Two New C_{20}-Diterpenoid Alkaloids from the Tibetan Medicinal Plant *Aconitum naviculare* STAPF

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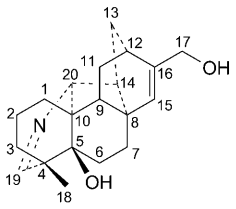
Two new C_{20}-diterpenoid alkaloids named naviculine A (**1**) and naviculine B (**2**), were isolated from *Aconitum naviculare* STAPF. Their structures were established by spectral methods, especially 2D-NMR spectra (^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY) and DFT methods (at the B3LYP/6-311++G(2d,p)//B3LYP/6-31G(d) level), respectively. They were assayed for their anti-HIV-1 activity.

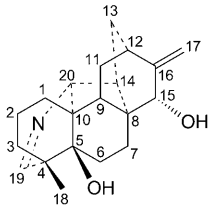
Introduction. – The plant genus *Aconitum* (Ranunculaceae) has over 100 species that are native to temperate regions of the northern hemisphere. Crude preparations from *Aconitum* plants were popularly used in Asia, Alaska, and Europe [1] in folklore and traditional medicine for the treatment of traumatic injury [2], as a febrifuge and bitter tonic [3], and as ingredients in intoxicating liquors [4]. Even in modern medicine, aconitine-containing liniments have been used for the treatment of rheumatism, neuralgia, and sciatica [5]. The whole plants of *Aconitum naviculare* STAPF are used in traditional Tibetan medicine for the treatment of gastricism, hepatitis, nephritis, and other diseases. However, only few works have been reported on *A. naviculare*. In this context, *A. naviculare* was analyzed for alkaloids.

Analysis of the alkaloids from this plant species, which is located in the area of elevation of 4100–5000 meters in Tibet, resulted in the isolation of two new C_{20}-diterpenoid alkaloids, which have been named naviculines A (**1**) and B (**2**). The details are reported in this paper.

Results and Discussion. – The dried material (20 kg) was extracted with MeOH. Evaporation of the solvent then afforded a crude MeOH extract (2.1 kg). The MeOH extract (2.1 kg) was carefully acidified with 0.1M H_2SO_4, and then extracted with $CHCl_3$. The acidic aqueous extract was basified to pH 10 with 20% aqueous Na_2CO_3 solution and then extracted with $CHCl_3$. The combined $CHCl_3$ extract was washed with H_2O and dried over anhydrous Na_2SO_4. The solvent was evaporated to afford a crude



1 [1])

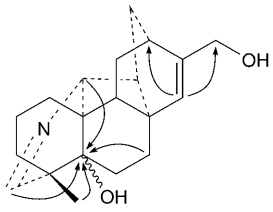


2 [1])

alkaloid extract (40 g), which was subjected to repeated column chromatography to afford **1** and **2**.

Naviculine A (**1**) was obtained as colorless crystals (MeOH). HR-ESI-MS experiments indicated the molecular formula $C_{20}H_{27}NO_2$ (calc. for $[M+H]^+$: 314.2120; found: 314.2105), with 8 degrees of unsaturation. The IR spectra exhibited absorption bands for OH (3417 cm^{-1}) and C=N (1647 cm^{-1}). The ^{13}C-NMR (DEPT) spectrum displayed the signals of 20 C-atoms, including five quaternary C-atoms, six CH and eight CH_2 groups, and one Me group. A sharp *singlet* resonating at δ(H) 1.05 (Me) was ascribed to the Me(18) group, and the signal ascribed to H–C(20) (br. *s*) was observed at δ(H) 3.51, which suggested that this alkaloid belongs to the C_{20}-diterpenoid alkaloids. Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with 7α-hydroxycossonidine [6] showed chemical shift values of **1** similar to those of the latter, which suggested that they have similar structures. The distinct differences between them is the following: the chemical shifts of **1** for H–C(19) (δ(H)) 7.39, δ(C) 172.9 (*d*)) and of the latter (δ(H–C(19a)) 2.49, δ(H–C(19b)) 2.43, δ(C(19)) 62.3 (*t*)), suggested that there is a C=N bond in **1**, instead of a C–N bond as in 7α-hydroxycossonidine. The chemical shifts of CH_2(17) δ(H) 4.09, δ(C) 63.3), suggested that there is a CH_2–OH group in **1** instead of C=CH_2. The chemical shifts of δ(C) 73.0 (*s*), suggested that there is one oxygenated quaternary C-atom in **1**. A *doublet* (δ(H) 5.50 (H–C)) in the ^{1}H-NMR spectrum, and two signals at δ(C) 128.8 (H–C) and δ(C) 151.6 (C) in the ^{13}C-NMR spectrum indicated the presence of a C=CH group.

The HMBC correlations in **1** (*Fig. 1*) of CH_2(7), Me(18), CH_2(19), and H–C(20) with the quaternary C(5) (δ(C) 73.0) suggested that one OH group was attached to



1a = (5*R*)-**1**
1b = (5*S*)-**1**

Fig. 1. *Key HMBC correlations for* **1**

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

C(5). In the HMQC spectrum, a correlation of the *doublet* (δ(H) 4.09 (CH_2)) to the signal C(17) (δ(C) 63.3 (*t*)) was found, which suggested that the second OH group should be attached to C(17). In the HMBC spectrum of **1** (*Fig. 1*), a correlation of H–C(15) with C(12) and C(17) suggested that the C=C bond is located between C(15) and C(16).

From the above data, the structure of **1** was established. The configuration of C(5) could not be identified by the current methods. There are two possible configurations at C(5), (5*R*)-**1** or (5*S*)-**1**. Thus, our previous computational methods were used in the configuration study [7–10]. The computations were performed at the B3LYP/6-311 + + G(2d,p)//B3LYP/6-31 + G(d) level using the Gauge-Independent Atomic Orbital (GIAO) method [11][12]. The calculated magnetic shieldings were finally converted into the corresponding chemical shifts and corrected. The detailed magnitudes are listed in *Table 1*.

Table 1. *The Calculated ^{13}C Chemical Shifts* (δ, ppm) *for Compounds* **1a** *and* **1b**, *and Their Relative Errors* ($\Delta\delta$) *between the Recorded* δ(**1**$_{exp.}$) *and Calculated* δ(**1a**) *or* δ(**1b**) *Values*

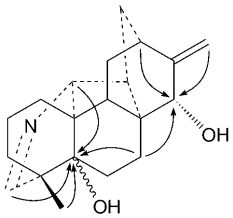
Position[1])	δ(**1**$_{exp.}$)	δ(**1a**)	δ(**1b**)	$\Delta\delta$(**1**$_{exp.}$ – **1a**)	$\Delta\delta$(**1**$_{exp.}$ – **1b**)
1	29.2	28.9	28.3	0.3	0.9
2	21.9	21.2	17.8	0.7	4.1
3	31.5	30.5	30.8	1.0	0.7
4	46.4	46.4	51.5	0.0	– 5.1
5	73.0	74.5	77.7	– 1.5	– 4.7
6	32.5	32.0	25.1	0.5	7.4
7	32.2	31.4	35.4	0.8	– 3.2
8	44.9	46.2	48.6	– 1.3	– 3.7
9	48.2	51.1	47.4	– 2.9	0.8
10	46.7	48.1	49.3	– 1.4	– 2.6
11	28.7	28.9	27.5	– 0.2	1.2
12	32.9	34.1	32.4	– 1.2	0.5
13	44.3	42.8	40.0	1.5	4.3
14	45.8	47.7	54.1	– 1.9	– 8.3
15	128.8	129.3	125.3	– 0.5	3.5
16	151.6	151.1	145.1	0.5	6.5
17	63.3	61.7	59.1	1.6	4.2
18	19.3	16.4	19.7	2.9	– 0.4
19	172.9	171.6	174.9	1.3	– 2.0
20	81.3	81.5	85.2	– 0.2	– 3.9

The maximum error of 8.3 ppm was found for C(14) in **1b**. Compared with this difference, (5*R*)-**1** presented much smaller errors than (5*S*)-**1**. Thus, the final configuration was identified as (5*R*)-**1**.

Naviculine B (**2**) was obtained as colorless crystals. HR-ESI-MS Experiments indicated the molecular formula $C_{20}H_{27}NO_2$ (calc. for $[M+H]^+$: 314.2120; found: 314.2133), with eight degrees of unsaturation. The IR spectra exhibited absorption bands for –OH (3442 cm^{-1}), C=N (1643 cm^{-1}). The ^{13}C-NMR (DEPT) spectrum displayed the signals of 20 C-atoms, including five quaternary C-atoms, six CH and eight CH_2 groups, and one Me group. Comparison of the ^1H- and ^{13}C-NMR data of **2**

with 7α-hydroxycossonidine [6] showed that the structure of **2** is very similar to the latter, except for the C=N bond in **2** replacing the C–N bond in 7α-hydroxycossonidine.

HMBC correlations of **2** (*Fig. 2*) of $CH_2(7)$, Me(18), $CH_2(19)$, and H–C(20) with the quaternary C(5) suggested that one OH group was attached to C(5). Other correlations of $CH_2(7)$, H–C(12), and $CH_2(17)$ with C(15) (δ(C) 73.9 (*d*)) suggested that one OH group was attached to C(15). This compound is similar to **1**. By computational calculations of the ^{13}C-NMR of **2** using the GAIO method, the C(5) configuration was identified as (5*R*), as in the case of **1**. However, the calculated ^{13}C-NMR could not point out whether C(15) has (*R*) or (*S*) configuration, since all the absolute error magnitudes were in the error window of 0–8.0 ppm, especially, the two error patterns between **2a** and **2b** were very similar. See *Table 2* for the details.



2a = (5*R*)-**2**
2b = (5*S*)-**2**

Fig. 2. *Key HMBC correlations for* **2**

Table 2. *The Calculated ^{13}C Chemical Shifts* (δ, ppm) *for Compounds* **2a** *and* **2b**, *and Their Relative Errors* ($\Delta\delta$) *between the Observed δ(**2**$_{exp.}$) and Calculated δ(**2a**) or δ(**2b**) Values*

Position[1])	δ(**2**$_{exp.}$)	δ(**2a**)	δ(**2b**)	$\Delta\delta$(**2**$_{exp}$ – **2a**)	$\Delta\delta$(**2**$_{exp.}$ – **2b**)
1	27.4	28.0	27.4	– 0.6	0.0
2	21.9	21.6	22.1	0.3	– 0.2
3	31.7	31.7	31.2	0.0	0.5
4	46.4	47.1	46.3	– 0.7	0.1
5	72.8	73.5	73.2	– 0.7	– 0.4
6	29.8	31.5	32.1	– 1.7	– 2.3
7	32.1	29.8	28.6	2.3	3.5
8	48.4	47.2	48.5	1.2	– 0.1
9	43.1	44.7	40.9	– 1.6	2.2
10	45.9	49.6	50.2	– 3.7	– 4.3
11	38.9	31.1	32.1	7.8	6.8
12	35.9	37.5	38.2	– 1.6	– -2.3
13	30.3	37.1	36.6	– 6.8	– 6.3
14	42.9	43.9	47.2	– 1.0	– 4.3
15	73.9	74.2	73.2	– 0.3	0.7
16	157.5	164.7	164.0	– 7.2	– 6.5
17	107.0	101.2	102.0	5.8	5.0
18	19.2	17.3	17.1	1.9	2.1
19	172.8	167.6	167.9	5.2	4.9
20	80.9	79.6	80.0	1.3	0.9

The NOESY spectrum of **2** (*Fig. 3*) showed correlations of H_β–C(9) with H–C(7) at δ(H) 1.47, which suggested that the H-atom at δ(H) 1.47 is in β-position. A correlation of H_β–C(7) with H–C(15) suggested that H–C(15) is in β-position as well; therefore, OH–C(15) is α-oriented, namely, C(15) had (*R*) configuration.

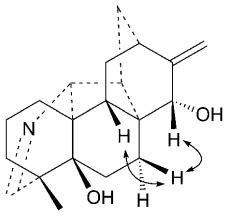


Fig. 3. *Key NOESY correlations for* **2**

Unfortunately, compounds **1** and **2** did not show any bioactivities in the anti-HIV-1 and neuraminidase inhibition assays (data not shown).

Experimental Part

General. M.p.: *YuHua-X-4* apparatus. Optical rotation: *Jasco-DIP-370* digital polarimeter. IR-Spectra: *Perkin-Elmer 577* spectrometer, with KBr pellets; in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker*

Table 3. *^1H- and ^{13}C-NMR Data of Compounds* **1** *and* **2**[1]). δ in ppm, *J* in Hz. Assignments were confirmed by ^1H,^1H-COSY, HMQC and HMBC experiments.

	1 (CD_3OD)		**2** (CD_3OD)	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.70–1.72 (*m*), 1.51–1.53 (*m*)	29.2	1.65–1.67 (*m*), 1.60–1.62 (*m*)	27.4
CH_2(2)	1.52–1.54 (*m*), 1.17–1.19 (*m*)	21.9	1.53–1.55 (*m*), 1.22–1.26 (*m*)	21.9
CH_2(3)	1.76–1.79 (*m*), 1.22–1.25 (*m*)	31.5	1.8 (*dd*, *J* = 14, 4.5), 1.24–1.28 (*m*)	31.7
C(4)		46.4		46.4
C(5)		73.0		72.8
CH_2(6)	1.68–1.71 (*m*), 1.46–1.48 (*m*)	32.5	1.67–1.69 (*m*), 1.35–1.39 (*m*)	32.1
CH_2(7)	2.00–2.04 (*m*), 1.71–1.73 (*m*)	32.2	2.00 (*dd*, *J* = 12.0, 4.5), 1.47 (*dd*, *J* = 12.0, 4.5)	30.3
C(8)		44.9		48.4
H–C(9)	1.67–1.69 (*m*)	48.2	1.74–1.76 (*m*)	43.1
C(10)		46.7		45.9
CH_2(11)	1.63–1.65 (*m*), 1.37 (*t*, *J* = 12.4)	28.7	1.88–1.91 (*m*), 1.50–1.52 (*m*)	29.8
H–C(12)	2.45 (*s*)	32.9	2.31 (*s*)	35.9
CH_2(13)	1.83 (*dd*, *J* = 12.0, 4.0), 1.53–1.55 (*m*)	44.3	2.16 (*dd*, *J* = 13.5, 8.5), 1.86–1.88 (*m*)	38.9
H–C(14)	1.55–1.57 (*m*)	45.8	1.90–1.92 (*m*)	42.9
H–C(15)	5.50 (*d*, *J* = 1.4)	128.8	3.89 (*s*)	73.9
C(16)		151.6		157.5
CH_2(17)	4.09 (*s*)	63.3	4.86 (br. *s*), 4.85 (br. *s*)	107.0
Me(18)	1.05 (*s*)	19.3	1.04 (*s*)	19.2
H–C(19)	7.39 (*d*, *J* = 2.6)	172.9	7.37 (*d*, *J* = 2.4)	172.8
H–C(20)	3.51 (*s*)	81.3	3.53 (*s*)	80.9

AM-400 and/or *DRX-500* spectrometers (δ in ppm, J in Hz). MS: *VG Autospec-3000* mass spectrometer (m/z (rel. %)).

Plant Material. Whole plants of *A. naviculare* were collected from Tibet (China) in June and August 2006.

Extraction and Isolation. The dried material (20 kg) was extracted with MeOH. The MeOH extract was filtered, and the process was repeated four times. Evaporation of the solvent then afforded a crude MeOH extract (2.1 kg). The MeOH extract (2.1 kg) was acidified with 0.1M H_2SO_4 and then extracted with $CHCl_3$. The acidic aq. extract was basified (pH 10) with 20% aq. Na_2CO_3 soln. and then extracted with $CHCl_3$. The combined $CHCl_3$ extract was washed with H_2O and dried with Na_2SO_4, and the solvent was evaporated to afford a crude alkaloid extract (40 g), which was subjected to repeated column chromatography (CC; SiO_2; $CHCl_3$/MeOH 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100). The fraction eluted with $CHCl_3$/MeOH 80:20 (3.6 g) was further purified by repeated CC (1. SiO_2, $CHCl_3$/MeOH 100:1; 2. *Sephadex LH-20*, $CHCl_3$/MeOH 1:1) to afford compounds **1** (102 mg) and **2** (15 mg).

Naviculine A (=*(1S,5S,8R,9S,11R,14S,17R)-12-(Hydroxymethyl)-5-methyl-7-azahexacyclo[9.6.2.0^{1,8}.0^{5,17}.0^{9,14}.0^{14,18}]nonadeca-6,12-dien-17-ol*; **1**). Colorless crystals from MeOH. M.p. 172–174°. $[\alpha]_D^{26} = +41.9$ ($c = 0.00438$, MeOH). IR (KBr): 3417, 2932, 1647, 1459, 1181, 1045, 1030. ^{1}H- and ^{13}C-NMR: *Table 3*. EI-MS: 313 (100, M^+), 298 (83), 296 (21), 209 (9), 107 (11), 136 (10). HR-ESI-MS: 314.2105 ($[M+H]^+$, $C_{20}H_{28}NO_2^+$; calc. 314.2120).

Naviculine B (=*(1S,5S,8R,9S,11R,13S,14S,17R)-5-Methyl-12-methylidene-7-azahexacyclo[9.6.2.0^{1,8}.0^{5,17}.0^{9,14}.0^{14,18}]nonadec-6-ene-13,17-diol*; **2**). Colorless crystals from MeOH. M.p. 200–202°. $[\alpha]_D^{26} = +30.6$ ($c = 0.00300$, MeOH). IR (KBr): 3442, 2933, 1643, 1459, 1181, 1026. ^{1}H- and ^{13}C-NMR: *Table 3*. EI-MS: 313 (100, M^+), 298 (86), 285 (58), 270 (27), 256 (16), 190 (8), 136 (9). HR-ESI-MS: 314.2133 ($[M+H]^+$, $C_{20}H_{28}NO_2^+$; calc. 314.2120).

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