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DISTRIBUTION OF SOME TRITERPENES AND PHENOLIC COMPOUNDS IN THE EXTRACTIVES OF ENDEMIC DIPTEROCARPACEAE SPECIES OF SRI LANKA*

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Key Word Index—Dipterocarpaceae; triterpenes; hexamethylcoruleoellagic acid; pentamethylflavellagic acid; tetramethylellagic acid; chrysophanol; scopoletin.

Abstract—Extractives of bark and/or timber of 11 species belonging to the genera *Cotylelobium*, *Hopea*, *Shorea*, *Vateria* and *Vatica* yielded a fatty-acid ester, a sitosteryl ester, β-amyrin acetate, β-amyrin, dipterocarpol, ursolic acetate, lupeol, sitosterol, ursolic acid, betulinic acid, hexamethyl-coruleoellagic acid, tetramethylellagic acid, chrysophanol and scopoletin. The distribution of these compounds in 18 other species was examined by TLC screening.

INTRODUCTION

Endemic Dipterocarp flora of Sri Lanka is represented by seven genera and 44 species [1]. They are the most abundant tree species in the lowland wet-zone forests [2] and are important for their durable timber, edible fruits and useful resins.

Out of the 44 endemic species, chemical work has been reported on the resins of five *Shorea* species [3] and three *Dipterocarpus* species [4]. Besides chemical studies on the bark and/or timber extractives of three *Dipterocarpus* species [5, 6], two *Shorea* (*Donna*) species [7] and eight *Stemonoporus* species [8] have also been reported so far. In the present study, bark and/or timber extractives of the 11 species were

Table 1. Triterpenes and phenolic compounds identified in Dipterocarpaceae species

		Sitosterol	Betulinic acid	Ursolic acid	β-Amyrinacetate	β-amyrin	Dipterocarpol	Fatty-acid esters	Chrysophanol	Hexamethylcoruleoellagic acid	Tetramethylellagic acid	Scopoletin	Sitosteryl ester	Ursolic acetate	Lupeol
Cotylelobium scabriusculum (THw.) Brandis	T		0.017	0.014	0.063	0.004		0.046				.001		0.026	
Hopea cordifolia (Thw.) Trimen	B	0.032		0.078		0.003	0.015	0.025							0.021
	T	0.018	0.015	0.011		0.029	0.007								0.052
H. jucunda var. *modesta* Thw.	T	0.051	0.016	0.018		0.003									0.007
Shorea dyeri Thw.	T	0.069	0.030	0.026	0.043		0.069		0.002				0.020		
S. lysophylla Thw.	T	0.051			0.034			0.050	0.001	0.001		0.006			
*S. stipularis*Thw.	B	0.071				0.001	0.002						0.016		
	T	0.058				0.023	0.001		0.001				0.080		
S. affinis (Thw.) Ashton	T	0.072	0.010	0.019	0.056	0.015			0.001						
S. affinis (Thw.) Ashton	T	0.018	0.075	0.011	0.024	0.036		0.013							
S. magistophylla Ashton	B	0.018		0.002		0.073	0.002		0.002	0.002		0.001	0.013		
Vateria copalifera (Retz.) Ashton	B	0.033			0.053	0.004	0.020								
Vatica affinis Thw.	T	0.074	0.030		0.047	0.013				0.001	0.001	0.010		0.020	

B = bark; T = timber; %yield on dry wt. basis.

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Table 2. TLC screening of Dipterocarpaceae

	Asiatic acid	Ursolic acid	Betulinic acid	Scopoletin	Betulin
R_f	0.10	0.13	0.16	0.21	0.32
TLC colour	Purple	Purple	Purple	UV fluorescent	Purple
Dipterocarpus glandulosus Thw.	+	+	+		+
D. hispidus Thw.		+	+		+
D. insignis Thw.		+	+		+
D. zeylanicus Thw.	+	+	+		+
Hopea brevipetiolaris (Thw.) Ashton		+	+		+
H. jucunda var. *modesta* Thw.		+	+		+
Shorea congestiflora (Thw.) Ashton		+	+		
S. oblongifolia Thw.		+	+		
S. ovalifolia (Thw.) Ashton		+	+		
S. trapezifolia (Thw.) Ashton		+	+	+	
S. zeylanica (Thw.) Ashton		+	+		
Stemonoporus affinis Thw.		+	+	+	
S. canaliculatus Thw.		+	+		
S. cordifolus (Thw.) Ashton		+	+		
S. lancifolius (Thw.) Ashton		+	+		
S. oblongifolius Thw.	+	+	+	+	
S. petiolaris Thw.		+	+	+	
S. reticulatus Thw.		+	+	+	

All timber extractives

separated by chromatography and the constituents identified. Eighteen other species were examined for the presence of phenolic and several other compounds by TLC screening.

RESULTS AND DISCUSSION

The presence of a non-crystalline polymeric substance in most of the extracts examined complicated the isolation and purification of terpenes. The polymer, which could be partially precipitated, was found to be different from the 'β-resene' which has been reported from the resins of these plants [9] in that the latter is a carbonyl compound, readily recognizable by the IR spectrum.

The distribution of the 14 compounds identified among the species examined is given in Table 1. The TLC screening results are given in Table 2. It is noticeable that in all the species examined here, and earlier, the bark and timber constituents are almost identical, but it was found that the timber extractives were free from tarry matter and, hence, they were selected for analysis.

Except for dipterocarpol, no other compounds belonging to the dammarane series which have been previously isolated from resins of *Dipterocarpus* [4] were identified in the extractives examined. Similarly, in the majority of the extractives examined, the amount of the acidic constituents was small compared to the resins on which work was reported.

Out of the triterpenes isolated, β-amyrin, dipterocarpol, betulinic acid and ursolic acid had a wide distribution while lupeol was isolated only from *Hopea cordifolia* and *H. jucunda*. The absence of lupeol in other genera may be due to the presence of an oxidative enzyme system specific to the conversion of lupeol to betulinic acid and lupeol could be considered as a marker for the genus *Hopea*. This is the first report of lupeol in the family.

The anthraquinone chrysophanol was isolated only from the timber extractives of *Shorea* species and can be considered as a marker of the genus *Shorea*. Bate-Smith describes a classification of *Shorea* timber according to their colour [10], which could be due to the presence of this quinone.

The co-occurrence of the methyl ethers of ellagic species for triterpenes and phenolic compounds

Hexamethylcoruleoellagic acid	Sitosterol	Tetramethylellagic acid	β-Amyrin/dipterocarpol/lupeol		β-Amyrin acetate	Chrysophanol	Fatty-acid ester
0.41	0.45	0.60	0.65		0.90	0.96	0.98
UV fluorescent	Pink-green/Purple/green	UV fluorescent	(a) Brown-pink (purple) (b) Pink-brown		Yellow-brown	Red	Brown
			(a)	(b)			
	+			+			
	+			+	+		
	+			+	+		
	+			+			
	+			+	+		
	+			+	+		
+	+	+	+		+	+	+
+	+	+	+		+		+
+	+	+	+		+		+
	+		+		+		+
+	+	+	+		+	+	+
+	+		+				
	+		+		+		
	+		+		+		
	+		+		+		
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+	+	+	+				
+	+	+	+				

acid, mono- and dihydrox-ellagic acids together with the isocoumarin, scopoletin, which is considered to be a phytoalexin, in the genera *Shorea*, *Vatica* and *Stemonoporous* is of biogenetic significance.

No marked chemical difference was observed between constituents present in the extractives of the species belonging to the subsections *Shoreae* and *Doonae*. However, it was reported earlier [8] that examination of the available data for the resins of the species of these two subsections gave different chemotaxonomic markers [7] in keeping with their earlier distinction as two genera. Therefore, these two genera need further detailed study if the chemical evidence is to provide more definitive information.

EXPERIMENTAL

All plant materials were collected from the Kanneliya Rain Forest in Sri Lanka. All dried, powdered bark and timber were extracted with boiling petrol (60–80°), C_6H_6 and MeOH or rectified spirit to give petrol, C_6H_6 and MeOH or rectified-spirit extracts. These extracts were separated by CC on Si gel (Merck) followed by PLC on Si gel, when necessary. All compounds isolated, except the sitosteryl palmitate and the octacosanol ester, were identified by mmp, co-TLC and IR comparison with authentic samples. For TLC screening, dried, powdered bark or timber parts (15 g) were refluxed with $CHCl_3$ for 6 hr and the concd extracts were chromatographed on 0.25 mm Si gel (Merck type G) plates (8 × 20 cm) using $CHCl_3$–MeOH (19:1) as the eluent. The plates were examined under UV light and then sprayed with ceric sulphate reagent and heated at 110°. The spots which appeared were identified by colour and R_f comparison with authentic samples.

Cotylelobium scabriusculum timber (3.1 kg) gave petrol extract (12 g, 0.4%) and hot rectified-spirit extract (588 g, 19.0%).

Hopea cordifolian bark (3.6 kg) gave petrol extract (26 g, 0.72%) and C_6H_6 extract (18 g, 0.5%). Timber (3.8 kg) gave petrol extract (28 g, 0.77%) and C_6H_6 extract (17 g, 0.47%).

H. jucunda timber (4.2 kg) gave petrol extract (2.5 g, 0.06%) and MeOH extract (107 g, 2.55%).

Shorea affinis timber (3.37 kg) gave petrol extract (17 g, 0.5%) and MeOH extract (117 g, 1.3%).

S. cordifolia timber (4.3 kg) gave petrol extract (11.0 g, 0.23%.

S. dyeri bark (4.3 kg) gave petrol extract (11.0 g, 0.26%).

S. lysophylla timber (6.05 kg) gave petrol extract (19 g, 0.3%) and hot MeOH extract (275 g, 4.6%).

S. magistophylla bark (3.75 kg) gave petrol extract (11.0 g, 0.3%).

S. stipularis bark (3.5 kg) gave petrol extract (10.0 g, 0.3%)and MeOH extract (225.0 g, 6.5%). Timber (2.2 kg) gave petrol extract (3.0 g, 0.09%) and MeOH extract (43.0 g, 1.4%).

Vateria copallifera bark (3.0 kg) gave petrol extract (16.0 g, 0.33%) and MeOH extract (270 g, 19%).

Vatica affinis bark (4.0 kg) and timber (2.9 kg) gave petrol extract (18 g, 0.45%) and (134g, 0.32%), respectively.

Octacosanol ester of hexacosanoic acid. Petrol or C_6H_6 extracts of most of the species when separated on a Si gel column gave an ester on elution with petrol. The ester had mp 78°, R_f 0.4 (C_6H_6–petrol, 1:9), IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}. 2900, 1745, 1440, 1340, 1110; MS *m/e* (rel.int.): 788 (M^+ 28), 760 (35), 736 (30), 708 (6), 678 (2), 454 (4), 437 (5), 426 (40), 396 (100), 368 (70), 125 (85), 96 (76), 84 (78). $C_{54}H_{107}O_2$ requires M, 788.4. Hydrolysis of the ester (0.1 g) gave hexacosanoic acid, mp 88° (lit. [11a] 88–89°), and octacosanol, mp 83° (lit. [12] 83.2°). Both were found to be identical with authentic samples.

Chrysophanol (*1,8-dihydroxy-3-methylanthraquinone*). Petrol or C_6H_6 extracts of the timber of all the *Shorea* species examined, when separated on a Si gel column, gave chrysophanol on elution with petrol. Red crystals from EtOH, mp 196° (lit. [11b] mp 196°). Mmp, IR and TLC identical with an authentic sample.

Sitosteryl palmitate. Elution of the Si gel column with C_6H_6–petrol (1:19) gave a white solid. White shiny plates from EtOH, mp 89° (lit. [11c] 89°), IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 2900, 1745, 1600, 1375, 1240, 1165 and 715; MS *m/e* (rel. int.): 652 (M^+ 20), 551 (25), 538 (11), 524 (10), 510 (31), 426 (35), 409 (8), 398 (24), 379 (20), 350 (22), 175 (14), 129 (68), 124 (30), 101 (72), 98 (100). Hydrolysis of the ester (0.15 g) gave sitosterol (0.06 g), mp 137° (from EtOH) (lit. [11c] mp 137°) and palmitic acid (0.005 g), mp 62° (lit. [11d] 62.6°) which were found to be identical with authentic samples.

β-Amyrin acetate. Elution of the Si gel column with C_6H_6–petrol (1:9) gave β-Amyrin acetate, mp 240°, $[\alpha]_D^{26}+$ 78° ($CHCl_3$) (lit. [13] mp 241°, $[\alpha]_0$(+80°). β-Amyrin acetate was found in all but one *Hopea* species.

β-Amyrin and dipterocarpol. Elution with C_6H_6 in most of the cases gave pure β-amyrin, mp 198°, $[\alpha]_D^{27}+85°$ ($CHCl_3$) (lit. [13] 200° $[\alpha]_D^{26}+88°$) and/or pure dipterocarpol, mp 135°, $[\alpha]_D^{27}+66°$ ($CHCl_3$) (lit. [14] 134–136°, $[\alpha]_D^{27}+66°$). In some cases, the two compounds were obtained as a mixture and were separated by PLC.

Lupeol. Petrol or C_6H_6 extracts of the *Hopea* species examined on elution with C_6H_6–petrol (1:3) gave lupeol, mp 215°, $[\alpha]_D^{27}+29°$ ($CHCl_3$) (lit. [15] 215°, $[\alpha]_D^{27}+26.4°$). Identical with an authentic sample.

Sitosterol. Elution of the column with C_6H_6 gave sitosterol, mp 136–137°.

Betulinic acid. Elution with MeOH–$CHCl_3$ (1:46) gave betulinic acid as crystals, mp 309–310° (petrol), $[\alpha]_D^{27}+12°$ ($CHCl_3$) (lit. [16] 309–310°, $[\alpha]_D^{26}+8°$), identical with an authentic sample.

Ursolic acid. Further elution with MeOH–$CHCl_3$ (1:19) gave ursolic acid, mp 280° (petrol), $[\alpha]_D^{27}+62°$ (Py) (lit. [17] 280°, $[\alpha]_D^{27}+65.4°$), identical with an authentic sample.

Hexamethylcoruleoellagic acid, pentamethylflavellagic acid, tetramethylellagic acid and scopoletin. Petrol or C_6H_6 extracts of some species examined on a Si gel column gave mixtures of compounds which gave bright blue fluorescence under UV light. These mixtures were separated by PLC on Si gel. The least polar fraction gave hexamethylcoruleoellagic acid, mp 229° (lit. [7] 229°). The next polar fraction gave pentamethylflavellagic acid, mp 245° (lit. [7] 243°). The next fraction gave tetramethylellagic acid, mp 300° (lit. [7] 300°) and the most polar fraction gave scopoletin, mp 204° (lit. [7] 204°). All four compounds were shown to be identical with authentic samples.

Ursolic acetate. Petrol extracts of *Cotylelobium scabriusculum* and *Vatica affinis* when chromatographed gave, with C_6H_6 petrol (3:17), ursolic acetate, mp 289–290° (EtOH), $[\alpha]_D^{27}+$ 63° ($CHCl_3$) (lit. [11e] mp 289–290°, $[\alpha]_D^{26}+53°$). It was identical with an authentic sample.

Asiatic acid. The Na_2CO_3-soluble fraction of the petrol extract of the bark of *Shorea magistophylla* on chromatography gave asiatic acid with MeOH–$CHCl_3$ (1:19), mp 301° (petrol), $[\alpha]_D^{27}+52.2°$ (Py) (lit [18] 300–305°, $[\alpha]_D^{26}+53°$). It was identical with an authentic sample.

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