Flavonoids from the Aerial Parts of *Diplomorpha canescens*

Hari Prasad Devkota,[a] Masato Watanabe,[a] Takashi Watanabe,[b] and Shoji Yahara*,[a]

[a] *Graduate School of Pharmaceutical Sciences, Kumamoto University; 5–1 Oe-honmachi, Kumamoto 862–0973, Japan: and* [b] *The Kochi Prefectural Makino Botanical Garden; 4200–6 Godaisan, Kochi 781–8125, Japan.*

Received February 14, 2010; accepted March 12, 2010; published online March 15, 2010

Two new *C*-methyl flavonoids, (2*R*,3*S*)-6,8-di-*C*-methyldihydrokaempferol (1) and (2*R*,3*R*)-6,8-di-*C*-methyldihydrokaempferol (2) were isolated from the aerial parts of *Diplomorpha canescens* (Meisn.) C. A. Meyer along with eleven known flavonoids such as genkwanin (3), rhamnocitrin 3-*O*-*β*-D-glucopyranoside (4), genkwanin 5-*O*-*β*-D-glucopyranoside (5), apigenin (6), kaempferol (7), kaempferol 3-*O*-*β*-D-glucopyranoside (8), rhamnetin 3-*O*-*β*-D-glucopyranoside (9), luteolin (10), quercetin (11), quercetin 3-*O*-*β*-D-glucopyranoside (12) and genkwanin 5-*O*-primeveroside (13). Structures of these compounds were elucidated on the basis of spectroscopic data.

Key words *Diplomorpha canescens*; Thymelaeaceae; flavonoid; (2*R*,3*S*)-6,8-di-*C*-methyldihydrokaempferol; (2*R*,3*R*)-6,8-di-*C*-methyldihydrokaempferol

Diplomorpha canescens (Meisn.) C. A. Meyer (Family: Thymelaeaceae) (Synonym: *Wikstroemia canescens* Meisn.) is widely distributed throughout Nepal, Afghanistan, northern India, Sri Lanka and China. Traditionally in Nepal, fiber from the bark of stem is used to prepare handmade Nepalese paper.[1)] This plant is said to be used in antitumour therapy in China.[2)] Previous phytochemical studies have reported tigliane type diterpene esters from the root of this plant.[2)] The present report deals with the isolation and structure elucidation of two new *C*-methyl flavonoids, along with eleven known flavonoids from the aerial parts of *Diplomorpha canescens*.

The fresh aerial parts of *D. canescens* were collected from Daman, Nepal and shade dried for one month. Then the shade dried aerial parts were extracted with 70% MeOH. The 70% MeOH extract was then subjected to repeated column chromatography on MCI gel CHP20P, Sephadex LH20, octadecyl silica (ODS) and silica gel to afford two new flavonoids, (2*R*,3*S*)-6,8-di-*C*-methyldihydrokaempferol (**1**), (2*R*,3*R*)-6,8-di-*C*-methyldihydrokaempferol (**2**) along with genkwanin (**3**),[3)] rhamnocitrin 3-*O*-*β*-D-glucopyranoside (**4**),[4)] genkwanin 5-*O*-*β*-D-glucopyranoside (**5**),[5,6)] apigenin (**6**),[4)] kaempferol (**7**),[4)] kaempferol 3-*O*-*β*-D-glucopyranoside (**8**),[4)] rhamnetin 3-*O*-*β*-D-glucopyranoside (**9**),[4)] luteolin (**10**),[7)] quercetin (**11**)[4)] quercetin 3-*O*-*β*-D-glucopyranoside (**12**)[8)] and genkwanin 5-*O*-primeveroside (**13**)[6)] (Fig. 1). Although Chen *et al.*[9)] have reported the chemical structure similar to that of **1** and **2**, there has been no report regarding the isolation, synthesis, physical and spectral data and absolute configuration of these compounds in literature. Hence, we report these compounds **1** and **2** as new natural compounds. All of the known compounds were identified by using physical data and spectroscopic data including melting point, optical rotation, NMR data and with comparison to literature data. All of these compounds were isolated for the first time from this plant.

Compound **1** was obtained as pale yellow amorphous powder, $[\alpha]_D^{20}$ −80.5° (*c*=0.52, MeOH). The HR-FAB-MS of **1** showed the quasi-molecular ion $[M+H]^+$ at *m*/*z*: 317.0997 supporting the formula $C_{17}H_{16}O_6$ (Calcd for $C_{17}H_{17}O_6$, 317.1025). The ^{1}H-NMR spectrum of **1** (Table 1) showed signals due to two aromatic methyl groups at δ_H 2.00 (3H, s) and 2.04 (3H, s), and the proton resonances at δ_H 6.79 (2H, d, *J*=8.2 Hz, $C_{3'}$-H, $C_{5'}$-H) and 7.35 (2H, d, *J*=8.2 Hz, $C_{2'}$-H, $C_{6'}$-H) were characteristic of *p*-substituted phenyl ring. Similarly, two resonances at δ_H 5.32 (1H, d, *J*=2.7 Hz, C_3-H) and 4.20 (1H, d, *J*=2.7 Hz, C_2-H) were characteristics of dihydroflavonol skeleton with *cis* stereochemistry. The ^{13}C-NMR also supported the presence of a dihydroflavonol moiety and two aromatic methyl groups at δ_C 7.4 and 8.1 ppm (Table 1). All of these assignments were made on the basis of heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) correlations. In the HMQC spectrum, proton signals at δ_H 2.00 and 2.04 had correlations with carbons at δ_C 7.4 and 8.1, respectively. In the HMBC spectrum, proton resonances at δ_H 6.79 ($C_{3'}$-H, $C_{5'}$-H) and 7.35 ($C_{2'}$-H, $C_{6'}$-H) had correlation with carbon resonance at δ_C 158.4 (C-4′). Similarly, the proton signal for C_2-H (δ_H 4.20) showed correlations with carbons at δ_C 128.6 (C-1′), 129.6 (C-2′, 6′) and with the carbons of the dihydroflavonol skeleton at δ_C 82.5 (C-3), 197.0 (C-4) and 158.7 (C-9). The C_6-methyl signal (δ_H 2.00) had correlation with carbons at δ_C 160.7 (C-5), 105.0 (C-6) and 164.4 (C-7). Similarly, C_8-methyl signal (δ_H 2.04) had correlation with car-

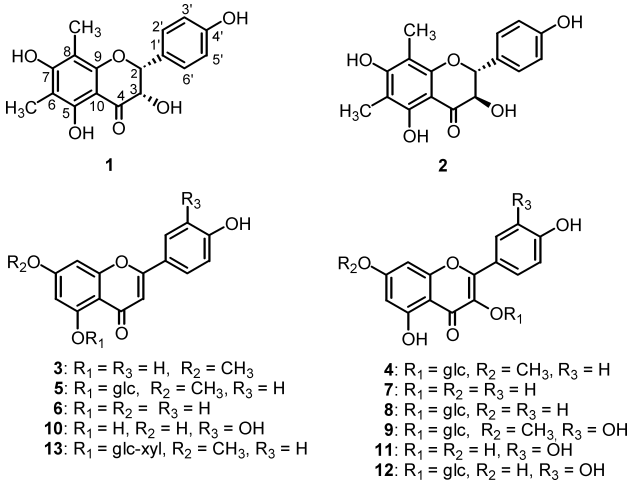
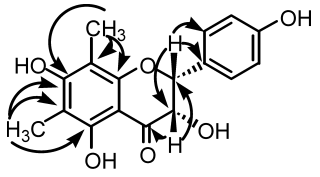


Fig. 1. Structures of Isolated Compounds

* To whom correspondence should be addressed. e-mail: yaharas1@gpo.kumamoto-u.ac.jp

Table 1. NMR Spectroscopic Data for **1** and **2** in CD_3OD

Position	**1**		**2**	
	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)
2	73.0	4.20, d (2.7)	73.7	4.49, d (11.7)
3	82.5	5.32, d (2.7)	84.8	4.92, d (11.7)
4	197.0		199.0	
5	160.7		160.1	
6	105.0		105.2	
7	164.4		164.5	
8	104.2		104.3	
9	158.7		159.1	
10	101.7		101.7	
1′	128.6		129.7	
2′	129.6	7.35, d (8.2)	130.3	7.37, d (8.3)
3′	115.9	6.79, d (8.2)	116.2	6.83, d (8.3)
4′	158.4		159.0	
5′	115.9	6.79, d (8.2)	116.2	6.83, d (8.3)
6′	129.6	7.35, d (8.2)	130.3	7.37, d (8.3)
6-CH_3	7.4	2.00, s	7.4	1.94, s
8-CH_3	8.1	2.04, s	8.0	2.01, s



1

Fig. 2. Key HMBC Correlations of **1**

bons at δ_C 158.7 (C-9), 104.2 (C-8) and 164.4 (C-7). The key HMBC correlations have been given in Fig. 2. The circular dichroism (CD) spectrum of **1** showed the positive Cotton effect at 349 nm suggesting the absolute configuration at the C-2 position to be *R*.[10] Depending upon the CD data and the coupling constant (J=2.7 Hz) of C_2-H and C_3-H, the configuration of dihydroflavonol was found to be 2*R*, 3*S* having 2α equatorial aryl group and 3α axial hydroxyl group. The structure of **1** was finally concluded as (2*R*,3*S*)-6,8-di-*C*-methyldihydrokaempferol.

Compound **2** was obtained as pale yellow amorphous powder, $[\alpha]_D^{21}$ +4.8° (c=0.48, MeOH). The HR-FAB-MS of **2** showed the quasi-molecular ion $[M+H]^+$ at *m/z*: 317.1065 supporting the formula $C_{17}H_{16}O_6$ (Calcd for $C_{17}H_{17}O_6$, 317.1025). The ^{1}H- and ^{13}C-NMR data of **2** were similar to that of **1** except that the resonance at δ_H 4.92 (1H, d, J=11.7 Hz, H-3) and 4.49 (1H, d, J=11.7 Hz, H-2) revealed the *trans* stereochemistry of the dihydroflavonol moiety between C_2-H and C_3-H. All other signals were assigned with comparison to those of **1**. The CD spectra of **2** also showed the positive Cotton effect at 347 nm suggesting 2*R* configuration.[10] Depending upon the CD data and the coupling constant (J=11.7 Hz) of C_2-H and C_3-H, the configuration of dihydroflavonol was found to be 2*R*, 3*R* having 2α equatorial aryl group and 3β equitorial hydroxyl group. On the basis of these data, the structure of **2** was concluded as (2*R*,3*R*)-6,8-di-*C*-methyldihydrokaempferol.

Experimental

General Experimental Procedures Optical rotations were measured with a JASCO DIP-1000KUY polarimeter. ^{1}H-, ^{13}C-NMR spectra were measured on a JEOL α-500 spectrometer. Chemical shifts are given in ppm with reference to tetramethylsilane (TMS). Mass spectra were recorded on JEOL JMS 700 MStation mass spectrometer. Column chromatography was carried out with silica gel 60 (0.040—0.063 mm, Merck), MCI gel CHP20P (75—150 mm, Mitsubishi Chemical Industries Co., Ltd.), Sephadex LH20 (Amersham Pharmacia Biotech) and Chromatorex ODS (30—50 μm, Fuji Silysia Chemical Co., Ltd.). TLC was performed on a precoated silica gel 60 F_{254} (0.2 mm, aluminum sheet, Merck). CD spectra were recorded on JASCO J-810 spectropolarimeter. Melting points were measured on Yanaco Micromelting point apparatus (MP-J3, MP-S3) and were uncorrected.

Plant Material Fresh aerial parts of *D. canescens* were collected in August, 2007 from Daman, Nepal and shade dried for one month. The specimen was identified by Mr. Kuber Jung Malla, Scientic Officer, Department of Plant Resources, Thapathali, Kathmandu, Nepal. The voucher specimen has been deposited at The Kochi Prefectural Makino Botanical Garden, Kochi, Japan.

Extraction and Isolation The shade dried aerial parts of *D. canescens* (492 g) were extracted with 70% MeOH (3 l) twice to give 70% MeOH extract (148.7 g) which was then subjected to MCI gel CHP20P column and eluted with water, 40% MeOH, 70% MeOH and 100% MeOH to give 8 fractions. Fraction 6 (6.4 g) was then subjected Sephadex LH20 column (MeOH) to afford 8 subfractions. Subfraction 6-3 (1.5 g) was then applied on silica gel column eluting with $CHCl_3$: MeOH : H_2O (9 : 1 : 0.1) and then with hexane : EtOAc (1 : 1) to give compounds **3** (6.4 mg), **2** (11.8 mg), **4** (102 mg) and **5** (79 mg). Compound **6** (156.5 mg) and **7** (444.7 mg) were obtained from the recrysatllization (MeOH : H_2O) of subfraction 6-4 (689.9 mg) and 6-5 (684.9 mg), respectively. Subfraction 6-8 (253.3 mg) was applied on silica gel column ($CHCl_3$: MeOH : H_2O=9 : 2 : 0.1) to obtain compound **5** (39 mg). Similarly, fraction 5 (2.5 g) was then subjected Sephadex LH20 column (MeOH) to obtain 9 subfractions. The subfraction 5-5 was then subjected to ODS column (45% MeOH) to give 11 subfractions. Subfractions 5-5-3, 5-5-7 and 5-5-10 afforded pure compounds **8** (45 mg), **9** (34 mg) and **4** (8 mg), respectively. Subfraction 5-5-6 (151 mg) was subjected to MCI gel column (30% MeOH), Sephadex LH20 column ($CHCl_3$: MeOH=3 : 1) and then silica gel column ($CHCl_3$: MeOH : H_2O= 9 : 2 : 0.1) to obtain compound **1** (52 mg). Compound **10** (27 mg) and **11** (68 mg) were obtained from the recrysatllization (MeOH : H_2O) of subfraction 5-7 (135.7 mg) and 5-8 (80 mg), respectively. Fraction 3 (10.9 g) was suspended in MeOH and filtered to separate MeOH soluble (3-1) and MeOH insoluble (3-2) parts. The MeOH soluble part (3-1, 8.5 g) was then applied on Sephadex LH20 column (MeOH) to give seven subfraction. Subfraction 3-1-4 was again applied on ODS column (30—50% MeOH) to give further 12 subfractions. Among them, subfraction 3-1-4-5 was applied on silica gel column eluting with $CHCl_3$: MeOH : H_2O (8 : 2 : 0.1) to obtain compound **12** (67 mg). Subfraction 3-1-4-10 was obtained as compound **13** (12.8 mg).

(2*R*,3*S*)-6,8-di-*C*-methyldihydrokaempferol (**1**): A pale yellow amorphous powder; $[\alpha]_D^{20}$ −80.5° (c=0.52, MeOH); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz), Table 1; HR-FAB-MS *m/z*: 317.0997 $[M+H]^+$ (Calcd for $C_{17}H_{17}O_6$, 317.1025); CD (MeOH, c=0.022): $\Delta\varepsilon$ (nm) −27.1 (297), +7.7 (349).

(2*R*,3*R*)-6,8-di-*C*-methyldihydrokaempferol (**2**): A pale yellow amorphous powder; $[\alpha]_D^{21}$ +4.8° (c=0.48, MeOH); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz), Table 1; HR-FAB-MS *m/z*: 317.1065 $[M+H]^+$ (Calcd for $C_{17}H_{17}O_6$, 317.1025); CD (MeOH, c=0.020): $\Delta\varepsilon$ (nm) −28.6 (297), +6.2 (347).

Acknowledgement Authors would like to thank the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan for the scholarship to Mr. Hari Prasad Devkota.

References

1) Manandhar N. P., "Plants and People of Nepal," Timber Press, Inc., Portland, 2002, p. 480.
2) Wu Dagang, Sorg B., Adolf W., Opferkuch H. J., Seip E. H., Hecker E., *Phytother. Res.*, **7**, 194—196 (1993).
3) Park Y., Moon B. H., Yang H., Lee Y., Lee E., Lim Y., *Magn. Reson. Chem.*, **45**, 1072—1075 (2007).
4) Haborne J. B., Marby T. J., "The Flavonoids, Advances in Research," Chapman and Hall, London, 1982, pp. 19—134.
5) Veit M., Geiger H., Czygan F. C., Markham K. R., *Phytochemistry*, **29**, 2555—2560 (1990).

6) Lin J. H., Lin Y. T., Huang Y. J., Wen K. C., Chen R. M., Ueng T. H., Liao C. H., *J. Food Drug Anal.*, **9**, 6—11 (2001).
7) Park Y., Moon B. H., Lee E., Lee Y., Yoon Y., Ahn J. H., Lim Y., *Magn. Reson. Chem.*, **45**, 674—679 (2007).
8) Kim M. R., Lee J. Y., Lee H. H., Aryal D. K., Kim Y. G., Kim S. K., Woo E. R., Kang K. W., *Food Chem. Toxicol.*, **44**, 1299—1307 (2006).
9) Chen Z., Hu Y., Wu H., Jiang H., *Bioorg. Med. Chem. Lett.*, **14**, 3949—3952 (2004).
10) Slade D., Ferreira D., Marais J. P. J., *Phytochemistry*, **66**, 2177—2215 (2005).