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MACROCYCLIC DITERPENE ESTERS OF THE CYTOTOXIC FRACTION FROM *EUPHORBIA KAMERUNICA*

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Key Word Index—*Euphorbia kamerunica*; Euphorbiaceae; ingol esters; macrocyclic diterpenes.

Abstract—The ether-soluble resin of the latex of *Euphorbia kamerunica* was separated into a non-polar and a polar fraction by gradient elution column chromatography. From the non-polar fraction three macrocyclic diterpene esters were isolated by adsorption and partition preparative TLC methods. These compounds were identified as 3,7,8-triacetyl-ingol-12-tigliate, 3,7-diacetyl-ingol-12-tigliate and ingol-tetra-acetate by means of spectroscopic data, together with hydrolysis and partial synthesis.

INTRODUCTION

Plants of the genus *Euphorbia* produce ingenol and phorbol esters, and these compounds are well known for both their acute and chronic toxic effects including pro-inflammatory and tumour-promoting activities [1]. A microchemical screen of several sections of the genus [2] has shown that ingenol is the most commonly occurring diterpene of these species. The genus *Euphorbia* also produces macrocyclic diterpenes [3,4] and it has been suggested that these substances are the immediate biosynthetic precursors of the phorbol and ingenol derivatives [5]. The genus has been less well investigated for macrocyclic diterpenes because these substances do not possess the toxic activities of the tetracyclic class of compounds.

During routine testing of column fractions from *E. kamerunica* it was found that the non-polar fractions which have no pro-inflammatory activity, demonstrated pronounced cytotoxic activity *in vitro* against mouse TLX/5 lymphoma cells. This communication describes the isolation of three macrocyclic esters based upon the diterpene ingol from this fraction.

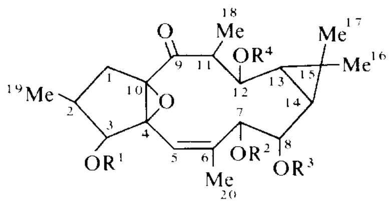
RESULTS AND DISCUSSION

Spectroscopic analysis demonstrated that the non-polar fractions contained tetra- and triesters of the macrocyclic diterpene ingol (Fig. 1). These esters were converted to the polyol **6** by means of base-catalysed hydrolysis from which the stable tetra-acetate **3** was obtained by synthesis. In the ^{1}H NMR spectrum of **3**, signals for four protons adjacent to acetate groups were exhibited. Assignments for these signals were facilitated by decoupling experiments. Irradiation of the signal at 2.9 ppm (1 H-11) caused the double doublet at 4.87 ppm to collapse to a sharp doublet ($J = 11$ Hz) and the doublet at 1.06 ppm to collapse to a sharp singlet, thereby confirming these signals as the 1 H-12 and 3 H-18, respectively. Furthermore, irradiation of the signal at 5.1 ppm (1 H-7) induced the double doublet at 4.57 ppm to collapse to a doublet ($J = 10.7$ Hz) indicating that this signal was due to the 1 H-8. Finally, irradiation of the signal at 2.48 ppm (1 H-2) caused the doublets at 5.33 and 0.97 ppm to collapse to sharp singlets thereby confirming the signal at 5.34 ppm as the 1 H-3 and the signal at 0.973 ppm as the 3 H-19. Although the mass and ^{1}H NMR spectra of **1** and **2** indicated the nature of the substituent ester functions no information was obtained about the positions of acylation. Assignments of the position of acylation in **1** and **2** were obtained by means of hydrolysis and *trans*-esterification reactions. In the ^{1}H NMR spectra of the isolated hydrolysis products there was a shift in the 1 H signals for the protons adjacent to a free OH group as compared to the signals of protons adjacent to an ester function. Compound **1** was accordingly confirmed as the new ingol ester 3,7,8-triacetyl-ingol-12-tigliate, **2**, as 3,7-diacetyl-ingol-12-tigliate, a new positional isomer of a compound previously obtained from a *Euphorbia* species [6] and **3** as ingol-tetra-acetate which has been obtained by partial synthesis from the polyol [3] but was not previously known as a natural product from plant sources.

EXPERIMENTAL

The latex of *E. kamerunica* was collected into an equal vol. of MeOH. Latex was obtained from plants growing near Vom in Nigeria.

Extraction. MeOH preserved latex (500 ml) was evapd to dryness below 45°. The residue was exhaustively extracted with Me_2CO at room temp. for 1 week. After evaporation of the Me_2CO the residue was dissolved in MeOH-H_2O (2:3) and the lipids and steroids removed by partition with hexane. The aq. phase was re-extracted with Et_2O and the Et_2O-soluble resin tested for biological activity using established methods [6, 7]. Resin (7 g) was separated by CC on 100 g of activated Florosil, eluting in the gradient, hexane-C_6H_6 (3/2, 100 ml), hexane-C_6H_6 (2:3, 100 ml), C_6H_6 (200 ml), C_6H_6-EtOAc (9:1, 100 ml), C_6H_6-EtOAc (6:1, 100 ml), C_6H_6-EtOAc (3:1, 100 ml), C_6H_6-EtOAc (1:1, 200 ml), C_6H_6-EtOAc (1:3, 100 ml),



	R^1	R^2	R^3	R^4
1	CO · Me	CO · Me	CO · Me	CO · C(Me) · CH · Me
2	CO · Me	CO · Me	H	CO · C(Me) · CH · Me
3	CO · Me	CO · Me	CO · Me	COMe
4	H	CO · Me	CO · Me	CO · C(Me) · CH · Me
5	H	H	CO · Me	CO · C(Me) · CH · Me
6	H	H	H	H

Fig. 1. The ingol esters of *Euphorbia kamerunica*.

C_6H_6–EtOAc (1:6, 100 ml), C_6H_6–EtOAc (1:9, 100 ml). EtOAc (300 ml). Fractions (10 ml) were collected and monitored by TLC and biological tests. The C_6H_6–EtOAc (9:1) fractions contained ingol esters and these were bulked for further purification.

TLC purification. The column residue (350 mg) was separated by adsorption TLC on Si gel GF_{254} 500 μm layers using hexane–Et_2O–C_6H_6 (1:2:1). Three zones were removed from the plates of R_f value, 0.14, 0.29 and 0.41. The Me_2CO–$CHCl_3$ (1:1) eluate from the three zones were separately purified by partition TLC using kieselguhr G 500 μm layers coated with 15% dipropylene glycol and using heptane–C_6H_6 (7:3) as solvent.

3,7,8-*Triacetyl-ingol*-12-*tigliate*, **1**, yield 200 mg, R_f 0.72 in the system above, exhibited the following spectral data: IR, ν_{max} 2930, 1740, 1720, 1650, 1440, 1375 and 1230 cm^{-1}; UV, λ_{max}^{MeOH} (log ε) nm 208 (4.23), 226 (4.15); EIMS (250°, 70 eV) m/z (rel. intensity): 574 ($M^{+\cdot}$, 4.5, $C_{31}O_{10}H_{42}$), 515 (2), 475 (1), 432 (2), 372 (1), 355 (1), 330 (2), 312 (1.5), 294 (1), 245 (4), 109 (8), 101 (2), 99 (3), 83 (100), 55 (55), 43 (99); ^{1}H NMR (250 MHz, $CDCl_3$, TMS), δ 6.89 (1 H, *q*, $J = 7$ Hz, tigliate), 5.58 (1 H, *s*, H-5), 5.24 (1 H, *d*, $J = 8.46$ Hz, H-3), 5.23 (1 H, *d*, $J = 1.84$ Hz, H-7), 4.88 (1 H, *dd*, $J = 11.03$, 4.04 Hz, H-12), 4.60 (1 H, *dd*, $J = 10.3$, 1.84 Hz, H-8), 2.95 (1 H, *q*, $J = 4.04$ Hz, H-11), 2.79 (1 H, *dd*, $J = 15.08$, 9.19 Hz, H-1), 2.50 (1 H, *q*, $J = 15.81$, 8.27 Hz, H-2), 2.02, 2.08, 2.11, 2.13, (12 H, 3 × $C\underline{H}_3CO$, 3 H-20), 1.86 (6 H, *m*, tigliate), 1.69 (1 H, *d*, $J = 15.08$ Hz, H-1), 1.31–1.22 (2 H, *dd*, H-13, H-14), 1.11, 0.86 (6 H,3 H-16, 3 H-17), 1.08 (3 H, *d*, $J = 6.99$ Hz, 3H-18), 0.93 (3 H, *d*, $J = 7.35$ Hz, 3H-18) ppm.

3,7-*Diacetyl-ingol*-12-*tigliate*, **2**, yield 80 mg, R_f value 0.28, in the partition system. The following spectral data were recorded: IR γ_{max} (KBr), 3430, 2920, 1730, 1710, 1650, 1450, 1370, 1240 cm^{-1}; UV λ_{max}^{MeOH} nm (log ε), 209 (4.48), 224 (4.20); MS, m/z (rel. intensity): 532 ($M^{+\cdot}$, 1, $C_{29}O_9H_{40}$), 472 (3), 390 (1), 330 (3), 312 (2), 245 (3), 211 (5), 165 (5), 137 (5), 109 (11), 83 (100), 69 (8), 55 (61), 43 (58); ^{1}H NMR (250 MHz, $CDCl_3$), δ 6.89 (1 H, *q*, $J = 6.98$ Hz, tigliate), 5.54 (1 H, *s*, H-5), 5.27 (1 H, *d*, $J = 8.46$ Hz, H-3), 5.10 (1 H, *d*, $J = 1.84$ Hz, H-7), 4.88 (1 H, *dd*, $J = 11.03$, 4.05 Hz, H-12), 3.54 (1 H, *dd*, $J = 9.93$, 1.84 Hz, H-8), 3.56 (1 H, exchangeable with D_2O), 2.95 (1 H, *q*, $J = 4.04$ Hz, H-11), 2.79 (1 H, *dd*, $J = 14.89$, 9.19 Hz, H-1), 2.48 (1 H, *q*, $J = 15.81$, 8.27 Hz, H-2), 2.12, 2.08, 2.08 (9 H, 2 × $C\underline{H}_3CO$, 3 H-20), 1.86 (6 H, *m*, tigliate), 1.69 (1 H, *d*, $J = 15.08$ Hz, H-1), 1.26–1.10 (2 H, *dd*, H-13, H-14), 1.14, 1.09 (6 H, 3 H-16, 3 H-17), 1.05 (3 H, *d*, $J = 7.35$ Hz, 3 H-18), 0.94 (3 H, *d*, $J = 7.35$ Hz, 3 H-19) ppm.

Ingol-3,7,8,12-*tetra-acetate*, **3**, yield 40 mg, R_f value 0.53 in the partition system. **3** exhibited the following spectral data: IR, ν_{max} (KBr), 2910, 1735, 1700, 1650, 1365, 1230 cm^{-1}; UV, λ_{max}^{MeOH} nm (log ε) 212 (3.41), 226 (3.15); MS, m/z (rel. intensity): 534 ($M^{+\cdot}$, 14, $C_{28}H_{38}O_{10}$), 475 (6), 432 (4), 372 (4), 312 (5), 294 (5), 245 (5), 181 (11), 165 (13), 139 (14), 109 (18), 83 (13), 69 (20), 55 (16), 43 (100); ^{1}H NMR (250 MHz, $CDCl_3$), δ 5.99 (1 H, *s*, H-5), 5.33 (1 H, *d*, $J = 8.46$ Hz, H-3), 5.10 (1 H, *d*, $J = 1.47$ Hz, H-7), 4.87 (1 H, *dd*, $J = 11.03$, 3.68 Hz, H-12), 4.57 (1 H, *dd*, $J = 10.66$, 1.84 Hz, H-8), 2.92 (1 H, *q*, $J = 3.68$ Hz, H-11), 2.79 (1 H, *dd*, $J = 15.08$, 9.19 Hz, H-1), 2.48 (1 H, *q*, $J = 8.09$, 15.81 Hz, H-2), 2.15, 2.11, 2.09, 2.10, 2.03 (15 H, 4 × $C\underline{H}_3CO$, 3 H-20), 1.70 (1 H, *d*, $J = 15.08$ Hz, H-1), 1.24–1.32 (2 H, *dd*, H-13, H-14), 1.11 and 0.85 (6 H, 3 H-16, 3 H-17), 1.06 (3 H, *d*, $J = 7.35$ Hz, 3 H-18), 0.97 (3 H, *d*, $J = 7.35$ Hz, 3 H-19) ppm.

Base-catalysed hydrolysis of **1**. **1** (40 mg) was hydrolysed with 0.1 M KOH in MeOH at room temp. for 45 min. Four hydrolysis products were isolated from the reaction mixture by means of TLC on Si gel G F_{254} as absorbent and hexane–Et_2O–EtOAc (1:1:1) as solvent.

8,7-*Diacetyl-ingol*-12-*tigliate*, **4** (R_f 0.41), MS, m/z (rel. intensity): 532 ($M^{+\cdot}$, 4, $C_{29}O_9H_{40}$), 472 (1), 372 (0.5), 330 (1), 312 (2), 245 (2), 221 (1), 205 (2), 181 (3), 165 (6), 137 (6), 111 (14), 100 (8), 83 (100), 59 (6), 55 (48), 43 (50); ^{1}H NMR (250 MHz, $CDCl_3$), δ 6.89 (1 H, *q*, $J = 6.98$ Hz, tigliate), 5.66 (1 H, *s*, H-5), 5.37 (1 H, *d*, $J = 1.84$ Hz, H-7), 4.88 (1 H, *dd*, $J = 11.03$, 4.05 Hz, H-12), 4.60 (1 H, *dd*, $J = 10.30$, 1.84 Hz, H-8), 4.29 (1 H, *d*, $J = 10.27$ Hz, H-3), 2.95 (1 H, *q*, $J = 4.04$ Hz, H-11), 2.79 (1 H, *dd*, $J = 14.89$, 9.19 Hz, H-1), 2.40 (1 H, *q*, $J = 15.81$, 8.27 Hz, H-2), 2.13, 2.11, 2.01 (9 H, 2 × $C\underline{H}_3CO$, 3 H-20), 1.86 (6 H, *m*, tigliate), 1.66 (1 H, *d*, $J = 14.89$ Hz, H-1), 1.67 (1 H, exchangeable with D_2O), 1.31–1.22 (2 H, *dd*, H-13, H-14), 1.11, 0.86 (6 H, 3 H-16, 3 H-17), 1.08 (3 H, *d*, $J = 6.99$ Hz, 3 H-18, 0.93 (3 H, *d*, $J = 7.35$ Hz, 3 H-19) ppm.

The reaction product of R_f 0.34 was identified as 3,7-diacetyl-ingol-12-tigliate **2** (TLC, MS, ^{1}H NMR).

8-*Acetyl-ingol*-12-*tigliate*, **5**, R_f 0.14, MS, m/z (rel. intensity): 490 ($M^{+\cdot}$, 3, $C_{27}H_{28}O_8$), 472 (2), 430 (2), 391 (5), 330 (4), 245 (5), 165 (19), 138 (20), 109 (30), 83 (100), 69 (22), 55 (100), 43 (100); ^{1}H NMR (250 MHz, $CDCl_3$), δ 6.90 (1 H, *q*, $J = 6.98$ Hz, tigliate), 5.89 (1 H, *s*, H-5), 4.88 (1 H, *dd*, $J = 11.03$, 4.05 Hz, H-12), 4.60 (1 H, *dd*, $J = 10.30$, 1.84 Hz, H-8), 4.30 (1 H, *d*, $J = 1.84$ Hz, H-7), 4.29 (1 H, *d*, $J = 10.27$ Hz, H-3), 2.95 (1 H, *q*, $J = 15.81$, 8.27 Hz, H-2), 2.79 (1 H, *dd*, $J = 14.89$, 9.19 Hz, H-1), 2.40 (1 H, *q*, $J = 15.81$, 8.27 Hz, H-2), 2.12, 2.11 (6 H, $C\underline{H}_3CO$, 3H-20), 1.86 (6 H, *m*, tigliate), 1.67 (1 H, exchangeable with D_2O), 1.66 (1 H, *d*, $J = 14.89$ Hz, H-1), 1.31–1.22 (2 H, *dd*, H-13, H-14), 1.11, 0.86 (6 H, 3 H-16, 3 H-17), 1.08 (3 H, *d*, $J = 6.99$ Hz, 3 H-18), 0.93 (3 H, *d*, $J = 7.35$ Hz, 3 H-19) ppm. The fourth reaction product was a TLC base-line compound **6** which was converted to its acetate by reaction in Ac_2O–pyridine (2:1). This product was identical to ingol-tetra-acetate **3** (TLC, MS, ^{1}H NMR.

Transesterification of **2**. **2** (30 mg) was reacted in 0.05 M KOMe in dry MeOH for 35 min. Two compounds were isolated from the reaction mixture by TLC as before. The compound R_f value 0.14 was identical to **5** (TLC, MS, ^{1}H NMR), whilst the base-line compound **6** after acetylation was found to be ingol-tetra-acetate **3** (TLC, MS, ^{1}H NMR).

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