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Novel Triterpenoid Saponins from *Mimusops elengi*

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Abstract ---Two novel triterpenoid saponins, mimusopin {3-*O*-β-D-glucopyranosyl-2β, 3β, 6β, 23-tetrahydroxy-olean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)[α-L-rhamnopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside}(**1**) and mimusopsin {3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-gluco-pyranosyl]-2β, 3β, 6β, 23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside}(**2**) were isolated from the seeds of *Mimusops elengi*. Their structures were elucidated by a combination of 2D-NMR (COSY, HOHAHA, HETCOR, HMBC and NOESY), FAB-MS/MS and strategic chemical degradation. In addition, molecular mechanics and dynamics studies showed that the lack of a ^{13}C glycosylation shift at the C-4 of the inner rhamnose in **1** could be correlated with distortion in the corresponding torsion angles.

Mimusops elengi Linné (Sapotaceae) is a small to large evergreen tree, widely distributed throughout the greater part of India. The bark and fruit enjoy a considerable reputation in Indian medicine as an astringent and tonic and are used in the treatment of diarrhoea and dysentery.[1] In previous communications[2-4], the isolation and characterization of several pentacyclic triterpenes and the saponins, mimusopsides A and B, from the seeds of the plant have been reported. Further work on the saponins of the plant led to the isolation of two novel triterpenoid saponins designated as mimusopin (**1**) and mimusopsin (**2**) from the seeds of the plant.

RESULTS AND DISCUSSION

Mimusopin (**1**), an amorphous solid, $[\alpha]_D$ -17.9°, has a molecular formula of $C_{64}H_{104}O_{31}$, as determined from its high resolution positive ion FAB-MS (*m/z* 1381.6459 $[M+Na]^+$) and negative ion FAB-MS (1367.6483 $[M-H]^-$). Its spectral features and physiochemical properties suggested **1** to be a triterpenoid saponin. Of the 64 carbons displayed in the ^{13}C-NMR, 30 were assigned to the aglycone part and 34 to the oligosaccharide moiety (Tables 1 and 2). The six sp^3 quaternary carbons at δ 16.7, 18.4, 18.9, 23.7, 26.2, 33.1, and the two sp^2 hybrid carbons at δ 123.4 (d) and 143.5 (s) coupled with the information from ^1H NMR

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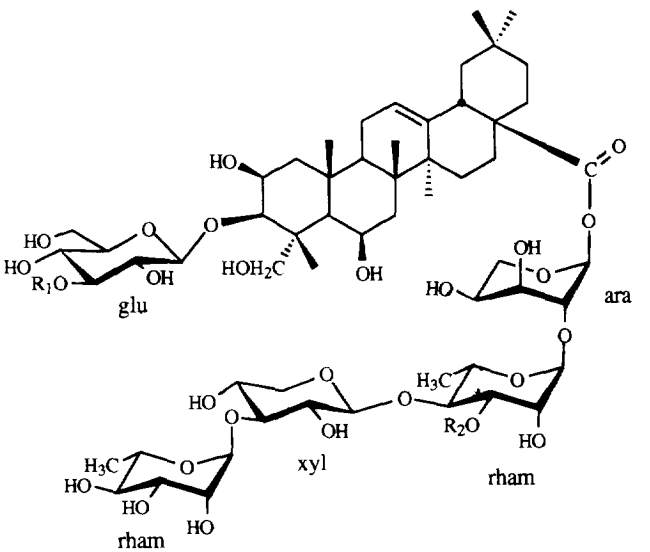
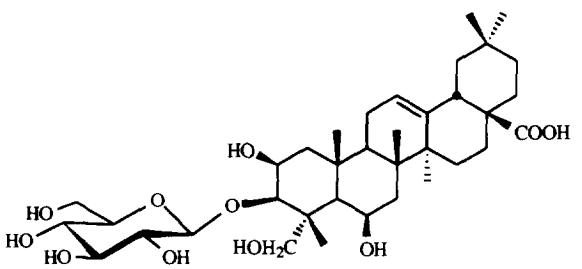
(six methyl proton singlets and a t-like vinyl proton at δ 5.54) indicated that the aglycone possesses an olean-12- ene skeleton. After an extensive 2D-NMR study, the aglycone was assigned to be protobassic acid (Table 1), a sapogenin already isolated from a number of sources.[5,6] Alkaline hydrolysis of **1** furnished the known prosapogenin, protobassic acid 3-*O*-β-D-glucopyranoside (**3**), based upon its spectral evidence.[7,8] Acidic hydrolysis of **1** under argon atmosphere yielded an artifactual aglycone, 2β, 3β, 23-trihydroxyolean-5,12-dien-28-oic acid (bassic acid, **4**), which was the dehydration product of protobassic acid.[9,10] The monosaccharides were shown to be L-arabinose, D-glucose, D-xylose and L-rhamnose (1:1:1:3) based on GLC analysis. The ^{1}H and ^{13}C NMR of **1** displayed six sugar anomeric signals at δ 4.99 (d, *J*=7.6 Hz), 5.07 (d, 7.9), 5.48 (s), 5.63 (s), 5.95 (s), 6.43 (s), and δ 92.9, 101.4, 102.6, 104.3, 104.9, and 105.4, respectively (Tables 2 and 3).



1 R_1= H R_2= Rham

2 R_1= Glu R_2= H



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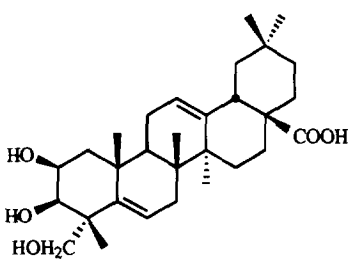

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The nature of the monosaccharides and the sequence of the oligosaccharide chain were determined by a combination of COSY, HOHAHA, DEPT, HETCOR, HMBC and phase-sensitive NOESY experiments. Starting from the anomeric protons of each sugar unit, all the hydrogens within each spin system were delineated using COSY with the aid of 2D-HOHAHA and NOESY spectra. Due to the highly overlapping nature of sugar proton signals within mainly a two-ppm region in the ^1H NMR spectrum, the assignment of all the sugar protons for a molecule like compound **1** was not an easy task. Information from COSY and 2D-HOHAHA furnished most of the assignment. A NOESY experiment, in addition to the NOEs across the glycosidic bonds, also revealed the 1,3 and 1,5-diaxial relationships for glucose and xylose residues, thus greatly simplifying the mapping of these two spin systems. On the basis of the assigned protons, the ^{13}C resonances of each sugar unit were identified by HETCOR and further confirmed by HMBC experiment. Interpretation of the COSY and 2D-HOHAHA spectra revealed the presence of six sugar units and three of them were assigned as α-L-rhamnoses from their typical patterns in the COSY spectrum. In the light of the assigned ^1H, and ^{13}C-NMR, the other three sugar units were identified as D-glucose, L-arabinose and D-xylose and confirmed by GLC analysis of the acid hydrolysate of **1**. From the above evidence, it was concluded that **1** was a bidesmosidic saponin with a glucose ether-linked to the C-3 position of the aglycone and the other five monosaccharides were connected to the C-28 position of the aglycone through an ester bond. The linkage of the sugar units at the side chain was established using the following HMBC correlations: H-1 of the terminal rhamnose (R') with the C-3 of xylose; H-1 of xylose with the C-4 of the inner rhamnose (R); H-1 of the other terminal rhamnose (R") with the C-3 of the inner rhamnose (R); and H-1 of the inner rhamnose (R) with the C-2 of arabinose, while the attachment of the pentasaccharide chain to the C-28 of the aglycone was based on a correlation between H-1 of arabinose and the C-28 of the aglycone (Figure 1).

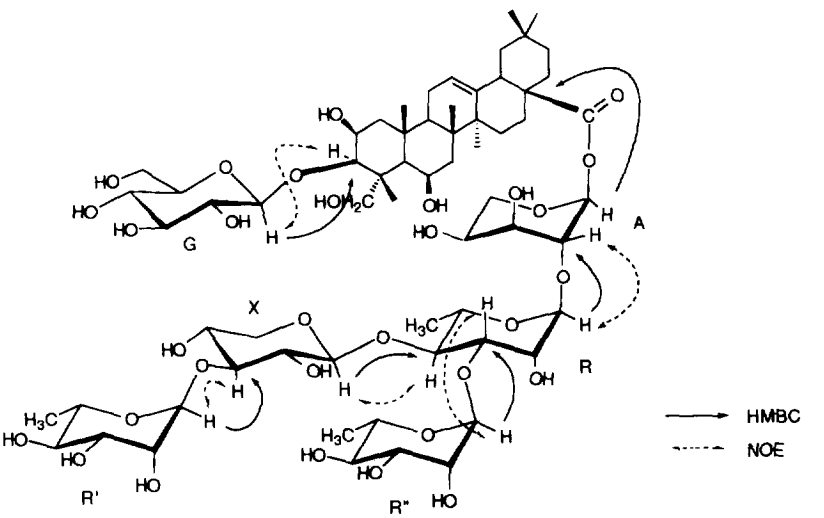


Fig. 1. Some key correlations of mimusopin (**1**) observed in HMBC and NOESY experiments.

The ^{13}C glycosylation shifts of these carbons also indicated that they were the linkage sites. The same conclusion was drawn from a NOESY experiment (Figure 1). The linkage assignment was further supported by the fragmentation patterns observed in the FAB-MS experiment. The negative FAB-MS/MS experiment on the deprotonated molecular ion $[M-H]^-$ (*m/z* 1367.6) provided the following structural information. In addition to the molecular ion ($[M-H]^-$, *m/z* 1367), the spectrum showed a daughter ion at *m/z* 1221 [$(M-H)^-$-146] from loss of one of the terminal rhamnoses and a prominent fragment at *m/z* 665 [$(M-H)^-$-702] due to the subsequent loss of a tetrasaccharide unit (Rham'-Xyl-Rham-Ara). Another ion at *m/z* 503 resulted from further loss of the glucose at C-3, corresponding to the aglycone [protobassic acid-H]$^-$. Other ions at *m/z* 483 and 455 from the dehydration and decarboxylation of the aglycone part were also observed. All the monosaccharides in the pyranose forms were determined from their ^{13}C NMR data. The β anomeric configurations for the glucose and xylose were based on their large $^3J_{H1,\,H2}$ coupling constants (7-8 Hz) and small $J_{C1,H1}$ coupling constants (159-160 Hz). The ^1H non-splitting patterns, the ^{13}C chemical shifts and large $J_{C1,H1}$ coupling constants (168-170 Hz) of the rhamnoses indicated α configurations. The anomeric proton of the arabinose was shown to be a broad singlet; thus no information was available from the ^1H NMR spectrum. However, a $J_{C1,H1}$ value of 171 Hz clearly indicated the presence of an α anomer and the NOESY experiment showed that there were NOEs between H-1, H-3 and H-5 and this could be used as an indication that the arabinose adopted an α configuration at its anomeric carbon.[11] The abnormality could be explained by the high conformational mobility of arabinopyranosides between $^4C_1$ and $^1C_4$ with the predominant $^1C_4$ conformation.[12,13] The absolute configurations of these monosaccharides were chosen in keeping with those mostly encountered among plant glycosides.

From the foregoing evidence, the structure of mimusopin (**1**) was elucidated to be 3-*O*-β-D-glucopyranosyl-2β, 3β, 6β, 23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)[α-L-rhamnopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

Mimusopsin (**2**), an amorphous solid, $[\alpha]_D + 30.7°$, has a molecular formula of $C_{64}H_{104}O_{32}$ based on its high resolution positive FAB-MS ion at *m/z* 1407.6408 $[M+Na]^+$ and negative FAB-MS 1383.6432 $[M-H]^-$. Its ^1H and ^{13}C NMR spectra indicated that compound **2** had the same aglycone as that of **1** but differed in the oligosaccharide part (Tables 1 and 2). On acidic hydrolysis, compound **2** afforded the same artifactual aglycone, bassic acid (**4**) as **1**, and the monosaccharides were identified to be xylose, glucose, rhamnose and arabinose in the ratio of 1:2:2:1 from GLC analysis. The overall structure assignment was accomplished using the same protocol as in **1**. The presence of six sugars in **2** was indicated from the six anomeric protons (δ 5.00, 5.09, 5.15, 5.66, 6.06 and 6.38) and carbons (δ 93.3, 101.1, 102.5, 105.1, 105.7 and 106.2). Interpretation of the COSY and HOHAHA spectra revealed the presence of six sugar units. Two of them were easily identified to be rhamnoses from their distinct COSY patterns. The other four were assigned to be two glucoses, one xylose, and one arabinose based upon their assigned ^1H, ^{13}C chemical shifts as well as evidence from acid hydrolysis. From the assigned aglycone (Table 1), it was apparent that the six sugars were present in two saccharide units, one attached to C-3 and the other at C-28. The sugar arrangement was initially determined by the fragmentation patterns observed in the negative FAB-MS/MS experiment. When the deprotonated molecular ion $[M-H]^-$ at *m/z* 1383 was subjected to MS/MS analysis, the daughter ion spectrum provided the following structural information. In addition to the molecular ion $[M-H]^-$ at *m/z* 1383, daughter ions at *m/z* 1237 [$(M-H)^-$-146] and 1221 [$(M-H)^-$-162] from loss of the terminal rhamnose and glucose, respectively, were observed.

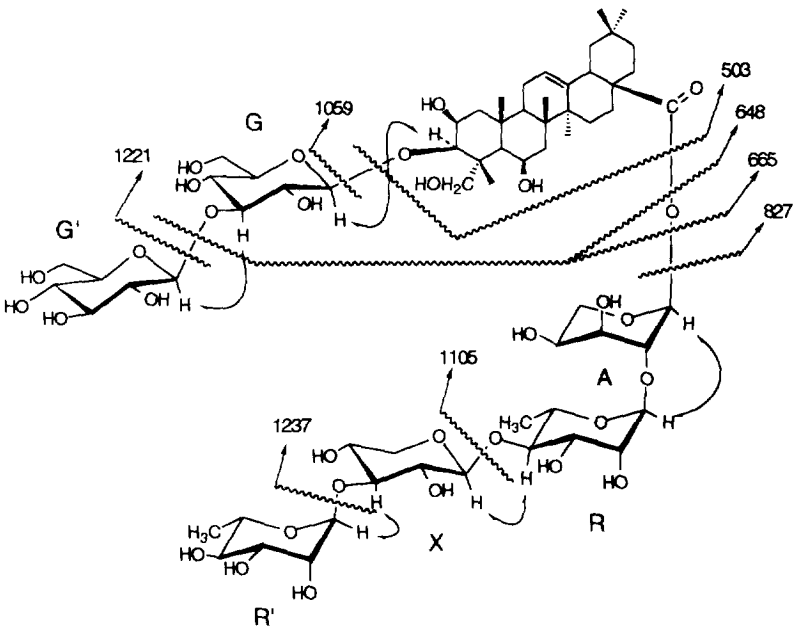


Figure 2. The fragmentation pattern (*m/z*) observed in negative FAB-MS/MS and interresidue NOEs (◄——►) from a phase-sensitive NOESY.

Other ions at *m/z* 1105 [(M-H)$^-$-278] and 1059 [(M-H)$^-$-324] corresponded to the loss of the terminal disaccharide Rham-Xyl and Glu-Glu'. A relatively strong ion at *m/z* 827 resulted from the loss of a tetrasaccharide unit (Rham'-Xyl-Rham-Ara, *m/z* 556) from the deprotonated molecular ion. Additional ions observed at *m/z* 665 and 503 were due to the further loss of a glucose or a Glu-Glu' fragment (corresponding to the aglycone [protobassic acid-H]$^-$) from the *m/z* 827 fragment. The foregoing fragmentation patterns indicated that the two glucoses were in one unit and the other four monosaccharides were in a linear chain with the Rham-Xyl unit as a terminal segment.

The exact linkage position for the tetrasaccharide unit was established using the following HMBC correlations: H-1 of the terminal rhamnose (R') with the C-3 of xylose; H-1 of xylose with the C-4 of the inner rhamnose (R); and H-1 of the inner rhamnose (R) with the C-2 of arabinose. The attachment of the tetrasaccharide chain to the C-28 of the aglycone was based on a correlation between H-1 of arabinose and the C-28 of the aglycone. The Glu'1→3 Glu arrangement at C-3 position was also identified from the same HMBC experiment. Supporting conclusion was inferred from a phase-sensitive NOESY experiment (Figure 2).

The stereochemistry of each anomeric carbon was determined from the same observation as that of **1**. Thus, mimusopsin (**2**) was elucidated to be 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-2β, 3β,6β,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamno-pyranosyl-(1→2)-α-L-arabinopyranoside.

Table 1. ^{13}C NMR Data for Aglycone Moieties (125 MHz in pyridine-d_5-methanol-d_4 (11:1))*

Carbon	1	2	3	Carbon	1	2	3
1	46.4 t	46.4 t	46.5 t	16	23.3 t	23.3 t	23.6 t
2	70.6 d	70.8 d	70.5 d	17	47.4 s	47.4 s	48.6 s
3	83.0 d	83.1 d	83.0 d	18	41.8 d	41.8 d	41.9 d
4	43.8 s	43.8 s	43.9 s	19	46.3 t	46.3 t	46.3 t
5	48.7 d	48.7 d	48.9 d	20	30.9 s	30.9 s	30.7 s
6	67.4 d	67.5 d	67.3 d	21	34.2 t	34.2 t	34.1 t
7	41.1 t	41.0 t	40.8 t	22	32.8 t	32.8 t	33.1 t
8	39.4 s	39.4 s	39.1 s	23	65.2 t	65.1 t	65.1 t
9	49.1 d	49.1 d	49.0 s	24	16.7 q	16.6 q	16.4 q
10	36.8 s	36.8 s	36.6 s	25	18.4 q	18.4 q	18.7 q
11	24.1 t	24.1 t	23.9 t	26	18.9 q	18.9 q	18.3 q
12	123.4 d	123.5 d	123.4 d	27	26.2 q	26.2 q	26.1 q
13	143.5 s	143.5 s	144.0 s	28	176.2 s	176.2 s	179.9 s
14	42.8 s	42.8 s	42.7 s	29	33.1 q	33.1 q	33.0 q
15	28.1 t	28.2 t	28.1 t	30	23.7 q	23.7 q	23.5 q

*Assignments based upon COSY, HOHAHA, HETCOR, DEPT, HMBC and NOESY experiments.

From the completely assigned ^{13}C NMR data of compounds **1** and **2**, we found a very interesting phenomenon. The C-3 of the inner rhamnose (designated as R) in **2** was substituted by the third rhamnose (designated as R") as in the case of compound **1**; the C-4 chemical shift of the inner rhamnose was experienced as an upfield shift of as much as 6.6 ppm. In addition, we also noticed that some unusual NOEs existed between H-2 of the third rhamnose (R") and H-1 of xylose, and H-1 of the third rhamnose (R") and H-2 of the inner rhamnose (R) besides the NOEs across the glycosidic bonds in compound **1**. The through-space interactions suggested that the substitution of the third rhamnose caused the branching point to be very crowded. These anomalies prompted us to carry out molecular mechanics and molecular dynamics studies on the two compounds. In this way, we hoped that we could explain this phenomenon from a deeper theoretical basis. The starting geometries of compounds **1** and **2** were generated by the Metropolis Monte Carlo search method[18] and submitted to energy minimization using the Discover-cff91 force field program.[20] The local minima, thus found, were taken as starting structures for molecular dynamic calculation *in vacuo* at 300 K. The calculation results showed that the glycosidic torsion angles ϕ and ψ (defined as H_1-C_1-O_1-C_x and C_1-O_1-C_x-H_x, respectively) of the preferred conformations of compounds **1** and **2** (Figure 3) were obviously different from the xylose1→3rhamnose fragments (Table 4). The conformations of the remaining parts were essentially the same. Previous studies[14] showed that the chemical shifts of carbon atoms on either side of the glycosidic linkage may be up to 12 ppm, depending on the conformation of the linkage. Various theoretical rationalizations concerning this effect have been postulated; namely the anomeric effect,[15] exo-anomeric effect,[16] and pseudo-anomeric effect.[14] These effects were believed to originate from the interactions of the lone-pair electrons on the ring or bridging oxygen with their neighboring carbons.

Table 2. ^{13}C NMR Data for the Sugar Units (125 MHz in pyridine-methanol-d_4 (11:1))*

Sugar unit	1	(J_{CH} Hz)	2	3
C_3-Glucose (G)				
G-1	105.4	(160)	105.1	105.2
G-2	75.4		74.0	75.2
G-3	78.4		88.6	78.1
G-4	71.5		69.6	71.2
G-5	78.1		77.7	77.9
G-6	62.6		62.2	62.4
Glucose (G')				
G'-1			105.7	
G'-2			75.3	
G'-3			78.5	
G'-4			71.5	
G'-5			78.0	
G'-6			62.4	
C_{28}-Arabinose (A)				
A-1	92.9	(171)	93.3	
A-2	75.8		75.4	
A-3	69.6		70.3	
A-4	65.4		66.0	
A-5	62.1		63.1	
Rhamnose (R)				
R-1	101.4	(170)	101.1	
R-2	71.8		72.4	
R-3	81.2		72.4	
R-4	76.9		83.5	
R-5	69.0		68.6	
R-6	18.4		18.2	
Xylose (X)				
X-1	104.9	(159)	106.2	
X-2	75.2		75.9	
X-3	83.9		83.4	
X-4	69.5		69.2	
X-5	66.7		65.1	
Rhamnose (R')				
R'-1	102.6	(170)	102.5	
R'-2	72.2		72.2	
R'-3	72.4		71.9	
R'-4	73.9		73.9	
R'-5	69.8		69.8	
R'-6	18.6		18.5	
Rhamnose (R")				
R"-1	104.3	(168)		
R"-2	71.9			
R"-3	72.7			
R"-4	73.9			
R"-5	70.0			
R"-6	18.4			

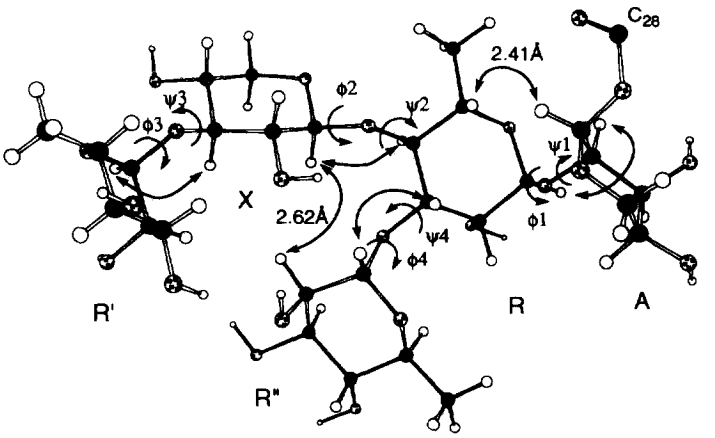
*Assignments based upon COSY, HOHAHA, HETCOR, DEPT and HMBC experiments.

Table 3. ^{1}H NMR Data for the Sugar Units (500 MHz in pyridine-d_5-methanol-d_4 (11:1))*

Sugar unit	1	2
C_3-Glucose (G)		
G-1	5.07 d (7.9)	5.09 d (7.0)
G-2	3.93	3.98
G-3	4.05	4.02
G-4	3.99	4.03
G-5	3.82 m	3.77 m
G-6	4.39	4.30
	4.21	4.17
Glucose (G')		
G'-1		5.15 d (7.9)
G'-2		3.98
G'-3		3.94
G'-4		4.09
G'-5		4.15
G'-6		4.45
		4.22
C_{28}-Arabinose (A)		
A-1	6.43 s	6.38 d (3.7)
A-2	4.37	4.45
A-3	4.43	4.40 m
A-4	4.34	4.30
A-5	4.47	4.44
	3.86 dd (10.4, 4.3)	3.87 dd (10.7, 3.7)
Rhamnose (R)		
R-1	5.48 s	5.66 s
R-2	4.56 m	4.46
R-3	4.39	4.46
R-4	4.36	4.24
R-5	4.24	4.32
R-6	1.61 d (6.1)	1.68 d (6.1)
Xylose (X)		
X-1	4.99 d (7.6)	5.00 d (7.6)
X-2	3.78	3.95
X-3	4.01	4.10
X-4	3.96	4.02
X-5	4.05	4.15
	3.28 dd (10.8, 10.3)	3.42 dd (11.0, 10.7)
Rhamnose (R')		
R'-1	5.95 s	6.06 s
R'-2	4.61 m	4.68 m
R'-3	4.41 m	4.47
R'-4	4.12 m	4.20
R'-5	4.71 m	4.81 m
R'-6	1.58 d (5.8)	1.63 d (6.4)
Rhamnose (R")		
R"-1	5.63 s	
R"-2	4.81 m	
R"-3	4.42	
R"-4	4.12	
R"-5	4.55 m	
R"-6	1.50 d (6.4)	

*Assignments based upon COSY, HOHAHA, HETCOR, DEPT, HMBC, and NOESY experiments. Coupling constants (in Hz) are given in parenthesis.

Such interactions may cause appreciable distortion in the involved torsion angles, resulting in a change in the electron density around pertinent carbon atoms. Evidence showed that the deshielding effects, causing a lack of glycosylation, were highly sensitive to the changing of the conformation of the atoms about the glycosidic linkage.[17] Results from our molecular modeling study and NOESY experiment showed that the non-bonded interactions among the sugars, arising from the substitution of the third rhamnose distorted the torsion angles between xylose1→3rhamnose and resulted in the abnormality observed in ^{13}C NMR spectrum. Therefore, the lack of a glycosylation shift at C-4 of xylose in **1** could be correlated with the change in corresponding torsion angles. Due to the limited models of such compounds, we could not explain the specific relationship between glycosidic conformations and ^{13}C chemical shifts. However, we suggested that application of the traditional rule of glycosylation shifts should be treated with caution, especially in the structural study of oligosaccharides with rhamnose involved in the branching points.



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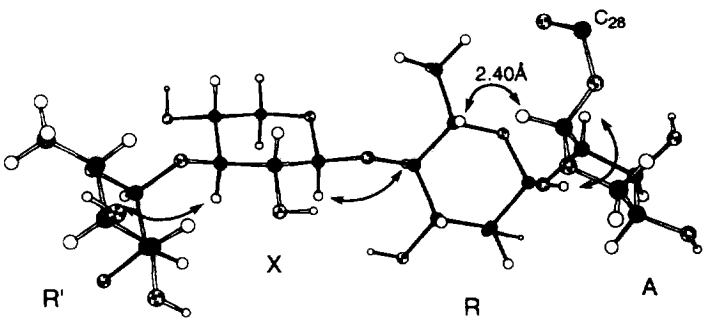

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Figure 3. The calculated preferred conformations of compounds **1** and **2**
(⟷ : NOEs from phase-sensitive NOESY)

Table 4. Selected torsion angles (°) for the saccharide part of saponins 1 and 2

ϕ	1	2	$\Delta\phi$ (1-2)	ψ	1	2	$\Delta\psi$ (1-2)
1	54.9	56.1	-1.2	1	19.4	16.4	3.0
2	36.3	46.1	-9.8	2	22.3	8.5	13.8
3	-42.3	-43.9	1.6	3	-13.3	-14.7	1.4
4	44.8			4	-12.3		

EXPERIMENTAL

***General Procedures*:** IR spectra were determined using a JASCO 7300 FTIR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. FABMS were conducted using JMS-SX-102A mass spectrometer. ^{1}H and ^{13}C NMR were recorded using a JEOL α-500 FT-NMR or a JEOL EX-400 FT-NMR spectrometer. Chemical shifts were expressed in δ (ppm) referring to solvent peaks: δ_H 7.20 and δ_C 135.50 for pyridine-d_5. TLC was carried out on silica gel 60 F_{254}, and spots were visualized by spraying with 10 % H_2SO_4. Diaion HP-20 (Mitsubishi Chemicals) and silica gel (Silica gel 60, Merck) were used for column chromatography. Preparative HPLC was performed using an ODS column (Capcell pak ODS, Shiseido, 10 mm i.d.x 250 mm, detector: UV 210 nm or reflective index). GLC: 2% SE-30 on Chromsorb W (60-80 mesh), 3 mm i.d. x 1.5 m, 150°C column temperature, N_2 carrier gas, 15 ml/min flow rate.

Extraction and isolation of the triterpenoid glycosides. The air dried powdered seeds of *M.elengi* (2 kg) were successively extracted with petroleum ether (60-80°C), $CHCl_3$ and MeOH under reflux conditions. The MeOH extract was partitioned between *n*-BuOH and H_2O. The organic layer was concentrated to dryness under reduced pressure to give a residue (30 g) which was chromatographed on silica gel (500 g). Elution was carried out with $CHCl_3$ followed by various mixtures of $CHCl_3$-MeOH and mixtures of $CHCl_3$-MeOH and according to their TLC behavior. Early fractions eluted with $CHCl_3$-MeOH (17:3) yielded previously reported minusopsides A and B.[4] The more polar fractions eluted with $CHCl_3$-MeOH (3:1) were combined and was applied to a column of Diaion HP (150 g) and washed with H_2O, 30, 40, 60, 80 and 100% MeOH to give 17 fractions. The fractions were again chromatographed on silica gel by medium pressure LC (solvent system: $CHCl_3$-MeOH-H_2O (6:4:1), flow rate 0.6 ml/min, detection RI) and then purified by ODS medium pressure LC (Lichroprep Rp-18, solvent system MeOH-H_2O (7:3), flow rate 0.5 ml/min, detection UV 210 nm). Further purification by HPLC on ODS with MeOH-H_2O (7:3) afforded mimusopin (**1**, 50 mg) and mimusopsin (**2**, 15 mg).

Mimusopin (1). An amorphous solid, $[\alpha]_D^{21}$ -17.9° (MeOH;*c*=0.12). IR ν^{KBr}max cm^{-1}: 3430, 2936, 1731, 1045. High resolution FAB-MS ($C_{64}H_{104}O_{31}$): *m/z* 1391.6459 $[M+Na]^+$ (in positive mode) and *m/z* 1367.6483 $[M-H]^-$ (negative). FAB-MS/MS (negative): *m/z* 1367 $[M-H]^-$, 1221[(M-H)$^-$-146], 797, 665, 503, 483, 455. ^{1}H-NMR (pyridine-d_5, 500 MHz): δ 0.93, 1.02, 1.24, 1.58, 1.88, 2.01 (each 3H, s, H_3 of C-30, C-29, C-27, C-25, C-24, C-26), 4.22 (1H, m, H-3), 4.78 (1H, m, H-2), 5.05 (1H, m, H-6), 5.54 (1H, t-like, H-12). ^{1}H-NMR data for the sugar moiety are given in Table 3. ^{13}C-NMR data: Tables 1 and 2.

Mimusopsin (2). An amorphous solid, $[\alpha]_D^{21}$ +30.7° (MeOH;*c*=0.08). IR ν^{KBr}max cm^{-1}: 3440, 2925, 1731, 1649, 1047. High resolution FAB-MS ($C_{64}H_{104}O_{32}$):*m/z* 1407.6408 $[M+Na]^+$ (positive) and 1383.6432 $[M-H]^-$ (negative). FAB-MS/MS (negative): *m/z* 1383 $[M-H]^-$, 1236[$(M-H)^-$-146], 1221[$(M-H)^-$-162], 1059[$(M-H)^-$-234], 1105[$(M-H)^-$-278], 827[$(M-H)^-$-556], 665, 648, 503, 483, 457. ^{1}H-NMR (pyridine-d_5, 500 MHz): δ 0.90, 1.01, 1.25, 1.60, 1.92, 2.13 (each 3H, s, H_3 of C-30, C-29, C-27, C-25, C-24, C-26), 3.30 (1H, dd, *J*=12.5,4.0 Hz, H-18), 4.28 (1H, m, H-3), 4.80 (1H, m, H-2), 5.10 (1H, m, H-6), 5.54 (1H, t-like, H-12). ^{1}H-NMR data for the sugar moiety are given in Table 3. ^{13}C-NMR data: Tables 1 and 2.

Alkaline hydrolysis of mimusopin (1) . A solution of **1** (30mg) in 0.5 M KOH was refluxed for 3 hr. After cooling, the reaction mixture was adjusted to pH 6 with diluted HCl and then extracted with *n*-BuOH saturated with H_2O. The BuOH layer was evaporated to dryness and the residue subjected to HPLC purification (column: Shiseido Capcell Pak C_{18}, solvent system: MeOH-$H_2$0 (8:2)) to give **3** (10 mg).

Protobassic acid 3-O-β-D-glucopyranoside (3). An amorphous solid, $[\alpha]_D^{23}$ +20.0° (MeOH;*c*=0.09). FAB-MS: *m/z* 1127 $[M+ Na]^+$. ^{1}H NMR (pyridine-d_5-methanol-d_4 (11:1), 500 MHz): δ 0.94, 1.00, 1.27, 1.57, 1.91, 2.12 (each 3H, s, H_3 of C-29, C-30, C-27, C-25, C-24, C-26), 3.27 (1H, dd, *J*=14.0, 4.0 Hz, H-18), 3.83 (1H, m, H-5 of glc.), 3.93 (1H, dd, *J*=8.0, 8.6 Hz, H-2 of glc.), 3.94, 4.42 (each 1H, d, *J*=10.9, H_2-23), 4.06 (1H, dd, *J*=8.6, 8.8 Hz, H-3 of glc.), 4.09 (1H, dd, *J*=8.2, 8.8 Hz, H-4 of glc.), 4.22 (1H, dd, *J*=11.6, 5.1 Hz, H-6 of glc.), 4.25 (1H, d, *J*=3.7 Hz, H-3), 4.36 (1H, dd, *J*=11.6, 2.7 Hz, H-6 of glc.), 4.80 (1H, m, H-2), 5.06 (1H, br.s, H-6), 5.08 (1H, d, *J*=8.0 Hz, H-1 of glc.), 5.55 (1H, br.t, H-12). ^{13}C NMR data: Table 1.

Acidic hydrolysis of 1 and 2. Compound **1** (10 mg) was heated in 1ml 1N HCl (Dioxane-H_2O, 1:1) at 80°C under argon atmosphere for 1 hr in a water bath. After dioxane was removed, the solution was extracted with EtOAc (1 ml x 3). The solvent was washed with water and then distilled off to give a white powder. Purification of the product over HPLC (column: Shiseido Capcell Pak C_{18}, solvent: MeOH (9:1)) afforded bassic acid (**4**, 3 mg). The monosaccharide portion was neutralized by passing through an anion-exchange resin (Amberlite MB-3) column, concentrated and then treated with 1-(trimethylsilyl) imidazole at room temperature for 2 hours. After the excess reagent was decomposed with water, the reaction product was extracted with hexane (1 ml x 3 times). The TMSi derivatives of the monosaccharides were identified to be L-arabinose, D-xylose, D-glucose and L-rhamnose (1:1:1:3) from GLC analysis.

Using the same method, **2** was hydrolyzed to give the same sapogenin and the monosaccharides were shown to be L-arabinose, D-xylose, D-glucose and L-rhamnose (1:1:2:2).

Bassic acid (5). ^{1}H NMR (pyridine-d_5, 500 MHz): δ 0.94 (3H, s, H-29), 1.01(3H, s, H-26), 1.20 (6H, s, H-24, 27), 1.71 (6H, s, H-25, 30), 1.52 (1H, dd, *J*=14.0, 3.9 Hz, H-1), 2.25 (1H, dd, *J*=14.0, 3.7 Hz, H-1), 3.32 (1H, dd, *J*=14.0, 4.2 Hz, H-18), 4.02, 4.20 (2H, d, *J*=10.4 Hz, H_2-23), 4.27 (1H, d, *J*=3.9 Hz, H-3), 4.51 (1H, ddd, *J*=3.7, 3.8, 3.9 Hz, H-2), 5.61 (1H, br.t, H-12), 5.86 (1H, dd, *J*=3.1, 5.2 Hz, H-6). ^{13}C NMR (pyridine-d_5, 100 MHz): δ 21.2 (q, C-24*), 22.9 (q, C-25*), 23.7 (t, C-16), 23.8 (q, C-26*), 23.8 (q, C-30), 24.2 (t, C-11), 26.2 (q, C-27), 27.8 (t, C-15), 30.9 (s, C-20), 33.2 (t, C-7), 33.2 (t, C-22), 33.3 (q, C-29), 34.4 (t, C-21), 37.6 (s, C-8), 38.6 (s, C-10), 42.6 (d, C-18), 43.2 (t, C-1), 43.2 (s, C-14), 45.5 (s, C-4), 46.0 (t, C-19), 46.1 (d, C-9), 47.1 (s, C-17), 69.7 (t, C-23), 70.8 (d, C-2), 73.1 (d, C-3),

121.0 (d, C-6), 123.4 (d, C-12), 145.2 (s, C-13), 148.7 (s, C-5), 180.3 (s, C-28). *Signals may be interchangeable.

Conformational calculation. The starting geometries were generated by the Metropolis Monte Carlo search method[18] using the CONFSEARCH program[19] and were submitted to energy minimization using the Discover-cff91 force field program.[20] The local minima thus found were taken as the starting structures for molecular dynamics calculated in vacuo at 300 K and at a time step of 1fs. The equilibration time was 1ps and the total time was 1000 ps. Trajectory frames were saved every 0.01 ps. The trajections were then examined with the Analysis module of Insight II.[21] Calculations were performed on an IRIS Indigo Elan computer.

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