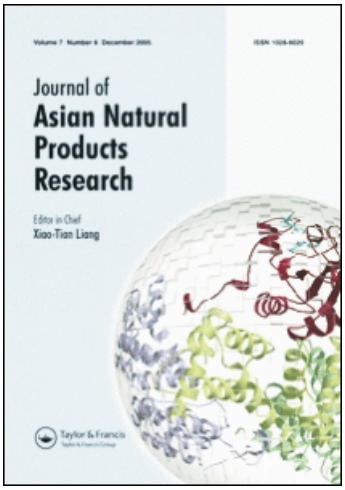
This article was downloaded by:
On: *22 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two new 18-carbon norditerpenoid alkaloids from *Aconitum sinomontanum*

J. -J. Tan[a]; C. -H. Tan[a]; B. -Q. Ruan[b]; S. -H. Jiang[a]; D. -Y. Zhu[a]
[a] State Key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China [b] Fujian Institute for Drug Control, Fuzhou, China

To cite this Article Tan, J. -J. , Tan, C. -H. , Ruan, B. -Q. , Jiang, S. -H. and Zhu, D. -Y.(2006) 'Two new 18-carbon norditerpenoid alkaloids from *Aconitum sinomontanum*', Journal of Asian Natural Products Research, 8: 6, 535 — 539
To link to this Article: DOI: 10.1080/10286020500175643
URL: http://dx.doi.org/10.1080/10286020500175643

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Asian Natural Products Research, Vol. 8, No. 6, September 2006, 535–539

Taylor & Francis
Taylor & Francis Group

Two new 18-carbon norditerpenoid alkaloids from *Aconitum sinomontanum*

J.-J. TAN†, C.-H. TAN†, B.-Q. RUAN‡, S.-H. JIANG† and D.-Y. ZHU†*

†State Key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 555 ZuChongZhi Road, Shanghai 201203, China
‡Fujian Institute for Drug Control, Fuzhou 350001, China

(Received 21 December 2004; revised 1 February 2005; in final form 5 February 2005)

Downloaded At: 19:16 22 January 2011

Two new 18-carbon norditerpenoid alkaloids, sinaconitines A (**1**) and B (**2**), together with two known alkaloids, ranaconitine (**3**) and lappaconitine (**4**), were isolated from the roots of *Aconitum sinomontanum*. Their structures were elucidated on the basis of spectral evidences and X-ray crystallographic analysis for **1**. Furthermore, the reversed ^{13}C NMR assignments for C-10 and C-13 of **3**, **4**, puberanine, puberanidine and demethyllappaconitine were revised. Compounds **1**–**4** were evaluated for cyclooxygenase-2 (COX-2) enzyme inhibitory activity. However, they did not show any inhibition at 10 μM.

Keywords: Sinaconitine A; Sinaconitine B; Norditerpenoid alkaloid; *Aconitum sinomontanum*

1. Introduction

The root of *Aconitum sinomontanum* Nakai (Ranunculaceae) has a long history as Chinese folk medicine for the treatment of bruises and injuries [1]. Previous studies showed that norditerpenoid alkaloids were the main constituents and effective components in the genus *Aconitum* [2–4], for instance, lappaconitine (**4**), crassiculine A and 3-acetylaconitine have been used clinically as analgesics in China [5]. As a continuous interest in the genus *Aconitum* [6–9], we conducted the phytochemical study on the titled plant, which led to the isolation of two novel 18-carbon norditerpenoid alkaloids, named sinaconitines A (**1**) and B (**2**), along with ranaconitine (**3**) [$[\alpha]_D^{25}$ + 40.2, (*c* 0.56, MeOH)] and **4** [$[\alpha]_D^{25}$ + 27.0 (*c* 0.22, $CHCl_3$)] [10]. The above new structures were elucidated on the basis of spectroscopic methods and X-ray diffraction analysis for **1**. Furthermore, the reversed ^{13}C NMR assignments for C-10 and C-13 of some related alkaloids reported in the literatures were revised.

*Corresponding author. Tel.: + 86-21-50806728. E-mail: dyzhu@mail.shcnc.ac.cn

Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2006 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020500175643

2. Results and discussion

Sinaconitine A (**1**), colorless prisms, gave a positive *Dragendorff* effect, possessing the molecular formula $C_{32}H_{42}N_2O_{10}$ deduced from the quasi molecular ion peak at *m/z* 613.2783 in the negative HR-ESI-MS. The EI-MS fragment peaks at *m/z* 435 $[M\text{-}C_6H_4NHCOCH_3COOH]^+$, 162 $[C_6H_4NHCOCH_3CO]^+$ and 120 $[C_6H_4CONH_2]^+$ demonstrated the existence of a subunit of $C_6H_4(NHCOCH_3)COÕ$. Its ^{13}C NMR and DEPT spectra (table 1) showed 32 carbon and 38 carbon-linked proton signals (5 × CH_3, 6 × CH_2, 11 × CH, and 10 × C), indicating the above-mentioned N-acetyl anthranoyl ester group (δ_c 169.3, 167.2, 141.9, 134.7, 131.0, 122.4, 120.4, 115.3, 25.6), three methoxyls (δ_c 55.9, 56.4, and 58.1), as well as an 18-carbon norditerpeniod alkaloid skeleton under the reference to **3** and **4** [11]. These ^{13}C NMR data were almost superposed with those of **3**, with the exception of an N-Et (δ 51.1 and 14.7) of **3** being replaced by an N-acetyl (δ 170.0 and 22.6), suggesting **1** to be an oxidated ranaconitine.

Downloaded At: 19:16 22 January 2011

Table 1. NMR data of compounds **1** and **2** ($CDCl_3$, 125 MHz).

Site	δ_C *of* ***1***	δ_C *of* ***2***	δ_H *of* ***1***	δ_H *of* ***2***
1	81.2 (d)	81.2 (d)	3.10 (dd, 9.0, 8.2)	3.24 (dd, 11.8, 6.4)
2α	26.4 (t)	26.4 (t)	1.55 (m)	1.50 (m)
β			2.25 (m)	2.29 (m)
3α	31.2 (t)	31.7 (t)	2.55 (m)	2.59 (m)
β			1.84 (m)	1.88 (m)
4	82.8 (s)	82.7 (s)	–	–
5	47.6 (d)	47.3 (d)	2.51 (d, 7.4)	2.59 (m)
6α	32.6 (t)	24.6 (t)	1.59 (d, 15.1)	1.88 (m)
β			3.16 (dd, 7.4, 15.1)	2.81 (m)
7	82.2 (s)	53.8 (d)	–	1.99 (m)
8	77.7 (s)	75.4 (s)	–	–
9	78.4 (s)	78.0 (s)	–	–
10	49.6 (d)	49.9 (d)	2.18 (m)	2.21 (m)
11	50.8 (s)	51.1 (s)	–	–
12α	25.4 (t)	25.5 (t)	2.47 (m)	2.43 (m)
β			1.99 (m)	1.99 (m)
13	37.1 (d)	36.9 (d)	2.41 (m)	2.43 (m)
14	89.9 (d)	90.0 (d)	3.50 (d, 4.3)	3.48 (d, 4.5)
15α	37.7 (t)	44.9 (t)	3.04 (dd, 8.4, 15.1)	2.48 (dd, 14.1, 7.3)
β			1.80 (dd, 7.8, 15.1)	2.15 (dd, 14.1, 8.5)
16	82.9 (d)	83.0 (d)	3.22 (m)	3.29 (m)
17	60.5 (d)	58.9 (d)	3.72 (s)	3.93 (br. S)
19α	47.9 (t)	47.3 (t)	4.88 (d, 14.4)	4.92 (d, 14.5)
β			3.33 (d, 14.4)	3.16 (d, 14.5)
N**CO**CH_3	171.0 (s)	169.3 (s)	–	2.18 (s)
NCO**CH_3**	22.6 (q)	22.5 (q)	2.15 (s)	–
1-OCH_3	55.9 (q)	55.9 (q)	3.24 (s)	3.26 (s)
14-OCH_3	58.1 (q)	58.1 (q)	3.43 (s)	3.43 (s)
16-OCH_3	56.4 (q)	56.4 (q)	3.32 (s)	3.31 (s)
NH**CO**CH_3	169.3 (s)	169.2 (s)	–	–
NHCO**CH_3**	25.6 (q)	25.6 (t)	2.20 (s)	2.23 (s)
N**H**$COCH_3$			11.0 (br. S)	11.0 (br s)
COO	167.2 (s)	167.2 (s)	–	–
1″	115.3 (s)	115.3 (s)	–	–
2″	141.9 (s)	142.0 (s)	–	–
3″	120.4 (d)	120.4 (d)	8.66 (d, 8.5)	8.68 (d, 8.5)
4″	134.7 (d)	134.7 (d)	7.49 (dd, 7.5, 8.5)	7.50 (dd, 8.5, 7.7)
5″	122.4 (d)	122.4 (d)	7.01 (dd, 7.5, 7.8)	7.02 (dd, 8.0, 7.7)
6″	131.0 (d)	131.0 (d)	7.90 (d, 7.8)	7.91 (d, 8.0)

Since **1** was obtained as nice prisms, it was subjected to X-ray crystallographic analysis. The ORTEP viewing was shown in figure 1, which confirmed **1** to be a 21-oxo ranaconitine. The complete ^{1}H and ^{13}C assignments were made by means of 2D NMR analysis (^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY). Two methine signals at δ 49.6 and 37.1 were unambiguously assigned for C-10 and C-13 rather than the contrary as in **3** [7] on the basis of the HMBC cross-peaks of H-1 ($\delta_H = 3.10$)/C-10 ($\delta_C = 49.6$), H-5 ($\delta_H = 2.51$)/C-10, as well as H-15α ($\delta_H = 3.04$)/C-13 ($\delta_C = 37.1$). As a corollary, the ^{13}C NMR assignments of C-10 and C-13 in the alkaloids with the same skeleton, such as **3**, **4** [10], puberanine, puberanidine [12] and demethyllappaconitine [13] in the quoted literatures need also to be reversed.

The molecular formula of sinaconitine B (**2**) was determined to be $C_{32}H_{42}N_2O_9$ by the HR-EI-MS spectrum at *m/z* 598.2891 [M^+]. Its ^{13}C- and ^{1}H-NMR spectra (table 1) were similar to those of **1**. The differences were the presence of an extra methine (δ_C 53.8 and δ_H 1.99) in **2** instead of the quaternary carbon ($\delta_{C\text{-}7}$ 82.2) in **1**, as well as downshifted chemical shifts of C-6, C-8, C-17 (-8.0, -2.3, and -1.6, respectively) and upshifted δ value of C-15 ($+7.2$) ongoing from **1** to **2**. It is in agreement with a hypothesis that **1** is the 7-hydroxylated derivative of **2** according to the β- and γ-effects theory [14]. Similarly, the same differences were observed between **3** and **4**. The above evidences proved sinaconitine B to have the structure **2**.

Although N-acetylated compounds were extensively reported from natural resources, to our knowledge, only two norditerpenoid alkaloids with N-acetyl group were found up to now [15]. However, 18-carbon norditerpeniod alkaloid with such a function group is disclosed for the first time. Taking into account that in this case the extraction and isolation were performed under mild conditions, it is apparent that compounds **1** and **2** should be natural products.

Downloaded At: 19:16 22 January 2011

3. Experimental

3.1 General experimental procedures

All melting points were determined on a BüCHI 510 melting point apparatus and are uncorrected. Optical rotations were measured using a Perkin–Elmer polarimeter 341. IR spectra were recorded on a Nicolet Magna 750 FTIR (KBr) spectrometer. EI-MS/HR-EI-MS, ESI-MS and HR-ESI-MS data were obtained with a MAT-95, LCQ-Deca and Q-Tof Ultima mass spectrometer, respectively. NMR spectra were recorded on a Bruker AV500 instrument with TMS as internal standard. Neutral Al_2O_3 (200–300 mesh, Shanghai Xin Cheng Fine

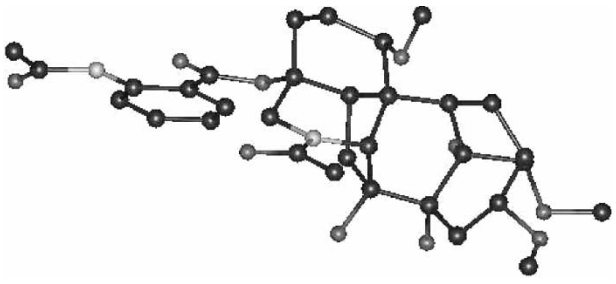
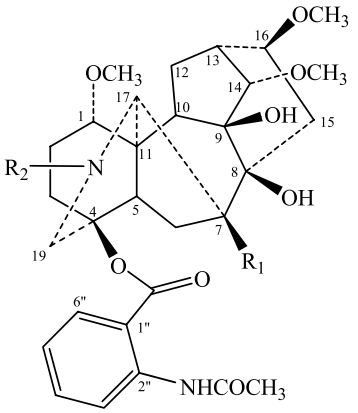


Figure 1. X-ray crystal structure of **1**.



1 R_1=OH, R_2=Ac; **2** R_1=H, R_2=Ac
3 R_1=OH, R_2=Et; **4** R_1=H, R_2=Et

Scheme 1. Structures of compounds **1**-**4**.

Downloaded At: 19:16 22 January 2011

Chemical Co. LTD, Shanghai, China), silica gel (200–300, 400 mesh) and precoated plates of silica gel ($HSGF_{254}$) (Qingdao Haiyang Chemical Group Co., Qingdao, China) were used for column chromatography (CC) and TLC, respectively.

3.2 Plant material

The dried roots of *A. sinomontanum* were collected in Datong county, Qinghai Province, China, in August 2003, and identified by Prof. Sheng-Xin Wang of Northwest Institute of Plateau Biology, Chinese Academy of Sciences. A voucher (No. 03–06) specimen is deposited in our laboratory.

3.3 Extraction and isolation

The roots (14 kg) of *A. sinomontanum* were chopped and extracted four times with 95% EtOH at room temperature. After removing solvent under reduced pressure, the extract was dissolved in 1% HCl solution then filtrated. The acidic solution was basified to pH 10 with ammonia (25%) and exhaustively extracted with $CHCl_3$ to get crude alkaloidal extract (143.3 g). The extract was chromatographed over neutral Al_2O_3 (3.3 kg, 200 − 300 mesh) column and eluted with gradient petroleum ether (PE)–EtOAc [8:2 (15L), 7:3 (15L), and 1:1 (30L)], EtOAc (20L) and EtOAc–MeOH (8:2, 6L) to give five fractions. Fractions 2 and 3 yielded crude crystalline solid, which afforded **4** (3.2 g) and **3** (5.8 g) after recrystallization, respectively. The remaining fraction 3 was subjected to neutral Al_2O_3 (350 g, 200–300 mesh) CC eluted with PE–EtOAc (7:3, 6:4, 5:5 and 4:6, each 2L) to afford four subfractions, fractions 3.1-3.4. The fraction 3.4 (0.9 kg) was further purified on a neutral Al_2O_3 (100 g) column with PE–EtOAc (1:1, 1.5 L) to give an amorphous alkaloid **1** (93 mg). Single crystals of **1** were finally obtained from PE–EtOAc solution. The fraction 3.3 (875 mg) was purified through silica gel (30 g, 300–400 mesh) column with n-hexane–Me_2CO–Et_3N (70:30:1) as eluent to furnish **2** (54 mg).

3.3.1 Sinaconitine A (1). Colorless prisms, mp 150–151.5°C, $[\alpha]_D^{20} + 35$ ($CHCl_3$, *c* 1.02). IR υ_{max} KBr cm^{-1}: 3435 (OH and NH), 2941, 2825, 1686 (C═O), 1624, 1525, 1448, 1371, 1313, 1267, 1090 (ether group), 756. EI-MS 70eV, *m/z* (rel. int.): 615 $[MH]^+$(1), 435 [M-$C_6H_4NHCOCH_3COOH]^+$(100), 421 (7), 385 (6), 344 (6), 162 $[C_6H_4NHCOCH_3CO]^+$(8), 120 $[C_6H_4CONH_2]^+$(7). HR-ESI-MS (negative), *m/z* 613.2783 $[M-1]^-$ (calcd for $C_{32}H_{41}N_2O_{10}$, 613.2761). ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data (see table 1).

3.3.2 X-ray crystal data of 1. $C_{32}H_{42}N_2O_{10}{\cdot}Et_2O{\cdot}MeOH$, $D_c = 1.318\ mg/m^3$, monoclinic, *C*2, $a = 31.614$ (9), $b = 7.602$ (2), $c = 15.114$ (4)Å, $\alpha = \gamma = 90°$, $\beta = 90.007$ (11)°, $V = 3632.6$ (17)Å^3, $Z = 4$; crystal size: 0.512 × 0.500 × 0.151 mm. A total of 6830 unique reflections were collected using graphite monochromated MoKα radiation ($\lambda = 0.71073$ Å) on a Rigaku AFCFR diffractometer. The structure was solved by direct methods (SHELXL-97) refined by full matrix least squares techniques based on F^2 to give $R = 0.2520$, $wR2 = 0.2169$. Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 256332. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk].

3.3.3 Sinaconitine B (2). Colorless amorphous powder, $[\alpha]_D^{20} + 17$ ($CHCl_3$, *c* 0.35). IR υ_{max} KBr cm^{-1}: 3446 (OH and NH). 2928, 1684 (C═O), 1647, 1589, 1525, 1448, 1371, 1267, 1124, 1086 (ether group), 757. EI-MS 70eV, *m/z* (rel. int.):599 $[MH]^+$(1), 419 [M-$C_6H_4NHCOCH_3COOH]^+$(100), 405 (33), 386 (28), 369 (19), 162 $[C_6H_4NHCOCH_3CO]^+$(13), 120 $[C_6H_4CONH_2]^+$(13), 71 (16). HR-EIMS, *m/z* 598.2891 $[M]^+$ (calcd for $C_{32}H_{42}N_2O_9$, 598.2890). ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data (see table 1).

Downloaded At: 19:16 22 January 2011

Acknowledgements

We thank Professor Sheng-Xin Wang of Northwest Institute of Plateau Biology, Chinese Academy of Sciences, for the plant's collection and identification.

References

[1] W.T. Wang, P.G. Xiao. *Flora Reipublicae Popularis Sinicae*, **27**, 168 (1979).
[2] F.P. Wang, C.S. Peng, X.X. Jian, D.L. Chen. *J. Asian Nat. Prod. Res.*, **3**, 15 (2001).
[3] C.S. Peng, F.P. Wang, J.Z. Wang, X.X. Jian. *Acta Pharm. Sin.*, **35**, 201 (2000).
[4] C.S. Peng, J.Z. Wang, X.X. Jian, F.P. Wang. *Nat. Prod. Res. Dev.*, **12**, 45 (2000).
[5] L.M. Gao, X.F. Mao, X.M. Wei, S.Z. Zheng. *J. Northwest Normal University (Natural Science)*, **35**, 98 (1999).
[6] S.H. Jiang, P.M. Yang, H. Zhou, D.Y. Zhu. *Planta Med.*, **68**, 1147 (2002).
[7] H.Q. Wang, S.H. Jiang, P.M. Yang, M.H. Ying, S.J. Lin, D.Y. Zhu. *Nat. Prod. Res. Dev.*, **14**, 13 (2002).
[8] X.Q. Ma, S.H. Jiang, D.Y. Zhu. *Chin. J. Chin. Mat. Med.*, **23**, 679 (1998).
[9] G. Wu, S.H. Jiang, D.Y. Zhu. *Phytochemistry*, **42**, 1253 (1996).
[10] S.W. Pelletier, N.V. Mody, A.P. Venkov. *Tetrahedron Lett.*, **50**, 5045 (1978).
[11] L.S. Ding, W.X. Chen. *Nat. Prod. Res. Dev.*, **1**, 42 (1989).
[12] D.Q. Yu, B.C. Das. *Planta Med.*, **49**, 85 (1983).
[13] A. Ulubelen, A.H. Mericli, F. Mericli, F. Yilmaz. *Phytochemistry*, **41**, 957 (1996).
[14] B.S. Joshi, S.W. Pelletier. *Tetrahedron*, **47**, 4299 (1991).
[15] C.S. Peng, F.P. Wang. *Acta Pharm. Sin.*, **35**, 932 (2000).