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BIOLOGICALLY ACTIVE DITERPENE ESTERS FROM *EUPHORBIA PEPLUS**

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Key Word Index—*Euphorbia peplus*; Euphorbiaceae; 20-deoxyingenol; ingenol; pro-inflammatory esters.

Abstract—By means of partition and preparative TLC, two pro-inflammatory diterpene esters were isolated from *Euphorbia peplus*. These compounds were identified as 20-deoxyingenol 3-*O*-angelate which exhibited an ID_{50} of 0.18 μg on mice and the new ester ingenol 20-*O*-octanoate which exhibited an ID_{50} of 1.0 μg also on mice skin.

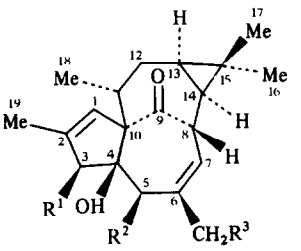
INTRODUCTION

Euphorbia peplus L., the most common *Euphorbia* growing in Egypt, is a small glabrous weed which invades cultivated land as secondary growth [1]. The plant has been used medicinally for the treatment of asthma and catarrh [2] but is known to induce painful vomiting and pergation, particularly in domestic animals [3]. Extracts of this plant are said to be C-mitotic poisons [4] and have reported proteolytic activities (5). *Euphorbia peplus* belongs to the section *Tithymalus*, subsection *esula* of the genus *Euphorbia* and a microchemical screen of this section of the genus has previously indicated that these species contain ingenane diterpenes [6]. This communication describes the isolation and identification of the irritant diterpene esters, based upon the ingenane skeleton.

RESULTS AND DISCUSSION

Two ingenane esters were isolated from *Euphorbia peplus* by means of conventional adsorption and partition TLC techniques. These compounds were shown to be pro-inflammatory toxins when applied to mammalian skin. At a dose level of 5 μg per animal the compounds induced erythema of skin in 100% of two groups of 36 mice each. The irritant dose 50% (ID_{50}) of the pure esters was ascertained by the method of Evans and Schmidt [7] as 0.18 μg per animal for **1** and 1.0 μg for **4**. The esters were accordingly less potent than the related group of phorbol esters also obtained from some *Euphorbia* species and the daphnane ortho-esters from several genera of Euphorbiaceae and Thymelaeaceae, but the inflammation induced was more prolonged and led to extensive tissue damage in the chronic phase.

A TLC-microanalysis of the section *Tithymalus* of this genus had previously indicated the presence of ingenane diterpenes in the latex of *Euphorbia peplus* [6]. This was later enforced by biological analysis of the extract of this plant [8] which exhibited pro-inflammatory activity *in vivo*. This is the first time, however, that individual naturally occurring esters have been isolated from this plant, thereby explaining the known toxicological properties of the herb [3]. The major toxin **4** was identified as a new ester of ingenol **5** (Fig. 1) after hydrolysis and subsequent acetylation to the more stable triacetate **6**. The absolute configuration of **6** was previously obtained by X-ray methods [9]. From the mass spectrum of **4**, this compound was a mono-ester of ingenol, exhibiting an octanoic acid moiety at either C-3, C-5 or C-20 of the nucleus. The octanoate residue of **4** would be assigned to the C-20 on the basis of the chemical shift of the 2H-20 exhibited in the ^1H NMR spectrum. It has been demonstrated that 20-*O*-acyl derivatives of ingenol exhibit the 2H-20 signal as an AB quartet at δ4.4–4.6 in their ^1H NMR spectra whilst compounds containing a primary hydroxyl moiety at this position exhibit the allylic proton signal at about δ4.0–4.1 as a broad singlet [10, 11]. The minor toxic ingenane ester **1** from this plant was identified as a previously known ester of 20-deoxyingenol [2]. The parent diterpene was identified subsequent to hydrolysis



	R^1	R^2	R^3
1	Angelate	OH	H
2	OH	OH	H
3	Acetate	Acetate	H
4	OH	OH	Octanoate
5	OH	OH	OH
6	Acetate	Acetate	Acetate

*Part 13 in the series "Constituents of Egyptian Euphorbiaceae"

followed by acetylation to the diacetate **3** and comparison to an authentic sample. Esters of this diterpene are rare in the genus *Euphorbia* to date having only been obtained previously from *E. kansui* [12] and *E. paralias* [13]. The mass spectrum of **1** indicated that this compound was a mono-ester of a deoxyingenol diterpene and that the acyl residue was either the tigliate or angelate of position 3 or 5 of the nucleus. In the ^{1}H NMR spectrum of **1** the characteristic chemical shift of the olefinic proton at δ6.16 confirmed that the acyl residue was the angelate isomer rather than the tigliate. Furthermore, due to the chemical shift of a proton adjacent to a secondary acyl group at δ5.46 this acyl residue was located at C-3 of 20-deoxyingenol **2**. The two major toxins of Egyptian *Euphorbia peplus* were assigned as ingenol 20-octanoate **4** and 20-deoxyingenol 3-angelate **1** respectively.

EXPERIMENTAL

Extraction. About 750 g dried powdered *Euphorbia peplus* L. was extracted three times with successive portions of acetone during 7 days. The acetone extracts were bulked and evaporated to dryness under red. pres. below 40°. The dry residue (44 g) was dissolved in MeOH–H_2O (17:13, 100 ml) and extracted by partition with *n*-hexane (3 × 50 ml). The methanolic phase was further extracted with ether (3 × 100 ml), the combined ether fraction washed with 0.25% w/v Na_2CO_3 (2 × 50 ml) followed by distilled water. After drying and filtering, the neutral ether phase was evaporated to dryness to produce a yellow-green resin (1.9 g). The ether soluble residue was separated into four zones by means of prep. TLC. Plates were prepared with silica gel 0.5 mm layers buffered at pH 7.0 with phosphate buffer and developed twice with $CHCl_3$–Et_2O–C_6H_6 (1:3:3). Zones of R_f 0.75 and 0.57 elicited inflammation in 100% of the mice in doses of 5 μg per ear. Other fractions were shown to be biologically inactive up to doses of 100 μg per animal.

20-Deoxy-ingenol-3-O-angelate **1** (R_f 0.75, yield 22 mg), was further purified by means of partition TLC using Kieselguhr G plates coated with 15% dipropylene glycol and in heptane–C_6H_6 (17:3), followed by adsorption TLC as before in $CHCl_3$–Et_2O (19:1) (R_f 0.38). IR ν_{max}^{KBr} cm^{-1}: 3460, 1640 and 1450. EIMS (70 eV, 170°) *m/z*: 414 $[M]^+$ ($C_{25}H_{34}O_5$), 314 ($C_{20}H_{26}O_3$), 296, 121 (base peak). ^{1}H NMR ($CDCl_3$, 80 MHz, TMS): δ6.16 (*m*, 1H, angelate), 6.05 (*s*, 1H-1), 5.75 (*d*, J = 5.7 Hz, 1H-7), 5.46 (*s*, 1H-3), 4.03 (*m*, 1H-8), 3.68 (*s*, 1H-5), 3.43 (1H, exchangeable D_2O), 2.42 (*m*, 2H-12), 1.91 (*s*, 3H-20), 1.80 (*m*, 3H-19, 6H-angelate), 1.60 (1H, exchangeable D_2O), 1.05–1.10 (*d*, 3H-16, 3H-17, 3H-18), 0.90 (*br s*, 1H-13, 1H-14). Compound **1** was hydrolysed with 0.5 M methanolic KOH at room temp. The polyol **2** was converted to a diacetate **3** with Ac_2O–pyridine (2:1). Compound **3** was identified as 20-deoxyingenol diacetate by comparison with an authentic sample (TLC, MS) [13].

Ingenol-20-O-octanoate **4** (R_f 0.57, yield 62 mg), was purified by partition TLC as before using heptane–C_6H_6 (7:3) as the developing solvent. EIMS (70 eV, 170°) *m/z* (rel. int.): 474 $[M]^+$ (1%, $C_{28}H_{42}O_6$), 456 $[M-18]^+$ (12%), 312 (30%), 294 (20%), 121 (100%). ^{1}H NMR (250 MHz, $CDCl_3$, TMS): δ6.04 (*d*, J = 2 Hz, 1H-7), 6.00 (*s*, 1H-7), 4.55 (ABq, 2H-20), 4.55 (*m*, 1H-8), 4.04 (*s*, 1H-5), 3.90 (*s*, 1H-3), 2.20–2.50 (*m*, 2H-12, –CH_2–CO–), 1.2–0.89 (Me, –$(CH_2)_5$–, 3H-16, 3H-17, 3H-18). Compound **4** was hydrolysed to its polyol **5** and acetylated to the triacetate **6** as before. Compound **6** was identified as ingenol-triacetate by comparison with an authentic sample (TLC, MS) [14].

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