Two New Polymethoxylated Flavones, a Class of Compounds with Potential Anticancer Activity, Isolated from Cold Pressed Dancy Tangerine Peel Oil Solids

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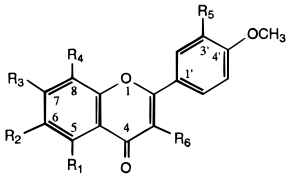
Ten polymethoxylated flavonoids were isolated and characterized from cold pressed tangerine oil solids; they are 5,6,7,3′,4′-pentamethoxyflavone (sinensetin) (**I**); 7-hydroxy-3,5,6,3′,4′-pentamethoxyflavone (**II**); 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone (**III**); 5,6,7,8,3′,4′-hexamethoxyflavone (nobiletin) (**IV**); 5,6,7,8,4′-pentamethoxyflavone (tangeretin) (**V**); 5,7,8,4′-tetramethoxyflavone (tetra-*O*-methylisoscutellarein) (**VI**); 7-hydroxy-3,5,6,8,3′,4′-hexamethoxyflavone (**VII**); 5,6,7,4′-tetramethoxyflavone (tetra-*O*-methylisoscutellarein) (**VIII**); 3,5,6,7,8,3′,4′-heptamethoxyflavone (**IX**); and 5,7,8,3′,4′-pentamethoxyflavone (**X**). Structures of the compounds were elucidated on the basis of spectroscopic methods and chemical data. Compounds **II** and **VII** are novel natural products; compounds **IV**, **V**, and **VIII** have been reported with significant activity against various strains of carcinoma cells; and compounds **I** and **IV** decrease erythrocyte aggregation and sedimentation *in vitro*. A biological activity screen of other compounds is in progress.

Keywords: *Polymethoxylated flavones; antitumor activity; Citrus; tangerine oils; Dancy tangerine*

INTRODUCTION

The treatment of cancer for some time has relied on the use of pharmaceuticals with selective toxicity to the cancer cells. This strategy has produced success in the treatment of some cancers; however, there is a need to reduce the toxicity of cancer chemotherapy. In the search for effective treatments of cancer in humans, edible plants may be a good source of new antitumor agents with reduced side effects. There has also been a resurgence of interest in the idea that cancer formation can be prevented by the consumption of certain foods. It is commonly accepted that citrus fruits and juices are health-promoting foods. In a recent literature review on citrus flavonoids, a broad spectrum of biological activity including anticarcinogenic and antitumor activities was discussed (Attaway, 1994). Polyhydroxylated flavonoids such as quercetin inhibit carcinogenesis in a number of models and selectively inhibit a variety of tumor cells growth. Polymethoxylated flavonoids such as *tangeretin* (**V**) and *nobiletin* (**IV**) are more potent inhibitors of tumor cell growth than hydroxylated flavonoids. This difference in activity may be due to greater membrane uptake of the polymethoxylated flavonoids (PMFs) since methoxylation of the phenolic groups decreases the hydrophilicity of the flavonoids (Kandaswami, 1991).

Citrus PMFs are minor components in citrus that are associated with the oil glands of the citrus peel flavedo. Composition of PMFs varies considerably between species and varieties (Mizuno et al., 1987, 1991; Tatum et al., 1978; Widmer and Barros, 1995). The tangerine variety Dancy has the highest total PMF content, containing approximately 5 times the amount found in the peel of sweet orange varieties (*Citrus sinensis* Osbeck.). In Dancy tangerines tangeretin (**V**) and nobiletin (**IV**) are predominant, while in sweet oranges



	R_1	R_2	R_3	R_4	R_5	R_6
I	OCH_3	OCH_3	OCH_3	H	OCH_3	H
II	OCH_3	OCH_3	OH	H	OCH_3	OCH_3
III	OH	OCH_3	OCH_3	OCH_3	OCH_3	H
IV	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	H
V	OCH_3	OCH_3	OCH_3	OCH_3	H	H
VI	OCH_3	H	OCH_3	OCH_3	H	H
VII	OCH_3	OCH_3	OH	OCH_3	OCH_3	OCH_3
VIII	OCH_3	OCH_3	OCH_3	H	H	H
IX	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3
X	OCH_3	H	OCH_3	OCH_3	OCH_3	H

Figure 1. Structures of compounds **I**−**X**.

nobiletin (**IV**), sinensetin (**I**), and heptamethoxyflavone (**IX**) predominate (Figure 1). Chromatographic separations of citrus peel PMFs have been done primarily with normal stationary phases. Swift (1967) reported separation of five PMFs from orange peel juice by thin layer chromatographic (TLC) with silica gel and hexane/butanol (85:15). Separation of citrus PMFs has also been accomplished with normal phase high-performance liquid chromatography (HPLC). Bianchini and Gaydou (1980) reported on the separation efficiency of 17 PMFs with heptane/ethanol compared to heptane/2-propanol using a 250 × 4 mm LiChrosorb Si 60 column. Eleven of the PMFs were isolated from orange peel. Gaydou et al. (1987) compared the PMF composition of oil extracted from eight mandarin and seven orange varieties. Six PMFs were separated with LiChrosorb silica. More recently, Mizuno et al. (1987, 1991) separated seven PMFs from the peel of many citrus varieties using normal phase HPLC for chemotaxonomic comparision. A comprehensive HPLC study of many citrus flavanoids includes the PMFs and takes into account the effect of

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Table 1. ^{1}H-NMR Data of Compounds I–X (δ, J = Hz)[a]

compd	H-3	H-5	H-6	H-7	H-8	H-2′	H-3′	H-4′	H-5′	H-6′	OCH_3
I	6.59 s				6.80 s	7.33 d (J = 2.1)			6.98 d (J = 8.4)	7.52 dd (J = 2.1, 8.4)	4.00 × 2, 3.98, 3.96, 3.92
II					6.77 s	7.80 d (J = 2.2)			6.99 d (J = 9.0)	7.79 dd (J = 2.2, 9.0)	3.91, 3.95, 3.97, 3.98, 4.02
III	6.61 s					7.42 d (J = 2.1)			6.99 d (J = 9.0)	7.58 dd (J = 2.1, 9.0)	4.12, 3.99 × 2, 3.97, 3.96
IV	6.62 s					7.41 d (J = 2.1)			6.99 d (J = 9.0)	7.57 dd (J = 2.1, 9.0)	4.11, 4.03, 3.98, 3.97, 3.96 × 2
V	6.60 s					7.88 d (J = 9.0)	7.02 d (J = 9.0)		7.02 d (J = 9.0)	7.88 d (J = 9.0)	4.10, 4.02, 3.95 × 2, 3.89
VI	6.60 s		6.44 s			7.90 d (J = 9.0)	7.02 d (J = 9.0)		7.02 d (J = 9.0)	7.90 d (J = 9.0)	4.10, 3.99, 3.89, 3.96
VII						7.90 d (J = 9.0)			7.02 d (J = 9.0)	7.91 dd (J = 2.1, 9.0)	4.13, 4.04, 4.00 × 2, 3.97, 3.96
VIII	6.59 s				6.80 s	7.78 d (J = 9.0)	6.97 d (J = 9.0)		6.97 d (J = 9.0)	7.78 d (J = 9.0)	4.10, 3.99, 3.94, 3.88
IX						7.80 d (J = 2.4)			7.01 d (J = 8.4)	7.84 dd (J = 2.4, 8.4)	4.10, 4.00, 3.97 × 3, 3.95, 3.89
X	6.61 s		6.44 s			7.42 d (J = 2.4)			6.98 d (J = 2.4, 8.4)	7.58 dd (J = 2.4, 8.4)	4.01, 3.99, 3.97, 3.96 × 2

[a] Solvent, $CDCl_3$; s, singlet; d, doublet; dd, doublet doublet.

chemical structure on separation (Castillo et al., 1994). There has been success in separating the polymethoxylated flavones using supercritical fluid technology (Morin et al., 1991).

The waste material produced during refining of cold pressed peel oils contains a high concentration of polymethoxylated flavones. Cold pressed peel oil solids are isolated from citrus oils by a process called winterizing. Citrus peel oil is placed in cold storage (−20 °C), which causes the solids to precipitate. The solids are removed and discarded as waste (Braddock et al., 1992). Citrus oil is a very important byproduct produced in citrus juice processing. The purpose of this study was to carry out a comprehensive chemical separation of Dancy tangerine cold pressed peel oil solids to determine if there were any new polymethoxylated flavones present.

MATERIALS AND METHODS

General Chromatography. Normal phase TLC was performed on high-performance silica gel plates (Whatman 4807-400), using two solvent systems: solvent A, chloroform/methanol (95:5); solvent B, benzene/ethyl acetate (90:10). Normal phase plates were visualized by observation under multiband UV lamp (UV-254/366 nm) and/or by spraying with H_2SO_4/ethanol (1:4) followed by charring with a heat gun. Reversed-phase TLC was performed on octadecyl (C_{18}) plates (Whatman 4803-600) with three solvent systems: solvent C, methanol/water (80:20); solvent D, ethanol/water (60:40); solvent E, acetonitrile/tetrahydrofuran (90:10). Reversed-phase plates were observed under multiband UV lamp (UV-254/366 nm). Flash chromatography was performed on silica gel (Baker Analyzed 40μ lot G42353) using solvent B.

HPLC. Preparative HPLC was carried out on an LDC analytical system (Thermo Separation Products Inc., Riviera Beach, FL) consisting of a ConstaMetric 3200 pump, a Model IV refractive index detector, a Rheodyne Model 7125 injection valve, and a Gilson FC203B fraction collector (Gilson Medical Electronics, Middleton, WI). The column used was a Rainin (Woburn, MA) Dynamax 60A 8 μm C_{18}, 21.4 mm i.d. × 25 cm, coupled to a Dynamax 60A guard column 8 μm C_{18}, 21.4 mm i.d. × 5 cm. The HPLC separations were done with either solvent C or solvent D described above.

Mass, Nuclear Magnetic Resonance, and Ultraviolet Spectroscopy. The mass spectral analyses were carried out on a Finnigan MAT 95Q magnetic sector mass spectrometer (Finnigan MAT, San Jose, CA), with electron ionization at 70 eV and low-resolution scan. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a General Electronic QE-300 spectrometer; chemical shifts in $CDCl_3$ are reported relative to internal TMS. UV spectra were recorded on a Perkin-Elmer (Norwalk, CT) Lambda 5 spectrophotometer.

Extraction and Isolation. The oil was extracted from mature fruit when the juice was extracted. The oil was then placed in a −20 °C storage facility for several months, a process known as winterizing. When the oil is chilled to −20 °C, a precipitate forms at the bottom of the holding tank. The sample of the Dancy tangerine cold pressed peel oil solids was obtained from Florida Flavors (Lakeland, FL). Twenty grams of dried tangerine oil solids was applied to a glass column (5 cm i.d.) filled with silica gel (200 g, 40 μm flash silica gel) for chromatography. The column was eluted with benzene/ethyl acetate 75:25 → ethyl acetate → ethyl acetate/2-propanol 90:10 → 2-propanol, increasing solvent polarity in linear gradient. Fractions (100 mL) were collected, and their content was monitored by TLC. Those fractions containing the same polymethoxylated flavones were pooled together, evaporated under vacuum, and further purified by using C_{18} preparative HPLC (prep-HPLC). Solvent systems C and D (listed above) were used for the prep-HPLC separation. Reversed-phase TLC was used to determine which solvent system, C or D, would be used for each separation. Peaks were collected using a Gilson FC203b fraction collector, and then they were concentrated *in vacuo*. The dried samples were stored in a freezer (−20 °C) for spectral analysis and biological testing.

RESULTS AND DISCUSSION

We performed exploratory analyses of polymethoxylated flavonoids of Dancy tangerine cold pressed peel oil solids and succeeded in isolating 10 polymethoxylated flavones. Compounds **IV**, **V**, and **VIII** have been reported to exhibit antitumor activities (Bracke et al., 1994; Machida et al., 1989). Compounds **I** and **IV** reportedly decrease erythrocyte aggregation and sedimentation *in vitro* and might be useful in the dietary control of high blood viscosity syndrome (Robbins, 1977). Compounds **II** and **VII** are novel natural products.

Cold pressed tangerine peel oil solids were obtained from the peel of processed Dancy tangerine fruit. After the fractionation with the silica gel column chromatography and prep-HPLC, 10 compounds were purified (Figure 1). ^{1}H-NMR, ^{13}C-NMR, and UV spectra revealed a great similarity in these compounds and indicated the structural features of polymethoxylated flavonoids.

Methoxylation patterns of all of the flavones were determined from their ^{1}H-NMR spectra (Table 1). Spectra of compounds **III**, **IV**, **V**, **VII**, and **IX** had no

Table 2. ^{13}C-NMR Data of Compounds I−X (δ)[a]

carbon no.	I	II	III	IV	V	VI	VII	VIII	IX	X
C-2	160.0	151.8	158.8	161.0	162.2	162.3	150.7	162.0	151.1	160.5
C-3	107.4	140.0	106.5	106.7	106.7	106.9	142.9	106.8	140.8	107.2
C-4	177.2	17136	179.3	177.4	177.3	177.9	171.8	176.8	173.9	177.9
C-5	154.5	137.4	149.1	144.0	144.0	152.0	143.6	154.3	143.9	152.0
C-6	140.4	142.8	136.2	138.0	138.1	92.6	147.7	140.5	137.8	92.6
C-7	157.6	158.2	152.9	151.4	151.3	156.3	146.9	157.5	151.3	156.3
C-8	96.3	96.1	132.9	138.0	148.4	130.8	137.4	96.2	137.8	130.7
C-9	152.6	153.7	142.9	147.7	147.7	156.3	146.9	152.5	148.2	156.3
C-10	112.9	109.8	107.5	114.8	114.8	109.1	111.7	112.4	115.1	109.1
C-1′	124.1	123.8	123.7	124.0	123.8	123.8	123.9	123.7	123.5	124.1
C-2′	108.7	110.6	114.6	108.7	127.8	127.7	110.6	127.4	110.9	108.7
C-3′	149.3	148.9	145.6	149.3	114.4	114.5	149.0	114.2	148.8	149.2
C-4′	151.9	150.5	149.0	151.9	161.1	160.7	151.7	160.9	153.0	151.5
C-5′	111.0	111.0	110.5	111.0	114.6	114.5	111.3	114.2	111.0	111.0
C-6′	119.7	120.8	121.6	119.6	127.6	127.7	121.1	127.4	121.9	119.6
OCH_3	62.2	62.3	62.1	62.3	62.3	61.5	62.3	61.9	62.3	61.5
	61.6	61.6	61.7	62.0	62.1	(8)	61.8	61.3	61.9	(8)
	(5,6)	(3,5)	61.2	61.8	61.9	56.6	61.7	(5,6)	61.8	56.5
	56.3	56.4	(6,7,8)	61.7	61.7	56.2	61.6	56.1	61.7	56.3
	56.1	56.1	56.1	(5,6,7,8)	(5,6,7,8)	55.0	(5,6,8,3)	55.3	59.9	56.1
	56.0	56.0	56.0	56.1	55.5	(5,7,4′)	56.0	(7,4′)	(3,5,6,7,8)	56.0
	(7,3′,4′)	(7,3′,4′)	(3′,4′)	56.0	(4′)		55.9		56.0	(5,7,3′,4′)
				(3′,4′)			(3′,4′)		55.9	
									(3′,4′)	

[a]Solvent, $CDCl_3$.

A-ring aromatic proton resonances, indicating methoxylation at C-5, C-6, C-7, and C-8. Spectra of each of the remaining five flavones had a signal aromatic A-ring proton absorption. In compounds **I**, **II**, and **VIII** the signal occurred $\Delta\delta$ 0.3 downfield from the like signal in the others, indicating that the proton was attached to C-8, whereas in the remainder (**VI** and **X**) the proton was at C-6. An olefinic proton signal at δ 6.6, which has been assigned and is attached to C-3, was recognized (Sugiyama et al., 1993). B-ring signals were, likewise, diagnostic for B-ring substitution patterns. Spectra of compounds **V**, **VI**, and **VIII** each had a pair of two-proton, *ortho* coupled doublets arising from two pairs of degenerate protons (H-2′, H-6′ and H-3′, H-5′), shoing the presence of an A_2B_2 pattern in the B-ring, typical of para-substituted benzene rings. Spectra in the B-ring of compounds **I**−**IV**, **VII**, **IX**, and **X** with ABX type aromatic proton signals showed a pattern of three protons, the multiplicity of which showed one proton coupled to the remaining two which are, in turn, not coupled to each other. The size of the coupling constants (approximately J= 2 and 8.5 Hz) is characteristic of meta and ortho coupling as found in 3′,4′-methoxylated flavonoids. Besides substitution patterns, ^{1}H-NMR spectra provided the number of methoxyl groups in each flavone and confirmed the absence of any other hydrogen-bearing substitutions. The strongly chelated 5-OH present in compound **III** gave rise to a low-field singlet (δ 12.55) in the spectra.

^{13}C-NMR spectral assignments of the polymethoxylated flavones are shown in Table 2. It is well-known (Panichpol et al., 1978; Roitman et al., 1985) that resonances of aromatic methoxyl groups attached to di-ortho-substituted carbons occur considerably downfield (at ca. δ 60) from signals of those attached to carbons bearing one or no ortho substituent (ca. δ 55), thus providing a useful diagnostic tool for structural analysis of polymethoxyflavones. Methoxyl groups at the C-3 position of the quasi-aromatic heterocyclic C-ring of the flavones are included with the other di-ortho-substituted methoxyls.

The B-ring signals of compounds **V**, **VI**, and **VIII** were readily assigned by consideration of symmetry and standard chemical shift effects of a single oxygen on an aromatic ring. The finding of two pairs of degenerate methine carbon signals parallels the ^{1}H-NMR data requiring the oxygen to be placed at C-4′. The lower field set of methine signals (δ 127.7) occurs at nearly the same chemical shift as that of benzene, and the absence of a sizeable oxygen-caused chemical shift indicates that those carbons are meta to the site of oxygenation and, hence, C-2′ and C-6′, the other pair of signals (C-3′ and C-5′), exhibits typical ortho shielding causing the shift upfield to 114.5. Compared with the B-ring signals of compounds **V**, **VI**, and **VIII**, the spectra of the dioxygenated B-rings (compounds **I**−**IV**, **VII**, **IX**, and **X**) have only two resonances (C-1′ and C-5′) that remain unchanged, hence meta, to the second oxygen, thereby locating it at C-3′. Of the two C−O signals, the one at lowest field was assigned to C-4′ (δ 150−153) because of the para-substituent effect of the C-ring on C-4′. As mentioned, assignments of C-1′ and C-5′ signals for the 3′,4′-dioxygenated flavones were readily made by comparison with the values observed for **V**, **VI**, and **VIII**. The effect of an additional meta substituent was expected to be minimal. This left two methine carbon resonances (C-2′ and C-6′) to be assigned. Both C-2′ and C-6′ are symmetrically disposed about the C-1′−C-4′ axis and, therefore, the chemical shift difference between their signals is due to the C-3′ substituent alone. Because oxygen shields ortho carbons more than para ones, the upfield methine signal was assigned to C-2′. All of the polymethoxylated flavones exhibited tri- and tetraoxygenated signals at A-ring and separation of oxygenated from nonoxygenated carbon signals based on their characteristic chemical shift ranges. The C-10 signal can be most readily identified because its multiplicity separates it from any methine carbon signals and its chemical shift is far upfield from the remaining quaternary carbon signals which arise from oxygenated carbons. Carbonyl resonance is influenced by hydrogen bonding interactions with the 5-OH group, causing a ca. $\Delta\delta$ 3.5 downfield shift. When the samples **II** and **VII** were treated with sodium methoxide, their UV spectra exhibited a bathochromic shift of 45 and 53 nm, respectively, in the major absorption band (band II)

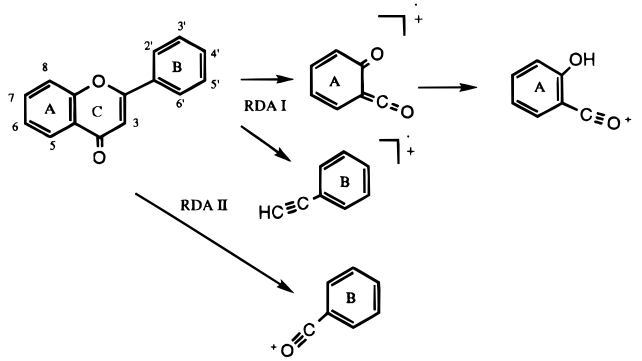

Figure 2. Mass spectral fragmentation of flavonic skeleton by retro-Diels–Alder reactions.

relative to their spectrum in methanol, which indicated the presence of an hydroxy group at the 7 position in **II** and **VII** (Scott, 1964).

The mass spectra of all the flavones provided intense molecular ions, accurate mass measurement of which provides elemental formulas confirming numbers of oxygens and methyl groups. The $[M - 15]^+$ fragment as base peak in 6- and (or) 8-methoxyflavones and [M − 19] species were specific for the 3-methoxyflavones (Berahia et al., 1994). The retro-Diels–Alder (RDA) fragments of these polymethoxylated flavones (Rizzi et al., 1984; Berahia et al., 1994) gives a good indication of the substitution patterns of the A- and B-rings (Figure 2).

Two new PMFs were isolated from the cold pressed peel oil solids of Dancy tangerine. This class of compounds has been known in the literature for some time, not only from the *Citrus* sp. but also from other species in the family Rutaceae (Harborne, 1984). The polymethoxylated flavones are not found in any other commonly consumed food product. The consumption of all the PMFs in orange juice is on the order of 1−2 mg/240 mL (8 fl oz) (Widmer et al., 1996). The level of the major PMFs in Dancy tangerine peel has been determined by HPLC (Widmer et al., 1995). The PMFs have an impact on *in vitro* animal and human cancer cell lines (Attaway, 1994; Bracke et al., 1994) and capillary fragility (Robbins, 1977). There are no direct data on whether these products have any beneficial effects on human health. It is widely accepted that citrus foods are health-promoting foods (National Research Council, 1989). The isolation of these natural products will provide materials for further testing. This will enable us to better determine the contribution made by the polymethoxylated flavones to human health.

Spectral and Chemical Characteristics of Compounds I–X. *Sinensetin [5,6,7,3′,4′-Pentamethoxyflavone (**I**)] (Sugiyama et al., 1993)*: colorless needles (MeOH); mp 178−179 °C; t_R 11.5 min (prep-HPLC with system D); R_f 0.45 (TLC with system D); UV (MeOH) λ_{max} 326, 261 (sh), 236 (sh), 213 nm; MS, *m/z* (%) 372 (M^+, 31.97), 357 ($M^+ - CH_3$, 100), 341 ($M^+ - OCH_3$, 13.6), 296 (2.16), 195 (4.55), 179 (10.92), 119 (7.94), 93 (14.66), 91 (13.21).

*7-Hydroxy-3,5,6,3′,4′-pentamethoxyflavone (**II**)*: pale yellow needles (MeOH); mp 183−184 °C; t_R 17.4 min (prep-HPLC with system C); R_f 0.32 (TLC with system C); UV (MeOH) λ_{max} 352, 251 (sh), 205 nm; λ_{max} (MeOH + NaOMe) 397, 254 (sh), 204 nm; MS, *m/z* (%) 388 (M^+, 100), 373 ($M^+ - CH_3$, 90.69), 357 ($M^+ - OCH_3$, 14.02), 343 (11.01), 331 (13.94), 327 (16.49), 299 (5.78), 195 (4.09), 194 (8.61), 181 (4.13), 167 (15.11), 165 (22.50), 163 (7.01), 149 (10.66), 93 (11.91).

*5-Hydroxy-6,7,8,3′,4′-pentamethoxyflavone (**III**) (Kinoshita et al., 1996)*: yellow needles; mp 144−145 °C (MeOH); t_R 13.8 min (prep-HPLC with system D); R_f 0.42 (TLC system D); UV (MeOH) λ_{max} 337, 280, 251 (sh), 205 nm; MS, *m/z* (%) 388 (M^+, 100), 373 ($M^+ - CH_3$, 95.83), 358 ($M^+ - OCH_3$, 5.50), 343 ($M^+ - CH_3 - OCH_3$, 6.42), 327 (4.21), 259 (3.31), 211 (16.92), 194 (4.37), 186 (7.56), 183 (19.84), 165 (9.79), 163 (9.41), 148 (5.33), 147 (5.61), 127 (13.01), 91 (11.44), 69 (57.07).

*Nobiletin [5,6,7,8,3′,4′-Hexamethoxyflavone (**IV**)] (Sugiyama et al., 1993)*: colorless needles (MeOH); mp 138−138 °C; t_R 25.0 min (prep-HPLC with system C); R_f 0.29 (TLC with system C); UV (MeOH) λ_{max} 328, 268 (sh), 246 (sh), 219 nm; MS, *m/z* (%) 402 (M^+, 43.32), 387 ($M^+ - CH_3$, 100), 371 ($M^+ - OCH_3$, 4.37), 359 (4.56), 344 (9.62), 326 (4.85), 225 (6.46), 197 (20.42), 194 (10.02), 182 (12.10), 162 (8.91), 153 (5.06), 147 (5.24), 119 (6.00), 91 (9.48), 83 (24.05).

*Tangeretin [5,6,7,8,4′-Pentamethoxyflavone (**V**)] (Sugiyama et al., 1993)*: colorless needles (MeOH); mp 156−157 °C; t_R 28.5 min (prep-HPLC with system C); R_f 0.60 (TLC with system C); UV (MeOH) λ_{max} 320, 269, 206 nm; MS, *m/z* (%) 372 (M^+, 38.32), 357 ($M^+ - CH_3$. 100), 341 ($M^+ - OCH_3$, 4.20), 329 (4.57), 314 (10.38), 296 (6.19), 225 (7.41), 197 (25.97), 182 (19.40), 167 (7.02), 153 (7.85), 135 (16.36), 132 (32.16), 117 (20.31), 89 (32.58), 83 (55.56)

*Tetra-O-methylisoscutellarein [5,7,8,4′-Tetramethoxyflavone (**VI**)] (Sugiyama et al., 1993)*: colorless needles (MeOH); mp 216−217 °C; t_R 19.4 min (prep-HPLC with system D); R_f 0.31 (TLC with system D); UV (MeOH) λ_{max} 310, 267, 206 nm; MS, *m/z* (%) 342 (M^+, 78.55), 327 ($M^+ - CH_3$, 100), 325 (4), 314 ($M^+ - CO$), 313 (16), 312 ($M^+ - OCH_3$), 300 (16), 298 (29), 296 (5), 211 (11), 195(11), 167 (24), 139 (18), 132 (10).

*7-Hydroxy-3,5,6,8,3′,4′-hexamethoxyflavone (**VII**)*: yellow needles (MeOH); mp 147−148 °C; t_R 30.5 min (prep-HPLC with system D); R_f 0.23 (TLC with system D); UV (MeOH) λ_{max} 363, 254, 207 nm; λ_{max} (MeOH + NaOMe) 416, 374, 254, 207 nm; MS, *m/z* (%) 418 (M^+, 58.16), 403 ($M - CH_3$, 100), 400 ($M^+ - H_2O$, 15.43), 390 (4.76), 388 ($M^+ - OCH_3$, 9.85), 227 (14.21), 226 (5.43), 211 (17.02), 196 (21.23), 161 (12.31), 146 (6.49), 134 (10.09), 106 (5.07).

*Tetra-O-methylscutellarein [5,6,7,4′-Tetramethoxyflavone (**VIII**)] (Sugiyama et al., 1993)*: colorless needles (MeOH); mp 141−142 °C; t_R 14.3 min (prep-HPLC with system C); R_f 0.41 (TLC with system C); UV (MeOH) λ_{max} 316, 264, d 216 nm; MS, *m/z* (%) 342 (M^+, 78.12), 327 ($M^+ - CH_3$, 100), 311 ($M^+ - OCH_3$, 5.43), 298 (21.62), 283 (16.51), 211 (16.02), 194 (9.15), 167 (21.39), 105 (15.31), 77 (4.98).

*3,5,6,7,8,3′,4′-Heptamethoxyflavone (**IX**) (Sugiyama et al., 1993)*: pale yellow needles (MeOH); mp 130−131 °C; t_R 22.5 min (prep-HPLC with system C); R_f 0.41 (TLC with system C); UV (MeOH) λ_{max} 339, 266 (sh), 251, 208 nm; MS, *m/z* (%) 432 (M^+, 61.01), 417 ($M^+ - CH_3$, 100), 404 ($M^+ - CO$, 6.78), 402 ($M^+ - OCH_3$, 13.22), 389 (19.21), 386 (7.51), 240 (6.51), 225 (16.78), 212 (4.13), 197 (29), 162 (8.04), 147 (4.05), 135 (18.28), 107 (10.23).

*Isosinensetin [5,7,8,3′,4′-Pentamethoxyflavone (**X**)] (Sugiyama et al., 1993)*: colorless needles (MeOH); mp 199−200 °C; t_R 15.4 min (prep-HPLC with system C); R_f 0.38 (TLC with system C); UV (MeOH) λ_{max} 337, 268, 246 (sh), 206 nm; MS, *m/z* (%) 372 (M^+, 85.02), 357 ($M^+ - CH_3$, 100), 371 ($M^+ - 1$, 15.02), 344 ($M^+ - CO$, 3.50), 343 (15.21), 341 ($M^+ - OCH_3$), 329 (21.10), 326 (6.21),

211 (5.00), 210 (4.50), 209 (3.21), 195 (12.46), 182 (2.56), 162 (5.04), 147 (5.03), 105 (16.89), 77 (9.03).

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