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A TRITERPENOID SAPONIN FROM *MAESA RAMENTACEA*

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Key Word Index—*Maesa ramentacea*; Myrsinaceae; triterpenoid saponin; piscicidal activity.

Abstract—The structure of a piscicidal triterpenoid saponin (saponin A) isolated from the leaves of *Maesa ramentacea* has been shown to be 3-*O*-{[(α-L-rhamnopyranosyl(1 → 2)-α-L-rhamnopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 3)]-[β-D-glucopyranosyl(1 → 2)]-β-D-glucuronopyranosyl}barringtogenol C21, 22-*O*-diangeloate. Extensive use was made of homo- and heteronuclear 2D NMR techniques. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

In a search for piscicidal compounds from Thai plants, an aqueous extract of the leaves of *Maesa ramentacea* was found to show good piscicidal activity (LD_{100} = 100 mg l^{-1} [1]. It occurred to us that these leaves could be potentially useful as a fish poison in commercial fish and prawn farming. *Maesa ramentacea* is a rapidly growing, common tree in the southern part of Thailand and should present no difficulty in cultivation. For this reason, chemical studies of this plant were undertaken. Quinones, flavonoids, triterpenoids and saponins have so far been found as constituents of *Maesa* spp. The benzoquinones, bhogatin and maesanin, have been isolated from *M. macrophylla* [2] and *M. lanceloata* [3], respectively, and quercitin 3-rhamnoside has been reported from the leaves of *M. indica* [4]. Three triterpenoids, camelliagenin and two derivatives, were isolated from the leaves of *M. chisia* var. *angustifolia* [5] and recently six new saponins having moderate virucidal activity were obtained from the leaves of *M. lanceolata* [6]. We now wish to report the isolation and structural elucidation of a new triterpenoid saponin from the leaves of *M. ramentacea*. The compound showed strong piscicidal activity.

RESULTS AND DISCUSSION

A crude saponin fraction was obtained by extraction of an aqueous extract of milled leaves with *n*-butanol, followed by chromatography on silica gel. This fraction showed significant piscicidal activity (LD_{100} = 1 mg l^{-1}). The fraction was further purified by TLC on silanized silica gel to give a single spot saponin fraction. HPLC gave pure saponin A(**1**).

The FAB (negative ion) mass spectrum gave an $[M-H]^-$ ion at *m/z* 1445, corresponding to a molecular formula $C_{70}H_{110}O_{31}$. Prominent fragment ions occurred at *m/z* 1299 $[M-H-146]^-$ and 1283 $[M-H-162]^-$, corresponding to the independent losses of a deoxyhexose and a hexose unit, indicating a branched oligosaccharide with terminal deoxyhexose and hexose. Other ions appeared at *m/z* 1153 $[M-H-146-146]^-$, 991 $[M-H-146-146-162]^-$, 829 $[M-H-146-146-162-162]^-$ and 653 $[M-H-146-146-162-162-176]^-$, corresponding to the subsequent loss of a deoxyhexose and a hexose unit and finally a hexuronic acid unit. The ^{13}C NMR spectrum of the saponin (Table 1) exhibited signals for five anomeric carbons (δ 101.2, 101.5, 103.1, 103.9 and 105.6) and three carbonyl carbons (δ 168.5, 169.1 and 176.0). The peak at δ 90.9, showing a significant glycosidation shift, was suggestive of the linkage of the sugar moiety to the aglycone at C-3. Methanolysis of the saponin using the method previously described [7] gave a sugar fraction containing L-rhamnose, D-glucose, D-galactose and D-glucuronic acid. The aglycone product was identified as 21,22-*O*-diangeloylbarringtogenol C. This was confirmed by its spectroscopic properties, by preparation of the diacetyl derivative and by alkaline hydrolysis to barringtogenol C (characterized as the pentaacetate).

The complete structure of **1** was determined by extensive 2D NMR experiments at 600 HMz, using a

Table 1. ^{13}C NMR chemical shifts of saponin A (**1**) in pyridine-d_5

Aglycone (δ)				Sugar (δ)					
C-1	39.5	C-16	69.4	Glu	C-1	105.6	Rha1	C-1	101.5
C-2	26.9	C-17	48.6		C-2	79.8		C-2	79.3
C-3	90.9	C-18	40.7		C-3	82.4		C-3	72.7
C-4	37.3	C-19	47.8		C-4	71.7		C-4	74.6
C-5	56.4	C-20	36.8		C-5	71.7		C-5	70.2
C-6	19.9	C-21	79.5		C-6	176.0		C-6	18.5
C-7	33.7	C-22	75.4	Glc	C-1	103.1	Rha2	C-1	103.9
C-8	40.2	C-23	28.5		C-2	76.6		C-2	72.4
C-9	47.5	C-24	17.2		C-3	78.6		C-3	73.1
C-10	36.8	C-25	16.1		C-4	73.1		C-4	74.5
C-11	24.4	C-26	17.5		C-5	78.4		C-5	70.5
C-12	124.6	C-27	28.1		C-6	64.0		C-6	18.7
C-13	143.2	C-28	64.5	Gal	C-1	101.2			
C-14	40.7	C-29	30.0		C-2	77.2			
C-15	35.1	C-30	20.7		C-3	76.1			
					C-4	71.6			
21,22-*O*-Diangeloyl (δ)					C-5	77.4			
					C-6	60.5			
C-1′	169.1	168.5							
C-2′	129.4	129.4							
C-3′	138.1	137.7							
C-4′	16.3	16.3							
C-5′	21.3	21.2							

pyridine-d_5 solution at the optimum temperature for resolution and dispersion of 333 K. The ^1H NMR spectrum (sugar region) was fully assigned from a combination of the 2D DQF–COSY, and 2D DQ spectra, and 1D TOCSY experiments involving selective excitation of the individual anomeric ^1H signals. The assignments are shown in Table 2. The ^{13}C NMR spectrum was then assigned by a combination of DEPT, 2D HMQC and 2D HMBC experiments (Table 1). Finally, the positions of the glycosidic linkages were established from the connectivities indicated in the 2D HMBC, NOE and ROESY experiments. The HMBC experiment showed, *inter alia*, coupling between genin C-3 (δ 90.9) and glucuronic H-1 (δ 4.83). The ^1H NMR signals of glucuronic H-2 and H-3 at δ 4.58 and 4.75 were directly correlated with the ^{13}C NMR peaks at δ 79.8 and 82.4, implying glycosidic substitutions at these positions. This was confirmed by long-range correlations between the ^1H NMR resonances at δ 4.58 and 4.75 and the anomeric ^{13}C NMR signals of glucose (δ 103.1) and of galactose (δ 101.2). Similar long-range correlations were also observed between galactose H-2 (δ 4.47) and rhamnose C-1 (δ 101.5), rhamnose H-2 (δ 4.64) and terminal rhamnose C-1 (δ 103.9), respectively. The ROESY experiment showed cross-peaks defining interglycosidic linkages. Besides providing sequencing information, the ROESY experiment showed correlations between H-1 and H-5 of glucose and of galactose, thus pointing to β-D-configurations for these sugars. The structure of **1** was then deduced as 3-*O*-{[(α-L-rhamnospyranosyl(1→2)-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl-(1→3)]-[β-D-glucopyranosyl(1→2)]-β-D-glucuronopyranosyl}barringtogenol C21, 22-*O*-diangeloate.

Table 2. ^1H NMR chemical shifts (δ) for the sugar moieties of saponin A (**1**) in pyridine-d_5

Glu	H-1	4.83	Rha1	H-1	5.99
	H-2	4.58		H-2	4.64
	H-3	4.75		H-3	4.55
	H-4	4.42		H-4	3.99
	H-5	4.49		H-5	4.70
				H-6	1.42
Glc	H-1	5.60	Rha2	H-1	5.68
	H-2	3.95		H-2	4.60
	H-3	4.22		H-3	4.35
	H-4	3.85		H-4	4.06
	H-5	4.17		H-5	4.31
	H-6a	4.11		H-6	1.56
	H-6b	4.49			
Gal	H-1	5.90			
	H-2	4.47			
	H-3	4.28			
	H-4	4.21			
	H-5	4.08			
	H-6a	4.02			
	H-6b	3.97			

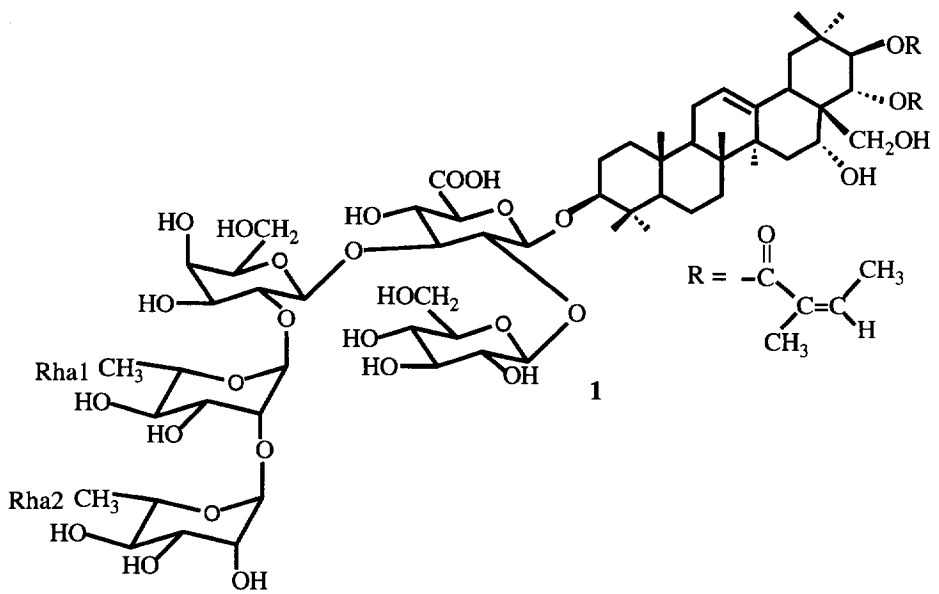
Barringtonia spp. have been known to be toxic to fish because of the presence of barringtogenol related sapogenins and saponins [8, 9]. Similar compounds have also been found in some other plant genera, e.g. *Aesculus* [10–16], *Pittosporum* [17, 18], *Eryngium* [19–21], *Thea* [22–24], *Camellia* [25, 26] and *Petersianthus* [27]. Only a few of these sapogenins and their glycosides have been biologically investigated. For example, sapogenols from *A. hippocastanum* have been reported to have cytotoxic activity [12]. Two barringtogenol C saponins from *Petersianthus macrocarpus*

were shown to have potential in stimulating hormone production in cultivated rat hypophysis cells [27]. In addition to having piscicidal activity the saponin fraction of the leaves of *M. ramentacea* also showed antifungal activity [28]. The present work thus extends the list of biologically active barringtogenol C saponins.

EXPERIMENTAL

Unless otherwise stated, microanalyses were carried out by the School of Chemistry, University of New South Wales, Sydney, Australia. Mps: micro mp apparatus. IR spectra: Perkin-Elmer 1600 FTIR spectrophotometer. ^{1}H NMR spectra of $CDCl_3$ and pyridine-d_5 solns: Bruker AMX 400 (400 MHz) and Bruker AMX 600 (600 MHz) spectrometers with TMS as int. standard. ^{13}C NMR spectra of pyridine-d_5 solns: 150.92 MHz; multiplicities were determined by DEPT experiments. Negative ion FAB MS: glycerol as the matrix. Optical rotations: Perkin-Elmer 241 digital polarimeter. TLC: precoated silica gel 60 F254 (Merck); spots were detected by spraying with 1% $CeSO_4$ in 10% aq. H_2SO_4 followed by heating. CC: silica gel (70–230 mesh, Merck) and silanized silica gel (RP18, 40–60 μm). Analyt. HPLC: Waters LC consisting of a 6000A pump, U6K injector, 450 nm variable wavelength UV detector and R401 RI detector, with a Zorbax ODS column (5 μm, 0.46 × 25 cm, Dupont) and MeOH–H_2O–HOAc (75:25:0.2) at a flow rate of 0.8 ml min^{-1}. Prep. HPLC: Waters LC consisting of a 510EF pump, U6K injector, 481 nm variable wavelength UV detector and R403 RI detector, using a Zorbax ODS column (8 μm, 2.12 × 25 cm, Dupont) and MeOH–H_2O–HOAc (75:25:0.2) at a flow rate of 11.25 ml min^{-1}. Silylated sugars were analysed by GC on a Hewlett-Packard 5890A instrument equipped with a split/splitless capillary injector and FID, using a SGE 25QC2/BP5 column (25 m × 0.22 mm), film thickness 0.25 μm, carrier gas He, inlet pressure 110 kPa, injection and detection temp. 270°, temp. programmed from 60 to 300° at 10° min^{-1}.

Extraction and isolation. Milled dry leaves of *M. ramentacea* (420 g) was blended with H_2O (4 l) and warmed. After filtration, the filtrate was extracted with *n*-BuOH and the extract evapd to give a brown powder (40 g). A portion of the *n*-BuOH extract (30 g) was subjected to CC on silica gel (1.5 kg) using CH_2Cl_2–



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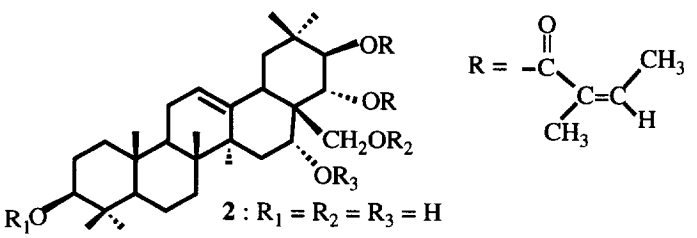

2 : $R_1 = R_2 = R_3 = H$

3 : $R_1 = R_2 = Ac$; $R_3 = H$

4 : $R = R_1 = R_2 = R_3 = H$ = barringtogenol C

5 : $R = R_1 = R_2 = R_3 = Ac$

MeOH–H_2O (7:3:1; 6:4:1) as eluent to give a crude saponin fr. as a brown solid (11.7 g). The crude saponin (6.0 g) was purified by CC over silanized silica gel, gradiently eluted with MeOH–H_2O (1:1; 3:2; 7:3) to give a single spot (TLC) saponin fr. (2.4 g). Purification of this fr. (1.48 g) by RP HPLC gave **1** (135 mg) as a powder, mp 255–260°. $[\alpha]_D$ − 43.3° (*c* 1.0, pyridine). IR ν_{max}^{Nujol} cm^{-1}: 3380, 2940, 2847, 1720, 1462, 1377, 1082, 1042. FAB MS *m/z* (rel. int.): 1445 $[M-H]^-$ (100%), 1299 $[M-H-146]^-$ (24), 1283 $[M-H-162]^-$ (17), 1153 $[M-H-146-146]-$ (30), 991 $[M-H-146-146-162]^-$ (80), 829 $[M-H-146-146-162-162]^-$ (21), 653 $[M-H-146-146-162-162-176]^-$ (7).

Acid hydrolysis of saponin A (**1**). A soln of **1** (251 mg) in 10% aq. HCl (20 ml) was refluxed for 4 hr. The mixt. was cooled and extracted with EtOAc. The organic layer was washed with H_2O, dried (Na_2SO_4) and evapd to give a brown solid (104 mg). The product was purified by CC silica gel using CH_2Cl_2–MeOH (50:1) to give [3*β*, 16*α*, 21*β*(*Z*), 22*α*(*Z*)]olean-12-ene-3, 16, 21, 22, 28-pentol 21, 22-bis(2-methyl-2-butenoate) (21,22-*O*-diangeloylbarringtogenol C) (**2**) (59 mg) as a slightly yellow solid which was crystallized from MeOH as needles (31 mg), mp 266–268° (lit. [13] 244°). $[\alpha]_D$ + 3.5° (*c*, 0.68, $CHCl_3$). IR ν_{max}^{nujol} cm^{-1}: 3414, 1720, 1703, 1650, 1461, 1378, 1234, 1168. ^1H NMR ($CDCl_3$): *δ* 0.79, 0.90, 0.92, 0.94, 1.00, 1.09, 1.45 (3H each, all *s*, 7 × Me), 1.82 (6H, *br t*, *J* = 1.5 Hz, 2 × Me-C=CH), 1.92 (3H, *dd*, *J* = 1.5, 7.0 Hz, Me-CH=C), 1.96 (3H, *dd*, *J* = 1.5, 7.0 Hz, MeCH=C), 2.57 (1H, *t*, *J* = 14.0 Hz, H-19), 2.72 (1H, *dd*, *J* = 4.0, 14.0 Hz, H-18), 2.89 (1H, *d*, *J* = 12.0 Hz, H-28), 3.22 (1H, *dd*, *J* = 4.5, 11.0 Hz, H-3), 3.27 (1H, *d*, *J* = 12.0 Hz, H-28), 3.93 (1H, *br s*, H-16), 5.41 (1H, *d*, *J* = 10.5 Hz, H-22), 5.46 (1H, *t*, *J* = 4.0 Hz, H-12), 5.85 (1H, *d*, *J* = 10.5 Hz, H-21), 6.01 (1H, *dq*, *J* = 1.5, 7.0 Hz, C=CH–Me), 6.11 (1H, *dq*, *J* = 1.5, 7.0 Hz, C=CH–Me). MS *m/z* (rel. int.): 654 (0.03), 636 (0.14), 554 (0.5), 536 (0.65), 506 (0.3), 491 (0.8), 454 (1), 446 (0.3), 428 (0.2), 346 (1.8), 207 (13), 189 (5), 83 (100).

[3*β*,16*α*,21*β*(Z),22*α*(Z)] - *Olean* - 12 - *ene* - 3,16,21,22,28 - *pentol* 3,28-*diacetate* 21,22-*bis*(2-*methyl*-2-*butenoate*) (**3**). A soln of **2** (21 mg) in pyridine (1.0 ml) and Ac_2O (1.0 ml) was stirred at room temp. Overnight. The mixt. was poured on to crushed ice and extracted with CH_2Cl_2, the crude product was purified by PLC with petrol–EtOAc (17:3) as mobile phase and the chromatogram was developed 2×. The diacetate (16 mg) was crystallized from MeOH–H_2O as needles (12 mg), mp 191–193° (lit. [13] 190°). $[\alpha]_D$ + 25.6° (*c*, 0.43, $CHCl_3$). IR ν_{max}^{nujol} cm^{-1}: 3483, 1719, 1416, 1377, 1242. ^1H NMR ($CDCl_3$): *δ* 0.86, 0.88, 0.89, 0.92, 0.96, 1.09, 1.45 (3H, each, all *s*, 7 × Me), 1.83 (6H, *br s*, 2 × Me–C=CH), 1.93 (6H, *dq*, *J* = 1.5, 7.0 Hz, 2 × Me–CH=C), 2.03 (3H, *s*, Ac), 2.05 (3H, *s*, Ac), 2.50 (1H, *dd*, *J* = 3.5, 13.5 Hz, H18), 2.60 (1H, *t*, *J* = 13.5 Hz, H-19), 3.68 (1H, *d*, *J* = 11.5 Hz, H-28), 3.72 (1H, *d*, *J* = 11.5 Hz, H-28), 4.24 (1H, *br s*, H-16), 4.50 (1H, *m*, H-3), 5.38 (1H, *t*, *J* = 3.5 Hz, H12), 5.58 (1H, *d*, *J* = 10.0 Hz, H-22), 5.71 (1H, *d*, *J* = 10.0 Hz, H-21), 5.99 (1H, *dq*, *J* = 1.5, 7.0 Hz, C=CH–Me), 6.04 (1H, *dq*, *J* = 1.5, 7.0 Hz, C=CH–Me). MS *m/z* (rel. int.): 738 (0.11), 720 (0.19), 678 (0.28), 638 (0.27), 488 (0.2), 470 (3.4), 388 (2), 370 (0.2), 249 (4), 83 (100).

Alkaline hydrolysis of 21,22-O-*diangeloylbarringtogenol C* (**2**). Compound **2** (91 mg) in a satd soln of K_2CO_3 in MeOH (5 ml) was refluxed for 2 hr. The mixt. was evapd, diluted with H_2O and extracted with EtOAc. The organic layer was washed with H_2O, dried (Na_2SO_4) and evapd to give a yellow residue. CH_2Cl_2 (2 ml) was added to the residue; the solid which formed (25 mg) was collected. Crystallization of the product from MeOH gave barringtogenol C (**4**) (18 mg) as needles, mp 288–293° (lit. [24] 278–284°). $[\alpha]_D$ + 4.0° (*c*, 0.50, pyridine) (lit. [24] + 11.0°). IR ν_{max}^{nujol} cm^{-1}: 3347, 1460, 1377. ^1H NMR (pyridine-d_5); *δ* 0.97, 0.98, 1.06, 1.24, 1.34, 1.39, 1.86 (21H, all *s*, 7 × Me), 2.81 (1H, *dd*, *J* = 4.0, 14.0 Hz, H-18), 3.05 (1H, *t*, *J* = 14.0 Hz, H-19), 3.47 (1H, *m*, H-3), 3.74 (1H, *d*, *J* = 10.0 Hz, H28), 4.04 (1H, *dd*, *J* = 4.0, 10.0 Hz, H-28), 4.63 (1H, *d*, *J* = 9.5 Hz, H-22), 4.78 (1H, *d*, *J* = 9.5 Hz, H-21), 5.05 (1H, *br s*, H-16), 5.45 (1H, *br t*, *J* = 3.5 Hz, H-12). MS *m/z* (rel. int.): 490 (2.2%), 472 (1.8), 454 (4.4), 282 (30), 264 (34), 246 (52), 215 (100), 207 (52), 189 (24).

[3*β*,16*α*,21*β*,22*α*] - *Olean* - 12 - *ene* - 3,16,21,22,28 - *pentol* 3,16,21,22,28-*pentaacetate* (*barringtogenol C pentaacetate*) (**5**). A soln of **4** (36 mg) and a catalytic amount of *p*-toluenesulphonic acid in Ac_2O (1.0 ml) was stirred at room temp. overnight. The mixt. was poured on to crushed ice and extracted with CH_2Cl_2. The organic layer was washed with aq. $NaHCO_3$ and H_2O, dried (Na_2SO_4) and evapd to dryness. The crude product was purified on a silica gel column with petrol–EtOAc (4:1, 3:2) to give **5** (41 mg). The product was crystallized from MeOH-H_2O as needles (32 mg), mp 146–148° (lit. [24] 147–150°). $[\alpha]_D$ − 12.9° (*c*, 0.45, $CHCl_3$) (lit. [24] − 10°). IR ν_{max}^{nujol} cm^{-1}: 1742, 1461, 1376, 1288, 1252. ^1H NMR ($CDCl_3$): *δ* 0.86, 0.87, 0.89, 0.91, 0.95, 1.05, 1.30 (21H, all *s*, 7 × Me), 1.95, 2.01, 2.05, 2.06, 2.26 (15H, all *s*, 5 × Ac), 2.40 (1H, *t*, *J* = 13.5 Hz, H-19), 2.48 (1H, *dd*, *J* = 3.5, 13.5 Hz, H-18), 3.75 (2H, 3, 2 × H-28), 4.50 (1H, *m*, H-3), 5.24 (1H, *br s*, H-16), 5.31 (1H, *t*, *J* = 10.0 Hz, H-22), 5.33 (1H, *d*, *J* = 10.0 Hz, H-21), 5.41 (1H, *t*, *J* = 3.0 Hz, H-12). MS *m/z* (rel. int.): 700 (0.1), 640 (9), 580 (3.4), 520 (4), 507 (1.2), 478 (1.2), 460 (1.2), 447 (2.8), 390 (36), 270 (19), 249 (14), 231 (2), 215 (30), 197 (34), 190 (52), 43 (100).

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