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DAPHSAIFNIN, A DIMERIC COUMARIN GLUCOSIDE FROM DAPHNE OLEOIDES

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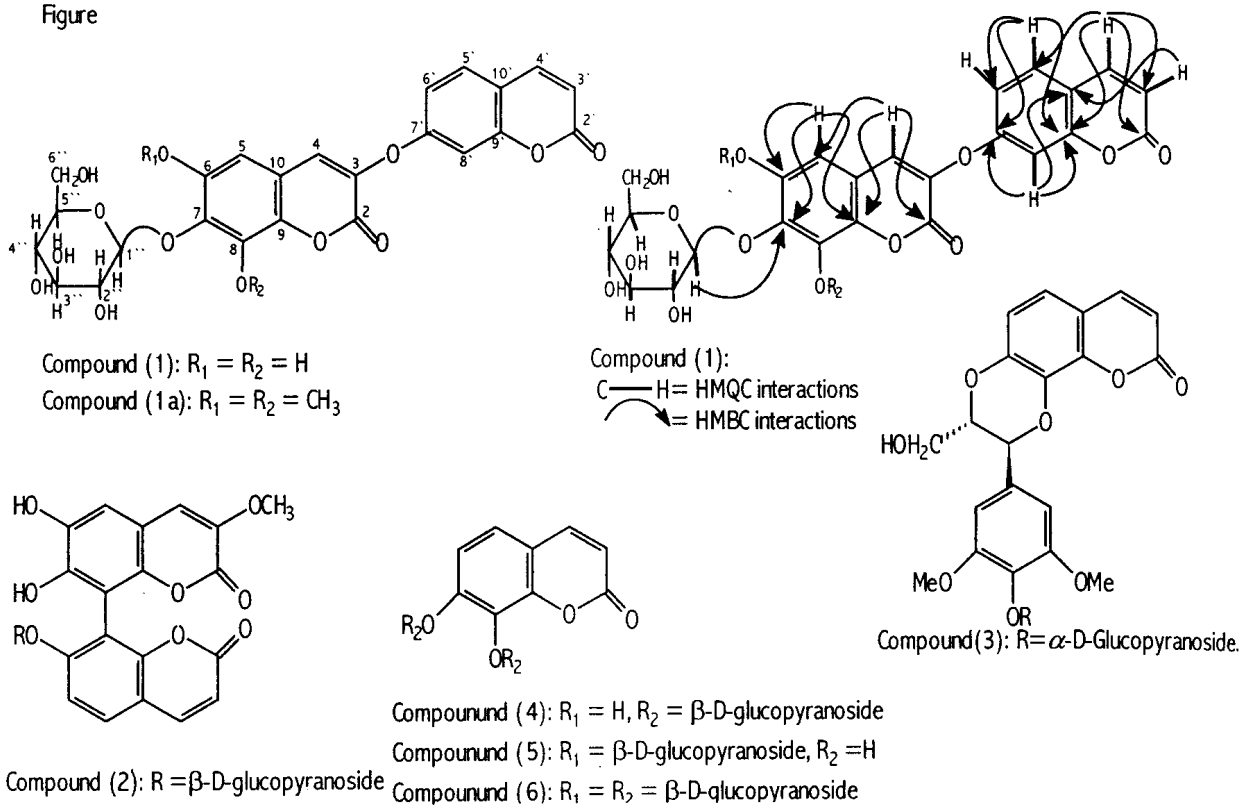
Abstract- Daphsafnin, A new dimeric coumarin glucoside, (**1**) was isolated from the roots of *Daphne oleoides* along with five known coumarins (**2-6**). The structure of **1** was established by the extensive spectroscopic studies including 2D-NMR and chemical analysis.

The family Thymelaeaceae is regarded as principal source of coumarins, their dimers and other derivatives.[1] *Daphne oleoides*, belonging this family is a small shrub, frequently found in the northern mountainous areas of Pakistan. It finds numerous traditional uses in folk medicines.[2] Our studies on the coumarin constituents from the roots of *D. oleoides* have resulted in the isolation and characterization of a new dimeric coumarin glucoside, daphsaifnin (**1**) along with five known coumarins (**2-6**).[3-7]

Daphsafnin (**1**) was obtained as colorless amorphous powder. The molecular formula $C_{24}H_{20}O_{13}$ was defined by molecular ion peak in HR-FABMS at *m/z* 515.0820 [M^+-H], calcd 515.0819). The IR spectrum of **1** exhibited the absorption at 3470-3085, 1723, 1607, 1570, 1475, 1220 and 1104 cm^{-1}, which indicated the presence the hydroxyl, aromatic and lactone functionalities. It gave characteristic blue spot on silica gel plates under UV light (365 nm) and the UV bands at 334 and 323 nm suggested the coumarin skeleton.[8]

The ^{13}C-NMR spectrum revealed the presence of twenty-four carbon signals, which were assigned by DEPT spectrum as one alcoholic methylene twelve methine and eleven quarternary carbons. It included the characteristic signal for two α, β–unsaturated carbonyl carbons of coumarins at δ 160.2 and 159.4.[8] The dimeric coumarin skeleton for **1**, with C-O-C linkage between C-3 and C-7$^{\backslash}$ was established through comparison of its ^{1}H and ^{13}C NMR spectral data with dephnoretin.[9] The occurrence of H-4 as singlet in downfielded region at δ 7.74 was due to the presence of oxygen substitutent at C-3. The other singlet at δ 7.63 was assigned to H-5. The pair of typical doublets for H-4$^{\backslash}$ and H-3$^{\backslash}$ appeared at δ 7.62 (J = 9.6 Hz) and 6.33 (J = 9.6 Hz), respectively. The remaining three resonances in the aromatic region of ^{1}H-NMR spectrum in ABX pattern at δ 7.43 (d, J = 8.6 Hz), 7.10 (dd, J = 2.4, 8.6 Hz) and 7.07 (d, J = 2.4 Hz) were assigned to H-5$^{\backslash}$, H-6$^{\backslash}$ and H-8$^{\backslash}$, respectively. The presence of a doublet at δ 5.42 (J = 7.2 Hz)

Figure



indicated the presence of a sugar moiety in **1**, which was also supported by the signals for its oxygen bearing carbons at δ 103.6, 75.13, 77.7, 70.8 and 77.8 in ^{13}C NMR spectrum. The *β*-configuration was proved by the larger coupling constant of its anomeric proton J = 7.24 Hz. The sugar moiety was identified as *β–D*-glucose by comparing of its carbons signals in ^{13}C NMR spectrum with reference data.[10] This was also confirmed by the comparative TLC of sugar moiety after the acid hydrolysis of **1**. The remaining two oxygen atoms in **1** were concluded to be present as phenolic groups at unit A which was supported by the chemical shifts of C-5, C-6, C-7, and C-8 carbon atoms.

It was further confirmed by the methylation of **1** with diazomethane, which resulted in the addition of two methoxy groups in **1a**. The methoxy protons at δ 3.87 and 3.85 showed the ^{3}J-interactions with C-6 (δ 145.4) and C-8 (136.2) carbons, respectively, in HMBC spectrum which confirmed the loci of these phenolic groups at C-6 and C-8 carbons. The locatioin of *D*-glucose at C-7 was confirmed by the interaction of its anomeric proton at δ 5.42 with C-7 (δ 133.4). All the ^{1}H-^{1}H COSY, HMQC and HMBC experiments (**Figure)** confirmed the structure of compound (**1**) as 6,7-dihydroxy-7-*O*-[*β-D*-glucopyranosyl]-3-[(2-oxo-2*H*-1-benzopyran-7\-yl)-2*H*-1-benzopyran-2-one.

EXPERIMENTAL

General Experimental Procedure – Column chromatography and flash chromatography were done by using silica gel 70-230 and 220-440 mesh, respectively. The TLC was done on precoated silica gel G-25-UV254 plates, the spots were detected by UV light and were visualized with ceric sulfate reagent. The UV spectra were obtained using a Hitachi-UV-3200 spectrophotometer whereas the IR spectra were measured on Jasco-320A spectrophotometer. The FD and HR-FABMS were performed on JMS-X-110 and JMS-DA-500 mass spectrometers, respectively. 1D and 2D-NMR spectrum were recorded on Brucker AMX-400 and 500 MHz spectrometers, respectively. The roots of *Daphne oleoides* were collected from Mansehra district of N.W.F.P., Pakistan in October 1999 and was identified by Prof. Manzoor Hussain (Plant Taxonomist), where a voucher specimen (No: 99/73) was deposited at the herbarium of Botany department of Govt. Postgraduate College-1, Abbottabad, N.W.F.P., Pakistan.

Extraction and Isolation

The air-dried ground plant material of *D. oleoides* (6 kg) was exhaustively extracted with methanol (25 L) at rt for 10 days. The methanolic extract (500 g) was suspended in water and successively extracted with n-hexane and ethyl acetate. The ethyl acetate fraction (135 g) was subjected to column chromatography with hexane/chloroform and chloroform/methanol gradient systms. The flash column chromatography of the fractions obtained with $CHCl_3$/MeOH 7.6:2.4 were combined and subjected to the flash chromatography, eluting with EtOAc/MeOH 9.9:1.1 to 8.0:2:0 to obtain fractions A to K. The flash chromatography of fraction F (0.4 g) and I (0.5 g) using EtOAc /MeOH 9.6:0.4 and 9.1:0.9 afforded daphneticin-4``-*O*-α-*D*-glucopyranoside (**3**)[4] (0.015 g), daphnin (**4**)[7] (0.020 g), daphnetin-*8–O–β-D*-glucopyranoside (**5**)[5] (0.025 g), (**1**) (0.014 g), gulsamanin (**2**)[3] (0.030 g) and daphneside (**6**)[6] (0.024g), respectively.

Daphsafnin (1)

Colorless amorphous powder; UV λ_{max}^{MeOH} nm: 334, 323, 285, 227; IR ν_{max}^{KBr} cm^{-1}: 3470-3085, 2915, 1723, 1607, 1570, 1475, 1220 and 1104; FDMS (*m/z*): 354; HR-FABMS (Neg.) *m/z* 515.0820 (calcd for $C_{24}H_{19}O_{13}$ 515.0819); ^{1}H NMR and ^{13}C NMR see **Table 1.**

Hydrolysis of **1-** A solution of **1** (8 mg) in methanol (4 mL) and 1N HCl (1 mL) was refluxed for 4 h. The solution was concentrated under reduced pressure and diluted with H_2O (5 mL). It was washed with ethyl acetate. The sugar in the aqueous phase was identified as glucose by comparative TLC with an authentic sample using solvent system n-BuOH/EtOAc/I-PrOH/HOAc/H_2O (7:20:12:7:6). The TLC was run thrice in the same direction and the spots were visualized with aniline phosphate reagent. The sugar was found to be glucose.

Methylation of **1**- To an ethereal solution of **1** (12 mg), freshly prepared CH_2N was added and the solution was kept at rt for overnight. Usual work up of the reaction mixture afforded **1a** (6 mg) which was precipitated as an amorphous powder on keeping its concentrated methanolic solution in the cold.

Compound (**1a**)

HR-FABMS (Neg): *m/z* 543.1133 [M^+-H] (calcd for $C_{26}H_{23}O_{13}$: 543.1131). The ^{1}H NMR and ^{13}C NMR spectrum (500 MHz, Pyridine-d_5) were similar to **1** except with two additional resonances at δ 3.87 and 3.85 (each 3H, s, OCH_3) and at δ 56.5 and 55.8, respectively. ^{1}H NMR and ^{13}C NMR see **Table 1.**

Table 1. ^{1}H NMR and ^{13}C NMR assignments of compounds (**1**) and (**1a**) in C_5D_5N.

	1		**1a**	
No.	**^{1}H***	**^{13}C**	**^{1}H***	**^{13}C**
2	-	159.4	-	160.0
3	-	138.4	-	136.8
4	7.74 (s)	129.5	7.76 (s)	129.4
5	7.63 (s)	102.4	7.66 (s)	102.2
6	-	143.4	-	145.4
7	-	133.4	-	132.9
8	-	134.6	-	142.1
9	-	133.8	-	132.9
10	-	116.6	-	116.7
2`	-	160.2	-	160.8
3`	6.33 (d, 9.6)	113.6	6.31 (d, 9.5)	113.9
4`	7.62 (d, 9.6)	144.3	7.65 (d, 9.5)	144.5
5`	7.43(d, 8.56)	129.9	7.51(d, 8.7)	129.6
6`	7.10 (dd, 2.4, 8.6)	114.0	7.16 (dd, 2.1, 8.7)	114.4
7`	-	157.2	-	157.6
8`	7.07 (d, 2.4)	104.3	7.10 (d, 2.1)	104.2
9`	-	156.5	-	156.8
10`	-	114.8	-	114.9
1``	5.42 (d, 7.2)	103.6	5.02 (d, 7.3)	102.5
2``	3.24 (m)	74.9	3.22 (m)	74.6
3``	3.30 (m)	76.9	3.29 (m)	75.4
4``	3.46 (m)	70.8	3.38 (m)	70.0
5``	3.32 (m)	77.1	3.30 (m)	76.9
6``	3.92 (m)	62.0	3.89 (m)	61.8
			3.87 (s)	56.5
			3.85 (s)	55.6

* Figures in parantheses represent multiplicities and *J* values in Hz.

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