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8-METHOXYINGOL ESTERS FROM THE LATEX OF *EUPHORBIA HERMENTIANA**

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Key Word Index—*Euphorbia hermentiana*; Euphorbiaceae; macrocyclic diterpenes; methoxylated ingol esters; 18-hydroxyingol ester.

Abstract—Two new ingol esters, 3,12-*O*-diacetyl-7-*O*-benzoyl-8-methoxyingol and 3,12-*O*-diacetyl-7-*O*-tigloyl-8-methoxyingol, were isolated from an acetone-soluble fraction of the latex of *Euphorbia hermentiana*. These macrocyclic diterpenes were identified on the basis of their spectroscopic parameters, as well as those of their hydrolytic derivatives. A third methoxylated ingol ester and a fourth new compound were tentatively identified as 3,12-*O*-diacetyl-7-*O*-angeloyl-8-methoxyingol and 3,7,12-*O*-triacetyl-8-*O*-benzoyl-18-hydroxyingol, respectively. All four new compounds, and three further ingol ester constituents of known structure, were found to be easily separable using a newly developed isolation procedure involving droplet counter-current chromatography.

INTRODUCTION

The ornamental succulent plant, *Euphorbia hermentiana* Lem., sold in certain plant stores in the United States, is indigenous to southwest Africa [1]. The poisonous nature of the latex of this species as a contact skin-irritant for humans has been documented [1, 2]. We have previously reported the isolation and identification of five ingenane derivatives from a dermatitis-producing acetone extract of *E. hermentiana* latex [3]. Reported here are the separation and characterization of further components of this extract, comprising six esters of the macrocyclic diterpene ingol and one ester of 18-hydroxyingol.

RESULTS AND DISCUSSION

Compounds **5**–**7** exhibited spectral properties (UV, IR, ^{1}H NMR, EIMS) that suggested them to be esters of the macrocyclic diterpene ingol [4–8]. On hydrolysis with 0.5 M methanolic potassium hydroxide and acetylation, the known compound 3,7,8,12-*O*-tetraacetylingol (**8**) was produced in each case. Compounds **5** and **6** have both been identified in mixtures of ingol esters obtained from the latices of *Euphorbia ingens* E. Mey [4] and *E. kamerunica* Pax [7], although the present study is the first to report the individual spectral characterization of these compounds as pure isolates. The identification of **5** as 3,7,12-*O*-triacetyl-8-*O*-benzoylingol was confirmed by stepwise hydrolysis experiments using methanolic potassium hydroxide, to afford, in turn, 8-*O*-benzoyl-12-*O*-acetylingol (**14**) and 12-*O*-acetylingol (**15**). Similarly, **6** was confirmed as 3,7,12-*O*-triacetyl-8-*O*-tigloylingol after the generation of 7,12-*O*-diacetyl-8-*O*-tigloylingol (**16**), 8-*O*-tigloyl-12-*O*-acetylingol (**17**) and 12-*O*-acetylingol (**15**), respectively. The ingol triester **7** exhibited closely comparable IR, ^{1}H NMR and EIMS spectral data to those described for 3,12-*O*-diacetyl-7-*O*-tigloylingol when isolated as a constituent of *E. kamerunica* latex, and the known products 8-*O*-tigloyl-12-*O*-acetylingol (**17**) and 12-*O*-acetylingol (**15**) were produced on alkaline hydrolysis of **7** [8].

Compounds **1**–**3** were identified as methoxylated ingol triesters on the basis of ^{1}H NMR and EIMS observations on the isolates themselves, as well as their hydrolytic products. A methyl ether substituent was suspected as being attached to C-8 in **1**, rather than either an ester function or a free hydroxy group at this position, because of the observation of a three-proton sharp singlet that resonated at δ3.35 in its ^{1}H NMR spectrum [9]. Also, no characteristic [5–8] double-doublet attributable to a C-8 methine proton attached to either an ester function (δ4.55–4.85) or a free hydroxy group (δ3.45–3.65) was apparent. The C-8 methine proton signal was not in fact observed in the ^{1}H NMR spectrum of **1**, although it would be expected to occur some 0.2 ppm further upfield than if adjacent to a free hydroxy group [9], in which region it was apparently obscured by other signals.

Further analysis of the spectroscopic data of **1** indicated the presence in the molecule of one benzoate and two acetate substituents affixed to C-3, C-7 and C-12. Among the ingol 7-esters, the C-7 methine proton has been found to resonate as a characteristic small doublet ($J_{7,8} < 2$ Hz) or a broad singlet [5–8]. In the case of **1**, this signal was observed in the ^{1}H NMR spectrum as far downfield as δ5.54, on account of the deshielding effect exerted by the neighboring *gem*-oriented benzoate substituent. Thus the two remaining acetate groups in **1** were tentatively placed at C-3 and C-12. Confirmation of these ester group position assignments was obtained as a result of selective hydrolysis experiments using 0.1 M methanolic potassium hydroxide, in which 7-*O*-benzoyl-8-methoxy-12-

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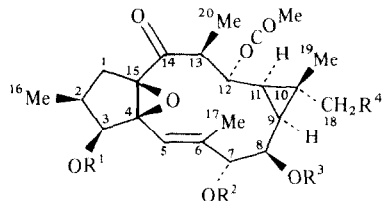
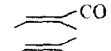
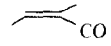
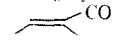
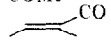
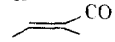
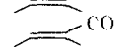


	R^1	R^2	R^3	R^4
1	COMe	COC_6H_5	Me	H
2	COMe		Me	H
3	COMe		Me	H
4	COMe	COMe	COC_6H_5	OH
5	COMe	COMe	COC_6H_5	H
6	COMe	COMe		H
7	COMe		H	H
8	COMe	COMe	COMe	H
9	COMe	COMe	COMe	OCOMe
10	COMe	COMe	COC_6H_5	OCOMe
11	H	COC_6H_5	Me	H
12	H	H	Me	H
13	H		Me	H
14	H	H	COC_6H_5	H
15	H	H	H	H
16	H	COMe		H
17	H	H		H

O-acetylingol (**11**) and 8-methoxy-12-*O*-acetylingol (**12**) were sequentially obtained. Acetylation of **11** resulted in the regeneration of **1**, thereby demonstrating that no ester group *trans*-esterification had occurred during the alkaline hydrolysis of **1**, as encountered during similar experiments on ingenol-3-esters [3, 10] and as shown in compound **7** [7].

The molecular formula of **1** was determined as $C_{32}H_{40}O_9$ by high-resolution mass spectrometry. Owing to the resistance of the C-8 methoxy group to alkaline hydrolysis, it was not possible to prove the stereochemistry of **1** by conversion to 3,7,8,12-*O*-tetraacetylingol (**8**), a compound recently elucidated by X-ray crystallography [11]. However, the small coupling constant ($J_{7,8}$ = 0.9 Hz) for the C-7 proton observed in the ^{1}H NMR spectrum of **1** was found to be comparable in magnitude to coupling constants exhibited by the analogous methine proton in compounds **5**–**7**, thus suggesting that the relative stereochemistry of the C-7 and C-8 substituents in **1** was the same as that of compounds **5**–**7**. Since other ^{1}H NMR chemical shifts and coupling constants were also comparable among these four compounds, the structure of **1** was established as 3,12-*O*-diacetyl-7-*O*-benzoyl-8-methoxyingol.

Compound **2** was assigned the molecular formula $C_{30}H_{42}O_9$ by high-resolution mass spectrometry, and analysis of its ^{1}H NMR and EIMS data demonstrated the presence of a methoxy group (δ3.32), and one tiglate (δ6.87, 1.85, 1.76) and two acetate (δ2.07) residues. Partial hydrolysis experiments with 0.1 M potassium hydroxide in methanol afforded 7-*O*-tigloyl-8-methoxy-12-*O*-acetylingol (**13**) and 8-methoxy-12-*O*-acetylingol (**12**). Accordingly, the structure of **2** was determined as 3,12-*O*-diacetyl-7-*O*-tigloyl-8-methoxyingol.

The ^{1}H NMR spectrum of **3**, an isomer of **2**, indicated the presence of one methoxy group (δ3.31), two acetates (δ2.07) and one angelate substituent (δ6.09, 1.93, 1.90). The EIMS fragment peak at *m/z* 515, corresponding to a loss of 31 amu from the molecular ion, provided further evidence for the presence of a methoxy group in **3**. Owing to the paucity of material isolated, it was not possible to prove the relative positions of the ether and acyl substituents in the molecule of **3**. However, on biogenetic grounds, and on the basis of the close correlation of the ^{1}H NMR spectra of compounds **2** and **3**, the latter compound was tentatively proposed as 3,12-*O*-diacetyl-7-*O*-angeloyl-8-methoxyingol.

In its ^{1}H NMR spectrum, compound **4** ($C_{33}H_{40}O_{11}$) was found to exhibit many similarities to that of **5**. However, an additional two-proton AB quartet, centered at δ3.31 and 3.65, as well as a D_2O-exchangeable singlet at δ1.84, was detected in the spectrum of the former compound. In contrast, the ^{1}H NMR resonances at δ0.84 and 1.16 assigned to the C-18 and C-19 tertiary methyl groups in **5**, were replaced by a more downfield methyl group singlet at δ1.25 in the spectrum of **4**. It was therefore apparent from its IR, ^{1}H NMR and EIMS data that the diterpene moiety of **4** contained four methyl groups and one hydroxymethyl group, and was esterified with one benzoate and three acetate substituents.

Close inspection of the ^{1}H NMR spectra of compounds **4** and **5** suggested that the only feasible positions for the insertion of an additional hydroxy group in **4** were the methyl groups at C-18 and C-19. Thus, the almost superimposable nature of the signals accorded to the C-16, C-17 and C-20 methyl groups in the two compounds appeared to preclude the possibility of the insertion of a hydroxy group in **4** at one of these three positions. Reference to molecular models indicated that the environment of a C-19 hydroxymethyl group would be somewhat more sterically crowded than that of a C-18 hydroxymethyl group, and less likely to account for the facile conversion of the hydrolysed product of **4** to the penta-acetate **9** that was produced in this study. The positioning in **4** of a primary alcoholic group at C-18 is also favored on biogenetic grounds [12]. It is pertinent to point out that Adolf and Hecker [12] have noted the occurrence of 18-hydroxyingol esters in *E. unispina* and *E. marginata* latices, although the spectroscopic characterization of these compounds does not appear to have been reported to date.

Owing to the extremely small amount of compound **4** isolated, it was not possible to prove the ester substituents by selective hydrolysis. However, the observation of a more downfield C-8 methine proton chemical shift in the ^{1}H NMR spectrum of **10** (obtained from **4** by direct acetylation) compared with that in **9** (δ4.89 vs 4.42) strongly suggested a C-8 benzoate group in **4**, in direct comparison in this respect to **5**. Compound **4** was therefore tentatively identified as 3,7,12-*O*-triacetyl-8-*O*-benzoyl-18-hydroxyingol.

Ingol esters, which are macrocyclic diterpenes based on the parent hydrocarbon lathyrane, are of interest in being biogenetically related to the tumor-promoting compounds of the tigliane, dephnane and ingenane types [11, 12]. Esters of ingol, which have only been found to date as constituents of the genus *Euphorbia* [4–8, 12–15], may prove to be of considerable systematic value. The occurrence of 8-methoxylation in ingol esters, as exemplified by the *E. hermentiana* constituents **1**–**3**, is also of potential chemotaxonomic importance.

EXPERIMENTAL

Mps: uncorr.; $[\alpha]_D^{25}$: $CHCl_3$; UV: MeOH; FT-IR: film; ^{1}H NMR: 60 MHz, $CDCl_3$, using the FT mode, with TMS as int. standard; EIMS: *ca* 20 eV; droplet counter current chromatography (DCCC): Tokyo Rikakikai Model A instrument; prep. TLC: silica gel (0.25 mm), using 60% (w/v) H_2SO_4 as visualizing reagent, and Me_2CO to elute zones from plates, solvent systems: 1, hexane–C_6H_6–Et_2O–EtOAc (2:2:1:1); 2, cyclohexane–Et_2O–EtOAc (1:1:1); 3, $CHCl_3$–Et_2O (19:1); 4, $CHCl_3$–MeOH (19:1).

Plant material. Specimens of *Euphorbia hermentiana* Lem. were purchased from plant stores in the Chicago area, and identified by Dr. D. D. Soejarto [1, 2]. An appropriate voucher specimen was deposited in the herbarium of the Field Museum of Natural History, Chicago (sheet No. 1870845). Latex aliquots (*ca* 10 ml) were collected from the specimens at twice-weekly intervals over a period of 4 months, and dried at 40° under red. pres.

Isolation of macrocyclic diterpene esters. The stages of solvent partition and DCCC fractionation of an Me_2CO extract (6.5 g) of dried *E. hermentiana* latex (27 g) have been described by us previously [3].

Fractions 51–109 from the DCCC separation exhibited three major zones by TLC when solvent 1 was used for development. Purification of the least polar zone by prep. TLC in solvent 3 yielded 6.1 mg **1** (R_f 0.38), 1.2 mg **3** (R_f 0.37), 17.3 mg **5** (R_f 0.42) and 2.5 mg **6** (R_f 0.41). The zone of intermediate polarity afforded 8.8 mg **2** on sequential prep. TLC in solvents 1 (R_f 0.42) and 3 (R_f 0.33), respectively. Fractions 110–174 from the DCCC apparatus were subjected to prep. TLC in solvents 1 and 3 to produce 1.5 mg **4** (R_f 0.25, 0.14, respectively) and 10.7 mg **7** (R_f 0.20, 0.08, respectively).

This isolation procedure was repeated on a further 56 g of dried latex to produce a further 15.1, 21.9, 0.3, 2.2, 32.2, 33.9 and 7.9 mg of compounds **1**–**7**.

Characterization of isolates **1**–**7**. 3,12-*O*-Diacetyl-7-*O*-benzoyl-8-methoxyingol (**1**, 17.4 mg, 0.026% w/w), exhibited the following data: resin; $[M]^+$ 568.2669, $C_{32}H_{40}O_9$ requires 568.2671; $[\alpha]_D^{25}$ −7.2° (*c* 0.08); UV λ_{max}^{MeOH} nm (log ε): 282 (2.88), 273 (2.93), 225 (4.23), 208 (4.32); IR ν_{max}^{film} cm^{-1}: 2956, 2920, 1741, 1736, 1732, 1716, 1450, 1370, 1250; ^{1}H NMR ($CDCl_3$): δ7.41–8.11 (5H, *m*, aromatic), 5.67 (1H, *s*, H-5), 5.54 (1H, *d*, *J* = 0.9 Hz, H-7), 5.17 (1H, *d*, *J* = 8.1 Hz, H-3), 4.92 (1H, *dd*, *J* = 10.0, 4.0 Hz, H-12), 3.35 (3H, *s*, –OC$\underline{H}_3$), 2.97 (1H, *m*, H-13), 2.82 (1H, *m*, H-1), 2.45 (1H, *m*, H-2), 2.12 (6H, *s*, 2 × –OCOC$\underline{H}_3$), 2.05 (3H, *s*, 3H-17), 1.14, 1.00 (6H, *s*, 3H-18, 3H-19), 1.07 (3H, *d*, *J* = 8.3 Hz, 3H-20), 0.93 (3H, *d*, *J* = 7.8 Hz, 3H-16); EIMS, *m/z* (rel. int.): 568 $[M]^+$ (90), 508 (3), 432 (1), 372 (2), 330 (2), 329 (3), 312 (6), 284 (3), 245 (6), 221 (8), 207 (9), 203 (8), 181 (5), 180 (5), 177 (8), 165 (7), 152 (14), 138 (12), 122 (9), 111 (40), 105 (100).

3,12-*O*-Diacetyl-7-*O*-tigloyl-8-methoxyingol (**2**, 30.7 mg, 0.037% w/w) exhibited the following data: mp 164–165° (recrystallized from Me_2CO); $[M]^+$ 546.2830, $C_{30}H_{42}O_9$ requires 546.2827; $[\alpha]_D^{25}$ +6.7° (*c* 0.08); UV λ_{max}^{MeOH} nm (log ε): 210 (4.26); IR ν_{max}^{film} cm^{-1}: 2920, 1715, 1700, 1435, 1360, 1240; ^{1}H NMR ($CDCl_3$): δ6.87 (1H, *m*, H-3′), 5.56 (1H, *s*, H-5), 5.32 (1H, *s* (*br*), H-7), 5.25 (1H, *d*, *J* = 8.4 Hz, H-3), 4.89 (1H, *dd*, *J* = 10.5, 3.8 Hz, H-12), 3.32 (3H, *s*, –OC$\underline{H}_3$), 2.95 (1H, *m*, H-13), 2.82 (1H, *m*, H-1), 2.46 (1H, *m*, H-2), 2.11, 2.07 (6H, *s*, 2 × –OCOC$\underline{H}_3$), 2.07 (3H, *s*, 3H-17), 1.85, 1.76 (6H, *m*, 3H-4′, 3H-5′), 1.11, 0.98 (6H, *s*, 3H-18, 3H-19), 1.05 (3H, *d*, *J* = 7.4 Hz, 3H-20), 0.93 (3H, *d*, *J* = 6.6 Hz, 3H-16); EIMS, *m/z* (rel. int.): 546 $[M]^+$ (74), 486 (2), 404 (5), 344 (5), 312 (5), 245 (4), 221 (6), 207 (8), 181 (5), 177 (4), 176 (4), 165 (4), 163 (5), 152 (9), 139 (5), 138 (9), 111 (26), 83 (100).

3,12-*O*-Diacetyl-7-*O*-angeloyl-8-methoxyingol (**3**, 1.5 mg, 0.0014% w/w) exhibited the following data: resin; $[M]^+$ 546.2826, $C_{30}H_{42}O_9$ requires 546.2827; $[\alpha]_D^{25}$ −6.5° (*c* 0.09); UV λ_{max}^{MeOH} nm (log ε): 213 (4.25); IR ν_{max}^{film} cm^{-1}: 2951, 2935, 2929, 1742, 1726, 1709, 1371, 1241, 1237; ^{1}H NMR ($CDCl_3$): δ6.04 (1H, *m*, H-3′), 5.55 (1H, *s*, H-5), 5.34 (1H, *d*, *J* = 2.2 Hz, H-7), 5.25 (1H, *d*, *J* = 8.6 Hz, H-3) 4.85 (1H, *m*, H-12), 3.31 (3H, *s*, –OC$\underline{H}_3$), 2.94 (1H, *m*, H-13), 2.81 (1H, *m*, H-1), 2.49 (1H, *m*, H-2), 2.11, 2.07 (6H, *s*, 2 × –OCOC$\underline{H}_3$), 2.07 (3H, *s*, 3H-17), 1.93, 1.90 (6H, *m*, 3H-4′, 3H-5′), 1.08, 0.97 (6H, *s*, 3H-18, 3H-19), 1.03 (3H, *d*, *J* = 7.2 Hz, 3H-20), 0.92 (3H, *d*, *J* = 6.2 Hz, 3H-16); EIMS, *m/z* (rel. int.): 546 $[M]^+$ (86), 515 (2), 486 (2), 404 (7), 344 (6), 329 (2), 312 (7), 262 (7), 245 (4), 221 (7), 207 (12), 181 (6), 177 (5), 176 (5), 165 (7), 163 (6), 152 (12), 139 (6), 138 (12), 111 (28), 83 (100).

3,7,12-*O*-Triacetyl-8-*O*-benzoyl-18-hydroxyingol (**4**, 3.7 mg, 0.0057% w/w) exhibited the following data: resin; $[M]^+$ 612.2571, $C_{33}H_{40}O_{11}$ requires 612.2570; $[\alpha]_D^{25}$ −45.1° (*c* 0.19); UV λ_{max}^{MeOH} nm (log ε): 282 (3.21), 275 (3.30), 233 (4.43), 209 (4.32); IR ν_{max}^{film} cm^{-1}: 3510, 2935, 1748, 1740, 1734, 1724, 1718, 1458, 1370, 1276, 1242, 1237, 1233, 1168, 1108, 1025; ^{1}H NMR ($CDCl_3$): δ7.40–8.08 (5H, *m*, aromatic), 5.64 (1H, *s*, H-5), 5.36 (1H, *d*, *J* = 8.1 Hz, H-3), 5.24 (1H, *d*, *J* = 1.7 Hz, H-7), 5.06 (1H, *m*, H-12), 4.90 (1H, *dd*, *J* = 10.2, 1.6 Hz, H-8), 3.65, 3.31 (2H, ABq, J_{AB} 12.1 Hz, 2H-18), 2.99 (1H, *m*, H-13), 2.84 (1H, *m*, H-1), 2.47 (1H, *m*, H-2), 2.15, 2.13, 2.12 (9H, *s*, 3 × –OCOC$\underline{H}_3$), 2.08 (3H, *s*, 3H-17), 1.84 (1H, *s*, 18-OH, exchangeable with D_2O), 1.25 (3H, *s*,

3H-19), 1.11 (3H, *d*, J = 7.3 Hz, 3H-20), 0.99 (3H, *d*, J = 7.2 Hz, 3H-16); EIMS, *m/z* (rel. int.): 612 $[M]^+$ (21), 554 (4), 491 (16), 490 (36), 448 (7), 430 (6), 388 (11), 371 (7), 370 (6), 328 (9), 311 (9), 310 (8), 294 (6), 293 (7), 282 (6), 245 (7), 207 (12), 181 (12), 165 (17), 151 (8), 136 (18), 135 (18), 123 (15), 122 (18), 105 (100).

3,7,12-*O*-Triacetyl-8-*O*-benzoylingol (**5**, 49.5 mg, 0.06% w/w) has previously been found to occur in mixtures of ingol esters isolated from the latices of *E. ingens* [4] and *E. kamerunica* [7]. The physical and spectroscopic data of **5**, which have not been reported previously, were: resin; $[\alpha]_D^{25}$ −62.9° (*c* 0.08); UV λ_{max}^{MeOH} nm (log ε): 282 (3.26), 274 (3.33), 232 (4.44), 210 (4.36); IR ν_{max}^{film} cm^{-1}: 3000, 2940, 1720, 1700, 1440, 1360, 1260; ^{1}H NMR ($CDCl_3$): δ 7.41–8.10 (5H, *m*, aromatic), 5.64 (1H, *s*, H-5) 5.38 (1H, *d*, J = 8.0 Hz, H-3), 5.21 (1H, *d*, J = 1.3 Hz, H-7), 4.94 (1H, *dd*, J = 10.0, 3.9 Hz, H-12), 4.85 (1H, *dd*, J = 11.4, 1.6 Hz, H-8), 3.00 (1H, *m*, H-13), 2.84 (1H, *m*, H-1), 2.46 (1H, *m*, H-2), 2.16, 2.12, 2.08 (9H, *s*, 3 × –OCOC<u>H</u>$_3$), 2.08 (3H, *s*, 3H-17), 1.16, 0.84 (6H, *s*, 3H-18, 3H-19), 1.10 (3H, *d*, J = 7.7 Hz, 3H-20), 0.99 (3H, *d*, J = 7.2 Hz, 3H-16); EIMS, *m/z* (rel. int.): 596 $[M]^+$ (71), 537 (7), 475 (13), 432 (7), 372 (9), 330 (8), 313 (11), 312 (14), 294 (11), 284 (11), 207 (13), 201 (13), 181 (13), 165 (12), 140 (13), 138 (20), 122 (23), 109 (15), 105 (100).

3,7,12-*O*-Triacetyl-8-*O*-tigloylingol (**6**, 10.4 mg, 0.013% w/w) is also known as a constituent of both *E. ingens* [4] and *E. kamerunica* [7] latices, but in both cases this compound was not isolated in a pure form. Compound **6** exhibited the following data: resin; $[\alpha]_D^{25}$ −41.9° (*c* 0.09); UV λ_{max}^{MeOH} nm (log ε): 218 (4.32); IR ν_{max}^{film} cm^{-1}: 3000, 2940, 1730, 1700, 1440, 1370, 1240, 1220; ^{1}H NMR ($CDCl_3$): δ 6.84 (1H, *m*, H-3′), 5.59 (1H, *s*, H-5), 5.35 (1H, *d*, J = 8.2 Hz, H-3), 5.10 (1H, *s* (*br*), H-7), 4.86 (1H, *dd*, J = 10.8, 4.3 Hz, H-12), 4.62 (1H, *dd*, J = 9.3, 1.5 Hz, H-8), 2.94 (1H, *m*, H-13), 2.82 (1H, *m*, H-1), 2.45 (1H, *m*, H-2), 2.16, 2.12, 2.08 (9H, *s*, 3 × –OCOC<u>H</u>$_3$), 2.08 (3H, *s*, 3H-17), 1.80 (6H, *m*, 3H-4′, 3H-5′), 1.11, 0.82 (6H, *s*, 3H-18, 3H-19), 1.06 (3H, *d*, J = 6.4 Hz, 3H-20), 0.98 (3H, *d*, J = 7.0 Hz, 3H-16); EIMS, *m/z* (rel. int.): 574 $[M]^+$ (30), 515 (4), 475 (4), 432 (5), 372 (4), 330 (5), 312 (6), 294 (5), 284 (5), 207 (6), 181 (5), 165 (6), 138 (9), 122 (11), 83 (100).

The resinous compound **7** (44.6 mg, 0.054% w/w) exhibited identical spectroscopic data (IR, ^{1}H NMR, MS) to 3,12-*O*-diacetyl-7-*O*-tigloylingol, a constituent of *E. kamerunica* latex [8]. Compound **7** also gave $[\alpha]_D^{25}$ −21.3° (*c* 0.05); UV λ_{max}^{MeOH} nm (log ε): 209 (4.13).

Complete hydrolysis and acetylation of compounds **4**–**7**. When 0.5–1.0 mg samples of compounds **5**–**7** were hydrolysed in 0.5 M KOH in MeOH for 30 min at 25°, and acetylated with C_5H_5N–Ac_2O (2:1, 0.5 ml) for 1 hr at 100°, the known compound 3,7,8,12-*O*-tetraacetylingol (**8**) was produced and identified (mp, EIMS, co-TLC), after work-up, by direct comparison with a sample of this compound obtained in earlier work on *E. hermentiana* latex [14].

Compound **4** (1.5 mg) was hydrolysed with 0.5 M KOH in MeOH for 1 hr at 25°, and acetylated as described for compounds **5**–**7**. Extraction into $CHCl_3$ and prep. TLC in solvent 2 (R_f 0.50) afforded 3,7,8,12,18-*O*-pentaacetyl-18-hydroxyingol (**9**, 1.2 mg): resin; $[\alpha]_D^{25}$ +16.1° (*c* 0.042); UV λ_{max}^{MeOH} nm (log ε): 209 (4.16); IR ν_{max}^{film} cm^{-1}: 2930, 1740, 1734, 1718, 1706, 1700, 1373, 1228, 1038, 1029; ^{1}H NMR ($CDCl_3$): δ 5.59 (1H, *s*, H-5), 5.23 (1H, *d*, J = 8.0 Hz, H-3), 5.06 (1H, *s* (*br*), H-7), 5.02 (1H, *m*, H-12), 4.42 (1H, *m*, H-8), 4.05, 3.89 (2H, AB_q, J_{AB} = 11.7 Hz, 2H-18), 2.98 (1H, *m*, H-13), 2.12, 2.08, 1.97, 1.85 (15H, *s*, 5 × –OCOC<u>H</u>$_3$), 2.08 (3H, *s*, 3H-17), 1.25 (3H, *s*, 3H-19), 1.02 (3H, *d*, J = 7.7 Hz, 3H-20), 0.96 (3H, *d*, J = 7.4 Hz, 3H-16); EIMS, *m/z* (rel. int.): $[M]^+$ missing, 491 $[M-101]^+$ (7), 448 (5), 433 (7), 430 (6), 391 (10), 373 (12), 349 (8), 331 (16), 313 (9), 289 (25), 271 (43), 264 (13), 233 (22), 222 (31), 207 (26), 165 (64), 147 (21), 125 (15), 43 (100).

Compound **4** (1.5 mg) was acetylated without prior hydrolysis using C_5H_5N–Ac_2O (2:1, 0.5 ml) for 1 hr at 100°. After extraction into $CHCl_3$ and purification by prep. TLC in solvent 1 (R_f 0.34), the product 3,7,12,18-*O*-tetraacetyl-8-*O*-benzoyl-18-hydroxyingol (**10**, 1.1 mg) was found to exhibit the following data: resin; $[\alpha]_D^{25}$ −68.4° (*c* 0.05); UV λ_{max}^{MeOH} nm (log ε): 282 (3.42), 275 (3.49), 233 (4.47), 209 (4.41); IR ν_{max}^{film} cm^{-1}: 2935, 1742, 1739, 1732, 1717, 1713, 1610, 1590, 1453, 1375, 1276, 1271, 1238, 1235; ^{1}H NMR ($CDCl_3$): δ 7.40–8.10 (5H, *m*, aromatic), 5.63 (1H, *s*, H-5), 5.35 (1H, *d*, J = 8.2 Hz, H-3), 5.22 (1H, *s* (*br*), H-7), 5.06 (1H, *m*, H-12), 4.89 (1H, *dd*, J = 7.5, 1.9 Hz, H-8), 4.25, 3.57 (2H, AB_q, J_{AB} = 11.7 Hz, 2H-18), 2.98 (1H, *m*, H-13), 2.83 (1H, *m*, H-1), 2.46 (1H, *m*, H-2), 2.13, 2.10 (15H, *s*, 4 × –OCOC<u>H</u>$_3$, 3H-17), 1.19 (3H, *s*, 3H-19), 1.11 (3H, *d*, J = 7.8 Hz, 3H-20), 0.99 (3H, *d*, J = 6.7 Hz, 3H-16); EIMS, *m/z* (rel. int.): 654 $[M]^+$ (73), 595 (16), 552 (11), 492 (11), 490 (16), 448 (9), 431 (8), 430 (6), 388 (10), 370 (15), 311 (13), 310 (11), 297 (13), 292 (12), 282 (18), 245 (12), 235 (19), 181 (17), 165 (18), 163 (18), 138 (17), 122 (17), 105 (100).

Partial hydrolysis of compound **1**. Compound **1** (2.5 mg) was hydrolysed with 0.1 M KOH in MeOH for 20 min at 25°. The hydrolysis product obtained, 7-*O*-benzoyl-8-methoxy-12-*O*-acetylingol (**11**, 1.4 mg), on purification by prep. TLC in solvent 1 (R_f 0.26) was characterized as follows: resin; $[\alpha]_D^{25}$ +4.9° (*c* 0.018); UV λ_{max}^{MeOH} nm (log ε): 282 (3.07), 274 (3.18), 230 (4.24), 210 (4.14); IR ν_{max}^{film} cm^{-1}: 3450, 2920, 1720, 1440, 1375, 1260, 1240; ^{1}H NMR ($CDCl_3$): δ 7.42–8.13 (5H, *m*, aromatic), 5.74 (1H, *s*, H-5), 5.64 (1H, *d*, J = 1.7 Hz, H-7), 4.89 (1H, *m*, H-12), 4.24 (1H, *d*, J = 8.2 Hz, H-3), 3.35 (3H, *s*, –OC<u>H</u>$_3$), 2.12 (6H, *s*, –OCOC<u>H</u>$_3$, 3H-17), 1.72 (1H, *s*, 3-OH, exchangeable with D_2O), 1.14, 1.00 (6H, *s*, 3H-18, 3H-19), 1.04 (3H, *d*, J = 6.0 Hz, 3H-20), 0.94 (3H, *d*, J = 6.7 Hz, 3H-16); EIMS, *m/z* (rel. int.): 526 $[M]^+$ (64), 466 (2), 404 (1), 372 (1), 312 (2), 245 (3), 233 (3), 165 (15), 152 (20), 138 (16), 122 (9), 111 (68), 105 (100). When compound **11** was acetylated and worked up as described for compound **4**, the product on purification was found to be identical (^{1}H NMR, TLC) to **1**, thus indicating that no ester group translocation had occurred during the alkaline hydrolysis of **1** to **11**.

Compound **1** was also hydrolysed for 1 hr with 0.1 M KOH at 25° to produce two reaction products. The less polar product was identified as compound **11** (^{1}H NMR, TLC), while the more polar product, 8-methoxy-12-*O*-acetylingol (**12**, 1.3 mg), on purification by prep. TLC in solvent 2 (R_f 0.09), was found to exhibit the following data: resin; $[\alpha]_D^{25}$ +46.5° (*c* 0.067); UV λ_{max}^{MeOH} nm (log ε): 208 (4.08); IR ν_{max}^{film} cm^{-1}: 3442, 2955, 2924, 1734, 1456, 1385, 1370, 1246; ^{1}H NMR ($CDCl_3$): δ 5.83 (1H, *s*, H-5), 4.85 (1H, *dd*, J = 10.8, 4.5 Hz, H-12), 4.36 (1H, *d*, J = 7.1 Hz, H-3), 4.35 (1H, *d*, J = 1.3 Hz, H-7), 3.35 (3H, *s*, –OC<u>H</u>$_3$), 2.94 (1H, *m*, H-13), 2.73 (1H, *m*, H-1), 2.44 (1H, *m*, H-2), 2.19 (1H, *s*, –OH, exchangeable with D_2O), 2.10 (3H, *s*, –OCOC<u>H</u>$_3$), 1.98 (3H, *s* (*br*), 3H-17), 1.07, 0.97 (6H, *s*, 3H-18, 3H-19), 1.03 (6H, *d*, J = 7.2 Hz, 3H-16, 3H-20); EIMS, *m/z* (rel. int.): 422 $[M]^+$ (32), 404 (18), 365 (5), 344 (5), 312 (4), 263 (7), 245 (13), 233 (8), 221 (11), 192 (18), 177 (21), 165 (45), 152 (50), 138 (95), 122 (77), 111 (100).

Partial hydrolysis of compound **2**. Compound **2** (3.0 mg) was hydrolysed with 0.1 M KOH in MeOH for 5 min at 25°. One hydrolysis product, 7-*O*-tigloyl-8-methoxy-12-*O*-acetylingol (**13**, 2.1 mg), was obtained after purification by prep. TLC in solvent 1 (R_f 0.23), and exhibited the following data: resin; $[\alpha]_D^{25}$ +3.7° (*c* 0.11); UV λ_{max}^{MeOH} nm (log ε) 218 (4.40); IR ν_{max}^{film} cm^{-1}: 3475, 2920, 1737, 1710, 1700, 1440, 1370, 1247; ^{1}H NMR ($CDCl_3$): δ 6.88 (1H, *m*, H-3′), 5.63 (1H, *s*, H-5), 5.45 (1H, *s* (*br*), H-7), 4.88 (1H, *dd*, J = 10.2, 4.0 Hz, H-12), 4.28 (1H, *d*, J = 8.0 Hz, H-3), 3.31 (3H, *s*, –OC<u>H</u>$_3$), 2.95 (1H, *m*, H-13), 2.82 (1H, *m*, H-1), 2.46 (1H, *m*, H-2), 2.11 (3H, *s*, –OCOC<u>H</u>$_3$), 2.06 (3H, *s*, 3H-17), 1.85, 1.74, (6H, *m*, 3H-4′, 3H-5′), 1.09, 0.98 (6H, *s*, 3H-18, 3H-19), 1.04 (6H, *d*, J = 7.0 Hz, 3H-17, 3H-16); EIMS, *m/z* (rel. int.): 504 $[M]^+$ (14), 444 (4), 386 (3), 345 (22), 329 (4), 312 (9), 301 (46), 299 (23), 284

(14), 256 (18), 245 (63), 183 (14), 165 (34), 163 (43), 151 (24), 139 (25), 125 (34), 111 (66), 99 (67), 97 (71), 83 (100). When **13** (1.2 mg) was acetylated and worked up as described for **4**, the resultant acetate was found to be identical (^{1}H NMR, TLC) to **2**.

When **2** (5.5 mg) was hydrolysed with 0.1 M KOH in MeOH for 1 hr at 25°, two hydrolysis products resulted, with the less polar compound being identified as compound **13** (^{1}H NMR, TLC). The more polar product (1.0 mg), after purification by prep. TLC in solvent 4 (R_f 0.25), was identified ($[\alpha]_D$, UV, IR, ^{1}H NMR, EIMS) as 8-methoxy-12-*O*-acetylingol (**12**).

Partial hydrolysis of compound **5**. Compound **5** (10 mg) was hydrolysed with 0.1 M KOH in MeOH for 15 min at 25°. Two hydrolysis products were isolated from the reaction mixture by prep. TLC in solvent 2. The less polar and major product, 8-*O*-benzoyl-12-*O*-acetylingol (**14**, 4.0 mg, R_f 0.29) exhibited the following data: resin; $[\alpha]_D^{25}$ −4.8° (*c* 0.14); UV λ_{max}^{MeOH} nm (log ε): 282 (3.22), 274 (3.26), 231 (4.39), 209 (4.32); IR ν_{max}^{film} cm^{-1}: 3450, 2930, 1725, 1698, 1693, 1275, 1256, 1252, 1247; ^{1}H NMR ($CDCl_3$): δ 7.42–8.11 (5H, *m*, aromatic), 5.92 (1H, *s*, H-5), 4.93 (1H, *dd*, *J* = 9.6, 4.0 Hz, H-12), 4.85 (1H, *dd*, *J* = 7.2, 1.8 Hz, H-8), 4.40 (1H, *s* (*br*), H-7), 4.36 (1H, *d*, *J* = 6.0 Hz, H-3), 2.96 (1H, *m*, H-13), 2.75 (1H, *m*, H-1), 2.40 (1H, *m*, H-2), 2.09 (6H, *s*, –OCOC$\underline{H}_3$, 3H-17), 1.13, 0.85 (6H, *s*, 3H-18, 3H-19), 1.07 (3H, *d*, *J* = 7.2 Hz, 3H-20), 1.06 (3H, *d*, *J* = 7.2 Hz, 3H-16); EIMS, *m/z* (rel. int.): 512 $[M]^+$ (29), 494 (10), 452 (6), 391 (12), 330 (11), 312 (5), 284 (6), 269 (6), 245 (11), 210 (12), 201 (21), 192 (25), 181 (19), 165 (44), 151 (17), 138 (72), 122 (91), 109 (30), 105 (100). When **14** was acetylated and worked up as described for **4**, the product was found to be identical (^{1}H NMR, TLC) to **5**.

The second and more polar product (1.0 mg, R_f 0.03) of the partial alkaline hydrolysis of **5**, $[\alpha]_D^{25}$ +40.2° (*c* 0.17); UV λ_{max}^{MeOH} nm (log ε): 210 (4.08): IR ν_{max}^{film} cm^{-1}: 3450, 3415, 2926, 1737, 1721, 1363, 1247, was identified as 12-*O*-acetylingol (**15**), on the basis of its closely comparable ^{1}H NMR and EIMS data to lit. data [5, 7].

Partial hydrolysis of compound **6**. Compound **6** was hydrolysed with 0.1 M KOH in MeOH for 5 min at 25°. The single reaction product, after purification by prep. TLC in solvent 2 (R_f 0.51), was identified as 7,12-*O*-diacetyl-8-O-tigloylingol (**16**, 1.1 mg). The resinous compound **16**, $[\alpha]_D^{25}$ −4.2° (*c* 0.08); UV λ_{max}^{MeOH} nm (log ε): 208 (4.22), exhibited closely comparable spectral data (IR, ^{1}H NMR, EIMS) to lit. values reported when obtained as a constituent of *E. lactea* latex [5].

When compound **6** (5.7 mg) was hydrolysed with 0.1 M KOH in MeOH for 30 min at 25°, two further known hydrolytic products were obtained, namely 8-*O*-tigloyl-12-*O*-acetylingol (**17**, R_f 0.26) ($[\alpha]_D^{25}$ +2.5°, *c* 0.12) and 12-*O*-acetylingol (**15**). These compounds were identified by comparison of spectroscopic data (IR, ^{1}H NMR, EIMS) obtained in the present investigation with lit. data [5, 7].

Partial hydrolysis of compound **7**. The known hydrolytic products [7], 8-*O*-tigloyl-12-*O*-acetylingol (**17**, 1.5 mg) and 12-*O*-acetylingol (**15**, 1.0 mg), were obtained after the hydrolysis of compound **7** (5.2 mg) with 0.1 M KOH in MeOH for 30 min at 25°.

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