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TRITERPENOID SAPONINS FROM *MIMUSOPS ELENGI*

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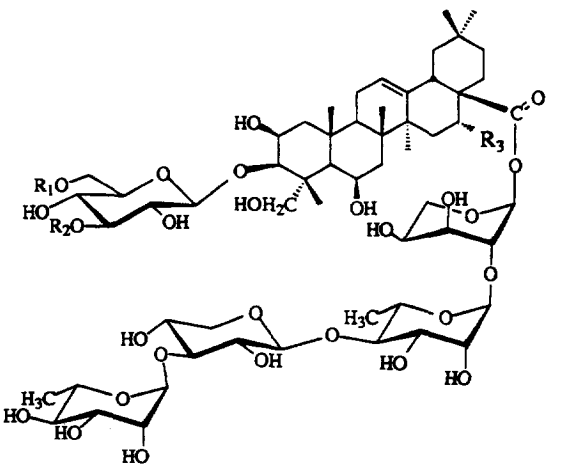
Key Word Index—*Mimusops elengi*; Sapotaceae; seeds; triterpenoid saponins; protobassic acid; mimusin; mimusopsin.

Abstract—A novel minor triterpenoid saponin mimusin {3-*O*-[β-D-glucopyranosyl-(1→6)-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→2)-α-L-arabinopyranoside} was isolated from the seeds of *Mimusops elengi*, in addition to two known triterpenoid saponins, Mi-saponin A and 16α-hydroxy Mi-saponin A. The structure of the minor saponin was established by comparing its ^{13}C NMR and LS-MS linked-scan, ESI-MS data with FAB-MS of the mimusopsin isolated earlier from the same source. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Mimusops elengi Linné is a small to large evergreen tree, widely distributed throughout the greater part of India. The plant enjoys a considerable reputation in Indian medicine as an astringent, tonic and in the treatment of diarrhoea [1]. In previous papers we reported the isolation and structure elucidation of four new triterpenoid saponins, mimusopsides A and B [2], mimusopin and mimusopsin [3], from the seeds of the plant. Continuation of investigations of the other saponin fraction of the plant has now led to the isolation and structural elucidation of a new minor saponin, mimusin (**1**), along with two known ones, Mi-saponin A (**3**) and 16α-hydroxy Mi-saponin A (**4**) from the defatted seeds of the plant.



1	R_1= Glc	R_2= H	R_3= H
2	R_1= H	R_2= Glc	R_3= H
3	R_1= H	R_2= H	R_3= H
4	R_1= H	R_2= H	R_3= OH

RESULTS AND DISCUSSION

Mimusin (**1**), an amorphous solid, $[\alpha]_D$ +20.0°, had a molecular formula of $C_{64}H_{104}O_{32}$ as determined from its high resolution positive LSI-MS ion at *m/z* 1385.6575 $[M + H]^+$ and negative LSI-MS 1383.6417 $[M - H]^-$. Its ^1H and ^{13}C NMR spectra indicated that **1** had the same aglycone, protobassic acid as mimusopsin (**2**) [3] but differed in the oligosaccharide part (Tables 1 and 2). The presence of six sugars in **1** was indicated from the six anomeric protons (δ 4.82, 4.94, 4.97, 5.59, 5.98, 6.34) and carbons (δ 93.0, 100.9, 102.3, 104.5, 104.6 and 106.3). 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 the comparison of their scalar-coupling patterns in COSY, HOHAHA and ^{13}C chemical shift data with that of mimusopsin (**2**). From the assigned aglycone

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Table 1. ^{13}C NMR data for aglycone moieties of compounds **1** and **2** (125 MHz in pyridine-d_5)

C	1	2	C	1	2
1	46.1 *t*	46.4 *t*	16	23.1 *t*	23.3 *t*
2	70.0 *d*	70.8 *d*	17	47.3 *s*	47.4 *s*
3	83.2 *d*	83.1 *d*	18	41.6 *d*	41.8 *d*
4	43.4 *s*	43.8 *s*	19	46.0 *t*	46.3 *t*
5	48.6 *d*	48.7 *d*	20	30.7 *s*	30.9 *s*
6	67.4 *d*	67.5 *d*	21	34.0 *t*	34.2 *t*
7	40.7 *t*	41.0 *t*	22	32.6 *t*	32.8 *t*
8	39.2 *s*	39.4 *s*	23	65.4 *t*	65.1 *t*
9	48.8 *d*	49.1 *d*	24	16.5 *q*	16.6 *q*
10	36.5 *s*	36.8 *s*	25	18.0 *q*	18.4 *q*
11	23.9 *t*	24.1 *t*	26	18.1 *q*	18.9 *q*
12	123.4 *d*	123.5 *d*	27	25.9 *q*	26.2 *q*
13	143.2 *s*	143.5 *s*	28	176.1 *s*	176.2 *s*
14	42.6 *s*	42.8 *s*	29	32.9 *q*	33.1 *q*
15	28.0 *t*	28.2 *t*	30	23.5 *q*	23.7 *q*

(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. Since this compound was available in minute amount, running hetero-correlation spectra like HETCOR or HMBC was not possible. Therefore, most of the effort in the structural study was by modern mass spectroscopy investigation. The low resolution LSI-mass spectrometry (liquid secondary ionization) and ESI (electron spray ionization) methods were employed to determine the molecular weight while the high resolution LSI-MS was used to furnish the chemical composition. The same fragmentation patterns observed in the negative LSI-MS and linked-scan studies of **1** and the FAB-MS of mimusopsin (**2**) (Figs 1 and 2) further confirmed the sugar identities and suggested that the two compounds had the same sugar sequences. Therefore, mimusin was an isomer of mimusopsin, with two glucoses being in one unit and the other four in a chain with the Rham-Xyl unit as a terminal segment.

The exact inter-glycosidic linkage positions for the two sugar subunits were established by comparing the ^{13}C NMR data with that of mimusopsin (Table 2). All the ^{13}C NMR data from the oligosaccharide parts were the same except for some of the resonances from one of the glucoses. The down field shift of the C-6 resonance of the glucose of δ69.8 and the disappearance of the resonance at δ88.6 from the ^{13}C NMR spectrum of mimusopsin suggested that the two glucose moieties in **1** were linked by a 1→6 linkage. The other oligosaccharide unit at C-28 had the same arrangement as that of mimusopsin. 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 constant (7–8 Hz). The ^1H non-splitting patterns and the ^{13}C chemical shifts of the rhamnoses indicated α-orientations. The anomeric proton of the arabinose was shown to be a broad singlet, thus no information was available from the ^1H NMR spectrum. However, an α-orientation can be inferred by the similarity of its ^{13}C NMR data with those of mimusopsin and other triterpenoid saponins from the same source [3]. The absolute configurations of these monosaccharides were chosen in keeping with those mostly encountered among plant glycosides. Thus, mimusin (**1**) was elucidated to be 3-*O*-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]-2β,3β,6β,23-tetrahydroxyloean-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.

The structural studies of compounds **3** and **4** were accomplished by spectral and chemical analyses. Compound **3** was identified as Mi-saponin A, 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→2)-α-L-arabinopyranoside, a compound first isolated from *Madhuca longifolia* [4]. Compound **4** was characterized as 16α-hydroxy Mi-saponin A, 3-*O*-β-D-glucopyranosyl-2β,3β,6β,23-pentahydroxyolean-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, a compound already isolated from different plant sources [5, 6].

EXPERIMENTAL

All mps were measured using a Yanaco microscope apparatus and were uncorr. IR spectra were determined using a JASCO 7300 FTIR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. FAB/LSI-MS and ESI-MS were conducted using VG Organic AutoSpec-Q and VG Organic Quattra-2 mass spectrometers, respectively. ^1H and ^{13}C NMR spectra were recorded using a JEOL α-500 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 Kasei), silica gel (Silica gel 60, Merck) were used for CC. ODS MPLC was performed on pre-packed column (LiChroprep RP-18, 40–63 μm, 25 × 310 mm; detector: UV 210 nm; solvent: MeOH–H_2O (7:3), 0.4 ml min^{-1}). Prep. HPLC was performed using an ODS column (Capcell pak ODS, Shiseido, 10 mm i.d. × 250 mm, detector: UV 210 nm or refractive index; solvent: MeOH–H_2O (7:3), 1 ml min^{-1}). GLC: 2% SE-30 on Chromsorb W (60–80 mesh), 3 mm i.d. × 1.5 m, 150° column temp, N_2 carrier gas, 15 ml min^{-1} flow rate.

Extraction and isolation. The air dried powdered seeds of *M. elengi* (2 kg) were successively extracted with petrol (60–80°), $CHCl_3$ and MeOH under reflux conditions. The MeOH extract was partitioned between *n*-BuOH and H_2O. The organic layer was concd to dryness under red. pres. to give a residue (30 g) which was chromatographed on silica gel (500 g).

Table 2. ^{13}C and ^{1}H NMR data for the sugar units (125 MHz in pyridine-d_5)

Sugar unit	1 (^{13}C)	1 (^{1}H)	Mimusopsin (^{13}C)
C_3-Glucose			
G-1	104.5	4.94 *d* (8.0)	105.1
G-2	74.8	3.89	74.0
G-3	77.9	4.01	88.6
G-4	71.4	4.01	69.6
G-5	76.3	3.95	77.7
G-6	69.8	4.05	62.2
		4.70 *dd* (8.2, 1.0)	
Glucose (G′)			
G′-1	104.6	4.82 *d* (7.7)	105.7
G′-2	74.9	3.88	75.3
G′-3	78.1	4.09	78.5
G′-4	71.3	4.03	71.5
G′-5	77.7	3.81	78.0
G′-6	62.4	4.38	62.4
		4.20	
C_{28}-Arabinose			
A-1	93.0	6.34 *d* (2.8)	93.3
A-2	75.2	4.40	75.4
A-3	69.6	4.28	70.3
A-4	65.8	4.25	66.0
A-5	62.7	3.85	63.1
		4.38	
Rhamnose (R)			
R-1	100.9	5.59 (*s*)	101.1
R-2	72.1	4.62	72.4
R-3	72.1	4.41	72.4
R-4	83.6	4.16	83.5
R-5	68.7	4.74 *m*	68.6
R-6	18.3	1.59 *d* (6.1)	18.2
Xylose			
X-1	106.3	4.97 *d* (7.9)	106.2
X-2	75.6	3.89	75.9
X-3	83.2	4.03	83.4
X-4	69.0	4.00	69.2
X-5	66.9	4.12	65.1
		3.41 *dd* (11.0, 0.5)	
Rhamnose (R′)			
R′-1	102.3	5.98 (*s*)	102.5
R′-2	72.0	4.40	72.2
R′-3	71.7	4.40	71.9
R′-4	73.6	4.18	73.9
R′-5	69.6	4.27 *m*	69.8
R′-6	18.7	1.64 *d* (5.8)	18.5

Elution was carried out with $CHCl_3$ followed by various mixts of $CHCl_3$–MeOH and the frs were combined according to their TLC behaviour. Early frs yielded the previously reported minusopsides A and B. The more polar frs were combined and applied to a column of Diaion HP (150 g) and washed with H_2O, 30, 40, 60, 80 and 100% MeOH to give 17 frs. Further purification over silica gel ($CHCl_3$–MeOH–H_2O, 6:4:1), ODS medium LC and finally HPLC afforded mimusopin, mimusopsin, Mi-saponin A (100 mg), 16-α-hydroxy Mi-saponin A (20 mg) and the minor saponin, mimusin (**1**, 9.0 mg).

Mimusin (**1**). An amorphous solid, mp 227° (dec.), $[\alpha]_D$ +20° (MeOH; $c = 0.09$). IR ν_{max}^{KBr} cm^{-1}: 3407, 2926, 1632, 1395, 1255, 1050, 643. ESI-MS (negative): m/z 1383.7 $[M - H]^-$, 691.6 $[M - 2H]^{-2}$, (positive)

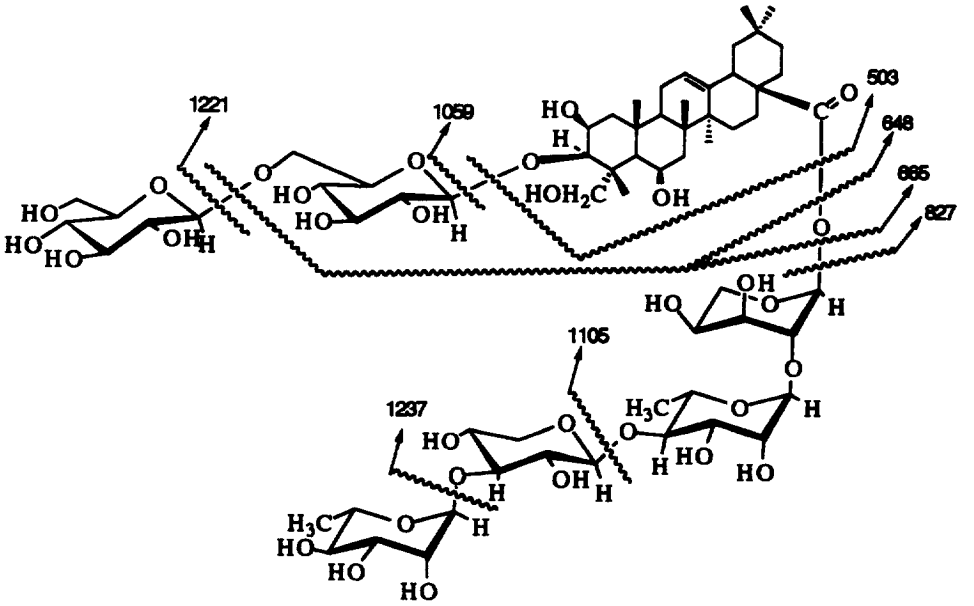

Fig. 1. The fragmentation pattern of misumin (**1**) observed in negative LSI-MS.

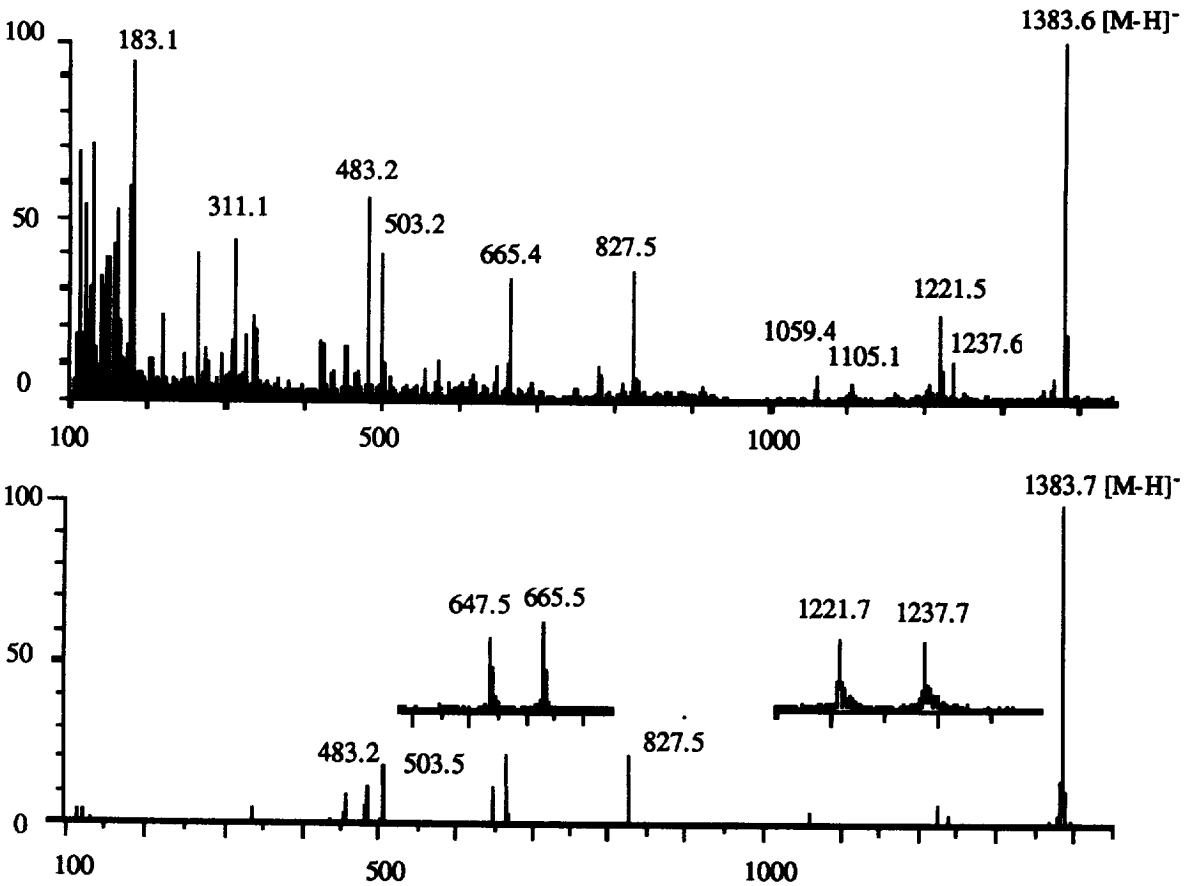

Fig. 2. The LSI-MS of misumin (**1**, upper) and FAB-MS of mimusopsin (**2**, lower).

m/z 1047.8 $[M + Na]^+$. High resolution LSI-MS ($C_{64}H_{104}O_{32}$): *m/z* 1385.6575 $[M + H]^+$ (in positive mode) and *m/z* 1383.6417 $[M - H]^-$ (negative). LSI-MS (negative): *m/z* 1383 $[M - H]^-$, 1237 $[M-H-146]^-$, 1221 $[M-H-162]^-$, 1059 $[M-H-324]^-$, 1105 $[M-H-278]^-$, 827 $[M-H-556]^-$, 665, 648, 503, 483, 457. ^1H NMR (pyridine-d_5, 500 MHz): δ0.91, 1.01, 1.20, 1.55, 1.85, 2.07 (each 3H, *s*, H_3 of C-30, C-29, C-27, C-25, C-24, C-26), 3.27 (1H, *dd*, $J = 12.5$, 4.0 Hz, H-18), 4.26 (1H, *m*, H-3), 4.88 (1H, *m*, H-2),

5.03 (1H, *m*, H-6), 5.48 (1H, *t*-like, H-12). ^{1}H NMR data for the sugar moiety are given in Table 2. ^{13}C NMR data: Tables 1 and 2.

Acid hydrolysis of **1**. Compound **1** (4 mg) was heated in 1 ml 1M HCl (dioxane–H_2O, 1:1) at 80° under argon atmosphere for 1 hr in a water bath. After dioxane was removed, the soln was extracted with EtOAc (1 ml × 3). The solvent was washed with H_2O and then distilled off to give bassic acid (0.5 mg). The monosaccharide portion was neutralized by passing through an ion-exchanged resin (Amberlite MB-3) column, concd and then treated with 1-(trimethylsilyl)imidazole at room temperature for 2 hr. After the excess reagent was decomposed with H_2O, the reaction product was extracted with hexane (1 ml × 3). The TMSi derivatives of the monosaccharides were identified to be L-arabinose, D-xylose, D-glucose and L-rhamnose (1:1:2:2) by GLC.

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