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MICROWAVE-ASSISTED EFFICIENT SYNTHESIS OF POLYMETHOXYACETOPHENONES AND NATURAL POLYMETHOXYFLAVONES, AND THEIR INHIBITORY EFFECTS ON MELANOGENESIS

Masao Tsukayama,[a*] Eiji Kusunoki,[a] Mohammad M. Hossain,[a] Yasuhiko Kawamura,[a] and Shinji Hayashi[b]

[a]Department of Chemical Science and Technology, Faculty of Engineering, The University of Tokushima, Minamijosanjima-cho, Tokushima 770-8506, Japan
[b]NOF Corporation Oleochemicals Research Laboratory, 1-56 Ohama-cho, Amagasaki-shi, Hyogo 660-0095, Japan
e-mail:tukayama@mb.tcn.ne.jp

Abstract – Microwave-assisted Friedel-Crafts acetylation of polymethoxybenzenes with acetic anhydride in the presence of $In(CF_3SO_3)_3$ under solvent-free conditions was achieved rapidly to give polymethoxyacetophenones in high yields. Microwave-assisted benzoylation of 2'-hydroxypolymethoxyacetophenones with polymethoxybenzoyl chlorides, followed by the rearrangement of the resulting benzoates and the final formation of natural polymethoxyflavones was achieved rapidly and efficiently. The polymethoxyflavones showed inhibitory effects on melanogenesis in human melanoma cells.

INTRODUCTION

Aromatic ketones are very useful and important as starting materials and/or synthetic intermediates in synthetic organic chemistry. They have been synthesized by Friedel-Crafts acylation of aromatic compounds, which needs usually stoichiometric amounts of a Lewis acid, such as $AlCl_3$.[1] Recently, the catalytic Friedel-Crafts acylation of various substituted benzenes have been carried out by use of hafnium(IV) triflate.[2] Aromatic ketones have also been synthesized by solvent-free Friedel-Crafts acylation of aromatic compounds in the presence of catalytic amounts of perfluoroalkanoic anhydride and bismath triflate.[3] Moreover, microwave(MW)-assisted Friedel-Crafts acylation of aromatic compounds has been carried out in the presence of catalytic Lewis acids under solvent-free conditions.[4-7] However, as far as we know, the synthesis of highly oxygenated acetophenones has not been conducted yet in the

presence of a catalytic amount of Lewis acids under microwave irradiation (MWI). The synthetic problems of polymethoxyacetophenones remain to be resolved. Therefore, we wish to report here on MW-assisted Friedel-Crafts acylation of polymethoxybenzenes with acetic anhydride in catalytic amounts of $In(CF_3SO_3)_3$ under solvent-free conditions.

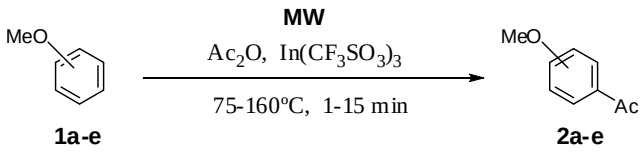
Flavonoids are present in a wide variety of edible plants, especially in *Citrus* species. The highly methoxylated flavones exhibit higher biological activity even though they occur in much lower concentrations.[8] Nobiletin (3',4',5,6,7,8-hexamethoxyflavone) and tangeretin (4',5,6,7,8-pentamethoxyflavone) in *Citrus aurantium* were easily synthesized under microwave irradiation and they have shown inhibitory effects on melanogenesis in human HM3KO melanoma cells.[9,10] Recently, new 5,6,7,8,3',4',5'-heptamethoxyflavone (*Eupatorium coelestinum*) (**4**),[11] 5,7,8,3',4',5'-hexamethoxyflavone (*Murraya paniculata*) (**5**),[12] and 5,7,8,3',4'-pentamethoxyflavone (*Citrus hassaku*) (**6**),[13] were isolated and their structures were confirmed by spectroscopic analysis. As a continuation of our studies on the MW-assisted synthesis of polymethoxyflavones, we have examined the efficient total synthesis of polyoxygenated flavones **4**, **5**, **6** and non-natural 5,7,8,2',6'-pentamethoxyflavone (**7**) from the obtained polymethoxyacetophenones under solvent-free conditions or with minimal use of organic solvents by MWI owing to the approach to an environmentally-friendly methodology. We also wish to report here for the first time on the inhibitory effects of these flavones on melanogenesis in human melanoma cells.

RESULTS AND DISCUSSION

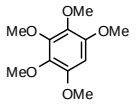
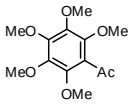
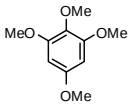
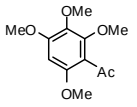
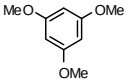
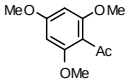
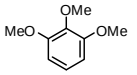
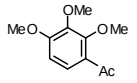
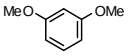
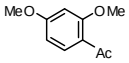
MW-assisted methylation of phloroglucinol, pyrogallol and resorcinol in a minimal acetone by $(MeO)_2SO_2$-K_2CO_3 method gave the methyl ethers **1c**, **1d** and **1e** for 4 min in 90, 87 and 91% yields, respectively. In a similar manner, MW-assisted methylation of 2,5-dihydroxy-1,3-dimethoxybenzene, which was prepared from 2,6-dimethoxy-1,4-benzoquinone,[14] and 2-hydroxy-1,3,4,5-tetramethoxybenzene (**2b"**), which was synthesized according to Scheme 1, gave methyl ethers **1b** (87% yield) and **1a** (88% yield) for 10 min, respectively.

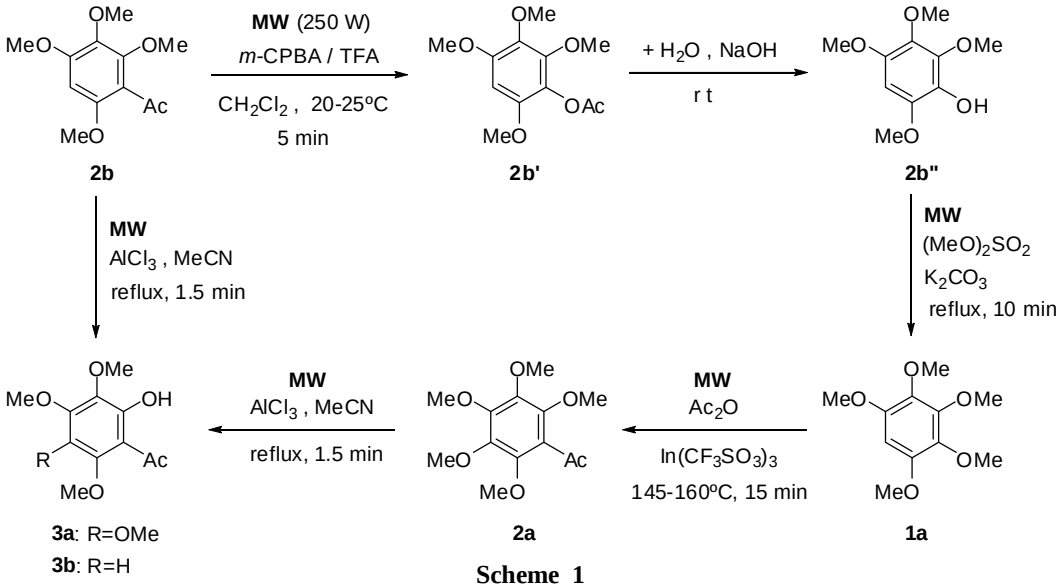
MW-assisted Friedel-Crafts acetylation of polymethoxybenzenes **1a** and **1b** with acetic anhydride in the presence of catalytic $In(CF_3SO_3)_3$ under solvent-free conditions was achieved for 3-15 min to give tetra- and pentamethoxyacetophenones **2a** and **2b** in high yields (Table 1). In a similar manner, MW-assisted Friedel-Crafts acetylation of the other methyl ethers **1c, 1d** and **1e** was carried out rapidly to give acetophenones **2c, 2d** and **2e** in good yields (Table 1). The above results revealed that the MW-assisted acetylation of polymethoxybenzenes with acetic anhydride in the presence of $In(CF_3SO_3)_3$ under solvent-free conditions is an excellent method for the synthesis of polymethoxyacetophenones, because of rapid reaction, clean experiments, high yields and solvent-free conditions. Pentamethoxyacetophenone

Table 1 Microwave-assisted synthesis of polymethoxyacetophenones under solvent-free conditions



1a-e **2a-e**

Methoxybenzene	$In(CF_3SO_3)_3$ (equiv)	Temp (°C)	Time (min)	Acetophenone (%)
 **1a**	0.015	145-160	15	 **2a** 77
 **1b**	0.006	85-95	3	 **2b** 87
 **1c**	0.009	110-120	1	 **2c** 80
 **1d**	0.012	75-90	7	 **2d** 73
 **1e**	0.012	85-100	6	 **2e** 60

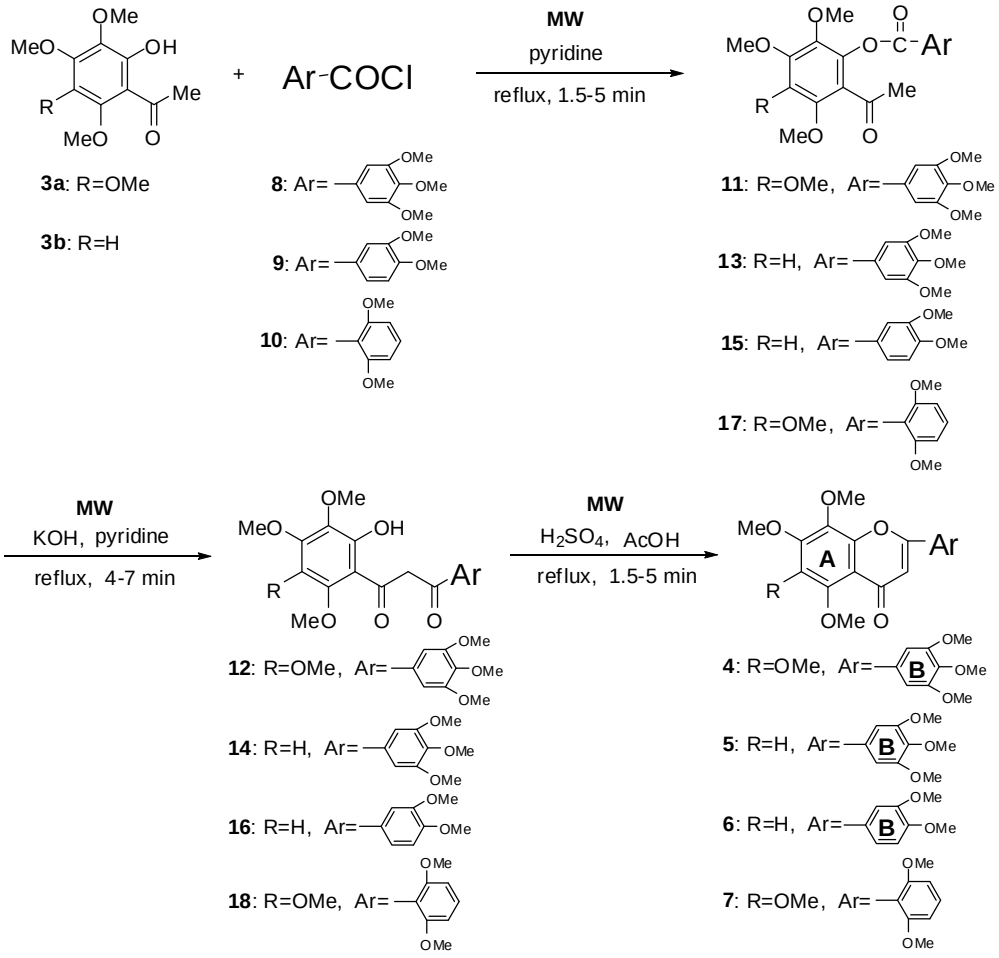


Scheme 1

2a was synthesized according to the pathway of Scheme 1. So far, pentamethoxybenzene (**1a**) in Table 1 has been synthesized by methylation of 2,3-dihydroxy-1,4,5-trimethoxybenzene, which has been synthesized in less than 60% yield by Dakin reaction of 2'-hydroxytrimethoxyacetophenone **3b** with 6% H_2O_2-NaOH.[9,15] The oxidative reaction of **2b** should be improved to obtain the corresponding phenol **2b"**

in a better yield. Baeyer-Villiger reaction of **2b** with *m*-chloroperbenzoic acid (*m*-CPBA) in the presence of trifluoroacetic acid (TFA) in CH_2Cl_2 gave the desired acetate **2b'** for 18 h at 25˚C in 63% yield. Furthermore, MW (250w)-assisted Baeyer-Villiger reaction of **2b** with *m*-CPBA/TFA in CH_2Cl_2 gave the corresponding acetate **2b'** for 5 min at 20-25˚C in 73% yield. From this result, MW-assisted conditions were found to be more effective for Baeyer-Villiger reaction of acetophenone **2b**. Hydrolysis of **2b'** with aqueous NaOH, followed by the MW-assisted methylation of the resultant phenol **2b"** gave the desired pentamethoxybenzene **1a**, which was efficiently converted into the pentamethoxyacetophenone **2a** (Scheme 1 and Table 1). MW-assisted demethylation of the acetophenones **2a** and **2b** with $AlCl_3$ in MeCN gave 2'-hydroxytetramethoxyacetophetone **3a** and 2'-hydroxytrimethoxyacetophetone **3b** for 1.5 min in 84% and 81% yield, respectively (Scheme 1).

MW-assisted benzoylation of acetophenones **3a** and **3b** with substituted benzoyl chlorides **8**-**10** in minimal pyridine was carried out from 1.5 to 5 min under reflux to give the corresponding benzoates **11**-**17** in 75-89% yields, respectively. MW-assisted Baker-Venkataraman rearrangement of benzoates **11**-**17** in the presence of KOH in minimal pyridine was achieved for 4-7 min under reflux to afford the corresponding diketones **12**-**18** in 86-96% yields, respectively. MW-assisted cyclization reaction of **12**-**18**



Scheme 2

with concd H_2SO_4 in minimal AcOH was conducted from 1.5 to 5 min under reflux to give the corresponding flavones **4-7** in 83-94% yields, respectively (Scheme 2). The ^{1}H-NMR spectra and other physical properties of the synthetic flavones **4**, **5** and **6** were identical with those of each natural sample of 3',4',5',5,6,7,8-heptamethoxy-, 3',4',5',5,7,8-hexamethoxy-, and 3',4',5,7,8-pentamethoxyflavone. From the results of the MW-assisted total synthesis of flavone **7**, three successive reactions were not shown to be affected by the steric hindrance of 2,6-dimethoxybenzoyl group.

The new synthetic methodology under microwave-enhanced conditions is also environmentally friendly and results in high yields of the polymethoxyacetophenones and polymethoxyflavones.

The cytotoxicity of the synthetic polymethoxyflavones (**4**, **5**, nobiletin and tangeretin) on human HM3KO melanoma cells[10] was investigated by MTT assay, and the results are shown in Figure 1. The inhibitory effects of the synthetic polymethoxyflavones (**4**, **5** and **6**) as well as nobiletin and tangeretin on melanogenesis in HM3KO melanoma cells LINE, HM3KO,[10] were carried out at different concentrations, and the results are shown in Figure 2. From Figure 1, it is obvious that the highly methoxylated B-ring (**4**, **5** and nobiletin; see Scheme 2) has a propensity to increase the cytotoxicity of polymethoxyflavones (**4**, **5** and nobiletin) at concentrations of 10-20 μM. In contrast, polymethoxyflavones having the completely methoxylated A-ring (**4**, nobiletin and tangeretin; see Scheme 2) show the upward tendency of inhibitory effect on melanogenesis. Figure 2 shows that the polymethoxflavones (**4**, **5**, nobiletin and tangeretin) suppress the production of melanin upto 40-60% at concentrations of 5-10 μM. These results clearly indicate that both the cytotoxicity and inhibitory effects of polymethoxflavones on melanogenesis are affected by the number of methoxy groups on a flavone skeletone. Therefore, hepta- and hexamethoxyflavones (**4** and **5**) as well as nobiletin and tangeretin would be utilizable as an agent for the treatment of skin pigmentation such as spots and freckles induced by UV light.[10]

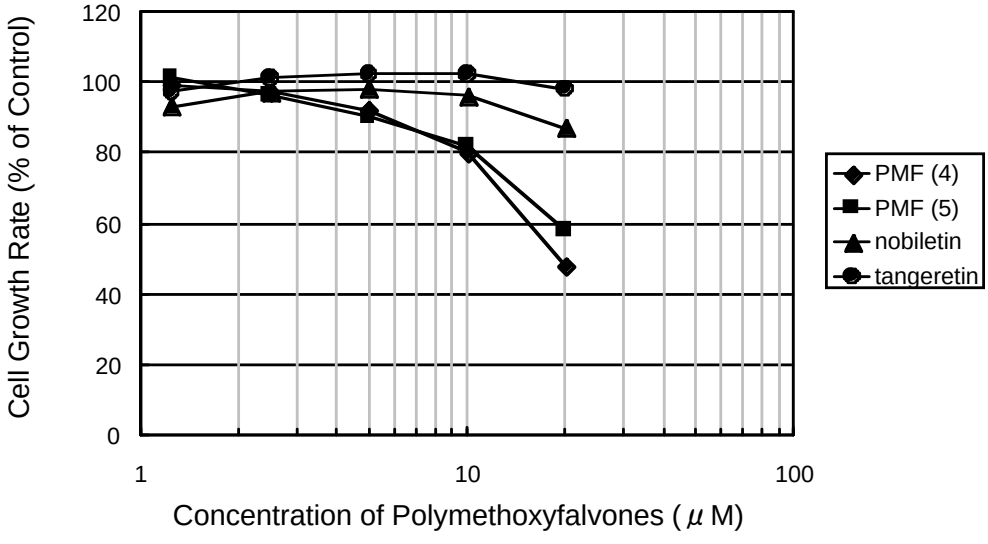


Fig 1 Cytotoxicity Assay of Polymethoxyflavones on Human Melanoma Cells

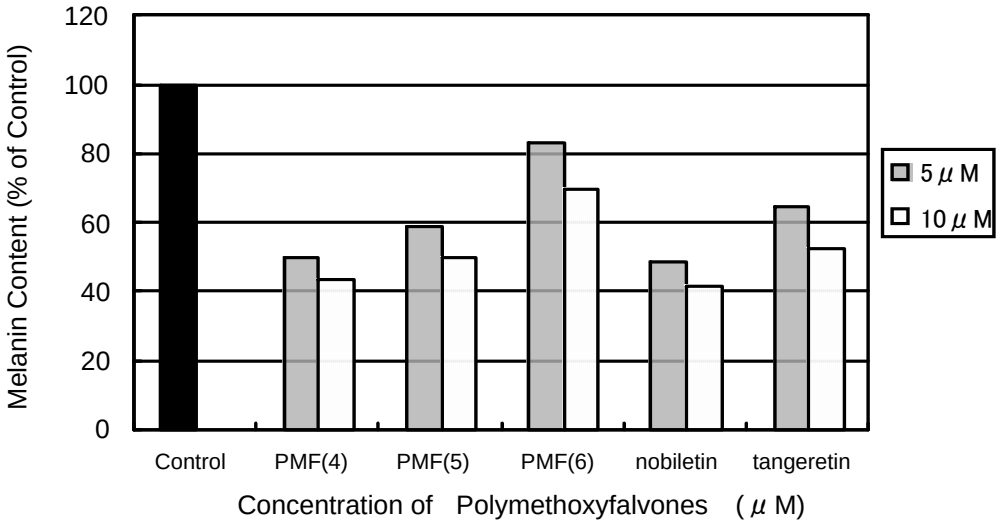


Fig 2 Inhibitory Effects of Polymethoxyflavones on Melanogenesis

EXPERIMENTAL

All the melting points were taken on a Yanaco MP-J3 micro melting-point apparatus and were uncorrected. The ^{1}H-NMR spectra were recorded with a JEOL EX-400 spectrometer (400 MHz), using tetramethylsilane as internal standard (δ, ppm). The IR spectra were recorded on an FT/IR-460 Plus (JASCO) spectrophotometer using KBr pellets, and the UV spectra were recorded on a Hitachi U-2000 spectrophotometer. Elemental analyses were performed with a J-Science Lab. CHN corder JM-10. A microwave oven (650 W and 2.45 GHz, modified properly by fitting a condenser and a thermo-sensor through the holes made in the roof; Shikoku Instrumentation Co., Ltd, Japan) was used as a reaction apparatus. Column chromatography and thin-layer chromatography (TLC) were carried out on Kieselgel 60 (70-230 mesh) and Kieselgel 60 F-254 (Merck).

Polymethoxybenzenes (1c-e) from Phloroglucinol, Pyrogallol and Resorcinol.

A mixture of polyphenols (each 500 mg, 3.96-4.45 mmol), $(MeO)_2SO_2$ (1.9-2.2 mL) and K_2CO_3 (25-27 mmol) in a minimal acetone (8-9 mL) was refluxed with stirring for 4 min (30 s × 8 times, 30 s interval/irradiation) under MWI. The reaction mixture was extracted with AcOEt, and the extract was washed with 6% HCl, water and dried (Na_2SO_4). The resulting compounds were chromatographed over a silica gel column to give polymethoxybenzenes (**1c**, **1d** and **1e**) in high yields: 1,3,5-trimethoxybenzene (**1c**, mp 51-53 ˚C, 91% yield as colorless needles); 1,2,3-trimethoxybenzene (**1d**, mp 43-45 ˚C, 87% yield as colorless plates); 1,3-dimethoxybenzene (**1e**, 91% yield as a pale yellow oil) (Table 1).

Polymethoxyacetophenones (2c-e).

A mixture of polymethoxybenzenes **1c-e** (each 500 mg, 2.97-3.62 mmol), Ac_2O (2 equiv) and $In(CF_3SO_3)_3$ (0.006-0.012 equiv) was stirred for 1-7 min (30 s × 2-14 times, 30 s interval/irradiation) at 75-120 ˚C under MWI. The resulting compounds were chromatographed over a silica gel column to give

the acetophenones (**2c-e**); acetophenone **2c**: mp 101-103 ˚C, acetophenone **2d**: a pale yellow oil and acetophenone **2e**: a pale yellow oil (Table 1).

1,2,3,5-Tetramethoxybenzene (1b).

A mixture of 2,5-dihydroxy-1,3-dimethoxybenzene[9] (5.00 g, 29.4 mmol), $(MeO)_2SO_2$ (11.2 mL, 118 mmol) and K_2CO_3 (12.2 g, 88.1 mmol) in acetone (25 mL) was refluxed with stirring for 4 min (30 s × 8 times, 30 s interval/irradiation) under MWI. The resulting compounds were chromatographed over a silica gel column to give the tetramethoxybenzene **1b**[14] (5.17 g, 87%) as a pale yellow oil (Table 1).

2',3',4',6'-Tetramethoxyacetophenone (2b).

A mixture of the tetramethoxybenzene **1b** (5.00 g, 25.2 mmol), Ac_2O (4.77 mL, 50.1 mmol) and $In(CF_3SO_3)_3$ (97 mg, 0.17 mmol) was irradiated with stirring for 3 min (15 s × 12 times, 30 s interval/irradiation) under MWI. The reaction mixture was extracted with AcOEt, and the extract washed with water, and dried (Na_2SO_4). The resulting compound was chromatographed over a silica gel column (AcOEt:hexane=1:2 as a solvent) to give the acetophenone **2b**[14] (2.25 g, 87%) as colorless needles, mp 49.5-51 ˚C.

2-Acetoxy-1,3,4,5-tetramethoxybenzene (2b').

CF_3CO_2H (0.11 ml, 1.5 mmol) was added into a solution of *m*-CPBA (460 mg, 1.99 mmol) in CH_2Cl_2 (3 mL) with stirring in an ice-water bath, and then the mixture was stirred for 30 min. The whole mixture, which tetramethoxyacetophenone **2b** (300 mg, 1.25 mmol) was added to the above mixture, was irradiated with stirring at 20-25 ˚C for 5 min (10 s × 30 times, 30 s interval cooling by ice/irradiation) under MWI (250W). The resulting compound was filtered and the filtrate was extracted with CH_2Cl_2, and the extract washed with aqueous $NaHCO_3$ solution and water, and dried (Mg_2SO_4). The resulting compound was chromatographed over a silica gel column to give the desired acetoxybenzene **2b'** (232 mg, 73%) as colorless needles, mp 60-62 ˚C. ^{1}H-NMR ($CDCl_3$) δ 2.33 (3H, s, $OCOCH_3$), 3.80, 3.83, 3.87 and 3.88 (each 3H, s, OCH_3), 6.33 (1H, s, Ar-H).

2,3,4,6-Tetramethoxyphenol (2b").

The acetate **2b'** (500 mg, 1.95 mmol) was hydrolyzed with 10% aqueous NaOH for 30 min at 25 ˚C to give the phenol **2b"** (368 mg, 87%) as pale yellow needles, mp 84-85˚C. ^{1}H-NMR ($CDCl_3$) δ 3.83, 3.84, 3.87 and 3.89 (each 3H, s, OCH_3), 5.25 (1H, s, OH), 6.33 (1H, s, Ar-H).

1,2,3,4,5-Pentamethoxybenzene (1a).

A mixture of **2b"** (500 mg, 2.33 mmol), $(MeO)_2SO_2$ (0.45 mL, 4.7 mmol) and K_2CO_3 (691 mg, 5.0 mmol) in acetone (5 mL) was irradiated with stirring for 10 min (30 s × 20 times, 30 s interval/irradiation) under MWI. The resulting compound was chromatographed over a silica gel column to give the pentamethoxybenzene **1a**[14] (471 mg, 88%) as pale yellow needles, mp 55.5-57 ˚C. ^{1}H-NMR ($CDCl_3$) δ 3.83 (6H, s, OCH_3 × 2), 3.85 (6H, s, OCH_3 × 2), 3.95 (3H, s, OCH_3), 6.30 (1H, s, Ar-H).

2',3',4',5',6'-Pentamethoxyacetophenone (2a).

A mixture of **1a** (500 mg, 2.19 mmol), Ac_2O (10 mL) and $In(CF_3SO_3)_3$ (8 mg, 0.015 mmol) was irradiatied with stirring for 15 min (30 s × 30 times, 30 s interval/irradiation) under MWI. The resulting compound was chromatographed over a silica gel column to give the acetophenone **2a**[14] (456 mg, 77%) as a pale yellow oil.

2'-Hydroxy-3',4',5',6'-tetramethoxyacetophenone (3a).

A solution of **2a** (1.06 g, 3.93 mmol) and $AlCl_3$ (1.05 g, 7.86 mmol) in MeCN (8 mL) was irradiated with stirring for 1.5 min (10 s × 9 times, 15 s interval/irradiation) under MWI. To the reaction mixture was added 6% HCl (10 mL) and the whole mixture was stirred for 30 min. The resulting compound was chromatographed over a silica gel column (AcOEt:hexane=2:3 as a solvent) to give the 2'-hydroxyacetophenone **3a**[14] (850 mg, 85%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 2.67 (3H, s, $COCH_3$), 3.81, 3.86, 3.95 and 4.08 (each 3H, s, OCH_3), 13.16 (1H, s, OH).

2'-Hydroxy-3',4',6'-trimethoxyacetophenone (3b).

A solution of **2b** (6.0 g, 25 mmol) and $AlCl_3$ (6.60 g, 49.9 mmol) in MeCN (30 ml) was irradiated with stirring for 1.5 min (10 sec × 9 times, 15 sec interval/irradiation) under MWI. The resulting compound was chromatographed over a silica gel column to give the 2'-hydroxyacetophenone **3b**[16] (4.49 g, 81%) as pale yellow needles, mp 111-113 ˚C. ^{1}H-NMR ($CDCl_3$) δ 2.62 (3H, s, $COCH_3$), 3.82, 3.90 and 3.94 (each 3H, s, OCH_3), 5.97 (1H, s, Ar-H), 13.80 (1H, s, OH).

2'-(3,4,5-Trimethoxybenzoyloxy)-3',4',5',6'-tetramethoxyacetophenone (11).

A mixture of **3a** (410 mg, 1.60 mmol), fresh 3,4,5-trimethoxybenzoyl chloride (**8**) (424 mg, 1.94 mmol) and pyridine (3 mL) was refluxed with stirring for 1.5 min (30 s × 3 times, 30 s interval/irradiation) under MWI. The resulting mixture was worked up in the same manner as in the case of **3a** to give the ester **11** (541 mg, 75%) as colorless needles, mp 73.5-75.5 ˚C. ^{1}H-NMR ($CDCl_3$) δ 2.49 (3H, s, $COCH_3$), 3.83 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.93 (6H, s, OCH_3 × 2), 3.94(3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 7.41 (2H, s, Ar-H × 2).

1-(2-Hydroxy-3,4,5,6-tetramethoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-1,3-propanedione (12).

A mixture of **11** (480 mg, 1.06 mmol), powdered KOH (300 mg, 5.33 mmol) and pyridine (5 mL) was refluxed with stirring for 5 min (30 s × 10 times, 30 s interval/irradiation) under MWI. The reaction mixture was extracted with AcOEt, after which the extract was washed with water and dried (Na_2SO_4). The resulting compound was chromatographed over a silica gel column to give the diketone **12** (460 mg, 92%) as pale yellow needles, mp 79-81 ˚C.

5,6,7,8,3',4',5'-Heptamethoxyflavone (4).

A solution of the diketone **12** (300 mg, 0.666 mmol) and concd H_2SO_4 (0.1 mL) in AcOH (4 mL) was irradiated with stirring for 3 min (15 s × 12 times, 30 s interval/irradiation) under MWI. The reaction

mixture was poured into ice-water and extracted with AcOEt, after which the extract was washed with 3% aqueous NaOH, then water, and dried (Na_2SO_4). The resulting compound was recrystallized from AcOEt/hexane to give the desired flavone **4** (251 mg, 87%) as pale yellow needles, mp 101.5-102.5 ˚C. IR (KBr) ν 3427, 2957, 2839, 1644, 1590 cm^{-1}; UV λ_{max} (log ε) (MeOH) 223 (4.33), 273 (4.19), 321 (4.27); ^{1}H-NMR δ3.93 (3H, s, OCH_3), 3.95 (9H, s, OCH_3 × 3), 3.96 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 4.11 (3H, s, OCH_3), 6.64 (1H, s, C_3-H), 7.17 (2H, s, Ar-H × 2); *Anal.* Calcd for $C_{22}H_{24}O_9$: C, 61.10; H, 5.59. Found: C, 60.95; H, 5.55.

2'-(3,4,5-Trimethoxybenzoyloxy)-3',4',6'-trimethoxyacetophenone (13).

A mixture of **3a** (1.00 g, 4.42 mmol), benzoyl chloride **8** (1.17 g, 5.08 mmol) and pyridine (3 mL) was irradiated with stirring for 2 min (30 s × 4 times, 30 s interval/irradiation) under MWI. The resulting compound was worked up in the same manner as in the case of **11** to give the ester **13** (1.52 g, 82%) as pale yellow needles, mp 122-124 ˚C. ^{1}H-NMR ($CDCl_3$) δ2.48 (3H, s, $COCH_3$), 3.77 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.93 (9H, s, OCH_3 × 3), 3.95 (3H, s, OCH_3), 6.45 (1H, s, Ar-H), 7.43 (2H, s, Ar-H × 2).

1-(2-Hydroxy-3,4,6-trimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-1,3-propanedione (14).

A mixture of **13** (500 mg, 1.19 mmol) and KOH (334 mg, 5.95 mmol) in pyridine (5 mL) was irradiated with stirring for 5 min (30 s × 10 times, 30 s interval/irradiation) under MWI. The resulting compound was worked up in the same method as in the case of **12** to give the diketone **14** (440 mg, 88%) as pale yellow needles, mp 164-166 ˚C.

5,7,8,3',4',5'-Hexamethoxyflavone (5).

A solution of the diketone **14** (300 mg, 0.714 mmol) and conc H_2SO_4 (0.1 mL) in AcOH (4 mL) was irradiated with stirring for 1.5 min (15 s × 6 times, 30 s interval/irradiation) under MWI. The resulting compound was worked up in the same method as in the case of **4** to give the flavone **5** (272 mg, 94%) as colorless needles, mp 197-199 ˚C IR (KBr) ν 3427, 2942, 2843, 1679, 1610 cm^{-1}; UV λ_{max} (log ε) (MeOH) 271 (4.19), 319 (4.15), 341 (4.13); ^{1}H-NMR ($CDCl_3$) δ3.93 (3H, s, OCH_3), 3.95 (6H, s, OCH_3 × 2), 3.97 (3H, s, OCH_3), 4.01 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 6.45 (1H, s, Ar-H), 6.65 (1H, s, C_3-H), 7.19 (2H, s, Ar-H × 2); *Anal.* Calcd for $C_{21}H_{22}O_8$: C, 62.68; H, 5.51. Found: C, 62.56; H, 5.52.

2'-(3,4-Dimethoxybenzoyloxy)-3',4',6'-trimethoxyacetophenone (15).

A solution of **3b** (1.00 g, 4.42 mmol), benzoyl chloride **9** (1.02 g, 5.08 mmol) in pyridine (3 mL) was irradiated with stirring for 2 min (30 s × 4 times, 30 s interval/irradiation) under MWI. The resulting compound was worked up in the same manner as in the case of **11** to give the ester **15** (1.53 g, 89%) as colorless needles, mp 201-203 ˚C. ^{1}H-NMR ($CDCl_3$) δ2.47 (3H, s, $COCH_3$), 3.76(3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.96 (3H, s, OCH3), 6.45 (1H, s, Ar-H), 6.93 (1H, d, *J*=8.6 Hz, Ar-H), 7.64 (1H, d, *J*=2.2 Hz, Ar-H), 7.84 (1H, dd, *J*=2.2 and 8.6 Hz, Ar-H).

1-(2-Hydroxy-3,4,6-trimethoxyphenyl)-3-(3,4-dimethoxyphenyl)-1,3-propanedione (16).

A mixture of **15** (1.00 g, 2.56 mmol) and KOH (720 mg, 12.8 mmol) in pyridine (10 mL) was irradiated with stirring for 4 min (30 s × 8 times, 30 s interval/irradiation) under MWI. The resulting compound was worked up in the same method as in the case of **12** to give the diketone **16** (860 mg, 86%) as pale yellow needles.

5,7,8,3',4'-Pentamethoxyflavone (6).

A solution of the diketone **16** (835 mg, 2.14 mmol) and conc H_2SO_4 (0.6 mL) in AcOH (8 mL) was irradiated with stirring for 2 min (30 s × 4 times, 30 s interval/irradiation) under MWI. The resulting compound was worked up in the same method as in the case of **4** to give the flavone **6** (660 mg, 83%, crystallized from MeOH) as pale yellow needles, mp 195-197 ˚C. IR (KBr) ν 3310, 2885, 2848, 1662, 1609 cm^{-1}; UV λ_{max} (log ε) (MeOH) 249 (4.34), 269 (4.39), 337 (4.27); ^{1}H-NMR ($CDCl_3$) δ 3.96 (6H, s, OCH_3 × 2), 3.98 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 6.44 (1H, s, Ar-H), 6.62 (1H, s, C_3-H), 7.00 (1H, d, *J*=8.6 Hz, $C_{5'}$-H), 7.42 (1H, d, *J*=2.0 Hz, $C_{2'}$-H), 7.59 (1H, dd, *J*=2.0 and 8.6 Hz, $C_{6'}$-H); ; *Anal.* Calcd for $C_{20}H_{20}O_7$: C, 64.51; H, 5.41. Found: C, 64.48; H, 5.46.

2'-(2,6-Dimethoxybenzoyloxy)-3',4',5',6'-tetramethoxyacetophenone (17).

A solution of **3a** (500 mg, 1.95 mmol) and benzoyl chloride **10** (467 mg, 2.33 mmol) in pyridine (2 mL) was irradiated with stirring for 3.5 min (30 s × 7 times, 30 s interval/irradiation) under MWI. The resulting compound was worked up in the same manner as in the case of **11** to give the ester **17** (620 mg, 76%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ 2.52 (3H, s, $COCH_3$), 3.88 (9H, s, OCH_3 × 3), 3.90, 3.94 and 3.98 (each 3H, s, OCH_3), 6.59 (2H, d, *J*=8.5 Hz, C_3-H and C_5-H), 7.33 (each 1H, d, *J*=8.5 Hz, C_4-H overlap).

1-(2-Hydroxy-3,4,5,6-tetramethoxyphenyl)-3-(2,6-dimethoxyphenyl)-1,3-propanedione (18).

A mixture of **17** (520 mg, 1.26 mmol) and KOH (353 mg, 6.29 mmol) in pyridine (5 mL) was irradiated with stirring for 7 min (30 s × 12 times, 30 s interval/irradiation) under MWI. The resulting compound was worked up in the same method as in the case of **12** to give the diketone **18** (494 mg, 95%) as a pale yellow oil.

5,6,7,8,2',6'-Hexamethoxyflavone (7).

A solution of the diketone **18** (470 mg, 1.12 mmol) and conc H_2SO_4 (0.2 mL) in AcOH (5 mL) was irradiated with stirring for 5 min (30 s × 10 times, 30 s interval/irradiation) under MWI. The resulting compound was worked up in the same method as in the case of **4** to give the flavone **7** (410 mg, 91%), which was chromatographed over a silica gel column (AcOEt:hexane=1:1 as a solvent), as colorless needles, mp 99-100.5 ˚C. IR (KBr) ν 3427, 2938, 2826, 1609, 1592 cm^{-1}; UV λ_{max} (log ε) (MeOH) 231 (4.19), 257 (4.25); ^{1}H-NMR ($CDCl_3$) δ 3.80 (6H, s, OCH_3 × 2), 3.92 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 4.08 (3H, s, OCH_3), 6.28 (1H, s, C_3-H), 6.63 (2H, d, *J*=8.6 Hz, $C_{3'}$-H and $C_{5'}$-H

overlap), 7.40 (1H, d, *J*=8.6 Hz, $C_{4'}$-H overlap); *Anal.* Calcd for $C_{21}H_{22}O_8$: C, 62.68; H, 5.51. Found: C, 62.66; H, 5.52.

Cell culture. Human HM3KO melanoma cells were maintained in culture medium consisting of Dulbecco's modified Eagle medium supplement with 10% fetal bovine serum, 100,000 U/L benzylpenicillin potassium and 100 mg/L kanamycin sulfate.

Cytotoxicity assay. HM3KO melanoma cells (10,000cells/well) were seeded on 96-well plates in 100 μL of culture medium and maintained for 8 h. Then culture medium containing test compound was applied for 3 days. The culture medium in the well was exchanged with 100 μL of culture medium containing 10 μL of 0.5% MTT solution and incubation was continued for 2 h. Then the culture medium containing the MTT solution in the well was exchanged with 100 μL of stop solution (0.04 M HCl in 2-propanol) and the absorbance was determined at 570 nm with a reference wavelength of 630 nm using a microplate reader. The cell growth rate was calculated from the absorbance compared with the control.

Melanin assay. HM3KO melanoma cells were seeded in 85 mm dishes at a density of 4×10^5 cells per dish and cultured. After incubation for 9 h, the cells were treated with various concentrations of test reagents. Three days later, the medium was changed and the test reagents at the same concentration were added to the medium. The cells were cultured for an additional 2 days, after which they were harvested by trypsinization, washed with phosphate-buffered saline (PBS). The cells were washed with 5% trichloroacetic acid aqueous solution, ethanol/ether (3/1) and ether in turn, and dissolved in 1 mL of 2 M NaOH. Inhibitory activity of melanogenesis was calculated by measuring the absorbance at 410 nm of the solution.

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