A NEW TRITERPENOID FROM *EUPHORBIA TIRUCALLI*

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A new tetracyclic triterpenoid has been isolated from the fresh latex of *Euphorbia tirucalli* and provisionally named as euphorbinol. The chemical and spectral evidence provided its structure as 13 α, 14 β, 17 α- lanost-8, 25-diene-3β-ol, 24 methyl.

The latex of *Euphorbia tirucalli* is known to contain many sterols and terpenes, among which the four triterpenoids namely, euphoron, euphorol, isoeuphorol and tirucallol, are the major constituents. Euphorone $C_{30}H_{48}O$ was first isolated from the dried latex of South Indian origin by Karimullah and Dutta in 1944 [1,2]. Isoeuphorol ($C_{30}H_{50}O$), was reported from the fresh latex by Siddiqui *et al.* [3], who also noted that reduction of euphoron mainly gives euphorol — the stereomer of isoeuphorol [4]. Later on both isoeuphorol and euphorol were found to be identical with euphol and isoeuphol obtained from euphorbia resinn of unverified source by Newbold and Spring [5]. Tirucallol was isolated from the resin of *Euphorbia tirucalli* [6] and subsequently characterized as 20-epimer of euphol [7]. The other subsidiary triterpenoids of euphol series reported from this and other species of *Euphorbia* are stereochemically related to either euphol (13α, 14β, 17α, 20α, lanost-8,24-diene-3β-ol) or tirucallol (13α, 14β, 17α, 20β-lanost-8,24-diene-3β-ol).

As a result of present studies in the fresh latex of *Euphorbia tirucalli* from the Karachi region, a new tetracyclic triterpenoid has been isolated and provisionally named as euphorbinol. It analysed for $C_{31}H_{52}O$, m.p. 112°, $[\alpha]^{17}_D$ = +54.5^O (as against euphorbol [5], $C_{31}H_{52}O$, m.p. 127°, $[\alpha]^{17}_D$ = 0°).

Its isolation was based on its sparing solubility in acetone, according to the procedure described in the experimental in detail, finally through fractional crystallisation from methanol.

The presence of an alcoholic group in euphorbinol was shown by the formation of monoacetate, m.p. 102–3° (euphorbenyl acetate m.p. 124–25°) [5] and monobenzoate, m.p. 145–46°. Titration of the acetate wih perbenzoic acid indicated the presence of two double bonds which were of unequal reactivity, only one of them being reduced with palladium catalyst giving dihydro-derivative — euphorbinenol, ($C_{31}H_{54}O$) m.p. 122–23°, $[\alpha]^{17}_D$ + 51.5^O in CHCl$_3$ (euphorbenol, m.p. 133–35° $[\alpha]^{15}_D$ = – 15°) [5]. It also formed a dibromo-derivative ($C_{31}H_{52}OBr_2$), m.p.

150°. Euphorbinenol, nevertheless, gives yellow colouration with tetranitromethane indicating the presence of an inert double bond. This conforms to the requirement that doubly unsaturated alcohol of molecular formula $C_{31}H_{52}O$ should have a tetracyclic structure. The IR spectrum of euphorbinenol acetate has only a slight shoulder in the ethylenic region indicating that the inert double bond is tetrasubstituted [8]. In addition the intensity values for the low wavelenght UV extinction (200 nm ϵ2800, 223 nm, ϵ 2400) were consistent with a tetrasubstituted double bond exocyclic to one ring. The UV absorption spectrum corresponded to that of cholest - $\Delta^{8:9}$-enol rather than that of cholest — $\Delta^{8:14}$-enol [9] and even greater resemblance is shown with the UV spectrum of euphorbenol acetate. This suggests that the position of the inert double bond is at 8 (9), as in the case of other triterpenoids of the euphol series.

The secondary nature of the alcoholic group was proved through selective oxidation of euphorbinol with chromic — acetic acid, to a ketone euphorbinadienone ($C_{31}H_{50}O$), m.p. 86–7°. Its IR spectrum showed ethylenic absorption at 1650 cm^{-1} and carbonyl stretching at 1708 cm^{-1}, which together with CH$_2$—CO stretching at 1427 cm^{-1}, showed a ketonic function at position 3 [10]. This was further substantiated by the mass spectrum. Aside of M$^+$ peak at 438, there was a moderate peak at m/e 352 which is characteristic of 3-ketones and arise from the fission of the 1–2 and 4–5 bonds, accompanied by the loss of 2 H atoms, mainly from positions 5 and 6 of the charged fragment [11]. On Meerwein–Pondorff reduction euphorbinadienone gives back the parent alcohol which established the presence of secondary OH at position 3 in euphorbinol. The molecular rotation changes on acetylation of euphorbinadienol is positive and roughly equals those observed for euphorbol, thereby indicating a β configuration for its hydroxyl group [12].

On the treatment with osmium tetraoxide euphorbinol acetate afforded a viccinal dihydroxy compound — euphorbinene triol ($C_{31}H_{54}O_3$), m.p. 170–71°. This on further
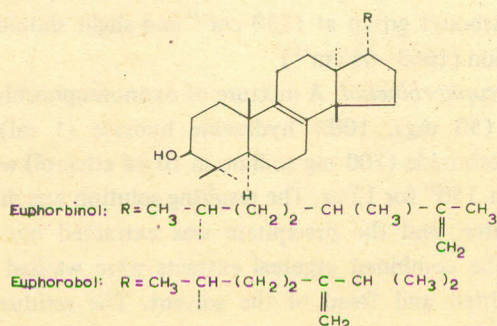
oxidation with lead tetraacetate eliminated formaldehyde and yielded oxonoreuphorbinenol ($C_{30}H_{50}O_2$), m.p. $101 - 2^O$, furnishing evidence for the position of the active double bond in the vinylidene group.

The IR spectrum of euphorbinol showed bands at 3400 cm^{-1} (alcoholic OH); 3071 cm^{-1} (olefinic C–H stretching of vinylidene group); 2860–2960 cm^{-1} (aliphatic C–H stretching) and 1650 cm^{-1} (C=C). A strong band at 1460 cm^{-1} represents C–H bending due to the side-chain while another band at 1376 cm^{-1} is attributed to the C–H bending of the *gem* dimethyl groups. The band at 1040 cm^{-1} is due to the C–OH stretching of β–OH in 6-member-ed ring. Additional bands between 935 and 1180 cm^{-1}, are fairly in agreement with 3β–OH, 5α structure [13]. A strong band at 887 cm^{-1} represents C–H out-of-plane bending of the vinylidene group. The UV spectrum in alcohol was characteristic of nonconjugated heteroannular diene with λ_{max} at 215.

The position 24 could have been considered for the active methylene group in analogy to euphorbol but this was shown to be untenable, because oxonoreuphorbinenol is reduced by the Wolff Kishner method to noreuphorbi-nenol ($C_{30}H_{52}O$), m.p. $90 - 91^O$, which is not identical with euphenol or tirucallenol, the latter being obtainable from euphorbol under parallel condition of working [5,14]. It ·may, therefore, be concluded that the location of the doulbe bond in the side-chain is different from that of· euphorbol.

On treatment with potassium hypobromite, oxonoreu-phorbinenyl acetate is slowly oxidized to an acid charac-terized as its methyl ester (methyl-3 β-acetoxy bisnoreu-phorbinenoate), showing that oxonoreuphorbinenol is a methyl ketone and that the side-chain in euphorbinadienol is terminated by an isopropenyl group.

Taking into account the foregoing observations and the stereochemical analogy with euphorbol and other related triterpenoids of the euphol series, the following structure is provisionally provided for euphorbinol (euphorbinadienol).

Euphorbinol: $R = CH_3 - CH - (CH_2)_2 - CH(CH_3) - C - CH_3$
                                                    ‖
                                                    CH_2

Euphorobol: $R = CH_3 - CH - (CH_2)_2 - C - CH(CH_3)_2$
                                        ‖
                                        CH_2

The mass spectrum showed M^+ peak at 440 (base peak),

followed by diagnostic peaks at *m/e* 425 $(M-CH_3)^+$; 422 $(M-H_2O)^+$ and 407 $(M-CH_3 + H_2O)^+$. The peak at 315 is due to the loss of C_9H_{17} side-chain. The loss of water from this ion gives another peak at 297, while the peak at 313 $(M-side-chain + 2H)^+$ is indicative of monounsatura-tion in the side-chain [15]. The formation of this ion is suppressed by the double bond at $\Delta^{8:9}$ and explains the low intensity of this peak [16]. Another abundant ion at 371 represents the cleavage of the 23–24 bond with hydro-gen transfer to the C_5H_8 neutral fragment. This is a known fragmentation of Δ^{25}-triterpenoids [16, 17] and the posi-tion of double band at Δ^{25} was further supported by peaks at 356 $(M-C_5H_9+ CH_3)^+$ and 353 $(M-C_5H_9 + H_2O)^+$.

The proton NMR spectrum in deuterated chloroform showed olefinic protons (2H, singlet at δ 4.75) *geminal* pro-ton at C-3 (1H, distroted multiplet at δ 3.32), while C-27 methyl group comes as a 3H singlet at δ 1.64. The three proton doublets at δ 0.75 and δ 0.71 represents C-20 and C-28 methyl group respectively. A 3 H singlet at δ 1.08 can be attributed to C-19 anglular methyl group. The peaks for other methyl groups were at δ 0.82, 0.88, 0.90 and 0.98.

In so far, however, as euphorbinenol differs from eu-phorbenol in melting point and optical rotation the two may be regarded as stereoisomers of each other possibly differing in stereochemistry at C-20 or C-24. The actual configuration at these asymmetric centres, therefore, needs further work for its elucidation.

EXPERIMENTAL

The fresh latex of *Euphorbia tirucalli* (ca. 500 ml) was directly tapped from the cuts into a flask containing 500 ml acetone. After keeping overnight in the cold the coagulated insoluble residue was sucked, repeatedly washed with acetone and extracted out with ether. The turbid ethereal extract was treated with a small quantity of metha-nol, and a little darkish yellow resinous mass that separated out in this operation was neglected. The clear light yellow ethereal solution was freed of the solvent, finally in vacuo, and the residue was subjected to a three-cornered fractional crystallization from methanol, when euphorbinol was obtained from the top fractions as colourless shinning need-les, m.p. $111-12^O$. It analyzed for $C_{31}H_{52}O$ with 8 C-methyl groups. (Found: C 84.41; H 11.98, O 3.61, C-methyl 27.19%, M^+ peak at 440. Calcd for $C_{31}H_{52}O$: C 84.545, H 11.912%; O 3.636; 8 C-methyl, 27.272%, mol. wt. 440).

Euphorbinadienyl Acetate. Euphorbinol (100 mg) was refluxed with 1 ml pyridine and 2 ml acetic anhydride for 2 hr. The reaction mixture was worked up in the usual

manner to yield euphorbinadienyl acetate — colourless fine needles from methanol, m.p. 102–3°; $[\alpha]_D^{23^O}$ = + 72.5^O. (Found: C 82.2, H 11.0. Calcd for $C_{33}H_{54}O_2$. C 82.157, H, 11.292%).

Euphorbinadienyl Benzoate. Euphorbinol (100 mg) was refluxed with 1 ml pyridine and 1 ml benzoyl chloride for 1 hr. The product was worked up to yield colourless needles from methanol, m.p. 141–3°. (Found: C 83.73, H 10.41%. Calcd for $C_{38}H_{56}O_2$: C 83.823, H 10.376%). The IR spectrum in KBr showed ethylenic absorption at 1650 cm^{-1} and carbonyl band at 1720 cm^{-1}.

Perbenzoic Acid Titration of Euphorbinadienyl Acetate. To 40 mg euphorbinadienyl acetate in 10 ml of CHCl$_3$ at 0° was added 5 ml 0.3N perbenzoic acid in benzene. The reaction mixture was allowed to stand at 0° in the dark. At the end of 48 hr an aliquot was withdrawn and added to 25 ml water containing 1.5 g KI and 1.5 ml glacial acetic acid. The liberated iodine was titrated against a standard solution of sodium thiosulphate. A blank was similarly treated. The sample consumed the equivalents of 1.99 double bond.

Euphorbinenol. Euphorbinol (100 mg) was taken up in absolute alcohol and hydrogenated over palladium catalyst at the atmospheric pressure for 24 hr. The solution was filtered, freed of solvent and the residue crystallized from methanol to afford fine colourless needles of euphorbinenol, m.p. 121–22°. (Found: C 84.3, H 12.27%, M$^+$ peak 442. Calcd for $C_{31}H_{54}O$: C 84.162, H 12.314%, mol. wt. 442). The IR spectrum in KBr showed OH group at 3420 cm^{-1} and slight shoulder in the ethylenic region at 1663–1665 cm^{-1}.

Euphorbinol Dibromide. Euphorbinol (100 mg) was dissolved in CHCl$_3$ and titrated against 0.5% solution of bromine in chloroform at 0°. The absorption was very rapid and the solution showed excess of bromine when 1.1 mole had been added. The solvent was carefully removed in rotary evaporator and the residue crystallized from methanol in cream-coloured slender needles of dibromide, m.p. 149–50°. On debromination with sodium idoide in alcohol it afforded back the parent alcohol. (Found: C 61.89; H 8.91, Br 26.34%. Calcd for $C_{31}H_{52}OBr_2$: C 62, H 8.73, Br 26.666%). The IR spectrum showed OH group at 3420 cm^{-1}, C–Br stretching at 670 cm^{-1} and slight shoulder in the region of double bond (1665 cm^{-1}).

Euphorbinadienone. Euphorbinol (100 mg) in 5 ml benzene was stirred for 48 hr at room temperature with chromium trioxide (150 mg) in water (2 ml) and acetic acid (10 ml). Methanol (2 ml) was added, and the lower layer was separated from the benzene solution and repeatedly extracted with fresh benzene. The combined benzene extracts were washed with water and aqueous sodium

bicarbonate. The solvent was removed and the residue chromatographed over alumina. Elution with petroleum ether—benzene gave a solid which on repeated crystallizations from methanol furnished fine colourless needles of euphorbinadienone, m.p. 86–87°. (Found: C 84.9, H 11.3, M peak at 438. $C_{31}H_{50}O$ requires: C 84.931, H 11.506%, mol. wt. 438). On Wolff Kishner reduction it yielded back the parent alcohol.

Euphorbinenetriol. To a solution of euphorbinadienyl acetate (182 mg) in ether was slowly added an ethereal solution of osmium tetraoxide (100 mg). The resulting blackish solution was kept for six days till the completion of the reaction which was checked from time to time on TLC, and the solvent was distilled off. The darkish residue was taken up in a mixture of benzene—ethanol and refluxed with the addition of alcoholic potash (0.4 g) and mannitol (0.6 g) for 6 hr. After removal of the solvent the residue was refluxed with alcoholic potash for a period of 4 hr. The alkaline alcoholic refluxate was stumped down with ammonia to about 8 pH and then freed of the solvent in vacuo. The residue was extracted out with ether and the ethereal solution was washed with water, dried and chromatographed over neutral alumina. Elution with ether yielded a crystalline residue which on repeated crystallizations from ether—petroleum ether gave euphorbinenetriol as colourless needles, m.p. 170–171°. (Found: C 78.1, H 11.52%. $C_{31}H_{54}O_3$ requires: C 78.48, H 11.483%). The IR spectrum showed OH group at 3400 cm^{-1} and slight shoulder in the region of C=C (1663–65 cm^{-1}).

Oxonoreuphorbinenol. Euphorbinenetriol (40 mg) in acetic acid (4 ml) was treated with lead teteracetate (60 mg) in acetic acid (6 ml). After keeping the reaction mixture at 25° for 6 hr, water was added, and the resulting precipitate filtered, dried and chromatographed over alumina. Elution with petroleum ether—benzene (1:1) gave a solid which crystallized from methanol to yield colourless silky needles of oxonoreuphorbinenol, m.p. 108–9°. (Found: C 81.75, H 11.21%. $C_{30}H_{50}O_2$ requires: C 81.447. H 11.402%). The IR spectrum showed OH group at 3400 cm^{-1}, carbonyl group at 1730 cm^{-1} and slight shoulder in C=C region (1663–65 cm^{-1})

Noreuphorbinenol. A mixture of oxonoreuphorbinenyl acetate (50 mg), 100% hydrazine hydrate (1 ml) and sodium ethoxide (100 mg sodium in 10 ml ethanol) was refluxed at 150° for 12 hr. The resulting solution was diluted with water, and the precipitate was extracted out with ether. The combined ethereal extracts were washed with water, dried and freed of the solvent. The residue was treated with acetic anhydride and pyridine. The acetylated product thereby obtained was subjected to column chromatography over neutral alumina. From the petroleum ether

eluate a crystalline acetylated product was obtained which on alkaline hydrolysis yielded noreuphorbinenol in colourless fluffy needles, on crystallization from methanol, m.p. 90–91°. (Found: C 84.3, H 12.15%. $C_{30}H_{52}O$ requires: C 84.112, H 12.246%). The IR spectrum showed OH group at 3400 cm^{-1} and slight shoulder in the region of C=C (1663–65 cm^{-1}).

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