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Chemical Constituents of *Urtica ardens* Leaves

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Abstract

From MeOH extract of leaves of *Urtica ardens* **3β-hydroxy-35-(cyclohexyl-5'-propan-7'-one)-33-ethyl-34-methyl-bacteriohopane** and **Glucopyranosyl-O-(1→2) fructofuranoside** (Sucrose) have been isolated .The structures of isolated compounds was confirmed by spectroscopic methods viz. UV, IR, NMR and Mass.

Keywords: Urtica ardens, Urticaceae, 3β-hydroxy-35-(cyclohexyl-5'-propan-7'- one) - 33 - ethyl - 34 - methyl - bacteriohopane and Glucopyranosyl - O-(1→2) fructofuranoside (Sucrose).

Introduction

Urticaceae is a large family of about 45 genera and 550 species found in tropical and temperate regions, 21 genera and 120 species in India. The plants of this family are herbs, undershrubs or rarely trees, without latex; epidermal cells often cystoliths, stem fibrous, leaves alternate or opposite, simple, stipulate or not. Flowers are minute, unisexual, regular, usually cymose, sometimes crowded on enlarged receptacle. Perianth lobes 3-5, sepaloids, free or united and stamens are equal to tepals, inflexed in buds, pollens 3-5 porate, spheroidal, stenopalynous. Ovary 1-locular, 1-erect ovulate, style simple and fruit achene or drupe.

Urtica ardens, vern. Stinging nettle, belong to family Urticaceae is a perennial, erect, pubescent herbs or shrubs often attaining to 2.5m high; stem greenish-pale, bark fibrous, petioles, leaves, branches covered with stinging bristles. Flowers are small, pale green, clustered on spreading, axillary 4-8cm long, paniculate cymes. Male flowers with 4-perianth segments and 4 stamens. Female perianth segments 4, unequal, inner ones twice longer than outer ones. Achenes ovoid, pale-brown, hairy, enclosed by persistent perianth. Flowering and fruiting season is August to January .The plants of genus Urtica is distributed throughout the world including Paraguay, Uruguay, Brazil, southwest of Hubei province, China, Asia, America, Europe, Iran, Greece and Turkey.

Materials and Methods

General

Melting points were recorded on Perfit melting point apparatus. UV spectra were measured on a Perkin-Elmer Lambda-25 spectrophotometer (methanol as solvent) and IR spectra were recorded on Perkin-Elmer spectrum RX I FT-IR spectrophotometer using KBr discs. NMR spectra were obtained on SFO1 300 MHz –Bruker NMR Spectrophotometer (300 MHz, for ^{1H} and ^{13}C - NMR, TMS as internal standard), HRMS micro OTOF - Q II 10328 (Bruker Compass data Analysis). Column chromatography was carried out on silica gel (Merck 10-40 µ) precoated plates (0.5 mm thick layer) were visualized by spraying with 7% H_2SO_4 as universal spraying reagent along with other specific reagents for particular class of natural products.

Plant Material

Whole plants (10 kg) of *Urtica ardens* were collected from the Auli, Chamoli Uttrakhand India in October 2014. The plant was identified from Department of Botany, HNB Garhwal University Srinagar Uttrakhand. A voucher specimen (GUH-6890) was deposited in the same section.

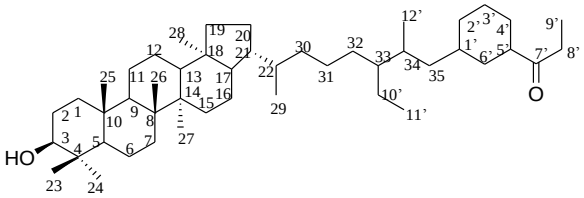
Extraction and Isolation

Shade dried coarsely powdered whole plant of *Urtica ardens* (10 kg) was extracted (three times) exhaustively with 95% ethanol (5L) at 30-50 °C temperature for 16-18 hours on a heating mantle. The extraction mixture was filtered and solvent evaporated to dryness under reduced pressure to yield black residue (400 g). The crude extract was fractionated with petroleum ether and ethyl acetate (repeatedly three times) by soxhlet

apparatus to yield petroleum ether (90 g), ethyl acetate (130 g), crude extracts. The ethyl acetate soluble (130 g) was pre-adsorbed onto silica gel (Merck, 60-120 mesh, 200 g) and then allowed to run over the silica gel (500 g) packed column. The elution was started with $CHCl_3$: MeOH by increasing polarity of MeOH (5-17%). The fractions (100 ml of each) obtained from column were collected and deposited in same conical on the basis of TLC analysis. The elution afforded two compounds in pure form (purified by recrystallization)

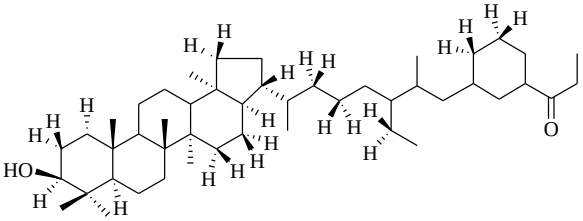
Compound -1

Shiny white crystals (90 mg); m.p. 258-260 °C; UV λ_{max} : 295 nm; IR $\bar{\nu}_{max}$:3417, 1715, 2850 cm^{-1}; EIMS (m/z): 679 $[M+H]^+$ (100%), 661 (10%), 607 (4%), 427 (9%), 219 (20%); NMR data: see Table -1; Elemental analysis: (found C, 83.12; H, 12.17; O, 4.71%; calcd. For $C_{47}H_{82}O_2$: C, 82.81; H, 12.06; O, 4.63%).

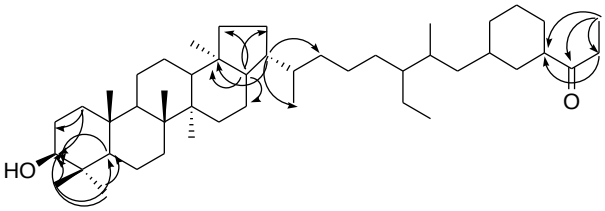


Chemical Structure of Compound -1

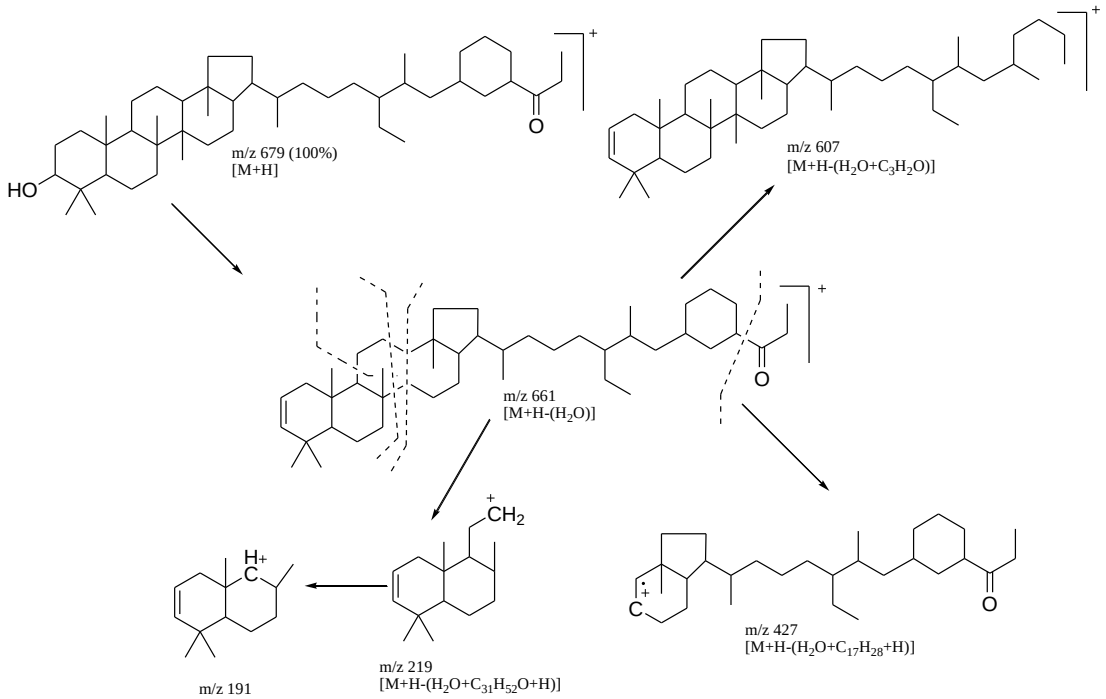
It was obtained as shiny white needle shaped crystals (90 mg); m.p. 258-260 °C and molecular formula $C_{47}H_{82}O_2$ was proposed on the basis of its quasi-molecular ion at m/z 679.6056 $[M+H]^+$ in HR-ESIMS (positive mode, calcd. 679.6387) and elemental analysis. The IR spectrum exhibited bands at 3417 cm^{-1} characteristic of hydroxyl group and a band at 1715 cm^{-1} was clearly expressive for the stretching in a carbonyl group. The ^{13}C and DEPT NMR spectra of compound showed 47 carbon signals including ten methyl, twenty methylene, eleven methine and six quaternary carbons. The ^{13}C NMR spectrum showed a highly downfield singlet at δ 213.2 indicating the presence of a carbonyl group whereas the signals appeared at δ 6.8, 11.6, 14.6, 15.7, 16.3, 17.5, 17.9, 18.2, 22.2, and 30.5 were due to the presence of ten methyl groups. A signal at δ 3.73 (overlap) for α H-3 in the ^1H NMR and its NOESY correlation with H-5 (δ 0.94) and H-24 δ (δ 0.95) confirmed the β position of hydroxyl group at C-3. The stereochemistry of various other key position in compound was determined by NOESY experiment. In NOESY spectrum the strong correlation of H-16 to H-29 and H-21 to H-30 determined the position of side chain at C-21. This fact was further confirmed by the HMBC correlation of H-21 to C-30 (δ 30.6). The above spectral studies led us to deduced that compound is hopane type triterpene consist a side chain (Tsuzuki et al., 2001; Ye et al., 2007). The long range correlation H-9' (δ 0.72) to C-5' (δ 59.4), C-7' (δ 213.2) and C-8' (δ 41.7) in HMBC was corroborated for a terminal propanoyl moiety. The index of hydrogen deficiency for $C_{47}H_{82}O_2$ (7) showed the presence of six ring and one double bond (carbonyl). Moreover, except carbonyl, there were no other multiple bonds present in compound (confirmed by IR and NMR spectra). Thus, it is clear that sixth ring must be present in the side chain. Relatively downfield values at δ 41.2 (C-1') and δ 59.4 (C-5') suggested bisubstituted aliphatic ring with a substitution of a propanoyl moiety. The HMBC correlation of H-9' and H-8' to H-5' confirmed the position of propanoyl at C-5'. The detailed study of HMBC, NOESY and DEPT spectra gave the evidences for bacteriohopane type structure of compound (Ourisson and Rohmer, 1992). The mass spectrum of compound was found very informative in support of the structure confirmation. It showed a molecular ion peak at m/z 679.6056 (100%), which loses H_2O to furnish an ion at 661.53 (10%) whereas the important ions at 219.20 and 427.40 were due to the rupture of ring-C (Ourisson et al., 1979). Hence, the structure of compound was elucidated as **3β-hydroxy-35-(cyclohexyl-5'-propan-7'-one)-33-ethyl-34-methyl-bacteriohopane**.



Key NOESY Correlation in Compound



Important HMBC Correlation in Compound



Proposed Mass Fragmentation in Compound

Table - 1

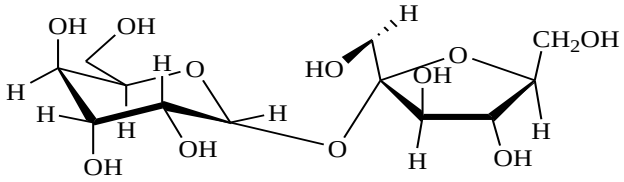
^{13}C, ^{1}H (300 MHz) NMR, NOESY and HMBC data of SS-1 in $CDCl_3$

Position	δC ppm	δC ppm	NOESY	HMBC
1	38.2	0.92(m)	H-2, H-25	C-2, 3
2	28.1	1.32(m), 2.33(m)	H-3	C-4, 10
3	72.2	3.73(broad)	H-4, H-24	C-1, 5
4	39.7	-	-	-
5	49.1	0.94(m)	H-24, H-3	C-1, 3, 7, 9, 23, 24
6	18.6	1.36(m),2.25(m)	H-24	C-8, 10
7	32.0	1.47(m), 2.25(m)	H-27	C-5, 9, 14, 26
8	42.8	-	-	
9	53.2	0.99(m)	-	C-1, 5, 7, 12, 14
10	37.8	-	-	
11	20.1	0.91(m),1.93(m)	H-25	C-8, 10, 13
12	20.2	0.91(m),1.93(m)	H-19	C-9, 14, 18
13	53.2	2.37(m)	-	C-8, 11, 15, 17
14	41.5	-	-	
15	32.8	0.96(m),2.38(m)	H-27	C-13, 17
16	36.0	0.96(m),2.38(m)	H-29	C-14, 18, 21
17	58.2	1.21(overlap)	H-29	C-16, 18, 19, 20
18	42.1	-	-	
19	39.2	0.90(m),2.23(m)	H-12, H-20	C-13, 17, 21
20	30.0	0.90(m),2.23(m)	-	C-17, 18, 22
21	61.3	1.40(overlap)	H-30	C-16, 18, 19, 30
22	35.0	1.98(m)	-	C-17, 20, 31
23	30.5	0.96 (overlap)	-	C-3, 5
24	22.2	0.95(overlap)	-	C-3, 5
25	16.3	0.88(S)	H-11	C-1, 9
26	18.2	0.90(S)	-	C-9, 14
27	17.5	0.91(s)	H-7, H-15	C-8, 15
28	14.6	0.85(s)	-	C-13, 17
29	17.9	1.18(s)	H-16, H-17	C-21, 30
30	30.6	1.94(m)	H-21	C-21, 29, 32
31	32.4	1.90(m)	H-10, H-29	C-22, 33

32	35.3	1.93(m)	-	C-30, 34, 10’
33	37.1	2.17(m)	-	C-31, 35, 11’, 12’
34	38.3	2.22(m)	-	C-32, 1’, 10’
35	37.4	1.47(m)	-	C-33, 2’, 12’
1'	41.2	2.12(m)	-	C-34, 3’, 5’
2'	35.1	1.37(m)	H-3',12'	C-35, 4’, 6’
3'	32.3	1.30(m)	H-4'	C-1’, 5’
4'	35.6	1.38(m)	H-3'	C-2’, 7’, 6’
5'	59.4	2.41(m)	-	C-1’, 3’, 8’
6'	32.7	1.90(m)	-	C-2’, 4’, 35
7'	213.2	-	-	
8'	41.7	2.38(m)	-	C-5’
9'	6.8	0.72(s)		C-5’, 7’
10'	31.7	1.08(m)	H-31,11'	C-11’, 32, 34
11'	11.6	0.85(m)	H-10'	C-10’, 33
12'	15.7	0.91(m)	H-2'	C-33, 35

Compound -2

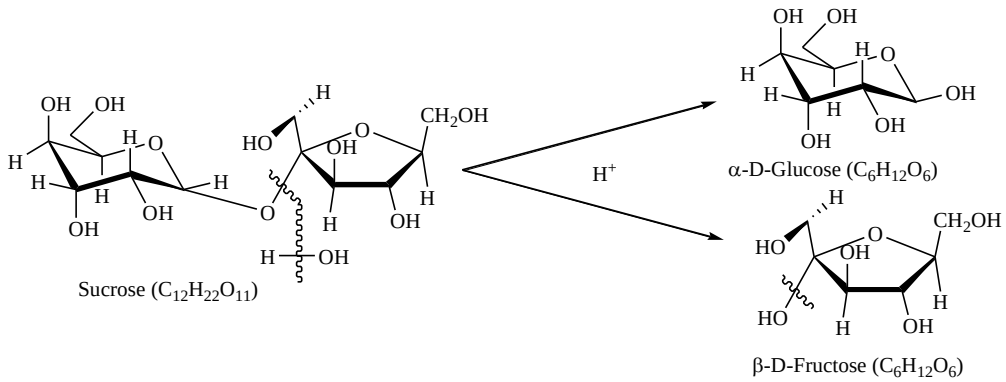
Brownish crystalline compound (10 gm); m. p. 187-189°C; $\bar{y}_{max}$ 3404 cm^{-1} (-OH), 2944 cm^{-1} (Aliphatic C-H), 1233 cm^{-1} (C-O streching), 989 cm^{-1} (OH bending); HR-ESIMS (m/z): 342 (calcd.) $[M]^+$, 365 $[M+Na]^+$ (100%), 366 $[M+Na+H]^+$ (10%), 282 (5%), 202 (13%); NMR data see Table 2.7; Elemental analysis: (found C, 42.42; H, 6.43; O, 51.46%; calcd. For $C_{30}H_{48}O_3$: C, 42.81; H, 06.66; O, 51.23%).



Chemical Structure of Compound

Acid Hydrolysis of Compound

Compound (1 mg) in 2 M HCl (0.2 mL) was refluxed at 70°C for 3 h (Joshi et al., 2014). Reaction mixtures were separately subjected to silica gel TLC and PC, together with the standard samples (Sucrose, Glucose and Fructose), using $CHCl_3$– MeOH–H_2O (6:4:1, v/v/v) and n-BuOH–AcOEt–H_2O (5:1:4, v/v/v, upper phase) as the developing solvents and using 10% aqueous H_2SO_4 as the detection reagent. Glucose and Fructose were detected from the compound.



Acid Hydrolysis Fragmentation of Compound

It was obtained as brownish crystalline compound (10 gm); m. p. 187-189°C; from methanolic soluble fraction (CHCl3: MeOH, 9:10) and its molecular formula $C_{12}H_{22}O_{11}$ was proposed on the basis of its quasi-molecular ion at m/z 342 (calcd.) $[M]^+$, 365 $[M+Na]^+$ (100%), 366 $[M+Na+H]^+$ (10%), 282 (5%), 202 (13%); in HR-ESIMS (positive mode,) and elemental analysis. It was not gave a positive test with Benedict's reagent so it is a non-reducing sugar (Simoni et al., 2002).IR spectrum exhibited bands at 3404 cm^{-1} (Broad) characteristic of hydroxyl group, 2944 cm^{-1} (Aliphatic C-H), 1233 cm^{-1} (C-O streching) and a band at 989 cm^{-1} was clearly expressive for the OH bending. The ^{1}H-NMR spectrum of compound exhibited one proton double doublet at δ 5.16 (dd, j=2.5, 3.2 Hz) and one proton doublet at δ 5.01 (d, j=6.0 Hz) corresponding to methine proton at C-4 and the anomeric proton at C-1. The large coupling constant (11 Hz) of the carbinyl proton indicates that it is in the axial position. A one

proton multiplet at δ 4.35 was assigned to oxygenated methine proton H-5. The carbinol protons H-2, H-3, H-4, H-3' and H-4' appeared as five one proton multiplets at δ 3.48, 3.37, 3.81, 3.34 and 5.16 respectively. The ^{13}C and DEPT NMR spectra of compound showed twelve carbon signals in the broad band decoupled spectrum; three methylene, eight methine and one quaternary carbon atoms. The ^{13}C-NMR spectrum of compound displayed anomeric carbons C-1 and C-2' at δ 91.85 and δ 104.12 respectively where as the oxygenated methylene carbons C-1', C-6 and C-6' resonated between δ 60.58, 62.15 and 62.23 (Bhat et al., 2010; Otsuka et al., 2008). The ^{1}H-NMR and ^{13}C-NMR spectral data and comparison of the melting point and the authentic sample along with a co TLC pattern confirmed compound to be a disaccharide (sucrose). Its structure has been designated as **Glucopyranosyl-O-(1→2) fructofuranoside** (Sucrose).

Table -2
^{13}C, ^{1}H and DEPT Data of Compound in $DMSOd_6$

S. N.	Position	δC ppm	δH ppm	DEPT
1	1	91.85	5.01(d)	CH
2	2	72.92	3.48(d)	CH
3	3	72.97	3.76(dd)	CH
4	4	69.92		CH
5	5	71.73	4.35(ddd)	CH
6	6	62.15		CH_2
7	1'	60.58	4.48(d)	CH_2
8	2'	104.12		C
9	3'	77.09		CH
10	4'	74.36	5.16(dd)	CH
11	5'	82.64	4.7(m,broad)	CH
12	6'	62.23	3.40(d)	CH_2

Conclusion

Urtica ardensis is an important medicinal plants of Garhwal Himalaya. It is locally known as Sishnu in Nepalese, Paharah Bichubuti in Bengali, Kandli in Garhwal and Nettle in English. The young leaves of the plant are very nutritious and are consumed as food in Western Himalayan region. This plant is used traditionally to cure various diseases and disorders. Leaves of the plant are used to cure goiter. The leaves are also used to treat cold and cough. The leaf extract is used in hair wash to prevent baldness. The leaves are applied to cure the dislocation of bone; and also boils. The decoction of the plant is used as febrifuge. Phytochemical investigation of leaves of plant showed the presence of some important secondary metabolites, which have a marked physiological effects on the body of individuals. Therefore, it suggests that the plant can be used as a source of oral drugs to be used in the treatment of cough and cold.

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