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# TRITERPENOID SAPOGENINS OF *PITTOSPORUM PHILLYRAEOIDES*

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**Key Word Index**—*Pittosporum phillyraeoides*; Pittosporaceae; leaf triterpenoids; sapogenin; 27-desoxyphillyrigenin; phillyrigenin; 23-hydroxyphillyrigenin; dihydropriverogenin A; 16-desoxybarringtogenol C; barringtogenol C; $R_1$-barrigenol.

**Abstract**— A re-examination of the sapogenins from *Pittosporum phillyraeoides* leaves revealed the presence of two new natural products 27-desoxyphillyrigenin (3β-hydroxytaraxastan-28,20β-olide) and 23-hydroxyphillyrigenin (3β,23,27-trihydroxytaraxastan-28,20β-olide) together with dihydropriverogenin A, 16-desoxybarringtogenol C, barringtogenol C and the previously reported constituents phillyrigenin and $R_1$-barrigenol. Spectral evidence is presented to establish the structure of phillyrigenin as 3β,27-dihydroxytaraxastan-28,20β-olide.

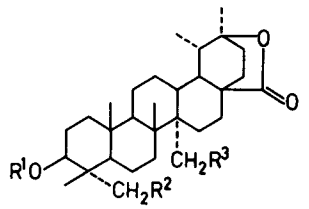
## INTRODUCTION

An earlier examination [1] of the sapogenin mixture obtained by acid hydrolysis and saponification of the aqueous ethanol extract of *Pittosporum phillyraeoides* leaves revealed two triterpenoids: phillyrigenin, a dihydroxylactone for which the alternative structures **1a** and **1b** were later proposed [2], and a hexol later identified [3] as $R_1$-barrigenol and shown [4, 5] to have structure **2**. This paper describes the isolation and identification of the five minor triterpenoid constituents **3**–**7** and describes spectral evidence that establishes **1a** as the structure of phillyrigenin.

## RESULTS AND DISCUSSION

The sapogenin mixture was obtained from *P. phillyraeoides* leaves by extraction with aqueous ethanol, hydrolysis of the extract with hydrochloric acid, saponification of the crude sapogenin and Soxhlet extraction with chloroform. The residues remaining in the mother liquors after removal of two crops of phillyrigenin yielded chromatography fractions showing a total of ten spots on TLC.

27-Desoxyphillyrigenin. (**3**) $C_{30}H_{48}O_3$ ($M^+$, *m/z* 456), mp 294–297° was the first compound eluted. The IR spectrum showed absorption bands at 3600 (hydroxyl) and 1740 $cm^{-1}$ (δ-lactone). The low field region of the $^1H$ NMR spectrum showed only a broad multiplet (δ3.21, 1H) expected for 3β-hydroxytriterpenoids. The spectrum also featured a downfield methyl signal at δ1.32 (3H, *s*), a secondary methyl resonance at δ0.98 (3H, *d*, *J* = 7 Hz) and resonances for five tertiary methyls (see Table 1). The mass spectrum included peaks at *m/z* 207, attributed [6] to fragment ion **8**, and 189 (**8** – $H_2O$). Compound **3**, which formed a monoacetate (**9**) under mild conditions, has not been found in nature before but a partial synthesis has been reported [7]. Identity was confirmed by direct comparison of the acetate **9** with an authentic specimen.

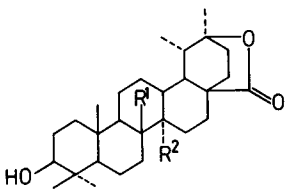


| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **1a** | H | H | OH |
| **3** | H | H | H |
| **4** | H | OH | OH |
| **9** | Ac | H | H |
| **10**[1] | Ac | H | OH |
| **11** | Ac | H | OAc |
| **13** | Ac | OAc | OAc |

Table 1. Chemical shifts of methyl groups in $^1H$ NMR spectra of *P. phillyraeoides* lactones

| H | 1a | 3 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|
| 23 | 0.96 | 0.97 | 0.85 | 0.84 | 0.85 | 0.95 | – |
| 24 | 0.77 | 0.77 | 0.85 | 0.84 | 0.85 | 0.75 | 0.81 |
| 25 | 0.86 | 0.84 | 0.85 | 0.88 | 0.88 | 0.85 | 0.91 |
| 26 | 0.94 | 0.94 | 0.95 | 0.95 | 0.96 | 1.01 | 0.96 |
| 27 | – | 0.94 | 0.92 | – | – | – | – |
| 29 | 0.97 | 0.98 | 0.98 | 0.98 | 0.97 | 1.02 | 0.99 |
| 30 | 1.32 | 1.32 | 1.32 | 1.32 | 1.32 | 1.31 | 1.32 |

The known constituent phillyrigenin (**1a**) was the second compound eluted. In earlier investigations [2] no



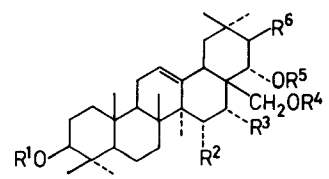
**1b** $R^1 = CH_2OH$, $R^2 = Me$

**12** $R^1 = Me$, $R^2 = COOMe$

choice could be made between C-26 (**1b**) and C-27 (**1a**) for the position of the primary hydroxyl group. However, the mass spectrum of **1a** shows peaks at *m/z* 207 (fragment **8**) [6] and 189 (**8** $-H_2O$, 100%) thereby establishing that the hydroxyl group is not at C-26. Also, the methyl resonances in the $^1H$ NMR spectra of phillyrigenin and its derivatives **10–12** are consistent with C-27 hydroxylation. In the spectrum of the previously described [2] ester **12**, the C-26 methyl is deshielded 0.07 ppm by the C-27 carboxymethyl, a figure similar to the 0.09 ppm noted in a previous lupene example [8]. A carboxymethyl at C-26 would be expected to shield the C-25 methyl (with which it has a 1,3-diaxial relationship) by *ca* 0.2 ppm [9].

23-Hydroxyphillyrigenin (**4**), $C_{30}H_{48}O_5$ ($M^+$, *m/z* 488), mp 330–332°, which was more polar than phillyrigenin, had IR absorption at 3350 (hydroxyl) and 1735 $cm^{-1}$ (δ-lactone) and readily formed the triacetate **13**. Although no peak corresponding [6] to fragment **8a** (*m/z* 223) was observed in the mass spectrum of **4**, peaks at *m/z* 205 (**8a** $-H_2O$) and 187 (**8a** $-2H_2O$, 100%) required two of the hydroxyl groups to be situated on the A and B rings. The NMR spectrum of the triacetate **13** included signals due to a downfield methyl (δ1.32), a secondary methyl (δ0.99, $J \sim 6$ Hz) and three tertiary methyls (see Table 1). One acetate was presumably 3β (3α-H at δ4.78, *m*, $w_{1/2}$ = 18 Hz). An AB quartet at δ4.13 and 4.61 ($J = 12.5$ Hz) was attributed to a primary acetate on C-27 (observed as a singlet at δ4.35 in **11**). A second AB quartet at δ3.65 and 3.89 ($J = 12$ Hz) had a chemical shift close to that expected [10] for an equatorial acetoxymethyl group (*ca* δ3.8) rather than that for an axial group (*ca* δ4.2) indicating that the second primary acetate was on C-23. This is supported by examination of the A-ring methyl resonances (Table 1). Comparison of the spectrum of the triacetate **13** with that of the diacetate **11** shows that the 24-methyl has been shielded by 0.04 ppm in good agreement with the predicted [11] value of 0.03 ppm. A C-24 acetate would be expected [11] to deshield a 23-methyl by *ca* 0.14 ppm.

Compound **5**, $C_{30}H_{50}O_4$ ($M^+$ at *m/z* 474) was shown to be a tetrol by the formation of the tetra-acetate **14** and was identified as dihydropriverogenin A (camelliagenin A) by direct comparison with an authentic specimen. **5** has also been obtained from *P. undulatum* [12]. Compound **6**, $C_{30}H_{50}O_4$ ($M^+$ at *m/z* 474) was also a tetrol, though the formation of a tetra-acetate (**15**) under mild conditions showed that no 16α-hydroxyl was present; direct comparison with 16-desoxybarringtogenol C confirmed its identity. Compound **7**, $C_{30}H_{50}O_5$ ($M^+$ at *m/z* 490) was recognised as barringtogenol C, the pentol which has also been recorded [13] with $R_1$-barrigenol in *P. tobira*. TLC comparisons showed that $A_1$-barrigenol (**16**), the major constituent of *P. undulatum* [4], was not one of the three unidentified trace components.



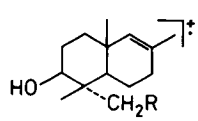
| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $R^6$ |
|---|---|---|---|---|---|---|
| **2** | H | OH | OH | H | H | OH |
| **5** | H | H | OH | H | H | H |
| **6** | H | H | H | H | H | OH |
| **7** | H | H | OH | H | H | OH |
| **14** | Ac | H | OAc | Ac | Ac | H |
| **15** | Ac | H | H | Ac | Ac | OAc |
| **16** | H | OH | OH | H | H | H |

## EXPERIMENTAL

Mps corr. $^1H$ NMR spectra were recorded at 60 MHz in $CDCl_3$ soln with TMS as int. std. The IR spectra were run in $CS_2$ soln. EIMS (70 eV) were measured with a direct inlet. Optical rotations were measured in $CHCl_3$ soln at 21° using a 1 dm cell.

*Isolation of sapogenins.* Leaves and terminal branches (15 kg) of *P. phillyraeoides* D.C. collected between Southern Cross and Koolyanobbing, Western Australia, were extd successively with $Et_2O$ and aq. EtOH. The concd EtOH ext. was hydrolysed with 6% aq. HCl for 6 hr at 100°. Crude sapogenin (750 g) was heated 1 hr with NaOH in EtOH. After diln with $H_2O$ two crops (total 286 g) of crude sapogenin were collected. The first crop (163 g) was extracted in a Soxhlet apparatus with $CHCl_3$ (6 × 500 ml for 8 hr). On cooling, the first ext. deposited two crops (total *ca* 60 g) shown by TLC to be almost pure phillyrigenin (**1a**). Extracts 2–6 (total 86 g) were shown by TLC to contain mostly **2**, **6** and **7** with traces of **1a**. Residues from the mother liquor of the first ext. were subjected to repeated CC. (i) Total residues (8.3 g) were adsorbed on $Al_2O_3$ (200 g). Petrol–$CHCl_3$ (1:1) eluted fractions containing **3** (1.4 g) and **1a** (1.96 g); $CHCl_3$ eluted **1a**+**5** (0.79 g); MeOH–$CHCl_3$ (1:19) eluted **4**–**6** and traces of three unidentified compounds (2.26 g); MeOH–$CHCl_3$ (1:9) eluted mostly **6**; MeOH–$CHCl_3$ (1:1) eluted **7** (1.04 g) and MeOH eluted $R_1$-barrigenol (**2** 0.53 g) as needles (MeOH) mp 314–319° lit. [1] mp 306–308°. (ii) $CHCl_3$ and MeOH–$CHCl_3$ (1:19) fractions were combined (1.86 g) and chromatographed on $Al_2O_3$ (50 g). $CHCl_3$ eluted a mixt. (0.99 g); MeOH–$CHCl_3$ (1:19 eluted **4**+**6** (0.69 g) and **6** (0.17 g). (iii) The $CHCl_3$ fractions (0.9 g) from (ii) were adsorbed on $Al_2O_3$ (25 g). Petrol–$CHCl_3$ (1:1) eluted solids (0.50 g); $CHCl_3$ eluted largely **5** (0.21 g) and MeOH–$CHCl_3$ (1:9) yielded solids (0.17 g).

*27-Desoxyphillyrigenin* (**3**). Crude **3**, a yellow glass, crystallized on addition of $Et_2O$ to give **3** as needles (MeOH) mp 294–297°, lit. [7] 292–296°. IR $\nu_{max}$ $cm^{-1}$: 3600, 1740. $^1H$ NMR: δ3.21 (1H, *m*, $W_{1/2} = 19$ Hz, H-3α). EIMS *m/z* (rel. int.): 456 $M^+$ (6), 438 [M



**8** R = H

**8a** R = OH

$-H_2O]^+$ (64), 423 [M $-H_2O$, Me]$^+$ (25), 395 [M $-H_2O$, Me, CO]$^+$ (100), 207 [**8**]† (13), 189 [**8** $-H_2O]^+$ (48).

*Acetylation of 27-desoxyphillyrigenin.* **3** (62 mg) in pyridine was stood 24 hr at room temp. with $Ac_2O$. Work-up in the usual way gave crystals (64 mg) which were recrystallized from MeOH to give **9** as short needles mp 312–316° undepressed on admixt. with an authentic sample, mp 313–317°. The IR spectra of the two compounds were identical. IR $\nu_{max}$ cm$^{-1}$: 1740 ($\delta$-lactone), 1730 (ester). $^1$H NMR: $\delta$ 4.52 (1H, *m*, $W_{1/2}$ = 20 Hz, H-3$\alpha$); 2.05 (3H, *s*, acetoxyl. EIMS *m/z* (rel. int.) 498 M$^+$ (3), 438 [M – AcOH]$^+$ 49), 423 [M – AcOH, Me]$^+$ (27), 395 [M – AcOH, Me, CO]$^+$ (100), 249 (11), 234 (23), 233 (23), 205 (32), 203 (34), 189 (94), 175 (45).

*Phillyrigenin* (**1a**). **1a** was recrystallized from MeOH to give phillyrigenin as needles mp 335–337° lit. [1] 339–341°. $^1$H NMR: $\delta$ 3.17 (1H, *m*, $W_{1/2}$ = 19 Hz), 3.72 and 4.18 (2H, *ABq J* = 12.5 Hz). EIMS *m/z*: 472 M$^+$ [$C_{30}H_{48}O_4$] (4), 454 [M $-H_2O]^+$ (69), 441 [M $-CH_2OH]^+$ (27), 439 [M $-H_2O$, Me]$^+$ )24), 436 [M $-2H_2O$ 25]$^+$, 423 (36), 411 [M $-H_2O$, Me, CO]$^+$ (33), 393 [M $-2H_2O$, Me, CO]$^+$ (18), 232 (51), 207 [**8**]$^+$ 31), 205 (34), 203 (30), 189 [**8** $-H_2O]^+$ (100), 175 (34), 173 (40).

*Acetylation of phillyrigenin.* **1a** was acetylated by treatment with $Ac_2O$ in pyridine at room temp. to give the diacetate (**11**), mp 248–250° lit. [1] 252°. $^1$H NMR: $\delta$ 4.35 (2H, *s*), ~4.5 (1H, *m*), 2.07 (3H, *s*), 2.03 (3H, *s*).

*23-Hydroxyphillyrigenin triacetate* (**13**). Crystals containing **4–6** from chromatography (i) were stood in pyridine for 15 hr with $Ac_2O$. Work-up in the usual way yielded a glass which, on addition of a few drops of MeOH, gave **13** as needles (MeOH), mp 195–196°, $[\alpha]_D$ +39° (*c* 0.28). IR $\nu_{max}$ cm$^{-1}$: 1740. $^1$H NMR: $\delta$ 4.78 (1H, *m*, $W_{1/2}$ = 18 Hz), 4.61 and 4.13 (2H, *ABq J* = 12.5 Hz), 3.89 and 3.65 (2H, ABq *J* = 12 Hz), 2.04 (6H, *s*), 2.02 (3H, *s*). (Found: C, 70.5; H 9.1. $C_{36}H_{56}O_8$ requires: C, 70.3; H, 8.85%).

*23-Hydroxyphillyrigenin* (**4**). Compound **13** (225 mg) was refluxed with 10% KOH in MeOH for 40 min. Work-up in the usual way yielded crude **4** (151 mg) which gave **4** as fine needles (MeOH), mp 330–332°, $[\alpha]_D$ +19° (*c* 0.23). IR $\nu_{max}$ cm$^{-1}$: 3400, 1730. EIMS *m/z*: 488 [M]$^+$ (12), 470 [M $-H_2O]^+$ (25), 457 [M $-CH_2OH]^+$ (38), 452 [M $-2H_2O]^+$ (51), 440, (47), 439 [M $-H_2O$, $CH_2OH]^+$ (42), 434 [M $-3H_2O]^+$ (30), 425 (39), 422 (43), 381 (26), 287 (26), 232 (30), 231 (32), 219 (33), 205 [**8a** $-H_2O]^+$ (68), 201 (62), 187 [**8a** $-2H_2O]^+$ (100), 175 (94), 173 (93).

*Dihydropriverogenin A* (**5**). The $CHCl_3$ fraction from chromatography (iii) was recrystallized from MeOH to give **5** as prisms, mp 283–285°, lit [12] mp 274–276°, [14] mp 282–283°, undepress on mixing with dihydropriverogenin A mp 285–288° isolated from *P. undulatum.*

*Dihydropriverogenin A tetra-acetate* (**14**). **5** in pyridine was heated for 3 hr at 100° with $Ac_2O$. The crude product (a mixt by TLC) in $Ac_2O$ (6 ml) was stood for 3 hr at room temp. with $HClO_4$ (1 drop). The crude product was filtered through $Al_2O_3$ to give **14**, amorphous (lit. [14] amorphous), identical (IR, TLC) with an authentic specimen.

*16-Desoxybarringtogenol C* (**6**). The $CHCl_3$–MeOH (1:19) fraction from chromatography (ii) was recrystallized (MeOH) to give **6** as prisms mp 287–289° undepressed on admixt. with authentic 16-desoxybarringtogenol C, mp 286–288°.

*16-Desoxybarringtogenol C tetra-acetate* (**15**). **6** in pyridine was stood at room temp for 18 hr with $Ac_2O$. The crude product (an oil) deposited crystals on addition of petrol. Filtration through $Al_2O_3$ gave **15** as needles (MeOH), mp 224–225°, lit. [15] mp 225–226.5°.

*Barringtogenol C*(**7**). The MeOH–$CHCl_3$ (1:1) fractions from chromatography (i) gave needles (MeOH), mp 314–319°, undepress on mixing with barringtogenol C, mp 317–322°, obtained from *Barringtonia acutangula* [16].

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