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TRITERPENOID SAPOGENINS FROM LEAVES OF *PITTOSPORUM UNDULATUM*

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Abstract—Three triterpenoid sapogenins were obtained on enzymatic hydrolysis of a saponin fraction from the leaves of *Pittosporum undulatum*. On the basis of chemical and spectral evidence they were determined as 22-*O*-(2-methylbutyroyl)- and 22-*O*-(3,3-dimethylacryloyl)-A_1-barrigenol, and 16-*O*-acetylcamelliagenin A. The first two compounds are new natural products.

INTRODUCTION

Pittosporum undulatum Vent. has been used as a medicinal plant in Australia [1]. The constituents of the fruits have been studied extensively and two monoterpenes [2], cyanidin [3] and two triterpenes [4, 5], of which the structures were established later as A_1- and R_1-barrigenol [6, 7], have been reported. A saponin mixture obtained from the leaves of a related plant *P. tobira* is reported to possess antibiotic activity [8] and three triterpenoid sapogenins, R_1-barrigenol, its 21-*O*-angelate and 21-*O*-angeloyl-barringtogenol, have been isolated from the leaves [9].

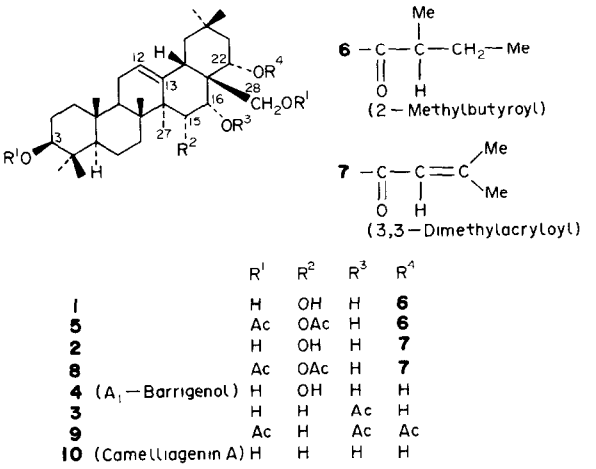
This paper describes the isolation and structure determination of three acylated triterpenoid sapogenins from the leaves of *P. undulatum*

RESULTS AND DISCUSSION

Hydrolysis of a saponin mixture, obtained from methanol extracts of the leaves with a crude hesperidinase, gave products which were separated by Si gel CC to give three homogeneous compounds, **1**–**3**.

By taking the elemental analytical and the EIMS data into account, **1** and **2** were assigned, respectively, the molecular formulae $C_{35}H_{58}O_6$ and $C_{35}H_{56}O_6$. Compound **1** possesses hydroxyl and ester groups, as shown by its IR spectrum, whereas **2** exhibited the IR absorptions of hydroxyl and α,β-unsaturated ester functions. The ^{13}C NMR spectra of **1** and **2** both showed two olefinic carbon signals (δ 124.5 and 144.1 in **1**, δ 124.5 and 144.2 in **2**) which were in good agreement with those of C-12 and C-13 of olean-12-ene derivatives [10]. On treatment with alkali, **1** and **2** gave the same product, A_1-barrigenol (**4**). Compounds **1** and **2** were presumed, therefore, to be the derivatives of **4**, which have, respectively, a C_4H_9CO and an α,β-unsaturated C_4H_7CO residue combined with a hydroxyl group of **4**.

The ^1H NMR spectrum of **1** showed a triplet at δ 0.83 (J = 7 Hz) and the triacetate of **1** (**5**) showed a doublet at δ 1.12 (J = 7 Hz), respectively, which resembled signals of the primary methyl and secondary methyl groups of the 2-methylbutyroyl function (**6**). Compound **1** exhibited, in the ^{13}C NMR spectrum, five signals (δ 11.8, 16.6, 27.1, 41.6 and 175.8) identical with those of **6**. The presence of a 3,3-dimethylacryloyl group (**7**) in **2** was suggested by the ^1H NMR spectrum of the triacetate of **2** (**8**) which showed the signals of one olefinic hydrogen (δ 5.68, *m*) and two vinylic methyl signals (δ 1.89 and 2.15, each *br s*) which were in good agreement with those of **7** [11] and different from those of angeloyl [9] and tigloyl [12] groups (Table 1).



		R¹	R²	R³	R⁴
1		H	OH	H	**6**
5		Ac	OAc	H	**6**
2		H	OH	H	**7**
8		Ac	OAc	H	**7**
4	(A_1–Barrigenol)	H	OH	H	H
3		H	H	Ac	H
9		Ac	H	Ac	Ac
10	(Camelliagenin A)	H	H	H	H

Therefore, **1** and **2**, were considered, respectively, to be the 2-methylbutyrate and the 3,3-dimethylacrylate of **4**

The location of the acyl moiety was determined as follows. The EIMS of both **1** and **2** exhibited the characteristic fragment peaks (*m/z* 366, 348, 207 in **1**; 364, 346, 207 in **2**) due to the retro-Diels–Alder cleavage of the olean-12-ene skeleton [13], suggesting that the acyl groups in **1** and **2** were located in a ring other than ring A. Formation of the triacetate and not the tetra-acetate from both compounds by usual acetylation suggested that the C-16 hydroxyl group in **1** and **2** was not acylated [9]. Compounds **1** and **2** showed in their ^1H NMR spectra a

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Table 1 ^{13}C and ^{1}H NMR data of acyl groups

(a) ^{13}C NMR data (C_5D_5N)

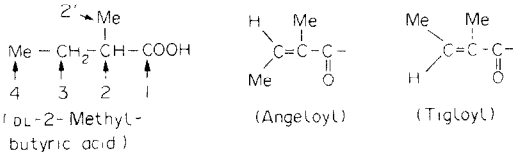
	C-1	C-2	C-3	C-4	C-2′
DL-2-Methylbutyric acid*	178 3	41 4	27 2	11 9	17 1
Acyl moiety of **1**	175 8	41 6	27 1	11 8	16 6

(b) ^{1}H NMR data ($CDCl_3$)

	Olefinic H	Me group
DL-2-Methylbutyric acid*	—	0 95 (*t*, J = 7 Hz)
		1 18 (*d*, J = 7 Hz)
Acyl moiety of **1**†	—	0 83 (*t*, J = 7 Hz)
Acyl moiety of **5**	—	1 12 (*d*, J = 7 Hz)
Angeloyl [9]	5 99 *q*, J = 7	1 83 (*br s*)
		1 99 (*d*, J = 7 Hz)
Tigloyl [12]	6 84 *m*	1 82 (*d*, J = 6 Hz)
		1 84 (*br s*)
3,3-Dimethylacryloyl [11]	5 65 *m*	1 89 (*br s*)
		2 14
Acyl moiety of **8**	5 68 *m*	1 89 (*br s*)
		2 15 (*br s*)

*Commercial

†In $C_5D_5N + D_2O$



signal of one proton quartet (J = 6, 12 Hz) at δ 6 13 and 6 15, respectively, which was attributable to the proton next to an acylated hydroxyl group, and the splitting pattern suggested that the signals were due to H-22. The above data indicated that the C-22 hydroxyl group of **4** was esterified both in **1** and **2** Consequently, **1** and **2** are 22-*O*-(2-methylbutyroyl)- and 22-*O*-(3,3-dimethylacryloyl)-A_1-barrigenol, respectively

Compound **3**, $C_{32}H_{52}O_5$, showed an ester carbonyl absorption in the IR and the signals of one acetoxy, one olefinic proton and seven tertiary methyls in the ^{1}H NMR spectrum, suggesting that **3** was the monoacetate of an oleanene derivative Compound **3** was acetylated to give the tetra-acetate, **9**, and hydrolysed with alkali to provide camelliagenin A (**10**) [14, 15]

The ^{1}H NMR spectrum of the 16-*O*-acetate* of **10** had been reported [16] and the signals due to the proton next to the acetoxy group were in good agreement with those of **3** Thus, **3** is 16-*O*-acetyl-camelliagenin A

There have been reported several kinds of 22-*O*-acylates of **4** [17–19], but **1** and **2** are the first natural products obtained in a pure state

Since **1**–**3** were isolated following enzymatic hydrolysis of a saponin fraction, it is assumed that they are not artefacts and that in the plant they are combined with sugar moieties to form the respective parent saponins

*According to the personal communication of Professor Kitagawa, this compound was obtained by acid hydrolysis of a saponin fraction of *Primula japonica*

EXPERIMENTAL

All mps were uncorr Optical rotations were measured at 19–21° using a 1 dm cell ^{1}H NMR spectra were taken at 100 MHz in $CDCl_3$ soln unless otherwise specified using TMS as int standard ^{13}C NMR spectra were recorded at 25 05 MHz in C_5D_5N (TMS as int standard) employing the FT mode The EIMS were run on double focusing mass spectrometers and were recorded electrically with an accelerating potential of 3 0–6 5 kV and an ionizing potential of 30–75 eV TLC was carried out on Si gel and the solvent systems were (a) $CHCl_3$–MeOH (20 1) and (b) *n*-hexane–EtOAc (3 2)

Plant material Foliage from a single tree of *Pittosporum undulatum* (voucher specimen No 77-070, Biological and Chemical Research Institute Herbarium) was collected in June at Berowra Heights near Sydney, New South Wales, Australia

Isolation of the saponin mixture Fresh material was extracted successively with *n*-hexane and MeOH The MeOH extractives were partitioned between EtOAc *n*-BuOH (2 1) and H_2O The H_2O layer was extracted with *n*-BuOH and the extracts evaporated *in vacuo* to give a saponin mixture (18 0 g)

Enzymatic hydrolysis of the saponin mixture The crude saponin (1 8 g) in 0 2 M KH_2PO_4 (450 ml) was incubated with a crude hesperidinase (1 1 g) at 37° for 2 days and the products extracted with EtOAc The extracts showing two major spots (R_f 0 37, 0 19) in TLC (solvent *a*) were evaporated and chromatographed on Si gel (eluant, $CHCl_3$–MeOH, 100 1 → 50 1) to give two fractions (fraction 1, R_f 0 37, fraction 2, R_f 0 19) Fraction 1 showed two spots (R_f 0 39, 0 35) in TLC using solvent *b* and it was subjected to prep TLC (solvent *b*) to afforded two homogeneous compounds, **1** (37 mg, R_f 0 39) and **2** (25 mg, R_f 0 35) Fraction 2 was

crystallized from *n*-hexane–EtOAc to yield **3** (40 mg) (R_f 0 19 in solvent *a*; 0 18 in solvent *b*).

Compound **1** Colourless plates (from $CHCl_3$–Me_2CO), mp 225–228°, $[\alpha]_D$ + 31 7° (MeOH, *c* 1 7) IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1700 (ester); EIMS *m/z* 574 4247 (M^+, calcd for $C_{35}H_{58}O_6$ 574 4233), 366, 348, 207, ^1H NMR ($C_5D_5N + D_2O$) δ 0 99, 1 14, 1 24 and 1 26 (3H each, *s*, Me × 4), 1 08 (6H, *s*, Me × 2), 1 86 (3H, *s*, H-27), 0.83 (3H, *t*, *J* = 7 Hz, Me of acyl group), 3 46 (1H, *t*, *J* = 8 Hz, H-3), 3 58 and 3 80 (1H each, *d*, *J* = 10 Hz, H_2-28), 4.26 (1H, *d*, *J* = 4 Hz, H-15), 4 50 (1H, *d*, *J* = 4 Hz, H-16), 5 56 (1H, *m*, H-12), 6 13 (1H, *q*, *J* = 6, 12 Hz, H-22), ^{13}C NMR δ 11 8 (*q*, Me of acyl group), 16.6 (*q*, Me of acyl group), 27.1 (*t*, $-CH_2-$ of acyl group), 41 6 (*d*, –CH– of acyl group), 124 5 (*d*, C-12), 144 1 (*s*, C-13), 175 8 (*s*, RCOO–) (Found: C, 71 39, H, 9 89 $C_{35}H_{58}O_6 \cdot H_2O$ requires C, 70.91, H, 10 20%.)

Triacetate (**5**) *of* **1** Compound **1** was acetylated with Ac_2O–pyridine at room temp for 1 day to give **5**, colourless prisms (from MeOH), mp 235–237° IR $\nu_{max}^{CCl_4}$ cm^{-1} 3500 (OH), 1730 (ester), EIMS *m/z* 700 $[M]^+$; ^1H NMR δ 0 84, 0 87, 0.93, 0.96, 0 99 and 1.02 (3H each, *s*, Me × 6), 1.53 (3H, *s*, H-27), 1 12 (3H, *d*, *J* = 7 Hz, Me of acyl group), 2.05, 2 07 and 2 10 (3H each, *s*, OAc × 3), 3 73 and 3 92 (1H each, *d*, *J* = 11 Hz, H_2-28), 4 20 (1H, *d*, *J* = 4 Hz, H-16), 4 49 (1H, *t*, *J* = 8 Hz, H-3), 5 13 (1H, *d*, *J* = 4 Hz, H-15), 5.28 (1H, *q*, *J* = 6, 12 Hz, H-22), 5.45 (1H, *m*, H-12)

Compound **2** Colourless plates (from $CHCl_3$–Me_2CO), mp 240–243°, $[\alpha]_D$ + 24 0° (MeOH, *c* 0 8) IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1690 and 1650 (conjugated ester), EIMS *m/z* 572 4013 (M^+, calcd for $C_{35}H_{56}O_6$ 572 4076), 364, 346, 207, ^1H NMR (C_5D_5N): δ 0.99, 1.12, 1 25 and 1 30 (3H each, *s*, Me × 4), 1 08 (6H, *s*, Me × 2), 1 87 (3H, *s*, H-27), 1 65 and 2 17 (3H each, *br s*, Me of acyl group), 3 47 (1H, *t*, *J* = 8 Hz, H-3), 3 58 and 3.79 (1H each, *d*, *J* = 10 Hz, H_2-28), 4 26 (1H, *d*, *J* = 4 Hz, H-15), 4 51 (1H, *m*, H-16), 5.54 (2H, *m*, vinylic H of acyl group and H-12), 6 15 (1H, *q*, *J* = 6, 12 Hz, H-22); ^{13}C NMR δ 124 5 (*d*, C-12), 144.2 (*s*, C-13) (Found: C, 71 20, H, 9 67. $C_{35}H_{56}O_6 \cdot H_2O$ requires C, 71.15; H, 9 90%.)

Triacetate (**8**) *of* **2** This was prepared by acetylation of **2** in the same manner as for **1** Powder (from *n*-hexane–Me_2CO), mp 145–148°. IR $\nu_{max}^{CCl_4}$ cm^{-1} 3500 (OH), 1740 (ester), EIMS *m/z* 698 $[M]^+$, ^1H NMR: δ 0.84, 0.87, 0.93, 0.95, 0.99 and 1.04 (3H each, *s*, Me × 6), 1.53 (3H, *s*, H-27), 2 01, 2 05 and 2.10 (3H each, *s*, OAc × 3), 1.89 and 2 15 (3H each, *br s*, Me of acyl group), 4 26 (1H, *d*, *J* = 4 Hz, H-16), 4 48 (1H, *t*, *J* = 8 Hz, H-3), 5 16 (1H, *d*, *J* = 4 Hz, H-15), 5 30 (1H, *m*, H-22), 5 44 (1H, *m*, H-12), 5.68 (1H, *m*, vinylic H of acyl group)

Alkaline hydrolysis of **1** *and* **2** *to give* A_1*-barrigenol* (**4**) Compound **1** (or **2**) was boiled with 4% (w/v) K_2CO_3 in MeOH for 10 min, the reaction mixture was diluted with H_2O and extracted with EtOAc. The organic layer was evaporated and the residue was crystallized from Me_2CO to give **4** as colourless needles, mp 278–280°, $[\alpha]_D$ + 27 9° (dioxane, *c* 0 7). IR ν_{max}^{KBr} cm^{-1}. 3500 and 3400 (OH); EIMS *m/z*: 490 3617 (M^+, calcd for $C_{30}H_{50}O_5$ 490.3658), 282, 207 It was identical with an authentic sample: mmp, IR, EIMS and TLC (solvent *a*)

Compound **3**. Colourless prisms (from *n*-hexane–EtOAc), mp 252–255°, $[\alpha]_D$ −1.9° (MeOH, *c* 1.6) IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1720 (ester); EIMS *m/z* 516 3826 (M^+, calcd for $C_{32}H_{52}O_5$. 516 3815), 308, 207, ^1H NMR δ 0 78 (3H, *s*, Me), 0 94 (12H, *s*, Me × 4), 0.98 (3H, *s*, Me), 1 34 (3H, *s*, H-27), 2 10 (3H, *s*, OAc), 3.20 (1H, *t*, *J* = 8 Hz, H-3), 3.34 and 3 66 (1H each, *d*, *J* = 11 Hz, H_2-28), 3 93 (1H, *q*, *J* = 6, 11 Hz, H-22), 5.30 (1H, *m*, H-12), 5.70 (1H, *m*, $W_{1/2}$ = 8 Hz, H-16) Compound **3** was acetylated with Ac_2O–pyridine at 80° for 1 hr to yield the tetra-acetate, **9**, as a powder (from *n*-hexane–Me_2CO), mp 108–110°. IR $\nu_{max}^{CCl_4}$ cm^{-1} no OH, 1740 (ester); EIMS *m/z* 642 $[M]^+$, ^1H NMR δ 0 87 (6H, *s*, Me × 2), 0.91 (3H, *s*, Me), 0 97 (6H, *s*, Me × 2), 1 03 (3H, *s*, Me), 1 31 (3H, *s*, H-27), 2.00 (3H, *s*, OAc), 2 06 (6H, *s*, OAc × 2), 2.10 (3H, *s*, OAc), 3 71 and 3.88 (1H each, *d*, *J* = 11 Hz, H_2-28), 4 48 (1H, *q*, *J* = 7, 9 Hz, H-3), 5.0–5 6 (3H, *m*, H-12, H-16, H-22)

Alkaline hydrolysis of **3** *to give camelliagenin A* (**10**) Compound **3** was hydrolysed as for **1** The reaction mixture was diluted with H_2O and the ppts were collected and crystallized from MeOH to give **10** as colourless needles, mp 274–276°, $[\alpha]_D$ + 25 7° (MeOH, *c* 0.8). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH); EIMS *m/z* 474 3726 (M^+, calcd for $C_{30}H_{50}O_4$ 474 3709), 266, 248, 207 It was identified with an authentic sample by mmp, IR, EIMS and TLC (solvent *a*)

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