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SAPONINS FROM THREE SPECIES OF *MIMUSOPS*

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Key Word Index—*Mimusops elengi*; *M. hexandra*; *M. manilkara*; Sapotaceae; seed kernel; triterpenoidal saponins; Mi-saponin A.

Abstract—Six saponins were isolated from the seed kernel of *Mimusops elengi*, *M. hexandra* and *M. manilkara*. Their structures were determined using a combination of ^{1}H NMR, ^{13}C NMR and mass spectroscopy. Three of them are new compounds: 3-*O*-(β-D-glucuronopyranosyl) 28-*O*-(α-L-rhamnopyranosyl (1 → 3) β-D-xylopyranosyl(1 → 4) [α-L-rhamnopyranosyl(1 → 3)] α-L-rhamnopyranosyl(1 → 2) α-L-arabinopyranosyl) protobassic acid, 3-O-(β-D-glucuronopyranosly) 28-O-(α-L-rhamnopyranosyl(1 → 3) β-D-xylopyranosyl(1 → 4) α-L-rhamnopyranosyl(1 → 2) α-L-arabinopyranosyl) 16-α-hydroxyprotobassic acid and 3-O-(β-D-glucopyranosyl(1 → 3) β-D-glucopyranosyl) 28-O-(α-L-rhamnopyranosyl(1 → 3) β-D-xylopyranosyl(1 → 4) α-L-rhamnopyranosyl(1 → 2) α-L-arabinopyranosyl) protobassic acid.

INTRODUCTION

The genus *Mimusops* (Sapotaceae) is widely distributed in India. *M. elengi* and *M. hexandra* are commonly used in Indian traditional medicine [1]. These species have been reported to contain triterpenoids and saponins [2–6]. Continuing studies on the genus *Mimusops*, we report the isolation and structural elucidation of Mi-saponin A [7–9], arganine C [8–10], butyroside C [11] and of three new triterpenoidal saponins from seed kernels of *M. elengi*, *M. hexandra* and *M. manilkara*.

RESULTS AND DISCUSSION

The defatted seed kernel of each species was extracted with ethanol. After dialysis against water, the ethanol extract was purified using a combination of silica gel column chromatography, preparative TLC or reversed-phase C-18 column. The extract from *M. elengi* contained the known compounds Mi-saponin A(**1**), arganine C(**2**), butyroside C(**3**) and a new saponin **4**. From *M. hexandra*, saponins **1** and **3** were isolated with the two novel compounds **5** and **6**. The extract of *M. manilkara* contained Mi-saponin A(**1**) and saponin **6**. Saponins **1**, **3**, **4** and **5** are bisdesmosides of protobassic acid while saponins **2** and **6** contain 16α-hydroxyprotobassic acid as aglycone.

Saponin **1**, common to the three species, showed in the positive-ion FAB mass spectrum, a pseudomolecular peak at *m/z* 1245.5 $[M + Na]^+$ analysed as $C_{58}H_{94}O_{27}Na$. The negative-ion spectrum displayed a $[M - H]^-$ ion at *m/z* 1221.7. The structures of the triterpene and of the five sugar units were determinated by ^{1}H NMR and ^{13}C NMR (see Tables 1 and 2) using connectivities observed in COSY, HOHAHA, HMQC and HMBC experiments [12]. The genin was thus identified as protobassic acid, and the sugars as β-D-glucopyranose, β-D-xylopyranose, α-L-rhamnopyranose, a desoxy-6-hexopyranose and a pentopyranose (with H-1 deshielded at δ5.64 corresponding to an ester position). Superimposition of five proton signals between δ3.83 and 3.87, precluded unambiguous determination of the axial or equatorial nature of these protons.

Preparation of the peracetylated derivative **1a** and analysis of its ^{1}H NMR spectrum confirmed the presence of arabinose as an ester and of inner rhamnose. The chemical shifts of osidic protons α to branching points were not affected by acetylation: H-2 of arabinose (δ3.98), H-4 of one rhamnose (δ3.65) and H-3 of xylose (δ3.81); glucose and the second rhamnose were fully peracetylated and thus terminal. In the absence of branching, the presence of two chains of sugars linked to protobassic acid was determinated. Sequencing of the sugar chains and linkage on the triterpene in **1** were determinated by ROESY and/or HMBC experiments. ROes were observed between H-1 of glucose (δ4.44) and H-3 of protobassic acid (δ3.57), between H-1 of xylose (δ4.52) and H-4 of a rhamnose (δ3.54). HMBC showed interglycosidic correlations between H-1 of the second rhamnose (δ5.13) and C-3 of xylose (δ84.1). This latter carbon being identified by a $^3J_{H-C}$ with H-4 (δ3.53). The overlap

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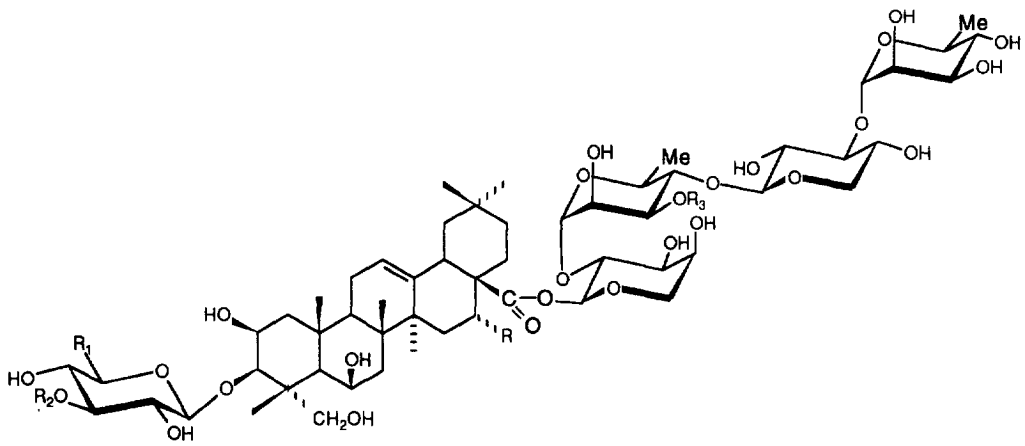
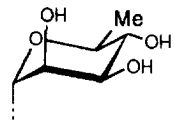
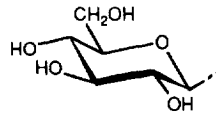


	R	R_1	R_2	R_3	
1	H	CH_2OH	H	H	Mi-saponin A
2	OH	CH_2OH	H	H	arganine C
3	H	COOH	H	H	butyroside C
4	H	COOH	H		
5	OH	COOH	H	H	
6	H	CH_2OH		H	

of H-2, H-3 and H-4 of arabinose did not allow separation of their rOes and HMBC correlations with H-1 (δ5.09) and C-1 (δ101.5) of the inner rhamnose; branching position on this sugar was cleared however by observation of a shielded H-2 on derivative **1a**. These observations allowed identification of saponin **1** as Mi-saponin A, previously isolated from other Sapotaceae plants: *Madhuca longifolia* [7], *Madhuca butyracea* [8] and *Argania spinosa* [9].

Structures of saponins **2** and **3** were attributed to arganine C and butyroside C, respectively, on a similar basis. Their proton and carbon NMR spectra were found to be in agreement with those described in the literature (Tables 1 and 2). Saponin **2** was previously isolated from *Crossopteryx febrifuga* (Rubiaceae) [10] and from *Argania spinosa* [9], and saponin **3** from *Madhuca butyracea* [11].

The negative FAB-mass spectrum of saponin **4** exhibited a pseudomolecular peak at *m/z* 1381.4 $[M - H]^-$ ($C_{64}H_{101}O_{32}$). Negative ion fragments at *m/z* 1205.7 and 1059.5 were attributed to the losses of a terminal uronic acid and of terminal uronic acid + desoxyhexose moieties. The ^{13}C NMR spectrum of **4** showed signals for protobassic acid as in Mi-saponin A(**1**) and displayed five resonances for six anomeric carbons at δ94.0, 101.6, 102.7, 104.3 (two carbons) and 105.2 (Table 1). These carbons were linked, respectively, to six protons at δ5.65 (*d*, $J = 4$), 5.05 (*br s*), 5.13 (*d*, $J = 1.5$), 5.06 (*br s*), 4.49 (*d*, $J = 7.5$) and 4.50 (*d*, $J = 7.5$) in the HMQC spectrum.

Analysis of the correlations in COSY and HOHAHA experiments showed the presence of one β-D-xylopyranose substituted in position C-3 and of two terminal α-L-rhamnopyranoses (Table 2), with carbon chemical shifts identical to those of Mi-saponin A(**1**). The H-H correlation maps and carbon resonances for the first sugar in the ester chain are similar in saponins **1–4** (and in saponins **5–6**; see Table 2); this similarity confirmed that an α-L-arabinose was linked to the C-28 acid function of the triterpene in all saponins isolated (with prominent $^1C_4$ conformation). The uronic acid residue was identified as β-D-glucuronopyranosyl by comparison of NMR data of **3** and **4** (Tables 1 and 2). The presence of

Table 1. ^{13}C NMR data for compounds **1–6** (in CD_3OD)

C	1	2	3	4	5	6	C	1	2	3	4	5	6	C	1	2	3	4	5	6
1	46.6	47.0	46.9	47.1	47.0	46.7		Arabinose (ester)							Glucose		Glucuronic acid			Glucose
2	71.3	71.3	71.0	71.6	70.9	71.1	1	94.0	94.0	94.1	94.0	94.1	94.0	1	105.3	106.4	104.4	104.3	104.4	105
3	83.7	83.9	83.0	82.4	83.2	83.8	2	75.4		75.4	75.4	75.4	75.5	2	75.7		75.0	75.0	75.0	74.8
4	44.4	44.0	44.1	44.2	44.0	44	3	67.4		67.2	67.3	67.4	67.4	3	76.3		78.0	78.1	78.0	88.0
5	48.9	nd	nd	47.5	nd	nd	4	72.4		72.5	72.3	72.4	72.4	4	71.1		73.6	73.6	73.5	69.6
6	68.6	68.6	68.7	70.2	68.8	68.6	5	64.1		64.4	65.2	64.2	64.1	5	78.2		76.3	76.1	74.6	77.4
7	41.2	42.7	41.3	41.3	41.4	41.2	6							6	62.2		177.1	177.2		62.6
8	39.8	39.9	39.9	39.9	40.0	37.2														
9	49.7	nd	49.6	49.6	48.7	49.6		Rhamnose (inner)									Rhamnose			Glucose
10	37.1	37.2	37.3	37.3	37.3	37.2	1	101.5	101.5	101.4	104.3	101.3	101.5	1				101.6		105.2
11	23.7	25.0	23.7	23.9	24.6	23.8	2	72.3		72.3	71.2	72.2	72.3	2				72.3		75.4
12	124.3	124.1	124.3	124.3	124.1	124.3	3	71.8		72.3	80.8	71.8	71.8	3				72.1		76.3
13	144.2	143.9	144.2	144.3	143.9	144.2	4	83.4		84.2	78.1	83.3	83.5	4				74.0		71.6
14	43.7	43.4	43.7	43.7	43.4	43.7	5	68.9		68.9	68.6	69.0	69.0	5				70.2		78.2
15	29	36.4	29.1	29.0	36.4	29	6	18.1	18.0	17.9	18.3	18.0	18.1	6				17.9		62.2
16	24.7	76.2	24.7	24.7	76.2	24.6														
17	48.3	50.4	nd	nd	50.4	nd		Xylose												
18	42.8	42.2	42.8	42.7	42.2	42.8	1	106.6	106.4	106.7	105.2	106.5	106.6							
19	47.1	47.5	47.1	nd	47.6	47.1	2	77.7		78.0	78.0	78.0	77.8							
20	31.6	31.3	31.6	31.6	31.3	31.6	3	84.1		83.6	84.8	84.0	84.2							
21	34.9	36.3	34.9	35.0	36.4	34.9	4	69.8		69.8	69.3	69.8	69.8							
22	33.4	31.8	33.4	33.4	31.8	33.4	5	67.2		67.2	66.9	67.2	67.2							
23	65.4	nd	65.5	65.2	65.5	64.1														
24	16.3	16.3	16.3	16.3	16.3	16.3		Rhamnose												
25	18.9	19.2	19.3	19.2	19.3	19.3	1	102.5	102.5	101.4	102.7	102.5	102.5							
26	18.1	19.0	19.0	18.9	19.0	18.9	2	72.3		72.3	72.3	72.2	72.3							
27	26.4	27.3	26.3	26.5	27.4	26.4	3	72.2		72.3	72.3	72.2	72.3							
28	177.9	177.1	177.9	nd	177.1	177.9	4	74.0		74.0	74.0	74.0	74.0							
29	33.5	33.4	33.5	33.5	33.4	33.5	5	70.0		70.0	70.3	70.0	70.0							
30	24.0	25.1	24.0	24.0	25.1	24.0	6	17.9	17.9	18.1	17.9	17.9	17.9							

Table 2. ^{1}H NMR data for osidic part of **1**–**6** (in CD_3OD)

H	1	2	3	4	5	6
	Arabinose (ester)					
1	5.64 (*d*; 4)	5.64 (*d*; 4)	5.61 (*d*; 4)	5.65 (*d*; 4)	5.59 (*d*; 4)	5.63 (*d*; 4)
2	3.84 (*m*)	3.84 (*m*)	3.83 (*m*)	3.83 (*m*)	3.81 (*m*)	3.83 (*m*)
3	3.85 (*m*)	3.85 (*m*)	3.86 (*m*)	3.86 (*m*)	3.86 (*m*)	3.84 (*m*)
4	3.83 (*m*)	3.83 (*m*)	3.85 (*m*)	3.83 (*m*)	3.86 (*m*)	3.84 (*m*)
5	3.51 (*dd*; 12, 2)	3.51 (*dd*; 12, 2)	3.53 (*dd*; 11, 2)	3.51 (*dd*; 11, 3)	3.51 (*brd*; 12.5)	3.50 (*d*; 9)
5	3.90 (*m*)	3.90 (*m*)	3.90 (*m*)	3.93 (*dd*; 11, 2)	3.92 (*d*; 12.5)	3.90 (*d*; 9)
	Rhamnose (inner)					
1	5.09 (*brs*)	5.07 (*brs*)	5.13 (*brs*)	5.06 (*brs*)	5.10 (*brs*)	5.10 (*brs*)
2	3.87 (*m*)	3.84 (*m*)	3.87 (*m*)	3.94 (*m*)	3.86 (*m*)	3.86 (*brs*)
3	3.87 (*m*)	3.84 (*m*)	3.86 (*m*)	3.90 (*m*)	3.86 (*m*)	3.85 (*d*; 6)
4	3.54 (*t*; 9)	3.58 (*t*; 9)	3.58 (*t*; 9)	3.72 (*t*; 9)	3.58 (*t*; 9.5)	3.56 (*t*; 9)
5	3.74 (*m*)	3.73 (*m*)	3.75 (*m*)	3.80 (*m*)	3.72 (*m*)	3.74 (*m*)
6	1.28 (*d*; 6)	1.30 (*d*; 6)	1.28 (*d*; 6)	1.27 (*d*; 6)	1.30 (*d*; 6)	1.28 (*d*; 6)
	Xylose					
1	4.52 (*d*; 7.5)	4.54 (*d*; 7.5)	4.51 (*d*; 8)	4.50 (*d*; 7.5)	4.54 (*d*; 7.5)	4.51 (*d*; 7.5)
2	3.34 (*t*; 8)	3.34 (*dd*; 9, 7.5)	3.32 (*t*; 8.5)	3.28 (*dd*; 8, 7.5)	3.34 (*t*; 8.5)	3.29 (*t*; 8)
3	3.45 (*t*; 8.5)	3.45 (*t*; 9)	3.45 (*t*; 9)	3.41 (*t*; 8)	3.46 (*t*; 9)	3.48 (*t*; 8)
4	3.53 (*m*)	3.53 (*td*; 9, 5)	3.51 (*m*)	3.53 (*td*; 9, 3)	3.53 (*td*; 9, 4)	3.52 (*m*)
5	3.21 (*dd*; 11, 10)	3.21 (*dd*; 12, 9)	3.21 (*dd*; 12, 9)	3.16 (*dd*; 11.5, 9.5)	3.22 (*dd*; 11, 9)	3.21 (*dd*; 12, 10)
5	3.88 (*dd*; 11, 5)	3.88 (*dd*; 12, 5)	3.87 (*dd*; 12, 5)	3.87 (*dd*; 11.5, 5)	3.87 (*dd*; 11, 5)	3.85 (*dd*; 12, 2)
	Rhamnose					
1	5.13 (*d*; 1.7)	5.13 (*d*; 1.5)	5.13 (*brs*)	5.13 (*d*; 1.5)	5.14 (*brs*)	5.13 (*d*; 1.5)
2	3.95 (*dd*; 3, 1.5)	3.95 (*dd*; 3.5, 1.5)	3.96 (*dd*; 3, 2)	3.97 (*dd*; 3, 2)	3.95 (*dd*; 3, 1.5)	3.95 (*dd*; 3.5, 1.5)
3	3.71 (9.5, 3.5)	3.70 (*dd*; 9, 3.5)	3.72 (*dd*; 9, 3)	3.70 (*dd*; 9, 3)	3.71 (*dd*; 9.5, 3.5)	3.70 (*dd*; 9.5, 3)
4	3.38 (*t*; 9.5)	3.38 (*t*; 9)	3.38 (*t*; 9)	3.39 (*t*; 9)	3.38 (*t*; 9.5)	3.39 (*t*; 9.5)
5	3.96 (*dq*; 9.5, 6)	4.00 (*dq*; 9, 6)	4.00 (*dq*; 9, 6)	3.98 (*m*)	3.96 (*dq*; 9.5, 6)	3.99 (*dq*; 9.5, 6)
6	1.24 (*d*; 6)	1.23 (*d*; 6)	1.24 (*d*; 6)	1.24 (*d*; 6)	1.24 (*d*; 6)	1.23 (*d*; 6)
	Glucose		Glucuronic acid			Glucose
1	4.44 (*d*; 7.5)	4.44 (*d*; 7.5)	4.48 (*d*; 7)	4.49 (*d*; 7.5)	4.50 (*d*; 8)	4.50 (*d*; 7)
2	3.28 (*dd*; 9.5, 7.5)	3.27 (*dd*; 8, 7.5)	3.35 (*m*)	3.32 (*t*; 8)	3.35 (*t*; 8.5)	3.48 (*t*; 8)
3	3.35 (*t*; 10)	3.35 (*t*; 8)	3.40 (*m*)	3.40 (*m*)	3.40 (*m*)	3.53 (*t*; 8)
4	3.70 (*m*)	3.70 (*dd*; 9, 8)	3.40 (*m*)	3.40 (*m*)	3.40 (*m*)	3.45 (*dd*; 9, 7)
5	3.31 (*m*)	3.30 (*m*)	3.63 (*d*; 10)	3.61 (*d*; 9)	3.65 (*brd*; 6.5)	3.32 (*m*)
6	3.68 (*dd*; 12, 4.5)	3.69 (*dd*; 12, 5)				3.71 (*dd*; 12, 4)
6	3.79 (*dd*; 12, 2)	3.80 (*dd*; 12, 3.5)				3.88 (*dd*; 12, 2)
				Rhamnose		Glucose (terminal)
1				5.05 (*brs*)		4.56 (*d*; 7.5)
2				4.06 (*dd*; 3, 1.5)		3.28 (*dd*; 9, 7.5)
3				3.74 (*dd*; 9.5, 3)		3.32 (*m*)
4				3.41 (*t*; 9)		3.35 (*t*; 9)
5				3.80 (*m*)		3.32 (*m*)
6				1.25 (*d*; 6)		3.63 (*dd*; 12, 6)
6						3.81 (*dd*; 12, 2.5)

a third 6-desoxyhexose in saponin **4** was revealed by the observation of three $^3J_{H-H}$ COSY correlations between three methyl doublets at δ1.24, 1.25 and 1.27 and sugar protons at δ3.80 (2H) and δ3.98. These results suggested that saponin **4** consisted of a butyroside C unit with a supplementary terminal rhamnose branched on the inner rhamnose, whose carbon resonances were displaced with regard to **3** (Table 1).

The peracetylated derivative **4a** was prepared in order to verify the hypothesis and to secure the identification and sequencing of the sugars. The measurement of H–H coupling constants confirmed the presence of one glucuronic acid, one arabinose ester, one xylose and three rhamnoses. The CHOR positions that were not acetylated were those of H-2 of arabinose (δ3.87), H-3 of xylose (δ4.02), H-3 and H-4 of inner rhamnose (δ3.95 and 3.67). The supplementry rhamnose was thus linked to position C-3 of the inner rhamnose residue of butyroside C. The signals of osidic protons in the ester chain of **4a** were similar to those described for the peracetylated tridesmosaponin A [13] where xylose replaced arabinose in **4a**.

The sequence of the sugars in saponin **4** was established through a ROESY experiment. It showed rOes between H-1 of glucuronic acid and H-3 of protobassic acid, between H-1 of xylose and H-4 of inner rhamnose, and between the H-1s of rhamnoses at δ5.06 ppm with H-2 of arabinose and H-3 of inner rhamnose. The relative equatorial and axial positions of H-1 and H-3 in rhamnose make observation of intraresidual Overhauser effects unlikely and thus, the rOe was assigned to cross-relaxation between H-1 of the terminal rhamnose and H-3 of the inner rhamnose. A rOe of weak intensity was also observed between the H-1 of the second terminal rhamnose (δ5.13) and H-3 of xylose.

Saponin **4** is thus 3-*O*-(β-D-glucuronopyranosyl) 28-*O*-(α-L-rhamnopyranosyl(1 → 3) β-D-xylopyranosyl (1 → 4) [α-L-rhamnopyranosyl(1 → 3)] α-L-rhamnopyranosyl(1 → 2) α-L-arabinopyranosyl) protobassic acid.

^{1}H and ^{13}C NMR spectra showed that saponins **5** and **3** had same sugar chains ($\Delta\delta^{13}C < 0.3$). One glucuronic acid was located at C-3 of aglycone as in butyroside C. The genin part was determined to be 16-α-hydroxyprotobassic acid on the basis of the observation of one supplementary CHOR signals in the ^{1}H NMR spectrum of **5**. The six CHOR signals belonging to the triterpene consisted of an AB quartet (doublets at δ3.41 and 3.73, J = 12.5) corresponding to H-23, two multiplets at δ4.35 and 3.60, part of an ABXY with two protons at high field (δ1.20 and 2.06) corresponding to H-2 and H-3, and two superimposed resonances (δ4.48) coupling with five high field protons in the COSY experiment. Four of those formed two AB systems and corresponded to H-7s (δ1.81 and 1.55) and H-15s (δ1.43 and 1.81). The extra CHOR signals thus belonged to H-16 and this was confirmed by the ^{13}C NMR, which showed similarities with that of arganine C(**2**). Other ^{13}C NMR data were similar to those described in the literature for 16-α-hydroxyprotobassic acid [9, 10]. Saponin **5** is thus 3-*O*-(β-D-glucuronopyranosyl) 28-*O*-(α-L-rhamnopyranosyl(1 → 3) β-D-xylopyranosyl(1 → 4) α-L-rhamnopyranosyl(1 → 2) α-L-arabinopyranosyl) 16-α-hydroxyprotobassic acid.

Saponin **6** showed a pseudomolecular peak at m/z 1607.6 $[M + Na]^+$ in the positive FAB-mass spectrum, suggesting branching by a supplementary hexose compared with Mi-saponin A(**1**). This was confirmed by the presence of a sixth anomeric proton at δ4.56, of an anomeric carbon at δ105 ppm and of a methylene carbon at δ 62.6.

The COSY analysis and the HOHAHA correlation system from this extra anomeric proton allowed us to identify a β-D-glucopyranose. ROESY effects in the ester chain were in accordance with those observed for Mi-saponin A(**1**); a rOe was observed between H-1 of glucose (δ4.50) with H-3 of protobasic acid and between H-1 of the second glucose (δ4.56) with one triplet proton near δ3.5 (H-3 or H-4 of the first glucose). To solve this ambiguity, the peracetylated saponin **6a** was prepared. Its COSY and relayed COSY confirmed structure of a glucoside of Mi-saponin A. Only H-2 of arabinose, H-4 of one rhamnose, H-3 of xylose and H-3 of one glucose were not deshielded by acetylation; rhamnose and glucose were terminal sugars. Saponin **6** is thus 3-*O*-(β-D-glucopyranosyl(1 → 3) β-D-glucopyranosyl) 28-*O*-(α-L-rhamnopyranosyl(1 → 3) β-D-xylopyranosyl(1 → 4) α-L-rhamnopyranosyl(1 → 2) α-L-arabinopyranosyl) protobassic acid.

The set of saponins from these *Mimusops* species all contain protobasic acid (or 16-α-OH protobassic acid) which may be considered as a chemical marker for the Sapotaceae family [14]. The four-sugar core (ara-rha-xyl-rha) is also common to a large number of saponins in many plant families (Sapotaceae, Rubiaceae) and the shorter chain (ara-rha-xyl) is even more common and found in Leguminosae (*Medicago*), Cucurbitaceae (*Cucurbita*) and Campanulaceae (*Platycodon*). This probably reflects similar biosynthetic pathways in these plants.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz. 2D experiments were performed using standard Bruker microprograms. Hardware modifications of the AC-300 spectrometer allowed acquisition of the C-H correlations in the reverse mode. The FAB spectra were recorded on a ZAB2-SEQ mass spectrometer (Fisons-VG) equipped with a caesium ion gun delivering about 3 μA of caesium ion current at 35 kV energy. Samples were dissolved in MeOH and 1–2 μl of the soln was mixed with 1–2 μl of the matrix (thioglycerol) already on the target.

Plant material. The mature fruits of *M. elengi*, *M. hexandra* and *M. manilkara* were collected from different parts of the country and voucher specimens preserved with the authors (India).

Extraction and isolation of saponins. Seeds were separated and decorticated to get the kernels (2 kg each). Defatted kernel powder of each species was repeatedly extracted with EtOH. The concentrated extract was pptd by addition of large excess of Me_2CO. The crude extracts were dissolved in H_2O and then purified by dialysis against pure water (20 ml for 1 g of extract). After 96 hr, the content of tube was frozen and lyophilized. For *M. elengi* and *M. hexandra*, 4 g of each crude extract gave 1.4 g and 1.2 g of dialysed saponin mixture respectively; for *M. manilkara*, 10 g of extract were subjected to dialysis to give 935 mg of saponins.

The crude saponin (1 g) of *M. elengi* was chromatographed on a silica gel column eluting with $CHCl_3$–MeOH (60:40) to give frs 1–13. Frs 2–5 were subjected to prep. TLC with $CHCl_3$–MeOH–H_2O (60:40:6) to yield **1** (70 mg). Frs 6–11 were chromatographed on a RP-18 CC eluting with MeOH–H_2O (70:30 or 65:35) to provide **2** (18 mg). Fr. 13 was separated with a RP-18 CC (MeOH–H_2O, 65:35) to give mixture of fractions **3** and **4** which were further purified on a silica gel CC ($CHCl_3$–MeOH–H_2O, 70:30:4 and 60:40:7) to yield **3** (28 mg) and **4** (14 mg).

The saponin extract of *M. hexandra* (1 g) was subjected to a RP-18 reversed phase CC using MeOH–H_2O

(65:35) as solvent and frs 1–13 were collected. Frs 3–4 and 5–6 were again subjected to silica gel CC ($CHCl_3$–MeOH–H_2O, 60:40:4) or RP-18 CC (MeOH–H_2O, 60:40), respectively, to yield **5** (24 mg). Frs 7–9 were purified by CC ($CHCl_3$–MeOH–H_2O, 70:30:2, 70:30:4, 60:40:4) and some collected frs were subjected to prep. TLC on normal or reversed phase to yield **1** (40 mg), **6** (15 mg) and **3** (7 mg). Frs 10–13 were purified by prep. TLC in $CHCl_3$–MeOH–H_2O (60:40:7) to give **1** (9 mg) amd **3** (16 mg).

The saponin residue from *M. manilkara* (900 mg) was chromatographed on silica gel column using $CHCl_3$–MeOH–H_2O (70:30:1) as solvent. Frs 7–17 were purified by a RP-18 CC with MeOH–H_2O (70:30) to afford **1** (10 mg). Frs 18–27 were subjected to prep. TLC ($CHCl_3$–MeOH–H_2O, 60:40:7) to yield **1** (3 mg) and **6** (10 mg).

Saponin **1**. Positive FABMS *m/z* (rel. int.): 1245.5 $[M + Na]^+$ (19); negative FABMS *m/z* (rel. int.): 1221.7 $[M - H]^-$ (69), 1075.6 (19), 1059.9 (15), 665.4 (54), 503.4 (19); ^1H NMR (CD_3OD): δ0.90 (H-29, *s*), 0.94 (H-30, *s*), 1.06 (H-26, *s*), 1.13 (H-27, *s*), 1.15 (H-19eq), 1.15 (H-lax), 1.30 (H-5), 1.31 (H-24, *s*), 1.51 (H-7, *brd*, J = 10 Hz), 1.61 (H-25, *s*), 1.72 (H-19, *t*, J = 12 Hz), 1.75 (H-7), 1.95 (H-11), 2.04 (H-1, *dd*, J = 14, 2 Hz), 2.11 (H-11), 2.93 (H-18, *dd*, J = 14, 4 Hz), 3.42 (H-23, *brd*, J = 11 Hz), 3.57 (H-3, *d*, J = 3.5 Hz), 3.71 (H-23, *d*, J = 11 Hz), 4.33 (H-2, *m*, $W_{1/2}$ = 10 Hz), 4.46 (H-6, *m*, $W_{1/2}$ = 10 Hz), 5.35 (H-12, *t*, J = 3 Hz).

Derivative **1a**. ^1H NMR ($CDCl_3$): δ0.92 (H-29, *s*), 0.93 (H-30, *s*), 1.07 (H-26, *s*), 1.10 (H-1), 1.12 (H-27, *s*), 1.16 (H-5), 1.17 (rha′-6, *d*, J = 6 Hz), 1.20 (H-19), 1.29 (rha-6, *d*, J = 6 Hz), 1.38 (H-24, *s*), 1.50 (H-7), 1.60 (H-7), 1.64 (H-25, *s*), 1.64 (H-19), 1.95 (H-11), 1.96–2.14 (14 $COCH_3$, 11*s*), 2.13 (H-11), 2.15 (H-1), 2.87 (H-18, *dd*, J = 13, 3.5 Hz), 3.30 (xyl-5, *dd*, J = 12, 8 Hz), 3.46 (H-3, *d*, J = 3.7 Hz), 3.70 (rha-4, *t*, J = 9 Hz), 3.72 (glc-5, *m*), 3.73 (ara-5, *dd*, J = 12, 2.5 Hz), 3.80 (xyl-3, *t*, J = 9.5 Hz), 3.82 (H-23, *d*, J = 12 Hz), 3.90 (rha′-4, *t*, J = 9 Hz), 3.92 (rha-5, *m*), 3.93 (ara-5, *dd*, J = 12, 4.5 Hz), 3.98 (ara-2, *dd*, J = 7, 5.5 Hz), 4.03 (H-23, *d*, J = 12 Hz), 4.11 (glc-6, *dd*, J = 12.5, 1.5 Hz), 4.12 (xyl-5, *dd*, J = 12, 5.5 Hz), 4.21 (glc-6, *dd*, J = 12.5, 5 Hz), 4.23 (H-2, *m*), 4.30 (H-6, *m*, $W_{1/2}$ = 8 Hz), 4.59 (glc-1, *d*, J = 8 Hz), 4.63 (xyl-1, *d*, J = 7.5 Hz), 4.86 (rha-1, *d*, J = 2 Hz), 4.93 (xyl-4, *td*, J = 8, 5.5 Hz), 4.98 (xyl-2, *dd*, J = 9.5, 7.5 Hz), 5.00 (rha′-1, *d*, J = 1.5 Hz), 5.06 (rha-2, *m*), 5.06 (rha-4, *t*, J = 9 Hz), 5.07 (glc-2, *dd*, J = 9.5, 8 Hz), 5.10 (glc-4, *t*, J = 9 Hz), 5.12 (rha-3, *dd*, J = 9, 2.5 Hz), 5.14 (rha′-2, and rha′-3, *m*), 5.15 (ara-3, *dd*, J = 7, 3.5 Hz), 5.22 (glc-3, *t*, J = 9.5 Hz), 5.26 (ara-4, *dt*, J = 5, 3.5 Hz), 5.40 (H-12, *t*, J = 3 Hz), 5.65 (ara-1, *d*, J = 5.5 Hz).

Saponin **2**. Positive FABMS *m/z* (rel. int.): 1261.4 $[M + Na]^+$ (3); ^1H NMR (CD_3OD): δ0.88 (H-29, *s*), 0.96 (H-30, *s*), 1.05 (H-26, *s*), 1.06 (H-19), 1.18 (H-1, *dd*, J = 14, 5 Hz), 1.30 (H-27, *s*), 1.33 (H-24, *s*), 1.41 (H-15, *dd*, J = 14, 3 Hz), 1.55 (H-7, *brd*, J = 14 Hz), 1.62 (H-25, *s*), 1.81 (H-7 and H-15), 1.99 (H-11), 2.05 (H-1, *dd*, J = 14, 2 Hz), 2.12 (H-11, *dd*, J = 15, 7 Hz), 2.28 (H-19, *brt*, J = 13 Hz), 3.08 (H-18, *dd*, J = 13, 3 Hz), 3.41 (H-23, *d*, J = 11 Hz), 3.58 (H-3, *d*, J = 3 Hz), 3.72 (H-23, *d*, J = 11 Hz), 4.33 (H-2, *m*, $W_{1/2}$ = 9 Hz), 4.48 (H-6 and H-16, *m*), 5.41 (H-12, *t*, J = 2 Hz).

Saponin **3**. Positive FABMS *m/z* (rel. int.): 1275.1 $[M + Na]^+$ (22); negative FABMS *m/z*: 1235.3 $[M - H]^-$, 1089.3, 679.1; ^1H NMR (CD_3OD): δ0.90 (H-29, *s*), 0.94 (H-30, *s*), 1.06 (H-26, *s*), 1.13 (H-27, *s*), 1.15 (H-1), 1.30 (H-24, *s*), 1.34 (H-5), 1.51 (H-7), 1.61 (H-25, *s*), 1.80 (H-7), 1.95 (H-11), 2.04 (H-1), 2.10 (H-11), 2.93 (H-18, *dd*, J = 12, 4 Hz), 3.40 (H-23, *brd*, J = 12 Hz), 3.60 (H-3, *d*, J = 4 Hz), 3.74 (H-23, *d*, J = 12 Hz), 4.32 (H-2, *m*), 4.47 (H-6, *m*), 5.34 (H-12, *brt*, J = 3 Hz).

Saponin **4**. $[\alpha]_D$ −37.7° (*c* 0.305; MeOH); negative FABMS *m/z* (rel. int.): 1381.4 $[M - H]^-$ (8), 1205.7 (1), 1059.5 (1), 701.4 (3), 634.4 (2); ^1H NMR (CD_3OD): δ0.90 (H-29, *s*), 0.94 (H-30, *s*), 1.06 (H-26, *s*), 1.13 (H-27, *s*), 1.16 (H-1, *brd*, J = 14 Hz), 1.30 (H-5), 1.30 (H-24, *s*), 1.49 (H-7, *brd*, J = 14 Hz), 1.61 (H-25, *s*), 1.80 (H-7, *brd*, J = 14 Hz), 1.99 (H-11), 2.06 (H-1), 2.13 (H-11), 2.93 (H-18, *dd*, J = 12, 4 Hz), 3.39 (H-23, *d*, J = 12 Hz), 3.60 (H-3, *d*, J = 4 Hz), 3.71 (H-23, *d*, J = 12 Hz), 4.32 (H-2, *m*, $W_{1/2}$ = 9.5 Hz), 4.46 (H-6, *m*), 5.35 (H-12, *t*, J = 3 Hz).

Derivative **4a**. ^1H NMR ($CDCl_3$): δ0.91 (H-29, *s*), 0.93 (H-30, *s*), 1.05 (H-26, *s*), 1.10 (H-27, *s*), 1.17 (rha′-6, *d*, J = 6 Hz), 1.19 (rha″-6, *d*, J = 6 Hz), 1.20 (H-19), 1.25 (rha-6, *d*, J = 6 Hz), 1.37 (H-24, *s*), 1.68 (H-7), 1.62 (H-25, *s*), 1.70 (H-19), 1.95 (H-11), 1.96–2.17 (15 $COCH_3$, 15*s*), 2.15 (H-11), 2.86 (H-18, *dd*, J = 14, 4 Hz), 3.35 (xyl-5, *dd*, J = 12, 10 Hz), 3.49 (H-3, *d*, J = 4 Hz), 3.67 (rha-4, *t*, J = 8 Hz), 3.69 (ara-5, *dd*, J = 11, 2 Hz), 3.73 (rha-5, *m*), 3.83 (rha″-5, *m*), 3.87 (ara-2, *dd*, J = 6, 4 Hz), 3.92 (ara-5, *dd*, J = 11, 4.5 Hz), 3.93 (rha′-5, *m*), 3.95 (rha-3, *dd*, J = 9, 3 Hz), 3.98 (H-23, *d*, J = 10 Hz), 4.02 (xyl-3, *t*, J = 9 Hz), 4.07 (H-23, *d*, J = 10 Hz), 4.07 (gluA-5, *d*, J = 7Hz), 4.09 (xyl-5, *dd*, J = 12, 6 Hz), 4.24 (H-2, *m*), 4.33 (H-6, *m*), 4.67 (gluA-1, *d*, J = 8 Hz), 4.78 (xyl-1, *d*, J = 8 Hz), 4.87 (rha′-1, *d*, J = 1.5 Hz), 4.93 (xyl-4, *m*), 4.93 (rha-1, *d*, J = 1.5 Hz), 4.95 (xyl-2, *dd*, J = 9, 8 Hz), 4.96 (rha″-1, *brs*), 4.97 (gluA-4, *m*), 5.01 (rha′-4, *t*, J = 9 Hz), 5.04 (rha-2, *dd*, J = 4, 1.5 Hz), 5.06 (rha′-2, *dd*, J = 4, 2 Hz), 5.07 (rha″-4, *t*, J = 9.5 Hz), 5.10 (rha′-3, *dd*, J = 9, 4 Hz), 5.11 (gluA-2, *t*, J = 8 Hz), 5.19 (rha″-3, *dd*, J = 9.5, 3.5 Hz), 5.21 (ara-3, *m*), 5.23 (ara-4, *m*), 5.24 (gluA-3, *t*, J = 8 Hz), 5.39 (H-12, *brt*, J = 2.5 Hz), 5.43 (rha″-2, *dd*, J = 3, 1 Hz), 5.78 (ara-1, *d*, J = 4 Hz).

Saponin **5**. $[\alpha]_D$ −49.4° (*c* 0.563; MeOH); Positive FABMS *m/z* (rel. int.): 1275.6 $[M + Na]^+$ (3); ^1H NMR (CD_3OD): δ0.88 (H-29, *s*), 0.98 (H-30, *s*), 1.04 (H-19), 1.05 (H-26, *s*), 1.20 (H-1), 1.31 (H-27, *s*), 1.32 (H-5), 1.34 (H-24, *s*), 1.43 (H-15), 1.55 (H-7), 1.62 (H-25, *s*), 1.81 (H-7 and H-15), 1.98 (H-11), 2.06 (H-1), 2.10 (H-11), 2.28 (H-19, *t*, J = 13.5 Hz), 3.08 (H-18, *dd*, J = 14, 3 Hz), 3.41 (H-23, *d*, J = 12.5 Hz), 3.60 (H-3, *d*, J = 4 Hz), 3.73 (H-23, *d*, J = 12.5 Hz), 4.35 (H-2, *m*, $W_{1/2}$ = 9.5 Hz), 4.48 (H-6 and H-16, *m*), 5.41 (H-12, *t*, J = 3 Hz).

Saponin **6**. $[\alpha]_D$ −31.5° (*c* 0.454; MeOH); Positive FABMS *m/z*: 1407.6 $[M + Na]^+$, ^1H NMR (CD_3OD): δ0.90 (H-29, *s*), 0.94 (H-30, *s*), 1.06 (H-26, *s*), 1.13 (H-27, *s*), 1.20 (H-19 and H-1), 1.30 (H-24, *s*), 1.32 (H-5), 1.55 (H-7), 1.61 (H-25, *s*), 1.75 (H-19), 1.80 (H-7), 1.98 (H-11), 2.05 (H-1), 2.13 (H-11), 2.93 (H-18, *dd*, J = 14, 4 Hz), 3.42

(H-23, *d*, J = 12.5 Hz), 3.58 (H-3, *d*, J = 4 Hz), 3.73 (H-23, *d*, J = 12.5 Hz), 4.32 (H-2, *m*, $W_{1/2}$ = 9 Hz), 4.46 (H-6, *m*), 5.35 (H-12, *t*, J = 3 Hz).

Derivative **6a**. ^{1}H NMR ($CDCl_3$): δ0.92 (H-29, *s*), 0.93 (H-30, *s*), 1.07 (H-26, *s*), 1.12 (H-27, *s*), 1.13 (H-5), 1.17 (rha′-6, *d*, J = 6 Hz), 1.18 (H-19), 1.29 (rha-6, *d*, J = 6 Hz), 1.36 (H-24, *s*), 1.40 (H-7), 1.60 (H-7), 1.63 (H-25, *s*), 1.70 (H-19), 1.90 (H-11), 1.97–2.18 (16 $COCH_3$, 11*s*), 2.10 (H-11), 2.87 (H-18, *dd*, J = 14, 4 Hz), 3.30 (xyl-5, *dd*, J = 12, 7.5 Hz), 3.34 (H-3, *d*, J = 4 Hz), 3.65 (rha-4, *t*, J = 9 Hz), 3.68 (glc-5, *m*), 3.69 (glc′-5, *dm*, J = 8 Hz), 3.73 (ara-5, *dd*, J = 12, 3 Hz), 3.81 (xyl-3, *dd*, J = 9, 8 Hz), 3.90 (H-23, *d*, J = 12 Hz), 3.90 (rha-5, *m*), 3.92 (ara-5, *dd*, J = 12, 3 Hz), 3.94 (rha′-5, *m*), 3.97 (H-23, *d*, J = 12 Hz), 3.98 (ara-2, *dd*, J = 7, 5 Hz), 4.05 (glc′-6, *dd*, J = 12, 2 Hz), 4.11 (xyl-5, *dd*, J = 12, 5 Hz), 4.14 (2H, glc-6, *brd*, J = 3.5 Hz), 4.20 (H-2, *m*), 4.32 (H-6, *m*, $W_{1/2}$ = 8 Hz), 4.37 (glc′-6, *dd*, J = 12, 4.5 Hz), 4.41 (glc-1, *d*, J = 8 Hz), 4.61 (glc′-1, *d*, J = 8 Hz), 4.63 (xyl-1, *d*, J = 7.5 Hz), 4.86 (rha-1, *d*, J = 2 Hz), 4.91 (xyl-4, *m*), 4.91 (glc-4, *t*, J = 8.5 Hz), 4.92 (glc′-2, *dd*, J = 9.5, 7 Hz), 4.98 (xyl-2, *dd*, J = 9, 7.5 Hz), 5.03 (rha′-4, *t*, J = 9 Hz), 5.06 (rha′-1, *d*, J = 1.9 Hz), 5.07 (glc-2, *t*, J = 9 Hz), 5.07 (rha-2, *dd*, J = 5, 2 Hz), 5.07 (glc′-4, *t*, J = 9 Hz), 5.09 (rha-3, *m*), 5.11 (rha′-2 and rha′-3, *m*), 5.12 (glc′-3, *t*, J = 9 Hz), 5.14 (ara-3, *dd*, J = 7, 5 Hz), 5.25 (ara-4, *m*), 5.40 (H-12, *m*, $W_{1/2}$ = 7 Hz), 5.65 (ara-1, *d*, J = 5.5 Hz).

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