**Supplementary material 2**

**Preparation and identification of pyroaconitine and 16-epi-pyroaconine**

**Methods**

*Preparation and identification of pyroaconitine*

1.5 g aconitine is put into a 100 mL round bottom flask and heat under vacuum at 100 ℃ for 1h. DAC dynamic preparation liquid chromatography system is used for separation. Two fractions are obtained and further purified. The solvent in the fraction is recovered under negative pressure. After removing solvation, the residues were dissolved in dichloromethane. Then, filter and recover the solvent to obtain brownish red oil samples. Freeze them in a refrigerator at -50 ℃, white crystals were precipitated, which are compound 1 and compound 2. About 10 mg of these two compounds are dissolved in deuterated dimethyl sulfoxide for nuclear magnetic analysis (AVANCE NEO-700 MHZ superconducting fourier Transform nuclear magnetic resonance spectrometry (Bruker, Germany)).

*Preparation and identification of 16-epi-pyroaconine*

0.5 g compound 1 is put into a 10 mL round bottom flask with nitrogen and put magnetic stirrer into it with heat at 120 ℃ and react for 40 min. DAC dynamic preparation liquid chromatography system is used for separation. Then the solvent in the fraction is recovered under negative pressure and obtained white amorphous powder (compound 3). About 10 mg compound 3 is dissolved in deuterated dimethyl sulfoxide for nuclear magnetic analysis.

**Results**

*Identification of pyroaconitine and 16-epi-pyroaconine*

The structure of compounds was determined by 1H NMR and 13C NMR analysis and comparison with the data in the published literature. The results are as follows:

Compound 1: white crystal; EI-MS，m/z 586 [M+]，molecular formula, C32H43NO9. 1H NMR (DMSO-*d6,* 700Mz):0.98 (3H, t, *J* = 7 Hz, NCH2CH3), 1.70 (1H, t, *J* = 13.6 Hz), 2.13-2.15 (2H, m), 2.19-2.21 (2H，m), 2.32-2.33 (1H, m), 2.33-2.34 (2H, m), 2.56-2.57 (1H, d, *J* = 12.6 Hz), 2.69 (1H, d, *J* = 10.5 Hz), 3.05 (1H, m), 3.22, 3.23, 3.25, 3.69 (each 3H, s, OCH3 × 4), 3.76 (1H, d, *J* = 9.8 Hz), 3.80 (1H, t, *J* =2.1 Hz), 3.96 (1H, d, *J* = 7 Hz), 4.48 (1H, d, *J* = 4.9 Hz), 5.32 (1H, d, *J* = 4.9 Hz), 7.60 (2H, t, *J* = 7.7 Hz), 7.73 (1H, t, *J* = 7 Hz), 7.98-8.01 (2H, m). 13C-NMR (DMSO-*d6*, 175Mz): 83.6 (C-1), 32.0 (C-2), 72.4, (C-3), 43.9 (C-4), 49 (C-5), 84.2(C-6), 42.4 (C-7)，49.0(C-8), 38.1(C-9), 44.1(C-10), 50.9(C-11), 35.5 (C-12), 77.4 (C-13), 78.1(C-14), 213.3 (C-15), 86.5 (C-16), 62.2 (C-17), 77.1 (C-18), 47.5(C-19), 49.1(C-21), 13.6 (C-22), 56.0 (1-OMe), 58.2(6-OMe), 61.4 (16-OMe), 58.7(18-OMe),165.6(OCO). 129.8 (1’’), 130.5(3’’,5’’), 129.3 (2’’,6’’). The above data are basically consistent with the literature reports, so compound 1 is identified as α-pyroaconitine([Jian, et al., 2012](#_ENREF_1)) (Figure. 4A and Figure. S1).

Compound 2: white crystal; EI-MS，m/z 586 [M+]，molecular formula, C32H43NO9. 1H NMR (DMSO-*d6,* 700Mz): 0.98 (3H, t, *J* = 6.3 Hz, NCH2CH3), 2.14-2.17 (3H, m), 2.21-2.23 (3H, m), 2.35 (2H, q, *J* = 14 Hz), 2.59 (1H, d, *J* = 6.3 Hz), 2.75 (1H, s), 3.10 (1H, q, *J* = 10.5 Hz), 3.23, 3.24, 3.29, 3.58 (each 3H, OCH3 × 4), 3.33 (1H, s), 3.49-3.53 (1H, m), 3.77 (1H, d, *J* = 8.4 Hz), 4.00 (1H, d, *J* = 7 Hz), 4.49 (1H, d, *J* = 5.6 Hz), 5.05 (1H, s), 5.24 (1H, d, *J* =4.9Hz), 7.56 (2H, t, *J*=7 Hz), 7.69 (1H, t, *J* = 7Hz)，7.99-8.00 (2H，m). 13C-NMR (DMSO-d6, 175Mz): 83.5(C-1), 35.5 (C-2), 72.3(C-3), 43.1(C-3), 47.9(C-5), 84.2(C-6), 42.8(C-7), 38.5(C-9), 44.0(C-10), 50.7(C-11), 36.2 (C-12), 76.1 (C-13), 78.9 (C-14), 212.9 (C-15), 90.0 (C-16), 61.5(C-17), 76.1 (C-18), 47.4(C-19), 49.0 (C-21), 13.3 (C-22), 56.0 (1-OMe), 58.3 (6-OMe), 61.5(16-OMe), 58.7 (18-OMe), 165.7 (OCO), 130.0 (1’’), 129.0 (2’’,6’’), 130.4 (3’’,5’’), 133.7 (4’’). The above data are basically consistent with the literature reports, so compound 2 is identified as β- pyroaconitine([She, et al., 2012](#_ENREF_2)) (Figure. 4B and Figure. S2).

Compound 3: white powder, EI-MS，m/z 482 [M+], molecular formula C25H39NO8. 1H NMR (DMSO-*d6*, 700 MHz): 0.82 (1H d, J = 6.3 Hz), 0.97 (3H, t, *J* = 7 Hz, NCH2CH3), 1.30 (1H, s), 1.58-1.52 (1H, m), 2.02-2.06 (1H, m), 2.08-2.12 (1H, m), 2.15 (2H, d, *J* = 6.3 Hz), 2.21 (1H, q, *J* = 12.6 Hz), 2.36-2.28 (2H, m), 2.43 (1H, q, *J* = 5.6 Hz), 2.70-2.62 (2H, m), 2.76-2.73 (2H, m), 2.99 (1H, dd, *J* = 11.2, 6.3 Hz), 3.19, 3.23, 3.28, 3.61 (each 3H, OCH3 × 4), 3.66 (1H, s), 3.79 (1H, d, *J* = 7.7 Hz), 3.95-3.92 (1H, m), 3.98 (1H, d, *J* = 6.3 Hz), 4.43 (1H, s), 4.79-4.70 (1H, m), 4.81 (1H, s), δ 5.30 (1H, d, *J* = 2.1 Hz). 13C-NMR (DMSO-*d6*, 175Mz): 83.6(C-1), 33.0(C-2), 72.5(C-3), 43.9(C-4), 47.9(C-5), 83.8(C-6), 42.4(C-7), 44.4(C-8), 48.9(C-9), 40.7(C-10), 50.9(C-11), 33.0(C-12), 78.1(C-13), 77.1 (C-14), 213.2 (C-15), 86.7 (C-16), 61.5 (C-17), 77.1 (C-18), 49.1 (C-19), 47.6(C-21), 13.6(C-13), 55.9(1-OCH3), 58.3 (6-OCH3), 62.0(16-OCH3), 58.7(18-OCH3 ). The above data are basically consistent with the literature reports, so compound 3 is identified as 16-epi-pyroaconitine([Ji and Wang, 2006](#_ENREF_3)) (Figure. 4C and Figure. S3). In fact, 16-epi-pyroaconine also has isomerism of R and S at position C-16. Unfortunately, the other configuration has not been separated.

**Reference**

[1] Jian X. X. and Tang P. and Liu X. X.*, et al.* 2012. Structure-cardiac activity relationship of C19-diterpenoid alkaloids. Nat Prod Commun. 7, 713-720.

[2] She X. K. and Jian X. X. and Chen D. L.*, et al.* 2012. Studies on the relative reactivity of three hydroxyl groups in aconitine. J Asian Nat Prod Res. 14, 665-677. https://doi.org/10.1080/10286020.2012.684684.

[3] Ji H. and Wang F. P. 2006. Structure of lasiansine from Aconitumnagarum var. lasiandrum. J Asian Nat Prod Res. 8, 619-624. https://doi.org/10.1080/10286020500208550.