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SAPONINS FROM *BOLBOSTEMMA PANICULATUM*: CYCLIC BISDESMOSIDES, TUBEIMOSIDES II AND III

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Key Word Index—*Bolbostemma paniculatum*; Cucurbitaceae; Chinese folk medicine; 3-hydroxy-3-methylglutarate; tubeimosides I, II, III, cyclic bisdesmoside; bayogenin; polygalacic acid; saponin.

Abstract—The isolation and structural determination of tubeimosides II and III, new cyclic bisdesmosides from tubers of *Bolbostemma paniculatum* are reported.

INTRODUCTION

The Chinese cucurbitaceous folk medicine, 'Tu Bei Mu', the tubers of *Bolbostemma paniculatum* (Maxim.) Franquet, is used as an anti-inflammatory agent for mastitis and as an antidote for snake venoms. From this folk medicine, an oleanane saponin named tubeimoside I (**1**) was isolated and the structure was reported in preliminary communications [1, 2]. This is the first example of a saponin having a novel cyclic structure with a 3-hydroxy-3-methylglutarate bridge and the name 'cyclic bisdesmoside' was proposed for saponins of this type It was noted that compound **1** is a potent solubilizer [1]. The present paper deals with the isolation and structure determination of two additional new cyclic bisdesmosides named tubeimosides II (**2**) and III (**3**) from the same source The experimental details of the study on compound **1** are also described.

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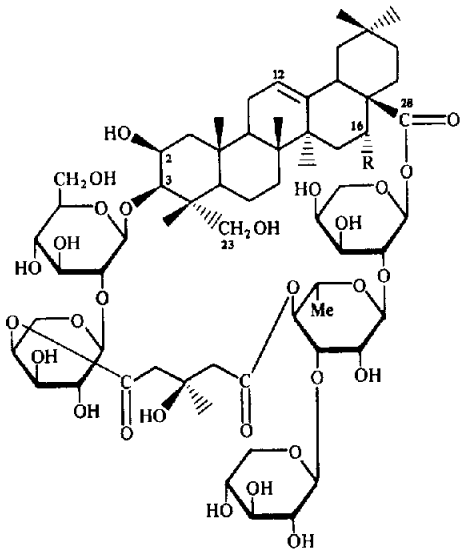
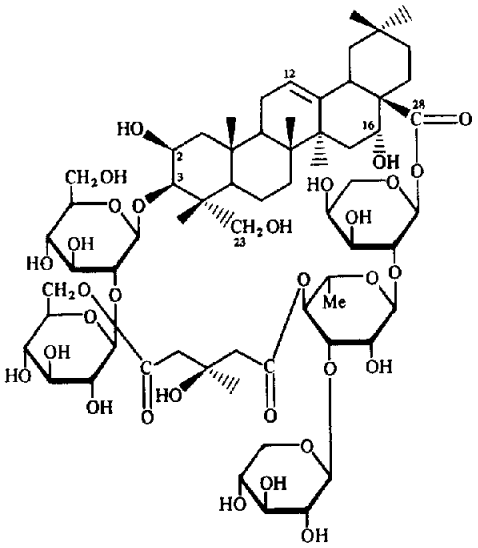
RESULTS AND DISCUSSION

A methanolic extract of the tubers was chromatographed on a column of highly porous polymer resin to give a mixture of saponins. The mixture was subjected to column chromatography on silica gel, reversed phase silica gel and finally on hydroxyapatite, affording compounds **1**–**3** in yields of 1.9, 0.5 and 1.0%, respectively



1 R = H
2 R = OH



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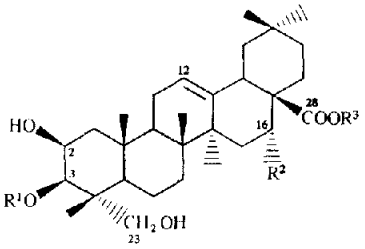
On mineral acid hydrolysis, compound **3** yielded an aglycone (**4**) and D-glucose, D-xylose, L-rhamnose and L-arabinose as sugar components. On the basis of spectral data, compound **4** was proved to be identical with polygalacic acid previously isolated from *Polygala paenea* [3] and *Platycodon grandiflorum* [4] as a sapogenin.

The ^{1}H and ^{13}C NMR spectra of compound **3** showed the presence of five monosaccharide units and an acyl moiety (Tables 1 and 2). Mild alkaline hydrolysis of **3** with 0.5% barium oxide–methanol and 0.5% potassium hydroxide–water yielded deacylated compound **5** and a dicarboxylic acid (**6**), respectively. The latter compound was identified as 3-hydroxy-3-methylglutaric acid. Comparison of the ^{13}C NMR spectrum of compound **5** with that of **4** showed the glycosylation shifts [5] for C-2, C-3, C-23 and C-28 (Table 1), indicating that **5** is a bisdesmoside of **4** with glycosyl linkage at both the 3-hydroxyl and 28-carboxyl groups. On selective cleavage of the ester glycoside linkage at C-28 with lithium iodide in methanol and 2,6-lutidine [6], compound **5** gave a monodesmoside (**7**) and a methyl glycoside (**8**). Acid hydrolysis of compound **7** yielded D-glucose. The ^{1}H and ^{13}C NMR spectra of compound **7** showed the presence of two β-glucosyl units. The EIMS of a peracetate of compound **7** exhibited the fragment ions characteristic of terminal glucose (*m/z* 331) and glucobiose (*m/z* 619). The methylation analysis [7] of the permethyl ether of compound **7** revealed the presence of terminal and 2-linked glucosyl residues. Consequently, compound **7** is formulated as the 3-*O*-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside (β-sophoroside) of compound **4**. The methyl glycoside (**8**) was identified as methyl β-D-xylopyranosyl (1→3)-α-L-rhamnopyranosyl (1→2)-L-arabinopyranoside by comparison of its ^{13}C NMR spectrum (Table 2) with that of an authentic sample obtained from **1** under the same conditions as above. The anomeric configuration of the arabinopyranosyl unit of compound **5** was confirmed as α based on the unusual ^{13}C NMR glycosylation shifts (Table 2) observed for the 2-linked arabinopyranosyl esters of the oleanane type triterpenes reported by Mizutani *et al* [8]. These results led to the formulation of compound **5** as shown.

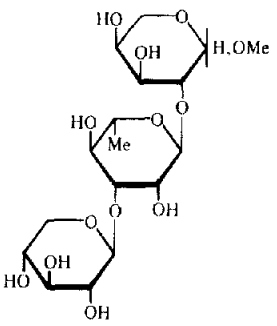
The location of the acyl moiety of compound **3** was elucidated from the following results. In the ^{13}C NMR spectrum of compound **3**, the signals due to the trisaccharide moiety at C-28 appeared at almost the same positions as those in the spectrum of **1** (Table 2). In addition, a proton signal of compound **3** in the range of an acylated carbinyl proton (δ 6.06) was assigned to H-4 of the rhamnose unit by means of ^{1}H ^{1}H 2D correlation spectroscopy (2D-COSY) as shown in Fig. 1. These results indicated that the 4-hydroxyl group of the rhamnose unit is linked with the acyl moiety.

In the ^{13}C NMR spectrum of compound **3** (Table 2), signals due to one of two hydroxymethyl carbons of the sophorosyl moiety appeared at lower field (δ 64.7) than the corresponding carbon signals in the spectrum of **5**, indicating that the 6-hydroxyl group of either the termi-



	R^1	R^2	R^3
4	H	OH	H
5	A	OH	B
7	A	OH	H
12	C	OH	B
13	H	H	H
14	C	H	B
15	C	H	H
16	C	OH	H

A -β-D-Glc(p)-[2-1]-β-D-Glc(p)
B -α-D-Ara(p)-[2-1]-α-L-Rha(p)-[3-1]-β-D-Xyl(p)
C -β-D-Glc(p)-[2-1]-α-L-Ara(p)



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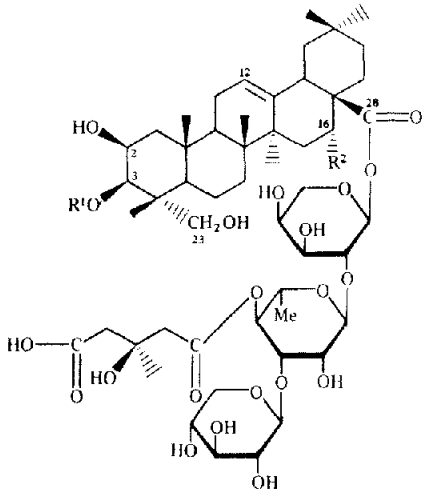
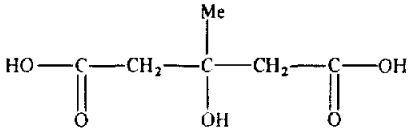


	R^1	R^2
9	H	OH
17	C	H
18	C	OH



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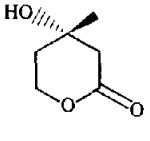

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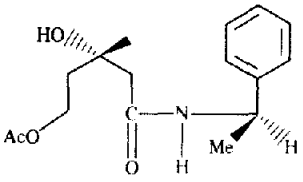


3(*S*)-**11**

Table 1 ^{13}C NMR chemical shifts of aglycone moieties in C_5D_5N

	1	14	17	15	13	2	12	18	16	4	3	5	7	9	6
C-1	44 1	44 1	44 0	44 1	44 9	44.3	44 2	44 2	44 2	44 9	44.1	44 0	44 0	45 0	
2	69 3	70 5	70 5	70 7	71 6	69 2	70.5	70 4	70 6	71.6	70.0	70 1	70 3	71 6	
3	82 9	82 5	82.5	82 5	73 0	83.6	82 6	82 8	82 6	73.2	82 9	83 0	83 0	73 2	
4	43 1	42 3	42.7	42 5	42 3	43 4	42 6	42.7	42 7	42.4	42 5	42 6	42 7	42 4	
5	48 6	48 5	48 5	48 5	48 5	47 9[a]	47 6[a]	47 9[a]	47 7[a]	48 3	47 7	48 1[a]	48.5	48 5	
6	18 6	18 1	18 2	17 9	18 3	19.0	18 0	18 4	17 9	18 3	18 7	18 1	18.1	18 6	
7	33 1	33 1	33 0	33 3	33.2	33 4	33 2	33 2	33 3	33 3	33 7	33 1	33 1	33 6	
8	40 4	40 0	40 1	39 9	39 9	40.4	40 2	40 3	40 1	40.1	40.3	40 1	40 0	40 3	
9	47 4[a]	47 7[a]	47 7[a]	47.7	48 1	47.3[a]	47 8[a]	47 7[a]	47 8[a]	47 7	47.7	47 6[a]	47 7	47 8	
10	37 2	36 9	36.9	37 0	37 2	37.4	36 9	37.0	37 0	37 3	37 0	36 9	37 0	37.3	
11	23 9	23 9	23 9	23 8	24 0	24.0	24 0	24 1	24 0	24 0	24 0	23 9	24 0	24 1	
12	122 7	123 1	122 5	122 7	122 7	122 7	123.0	122 5	122 5	122 6	122 9	122.9	122 6	123.0	
13	144 2	144 2	144 2	144 8	144.8	144 6	144 4	144 5	145 1	145 1	144 6	144 4	145 1	144 4	
14	41 8	42 7	42 5	42 3	42.4	41 8	42.2	42 4	42 3	42 3	42.1	42 1	42 3	42 4	
15	29 1	28 2	28 2	28 3	28.3	36 8	35 9	36 3	36 2	36 1	37 8	35.9	36 1	36 4	
16	22 7	23 2	23 0	23 8	23.8	75 0	74 6	74 1	74 8	74 8	73 4	73 9	74 8	74 0	
17	47 0[a]	47 3[a]	47 3[a]	46 7	46 6	49 1	49 6	49 5	48 9	48 9	49 3	49 5	48 9	49 6	
18	41 3	41 7	41 3	42 0	42.0	40 4	41.2	41 3	41 4	41 5	40 9	41.2	41 5	41 4	
19	46 3[b]	46 2	46.4[b]	46 4	46.4	46 6[b]	47.0	47.3[b]	47 2	47 2	46 5[a]	47 0	47 2	47 2	
20	30 8	30 8	30 8	31 0	30 9	30 8	30 8	30.9	31 0	31 0	30 8	30 8	31 0	30 9	
21	33 9	34 2	33 7	34 2	34 2	35.9	36 1	35 8	36 2	36 1	36 0	36 0	36 2	35 9	
22	32 2	32 7	32.6	33 0	33.0	32 4	32.0	32 2	32 8	32 8	32 4	31 9	32 9	32 1	
23	64 6	64 6	64.4	64 6	67.6	65 4	64.6	64 8	64 6	67 8	64.3	65 8	65 9	67 8	
24	15 6	14 7	14 6	14 7	14 5	15 8	14 6	14 7	14 7	14 6	15.3	14.7	14 8	14 5	
25	17 4	17 3[b]	17 3[c]	17 2[a]	17 3[a]	17 8[c]	17 4[b]	17 5	17 3[b]	17 4[a]	17 5[b]	17 3[b]	17 3[a]	17.5[a]	
26	17 4	17 6[b]	17 6[c]	17 5[a]	17 5[a]	17 9[c]	17 6[b]	17 5	17 5[b]	17 6[a]	17 8[b]	17 5[b]	17 6[a]	17 8[a]	
27	26 0[c]	26.1	26 1	26 3	26 2	27 5	27.2	27 3	27 3	27 2	27 3	27 1	27 3	27 3	
28	176 0	176 2	176 3	180 2	180.2	175 5	175 9	175 8	180 0	180 0	175 6	175 9	180 0	175 9	
29	33 1	33 1	33 1	33 3	33.2	33.2	33 2	33.2	33 3	33 3	33 1	33 1	33 3	33 2	
30	23 7	23 7	23 6	24 0	23 8	24 6	24 8	24 7	24 8	24.8	24 3	24 7	24.8	24 7	
C-1′	171 2[d]		171.7			171 2		172 0			171 2[c]			171 7	174 7
2′	46 6[b]		46.3[b]			46 9[b]		47 1[b]			47 2[a]			46 4[b]	46 4
3′	70 1		70 1			70 2		70.4			70 1			70 1	70 0
4′	47 9[b]		46 3[b]			47.7[b]		47 6[b]			46 9[a]			46 7[b]	46 4
5′	171 4[d]		174 9			171 4[d]		174.8			171 4[c]			174 8	174 7
6′	26.4[c]		28.2			26 1		28 2			26.0			28 3	28 3

[a–d] Signals may be interchangeable in each vertical column

nal or inner glucose unit is acylated. By the ^{13}C–^{1}H 2D-COSY spectrum of compound **3** (Fig. 2), this acylated carbinyl carbon signal was correlated to the proton signals at δ4.98 and 4.61 which were assigned to the H_2-6 of the terminal glucose unit by means of 2D-NOESY (Fig 3) and ^{1}H–^{1}H COSY (Fig. 1) In addition, the EIMS of a trimethylsilyl ether of compound **3** showed no fragment ion due to a terminal glucose unit, which was observed in the spectrum of a trimethylsilyl ether of **5**. It follows that in compound **3**, another carboxyl group of the acyl moiety must be linked to the 6-hydroxyl group of the terminal glucose unit

Finally, the chirality of the asymmetric carbon of the acyl moiety of compound **3** was determined as the *S*-configuration as follows. Enzymatic hydrolysis of **3** with β-glucuronidase gave compound **9**. The free carboxyl group of acetylated **9** was reduced with diborane and the product was saponified by alkali to give mevalonolactone (**10**). Hirai [9] reported the micro-scale identification of the chirality of mevalonolactone by HPLC analysis as 3(*R* or *S*)-5-*O*-acetyl-1[(*R*)-phenylethyl]-mevalonamide (**11**). We revealed that 3-*O*-trimethylsilyl ethers of the 3-epimers of compound **11** can be distinguished from each other by GC-MS By means of this modified procedure, the chirality of C-3 of compound **9** was found to be the *S*-configuration as in the case of **1**. Based on these results, the structure of compound **3** was established.

On mineral acid hydrolysis, compound **2** also afforded **4** as an aglycone and D-glucose, D-xylose, L-rhamnose and L-arabinose as sugar components. On mild alkaline saponification, compound **2** gave a deacylated compound (**12**) and 3-hydroxy-3-methylglutaric acid (**6**). In the ^{13}C NMR spectrum of compound **2**, signals due to the aglycone moiety were almost superimposable over those in the spectrum of **3**, while those associated with sugar and acyl moieties appeared at almost the same positions as those of **1** (Tables 1 and 2). This indicated that compound **2** must be a 16α-hydroxylated derivative of **1**. The allocation of the acyl moiety of compound **2** was

Table 2 ^{13}C NMR chemical shifts of sugar moieties in C_5D_5N

	1	14	17	15	2	12	18	16		3	5	7	9	8	
3-*O*-									3-*O*-						
Glc-1	103 0	103 4	103 4	103 7	103 3	103 4	103 3	103 5	glc-1	103 3	102 9	103 0			
-2	79 8	83 5	83.4	83 6	79 9	83 4	82 9	83 5	-2	83 9	83 2	83 6			
-3	78 7[a]	77 9[a]	78 0	78 0[a]	78 9[a]	78 0[a]	78 0[a]	78 0	-3	77 3[a]	78 2[a]	78 0[a]			
-4	71 4	71 8[b]	71 6[b]	71 3	71 5	71 7[b]	71 8[b]	71 3	-4	70 9	70 8[b]	71 2			
-5	78 5[a]	78 0[a]	78 0	78 1[a]	78 5[a]	77 9[a]	77 9[a]	78 0	-5	78 1[b]	77 9[a]	78 3[a]			
-6	62 5	62 4	62 3	62 5	62 5	62 3	62 3	62 4	-6	62 4	62 7[c]	62 5[b]			
Ara-1	104 4	106 5	106 4	106 6	104 6	106 4	106 0	106 5	Glc-1	105 5	105 5	105 8			
-2	73 7	73 8	73 7	73 9	73 8	73 7	73 7	73 8	-2	77 1[a]	76 4	76 8			
-3	72 5	74 3	74 2	74 4	72 6	74.2	73 7	74 3	-3	77 8[b]	78 1[a]	78 0[a]			
-4	72 3	69 2	69 2	69 3	72 3	69 2	69 3	69 3	-4	70 9	70 9[b]	71 2			
-5	64 6[b]	67 2	67 1	67 3	64 6[b]	67 1	67 3	67 2	-5	75 7	78 0[a]	78 2[a]			
28-*O*-									-6	64 7	62 3[c]	62 4[b]		α type	β type
Ara-1	94 0	93 5	93 7		94.1	93 4	93 8			94 5	93 4		93 8	103 7	101 1
-2	74 7	75 6	74 6		74 7	75 6	74 2			76 6	75 3[d]		75 2	76 8	78 9
-3	70 9	70 1	70 8		70 7	70 1	70 8			74 8	70 3		71 6	74 3	70 6[a]
-4	67 5	66 2	67 1		67 4	65 8	67 1			69 5	65 8		66 7	69 4	69 6[a]
-5	64 4[b]	63 1	64 3		64 5[b]	62 8	64 8			67 4[c]	62 5[c]		65 2	66 0	63 4
Rha-1	100 6	101 4	101 5		100 7	101 4	101 4			102 4	101 3		101 5	102 5	104 6
-2	72 4	71 2[b]	72 0[b]		72 3	71 2[b]	71 9[b]			72 5	71 5[b]		72.0	70 6	71 5
-3	78 0[a]	83 2	78 9		78 2[a]	83 0	78 8			78 2[b]	82 8		79 0	83 1	83 1
-4	73 2	72 8	73 1		73 3	72 7	73 2			73 4	72 5		73 2	71 6	72 8
-5	68 0	70 1	68 0		68 1	69 6	67 9			68 0	69 6		68 1	69 1	68 9
-6	18 3	18 4	18 0		18.3	18 4	18 0			18 3	18 3		18 0	17 8	18 3
Xyl-1	106 6	107 2	106 7		106 5	107 0	106 4			106 3	106 8		106 7	106 9	106 7
-2	74 6	75 0	75 0		74 7	75 3	75 3			74 8	75 4[d]		74 7	75 5	75 5
-3	78 5[a]	78 3[a]	78 0		78 5[a]	78 1[a]	77 9[a]			78 0[b]	77 9[a]		78 1	78 1	78 1
-4	70 9	71 0[b]	71 2[b]		70 9	70 9[b]	71 2[b]			70 9	71 3[b]		70 9	70 8	70 8
-5	66 9	67 2	67 1		66 9	67 1	67 5			67 0[c]	67 0		67 0	67 0	67 0
														-OMe	56 0	55 1

[a–d] Signals may be interchangeable in each vertical column

supported by means of 2D-NMR analysis The chirality of the acyl moiety of compound **2** was also assigned *S*-configuration by the same procedure as used for **1** and **3**. Consequently, the structure of compound **2** was formulated as shown

We have been informed recently that Dr R.-S. Xu and his group, have also isolated **2** and **3** from the same plant and reached the same conclusions regarding the structure elucidations [10].

EXPERIMENTAL

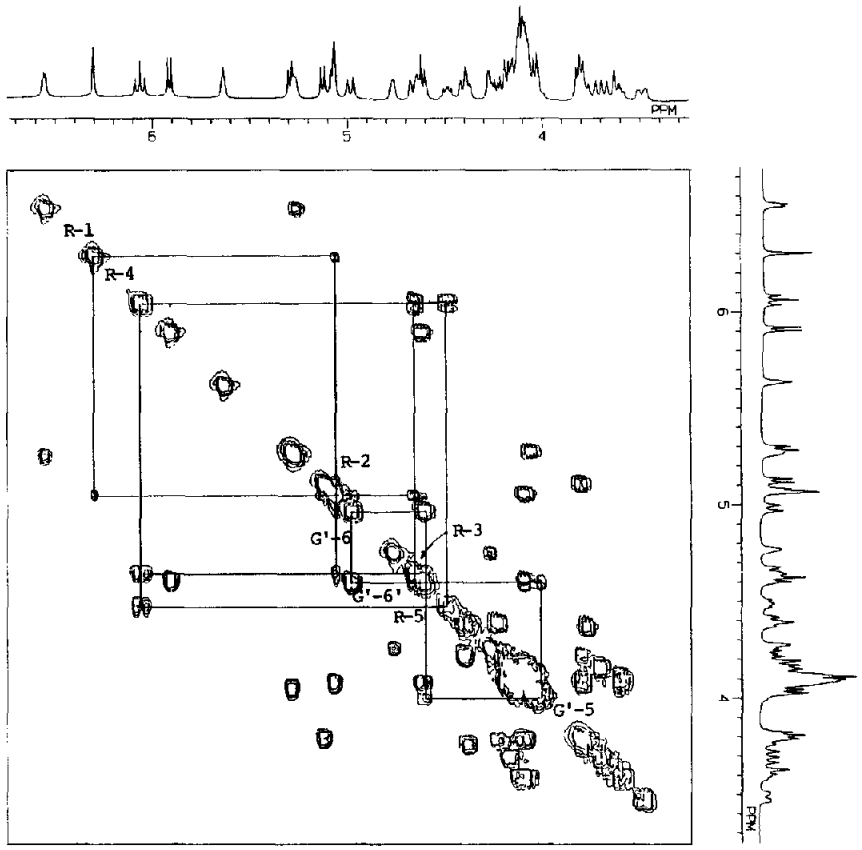
Mps uncorr ^{1}H NMR spectra were measured at 100, 270 and 400 MHz and ^{13}C NMR spectra at 25, 67 8 and 100 MHz The solvents used for spectral determination were, C_5D_5N–TMS (NMR), Nujol (IR), MeOH ($[\alpha]_D$), unless otherwise stated MS were recorded at 75 eV (EI) Acid hydrolysis of saponins followed by identification of the resulting monosaccharides including absolute configuration [11], and the methylation analysis of the sugar moieties by GC-MS were carried out as described in the previous paper [7] For CC, silica gel 60 and LiChroprep RP-8 were used The solvent system for CC on silica gel was $CHCl_3$–MeOH–H_2O (6 4 1 homogeneous)

Plant material The plant was collected at Shanxi (China) and identified by Kunming Institute of Botany, Chinese Academy of Science A specimen has been deposited in the Herbarium of this Institute

Extraction and separation The dried and powdered tubers (500 g) were extracted with hot MeOH and then hot 50% MeOH After removal of the solvents by evapn, the combined extracts (261 g) were chromatographed on highly porous polymer, DIAION HP-20 (Mitsubishi Chem. Ind Tokyo, Japan) (H_2O, MeOH and Me_2CO, successively) The MeOH eluate (50 g) was separated by CC on silica gel and then reversed-phase silica gel, LiChroprep RP-8 (65% aq MeOH and 58% aq MeOH) to give compound **1** (1 9%) and a mixture of **2** and **3** The mixture of compounds **2** and **3** was separated by CC on hydroxyapatite, PENTAX HP-40 (Asahi Optical Ind Tokyo, Japan) (80% aq MeCN) to give **2** and **3** in yields of 0 5% and 1 0%, respectively

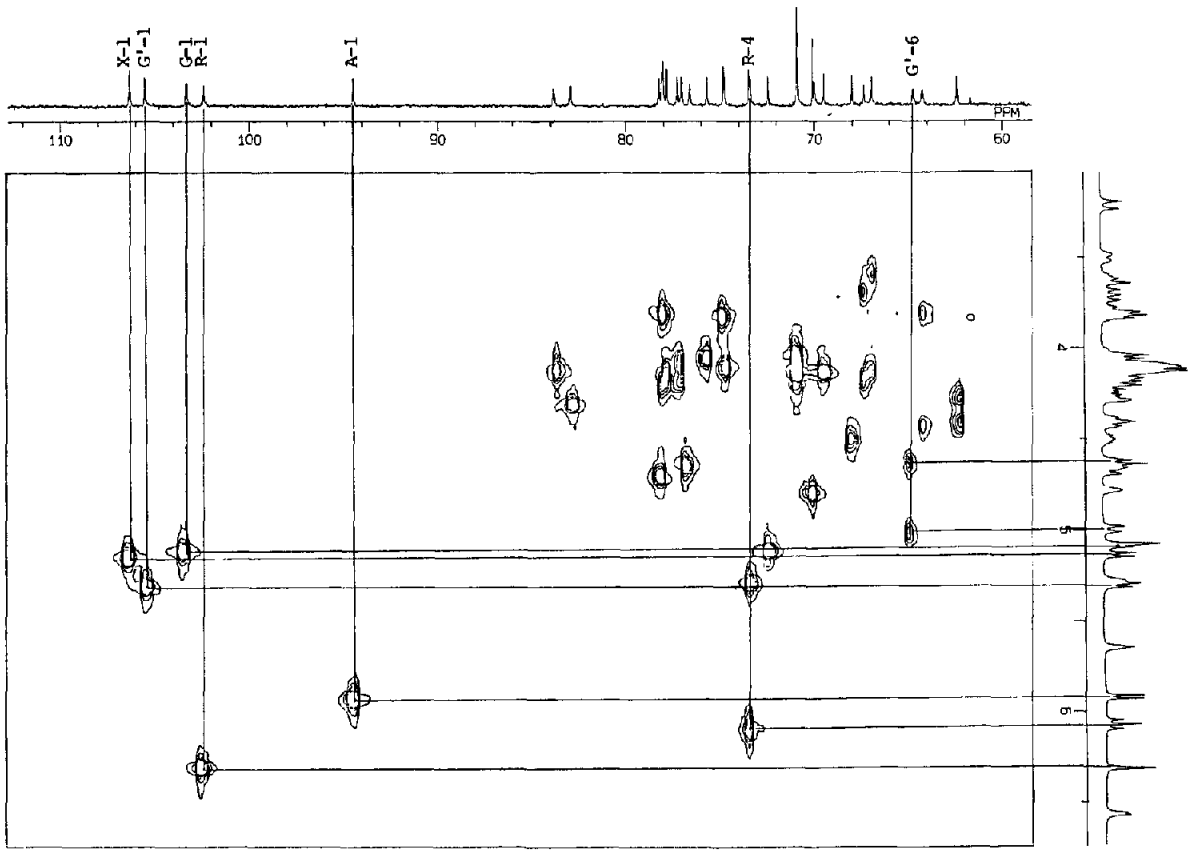
Tubeimoside I (**1**) A white powder, $[\alpha]_D^{17}$ + 14 6° (*c* 1 09) IR ν_{max} cm^{-1} 3300 (OH), 1740 (COOR), 1725 (COOR). FDMS *m/z* 1341 $[M+Na]^+$ EIMS (TMSi deriv) *m/z* 349 (Xyl) $TMSi_3$ ^{1}H NMR (270 MHz) δ 6 18 (1H, *s*, Rha H-1), 6 17 (1H, *d*, *J* = 3 3 Hz, ester Ara H-1), 5 59 (1H, *d*, *J* = 7 3 Hz Ara H-1), 5 06 (1H, *d*, *J* = 8 0 Hz, Glc H-1). 5 05 (1H, *d*, *J* = 8 0 Hz, Xyl H-1) (Found C, 55 89. H,7 56 $C_{63}H_{98}O_{29}$ $2H_2O$ requires C, 55 82, H, 7 58%)

Tubeimoside II (**2**) Colourless fine needles, mp 262 0–264 0° (from MeOH). $[\alpha]_D^{27}$ − 5 54° (*c* 0 99) IR ν_{max}cm^{-1} 3400 (OH), 1720 (COOR) FABMS (negative) *m/z* 1334 $[M]^-$ EIMS (TMSi deriv) *m/z* 349 (Xyl)$TMSi_3$ ^{1}H NMR (400 MHz) δ 6 21 (1H, *d*, *J* = 4 3 Hz, ester Ara H-1), 6 09 (1H, *s*, Rha H-1), 5 60 (1H, *d*, *J* = 7 0 Hz, Ara H-1), 5 06 (1H, *d*, *J* = 7 8 Hz, Glc H-1), 5 05



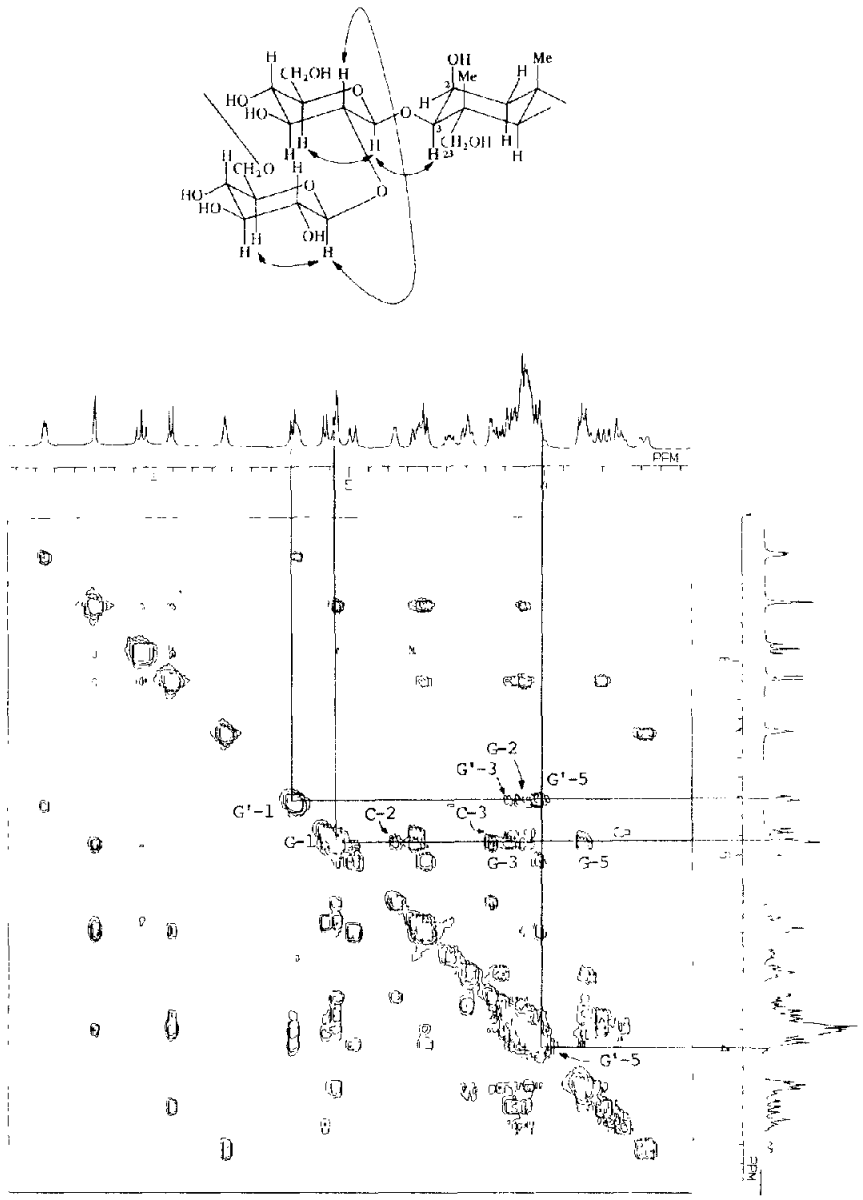
R=rhamnose, G'=terminal glucose

Fig 1.



X=xylose, G'=terminal glucose, G=inner glucose, R=rhamnose, A=arabinose

Fig. 2.



G'=terminal glucose, G=inner glucose, C=aglycone

Fig 3

(1H, *d*, $J=7.5$ Hz, Xyl H-1) (Found: C, 54.84, H, 7.50 $C_{63}H_{98}O_{30}$ $5/2H_2O$ requires C, 54.81, H, 7.52%)

Tubeimoside III (**3**) Colourless prisms, mp 261.0–262.0° (from MeOH) $[\alpha]_D^{18}$ +0.2° (*c* 1.07) IR $\nu_{max}cm^{-1}$ 3300 (OH), 1730 (COOR) FABMS (negative) *m/z* 1363 $[M-H]^-$ EIMS (TMSi deriv.) *m/z* 349 (Xyl) $TMSi_3$ ^1H NMR (400 MHz) δ 5.07 (1H, *d*, $J=8.0$ Hz, inner Glc H-1), 5.12 (1H, *d*, $J=7.3$ Hz, Xyl H-1), 5.29 (1H, *d*, $J=7.9$ Hz, terminal Glc H-1), 5.91 (1H, *d*, $J=7.3$ Hz, Ara H-1), 6.30 (1H, *s*, Rha H-1) (Found: C, 53.96, H, 7.56 $C_{64}H_{100}O_{31}$ $3H_2O$ requires C, 54.15, H, 7.53%)

Acid hydrolysis of saponins and identification of sapogenins A soln of **1** (287 mg) in N HCl–50% dioxane (20 ml) was refluxed for 3 hr. After cooling, deposited crystals were collected by filtration, washed with H_2O and recrystallized from MeOH–H_2O to give compound **13** as colourless needles (76 mg), which was identified as bayogenin by comparison of spectral and physical data with those of reference data [12].

Compound **13** Colourless needles, mp 333–335° (MeOH–H_2O) $[\alpha]_D^{17}$ +98.8° (C_5H_5N, *c* 0.43) IR $\nu_{max}cm^{-1}$ 3450 (OH), 1670 (COOH) ^1H NMR (100 MHz) δ 0.93, 1.00, 1.09, 1.26, 1.35, 1.58 (3H, each *s*, *t* Me × 6), 3.29 (1H, *dd*, $J_{18,19\alpha}=14$ Hz, $J_{18,19\beta}=4$ Hz), 3.69, 4.17 (each 1H, each *d*, $J=12$ Hz H-23), 4.25 (1H, *d*, $J=3.9$ Hz. H-3), 4.52 (1H, *m*, H-2), 5.29 (1H, *t-like*, H-12)

A soln of compound **2** (62 mg) in NH_2SO_4–50% EtOH (20 ml) was refluxed for 14 hr. After cooling, the soln was concd to 10 ml under red. pres., and the ppts formed were collected by filtration, washed with H_2O and purified by reprecipitation from EtOH–H_2O to give compound **4** as a white powder (17 mg). Compound **3** also afforded an aglycone **4**. Compound **4** was identified as polygalacic acid by comparison of the spectral and physical data with those of reference data [3, 4].

Compound **4** A white powder, $[\alpha]_D^{19}$ +40.9° (C_5H_5N, *c* 0.85). IR $\nu_{max}cm^{-1}$ 3350 (OH), 1670 (COOH). ^1H NMR (100 MHz) δ 1.04, 1.14, 1.18, 1.37, 1.64, 1.82 (3H, each *s*, *t*Me × 6), 3.70, 4.17 (each 1H, each *d*, *J* = 11 Hz, H-23), 4.28 (1H, *d*, *J* = 4.1 Hz, H-3), 4.56 (1H, *m*, H-2), 5.26 (1H, *br s*, H-16), 5.68 (1H, *t-like*, H-12)

Deacylation of saponins A soln of compound **1** (123 mg) in 0.5 N BaO–dry MeOH (20 ml) was stirred for 1 hr at room temp The reaction mixture was neutralized with ion exchange resin (Amberlite IR-120 B, H^+ form) and concd to dryness *in vacuo* The residue was chromatographed on silica gel to give compound **14** (38 mg) along with the monomethyl ester of **17** (**19**) (27 mg)

Compound **14** A white powder, $[\alpha]_D^{17}$ −5.8° (*c* 0.97). IR $\nu_{max}cm^{-1}$ 3350 (OH), 1725 (COOR) FDMS *m/z* 1215 $[M+Na]^+$ EIMS (TMSi deriv) *m/z* 915 (Ara–Rha–Xyl)$TMSi_7$, 639 (Rha–Xyl)$TMSi_5$, 727 (Glc–Ara)$TMSi_5$, 349 (Ara or Xyl)$TMSi_3$ (Found C, 54.65; H, 7.69, $C_{57}H_{92}O_{26}$ 7/2H_2O requires C, 54.49, H, 7.94%).

Deacylation of compounds **2** (206 mg) and **3** (101 mg) in the same way as above gave **12** (127 mg) and **5** (57 mg), respectively.

Compound **12** A white powder, $[\alpha]_D^{24}$ −19.9° (*c* 1.16) IR $\nu_{max}cm^{-1}$ 3400 (OH), 1725 (–COOR) EIMS (TMSi deriv.) *m/z* 915 (Ara–Rha–Xyl)$TMSi_7$, 737 (Glc–Ara)$TMSi_6$, 639 (Rha–Xyl)$TMSi_5$, 349 (Ara or Xyl)$TMSi_3$. ^1H NMR (270 MHz) δ 5.12 (1H, *d*, *J* = 7.0 Hz, Xyl or inner Glc H-1), 5.15 (1H, *d*, *J* = 7.0 Hz, inner Glc or Xyl H-1), 5.26 (1H, *d*, *J* = 7.3 Hz, terminal Glc H-1), 5.76 (1H, *s*, Rha H-1), 6.54 (1H, *d*, *J* = 3.5 Hz, Ara H-1) (Found C, 55.58, H, 7.95, $C_{57}H_{92}O_{27}$ H_2O requires: C, 55.78, H, 7.72%)

Compound **5** A white powder, $[\alpha]_D^{25}$ −26.9° (*c* 1.67) IR $\nu_{max}cm^{-1}$ 3300 (OH), 1720 (COOR) EIMS (TMSi deriv) *m/z*: 915 (Ara–Rha Xyl)$TMSi_7$, 829 (Glc–Glc)$TMSi_7$, 639 (Rha–Xyl)$TMSi_5$, 451 (Glc)$TMSi_4$, 349 (Xyl)$TMSi_3$ ^1H NMR (270 MHz) δ 5.15 (1H, *d*, *J* = 8.0 Hz, inner Glc H-1), 5.25 (1H, *d*, *J* = 8.0 Hz, Xyl H-1), 5.38 (1H, *d*, *J* = 8.0 Hz, terminal Glc H-1), 5.78 (1H, *s*, Rha H-1), 6.56 (1H, *d*, *J* = 3.5 Hz, Ara H-1) (Found C, 54.21, H, 7.69, $C_{58}H_{94}O_{28}\cdot 5/2H_2O$ requires C, 54.24, H, 7.77%).

Partial alkaline hydrolysis of compound **1** *and* **2** A soln of compound **1** (125 mg) in 0.25% aq KOH (20 ml) was stirred for 1 hr at 0° under N_2 atmosphere The reaction mixture was neutralized with Amberlite IR-120B (H^+ form) and concd to dryness *in vacuo* The residue was chromatographed on silica gel to give compounds **17** (43 mg) and recovered **1** (73 mg) Partial alkaline hydrolysis of **2** (115 mg) afforded **12** (37 mg) and **18** (52 mg).

Compound **17** A white powder, $[\alpha]_D^{20}$ +10.3° (*c* 1.00) IR $\nu_{max}^{KBr}cm^{-1}$ 3400 (OH), 1725 (COOR) FDMS (monomethyl ester) *m/z* 1373 $[M+Na]^+$ EIMS (monomethyl ester TMSi deriv.) *m/z* 797 [(Rha–Xyl)–OCO–CH_2–CMe(OH)–CH_2–COOMe]$TMSi_5$, 549 (Rha–Xyl)$TMSi_4$, 717 (Glc–Ara)$TMSi_6$, 349 (Ara or Xyl)$TMSi_3$ (Found C, 54.93; H, 7.75, $C_{63}H_{100}O_{30}$ 2H_2O requires: C, 55.09, H, 7.63%)

Compound **18** A white powder, $[\alpha]_D^{20}$ −13.7° (*c* 1.80) IR $\nu_{max}cm^{-1}$ 3250 (OH), 1720 (COOR) FABMS (negative) *m/z*. 1351 $[M-H]^-$ ^1H NMR (400 MHz) δ 5.03 (1H, *d*, *J* = 7.9 Hz, Xyl H-1), 5.13 (1H, *d*, *J* = 7.3 Hz, inner Glc H-1), 5.16 (1H, *d*, *J* = 6.7 Hz, terminal Glc H-1), 5.96 (1H, *s*, Rha H-1), 6.43 (1H, *d*, *J* = 3.7 Hz, Ara H-1) (Found. C, 54.18, H, 7.79, $C_{63}H_{100}O_{31}$ 2H_2O requires C, 54.45, H, 7.54%.)

Enzymatic hydrolysis of compound **3**. To a soln of compound **3** (46 mg) in acetate buffer (pH 5.0, 10 ml) was added β-glucuronidase (Sigma Co 1 ml) and the mixture was incubated for 5 days at 37°. The reaction mixture was dild with an equiv vol. of MeOH and passed through a column of Diaion HP-20 eluting with 50% aq. MeOH and MeOH, successively. The fraction eluted with MeOH was concd to dryness *in vacuo* The residue was chromatographed on silica gel to give compound **9** (22 mg)

Compound **9** A white powder, $[\alpha]_D^{21}$ −16.6° (*c* 1.00), IR $\nu_{max}cm^{-1}$ 3300 (OH), 1720 (COOR) FABMS (negative) *m/z* 1057 $[M-H]^-$, (positive) *m/z* 1081 $(M+Na)^+$ ^1H NMR (400 MHz) δ 5.03 (1H, *d*, *J* = 7.6 Hz, Xyl H-1), 5.98 (1H, *s*, Rha H-1), 6.38 (1H, *d*, *J* = 4.3 Hz, Ara H-1) (Found C, 56.64, H, 7.83, $C_{52}H_{82}O_{22}\cdot 5/2H_2O$ requires: C, 56.56, H, 7.94%)

Selective cleavage of the ester glycoside linkage of compounds **5**, **12** *and* **14** A soln of **14** (106 mg) and LiI (112 mg) in 2,6-lutidine (2 ml) and dry MeOH (2 ml) was refluxed for 16 hr under N_2 atmos. After cooling, the reaction mixture was dild with 50% aq MeOH (2 ml), deionized with Amberlite MB-3 (H^+, OH^- form) and evapd to dryness The residue was chromatographed on a column of silica gel to give compounds **15** (11 mg) and **8** (18 mg) together with **14** (66 mg, *vide supra*) By the same reaction, compound **12** (131 mg) afforded **16** (16 mg) and **8** (28 mg), and **5** (91 mg) gave **7** (17 mg) and **8** (27 mg) On the basis of EIMS, ^1H and ^{13}C NMR (Table 2) spectral data, as well as methylation analysis, compound **8** was formulated as methyl β-D-xylopyranosyl (1→3)-α-L-rhamnopyranosyl(1→2)-L-arabinopyranoside

Compound **8** ^1H NMR (100 MHz) δ α type; 6.02 (*s*, Rha H-1), 5.09 (*d*, *J* = 6.3 Hz, Xyl H-1). β type, 5.32 (*d*, *J* = 2.2 Hz, Ara H-1), 5.65 (*s*, Rha H-1), 5.09 (*d*, *J* = 6.3 Hz, Xyl H-1) Methylation analysis, 2-linked arabinopyranose unit, 3-linked rhamnopyranose unit, terminal xylopyranose unit

Compound **15** A white powder, $[\alpha]_D^{15}$ +55.1° (*c* 1.01), IR $\nu_{max}cm^{-1}$ 3350 (OH), 1690 (COOH). Methylation analysis, 2-linked glucopyranose unit, terminal arabinopyranose unit, EIMS (acetate) *m/z*, 547 (Glc–Ara)Ac_6, 259 (Ara)Ac_3 ^1H NMR (100 MHz) δ 5.17 (1H × 2, *d*, *J* = 6.6 Hz, Ara and Glc H-1) (Found C, 59.21, H, 8.35 $C_{41}H_{66}O_{14}$ 5/2H_2O requires C, 59.47, H, 8.64%)

Compound **16**. A white powder, $[\alpha]_D^{20}$ +32.8° (*c* 1.06), IR $\nu_{max}cm^{-1}$ 3250 (OH), 1670 (COOH) EIMS (monomethyl ester TMSi deriv) *m/z*, 737 (Glc–Ara)$TMSi_6$, 349 (Ara)$TMSi_3$. ^1H NMR (100 MHz) δ 5.14 (1H × 2, *d*, *J* = 7.3 Hz, Ara and Glc H-1) (Found C, 60.09, H, 8.50, $C_{41}H_{66}O_{15}$ H_2O requires C, 60.27; H, 8.39%)

Compound **7** A white powder, $[\alpha]_D^{17}$ + 22.2° (*c* 1.01), IR $\nu_{max}cm^{-1}$ 3300 (OH), 1680 (COOH). EIMS (acetate) *m/z*, 619 (Glc–Glc)Ac_7, 331 (Glc)Ac_4, Methylation analysis: 2-linked glucopyranose unit, terminal glucopyranose unit. ^1H NMR (100 MHz) δ. 5.12 (1H, *d*, *J* = 6.4 Hz, inner Glc H-1), 5.36 (1H, *d*, *J* = 6.3 Hz, terminal Glc H-1) (Found. C, 59.13; H, 8.48, $C_{42}H_{68}O_{16}$ 3/2H_2O requires C, 58.93; H, 8.36%)

Alkaline hydrolysis of saponins and identification of resulting dicarboxylic acid A soln of saponin in 0.5% aq KOH (80 ml) was stirred for 3 hr at 0° under N_2 atmosphere After acidification with dil HCl, the reaction mixture was conc to 40 ml *in vacuo* The soln was extracted with Et_2O by using an automatic extractor for 3 days The Et_2O extracts were conc to give a yellow gum, which was chromatographed on Sephadex LH-20 (MeOH) to give compound **6** as a colourless syrup which was identified as 3-hydroxy-3-methylglutaric acid by direct comparison of ^1H and ^{13}C NMR spectra and GC-MS analysis (dimethyl ester) with those of a commercial authentic sample. GC-MS conditions. 2 m × 2.6 mm glass column packed with 40% DEGS, He at 20 ml/min, isothermal, 165°, injection temp 230°, separator temp., 260°, ionizing voltage 70 eV

Compound **6** IR $\nu_{max}cm^{-1}$ 3250 (OH), 1690 (COOH) ^1H NMR (100 MHz) δ 1.80 (3H, *s*, *tert*-Me), 3.22 (4H, *s*, $-CH_2-$ × 2) GC-MS (dimethyl ester) (rel int) *m/z*. 175 $[M-Me]^+$ (9), 159 $[M-OMe]^+$ (5), 143 (30), 141 (14), 117 $[CMe(OH)-CH_2-COOMe]^+$ (100), 101 (38), 85 (69), 75 (13), 74 (17), 69 (10), 59 (15), 58 (14), 57 (11), 56 (51), 43 (100), 42 (21), 41

(10), 39 (10), 31 (25), 29 (28), 27 (29), 15 (24)

Reduction and alkaline hydrolysis of acetylated compound **9** *and identification of resulting mevalonolactone* (**10**) A soln of acetylated compound **8** (112 mg) in 0·1 M BH_3–dry THF (20 ml) was stirred at −18° for 30 min and at room temp overnight under Ar atmosphere To the reaction mixture was added H_2O (0·1 ml) at 0° and the mixture was taken to dryness The residue was subjected to repeated co-distillation with MeOH to remove H_3BO_3 A soln of the product in 2% KOH MeOH (20 ml) was stirred for 6 hr at room temp The reaction mixture was diluted with H_2O and acidified with 1 N HCl The mixture was conc to remove MeOH and then extracted with Et_2O for 4 days by using an automatic extractor The Et_2O layer was evapd to dryness to give crude mevalonolactone (**10**) (17 mg), which was converted to 5-*O*-acetyl-(phenylethyl)-mevalonamide (**11**) according to the method of Hirai [9], and then trimethylsilylated with 2·5% *N,O*-bis-trimethylsilylacetoamide (BSA) in dry MeCN The resulting TMSi ether of compound **11** was compared with the same derivative of authentic (*R*)- and (*S*)-mevalonolactone by GC-MS From the R_t [(*S*)-mevalonolactone, 40·5 min (*R*)-mevalonolactone, 42·5 min, **10**, 40·4 min], compound **10** was identified as (*S*)-mevalonolactone GC-MS conditions 2 m × 2·6 mm glass column containing 5% SE-52; He at 14 ml/min; isothermal, 220°, separator temp, 260°, injection temp 230°, ionizing voltage 70 eV

3(*S*)-5-*O*-*Acetyl*-3-*O*-*trimethylsilyl*-1-[(*R*)-*phenylethyl*-]-*mevalonamide* GC-MS (rel. int.) *m/z*: 365 $[M]^+$ (15), 350 $[M-15]^+$ (12), 290 $[M-AcOH-15]^+$ (3), 278 (14), 275 $[M-TMSiOH]^+$ (12), 215 $[M-TMSiOH-AcOH]^+$ (10), 186 (5), 174 (17), 143 (26), 132 (5), 120 (73), 119 (6), 117 (8), 116 (9), 115 (9), 112 (27), 106 (18), 105 (100), 104 (14), 103 (8), 79 (12), 77 (11), 75 (28), 74 (7), 73 (37), 69 (12), 68 (7), 45 (5), 43 (Ac, 30).

From compounds **1** and **2**, (*S*)-mevalonolactone was also identified by the same procedure

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