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ISOLATION AND STRUCTURE OF CYCLOEUPHORNOL, A NEW TRITERPENE FROM *EUPHORBIA TIRUCALLI*

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Apart from lanosterol, a new triterpene has been isolated from the fresh latex of *Euphorbia tirucalli* and provisionally named as cycloeuphornol. Chemical and spectral studies have provided evidence for its structure as 9:19-cyclolanost - 9 β .20(22),25-diene-3 β -ol, 24 methyl-[20(22)-dehydro cyclolaudenol].

The isolation and structure of a new triterpene (euphorbinol) from the fresh latex of *Euphorbia tirucalli*, has been communicated earlier [1]. Following further studies in the constituents of the latex, another new triterpenoid has been isolated and provisionally named as cycloeuphornol. The procedure of its isolation, details of which are described in the experimental, was based on column chromatography of the acetone-soluble factor of the latex, which also yielded lanosterol, the presence of which in the E.T. latex has not been reported earlier. Cycloeuphornol forms slender needles, melts at 95 $^{\circ}$; $[\alpha]_D + 46^{\circ}$ (CHCl₃) and analyzed for C₃₁H₅₀O.

The presence of an alcoholic group in cycloeuphornol was shown by the formation of monoacetate, m.p. 138 $^{\circ}$, and monobenzoate, m.p. 152 $^{\circ}$. Titration of the acetate with perbenzoic acid indicated the presence of two double bonds. On reduction with palladium catalyst, it absorbs two moles of hydrogen, giving the saturated alcohol-cycloeuphornanol. C₃₁H₅₄O, m.p. 133–34 $^{\circ}$, $[\alpha]_D + 43^{\circ}$. It was further characterized through its acetate, m.p. 132 $^{\circ}$, $[\alpha]_D + 50^{\circ}$. Cycloeuphornadienol also forms a tetrabromo-derivative C₃₁H₅₀OBr₄, m.p. 126–27 $^{\circ}$.

The nature of the alcoholic group was shown to be secondary through selective oxidation of cycloeuphornanol with CrO₃-acetic acid to a ketone-cycloeuphornanone C₃₁H₅₂O, m.p. 122–23 $^{\circ}$. Its IR spectrum showed carbonyl stretching at 1710 cm⁻¹, which together with CH₂-CO stretching at 1426 cm⁻¹, showed a ketonic function at position 3 [2]. On Meerwein-Ponndorf reduction cycloeuphornanone gives back the parent alcohol which established the presence of secondary OH at position 3 in cycloeuphornadienol and cycloeuphornanol. Cycloeuphornadienone could not be prepared by this method due to the facile migration of double bonds in cycloeuphornol. On acetylation the

molecular rotation of cycloeuphornadienol changes from +201 to +259 and of cycloeuphornanol from +190 to +242. The positive direction roughly equals those observed for cyclolaudenol [3](from +206 to +265) thereby indicating a β -configuration for their hydroxyl group.

The presence of a cyclopropane ring in cycloeuphornadienol was confirmed by treatment of cycloeuphornanyl acetate with hydrogen chloride which gave an acetate mixture, m.p. 147–53 $^{\circ}$. In contrast to cycloeuphornanyl acetate it gives a strong yellow colour with tetranitromethane and shows ethylenic absorption in UV. From this mixture, through fractional crystallization, a homogenous compound was isolated which gives a strong yellow colour with tetranitromethane reagent, melted at 173–74 $^{\circ}$, $[\alpha]_D + 81^{\circ}$ and analyzed for C₃₃H₅₆O₂. It could be identified as laudenyl acetate, obtained from cyclolaudenol under parallel conditions of working [3]. The IR spectra of cycloeuphornadienol and its derivatives in CCl₄, show a band at 3040 cm⁻¹ which is characteristic of a methylene group included in a cyclopropane ring [4].

The UV spectrum of cycloeuphornadienol showed λ_{max} in ethanol at 212 nm which is typical of nonconjugated heteroannular dienes. The IR spectrum of cycloeuphornadienol showed bands at 3400 cm⁻¹ (OH group); 3071 cm⁻¹ (C-H olefinic stretching); 3040 cm⁻¹ (CH₂ asym. stretching in cyclopropane ring); 2900–2940 cm⁻¹ (aliphatic C-H stretching) and 1640–50 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 six-membered ring, while that at 1015 cm⁻¹ represents ring vibration of alkyl cyclopropane. Additional bands between 935–1180 cm⁻¹ are fairly in agreement with 3 β -OH, 5 α structure

[5]. Evidence for a vinylidene group was provided by the bands at 3071 and 887 cm^{-1} , the latter representing C—H out-of-plane bending in the grouping $\text{C}=\text{CH}_2$. Other bands at 840, 820 and 800 cm^{-1} indicate the presence of additional double bond in the grouping $\text{C}=\text{C}$.

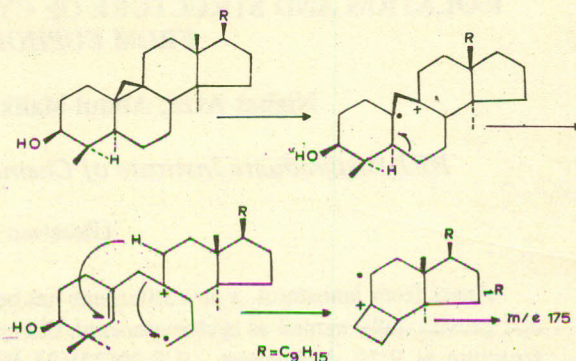
The proton NMR spectrum of cycloephornadienol in deuterated chloroform showed a one-proton triplet at δ 5.2 for the trisubstituted double bond present in the environment $\text{C}=\text{CH}-\text{CH}_2$. The vinylidene group comes as a 2-proton singlet at δ 4.7. A distorted triplet at δ 3.32 represents the *geminal* proton at C-3, while six-proton singlet at δ 1.66 is attributed to two methyl groups shifted downfield due to double bond. A pair of doublets at δ 0.33 and 0.58 is indicative of the cyclopropane ring bearing two non-equivalent hydrogen atoms [6]. Other methyl groups occur in the high field region from δ 0.8 to 1.1 and the integration curve counts 15 protons corresponding to five methyl groups.

The key to the structure of cycloephornadienol was provided by its tetrahydro product—cycloephornanol. Its physical data were found to be identical with that of cyclolaudenol ($\text{C}_{31}\text{H}_{54}\text{O}$) which is the corresponding reduction product of cyclolaudenol ($\text{C}_{31}\text{H}_{52}\text{O}$) reported from the alkaloid-free fraction of opium by Spring *et al.* [3]. The melting point and optical rotation of cycloephornanyl acetate agree with those reported for cyclolaudanyl acetate [3]. Moreover, the acetates of both cycloephornanol and cyclolaudenol yield the same laudenyl acetate on treatment with hydrogen chloride. In view of these findings it may be concluded that cycloephornadienol has the same basic skeleton and stereochemistry as cyclolaudenol, the former differing from the latter in having two double bonds instead of one.

The structural similarity of cycloephornol and cyclolaudenol was also indicated by the mass spectrum of cycloephornol. Aside of M^+ peak at m/e 438, it showed diagnostic peaks at m/e 423 ($\text{M}-\text{CH}_3$) $^+$; 420 ($\text{M}-\text{H}_2\text{O}$) $^+$; and 407 ($\text{M}-\text{H}_2\text{O} + \text{CH}_3$). The peak at m/e 315 is common to cyclolaudenol and results from the direct loss of C_9H_{15} side chain. The loss of water molecule from this ion gives another peak at 297. This suggested that cycloephornol has the same nuclear structure and differs only in the nature of the side chain.

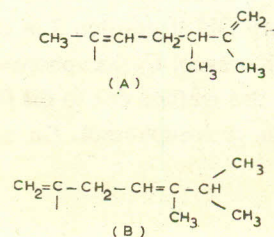
An abundant ion at m/e 298 represents the fragmentation induced by a cyclopropane ring in 9,19-cyclosterols and related tetracyclic teriterpenes [7, 8]. The strain imposed on ring B is relieved by opening of the 9, 10 - bond followed by cleavage of the 5,6 - bond and McLafferty rearrangement. The loss of the side chain from this ion gives a pronounced peak at m/e 175 as in the case of cyclolaudenol. The formation of such ions may be represented

on the following pattern.

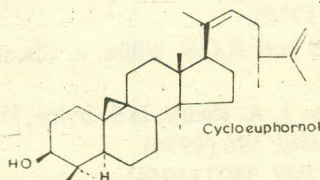


The presence of unsaturation in the side chain is indicated by the peak at 313, due to the loss of the side chain and 2 H atoms [8] from the nucleus. The elimination of a molecule of water from this ion gives another peak at 295. The greater intensity of these peaks as compared to cyclolaudenol is due to the presence of two double bonds in the side chain of cycloephornol in contrast to one in cyclolaudenol.

The presence of a free methylene group in the side chain was confirmed through hydroxylation of cycloephornadienol with osmium tetroxide followed by oxidative cleavage with lead tetraacetate. A mixture of oxidation products was formed from which formaldehyde was isolated by steam distillation and identified through formaldehyde-dimedone compound, m.p. 189 $^{\circ}$ (lit. 189–90 $^{\circ}$). [3]. The possible loci for this methylene group are positions 20, 24 and 25. The position 24 was ruled out by the behaviour of cycloephornol in conditions which promote isomerization of such a double bond. The 24 methylene triterpenes and steroids are reportedly converted into their Δ^{24} isomers on chromatography over silica gel [9] or treatment with H_2SO_4 and isopropanol [10]. No such conversion could be noted for cycloephornol. Taking into account the remaining positions 20 and 25 and also considering the presence of an additional nonconjugated trisubstituted double bond in the side chain, the following two possible structures can be considered for the side chain of cycloephornol.



The structure B was excluded on the basis of spectral data. The NMR spectrum did not show the characteristic doublet due to 6-methyl protons of the isopropyl group. However, 3H doublet for 24-methyl group appears at the same position (δ 0.9) as in cyclolaudenol. Moreover, six protons are found downfield in the vinyl methyl region at δ 1.06 which corresponds to structure A rather than B. Absence of an isopropyl group was indicated by the lack of peaks in the mass spectrum at m/e 395 (M-isopropyl)⁺ and 337 (M-isopropyl + H₂O)⁺. On the basis of the findings recorded above, the following structure may be provisionally assigned to cycloeuphornol (cycloeuphornadienol).



This structure carries the free methylene group at Δ^{25} which is identical with cyclolaudenol, the structural similarity with which has already been established. It may be stated that recently Yaukov and Khusein [11] have reported a new triterpene from the seeds of *Citrullus coleocynthis* which they provisionally named as citrullanol. It has the same position of two double bonds in the side chain as in cycloeuphornol, providing biogenetic evidence for the structure of cycloeuphornol.

EXPERIMENTAL

The fresh latex of *Euphorbia tirucalli* (ca. 500 ml) was directly tapped from incision into a flask containing 500 ml acetone. After keeping overnight in the cold, the light yellow supernatant was decanted from the coagulated insoluble residue and kept overnight at room temperature for slow evaporation, and then sucked. The residue thereby obtained, was taken up in 1:1 acetone-methanol and cooled, when a colourless crystalline mixture of triterpenes was obtained which was filtered, dissolved in benzene with the help of little methanol, and chromatographed over alumina. The elution was carried out with hexane, 1:1 hexane-benzene, benzene, 1:1 benzene-ether and ether. The last two eluates gave a solid which crystallized out from ethanol as colourless shining needles, which on repeated crystallizations finally melted at 139–40°; showed $[\alpha]_D +61.5^\circ$ and analyzed for C₃₀H₅₀O. (Found: C 84.581, H 11.819%; M⁺ peak at 426. Calcd. for C₃₀H₅₀O: C 84.5070, H 11.7370%; mol. wt. 426). The analytical and physical data corresponded to lanosterol and the identity was further substantiated through superimposable spectral data as well as preparation of its crystalline

acetate m.p. 118–19° (lit [12] 118°); benzoate m.p. 194° (lit [12] 194–95°) and dibromide m.p. 181–82° (lit [12] 180–82).

The column was further eluted with various proportions of benzene-chloroform, benzene-methanol and finally with methanol. The residue from the last two eluates was taken up in methanol and kept in the cold whereby cycloeuphornol was obtained as colourless fine needles m.p. 95–6°. It analyzed for C₃₁H₅₀O with 7 C-methyl groups. (Found: C 85.031, H 11.292, O 3.67%, C-methyl 23.91%; M⁺ peak at 438. Calcd. for C₃₁H₅₀O: C 84.93, H 11.41, O 3.65%, 7 C-methyl 23.97%, mol. wt. 438).

Cycloeuphornadienyl Acetate. Cycloeuphornol (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 cycloeuphornadienyl acetate-colourless slender needles from methanol, m.p. 138°; $[\alpha]_D +54^\circ$. (Found: C 81.48, H 10.801%. Calcd. for C₂₃H₅₂O₂: C 81.5, H 10.83%). The IR spectrum showed bands at 3071 cm⁻¹ (olefinic C-H stretching); 3040 cm⁻¹ (cyclopropane ring); 1720 cm⁻¹ (C=O); 1640–50 cm⁻¹ (C=C); 887 cm⁻¹ (HC=C); 840, 820 and 800 cm⁻¹ (HC=C).

Cycloeuphornadienyl Benzoate. One hundred mg cycloeuphornol was refluxed with 1 ml pyridine and 1 ml benzoyl chloride for 1 hr. On working up in the usual manner the reaction product yielded colourless needles of cycloeuphornadienyl benzoate from methanol, m.p. 150°. (Found: C 84.22, H 10.01%. Calcd. for C₃₈H₅₄O₂: C 84.13, H, 9.96%).

Perbenzoic Acid Titration of Cycloeuphornadienyl Acetate. To 40 mg of cycloeuphornadienyl acetate in 10 ml of CHCl₃ 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.

Cycloeuphornanol. Cycloeuphornadienol was taken up in absolute alcohol and hydrogenated over palladium catalyst at the atmospheric pressure for 24 hr. The residue left on removal of the solvent from the filtered solution, was taken up in hot methanol. On keeping the alcoholic solution in the cold cycloeuphornanol was obtained in the form of colourless needles which melted at 133–34°, $[\alpha]_D +43^\circ$. (Found: C 83.09, H 12.5%; M⁺ peak 442. C₃₁H₅₄O requires: C 84.1, H 12.3%; mol. wt. 442). The IR spectrum showed diagnostic bands at 3420 cm⁻¹ (OH group), 3040 cm⁻¹ (cyclopropane ring) and no absorption in the ethylenic region.

Cycloephornanyl Acetate. Cycloephornanol (40 mg) was refluxed with 0.5 ml pyridine and 1 ml acetic anhydride for 2 hr. The reaction product was worked up to yield cycloephornanyl acetate in colourless needles, m.p. 132°, $[\alpha]_D + 50^\circ$. (Found: C 81.96, H 11.48%. $C_{33}H_{56}O_2$ requires: C 81.81, H 11.57%).

Cycloephornol Tetrabromide. Cycloephornol (100 mg) was dissolved in chloroform 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 2.1 mole had been added. The residue, left on carefully removing the solvent in rotary evaporator, crystallized from methanol in cream-coloured needles of tetrabromide, m.p. 126-27°. On debromination with NaI in alcohol it afforded back the parent alcohol. (Found: C 48.98, H 6.39 and Br 42.32%. Calcd for $C_{31}H_{50}OBr_4$: C 49.0765, H 6.59, Br 42.216%).

Cycloephornanone. Cycloephornanol (50 mg) in acetic acid (2 ml) was stirred at room temperature and chromium trioxide (75 mg) in acetic acid (5 ml) added during half-an-hour. The neutral product was isolated in the usual way and chromatographed over alumina. The 1:1 petroleum ether-benzene eluates gave a fraction which after crystallization from methanol yielded cycloephornanone as colourless fluffy needles, m.p. 84°. (Found: C 84.62, H 11.73%; M^+ peak at 440. $C_{31}H_{52}O$ requires: C 84.545, H 11.81%; mol. wt. 440). The IR spectrum showed bands at 3071 cm^{-1} (olefinic C-H stretching); 3040 cm^{-1} (cyclopropane ring); 1071 cm^{-1} (C = O); 1640-50 cm^{-1} (C = C); 887 cm^{-1} (C = CH₂); 840, 820 and 800 cm^{-1} (C = C).

Treatment of Cycloephornanyl Acetate with Hydrogen Chloride. A solution of the acetate in dry chloroform at 0° was treated with a stream of hydrogen chloride for 2 hr. The product, isolated in the usual manner, crystallized from chloroform-methanol as needles, m.p. 147-53°, showing a strong yellow colour with tetranitromethane. On repeated crystallizations from chloroform-methanol,

a homogenous crystallizate was obtained which melted at 173-74°, $[\alpha]_D + 81^\circ$ and analyzed for $C_{33}H_{56}O_2$. (Found: C 81.6, H 11.3%. $C_{33}H_{56}O_2$ requires: C 81.8%, H 11.6%). It could be identified as laudenyl acetate through superimposable spectral data.

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