Diterpenoid Alkaloids from *Aconitum tanguticum*

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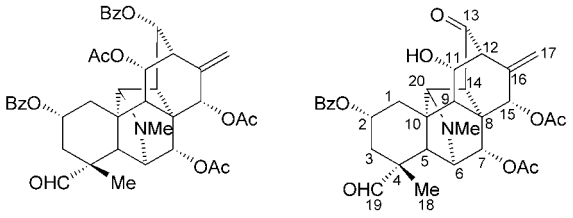
A new 19,21-secohetisan diterpenoid alkaloid, tangutisine B (**1**), was isolated from the aerial parts of *Aconitum tanguticum* (MAXIM.) STAPF., together with pacidine, 14-deacetylajadine. The structure of **1** was determined by an interpretation of 1D- and 2D-NMR together with X-ray crystallographic data. Bioactivities of the isolates were tested in the P-388, Bel-7402, Cox-2, and caspase-3 assays.

Introduction. – Plants in the family Ranuculaceae, genus *Aconitum* L., have been recognized to be rich in diterpenoid alkaloids that display analgesic, anti-inflammatory, and curane-like activities [1]. *Aconitum tanguticum* (MAXIM.) STAPF. is used in Tibetan folk medicine for the treatment of fever, pneumonia, inflammation, as well as flu [2]. Recently, we reported a novel 19,21-secohetisan diterpenoid alkaloid called tangutisine A (*Fig. 1*), in which the carbonyl group at C(13) is reduced to an (esterified) OH group [3]. Being interested in this type of compound, we further investigated the residue remaining from the EtOH extraction of the aerial parts of *A. tanguticum* to isolate another new 19,21-secohetisan diterpenoid alkaloid, namely tangutisine B (**1**; *Fig. 1*), together with the two known compounds pacidine [4] and 14-deacetylajadine [5], which were isolated for the first time from the title plant.



Tangutisine A Tangutisine B (**1**)

Fig. 1. *Structures of tangutisine A and tangutisine B* (**1**)

Results and Discussion. – Tangutisine B (**1**) was isolated as colorless needles. The molecular formula $C_{32}H_{35}NO_9$ was established by HR-ESI-MS ($[M+1]^+$ at *m/z* 578.2389; calc. 578.2390) and the structure was established as (2α,7α,11α,15α)-7,15-

bis(acetyloxy)-2-benzyloxy-11-hydroxy-13-oxo-21-methyl-19,21-secohetisan-19-al[1]) by 1D- and 2D-NMR spectroscopy and X-ray crystallography.

That **1** is a 19,21-secohetisan diterpenoid alkaloid can be recognized by the presence of an exocyclic CH_2 group (δ(H) 5.53, 5.38; δ(C) 136.2, 123.8), a tertiary Me group (δ(H) 1.05; δ(C) 26.2), a tertiary aldehyde function (δ(H) 9.50; δ(C) 198.6), a MeN group (δ(H) 2.40; δ(C) 33.7), an isolated keto group (δ(C) 205.3), two AcO groups (δ(H) 2.13, 2.07), and a BzO group (δ(H) 7.28–7.50) in the NMR spectrum. IR Maxima at 2939, 1700, and 1717 cm^{-1} due to the presence of the aldehyde and C=O groups were observed. The DEPT spectrum indicated two CH_2 groups at δ(C) 31.8 and 34.3 ppm. In the $^1H,^1H$-COSY plot, a C–CH_2–CH(OR)–CH_2–C fragment was observed (a CH proton at δ 5.55 (H–C(2)) exhibited vincinal coupling patterns with the two CH_2 groups (δ(H) 2.23, 1.95; 2.47, 1.53)). A quaternary Me group (δ(H) 1.05) showed correlations with δ 44.1 (C(4)), δ 198.6 (C(19)), and δ 58.3 (C(5)) in HMBC plot. Based on these spectral data, the aldehyde group and the quaternary Me group are located at C(4) (δ 44.1), and the other quaternary C-atom is attached to C(1) and was assigned as C(10) (δ 50.3). HMQC and HMBC Spectra established that the NMe group bridges C(6) (δ 66.1) and C(20) (δ 70.9). The $^1H,^1H$ correlations of H–C(5) with H–C(6) and H–C(6) with H–C(7) indicated another C–CH–CH–CH–C partial fragment in compound **1**. In the HMBC plot, correlations of H–C(9) (δ 2.76) with C(10), C(8), and C(11) established another cyclic bridging fragment (C(10)–C(9)–C(11)). These data together with the HMBC correlations of H–C(12) with C(13), C(16), and C(14), and of H–C(17) with C(12) and C(15) enabled us to establish the cyclic partial structure C(12)–C(13)–C(14)–C(8)–C(15)–C(16) of **1**. HMBC correlations of H–C(7) and H–C(15) with the C=O groups of the AcO units and of H–C(2) with the benzoate carboxy C-atom together with the IR maximum absorption at 3440 cm^{-1} established that the remaining O-atom is a free OH group located at C(11).

The relative configuration of **1** was determined by a 2D NOESY experiment and further confirmed by X-ray crystallographic data (*Fig. 2*). The absolute configuration of **1** is believed to be as shown in *Fig. 1* (with the Me group at C(4) in the β-orientation) on biogenetic grounds. NOE Correlations for Me–C(4) with H–C(6), H–C(6) with

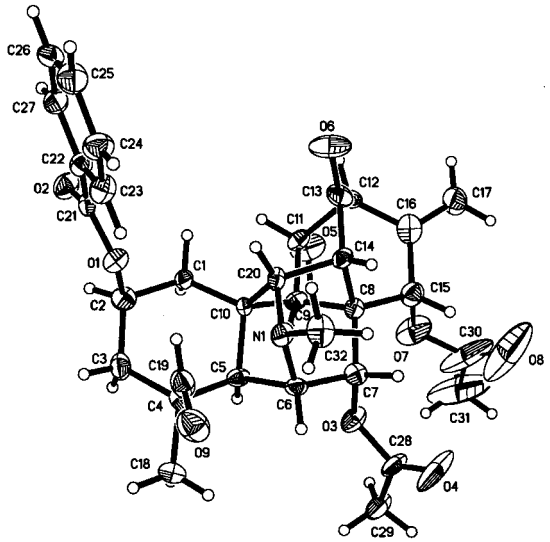


Fig. 2. *X-Ray crystal structure of* **1**

[1]) Trivial name. See *Exper. Part* for the systematic name.

H–C(7), and H–C(6) with H–C(15) and ^{1}H,^{1}H correlations for H–C(5) with H–C(9), H–C(9) with H–C(11), and H–C(11) with H–C(12) indicated that all of these H-atoms occupy β-positions.

Experimental Part

General and Plant Materials. See [3]. ESI-MS: *Applied Biosystems QSTAR Pulsar* instrument. X-Ray analysis was performed on a *Bruker SMART APEX CCD* instrument.

Extraction and Isolation. The residue (18.1 g) remaining after EtOH extraction of the aerial parts of *A. tanguticum* [3] was subjected to CC (silica gel, petroleum ether/Me_2CO/Et_3N 10:1:0.1 to 1:2:0.01). On the basis of TLC R_f values (detection with *Dragendorff* reagent), six crude fractions were pooled. 14-Deacetylajadine (1.21 g) was isolated from *Fr. 2* by recrystallization from $CHCl_3$/Me_2CO after repeated CC (silica gel). *Fr. 4* (1.4 g) was isolated by CC on silica gel (petroleum ether/Me_2CO/Et_3N 6:1:0.1) to yield pacidine (21 mg). *Fr. 5* and *6* were combined to provide **1**. CC Elutes were analyzed by TLC (petroleum ether/Me_2CO/Et_3N 2:1:0.05) R_f Values: **1**: 0.20, pacidine: 0.34, 14-deacetylajadine: 0.53.

Tangutisine B (= *(2*S*,4*R*,4a*S*,5*R*,7*S*,8*R*,10*R*,11*R*,11a*S*,11b*S*,13*R*)-8,13-Bis(acetyloxy)-2-(benzoyloxy)dodecahydro-11-hydroxy-4,6-dimethyl-9-methylene-12-oxo-8*H*-5,7a:7,10-dimethano-1*H*-indeno[2,1-c]isoindole-4-carboxaldehyde* = *(2*S*,3*S*,5*S*,7*R*,8*S*,9*R*,12*S*,14*R*,16*R*,17*R*,18*R*)-16,17-Bis(acetyloxy)-7-formyl-18-hydroxy-7,10-dimethyl-15-methylidene-13-oxo-10-azahexacyclo[7.7.1.1^{2,14}.0^{4,12}.0^{3,8}.0^{3,11}]octadec-5-yl Benzoate*; **1**). Colorless needles. M.p. 242–244°. $[\alpha]_D^{24} = -61.98$ ($c = 0.121$, $CHCl_3$). UV ($CHCl_3$): 241 (4.0), 253 (2.3). IR (KBr): 3440, 2939, 1744, 1717, 1700, 1651, 1276, 1253, 728. ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz): see the *Table*. EI-MS: 576 (1), 549 (33), 428 (44), 340 (8), 268 (9), 146 (27), 105 (100), 77 (29); HR-ESI-MS: 578.2389 ($C_{32}H_{36}NO_9$; calc. 578.2390).

Table. *^{1}H- and ^{13}C-NMR Data of Tangutisine B* (**1**). In $CDCl_3$; δ in ppm, J in Hz.

Position[a])	δ(H)	δ(C)	Position[a])	δ(H)	δ(C)
H_α–C(1)	2.23 (br. *d*, *J* = 14.5)	31.8	H_a–C(17)	5.53 (*s*)	123.8
H_β–C(1)	1.95 (*dd*, *J* = 3.6, 15.0)		H_b–C(17)	5.38 (*s*)	
H–(2)	5.55 (*m*)	68.1	Me–C(18)	1.05 (*s*)	26.2
H_α–C(3)	2.47 (br. *s*)	34.3	CH(19)=O	9.50 (*s*)	198.6
H_β–C(3)	1.53 (*dd*, *J* = 3.2, 15.5)		H–C(20)	3.38 (br. *s*)	70.9[c])
C(4)	–	44.1	MeN	2.40 (*s*)	33.7
H–C(5)	2.08 (*s*)	58.3	BzO:		
H–C(6)	3.18 (*d*, *J* = 3.3)	61.1	C(1′)	–	130.1
H–C(7)	5.30 (*d*, *J* = 3.5)	69.6	H–C(2′), H–C(6′)	7.89 (*dd*, *J* = 1.1, 7.2)	129.5
C(8)	–	50.3[b])	H–C(3′), H–C(5′)	7.44 (overlap)	128.6
H–C(9)	2.76 (*d*, *J* = 2.2)	52.5	H–C(4′)	7.57 (*m*)	133.7
C(10)	–	50.3[b])	C=O	–	166.2
H–C(11)	4.14 (*d*, *J* = 4.5)	63.6	AcO–C(7):		
H–C(12)	3.34 (*d*, *J* = 4.5)	60.3	Me*C*O	–	169.5
H–C(13)	–	205.3	*Me*CO	2.13 (*s*)	21.5
H–C(14)	2.51 (br. *d*, *J* = 2.3)	57.4	AcO–C(15):		
H–C(15)	5.94 (*s*)	71.0[c])	Me*C*O	–	169.4
C(16)	–	136.2	*Me*CO	2.07 (*s*)	21.0

[a]) Trivial numbering. [b]) ^{13}C Signals of C(8) and C(10) overlapping. [c]) Assignments interchangeable.

X-Ray Crystal Data of **1**. A colorless crystal obtained from acetone (0.448 × 0.128 × 0.120 mm) was selected for X-ray analysis. The crystallographic data were collected on a CCD diffractometer with graphite-monochromated Mo/K_α radiation. Structure analysis was made with the SHELXL-97 program on a PC. The compound crystallized in the space group $P4_3$, a = 9.414(4) Å, b = 9.414(4) Å, c = 32.048(17) Å, tetragonal, β = 90°, V = 2840(2) Å^3, Z = 4, $D_{calc.}$ = 1.351 g/cm^3, λ = 0.71073 Å, μ(Mo K_α) = 0.099 mm^{-1}, F (000) = 1224, and

$T = 293$ (2) K. A total of 14854 reflections were collected in the range $2.16 \leq \theta \leq 25.49°$, of which 5211 unique reflections with $I > 2\sigma(I)$ were used for the analysis. The structure was solved by direct methods and refined by full-matrix least-squares on the F^2 values. Non-H-atoms were refined anisotropically. H-Atoms were fixed at calculated positions and refined according to a riding mode. The final indices were $R = 0.0842$, $R_w = 0.1906$ with a goodness-of-fit = 0.935. Scattering factors were taken from [6]. Crystallographic data for compound **1** have been deposited with the *Cambridge Crystallographic Data Centre* (deposition number CCDC-223380). Copies of the data can be obtained, free of charge, on application to the director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +044-(0)1223-330633); e-mail: deposit@ccdc.cam.ac.uk)

Bioassay. Tangutisin A and **1** were found to be inactive towards P-388 [7] and Bel-7402 [8] on the basis of inhibition ratios $< 85\%$ at several concentrations when evaluated in bioactivity assays. All four isolates – **1**, tangutisin A, pacidine, and 14-deacetylajadine – were pharmacologically inactive in bioassay screens toward Cox-2 [9] and caspase-3 [10].

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