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Synthesis of Naturally Occurring 5-Hydroxy-7,2′,4′,5′-tetramethoxyflavone and Related Compounds

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The synthesis of the title compound is described starting from 2,4,6-trihydroxyacetophenone and 2,4,5-trimethoxybenzoyl chloride. In addition 5,7-dihydroxy-2′,4′,5′-trimethoxyflavone, 5-hydroxy-6-methyl-7,2′,4′,5′-tetramethoxyflavone, 5,7,2′,4′,5′-pentamethoxyflavone and 8-methyl-5,7,2′,4′,5′-pentamethoxyflavone were prepared and characterized.

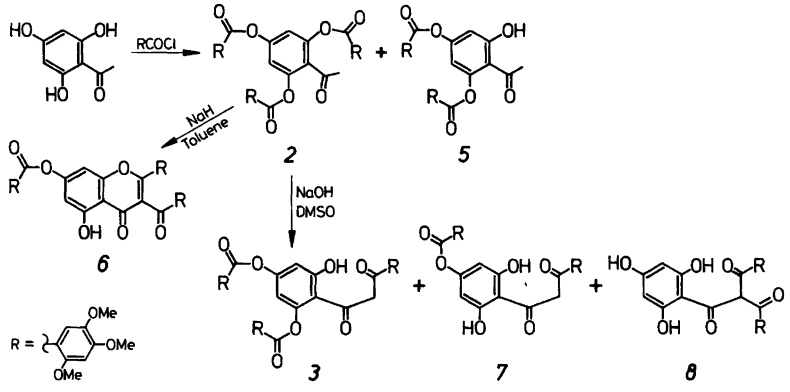
Recently two new flavones, 7,2′,4′,5′-tetramethoxyflavone and 5-hydroxy-7,2′,4′,5′-tetramethoxyflavone (**1**), have been isolated from the Mexican medicinal plant *Calliandra californica* Benth.[1] The former compound was synthesized and found to be inactive in antibiotic assays. The latter flavone (**1**) exhibited antibacterial activity and, in order to confirm the structure and gain more compound, the synthesis was undertaken.

Results and discussion

The strategy adopted for the synthesis of 5-hydroxy-7,2′,4′,5′-tetramethoxyflavone (**1**) includes the preparation of 2,4,6-tris(2,4,5-trimethoxybenzoyloxy)acetophenone (**2**) followed by base-catalyzed rearrangement to give 1-[2-hydroxy-4,6-bis(2,4,5-trimethoxybenzoyloxy)-phenyl]-3-(2,4,5-trimethoxyphenyl)propane-1,3-dione (**3**). Acid-catalyzed cyclisation of the latter compound (**3**) with subsequent hydrolysis would leave 5,7-dihydroxy-2′,4′,5′-trimethoxyflavone (**4**) to be partially methylated to the title compound (**1**).

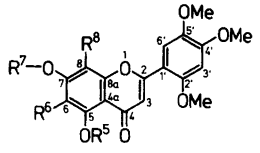
The reaction between 4.5–5 equivalents of 2,4,5-trimethoxybenzoyl chloride[1,2] and the monohydrate of 2,4,6-trihydroxyacetophenone gave a fair yield of **2** purified by recrystallization. If 3 equivalents were added, corresponding to the analogous reaction with 3,4,5-trimethoxybenzoyl chloride,[3] a mixture of **2** and 2-hydroxy-4,6-bis(2,4,5-trimethoxybenzoyloxy)acetophenone (**5**) was formed and separated chromatographically. The structure of **5** was inferred from the sharp singlet at very low field (13.65 ppm) in the NMR spectrum due to the proton of the 5-hydroxy group strongly hydrogen bonded to the carbonyl group.

Base-catalyzed rearrangement of **2** was expected to give **3** which is set up for ring closure to the flavone di-



Scheme 1.

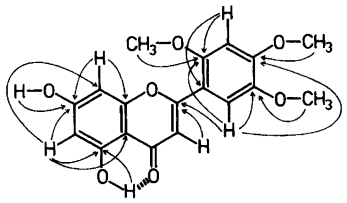
ester. However, the rearrangement reaction is strongly dependent on reaction conditions and failed to give the desired product under a variety of experimental conditions which have proved satisfactory in the synthesis of less hydroxylated flavones. Thus the use of potassium hydroxide in dry pyridine or toluene resulted in degradation products. Sodium ethoxide in ethanol almost quantitatively furnished 2,4,5-trimethoxybenzoic acid ethyl ester and 2,4,6-trihydroxyacetophenone. Sodium hydride in toluene resulted in the formation of 2′,4′,5′-trimethoxy-3-(2,4,5-trimethoxybenzoyl)-5-hydroxy-7-(2,4,5-trimethoxybenzoyloxyflavone (**6**) as the main product. Finally sodium hydroxide in dimethyl sulfoxide at room temperature gave as the main product 1-[2,6-dihydroxy-4-(2,4,5-trimethoxybenzoyloxy)phenyl]-3-(2,4,5-trimethoxyphenyl)propane-1,3-dione (**7**) with smaller amounts of **3** and the diacylated 2,2-bis(2,4,5-trimethoxybenzoyl)-2′,4′,6′-trihydroxyacetophenone (**8**).



1 $R^5=R^6=R^8=H$, $R^7=Me$
4 $R^5=R^6=R^7=R^8=H$
9 $R^5=R^8=H$, $R^6=R^7=Me$
10 $R^5=R^7=Me$, $R^6=R^8=H$
11 $R^5=R^7=R^8=Me$, $R^6=H$

Since **7** is as well suited for cyclisation as **3** (**3** was not purified and therefore is not included in the Experimental section), the raw material was treated with acid to give **4** in fair yield. Methylation of **4** with methyl iodide in acetone–potassium carbonate afforded a mixture of **1** and starting material **4**. Extraction with chloroform removed **1** and left the less soluble **4**. The isomer **1** was identical in all respects with the natural compound.[1] Several other methylation procedures (methyl iodide and aqueous sodium hydroxide, dimethyl sulfate and aqueous sodium hydroxide, acetone–potassium carbonate, respectively) also gave **1**. Methyl iodide and the sodium salt of **4** in dimethyl formamide yielded **9** also formed from **4** with methyl iodide and silver hydroxide in chloroform. The latter experiment further gave rise to the formation of **11**. Methylation of **1** with dimethyl sulfate and potassium carbonate in acetone afforded a mixture of **1** and **10** which was separated chromatographically.

Assignments of the NMR resonances were hampered by the similarity of the chemical shifts (^1H) and the many quaternary resonances of comparable magnitude (^{13}C). In order to aid future structure elucidations of this type of compound, assignments of the data from the naturally occurring compound (**1**), the key dihydroxy compound (**4**), the C-6-methylated compound (**9**) and the C-8-methylated compound (**11**) are presented in Tables 1 and 2. The assignments of these data for **1**, **4** and **9** are based



4

Fig. 1. Connectivities established in **4** by long-range couplings optimized for J_{C-H} 7 Hz.

Table 1. ^1H NMR assignments.

	Compound			
Position	**1**	**4**[a]	**9**	**11**[b]
3	7.04	6.85	7.04	6.70
6	6.36	6.19	–	6.85
8	6.45	6.50	6.44	–
3′	6.59	6.84	6.59	6.63
6′	7.41	7.43	7.41	7.45
OH(5)	12.92	12.93	13.0	–
OH(7)	–	10.77	–	–

[a] In DMSO-d_6, $J_{6,8}=2$ Hz, Me(2′) δ 3.81, Me(4′) 3.95, Me(5′) 3.91. **9**: Four methoxy groups at δ 3.92, 3.93, 3.95, 3.98 and Me(6) at δ 2.12. [b] In DMSO-d_6, Me(8) δ 2.24, Me(5.7) 3.90, 3.94, Me(2′,4′) 3.96, 3.88, Me(5′) 3.80.

on HETCOR experiments and connectivity studies using long-range HETCOR techniques optimized for proton–carbon coupling constants of 7 Hz. The resulting connectivities in the case of **4** are depicted in Fig. 1. In the case of **1** the assignments were further substantiated by

Table 2. ^{13}C NMR assignments.

	Compound			
Position	**1**[a]	**4**[b]	**9**[c]	**11**[d]
2	161.1	161.0	160.6	160.9
3	109.7	108.2	109.8	110.4
4	182.8	181.7	182.7	176.4
4a	105.5	103.6	105.7	107.7
5	162.1	161.3	158.4	157.3
6	97.8	98.7	108.6	104.5
7	163.3	164.1	163.2	158.4
8	92.4	94.1	89.0	92.5
8a	157.7	157.4	155.9	155.9
1′	111.4	110.0	111.4	111.1
2′	143.1	142.8	143.0	142.7
3′	97.1	98.3	97.0	98.4
4′	152.8	153.8	152.6	152.2
5′	154.0	153.1	153.8	153.4
6′	111.9	112.0	111.9	111.4

[a] Me(7) δ 55.8, Me(4′) 56.8, Me(5′,2′) 56.1, 56.3. [b] In DMSO-d_6, Me(2′) δ 56.4, Me(4′) 56.6, Me(5′) 56.0. [c] Four methoxy groups at δ 56.8, 56.1, 56.0, 55.8 and Me(6) at δ 7.18. [d] The assignments are tentative. Me(8) δ 7.9, five methoxy groups at δ 56.0, 56.1, 56.1, 56.2, 56.5.

a 2% NOE effect in the signals originating from H(8) and H(3), respectively, on saturation of the hydroxy group proton signal at δ 12.92. Analogously, in the case of **9**, an NOE effect of 1% was observed for H(3) on irradiating at the frequency corresponding to the hydroxy group proton (δ 13.00). The assignments of the proton resonances in **11** result from NOE difference data. Consecutive saturation of the signals originating from the protons at position 3, 6, 3′ and 6′ produced enhancements of 2%(H6′); 2%(Me5,7) each; 2%(Me2′,4′) each and 4%(H3), 4%(Me5′), 2%(Me8), respectively. These enhancements are only compatible with an 8-methyl derivative. The carbon data for **11** were assigned by comparison with values from the reference compounds **1**, **4** and **9**.

Experimental

The ^1H and ^{13}C NMR spectra were recorded on a Bruker 250 AM or a Varian XL-400 spectrometer, operating at 250 or 400 MHz for protons and at 62.9 or 100.6 MHz for carbons. Unless otherwise stated the NMR spectra were recorded of $CDCl_3$ (99.8% D) solutions with TMS (0.03%) as an internal standard. Mass spectra were obtained on a Masslab VG20-250 (IP 70eV) quadrupole or a JEOL JMS-HX/HX110A spectrometer (IP 70eV). Preparative chromatographic separations were performed using Merck silica gel 40–63 μm. Melting points were determined on a Büchi 535 apparatus and are uncorrected. Chemicals were purchased from Aldrich. All intermediates exhibited correct elementary analysis and molecular ions in the mass spectra as well as the expected ^1H and ^{13}C NMR patterns.

*2,4,6-Tris(2,4,5-trimethoxybenzoyloxy)acetophenone (**2**).* To a solution of 2,4,6-trihydroxyacetophenone hydrate (930 mg, 5 mmol) in dry pyridine was added 2,4,5-trimethoxybenzoyl chloride[1] (8 g, 25 mmol) in one portion. The reaction mixture was heated to 60°C for 1 h and poured onto ice. Recrystallization from EtOH gave **2** in 48% yield (3.6 g), m.p. 164–165°C. Anal. $C_{38}H_{38}O_{16}$: C, H. EIMS *m/z* (% rel. int.): 750 (1, M^+), 195 (100, $[C_{100}H_{11}O_4^+]$). ^1H NMR: δ 2.51 (s, 3 H, CH_3), 3.90 (s, 6 H, 2 MeO), 3.91 (s, 3 H, MeO), 3.92 (s, 6 H, 2 MeO), 3.93 (s, 3 H, MeO), 3.97 (s, 6 H, 2 MeO), 3.98 (s, 3 H, MeO), 6.56 (s, 2 H), 6.57 (s, 1 H), 7.20 (s, 2 H), 7.53 (s, 2 H), 7.55 (s, 1H).

*2-Hydroxy-4,6-bis(2′,4′,5′-trimethoxybenzoyloxy)acetophenone (**5**).* The preparation was identical with the one described above for **2** except that only 15 mmol of 2,4,6-trimethoxybenzoyl chloride were used. Separation of **2** and **5** was effected by column chromatography on silica gel with AcOEt–heptane–EtOH (7:2:1) as the eluent. Yield 10%, m.p. 141–142°C. Anal. $C_{28}H_{28}O_{12}$: C, H. EIMS *m/z* (% rel. int.): 556 (10, M^+), 362 (80), 344 (30), 212 (80), 195 (100 $[C_{10}H_{11}O_4]^+$). ^1H NMR: δ 2.61 (s, 3 H, CH_3), 3.90 (s, 6 H, 2 MeO), 3.92 (s, 3 H, MeO), 3.94 (s, 3 H, MeO), 3.97 (s, 3 H, MeO), 3.99 (s, 3 H, MeO), 6.56 (s, 1 H), 6.58 (s, 1 H), 6.67 (d, $J = 2.2$ Hz, 1 H), 6.79 (d, $J = 2.2$ Hz), 7.52 (s, 1 H), 7.58 (s, 1 H), 13.65 (s, 1 H, OH).

*Rearrangement of 2,4,6-tris(2,4,5-trimethoxybenzoyloxy)acetophenone (**2**)*

*2′,4′,5′-Trimethoxy-3-(2,4,5-trimethoxybenzoyl)-5-hydroxy-7-(2,4,5-trimethoxybenzoyloxyflavone (**6**).* To a suspension of (**2**) in toluene was added the equivalent amount of NaH (80% in mineral oil) at room temperature. A yellow color appeared within 15 min and the suspension was stirred for a further 1.5 h. After evaporation and acidification with acetic acid the precipitated product was recrystallized from ethanol. Yield 30%, m.p. 196–197°C. Anal. $C_{38}H_{36}O_{15}$: C, H. EIMS *m/z* (% rel. int.): 732 (20, M^+), 507 (35), 195 (100, $[C_{10}H_{11}O_4^+]$). ^1H NMR: δ 3.42 (s, 3 H, MeO), 3.73 (s, 3 H, MeO), 3.79 (s, 3 H, MeO), 3.86 (s, 3 H, MeO), 3.88 (s, 3 H, MeO), 3.90 (s, 3 H, MeO), 3.94 (s, 3 H, MeO), 3.95 (s, 3 H, MeO), 3.97 (s, 3 H, MeO), 6.39 (s, 1 H), 6.50 (s, 1 H), 6.57 (s, 1 H), 6.65 (d, $J = 3.2$ Hz, 1 H), 6.93 (d, $J = 3.2$ Hz, 1 H), 7.04 (s, 1 H), 7.46 (s, 1 H), 7.54 (s, 1 H), 12.68 (s, 1 H, OH).

2,4,5-Trimethoxybenzoic acid ethyl ester. Treatment of (**2**) with sodium ethoxide in ethanol at room temperature caused ethanolysis producing the starting material, 2,4,6-trihydroxyacetophenone, and 2,4,5-trimethoxybenzoic acid ethyl ester in almost quantitative yield, m.p. 67–68°C (Lit.[2] 72°C). Anal. $C_{12}H_{16}O_5$: C, H. EIMS *m/z* (% rel. int.): 240 (80, M^+), 195 (100, $[C_{10}H_{11}O_4]^+$). ^1H NMR: δ 1.38 (t, $J = 7.2$ Hz, 3 H), 3.88 (s, 3 H, MeO), 3.90 (s, 3 H, MeO), 3.94 (s, 3 H, MeO), 4.35 (q, $J = 7.2$ Hz, 2 H), 6.53 (s, 1 H), 7.41 (s, 1 H).

*Base-catalyzed rearrangement of **2** in DMSO.* To a solution of **2** (3.6 g) in DMSO (25 ml) was added solid finely crushed NaOH (3 g) and the suspension was stirred for 1 h at ambient temperature. The reaction mixture was poured into ice, neutralized with acetic acid and the product isolated by filtration.

*1-[2,6-Dihydroxy-4-(2,4,5-trimethoxybenzoyloxy)phenyl]-3-(2,4,5-trimethoxyphenyl)propane-1,3-dione (**7**).* Extraction of the filtered acidified solution with ethyl acetate (4 × 50 ml) gave, after drying ($MgSO_4$) and concentration at reduced pressure, 3.1 g of raw material (yield 86%). A sample (200 mg) was chromatographed on a silica gel column eluted with ethyl acetate–heptane (9:1). The main fraction was identified as **7**. Yield 55 mg (28%), m.p. 275–280°C (decomp.). Anal. $C_{28}H_{28}O_{12}$: C, H. EIMS *m/z* (% rel. int.): 556 (5, M^+), 538 (10), 195 (100). ^1H NMR: δ 3.51, 3.56, 3.66, 3.70, 3.81, 3.88 (s, 6 × 3 H, CH_3O), 4.02 (s, 1 H), 4.04 (s, 1 H), 6.23 (d, $J = 2.2$ Hz, 1 H), 6.40 (d, $J = 2.2$ Hz, 1 H), 6.65 (s, 1 H), 6.71 (s, 1 H), 6.91 (s, 1 H), 7.17 (s, 1 H), 12.59 (s, 1 H, OH).

1-(2,4,6-Trihydroxyphenyl)-2-(2,4,5-trimethoxybenzoyl)-3-(2,4,5-trimethoxyphenyl)propane-1,3-dione (**8**). Filtration of the acidified solution left 2,2-bis(2,4,5-trimethoxybenzoyl)-2′,4′,6′-trihydroxyacetophenone (**8**) which was recrystallized from ethanol. Yield 240 mg (9%), m.p. 192–193 °C (decomp.) with evolution of gas. Anal. $C_{28}H_{28}O_{12}$: C, H. EIMS *m/z* (% rel. int.): 538 (100) $[M-H_2O]^+$; FABMS *m/z* 557 $(M+H)^+$, 539 $(M-H_2O+H)^+$, collision activation of the ion *m/z* 557 resulted in a spectrum with prominent *m/z* 539 indicating this fragment to be formed from the pseudomolecular ion; ^{1}H NMR (DMSO-d_6): δ 3.53, 3.82, 3.95 (6 × CH_3), 5.86, 7.77, 7.47 (6 aromatic protons), ca. 10 (2 H, br s) and ca. 13 (2 H, br s). The remaining proton signal could not be located but was presumably hidden in the solvent signal.

5,7-Dihydroxy-2′,4′,5′-trimethoxyflavone (**4**). Impure raw material (3.1 g) from the synthesis of **7** was used directly for the cyclisation reaction. A solution in AcOH (30 ml) and conc. sulfuric acid (1 ml) was boiled under reflux for 1 h and poured onto ice. The product was isolated by centrifugation. Recrystallization from EtOH yielded 0.960 g (68%), m.p. 303–304 °C. Anal. $C_{18}H_{16}O_7$: C, H. Positive FABMS *m/z* 345 $[M+H]^+$ and EIMS *m/z* 344 (M^+), 301, 153. ^{1}H NMR and ^{13}C NMR are given in Tables 1 and 2. UV (EtOH) λ_{max}/nm (log ε): 210 (4.57), 258 (4.29), 288 (3.97), 364 (4.29).

Methylation of 5,7-dihydroxy-2′,4′,5′-trimethoxyflavone (**4**) *and 5-hydroxy-7,2′,4′,5′-tetramethoxyflavone* (**1**)

5-Hydroxy-7,2′,4′,5′-tetramethoxyflavone (**1**). To a suspension of 5,7-dihydroxy-2′,4′,5′-trimethoxyflavone (**4**) (344 mg, 1.0 mmol) and anhydrous K_2CO_3 (140 mg, 1.0 mmol) in acetone (50 ml) was added methyl iodide (93 µl, 1.5 mmol). The mixture was boiled under reflux for 1 h and concentrated *in vacuo*. After addition of hydrochloric acid, extraction with $CHCl_3$ and evaporation of the dried $CHCl_3$ phase, the yield of crude product was 160 mg (45%). This was recrystallized from AcOEt–heptane (1:1) to give colorless crystals, m.p. 181–182 °C. The ^{1}H and ^{13}C NMR assignments are given in Tables 1 and 2. In the EIMS, which was identical with that of the natural product,[1] the prominent fragment ion *m/z* 167 (37%), according to HRMS (167.0359) does not represent the trimethoxyphenylium ion $(C_9H_{11}O_3)^+$, but rather the 2,6-dihydroxy-4-methoxybenzoylium $(C_8H_7O_4)^+$ ion (Δppm = 9.1) derived from A ring. The product was identical in all aspects with the natural product.[1]

5-Hydroxy-6-methyl-7,2′,4′,5′-tetramethoxyflavone (**9**). The residue left after concentration of a solution of 5,7-dihydroxy-2′,4′,5′-trimethoxyflavone (60 mg, 0.17 mmol) and NaOH (14 mg, 0.34 mmol) in EtOH was redissolved in DMF (4 ml) and excess methyl iodide was added. After being stirred for 72 h at room temperature, the reaction mixture was poured into water and the aqueous phase lyophilized. Column chromatography (silica gel) with ethyl acetate and heptane (3:7) yielded 76% **9**, recrystallized (ethyl acetate–heptane 1:1) to give m.p. 193–194 °C. Anal. $C_{20}H_{20}O_7$: C, H. EIMS *m/z* (% rel. int.) 372 (100 M^+), 343 (10). ^{1}H NMR assignments are given in Table 1 and ^{13}C NMR assignments in Table 2. UV (EtOH) λ_{max}/nm (log ε) 216 (4.41), 242 (4.14), 258 (4.21), 360 (4.25).

5,7,2′,4′,5′-Pentamethoxyflavone (**10**). To a suspension of **1** (40 mg, 0.11 mmol) and anhydrous K_2CO_3 (17 mg, 0.11 mmol) in acetone (25 ml) was added dimethyl sulfate (20 µl, 0.2 mmol) and the mixture was boiled under reflux for 4 h. After concentration *in vacuo* the residue was chromatographed on silica gel with EtOH–$CHCl_3$–CH_3CN (10:65:25) yielding 22 mg **1** and 8 mg **10** (20%), m.p. 212–215 °C. Anal. $C_{20}H_{20}O_7$: C, H. EIMS *m/z* (% rel. int.) 372 (100 M^+), 326 (30), 177 (15), 151 (20). ^{1}H NMR: δ 3.90, 3.91, 3.92, 3.94, 3.95 (s, 5 × 3 H, OCH_3), 6.35 (d, J = 2.2 Hz, 1 H), 6.50 (d, J = 2.2 Hz, 1 H), 6.57 (s, 1 H), 7.04 (s, 1 H), 7.38 (s, 1 H). ^{13}C NMR: δ 55.62, 55.97, 56.02, 56.29, 56.78, 92.58, 95.80, 97.15, 111.5, 111.94, 112.71, 142.9, 152.2, 153.6, 157.1, 158.0, 159.8, 160.7, 163.7, 177.9. UV (EtOH) λ_{max}/nm (log ε) 210 (4.64), 254 (4.38), 293 (4.06), 350 (4.34).

8-Methyl-5,7,2′,4′,5′-pentamethoxyflavone (**11**). To a suspension of **4** (56 mg, 0.16 mmol) in $CHCl_3$ (10 ml) were added excess methyl iodide (> 200 mmol) and silver oxide. The reaction mixture was shaken for 2 h and after filtration the $CHCl_3$ phase was evaporated to yield 45 mg solid material. Chromatographic separation on silica gel with ethyl acetate as the eluent yielded 15 mg which on repeated purification yielded 9 mg (15%) **11**, m.p. 221–224 °C. The structure was inferred from the NMR spectra which are given in Tables 1 and 2. UV (EtOH) λ_{max}/nm (log ε) 210 (4.46), 258 (4.25), 268 (4.23), 288 (3.90), 356 (4.21). EIMS *m/z* (% rel. int.) 386 (100 M^+).

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