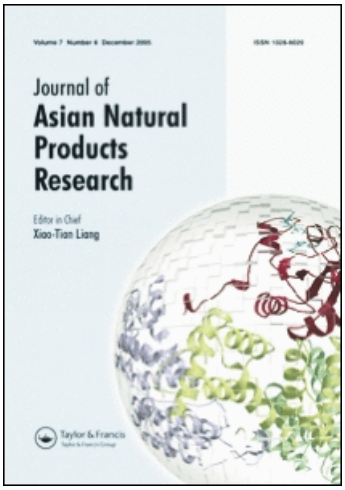

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Antibacterial sesquiterpene lactone glucoside from seed pods of *Bauhinia retusa*

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Antibacterial sesquiterpene lactone glucoside from seed pods of *Bauhinia retusa*

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From the seed pods of *Bauhinia retusa*, a new eudesmane sesquiterpene glucoside, 1-*O*-β-D-glucopyranosyl-9β,15-dihydroxy-5α,6βH-eudesma-3-ene-6α,12-olide (**1**), has been isolated together with three known compounds, 4′-hydroxy-7-methoxy flavane (**2**), β-sitosterol (**3**), and stigmasterol (**4**). The structures of isolated compounds were verified with the help of 1D, 2D NMR, and HR-ESI-MS spectroscopies. Compound **1** showed moderate antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli* when a disc diffusion method is used.

Keywords: Caesalpiniaceae; eudesmane glucoside; *Bauhinia retusa*; antibacterial activity

1. Introduction

Bauhinia is a genus of deciduous trees or shrubs of Caesalpiniaceae family having about 300 species distributed in the warmer parts of world, out of which 30 species are found in India [1]. Various *Bauhinia* species are traditionally used in treating ailments such as asthma, wounds, ulcers, and diabetes, and as a diuretic agent [2–4]. Characteristic constituents of the genus are flavone glycosides [5], phenanthraquinone [6], steroidal glycosides [7], and lignans [8]. The previous chemical studies on *Bauhinia retusa* have resulted in the isolation of flavone glycosides [9], quercetin-3-*O*-glucoside [10], and amino acids [11]. As a part of our continuous research on bio-active constituents from Garhwal Himalayan flora, we focused our attention on the chemical investigation of seed pod of *B. retusa*, which led to the isolation of new eudesmane sesquiterpene glucoside **1** with three known compounds. In this paper, we report the isolation, structural elucidation, and antibacterial activity of the eudesmane sesquiterpene glucoside from the ethanolic extract of seed pods of *B. retusa*.

2. Results and discussion

Compound **1** was isolated as a yellow amorphous powder and showed quasi-molecular ion peak at *m/z* 467.3018 $[M + Na]^+$ in HR-ESI-MS, corresponding to the molecular formula $C_{21}H_{32}O_{10}$. The IR spectrum of the isolated compound afforded absorption bands typical of hydroxyl (3457 cm^{-1}) and lactone carbonyl (1768 cm^{-1}) groups. The ^1H NMR spectrum of **1** showed characteristic signals of one olefinic proton at δ 5.31 (brs), three oxygenated methines at δ 4.28 (dd, $J = 6.2$, 9.8 Hz), 4.01 (dd, $J = 9.8$, 11.4 Hz), 4.12 (dd, $J = 6.8$, 10.2 Hz), one oxygenated methylene at δ 4.68 (brs), two methyls with one at δ 0.89(d, $J = 7.2$ Hz)

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Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of **1** in CD_3OD.

Position	δ_C	$\delta_H(\alpha/\beta$ J in Hz)
1	72.4	4.28 (dd,6.2,9.8)
2	30.2	2.51 (dd,6.2,17.4) 2.38(dd,9.8,17.4)
3	114.1	5.31 brs
4	132.2	–
5	48.7	2.41 (d,11.4)
6	79.2	4.01 (dd,9.8,11.4)
7	38.7	2.63 (dddd,5.8,7.2,9.8,15.1)
8	29.2	1.52 (ddd,5.8,6.8,15.2) 2.19 (ddd,10.2,15.1,15.2)
9	83.3	4.12 (dd,6.8,10.2)
10	41.3	–
11	40.1	2.76 (dq,7.2,8.8)
12	177.7	–
13	12.4	0.89 (d,7.2)
14	21.8	0.81 s
15	66.7	4.68 brs
1′	102.1	5.11 (d,7.4)
2′	73.4	3.92 m
3′	77.4	4.11 m
4′	71.2	4.18 m
5′	76.3	3.96 m
6′	62.9	4.42 (dd,6.2,11.2) 4.68[a]

Note: [a] Multiplicity not determined due to overlap.

and the other at δ 0.81 (s) (Table 1). The ^{13}C NMR and DEPT spectra indicated the presence of 21 carbons including 2 methyls, 4 methylenes, 12 methines, 1 quaternary, 1 tri-substituted double bond, and 1 carbonyl carbon suggested that compound **1** had a sesquiterpene lactone glycoside skeleton [12]. In addition, one quaternary carbon signal at δ 41.3 and one methyl group signal at δ 12.4 indicated that this compound was eudesmanolide-type sesquiterpene having an α-methyl group at C-11 position [13]. In ^{1}H NMR spectrum, a doublet at δ 5.11 ($J = 7.4$ Hz) for anomeric H-1′ along with the carbon resonance at δ 102.1 indicated the presence of β-linked sugar unit. Typical signals for β-D-glucopyranoside were readily recognized from NMR spectra, which were further confirmed as D-glucose after acid hydrolysis of **1** and PC comparison with authentic sample.

The ^{1}H–^{1}H COSY spectrum of **1** showed the correlations between the signal at δ 4.01 (H-6) and the signals at δ 2.41 (H-5) and 2.63 (H-7), suggesting the presence of a C_5H—$C_6H(O)$—C_7H moiety. The other correlations between the signal at δ 1.52 (H-8), and the signals at δ 2.63 (H-7) and 4.12 (H-9) indicated the existence of a C_7H—C_8H—C_9H moiety in the molecule. The attachment of sugar at C-1 was deduced from the HMBC correlation between H-1′ and C-1 (72.4) (Table 1). The relative stereochemistry of **1** was determined by the combination of coupling constant values and NOESY experiment. The large coupling constant of H-1 with H-2 ($J_{1\alpha,2\beta} = 9.8$ Hz), H-9 with H-8 ($J_{9\alpha,8\beta} = 10.2$ Hz), H-6 with H-5 ($J_{6\beta,5\alpha} = 11.4$ Hz), and H-6 with H-7 ($J_{6\beta,7\alpha} = 9.8$ Hz) allowed the relative stereochemistry of H-1 and H-9 in α orientation and the lactone group at C-6 and C-7 as *trans* fused (6β, 7α). In the NOESY experiment, cross peaks between H-11 and H-6β; H_3-14 and H-6β; H-5 and H-7 confirmed 11-methyl in α and 14-methyl group in β configuration, while H-5 and H-7 were α oriented. On the basis

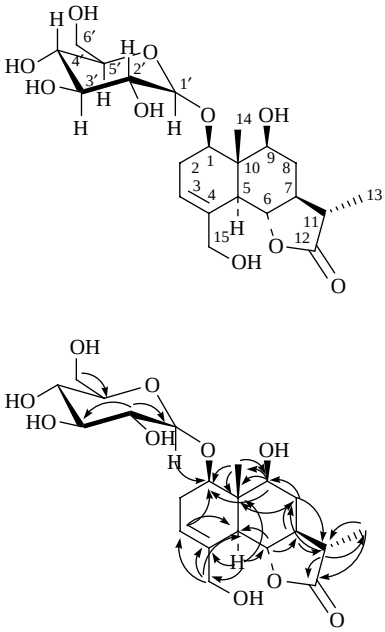

Figure 1. Structure and selected HMBC correlations of compound **1**.

of these findings, the structure of **1** was established as 1-*O*-β-D-glucopyranosyl-9β,15-dihydroxy-5α, 6βH-eudesma-3-ene-6α,12-olide and is reported for the first time from nature (Figure 1).

In addition, 4′-hydroxy-7-methoxy flavane (**2**) [14], β-sitosterol (**3**) [15], and stigmasterol (**4**) [16] were also characterized by comparison of their spectral data with those reported in the literature.

Compound **1** was evaluated for antibacterial activity against four strains using the agar diffusion method [17], and a moderate activity against *Pseudomonas aeruginosa* and *Escherichia coli* was found at a concentration of 250 μg/ml (Table 2).

3. Experimental

3.1 General experimental procedures

Melting points were obtained in an open capillary tube and are uncorrected. Optical rotations were checked with Rudolph's Autopol III polarimeter. UV spectra were obtained using Perkin-Elmer Lambda 15 UV/VIS spectrophotometer. The IR spectra were recorded on Perkin-Elmer Infrared 15 spectrophotometer with KBr and expressed in cm^{-1}. The ^{1}H and ^{13}C NMR spectra were performed on the Bruker AVANCE 400 spectrometer with TMS as internal standard. MS were recorded on the Jeol SX 102 mass spectrometer. Chemical shifts (δ) were expressed in ppm with reference to solvent signals. Column chromatography was performed on silica gel (60–120 mesh, Merck Ltd., Mumbai, India).

3.2 Plant material

B. retusa was collected from High Altitude Plant Physiology Research Centre

Table 2. *In vitro* antibacterial activities of *B. retusa* extract and compound **1** against bacterial strains.

Particular	Zone of inhibition (mm)[a]			
	S. aureus	*E. coli*	*P. aeruginosa*	*S. typhi*
B. retusa hexane extract[b]	NA	6	NA	NA
B. retusa ethyl acetate extract[b]	NA	8	9	NA
B. retusa methanol extract[b]	NA	12	7	NA
Compound **1**[c,d]	NA	9.30 ± 2.0	13.10 ± 1.0	NA
Positive control	20.4 ± 2.4	17.2 ± 2.1	24.6 ± 1.8	25.40 ± 0.6

Notes: [a] Inhibitory zone including the diameter of paper disc (6.0 mm).
[b] 500 mg/ml.
[c] 250 μg/ml.
[d] Data are means ± standard deviation of triplicate determinations, and 'NA' means ' Not Active'.

(HAPPRC), HNB Garhwal Central University, Srinagar Garhwal, Uttarakhand, India in February 2006 and identified by Prof. R. D. Gaur (Taxonomist, HNB University). A voucher specimen (06-011) is deposited in the Natural Product Laboratory, Chemistry Department, HNB Garhwal Central University, Srinagar Garhwal, Uttarakhand, India.

3.3 Extraction and isolation

Air-dried seed pods of *B. retusa* (4 kg) were grinded and extracted with 90% EtOH (5 × 5 l, 24 h) at room temperature. The crude concentrated viscous mass (110.4 g) was partitioned with *n*-hexane, EtOAc, and CH_3OH to give *n*-hexane(25.4 g), ethyl acetate (47.2 g), and methanol (20.4 g) fractions, respectively. The EtOAc fraction (47.2 g) was charged with silica gel column chromatography (60–120 mesh, 7 × 80 cm, 800 g) and eluted with a binary mixture of $CHCl_3$: MeOH (99:1 → 8:2) to give four fractions (99:1, 0.8 l; 95:5, 0.25 l; 9:1, 0.2 l; 8:2, 0.4 l; fractions of 25 ml each).

Fraction A (2.1 g) was rechromatographed using silica gel (60–120 mesh, 60 × 3.2 cm, 150 g) eluting with *n*-hexane:EtOAc (95:5, 0.14 l; 9:1, 0.08 l; fractions of 10 ml) to afford **3** (70 mg) and **4** (98 mg). Fraction C (0.800 g) was applied to silica gel column (60–120 mesh, 60 × 2.8 cm, 100 g) and eluted with *n*-hexane:EtOAc (9:1, 0.2 l; 1:1, 0.08 l; 1:4, 0.3 l; fractions of 10 ml) to give fractions CI–CIII. Fraction CII (52 mg) was purified with acetone to yield **2** (36 mg). Fraction CIII (109 mg) was again rechromatographed with $CHCl_3$:EtOAc (7:3) and afforded **1** (67 mg).

3.3.1 1-β-O-D-Glucopyranosyl-9β,15-dihydroxy-5α,6βH-eudesma-3-ene-6α,12-olide (1)

Yellow amorphous powder; mp 210–213°C; $[\alpha]_D$ -16 ($C = 1.2$, MeOH); IR (KBr) ν_{max} cm^{-1}: 3457, 1768, 1735, 1390, 1280, 898; ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) spectral data see Table 1; HR-ESI-MS *m/z*: 445.3871 $[M + H]^+$ (calcd for $C_{21}H_{33}O_{10}$, 445.2074).

3.3.2 Acid hydrolysis

Compound **1** (5 mg) was added to the mixture of aqueous H_2SO_4 (2 mol/l, 3 ml) and toluene (3 ml) and refluxed for 3 h, respectively. After cooling, the reaction mixtures were neutralized with 10% NaOH and partitioned between H_2O and $CHCl_3$. The H_2O-soluble phase was concentrated and examined by paper chromatography (PC) (EtOAc–Pyridine–H_2O (2:1:5)) with β-D-glucose.

3.4 Antibacterial assay

The micro-organisms, *Staphylococcus aureus* MTCC7405, *E. coli* MTCC1683, *P. aeruginosa* MTCC4676, and *Salmonella typhi* MTCC3917, were procured from IMTECH, India. Antibacterial activity of *B. retusa* fractions and isolated compound were investigated by the disk diffusion method. The Mueller–Hinton II agar plates, containing an inoculum size of 10^6 colony forming units/ml were spread on the solid plates with an L-shaped glass rod. The filter paper discs (6 mm) impregnated with 25 μl of hexane, ethyl acetate, methanol extracts (500 mg/ml each), and compound **1** (250 μg/ml) were placed on inoculated plates. Similarly, a blank disk with solvent for solvent control and an antibiotic disk of chloramphenicol (30 μg/ml) were used as a positive control. Plates were incubated at 37°C for 18 h. The sensitivity of the micro-organism species to the plant extracts and isolated compound was measured by the size of inhibitory zones (including the disk diameter) on the agar surface around the disks. Experiments were performed in triplicate and results are reported as the average of three experiments.

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