Biflavonoids, Lignans, and Related Compounds from the Roots of *Diplomorpha canescens*

by **Hari Prasad Devkota***[a][b], **Khem Raj Joshi**[a][b], **Takashi Watanabe**[c], and **Shoji Yahara***[b]

[a]) Program for Leading Graduate Schools, Health Life Sciences: Interdisciplinary and Glocal Oriented (HIGO) Program, Kumamoto University, 5-1 Oe-honmachi, Chuo-ku, Kumamoto 862-0973, Japan
[b]) Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Chuo-ku, Kumamoto 862-0973, Japan (phone/fax: +81-96-3714381; e-mail: devkotah@kumamoto-u.ac.jp (*H. P. Devkota*), yaharas1@gpo.kumamoto-u.ac.jp (*S. Yahara*))
[c]) Research Organization for Regional Alliances, Kochi University of Technology, 185 Miyanokuchi, Tosayamada, Kami City, Kochi 782-0003, Japan

Two new biflavonoids, 14″-*O*-methyldihydrodaphnodorin B (**1**) and 14″-*O*-methyldaphnodorin J (**2**), along with 16 known compounds, *i.e.*, dihydrodaphnodorin B (**3**), daphnodorin J (**4**), 3″-epidihydrodaphnodorin B (**5**), daphnodorin B (**6**), neochamaejasmin B (**7**), sikokianin B (**8**), (−)-syringaresinol (**9**), (−)-syringaresinol 4-*O*-*β*-D-glucopyranoside (**10**), (+)-nortrachelogenin (**11**), (−)-lariciresinol (**12**), (−)-pinoresinol (**13**), syringin (**14**), syringinoside (**15**), daphnoretin (**16**), phorbol 13-acetate (**17**), and methyl paraben (**18**) were isolated from the roots of *Diplomorpha canescens* (MEISN.) C.A. MEYER. The structures were determined on the basis of spectroscopic data.

Introduction. – *Diplomorpha canescens* (MEISN.) C.A. MEYER (Synonym: *Wikstroemia canescens* MEISN.), belonging to Thymelaeaceae family, is widely distributed throughout Nepal, Afghanistan, northern India, Sri Lanka, and China [1]. In Nepal, it is locally known as '*Phurkepaat*', and the stems are used against toothache in Nepal [2]. Roots are called as '*Sanhijyou*' in traditional Chinese medicine and used for the treatment of several disorders [3] and in antitumor therapy [4]. Previous phytochemical studies have revealed two tigliane-type diterpene esters, wikstroemia factors C_1 and C_2, from the root of this plant [4]. We have previously reported six new and 26 known compounds from the aerial parts of *D. canescens* [5]. In this article, we report the isolation and structure elucidation of two new biflavonoids, 14″-*O*-methyldihydrodaphnodorin B (**1**) and 14″-*O*-methyldaphnodorin J (**2**; *Fig. 1*), along with 16 known compounds from the roots of *D. canescens.*

Results and Discussion. – The dried roots of *D. canescens* (500 g) were extracted twice with 70% MeOH (4.5 l), and extracts were evaporated under reduced pressure to give 70% MeOH extract (104 g). The extract was then separated into the H_2O soluble (45 g) and H_2O-insoluble parts (59 g). The H_2O-insoluble part was subjected to repeated column chromatography on *MCI* gel *CHP20P*, *Sephadex LH-20*, octadecyl silica (*ODS*), and silica gel to afford two new biflavonoids, **1** and **2**, along with 16 known compounds. From the detailed spectral analysis and comparison with literature data, the known compounds were identified as six biflavonoids, *i.e.*, dihydrodaphnodorin B

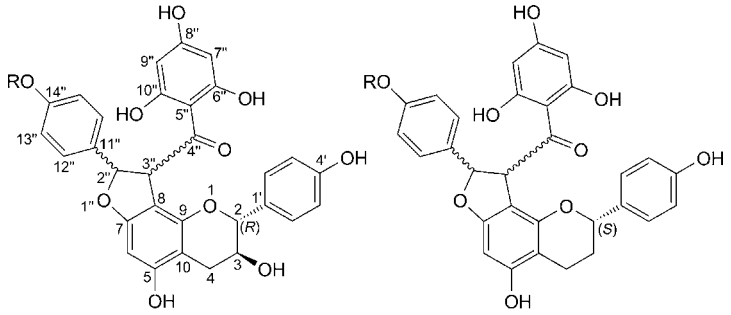


Fig. 1. *Structures of compounds* **1**–**4**

(**3**) [6][7], daphnodorin J (**4**) [6][7], 3″-epidihydrodaphnodorin B (**5**) [8], daphnodorin B (**6**) [9–11], neochamaejasmin B (**7**) [12][13], sikokianin B (**8**) [12]; five lignans, *i.e.*, (−)-syringaresinol (**9**) [14], (−)-syringaresinol 4-*O*-*β*-D-glucopyranoside (**10**) [15], (+)-nortrachelogenin (**11**) [16], (−)-lariciresinol (**12**) [17], (−)-pinoresinol (**13**) [18]; two phenylpropanoids, *i.e.*, syringin (**14**) [19], syringinoside (**15**) [19][20]; a coumarin, *i.e.*, daphnoretin (**16**) [21]; a phorbol derivative, *i.e.*, phorbol 13-acetate (**17**) [22]; and methyl paraben (**18**) [23]. All of these compounds were isolated for the first time from *D. canescens* except **12** and **13** which were previously isolated from the aerial parts [5b].

Compound **1** was obtained as pale-yellow amorphous powder. The HR-FAB-MS of **1** showed a *quasi*-molecular ion ($[M-H]^-$) at *m*/*z* 557.1475, suggesting the molecular formula $C_{31}H_{26}O_{10}$. The ^{1}H-NMR spectrum of **1** (*Table*) showed signals due to two pairs of *p*-substituted phenyl groups (7.19, 6.68 (2*d*, *J* = 8.5, each 2 H) and 7.09, 6.67 (2*d*, *J* = 8.5, each 2 H)); a 2,4,6-trihydroxyphenyl group (5.65 (*s*, 2 H)); a pair of coupled H-atoms (6.07, 5.96 (2*d*, *J* = 10.3, each 1 H)); a set of H-atoms attributed to those of the *C*-ring of flavan-3-ol moiety (4.71 (*d*, *J* = 7.3, 1 H), 3.92 (br. *d*, *J* = 7.3, 12.1, 1 H), 2.83 (*dd*, *J* = 4.8, 16.1, 1 H), and 2.57 (*dd*, *J* = 7.3, 16.1, 1 H)); an aromatic *singlet* (*δ*(H) 6.04)), and a MeO signal (3.67 (*s*, 3 H)). All these ^{1}H- and ^{13}C-NMR data were similar to those of dihydrodaphnodorin B (**3**) [6] except those of the MeO group, suggesting that **1** was a methyl ether derivative of **3**. The location of the Me group at 14″-*O*-position was confirmed on the basis of 2D-NMR data including ^{1}H,^{1}H-COSY, HMBC, and HMQC. In the HMBC spectra, the signal of the MeO group at *δ*(H) 3.67 showed a correlation with that of the C-atom at *δ*(C) 160.6 (C(14″)), which also correlated to signals at *δ*(H) 7.19 (H–C(12″), H–C(16″)) and 6.68 (H–C(13″), H–C(15″)). Similarly, the signal at *δ*(H) 7.19 (H–C(12″), H–C(16″)) also correlated with the signal at *δ*(C) 88.8 (C(2″)). Key HMBCs are depicted in *Fig. 2*. The CD data of compound **1** (see *Exper. Part*) were similar to those of dihydrodaphnodorin B (**3**), suggesting the (*R*)-configuration at C(2) [6]. The *trans* configuration of H–C(2) and H–C(3) was concluded on the basis of large coupling constant (7.3 Hz) between these two H-atoms. The relative configuration between C(2″) and C(3″) was determined as *cis* on the basis of the coupling constant (10.3 Hz) [6] in ^{1}H-NMR spectra of **1**, but the absolute configuration is yet to be

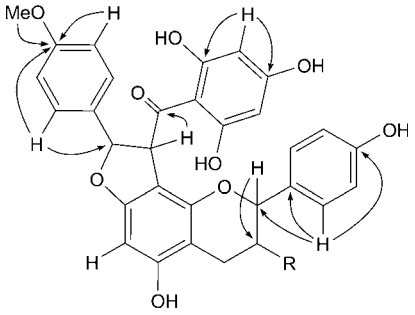
Table. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** *and* **2** *in CD_3OD.* δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
2	4.71 (*d*, $J = 7.3$)	82.2	4.87 (br. *d*, $J = 8.5$)	78.4
3	3.92 (br. *dd*, $J = 7.3, 12.1$)	69.1	2.07–2.13 (*m*), 1.72–1.85 (*m*)	31.7
4	2.83 (*dd*, $J = 4.8, 16.1$), 2.57 (*dd*, $J = 7.3, 16.1$)	28.2	2.67–2.74 (*m*), 2.52–2.65 (*m*)	20.4
5		166.0[a])		166.0[a])
6	6.04 (*s*)	90.2	6.02 (*s*)	89.9
7		166.1[a])		166.1[a])
8		106.5		106.5
9		161.8		161.5
10		101.3		103.3
1′		131.6		134.6
2′, 6′	7.09 (*d*, $J = 8.5$)	128.8	7.02 (*d*, $J = 8.5$)	127.5
3′, 5′	6.67 (*d*, $J = 8.5$)	115.8	6.63 (*d*, $J = 8.5$)	115.8
4′		152.2		153.2
2″	6.07 (*d*, $J = 10.3$)	88.8	6.08 (*d*, $J = 10.3$)	88.7
3″	5.96 (*d*, $J = 10.3$)	57.2	5.95 (*d*, $J = 10.3$)	57.2
4″		203.1		203.3
5″		105.6		106.1
6″,10″		157.6[b])		157.7[b])
7″,9″	5.65 (*s*)	95.6	5.69 (*s*)	95.6
8″		157.7[b])		157.8[b])
11″		131.3		131.5
12″,16″	7.19 (*d*, $J = 8.5$)	129.5	7.19 (*d*, $J = 8.5$)	129.5
13″,15″	6.68 (*d*, $J = 8.5$)	113.9	6.68 (*d*, $J = 8.5$)	113.9
14″		160.6		160.5
MeO	3.67 (*s*)	55.5	3.67 (*s*)	55.5

[a]), [b]) Assignments with same superscript may be interchanged within same column.

determined. Finally, the structure for **1** was elucidated as 14″-*O*-methyldihydrodaphnodorin B as shown in *Fig. 1*.

Compound **2** was obtained as pale-yellow amorphous powder. The HR-FAB-MS of **2** showed a *quasi*-molecular ion ($[M-H]^-$) peak at m/z 541.1524, providing the molecular formula $C_{31}H_{26}O_9$. The ^{1}H-NMR spectrum of **2** (*Table*) exhibited signals due to two pairs of *p*-substituted phenyl groups (δ(H) 7.19, 6.68 (2*d*, $J = 8.5$, each 2 H) and 7.02, 6.63 (2*d*, $J = 8.5$, each 2 H)); a 2,4,6-trihydroxyphenyl group (δ(H) 5.69 (*s*); a pair of coupled H-atoms (δ(H) 6.08, 5.95 (*d*, $J = 10.3$, each 1 H)); and a set of H-atoms attributed to those of the *C*-ring of flavan moiety (4.87 (br. *d*, $J = 8.7$, 1 H), 2.67–2.74, 2.52–2.65, 2.07–2.13, 1.72–1.85 (4*m*, each 1 H)), and an aromatic *singlet* (6.04) and a MeO signal (3.67 (*s*, 3 H)). All these ^{1}H- and ^{13}C-NMR data except those for Me group were similar to those of dihydrodaphnodorin A or daphnodorin J (**4**), evidencing that **2** was a methyl ether derivative of **4**. Comparing the spectral data of **2** with those of **1**, the presence of a CH_2 groups (δ(C) 31.7) in **2** instead of CH groups (δ(C) 69.1) in **1** also suggested the above statement. The location of the Me group at 14″-*O*-position was confirmed on the basis of 2D-NMR data including ^{1}H,^{1}H-COSY, HMBC and HMQC



1 R = OH
2 R = H

Fig. 2. *Key HMBCs observed in the spectra of* **1** *and* **2**

as for **1**. Key HMBCs are shown in *Fig. 2*. The CD data of **2** (see *Exper. Part*) were also similar to those of daphnodorin J (**4**), suggesting the (*S*)-configuration at C(2) [6]. The relative configuration between C(2″) and C(3″) was deduced as *cis* on the basis of the coupling constant (10.3 Hz) [6] in ^{1}H- NMR spectra of **2**, but the absolute configuration is yet to be determined. Finally, the structure for **2** was elucidated is 14″-*O*-methyldaphnodorin J as shown in *Fig. 1*.

Experimental Part

General. TLC: Precoated silica gel *60 F$_{254}$* (0.2 mm, aluminum sheet, *Merck*). Column chromatography (CC): silica gel 60 (SiO_2; 0.040–0.063 mm; *Merck*), *MCI* gel *CHP20P* (75–150 μm, *Mitsubishi Chemical Industries Co., Ltd.*), *Sephadex LH-20* (*Amersham Pharmacia Biotech*), and *Chromatorex ODS* (30–50 μm, *Fuji Silysia Chemical Co., Ltd.*). Optical rotations: *JASCO DIP-1000KUY* polarimeter. CD Spectra: *JASCO J-810* spectropolarimeter. ^{1}H- and ^{13}C-NMR spectra: *JEOL* α-500 spectrometer; chemical shifts, δ, are in ppm with reference to TMS; coupling constants (*J*) in Hz. MS: *JEOL JMS 700 MStation* mass spectrometer.

Plant Material. Fresh roots of *D. canescens* were collected in January, 2009, from Chisapani Area (2300 m), Nepal, and shade-dried for one month. The specimen was identified by Mr. *Kuber Jung Malla*, Scientific Officer, Department of Plant Resources, Thapathali, Kathmandu, Nepal. A voucher specimen (No. 1KUNP 20090621-02) has been deposited with the Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan.

Extraction and Isolation. The dried roots of *D. canescens* (500 g) were extracted twice with 70% MeOH (4.5 l; 1 week for each time), and the extracts were evaporated under reduced pressure to give the 70% MeOH extract (104 g). The extract was then separated into the H_2O-soluble part (45 g) and the H_2O-insoluble part (59 g). The H_2O-insoluble part was dissolved in 40% MeOH and subjected to CC (*MCI CHP20P*; 40, 60, 80, and 100% MeOH) to give 16 fractions. *Fr. 2* (2.7 g) was submitted to CC (*MCI* gel *CHP20P* (10–20% MeOH), *Sephadex LH-20* (MeOH), and *ODS* (20–40% MeOH)) to give **14** (205 mg) and **15** (284 mg). *Fr. 5* (7.0 g) was subjected to CC (*Sephadex LH-20* (MeOH) to afford seven subfractions, *Subfr. 5-1–5-7. Subfr. 5-2* (1.0 g) was separated by CC (SiO_2; $CHCl_3$/MeOH/H_2O 9 : 1 : 0.1) to furnish compounds **10** (32 mg) and **17** (47 mg). *Subfr. 5-6* (2.7 g) was purified by CC (*ODS*; 30–60% MeOH) to give compounds **1** (116 mg) and **5** (799 mg). *Subfr. 5-7* (802 mg) was subjected to CC (*ODS* 40–70% MeOH) to afford compounds **3** (182 mg), **4** (129 mg), and **6** (93 mg). *Fr. 7* (4.4 g) was subjected to CC (*Sephadex LH-20* (MeOH) and SiO_2 ($CHCl_3$/MeOH/H_2O 9 : 1 : 0.1 and $CHCl_3$/MeOH 20 : 1) to afford **12** (48 mg), **11** (28 mg), and **18** (1 mg). *Fr. 10* (3.1 g) was subjected to CC (*Sephadex LH-*

20; MeOH) to give ten subfractions, *Subfr. 10-1 – 10-10*). *Subfrs. 10-2* (371 mg) and *10 – 4* (129 mg) were subjected to CC (SiO_2; $CHCl_3$/MeOH 20 : 1) to give **9** (243 mg) and **13** (77 mg), resp. *Subfrs. 10-8* and *10-10* furnished **2** (469 mg) and **8** (465 mg), resp. *Frs. 13* (787 mg) and *14* (2.6 g) were subjected to CC (*Sephadex*; MeOH) to afford **16** (179 mg) and **7** (1080 mg), resp.

14″-O-Methyldihydrodaphnodorin B (= *[(2*R,3S*)-3,4,8,9-Tetrahydro-3,5-dihydroxy-2-(4-hydroxyphenyl)-8-(4-methoxyphenyl)-2*H*-furo[2,3-*h*]chromen-9-yl](2,4,6-trihydroxyphenyl)methanone*; **1**). Pale-yellow amorphous powder. $[\alpha]_D^{21} = +25.5$ ($c = 0.84$, MeOH). CD (MeOH, $c = 0.14$): -0.26 (260), $+3.07$ (280), -6.02 (309). ^{1}H- and ^{13}C-NMR (CD_3OD): see the *Table*. HR-FAB-MS: 557.1475 ($[M-H]^-$, $C_{31}H_{25}O_{10}^-$; calc. 557.1448).

14″-O-Methyldaphnodorin J (= *[(2*S*)-3,4,8,9-Tetrahydro-5-hydroxy-2-(4-hydroxyphenyl)-8-(4-methoxyphenyl)-2*H*-furo[2,3-*h*]chromen-9-yl](2,4,6-trihydroxyphenyl)methanone*; **2**). Pale-yellow amorphous powder. $[\alpha]_D^{21} = +37.7$ ($c = 0.71$, MeOH). CD (MeOH, $c = 0.10$): -0.03 (264), $+1.28$ (282), -4.09 (309). ^{1}H- and ^{13}C-NMR (CD_3OD): see the *Table*. HR-FAB-MS: 541.1524 ($[M-H]^-$, $C_{31}H_{25}O_9^-$; calc. 541.1499).

Dihydrodaphnodorin B (= *[(2R,3S)-3,4,8,9-Tetrahydro-3,5-dihydroxy-2,8-bis(4-hydroxyphenyl)-2*H*-furo[2,3-*h*]chromen-9-yl](2,4,6-trihydroxyphenyl)methanone*; **3**). Pale-yellow amorphous powder. $[\alpha]_D^{21} = +10.8$ ($c = 0.50$, MeOH). CD (MeOH, $c = 0.14$): -0.34 (261), $+3.50$ (282), -6.10 (309).

Daphnodorin J (= *[(2*S*)-3,4,8,9-Tetrahydro-5-hydroxy-2,8-bis(4-hydroxyphenyl)-2*H*-furo[2,3-*h*]-chromen-9-yl](2,4,6-trihydroxyphenyl)methanone*; **4**). Pale-yellow amorphous powder. $[\alpha]_D^{21} = +37.3$ ($c = 0.74$, MeOH). CD (MeOH, $c = 0.10$): -0.44 (264), $+2.53$ (281), -6.90 (308).

REFERENCES

[1] N. P. Manandhar, 'Plants and People of Nepal', Timber Press, Inc., Portland, 2002, pp. 411 – 412.
[2] T. Watanabe, K. R. Rajbhandari, K. J. Malla, H. P. Devkota, S. Yahara, 'A Handbook of Medicinal Plants of Nepal Supplement I', Ayurseed Life Environmental Institute, Kanagawa, 2013, pp. 242 – 243.
[3] P. G. Xiao, 'A Pictorial Encyclopedia of Chinese Medical Herbs', Chuokoron-Sha, Inc., Tokyo, 1993, Vol. 8, p. 91.
[4] D. Wu, B. Sorg, W. Adolf, H. J. Opferkuch, E. H. Seip, E. Hecker, *Phytother. Res.* **1993**, *7*, 194.
[5] a) H. P. Devkota, M. Watanabe, T. Watanabe, S. Yahara, *Chem. Pharm. Bull*. **2010**, *58*, 859; b) H. P. Devkota, M. Watanabe, T. Watanabe, S. Yahara, *Chem. Pharm. Bull*. **2012**, *60*, 554; c) H. P. Devkota, M. Watanabe, T. Watanabe, S. Yahara, *Phytochem. Lett*. **2012**, *5*, 284; d) H. P. Devkota, M. Watanabe, T. Watanabe, S. Yahara, *Chem. Pharm. Bull*. **2013**, *61*, 242.
[6] M. Taniguchi, A. Fujiwara, K. Baba, *Phytochemistry* **1997**, *45*, 183.
[7] M. Taniguchi, K. Baba, *Phytochemistry* **1996**, *42*, 1447.
[8] S. Liang, J.-M. Tian, Y. Feng, X.-H. Liu, Z. Xiong, W.-D. Zhang, *Chem. Pharm. Bull*. **2011**, *59*, 653.
[9] K. Baba, K. Takeuchi, F. Hamasaki, M. K. Kozawa, *Chem. Pharm. Bull*. **1985**, *33*, 416.
[10] K. Baba, K. Takeuchi, F. Hamasaki, M. Kozawa, *Chem. Pharm. Bull*. **1986**, *34*, 595.
[11] K. Baba, K. Takeuchi, M. Doi, M. Inoue, M. Kozawa, *Chem. Pharm. Bull*. **1986**, *34*, 1540.
[12] M. Niwa, P.-F. Jiang, Y. Hirata, *Chem. Pharm. Bull*. **1986**, *34*, 3631.
[13] M. Niwa, H. Tatematsu, G.-Q. Liu, Y. Hirata, *Chem. Lett.* **1984**, *13*, 539.
[14] Y. W. Leong, L. J. Harrison, A. D. Powell, *Phytochemistry* **1999**, *50*, 1237.
[15] S. Yahara, T. Nishiyori, A. Kohda, T. Nohora, I. Nishioka, *Chem. Pharm. Bull*. **1991**, *39*, 2024.
[16] S. Yamauchi, T. Sugahara, Y. Nakashima, A. Okada, K. Akiyama, T. Kishida, M. Maruyama, T. Masuda, *Biosci. Biotechnol. Biochem.* **2006**, *70*, 1934; A. Kato, Y. Hashimoto, M. Kidokoro, *J. Nat. Prod.* **1979**, *42*, 159.
[17] L.-H. Xie, T. Akao, K. Hamasaki, T. Deyama, M. Hattori, *Chem. Pharm. Bull*. **2003**, *51*, 508.
[18] T. Konishi, S. Wada, S. Kiyosawa, *Yakugaku Zasshi* **1993**, *113*, 670.
[19] M. Niwa, Y. Iwadare, Y.-C. Wu, Y. Hirata, *Chem. Pharm. Bull*. **1988**, *36*, 1158.
[20] N. Ullah, S. Ahmed, A. Malik, *Chem. Pharm. Bull*. **1999**, *47*, 1237.

[21] G. A. Cordell, *J. Nat. Prod.* **1984**, *47*, 84.
[22] G. T. Marshall, A. D. Kinghorn, *J. Am. Oil Chem. Soc.* **1984**, *61*, 1220.
[23] H. A. Almahy, H. E. Khalid, *Int. J. Trop. Med.* **2006**, *1*, 58.

Received September 19, 2014