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TERPENOIDS OF *CANARIUM ZEYLANICUM*

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Key Word Index—*Canarium zeylanicum*; Burseraceae; oleoresin; bark; timber; monoterpenoids; sesquiterpenoids; triterpenoids; 3β-hydroxyurs-12-en-11-one (neoilexonol); 3β-hydroxyolean-12-en-11-one; olean-12-en-3,11-dione; urs-12-en-3,11-dione; elemene; elemol; α-amyrin and β-amyrin; α- and β-amyrenone; canaric acid; taraxerol; sitosterol.

Abstract—The following terpenoids were identified in the oleoresin, bark and timber of *Canarium zeylanicum*: 3β-hydroxyurs-12-en-11-one, 3β-hydroxyolean-12-en-11-one, olean-12-en-3,11-dione, urs-12-en-3,11-dione, α- and β-amyrin, α- and β-amyrenone, taraxerol, sitosterol, canaric acid, elemene, elemol, α-pinene, α- and β-phellandrene, limonene, terpineol and carvone.

INTRODUCTION

Canarium zeylanicum Thw. (Sinhalese-Kekuna) is a very branched tree and is endemic to Sri Lanka. The whole tree is fragrant when bruised and a clear fragrant balsamic gum-like resin, similar to the elemi of commerce, exudes from the bark. The gum was burnt by the poorer Ceylonese peasants for light in the house and was commonly used for fumigation.

Chandrasena and Lorenz [1] investigated the resin and found it to yield 10–15% of essential oil. They isolated as the major components α-phellandrene and a pale yellow oil which, on cooling, yielded a crystalline solid, $C_7H_{16}O$, mp 164°. The isolation of terpenoid constituents from the resin, timber and bark is now reported.

RESULTS AND DISCUSSION

The bark and timber were extracted with hot C_6H_6. Steam distillation of the gums obtained and of the oleoresin yielded liquids which were found (by GLC) to contain α-pinene, α-phellandrene, β-phellandrene, limonene, terpineol and carvone.

The non-volatile fraction of the oleoresin was extracted with ether and the ether-soluble fraction was separated by partitioning into acidic and neutral fractions. The acidic fraction on chromatography yielded canaric acid.

The neutral material consisted mainly of mixed triterpenoids (Liebermann–Burchard test) and was chromatographed on Si gel to give the above mono- and sesquiterpene mixture, followed by elemene. Further elution of the column yielded five major fractions.

Fraction 1 yielded olean-12-en-3-one and urs-12-en-3-one while fraction 5 gave elemol and sitosterol.

Fraction 3 was a mixture of α- and β-amyrin. The mixture was separated by the method of Bannon *et al.* [2]. Chlorination and repeated PLC yielded α-amyrin and 12-chloroolean-12-en-3β-ol.

PLC of fraction 4 gave the products A (highest R_f), B and E (lowest R_f). All compounds responded to a Liebermann–Burchard test for triterpenoids and products A and B were shown by high resoltuion MS to be isomers ($C_{30}H_{48}O_2$). That they were hydroxyketones followed from the preparation of acetates and IR spectra. The IR spectrum of A showed strong absorptions at 3300 (OH) and 1659 (C=O) and a weak absorption at 1610 (C=C) cm^{-1}. Conjugation of the carbonyl groups was apparent from IR and UV spectra (λ_{max} 252 nm). The likelihood that A and B were 11-oxo derivatives of α-and β-amyrin was supported by ^1H NMR [3] and MS data. ^1H NMR spectra showed an olefinic one-proton singlet at δ 5.59 for both A and B. The chemical shift of the olefinic proton compared with that of α- or β-amyrin (δ 5.35) suggested that it was adjacent to a carbonyl group. The one-proton singlet at δ 5.50 for A and 3.53 for B exchanged with D_2O and were due to OH groups. A one-proton triplet at 3.22 for A and at 2.88 for B was attributed to CH-OH. The singlets at 0.82, 0.98, 0.99, 1.18, 1.46 for A and at 0.84, 0.96, 1.04, 1.22, 1.33 for B indicated the presence of eight methyl groups in both A and B.

Compounds A and B and their acetates showed fragmentation patterns analogous to derivatives of glycyrrhetinic acid [3]. Thus both compounds showed peaks at *m/e* 232, attributable to the retro-Diels–Alder fragmentation [4], and both A and B similarly gave a prominent fragment at *m/e* 273 [3].

The base peak in the MS of A and its acetate and a prominent peak in the MS of B and its acetate occurred at *m/e* 135 and was shown to arise from a fragment of *m/e* 273 [3]. That these substances were in fact 3β-hydroxyolean-12-en-11-one and 3β-hydroxyurs-12-en-11-one was shown by NBS oxidation of β-amyrin and α-amyrin and their acetates by

the method of Finucane and Thomson [5] and hydrolysis of authentic 3β-acetoxyolean-12-en-11-one and 3β-acetoxyurs-12-en-11-one.

Comparison of the MS of natural and synthetic 3β-hydroxyolean-12-en-11-one and 3β-hydroxyurs-12-en-11-one and their acetates indicated that the fragment at *m/e* 273 was the base peak in the 11-oxours-12-ene series while the fragment at *m/e* 135 was the base peak in the 11-oxoolean-12-ene series.

Fraction 2 was separated into products C, mp 234–235°, and D, mp 198–199°. They were shown to be triterpenoids and analysed for $C_{30}H_{46}O_2$ (confirmed by high resolution MS). The presence of a base peak at *m/e* 135 indicated that C was a 11-oxoolean-12-ene-type triterpene, and this was established by its ^1H NMR spectrum. Methyl group signals in the ^1H NMR spectra of C and methyl 3,11-dioxo-18β-olean-12-en-30-oate [3] showed good agreement. The singlets at δ 0.88, 0.98, 1.07, 1.11, 1.25, 1.37 in D were due to eight methyl groups. The presence of a base peak at *m/e* 273 showed that D was a 11-oxours-12-ene-type triterpene. The presence of a sharp carbonyl peak at 1640 and an olefinic absorption at 1610 cm^{-1} for C, and similar absorptions at 1646 and 1610 for D, and the intense UV absorption at λ_{max} 252 nm confirmed that the compounds were α,β-unsaturated ketones. The C-12 proton appeared at δ 5.59 for C and at 5.56 for D. The presence of a sharp carbonyl absorption at 1702 cm^{-1} in C and D, the absence of OH absorptions in the IR and ^1H NMR spectra, and the absence of the broad triplet assigned to the 3β-proton in the ^1H NMR spectra of A and B confirmed the presence of an oxo group at C-3 in C and D. This evidence led to the identification of C as olean-12-en-3,11-dione and D as urs-12-en-3,11-dione. Oxidation of 3-hydroxyolean-12-en-11-one and 3-hydroxyurs-12-en-11-one with Jones' reagent gave C and D, respectively.

Compounds A, C and D have previously been synthesized [5], but this is the first report of their occurrence in nature. Compound B, 3β-hydroxyurs-12-en-11-one (neoilexonol) has been previously isolated (as its acetate) from the barks of *Ilex goshiensis* and *I. buergeri* (Aquifoliaceae) [6].

The distribution of the terpenoids in the oleoresin, bark, and timber is listed in Table 1.

EXPERIMENTAL

General. All mps are uncorr. IR spectra were recorded for nujol mulls. ^1H NMR spectra were determined in $CDCl_3$ at 60 MHz with TMS as int. stand. MS were determined at 70 eV. UV spectra were in 95% EtOH and optical rotations were determined for *ca* 1% solns in $CHCl_3$ at 21°. GLC was carried out with FID on a 2 m × 4 mm stainless steel column of 5% dinonyl phthalate on 60–100 mesh, acid washed Embacel; flow rate of N_2 30 ml/min. Identification of compounds was made by co-injection and TLC, mmp, IR, ^1H NMR and MS comparisons with authentic specimens.

Isolation. The timber, bark and oleoresin of *Canarium zeylanicum* were obtained from the Royal Botanical Gardens, Peradeniya, Sri Lanka. After the preliminary defatting of the powdered bark (10 kg) and timber (10 kg) with petrol (60–70°), extractives were obtained successively with hot C_6H_6 and hot MeOH (Soxhlet). The C_6H_6 extract of the bark gave, on concn, a pale yellow gum (15 g); the timber gave 9 g of a dark brown gum. Steam distillation of the C_6H_6 extracts of the bark and timber gave respectively 6 and 4 g of the steam-volatile fraction. Steam distillation of the oleoresin (50 g) yielded a steam-volatile fraction as an oil (12 g).

GLC analysis of the steam-volatile fractions of the timber, bark and oleoresin. GLC analysis of the steam-volatile fractions of the oleoresin timber and bark indicated the presence of α-pinene (R_{rel} 0.88), α-phellandrene (R_{rel} 2.00), β-phellandrene (R_{rel} 3.75), and limonene (R_{rel} 4.13) at 110°; terpineol (R_{rel} 12.00) and carvone (R_{rel} 14.13) and two unidentified terpenes (R_{rel} 3.55 and 22.25) at 140°.

Terpenes of the non-steam-volatile fraction of the oleoresin. The residue left after steam distillation of the oleoresin was extracted with Et_2O and dried. Evapn of the Et_2O yielded a pale yellow gum (21 g) which was partitioned into acidic (1 g) and neutral (19 g) fractions.

Acidic fraction canaric acid. The acid fraction (1.0 g) on chromatography on a column of Si gel and repeated PLC of one of the fractions yielded canaric acid as a white amorphous solid (10 g), mp 213–215°, (lit. [7] 215–216°).

Chromatography of the neutral fraction. The pale yellow neutral mixture (19 g) was chromatographed on Si gel (700 g). Elution of the column with petrol (40–50°) gave the monoterpenes and the sesquiterpenes as a mixture (3 g).

Elemene. Elution of the column with petrol (60–70°) afforded elemene as a colourless oil (35 mg). Gradient elution

Table 1. Terpenoid components identified in the oleoresin, bark and timber of *Canarium zeylanicum*

	Oleoresin	Bark	Timber		Oleoresin	Bark	Timber
α-Pinene	***	—	**	β-Amyrin (olean-12-en-3β-ol)	*	*	*
α-Phellandrene	***	***	***	α-Amyrenone (urs-12-en-3-one)	*	—	—
β-Phellandrene	**	**	**	β-Amyrenone (olean-12-en-3-one)	*	—	—
Limonene	*	—	—	3β-Hydroxyolean-12-en-11-one	*	*	—
Terpineol	*	*	—	3β-Hydroxyurs-12-en-11-one	*	*	—
Carvone	**	—	—	Olean-12-en-3,11-dione	*	*	—
				Urs-12-en-3,11-dione	*	*	—
Elemene	*	*	—	Taraxerol	—	*	—
Elemol	*	*	—	Canaric acid	*	—	—
α-Amyrin (urs-12-en-3β-ol)	***	***	***	Sitosterol	**	**	**

*** Most abundant, ** major components, * minor components, — not detected.

of the column with petrol–C_6H_6 gave fractions 1 (650 mg) and 2 (200 mg) as oils and fraction 3 (6.90 g) as a solid. Elution with $CHCl_3$–C_6H_6 (9:1) gave fraction 4 (480 mg) as a gum while fraction 5 (1.8 g) was obtained as an oil when eluted with C_6H_6–$CHCl_3$ (2:3).

Products from fraction 1: *urs-12-en-3-one and olean-12-en-3-one.* Fraction 1 (650 mg) was rechromatographed over Si gel. Elution with petrol–C_6H_6 (4:1) gave the amyrenones as a mixture (390 mg). Repeated PLC ($CHCl_3$) afforded urs-12-en-3-one (180 mg), mp 126–127° (lower R_f) and olean-12-en-3-one (40 mg), mp 176–178° (higher R_f) identical with samples obtained by the oxidation of α-amyrin and β-amyrin respectively with Jones' reagent.

Products from fraction 5: *elemol and sitosterol.* Fraction 5 (1.8 g) was rechromatographed over Si gel followed by repeated PLC to give elemol (56 mg) (higher R_f), mp 53° and sitosterol (900 mg) (lower R_f), mp 136–137°.

Products from fraction 3: *α-amyrin and 12-chloroolean-12-en-3β-ol.* The gas mixture generated by warming fuming HNO_3 (15 ml) and NaCl (5 g) was passed into a soln of fraction 3 (3.45 g) in Py (75 ml) at 0° until a yellow-brown colour persisted (12 min). The mixture was poured into H_2O and the usual work-up afforded a brown solid. Analytical TLC showed two major bands. The band with the higher R_f afforded α-amyrin (2.9 g). The compound of lower R_f crystallized from C_6H_6 as 12-chloroolean-12-en-3β-ol, pale yellow needles (32 mg), mp 160°. (Found: C, 78.0; H, 10.2; Cl, 7.2. $C_{30}H_{49}ClO$ requires: C, 78.3; H, 10.7; Cl, 7.7%). IR ν_{max}^{Nujol} cm^{-1}: 3280, 1625, 1460, 1378, 1192, 1095, 1030, 995, 900, 858, 700. ^1H NMR ($CDCl_3$): δ 0.83 (*s*, Me), 0.86 (*s*, Me), 0.97 (*s*, Me), 0.99 (*s*, Me), 1.03 (*s*, Me), 3.25 (*bt*, C-3). MS *m/e* (rel. int.): 562 (M^+, 8), 460 (25), 281 (9), 255 (11), 253 (20), 254 (33), 252 (100), 237 (10), 217 (31), 208 (45), 207 (30), 190 (50), 168 (6), 154 (6).

Products from fraction 2. Fraction 2 (200 mg) was subjected to multiple-development PLC ($CHCl_3$) allowing the ilsolation of one band containing the two major components. Repeated PLC in $CHCl_3$ and finally in C_6H_6–EtOAc (4:1) gave two bands C and D, C with the higher R_f value.

Compound C: *olean-12-en-3,11-dione.* Band C gave compound C, olean-12-en-3,11-dione (22 mg), mp 234–235°, $[\alpha]_D$ +132° (lit. [5] mp 237–238°, $[\alpha]_D^{30}$ +141°). (Found: C, 82.1; H, 10.7. $C_{30}H_{46}O_2$ requires: C, 82.2; H, 10.5%) Found: M^+ 438.3502, *m/e* 273.2231, 232.1825, 135.0809. $C_{30}H_{46}O_2$, $C_{19}H_{29}O$, $C_{16}H_{24}O$ and $C_9H_{11}O$ require: M^+ 438.3497, *m/e* 273.2218, 232.1827 and 135.0809, respectively). IR ν_{max}^{Nujol} cm^{-1}: 1702, 1640, 1610. UV λ_{max}^{MeOH} nm: 252 (ε 11 200). MS *m/e* (rel. int.): 438 (M^+, 36), 273 (85), 232 (90), 135 (100).

Compound D: *urs-12-en-3,11-dione.* Band D gave urs-12-en-3,11-dione as colourless needles (36 mg), mp 198–199°, $[\alpha]_D$ +136° (lit. [5] mp 201–202°, $[\alpha]_D^{30}$ +143°). (Found: C, 82.2; H, 10.8. $C_{30}H_{46}O_2$ requires: C, 82.2; H, 10.5%). (Found: M^+ 438.3531, *m/e* 273.2245, 232.1825, 135.0809. $C_{30}H_{46}O_2$, $C_{19}H_{29}O$, $C_{16}H_{24}O$, $C_9H_{11}O$ require: M^+ 438.3497, *m/e* 273.2218, 232.1827 and 135.0809, respectively). IR ν_{max}^{Nujol} cm^{-1}: 1702, 1646, 1610. UV λ_{max}^{MeOH} nm: 252 (ε 10 550). MS *m/e* (rel. int.): 438 (M^+, 34%), 273 (100), 232 (56), 135 (71).

Products from fraction 4. Multiple development PLC (480 mg) gave two major bands, band 1, containing the compounds A and B, and band 2, containing the compounds B and E. Repeated PLC (C_6H_6–EtOAc, 4:1) of band 1 gave compounds A (higher R_f value) and B.

Compound A: *3β-hydroxyolean-12-en-11-one.* Crystallization of A (2×) from MeOH gave 3β-hydroxyolean-12-en-11-one as feathery needles (20 mg), mp 229–232°, $[\alpha]_D$ + 100.5° (lit. [5] mp 231–233°, $[\alpha]_D^{30}$ + 103°). (Found: C, 81.6; H, 10.8. $C_{30}H_{48}O_2$ requires: C, 81.8; H, 10.9%). (Found: M^+ 440.3663, *m/e* 273.2199, 232.1825, 135.0809. $C_{30}H_{48}O_2$, $C_{19}H_{29}O$, $C_{16}H_{24}O$, $C_9H_{11}O$ require: M^+ 440.3654, *m/e* 273.2218, 232.1827 and 135.0809, respectively). UV λ_{max}^{MeOH} nm: 252 (ε 10 800). IR ν_{max}^{Nujol} cm^{-1}: 3300, 1659, 1610. MS *m/e* (rel. int.): 440 (M^+, 0.12), 273 (53), 232 (71), 135 (100).

Compound B: *3β-hydroxyurs-12-en-11-one.* Recrystallization of B from MeOH allowed the isolation of 3β-hydroxyurs-12-en-11-one as colourless needles (32 mg), mp 206–208°, $[\alpha]_D$ + 96° (lit. [5] mp 207.5–208.5°, $[\alpha]_D^{30}$ + 100°). (Found: C, 81.8; H, 11.0. $C_{30}H_{48}O_2$ requires: C, 81.8; H, 10.9%). (Found: M^+ 440.3634, *m/e* 273.2234, 232.1843, 135.0826. $C_{30}H_{48}O_2$, $C_{19}H_{29}O$, $C_{16}H_{24}O$, $C_9H_{11}O$ require: M^+ 440.3654, 273.2218, 232.1827 and 135.0809, respectively). UV λ_{max}^{MeOH} nm: 252 (ε 10 650); IR ν_{max}^{Nujol} cm^{-1}: 3400, 1655, 1610. MS *m/e* (rel. int.): 440 (M^+, 22) [illegible] (100), 232 (53), 135 (44).

Acetylation of A. Acetylation of a with Ac_2O in Py at room temp. afforded 3β-acetoxyolean-12-en-11-one as white needles (16 mg), mp 269–272°, $[\alpha]_D$ + 98° (lit. [5] mp 268–269°, $[\alpha]_D^{30}$ + 102°). (Found: M^+ 482.3762, *m/e* 273.2211, 232.1821 and 135.0809. $C_{32}H_{50}O_3$, $C_{19}H_{29}O$, $C_{16}H_{24}O$ and $C_9H_{11}O$ require: M^+ 482.3759, *m/e* 273.2218, 232.1827 and 135.0809, respectively). IR ν_{max}^{Nujol} cm^{-1}: 1728, 1655, 1610. UV λ_{max}^{MeOH} nm: 252 (ε 11 650). MS *m/e* (rel. int.): 482 (M^+ 8), 466 (54), 273 (76), 232 (97), 135 (100).

Acetylation of B. B (20 mg) when acetylated as before afforded a solid which crystallized from MeOH–$CHCl_3$ to give 3β-acetoxy-urs-12-en-11-one as shiny flakes (12 mg), mp 286–288°, $[\alpha]_D$ + 101° (lit. [5] mp 289–290°, $[\alpha]_D^{30}$ + 89°). (Found: M^+ 482.3781, *m/e* 273.2231, 232.1831 and 135.0801. $C_{32}H_{50}O_3$, $C_{19}H_{29}O$ and $C_9H_{11}O$ require: M^+ 482.3759, *m/e* 273.2218, 232.1827 and 135.0809, respectively). IR ν_{max}^{Nujol} cm^{-1}: 1730, 1628, 1610. UV λ_{max}^{MeOH} nm: 252 (ε 11 900). MS: *m/e* 482 (M^+, 8), 273 (100), 232 (66), 135 (88).

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