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CYCLOCADUCINOL, A CYCLOARTANE TYPE TRITERPENE FROM *EUPHORBIA CADUCIFOLIA*

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Key Word Index—*Euphorbia caducifolia*; Euphorbiaceae; latex; cycloartane triterpenoid; cyclocaducinol; (24R)-24-ethyl-31-norcycloart-20-en-3β-ol.

Abstract—A new 31-norcycloartane type triterpene, cyclocaducinol, has been isolated from the latex of *Euphorbia caducifolia*. Its absolute structure was determined as (24*R*)-24-ethyl-31-norcycloart-20-en-3β-ol by the use of chemical and spectroscopic methods including 2D NMR. The known triterpenes euphol, tirucallol and cycloartenol have also been isolated as major constituents.

INTRODUCTION

Euphorbia caducifolia Haines is a short shrub growing mainly in the dry regions of India and Pakistan. Its latex is reputed in the indigenous system of medicine as a purgative, rubefacient and expectorant [1], while the root is antispasmodic [1, 2]. Only a few terpenoidal constituents have been reported from various parts of this plant [3, 4]. In this paper we report the isolation and structure elucidation of a new cycloartane type triterpenoid, cyclocaudicinol (**1**) from the fresh and undried latex of this plant.

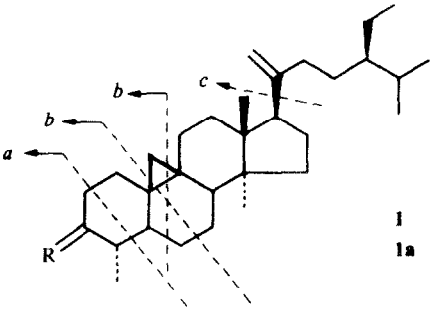
RESULTS AND DISCUSSION

Cyclocaducinol was isolated as a colourless crystalline solid. The molecular formula was established as $C_{31}H_{52}O$ by its high resolution mass spectrum which showed the $[M]^+$ peak at *m/z* 440.6942 (calcd. 440.7561). Its UV spectrum showed ν_{max} at 207 nm. The IR spectrum indicated the presence of a hydroxyl (3450 cm^{-1}), cyclopropane ring (3045 cm^{-1}), terminal methylene (1645 and 890 cm^{-1}) and *gem* dimethyl (twin bands at 1325 and 1315 cm^{-1}) groups.

The ^1H NMR spectrum showed signals due to two tertiary and three secondary methyl groups besides an ethyl group (3H triplet at δ0.84 and 2H multiplet at δ1.3). In addition there were characteristic doublets at δ0.14 and 0.47 ($J = 4.6$ Hz) for the non-equivalent protons of a cyclopropyl methylene group. The terminal methylene group formed broad singlets at δ4.62 and 4.70 while the carbinylic proton resonated as a sextet at δ3.20 ($J_{ax,ax} = 8.6$ Hz, $J_{ax,eq} = 3.5$ Hz). The ^{13}C NMR spectrum showed 31-carbon atoms in the molecule. The multiplicity assignments were made by DEPT pulse sequence with the last polarization pulse angle θ 45°, 90° and 135°.

The secondary nature of the hydroxyl group in compound **1** followed from its oxidation to a ketone **1a**. The ketone gave a positive Zimmermann test, indicating the presence of a 3-oxo group. The ketone could be reduced back to the parent alcohol confirming the equatorial configuration of the hydroxylic group at C-3 of **1**. The fragmentation pattern in the EI mass spectrum was similar to cyclotrichosantol [5], cycloeuphordenol [6] and cycloeucalenol [7]. The strong peak at *m/z* 422 represented loss of a water molecule. It formed a daughter fragment at *m/z* 353 by cleavage '*a*' which is characteristic of 4-methyl-9β, 19-cyclosterols [8]. Another intense ion peak at *m/z* 300 is formed by cleavage '*b*' which is also typical of 4-methyl-9β,19-cyclosterols [8, 9]. These facts were consistent with the presence of one methyl group at C-4. The ORD curve of **1a** exhibited a positive Cotton effect which is characteristic of 3-keto-4α-methyl-5α-steroids [10]. The presence of monounsaturated $C_{10}H_{19}$ side chain was evident from the fragment $[M-139]^+$ in the spectra of **1**–**1a** which is rationalized by cleavage '*c*'.

In the ^{13}C NMR spectrum the chemical shifts of the nucleus carbon atoms showed close agreement with the published spectra of cycloeucalenol and cycloeuphordenol [11, 6] but differed in the chemical shifts of the side chain carbon atoms and also in having one carbon atom more than cycloeucalenol and cycloeuphordenol. Cyclo-



1 R = βOH, αH
1a R = O

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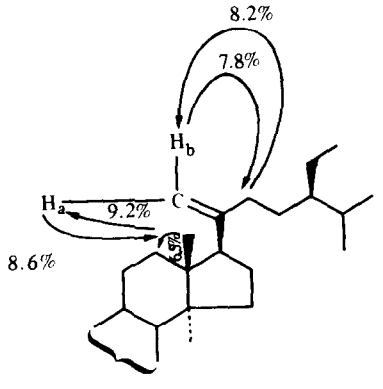
caducinol has, therefore, the same basic skeleton and stereochemistry as cycloeucalenol and cycloeuphordinol but differs only in the structure of side chain.

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 ^{1}H NMR spectra of **1**–**1a**. The occurrence of two broad singlets instead of one in the olefinic region was also in agreement with the absence of a double bond at C-25 [12]. The $\Delta^{24(28)}$ sterols give a diagnostic peak at $[M-84]^+$ which arises from the cleavage of C-22, C-23 bond with hydrogen transfer [13]. This fragment, as well as the peak resulting from the loss of the side chain plus two hydrogen atoms were absent in the mass spectra of **1**–**1a** providing strong evidence for the presence of a vinylic double bond at C-20(21). The ethyl group was assigned to C-24 in biogenetic analogy to the related compounds. The structure of cyclocaudicinol and its oxidation product can, therefore, be represented by structures **1** and **1a**, respectively.

2D heteronuclear ^{1}H–^{13}C chemical shift correlated spectroscopy (Hetero COSY) [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, C-24, C-25, and C-28 could easily be correlated with the chemical shifts of their respective protons. The protons connectivity was also determined by 2D-homonuclear ^{1}H–^{1}H chemical shifts correlated spectroscopy (COSY 45°) [14] which showed connectivity of 3α-H 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 connectivity of 25-H with 26-H_3 and 27-H_3, that of 24-H with 28-H_2 and 28-H_2 to 31-H_3 were also observed. Irradiation at δ1.19 (4β-H) caused the doublet of the *methyl group at δ1.01 (30-H_3) to collapse into a singlet.* The secondary methyl groups could, therefore, be assigned to C-4, C-25 and the ethyl group was established at C-24.

The position of the double bond at C-20(21) was authenticated by NOE difference measurements. Irradiation at δ4.7 (20-H_a) resulted 8.6% NOE at 0.94 (18-H_3) while irradiation at 4.62 (20-H_b) resulted 7.8% NOE at 2.1 (22-H_2). Irradiation at δ0.94 (18-H_3) resulted 9.2% NOE at 4.7 (20-H_a) and 6.5% NOE at 1.4 (12-H_2). These NOE interactions are summarized in Scheme 1.



Scheme 1.

The *R*-configuration at C-24 was established by comparing chemical shifts of carbons and protons in the ^{1}H and ^{13}C NMR spectra of **1** with a series of triterpenoids and sterols possessing *R* and *S*-configurations at C-24. The values showed very close agreement with compounds of *R*-configuration including sitosterol, stigmasterol [15, 16] and cycloeuphordenol [6], providing strong evidence for the (24*R*)-orientation of the ethyl group at C-24.

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

EXPERIMENTAL

General. UV spectra were recorded in MeOH, IR in $CHCl_3$. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS as int. ref. DEPT experiments were carried with $\theta = 45°, 90°$ and 135°. For NOE measurement, the sample was frozen under liquid nitrogen and degassed, using a decoupler power of 35 l and pre-irradiation time of 11 sec. An impulse length of 10 msec was maintained to avoid satn. The 2D COSY-45° experiment was performed at 300 MHz with a sweep width of 4000 Hz (2000 data points in ω_2). The heteronuclear two dimensional ^{1}H–^{13}C chemical shift correlation experiments were carried out at 300 MHz with a sweep width of 1282 Hz (2000 data point in ω_2) and 1024 Hz (25t_1 values zero-filled to 2000) in ω_1. In both the 2D experiments 2 sec relaxation delay was used and 16 transients were performed for each t_1 value.

Plant material. The plant material (latex) was collected in Karachi, Pakistan and was identified by Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen is deposited.

Extraction and isolation. Latex (2 kg) was directly tapped from incisions into a flask containing Me_2CO. After keeping overnight in an ice chest, the supernatant was removed and the solid residue repeatedly washed with Me_2CO. The supernatant was kept for slow evapn at room temp. The thick sticky crystallizate formed (50 g) was filtered off and part of it (22 g) subjected to column chromatography over 750 g of activated silica gel. The elution was carried out with solvent gradient of increasing order of polarity. The eluate obtained in hexane–$CHCl_3$ (9.8:0.2) yielded on crystallization from MeOH–Me_2CO pure euphol (2.1 g), mp 114–116°, $[\alpha]_D$ +31.8° ($CHCl_3$; *c* 0.120). The fraction obtained from hexane–$CHCl_3$ (19:1) consisted of a binary mixture of triterpenes which could be separated by prep. TLC to provide tirucallol (0.81 g), mp 133–135°, $[\alpha]_D$ +5° ($CHCl_3$; *c* 0.21) and cycloartenol (0.49 g), mp 115–117°, $[\alpha]_D$ +53.8° $CHCl_3$; *c* 0.106). All these known compounds were identified through comparison of physical and spectral data with those mentioned in the literature. The eluate obtained in hexane–$CHCl_3$ (9:1) was further purified on prep. silica gel plates impregnated with 20% $AgNO_3$, using hexane–$CHCl_3$ MeOH (17:5:3) as solvent system to yield compound **1**.

Cyclocaducinol (**1**) was obtained as colourless needles from Me_2CO (0.054 g, 0.006% on the basis of undried latex), mp 96–98%, $[\alpha]_D$ +45.89° ($CHCl_3$, *c*, 0.16). For UV and IR see results and discussion; HRMS: $[M]^+$ at *m/z* 440.6942 ($C_{31}H_{52}O$). EIMS *m/z*: 440 $[C_{31}H_{52}O]^+$, 425 $[C_{31}H_{52}O-Me]^+$, 422 $[C_{31}H_{52}O-H_2O]^+$, 407 $[C_{31}H_{52}O-Me-H_2O]^+$, 367 $[(C_{31}H_{52}O-H_2O)-C_4H_7]^+$, 301 $[C_{31}H_{52}O-C_{10}H_{19}$ (entire substituent at C-17)$]^+$, 314 $[C_{31}H_{52}O-C_8H_{14}O$ (loss of ring A along with C-6 or C-19)$]^+$, 299

$[C_{31}H_{52}O-C_8H_{14}O-Me]^+$, 283 $[C_{31}H_{52}O-C_{10}H_{19}-H_2O]^+$ and 175 $[C_{31}H_{52}O-C_8H_{14}O-C_{10}H_{19}]^+$; ^{1}H NMR: ($CDCl_3$): δ0.14, 0.47 (a pair of *d*, $J=4.6$ Hz, 19-H_2) 4.62, 4.7 (*br s*, 21-H_2), 3.2 (sextet, $J_{ax,ax}=8.6$ Hz, $J_{ax,eq}=3.5$ Hz, 3-αH), 1.03 (*d*, $J=7.2$ Hz, 26 and 27-Me), 1.01 (*d*, $J=6.3$ Hz, 30-Me), 0.94 (*s*, 18-Me), 0.88 (*s*, 29-Me), 0.84 (*t*, $J=7.0$ Hz, 31-Me). ^{13}C NMR ($CDCl_3$): δ30.4 (C-1), 34.8 (C-2), 76.3 (C-3), 44.5 (C-4), 43.2 (C-5), 24.6 (C-6), 28.0 (C-7), 46.7 (C-8), 23.5 (C-9), 29.5 (C-10), 25.1 (C-11), 35.2 (C-12), 45.2 (C-13), 48.7 (C-14), 32.9 (C-15), 26.5 (C-16), 52.0 (C-17), 17.7 (C-18), 26.9 (C-19), 156.0 (C-20), 106.9 (C-21), 35.0 (C-22), 31.31 (C-23), 50.3 (C-24), 26.2 (C-25), 18.8 (C-26), 19.8 (C-27), 23.1 (C-28), 19.0 (C-29), 14.4 (C-30), 11.9 (C-31).

Oxidation of compound **1**. Compound **1** (20 mg) was dissolved in Me_2CO (40 ml) and treated with Jones reagent (5 ml). The reaction mixture was stirred at room temp. till the reaction was completed (TLC monitoring). Usual work-up and crystallization from hexane–Me_2CO provided the ketone **1a** (9.6 mg); mp 192–194°; $[\alpha]_D+15°$ ($CHCl_3$; *c* 0.21); IR ν_{max} cm^{-1}: 3040, 1705, 1640, 890, 1325 and 1315; HRMS: M^+ 438.3861 ($C_{31}H_{50}O$). EIMS *m/z*: 438 $[C_{31}H_{50}O]^+$, 423 $[C_{31}H_{50}O-Me]^+$, 314 $[C_{31}H_{50}O-C_8H_{12}O$ (loss of ring A along with C-6 or C-9)$]^+$, 299 $[C_{31}H_{50}O-C_{10}H_{19}$ (entire substituent at C-17)$]^+$ and 175 $[C_{31}H_{50}O-C_{10}H_{19}-C_8H_{12}O]^+$; ^{1}H NMR: ($CDCl_3$): δ0.13, 0.45 (a pair of *d*, $J=4.2$ Hz, 19-H_2), 4.6, 4.68 (*br s*, 21-H_2), 1.04 (*d*, $J=6.8$ Hz, 26 and 27-Me), 1.02 (*d*, $J=6.7$ Hz, 30-Me) 0.96 (*s*, 18-Me), 0.87 (*s*, 29-Me), 0.84 (*t*, $J=7.0$ Hz, 31-Me).

Reduction of **1a**. The ketone **1a** (10 mg) was dissolved in 2 ml of EtOH and 0.5 mg of $NaBH_4$ was then added with stirring (1 hr). After dilution with H_2O the mixture was extracted with EtOAc, washed with H_2O, dried and evapd. Crystallization from Me_2CO yielded **1** mp 96–98°, $[\alpha]_D+44.9°$ ($CHCl_3$; *c* 0.19).

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