Isolation of Five New Flavonoids from *Melicope triphylla*

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Received October 25, 2012; accepted February 1, 2013

Five new flavonoids, 5,8-dihydroxy-3,7-dimethoxy-3',4'-methylenedioxyflavone (**1**), 7-hydroxy-3,5-dimethoxy-3',4'-methylenedioxyflavone (**2**), 7-(2,3-dihydroxy-3-methylbutoxy)-3,5-dimethoxy-3',4'-methylenedioxyflavone (**3**), 7-(2,3-dihydroxy-3-methylbutoxy)-3,3',4',5-tetramethoxyflavone (**4**), and 7-(2,3-dihydroxy-3-methylbutoxy)-3,3',4',5,8-pentamethoxyflavone (**5**), were isolated from the leaves of *Melicope triphylla*. In addition, six known flavonoids were detected: 3,4',5-trihydroxy-3',7,8-trimethoxyflavone (**6**), 5,7-dihydroxy-3-methoxy-3',4'-methylenedioxyflavone (**7**), 4',5,7-trihydroxy-3,3'-dimethoxyflavone (**8**), 4',7-dihydroxy-3,3',5,8-tetramethoxyflavone (**9**), 4',7-dihydroxy-3,3',5-trimethoxyflavone (**10**), and 4',5,7-trihydroxy-3,3',8-trimethoxyflavone (**11**). The new compound structures were determined by spectroscopic methods. Compounds **1–5** did not exhibit any ichthyotoxic activity against Japanese killifish (*medaka* in Japanese) (*Oryzias latipes* var.) at 10 ppm.

Key words *Melicope triphylla*; Rutaceae; prenylated flavonoid; ichthyotoxic activity

Melicope triphylla MERR. (Rutaceae) (*Awadan* in Japanese), a small evergreen tree, is widely distributed in Southeast Asia and is the only species of *Melicope* native to Japan. Many flavonoids have been isolated from the leaves,^{1–4} root, and stem bark^{1,5,6} of this plant. Some have been reported to show antiplatelet aggregation,^{2,6} ichthyotoxic,³ cytotoxic,⁴ and vasorelaxation activities.⁶ We have re-examined the constituents of this plant to obtain new biologically active flavonoids. Previously, we reported the isolation of ten new flavonoids.^{7,8} In a follow-up investigation, we obtained five new compounds, **1–5**, and six known compounds, **6–11**, from the leaves. In this study, we report the isolation and structure elucidation of the new compounds and those ichthyotoxicities.

Results and Discussion

After chromatographic separation, the remaining fractions from the extraction and isolation previously described⁷ yielded six known compounds, 3,4',5-trihydroxy-3',7,8-trimethoxyflavone (**6**),⁹ 5,7-dihydroxy-3-methoxy-3',4'-methylenedioxyflavone (**7**),¹⁰ 4',5,7-trihydroxy-3,3'-dimethoxyflavone (**8**),¹¹ 4',7-dihydroxy-3,3',5,8-tetramethoxyflavone (**9**),¹² 4',7-dihydroxy-3,3',5-trimethoxyflavone (**10**),¹³ and 4',5,7-trihydroxy-3,3',8-trimethoxyflavone (**11**),⁹ and five new compounds, **1–5**.

This is the first report of compound **7** as a natural product.

Compounds **1–5** showed positive responses to the magnesium–hydrochloric acid test for flavonoids. Additionally, their IR (α,β -unsaturated carbonyl group) and UV spectral data (bands I and II) were typical of flavonoids.

Compound **1** was obtained as yellow needles (melting point (mp)) 256–257°C. Its molecular formula was determined to be C₁₈H₁₄O₈ by high-resolution electrospray ionization mass spectrum (HR-ESI-MS) showing the pseudomolecular ion [M+H]⁺ at *m/z* 359.0781 (Calcd for C₁₈H₁₅O₈: 359.0767). The IR spectrum showed a band at 3300 cm^{–1}, which was assigned to the hydroxyl group. In the UV spectrum of **1** in methanol, band I showed a large bathochromic shift ($\Delta\lambda_{\text{max}}=60$ nm) upon addition of an AlCl₃–HCl mixture, suggesting the presence of a 5-hydroxyl group and the absence of an oxygen moiety at

C-6.¹⁴ The ¹H-NMR spectrum of **1** (Table 1) revealed the presence of two methoxy groups [3.79 ppm (3H, s) and 3.89 ppm (3H, s)], one methylenedioxy group [6.15 ppm (2H, s)], one A-ring proton [6.55 ppm (1H, s)], and one hydrogen-bonded hydroxyl group [12.10 ppm (1H, s, OH-5)]. In addition, it displayed three ABX-type protons [7.13 ppm (1H, d, *J*=8.0 Hz, H-5'), 7.63 ppm (1H, d, *J*=2.0 Hz, H-2'), and 7.71 ppm (1H, dd, *J*=2.0, 8.0 Hz, H-6')], suggesting that C-3' and C-4' were substituted in the B-ring. The electron ionization (EI)-MS spectrum showed a diagnostic peak at *m/z* 149 corresponding to the (OCH₂O)C₆H₃–C≡O⁺ fragment, consistent with a 3',4'-methylenedioxy substitution pattern in the B-ring.¹⁵ The ¹³C-NMR spectrum (Table 2) showed one methoxy carbon signal resonating at lower magnetic field (59.8 ppm). This suggested that both positions *ortho* to the methoxy group were substituted.^{16–19} The carbon shifts of OCH₃ substituents usually occur between 55.0 and 56.5 ppm but in some cases they are observed further downfield between 59.5 and 63.0 ppm. This deshielding effect is seen only when the OCH₃ is *diortho* substituted by two bulky substituents such as ring junction. Therefore, the signal was assigned to the 3-methoxy group. It also exhibited a signal for another methoxy carbon at higher magnetic field (56.4 ppm), which suggested that only one or no position *ortho* to this methoxy group was substituted.^{16–19} This signal was assigned to the A-ring methoxy group. The heteronuclear multiple-bond correlation (HMBC) spectrum of **1** (Fig. 1) showed long-range correlations between the A-ring proton and C-10, indicating that the A-ring proton is located at C-6 or C-8. Thus, the substitution pattern in the A-ring is 5,6-dihydroxy-7-methoxy or 5,8-dihydroxy-7-methoxy. The ¹³C-NMR chemical shifts assigned to the A-ring of **1** agreed well with the ¹³C-NMR spectrum of 5,8-dihydroxy-3,4',7-trimethoxyflavone,²⁰ suggesting that the substitution pattern in the A-ring is 5,8-dihydroxy-7-methoxy. The nuclear Overhauser enhancement spectroscopy (NOESY) spectrum showed a relationship between the A-ring proton and both the OCH₃-7 and OH-5 protons (Fig. 1), confirming that the A-ring proton is located at C-6. Therefore, **1** was characterized as 5,8-dihydroxy-3,7-dimethoxy-3',4'-methylenedioxyflavone. All correlations in the HMBC spectrum (Fig. 1) were in complete agreement with the proposed structure.

The authors declare no conflict of interest.

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Chart 1

Table 1. ¹H-NMR Spectral Data for Compounds **1–5** (500 MHz, δ)

	1 ^{a)}	2 ^{a)}	3 ^{b)}	4 ^{b)}	5 ^{b)}
H-6	6.55 (s)	6.38 (d, 2.0)	6.34 (d, 2.0)	6.36 (d, 2.0)	6.42 (s)
H-8		6.51 (d, 2.0)	6.49 (d, 2.0)	6.52 (d, 2.0)	
H-2'	7.63 (d, 2.0)	7.53 (d, 2.0)	7.59 (d, 2.0)	7.69 (d, 2.0)	7.82 (d, 2.0)
H-5'	7.13 (d, 8.0)	7.10 (d, 8.0)	6.92 (d, 8.0)	6.97 (d, 8.5)	7.01 (d, 8.5)
H-6'	7.71 (dd, 2.0, 8.0)	7.59 (dd, 2.0, 8.0)	7.66 (dd, 2.0, 8.0)	7.70 (dd, 2.0, 8.5)	7.84 (dd, 2.0, 8.5)
H-1''			4.10 (dd, 8.0, 9.5)	4.11 (dd, 8.0, 9.5)	4.22 (dd, 7.0, 9.5)
			4.23 (dd, 3.0, 9.5)	4.24 (dd, 3.0, 9.5)	4.39 (dd, 2.5, 9.5)
H-2''			3.88 (dd, 3.0, 8.0)	3.88 (dd, 3.0, 8.0)	3.83 (dd, 2.5, 7.0)
H-4''			1.32 (s) ^{c)}	1.32 (s) ^{c)}	1.33 (s) ^{c)}
H-5''			1.37 (s) ^{c)}	1.37 (s) ^{c)}	1.37 (s) ^{c)}
OH-5	12.10 (s)				
OH-8	8.88 (s)				
OCH ₃ -3	3.79 (s)	3.72 (s)	3.86 (s)	3.87 (s)	3.90 (s)
OCH ₃ -5		3.81 (s)	3.94 (s)	3.95 (s)	3.98 (s)
OCH ₃ -7	3.89 (s)				
OCH ₃ -8					3.95 (s)
OCH ₃ -3'				3.96 (s) ^{c)}	3.967 (s) ^{c)}
OCH ₃ -4'				3.97 (s) ^{c)}	3.972 (s) ^{c)}
OCH ₂ O-3',4'	6.15 (s)	6.14 (s)	6.06 (s)		

Mesurements *a*) in DMSO-*d*₆, *b*) in CDCl₃, *c*) Signals with the same superscript may be interchanged.

Compound **2** was obtained as colorless needles. Its molecular formula was determined to be C₁₈H₁₄O₇ by high resolution-electro spray ionization (HR-ESI)-MS. The IR spectrum of **2** showed a band at 3125 cm⁻¹, which was assigned to the hydroxyl group. In the UV spectrum of **2** in methanol, band II showed a bathochromic shift ($\Delta\lambda_{\text{max}}=6\text{nm}$) upon NaOAc addition, suggesting the presence of a 7-hydroxyl group.¹⁴⁾ The ¹H-NMR spectrum of **2** (Table 1) revealed the presence

of two methoxy groups [3.72 ppm (3H, s) and 3.81 ppm (3H, s)], one methylenedioxy group [6.14 ppm (2H, s)], and two *meta*-coupled A-ring protons [6.38 ppm (1H, d, *J*=2.0 Hz, H-6) and 6.51 ppm (1H, d, *J*=2.0 Hz, H-8)]. In addition, it displayed signals for three ABX type B-ring protons [7.10 ppm (1H, d, *J*=8.0 Hz, H-5'), 7.53 ppm (1H, d, *J*=2.0 Hz, H-2'), and 7.59 ppm (1H, dd, *J*=2.0, 8.0 Hz, H-6')]. The EI-MS spectrum showed a diagnostic peak at *m/z* 149 corresponding to the

Table 2. ^{13}C -NMR Spectral Data for Compounds **1–5** (125 MHz, δ)

	1 ^{a)}	2 ^{a)}	3 ^{b)}	4 ^{b)}	5 ^{b)}
C-2	154.9	151.6	152.5	152.8	152.5
C-3	137.9	140.6	141.2	141.2	141.0
C-4	178.5	172.5	174.1	174.0	174.2
C-5	152.8	161.2	161.0	161.1	156.5
C-6	95.5	96.7	96.0	96.0	93.8
C-7	154.2	163.1	162.8	162.8	155.2
C-8	126.0	95.4	93.1	93.2	131.0
C-9	143.7	158.5	158.6	158.7	151.0
C-10	104.5	107.8	109.6	109.7	110.0
C-1'	123.7	124.5	124.5	123.3	123.4
C-2'	108.1	108.2	108.4	111.3	111.0
C-3'	147.6	148.0	147.9	148.8	148.7
C-4'	149.6	149.4	149.4	151.0	151.0
C-5'	108.6	109.0	108.4	110.9	111.0
C-6'	123.8	123.3	123.1	121.7	121.8
C-1''			69.7	69.7	71.5
C-2''			75.8	75.8	75.4
C-3''			71.7	71.7	71.8
C-4''			25.1	25.1	26.6
C-5''			26.6	26.6	25.5
OCH ₃ -3	59.8	59.8	59.9	59.9	59.9
OCH ₃ -5		56.3	56.4	56.4	56.6
OCH ₃ -7	56.4				
OCH ₃ -8					61.7
OCH ₃ -3'				56.0	55.9
OCH ₃ -4'				56.1	56.0
OCH ₂ O-3',4'	102.0	102.2	101.6		

Measurements a) in DMSO-*d*₆, b) in CDCl₃.

(OCH₂O)C₆H₃–C≡O⁺ fragment, consistent with a 3',4'-methylenedioxy substitution pattern in the B-ring.¹⁵⁾ It also showed a peak at *m/z* 323 corresponding to the (M⁺–OH₃) fragment, suggesting a 3,5-dimethoxy substitution pattern.¹⁵⁾ The ^{13}C -NMR spectrum (Table 2) showed one methoxy carbon signal at 59.8 ppm, which was assigned to the 3-methoxy group by the same manner as **1**. It also exhibited a signal for another methoxy carbon at 56.3 ppm, which was assigned to the 5-methoxy group^{16–19)} by observation of a correlation between a singlet at 3.81 ppm due to the methoxy proton and a signal at 161.2 ppm due to C-5 in the HMBC spectrum. This supported by the comparison of the chemical shifts for **2** with those described in literatures.^{16–19)} Therefore, **2** was characterized as 7-hydroxy-3,5-dimethoxy-3',4'-methylenedioxyflavone. All correlations in the HMBC spectrum (Fig. 1) were in complete agreement with the proposed structure.

Compound **3** was obtained as a colorless amorphous powder. Its molecular formula was determined to be C₂₃H₂₄O₉ by HR-ESI-MS. Its spectral data were closely comparable to those of **2**. The substitution pattern in the B-ring was determined to be 3',4'-methylenedioxy by its ^1H -NMR and EI-MS spectra by the same manner as **1** and **2**. The ^1H -NMR spectrum of **3** (Table 1) was similar to that of **2**, except for the presence of signals for the 2,3-dihydroxy-3-methylbutoxy group²¹⁾ on A ring [1.32, 1.37 ppm (each 3H, s, –OCH₂CH(OH)C(CH₃)₂OH), 3.88 ppm (1H, dd, *J*=3.0, 8.0 Hz, –OCH₂CH(OH)C(CH₃)₂OH), 4.10 ppm (1H, dd, *J*=8.0, 9.5 Hz, –OCH₂CH(OH)C(CH₃)₂OH), and 4.23 ppm (1H, dd, *J*=3.0, 9.5 Hz, –OCH₂CH(OH)C(CH₃)₂OH)] in **3**. The EI-MS spectrum showed a peak at *m/z* 341 that was assigned to the fragment ion produced by the loss of the 2,3-dihydroxy-3-methylbutyl radical from the molecular ion,

confirming the presence of the 2,3-dihydroxy-3-methylbutoxy group.²²⁾ It also showed a peak at *m/z* 425 suggesting a 3,5-dimethoxy substitution pattern.¹⁵⁾ The ^{13}C -NMR spectrum (Table 2) showed one methoxy carbon signal at 56.4 ppm, which was assigned to the 5-methoxy group. It also exhibited a signal for another methoxy carbon at 59.9 ppm, which was assigned to the 3-methoxy group.^{16–19)} The HMBC spectrum of **3** (Fig. 1) showed correlations between C-7 and three proton signals: the oxymethylene protons (H₂-1''), H-6, and H-8, confirming that a prenyloxy group is located at C-7. Therefore, **3** was characterized as 7-(2,3-dihydroxy-3-methylbutoxy)-3,5-dimethoxy-3',4'-methylenedioxyflavone. All correlations in the HMBC spectrum (Fig. 1) were in complete agreement with the proposed structure.

Compound **4** was obtained as a colorless amorphous powder. Its molecular formula was determined to be C₂₄H₂₈O₉ by HR-ESI-MS. Its spectral data were closely comparable to those of **3**. The ^1H -NMR spectrum of **4** (Table 1) was similar to that of **3**, except for the presence of two methoxy group signals [3.96 ppm (3H, s) and 3.97 ppm (3H, s)] instead of the methylenedioxy group signal present in **3**. The EI-MS spectrum showed fragment ion peaks at *m/z* 411 and 357 due to [M⁺–OH₃] and [M⁺–C₅H₁₁O₂] ions, respectively, suggesting the presence of a 3,5-dimethoxy substitution pattern¹⁵⁾ and a 2,3-dihydroxy-3-methylbutyl group.²²⁾ It also showed a diagnostic peak at *m/z* 165 corresponding to the (OCH₃)₂C₆H₃–C≡O⁺ fragment, consistent with a 3',4'-dimethoxy substitution pattern in the B-ring.¹⁵⁾ The ^{13}C -NMR spectrum (Table 2) showed three methoxy carbon signals at 56.0, 56.1, and 56.4 ppm, which were assigned to the 3'-, 4'-, and 5-methoxy groups by observations of correlations

Fig. 1. Key HMBC Correlations (—) and NOEs (---) for Compounds 1–5

between each singlet due to methoxy proton and each signal due to corresponding carbon atoms bonded to the methoxy groups in HMBC spectrum of **4**. It also exhibited a signal for another methoxy carbon at 59.9 ppm, which was assigned to the 3-methoxy group.^{16–19} The HMBC spectrum of **4** (Fig. 1) showed correlations between C-7 and three proton signals: the oxymethylene protons (H₂-1'', H-6, and H-8), confirming that a prenyloxy group is located at C-7. Therefore, **4** was characterized as 7-(2,3-dihydroxy-3-methylbutoxy)-3,3',4',5-tetramethoxyflavone. All correlations in the HMBC spectrum (Fig. 1) were in complete agreement with the proposed structure.

Compound **5** was obtained as a colorless amorphous powder. Its molecular formula was determined to be C₂₅H₃₀O₁₀ by HR-ESI-MS. Its spectral data were closely comparable to those of **4**. The substitution pattern in the B-ring was determined to be 3',4'-dimethoxy by its ¹H-NMR and EI-MS spectra by the same manner as **3** and **4**. The ¹H-NMR spectrum of **5** (Table 1) was similar to that of **4**, except for the presence of one methoxy group signal [3.95 ppm (3H, s)] instead of one of the two A-ring proton signals in **4**. The EI-MS spectrum

suggested the presence of the 2,3-dihydroxy-3-methylbutyl group (*m/z* 387, M⁺–C₅H₁₁O₂)²² and a 3,5-dimethoxy substitution pattern (*m/z* 471, M⁺–OH₃).¹⁵ The ¹³C-NMR spectrum (Table 2) showed five methoxy carbon signals, 55.9, 56.0, 56.6, 59.9, and 61.7 ppm. The signals of 55.9, 56.0, 56.6, and 59.9 ppm were assigned to the 3', 4', 5-, and 3-methoxy groups, respectively, by the same manner as **3** and **4**. The remaining methoxy group was assigned to A-ring by observation of a fragment ion peak at *m/z* 165 due to [(CH₃O)₂C₆H₃–C≡O⁺] ion corresponding to B-ring in the EI-MS spectrum. The HMBC spectrum of **5** (Fig. 1) showed a correlation between the A-ring proton and C-10, indicating that the A-ring proton is located at either C-6 or C-8. Thus, the substitution pattern in the A-ring is 6-(2,3-dihydroxy-3-methylbutoxy)-5,7-dimethoxy or 7-(2,3-dihydroxy-3-methylbutoxy)-5,8-dimethoxy. The ¹³C-NMR chemical shifts assigned to the A-ring of **5** agreed well with the ¹³C-NMR spectrum of 7-(2-hydroxy-3-methylbut-3-enyloxy)-3,3',4',5,8-pentamethoxyflavone,⁸ suggesting that the substitution pattern in the A-ring is 7-(2,3-dihydroxy-3-methylbutoxy)-5,8-dimethoxy. The NOESY spectrum (Fig. 1) showed a relationship between the A-ring proton

and two proton groups: the oxymethylene protons (H_2-1'') and the methoxy group protons at 3.98 ppm. NOE effects were also observed between H_2-1'' and OCH_3-8 at 3.95 ppm, confirming that a prenyloxy group is located at C-7. Therefore, **5** was characterized as 7-(2,3-dihydroxy-3-methylbutoxy)-3,3',4',5,8-pentamethoxyflavone. All correlations in the HMBC spectrum (Fig. 1) were in complete agreement with the proposed structure, although a part of correlativity of 2,3-dihydroxy-3-methylbutoxy was not observed. This is the first report of flavonoids bearing 2,3-dihydroxy-3-methylbutoxy substituent.

Compounds **1–5** did not display any ichthyotoxic activity against Japanese killifish (*Oryzias latipes* var.) when tested at 10 ppm, in spite that the activity was appeared by 3,3',4',5-tetramethoxy-7-(3-methylbut-3-enyloxy)flavone which isolated from this plant at the same concentration.⁸⁾ The reason for these facts seem to be due to the presence of hydroxyl substituents at C-5, C-7, C-8, C-2'', or C-3''. On the basis of the results in this study and in previous experiment,⁸⁾ the hydroxy group at C-2'' was judged to have no effect on the ichthyotoxicity. Therefore, the absolute configuration at C-2'' in **3–5** were not determined by this research as well as the previous research. Further extensive scope for the determination of stereochemistry of the **3–5** and the evaluation to the other biological activities are in progress in our lab.

Experimental

General Procedures Melting points were measured on a Yanagimoto micro melting point apparatus MP-S3 and were not corrected. IR spectra were acquired using a JASCO FT/IR-4100 spectrometer; UV spectra were measured on a JASCO V-630 instrument; EI-MS were determined on a Hitachi M-2500 apparatus (70 eV, direct inlet system) and HR-ESI-MS were obtained on a JEOL JMS-T100LP mass spectrometer. 1H -, ^{13}C -, and two-dimensional NMR spectra were acquired on a JEOL $\alpha 500$ (1H : 500 MHz, ^{13}C : 125 MHz) and a Bruker Avance III 500 (1H : 500 MHz, ^{13}C : 125 MHz) spectrometers. Chemical shifts were given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The symbols s, d, t, q, dd and m denote singlet, doublet, triplet, quartet, double doublet and multiplet, respectively. Flash-column chromatography (FC) were carried out on Kieselgel 60N 40–50 μm (Merck). Preparative thin layer chromatography (PTLC) was performed on a precoated Kieselgel 60 F₂₅₄ plate (Merck). Ichthyotoxicity tests were performed in 150 mL of a test sample aqueous solution using male Japanese killifish (*Oryzias latipes* var.).²³⁾

Extraction and Isolation The extraction and isolation of the constituents of the fresh leaves of *M. triphylla* were described in our previous paper.⁷⁾ Fraction III (26.90 g) was divided into C_6H_6 -soluble and C_6H_6 -insoluble portions (3.80 g). The C_6H_6 -soluble portion was purified by FC on a silica gel (C_6H_6 –EtOAc gradient) and PTLC on a silica gel (8:2 $CHCl_3$ –EtOAc) to yield **5** (11 mg), **3** (5 mg), **4** (12 mg), **6** (18 mg), **7** (18 mg), **1** (36 mg), and **8** (25 mg). The C_6H_6 -insoluble portion was purified by FC on a silica gel (C_6H_6 –EtOAc gradient) and PTLC on a silica gel (7:3 $CHCl_3$ –EtOAc) to yield **9** (15 mg), **10** (28 mg), **11** (17 mg), and **2** (53 mg).

5,8-Dihydroxy-3,7-dimethoxy-3',4'-methylenedioxyflavone (1): Yellow needles (C_6H_6), mp 256–257°C. Mg–HCl test: +. HR-ESI-MS m/z : 359.0781 ($[M+H]^+$, $C_{18}H_{15}O_8$, Calcd 359.0767). IR ν_{max} (KBr) cm^{-1} : 3300 (OH), 1655 (C=O). UV

λ_{max} (MeOH) nm (log ϵ): 259 (4.09), 280 (4.14), 335 (4.00), 373 sh (3.75); λ_{max} (MeOH+ $AlCl_3$) nm (log ϵ): 274 sh (4.09), 288 (4.13), 328 (3.89), 364 (4.03), 438 (3.66); λ_{max} (MeOH+ $AlCl_3$ +HCl) nm (log ϵ): 271 sh (4.04), 288 (4.11), 329 (3.92), 358 (4.01), 433 (3.58). EI-MS m/z (rel. int. %): 358 [M^+] (100), 149 [$(OCH_2O)C_6H_3-C\equiv O^+$] (11). 1H -NMR: Table 1. ^{13}C -NMR: Table 2.

7-Hydroxy-3,5-dimethoxy-3',4'-methylenedioxyflavone (2): Colorless needles (MeOH), mp 274–276°C. Mg–HCl test: +. HR-ESI-MS m/z : 365.0642 ($[M+Na]^+$, $C_{18}H_{14}NaO_7$, Calcd 365.0637). IR ν_{max} (ATR: attenuated total reflection) cm^{-1} : 3125 (OH), 1609 (C=O). UV λ_{max} (MeOH) nm (log ϵ): 269 (4.11), 339 (4.00), 372 (4.00); λ_{max} (MeOH+NaOAc) nm (log ϵ): 275 (4.44), 326 (4.29), 383 (4.25). EI-MS m/z (rel. int. %): 342 [M^+] (100), 323 [M^+-OH_3] (27), 149 [$(OCH_2O)C_6H_3-C\equiv O^+$] (9). 1H -NMR: Table 1. ^{13}C -NMR: Table 2.

7-(2,3-Dihydroxy-3-methylbutoxy)-3,5-dimethoxy-3',4'-methylenedioxyflavone (3): A colorless amorphous powder. Mg–HCl test: +. $[\alpha]_D^{21}$ -10.1° ($c=0.10$, $CHCl_3$). HR-ESI-MS m/z : 467.1325 ($[M+Na]^+$, $C_{22}H_{24}NaO_9$, Calcd 467.1318). IR ν_{max} (ATR) cm^{-1} : 3403 (OH), 1608 (C=O). UV λ_{max} (MeOH) nm (log ϵ): 255 sh (4.05), 264 sh (4.01), 292 (3.92), 330 (3.85). EI-MS m/z (rel. int. %): 444 [M^+] (87), 425 [M^+-OH_3] (51), 341 [$M^+-C_5H_{11}O_2$] (100), 149 [$(OCH_2O)C_6H_3-C\equiv O^+$] (49). 1H -NMR: Table 1. ^{13}C -NMR: Table 2.

7-(2,3-Dihydroxy-3-methylbutoxy)-3,3',4',5-tetramethoxyflavone (4): A colorless amorphous powder. Mg–HCl test: +. $[\alpha]_D^{21}$ -1.50° ($c=0.43$, $CHCl_3$). HR-ESI-MS m/z : 483.1627 ($[M+Na]^+$, $C_{24}H_{28}NaO_9$, Calcd 483.1631). IR ν_{max} (ATR) cm^{-1} : 3421 (OH), 1615 sh (C=O). UV λ_{max} (MeOH) nm (log ϵ): 250 (4.27), 264 sh (4.21), 300 sh (4.04), 338 (4.17). EI-MS m/z (rel. int. %): 460 [M^+] (100), 441 [M^+-OH_3] (17), 357 [$M^+-C_5H_{11}O_2$] (20), 165 [$(OCH_3)_2C_6H_3-C\equiv O^+$] (15). 1H -NMR: Table 1. ^{13}C -NMR: Table 2.

7-(2,3-Dihydroxy-3-methylbutoxy)-3,3',4',5,8-pentamethoxyflavone (5): A colorless amorphous powder. Mg–HCl test: +. $[\alpha]_D^{23}$ -5.27° ($c=0.06$, $CHCl_3$). HR-ESI-MS m/z : 513.1750 ($[M+Na]^+$, $C_{25}H_{30}NaO_{10}$, Calcd 513.1737). IR ν_{max} (ATR) cm^{-1} : 3420 (OH), 1618 (C=O). UV λ_{max} (MeOH) nm (log ϵ): 251 (4.28), 272 (4.26), 299 sh (4.08), 348 (4.15). EI-MS m/z (rel. int. %): 490 [M^+] (100), 471 [M^+-OH_3] (21), 387 [$M^+-C_5H_{11}O_2$] (35), 165 [$(OCH_3)_2C_6H_3-C\equiv O^+$] (8). 1H -NMR: Table 1. ^{13}C -NMR: Table 2.

3,4',5-Trihydroxy-3',7,8-trimethoxyflavone (6): A pale yellow amorphous powder. The 1H -NMR spectral data were identical with those in ref. 9.

5,7-Dihydroxy-3-methoxy-3',4'-methylenedioxyflavone (7): Pale yellow needles (MeOH), mp 270–272°C (lit.¹⁰⁾ 274–275°C). Compound **7** was identified by examination of its IR, UV, EI-MS, 1H , and ^{13}C -NMR spectra.

IR ν_{max} (KBr) cm^{-1} : 3145 (OH), 1651 (C=O). UV λ_{max} (MeOH) nm (log ϵ): 256 (4.26), 351 (4.23); λ_{max} (MeOH+NaOAc) nm (log ϵ): 276 (4.35), 320 (4.08), 374 (4.13); λ_{max} (MeOH+ $AlCl_3$ +HCl) nm (log ϵ): 271 (4.24), 355 (4.15), 399 (4.10). EI-MS m/z (rel. int. %): 328 [M^+] (100), 149 [$(OCH_2O)C_6H_3-C\equiv O^+$] (10). 1H -NMR (DMSO- d_6 , 270 MHz) δ : 3.78 (3H, s, OCH_3-3), 6.15 (2H, s, $OCH_2O-3',4'$), 6.20 (1H, d, $J=2.0$ Hz, H-6), 6.46 (1H, d, $J=2.0$ Hz, H-8), 7.12 (1H, d, $J=8.5$ Hz, H-5'), 7.56 (1H, d, $J=2.0$ Hz, H-2'), 7.63 (1H, dd, $J=8.5$, 2.0 Hz, H-6'), 12.59 (1H, s, OH-5). ^{13}C -NMR (DMSO- d_6 , 67.8 MHz) δ : 154.8 (C-2), 138.1 (C-3), 177.9 (C-4), 161.2

(C-5), 98.7 (C-6), 164.5 (C-7), 93.9 (C-8), 156.4 (C-9), 104.2 (C-10), 123.6 (C-1'), 108 (C-2'), 147.7 (C-3'), 149.6 (C-4'), 108.6 (C-5'), 123.5 (C-6'), 59.8 (OCH₃-3), 101.9 (OCH₂O-3',4').

4',5,7-Trihydroxy-3,3'-dimethoxyflavone (**8**): Pale yellow plates (CHCl₃), mp 248–250°C (lit.¹¹) 230–232°C). The ¹³C-NMR spectral data were identical with those in ref. 11.

4',7-Dihydroxy-3,3',5,8-tetramethoxyflavone (**9**): Pale yellow needles (MeOH), mp 240–242°C (lit.¹²) 244–246°C). The ¹H-NMR spectral data were identical with those in ref. 12. ¹H-NMR (DMSO-*d*₆, 400 MHz) δ : 3.75, 3.78, 3.84, 3.86 (each, 3H, s, OCH₃), 6.45 (1H, s, H-6), 6.97 (1H, d, *J*=8.5 Hz, H-5'), 7.59 (1H, dd, *J*=8.5, 2.0 Hz, H-6'), 7.66 (1H, d, *J*=2.0 Hz, H-2').

4',7-Dihydroxy-3,3',5-trimethoxyflavone (**10**): Pale yellow needles (MeOH), mp 256–258°C. The ¹H-NMR spectral data were identical with those in ref. 13.

4',5,7-Trihydroxy-3,3',8-trimethoxyflavone (**11**): Yellow needles (MeOH), mp 216–218°C (lit.⁹) 215–217°C). The ¹H-NMR spectral data were identical with those in ref. 9.

Acknowledgements We are grateful for the financial support from the Research Seeds Program of Faculty of Science, University of the Ryukyus and Okinawa Science and Technology Promotion Center.

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