Isolation of Four New Flavonoids from *Melicope triphylla*

Matsutake Higa,* Eisaku Nakadomari, Megumi Imamura, Kazuhito Ogihara, and Toshimasa Suzuka

College of Science, University of the Ryukyus; Nishihara, Okinawa 903–0213, Japan.
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Four new flavonoids, 3,5-dihydroxy-7,8-dimethoxy-3′,4′-methylenedioxyflavone (1), 3,5-dihydroxy-7-methoxy-3′,4′-methylenedioxyflavone (2), 3,5-dihydroxy-7-isopentenyloxy-8-methoxy-3′,4′-methylenedioxyflavone (3) and 5-hydroxy-3-isopentenyloxy-7-methoxy-3′,4′-methylenedioxyflavone (4), were isolated from the leaves of *Melicope triphylla*. In addition, two known flavonoids were detected including 5-hydroxy-3,7-dimethoxy-3′,4′-methylenedioxyflavone (5) and 5-hydroxy-7-isopentenyloxy-3,8-dimethoxy-3′,4′-methylenedioxyflavone (6). The structures of the new compounds were established by spectroscopic methods.

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

Melicope triphylla Merr. (Rutaceae) (Awadan in Japanese) is a shrub growing in Southeast Asia. Many flavonoids have been isolated from the leaves[1—4] and the root and stem bark[1,5—7] of this plant. Some of them have been reported to show piscicidal,[2] antiplatelet aggregating,[3] cytotoxic[4] and vasorelaxing activities.[7] In a follow-up investigation, we obtained four new compounds (**1**—**4**) and two other known compounds **5** and **6** from the leaves. In this study, we report the isolation and structure elucidation of these new compounds.

Results and Discussion

After chromatographic separation, the methanol extract of the *M. triphylla* fresh leaves yielded 5-hydroxy-3,7-dimethoxy-3′,4′-methylenedioxyflavone (**5**)[2] and 5-hydroxy-7-isopentenyloxy-3,8-dimethoxy-3′,4′-methylenedioxyflavone (**6**),[2] and four new compounds **1**—**4**, in addition to methyl *p*-geranyloxy-*trans*-cinnamate and β-amyrin.

Compound **1** (yellow needles) showed a positive response to the magnesium–hydrochloric acid test for flavonoids. The IR spectrum of **1** showed peaks at 3300 and 1649 cm^{-1} assigned to the hydroxy and α,β-unsaturated carbonyl groups, respectively. The molecular formula of **1** was deduced as $C_{18}H_{14}O_8$ by high resolution (HR) electron ionization (EI)-MS (M^+ *m/z* 358.0685) (Calcd 358.0687). The UV spectrum of **1** in methanol displayed a band I absorption maximum at a longer wavelength (λ_{max}=378 nm), suggesting that compound **1** is a flavonol. Band I showed a bathochromic shift ($\Delta\lambda_{max}$=40 nm) upon NaOMe addition, supporting the presence of a 3-hydroxy group. It also exhibited a bathochromic shift ($\Delta\lambda_{max}$=63 nm) upon addition of $AlCl_3$–HCl mixture, consistent with the presence of a 5-hydroxy group.[8] The ^{1}H-NMR spectrum of compound **1** (Table 1) revealed the presence of two methoxy groups [3.90 ppm (3H, s), 3.80 ppm (3H, s)], one methylenedioxy group [6.13 ppm (2H, s)], one A-ring proton [6.57 ppm (1H, s)], one phenolic hydroxy group [9.71 ppm (1H, s, OH-3)] and one hydrogen-bonded hydroxy group [12.19 ppm (1H, s, OH-5)]. In addition, it displayed three ABX type protons at 7.13, 7.66 and 7.76 ppm [7.13 ppm (1H, d, *J*=8.3 Hz, H-5′), 7.66 ppm (1H, d, *J*=2.0 Hz, H-2′), 7.76 ppm (1H, dd, *J*=2.0, 8.3 Hz, H-6′)], suggesting that C-3′ and C-4′ were substituted in the B-ring. The EI-MS spectrum showed a diagnostic peak at *m/z* 149 corresponding to the $(OCH_2O)C_6H_3$–$C{\equiv}O^+$ fragment (Chart 1), consistent with a 3′,4′-methylenedioxy substitution pattern for the B-ring.[9] The ^{13}C-NMR spectrum (Table 2) showed that one methoxy carbon resonated at 56.5 ppm suggesting that none or only one of the *ortho* positions was substituted. It also showed a peak for another methoxy carbon at 61.0 ppm indicating that both positions *ortho* to the methoxy were substituted.[10—13] Thus, the substitution pattern of A-ring is 5-hydroxy-6,7-dimethoxy or 5-hydroxy-7,8-di-

Table 1. ^{1}H-NMR Spectral Data for Compounds **1**—**4** (δ)

	1[a]	**2**[b]	**3**[b]	**4**[b]
H-6	6.57 (s)	6.38 (d, 2.2)	6.44 (s)	6.35 (d, 2.3)
H-8		6.49 (d, 2.2)		6.43 (d, 2.3)
H-2′	7.66 (d, 2.0)	7.71 (d, 1.6)	7.78 (d, 1.8)	7.63 (d, 1.9)
H-5′	7.13 (d, 8.3)	6.96 (d, 8.3)	6.98 (d, 8.5)	6.92 (d, 8.4)
H-6′	7.76 (dd, 2.0, 8.3)	7.81 (dd, 1.6, 8.3)	7.88 (dd, 1.8, 8.5)	7.71 (dd, 1.9, 8.4)
H-1″			4.67 (d, 6.5)	4.59 (d, 7.5)
H-2″			5.51 (t, 6.5)	5.41 (t, 7.5)
H-4″			1.81 (s)	1.70 (s)
H-5″			1.78 (s)	1.64 (s)
OCH_3-7	3.90 (s)	3.89 (s)		3.87 (s)
OCH_3-8	3.80 (s)		3.94 (s)	
OH-3	9.71 (s)	6.65 (s)	6.64 (s)	
OH-5	12.19 (s)	11.69 (s)	11.51 (s)	12.68 (s)
OCH_2O-3′,4′	6.13 (s)	6.07 (s)	6.07 (s)	6.07 (s)

Multiplicity and coupling constants (*J*, Hz) are shown in parentheses. Measurements *a*) in DMSO-d_6 at 400 MHz, *b*) in $CDCl_3$ at 500 MHz.

* To whom correspondence should be addressed. e-mail: matuhiga@sci.u-ryukyu.ac.jp

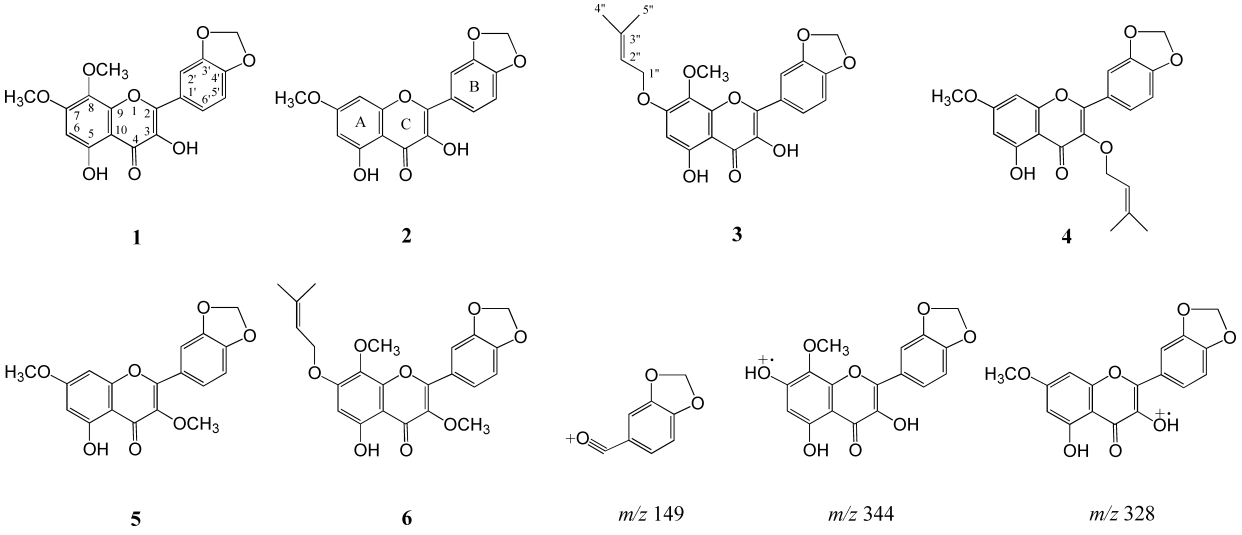

Chart 1. Structures and Significant Ions in the EI-MS of Flavonoids Isolated from *Melicope triphylla*

Table 2. ^{13}C-NMR Spectral Data for Compounds **1**—**4** (δ)

	1[a)]	**2**[b)]	**3**[b)]	**4**[b)]
C-2	146.1	145.3	145.2	156.0
C-3	136.4	135.9	135.5	137.7
C-4	176.5	175.2	175.5	179.1
C-5	155.9	160.9	156.1	162.1
C-6	95.3	98.0	96.4	97.8
C-7	158.0	165.9	158.0	165.4
C-8	128.3	92.2	129.5	92.1
C-9	147.8	156.8	148.5	156.7
C-10	103.4	103.9	103.2	106.0
C-1′	124.8	124.7	124.9	124.6
C-2′	107.2	107.8	107.8	108.8
C-3′	147.5	148.1	148.1	147.8
C-4′	148.8	149.3	149.3	149.7
C-5′	108.6	108.6	108.6	108.3
C-6′	122.6	122.9	123.0	123.8
C-1″			66.2	69.0
C-2″			118.8	119.7
C-3″			138.9	139.7
C-4″			25.9	25.8
C-5″			18.3	18.0
OMe-7	56.5	55.9		55.8
OMe-8	61.0		61.5	
OCH_2O-3′,4′	101.8	101.6	101.6	101.7

Measurements *a*) in DMSO-d_6 at 100 MHz *b*) in $CDCl_3$ at 125 MHz.

methoxy. These two patterns could be distinguished by comparing the intensities of the $[M^+]$ and $[M^+-CH_3]$ ion peaks in the MS spectrum.[14)] In 5-hydroxy-6,7-dimethoxyflavone derivatives, the relative intensity of $[M^+]$ is usually the base peak and is higher than that of $[M^+-CH_3]$, which is the base peak in 5-hydroxy-7,8-dimethoxyflavone derivatives.[14)] In the EI-MS spectrum of compound **1**, the relative intensity of $[M^+-CH_3]$ peak amounted to 100%, while that of $[M^+]$ peak was 65% indicating that the substitution pattern of the A-ring is 5-hydroxy-7,8-dimethoxy. The ^{13}C-NMR chemical shifts assigned to the A-ring of **1** agreed with the ^{13}C-NMR spectrum of 3,5-dihydroxy-4′,7,8-trimethoxyflavone.[15)] Nuclear Overhauser effect (NOE) experiments showed NOE effects between the methoxy protons at 3.90 ppm and H-6, and between H-6 and OH-5 (Fig. 1), thus confirming that the substitution pattern of the A-ring is 5-hydroxy-7,8-dimethoxy. Therefore, compound **1** was characterized as 3,5-dihydroxy-7,8-dimethoxy-3′,4′-methylenedioxyflavone. The assigned structure of compound **1** was fully supported by the heteronuclear multiple-bond correlation (HMBC) spectrum (Fig. 1).

Compound **2** (yellow powder) was shown to be a flavonoid from its UV absorption band maxima at 257 and 369 nm and its IR peak at 1664 cm^{-1}. The molecular formula of **2** was deduced as $C_{17}H_{12}O_7$ by HR-EI-MS. Its spectral data were closely comparable to those of **1**. The presence of 3- and 5-hydroxy groups in **2** was shown by its IR and ^{1}H-NMR spectra, and by the analysis of the UV spectrum utilizing shift reagents.[8)] The substitution pattern for the B-ring was shown to be 3′,4′-methylenedioxy by its ^{1}H-NMR and EI-MS[9)] spectra. The ^{1}H-NMR spectrum of **2** (Table 1) differed from that of **1** only by the absence of one of two methoxy signals in **1**. The peak of the methoxy group observed in the ^{1}H-NMR spectrum in C_6D_6 shifted by 0.62 ppm in $CDCl_3$, suggesting the presence of a 7-methoxy group.[16)] The ^{13}C-NMR spectrum (Table 2) showed that one methoxy carbon resonated at a higher magnetic field (δ=55.9 ppm), indicating that none or only one of the *ortho* positions was substituted.[10—13)] Thus, the substitution pattern of the A-ring is 5-hydroxy-7-methoxy. The ^{13}C-NMR chemical shifts observed for the A-ring of **2** agreed with the ^{13}C-NMR of 3,3′,4′,5-tetrahydroxy-7-methoxyflavone (rhamnetin).[17)] NOE experiments showed NOE effects between the methoxy protons and both H-6 and H-8 (Fig. 1), indicating that the methoxy group is at C-7. Therefore, compound **2** was characterized as 3,5-dihydroxy-7-methoxy-3′,4′-methylenedioxyflavone. The structure assigned to compound **2** was fully confirmed by the HMBC spectrum (Fig. 1).

Compound **3** (yellow powder) was shown to be a flavonoid from its UV and IR spectra. The molecular formula of **3** was deduced as $C_{22}H_{20}O_8$ by HR-EI-MS. Its spectral data were closely comparable to those of **1**. The presence of 3- and 5-hydroxy groups in **3** was shown by its IR and ^{1}H-NMR spectra, and by the analysis of the UV spectrum utilizing shift reagents.[8)] The substitution pattern for the B-ring was shown to be 3′,4′-methylenedioxy by its ^{1}H-NMR and EI-MS[9)] spectra. The ^{1}H-NMR spectrum of **3** (Table 1) were similar

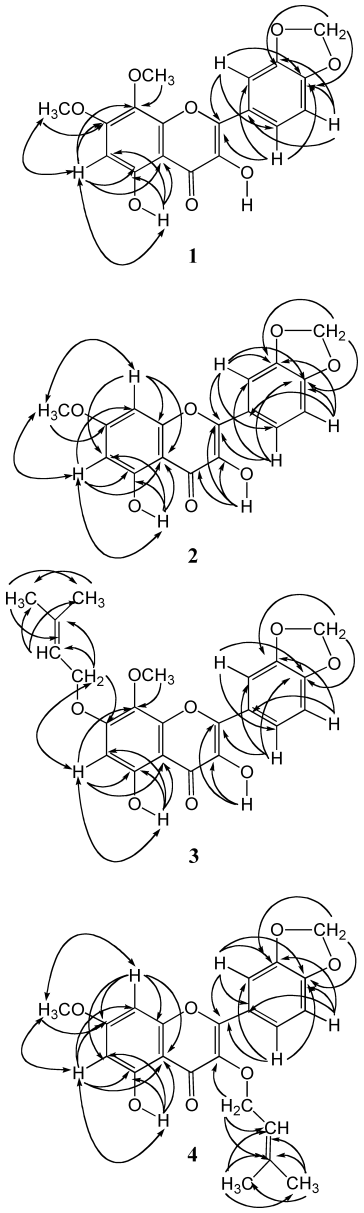

Fig. 1. HMBC Correlations (⟶) and the Key NOEs (⟷) for Compounds **1**—**4**

to those of **1**, except for the presence of an isopentenyloxy group signals [1.78 ppm, 1.81 ppm (each 3H, s, –CH=C(C$\underline{H}_3$)$_2$), 4.67 ppm (2H, d, J=6.5 Hz, –OC$\underline{H}_2$CH=C(CH$_3$)$_2$), 5.51 ppm (1H, t, J=6.5 Hz, –OCH$_2$C$\underline{H}$=C(CH$_3$)$_2$)] instead of one of two methoxy signals in **1**. The EI-MS spectrum showed a peak at *m*/*z* 344 that was assigned to the fragment ion produced by the loss of 2-methylbutadiene from molecular ion [M$^+$−CH$_2$=CCH$_3$–CH=CH$_2$] (Chart 1), supporting the presence of the isopentenyloxy group.[18] The ^{13}C-NMR spectrum (Table 2) showed one methoxy carbon at lower magnetic field (61.5 ppm), indicating the presence of two substituents at both positions *ortho* to the methoxy group.[10—13] Thus, the substitution pattern of A-ring is 5-hydroxy-6-methoxy-7-isopentenyloxy or 5-hydroxy-7-isopentenyloxy-8-methoxy. Comparison between the ^{13}C-NMR chemical shifts of the A-ring of **3** and those of 3,4′,5-trihydroxy-7-isopentenyloxy-3′,8-dimethoxyflavone[19] suggested that the substitution pattern of the A-ring was 5-hydroxy-7-isopentenyloxy-8-methoxy. NOE experiments showed NOE effects between the oxymethylene protons of the isopentenyloxy group and H-6 and between H-6 and OH-5 (Fig. 1), indicating that the isopentenyloxy group is at C-7 and that the substitution pattern of the A-ring is 5-hydroxy-7-isopentenyloxy-8-methoxy. Therefore, compound **3** is characterized as 3,5-dihydroxy-7-isopentenyloxy-8-methoxy-3′,4′-methylenedioxyflavone. This assigned structure was fully supported by the HMBC spectrum (Fig. 1). A prenylated flavone similar to compound **3** has previously been reported from *Comtonella microcarpa* (Rutaceae).[20]

Compound **4** (yellow powder) was shown to be a flavonoid from its UV and IR spectra. The molecular formula of **4** was deduced as $C_{22}H_{20}O_7$ by HR-electrospray ionization (ESI)-MS. The UV spectrum of **4** in methanol, exhibited a band I absorption maximum at a relatively shorter wavelength (λ_{max}=351 nm), suggesting that compound **4** was a 3-substituted flavonol.[8] The presence of a 5-hydroxy group in **4** was shown by its ^{1}H-NMR spectrum and by the analysis of the UV spectrum utilizing shift reagents.[8] The substitution pattern for the B-ring was shown to be 3′,4′-methylenedioxy by its ^{1}H-NMR and EI-MS[9] spectra. The ^{1}H-NMR spectrum of **4** (Table 1) were similar to those of **2**, except for the presence of an isopentenyloxy group signals instead of 3-hydroxy group signal in **2**. The EI-MS spectrum showed a peak at *m*/*z* 328 assigned to [M$^+$−CH$_2$=CCH$_3$–CH=CH$_2$] (Chart 1), supporting the presence of the isopentenyloxy group.[18] These spectral results suggest that the substitution pattern of the A- and C-rings is 3-isopentenyloxy-5-hydroxy-7-methoxy or 3-methoxy-5-hydroxy-7-isopentenyloxy. The ^{1}H-NMR spectrum displayed a 0.70 ppm shift of the methoxy group peak in C_6D_6 relative to the ^{1}H-NMR spectrum in $CDCl_3$ suggesting the presence of a 7-methoxy group.[16] The ^{13}C-NMR spectrum (Table 2) showed one methoxy carbon at a higher magnetic field (55.8 ppm), indicating that none or only one of the *ortho* positions was substituted, consistent with a 3-isopentenyloxy-5-hydroxy-7-methoxy substitution pattern for the A- and C-rings. NOE experiments showed NOE effects between the methoxy protons and both H-6 and H-8, and between H-6 and OH-5 (Fig. 1), indicating that the methoxy group is at C-7 and that the substitution pattern of the A- and C-rings is 3-isopentenyloxy-5-hydroxy-7-methoxy. Therefore, compound **4** is characterized as 5-hydroxy-3-isopentenyloxy-7-methoxy-3′,4′-methylenedioxyflavone. This assigned structure was fully supported by the HMBC spectrum (Fig. 1).

The flavonoids having a 7-hydroxy group are known to show a bathochromic shift of the band II (5—20 nm) upon sodium acetate addition.[8] This shift provides an excellent method for the characterization of 7-hydroxy groups.[8] Although compounds **1**—**3** did not contain 7-hydroxy groups, they exhibited a bathochromic shift of the band II (6—10 nm) upon sodium acetate addition. Horie *et al.* reported that 3-hydroxyflavones exhibited a bathochromic shift of the band I upon sodium acetate addition in the absence of a hydroxy group at C-7, but they did not describe the behavior of the band II.[21] In 3-hydroxyflavones such as compounds **1**—**3**, specification of the 7-hydroxy group by the addition of sodium acetate must be conducted with scrupulous care.

Compound **1** did not show any ichthyotoxic activity

against guppies (*Poecilia* (*Lebites*) *reticulate* PETERS) when tested at 10 ppm, which is attributable to the presence of hydroxy substituents at C-3 and C-5.[2] The ichthyotoxic activity of compounds **2**—**4** could not be determined due to the scarcity of the samples. These compounds are also assumed to be inactive in ichthyotoxicity tests because of the presence of hydroxy substituents.[2]

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 Shimadzu FTIR-8200A spectrometer; UV spectra were measured on a Hitachi 100-50 instrument; EI-MS and HR-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. ^{1}H- and ^{13}C-NMR spectra were acquired on a JEOL EX-270 (^{1}H: 270 MHz, ^{13}C: 67.8 MHz), a JEOL 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) and a JEOL α500 (^{1}H: 500 MHz, ^{13}C: 125 MHz) spectrometer. Two-dimensional NMR were acquired using a JEOL α500. Chemical shifts were given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The symbols s, d, t, q, dd, dq, m, and br denote singlet, doublet, triplet, quartet, double doublet, double quartet, multiplet, and broad, respectively.

Column chromatography (CC) and flash-column chromatography (FC) were carried out on Wakogel C-300 (Wako Pure Chemical) and Kieselgel 60H (Merck), respectively. HPLC was performed on Shimadzu LC-8A (Column, Waters μ Bondasphere 5μ C_{18} 100 Å [19×150 mm]; flow rate, 10 ml/min; detection, 358 nm).

Ichthyotoxicity tests were performed in 150 ml of a test sample aqueous solution against male guppies (*Poecilia* (*Lebites*) *reticulata* PETERS).[22]

Extraction and Isolation The fresh *M. triphylla* leaves (13.1 kg) collected at Tamagusuku, Okinawa in April 1992, were soaked in MeOH (40 l) for 3 months. The extract was evaporated to dryness and the residue was partitioned between EtOAc and H_2O. The EtOAc layer was evaporated and the residue was divided into C_6H_6-soluble and C_6H_6-insoluble portions (6.30 g). The C_6H_6-soluble portion (292.5 g) was subjected to CC on silica gel. Elution with C_6H_6, $CHCl_3$, $CHCl_3$–EtOAc (7 : 3), EtOAc and MeOH gave five fractions I (50.22 g), II (159.60 g), III (26.90 g), IV (6.46 g) and V (42.40 g), respectively.

Fraction I was divided into hexane-soluble and hexane-insoluble portions (0.27 g). The hexane-soluble portion was purified by CC on a silica gel (hexane–C_6H_6 gradient) to yield methyl *p*-geranyloxy-*trans*-cinnnamate, **4** (12 mg), β-amyrin, 5-hydroxy-3,7-dimethoxy-3′,4′-methylenedioxyflavone (**5**) and 5-hydroxy-7-isopentenyloxy-3,8-dimethoxy-3′,4′-methylenedioxyflavone (**6**). The hexane-insoluble portion was purified by FC on a silica gel (hexane–C_6H_6 gradient) and HPLC on ODS (8 : 2 MeOH/H_2O) to yield **2** (8 mg), **3** (6 mg) and **1** (45 mg).

Compounds **5** and **6** were identified by direct comparisons with authentic samples.

3,5-Dihydroxy-7,8-dimethoxy-3′,4′-methylenedioxyflavone (**1**): Yellow needles (C_6H_6–EtOH), mp 265—266 °C. Mg–HCl test: +. HR-EI-MS *m/z*: 358.0685 (Calcd for $C_{18}H_{14}O_8$: 358.0687). IR ν_{max} (KBr) cm^{-1}: 3300 (OH), 1649 (C=O). UV λ_{max} (MeOH) nm (log ε): 257 (4.36), 378 (4.23); λ_{max} (MeOH+NaOMe) nm (log ε): 267 (4.37), 418 (4.28); λ_{max} (MeOH+NaOAc) nm (log ε): 263 (4.35), 421 (4.25); λ_{max} (MeOH+$AlCl_3$+HCl) nm (log ε): 259 (4.49), 364 (4.05), 441 (4.30). MS *m/z* (rel. int. %): 358 [M^+] (55), 343 [M^+-CH_3] (100), 329 (5), 164 (6), 153 (7), 149 [$(OCH_2O)C_6H_3$–C≡O^+] (7), 69 (4). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2.

3,5-Dihydroxy-7-methoxy-3′,4′-methylenedioxyflavone (**2**): Yellow powder. HR-EI-MS *m/z*: 328.0579 (Calcd for $C_{17}H_{12}O_7$: 328.0581). IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 1664 (C=O). UV λ_{max} (MeOH) nm (rel. A: relative absorbance): 257 (1.00), 369 (0.95); λ_{max} (MeOH+NaOMe) nm (rel. A): 266 (1.00), 410 (0.83); λ_{max} (MeOH+NaOAc) nm (rel. A): 267 (1.00), 411 (0.89); λ_{max} (MeOH+$AlCl_3$+HCl) nm (rel. A): 267 (1.00), 361 (0.65), 424 (0.94). MS *m/z* (rel. int. %): 328 [M^+] (100), 299 (4), 285 (7), 149 [$(OCH_2O)C_6H_3$–C≡O^+] (9), 135 (3). ^{1}H-NMR (C_6D_6) δ: 3.27 ppm (3H, s, OCH_3-7); ($CDCl_3$): Table 1. ^{13}C-NMR: Table 2.

3,5-Dihydroxy-7-isopentenyloxy-8-methoxy-3′,4′-methylenedioxyflavone (**3**): Yellow powder. HR-EI-MS *m/z*: 412.1160 (Calcd for $C_{22}H_{20}O_8$: 412.1156). IR ν_{max} (KBr) cm^{-1}: 3340 (OH), 1651 (C=O). UV λ_{max} (MeOH) nm (rel. A): 260 (1.00), 378 (0.87); λ_{max} (MeOH+NaOMe) nm (rel. A): 268 (1.00), 423 (0.84); λ_{max} (MeOH+NaOAc) nm (rel. A): 267 (1.00), 422 (0.89); λ_{max} (MeOH+$AlCl_3$+HCl) nm (rel. A): 271 (1.00), 364(0.77), 440 (0.88). MS *m/z* (rel. int. %): 412 [M^+] (34), 344 [$M^+-CH_2=C(CH_3)$–CH=CH_2] (94), 329 (100), 315 (17), 149 [$(OCH_2O)C_6H_3$–C≡O^+] (8), 69 (12), 41 (8). ^{1}H-NMR: Table 1. ^{13}C-NMR: Table 2.

5-Hydroxy-3-isopentenyloxy-7-methoxy-3′,4′-methylenedioxyflavone (**4**): Yellow powder. HR-ESI-MS *m/z*: 419.1106 (Calcd for $C_{22}H_{20}NaO_7$: 419.1106). IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 1662 (C=O). UV λ_{max} (MeOH) nm (rel. A): 254 (1.00), 351 (0.91); λ_{max} (MeOH+NaOMe) nm (rel. A): 279 (1.00), 362 (0.84); λ_{max} (MeOH+NaOAc) nm (rel. A): 253 (1.00), 352 (0.88); λ_{max} (MeOH+$AlCl_3$+HCl) nm (rel. A): 272 (1.00), 358(0.83), 400 (0.84). MS *m/z* (rel. int. %): 396 [M^+] (18), 328 [$M^+-CH_2=C(CH_3)$–CH=CH_2] (100), 149 [$(OCH_2O)C_6H_3$–C≡O^+] (9). ^{1}H-NMR (C_6D_6) δ: 3.17 ppm (3H, s, OCH_3-7); ($CDCl_3$): Table 1. ^{13}C-NMR: Table 2.

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