A new diterpenoid alkaloid from a Tibetan medicinal herb *Aconitum naviculare* Stapf

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A new diterpenoid alkaloid, named as navirine (**1**), was isolated along with five known alkaloids, *i.e.*, isoatisine (**2**), hordenine (**3**), atisine (**4**), hetisinone (**5**) and delfissinol (**6**), from the ethanol extract of the whole plant of Tibetan medicinal plant *Aconitum naviculare* Stapf. The structure of the new compound was established on the basis of HR-MS, ^{1}H, ^{13}C and 2D NMR spectroscopic methods.

Keywords: *Aconitum naviculare*, alkaloid, diterpenoid, navirine

Aconitum naviculare Stapf is a perennial herb distributed around an altitude of 2000–3000 m in Tibet and the surroundings. The whole plant has been used in Tibetan folk medicine as a sedative, analgesic balm, and/or febrifuge.[1] Although the chemical constituents of plants of the genus *Acotinum* have been extensively studied,[2,3] the chemical constituents and biological activities of *Aconitum naviculare* have not been reported previously. In our effort to find biologically active components from Chinese medicinal plants,[4] we obtained from the ethanol extract of the whole plant of *Aconitum naviculare* a new diterpenoid alkaloid, (**1**), as well as five known alkaloids, *i.e.*, isoatisine (**2**), hordenine (**3**), atisine (**4**), hetisinone (**5**) and delfissinol (**6**). This is the first report on the isolation of diterpenoid alkaloids from *Aconitum naviculare* Stapf. The structure of the new compound was elucidated by spectroscopic methods and the total ^{1}H and ^{13}C NMR chemical shifts were assigned.

The crushed whole plant of *Aconitum naviculare*, collected from the National Forest Park of Huzhubei Mountain, Qinghai province, China was extracted with ethanol followed by silica gel column chromatographic separation to give compounds **1–6**.

Compound **1** was obtained as white needles, m.p. 175–176 °C. $[a]_D^{19}$ +22°(c 0.6, $CHCl_3$). The HR-SIMS-MS spectrum exhibited an M+H ion peak at *m/z* 461.3163, corresponding to a molecular formula of $C_{30}H_{40}N_2O_2$ (calcd. for M+H: 461.3163). Its IR spectrum showed absorption bands for hydroxyl groups (3347 cm^{-1}), C=C double bond (3026 and 1641 cm^{-1}), C=N double bond (1672 cm^{-1}) and a *p*-disubstituted benzene ring (822, 1510, 1610 and 3028 cm^{-1}). The ^{13}C NMR and DEPT spectra of **1** exhibited 30 carbon signals (3×CH_3, 10×CH_2, 10×CH, 7×C). In conjunction with its ^{1}H NMR spectrum it was clear that compound **1** possessed one methyl group (δ_C 18.9 and δ_H 1.04), two identical *N*-methyl groups (δ_C 45.4 and δ_H 2.30), a *p*-disubstituted phenyl moiety attached to oxygen (δ_C 114.7, 129.4, 132.3 and 157.3; δ_H 6.84, 2H, *d*, *J* = 8.4 Hz; d_H 7.10, 2H, *d*, *J* = 8.4Hz), an endocyclic double bond (δ_C 130.5, δ_H 5.67, and δ_C 146.2) and two carbons attached to oxygen (δ_C 72.4 and δ_C 68.3, δ_H 4.54). In addition, the extremely low field methine carbon (δ_C 169.5, δ_H 7.41) suggested the presence of a –CH=N– moiety. This was also supported by the IR absorption at 1672 cm^{-1}. Comparison of the ^{1}H and ^{13}C NMR spectra with those of tongolinine,[5] tangirine[6] and hordenine[7] suggested that compound **1** contained molecule of a C_{20}-diterpenoid alkaloid (**DA**) and hordenine. This is supported by its EI-MS spectrum which gave three principal fragments at *m/z* 460, 296 and 58, corresponding to the M^+, $\mathbf{DA}^+$ and $[CH_2{=}N(CH_3)_2]^+$ from the hordenine. The ^{1}H and ^{13}C chemical shifts of the partial structure **DA** were similar to those of tongolinine[5] except that the 17-exocyclic double bond (δ_C 104.5 and 158.0, δ_H 4.94, 2H) in tongolinine[5] was replaced by an endocyclic double bond in **DA** (δ_C 130.5 and 146.2, δ_H 5.67, 1H) and the location of hydroxyl groups was different in the two structures. The ^{1}H and ^{13}C chemical shifts of the partial structure **DA** were almost identical with those of the alkaloidal portion in tanirine[6] except that the chemical shifts of H-15 and H-17 in **DA** (δ_H 5.67 and 4.54 respectively) were shifted down-field compared to those in the alkaloidal portion of tanirine (δ_H 4.98 and 3.82 respectively[6]) due to the deshielding effect of the phenyl ring in **1**. Therefore, the structure of compound **1** was established as shown in Fig. 1 and named as navirine in which the diterpene alkaloid was connected with hordenine at C-17. This structure was confirmed by H, H COSY, HMBC and NOESY correlations as shown in Table 1. Briefly, the extremely low-field methine (δ_C 169.5, δ_H 7.41, *d*, *J* = 2.5 Hz) can be assigned as C-19 which showed clear HMBC correlations with H-5, H-18 and H-20, and H, H COSY correlation with H-20. The oxygen-connecting methylene (δ_C 68.3, δ_H 4.54, br, 2H) was assigned as C-17 which showed HMBC and H, H COSY correlations with H-15, and the phenyl quaternary carbon

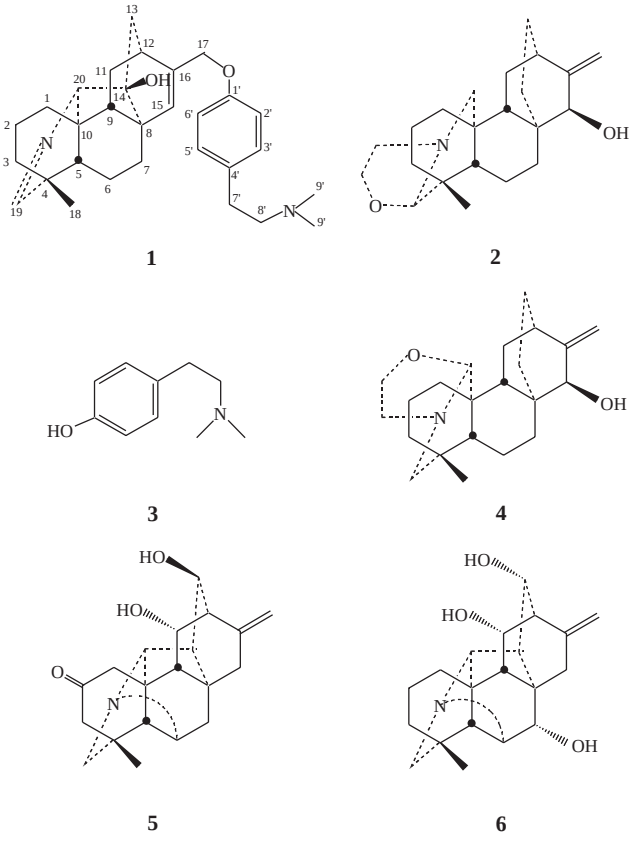

Fig. 1

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Table 1 ^{1}H (400 MHz) and ^{13}C (100 MHz) chemical shifts and 2D NMR correlations of compound (**1**)[a]

No.	δ_H (*J*, Hz)	δ_C	H-H COSY	HMBC (H→C)	NOESY
1	1.27 (β) m	30.9 t	H-2	H-5, H-9	
	1.73 (α) m				
2	1.40 (β) dtt (11.6, 10.8, 3.6)	27.8 t	H-1, H-3		H-20
	1.65 (α) m				
3	1.80 (α) ddd (13.2, 3.6, 2.4)	30.9 t	H-2	H-2α, H-18	H-1β
	1.98 (β) ddd (13.2, 10.8, 6.8)				
4	–	44.9 s		H-2α,β, H-5, H-18, H-19	
5	1.68 (β)m	44.3 s	H-20 (w)	H-1, H-3, H-6, H-9, H-20	
6	1.26 (α) m	20.6 t	H-6β, H-7	H-5, H-7	
	1.58 (β) m		H-6α		
7	1.56 (β) m	31.6 t	H-7α, H-6		
	1.63 (α) m		H-7β	H-15	
8	–	43.8 s		H-7α, H-9, H-11, H-13α, H-15	
9	1.66 (β)m	47.0 d	H-11	H-1β, H-5	
10	–	45.3 s		H-2, H-6, H-9	
11	1.54 (α) m	28.4 t	H-9, H-12	H-1 (^{4}J), H-20 (^{4}J)	
	1.68 (β) m				
12	2.54 (α) br	31.6 d	H-11, H-13	H-13α,β, H-15, H-17	H-13α, H–17, H–20α
13	1.60 (β) m	43.2 t	H-13β, H-12	H-20	H-11α
	1.90 (α) dd (12.4, 4.0)		H-13α		
14	–	72.4 d		H-7β, H-9, H-20	
15	5.67 br s	130.5 d	H-12, H-17	H-17	H-17
16	–	146.2 s		H-11α,β, H-13α,β, H-17	
17	4.54 br	68.3 t	H-15	H-15	H-15
18	1.04 s	18.9 q		H-5, H-19	
19	7.41 d (2.5)	169.5 d	H-20	H-5, H-18, H-20	
20	3.55 (α) br	80.4 d	H-5, H-19	H-5, H-9, H-13α,β	H-2α, H–12α, H–13α
1'	–	157.3 s		H-2', H-6', H-3', H-5', H-17	
2'	6.84 d (8.4)	114.7 d	H-3'	H-3', H-5′	H-17
3'	7.10 d (8.4)	129.4 d	H-2'	H-2', H-6′, H-7'	H-7'
4'	–	132.3 s		H-2', H-6′, H-7', H-8'	
5'	7.10 d (8.4)	129.4 d	H-6'	H-2', H-6′, H-7'	H-7'
6'	6.84 d (8.4)	114.7 d	H-5'	H-3', H-5'	H-17
7'	2.72 m	33.4 t	H-8'	H-3', H-5', H-8'	
8'	2.52 m	61.7 t	H-7'	H-7', H-9'	
9'	2.30 s	45.4 q		H-8'	

[a]Determined in $CDCl_3$. ^{13}C NMR multiplicities were established by DEPT.

(δ_C 157.3) showed HMBC correlation with H-17. The complete ^{1}H and ^{13}C NMR chemical shift assignments together with 2D NMR correlations are listed in Table 1.

Compounds **2**–**6** were identified by comparison of their ^{1}H and ^{13}C NMR, MS and IR spectroscopic data with those reported in literatures as isoatisine (2),[8] hordenine (3),[7] atisine (4),[7,8] hetisinone (5)[9] and delfissinol (6).[10]

Experimental

Optical rotation was measured on a Perkin-Elmer 241 polarimeter. IR spectra were taken on a Nicolet 170 SX IR spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker AM 400 NMR spectrometer with TMS as internal standard. HR-ESI-MS and EI-MS spectra were obtained on a Bruker APEX II FT-MS and HP 5988 MS spectrometers respectively.

Extraction and isolation procedures

The air-dried whole plant of *Aconitum naviculare* (10.5kg) was crushed and extracted with 5 dm^{-3} of 90 $^c/_o$ EtOH at room temperature for 5 days. The EtOH extract was treated with 5 $^c/_o$ HCl and the acidic solution was basified with 28 % NH_4OH to pH 11 and extracted with $CHCl_3$ to give the crude alkaloids (143g) after removing the solvent. This residue was separated by silica gel (100-200 mesh) column chromatography (CC) with gradient elution of PE-Me_2CO-Et_2NH, giving, in order of the increasing polarity, navirine (**1**, 12 mg), isoatisine (**2**, 72 mg), hordenine (**3**, 230 mg), atisine (**4**, 40 mg), hetisinone (**5**, 38 mg) and delfissinol (**6**, 15 mg). The structures of compounds **2**–**6** were characterised by their m.p., IR, MS, ^{1}H and ^{13}C NMR chemical shifts and compared with those reported in literatures.[5-10]

Navirine (**1**): White needles, m.p. 175–176 ^{o}C, $[\alpha]_D^{19}$+22°(c 0.6, $CHCl_3$). Positive-SIMS-MS: Found: 461.3163, Calcd. for $C_{30}H_{40}N_2O_2$ + H: 461.3163). EI-MS *m/z* (rel. int.): 460 (M^+, 25), 368 (5), 296 (M-164, 34), 162 (25), 121 (48), 91 (66), 77 (59), 58 (100). ν_{max}/cm^{-1}: 3347, 3028, 3026, 1672, 1641, 1610, 1510, 822. For ^{1}H and ^{13}C NMR data see Table 1.

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