Studies on the Differentiation Inducers of Myeloid Leukemic Cells from *Citrus* Species

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An attempt was made to isolate differentiation inducers from Aurantii Nobilis Pericarpium and the fruit peel of *Citrus reticulata* BLANCO (Rutaceae). Twenty-seven kinds of flavones, including five new flavones, were isolated after repeated chromatography from methanol extracts of these plants and their structures were established, from their physicochemical data, to be highly methoxylated flavones. Each compound, except for two flavone glucosides, showed the differentiation inducing activity toward mouse myeloid leukemia cells (M1), and the cells came to have phagocytic activity. Furthermore, differentiation inducing activity was tested using human acute promyelocytic leukemia cell line (HL-60).

Keywords differentiation; flavone; Aurantii Nobilis Pericarpium; *Citrus reticulata*; M1 cell; HL-60 cell

In the previous paper, we reported the differentiation inducing activity of triterpenes.[1] Some triterpenes induced leukemic cells (M1 and HL-60) into differentiated cells and the cells came to have phagocytic activity. From further screening, the methanol extracts of *Citrus* sp. were recognized to have differentiation inducing activity. The active constituents of Aurantii Pericarpium and *Citrus reticulata* were investigated.

The suspension of methanolic extract of Aurantii Nobilis Pericarpium in water was extracted with $CHCl_3$. The $CHCl_3$ layer showing the differentiation inducing activity toward M1 cells was subjected to repeated chromatography on silica gel and high performance liquid chromatography (HPLC) to afford active components **1**—**13** containing new flavones (**1** and **5**) and flavone glucosides (**14** and **15**) (Chart 1). *Citrus reticulata* was extracted with hot MeOH and the MeOH extract was partitioned with benzene and water. M1 cells were induced into phagocytic cells by treating with the benzene layer, then the benzene layer was chromatographed on silica gel and HPLC to give active compounds **4**, **6**, **7**, **10**, **12**, **13**, **16**—**27** including new flavone **24**. The structures of twenty-two known compounds (**2**—**4**, **6**—**13**, **16**—**23**, **25**—**27**) were identified by comparing their spectral data with previously reported values.[2-14]

Compound **1**, $C_{21}H_{22}O_8$, mp 128.5—129.5 °C was obtained as colorless prisms. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed six methoxyl signals (δ 3.87, 3.90, 3.95, 3.97, 4.00, 4.09) and AX type

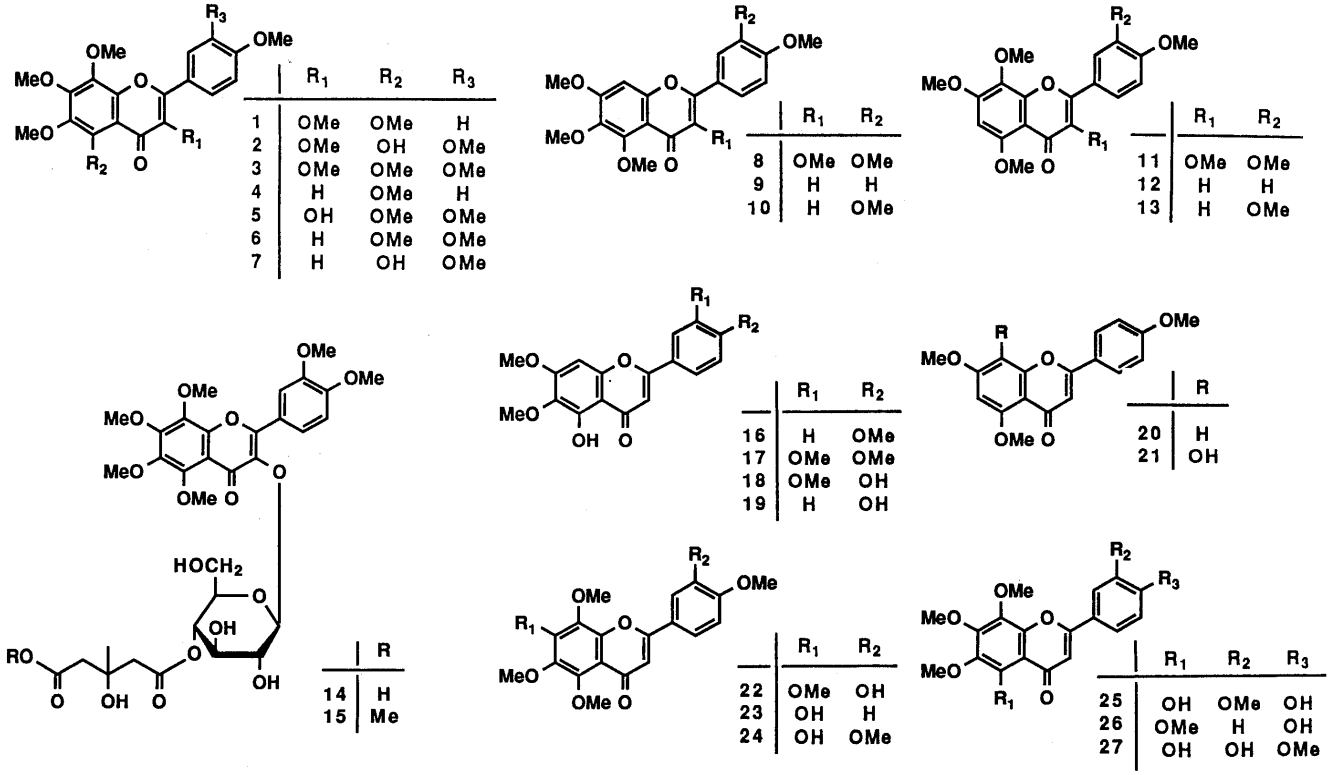

Chart 1

aromatic proton signals [δ 7.04 (2H, d, J=9 Hz), 8.14 (2H, d, J=9 Hz)] (Table I). In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) the characteristic signals of flavonol and six methoxyl groups were observed (Table II). These data suggested the structure of **1** as 3,5,6,7,8,4′-hexamethoxyflavone shown in Chart 1.

Compound **5**, $C_{21}H_{22}O_9$, mp 146—148 °C was obtained as yellow plates. The ^{1}H-NMR spectrum of **5** indicates the presence of six methoxyl groups (δ 3.96, 3.97, 3.99×2, 4.03, 4.12) and aromatic protons coupled with ABX type at δ 7.90 (d, J=2 Hz), 7.03 (d, J=9 Hz) and 7.91 (dd, J=9, 2 Hz) assignable to H-2′, H-5′ and H-6′, respectively. Furthermore, four methoxyl signals observed at δ 61.8—62.4 in the ^{13}C-NMR were characteristic for the methoxyl groups having substituents in both *ortho* positions, and were indicating 5,6,7,8-tetramethoxy structure. The structure of **5** was identified by comparison of various data (mp, UV, ^{1}H-NMR spectra) with reported values previously derived from 5,6,7,8,3′,4′-hexamethoxy-3-hydroxyflavone 3-β-D-glucoside.[15]

Compound **14**, $C_{33}H_{40}O_{18}$, $[\alpha]_D$ −6.3° was obtained as an amorphous powder. In the ^{1}H-NMR spectrum, singlet methyl signal (δ 0.87) and two methylene signals [δ 2.09 (d, J=13 Hz), 2.14 (d, J=13 Hz) and 1.86 (d, J=15 Hz), 2.06 (d, J=15 Hz)] were observed in addition to six methoxyl signals and ABX type aromatic proton signals. After methanolysis of **14**, it gave **5** as an aglycone and methyl glucose which was identified by microanalysis using HPLC and CD spectroscopy.[16] The ^{13}C-NMR spectrum of **14** also suggested the structure of **14** should be flavone glucoside having 3-hydroxy-3-methylglutaric acid which was assumed to bond to glc-4 from the upfield shift of glc-3 (Δ −2.2) and glc-5 (Δ −1.0) by comparing with 3′,4′,5,6,7,8-hexamethoxy-3-hydroxyflavone 3-β-D-glucoside.[15,17] These data led us to conclude the structure of **14** to be 5,6,7,8,3′,4′-hexamethoxy-3-hydroxyflavone-3-*O*-β-D-[4-*O*-(3-hydroxy-3-methylglutaroyl)]glucoside as shown in Chart 1.

Compound **15**, $C_{34}H_{43}O_{18}$, $[\alpha]_D$ −2.3° was isolated as an amorphous powder. The ^{1}H- and ^{13}C-NMR spectra of **15** were almost the same as those of **14** except for the presence of an extra methoxyl group. Compound **15** was methanolysed to give **5** as an aglycone and methyl glucose which was identified in the same manner as employed in **14**. The carbonyl carbon signal presumed to 5‴ shifted to upfield (Δ −5.7) compared with **14** in the ^{13}C-NMR spectrum. These data suggested the structure of **15** as 5,6,7,8,3′,4′-hexamethoxy-3-hydroxyflavone-3-*O*-β-D-[4-*O*-(3-hydroxy-3-methyl-methylglutaroyl)]glucoside. This compound was not disregarded as a possible artifact of **14**.

Compound **24**, $C_{20}H_{20}O_8$, mp 190—191 °C was isolated as colorless needles. In the ^{1}H-NMR spectrum, five methoxyl signals (δ 3.97, 3.98, 4.00, 4.04, 4.14), ABX type aromatic proton signals and olefinic proton signal at δ 6.63 assignable to H-3 were recognized. Three of the five methoxyl groups were estimated to have substituents in both *ortho* positions because their signals were observed in the lower field (δ 61.5, 62.1, 62.8) in the ^{13}C-NMR spectrum. The ultraviolet (UV) spectrum of **24** in MeOH showed

TABLE I. Cell Growth and Phagocytosis of M1 Cells Treated with Flavones

Compound[a]	Concentration (μM)	Growth rate (%)	Phagocytic activity[b]	Compound[a]	Concentration (μM)	Growth rate (%)	Phagocytic activity[b]
Cont.		100	−	Cont.		100	−
Dex.	1	65	++	Dex.	1	63	++
1	50	55	++	**8**	50	77	++
	5	81	+		5	69	+
2	50	35	+	**9**	50	53	++
	5	55	+		5	99	+
3	50	44	++	**10**	50	62	+
	5	71	+		5	81	+
4	50	48	++	**11**	50	75	+
	5	52	+		5	71	−
5	50	18	+	**12**	50	47	++
	5	51	+		5	78	+
6	50	44	++	**13**	50	78	++
	5	74	+		5	89	−
7	50	29	+	Apigenin	50	20	++
	5	51	−		5	66	+
Cont.		100	−	Cont.		100	−
Dex.	1	71	++	Dex.	1	66	+++
16	50	67	+	**22**	50	37	++
	5	76	−		5	50	+
17	50	63	+	**23**	50	22	+++
	5	93	−		5	56	+
18	50	12	+	**24**	50	32	+++
	5	60	+		5	65	+
19	50	28	+	**25**	50	2	+
	5	69	−		5	55	+
20	50	35	++	**26**	50	48	++
	5	85	−		5	77	+
21	50	42	++	**27**	50	14	++
	5	80	−		5	20	+

a) Cont., control; Dex., dexamethasone. *b*) +, >10%; ++, >25%; +++, >50%; uc, uncountable.

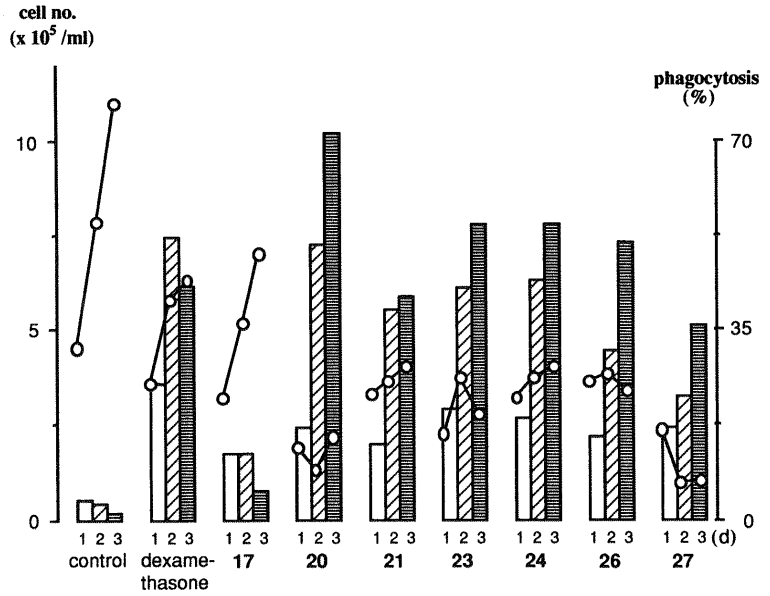

Fig. 1. Time Course of Cell Growth and Phagocytosis of M1 Cells Treated with Flavones from *C. reticulata*

Concentration: dexamethasone, 1 μM; flavones (**17**—**27**), 50 μM. ——, cell no.; □, phagocytosis.

maximum absorptions at 251, 281 and 334 which exhibited a bathochromic shift of 68 nm in band II (334 nm) when sodium methoxide was added, thus suggesting the presence of a free hydroxyl group at C-7 in **24**.[18] From these data, the structure of **24** was confirmed to be 7-hydroxy-5,6,8,3′,4′-pentamethoxyflavone.

All active compounds isolated from Aurantii Nobilis Pericarpium were highly methoxylated flavones. 50 μM of these polymethoxyflavones induced the differentiation of M1 cells at a high percentage, and 5 μM of these compounds could induce M1 cells into phagocytic cells (Table I). In the polymethoxyflavones, their differentiation inducing activity was not altered by numbers or the attached position of methoxyl groups. Compounds **2** and **7**, which have a 5-hydroxyl group, showed higher anti-proliferative activity and lower differentiation-inducing activity compared with non-5-hydroxyl compounds. Flavonol glycosides **14** and **15** were inactive in inducing the differentiation of M1 cells and exhibited no anti-proliferative activity.

From *C. reticulata*, highly oxygenated flavones were isolated as active compounds. The five flavones (**16**—**19** and **25**) having a hydroxyl group at C-5 showed lower activity toward M1 cells than other flavones as observed in the cases of **2** and **7** (Table I). Compounds **23** and **24**, which have hydroxyl groups at C-7, induced more than 50% of the M1 cells to phagocytic cells and were the most potent flavones of all those we isolated from the *Citrus* species.

Active flavones isolated from *C. reticulata* were classified into two groups from their differentiation inducing activity as higher activity groups (**20**—**24**, **26** and **27**) and lower activity groups (**16**—**19** and **25**). M1 cells were incubated at various times with 7 flavones (**17**, **20**, **21**, **23**, **24**, **26** and **27**) at 50 μM. Higher activity group flavones (**20**, **21**, **23**, **24**, **26** and **27**) induced M1 cells into phagocytic cells at a high percentage depending on their incubation time, but lower activity flavone (**17**) did not (Fig. 1). It was revealed that the low activity of the lower activity group having 5-OH did not contribute to their insufficient incubation time.

In our previous paper, we reported the differentiation

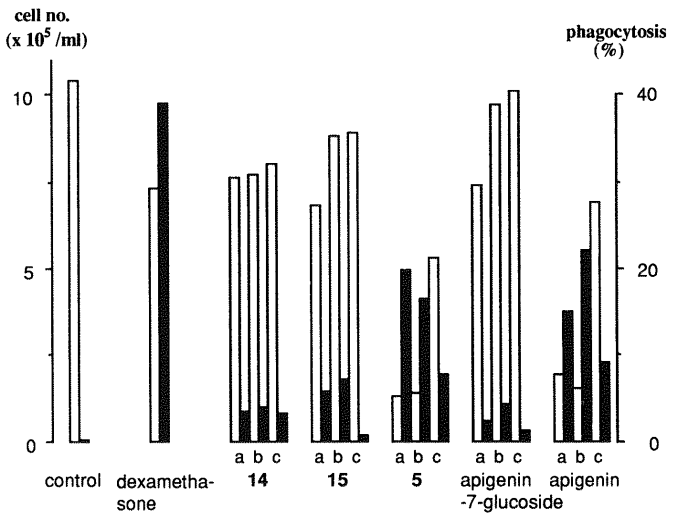

Fig. 2. Effect of Flavonoid Glucosides on the Growth and Induction of Phagocytic Activity of M1 Cells

Concentration: dexamethasone, 1 μM; a, 100 μM; b, 50 μM; c, 5 μM. □, cell no.; ■, phagocytosis.

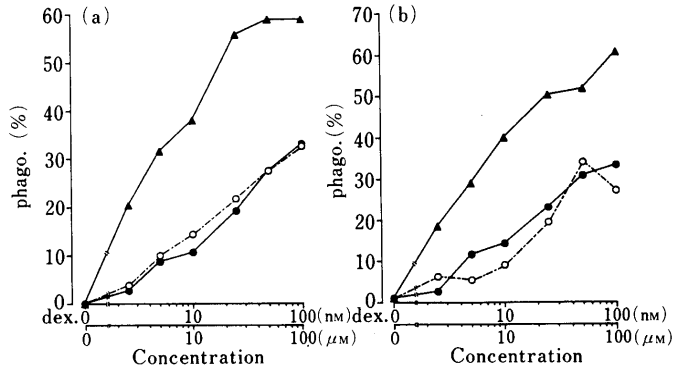

Fig. 3. Differentiation Inducing Effect of **6** (a) and **24** (b) Employed with Dexamethasone on M1 Cells

dex., dexamethasone; phago., phagocytosis. (a) —●—, dex.; ---○---, **6**; —▲—, dex. + **6**. (b) —●—, dex.; ---○---, **24**; —▲—, dex. + **24**.

TABLE II. Cell Growth and Phagocytosis of HL-60 Cells Treated with Flavones from Aurantii Pericarpium

Compound[a]	Concentration (μM)	Growth rate (%)	Phagocytic activity[b]	Compound[a]	Concentration (μM)	Growth rate (%)	Phagocytic activity[b]
Control		100	−	Control		100	−
TPA	0.01	54	++	TPA	0.01	56	++
1	100	15	+	**2**	100	53	+
	50	35	++		50	56	−
3	100	38	+	**5**	100	30	+
	50	64	++		50	42	−
4	100	40	+	**7**	100	67	−
	50	26	++		50	56	−
6	100	21	++	Apigenin	100	16	++
	50	48	++		50	53	+
8	100	58	+	Luteolin	100	7	uc
	50	69	++		50	37	++
9	100	10	++	**14**	100	91	−
	50	44	++		50	100	−
10	100	50	++	**15**	100	100	−
	50	73	+		50	100	−
11	100	33	+	Apigenin-7-glucoside	100	97	−
	50	54	+		50	100	−
12	100	65	−				
	50	79	−				
13	100	42	+				
	50	62	+				

a) TPA, 12-*O*-tetradecanoylphorbol-13-acetate. *b*) +, >10%; ++, >25%; uc, uncountable.

TABLE III. ^{1}H-NMR Spectral Data of Flavones from *Citrus* sp.

Compound	H-3	H-2′	H-3′	H-5′	H-6′	OMe	6‴-CH_3	H-2‴, H-4‴[a]			5‴-OMe
1[b]	—	8.14 (d, J=9)	7.04 (d, J=9)	7.04 (d, J=9)	8.14 (d, J=9)	4.09, 4.00, 3.97, 3.95, 3.90, 3.87					
5[b]	—	7.90 (d, J=2)	—	7.03 (d, J=9)	7.91 (dd, J=9, 2)	4.11, 4.03, 3.98, 3.97, 3.96, 3.95					
14[c]	—	7.82 (d, J=2)	—	7.13 (d, J=9)	7.71 (dd, J=9, 2)	4.03, 3.95, 3.86×2, 3.84, 3.82	0.87 (s)	2.14, (1H, d′)	2.09, (1H, d′)	2.06, (1H, d″)	1.86 (1H, d″)	—
15[c]	—	7.83 (d, J=2)	—	7.12 (d, J=9)	7.70 (dd, J=9, 2)	4.02, 3.95, 3.85×2, 3.84, 3.82	1.02 (s)	2.39, 2.28 (4H, m)				3.48
24[b]	6.63 s	7.42 (d, J=2)	—	6.99 (d, J=9)	7.57 (dd, J=9, 2)	4.14, 4.04, 4.00, 3.98, 3.97						

a) d′, J=13 Hz; d″, J=15 Hz. Spectra were recorded in *b*) $CDCl_3$ or *c*) dimethylsulfoxide-d_6 at 500 MHz.

inducing activity of some triterpenes and low activity of triterpene glycosides.[1] Flavonol glycosides (**14**, **15** and apigenin-7-*O*-glc) and their aglycones were investigated for their differentiation inducing activity using M1 cells (Fig. 2). The aglycones (**5** and apigenin) induced M1 cells to phagocytic cells at 5 μM but their glucosides failed to induce the differentiation of M1 cells. Low activity in the water layer of the methanol extracts is appropriate for their constituents.

Some differentiation inducers (such as proteins and steroids) are known to have enhanced activity when employed with interferon, retinoids, prostaglandin-E and so on.[19] In this study we investigated the effect of flavones (**6** and **24**) employed with dexamethasone. As shown in Fig. 3, 5 μM of flavones employed with 5 nM of dexamethasone (at that concentration, they were scarcely positive in the induction of the differentiation of M1 cells) induced M1 cells into phagocytic cells and their effects were nearly equal to the effect of 20 times that of a single dosage of the flavones or dexamethasone. A high dosage of flavones increased the activity of dexamethasone synergistically.

Highly methoxylated flavones exhibited the differentiation inducing activity toward human promyelocytic leukemia cells (HL-60) at 50 μM (Table II). Their activity was not affected by the number of methoxyl groups of the B-ring, but substituted the pattern of those of the A-ring. Compounds **8**, **9** and **10** (those that have a 5,6,7-trimethoxy A-ring) suppressed proliferation of HL-60 cells and induced the cells into differentiated cells, but **11**, **12** and **13** (5,7,8-trimethoxy A-ring) showed weak anti-proliferation activity and differentiation inducing activity. Flavones having a free hydroxyl group in their A- or C-ring (**2**, **5** and **7**) and glucosides (**14** and **15**) had poor activity as observed in the case of M1 cells. Flavones having free hydroxyl groups isolated from *C. reticulata* represented no activity (data were not shown).

Experimental

General Procedures All melting points were determined on a Yanagimoto MP-500 micro melting point apparatus and are uncorrected. UV spectrum were measured on a Hitachi U3410 spectrophotometer. Mass spectrum (MS) were taken on a JEOL JNM-SX 102 mass spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were taken on JEOL JNM-GSX 270 and JNM-GSX 500 spectrometer (270.05 and 67.8 MHz, 500.00 and

TABLE IV. ^{13}C-NMR Data of Flavones from *Citrus* sp.

Carbon No.	1[a]	5[a]	14[b]	15[b]	24[a]
2	151.3	146.9	151.0	151.0	161.4
3	140.7	143.1	146.2	146.2	106.7
4	174.0	172.0	172.2	172.2	177.3
5	143.9	143.6	143.5	143.5	145.1
6	138.0	137.9	137.4	137.4	140.1
7	153.5	150.6	151.0	151.0	140.3
8	138.0	137.5	135.5	135.5	138.1
9	148.2	147.7	148.1	148.2	145.6
10	115.2	111.8	114.2	114.2	114.2
1′	123.4	123.9	122.7	122.7	124.2
2′	130.0	111.2	111.6	111.5	108.7
3′	114.2	149.0	147.3	147.3	149.4
4′	161.5	151.7	153.6	153.6	152.0
5′	114.2	110.3	112.5	112.5	111.3
6′	130.0	121.1	121.8	121.7	119.7
OMe	62.4, 62.1	62.4, 62.0	61.9, 61.8	61.9, 61.7	62.8, 62.1
	61.9, 61.7	61.9, 61.8	61.5, 61.4	61.5, 61.4	61.5
	58.0	(5, 6, 7, 8)	(5, 6, 7, 8)	(5, 6, 7, 8)	(5, 6, 8)
	(3, 5, 6, 7, 8)	56.1, 55.8	55.7, 55.6	55.7, 55.6	56.5, 56.0
	55.0 (4′)	(3′, 4′)	(3′, 4′)	(3′, 4′)	(3′, 4′)
glc-1″			101.0	101.0	
2″			74.3	74.6	
3″			74.3	74.3	
4″			70.1	70.2	
5″			76.4	76.4	
6″			62.8	63.3	
1‴, 5‴			170.3, 175.7	170.7[c], 170.0[c]	
2‴, 4‴			47.0[d], 46.7[d]	45.3[e], 45.0[e]	
3‴			68.5	68.7	
6‴			27.9	27.1	
5‴-OMe			—	50.8	

Spectra were recorded in *a*) $CDCl_3$ or *b*) dimethylsulfoxide-d_6 at 67.8 MHz. *c*—*e*) The assignment may be interchanged in each column.

125.00 MHz, respectively) and chemical shifts are given in δ (ppm) with tetramethylsilane (TMS) as an internal standard. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. Column chromatography was carried out on silica gel (Merck Kieselgel 60). HPLC was conducted on a JASCO model 800 series with HPLC column PRO-10 Zorbax (20 mm × 25 cm), D-ODS-7 YMC (20 mm × 25 cm) and C8-10 Develosil (20 mm × 25 cm) were used.

Extraction and Isolation Commercially available Aurantii Nobilis Pericarpium (3 kg from Niiya in Shimizu, Shizuoka prefecture) were extracted with hot MeOH, and the MeOH extract was partitioned with $CHCl_3$ and water. The $CHCl_3$ layer was chromatographed repeatedly on a silica gel column with $CHCl_3$–MeOH and *n*-hexane–AcOEt solvents system to give active components **1** (15 mg), **2** (2 mg), **3** (600 mg), **4** (300 mg), **5** (30 mg), **6** (600 mg), **7** (15 mg), **8** (8 mg), **9** (5 mg), **10** (300 mg), **11** (6 mg), **12** (32 mg), **13** (11 mg) with two new flavone glucosides **14** (60 mg) and **15** (25 mg). *Citrus reticulata* BLANCO (1.4 kg supplied from Shizuoka Citrus Growers Cooperative Association in Shimizu) were extracted with hot MeOH and the MeOH extract was partitioned between benzene and water. The benzene layer was chromatographed repeatedly on a silica gel column to give eighteen flavones: **4** (1.7 g), **6** (5 g), **7** (360 mg), **10** (50 mg), **12** (260 mg), **13** (190 mg), **16** (14 mg), **17** (50 mg), **18** (5 mg), **19** (2 mg), **20** (8 mg), **21** (4 mg), **22** (30 mg), **23** (7 mg), **24** (14 mg), **25** (17 mg), **26** (2 mg), **27** (3 mg).

3,5,6,7,8,4′-Hexamethoxyflavone (**1**): Colorless prisms, mp 128.5—129.5 °C (MeOH). UV λ_{max}^{MeOH} nm (log ε): 222 sh (4.45), 268 (4.37), 332 (4.42). *Anal.* Calcd for $C_{21}H_{22}O_8$: C, 62.68; H, 5.51. Found: C, 62.64; H, 5.46. FAB-MS *m/z*: 403 $[M+H]^+$. ^{1}H-NMR: Table III and ^{13}C-NMR: Table IV.

5,6,7,8,3′,4′-Hexamethoxy-3-hydroxyflavone (**5**): Yellow plates, mp 146—148 °C (EtOH). UV λ_{max}^{MeOH} nm (log ε): 256 (4.44), 367 (4.36). *Anal.* Calcd for $C_{21}H_{22}O_9$: C, 60.28; H, 5.30. Found: C, 60.40; H, 5.23. EI-MS *m/z*: 418 $[M]^+$, 403 $[M-CH_3]^+$. ^{1}H-NMR: Table III and ^{13}C-NMR: Table IV.

5,6,7,8,3′,4′-Hexamethoxyflavone-3-*O*-β-D-[4-*O*-(3-hydroxy-3-methylglutaroyl)]glucoside (**14**): Colorless amorphous powder, $[\alpha]_D$ −6.3° (*c* = 0.32, MeOH). UV λ_{max}^{MeOH} nm (log ε) 254 (4.38), 270 sh (4.26), 344 (4.31). FAB-MS *m/z*: 747 $[M+Na]^+$, 603 $[M+Na-(3\text{-hydroxy-3-methylglutarate})]^+$, 419 $[\text{aglycone}+H]^+$. ^{1}H-NMR: Table III and ^{13}C-NMR: Table IV.

5,6,7,8,3′,4′-Hexamethoxyflavone-3-*O*-β-D-[4-*O*-(3-hydroxy-3-methylmethylglutaroyl)]glucoside (**15**): Colorless amorphous powder, $[\alpha]_D$ −2.3° (*c* = 0.43, MeOH). UV λ_{max}^{MeOH} nm (log ε): 254 (4.49), 270 sh (4.38), 340 (4.46). FAB-MS *m/z*: 761 $[M+Na]^+$, 739 $[M+H]^+$, 419 $[\text{aglycone}+H]^+$. ^{1}H-NMR: Table III and ^{13}C-NMR: Table IV.

7-Hydroxy-5,6,8,3′,4′-pentamethoxyflavone (**24**): Colorless needles, mp 190—191 °C (MeOH). UV λ_{max}^{MeOH} nm (log ε): 251 (4.88), 281 (4.97), 334 (5.10); λ_{max}^{+NaOMe} nm (log ε): 291 sh (5.01), 315 (5.14), 342 sh (4.97), 403 (4.27). *Anal.* Calcd for $C_{20}H_{20}O_8$: C, 61.85; H, 5.19. Found: C, 61.82; H, 5.46. FAB-MS *m/z*: 389 $[M+H]^+$. ^{1}H-NMR: Table III and ^{13}C-NMR: Table IV.

Acid Hydrolysis and *p*-Bromobenzoylation of 14 and 15[16] **14** (16 mg) in 0.5 N HCl (1 ml) was refluxed for 1 h. The reaction mixture was poured into ice-cold water and extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated off, and the residue was recrystallized from EtOH to give aglycone (6.5 mg). Thin layer chromatographic (TLC) and HPLC behavior and melting point were identical with those of **5**. The water layer was evaporated off to dryness and part of the residue (2 mg) dissolved in 10% HCl–MeOH (excess) was refluxed for 1 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated off to dryness and then heated at 80 °C with pyridine (1 ml), *p*-bromobenzoyl chrolide (excess) overnight. The reaction mixture was poured into water and extracted with *n*-hexane. The *n*-hexane layer was chromatographed using a Pro-10 Zorbax column to give per-*p*-bromobenzoate.

HPLC Conditions: column, PRO-10 Zorbax (4.6 mm × 250 mm); solvent, CH_3CN–H_2O (80 : 20); flow rate, 1 ml/min; t_R, α-Me glc perbenzoate 10.4 min, β-Me glc perbenzoate 7.6 min. Acid hydrolysis and *p*-bromobenzoylation of **15** was performed by the same manner used for **14**.

Determination of the *A*-Values[16] The UV and CD spectra of per-*p*-bromobenzoate in CH_3CN were measured. The concentration of the benzoate was calculated from the absorbance related to the reported extinction coefficiences (76400 for tetra-*p*-bromobenzoate). The *A*-values were calculated from the CD data of *p*-bromobenzoate of methyl sugar (α-Me-glc perbenzoate, +19.7; β-Me-glc perbenzoate, +22.7) and they were identical with reported values (α-Me-glc perbenzoate, +24; β-Me-glc perbenzoate, +32).

Materials Eagle's MEM, RPMI 1640 medium, Eagle's MEM amino acids and vitamins medium were purchased from Nissui Pharmaceutical Co., Ltd. Dexamethasone was from Nakarai Chemicals, Ltd. TPA was from Sigma Chemical Co. Polystyrene latex particles were from The Dow Chemical Company. Apigenin, luteolin and apigenin-7-glucoside were from Funakoshi Co., Ltd.

Cell Culture M1 cells were grown in Eagle's MEM medium containing 10% heat-inactivated calf serum and diluted when the cell density reached about 2×10^6 cells per ml in a 5% CO_2 humidified atmosphere at 37 °C. HL-60 cells were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum at 37 °C in a humidified 5% CO_2 incubator.

Measurement of Phagocytosis Phagocytic activity was assayed as reported previously.[1] Cells were inoculated at a concentration of 2×10^5 cells/ml into 2 ml of culture medium and incubated with 20 μl of sample solution diluted with ethanol. After 48 h, the cells were washed and incubated for 4 h with a suspension of polystyrene latex particles (2 μl/ml of serum free medium). Then the cells were washed thoroughly 3 or 4 times with phosphate-buffered saline and the percentage of phagocytic cells was calculated.

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