Novel Coumarin Glycosides from *Daphne oleoides*

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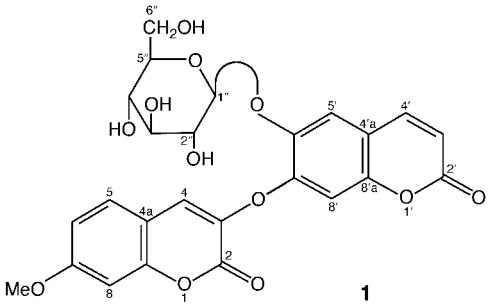
'Dimeric' coumarin glycoside **1** and 'trimeric' coumarin fucosides **2** and **3** were isolated from *Daphne oleoides*. Their structures were established by means of different spectroscopic techniques, including 2D-NMR spectroscopy.

Introduction. – The family Thymelaeaceae is an important source of coumarins and their dimers. *Daphne oleoides* SCHREB., a xerophytic shrub, belongs to this family and is found in northern hilly areas of Pakistan. It finds a variety of uses in folk medicine [1]. Previous studies established the occurrence of lignans [2], monomeric coumarins [3], a 'dimeric' coumarin glycoside [4], and a coumarin lignoid [5] in this plant. Reinvestigations of the MeOH extract of the roots of *D. oleoides* have now led to the isolation and structural elucidation of a 'dimeric' coumarin glucoside **1** and of **2** and **3** belonging to a very rare class of 'trimeric' coumarin fucosides. Besides these new compounds, the coumarins **4** and **5** [6] are reported for the first time from this species.

Results and Discussion. – The crude MeOH extract of roots of *D. oleoides* were defattened with hexane and partitioned between AcOEt and H_2O. The AcOEt fraction was subjected to column and flash chromatography with different mobile phases. Compounds **1**–**3** were finally obtained by low-pressure liquid chromatography, and their structures were established by UV, IR, mass, and NMR spectroscopy.

Compound **1**. The HR-FAB-MS of **1** provided the $[M-H]^+$ ion at *m*/*z* 513.1014 indicating the molecular formula $C_{25}H_{22}O_{12}$. The IR spectrum exhibited the characteristic bands for OH (3412 cm^{-1}), CO (1722 cm^{-1}), aromatic C=C (1606, 1576 and 1470 cm^{-1}), and C(O)O (1223 and 1106 cm^{-1}) groups. Compound **1** gave a characteristic greenish-blue spot on TLC (silica gel) under UV light (365 nm), and the UV bands at 335 and 324 nm suggested the presence of a coumarin skeleton [7]. The assignments of the ^1H- and ^{13}C-NMR data (*Table 1*) were made by comparison with the data of daphnoretin [8] and confirmed by COSY, HMQC, and HMBC experiments. The presence of a D-glucose moiety in the structure of **1** was established by comparison of its ^{13}C-NMR data with those of standard reference data [9], and also by acid hydrolysis of **1** which provided glucose, identified by TLC comparison with an authentic sample. Thus, the structure of **1** is proposed to be 3-({6-[(β-D-glucopyranosyl)oxy]-2-oxo-2*H*-1-benzopyran-7-yl}oxy)-7-methoxy-2*H*-1-benzopyran-2-one.

The ^{13}C-NMR (broad band and DEPT) of **1** revealed the presence of 25 C-atoms including 1 Me, 1 CH_2, 13 CH and 10 quaternary C-atoms, as well as signals for two α,β-unsaturated ketone moieties characteristic of



1

HMBC Interactions:

H-C(3′)/C(2′), C(4′a)
H-C(4′)/C(2′), C(3′), C(5′), C(8′a)
H-C(5′)/C(4′a), C(7′), C(8′a)
H-C(8′)/C(4′a), C(6′), C(7′)
H-C(4)/C(2), C(3), C(5), C(8a)
H-C(5)/C(7), C(8a)
H-C(6)/C(4a), C(7)
H-C(8)/C(6), C(8a)
C*H*$_3$O-C(7)/C(7)
H-C(1″)/C(6′)

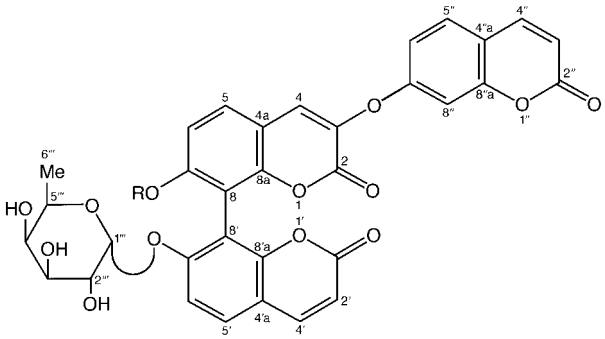
Table 1. *^{1}H-NMR* (400 MHz) *and ^{13}C-NMR* (125 MHz) *Data* (CD_3OD + a few drops of CD_3Cl) *of Compound* **1** (δ in ppm, *J* in Hz)

Atom	^{13}C-NMR	^{1}H-NMR (HMBC)
C(2)	160.0	–
C(3)	136.4	–
H–C(4)	129.6	7.76 (*s*)
H–C(5)	131.1	7.46 (*d*, *J* = 8.48)
H–C(6)	113.5	7.10 (*dd*, *J* = 1.8, 8.4)
C(7)	157.3	–
H–C(8)	104.3	7.05 (*d*, *J* = 1.8)
C(8a)	155.2	–
C(4a)	112.6	–
C(2′)	160.1	–
H–C(3′)	114.3	6.23 (*d*, *J* = 9.5)
H–C(4′)	143.3	7.69 (*d*, *J* = 9.5)
H–C(5′)	104.3	7.29 (*s*)
H–C(6′)	144.4	–
C(7′)	157.7	–
H–C(8′)	103.7	7.19 (*s*)
C(8′a)	152.2	–
C(4′a)	114.2	–
H–C(1″)	104.2	4.89 (*d*, *J* = 7.2)
H–C(2″)	74.6	3.14 (*m*)
H–C(3″)	78.1	3.20 (*m*)
H–C(4″)	70.9	3.34 (*m*)
H–C(5″)	79.0	3.23 (*m*)
CH_2(6″)	62.1	3.81, 3.41 (*m*)
MeO	52.4	3.68 (*s*)

coumarins (δ 160.0 and 160.1) [7]. In the ^{1}H-NMR, a pair of *d* at δ 7.69 and 6.23 (*J* = 9.5 hz, each 1 H) were assigned to H–C(4′) and H–C(3′), respectively. The occurrence of H–C(4) as *s* downfield at δ 7.76 revealed the presence of an O-substituent at C(3). The *s* at δ 3.68 (3 H) was due to aromatic MeO. The presence of the sugar moiety was revealed by the signal of the anomeric proton at δ 4.89, the protons geminal to an OH group at δ 3.14–3.81, the anomeric C-atom at δ 104.2, and further OH-bearing C-atoms at δ 79.0, 78.1, 74.6, 70.9, and 62.1. The β-D-configuration of the glucose moiety was confirmed by the large coupling constant for the anomeric proton (*J* = 7.2 Hz). The nonreactivity with diazomethane confirmed the absence of a phenolic group in **1**. Since the sugar moiety, the MeO group, and the coumarin skeletons already accounted for 11 of the 12 O-atoms, the remaining O-atom must be involved in the O-linkage between the two coumarin units. The position of the O-linkage between C(7′) and C(3) of the coumarin units was established by comparison of the chemical shifts of

various protons and C-atoms with those of daphnoretin [8]. The aromatic protons were assigned with the help of coupling constants and proton-correlated spectroscopy (COSY). The shielded and noncoupled protons at δ 7.29 and 7.19 could be assigned to H–C(5′) and H–C(8′), respectively, the *dd* at δ 7.10 ($J = 1.8$, 8.4 Hz, 1 H) to H–C(6), the *d* at δ 7.05 ($J = 1.8$ Hz, 1 H) to H–C(8), and the *d* at δ 7.46 ($J = 8.48$ Hz, 1 H) to H–C(5). Final evidence of the structure was provided by a series of HMBC interactions.

Compound **2**. The molecular formula of **2** was assigned as $C_{33}H_{24}O_{13}$ by HR-FAB-MS in which the $[M-H]^+$ peak was at *m/z* 627.1082. Compound **2** showed a blue spot on TLC under UV light (365 nm) and the characteristic UV spectrum for coumarins with absorptions at 325 and 268 nm [7]. The IR spectrum exhibited the absorbance at 1724 cm^{-1} due to the presence of a lactone carbonyl group. The ^{1}H- and ^{13}C-NMR assignments (*Table 2*) were made by comparison with edgeworoside [10]. The presence of the sugar moiety D-fucose in **2** was confirmed by comparison of its ^{13}C-NMR resonances with standard reference data [9], and also by acid hydrolysis of **2** to provide fucose, identified by TLC comparison with an authentic sample. 1D-NOE, HMQC, and HMBC experiments confirmed the structure of **2** as 8-{7-[(α-D-fucopyranosyl)oxy]-2-oxo-2*H*-1-benzopyran-8-yl}-7-hydroxy-3-[(2-oxo-2*H*-1-benzopyran-7-yl)oxy]-2*H*-1-benzopyran-2-one.



2 R = H
3 R = MeO

The ^{13}C-NMR and DEPT experiment revealed the presence of 33 C-atoms including 1 Me, 17 CH, and 15 quaternary C-atoms, as well as the signals for three α,β-unsaturated ketone moieties characteristic of coumarins (δ 161.9, 161.1, and 158.9) [7]. In the ^{1}H-NMR, the *d* at δ 7.54 ($J = 9.5$ Hz), 6.26 ($J = 9.5$ Hz), 7.52 ($J = 9.6$ Hz), and 6.30 ($J = 9.6$ Hz) could be assigned to H–C(4′), H–C(3′), H–C(4″), and H–C(3″), respectively. The occurrence of the H–C(4) *s* downfield at δ 7.78 revealed the presence of an O-substituent at C(3). The sugar moiety gave rise to the signals of the anomeric proton at δ 5.50, the protons geminal to an OH group at δ 3.89–4.66, the anomeric C-atom at δ 97.7, and further OH-bearing C-atoms at δ 97.7, 72.2, 69.9, 68.8, 67.0, and 16.5. The α-D-configuration of the fucose moiety was confirmed by a small coupling constant for its anomeric proton ($J =$ 2.0 Hz). The position of the fucose moiety at C(7′), the O-linkage between C(3) and C(7″), and the C–C linkage between C(8) and C(8′) were inferred by comparison of the ^{13}C-NMR chemical shifts with those of edgeworoside [10]. The linkage position of the fucose unit to the tricoumarin aglycone of **2** could also be determined by 1D-NOE measurements (NOEDIF). Irradiation of the anomeric proton (δ 5.50) resulted in a 10.5% NOE on H–C(6). The attachment of the sugar at C(7′) was confirmed by the ^3J-interactions of its anomeric proton at δ 5.50 with C(7′) at δ 157.1.

All the assignments were also confirmed by the ^{1}H,^{13}C interactions in the HMQC and HMBC spectra of **2**. The most important HMBC interactions were between *H*–C(4) (7.78) and C(2) (δ 158.9), C(5), (δ 128.4), and C(8a) (δ 150.8) and between *H*–C(5) (δ 7.24) and C(7) (δ 158.4) and C(4a) (δ 110.0). *H*–C(6) (δ 7.03) showed

Table 2. *^{1}H-NMR* (400 MHz) *and ^{13}C-NMR* (125 MHz) *Data* (CD_3OD + a few drops of CD_3Cl) *of Compounds* **2** *and* **3** (δ in ppm, J in Hz)

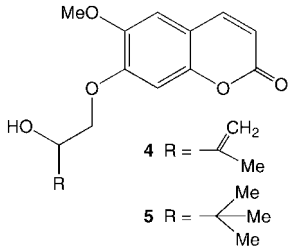
	Compound **2**			Compound **3**		
	DEPT	^{13}C-NMR	^{1}H-NMR (HMQC)	DEPT	^{13}C-NMR	^{1}H-NMR (HMQC)
C(2)	C	158.9	–	C	160.1	–
C(3)	C	137.5	–	C	138.6	–
H–C(4)	CH	129.1	7.78 (*s*)	CH	130.1	7.75 (*s*)
H–C(5)	CH	128.4	7.24 ($d, J = 8.5$)	CH	130.8	7.63 ($d, J = 8.6$)
H–C(6)	CH	110.9	7.03 ($d, J = 8.5$)	CH	112.0	7.36 ($d, J = 8.6$)
C(7)	C	158.4	–	C	157.8	–
C(8)	C	105.9	–	C	106.0	–
C(8a)	C	150.8	–	C	152.0	–
C(4a)	C	110.0	–	C	112.1	–
C(2′)	C	161.9	–	C	162.1	–
H–C(3′)	CH	112.8	6.26 ($d, J = 9.5$)	CH	112.1	6.15 ($d, J = 9.5$)
H–C(4′)	CH	144.5	7.54 ($d, J = 9.5$)	CH	145.1	7.79 ($d, J = 9.5$)
H–C(5′)	CH	128.6	7.31 ($d, J = 8.7$)	CH	130.6	7.63 ($d, J = 8.7$)
H–C(6′)	CH	112.6	7.26 ($d, J = 8.7$)	CH	114.4	7.20 ($d, J = 8.7$)
C(7′)	C	157.1	–	C	158.6	–
C(8′)	CH	111.0	–	CH	111.3	–
C(8′a)	C	152.8	–	C	154.3	–
C(4′a)	C	113.8	–	C	113.2	–
C(2″)	C	161.1	–	C	161.7	–
H–C(3″)	CH	113.7	6.30 ($d, J = 9.6$)	CH	114.7	6.29 ($d, J = 9.6$)
H–C(4″)	CH	143.5	7.52 ($d, J = 9.6$)	CH	144.9	7.76 ($d, J = 9.6$)
H–C(5″)	CH	128.6	6.74 ($d, J = 8.8$)	CH	131.8	7.35 ($d, J = 8.6$)
H–C(6″)	CH	111.8	6.73 ($dd, J = 2.0, 8.8$)	CH	113.0	6.73 ($dd, J = 1.8, 8.6$)
C(7″)	C	158.8	–	C	157.8	–
H–C(8″)	C	104.6	6.72 ($d, J = 2.0$)	CH	105.9	7.02 ($d, J = 1.8$)
C(8″a)	C	154.8	–	C	156.6	–
C(4″a)	C	114.7	–	C	114.9	–
H–C(1‴)	CH	97.7	5.50 ($d, J = 2.0$)	CH	98.8	5.56 ($d, J = 2.1$)
H–C(2‴)	CH	68.8	4.66 (*m*)	CH	69.2	4.30 (*m*)
H–C(3‴)	CH	69.9	4.03 (*m*)	CH	70.4	4.08 (*m*)
H–C(4‴)	CH	72.2	4.21 (*m*)	CH	73.0	4.16 (*m*)
H–C(5‴)	CH	67.0	3.89 (*m*)	CH	67.8	3.74 (*m*)
Me(6‴)	Me	16.5	1.5 ($d, J = 6.0$)	Me	17.5	1.20 ($d, J = 6.5$)
MeO					51.9	3.79

interaction with C(5) (δ 128.4) and C(7) (δ 158.4), *H*–C(3′) (δ 6.26) with C(2′) (δ 161.9) and C(4′) (δ 144.5), *H*–C(4′) (δ 7.54) with C(3′) (δ 112.8), C(4′a) (δ 113.8), and C(5′) (δ 128.6), and *H*–C(5′) (δ 7.31) with C(6′) (δ 112.6), C(7′) (δ 157.1), and C(8′a) (δ 152.8). Other important connectivities in the HMBC spectrum were between *H*–C(4″) (δ 7.52) and C(2″) (δ 161.1), C(5″) (δ 128.6), and C(8″a) (154.8) and between *H*–C(5″) (δ 6.74) and C(6″) (δ 111.8), C(7″) (δ 158.8), and C(8″a) (154.8).

Compound **3**. The molecular formula of **3** was assigned as $C_{34}H_{26}O_{13}$ by HR-FAB-MS showing the $[M-H]^+$ peak at m/z 641.1175. The characteristic absorptions in the UV and IR spectrum indicated the presence of the coumarin moiety [8]. The ^{1}H- and ^{13}C-NMR, and HMBC spectra of **3** were similar to those of **2**, the main difference being the presence of a MeO group in **3** instead of an OH function, in accordance also with the molecular formula. By NOE measurements, the MeO group was assigned to be at

C(7) irradiation at δ 3.79 (MeO) → NOE at δ 7.36 H–C(6)). Further confirmation was provided by a HMBC experiment that showed ^3J interactions of the MeO protons (δ 3.79) with C(7) (δ 157.8). The structure of **3** was, therefore, assigned as 8-{7-[(α-D-fucopyranosyl)oxy]-2-oxo-2*H*-1-benzopyran-8-yl}-7-methoxy-3-[(2-oxo-2*H*-1-benzopyran-7-yl)oxy]-2*H*-1-benzopyran-2-one.

Compounds **4** *and* **5**. The structures of the known compounds **4** and **5** have been established by *Debenedetti et al.* [6].



Experimental Part

General. Column chromatography (CC): silica gel, 70–230 mesh. Flash chromatography (FC): silica gel, 220–440 mesh. TLC: precoated silica gel *G-25-UV$_{254}$* plates; detection at 254 and 365 nm and by the ceric sulfate reagent. UV Spectra: *Hitachi UV-3200* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Jasco 320-A* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR, COSY, HMQC, and HMBC: *Bruker* spectrometers operating at 400 and 500 MHz; chemical shifts δ in ppm and coupling constants *J* in Hz. EI-, FAB-MS, and HR-FAB-MS (negative-ion mode): *JMS HX-110* with a data system and *JMS DA-500* mass spectrometers, resp.; *m/z* (rel. %).

Plant Material. The roots of *Daphne oleoides* SCHREB. were collected from Mansehra district of NWFP (Pakistan) in October 1999. The plant was identified by Prof. *Manzoor Hussain* (plant taxonomist) at the Department of Botany, Govt., Postgraduate College-1, Abbottabad, NWFP, Pakistan. The voucher specimen (No: 99/73) was deposited at the herbarium of that department.

Extraction and Isolation. The air-dried ground roots of *D. oleoides* (6 kg) were exhaustively extracted with MeOH at r.t. The extract was evaporated and the residue (500 gms) defatted by extracting with hexane. The defatted extract was partitioned between AcOEt and H_2O. The AcOEt fraction was submitted to CC (hexane/$CHCl_3$ and $CHCl_3$/MeOH gradients). The fractions obtained with $CHCl_3$/MeOH 7.5 : 2.5 were combined and further subjected to low-pressure liquid chromatography (AcOEt/MeOH 9.8 : 0.2 → 8.0 : 2.0): *Fractions A–K*. FC (AcOEt/MeOH 9.2 : 0.8 and 8.5 : 1.5) of Fr. *E* and *H* resp., afforded **4** (79 mg), **5** (10 mg), and **1** (20 mg), and **2** (18 mg) and **3** (15 mg), resp.

*3-({6-[(β-*D*-Glucopyranosyl)oxy]-2-oxo-2*H*-1-benzopyran-7-yl}oxy)-7-methoxy-2*H*-1-benzopyran-2-one* (**1**). Amorphous solid (20 mg). UV (MeOH): 335 (4.21), 324 (4.48), 285 (4.99), 227 (4.25). IR (KBr): 3480–3090, 2910, 1722, 1608, 1500, 1470. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-FAB-MS: 513.1014 ([*M* – H]$^+$, $C_{25}H_{21}O_{12}^+$; calc. 513.1026). EI-MS: 352 (8; [*M* + H – sugar]$^+$), 338 (45), 337 (12), 324 (6), 310 (41), 281 (9), 177 (60), 167 (30), 166 (70), 165 (100), 151 (70), 145 (8), 89 (40), 60 (33).

Hydrolysis of **1**. A soln. of **1** (8 mg) in MeOH (7 ml) and 1N HCl (7 ml) was refluxed for 2 h. The soln. was diluted with H_2O (12 ml) and extracted with AcOEt. The sugar in the aq. phase was identified as glucose by comparison with an authentic sample on TLC (BuOH/AcOEt/^{i}PrOH/AcOH/H_2O 7 : 20 : 12 : 7 : 6). The TLC was developed thrice in the same direction, and spots were visualized with the aniline phosphate reagent.

*8-{7-[(6-Deoxy-α-*D*-galactopyranosyl)oxy]-2-oxo-2*H*-1-benzopyran-8-yl}-7-hydroxy-3-[(2-oxo-2*H*-1-benzopyran-7-yl)oxy]-2*H*-1-benzopyran-2-one* (**2**). Amorphous powder (18 mg). UV (MeOH): 339 (4.36), 325 (4.32), 310 (4.21), 268 (3.92), 209 (3.90), 190 (3.56). IR (KBr): 3470–3110, 2930, 1724, 1600, 1590, 1480, 1375, 1300, 1200. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-FAB-MS: 627.1082 ([*M* – H]$^+$, $C_{33}H_{22}O_{13}^+$; calc. 627.1131). FAB-MS 627 ([*M* – H]$^+$), 461 ([*M* – H – sugar]$^+$), 367, 325. EI-MS: 482 (36, [*M* + H – sugar]$^+$), 464 (16), 352 (30), 337 (55), 321 (45), 310 (41), 281 (9), 291 (32), 277 (20), 265 (12), 263 (12), 251 (16), 237 (12), 208 (21), 153 (81), 111 (100), 89 (34), 83 (9), 82 (15).

Hydrolysis of **2**. Acid hydrolysis and identification of the sugar moiety were performed as described for **1**, and the sugar was identified as 6-deoxygalactose.

*8-[7-[(α-*D*-6-Deoxy-α-*D*-galactopyranosyl)oxy]-2-oxo-2*H*-1-benzopyran-8-yl]-7-methoxy-3-[(2-oxo-2*H*-1-benzopyran-7-yl)oxy]-2*H*-1-benzopyran-2-one* (**3**). Amorphous powder (15 mg). UV (MeOH): 345 (4.39), 327 (4.33), 312 (4.22), 266 (3.91), 204 (4.22), 194 (4.75). IR (KBr): 3510 – 3120, 2940, 1720, 1635, 1570, 1500, 1475, 1410, 1320, 1240, 1130. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-FAB-MS 641.1175 ($[M-H]^+$, $C_{34}H_{25}O_{13}^+$; calc. 641.1287). EI-MS: 496 (9, $[M+H-\text{sugar}]^+$), 481 (22), 351 (20), 336 (70), 335 (65), 321 (28), 309 (15), 265 (70), 180 (45), 164 (18), 163 (17), 162 (90), 161 (100), 145 (35), 134 (90), 105 (25), 89 (30), 79 (9), 78 (37).

Hydrolysis of **3**. Acid hydrolysis and identification of the sugar moiety were performed as described for **1**, and the sugar was identified as 6-deoxygalactose.

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