Two New Isoflavones from *Ceiba pentandra* and Their Effect on Cyclooxygenase-Catalyzed Prostaglandin Biosynthesis

Ylva Noreen, Hesham El-Seedi, Premila Perera, and Lars Bohlin*

Division of Pharmacognosy, Department of Pharmacy, Biomedical Centre, Uppsala University, Box 579, S-751 23 Uppsala, Sweden

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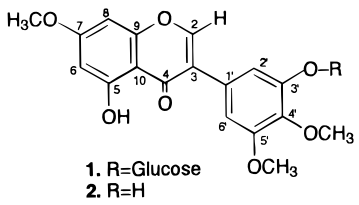
The new isoflavone glucoside vavain 3′-*O*-*β*-D-glucoside (**1**) and its aglycon, vavain (**2**), were isolated from the bark of *Ceiba pentandra*, together with the known flavan-3-ol, (+)-catechin. These novel structures were elucidated by one- and two-dimensional NMR experiments and by MS, IR, and UV spectroscopy as 5-hydroxy-7,4′,5′-trimethoxyisoflavone 3′-*O*-*β*-D-glucoside (**1**) and 5,3′-dihydroxy-7,4′,5′-trimethoxyisoflavone (**2**), respectively. The compounds were isolated following bioactivity-directed fractionation, using a cyclooxygenase-1-catalyzed prostaglandin biosynthesis assay in vitro, in which compounds **1** and **2** and (+)-catechin exhibited IC_{50} values of 381, 97, and 80 μM, respectively (standard: indomethacin, IC_{50} 1.1 μM). When further tested for their inhibitory effects on cyclooxygenase-2-catalyzed prostaglandin biosynthesis, **1** and **2** were found to be inactive (IC_{50} > 1200 and >900 μM, respectively).

Ceiba pentandra (L.) Gaertner (Bombacaceae) is a tropical tree of economic importance due to the elastic and water resistant floss of the capsules commonly known as kapok, used in, e.g., cushions and lifejackets.[1,2] This species is also widely used in traditional medicine. In Asia, Oceania, Africa, and Central America it is used for a variety of disorders including diarrhea, fever, gonorrhea, parasitic infections, and wounds and as a diuretic and an emollient.[3] In the Samoan Islands, a cold water infusion of the bark of *C. pentandra* (vernacular name *vavae*) is used for asthma.[4] Two studies have shown antiinflammatory properties of the bark extract, both in vitro and in vivo,[3,5] which can be related to several reports of traditional use in the treatment of ailments of an inflammatory nature such as asthma and cough.[3,6−8] In these studies, extracts showed suppression of carrageenan-induced rat paw edema as well as EPP-induced rat ear edema,[3] two in vivo experimental models of inflammation. Further, an extract was found to inhibit cyclooxygenase-1 (COX-1)-catalyzed prostaglandin biosynthesis in vitro.[3]

These results prompted us to further investigate the species phytochemically by bioactivity-directed fractionation using a prostaglandin biosynthesis assay in vitro. Previously reported constituents of this species include polysaccharides and xylans,[9,10] the naphthoquinone, 7-hydroxycadelene, sesquiterpene lactones,[11] and fatty acids.[12−14] This paper deals with the isolation and characterization of three flavonoids from the bark and the assessment of their inhibitory properties on COX-1 and cyclooxygenase-2 (COX-2)-derived prostaglandin biosynthesis.

Results and Discussion

Guided by an in vitro assay testing for inhibition of COX-1-catalyzed prostaglandin biosynthesis, a 70% aqueous ethanol extract of the bark of *C. pentandra* was fractionated mainly by combining medium-pressure liquid chromatography (MPLC), high-speed countercurrent chromatography (HSCCC), and Sephadex LH-20 chromatographic techniques. The fractionation procedure resulted in the isolation of two new isoflavones, vavain 3′-*O*-*β*-D-glucoside (**1**) and vavain (**2**), along with the known flavan-3-ol, (+)-catechin. To our knowledge, isoflavonoids have so far not been reported before from the genus *Ceiba*.



1. R=Glucose
2. R=H

The FABMS of **1** showed a peak at *m*/*z* 507 $[M + H]^+$, suggesting the molecular formula $C_{24}H_{26}O_{12}$. The ^1H NMR spectrum exhibited a flavonoid pattern and showed a signal at δ 8.15 (1H, s), typical of the proton at C-2 of an isoflavonoid skeleton.[15,16] A singlet at δ 12.83, which disappeared on deuterium exchange, indicated a C-5 hydroxyl.[17] This functionality was further confirmed by a bathochromic shift in the UV spectrum in the presence of $AlCl_3$.[16,18,19] Two doublets at δ 6.96 and 6.93, integrating for one proton each, suggested a trioxygenated unsymmetrical B-ring. Two *meta*-coupled doublets at δ 6.54 and 6.38 (1H, J = 2.0 Hz each) represented H-8 and H-6, respectively. ^1H NMR resonances at δ 3.8 to 3.4 and signals in the ^{13}C NMR spectrum just below δ 70 indicated the presence of a sugar moiety. A loss of 162 mass units from the molecular ion in the FABMS and a signal at δ 61.71, shown by DEPT to represent a CH_2 group, suggested glucose or galactose. The carbohydrate moiety was confirmed as glucose by acid hydrolysis and subsequent TLC sugar analysis. The glucose ^{13}C NMR spectral data were in agreement with

* To whom correspondence should be addressed. Tel.: + 46 18 4714492. Fax: + 46 18 509101. E-mail: lars.bohlin@pharmacog.uu.se.
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Table 1. ^{1}H-NMR Spectral Data of Compounds **1** and **2**

	compound					
	1			**2**		
	(AcCN-d_3 + D_2O) 20 °C	(AcCN-d_3) 60 °C		(AcCN-d_3 + D_2O) 20 °C	(AcCN-d_3) 60 °C	
proton	^{1}H δ (integral, mult, J (Hz))	^{1}H δ (integral, mult, J (Hz))	long-range COSY	^{1}H δ (integral, mult, J (Hz))	^{1}H δ (integral, mult, J (Hz))	long-range COSY
H-2	8.15 (1H, s)	8.12 (1H, s)		8.10 (1H, s)	8.07 (1H, s)	
H-6	6.38 (1H, d, 2.0)	6.40 (1H, d, 2.3)	H-8, 7-OMe	6.39 (1H, d, 2.3)	6.39 (1H, d, 2.2)	H-8, 7-OMe
H-8	6.54 (1H, d, 2.0)	6.54 (1H, d, 2.3)	H-6, 7-OMe	6.55 (1H, d, 2.3)	6.53 (1H, d, 2.3)	H-6, 7-OMe
H2′	6.96 (1H, d)	7.07 (1H, d, 2.0)	H-6′, H-1″	6.71 (1H, d, 2.0)	6.75 (1H, d, 1.9)	
H-6′	6.93 (1H, d)	6.97 (1H, d, 2.0)	H-2′, 5′-OMe	6.73 (1H, d, 2.0)	6.76 (1H, d, 2.0)	5′-OMe
H-1″	4.99 (1H, d, 7.3)	4.94 (1H, d, 7.3)	H-2′, H-2″−H-5″			
H-2″−H-5″	3.46−3.57 (4H, m)	3.36−3.55 (4H, m)	H-1″, H-2″−H-5″			
H-6_A″	3.80 (1H, dd)	3.81 (1H, dd, 2.5, 2.7, 11.9)	H-2″−H-5″			
H-6_B″	3.67 (1H, dd, 2.6, 3.6, 12.7)	3.66 (1H, dd, 5.3, 5.5, 11.8)	H-2″−H-5″			
OMe	3.86 (6H, s); 3.83 (3H, s)	pos. 7: 3.90 (3H, s)	H-6, H-8	3.88 (3H, s);	pos. 7: 3.90 (3H, s)	H-6, H-8
OMe		pos. 4′: 3.86 (3H, s)		3.85 (3H, s);	pos. 4′: 3.87 (3H, s)	
OMe		pos. 5′: 3.88 (3H, s)	H-6′	3.82 (3H, s)	pos. 5′: 3.88 (3H, s)	H-6′

Table 2. ^{13}C-NMR Spectral Data of Compounds **1** and **2**

	compound								
	1						**2**		
	(AcCN-d_3 + D_2O) 20 °C			(AcCN-d_3) 60 °C			(AcCN-d_3) 60 °C		
carbon	^{13}C	DEPT	C−H COSY (δ ^{1}H)	^{13}C	HMBC	HMQC	^{13}C	HMBC	HMQC
2	155.90	CH	8.15	155.88			155.98	H-2′	H-2
3	123.75	C		124.45	H-2, H-2′, H-6′		125.02	H-2, H-2′, H-6′	
4	181.60	C		182.16	H-2		182.56	H-2	
5	162.62	C		164.06	H-6		164.41	H-6	
6	99.30	CH	6.38	99.67	H-8	H-6	99.89	H-8	H-6
7	166.74	C		167.47			167.71	H-6, H-8	
8	93.58	CH	6.54	93.89		H-8	94.11	H-6	H-8
9	158.92	C		159.52	H-2, H-8		159.84	H-2, H-8	
10	106.74	C		107.50	H-6, H-8		107.82	H-6, H-8	
1′	127.89	C		127.91			128.53	H-2	
2′	111.01	CH	6.96	113.10	H-6′	H-2′	111.29	H-6′	H-2′
3′	151.26	C		152.02			151.52	H-2′	
4′	139.12	C		141.19	H-2′, H-6′		138.30	H-2′, H-6′	
5′	154.01	C		154.94			154.66	H-6′	
6′	109.00	CH	6.93	110.21	H-2′	H-6′	107.58	H-2′	H-6′
1″	101.81	CH	4.99	103.15		H-1″			
2″	74.05	CH	3.4−3.6	75.22		H-2″, H-3″,			
3″	76.85[a]	CH	3.4−3.6	78.19[a]		H-4″, H-5″			
4″	70.30	CH	3.4−3.6	71.98					
5″	77.28[a]	CH	3.4−3.6	78.31[a]					
6″	61.71	CH_2	3.8	63.31		H-6″			
OMe	56.93	CH_3	3.85	57.58		MeO-7	57.55		MeO-7
OMe	61.87	CH_3		61.89		MeO-4′	61.93		MeO-4′
OMe	56.93	CH_3	3.85	57.27		MeO-5′	57.63		MeO-5′

[a] Assignments with the same superscript letter may be interchanged.

the literature.[20−22] The anomeric glucose proton appeared at δ 4.99 as a doublet with a coupling constant of J = 7.3 Hz, indicating a β-linkage of the glucose unit to the aglycon.[16] Signals attributed to three methoxy groups were observed at δ 3.83 (3H, s) and δ 3.86 (6H, s). The above data in conjunction with the ^{13}C NMR spectrum (DEPT sequence) were found to be consistent with an isoflavone glucoside structure. Assignments of the protonated carbons were made using C−H COSY (HETCOR). The results are shown in Tables 1 and 2.

In the ^{13}C NMR spectrum of **1** one methoxyl group was found at δ 61.87, and there were another two at δ 56.93. The shift of the former below δ 60 indicated its placement between two oxygenated carbons[23] and suggested the following configuration of the B-ring: the glucose unit occurred at position C-3′ and two methoxyl groups were affixed to positions C-4′ and C-5′.[17] To confirm this, HMBC and long-range COSY experiments were performed (Figure 1). Because of viscosity problems (the compound had tendencies to form a gel in solution), these spectra were recorded at an elevated temperature (60 °C). ^{1}H and ^{13}C NMR spectra were rerun at this temperature. From long-range COSY connectivities (Figure 3) the proposed configuration of the B-ring was confirmed, since cross-peaks from H-2′ to H-1″ were found, as well as from H-6′ to OMe. Furthermore, connectivities from both H-6 and H-8 to OMe were observed, which placed the third methoxyl group at position C-7. The structure of **1** was thus

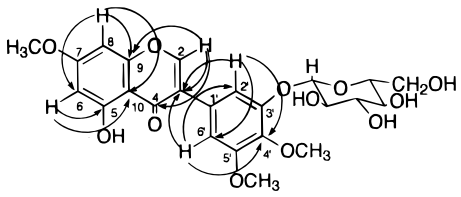

Figure 1. HMBC connectivities of **1**.

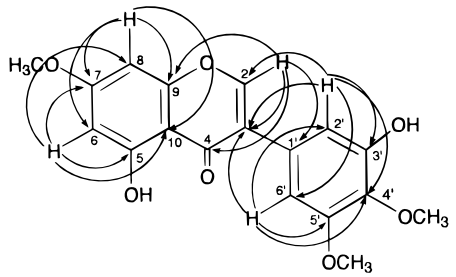

Figure 2. HMBC connectivities of **2**.

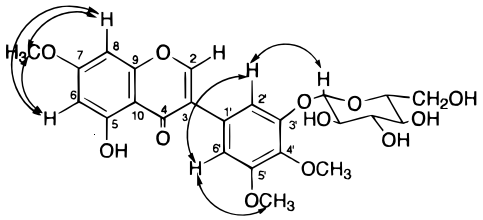

Figure 3. Important long-range COSY connectivities observed for **1**.

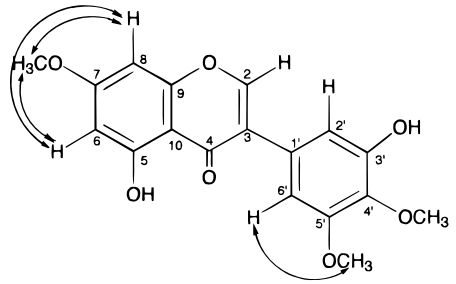

Figure 4. Long-range COSY connectivities for **2**.

established as 5-hydroxy-7,4′,5′-trimethoxyisoflavone 3′-*O*-*β*-D-glucoside (vavain 3′-*O*-*β*-D-glucoside).

The ^{1}H NMR and ^{13}C NMR data of **2** were similar to **1** apart from the absence of sugar resonances and the fact that three separate methoxy signals could be observed in the ^{1}H NMR spectrum when run at 20 °C [*δ* 3.81 (3H, s), 3.85 (3H, s), 3.88 (3H, s)]. The ^{1}H NMR spectrum exhibited the same indication of a C-5 hydroxyl, which was confirmed by UV data with $AlCl_3$ shift reagent, as performed for **1**. The EIMS and FABMS of **2** showed peaks at *m*/*z* 344 and 345 $[M + H]^+$, respectively, suggesting the molecular formula $C_{18}H_{16}O_7$. The presence of a $[M - 15]^+$ ion in the MS indicated the loss of a methyl group. Ions at *m*/*z* 315 and 301 indicated two other methyl groups. An ion at *m*/*z* 167 was assigned to a *retro*-Diels−Alder fragmentation product. Ring A was thus substituted with one methoxyl and one hydroxy group. The long-range COSY spectrum (Figure 4) in conjunction with the HMQC spectrum allowed the assignment of the individual methoxyl units to positions C-4′, C-5′, and C-7, respectively (Tables 1 and 2). The HMBC spectrum (Figure 2) confirmed the structure. Thus, **2** was assigned as 5,3′-dihydroxy-7,4′,5′-trimethoxyisoflavone, which has been given the trivial name vavain.

The antiinflammatory effect of the isolated compounds was assessed employing an in vitro assay testing for inhibition of COX-catalyzed prostaglandin biosynthesis. Prostaglandins are important mediators in the inflammatory process, and the inhibition of COX is considered to be involved in the mechanism of action of many antiinflammatory drugs, although the effects observed in vitro may not have an in vivo counterpart.[24−26] With the COX-1-catalyzed prostaglandin biosynthesis assay in vitro, as employed in the bioactivity-directed fractionation, vavain 3′-*O*-*β*-D-glucoside (**1**), vavain (**2**), and (+)-catechin exhibited IC_{50} values of 381, 97, and 80 *μ*M, respectively. The values can be compared with indomethacin (IC_{50} of 1.1 *μ*M), one of the strongest inhibitors of prostaglandin biosynthesis[27] and acetylsalicylic acid (aspirin), a much weaker inhibitor in this test system, exhibiting an IC_{50} value of 1 mM. Compounds **1**, **2**, and (+)-catechin were further tested for their inhibitory activities on COX-2-catalyzed prostaglandin biosynthesis, with **1** and **2** found to be essentially inactive. The IC_{50} value for **1** was over 1.2 mM and that for **2** over 900 *μ*M (maximum dissoluble concentration of **2** in solvents available to the assay). The isoflavones **1** and **2** thus exhibited a certain COX-1 selectivity. Further studies of the inhibitory activities on the COX-1- and COX-2-catalyzed prostaglandin biosynthesis by (+)-catechin are described in the accompanying paper.[28]

It cannot be ruled out that the aglycon, vavain (**2**), is an artifact of the glucoside, **1**, and thus formed by hydrolysis during extraction. Simultaneous occurrence of isoflavonoid aglycons and their *O*-glycosides have, however, been reported previously from several plant species.[29−31] Vavain 3′-*O*-*β*-D-glucoside (**1**) did not decompose under any of the laboratory conditions applied.

Experimental Section

General Experimental Procedures. EIMS, HR-FABMS, and positive-ion FABMS (with glycerol as matrix) spectra were recorded with a JEOL JMS SX/SX102A instrument.

^{1}H and ^{13}C NMR, DEPT, and C−H COSY (HETCOR) and further spectra were obtained in acetonitrile-d_3 + D_2O (5−10%) at 20 °C with TMS as internal standard using a 270 MHz JEOL JNM Ex-270/4000 NMR instrument. ^{1}H and ^{13}C NMR, inverse heteronuclear correlations HMBC and HMQC, as well as long-range COSY spectra were recorded at 60 °C in acetonitrile-d_3 on a 400 MHz Varian VXR-400 NMR instrument, IR spectra were recorded with a Nicolet MX-S spectrophotometer, and UV spectra were recorded with a Perkin-Elmer Lambda 2 UV/vis spectrophotometer. Melting points are uncorrected and were determined using a Digital melting point apparatus (Model IA 8103, Electrothermal Engineering Ltd, Southend-on-Sea, Essex, U.K.). The optical rotation was determined in Me_2CO or EtOH at ambient temperature using a Perkin-Elmer Model 241 polarimeter.

TLC was performed on precoated aluminum sheets [silica 60 F_{254}, 0.25 mm (Merck, Darmstadt, Germany)] and preparative TLC on precoated glass sheets [silica 60 F_{254}, 0.5 mm (Merck)], with detection provided by UV light (254 and 366 nm) and by spraying with vanillin−sulfuric acid reagent followed by heating (120 °C).

A three-phase liquid extraction procedure has been described by Nyiredy et al.[32] and was carried out in this investigation using a separatory funnel, partitioning the plant extract, and retrieving each phase separately.

MPLC was performed with SEPARO MPLC equipment (Bæckström Separo AB, Lidingö, Sweden).[33] SEPARO variable-length glass columns with an inner diameter of 1.5 or 2.5 cm, packed with silica gel 60, 40−63 μm (Merck), were used. An FMI Lab pump, Model QD (Fluid Metering, Inc., Oyster Bay, NY) was used at a flow of 20−30 mL/min. Fractions of 9 mL were collected with a Gilson Model 201 fraction collector. Columns were eluted with continuous gradients running from hexane, over CH_2Cl_2 to MeOH, and H_2O afforded by a SEPARO constant-volume mixing chamber combined with an open reservoir. Initially, the mixing chamber contained 50 mL of nonpolar solvent and the reservoir the first of 15−20 premixed binary (less polar/more polar solvent) gradient mixtures, of 20−40 mL each, which were successively fed to the reservoir during separation. High-speed counter-current chromatography (HSCCC) was carried out using an Ito Multi-Layer Coil CCC instrument (PC Inc., Potomac, MD). Coil #10 with a volume of 370 mL was used at 800 rpm. The flow was 3 mL/min provided by an LKB 2150 HPLC pump (LKB, Bromma, Sweden). Fractions collected ranged from 9 to 15 mL. Column chromatography was performed over Sephadex LH-20 (Pharmacia, Uppsala, Sweden).

During bioactivity-directed fractionation, extracts and crude and semi-purified fractions, dissolved in 10% EtOH or 0.5−20% DMSO to a final concentration of 100 μg/mL, were tested for inhibitory effects in a COX-1-catalyzed prostaglandin biosynthesis assay *in vitro*.[34]

For IC_{50} value determinations compound **1** and (+)-catechin were dissolved in 10% aqueous EtOH, while **2** was dissolved in 20% DMSO (yielding final concentrations of 2% and 4% of the respective solvent under the assay conditions). Materials for COX-1 and COX-2 prostaglandin biosynthesis assay were as follows: [^{14}C]-arachidonic acid (Amersham), *l*-epinephrine (Apoteksbolaget, Stockholm, Sweden), arachidonic acid, aspirin (acetylsalicylic acid), indomethacin, prostaglandins E_2 and $F_{2\alpha}$, and reduced glutathione (Sigma). For chromatographic separation, silica gel 60, 63−200 μm (Merck) was used. Chemicals and solvents were of analytical grade.

Plant Material. *C. pentandra* (L.) Gaertner bark was collected at the island of Savai'i of Western Samoa in November 1991 and authenticated by Prof. Paul A. Cox, Department of Botany and Range Science, Brigham Young University, Provo, UT. A voucher specimen (SA 8) is deposited at the Division of Pharmacognosy, Department of Pharmacy, Uppsala University, Sweden.

Extraction and Isolation. Powdered bark of *C. pentandra* (1.25 kg) was extracted three times in aqueous 70% EtOH (1 kg of material dry weight to 10 L of aqueous EtOH first extraction, 1:5 second and third extractions) at room temperature overnight with occasional stirring. The dried crude extract (68.8 g) was subjected to a three-phase liquid extraction procedure with a solvent system consisting of *n*-hexane−CH_2Cl_2−AcCN−H_2O (26:13:44:13) (910 mL/455 mL/1540 mL/455 mL × 2) yielding three extracts: hexane (2.3 g), AcCN/CH_2Cl_2 (3.9 g), and H_2O. The H_2O extract was further extracted with EtOAc (120 × 3 mL), thus yielding an EtOAc extract (4.5 g) and an H_2O extract (46.9 g).

A portion of the EtOAc extract (1.5 g) was adsorbed onto silica gel and chromatographed on a SEPARO column eluted with a continuous gradient from hexane over CH_2Cl_2 to MeOH of increasing polarity. Eluted fractions were evaluated by TLC and combined upon similar appearance, yielding 13 fractions. Fraction 8 (170 mg) was combined with several fractions (ca. 70 mg) exhibiting similar TLC profiles from two earlier HSCCC separations of portions of the EtOAc extract; the solvent system used in both cases was *n*-hexane−EtOAc−MeOH−H_2O (1:8:4:10) [first separation, EtOAc extract (500 mg), aqueous phase was used as mobile phase, retention of stationary phase was 32%; second separation, EtOAc extract (400 mg), organic phase as mobile phase, 62% retention of stationary phase]. The combined fractions (240 mg) were subjected to a third HSCCC separation [$CHCl_3$−MeOH−1-BuOH−H_2O (10:10:1:6), organic phase as mobile phase, 70% retention of stationary phase] from which 10 fractions were obtained. Fraction 2 contained (+)-catechin, which was further purified over Sephadex LH-20, eluted by MeOH−1-PrOH (1:1), yielding 19 mg. Fraction 10 (the contents remaining in the stationary phase after completion of the separation) was purified over Sephadex LH-20 (1-PrOH), which afforded vavain 3′-*O*-β-D-glucoside (**1**, 36 mg).

The AcCN/CH_2Cl_2 extract was separated by MPLC, with a continuous gradient from *n*-hexane to CH_2Cl_2 over MeOH to H_2O, which afforded 20 fractions. Fraction 11 (180 mg) was chromatographed over Sephadex LH-20 eluted with 1-PrOH and further separated by preparative TLC (CH_2Cl_2−MeOH, 9:1), which yielded 4 mg of vavain (**2**). Fraction 12 (300 mg) was rechromatographed on a SEPARO column with the same continuous gradient as employed previously, followed by chromatography over Sephadex LH-20 with 1-PrOH as eluent, which yielded 3.7 mg of **2**.

Acid Hydrolysis of 1. Compound **1** (24 mg) was refluxed for 75 min with 4 N HCl in MeOH (35 mL). The acid hydrolysate was concentrated, extracted with EtOAc, and examined by TLC on silica gel in $CHCl_3$ and ^{1}H NMR for the liberated aglycon, which was identified by direct comparison (^{1}H NMR, TLC) with the isolated naturally occurring **2**. The acidic mother liquor was neutralized with Na_2CO_3, filtered, and evaporated to dryness for examination of the sugar moiety, which proved to be glucose by detection on TLC [EtOAc−isopropanol−H_2O (65:23:12)], and sprayed with freshly prepared anisaldehyde−H_2SO_4 reagent followed by heating.[35]

5-Hydroxy-7,4′,5′-trimethoxyisoflavone 3′-*O*-β-D-glucoside (vavain 3′-*O*-β-D-glucoside) (1) was crystallized from EtOH as light yellow crystals: mp 140−141 °C; $[\alpha]^{20}_D$ −8.0° (*c* 0.1, EtOH); UV (MeOH) λ_{max} (log ϵ) 261 (+$AlCl_3$) 270 nm; IR (KBr) ν_{max} 3390 (OH), 2931−2852 (CH, aliphatic), 1660 (C=O), 1618, 1585, 1571, 1512, 1447, 1349, 1305, 1249, 1212, 1200, 1170, 1155, 1104, 1077, 1054, 993, 831; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HR-positive FABMS *m/z* $[M + Na]^+$ 529.1529 ($C_{24}H_{26}O_{12}Na$ requires 529.1322); FABMS *m/z* 507 $[M + H]^+$, 491, 437, 373, 345 $([M + H] - 162)^+$, 315, 285, 257, 193, 167, 151.

5,3′-Dihydroxy-7,4′,5′-trimethoxyisoflavone (vavain) (2) was crystallized from AcCN−CH_2Cl_2 as a

white amorphous solid: mp 169−170 °C; UV (MeOH) λ_{max} (log ϵ) 260 (+$AlCl_3$) 270 nm; IR (KBr) ν_{max} 3423 (OH), 2966−2854 (CH, aliphatic), 1665 (C=O), 1624, 1588, 1511, 1447, 1377, 1357, 1293, 1202, 1167, 1061, 998, 829 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HR-positive FABMS *m/z* $[M + H]^+$ 345.0979 ($C_{18}H_{17}O_7$ requires 345.0974); EIMS (70 eV) *m/z* 344 $[M^+]$ (100), 329 (46), 315 (4), 301 (24), 297 (7), 269 (5), 257 (4), 230 (13), 192 (2), 167 (17), 142 (8), 135 (7), 115 (5); FABMS *m/z* 345 $[M + H]^+$, 331, 299, 275, 245, 215, 207, 183, 167, 115.

(+)-Catechin was crystallized from AcCN−CH_2Cl_2 (2:1) (19 mg) and identified by comparison of its ^1H and ^{13}C NMR (MeOH-d_4) spectra with literature data,[36] $[\alpha]^{20}_D$ +5.9° (*c* 0.1, Me_2CO).

COX-1 and COX-2-Catalyzed Prostaglandin Biosynthesis Assay. The assay followed the method developed by White and Glassman.[34] Bovine cyclooxygenase-1 (prostaglandin endoperoxide H synthase-1) was derived as described by Takeguchi et al.[37] COX-2 (prostaglandin endoperoxide H synthase-2) enzyme purified from sheep placental cotyledons was procured from Cayman Chemical Company, Ann Arbor, MI.

Bovine seminal vesicle microsomes (10 μL, 20−30 μg protein) or purified sheep placental COX-2 enzyme (10 μL, 5 units, 0.8 μg) were preincubated with 50 μL of cofactor solution (reduced glutathione and *l*-epinephrine, 0.3 mg/mL each in Tris buffer, pH 8.2) in an ice−water bath for 15 min. Vehicle or test solution (20 μL) and 20 μL of [^{14}C]arachidonic acid (16 Ci/mol, 30 μM) were added and incubated at 37 °C for 10 min. A blank was kept in the ice−water bath. After incubation, the reaction was terminated by adding 10 μL of 2 N HCl followed by 5 μL of a carrier solution (PGE_2 and $PGF_{2\alpha}$ 0.2 mg/mL of each in EtOH). The unmetabolized arachidonic acid was separated from the prostaglandin products by column chromatography and eluted with *n*-hexane−dioxane−glacial acetic acid (70:30:1). The prostaglandin products were then eluted with EtAOc−MeOH (85:15), and the samples were counted in a Packard scintillation spectrometer. Indomethacin (2 μg/mL) was used as a positive control, yielding 70−90% inhibition. IC_{50} values were determined by regression analysis.

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