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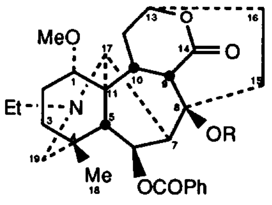
Tangirine, A Novel Dimeric Alkaloid from *Aconitum Tanguticum* (Maxim.) Stapf, W.T.Wang

Balawant S. Joshi[a*], Yili Bai[a], Di Hua Chen[b] and S. William Pelletier[a*]
[a]Institute for Natural Products Research and Department of Chemistry, University of Georgia, Athens, GA 30602-2556, U.S.A.; [b]Chinese Academy of Medical Sciences, Beijing, China

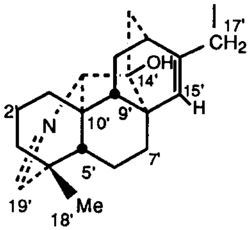
Abstract: A novel diterpenoid alkaloid tangirine (**1**) has been isolated from *Aconitum tanguticum.* Its structure was elucidated by multidimensional ^1H and ^{13}C nmr spectroscopy.

In an earlier communication, the isolation and structure determination of tangutisine from *Aconitum tanguticum* (Maxim.) Staff, W.T.Wang were described.[1] We report herein the structure of a dimeric diterpenoid alkaloid tangirine, isolated in 0.001% yield from the neutral $CHCl_3$ fraction obtained during the isolation of tangitisine by chromatography over Al_2O_3. The molecular formula of tangirine[2] (**1**) was established to be $C_{49}H_{62}N_2O_7$ by HRFAB-MS (m/z 790.4558, calc. 790.4557). Sixty-one protons and 49 carbons were visible in the ^1H and ^{13}C nmr spectra of **1** in $CDCl_3$ and all one bond ^1H-^{13}C connectivities were established by a HETCOR experiment (Table1). The DEPT spectrum indicated that tangirine possessed 11 quaternary carbons (*s*), 19 methines (*d*), 15 methylenes (*t*) and 4 methyls (*q*). Out of these, the seven carbon singlets at δ 173.3, 166.6, 130.8, 78.7, 48.8, 34.7, the eleven doublets at δ 132.3, 130.2 (2C), 128.2 (2C), 82.3, 75.1, 74.0, 62.9, 55.9, 48.2, 44.7, 43.0, the seven triplets at δ 57.4, 48.8, 36.3, 31.2, 29.7, 29.2, 26.7 and the three quartets at δ 55.0, 25.9 and 13.5 appeared to belong to 6-benzoylheteratisine[3] (**1**; R=H). The up field shifts of C-7 and C-15 by ~ 4-5 ppm when compared with the values in 6-benzoylheteratisine can be attributed to the bulky substituent group attached at the C-8 position. The ^1H-^1H COSY, the NOESY, and the HMBC spectral results (Table 1) are consistent with this partial structure for tangirine. The mass spectral fragmentations[2] also support the presence of a methoxyl group at C-1[5] and a benzoate ester group in **1**.

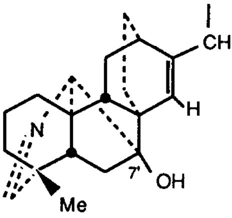
The group R in tangirine is attached at C-8 through an oxygen function forming an ether. This portion $C_{20}H_{26}NO$ observed as a fragment in the ms (m/z 296, R^+; m/z 494 M^+–R), showed in the ^{13}C nmr spectrum, one sp^2 singlet at δ 146.0, four sp^3 singlets at δ 72.5, 45.0, 44.9, 43.1, two sp^2 doublets at δ 169.2, 127.8, four sp^3 doublets at δ 80.3, 46.4, 44.3, 31.5, eight methylenes at δ 60.4, 42.8, 31.5, 30.6, 30.5, 28.4, 27.4, 20.6 and one methyl at δ 19.0. The ^1H nmr signal at δ 3.82 (2H, *dd*, AB, J=12.0 Hz; ^{13}C, 60.4 ppm), showing a connection (COSY, nOe) with δ 4.98 (1H, br *s;* ^{13}C, 127.8 ppm) suggested the presence of the grouping, $-CH=C-CH_2-O-$ in R. Also the signal at δ 7.32 (1H, *d*, J=2.5 Hz; ^{13}C, 169.2 ppm) was coupled (COSY) to δ 3.30 (1H, br *s* ; ^{13}C, 80.3 ppm) indicative of the system -C-CH=N-CH- in R. The three-proton singlet at δ 1.00 (^{13}C, 19.0 ppm) suggested the presence of a tertiary methyl group. The molecular formula $C_{20}H_{26}NO$, biogenetic considerations and the functional groups described, suggested one of the three possible structures A, B or C for R.



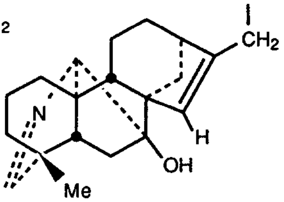
1 : R = A



A



B

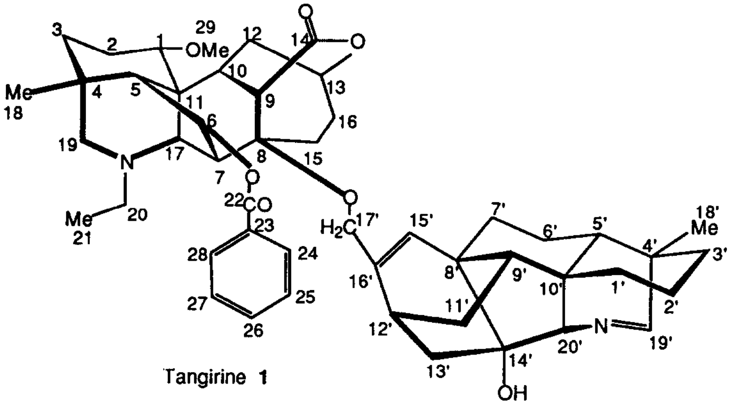


C

The quaternary carbon singlet at 72.5 ppm can be assigned to C-14' in **A**, or to C-7' in **B** or **C**. The other singlets at 45.0, 44.9 and 43.1 ppm were assigned to C-4', C-10' and C-8', respectively. Of the two closely appearing signals, 45.0 ppm was assigned to C-4' as this signal showed a two-bond correlation to H-19' (δ 7.32; ^{13}C, 169.2 ppm) in the HMBC[6] spectrum (Table 1). Also, C-10' showed a three-bond correlation with the proton at δ 1.32 (^{13}C at 27.4 ppm, HMBC) which may be assigned to C-2' or C-6'. The methine proton at δ 1.18 (^{13}C, 44.3 ppm) was assigned to C-5' as this correlated to H-20' (δ 3.30; ^{13}C, 80.3 ppm) in the long range COSY (LRCOSY) spectrum and showed a three-bond correlation with the tertiary methyl CH_3-18' (δ ^{1}H 1.00; ^{13}C, 19.0 ppm, HMBC). H-5' exhibited an nOe to H-3' (δ, ^{1}H 1.22; ^{13}C, 30.6 ppm). The most down field quaternary carbon at 146.0 ppm is clearly assigned to C-16'. The 2D COSY spectrum showed a correlation of H-15' (δ 4.98) to H-12' (δ 2.00) and to the methylene at C-17' (δ 3.82, ^{13}C, 60.4 ppm). An important clue to the attachment of the two halves of this dimeric alkaloid is an nOe observed between H-7 proton of 6-benzoylheteratisine and the H-17' methylene of the remaining part of the molecule. In the HMBC spectrum, H-15' (δ 4.98) showed a two-bond correlation to C-8', and a three bond correlation to C-17', which is in agreement with all the three structures **A**, **B**, or **C**. H-15' also showed a correlation to 31.5 ppm (overlapping signals for a CH_2 and a CH). In the case of **A**, these carbons are assigned to C-7' and C-12' or C-9', whereas in **B** these have to be assigned to C-14' and C-9' or C-12' and in **C** to C-14', C-9' or C-13'. *The crucial evidence in support of structure **A** was the observed correlations of H-13' in the HMBC spectrum.* One of the methylene protons at δ 1.52 ascribed to H-13'a (^{13}C, 42.8 ppm) is related to C-8' (43.1 ppm, 3 bonds separated), C-12' (31.5 ppm, 2 bonds), C-16' (146.0 ppm, 3 bonds) and C-20' (80.3 ppm, 3 bonds). A correlation of any methylene proton, two or three bonds separated from C-16' (146.0 ppm) to C-20' (80.3 ppm) is possible only in the case of structure **A**. This evidence excludes the alternative structures **B** and **C** for R. After assigning five methine carbons as discussed, the remaining signal at 46.4 ppm was assigned to C-9'. In the HMBC spectrum, the methylene protons at δ 1.42 (^{13}C, 30.5 ppm) showed a relation to C-5' (44.3 ppm), three-bonds separated and were assigned to H-1'. The methylene carbons appearing at 27.4 and 20.6 ppm were assigned to C-2' and C-6', respectively, by comparison with the values assigned to dihydroatisine (C-2 23.2, C-6 17.6 ppm),[7] and dihydroajaconine (C-2 23.1, C-6 17.6 ppm).[8] The ^{13}C assignments, the ^{1}H COSY and NOESY results, and the HMBC data for R given in Table 1 confirm the structure **1** for tangirine. Tangirine is the only example of a dimeric alkaloid in which a norditerpenoid and a diterpenoid alkaloid are joined together. Six other dimeric alkaloids of this class [9], e.g. staphidine, staphisine, etc., all isolated from *D. staphisagria*, are C_{20}-diterpenoid alkaloids of the atisane-type, dimerized at the C-17, C-17', 15'(O), 16 positions to form spiro ethers.

Table 1. nmr data for tangirine (**1**).

Position	δ ^{13}C	δ ^{1}H (J=Hz)	COSY	HMBC (H→C)	nOe
(6-benzoylheteratisine part)					
1	82.3 (d)	3.18 (m)	-	29-OMe, 17	-
2	26.7 (t)	2.13 (m)	-	-	-
3	36.3 (t)	H3a: 1.22 (m)	-	-	H3b
		H3b: 1.59 (m)			H3a
4	34.7 (s)	-	-	-	-
5	55.9 (d)	1.58 (br *s*)	H17, H6	4, 6, 10, 11, 17, 18, 19	18-Me
6	74.0 (d)	5.64 (d, 7.2)	H7, H5	4, 5, 8, 11, 22	18-Me, 7, 19
7	44.7 (d)	2.95 (d, 7.2)	H6	5, 8, 9, 11, 17	20
8	78.7 (s)	-	-	-	-
9	48.2 (d)	4.19 (d, 8.0)	H10	8, 10, 12, 14, 15	5, 10
10	43.0 (d)	2.45 (m)	H12a	8	5
11	48.8 (s)	-	-	-	-
12	29.2 (t)	2.13 (m), 3.15 (m)	H10, H13	10, 11, 13	10
13	75.1 (d)	4.73 (dd, 5.5)	H12a, H16	10	-
14	173.2 (s)	-	-	-	-
15	31.2 (t)	H15a: 1.82	H15b	-	-
		H15b: 2.04	H15a	-	-
16	29.7 (t)	H16a: 1.83	H16b	8	16b
		H16b: 2.40	H16a	-	16a
17	62.9 (d)	3.60 (br s)	H5	5, 6, 11, 19, 20	7, 16a, 20
18-Me	25.9 (q)	0.86 (s)	-	3, 4, 5, 19	6
19	57.4 (t)	H19a: 2.19	H19b	3, 18, 20	19b
		H19b: 2.65 (d, 12.0)	H19a	3, 4, 5, 17	19a
20	48.8 (t)	2.52 (AB, 7.2)	21-Me	21-Me, 17, 19	21-Me
21-Me	13.5 (q)	1.08 (t, 7.2)	H20	20	20
22	166.6 (s)	-	-	-	-
23	130.8 (s)	-	-	-	-
24,28	130.2 (d)	8.05 (dd, 7.0, 1.4)	H25, H27	22, 26	9, 25, 27
25,27	128.2 (d)	7.43 (t, 7.1)	H24, H26, H28	23, 24, 28	24, 26, 28
26	132.3 (d)	7.53 (t, 7.1)	H25, H27	24, 28	25, 27
29-OMe	55.0 (q)	3.29 (s)	-	1	-
			(A)		
1'	30.5 (t)	1.42 (m)	-	5'	-
2'	27.4 (t)	1.07, 1.32 (m)	-	10', 4'	-
3'	30.6 (t)	1.22 (m)		-	5'
4'	45.0 (s)	-	-	-	-
5'	44.3 (d)	1.18 (br s)	H20'	18'	3'
6'	20.6 (t)	1.48 (m)	-	-	-
7'	31.5 (t)	H7'a: 1.36 (m)	H7'b	8', 14'	-
		H7'b: 1.48 (m)	H7'a		-
8'	43.1 (s)	-	-	-	–
9'	46.4 (d)	0.87 (m)	-	-	-
10'	44.9 (s)	-	-	-	-
11'	28.4 (t)	1.43, 1.50 (m)	-	-	-
12'	31.5 (d)	2.00 (m)	H13'b	-	-
13'	42.8 (t)	H13'a: 1.05 (m)	H13'b	-	
		H13'b:1.52 (m)	H13'a	8', 12', 16', 20'	-
14'	72.5 (s)	-	-	-	-
15'	127.8 (d)	4.98 (br s)	H12', H17'	7', 8', 12', 17'	7,' 17'
16'	146.0 (s)	-	-	-	-
17'	60.4 (t)	3.82 (AB,12.0)	H15'12', 15', 16'	7, 12'	
18'-Me	19.0 (q)	1.00 (s)	-	3', 4', 19'	19'
19'	169.2 (d)	7.32 (d, 2.5)	H20'	4', 18', 20'	18'-Me
20'	80.3 (d)	3.30 (br s)	H19'	-	-



Tangirine **1**

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