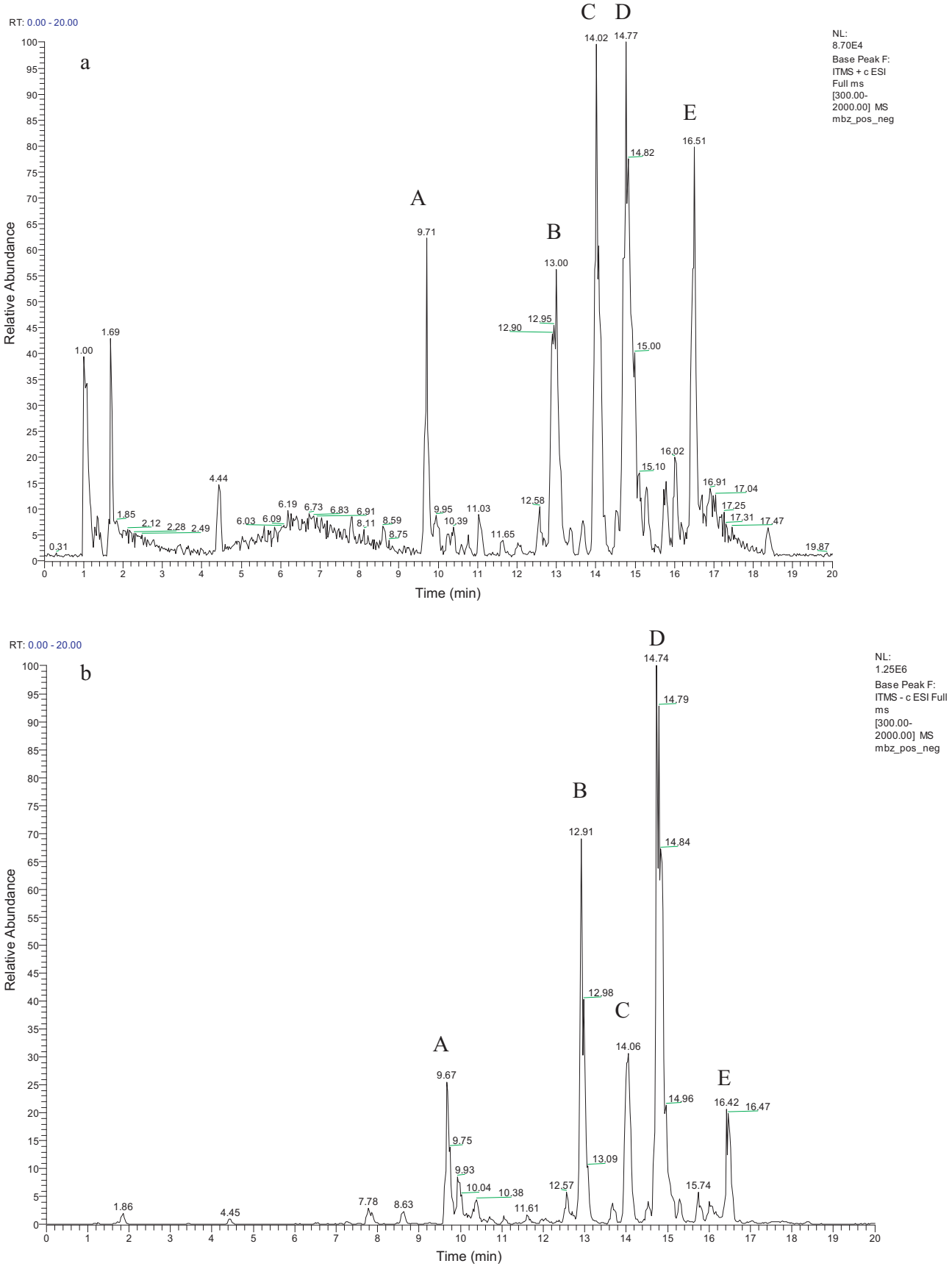


Fig. 2. LC/MS chromatograms of F3 (a) positive ion LC/ESI-MS total ion current (TIC) and (b) negative ion LC/ESI-MS total ion current (TIC). The total ion chromatograms acquired in the positive and negative ionization modes from the LC/MS^n analysis of F3 was shown above and labeled the five *Momordica cochinchinensis* saponins as "A", "B", "C", "D" and "E".

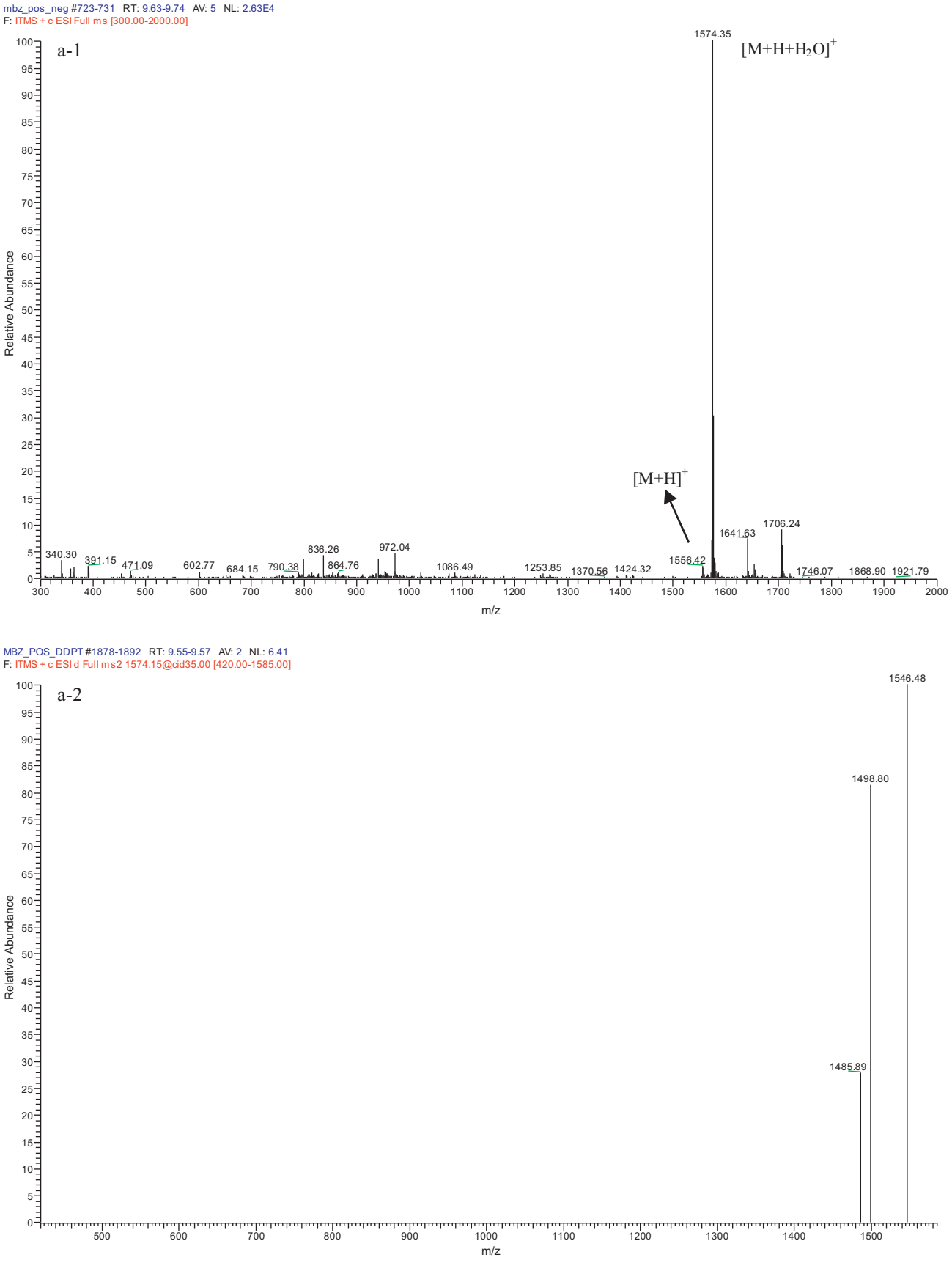
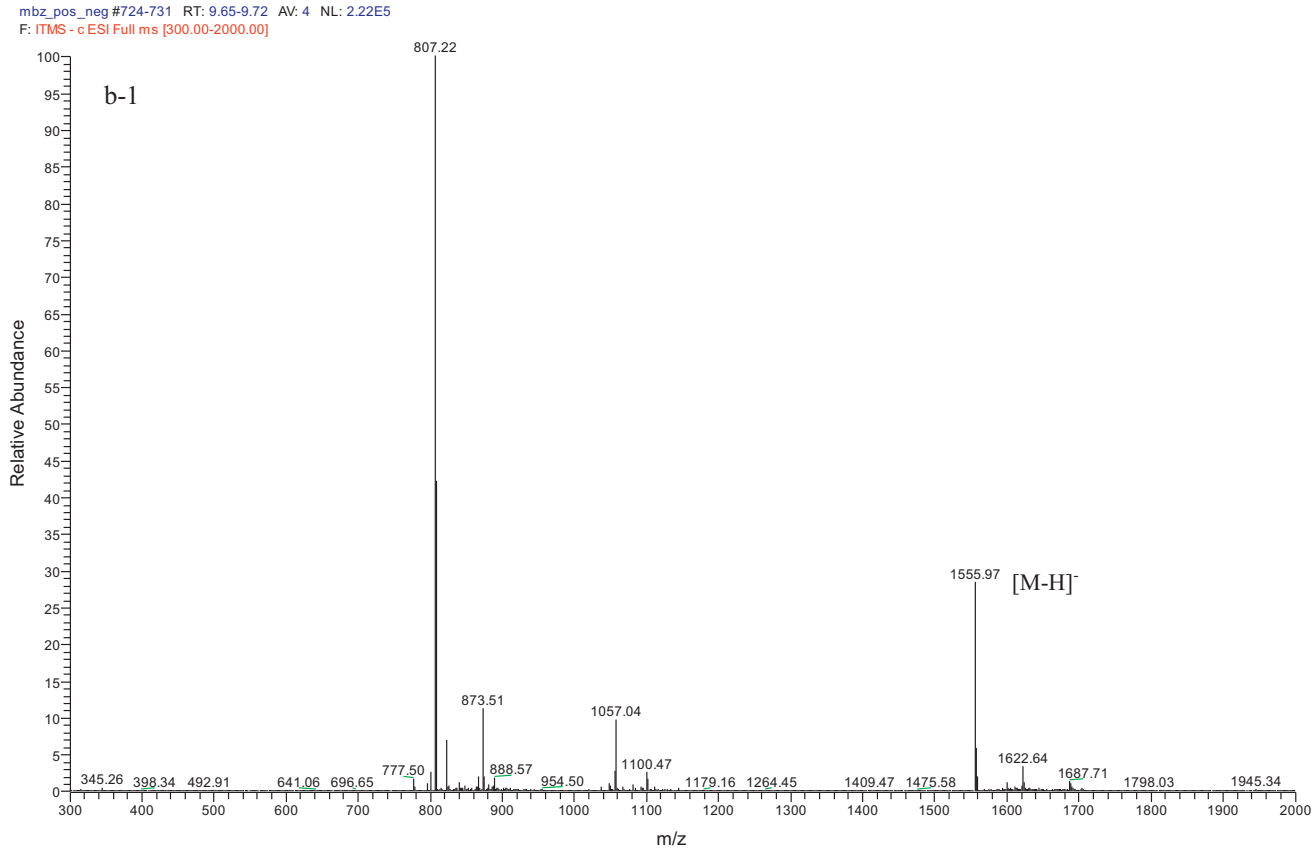


Fig. 3. MS^n spectra of A in positive ion mode (a-1, a-2) and in negative ion mode (b-1, b-2).



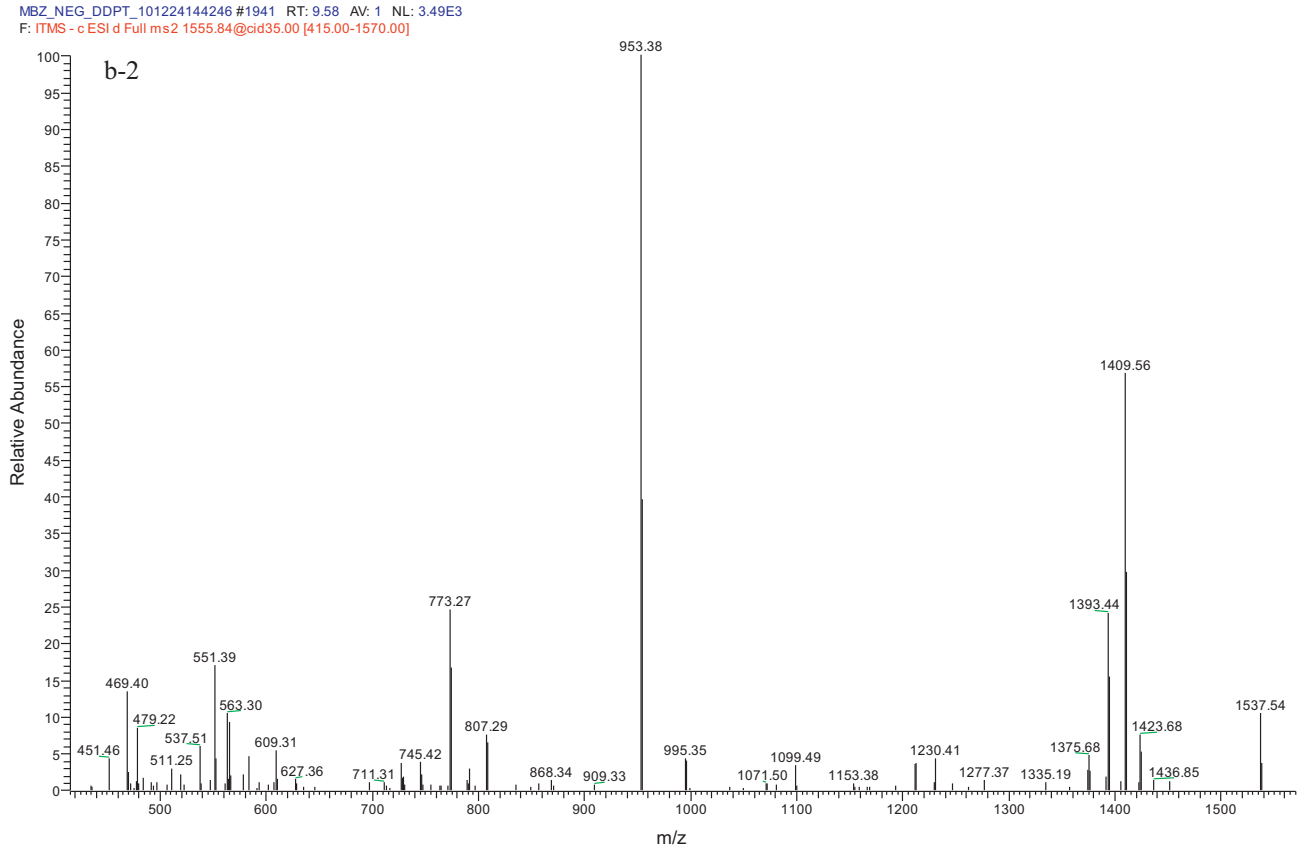


Fig. 3. (*Continued*)

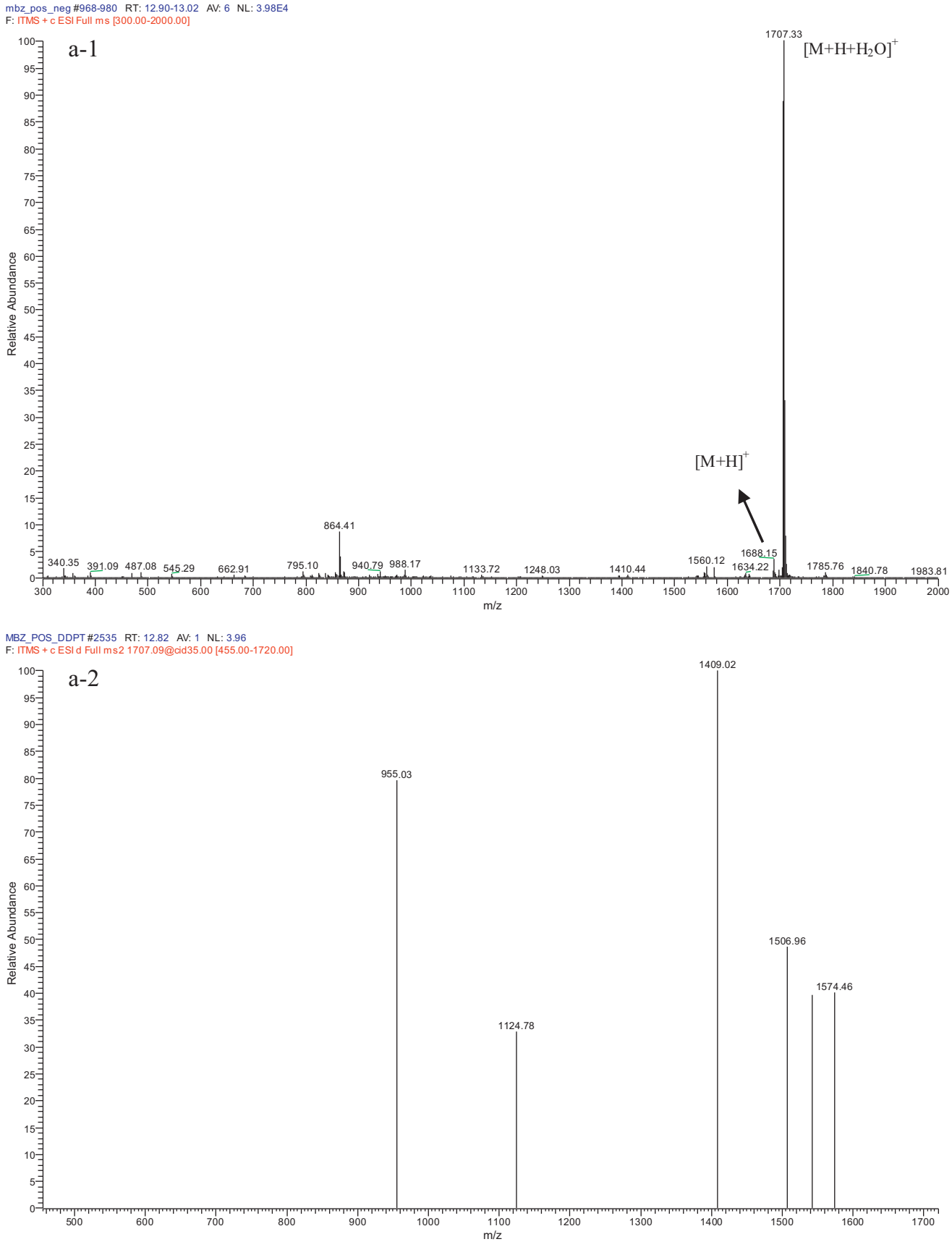


Fig. 4. MS^n spectra of B in positive ion mode (a-1, a-2) and in negative ion mode (b-1, b-2).

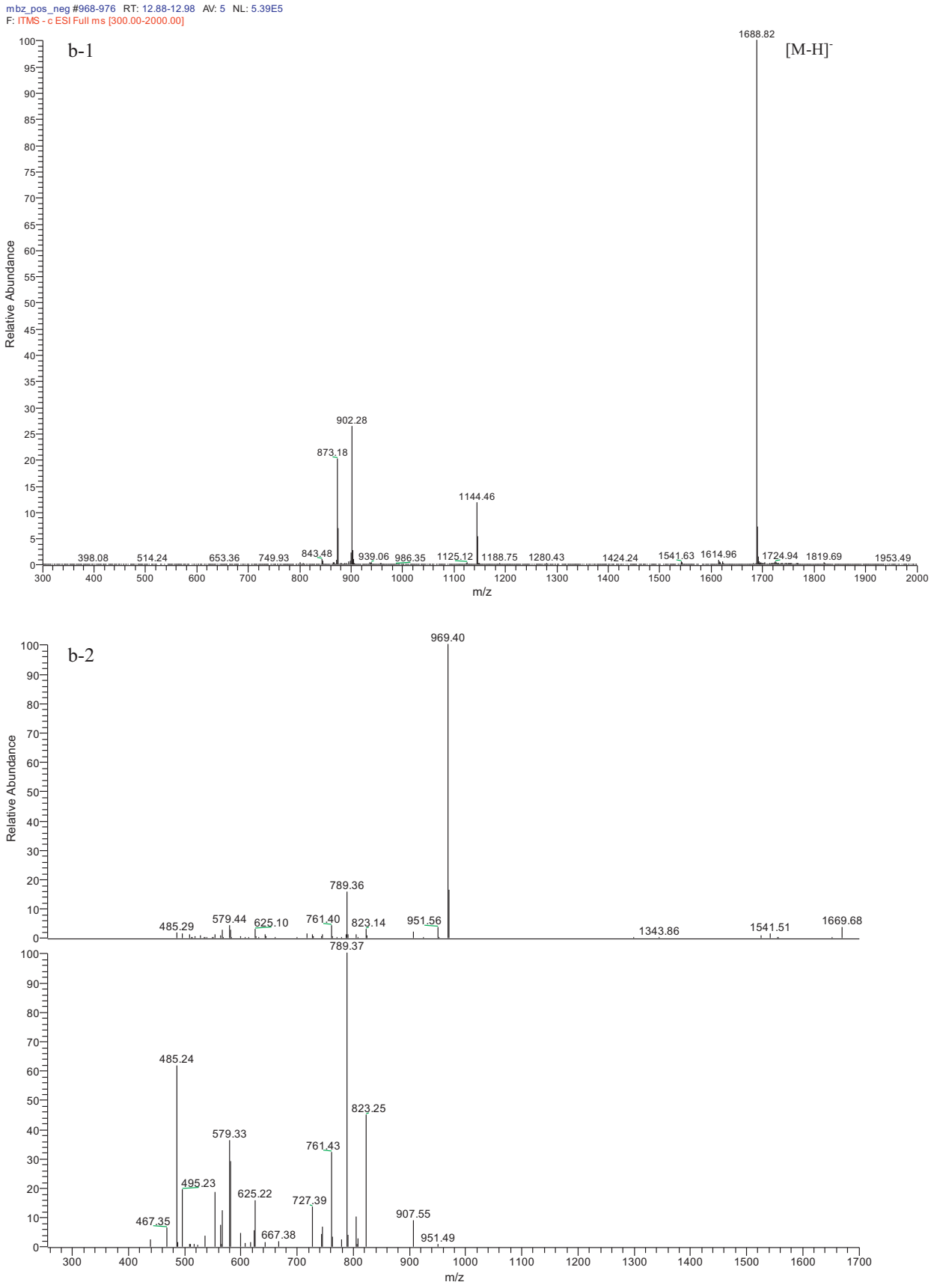


Fig. 4. (*Continued*)

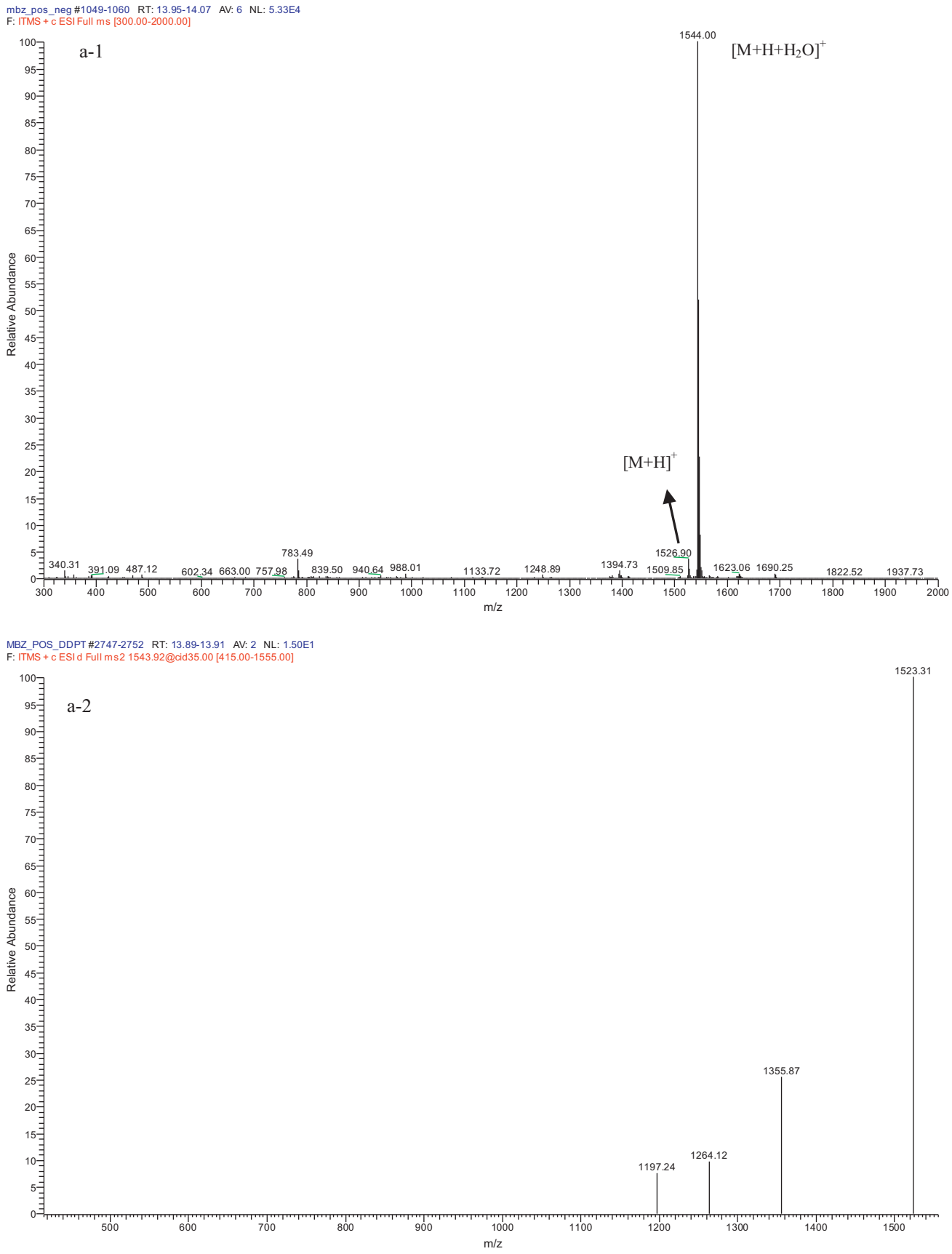


Fig. 5. MS^n spectra of C in positive ion mode (a-1, a-2) and in negative ion mode (b-1, b-2).

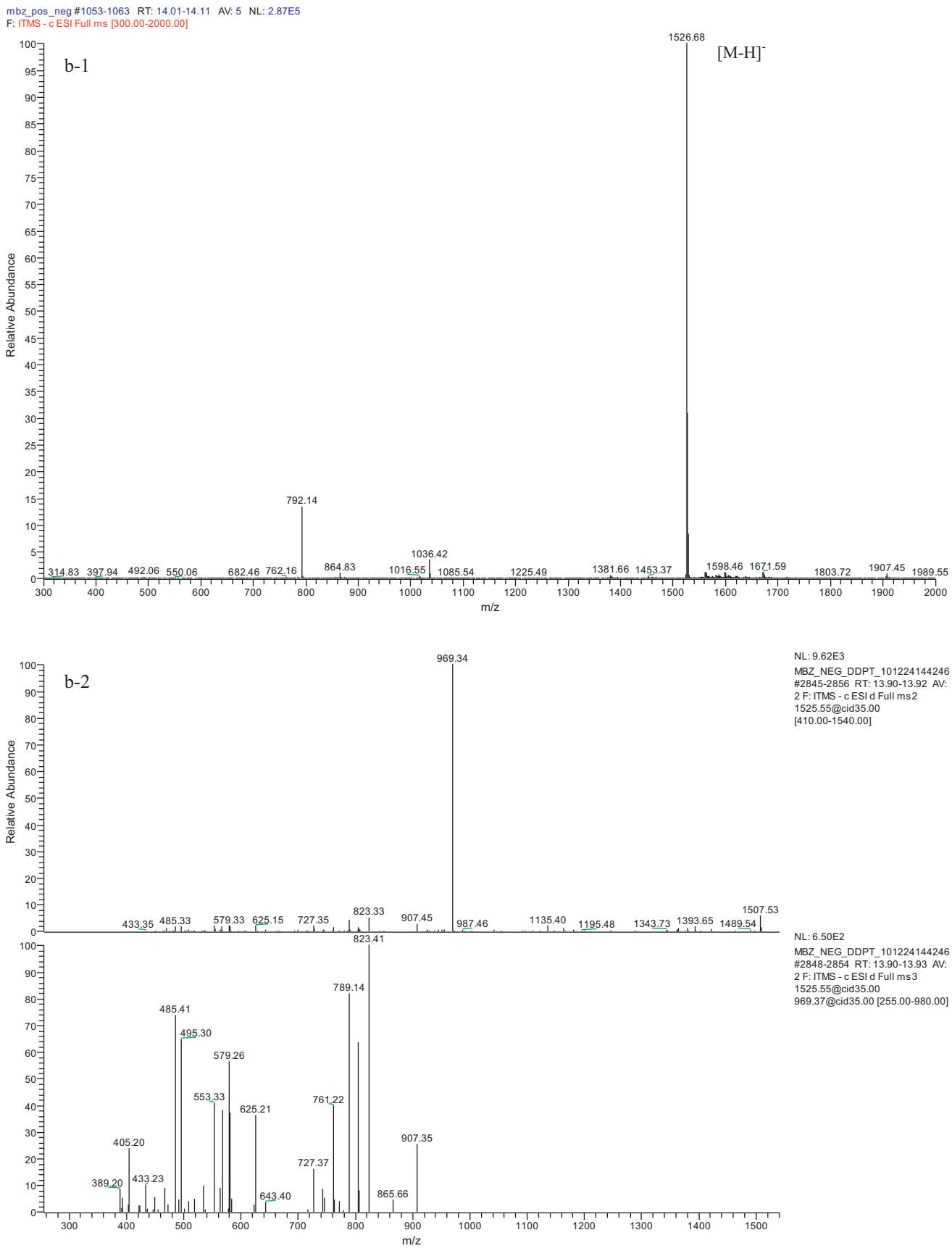


Fig. 5. (*Continued*)

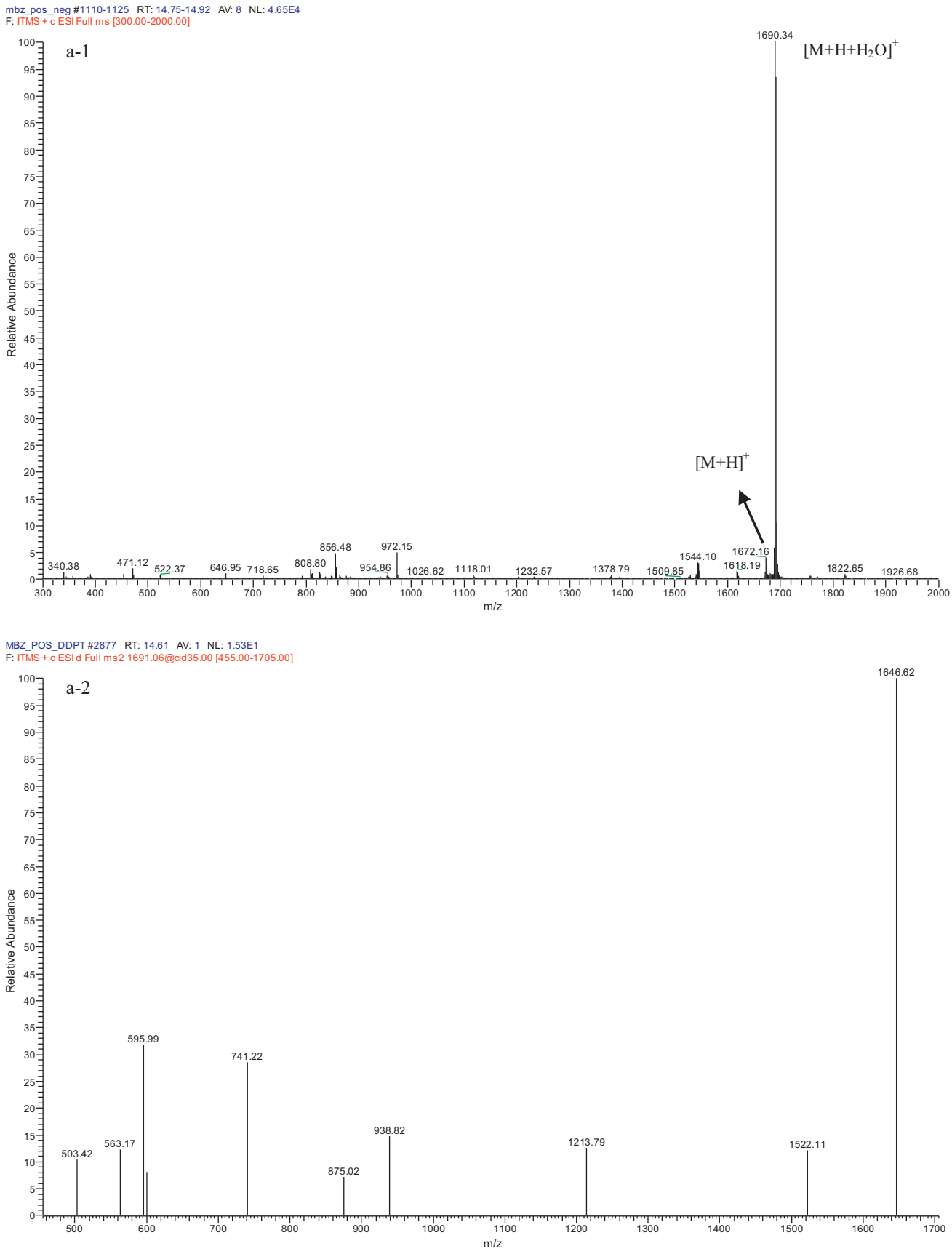


Fig. 6. MS^n spectra of D in positive ion mode (a-1, a-2) and in negative ion mode (b-1, b-2).

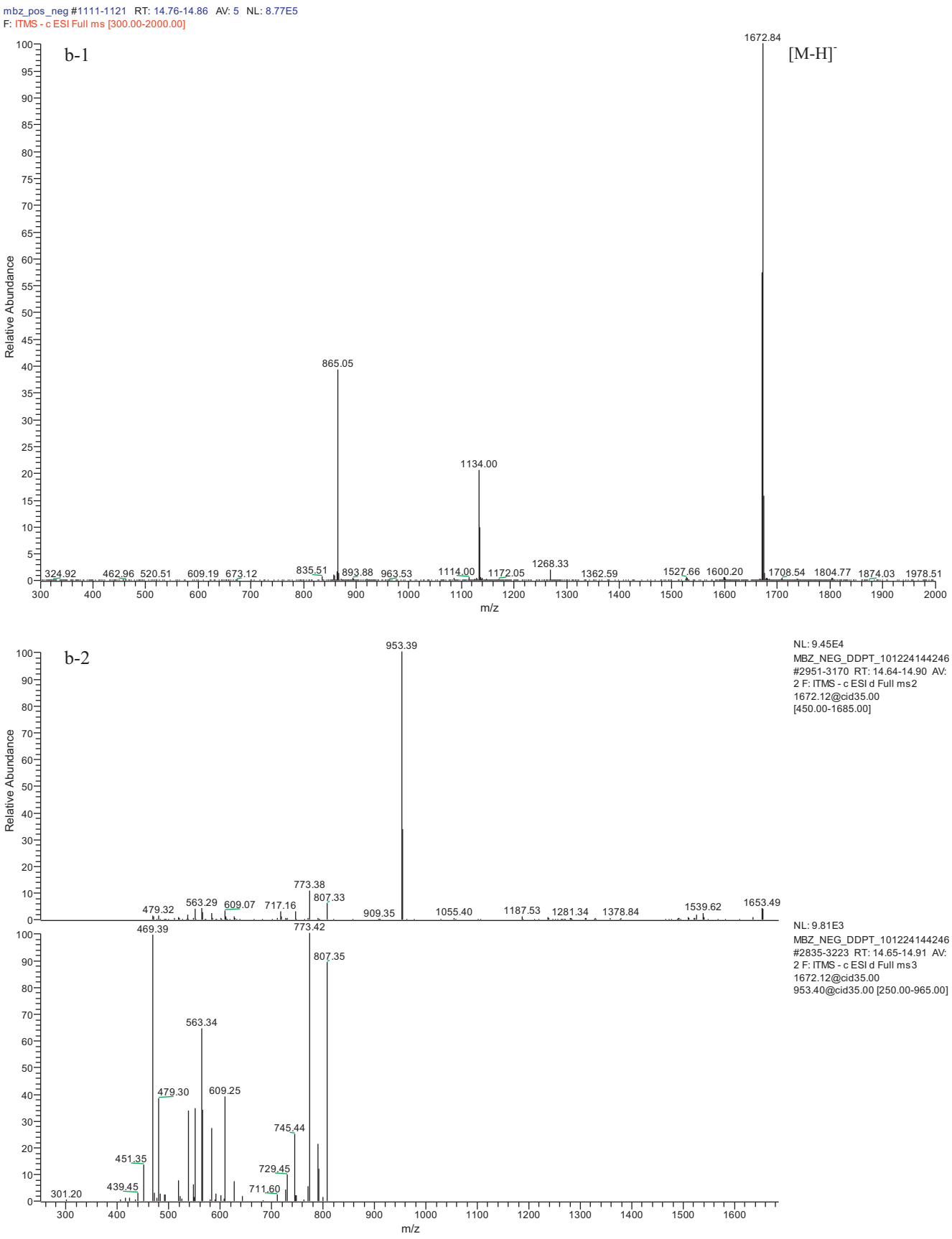


Fig. 6. (*Continued*)

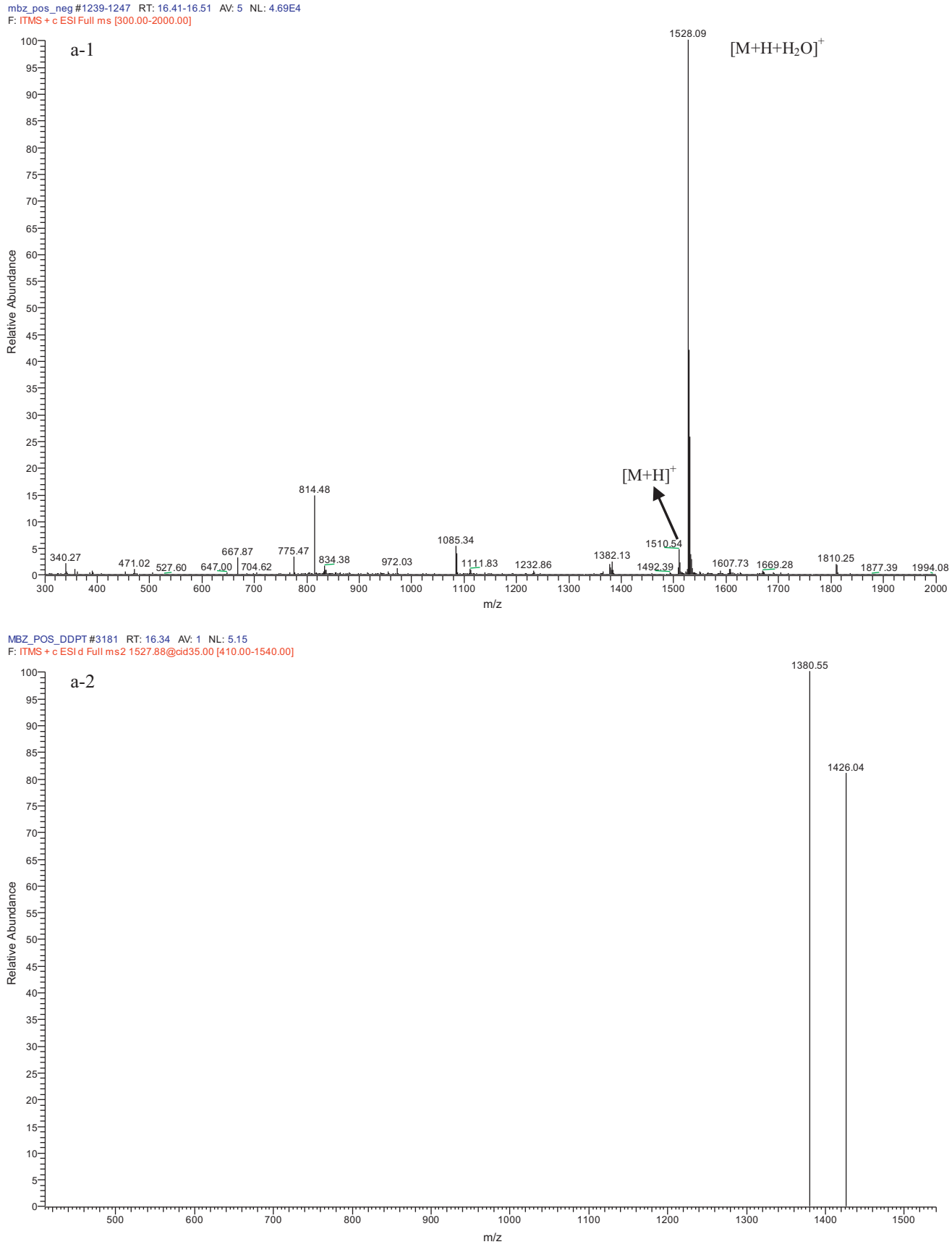


Fig. 7. MS^n spectra of E in positive ion mode (a-1, a-2) and in negative ion mode (b-1, b-2).

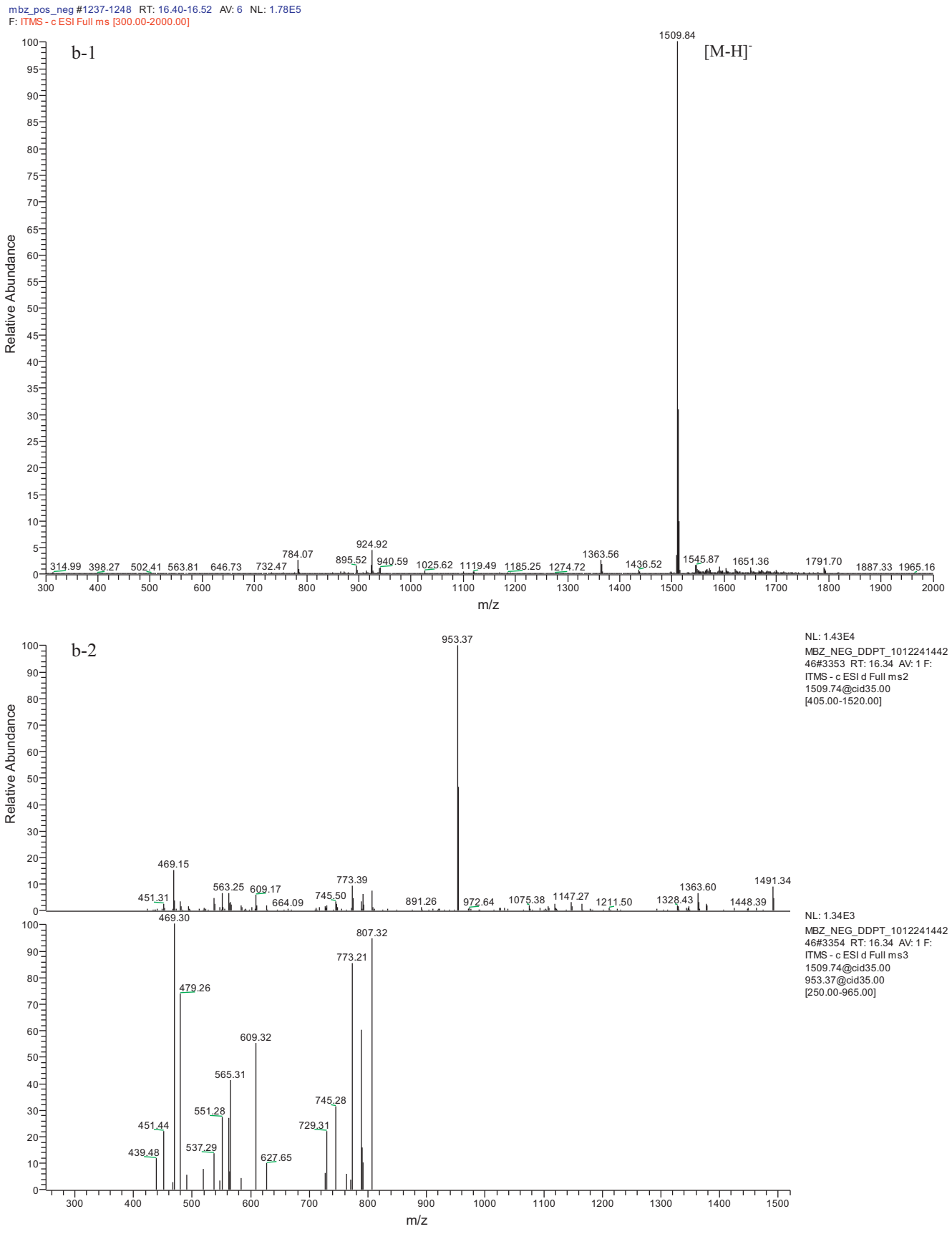


Fig. 7. (*Continued*)

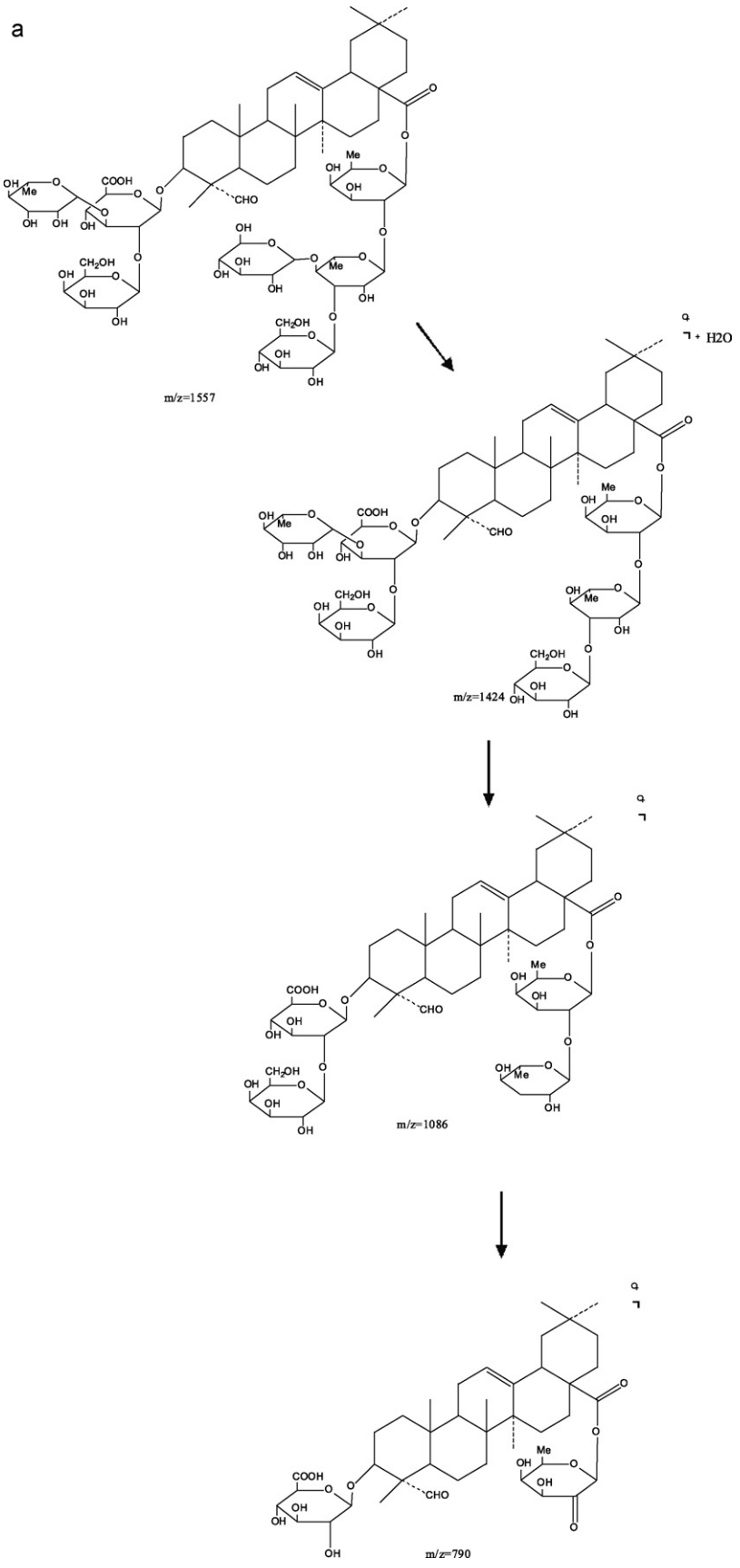


Fig. 8. Proposed fragmentation pathways of A in (a) positive ion mode and (b) negative ion mode.

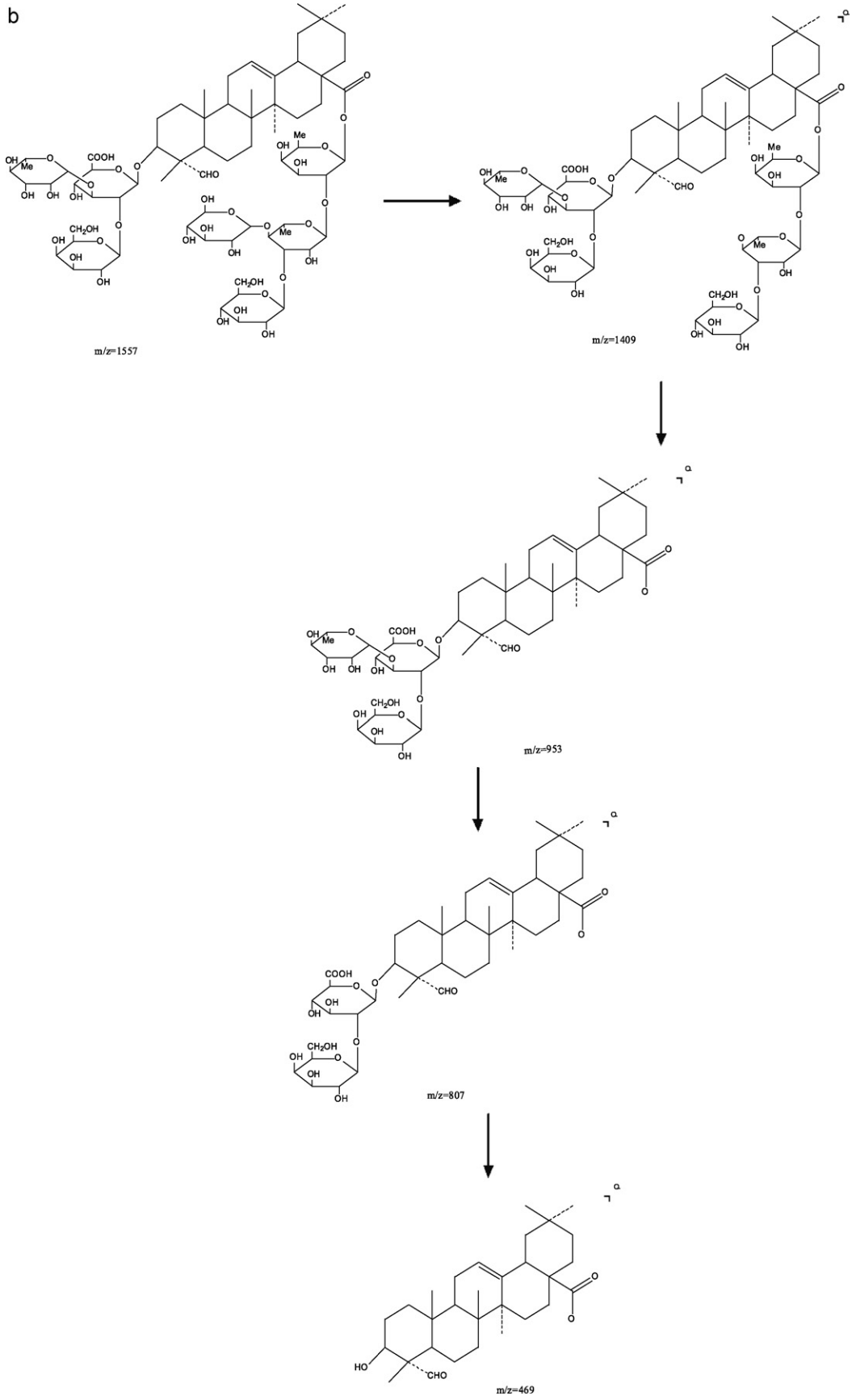


Fig. 8. (*Continued*)

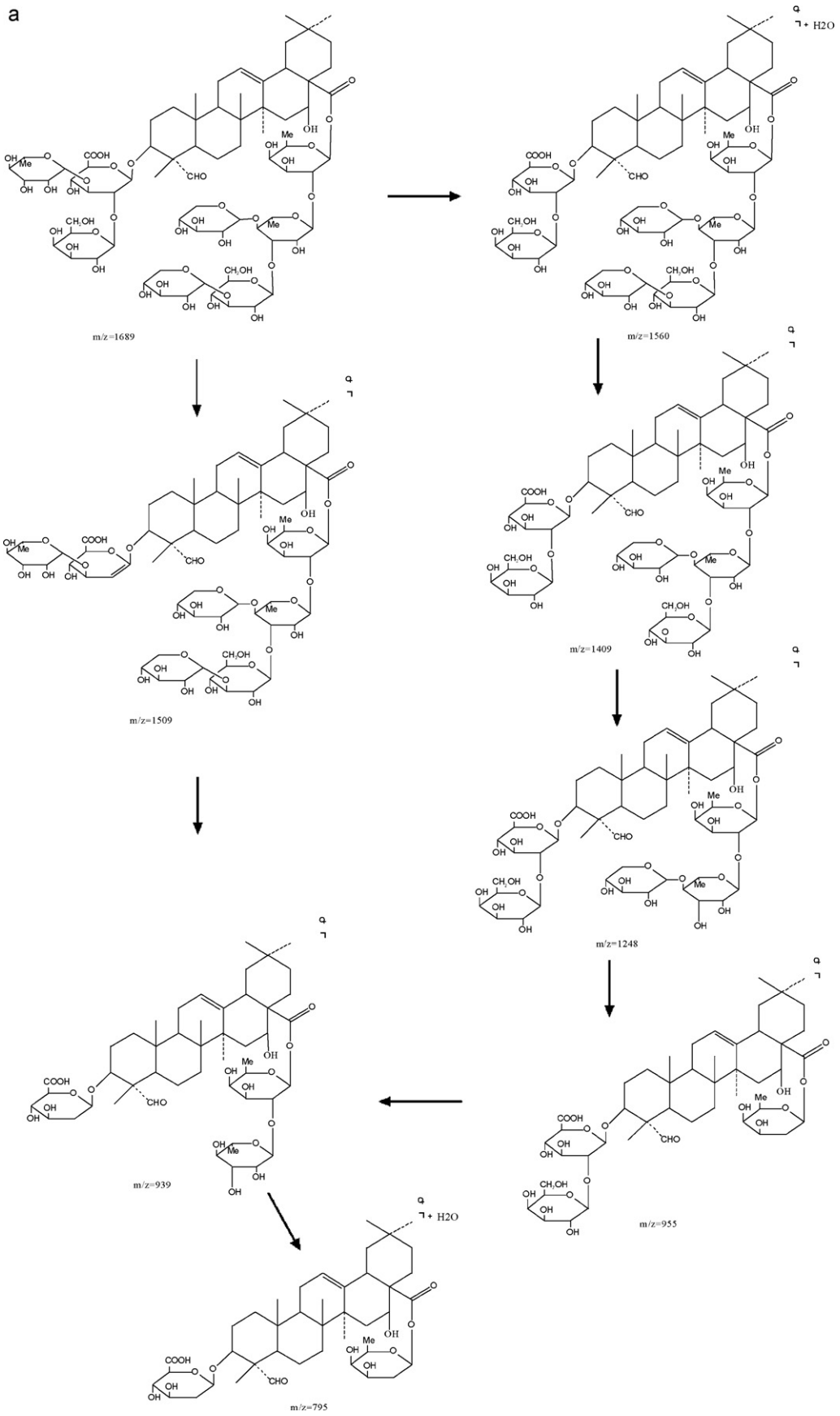


Fig. 9. Proposed fragmentation pathways of B in (a) positive ion mode and (b) negative ion mode.

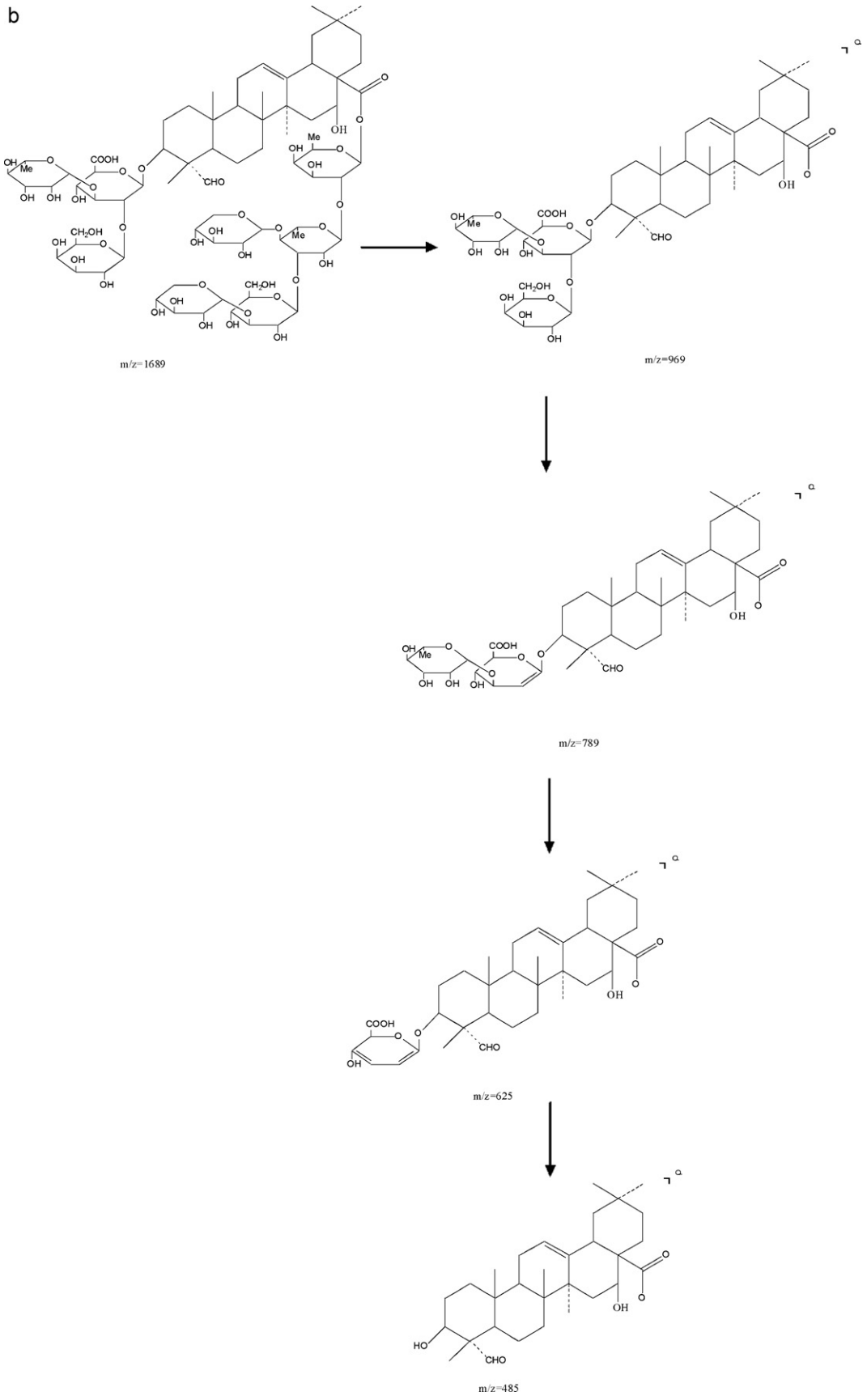


Fig. 9. (*Continued*)

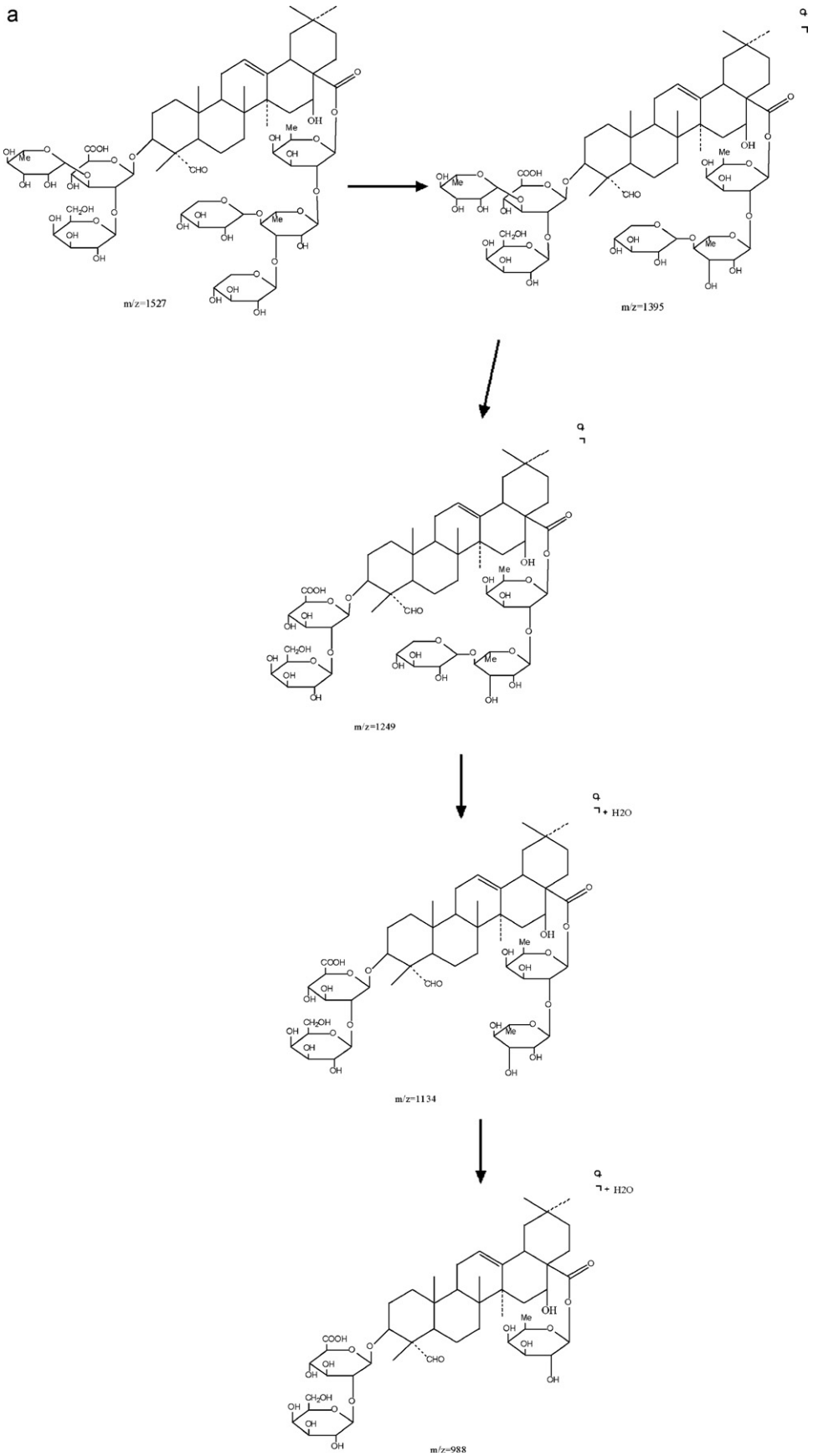


Fig. 10. Proposed fragmentation pathways of C in (a) positive ion mode and (b) negative ion mode.

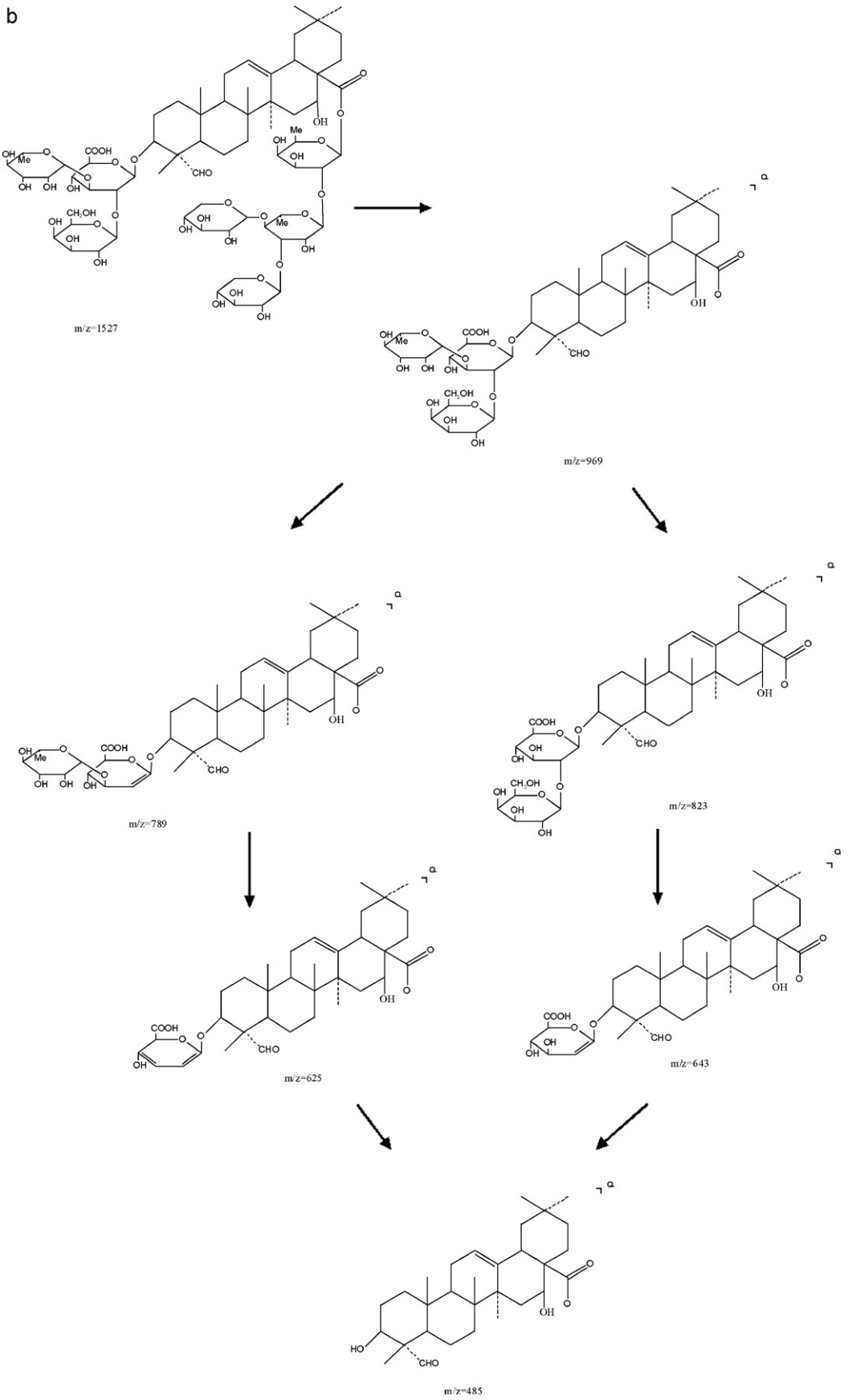


Fig. 10. (*Continued*)

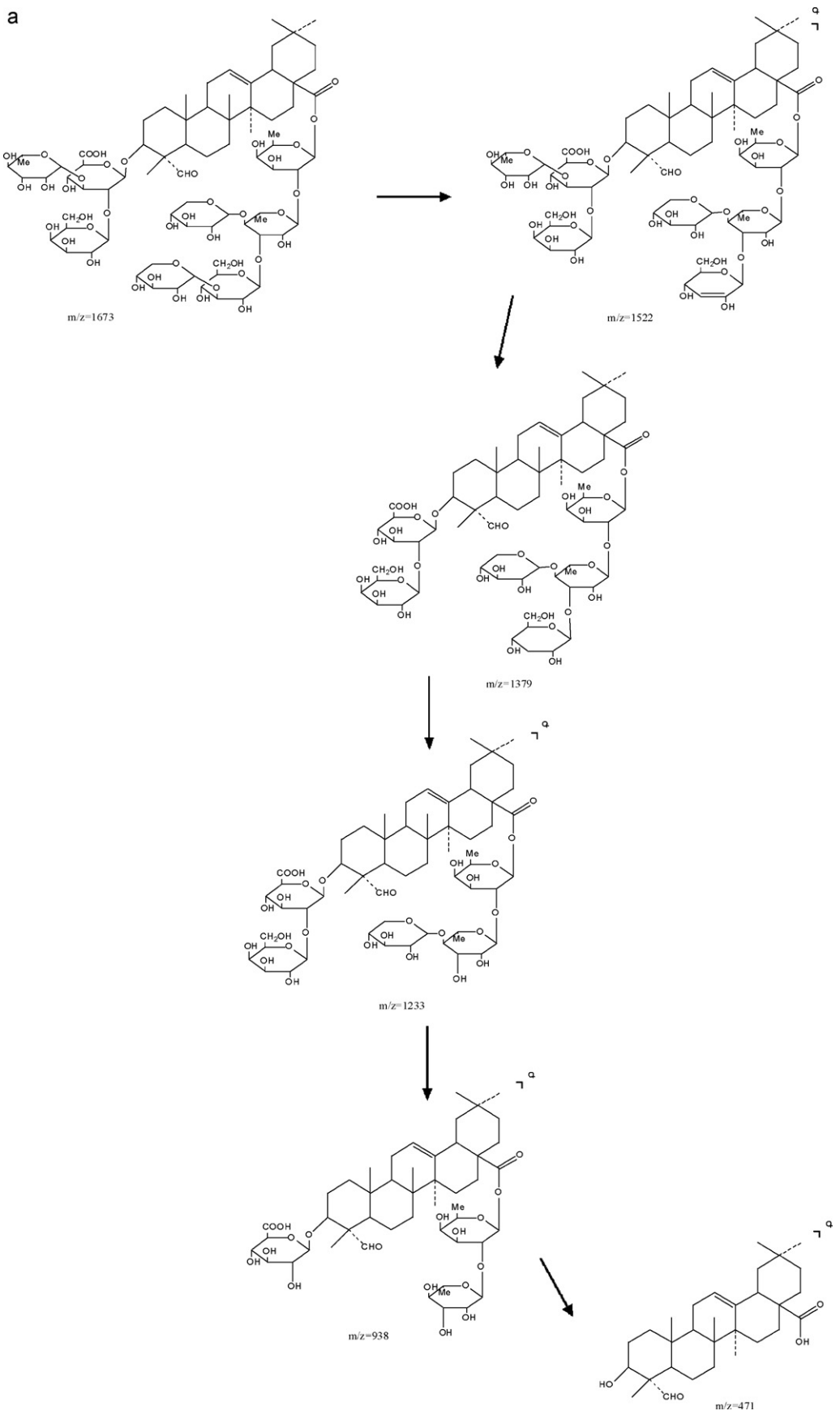
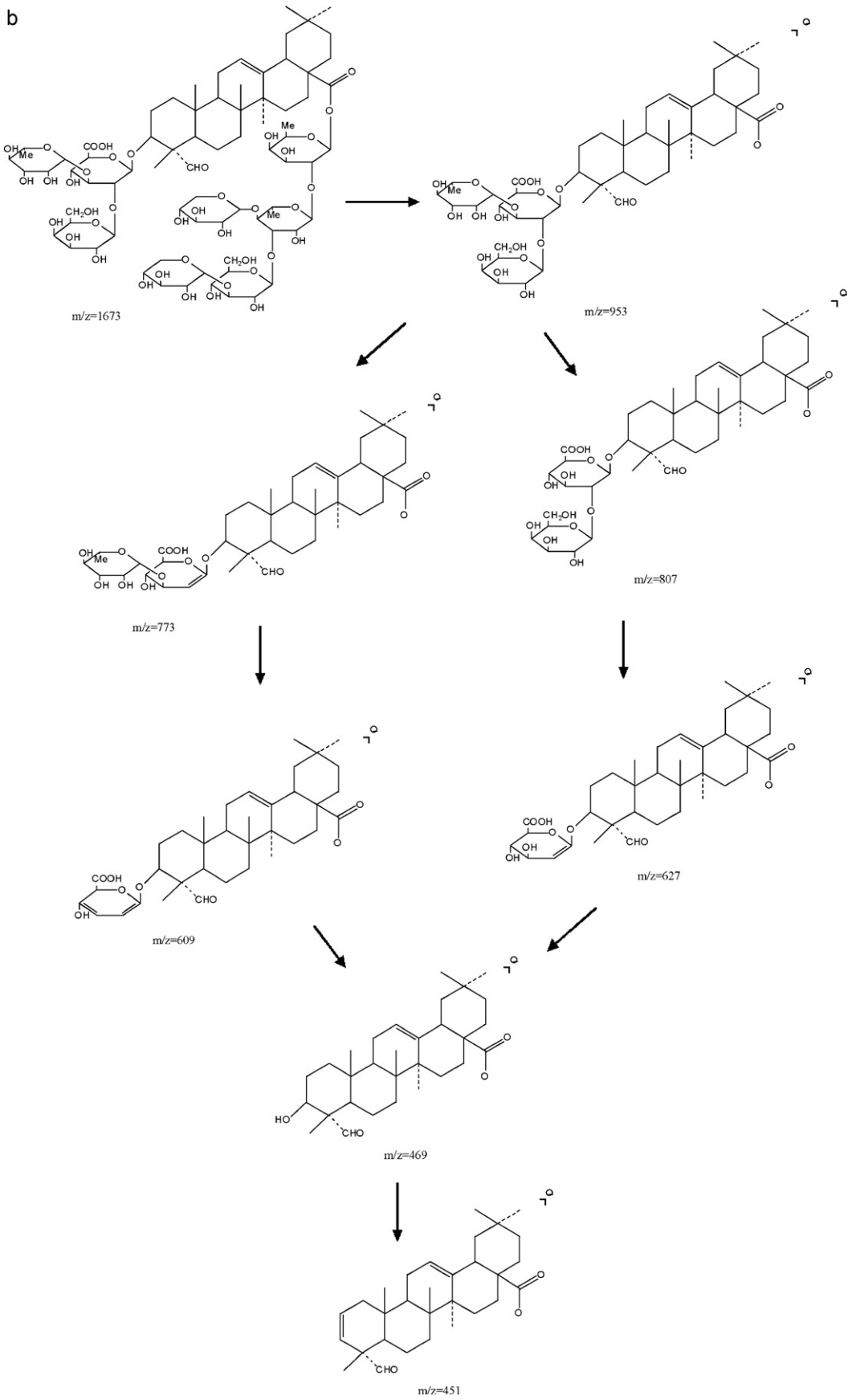


Fig. 11. Proposed fragmentation pathways of D in (a) positive ion mode and (b) negative ion mode.



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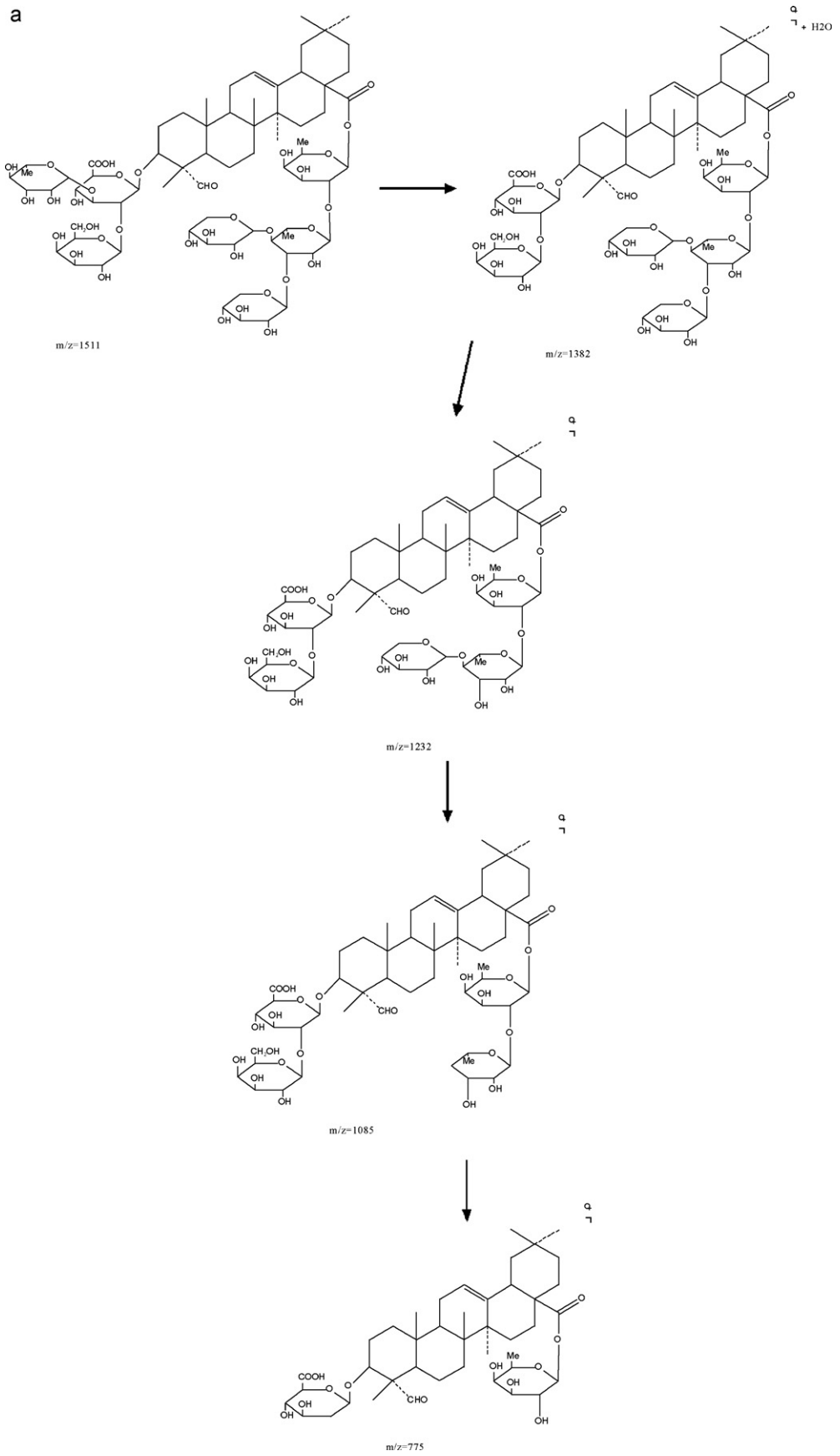

Fig. 12. Proposed fragmentation pathways of E in (a) positive ion mode and (b) negative ion mode.

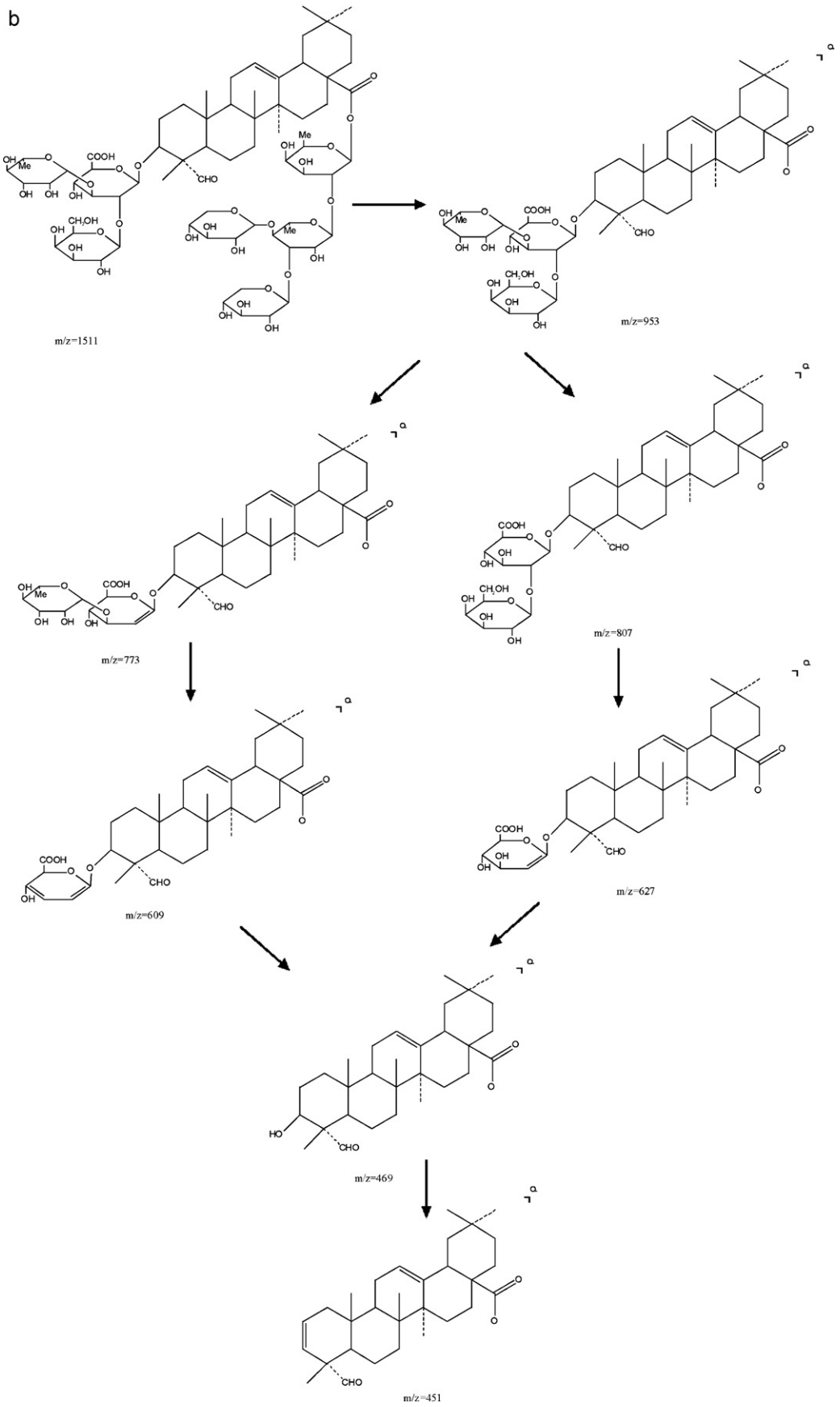

Fig. 12. (*Continued*)

The structure of *M. cochinchinensis* saponins should be determinated by the fragmentation of product ions from both positive ion mode and negative ion mode.

4. Conclusion

The ionization of five *M. cochinchinensis* saponins in F3 was investigated in both the positive and negative ESI ion modes. The $[M+H+H_2O]^+$ ion was the base peak in the positive ion mode, whereas the $[M-H]^-$ ion was the base peak in the negative ESI ion mode. The LC/MS^n experiments also confirmed that *M. cochinchinensis* saponins followed two distinct fragmentation pathways in two ESI ion modes. In negative ion mode, the bond between C28 and O was preferred cleaved and generated high abundance of fragment ions at m/z 953 or m/z 969. MS^n information of the ions at m/z 953 or m/z 969 were helpful to presume the structure of saccharic chain on C3 bond. In contrast, the fragmentation information of product ions in positive ion mode were conduced to infer the structure of saccharic chain on C28 bond. The final structure of the triterpenoid saponins could be concluded by the fragmentation information of product ions from both ESI ion modes. Comparison of different fragmentation patterns showed that the results from positive and negative ion ESI-MS^n were complementary. Both modes can yield structurally different information for the characterization of triterpenoid saponins. This study provides a powerful and rapid approach for the online structural elucidation and identification of triterpenoid saponins from *M. cochinchinensis* and other triterpenoid saponins distributed in related Traditional Chinese Medicines.

Acknowledgments

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