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Saponins from the seeds of *Mimusops laurifolia*

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Dedicated to Professor Rod Croteau on the occasion of his 60th birthday.

Abstract

Nine saponins were isolated from the seeds of *Mimusops laurifolia*. Their structures were established using one- and two-dimensional NMR spectroscopy and mass spectrometry. Three of them are identified as: 3-*O*-(β-D-apiofuranosyl-(1 → 3)-β-D-glucuronopyranosyl)-28-*O*-(α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl)-16α-hydroxyprotobassic acid, 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)-16α-hydroxyprotobassic acid and 3-*O*-(β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl)-28-*O*-(α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl)-16α-hydroxyprotobassic acid.
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1. Introduction

Species belonging to genus *Mimusops* are widely distributed in India and are reported to be used in traditional medicine (Sahu et al., 1995; Shah et al., 2003). In an effort to understand the miscellaneous biological activities displayed by these plants, several phytochemical investigations were launched, leading mostly to the isolation of triterpenoid saponins (Nigam et al., 1992; Sahu et al., 1995; Lavaud et al., 1996; Sahu, 1996). *Mimusops laurifolia* (Forssk.) Friis, also known as *Binectaria laurifolia* Forssk. and *M. schimperi* A. Rich. (Friis, 1981), is one of these species, cultivated in Egypt, Uganda and the Sudan. It is an evergreen tree, with small fruits containing one to three shiny hard

seeds and which was long been used in ancient Egypt since the seeds and leafy twigs, used as funeral bouquets, were found in pharaonic tombs. More recently, this plant regained popularity since the extract was patented for skin-conditioning and -moisturizing effects, as part of preparations used in cosmetics, bath formulations and detergents (Ohara et al., 2001). These activities were certainly due to the presence of saponins although this was not part of the claims. As a continuation of a search for bioactive saponins from plant species growing in Egypt, and because of the absence of any phytochemical report on this species and its reported cosmetic activity, we decided to investigate its saponin constituents. We report here the isolation and structural elucidation of three new bidesmosidic saponins possessing 16α-hydroxyprotobassic acid as aglycone, along with six known saponins: butyroside C, arganines A, C and D, Mi-saponin A and saponin 5 from *M. hexandra* later named tieghemelin A, from the seed extract.

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2. Results and discussion

The defatted seed powder of *M. laurifolia* was extracted with MeOH, and then the MeOH extract was precipitated by acetone. After dialysis against water, the crude saponin mixture was purified using a combination of reversed phase C-18 column and silica gel column chromatography. Final steps of purification were either preparative TLC or semi-prep. HPLC over C-18 reversed phase and afforded nine saponins (1–9). The sugar composition was determined by TLC after acid hydrolysis of the crude saponin mixture as D-glucose, D-xylose, L-rhamnose, L-arabinose, D-apiose and D-glucuronic acid, configurations were established by polarimetry. The extract from *M. laurifolia* seeds contained known compounds (1–6) whose structural assignments were made by analysis of ESI-MS and 2D NMR spectral data including COSY, TOCSY, ROESY, HSQC and HMBC. Characterization was carried out by comparison of their data with those reported in literature.

Saponin 1 (ESI-MS: m/z 1235 [M−H]$^{-}$), saponin 2 (ESI-MS: m/z 1385 [M+H]$^{+}$) and saponin 3 (ESI-MS: m/z 1245 [M+Na]$^{+}$) were bidesmosides of protobassic acid and identified as butyroside C, arganine D and Mi-saponin A, respectively. Butyroside C (1) was previously isolated from *Madhuca butyraceae* (Li et al., 1994) and from three species of *Mimusops* (Lavaud et al., 1996), while arganine D (2) was identified first in *Argania spinosa* (Charrouf et al., 1992) and then in *Mimusops elengi* (Sahu et al., 1997). Mi-saponin A (3) is an ubiquitous compound found in seeds of Sapotaceae and isolated on many instances and for example from *Madhuca longifolia* (Kitagawa et al., 1975; Ishii et al., 1981), *Argania spinosa* (Charrouf et al., 1992), *Madhuca butyraceae* (Nigam et al., 1992) and *Mimusops* species (Lavaud et al., 1996; Sahu et al., 1997).

Saponin 4 (ESI-MS: m/z 1251 [M−H]$^{-}$), saponin 5 (ESI-MS: m/z 1401 [M+H]$^{+}$) and saponin 6 (ESI-MS: m/z 1261 [M+Na]$^{+}$) were bidesmosides of 16α-hydroxyprotobassic acid and identified as 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)-16α-hydroxyprotobassic acid, arganine A and arganine C, respectively. Saponin 4 was initially isolated from
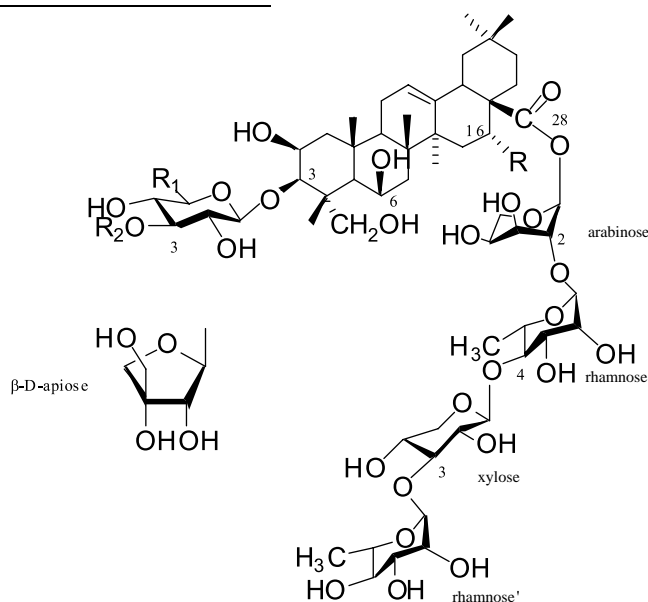
	R	R₁	R₂	
1	H	COOH	H	**butyroside C**
2	H	CH₂O-β-D-glucose	H	**arganine D**
3	H	CH₂OH	H	**Mi-saponin A**
4	OH	COOH	H	**tieghemelin A**
5	OH	CH₂O-β-D-glucose	H	**arganine A**
6	OH	CH₂OH	H	**arganine C**
7	OH	COOH	β-D-apiose	
8	OH	CH₂OH	β-D-glucose''	
9	OH	CH₂O-β-D-glucose''(6–> 1)β-D-glucose'''	H	

M. hexandra and *M. manilkara* (Lavaud et al., 1996) and more recently from *Tieghemella heckelii* and claimed there to be a new compound which was named tieghemelin A (Gosse et al., 2002). Arganines A (**5**) and C (**6**) were previously isolated from *Argania spinosa* (Sapotaceae) (Charrouf et al., 1992). Arganine C was described for the first time in a Rubiaceae species: *Crossopteryx febrifuga* (Gariboldi et al., 1990) and it was since identified in three other species of Sapotaceae *Madhuca butyraceae* (Nigam et al., 1992), *Mimusops elengi* (Lavaud et al., 1996; Sahu et al., 1997) and *Tieghemella heckelii* (Gosse et al., 2002).

The 16α-hydroxyprotobassic acid was identified as the aglycone of new compounds **7**–**9** owing to the spectroscopic data which were in good agreement with those reported in the literature [δ_{H-16} 4.52 ± 0.05, δ_{C-16} 73.1 ± 0.1 ppm]. (Sahu et al., 1995; Lavaud et al., 1996; Nigam et al., 1992). The ^{13}C chemical shifts of C-28 at δ 175.6 and of C-3 at δ 81.9 ± 0.2 ppm suggested the presence of two oligosaccharidic chains attached to these positions of the 16α-hydroxyprotobassic acid.

The positive ESI-MS of saponin **7** gave a quasi-molecular ion peak at m/z 1429.7 [M−2H+2Na]$^+$ while, in the negative mode, a quasi-molecular ion was detected at m/z 1383.8 [M−2H]$^-$ corresponding to a molecular formula of $C_{63}H_{100}O_{33}$. The MS^2 experiment of the [M−2H+2Na]$^+$ ion, gave a positive fragment at m/z 873.5 [M−2H+2Na−2×132−2×146]$^+$, attributed to the loss of a tetraglycosidic chain made of two pentoses and of two desoxyhexoses. The MS^3 experiment gave positive fragments at m/z 741.4 [M−2H+2Na−3×132−2×146]$^+$ and at m/z 565.4 [M−2H+2Na−3×132−2×146−176]$^+$ due to the successive losses of a terminal pentose and of an uronic acid (Table 1). The ^{13}C NMR spectrum showed six signals for anomeric carbons at δ 92.5, 99.9, 101.1, 103.2, 105.0 and 109.3 respectively linked to anomeric protons at δ 5.62, 5.12, 5.17, 4.51, 4.57 and 5.39 in the HSQC experiment (Table 2). The analysis of the COSY and TOCSY spectra revealed the presence of a β-D-xylose ($J_{H-1,H-2}$ = 7.7Hz), a β-D-erythroapiofuranose ($J_{H-1,H-2}$ = 2.3 Hz) (Ishii and Yanagisawa, 1998), an α-L-arabinose in a predominant $^1C_4$ conformation ($^3J_{H-1, H-2}$ = 4.2 Hz and $^3J_{H-2,H-3}$ = 5.2 Hz) (Ishii et al., 1981) and two α-L-rhamnoses identified through the observation of COSY correlations between two methyl doublets at δ 1.32 and 1.27 and glycosidic protons at δ 3.78 and 4.03, respectively. The uronic acid residue was identified as a β-D-glucuronic acid ($J_{H-1,H-2}$ = 7.3 Hz). Sequencing of the glycosidic chains was achieved by analysis of HMBC and ROESY experiments. For the tetraglycosidic chain linked to the carboxylic C-28 of the aglycone, the HMBC showed cross-peaks between H-1 (δ 5.17) of terminal rhamnose and C-3 (δ 82.6) of xylose, between H-1 of xylose (δ 4.57) and C-4 (δ 81.7) of inner rhamnose, between H-1 (δ 5.12) of inner rhamnose and C-2 (δ 74.0) of arabinose and between H-1 (δ 5.62) of arabinose and C-28 (δ 175.6). Another correlation was observed between H-1 (δ 5.39) of the apiose and C-3 (δ 82.2) of the glucuronic acid, thus building a disaccharidic chain ether which was attached to C-3 of

the aglycone as shown by a correlation between H-1 (δ 4.51) of glucuronic acid and C-3 (δ 81.9) of the aglycone. Compared with tieghemelin A (**4**), saponin **7** thus possessed a supplementary apiose unit linked to C-3 of the glucuronic acid and was identified as 3-*O*-(β-D-apiofuranosyl(1 → 3)-β-D-glucuronopyranosyl)-28-*O*-(α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl)-16α-hydroxyprotobassic acid.

Saponin **8** exhibited a quasi-molecular ion peak at m/z 1424.4 [M+Na]$^+$ in the positive mode and at m/z 1400.4 [M−H]$^-$ in the negative mode, in agreement with a molecular formula of $C_{64}H_{104}O_{33}$ same as measured for arganine A (**5**). The MS^2 fragmentation of [M+Na]$^+$ gave an ion peak at m/z 867.7 [M+Na−2×132 − 2×146]$^+$ indicating the loss of a tetrasaccharidic chain ester-linked to the aglycone identical to that of saponin **7**. The MS^4 experiment gave a positive fragment at m/z 661.5 [M+Na−2×132 − 2×146 − 162 − CO$_2$]$^+$ attributed to the loss of a terminal hexose unit (Table 1). The 2D experiments (COSY, TOCSY and HSQC) allowed the assignment of all the ^{1}H and ^{13}C signals of the sugars (Table 2), identified as two β-D-glucoses (δ_{H-1} 4.60 and 4.53, δ_{C-1} 103.8 and 103.5), two α-L-rhamnoses (δ_{H-1} 5.17 and 5.10, δ_{C-1} 101.1 and 99.9), a β-D-xylose (δ_{H-1} 4.57, δ_{C-1} 105.0) and an α-L-arabinose (δ_{H-1} 5.63, δ_{C-1} 92.6). The analysis of HMBC correlations showed that the four sugar residues involved in the ester-linked chain were attached in the same way that in the previous and other saponins. In saponin **8**, the ether-linked sugar chain was composed of two glucose moieties and HMBC correlations were observed between H-1 (δ 4.60) of the outer glucose and C-3 (δ 86.5) of the inner glucose and between H-1 (δ 4.53) of the inner glucose and C-3 (δ 82.1) of the aglycone. ROEs observed across the glycosidic bonds confirmed the previous interpretation of the HMBC spectrum. Thus, the new saponin **8** was dentified as being 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)-16α-hydroxyprotobassic acid.

A quasi-molecular ion peak was observed at m/z 1586.6 [M+Na]$^+$ in the positive ESI-MS of saponin **9** and in the negative mode, a quasi-molecular ion peak was detected at m/z 1562.5 [M−H]$^-$ corresponding to a molecular formula of $C_{70}H_{114}O_{38}$. The MS^2 fragmentation of [M+Na]$^+$ gave the expected ion peak at m/z 1030.0 [M+Na−2×132 − 2×146]$^+$ due to the loss of the ester glycosidic chain. The MS^4 experiment gave an intense ion peak at m/z 823.6 attributed to the loss of a terminal hexose unit. Finally, the MS^5 fragmentation gave ion peaks at m/z 661.5 [M+Na−2×132 − 2×146 − 2×162 − CO$_2$]$^+$ due to the loss of a second hexose unit and 519.3 [Aglycone+H]$^+$ indicating the presence of three hexose units in the ether-linked chain at C-3 of aglycone (Table 1). The ^{13}C NMR spectrum showed seven anomeric carbons at δ 92.6, 99.9, 101.1, 102.7, 103.4, 103.5 and 105.0 linked respectively to anomeric protons (HSQC experiment) at δ 5.62 (d, J = 4.1 Hz), 5.11 (s), 5.17 (d, J = 1.6 Hz), 4.51 (d, J = 7.6 Hz), 4.39 (d,

Table 1
Positive and negative ESI-MS fragmentation of saponins **7–9**

	Fragments[a]	**7** ($M_w = 1384$)	**8** ($M_w = 1400$)	**9** ($M_w = 1562$)
		$C_{63}H_{100}O_{33}$	$C_{64}H_{104}O_{33}$	$C_{70}H_{114}O_{38}$
ESI-MS$^+$	[M+Na]$^+$	1429.7 (−2H+Na)	1423.7	1586.6
MS^2 [M+Na]$^+$	[M+Na−2×132−2×146]$^+$	873.5 (−2H+Na)	867.7	1030.0
MS^3 [M+Na−2×132−2×146]$^+$	[M+Na-2×132−2×146−CO$_2$]$^+$		823.6	985.6
	[M+Na−3×132−2×146]$^+$	741.4 (−2H+Na)		
	[M+Na−3×132−2×146−176]$^+$	565.4 (−2H+Na)		
MS^4[M+Na−2×132−2×146−CO$_2$]$^+$	[M+Na−2×132−2×146−162−CO$_2$]$^+$		661.5	823.6
MS^5[M+Na−2×132−2×146−162−CO$_2$]$^+$	[M+Na−2×132−2×146−2×162−CO$_2$]$^+$			661.4
	[Aglycone + H]$^+$			519.3
ESI-MS$^-$	[M−H]$^-$	1383.8 (−H)	1400.4	1562.5
MS^2[M−H]$^-$	[M−H − 2×132−2×146]$^-$	827.4 (−H)	843.3	1005.6
MS^3[M−H − 2×132−2×146]$^-$	[M−H − 2×132−2×146−162]$^-$		681.3	843.2
	[M−H − 3×132−2×146]$^-$	695.2 (−H)		
	[M−H − 3×132−2×146−176]$^-$	519.2 (−H)		
	[M−H − 2×132−2×146 − H$_2$O − CO$_2$]$^-$			943.4
MS^4[M−H − 2×132−2×146 − 162]$^-$	[M−H − 2×132−2×146−162 − CO$_2$]$^-$			799.2
	[M−H − 2×132−2×146 − 2×162]$^-$		519.2	681.2
	[M−H − 2×132−2×146 − 3×162]$^-$			519.2
MS^4[M−H − 2×132−2×146 − H$_2$O − CO$_2$]$^-$	[M−H − 2×132−2×146−162 − H$_2$O − CO$_2$]$^-$			781.3
MS^5[M−H − 2×132−2×146 − 162 − H$_2$O − CO$_2$]$^-$	[M−H − 2×132−2×146 − 2×162 − H$_2$O − CO$_2$]$^-$			619.3

[a] Accurate mass; losses of 132 = pentose, 146 = desoxy-hexose, 162 = hexose and 176 = uronic acid.

Table 2
^{1}H and ^{13}C spectroscopic data for glycosidic part of saponins **7**–**9** in CD$_3$OD

	7		**8**		**9**	
	δH	δC	δH	δC	δH	δC
Sugar chain linked to C-28						
α-L-Arabinose (ester)						
1	5.62 (*d*; 4.2)	92.5	5.63 (*d*; 4)	92.6	5.62 (*d*; 4.1)	92.6
2	3.83 (*dd*; 5.2,4.1)	74.0	3.83 (*dd*; 5.2,4)	74.1	3.83 (*dd*; 5.6,4.1)	74.0
3	3.88 (*dd*; 5.5,3)	70.3	3.89 (*t*; 5)	70.1	3.89 (*t*; 4)	70.3
4	3.84 (*m*)	65.9	3.84 (*m*)	65.8	3.84 (*m*)	65.9
5	3.54 (*dd*; 10.5,4)	62.8	3.54 (*dd*; 11,4.2)	62.7	3.54 (*dd*; 10.9,3.3)	62.7
5	3.93 (*dd*; 11.2,7.1)		3.94 (*dd*; 11.3,7.5)		3.93 (*dd*; 11.4,7.4)	
α-L-Rhamnose (inner)						
1	5.12 (*s*)	99.9	5.10 (*brs*)	99.9	5.11 (*s*;1)	99.9
2	3.88 (*brs*)	70.8	3.88 (*m*)	70.8	3.88 (*brs*)	70.8
3	3.87 (*m*)	70.8	3.87 (*dd* like; 9.5,3.2)	70.9	3.87 (*dd*; 9,3.1)	70.9
4	3.61 (*t*; 9)	81.7	3.61 (*t*; 9.2)	81.6	3.61 (*t*; 9)	81.6
5	3.78 (*dq*; 9.5,6.5)	67.5	3.75 (*dq*; 9.5,6.3)	67.5	3.75 (*m*)	67.5
6	1.32 (*d*; 6.2)	16.6	1.32 (*d*; 6.1)	16.6	1.32 (*d*; 4.8)	16.6
β-D-Xylose (inner)						
1	4.57 (*d*; 7.7)	105.0	4.57 (*d*; 7.8)	105.0	4.57 (*d*; 7.7)	105.0
2	3.37 (*dd*; 9, 7.8)	74.8	3.37 (*t*; 8)	74.8	3.37 (*t*; 8)	74.8
3	3.49 (*t*; 8.9)	82.6	3.49 (*t*; 8.9)	82.5	3.49 (*t*; 9)	82.5
4	3.56 (*ddd*; 10.2. 8.8,6)	68.3	3.55 (*m*)	68.3	3.55 (*m*)	68.3
5	3.25 (*dd*; 11.5,10.4)	65.8	3.25 (*dd*; 11.5,10.4)	65.7	3.25 (*dd*; 12, 10)	65.6
5	3.90 (*dd*; 11.5,5.7)		3.90 (*dd*; 11.7, 5.4)		3.90 (*dd*; 11.3,5.4)	
α-L-Rhamnose′(outer)						
1	5.17 (*d*; 1.5)	101.1	5.17 (*d*; 1.7)	101.1	5.17 (*d*; 1.6)	101.1
2	3.99 (*dd*; 3.3,1.7)	70.7	3.98 (*dd*; 3.3,1.7)	70.8	3.98 (*dd*; 3.4,1.7)	70.7
3	3.73 (*dd*; 9.6,3.3)	70.8	3.73 (*dd*; 9.4,3.4)	70.7	3.74 (*dd*; 9.5,3.5)	70.8
4	3.41 (*t*; 9.6)	72.6	3.41 (*t*; 9.5)	72.5	3.41 (*t*; 9.4)	72.5
5	4.03 (*dq*; 9,6)	68.5	4.03 (*dq*; 9.5,6.1)	68.5	4.03 (*dq*; 9.5,6)	68.5
6	1.27 (*d*; 6.2)	16.4	1.27 (*d*; 6.2)	16.4	1.27 (*d*; 6.2)	16.4
Sugar chain linked to C-3						
3-*O*-β-D-Glucose′						
1			4.53 (*d*; 7.7)	103.5	4.51 (*d*; 7.6)	102.7
2			3.52 (*t*; 8)	73.3	3.33 (*t*; 8)	73.8
3			3.59 (*t*; 8.8)	86.5	3.40 (*t*; 8.5)	76.9
4			3.51 (*t*; 9)	68.0	3.28 (*t*; 9)	70.8
5			3.34 (*m*)	75.9	3.63 (*brt*; 9.5, 1.7)	75.0
6			3.75 (*dd*; 11.4, 5.9)	60.7	3.68 (*dd*; 10.8, 8)	69.7
6			3.84 (*dd*; 12,1.7)		4.19 (*dd*; 10.5,1.5)	
β-D-Glucose″						
1			4.60 (*d*; 7.9)	103.8	4.34 (*d*; 7.7)	103.5
2			3.31 (*t*; 8.2)	74.0	3.25 (*t*; 8.5)	73.6
3			3,41 (*t*; 9.5)	76.3	3.40 (*t*; 8.5)	76.2
4			3.29 (*t*; 9.7)	70.1	3.35 (*t*; 8.8)	70.0
5			3.35 (*m*)	76.7	3.49 (*brdd*; 9,6.2)	75.4
6			3.65 (*dd*; 11.9,6.3)	61.2	3.79 (*dd*; 11.6,6.1)	68.6
6			3.91 (*dd*; 11.7,1.9)		4.18 (*dd*; 11.5,1.8)	
β-D-Glucose‴						
1					4.39 (*d*; 7.8)	103.4
2					3.25 (*t*; 8.5)	73.6
3					3.38 (*t*; 8.7)	76.5
4					3.34 (*t*; 8.3)	69.9
5					3.27 (*m*)	76.5
6					3.72 (*dd*; 12.1,5.4)	61.1
6					3.89 (*brd*; 12)	
β-D-Glucuronic acid						
1	4.51 (*d*; 7.3)	103.2				
2	3.49 (*brt*; 9)	73.7				
3	3.55 (*t*; 8.9)	82.2				
4	3.49 (*t*; 9.4)	70.9				

Table 2 (continued)

	7		8		9	
	δH	δC	δH	δC	δH	δC
5	3.65 (d; 9.6)	75.6				
6		175.3				
β-D-Apiose						
1	5.39 (d; 2.3)	109.3				
2	4.00 (d; 2.3)	76.5				
3	–	79.1				
4	3.78 (d; 9.7)	73.7				
4	4.16 (d; 9.6)					
5	3.65(s)	64.2				
5						

$J = 7.8$ Hz), 4.34 (d, $J = 7.7$ Hz) and 4.57 (d, $J = 7.7$ Hz), (Table 2). The analysis of the COSY and TOCSY spectra confirmed the mass spectrometry results showing the presence of three β-D-glucose moieties in addition to the fixed four sugars oligosaccharidic ester chain. Sequencing of the ether-linked sugar chain to C-3 of the aglycone composed of these three glucose units, was established using the following HMBC correlations: H-1 (δ 4.39) of outer terminal glucose‴ with C-6 (δ 68.6) of inner glucose″, H-1 (δ 4.34) of inner glucose″ with C-6 (δ 69.7) of the first glucose′ and H-1 (δ 4.51) of the first glucose′ with C-3 (δ 82.1) of the aglycone. ROEs observed between H-1 (δ 4.39) of terminal glucose‴ and the two H-6 (δ 3.79,4.18) of the second glucose″ and between H-1 (δ 4.34) of glucose′ and the two H-6 (δ 3.68,4.19) of glucose′ confirmed the sequencing of the trisaccharidic chain as β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl. Thus, saponin **9** was composed of arganine A bearing a supplementary terminal glucose unit in the ether-linked glycosidic chain. Consequently, saponin **9** was deduced as 3-O-(β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl)-28-O-(α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl)-16α-hydroxyprotobassic acid.

All isolated saponins from *M. laurifolia* contain protobassic acid and 16α-hydroxyprotobassic acid glycosides as major saponins and among these, compounds **7–9** are new derivatives. These results confirm the fact that bassic acid itself or its derivatives are not genuinely natural but dehydration products of protobassic acid. Given this isolation and previous work on plants of Sapotaceae family precisely *Mimusops*, *Argania* and *Madhuca* (Sahu, 1996; Lavaud et al., 1996). Mi-saponin A (**3**) with protobassic acid as genin can be considered as a chemical marker of species belonging to Sapotaceae, especially of the seed part. The occurrence of arganine C(**6**) with 16α-hydroxyprotobassic acid in *M. laurifolia* suggests that this compound could be also considered as another chemical marker in the seed saponin mixture. The isolation of these two minor compounds from other families (Rubiaceae and Verbenaceae) confirms the phylogenetic relationship of these three families in Asterids and suggests similar biosynthetic pathways in these plants.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded in CD_3OD on a Bruker DRX-500 at 500 and 125 MHz. 2D experiments were performed using standard Bruker microprograms. ESI-MS and MS-MS experiments were recorded on Esquire-LC mass spectrometer; samples were introduced by infusion in a MeOH solution. Optical rotations were determined in MeOH or H_2O with a Perkin–Elmer 241 polarimeter. TLC was carried out on precoated silica gel 60 F_{254} (Merck) and spots were visualized by spraying with 50% aqueous H_2SO_4. Kieselgel 60 (63–200 μm, Merck) and Lichroprep RP-18 (40–63 μm, Merck) were used for CC. HPLC was performed on a DIONEX apparatus equipped with ASI-100 automated sample injector, a P580 pump, a STH 585 column oven, a diode array detector UVD 340S and a Chromeleon software. Pre-packed column (Dionex RP-18, 201SP, 10×250 mm, 5 μm) was used for semi-prep. HPLC with a binary gradient (solvent A: H_2O/TFA, 0.025%, solvent B: MeCN) and a flow rate of 3 ml min^{-1}; the chromatogram was monitored at 205 nm.

3.2. Plant material

The seeds of *M. laurifolia* were collected from the Egyptian museum garden, Cairo, Egypt from July to August 2001. The plant was identified by Dr. R. Hamdy, lecturer of plant taxonomy, faculty of science, Cairo University (Hemsley, 1968).

3.3. Extraction and isolation

The air dried powdered seeds of *M. laurifolia* (500 g) was defatted by successive extraction with petroleum ether, hexane, ethyl acetate and $CHCl_3$. Then, the defatted powder was extracted with MeOH to obtain a concentrated extract (70 g), which was suspended in MeOH (150 ml) and precipitated by addition of a large excess of Me_2CO (850 ml). The resulting ppt. was filtered and dried over KOH. The dried ppt. (39 g) was dissolved in H_2O and dialysed against pure distilled H_2O in an agi-

tated seamless cellulose tubing. After 72 h the content of the tube was freeze-dried to afford the dialysed saponin mixture (5.3 g). The crude saponin mixture (3 g) was subjected to RP-18 CC using a gradient of $MeOH:H_2O$ (40:60 to 60:40) to give 88 fractions. Fractions [7–8] were purified on silica gel CC eluted with $CHCl_3:MeOH$ (60:40) to give **4** (81 mg) and 7 (20 mg). Fractions [15–17] afforded saponin **1** (106 mg). Fractions [20–21] were applied to silica gel CC eluted with $CHCl_3:MeOH:H_2O$ (70:30:5) to give saponin **9** (10 mg). Fractions [27–30] were passed over cationic ion exchange resin column, then subjected to reversed phase RP-18 CC eluted with $MeOH:H_2O$ (45:55) to yield **1** (37 mg) and **5** (114 mg). Fractions [43–47] were purified on prep. TLC with $CHCl_3:MeOH:H_2O$ (50:50:7) to afford saponin **8** (18 mg) and **6** (16 mg). Fractions [70–76] contained pure saponin **2** (142 mg). Fractions [80–88] were purified by semi-prep. HPLC with the gradient elution program: 25–30% B (0–30 min) to give saponin **3** (22 mg).

3.4. Saponin 7

White powder, $[\alpha]_D^{21}$ −50.8° (c 0.25, MeOH). ^1H and ^{13}C NMR of the glycosidic part: see Table 2, ^1H NMR of the aglycone part (CD_3OD): δ 0.91 (H-29, s), 0.99 (H-30, s), 1.07 (H-26, s), 1.33 (H-24, s), 1.36 (H-27, s), 1.64 (H-25, s), 3.10 (H-18, dd, 14.1, 4.0 Hz), 3.43 (H-23, d, 12.0 Hz), 3.61 (H-3, d, 4.4 Hz), 3.76 (H-23, d, 12.5 Hz), 4.36 (H-2, q, 4.0 Hz), 4.51 (H-6, brt, 4.3 Hz), 4.51 (H-16, brs), 5.44 (H-12, brt, 3.5 Hz). ^{13}C NMR of the aglycone part (CD_3OD): δ 14.8 (C-24), 17.6 (C-26), 17.8 (C-25), 23.6 (C-30), 25.9 (C-27), 31.9 (C-29), 40.7 (C-18), 63.9 (C-23), 67.3 (C-6), 69.7 (C-2), 73.2 (C-16), 81.9 (C-3), 122.7 (C-12), 142.5 (C-13), 175.6 (C-28). ESI-MS: see Table 1.

3.5. Saponin 8

White powder, $[\alpha]_D^{21}$ −41.7° (c 0.27, MeOH). ^1H and ^{13}C NMR of the glycosidic part: see Table 2, ^1H and ^{13}C NMR chemical shift values of the aglycone are identical to those described for saponin **7** to ±0.02 and ±0.2 ppm, respectively. ESI-MS: see Table 1.

3.6. Saponin 9

White powder, $[\alpha]_D^{21}$ −45.0° (c 0.4, MeOH). ^1H and ^{13}C NMR of the glycosidic part: see Table 2, ^1H and ^{13}C NMR chemical shift values of the aglycone are identical to those described for saponin 7 to ±0.06 and ±0.2 ppm, respectively. ESI-MS: see Table 1.

3.7. Acid hydrolysis of saponin

The crude saponin mixture (200 mg) was refluxed with 30 ml of 0.02 N H_2SO_4 and 6% $HClO_4$, (1:1) at 100° for 5 h. After filtration the acid aqueous layer was neutralised

with 1 N KOH and freeze-dried. Six sugars were identified and compared with authentic samples by TLC using solvent MeCOEt–iso-PrOH–Me_2CO–H_2O (20:10:7:6) as glucose, xylose, arabinose, rhamnose, apiose and glucuronic acid. The purification of these sugars was achieved by Prep. TLC using Kieselgel 60 plates which were eluted three times with solv., $CHCl_3:MeOH:H_2O$ (70:30:3) to afford xylose (R_f 0.48, $[\alpha]_D^{21}$ +9), arabinose (R_f 0.38, $[\alpha]_D^{21}$ +25.5), glucose (R_f 0.26, $[\alpha]_D^{21}$ +24), rhamnose (R_f 0.62, $[\alpha]_D^{21}$ +12), glucuronic acid (R_f 0.15, $[\alpha]_D^{21}$ +4.2) and apiose (R_f 0.58).

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