Research article

Drug Testing and Analysis

Received: 28 August 2011 Revised: 20 December 2011 Accepted: 20 December 2011 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/dta.416

Study on the aconitine-type alkaloids of *Radix Aconiti Lateralis* and its processed products using HPLC-ESI-MS^n

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The type and content change of alkaloids of *Radix Aconiti Lateralis* (Lateral root of *Aconitum carmichaeli Debx*, an important and popular medicinal herb in Traditional Chinese Medicine) in processing were studied using high performance liquid chromatography-electrospray ionization-multi-stage mass spectrometry (HPLC-ESI-MS^n). Extract containing alkaloids, which were known to be the main bioactive components of the herb, was prepared by 1% (*v/v*) hydrochloric acid solution. An HPLC method which can simultaneously separate these alkaloids was established with gradient elution mode. Forty-eight compounds were structurally identified by employing LC-MS^n techniques; the MS^n spectra of most alkaloids displayed a characteristic behaviour of loss of CH_3COOH (60 u), CH_3OH (32 u), C_6H_5COOH (122 u), CO (28 u) and H_2O (18 u). Among them, the fragmentation ion C6H5COOH (122 u) was reported for the first time. By comparison, 22 compounds were found both in the crude materials and the processed products; 17 alkaloids were only found in the processed products and 9 alkaloids,which existed in crude material, could not be detected after processing. In the process of identification, we found new kinds of alkaloids, with protonated molecules at *m/z* 452, 468, and 482. We called these compounds dehydra-hypaconine, dehydra-mesaconine, and dehydra-aconine, respectively. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: *Radix Aconiti Lateralis*; alkaloids; electrospray ionization; mass spectrometry

Introduction

The processing (Praeparata) of drugs, termed as *paozhi* in Traditional Chinese Medicine (TCM), is a pharmacy technology, which was taken according to the theory of TCM, the need of differentiation of symptoms and signs,the nature of the drugs themselves, and the different needs of dispensing or preparing, in order to meet therapeutic requirements to ensure safety and produce satisfactory medicinal effects in clinical practice. *Radix Aconiti Lateralis* (root of *Aconitum carmichaeli Debx*.), is an important Chinese medicinal herb used in clinical practice for treating rheumatosis, rheumatoid arthritis, and other inflammations.[1] Over the last several decades, studies have shown that alkaloids were the major bioactivity constituents of this herb.[2]

Crude *Radix Aconiti Lateralis* has great toxicity. TCM practice proved that processing may decrease its toxicity. The processed products were strong in their effect of restoring yang to save from collapse and to tonify fire and assist yang, etc. However, how could we reveal the difference between the crude drug and the processed drug? In this paper, HPLC hyphenated with ion trap MS, a relatively new technique growing fast in popularity which has been successfully applied to elucidate the structures of active compounds in herbal extracts,[3] was employed to explain the mechanism by identifying the alkaloids.

Materials and methods

Standards and samples

Standards of acotinine, mesacotinine, and hypaconitine (>98%) were purchased from the National Institute for the Control of Pharmaceutical & Biological Products (Beijing, China). Samples of Radix *Aconiti Lateralis* were collected from the cultivation base of *Aconitum carmichaeli Debx*. (Jiangyou, China), and were air-dried according to the procedure described in China Pharmacopoeia.[4] All the samples were authenticated by Prof. Yuting Chen.

Solvents and reagents

HPLC-grade acetonitrile (MeCN) was purchased from E. Merck (Darmstadt, Germany) and ammonia (AR grade) was obtained from Beihua Fine Chemicals Co., Ltd. (Beijing, China). Ammonia and the water used for HPLC were purified by a Milli-Q system (Millipore, Milford, MA, USA).

Sample preparation

Approximately 0.5 g of powdered *Radix Aconiti Lateralis* and 15 ml of 1% (*v/v*) hydrochloric acid solution were mixed in a 50-ml flat-bottomed flask. After circumfluence extraction for 1 h, the sample solution was filtered through a 0.45 μm Nylon filter (Iwaki Glass, Tokyo, Japan) into an HPLC amber sample vial for HPLC analysis.

HPLC method

HPLC analysis was carried out on an Agilent Series 1100 (Agilent, Wilmington, Germany) equipped with a binary pump,

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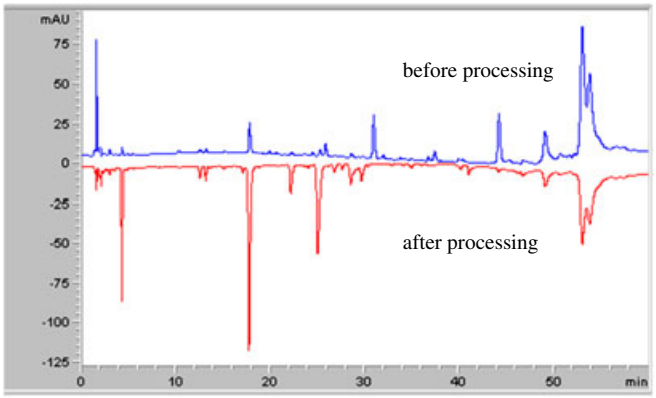


Figure 1. The HPLC of fuzi before and after processing.

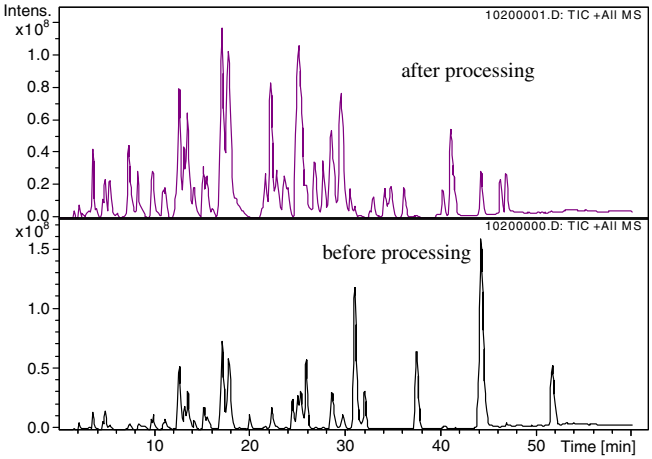


Figure 2. The TIC of fuzi before and after processing.

Table 1. Compounds identified in fuzi before processing

No.	t_R	MS	MS^2	MS^3	Identification
1	2.3	502.4	454.3	436.1	10-OH-mesaconine
2	3.7	486.4	436.5	404.1	mesaconine
3	5.4	500.6	450.9	418.1	aconine
4	7.5	470.7	438.4	406.1	hypaconine
5	8.5	470.1	453.0	420.1	deoxymesaconine◆
6	9.3	469.0	436.2	404.1	3,13-deoxyaconine◆
7	9.8	484.4	452.2	420.1	deoxyaconine
8	11.2	394.6	376.2	358.1	karakolidine
9	12.7	454.5	436.3	404.1	fuziline
10	12.8	468.4	419.4	386.1	dehydrated 10-OH-hypaconine
11	13.6	408.6	390.3	358.1	isotalatizidine
12	13.7	344.6	326.1	250.9	bullatine A
13	15.3	394.5	376.1	358.1	chuanfumine
14	15.6	378.8	360.1	342.1	carmichaeline
15	17.2	438.7	420.4	388.1	neoline
16	17.6	452.8	421.8	388.2	dehydrated hypaconine
17	17.7	590.6	541.5	508.3	14-benzoylmesaconine
18	18	378.7	360.2	342.1	karakoline
19	21.7	554.9	524.2	399.1	dehydrated 3,13-deoxyaconine◆
20	22.4	604.9	555.6	522.2	14-benzoylaconine
21	24.9	632.8	573.1	540.1	mesaconitine◆
22	25	422.5	390.2	358.1	talatizamine
23	25.3	574.5	542.6	510.3	14-benzoylhypaconine
24	26	648.8	588.7	529.1	10-OH-mesaconitine◆
25	28.7	358.4	340.2	322.0	songorine
26	29.6	466.2	433.0	400.2	dehydrated deoxyaconine◆
27	31.4	632.6	572.6	513.2	10-OH-hypaconitine
28	32	662.7	602.8	543.6	10-OH-aconitine◆
29	37.8	646.7	586.6	526.8	aconitine◆
30	44.5	616.6	556.7	525.0	hypaconitine
31	51.7	630.9	570.7	511.6	deoxyaconitine◆

◆ these compounds were not detected after processing.

automatic sample injector, and diode array detector (DAD). Separation was performed on an Agilent ZORBAX Eclipse extend-C_{18}(4.6 mm × 250 mm,5 μm) at ambient temperature with a sample injection volume of 10 μl and the detection wavelength was at 240 nm. Solvent A (water/ammonia, 99 : 1 *v/v*) and solvent B (MeCN) were used as mobile phase (1 ml/min). A gradient program of 15–45% (B) in 0–45 min, 45–60% (B) in 45–60 min was used.

Table 2. Compounds identified in fuzi after processing

No.	t_R	MS	MS^2	MS^3	Identification
1	1.8	502.7	454.3	436.1	10-OH-mesaconine
2	3.6	486.1	436.6	404.1	mesaconine
3	4	516.4	466.9	434.1	10-OH-aconine▲
4	5.4	500.6	450.5	418.1	aconine
5	7.6	470.4	438.3	406.1	hypaconine
6	8.3	468.6	418.5	386.1	dehydrated mesaconine▲
7	9.8	484.9	452.4	420.1	deoxyaconine
8	10.8	498.4	448.3	416.1	dehydrated 10-OH-aconine▲
9	11.1	394.6	376.3	358.1	karakolidine
10	12.2	482.6	432.5	400.1	dehydrated aconine▲
11	12.5	454.9	436.3	404.1	fuziline
12	12.8	468.5	418.7	386.1	dehydrated 10-OH-hypaconine
13	13.1	606.7	558.5	540.4	14-benzoyl-10-OH-mesaconine▲
14	13.5	408.5	390.3	358.1	isotalatizidine
15	13.6	344.7	326.1	298.0	bullatine A
16	15.5	378.4	360.1	342.1	carmichaeline
17	15.6	394.3	376.1	358.1	chuanfumine
18	15.7	360.9	253.0	210.8	napelline▲
19	17.3	438.5	420.2	388.1	neoline
20	17.6	452.6	420.6	388.1	dehydrated hypaconine
21	18	590.6	542.7	508.3	14-benzoylmesaconine*
22	18.1	378.8	360.1	342.1	karakoline
23	21.7	480.7	462.3	430.1	14-acetylneoline▲
24	21.9	616.6	566.6	534.1	deoxymesaconitine▲
25	22.5	604.6	555.5	522.3	14-benzoylaconine
26	23.6	360.6	342.2	324.0	12-epinapelline▲
27	24.9	422.7	390.3	358.1	talatizamine
28	25.5	574.7	542.5	510.3	14-benzoylhypaconine
29	28.2	602.9	553.0	520.1	dehydrated 10-OH-aconitine▲
30	28.5	358.5	340.1	322.0	songorine
31	29.5	464.9	432.4	400.1	14-acetyltalatizamine▲
32	30.5	558.8	540.6	508.2	14-benzoyl-deoxyhypaconine▲
33	31	632.8	572.5	512.3	10-OH-hypaconitine
34	32.9	586.7	536.6	504.2	dehydrated aconitine▲
35	34.1	542.7	510.5	492.2	14-benzoylnoeline▲
36	34.3	584.7	566.6	534.2	8-acetyl-14-benzoylnoeline▲
37	41.1	556.7	524.5	492.3	dehydrated benzoylhypaconine▲
38	44.2	616.8	556.7	497.4	hypaconitine
39	46.2	571.0	538.5	506.3	dehydrated deoxyaconitine▲

▲ these compounds were detected after processing.

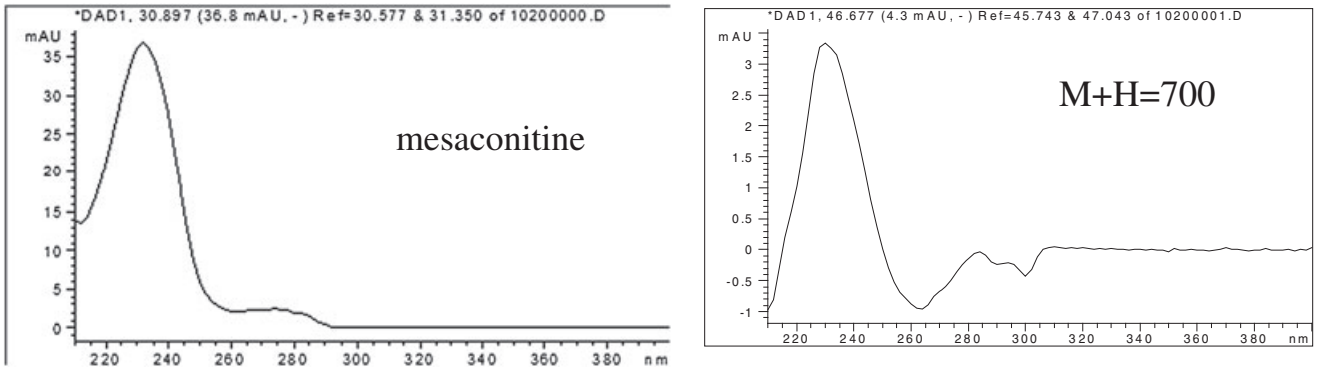


Figure 3. The UV wavelength scan.

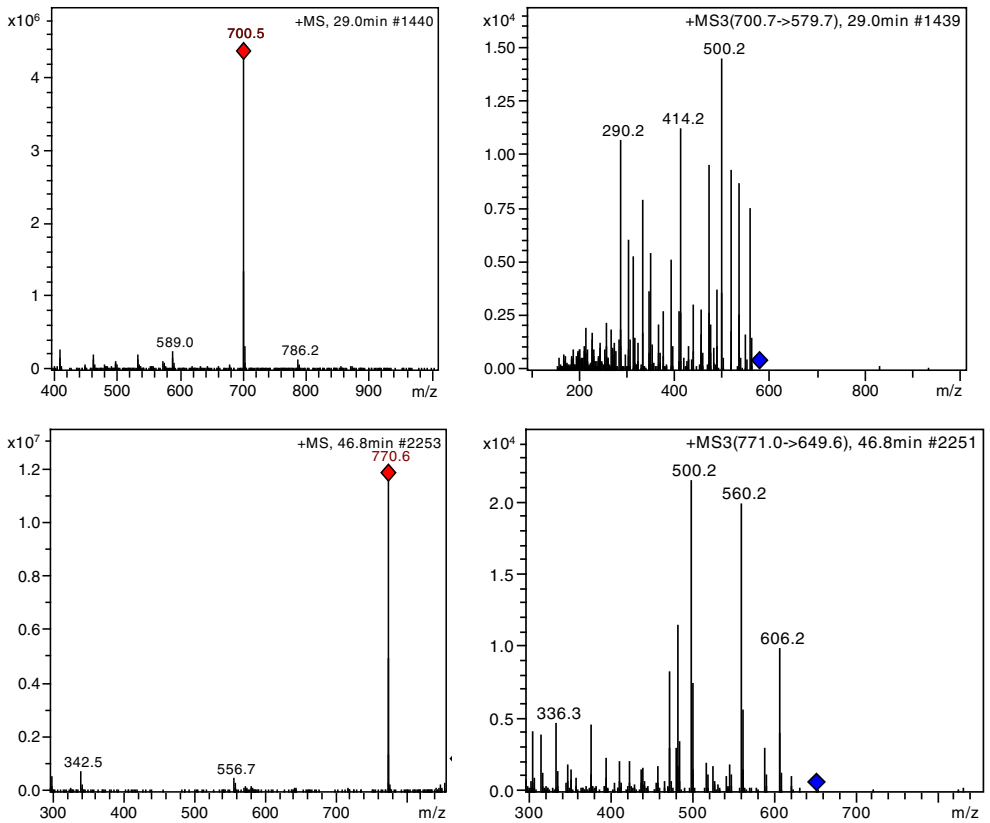


Figure 4. The MS^{1-3} of unknown alkaloids.

LC-MS^n identification

The analytical conditions for the LC-MS^n were the same as those used for the HPLC analysis, except for that one-fourth of the eluant was introduced into the MS system by using split technique. Agilent 1100 HPLC/MSD Trap XCT/plus mass spectrometer (Wilmington, Germany) equipped with an ESI source was used. An HPLC system coupled with DAD was controlled by an HPLC-MS^n ChemStation software system. Auto MS^3 mode of mass spectrometer was chosen to analyze the sample.

Results and discussion

Selection of extraction methods

The effective compositions of *Radix Aconiti Lateralis* were alkaloids. Alkaloids were easy to dissolve in acid water, so 1% (*v/v*) hydrochloric acid was chosen, as this solvent can extract most alkaloids in this herb.

Optimization of LC condition

In the mobile phase, ammonia was added to depress the tailing of the peaks of aconitum alkaloids. The effect of a series of concentrations of ammonia in mobile phase A on the separation of alkaloids was investigated. It was found that the distortion of peaks of benzoylaconine and benzoylmesaconine would not separate if the concentration of ammonia was less than 1%. Finally, the concentration of 1% ammonia was selected to ensure the separation of the fingerprints of *Radix Aconiti Lateralis* (Figure 1).

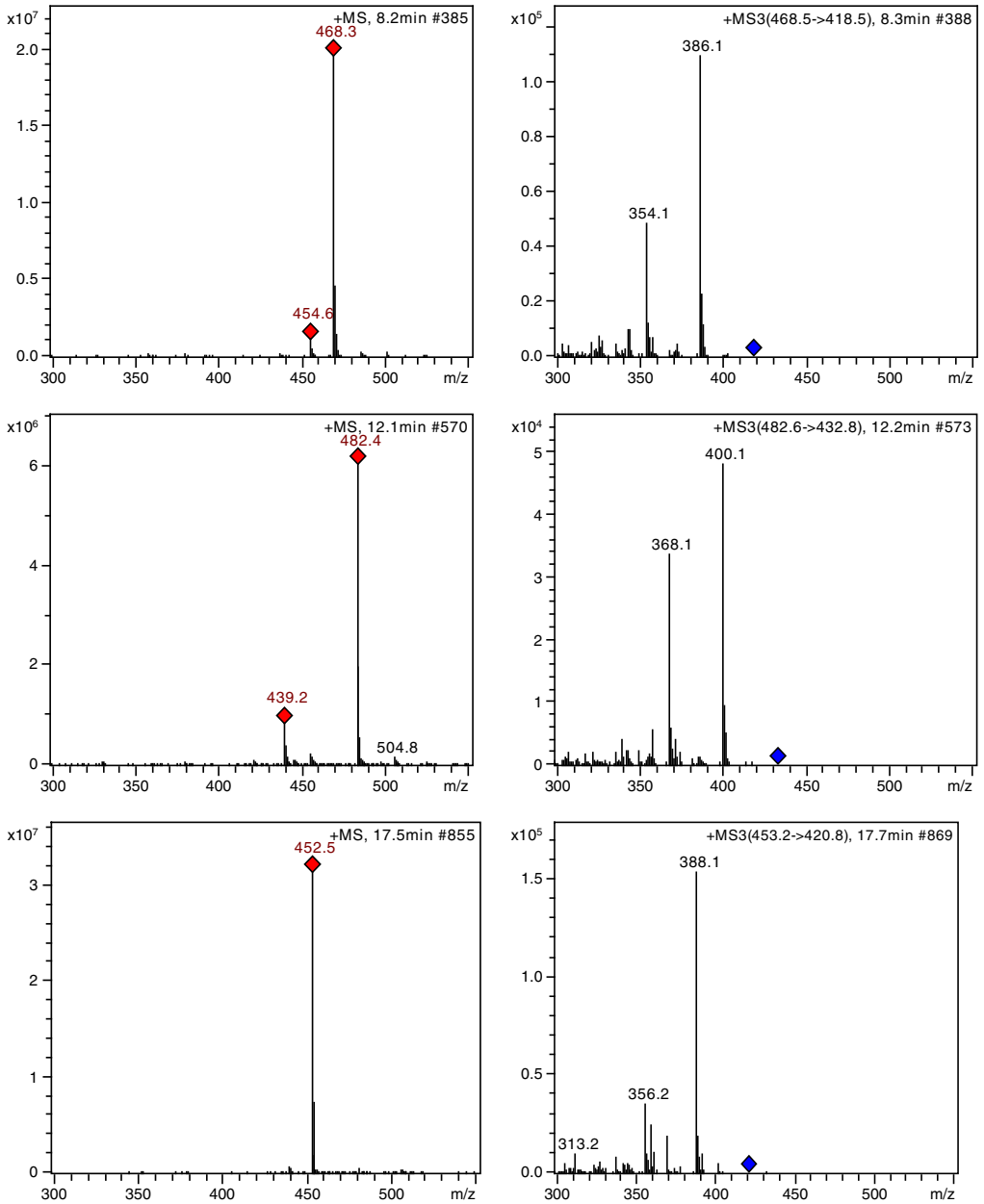
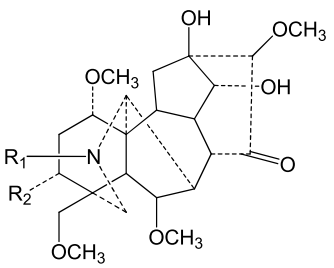


Figure 5. The MS^{1-3} dehydra-aconitine, dehydra-mesaconitine and dehydra-hypaconitine



	R_1	R_2
dehydra-aconine	C_2H_5	OH
dehydra-mesaconine	CH_3	OH
dehydra-hypaconine	CH_3	H

Figure 6. The structure of dehydra-aconine,dehydra-mesaconine, and dehydra-hypaconine.

Identification of alkaloids in *Radix Aconiti Lateralis* by LC-MS^n

The multi-alkaloid HPLC of authenticated herbal material was obtained according to the developed HPLC method described above. Identification of the peaks in this fingerprint profile was carried out by using the LC-MS^n technique. The reference standard aconitine was analyzed by direct injection in order to optimize ESI conditions. This experiment aimed at improving MS parameters showed that the positive ion mode is more sensitive than the negative ion mode for identifying alkaloids. The condition is list in Table 1. The LC chromatogram exhibited good agreement with that obtained from HPLC analysis (Figure 2).

Forty-eight aconitine-type alkaloids (Tables 1 and 2)[5–17] were tentatively identified by detailed study of their MS^n spectral data (Table 1). By comparison with published data and hydrolyzates of aconitine, mesaconitine, and hypaconitine, according to tR and MS, we identified protonated molecules at 646, 616,632, 604, 590, 574, 500, 486, and 470 representing aconitine, mesaconitine, hypaconitine, benzoylaconine, benzoylmesaconine, benzoylhypaconine, aconine, mesaconine,

and hypaconine. The others were identified by reference. The aconitum alkaloids were complicated, so some components could not be identified completely by MS^n. The ESI-MS fragmentation of alkaloids mainly involved in the loss of a molecule of H_2O (18 u), CH_3COOH (60 u), C_6H_5COOH (122 u), CH_3OH (32 u) and CO (28 u); among them C_6H_5COOH (122 u) was reported for the first time, its parent ion was 700 and 770.[8] A further study of these two compounds showed that they both had the same ultraviolet (UV) wavelength scan (Figure 3) with aconitine, and they all have the same MS fragmentation with aconitine (Figure 4). Based on this data, we speculated that they were aconitum alkaloids; furthermore, there was a substituent on C_{15} position which reduced the activity of C_8-acetoxyl group. The structure of these compounds was not identified, but our data indicated that they were a new class of aconitum alkaloids.

Different alkaloids show different cleavage rules. Aconitine-type alkaloids share a common C_{19}-norditerpenoid skeleton. Traditionally, they can be divided into three major types according to the substitute at the C_8 position of the diterpenoid-skeleton. These are monoester-diterpenoid alkaloids (MDA), diester-diterpenoid alkaloids (DDA), and lipo-alkaloids. According to the fragmentation pathways, we found new kinds of alkaloids, with protonated molecules at *m/z* 452, 468, and 482. We named these compounds dehydra-hypaconine, dehydra-mesaconine, and dehydra-aconine, respectively. The MS^n spectra of these compounds were different (Fig. 5). The ESI-MS fragmentation of dehydra-hypaconine, and dehydra-mesaconine mainly involved in the loss of a molecule of H_2O (18 u) and CH_3OH (32 u), but, dehydra-hypaconine was only involved in the loss of CH_3OH (32 u). By comparing the structure of aconine, mesaconine, and hypaconine, we found that their difference was the substitute of C_3 position. There was a hydroxyl group on C_3 position of aconine and mesaconine, while, there was no substitute on C_3 position of hypaconine. By speculation, we ensured the structure (Figure 6).

By comparing the types of alkaloids before and after processing, we found that the content of MDA and amine alkaloids increased, while the content of DDA reduced. Especially, diester-diterpenoid alkaloids, aconitine, and mesaconitine could not be detected. The result indicated that hypaconitine was more stable than aconitine and mesaconitine.

Conclusions

In clinical use of TCM, the decoction solvent of *Radix Aconiti Lateralis* is usually water. Furthermore, it is well known that alkaloids dissolve in acid water easily, so we choose 1% (*v/v*) hydrochloric acid solution as the solvent of extract. In our study, the kinds of diester-diterpenoid alkaloids were much less compared to the literature,[8] which could be due to the polarity difference of the decoction solvent.

In processed products of Radix *Aconiti Lateralis*, several new alkaloids were identified, these alkaloids revealed that some new chemical reactions occurred during processing. These data are useful to reveal the mechanism of toxicity reduction and efficacy enhancement after processing.

In this paper, we found that the types of alkaloids in *Radix Aconiti Lateralis* were very complicated. Chemical components change significantly and the concentration changes significantly after processing. However, it is difficult to obtain all kinds of standard components, so the content change of all alkaloids will be studied in the future.

Acknowledgements

This study was supported by National Natural Science Foundation of China (No. 30901959) and Key Project of Chinese National Programs for Fundamental Research and Development (2009CB522805).

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