TIRUCALICINE - A NEW MACROCYCLIC DITERPENE FROM EUPHORBIA TIRUCALLI

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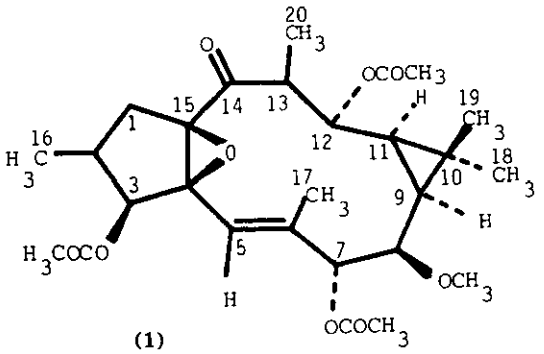
Abstract - A new macrocyclic diterpene, tirucalicine has been isolated from the fresh and undried latex of Euphorbia tirucalli. Its structure has been assigned as (1) on the basis of chemical and spectral studies.

Ingol is one of a recent new class of macrocyclic diterpenes which have been isolated from plants belonging to genus Euphorbia[1]. Esters of ingol are deviod of the toxic tumour-promoting and pro-inflammatory activity of phorbol and ingenol esters which have also been isolated from species of Euhporbia and are considered to be biosynthetic precursors of these polycyclic compounds. Recently tetraacyl-ingol derivatives were shown to possess cytotoxic activity in vitro against mouse lymphoma and rat basophillic leukemia cells[2]. This communication describes the structure of a new tri-ester of ingol isolated from the latex of Euphorbia tirucalli (Linn).

RESULTS AND DISCUSSION

Tirucalicine formed colourless needles from ethanol, mp 148-150°C, $[\alpha]_D$ + 16.13°. Its ir spectrum indicated the presence of ester carbonyl at 1730 cm^{-1}, ketone 1705 cm^{-1}, a trisubstituted double bond at 3055, 1650, 810 cm^{-1} and a methoxy group at 1250 cm^{-1}. High resolution mass spectrum (HRMS) of the diterpene afforded the molecular ion peak at m/z 506.2513, correspondig to the molecular formula $C_{27}H_{38}O_9$, revealing nine degree of unsaturation in the molecule. The molecular ion peak was confirmed by FD mass spectrometry[3].



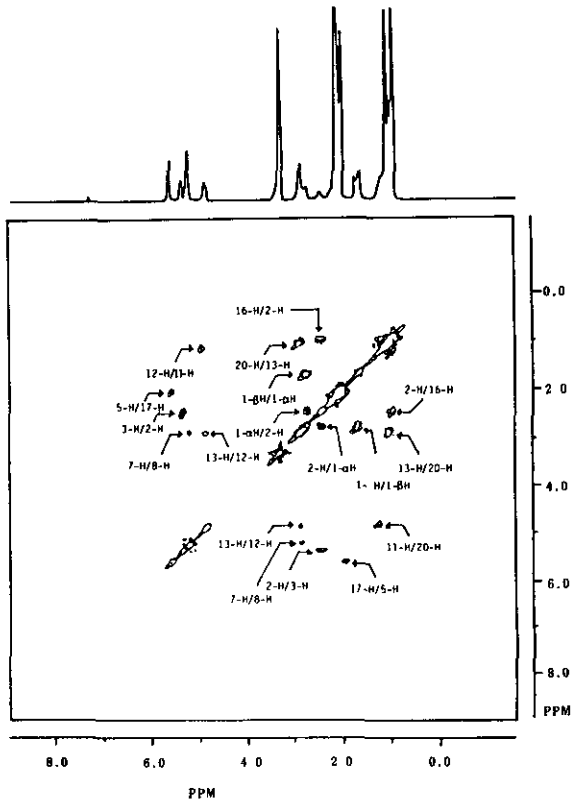
(1)

The ^{1}H-nmr spectrum of tirucalicine (1) ($CDCl_3$, 300MHz) showed characteristic features of ingol derivatives with characteristic double doublets of cyclopropyl protons at δ 1.21, 1.30, a multiplet of proton α to the carbonyl group at δ 2.90 and broad singlet of vinylic proton at δ 5.60[4]. The presence of three acetate group was evident by characteristic methyl singlets at δ 2.10, 2.15 and 2.08. Another three protons singlet at δ 3.30 was indicative of methoxy group. It further showed the presence of two secondary methyl groups as doublets at δ 1.08, 0.93 and three tertiary methyl groups as singlets at δ 2.01, 1.11, and 0.86, while the signals at δ 5.35, 5.21, and 4.88 were assigned to protons geminal to ester functions. Alkaline hydrolysis of 1 followed by acidification yielded tri-ol as major product, providing chemical evidence for the presence of three acetate group in 1. In its ^{1}H-nmr spectrum the signals of protons geminal to the hydroxy group showed an upfield shift comparing to the parent compound.

Various functional groups were located by a series of homodecoupling experiments. Irradiation at δ 1.21 induced the double doublets at δ 4.88 and δ 1.30 to collapse into doublets (J = 4.1 and 10.4 Hz). On the other hand, irradiation at δ 4.88 caused the double doublet at δ 1.21 to collapse into doublet (J = 8.7Hz) and simplified the multiplet at δ 2.90 into quartet. Irradiation at δ 2.90 reduced the doublet of one secondary methyl group into a singlet and also reduced the double doublet at δ 4.88 into a doublet (J = 11.0Hz). The signals at δ 1.30, 1.21, 4.88 and 2.90 could, therefore, be assigned to protons at C-9, C-11, C-12, and C-13. Although the vinylic proton appeared as broad singlet but the allylic coupling with the methyl group at δ 2.01 was evident in 2D J-resolved spectrum and further confirmed by irradiation at δ 5.60 which converted the broad singlet at δ 2.01 into sharp singlet and vice versa. Irradiation at δ 1.30 caused double doublet at δ 2.87 to collapse into double (J = 1.8Hz) while irradiation at δ 2.87 collapsed double doublets at δ 1.30 into doublet (J = 8.7Hz) and doublet at δ 5.21 into a sharp singlet. On the other hand, irradiation at δ 5.21 caused the double doublet at δ 2.87 to collapse into doublet (J = 10.4Hz). The signals at δ 5.21 and 2.87 were, therefore, assigned to protons at C-7 and C-8.

Since the cyclopropyl protons at C-9 and C-11 show coupling with each other as well as vicinal coupling with neighbouring protons, the C-10 must be fully substituted. The remaining tertiary methyl groups were assigned to this carbon in analogy to lathyrol and ingol derivatives. Similar analogy allowed us to place the acetyl group and secondary methyl group at C-3 and C-2, respectively[5]. This was further confirmed by homodecoupling experiments. Irradiation at δ 5.35 simplified the multiplet at δ 2.45 while irradiation at δ 2.45 caused the doublet at δ 5.35 to collapse into singlet and double doublet at δ 2.76 into doublet (J = 14.3Hz). On the other hand, irradiation at δ 2.76 simplified the multiplet at δ 2.45, and doublet at δ 1.67 collapsed into

singlet. The signals at δ 2.45, 2.76 and 5.35 could be confirmed as those of protons at C-2, C-α1 and C-3. The doublet at δ 1.67 was assigned to C-1βH on the basis of geminal coupling constant of the same magnitude. The non-coupling interaction between this proton and the proton at C-2 has already been reported in literature for ingol and its derivatives.[5] The evidences provided with above further revealed that C-4 and C-15 are fully substituted. In the absence of hydroxyl group, the remaining oxygen must be accounted in epoxide ring which must be at these positions in accordance to the structure (1) of tirucalicine. All the coupling interactions established through selective decoupling experiment were also illustrated by correlated spectroscopy (COSY-45°)[6] Fig.1.



(Fig 1)

The ^{13}C-nmr spectrum ($CDCl_3$, 75MHz) showed the presence of 27-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°[7-8]. The assignments were made by compairing with published ^{13}C-nmr spectra of ingol derivatives[9] and confirmed by ^{1}H-^{13}C heteronuclear chemical shift correlated spectroscopy (Hetero-COSY)[6].

Table-1: ^{13}C-Nmr. chemical shifts of tirucalicine (1).

Carbon No.	δ	Carbon No.	δ
1	31.42	15	71.24
2	29.37	16	16.95
3	76.31	17	17.59
4	73.52	18	16.56
5	117.19	19	29.22
6	139.78	20	16.21
7	74.10	8-OCH_3	56.47
8	78.45	3-$OCO\underline{C}H_3$	21.08
9	27.09	7-$OCO\underline{C}H_3$	21.03
10	19.41	12-$OCO\underline{C}H_3$	20.48
11	30.72	3-$O\underline{C}OCH_3$	170.50
12	71.10	7-$O\underline{C}OCH_3$	170.11
13	42.87	12-$O\underline{C}OCH_3$	170.63
14	207.36		

The structure of tirucalicine (1) has close resemblance to ingol tetraacetate differing only in acetat group at C-8 been replaced by a methoxy function. Very close agreement of the chemical shifts of protons and carbons in ^{1}H- and ^{13}C-nmr spectra of these two compounds led us to assign same stereochemistry at the chiral centres as of (1) as of ingol tetraacetate. The absolute structure of which has already been established by X-ray methods[10].

EXPERIMENTAL

Melting points are uncorrected, ir spectra were recorded on JASCO A-302 spectrophotometer. HRMS were recorded on Finnigan MAT-312 mass spectrometer connected to PDP 11/34 (DEC) computer system. The ^{1}H-nmr spectra were recorded at 300MHz on Bruker AM-300 spectrometer with TMS as internal reference. TLC experiment were performed on silica gel (GF-254, 0.2mm) plates (E.Merck). Two dimensional COSY-45° experiment was acquired at 300MHz with sweep width of 4000Hz (2k data points in ω_2) and 2000Hz (256t_1 values zero-filled to 1k) in ω_1. The heteronuclear two dimensional ^{1}H-^{13}C chemical shift correlation experiments were carried out at 300MHz with sweep width of 12820Hz (2k data points in ω_2) and 1024Hz (256t_1 values zero-filled to 1k) in ω_1. In both the 2D-experiments a 2 sec. relaxation delay was used 16 transients were performed for each t_1 value.

Isolation of Tirucalicine (1): The plant material (latex) was collected in Karachi, Pakistan and was identified by Plant Texonomist, Department of Botany, University of Karachi, where a voucher specimen is deposited. The fresh latex (2kg) was directly tapped from incision into a flask containing acetone. It was stored over night at 4°C. The coagulated residue then formed was sucked. The ether soluble fraction obtained from acetone insoluble residue was subjected column chromatography over activated silica gel. Elution was carried out with a mixture hexane-ether. The eluate from hexane-ether (55:45) was further subjected to preparative TLC on silica gel (GF-254) precoated plates with hexane-ethyl acetate-methanol (7:2.5:0.5) as solvent system. The major diterpene, tirucalicine (1) (Rf = 0.3) 212mg, was cystralized out as colourless needles from ethanol.

Mp : 148-150°C, $[\alpha]_D$ + 16.13°(c, 3.41, $CHCl_3$). Ir: ($CHCl_3$) ν_{max}, 1730 cm^{-1} (ester carbonyl), 1705 cm^{-1} (ketone), 3055, 1650, 810 cm^{-1} (vinylic) and 1250 cm^{-1} (methoxy). HRMS: M^+ 506.2513 ($C_{27}H_{38}O_9$). Ms: m/z (rel.intens). 506 (M^+, 52%), 446 (15), 403 (20), 344 (25), 312 (10), 245 (7), 181 (20), 165 (22), 111 (100). ^{1}H-Nmr: ($CDCl_3$, 300MHz, δ ppm): 5.60 (1H, br s, C-5H), 5.35 (1H, d, J =8.7Hz, C-3H), 5.21 (1H, d, J = 1.8Hz, C-7H), 4.88 (1H, dd, J = 11.0, 4.1Hz, C-12H), 3.30 (3H, s, 8-OCH_3), 2.90 (1H, m, C-13H), 2.87 (1H, dd, J = 10.4, 1.8Hz, C-8H), 2.76 (1H, dd, J = 14.3, 8.7Hz, C-1αH), 2.45 (1H, m, C-2H), 2.01 (3H, br s, C-17H), 2.10, 2.15, 2.08 (9H, s, 3-$OCOCH_3$), 1.67 (1H, d, J = 14.3, C-1βH), 1.11 (3H, s, C-19H), 1.08 (3H, d, J = 7.1Hz, C-20H), 0.93 (3H, d, J = 6.3Hz, C-16H), 0.86 (3H, s, C-18H). ^{13}C-Nmr ($CDCl_3$, 75MHz) (δ ppm): Table-1.

Base-Catalysed Hydrolysis of Tirucalicine (1):

1 (40mg) was hydrolysed with 0.5M KOH in methanol (1.4 gm KOH in 50ml of MeOH) at 60°C for 1 hr. The reaction mixture was acidified with 5% HCl and workup in the usual manner. The parent alcohol has been isolated in pure state by TLC on silica gel (GF-254) as absorbent and hexane-chloroform (4:6) as solvent, mp: 188-190°C, $[\alpha]_D$ + 35.1° (c,0.51, $CHCl_3$), ir: ($CHCl_3$) ν_{max}, 3450 cm^{-1} (OH), 1705 cm^{-1} (ketone) 3050, 1640, 810 cm^{-1} (trisubstituted double bond), 1250 cm^{-1} (OCH_3). HRMS: M^+ 380.2198 ($C_{21}H_{32}O_6$). Ms: m/z (rel.intens.) 380 (M^+, 25), 312 (32), 245 (15), 181 (30), 165 (48), 85 (100), 69 (60). ^{1}H-nmr: ($CDCl_3$, 300MHz, δ ppm) 5.72 (1H, br s, C-5H), 4.30 (1H, d, J = 8.6Hz, C-3H), 4.27 (1H, d, J = 1.6Hz, C-7H), 3.87 (1H, dd, J = 10.7, 3.9Hz, C-12H), 3.27 (3H, s, 8-OCH_3), 2.90 (1H, m, C-13H), 2.84 (1H, dd, J = 10.5, 1.6Hz, C-8H), 2.74 (1H, dd, J = 14.7, 8.8Hz, C-1αH), 1.90 (3H,br s, C-17H), 1.67 (1H, d, J = 14.7, C-1βH), 1.11 (3H, s, C-19H), 1.07 (3H, d, J = 6.9Hz, C-20H), 0.96 (3H, d, J = 6.5Hz, C-16H), 0.88 (3H, s, C-18H).

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