Tetrahedron Letters,Vol.27,No.47,pp 5765-5768,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

STRUCTURAL STUDY OF TUBEIMOSIDE I, A CONSTITUENT OF TU-BEI-MU

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The structure of Tubeimoside I isolated from the bulb of *Bolbostemma paniculatum* [Maxim] Franquet (Tu-bei-mu) was determined as **1** mainly by comparative NMR studies of **1** and its degradation products. The compound **1** has inter-saccharidechain bridging by a dicrotalic acid to form a unique macrocyclic structure.

Tu-bei-mu, the bulb of *Bolbostemma paniculatum* [Maxim] Franquet, is one of the Chinese folk medicines often used for the treatment of tumors as well as for detoxication. It has also been used to cure warts.[1,2] Maltose has been isolated from these bulbs, but no other chemical components have been isolated and identified.[3] We have isolated and identified seven chemical constituents of Tu-bei-mu including maltol and $\Delta^{7,16,25(26)}$-stigmastatrien-3-ol. The structure of one of the new triterpenoid saponins, tubeimoside I[4] **1**, which has an unusual dicrotalic acid bridging between two sugar chains, was determined as follows.

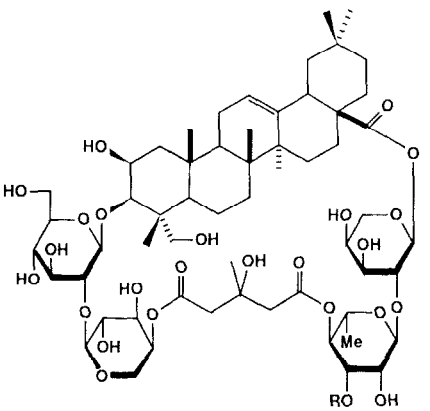
Tubeimoside I, white needles, mp; 250-2°C, $[\alpha]_D$ +13.0°(*c* 0.8, MeOH), has the molecular formula $C_{63}H_{98}O_{29}$, MW 1318 (SIMS; *m/z* 1319, M+H, and 1341, M+Na). Mild acid hydrolysis of **1** gave bayogenin[5] and 4 sugars: L-arabinose, D-glucose, L-rhamnose and D-xylose. However, the ^{13}C-NMR spectrum of **1** (Table 1), shows five sugar anomeric carbons. The presence of a dicrotalic acid moiety[6] was also suggested from the NMR data (^{1}H-NMR: δ2.03, 3H, s; 3.46 and 3.05, 1H each, AB-system, *J*=15.8Hz; 3.31 and 3.07, 1H each, AB-system, *J*= 15.6Hz; ^{13}C-NMR: see Table 1). The signals at δ94.1 and 175.9 indicated the presence of a glycosidic ester linkage at C-28 of bayogenin.[7]

Hydrolysis of **1** with 1N KOH afforded prosapogenin **2**(FDMS, *m/z* 783, M+H) and trisaccharide **3**, which was originally linked to C-28. The prosapogenin **2** was subjected to acid hydrolysis to give bayogenin, arabinose and glucose, while the trisaccharide **3** gave arabinose, rhamnose and xylose. By comparison of the ^{13}C-NMR spectrum of **2** with those of bayogenin methyl ester and methyl β-D-gluco- and

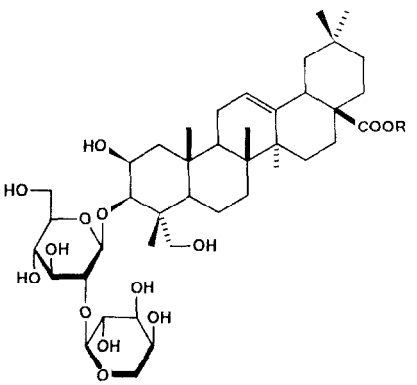
α-L-arabinopyranosides,[8] two glycosidation shifts by 10.3 ppm (C-3 of aglycone, from δ72.9 to 83.2) and 8.6 ppm (C-2 of glucose, from δ74.9 to 83.5) were observed. Therefore, in prosapogenin **2**, the O-3 of bayogenin should be glycosylated with β-D-glucopyranose bearing an α-L-arabinopyranosyl unit on O-2.[9,10] The anomeric configurations of glucose and arabinose were confirmed as β and α(L), respectively, based on the coupling constants of their anomeric protons; *J*=7.7Hz(glucose, δ5.09, d) and *J*=6.9Hz(arabinose, δ5.11, d). Hence, the structure of prosapogenin **2** was determined as 3-O-[α-L-arabinopyranosyl(1→2)-β-D-glucopyranosyl]-bayogenin.

A cellulase treatment of **1** gave prosapogenin **6** and xylose.[11] It was clearly shown that **6** still possesses three ester carbonyl groups(δ176.0, 171.2 and 171.1) as does **1**. Treatment of **6** with 5% K_2CO_3 afforded a deacylated compound **7** containing only one ester carbonyl group (δ176.1, C-28 of bayogenin). Compound **7** gave a ^{13}C-NMR spectrum that was almost superimposable on that of **2** except that the C-28 of bayogenin was shifted and two extra sugar moieties, arabinose and rhamnose, were present. Furthermore, comparison of the ^{13}C-NMR spectrum of **7** with that of [α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl] 3-O-acetyl-oleanolate(**8**),[12] in which the carbons of the sugar moieties has been assigned, allowed us to deduce the sequence of the sugar chain on the C-28 carbonyl group of **7** to be an α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl group.

A mild alkaline hydrolysis of **1** with 0.5% KOH gave dibasic acid **10** and deacylated compound **9**, needles from MeOH, mp 233-5°C. The ^{13}C-NMR spectrum of **9** was devoid of signals corresponding to the dicrotalic acid moiety. A glycosidation shift (**7** *vs.* **9**) by 10.8 ppm (from δ72.3 to 83.1) at C-3 of rhamnose indicating that the terminal xylose was attached through O-3 of rhamnose.[10] The anomeric configuration of the xylose was deduced to be β from the coupling constant of its anomeric proton (δ5.24, d, *J*=7.6Hz) as well as the ^{13}C-NMR. Thus the structure of **9** can be characterized as 3-O-[α-L-arabinopyranosyl(1→2)-β-D-glucopyranosyl]-bayogenin 28-[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl] ester. The dibasic acid **10**, FAB-MS, *m/z* 163(M+H) and 185(M+Na), ^{1}H-NMR, two singlets at δ2.50 and 1.22, was crystallized from MeOH as white plates, mp 106-8°C, and identified as dicrotalic acid.[6]



1 R= β-D-Xyl
6 R= H



2 R= H
7 R= α-L-Rha(1→2)-α-L-Ara-
9 R= β-D-Xyl(1→3)-α-L-Rha(1→2)-α-L-Ara-

Table 1. Carbon Chemical Shifts of Tubeimoside I, **1**, and Related Compounds (in Pyridine-d_5)

	C.No.	Me.Bay[a]	**1**	**9**	**6**	**7**	**2**	**8**[12]	Me.Gly[b]
Bayogenin Moiety									
	1.	44.8	44.1	44.1	44.7	44.1	44.0		
	2.	71.5	69.1	70.8[g]	69.2	70.4[m]	70.3		
	3.	72.9	83.2	82.6[h]	83.9	82.7[n]	83.2[p]		
	4.	42.4	43.0	42.7	43.3	42.7	42.3		
	5.	48.0	47.7	47.7	47.6	47.7	47.9		
	6.	18.2	18.7	18.5	18.7	18.5	18.1		
	7.	32.8	33.1	33.0	33.0	33.0	33.2		
	8.	39.7	40.1	40.0	40.0	40.0	40.1		
	9.	48.4	47.7	47.7	47.6	47.7	47.9		
	10.	37.1	37.2	36.9	37.2	36.9	37.0		
	11.	23.9	23.9	24.0	23.8	24.0	23.9		
	12.	123.1	123.2	123.1	123.1	123.1	123.1		
	13.	144.1	144.2	144.2	144.0	144.2	144.9		
	14.	42.0	41.9	42.3	41.9	42.3	42.5		
	15.	28.0	28.2	28.3	28.8	28.5	28.3		
	16.	23.3	22.8	23.2	23.8	23.7	23.9		
	17.	46.9	47.0	47.3	47.0	47.3	46.7		
	18.	41.8	41.3	41.7	41.4	41.8	42.1		
	19.	46.0	46.0[c]	46.3	45.9[k]	46.3	46.7		
	20.	30.8	30.7	30.9	30.6	30.9	30.9		
	21.	33.9	34.3	34.2	33.9	34.2	34.0		
	22.	32.8	32.2	32.7	32.1	32.7	33.2		
	23.	67.5	64.7[d]	64.6	65.7	66.1	65.1		
	24.	14.6	15.5	14.7	15.7	14.6	14.6		
	25.	17.2	17.7	17.5[i]	17.7[l]	17.5[o]	17.5[q]		
	26.	17.2	17.7	17.3[j]	17.5[l]	17.3[o]	17.2[q]		
	27.	26.2	26.0[e]	26.1	26.3	26.1	26.3		
	28.	177.9	175.9	176.2	176.0	176.1	180.0		
	29.	33.1	33.1	33.1	33.0	33.1	33.2		
	30.	23.6	23.7	23.7	23.6	23.7	23.9		
Dicrotalic Acid Moiety									
	1.		171.2		171.2				
	2.		47.0[c]		47.4[k]				
	3.		70.1		70.1				
	4.		46.8[c]		46.0[k]				
	5.		171.1		171.1				
	3-Me		26.3[e]		26.4				
Saccharide Chains									
Glc	1.		103.5	103.5	103.6	103.3	103.4		105.4
	2.		80.2	83.5[h]	80.2	83.4[n]	83.5[p]		74.9
	3.		78.8[f]	78.0[j]	79.0	77.8	77.9		78.1
	4.		71.3	71.7	71.5	71.3	71.8		71.4
	5.		78.3[f]	78.2[j]	78.3	78.0	78.3		78.1
	6.		62.4	62.4	62.7	62.4	62.9		62.5
Ara	1.		104.4	106.5	104.7	106.3	106.6		105.9
	2.		73.7	73.8	73.8	73.8	73.8		72.2
	3.		72.3	74.3	72.6	74.3	74.5		74.4
	4.		72.3	69.2	72.6	69.2	69.2		69.1
	5.		64.7[d]	67.2	64.4	67.2	67.2		66.6
Ara	1.		94.1	93.5	94.4	93.4		93.3	105.9
	2.		74.6	75.6	74.9	75.2		74.9	72.2
	3.		70.8	70.1[g]	70.9	70.1[m]		70.2	74.4
	4.		67.6	66.2	67.5	66.1		66.0	69.1
	5.		64.3[d]	63.1	64.4	62.9		62.9	66.6
Rha	1.		100.6	101.3	100.6	101.3		101.2	102.4
	2.		72.3	71.2	72.6	72.3		72.1	72.2
	3.		77.8[f]	83.1[h]	70.1	72.3		72.4	72.5
	4.		73.2	72.8	75.4	73.7		73.7	73.6
	5		67.8	70.5[g]	68.0	70.3[m]		70.2	69.4
	6.		18.2	18.5	18.4	18.5		18.4	18.4
Xyl	1.		106.4	107.1					106.1
	2.		74.6	75.0					74.6
	3.		78.5[f]	78.2[j]					78.1
	4.		71.1	71.0[g]					70.9
	5.		66.8	67.2					66.9

a: Bayogenin Methyl ester, b: Methyl Glycosides, (Skeletal carbons only)
c - p: assignments might be interchangable.

In order to determine the linkage positions of dicrotalic acid, a comparative ^{1}H-NMR study was carried out on **6** and its deacylated derivative **7**. All proton signals of the sugar moieties of **6** and **7** were assigned using the COSY technique. Significant downfield shifts[13] of the H-4 signals of rhamnose and the terminal arabinose by 1.57ppm (from δ4.32 to 5.89) and 1.23 ppm(from δ4.33 to 5.56), respectively, indicated that the dicrotalic acid was linked to the O-4 of rhamnose and the O-4 of the terminal arabinose. This was corroborated by a set of acylation shifts[11] in ^{13}C-NMR(**6** *vs.* **7**) involving C-3(upfield by 1.7 ppm from δ74.3 to 72.6), C-4 (downfield by 3.4 ppm from δ69.2 to 72.6) and C-5 (upfield by 2.8 ppm from δ67.2 to 64.4) of the terminal arabinose. A similar shift pattern was observed with the C-3, 4 and 5 of rhamnose. Therefore, it is obvious that one end of the dicrotalic acid bridge is bound to the O-4 of the terminal arabinose, and the other end, to the O-4 of rhamnose. Thus, the structure of **1** was determined unambiguously. Due to the influence of the dicrotalic acid bridging in the molecule, some difficulties were experienced to assign the carbon signals of **1** and its prosapogenin **6**, by comparing them with similar compounds with no inter-saccharidechain bridging. Therefore, C-H COSY experiments had to be performed on **6** to obtain the assignment of the carbon signals of the sugar moieties. Two notable upfield shifts by 3.2ppm(C-2 of glucose, from δ83.4 to 80.2) and 1.6 ppm(C-1 of arabinose, from δ106.3 to 104.7) were observed(**6** *vs.* **7**). These can be explained in terms of orientational changes arround the glycosidic linkage(s) in the structure of **6**, and also in **1**, caused by the inter-saccharidechain bridge. It should be noted that in special cases like these, much care should be taken when employing empirical glycosidation and acylation shift rules for sequencing of sugar chains by ^{13}C-NMR.

It is noteworthy that purified tubeimoside I showed moderate antitumor activity in primary *in vivo* pharmacological test.[14]

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4. Independent from our work, the compound named tubeimoside I has been isolated from the same source by O. Tanaka *et. al.* (R. Kasai, M. Miyakoshi, K. Matsumoto, T. Morita, O. Tanaka, J. Zhou, 27th Symposium on the Chemistry of Natural Products, Hiroshima, Oct 1985, Abstract p749; *idem.*, *Chem. Pharm.Bull.*,submitted). Comparison of the physical data of both compounds revealed them to be identical. Therefore, in order to avoid further confusions in naming of a series of compounds, we have adopted the name tubeimoside. We are grateful to Professor Tanaka and co-workers for sharing their results with us prior to the publication.
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(Received in Japan 30 August 1986)