Two New Alkaloids from the Roots of *Aconitum sinomontanum* NAKAI

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Two new alkaloids, ranaconidine (=20-ethyl-1α,14α,16β-trimethoxyaconitane-4,7,8,10-tetrol; **1**) and *N*-(chloromethyl)taspine (=1-[2-(*N*-(chloromethyl)-*N*,*N*-dimethylaminium)ethyl]-3,8-dimethoxybenzopyrano[5,4,3-*cde*]benzopyran-5,10-dione; **2**), together with two known compounds, *i.e.*, benzethonium chloride (**3**) and ranaconine (**4**) were isolated from the roots of Aconitum sinomontanum NAKAI. The structures of the compounds were elucidated on the basis of spectral evidences and in the comparison with previously published data.

Introduction. – *Aconitum sinomontanum* NAKAI is distributed mainly in western China. The root of *A. sinomontanum* NAKAI has a long history due to its use as folk medicine for the treatment of injuries, bruises, rheumatism, acute or chronic bacterial dysentery, enteritis *etc.* [1][2]. The main constituents of the genus *Aconitum* are norditerpenoid alkaloids. So far, 23 norditerpenoid alkaloids have been isolated from the roots of *A. sinomontanum* NAKAI [3]. Lappaconitine, the main alkaloid constituent in *A. sinomontanum* NAKAI, is used as the source for the industrial production of lappaconitine hydrobromide, a non-addictive analgesic drug used in China [3][4]. Due to our continuous interest in this plant, we have carried out a phytochemical investigation resulting in the isolation of a novel ranaconitine-type norditerpenoid alkaloid, ranaconidine, and a novel ellagic acid-type alkaloid, *N*-(chloromethyl)taspine, which is the first non-diterpenoid alkaloid isolated from *A. sinomontanum* NAKAI (*Fig. 1*), together with two known compounds isolated for the first time from this plant. The new structures were determined by analyses of the UV, IR, 1D- and 2D-NMR, and MS data.

Results and Discussion. – Repeated column chromatography (CC) and preparative TLC (PTLC) of the 95% EtOH extract of the dried roots of *A. sinomontanum* NAKAI yielded two new compounds, ranaconidine (**1**) and *N*-(chloromethyl)taspine (**2**), and two known compounds.

Compound **1** was obtained as white powder. Its molecular formula was deduced as $C_{23}H_{37}NO_7$ by HR-ESI-MS ($[M+H]^+$ signal at m/z 440.2660; calc. 440.2648). The IR spectrum showed absorptions at 3390 cm^{-1} (OH). The ^{1}H-NMR spectrum revealed the presence of Me group (δ(H) 1.30, *t*, $J=7.1$, 3 H) and three MeO groups (δ(H) 3.33, 3.39, 3.40 (3*s*); δ(C) 56.5, 56.5, 58.2). A signal at δ(H) 3.70 (*dd*, $J=4.9$, 1.1, 1 H) indicated the presence of H_β–C(14) [5]. The ^{13}C-NMR spectrum revealed the presence of 23 C-atoms, which were identified with the aid of DEPT-135 experiment as one

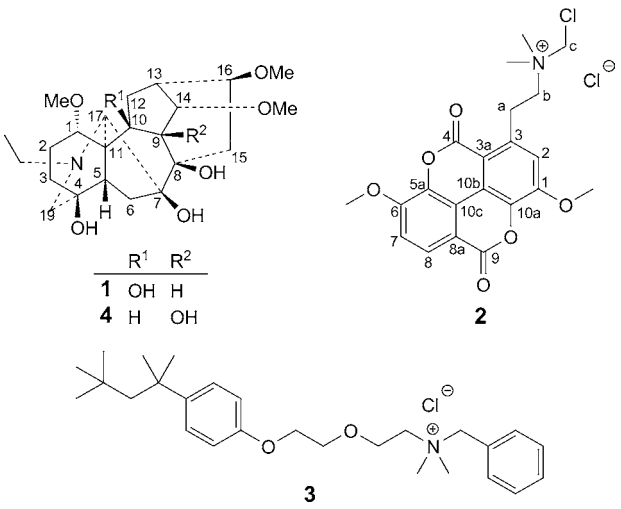

Fig. 1. *Structures of compounds* **1**–**4**

Me, three MeO, seven CH_2, and seven CH groups, and five quaternary C-atoms (*Table 1*).

The ^{13}C-NMR spectrum of **1** was very similar to that of the known compound **4** except the signals of C(9) and C(10) and signals of C-atoms close to C(9) and C(10). In the ^{13}C-NMR of **1**, C(10) signal was at 81.3 and that of C(11) at 57.1, compared to 49.9 and 52.0, respectively of compound **4**, indicating that C(10) of **1** had an O-containing substituent. In addition, in the ^{13}C-NMR of **1**, C(9) signal appeared at 49.9 and that of C(8) at 80.1, compared with 79.3 and 88.1 of **4**, suggesting that C(9) of **1** is not connected with an O-carrying group [5]. The presence of the cross-peak between H–C(14) and H–C(9) in the ^{1}H,^{1}H-COSY spectrum also confirmed that C(9) is a tertiary C-atom without an O-containing substituent. Therefore, **1** is a new ranaconine-type alkaloid, which can be considered as 9-deoxy-10-hydroxyranaconine and named ranaconidine (**1**), a novel C_{18}-diterpenoid alkaloid [5][6].

The relative configuration of **1** was assigned by comparison of the chemical shifts with those of known compounds and through a NOESY experiment. The chemical shifts of most H- and C-atoms in **1** were close to those of compound **4**, indicating that the configurations of most C-atoms of **1** were the same as those of **4**. Furthermore, in the NOESY spectrum of **1**, the cross-peaks H–C(1)/H_β–C(2), H_β–C(2)/H–C(5), H–C(9)/H_β–C(14), and H_β–C(14)/H–C(13) evidenced that the H-atoms were β-oriented. In addition, the correlations of MeO–C(16) with H–C(9) and H–C(13), respectively, also indicated the β-position of the MeO group (*Fig. 2*). By comparison with the previously reported data, HO–C(10), HO–C(8), HO–C(7), and HO–C(4) were deduced to be β-oriented [7–9]. The correlation between H_α–C(12) and H–C(17) indicated that the CH_2–N(Et)–CH (C(19)–N–C(17)) bridge was below the fragments A and B (*Fig. 3*). Other important NOEs are compiled in *Table 1*. Therefore, the structure of **1** was identified as 20-ethyl-1α,14α,16β-trimethoxyaconitane-4,7,8,10-tetrol and named ranaconidine.

Table 1. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; CD_3OD) *of Compound* **1**. δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1*.

Position	δ(H)	δ(C)	HMBC(C → H)	^{1}H,^{1}H-COSY	NOESY
1	3.95 (*t*, *J* = 4.5)	80.8	1-MeO, 3β	2α, 2β	2β
2α	1.82 (*s*)	23.9	–	1, 2β	3α
2β	2.08–2.17 (*m*)			1, 2α, 3β	1, 5, 19β
3α	2.33–2.42 (*m*)	39.4	1	3β	2α
3β	2.20–2.23 (*m*)			2β, 3α	5
4	–	70.3	6α, 6β	–	–
5	2.23–2.27 (*m*)	48.3	3α, 3β, 6α, 6β	6α	2β, 3β
6α	1.72 (*dd*, *J* = 15.4, 8.2)	23.9	–	5, 6β	–
6β	2.76 (*d*, *J* = 6.4)			6α	5
7	–	75.2	5, 6α, 6β, 14[b]), 15α, 15β	–	–
8	–	80.1	5[b]), 14	–	–
9	3.31–3.33 (*m*)	49.9	6α[b]), 6β[b]), 15α, 15β	14, 15α[a]), 15β[a])	13, 14, 16-MeO
10	–	81.3	1, 12α, 12β, 13	–	–
11	–	57.1	1, 5, 6α, 9	–	–
12α	2.01–2.05 (*m*)	44.0	9, 13, 16	12β, 13	16, 17
12β	2.00 (*d*, *J* = 7.9)			12α, 13	13, 14
13	2.52–2.57 (*m*)	36.1	9, 15α	12α, 12β, 14	12β, 14, 16-MeO
14	3.70 (*dd*, *J* = 4.9, 1.1)	88.5	9, 13, 16, 4-MeO	9, 13, 16[a])	9, 12β, 13, 16-MeO
15α	2.23–2.27 (*m*)	39.4	9, 13	15β, 16	15β, 16
15β	2.33–2.42 (*m*)			15α, 16	15α
16	3.29–3.30 (*m*)	83.9	12α, 12β, 13, 14, 15α, 15β, 16-MeO	14[a]), 15α, 15β	12α, 15α
17	3.09 (*s*)	63.4	1, 5	5[a])	12α, 15α, 16
19α	2.72 (*d*, *J* = 8.0)	59.6	–	5[a]), 19β	19β
19β	3.01 (*d*, *J* = 8.0)			19α	2β,19α
CH_2N	3.10–3.26 (*m*)	50.1		*Me*–CH_2–N	*Me*–CH_2–N, 5
MeCH_2	1.30 (*t*, *J* = 7.1)	11.4	Me–*CH_2*–N	Me–*CH_2*–N	Me–*CH_2*–N, 5
1-MeO	3.39 (*s*)	56.5	1	–	1
14-MeO	3.40 (*s*)	58.2	14	–	14
16-MeO	3.33 (*s*)	56.5	16	–	9, 13

[a]) ^4J(H,H) coupling. [b]) ^4J(C,H) coupling.

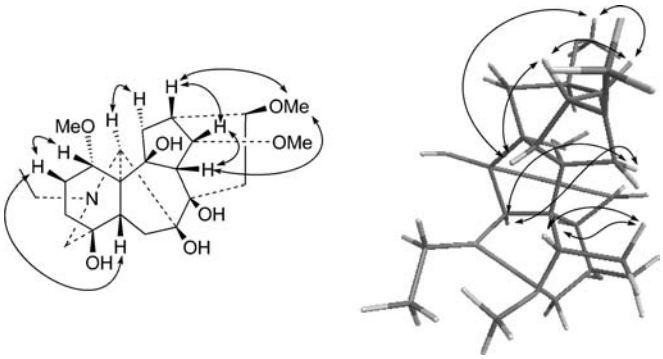

Fig. 2. *Key NOE correlations* (H ↔ H) *of compound* **1**

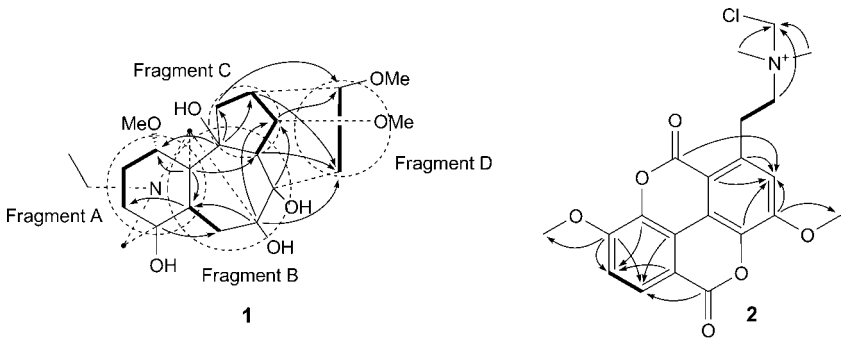

Fig. 3. *Key* $^1H,^1H$*-COSY* (—) and *HMB* (C → H) *correlations of compounds* **1** *and* **2**

Compound **2** was obtained as white powder. Its molecular formula was determined as $C_{21}H_{21}ClNO_6$ by HR-ESI-MS (M^+ signal at *m*/*z* 418.1065 (100%; calc. 418.1057), 420.1145 (33.40%)). This isotope pattern suggested that there is one Cl-atom in the molecule. A UV maximum was observed at 270 nm. The IR spectrum showed absorptions at 3037 (C(sp^2)–H), 1751, 1727 (O=C–O–C), 1139, 1089, 1290 cm^{-1}. The ^{1}H-NMR spectrum exhibited the signals due to two MeN (δ(H) 3.31 (*s*, 6 H); δ(C) 48.8, 2 C), three CH_2 (δ(H) 3.61 – 3.69, 3.69 – 3.79, 5.54; δ(C) 62.1, 27.5, 69.1), and two MeO (δ(H) 4.08, 4.10; δ(C) 56.9, 56.9) groups. The ^{13}C-NMR spectrum showed the presence of 21 C-atom signals which were ascribed, with the help of DEPT-135 experiment, to two Me, two MeO, three CH_2, three CH groups, and eleven quaternary C-atoms (*Table 2*). By comparison with the published data [10], the ^{1}H- and ^{13}C-NMR spectra of **2** revealed characteristic features of an ellagic acid-type alkaloid skeleton (ellagic acid = 2,3,7,8-tetrahydroxychromeno[5,4,3-*cde*]chromene-5,10-dione). The skeleton was further confirmed by HMBC and ^{1}H,^{1}H-COSY experiments (*Fig. 3*). Besides the skeleton C-atoms, there were five additional C-atoms in **2**. The ^{1}H,^{1}H-COSY correlation of CH_2(a) with CH_2(b) and the HMBC cross-peaks of CH_2(b) to CH_2(c) and cross-peaks of two MeN to CH_2(c) evidenced that these five C-atoms formed a side chain. The δ(C) of C(c) at 69.1 suggested that C(c) was connected with N- and Cl-atoms [11]. The HMBC cross-peak between C(a) and C(3) showed that the side chain was connected to the skeleton at C(3). Based on the above evidence, **2** was identified as 1-[2-(*N*-(chloromethyl)-*N*,*N*-dimethylaminium)ethyl]-3,8-dimethoxybenzopyrano[5,4,3-*cde*]benzopyran-5,10-dione and named *N*-(chloromethyl)taspine.

The known compounds benzethonium chloride (**3**) [12] and ranaconine (**4**) [13] were also isolated for the first time from *A. sinomontanum* NAKAI, and identified on the basis of ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments and the reported data.

Experimental Part

General. Reagents used were of anal. grade. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia Biotech AB*, SE-Uppsala), *ODS* (*YMC Co.*, Japan). *MCI GEL* (*Mitsubishi Chemical Co.*, Japan), and neutral Al_2O_3 (*Sinapharm Chemical Reagent Co., Ltd.*, P. R. China). TLC: Prep. TLC silica gel (*HSGF$_{254}$*, *Yantai Jiangyou Guijiao Kaifa Co.*, P. R. China). Chromogenic agent: *Dragendorff*

Table 2. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; (D_6)DMSO) *of Compound* **2**. δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1.*

Position	δ(H)	δ(C)	HMBC(C → H)	^{1}H,^{1}H-COSY	NOESY
1	–	150.5	2, 1-MeO	–	–
2	7.72 (*s*)	118.1	–	–	a, b, 1-MeO
3	–	137.9	2	–	–
3a	–	109.4	2	–	–
4	–	157.9	2[b])	–	–
5a	–	136.8	7	–	–
6	–	150.8	7, 8, 6-MeO	–	–
7	7.62 (*d*, J = 8.8)	114.9	–	8	8, 6-MeO
8	8.10 (*d*, J = 8.8)	126.7	–	7	7
8a	–	111.0	7	–	–
9	–	158.1	8	–	–
10a	–	137.0	2	–	–
10b	–	118.1	–	–	–
10c	–	119.1	8	–	–
a	3.69–3.79 (*m*)	27.5	–	b	2, c
b	3.61–3.69 (*m*)	62.1	c	a	2, c
c	5.54 (*s*)	69.1	–	–	a, b
2 MeN	3.31 (*s*)	48.8	c	–	–
1-MeO	4.08 (*s*)	56.9	–	–	2
6-MeO	4.10 (*s*)	56.9	–	–	7

[a]) ^4J(H,H) coupling. [b]) ^4J(C,H) coupling.

reagent. Optical rotations: *Perkin-Elmer 341* polarimeter. IR Spectra: *Nicolet-Magna-750-FTIR* spectrometer; KBr pellets; in cm^{-1}. NMR Spectra: *Bruker AV-400* instrument at 400 MHz (^{1}H) and 100 MHz (^{13}C); in (D_6)DMSO or CD_3OD; δ in ppm rel. to Me_4Si; J in Hz. ESI-MS and HR-ESI-MS: *Bruker Esquire 3000 plus* and *Finnigan LCQ DECA* mass spectrometers, resp.; in m/z (rel. %).

Plant Material. The roots of *Aconitum sinomontanum* NAKAI were collected in Tianshui City, Gansu Province, in October, 2011, and identified by Prof. *Shanhao Jiang*, Chinese Academy of Sciences Shanghai Institute of Materia Medica. A voucher specimen (No. 612) has been deposited with the Plant Laboratory of the Shanghai University of Traditional Chinese Medicine, Shanghai, P. R. China.

Extraction and Isolation. The roots (95 kg) of *A. sinomontanum* NAKAI were extracted with 95% EtOH to give the crude extracts. The crude extracts were acidified with 0.5% aq. HCl soln., basified with aq. NH_3 soln., extracted with CH_2Cl_2, to obtain the crude alkaloid extracts (1.6 kg). This method was repeatedly applied to furnish the total alkaloid content (700 g). Total alkaloid content was subjected to CC (neutral Al_2O_3 (4.25 kg); petroleum ether (PE)/CH_2Cl_2 10:1, then CH_2Cl_2, CH_2Cl_2/MeOH 100:1, 50:1, 30:1, 10:1, 5:1, 2:1, and 1:1, and finally MeOH) to afford *Fractions 1–22*. *Fr. 22* was submitted to CC (*Sephadex LH-20*; MeOH) to give *Frs. 22.1–22.4*. *Fr. 22.3* was subjected to CC (*MCI*; 45% MeOH; and *C18* (CC; 35% → 70% MeOH) to furnish *Frs. 22.3.1–22.3.3*. *Fr. 22.3.1* was further purified by CC (*Sephadex LH-20*; 35% MeOH; and *C18*; 35% → 40% MeOH) to afford **4** (11 mg). *Fr. 22.3.3* was purified by CC (*C18*; 32.5% MeOH) to give **1** (26 mg). *Fr. 5* (1 g) was subjected to CC (neutral Al_2O_3; PE/Me_2CO (6:1 → 1.5:1) to give *Frs. 5.1* and *5.2*. *Fr. 5.2* was purified by CC (*C18*; 70% → 90% MeOH) to give **3** (24 mg). *Fr. 11* was dissolved in CH_2Cl_2 to yield a crude deposit, which afforded **2** (30 mg) after purification with Me_2CO and H_2O.

Ranaconidine (=*(1α,14α,16β)-20-Ethyl-1,14,16-trimethoxyaconitane-4,7,8,10-tetrol*; **1**). White powder. $[\alpha]_D^{25} = +22.3$ (c = 0.1, MeOH). IR: 3390 (OH). ^{1}H- and ^{13}C-NMR: see *Table 1.* HR-ESI-MS: 440.2660 ($[M+H]^+$, calc. 440.2648).

N-*Chloromethyltaspine* (= N-*(Chloromethyl)-2-(3,8-dimethoxy-5,10-dioxo-5,10-dihydrochromeno[5,4,3-*cde*]chromen-1-yl)*-N,N-*dimethylethanaminium Chloride*; **2**). White powder. $[\alpha]_D^{25} = +1.3$ ($c =$ 0.1, MeOH). UV: 270. IR: 3037, 1751, 1727 (O=C–O–C), 1139, 1089, 1290. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 418.1065 (100, M^+, calc. 418.1057), 420.1145 (33.40).

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