TANGUTISINE, A NEW DITERPENOID ALKALOID FROM ACONITUM TANGUTICUM (MAXIM.) STAPF, W. T. WANG

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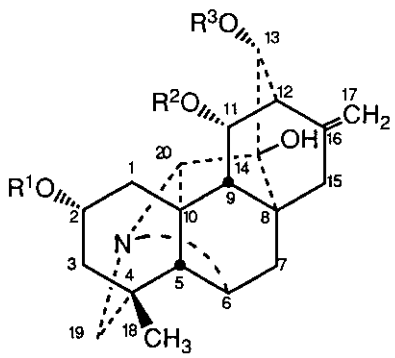
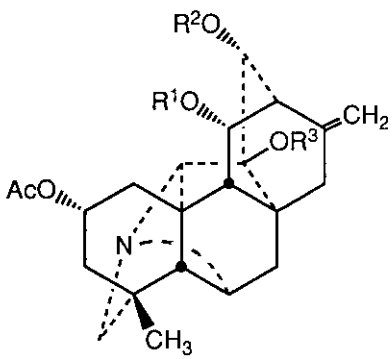
Abstract – Tangutisine, isolated from Aconitum tanguticum, has been assigned the structure (**1**) on the basis of homonuclear ^{1}H COSY, HETCOR, two dimensional nOe, ^{1}H – ^{13}C long range correlations (FLOCK) and selective INEPT nmr techniques.

Aconitum tanguticum (Maxim.) Stapf, W. T. Wang had previously been reported to contain the known diterpenoid alkaloids atisine, heteratisine, benzoylheteratisine, and a new alkaloid, tanwusine, the structure of which was not established.[1] In this paper we describe the isolation and structure determination of tangutisine (**1**), a diterpene alkaloid, of the hetisane-type.





1 R^1; R^2; R^3 = H

3 R^1; R^3; = Ac; R^2 = H

6 R^1 = COCH $(CH_3)_2$; R^2; R^3 = H

2 R^1; R^2; R^3 = Ac

4 R^1; R^2 = Ac; R^3 = H

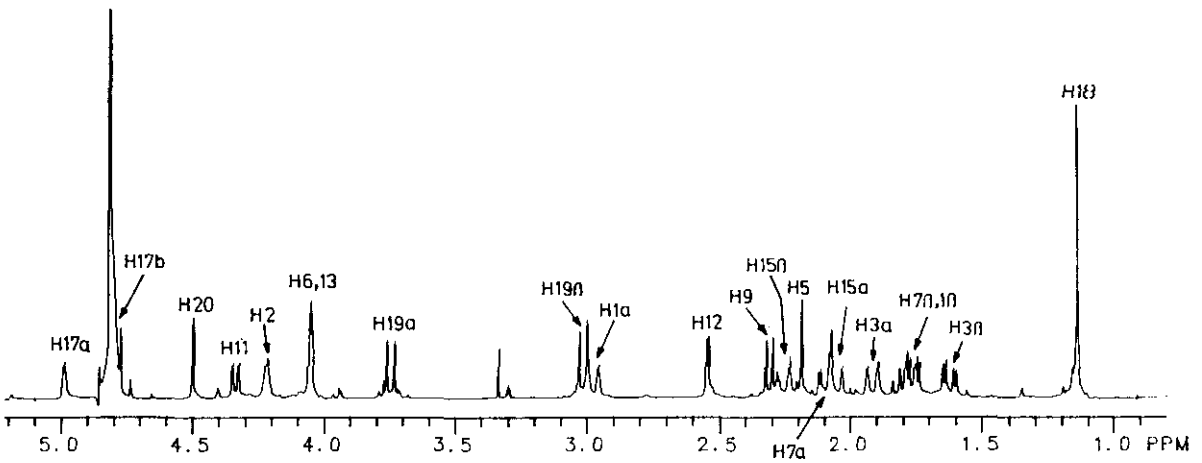
5 R^1; R^2; R^3 = H

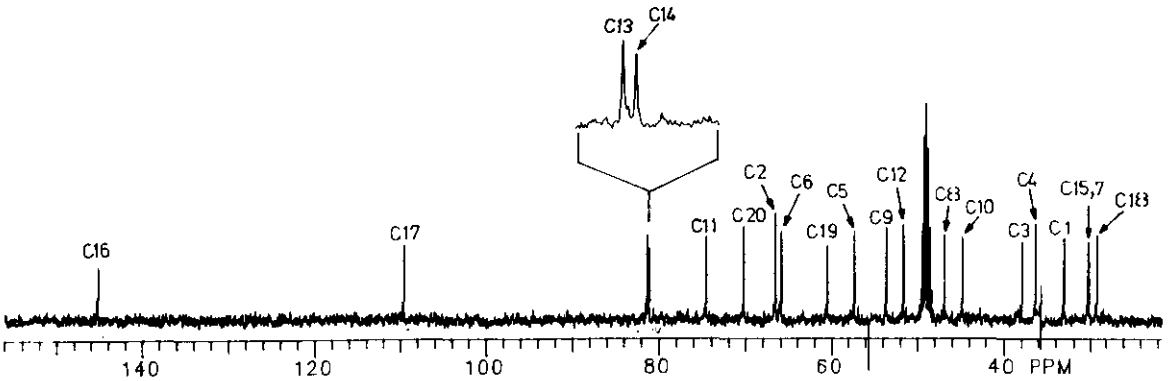
The molecular formula, $C_{20}H_{27}NO_4$, originally derived from the low resolution mass spectrum (EIMS, m/z 345, M^+), ^{1}H and ^{13}C nmr spectra of the hydrochloride salt (in D_2O with three drops of CD_3OD, Figure 1 and Table 1), was confirmed by HRMS (EI, 40 eV: m/z 345.1939, M^+; calcd for $C_{20}H_{27}NO_4$, 345.1940). A DEPT experiment revealed that the twenty carbons included five nonprotonated carbons (δ 145.2, 81.4, 46.9, 44.9, 36.4), eight methines (δ 81.8, 74.6, 70.3, 66.7, 66.0, 57.4, 53.7, 51.7),

six methylenes (δ 109.6, 60.6, 38.1, 33.2, 30.4, 30.3), and one methyl group (δ 29.2). The two most downfield carbon signals (δ 145.2, s, and 109.6, t) and the two broad singlets in the ^{1}H nmr spectrum (δ 4.78 and 4.99) which correlated with the olefinic methylene carbon in the HETCOR spectrum clearly indicated that tangutisine possessed an exocyclic methylene group, typical of the hetisane-type alkaloids without a hydroxyl group at C-15. Hetisane-type alkaloids with a hydroxyl group at C-15 have the C-16 carbon resonance between δ 154–156.[2,3] The absence of a methoxyl group, or an *N*-methyl or *N*-ethyl group, which required that all twenty carbons were part of the parent alkaloidal skeleton, further supported the conclusion that tangutisine was a hetisane-type alkaloid. All the four oxygenated carbons were due to hydroxyl groups, since the eight units of unsaturation required by the molecular formula were accounted for by the hetisane skeleton with the exocyclic double bond; i.e., there were no cyclic ether units in **1**. Acetylation of tangutisine with Ac_2O-TsOH gave the tetraacetate (**2**).

Of the four hydroxyl groups, one was a tertiary alcohol, and therefore located at C-5, C-6, C-9, C-12, C-14 or C-20 of the hetisane skeleton, to form the quaternary carbon appearing at δ 81.4. Location of a hydroxyl group at C-6 (carbinolamine) was discounted as this would have shifted the C-6 resonance downfield to 97–101 ppm (geyerinine: δ 96.9, geyeridine: δ 100.3, and geyerine: δ 99.2;[4] tatsirine: δ 98.0[5]). Location of a hydroxyl group at C-5 or C-20 was considered unlikely (no other alkaloids have been isolated with this substitution except anopterine, which has a different ring system[6]). A hydroxyl group at C-12 should also have produced a downfield shift on C-16 to δ 154–156, analogous to the β-effect produced by hydroxylation of C-15. This was not observed, and a C-12 hydroxyl group was considered unlikely. The C-10 chemical shift in alkaloids bearing a hydroxyl group at C-9 appears around δ 51–55 (sadosine: δ 51.4,[7] and hypognavine: δ 54.9[8]), 5 to 8 ppm downfield compared to the hetisane-type alkaloids not bearing a hydroxyl group at C-9 (Guan-fu base Z: δ 46.3,[9] tatsirine: δ 45.9[5]). Hence, the hydroxyl group most likely is located at C-14 rather than C-9 since no singlet carbon appeared in this region.

The remaining three hydroxyl groups were all secondary alcohols as concluded from the DEPT experiment. A hydroxyl group at C-19 was excluded due to the lack of a carbinolamine carbon resonance (δ 95–100 region). One of three hydroxyl groups should be located at C-1, C-2 or C-3 because no triplet carbon signal appeared at around δ 19.8 where the C-2 resonance would appear if the A-ring was not oxygenated. A hydroxyl group at C-1 was considered unlikely because such substitution also induces a downfield shift in the C-10 quaternary carbon (β-effect as in hanamisine: δ 52.2;[10] hypognavine: δ 54.9 and hypognavinol: δ 55.5[2,8]), and no quaternary carbon signal appeared between δ 46.9 and 81.4. Similar to tatsirine, C-2 was thought to bear a hydroxyl group.[5] The other two hydroxyl groups had to be located on C-3, C-7, C-11 or C-13. A comparison of the chemical shifts of all methine carbinol carbons in hetisane-type alkaloids indicated that only a C-13 bearing a hydroxyl group with an adjacent hydroxyl group on C-14 would appear around 80 ppm (Guan-fu base Z:[9] C-13, δ 80.0, d, C-14, δ 80.2, s), otherwise a hydroxylated C-13 would appear around δ 75.0 (tatsirine:[5] C-13 δ 75.4). As there were two signals around this region, δ 81.8 (d) and δ 81.4 (s), C-13 was thought to bear a hydroxyl group. The location of the fourth hydroxyl group could not be deduced from the ^{13}C nmr data. The candidate structure **1** for tangutisine was therefore a





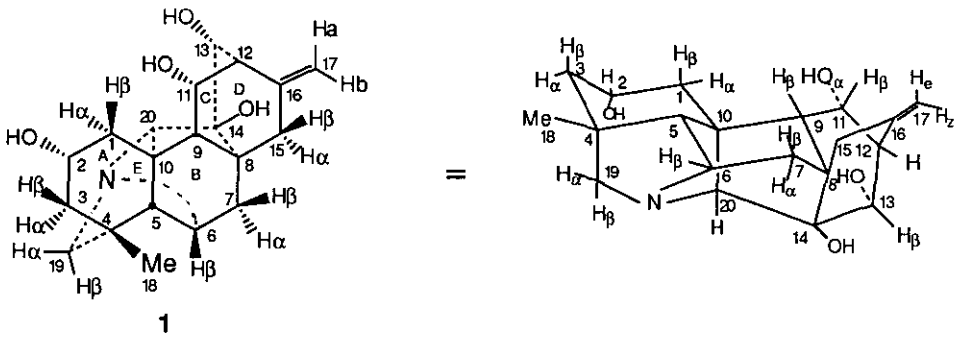


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Figure 1. ^{1}H and ^{13}C nmr spectra of tangutisine **1** in D_2O (with 3 drops of CD_3OD). Labelled peaks indicate the complete proton and carbon assignments as listed in Table 1. For the convenience of depiction in the diagram **1**, H-19α and H-19β indicate the pseudo-axial and pseudo-equatorial protons, respectively, in the chair conformation of the E ring formed by C-4, C-5, C-10, C-20, N and C-19. Also Hα and Hβ in the ring C are designated for the pseudo-axial and pseudo-equatorial protons of the twist boat conformation of the ring formed by C-8, C-9, C-11, C-12, C-13 and C-14.

Table 1. ^{13}C and ^{1}H Chemical Shifts Assignments of Tangutisine **1**[a]

Carbon	d (ppm)	Proton	d (ppm)	J (Hz)
1	33.2 (t)	1β	1.76	dd, $J_{1\beta,1\alpha}$=15.4, $J_{1\beta,2\beta}$=4.1
		1α	2.97	br d, $J_{1\alpha,1\beta}$=15.4, $J_{1\alpha,3\alpha}$=1.7, $J_{1\alpha,2}$=1.7
2	66.7 (d)	2β	4.21	br s, $W_{1/2}$=10.4
3	38.1 (t)	3β	1.62	dd, $J_{3\beta,3\alpha}$=15.4, $J_{3\beta,2\beta}$=4.3
		3α	1.92	br d, $J_{3\alpha,3\beta}$=15.4, $J_{3\alpha,1\alpha}$=1.7, $J_{3\alpha,2}$=2.1
4	36.4 (s)			
5	57.4 (d)	5	2.19	s, $W_{1/2}$=4.0
6	66.0 (d)	6	4.05	br s, $W_{1/2}$=6.8, overlapped with H-13.
7	30.3 (t)	7β	1.77	br d, $J_{7\beta,7\alpha}$=15.3
		7α	2.10	dd, $J_{7\beta,7\alpha}$=15.3, $J_{7\alpha,6}$=3.4
8	44.9 (s)			
9	53.7 (d)	9	2.31	d, $J_{9,11\beta}$=8.8
10	46.9 (s)			
11	74.6 (d)	11β	4.33	d, $J_{11\beta,9}$=8.8, $J_{11\beta,13\beta}$=1.8, $J_{11\beta,12}$<1.0
12	51.7(d)	12	2.55	d, $J_{12,13\beta}$=3.0, $J_{12,11\beta}$<1.0
13	81.8(d)	13β	4.05	br s, $W_{1/2}$=6.8, overlapped with H-6.
14	81.4(s)			
15	30.4(t)	15β	2.26	AB, J_{gem}=18.1
		15α	2.06	AB, J_{gem}=18.1
16	145.2(s)			
17	109.6(t)	17a	4.99	br s, $W_{1/2}$=7.0
		17b	4.78	br s, under the solvent peak.
18	29.2(q)	18	1.16	s
19	60.6(t)	19β	3.01	d, J_{gem}=11.6
		19α	3.74	d, J_{gem}=11.6
20	70.3(d)	20	4.50	s, $W_{1/2}$=3.9

[a] Spectra were run in D_2O with 3 drops CD_3OD.

hetisane-type diterpenoid alkaloid with hydroxyl groups at C-2, C-13 and C-14, and a fourth hydroxyl group at C-3, C-7 or C-11. The correct structure **1** was established by one and two dimensional nmr techniques.

A broad signal at δ 4.21 coupled to four upfield protons at δ 2.97, 1.92, 1.76, and 1.62 in the COSY spectrum. The HETCOR experiment revealed that among the four protons, the two protons at δ 1.76 and 2.97 were nonequivalent methylene gem-partners, as were the two protons at δ 1.62 and 1.92. Therefore, this carbinol proton (δ 4.21) had to be assigned to H-2 since this was the only position at which a proton could have scalar coupling with two adjacent methylene groups. Thus, the A-ring bears only one hydroxyl group at C-2, and the possibility of locating a second A-ring hydroxyl group at C-3 was excluded. The appearance of H-2 as a broad singlet ($W_{1/2}$ = 10.4 Hz) required that H-2 must be β-oriented. The coupling constants between H-2 and H-1β, and H-2 and H-3β were 4.1 and 4.3 Hz, respectively, as determined by decoupling experiments, typical axial-equatorial coupling constants. (The C-1 and C-3 methylene protons were distinguished as discussed below.) Therefore, the α-orientation of the hydroxyl group at C-2 was confirmed.

A nonequivalent methylene gem-pair at δ 1.77 and 2.10, correlated with the carbon signal at δ 30.3 in the HETCOR spectrum, and showed scalar coupling to an overlapped signal at δ 4.05 in the COSY spectrum. The latter signal integrated to two protons, one of which was assigned to H-6 (assigned

by nOe's with H-18, H-19β and H-5, as discussed below) correlating with a carbon at δ 66.0 in the HETCOR spectrum. The second signal was assigned to H-13 as discussed below. Since it was not possible for H-13 to have vicinal scalar coupling with a methylene group, the two protons at δ 1.77 and 2.10 must have coupled to H-6 (δ 4.05). Thus, the δ 1.77 and 2.10 resonances belonged to the C-7 methylene protons. This spin system indicated that C-7 is not oxygenated. Moreover, W-coupling was observed between H-6 and H-20 (^{1}H: δ 4.50, ^{13}C: δ 70.3) in a long range COSY (LRCOSY) experiment (Figure 2, delay = 0.2 s) and enabled the assignment of the C-20 nitrogenated methine group.

Allylic coupling from both exocyclic methylene protons (δ 4.99 and 4.78) to both nonequivalent C-15 methylene protons (δ 2.26 and 2.06) proved that C-15 was not substituted by a hydroxyl group, as suggested by the chemical shift rationale based on the shift of C-16. Therefore, the remaining three hydroxyl groups must be located among C-9, C-11, C-12, C-13 or C-14.

There were only four proton resonances involved in the C-9 through C-14 subunit: signals "A" at δ 2.31 (d, 8.8 Hz), "B" at δ 4.33 (d, 8.8 Hz), "C" at δ 2.55 (d, 3.0 Hz) and "D" at δ 4.05 (br s, overlapped with H-6), all shown to be methines by the DEPT and HETCOR spectra. Proton "A" showed a strong vicinal scalar coupling with proton "B", as did proton "C" with proton "D" in the COSY spectrum. Weak coupling between the two carbinol carbons ("B" and "D") was also observed in the COSY spectrum though coupling between "B" and "C" was not observed. This weak coupling between "B" and "D" could have arisen from a long range coupling (four bonds) if the connection was "A-B-C-D" or "B-A-D-C", or from very small vicinal coupling due to a near 90° dihedral angle if they were connected by "A-B-D-C". In the LRCOSY experiment, using a delay of 0.2 s, the intensity of the scalar coupling between protons "B" and "D" was enhanced, while two new cross peaks between protons "B" and "C", and protons "A" and "C" appeared. The existence of long range coupling between "A" and "C" suggested that the weak coupling between "B" and "D" was due to W-type coupling, while the weak coupling between "B" and "C" was due to a dihedral angle near 90°. If "B" and "D" had a vicinal relationship, the coupling from "A" to"C" (five bonds) would have been difficult to see in the LRCOSY spectrum, therefore the connection "A-B-D-C" was ruled out. Similarly, the observation of the weak scalar interaction between protons "B" and "C" excluded the connection "B-A-D-C". (Furthermore, no coupling was observed between "A" and "D"). For all spin coupling patterns of these four methine protons, the only possible connection was "A-B-C-D", and a hydroxyl group at C-12 was discounted since four contiguous methines were required.

Two possible four-spin systems exist to explain this pattern, however: H-9-H-11-H-12-H-13 and H-11-H-12-H-13-H-14. Since these two possibilities could not be distinguished by the pattern of scalar couplings, it was necessary to find the spatial relationship with other protons. The resonance of H-5 appeared as a sharp singlet (^{1}H nmr: δ 2.19, ^{13}C nmr: δ 57.4), a characteristic signal in hetisine-type diterpenoid alkaloids. As seen in the NOESY spectrum (Table 2), a strong nOe between H-5 and signal "A" was observed (mixing time = 0.6 s), thus proton "A" must be oriented on the same molecular face as H-5, in a 1,3-diaxial relationship, and was assigned to H-9. This assignment was confirmed by the nOe from H-9 to a proton previously assigned to H-1, and which therefore must be

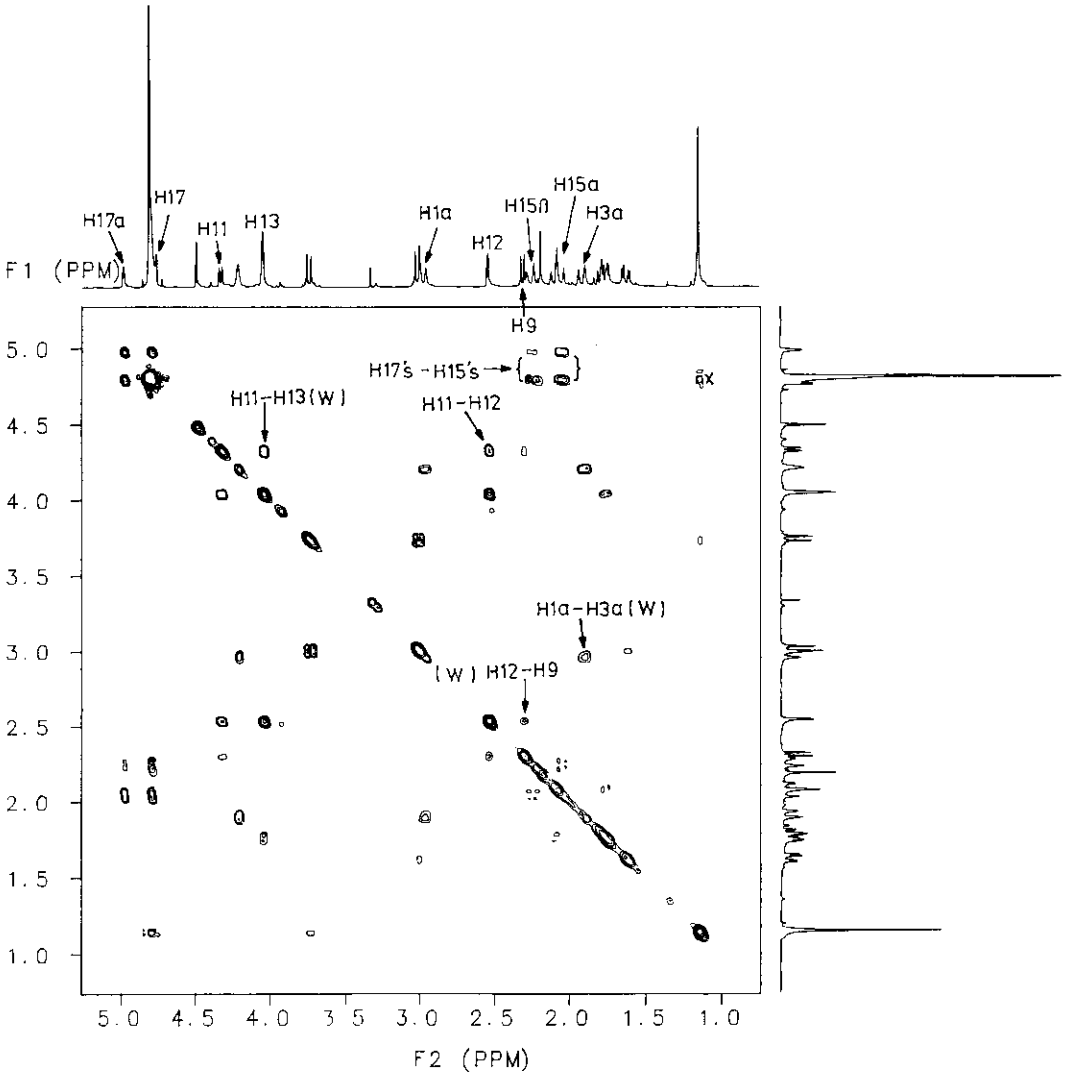

Figure 2. Long range COSY of **1** using a delay of 0.2 s in D_2O (with 3 drops of CD_3OD). The capital letter 'W' indicates the important W-couplings. Other labelled peaks are the vicinal coupling between H-11 and H-12 which was not observed in COSY, and the characteristic long range couplings between H-17's and H-15's.

H-1β. Consequently, the signals of protons "B", "C" and "D" were assigned to H-11, H-12 and H-13, respectively, and C-11, C-13 and C-14 bear the hydroxyl groups as predicted by the chemical shift rationale.

The stereochemistry of the C-11 and C-13 hydroxyl groups was easily determined by the analysis of observed nOe's and vicinal coupling constants. The H-9 signal appeared as a doublet with a coupling constant of 8.8 Hz. This large scalar coupling must result from a near 0° dihedral angle between H-9 and H-11, which is only possible if the 11-OH group possesses an α-orientation (a dihe-

dral angle of 180° between H-9 and an H-11 proton is impossible in the hetisane skeleton). The β-orientation of H-11 was confirmed by the observation of a dipolar interaction (nOe) between H-11 and H-15β (see the discussion later in this manuscript for this assignment) at 35°C (Table 2). One strong piece of evidence to establish the stereochemistry of 13-OH group was the observation of W-coupling between H-11 and H-13 in the COSY and LRCOSY spectra. The H-13 proton has to be β-orientated to form the W-shape with H-11. The dipolar interaction observed in the NOESY spectrum

Table 2. ^{1}H-^{1}H Correlations and nOe's of **1**. (In D_2O with 3 drops CD_3OD)

Proton	nOe's (NOESY)	Correlations (COSY)
H-1β	H-1α, H-2β, H-5, H-9	H-1α, H-2β
H-1α	H-1β, H-2β, H-20	H-2β, H-1β, H-3α*
H-2β	H-1α, H-1β, H-3α, H-3β	H-1α, H-1β, H-3α, H-3β
H-3β	H-3α, H-2β, H-18, H-5	H-3α, H-2β
H-3α	H-3β, H-2β, H-18, H-19α	H-3β, H-2β, H-1α*
H-5	H-6, H-7β, H-9, H-18, H-1β, H-3β	
H-6	H-5, H-19β, H-7α, H-7β, H-18	H-7α, H-7β, H-20*
H-7β	H-7α, H-6, H-9, H-5, H-15β#	H-7α, H-6
H-7α	H-7β, H-6	H-7β, H-6
H-9	H-5, H-7β, H-1β, H-11β	H-11β
H-11β	H-9, H-12, H-15β#	H-9, H-13β*
H-12	H-17α, H-11β, H-13β	H-13β
H-13β	H-12, H-17a	H-12, H-11β*
H-15β	H-15α, H-7β#, H-11β#, H-17b#	H-15α, H-17a,b**
H-15α	H-15β, H-17β#	H-15β, H-17a,b**
H-17β	H-17a, H-15α#, H-15β#	H-17a, H-15α**, H-15β**
H-18	H-3β, H-3α, H-5, H-19β, H-6	
H-19β	H-19α, H-18, H-6	H-19α
H-19α	H-19β, H-3α, H-20	H-19β
H-20	H-19α, H-1α	H-6*

\# nOe's that only showed up in NOESY at 35°C.
* W-couplings.
** Long range couplings.

between H-13 and H-17a confirmed this stereochemical assignment. Thus, the structure of tangutisine was established as **1**.

The two vinyl protons at δ 4.99 and 4.78 showed significant long range scalar couplings to an isolated AB system at δ 2.26 and 2.06 (J_{AB} = 18.1 Hz) in the COSY spectrum. As these correlations were enhanced in the LRCOSY experiment, these characteristic spin systems were the H-17's and H-15's with significant allylic coupling. The nOe's from both H-12 and H-13 to the more downfield vinyl proton at δ 4.99 shown in the NOESY spectrum enabled us to distinguish the two methylene protons at C-17. This downfield resonance was therefore assigned to H-17a. The NOESY spectrum of tangutisine **1** also revealed that H-5 had dipolar interactions with five protons. One of them had been assigned to H-9 (δ 2.31) as discussed previously. The nOe's between H-5 and H-18 (the only methyl group in the molecule) with a methine proton at δ 4.05 indicated that this latter resonance is H-6. The methylene pair at C-3 (δ 1.62, 1.92) was distinguished from methylene protons at C-1 (δ 1.76, 2.97) because the former showed nOe's with H-18. The observation of nOe's from H-5 to the

upfield signal of C-3 methylene pair enabled the distinction of H-3β (δ 1.62) and H-3α (δ 1.92) to be made. The two geminal protons at C-1 were distinguished by the observed nOe between H-20 and the more downfield signal (δ 2.97). The latter was assigned to H-1α since it was spatially close to H-20. The strong W-coupling between H-1α and H-3α observed in the COSY and LRCOSY spectra confirmed the assignments of these two methylene pairs. The assignment of H-19α and H-19β was easily done by the observation of nOe's from H-19β to H-6 and H-18.

Both H-5 and H-9 showed significant dipolar interactions with the two severely overlapped protons around δ 1.77 which are the resonances of H-1β and the upfield signal of the C-7 proton of methylene pair. Since H-5 and H-9 have a 1,3-diaxial relation with both H-1β and H-7β, the observed nOe's could not be used to assign the resonance at δ 1.77 to be H-7β. That is, the observed nOe's may result from the dipolar interaction with H-1β. Thus the assignments of the methylene protons at C-7 relied upon the assignments of the C-15 methylene protons. Unfortunately, the downfield signal of the C-7 methylene protons was overlapped with the upfield resonance of the C-15 pair around δ 2.10, and no nOe's were observed between the upfield H-7 signal and the downfield H-15 signal in the NOESY spectrum at 20°C. Furthermore, no nOe's were observed from H-15 protons to any neighboring protons in the NOESY spectrum at 20°C, resulting in difficulties in assigning the nonequivalent protons at C-15 as well as C-7.

Since the spatial relationships of the H-15 protons with H-17b, H-7α,β and H-11β in tangutisine were very similar to those in tatsirine where these crucial nOe's were easily observed,[5] the lack of nOe's *in* **1** *was most likely caused by nonoptimized experimental conditions, especially mixing time and* correlation time, rather than the possibility that these protons were too far apart to interact through space. Failing to observe any nOe's with the C-15 protons in 1D nOe difference experiments using different delays for nOe build up by irradiating H-11β, the only available proton which could be safely saturated without perturbing an adjacent resonance, an attempt was made to change the correlation time of these protons by changing the temperature in the NOESY experiment. The first NOESY experiment was performed at 7.5°C with the same mixing time (0.8 s) as the one originally recorded at room temperature. However, most of the cross peaks in the NOESY spectrum were either reduced or vanished. Reducing the temperature, and thereby increasing correlation times, was therefore the wrong variation for enhancing dipole-dipole interactions. (That is, the double quantum relaxation pathway must be dominant at room temperature). Therefore, another NOESY experiment was done at 35°C, also using the same mixing time (0.8 s). New cross peaks from the more downfield signal (δ 2.26) of the methylene pair at C-15, to H-11β and H-17b appeared (Table 2). Thus, the signal at δ 2.26 was assigned to H-15β. Another important new nOe was from H-15β to the upfield H-7 resonance, enabling assignment of the latter signal to H-7β (δ 1.77). The α-protons of C-15 and C-7 are overlapped around δ 2.10. The nOe observed from H-11β to H-15β further confirmed the stereochemistry of 11-OH.

The chemical shift assignments of all methyl, methylene and methine carbons were done by DEPT and HETCOR, selective INEPT[11] and FLOCK[12] (Table 3) experiments after the complete proton assignments were made, with the exception of C-13. The resonances of C-13 and C-14 (δ 81.4 and

81.8) were too close to be distinguished by a HETCOR experiment. Although one of these resonances appeared as a methine carbon and the other disappeared as a quaternary carbon in the DEPT experiment, it was still not reliable to assign these two carbon signals by comparing resonance frequencies obtained from the DEPT or selective INEPT spectra with the normal ^{13}C spectrum. (In other words, when only one of these two signals appeared in either the DEPT or selective INEPT

Table 3. ^{1}H-^{13}C Long Range Correlations (FLOCK) of **1**[a]

Observed H	Two or three bond correlated to
H-17a	C-15, C-12
H-17b	C-12
H-20	C-8, C-6
H-11	C-10, C-13
H-13	C-11
H-6	C-10, C-8
H-19α	C-6
H-19β	$\underline{C}H_3$, C-20
H-12	C-9, C-14, C-17
H-9	C-12, C-20, C-11, C-14
H-7β	C-9, C-6
H-15α	C-17
H-3α	C-2
H-7α	C-14
H-1β	C-2
$C\underline{H}_3$	C-4, C-5, C-19

[a]Spectrum run in D_2O with 3 drop CD_3OD.

spectra, it was impossible to unambiguously say which one it was! The two carbon resonance frequencies were simply too close). A simple APT experiment was utilized to solve this problem. Two very close but resolved carbon signals were observed for C-13 and C-14. The more downfield signal (δ 81.8) was a tertiary carbon, therefore it was assigned to C-13, while the signal at δ 81.4 was a quaternary carbon, the resonance of C-14. One of the three (C-4, C-8, C-10) remaining quaternary carbons, the signal at δ 36.4 showed long range heteronuclear correlation to H-18 in the FLOCK experiment and was assigned to C-4 (two bond coupling). The quaternary carbon at δ 44.9 had long range heteronuclear coupling with H-6 and H-20, while the remaining carbon singlet at δ 46.9 correlated with H-6 and H-11 in the FLOCK spectrum. Since all these long range correlations were in potential range (two or three bonds) for both C-8 and C-10, these two carbon signals could not be discriminated by the FLOCK experiment. The assignments of the last two quaternary carbons (C-8, C-10) were completed by selective INEPT experiments. Polarization transfer from H-18 in a selective INEPT experiment resulted in two strongly enhanced carbon signals at δ 36.4 (s) and δ 57.4 (d). These were the resonances of C-4 (two bond coupling with H-18) and C-5 (three bond coupling with H-18), respectively. When H-2 was irradiated, C-10 (δ 46.9) was strongly enhanced (three bond coupling with H-2), while C-4 (δ 36.4) also appeared in the same experiment. A third selective INEPT experiment was run to detect C-8 by irradiating H-20 (three bond coupling). As predicted, the polarization transfer from H-20 to C-8 (δ 44.8) was successfully detected (Figure 3). The complete proton and carbon assignments are listed in Table 1.

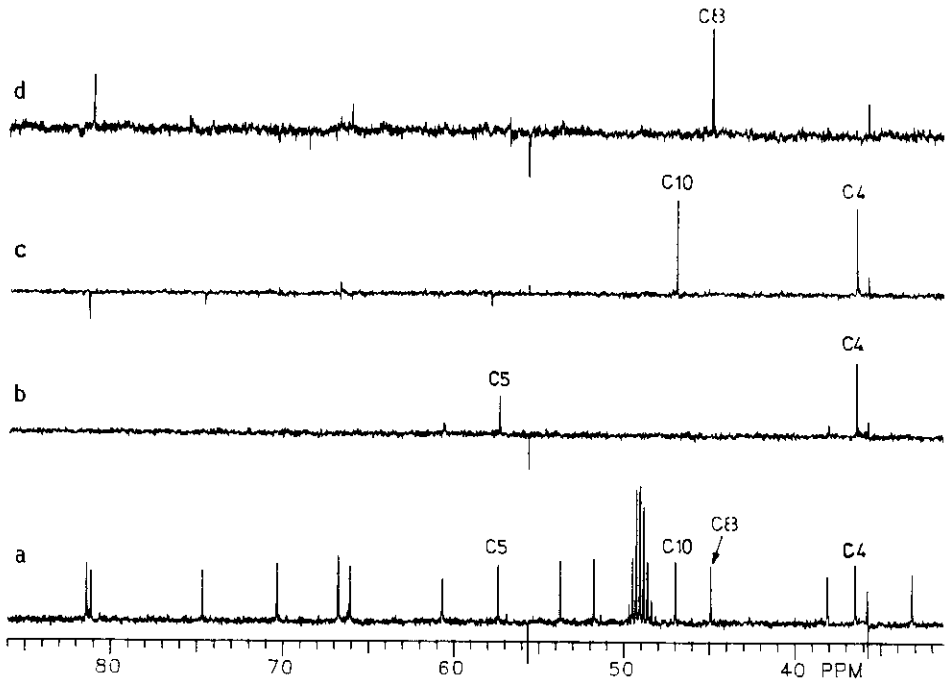

Figure 3. Selective INEPT spectra of **1** in D_2O (with 3 drops of CD_3OD). (a) The normal ^{13}C nmr spectrum for comparison. The quaternary carbons were assigned by the observations of the polarization transfer from (b) H-18, (c) H-2 and (d) H-6.

A literature search indicated that four alkaloids which are acylated derivatives of tangutisine **1** have been isolated from A. bullatofolium var. homotorichum and A. koreanum. These are: Guan-fu base A (**3**),[13,14] Guan-fu base G (**4**),[13,15] Guan-fu base Y (**5**),[14] and Guan-fu base Z (**6**).[9,14] The structure of Guan-fu base B has not been fully established.[9,16]

EXPERIMENTAL

General: – Melting points are uncorrected and were taken on a Thomas-Kofler hot stage equipped with a microscope. Infrared spectra were obtained on a Perkin-Elmer model 399 spectrophotometer. The nmr spectra were recorded on a Varian XL-400 spectrometer (93.94 kG, 400 MHz for ^1H, 100 MHz for ^{13}C). The pulse sequences employed in the one and two dimensional nmr experiments were the standard Varian software, version 6.1c, except the FLOCK pulse sequence which was added to the Varian nmr pulse sequence library based on Reynold's program.[12] The nmr sample (5 mg) of **1** was of the hydrochloride salt. All nmr spectra were obtained in D_2O with 3 drops of CD_3OD. Residual CHD_2OD, and $^{13}CD_3OD$ were used as internal references (^1H: δ 3.30, ^{13}C: δ 49.0) for ^1H and ^{13}C nmr, respectively. The HRMS spectrum was recorded on a Finnigan MAT 90 instrument; (EI, 40 eV).

The whole plant of A. tanguticum was collected in Western, Si–Chuan province, China, in August 1981 and a voucher specimen of the plant has been deposited in the Herbarium House of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing.

Extraction and isolation of tangutisine (1): – The dried plant material (roots, stem and leaves) (4.35 kg) was exhaustively extracted at room temperature with EtOH and evaporated under vacuum to give the ethanolic extract (250 g). This was stirred with 1% HCl (1080 ml) and filtered. The acidic filtrate was extracted with $CHCl_3$ (4 x 500 ml) and the aqueous layer was basified with 10% NH_4OH to pH 5. The mixture was extracted with $CHCl_3$ and the aqueous layer was basified to pH 9-10 by addition of solid Na_2CO_3. This was washed with $CHCl_3$ to afford a crude alkaloid (11.24 g) which was treated with MeOH: Me_2CO (1:1) (15 ml) and filtered. The filtrate was chromatographed over a column of silica gel and eluted with ethanol containing 1% HCl to afford **1** (150 mg) as the hydrochloride salt, recrystallized from water, mp 310–315° (decomp.); ir ν_{max} (KBr): 3380, 3300, 3260, 3220, 1657 cm^{-1}; ms: m/z (%), 345 (M^+, 34), 330 (M^+-CH_3, 60), 328 (M^+-OH, 100), 316 (34), 300 (65). Hrms: m/z 345.1939 (M^+, calcd for $C_{20}H_{27}NO_4$, 345.1040)

Tetraacetyltangutisine (2): – Treatment of tangutisine with Ac_2O-p-toluenesulfonic acid gave the tetraacetate as an amorphous powder; ms: m/z (%): 513 (M^+, 30), 470 (M^+-Ac, 100), 454 (M^+-OAc, 74), 410 (470-AcOH, 6), 394 (454-AcOH, 15), 352 (394-Ac, 5), 43 (Ac, 71); ^1H nmr ($CDCl_3$): δ 1.00 (3H, s), 2.04, 2.08, 2.12, 2.12 (each 3H, s, $COCH_3$), 2.64, 2.90 (each 1H, AB-type, J = 13 Hz), 3.30 (1H, br s, $W_{1/2}$ 7 Hz), 4.25 (1H, s), 4.83, 5.05 (each 1H, s), 5.03–5.16 (3H, m).

Nmr multiple sequence: – One dimensional ^1H nmr spectra were recorded with a spectral window of 4000 Hz and an acquisition time of 2.0 s, giving a digital resolution of 0.5 Hz per point. The spectral window and the acquisition time for one dimensional ^{13}C nmr spectra were 20000 Hz and 0.5 s, respectively, giving a digital resolution of 2.0 Hz per point. The ^{13}C multiplicities were assigned by APT and DEPT experiments. The APT experiment was run with a spectral window 9166 Hz, and acquisition time of 0.279 s, since the region of interest only covered the sp^3-hybridized carbon resonant frequencies. A refocusing delay of 0.007 s optimized for $^1J_{CH}$ of 142 Hz was generally used in the APT experiment. The DEPT experiment was performed with a narrowed spectral window of 12642 Hz and an acquisition time of 0.5 s, optimized for one bond heteronuclear coupling of 140.0 Hz, in order to get better phased sub-spectra. Long range (two and three bond) heteronuclear ^1H,^{13}C couplings were detected with one dimensional selective INEPT and two dimensional FLOCK experiments. The selective INEPT spectra were recorded with the excitation and refocusing delays optimized for different long range heteronuclear coupling constants according to the formulae t = 1/2J and Δ = 1/3J, respectively.[11] The excitation and refocusing delays used in the FLOCK experiment were the same as those in a normal HETCOR. The parameters for the two dimensional experiments are lised in Table 4.

Abbreviations for the acquisition parameters for the 2D nmr spectra listed in Table 4 are:

SW1	Spectral window in the second dimension.
SW2	Spectral window in the first dimension.
AT	Acquisition time (unit: second).

NP Number of data points in the second dimension.
NT Number of transients per increment on the first dimension.
NI Number of increments in the first dimension.
D_3 Refocusing delay prior to detection (unit: second).
MIX Mixing time in NOESY experiment (unit: second).
TAU Excitation delay (unit: second).

Table 4. Parameters For Recording 2D nmr Spectra of **1**

Parameters	COSY	LRCOSY	NOESY[a]	NOESY[b]	FLOCK
SW1 (Hz)	1687.2	1810.6	1810.6	1763.4	12610.3
SW2 (Hz)	1687.2	1810.6	1810.6	1763.4	1907.5
AT (s)	0.152	0.141	0.141	0.145	0.020
NP	512	512	512	512	512
NT	32	32	32	32	288
NI	256	256	256	256	128
D_3 (s)		0.200			0.040
MIX (s)			0.600	0.600	
TAU (s)					0.072

[a] The spectrum was obtained at the room temperature
[b] The spectrum was obtained at 35°C.

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