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TUBEIMOSIDE I, A NEW CYCLIC BISDESMOSIDE FROM CHINESE CUCURBITACEOUS FOLK MEDICINE "TU BEI MU", A TUBER OF *Bolbostemma paniculatum*

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An oleanane type triterpenoid saponin, named tubeimoside I was isolated from tubers of *Bolbostemma paniculatum* (Maxim.) Franquet (Cucurbitaceae), a Chinese folk medicine, "Tu Bei Mu". Its novel cyclic structure, including the chirality of the 3-hydroxy-3-methylglutarate moiety, is reported. The name cyclic bisdemoside is proposed for this type of saponin. The potent solubilizing effect of this compound is also described.

KEYWORDS——— tubeimoside I; cyclic bisdesmoside; bayogenin; saponin; *Bolbostemma paniculatum*; Cucurbitaceae; Chinese folk medicine; 3-hydroxy-3-methylglutarate; mevalonolactone chirality; solubilizing effect

A Chinese crude drug "Tu Bei Mu", steamed and dried tubers of *Bolbostemma paniculatum* (Maxim.) Franquet (Cucurbitaceae), has been used in China as an anti-inflammatory agent for mastitis and an antidote for snake poison.

A suspension of a methanolic extract of the tubers in water was passed through a column of highly porous polymer resin to give a mixture of glycosides which was chromatographed on silica gel and then on reversed phase silica gel. This afforded a new glycoside named tubeimoside I (**1**), a white powder, $[\alpha]_D^{17}$ +14.6° (c=1.09, MeOH), $C_{63}H_{98}O_{29}\cdot 2H_2O$, yield 1.93%.

The ^{1}H- and ^{13}C-NMR of **1** showed the presence of five monosaccharide units. On acid hydrolysis, **1** afforded an aglycone (**2**), D-glucose, L-arabinose, L-rhamnose and D-xylose. The identification of these monosaccharides, including their absolute configuration, was established according to the method recently reported by Oshima *et al.*[1] The aglycone **2** was obtained as colorless needles (from MeOH-H_2O), mp 333-335°C, $[\alpha]_D^{17}$ +98.8° (c=0.43, pyridine). On inspection of the ^{13}C-NMR spectral data, **2** was proved to be identical with bayogenin, the oleanane type triterpene already isolated from *Castanospermum australe* Cunn. *et* Fras. as a minor sapogenin.[2]

The IR spectrum (in Nujol) of **1** revealed the presence of ester groups (1730cm^{-1}) and the ^{13}C-NMR spectrum (in pyridine-d_5) of **1** showed three ester carbonyl signals at δ 171.2, 171.4 and 176.0, the last of which can be assigned as the 28-*O*-glycosyl ester.[3] Mild alkaline treatment of **1** with 0.5% KOH-H_2O yielded **3** and a dicarboxylic

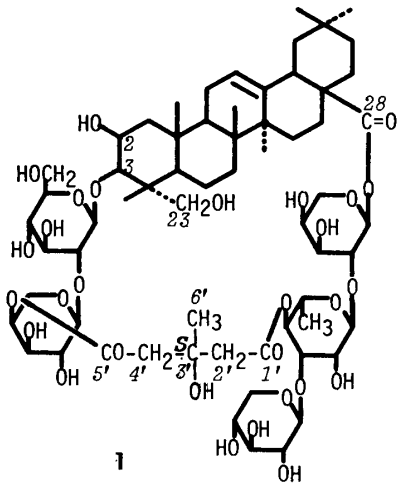
acid (**4**). The latter was identified as 3-hydroxy-3-methylglutaric acid by comparison of spectral data and GC-MS with an authentic sample.

In comparing the ^{13}C-NMR spectra (in pyridine-d_5) of **3** and **2**, glycosylation shifts[4] were observed for the signals due to C-2 (-1.1ppm), -3 (+9.5ppm), -23 (-3.0ppm)[4] and -28 (-4.0ppm)[3]. This indicates that **3** is a bisdesmoside of **2** with glycosyl linkage at both 3-OH and 28-COOH. The EI-MS spectrum of a trimethylsilyl (TMSi) ether of **3** exhibited fragment ions due to sugar moieties at *m/z* 915 (pentose-methylpentose-pentose), 727 (pentose-hexose), 639 (pentose-methylpentose) and 349 (terminal pentose). But there were no ions associated with the terminal hexosyl and the terminal methylpentosyl units. On selective cleavage of an ester glycoside linkage with LiI in a mixture of abs. MeOH and 2,6-lutidine,[5] **3** gave a monodesmoside **5** and a methyl trisaccharide **6**. On acid hydrolysis, **5** gave arabinose and glucose. The EI-MS spectrum of a peracetate of **5** exhibited the fragment ions characteristic of terminal arabinose (*m/z* 259) and arabinosyl-glucose (*m/z* 547). The sequencing analysis of a permethyl ether of **5** indicated the presence of a terminal arabinopyranosyl unit and a 2-linked glucopyranosyl unit. These results coupled with the inspection of carbon signals due to the sugar moiety (Table I) led to the formulation of **5** as shown in Chart 1. The sequencing analysis of a permethyl ether of **6** showed the presence of a 2-linked arabinopyranosyl unit, a 3-linked rhamnopyranosyl unit and a terminal xylopyranosyl unit. Coupled with the fragmentation of the EI-MS of TMSi ether of **3** (*vide supra*), **6** was formulated as methyl D-xylopyranosyl-(1→3)-L-rhamnopyranosyl-(1→2)-L-arabinopyranoside. By reference to the study on the unusual glycosylation shifts observed for the 2-linked arabinopyranosyl esters of oleanane type triterpenes,[6] the sugar carbon signals of **3** were assigned as shown in Table I. Based on these results, the structure of **3** was established as shown in Chart 1.

On very mild alkaline hydrolysis with 0.25% KOH-H_2O at 0°C, **1** gave **7**. The ^{13}C-NMR spectrum (in pyridine-d_5) of **7** showed signals due to two ester carbonyl carbons at δ 176.3 (28-glycosyl ester) and 171.7 as well as a signal due to a free carboxyl carbon at δ 174.9, indicating that one of two ester groups of the hydroxymethylglutarate moiety of **1** was saponified by this reaction. Hydrolysis of **1** with 0.5N BaO in MeOH at room temperature yielded a methyl ester (**8**) of **7**. The EI-MS spectrum of a TMSi ether of **8** exhibited the fragment ions associated with terminal pentose (*m/z* 349), arabinosyl-glucose (*m/z* 727) and (xylosyl-rhamnose)-OCO-CH_2-CCH_3(OH)-CH_2-$COOCH_3$ (*m/z* 797). This indicated that the hydroxymethylglutarate moiety of **8** (and **7**) must be located on either the rhamnosyl unit or the terminal xylosyl unit. Comparison of the ^{13}C-NMR spectrum of **7** with that of **3** (Table I) in view of the acylation shift[7] revealed the allocation of the acyl group at the 4-hydroxyl group of the rhamnosyl unit. Recently, R.-S. Xu and his co-workers also isolated **1** from the same crude drug as ours and found by 2D-NMR analysis that another ester linkage of the hydroxymethylglutarate moiety of **1** must be located at the 4-hydroxyl group of the terminal arabinosyl unit.[8,9]

Finally, the chirality of the asymmetric carbon of the acyl moiety of **1** was established as follows. In order to reduce free COOH of **7** to CH_2OH, a peracetate of **7** was treated with diborane in tetrahydrofuran. Alkaline saponification of the product gave mevalonolactone (**9**) and **3**. Hirai *et al.*[10] reported the micro-scale identification of the chirality of optically active mevalonolactone by conversion into 3(*R* or *S*)-5-*O*-acetyl-1-[(*R*)-phenylethyl]-mevalonamide (**10**) and subsequent HPLC analysis. We found that TMSi ethers (**11**) of the isomers of **10** can be distinguished from each

Chart 1



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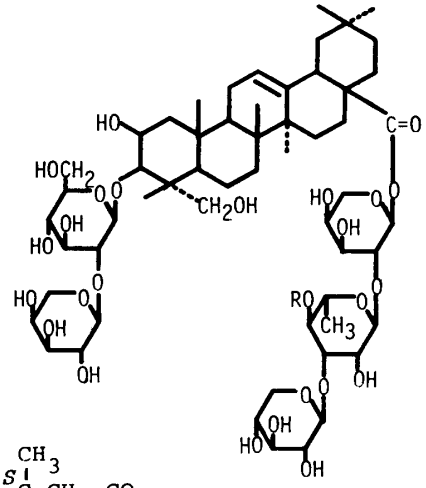
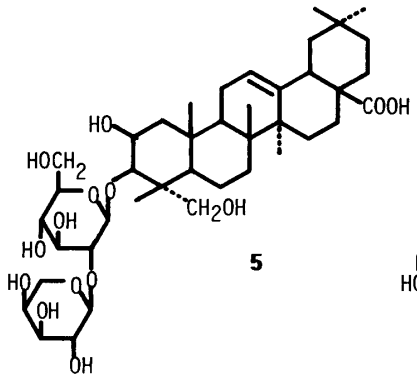


R

3 : H-

7 : HO-CO-CH_2-C(CH$_3$)(OH)-CH_2-CO-

8 : CH_3O-CO-CH_2-C(CH$_3$)(OH)-CH_2-CO-



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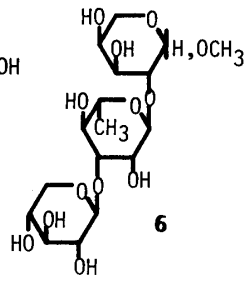

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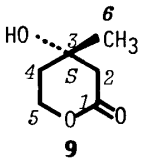

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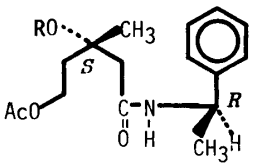
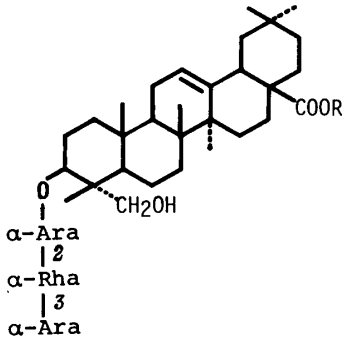


3(*S*)-**10**: R=-H

3(*S*)-**11**: R=-TMSi



α-Ara
|2
α-Rha
|3
α-Ara

12: R=-H

13: R=-β-Glc$\overset{2}{-}$β-Glc

Table I. ^{13}C-Chemical Shifts of Sugar Moieties in Pyridine-d_5

	3	5	7		3	7
3-O-				28-O-		
Glc-1	103.4	103.4	103.4	Ara-1	93.5	93.7
2	83.5	83.6	83.4	2	75.6	74.6
3	77.9[a]	78.0[a]	78.0	3	70.1	70.8
4	71.8[b]	71.8	71.6[b]	4	66.2	67.1
5	78.0[a]	78.1[a]	78.0	5	63.1	64.3
6	62.4	62.5	62.3			
				Rha-1	101.4	101.5
Ara-1	106.5	106.6	106.4	2	71.2[b]	72.0[b]
2	73.8	73.9	73.7	3	83.2	78.9
3	74.3	74.4	74.2	4	72.8	73.1
4	69.2	69.3	69.2	5	70.1	68.0
5	67.2	67.3	67.1	6	18.4	18.0
				Xyl-1	107.2	106.7
				2	75.0	75.0
				3	78.3[a]	78.0
				4	71.0[b]	71.2[b]
				5	67.2	67.1

a.b: These assignments may be reversed.

Table II. Solubilizing Effect of **1**, **3** and **13** (1mg/ml) on Saponin A (**12**) in H_2O

Compd.	A(mg/ml)	B(mg/ml)	C
None	1.6	0.017	-
13	24	16*	22
3	24	21*	28
1	30	30	44

A:amount of **12**, B:solubility of **12**, C:mol ratio of solubilized **12** to bisdesmosides, at 37°C, *:saturated soln.

Table III. Solubilizing Effect of **1**, **3** and **13** (1mM) on Yellow OB in Buffer

Compd.	solubility of Yellow OB
None	Insoluble
13	13.0μM
3	12.3μM
1	78.4μM

M/80 phosphate buffer (pH 6.5 ionic strength 0.02). at 37°C.

other by GC-MS (on a column packed with 5% SE-52 on Chromosorb-W). By this modified procedure, the chirality of the C-3 of **9** was found to be the *S*-configuration. Consequently, the structures of **1**, **7** and **8** including the stereochemistry of the acyl moiety were established as shown in Chart 1. The name, cyclic bisdesmoside is proposed for glycosides of this type.

Recently, it has been found that the solubility in water of the monodesmosides of pericarps of *Sapindus mukurossi* Gaertn., such as saponin A (**12**) is markedly increased by the bisdesmosides such as mukurozi-saponin Y_2 (**13**)[11] and the sesquiterpene-oligoglycosides[12] which co-occur in this plant. It was also found that the water solubility of a synthetic dye, Yellow OB was also increased by **13**.[13] As shown in Table II and Table III, **1** is a more potent solubilizer than non-cyclic bisdesmosides **3** and **13**. This indicates the significance of the cyclic structure of this type in the surface activities of saponins.

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