

Characterization of Bufadienolides and Ferulic Acid Ester from *Urginea Indica*

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Abstract - Phytochemical study of the methanolic extract of the bulb of *Urginea indica*, four compounds were isolated from the chloroform soluble fraction. 14 β -hydroxy-3,5,20,20-tetraenolide, 12 β -14 β -dihydroxybufa-3,5,20,22-tetraenolide, 16- β -Oacetyl-3 β -14 β -dihydroxy-19-formyl-bufa-4,20,22-trienolide and 32-hydroxy-dotriacontayl-p-ferulate were isolated and characterized on the basis of spectral (IR, ¹H, ¹³CNMR and Mass) data. Isolation and characterization of compound 4 as 32-hydroxy-dotriacontayl-p-ferulate being reported for the first time in this plant.

Keywords - Ferulic acid ester, *Urginea indica*, Bufadienolides, 14 β -hydroxy-3,5,20,20-tetraenolide, 12 β -14 β -dihydroxybufa-3,5,20,22-tetraenolide, 16- β -Oacetyl-3 β -14 β -dihydroxy-19-formyl-bufa-4,20,22-trienolide and 32-hydroxy-dotriacontayl-p-ferulate.

I. INTRODUCTION

Urginea indica belongs to the family Hyacinthaceae [1,2] called as Jungli Piyaz or Indian squill. It is also known as sea onion. The plant grows in sandy soil and dry hills. About 100 species of the genus *Urginea* are known in India [3]. It is an annual herb containing fibrous roots from the base of globular bulb. The outermost layer of the bulb is thin and the colour is reddish, orange to brown [4]. The bulbs are reported as a good source for various medicinal applications e.g. as cardiogenic, stimulant, expectorant, digestive and also reported in the treatment of various diseases like asthma, rheumatism, hypoglycemic, anticancer, leprosy and skin diseases. The bulb mainly contains good percentage of

glycoproteins, bufadienolides [5,6] which possess cardiogenic, insecticidal and antimicrobial properties [7,8]. *Urginea indica* preparation is also useful in dermatological problems and psoriasis, an inflammatory diseases as the treatment of psoriasis is not available in allopathic system [5]. Dry ethanolic extract of bulbs are also reported for anticancerous activity especially against human epidermal carcinoma, antioxidant, gastrointestinal stimulant and antiangiogenic activities [9, 10]. Moderate analgesic and significant anti-inflammatory activities have been reported from ethanolic extract on rats [11]. *Urginea indica* is specially useful and well known for its cardiogenic activity and this is the characteristic feature of the bufadienolides which are present in the plant in good amount [12].

II. MATERIAL AND METHOD

IR spectra (Cm⁻¹) recorded on Shimadzu FTIR-84ROS Spectrometer using KBr pellets. ¹HNMR and ¹³CNMR spectra (δ ppm) were recorded on JEOL-AL-300 spectrometer using CDCl₃ as solvent and TMS as an internal reference. Mass spectrum were obtained from SIC, CDRI, Lucknow, India.

III. EXPERIMENTAL SET UP

The bulbs of *Urginea indica* were collected from Dholpur, Rajasthan, India and identified by Prof. Navjyot Sarna, Department of Botany, University of Rajasthan, Jaipur.

Extraction and Isolation

The shade dried bulbs (2.8 kg) were powdered and extracted with methanol on steam bath for 48 Hours. The extract was filtered off and solvent was removed under reduced pressure the crude extract so obtained was dissolved in chloroform. The chloroform soluble portion after removal of solvent was chromatographed over silica gel column using solvents of increasing polarity with petroleum ether as starting solvent. After that increasing ratio of ethylacetate was used and compounds 1,2,3, and 4 were isolated and characterized.

Isolation of Compound 1

Elution of column with petroleum ether-ethyl acetate (9:1) yielded 14- β -O-acetylbufa-3,5,20,22-tetraenolide and purified from acetone gave cream coloured compound. The spectral studies on as UV λ_{\max} (CH₃OH) 235 and 290 nm, IR (KBr) 3410, 1640, 1456, 1338, 1240, 1180, 1130 cm⁻¹ and mass (m/z) 366.3 (M⁺).

Isolation of Compound 2

Compound 2 was obtained as yellow mass on elution the column with petroleum ether-ethylacetate (4:1) yielded 12- β , 14 β -dihydroxy bufa-3,4,20,22-tetraenolide and purified by PTLC with chloroform gave yellowish amorphous compound. It showed homogenous behaviour and analysed as UV λ_{\max} (CH₃OH) 235 and 288 nm. IR (KBr) 3340, 2910, 1645, 1460, 1300, 1235, 1160 Cm⁻¹ and mass (m/z) 382.3(M⁺).

Isolation of Compound 3

Elution of column with ethyl acetate yielded scillicyanosidin and purified from acetone to give white crystalline (5.6 mg) compound. The spectral studies on as UV λ_{\max} [M⁺CH₃COOH-H₂O] (25), 378(M⁺ CH₃COOH-H₂O] (8), 368 (7), 350(12), 307(23), 91(100).

Isolation of Compound 4

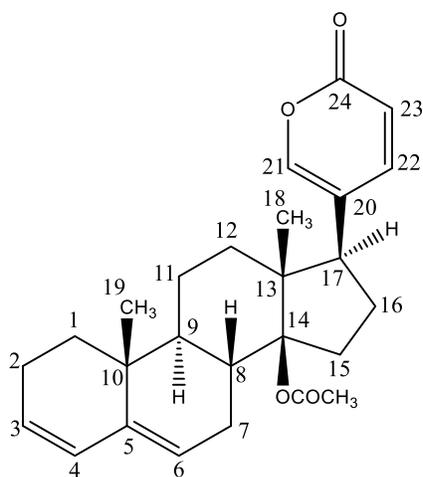
Compound 4 was isolated by eluting the column with ethylacetate – methanol (95:5). The molecular weight was calculated by mass spectrum, m/z 658(M⁺).

IV. RESULT AND DISCUSSION

Compound 1 was obtained after the elution of column with petroleum ether-ethylacetate (9:1) as cream coloured compound. Which was crystallized from acetone (4.3mg) cream coloured powder. The UV spectrum of the compound showed absorptions λ_{\max} (CHCl₃) at 237 nm which is characteristic for a conjugated diene system [12] and at λ_{\max} 290 nm due to the carbonyl group of conjugated diene system [5].

In ¹HNMR spectrum presence of two singlets for three protons at δ 0.70 and 0.88 were observed and assigned to C-18 and C-19 methyl groups respectively. The presence of bufadienolide system containing δ lactone ring attached at C-17 position was characterized by observing doublets, at 6.20 ($J=9.7$ Hz) for one proton present at C-23 position, 7.20 (d, $J=2.7$ Hz) assigned for the proton at C-21 and 7.80 as a double doublet ($J=9.6, 2.7$ Hz) assigned to the proton at C-22 position. The PMR spectrum showed signals at 5.44, 5.62, 5.93 for one proton each in the olefinic region confirming the presence of conjugated diene system (which was also confirmed by UV spectrum) for one proton each present at C-6, C-3 and C-4 positions respectively.

The ¹³C NMR spectrum of compound 1 suggested the presence of hydroxyl group by indicating the absorption at δ 86.81 which is characteristic for the tetra substituted carbon with β orientation of hydroxyl group, confirmed by comparing the ¹HNMR and ¹³CNMR data with the reported values. The spectral data were found in good agreement with reported value for conjugated, diene. Thus on the above basis and comparing the data with reported values compound 1 was identified as 14 β -hydroxybufa-3,5,20,22-tetraenolide, which is supported by mass Spectrum m/z 366.z(M⁺). Thus, confirming the molecular formula C₂₄H₃₀O₃.

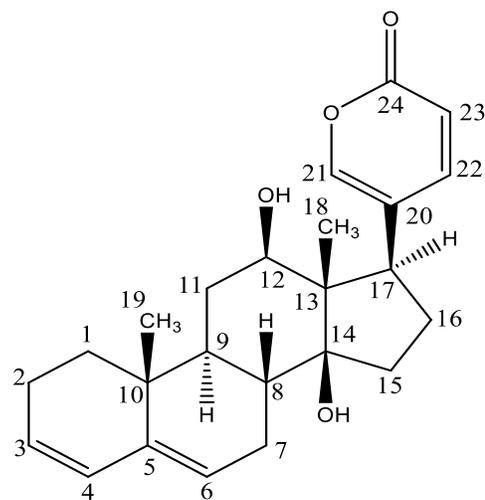


Compound 1

Compound 2 was obtained as white powder. The molecular formula was assigned on the basis of PMR, CMR and mass spectrum as C₂₄H₃₀O₄ (m/z 382.3). Other prominent ions in mass spectrum were observed at m/z, 365, 342, 267 which can be explained by the loss of OH, CHCO and γ -pyron. In the ¹HNMR spectrum presence of two methyl groups was confirmed by locating the absorptions at 0.86(s,3H,C-18) and 0.71(s,3H,C-19). The presence of lactone ring at C-17 position was confirmed by showing a multiplet at 2.50 for one proton and the geometry of the lactone ring was confirmed in β -orientation⁵. The protons present as positions C-21, 22 and 23 were confirmed by the absorptions at 7.20 (d, J=2.7Hz), 7.78(dd, J=9.8,2.7Hz) & 6.21(d, J=9.8) for one proton each respectively. The absorption observed at 3.45 as a doublet (J=7.2Hz) for one proton assigned for a hydroxyl group at C-12 position with β -orientation [13,14]. The presence of conjugated diene system was also confirmed by observing the olefinic proton at 5.60 as a multiplet, 5.88 as a doublet (J=9.6) and 5.38 as a triplet for one proton each attached at C-3, C-4 and C-6 positions respectively. In ¹³CNMR two signals located at δ 85.78 and 77.23 clearly indicates the nature of carbon atom as quaternary and tertiary respectively.

The presence of hydroxyl groups at these positions with β -orientation was confirmed [15, 16] by comparing the NMR Spectra of the compound with

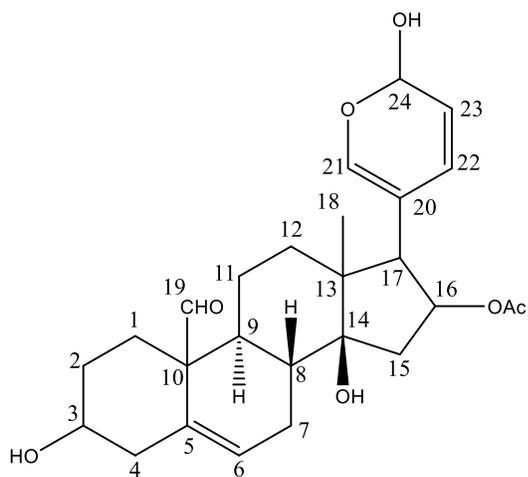
reported value and β -orientation is characteristic for most of the bufadienolides isolated from the genus *Urginea*. The β -orientation of the hydroxyl group at C-12 position was also confirmed by comparing the ¹HNMR data [17-21] on the basis of above description compound 2 was identified as 12 β , 14 β - dihydroxybufa -3, 5,20,22-tetraenolide



Compound 2

Compound 3 was obtained as colourless powdered. The molecular formula for compound 3 was established as C₂₆H₃₂O₇. On the basis of FAB-MS (M/Z 457 (M+H)⁺, proton and carbon NMR spectra. The compound was also indicated the presence of bufadienolide by showing the absorption at δ 7.20 (d, J= 2.6Hz), 7.95 (dd, J= 9.8, 2.6H), and 6.17 (d, J= 9.8H respectively for 6.17 one proton each attached on C-21, C-22 and C-23 position respectively. Proton NMR spectrum showed the presence of an absorption at δ 0.77 (s,3H) which was assigned for the methyl group present at C-18 position. In the NMR spectrums of title compound one more methyl group which is present in bufadienolides, the signal was not observed, but a downfield signal at δ 9.72 was observed as sharp singlet accounted for one proton, and it was assigned for the proton of -CHO group. The presence of aldehyde group was also confirmed by locating a signal at δ 202.4 and sharp peak at 1715 Cm⁻¹ in the IR spectrum of the compound. Other characteristic signals in PMR

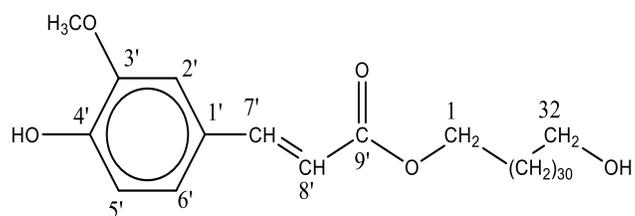
spectrum were observed at 4.12 for one proton as a broad singlet and account and assigned to the proton present at position-3. The downfield shift of the proton confirms the presence of hydroxyl group attached at C-3 position. In ^{13}C NMR the characteristic signals were observed at 5.69 for one proton as a singlet confirming the presence of olefinic proton. The signals for C=C double bond was located at 130.0 and 136.9 for the carbons at C-4 & C-5 respectively. The presence of a signal at 84.0 was due to presence of a quaternary carbon containing hydroxyl group with β -configuration. The carbon of δ pyrone ring resonates as δ 151.3, 150.0 and 113.6 and accounted for C-21, C-22, and C-23 respectively. The ^1H NMR and ^{13}C NMR data for the title compound was found in good agreement with the bufadienolide characterized earlier [21] and characterized as 16 β -acetoxy-3 β , 14 β -dihydroxy-19-formyl-bufa-4,20,22-trienolide.



Compound 3

Compound 4, Elution of column with ethylacetate-methanol (95:5) afforded dotriacontanylferulate, m.p. 93°C, IR (KBr) ν_{max} (Cm^{-1}) 3450, 2920, 2840, 1715, 1690, 1620, 1600, 1590, 1500, 1050, 955 etc. Compound 4 showed M^+ ion at m/z 658. Corresponding to molecular formula $\text{C}_{42}\text{H}_{74}\text{O}_5$ which was also supported by ^1H NMR and ^{13}C NMR spectra. The IR spectrum of the compound confirmed the presence of hydroxyl and ester groups by observing the absorptions at 3450 and 1715 cm^{-1} respectively. The presence of ester group conjugated with

carbon-carbon double bond was established by the sharp absorption at 1620 and 955 cm^{-1} . The presence of phenyl ring was supported by the absorptions at 1600, 1590 and 1500 cm^{-1} . The ^1H NMR spectrum showed prominent signals for trans-ferulate moiety at δ 7.60, 7.57 and δ 6.30, 6.26 with a coupling constant $J=15$ Hz, confirming the trans geometry at C-7' and C-8' positions. The aromatic protons were observed at 7.06 (dd), 7.00 (d) and 6.88 (d), while in the aliphatic region broad singlet at 5.82 confirmed the presence of phenolic hydroxyl group. A sharp singlet at δ 3.92 for three protons confirmed the presence of methoxy group. Two triplets at 4.18 and 3.64 for two protons each were observed and assigned for the methylene protons attached to ester and hydroxyl groups respectively. The ^{13}C NMR spectrum is also in agreement with the above values [22]. On the basis of above spectral data compound 4 was characterized as 32-hydroxy-dotriacontonyl-p-ferulate.



Compound 4

V. ACKNOWLEDGEMENT

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