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A TARAXERANE TYPE TRITERPENE FROM *EUPHORBIA TIRUCALLI*

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Key Word Index—*Euphorbia tirucalli*, Euphorbiaceae, euphorginol, taraxer-14-en-6α-ol

Abstract—A new triterpene, euphorginol, has been isolated from the fresh and undried stem bark of *Euphorbia tirucalli*. Its stereostructure has been elucidated as taraxer-14-en-6α-ol through chemical and spectral studies including 2D NMR and NOE difference measurements

INTRODUCTION

Euphorbia tirucalli Linn commonly grows in Asia and Africa. Its latex is used in indigenous medicine as a purgative and a remedy for rheumatism, neuralgia and toothache [1] while the stem bark has long been used in the treatment of colic, asthma and gastralgia [1] Previously we have reported glut-5-en-3β-ol and cycloart-23-en-3β, 25-diol from the fresh and undried stem bark of this plant [2] The present work describes the isolation and stereostructure of a new taraxerane type triterpene named euphorginol

RESULTS AND DISCUSSION

Euphorginol (**1**) gave a molecular ion peak at *m/z* 426.3834 corresponding to the molecular formula $C_{30}H_{50}O$ (calcd 426.3861). The triterpenoid nature of the compound was indicated by a positive Liebermann–Burchard test and a violet coloration with ceric sulphate. The IR spectrum showed absorption for a hydroxyl group (3410 cm^{-1}) and a trisubstituted double bond (3055, 1650 and 810 cm^{-1}). The ^1H NMR spectrum of **1** gave resonances for eight tertiary methyl groups at δ1 12, 0.97, 0.95, 0.92, 0.90, 0 87, 0 84 (all singlets), a pair of doublets at δ5.45 was assigned to olefinic proton while another one proton sextet at δ3.71 could be attributed to a carbinylic proton The ^{13}C NMR spectrum showed thirty carbon atoms The multiplicity assignments were made by DEPT experiments [3, 4] which revealed the presence of 8-methyl, 10-methylene and 5-methine carbon atoms.

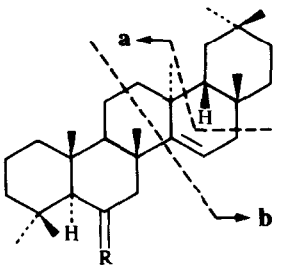
The secondary nature of the alcoholic group in **1** was proved through formation of a crystalline acetate **1a** and also through its oxidation to a ketone **1b** The latter gave a negative Zimmermann test showing the absence of an oxo group at C-3 It could be reduced back to the parent alcohol with sodium isoamyl alcohol indicating the equatorial configuration of the hydroxyl group in **1**.

The mass spectrum of euphorginol was characteristic of the Δ^{14}-taraxerene series of triterpenes [5]. The major fragment (a) at *m/z* 302 was due to retro-Diels–Alder decomposition with collapse of ring D and charge retention on the diene position [5] The ion *a* was accompanied by other fragments resulting from the loss of a methyl group and water, respectively Another abundant ion (**b**) at *m/z* 204 resulted from the removal of an electron from the carbon–carbon double bond, migration of the C-13 methyl group and fission of the 11-12 and 8-14 bonds [5] This ion was accompanied by a satellite 15 mass unit lower due to loss of a methyl group. However, no peak was observed due to loss of a water molecule from ion **b** which confirms the absence of a hydroxyl group in rings D and E [5].

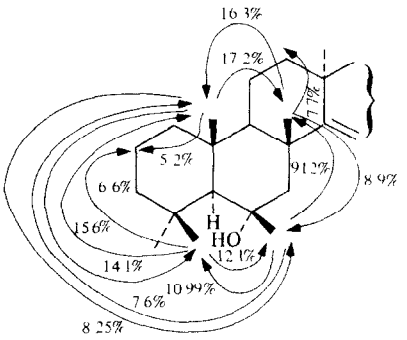
The chemical shifts in the ^1H and ^{13}C NMR spectra of **1** closely resembled those of Δ^{14}-taraxerene derivatives, particularly the chemical shift and multiplicities of the olefinic proton [6, 7] and the chemical shifts of ring C, D and E carbon atoms

The remaining problem was to locate the position of the equatorial hydroxyl group which must be in ring A or B. In the ^1H NMR spectrum of ketone **1b** three protons were exchangeable with D_2O. This suggested the presence of carbonyl group in **1b** and hence the hydroxyl group in **1** at C-6 This was further confirmed by the coupling interaction of the axial carbinylic proton with neighbouring axial and equatorial protons at C-5 and C-7 thereby giving rise to a sextet which was well defined in the 2D *J*-resolved spectrum. The structures of euphorginol and its derivatives could, therefore, be represented by formulae **1**, **1a** and **1b**.

Conclusive evidence for the structure of euphorginol was provided by (i) the heteronuclear ^1H–^{13}C chemical



1 R = αOH, βH
1a R = αOAc, βH
1b R = O



shift correlation spectrum (hetero-COSY) [8] and (ii) the homonuclear ^1H-^1H chemical shift correlated spectrm (COSY 45°) [8] which showed the connectivity of the axial proton at C-6 to protons at C-5 and C-7 and also the connectivity of the olefinic proton at C-15 to the methylenic protons at C-16.

The structure and the stereochemistry of **1**, particularly the α configuration of the hydroxyl group, was confirmed by NOE difference measurements Irradiation at δ 3 71 (6-β H) resulted in a 9.12% NOE at δ 1 10 (26-H_3), a 7 6% NOE at δ 0 97 (25-H_3) and a 10 99% NOE at δ 0 90 (24-H_3) Irradiation at δ 0 90 (24-H_3) resulted in a 12 10% NOE at δ 3 71 (6-β H), a 15 6% NOE at δ 0 97 (25-H_3) and a 6.6% NOE at δ 1 4 (2-H_2). Irradiation at δ 0.97 (25-H_3) resulted in a 8 25% NOE at δ 3 71 (6-β H), a 17 2% NOE at δ 1.10 (26-H_3), a 14 1% NOE at δ 0 90 (24-H_3) and a 5.2% NOE at δ 1.4 (2-H_2) Finally irradiation at δ 1 10 (26-H_3) resulted in a 8 9% NOE at δ 3 71 (6-β H), a 16 3% NOE at δ 0 97 (25-H_3) and a 7 7% NOE at δ 1 37 (12-H_2).

EXPERIMENTAL

General Mps uncorr, HRMS, Finnigan MAT 312 double focusing mass spectrometer with a PDP 11/34 computer system, ^1H and ^{13}C NMR TMS as int ref The DEPT experiments were carried out with θ 45°, 90 and 135 , the quaternary carbons were determined by subtraction of these spectra from the broad band ^{13}C NMR spectrum For NOE measurements, the sample was frozen under liquid nitrogen and degassed A lower decoupler power of maximum 0.2 W with 35 attenuation in dbs was used The pre-irradiation time was 11 sec which is the sum of three delays as used in the NOE difference programme of Bruker The impulse length of 10 μsec was maintained to avoid saturation The 2D COSY-45° data was acquired at 300 MHz with a sweep width of 4000 Hz (2K data points in ω_2) and 2000 Hz (256t_1 values zero-filled to 1K) in ω_1 The heteronuclear 2D ^1H-^{13}C chemical shift correlation experiments were carried out at 300 MHz with a sweep width of 12 820 Hz (2K data point in ω_2) and 1024 Hz (256 t_1 values zero-filled to 2K) in ω_1 In both 2D experiments a 2 sec relaxation delay was used and 16 transients were performed for each t_1 value

Plant material. The plant material was collected from the Karachi region and was identified by the Plant Taxonomist, Department of Botany, University of Karachi where a voucher specimen has been deposited

Extraction and isolation The freshly collected stem bark (20 kg) of *E. tirucalli* was cut in small pieces and extracted with EtOH The EtOH extract was partitioned between EtOAc and H_2O The former fraction was further partitioned into hexane soluble and insoluble portions The hexane soluble portion was subjected to CC over silica gel The elution was carried out with solvent gradient of increasing polarity The eluate from $CHCl_3$–MeOH (49 1) was further subjected to prep TLC on silica gel plates ($CHCl_3$–Et_2O–MeOH, 45 4 1) to yield **1** (95 mg)

Euphorginol (**1**) was obtained as colourless needles by repeated crystallizations from MeOH Mp 168–170°, $[\alpha]_D$ +22 35° ($CHCl_3$, *c* 0 19), IR ν_{max}, cm^{-1} 3410 (OH), 3055, 1650 and 810 (trisubstituted double bond), MS *m/z* (rel int), 426 $[M]^+$ (12), 411 $[M-Me]^+$ (20), 408 $[M-H_2O]^+$ (23), 393 $[M-Me-H_2O]^+$ (10), 302 $[a]^+$ (30), 287 $[a-Me]^+$ (26), 269 $[a-Me-H_2O]^+$ (12), 204 $[b]^+$ (100), 189 $[b-Me]^+$ (42); ^1H NMR ($CDCl_3$, 300 MHz) δ 5 45 (1H, *dd*, *J* = 8.9 and 4 1 Hz, H-15), 3 71 (1H, sext , $J_{ax,ax}$ = 10 4 Hz, $J_{ax\ eq}$ = 4 4 Hz, H-6), 1 12 (3H, *s*, H-26), 0 97 (3H, *s*, H-25), 0 95 (3H, *s*, H-29), 0 92 (6-H, *s*, H-30, H-27), 0 90 (3H, *s*, H-24), 0 87 (3H, *s*, H-23), 0 84 (3H, *s*, H-28), ^{13}C NMR ($CDCl_3$, 75 43 MHz) δ 37 10 (C-1), 26 30 (C-2), 21 70 (C-3), 38.00 (C-4), 56 70 (C-5), 78 98 (C-6), 36 21 (C-7), 38 81 (C-8), 48.63 (C-9), 37 00 (C-10), 17 39 (C-11), 32 98 (C-12), 37 08 (C-13), 158 12 (C-14), 116 81 (C-15), 36 56 (C-16), 38 81 (C-17), 49 18 (C-18), 41 21 (C-19), 28 84 (C-20), 33 58 (C-21), 27 04 (C-22), 27 88 (C-23), 18 11 (C-24), 18 71 (C-25), 30 10 (C-26), 25 80 (C-27), 29 88 (C-28), 33 25 (C-29), 21 21 (C-30)

Acetylation of **1** **1** (25 mg) was treated with a mixture of Ac_2O (5 ml) and pyridine (2 0 ml) at room temp overnight The reaction mixture was then worked-up in usual manner to yield monoacetate **1a** (18 mg), which was crystallized from MeOH Mp 210–212°, $[\alpha]_D$ +32 5° ($CHCl_3$, *c* 0 14), IR ν_{max} cm^{-1} 1735, 1130 (OAc), 3050, 1635 and 815 (trisubstituted double bond), MS *m/z* (rel int) 468 $[M]^+$ (4), 453 $[M-Me]^+$ (6), 408 $[M-AcOH]^+$ (15), 393 $[M-Me-HOAc]^+$ (10), 344 $[a]^+$ (45), 329 $[a-Me]^+$ (23), 269 $[a-Me-HOAc]^+$ (17), 204 $[b]^+$ (90), 189 $[b-Me]^+$ (55), ^1H NMR ($CDCl_3$, 300 MHz) δ 5 57 (1H, *dd*, *J* = 8 6 and 4 6 Hz, H-15), 4 82 (1H, sext , $J_{ax\ ax}$ = 11 0 Hz, $J_{ax\ eq}$ = 4 7 Hz, H-6), 1 13 (3H, *s*, H-26), 1 08 (3H, *s*, H-25), 0 96 (3H, *s*, H-29), 0 91 (6H, *s*, H-30, H-27), 0 90 (3H, *s*, H-24), 0 89 (3H, *s*, H-23), 0 85 (3H, *s*, H-28)

Oxidation of **1** Compound **1** (50 mg) in pyridine (5 ml) was stirred with a suspension of Cr_2O_3 (75 mg) in pyridine (3 ml) at 0° for 3 hr, then at room temp for 12 hr The product was extracted into $CHCl_3$ and recrystallized from Me_2CO to give **1b** as needles (39 mg), mp 285–287°, $[\alpha]_D$ +29 5° ($CHCl_3$, *c* 0 21), IR ν_{max} cm $^{-1}$ 1708 (carbonyl), 3040, 1630 and 815 (trisubstituted double bond), MS *m/s* (rel int) 424 $[M]^+$ (10), 409 $[M-Me]^+$ (16), 300 $[a]^+$ (32), 285 $[a-Me]^+$ (28), 204 $[b]^+$ (45), 189 $[b-Me]^+$ (100), ^1H NMR ($CDCl_3$, 300 MHz) δ 5 29 (1H, *dd*, *J* = 9 1 and 4 4 Hz, H-15), 1 15 (3H, *s*, H-26), 0 99 (3H, *s*, H-25), 0 95 (3H, *s*, H-29), 0 93 (6H, *s*, H-30 and H-27) 0 88 (3H, *s*, H-24), 0 87 (3H, *s*, H-23), 0 84 (3H, *s*, H-28)

Reduction of **1b** Na (100 mg) was added over 1 hr to a refluxing soln of **1b** (20 mg) in isoamyl alcohol (4 ml) and refluxing continued until all the Na had dissolved After steam distillation the ppt was extracted with EtOAc The organic layer was washed with H_2O, dried (Na_2SO_4) and evapd, the residue was crystallized from Et_2O–MeOH to yield (17 mg) euphorginol (**1**), mp 166–170°, $[\alpha]_D$ +21 99° ($CHCl_3$, *c* 0 16)

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