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A fragmentation study on four C_{19}-diterpenoid alkaloids by electrospray ionization ion-trap time-of-flight tandem mass spectrometry

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High-resolution electrospray ionization ion-trap time-of-flight tandem mass spectrometry (HR-ESI-IT-TOF-MS) in positive-ion mode was used to determine the accurate masses and fragmentation pathways of four C_{19}-diterpenoid alkaloids, aconitine (1), yunnanconitine (2), crassicauline A (3), and benzoylmesaconine (4). The [M + H]^+ ions of compounds 1–4 were readily observed in conventional single-stage mass spectrometry. Based on the MS{sup 1–6} analyses, detailed fragmentation rules of the four compounds were proposed. The neutral losses of AcOH, MeOH, H_{2}O, CO, C_{2}H_{4}, PhCOOH and p-OMePhCOOH segments were the characteristic eliminations from the precursor ions due to the presence of acetyl, methoxyl, hydroxyl, N-ethyl, benzoyl and p-methoxyl-benzoyl units in the structures. Benefited from the high resolution of the mass analyzer, the loss of 28 Da corresponding to CO or CH_{4} segment in product ions was unambiguously distinguished. The losing sequence of the main substituent groups was summarized as: C(8)-acetyl > C(16)-methotyl > C(15)-hydroxyl > C(6)-methoxyl > C(1)-methoxyl/C(3)-hydroxyl > C(18)-methoxyl > C(13)-hydroxyl. The sequential loss of (16)-methoxyl moiety and CO (generating from enol–ketone tautomerism) groups could be recognized as the characteristic eliminations for the compounds with C(16)-methoxyl and C(15)-hydroxyl groups simultaneously. The application of HR-ESI-IT-TOF-MS technique to investigate the fragmentation of C_{19}-diterpenoid alkaloids provided useful information to understand their fragmentation behaviors.

Keywords: Ranunculaceae; Aconitum; HR-ESI-IT-TOF-MS; Fragmentation rules; C_{19}-diterpenoid alkaloids

1. Introduction

C_{19}-diterpenoid alkaloids as the main substance of plants Aconitum L. (Ranunculaceae) show wide range of bioactivities [1–3], such as anti-inflammatory, cardiotonic, and analgesic effects; however, the obvious toxicity limits their applications in clinic [4]. According to the previous reports [4], diester-diterpenoid alkaloids are the major constituents of the root of Aconitum kusnezoffii Reichb. and cause toxicities and side effects. Therefore, many strategies were attempted to reduce the toxicity or establish a fast and effective method for detecting diester-diterpenoid alkaloids [5–8].

Mass spectrometry (MS) with high sensitivity and resolution has become the routine method in various aspect of analytic chemistry. In particular, the tandem mass spectrometry (MS^n) techniques make it possible to determine the relationship between the precursor and product ions, by which the fragmentation
rules can be resolved. Cui et al. [9] investigated the mass spectrometric behavior of eight phenanthroindolizidine alkaloids by ESI-MS$^n$ and established a fast method by the rules to identify the trace-level alkaloids in the crude of Tylophora atrfolliculata. Nagy et al. [10] reported the detailed fragmentation behavior of protonated noscapines under electrospray condition. These studies have demonstrated the advantages of using the MS/MS technique for identifying natural products. Although some papers reported the fragmentation rules of C$_{19}$-diterpenoid alkaloids [11–15], the MS$^n$ analyses were performed by low-resolution mass analyzer and the investigation based on high resolution mass analyzer were scarce.

Aconitine (1), yunnaconitine (2), crassicauline A (3), and benzoylmesaconine (4) (as shown in Figure 1) are four aconitine-type alkaloids with a C$_{10}$ skeleton, of which aconitine (1), yunnaconitine (2), and crassicauline A (3) belong to the diester-diterpenoid alkaloids. Compounds 1–4 are appropriate candidates for tandem mass spectrometry (MS$^n$) study due to the suitable molecule masses (>500 Da) and the prolific substituent groups. To our knowledge, there are no systematic reports about the high-resolution MS$^n$ fragmentation rules of the four compounds. In this paper, the LC/MS-IT-TOF mass spectrometer equipped with an electrospray ionization source linked to ion-trap and time-of-flight mass analyzers (ESI-IT-TOF) which enables fast acquisition of multistage tandem spectrometry (MS$^1$–$^{10}$) was used to investigate the fragmentation rules of compounds 1–4. It is important to use HR-ESI-IT-TOF-MS$^n$ technique to investigate the fragmentation of C$_{19}$-diterpenoid alkaloids, which will provide useful information to understand their fragmentation behaviors.

2. Results and discussion

Aconitine (1), yunnaconitine (2), crassicauline A (3), and benzoylmesaconine (4) are four natural C$_{19}$-diterpenoid alkaloids with alkalinity so that MS$^n$ experiments in positive mode were applied to characterize their fragmentation behaviors. The proposed fragmentation pathways are shown in Figures 2, 3, S2 and S3 and data for accurate masses and elemental compositions from tandem mass spectrometry are shown in Tables 1–4.

2.1. ESI-MS$^n$ results

2.1.1. ESI-IT-TOF MS$^n$ fragmentations of aconitine (1) in positive mode (as shown in Figure 2 and Table 1)

The full-scan mass spectrometry for aconitine (1) in positive mode was analyzed to afford the [M + H]$^+$ ion (1a) at m/z 646.3220, corresponding to the molecular formula C$_{34}$H$_{48}$NO$_{11}$. Ion 1a was selected as precursor ion to yield versatile product ions (1b–1k) in MS$^2$ experiment, and the [M + H – AcOH]$^+$ ion at m/z 586 (1b) with high intensity (90%) further yielded the fragment at m/z 554 (1c) due to the departure of MeOH at C(16) position. The ketone-enol tautomers (between Δ$^{15,16}$ and

![Figure 1. Structures of compounds 1–4.](image-url)
C(15)-hydroxyl group] of the unstable ion 1c lost a carbonyl moiety as one molecule of CO to give rise to the ion 1d (90%) at m/z 526 or eliminated C(6)-methoxyl unit directly to produce the ion 1e at m/z 522. Then the ion 1d lost C(6)-methoxyl group to yield the ion at m/z 494 (1f, 100%). After that, 1e and 1f both lost the methoxyl moiety at C(1) position to generate ions 1g (m/z 490) and 1i (m/z 462), respectively. The ion 1h at m/z 476 could be interpreted by the departure of C(3)-hydroxyl group from 1f, and the elimination of a 122 Da (PhCOOH) from 1d and 1g was happened to afford ions 1j (m/z 404) and 1k (m/z 368) due to the presence of C(14)-benzoyl unit.

Ions 1b (m/z 586), 1h (m/z 476), 1j (m/z 404), and 1k (m/z 368) were selected as precursor ions to perform MS³ experiments, which provided ions 1c, 1d, 1f, 1h, 1i, 1j, and 1l–1q. The fragments 1c, 1d, 1f, 1h, 1i, and 1j could be also detected in the MS² analysis. The ion 1l (m/z 354, 100%) was the most abundant product ion due to the loss of PhCOOH from precursor ion 1h. Precursor ion 1j eliminated C(6)-methoxyl.
group to generate the ion Im at m/z 372 due to the formation of a stably conjugated structure. Then Im gave rise to ions Ii (m/z 354) and In (m/z 340) in agreement with the departure of C(3)-hydroxyl and C(1)-methoxyl units, and the ion Io (m/z 340)
Table 1. Data for accurate masses and elemental composition of aconitine (I) observed from tandem mass spectrometry in positive mode.

| MS<sup>+</sup> | Precursor ion (m/z) | Product ion (m/z) | Elemental composition | Measured (m/z) | Calculated (m/z) | Error (mDa) | Ion name | Intensity (%) | Assignment |
|----------------|---------------------|------------------|----------------------|----------------|------------------|------------|----------|--------------|------------|
| (+)MS 645      | 646                 | C<sub>34</sub>H<sub>48</sub>N<sub>11</sub> | 646.3220             | 646.3222        | 0.2              | I<sub>a</sub>  | 100      | [M + H]<sup>+</sup> |
| MS<sup>2</sup> 586 | 554                 | C<sub>32</sub>H<sub>44</sub>N<sub>9</sub> | 554.2739             | 554.2748        | 0.9              | I<sub>c</sub>  | 20       | I<sub>b</sub> - AcOH |
|                | 522                 | C<sub>30</sub>H<sub>38</sub>N<sub>7</sub> | 522.2435             | 522.2486        | 5.1              | I<sub>e</sub>  | 15       | I<sub>c</sub> - MeOH |
|                | 494                 | C<sub>28</sub>H<sub>34</sub>N<sub>6</sub> | 494.2541             | 494.2537        | 0.4              | I<sub>f</sub>  | 100      | I<sub>d</sub> - MeOH |
|                | 490                 | C<sub>26</sub>H<sub>32</sub>N<sub>6</sub> | 490.2259             | 490.2224        | 3.5              | I<sub>g</sub>  | 15       | I<sub>e</sub> - MeOH |
|                | 476                 | C<sub>24</sub>H<sub>30</sub>N<sub>5</sub> | 476.2433             | 476.2431        | 0.2              | I<sub>h</sub>  | 45       | I<sub>f</sub> - H<sub>2</sub>O |
|                | 462                 | C<sub>22</sub>H<sub>28</sub>N<sub>5</sub> | 462.2271             | 462.2275        | 0.4              | I<sub>i</sub>  | 20       | I<sub>f</sub> - MeOH |
|                | 404                 | C<sub>23</sub>H<sub>24</sub>N<sub>5</sub> | 404.2432             | 404.2431        | 0.1              | I<sub>j</sub>  | 45       | I<sub>d</sub> - PhCOOH |
|                | 368                 | C<sub>22</sub>H<sub>26</sub>N<sub>4</sub> | 368.1849             | 368.1856        | 0.7              | I<sub>k</sub>  | 55       | I<sub>g</sub> - PhCOOH |
|                | 554                 | C<sub>31</sub>H<sub>40</sub>N<sub>8</sub> | 554.2719             | 554.2748        | 2.9              | I<sub>c</sub>  | 15       | I<sub>b</sub> - MeOH |
|                | 526                 | C<sub>30</sub>H<sub>40</sub>N<sub>7</sub> | 526.2770             | 526.2799        | 2.9              | I<sub>d</sub>  | 35       | I<sub>c</sub> - CO |
|                | 494                 | C<sub>29</sub>H<sub>34</sub>N<sub>6</sub> | 494.2510             | 494.2537        | 2.7              | I<sub>f</sub>  | 100      | I<sub>d</sub> - MeOH |
|                | 476                 | C<sub>28</sub>H<sub>32</sub>N<sub>6</sub> | 476.2409             | 476.2431        | 2.2              | I<sub>h</sub>  | 30       | I<sub>f</sub> - H<sub>2</sub>O |
|                | 462                 | C<sub>27</sub>H<sub>30</sub>N<sub>5</sub> | 462.2263             | 462.2275        | 1.2              | I<sub>i</sub>  | 20       | I<sub>f</sub> - MeOH |
|                | 404                 | C<sub>23</sub>H<sub>24</sub>N<sub>4</sub> | 404.2398             | 404.2431        | 3.3              | I<sub>j</sub>  | 30       | I<sub>d</sub> - PhCOOH |
|                | 354                 | C<sub>22</sub>H<sub>28</sub>N<sub>3</sub> | 354.2048             | 354.2064        | 1.6              | I<sub>l</sub>  | 100      | I<sub>h</sub> - PhCOOH |
|                | 354                 | C<sub>22</sub>H<sub>28</sub>N<sub>3</sub> | 354.2047             | 354.2064        | 1.6              | I<sub>l</sub>  | 25       | I<sub>j</sub> - MeOH |
|                | 340                 | C<sub>21</sub>H<sub>26</sub>N<sub>3</sub> | 340.1901             | 340.1907        | 0.6              | I<sub>n</sub>  | 25       | I<sub>m</sub> - H<sub>2</sub>O |
|                | 322                 | C<sub>21</sub>H<sub>28</sub>N<sub>3</sub> | 322.1727             | 322.1802        | 7.5              | I<sub>o</sub>  | 15       | I<sub>n</sub> - MeOH or I<sub>n</sub> - H<sub>2</sub>O |
|                | 218                 | C<sub>19</sub>H<sub>22</sub>N<sub>2</sub> | 218.1542             | 218.1539        | 0.3              | I<sub>p</sub>  | 100      | I<sub>o</sub> - C<sub>7</sub>H<sub>4</sub>O |
|                | 368                 | C<sub>21</sub>H<sub>28</sub>N<sub>3</sub> | 360.1890             | 340.1907        | 1.7              | I<sub>n</sub>  | 30       | I<sub>k</sub> - CO |
|                | 296                 | C<sub>19</sub>H<sub>22</sub>N<sub>2</sub> | 296.1639             | 296.1645        | 0.6              | I<sub>q</sub>  | 100      | I<sub>n</sub> - C<sub>2</sub>H<sub>4</sub>O |
| MS<sup>3</sup> 494 | 476                 | C<sub>29</sub>H<sub>34</sub>N<sub>5</sub> | 476.2415             | 476.2431        | 1.6              | I<sub>h</sub>  | 45       | I<sub>f</sub> - H<sub>2</sub>O |
|                | 462                 | C<sub>28</sub>H<sub>32</sub>N<sub>5</sub> | 462.2257             | 462.2275        | 1.8              | I<sub>i</sub>  | 100      | I<sub>f</sub> - MeOH |
|                | 354                 | C<sub>21</sub>H<sub>28</sub>N<sub>2</sub> | 322.1781             | 322.1802        | 2.1              | I<sub>o</sub>  | 25       | I<sub>i</sub> - MeOH |
Table 2. Data for accurate masses and elemental composition of yunnaconitine (2) observed from tandem mass spectrometry in positive mode.

| MS<sup>n</sup> | Precursor ion (m/z) | Product ion (m/z) | Elemental composition | Measured (m/z) | Calculated (m/z) | Error (mDa) | Ion name | Intensity (%) | Assignment |
|----------------|---------------------|-------------------|-----------------------|---------------|-----------------|------------|----------|--------------|------------|
| (+)MS 659      | 660                 | C<sub>35</sub>H<sub>40</sub>N<sub>11</sub> | 660.3405              | 660.3378       | +2.7            | 2a         | 100      | [M + H]<sup>+</sup> |
| MS<sup>2</sup> 660 | 600                 | C<sub>33</sub>H<sub>36</sub>N<sub>9</sub> | 600.3196              | 600.3167       | +2.9            | 2b         | 100      | 2a – AcOH |            |
| 568            | C<sub>32</sub>H<sub>42</sub>N<sub>8</sub> | 568.2925          | 568.2905              | +2.0           | 2c              | 70         | 2b – MeOH |
| 550            | C<sub>32</sub>H<sub>40</sub>N<sub>7</sub> | 550.2841          | 550.2799              | +4.2           | 2d              | 100        | 2c – H<sub>2</sub>O |
| 536            | C<sub>31</sub>H<sub>38</sub>N<sub>7</sub> | 536.2647          | 536.2643              | +0.4           | 2e              | 50         | 2c – MeOH |
| 518            | C<sub>31</sub>H<sub>36</sub>N<sub>6</sub> | 518.2539          | 518.2537              | +0.2           | 2f              | 30         | 2d – MeOH or 2e – H<sub>2</sub>O |
| 486            | C<sub>30</sub>H<sub>32</sub>N<sub>6</sub> | 486.2264          | 486.2275              | -1.1           | 2g              | 20         | 2f – MeOH |
| 366            | C<sub>29</sub>H<sub>32</sub>N<sub>5</sub> | 366.2047          | 366.2064              | -1.7           | 2h              | 20         | 2f – p-OMePhCOOH |
| 334            | C<sub>29</sub>H<sub>32</sub>N<sub>2</sub> | 334.1787          | 334.1802              | -1.5           | 2i              | 50         | 2g – p-OMePhCOOH or 2h – MeOH |
| 500            | 600                 | C<sub>35</sub>H<sub>40</sub>N<sub>11</sub> | 600.3196              | 600.3167       | +2.9            | 2b         | 100      | 2a – MeOH |
| MS<sup>3</sup> 600 | 550                 | C<sub>33</sub>H<sub>36</sub>N<sub>9</sub> | 550.2807              | 550.2799       | +0.8            | 2d         | 100      | 2b – H<sub>2</sub>O |
| 536            | C<sub>31</sub>H<sub>38</sub>N<sub>7</sub> | 536.2652          | 536.2643              | +0.9           | 2e              | 20         | 2c – MeOH |
| 518            | C<sub>31</sub>H<sub>36</sub>N<sub>6</sub> | 518.2550          | 518.2537              | +1.3           | 2f              | 20         | 2d – MeOH or 2e – H<sub>2</sub>O |
| 486            | C<sub>30</sub>H<sub>32</sub>N<sub>5</sub> | 486.2287          | 486.2275              | +1.2           | 2g              | 15         | 2f – MeOH |
| 447            | C<sub>29</sub>H<sub>27</sub>O<sub>2</sub> | 447.1840          | 447.1802              | +3.8           | 2j              | 10         | 2d – C<sub>6</sub>H<sub>10</sub>N<sub>2</sub>NO |
| 366            | C<sub>23</sub>H<sub>38</sub>N<sub>3</sub> | 366.2060          | 366.2064              | -0.4           | 2h              | 15         | 2f – p-OMePhCOOH |
| 334            | C<sub>22</sub>H<sub>32</sub>N<sub>2</sub> | 334.1789          | 334.1802              | -1.3           | 2i              | 40         | 2g – p-OMePhCOOH or 2h – MeOH |
| 366            | 334                 | C<sub>22</sub>H<sub>32</sub>N<sub>2</sub> | 334.1792              | 334.1802       | -1.0            | 2i         | 100      | 2h – MeOH |
| MS<sup>4</sup> 350 | 550                 | C<sub>31</sub>H<sub>36</sub>N<sub>6</sub> | 518.2541              | 518.2537       | +0.4            | 2f         | 65       | 2d – MeOH |
| 518            | C<sub>30</sub>H<sub>32</sub>N<sub>5</sub> | 486.2280          | 486.2275              | +0.5           | 2g              | 30         | 2f – MeOH |
| 486            | C<sub>29</sub>H<sub>30</sub>N<sub>4</sub> | 472.2111          | 472.2118              | -0.7           | 2k              | 15         | 2f – C<sub>2</sub>H<sub>5</sub>O |
| 472            | C<sub>29</sub>H<sub>27</sub>O<sub>2</sub> | 447.1759          | 447.1802              | -4.3           | 2j              | 20         | 2d – C<sub>6</sub>H<sub>10</sub>N<sub>2</sub>NO |
| 366            | C<sub>23</sub>H<sub>38</sub>N<sub>3</sub> | 366.2046          | 366.2064              | -1.8           | 2h              | 30         | 2f – p-OMePhCOOH |
| 334            | C<sub>22</sub>H<sub>32</sub>N<sub>2</sub> | 334.1782          | 334.1802              | -2.0           | 2i              | 65         | 2g – p-OMePhCOOH or 2h – MeOH |
| 518            | 486                 | C<sub>20</sub>H<sub>32</sub>N<sub>4</sub> | 486.2266              | 486.2275       | -0.9            | 2g         | 100      | 2f – MeOH |
| MS<sup>5</sup> 486 | 454                 | C<sub>20</sub>H<sub>32</sub>N<sub>4</sub> | 454.2005              | 454.2013       | -0.8            | 2i         | 56       | 2g – MeOH |
| 334            | 302                 | C<sub>21</sub>H<sub>32</sub>N<sub>4</sub> | 302.1587              | 302.1539       | +4.8            | 2m         | 100      | 2i – MeOH |
| 290            | C<sub>20</sub>H<sub>28</sub>N<sub>4</sub> | 290.1588          | 290.1539              | +4.9           | 2n              | 66         | 2i – C<sub>2</sub>H<sub>5</sub>O |
| 288            | C<sub>20</sub>H<sub>18</sub>N<sub>4</sub> | 288.1329          | 288.1383              | -5.4           | 2o              | 66         | 2i – C<sub>2</sub>H<sub>6</sub>O |
| Precursor ion (m/z) | Product ion (m/z) | Elemental composition | Measured (m/z) | Calculated (m/z) | Error (mDa) | Ion name | Intensity (%) | Assignment |
|---------------------|-------------------|-----------------------|----------------|----------------|-------------|----------|--------------|------------|
| (+)MS 643 | 644 | C_{35}H_{50}NO_{10} | 644.3476 | 644.3429 | +4.7 | 3a | 100 | [M + H]^+ |
| MS^2 644 | 584 | C_{33}H_{46}NO_{8} | 584.3251 | 584.3218 | +3.3 | 3b | 76 | 3a – AcOH |
| 552 | 524 | C_{32}H_{42}NO_{7} | 524.2643 | 524.2643 | 0.0 | 3c | 100 | 3b – MeOH |
| 520 | 492 | C_{29}H_{34}NO_{6} | 492.2380 | 492.2381 | -0.1 | 3f | 15 | 3d – MeOH or 3e – C_2H_4 |
| 488 | 458 | C_{30}H_{34}NO_{6} | 488.2447 | 488.2431 | +1.6 | 3g | 30 | 3e – MeOH |
| 400 | 368 | C_{29}H_{33}NO_{6} | 368.2218 | 368.2220 | -0.2 | 3j | 30 | 3e – p-OMePhCOOH or 3h – MeOH |
| 336 | 304 | C_{21}H_{25}NO_{3} | 304.1680 | 304.1696 | -1.6 | 3k | 15 | 3j – MeOH |
| MS^3 584 | 552 | C_{29}H_{33}NO_{7} | 552.2970 | 552.2961 | +1.4 | 3e | 100 | 3b – MeOH |
| 524 | 492 | C_{29}H_{33}NO_{6} | 492.2340 | 492.2381 | +2.5 | 3f | 23 | 3d – MeOH or 3e – C_2H_4 |
| 488 | 428 | C_{30}H_{34}NO_{3} | 428.2340 | 428.2341 | +1.9 | 3g | 16 | 3e – MeOH |
| 400 | 368 | C_{29}H_{33}NO_{6} | 368.2218 | 368.2220 | +0.3 | 3h | 10 | 3e – p-OMePhCOOH |
| 336 | 304 | C_{21}H_{25}NO_{3} | 304.1680 | 304.1696 | -1.6 | 3k | 15 | 3j – MeOH |
| 304 | 214 | C_{21}H_{25}NO_{2} | 214.1530 | 214.1531 | -1.0 | 3l | 15 | 3i – MeOH |
| 520 | 492 | C_{29}H_{33}NO_{6} | 492.2356 | 492.2381 | -2.5 | 3f | 18 | 3e – C_2H_4 |
| 488 | 428 | C_{30}H_{34}NO_{3} | 428.2340 | 428.2341 | +1.9 | 3g | 100 | 3e – MeOH |
| 368 | 336 | C_{22}H_{26}NO_{2} | 336.1947 | 336.1958 | -0.1 | 3j | 54 | 3g – p-OMePhCOOH or 3i – MeOH |
| 336 | 304 | C_{21}H_{25}NO_{3} | 304.1680 | 304.1696 | -1.6 | 3k | 15 | 3j – MeOH |
| 400 | 372 | C_{22}H_{26}NO_{2} | 372.2158 | 372.2169 | -1.1 | 3l | 15 | 3i – MeOH |
| 354 | 324 | C_{21}H_{26}NO_{2} | 324.1956 | 324.1958 | -0.2 | 3o | 25 | 3i – C_2H_4O |

(Continued)
| MS\(^n\) | Precursor ion (m/z) | Product ion (m/z) | Elemental composition | Measured (m/z) | Calculated (m/z) | Error (mDa) | Ion name | Intensity (%) | Assignment |
|--------|------------------|------------------|----------------------|---------------|-----------------|-------------|----------|--------------|------------|
| 322    | C\(_{21}\)H\(_{24}\)NO\(_2\) | 322.1755         | 322.1802             | −4.7          | 3p              | 42          | 3m – MeOH |
| 308    | C\(_{20}\)H\(_{22}\)NO\(_2\) | 308.1656         | 308.1645             | +1.1          | 3q              | 25          | 3j – C\(_2\)H\(_4\) |
| 304    | C\(_{19}\)H\(_{18}\)NO | 276.1372         | 276.1383             | −1.1          | 3r              | 100         | 3k – C\(_2\)H\(_4\) |
| 520    | C\(_{30}\)H\(_{38}\)NO\(_7\) | 524.2642         | 524.2643             | −0.1          | 3d              | 100         | 3e – C\(_2\)H\(_4\) |
| 492    | C\(_{29}\)H\(_{34}\)NO\(_6\) | 492.2399         | 492.2381             | +1.8          | 3f              | 37          | 3d – MeOH or 3e – C\(_2\)H\(_4\) |
| 488    | C\(_{22}\)H\(_{26}\)NO\(_2\) | 336.1969         | 336.1958             | +1.1          | 3j              | 100         | 3g – p-OMePhCOOH |
| 368    | C\(_{22}\)H\(_{26}\)NO\(_2\) | 336.1955         | 336.1988             | +0.3          | 3j              | 64          | 3i – MeOH |
| 336    | C\(_{22}\)H\(_{22}\)NO\(_2\) | 308.1646         | 308.1645             | +0.1          | 3q              | 43          | 3j – C\(_2\)H\(_4\) |
| 304    | C\(_{21}\)H\(_{22}\)NO | 304.1662         | 304.1696             | −3.4          | 3k              | 60          | 3j – MeOH |
| 290    | C\(_{20}\)H\(_{20}\)NO | 290.1542         | 290.1539             | +0.3          | 3s              | 100         | 3k – CH\(_2\) |
| 276    | C\(_{19}\)H\(_{18}\)NO | 276.1398         | 276.1383             | +1.5          | 3r              | 87          | 3q – MeOH or 3k – C\(_2\)H\(_4\) |
| 262    | C\(_{18}\)H\(_{16}\)NO | 262.1221         | 262.1268             | −0.5          | 3t              | 65          | 3s – C\(_2\)H\(_4\) or 3r – CH\(_2\) |
| 524    | C\(_{28}\)H\(_{34}\)NO\(_6\) | 492.2362         | 492.2381             | −1.9          | 3f              | 46          | 3d – MeOH |
| 460    | C\(_{28}\)H\(_{30}\)NO\(_5\) | 460.2131         | 460.2118             | +1.3          | 3u              | 20          | 3f – MeOH |
| 492    | C\(_{28}\)H\(_{30}\)NO\(_5\) | 460.2117         | 460.2118             | −0.1          | 3u              | 40          | 3f – MeOH |
Table 4. Data for accurate masses and elemental composition of benzoylmesaconine (4) observed from tandem mass spectrometry in positive mode.

| MS<sup>n</sup> | Precursor ion (m/z) | Product ion (m/z) | Elemental composition | Measured (m/z) | Calculated (m/z) | Error (mDa) | Ion name | Intensity (%) | Assignment |
|----------------|------------------|------------------|----------------------|----------------|------------------|------------|----------|-------------|------------|
| (+)MS          | 589              | 590              | C<sub>31</sub>H<sub>44</sub>N<sub>10</sub>O<sub>10</sub> | 590.2966       | 590.2960         | +0.6        | 4a       | 100         | [M + H]<sup>+</sup> |
| MS<sup>3</sup> | 590              | 572              | C<sub>31</sub>H<sub>43</sub>N<sub>9</sub>O<sub>9</sub> | 572.2894       | 572.2854         | +4.0        | 4b       | 19          | 4a – H<sub>2</sub>O |
|                | 558              | C<sub>30</sub>H<sub>46</sub>N<sub>9</sub>O<sub>9</sub> | 558.2674       | 558.2698         | -2.4        | 4c       | 19          | 4a – MeOH   |
|                | 540              | 540              | C<sub>30</sub>H<sub>38</sub>N<sub>9</sub>O<sub>9</sub> | 540.2591       | 540.2592         | -0.1        | 4d       | 100         | 4b – MeOH or 4c – H<sub>2</sub>O |
|                | 526              | 526              | C<sub>29</sub>H<sub>36</sub>N<sub>9</sub>O<sub>9</sub> | 526.2425       | 526.2435         | -1.0        | 4e       | 52          | 4c – MeOH   |
|                | 522              | 522              | C<sub>30</sub>H<sub>36</sub>N<sub>9</sub>O<sub>7</sub> | 522.2505       | 522.2486         | +1.9        | 4f       | 10          | 4d – H<sub>2</sub>O |
|                | 508              | 508              | C<sub>29</sub>H<sub>36</sub>N<sub>9</sub>O<sub>7</sub> | 508.2330       | 508.2330         | 0           | 4g       | 90          | 4d – MeOH or 4e – H<sub>2</sub>O |
|                | 494              | 494              | C<sub>28</sub>H<sub>36</sub>N<sub>9</sub>O<sub>7</sub> | 494.2193       | 494.2173         | +2.0        | 4h       | 74          | 4e – MeOH   |
|                | 490              | 490              | C<sub>29</sub>H<sub>36</sub>N<sub>9</sub>O<sub>6</sub> | 490.2231       | 490.2224         | +0.7        | 4i       | 37          | 4f – MeOH or 4g – H<sub>2</sub>O |
|                | 476              | 476              | C<sub>28</sub>H<sub>36</sub>N<sub>9</sub>O<sub>6</sub> | 476.2060       | 476.2068         | -0.8        | 4j       | 74          | 4g – MeOH or 4h – H<sub>2</sub>O |
| MS<sup>3</sup> | 558              | 540              | C<sub>30</sub>H<sub>36</sub>N<sub>9</sub>O<sub>8</sub> | 540.2581       | 540.2592         | -1.1        | 4d       | 25          | 4c – H<sub>2</sub>O |
|                | 526              | 526              | C<sub>29</sub>H<sub>36</sub>N<sub>9</sub>O<sub>8</sub> | 526.2432       | 526.2435         | -0.3        | 4e       | 90          | 4c – MeOH   |
|                | 508              | 508              | C<sub>29</sub>H<sub>36</sub>N<sub>9</sub>O<sub>8</sub> | 508.2312       | 508.2330         | -1.8        | 4g       | 15          | 4d – MeOH or 4e – H<sub>2</sub>O |
|                | 494              | 494              | C<sub>28</sub>H<sub>36</sub>N<sub>9</sub>O<sub>8</sub> | 494.2160       | 494.2173         | -1.3        | 4h       | 55          | 4e – MeOH   |
|                | 476              | 476              | C<sub>28</sub>H<sub>36</sub>N<sub>9</sub>O<sub>8</sub> | 476.2050       | 476.2068         | -1.8        | 4j       | 15          | 4g – MeOH or 4h – H<sub>2</sub>O |
|                | 466              | 466              | C<sub>27</sub>H<sub>36</sub>N<sub>9</sub>O<sub>8</sub> | 466.2208       | 466.2224         | -1.6        | 4k       | 15          | 4h – CO     |
| 540            | 522              | 522              | C<sub>30</sub>H<sub>36</sub>N<sub>9</sub>O<sub>7</sub> | 522.2471       | 522.2486         | -1.5        | 4f       | 10          | 4d – H<sub>2</sub>O |
|                | 508              | 508              | C<sub>29</sub>H<sub>36</sub>N<sub>9</sub>O<sub>7</sub> | 508.2326       | 508.2330         | -0.4        | 4g       | 100         | 4d – MeOH   |
|                | 490              | 490              | C<sub>29</sub>H<sub>36</sub>N<sub>9</sub>O<sub>7</sub> | 490.2209       | 490.2224         | -1.5        | 4i       | 20          | 4f – MeOH or 4g – H<sub>2</sub>O |
|                | 476              | 476              | C<sub>28</sub>H<sub>36</sub>N<sub>9</sub>O<sub>7</sub> | 476.2075       | 476.2068         | +0.7        | 4h       | 66          | 4g – MeOH   |
|                | 526              | 526              | C<sub>28</sub>H<sub>36</sub>N<sub>9</sub>O<sub>7</sub> | 526.2425       | 526.2435         | -3.4        | 4j       | 10          | 4h – H<sub>2</sub>O |
|                | 508              | 508              | C<sub>29</sub>H<sub>36</sub>N<sub>9</sub>O<sub>7</sub> | 508.2326       | 508.2330         | -0.4        | 4g       | 100         | 4d – MeOH   |
|                | 476              | 476              | C<sub>28</sub>H<sub>36</sub>N<sub>9</sub>O<sub>7</sub> | 476.2075       | 476.2068         | +1.4        | 4j       | 100         | 4g – MeOH   |
|                | 476              | 476              | C<sub>28</sub>H<sub>36</sub>N<sub>9</sub>O<sub>7</sub> | 458.1934       | 458.1962         | -2.8        | 4l       | 25          | 4j – H<sub>2</sub>O |
|                | 444              | 444              | C<sub>27</sub>H<sub>36</sub>N<sub>9</sub>O<sub>7</sub> | 444.1799       | 444.1805         | -0.6        | 4m       | 58          | 4j – MeOH   |
|                | 354              | 354              | C<sub>21</sub>H<sub>36</sub>N<sub>9</sub>O<sub>7</sub> | 354.1691       | 354.1690         | -0.9        | 4n       | 60          | 4j – PhCOOH |
| MS<sup>3</sup> | 494              | 476              | C<sub>28</sub>H<sub>36</sub>N<sub>9</sub>O<sub>6</sub> | 476.2057       | 476.2068         | -1.1        | 4j       | 60          | 4h – H<sub>2</sub>O |
|                | 490              | 458              | C<sub>28</sub>H<sub>36</sub>N<sub>9</sub>O<sub>5</sub> | 458.1926       | 458.1962         | -3.6        | 4l       | 80          | 4i – MeOH   |

(Continued)
322) could be interpreted by eliminating C(1)-methoxyl group from 1l or C(3)-hydroxyl moiety from 1n. Besides 1l, 1n, and 1o, the ion 1p (m/z 218) was detected with high abundance, which could be interpreted by the loss of C7H4O segment from 1o. In another route, the precursor ion 1k produced the ion 1n (m/z 340) by losing one molecule of CO and further gave rise to the ion 1q (m/z 296), which was attributed to the departure of C2H4O segment at C(4) position.

In MS4 scan of 1f at m/z 494, two product ions, 1h at m/z 476 and 1i at m/z 462 (100%), were observed. At the same time ion, 1l at m/z 354 was selected as precursor ion to generate a fragment at m/z 322 (1o), which was assigned to the elimination of C(1)-methoxyl moiety on 1l.

Besides, we found that the losses of moieties at C(8), C(15), C(6), C(1), and C(14) positions generated ions 1b, 1d, 1f, 1i, and 1l with high intensity which suggested these positions were active in mass spectrometry.

2.1.2. ESI-IT-TOF MSn fragmentations of yunnaconitine (2) in positive mode (as shown in Figure S2 and Table 2)

Unlike aconitine (1), yunnaconitine (2) showed simple fragmentation characteristics relatively. In the single-stage mass spectrometry of yunnaconitine (2) in positive mode, the [M + H]+ ion at m/z 660.3405 (2a) was found and its molecular formula was C38H50NO11. In MS3 analysis, 2a produced ions 2b–2i in which 2b (m/z 600, 100%) and 2c (m/z 568, 70%) with high abundance were assigned to the elimination of C(8)-acetyl and C(16)-methoxyl units just as the fragmentation rules of 1b and 1c in aconitine (1). Then 2c lost C(3)-hydroxyl or C(6)-methoxyl group to generate ions 2d (m/z 550, 100%) and 2e (m/z 536). The ion 2f (m/z 518) arose from two originations, 2d losing C(6)-methoxyl group and 2e losing C(3)-hydroxyl unit. Fragments 2g at m/z 486 and 2h at m/z 366.
both derived from the ion $2f$ by losing C(6)-methoxyl unit for the former and eliminating C(14)-p-methoxyl-benzoyl group for the latter. Then $2g$ lost C(14)-p-methoxyl-benzoyl unit and $2h$ split C(1)-methoxyl group to generate the same product ion $2i$ at $m/z$ 334.

Precursor ion $2b$ ($m/z$ 600) was analyzed in MS$^3$ experiment to afford product ions $2c-2i$ in which ion $2j$ at $m/z$ 447 had a non-nitrogen molecular formula as C$_{27}$H$_{27}$O$_6$ that predicted the nitrogen heterocyclic ring on $2d$ split to lose a C$_5$H$_{13}$NO segment. The ion $2i$ was also observed when $2h$ ($m/z$ 366) acted as precursor ion due to the departure of C(1)-methoxyl group.

In MS$^4$ analysis, the product ions $2f-2j$ were detected from precursor ion $2d$ ($m/z$ 550) just as above-mentioned interpretations. Another precursor ion $2f$ ($m/z$ 518) afforded four fragments, $2g$ at $m/z$ 486 (100%), $2k$ at $m/z$ 472, $2h$ at $m/z$ 366, and $2i$ at $m/z$ 334, in which $2k$ was arose from the elimination of C(4)-methoxymethyl unit as a C$_2$H$_4$O segment from $2f$.

Via MS$^5$ analysis, the ion $2l$ at $m/z$ 454 was observed from precursor ion $2g$ ($m/z$ 486) attributed to the loss of C(18)-methoxyl group. Precursor ion $2i$ ($m/z$ 334) gave rise to three fragments, the ion $2m$ at $m/z$ 302, $2n$ at $m/z$ 290, and $2o$ at $m/z$ 288, based on losing C(18)-methoxyl group or directly eliminating C(4)-methoxymethyl unit as C$_2$H$_4$O or C$_2$H$_2$O segment, respectively.

In the MS$^n$ analyses of yunnanconitine (2), the losses of C(8), C(16), C(3), C(1)-moieties gave rise to a series of ions with high intensity such as $2b$, $2c$, $2d$, and $2g$ which were similar as aconitine (1).

2.1.3. ESI-IT-TOF MS$^n$ fragmentations of crassicauline A (3) in positive mode (as shown in Figure S3 and Table 3)

The first-stage mass spectrometry of crassicauline A (3) generated the [M + H]$^+$ ion at $m/z$ 644.3476 (3a), corresponding to the molecular formula C$_{33}$H$_{50}$NO$_{10}$. For MS$^2$ analysis of 3a ($m/z$ 644), ions 3b at $m/z$ 584 (76%), and 3c at $m/z$ 552 (100%) with high intensity were observed and assigned with the losses of C(8)-acetyl and C(16)-methoxyl groups sequentially from 3a. Because of the absence of C(3)-hydroxyl group, the ion 3c lost N-ethyl unit easily to produce the ion 3d ($m/z$ 524) and then 3d lost C(6)-methoxyl group to yield the ion 3f at $m/z$ 492. Besides, the ion 3e afforded the ion 3e at $m/z$ 520 with high abundance (76%) relatively by eliminating C(6)-methoxyl unit to form a stable conjugated structure. The ion 3h at $m/z$ 400 was detected readily corresponding to the elimination of C(14)-p-methoxyl-benzoyl group from 3c, too, and 3h further lost C(6)-methoxyl unit to afford the ion 3i at $m/z$ 368. Certainly, the loss of N-ethyl or C(14)-p-methoxyl-benzoyl unit from 3e was the pathway to generate the fragments 3f and 3i as well. 3e also gave rise to the ion 3g at $m/z$ 488 by eliminating C(1)-methoxyl group. Thus the ion 3j ($m/z$ 336) could be explained by the loss of C(14)-p-methoxyl-benzoyl unit from 3g or the loss of C(1)-methoxyl group from 3i, and then 3j eliminated one molecule of MeOH at C(18) position to produce the ion 3k at $m/z$ 304.

In agreement with MS$^2$ analysis, the ions 3e-3k and 3f, 3g, 3i, 3j were all found when 3b ($m/z$ 584), 3e ($m/z$ 520), and 3h ($m/z$ 400) were selected as precursor ions in MS$^3$ experiments. Ions 3i, 3j, and 3l-3q were obtained in the MS$^3$ analysis of 3h in which 3l-3q were interpreted as follows: the ion 3h ($m/z$ 400) eliminated N-Et or C(4)-methoxymethyl group to generate the ions 3i ($m/z$ 372) and 3m ($m/z$ 354), and then 3i and 3m deducted C(6)-methoxyl unit relatively to yield the ions 3n ($m/z$ 340) and 3p ($m/z$ 322); the ion 3o ($m/z$ 324) derived from 3i due to the loss of C(4)-methoxymethyl group as C$_2$H$_2$O segment; the base peak ion 3q ($m/z$ 308) was formed by losing N-ethyl unit from 3j directly. Whilst, 3r
(m/z 276) was the base peak ion in the MS³ analysis of precursor ion 3k which was owed to the elimination of N-ethyl group as CH₃N unit. The MS⁴ analysis of four precursor ions, 3c (m/z 552), 3g (m/z 488), 3i (m/z 368), and 3j (m/z 336), yield nine product ions, 3d–3j, 3k, 3q, and 3r–3t, in which the fragmentation pathways of 3d–3j, 3q, and 3k had been elucidated in MS² and MS³ experiments. The ion 3r at m/z 276 could be explained by the loss of MeOH from 3q at C(18) position or the elimination of C₂H₄ segment from 3k on nitrogen-atoms. The ion 3s was attributed to the loss of tertiary carbon as CH₂ group at C(4) position from 3k. Then 3s gave rise to the ion 3t by eliminating N-ethyl group as CH₃N unit. Another origination of 3t was the ion 3r, which lost the tertiary carbon moiety as CH₂ segment at C(4) position to generate fragment 3i.

In MS⁵ analysis of precursor ion 3d (m/z 524), the ion 3f (m/z 492) was detected due to the elimination of C(6)-methoxyl unit and then 3f generated the ion 3u (m/z 460) owing to the loss of C(1)-methoxyl group.

To study the fragmentation route of the ion 3f (m/z 492) in depth, the MS⁶ analysis of ion 3f was carried out from which product ion 3u at m/z 460 was detected and attributed to the loss of C(1)-methoxyl unit.

We could find that ions 3b, 3c, 3e, and 3g with high abundance, mentioned in Table 3 were generated by the elimination of moieties at C(8), C(16), C(6), and C(1) positions.

2.1.4. ESI-IT-TOF MSⁿ fragmentation of benzoylmesaconine (4) in positive mode (as shown in Figure 3 and Table 4)

It was easy to detect the [M + H]⁺ ion (4a) at m/z 590.2966 of benzoylmesaconine (4) in the positive full-scan mass spectrometry concurring with the molecular formula C₃₁H₄₄NO₁₀. A series of ions (4b–4j) were found when 4a acted as precursor ion in MS² experiment. The loss of C(8)-hydroxyl or C(16)-methoxyl unit from 4a leaded to the appearance of the ions 4b at m/z 572 and 4c at m/z 558 relatively. The ion 4d (m/z 540, 100%) could be explained by the elimination of C(16)-methoxyl group on 4b or C(8)-hydroxyl unit on 4c and then 4d generated the ion 4f at m/z 522 by losing C(3)-hydroxyl. Ions 4c, 4d, and 4f all eliminated one molecule of MeOH to produce the [P-MeOH]⁺ (P: product ion) ions, 4e at m/z 526, 4g at m/z 508 (90%), and 4i at m/z 490 due to the existence of C(6)-methoxyl group, respectively. The ions 4e and 4g gave rise to the ions 4h (m/z 494) and 4j (m/z 476) relatively due to the further splitting of C(1)-methoxyl unit. Furthermore, the ion 4e generated the ion 4g by eliminating C(8)-hydroxyl group, as well as 4h to 4j. Similarly, 4j could be interpreted by the loss of C(3)-hydroxyl unit from 4g, too.

In MS³ analysis, ions 4d–4j were detected when 4c (m/z 558), 4d (m/z 540), 4e (m/z 526), and 4g (m/z 508) acted as precursor ions, in which the ion 4k at m/z 466 was caused by the loss of CO from 4h. The MS³ experiment of the ion 4j at m/z 476 was carried out to yield fragments 4l–4n, in which 4l at m/z 458 arose from the scission of C(3)-hydroxyl group, 4m at m/z 444 originated from the elimination of C(18)-methoxyl unit, and 4n at m/z 354 derived from the loss of C(14)-benzoyl group.

Based on MS⁴ experiment, the precursor ion 4h at m/z 494 eliminated C(8)-hydroxyl moiety to produce the ion 4j at m/z 476. Precursor ion 4i at m/z 490 afforded ions 4l at m/z 458 and 4o at m/z 368 by losing C(6)-methoxyl or C(14)-benzoyl group. From precursor ion 4m at m/z 444, ions 4p at m/z 426 and 4r at m/z 322 were easily explained by the eliminations of H₂O at C(3) position and PhCOOH at C(14) position, and the ion 4q at m/z 394 derived from 4p by losing C(4)-tertiary carbon and C(13)-hydroxy groups consecutively.
According to MS\textsuperscript{5} analysis, the ion 4j (m/z 476) was selected as precursor ion to give rise to the ions 4m at m/z 444, 4p at m/z 426, and 4q at m/z 394. All of them had been explained in MS\textsuperscript{3} and MS\textsuperscript{4} experiments.

Ultimately, the ion 4q at m/z 394 was the only precursor ion in MS\textsuperscript{6} analysis to afford the ion 4s at m/z 366 by losing one molecule of CO.

For benzoylmesaconine (4), ions with high intensity also arose from the losses of moieties at C(16), C(6), C(1) and C(8) positions such as 4d, 4e, 4g, 4h, and 4j whose abundance was more than 90%.

### 2.2. Discussion

#### 2.2.1. The losses of C(8), C(15), and C(16) substituent groups

Aconitine (1), yunnaconitine (2), crassicauline A (3), and benzoylmesaconine (4) with C(8)-acetyl or C(8)-hydroxyl group first generate the product ion as [M + H-AcOH]\textsuperscript{+} (1b, 2b, and 3b) or [M + H-H\textsubscript{2}O]\textsuperscript{+} (4b) in MS\textsuperscript{2} spectrometry. It suggested that C(8) position was the active location at which moieties were easily to be lost. However, the position for proton leaving when C(8)-moiety was eliminated as neutral loss was disputed. Many articles tended to the positions at C(15) or C(7) [18,19]. In this paper, the position C(7) was proposed so that the fragments could lose its C(16)-methoxyl moiety to form a double bond between C(15) and C(16) positions and after that ketone–enol tautomerism occurred to form a carbonyl at C(15) position. In MS\textsuperscript{2} analysis, the [M + H-AcOH-MeOH-CO]\textsuperscript{+} (1d) ion from aconitine (1) was obtained that could demonstrate the above-mentioned conjecture. The high intensity ions 1b, 1d, 2b, 3b, 3c, and 4d were all come from the loss of C(8) or C(16)-moiety. The discussions indicated that C(8) was the most active position and the activity of C(16) is slightly inferior in MS\textsuperscript{n} experiments, the eliminations of moieties at C(8) and C(16) positions were the originations to generate base peak ions.

#### 2.2.2. The losses of C(6)-methoxyl, C(1)-methoxyl, C(3)-hydroxyl units, and C(14) substituent groups

The fragmentation routes of losing C(6)-methoxyl group after losing the C(8) and C(16) moieties were proposed in MS\textsuperscript{5} analyses of the candidates. It was benefited from forming more stable ions with high conjugated structures such as 1e, 1f, 2e, 2f, 3e, 3f, 3i, 4e, 4g, and 4i, in which the ions 1f at m/z 494 and 4g at m/z 508 in MS\textsuperscript{2} analysis were base peak ions simultaneously. After that, the eliminations of C(1)-methoxyl and C(3)-hydroxyl units occurred to yield fragments with longer conjugated structures. The loss of C(1)-methoxyl moiety generated a series of ions as base peaks, for example, the ion 1i at m/z 462 from aconitine (1), the ion 2i at m/z 334 from yunnaconitine (2), the ion 3g at m/z 488 from crassicauline A (3) and the ion 4j at m/z 476 from benzoylmesaconine (4) in MS\textsuperscript{3} experiments. In addition, the ions originated from the elimination of C(14) substituent groups as PhCOOH or p-OMePhCOOH segment were also base peak ions, for instance, the ion 1l at m/z 354 from aconitine (1) in MS\textsuperscript{3} analysis and the ion 3j at m/z 336 from crassicauline A (3) in MS\textsuperscript{4} experiments. The discussions showed that the losses of C(6)-methoxyl, C(1)-methoxyl moieties and C(14) substituent groups were easily obtained and generated base peak ions readily following with C(8), C(15), and C(16) substituent groups.

#### 2.2.3. The losses of N-ethyl/methyl

The loss of N-ethyl/methyl moiety was unfavorable on aconitine (1), yunnaconitine (2), and benzoylmesaconine (4). For crassicauline A (3), N-ethyl unit displayed as an active group to be eliminated easily which gave rise to the ions 3d at m/z 524
and 3r at m/z 276 in MS$^3$ experiments with high abundance. It could be explained that the absence of C(3)-hydroxyl influenced the activity of N-ethyl moiety and drove N-ethyl group much more active in mass spectrometry. Meanwhile, the types of substituent groups at nitrogen atom impacted on the activity of moieties as well. For example, it was hard to detect the [$P-CH_4]^+$ (P: product ion) ion in MS$n$ analyses of benzoylmesaconine (4) with N-methyl moiety. It suggested that N-ethyl was more active than N-methyl in MS$n$ analyses of C$_{19}$-diterpenoid alkaloids. Thus, N-ethyl moiety of C$_{19}$-diterpenoid alkaloids without C(3)-hydroxyl group was more active than the same moiety on the compounds with C(3)-hydroxyl unit or more active than N-methyl group.

3. Experimental

3.1. Chemicals and samples

Acetonitrile of HPLC grade was purchased from Merck Co. Ltd. (Darmstadt, Germany). Formic acid was purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China). Deionized water was purified using a MingChe$^\text{TM}$-D 24UV Merck Millipore system (Shanghai, China).

Compounds 1–4 were isolated from the root of A. kusnezoffii Reichb. in our laboratory, whose structures were unambiguously determined by MS and NMR data. Sample solutions were prepared by dissolving each sample in a solution of 85% CH$_3$CN/H$_2$O containing 0.05% formic acid to a final concentration of 0.2 mg ml$^{-1}$. The samples were introduced into the source via a syringe pump at a flow rate of 2 µl min$^{-1}$.

3.2. Apparatus and analytical conditions

MS$n$ analyses were applied on the LC–MS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan). Accurate masses were corrected by calibration using the sodium trifluoroacetate (CF$_3$CO$_2$Na) clusters. The mass resolution was about 10 000 full width at half maximum (FWHM). The Shimadzu Composition Formula Predictor was used to speculate the molecular formula. MS experiments were achieved in automatic pattern, and MS$^2$–$^6$ experiments were performed in manual mode. The ESI-MS analytical conditions were as follows: drying gas pressure, 100.0 kPa; nebulizing gas (N$_2$) flow, 0.5 L min$^{-1}$; spray voltage, +4.5 KV; detector voltage, 1.60 kV; equipment temperature, 40.0°C; heat block temperature, 200.0°C; curved desolvation line temperature, 200.0°C; precursor ion selected width, m/z ± 3.0 Da, and selected time, 20 ms; collision induced dissociation collision time, 30 ms; ion accumulation time, 10 ms; collision energy, 50%; collision gas, 50%; and $q = 0.251$; scan range, m/z 100–1000 for MS.

4. Conclusions

Using HR-ESI-IT-TOT-MS$^n$ (MS$^1$–$^6$) to investigate the fragmentation pathways of aconitine (1), yunnaconitine (2), crassicauline A (3), and benzoylmesaconine (4) provided useful information and the finding rules were concluded: the [M $+$ H]$^+$ ion was readily detected under the acidic analytical conditions. The neutral losses of AcOH (−60 Da), MeOH (−32 Da), H$_2$O (−18 Da), CO (−28 Da), C$_2$H$_4$ (−28 Da) and PhCOOH (−122 Da) or p-OMePhCOOH (−152 Da) due to the presence of acetyl, methoxyl, hydroxyl, N-ethyl and benzoyl or p-methoxyl-benzoyl groups are the characteristic eliminations from the precursor ions. The losing sequence of the main substituent groups was: C(8)-acetyl $>$ C(16)-methoxyl $>$ C(15)-hydroxyl $>$ C(6)-methoxyl $>$ C(1)-methoxyl/C(3)-hydroxyl $>$ C(18)-methoxyl $>$ C(13)-hydroxyl. The absence of C(3)-hydroxyl made N-ethyl group much more active on the compounds. The eliminations of moieties at C(8), C(16), C(6), C(1) and...
C(14) positions were the originations to generate ions with high intensity.

**Supplementary material**

Supplementary material related to this article is available online.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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