A Fragmentation Study on Four Unusual Secoiridoid Trimers, Swerilactones H–K, by Electrospray Tandem Mass Spectrometry

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Abstract Swerilactones H–K (1–4) as four unprecedented secoiridoid trimers represent a new type of natural product, which has attracted much interest of natural chemists due to their novel skeletons and promising bioactivity. In order to well understand their MS fragmentation behaviors, they were investigated by electrospray ionization ion-trap time-of-flight multistage product ion mass spectrometry (ESI-IT-TOF-MS^n) for the first time. The protonated molecules ([M+H]^+) of swerilactones J and K, and deprotonated molecules ([M-H]-) of swerilactones H, J and K were readily observed in the conventional single-stage mass spectra (MS); however only the [M+Cl]^− ion for swerilactone I was obtained in negative mode. Based on the MS^n study, the fragmentation pathways of swerilactones H and I in negative mode, and swerilactones J and K in both positive and negative modes were proposed. The neutral losses of H2O, CO, CO₂ and C₂H₄O moieties are the particular elimination from the precursor ions due to the presence of hydroxyl, δ-lactone and 1-O-ethyl moieties in their structures, of which the retro-Diels–Alder cleavage was the most particular dissociation. The fragment ions at m/z 341 and 291 in negative mode can be considered as the diagnostic ions for secoiridoid trimers. This investigation will provide valuable information for their fast characterization from complicated natural mixtures and extensive understanding their structural architectures.

Keywords ESI-IT-TOF-MS^n · Fragmentation rules · Secoiridoid trimers · Swerilactones H–K · Retro-Diels–Alder (RDA) cleavage

1 Introduction

Natural products with diversities in chemical structures and pharmacological activities provide versatile candidates in drug discovery. Many natural chemists are committed to searching for novel compounds to enrich this library. Swerilactones H–K (1–4) (Fig. 1), unprecedented secoiridoid trimers from the traditional Chinese herb Swertia mileensis, represent a new type of natural product, which has attracted much interest of natural chemists due to their novel skeletons and promising bioactivity [1–2]. However, this type of compound exists as minor components in plants, which presents challenges for their fast and reliable characterization [3]. Mass spectrometry (MS) with the associated high sensitivity and resolution well meets this requirement and has become the routine method in various aspects of medicinal chemistry [4–8]. Tandem MS techniques are particularly useful for ascertaining the relationship between precursor and product ions, by which the fragmentation rules and diagnostic ions of complicated...
compounds can be easily deduced [9–14]. The LCMS-IT-TOF mass spectrometer equipped with an electrospray ionization source linked to ion-trap and time-of-flight mass analyzers (ESI-IT-TOF) allows fast acquisition of multi-stage product ion spectra (MS\textsuperscript{n}) with high accuracy and resolution in both positive and negative modes [15–17]. This feature leads to easier interpretation of the origin of product ions, which is suitable for investigating the structures of natural products. In this paper, we report for the first time a high-resolution MS\textsuperscript{n} fragmentation study on swerilactones H–K (1–4) by ESI-IT-TOF mass spectrometer, which will provide valuable information not only for their fast characterization from complicated natural mixtures but also for a better understanding of their structural architectures.

2 Experimental

2.1 Apparatus and Analytical Conditions

MS\textsuperscript{n} analyses were acquired on the LCMS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan). The mass resolution was about 10000 full width at half maximum (FWHM). Accurate masses were corrected by calibration using sodium trifloroacetate (CF\textsubscript{3}CO\textsubscript{2}Na) clusters. MS experiments were achieved in automatic pattern, and MS\textsuperscript{n} experiments were performed in direct mode. Unless specified otherwise, analytical conditions were as follows: spray voltage, 4.50 and −3.50 kV; detector voltage, 1.60 kV; drying gas pressure, 100.0 kPa; nebulizing gas (N\textsubscript{2}) flow, 0.5 L/min; curved desolvation line (CDL) temperature, 200.0 °C; heat block temperature, 200.0 °C; equipment temperature, 40.0 °C; ion accumulation time, 10 ms; precursor ion selected width, m/z ± 3.0 Da, and selected time, 20 ms; collision induced dissociation (CID) collision time, 30 ms; collision energy, 50%; collision gas, 50%; and q = 0.251; scan range, m/z 100–1000 for MS.

The Shimadzu Composition Formula Predictor was used to determine the molecular formula.

2.2 Chemicals and Samples

HPLC grade acetonitrile (CH\textsubscript{3}CN) was purchased from Merck (Merck Co. Ltd., Germany). HPLC grade formic acid was purchased from Aladdin (Aladdin Chemistry Co. Ltd. China). Deionized water was purified using a MingChe\textsuperscript{TM}-D 24UV Merck Millipore system (Merck Millipore, Shanghai, China).

Swerilactones H–K (1–4) were isolated from S. mileensis in our previous investigation, whose structures were unambiguously determined by extensive spectroscopic data and X-ray analyses [1]. Sample solutions were prepared by dissolving each sample in a solution of 85% CH\textsubscript{3}CN/H\textsubscript{2}O containing 0.05% formic acid to a final concentration of 0.2 mg/mL. The samples were introduced into the source via a syringe pump at a flow rate of 2 μL/min.

3 Results and Discussion

Before MS\textsuperscript{n} investigation, the full-scan MS of compounds 1–4 in both positive and negative ion modes were acquired in automatic pattern. The protonated molecule ([M+H]\textsuperscript{+}) and deprotonated molecule ([M−H]\textsuperscript{−}) ions for swerilactones J (3) and K (4) were readily detected. However, swerilactones H (1) and I (2) only displayed [M−H]\textsuperscript{−} or [M+Cl]\textsuperscript{−} ion in negative mode. Therefore, the subsequent MS\textsuperscript{n} study for swerilactones H and I (1 and 2) in negative mode, and for swerilactones J and K (3 and 4) in both positive and negative modes was performed, from which their fragmentation pathways were proposed (Figs. 2, 3, 4, 5). It should be noted that alternative ways of fragmentation that can reasonably interpret the product ions are also possible in addition to the proposed pathway.
3.1 ESI-IT-TOF MS\textsuperscript{n} Fragmentations of Swerilactone H (1) in Negative Mode

In the single-stage mass spectrum of swerilactone H (1), the deprotonated molecular [M–H]\textsuperscript{−} ion at \textit{m/z} 567.1871 (1a) was readily obtained, corresponding to the molecular formula C\textsubscript{30}H\textsubscript{32}O\textsubscript{11}. When [M–H]\textsuperscript{−} (1a) was selected as the precursor ion to perform MS\textsuperscript{2} experiment, multiple product ions (1b–1k) were observed. Among them, the ions at \textit{m/z} 549 (1b) and 535 (1c) were deduced to be generated by the neutral losses of H\textsubscript{2}O and CH\textsubscript{4}O from 1a due to the presence of vicinal hydroxyl and methoxy groups [18–19]. The product ions at \textit{m/z} 519 (1d) and 505 (1e) were assigned to be the elimination of CH\textsubscript{2}O and the retro-Diels–Alder (RDA) cleavage of C\textsubscript{2}H\textsubscript{4}O from 1b [20]. Similarly, the cleavage of ring H by losing one C\textsubscript{3}H\textsubscript{6}O\textsubscript{2} fragment from 1e generated ion 1f at \textit{m/z} 431, and the most abundant ion at \textit{m/z} 363 (1g) could be explained by subsequent loss of a C\textsubscript{3}H\textsubscript{6}O molecule by an RDA-like process [21]. It is noteworthy that two abundant ions at \textit{m/z} 341 (1h) and 297 (1j/1j\textsuperscript{0}) were readily observed in the MS\textsuperscript{2} spectrum, of which the ions at \textit{m/z} 297 were present as double peaks at \textit{m/z} 297.0792 (1j) and 297.1105 (1j\textsuperscript{0}), corresponding to the chemical composition of C\textsubscript{17}H\textsubscript{13}O\textsubscript{5} and C\textsubscript{18}H\textsubscript{17}O\textsubscript{4} (Fig. 2). The ion at \textit{m/z} 341 (1h) could be well interpreted by the RDA cleavage of ring F to lose a C\textsubscript{11}H\textsubscript{14}O\textsubscript{5} part [22–23]. Thus, the elimination of 44 Da can be attributed to the losses of C\textsubscript{2}H\textsubscript{4}O and CO\textsubscript{2} to yield product ions 1j (\textit{m/z} 297.0792) and 1j\textsuperscript{0} (\textit{m/z} 297.1105) [24]. Likewise, the ion 1j could further lose a molecule of CO\textsubscript{2} to generate ion 1k (\textit{m/z} 253). In the MS\textsuperscript{3} experiment from the precursor ion 1g (\textit{m/z} 363), two product ions at \textit{m/z} 319 (1i) as base peak and 275 (1l) were detected, which were proposed to be arisen from the sequential loss of two CO\textsubscript{2} molecules.

3.2 ESI-IT-TOF MS\textsuperscript{n} Fragmentations of Swerilactone I (2) in Negative Mode

Compared to swerilactones H, J and K, swerilactone I (2) was more unstable in this MS\textsuperscript{n} study, and thus, gave rise to less MS\textsuperscript{n} information, which might be due to the presence

Fig. 2 Proposed fragmentation pathways of swerilactone H (1) in negative mode

Fig. 3 Proposed fragmentation pathways of swerilactone I (2) in negative mode
of aldehyde group in the structure. In the full-scan mass spectrum, swerilactone I (2) displayed neither [M+H]+ nor [M−H]− ions, but an ion at m/z 571.1383 (2a) was readily obtained in the negative ion mode. This ion was ascribed with the chemical composition of C_{29}H_{28}O_{10}Cl ([M−Cl]−) based on its high accordance in both accuracy (0.7 mDa) and isotopic abundance (83.9%) with those of the theoretical values. However, the origin of Cl− ion was unclear, which was always encountered in negative ESIMS investigation [25–26]. In addition to the [M+Cl]− ion, two fragments at m/z 341 (2b) and 297 (2c) were observed with high abundance. The ion 2b corresponding to the loss of a C_{10}H_{11}O_{4}Cl motif (rings G and H) from ion 2a could be explained by the RDA ring-opening of ring F, and the ion 2c was proposed to be generated by a further RDA process leading to the loss of a C_{9}H_{8}O part from ion 2b (Fig. 3). The above deduction was confirmed by the MS\textsuperscript{2} experiment on 2a, which gave rise to the expected ions 2b (m/z 341) and 2c (m/z 297), and the subsequent MS\textsuperscript{3} experiment on 2b in which the fragment ion at m/z 297 (2c) was further detected. Combined with the observation that the fragmentation ion 2c showed much higher abundance compared to the parent [M+Cl]− ion in the first stage mass spectrum, the following MS\textsuperscript{2} experiment was further performed on ion 2c (m/z 297) to generate three characteristic ions at m/z 253 (2d), 223 (2e) and 209 (2f). The ion 2d was attributed to the neutral loss of a CO\textsubscript{2} moiety from ion 2c, and the ions 2e and 2f were corresponding to the elimination of one CH\textsubscript{2}O or CO\textsubscript{2} parts from 2d. In the MS\textsuperscript{3} experiment on ion 2d, the expected fragment ions 2e (m/z 223) and 2f (m/z 209) were readily displayed, which was in accordance with the above deduction. When ion 2e was further selected for MS\textsuperscript{4} experiment, a fragment ion at m/z 195 (2g) corresponding to a 28 Da loss was obtained.
which was deduced as the elimination of one CO moiety from the ion 2e [18].

### 3.3 ESI-IT-TOF MS<sup>5</sup> Fragmentations of Swerilactone J (3) in Positive and Negative Modes

In the positive full-scan mass spectrum, the [M+H]<sup>+</sup> ion (3A) at m/z 537.1732 was readily detected, as well as the fragment ion (3B) at m/z 519 ([M+H–H<sub>2</sub>O]<sup>+</sup>) which was displayed as base peak in the subsequent MS<sup>2</sup> experiment from 3A. The MS<sup>3</sup> product ion at m/z 493 (3D) was designated as the RDA elimination of C<sub>2</sub>H<sub>4</sub>O moiety from 3A due to the presence of 1-O-ethyl group in the structure (Fig. 4). Similarly, the ion 3F (m/z 475) was formed by losing a C<sub>2</sub>H<sub>2</sub>O part from 3B, and further gave rise to ions 3H (m/z 447) and 3J (m/z 403) via consecutive elimination of one CO and one CO<sub>2</sub> molecule [20]. The loss of a C<sub>2</sub>H<sub>2</sub>O segment was characteristic, by which the fragments 3E (m/z 477), 3I (m/z 433) and 3L (m/z 361) were formed from their respective parent ions 3B, 3F and 3J. In the MS<sup>5</sup> spectrum from 3B, the ion 3C (m/z 501) corresponding to the loss of a H<sub>2</sub>O molecule was observed. With the elimination of a C<sub>3</sub>H<sub>4</sub>O<sub>2</sub> part, the ion 3K (m/z 387) was produced, and further generated ions 3M (m/z 343) and 3N (m/z 315) by successive losses of CO<sub>2</sub> and CO molecules. The ion 3O (m/z 307) in the MS<sup>3</sup> spectrum was correspondent to the elimination of C<sub>6</sub>H<sub>6</sub>O<sub>3</sub> moiety from precursor 3I.

The MS<sup>5</sup> investigation on swerilactone J (3) in negative mode provided more valuable information than that in positive mode. The first-stage mass spectrum displayed the [M–H]<sup>–</sup> ion at m/z 535.1602, assigned to the molecular formula C<sub>29</sub>H<sub>28</sub>O<sub>10</sub>. It should be noted that two fragmentation ions at m/z 491 (3b) and m/z 341 (3j)<sup>–</sup> were readily obtained with high abundance in addition to the [M–H]<sup>–</sup>
ion, assigned to the molecular formula C_{28}H_{27}O_{8} and C_{19}H_{27}O_{6}, respectively. The ion 3b was explained by the neutral loss of CO_{2} from the precursor ion 3a, and further confirmed by MS\textsuperscript{2} analysis in which the ion at m/z 491 was obtained as base peak. The ion 3j\textsuperscript{\textprime} (C_{19}H_{17}O_{6}) was proposed to be derived from 3a by neutral loss of a C_{10}H_{12}O_{4} part, due to the RDA cleavage of ring F \[20\]. When ion 3b (m/z 491) was selected as the precursor ion to perform MS\textsuperscript{2} experiment, prolific fragment ions were obtained, from which their fragmentation rules were proposed as shown in Fig. 4. Due to the high abundance of ion 3j\textsuperscript{\textprime} (m/z 341.1030, C_{19}H_{17}O_{6}) in the first-stage mass spectrum, subsequent MS\textsuperscript{2-4} experiments were applied on ion 3j\textsuperscript{\textprime}, from which a parallel fragmentation pathway was recognized. The neutral loss of CO_{2} from 3j\textsuperscript{\textprime} provided ion 3m\textsuperscript{\textprime} (m/z 297.1081), and further produced ions 3n (m/z 279) and 3o (m/z 253) through the elimination of H_{2}O or C_{2}H_{4}O part. In the MS\textsuperscript{3} experiment from the precursor ion 3n (m/z 279), characteristic product ions at m/z 235, 220 and 193 were obtained, of which the ion at m/z 235 was consist of two closed peaks at m/z 235.1087 (C_{17}H_{15}O, 3p) and 235.0749 (C_{16}H_{11}O_{2}, 3p\textsuperscript{\textprime}), attributed to the neutral losses of CO_{2} or C_{2}H_{4}O moiety from 3n.

3.4 ESI-IT-TOF MS\textsuperscript{n} Fragmentations of Swerilactone K (4) in Positive and Negative Modes

Structurally, swerilactone K (4) with an aromatic ring is obviously different from swerilactones H–J (1–3). The first-stage mass spectrum in positive mode displayed [M+H]\textsuperscript{+} ion (4a) at m/z 519.1651, corresponding to the molecular formula C_{28}H_{26}O_{9}. The subsequent MS\textsuperscript{2} experiment from 4a yielded two high-abundance ions 4b (m/z 475) and 4c (m/z 457, base peak), attributed to the successive losses of C_{2}H_{4}O and H_{2}O parts, in combination with three minor ions at m/z 299 (4j), 281(4k) and 253 (4l). The ion 4j was interpreted by the neutral loss of C_{10}H_{8}O_{3} from the precursor 4b due to the RDA cleavage of ring F, and further gave rise to ions 4k (m/z 281) and 4l (m/z 253) by the elimination of a molecule of H_{2}O and CO (Fig. 5). This deduction was also confirmed by the MS\textsuperscript{3} analysis from the parent ion 4b. When ion 4c (m/z 457) was applied for the MS\textsuperscript{3} experiment, the most intensive ion at m/z 439 (4d) was readily detected, ascribe to the loss of H_{2}O, together with a series of fragment ions 4e–4i.

In the negative ion mode, sweilactone K (4) gives rise to the deprotonated ion at m/z 517.1511, correlated to the molecular formula C_{28}H_{26}O_{9}. The following MS\textsuperscript{2} experiment on 4a provided versatile fragments with ion at m/z 473 (4d) as base peak which was further applied for MS\textsuperscript{3} spectrum. Based on the above experiments, the fragmentation rules for swerilactone K (4) in negative mode were concluded. The minor ions at m/z 499 (4b) and 489 (4c) in MS\textsuperscript{2} spectrum were derived from neutral loss of H_{2}O and CO from the precursor 4a. The most abundant ion 4d (m/z 473) generated from 4a by the RDA elimination of C_{2}H_{4}O segment, can further give rise to ions at m/z 427 (4e), 383 (3f) and 339 (4h) by sequential losses of CH_{2}O_{2}, CO_{2} and CO_{2} parts. In the MS\textsuperscript{3} spectrum from 4d, the product ions 4j (m/z 268), 4k (m/z 267), 4m (m/z 239) and 4n (m/z 211) could be explained by the consecutive elimination of C_{11}H_{9}O_{4} radical, hydrogen radical, CO and CO, respectively.

4 Conclusion

The ESI multistage product ion mass spectra (MS\textsuperscript{n}) of swerilactones H–K were obtained for the first time by LCMS-IT-TOF, from which their fragmentation pathways were deduced. This investigation suggested that these molecules were unstable in this MS\textsuperscript{5} study, especially for sweilactone I. The losses of H_{2}O, CO_{2}, CO and C_{2}H_{4}O moieties were the particular elimination from the precursor ions due to the presence of hydroxyl, 6-lactone and 1-O-ethyl groups. In particular, the RDA dissociation was the most common fragmentation rule which might correspond to the fused six-membered rings in their structures. It is important to note that the loss of CO_{2} and C_{2}H_{4}O can be unambiguously distinguished by high-resolution mass spectrometry. Structurally, swerilactones H–K share a closely related skeleton with the main difference located at rings F, G and H. Therefore, the conservative moiety (rings A to E) leads to the common fragments at m/z 341 and 291 in negative mode, which can be considered as the diagnostic ions for secoiridoid trimers. The present MS\textsuperscript{n} fragmentation study on swerilactones H–K (1–4) by ESI-IT-TOF mass spectrometer will provide valuable information not only for their fast characterization from complicated natural mixtures but also for a better understanding of their structural architectures.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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