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Research Article

Isolation and Characterization of New Bufadienolides from *Urginea indica*

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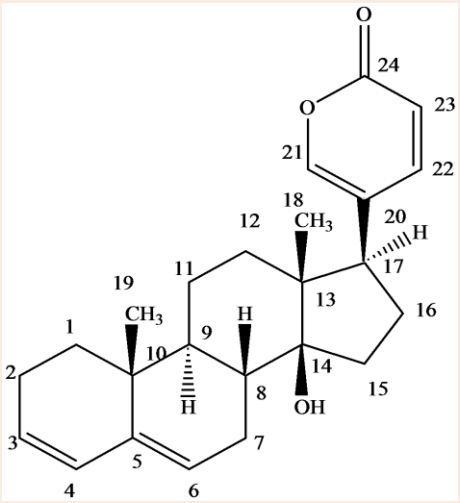
Abstract

In the course of phytochemical study of the methanolic extract of the bulb of *Urginea indica*, nine compounds were isolated from the chloroform soluble fraction. In addition to four bufadienolides, viz. scillaridine (1), 12*β*-hydroxy scillaridine (2), 5α-4,5-dihydro-8*β*-hydroxy scillicyanosidin (3), 14*β*-hydroxybufa-20, 22-dienolide-3-O-*α*-L-thevetoside (4); three steroids: stigmasterol, *β*-sitosterol and *β*-sitosterol-3-O-*β*-D-glucoside; and two waxy components: lauric acid and pentatricontanol were isolated through column chromatography. Scillaridine (1) have been isolated for the first time from the bulb of the title plant. Compounds (2-4) are new and are being reported for the first time. The compounds were identified on the basis of spectral (IR, ^{1}H NMR, ^{13}C NMR and Mass) data.

Keywords: *Urginea indica*; Hyacinthaceae; Bufadienolide; Scillaridine; 12*β*-hydroxy scillaridine; 5α-4,5-dihydro-8*β*-hydroxy scillicyanosidin; 14*β*- hydroxybufa-20, 22-dienolide-3-O-*α*-L-thevetoside.



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Introduction

The plant belongs to the family Hyacinthecae and it is commonly known as 'Jungli piyaz' (Indian squill). *Urginea* is a polytypic genus with about hundreds of species occurring in India, Africa and Mediterranean region [1]. According to taxonomic study five species of the genus were recognised in India [2]. *Urginea indica* is a perennial herb with fibrous roots protruding from the base of a large, tunicated, nearly globular bulb. The outer scales of bulb are thin and papery with red or orange-brown colour. According to the Dioscorides Encyclopedia the extracts of bulbs are used for recovering corns and warts. Indian drug squill in small doses, is also used as an expectorant, stimulant and cardio tonic agent. Psoriasis an inflammatory disease of the skin, where treatments are not available in allopathic medicine but *Urginea indica* preparations are known to be used traditionally by many tribes and aborigines against it and many more dermatological diseases.

Ethanolic extract of bulb contains anti-cancerous properties, especially against human epidermoid carcinoma. In the literature, plant has been reported as an antifungal [3], antioxidant [4], gastrointestinal stimulant [5], antiangiogenic and proapoptotic activities [6]. Ethanolic extract at dose of 1.5 g/kg, orally was given to each rat and reported for high anti-inflammatory activity, anti arthritic activity and moderate analgesic effects [7]. *Urginea* is also known to possess cardio tonic activity, a characterstic feature ascribed to the presence of bufadienolides [8].

Materials and Methods

General experimental procedure

IR spectra were recorded on SHIMADZU FTIR-8400S spectrometer using KBr pellets. ^{1}H NMR and ^{13}C NMR spectra (300 MHz and 75 MHz respectively) were recorded on JEOL AL-300 spectrometer in parts per million (δ) in $CDCl_3$ with TMS as an internal reference. FAB Mass spectra were recorded on JEOL SX 102/BA-600 mass spectrometer. Column chromatography was performed on column (length 120 & diameter 2.5 cm), silica gel (60-120 Mesh) and TLC on Merck's silica gel 60F254 pre -coated glass plates.

Extraction and isolation

The bulb of the plant was collected from Chambal region, Dholpur (Rajasthan, India). The species were identified by Mr. Vinod Kumar Sharma (voucher no. RUBL 21067) and a voucher specimens was deposited at the Herbarium of the Department of Botany, University of Rajasthan, Jaipur, India. Shade dried bulbs (5kg) of *U. Indica* were powdered and extracted with methanol on a water bath for (12×3) hrs. The extract was concentrated under reduced pressure to yield 542.4 g and then re-extracted with petroleum ether (5.2 g), chloroform (17.6 g) and ethyl acetate (31.3 g). The petroleum ether and chloroform extracts were mixed together and preceded for the isolation of constituents through column chromatography over silica gel column and eluted with the solvent of increasing polarity. After elution of column, compounds 1 to 4 were isolated, purified and characterized. The isolated compounds were purified with help of crystallization and PTLC method. All these compounds were identified by ^{1}H NMR, ^{13}C NMR and mass spectral data (**table 1**.)

Isolation of 14-*β*-hydroxybufa-3, 5, 20, 22-tetraenolide (1)
On eluting the column with petroleum ether - chloroform (1:3) yielded 14-*β*-hydroxybufa-3, 5, 20, 22-tetraenolide and purified from acetone-methanol mixture gave cream colored amorphous compound. It showed homogeneous behavior on TLC. The spectral data are as : UV λ_{max} (CH_3OH) 237 and 290 nm; IR (KBr) 3390, 1650, 1452, 1325, 1235, 1190, 1120 cm^{-1}; API-Mass (m/z) 366.3 (M^+), 322.3, 284.3, 256.3, 141.0 ($C_{24}H_{30}O_3$ required 366.219).

Isolation of 12-*β*, 14-*β*-dihydroxybufa-3, 5, 20, 22-tetraenolide (2)
Further elution of column with petroleum ether - chloroform (1:1) as the eluent yielded the new bufadienolide named as 12-*β*, 14-*β*-dihydroxybufa-3, 5, 20, 22-tetraenolide and purified by PTLC using petroleum ether-chloroform (1:1. After usual work up a cream colored amorphous compound was obtained. It showed homogeneous behavior on TLC and analysed as : UV λ_{max} (CH_3OH) 237, 290 nm; IR (KBr) 3350, 2930, 1640, 1465, 1305, 1237, 1166 cm^{-1}; API-Mass (m/z) 382.3 (M^+), 366.2, 322.3 [M^+-CH_2CO-H_2O], 284.3 [M^+-α-pyrone], 256.3.

Isolation of 16-*β*-O-acetyl-10-*β*-formyl-3-*β*, 8-*β*, 14-*β*-trihydroxybufa-20, 22-dienolide (3)
Elution of column with chloroform - ethyl acetate (4:1) as eluent yielded another novel bufadienolide i.e. 16-*β*-O-acetyl-10-*β*-formyl-3-*β*,8-*β*,14-*β*-trihydroxybufa-20,22-dienolide and further purified through PTLC. It showed homogeneous behavior on TLC plate. The spectral data are obtained as : UV λ_{max} (CH_3OH) 290 nm; IR (KBr) 3410; 2940; 1728; 1630; 1455; 1237; 1190, cm^{-1}; API-Mass (m/z) 474.3 (M^+), 456.3 [M^+-H_2O], 414.3 [M^+-CH_3COOH], 382.3, 340.3 [M^+-α-pyrone-$2H_2O$].

Isolation of 14-*β*-hydroxybufa-20, 22-dienolide-3-O-α-L-thevetoside (Compound 4)
Further elution of column with chloroform-ethyl acetate (4:1) yielded the new chemical entity named as 14-*β*-hydroxybufa-20, 22-dienolide-3-O-α-L-thevetoside after purification through PTLC. The spectral data are recorded as: UV λ_{max} (CH_3OH) 290 nm; IR (KBr) 3450 (*br*), 2930, 1640, 1485, 1305, 1180, API-Mass (m/z) 530.3 (M^+), 512.3 [M^+-H_2O], 370.3 [M^+-160], 272.3 [M^+-160-α- pyrone].

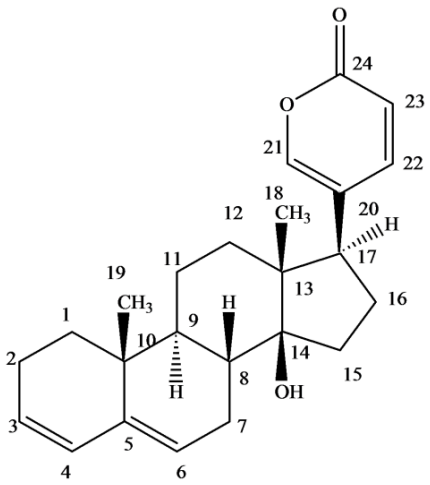
Results and discussion

Compound (1) was obtained as cream colored amorphous powder. Its molecular formula $C_{24}H_{30}O_3$ was determined by API-ES mass spectrum showing M^+ at m/z 366.3 [M^+]. The UV, IR, ^{1}H and ^{13}C NMR spectral data of Compound were identical to those of compound isolated from the bulb of *Urginea epigea* [9].

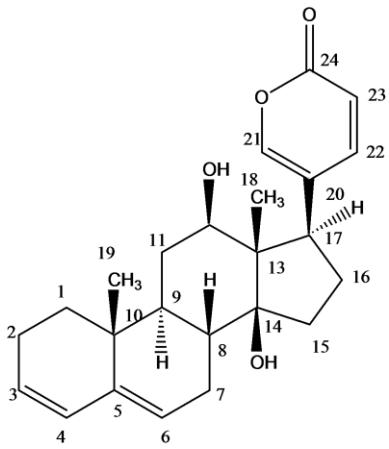
The UV spectrum of this bufadienolide gave a distinct absorption λ_{max} (CH_2Cl_2) at 237 nm, which is in good agreement with that observed for a conjugated diene system [10]. The lactone ring also contains a conjugated diene system but this is affected by the carbonyl group and hence absorption occurs at 290 nm.

^{1}H NMR spectrum two singlets appeared at δ 0.693 and δ 0.861, were assigned to C-18 and C-19 methyl group protons respectively. The presence of bufadienolide-type δ-lactone ring at C-17 was confirmed by the coupled resonances at δ 7.772 (*dd*, *J*=2.7, 9.6 Hz, 1H, C-22), δ 7.178 (*d*, *J*=2.7 Hz, 1H, C-21) and δ 6.195 (*d*, *J*=9.7 Hz, 1H, C-23) and confirmed its *β*-orientation. The presence of a conjugated diene system was established by the olefinic proton resonating at δ 5.557 (*m*, 1H), δ 5.869 (*d*, *J*=9.3 Hz, 1H) and δ 5.386 (*m*, 1H) and assigned to the proton present at C-3, C-4 and C-6, respectively [11, 12].

The ^{13}C NMR spectrum of Compound (1) suggested a fully substituted carbon by showing the absorption at δ 86.77 and it was assigned to C-14 position indicating that a hydroxyl group is present at this position. The stereochemistry at C-14 could not be determined from the NMR spectrum but on the basis of literature and comparison of the ^{1}H NMR spectra of various bufadienolides reported in the literature, it was concluded that the hydroxyl group present at position C-14 have *β*-orientation [13, 14].



14β-Hydroxybufa-3, 5, 20, 22-tetraenolide (1)



12*β*, 14*β*-dihydroxybufa-3, 5, 20, 22-tetraenolide (2)

Compound (2) was obtained as cream-white powder. Its molecular formula was determined by API-ES mass spectrum as $C_{24}H_{30}O_4$, showing M^+ at m/z 382.3. The other prominent ions were observed at m/z 366.3, 322.3 [M^+-CH_2CO-H_2O], 284.3 [M^+-α-pyrone] indicating the steroidal skeleton of the molecule.

^{1}H NMR spectrum showed the presence of two methyl groups by showing the signal at δ 0.858 (*s*, C-18) and δ 0.704 (*s*, C-19) for three protons each. The δ-lactone ring attached at C-17 position of the bufadienolide with *β*-orientation was established by the presence of the absorptions at δ 7.769 (*dd*, *J* = 2.7, 9.9 Hz, 1H, C-22), δ 7.176 (*d*, *J* = 2.7 Hz, 1H, C-21) and δ 6.190 (*d*, *J* = 9.9 Hz, 1H, C-23). A downfield absorption was observed at δ 3.400 (*d*, *J* = 7.2 Hz, 1H) due to the presence a hydroxyl group with β-orientation at C-12 position. The presence of a conjugated diene system was also evident from the olefinic proton at δ 5.559 (*m*, 1H), δ 5.862 (*d*, *J*=9.6, 1H) and δ 5.384 (*t*, 1H) attached at C-3, C-4 and C-6, respectively [11, 12].

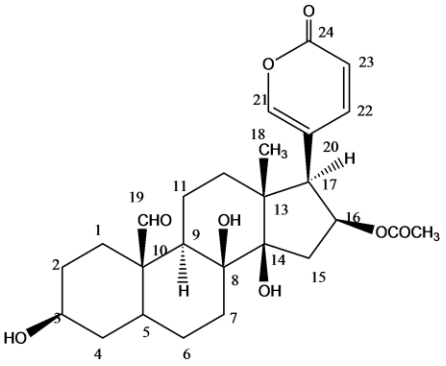
The quaternary carbon appeared at δ 85.75 in the ^{13}C NMR spectrum was assigned to C-14 indicating the presence of a hydroxyl group at this position with *β*-orientation which is a characteristic for most of the bufadienolides isolated so far from the genus [13,14]. Carbons C-11, C-12, C-13 and C-18 were observed in the

downfield, due to the presence of hydroxyl group at C-12 position [15]. The presence of C-12 hydroxy group with *β*-orientation was confirmed by its H^1 NMR data [16].

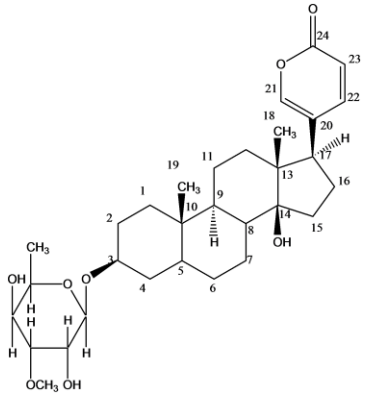
Compound (3) was obtained as white powder. Molecular formula was established on the basis of API-ES mass spectrum as $C_{26}H_{34}O_8$ by showing M^+ at m/z 474.3 with important ions at m/z 456.3 [M^+-H_2O], 414.3 [M^+-CH_3COOH], 382.3, 340.3 [M^+-α-pyrone-$2H_2O$] confirming the steroidal skeleton.

The ^{1}H NMR spectrum of Compound (3) showed the two singlet for methyl and an aldehydic protons appeared at δ 0.842 (*s*, 3H, C-18) and δ 9.754 (*s*, 1H, C-19) respectively. This compound was also characterized as bufadienolide-type δ-lactone or δ-pyrone ring with *β*- orientation at C-17 position and it was established by the presence of the signals at δ 7.952 (*dd*, *J* = 2.4, 9.6 Hz, 1H, C-22), δ 7.295 (*d*, *J* = 2.4 Hz, 1H, C-21) and δ 6.203 (*d*, *J* = 9.6 Hz, 1H, C-23). A downfield signal was observed at 5.398 (*t*, *J* = 8.7 Hz, 1H, C-16) due to presence of acetoxy group at this position. Its location and orientation was also confirmed as *β*-configuration at C-16, due to the downfield shift of C-17 proton [17]. The proton on C-17 was observed as a doublet at 2.781 (*d*, *J* = 8.7 Hz, 1H). A singlet at δ 1.818 for three protons was due to presence of O- acetyl moiety.

The two quaternary carbons showed signal at δ 87.20 and 83.85 were assigned to C-8 and C-14 respectively in ^{13}C NMR spectrum indicated that two hydroxyl groups are present at these positions with *β*-orientation [13, 14]. In ^{13}C NMR spectrum, three downfield signals were observed at δ 202.92, 169.92 and 161.86 due to aldehydic, acetyl and lactone carbons respectively.



16-*β*-O-acetyl-10-*β*-formyl-3-*β*,8-*β*, 14-*β*-trihydroxybufa-20, 22-dienolide (3)



14-*β*- hydroxybufa-20, 22-dienolide-3-O-α-L-thevetoside (4)

Compound (4) was obtained as white powder. Its molecular formula was determined by API-ES mass spectrum as $C_{31}H_{46}O_7$, showing M^+ at m/z 530.3. The other important peaks were observed at m/z 512.3 [M^+-H_2O], 370.3 [M^+-160], 272.3 [M^+-α-pyrone] indicating the steroidal skeleton.

^{1}H NMR spectrum showed the presence of two singlets at δ 0.735 (*s*) and δ 0.924 (*s*) for three protons each and assigned for C-18 and C-19 methyl groups respectively. Absorptions at δ 7.750 (*dd*, *J* = 2.4, 9.6 Hz, 1H, C-22), δ 7.162 (*d*, *J* = 2.4 Hz, 1H, C-21) and δ 6.194 (*d*, *J* = 9.6 Hz, 1H, C-23) confirmed the presence of δ-lactone ring at C-17 position with β -orientation. Methyl protons were observed at 1.262 (*d*) due to presence of its 6'- position of thevetose ring.

The ^{13}CNMR spectrum confirmed the presence of thevetose sugar carbons by showing the absorption at δ 97.71, 72.75, 84.54, 74.92, 68.41 and 17.82 for C 1'-6' respectively in the title compound. The quaternary carbon observed at δ 85.21 was assigned to C-14 position that contains a hydroxyl group at this position with *β*-orientation [13, 14].

Table 1 ^{1}H, ^{13}C NMR data of Compound 1, 2, 3 and 4

C-atom	1		2		3		4	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1.	*α*, 2.332, *m* *β*, 1.130, *m*	33.66	*α*, 2.329, *m* *β*, 1.118, *m*	35.69	2.318, *m* (2H)	32.67	2.280, *m* (2H)	35.34
2.	*α*, 1.721, *m* *β*, 2.055, *m*	34.50	*α*, 1.723, *m* *β*, 2.057, *m*	33.52	*α*, 2.083, *m* *β*, 2.074, *m*	29.55	*α*, 2.072, *m* *β*, 1.336, *m*	29.36
3.	5.557, *m*	126.20	5.559, *m*	126.20	4.007 (*d*, *J*=6.6 Hz)	66.50	3.662, *m*	76.28
4.	5.869 (*d*, *J*=9.3 Hz)	129.37	5.862 (*d*, *J*=9.6 Hz)	129.37	*α*, 1.232, *m* *β*, 1.533, *m*	32.25	*α*, 1.230, *m* *β*, 1.534, *m*	32.23
5.	-	141.31	-	141.31	1.321, *m*	30.52	1.318, m	31.92
6.	5.386, *m*	122.64	5.384, *t*	122.42	*α*, 1.782, *m* *β*, 2.264, *m*	27.73	*α*, 1.828, *m* *β*, 2.220, *m*	32.68
7.	*α*, 2.082, *m* *β*, 2.423, *m*	27.61	*α*, 2.078, *m* *β*, 2.422, *m*	28.62	*α*, 2.178, *m* *β*, 1.126, *m*	28.81	*α*, 2.108 *m* *β*, 1.124, *m*	40.68
8.	1.778, *m*	39.52	1.769, *m*	39.54	-	87.20	1.876, *m*	42.78
9.	1.232, *m*	45.52	1.292, *m*	45.56	1.227, *m*	49.46	1.408, *m*	51.08
10.	-	36.28	-	36.26	-	53.17	-	54.24
11.	*α*, 1.522, *m* *β*, 1.334, *m*	23.76	*α*, 1.618, *m* *β*, 1.333, *m*	27.72	*α*, 1.504, *m* *β*, 1.886, *m*	21.42	*α*, 1.495, *m* *β*, 1.960, *m*	22.68
12.	*α*, 1.454, *m* *β*, 1.371, *m*	40.99	3.400 (*q*, *J*=7.2 Hz)	77.23	*α*, 1.245, *m* *β*, 1.594, *m*	40.01	*α*, 1.324, *m* *β*, 1.987, *m*	37.65
13.	-	49.18	-	48.15	-	49.23	-	50.24
14.	-	86.77	-	85.75	-	83.85	-	85.21
15.	2.128, *m*	22.08	2.117, *m*	22.08	*α*, 2.438, *m* *β*, 1.621, *m*	39.78	*α*, 2.392, *m* *β*, 1.716, *m*	33.90
16.	*α*, 2.142, *m* *β*, 1.755, *m*	29.03	*α*, 2.141, *m* *β*, 1.751, *m*	29.03	5.398 (*t*, *J*=8.7 Hz)	73.25	*α*, 2.122, *m* *β*, 1.702, *m*	29.70

17.	2.452, *m*	52.23	2.455, *m*	53.25	2.781 (*d*, *J*=8.7 Hz)	56.87	2.403, *m*	55.48
18.	0.693, *s*	17.17	0.858, *s*	12.19	0.842, *s*	16.35	0.735, *s*	21.17
19.	0.861, *s*	19.54	0.704, *s*	19.54	9.754, *s*	202.92	0.924, *s*	19.16
20.	-	123.24	-	123.47	-	116.56	-	122.66
21.	7.178 (*d*, *J*=2.7 Hz)	149.44	7.176 (*d*, *J*=2.7 Hz)	149.44	7.295 (*d*, *J*=2.4 Hz)	151.07	7.162 (*d*, *J*=2.4 Hz)	148.56
22.	7.772 (*dd*, *J*=2.7, 9.6 Hz)	147.55	7.769 (*dd*, *J*=2.7, 9.9 Hz)	147.55	7.952 (*dd*, *J*=2.4, 9.6 Hz)	148.56	7.750 (*dd*, *J*=2.4, 9.6 Hz)	146.79
23.	6.195 (*d*, *J*=9.6 Hz)	116.17	6.190 (*d*, *J*=9.9 Hz)	116.27	6.203 (*d*, *J*=9.6 Hz)	113.27	6.194 (*d*, *J*=9.6 Hz)	115.36
24.	-	162.31	-	162.32	-	161.86	-	162.49
CO*CH*$_3$					1.818, *s*	20.92		
*CO*CH$_3$					-	169.92		
1'							5.312 (*d*, *J*=1.2 Hz)	97.71
2'							3.881 (*d*, *J*=1.2 Hz)	72.75
3'							3.422, *s* (3H)	84.54
4'							3.598, *m*	74.92
5'							3.288, *m*	68.41
6'							1.262 (*d*, *J*=6.0 Hz)	17.82

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