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# Coumarinolignoid glycoside from *Daphne oleoides*

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**Abstract**

A new coumarinolignoid glycoside, daphneticin-4″-*O*-α-D-glucopyranoside, along with the known, daphneticin, have been isolated from the whole plant extract of *Daphne oleoides*. The structures of these compounds were elucidated on the basis of chemical and spectroscopic evidence. 

*Keywords: Daphne oleoides*; Thymelaeaceae; Coumarinolignoid; Daphneticin-4″-*O*-α-D-glucopyranoside

## 1. Introduction

As a part of our ongoing phytochemical studies on *Daphne oleoides*, we have recently reported some lignans and triterpenoids from this species (Ullah et al., 1998; Ullah, Ahmed, Anis, & Malik, 1998). In this paper, we now wish to report the isolation and structural elucidation of a new coumarinolignoid glycoside (**1**) from the whole plant extract of *D. oleoides*. This plant is a small multibranched shrub, found on the western Himalayas, from Garhwal Westward to Murree, occurring at an altitude of 3000–9000 feet (Watt, 1972). The root of this species is a purgative and the bark and leaves are given in cutaneous affections, whereas infusion of leaves is given in gonorrhoea and applied to abscesses (Baquar, 1989).

## 2. Results and discussion

Chromatographic resolution of the $CHCl_3$-soluble fraction of the methanolic extract afforded **1** as colourless needles. The positive ($[M+H]^+$ *m/z* 549) and negative ($[M-H]^-$ *m/z* 547) FAB mass spectrum showed the $M_r$ of 548. It was assigned the molecular formula $C_{26}H_{28}O_{13}$ by negative HR-FAB mass spectrometry, showing the $[M-H]^+$ peak at *m/z* 547.1121 (calcd. for $C_{26}H_{27}O_{13}$ 547.1120). The UV maximum (322 nm), IR bands (1720 and 1620 $cm^{-1}$) and $^1H$ NMR (two doublets with AB pattern at $\delta$ 6.32 and 7.95, $J=9.5$ Hz characteristic of H-3 and H-4 protons, along with another AB pattern resonating at $\delta$ 7.10 and 7.35, $J=8.6$ Hz corresponding to protons H-6 and H-5) indicated the existence of a coumarin skeleton with a 7,8-substitution pattern (Lin-Gen, Seligmann, & Wagner, 1983). It was confirmed by the nearly identical $^1H$ NMR spectrum in the corresponding region of 7,8-dihydroxy coumarin (daphnetin) isolated from the same species (Thusoo, Raina, Minhaj, Shakti, & Zaman, 1981). No methylation was observed when **1** was treated with $CH_2N_2$, indicating the absence of phenolic group in **1**, whereas acetylation of **1** with acetic anhydride and pyridine yielded a pentaacetate (**2**), revealing the presence of five hydroxyl groups.

The $^1H$ NMR also showed resonances for one primary hydroxyl group at $\delta$ 3.65 (2H, m), two methoxy groups at $\delta$ 3.80 (6H, s), one oxymethine proton at $\delta$ 4.30 (1H, m), a mono-oxybenzylic proton at $\delta$ 4.85 (1H, d, $J=7.5$ Hz) and an anomeric proton signal at $\delta$ 5.10 (1H, d, $J=2.4$ Hz), the latter suggesting the presence of a sugar moiety in **1** in the α configuration. Acid hydrolysis of **1** gave an aglycone (**3**) and a sugar, which was identified as D-glucose by PC and also by the GC retention time of its TMS ether (Markham, 1982). The EI-mass spectrum of **3** showed a $[M]^+$ at *m/z* 386. The $^1H$ and $^{13}C$ NMR identified compound **3** as the corresponding aglycone of **1**. Since five of the hydroxyl groups have already been accounted for, the remaining two oxygens must, therefore, be present as ether linkages, probably constituting the required additional dioxane ring with the remaining $C_{17}$-fragment. This was deduced by the presence of fragment at *m/z* 178 (A) in the mass spectrum of **1**.

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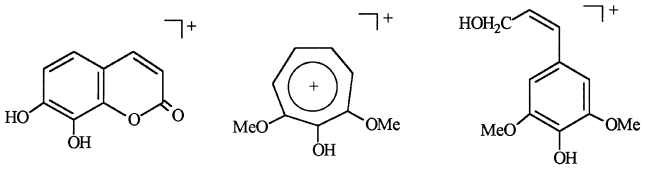


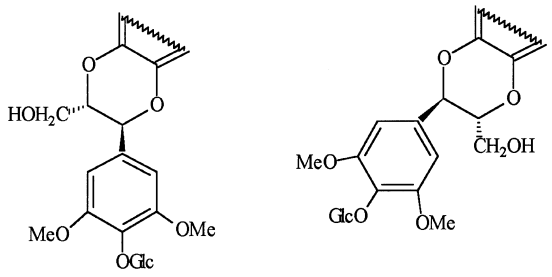
A *m/z* 178 B *m/z* 167 C *m/z* 210

Further evidence about the structure of the $C_{17}$-fragment was provided by $^{13}C$ and $^{1}H$ NMR spectra of **1**. The $^{13}C$ NMR (BB and DEPT) exhibited the presence of 12 methine, two methylene, one methyl and eight quaternary carbon atoms, which were assigned with the help of HMQC experiments and by comparison with the reported literature data on related compounds (Wenkert, Gottlieb, Gottlieb, Pereira, & Formiga, 1976; Ray, Chattopadhyay, Konno, & Hikino, 1980). In the $^{1}H$ NMR, a singlet at $\delta$ 6.88 integrating for two aromatic protons, together with the presence of two identical methoxyl groups indicated either a 4″-*O*-glucosyl-3,5- or 4″-*O*-glucosyl-2,6-dimethoxy substituted benzene ring.

The aglycone **3** was treated with $CH_2N_2$ to obtain the monomethyl ether **4**, whereas acetylation provided a diacetate **5**, suggesting a phenolic and an alcoholic group in **3**. Since the phenolic group was absent in **1**, it was concluded that the glucose moiety must be connected through a phenolic-*O*-linkage. Moreover, the singlet at $\delta$ 6.75 (2H, s) in **3** was shifted to low-field [$\Delta\delta$ 0.13 (DMSO-$d_6$)] in the corresponding diacetate **5**, providing evidence for the 4″-hydroxy-3,5-dimethoxy substitution pattern in **3** (Lin-Gen et al., 1983) and, hence a 4″-*O*-glucosyl-3,5-dimethoxyl substitution pattern in **1**. Irradiation of the singlet at $\delta$ 3.80 (6H, s) in **1** caused 16.85% enhancement of the signal at $\delta$ 6.88 (2H, s), confirming the above assignment. The existence of the $C_3$-unit came from the $^{1}H$ NMR spectrum which showed two vicinal aliphatic oxymethines at $\delta$ 4.85 (1H, d, $J=7.5$ Hz, H-1′) and 4.30 (1H, m, H-2′) linked to a phenyl and to a -$CH_2OH$ group (Gottlieb, Maia, & Mourao, 1976); this was confirmed by the presence of the most abundant retro-Diels-Alder fragment ion at *m/z* 210 (C) (Ray et al., 1980; Zoghbi, Roque, & Gottlieb, 1981) in the mass spectrum of **1**.

The coupling constant between H-1′ and H-2′ signals in **1** was 7.4 Hz, demonstrating that the two hydrogens are trans-oriented. This type of skeleton is similar to that observed for cleomiscosins A and B, isolated from *Cleome viscosa* (Ray et al., 1980; Ray, Chattopadhyay, Konno, & Hikino, 1982), and cleomiscosin B, recently found in *Simaba multiflora* (Simaroubaceae), *Soulamea soulameoides* (Simaroubaceae) and *Matayaba arborescens* (Sapindaccae) (Lin-Gen et al., 1983). Compound **1**, therefore, was a similar skeleton to cleomiscosin A and B, with methoxyl groups present in the isolated phenyl ring forming a syringyl residue (with a 4″-*O*-α-D-glucopyranoside moiety), rather than at C-6 of a coumarin moiety and, thus, could be represented by either structure **1a** or **1b**, bearing in mind the *trans*-disposition.

The final evidence in favour of **1a** was made with the help of measurements of the selective $^{13}C\{^{1}H\}$ heterodecouplings of diacetate **5**, by analog with the structural determination of cleomiscosins A and B (Lin-Gen et al., 1983). Irradiation of the H-2′ signal at $\delta$ 4.42 sharpened the doublet at $\delta$ 146.4 attributed to C-7. The same observation was made for the C-8 signal at $\delta$ 133.5, when the irradiation was carried out on H-1′ at $\delta$ 4.94. These spectroscopic results were identical to those observed for cleomiscosin B (Lin-Gen et al., 1983). The above evidence and the biogenetic evidence provided by the cooccurrence of daphneticin, which has the same stereochemistry, established the structure of **1** as daphneticin 4″-*O*-α-D-glucopyranoside. The corresponding aglycone, daphneticin, has earlier been isolated from *D. tangutica* (Lin-Gen et al., 1983).



**1a** **1b**

## 3. Experimental

### 3.1. General

Mps are uncorr. $^{1}H$ NMR and $^{13}C$ NMR were obtained on a Bruker AM-500 spectrometer. DEPT expts were carried out with $\theta=45$, 90 and 135°. Chemical shifts are reported in $\delta$ ppm, with TMS as int. standard. Kieselgel 60 (35–70) mesh was used for CC. Precoated Kieselgel 60, $F_{254}$ aluminium sheets (Merck) was used to check the purity. Spots were visualized by spraying with ceric sulphate sn in 10% $H_2SO_4$, followed by heating.

### 3.2. Plant material

Whole plants of *D. oleoides* were collected from Hazara division of N.W.F.P., Pakistan, in February, 1995. A voucher specimen was identified by Professor Iftikhar Hussain Shah and is deposited in the Herbarium of the Faculty of Pharmacy, Gomal University, D.I. Khan, Pakistan.

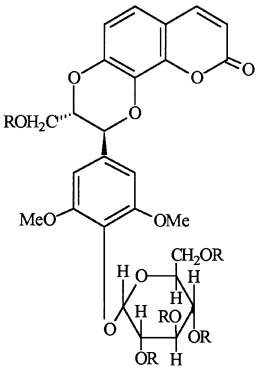
### 3.3. Extraction and isolation

Shade-dried plant material (16 kg) was extracted three times with MeOH. The combined extract was evapd under red. press. The residue was suspended in $H_2O$ and

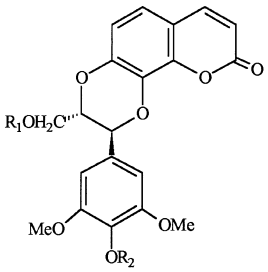
extracted successively with petrol, EtOAc, $CHCl_3$ and *n*-BuOH. The $CHCl_3$ extract was evapd to obtain 70 g of residue, which was subjected to CC on silica gel using a gradient of MeOH in $CHCl_3$, to afford a mixture of compounds at polarity of (9:1). This mixt. was further resolved by repeated CC on silica gel, to obtain **1** (33 mg), using the same gradient system of polarity (79.5:20.5), as colourless needles, whereas the fr. eluted at (9.4:0.6) gave daphneticin **3** after crystallization from MeOH–$Me_2CO$ (37 mg).

### 3.4. Compound *1*

Mp 254–255°. $[\alpha]_D$ +23.5 (DMSO; $c=0.10$). $C_{26}H_{28}O_{13}$ (neg. HR-FAB-MS, $[M]^+$ $m/z$ 547.1121). UV $\lambda_{max}^{MeoH}$ nm ($\varepsilon$): 240 (9150), 265 (8790), 322 (11310). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3440, 3245, 1720, 1620, 1575, 1442, 1330, 1265. EIMS $m/z$ (rel. int): 386 $[M]^+$ (8), 368 $[M–H_2O]^+$ (6), 354 (10), 311 (15), 210 (70), 178 (100), 167(65), 150 (75), $^1$H NMR (500 MHz, DMSO-$d_6$): (see Section 2). $^{13}$C NMR (500 MHz, DMSO-$d_6$): $\delta$ 160.3 (C-2), 150.1 (C-3″, C-5), 149.4 (C-9), 147.4 (C-7), 144.4 (C-4), 138.9 (C-8), 133.8 (C-4″), 127.1 (C-1″), 119.6 (C-5), 113.7 (C-10), 113.5 (C-3), 113.1 (C-6), 105.8 (C-2″, C-6″), 100.4 (C-1‴), 79.6 (C-2′), 77.4 (C-1′), 74.0 (C-3‴), 72.8 (C-2‴), 72.5 (C-5‴), 70.8 (C-4‴), 61.8 (C-6‴), 60.9 (C-3′), 56.2 (2× -$OCH_3$).




(**1**): R = H
(**2**): R = Ac




(**3**): $R_1 = R_2 = H$
(**4**): $R_1 = H$, $R_2 = CH_3$
(**5**): $R_1 = R_2 = Ac$

### 3.5. Acetylation of *1*

A mixt. of 4 mg of **1**, $Ac_2O$ (1 ml) and pyridine (1 ml) was stirred overnight at room temp. and worked-up in the usual way, to afford **2** as a colourless oil (4.2 mg). $C_{36}H_{38}O_{18}$ (MS $m/z$ 758 $[M]^+$). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1725, 1625, 1570, 1450, 1355, 1210. $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.90 (1H, d, $J=9.5$ Hz, H-4), 7.30 (1H, d, $J=8.6$ Hz, H-5), 7.06 (1H, d, $J=8.6$ Hz, H-6), 6.92 (2H, s, H-2″, H-6″), 6.35 (1H, d, $J=9.5$ Hz, H-3), 5.24 (1H, d, $J=2.4$ Hz, H-1‴), 5.12 (1H, d, $J=7.4$ Hz, H-1′), 4.65 (1H, m, H-2′), 4.15 (2H, m, H-3′), 3.82 (6H, s, -$OCH_3$ × 2), 2.27 (9H, s, -$COOCH_3$ × 3) and 2.24 (6H, s, -$COOCH_3$ × 2). $^{13}$C NMR (500 MHz, DMSO-$d_6$): $\delta$ 160.3 (C-2), 150.6 (C-3″), 150.5 (C-9), 150.4 (C-5″), 146.6 (C-7), 144.1 (C-4), 133.8 (C-8), 133.2 (C-4″), 128.0 (C-1″), 119.9 (C-5), 113.8 (C-6, C-8), 113.7 (C-3), 113.5 (C-10), 105.4 (C-2″, C-6″), 98.3 (C-1‴), 77.1 (C-1′), 75.4 (C-2′), 71.5 (C-3‴), 70.1 (C-2‴), 68.8 (C-5‴), 68.6 (C-4‴), 62.5 (C-3′), 60.2 (C-6‴), 56.0 (2 × $OCH_3$), 170.5, 170.3, 170.2, 169.2, 168.8 (5× -$COOCH_3$), 20.8, 20.6, 20.4, 20.3, 20.2 (5× -$COOCH_3$).

### 3.6. Acetylation of aglycone *3*

Aglycone **3** was acetylated by same procedure as described above for **1**, to obtain **5**, mp 208–209°C. $C_{24}H_{22}O_{10}$ (MS $m/z$ 470, $[M]^+$). All the physical and spectral data coincided with the lit. data for daphneticin diacetate (Wenkert et al., 1976).

### 3.7. Daphneticin *3*

Colourless crystals, mp 235–238°C. $C_{20}H_{18}O_8$ (MS, $[M]^+$ $m/z$ 386.1005). Physical and spectral data identical with lit. (Wenkert et al., 1976).

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