Dapholidins A – C, New Isomeric Biisoflavonoids From *Daphne oleoides*

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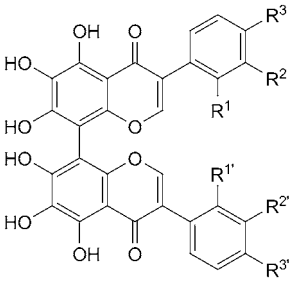
Three new isomeric biisoflavonoids, dapholidins A – C (**1** – **3**, resp.), have been isolated from the AcOEt-soluble fraction of the MeOH-soluble extract of the roots of *Daphne oleoides*, along with the known compounds daphwazirin (**4**), daphnetin 8-*O*-*β*-D-glucopyranoside (**5**), daphnin (**6**), daphneticin 4″-*O*-*β*-D-glucopyranoside (**7**), and 6,7-dihydroxy-3-methoxy-8-[2-oxo-2*H*-1-benzopyran-7-(*O*-*β*-D-glucopyranosyl)-8-yl]-2*H*-1-benzopyran-2-one (**8**). The structures of the new compounds were determined by spectroscopic analyses, including 1D- and 2D-NMR.

Introduction. – The genus *Daphne* (Thymelaeaceae) consists of *ca.* 70 species occurring in Europe, Mediterranean region, temperate and subtropical Asia, Indo-Malayan region, Philippine Islands, North Africa, Australia, and Pacific. The genus is represented in Pakistan by three species [1][2]. Various plants belonging to this genus are used in traditional medicine for the treatment of rheumatism, ulcers, toothache, and as abortifacient and purgative [3]. Antileukemic diterpenoid mezerein has been isolated from *D. mezereum* [4][5]. The roots of *D. odora* are used to treat bites by venomous snakes, while flowers have been used to treat sore throat and neuralgic pain [6]. *D. oleoides* grows in northern areas of Pakistan. Literature survey revealed that the previous phytochemical studies carried out over the years by different workers on this species have resulted in the isolation of different classes of compounds including coumarins [7], lignans [8], flavonoids [9], diterpenes [10], and steroids [5]. The chemotaxanomic and ethnopharmacological importance of the genus *Daphne* prompted us to carry out further studies on *D. oleoides*. Chromatographic studies on the AcOEt-soluble fraction have resulted in the isolation of three new isomeric biisoflavonoides named as dapholidins A – C (**1** – **3**, resp.; *Fig. 1*), in addition to the known compounds daphwazirin (**4**), daphnetin 8-*O*-*β*-D-glucopyranoside (**5**), daphnin (**6**), daphneticin 4″-*O*-*β*-D-glucopyranoside (**7**), and 6,7-dihydroxy-3-methoxy-8-[2-oxo-2*H*-1-benzopyran-7-(*O*-*β*-D-glucopyranosyl)-8-yl]-2*H*-1-benzopyran-2-one (**8**).

Results and Discussion. – The MeOH-soluble extract of the roots of *D. oleoides* was processed as described in the *Exper. Part* to afford three new dimeric isoflavonoids named as dapholidins A, B, and C (**1**, **2**, and **3**, resp.) along with five known compounds **4** – **8**. Compounds **1** – **3** gave violet coloration with $FeCl_3$ for a phenol.



	R^1	R^2	R^3
1	H	H	OH
2	H	OH	H
3	OH	H	H

Fig. 1. *Structures of dapholidins A – C* (**1** – **3**, resp.)

Dapholidin A (**1**) was obtained as yellow amorphous powder. The UV spectrum showed characteristic absorptions at 264 and 336 nm for an isoflavone [11][12]. On addition of $AlCl_3$ and $AlCl_3$/HCl, it showed a bathochromic shift of 28 nm in band II, revealing the presence of chelated OH group at C(3) or C(5). The IR spectrum revealed the presence of OH (3500 cm^{-1}) and α,β-unsaturated CO (1677 cm^{-1}) groups, and aromatic moiety (1600, 1535, 1510 cm^{-1}). The positive-ion mode fast atom bombardment mass spectrum (FAB-MS) of **1** exhibited the *quasi*-molecular-ion peak at *m*/*z* 571, while negative-ion mode FAB-MS showed a *quasi*-molecular-ion peak at *m*/*z* 569. The molecular formula was established as $C_{30}H_{18}O_{12}$ on the basis of elemental analysis and HR-EI-MS (*m*/*z* 570.0792 (M^+; calc. 570.0798)). The ^{13}C-NMR spectra (broad-band (BB) and distortionless enhancement by polarization transfer (DEPT)) showed 15 signals attributed to five CH groups and ten quaternary C-atoms (*Table 1*). The signals at δ(C) 150.8, 125.1, 179.8, 166.5, and 103.2 were characteristic signals of C(2), C(3), C(4), C(9), and C(10) of an isoflavone. Four other downfield signals were due to the O-bearing aromatic C atoms.

Table 1. *^{13}C-NMR Data of* **1** – **3**. At 100 MHz, in (D_6)DMSO; δ in ppm.

C-Atom	**1**	**2**	**3**	C-Atom	**1**	**2**	**3**
C(2)	150.8	151.2	151.6	C(10)	103.2	102.6	103.5
C(3)	125.1	129.1	121.9	C(1′)	123.1	135.2	125.4
C(4)	179.8	178.6	179.2	C(2′)	130.7	119.1	156.2
C(5)	153.2	152.8	153.1	C(3′)	118.5	156.3	117.2
C(6)	122.7	122.6	122.5	C(4′)	160.5	120.3	134.4
C(7)	155.1	153.8	155.2	C(5′)	118.5	126.8	125.8
C(8)	98.6	99.1	99.5	C(6′)	130.7	121.3	131.5
C(9)	166.5	166.5	166.5				

In the ^{1}H-NMR spectrum of **1** (*Table 2*), the most downfield signal at δ(H) 12.03 could be assigned to chelated OH group. A further downfield *singlet* at δ(H) 8.67 showed a HMQC with the C-atom signal at δ(C) 150.8, as well as ^3J correlation with

C(4) in an HMBC experiment, allowing us to assign it to H–C(2) of the isoflavonoidal skeleton [13]. The H-atoms of *para* substituted ring *B* led to an *AA′BB′* system exhibiting two *doublet*s at δ(H) 7.96 (*d*, $J = 8.4$, 2 H) and 7.65 (*d*, $J = 8.4$, 2 H). The *retro-Diels–Alder* (RDA) fragments at *m*/*z* 166 and 118 confirmed the presence of one OH group in ring *B* and three OH groups in ring *A*, one of these already been assigned to C(5).

Table 2. *^{1}H-NMR Data of* **1**–**3**. At 400 MHz, in (D_6)DMSO; δ in ppm; J in Hz.

H-Atom	**1**	**2**	**3**
H–C(2)	8.67 (*s*)	8.60 (*s*)	8.60 (*s*)
H–C(2′)	7.96 (*d*, $J = 8.4$)	7.10 (*dd*, $J = 2.7, 2.1$)	–
H–C(3′)	7.65 (*d*, $J = 8.4$)	–	7.32 (*dd*, $J = 8.2, 2.1$)
H–C(4′)	–	7.56 (*dd*, $J = 7.5, 2.1$)	7.80 (*dd*, $J = 8.2, 2.1$)
H–C(5′)	7.65 (*d*, $J = 8.4$)	7.84 (*dd*, $J = 7.9, 7.5$)	7.61 (*dd*, $J = 8.2, 2.1$)
H–C(6′)	7.96 (*d*, $J = 8.4$)	7.80 (*dd*, $J = 7.5, 2.1$)	7.15 (*dd*, $J = 8.2, 2.1$)
HO–C(5)	12.03 (*s*)	12.01 (*s*)	12.10 (*s*)

The NMR data, ^{1}H,^{1}H-COSY correlations, and HMBCs (*Fig. 2*) showed close resemblance to those of 6-hydroxygenistein [14]. In so far, however, since there are twice as many C-atoms, compound **1** is a symmetrical biisoflavonoid derived from 6-hydroxygenistein. The absence of H-atom at C(8) indicated that compound **1** is a 8–8″ linked biisoflavonoid. Comparison of ^{13}C-NMR data of 6-hydroxygenistein with compound **1** showed a downfield shift of C(8) by 5 ppm, confirming the assigned structure of dapholidin A (**1**; *Fig. 1*).

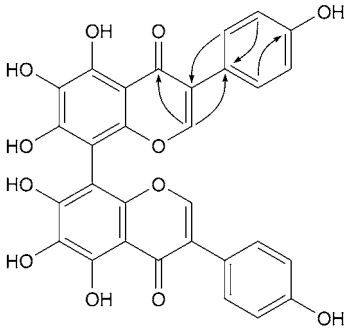


Fig. 2. *HMBCs of compound* **1**

Dapholidin B (**2**) was also obtained as yellow amorphous powder. Its UV and IR spectra were very similar to those of **1**. The FAB-MS in positive- and negative-ion modes showed *quasi*-molecular-ion peaks at *m*/*z* 571 and 569, respectively. The molecular formula was established as $C_{30}H_{18}O_{12}$ on the basis of elemental analysis and HR-EI-MS (M^+ at *m*/*z* 570.0790 (calc. 570.0798)). The ^{13}C-NMR (BB and DEPT) spectra exhibited 15 signals attributed to five CH groups and ten quaternary C-atoms (*Table 1*). They showed a close similarity to those of **1** except minor differences in the chemical shifts of C-atoms of the ring *B*. The ^{1}H-NMR spectrum of **2** (*Table 2*) was also very similar to that of **1** except the notable differences in the chemical shifts and

coupling pattern of the H-atoms of the ring *B*. These were characteristic of *meta*-substituted ring *B* with signals appearing at δ(H) 7.84 (*dd*, *J* = 7.9, 7.5, 1 H), 7.80 (*dd*, *J* = 7.5, 2.1, 1 H), 7.56 (*dd*, *J* = 7.5, 2.1, 1 H), and 7.10 (*dd*, *J* = 2.7, 2.1, 1 H). ^{1}H,^{1}H-COSY Correlations and HMBCs (*Fig. 3*) were in conformity to *meta*-hydroxylated ring *B*. The NMR spectral data closely resembled those of 3′,5,6,7-tetrahydroxyisoflavone, indicating that the compound **2** is a regioisomer of compound **1**. It could also be confirmed by downfield shift of C(8) by 6 ppm compared to 3′,5,6,7-tetrahydroxyisoflavone. Thus, dapholidin B (**2**) is a 8–8′ linked biisoflavone derived from 3′,5,6,7-tetrahydroxyisoflavone (*Fig. 1*).

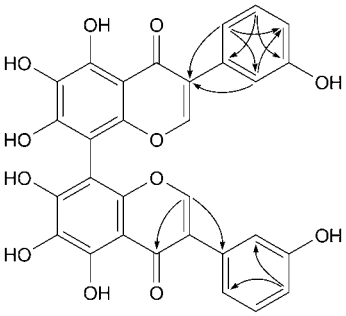


Fig. 3. *HMBCs of compound* **2**

Dapholidin C (**3**) was also obtained as yellow amorphous powder. The UV and IR spectra were similar to those of compound **1**. The FAB-MS in positive- and negative-ion modes showed *quasi*-molecular-ion peaks at *m*/*z* 571 and 569, respectively. Its molecular formula was established as $C_{30}H_{18}O_{12}$ on the basis of elemental analysis and HR-EI-MS (M^+ at *m*/*z* 570.0795 (calc. 570.0798)). The ^{13}C-NMR (BB and DEPT) spectra (*Table 1*) were closely similar to those of compound **1** except minor differences in the chemical shifts of C-atoms of ring *B*. The ^{1}H-NMR spectrum (*Table 2*) also showed a close resemblance to that of **1** with notable differences in the chemical shifts and coupling pattern of the H-atoms of ring *B*. The signals were characteristic of *ortho*-substituted aromatic ring *B* with signals at δ(H) 7.80 (*dd*, *J* = 8.2, 2.1, 1 H), 7.61 (*dd*, *J* = 8.2, 2.1, 1 H), 7.32 (*dd*, *J* = 8.2, 2.1, 1 H), and 7.15 (*dd*, *J* = 8.2, 2.1, 1 H). ^{1}H,^{1}H-COSY Correlations and HMBCs (*Fig. 4*) were in conformity to *ortho*-hydroxylated ring *B*. Thus, compound **3** is another regioisomer of compound **1** (*Fig. 1*).

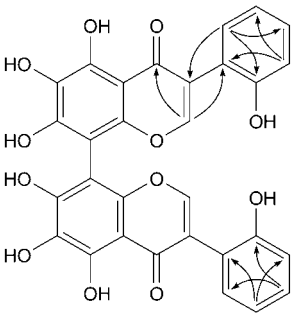


Fig. 4. *HMBCs of compound* **3**

Compounds **4**–**8** were identified by comparison of physical and spectral data with those reported in the literature [15–19] (*Fig. 5*).

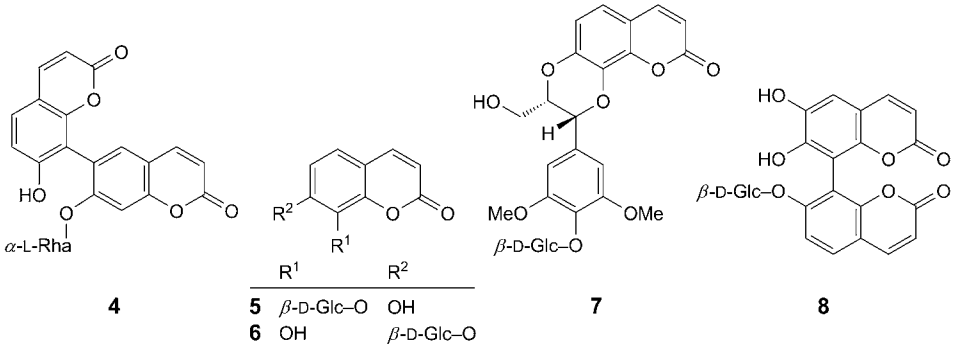


Fig. 5. *Structures of compounds* **4**–**8**

Experimental Part

General. Column and flash chromatograpy (CC and FC, resp.): silica gel (SiO_2; 230–400 mesh; *E. Merck*). TLC: Precoated silica gel F_{254} plates; detection at 254 nm and by spraying with ceric sulfate reagent. HPLC: *JAIGEL*, *ODS-M 80* column; M.p.: *Gallenkamp* apparatus; uncorrected. UV Spectra: *Hitachi-UV-3200* instrument. IR Spectra: *Jasco-302-A* spectrophotometer, in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker AMX 400* spectrometers at 400 and 100 MHz, resp.; δ in ppm, J in Hz. 2D-NMR Spectra: *Bruker AMX-500* spectrometer. FAB-MS: *Jeol-JMS HX-110* mass spectrometer. EI-MS and HR-EIMS: *Jeol JMS-DA-500* mass spectrometer, in *m*/*z* (rel.%). Elemental analyses: *Carlo Elba MOD-1106* elemental analyzer.

Plant Material. The roots of *Daphne oleoides* (15 kg) were collected from Peshawar (Pakistan) in May 2010 and identified by Prof. *Manzoor Husain*, Plant Taxonomist, Govt. Postgraduate College-1, Abbotabad, KPK, Pakistan, where a voucher specimen (No. GPCA-34) has been deposited.

Extraction and Isolation. The roots of *Daphne oleoides* were shade-dried, ground, and extracted in MeOH (3 × 50 l, 10 d each) at r.t. The combined MeOH extract was evaporated under reduced pressure to yield a gummy residue (800 g), which was suspended in H_2O and fractionated into hexane-, AcOEt-, and BuOH-soluble fractions. The AcOEt-soluble fraction (60 g) was subjected to CC (SiO_2; hexane, hexane/$CHCl_3$, $CHCl_3$, and $CHCl_3$/MeOH gradient systems). The fraction obtained from $CHCl_3$/MeOH 9.5 : 0.5 was subjected to recycling reversed-phase (RP) HPLC (*ODS-M 80*; H_2O/MeOH 1 : 1; flow rate: 2 ml/min) resulting in the isolation of *dapholidin A* (**1**; t_R 24 min, 14 mg), *dapholidin B* (**2**; t_R 32 min, 12 mg), and dapholidin C (**3**; t_R 40 min, 10 mg), resp. The fractions eluted with $CHCl_3$/MeOH 7 : 3 were combined and subjected to FC (AcOEt/MeOH 9 : 1) to furnish compounds (**5**; 25 mg), (**6**; 20 mg), and (**7**; 15 mg). The elution with $CHCl_3$/MeOH 8.5 : 1.5 resulted in the isolation of compounds **4** (30 mg) and **8** (25 mg).

Dapholidin A (*=5,5′,6,6′,7,7′-Hexahydroxy-3,3′-bis(4-hydroxyphenyl)-4*H,*4′*H*-8,8′-bichromene-4,4′-dione*; **1**). Yellow amorphous powder. M.p. 278–280°. UV: (MeOH): 264, 336 (sh). IR (KBr): 3500, 1677, 1600, 1535, 1510. ^1H- and ^{13}C- NMR: see *Tables 2* and *1*, resp. FAB-MS (pos.): 571 ($[M+H]^+$). FAB-MS (neg.): 569 ($[M-H]^-$). EI-MS: 570.4 (3, M^+), 285 (39), 166 (72), 118 (70). HR-EI-MS: 570.0792 (M^+, $C_{30}H_{18}O_{12}^+$; calc. 570.0798). Anal. calc. for $C_{30}H_{18}O_{12}$ (570.46): C 63.16, H 3.18; found C 63.25, H 3.15.

Dapholidin B (*=5,5′,6,6′,7,7′-Hexahydroxy-3,3′-bis(3-hydroxyphenyl)-4*H,*4′*H*-8,8′-bichromene-4,4′-dione*; **2**). Yellow amorphous powder. M.p. 297–299°. UV: (MeOH): 262, 332 (sh). IR (KBr): 3550, 1670, 1610, 1530, 1500. ^1H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. FAB-MS (pos.): 571 ($[M+H]^+$). FAB-MS (neg.): 569 ($[M-H]^-$). EI-MS: 570.4 (5, M^+), 285 (45), 166 (70), 118 (77). HR-EI-MS:

570.0790 (M^+, $C_{30}H_{18}O_{12}^+$; calc. 570.0798). Anal. calc. for $C_{30}H_{18}O_{12}$ (570.46): C 63.16, H 3.18; found C 63.20, H 3.10.

Dapholidin C (*=5,5′,6,6′,7,7′-Hexahydroxy-3,3′-bis(2-hydroxyphenyl)-4*H,*4′*H-*8,8′-bichromene-4,4′-dione*; **3**). Yellow amorphous powder. M.p. 310–312°. UV: (MeOH): 266, 333 (sh). IR (KBr): 3545, 1675, 1605, 1537, 1510. ^{1}H- and ^{13}C-NMR: see *Tables 2* and *1*, resp. FAB-MS (pos.): 571 ($[M+H]^+$). FAB-MS (neg.): 569 ($[M-H]^-$). EI-MS: 570.4 (4, M^+), 285 (40), 166 (79), 118 (72). HR-EI-MS: 570.0795 (M^+, $C_{30}H_{18}O_{12}^+$; calc. 570.0798). Anal. calc. for $C_{30}H_{18}O_{12}$ (570.46): C 63.16, H 3.18; found C 63.15, H 3.35.

REFERENCES

[1] E. Nasir, S. I. Ali, 'Flora of West Pakistan', Ferozsons, Karachi, Pakistan, Vol. 1–10, 1971, p. 12.
[2] L.-G. Zhuang, O. Seligmann, H. Wagner, *Phytochemistry* **1983**, *22*, 617.
[3] A. Ronlán, B. Wickberk, *Tetrahedron Lett.* **1970**, *11*, 4261.
[4] S. M. Kupchan, R. L. Baxter, *Science* **1975**, *187*, 652.
[5] B. Kreher, H. Wagner, A. Neszmélyi, *Phytochemistry* **1990**, *29*, 3633.
[6] G. Watt, 'Dictionary of the Economic Product of India', Cosmo Publication, Delhi-6, India, Vol. 3, 1972, p. 29.
[7] P. C. Maiti, *Curr. Sci.* **1957**, 99.
[8] M. Riaz, N. Ullah, A. Mehmood, H. R. Nawaz, A. Malik, N. Afza, *Z. Naturforsch. B* **2000**, *55*, 1216.
[9] M. Taniguchi, K. Baba, *Phytochemistry* **1996**, *42*, 1447.
[10] N. Ullah, S. Ahmad, A. Malik, *Chem. Pharm. Bull.* **1999**, *47*, 114.
[11] K. R. Markham, 'Techniques of Flavonoids Identification', New York, Academic press, 1982.
[12] E. H. Anouar, J. Gierschner, J.-L. Duroux, P. Trouillas, *Food Chem.* **2012**, *131*, 79.
[13] S.-M. Wong, C. Konno, Y. Oshima, J. M. Pezzuto, H. H. S. Fong, N. R. Farnsworth, *J. Nat. Prod.* **1987**, *50*, 178.
[14] K. R. Markham, T. J. Mabry, T. W. Swift III, *Phytochemistry* **1968**, *7*, 803.
[15] M. Riaz, A. Malik, S. K. Sadhozai, M. Hussain, N. Ullah, *Nat. Prod. Lett.* **2001**, *15*, 433.
[16] N. Ullah, S. Ahmed, P. Mohammad, H. Rabnawaz, A. Malik, *Fitoterapia* **1999**, *70*, 214.
[17] H. R. D. Murray, J. Medez, A. S. Brown, 'The Natural Coumarins; Occurance; Chemistry and Biochemistry', John Wiley & Sons, New York, 1982, p. 27.
[18] N. Ullah, S. Ahmed, P. Muhammad, Z. Ahmed, H. Rab Nawaz, A. Malik, *Phytochemistry* **1999**, *51*, 103.
[19] N. Ullah, S. Ahmed, A. Malik, *Phytochemistry* **1999**, *51*, 99.

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