The Structure and Absolute Configuration of Cyclotirucanenol, a New Triterpene from Euphorbia tirucalli Linn

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The structure and absolute configuration of “cyclotirucanenol” a new triterpene isolated from Euphorbia tirucalli has been established as 24β-methyl-9β-19-cyclolanost-20-en-3β-ol through chemical and spectroscopic studies including 2D-NMR.

Introduction

Euphorbia tirucalli Linn (Euphorbiaceae) is a small tree, 15–20 feet high, with cylindrical branches and a few minute leaves at the extremities. It grows wildly in Pakistan as well as other parts of Asia and Africa and is reported to be useful for the treatment of rheumatism, neuralgia, colic, asthma and gastralgia [1]. We have earlier reported the isolation of two new triterpenes in the fresh and undried latex of E. tirucalli [2, 3]. The present paper describes the isolation of a new triterpene “cyclotirucanenol”. The structure and absolute configuration of this compound has been elucidated with the help of chemical and spectroscopic methods including extensive 2D-NMR studies.

Results and Discussion

Cyclotirucanenol (1), m.p. 94 °C, showed the molecular ion peak in its mass spectrum at m/z 440.694 corresponding to the molecular formula C_{31}H_{52}O (calcd 440.756). The UV spectrum of 1 showed only end absorption at 205 nm, and IR spectrum (chloroform) showed peaks at 3430 (OH group), 3075 (C=H olefinic stretching), 3040 (CH_{2}) showed the presence of four quaternary methyl groups as singlets at δ 0.81 (3H), δ 0.89 (3H), δ 0.96 (3H) and δ 0.965 (3H) along with three tertiary methyl groups as doublets at δ 0.99 (3H, J = 7 Hz) and 1.03 (6H, J = 6.7 Hz). A pair of doublets at δ 0.32 and 0.55 (J = 4 Hz) was indicative of cyclopropane ring bearing two non-equivalent hydrogen atoms. The broad singlets at δ 4.6 and 4.7 (1H each) could be assigned to the vinylidene group, while the double doublet at δ 3.2 (J_{ax,eq} = 10.7 Hz, J_{ax,ax} = 5.4 Hz) was due to the proton attached to the carbon bearing the hydroxyl group. 13C NMR spectrum showed 31 carbon atoms. The multiplicity of each was determined by using DEPT experiments [4] with the polarization pulse θ = 45°, 90° and 135°. The experiment revealed the presence of 7 methyls, 12 methylene and 6 methine carbons.

The nature of the alcoholic group in 1 was shown to be secondary from its oxidation to a ketone, cyclotirucanenone 1b. The ketone gave a positive Zimmermann test, indicating the presence of a 3-oxo group. It could be reduced back to the parent alcohol which established the presence of secondary alcoholic group at position 3 in β and equatorial configuration. The positive increment in the molecular rotation differences between 1 and its corresponding acetate was also in conformity to the β configuration of the hydroxyl group [5]. Perbenzoic acid titration of acetate 1a indicated the presence of one double bond [2]. On reduction with platinum catalyst, it absorbed one mole of hydrogen giving the saturated alcohol, cyclotirucanol through its acetate. The presence of double bond as free methylene group was confirmed through ozonolysis of 1a which gave a mixture of products from which formaldehyde was shown to allow reuse in the area of future scientific usage.

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isolated and identified through the formation of crystalline dimedone derivative.

Further information on the structure of cyclotirucanenol was provided by the mass spectra of 1–1b. The mass spectra of 1 and 1a showed a peak corresponding to the elimination of 69 mass unit from the ion M-18 and M-60. This process is typical of 4,4-dimethyl-9:19-cyclosterols [6] and results from the loss of carbons 2, 3 and 4 along with methyl groups at C-4. The second characteristic process involving elimination of ring A [6—7] was also visible in the spectra: M-140 in 1, M-182 in 1a and M-138 in 1b. The presence of monounsaturated C₁₇H₃ side chain is evident from the fragment M-125 in the spectra of 1–1b. The loss of water molecule in 1 and a molecule of acetic acid in 1a from this ion gave peak at m/z 297. Similar peaks were observed in the mass spectra of cyclolaudenol and its derivatives, and revealed the close similarity in the basic skeleton of these compounds.

The key evidence to the structure of cyclotirucanenol was provided by its dihydro product-cyclotirucananol. Its physical data were found to be identical with that of cyclolaudanol which is the corresponding reduction product of cyclolaudenol reported from the alkaloid-free fraction of opium by Spring et al. [8]. The melting point and optical rotation of cyclotirucanananyl acetate agree with those reported for cyclolaudanyl acetate [8]. Moreover, in the ¹³C NMR spectrum of 1 the chemical shifts of the nucleus carbon atoms showed close agreement with the published spectrum of cyclolaudenol [9] but differed in the chemical shifts of the side chain carbon atoms. In view of these findings it may be concluded that 1 has the same basic skeleton and stereochemistry as cyclolaudenol, the former differing from the latter in having different position of double bond. In cyclolaudenol the double bond is at C-25. This possibility could be eliminated by the absence of vinyl methyl group and the presence of characteristic isopropyl doublet in the ¹H NMR spectrum of 1. 24-Methylene cycloartenol gives a diagnostic peak at M-84 which is typical of Δ²⁴(28)-sterols and arises from the cleavage of C-22, C-23 bond with hydrogen transfer [6—7]. Such a fragment was not visible in the mass spectrum of 1–1b. Moreover, the peak resulting from the loss of the side chain plus two hydrogen atoms was also absent. This allowed us to place the double bond at C-20, the absolute structure of 1–1b can, therefore, be represented as follows:

The structure of cyclotirucanenol was fully supported by extensive 2D NMR experiments. The chemical shifts of various portions were located by heteronuclear ¹H—¹³C chemical shift correlation spectrum (Heterocosy) [10] as shown in Fig. 1. The proton connectivity was determined by homonuclear ¹H—¹H chemical shift correlation measurements (COSY 45°) [10] which showed connectivity of 3α-H to both the proton at C-2. In addition, the connectivity of 25-H with 26- and 27-H, and that of 24-H with 28-H, were also observed. Irradiation at δ 1.4 (24-H) and δ 1.45 (25-H) caused the doublets of methyl groups at δ 0.99 (28-H₃) and δ 1.03 (26- and 27-H₃) to collapse into singlets. The tertiary methyl groups could therefore be assigned to C-24 and C-25, providing indirect evidence for the presence of vinyl group at C-20.

The position of double bond and stereochemistry at C-24 was finally confirmed by NOE difference measurements. Irradiation at δ 4.7 (21-H) resulted in 9.7% NOE at δ 0.96 (18-H₃) and 2.9% NOE at δ 2.23 (22-H₂). Irradiation at δ 2.23 resulted in 5.5% NOE at δ 4.7 and 11.1% NOE at δ 0.99 (28-H₃). Irradiation at δ 1.4 (24-H) caused 6.75% NOE at δ 1.45 (25-H) and 17.8% NOE at δ 1.95 (16-H). Irradiation at δ 1.95 (16-H) caused 11.7% NOE at δ 1.4 (24-H). The strong NOE interaction between 24-H and 16-H is due to free rotation of the bond between C-22 and C-23 which results in close proximity of these two protons and indicate α orientation of 24-H. The β configuration of 24-Me was also authenticated by formation of cyclolaudanol from 1. The β configuration of 24-Me in former has already been established by Spring et al. [11]. The NOE interactions are summarized below:
Experimental

IR spectra were recorded with a Jasco A-302 spectrometer and the UV spectra on Pye-Unicam SP 800 spectrometer. The mass spectra were recorded on a Finnigan MAT 312 double focusing mass spectrometer with PDP 11/34 Computer system. The $^1$H and $^{13}$C NMR recorded on a Bruker Aspect AM-300 spectrometer with TMS as internal reference.

Extraction and isolation

The plant material (latex) was collected in Karachi, Pakistan, and identified in the Department of Botany, University of Karachi. A voucher specimen has been deposited in the herbarium of the Department of Botany, University of Karachi.

The fresh latex (2 kg) was directly tapped from incisions into a flask containing acetone. After standing overnight at 4 °C, the coagulated residue was sucked. The filtrate was kept at room temperature for slow evaporation and the resulting crystalline sticky mass was recrystallized from 1:1 acetone-methanol to provide a complex mixture of triterpenes. It was chromatographed on a column of silica gel. Elution was carried out with a mixture of hexane and chloroform using increasing order of polarity. The eluate obtained from 80:20 hexane-chloroform was rechromatographed over silica gel impregnated with silver nitrate. Elution was carried out with solvent gradient of increasing polarity. The fraction eluted with hexane-ethylacetate (8.5:1.5) was recrystallized from acetone to yield cyclotirucanenol 1 (118 mg).

Spectral data of cyclotirucanenol

$[\alpha]_D + 42.3^\circ$ (CHCl$_3$), for UV and IR see Results and Discussion. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 0.32–0.55 (dd, 2H, $J = 4$ Hz, 19-H$_2$), 3.2 (dd, 1H, $J_{ax,ax} = 10.7$ Hz, $J_{ax,eq} = 5.4$ Hz, 3-H), 0.81 (s, 3H, 4$\beta$Me), 0.89 (s, 3H, 14-Me), 0.965 (s, 3H, 4$\alpha$Me), 0.96 (s, 3H, 13-Me), 0.99 (d, 3H, $J = 7$ Hz, 24-Me), 1.03 (d, 6H, $J = 6.7$ Hz, 25-Me$_2$), 4.7–4.6 (br s, 2H, 21-H$_2$). MS: m/z 440 (M$^+$), 425 (M–Me)$^+$, 422 (M–H$_2$O)$^+$, 407 (M–Me–H$_2$O)$^+$, 300 (M-ring A)$^+$, 285 (M-ring A–Me)$^+$, 315 (M-side chain)$^+$, 297 (M-side chain–H$_2$O)$^+$, 353 [(M–H$_2$O)–69]$^+$ and 175 (M-ring A-side chain)$^+$. $^{13}$C NMR (CDCl$_3$,
The Structure and Absolute Configuration of Cyclotirucananol

75.43 MHz): C-1 (32.0), C-2 (30.46), C-3 (79.06), C-4 (40.60), C-5 (47.20), C-6 (21.16), C-7 (28.10), C-8 (40.10), C-9 (20.1), C-10 (26.2), C-11 (26.05), C-12 (35.6), C-13 (45.7), C-14 (34.6), C-15 (33.0), C-16 (26.5), C-17 (52.9), C-18 (18.05), C-19 (29.9), C-20 (156.5), C-21 (106.5), C-22 (32.3), C-23 (34.6), C-24 (36.17), C-25 (30.9), C-26 (22.03), C-27 (21.9), C-28 (19.3), C-29 (25.49), C-30 (14.8), C-31 (18.37).

Cyclotirucanenyl acetate (1a)

40 mg of 1 was refluxed with acetic anhydride (20 ml) in pyridine (6 ml) for 45 min. Usual workup provided acetate 1a (34.7 mg) which was recrystallized from alcohol m.p. 107–109 °C; [α]D + 36.5° (c = 0.5, CHCl3; IR (CHCl3): 3040, 1715 and 1210 cm⁻¹; ¹H NMR (CDCl₃): δ 0.34–0.54 (dd, 2H, J = 4 Hz, 19-H₂), 4.4 (1H, dd, Jaxax = 9.98 Hz, Jax. = 4.89 Hz, 3aH), 4.63–4.68 (br s, 2H, 21-H₃), 2.02 (3H, s, OAc), 0.86 (3H, s, 4ßMe), 0.84 (3H, s, 4αMe), 0.87 (3H, s, 14-Me), 0.95 (3H, s, 13-Me), 0.99 (3H, d, J = 6.8 Hz, 24-Me), 1.01 (6H, d, J = 6.0 Hz, 25-Me₂). MS: m/z 482 (M⁺), 467 (M-Me)⁺, 300 (M-ring A)⁺, 285 (M-ring A-Me)⁺, 313 (M-side chain)⁺, 175 (M-ring A-side chain)⁺.

Cyclotirucanenone (1b)

1 (10 mg) was dissolved in acetone (20 ml) and treated with freshly prepared Jone’s reagent (2.5 ml) at room temperature. Usual workup and crystallization from methanol provided the ketone 1b (7.6 mg); m.p. 87–88 °C; [α]D + 31.5° (c = 0.1, CHCl₃); IR (CHCl₃): 3045 and 1710 cm⁻¹; ¹H NMR (CDCl₃): δ 0.34–0.54 (dd, 2H, J = 4 Hz, 19-H₂), 4.6–4.7 (br s, 2H, 21-H₃), 1.05 (3H, s, 4ßMe), 1.01 (3H, s, 4αMe), 0.89 (3H, s, 14-Me), 0.97 (3H, s, 13-Me), 1.0 (3H, d, J = 7.9 Hz, 24-Me), 1.04 (6H, d, J = 7.1 Hz, 25-Me₂). MS: m/z 426 (M⁺), 411 (M-Me)⁺, 300 (M-ring A)⁺, 285 (M-ring A-Me)⁺, 313 (M-side chain)⁺, 175 (M-ring A-side chain)⁺.

Cyclotirucananol

Cyclotirucanenyl acetate 1a (10 mg) in glacial acetic acid (30 ml) was shaken with hydrogen and platinum (from 60 mg of PtO₂) for 1 h. The product was crystallized from methanol to give cyclotirucanenal acetate (5.8 mg) m.p. 131–132 °C; [α]D + 36.5° (c = 0.5, CHCl₃). Hydrolysis of the acetate with 3% ethanolic potassium hydroxide gave cyclotirucananol which crystallized from methanol m.p. 133–136 °C; [α]D + 43° (c = 0.1, CHCl₃).

The physical and spectral data agreed with the published data of cyclolaudanol [10].

Ozonolysis

The acetate (20 mg) was dissolved in glacial acetic acid (10 ml) cooled in an ice bath. Ozone (approx 10%) was passed through the solution for 20 min. The volatile product was steam distilled into a solution of dimedone. The pH of the dimedone solution was maintained just below 7 by the addition of alkali. The product was crystallized from methanol-water (m.p. 187–189 °C) and compared with an authentic sample of formaldehyde-dimedone adduct.