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TERPENOIDS FROM *EUPHORBIA ANTIQUORUM*

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Key Word Index—*Euphorbia antiquorum*; Euphorbiaceae, terpenoids

Abstract—Three new triterpenoids, namely friedelane-3β,30-diol diacetate, 30-acetoxyfriedelan-3β-ol and 3β-acetoxyfriedelan-30-ol, together with several known compounds have been isolated from the stems of *Euphorbia antiquorum*. The structures were elucidated by spectroscopic and chemical methods.

INTRODUCTION

Euphorbia antiquorum L. is grown throughout the hotter parts of India. All parts of this plant find frequent use in indigenous systems of medicine [1]. Previous investigations on this plant showed the presence of taraxerol and *epi*-friedelanol in the stem-bark [2], friedelan-3β-ol and 3α-ol, taraxerol and taraxerone in the stems [3, 4], euphol, euphorbol, β-amyrin, cycloartenol [5] and ingenol type diterpenoids [6, 7] in the latex.

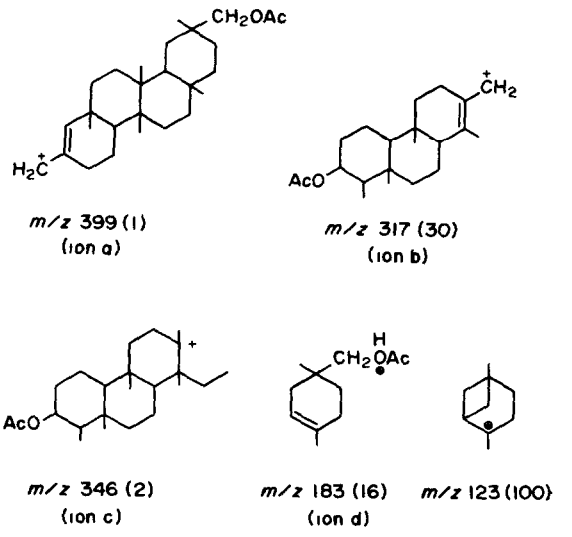
As part of our continued interest in the triterpenoids of *Euphorbia* species, we undertook a systematic re-examination of the stems of *E. antiquorum* with the aim of isolating minor constituents. We now report three new and several known terpenoids from this source.

RESULTS AND DISCUSSION

The column chromatograph of the *n*-hexane extract of the stems of *E. antiquorum* yielded three new friedelane derivatives (**1**–**3**) and several known compounds. The known compounds were identified as taraxeryl acetate, friedelan-3β-yl acetate, lupeol cinnamate, moretenone, lupenone, taraxerone, friedelan-3β-ol, β-amyrin, lupeol, cycloartenol, euphol, taraxerol, friedelan-3α-ol, β-sitosterol, the C-24 epimers of cycloart-25-ene-3β,24-diol [8], cycloartane-3β,25-diol [9, 10], ψ-taraxastane-3β,20-diol [11] and a diterpenoid 7-hydroxyingol-3,12-di-O-acetyl-8-tigliate [12]. Among the known compounds cycloartane-3β,25-diol and ψ-taraxastane-3β,20-diol have been reported for the first time from *Euphorbia* species.

Compound **1** analysed for $C_{34}H_{56}O_4$. Its IR spectrum indicated acetate carbonyl (1730 cm^{-1}). Alkaline hydrolysis of compound **1** afforded a new diol (**1a**). The ^1H NMR spectrum of compound **1** showed no olefinic protons and exhibited seven *C*-methyl groups (δ0.76–1.21), two acetate methyl groups (δ1.96 and 1.99), acetoxymethylene protons at δ3.67 [moved to δ3.26 in the spectrum of the diol (**1a**)] and an acetoxymethyne proton at δ4.83 [moved to δ3.72 in the spectrum of the diol (**1a**)].

These data plus the mass spectral fragmentation pattern (Scheme 1) suggested that compound A was a new pentacyclic triterpenoid diol diacetate with a friedelane carbon skeleton [13, 14]. Ion **a** at *m/z* 399, ion **b** at *m/z* 317 and the fragment at *m/z* 257 (*m/z* 317 − 60) indicated the secondary acetoxyl group in ring A probably at C-3 and acetoxymethylene in ring D or E. Therefore, the point of attachment of acetoxymethylene is limited to C-17 or C-20. The possibility that the acetoxymethylene was at C-17 was eliminated and located at C-20 by taking into account the ion **d** at *m/z* 183 and the low intensity of the fragment at *m/z* 455 $[M-CH_2OAc]^+$ [14]. The base peak at *m/z* 123 might be formed by the loss of acetic acid from ion **d**. The two proton singlet at δ3.67 in the ^1H NMR spectrum of compound A can be assigned to C-20α acetoxymethylene protons [14]. Thus, the structure of Compound **1** was assigned as friedelane-3β,30-diol diacetate (**1**). This structure was confirmed by oxidation of **1a** with Jones reagent to give a keto acid, identified as polpunonic acid (**1b**) [15] by direct comparison with an authentic sample.



Scheme 1

Compound **2** analysed for $C_{32}H_{54}O_3$. Its IR spectrum showed absorption bands at 3500 cm^{-1} (OH) and 1730 cm^{-1} (CO). The ^1H NMR spectrum showed seven C-methyl groups between δ0.88 and 1.21, an acetate methyl group at δ1.98 and a broad signal for three protons at δ3.68 (H-3 and H_2-30) Compound **2** formed a diacetate [^1H NMR· δ1.96, 2.0, 3 67 (*s*, H_2-30) and 4.82 (*br s*, H-3)] and on alkaline hydrolysis afforded a diol [^1H NMR: δ3 25 (*s*, H_2-30), 3 70 (*br s*, H-3)]. The diacetate and the diol of compound **2** were found to be identical with **1** and **1a** respectively by direct comparison. Therefore, compound **2** was a friedelane diol monoacetate with a secondary hydroxyl and a primary acetoxyl groups Thus, the structure of compound **2** was assigned as 30-acetoxyfriedelan-3β-ol (**2**).

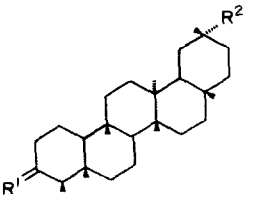
Compound **3**, analysed for $C_{32}H_{54}O_3$ and was found to be isomeric with compound **2**. The IR spectrum showed absorption bands at 3450 cm^{-1} (OH) and 1735 cm^{-1} (CO). The ^1H NMR spectrum showed signals for seven C-methyl groups (δ0.78–1 21), a secondary acetoxyl [δ2.04 and 4.88 (H-3)] and a hydroxymethylene [δ3 27 (*s*, H_2-30)]. The acetylated and hydrolysed products of Compound **3** were identified as **1** and **1a** respectively by direct comparison. Therefore, compound **3** was assigned the structure 3β-acetoxyfriedelan-30-ol (**3**).

It is very interesting to note that although all of the possible acetates of friedelane-3β,30-dol (**1a**) have been isolated from the *n*-hexane extract of the stems of *E. antiquorum*, we could not detect the presence of the diol **1a** itself

EXPERIMENTAL

Mps: uncorr ^1H-NMR 90 MHz as $CDCl_3$ soln with TMS as int. standard. Acme's silica gel (100–200 mesh) was used for CC

Isolation procedure. The stems of *E antiquorum* collected near Ongole in Andhra Pradesh, India were dried and powdered The powder (4 kg) was extracted continuously with *n*-hexane (bp 60–80°) in a large aspirator bottle The dark brown *n*-hexane extract yielded a solid (5 g) which was removed by filtration and sepd into friedelan-3β-ol (1 8 g), taraxerol (1.2 g) and friedelan-3α-ol (0 4 g) by CC over silica gel (80 g) The filtrate was evapd under red pres and the resultant gummy residue (35 g) was chromatographed over a column of silica gel (600 g). In all 205 fractions (each 800 ml) were collected and monitored by TLC The CC fractions were as follows· 1 (*n*-hexane), 2 (*n*-hexane), 3 (*n*-hexane–C_6H_6, 19.1), 4 (*n*-hexane–C_6H_6, 9 1), 5 (*n*-hexane –C_6H_6, 4 1), 6 (*n*-hexane–C_6H_6, 7·3), 7 (*n*-hexane–C_6H_6, 1 1), 8 (*n*-hexane–C_6H_6, 1·3), 9 (C_6H_6), 10 (C_6H_6), 11 (C_6H_6), 12 (C_6H_6–EtOAc, 19 1), 13 (C_6H_6–EtOAc, 19 1), 14 (C_6H_6–EtOAc, 4 1), 15 (EtOAc). Crystallization of fractions 1 and 2 ($CHCl_3$–MeOH) afforded taraxeryl acetate (120 mg) and friedelan-3β-yl acetate (80 mg) respectively. Fraction 3, upon rechromatography over a column of silica gel followed by repeated fractional crystallization ($CHCl_3$–MeOH), yielded lupeol cinnamate (20 mg), moretenone (15 mg), taraxerone (40 mg) and lupenone (10 mg) Fraction 4 gave friedelan-3β-ol (2 g). Fraction 5 was acetylated with Ac_2O–pyridine on a steam-bath for 3 hr The usual work-up followed by fractional crystallization ($CHCl_3$–MeOH) afforded β-amyrin acetate (1.4 g) and lupeol acetate (600 mg) The residue, on CC, yielded euphyl acetate (160 mg) and cycloartenyl acetate (80 mg) Fractions 6–8 yielded taraxerol (1.2 g), friedelan-3α-ol (700 mg) and β-sitosterol (2 g) respectively. Fractions 9–11 gave compounds **1** (50 mg), **2** (30 mg) and **3** (35 mg) respectively Fractions 12–14 afforded the C-24 epimers of cycloart-25-ene-3β,24-diol (40 mg), cycloartane-3β,25-diol (20 mg) and ψ-taraxastane-3β,20-diol (60 mg) respectively Fraction 15 was crystallized from C_6H_6 to give colourless crystals identified as a diterpenoid. 7-hydroxyingol-3,12-di-*O*-acetyl-8-tigliate (450 mg)



	R¹	R²
1	OAc, H	CH_2OAc
1a	OH, H	CH_2OH
1b	O	COOH
2	OH, H	CH_2OAc
3	OAc, H	CH_2OH

Identification of all the known compounds is based on their physical and spectroscopic characteristics (IR, ^1H NMR and MS) and also by direct comparison with the authentic samples wherever possible

Compound **1** (friedelane-3β,30-diol diacetate, **1**). Crystallized from $CHCl_3$–MeOH as colourless plates (R_f 0 37 in C_6H_6), mp 268–269°, $[\alpha]_D^{30}$ +23.5° ($CHCl_3$, *c* 0 85). (Found C· 77 40, H, 10.52 $C_{34}H_{56}O_4$ requires C, 77 27, H, 10 61%), IR ν_{max}^{KBr} cm^{-1} 1730 ^1H NMR δ0 76, 0 87, 0 91, 1 02, 1.21 (21H, 7 × Me), 1 96 (3H, *s*), 1 99 (3H, *s*), 3.67 (2H, *s*), 4 83 (1H, *br s*), MS *m/z* (rel int.). 528 $[M]^+$ (6), 514 (3), 513 (8), 469 (8), 468 $[M-AcOH]^+$ (20), 455 $[M-CH_2OAc]^+$ (0.4), 454 (5), 453 (12), 399 (1), 346 (2), 331 (2), 318 (16), 317 (30), 292 (13), 286 (3), 278 (88), 275 (7), 263 (2), 257 (5), 231 (31), 217 (19), 267 (18), 205 (10), 203 (18), 189 (24), 183 (17), 177 (48), 175 (26), 161 (19), 149 (32), 147 (43), 145 (24), 135 (43), 123 (100), 109 (81), 107 (62), 95 (84), 81 (67), 67 (36), 55 (38), 43 (86)

Alkaline hydrolysis of compound **1** Compound **1** (25 mg) in C_6H_6 (5 ml) was refluxed with 6% alcoholic KOH for 4 hr on a steam bath. The usual work-up followed by crystallization from $CHCl_3$–MeOH afforded colourless needles (**1a**, 18 mg), mp 294–296°, $[\alpha]_D^{30}$ +13 9° ($CHCl_3$, *c* 1), (Found C, 81 00, H, 11 68 $C_{30}H_{52}O_2$ requires· C, 81 08; H, 11 71%), IR ν_{max}^{KBr} cm^{-1} 3500 (*br*); ^1H NMR: δ0 87, 0 97, 1 03, 1 22 (21H, 7 × Me), 3 26 (2H, *s*), 3.72 (1H, *br s*)

Jones oxidation of **1a**. The diol **1a** (10 mg) was dissolved in Me_2CO (5 ml) and treated with Jones reagent at room temp for 6 hr The usual work-up followed by crystallization (C_6H_6) gave colourless crystals (7 mg), mp 260–261°, $[\alpha]_D^{30}$ −40 2° ($CHCl_3$, *c* 0.6) identical with authentic polpunonic acid (**1b**)

Compound **2** (30-*acetoxyfriedelan-3β-ol*, **2**) Crystallized from $CHCl_3$–MeOH as colourless plates, (R_f 0 23 in C_6H_6), mp 238–240°, $[\alpha]_D^{30}$ +27 5° ($CHCl_3$, *c* 0 8) (Found. C, 78.92; H, 11 15 $C_{32}H_{54}O_3$ requires· C, 79 01, H, 11 11%), IR ν_{max}^{KBr} cm^{-1} 3500, 1730, ^1H NMR· δ0.88, 0 94, 1 00, 1 02, 1 21 (21H, 7 × Me), 1 98 (3H, *s*), 3.68 (3H, *br s*)

Acetylation of compound **2** Compound **2** (8 mg) was acetylated with Ac_2O–pyridine (0 5 ml, 1 1) at room temp overnight The usual work-up followed by crystallization ($CHCl_3$–MeOH) gave colourless plates (6 mg), mp 266–267° identical with friedelane-3β,30-diol diacetate (**1**)

Alkaline hydrolysis of compound **2**. Compound **2** (10 mg) in C_6H_6 (2 ml) was refluxed with 6% alcoholic KOH soln for 4 hr on a steam bath. The usual work-up followed by crystallization ($CHCl_3$–MeOH) gave colourless needles, mp 291–293°, identical with friedelane-3β,30-diol (**1a**).

Compound **3** (3β-*acetoxyfriedelan*-30-*ol*, **3**). Crystallized from $CHCl_3$–MeOH as colourless plates (R_f 0.12 in C_6H_6), mp 311–312°, $[\alpha]_D^{30}$ +62.8° ($CHCl_3$, *c* 0.8). (Found: C, 79.12, H, 11.15. $C_{32}H_{54}O_3$ requires: C, 79 01; H, 11.11%); IR ν_{max}^{KBr} cm^{-1}. 3450, 1735; ^{1}H NMR· δ0.78, 0.88, 0 95, 1 02, 1.21 (21H, 7 × Me), 2.04 (3H, *s*), 3.27 (2H, *s*), 4 88 (1H, *br s*) Compound **3**, on acetylation and alkaline hydrolysis as mentioned above, afforded **1** and **1a** respectively.

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