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New Metabolites of Aconitine in Rabbit Urine

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Abstract: A sensitive analytical method to identify and determine aconitine and its metabolites in rabbit urine was developed by liquid chromatography – electrospray ionization mass spectrometry (LC/ESI-MS^n). In this method, aconitine and its four metabolites in rabbit urine were isolated and deduced as 16-*O*-demethylaconine (M**1**), benzoylaconine (M**2**), 16-*O*-demethylbenzoylaconine (M**3**) and aconine (M**4**). M**1** and M**3** are new metabolites of aconitine and M**2** and M**4** are first identified in rabbit urine.

Keywords: *Aconitum*, metabolites, aconitine, LC/MS.

Chuanwu(*Aconitum carmichaeli Debx*.), Caowu(*Aconitum kusnezoffii Reichb*.) and Fuzi(*Aconitum carmichaeli Debx*.) in Chinese traditional medicine belong to plants of genus *Aconitum* in family of Ranunculaceae, which were collected in the Pharmacopoeias of China and some other countries. Combining with wumei, wushe and other chinese traditional medicines, they were used to deal with rheumatic arthritis.

These Chinese traditional medicines have very good effects on the aforementioned disease, but sometimes are highly toxic[1]. Aconitine frequently occurred poisoning. The toxicity is caused by alkaloids of the aconitines from the above plants. Studies on the metabolites of aconitine have many senses in medical science and phar-macology.

Experimental

The Finnigan LCQ ion trap mass spectrometer (Finnigan Mat, San Jose, CA, USA) with electrospay interface (ESI) was used for the seperation and determination of aconitine and its metabolites. Five rabbits were orally administered aconitine 0.5mg • kg^{-1}. The urine was collected in 24 h and filtered before it was loaded onto the SPE (solid-phase extraction) column, which was activated by washing with 3 mL of methanol and then 3 mL of water. The sample was eluted with 2 mL methanol and the elution was injected

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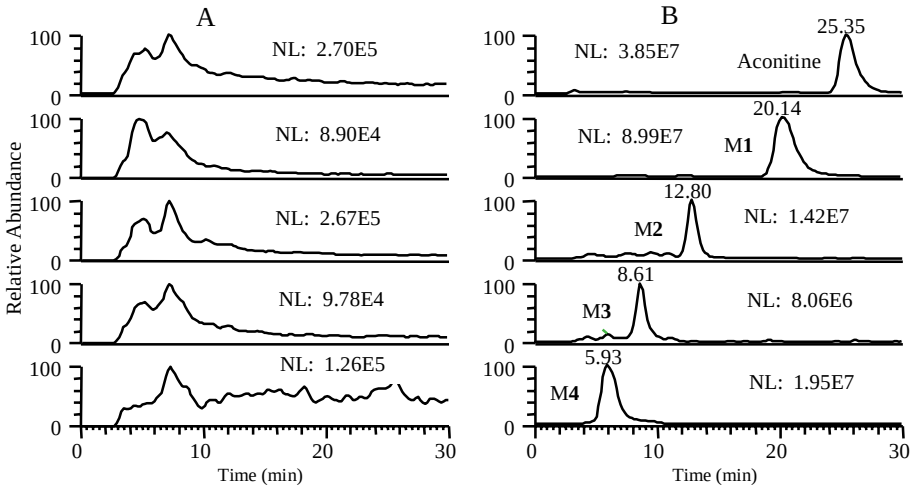
into the LC/MS system for analysis. The samples were loaded analyzed on a Hypersil C_{18} column (150 mm × 4.6 mm, particle size 5 μm, Elite Scientific Instrument Corporation, Dalian, China). The mobile phase consisted of acetonitrile-water-formic acid (105:95:1, v/v). The flow rate was 0.5 mL·min^{-1}.

Results and Discussion

Comparing with the blank rabbit urine, aconitine and its metabolites were found in the urine of rabbits after oral administration of aconitine (**Figure 1**). Their protonated molecular ions at *m/z* 646, 632, 604, 590, 500 and multistage fragment ions with neutral loss of 60 u, 32 u, 28 u and 18 u were monitored. The data of aconitine and its metabolites are given in **Table 1**.

Figure 1 Full scan MS^2 chromatograms of aconitine and its metabolites in the urine of rabbit



* NL is a shortened form of normalize *Y*-axis normalization mode, in which LCQ automatically sets the vertical scale equal to the height of the largest peak in a mass spectrum

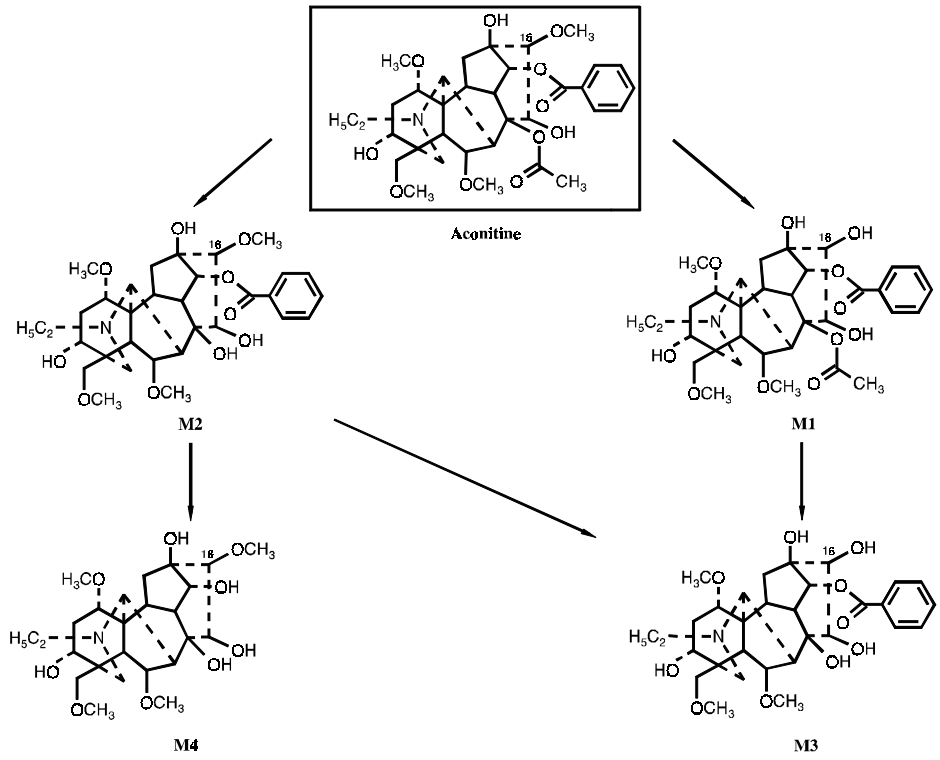
A: blank rabbit urine, B: the urine of rabbits after oral administration of aconitine

Table 1 LC/MS^n data of aconitine and its major metabolites

Compounds	t_R/min	$[M+H]^+$	MS^2	MS^3
Aconitine	25.35	646	586(– CH_3COOH)	554(– CH_3OH), 526(– CH_2=CH_2)
M1	20.14	632	572(– CH_3COOH)	540(– CH_3OH), 512(– CH_2=CH_2)
M2	12.80	604	554(– CH_3OH, – H_2O)	522(– CH_3OH)
M3	8.61	590	540(– CH_3OH, – H_2O)	508(– CH_3OH)
M4	5.93	500	450(– CH_3OH, – H_2O)	418(– CH_3OH)

Their relative concentrations were M**1** > aconitine > M**4** > M**2** > M**3**. According to the protonated molecule and multistage fragment ions, furthermore, compared with the metabolites of lappaconitine[2], M**1**–M**4** were deduced as 16-*O*-demethylaconine, benzoylaconine, 16-*O*-demethylbenzoylaconine and aconitine, respectively. M**1** and M**3** are two new metabolites of aconitine. The metabolic route was proposed in **Figure 2**.

Figure 2 Proposed metabolic pathways of aconitine in rabbit



Acknowledgments

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