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Potential Cancer Chemopreventive Constituents of the Seeds of *Dipteryx odorata* (Tonka Bean)

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A new cassane diterpene, dipteryxic acid (**1**), and a new isoflavonolignan, 5-methoxyxanthocercin A (**2**), as well as four known active compounds, isoliquiritigenin (**3**), 6,4′-dihydroxy-3′-methoxyaurone (**4**), sulfuretin (**5**), and (±)-balanophonin (**6**), and five known inactive compounds, butin, eriodictyol, 7-hydroxychromone, 7,3′-dihydroxy-8,4′-dimethoxyisoflavone, and (−)-lariciresinol, were isolated from an ethyl acetate-soluble extract of the seeds of *Dipteryx odorata*, using a bioassay based on the induction of quinone reductase (QR) in cultured Hepa 1c1c7 mouse hepatoma cells to monitor chromatographic fractionation. The structures of compounds **1** and **2** were elucidated by spectroscopic data interpretation. Single-crystal X-ray diffraction analysis was used to confirm the relative stereochemistry of compound **1**. Selected compounds (**3**−**5**) were evaluated in a mouse mammary organ culture assay, with isoliquiritigenin (**3**) found to exhibit 76% inhibition at a dose of 10 μg/mL.

Dipteryx odorata (Aubl.) Willd. (syn. *Coumarouna odorata* Aubl.; Leguminosae) is a tall arboreal species native to Central America and northern South America and commonly known as the "tonka bean" tree.[1] A commercial market exists for the use of extractives of tonka beans in flavoring snuff, cigarettes, cigars, cocoa, and confectionery and as an ingredient of perfumes, liqueurs, sachet powders, and cosmetics.[1,2] The beans also yield a high percentage of a solid fat known as "tonka butter", which is used in flavoring foods.[1] Previous phytochemical investigations on this plant have resulted in the isolation of coumarins,[2,3] cassane diterpenoids,[4] isoflavonoids,[5,6] fatty acids,[7] and lupane triterpenoids.[7] In an ongoing project directed toward the discovery of novel, naturally occurring cancer chemopreventive agents from plants,[8,9] the seeds of *Dipteryx odorata* were chosen for more detailed investigation, since its ethyl acetate-soluble extract was found to induce the enzyme quinone reductase (QR) in cultured Hepa 1c1c7 (mouse hepatoma) cells. Phase II drug-metabolizing enzymes, such as QR and glutathione *S*-transferase in rodent tissues, are primarily responsible for the metabolic detoxification of chemical carcinogens and other harmful oxidants. Therefore, induction of QR is a major mechanism of protection against tumor initiation.[10]

Bioassay-guided fractionation of the ethyl acetate-soluble residue of the seeds of *Dipteryx odorata* using the QR induction assay led to the isolation and characterization of a new cassane diterpene (**1**) and a new isoflavonolignan (**2**) and the identification of nine known constituents. Single-crystal X-ray diffraction analysis was used to confirm the relative stereochemistry of compound **1**. These isolates were evaluated for their potential to induce QR in the Hepa 1c1c7 model. Selected compounds (**3**−**5**) were

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Table 1. NMR Data for Compound **1**

position	δ_C[a]		δ_H multiplicity (J, Hz)[b]	HMBC correlations[b]
1	38.1	t	1.00 m	3
			1.69 m	
2	17.5	t	1.49 m	
3	36.3	t	1.48 m	5, 18
			1.67 m	
4	46.2	s		
5	48.7	d	1.63 m	3, 4, 7, 10, 18, 19, 20
6	23.5	t	1.18 m	
			1.40 m	
7	29.8	t	1.30 m	
			1.53 m	
8	40.0	d	1.51 m	
9	44.9	d	1.42 m	
10	35.7	s		
11	37.5	t	2.25 dd (12.7, 2.6)	8, 9, 12, 13
			1.17 m	
12	105.6	s		
13	173.2	s		
14	35.8	d	2.88 dq (7.0, 4.9)	8, 9, 12, 13, 17
15	112.5	d	5.79 s	12, 13, 14, 16
16	170.3	s		
17	12.5	q	1.06 d (7.0)	8, 14, 13
18	16.7	q	1.07 s	3, 4, 5, 19
19	179.5	s		
20	14.0	q	0.78 s	1, 5, 9, 10, 14
OH-12			7.25 s	11, 12, 13
COOH-4			12.13 s	

[a] Spectrum was run in DMSO-d_6 at 125 MHz, and values are reported in ppm relative to TMS. Multiplicities were determined by a DEPT ^{13}C NMR experiment. [b] Spectrum was run in DMSO-d_6 at 500 MHz. Assignments are based on COSY, HMQC, and HMBC experiments.

then chosen for evaluation in a mouse mammary organ culture assay, which is used as a secondary discriminator in our program on cancer chemoprevention.[8,9] The structure elucidation of **1** and **2**, and the biological evaluation of the isolates obtained from *D. odorata* seeds, are described herein.

Results and Discussion

Purification of the ethyl acetate-soluble fraction of the methanol extract of the seeds of *Dipteryx odorata* using a quinone reductase induction assay to monitor fractionation led to the isolation of a new cassane diterpene, dipteryxic acid (**1**), and a new isoflavonolignan, 5-methoxyxanthocercin A (**2**), as well as nine known compounds, isoliquiritigenin (**3**),[11] 6,4′-dihydroxy-3′-methoxyaurone (**4**),[12] sulfuretin (**5**),[13,14] (±)-balanophonin (**6**),[15] butin,[16] eriodictyol,[17] 7-hydroxychromone,[18] 7,3′-dihydroxy-8,4′-dimethoxyisoflavone,[19] and (−)-lariciresinol (Figure 1).[20] Isoliquiritigenin (**3**), 6,4′-dihydroxy-3′-methoxyaurone (**4**), sulfuretin (**5**), (±)-balanophonin (**6**), 7-hydroxychromone, and (−)-lariciresinol have not been isolated from a *Dipteryx* species previously, and no NMR data have been reported for **4**. The structures of the known compounds were identified by physical (mp, $[\alpha]_D$) and spectroscopic data (^{1}H NMR, ^{13}C NMR, 2D NMR, and MS) measurement and by comparison with published values.

Compound **1** was obtained as colorless crystals and gave a protonated molecular ion $[M]^+$ at m/z 348.1949 by HREIMS, consistent with an elemental formula of $C_{20}H_{28}O_5$. It exhibited IR maxima at 3347, 1733, and 1682 cm^{-1}, suggesting the presence of a hydroxy group and an α,β-butenolide moiety.[21] Unambiguous NMR assignments (Table 1) were made by application of 1D- and 2D-homo- and heteronuclear NMR experiments (^{1}H NMR, ^{13}C NMR, DEPT, COSY, NOESY, HMQC, and HMBC). The ^{1}H NMR

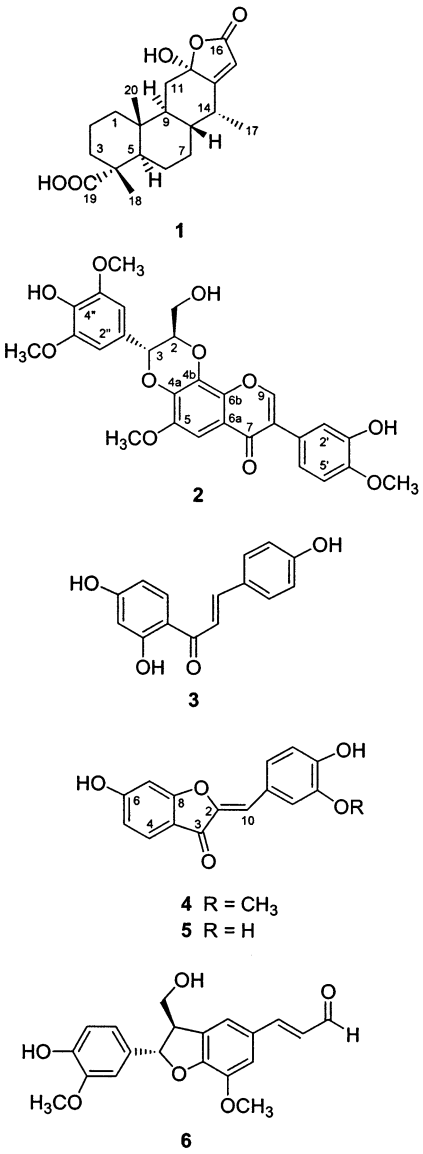

Figure 1. Structures of compounds **1**−**6** isolated from *D. odorata*.

spectrum of **1** showed a resonance for an olefinic proton at 5.79 (1H, s), which was assigned to H-15, as well as signals for three methyl groups, with two attached to quaternary saturated carbons (δ 0.78 and 1.07, both s) and one to a tertiary carbon (δ 1.06, d, J = 7.0 Hz). The ^{13}C NMR and DEPT spectra of **1** (Table 1) showed six quaternary carbons including two carbonyl carbons (δ 170.3 and 179.5), one sp^2 carbon (δ 173.2), and a characteristic sp^3 carbon of a hemiketal (δ 105.6), as well as five tertiary carbons, comprising one sp^2 carbon (δ 112.5), six secondary carbons, and three methyl groups (δ 12.5, 14.0, and 16.7).

From the HMBC spectrum (Table 1), in which cross-peaks were observed between δ_H 5.79 (s, H-15) and δ_C 105.6 (C-12), 173.2 (C-13), 35.8 (C-14), and 170.3 (C-16), it was inferred that compound **1** is a cassane-type diterpene acid containing an α,β-butenolide moiety connected to the carbon of a hemiketal unit.[21,22] The position of the carboxylic group at C-4 was determined from HMBC correlations between δ_H 1.07 (s, H-18) and δ_C 36.3 (C-3), 46.2 (C-4), 48.7 (C-5), and 179.5 (C-19). All of these data were in accordance with the structure proposed for **1**, and its structure and relative stereochemistry were confirmed unambiguously by X-ray diffraction analysis (Figure 2). Thus, dipteryxic acid (**1**) was elucidated as *rel*-(4*R*,5a*R*,8a*S*,9*S*,10*R*,12*R*,14*R*)-12-

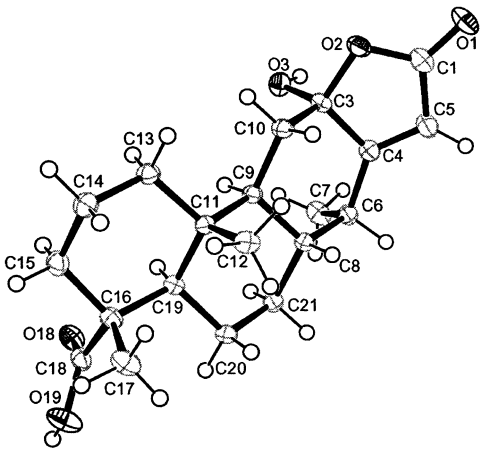

Figure 2. X-ray structure of compound **1** drawn by ORTEP. (The numbering system does not follow that of *Chemical Abstracts*.)

hydroxycass-(13)15-en-16,12-olid-19-oic acid. Cassane diterpenes fused with an α,*β*-butenolide moiety appear to be rare in the plant kingdom.[21,22] This is first report of the isolation of this type of diterpene from a *Dipteryx* species.

Compound **2** was obtained as colorless crystals and gave a protonated molecular ion $[M + 1]^+$ at *m*/*z* 539.1563 by HRFABMS, consistent with an elemental formula of $C_{28}H_{26}O_{11}$. The ^{1}H NMR spectrum showed resonances for an isoflavone moiety in **2** which exhibited a diagnostic vinylic singlet at *δ* 8.45 (H-9, 1H, s).[19] The aromatic region of the spectrum displayed a two-proton singlet at *δ* 6.70, indicative of a symmetrically tetrasubstituted aromatic ring and a one-proton singlet at *δ* 7.16, which was assigned to H-6 and was consistent with the substitution pattern in ring A of dipteryxin (7,8-dihydroxy-6,4′-dimethoxyisoflavone).[6] Also observed were a two-proton signal at *δ* 6.98(overlap) and a broad one-proton singlet at *δ* 7.13, which were assigned to the protons of the B ring of the isoflavonoid part of **2**. Besides four aromatic methoxy signals [*δ* 3.89, 3.80, and 3.78 (×2)], the aliphatic region also displayed signals for an AMXY system (*δ* 5.01, 1H, d, *J* = 6.9 Hz; *δ* 4.44, 1H, m; *δ* 3.67, 1H, brd, *J* = 12.2 Hz; *δ* 3.42, 2H, m). Careful analysis of the COSY, HMQC, and HMBC NMR data indicated that **2** contains a phenylpropanoid unit.[23,24] The deshielded doublet at δ_H 5.01 (H-3), typical of a benzylic methine substituted by an oxygen, and the multiplet at δ_H 4.44 (H-2), which were coupled to each other, implied the presence of a 1,4-dioxane ring between an isoflavonoid moiety and a phenyl ring.[23,25] On the basis of this observation, it could be proposed that compound **2** is composed of a 3,4,5-trioxygenated phenylpropanoid unit coupled via the dihydroxy functionality of a pentaoxygenated isoflavonoid moiety. The linkage of the isoflavonoid and phenylpropanoid units through a 1,4-dioxane bridge was deduced by HMBC NMR experiments (Figure 3). Thus, after optimizing the *J* value [$^{2,3}J$(C,H)] for a long-range correlation to 4 Hz,[24] a HMBC cross-peak for H-3/C-4a was observed. All of the positions of the methoxy and hydroxyl groups were determined unambiguously by HMBC and NOESY NMR experiments (Figure 3). Comparison of the above data with those in the literature[25] indicated that the structure of **2** is very closely related to that of an isoflavonolignan, xanthocercin A, except for the presence of an additional methoxy group at C-5 in **2**. The coupling constant (*J* = 6.9 Hz) between H-2 and H-3 and a NOE correlation between H-2 and H-2″ (Figure 3) clearly indicated a *trans* configuration of the chiral centers of the dioxane ring.[23−25] Therefore, the structure of this new isoflavonolignan, 5-methoxyxanthocercin A (**2**), was elucidated as 2,3-*trans*-3-(4-hydroxy-3,5-dimethoxyphenyl)-8-(3-hydroxy-4-methoxyphenyl)-2-hydroxymethyl-5-methoxy-2,3-dihydro-7*H*-1,4-dioxino[2,3-*h*]chromen-7-one.

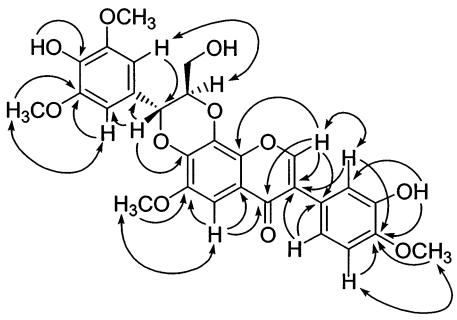

Figure 3. Selected correlations observed in the HMBC (→) and NOESY (↔) NMR spectra of **2**.

Table 2. NMR Data for Compound **2**

position	δ_C[a]		δ_H multiplicity (*J*, Hz)[b]
CH_2OH	59.8	t	3.42 m 3.67 brd (12.2)
2	77.8	d	4.44 m
3	76.6	d	5.01 d (6.9)
4a	138.0	s	
4b	132.6	s	
5	146.6	s	
6	95.7	d	7.16 s
6a	116.9	s	
6b	141.1	s	
7	174.1	s	
8	124.6	s	
9	152.8	d	8.45 s
1′	123.0	s	
2′	116.4	d	7.13 brs
3′	146.0	s	
4′	147.5	s	
5′	111.9	d	6.98 (overlap)
6′	119.7	d	6.98 (overlap)
1″	125.5	s	
2″/6″	105.6	d	6.70 s
3″/5″	147.9	s	
4″	136.2	s	
OMe-5	55.58	q	3.89 s
OMe-4′	55.61	q	3.80 s
OMe-3″/5″	56.0	q	3.78 s
CH_2OH			5.08 brt
OH-3′			9.05 brs
OH-4″			8.60 brs

[a] Spectrum was run in DMSO-d_6 at 125 MHz, and values are reported in ppm relative to TMS. [b] Spectrum was run in DMSO-d_6 at 500 MHz. Assignments are based on COSY, HMQC, and HMBC experiments.

The potential of all of the isolates from *D. odorata* seeds to induce QR activity in Hepa 1c1c7 cells was evaluated, and the results obtained for the only active compounds, **3**−**6**, are summarized in Table 3. The known chalcone, isoliquiritigenin (**3**), exhibited the most potent QR activity [concentration to double activity (CD) value 3.8 *μ*M], while the known aurones, 6,4′-dihydroxy-3′-methoxyaurone (**4**) and sulfuretin (**5**), and the lignan, (±)-balanophonin (**6**), induced QR activity, with CD values ranging from 6.4 to 20.7 *μ*M. The two new compounds (**1** and **2**) and known compounds, butin, eriodictyol, 7-hydroxychromone, 7,3′-dihydroxy-8,4′-dimethoxyisoflavone, and (−)-lariciresinol, were inactive (CD value >10 *μ*g/mL) as inducers of QR. Sulfuretin (**5**) was recently isolated by our group as a potent antioxidative substance from *Cotinus coggygria*.[14] Compounds **3**−**5** were chosen for evaluation in a mouse mammary organ culture (MMOC) assay to evaluate the potential of inhibiting carcinogen-induced preneoplastic lesion formation (Table 3).[26,27] As a result, isoliquiritigenin (**3**) also

Table 3. Biological Activity of Compounds **3**−**6** from *D. odorata* in the Quinone Reductase (QR) Induction and Mouse Mammary Organ Culture (MMOC) Bioassays

	QR[a]			
compound	CD (μM)	IC_{50} (μM)	CI	MMOC (%)[b]
3	3.8	27.3	7.2	76.0
4	6.4	34.6	5.4	48.2
5	20.7	78.5	3.8	58.5
6	15.1	48.5	3.2	ND[c]
sulforaphane[d]	0.43	11.0	25.0	83.7

[a] CD, concentration required to double QR activity; IC_{50}, concentration inhibiting cell growth by 50%; CI, chemoprevention index (= IC_{50}/CD). The new compounds **1** and **2** and the known compounds butin, eriodictyol, 7-hydroxychromone, 7,3′-dihydroxy-8,4′-dimethoxyisoflavone, and (−)-lariciresinol were inactive (CD values >10 μg/mL) in the QR assay. [b] Inhibition of 7,12-dimethylbenz[*a*]anthracene-induced preneoplastic lesions in a mouse mammary organ culture model. Selected compounds from *D. odorata* were tested at a concentration of 10 μg/mL. On the basis of historical controls, inhibition of >60% (at 10 μg/mL) is considered significant. [c] Not determined since the amount of available compound was insufficient. [d] Sulforaphane was used as a positive control and was tested at a concentration of 1 μg/mL in the MMOC assay.[10,35,36]

exhibited a significant response in the MMOC assay (76% inhibition at 10 μg/mL). Therefore, this compound, also a constituent of licorice and shallots, is worthy of consideration as a potential cancer chemopreventive agent through additional biological evaluation. Our data on isoliquiritigenin (**3**) are consistent with recent results by Baba et al. on the inhibition of azoxymethane (AOM)-induced murine colon carcinogenesis and AOM-induced murine colon aberrant crypt focus formation by this compound.[28] Isoliquiritigenin (**3**) has also been found to suppress metastasis in a pulmonary metastasis model of mouse renal cell carcinoma and to prevent severe 5-fluorouracil-induced leukocytopenia in this model.[29]

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. UV spectra were recorded with a Beckman DU-7 spectrometer. IR spectra were run on an ATI Mattson Genesis Series FT-IR spectrometer. NMR experiments were conducted on Bruker DPX-300 and DRX-500 spectrometers with tetramethylsilane (TMS) as internal standard. FABMS and HRFABMS were obtained on a VG 7070E-HF sector-field mass spectrometer, and EIMS and HREIMS on a Finnigan MAT 90/95 sector-field mass spectrometer or a JEOL GCmate II mass spectrometer, operating at 70 eV. Thin-layer chromatographic (TLC) analysis was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by dipping plates into 10% (v/v) H_2SO_4 reagent (Aldrich, Milwaukee, WI) followed by charring at 110 °C for 5−10 min. Silica gel (Merck 60A, 70−230 or 200−400 mesh ASTM) was used for column chromatography. Preparative TLC was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness). All solvents used for chromatographic separations were purchased from Fisher Scientific (Fair Lawn, NJ) and distilled before use.

X-ray crystallographic analysis data collection for compound **1** was carried out on an Enraf-Nonius Kappa CCD area detector equipped with a rotating anode X-ray generator and Mo Kα radiation.[30] The direct methods program SIR-92 was used to locate the non-hydrogen atoms.[31] Repeated cycling with least-squares refinement on F^2 with SHELX-97 and difference Fourier maps yielded the final structure and was useful to identify hydrogen atom positions.[32] All non-hydrogen atoms were refined with anisotropic Gaussian displacement parameters. The ORTEP diagram was drawn with 50% probability ellipsoids.[33]

Plant Material. The seeds of *Dipteryx odorata* (Aubl.) Willd. were collected in Rio Huyabamba, Dos de Mayo district, Mariscal Caceres Province, Peru, in September 2001 by two of us (J.S.V. and J.G.G.). A voucher specimen has been deposited at the Field Museum of Natural History, Chicago, IL (accession no. Schunke 14458).

Quinone Reductase Assay. For the evaluation of plant extracts, fractions, and pure isolates as inducers of quinone reductase (QR), cultured mouse Hepa 1c1c7 cells were used as described previously.[10,34−36]

Mouse Mammary Organ Culture Assay. The inhibition of lesion formation in mouse mammary organ culture was performed as previously described.[26,27]

Extraction and Isolation. The dried and milled plant material (3.3 kg) was extracted with MeOH (3 × 8 L) by maceration. The extracts were combined and concentrated in vacuo at 40 °C. The concentrated extract was suspended in 90% MeOH and then partitioned with petroleum ether (3 × 2 L) to afford a petroleum ether-soluble syrup (D001) on drying. Next, the aqueous methanol extract was concentrated and suspended in H_2O (2 L) and partitioned again with EtOAc (3 × 2 L) to give an EtOAc-soluble extract (D002) and an aqueous residue (D003). The CD values (μg/mL) of the solvent partitions, D001, D002, and D003, were >10, 9.8, and >10, respectively.

On the basis of the above activity results, the EtOAc-soluble extract (D002, 185 g) was chromatographed over silica gel as stationary phase using a $CHCl_3$−MeOH gradient (from 1:0 to 0:1 v/v) as mobile phase to afford 18 pooled fractions (F004−F021). Of these, fractions F016 and F018 showed the most potent QR-inducing activity (CD values 9.1 and 6.3 μg/mL, respectively). Fraction F016 [eluted with $CHCl_3$−MeOH (19:1 v/v); 10.3 g] was chromatographed over silica gel ($CHCl_3$−acetone, 19:1 → 4:1 v/v), resulting in nine subfractions (F033−F042). Of these, fraction F037 [eluted with $CHCl_3$−acetone (9:1 v/v)], which showed the most potent QR-inducing activity (CD value <2.5 μg/mL), was chromatographed over silica gel ($CHCl_3$−MeOH, 99:1 v/v), to afford 10 further subfractions (F045−F052). (−)-Lariciresinol (3.0 mg, 0.000091%) {$[\alpha]^{20}_D$ −15.8° (*c* 0.1, MeOH); lit.[37] $[\alpha]^{25}_D$ −17.8° (*c* 1.4, Me_2CO)}, 7,3′-dihydroxy-8,4′-dimethoxyisoflavone (4.8 mg, 0.00015%), and (±)-balanophonin (**6**; 1.4 mg, 0.000042%) were isolated from fractions F043, F045, and F046, respectively, by preparative TLC, developed with petroleum ether−EtOAc−2-propanol (5:4:1) (R_f = 0.42, 0.50, and 0.35, respectively). Three further known compounds, 6,4′-dihydroxy-3′-methoxyaurone (**4**; 12 mg, 0.00036%), 7-hydroxychromone (23 mg, 0.0007%), and isoliquiritigenin (**3**; 210 mg, 0.0064%) were purified from fractions F049, F050, and F051, respectively, by recrystallization (in petroleum ether−EtOAc, 1:1). The new compounds **1** (300 mg, 0.0091%) and **2** (4.2 mg, 0.00013%), along with the known compound butin (11 mg, 0.00033%), were obtained from fractions F038, F041, and F018, respectively, by recrystallization from MeOH.

The second active fraction, F018 [eluted with $CHCl_3$−MeOH (14:1 v/v), 12.1 g], was purified over a further silica gel column, with petroleum ether−EtOAc−MeOH (6:3.5:0.5 → 5:4:1 v/v) used as solvent system, yielding in turn the known compounds eriodictyol (400 mg, 0.012%)[17] and sulfuretin (**5**; 19.5 mg, 0.00059%).[13,14]

Dipteryxic acid (1): colorless crystals; mp 255−257 °C; $[\alpha]^{20}_D$ −64° (*c* 0.1, $CHCl_3$−MeOH, 1:1); UV (EtOH) λ_{max} (log ϵ) 237 (3.84), 284 (3.10) nm; IR ν_{max} (NaCl) 3347, 2929, 2359, 2342, 1733, 1682, 1215, 1191, 1129, 873, 635 cm^{-1}; ^1H NMR, ^{13}C NMR, and HMBC data, see Table 1; EIMS *m*/*z* 348 ($[M]^+$, 12), 330 (27), 320 (39), 304 (100), 289 (31), 274 (24), 257 (16), 235 (19), 215 (32), 207 (40), 189 (30), 161 (58); HREIMS *m*/*z* 348.1949 $[M]^+$, $C_{20}H_{28}O_5$, calcd 348.1937).

X-ray Crystallography of 1. A colorless crystal was obtained from MeOH and selected for data collection. Crystal size: 0.29 × 0.35 × 0.40 mm. Cell parameters: a = 11.0783(3) Å, b = 7.4287(2) Å, c = 11.1597(3) Å, β = 102.3950(10)°, V =

897.01(4) Å^3, $Z = 2$, $D_{calc} = 1.290$ Mg/m^3, $T = 150(2)$ K, $\lambda = 0.71073$ Å, space group = $P2_1$, μ(Mo Kα) = 0.091 mm^{-1}, $F(000) = 376.0$, refinement with 3607 reflections ($3423 > 2\sigma I$) led to final R, R (all), and GOF values of 0.0286, 0.0309, and 1.028, respectively. Crystallographic data (excluding structure factors) for the structure of this compound have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC195044. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

5-Methoxyxanthocercin A (2): colorless crystals; mp 173−174 °C; $[\alpha]^{20}_D$ +11° (*c* 0.1, $CHCl_3$−MeOH, 1:1); UV (EtOH) λ_{max} (log ϵ) 256 (4.16), 295 (3.96), 313 (3.81, sh) nm; IR ν_{max} (NaCl) 3365, 3021, 2926, 2948, 1607, 1510, 1446, 1363, 1265, 1236, 1110, 1056, 832, 756 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 1; LRFABMS *m/z* 539 ([M + 1]$^+$, 6), 482 (5), 307 (32), 289 (15), 154 (100), 136 (76); HRFABMS *m/z* 539.1563 ([M + 1]$^+$, calcd for $C_{28}H_{27}O_{11}$, 539.1553).

Isoliquiritigenin (3): pale yellow powder; mp 188−191 °C; EIMS *m/z* 256 ([M]$^+$, 100), 239 (15), 163 (29), 137 (73), 120 (35), 107 (13); ^{1}H NMR data were in agreement with reported values.[11]

6,4′-Dihydroxy-3′-methoxyaurone (4): orange crystals; mp 233−235 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.60 (1H, overlap, H-2′), 7.56 (1H, d, $J = 8.4$ Hz, H-4), 7.49 (1H, dd, $J = 8.3$, 1.8 Hz, H-6′), 6.93 (1H, d, $J = 8.2$ Hz, H-5′), 6.80 (1H, d, $J = 1.6$ Hz, H-7), 6.75 (1H, dd, $J = 8.3$, 1.6 Hz, H-5), 6.66 (1H, s, H-10), 3.94 (3H, s, MeO-4′); ^{13}C NMR (CD_3OD, 75 MHz) δ 182.2 (C-3), 169.2 (C-6), 168.6 (C-8), 149.5 (C-4′), 148.6 (C-3′), 147.6 (C-2), 126.4 (C-4 or C-6′), 126.3 (C-4 or C-6′), 125.5 (C-1′), 116.4 (C-5), 115.1 (C-2′), 114.2 (C-5′), 114.1 (C-9), 111.7 (C-10), 99.6 (C-7), 56.3 (MeO-4′); EIMS *m/z* 284 ([M]$^+$, 100), 269 (7), 253 (6), 241 (11), 213 (6), 162 (5), 148 (7), 137 (10).

Sulfuretin (5): orange crystals; mp 192−195 °C (dec); EIMS *m/z* 270 ([M]$^+$, 100), 253 (17), 213 (10), 137 (22), 112 (11); ^{1}H and ^{13}C NMR data were in agreement with reported values.[13,14]

(±)-Balanophonin (6): colorless oil; $[\alpha]^{20}_D$ −0.2° (*c* 0.1, MeOH) {lit.[38] (−)-balanophonin, $[\alpha]^{20}_D$ −114° (*c* 0.34, $CHCl_3$); lit.[38] (+)-balanophonin, $[\alpha]^{25}_D$ +108° (*c* 0.41, $CHCl_3$)}; EIMS *m/z* 356 ([M]$^+$, 55), 338 (100), 326 (48), 323 (40), 306 (17), 278 (10), 165 (12), 149 (15), 137 (21), 129 (16); ^{1}H and ^{13}C NMR data were in agreement with reported values.[15,38]

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Supporting Information Available: Experimental details of X-ray analysis of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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