Synthesis of a Complete Series of *O*-Methyl Analogues of Naringenin and Apigenin

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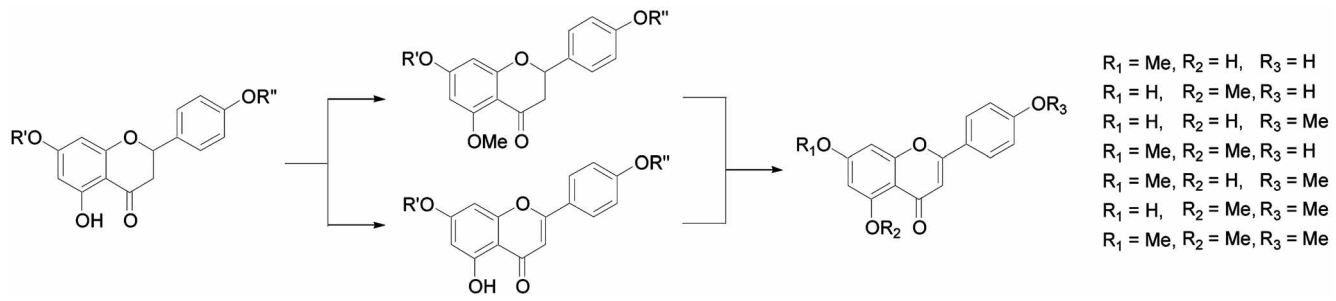
Flavonoids have a variety of biological effects in numerous mammalian systems *in vitro* as well as *in vivo*.[1] Most of the *in vitro* studies are performed on flavonoid itself and not on its metabolites as these latter are not readily available from commercial sources. However, no systematic preparation of flavonoid metabolites has been reported presumably due to the difficulty in controlling the regioselectivity. Particularly, regioselective synthesis of the 5-*O*-methylflavones or 5-*O*-methylflavanones has rarely been accomplished. It is well-known that the hydroxyl function on the 5 position of flavonoids resists to alkylation under basic conditions due to the acid-weakening effect of an intramolecular H-bond between the 5-hydroxyl group and the 4-keto group.[2] The aim of this study was thus to prepare a complete series of *O*-methyl naringenins (flavanones) and apigenins (flavones) with emphasis on the syntheses of 5-*O*-methyl analogues.

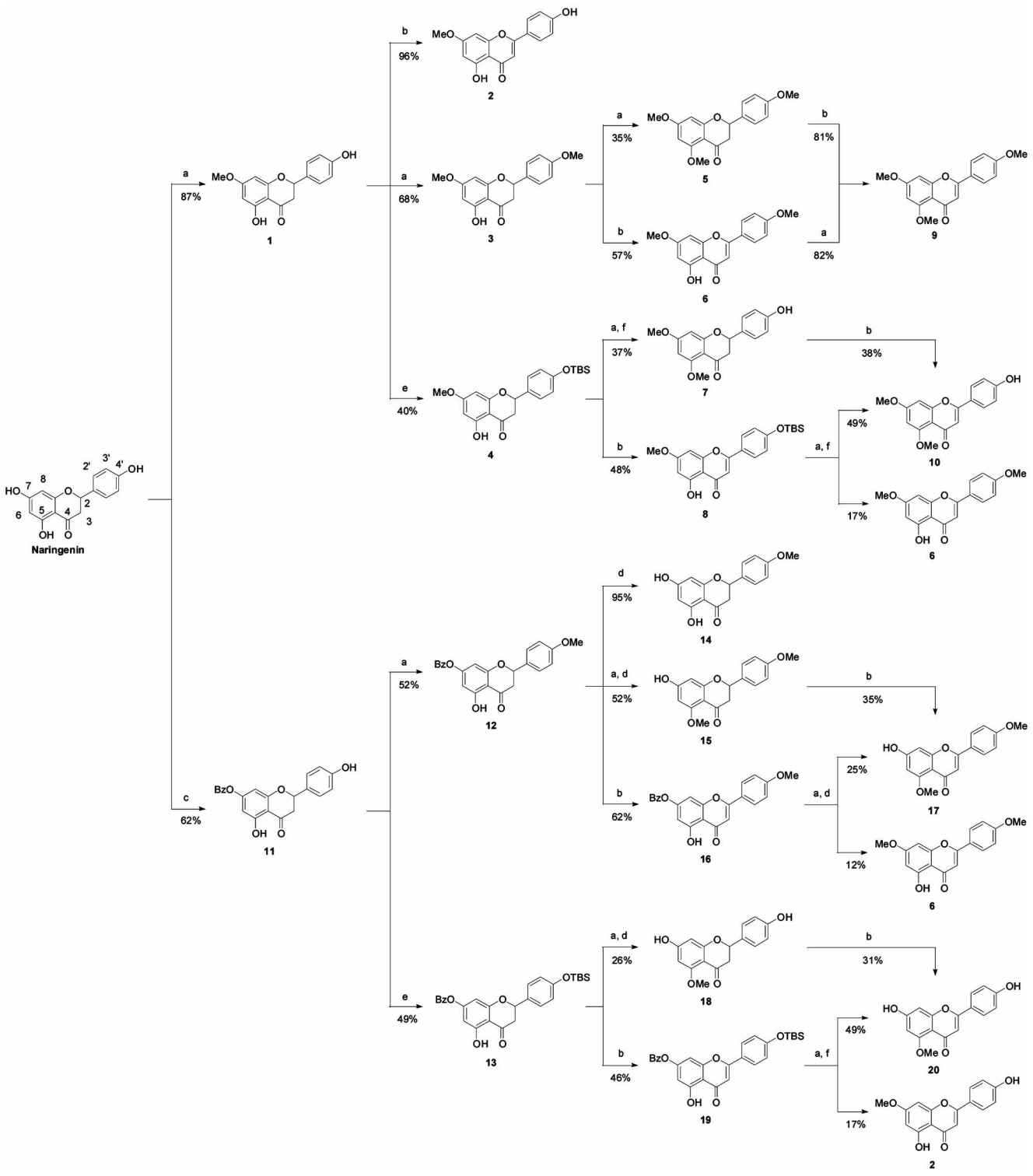
For the purpose of preparing every possible *O*-methyl analogues of naringenin and apigenin, various regioselective methylation and protection was attempted by using naringenin as a starting material (Scheme 1). The hydroxyl function on the 7 position of naringenin is the most acidic site among its three hydroxyl groups (7-OH, 5-OH and 4'-OH).[3] Thus, treatment of naringenin with equimolar amount of the alkylating reagents such as Me_2SO_4 and BzCl under basic conditions gave the corresponding 7-*O*-alkylated products **1**[4] and **11**,[5] from which a variety of *O*-methyl naringenins and apigenins were prepared. Oxidation of **1** with DDQ provided 7-*O*-methylapigenin **2**. On the other hand, treatment of **1** with Me_2SO_4 and TBDMSCl provided 7,4'-di-*O*-methyl naringenin **3** and 7-*O*-methyl-4'-*O*-*tert*-butyldimethylsilyl naringenin **4**, respectively. It is worth to note that, compared with dimethylation of naringenin by use of excess amount of Me_2SO_4, stepwise methylation of naringenin *via* 7-*O*-methyl analogue **1** always provided the 7,4'-*O*-dimethylated naringenin **3** in higher yield. With these mono- and dimethylated naringenin analogues **3** and **4** in our hands, we set out divergent syntheses of 5-*O*-methyl apigenins **9** and **10**: methylation followed by oxidation (upper routes: **3** → **5** → **9** and **4** → **7** → **10**; Scheme 2) and the other way around (lower routes: **3** → **6** → **9** and **4** → **8** → **6**,**10**; Scheme 2). First, treatment of **3** and **4** with excess amount of Me_2SO_4 provided the corresponding 5-*O*-methyl naringenin analogues **5** and **7**, respectively, which were then treated with DDQ in refluxing 1,4-dioxane to give clean conversions to the corresponding 5-*O*-methyl apigenin analogues **9** and **10**. 5,7,4'-Trimethyl apigenin **9** was also prepared by methylation of **6** which was obtained by DDQ-oxidation of **3**. The ^1H NMR spectrum of **9** was found identical to the literature data.[6]

The structure of **9** was further characterized by 2D-NOESY experiment in which irradiations of 5-*O*-*Me*, 7-*O*-*Me* and 4'-*O*-*Me* resulted in increases of H-6, H-6/H-8, and H-3' resonance frequencies, respectively. Throughout this study, these characteristic nuclear Overhauser effects between adjacent protons were used to unequivocally identify the positions of the *O*-methyl groups.

On the other hand, methylation of **8** gave an inseparable mixture of apigenin analogues which were purified after removal of protecting groups by TBAF in THF to give two dimethylated apigenin analogues **6** and **10**. Presumably, the compound **6** was formed by partial loss of the 4'-*O*-TBS group followed by methylation. Thus, we tried to install other protecting groups such as benzyl or benzoyl groups at the 4' position but, in these cases, DDQ-oxidation did not proceed at all (data not shown), which suggests the 4'-*O*-TBS group as the orthogonal protecting group of choice to the 7-*O*-Me of naringenin.



Scheme 1



Scheme 2. Preparation of a complete set of *O*-methyl naringenins and apigenin analogues (For the sake of regioselective alkylation, reactions were monitored by TLC and stopped before complete consumption of the starting materials and appearance of regioisomers. Thus, starting materials were recovered and the yields were not optimized). Reagents and Conditions: a) Me_2SO_4, K_2CO_3, acetone, rt; b) DDQ, 1,4-dioxane, reflux; c) BzCl, pyr, rt; d) NH_4OH, MeOH; e) TBDMSCl, pyr, rt; f) TBAF, THF.

The same protocol of divergent synthesis was applied for the preparation of 5,4'-di-*O*-methyl and 5-*O*-methyl analogues of naringenin and apigenin. Thus, 5,4'-di-*O*-methyl naringenin **15** and 5-*O*-methyl naringenin **18** obtained by sequential methylation and hydrolysis of the 7-*O*-Bz naringenin **11** and 7-*O*-Bz-4'-*O*-TBS naringenin **13** were oxidized to give the corresponding apigenin analogues **17** and **19**, respectively. Also, methylation followed by desilylation of the 7-*O*-Bz-4'-*O*-methyl naringenin **16** and 7-*O*-Bz-4'-*O*-TBS apigenin **19** gave mixtures of dimethyl apigenins (**17**

Table 1. Spectral data of methylated naringenin and apigenin analogues

Compd	^{1}H NMR	^{13}C NMR	HRMS (M+H)
1	$(CD_3)_2CO$, 400 MHz δ 2.63 (dd, J = 17.2, 3.0 Hz, 1H), 3.09 (dd, J = 17.1, 12.9 Hz, 1H), 3.72 (s, 3H), 5.35 (dd, J = 12.8, 3.0 Hz, 1H), 5.90 (d, J = 2.3 Hz, 1H), 5.92 (d, J = 2.3 Hz, 1H), 6.77 (d, J = 8.6 Hz, 2H), 7.27 (d, J = 8.5 Hz, 2H), 8.40 (s, 1H)	$(CD_3)_2CO$, 100 MHz δ 198.0, 169.3, 165.4, 164.6, 159.1, 131.1, 129.5, 116.6, 104.1, 95.9, 95.0, 80.4, 56.7, 43.9	calcd. for $C_{16}H_{15}O_5$, 287.092; found, 286.740.
2	DMSO-d6, 400 MHz δ 3.75 (s, 3H), 6.25 (s, 1H), 6.38 (s, 1H), 6.44 (s, 1H), 6.88 (d, J = 8.7 Hz, 2H), 7.80 (d, J = 8.7 Hz, 2H)	DMSO-d6, 100 MHz δ 187.6, 175.6, 162.3, 154.1, 159.1, 159.0, 142.5, 133.9, 126.9, 116.7, 106.8, 97.3, 56.5	calcd. for $C_{16}H_{15}O_5$, 285.076; found, 284.732 .
3	$(CD_3)_2CO$, 400 MHz δ 2.79 (dd, J = 17.2, 3.0 Hz, 1H), 3.12 (dd, J = 17.1, 13.1 Hz, 1H), 3.80 (s, 3H), 3,89 (s, 3H), 5.37 (dd, J = 13.0, 2.8 Hz, 1H), 6.04 (d, J = 2.2 Hz, 1H), 6.07 (d, J = 2.2 Hz, 1H), 6.96 (d, J = 8.7 Hz, 2H), 7.39 (d, J = 8.6 Hz, 2H)	$(CD_3)_2CO$, 100 MHz δ 197.3, 168.7, 164.3, 163.9, 160.8, 131.6, 128.7, 114.5, 103.5, 95.3, 94.4, 79.7, 56.0, 55.4, 43.3	calcd. for $C_{17}H_{17}O_5$, 301.108; found, 300.778
5	$CDCl_3$, 400 MHz δ 2.77 (dd, J = 16.5, 2.9 Hz, 1H), 3.06 (dd, J = 16.5, 13.2 Hz, 1H), 3.09 (s, 3H), 5.36 (dd, J = 13.2, 2.8 Hz, 1H), 6.09 (d, J = 2.3 Hz, 1H), 6.14 (d, J = 2.3 Hz, 1H), 6.94 (d, J = 6.9 Hz, 3H), 7.39 (d, J = 6.8 Hz, 2H)	$CDCl_3$, 100 MHz δ 187.9, 166.4, 165.5, 163.0, 160.6, 132.2, 128.5, 114.5, 106.5, 94.2, 93.4, 79.5, 56.0, 57.8, 57.4, 46.0	calcd. for $C_{18}H_{19}O_5$, 315.123; found, 314.800.
6	400 MHz, DMSO-d6 δ = 3.83 (s, 3H), 3.90 (s, 3H) 6.50 (s, 1H), 6.59 (s, 1H), 6.84 (s, 1H), 6.91 (d, J = 8.7 Hz, 2H), 7.89 (d, J = 8.7 Hz, 2H)	DMSO-d6, 100 Hz δ 182.3, 165.6, 164.0, 162.8, 161.6, 157.7, 128.8, 123.1, 115.0, 105.1, 104.1, 98.4, 93.1, 56.4, 55.9	calcd. for $C_{17}H_{15}O_5$, 299.092; found, 298.764.
7	$(CD_3)_2CO$, 400 MHz δ 2.78 (dd, J = 16.7, 2.0 Hz, 1H), 3.24 (dd, J = 16.8, 12.8 Hz, 1H), 4.01 (s, 3H), 4.04 (s, 3H), 5.53 (dd, J = 12.7, 2.0 Hz, 1H), 6.05 (s,1H), 6.60 (s, 1H), 7.00 (d, J = 8.5 Hz, 2H), 7.50 (d, J = 8.5 Hz, 2H)	$(CD_3)_2CO$, 100 MHz δ 196.3, 166.7, 161.3, 160.9, 160.8, 130.6, 127.7, 115.5, 104.2, 94.9, 94.0, 76.1, 56.4, 53.6, 44.7	calcd. for $C_{17}H_{17}O_5$, 301.108; found, 300.724
9	400 MHz, DMSO-d6 δ 3.83 (s, 3H), 3.85 (s, 3H), 3.90 (s, 3H), 6.51 (s, 1H), 6.68 (s, 1H), 6.86 (s, 1H), 7.10 (d, J = 8.9 Hz, 2H), 8.0 (d, J = 8.9 Hz, 2H)	DMSO-d6, 100 Hz δ 176.0, 164.0, 162.1, 160.6, 160.0, 159.5, 128.1, 123.4, 114.8, 108.6, 107.1, 96.6, 93.7, 56.4, 56.3, 55.9	calcd. for $C_{18}H_{17}O_5$, 313.108; found, 312.777
10	DMSO-d6, 400 MHz δ 3.87 (s, 6H), 6.39 (s, 1H), 6.59 (s, 1H), 6.80 (s, 1H), 7.13 (d, J = 8.4 Hz, 2H), 8.07 (d, J = 8.5 Hz, 2H)	DMSO-d6, 100 MHz δ 176.7, 164.2, 162.1, 161.3, 161.1, 160.2, 128.4, 122.6, 116.8, 109.7, 107.5, 96.7, 93.7, 56.2, 55.9	calcd. for $C_{17}H_{15}O_5$, 299.092; found, 298.756
14	$(CD_3)_2CO$, 400 MHz δ 2.76 (dd, J = 17.1, 3.0 Hz, 1H), 3.13 (dd, J = 17.1, 13.0 Hz, 1H), 3.83 (s, 3H), 5.80 (dd, J = 12.9, 2.9 Hz, 1H), 5.99 (s, 2H), 6.96 (d, J = 8.7 Hz, 2H), 7.43 (d, J = 8.6 Hz, 2H)	DMSO-d6, 100 MHz δ 196.1, 166.6, 163.5, 162.7, 159.4, 130.5, 128.6, 128.5, 113.6, 113.5, 101.7, 95.8, 94.9, 77.8, 55.0, 41.9	calcd. for $C_{16}H_{15}O_5$, 287.092; found, 286.746
15	$(CD_3)_2CO$, 400 MHz δ 2.82 (dd, J = 17.1, 2.1 Hz, 1H), 3.22 (dd, J = 17.2, 12.8 Hz, 1H), 3.81 (s, 3H), 3.84 (s, 3H), 5.51 (dd, J = 12.8, 2.0 Hz, 1H), 6.03 (s, 1H), 6.04 (s, 1H), 6.99 (d, J = 8.5 Hz, 2H), 7.48 (d, J = 8.5 Hz, 2H)	DMSO-d6, 100 MHz δ 188.4, 166.7, 166.3, 164.1, 160.3, 132.3, 129.2, 125.4, 107.7, 97.1, 95.8, 80.2, 57.0, 55.3, 36.4	calcd. for $C_{16}H_{15}O_5$, 301.108; found, 300.775
17	DMSO-d6, 400 MHz δ 3.80 (s, 3H), 3.84 (s, 3H), 6.38 (d, J = 1.9 Hz, 1H), 6.55 (d, J = 1.9 Hz, 1H), 6.61 (s, H), 7.09 (d, J = 8.9 Hz, 2H), 7.96 (d, J = 8.9 Hz, 2H)	DMSO-d6, 100 MHz δ 175,6, 162.6, 161.7, 159.4, 159.1, 127.7, 123.2, 123.0, 120.6, 114.4, 107.1, 106.6, 96.5, 95.8, 95.2, 55.8, 55.5	calcd. for $C_{17}H_{14}O_5Na$, 321.074; found, 320.778
18	DMSO-d6, 400 MHz δ 2.53 (dd, J = 16.3, 2.3 Hz, 1H), 3.00 (dd, J = 16.4, 12.6 Hz, 1H), 3.73 (s, 3H), 5.34 (dd, J = 12.5, 2.4 Hz, 1H), 5.95 (d, J= 2.0 Hz, 1H), 6.05 (d, J = 1.9 Hz, 1H), 6.78 (d, J = 8.5 Hz, 2H), 7.29 (d, J = 8.5 Hz, 2H)	DMSO-d6, 100 MHz δ 188.5, 164.3, 162.5, 157.6, 129.3, 128.1, 115.1, 104.9, 95.60, 93.20, 78.06, 55.59, 44.78	calcd. for $C_{16}H_{15}O_5$, 287.092; found, 286.736
20	DMSO-d6, 400 MHz δ 3.83 (s, 3H), 6.35 (s, 1H), 6.51 (s, 2H), 6.90 (d, J = 8.6 Hz, 2H), 7.84 (d, J = 8.6 Hz, 2H)	DMSO-d6, 100 MHz δ 175.5, 162.3, 160.4, 160.2, 127.6, 125.0, 124.7, 121.3, 115.7, 106.9, 105.7, 93.9, 55.6	calcd. for $C_{17}H_{15}O_5$, 185.076; found, 284.720

and **6**, **20** and **2**) which were easily separated by column chromatography on silica gel. The spectral data of the methylated naringenin and apigenin analogues prepared in this study are summarized in Table 1.

Experimental Section

Methylation of flavonoids: Synthesis of 7-*O*-methylnaringenin (1) from naringenin is representative. A mixture of naringenin (10 g, 36.8 mmol), K_2CO_3 (5.1 g, 36.8 mmol) and Me_2SO_4 (4.7 g, 36.8 mmol) in acetone was refluxed for 12 hrs. After cooling to room temperature, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure to give a dark yellow residue which was purified by column chromatography on silica gel (hexanes:ethyl acetate = 5:1) to give 7-*O*-methylnaringenin (**1**) as a yellow powder (9.2 g, 87% yield).

DDQ-oxidation of C2-C3 bond: Synthesis of 7-*O*-meth-

ylapigenin (2) from 7-*O*-methylnaringenin (1) is representative. A mixture of 7-*O*-methylnaringenin (**1**) (266 mg, 0.93 mmol) and DDQ (633 mg, 2.79 mmol) in 1,4-dioxane (10 mL) was refluxed for 12 hrs. The reaction mixture was cooled to room temperature and the resulting precipitate was filtered washing with cold water. The filtrate was concentrated under reduced pressure to give a dark green residue, which was purified by column chromatography on silica gel (hexanes:acetone = 4:1) to give 7-*O*-methylapigenin (**2**) as a green powder (254 mg, 96% yield).

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