A TRI-ESTER OF INGOL FROM THE LATEX OF *EUPHORBIA KAMERUNICA*

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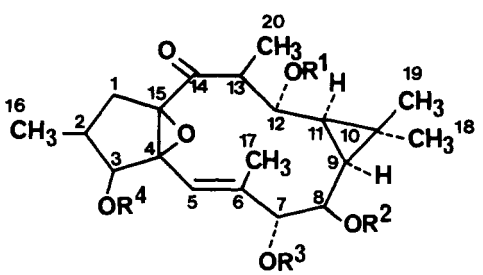
The macrocyclic diterpene, ingol, has been isolated as a series of esters from *Euphorbia ingens*, *E. resinifera* and *E. lactea* (1, 2, 3), and its configuration as the tetracetate was obtained by X-ray methods (4). Esters of ingol are devoid of the toxic tumor-promoting and pro-inflammatory activities of phorbol and ingenol esters which have been isolated from species of *Euphorbia*, and are considered to be biosynthetic precursors of these polycyclic compounds (5). Recently tetra-acyl ingol derivatives were shown to possess cytotoxic activity *in vitro* against TLX/5 mouse lymphoma and rat basophillic leukemia cells (6). This communication describes the structure of a new tri-ester of ingol isolated from the latex of *E. kamerunica* Pax. This ester, **1**, is the isomeric compound of a tri-ester previously obtained from *E. lactea* (3). The mass-spectrum (ms) of **1** (figure 1) suggested that this compound was a diacetate, tigliate ester of ingol. In the nmr spectra of ingol esters, the chemical shifts of the protons adjacent to the secondary acyl groups of C–3, 7, 8 and 12 (1) are diagnostic. Removal of an acyl substituent results in the upfield shift of the IH signal in its spectrum. The signal for the IH–8 in the nmr spectrum of **1** was observed at 3.55 ppm indicating that the three acyl groups present in this structure were located at C–3, 7 and 12 of ingol. Alkaline hydrolysis of **1** produced ingol-12-acetate as the major product. In addition the polyol, ingol, was obtained as a base line product and its structure after acetylation was confirmed as ingol-tetra-acetate by comparison to authentic material. The

FIGURE 1



	R^1	R^2	R^3	R^4
1	O.C.CH_3	H	OC.C(CH_3).CH(CH_3)	O.C.CH_3
2	O.C.CH_3	O.C.CH_3	O.C.CH_3	O.C.CH_3
3	O.C.CH_3	H	H	H
4	O.C.CH_3	OC.C(CH_3).CH(CH_3)	H	H

tigliate and second acetate groups of **1** were therefore located at C-3 or 7 of the structure. Transesterification of **1** produced a single diester **4** (figure 1). From the chemical ionization-ms of **4**, the two acyl groups were identified as acetate and tigliate, respectively. Observations of the chemical shifts of the signals in the nmr spectrum of **4** demonstrated an upfield shift for the 1H-3 from 5.26 ppm in **1** to 4.36 ppm in **2** and for the 1H-7 from 5–10 ppm in **1** to 4.30 ppm in **4**. The oxygen functions at C-3 and 7 in compound **4** were accordingly present in the form of free secondary alcohols. Furthermore the downfield shift for the 1H-8 indicated that the tigliane residue in **4** was present in this position. The C-7,8 diol system of ingol undergoes acyl rearrangement during the alkaline-catalyzed transesterification reaction. Accordingly compound **1** was assigned as 3,12-diacetyl-ingol-7-tigliate.

EXPERIMENTAL

PLANT MATERIAL.—The latex of *Euphorbia kamerunica* Pax. (Euphorbiaceae) was collected from plants growing near Jos in Nigeria and put directly into methanol. The plant material was authenticated by Professor Harris of Ahmadu Bello University, Zaria, and retained at the School of Pharmacy in London. Latex samples were deep-frozen under an atmosphere of nitrogen until required for analysis.

EXTRACTION.—Latex samples, in batches of 500 ml, were dried by evaporation and extracted with acetone and an ether soluble fraction was prepared (6). A gradient of hexane-benzene-ethylacetate was used to separate this resin by column chromatography (6). Compound **1** was eluted with benzene-ethylacetate (1:1).

PURIFICATION.—Compound **1** was initially purified by adsorption tlc with silica gel G adsorbent and developing twice with ethylacetate-benzene-ether (1:1:2) (R_f 0.42), followed finally by partition tlc on kieselguhr G coated with 15% dipropylene glycol and developing with heptane-benzene (70:30) (R_f 0.38). Final purification was by tlc on silica gel as before with cyclohexane-ether-ethyl acetate-benzene (40:15:30:20) (R_f 0.23) as solvent.

COMPOUND **1**.—*3,12-diacetyl-ingol-7-tigliate*, yield 12 mg per 500 ml of latex. The following data resulted: ms (electron-impact, 70 e.v. 200°), *m/z* 532 (M^+, 0.7%), 472 (4%), 432 (0.3%), 390 (1%), 372 (0.5%), 330 (4%), 312 (2%), 294 (2%), 211 (8%), 109 (13%), 105 (22%), 85 (83%), 83 (100%); ir (KBr disc) ν max, cm^{-1}, 3420, 2910, 1730, 1705, 1650, 1365, 1230; ^{1}H-nmr (250 MHz, $CDCl_3$) δ 6.876 (q, J=6.99 Hz, 1H, tigliate), 5.540 (s, 1H-5), 5.263 (d, J=8.46 Hz, 1H-3), 5.100 (d, J=1.84 Hz, 1H-7), 4.902 (d.d., J=4.05, 11.03 Hz, 1H-12), 3.788 (s, 1H, exchangeable with D_2O), 3.547 (d.d., J=1.84, 9.93 Hz, 1H-8), 2.945 (q, J=4.04 Hz, 1H-13), 2.781 (d.d., J=8.83, 14.71 Hz, 1H-1), 2.474 (q, J=7.35 Hz, 1H-2), 2.123 (s, CH_3CO·), 2.074 (s, CH_3CO·, 3H-17), 1.865 (m, 6H, tigliate), 1.633 (d, J=13.97 Hz, 1H-1), 1.260–1.00 (m, 1H-11, 1H-9), 1.119, 1.088 (3H-18, 3H-19), 1.055 (d., J=7.35 Hz, 3H-20), 0.943 (d, J=7.72 Hz, 3H-16) ppm.

BASE CATALYZED HYDROLYSIS.—Compound **1** was hydrolyzed with 0.5M KOH in methanol. The reaction mixture was separated into two fractions by tlc on silica gel G with hexane-ether-ethyl acetate (1:1:1) as solvent. The base line product was eluted and acetylated with pyridine-acetic anhydride (2:1). The resulting acetate was identical to ingol-tetra-acetate (1) **2** (^{1}H-nmr, ms, tlc). The second product from the hydrolysis **3** of R_f 0.15 exhibited an identical ms and ir spectrum to ingol-12-acetate (3). Signals were evident in the ^{1}H-nmr spectrum (250 MHz, $CDCl_3$-D_2O) at δ 5.832 (s, 1H-5), 4.837 (d.d., J=4.05, 11.03 Hz, 1H-12), 4.364 (d, J=8.46 Hz, 1H-3), 4.347 (d., J=1.84 Hz, 1H-7), 2.950 (q, J=4.05 Hz, 1H-13), 2.865 (d.d., J=1.84, 9.93 Hz, 1H-8), 2.763 (d.d., J=14.71, 9.19 Hz, 1H-1), 2.375 (q, J=8.46 Hz, 1H-2), 2.118 (s, CH_3CO·), 1.996 (s, 3H-17), 1.639 (d, J=14.71 Hz, 1H-1), 1.240–1.100 (m, 1H-11, 1H-9), 1.072–0.969 (12H, 3H-19, 3H-20, 3H-16) ppm.

TRANSESTERIFICATION.—Compound **1** was reacted in methanol with 0.05 M KOH for 40 mins. A single diester **4** was isolated from the reaction on silica gel G as before (R_f 0.2). Compound **4** exhibited mass and infrared spectra identical to 12-acetyl-ingol-8-tigliate (3), ^{1}H-nmr (250 MHz, $CDCl_3$-D_2O) signals were evident at δ 6.876 (q, J=6.99 Hz, 1H, tigliate), 5.889 (q, J=1.10 Hz, 1H-5), 4.874 (d.d., J=4.05, 11.40 Hz, 1H-12), 4.580 (d.d., J=1.47, 10.66 Hz, 1H-8), 4.356 (d, J=8.47 Hz, 1H-3), 4.304 (b.s., 1H-7), 2.926 (d.d., J=4.04, 7.35 Hz, 1H-13), 2.794 (d.d., J=8.19, 14.71 Hz, 1H-1), 2.425 (q, J=7.354, 1H-2), 2.176 (s, CH_3CO·), 2.099 (s, 3H-17), 1.849 (m, 6H, tigliate), 1.652 (d, J=15.08 Hz, 1H-1), 1.445 (d.d., J=9.19, 10.66 Hz, 1H-9), 1.188 (d.d., J=8.82, 11.03 Hz, 1H-11), 1.086, 0.834 (6H, 3H-18, 3H-19), 1.075 (d., J=3.67 Hz, 3H-20), 1.044 (d., J=3.31 Hz, 3H-16) ppm.

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