Semisynthesis of polymethoxyflavonoids from naringin and hesperidin

Yue Li, Shuanglian Cai, Kailin He and Qiuan Wang*

College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, P.R. China

Polymethoxyflavonoids (PMFs) possess important biological activities, notably as anticancer agents. Semisynthesis of a series of PMFs were performed by glycoside hydrolysis, dehydrogenation, bromination, aromatic nucleophilic substitution, *O*-methylation, dimethyldioxirane oxidation and regioselective demethylation, starting from abundant and inexpensive natural sources naringin and hesperidin. A new synthetic method for selective methylation using CuBr catalysed and microwave-assisted reaction was developed, and the dimethyl dioxirane oxidation of flavones to flavonols was much improved. The new semisynthetic route has the advantages of easy availability of starting materials, simple operation and good yields.

Keywords: polymethoxyflavonoids, naringin, hesperidin, semisynthesis, microwave-assisted Ullmann reaction

Polymethoxyflavonoids (PMFs) are a class of natural products, which almost exclusively exist in the citrus genus, particularly in the peel of sweet orange [*Citrus sinensis* (L.) Osbeck] and mandarin (*Citrus reticulate* Blanaco).[1] PMFs are of particular interest due to their broad spectrum of biological activities, including anticarcinogenic, anti-inflammatory and antiviral activities.[2–4]

Many *in vitro* and *in vivo* studies have indicated the protective effects of PMFs against the occurrence of cancer. For example, tangeretin (5,6,7,8,4′-pentamethoxyflavone), a very abundant PMF in citrus peels, exerts anticancer effects by multiple mechanisms. It can inhibit tumour cell growth through inhibiting the activities of cyclin-dependent kinase 2 (CdK2) and CdK4 as well as increasing the content of CdK protein inhibitors p21 and p27 in human colorectal carcinoma cells.[5] Moreover, its antitumour activity has also been demonstrated in lung tumour by repressing induced and constitutively expressed cyclooxygenase-2 (COX-2).[6] Nobiletin (3′,4′,5,6,7,8-hexamethoxyflavone), the most abundant PMF in citrus peels, shows strong anti-proliferative activity against six human cancer cell lines including lung, prostate, colon, melanoma, oestrogen receptor positive and oestrogen receptor negative breast cancer cells.[7] In particular, increasing attention has been paid to its antitumour metastatic activity due to the inhibition of gene expression and the production of some matrix metalloproteinases (MMP-1, -3 and -9).[8] 3,5,6,7,8,3′,4′-Hepamethoxy-flavonoid also shows a large inhibitory effect on mouse skin tumour promotion in an *in vivo* two-stage carcinogenesis test.[9] Furthermore, screening against cell proliferation and the induction of apoptosis in HL-60 leukaemia cells revealed the strong activity of 5-hydroxylated PMFs.[10]

Despite many recent reports on PMFs and their biological activities, the synthesis of these compounds has been much less studied.[11,12] The full potential of this class of compounds to be used as drugs or bioactive molecules has yet not been realised. Recently, as a continuation of our investigation of bioactive flavonoids and the development of new antitumour active compounds, we have reported the total synthesis of tangeretin and nobiletin.[13,14] However, these multistep processes are excessively tedious, partly due to the tetramethoxylation pattern of ring A. Thus, we turned to a semisynthetic method starting from readily available natural sources. We now report the facile synthesis of a series of polymethoxyflavonoids **1**–**12** (Scheme 1) with good yields from abundant and inexpensive natural sources naringin and hesperidin, the predominant flavonoids in sweet orange.

* Correspondent. E-mail: wangqa@hnu.edu.cn

Result and discussion

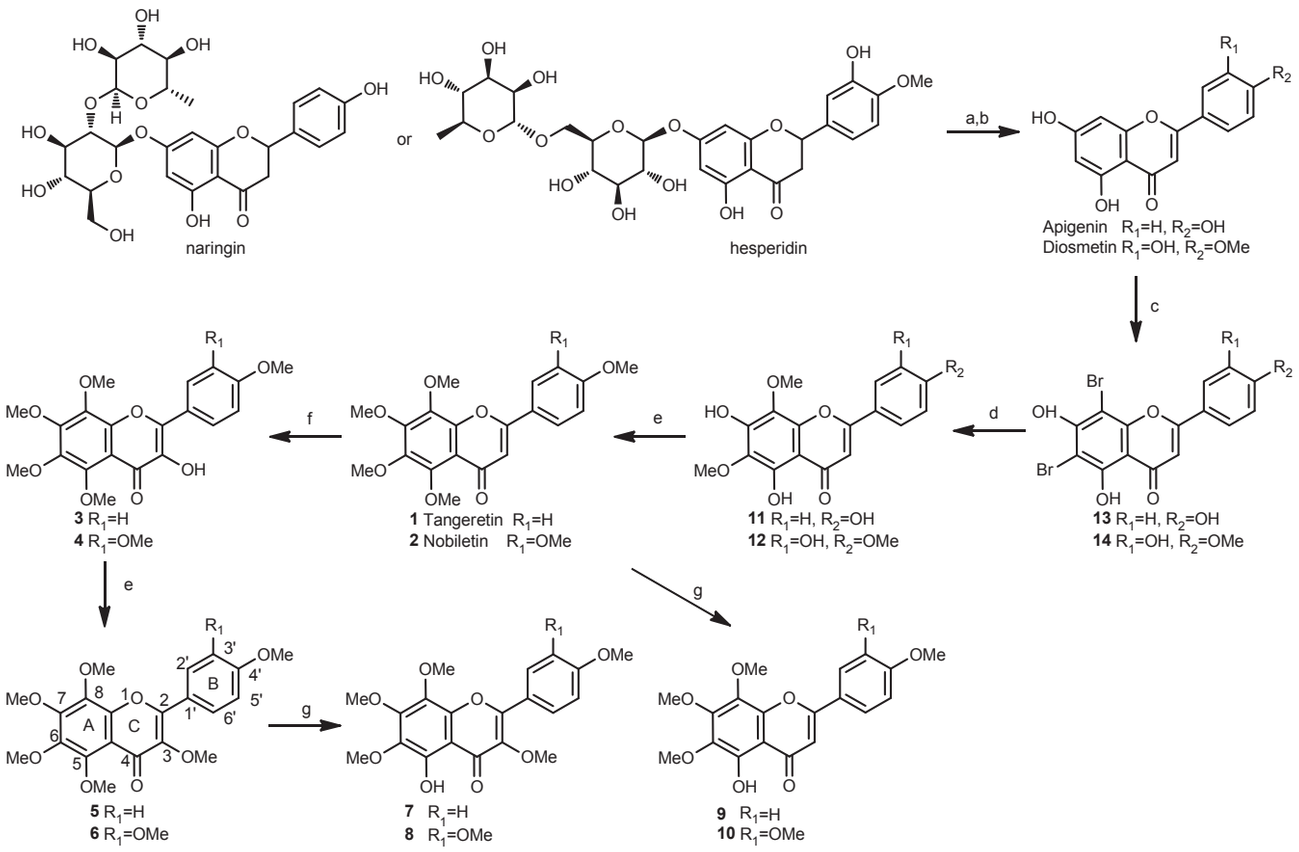
Scheme 1 outlines the synthesis route of a series of polymethoxyflavonoids **1**–**12** using low-priced naringin or hesperidin as the starting materials. Apigenin and diosmetin were readily obtained from naringin and hesperidin by acid hydrolysis of their glycosidic bonds followed by dehydrogenation with I_2/Py according to the method previously described by us.[15,16]

Next, our efforts were concentrated on the methoxylation of apigenin or diosmetin at the 6,8-positions. We planned to use a two-stage procedure, firstly brominating with 2 equiv. of *N*-bromosuccinimide (NBS) in trifluoroacetic acid and then using an Ullmann-type reaction to replace the bromo groups with methoxyl groups. Gratifyingly, apigenin and diosmetin were brominated in 91–93% yield. Bromination at both C-6 and C-8 was confirmed by ^{1}H NMR spectroscopy (loss of H-6 and H-8 signals, unchanged signals for the spin–spin coupling system of the B ring).

Although an efficient procedure with a copper-catalysed Ullmann-type reaction has been reported in the literature.[17,18] Unfortunately, the same reaction could not be used with sensitive compounds such as polyhydroxyflavones. Therefore we decided to optimise the method of CuBr-promoted methanolysis of 6,8-dibrominated apigenin **13** and 6,8-dibrominated diosmetin **14** using a mixture of MeOH/DMF and the results are shown in Table 1. Firstly, we chose different copper (I) salts (CuCl, CuBr or CuI) as the catalyst using the same reagents and solvents at 100 °C. But only the CuBr-catalysed reaction gave a new spot on TLC plate, which, however, could not be isolated from the raw mixture in sufficiently pure form for characterisation.

Table 1 Yields of methoxylation products of 6,8-dibrominated flavones **13** and **14** *via* a methanolysis reaction in DMF/MeOH catalysed by copper (I) halides with and without microwave irradiation at different reaction times and temperatures

Entry	Compound	CuX (0.01 equiv.)	T/°C	Time/h	Microwave power/W	Yield/%
1	**13**	CuCl	100	12	0	None
2	**13**	CuBr	100	12	0	Trace
3	**13**	CuI	100	12	0	None
4	**13**	CuCl	120	12	0	5
5	**13**	CuBr	120	12	0	70
6	**13**	CuI	120	12	0	7
7	**13**	CuBr	120	5	300	53
8	**13**	CuBr	120	3	500	60
9	**13**	CuBr	120	1	700	90
10	**14**	CuCl	120	12	0	Trace
11	**14**	CuBr	120	14	0	33
12	**14**	CuI	120	14	0	4
13	**14**	CuBr	120	1	700	83



Scheme 1 Semisynthetic route of polymethoxyflavones **1**–**12** Reagents and conditions: (a) concentrated H_2SO_4, ethanol (or methanol), reflux; (b) I_2/pyridine, reflux; (c) NBS, TFA, r.t.; (d) MeONa (10 equiv.), CuBr, DMF, N_2, MV, reflux; (e) Me_2SO_4, K_2CO_3, acetone, reflux; (f) oxone, acetone, CH_2Cl_2/$NaHCO_3$/Na_2CO_3; (g) $AlCl_3$, MeCN, reflux.

Secondly, we ran the reaction using anhydrous DMF as a solvent under an N_2 atmosphere. MeONa in MeOH/DMF was mixed with CuBr at 60 °C, and when a blue solution was formed, an anhydrous DMF solution of 6,8-dibromoapigenin was added under N_2. After keeping the reaction for 12 h at 120 °C, 6,8-dimethoxyapigenin was isolated in 70% yield. However, when the reaction was catalysed by CuCl or CuI, the yields of 6,8-dimethoxyapigenin were only about 5 and 7%, respectively. So we could confirm that CuBr is the most efficient catalyst in this reaction.

In the above procedure, we suspected that decomposition of DMF caused by prolonged heating at 120 °C had affected the yield. Therefore, a microwave-assisted reaction was tried in an attempt to shorten the reaction time. Gratifyingly, a very good yield (90%) of 6,8-dimethoxyapigenin was obtained at 700 W within only 1 h. *O*-Methylation of **11** and **12** with Me_2SO_4 provided the PMFs tangeretin **1** and nobiletin **2**, respectively. Oxidation by dimethyl dioxirane (DMDO) has been reported by several groups.[19] Recently we have developed the syntheses of kaempferol and 5,7,3′,4′-tetramethoxyflavonol,[20,21] which involved a C-3-hydroxylation of flavones by DMDO. Following this procedure, 3-hydroxytangeretin **3** and 3-hydroxynobiletin **4** were synthesised in one-step by reacting **1** or **2** with dimethyldioxirane (DMDO) in acetone at low temperature. The oxidation reaction was regioselective with high yield. Note that oxone was selected as an oxidant because it is stable enough and commercially available in large quantities, while DMDO can be generated *in situ* from oxone and acetone.

Finally, polymethoxyflavonoids **1**, **2**, **5** and **6** underwent regioselective demethylation of the C-5-methoxy group to give 5-hydroxylated polymethoxyflavonoids **7**–**10** in good yields due to the neighbouring-group participation of the C-4-carbonyl oxygen.

In summary, 12 bioactive natural polymethoxyflavonoids were synthesised. A new synthetic method for aromatic nucleophilic substitution of bromide by methoxy group was developed using CuBr-catalysed and microwave-assisted heating. Also DMDO oxidation of flavones to flavonols was much improved. This methodology provides the convenient way of synthesising PMFs from easily available starting materials, in simple operations and good yields.

Experimental

Melting points were determined with a XRC-I apparatus and are uncorrected. NMR spectra were obtained on a Bruker AM-400 instrument (^{1}H NMR at 400 Hz, ^{13}C NMR at 100 Hz) in $CDCl_3$ or DMSO-d_6 using tetramethylsilane as internal standard. Chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hz. IR spectra were measured on a Bruker Tensor-27 spectrometer. MS and HRMS were determined with VG Autospec-3000 or MAT 95 XP spectrometer by the EI or ESI method. Microwave-oven XH-MC-1 has power from 50 to 900 W. Naringin and hesperidin were obtained from Sichuan Herbal Medicine Standards Company.

Naringenin: Naringin (20 g) was dissolved in ethanol (150 mL, 80%), and conc. H_2SO_4 (10 mL) was added dropwise. The reaction mixture was heated under reflux for 4 h, then cooled to room temperature and poured into ice water. The resulting precipitate was filtered, recrystallised from methanol, and dried under vacuum to afford naringenin as a yellow solid: 9.12 g; 90%; m.p. 247–250 °C (lit.[22] 252–253 °C).

Apigenin: A solution of naringenin (5.0 g, 18 mmol) and iodine (5.0 g, 19 mmol) in pyridine (50 mL) was heated to 95 °C for 5 h. The mixture was poured into ice water. The resulting precipitate was filtered, and then washed with water, dilute hydrochloric acid and saturated sodium thiosulfate. Recrystallisation of the dried residue from ethanol afforded apigenin: 3.20 g; 66%; m.p. 345–350 °C (lit.[23] 344–346 °C).

Hesperetin from hesperidin: The same procedure as for naringenin was used. Hesperetin was obtained as a yellow solid: 88%; m.p. 224–226 °C (lit.[24] 224–226 °C).

Diosmetin: The same procedure as for apigenin was used. diosmetin was obtained as a yellow solid: 60%; m.p. 253–255 °C (lit.[25] 253–254 °C).

6,8-Dibromoapigenin **(13):** NBS (2.88 g, 16.2 mmol) was added to a solution of apigenin (2.16 g, 8 mmol) in TFA (22 mL). The reaction mixture was stirred for 5 h at room temperature, and then poured into ice water. The resulting precipitate was filtered, washed with water and dried under vacuum to afford a green crude solid which was chromatographed on silica gel using petroleum ether/ethyl acetate (4 : 1) as the eluent to afford **13** as a yellow solid: 3.15 g; 93%; m.p. 311–313 °C; ^{1}H NMR (DMSO-d_6) δ 12.96 (s, 1H), 10.81 (s, 1H), 10.37 (s, 1H), 7.93 (d, *J* = 8.4 Hz, 2H, H-2′ and 6′), 6.93 (d, *J* = 8.3 Hz, 2H, H-3′ and 5′), 6.49 (s, 1H, H-3).

6,8-Dibromodiosmetin **(14):** The same procedure as for **13** was used. **14** was obtained as a yellow solid: 93%; m.p. 284–285 °C (lit.[26] 284–287 °C); ^{1}H NMR (DMSO-d_6) δ 9.56 (s, 1H), 7.65 (dd, *J* = 8.6, 2.1 Hz, 1H, H-6′), 7.56 (d, *J* = 2.2 Hz, 1H, H-2′), 7.13 (d, *J* = 8.7 Hz, 1H, H-5′), 7.00 (s, 1H, H-3), 3.89 (s, 3H, OCH_3-4′).

6,8-Dimethoxyapiginin **(11):** Small pieces of clean sodium (Na) (0.23 g) were added to methanol (12 mL) slowly. CuBr (0.002 g) was mixed with MeONa/MeOH. When the colour of reaction solution changed to blue, a solution of 6,8-dibromoapigenin (0.425 g) in anhydrous DMF (20 mL) was added into the reaction solution under nitrogen. The mixture was exposed to microwave irradiation (700 W) at 120 °C for 1 h, and then cooled to room temperature. After adjustment of the pH to 6 with hydrochloric acid (5%, 200 mL), the mixture was filtered. The filtrate was extracted with ethyl acetate (3 × 50 mL) and the organic layers were dried over anhydrous Na_2SO_4. The solvent was evaporated to yield a crude oil that was chromatographed on silica gel using petroleum ether/ethyl acetate (3 : 1) as eluent to afford **11** as a yellow solid: 200 mg; 90%; m.p. 270–271 °C (lit.[27] 271–273 °C); ^{1}H NMR (DMSO-d_6) δ 12.81 (s, 1H), 10.41 (s, 1H), 10.03 (s, 1H), 7.80 (d, *J* = 8.8 Hz, 2H, H-2′ and 6′), 6.95 (d, *J* = 8.8 Hz, 2H, H-3′ and 5′), 6.80 (s, 1H, H-3), 3.89 (s, 3H), 3.78 (s, 3H); MS (*m/z*, EI): 330 (M^+), 315, 287, 272, 197, 169, 113, 69; HRMS (EI): *m/z* [M^+] calcd for $C_{17}H_{14}O_7$: 330.0740, found: 330.0745.

6,8-Dimethoxydiosmetin **(12):** The same procedure as for **11** was used. **12** was obtain as pale yellow solid: 83%; m.p. 243–245 °C (lit.[28] 239–241 °C); ^{1}H NMR (DMSO-d_6) δ 12.79 (s, 1H), 10.45 (s, 1H), 9.59 (s, 1H), 7.57 (dd, *J* = 8.6 and 2.3 Hz, 1H, H-6′), 7.48 (d, *J* = 2.2 Hz, 1H, H-2′), 7.12 (d, *J* = 8.7 Hz, 1H, H-5′), 6.81 (s, 1H, H-3), 3.87 (d, *J* = 3.8 Hz, 6H), 3.77 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 182.7, 163.8, 151.7, 151.3, 148.8, 147.3, 145.9, 132.0, 128.4, 123.5, 119.1, 113.3, 112.7, 103.5, 103.5, 61.8, 60.6, 56.2; MS (*m/z*, EI): 360 (M^+), 345 (100), 330, 315, 300, 287, 272, 229, 216, 197, 169, 149, 113, 83, 69; HRMS (EI): *m/z* [M^+] calcd for $C_{18}H_{16}O_8$: 360.0845, found: 360.0845.

Tangeretin (5,6,7,8,4′-pendamethoxyflavone) **(1):** Me_2SO_4 (0.1 mL, 3 equiv.) was added dropwise to stirred solution of 6, 8-dimethoxydiosmetin (120 mg) in acetone and K_2CO_3 (0.91 g, 6.48 mmol) at 65 °C. The reaction was terminated after 5 h, and the mixture was then filtered. The solution was evaporated to afford a solid residue. The crude solid was chromatographed on silica gel using petroleum ether/ethyl acetate (3 : 1) as eluent to afford light yellow crystals of **1**: 115 mg; 86%; m.p. 151–153 °C (lit.[13] 152–153 °C); ^{1}H NMR ($CDCl_3$) δ 7.80 (d, *J* = 8.8 Hz, 2H, H-2′ and 6′), 6.94 (d, *J* = 8.8 Hz, 2H, H-3′ and 5′), 6.52 (s, 1H, H-3), 4.03 (s, 3H), 3.94 (s, 3H), 3.87 (s, 6H), 3.81 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 176.3, 161.3, 160.1, 150.3, 147.3, 146.7, 143.0, 137.1, 126.7, 122.8, 113.8, 113.5, 105.6, 61.2, 61.0, 60.8, 60.6, 54.5; IR (KBr): ν 2946, 2843, 1650, 1607, 1587, 1512, 1462, 1363, 1265, 1181, 1074, 968, 830 cm^{-1}; MS (*m/z*, EI): 372 (M^+), 327, 315, 287, 259, 194, 135, 77, 57; HRMS (EI): *m/z* [M^+] calcd for $C_{20}H_{20}O_7$: 372.1209, found: 372.1204.

Nobiletin (5,6,7,8,3′,4′-hexamethoxyflavone) **(2):** The same procedure as for **1** was used. **2** was obtained as yellow crystals: 80%; m.p. 112–113 °C (lit.[13] 112–113 °C); ^{1}H NMR ($CDCl_3$) δ 7.50 (dd, *J* = 8.5 and 1.9 Hz, 1H, H-6′), 7.34 (s, 1H, H-2′), 6.92 (d, *J* = 8.5 Hz, 1H, H-5′), 6.55 (s, 1H, H-3), 4.03 (s, 3H), 3.96 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H), 3.88 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 177.3, 161.1, 151.9, 151.5, 149.2, 148.4, 147.7, 144.1, 123. 9, 119.9, 114.7, 111.2, 108.5, 106.7, 62.3, 62.0, 61.8, 61.7, 56.1, 55.9; MS (*m/z*, EI): 402 (M^+), 387 (100), 371, 344, 326, 298, 283, 239, 225, 198, 197, 182, 162, 153, 139, 91, 83, 77; HRMS (EI): *m/z* [M^+] calcd for $C_{21}H_{22}O_8$: 402.1315, found: 402.1314.

3-Hydroxytangeretin (3-hydroxy-5,6,7,8,4′-pentamethoxyflavone) **(3):** To a solution of tangeretin (500 mg) and buffer $NaHCO_3$ (1.9 g $NaHCO_3$, 4 g Na_2CO_3, 100 mL H_2O) in CH_2Cl_2 (30 mL) and acetone (25 mL), potassium hydrogen sulfate solution was added dropwise. The mixture was left for 12 h at 5 °C ± 1 °C, and then recharged with acetone (25 mL) and buffer $NaHCO_3$ (1.9 g $NaHCO_3$, 4 g Na_2CO_3, 100 mL H_2O). After potassium hydrogen sulfate solution was added dropwise, the mixture was kept again at 5 °C ± 1 °C for another 12 h. The water layer was then extracted with dichloromethane (3 × 50 mL). The organic layers were combined and dried with anhydrous $MgSO_4$. The solution was filtered and *p*-toluenesulfonic acid (5 mg) was then added. After stirring for 1 h, the mixture was evaporated and crystallised by dichloromethane/methanol to afford **3** as yellow crystals: 350 mg (67%); m.p. 130–132 °C (lit.[13] 134–135 °C); ^{1}H NMR ($CDCl_3$) δ 8.25 (d, *J* = 9.1 Hz, 2H, H-2′ and 6′), 7.31 (s, 1H, OH-3), 7.06 (d, *J* = 9.1 Hz, 2H, H-3′ and 5′), 4.13 (s, 3H), 4.04 (s, 3H), 3.99 (s, 3H), 3.96 (s, 3H), 3.90 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.8, 159.8, 150.5, 146.5, 145.9, 142.4, 142.2, 136.9, 136.2, 128.1, 122.6, 113.1, 110.7, 61.3, 61.0, 60.8, 60.7, 54.4; IR (KBr): ν 3452, 3292, 1564, 1602, 1462, 1402, 4264, 1209, 1184, 1057, 985, 835 cm^{-1}; MS (*m/z*, ESI): 389 [M + 1] $^+$.

3-Hydroxynobiletein (3-hydroxy-5,6,7,8,3′,4′-hexamethoxyfavlone) **(4):** The same procedure as for **3** was used. **4** was obtained as yellow crystals: 64%; m.p. 133–134 °C (lit.[11] 129–130 °C); ^{1}H NMR ($CDCl_3$) δ 7.85 (dd, *J* = 10.6, 1.9 Hz, 2H, H-2′ and 6′), 6.96 (d, *J* = 8.4 Hz, 1H, H-5′), 5.23 (s, 1H, OH-3), 4.05 (s, 3H), 3.97 (s, 3H), 3.93 (s, 6H), 3.90 (s, 3H), 3.88 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 171.804, 151.572, 150.366, 148.719, 147.521, 146.781, 143.417, 142.905, 137.718, 137.306, 123.704, 120.919, 111.589, 110.880, 109.964, 62.284, 61.910, 61.826, 61.681, 55.914, 55.784; IR (KBr): ν 3435, 3299, 2942, 2844, 1597, 1561, 1515, 1438, 1409, 1268, 1208, 1146, 1051, 1023, 975 cm^{-1}; MS (*m/z*, ESI): 419 [M + 1]$^+$.

3,5,6,7,8,4′-Hexamethoxyflavone **(5):** Me_2SO_4 (0.2 mL, 1 equiv.) was added dropwise to a stirred solution of 6, 8-dimethoxydiosmetin (100 mg) in acetone (20 mL) and K_2CO_3 (144 mg) at room temperature for 2 h. The mixture was filtered, and the filtrate was evaporated to afford a crude solid which was crystallised from petroleum ether/ethyl acetate (3 : 1) to afford yellow crystals: 84 mg; 81%; m.p. 133–135 °C (lit.[29] 133–134 °C); ^{1}H NMR ($CDCl_3$) δ 8.16 (d, *J* = 9.0 Hz, 2H, H-2′ and 6′), 7.04 (d, *J* = 9.0 Hz, 2H, H-3′ and 5′), 4.10 (s, 3H), 4.01 (s, 3H), 3.98 (s, 3H), 3.95 (s, 3H), 3.90 (s, 3H), 3.88 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.8, 150.6, 149.5, 147.9, 146.6, 145.8, 142.5, 142.0, 136.8, 136.4, 122.8, 120.0, 110.7, 110.0, 109.2, 61.3, 60.9, 60.8, 60.7, 55.0, 54.9; IR (KBr): ν 2946, 2843, 1650, 1607, 1587, 1512, 1462, 1363, 1265, 1181, 1074, 968, 830 cm^{-1}; MS (*m/z*, EI): 402 (M^+), 387 (100), 359, 344, 298, 225, 197, 182, 150, 136, 109, 83, 65.

3,5,6,7,8,3′,4′-Heptamethoxylflavone **(6):** The same procedure as for **5** was used. **6** was obtained as yellow crystals: 83%; m.p. 130–131 °C (lit.[30] 130.5–131.8 °C); ^{1}H NMR ($CDCl_3$) δ 7.77 (dd, *J* = 8.5, 2.0 Hz, 1H, H-6′), 7.74 (d, *J* = 2.0 Hz, 1H, H-2′), 6.94 (d, *J* = 8.6 Hz, 1H, H-5′), 4.02 (s, 3H), 3.93 (s, 3H), 3.90 (s, 3H), 3.90 (s, 3H), 3.87(s, 3H), 3.81 (s, 3H), 3.66 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.9, 152.1, 150.3, 150.0, 147.7, 147.1, 145.7, 142.8, 139.7, 136.8, 122.4, 120.9, 114.0, 109.9, 99.0, 61.3, 60.9, 60.8, 60.7, 58.8, 55.0, 54.9; IR (KBr): ν 2938, 2840, 1648, 1590, 1521, 1463, 1363, 1273, 1217, 1177, 1138, 1049, 961 cm^{-1}; MS (*m/z*, EI): 432 (M^+), 404, 388, 373, 253, 236, 195, 181, 151 (100), 133, 108, 83, 69,

57; HRMS (EI): *m/z* [M^+] calcd for $C_{22}H_{24}O_9$: 432.1420[M], found: 432.1427[$M]^+$.

5-Hydroxy-6,7,8,4′-tetramethoxyflavone **(9):** Tangeretin (45 mg) was dissolved in anhydrous acetonitrile (13 mL) and anhydrous $AlCl_3$ (128 mg, 0.96 mmol, 8 equiv.) was added to the mixture which was kept at 60 °C for 3 h. Then acetonitrile was evaporated, and hydrochloric acid (3%, 15 mL) was added. The mixture was heated under reflux for 30 min. The mixture was cooled and then extracted with ethyl acetate (3 × 5 mL) and dried with anhydrous $MgSO_4$. Filtration and evaporation of the solvent afforded the crude solid which was purified by silica gel chromatography with petroleum ether/ethyl acetate (2 : 1) to afford yellow crystals of **9**: 40 mg; 93%; m.p. 175–176 °C (lit.[11] 174 °C); ^{1}H NMR ($CDCl_3$) δ 12.51 (s, 1H, OH-5), 7.83 (d, *J* = 9.0 Hz, 2H, H-2′ and 6′), 6.97 (d, *J* = 9.0 Hz, 2H, H-3′ and 5′), 6.54 (s, 1H, H-3), 4.04 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H), 3.83 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 182.0, 163.0, 161.7, 151.9, 148.5, 144.7, 135.5, 132.0, 127.0, 122.4, 113.6, 105.9, 102.7, 61.1, 60.7, 60.1, 54.5; MS (*m/z*, EI): 358 (M^+), 343 (100), 391, 297, 271, 269, 229, 211, 201, 183, 168, 157, 133, 127, 89, 69, 53; HRMS (EI): *m/z* [M^+] calcd for $C_{19}H_{18}O_7$: 358.1053, found: 358.1039.

5-Hydroxy-3,6,7,8,4′-pentamethoxyflavone **(7**): The same procedure as for **9** was used. **7** was obtained as yellow crystals: 90%; m.p. 123–125 °C (lit.[31] 125–127 °C); ^{1}H NMR ($CDCl_3$) δ 12.34 (s, 1H, OH-5), 8.07 (d, *J* = 9.0 Hz, 2H, H-2′ and 6′), 6.96 (d, *J* = 9.0 Hz, 2H, H-3′ and 5′), 4.03 (s, 3H), 3.87 (d, *J* = 1.8 Hz, 6H), 3.82 (s, 3H), 3.79 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 178.3, 160.8, 155.1, 151.8, 148.1, 143.9, 137.6, 135.1, 131.8, 129.2, 121.8, 113.2, 106.4, 61.1, 60.7, 60.1, 59.1, 54.4; MS (*m/z*, EI): 388.2 (M^+), 373 (100), 343, 330, 315, 287, 269, 259, 229, 211, 194, 183, 165, 135, 119, 91, 77, 69, 57; HRMS (EI): *m/z* [M^+] calcd for $C_{20}H_{20}O_8$: 388.1158, found: 388.1146.

5-Hydroxy-6,7,8,3′,4′-pentamethoxyflavone **(10):** The same procedure as for **9** was used. **10** was obtained as yellow crystals: 89%; m.p. 145–146 °C (lit.[13] 146–147 °C); ^{1}H NMR ($CDCl_3$) δ 12.47 (s, 1H, OH-5), 7.51 (dd, *J* = 8.5 and 1.9 Hz, 1H, H-6′), 7.34 (d, *J* = 1.7 Hz, 1H, H-2′), 6.93 (d, *J* = 8.5 Hz, 1H, H-5′), 6.53 (s, 1H, H-3′), 4.04 (s, 3H), 3.91 (s, 6H), 3.90 (s, 3H), 3.88 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 183.0, 163.9, 153.0, 152.5, 149.5, 149.4, 145.8, 136.6, 132.9, 123.7, 120.1, 111.3, 108.7, 107.0, 104.0, 62.1, 61.7, 61.1, 56.1, 56.0; MS (*m/z*, EI): 418 (M^+), 403 (100), 373, 357, 345, 317, 289, 256, 233, 211, 183, 165, 149, 119, 77, 57; HRMS (EI): *m/z* [M^+] calcd for $C_{20}H_{20}O_8$: 388.1158, found: 388.1158.

5-Hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone **(8):** Procedure as for **9** was used. **8** was obtained as yellow crystals: 89%; m.p. 160–162 °C (lit.[13] 164–165 °C); ^{1}H NMR ($CDCl_3$) δ 12.31 (s, 1H, OH-5), 7.77 (dd, *J* = 8.6 and 1.8 Hz, 1H, H-6′), 7.72 (d, *J* = 1.6 Hz, 1H, H-2′), 6.94 (d, *J* = 8.6 Hz, 1H, H-5′), 4.03 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 178.2, 154.8, 151.9, 150.6, 148.1, 147.8, 143.8, 137.7, 135.1, 131.8, 122.0, 121.3, 110.1, 110.0, 106.4, 61.0, 60.7, 60.1, 59.1, 55.0, 54.9; MS (*m/z*, EI): 418 (M^+), 403 (100), 373, 357, 345, 317, 289, 273, 233, 211, 183, 165, 149, 137, 92, 77, 57; HRMS (EI): *m/z* [M^+] calcd for $C_{21}H_{22}O_9$: 418.1264, found: 418.1254.

We thank the National Natural Science Foundation of China (no. J21342015) and the Natural Science Foundation of Hunan Province (no. 14JJ2048) for financial support.

Received 31 January 2014; accepted 11 March 2014
Paper 1402439 doi: 10.3184/174751914X13966139490181
Published online: 6 May 2014

References

1 G. Lewin, A. Maciuk, S. Thoret, G. Aubert, J. Dubois and T. Cresteil, *J. Nat. Prod*., 2010, **73**, 702.
2 K.L. Morley, P.J. Ferguson and J. Koropatnick, *Cancer Lett*., 2007, **251**, 168.
3 J.A. Manthey and P. Bendele, *J. Agric. Food Chem*., 2008, **56**, 9399.
4 T. Itoh, K. Ohguchi, M. Iinuma, Y. Nozawa and Y. Alkao, *Bioorg. Med. Chem*., 2008, **16**, 7592.
5 M.H. Pan, W.J. Chen, S.Y. Lin-Shiau, C.T. Ho and J.K. Lin, *Carcinogenesis*, 2002, **23**, 1677.
6 K.H. Chen, M.S. Weng and J.K. Lin, *Biochem. pharmacol*., 2007, **73**, 215.
7 J.A Manthey and N. Guthrie, *J. Agric. Food Chem*., 2002, **50**, 5837.
8 T. Oshitari, Y. Okuyama, Y. Miyata, H. Kosano, H. Takahashi and H. Natsugari, *Bioorg. Med. Chem. Lett*., 2011, **21**, 4520.
9 Y. Iwase, Y. Takemura, M. Ju-ichi, C. Ito, H. Furukawa, S. Kawaii, M. Yano, X.Y. Mou, J. Takayasu, H. Tokuda and H. Nishino, *Cancer Lett*., 2000, 154, 101.
10 S. Li, M.H. Pan, C.S. Lai, C.Y. Lo, S. Dushenkov and C.T. Ho, *Bioorg. Med. Chem*., 2007, **15**, 3381.
11 H.W. Chu, H.T. Wu and Y.J. Lee, *Tetrahedron*., 2004, **60**, 2647.
12 Y. Miyata, T. Oshitari, Y. Okuyama, A. Shimada, H. Takahashi, H. Natsugari and H. Kosano, *Bioorg. Med. Chem. Lett*., 2013, **23**, 183.
13 Q.A. Wang, Z. Wu, L. Liu, L.H. Zou and M. Luo, *Chin. J. Org. Chem*., 2010, **30**, 1682.
14 S.L. Cai, S. Liu, L. Liu and Q.A. Wang, *Chem. Res. Chinese Universities*., 2012, **28**, 631.
15 J.D. Liu, L. Chen, S.L. Cai and Q.A. Wang, *Carbohydrate Res*., 2012, **357**, 41.
16 Y. Shan, G.Y. Li, Q.A. Wang and Z.H. Li, *Chin. J. Org. Chem*., 2008, **28**, 1024.
17 P. Bovicelli, R. Antonioletti, M. Barontini, G. Borioni, R. Bernini and E. Mincione, *Tetrahedron Lett*., 2005, **46**, 1255.
18 E.R.S. Jessie and C.W. Michael, *Synthesis*., 2011, **1**, 1.
19 T.E. Adams, M.E. Sous, B.C. Hawkins, S. Hirner, G. Holloway, M.L. Khoo, D.J. Owen, G.P. Savage, P.J. Scammells and M.A. Rizzacasa, *J. Am. Chem. Soc*., 2009, **131**, 1607.
20 Z. Wu, S.L. Cai, W.J. Fan and Q.A. Wang, *Chin. J. Org. Chem*., 2012, **32**, 1296.
21 D.H. Lou, G.Q. Wang, L. Xia, L. Chen and Q.A. Wang, *Chin. J. Org. Chem*., 2013, **33**, 535.
22 T.R. Gormley and W.I. O'sullivan, *Tetrahedron*, 1973, **29**, 369.
23 H.H. Ko, W.L. Chang and T.M. Lu, *J. Nat. Prod*., 2008, **71**, 1930.
24 B.S. Zhang, X.L. Ye, Z. Chen, X.F. Jiang, L.J. Yuan, J. Yi and X.G. Li, *Med. Chem. Res*., 2011, **20**, 1200.
25 M. Correia-da-Silva, E. Sousa, B. Duarte, F. Marques, F. Carvalho, L.M. Cunha-Ribeiro and M. Pinto, *J. Med. Chem*., 2011, **54**, 95.
26 J. Quintin and G. Lewin, *Tetrahedron Lett*., 2005, **46**, 4341.
27 H. Tokunaru, S. Hiroshige, M. Mitsuo and O. Shigeo, *Nippon Kagaku Zasshi*, 1962, **83**, 602.
28 G. Janzso, F. Kallay and I. Koczor, *Tetrahedron Lett*., 1965, **27**, 2269.
29 S. Rajagopalan, R.K. Visweswara and T.R. Seshadri, *Proc.-Ind. Acad. Sci*., 1947, **26**, 18.
30 T. Patoomratana, R. Vichai, P. Manat, Y. Chalobon, K. Natedao, S. Samaisukh, S. Kulawee, E.U. Suchart and S.T. Thawatchai, *Tetrahedron*, 2002, **58**, 8073.
31 P.S. Sarin and T.R. Seshadri, *Tetrahedron*, 1960, **8**, 64.