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C-ALKYLATED COUMARIN AND COUMARIN GLYCOSIDE FROM *DAPHNE OLEOIDES*

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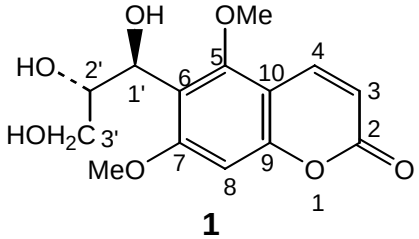
Abstract- New C-alkylated coumarin (**1**) and coumarin glucoside (**2**) have been isolated from the roots of *Daphne oleoides.* The structures of **1** and **2** were established through spectroscopic and chemical studies.

The genus *Daphne* (Thymelaeceae) is a principal source of coumarins. It comprises of many species, represented in Pakistan by *Daphne oleoides*. It is a small shrub, frequently found in the northern areas of Pakistan. The roots of this species are purgative, its bark and leaves are given in cutaneous infections, whereas infusion of leaves is given in gonorrhea.[1] Previously triterpenoids,[2] lignan glycosides,[3] biscoumarins,[4] and tricoumarins,[5] have been reported from this species. Reinvestigations on the coumarin constituents of the roots of this plant have now resulted in the isolation and characterization of new C-alkylated coumarin (**1**) and coumarin glycoside (**2**), respectively.

Compound (**1**) was obtained as colorless amorphous powder. The molecular formula was established as $C_{14}H_{16}O_7$ on the basis of ion peak at *m/z* 296.2732 (calcd 296.2726 for $C_{14}H_{16}O_7$) in HREIMS. It gave characteristic blue spot on silica gel plates under UV light (365 nm) and the UV absorption bands at 204, 258 and 320 nm suggested the coumarin skeleton.[6] The IR spectrum of **1** exhibited the absorption at 3490, 1718, 1605, 1562 and 1250 cm^{-1}, which indicated the presence of hydroxyl, lactone, aromatic and methoxyl functionalities.

The ^{13}C-NMR spectrum revealed the presence of fourteen carbon atoms, which were assigned by DEPT spectrum as one alcoholic methylene, five methine, two methyl, and six quaternary carbons. It included the characteristic signal for α,β-unsaturated carbonyl carbon of coumarins at δ 160.3 and 144.3 and three oxygenated aromatic carbons at 161.2, 158.9 and 156.3, respectively. The ^{1}H-NMR spectrum howed olefinic protons ad δ 7.78 and 6.39 (d, J = 9.5 Hz), aromatic proton at 6.91 and two methoxyl groups at δ 3. 79. The presence of trihydroxypropanoid moiety could be inferred by the signals of two vicinal protons at δ 5.58 (d, J = 8.3 Hz), 4.44 (dt, J = 8.3, 4.0 Hz) corresponding to the oxygenated methines and two further protons of a hydroxymethylene at δ 4.59 (m).

The EIMS spectrum showed a very weak ion peak due to the fission of the 1′-2′ bond, α to both oxygens giving rise to the base peak at *m/z* 205. The above data revealed that **1** is a coumarin with two methoxyls and a trihydroxypropanoid moiety. Their positions were established through ^{1}H-^{1}H COSY and ^{13}C-^{1}H long range couplings. In HMBC experiments the methoxyl protons showed ^{3}J interactions with C-5 and C-7 the methylene protons showed ^{3}J correlation with C-1′ and ^{2}J correlation with C-2′. The H-2′ methine proton showed ^{3}J correlation with C-6 and ^{2}J interaction with C-3′ and C-1′. The H-1′ methine proton showed ^{3}J interactions with C-7, C-5, C-3′ and ^{2}J interactions with C-2′ and C-6. The H-8 showed ^{3}J correlation with C-6 and ^{2}J correlation with C-9. Thus compound (**1**) was assigned the structure 5,7-dimethoxy-6-(1′,2′,3′-trihydroxypropyl)-2*H*-1-benzopyran-2-one. The *erythro* configuration was reflected by the larger coupling constant between H-1′ and H-2′. This could be further confirmed by comparison of ^{1}H NMR and ^{13}C NMR spectral data with those of *C*-alkylated coumarins isolated from the genus *Micromelum* and *Murraya*[7] suggesting the stereochemistry as **1** or its mirror image. The absolute stereochemistry cannot be established without chemical transformation that would require much more material.



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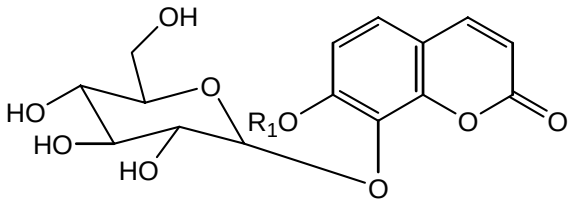

2. R_1 = H

2a. R_1 = Me

Compound (**2**) was obtained as yellow amorphous powder. The molecular formula $C_{15}H_{16}O_9$ was defined by molecular ion peak in HR-FABMS at *m/z* 339.2752 [M^+-H], calcd 339.2748. The IR spectrum of **2** exhibited the absorptions at 3406, 1716, 1605 and 1562 cm^{-1}, which indicated the presence of hydroxyl, lactone and aromatic functionalities. The UV absorption bands at 206, 258 and 325 nm suggested the coumarin skeleton. The bathochromic shift observed in the alkatine medium suggested the presence of a hydroxyl group at C-7[8]. It was confirmed through methylation of **2** to a monomethyl ether (**2a**).

The ^{1}H-NMR spectrum showed a pair of typical doublets for H-3 and H-4 at δ 6.45 (*J* = 9.5 Hz) and 7.91 (*J* = 9.5 Hz), and *ortho* coupled aromatic protons at δ 7.18 (d, *J* = 8.6 Hz) and 7.08 (d, *J* = 8.6 Hz). The signal of an anomeric proton at δ 5.64 (d, *J* = 3.5 Hz) suggested the presence of a sugar moiety in the α-configuration. Acid hydrolysis of **2** provided 7,8- dihydroxy-2 *H*-benzopyran-2-one (daphnetin) and the sugar which could be identified as D-glucose through sign of its optical rotation and comparison of retention time of its TMS ether with that of standard in GLC. The ^{13}C-NMR spectrum revealed the presence of fifteen carbon signals which were assigned by DEPT spectrum as one alcoholic methylene, nine methine and five quaternary carbons. It included the characteristic signals for α, β-unsaturated

carbonyl of coumarins at δ 160.8 and 144.8, an anomeric carbon at δ 99.9 and two oxygen bearing aromatic carbons at δ 153.7 and δ 131.9, respectively. The unambiguous locations of hydroxyl at C-7 and the glucose residue at C-8 were made on the basis of HMBC experiments. The typical two and three bond correlations were observed between (i) the anomeric proton and C-8 (ii) H-5 proton with C-7, C-6, C-9 and C-10, (iii) H-6 proton with C-5 , C-7, C-8 and C-10. Thus compound (**2**) was assigned the structure 7-hydroxy-8-*O*-[α-D-glucopyranosyl]-2*H*-benzopyran-2-one (daphnetin-8-*O*-α-D-glucopyranoside), which is a new compound following the earlier isolation of the corresponding β-isomer from various *Daphne* species.[2,8]

Table **1** ^{1}H and ^{13}C NMR spectral assignments of **1** and **2**

1			**2**		
Position	δ ^{1}H (*J*)	δ ^{13}C	Position	δ ^{1}H (*J*)	δ ^{13}C
2	-	160.3	2	-	160.8
3	6.39 d (9.5)	113.4	3	6.45 d (9.5)	113.6
4	7.78 d (9.5)	144.3	4	7.91 d (9.5)	144.8
5	-	158.9	5	7.18 d (8.6)	124.7
6	-	106.4	6	7.08 d (8.6)	111.5
7	-	161.2	7	-	153.7
8	6.91 s	98.4	8	-	131.9
9	-	156.3	9	-	145.2
10	-	104.9	10	-	112.7
1′	5.58 d (8.3)	77.9	1′	5.64 d (3.5)	99.9
2′	4.44 dt (8.3,4.0)	80.1	2′	3.72 dd (9.5, 3.5)	71.8
3′	4.59 m	60.8	3′	3.84 t (9.5)	73.9
OCH_3	3.79 (6H, s)	56.2	4′	3.47 t (9.5)	70.1
			5′	3.58 m	74.4
			6′	3.69 (2H m)	61.2

EXPERIMENTAL

General

Optical rotations were measured on JASCO DIP-360 digital polarimeter. IR spectra were measured on Schimadzu Infrared spectrophotometer IR 460. UV spectra were recorded in methanol on Hitachi U-3200 spectrophotometer. EI-MS were recorded on a Varian MAT 311, FAB-MS measurements were done on a JEOL-HX 110 mass spectrometer. NMR spectral experiments were carried out on a Bruker AMS-300 instrument (^{1}H: 300 MHz; ^{13}C:75 MHz). 2D experiments were done on a Bruker AMX-500 instrument. Silica gel 60 (70-230 mesh) and Silica gel (230-400 mesh) were respectively used for column and flash chromatograph. TLC was conducted on a Precoated Kieselgel 60, F_{254} aluminum sheet and RP-18 F_{254} plates (Merck).

Plant material. The roots of Daphne oleoides were collected from the Bhinghra mountains of Manshra district of N. W. F. P., in Nov 1999 and identified by Prof. Manzoor Hussain (Plant Taxonomist) at

Department of Botany, Govt. Postgraduate College-1, Abottabad, N. W. F. P., Pakistan The voucher specimen (99/73) was deposited at the herbarium of that department.

Extraction and isolation. The air dried and chopped roots (6 kg) of *D. oleoides* were exhaustively extracted with MeOH (18 L) for 6 days at rt. The crude extract (500 g) was partitioned between water, *n*-hexane, EtOAc and *n*-butanol. The EtOAc fraction (135 g) was subjected to column chromatography over silica gel eluting with *n*-hexane-$CHCl_3$ and $CHCl_3$-MeOH gradient systems. The flash column chromatography of the fraction obtained with *n*-hexane-$CHCl_3$ (1:9) and elution with the same solvent system afforded the compound (**1**) (0.046 g). The flash column chromatography of the fraction obtained with $CHCl_3$-MeOH 8:2 and elution with EtOAc-MeOH 8.9 : 1.1 provided the compound (**2**) (0.033 g).

5,7-Dimethoxy-6-(1´,2´,3´-trihydroxypropyl)-2*H*-1-benzopyran-2-one (1):
Amorphous powder; $[\alpha]_D^{25}$ – 9° (*c* 0.61, MeOH); UV max (MeOH) nm(log ε) : 204 (3.21), 258 (3.58) and 320 (4.21); IR (KBr): 3490, 1718, 1605, 1562, 1250, 1266 and 1126 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD) Table **1**; EIMS *m/z* (rel. int. %): 296 (0.31), 205 (100), 206 (78), 175 (35), 144 (21), 131 (6), 91 (12).

7-Hydroxyl-8-*O*-[α-D-glucopyranosyl]-2*H*-benzopyran-2-one (2): Yellow amorphous powder; $[\alpha]_D^{25}$ – 7° (*c* 0.49, MeOH); UV max (MeOH) nm(log ε) : 206 (3.21), 258 (3.58) and 325 (4.21); IR (KBr): 3406, 1716, 1605 and 1562 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD) Table **1**; EIMS *m/z* (rel. int. %): 340 (0.8), 161 (100), 147 (14), 119 (6).
Methylation of (2): Freshly prepared solution of CH_2N_2 in ether was added to **2** in MeOH (6 mg) in excess and the solution was kept overnight at rt. Usual work-up afforded monomethyl ether (**2a**) which settled down as an amorphous powder on keeping its methanolic solution in cold. The molecular formula $C_{16}H_{18}O_9$ was defined by molecular ion peak in EIMS at *m/z* 354.

Acid Hydrolysis of (2): A solution of **2** (4 mg) in MeOH (2.5 mL) containing 1N HCl (2.5 mL) was refluxed for 3 h, concentrated under reduced pressure and diluted with H_2O (6 mL). It was extracted with ethyl acetate and the residue recovered from the organic phase was subjected to preparative TLC to afford 7,8- dihydroxy-2 *H*-benzopyran-2-one as pale yellow needles mp 257 °C. Its molecular formula was established as $C_9H_6O_4$ on the basis of ion peak at *m/z* 178.0258 (calcd 178.0266 for $C_9H_6O_4$). The ^{1}H NMR spectrum showed similar resonances as **2** except for the now absent signals of the sugar moiety. It could be identified as daphnetin through physical and spectral data as well as color reactions and comparison of R_f value with literature.[9] The sugar was identified as D-glucose through sign of its optical rotation and comparison of retention time of its TMS ether with that of standard in GLC.

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