Dicoumarinyl Ether Glycoside from the Roots of *Daphne oleoides*

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Dedicated to Prof. W. Kreiser on the occasion of his 65th birthday

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A new dicoumarinyl ether glycoside (**1**), belonging to a very rare class of "dimeric coumarins", was isolated from the ethyl acetate fraction of the roots of *Daphne oleoides*. Its structure was established by extensive spectroscopic techniques including 1D and 2D NMR spectroscopy. Two known coumarin glycosides (**2** and **3**) were also isolated from the same source.

Introduction

The family Thymelaeaceae is an important source of different classes of coumarins and is regarded as the only source of the very rare class of dicoumarinyl ether glycosides represented in **1**.[1−3] *Daphne oleoides*, belonging to this family, is a multi-branched shrub, and is found abundantly in the northern hilly areas of Pakistan and many other Asian, African, and European countries. It has significant medicinal importance and a variety of uses in traditional folk medicine.[4] Previous studies on this species resulted in the isolation of coumarins,[1−3,5] coumarin lignanoids,[6] lignans,[7,8] and other aromatic compounds.[9] This paper describes the isolation and structure elucidation of a new dicoumarinyl ether glycoside (**1**) and two other known coumarin glycosides (**2** and **3**).

Results and Discussion

The defatted crude methanolic extract was partitioned between ethyl acetate and water. Repeated column chromatographic separation resulted in the isolation of compounds **1**−**3** (Exp. Sect.).

Compound **1** was obtained as a white powder. Its empirical formula was confirmed as $C_{24}H_{20}O_{12}$ by EI MS, from its $[M + H]^+$ peak at *m/z* = 501, together with ^{13}C, DEPT and ^1H NMR spectroscopic data.

The IR spectrum showed absorption bands at 1444, 1558, 1620, 1719, 1739, 3281, and 3472 cm^{-1}, caused by C=C, C=O, and O−H functions. On the TLC plate, the compound exhibited a blue fluorescence under 365-nm UV light. In conjunction with the diagnostic UV absorption bands at 349, 320, and 258 nm, this suggested the presence of a coumarin skeleton, which was further supported by the presence of a typical pair of doublets in the ^1H NMR spectrum at δ = 8.04 (*J* = 9.5 Hz) and 6.37 (*J* = 9.5 Hz) for the 3′-H and 4′-H protons.[10]

The ^{13}C NMR (broad band and DEPT) spectra revealed the presence of 24 carbon atoms: 1 methylene, 13 methine, and 10 quaternary carbon atoms. It also included the characteristic signals for α,β-unsaturated lactone carbonyl carbon atoms at δ = 159.9 and 159.6 The "dimeric" coumarin skeleton with the C−O−C linkage between C-3 and C-7′ was established by comparison of its ^1H and ^{13}C NMR spectroscopic data with those of the structurally related daphnoretin.[11]

The assignment of the chemical shifts to different protons and carbon atoms was performed by careful analysis of the 2D COSY − 45°, HMQC, and HMBC spectra. The pair of typical doublets appearing at δ = 8.04 (*J* = 9.5 Hz) and δ = 6.37 (*J* = 9.5 Hz) was assigned to 4′-H and 3′-H of unit **B** (Scheme 1). A further three resonances appearing as an ABX spin system at δ = 7.70 (d, *J* = 8.6 Hz), 7.19 (d, *J* = 2.4 Hz), and 7.11 (dd, *J* = 8.6, 2.4 Hz) were assigned to 5′-H, 8′-H and 6′-H, respectively. The remaining two singlets in the aromatic region of the ^1H NMR spectrum at δ = 7.44 and 6.94 were resonances for 5-H and 8-H.

A doublet at δ = 4.75 (*J* = 7.2 Hz), characteristic of an anomeric proton, indicated the presence of a sugar moiety in **1**, which was further confirmed by the presence of other characteristic signals for the sugar protons and carbon atoms in the ^1H and ^{13}C NMR spectra. The β configuration

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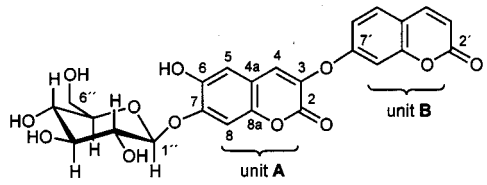
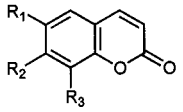


Structure of dicoumarinyl ether glycoside 1



2 : R_1 = H, R_2 = O-β-D-Glu, R_3 = OH
3 : R_1 = OH, R_2 = O-β-D-Glu, R_3 = H

Scheme 1. Structures of coumarin glucosides **1**−**3**

of the sugar moiety was confirmed by the typical large coupling constant, J = 7.2 Hz, of the anomeric proton. The sugar moiety was shown to be D-glucose by comparison of the ^{1}H and ^{13}C NMR chemical shifts and coupling patterns with standard reference data.[12] The appearance of singlets for the ^{1}H signals for 5-H and 8-H indicated their *para* disposition, and their chemical shifts were indicative of oxygen substitution at C-6 and C-7. Finally, the location of the glucose at C-7 rather than at C-6 was evident from the $^3J_{CH}$ interaction of the anomeric proton with C-7 (δ = 148.4) in the ^{13}C-^{1}H heteronuclear multiple bond correlation (HMBC). As one oxygen atom is involved in the C−O−C linkage between the **A** and **B** units, the remaining oxygen atom in the molecular formula had to be a phenolic group at C-6. This was confirmed by the ^{13}C resonances of the C-5, C-6, and C-7 carbon atoms, and was also in accord with the appearance of 5-H and 8-H signals as singlets.

The important connectivities in the ^{1}H-^{1}H COSY, HMQC, and HMBC spectra are shown in Table 1. All the accumulated data confirmed the structure of **1** as 7-*O*-[β-D-glucopyranosyl]-6-hydroxy-3-[(2-oxo-2*H*-1-benzopyran-7′-yl)-2*H*-1-benzopyran-2-one. Two known compounds, daphnin (**2**)[13] and cichoriin (**3**),[14] were also identified by comparison with literature data. Structurally related coumarins show anti-HIV-1 activity,[15] and investigation of the biological properties of **1**−**3** is in progress.

Experimental Section

Instrumentation: ^{1}H, 2D ^{1}H-^{1}H COSY, ^{13}C, and 2D HMBC spectra were recorded with a Bruker ARX 400 MHz spectrometer and 2D HMQC spectra with a Bruker ARX 200 NMR spectrometer. Chemical shifts are referenced to internal TMS (δ = 0) and coupling constants J are reported in Hz. Optical spectra were recorded with a NICOLET 510P FT-IR spectrophotometer, a UV-2101PC spectrophotometer, and a Perkin−Elmer 241 polarimeter.

Plant Material: The roots of *Daphne oleoides* were collected from the Mansehra district of N.W.F.P., Pakistan, in October 1999. The plant was identified by Professor Manzoor Hussain (plant taxonomist) at the department of Botany, Government Postgraduate College-1, Abbottabad, N.W.F.P., Pakistan. The voucher specimen (No. 99/73) was deposited at the herbarium of this department.

Table 1. ^{1}H, ^{13}C NMR, ^{1}H-^{1}H COSY, HMBC (400 MHz) and HMQC (200 MHz) data ([D$_6$]DMSO) of compound **1**

Position	δ (^{1}H) (HMQC)	δ (^{13}C)	^{1}H-^{1}H COSY[a]	HMBC[a]
2	−	159.6	−	−
3	−	135.7	−	−
4	7.81(s)	130.7	−	C-2, C-3, C-4, C-5
4a	−	114.3	−	−
5	7.44 (s)	110.9	−	C-4, C-8a, C-6, C-7
6	−	143.9	−	−
7	−	148.4	−	−
8	6.94 (s)	103.9	−	C-8a, C-6, C-7
8a	−	150.5	−	−
2′	−	159.9	−	−
3′	6.37 (d, 9.5 Hz)	114.3	4′-H	C-2′, C-4a′
4′	8.04 (d, 9.5 Hz)	143.0	3′-H	C-2′, C-3′, C-5′, C-8a′
4a′	−	111.3	−	−
5′	7.70 (d, 8.6 Hz)	129.8	6′-H	C-4′, C-6′, C-7′, C-8a′
6′	7.11 (dd, 8.6, 2.4 Hz)	113.3	5′-H, 8′-H	C-5′, C-7′, C-8′
7′	−	157.9	−	−
8′	7.19 (d, 2.4 Hz)	103.0	6′-H	C-6′, C-7′, C-8a′
8a′	−	154.9	−	−
1′′	4.75 (d, 7.27 Hz)	102.3	2′′-H	C-7
2′′	3.23 (m)	73.0	1′′-H, 3′′-H	−
3′′	3.72 (m)	76.0	−	−
4′′	3.55 (m)	69.4	−	−
5′′	3.46 (m)	77.1	−	−
6′′	3.24 and 3.34 (m)	60.5	−	−

[a] Only the diagnostic correlations with the protons in column 2 are listed.

Extraction and Isolation: The air-dried, ground roots of *D. oleoides* (6 kg) were exhaustively extracted with MeOH at room temperature. The extract was concentrated and the residue (500 g) was defatted with hexane. The defatted extract was partitioned between EtOAc and H_2O. The EtOAc fraction was subjected to silica gel column chromatography (with hexane/$CHCl_3$ and $CHCl_3$/MeOH gradient systems). The fractions obtained with $CHCl_3$/MeOH (85:15) were combined and again subjected to low-pressure column chromatograpy with MeOH/$CHCl_3$ (97:3−80:20) to obtain sub-fractions A−F. Preparative TLC of sub-fractions E and D with MeOH/$CHCl_3$ (97:3−80:20) resulted in the isolation of compounds **1** (13 mg), **2** (20 mg), and **3** (18 mg), together with a mixture of still unresolved compounds (**2** and **3**, 120 mg).

7-*O*-[β-D-Glucopyranosyl]-6-hydroxy-3-[(2-oxo-2*H*-1-benzopyran-7′-yl)-2*H*-1-benzopyran-2-one (1): M.p. 224−226 °C (decomp). ^1H and ^{13}C NMR spectroscopic data see Table 1. $[\alpha]_D^{20} = -21.4$ ($c = 0.70$, MeOH/$CHCl_3$, 4:1). UV (MeOH/$CHCl_3$, 4:1): λ_{max} (log ε) = 258 (3.37), 320 (4.03), 349 (3.96) nm. IR (KBr): $\tilde{\nu}$ = 3472, 3281, 3100, 3058, 2960, 2918, 2872, 1739, 1719, 1620, 1558, 1527, 1444, 1393, 1263, 1072 cm^{-1}. EI MS: *m/z* (%) = 501 (5) [$M^+ + 1$ ($C_{24}H_{20}O_{12} + H$)], 338 (100) [M^+ − sugar ($C_{18}H_{10}O_7$)], 310 (35) [M^+ − sugar − CO ($C_{17}H_{10}O_6$)], 281 (20), [M^+ − sugar − CO−HC ($C_{16}H_9O_5$)], 253 (8) [M^+ − sugar − CO−HCO−C ($C_{15}H_9O_4$)], 193 (5) ($C_9H_5O_5$), 165 (60) ($C_8H_5O_4$), 146 (40) ($C_9H_5O_3$).

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