# A Lignoid Glycoside and Dimeric Phenylpropanoids from *Daphne oleoides*

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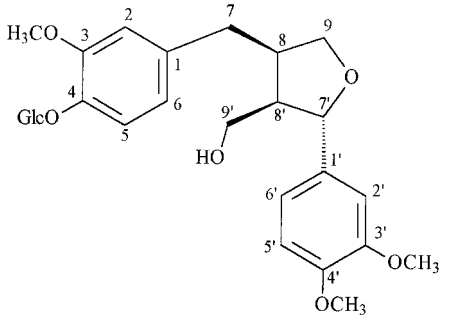
Three new natural products, a lignoid glycoside **1** and two dimeric phenylpropanoids **2** and **3**, along with two known lignans **4** and **5**, were isolated from the BuOH- and $CHCl_3$-soluble fractions of the whole plant of *Daphne oleoides* (Thymelaeaceae). The structures of the new compounds were established by spectroscopic techniques, including 2D NMR, as 4-(*β*-D-glucopyranosyloxy)-9′-hydroxy-3,3′,4′-trimethoxy-7′,9-epoxylignan (**1**), (1*R*,2*S*,5*R*,6*R*)-6-(3-ethyl-4-hydroxy-5-methoxyphenyl)-2-(4-hydroxy-3,5-dimethoxyphenyl)-3,7-dioxabicyclo-[3.3.0]octane (**2**) and (1*R*,2*S*,5*R*,6*S*)-2,6-bis(3-ethyl-4-hydroxy-5-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane (**3**). The other lignans were identified as (+)-pinoresinol *O*-(*β*-D-glucopyranoside) (**4**) and (+)-medioresinol (**5**).

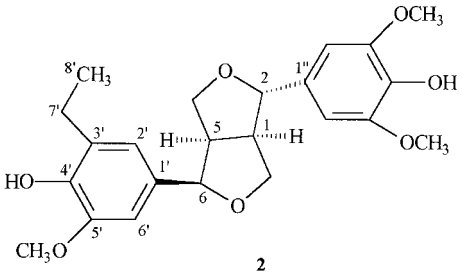
**Introduction.** – In previous publications [1][2], we have reported the isolation and structure elucidation of lignans and phenylpropanoid glycosides from the whole plant of *Daphne oleoides*. In continuation of our phytochemical investigation, this paper deals with the isolation and structural elucidation of compounds **1**–**5** from the whole plant of the same species. *Daphne oleoides* L. belongs to the family Thymelaeaceae and is a small multi-branched shrub found in the Western Himalayas, from Garhwal westward to Murree, occurring at an altitude of 3000 to 9000 feet [3]. The root of this plant is used as purgative, while the bark and leaves are used to treat cutaneous affections. An infusion of leaves is also used against gonorrhoea and applied to abcesses [4].

**Results and Discussion.** – The MeOH extract of the whole plant of *Daphne oleoides* was divided into hexane-, $CHCl_3$-, AcOEt- and BuOH-soluble fractions. Compounds **1** and **4** were isolated from the BuOH-partitioned extract, while **2**, **3**, and **5** were obtained from the $CHCl_3$-soluble fraction by means of repeated chromatographic purification.

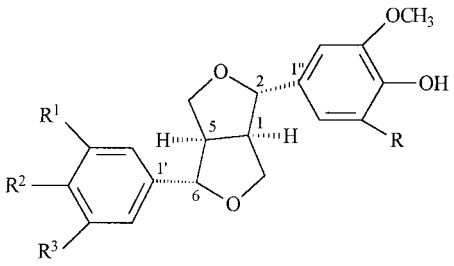
The molecular formula of compound **1** was established as $C_{27}H_{36}O_{11}$ by HR-FAB-MS (negative mode) showing $[M-H]^-$ at *m*/*z* 535.1922. Acid hydrolysis of **1** yielded the aglycone daphneligin, which has been isolated earlier from the same species [1], and a sugar, the latter being identified as D-glucose by paper chromatography (PC) and also by the $t_R$ of its trimethylsilyl ether in the GC [5]. Spectral analysis of **1** by IR, $^1$H-



**1**



**2**



**3** R = $R^1$ = Et, $R^2$ = OH, $R^3$ = MeO
**4** R = $R^3$ = H, $R^1$ = MeO, $R^2$ = GlcO
**5** R = H, $R^1$ = $R^3$ = MeO, $R^2$ = OH

and $^{13}$C-NMR, HMBC, NOE, and MS allowed us to elucidate its structure as 4-(glucopyranosyloxy) 9′-hydroxy-3,3′,4′-trimethoxy-7′,9-epoxylignan.[1])

The IR (KBr) spectrum of **1** displayed absorptions for an OH function at 3555 and 3250 and for an aromatic moiety at 1615 and 1510 cm$^{-1}$. The $^1$H-NMR spectrum showed the presence of 4 *d* in the aromatic region at $\delta$ 6.62 ($J$ = 7.2 Hz), 6.75 ($J$ = 1.8 Hz), 6.80 ($J$ = 7.6 Hz), and 6.92 ($J$ = 7.4 Hz), and, in addition, two *dd* at $\delta$ 6.57 ($J$ = 1.8, 7.2 Hz) and 6.70 ($J$ = 1.6, 7.4 Hz). The spectrum further showed a *d* due to an anomeric proton at $\delta$ 4.80 ($J$ = 7.2 Hz), indicating the presence of a sugar moiety in the $\beta$-D-configuration. The $^{13}$C-NMR chemical shifts and multiplicities confirmed the presence of a glycosidic unit and a lignan moiety. Their connection was determined by HMBC, which showed a long-range correlation between H–C(1″) and C(4)[1]) (*Fig. 1*). The $^{13}$C-NMR spectrum of the aglycone was identical to that of daphneligin [1]. The $M^{+\cdot}$ at $m/z$ 374 as well as the

[1]) For systematic numbering and names, see *Exper. Part.*

fragmentation pattern in the EI-MS of the aglycone were also identical to those of daphneligin. The configuration at the chiral centers were confirmed by NOEs between H–C(8) and H–C(8′) as well as between H–C(7), H–C(7′), and H–C(9′).

Compound **2** showed the $M^{+\cdot}$ at *m/z* 416 in the EI-MS. The HR-EI-MS established the molecular formula as $C_{23}H_{28}O_7$, indicating eight double-bond equivalents. Comparison of the $^1$H-NMR spectrum of **2** with that of (+)-sringaresinol [6] revealed very much similarity and suggested that a MeO group at one of the aryl substituents of the latter had been replaced by an Et group in the case of **2**. On the basis of the spectral evidences, the structure of **2** was established as (1*R*,2*S*,5*R*,6*R*)-6-(3-ethyl-4-hydroxy-5-methoxyphenyl)-2-(4-hydroxy-3,5-dimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]-octane[1]).

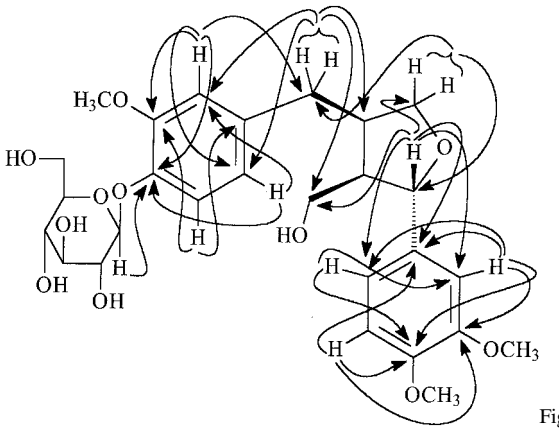



Fig. 1. *HMBC Connectivities of* **1**

The IR (KBr) spectrum of **2** showed absorptions for an OH function at 3410 and 3425 cm$^{-1}$ and for an aromatic moiety at 1620 and 1522 cm$^{-1}$. In the $^1$H-NMR spectrum, 2 *d* in the aromatic region at $\delta$ 6.82 ($J$ = 2.2 Hz) and 6.80 ($J$ = 2.3 Hz) and another *s* at $\delta$ 6.60 (2 H) were present. Furthermore, 2 broad *s* at $\delta$ 5.52 and 5.62 were assigned, by HMQC, to 2 OH groups, and in addition, 2 *s* at $\delta$ 3.86 (6 H) and 3.88 (3 H) were attributed to 3 MeO groups. Instead of the additional MeO group of (+)-sringaresinol, a *q* in the aliphatic region at $\delta$ 2.95 (2 H) and a *t* at $\delta$ 1.36 (3 H) appeared for the Et group of **2**. In the JMBC spectrum, H–C(7′) showed cross-peaks to C(3′), C(2′), and C(8′)[1]) (*Fig. 2*). The configuration of **2** was deduced from the observation that the signals of H–C(2) and H–C(6) were not identical. It is estimated that the axial proton H–C(2) is held close to the axial aryl group at C(6) and shifted upfield to $\delta$ 4.30, while H–C(6) adjacent to the equatorial aromatic ring is shifted downfield at $\delta$ 4.75 [7]. In the HMBC spectrum, the H–C(6) signal showed a cross-peak to C(1′). The upfield shift of C(3′) as well as downfield shifts of C(2′) and C(6′) relative to (+)-sringaresinol agreed well with the Et group linked at C(3′) of the axial ring.

Compound **3** was assigned the molecular formula $C_{24}H_{30}O_6$ by HR-EI-MS, showing the $M^{+\cdot}$ at *m/z* 414.1822. Comparison of the $^1$H-NMR spectrum of **3** with that of **2** indicated the presence of 2 aromatic MeO groups in the former, instead of 3 as in **2**, and suggested that one of the MeO groups of **2** had been replaced by an Et group. From the spectral data, the structure of **3** was established as (1*R*,2*S*,5*R*,6*S*)-2,6-bis(3-ethyl-4-hydroxy-5-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane[1]).

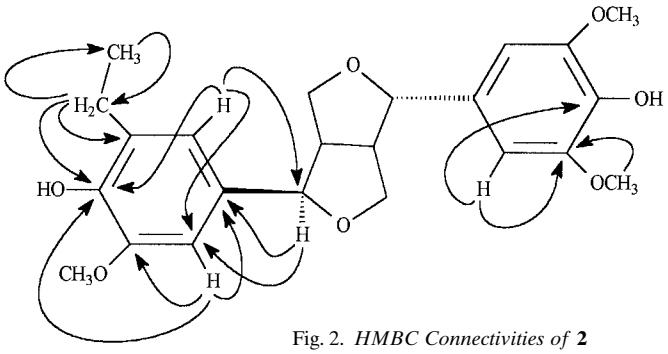



Fig. 2. *HMBC Connectivities of* **2**

The IR (KBr) spectrum of **3** showed absorptions for an OH function at 3415 $cm^{-1}$ and for an aromatic moiety at 1625 and 1526 $cm^{-1}$. The $^1$H-NMR of **3**, two pairs of equivalent aromatic protons appeared at $\delta$ 6.84 ($d$, $J = 2.5$ Hz) and 6.80 ($J = 2.5$ Hz), as well as a broad $s$ at $\delta$ 5.50 (2 H), which was assigned, by HMQC, to 2 OH groups. The resonances of H–C(2) and H–C(6) were identical ($\delta$ 4.78 ($J = 6.8$ Hz)), suggesting a diequatorial arrangement of the aryl substituents at C(2) and C(6) [6]. The presence of a $q$ in the aliphatic region at $\delta$ 2.98 (4 H) and a $t$ at $\delta$ 1.34 (6 H) was consistent with the presence of two equivalent Et groups in **3**. For HMBC, see *Fig. 3*.

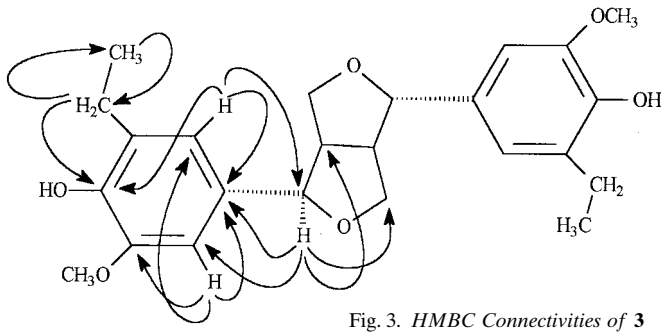



Fig. 3. *HMBC Connectivities of* **3**

Compounds **4** and **5** were identified as (+)-pinoresinol *O*-$\beta$-D-glucopyranoside and medioresinol, respectively, by comparison of the physical and $^{13}$C-NMR data with reported ones [6].

## Experimental Part

*General*. M.p.: open capillaries; electrothermal melting apparatus. Optical rotations: *Perkin-Elmer 241-MC* polarimeter. UV Spectra: *Shimadzu UV-160A*-UV/VIS-recording spectrophotometer; $\lambda_{max}$ ($\varepsilon$) in nm. IR Spectra: *Perkin-Elmer 881-IR* spectrometer; in $cm^{-1}$. $^1$H- and 2D-NMR Spectra: *Bruker AM-400*; $\delta$ in ppm, $J$ in Hz. MS: EI at 70 eV; LSI at 5 kV neg.; ESI, platform *LZA* (*Micromass*), $H_2O$/MeCN 1:1 with 0.1% $CF_3COOH$ as a solvent for loop injection (cone voltage 40 eV); in $m/z$ (rel %).

*Plant Material*. The whole plant of *D. oleoides* was collected in the division of N.W.F.P province in February, 1995. A voucher specimen (D-16995) was identified by Professor *Iftikhar Hussain Shah* and deposited in the Herbarium of the Faculty of Pharmacy, Gomal University, D.I. Khan, Pakistan.

*Extraction and Isolation.* Shade-dried and ground plant material (16 kg) was extracted with MeOH (3×). The resulting residue was suspended in $H_2O$ and extracted successively with petroleum ether, AcOEt, $CHCl_3$, and BuOH. The BuOH extract was subjected to VLC ($CHCl_3$/MeOH gradient of increasing polarity). *Fractions*

*A – F. Fr. D* was eluted with $CHCl_3$/MeOH 9 :1 and further subjected to repeated CC ($CHCl_3$/MeOH 8.4 :1.6): **1** (21 mg) and **4** (16 mg). The $CHCl_3$ extract was submitted to VLC ($CHCl_3$/MeOH gradient): *Fractions A′ – G′*. *Fr. B′* ($CHCl_3$/MeOH 9.6 :0.4) was subjected to CC: **2** (14 mg), **3** (18 mg), and **5** (14 mg).

*4-(β-*D*-Glucopyranosyloxy)-9′-hydroxy-3,3′,4′-trimethoxy-7′,9-epoxylignan* (=*(2*S*,3*R*,4*R*)-2-(3,4-Dimethoxyphenyl)-4-[[4-(β-*D*-glucopyranosyloxy)-3-methoxyphenyl]methyl]tetrahydrofuran-3-methanol*; **1**). Amorphous powder. M.p. 192 – 193°. $[\alpha]_D^{20} = -30.5$ ($c = 0.1$, MeOH). UV (EtOH): 229 (186). IR (KBr): 3555, 3250, 1615, 1510. $^1$H-NMR ($CD_3OD$): 6.92 (*d*, $J = 7.4$, H–C(5′)); 6.80 (*d*, $J = 1.6$, H–C(2′)); 6.75 (*d*, $J =$ 1.8, H–C(2)); 6.70 (*dd*, $J = 7.4$, 1.6, H–C(6′)); 6.62 (*d*, $J = 7.2$, H–C(5)); 6.57 (*dd*, $J = 7.2$, 1.8, H–C(6)); 4.80 (*d*, $J = 7.2$, H–C(1″)); 4.70 (*d*, $J = 6.8$, H–C(7′)); 3.86 (*dd*, $J = 9.2$, 6.2, $H_a$–C(9)); 3.74 (*s*, MeO–C(3)); 3.72 (*s*, MeO–C(3′), MeO–C(4′)); 3.65 (*dd*, $J = 11.4$, 6.4, $H_a$–C(9′)); 3.52 (*dd*, $J = 9.2$, 4.2, $H_b$–C(9)); 3.45 (*dd*, $J =$ 11.4, 4.1, $H_b$–C(9′)); 2.80 (*dd*, $J = 13.2$, 4.6, $H_a$–C(7)); 2.72 (*m*, H–C(8)); 2.42 (*dd*, $J = 13.2$, 8.8, $H_b$–C(7)); 2.20 (*m*, H–C(8′)). $^{13}$C-NMR ($CD_3OD$): aglycone: 149.40 (C(3′)); 148.60 (C(3)); 147.25 (C(4′)); 145.80 (C(4)); 137.52 (C(1)); 131.72 (C(1′)); 121.42 (C(6)); 118.0 (C(6′)); 116.25 (C(5)); 115.20 (C(5′)); 113.60 (C(2)); 108.76 (C(2′)); 82.45 (C(7′)); 72.25 (C(9)); 59.35 (C(9′)); 56.60 (*Me*O–C(3)); 56.42 (*Me*O–C(3′), MeO–C(4′)); 52.70 (C(8′)); 42.35 (C(8)); 32.40 (C(7)); Glc: 102.20 (C(1″)); 76.12 (C(5″)); 75.32 (C(3″)); 74.0 (C(2″)); 70.52 (C(4″)); 61.95 (C(6″)). EI-MS: 535 (100, $[M-1]^{+\bullet}$), 373 (72, $[M-1-\text{Glc}]^+$), 359 (19, $[M-\text{Glc}-CH_2]^+$), 342 (32), 192 (31), 137 (80).

*Acid Hydrolysis of* **1**. Compound **1** (15 mg) was refluxed for 4 h with 1N HCl in MeOH (5 ml). The soln. was evaporated, the residue diluted with $H_2O$ (5 ml), the mixture extracted with AcOEt, the extract evaporated, and the residue subjected to prep. TLC: daphneligin [1]. The aq. phase was concentrated, and D-glucose was identified by PC (*Schleicher & Schuell 2043b*, BuOH/AcOH/$H_2O$ 4 :1 :5, detection with aniline/phthalic acid). Identification was further confirmed by comparing the $t_R$ of its trimethylsilyl ether (see below) with an authentic sample by GC.

*Trimethylsilylation of* **1**. The sugar (1 mg) was dissolved in dry pyridine (0.4 ml) and hexamethyldisilazane (0.20 ml) added by syringe. Chlorotrimethylsilane (0.20) was then added and the flask stoppered, left for 30 min, dried under vacuum, and washed with dry heptane (0.20 ml). This soln. (2 µl) was used for GC (3% *OV-1* silanized *Chromosorb W*, column temp. 180°, injection port and detector temp. 275 – 300°, flow rate 35 ml/min, flame-ionization detector).

*(1*R*,2*S*,5*R*,6*R*)-6-(3-Ethyl-4-hydroxy-5-methoxyphenyl)-2-(4-hydroxy-3,5-dimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane* (=*2-Ethyl-4-[(1*R*,3a*R*,4*S*,6a*R*)-tetrahydro-4-(4-hydroxy-3,5-dimethoxyphenyl)-1*H*,3*H*-furo[2,3-*c*]furan-1-yl]-6-methoxyphenol*; **2**). Amorphous powder. M.p. 162 – 163°. $[\alpha]_D^{20} = +36.5$ ($c = 0.1$, $CHCl_3$). UV ($CH_2Cl_2$): 220 (192), 275 (186). IR (KBr): 3410, 3425, 1620, 1522. $^1$H-NMR ($CDCl_3$): 6.82 (*d*, $J = 2.2$, H–C(2′)); 6.80 (*d*, $J = 2.3$, H–C(6′)); 6.60 (*s*, H–C(2″), H–C(6″)); 4.75 (*d*, $J = 2.4$, H–C(6)); 4.30 (*m*, H–C(8), H–C(4), H–C(2)); 3.90 – 3.95 (*m*, H–C(8), H–C(4)); 3.85 (*s*, MeO–C(5′)); 3.80 (*s*, MeO–C(5″), MeO–C(3″)); 3.15 (*m*, H–C(1), H–C(5)); 2.95 (*q*, H–C(7′)); 1.36 (*t*, H–C(8′)). $^{13}$C-NMR ($CDCl_3$): *Table*. EI-MS: 416 (100, $M^{+\bullet}$), 386 (30, $[M-OCH_2]^+$), 388 (21, $[M-C_2H_4]^+$).

Table. *$^{13}$C-NMR* ($CDCl_3$; DEPT) *of* **2** *and* **3**

| | **2** | **3** |
|---|---|---|
| CH(1) | 54.12 | 54.25 |
| CH(2) | 85.83 | 85.85 |
| $CH_2$(4) | 71.57 | 71.40 |
| CH(5) | 54.31 | 54.25 |
| CH(6) | 86.04 | 85.85 |
| $CH_2$(8) | 71.63 | 71.40 |
| C(1′)(C(1″)) | 132.87 (132.04) | 132.24 (134.24) |
| CH(2′)(CH(2″)) | 118.92 (102.67) | 119.0 (119.0) |
| C(3′)(C(3″)) | 136.5 (147.13) | 136.8 (136.8) |
| C(4′)(C(4″)) | 145.20 (134.27) | 145.0 (145.0) |
| C(5′)(C(5″)) | 146.67 (147.13) | 146.8 (146.8) |
| CH(6′)(CH(6″)) | 108.57 (102.67) | 109.20 (109.20) |
| $CH_2$(7′)($CH_2$(7″)) | 38.5 (–) | 38.2 (38.2) |
| Me(8′)(Me(8″)) | 18.4 (–) | 18.6 (18.6) |

*(1*R,*2*S,*5*R,*6*S*)-2,6-Bis(3-ethyl-4-hydroxy-5-methoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane* (=*4,4′-[(1*S,*3a*R,*4*S,*6a*R*)-Tetrahydro-1*H,*3*H-*furo[3,4-*c*]furan-1,4-diyl]bis[2-ethyl-6-methoxyphenol]*; **3**): Amorphous powder. M.p. 151–153°. $[\alpha]_D^{20} = +30.5$ ($c = 0.1$, $CHCl_3$). UV ($CH_2Cl_2$): 225 (198), 272 (188). IR (KBr): 3415, 1625, 1526. $^1$H-NMR ($CDCl_3$): 6.84 (*d*, $J = 2.5$, H–C(2′), H–C(2″)); 6.80 (*d*, $J = 2.5$, H–C(6′), H–C(6″)); 4.78 (*d*, $J = 6.8$, H–C(2), H–C(6)); 4.25 (*m*, H–C(4), H–C(8)); 3.92–3.95 (*m*, H–C(4), H–C(8)); 3.82 (*s*, MeO–C(5′), MeO–C(5″)); 3.10 (*m*, H–C(1), H–C(5)); 2.98 (*q*, H–C(7′), H–C(7″)); 1.30 (*t*, H–C(8′), H–C(8″)). $^{13}$C-NMR ($CDCl_3$): *Table*. EI-MS: 414 (100, $M^{+\bullet}$), 386 (37, $[M - C_2H_4]^+$), 384 (29, $[M - OCH_2]^+$).

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