separated distinct species. Voucher specimens of the samples previously investigated in this laboratory [2,3] have been redetermined and found to be of *C. gonecha*. The determination of the lichen used in this investigation and the redeterminations were done by Mr. H. Østhagen Botanisk Museum, Oslo.

Previous investigations. C. gonecha: Bellidiflorin, squamatic acid, usnic acid, rhodocladonic acid [4]. *S. tomentosum*: Atranorin, lobaric acid, stictic acid, [4, p, 522], bourgeanic acid [5].

Present investigation. Brassicasterol has been isolated from both the lichens examined and identified by mp. of the compound and its acetate and by MS. To ensure authenticity of origin, specimens of *C. gonecha* were picked individually, whilst *S. tomentosum* occurred in a pure or almost pure stand. Check on purity therefore mostly consisted in removal of plant debris.

EXPERIMENTAL

Stereocaulon tomentosum. For origin of the material, see [5]. The dried neutral fraction (15 g) was chromatographed on Al_2O_3 to give 10.3 g of dark coloured mass eluted with Et_2O–MeOH (9:1), which slowly deposited colourless crystals, isolated with MeOH (60 mg). Recrystallised from $CHCl_3$–MeOH, mp 146–147°, $[\alpha]_D$ −63° (*c*, 1.47, $CHCl_3$), no selective absorption above 220 nm; acetate, mp 151–152° from MeOH, $[\alpha]_D$ −65° (*c*, 1.00). Observed data were in agreement with those reported for brassicasterol and its acetate [6]; MS identical with published MS [7].

Cladonia gonecha. Origin of the material, see [2]. Usnic acid, $[\alpha]_D$ −480° (*c*, 1.11); rotation not reported in [4]. The neutral fraction (30.5 g) was chromatographed on Al_2O_3. Et_2O eluted 2.1 g, which was acetylated and crystallised to give brassicasterol acetate, mp 152–153° from petrol (bp 40–70°); $[\alpha]_D$ −63° (*c*, 1.50), undepressed on admixture with the acetate above; their MS were identical. More recently another sample from the same locality (1.0 kg) furnished 6.2 g of neutral material. Chromatographed on Si gel elution with C_6H_6–Et_2O (3:1) afforded a late, viscous fraction (153 mg) which gave 22 mg of crude brassicasterol, recrystallised from MeOH, mp 142–143°, no depression on admixture with brassicasterol; their MS were identical.

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Phytochemistry, 1976, Vol. 15, pp 1180–1182 Pergamon Press Printed in England.

POLYPHENOLS OF *EUCALYPTUS GLOBULUS*, *E. REGNANS* AND *E. DEGLUPTA*

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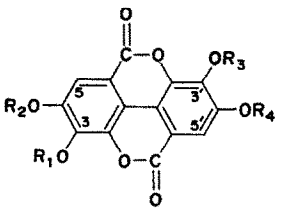
(*Revised received* 10 *February* 1976)

Key Word Index—*Eucalyptus*; *E. globulus*; *E. regnans*; *E. deglupta*; Myrtaceae; 3-mono-*O*-methyl ellagic acid-4′-rhamnoside, methylellagic and ellagic acids.

Eucalyptus deglupta Blume (Myrtaceae, "Kamarere"), *E. globulus* Labill ("Blue Gum") and *E. regnans* F. Muell ("Mountain Ash") are fast-growth species in appropriate climate zones.

The amount of MeOH solubles in both bark and wood from young trees of *E. globulus* and *E. regnans* and the composition of the wood extractives of both species is very similar (Table 1). Chromatographic examination of *E. globulus* bark extractives revealed at least 10 major components excluding polymers (Table 1). The ellagitannins D-1, D-2, D-6 and D-13, catechin and ellagic (**1a**) and gallic acids were identified. Proanthocyanins were present in only very small amounts. A new compound 3-*O*-methylellagic acid-4′-rhamnoside (**1b**), β-diketone(tritriacontane-16,18-dione), calcium oxalate and chlorogenic acid were isolated and identified from their physical and spectral data, and the preparation of derivatives. **1b** was hydrolyzed to 3-*O*-methylellagic acid and rhamnose in equimolar proportions. Methylation followed by hydrolysis yielded 3,4,3′-tri-*O*-methylellagic acid (**1c**), the NMR spectrum of **1b** indicated that it is an α-rhamnopyranoside.



(**1a**) R_1, R_2, R_3, R_4 = H
(**1b**) R_1 = Me, R_2, R_3 = H; R_4 = rhamnosyl
(**1c**) R_1, R_2, R_3 = Me; R_4 = H
(**1d**) R_1, R_3 = Me; R_2, R_4 = H
(**1e**) R_1, R_2, R_3, R_4 = Me

The most notable features of the extractives in the samples of *E. deglupta* collected in New Britain, New

Guinea and Fiji Islands are the relatively large portions of **1c**, **1d** and **1e**, the small number and amounts of other phenolics (mainly **1a** and gallic acid) and the trace amounts of ellagitannins. The methylated ellagic acids were isolated from *E. deglupta* wood along with gel substances A and B which were hydrolyzed to ferulic acid and 5 hydrolysis products, two of which were identified as behenyl and lignoceryl alcohols. GLC results indicated that the three unknown hydrolysis products were mainly long chain alcohols of chain length greater than n-C_{24}.

The amount (5.4%) of MeOH solubles in the heartwood of a large (80 cm diam), normally-coloured log of *E. deglupta* was higher than that (1.4–1.0%) in a smaller (26 cm diam) log which contained discoloured portions. The amount of total **1a**, **1c**, **1d** and **1e** is low so that most MeOH solubles are chromatographically unresolved or polymeric material. Extractives shown to be fungitoxic [1] are present in such small quantities, if at all, that their effect would probably be minimal. Wetwood shows an increase in MeOH solubles content affected by bacterial infection [2] and in this case also the content in discoloured outer heartwood (2.8%) was higher than that in the adjacent normal-coloured heartwood (1.4%).

Table 1. The amount and composition of the extracts of *E. regnans* and *E. globulus* bark and wood

	E. regnans		*E. globulus*	
	Bark	Wood	Bark	Wood
MeOH solubles* (%)	19.5	3.3	18.5	3.8
Compounds†				
Ellagic acid	+	*t*	+	+
D-1	+	*t*	+	+
D-2	++	++	+++	+++
D-3	−	+	−	−
D-4	+	*t*	*t*	*t*
D-6	+++	++	++	+
D-9	−	*t*	−	−
D-13	+++	+	++	*t*
3-Me-EA rhamnoside	−	*t*	+++	*t*
3-Me-EA glucoside (?)	++	*t*	+	*t*
Gallic acid + catechin	+	+	+	*t*
Gallocatechin	*t*	*t*	+	*t*
Chlorogenic acid	−	−	++	*t*
Chlorogenic acid isomer	−	−	*t*	*t*
Catechin polymer	+	*t*	+	*t*

* On dried samples of complete cross-sections. † Observed in 2-D chromatograms and visualized in UV light and with chromogenic sprays with the exception of β-diketone. +++ = large amount; ++ = medium; + = small; *t* = trace; − = not detected.

EXPERIMENTAL

Chromatographic examination on extractives from E. globulus *and* E. regnans *bark and wood*

E. globulus (3-yr old: 15 cm diam) was grown in Gippsland, Victoria, and *E. regnans* (4-yr old: 20 cm diam) was grown in Powelltown, Victoria. The bark and wood were freeze-dried, ground, extd. with MeOH and the extract examined chromatographically. General methods used have been previously reported [3,4]. The results were shown in Table 1.

Isolation and identification of extractives from E. globulus *bark.* The dried powdered bark (30 g) was extd successively with petrol (bp 40–60°), $CHCl_3$, Et_2O and MeOH to obtain 6.9, 0.9, 0.4 and 17.6% extractives respectively.

(i) *Tritriacontane-16,18-dione.* White, fine crystals were obtained after repeated crystallization of the yellow oily petrol solubles from MeOH, mp and mmp 66–67° (lit. [5] 67.5–68°). The compound was further characterized as the -dione (n-$C_{15}H_{31}COCH_2CO$ $C_{15}H_{31}$) with λ_{max}^{EtOH} (nm): 277 and $\lambda_{max}^{EtOH+NaOH}$ (nm): 297 nm. γ_{max}^{KBr} (cm^{-1}): 3440 *w*, 2950 *s*, 2910 *s*, 2840 *s*, 1635 *s*, 1470 *s*, 1460 *s*, 1415 *m*, 1405 *m*, 1365 *m*, 1340 *w*, 1320 *w*, 1305 *w*, 1280 *w*, 1260 *w*, 1240 *w*, 1220 *w*, 1200 *w*, 1180 *w*, 1135 *w*, 1095 *w*, 1010 *w*, 945 *w*, 925 *w*, 900 *w*, 850 *w*, 810 *w*, 785 *m*, 770 *m*, 750 *w*, 725 *m*, 715 *m*.

(ii) *3-O-methylellagic acid-4′-rhamnoside* (**1b**). The MeOH extract (5.3 g) was suspended in H_2O (500 ml), extd. with EtOAc in a liq.–liq. extractor. The extract (2.65 g) was crystallized from 70% EtOH (×7) to obtain white needles (75 mg), mp > 360° (Found: C, 54.5; H, 4.0; OMe, 6.4; $C_{21}H_{18}O_{12}$ requires: C, 54.5; H, 3.9; OMe, 6.7%). λ_{max}^{MeOH} (nm): 252, 350 (shoulder), 364; $\lambda_{max}^{MeOH+NaOAc}$ (nm): 256, 273, 353: $\lambda_{max}^{MeOH+NaOEt}$: 270, 292 (shoulder), 386; $\lambda_{max}^{MeOH+AlCl_3}$: 250, 349 (shoulder), 363. The fluorescence on paper was weak mauve in UV, green–yellow in UV–NH_4OH with R_f (×100) of 53 in BAW, 15 in 6% HOAc and 84 in HCl–HOAc–H_2O (3:30:10). γ_{max}^{KBr} (cm^{-1}): 3420 *s*, 2960 *w*, 2840 *w*, 1730 *s*, 1600 *s*, 1570 *m*, 1490 *s*, 1430 *m*, 1345 *s*, 1285 *m*, 1240 *m*, 1205 *m*, 1100 *s*, 1055 *s*, 995 *w*, 965 *m*, 915 *m*, 895 *w*, 835 *w*, 810 *w*, 800 *w*, 755 *m*, 690 *w*, 665 *w*, 600 *w*, 570 *w*, 530 *w*. NMRδ (DMSO-d_6): 7.65(*s*); 7.42 (*s*); 4.00 (*s*); 5.41 (*d*, J = 2); 1.105(*d*, J = 5). **1b** was hydrolyzed with 4% H_2SO_4 in 70% EtOH (1 hr) under reflux to give the aglycone (33.5 mg: theory 34.0 mg), mp > 360° (Found: C, 56.6; H, 2.8; OMe, 9.6. $C_{15}H_8O_8$ requires: C, 57.0; H, 2.5; OMe, 9.8%), acetate (Ac_2O, C_6H_5N, room temp.), mp 310–312° (Found: C, 57.3; H, 3.3; OMe, 6.7; COMe, 27.7. $C_{21}H_{14}O_{11}$ requires: C, 57.0; H, 3.2; OMe, 7.0; COMe, 29.2%). The aq. filtrate from the hydrolysis was passed through Amberlite IR-45 (–OH form) and examined with PC. The sugar was identical with authentic rhamnose. **1b** acetate (Ac_2O, C_6H_5N, room temp.), mp 135–136° (Found: C, 55.0; H, 4.1; OMe, 4.7; COMe, 29.9. $C_{31}H_{28}O_{17}$ requires: C, 55.4; H, 4.2; OMe, 4.6; COMe, 32.0%). **1b** was methylated (CH_2N_2, room temp.) to give the tri-*O*-methyl rhamnoside, mp 246–7°. R_f in BAW was 69 and in 6% HOAc 33. λ_{max}^{MeOH} (nm): 248, 288 (shoulder), 350 (shoulder), 367. γ_{max}^{KBr} (cm^{-1}): 3420 *s*, 2930 *w*, 2850 *w*, 1740 *s*, 1605 *s*, 1565 *w*, 1510 *w*, 1480 *s*, 1460 *m*, 1445 *m*, 1435 *m*, 1400 *s*, 1350 *s*, 1315 *s*, 1285 *w*, 1250 *s*, 1200 *m*, 1165 *m*, 1150 *m*, 1110 *s*, 1085 *s*, 1055 *s*, 990 *s*, 955 *s*, 905 *m*, 860 *w*, 835 *w*, 810 *w*, 770 *w*, 755 *m*, 740 *w*, 675 *w*. It was hydrolyzed to **1c** and rhamnose.

Identification of other polyphenols. Main components of the Et_2O extract (0.133 g) were gallic acid and catechin (identified by PC) and the EtOAc extract of MeOH solubles contained chlorogenic acid (purified by thick PC and identified by UV and PC). The aq. fraction remaining after the MeOH extract had been extd. with EtOAc contained **1a**, D-1, D-2, D-6 and D-13 (identified by co-chromatography with authentic compounds [4]).

Isolation of methylated ellagic acids. A log (80 cm diam) of *E. deglupta*, collected near Balima River in New Britain, was peeled into veneers (5 mm thick) and dried heartwood strips were ground and 2.2 kg extd. with cold MeOH. The extract (5.4% solids on wood basis) was conc., the ppt. refluxed with MeOH (1 hr), recentrifuged and the residue mixed with 5% Na_2CO_3. Insolubles were crystallized from DMF to give **1e**, mp 355° (decomp), the Na_2CO_3 solubles were acidified, the precipitate crystallized from DMF to give **1a** mp > 360°. Supernatant liquors obtained by centrifuging were dried, and after the addition of H_2O extd. with Et_2O in a liq.–liq. extractor (30 hr). During extraction, the froth which appeared at the aq. Et_2O boundary was collected. The pale yellow powder (0.9 g) from the Et_2O extract was chromatographed on a Si gel column (2 × 40 cm) with $CHCl_3$ and EtOAc–$CHCl_3$–HCOOH (2:10:1). Three fractions were repeatedly crystallized from DMF to remove a gel substance and finally, **1c** (6.2 mg),

1d (3.3 mg) and **1e** (15.4 mg) were obtained (identified by mp, mmp, TLC, UV and IR [6].

Wax alcohol esters of ferulic acid (Gel substances A and B). The froth obtained during liq.–liq. extraction was washed successively with Et_2O, 5% $NaHCO_3$ and H_2O and crystallized from MeOH to give gel substance A (10 mg). mp 95–105°. γ_{max}^{KBr} (cm^{-1}): 3430 *s*, 2920 *s*, 2850 *s*, 1710 *s*, 1630 *m*, 1600 *m*, 1585 *w*, 1510 *m*, 1465 *s*, 1435 *m*, 1380 *m*, 1310 *m*, 1270 *m*, 1170 *s*, 1120 *m*, 1030 *w*, 975 *w*, 845 *w*, 810 *w*, 720 *m*. λ_{max}^{MeOH} (nm): 296, 325. λ_{min}^{MeOH} (nm): 265. Gel substance B (mp 75–85°) isolated during recrystallization of **1c–1e** had the same UV and IR spectra as above. Saponification of the gel substance (*N* ethanolic NaOH, N_2, refluxing 2 hr) gave ferulic acid (identified by UV, PC and TLC) and 5 alcohols, which were acetylated (Ac_2O, C_6H_5N, room temp.), gas chromatographed on a 3% SE-30 column and 2 peaks were identified as behenyl and lignoceryl alcohols. The composition of wax alcohols in gel substance A were 5.47 (behenyl), 27.37 (lignoceryl), 36.23 (unknown (i)), 18.03 (unknown (ii)) and 12.88% (unknown (iii)) and in gel substance B, 24.54, 28.88, 21.66, 8.66 and 16.24%, respectively.

Examination of discoloured cross sections of E. deglupta. Discs (26 cm dia) from *E. deglupta* (11 yr old) which contained dark areas in the heartwood, were taken from the Keravat plantation in New Britain. One cross-section was divided into 7 parts: (a) sapwood and heartwood into (b) outermost and discoloured, (c) outer, (d) middle, (e) inner, (f) innermost, (g) innermost and discoloured. The amount of methanol soluble material and total ellagic acids [7] in these portions were respectively: (1) 1.2; 0.03; (2) 2.8, 0.18; (3) 1.4, 0.12; (4) 1.6, 0.12; (5) 1.3, 0.11; (6) 1.0, 0.09; (7) 0.7, 0.8%.

Acknowledgements—We thank the Department of Forests, Papua, New Guinea and Australian Paper Manufacturers Ltd., for supply logs of *E. deglupta* and *E. globulus* respectively and Dr. J. A. Lamberton and Dr. J. W. Rowe for samples of β-diketone and lignoceryl and behenyl alcohols.

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Phytochemistry, 1976, Vol. 15, pp. 1182–1183. Pergamon Press. Printed in England.

LIQCOUMARIN, A NOVEL COUMARIN FROM *GLYCYRRHIZA GLABRA*

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(*Received* 17 *February* 1976)

Key Word Index—*Glycyrrhiza glabra*; Leguminosae; liqcoumarin; 6-acetyl-5-hydroxy-4-methylcoumarin.

Plant. Glycyrrhiza glabra L.; *Source*: Dr. S. C. Sankhyadhar, experimental garden of Govt. Ayurvedic College, Jammu, (India). *Uses*: [1]. *Present work.* We earlier reported the occurrence of 2-methylisoflavones and other polyphenols from indigenous *Glycyrrhiza glabra* roots [2]. In the present communication we report a novel 4-methylcoumarin, liqcoumarin.

The solvent-free EtOH extract of air dried roots (1.5 kg) was repeatedly extracted with Et_2O. The Et_2O-soluble fraction was chromatographed over silica gel. Elution with C_6H_6–EtOAc in different proportions gave various compounds earlier reported [2]. The C_6H_6–EtOAc (9:1) eluate on preparative TLC purification using benzene gave liqcoumarin (35 mg), mp 165–6°, $C_{12}H_{10}O_4$ (M^+ 218, found C, 66.03, H, 4.55; required C, 66.05, H, 4.62%). It gave green colour with EtOH–$FeCl_3$, had UV fluoresence and had ν_{max}^{KBr}: 1720, 1650 cm^{-1}; λ_{max}^{MeOH} 255, 265, 310; +$AlCl_3$ 245, 285, 320; *MS* 218 (M^+ 100%), 203 (100%), 190 (98%), 175 (77%), 147 (60%), 119 (64%), 91 (98%), 77 (62%). *NMR* (δ $CDCl_3$, TMS as internal standard): 2.48 (3H, *d*, *J* 1 Hz, $-CH_3$), 2.95 (3H, *s*, –COMe), 6.15 (1H, *bs*), 6.80 (1H, *d*, *J* 10 Hz), 7.65 (1H, *d*, *J* 10 Hz) and 13,43 (1H, *s*, –OH). The spectral data showed it to be a coumarin with a chelated hydroxyl, a *C*-methyl and a *C*-acetyl substituents. The low field doublet at δ 7.65 could be either due to 4-proton of the coumarin or due to an aromatic proton adjacent to the *C*-acetyl unit and also ortho coupled with another proton (δ 6.80). The signal at δ 2.48 due to *C*-methyl shows allylic coupling (*J* 1 Hz) and hence the methyl group seems to be at C4. The signal at δ 7.65 is thus more likely due to an aromatic proton ortho-coupled with another proton (δ 6.80; *J* 10 Hz). Moreover, these signals have values which are lower than those of coumarin protons at C_3 and C_4 positions. *A priori*, liqcoumarin could be either a 6-acetyl-5-hydroxy-4-methylcoumarin or 7-acetyl-8-hydroxy-4-methylcoumarin. Liqcoumarin is assigned the structure of the former compound since it has been found to be identical with the synthetic sample obtained by condensing resacetophenone and ethyl acetoacetate in the presence of $AlCl_3$ in nitrobenzene [3], mp 164–5° (mp, mmp, TLC, superimposable IR and NMR).

Liqcoumarin seems to be of novel type and is presum-