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Reaction product analysis of aconitine in dilute ethanol using ESI-Q-ToF-MS

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The study was done to identify the reaction products of aconitine in dilute ethanol using electrospray ionization-triple quad time-of-flight mass spectrometry (ESI-Q-TOF-MS). Five hydrolysates were detected, their pseudo-molecules are 632, 604, 586, 570, 500**,** they are 8-ethyoxyl-14-benzoylaconitine, benzoylaconine, pyraconitine, 8-acetyl-14-ethyoxylaconitine, aconine, respectively. Among them, 8-ethyoxyl-14-benzoylaconitine and 8-acetyl-14-ethyoxylaconitine were identified firstly as reaction products of aconitine in dilute ethanol, and can thus be used as indicators in quality control of medicinal *Aconitum* preparations used in Traditional Chinese Medicine.

1. Introduction

Aconitine (AC) is one of the important alkaloids in Traditional Chinese Medicines prepared from *Aconitum* species and has potential toxicity and wide bioactivity. It belongs to the diester diterpenoid alkaloids that share a common C19-norditerpenoid skeleton and has anti-inflammatory and analgesic activities with spicy flavor and strong toxicity. The structure/activity relationship shows that alkaloids that activate or block Na^+ channels have a benzoyl ester side chain in the C-14 or C-4 positions respectively (Zhao et al. 2008). Levels of aconitine are reduced in Radix Aconiti Lateralis Preparata after processing (Liu et al. 2006). The toxicity and bioactivity of Radix Aconiti Lateralis Preparata relates to the structure and level of reaction products. Aconitine was eluated in a gradient setting by HPLC and then analyzed by ESI-MS^n. Four hydrolysates were found with protonated molecular ions at m/z 604, 500, 586 and 482 (Liu et al. 2007). Aconite root was processed with wine as assistant materials since Song dynasty and the processing method include wine soaked, wine and vinegar boiling, water soaked and wine boiling, wine boiling, wine and salt soaked, etc. Aconite root and Radix Aconiti Kusnezoffii were used to make a medicinal liquor, for example like chuanwu liquor in Puji Benshi Fang, but there is no study on the reaction products of aconitine in dilute ethanol. Electrospray ionization mass spectrometry (ESI-MS) is a soft ionization technique developed recently, particularly suitable for fast sample analysis and identification of products in complex systems, and has a high sensitivity, strong specificity, is simple and fast. The hydrolyzation of aconitum diester diterpenoid alkaloids was analysed by electrospray ionization mass spectrometry (Bao et al. 2009). Electrospray ionization-quadrupole time-of-flight mass spectrometry has the advantage of ultrahigh sensitivity and multistage tandem, in the present study, and was used to explore the reaction products of aconitine in 10% ethanol. Characteristic components was found in the reaction products, and this provides the basis for further study of medicinal liquor's preparation and wine processing of Aconitum containing Traditional Chinese Medicine.

2. Investigations, result*s* and discussion

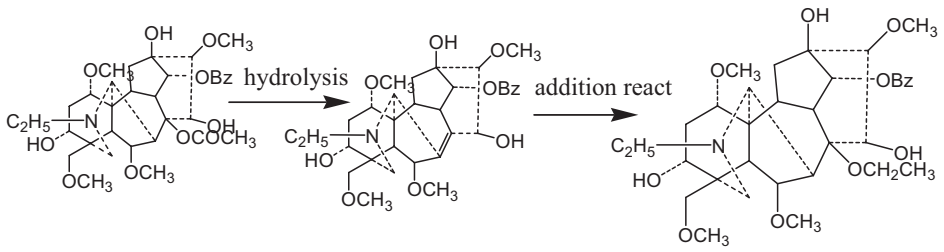
Under the positive ion mode, pseudo-molecules $[M+H]^+$ of aconitine-type alkaloids were often detected easily (Zhao et al. 2008). The pseudo-molecular of reaction products of aconitine were at m/z 500, 570, 586, 604, 632 respectively.

The pseudo-molecular of 632 should be 8-ethyoxyl-14-benzoylaconitine. Its MS^2 was at m/z 582, according to the literature (Desai et al. 1989). After the loss of acetyl, aconitine addition reaction occurs, the reaction equation is shown in Scheme 1. The fragmentation ion was loss of $[AC+H\text{-}AcOH+CH_3CH_2OH]^+$. The fragmentation was reported for the first time.

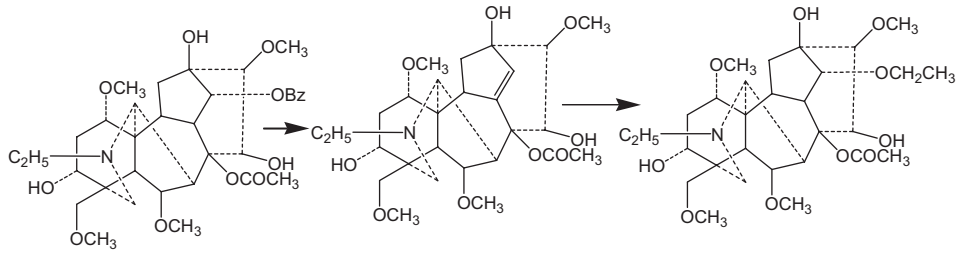
The pseudo-molecular of 570, is assigned to 8-acetyl-14-ethyoxylaconitine. Its MS^2 was at m/z 538 and 506, 538 was loss of CH_3OH and CH_3OH, while 506 was loss of CH_3OH from 538. The reaction equation is shown in Scheme 2. According to the literature (Desai et al. 1989), the fragmentation ion was loss of $[AC+H\text{-}C_6H_5COOH+CH_3CH_2OH]^+$. The fragmentation was reported for the first time.

The pseudo-molecular of 586, according to the literature (Wei et al. 2009), should be pyraconitine. Its MS^2 was at m/z 568 and 536, 568 was loss of H_2O, while 536 was loss of H_2O and CH_3OH. The pseudo-molecular of 604, according to the literature (Liu et al. 2007), is benzoylaconine. Its MS^2 was at m/z 572 and 554, 572 was loss of CH_3OH, while 554 was loss of H_2O. The pseudo-molecular of 500, according to the literature (Liu et al. 2007), is aconine. Its MS^2 was at m/z 450, 450 was loss of CH_3OH and H_2O. Through the above analysis of experimental results, its reaction process is shown in Scheme 3.

Aconitine belongs to the diester diterpenoid alkaloids (Scheme 3) and two esters are astable. 8-Ethyoxyl-14-



Scheme 1: Reaction of 8-acetyl-14-benzoylaconitine



Scheme 2: Reaction of 8-acetyl-14-ethoxy aconitine

benzoylaconine and 8-acetyl-14-ethyoxylaconine are two characteristic reaction products of aconitine in 10% ethanol, which can be used as indicators in quality control of medicinal liquor's preparation and wine processing of *Aconitum* preparations in Traditional Chinese Medicine. The toxicity and bioactivity of *Aconitum* diester diterpenoid alkaloids relate to their esters, and the studies on the toxicity and bioactivity of new reaction products and the quantitative change during the course of processing can help to elucidate processing mechanism, control processing or control quality, safety and effectivity of drugs in clinical use.

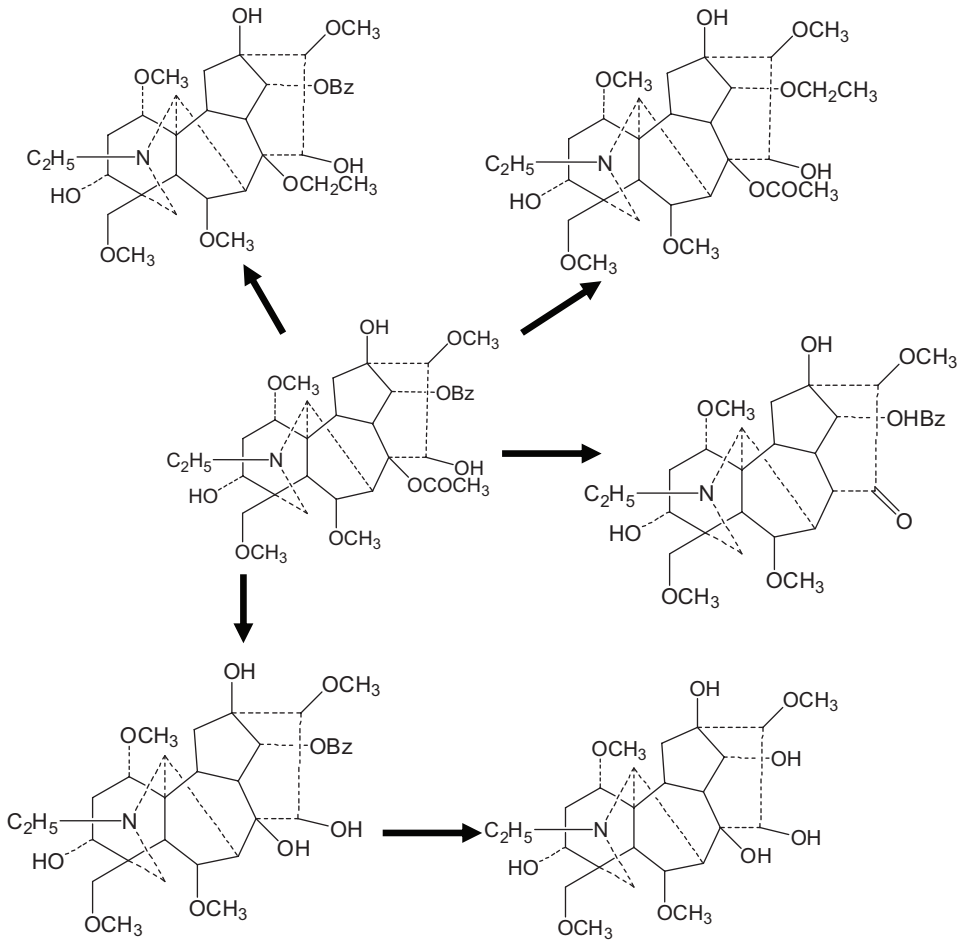
3. Experimental

3.1. Standards and samples

Standards of aconitine (> 98%) were purchased from the National Institute for the Control of Pharmaceutical & Biological Products (Beijing, China).

3.2. Solvents and reagents

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



Scheme 3: Reaction of aconitine in dilute ethanol

3.3. Sample preparation

Aconitine was dissolved in ethanol to obtain a solution of approx. 0.5 μg/ml, it was mixed with 18 ml water in a 50 ml flat-bottomed flask. After circumfluence extraction for 30 min. The sample solution was filtered through a 0.45 μm Nylon filter (Iwaki Glass, Tokyo, Japan) into a HPLC amber sample vial for HPLC-MS^n analysis.

3.4. Mass spectrometry analysis

All experiments were performed using an LCQ ion trap mass spectrometer, equipped with an electrospray source. The spray voltage was 4.0 KV in the positive ion mode and capillary temperature was set 350 °C, capillary voltage of 4000 eV, positive ion mode detection, atomizing pressure: 30 psi, dry nitrogen flow rate: 10 L/min, scan range: 100 ∼ 1000 u.

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References

Bao Yi, Song Feng-rui, Liu Zhi-qiang, Liu Shu-ying, Yin Jun (2009) Analysis on hydrolyzation of Aconitum diester diterpenoid alkaloids by Electrospray Ionization Mass Spectrometry. J Chin Mass Spectrometry Soc 30: 1–5.

Desai HK, Joshi BS, Ross SA, Pelletier SW (1989) Methanolysis of the C-8 acetoxyl group in aconitine-type alkaloids: a partial synthesis of Hokbusine A. J Nat Prod 52: 720–725.

Liu Fang, Yu Xiang-hong, Li Fei, Tan Yu-yuan, Qiao Yan-jiang (2006) Determination of three kind of diester diterpenoid alkaloids(DDAs) in Aconitum carmichaeli and its processed products by HPLC. China J Chin Mater Med 31: 1160–1161.

Liu Yong-gang, Liu Qian, Zhang Hong-gui, Qiao Yan-jiang (2007) Studies on hydrolysates of aconitine by HPLC-MS^n. Chin J New Drugs 16: 303–305.

Wei Wei, Li Xun-wen, Jin Yong-ri (2009) Study on hydrolysis product of aconitine. Chin J Anal Chem 37: D067–D068.

Zhao Yu-feng, Song Feng-rui, Wang Xi-ye, Guo Xin-hu, Liu Zhi-qiang, Liu Shu-ying (2008) Studies on the biotransformation of 16-O-demethylaconitine and electrospray Ionization Tandem Mass Spectrometry. Acta Chim Sin 66: 525–530.