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TRITERPENOIDS AND STEROIDS OF SOME SAPOTACEAE AND THEIR CHEMOTAXONOMIC SIGNIFICANCE*

SARATH P. GUNASEKERA†, VIJAYA KUMAR†, M. UVAIS S. SULTANBAWA†‡ and SINNATHAMBY BALASUBRAMANIAM§

†Department of Chemistry, University of Sri Lanka, Peradeniya Campus; and §Department of Botany, University of Sri Lanka, Peradeniya Campus, Sri Lanka

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Key Word Index—*Madhuca*; *Palaquium*; Sapotaceae; triterpenoids; α-spinasterol; chemotaxonomy.

Abstract—Extraction of the bark and timber of four *Madhuca* and five *Palaquium* species has yielded β-amyrin, β-amyrin acetate, β-amyrin cinnamate, β-amyrin decanoate, β-amyrenone, betulinic acid, friedelin, hederagenin, isoarborinol, ursolic acid, α-spinasterol and α-spinasterol-β-D-glucoside. Chemotaxonomically, the presence of α-spinasterol in these and other species of Sapotaceae is of significance; the predominance of β-amyrin and its derivatives and the absence of taraxeranes characterizes the subfamily Madhucoideae.

INTRODUCTION

The Sapotaceae is divided into three subfamilies Sideroxyloideae, Achradoideae (Mimusopoideae) and Madhucoideae. There are over 600 tropical species grouped in about forty genera and its members are important for their durable timber, edible fruits and fatty seed kernels.

Chemical work on six species of *Mimusops* [1–16], three each of *Madhuca* [1, 16–19] and *Planchonella* [14, 21, 22] and one of *Tieghmella* [23] has been reported and triterpenoids and steroids have been identified. The bark and timber constituents of Sapotaceae endemic to Sri Lanka have now been investigated. The eleven species are restricted to the subfamily Madhucoideae and are *Madhuca neriifolia* (Thw.) H. J. Lam, *M. moonii* (Thw.) H. J. Lam, *M. fulva* (Thw.) H. J. Lam and *M. microphylla* (Thw.) J. F. Macbr of the tribe Madhuceae and *Palaquium canaliculatum* (Thw.) Engl., *P. grande* (Thw.) Engl., *P. paucifolium* (Thw.) Engl., *P. petiolare* (Thw.) Engl., *P. laevifolium* (Thw.) Engl. *P. rubiginosum* (Thw.) Engl. and *P. thwaitesii* Trim. [25] of the tribe Palaquieae. Of the endemic *Palaquium* species *P. paucifolium* and *P. thwaitesii* are rare and have not been available for investigation.

RESULTS

Examination of the bark and timber of the endemic Sapotaceae showed the presence of ten triterpenoids and two steroids, which were identified in all but one case by comparison with authentic samples. β-Amyrin decanoate whose spectral characteristics suggested it to be the ester of a triterpenoid alcohol and a long chain fatty acid was hydrolysed with alkali to β-amyrin and decanoic acid, the latter being characterised as its *p*-bromophenacyl derivative.

Friedelin (**1**) belonging to the friedelane series and isoarborinol (**2**) belonging to the arborane series were isolated only from *M. neriifolia*, the former from both bark and timber extracts, the latter from the bark alone. Betulinic acid (**3**) belonging to the lupane series was isolated from the barks of *M. neriifolia*, *M. microphylla* and *Palaquium grande* These compounds have not been reported from any Sapotaceae species previously examined, although sodium betulinate was isolated from the bark of *Mimusops elengi*. Ursolic acid (**4**) belonging to the ursane series was found in the bark and timber of *P. canaliculatum* and the bark of *P. grande*. Ursolic acid has previously been isolated from the bark of *Mimusops manilkara* and *M. hexandra*. α-Spinasterol (**5**) is a constituent of the timber of *Madhuca neriifolia* and *M. fulva* and all but one of the *Palaquium* species studied. It is also present in the bark of *M. fulva*, *P. canaliculatum* and *P. grande* while its β-D-glucoside is present in the timber of *M. neriifolia*. Misra *et al.* [1] suggested that the characteristic phytosterol of the genus *Mimusops* is α-spinasterol. The widespread occurrence of this sterol in the bark and timber of the Madhucoideae now studied gives the sterol a more general significance within the family.

The bark and timber studied also contained a wide range of triterpenoids belonging to the oleanane series. These included β-amyrin (**6a**) β-amyrenone (**6e**), the ester of β-amyrin with acetic (**6b**), cinnamic (**6c**) and decanoic (**6d**) acids and the terpenoid acid hederagenin (**7**).

DISCUSSION

The endemic species investigated in the present work belong exclusively to the subfamily Madhucoideae. Although studies on the seed coat and the seed kernel of *M. longifolia* (L) J. F. Macbr [1, 18], *M. butyraceae* Macbr [1, 20] and *M. latifolia* (Roxb) Macbr [16, 19], the leaves [17] and mesocarp [16] of *M. longifolia* and the leaves of *M. latifolia* [17] have been reported earlier, no work has previously been carried out on the bark or

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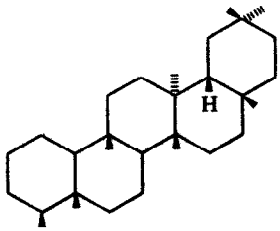
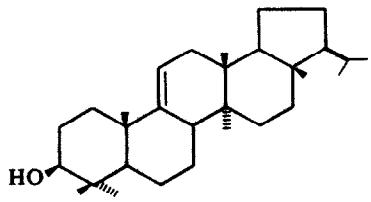
‡ For correspondence.



(1) Friedelin



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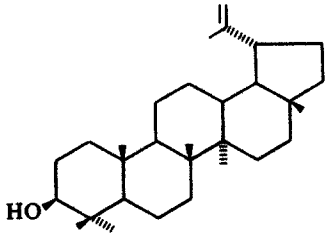
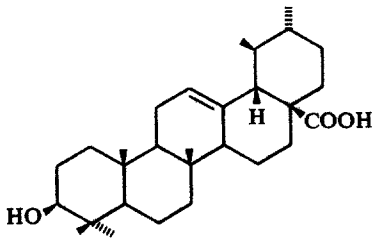
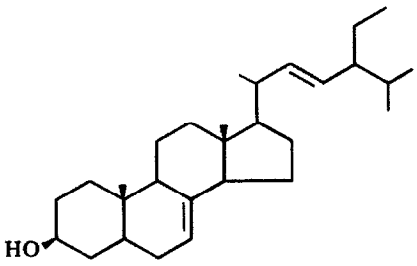


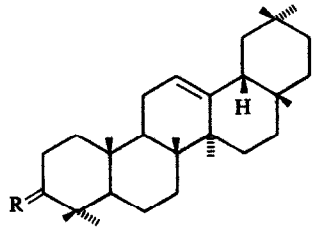
(3) Betulinic acid



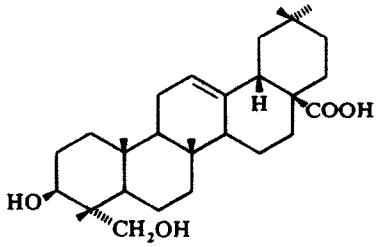
(4) Ursolic acid



(5) α-Spinasterol



(6a) β-amyrin R = αH, βOH
b β-amyrin acetate R = αH, βOAc
c β-amyrin cinnamate R = αH, β-OCOCH=CHPh
d β-amyrin decanoate R = αH, β-$OCOC_9H_{19}$
e β-amyrenone R = O



(7) Hederagenin

timber of any member of this subfamily. As in the other Sapotaceae, the characteristic constituents of the bark and timber are mainly triterpenoids of the oleanane series. β-Amyrin and β-amyrin acetate were isolated from all the endemic species studied while β-amrenone was absent only from *Madhuca moonii*. While β-amyrin and its simple derivatives have also been isolated from the members of the subfamily Achradoideae, i.e. in the genera *Mimusops* [1–9] and *Tieghmella* [23], they have not been reported in *Planchonella* species (subfamily Sideroxyloideae). Of the oleananes only the acids bayogenin and hederagenin were found to be present in one of the *Planchonella* species studied, *P. pohlmaniana* [21].

It is of interest that while taraxerol and its derivatives were present in the bark as well as the leaves and roots of *Mimusops* species [19], no members of the taraxerane series have been isolated in the present study. Similarly while α-amyrin and other ursanes occurred in all the *Mimusops* [1–3,5] studied they were absent in all the *Madhuca* [1, 16–19] species investigated so far. Ursolic acid was however isolated from *Palaquium canaliculatum* and *P. grande*.

Sitosterol, which is a common phytosterol, was absent in all the bark and timber studied. Although its presence has been noted in the mesocarp of *Mimusops manilkara* [3], its β-D-glucoside has been found to occur in the barks of some of the *Mimusops* [3, 9] species studied. The absence of sitosterol contrasts with its widespread

occurrence in the bark and timber of the endemic *Diospyros* [26] of Sri Lanka. The genus *Diospyros* belongs to the Ebenaceae which, with the Sapotaceae, is in the order Ebenales. Taraxerone and lupane derivatives which were found to generally co-occur with sitosterol in *Diospyros*, have been isolated from the barks of the *Mimusops* species [3, 9] but not from the subfamily Madhucoideae.

α-Spinasterol was isolated from *Madhuca neriifolia* and *M. fulva* and all the *Palaquium* studied except *P. rubiginosum*. Its co-occurrence in *Mimusops* species [1–3, 5] and in *Planconella novoceylandica* [21] and its absence from the bark of *Diospyros* species [26] makes it the characteristic phytosterol of the Sapotaceae.

EXPERIMENTAL

The bark and timber were separately collected, ground and extracted with boiling light petrol (bp 60–80°), C_6H_6 and MeOH to give the petrol, C_6H_6 and MeOH extracts. They were then subjected to chromatography on Si gel (Merck) columns and further purification, where necessary, was carried out using preparative TLC on Si gel $PF_{254+366}$ plates. IR was carried out on KBr discs while NMRs were in $CDCl_3$ solution at 100 MHz. All compounds isolated except α-spinasterol-β-D-glucoside were identified by comparison with authentic samples (TLC, IR, mp, mixed mp.)

Madhuca neriifolia. Collection was from Atveltota near Pallegama. Bark (4.0 kg) gave (a) petrol extract (53.5 g, 1.35%), (b) C_6H_6 extract (41.0 g, 1.0%), (c) MeOH extract (210 g, 5.2%). Timber (5.0 kg) gave (a) petrol extract (15 g, 0.3%), (b) C_6H_6 extract (2.2 g, 0.04%) and (c) MeOH extract (160 g, 3.2%).

Bark extractives. The petrol and C_6H_6 extracts were shown to be similar by TLC. The petrol extract (2 g) was chromatographed on Si gel (100 g). Elution with C_6H_6–petrol (1:19) gave β-amyrin decanoate, crystals from MeOH (0.09 g) mp 103–4°, $[\alpha]_D^{27}$ + 37.9°; ν_{max} 2925, 2860, 1730, 1241 and 1205 cm^{-1}; τ 4.88 (1H, *t*, *J* 4Hz, 12-H'), 5.49 (1H, *t*, *J* 8Hz, 3-H), 7.70 (2H, *t*, *J* 7Hz, R—CH_2—CO), 7.84–8.82 (saturated CH_2), 8.92, 8.98, 9.01, 9.07, 9.11 (×3) and 9.18 (24H, *s*, methyls), *m/e* 580 (M^+ 0.5%), 553 (8), 408 (40), 393 (15), 365 (34), 339 (10), 271 (15), 257 (8), 218 (100), 203 (35), 189 (45), 175 (20), 161 (21), 148 (18), 119 (20). Hydrolysis of β-amyrin decanoate (0.045 g) with 5% alcoholic KOH (20 ml) in the usual manner gave β-amyrin (0.030 g) mp 199–200°, $[\alpha]_D^{27}$ + 86.3° (lit. [27] mp 200°, $[\alpha]_D$ + 80°). From the aqueous solution decanoic acid (0.010 g) was isolated and converted to its *p*-bromophenacyl ester mp 66–67° (from MeOH) (lit. [28] mp 67°). It was identical with an authentic sample.

Isolation of β-amyrin acetate and friedelin. Further elution of the column with (a) C_6H_6–petrol (1:4) gave β-amyrin acetate (0.230 g) mp 240–1° (from petrol) $[\alpha]_D^{27}$ + 79.2° (lit. [29] mp 241°, $[\alpha]_D$ + 80°) (b) with C_6H_6–petrol (9:11) gave friedelin (0.165 g) mp 264–5° $[\alpha]_D^{27}$ − 24.2° (lit. [30] mp 264°, $[\alpha]_D$ − 22.1°) (c) C_6H_6 and then with $CHCl_3$ gave (i) white solid A (0.24 g) and (ii) a greenish solid B (0.80 g).

Isolation of isoarborinol. The solid A (0.24 g) on Si gel (20 g) with $CHCl_3$–C_6H_6 (1:3) gave isoarborinol (0.035 g) mp 299–300° (from petrol), $[\alpha]_D^{27}$ + 46.3° (lit. [31] mp 299°, $[\alpha]_D$ + 47°); ν_{max} 3495 and 1105 cm^{-1} τ 4.76 (1H, *m*, $W^1_{/2}$ 9Hz, 11-H), 6.80 (1H, *m*, $W_{1/2}$ 14Hz, 3-H), 8.12–8.80 (sat.–CH_2) and 8.91–9.25 (8 methyls); *m/e* 426 (M^+ 80%), 411 (100), 393 (75) and 259 (58).

Isolation of betulinic acid. The fraction (B) (0.5 g) on Si gel with $CHCl_3$ gave betulinic acid (0.085 g) mp 302–3°, $[\alpha]_D^{27}$ + 13.1° (lit. [32] mp 306°, $[\alpha]_D$ + 8°). The MeOH extract (200 g) was extracted with EtOAc. Concentration gave a brown solid (0.68) which before and after hydrolysis (10% HCl) did not show any characteristic TLC spots.

Timber extractives. The petrol extract (5.0 g) on Si gel gave (a) with C_6H_6–petrol, β-amyrin decanoate (0.55 g) mp 103–4°, (b) with C_6H_6–petrol (1:4) β-amyrin acetate (0.350 g) mp 240–1°, (c) with C_6H_6–petrol (2:3) a solid C (0.090 g). The above on PLC with $CHCl_3$ gave (i) friedelin (0.023 g) mp 264–5°, $[\alpha]_D^{27}$ − 25.2° and (ii) β-amyrenone (0.048 g) mp 178–9° (from petrol), $[\alpha]_D^{27}$ + 122.1° (lit. [28] mp 177–9°, $[\alpha]_D$ + 107°), (d) with C_6H_6, β-amyrin (0.043 g) mp 199–200°, (e) with $CHCl_3$ α-spinasterol (0.120 g) mp 162–3° (from petrol), $[\alpha]_D^{27}$ − 2.9° (lit. [5] mp 164°, $[\alpha]_D$ − 3.7°), M^+ 412. Calc. for $C_{29}H_{40}O$, M 412, (f) with MeOH–$CHCl_3$ (1:19) hederagenin (0.036 g) mp 323–4° (from EtOH $[\alpha]_D^{27}$ + 78° (pyridine) (lit. [28] mp 322–4°, $[\alpha]_D$ + 80°) M^+ 472. Calc. for $C_{30}H_{48}O_4$ M, 472 (g) with MeOH–$CHCl_3$ (3:17) α-spinasterol-β-D-glucoside (0.120 g) mp 291–2° (from petrol) $[\alpha]_D^{27}$ − 35.0° (lit. [28] mp 292°, $[\alpha]_D$ − 34.1°), M^+ 574. Calc. for $C_{35}H_{58}O_6$ M 574. α-spinasterol-β-D-glucoside (0.041 g) on hydrolysis with 5% HCl gave (i) α-spinasterol (0.025 g) mp 163–4° (ii) from the aqueous layer, D-glucose (0.008 g) was isolated. It was characterised by PC with authentic D-glucose and also by the preparation of glucosazone and comparison with authentic glucosazone.

Madhuca moonii. Collection was from Sinharajah forest in Kanneliya. The bark (5.0 kg) gave (a) petrol extract (340 g, 6.8% and (b) MeOH extract (2 kg, 40%). The timber (5.0 kg) gave (a) petrol extract (6 g, 0.12%), (b) C_6H_6 extract (0.5 g, 0.01%) and (c) MeOH extract (450 g, 9%).

Bark extractives. Steam distillation of the petrol extract (5 g) gave no volatile products.

Isolation of β-amyrin trans*-cinnamate.* The petrol extract (4 g) on Si gel (150 g), (a) with C_6H_6–petrol (1:9) gave a mixture (2.280 g). The mixture (0.1 g) on PLC and usual work up gave a less polar fraction β-amyrin *trans*-cinnamate (0.066 g) mp 228–9° (from petrol), $[\alpha]_D^{27}$ + 66.2° (lit. [5] mp 230°, $[\alpha]_D$ + 63°), M^+ 556.4256. Calc. for $C_{39}H_{56}O_2$, M 556 and the more polar β-amyrin acetate (0.023 g) mp 240–1°. Hydrolysis of β-amyrin *trans* cinnamate (0.025 g) with 5% alcoholic NaOH gave β-amyrin (0.011 g) and *trans* cinnamic acid (0.005 g) mp 133° (lit. [28c] mp 133°).

Isolation of β-amyrin acetate and β-amyrin. The above column gave on elution with (b) C_6H_6–petrol (1:3) β-amyrin acetate (0.540 g) mp 240–1°, (c) C_6H_6, β-amyrin (0.879 g) mp 199–200°.

Timber extractives. The petrol and C_6H_6 extracts were similar on TLC. The petrol extract (1 g) on Si gel gave with (a) C_6H_6–petrol (1:4) a mixture of β-amyrin *trans*-cinnamate and β-amyrin acetate (0.270 g) identified by Co-TLC with authentic samples, (b) C_6H_6–$CHCl_3$ (1:1) β-amyrin (0.406 g) mp 199–201°. The MeOH extract of bark and timber before and after hydrolysis did not show any characteristic TLC spots.

Madhuca fulva. Collection was from Sinharajah forest near Kanneliya. The bark (3.5 kg) gave (a) petrol extract (15 g, 0.4%), (b) MeOH extract (251 g, 7.1%). The timber (3.9 kg) gave (a) petrol extract (6.3 g, 0.16%), (b) C_6H_6 extract (1 g, 0.03%), (c) MeOH extract (70 g, 1.8%).

Bark extractives The petrol extract (1 g) on Si gel gave with (a) C_6H_6–petrol (1:19) β-amyrin decanoate (0.065 g) mp 103–4°, (b) C_6H_6–petrol (1:4) β-amyrin acetate (0.215 g) mp 240–1°, (c) C_6H_6–petrol (9:11) β-amyrenone mp 179–80° (d) C_6H_6, β-amyrin (0.36 g) mp 199–200°, (e) $CHCl_3$, α-spinasterol (0.175 g) mp 162–3°.

Timber extractives. The petrol and C_6H_6 extracts were similar on TLC. The petrol extract (1 g) on Si gel gave with (a) C_6H_6–petrol (1:4) a mixture of β-amyrin *trans*-cinnamate and β-amyrin acetate. The mixture (0.500 g) on PLC gave β-amyrin *trans*-cinnamate (0.043 g) mp 228° and β-amyrin acetate (0.040 g) mp 240–1°, (b) C_6H_6–petrol (3:7) β-amyrenone (0.120 g) mp 179–80°, (c) C_6H_6, β-amyrin (0.260 g) mp 199–200°, (d) $CHCl_3$–C_6H_6 (3:2) α-spinasterol mp 162–3°.

Madhuca microphylla. Collection was from Sinharajah forest near Kanneliya. Bark (3.0 kg) gave (a) petrol extract (14.5 g, 0.48%), (b) C_6H_6 extract (6.7 g, 0.22%), (c) 95% EtOH extract (350 g, 11.7%). Timber (5.6 kg) gave (a) petrol extract (10.0 g, 0.18%), (b) 95% EtOH extract (140 g, 2.5%).

Bark extractives. The petrol extract (1.4 g) on Si gel gave with (a) Et_2O–petrol (1:19) β-amyrin acetate (0.140 g) mp 240–1°, (b) Et_2O–petrol (1:9), β-amyrenone (0.080 g) mp 179–200°, (c) Et_2O–petrol (7:13) betulinic acid (0.280 g) mp 310–311°.

Timber extractives. The petrol extract (1 g) on separation on a column gave with C_6H_6 β-amyrin (0.070 g) mp 199–200°. The EtOH extract before and after hydrolysis showed no characteristic TLC spots.

Palaquium petiolare. Collection was from Morabotuwa forest near Agalawatte. The bark (4.25 kg) gave (a) petrol extract (230 g, 5.4%), (b) MeOH extract (460 g, 10.8%). The timber (4.5 kg) gave (a) petrol extract (16.5 g, 0.36%), (b) MeOH extract (160 g, 3.5%).

Bark extractives. The petrol extract (2 g) on Si gel gave with (a) C_6H_6–petrol (1:19) β-amyrin *trans*-cinnamate (0.115 g) mp 228–9°, (b) C_6H_6–petrol (1:9), β-amyrin acetate (0.215 g) mp 238–40°, (c) C_6H_6–petrol (1:1) β-amyrenone (0.045 g), (d) C_6H_6, β-amyrin (0.160 g) mp 199–200°.

Timber extractives. The petrol extract was separated on a column to give with (a) C_6H_6, a waxy product (2.90 g). The mixture was identified by TLC with authentic samples and shown to consist of β-amyrin *trans*-cinnamate, β-amyrin acetate and β-amyrin as the major constituents and β-amyrenone as a minor constituent. (b) $CHCl_3$–C_6H_6 (3:1) α-spinasterol (0.045 g) mp 163–4°.

Palaquium rubiginosum. Collection was from Kanneliya forest. The bark (4 kg) gave (a) Petrol extract (380 g, 9.5%), (b) C_6H_6 extract (8.5 g, 0.21%), (c) MeOH extract (325 g, 8.1%). Timber (6.0 kg) gave (a) petrol extract (21.5 g, 0.35%), (b) C_6H_6 extract (2.3 g, 0.04%) and (c) MeOH extract (214 g, 3.6%).

Bark extractives. The petrol extract (3 g) on Si gel gave with (a) C_6H_6–petrol (1:4) β-amyrin acetate (0.130 g) mp 238–40°, (b) C_6H_6–petrol (1:1) β-amyrenone (0.110 g) mp 178–9°, and (c) C_6H_6, β-amyrin (1.201 g) mp 199–200°.

Timber extractives. The petrol extract (5.0 g) on similar separation gave β-amyrin acetate (2.12 g) β-amyrenone (0.060 g) and β-amyrin (0.245 g).

Palaquium canaliculatum. Collection was from Kanneliya forest. Bark (2.0 kg) gave (a) petrol extract (33 g, 1.65%), (b) MeOH extract (150 g, 7.5%). Timber (3.15 kg) gave (a) petrol extract (5 g, 0.16%), (b) MeOH extract (110 g, 3.5%).

Bark extractives. Petrol extract (2 g) on Si gel (80 g) gave with (a) C_6H_6–petrol (1:19) β-amyrin decanoate (0.065 g) mp 103–4°, (b) C_6H_6–petrol (1:4) β-amyrin acetate (0.126 g) mp 240–1°, (c) C_6H_6–petrol (1:1) β-amyrenone (0.032 g), (d) C_6H_6, β-amyrin (0.285 g) mp 198–99°, (e) $CHCl_3$, α-spinasterol (0.090 g) mp 162–3° and (f) MeOH–$CHCl_3$ (1:99) ursolic acid (0.022 g) mp 282–3°.

Timber extractives. Petrol extract (1 g) on Si gel (40 g) gave with (a) C_6H_6–petrol (1:3), β-amyrin acetate (0.073 g) mp 240–1°, (b) C_6H_6, β-amyrin (0.115 g) mp 199–200°, (c) $CHCl_3$, α-spinasterol (0.032 g) mp 162–3°, (d) MeOH–$CHCl_3$ (1:99) ursolic acid (0.011 g) mp 282–3°.

Palaquium grande. Collection was from Gilimalle forest. The bark (2.4 kg) gave (a) petrol extract (14 g, 0.58%), (b) MeOH extract (260 g, 10.8%). Timber (0.200 kg) gave (a) petrol extract (1.1 g, 0.55%), (b) MeOH extract (3.6 g, 1.8%).

Bark extractives. The petrol extract (1 g) on Si gel (50 g) with C_6H_6–petrol mixtures gave in order of increasing polarity, (a) β-amyrin decanoate (0.036 g) mp 103–4°, (b) β-amyrin acetate (0.061 g) mp 240–1°, (c) β-amyrenone (0.018 g) mp 178–9°, (d) β-amyrin (0.078 g) mp 198–99°, (e) α-spinasterol (0.027 g) mp 162–63°, (f) betulinic acid (0.016 g) mp 308–9°, (g) ursolic acid (0.012 g) mp 282–83°.

Timber extractives. Petrol extract (1 g) chromatographed on Si gel (40 g), similarly gave (a) β-amyrin acetate (0.043 g), (b) β-amyrin (0.048 g), (c) α-spinasterol (0.036 g).

Palaquium laevifolium. Collection was from Kanneliya forest. The bark (4.5 kg) gave (a) petrol extract (310 g, 6.8%), (b) MeOH extract (140 g, 3.11%). Timber (6.5 kg) gave (a) petrol extract (2.5 g, 0.039%), (b) MeOH extract (260 g, 4.0%).

Bark extractives. The petrol extract (1 g) on Si gel (50 g) similarly gave (a) β-amyrin cinnamate (0.058 g), (b) β-amyrin acetate (0.067 g), (c) β-amyrenone (0.017 g) and (d) β-amyrin (0.086 g).

Timber extractives. The petrol extract (1 g) chromatographed similarly gave (a) β-amyrin acetate (0.046 g), β-amyrin (0.073 g), (c) α-spinasterol (0.061 g).

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