Bis-Diterpenoid Alkaloids from *Aconitum tanguticum* var. *trichocarpum*

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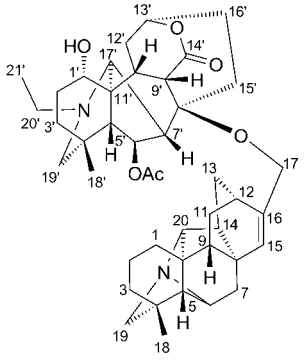
Phytochemical investigation of the whole herbs of *Aconitum tanguticum* (MAXIM.) STAPF var. *trichocarpum* HAND.-MAZZ. led to the isolation of one heteratisine-hetidine-type bis-diterpenoid alkaloid, trichocarpidine (**1**), three hetidine-hetisine type bis-diterpenoid alkaloids, trichocarpinine A – C (**2** – **4**, resp.), together with nine known compounds. Their structures were elucidated by spectroscopic analyses.

Introduction. – The genus *Aconitum* (Ranunculaceae) comprises *ca.* 400 species, crude prepararations of which were popularly used in Asia, Alaska, and Europe [1]. Diterpenoid alkaloids are structurally classified as C_{18}-, C_{19}-, and C_{20}-diterpenoid alkaloids, and the bis-diterpenoid alkaloids belong to C_{20}-diterpenoid alkaloids based on the new classification criteria of the C_{20}-diterpenoid alkaloids [2].

Aconitum tanguticum (MAXIM.) STAPF var. *trichocarpum* HAND.-MAZZ. is mainly distributed at an altitude of over 4000 m in the alpine meadows in Tibet of China, and characteristically contains lactone-type C_{19}-diterpenoid alkaloids and bis-diterpenoid alkaloids [3 – 5]. The whole herbs of this plant have been used in the traditional medicine for the treatment of pneumonia and fever. As part of our ongoing search for new and bioactive bis-diterpeniod alkaloids [6][7], we isolated new compounds **1** – **4** together with nine known compounds, *i.e.*, 6-acetylheteratisine [3], 6-benzoylheteratisine [8], dehydroheteratisine [8], heteratisine [8], guan-fu base Z [9], heterophyllidine [10], tangerine [4], tongolinine [11], and trichocarpinine [6].

Results and Discussion. – Trichocarpidine (**1**) was obtained as colorless amorphous powder. Its molecular formula, $C_{43}H_{58}N_2O_6$, was deduced from the HR-ESI-MS (*m*/*z* 699.4350 ($[M+H]^+$)). In addition to the signals of an Ac group (δ(H) 1.95(*s*); δ(C) 170.9(*s*) and 21.7(*q*)), those for an *N*-Et group (δ(H) 1.12 (*t*, *J* = 7.2, 3 H); δ(C) 13.0 (*q*) and 48.4 (*t*)), two tertiary Me groups (δ(H) 1.01, 0.97 (2*s*, each 3 H); δ(C) 27.2, 28.8 (2*q*)), a trisubstituted C=C bond (δ(H) 5.67 (*s*, 1 H); δ(C) 126.9 (*d*) and 145.1 (*s*)), a lactone group (δ(C) 172.3 (*s*)), together with the signals of six quaternary C-atoms, were displayed in the NMR spectra of **1** (*Table 1*). Seven structural fragments were indicated by the ^{1}H,^{1}H-COSY and HMQC experiments (*Fig. 1*). In the HMBC experiment, the correlations between H–C(1′) and C(11′), H–C(3′) and C(4′), H–C(17′) and C(11′), H–C(5′) and C(4′), H–C(7′) and C(8′), H–C(15′) and C(8′), H–C(16′) and C(13′), identified fragment A as C(1′) – C(3′), fragment B as C(5′)–C(7′),



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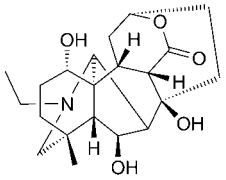
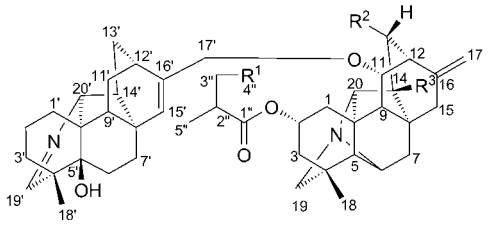
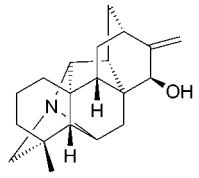


Heterophyllidine



Trichocarpinine A (**2**) R^1 = Me, R^2= AcO, R^3 = OH
Trichocarpinine B (**3**) R^1 = Me, R^2 = AcO, R^3 = H
Trichocarpinine C (**4**) R^1 = H, R^2 = AcO, R^3 = OH
Trichocarpinine (**5**) R^1 = Me, R^2 = OH, R^3 = OH



Nominine

fragment C as C(9′), C(10′), C(12′), and C(13′), fragment D as C(15)–C(16), respectively. HMBCs suggested that fragment A was attached to fragments B and C *via* C(4′) and C(11′) respectively, fragment B to fragments C and D *via* C(8′), and fragment C to fragment D *via* C(13′). Similarly, fragments E–G were identified as C(1)–C(3), C(5)–C(7), and C(9), C(11), C(12), C(13), C(14), C(20), respectively, and they were attached to each other *via* the quaternary C-atoms C(4), C(8), and C(10). Hence, all these informations evidenced that **1** is a bis-diterpenoid alkaloid. Comparison of the ^{13}C-NMR, HMQC, and DEPT data with those of the the known compound heterophyllidine led to the deduction that one moiety of **1** was a heteratisine-type alkolid. Apart from the heteratisine-type alkaloid skeleton, comparing further NMR data with those of the known compound nominine [11], we elucidated the left structure as a hetisine-type diterpenoid alkaloid. HMBCs of H–C(17) with C(8′), C(12), C(15), and C(16), and of H–C(15) with C(9), C(12), and C(17), implied that the heteratisine part was linked with the hetisine part *via* C(8′)–O–C(17), and additionally asserted the presence of a trisubstituted C=C bond. According to HMBC of **1**, the AcO and OH groups should be positioned at C(1) and C(6) of heteratisine part, respectively. The relative configurations at the stereogenic centers of **1** were deduced from the ^{1}H,^{1}H-COSY correlations (*Fig. 1*) and corresponding correlations from the NOEDS spectrum (*Fig. 2*). A correlation between H–C(1) and H_β–C(10) in the selective NOE experiment indicated that the OH group at C(1) was α-oriented. No correlation between H_β–C(5) and H–C(6) was observed in the ^{1}H,^{1}H-COSY spectrum (*Fig. 1*), indicating a dihedral angle of 90° between the two H-atoms, which evidenced the β-orientation of the 6-AcO group. All the available data led to the elucidation of the structure of trichocarpidine **1** as depicted.

Table 1. *^{1}H- and ^{13}C-NMR* (400 and100 MHz, resp.) *Data for* **1**. Atom numbering as indicated in the *Formulae*. δ in ppm, J in Hz.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
1	1.57–1.65 (*m*)	29.5 (*t*)	1′	3.86 (*t*, *J* = 5.6)	72.4 (*d*)
2	1.00–1.07 (*m*)	19.5 (*t*)	2′	1.70–1.78 (*m*, H_α), 2.23–2.33 (*m*, H_β)	27.6 (*t*)
3	1.44–1.52 (*m*)	34.0 (*t*)	3′	0.98–1.02 (*m*, H_α), 1.80–1.86 (*m*, H_β)	31.3 (*t*)
4		37.8 (*s*)	4′		33.1 (*s*)
5	2.22–2.30 (*m*)	61.7 (*d*)	5′	1.60–1.66 (*m*)	51.3 (*d*)
6	3.20 (*s*)	65.3 (*d*)	6′	5.45 (*d*, *J* = 7.2)	72.0 (*d*)
7	0.98–1.04 (*m*)	33.2 (*t*)	7′	2.83 (*d*, *J* = 7.2)	44.5 (*d*)
8		44.6 (*s*)	8′		79.2 (*s*)
9	1.62–1.70 (*m*)	31.5 (*d*)	9′	3.85–3.90 (*m*)	48.0 (*d*)
10		49.8 (*s*)	10′	2.34–2.40 (*m*)	40.1 (*d*)
11	1.70–1.80 (*m*)	29.4 (*t*)	11′		49.4 (*s*)
12	1.33–1.43 (*m*)	50.1 (*d*)	12′	1.02–1.10 (*m*, H_α), 2.35–2.43 (*m*, H_β)	30.4 (*t*)
13	1.82–1.87 (*m*, H_α), 2.00–2.06 (*m*, H_β)	31.6 (*t*)	13′	4.82 (*t*, *J* = 8.0)	74.2 (*d*)
14	1.70–1.78 (*m*)	48.5 (*d*)	14′		172.3 (*s*)
15	5.67 (*s*)	126.9 (*d*)	15′	1.84–1.92 (*m*, H_α), 2.03–2.08 (*m*, H_β)	34.2 (*t*)
16		145.1 (*s*)	16′	2.40–2.46 (*m*, H_α), 2.44–2.50 (*m*, H_β)	30.6 (*t*)
17	3.79–3.86 (*m*)	61.3 (*t*)	17′	3.41 (*s*)	64.2 (*d*)
18	1.01 (*s*)	27.2 (*q*)	18′	0.97 (*s*)	28.8 (*q*)
19	2.35–2.42 (*m*, H_α), 2.45–2.52 (*m*, H_β)	62.8 (*t*)	19′	2.42–2.50 (*m*)	61.5 (*t*)
20	2.48–2.54 (*m*)	73.9 (*d*)	20′	2.44–2.52 (*m*)	48.4 (*t*)
AcO	1.95 (*s*)	170.9 (*s*), 21.7 (*q*)	21′	1.12 (*t*, *J* = 7.2)	13.0 (*q*)

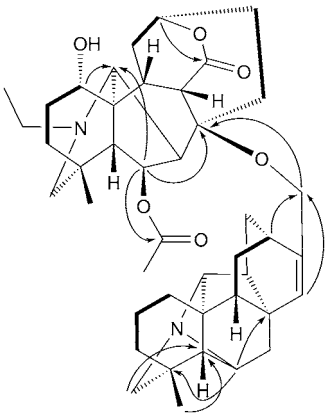


Fig. 1. *^{1}H,^{1}H-COSY* (—) *and key HMB* (H → C) *correlations of trichocarpidine* (**1**)

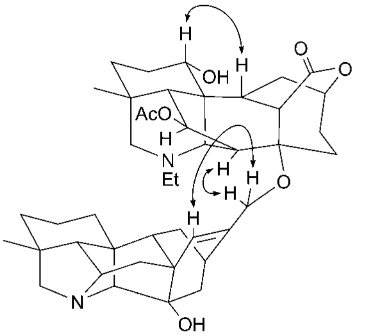


Fig. 2. *Key NOE correlations* (H ↔ H) *of trichocarpidine* (**1**)

For trichocarpinine A (**2**), the molecular formulas $C_{47}H_{62}N_2O_7$ deduced from its HR-ESI-MS. The NMR data of **2** (*Table 2*) were similar to those of the known compound trichocarpinine **5** [6], except an AcO group (δ(H) 1.96 (*s*), δ(C) 169.9 (*s*), and 21.2 (*q*)) in **2** replaced OH group in **5**, and this was supported by ESI-MS of **2** (increase by 42 amu). The AcO be at C(13) due to the HMBC of **2** (*Fig. 3*).

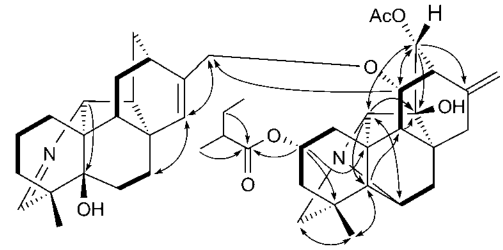


Fig. 3. *¹H,¹H-COSY* (—) *and key HMB* (H → C) *correlations of trichocarpinine A* (**2**)

The relative configuration of trichocarpinine A (**2**) was then established by combination of its vicinal coupling constants with the key NOESY correlations (*Fig. 4*). The signal of H–C(2) as a *multiplet* ($w_{1/2}$ = 10.2) indicated no axial–axial coupling relationship between H–C(2) and H_β–C(1), and H–C(2) and H_β–C(3), leading to the assignment of H–C(2) as equatorial, *i.e.*, β-oriented. The large coupling constant (*J* = 8.4) between H–C(11) and H_β–C(9) revealed a dihedral angle of *ca.* 0° for these two H-atoms, which implied that H–C(11) was β-oriented. Furthermore, a strong evidence to establish the orientation of HO–C(13) group was the observation of a characteristic W-shape coupling between H–C(11) and H–C(13) in the ¹H,¹H-COSY

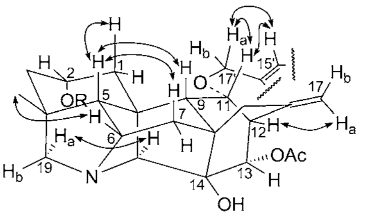


Fig. 4. *Key NOE correlations* (H ↔ H) *of trichocarpinine A* (**2**)

Table 2. *^{1}H- and ^{13}C-NMR* (400 MHz and 100 MHz, resp.) *Data for* **2** *and* **5**. Atom numbering as indicated in *Formulae*. δ in ppm, J in Hz.

Position	δ(H) of **2**	δ(C)		Position	δ(H) of **2**	δ(C)	
		2	**5**			**2**	**5**
1	1.66–1.74 (m, H_α), 3.02 (br. d, J = 16.4, H_β)	31.5 (t)	31.6(t)	1′	1.50–1.56 (m, H_α), 1.74–1.80 (m, H_α)	30.8 (t)	30.8 (t)
2	5.13 (br. s, $w_{1/2}$ = 10.2)	69.7 (d)	68.7 (d)	2′	1.50–1.60 (m)	20.5 (t)	20.6 (t)
3	1.54–1.58 (m, H_α), 1.79–1.83 (m, H_β)	28.3 (t)	28.4 (t)	3′	1.22–1.29 (m)	30.5 (t)	30.6 (t)
4		37.4 (s)	37.6 (s)	4′		44.7 (s)	44.9 (s)
5	1.56–1.62 (m)	59.8 (d)	60.1 (d)	5′		72.4 (s)	72.4 (s)
6	3.10 (s)	63.0 (d)	63.0 (d)	6′	1.56–1.62 (m, H_α), 1.80–1.88 (m, H_β)	36.7 (t)	36.9 (t)
7	1.30–1.36 (m)	31.8 (t)	31.3 (t)	7′	1.39–1.44 (m, H_α), 1.50–1.57 (m, H_β)	27.5 (t)	27.7 (t)
8		44.9 (s)	44.2 (s)	8′		43.6 (s)	43.8 (s)
9	2.00–2.06 (m)	52.5 (d)	52.2 (d)	9′	1.60–1.66 (m)	46.9 (d)	47.0 (d)
10		46.0 (s)	46.2 (s)	10′		45.2 (s)	45.3 (s)
11	3.76 (s)	81.0 (d)	82.1 (d)	11′	1.34–1.42 (m, H_α), 1.78–1.84 (m, H_β)	43.1 (t)	43.2 (t)
12	2.84 (s)	44.6 (d)	48.6 (d)	12′	2.09 (br. s)	31.6 (d)	31.8 (d)
13	4.93 (s)	80.8 (d)	79.8 (d)	13′	1.50–1.56 (m, H_α), 1.76–1.82 (m, H_β)	30.9 (t)	31.9 (t)
14		78.6 (s)	80.1 (s)	14′	1.60–1.66 (m)	44.2 (d)	44.3 (d)
15	1.99–2.02 (m, H_α), 2.14 (br. s)	30.9 (t)	31.0 (t)	15′	5.43 (s)	129.9 (d)	130.9 (d)
16		143.8 (s)	144.7 (s)	16′		147.2 (s)	146.7 (s)
17	4.72 (s), 4.93 (s)	109.2 (t)	108.1 (t)	17′	3.74, 3.92 (ABq, J = 12.4)	69.9 (t)	70.3 (t)
18	0.96 (s)	29.5 (q)	29.7 (q)	18′	1.00 (s)	18.9 (q)	19.0 (q)
19	2.51, 2.83 (ABq, J = 11.6)	62.6 (t)	62.9 (t)	19′	7.36 (s)	169.5 (d)	169.5 (d)
20	3.43 (s)	69.5 (d)	68.8 (d)	20′	3.49 (s)	80.2 (d)	80.4 (d)
1″		175.7 (s)	175.7 (s)	AcO	1.96 (s)	169.9 (s), 21.2 (q)	
2″	2.24–2.29 (m)	41.3 (d)	41.5 (d)				
3″	1.59–1.64 (m)	26.2 (t)	26.5 (t)				
4″	0.85 (t, J = 7.2)	11.5 (q)	11.6 (q)				
5″	1.13 (d, J = 7.2)	16.5 (q)	16.6 (q)				

spectrum. It was, therefore, concluded that the H–C(13) had to be β-oriented to retain the W-shape coupling with H–C(11). As a result, the substituents at C(2), C(11), and C(13) were all α-oriented. All the key NOESY correlations were observed as shown in *Fig. 4*. Accordingly, the structure of trichocarpinine A (**2**) was established as depicted.

For trichocarpinines B and C (**3** and **4**, resp.), molecular formulae $C_{47}H_{62}N_2O_6$ and $C_{46}H_{60}N_2O_7$ were determined by HR-ESI-MS, respectively. The NMR data of **3** (*Table 3*) were very similar to those of (**2**) except that a CH group (δ(C) 44.2) replaced on oxgenated quaternary C-atom (δ(C) 78.6) in **2**. The CH group C-atom was assigned as C(14), since a correlation of H–C(14) to C(9), C(10), C(13), and C(20) was observed in HMBC (*Fig. 5*). The AcO group was located at C(13) on the basis of correlations shown in *Fig. 5*. Therefore, the structure of trichocarpinine B (**3**) was established as depicted.

The NMR and HR-ESI-MS data of **4** were similar to those of **2**, except that **4** displayed 14 amu less than **2** (*Table 4*). The *triplet* at δ(H) 0.89 ($J = 7.2$, 3 H)) observed

Table 3. *^{1}H- and ^{13}C-NMR* (400 MHz and 100 MHz, resp.) *Data for* **3**. Atom numbering as indicated in *Formulae*. δ in ppm, J in Hz.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
1	1.66–1.74 (*m*, H_α), 3.08 (br. *d*, $J = 16.4$, H_β)	31.5 (*t*)	1′	1.79–1.84 (*m*, H_α), 1.84–1.88 (*m*, H_β)	30.8 (*t*)
2	5.18 (br. *s*, $w_{1/2} = 10.2$)	69.8 (*d*)	2′	1.50–1.60 (*m*)	20.5 (*t*)
3	1.50–1.58 (*m*, H_α), 1.70–1.80 (*m*, H_β)	28.2 (*t*)	3′	1.65–1.74 (*m*)	30.5 (*t*)
			4′		44.8 (*s*)
4		36.5 (*s*)	5′		72.2 (*s*)
5	1.54–1.62 (*m*)	61.3 (*d*)	6′	1.62–1.68 (*m*, H_α), 1.80–1.86 (*m*, H_β)	36.7 (*t*)
6	3.23 (*s*)	64.1 (*d*)			
7	1.47–1.53 (*m*, H_α), 1.60–1.68 (*m*, H_β)	36.1 (*t*)	7′	1.43–1.50 (*m*, H_α), 1.50–1.57 (*m*, H_β)	27.5 (*t*)
8		43.7 (*s*)	8′		43.6 (*s*)
9	1.97–2.05 (*m*)	54.0 (*d*)	9′	1.60–1.65 (*m*)	46.9 (*d*)
10		50.3 (*s*)	10′		45.2 (*s*)
11	3.80 (*s*)	81.3 (*d*)	11′	1.40–1.50 (*m*, H_α), 1.84–1.90 (*m*, H_β)	43.1 (*t*)
12	2.75 (*d*, $J = 2.4$)	43.1 (*d*)			
13	5.06 (*dd*, $J = 9.6$, 2.4)	73.5 (*d*)	12′	2.01 (br. *s*)	31.6 (*d*)
14	2.20–2.28 (*m*)	49.9 (*d*)	13′	1.50–1.56 (*m*, H_α), 1.76–1.82 (*m*, H_β)	30.6 (*t*)
15	1.99–2.02 (*m*, H_α), 2.09 (br. *s*, H_β)	33.9 (*t*)	14′	1.57–1.63 (*m*)	44.2 (*d*)
			15′	5.48 (*s*)	129.7 (*d*)
16		144.7 (*s*)	16′		147.3 (*s*)
17	4.73 (*s*), 4.94 (*s*)	108.8 (*t*)	17′	3.78, 3.94 (*ABq*, $J = 12.0$)	69.9 (*t*)
18	0.99 (*s*)	29.4 (*q*)	18′	1.04 (*s*)	18.9 (*q*)
19	2.52, 2.93 (*ABq*, $J = 12.4$)	63.2 (*t*)	19′	7.41 (*s*)	169.5 (*d*)
20	3.54 (*s*)	68.5 (*d*)	20′	3.54 (*s*)	80.2 (*d*)
1″		175.7 (*s*)	AcO	1.98 (*s*)	170.3 (*s*), 21.3 (*q*)
2″	2.20–2.28 (*m*)	41.3 (*d*)			
3″	1.59–1.65 (*m*)	26.2 (*t*)			
4″	0.89 (*t*, $J = 7.2$)	11.5 (*q*)			
5″	1.17 (*d*, $J = 6.8$)	16.5 (*q*)			

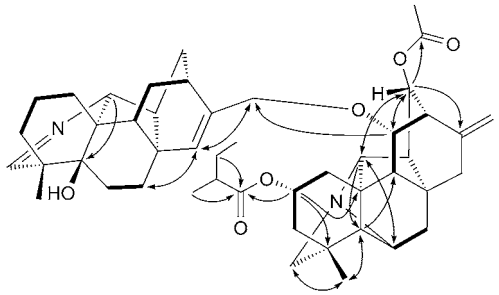


Fig. 5. *^{1}H,^{1}H-COSY* (—) *and key HMB* (H → C) *correlations of trichocarpinine B* (**3**)

in the ^{1}H-NMR spectrum disappeared, and two *doublet*s at δ(H) 1.16 ($J = 6.8$) and 1.20 ($J = 7.2$, each 3 H)) emerged in the ^{1}H-NMR spectrum of **4**, indicating the 2-methylbutanoyloxy group in **2** was replaced by an isobutanoyloxy group in **4**, which could be confirmed by the correlation H–C(2)/C(2″) in HMBC of **4** (*Fig. 6*). Thus the trichocarpinine C (**4**) was deduced as depicted.

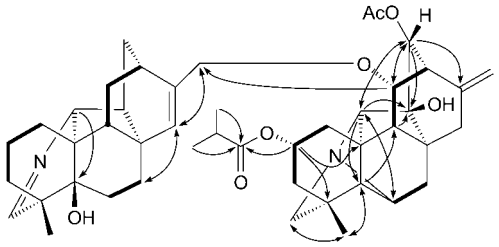


Fig. 6. *^{1}H,^{1}H-COSY* (—) *and key HMB* (H → C) *correlations of trichocarpinines C* (**4**)

Experimental Part

General. TLC and column chromatography (CC): silica gel *G* and *H*, resp. (SiO_2; *Qingdao Haiyang Chemical Group Co.*, P. R. China), The spots on TLC were visualized by using the modified *Dragendorff*'s reagent. Optical rotations: *Perkin-Elmer 241* polarimeter. IR Spectra: *Nicolet FT-IR 200 SXV* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Varian Unity INOVA 400/45* NMR spectrometer; in $CDCl_3$; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS and HR-ESI-MS: *VG Auto Spec 3000* or a *Finnigan-MAT 90* instrument; in *m*/*z*.

Plant Material: The whole herbs of *A. tanguticum* var. *trichocarpum* were collected in Naqu County, Tibet, P. R. China, in August 2009. The plant was identified by Assoc. Prof. *S. Ge Sang* 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 (5 kg) were percolated with 0.1M HCl (100 l). The acidic soln. was alkalized with 10% aq. NH_4OH (500 ml) to pH > 10 and then was extracted with AcOEt (25 l × 3). The combined extracts were concentrated to yield the total crude alkaloids (42 g), which were subjected to CC (SiO_2 *H* (500 g); $CHCl_3$/MeOH/Et_2NH 100:0:1–0:100:1) to give 5 fractions: *Fr. I* (6.0 g), *Fr. II* (6.5 g), *Fr. III* (2.2 g), *Fr. IV* (1.8 g), and *Fr. V* (16.5 g).

Fr. I afforded three parts, *A* (0.51 g), *B* (0.35 g), and *C* (1.65 g), by CC ($CHCl_3$/MeOH/Et_2NH 100:1:1). The part *A* was separated by CC (petroleum ether (PE)/acetone 10:1) to yield dehydroheteratisine. Part *B* was subjected to CC ($CHCl_3$/MeOH/Et_2NH (100:1:1) to afford 6-benzoylheteratisine (98 mg). CC of Part *C* ($CHCl_3$/MeOH/Et_2NH 150:1:1–100:1:1) gave *Frs. C-1*

Table 4. *^{1}H- and ^{13}C-NMR* (400 MHz and 100 MHz, resp.) *Data for* **4**. Atom numbering as indicated in *Formulae*. δ in ppm, J in Hz.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
1	1.69–1.78 (*m*, H_α), 3.05 (br. *d*, $J=16.0$, H_β)	31.5 (*t*)	1′	1.53–156 (*m*, H_α), 1.68–1.77 (*m*, H_β)	30.8 (*t*)
2	5.17 (br. *s*, $w_{1/2}=10.2$)	69.7 (*d*)	2′	1.58–1.62 (*m*)	20.5 (*t*)
3	1.53–1.56 (*m*, H_α), 1.79–1.83 (*m*, H_β)	28.3 (*t*)	3′	1.25–1.29 (*m*)	30.5 (*t*)
4		37.4 (*s*)	4′		44.7 (*s*)
5	1.58–1.62 (*m*)	59.8 (*d*)	5′		72.4 (*s*)
6	3.13 (*s*)	63.0 (*d*)	6′	1.59–1.64 (*m*, H_α), 1.84–1.88 (*m*, H_β)	36.7 (*t*)
7	1.28–1.32 (*m*)	31.8 (*t*)	7′	1.41–1.49 (*m*, H_α), 1.50–1.57 (*m*, H_β)	27.5 (*t*)
8		44.9 (*s*)			
9	2.03–2.05 (*m*)	52.5 (*d*)	8′		43.6 (*s*)
10		46.0 (*s*)	9′	1.62–1.66 (*m*)	46.9 (*d*)
11	3.77 (*s*)	81.0 (*d*)	10′		45.2 (*s*)
12	2.87 (*s*)	44.6 (*d*)	11′	1.35–1.38 (*m*, H_α), 1.81–1.83 (*m*, H_β)	43.1 (*t*)
13	4.96 (*s*)	80.8 (*d*)			
14		78.6 (*s*)	12′	2.12 (br. *s*)	31.6 (*d*)
15	2.03–2.05 (*m*, H_α), 2.12 (br. *s*, H_β)	30.9 (*t*)	13′	1.53–1.56 (*m*, H_α), 1.77–1.81 (*m*, H_β)	30.9 (*t*)
16		143.8 (*s*)	14′	1.60–1.65 (*m*)	44.2 (*d*)
17	4.75 (*s*), 4.96 (*s*)	109.2 (*t*)	15′	5.46 (*s*)	129.9 (*d*)
18	1.00 (*s*)	29.5 (*q*)	16′		147.2 (*s*)
19	2.54, 2.89 (*ABq*, $J=12.4$)	62.6 (*t*)	17′	3.77, 3.93 (*ABq*, $J=12.0$)	69.9 (*t*)
20	3.46 (*s*)	69.5 (*d*)	18′	1.04 (*s*)	18.9 (*q*)
1″		176.0 (*s*)	19′	7.40 (*s*)	169.5 (*d*)
2″	2.49 (*q*, $J=6.8$)	34.2 (*d*)	20′	3.53 (*s*)	80.2 (*d*)
3″	1.15 (*d*, $J=6.8$)	18.6 (*q*)	AcO	1.96 (*s*)	169.9 (*s*), 21.2 (*q*)
5″	1.19 (*d*, $J=6.8$)	19.3 (*q*)			

(800 mg), *C-2* (320 mg), and *C-3* (1.05 g). Further separation of fraction *C-3* by CC (PE/acetone/Et_2NH 5:1:0.05) yielded **2** (40 mg) and **4** (58 mg). Separation of *Fr. II* by CC (PE/AcOEt 1:1–1:3) afforded dehydroheteratisine (85 mg), tongolinine (40 mg), heteratisine (1.10 g), and subfraction *Fr. II-1* (1.21 g). Compound **3** (50 mg) was obtained from *Fr. II-1* by CC (PE/acetone/Et_2NH 5:1:0.05). *Fr. III* was separated by CC to yield *Frs. III-1* (1.44 g) and *III-2* (1.02 g). Further purification of *Frs. III-1* and *III-2* by CC (PE/acetone/Et_2NH 5:1:0.05) provided trichocarpine A (45 mg), heterophyllidine (96 mg), and **1** (78 mg). *Fr. IV* was submitted to CC (PE/acetone 5:1) to yielded heteratisine (38 mg) and *Frs. IV-1* (210 mg) and *IV-2* (400 mg). Further CC of *Fr. IV-2* ($CHCl_3$/MeOH 200:1) afforded 6-acetylheteratisine (50 mg), 6-benzoylheteratisine (13 mg). *Frs. V-1* (2.0 g) and *V-2* (5.5 g) were obtained from *Fr. V* by CC (PE/AcOEt 2:1–0:1). Guan-fu base Z (55 mg) was obtained from *Fr. V-2* by CC (PE/acetone 7:1–2:1).

Trichocarpidine (=*(1*S*,2*R*,3*S*,6*S*,9*S*,10*S*,11*R*,14*R*,17*S*,18*R*,19*S*)-12-Ethyl-17-hydroxy-14-methyl-9-{[(1*R*,5*R*,8*R*,9*S*,11*R*,14*S*,16*S*,17*R*,18*R*)-5-methyl-7-azaheptacyclo[9.6.2.$0^{1,8}$.$0^{5,17}$.$0^{7,16}$.$0^{9,14}$.$0^{14,18}$]nonadec-12-en-12-yl]methoxy}-4-oxo-5-oxa-12-azahexacyclo[8.7.2.$1^{2,6}$.$0^{1,11}$.$0^{3,9}$.$0^{14,18}$]icos-19-yl Acetate*; **1**). White amorphous powder. $[\alpha]_D^{20}=+68.6$ ($c=0.86$, $CHCl_3$). IR (KBr): 3436, 2929, 1738, 1249. ^{1}H- (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz; $CDCl_3$): see *Table 1*. ESI-MS: 699.57 ($[M+H]^+$). HR-ESI-MS: 699.4350 ($[M+H]^+$, $C_{43}H_{59}N_2O_6^+$; calc. 699.4358).

Trichocarpinine A (= *(1S,3S,5R,8S,9S,10R,11R,14R,16S,17R,18S,19S)-10-(Acetyloxy)-9-hydroxy-19-{[(1S,5S,8R,9R,11R,14S,17R,18R)-17-hydroxy-5-methyl-7-azahexacyclo[9.6.2.0^{1,8}.0^{5,17}.0^{9,14}.0^{14,18}]nonadeca-6,12-dien-12-yl]methoxy}-5-methyl-12-methylidene-7-azaheptacyclo[9.6.2.0^{1,8}.0^{5,17}.0^{7,16}.0^{9,14}.0^{14,18}]nonadec-3-yl 2-Methylbutanoate*; **2**). White amorphous powder. $[\alpha]_D^{20} = +169.8$ ($c = 1.16$, $CHCl_3$). IR (KBr): 3421, 2932, 1728, 1238. ^{1}H- (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz. $CDCl_3$): see *Table 2*. ESI-MS: 767.44 ($[M+H]^+$). HR-ESI-MS: 767.4614 ($[M+H]^+$, $C_{47}H_{62}N_2O_7^+$; calc. 767.4628).

Trichocarpinine B (= *(1S,3S,5R,8S,9S,10R,11R,14R,16S,17R,18S,19S)-10-(Acetyloxy)-19-{[(1S,5S,8R,9R,11R,14S,17R,18R)-17-hydroxy-5-methyl-7-zahexacyclo[9.6.2.0^{1,8}.0^{5,17}.0^{9,14}.0^{14,18}]nonadeca-6,12-dien-12-yl]methoxy}-5-methyl-12-methylidene-7-azaheptacyclo[9.6.2.0^{1,8}.0^{5,17}.0^{7,16}.0^{9,14}.0^{14,18}]nonadec-3-yl 2-Methylbutanoate*; **3**). White amorphous powder. $[\alpha]_D^{20} = +164.1$ ($c = 1.2$, $CHCl_3$). IR (KBr): 3424, 2932, 1728, 1645, 1242. ^{1}H- (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): see *Table 3*. ESI-MS: 751.52 ($[M+H]^+$). HR-ESI-MS: 751.4689 ($[M+H]^+$, $C_{47}H_{63}N_2O_6^+$; calc. 751.4696).

Trichocarpinine C (= *(1S,3S,5R,8S,9S,10R,11R,14R,16S,17R,18S,19S)-10-(Acetyloxy)-9-hydroxy-19-{[(1S,5S,8R,9R,11R,14S,17R,18R)-17-hydroxy-5-methyl-7-zahexacyclo[9.6.2.0^{1,8}.0^{5,17}.0^{9,14}.0^{14,18}]nonadeca-6,12-dien-12-yl]methoxy}-5-methyl-12-methylidene-7-azaheptacyclo[9.6.2.0^{1,8}.0^{5,17}.0^{7,16}.0^{9,14}.0^{14,18}]nonadec-3-yl 2-Methylpropanoate*; **4**). White amorphous powder. $[\alpha]_D^{20} = +182.1$ ($c = 1.12$, $CHCl_3$). IR (KBr): 3421, 2932, 1727, 1240. ^{1}H- (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): see *Table 4*. ESI-MS: 753.36 ($[M+H]^+$). HR-ESI-MS: 753.4476 ($[M+H]^+$, $C_{46}H_{60}N_2O_7^+$; calc. 753.4491).

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