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IMPROVED, RAPID AND EFFICIENT SYNTHESIS OF POLY-METHOXYFLAVONES UNDER MICROWAVE IRRADIATION AND THEIR INHIBITORY EFFECTS ON MELANOGENESIS

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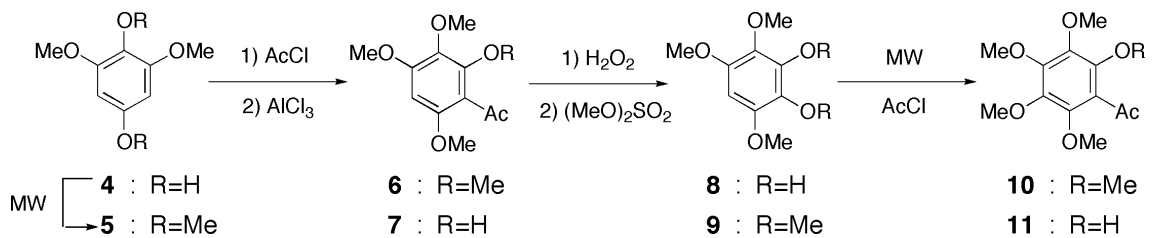
Abstract - The microwave-assisted methylation of 2,5-dihydroxy-1,3-dimethoxybenzene with $(CH_3O)_2SO_2$ for 5 min gave easily 1,2,3,5-tetramethoxybenzene, which was converted into pentamethoxybenzene. The microwave-assisted Friedel-Crafts acylation of pentamethoxybenzene in the presence of $In(CF_3SO_3)_3$ gave pentamethoxyacetophenone for 15 min under solvent-free conditions in high yield. The microwave-assisted cyclization reaction of the diketones, which were synthesized from the acetophenone *via* three steps under microwave irradiation, gave the desired polymethoxyflavones for 1.5-3 min in high yields. The polymethoxyflavones showed inhibitory effects on melanogenesis in a human melanoma.

Flavones are widely distributed in nature and have various biological properties.[1] The flavones contained in *Citrus* species have been found to exhibit antitumor, antioxidant and antiinflammatory activities.[2] Among these compounds, Nobiletin (3',4',5,6,7,8-hexamethoxyflavone) and tangeretin (4',5,6,7,8-pentamethoxyflavone) in *Citrus aurantium* have shown antiallergic activity and a cell-growth inhibitory effect,[3] while we have recently extracted nobiletin and tangeretin as a small amount of components from the pericarps of *Citrus aurantium tachibana* (Makino) Tanaka and have discovered that both these compounds also suppressed tyrosinase activity.[4] Not surprisingly, flavone derivatives have been receiving much attention in the fields of preventive medicine and pharmaceutics, as well as in food science and cosmetics in recent years. While polymethoxyflavones such as nobiletin have been prepared using a variety of methods,[5] many of the procedures are somewhat unsatisfactory with regard to operational simplicity, reaction times, yields or cost of the reagents. Microwave irradiation has been

recently used for a variety of applications including organic synthesis,[6] and the new microwave irradiation methodology could also be applicable to the synthesis of polymethoxyflavones. Improvement of polymethoxyflavone synthesis methods would further the continuation of our work and facilitate examinations of biological activities of these and other related compounds. We wish to report for the first time on the efficient synthesis of 3',4',5,6,7,8-hexamethoxyflavone (nobiletin) (**1**), 4',5,6,7,8-pentamethoxyflavone (tangeretin) (**2**) and 5,6,7,8-tetramethoxyflavone (**3**) under microwave-assisted conditions, and on the inhibitory effects of the obtained compounds on melanogenesis in a human melanoma.

RESULTS AND DISCUSSION

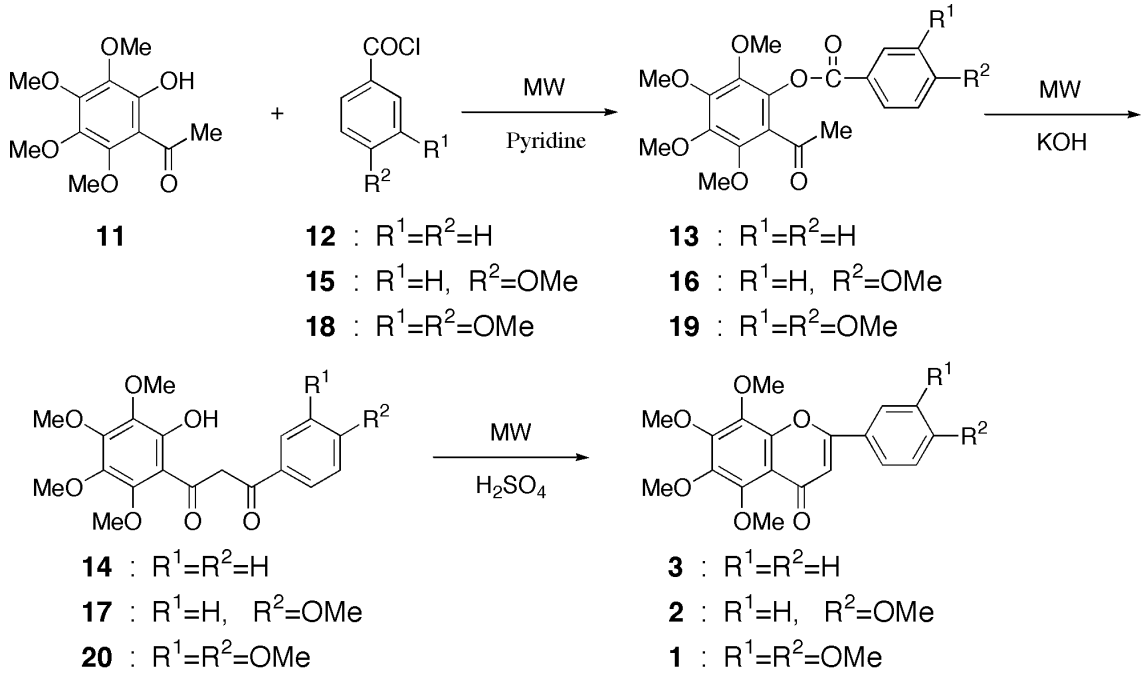
The catalytic hydrogenation of 2,6-dimethoxy-1,4-benzoquinone[7] over Raney nickel gave 2,5-dihydroxy-1,3-dimethoxybenzene (**4**). Microwave-assisted methylation of **4** with $(CH_3O)_2SO_2$ in the presence of K_2CO_3 gave the tetramethoxybenzene (**5**) for 5 min in minimal acetone in 84% yield. The reaction of **5** with acetyl chloride in the presence of $AlCl_3$ for 1.5 h gave easily tetramethoxyacetophenone (**6**), which was partially demethylated with $AlCl_3$ in acetonitrile to give 2'-hydroxyacetophenone (**7**) in 83% yield. However, the published method gave **7** (66%) and 3'-ethoxy-2'-hydroxy-4',6'-dimethoxyacetophenone (7 %) at 30 °C for 6 h.[8] Therefore, the above-mentioned improved synthesis of **6** and **7** is superior to the published method in reaction time, operational simplicity, yield and absence of 3'-ethoxy-2'-hydroxy-4',6'-dimethoxyacetophenone formation. Microwave-assisted acetylation of **5** with acetyl chloride in the presence of $In(CF_3SO_3)_3$ (2%)[9] gave the desired acetophenone (**6**) as a major product and 2'-acetoxy-3',4',6'-trimethoxyacetophenone, which was readily converted into the 2'-hydroxycetophenone (**7**), as a minor product for 6 min under solvent-free conditions. The oxidative rearrangement of **7** with 6% H_2O_2 gave 2,3-dihydroxybenzene (**8**),[10] which was readily converted into pentamethoxybenzene (**9**) by the $(CH_3O)_2SO_2$-K_2CO_3 method. Microwave-assisted acetylation of **9** with acetyl chloride in the presence of $In(CF_3SO_3)_3$ (0.5%) for 15 min without solvent gave pentamethoxyacetophenone (**10**) in 75% yield, and no by-product was obtained. The pentamethoxyacetophenone (**10**) had already been prepared in low yield by an alternative method.[7] The described microwave-assisted Friedel-Crafts acetylation was found to proceed quite rapidly and efficiently in spite of the existence of many methoxy groups (**Scheme 1**).



Scheme 1

The partial demethylation of **10** with $AlCl_3$ in acetonitrile was carried out to give 2'-hydroxyacetophenone (**11**) in 83% yield. The above results revealed that the microwave-assisted acetylation of polymethoxybenzenes in the presence of $In(CF_3SO_3)_3$ is an excellent method for the synthesis of polymethoxyacetophenones because of rapid reaction, high yield and solvent-free conditions.

The esterification of **11** with benzoyl chloride (**12**) in minimal pyridine was carried out for 4 min under microwave irradiation to give benzoate (**13**) in 75% yield. The Baker-Venkataraman rearrangement of **13** in the presence of KOH was carried out in minimal pyridine for 4 min under microwave irradiation to give easily the desired 1,3-diketone (**14**). The microwave-assisted cyclization reaction of **14** with concd H_2SO_4 in minimal acetic acid was achieved for 1.5 min to give the desired tetramethoxyflavone (**3**) in 81% yield (**Scheme 2**).



11

12 : R^1=R^2=H
15 : R^1=H, R^2=OMe
18 : R^1=R^2=OMe

13 : R^1=R^2=H
16 : R^1=H, R^2=OMe
19 : R^1=R^2=OMe

14 : R^1=R^2=H
17 : R^1=H, R^2=OMe
20 : R^1=R^2=OMe

3 : R^1=R^2=H
2 : R^1=H, R^2=OMe
1 : R^1=R^2=OMe

Scheme 2

In the same manner as in the case of **13**, the esterification of **11** with 4-methoxybenzoyl chloride (**15**) gave 4-methoxybenzoate (**16**) for 2.5 min under microwave irradiation. The rearrangement of the crude benzoate (**16**) with KOH in pyridine gave the desired diketone (**17**) for 5 min under microwave irradiation. The microwave-assisted cyclization reaction of **17** with concd H_2SO_4 was achieved in acetic acid for 3 min to give the desired pentamethoxyflavone (**2**) in 60% yield *via* three steps from **11**.

The esterification of **11** with 3,4-dimethoxybenzoyl chloride (**18**) also gave easily 3,4-dimethoxybenzoate (**19**) for 2 min under microwave irradiation. In the same manner as in the case of **2**, the rearrangement of the crude dimethoxybenzoate (**19**) into diketone (**20**), followed by the cyclization reaction of **20** was achieved for 3 min under microwave irradiation to give the desired hexamethoxyflavone (**1**) in 63% yield *via* three steps from **11**.

^{1}H-NMR spectral data for the synthetic hexa- and pentamethoxyflavones (**1** and **2**) were identical with those of natural nobiletin and tangeretin, respectively. Each melting point of the synthetic compounds (**1** and **2**) was not lowered by each admixture with natural nobiletin and tangeretin. On the basis of these results, structures of natural nobiletin and tangeretin in the pericarps of *Citrus aurantium tachibana* (Makino) Tanaka were unequivocally established to be 3',4',5,6,7,8-hexamethoxyflavone and 4',5,6,7,8-pentamethoxyflavone, respectively.

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

Inhibitory effects on melanogenesis in a human melanoma cell line, HM3KO,[11] were carried out at different concentrations of the synthetic polymethoxyflavones (**1**, **2** and **3**), and the results are shown in Figure 1. These compounds suppressed melanin production according to dosedependence. Nobiletin (**1**) and tangeretin (**2**) suppressed up to 50-61% of the melanin production at a concentration of 10 μM, but the tetramethoxyflavone (**3**) showed as much as 77% inhibition at the same concentration. The inhibitory effects of polymethoxyflavones on melanogenesis in HM3KO were consistent with those of natural nobiletin and tangeretin. On the other hand, polymethoxyflavones did not affect the cell growth at the concentration of less than 10 uM. From these results, the flavones containing more

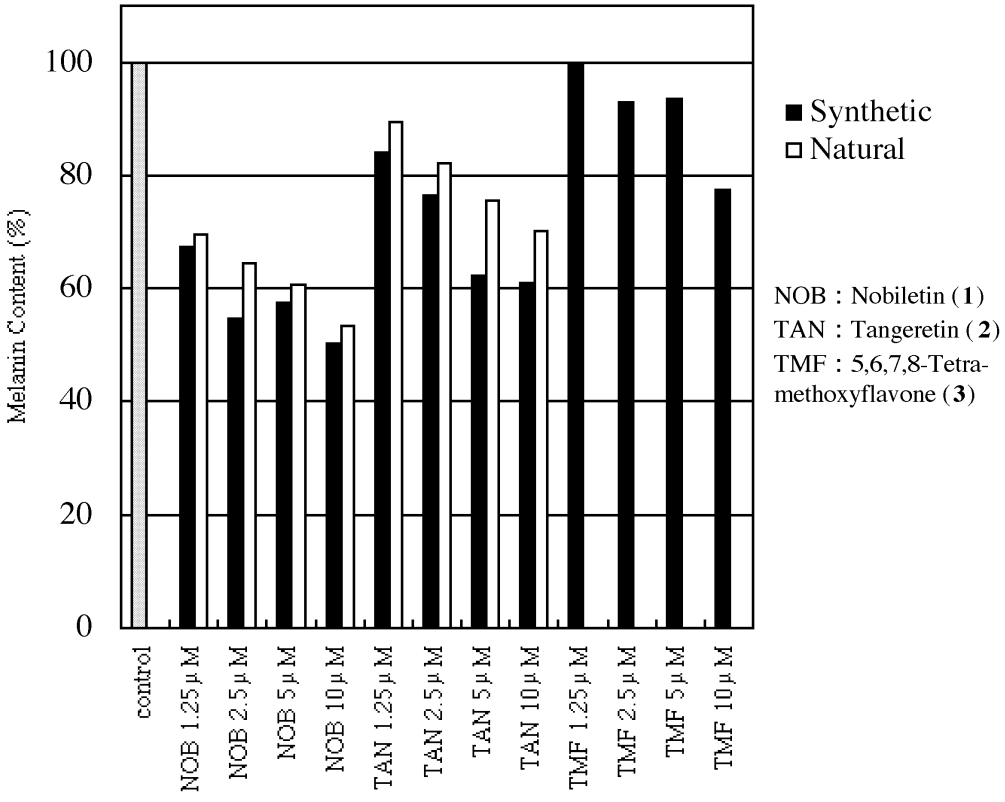


Figure 1. Inhibitory effect of polymethoxyflavones on melanogenesis

methoxy groups were found to show higher inhibitory effects on melanogenesis. Polymethoxyphenols (pentamethoxyacetophenone and pentamethoxybenzene) except for polymethoxyflavones showed no inhibitory effect. From these results, hexa- and pentamethoxyflavones (**1** and **2**) are utilizable as an agent for the treatment of skin pigmentation such as spots and freckles induced by UV light.

The improved, rapid synthesis of polymethoxyflavones under microwave irradiation would be useful for various examinations of biological activities, because the synthesis of these compounds is both simple and efficient.

EXPERIMENTAL

The melting points were measured on a Yanaco MP-J3 micro melting-point apparatus and are uncorrected. The ^{1}H-NMR spectra were measured with a JEOL EX400 spectrometer (400 MHz), using tetramethylsilane as internal standard (δ, ppm). Elemental analyses were performed with a Yanaco CHN corder model MT-5. A domestic microwave oven (Sanyo Co., 650 W and 2.45 GHz, modified properly by fitting a condenser and a thermosensor through the holes made in the roof) was used as a reaction apparatus. Column chromatography and thin-layer chromatography (TLC) were carried out on Kieselgel 60 (70-230 mesh) and with Kieselgel 60 F-254 (Merck).

2,6-Dimethoxy-1,4-hydroquinone (**4**)

2,6-Dimethoxy-1,4-benzoquinone (20.18 g, 0.12 mol), prepared by published method,[7)] was hydrogenated over Raney Ni (6.23 g) in methanol (200 mL) at rt with stirring until the uptake of hydrogen ceased. The resulting compound was chromatographed over a short cilica gel column (AcOEt : hexane = 1:1 as a solvent) to give the hydroquinone (**4**)[7] (19.32 g, 96%) as brown needles, mp 164-165 ˚C, from AcOEt).

1,2,3,5-Tetramethoxybenzene (**5**)

A mixture of **4** (20.1 g, 0.12 mol), $(CH_3O)_2SO_2$ (28.4 mL, 0.30 mol) and K_2CO_3 (83 g, 0.60 mol) in acetone (200 mL) was refluxed at 70 ˚C for 3 h with stirring. The resulting compound was purified by silica gel column chromatography (AcOEt:hexane =1:1) to give **5**[7] (17.8 g, 75% from 2,6-dimethoxy-1,4-benzoquinone) as colorless needles, mp 32-33 ˚C (crystallized from AcOEt-hexane); ^{1}H-NMR ($CDCl_3$) δ 3.79 (6H, s, OCH_3 x 2), 3.85 (6H, s, OCH_3 x 2), 6.15 (2H, s, Ar-H x 2).

1,2,3,5-Tetramethoxybenzene (**5**) (under microwave irradiation).

A mixture of the hydroquinone (**4**) (5 g, 29.4 mmol), $(CH_3O)_2SO_2$ (11.2 mL, 0.12 mol) and K_2CO_3 (20 g, 0.15 mol) in acetone (24 mL) was stirred at 73 ˚C for 5 min (1 min x 5 with intermissions of 20 sec in between) under microwave irradiation. The resulting compound was purified by silica gel column chromatography to give **5** (4.6 g, 84%) as a colorless oil.

2',3',4',6'-Tetramethoxyacetophenone (**6**)

To a mixture of **5** (3.01 g, 15.18 mmol) and $AlCl_3$ (6.71 g, 50.32 mmol) in ether (50 mL) was gradually

added acetyl chloride (1.49 g, 18.98 mmol) in ether (10 mL) and the whole mixture was stirred at 0 ˚C for a further 1.5 h. To the reaction mixture was added 6% HCl (50 mL), and then the resultant mixture was stirred for 30 min at rt. The resulting compound was extracted with ether and washed with 5% aqueous NaOH and water, and dried (Na_2SO_4). The resulting compound gave the acetophenone (**6**)[7] (3.66 g) as a pale paste, which was used for the next experiment without purification.

2'-Hydroxy-3',4',6'-trimethoxyacetophenone (**7**)

A mixture of **6** (3.66 g) and $AlCl_3$ (6.31 g, 47.3 mmol) in CH_3CN (20 mL) was stirred at 70 ˚C for 1 h. To the reaction mixture was added 6% HCl (50 mL) and the resultant mixture was stirred at 60 ˚C for 30 min. The resulting compound was recrystallized from methanol to give the 2'-hydroxyacetophenone (**7**)[8] (2.85 g, 83% from **5**) as pale yellow prisms, mp 112-114 ˚C; ^{1}H-NMR ($CDCl_3$) δ 2.62 (3H, s, CH_3CO), 3.82 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 5.97 (1H, s, Ar-H), 13.81 (1H, s, OH).

2',3',4',6'-Tetramethoxyacetophenome (**6**) (under microwave irradiation)

A mixture of **5** (1.5 g, 7.65 mmol), CH_3COCl (1.08 mL, 15.1 mmol) and $In(CF_3SO_3)_3$ (75 mg, 0.13 mmol) was stirred for 6 min (1 min x 6 with intermissions of 1 min in between) at 75-90 ˚C under microwave (350 W) irradiation. The resulting compound was chromatographed over a silica gel column to give the acetophenone (**6**) (0.963 g, 53%) and 2'-acetoxy-3',4',6'-trimethoxyacetophenone (0.13 g, 6%), which was hydrolyzed with 10% aqueous NaOH (5 mL) at rt for 30 min to give the 2'-hydroxyacetophenone (**7**) (90 mg, 80% from AcOEt-hexane), mp 112-114 ˚C.

2,3-Dihydroxy-1,4,5-trimethoxybenzene (**8**)

To a mixture of **7** (15.0 g, 66.31 mmol) and 5% aqueous NaOH (67.5 mL, 67 mmol) in methanol (130 mL) was drop by drop added 6% H_2O_2 (135 mL, 0.20 mol) at 3-5 ˚C for 1 h, and then the whole mixture was stirred at rt for 1 h. After the reaction mixture was acidified with 10% HCl, the organic solvent was removed under reduced pressure. The residue was extracted with ether and washed with water and dried (Na_2SO_4). The resulting compound gave the dihydroxybenzene (**8**)[7,10] (7.77 g, 59%) as pale yellow needles (mp 90-92 ˚C; part of which was crystallized from hexane-benzene), which was used for the next experiment without purification.

1,2,3,4,5-Pentamethoxybenzene (**9**)

A mixture of **8** (7.76 g, 38.76 mmol), $(CH_3O)_2SO_2$ (17.0 mL, 0.18 mol) and K_2CO_3 (53.9 g, 0.96 mol) was refluxed in acetone (150 mL) at 60 ˚C for 1.5 h with stirring. The resulting compound gave the pentamethoxybenzene (**9**)[7] (7.52 g) as a colorless solid, which was used for the next experiment without purification.

2',3',4',5',6'-Pentamethoxyacetophenone (**10**)

In a 50 mL round-bottomed flask were placed **9** (10.33 g, 45.28 mmol), CH_3COCl (6.50 mL, 91.4 mmol) and $In(CF_3SO_3)_3$ (128.4 mg, 0.22 mmol). The whole mixture was stirred by a magnetic stirrer for uniform mixing and after that the mixture was stirred for 15 min (20 sec x 45 with intermissions of 30 sec in between) under microwave irradiation. The reaction mixture was poured into ice water and then was extracted with ethyl acetate, after which the extract was washed with 3% aqueous NaOH, then water, and dried (Na_2SO_4). The resulting compound was chromatographed over a silica gel column to give the acetophenone (**10**)[7] (9.20 g, 75%, crystallized from hexane) as pale needles, mp 42-45 °C; ^{1}H-NMR ($CDCl_3$) δ 2.50 (3H, s, CH_3CO), 3.84 (6H, s, OCH_3 x 2), 3.88 (6H, s, OCH_3 x 2), 3.95 (3H, s, OCH_3).

2'-Hydroxy-3',4',5',6'-tetramethoxyacetophenone (**11**)

A mixture of **10** (5.49 g, 20.29 mmol), $AlCl_3$ (13.51 g, 101.31 mmol) and CH_3CN (20 mL) was stirred at 60 °C for 30 min. The resulting compound was extracted with ether, washed with sodium carbonate and water, and dried (Na_2SO_4). The obtained compound was chromatographed over a short silica gel column to give the 2'- hydroxyacetophenon (**11**)[7] (4.31 g, 83%) as a pale yellow oil.

2'-Benzoyloxy-3',4',5',6'-tetramethoxyacetophenone (**13**)

A mixture of **11** (1.01 g, 3.95 mmol), benzoyl chloride (0.54 mL, 4.65 mmol) and pyridine (1.2 mL) was stirred for 2 min (1 min x 2 with an intermission of 1 min in between) under microwave irradiation. The whole mixture was extracted with ether, and the extract was washed with 6% HCl and water, and dried (Na_2SO_4). The resulting compound was recrystallized from methanol to give the ester (**13**) (1.05 g, 74%) as pale brown needles, mp 78-79 °C; ^{1}H-NMR ($CDCl_3$) δ 2.48 (3H, s, CH_3CO), 3.82 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 7.50 (2H, t, J=8.1 Hz, Ar-H x 2), 7.63 (1H, m, Ar-H), 8.11-8.17 (2H, m, Ar-H x 2).

1-(2-Hydroxy-3,4,5,6-tetramethoxyphenyl)-3-phenyl-1,3-propanedione (**14**)

A mixture of **13** (2.75 g, 7.13 mmol), KOH (2.07 g, 35.64 mmol) and pyridine (15 mL) was stirred for 4 min (1 min x 4 with intermissions of 1 min in between) under microwave irradiation. The whole mixture was poured into a solution of ice-hydrochloric acid and stirred for 30 min at rt. The mixture was slightly acidified with 3% hydrochloric acid and extracted with ether, after which the extract was washed with water and dried (Na_2SO_4). The resulting compound gave the desired diketone (**14**) (2.20 g) as a pale brown oil, which was used for the next experiment without purification.

5,6,7,8-Tetramethoxyflavone (**3**)

A mixture of **14** (2.20 g, 6.11 mmol), concd H_2SO_4 (2 mL) and acetic acid (15 mL) was stirred for 1.5 min (1 min + 0.5 min with intermissions of 1 min in between) under microwave irradiation. The reaction mixture was poured into ice water and extracted with $CHCl_3$, after which the extract was washed with 3% aqueous NaOH, then water, and dried (Na_2SO_4). The resulting compound was recrystallized from

methanol to give the flavone (**3**) (1.69 g, 81%) as colorless needles, mp 107-108 °C; ^{1}H-NMR ($CDCl_3$) δ 3.96 (6H, s, OCH_3 x 2), 4.03 (3H, s, OCH_3), 4.11 (3H, s, OCH_3), 6.70 (1H, s, 2-H), 7.54 (3H, m, Ar-H x 3), 7.94 (2H, m, Ar-H x 2); *Anal.* Calcd for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30. Found: C, 66.58; H, 5.36.

2'-(4-Methoxybenzoyloxy)-3',4',5',6'-tetramethoxyacetophenone (**16**)

A solution of **11** (1.98 g, 7.73 mmol) and anisoyl chloride (**15**) (1.61 g, 9.41 mmol) in pyridine (5 mL) was stirred for 1.5 min (1 min + 0.5 min with an intermission of 1 min in between) under microwave irradiation. The reaction mixture was worked up in the same manner as in the case of the benzoate (**13**) to give the anisoate (**16**) (3.37 g) as a pale yellow solid, which was used for the next examination without purification.

1-(2-Hydroxy-3,4,5,6-tetramethoxyphenyl)-3-(4-methoxyphenyl)-1,3-propanedione (**17**)

A mixture of the crude **16** (3.37 g), KOH (1.38 g, 24.59 mmol) and pyridine (10 mL) was stirred for 5 min (1 min x 5 with intermissions of 1 min in between) under microwave irradiation. The reaction mixture was worked up in the same manner as in the case of **13** to give the crude diketone (**17**) (3.10 g) as a pale brown solid, which was used for the next experiment without purification.

4',5,6,7,8-Pentamethoxyflavone (**2**)

A solution of the crude **17** (3.10 g) and concd H_2SO_4 (2 mL) in acetic acid (15 mL) was stirred for 3 min (1 min x 3 with intermissions of 1 min in between) under microwave irradiation. The reaction mixture was worked up in the same manner as in the case of **3** to give the flavone (**2**)[4] (1.75 g, 60% *via* three steps from **11**) as a pale brown solid, which was recrystallized from methanol as colorless needles, mp 150-151 °C; ^{1}H-NMR ($CDCl_3$) δ 3.89 (3H, s, OCH_3), 3.95 (6H, s, OCH_3 x 2), 4.03 (3H, s, OCH_3), 4.10 (3H, s, OCH_3), 6.61 (1H, s, 2-H), 7.03 (2H, d, J=8.8 Hz, Ar-H x 2), 7.88 (2H, d, J=8.8 Hz, Ar-H x 2); *Anal.* Calcd for $C_{20}H_{20}O_7$: C, 64.51; H, 5.41. Found: C, 64.57; H, 5.44.

2'-(3,4-Dimethoxybenzoyloxy)-3',4',5',6'-tetramethoxyacetophenone (**19**)

A solution of **11** (2.15 g, 8.39 mmol) and fresh 3,4-dimethoxybenzoyl chloride (**18**) (1.61 g, 9.41 mmol) in pyridine (10 mL) was stirred for 2 min (1 min x 2 with an intermission of 1 min in between) under microwave irradiation. The reaction mixture was worked up in the same manner as in the case of **13** to give the dimethoxybenzoate (**19**)[5a] (3.78 g) as a pale brown oil, which was used for the next experiment without purification.

1-(2-Hydroxy-3,4,5,6-tetramethoxyphenyl)-3-(3,4-dimethoxyphenyl)-1,3-propanedione (**20**)

A mixture of the crude **19** (3.78 g), KOH (2.40 g, 42.7 mmol) and pyridine (30 mL) was stirred for 6 min (1 min x 6 with intermissions of 1 min in between) under microwave irradiation. The reaction mixture was worked up in the same manner as in the case of **13** to give the crude diketone (**20**)[5a] (4.58 g) as a pale brown solid, which was used for the experiment without purification.

3',4',5,6,7,8-Hexamethoxyflavone (1)

A solution of the crude **20** (4.58 g) and concd H_2SO_4 (3 mL) in acetic acid (30 mL) was stirred for 3 min (1 min x 3 with intermissions of 1 min in between) under microwave irradiation. The reaction mixture was worked up in the same manner as in the case of **3** to give the desired hexamethoxyflavone (**1**)[4, 5a] (3.53 g) as pale yellow needles, which were recrystallized from ethyl acetate to afford colorless needles (2.03 g, 63% *via* 3 steps from **11**), mp 136-137 °C; ^{1}H-NMR ($CDCl_3$) δ 3.96 (6H, s, OCH_3 x 2), 3.97 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 4.03 (3H, s, OCH_3), 4.11 (3H, s, OCH_3), 6.63 (1H, s, 2-H), 7.00 (1H, d, J=8.6 Hz, Ar-H), 7.42 (1H. s, Ar-H), 7.57 (1H, d, J=8.6 Hz, Ar-H); *Anal.* Calcd for $C_{21}H_{22}O_8$: C, 62.68; H, 5.51. Found: C, 62.70; H, 5.54.

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

Melanin assay. Melanoma cells were seeded in 85 mm dishes at a density of 4 x 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), suspended in 1 mL of PBS, and the number of them was counted. 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 absorbance at 410 nm of the solution.

REFERENCES

1. J. B. Harborne, 'The Flavonoids: Advances in Research Since 1986,' Chapman & Hall, New York, 1994.
2. O. Benavente-Garcia, J. Castillo, F. R. Marin, A. Ortuno, and J. A. Del Rio, *J. Agric. Food Chem.,* 1997, **45**, 4505.
3. a) Y. Chun and U. Sankawa, *Syoyakugaku Zasshi*, 1989, **43**, 314. b) Y. Satoh, S. Tashiro, M. Satoh, Y. Fujimoto, J-Y. Xu, and T. Ikekawa, *Yakugaku Zasshi*, 1996, **116**, 244.
4. R. Hashizume and S. Hayashi, *Fragrance Journal*, 2003, **18**, 103.
5. a) Z. Horii, *J. Pharm. Soc. Japan*, 1940, **60**, 614. b) V. V. S. Murti and T. R. Seshadri, *Proc. Indian Acad. Sci.*, 1949, **30A**, 12. c) A. Oliverio and C. Casinovi, *Gazz. Chim. Ital.*, 1950, **80**, 798. d) M. Krishamurti, T. R. Seshadri, and D. Sharma, *Indian J. Chem.*, 1970, **8**, 575. e) C. Wan Mu, *Indian J. Heterocycl. Chem.*, 1997, **6**, 221.

6. a) 'Microwaves in Organic Synthesis,' ed. By A. Loupy, Wiley-VCH, New York, 2002. b) 'Microwave-Enhanced Chemistry,' ed. by H. M. Kingstone and S. J. Haswell, Am. Chem. Soc., Washington, DC, 1997.
7. W. Baker, *J. Chem. Soc.*, 1941, 662.
8. G. J. Bennet and H.-H. Lee, *J. Chem. Soc., Perkin Trans. 1*, 1986, 633.
9. H. Koshima and M. Kubota, *Synth. Commun.*, 2003, in press.
10. T. Horie, T. Kitou, Y. Kawamura, and K. Yamashita, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 1033.
11. M. Oka, K. Ogita, H. Ando, T. Horikawa, K. Hayashibe, N. Saito, U. Kikkawa and M. Ichihashi, *J. Cell Physiol.*, 1996, **167**, 406.