Assay for pyrazole. From 0·2 to 0·5 ml extract was dil to 1·0 ml with 65% C_2H_5OH; 1·0 ml H_2O, 1·0 ml 0·2% trisodium pentacyanoaminoferrate, 0·1 ml 10% $NaNO_2$, and 0·1 ml conc HOAc were added with mixing. The absorptivity was determined at 458 nm.

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ESTERIFIED STEROL GLUCOSIDE AND METHYLELLAGIC ACIDS FROM *ALEURITES FORDII* FRUITS

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Key Word Index—*Aleurites fordii*; Euphorbiaceae; 6′-*O*-acylsterolglucoside; stigmasterol; campesterol; ellagic acid; 3,3′,4-tri-*O*-methylellagic acid; 3.3′-di-*O*-methylellagic acid; methyl gallate.

Plant. Aleurites fordii Hemsl. *Source.* Okayama University campus and Handayama Botanical Garden. *Uses.* Tung oil has been used in paints, varnishes, etc. *Previous work.* On the toxic principle of fruits [1], on the amino acids of seeds [2], and on the sterols of tung oil [3,4].

Present work. Fresh fruits were separated into seeds and residual part (outer part), and chemical constituents of each part were examined.

Constituents of seeds. Powdered seeds were extracted with petrol. After removal of solvent, the residual oil was shaken vigorously with MeOH and decanted. This procedure was repeated several times. The combined MeOH solutions were evaporated and chromatographed over Si gel. Elution with $CHCl_3$ gave a crystalline *sterol*, mp 136° (EtOH), $[\alpha]_D$ −40° ($CHCl_3$). GLC on a column of 2% OV-17 on Chromosorb W showed it to be a mixture of sitosterol (84%), stigmasterol (11) and campesterol (5). The identities with authentic samples were confirmed on GC–MS. Elution with $CHCl_3$–Me_2CO (9:1) gave another substance positive to Liebermann–Burchard reaction, which was further purified by PLC on Si gel developing with $CHCl_3$–Me_2CO (3:1) to afford an *esterified sterol glucoside* (ESG) as a colourless syrup. It was homogeneous on TLC ($CHCl_3$–Me_2CO 3:1, R_f 0·11; C_6H_6–Me_2CO 3:2, R_f 0·33). $\nu_{max}^{CHCl_3}$: 3550, 3400, 1725, 1630(*sh*) cm^{-1}. Treatment with Ac_2O-pyridine gave triacetate, mp 126–128° (MeOH), $[\alpha]_D$ −20° ($CHCl_3$), analysed for $C_{57}H_{96}O_{10}$, ν_{max}^{KBr}: 1747, 1635, 1250 cm^{-1}. NMR (90 MHz, $CDCl_3$) δ 1·98, 2·00, 2·02 (3 OAc). ESG and its acetate were methanolysed to give mixed sterol glucosides, mp 283° (decomp.), and fatty acid methyl esters mixture which was shown by GLC (1% OV-1) to be composed by methyl palmitate (58%), methyl linolate (27), and methyl stearate (15). Acid hydrolysis of the sterol glucosides mixture yielded glucose (PC, TMSi–GLC) and mixed sterols, mp 136°, which was shown by GLC and GC–MS to comprise sitosterol (78%), stigmasterol (18) and campesterol (4). The location of the ester group in ESG was shown to be mainly at C-6′ by NMR (90 MHz, $CDCl_3$) spectrum; methylene signal of C-6′ overlapped with the anomeric proton signal at δ 4·31, and no downfield shift of C-6′ protons was shown by the acetate (δ 4·13, *m*). The major component of ESG is, therefore, 6′-acylglucoside of sitosterol. Elution of the column with $CHCl_3$–Me_2CO (7:3) gave mixed *sterol glucoside*, mp 293° (decomp.) (pyridine–EtOH), $[\alpha]_D$ −42° (pyridine), analysed for $C_{35}H_{60}O_6$; tetraacetate, mp 164–165° (MeOH), $[\alpha]_D$ −24° ($CHCl_3$), analysed for $C_{43}H_{68}O_{10}$. Acid hydrolysis yielded glucose (PC, TMSi–GLC) and sterol mixture, mp 138° (EtOH), which is shown by GC–MS to be composed by sitosterol (85%), stigmasterol (12) and campesterol (3).

Constituents of outer part. MeOH extract was diluted with H_2O and extracted with EtOAc. Concentration of the EtOAc layer gave *ellagic acid*, $C_{14}H_6O_8$, mp >360° (pyridine); tetraacetate $C_{22}H_{14}O_{12}$, mp 333° (decomp.) (Ac_2O). The identity with authentic specimen was confirmed (IR). The extract after removal of ellagic acid was chromatographed on Si gel. Elution with $CHCl_3$ gave *3,3',4-tri-O-methylellagic acid*, $C_{17}H_{12}O_8$, mp 292–294° (DMF) (lit. [5] 294–295°, [6] 283°), ν_{max}^{KBr} 3430, 1750, 1610 cm^{-1}, λ_{max}^{EtOH} 247, 285(*sh*), 356(*sh*), 370 nm; monoacetate, $C_{19}H_{14}O_9$, mp 247° (dioxane–MeOH) (lit. [6] 251°), ν_{max}^{KBr}: 1773, 1733, 1608 cm^{-1}, λ_{max}^{EtOH} 247, 287(*sh*), 340, 357 nm. The identity with synthetic sample [6] was confirmed (mmp and IR). Elution with $CHCl_3$–Me_2CO (95:5) gave *3,3'-di-O-methylellagic acid*, $C_{16}H_{10}O_8$, mp 323–325° (DMF) (lit. [6] 330–331°), ν_{max}^{KBr} 3235, 1725, 1610 cm^{-1}, ν_{max}^{EtOH} 245, 287(*sh*), 359(*sh*), 374 nm. Diacetate, $C_{20}H_{14}O_{10}$, mp 303° (dioxane–MeOH) (lit. [6] 304–305°), which was identified with the synthetic sample [6]. Acid fraction from $CHCl_3$–Me_2CO (8:2) eluate gave *methyl gallate*, $C_8H_8O_5$, mp 195–196° (Et_2O–*n*-hexane) (lit. [7] 195°); triacetate, $C_{14}H_{14}O_8$, mp 122–123° (MeOH) (lit. [7] 122°). NMR ($CDCl_3$) δ 2·29 (3 OAc), 3·90 (OMe), 7·78 (2H, arom. H). Identity of the acetate with synthetic sample was confirmed.

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DITERPENE ESTERS OF THE IRRITANT AND COCARCINOGENIC LATEX OF *EUPHORBIA LACTEA*

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Key Word Index—*Euphorbia lactea*; Euphorbiaceae; diterpene esters; 3,12-di-*O*-acetylingol 8-tigliate, 16-hydroxyingenol 3,5,16,20-tetraacetate; skin irritants; cocarcinogens.

The branches of *Euphorbia lactea* exude a milky, sticky sap, which is extremely caustic and internally irritant, emetic and purgative [1]. It causes dermatitis and severe irritation, e.g. to the mucous membranes [2]. Interestingly, skin irritant latexes of several species of *Euphorbia* exhibit cocarcinogenic activity on mouse skin [3,4], in a manner similar to seed oil of *Croton tiglium* L. (Euphorbiaceae) [5]. Their active priciples are esters of the polyfunctional tetracyclic diterpenes phorbol [4,5] and ingenol [4,6] and derivatives thereof. Also, esters of new macrocyclic diterpenes such as the lathyrols [4] and ingol [7,9] are present. Although they are inactive as irritants, their particular chemical structures suggest that they play a role in the biogenesis of the diterpene moiety of the active principles [4]. The isolation and chemical characterisation of a new ester of ingol and of a diterpene parent alcohol from the irritant fraction of the latex of *Euphorbia lactea* is now reported.

Table 1. Acetone extracts from latex of *E. lactea* of various collections around Kingston (Jamaica). Irritant activity in irritant units (IU) and irritant dose 50 (ID_{50}) according to [Ref. 5]

Methanolic latex preparation		Acetone	Irritant activity	
Batch no.	Volume (ml)	Extract (g)	IU (μg/ear)	ID_{50}* (μg/ear)
1	500	70	25	1·2
2	650	45	88	9·8
3	750	70	500	62·0

* Level of significance $\alpha = 0{\cdot}05$; s.d. δ: 1·3.

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