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FURTHER STUDIES IN THE FRESH LATEX OF *EUPHORBIA TIRUCALLI*

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Apart from α-amyrine and cycloartenol, a new sterol has been isolated from the fresh latex of *Euphorbia tirucalli* and provisionally named as euphorbosterol. The chemical and spectral evidence established it as a distinct stereomer of stigmasterol.

INTRODUCTION

The isolation and structures of two new triterpenes (euphorbinol and cycloeuphornol) from the fresh latex of *Euphorbia tirucalli,* has been communicated earlier [1,2]. Following further studies in the constituents of the latex, a new sterol has been isolated and provisionally named as euphorbosterol. Its isolation was based on column chromatogrphy of the acetone insoluble factor of the latex, which in *Euphorbia tirucalli,* has not been reported earlier. Euphorbosterol forms colourless shining needles analyzed for $C_{29}H_{48}O$, m.p. 155°; $(\alpha)_D^{22} = -32°$ in $CHCl_3$.

The presence of an alcoholic group in euphorbosterol was shown by the formation of monoacetate: m.p. 132°, $(\alpha)_D^{22} = -42°$ in $CHCl_3$ and monobenzoate: m.p. 126°, $(\alpha)_D^{22} = -28°$ in $CHCl_3$. Titration of the acetate with perbenzoic acid indicated the presence of two double bonds. The bromination of the acetate in ether–acetic acid afforded tetrabromo euphorbosteryl acetate m.p. 170°.

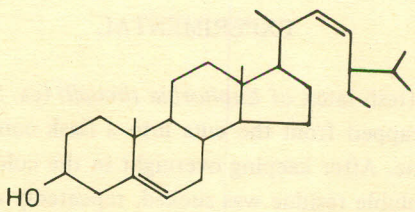
The nature of the alcoholic group was shown to be secondary through selective oxidation of euphorbosterol with CrO_3– acetic acid to a ketone which gave a single spot on TLC and M^+ peak at 410 corresponding to the formula $C_{29}H_{46}O$.

The IR spectrum of euphorbosterol showed OH stretching 3380–3400 cm^{-1}, olefinic C–H stretching at 3070 cm^{-1}, aliphatic C–H stretching 2900–2960 cm^{-1}, and C=C at 1650 cm^{-1}. A strong band at 1460 cm^{-1} represents C–H bending vibration due to the aliphatic side chain while another band at 1370–1380 cm^{-1} is attributed to C–H bending vibration of terminal isopropyl group. The band at 1070–1080 cm^{-1} is due to C–OH stretching of secondary carbinol in 6 membered ring. Additional peaks at 1200 cm^{-1}, 1020 cm^{-1}, 970–72 cm^{-1}, 960, 840 and 800 cm^{-1}, are farily in agreement with 3 β hydroxy Δ^5 sterolic skeleton [3,4].

Mass spectral studies provided molecular ion peak at m/e 412 and other common sterolic peaks at m/e 397 $(M\text{-}CH_3)^+$, 394 $(M-H_2O)^+$, 379 $(M\text{-}H_2O+CH_3)^+$, 369 (M–terminal isopropyl group)$^+$ and 351 (M–isopropyl group + $H_2O)^+$. The base peak at m/e 273 and strong peaks at m/e 246 (M–side chain+27)+; 228 (M–side chain + 27–H_2O); 231 (M–side chain+42)$^+$; 213 (M–side chain+ 42+$H_2O)^+$, supported the presence of 1 double bond in the nucleus [5,6]. The prominent peaks at m/e 355 $(M-57)^+$ and 57 allowed the placement of double bond in the position Δ^5 [7,8].

The strong peaks at m/e 271 (M–side chain+2H)$^+$ and 253 (M–side chain +2H+$H_2O)^+$ are indicative of monounsaturation in the $C_{10}H_{19}$ side chain [5,6]. Another abundant ion at m/e 300 represents the cleavage of the C–20 to C–22 bond with the hydrogen transfer to the C_8H_{16} neutral fragment. This is a known fragmentation of Δ^{22} sterols [5,6], and the position of double bond at Δ^{22} was further supported by peaks at m/e 285 $(M-C_8H_{16}+CH_3)$ and 282 $(M-C_8H_{16}+H_2O)^+$.

On the basis of foregoing evidence the structure of euphorbosterol can be represented as:



Proton NMR spectrum in deuterated $CHCl_3$ lends further support to the above structure. A one proton triplet at δ 5.38 is due to olefinic proton at C–6, split by

adjacent methylene protons at C–7. A slightly upfield two proton signal comes at δ 5.18 and represents C–22 and C–23 olefinic protons of the side chain. The one proton distorted multiplet at δ 3.68 is attributed to geminal proton at C–3, shifted downfield due to electron withdrawing hydroxyl group. The sharp 3 proton singlets at δ 0.7 and δ 1.02 represent C–18 and C–19 methyl groups [9,10] while C–21 methyl group comes as a distorted doublet at δ 0.94 [9,10]. The six methyl protons of isopropyl group occur together as undifferentiated signals between δ0.85–0.89. Another distorted triplet at δ 0.87 is of C–29 methyl group [9,10].

The molecular formula and spectral data corresponded to those of stigmasterol. In view of the fact, however, that the physical properties of euphorbosterol and its derivatives widely varied from those of stigmasterol and other related sterols [11–13] as brought out in Table 1, euphorbosterol appears to be distinct stereoisomer of stigmasterol. The heavy depression of its mixed melting point with an authentic sample of stigmasterol lends support to this conclusion.

Table I

Substance	M.P. (°C)	$(\alpha)_D$ in $CHCl_3$
Euphorbosterol	154	–32°
Poriferasterol	156	–46°
Stigmasterol	170	–51°
Euphorbosteryl acetate	132	–42°
Poriferasteryl acetate	147	–53°
Stigmasteryl acetate	144	–55.50°
Euphorbosteryl benzoate	126	–28°
Poriferasteryl benzoate	140	–22°
Stigmasteryl benzoate	160	–

The actual configuration of euphorbosterol, therefore, needs further work for its elucidation.

EXPERIMENTAL

The fresh latex of *Euphorbia tircualli* (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 ether fraction afforded euphorbinol by following the procedure recorded in the previous communication [1]. The gummy residue left on removal of solvent from its mother liquors was acetylated with acetic anhydride and pyridine. The acetylated product was taken up in light petrol and chromatographed over neutral alumina. The elution was successively carried out with petroleum ether, petroleum ether-benzene, benzene, benzene-ether, and acetone.

The residue left on removal of the solvent from combined pet–ether–benzene fractions gave a crystallizate which on repeated crystallizations furnished colourless leaflets of acetate melting at 223–25°. Its alkaline hydrolysis gave the free alcohol which crystallized out from ethanol as colourless needles m.p. 186°, $(\alpha)_D$=83° in $CHCl_3$. It analyzed for $C_{30}H_{50}O$ (Found: C, 84.6; H, 11.9 and M^+ peak 426. Calcd. for $C_{30}H_{50}O$: C, 84.4; H, 11.8 and mol. wt. 426). The molecular formula, melting point and optical rotation corresponded to those of α-amyrine. The identity was further substantiated through superimposable spectral data, mixed melting point as well as formation of crystalline acetate: m.p. 223–25° (lit. m.p. 225–26° [14]); benzoate: m.p. 194–95° (lit m.p. 195–96° [14]); and methyl ether: m.p. 219–21° (lit. m.p. 221–222° [15]).

The benzene–ether fractions gave a crystalline residue, repeated crystallizations of which from chloroform methanol afforded a crystalline acetate as plates m.p. 122°. Its alkaline hydrolysis with methanolic alkali gave the free alcohol which crystallized from ethanol as colourless needles m.p. 115° (after drying *in vacuo* at 65°). It showed $(\alpha)_D$ + 54° in $CHCl_3$ and analyzed for $C_{30}H_{50}O$ (Found: C, 84.3; H, 11.7 and M^+ peak 426. Calcd. for $C_{30}H_{50}O$: C, 84,4; H, 11.8 and mol. wt. 426). The molecular formula, melting point and optical rotation corresponded to cycloartenol. The identity was confirmed through superimposable spectral data as well as formation of crystalline acetate m.p. 122° (lit. m.p. 122–23° [16,17]); benzoate: m.p. 128–29° (lit. m.p. 129–30° [16,17]); dibromo derivative: m.p. 180–81° (lit.m.p. 182–83° [16,17]).

The acetonic eluates gave a yellowish residue, the acetylated product of which failed on hydrolysis to give a uniform product. It was, therefore, subjected to rechromatography following the procedure described above. Repeated crystallizations of the acetate from methanol finally yielded a uniform product with a single TLC spot, melting at 122°. Alkaline hydrolysis with methanolic alkali afforded euphorbosterol as colourless shining needles from methanol, m.p. 154°. It analyzed for $C_{29}H_{48}O$ (Found C, 84.5; H, 11.5 and M^+ peak at 412. Calcd. for $C_{29}H_{48}O$: C, 84.4; H, 11.6 and mol. wt. 412).

Characterization of Euphorbosterol

Euphorbosteryl Acetate. Euphorbosterol (100 mg) was refluxed with 1 ml pyridine and 2 ml freshly distilled acetic anhydride for 2 hr. The reaction mixture was worked up in

the usual manner to yield euphorbosteryl acetate—colourless needles m.p. 122°; $(\alpha)_D = -42°$. (Found: C, 81.8; H, 11.1. Calcd. for $C_{31}H_{50}O_2$: C, 81.9; H, 11.0). The IR spectrum showed bands at 3071 cm^{-1} (olefinic C–H stretching); 1720 cm^{-1} (C=O) and 1640–50 cm^{-1} (C=C).

Euphorbosteryl Benzoate. 100 mg of euphorbosterol was refluxed with 1 ml pyridine and 1 ml benzoyl chloride for 2 hr. On working up in the usual manner the reaction product yielded colourless needles of euphorbosteryl benzoate from methanol, m.p. 126°; $(\alpha)_D$ –28° (Found: C, 83.8; H, 10.2. Calcd. for $C_{36}H_{52}O_2$: C, 83.7; H, 10.0).

Perbenzoic Acid Titration of Euphorbosterol Acetate. To 40 mg of euphorbosteryl acetate in 10 ml of $CHCl_3$ at 0° was added 1 ml of 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 of 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 two double bonds.

Tetrabromo Euphorbosteryl Acetate. Euphorbosteryl acetate (100 mg) was dissolved in ether and titrated against 0.5% ethereal solution of bromine with the addition of some glacial acetic acid at 0°. The absorption was very rapid and the solution showed excess of bromine when 2.1 mole had been added. The residue, left on carefully removing the solvent in rotary evaporator, crystallized from ethanol in colourless needles of tetrabromo euphorbosteryl acetate, m.p. 170°. (Found: Br, 41.6. Calcd. for $C_{31}H_{50}O.Br_4$; Br, 41.3).

Oxidation of Euphorbosterol. Euphorbosterol (50 mg) in acetic acid (2 ml) was stirred at room temperature with chromium trioxide (75 mg) in acetic acid (4 ml) added during half an hour. The product was worked up in the usual way, and the pure ketonic derivative was obtained through preparative thin layer chromatography. It showed M^+ peak at 410 corresponding to molecular formula $C_{29}H_{46}O$. The quantity was not sufficient for further characterization.

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