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New Iridoid glycosides from *Wendlandia puberula*

R. K. JOSHI*

*Department of Chemistry, Govt. P.G. College, Gopeshwar- 246401, Uttarakhand, **INDIA**

Email: joshi.shanti@rediffmail.com

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ABSTRACT

An extract of the leaves of Wendlandia puberula has yielded three new iridoid glycosides namely 8-acetylharpagide, loganin and sweroside.

Keywords: 8-acetylharpagide, loganin and sweroside.

INTRODUCTION

Wendlandia puberula (rubiaceae) is known to yield iridoid glycosides[1-2]. The present communication deals with the isolation and characterization of three new iridoid glycosides designated as 8-acetylharpagide,1, loganin 2 and sweroside3 along with known compounds β-sitosterol and β-sitosterol-β-D-Glycoside. The structures of the compounds were determined from the studies of UV, IR and NMR spectra and chemical transformations.

MATERIALS AND METHODS

Extraction and isolation: The air-dried well chopped leaves of *Wendlandia puberula* (collected from Tharali, district Chamoli) was exhaustively defatted with light petroleum ether ($60\text{-}80^0$). The petroleum free mass was then extracted with 90% aqueous ethanol. The ethanol extract was concentrated to dry under reduced pressure in vacuum. A suspension of the residue was made with water, which was washed with diethyl ether for several times and then extracted with chloroform. The aqueous layer was successively extracted with EtOAc and BuOH (saturated with water). The EtOAc and BuOH layers were concentrated under reduced pressure to give EtOAc and BuOH extracts respectively. The BuOH extract was further treated with methanol: water (8:2) and filtered. The filtrate was evaporated to dry to give methanol extract. The light chloroform extract, ethyl acetate extract and BuOH were subjected to column chromatography over silica gel-G with various solvents in order to their increasing polarity. The $CHCl_3$ extract was subjected to CC over Sigel-G using gradient elution with C_6H_6-EtOAc (100:0 → 9:1) afforded β-sitosterol and β-Sitosterol-β-D-glycoside. The ethyl acetate extract was subjected to CC over Si-gel using gradient elution with $CHCl_3$:MeOH (1:0 → 17:3) to get various fractions. The $CHCl_3$:MeOH (90:10) fraction was subjected to CC over Si-gel using gradient elution with $CHCl_3$:MeOH (98:2→90:10) afforded compound **1** and various other fractions having mixture of compounds. The fractions obtained with $CHCl_3$:MeOH (93:7) were mixed and evaporated to dryness. The extract obtained was further chromatographed over

silica gel eluted with MeOH:$CHCl_3$ (9:1) afforded two fractions. Fraction **1** was subjected to CC over Si-gel eluted with MeOH:$CHCl_3$ (9:1) afforded compound **1** and compound **2** . Fraction **2** was subjected to repeated CC over silica gel eluted with MeOH:$CHCl_3$ (9:1) afforded compound **2** and compound **3**.

Experimental: Melting points were determined on a MAC (MSW-403) apparatus and are uncorrected. Electronic spectra (EtOH) were obtained on a SP-3-200 Pye Unicam spectrophotometer as KBr pellets, NMR spectra were recorded in BRUKER WM 400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR). Chemical shifts are given on a ppm scale with TMS as an internal standard. The analytical data of the compounds were found satisfactory.

RESULTS AND DISCUSSION

The spectral results of three compounds are given below.

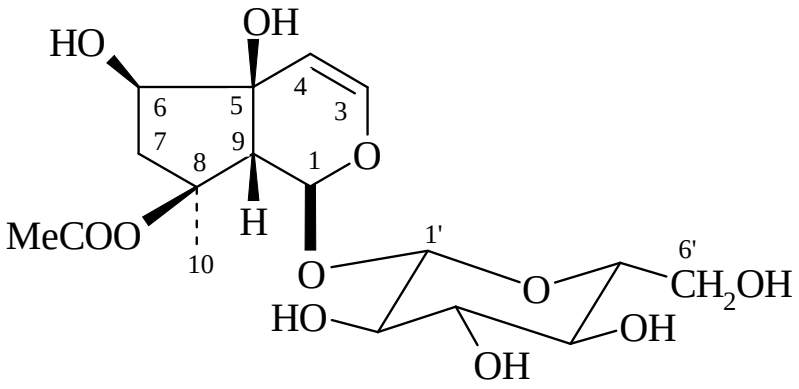
Compound **1**: λ_{max} 224 and 308nm; ν_{max} 3500 (chelated OH), 2900 (C-H stretching of saturated carbon atom), 1700 and 1650 cm^{-1} (α,β-unsaturated carbonyl function). ^{1}H-NMR (400 MHz, C_5D_5N): δ 6.71 (1H, d, J = 1.2 Hz, H-1), 6.53 (1H, d, J = 6.4 Hz, H-3), 5.15 (1H, dd, J = 6.4, 1.2 Hz, H-4), 4.03 (1H, d, J = 4.4 Hz, H-6), 2.06 (1H, dd, J = 14.8, 4.4 Hz, H-7α), 2.54 (1H, d, J = 14.8 Hz, H-7β), 3.54 (1H, brs, H-9), 1.59 (3H, s, H-10), 1.85 (3H, s, OAc), 5.30 (1H, d, J = 8 Hz, H-1'), 4.00 (1H, dd, J = 8.0, 8.8 Hz, H-2'), 3.99 (1H, t, J = 8.8 Hz, H-3'), 4.23 (1H, m, H-4'), 4.26 (1H, dd, J = 11.6, 2.4 Hz, H-5'), 4.50 (1H, dd, J = 11.6, 2.4 Hz, H-6'a), 4.32 (1H, dd, J = 11.6, 5.2 Hz, H-6'b). ^{13}C-NMR (100 MHz, C_5D_5N): δ 94.7 (C-1), 142.2 (C-3), 108.1 (C-4), 73.2 (C-5), 78.7 (C-6), 45.8 (C-7), 87.2 (C-8), 55.1 (C-9), 22.6 (C-10), 99.1 (C-1'), 74.8 (C-2'), 78.4 (C-3'), 71.6 (C-4'), 76.8 (C-5'), 62.8 (C-6'), 22.1 ($\underline{C}H_3COO$-) 170.9 ($CH_3\underline{C}OO$-).

Compound **2** : λ_{max} 224 and 308nm; ν_{max} 3450 (OH), 2900 (C-H stretching of saturated carbon atom), 1715 (unsaturated ester) and 1650 cm^{-1} (enol ether).^{1}H-NMR (400 MHz, CD_3OD): δ 5.26 (1H, d, J = 4.4 Hz, H-1), 7.38 (1H, s, H-3), 3.09 (1H, m, H-5), 1.62 (1H, m, H-6a), 2.03 (1H, m, H-6b), 4.04 (1H, t, J = 4.4 Hz, H-7), 1.87 (1H, m, H-8), 2.23 (1H, m, H-9), 1.08 (3H, d, J = 6.8 Hz, H-10), 3.68 (3H, s, -OCH_3), 4.64 (1H, d, J = 8.0 Hz, H-1'), 3.17-3.39 (4H, m, H-2', H-3', H-4', H-5'), 3.66 (1H, dd, J = 11.8, 6.0 Hz, H-6'a), 3.89 (1H, dd, J = 11.8, 5.2 Hz, H-6'b). ^{13}C-NMR (100 MHz, CD_3OD): δ 97.7 (C-1), 152.1 (C-3), 114.1 (C-4), 32.2 (C-5), 42.6 (C-6), 74.7 (C-7), 42.2 (C-8), 46.6 (C-9), 13.4 (C-10) 169.6 (-$\underline{C}$-OO-), 51.7 (-COO$\underline{C}H_3$), 99.9 (C-1'), 75.1 (C-2'), 78.3 (C-3'), 71.6 (C-4'), 78.0 (C-5'), 62.1 (C-6')

Compound **3** : λ_{max} 244 (iridoid enol ether system conjugated with a C-4 carbonyl group; ^{1}H-NMR (400 MHz, CD_3OD): δ 5.43 (1H, d, J = 1.2 Hz, H-I), 7.56 (1H, d, J = 2.4 Hz, H-3), 3.11 (1H, m, H-5), 1.71 (2H, m, H-6), 4.41 (2H, m, H-7), 5.56 (1H, m, H-8), 2.72 (1H, m, H-9), 5.33 (1H, dd, J = 2.0, 16.8 Hz, H-10a), 5.25 (1H, dd, J = 2.0, 9.0 Hz, H-10b), 4.63 (1H, d, J = 8.0 Hz, H-1'), 3.22 (1H, dd, J = 8.0, 8.8 Hz, H-2'), 3.71 (1H, t, J = 8.8 Hz, H-3'), 3.31 (1H, m, H-4'), 3.44 (1H, m, H-5'), 3.65 (1H, dd, J = 12.0, 6.0 Hz, H-6'a), 4.02 (1H, dd, J = 12.0, 6.4 Hz, H-6'b). ^{13}C-NMR (100 MHz, CD_3OD): δ 98.1 (C-1), 154.3 (C-3), 105.3 (C-4), 27.3 (C-5), 25.1 (C-6), 70.1 (C-7), 132.1 (C-8), 42.6 (C-9), 121.2 (C-10), 169.7 (C-11), 99.4 (C-1'), 73.7 (C-2'), 76.2 (C-3'), 70.2 (C-4'), 72.1 (C-5'), 61.0 (C-6').

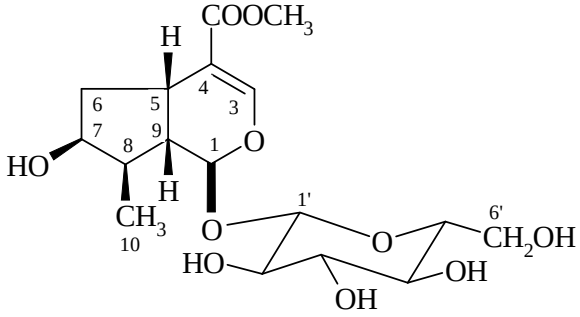
Compound **1:** The molecular formula is $C_{17}H_{25}O_{11}$ and m.p. 191-193^{o}C. The IR spectrum of displayed characteristic abosorption maxima at 3500 cm^{-1} for a chelated OH group at 2900 cm^{-1} for C-H stretching of saturated carbon atom, and at 1700 and 1650 cm^{-1} for α,β-unsaturated carbonyl function. Its UV-spectrum showed absorption bands characteristic to an iridoid enol ether system[3] at 224 and 308 nm. The molecular formula of the compound **1** showed presence of five double bond equivalence in the molecule. The ^{1}H-NMR spectrum of coupled with detailed analysis of ^{1}H-^{1}H COSY indicated presence of two integrated protons signals each for 1H at δ 6.53 (d, J = 6.4 Hz, H-3), and 5.15 (dd, J = 6.4, 1.2 Hz, H-4), two oxygen bearing methine signal at δ 6.71 (d, J = 1.2 Hz, H-1), methylene protons at δ 2.06 (1H, dd, J

= 14.8, 4.4 Hz, H-7α), and 2.54 (1H, d, J = 14.8 Hz, H-7β), one oxygen bearing methine proton signal at δ 4.03 (1H, d, J = 4.4 Hz, H-6), and methine proton signal at 3.54 (1H, brs, H-9). In addition to this a three protons singlet at δ 1.85 assignable for acetoxy group, methyl protons singlet at δ 1.59, and seven protons due to the sugar moiety were observed. A methylene proton signal at δ 2.06 (dd) showed coupling with another methylene proton at δ 2.54 and with an oxygen bearing methine proton at δ 4.03. A methine proton signal (brs) showed weak coupling with the proton signal appeared at δ 6.71 which might be due to a methine proton bearing two oxygen atoms and is compatible with the H-1 proton signal of most of the iridoids having O-glycosylation at C-1 carbon [3-6]. The ^{13}C-NMR spectrum and distortion less enhancement by polarization transfer (DEPT) spectrum showed 17 carbon signals; three quaternary carbon, 10 methine carbons, two methylene carbon and two methyl carbons. Acid hydrolysis of compound **1** with 5% HCl gave a sugar which was identified as D-glucose by paper chromatography. The glycosidic nature of the compound was supported by a doublet at δ 5.30 (J = 8.0 Hz) assignable to the anomeric proton of β-D-glucose. The usual location of sugar moiety at position O-1 of the aglycone was shown by the downfield shifted signal[4] of H-1 (δ 6.71, d, J = 1.2 Hz). The ^{13}C-NMR chemical shifts of anomeric carbon atom (C-1') at δ 99.1 and the chemical shifts of other carbon atoms of sugar moiety [δ 74.8 (C-2'), 78.4 (C-3'), 71.6 (C-4'), 76.8 (C-5'), 62.8 (C-6')] are in agreement with the NMR spectrum and thus confirmed the presence of glucose in the molecule. The ^{13}C-NMR spectrum confirmed the presence of methyl function (δ 22.6), a methylene carbon [δ 45.8 (C-7)], two methine carbons [(δ 78.7 (C-6) having oxygen function and 55.1 (C-9)], a secondary carbinyl carbon [δ 94.7 (C-1)], two quaternary carbons having an oxygen function [δ 73.2 (C-5), and 87.2 (C-8)], a di-substituted double bond [δ 142.2 (C-3), 108.1 (C-4),] and an acetate function [(δ 22.1 ($\underline{C}H_3COO$-) 170.9 (-$\underline{C}OO$-)]. These spectral data was strongly reminiscent of that reported for 8-acetylharpagide [4]. On the basis of above discussed spectral data compound **1** was identified as 8-acetylharpagide (structure **1**).



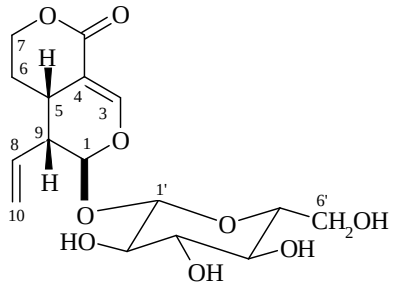
Structure of **Compound 1**

Compound 2: The molecular formula is $C_{17}H_{26}O_{10}$, m.p. 220-222^{0}C. The exhibited IR absorption characteristic for hydroxyl group (at 3450 cm^{-1}), C-H stretching of saturated carbon atom (2900 cm^{-1}), unsaturated ester (1715 cm^{-1}) and enol ether at 1650 cm^{-1}. UV-spectrum showed characteristic absorption bands at 224 and 308 nm for an iridoid enol ether system conjugated with a C-4 carboxyl group[3]. The ^{1}H-NMR spectrum of compound 2 indicated presence of 26 proton signals. An isolated singlet (1H) at δ 7.38 was assigned for a proton attached at tri-substituted double bond. An oxygen bearing methine proton signal at δ 4.04 for 1H appeared as a triplet (*J* = 4.4 Hz, H-7), and three methine signals at δ 3.09 (1H, *m*, H-5), 1.87 (1H, *m*, H-8), and 2.23 (1H, *m*, H-9), was assignable for H-5, H-8 and H-9 protons of iridoids respectively. Two methylene proton at δ 1.62 and 2.03 each for 1H appeared as a multiplet was assigned for H-6a and H-6b. In addition to this a three protons singlet at δ 3.68 assinable for protons of a carboxyl group, a doublet for three protons of a methyl function at δ 1.08, and seven protons due to the sugar moiety were observed. A doublet (*J* = 4.4 Hz) appeared at δ 5.26 was corroborated with the H-1 proton signal of most of the iridoids having O-glycosylation at C-1 carbon[4-6]. The ^{13}C-NMR spectrum and distortion less enhancement by polarization transfer (DEPT) spectrum showed 17 carbon signals; two quaternary carbons, 11 methine carbons, two methylene carbon and two methyl carbons. Acid hydrolysis of compound 2 with 5% HCl gave a sugar, which was identified as D-glucose by paper chromatography. The glycosidic nature of the compound was supported by a doublet at δ 4.64 (*J* = 8.0 Hz) assignable to the anomeric proton of β-D-glucose. The ^{13}C-NMR chemical shifts of anomeric carbon atom (C-1') at δ 99.9 and the chemical shifts of other carbon atoms of sugar moiety [δ 75.1 (C-2'), 78.3 (C-3'), 71.6 (C-4'), 78.0 (C-5'), 62.1 (C-6')] are in agreement with the ^{1}H-NMR spectrum and thus confirmed the presence of glucose in the molecule. The usual location of sugar moiety at position O-1 of the aglycone was shown by the downfield shifted signal of H-1 (δ 5.26, *d*, *J* = 4.4 Hz). The ^{13}C-NMR spectrum confirmed the presence of methyl function (δ 13.6), a methylene carbon [δ 42.6 (C-6)], three methine carbons [(δ 32.2 (C-5), 42.2 (C-8), and 46.6 (C-9)], a methine group having oxygen function at δ 74.7, a secondary carbinyl carbon [δ 97.7 (C-1)], a *tri*-substituted double bond [δ 152.1 (C-3), 114.1 (C-4),] and an acetate function [(δ 13.6 (-COO<u>C</u>H$_3$) 169.6 (-<u>C</u>OO-)]. The location of methyl group was determined at position C-8 by the 1H-NMR spectrum in which a methine proton appeared as a multiplet due to coupling with three methyl protons, and two methine protons (H-7 and H-9). The downfield chemical shifts of a methine proton at δ 4.04 attributed for H-7 indicated that hydroxyl function was attached at C-7 position. The UV absorption coupled with the ^{1}H- and ^{13}C-NMR data establishes that a carboxyl group is located at C-4 carbon. On the basis of above discussed spectral data compound 2 was identified as loganin (Structure-2) which was further confirmed comparison of spectral data with that of reported data [7-9].



Structure of **compound 2**

Compound 3 : The molecular formula is $C_{16}H_{22}O_9$ showed a typical iridoid colour reaction with hydrochloric acid. UV-spectrum of compound 3 showed characteristic absorption bands at 244 nm for an iridoid enol ether system conjugated with a C-4 carbonyl group [3]. The detailed analysis of ^{1}H-NMR spectrum coupled with of ^{1}H-^{1}H COSY indicated presence of two methylene protons at δ 1.71 (multiplet for 2 protons, H-6) which showed coupling with a methylene proton signals (multiplet for 2 protons) resonated downfield (δ 4.41, H-7) in comparison with H-6 indicated that the later is attached with an oxygen function. The methylene proton signal at δ 1.71 also showed coupling with a methine proton signal at δ 3.11 (*m*, 1H, H-5) which in turn showed coupling with a methine proton signal at δ 5.43 (*d*, *J* = 1.2 Hz, H-1) and at δ 2.72 (1H, *m*, H-9). Detailed analysis of ^{1}H-^{1}H-COSY showed that the methine proton signal attached to a mono-substituted double bond resonated at δ 5.56 (1H, *m*, H-8) showed coupling with a methine proton appeared at δ 2.72 (H-9) and with two methylene protons appeared at δ 5.33 (1H, *dd*, *J* = 2.0, 16.8 Hz, H-10a), and δ 5.25 (1H, *dd*, *J* = 2.0, 9.0 Hz, H-10b). These data indicated presence of a vinyl group in the molecule. An integrated methine proton signal which appeared as a double (*J* = 2.4) at δ 7.56 showed long range coupling in ^{1}H-^{1}H COSY spectrum with the methine proton signal appeared as a multiplet at δ 3.11. A doublet (*J* = 1.2 Hz) appeared at δ 5.43 was corroborated with the H-1 proton signal of most of the iridoids having O-glycosylation at C-1 carbon [4-6]. Besides these protons signals the ^{1}H-NMR showed a doublet at δ 4.63 (1H, *d*, *J* = 8.0 Hz, H-1'), which was corroborated with the presence of a β-D-glucose moiety in the molecule. The above discussed ^{1}H-NMR data are strongly reminiscent with those reported for sweroside [7-9]. The ^{13}C-NMR spectrum of compound 3 and DEPT spectrum showed 16 carbon signals out of which 2 are quaternary, 10 methine, and four methylene carbons. The assignment of methylene, methine and quaternary carbon signals readily made by HMQC experiment. On acid hydrolysis with acid, yielded an aglycon and D-glucose. The glycosidic nature of the compound was supported by a doublet at δ 4.63 (*J* = 8.0 Hz) assignable to the anomeric proton of β-D-glucose. The ^{13}C-NMR chemical shifts of anomeric carbon atom (C-1') at δ 99.4 and the chemical shifts of other carbon atoms of sugar moiety are in agreement with the ^{1}H-NMR spectrum and thus confirmed the presence of glucose in the molecule. The usual location of sugar moiety at position O-1 of the aglycone was shown by the downfield shifted signal of H-1 (δ 5.43). The ^{13}C-NMR data of compound 3 are in agreement with the ^{1}H-NMR data. The presence of a vinyl function as deduced by ^{1}H-NMR was confirmed by ^{13}C chemical shifts of unsaturated carbon atom at δ132.1 (C-8) and δ 121.2 (C-10). The ^{13}C-NMR spectrum also showed presence of two methine carbons at δ 27.3 (C-5) and 42.6 (C-9), a methylene carbon at δ 25.1 (C-6), a methylene carbon bearing an oxygen at δ 70.1 (C-7), a secondary carbinyl carbon [δ 98.1 (C-1)], a *tri*-substituted double bond [δ 154.3 (C-3), 105.3 (C-4),] and a carbonyl function at δ 169.7. The location of vinyl group was determined at position C-9 by the ^{1}H-NMR spectrum in which a methine proton appeared as a multiplet at δ 2.72 (H-9) showed coupling with three methine protons appeared at δ 5.43 (1H, *d*, *J* = 1.2 Hz, H-1), 3.11 (1H, *m*, H-5), and 5.56 (1H, *m*, H-8). The UV absorption coupled with the ^{1}H- and ^{13}C-NMR data establishes that a carbonyl group is located at C-4 carbon. On the basis of above discussed spectral data compound 3 was characterized as sweroside (Structure-3) which was confirmed by comparison of spectral data with the reported data [7-9].



Structure of **Compound 3**

APPLICATIONS

The extracted compounds loganin, sweroside are used in traditional Chinese medicine. They are also used for the treatment of such ailments as acute fever, headache, respiratory infection, and epidermic diseases. 8-O-acetylharpagide has antioxidant Activity and Vasoconstrictor activity.

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