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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Table 2. Irritant and cocarcinogenic activities of the acetone extract of latex of *Euphorbia lactea* as compared to that of an acetone extract of latex of *Euphorbia ingens*, assays on the mouse ear and on the back skin of mice, respectively

			Cocarcinogenic activity†							
									Histologic diagnoses tumors in treated area	
Exp. no.	Acetone extract from latex of	Irritant* activity ID_{50} (μg/ear)	Single dose *p* (μg/app)	Tumor rate (tumor bear./surv.) after weeks 12	24	Average tumor yield (tumors/surv.) After weeks 12	24	Death rate at end of exp.‡ (%)	Total/ mice investigat. histol.	Malig. tumors in total§
178	*E. ingens*	0·74	5000	0/26	1/22	0/26	1/22	57	6/3	1 FISA
642	*E. lactea* batch No. 2	9·8	10000	0/27	6/27	0/27	7/27	4	18/8	1 PEC

* Standard assay [5] level of significance $\alpha = 0{\cdot}05$, s.d. δ 1·3.

† Standard assay [5]: start: 28 male: female (no. 172) and 28 female 1:1 (no. 642) NMRI mice, initiator: 0·1 μM 7,12-dimethylbenz[a]anthracene; promoter: dose *p* twice weekly, after 12 and 24 weeks 24 and 48 applications, respectively.

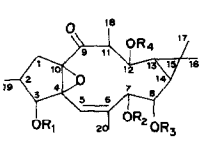
‡ No. 178 was ended at 48 weeks; no. 642 was ended at 28 weeks, because of lack of material.

§ FISA = Fibrosarcoma; PEC = squamous cell carcinoma.

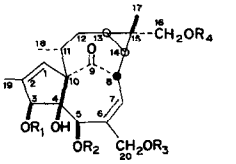
A methanolic preparation of fresh latex yielded an acetone extract containing the irritant and cocarcinogenic activities. The irritant activity of the extract appears to be subject to seasonal variation (Table 1). As compared to the acetone extract from latex of *E. ingens* E. Mey [7], the latex of *E. lactea* exhibits less irritant and similar weak cocarcinogenic activities (Table 2). At twice the single dose *p* and at a relatively short period of treatment with the extract (28 weeks = 56 doses *p*), benign tumours and one malignant squamous cell carcinoma were formed (see Table 2).

Fractionation of the acetone extract by column chromatography (see Experimental) yields a non-irritant diterpene fraction A, comprising 1·7% and an irritant diterpene fraction B, comprising 0·02% of the dry weight of the Me_2CO extract. Other fractions showed no increase of irritant activity after treatment with methanolic perchloric acid and thus do not contain cryptic [5] irritant or cocarcinogenic activity; they were not investigated further.

From fraction A by preparative TLC the non irritant diterpene ester **1b** is obtained. Spectrometric analyses of the compound and of the products obtained after both mild (**1c**) and rigorous (**1d**) base catalysed transesterification and after acetylation (**1e** from **1d**) show that it is the hitherto unknown 3,12-di-*O*-acetylingol 8-tigliate (**1b**). Various other esters of the macrocyclic diterpene ingol (**1a**) were isolated recently from *Euphorbia ingens* E. Mey. [8] and from the drug 'Euphorbium' obtained by air drying of latex of *Euphorbia resinifera* Berg. [9].



(1a) $R_1 - R_4 = H$
(1b) $R_1 = R_4 = Ac$; $R_2 = H$; $R_3 =$ Tiglyl
(1c) $R_1 = R_2 = H$; $R_3 =$ Tiglyl; $R_4 = Ac$
(1d) $R_1 - R_3 = H$; $R_4 = Ac$
(1e) $R_1 - R_4 = Ac$
(1f) $R_1 = R_2 = R_4 = Ac$; $R_3 =$ Tiglyl



(2a) $R_1 - R_4 = H$
(2b) $R_1 - R_4 = Ac$

The irritant fraction B is still contaminated. In an effort to isolate the irritant principle(s), various trials to remove the accompanying material by TLC and by column chromatography were only partially succesful with concomitant loss of most of the active material. Finally, by base catalysed transesterification of the entire fraction, followed by acetylation and TLC purification, the 3,5,16,20-tetraacetate **2b**, of the polyfunctional, tetracyclic diterpene 16-hydroxyingenol (**2a**) [10] was obtained. As compared to phorbol-12,13,20-triacetate (ID_{50} 3·8 μg/ear [4]) and to the preceding ester fraction B, **2b** is inactive as an irritant.

EXPERIMENTAL

Plant material. Methanolic latex preparations were kindly supplied by Dr. C. D. Adams, Department of Botany, Universtiy of the West Indies, Mona, Jamaica. For details of preparation see [11].

General methods. Chemical shifts in the NMR spectra refer to tetramethylsilane ($\delta = 0{\cdot}00$ ppm) as internal standard. TLC Merck Sil gel PF_{254} is used. Spots on TLC were visualized with vanillin–H_2SO_4 [11]. Columns were prepared with Merck Sil gel (0·05–0·20 mm) deactivated with 13% of H_2O.

Assays. Irritation units (IU) and the irritation doses 50 (ID_{50}) on the mouse ear were determined according to standard procedures [5]. During the fractionation, the ID_{50} was determined only for the fractions containing the main activity. To allow comparison with the inactive minor fractions, for the active fractions the IU's are given additionally. Cocarcinogenic activity was determined in the standard experiment on the back skin of mice [5] and expressed as tumor rate and average tumor yield at 12 and 24 weeks after initiation (see Table 2).

Acetone extracts. Methanolic latex preparation was filtered from the precipitate and residue remaining on the filter paper washed repeatedly with Me_2CO. Insoluble residue was discarded. The methanolic filtrate and the Me_2CO washings were combined and the solvents evaporated under red pres. After thorough drying the "acetone extract" is obtained as a pale yellowish amorphous powder. Irritant activities for Me_2CO extracts of various latex collections are summarized in Table 1. The assay for cocarcinogenic activity (Table 2) was performed with the Me_2CO extract of batch number 2.

Fractionation. 70 g (100% by wt) of Me_2CO extract of batch No. 3 (Table 1) was chromatographed on a Sil gel column and eluted with C_6H_6 (fraction 1; 48·6 g, 69·4%, IU: > 1000 μg/ear), followed by C_6H_6–EtOAc (1:1)(irritant fraction 2; 11·5 g, 16·4%, IU: 950 μg/ear, ID_{50} 16·6 μg/ear) and EtOAc (fraction 3; 2·8 g, 4%, IU: > 100 μg/ear). The highly polar fraction was eluted with MeOH (fraction 4; 6·9 g, 9·9%, insoluble in Me_2CO, no IU). The irritant fraction 2 was chromatographed on a further column eluted with Et_2O–petrol (1:1). After fraction 2a (6·3 g, 9%, IU: > 1200 μg/ear) fraction A (1·2 g, 1·7%, IU: > 60 μg/ear) was obtained, followed by fraction 2c (2·45 g, 3·5%, IU: > 200 μg/ear and finally by fraction B containing irritant materials (150 mg, 0·02%, IU: 75 μg/ear, ID_{50}: 6·02 μg/ear).

Isolation of 3,12-*di*-O-*acetylingol* 8-*tigliate* (**1b**). After repeated preparative TLC of fraction A with $CHCl_3$–EtOAc (1:2), 195 mg of an amorphous substance was isolated from the upper zone. MS: *m/e* 532 (11·6), 472 (10·6), 433 (11·6), 412 (2·9), 372 (4·7), 330 (13·4), 245 (15·5), 221 (11·6), 203 (13·5), 83 (100), 43 (37·8). IR (KBr): 3460, 1730, 1645 cm^{-1}. NMR ($CDCl_3$, 60 MHz): H–C = C (tigliate) 6·8–6·9 (*m*), 5-H 5·8 (*s*, broad), 3-H 5·23 (*d*), 12-H 4·88 (*dd*), 8-H 4·58 (*d*, broad), 7-H 4·28 (*s*, broad, 2 Me –CO, 20-H_3 2·1 (*s*, broad), Me tigliate 1·88 (*s*, broad), 1·78 (*d*); 16-H_3, 17-H 1·0 (*s*), 0·88 (*s*).

Mild transesterification of (**1b**) *to yield* **1c** *and* **1d**. 75 mg **1b** was treated with NaOMe in MeOH overnight at room temp. After neutralization, TLC in CH_2Cl_2–MeOH (10:1) gave 2 zones R_f 0·49 and 0·16. The zone with R_f 0·49 gave, after further TLC, 50 mg of an amorphous substance. MS: *m/e*, 490 (28·7), 472 (6·06), 430 (13), 391 (12·1), 330 (10·6), 245 (13·6), 83 (100), 43 (41·5) IR (KBr): 3490, 1730, 1700, 1645, 1385, 1370 cm^{-1}. Mass and IR spectra are identical with those of 12-O-acetylingol 8-tigliate (**1c**) [7].

Preparative TLC of the zone, R_f 0·16, yielded 30 mg of amorphous substance. MS: *m/e*, 408 (17·5), 390 (1·08), 361 (1·08), 348 (15·3), 330 (28·5), 301 (8·7), 245 (29·6) 221 (17·5), 210 (42·9), 43 (100) IR (KBr): 3440, 1720, 1705, 1380, 1370 cm^{-1}. IR and mass spectra, are identical with those of 12-O-acetylingol (**1d**) [7].

Transesterification of (**1b**) *and acetylation of the product.* 45 mg **1b** were dissolved in 2 ml MeOH, 2 ml 10^{-1}m $NaOCH_3$ added and the soln kept at room temp. After 20 hr the reaction was worked up and the product acetylated.

The acetate (16 mg) was purified by TLC. MS: *m/e* 534 (29·5), 475 (6·6), 432 (4·7), 414 (1·04), 372 (4·7), 346 (7·6), 330 (5·8), 312 (6·6), 43 (100) IR (KBr): 3445, 1730, 1705, 1365 cm^{-1}. These data are in agreement with those of authentic ingol-3,7,8,12-tetraacetate (**1e**) [7].

Direct acetylation of **1b** with Ac_2O–pyridine gave the triacetate (**1f**) MS: *m/e* 574 (3·2), 515 (4·8), 475 (11·2), 432 (5·5), 314 (10·6), 83 (100), IR (KBr): 3440, 1735, 1700, 1645, 1370, 1365 cm^{-1}. The data are identical with those of 3,7,12-tri-O-acetylingol 8-tigliate [8].

Isolation of 16-*hydroxy-ingenol*-3,5,16,20-*tetraacetate.* 150 mg of the irritant fraction B was dissolved in 200 ml of 10^{-3} NaOMe in MeOH and left for 48 hr at room temp. The product was amorphous (5 mg, 0·007%, IU: > 100 μg/ear, ID_{50} > 100 μg/ear), R_f 0·14, was obtained. MS: *m/e* 532 (12·5), 472 (31·7), 412 (75), 370 (62·5), 310 (100), 292 (87·5), 43 (37·7) IR (KBr): 3440, 1740, 1440, 1370, 1230 cm^{-1} NMR ($CDCl_3$, 90 MHz, PFT): 7-H 6·19 (*d*), 1-H 6·05 (*s*), 5-H 5·37 (*s*), 3-H 4·93 (*s*), 20-H_2 4·35 + 0·2 TAB = 15 Hz), 16-H_2 4·16, 8-H 4·18 (*d*), 19-H_3 1·74; 17-H_3 1·12, 18-H_3 0·99; ME–CO 2·2, 2·17; 2·1, 1·98. Mass NMR and IR spectra are identical with those of 16-hydroxy ingenol-3,5,16,20-tetraacetate (**2b**) [10].

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