Isolation and Identification of a Coumarin Glycoside from leaves of *Skimmia laureola*.

D. P. Pandey
Department of Chemistry, Govt. Degree College, Dehradun-248007, India
Email: pandeydp_123@rediffmail.com

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Abstract- The leaves of *S. laureola* were collected from Nachiketa Tal at an altitude of 2450-2500m, District Uttarkashi, Uttarakhand, India. The air-dried and powdered leaves of *S. laureola* were exhaustively defatted with light petroleum ether (60-80^0). The petroleum free mass extracted with 90% ethanol. The ethanol extract was concentrated under reduced pressure and a suspension of the residue was made with water, and then partitioned sequentially with $CHCl_3$ and n-butanol in a separatory funnel. The butanol layer was separated and concentrated under reduced pressure to give BuOH extract. The n-butanol extract was subjected to column chromatography over Si-gel eluted with different proportion of $CHCl_3$ and MeOH afforded 6-O-β-D-glucopyranoside-2H-1-benzopyran-2-one.

Key Words: *Skimmia laureola*, n-butanol extract, coumarin, coumarin glycosides.

Introduction

Skimmia belongs to the family *Rutaceae* is a large genus of strongly scented unarmed shrubs, distributed throughout the temperate Himalayas from north to south east[1,2]. The flowers are sweetly and leaves are strongly aromatic[3]. The leaves are often used as incense and burnt near small-pox patients for their supposed curative effects. The smoke produced by burning them is said to purify the air[4]. *Skimmia* species have been reported to possess antifungal, anti-fertility, anti-platelet, and spasmolytic activity[5-7]. Phytochemical studies on *Skimia* species resulted in the isolation of flavonoids, terpenoids, iridoids, coumarins, alkaloids, and some fatty esters[8-10]. From *S. laureola f*atty ester, terpenoids and quinoline alkaloids have been reported so far[11-14]. The present study deals with the isolation and identification of a new coumarin glycoside; 6-O-β-D-gluco pyranoside-2H-1-benzopyran-2-one from n-butanol extract of air dried leaves of *S. laureola*. Identification of these compounds was made by the concerted use of 1D and 2D spectral data.

Material and Methods

Plant Material

The leaves of *S. laureola* were collected from Nachiketa Tal (at an altitude of 2450-2500m), District Uttarkashi, Uttarkhand, India (Garhwal Himalaya) in September 2017. The plant species were identified by Dr. Jai Laxmi Rawat Department of Botany, RCU Govt. PG College Uttarkashi, Uttarakhand. A Voucher specimen (DOC 12/2009) was deposited in the Department of Botany, Govt. P.G. College, Uttarkashi, Uttarakhand, India.

Extraction and Isolation

The air-dried and powdered leaves (3 kg) of *S. laureola* were exhaustively defatted

with light petroleum ether (60-80^0). The petroleum free mass extracted with 90% ethanol. The ethanol extract was concentrated under reduced pressure and was washed with diethyl ether for several times. A suspension of the ethanol residue was made with water which was first partitioned with $CHCl_3$:H_2O:MeOH (6:4:4) and then with H_2O:n-BuOH; 1:1 in a separatory funnel. The n-butanol layer was separated and concentrated under reduced pressure to give n-BuOH extract. The n-butanol extract (12g) was subjected to column chromatography over S-gel eluted with $CHCl_3$:MeOH (100:0→1:1) afforded various fractions. The various fractions obtained with $CHCl_3$:MeOH (9:1) and (17:3) were mixed together and dried to get two fractions. Fraction **I** was again subjected to repeated CC over Si-gel eluted with $CHCl_3$:MeOH (9:1) gives various fractions. First few fractions were mixed together and subjected to CC over Si-gel eluted with $CHCl_3$:MeOH (4:1) and like fractions were collected and subjected to preparative TLC using $CHCl_3$:MeOH (3:1) which afforded compound **1**.

Results and Discussion

6-O-β-D-glucopyranoside-2H-1-benzo pyran-2-one (**1**): white amorphous solid, m.p. 197-199^{0}C; $[\alpha]_D^{25}$: +89^0 (c=0.1, $CHCl_3$); **HREI-MS:** m/z 324.0818, calculated for $C_{15}H_{16}O_8$; 324.0845; **IR (ν_{max}^{KBr}):** cm^{-1} 3455 (OH), 1722, 1622, 1463, 1326, 810 etc.; **UV (λ_{max}MeOH):** nm (log ∈) 205 (4.2), 230 (3.71), 256 (3.04), 317 (4.01); **^{1}H-NMR (400 MHz, CD_3OD):** δ 6.29, (1H, *d*, J=9.4 Hz, H-3), 7.92, (1H, *d*, J=9.4Hz, H-4), 7.07 (1H, *d*, *J*= 2.5 Hz, H-5, 7.15 (H-7, *dd*, J = 8.4, 2.7 Hz, H-7), 7.61 (1H, *d*, *J* = 8.4 Hz, H-8), 5.05 (1H, *d*, *J*=7.8 Hz, H-1'), 3.61 (1H, m, H-2'), 3.60 (1H, *m*, H-3'), 3.53 (1H, *m*, H-4'), 3.57 (1H, *m*,H-5'), 4.02 (1H, *dd*, *J* = 2.1, 9.4 Hz, H-6'a), 3.85 (1H, *dd*, *J* = 6.3, 9.4 Hz, H6'b) (Table-1).

^{13}C-NMR (100 MHz, CD_3OD): δ 162.82^s (C-2), 113.83^d (C-3), 144.81^d (C-4)), 115.65^d (C-5), 155.42^s (C-6), 121.83^d (C-7), 117.81^d (C-8), 149.66^s (C-9), 123.68^s (C-10), 101.35^d (C-1'), 73.91^d (c-2'), 78.10^d (C-3'), 70.77^d (C-4'), 76.83^d (C-5'), 62.25^t (C-6'). (Multiplicity of signals is given by DEPT spectroscopy, Table-1).

Acid Hydrolysis of Compound 1: 5 mg of compound **1** was refluxed with 5% aqueous HCl (5 ml) at 80˚C for 3 h. After cooling, the reaction mixture was neutralized with $AgNO_3$. The aqueous layer after concentration under reduced pressure was subjected to PC using BuOH:AcOH:H_2O (5:1:4) with authentic sugars. The sugar was identified as D-glucose.

The molecular formula of compound **1** was determined to be $C_{15}H_{16}O_8$ by high resolution EI-mass spectrum which showed a quasi-molecular ion peak at m/z 324.1618. Its UV spectrum displayed absorption maxima at 205, 230, 256 and 317 nm indicating the presence of coumarin skeleton[15]. The IR spectrum displayed absorption band at 3455 cm^{-1} for OH, 1722 cm^{-1} for six membered lactone carbonyl carbon, and 1622 cm^{-1} for olifinic carbon. The ^{1}H-NMR spectrum of **1** displayed signals for 16 protons and ^{13}C-spectrum displayed presence of fifteen carbons. The DEPT spectrum revealed the presence of four quaternary, 10 methine, and one methylene carbon atoms in the molecule. Assignment of all protons and carbon atoms were made by ^{1}H-^{1}H COSY, HSQC and HMBC spectral data [Table-1].

The ^{1}H-NMR spectrum of **1** showed two doublets (*J* = 9.4 Hz), each for one proton, at δ 6.29, and 7.92 which were assigned for H-3 and H-4 protons of the α,β-

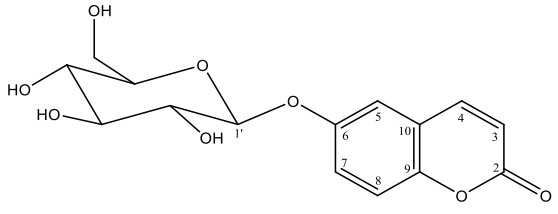
unsaturated lactone ring of coumarin [16]. In aromatic region presence of three signals, each for one proton, at δ 7.07 (*d, J* = 2.5, Hz, H-5), 7.15 (*dd, J* = 8.4 & 2.7 Hz, H-7) and 7.61 (*d, J* = 8.4 Hz, H-8) indicated presence of tri-substituted benzene ring in the molecule. The signal pattern of these signals clearly indicated substitution at C-6 position of benzene ring of coumarin. The ^{1}H-NMR spectrum also displayed presence of anomeric proton at δ 5.05 (d, *J* = 7.8 Hz) together with other sugar protons assignable to D-glucopyranoside[17]. Compound **1** on acid hydrolysis afforded sugar which was identified by PC with an authentic sugar. The β-orientation of the sugar was determined by the value of coupling constant (*J* = 7.8 Hz) of anomeric proton.

Table ^{1}H-NMR and ^{13}C-NMR data of compound **1** in CD_3OD

C/H	$^{\delta}$C	$^{\delta}$H (*J* in hertz)	HMBC (H→→C)
2	162.82^{s}	--	
3	113.83^{d}	6.29, *d* (9.4)	C-2, C-4, C-10 ,C-9
4	144.81^{d}	7.92, *d* (9.4)	C-2, C-5, C-8, C-9
5	115.65^{d}	7.07, *d* (2.5)	C-4, C-7, C-9, C-10
6	155.42^{s}	--	--
7	121.83^{d}	7.15, *dd* (8.4, 2.7)	C-5, C-6, C-8, C-9
8	117.81^{d}	7.61, *d* (8.4)	C-6, C-7, C-9, C-10
9	149.66^{s}	--	--
10	123.68^{s}	--	--
1’	101.35^{d}	5.05, *d* (7.8)	C-6, C-2’, C-3’
2’	73.91^{d}	3.61, m	C-1’, C-3’
3’	78.10^{d}	3.60, *m*	C-2’
4’	70.77^{d}	3.53, *m*	C-3’, C-5’
5’	76.83^{d}	3.57, *m*	C-4’, C-6’
6’	62.25^{t}	4.02, *dd* (2.1, 9.4)	C-4, C-5
		3.85, *dd* (6.3, 9.4)	C-4’, C-3’

The ^{13}C-NMR spectrum indicated presence of 15 carbon atoms, and resembled with ^{13}C-NMR data of other known coumarins [18]. The position of glucose moiety was ascertained by ^{13}C-chemical shift of benzene ring and HMBC experiment. The up-field chemical shift of C-6 carbon at δ 155.42 indicated that glucose is attached with C-6 position, which was confirmed by HMBC which displayed long-range correlation of H-1’ (anomeric proton) with C-6 of ring A of coumarin. Other long range correlations identified by HMBC are given in table 1.

On the basis of above discussed spectral data the structure of compound **1** was assigned as a coumarin glycoside; 6-O-β-D-glucopyranoside-2H-1-benzopyran-2-one which was first time reported from *S. laureola*.



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Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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