Trichocarpinine, a Novel Hetidine–Hetisine Type Bisditerpenoid Alkaloid from *Aconitum tanguticum* var. *trichocarpum*

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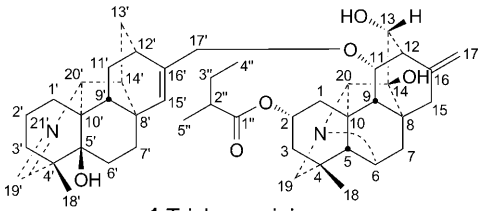
Trichocarpinine (**1**), the first hetidine–hetisine type bisditerpenoid alkaloid, was isolated from the whole herbs of *Aconitum tanguticum* var. *trichocarpum*. Its structure was determined by a combination of spectroscopic techniques, including HR-ESI-MS and 1D- and 2D-NMR experiments. Its plausible biogenetic pathway was proposed as well (*Scheme*).

Introduction. – The genus *Aconitum* is widely distributed throughout the northern hemisphere region and has been used as traditional medicines and arrow poisons by various civilizations from antiquity [1]. Subgen. *Aconitum* as the largest subgenus of the *Aconitum* plants consists of about 250 species, among which the ser. *Tangutica* W. T. WANG features chemotaxonomic primitiveness [2]. From a phytochemical perspective, only *Aconitum tanguticum* (MAXIM.) STAPF W. T. WANG from this series has been investigated to date, which is characteristic of two lactone-type diterpenoid alkaloids and a rare bisditerpenoid-type alkaloid [3].

Aconitum tanguticum (MAXIM.) STAPF var. *trichocarpum* HAND.-MAZZ. grows mainly at the altitude of over 4000 m in the alpine meadows in Tibet, China, and falls taxonomically into the ser. *Tangutica* of subgen. *Aconitum* [4]. The whole herbs of this plant have been used as a traditional medicine for the treatment of pneumonia and fever. As part of our ongoing research program to study comparatively the diterpenoid alkaloids from *Aconitum* and *Delphinium* species and to find the chemotaxonomic characteristics of the diterpenoid alkaloids, we investigated the whole herbs of *A. tanguticum* var. *trichocarpum*. The investigation led to the isolation of trichocarpinine (**1**), which represents the first example of the hetidine–hetisine type bisditerpenoid alkaloid. The isolation and structure determination of trichocarpinine (**1**) are described herein, and its plausible biogenetic pathway is proposed as well.



1 Trichocarpinine

Results and Discussion. – Trichocarpinine (**1**) was obtained as a white amorphous powder. Its molecular formula, $C_{45}H_{60}N_2O_6$, was inferred from the HR-ESI-MS ($[M+H]^+$ at m/z 725.4532) and ^{13}C-NMR spectrum, suggesting that **1** might be a bisditerpenoid alkaloid [5]. The ^{1}H- and ^{13}C-NMR data (*Table*) of **1** exhibited characteristic signals for a trisubstituted C=C bond (δ(H) 5.49 (br. *s*); δ(C) 146.7 (*s*) and 130.9 (*d*)), an exocyclic C=C bond (δ(H) 4.88 (*s*) and 4.70 (*s*); δ(C) 144.7 (*s*) and 108.1 (*t*)), a C=N moiety (δ(H) 7.40 (*d*); δ(C) 169.5 (*d*)), one (2-methylbutanoyl)oxy group (δ(H) 2.32–2.38 (*m*), 1.72–1.78 (*m*), 0.90 (*t*, $J=7.2$), and 1.16 (*d*, $J=7.2$); δ(C) 175.7 (*s*), 41.5 (*d*), 26.5 (*t*), 11.6 (*q*), and 16.6 (*q*)), and two tertiary Me groups (δ(C) 29.7 (*q*), δ(H) 1.01 (*s*); δ(C) 19.0 (*q*), δ(H) 1.05 (*s*)). The above-mentioned evidence suggested that trichocarpinine (**1**) consists of two C_{20}-diterpenoid alkaloid moieties, which could be deduced as a hetidine-type and a hetisine-type alkaloid on the basis of the chemical-shift values of the Me(18)/Me(18′) and CH_2(19)/H–C(19′) (*Table*), in conjunction with two typical sets of chemical-shift values of four non-O-bearing quaternary C-atoms (*i.e.*, C(4)/C(4′), C(8)/C(8′), C(10)/C(10′), and C(16)/C(16′)) (*Table*) [5].

Table. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$, 400 and 100 MHz) *of Trichocarpinine* (**1**). δ in ppm. J in Hz.

	δ(C)	δ(H)		δ(C)	δ(H)
H_α–C(1)	31.6 (*t*)	2.91 (br. *d*, $J=16.0$)	CH_2(3′)	30.6 (*t*)	1.25–1.30 (*m*)
H_β–C(1)		1.80 (hidden)	C(4′)	44.9 (*s*)	–
H–C(2)	68.7 (*d*)	5.16–5.20 (*m*, $w_{1/2}=10.2$)	C(5′)	72.4 (*s*)	–
CH_2(3)	28.4 (*t*)	1.57 (hidden)	H_a–C(6′)	36.9 (*t*)	1.74–1.80 (*m*)
C(4)	37.6 (*s*)	–	H_b–C(6′)		1.64 (hidden)
H–C(5)	60.1 (*d*)	1.51 (*s*)	CH_2(7′)	27.7 (*t*)	1.39 (hidden)
H–C(6)	63.0 (*d*)	3.14 (br. *s*)	C(8′)	43.8 (*s*)	–
H_α–C(7)	31.3 (*t*)	1.63 (hidden)	C(9′)	47.0 (*d*)	1.60 (hidden)
H_β–C(7)		1.80–1.86 (*m*)	C(10′)	45.3 (*s*)	–
C(8)	44.2 (*s*)	–	H_a–C(11′)	43.2 (*t*)	1.85 (hidden)
H–C(9)	52.2 (*d*)	2.01 (*d*, $J=9.2$)	H_b–C(11′)		1.46 (hidden)
C(10)	46.2 (*s*)	–	H–C(12′)	31.8 (*d*)	2.43–2.47 (*m*)
H–C(11)	82.1 (*d*)	3.88 (*d*, $J=8.8$)	CH_2(13′)	31.9 (*t*)	1.34–1.39 (*m*)
H–C(12)	48.6 (*d*)	2.62 (*d*, $J=2.4$)	H–C(14′)	44.3 (*d*)	1.62 (hidden)
H–C(13)	79.8 (*d*)	3.99 (br. *s*)	H–C(15′)	130.9 (*d*)	5.49 (br. *s*)
C(14)	80.1 (*s*)	–	C(16′)	146.7 (*s*)	–
CH_2(15)	31.0 (*t*)	2.07–2.12 (*m*)	CH_2(17′)	70.3 (*t*)	4.05, 3.90 (*AB*, $J=12.4$)
C(16)	144.7 (*s*)	–	Me(18′)	19.0 (*q*)	1.05 (*s*)
H_a–C(17)	108.1 (*t*)	4.88 (*s*)	H–C(19′)	169.5 (*d*)	7.40 (*d*, $J=2.8$)
H_b–C(17)		4.70 (*s*)	H–C(20′)	80.4 (*d*)	3.54 (br. *s*)
Me(18)	29.7 (*q*)	1.01 (*s*)	C(1″)	175.7 (*s*)	–
CH_2(19)	62.9 (*t*)	2.95, 2.55 (*AB*, $J=12.0$)	H–C(2″)	41.5 (*d*)	2.32–2.38 (*m*)
H–C(20)	68.8 (*d*)	3.68 (br. *s*)	CH_2(3″)	26.5 (*t*)	1.72–1.78 (*m*)
H_a–C(1′)	30.8 (*t*)	1.90–1.95 (*m*)	Me(4″)	11.6 (*q*)	0.90 (*t*, $J=7.2$)
H_b–C(1′)		1.70 (hidden)	Me(5″)	16.6 (*q*)	1.16 (*d*, $J=7.2$)
CH_2(2′)	20.6 (*t*)	1.50 (hidden)			

Starting from two typical Me(18) *s*, the signal at δ(C) 62.9 (*t*) was attributed to C(19), while the C=N could be assigned to C(19′) due to the HMBC data of **1** (*Fig. 1*).

Correlations from H–C(19′) to C(20′), CH_2(19) to C(20), H–C(6) to C(20), and H–C(20) to C(6) in the HMBC spectrum confirmed the existence of a hetidine-type alkaloid moiety and a hetisine counterpart in **1**. The critical ether linkage was positioned between C(11) and C(17′) based on the correlations from H–C(11) (δ(H) 3.88 (*d*)) to C(13), C(16), and C(17′) in the HMBC spectrum. Strong evidence also arose from the NOESY correlations H–C(11)/H_a–C(17′) and H–C(11)/H–C(15′) (*Fig. 2*). The observation of a signal at δ(H) 5.16–5.20 (H–C(2)) correlating with those of C(4) (δ(C) 37.6 (*s*)) and C(10) (δ(C) 46.2 (*s*)) demonstrated the location of the (2-methylbutanoyl)oxy group at C(2), which could be further supported by the $^1H,^1H$-COSY cross-peaks H–C(2)/CH_2(1) and H–C(2)/CH_2(3) (*Fig. 1*). The ^{13}C-NMR and DEPT spectra displayed six O-bearing C-atoms (δ(C) 82.1 (*d*), 80.1 (*s*), 79.8 (*d*), 72.4 (*s*), 70.3 (*t*), and 68.7 (*d*)), showing that **1** possesses three OH groups in addition to the ether linkage and an ester group. The secondary OH group was attributed to C(13) in the hetisine part because of the cross-peaks H–C(13)/C(20), H–C(13)/C(16), and H–C(20)/C(13). The two tertiary OH groups were located at C(14) and C(5′), respectively, based on the HMBCs shown in *Fig. 1*. In addition, the typical exocyclic C=C bond was isomerized to the endocyclic C(15′)=C(16′) bond in the hetidine section, which was strongly supported by the reciprocal HMBC cross-peaks H–C(15′)/C(7′), H–C(15′)/C(17′), CH_2(17′)/C(15′), and CH_2(7′)/C(15′).

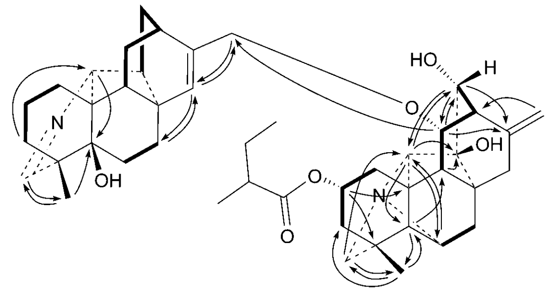


Fig. 1. *$^1H,^1H$-COSY* (—) *and key HMBC* (→) *data of trichocarpinine* (**1**)

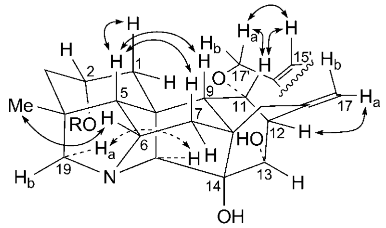


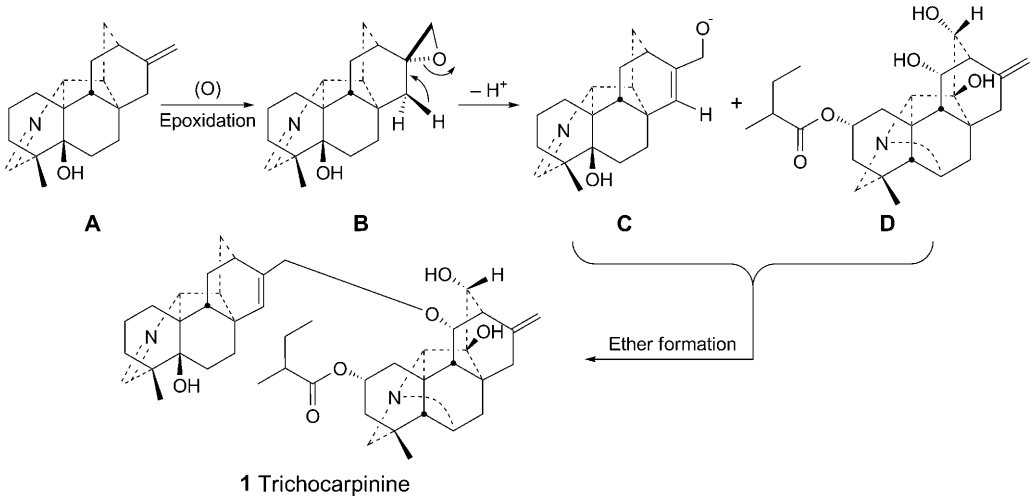
Fig. 2. *Key NOE correlations* (↔) *of trichocarpinine* (**1**)

The relative configuration of trichocarpinine (**1**) was then established by a combination of its vicinal coupling constants with the key NOESY correlations (*Fig. 2*). The appearance of H–C(2) as a *m* ($w_{1/2}$ = 10.2 Hz) indicated that no axial–axial coupling relationship existed between H–C(2) and $H_β$–C(1) or $H_β$–C(3), which led to the assignment of H–C(2) in an equatorial position, namely a β-

orientation. The large coupling constant between H–C(11) and H_β–C(9) ($J = 8.8$ Hz) revealed a dihedral angle of *ca.* 0° of these two H-atoms, which implied that H–C(11) was β-oriented. Furthermore, a strong piece of evidence to establish the orientation of the OH–C(13) group was the observation of a characteristic *W*-shape coupling between H–C(11) and H–C(13) in the ^{1}H,^{1}H-COSY plot. It was, therefore, concluded that H–C(13) had to be β-oriented to keep the *W*-shape with H–C(11). As a result, the substituents at C(2), C(11), and C(13) were all arranged in α orientations, which are consistent with those in the related hetisine-type alkaloids such as tangutisine (=(2α,11α,13*R*)-hetisan-2,11,13,14-tetrol) [3b] and guan fu base A (=(2α,11α,13*R*)-hetisan-2,11,13,14-tetrol 2,13-diacetate) [6]. All the key NOESY correlations were observed as shown in *Fig. 2*. Accordingly, the structure of trichocarpinine (**1**) was established as (2α,11α,13*R*)-13,14-dihydroxy-11-[(5-hydroxy-19,21-didehydrohetidan-17-yl)oxy]hetisan-2-yl 2-methylbutanoate.

A plausible biogenetic pathway for trichocarpinine (**1**) was proposed as shown in the *Scheme*. A hetidine-type C_{20}-diterpenoid alkaloid **A** could be oxidized to the corresponding epoxide **B**, which might be subsequently converted to the allyl alcohol anion **C** through an epoxy ring opening process. The hetisine part **D** could then react with fragment **C** *via* the formation of the ether linkage to yield the ultimate bisditerpenoid alkaloid **1**.

Scheme. *Plausible Biogenetic Pathway of Trichocarpinine* (**1**)



In nature, bisditerpenoid-class alkaloids which consist of two C_{20}-diterpenoid alkaloid moieties or of a C_{20}- and a C_{19}-diterpenoid alkaloid moiety are of rare occurrence. The naturally occurring bisditerpenoid-class alkaloids reported so far are about ten in number, including the atisine-hetidine-type, rearranged atisine–hetidine-type, denudatine–denudatine-type, and heteratisine–hetidine-type alkaloids. Trichocarpinine (**1**) presented in this article is the first example of a hetidine–hetisine-type bisditerpenoid alkaloid, which combines a hetidine moiety with a hetisine counterpart through an ether linkage between C(17′) and C(11). To the best of our knowledge, only

three bisditerpenoid alkaloids were isolated so far from the genus *Aconitum* L., and most of them are distributed in the primitive plants of the subgenus *Aconitum*, such as ser. *Tangutica*. There is a clear relationship between their structures and the systematic position of the corresponding primitive plants of subgenus *Aconitum*. Therefore, the presence of the bisditerpenoid alkaloids may serve as a reliable taxonomic character of the subgenus *Aconitum*.

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Experimental Part

General. TLC: silica-gel plates; detection by spraying with *Dragendorff* reagent. Column chromatography (CC): silica gel (SiO_2; 300–400 mesh, 10–40 m; *Qindao Sea Chemical Inc.*). Optical rotations: *Perkin-Elmer-341* polarimeter. IR Spectra: *Nicolet-FT-IR-200S* spectrometer; KBr pellets; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Varian-Unity-INOVA-400/54* spectrometers; at 400/100 or 200/50 MHz, resp.; δ in ppm rel. to Me_4Si, *J* in Hz. ESI-MS: *Finnigan LCQ*; in *m/z* (rel.%). HR-ESI-MS: *Micromass-Auto-Ultima-Tof* spectrometer.

Plant Material. The whole herbs of *A. tanguticum* var. *trichocarpum* were collected in Nagqu County, Tibet, P. R. China, in August 2008. The plant was identified by Associate Professor *S. Gesang* at the Tibet Institute for Food and Drug Control, where a voucher specimen (No. 005612) has been deposited.

Extraction and Isolation. Air-dried and powdered whole herbs of *A. tanguticum* var. *trichocarpum* (5.0 kg) were percolated with 0.1N HCl (75 l). The acidic soln. was alkalinized with 10% aq. NH_3 soln. to pH $\geq$ 10 and then extracted with AcOEt (30 l × 3). The combined extracts were concentrated to yield the total crude alkaloids (30.1 g), which were dissolved in $CHCl_3$ to yield the soluble part *I* (18.7 g) and the insoluble part *II* (12.0 g). Part *I* was subjected to CC (SiO_2 *H* (300 g), gradient cyclohexane/Me_2CO 5:1 → 1:1): *Fractions A–E*. Repeated subjection of *Fr. E* (2.8 g) to CC (SiO_2, $CHCl_3$/MeOH/Et_2NH 95:5:1) afforded trichocarpinine (**1**; 9 mg).

Trichocarpinine (=*(2α,11α,13*R*)-13,14-Dihydroxy-11-[(5-hydroxy-19,21-didehydrohetidan-17-yl)oxy]hetisan-2-yl 2-Methylbutanoate*; **1**): White amorphous powder. $[\alpha]_D^{20} = +17.7$ ($c = 0.14$, $CHCl_3$). IR (KBr): 3425, 2931, 1724, 1045. ^1H- and ^{13}C-NMR: *Table*. HR-ESI-MS 725.4532 ($[M+H]^+$, $C_{45}H_{61}N_2O_6^+$; calc. 725.4530).

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