Phenolic Glycosides from *Dirca palustris*

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Five novel phenolic glycosides (**1**−**5**) were isolated from the MeOH extract of the dried twigs of *Dirca palustris*, as confirmed by their ^{1}H NMR, ^{13}C NMR, and MS data. Compounds **1**−**3** were not active against cyclooxygenase I (COX-I), but compound **4** (200 μg/mL) and compound **5** (125 μg/mL) showed 12.5 and 9.2% inhibition of the COX-I enzyme, respectively. Compounds **1**−**5** did not exhibit cyclooxygenase II (COX-II) enzyme inhibition. Compound **5** did not show any antioxidant activity using the liposome assay; however, compounds **1**−**4** displayed antioxidant activity at 60 μg/mL, with compound **2** being the most efficacious.

The genus *Dirca* (Thymelaeaceae) contains only two species, *D. occidentalis* and *D. palustris*, both of which are shade-tolerant shrubs indigenous to North America. *D. occidentalis* is native to the Pacific States, where it is known by the colloquial name of western leatherwood.[1] *D. palustris*, on the other hand, is found in eastern North American mesic, deciduous forests, where it is known as leatherwood or moosewood. Except for two reports on biologically active compounds from *D. occidentalis*,[1,2] there are no reports on the chemistry of *D. palustris*.

Our investigation of the winter dormant twigs of *D. palustris* led to the isolation of five novel phenolic glycosides (**1**−**5**). The pure compounds were bioassayed for their antioxidant and antiinflammatory activities. This is the first chemical investigation of this plant, and all the compounds reported here are novel.

Results and Discussion

Fresh twigs of *D. palustris* were lyophilized, milled, and extracted sequentially with hexane, ethyl acetate, and methanol, respectively. The MeOH extract was then partitioned into $CHCl_3$−MeOH-soluble and -insoluble portions. The $CHCl_3$−MeOH-soluble portion was subjected to vacuum liquid chromatography (VLC) yielding five fractions. MPLC was performed on fraction D to yield seven fractions. Purification of fraction IV by reversed-phase preparative TLC yielded compounds **1** and **2**. Reversed-phase preparative TLC of fraction III afforded two major bands that were characterized as compounds **3** and **5**; similarly, fraction V gave one major band, which was identified as compound **4**.

The FABMS of **1** showed a quasimolecular ion $[M + H]^+$ at *m/z* 593, indicating a molecular formula of $C_{28}H_{33}O_{14}$. Detailed analyses of the ^{1}H, ^{13}C, and DEPT NMR spectra allowed us to fully characterize the structure of **1**. In the ^{1}H NMR spectrum of **1**, an AB pair of doublets at δ 8.04 (2H, d, J = 8.9 Hz) and 7.11 (2H, d, J = 8.9 Hz), respectively, suggested that the B ring was substituted at C-4 with either an OMe or an O-sugar group. The signal at δ 6.79, a singlet and integrated for one proton, was assigned to the H-3. The other two aromatic protons (δ 6.87 and 7.05), both doublets with a coupling constant of 2.4 Hz, indicated a meta coupling of these two protons and thus were assigned to H-6 and H-8, respectively, of the A ring. Two doublets at δ 4.78 and 4.18 were assigned to anomeric protons of the glucose and xylose, respectively. The large coupling constants of these protons, 7.6 and 7.2 Hz, respectively, indicated a β-linkage of the sugar moieties. The sugar identities were further confirmed by ^{13}C NMR and MS analyses as glucose and xylose. The presence of a 1,6-xylose−glucose glycosidic linkage was confirmed from the ^{13}C NMR downfield shifts of the signals assigned to glucose C-6 and xylose C-1. Downfield shifts of C-5 and C-7 of the A ring also indicated that these were oxygenated aromatic carbons. A survey of the literature shows that the glycosidic moiety occurs most frequently at C-7 or C-3 and to a much lesser extent at C-5 or C-8.[3,4] Thus, the sugar moiety and one of the OMe groups were assigned to C-7 and C-5, respectively, with the remaining OMe group being assigned to C-4′.[3−9] Thus, compound **1** was identified as 5,4′-dimethoxyflavone-7-*O*-glucoxyloside.

Compound **2** gave an $[M + H]^+$ at *m/z* 623 in the FABMS corresponding to a molecular formula of $C_{29}H_{34}O_{15}$. The ^{1}H NMR spectrum of **2** differed from that of **1** in that three of the aromatic protons were at δ 7.11, 7.56, and 7.68. This suggested that the B ring in **2** was substituted at C-3 and C-4. Three OMe groups were also present in **2**. Compound **2** also contained two anomeric protons at δ 4.79 and 4.19, respectively. The ^{1}H and ^{13}C NMR data, indicated that the sugar moieties were identical to those in compound **1**. Hence, compound **2** was identified as 5,7,4′-trimethoxyflavone-7-*O*-glucoxyloside.

The ^{1}H and ^{13}C NMR spectra for compound **3** were identical to those of **2**, except for the presence of a three-proton, broad singlet at δ 1.20 in the ^{1}H NMR spectrum and a signal at δ 18.2 in the ^{13}C NMR spectrum. This suggested that **3** is a glucorhamnoside, a conclusion supported further by FABMS data. Compound **3** gave an $[M + H]^+$ at *m/z* 637, which corresponded to a molecular formula of $C_{30}H_{36}O_{15}$. Thus, **3** was confirmed to be 5,7,4′-trimethoxyflavone-7-*O*-glucorhamnoside.

The structure of compound **4** was also elucidated from its ^{1}H NMR, ^{13}C NMR, and MS data. The ^{1}H NMR spectrum of **4** was very similar to that of **1**, with an AB pair of doublets, two aromatic proton doublets, an sp^2 proton singlet, and two anomeric protons. The differences

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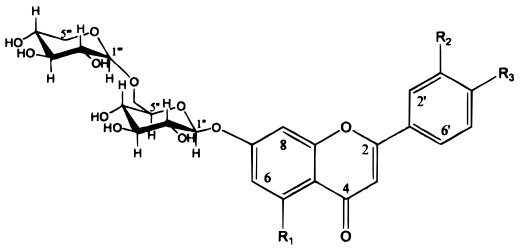
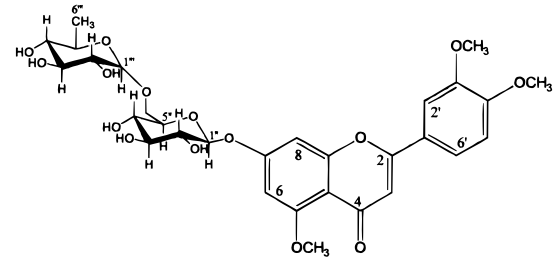


Compound	R_1	R_2	R_3
1	OCH_3	H	OCH_3
2	OCH_3	OCH_3	OCH_3
4	OCH_3	H	OH



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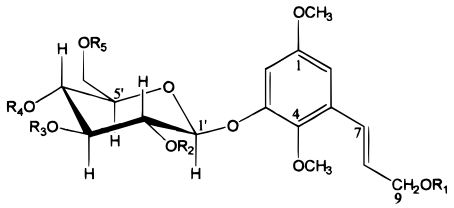

Compound	R_1	R_2	R_3	R_4	R_5
5	H	H	H	H	H
6	Ac	Ac	Ac	Ac	Ac

were that there was only one OMe group present in **4** and an exchangeable proton at δ 8.01, suggesting the presence of a phenoxyl moiety. The ^{13}C NMR spectrum supported these conclusions. The FABMS of **4** revealed an $[M + H]^+$ at *m/z* 579, supporting the sugar identities as determined from the ^{13}C NMR and a molecular formula of $C_{27}H_{30}O_{14}$. Thus, **4** was determined to be 4′-hydroxy-5 methoxyflavone-7-*O*-glucoxyloside.

Compound **5** exhibited significant differences in its proton and carbon spectra when compared to those of **1**−**4**. A singlet at δ 6.72 integrating for two protons, a doublet at δ 6.46, a doublet of triplets at δ 6.33, a multiplet at δ 4.09 integrating for two protons, an anomeric proton at δ 4.90, and a 6H singlet at δ 3.76. There was also a multiplet between δ 2.98 and 3.62 (6H), suggesting the presence of a sugar moiety. The ^{13}C NMR spectrum revealed 14 peaks, which were assigned to one oxygenated sp^2 (δ 152.7), two fully substituted sp^2 peaks (δ 132.6 and 133.9), three sp^2 methine peaks (δ 104.5, 128.5, and 130.2), an anomeric carbon signal at δ 102.6, four oxymethine carbon peaks, two oxymethylenes, and an OMe signal based on the DEPT analysis. The NMR data determined that **5** contained a β-glucose unit. A $[M - H]^+$ peak at *m/z* 371 in the FABMS ($C_{17}H_{24}O_9$) suggested that **5** possessed an aromatic ring. The number of OH groups in **5** was confirmed by acetylation to **6**. The NMR analysis of **6**, showed the presence of five acetoxy groups. The MS of **6** gave a molecular ion at *m/z* 582 corresponding to a molecular formula of $C_{27}H_{34}O_{14}$. Also, the fragment ion at 209 for **5**, which corresponded to the aglycon portion of this molecule, was also the base peak. HREIMS of the base peak in the mass spectrum of **5** was determined to be 209.0828 and confirmed a fragment ion of $C_{11}H_{13}O_4$.

Compounds **1**−**5** were tested for their antiinflammatory activities using the cyclooxygenase (COX)-I and -II enzymes. Compounds **1**−**3** were not active against COX-I. However, compound **4** (200 μg/mL) and compound **5** (125 μg/mL) showed 12.5 ± 2 and 9.2 ± 2.2% inhibition of the COX-I enzyme, respectively. The positive controls aspirin (180 μg/mL), ibuprofen (2.06 μg/mL), and naproxen (2.52 μg/mL) showed 52.13 ± 3.1%, 53.26 ± 1.9%, and 57.96 ± 2.7% inhibition, respectively, against COX-I. Compounds **1**−**5** gave no inhibition of COX-II at the concentrations tested (200 μg/mL for **1**−**4** and 125 μg/mL for compound **5**).[10,11]

Antioxidant properties of compounds **1**−**5** were assayed using the liposome oxidation assay.[12] Compound **5** showed no inhibition of peroxidation of the liposome and thus is

not considered as an antioxidant. However, compounds **1**−**4** showed significant antioxidant activities at 60 μg/mL. Compound **2** showed the best inhibition of liposome oxidation followed by compounds **1**, **4**, and **3** at 98, 78, 71, and 67%, respectively. The positive controls TBHQ (1.66 μg/mL), BHA (1.80 μg/mL), BHT (2.20 μg/mL), and vitamin E (4.31 μg/mL) showed 90, 91, 81, and 7%, respectively.

Experimental Section

General Experimental Procedures. CD measurements were carried out using a JASCO J-710 CD-ORD spectropolarimeter. Test compounds were dissolved in MeOH to give a final concentration of 1 mg/mL. UV spectra were taken on a Shimadzu UV/VIS spectrophotometer in MeOH and were serially diluted to a final concentration of 0.1 mg/mL. IR spectra (KBr disk) were recorded on a Mattson Galaxy Series 3000 FTIR spectrophotometer. NMR spectra (^{1}H, ^{13}C, DEPT) were recorded on a Varian INOVA 300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) or a Varian VXR 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C). Chemical shifts were recorded in DMSO-d_6 and the values are in δ (ppm) based on δ residual of DMSO-d_6 2.49 and 39.5 for ^{1}H and ^{13}C NMR, respectively. Coupling constants (*J*) are in Hertz. EIMS data were recorded on a JEOL JMS-AX505 H mass spectrometer operating at 70 eV. FABMS data were recorded on a JEOL JMS−HX 110 mass spectrometer, where the ions were produced by bombardment with a beam of Xe atoms at 6 keV and an accelerating voltage of 10 kV. The Si gel used for VLC and MPLC was Merck Si gel 60 (35−70 μm particle size). Reverse phase preparative TLC was done on Whatman KC 18F Si gel 60 glass plates (20 × 20 cm). All positive controls and chemicals used in the antioxidant and antiinflammatory assays were purchased from Sigma Chemical Company unless otherwise stated. All solvents were ACS reagent grade and were purchased from Aldrich Chemical Co., Inc.

Plant Material. Fresh twigs of *D. palustris* (1.42 kg) were harvested in January from 1- to 2-m tall plants growing as understory shrubs in sugar maple (*Acer saccharum*) forests in eastern Gogebic County of the Upper Peninsula of Michigan. A voucher specimen (Z99-4324-1) of this plant material was deposited at The Herbarium, University of Wisconsin, Madison, WI 53706.

Extraction and Isolation. The twigs (1.42 kg) were lyophilized and milled to yield a fine powder (560 g). This powder was extracted exhaustively with hexane (2 × 4 L, 48 h) to yield a hexane extract (6.13 g), followed by EtOAc (2 × 4 L, 48 h) to afford an EtOAc extract (2.82 g), and finally with MeOH (2 × 4 L, 48 h) to yield a MeOH extract (53.88 g). The MeOH extract (15.19 g) was triturated with a $CHCl_3$−MeOH mixture (15:1, 75 mL x 3) to yield a soluble fraction (5.18 g) and an insoluble fraction (9.81 g). Fractionation of the $CHCl_3$−MeOH-soluble extract (5.12 g) was carried out by VLC on Si (250 g) using $CHCl_3$ with increasing amounts of MeOH and finally MeOH as the eluting solvents. Five fractions, A−E, were collected: A (0.37 g, $CHCl_3$−MeOH, 15:1, 160 mL); B (0.13 g, $CHCl_3$−MeOH, 8:1, 500 mL); C (0.24 g, $CHCl_3$−MeOH, 8:1, 400 mL); D (3.49 g, $CHCl_3$-MeOH, 1:1, 1000 mL); and E (0.53 g, 100% MeOH, 1000 mL).

MPLC was performed on fraction D (3.37 g), on Si gel (150 g) and $CHCl_3$ with increasing amounts of MeOH, and finally with MeOH as the eluting solvents, to give seven fractions. Fractions I (55.1 mg), II (74.7 mg), and III (378.8 mg) eluted with $CHCl_3$−MeOH (5:1, 500 mL); fraction IV (997.5 mg, $CHCl_3$−MeOH, 3:1, 400 mL); fraction V (401.5 mg, $CHCl_3$−MeOH, 1:1, 400 mL), fraction VI (599.2 mg, $CHCl_3$−MeOH, 1:3, 300 mL), and fraction VII (443.6 mg, 100% MeOH, 800 mL).

Reversed-phase preparative TLC on fraction IV (161.2 mg) using MeOH−H_2O (60:40) as the developing solvent afforded two major bands: **1** (7.7 mg, R_f 0.25) and **2** (104.8 mg, R_f 0.35). Reversed-phase preparative TLC on fraction III (141.2 mg) using MeOH−H_2O (60:40) as the developing solvent also afforded two major bands: **3** (4.7 mg, R_f 0.40) and **5** (67.8 mg, R_f 0.85). Reversed-phase preparative TLC on fraction V (131.2 mg) using MeOH−H_2O (60:40) as the developing solvent afforded compound **4** (37.7 mg, R_f 0.45).

Compound 1: thin glass-like film; CD (MeOH) λ nm ($\Delta\epsilon$) 213 (+5.39), 221 (+5.25), 382 (−4.08); UV (MeOH) λ_{max} nm (log ϵ) 209.4 (3.57), 240.4 (3.28), 333.1 (3.30); IR (KBr, cm^{-1}) ν_{max} 3517, 3475, 3401, 3297, 2959, 2920, 2891, 1649, 1636, 1613, 1522, 1442, 1360; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.04 (2H, d, J = 8.9 Hz, H-2′, H-6′), 7.11 (2H, d, J = 8.9 Hz, H-3′, H-5′), 7.05 (1H, d, J = 2.4 Hz, H-8), 6.87 (1H, d, J = 2.4 Hz, H-6), 6.79 (1H, s, H-3), 4.78 (1H, d, J = 7.6 Hz, H-1″), 4.18 (1H, d, J = 7.2 Hz, H-1‴), 3.90 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 2.93 B 4.00 (11H, m, H-2″− H-6″, H-2‴− H-5‴); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 176.8 (C-4), 163.6 (C-2), 162.0 (C-4′), 160.8 (C-7), 158.4 (C-5), 158.1 (C-9), 128.0 (C-2′, C-6′), 122.7 (C-1′), 114.5 (C-3′, C-5′), 106.5 (C-10), 104.1 (C-3, C-1‴), 103.7 (C-6), 102.9 (C-8), 96.6 (C-1″), 76.5 (C-2″), 75.9 (C-3″), 75.6 (C-5″), 73.4 (C-2‴), 73.3 (C-3‴), 69.8 (C-4″), 69.5 (C-4‴), 68.6 (C-5‴), 65.6 (C-6″), 56.1 (OCH_3), 55.5 (OCH_3); positive ion FABMS m/z 593.63 [M + H]$^+$; HRFABMS m/z 593.5617 (calcd for $C_{28}H_{33}O_{14}$, 593.5604).

Compound 2: white amorphous solid; CD (MeOH) λ nm ($\Delta\epsilon$) 211 (+6.79), 224 (+4.63), 380 (−4.97); UV (MeOH) λ_{max} nm (log ϵ) 210.4 (3.40), 244.8 (3.12), 336.2 (3.15); IR (KBr, cm^{-1}) ν_{max} 3529, 3457, 3361, 2955, 2922, 2889, 1653, 1631, 1603, 1519, 1437, 1355; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.68 (1H, dd, J = 8.7, 1.5 Hz, H-6′), 7.56 (1H, d, J = 1.5 Hz, H-2′), 7.11 (1H, d, J = 8.7 Hz, H-5′), 7.08 (1H, d, J = 2.2 Hz, H-8), 6.89 (1H, s,H-3), 6.87 (1H, d, J = 2.2 Hz, H-6), 4.79 (1H, d, J = 7.2 Hz, H-1″), 4.19 (1H, d, J = 7.8 Hz, H-1‴), 3.90 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 2.94 B 3.90 (11H, m, H-2″−H-6″, H-2‴− H-5‴); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 176.9 (C-4), 163.6 (C-2), 160.9 (C-7), 158.5 (C-5), 158.1 (C-9), 151.9 (C-4′), 149.0 (C-3′), 122.8 (C-1′), 119.7 (C-6′), 111.6 (C-5′), 109.2 (C-2′), 106.7 (C-10), 104.1 (C-3, C-1‴), 103.7 (C-6), 102.9 (C-8), 96.7 (C-1″), 76.6 (C-2″), 75.9 (C-3″), 75.6 (C-5″), 73.5 (C-2‴), 73.4 (C-3‴), 69.7 (C-4″), 69.5 (C-4‴), 68.6 (C-5‴), 65.6 (C-6″), 56.1 (OCH_3), 55.9 (OCH_3), 55.7 (OCH_3); positive ion FABMS m/z 623.25 [M + H]$^+$; HRFABMS m/z 623.1995 (calcd for $C_{29}H_{35}O_{15}$, 623.1976).

Compound 3: thin glass-like film; CD (MeOH) λ nm ($\Delta\epsilon$) 215 (+8.37), 226 (+5.05), 389 (−5.33); UV (MeOH) λ_{max} nm (log ϵ) 210.9 (3.61), 243.2 (3.32), 334.1 (3.34); IR (KBr, cm^{-1}) ν_{max} 3510, 3476, 3279, 2956, 2925, 2895, 1645, 1633, 1610, 1525, 1444, 1357; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.69 (1H, dd, J = 8.5, 1.8 Hz, H-6′), 7.57 (1H, d, J = 1.8 Hz, H-2′), 7.12 (1H, d, J = 8.5 Hz, H-5′), 7.08 (1H, d, J = 2.4 Hz, H-8), 6.90 (1H, s, H-3), 6.87 (1H, d, J = 2.4 Hz, H-6), 4.79 (1H, d, J = 7.0 Hz, H-1″), 4.18 (1H, d, J = 7.0 Hz, H-1‴), 3.90 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 2.93−3.98 (10H, m, H-2″−H-6″, H-2‴−H-5‴), 1.20 (3H, br s, H-6‴); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 176.8 (C-4), 163.6 (C-2), 161.1 (C-7), 158.6 (C-5), 158.2 (C-9), 151.9 (C-4′), 149.2 (C-3′), 122.9 (C-1′), 119.9 (C-6′), 111.8 (C-5′), 109.2 (C-2′), 106.2 (C-10), 104.1 (C-3), 103.7 (C-6), 103.2 (C-1‴), 103.0 (C-8), 96.6 (C-1″), 76.4 (C-2″), 75.9 (C-3″), 75.6 (C-5″), 73.7 (C-4‴), 72.4 (C-3‴), 72.1 (C-2‴), 69.7 (C-4″), 69.4 (C-5‴), 66.4 (C-6″), 56.2 (OCH_3), 55.9 (OCH_3), 55.6 (OCH_3), 18.2 (C-6‴); positive ion FABMS m/z 637.72 [M + H]$^+$; HRFABMS m/z 637.6149 (calcd for $C_{30}H_{37}O_{15}$, 637.6134).

Compound 4: yellow amorphous solid; CD (MeOH) λ nm ($\Delta\epsilon$) 212 (+6.27), 223 (+5.47), 383 (−4.28); UV (MeOH) λ_{max} nm (log ϵ) 208.8 (3.43), 241.6 (3.13), 334.2 (3.15); IR (KBr, cm^{-1}) ν_{max} 3509, 3490−3250 (broad, intense peak), 2953, 2928, 2878, 1647, 1638, 1613, 1560, 1514, 1494, 1320; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.01 (1H, br s, ex. with D_2O, OH), 7.87 (2H, d, J = 8.6 Hz, H-2′, H-6′), 7.02 (2H, d, J = 8.6 Hz, H-3′, H-5′), 6.87 (1H, d, J = 2.1 Hz, H-8), 6.86 (1H, s, H-3), 6.84 (1H, d, J = 2.1 Hz, H-6), 4.76 (1H, d, J = 7.4 Hz, H-1″), 4.19 (1H, d, J = 7.4 Hz, H-1‴), 3.87 (3H, s, OCH_3), 2.95 B 4.00 (11H, m, H-2″−H-6″, H-2‴−H-5‴); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 176.9 (C-4), 163.5 (C-2), 162.8 (C-4′), 161.7 (C-7), 158.4 (C-5), 158.1 (C-9), 128.2 (C-2′, C-6′), 119.6 (C-1′), 116.4 (C-3′, C-5′), 105.3 (C-10), 104.1 (C-3, C-1‴), 103.8 (C-6), 103.0 (C-8), 96.7

(C-1″), 76.6 (C-2″), 75.9 (C-3″), 75.7 (C-5″), 73.5 (C-2‴), 73.4 (C-3‴), 69.8 (C-4″), 69.6 (C-4‴), 68.7 (C-5‴), 65.7 (C-6″), 56.0 (OCH_3); positive ion FABMS *m/z* 579.20 $[M + H]^+$; HRFABMS *m/z* 579.1727 (calcd for $C_{27}H_{31}O_{14}$, 579.1714).

Compound 5: off white amorphous solid; CD (MeOH) λ nm ($\Delta\epsilon$) 223 (+2.69), 227 (+2.14), 273 (−3.62), 314 (+1.34); UV (MeOH) λ_{max} nm (log ϵ) 221.1 (2.93), 265.2 (2.60); IR (KBr, cm^{-1}) ν_{max} 3391 (strong), 3022, 2935, 2847, 1584, 1508, 1465, 1419, 1340, 1243; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 6.72 (2H, s, H-2, H-6), 6.46 (1H, d, J = 15.6 Hz, H-7), 6.33 (1H, dt, J = 15.6, 6.0 Hz, H-8), 4.90 (1H, d, J = 8.1 Hz, H-1′), 4.09 (2H, m, H-9), 3.76 (6H, s, 2 × OCH_3), 2.98−3.62 (6H, m, H-2′−H-6′); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 152.7 (C-1, C-4), 133.9 (C-5), 132.6 (C-4), 130.2 (C-8), 128.5 (C-7), 104.5 (C-2, C-6), 102.6 (C-1′), 77.2 (C-2′), 76.6 (C-3′), 74.2 (C-5′), 69.9 (C-4′), 61.5 (C-9), 60.9 (C-6′), 56.4 (2 × OCH_3); negative ion FABMS *m/z* 371.21 $[M - H]^+$, 209; HRFABMS *m/z* 371.1341 (calcd for $C_{17}H_{24}O_9$, 371.1342) and HREIMS (positive mode) *m/z* 209.0828 (calcd for $C_{11}H_{13}O_4$).

Acetylation of 5. A mixture of **5** (10.2 mg), Ac_2O (1 mL), and pyridine (0.5 mL) was stirred continuously at room temperature for 24 h. The reaction was monitored via TLC, and the reaction was terminated after the disappearance of the starting material, **5**, by pouring the mixture into ice-cold H_2O. Extraction with EtOAc and evaporation of the solvent yielded **6** (13.2 mg), as a pale yellow gum.

Compound 6: pale yellow gum; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.58 (2H, s), 6.54 (1H, d, J = 15.6 Hz), 6.19 (1H, dt, J = 15.6, 6.3 Hz), 5.26 (4H, m), 5.05 (1H, d, J = 7.0 Hz), 4.69 (2H, dd, J = 6.3, 1.3 Hz), 4.16 (2H, m), 3.80 (6H, s), 2.08 (3H, s), 2.01 (3H, s), 2.00 (9H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.8, 170.6, 170.4, 169.4, 169.3, 153.0, 153.0, 134.4, 133.9, 133.0, 123.2, 103.8, 103.8, 101.2, 73.0, 71.9, 71.9, 68.4, 64.9, 62.2, 56.2, 56.2, 21.0, 20.7, 20.7, 20.7, 20.6; positive ion FABMS *m/z* 582.20 $[M]^+$; HRFABMS *m/z* 582.1943 (calcd for $C_{27}H_{34}O_{14}$, 582.1949).

Antiinflammatory Assay.[10,11] COX-I activity was measured using an enzyme preparation from ram seminal vesicles and purchased from Oxford Biomedical Research, Inc., Oxford, MI (ca. 0.46 mg protein/mL in 30 mM Tris buffer, pH 7.0). COX-II activity was measured using an enzyme preparation from insect cell lysate and diluted with Tris buffer (pH 7.0) to give an approximate final concentration of 1.5 mg protein/mL (supplied by Dr. Dave DeWitt, Department of Biochemistry, Michigan State University). COX assays were performed at 37 °C by monitoring the initial rate of O_2 uptake using an Instech micro oxygen chamber and electrode (Instech Laboratories, Plymouth Meeting, PA) attached to a YSI model 5300 biological oxygen monitor (Yellow Springs Instrument, Inc., Yellow Springs, OH). Each assay mixture contained 0.6 mL of 0.1 M Tris buffer (pH 7.0), 1 mmol phenol, 85 μg hemoglobin, and 100 μmol arachidonic acid. DMSO solutions containing the pure compounds or DMSO alone (20 μL) were added to the reaction chamber. Reactions were initiated by adding 5−25 μg of microsomal protein in a volume of 10−20 μL. The data were recorded using QuickLog for Windows data acquisition and control software (Strawberry Tree, Inc., Sunnyvale, CA).

Antioxidant Assay.[12] Antioxidant bioassays were conducted on the purified compounds by analysis of model liposome oxidation using fluorescence spectroscopy. A mixture containing 5 μmol of 1-steroyl-2-linoleoyl-*sn*-glycerol-3-phosphocholine (Avanti Polar Lipids, Inc., Alabaster, AL) and 5 μmol of the fluorescence probe 3-[*p*-(6-phenyl)-1,3,5-hexatrienyl]phenylpropionic acid (Molecular Probes, Inc., Eugene, OR) was dried under vacuum using a rotary evaporator at room temperature. The resulting lipid film was suspended in 500 μL of a buffer solution containing 0.15 M NaCl, 0.1 mM EDTA, and 0.01 M MOPS and subjected to 10 freeze−thaw cycles using a dry ice−EtOH bath. This buffer solution was treated with chelating resin Chelex 100 to remove trace-metal ions. The lipid−buffer suspension was then extruded 29 times through a LiposoFast extruder (Avestin, Inc., Ottawa, Canada) containing a polycarbonate membrane with a pore size of 100 nm to produce large unilamellar liposomes (LUVs). A 20-μL aliquot of this liposome suspension was diluted to a final volume of 2 mL in Chelex 100-treated HEPES buffer (100 μL, pH 7.0), 1 M NaCl (200 μL), N_2-sparged water (Millipore, 1.64 mL), and DMSO solution containing the test compound (20 μL), vortexed, and placed in the cuvette holder (23 °C) of the spectrophotometer. Peroxidation was then initiated by the addition of 20 μL of 2 mM stock $FeCl_2$ solution to achieve a final concentration of 20 μM of Fe^{2+} in the absence or presence of test compounds. The control sample did not contain either Fe^{2+} or the test compounds. The positive controls BHA, BHT, TBHQ, and α-tocopherol (vitamin E) were all tested at a final concentration of 10 μM. Fluorescence intensities of these liposome solutions were measured at an excitation wavelength of 384 nm every 3 min over a period of 21 min using a Turner model 450 digital fluorometer (Barnstead Thermolyne, Dubuque, IA). The decrease of relative fluorescence intensity with time indicated the rate of peroxidation. Relative fluorescence (F_t/F_0) was calcd by dividing the fluorescence value at a given time point (F_t) by that at t = 0 min (F_0).

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