# Effect of pH on the Metabolism of Aconitine under Rat Intestinal Bacteria and Analysis of Metabolites Using HPLC/MS-MS$^n$ Technique

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A semi-quantitative method of mass spectrometry (MS) has been described for the analysis of metabolites of aconitine by rat intestinal bacteria at different pH. At pH 7.0, the rat intestinal bacteria exhibit optimal activity for the metabolism of aconitine. A high-performance liquid chromatography-electrospray ionization multiple-stage mass spectrometry (HPLC/ESI-MS$^n$) method has been applied to investigate the characteristic product ions of metabolites. Then, the logical fragmentation pathways of metabolites have been proposed. By comparing the retention time ($t_R$) of HPLC and the ESI-MS$^n$ data with the data of standard compounds and reports from literature, ten metabolites have been identified and a distinctive metabolite (15-deoxyaconitine) has been deduced first time. The experimental results demonstrate that HPLC/ESI-MS$^n$ is a specific and useful method for the identification of metabolites of aconitine. Also, in the present paper, the HPLC-MS method was introduced to determine the synthetical metabolite prior to the study of the toxicity by the method of Bliss.

**Keywords** aconitine, metabolism, intestinal bacteria, mass spectrometry, 8-butyryl-benzoylmesaconine

## Introduction

Aconite tuber is an important and toxic crude drug used in traditional Chinese medicine. Aconitine-type alkaloids isolated from aconite have been shown to have potential toxicity and wide bioactivity. The majority of aconite alkaloids have an aconitane skeleton where aconitine is the most important representative alkaloid. As an analgesic and anaesthetic agent for the treatment of rheumatism and neuralgia, it has been reported that the toxicity of this herb apparently decreases after processing because the fatally toxic alkaloids transform to less toxic alkaloids, such as aconine, benzoylaconine and lipo-aconitine in processing.[1-5] Lipomesaconitine, one of the lipo-alkaloids, was found to exhibit anti-inflammatory and analgesic activities.[4] Deoxyaconitine is also one of the toxic and active ingredient in aconite tuber.[6]

Studies of fragmentation pathways and esterified reactions of aconitine-type alkaloids had been published by means of ESI-MS$^n$ several yeas ago.[7-9] A method for the direct determination of alkaloid profiling in plant tissue by using MALDI-TOFMS has been developed.[10] Quantum chemistry calculations were carried out to theoretically analyze the stability of fragment ions and the elimination rules of substituents in ESI-MS$^n$ for six DDAs.[11]

Aconitine was found to be converted to lipo-aconitine by GC-MS method after incubation with human intestinal bacteria.[12] In our laboratory, the biotransformation of aconitine in human intestinal bacteria has been explored by using electrospray ionization mass spectrometry.[13,14] However, as the most previous studies, the metabolism of aconitine was conducted at a fixed pH. As a matter of fact, the pH of the intestinal contents always changes with the different regions in rat.[15,16] On the other hand, although HPLC, LC/MS method are the most common tools for the quantitative analysis of natural products or metabolites in blood and urine,[17-22] the analysis of lipo-alkaloids is difficult by chromatography owing to their structural similarity.

In the present paper, the effect of pH on the metabolism of aconitine under rat intestinal bacteria has been explored by the semi-quantitative MS method. The reference metabolites of aconitine have been determined by LC/MS$^n$ in the positive ion mode. The LC/MS$^n$ study of the retention time and ESI-MS$^n$ data of aconitine and its metabolites have been carried out. Also, two isomers

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have been detected by HPLC/ESI-MS$^n$. The application of HPLC-MS method was illustrated in the determination of synthetical metabolite prior to the study of the toxicity by the method of Bliss.

## Experimental

### Instrumentations and reagents

Anaerobic Chamber system (Thermo, America). The standard sample of reserpine was purchased from Sigma Corporation (USA). Methanol and acetonitrile were of HPLC grade (Fisher). 28% ammonia solution, diethyl ether, chloroform and dichloromethane were of analytical grade, and butyric acid, toluene and pyridine were of chemical pure. Deionized water was prepared using a Millipore (Billerica, MA, USA) water purification system. General anaerobic medium (GAM) was purchased from Nissui Co. (Tokyo, Japan). In the experiments of rat intestinal bacteria, 1.29 mg of aconitine was dissolved in 200 μL of acetonitrile (0.02 mmol/L), and 1 mg of reserpine was dissolved in 100 mL of methanol.

### Animals

Wister rats and Kunming mice were purchased from Laboratory Animal Center of Jilin University. They were housed under normal laboratory conditions (21±2 ℃, 12 h light-dark cycle) with free access to standard rodent chow and water. The experiments were conducted in accordance with the guidelines and with the approval of Animal Research Ethics Committee of Jilin University.

### Methods of metabolism

Rat feces (0.2 g) was anaerobically cultured in GAM broth (10 mL×4) for 24 h at 37 ℃ and then centrifuged at 5000 r/min for 10 min. The precipitate of four broths were suspended in 0.2 mol•L$^{-1}$ phosphate buffer according to pH 5.8, 6.2, 6.6 and 7.0, respectively. 1.29 mg of aconitine was added to the suspension of different pH, respectively, and the mixture was anaerobically incubated at 37 ℃ for 5 d. 200 μL of the incubation mixture was extracted with dichloromethane (1 mL, twice). 5 μL of the dichloromethane extract was diluted to 500 μL with chloroform and methanol (95∶5, *V*∶*V*) and then 70 μL of reserpine ($M_r$ 608.7) was added to the 500 μL of mixture . The mixture was filtrated and analyzed by ESI-MS/MS$^n$. In addition, 2 mL of the dichloromethane extracted from pH 7.0 phosphate buffer was evaporated to give a residue. The residue was dissolved in 200 μL of acetonitrile, then was filtered through a 0.45 μm membrane and 10 μL of the filter solution was used for the HPLC/ESI-MS$^n$ analysis.

### Synthetical methods of metabolite

Butyric acid (1 mL) was added to a solution of aconitine (40 mg) in toluene (50 mL) and pyridine (0.5 mL) was used as catalyst. The mixture was refluxed at 100 ℃ for 10 h. After the vacuum distillation at 60 ℃, the residue was dissolved in ether (10 mL×3). The reaction mixture was evaporated to dryness, and dissolved in 15% $NaHCO_3$ and $H_2O$ (2.5 mL each), the pH of which was adjusted to 7.0. Then the solution was extracted with $CHCl_3$ (5 mL×3). After $CHCl_3$ was evaporated to dryness, the residue was dissolved in acetonitrile (10 μL) for HPLC-ESI/MS$^n$ analysis and the measurement of LD50.

### Apparatus

The high performance liquid chromatograph (HPLC) system consisted of a Waters (Milford, MA, USA) 2695 HPLC with a photodiode-array detector Waters 2996 set at 235 nm. The chromatographic conditions were as follows: column, Agilent Extent $C_{18}$, 150 mm×4.6 mm, 5 μm; eluent: (A) water (containing 35 mmol ammonium acetate and adjusted with 28% ammonia solution to pH 10.5); (B) acetonitrile; (C) methanol. Gradient elution was with 65%—55% A and 17.5%—22.5% B and 17.5%—22.5% C from 0 to 20 min, 55%—40% A and 22.5%—30% B and 22.5%—30% C from 20 to 40 min, 40%—10% A and 30%—0% B and 30%—90% C from 40 to 60 min, 10%—5% A and 90%—95% C from 60 to 120 min, 5%—0% A and 95%—100% C from 120 to 150 min. The flow-rate was 0.5 mL/min and the temperature was 30 ℃. Mass spectrometry instrument was connected to the HPLC system via the UV cell outlet. Table 1 shows the linear gradient of mobile phase.

**Table 1** The linear gradient of mobile phase

| Time/min | Mobile phase/% | | |
|---|---|---|---|
| | A | B | C |
| 0—20 | 65—55 | 17.5—22.5 | 17.5—22.5 |
| 20—40 | 55—40 | 22.5—30 | 22.5—30 |
| 40—60 | 40—10 | 30—0 | 30—90 |
| 60—120 | 10—5 | 0—0 | 90—95 |
| 120—150 | 5—0 | 0—0 | 95—100 |

Both MS$^n$ and LC/MS$^n$ injected by syringe pump and LC/MS$^n$ were performed using an LCQ ion trap mass spectrometer (Finnigan, San Jose, CA, USA) equipped with an electrospray source and capable of analyzing ions up to *m*/*z* 2000. The spray voltage was 5.0 kV in the positive ion mode. The sheath gas flow rate was fixed at 60 a.u. and capillary temperature was maintained at 240 ℃. Collision energies for the MS$^n$ analyses ranged from 25% to 40% arbitrary units depending on the mass of the precursor ion, the collision gas used for MS/MS was He, and isolation width was 2.

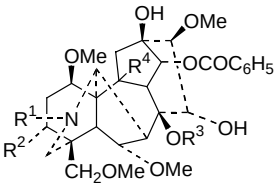
## Results and discussion

### Effect of pH on the metabolic activity of aconitine

Table 2 showed some possible structures of metabolites of aconitine by rat intestinal bacteria.

**Table 2** Some possible structures of aconitine and its metabolites by rat intestinal bacteria[a]




| $R^1$ | $R^2$ | $R^3$ | $R^4$ | Compound | *m/z* ($MH^+$) |
|---|---|---|---|---|---|
| $C_2H_5$ | OH | Ac | H | Aconitine | 646 |
| $C_2H_5$ | H | Ac | H | Deoxyaconine | 630 |
| $C_2H_5$ | OH | Ac | OH | 10-OH-Aconitine | 662 |
| $C_2H_5$ | OH | H | H | Benzoylaconine | 604 |

[a] If $R^3$ are occupied by octenoyl, hexenoyl, propionyl, butyryl, and 3-hyrdoxy-butyryl groups, the corresponding s-DDAs will be formed.

The pH of the contents of the regions including duodenum, upper small intestine, lower small intestine, cecum and colon in rat corresponded to about 6.2, 6.6, 6.6, 6.2 and 6.6,[15,16] respectively, and a pH of 7.0 was also used because this was the pH of intestinal contents, therefore, four spots were selected in the approximate range 6.0—7.0.

Figure 1 was the ESI mass spectra in positive ion mode for metabolites of aconitine at different pH. The ion at *m/z* 646 corresponding to aconitine was detected as the base peak at pH 5.8 and 6.2, which meant that large amount of aconitine was not metabolized at lower pH regions. While the base peaks were altered into the 586 and 604 at pH 6.6 and 7.0, respectively. Ion of 604 is the hydrolysis product, and 586 is the dissociating product of losing an acetic acid from aconitine.[7] In addition, although the relative abundance of the ion at *m/z* 604 was very low at pH 5.8, 6.2 and 6.6, *m/z* 604 became the base peak, and 586 was a pretty large one at pH 7.0.

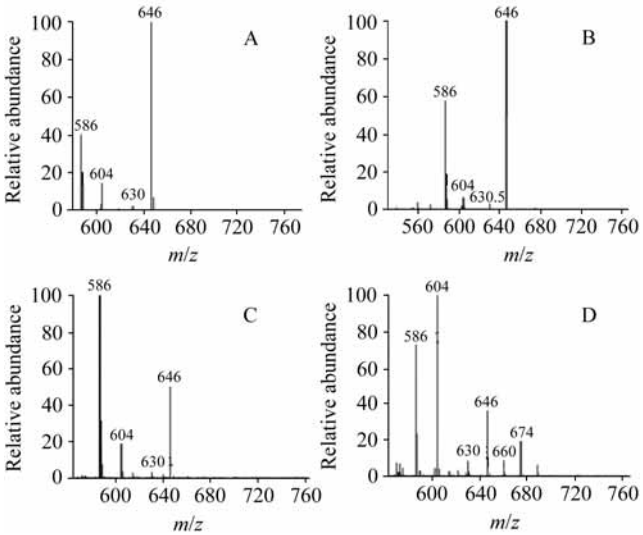



**Figure 1** ESI mass spectra of metabolites of aconitine by rat intestinal flora at pH 5.8 (A), 6.2 (B), 6.6 (C) and 7.0 (D).

It was interesting that some metabolites whose molecular mass was bigger than that of original aconitine (*m/z* 646), such as, *m/z* 674 corresponding to 8-butyryl-benzoylaconine were produced at pH 7.0. This may be generated by adding a group on the C-8. Therefore the metabolism is a complicated process, which involved not only decomposition but also addition reactions.

One possible explanation is that the rat intestinal bacteria can exhibit a better activity for the metabolism of aconitine with the escalation of pH (in the range between 5.8 and 7.0), while the reduction of pH will result in less metabolites quantitatively and qualitatively.

To confirm above point, we introduced the semi-quantitative analysis of reserpine as internal standard. As shown in Figure 2, the relative abundance of the reserpine ion was considered as 100% and we scaled the metabolites analyzed at $100\times[\text{metabolite}]^+/[\text{reserpine}]^+$ ratio. Thus, the relative abundance of the ion at *m/z* 646 according to residual aconitine standard after incubation with rat intestinal flora for 5 d at pH 5.8, 6.2, 6.6 and 7.0 corresponded to 103.22, 81.09, 21.49 and 10.15, respectively. In addition, with the escalation of pH, the relative abundance of ion at *m/z* 604 corresponding to benzoylaconine increased markedly from the values 16.04, 4.71, 9.84 to 39.72, and the relative abundance of the ion at *m/z* 674 corresponding to 8-butyryl-benzoylaconine increased from 0.66, 0.89, 1.04 to 6.13, respectively.

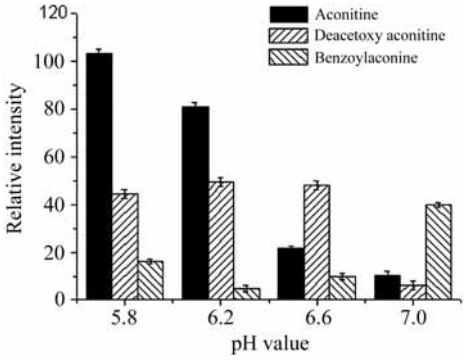



**Figure 2** The semi-quantitative analysis of aconitine and its metabolites by reserpine.

The relative intensity of ion at *m/z* 586 corresponding to deacetoxy aconitine, which in fact was a decomposition product via losing a neutral acetic acid molecule from aconitine, showed the change from 44.38, 49.36, 48.10 to 19.74 with the escalation of pH. At the pH 5.8, 6.2 and 6.6, the relative abundances of deacetoxy aconitine did not change obviously, but at pH 7.0, the relative abundance of deacetoxy aconitine decreased intensely. The dissociation reaction mentioned above depends on pH and the reaction may be rather slow at neutral condition under rat intestinal flora.

The results provide a direct and credible evidence

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that the metabolic activity of rat intestinal bacteria is highest for metabolism of aconitine, and most amounts of metabolites can be obtained at pH 7.0. Furthermore, from above study, it can be speculated that with the prolongation of metabolic time, the acids released by rat intestinal bacteria will increase the acidity of the phosphate buffer and negatively affect the conversion rate of aconitine to its metabolites.[12]

### HPLC/MS-MS$^n$ analysis of metabolites of aconitine at pH 7.0

Figure 3 showed the HPLC spectrum of metabolites of aconitine at pH 7.0.

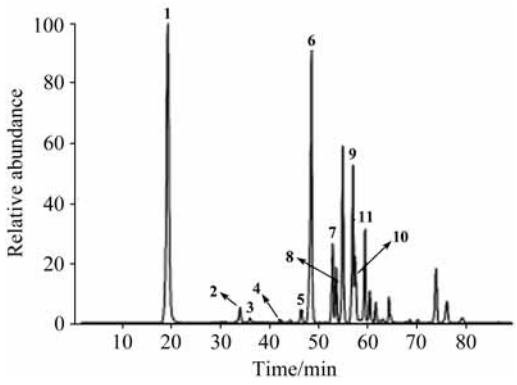
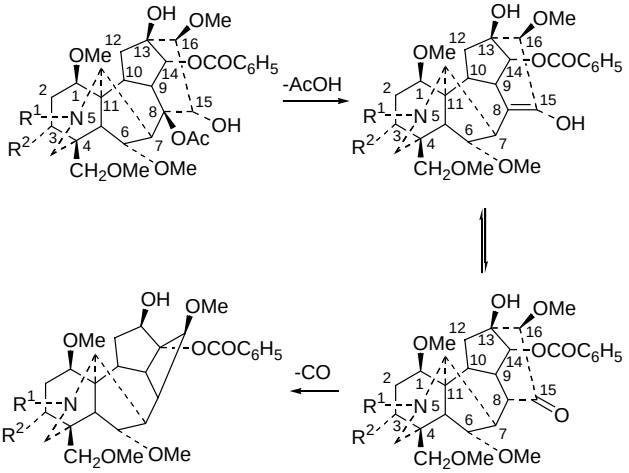



**Figure 3** HPLC chromatogram of metabolites of aconitine at pH 7. **1**. benzoylaconine, **2**. 15-deoxybenzoylaconine, **3**. 8-octenoyl-benzoylaconine, **4**. 10-OH-aconitine, **5**. 15-deoxyaconine, **6**. aconitine, **7**. 8-hexenoyl-benzoylaconine, **8**. 8-propionyl-benzoylaconine, **9**. 8-butyryl-benzoylaconine, **10**. deoxyaconitine, **11**. 8(3-hydroxyl)-butyryl-benzoylaconine.

Peak 5 and peak 10 were eluted at 46.4 and 57.9 min, respectively, and they both showed the $[M+H]^+$ at *m/z* 630 corresponding to the loss of 16 Da of aconitine. Furthermore, two ions both produced the fragment ions $[M+H-AcOH]^+$ at *m/z* 570 as the base peaks in the $MS^2$ spectra. In the $MS^2$ spectrum of peak 10, it was worth noticing that there was no fragment ions $[M+H-H_2O]^+$ at *m/z* 612 (data not shown). In the $MS^3$ spectrum of the ion (peak 10, showed in Figure 5), the data were similar to deoxyaconitine.[24] Thus, peak 10 was identified as deoxyaconitine. It was sure that there were three OH groups on the C-3, C-13 and C-15 position on the skeleton of aconitine molecule, respectively. Since C-3 hydrolysate of aconitine according to peak 10 was identified as deoxyaconitine, estimating the hydrolytic reaction to occurr at C-15 or C-13 became the next step.

As far as we know, the characteristic fragmentation pathway of protonated aconitine-type alkaloids in the $MS^3$ spectra was shown in Scheme 1[7] and the $[F-CH_3OH-CO]^+$ ion was detected as the base peak for almost all the aconitine-type alkaloids except the hypaconitine whose $[F-CH_3OH-CO]^+$ ion was observed as the second major peak. But in the $MS^3$ spectrum of the ion (peak 5), only the ions at *m/z* 538 $[F-CH_3OH]^+$ and 520 $[F-H_2O-CH_3OH]^+$ were detected. If the hydrolytic reaction happened at C-13, the ion at *m/z* 510 $[F-CH_3OH-CO]^+$ could be detected as base peak. But the $MS^3$ spectrum of peak 5 did not find *m/z* 510 ion at all. Therefore, this result strongly suggested that the hydrolytic reaction occurred at C-15, and the C=C bond between C-8 and C-15 could not conjugate with the OH group at C-15, resulting in the impossible elimination of CO ion. Thus peak 5 was identified as 15-deoxyaconitine and it was found in the metabolites of aconitine for the first time.

**Scheme 1** Proposed mechanism for successive losses of AcOH and CO from protonated aconitine-type alkaloids




The retention time ($t_R$) of peak 6 was at 48.5 min and it corresponded to the protonated molecule $[M+H]^+$ at *m/z* 646. The *m/z* 646 ion produced the fragment ion at *m/z* 586 corresponding to $[M+H-AcOH]^+$ as the base peak in its $MS^2$ spectrum. The further CID spectrum of the fragment ion of *m/z* 586 included product ions at *m/z* 568 $[F-H_2O]^+$, 554 $[F-CH_3OH]^+$, 536 $[F-H_2O-CH_3OH]^+$, and 526 $[F-CH_3OH-CO]^+$. Comparing above results with the data of standard component, peak 6 was confirmed as aconitine.[7] (F stands for M+H−AcOH for the sake of convenience).

From Figure 3, it could be seen that the major metabolites were compounds **1**, **7**—**10** and **2**—**5** were minor ones. The retention time of peak 1 was 19.2 min, and it corresponded to the predominate ion $[M+H]^+$ at *m/z* 604 which was the hydrolysis product. The $MS^2$ spectrum showed that the ion of *m/z* 604 produced fragment ions at *m/z* 586, 572 and 554, corresponding to neutral losses of $H_2O$, $CH_3OH$, and $H_2O+CH_3OH$, respectively. Thus, peak 1 was confirmed as benzoylaconine derived from the hydrolysis of aconitine.

Peak 9 was eluted at 57.0 min and it showed the intense ion $[M+H]^+$ at *m/z* 674. The *m/z* 586 ion from neutral loss of 88 Da was observed as base peak in the $MS^2$ spectrum of the *m/z* 674 ion and the $MS^3$ spectrum of the 586 ion was consistent with that of aconitine (showed in Figure 4), suggesting the presence of 8-butyryl-benzoylaconine[23] corresponding to peak 9.

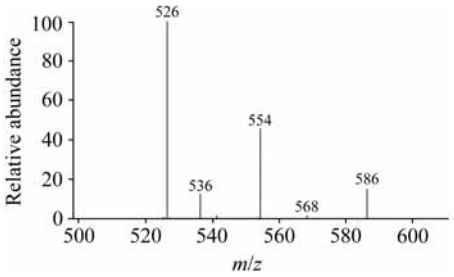



**Figure 4** The $MS^3$ spectrum of the 586 ion of peak 9.

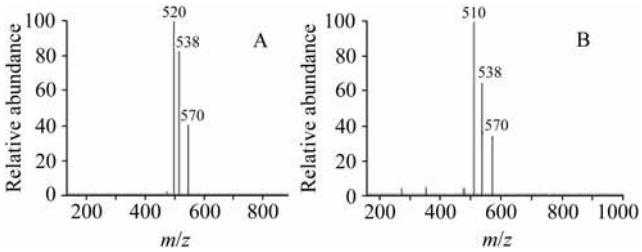



**Figure 5** The $MS^3$ spectra of peak 5 (A) and peak 10 (B).

In the same way as peak 9, peaks 3, 7, 8 and 11 eluted at 36.0, 52.8, 54.9 and 59.4 min, could be confirmed as 8-octenoyl-benzoylaconine, 8-hexenoyl-benzoylaconine, 8-propionyl-benzoylaconine and 8(3-hydroxyl)-butyryl-benzoylaconine,[23] respectively.

Peak 4 was eluted at 44.1 min and showed the protonated molecular ion $[M+H]^+$ at *m/z* 662. In the $MS^2$ spectrum of the ion, the 662 ion produced the fragment ion at *m/z* 602 $[M+H-AcOH]^+$ as the base peak. In the $MS^3$ spectrum at *m/z* 662>602, the product ions at *m/z* 584 $[F-H_2O]^+$, 570 $[F-CH_3OH]^+$ and 542 $[F-CH_3OH-CO]^+$ were found. The difference between peak 6 (aconitine) and peak 4 was the simultaneous neutral losses of $H_2O$ and $CH_3OH$ in the $MS^3$ spectra and peak 4 was deduced as 10-OH-aconitine.

Peak 2 was eluted at 33.9 min and showed the protonated molecule $[M+H]^+$ at *m/z* 588. The *m/z* 588 ion produced the fragment ions at *m/z* 570 $[M+H-H_2O]^+$, 556 $[M+H-CH_3OH]^+$ and 538 $[M+H-CH_3OH-H_2O]^+$ and the ion at *m/z* 528 $[M+H-CH_3OH-CO]^+$ was not present in the $MS^2$ spectrum. This result was similar to the fragmentation pathways of peak 5 in the $MS^3$ spectrum and it was proposed that the *m/z* 588 ion corresponding to neutral loss of 42 Da of 15-deoxyaconitine was identified as 15-deoxybenzoylaconine.

Proposed mechanism for all the metabolites of aconitine was shown in Scheme 2. Table 3 showed the HPLC/ESI-$MS^n$ data of aconitine and its metabolites in the positive ion mode.

## Study of toxicity of the metabolite by HPLC-MS method

Based on above HPLC-MS/$MS^n$ analysis, it could be speculated that aconitine could be converted into some alkaloids in which the C-8 positions were occupied by short-chain acid acyl groups, such as propionyl, butyryl, but not by long-chain fatty acid acyl group, such as oleoyl, stearoyl. Actually, the properties of these special alkaloids were similar to the diester-diterpenoid alkaloids (DDAs) and they were conveniently denoted

**Scheme 2** Proposed mechanism for all the metabolites of aconitine (R denote octenoyl, hexenoyl, propionyl, butyryl, and 3-hydroxy-butyryl)

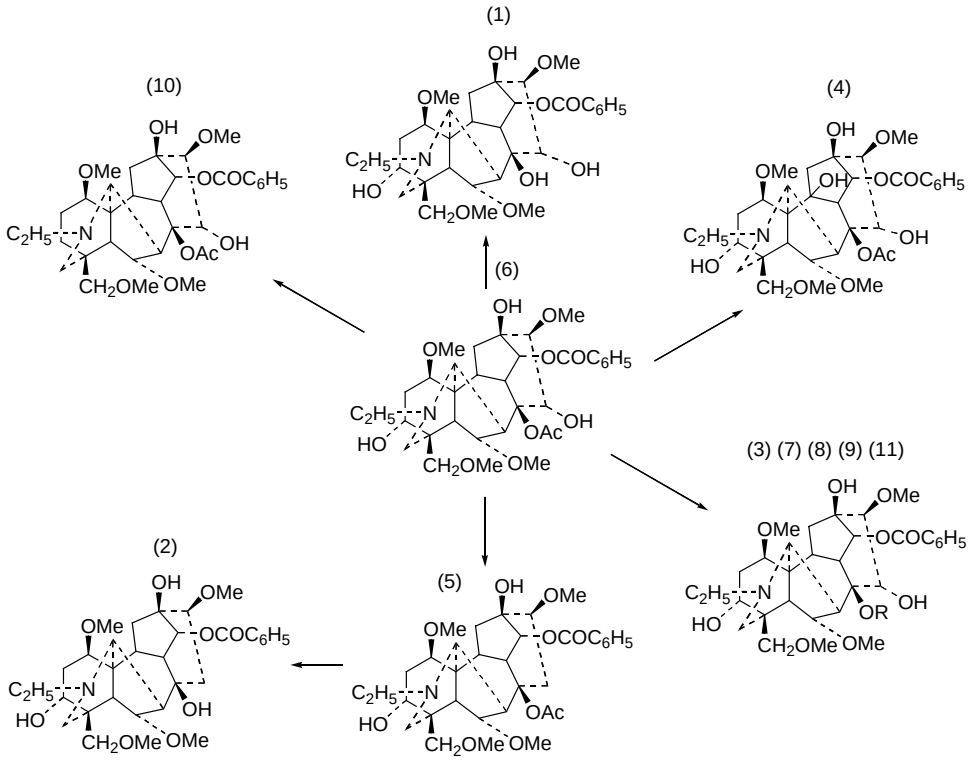


**Table 3** HPLC-MS/MS$^n$ data of aconitine and its metabolites in the positive ion mode

| No. | $t_R$/min | MS$^n$ ($m/z$) and relative abundance (%) | Identification |
|---|---|---|---|
| 1 | 19.2 | MS: 604 (100)<br>MS$^2$ [604]: 586 (22), 572 (58), 554 (100) | Benzoylaconine |
| 2 | 33.9 | MS: 588 (100)<br>MS$^2$ [588]: 570 (36), 556 (72), 538 (100) | 15-Deoxybenzoylaconine |
| 3 | 36.0 | MS: 728 (100)<br>MS$^2$ [728]: 586 (100)<br>MS$^3$ [728→586]: 568 (1), 554 (43), 536 (13), 526 (100) | 8-Octenoyl-benzoylaconine |
| 4 | 44.1 | MS: 662 (100)<br>MS$^2$ [662]: 602 (100)<br>MS$^3$ [662→602]: 584 (10), 570 (83), 542 (100) | 10-OH-Aconitine |
| 5 | 46.4 | MS: 630 (100)<br>MS$^2$ [630]: 570 (100)<br>MS$^3$ [630→570]: 538 (94), 520 (100) | 15-Deoxyaconine |
| 6 | 48.5 | MS: 646 (100)<br>MS$^2$ [646]: 586 (100)<br>MS$^3$ [646→586]: 568 (2), 554 (36), 536 (13), 526 (100) | Aconitine |
| 7 | 52.8 | MS: 700 (100)<br>MS$^2$ [700]: 586 (100)<br>MS$^3$ [700→586]: 568 (2), 554 (37), 536 (12), 526 (100) | 8-Hexenoyl-benzoylaconine |
| 8 | 54.9 | MS: 660 (100)<br>MS$^2$ [660]: 586 (100)<br>MS$^3$ [660→586]: 568 (1), 554 (43), 536 (13), 526 (100) | 8-Propionyl-benzoylaconine |
| 9 | 57.0 | MS: 674 (100)<br>MS$^2$ [674]: 586 (100)<br>MS$^3$ [674→586]: 568 (1), 554 (41), 536 (12), 526 (100) | 8-Butyryl-benzoylaconine |
| 10 | 57.9 | MS: 630 (100)<br>MS$^2$ [630]: 570 (100)<br>MS$^3$ [630→570]: 538 (64), 510 (100) | Deoxyaconitine |
| 11 | 59.4 | MS: 690 (100)<br>MS$^2$ [690]: 586 (100)<br>MS$^3$ [690→586]: 568 (1), 554 (40), 536 (12), 526 (100) | 8(3-Hydroxyl)-butyryl-benzoylaconine |

as s-DDAs. On the one hand, in order to investigate the toxicity of these s-DDAs, it was necessary to chose one of them to synthesize to study the LD50 which has not been reported before. On the other hand, owing to the comparability of structures and properties, mesaconitine showed similar metabolism pathway and metabolites with aconitine in the previous study.[13,14] From newly generated metabolites, we chose 8-butyryl-benzoylmesaconine to synthesize and test the LD50.

The synthetic method of 8-butyryl-benzoylmesaconine was improved on the basis of that by Yili *et al.*[25]

Figure 6 showed the full-scan mass spectrum of 8-butyryl-benzoylmesaconine synthesized. Figure 7 (A) and (B) showed the HPLC chromatograms of mesaconitine and 8-butyryl-benzoylmesaconine under the same HPLC condition, respectively. From Figure 7 (A), mesaconitine was eluted at 48.5 min and in Figure 7 (B), 8-butyryl-benzoylmesaconine was eluted at 51.5 min with nothing eluted at 48.5 min after mesaconitine was converted into 8-butyryl-benzoylmesaconine. Volatile butyric acid was eliminated in distillation, so it was obvious that mesaconitine was converted into 8-butyryl-benzoylmesaconine without impurity.

According to the method of Bliss, the LD50 of mesaconitine was 2.86 mg/kg and 95% confidence interval

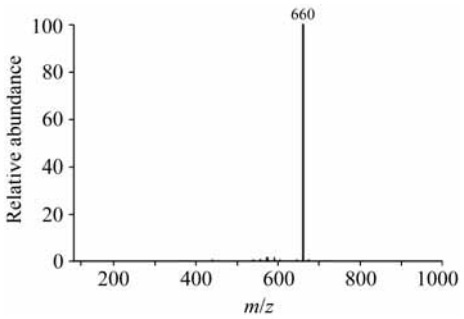



**Figure 6** The full-scan mass spectra of 8-butyryl-benzoylmesaconine.

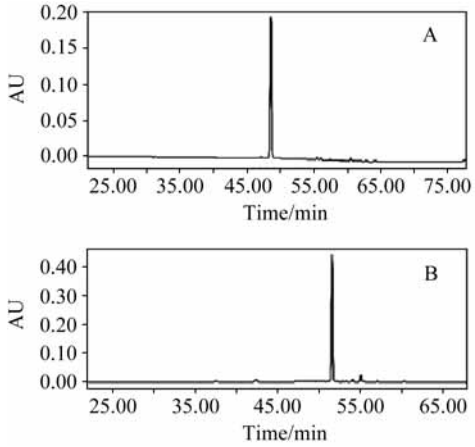



**Figure 7** The HPLC-UV chromatogram of mesaconitine (A) and 8-butyryl-benzoylmesaconine (B).

was 2.38—3.43 mg/kg; the LD50 of 8-butyryl-benzoylmesaconine was 15.78 mg/kg and 95% confidence interval was 12.85—19.38 mg/kg.

It is demonstrated that although the LD50 of 8-butyryl-benzoylmesaconine is less than that of mesaconitine, it still possesses considerable toxicity. The results suggest that HPLC along with MS method play a significant role in the determination of metabolite synthesized.

## Conclusion

In the present study, it was found that pH was an important factor to affect the metabolic process and metabolites of aconitine. By the semi-quantitative analysis of ESI-MS with reserpine as internal standard, it is concluded that the metabolic activity of rat intestinal bacteria is the highest for aconitine at pH 7.0, which is close to the pH condition of rat intestinal. The identification and elucidation of ten metabolites have been carried out by HPLC/ESI-$MS^n$. One pair of isomeric compounds has been identified and 15-deoxyaconitine has been found for the first time. Aconitine is able to be converted into other diester-diterpenoid alkaloids, monoester-diterpenoid alkaloids, s-DDAs by rat intestinal bacteria and some of them still reveal great toxicity.

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