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TRITERPENES OF FIVE EUPHORBIACEAE SPECIES OF SRI LANKA*

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Key Word Index—*Podadenia thwaitesii; Apurosa cardiosperma; Glochidion* spp.; *Bridelia moonii*; Euphorbiaceae; aleuritolic acid derivatives; glochidonol; lup-20(29)-ene-3α,23-diol; glochidiol; glochidone; lup-20(29)-ene-1α,3α-23-triol.

Abstract—Five endemic species of Euphorbiaceae have been investigated. Aleuritolic acid and its derivatives were isolated from *P. thwaitesii*. *A. cardiosperma* had friedelan derivatives. The *Glochidion* species contained the same triterpenes, viz. glochidonol, lup-20(29)-ene-3α,23-diol and glochidiol. The bark extractives of a new *Glochidion* species had, in addition, glochidone and probably lup-20(29)-ene-1α,3α,23-triol. The latter is a new natural product. Glochidone was isolated for the first time outside the genus *Glochidion* from *Bridelia moonii*.

INTRODUCTION

Forty-seven Euphorbiaceae species distributed amongst nineteen genera are endemic to Sri Lanka [1]. A detailed chemical investigation has been initiated and this paper describes the work on five species, namely *Podadenia thwaitesii* (Baill) Muell Arg., *Apurosa cardiosperma* Merr., *Glochidion moonii* Thw., *Bridelia moonii* Thw., and a new *Glochidion* species. Chemical investigation of several species belonging to the genera *Apurosa*, *Glochidion* and *Bridelia* have been undertaken by Hui and Li [2], Talapatra *et al.* [3] Ganguly *et al.* [4] and Johns and Lamberton [5]. The genus *Podadenia* is recorded only in Sri Lanka. Friedelane triterpenoids are common to the species of *Apurosa* and *Bridelia* whereas lupene triterpenoids are characteristic of *Glochidion* species.

RESULTS AND DISCUSSION

The major component of *P. thwaitesii* bark extractives was shown to be the acetate of aleuritolic acid, $C_{32}H_{50}O_4$ by NMR, IR and MS studies. Aleuritolic and aleuritolonic acids were shown to be minor components of the petrol extractives. Table 1 gives the percentages of the acids isolated. The acetate of aleuritolic acid and aleuritolic acid were isolated from the other Euphorbiaceae species, *Aleurites montana* [6] and *Sapium baccatum* [7].

The bark extractives of *A. cardiosperma* gave friedelan-3-one and friedelan-3β-ol besides sitosterol. The bark and timber extractives of the new *Glochidion* species were similar. Glochidonol and glochidiol were not only present in these but also in the stem extractives of *Glochidion moonii*. Glochidone, a common constituent of many *Glochidion* species, was isolated from the bark extractives of the new *Glochidion* species but was absent in the stem of *G. moonii*. Another triterpene present in all the *Glochidion* extracts was lup-20(29)-ene-3α,23-diol. This is a rare triterpene alcohol and has been isolated only once before from *Glochidion macrophyllum* [2]. It has been identified by mp, $[\alpha]_D$, MS, NMR and conversion to lupa-2,20(29)-diene.

The benzene extractives of bark of the new *Glochidion* species gave a new triterpene which was assigned the formula $C_{30}H_{50}O_3$. The signals at δ 4.75 (2H,*d*) and 1.70 (3H,*s*) in the ^{1}H NMR spectrum of the triol showed that it belonged to the lupene class. Acetylation gave a triacetate as evidenced by the ^{1}H NMR signals at δ 2.03 (3H,*s*), 1.96 (3H,*s*) and 1.93 (3H,*s*). The ^{1}H NMR spectra of the triol and triacetate had only signals for five tertiary methyl groups. The presence of the signals at 3.80 (2H,*d*), 4.43 (1H,*m*, $W_{1/2}$ = 8 Hz) and 4.03 (1H,*m*, $W_{1/2}$ = 8Hz) indicated the presence of a CH_2OH and two —CHOH protons, respectively. MS data were useful in arriving at a structure for this lupenetriol. Intense ions were recorded at *m/e* 440 ($M^+ - H_2O$) and 422 ($440 - H_2O$). The base peak in the MS of the triol was at *m/e* 189 as in the case of other lupenes with unsubstituted D- and E-rings (Scheme 1). Another significant fragment ion at *m/e* 218 also confirmed that rings D and E in the triol are unsubstituted. The location of all three hydroxyl groups in rings A and/or B was indicated by the fragment **a** (*m/e* 239). This fragment also readily lost molecules of water to give

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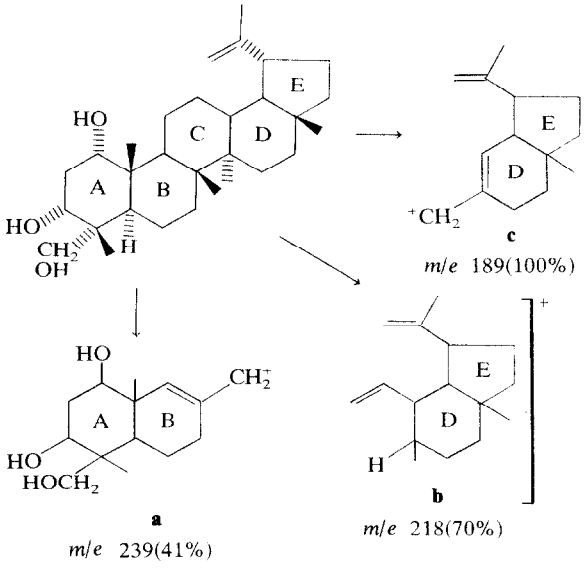
Table 1. Triterpenoids and steroids identified in Sri Lankan Euphorbiaceae species

Podadenia thwaitesii: sitosterol (0.02%); aleuritolic acid acetate (0.154%); aleuritolic acid (0.0015%); aleuritolonic acid (not isolated)
Apurosa cardiosperma: friedelan-3-one (0.04%); friedelan-3β-ol (0.06%); sitosterol (0.035%)
New *Glochidion* species: glochidone (0.0043%); glochidonol (0.03%); lup-20(29)-ene-3α,23-diol (0.06%), glochidiol (0.111%); lup-20(29)-ene-1α,3α,23-triol (0.0027%)
Glochidion moonii: sitosterol (0.018%); glochidonol (0.094%); lup-20(29)-ene-3α,23-diol (0.044%); glochidiol (0.011%)
Bridelia moonii: friedelan-3-one (0.026%); friedelan-3β-ol (0.009%); friedelan-3α-ol (0.001%); sitosterol (0.0051%); glochidone (0.00081%)

fragment ions at *m/e* 221 (239 − H_2O) and 203 (221 − H_2O). The absence of a fragment at *m/e* (M^+ − 31) for this triol showed that the —CH_2OH group in the triol was not at an angular position. The ^1H NMR data for the —CH_2OH and —CH_2OAc methylene groups of the triol and triacetate suggested that the —CH_2OH group should be in the C-23 position [8]. The half band widths of the —CHOH signals ($W_{1/2}$ = 8 Hz) coupled with the biogenetic consideration indicated that the triol is probably lup-20(29)-ene-1α,3α,23-triol. Lack of material precluded further work on the structure. This is the first report of the isolation of a trihydroxy triterpene belonging to the lup-20(29)-ene series.

The bark extractives of *Bridelia moonii* gave friedelan-3-one, friedelan-3β-ol and friedelan-3α-ol. Another triterpene ketone isolated was shown to be glochidone. This is the first report of the occurrence of glochidone outside the genus *Glochidion*. Friedelan-3α-ol is also reported for the first time from the genus *Bridelia*. The yields of the triterpenes isolated are given in Table 1. Timber extractives of *Bridelia*, *Apurosa* and *Podadenia* have been shown to be rich in hydrocarbons.



Scheme 1. MS fragmentation of lup-20(29)-ene-1α,3α,23-triol.

EXPERIMENTAL

Bark and timber of *Podadenia thwaitesii*, *Apurosa cardiosperma* and *Bridelia moonii* were collected from the Kanneliya Forest in the south of Sri Lanka. *Glochidion moonii* and the unidentified *Glochidion* species were collected by Dr. L. C. Wheeler from the Hantane hills in the Kandy District in central Sri Lanka. Bark and timber were separately collected, chipped and dried. The powdered plant parts were separately extracted with petrol, C_6H_6 and MeOH. The solvents were removed under red. pres. CC separations were on Si gel (30–70 mesh). Si gel was used for TLC and PLC separations. Mps were obtained using a Kofler hot stage melting point apparatus and are uncorr. Unless otherwise stated, identity of the isolated compounds was confirmed by comparison with an authentic sample (mp, mmp, co-TLC and IR).

Isolation of aleuritolic acid acetate. All extracts (petrol, C_6H_6 and MeOH) of the bark of *P. thwaitesii* had aleuritolic acid acetate. The petrol extract (37 g) from 7.9 kg of the bark of *P. thwaitesii* on cooling deposited 7.0 g of a white solid which was essentially 90% aleuritolic acid acetate. TLC gave pure aleuritolic acid acetate, mp 297–298° (lit. [6] 278–281°), $[\alpha]_D$ + 25.2° ($CHCl_3$).

Hydrolysis of aleuritolic acid acetate. The acetate (40 mg) was dissolved in EtOH and was heated under reflux with excess 10% ethanolic KOH (5 ml) for 4 hr. EtOH was evapd and the soln was acidified and extracted with $CHCl_3$. The dried solvent was evapd and the residue was recrystallized from EtOH to give pure aleuritolic acid (26 mg), mp 298° (lit. [6] 300–302°), $[\alpha]_D$ + 18° ($CHCl_3$).

Oxidation of aleuritolic acid. The hydroxy compound (20 mg) was treated with CrO_3 (14 mg) in Py (3.0 ml) at room temp. and the mixture was worked up in the usual manner. The pure oxidation product (10 mg) had mp 280° (lit. [6], 280–282°).

Methylation of aleuritolic acid acetate. The acetate (50 mg) was methylated using CH_2N_2 to give the methyl ester (35 mg), mp 241–242° (lit. [6] 241–243°), $[\alpha]_D$ + 22° ($CHCl_3$) (lit. [6] + 23°).

Methylation of aleuritolic acid. Aleuritolic acid (20 mg) was methylated using CH_2N_2 to give pure methyl ester (15 mg), mp 208–210° (lit. [7] 208–210°), $[\alpha]_D$ + 16° ($CHCl_3$) (lit. [7] + 11°).

Isolation of aleuritolic acid. The mixture of the white solid ppt. (see above) when separated by PLC gave aleuritolic acid, mp 298° (lit. [6] 300–302° dec.), $[\alpha]_D$ + 18° ($CHCl_3$). Aleuritolic acid was also isolated from the C_6H_6 extract.

Isolation of sitosterol. A MeOH extract (70 g) was obtained from 7.9 kg of the bark of *P. thwaitesii* after first extracting with petrol and C_6H_6 successively. The MeOH extract was re-extracted with C_6H_6 to give 6 g of material. This was chromatographed over Si gel to give aleuritolic acid acetate and sitosterol. Analytically pure sitosterol was obtained by PLC separation of the above mixture to give 37 mg, mp 136–137° (lit. [9] 136–137°), $[\alpha]_D$ − 36.1° (lit. [9] − 35°).

Isolation of friedelan-3-one. The bark of *Apurosa cardiosperma* (4.85 kg) gave 40 g petrol-soluble fraction. This fraction on CC and elution with petrol–C_6H_6 (1:1) gave a white solid, which on recrystallization from petrol gave friedelan-3-one, mp 264–265° (lit. [10] 264–265°), $[\alpha]_D$ − 21.6° ($CHCl_3$) (lit. [10] − 28°).

Isolation of friedelan-3β-ol. The above column separation gave, on elution with petrol–$CHCl_3$ (1:1), friedelan-3β-ol, mp 282–283° (lit. [10] 279–283°), $[\alpha]_D$ +22° ($CHCl_3$) (lit. [10] +24°). Continued elution of the column with the same solvent gave a mixture containing friedelan-3β-ol and sitosterol from which pure sitosterol was obtained by PLC.

Isolation of glochidone. The bark (3.70 kg) of the new *Glochidion* species gave 32 g petrol-soluble fraction. This extract (6 g) was chromatographed over Si gel and elution with petrol–$CHCl_3$ (9:1) yielded a white solid which was recrystallized from a mixture of $CHCl_3$–MeOH to give glochidone (12 mg) mp 164–165° (lit. [3] 164°), $[\alpha]_D$ +72° ($CHCl_3$) (lit. [3] +70°).

Isolation of glochidonol. Continued elution of the above column with petrol–$CHCl_3$ (7:3) gave a mixture of two compounds which was separated by PLC. The less polar component was isolated as an amorphous powder (180 mg) and was identified as glochidonol mp 228° (lit. [11] 228°), $[\alpha]_D$ +23° ($CHCl_3$).

Isolation of lup-20(29)-ene-3α,23-diol. After separating glochidonol, elution of the column with the same solvent gave a mixture of two compounds which was separated by PLC to give a terpene (350 mg) which was recrystallized from a mixture of $CHCl_3$ and petrol as white needles, mp 214–215°, $[\alpha]_D$ +15.0° ($CHCl_3$) (lit. [8] 209–213°), $[\alpha]_D$ +13.2°, IR ν_{max}^{KBr} cm^{-1} 880, 1030, 1380, 1450, 1640, 2900 and 3210. ^1H NMR ($CDCl_3$, 60 MHz), δ 4.62 (2H,*m*), 3.63 (1H,*t*), 3.47 (2H,*m*), 1.70 (3H,*s*), 1.03–0.66 (15H, 5×*s*, Me), MS *m/e* (rel. int.): 442 (M^+ 80), 424 (56), 406 (36), 257 (65), 250 (60), 236 (27), 232 (56), 231 (62), 229 (72), 223 (65), 218 (83), 206 (87), 205 (98), 201 (78), 189 (100) and 187 (90). MW: 442.3810; Calc. MW for $C_{30}H_{50}O_2$: 442.7268.

Tosylation of lup-20(29)-ene-3α,23-diol. The diol (100 mg) was treated with toluene-*p*-sulphonyl chloride (400 mg) in dry Py at 0° for 2 days. The usual work-up gave the ditosylate (110 mg), mp 158°. ^1H NMR (CCl_4, 60 MHz): δ 7.53 (8H,*d*, *J* = 8 Hz), 4.53 (2H,*d*), 3.66 (1H), 2.43 (6H,*s*), 1.63 (3H,*s*), 0.96–0.73 (15H, 5×*s*, Me).

$LiAlH_4$ *reduction of tosylate.* The above tosylate (50 mg) was heated under reflux with $LiAlH_4$ (500 mg) in dry THF (30 ml) for 3 days. Excess $LiAlH_4$ was destroyed by adding EtOAc and then H_2O gradually. Et_2O extraction and purification of the reaction product by PLC gave lupa-2, 20(29)-diene (20 mg) which was recrystallized from petrol to yield needles, mp 162–164° (lit. [8] 164°). This was shown to be identical (mmp, co-TLC, IR) with lupa-2,20(29)-diene obtained from lupeol when treated with $POCl_3$–Py for 2 hr under reflux.

Isolation of glochidiol. After separation of lup-20(29)-ene-3α,23-diol from the column (see above) continued elution with the same solvent gave glochidiol (520 mg), mp 266–267° (lit. [12] 266–267°), $[\alpha]_D$ +18.5° ($CHCl_3$) (lit. [12] +17.0°).

Isolation of lup-20(29)-ene-1α,3α,23-triol. The bark (3.78 kg) of the new *Glochidion* species gave 5.0 g of C_6H_6 extract which when chromatographed over Si gel and eluted with petrol–$CHCl_3$ (7:3) gave the following compounds—glochidion, lup-20(29)-ene-3α,23-diol and glochidiol. When the elution of these were completed and the solvent polarity was increased ($CHCl_3$–MeOH, 19:1) a yellow–white solid was obtained which was purified by PLC to give 100 mg of the triol, mp 266°; $[\alpha]_D$ +17.5° (Py); IR ν_{max}^{KBr} cm^{-1}: 880, 1020, 1270, 1380, 1450, 1640, 2910 and 3400. ^1H NMR (Py-d_5, 60 MHz): δ 4.75 (2H,*d*), 4.43 (1H,*m*, $W_{1/2}$ = 8 Hz), 4.03 (1H,*m*, $W_{1/2}$ = 8 Hz), 3.80 (2H,*d*), 1.70 (3H,*s*), 1.27–0.80 (15H, 5 Me). MS *m/e* (rel. int.): 458 (M^+ 54), 440 (57), 422 (48), 415 (22), 252 (22), 239 (41), 234 (30), 229 (83), 221 (65), 218 (70), 216 (61), 205 (78), 203 (89) and 189 (100). MW: 458.3719 (MS); Calc. MW for $C_{30}H_{50}O_3$: 458.7262.

Acetylation of lup-20(29)-ene-1α,3α,23-triol. The triol (10 mg) was dissolved in Py and refluxed with Ac_2O–Py for 4 hr. The major, less polar compound was purified by PLC to give 5 mg of the acetate, mp 248°. ^1H NMR ($CDCl_3$, 60 MHz): δ 4.80 (2H,*d*), 4.60 (1H,*m*), 4.06 (1H,*m*), 3.90 (2H), 2.03 (3H,*s*), 1.96 (3H,*s*), 1.93 (3H,*s*), 1.63 (3H,*s*), 1.23–0.73 (15H,*s*, Me). The timber of the new *Glochidion* species gave 6.0 g petrol extractive which, when chromatographed over Si gel and eluted with solvents of increasing polarity, gave the following compounds: sitosterol, glochidonol, lup-20(29)-ene-3α,23-diol, and glochidiol. The stem (1.70 kg) of *Glochidion moonii* gave 7.0 g petrol soluble fraction which, when chromatographed over Si gel and eluted with solvents of increasing polarities, gave the following: sitosterol, glochidonol, lup-20(29)-ene-3α,23-diol and glochidiol. The bark (2.65 kg) of *Bridelia moonii* gave 13.0 g petrol soluble fraction which, when separated on a column of Si gel, gave the following: glochidone, friedelan-3-one, friedelan-3β-ol and sitosterol. Further elution of the column with petrol–$CHCl_3$ mixture gave pure friedelan-3α-ol, mp 298–300° (lit. [10] 299–302°), $[\alpha]_D$ +16.9° ($CHCl_3$) (lit. [10] +18°).

Isolation of a hydrocarbon from the timber extractives. The timber (4.70 kg) of *Bridelia moonii* gave 8.5 g petrol fraction which on Si gel CC gave a hydrocarbon, 120 mg, mp 80–81°. IR ν_{max}^{KBr} cm^{-1}: 720, 730, 1060, 1460 and 2900. ^1H NMR ($CDCl_3$, 60 MHz): δ 1.27 (saturated CH_2) and 0.87 (Me groups). MS *m/e* (rel. int.): 392 (M^+ 25), 364 (30), 336 (23), 322 (13), 308 (20), 294 (17), 280 (20), 266 (23), 252 (35), 238 (30), 224 (33), 210 (70), 196 (40), 182 (43), 168 (47), 154 (53), 140 (58), 126 (65), 112 (73), 98 (80), 84 (90) and 70 (100). MW: 392.4381 (MS) MW calc. for $C_{28}H_{56}$: 392.7532. The timber (4.80 g) of *Apurosa cardiosperma* gave 13.5 g petrol-soluble fraction. The neutral fraction of this (10 g) when separated on Si gel gave a colourless liquid (4.0 g). GLC analysis of this revealed that it was a mixture of at least 20 components. Comparison of the chromatogram with that of standard hydrocarbon mixtures showed that the isolated oil contained C_{11}–C_{14} and also higher carbon residues. The timber (4.55 kg) of *Podadenia thwaitesii* gave 13.0 g petrol-soluble part which gave 5.5 g of a neutral fraction. This on Si gel CC gave 1.3 g of a mixture of hydrocarbons. GLC studies revealed that it was a mixture of at least 19 components, 4 of which were major. It was also found that the hydrocarbon mixture did not contain hydrocarbons with less than C_{13}.

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