Four Ingol Type Diterpenes from *Euphorbia antiquorum* L.

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From the latex of *Euphorbia antiquorum* L. (Euphorbiaceae), four macrocyclic diterpenes, 3,12-di-*O*-acetyl-8-*O*-benzoylingol (1), 3,12-di-*O*-acetyl-8-*O*-tigloylingol (2), 12-*O*-acetyl-8-*O*-tigloylingol (3) and 8-*O*-tigloylingol (4), were isolated and characterized. Compound 1 was previously reported as a component of a mixture, but was obtained in pure form in this experiment. Compounds 3 and 4 were isolated for the first time from a natural source.

Keywords *Euphorbia antiquorum*; 3,12-di-*O*-acetyl-8-*O*-benzoylingol; 3,12-di-*O*-acetyl-8-*O*-tigloylingol; 12-*O*-acetyl-8-*O*-tigloylingol; 8-*O*-tigloylingol

Euphorbia antiquorum L. (Euphorbiaceae) produces a milky latex which has found several applications in folk medicines. The latex is irritant, emetic, purgative and diuretic.[1] It is used to treat toothache, earache, nervous disease, dropsy, palsy, deafness and amaurosis.[2] The dried latex, a resin, is considered to be an aphrodisiac.[3] However, its most notable application is found in the "Kshara Sutra", a surgical treatment for fistula in the Ayurvedic medicinal system. The Kshara Sutra procedure consists in the preparation of a surgical thread dipped in an alkaline solution of the ash of *Achyranthes aspera* L. (Amaranthaceae) and in latex of *E. antiquorum*, followed by coating with the powder of *Curcuma longa* L. (Zingiberaceae). The thread is then pierced through the fistula and healing of the wound occurs. Recently, this treatment was introduced into Japan by Pilapitiya *et al.* and successfully applied to over 100 patients.[4]

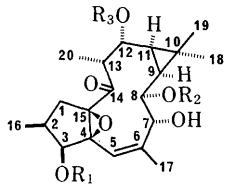
In a preceding paper,[5] we reported a stimulatory effect of the extract of *E. antiquorum* latex on blastogenesis of lymphocytes.

In this paper, we describe the isolation and characterization of four ingol-type diterpenes.[6] The only previous report describes the isolation of a single ingenol diterpene from the latex.[3]

Results and Discussion

The acetone extract was partitioned between aqueous and organic (*n*-hexane and then ether) phases. The ether-soluble portion was subjected to column chromatography, followed by preparative high-performance liquid chromatography (pHPLC) and preparative thin-layer chromatography (pTLC), to give four pure ingol esters (**1**—**4**).

Compound **1** showed characteristic signals of an ingol nucleus with two acetyl and one benzoyl residues in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum.[6-8] The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum also showed signals due to three ester carbonyls (δ 170.7, 170.4 and 166.1) indicating that out of four hydroxyl groups of the ingol nucleus, one was free. The broad singlet signal due to H-7 at δ 4.39 in the ^{1}H-NMR spectrum demonstrated that the hydroxyl group at C-7 was free and the double doublet at δ 4.77, due to H-8, strongly indicated that the hydroxy group at C-8 was benzoylated.[6] Furthermore, an appreciable long-range chemical shift correlation was observed between H-12 (δ 4.92) and –O-CO-Me (δ 170.4) and between H-8 (δ 4.77) and –O-CO-Ph (δ 166.1) by long-range ^{13}C–^{1}H shift correlated spectroscopy (long-range ^{13}C–^{1}H COSY). These findings indicated compound **1** to be 3,12-di-*O*-acetyl-8-*O*-benzoylingol. This structure was further confirmed by both ^{1}H–^{1}H and ^{13}C–^{1}H COSY experiments. Since all ^{1}H- and ^{13}C-NMR chemical shifts and vicinal spin–spin coupling values between skeletal protons (Tables I and II) were essentially similar to those of ingol tetraacetate,[8-10] whose absolute configuration had been established by X-ray crystallographic analysis, the stereochemistry of **1** was concluded to be as shown in Fig. 1. Compound **1** was first found in a mixture together with three other ingol-esters by Rizk *et al.*[11] However, this compound was isolated in pure form for the first time in the present work.



1: $R_1=R_3=$Ac, $R_2=$Bz
2: $R_1=R_3=$Ac, $R_2=$Tig
3: $R_1=$H, $R_2=$Tig, $R_3=$Ac
4: $R_1=R_3=$H, $R_2=$Tig

Fig. 1. Structures of Ingol Esters

Compound **2** had a similar ^{1}H-NMR profile to **1** except for the obvious difference of a tigloyl substituent (δ 1.82, dq, J=7.0 and 1.3 Hz; δ 1.85, dq, J=1.3 and 1.3 Hz; δ 6.90, qq, J=7.0 and 1.3 Hz) instead of a benzoyl group in **1**. The signal at δ 6.90 readily enabled us to distinguish a tigloyl group from an angeloyl group, whose olefinic proton would resonate at δ *ca.* 6.1.[11] Consequently, this compound was identified as 3,12-di-*O*-acetyl-8-*O*-tigloylingol. This compound was first isolated from the latex of *Euphorbia lactea*[12] and was also detected in an admixture of ingol esters from the latex of *E. royleana*.[9]

Compound **3** was isolated in pure form after repeated pTLC. The ^{13}C-NMR spectrum showed two signals (δ 170.5 and 167.5) due to an acetyl carbonyl and a tigloyl carbonyl, indicating that the two hydroxy groups of the ingol nucleus were free. In the ^{1}H-NMR spectrum, the signal due to H-7 at δ 4.29 indicated that the hydroxy group at C-7 was free. Furthermore, comparison of the ^{1}H-NMR of **3** with that of **2** showed that the doublet of H-3 at δ 5.21 moved to upper field (δ 4.37 in **3**) and the doublet of H-16 at δ 0.91 shifted downfield (δ 1.06 in **3**), demonstrating that the hydroxy group at C-3 was also free. These findings revealed

TABLE I. ^{1}H-NMR Spectral Data for Ingol Esters **1**—**4** (in $CDCl_3$)

Proton	**1**[a]	**2**[b]	**3**[b]	**4**[b]
H-1β	1.69 (br d, 15.0)	1.68 (br d, 14.9)	1.65 (dd, 14.9, 1.9)	1.66 (dd, 15.0, 1.9)
H-1α	2.84 (dd, 15.0, 9.2)	2.83 (dd, 14.9, 9.0)	2.79 (dd, 14.9, 9.0)	2.72 (dd, 15.0, 9.3)
H-2	2.58 (m)	2.58 (m)	2.42 (m)	2.41 (m)
H-3	5.23 (d, 8.2)	5.21 (d, 8.3)	4.37 (br t)	4.35 (br d, *ca.* 8)
H-5	5.85 (br s)	5.81 (br s)	5.88 (s)	5.89 (s)
H-7	4.39 (br s)	4.28 (br s)	4.29 (br s)	4.27 (s)
H-8	4.77 (dd, 10.7, 1.5)	4.57 (dd, 10.5, 1.8)	4.58 (dd, 10.7, 1.4)	4.49 (d, 11.0)
H-9	1.55 (dd, 10.7, 9.3)	1.43 (dd, 10.5, 9.3)	1.44 (dd, 10.7, 9.5)	1.66 (dd, 11.0, 8.8)
H-11	1.18 (dd, 11.0, 9.3)	1.12 (dd, 11.0, 9.3)	1.11 (dd, 11.0, 9.5)	0.73 (dd, 10.5, 8.8)
H-12	4.92 (dd, 11.0, 4.0)	4.87 (dd, 11.0, 3.9)	4.87 (dd, 11.0, 3.9)	3.23 (br t)
H-13	2.96 (dq, 4.0, 7.3)	2.92 (dq, 3.9, 6.9)	2.93 (dq, 3.9, 7.3)	2.71 (m)
H-16	0.92 (d, 7.3)	0.91 (d, 7.6)	1.06 (d, 7.6)	1.06 (d, 7.6)
H-17	2.12 (d, 0.9)	2.07 (d, 1.2)	2.08 (d, 1.0)	2.07 (d, 1.3)
H-18	1.14 (s)	1.09 (s)	1.08 (s)	1.15 (s)
H-19	0.85 (s)	0.83 (s)	0.83 (s)	0.95 (s)
H-20	1.10 (d, 7.3)	1.08 (d, 6.9)	1.05 (d, 7.3)	1.26 (d, 7.3)
Acetyl	2.09 (s)[c]	2.10 (s)	2.08	
	2.11 (s)[d]	2.11 (s)		
Benzoyl	7.47 (t, 7.5)			
	7.59 (tt, 7.5, 1.0)			
	8.06 (dd, 7.5, *ca.* 1)			
Tigloyl		1.82 (dq, 7.0, 1.3)	1.82 (dq, 6.0, 1.6)	1.83 (dq, 6.8, 1.2)
		1.85 (dq, 1.3, 1.3)	1.85 (dq, 1.6, 1.6)	1.86 (dq, 1.2, 1.2)
		6.90 (qq, 7.0, 1.3)	6.91 (qq, 6.0, 1.6)	6.90 (qq, 6.8, 1.2)

a) Measured at 400 MHz. *b*) Measured at 270 MHz. Multiplicities and *J*-values (Hz) in parentheses.

TABLE II. ^{13}C-NMR Spectral Data for Ingol Esters **1**—**4** (in $CDCl_3$)

Carbon	**1**[a]	**2**[b]	**3**[b]	**4**[b]
C-1	31.8	31.8	32.0	31.7
C-2	29.8	29.8	29.3	29.8
C-3	77.7	77.8	76.1	76.4
C-4	73.8	73.8	74.4	74.2
C-5	116.8	116.7	117.2	117.4
C-6	141.3	141.5	141.6	142.0
C-7	76.4	76.5	76.5	76.7
C-8	75.1	74.5	74.4	74.2
C-9	23.6	23.7	24.0	24.0
C-10	19.3	19.2	19.2	18.7
C-11	31.0	31.1	31.2	31.5
C-12	70.8	70.9	71.1	71.6
C-13	43.3	43.4	43.2	43.2
C-14	207.6	207.8	207.8	206.8
C-15	71.5	71.5	73.0	72.7
C-16	17.0	17.1	16.4	16.2
C-17	17.5	17.6	17.7	17.9
C-18	29.2	29.2	29.3	29.5
C-19	16.4	16.5	16.2	15.9
C-20	13.3	13.4	13.5	14.6
Acetyl				
Me	20.7	20.8	21.1	
	21.0	21.1		
CO	170.4	170.6	170.5	
	170.7	170.8		
Tigloyl				
3′-Me		12.1	12.1	12.1
2′-Me		14.6	14.6	14.6
C-3′		128.5	128.6	128.6
C-2′		138.3	138.2	138.2
C-1′		167.6	167.5	167.7
Benzoyl				
C-2′,6′	129.7			
C-3′,5′	128.6			
C-4′	129.9			
C-1′	133.3			
CO	166.1			

a) Measured at 100 MHz. The assignment was performed on the basis of ^{1}H–^{1}H, ^{13}C–^{1}H and long range ^{13}C–^{1}H COSY experiments. *b*) Measured at 22.5 MHz.

that **3** was 12-*O*-acetyl-8-*O*-tigloylingol. This compound had been obtained by mild hydrolysis of 3,12-di-*O*-acetyl-8-*O*-tigloylingol.[7,12] However, this compound was isolated for the first time from a natural source in the present work.

Compound **4** was assigned the molecular formula $C_{25}H_{36}O_7$ by high-resolution mass spectrometry (MS). The ^{13}C-NMR spectrum showed only one ester carbonyl peak (δ 167.7) ascribable to a tigloyl group. As in **2** and **3**, the tigloyl group was present at C-8 as evidence by the doublet at δ 4.49 due to H-8. Therefore, **4** was identified as 8-*O*-tigloylingol. This compound was isolated for the first time in the present work.

E. antiquorum latex has a potent inflamatory action in mice and a stimulatory action on blastogenesis of lymphocytes.[5] Our preliminary investigation of the latex showed that the major components were euphol (*ca.* 70%) and other triterpenes,[13] but they had no inflamatory or mitogenic actions. On the other hand, Adolf *et al.* have isolated 3-*O*-angeloylingenol as one of the toxic and skin irritant factors from the same latex.[3] Some biological activities of newly isolated ingol-esters **1**—**4** and the role of the latex in the Kshara Sutra treatment are under investigation.

Experimental

Apparatus ^{1}H- and ^{13}C-NMR were measured with JEOL GX-270 (^{1}H, 270 MHz), JEOL JNM GX-400 (^{1}H, 400 MHz, ^{13}C, 100 MHz) and JEOL FX-90Q (^{13}C, 22.5 MHz) spectrometers, with tetramethylsilane as an internal standard. The multiplicities of signals are represented by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; dd, double doublet; dq, double quartet. MS were measured with a JMS DX-300 mass spectrometer under the following conditions: ionization voltage for electron impact mass spectrometry (EI-MS), 70 eV; accelerating energy for fast atom bombardment mass spectrometry (FAB-MS), 6 kV; Xe and glycerol as the neutral gas and matrix, respectively. Optical rotation was measured with a JASCO DIP-360 digital polarimeter at 25 °C and concentrations (*c*) are presented as the weight (g) of compounds in 100 ml of solvent. HPLC was carried out on a Trirotar V (JASCO Co., Tokyo) equipped with a UVIDEC-100 detector (JASCO).

Chemicals Wako gel C-200 was purchased from Wako Pure Chem. Co., Osaka, for column chromatography. Merck Kieselgel 60 F_{254} (Merck, FRG) was used for TLC.

Plant Material The plant *Euphorbia antiquorum* L. was identified by U. Pilapitiya (Bandaranayake Memorial Ayurvedic Research Institute, Sri Lanka) and the latex was collected by him from plants growing wild in Sri Lanka.

Isolation Latex of *E. antiquorum* (800 g) was put in MeOH and the solid part was separated by filtration. The solid part was then extensively extracted with acetone below 40 C. The extract was dissolved in MeOH–H_2O (1 : 1) and this solution in turn was extracted three times with *n*-hexane and ether (0.8 l each). The ether fraction (900 mg) was chromatographed on silica gel (30 g) with a mixed solvent of C_6H_6 with increasing amounts of EtOAc (0—100%). Compounds **1** and **2** were obtained as a mixture, which was subjected to pHPLC (Chemosorb 5 Si (Chemco Co.); column size, 10 mm i.d. × 500 mm; solvent system, *n*-hexane–dichloroethane–EtOH (5 : 2 : 0.2); detection, 203 nm) to afford pure **1** (25 mg) and **2** (20 mg). Repeated pTLC (C_6H_6–EtOAc, 6 : 10) gave **3** (13 mg) and **4** (7.5 mg).

3,12-Di-*O*-acetyl-8-*O*-benzoylingol (1) $[\alpha]_D = -21.8$ ($c = 0.16$, $CHCl_3$). MS *m/z* (rel. int.): 553 (7%) $(M-1)^+$ 495 (31%) $(M-CH_3COO)^+$, 433 (62%) $(M-C_6H_5COO)^+$, 121 (100%) $(C_6H_5COO)^+$, 105 (100%) $(C_6H_5CO)^+$. IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1735 (C=O), 1710 (C=O). Long-range ^1H-^1H shift correlations were observed for H-7 *vs.* H-5, H-17 *vs.* H-7 and H-18 *vs.* H-19 in a ^1H–^1H COSY experiment, and long-range ^{13}C–^1H shift correlations were also seen for C-5 *vs.* H-7,17, C-9 *vs.* H-7,8, C-6 *vs.* H-5,7,17, and C-4,15 *vs.* H-1 in a long-range ^{13}C–^1H COSY experiment.

3,12-Di-*O*-acetyl-8-*O*-tigloylingol (2) $[\alpha]_D = -16.2$ ($c = 0.13$, $CHCl_3$). MS *m/z* (rel. int.): 531 (9%) $(M-1)^+$, 472 (56%) $(M-CH_3COOH)^+$, 433 (100%) $(M-CH_3CH=C(CH_3)COO)^+$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 1722 (C=O), 1700 (C=O), 1640 (C=C).

12-*O*-Acetyl-8-*O*-tigloylingol (3) $[\alpha]_D = +10$ ($c = 0.16$, $CHCl_3$). MS *m/z*: 491 $(M-1)^+$, 431 $(M-CH_3COOH-1)^+$, 391 (100%) $(M-CH_3CH=C(CH_3)COO)^+$. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1725 (C=O), 1702 (C=C), 1642 (C=C).

8-*O*-Tigloylingol (4) $[\alpha]_D = +21.8$ ($c = 0.12$, $CHCl_3$). High-resolution MS *m/z*: 448.2504 (Calcd for $C_{25}H_{36}O_7$: 448.2461). FAB-MS *m/z*: 449 $(M+1)^+$, 431 $(M+1-H_2O)^+$, 349 $(M-CH_3CH=C(CH_3)COO)^+$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1690 (C=O), 1640 (C=C), 1255 (C–O).

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