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Full Length Research Paper

Study on the reaction products of hypaconitine in methanol and water using electrospray ionization mass spectrometry

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To identify the products of hypaconitine in water and methanol, electrospray ionization mass spectrometry (ESI-MS) method is used to trigger a full scan product ion experiment for confirmation. Six hydrolysates were detected, and their pseudo-molecules are 470, 556, 574, 484, 452 and 588, respectively, indicating hypaconine, pyrhypaconitine, benzoylhypaconine, 8-methoxyl-hypaconine, dehydration hypaconine and 8-methoxyl-14-benzoylhypaconine, respectively. Among them, 8-methoxyl-hypaconine, 8-methoxyl-14-benzoyl-hypaconine and dehydration hypaconine are identified first in the reaction products of hypaconitine in methanol.

Key words: Hypaconitine, electrospray ionization, mass spectrometry.

INTRODUCTION

Aconite plants are widespread throughout Europe, Northern Asia and North America. They contain aconitum alkaloids (AAS) and other minor components, such as chasmanine, kobusine and higenamine (Hideki et al., 2009). AAS consist of aconitines, aconines and benzoylaconitines, among which the most toxic group includes aconitine, mesaconitine and hypaconitine. In China, Chinese herbs, which contain aconitum alkaloids, were used in clinical after processing. According to literatures (Liu et al., 2007; Tan et al., 2011; Wei et al., 2009; Liu et al., 2006), AAS were unstable in the course of processing. Usually, there are two ways of processing the aconite plants. One way is to be boiled in water, and the other is to be soaked in wine. However, how to find out the changes of chemical composition in the process-sing is under study. In this paper, hypaconitine is the main effective constituent of aconitum plants, that is of effects; cardiotonic, analgesia, anti-inflammatory, anti-tumor, etc (Liu et al., 2011), and was selected as a research object. On the basis of previous studies (Liu et al., 2007; Tan et al., 2011; Wei et al., 2009), several kinds of products of products were reported for the first time using electrospray ionization mass spectrometry, meanwhile, the reaction of hypaconitine in water and methanol was deduced.

MATERIALS AND METHODS

Instruments and reagents

Agilent 6430 triple Quad LC-MS (Agilent USA); Standard of hypaconitine was purchased from the Chinese Authenticating Institute of Material and Biological Products (Beijing, China). Analytical grade methanol, HPLC grade acetonitrile and ultra-pure water were used.

Sample preparation

Two 5.5 mg of powdered sample were weighed accurately and put into two10 ml volumetric flasks separately. Then, 10 ml of methanol or 10 ml of water were added into each flask accurately. Next, the mixture was heated (100°C for 30 min, and filtered through a 0.45 µm resistor-capacitor (RC) filter.

MS Parameters

Instrument parameters were as follows: ionization mode: ESI; capillary voltage: 3.5 kV; extraction cone voltage: 5 V; radio frequency (RF) lens voltage: 0.5 V; cone gas 50 L/h; desolvation

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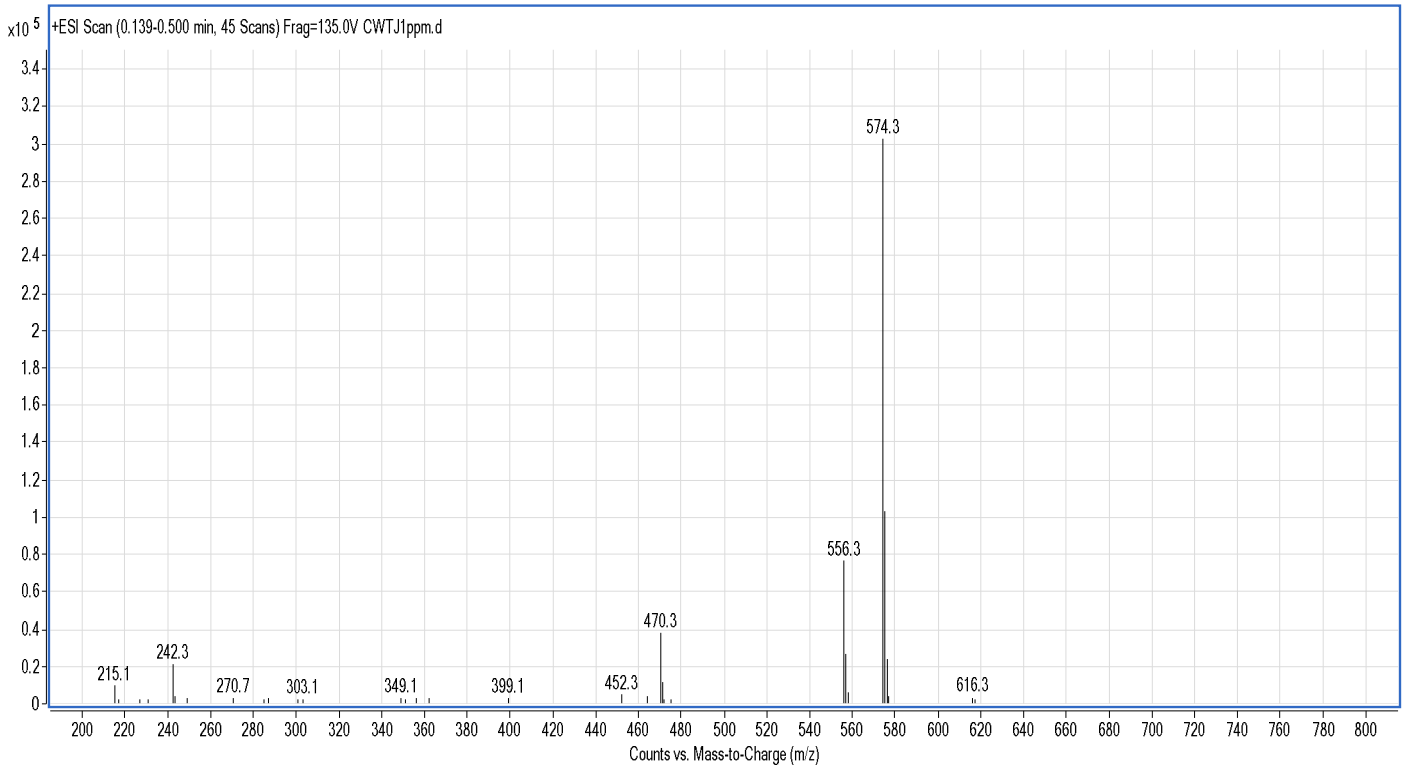

Figure 1. The MS of products of hypaconitine in water.

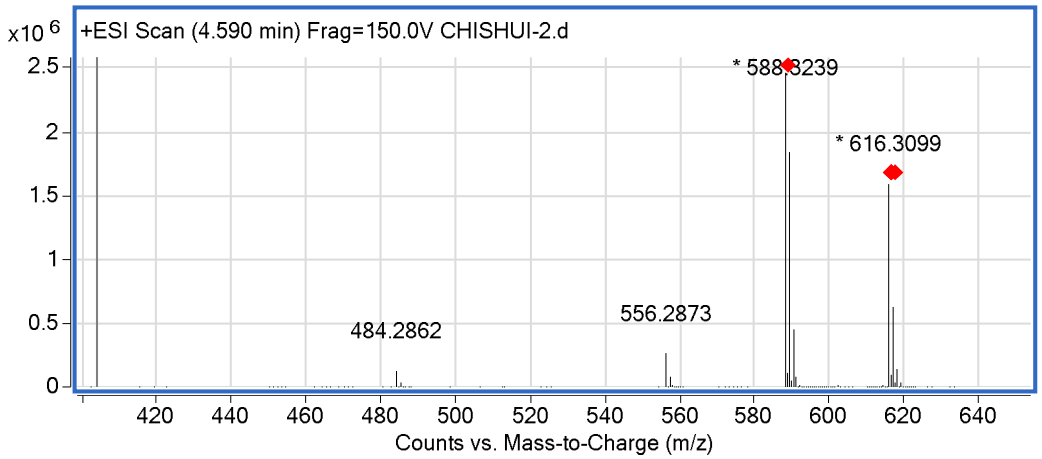

Figure 2. The MS of products of hypaconitine in methanol.

gas flow: 40 L/h; collision energy: 15 eV. Figures 1 and 2 are the MS^{1-2} of valtrate and acevaltrate.

RESULTS AND DISCUSSION

Under the positive ion mode, pseudo-molecules $[M+H]^+$ of aconitine-type alkaloids were often detected easily (Hideki et al., 2009). The mass spectrum of products of hypaconitine in water are as shown in Figure 1, the mass spectrum of products of hypaconitine in water are as shown in Figure 2. The pseudo-molecular of reaction products of aconitine were at m/z 588, 574, 470, 556, 452 and 484, respectively. In order to validate the experiment, we conducted a high resolution mass spectrometry (MS) test, and MS^1 is as shown in Figure 3 and the MS^2 is

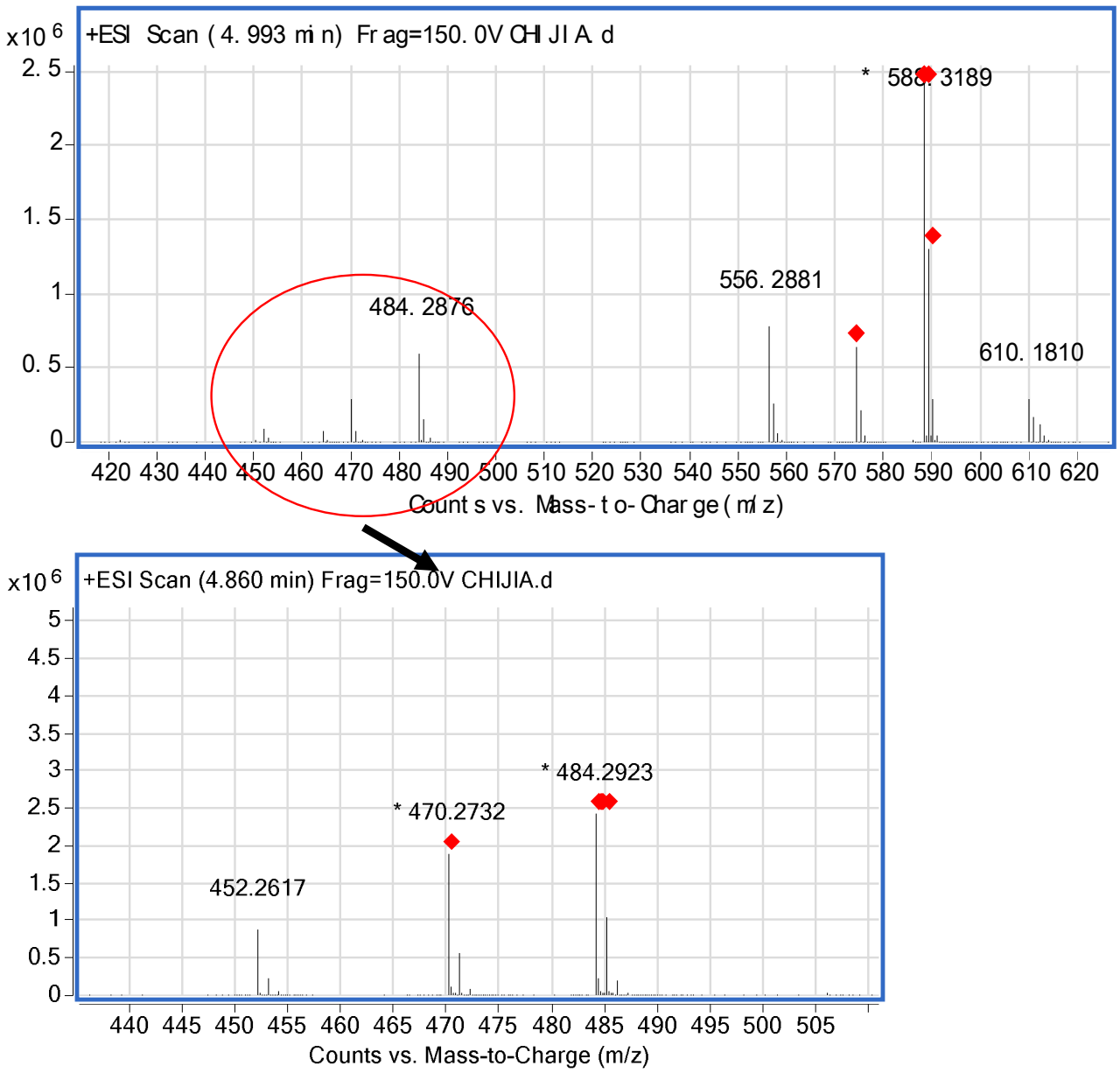

Figure 3. The MS of products of hypaconitine in methanol.

shown in Figure 4.

The pseudo-molecular of 556, according to the literature (Zhao et al., 2008) was identified as pyrhypaconitine. Its MS^2 was at m/z 524 and 492, indicating loss of CH_3OH and CH_3OH, respectively. The pseudo-molecular of 588, was referred to be 8-acetyl-14-benzoylaconine. Its MS^2 was at m/z 556, according to the literature (Desai et al., 1989). After loss of acetyl, carbonium ion was formed and substitution nucleophilic 1(SN1) (CH_3O^-) occurred, the reaction equation is as shown in Figure 5. The fragmentation ion was loss of $[AC+H-AcOH+CH_3OH]^+$, which was reported for the first time. The pseudo-molecular of 484, was identified as 8-acetyl-hypaconine. The reaction equation is as shown in Figure 6. Its MS^2 was at m/z 452, indicating loss of CH_3OH. According to literature (Desai et al., 1989), the reaction was reported for the first time.

The pseudo-molecular of 470, according to the literature (Tan et al., 2011), was referred to be hypaconine. The pseudo-molecular of 574, according to the literature (Tan et al., 2011), was referred to be benzoylhypaconine. The pseudo-molecular of 452, according to the literature (Tan et al., 2011), was identified as dehydration hypaconine. Its MS^2 was at m/z 420, indicating loss of CH_3OH. Through the afore-mentioned analysis of experimental data, the reaction process is as

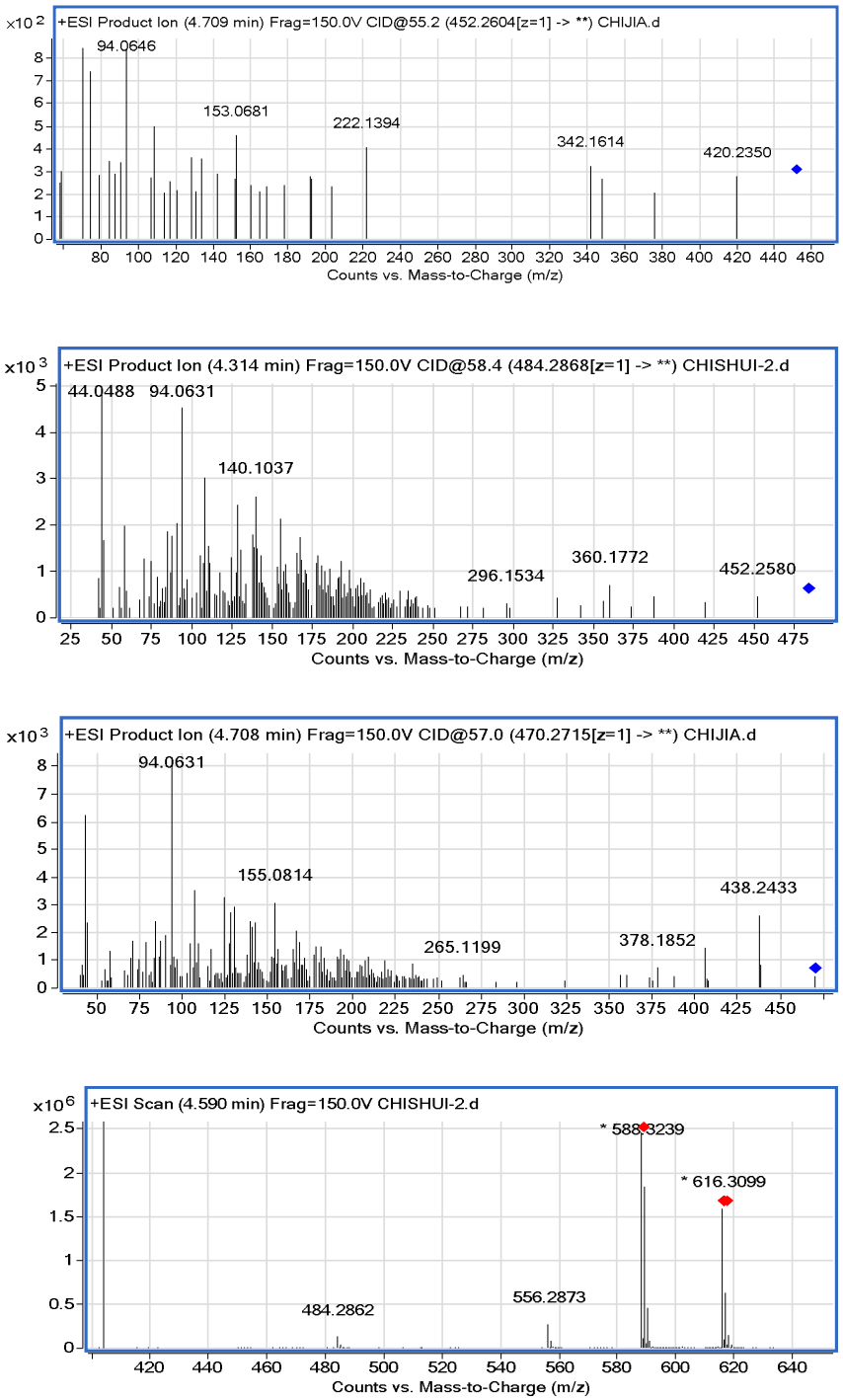

Figure 4. The MS^2 of products.

shown in Figure 7.

Conclusion

In this paper, we used methanol and water as extraction solvent, and obtained hydrolyzate and alcoholysis product. The process is similar to the processing of Fuzi, so the changes of these products could be similar in the processing of aconite plants. The experimental results can provide some data for the processing of aconite plants in the future. 8-methoxyl-hypaconine, 8-methoxyl-14-benzoylhypaconine and dehydration hypaconine were reported for the first time. We will spend lots of time to

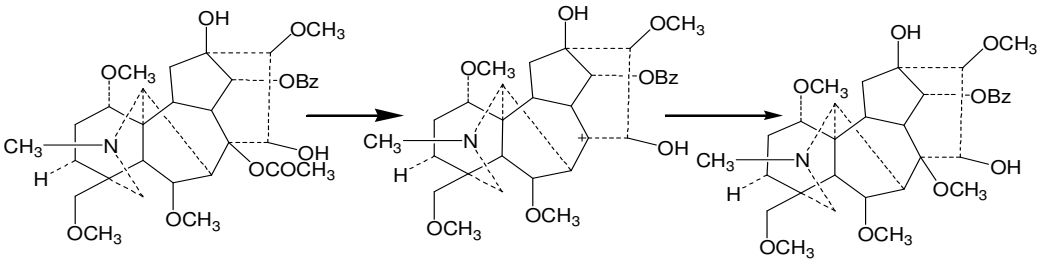

Figure 5. The possible reaction of 8-acetyl-14-benzoylaconitine.

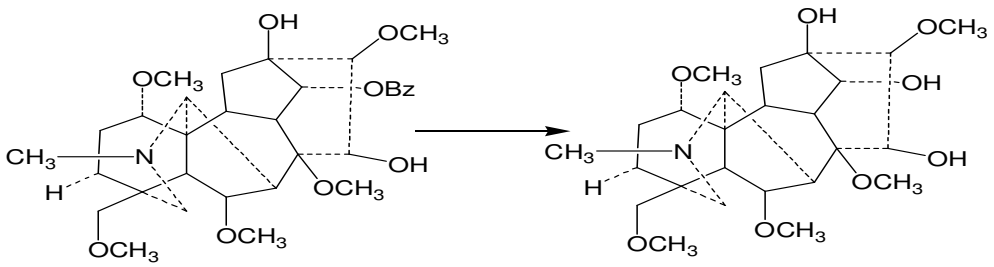

Figure 6. The possible reaction of 8-acetyl-hypaconnine.

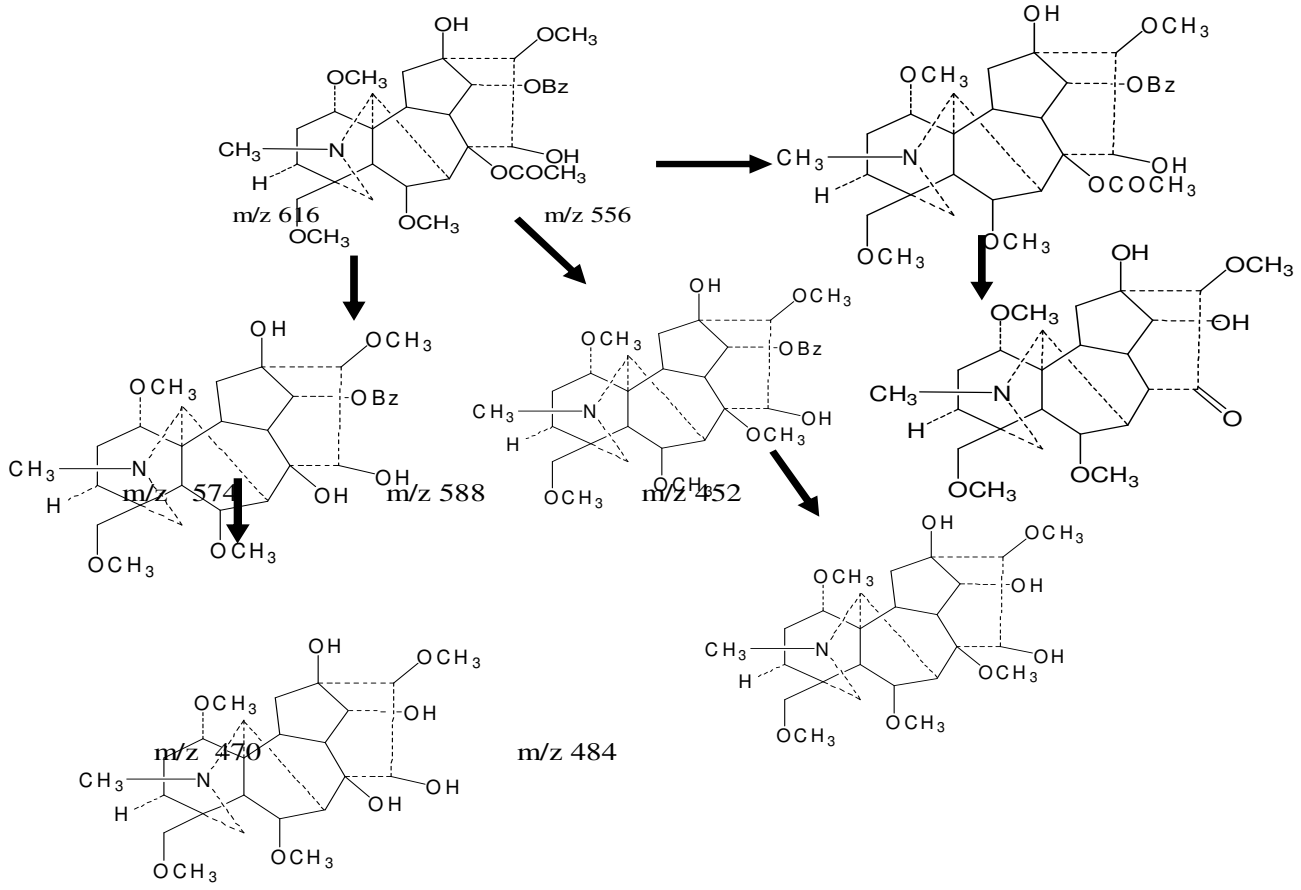

Figure 7. The reaction pathway of hypaconitine.

identify the structure by NMR in the future.

ACKNOWLEDGEMENTS

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