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CYCLOEUPHORDENOL, A NEW TRITERPENE FROM *EUPHORBIA TIRUCALLI*

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Key Word Index—*Euphorbia tirucalli*; Euphorbiaceae; latex; 31-nortriterpene; cycloeuphordenol; 4α,14α,24β-trimethyl-9β: 19-cyclocholest-20-en-3β-ol.

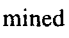
Abstract—A new 31-nortriterpene, cycloeuphordenol, has been isolated from the latex of *Euphorbia tirucalli*. Its absolute structure was determined as 4α,14α,24β-trimethyl-9β: 19-cyclocholest-20-en-3β-ol by the use of chemical and spectroscopic methods, including 2D NMR.

INTRODUCTION

Euphorbia tirucalli L. is widely distributed in Africa and Asia. Its various parts are highly reputed for their medicinal properties in the indigenous system of medicine [1]. Previous phytochemical studies on various parts of this plant led to the isolation of cycloeuphornol, euphorbinol, euphorbosterol, tirucallol, euphol, euphoron, taraxerone and taraxasterol [2–5]. In this paper we report the isolation and structure elucidation of a new cyclopropane containing 31-nortriterpene, cycloeuphordenol **1** from the fresh and undried latex of this plant.

RESULTS AND DISCUSSION

Compound **1** showed a $[M]^+$ peak at m/z 426.3834 (HR mass spectrum) which corresponded to the molecular formula $C_{30}H_{50}O$ (clacd 426.3861) and indicated that **1** may be a triterpenoid. Its UV spectrum showed λ_{max} at 205 nm. The IR spectrum revealed the presence of an OH group (3400 cm^{-1}), a cyclopropane ring (3045 cm^{-1}) and



$R_2C{=}CH_2$ (1640 and 892 cm^{-1}).

The ^1H NMR spectrum of **1** showed signals due to four secondary and two tertiary methyl groups besides the characteristic doublets at δ0.13 and 0.43 ($J = 4$ Hz) for the non-equivalent protons of a cyclopropyl methylene group. In addition there were signals due to a terminal methylene group (*br s* at δ4.6 and 4.7, 1H each) and a carbinylic proton (*sext* at δ3.2, $J_{ax,ax} = 8.9$ Hz, $J_{ax,eq} = 3.9$ Hz). The ^{13}C NMR spectrum showed 30 carbon atoms. The multiplicity of each carbon atom was determined using DEPT experiments [6] which revealed the presence of six methyl, twelve methylene and seven methine protons.

The presence of a secondary hydroxyl group in **1** followed from the preparation of an acetate **1a**, benzoate **1b** and from its oxidation to a ketone **1c**. The ketone gave a positive Zimmermann test indicating the presence of a 3-oxo group. The ketone can be reduced back to the parent alcohol confirming the β and equatorial configuration of the hydroxyl group at C-3 of **1**. Ozonolysis of **1a** provided a mixture of products from which formaldehyde could be separated and identified through the formation of its crystalline dimedone derivative, confirming the presence of a vinyl group.

The mass spectrum of **1** showed a strong peak at m/z 408 due to the loss of H_2O. It formed a daughter fragment at m/z 353 by cleavage 'a' which is characteristic of 4-methyl-9:19-cyclosterols [7]. In **1**–**1b** the fragment ion peak at m/z 300 is formed by cleavage 'b' which is also typical of 4-methyl-9:19-cyclosterols [7, 8]. These facts are consistent with the presence of one methyl group at C-4. The ORD curve of **1c** exhibited a positive Cotton effect which is also characteristic of 3-keto-4α-methyl-5α-steroids [9].

The presence of a monounsaturated C_9H_{17} side chain was evident from the fragment $[M-125]^+$ in the spectra of **1**–**1c**, which is rationalized by cleavage 'c'. The possible loci of the vinylic double bond are at C-20, C-24 and C-25. The latter probability could be eliminated by the absence of a vinyl methyl group and the presence of a characteristic isopropyl doublet in the ^1H NMR spectrum of **1**. The occurrence of two broad singlets instead of one in the olefinic region was also in confirmity with the absence of a double bond at C-25 [10].

The above data of **1** closely resemble that of cycloeucalenol which bears the double bond at C-24(28) [11]. The two compounds, however, differ widely in their respective melting points and optical rotations. Their IR spectra also show common features but they could not be superimposed. In the ^{13}C NMR spectrum the chemical shifts of the nucleus carbon atoms showed close agreement with the published spectrum of cycloeucalenol [12] but differed in the chemical shifts of the side chain carbon atoms. Cycloeuphordenol has, therefore, the same basic skeleton and stereochemistry as cycloeucalenol and differs only in the structure of side chain. The latter gives a diagnostic peak at $[M-84]^+$ which is typical of $\Delta^{24(28)}$-sterols and arises from the cleavage of the C-22, C-23 bond with hydrogen transfer [13]. This fragment, as well

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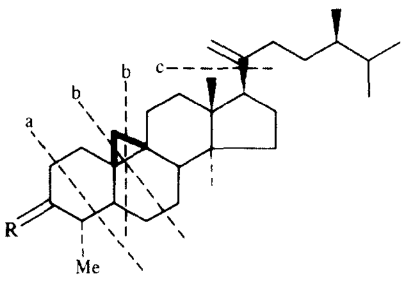

1 R = βOH, α-H
1a R = βOAc, α-H
1b R = βOBz, α-H
1c R = O

Scheme 1.

as the peak resulting from the loss of the side chain plus two hydrogen atoms, were absent in the mass spectrum of **1**–**1c** providing strong evidence for the presence of a vinylic double bond at C-20 (21); the structure of cycloeuphordenol and its derivatives can, therefore, be represented as shown in Scheme 1.

A 2D heteronuclear ^1H–^{13}C chemical shift correlation spectrum (heterocosy) [14] was recorded to locate the chemical shifts of the various protons. The signals of C-3, C-4, C-19, C-21, C-22 and C-25 could easily be correlated with chemical shifts of their respective protons. The proton connectivity was also determined by 2D homonuclear ^1H–^1H chemical shift correlation measurements (COSY 45°) [14] which showed connectivity of 3αH to both the protons at C-2 but to only one proton at C-4 because of the presence of the methyl group at this position. In addition, the connectivities of 25-H with 26- and 27-H_3 and that of 24-H with 28-H_3 were also observed. Irradiation at δ1.16 (4-H) caused the doublet of the methyl group at δ1.02 (30-H_3) to collapse into a singlet. The secondary methyl groups could therefore be assigned to C-4, C-24 and C-25, providing indirect evidence for the presence of a vinyl group at C-20.

The position of the double bond was finally confirmed by NOE difference measurements at certain points in the side chain. Irradiation at δ4.7 (21-H_2) resulted in 12.5% NOE at δ0.97 (18-H_3) and 9.37% NOE at δ2.2 (22-H_2). Irradiation at δ2.2 resulted in a 7.2% NOE at δ4.7 (21-H_2) and one of 14.06% at δ0.93 (28-H_3). Irradiation at δ0.93 (28-H_3) caused a 15.37% NOE at δ2.2 (22-H_2). Irradiation at δ1.4 (24-H) caused a 6.25% NOE at δ1.48 (25-H) and one of 17.5% at δ1.98 (16-H_2). Irradiation at δ1.98 (16-H_2) caused 12.5% NOE at δ1.4 (24-H). These interactions are summarized in Fig. 1.

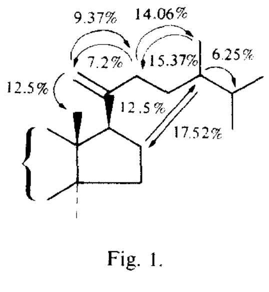

Fig. 1.

The strong NOE interaction between 16-H_2 and 24-H may be attributed to the R configuration at C-24 in which the 24-H comes closer to 16-H_2 owing to free rotation across the bond between C-22 and C-23. Such strong interactions are also observed in the case of stigmasterol and β-sitosterol which also have the 24(*R*) configuration. On the other hand, a similar interaction could not be observed in the case of 24(*S*) stigmast-5-en-3β-ol. However, further studies are required in a series of related compounds to establish whether it is a dependable method for the determination of the orientation of alkyl groups at C-24 in sterols and triterpenes. The 24(*R*) configuration of cycloeuphordenol was, however, established by molecular rotation relations. Bergmann and Low [15] have shown that the introduction of a 24α-methyl into cholesterol, cholestanol and their esters causes a substantial positive increment in molecular rotation, whereas a negative change accompanies the introduction of a 24β-methyl group. Cyclolaudenol and its derivatives which have a β-configuration of the methyl group at C-24 show negative increments in molecular rotations when compared to cycloartenol and its derivatives [16]. A similar relationship exists between cycloeuphordenol, its derivatives, and the corresponding 24-demethylated compounds reported in the literature [17], revealing that cycloeuphordenol has the 24(*R*) configuration like cyclolaudenol.

To the best of our knowledge this is the first report of the natural occurrence of a cyclopropanated 4,24-dimethylated sterol with a double bond at C-20 (21); its isolation may be of chemotaxonomic significance.

EXPERIMENTAL

General. UV spectra were recorded in MeOH, IR spectra in $CHCl_3$. DEPT expts were carried out with $\theta = 45°$, 90° and 135°; the quaternary carbons were determined by subtraction of these spectra from the broad band ^{13}C NMR spectrum. HRMS were recorded on a double focusing instrument coupled to a PDP 11/34 computer system. ^1H NMR spectra ($CDCl_3$) were recorded at 300 MHz with TMS as int. ref. For NOE measurements degassing was done under liquid N_2, using a decoupler power of 35 l and preirradiation time of 11 sec. An impulse length of 10 msec was maintained to avoid spin population effect. The 2D COSY-45° expt 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 two dimensional ^1H–^{13}C chemical shift correlation expts were carried out at 300 MHz with a sweep width of 12 820 Hz (2K data point in ω_2) and 1024 Hz (256t_1 values zero-filled to 2K) in ω_1. In both the 2D expts a 2 sec relaxation delay was used and 16 transients were performed for each t_1 value.

Plant material. Material was collected from the Karachi region and was identified by the Department of Botany, University of Karachi. A voucher specimen has been deposited in the herbarium of the Department of Botany, University of Karachi.

Extraction and isolation. Fr undried latex (2 kg) was directly tapped from incisions into a flask containing 2.5 l Me_2CO. After standing overnight at 4° the supernatant was decanted from the coagulate and the latter repeatedly washed with Me_2CO. The combined supernatants and washings were kept for slow evapn at room temp when a thick sticky crystallizate settled down, which was filtered off and recrystallized from $(Me)_2CO$–MeOH (1:1). The crystalline mixt. thereby obtained was subjected to CC. Elution was carried out with a mixt. of hexane–$CHCl_3$ of increasing polarity. The hexane–$CHCl_3$ (3:1) eluate was further subjected to flash CC on silica gel. Elution was carried out with gradients of increasing polarity using mixts of *n*-hexane–Et_2O.

Cycloeuphordenol was eluted by hexane–Et_2O (4:1) and purified by repeated crystallization from MeOH, whereby colourless fine needles of **1** (112 mg) were obtained, mp 105–106°, $[\alpha]_D + 39°$ ($CHCl_3$; *c* 0.127). For UV and IR see Results and Discussion; MS *m/z*: 426 $[C_{30}H_{50}O]^+$, 411 $[C_{30}H_{50}O-Me]^+$, 408 $[C_{30}H_{50}O-H_2O]^+$, 393 $[C_{30}H_{50}O-Me-H_2O]^+$, 353 $[(C_{30}H_{50}O-H_2O)-C_4H_7]^+$, 301 $[C_{30}H_{50}O-C_9H_{17}$ (entire substituent at C-17)$]^+$, 300 $[C_{30}H_{50}O-C_8H_{14}O$ (loss of ring A along with C-6 or C-19)$]^+$, 285 $[C_{30}H_{50}O-C_8H_{14}O-Me]^+$, 283 $[C_{30}H_{50}O-C_9H_{17}-H_2O]^+$ and 175 $[C_{30}H_{50}O-C_8H_{14}O-C_9H_{17}]^+$; ^1H NMR: δ0.13, 0.43 (pair of *d*, $J = 4$ Hz, 19H_2), 4.6, 4.7 (*br s*, 21-H_2), 3.2 (*sextet*, $J_{ax,ax} = 8.9$ Hz, $J_{ax,eq} = 3.9$ Hz, 3-H), 1.03 (*d*, $J = 6.2$ Hz, 26- and 27-Me), 1.02 (*d*, $J = 6.6$ Hz, 30-Me), 0.97 (*s*, 18-Me), 0.93 (*d*, $J = 6.4$ Hz, 28-Me), 0.89 (*s*, 29-Me). ^{13}C NMR ($CDCl_3$, 75.43 MHz): δ30.8 (C-1), 34.8 (C-2), 76.2 (C-3), 44.5 (C-4), 43.4 (C-5), 24.69 (C-6), 28.12 (C-7), 46.8 (C-8), 28.71 (C-9), 29.62 (C-10), 25.11 (C-11), 35.32 (C-12), 45.02 (C-13), 49.1 (C-14), 32.92 (C-15), 27.0 (C-16), 52.97 (C-17), 17.70 (C-18), 27.12 (C-19), 156.00 (C-20), 106.00 (C-21), 34.80 (C-22), 21.76 (C-23), 36.1 (C-24), 34.50 (C-25), 21.91 (C-26), 22.09 (C-27), 19.00 (C-28), 18.30 (C-29) and 14.38 (C-30).

Acetylation. **1** (70 mg) was refluxed with Ac_2O (35 ml) in C_5H_5N (8.5 ml) for 45 min. The reaction mixt was worked-up in usual manner to yield acetate **1a** (50.7 mg) which was crystallized from Me_2CO, mp 120–122°; $[\alpha]_D + 34°$ ($CHCl_3$; *c* 0.205); IR λ_{max}cm^{-1}: 3040 (cyclopropyl CH_2), 1720 and 1240 (ester), 1640 and 892 ($=CH_2$): MS *m/z*:468 $[C_{32}H_{52}O_2]^+$, 453 $[C_{32}H_{52}O_2-Me]^+$, 408 $[C_{32}H_{52}O_2-HOAc]^+$, 393 $[C_{32}H_{52}O_2-HOAc-Me]^+$, 353 $[C_{32}H_{52}O_2-HOAc)-C_4H_7]^+$, 343 $[C_{32}H_{52}O_2-C_9H_{17}$ (entire substituent at C-17)$]^+$, 300 $[C_{32}H_{52}O_2-C_{10}H_{16}O_2$ (loss of ring A along with C-6 or C-19)$]^+$, 285 $[C_{32}H_{52}O_2-C_{10}H_{16}O_2-Me]^+$, 283 $[C_{32}H_{52}O_2-C_9H_{17}-HOAc]^+$ and 175 $[C_{32}H_{52}O_2-C_{10}H_{16}O_2-C_9H_{17}]^+$. ^1H NMR: δ0.14, 0.4 (pair of *d*, $J = 4$ Hz, 19-H_2), 4.6, 4.7 (*br s*, 21-H_2), 2.04 (*s*, OAc), 4.5 (*sext*, $J_{ax,ax} = 9.4$ Hz, $J_{ax,eq} = 4.1$ Hz, 3-H), 1.029 (*d*, $J = 6$ Hz, 26- and 27-Me), 1.02 (*d*, $J = 6.3$ Hz, 30-Me), 0.96 (*s*, 18-Me), 0.93 (*d*, $J = 6.8$ Hz, 28-Me), 0.89 (*s*, 29-Me).

Benzoylation. **1** (10 mg) was refluxed with benzoyl chloride (2 ml) and C_5H_5N (0.5 ml) for 45 min. The reaction mixture was worked-up in usual manner. Crystallization from MeOH–C_6H_6 (1:1) yielded benzoate **1b** (4.98 mg), mp 118–120°; $[\alpha]_D + 25°$ ($CHCl_3$; *c* 0.16); IR λ_{max}cm^{-1}: 3040, 2910, 1710, 1240; MS *m/z*:530 $[C_{37}H_{54}O_2]^+$, 515 $[C_{37}H_{54}O_2-Me]^+$, 408 $[C_{37}H_{54}O_2-HOBz]^+$, 393 $[C_{37}H_{54}O_2-HOBz-Me]^+$, 353 $[(C_{37}H_{54}O_2-HOBz)-C_4H_7]^+$, 405 $[C_{37}H_{54}O_2-C_9H_{17}$ (entire substituent at C-17)$]^+$, 300 $[C_{37}H_{54}O_2-C_{15}H_{18}O_2$ (loss of ring A along with C-6 or C-19)$]^+$, 285 $[C_{37}H_{54}O_2-C_{15}H_{18}O_2-Me]^+$, 283 $[C_{37}H_{54}O_2-C_9H_{17}HOBz]^+$ and 175 $[C_{37}H_{54}O-C_{15}H_{18}O_2-C_9H_{17}]^+$.

Oxidation. **1** (10 mg) was dissolved in Me_2CO (20 ml) and treated with freshly prepared Jones reagent (2.5 ml) at room temp. Usual work-up and crystallization from $CHCl_3$ provided ketone **1c** (5.7 mg), mp 125–128°; $[\alpha]_D + 32°$ ($CHCl_3$; *c* 0.166); IR λ_{max}cm^{-1}: 3040, 1705; MS *m/z*: 424 $[C_{30}H_{48}O]^+$, 409 $[C_{30}H_{48}O-Me]^+$, 300 $[C_{30}H_{48}O-C_8H_{12}O$ (loss of ring A along with C-6 or C-19)$]^+$, 299 $[C_{30}H_{48}O-C_9H_{17}$ (entire substituent at C-17)$]^+$, 285 $[C_{30}H_{48}O-C_8H_{12}O-CH_3]^+$ and 175 $[M-C_8H_{12}O-C_9H_{16}]^+$.

Ozonolysis. Acetate **1a** (50 mg) was dissolved in HOAc (15 ml) and cooled to 0°. Ozone (*ca* 10%) was passed through the soln for 20 min. The volatile product formed was steam distilled into a soln of dimedone. The pH of the dimedone soln was maintained just below 7 by the addition of alkali. The product was crystallized from MeOH–H_2O (mp 189°) and was identical to an authentic sample of formaldehyde–dimedone adduct.

Reduction. Ketone **1c** (5 mg) was dissolved in 0.5 ml of THF. This soln was added slowly to a slurry of 6.5 mg of $LiAlH_4$ in 1 ml of the same solvent. The reaction mixt. was stirred while being cooled at 0°. When the reaction was complete (TLC monitoring), Et_2O–EtOAc (1:1) was added. After standing overnight, H_2O was then added and then concd HCl. The mixt. was extracted several time with Et_2O. Removal of Et_2O after washing the extracts with H_2O and recrystallization of the residue from MeOH yielded **1**, mp 104–106°, $[\alpha]_D + 39.8$.

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