γ-Pyrone Compounds. II: Synthesis and Antiplatelet Effects of Tetraoxygenated Xanthones

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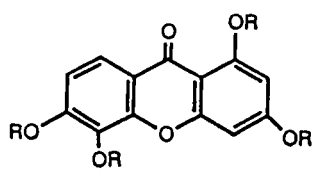
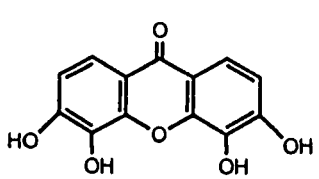
Abstract □ Norathyriol and its analogues, 1,3,5,6-, 3,4,5,6-, 3,4,6,7- and 2,3,6,7-tetrahydroxyxanthone, were synthesized from benzophenone precursors by Friedel–Crafts acylation and subsequent base-catalyzed cyclization to eliminate methanol. Both 3,4,6,7- and 2,3,6,7-tetrahydroxyxanthone tetraacetate showed potent anti-platelet aggregation effects on arachidonic acid-induced platelet aggregation. 3,4,6,7-Tetrahydroxyxanthone tetraacetate and 1,3,5,6-tetrahydroxyxanthone showed potent and significant anti-platelet aggregation effects on collagen-induced platelet aggregation.

Natural xanthone derivatives inhibit the aggregation and adenosine triphosphate-induced release of rabbit platelets induced by adenosine diphosphate (ADP), arachidonic acid, platelet-activating factor (PAF), collagen, ionophore A23187 (a naturally occurring antibiotic that acts as chelating agent with a high affinity for calcium ion[1]), and thrombin. The antiplatelet action is due to both inhibition of thromboxane formation and phosphoinositide breakdown.[2] In a study of structure–activity relationships, we found that xanthone skeletons with 1,3,7-trioxygenated and 1,3,6,7-tetraoxygenated xanthones were effective and that norathyriol (7) acetate and tripteroside (1) acetate were the most potent inhibitors of platelet aggregation.[2] As part of ongoing work on the development of drugs with anti-platelet aggregation activity, we synthesized norathyriol (7) and its analogues and evaluated their antiplatelet actions.



12: R=H

12 acetate: R=OAc



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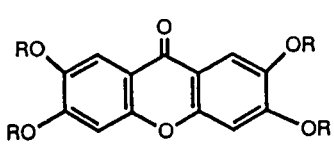
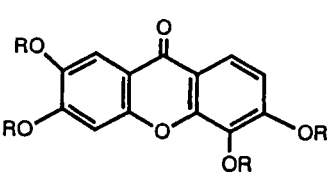


20: R=H

20 acetate: R=OAc

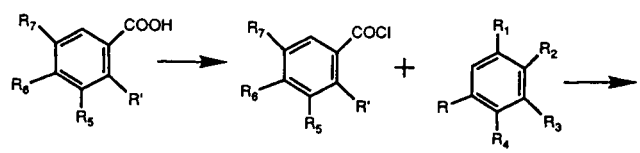


23: R=H

23 acetate: R=OAc

Results and Discussion

Two known xanthones [norathyriol (1,3,6,7-tetrahydroxyxanthone; 7) and 1,3,5,6-tetrahydroxyxanthone (12)] and three new xanthones [3,4,5,6- (16), 2,3,6,7- (20), and 3,4,6,7-tetrahydroxyxanthone (23)] were synthesized (Scheme I) by Friedel–Crafts acylation of the appropriate trimethoxybenzene[3] with another appropriate trimethoxybenzoyl chloride generated in situ. These acylations afforded a mixture of two isomeric benzophenones in high yield. Because the two benzophenones both lead to the same xanthone on cyclization, refluxing with tetramethylammonium hydroxide in pyridine afforded the appropriate tetramethoxyxanthones in good yield.[3,4] Demethylation of the appropriate tetramethoxyxanthone with HI gave the tetrahydroxyxanthone. The physical,



2: R'=R6=R7=OMe R5=H

3: R'=R6=R7=OMe R5=H

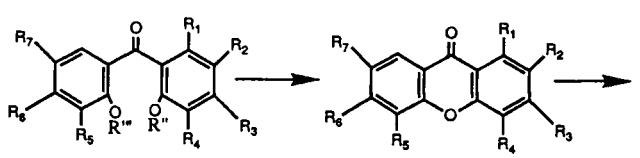
4: R=R1=R3=OMe R2=R4=H

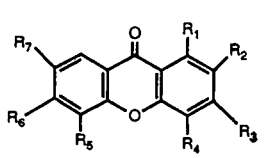
8: R'=R5=R6=OMe R7=H

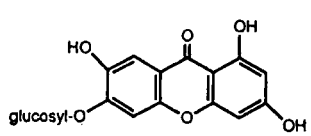
9: R'=R5=R6=OMe R7=H

13: R=R3=R4=OMe R1=R2=H

17: R=R2=R3=OMe R1=R4=H







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Scheme I

0022-3549/92/1100-1109$02.50/0

spectral, and analytical data for the three new benzophenones, the xanthones, and their derivatives are given in *Experimental Section*.

The anti-platelet aggregation effects of desmethylbellidifolin (1,3,5,8-tetrahydroxyxanthone), isolated from *Swertia randaiensis*,[5] **12**, **16**, **20**, **23**, and their acetylated derivatives (see structures) were studied on the aggregation of washed rabbit platelets induced by ADP (20 μM), arachidonic acid (100 μM), PAF (2 ng/mL), and collagen (10 μg/mL). As shown in Table I, desmethylbellidifolin tetraacetate (DMBA; 50 μg/mL), **16** (100 μg/mL), and **20** (100 μg/mL) did not show significant anti-platelet aggregation effects on ADP-, arachidonic acid-, collagen-, or PAF-induced aggregation, although **16** (100 μg/mL) slightly but significantly enhanced platelet aggregation induced by PAF. Compound **12** (100 μg/mL) significantly inhibited collagen-induced platelet aggregation, but its acetate (**12** acetate) had less anti-platelet aggregation effect. Although **20** and **23** did not inhibit platelet aggregation induced by arachidonic acid at concentrations as high as 100 μg/mL, the esterification of **20** and **23** very markedly enhanced the anti-platelet aggregation effects. The acetate derivatives of **22** and **23** completely inhibited arachidonic acid-induced platelet aggregation at 25 μg/mL. More experiments were then performed to study the effects of these xanthones and their derivatives on arachidonic acid- or collagen-induced platelet aggregation at various concentrations. Compared with norathyriol (**7**) acetate,[2] **23** acetate and **20** acetate had less potent anti-platelet aggregation effects when arachidonic acid (100 μM) was used as the aggregation agent (Figure 1). In collagen-induced platelet aggregation, **23** acetate was more potent, whereas **12** or **12** acetate was less potent than **7** or **7** acetate (Figure 2). On the basis of these results we suggest that these xanthones and their derivatives may have different mechanisms of inhibiting platelet aggregation and selectivities. Further experiments are required to elucidate the differences in the mechanism of action.

Experimental Section

All melting points were uncorrected. IR spectra were recorded on a Hitachi model 260-30 IR spectrophotometer. UV absorption spectra were measured on a Beckmann model 34 spectrophotometer. ^1H and ^{13}C NMR spectra [δ (ppm), *J* (Hz)] were run on a Bruker 100-MHz FT-NMR spectrometer. Mass spectra were determined on a Jeol JMS-D-100 mass spectrometer. Elemental analyses were within ±0.4% of the theoretical values, unless otherwise noted.

Procedure I: 4,6-Dimethoxy-2-hydroxy-2′,4′,5′-trimethoxybenzophenone (5a) and 2,4,6-Trimethoxy-2′-hydroxy-4′,5′-dimethoxybenzophenone (5b)—2,4,5-Trimethoxybenzoic acid (**2**; 1.0 g, 4.71 mmol) in dry C_6H_6 (30 mL) was treated with 2.0 mL of oxalyl chloride under an argon atmosphere and thoroughly stirred at room temperature.[4] After 2 h, the solvent and the excess reagent were removed under reduced pressure. The residue (**3**) was dissolved in anhydrous Et_2 (40 mL), and 1,3,5-trimethoxybenzene (**4**; 0.7 g, 4.14 mmol) and $AlCl_3$ (2.0 g) were added.[4] After being stirred for 8 h at room temperature, the mixture was hydrolyzed with ice water (250 mL) containing concentrated HCl (20 mL) and extracted with $CHCl_3$. Solvent removal gave a crude product that was purified by column chromatography (silica gel and $CHCl_3$) to yield, after crystallization from MeOH, yellow needles of **5** (1.2 g, 73%); mp, 152–154 °C; ^1H NMR ($CDCl_3$): δ 3.63, 3.70, 3.86, and 3.92 (4 singlets, 30 H, 10 OMe); 6.09 (d, *J* = 2.5 Hz, 2 H, H-3 and H-5 of **5a**); 6.14 (d, *J* = 2.5 Hz, 2 H, H-3 and H-5 of **5b**); 6.47 (s, 2 H, H-3′ of **5a** and **5b**); 6.70 (s, 2 H, H-6′ of **5a** and **5b**); and 12.50 (s, 2 H, 2 OH of **5a** and **5b**, exchangeable with D_2O).

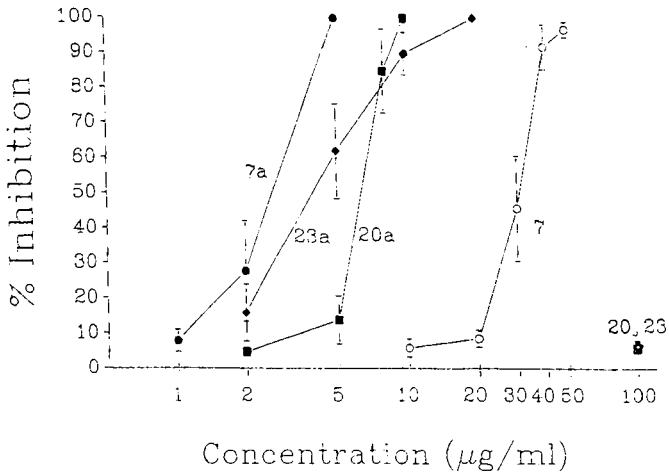

Figure 1—Effect of xanthone derivatives on platelet aggregation induced by arachidonic acid. Washed rabbit platelets were incubated with various concentrations of **7** (○), **20** (◇), and **23** (□) and their acetate derivatives **7a** (●), **20a** (■), **23a** (◆), respectively, and then arachidonic acid (100 μM) was added to trigger aggregation. Percent inhibitions are presented as means ± standard errors (n = 4–6).

Procedure II: 1,3,6,7-Tetramethoxyxanthone (6)—Compound **5** (1.2 g, 3.45 mmol) was treated with pyridine (20 mL), H_2O (10 mL), and aqueous 10% tetramethylammonium hydroxide (6.8 mL). The mixture was refluxed for 36 h,[4] poured into ice, acidified with HCl, and extracted with Et_2O. The reaction yielded an oil, which after purification by column chromatography (silica gel and $CHCl_3$) and crystallization from $CHCl_3$ yielded a colorless powder, **6** (0.90 g, 2.85 mmol, 83%); mp, 196–198 °C; UV, IR, ^1H NMR, and mass spectra were identical to those of an authentic sample of **6**.

Procedure III: 1,3,6,7-Tetrahydroxyxanthone (7)—A mixture of **6** (0.90 g, 2.85 mmol), phenol (18 mL), and HI (15 mL) was refluxed at 160 °C for 8 h, and the reaction mixture was poured into an aqueous $NaHSO_3$ solution. The resulting yellow precipitate was collected, purified by silica gel column chromatography ($CHCl_3$:MeOH, 4:1), and crystallized from MeOH to give yellow needles (0.70 g, 2.69 mmol,

Table I—Effects of Various Xanthone Derivatives on the Platelet Aggregation Induced by ADP, Arachidonic Acid, Collagen, and PAF[a]

Aggregation Inducer	Aggregation (%) in the Presence of:								
	Me_2SO (Control)	DMBA	**12**	**12** Acetate	**16**	**20**	**20** Acetate	**23**	**23** Acetate
ADP	84.5 ± 2.4 (11)	72.8 (2)	59.8 ± 5.1 (3)[b]	55.0 ± 8.8 (3)[b]	86.3 ± 4.8 (3)	77.6 ± 5.1 (3)	53.8 ± 8.3 (3)[b]	54.5 ± 10.7 (3)[b]	19.5 ± 14.2 (3)[b]
AA	88.1 ± 1.7 (11)	63.7 (1)	79.8 ± 7.3 (3)	77.1 ± 11.7 (3)	83.6 ± 3.6 (3)	87.4 ± 1.8 (3)	0 ± 0 (7)[b]	87.8 ± 1.7 (3)	0 ± 0 (7)[b]
Collagen	89.7 ± 1.5 (9)	87.9 (2)	23.6 ± 4.4 (5)[b]	77.1 ± 4.0 (5)[c]	92.1 ± 0.6 (3)	85.8 ± 3.0 (3)	79.0 ± 3.7 (3)[c]	80.5 ± 4.0 (3)[d]	14.1 ± 3.2 (6)[b]
PAF	90.9 ± 0.9 (9)	71.4 (1)	92.6 ± 1.5 (3)	91.1 ± 1.0 (3)	95.9 ± 1.0 (3)[c]	87.6 ± 1.3 (3)	86.3 ± 1.4 (3)[d]	84.7 ± 4.6 (3)[d]	82.6 ± 4.3 (3)[b]

[a] Platelets were preincubated with DMBA (50 μg/mL), **12** (100 μg/mL), **12** acetate (100 μg/mL), **16** (100 μg/mL), **20** (100 μg/mL), **20** acetate (25 μg/mL), **23** (100 μg/mL), **23** acetate (25 μg/mL), or DMSO (0.5%, control) at 37 °C for 3 min; then, ADP (20 μM), arachidonic acid (AA; 100 μM), collagen (10 μg/mL), or PAF (2 ng/mL) was added. Percentages of aggregation are presented as means ± standard errors of the mean (n). [b] Significantly different from control value at $p < 0.001$. [c] Significantly different from control value at $p < 0.01$. [d] Significantly different from control value at $p < 0.05$.

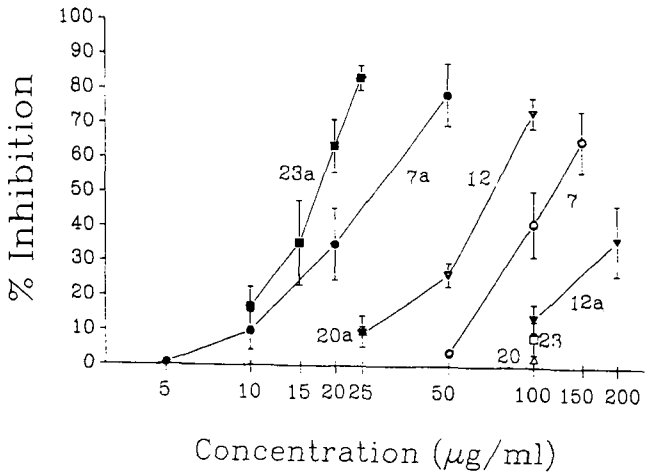

Figure 2—Effect of xanthone derivatives on platelet aggregation induced by collagen. Washed rabbit platelets were incubated with various concentrations of **7** (○), **12** (▽), **20** (△), and **23** (□) and their acetate derivatives **7a** (●), **12a** (▼), **20a** (▲), and **23a** (■), and (■) **23a**, respectively, and then collagen (10 μg/mL) was added to trigger aggregation. Percent inhibitions were are as means ± standard errors (n = 4–6).

94%); mp, >300 °C; UV, IR, ^{1}H NMR, and mass spectra were identical to those of an authentic sample of **7**.

4,6-Dimethoxy-2-hydroxy-2′,3′,4′-trimethoxybenzophenone (10a) and 2,4,6-Trimethoxy-2′-hydroxy-3,4-dimethoxybenzophenone (10b)—Treatment of 2,3,4-trimethoxybenzoic acid (**8**; 1.0 g, 4.71 mmol) by procedure I and reaction of the residue with **4** (0.7 g, 4.14 mmol) gave yellow needles (recrystallized from MeOH; 1.30 g, 79%); mp, 126–128 °C; ^{1}H NMR ($CDCl_3$): δ 3.65, 3.82, and 3.85 (3 singlets, 30 H, 10 OMe); 6.18 (d, J = 2.5 Hz, 4 H, H-3 and H-5 of **10a** and **10b**); 6.38 (d, J = 8.5 Hz, 2 H, H-5′ of **10a** and **10b**); 7.11 (d, J = 8.5 Hz, 2 H, H-6′ of **10a** and **10b**); and 12.47 (s, 2 H, 2 OH of **10a** and **10b**, exchangeable with D_2O).

1,3,5,6-Tetramethoxyxanthone (11)—Treatment of compound **10** (1.30 g, 3.74 mmol) by procedure II yielded a colorless powder (recrystallized from $CHCl_3$; 1.0 g, 3.16 mmol, 84%); mp, 78–80 °C; UV: λ_{max} (MeOH) 244, 287, 306, and 330 (sh) nm; IR (KBr): 1630 and 1610 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.90 (s, 3 H, OMe), 3.96 (s, 6 H, 2 OMe), 4.00 (s, 3 H, OMe), 6.30 (d, J = 2.5 Hz, 1 H, H-2), 6.53 (d, J = 2.5 Hz, 1 H, H-4), 6.90 (d, J = 8.5 Hz, 1 H, H-7), and 7.97 (d, J = 8.5 Hz, 1 H, H-8).

1,3,5,6-Tetrahydroxyxanthone (12) and 12 Acetate—Treatment of compound **11** (1.0 g, 3.16 mmol) by procedure III yielded yellow needles (0.80 g, 3.10 mmol, 98%); mp, >300 °C; MS: m/z 260 (M^+); UV: λ_{max} (MeOH) 249, 282, and 325 nm; λ_{max} (MeOH + $AlCl_3$) 244 (sh), 266, 393 (sh), and 386 nm; λ_{max} (MeOH + NaOAc) 246, 284 (sh), and 350 nm; λ_{max} (MeOH + NaOAc + H_3BO_3) 252, 386 (sh), and 342 nm; IR (KBr): 3450, 1660, 1620, and 1600 cm^{-1}; ^{1}H NMR (Me_2SO-d_6): δ 6.28 (d, J = 2.5 Hz, 1 H, H-2), 6.54 (d, J = 2.5 Hz, 1 H, H-4), 6.99 (d, J = 8.5 Hz, 1 H, H-7), 7.96 (d, J = 8.5 Hz, 1 H, H-8), and 12.80 (s, 1 OH, C-1). A solution of **12** (0.10 g, 0.38 mmol) in 5 mL of anhydrous pyridine was treated with 5 mL of acetic anhydride in a water bath for 4 h. The reaction mixture was concentrated under reduced pressure to remove excess solvent, and water was added to destroy excess acetic anhydride. The residue was concentrated under reduced pressure to give crude **12** acetate. Purification by column chromatography and crystallization from MeOH gave a colorless powder (**12** acetate; 0.15 g, 0.35 mmol, 92%); mp, 221–223 °C; IR (KBr): 1780, 1660, and 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.27, 2.28, 2.37, and 2.41 (4 singlets, 12 H, 4 OAc); 6.79 (d, J = 2.5 Hz, 1 H, H-2); 7.15 (d, J = 8.5 Hz, 1 H, H-7); 7.21 (d, J = 2.5 Hz, 1 H, H-4); and 8.07 (d, J = 8.5 Hz, 1 H, H-8).

3,4-Dimethoxy-2-hydroxy-2′,3′,4′-trimethoxybenzophenone (14a) and 2,3,4-Trimethoxy-2′-hydroxy-3′,4′-dimethoxybenzophenone (14b)—Treatment of compound **8** (1.0 g, 4.71 mmol) by procedure I and reaction of the residue with 1,2,3-trimethoxybenzene (**13**; 0.7 g, 4.14 mmol) yielded a pale yellow oil (1.20 g, 73%); ^{1}H NMR ($CDCl_3$): δ 3.77, 3.80, and 3.90 (4 singlets, 30 H, 10 OMe); 6.37 (d, J = 8.5 Hz, 2 H, H-5 of **14a** and H-5′ of **14b**); 6.53 (d, J = 8.5 Hz, 2 H, H-5′ of **14a** and H-5 of **14b**); 6.66 (d, J = 8.5 Hz, 2 H, H-6 of **14a** and H-6′ of **14b**); 6.97 (d, J = 8.5 Hz, 2 H, H-6′ of **14a** and H-6 of **14b**); and 12.50 (s, 2 H, 2 OH of **14a** and **14b**, exchangeable with D_2O.

Anal.—Calcd for $C_{18}H_{20}O_7$: C, H.

3,4,5,6-Tetramethoxyxanthone (15)—Treatment of compound **14** (1.20 g, 3.45 mmol) by procedure II yielded a colorless powder (recrystallized from $CHCl_3$; 0.7 g, 2.22 mmol, 64%), mp, 184–185 °C; IR (KBr): 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.93 (s, 6 H, 2 OMe), 4.03 (s, 6 H, 2 OMe), 6.97 (d, J = 8.5 Hz, 2 H, H-2 and H-7), and 8.05 (d, J = 8.5 Hz, H-1 and H-8).

Anal.—Calcd for $C_{17}H_{16}O_6$: C, H.

3,4,5,6-Tetrahydroxyxanthone (16)—Treatment of compound **15** (0.7 g, 2.22 mmol) by procedure III yielded pale green needles (recrystallized from MeOH; 0.55 g, 2.12 mmol, 95%); mp, >300 °C; MS: m/z (%) 260 (46) (M^+); UV: λ_{max} in MeOH (log ε) 250 (4.25), 283 (3.67), and 325 (3.87) nm; λ_{max} (MeOH + NaOAc) 250, 293, 337, and 285 (sh) nm; λ_{max} (MeOH + NaOAc + H_3BO_3) 255, 293, and 345 nm; IR: (KBr): 3300 and 1625 cm^{-1}; ^{1}H NMR (Me_2SO-d_6): δ 6.99 (d, J = 9.0 Hz, 2 H, H-2 and H-7) and 7.50 (d, J = 9.0 Hz, 2 H, H-1 and H-8); ^{13}C NMR (Me_2SO-d_6): δ 116.5 (C-1 and C-8), 114.3 (C-2 and C-7), 150.5 (C-3 and C-6), 132.8 (C-4 and C-5), 145.5 (C-4a and C-4b), 113.3 (C-8a and C-8b), and 175.0 (C-9).

Anal.—Calcd for $C_{13}H_8O_6$: C, H.

3,4,5,6-Tetrahydroxyxanthone (16) Acetate—As described for **12**, conversion of **16** (0.1 g, 0.38 mmol) to its tetraacetate yielded colorless needles (0.15 g, 0.35 mmol, 92%); mp, 235–238 °C; IR (KBr): 1790, 1750, and 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.33 (s, 6 H, 2 OAc), 2.37 (s, 6 H, 2 OAc), 7.27 (d, J = 8.5 Hz, 2 H, H-2 and H-7), and 8.23 (d, J = 8.5 Hz, 2 H, H-1 and H-8).

Anal.—Calcd for $C_{21}H_{16}O_{10}$: C, H.

4,5-Dimethoxy-2-hydroxy-2′,4′,5′-trimethoxybenzophenone (18a) and 2,4,5-Trimethoxy-2′-hydroxy-4′,5′-dimethoxybenzophenone (18b)—Treatment of compound **2** (1.0 g, 4.71 mmol) by procedure I and reaction of the residue with 1,2,4-trimethoxybenzene (**17**; 0.7 g, 4.14 mmol) yielded an orange-red oil (1.2 g, 73%); ^{1}H NMR ($CDCl_3$): δ 3.62, 3.68, 3.80, 3.87, and 3.91 (5 singlets, 30 H, 10 OMe); 6.47 (s, 2 H, H-3 of **18a** and H-3′ of **18b**); 6.53 (s, 2 H, H-3 of **18b** and H-3′ of **18a**); 6.73 (s, 2 H, H-6 of **18a** and H-6′ of **18b**); 6.80 (s, 2 H, H-6 of **18b** and H-6′ of **18a**); and 12.50 (s, 2 H, 2 OH of **18a** and **18b**, exchangeable with D_2O).

Anal.—Calcd for $C_{18}H_{20}O_7$: C, H.

2,3,6,7-Tetramethoxyxanthone (19)—Treatment of compound **18** (1.20 g, 3.45 mmol) by procedure II yielded a colorless powder (recrystallized from $CHCl_3$; 0.85 g, 2.69 mmol, 78%); mp, 227–229 °C; IR (KBr): 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.01 (s, 12 H, 4 OMe), 6.87 (s, 2 H, H-4 and H-5), and 7.66 (s, 2 H, H-1 and H-8).

Anal.—Calcd for $C_{17}H_{16}O_6$: C, H.

2,3,6,7-Tetrahydroxyxanthone (20)—Treatment of compound **19** (0.85 g, 2.69 mmol) by procedure III yielded a yellow powder (recrystallized from $CHCl_3$–MeOH; 0.67 g, 2.57 mmol, 96%); mp, >300 °C; MS; m/z (%) 260 (48) (M^+); UV: λ_{max} in MeOH (log ε) 242 (4.20), 274 (3.60), and 330 (4.00) nm; λ_{max} (MeOH + NaOAc) 235 and 363 nm; λ_{max} (MeOH + NaOAc + H_3BO_3) 250, 315 (sh), and 355 nm; IR (KBr): 1620 and 3250 cm^{-1}; ^{1}H NMR (Me_2SO-d_6): δ 6.84 (s, 2 H, H-4 and H-5) and 7.39 (s, 2 H, H-1 and H-8); ^{13}C NMR (Me_2SO-d_6): δ 109.4 (C-1 and C-8), 143.8 (C-2 and C-7), 153.4 (C-3 and C-6), 103.3 (C-4 and C-5), 151.7 (C-4a and C-4b), 113.9 (C-8a and C-8b), and 175.0 (C-9).

Anal.—Calcd for $C_{13}H_8O_6$: C, H.

2,3,6,7-Tetrahydroxyxanthone (20) Acetate—As described for **12**, conversion of **20** (0.1 g, 0.38 mmol) to its tetraacetate yielded a colorless powder (recrystallized from MeOH; 0.14 g, 0.33 mmol, 87%); mp, 263–265 °C; IR (KBr): 1680 and 1780 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.35 (s, 12 H, 4 OAc), 7.45 (s, 2 H, H-4 and H-5), and 8.10 (s, 2 H, H-1 and H-8).

Anal.—Calcd for $C_{21}H_{16}O_{10}$: C, H.

3,4-Dimethoxy-2-hydroxy-2′,4′,5′-trimethoxybenzophenone (21a) and 2,3,4-Trimethoxy-2′-hydroxy-4′,5′-dimethoxybenzophenone (21b)—Treatment of compound **2** (1.0 g, 4.71 mmol) by procedure I and reaction of the residue with **13** (0.7 g, 4.14 mmol) yielded a pale yellow powder (recrystallized from MeOH; 1.10 g, 67%); mp, 122–123 °C; ^{1}H NMR ($CDCl_3$): δ 3.70, 3.78, 3.81, 3.82, 3.85, 3.86, and 3.90 (7 singlets, 30 H, 10 OMe); 6.36 (d, J = 9.0 Hz, 2 H, H-5 of **21a** and H-5′ of **21b**); 6.53 (s, 2 H, H-3 of **21a** and H-3′ of **21b**); 6.80 (s, 2 H, H-6 of **21a** and H-6′ of **21b**); 7.13 (d, J = 9.0 Hz, 2 H, H-6 of **21a** and H-6′ of **21b**); and 12.47 (s, 2 H, 2 OH of **21a** and **21b**, exchangeable with D_2O).

Anal.—Calcd for $C_{18}H_{20}O_7$: C, H.

3,4,6,7-Tetramethoxyxanthone (22)—Treatment of compound **21** (1.10 g, 3.16 mmol) by procedure II yielded a colorless powder, **22** (recrystallized from $CHCl_3$; 0.70 g, 2.22 mmol, 70%); mp, 210–212 °C; IR (KBr): 1645 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.01 (s, 6 H, 2 OMe), 4.02 (s, 6 H, 2 OMe), 7.02 (d, J = 9.0 Hz, 1 H, H-2), 7.03 (s, 1 H, H-5), 7.66 (s, 1 H, H-8), and 8.09 (d, J = 9.0 Hz, 1 H, H-1).

Anal.—Calcd for $C_{17}H_{16}O_6$: C, H.

3,4,6,7-Tetrahydroxyxanthone (23)—Treatment of compound **22**) (0.70 g, 2.22 mmol) by procedure III yielded a pale yellow powder, **23** (recrystallized from MeOH–$CHCl_3$; 0.55 g, 2.12 mmol, 95%); mp, >300 °C; MS: m/z (%) 260 (14) (M^+); UV: λ_{max} in MeOH (log ε) 240 (sh) (4.06), 255 (4.21), 275 (sh) (3.81), 318 (3.80), and 358 (3.70) nm; λ_{max} (MeOH + NaOAc) 257 and 373 nm; λ_{max} (MeOH + NaOAc + H_3BO_3) 265, 280 (sh), 310, and 380 nm; IR (KBr): 1625 and 3400 cm^{-1}; ^1H NMR (Me_2SO-d_6): δ 6.86 (d, J = 8.5 Hz, 1 H, H-2), 6.87 (s, 1 H, H-5), and 7.38 (s, 1 H, H-8), 7.48 (d, J = 8.5 Hz, 1 H, H-1); ^{13}C NMR (Me_2SO-d_6): δ 116.2 (C-1), 114.3 (C-2), 150.7 (C-3), 132.7 (C-4), 146.5 (C-4a), 151.2 (C-4b), 102.8 (C-5), 154.1 (C-6), 144.0 (C-7), 108.5 (C-8), 112.8 (C-8a and C-8b), and 174.8 (C-9).

Anal.—Calcd for $C_{13}H_8O_6$: C, H.

3,4,6,7-Tetrahydroxyxanthone (23) Acetate—As described for **12**, conversion of **23** (0.1 g, 0.38 mmol) to its tetraacetate yielded colorless needles (recrystallized from MeOH; 0.14 g, 0.33 mmol, 87%); mp, 234–236 °C; IR (KBr): 1665, 1760, and 1780 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.35, 2.45 (2s, 12 H, 4 OAc), 7.25 (d, J = 8.5 Hz, 1 H, H-2), 7.46 (s, 1 H, H-5), 8.13 (s, 1 H, H-8), and 8.22 (d, J = 8.5 Hz, 1 H, H-1).

Anal.—Calcd for $C_{21}H_{16}O_{10}$: C, H.

Platelet Aggregation—Washed rabbit platelets were obtained from EDTA-anticoagulated platelet-rich plasma according to washing procedures described previously.[6] Platelet numbers were counted (Coulter Counter, model ZM) and adjusted to 4.5×10^8 platelets/mL. The platelet pellets were finally suspended in Tyrode's solution containing (mM) NaCl (136.8), KCl (2.8), $NaHCO_3$ (11.9), $MgCl_2$ (2.1), NaH_2PO_4 (0.33), $CaCl_2$ (1.0), and glucose (11.2) with bovine serum albumin (0.35%). Aggregation was measured by the turbidimetric method[7]; the absorbance of platelet suspension was designated as 0% aggregation, and the absorbance of platelet-free Tyrode's solution was designated as 100% aggregation. The aggregation was measured by a Lumi aggregometer (Chrono-Log Co.) connected to dual-channel recorders. The platelet suspension was stirred at 1200 rpm. To eliminate the effect of the solvent on aggregation, the final concentration of Me_2SO was fixed at 0.5%.

References and Notes

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