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Activity-Guided Isolation of Constituents of *Tephrosia purpurea* with the Potential to Induce the Phase II Enzyme, Quinone Reductase

Leng Chee Chang, Clarissa Gerhäuser,† Lynda Song, Norman R. Farnsworth, John M. Pezzuto, and A. Douglas Kinghorn*

Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

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An isoflavone, 7,4′-dihydroxy-3′,5′-dimethoxyisoflavone (**1**), and a chalcone, (+)-tephropurpurin (**2**), both novel compounds, as well as six constituents of known structure, (+)-purpurin (**3**), pongamol (**4**), lanceolatin B (**5**), (−)-maackiain (**6**), (−)-3-hydroxy-4-methoxy-8,9-methylenedioxypterocarpan (**7**), and (−)-medicarpin (**8**), were obtained as active compounds from *Tephrosia purpurea*, using a bioassay based on the induction of quinone reductase (QR) activity with cultured Hepa 1c1c7 mouse hepatoma cells. Additionally, three inactive compounds of known structure, 3′-methoxydaidzein, desmoxyphyllin B, and 3,9-dihydroxy-8-methoxycoumestan, were isolated and identified. The structure elucidation of compounds **1** and **2** was carried out by spectral data interpretation.

Tephrosia purpurea Pers. (Leguminosae) is a pantropical coastal shrub that grows up to 1 m high.[1] In India, various plant parts are used as a remedy for impotency and to treat asthma, diarrhea, gonorrhea, rheumatism, and urinary disorders.[2] A *T. purpurea* seed extract has shown insecticidal and insect repellant properties,[3] while an ethanolic extract of the entire plant was found to exhibit cytotoxic activity with KB cells in culture.[4] Significant in vivo hypoglycemic activity was demonstrated in diabetic rabbits using an aqueous extract of *T. purpurea* seeds.[5] Previous phytochemical investigations on this plant have resulted in the isolation of coumarins,[6] flavonoids and rotenoids,[7,8] flavanones,[9,10] and isoflavones.[11]

As part of our search for cancer chemopreventive natural products, the whole flowering and fruiting parts of *Tephrosia purpurea* were chosen for activity-guided fractionation, because their petroleum ether- and ethyl acetate-soluble extracts were both found to significantly induce quinone reductase (QR) activity with cultured Hepa 1c1c7 mouse hepatoma cells.[12] Induction of Phase II drug-metabolizing enzymes such as QR is considered a major mechanism of protection against tumor initiation.[13,14] In the present investigation, bioassay-guided fractionation of *T. purpurea* using the QR induction assay led to the isolation of eight active compounds, namely, 7,4′-dihydroxy-3′,5′-dimethoxyisoflavone (**1**) and (+)-tephropurpurin (**2**), both of novel structure, as well as six constituents of previously known structure: (+)-purpurin (**3**), pongamol (**4**), lanceolatin B (**5**), (−)-maackiain (**6**), (−)-3-hydroxy-4-methoxy-8,9-methylenedioxypterocarpan (**7**), and (−)-medicarpin (**8**). Additionally, three known compounds, 3′-methoxydaidzein, desmoxyphyllin B, and 3,9-dihydroxy-8-methoxycoumestan were isolated and found to be inactive in the process of QR induction. The structure elucidation of

* Address reprint requests to A. D. Kinghorn. Phone: (312) 996-0914. FAX: (312) 996-7107. E-mail: U11239@uicvm.uic.edu.

† German Cancer Research Center, Division of Toxicology and Cancer Risk Factors, Im Neuenheimer Feld 280, D69120 Heidelberg, Germany.

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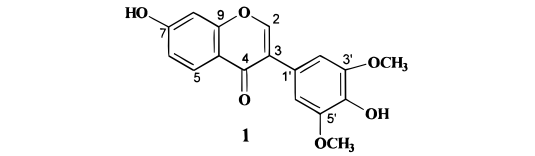
Table 1. ^{1}H- and ^{13}C-NMR Assignments and Selective INEPT Enhancements Observed for Compound **1** in DMSO-d_6[a]

position	δ_H (mult.)	δ_C	carbon signal enhanced[b]
2	8.32 s	153.3	174.6 (C-4), 157.3 (C-9),
3		123.6	121.9 (C-1′)
4		174.6	
5	7.97 (J = 8.7 Hz)	127.3	174.6 (C-4), 157.3 (C-9), 162.5 (C-7)
6	6.94 dd (J = 8.7, 1.4 Hz)	115.2	102.1 (C-8), 116.7 (C-10)
7		162.5	
8	6.87 (J = 1.4 Hz)	102.1	115.2 (C-6), 116.7 (C-10)
9		157.3	
10		116.7	
1′		121.9	
2′	6.87 s	106.6	123.6 (C-3), 147.6 (C-3′), 135.6 (C-4′) 106.6 (C-6′)
3′		147.6	
4′		135.6	
5′		147.6	
6′	6.87 s	106.6	
MeO-3′	3.78 s	56.1	147.6 (C-3′, C-5′)
MeO-5′	3.78 s	56.1	147.6 (C-3′, C-5′)

[a] TMS was used as internal standard; chemical shifts are shown in the δ scale with J values in parentheses. [b] Data show carbon atoms enhanced on irradiation ($^3J_{CH}$ = 7 Hz) of the proton indicated.

1 and **2** and the biological evaluation of **1**−**8** are the subject of this report.



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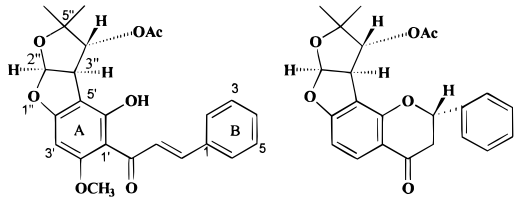

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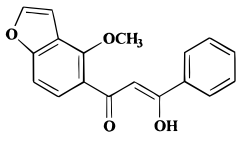

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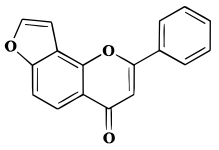

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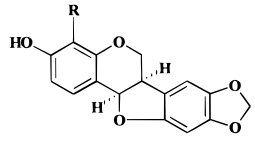
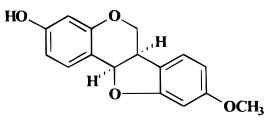


6 R=H
7 R=OCH$_3$



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Results and Discussion

Compound **1** was assigned a molecular formula of $C_{17}H_{14}O_6$ from its HR-EIMS (m/z 314.0798). Comparison of its UV, IR, and ^{1}H- (Table 1) and ^{13}C-NMR (Table 1) data with literature values indicated it was an isoflavone.[15−17] Furthermore, it was apparent that this compound contained two hydroxyl groups (IR, ν_{max} 3600−3400 cm^{-1}; δ_C 162.5, 135.6) and two magnetically equivalent OMe groups (δ_C 56.1, δ_H 3.78). The relative positions of these functional groups were established from the following observations. In the ^{1}H-NMR spectrum of **1**, a doublet of doublets (J = 8.7, 1.4 Hz) at δ_H 6.94 (H-6), a doublet (J = 1.4 Hz) at δ_H 6.87 (H-8), and a doublet (J = 8.7 Hz) at δ_H 7.97 (H-5) were observed as an ABX system, suggesting that the A ring was only functionalized at C-7 and that the other three oxygenated substituents were all present in ring B. In the ^{1}H−^{13}C HETCOR NMR spectrum, the peaks at δ_C 106.6 (C-2′ and C-6′) and δ_C 102.1 (C-8) all correlated to the same resonance at δ_H 6.87 (H-8, H-2′ and H-6′). In a 1D NOE NMR experiment on **1**, irradiation at δ_H 6.87 (H-2′ and H-6′) gave 6.9 and 5.3% NOE effects for the resonances at δ_H 8.32 (H-2) and δ 3.78 (OMe), respectively. Irradiation at δ_H 3.78 gave a 6.8% NOE effect at the δ_H 6.87 signal only. Therefore, the two OMe groups in the molecule of **1** could be tentatively assigned to the C-3′ and C-5′ positions, respectively. This inference was confirmed by a series of selective-INEPT NMR experiments, which are summarized in Table 1. The ring B ^{13}C-NMR chemical shifts of **1** were comparable with published values for those of a model compound with a symmetrical 4′-hydroxy-3′,5′-dimethoxy substituted B ring, pumilaisoflavone D.[18] Therefore, the structure of compound **1** was assigned as the novel compound 7,4′-dihydroxy-3′,5′-dimethoxyisoflavone.

Table 2. ^{1}H- and ^{13}C-NMR Assignments and HMBC Correlations for Compound **2** in $CDCl_3$[a]

position	δ_H (mult.)	δ_C	carbon signal correlated[b]
1		135.5	
2	7.61 m	128.4	C-β, C-4, C-3, C-1
3	7.39 m	128.9	C-5, C-4, C-1
4	7.39 m	130.1	C-6, C-5
5	7.39 m	128.9	C-4, C-3, C-1
6	7.61 m	128.4	C-β, C-5, C-4, C-1
1′		106.9	
2′		164.7	
3′	5.99 s	86.2	C-5′, C-4′, C-1′
4′		165.5	
5′		104.6	
6′		162.5	
2″	6.47 d (J = 6.2 Hz)	112.7	C-4′, C-5″, C-3″
3″	4.03 d (J = 6.2 Hz)	52.3	C-5′, C-4″
4″	5.43 s	79.8	C-2″, C-5″, C-5′
5″		87.9	
α	7.85 d (J = 15.5 Hz)	127.4	β′-C=O, C-β, C-1
β	7.78 d (J = 15.5 Hz)	142.4	β′-C=O, C-α, C-1
β′-C=O		193.0	
MeO-2′	3.94 s	56.2	
OAc	2.16 s	20.9	
OAc-4″		169.8	
Me_2	1.12 s	27.4	C-5″, C-4″, 23.2
	1.27 s	23.2	C-5″, C-4″, 27.4
OH	14.28 s		C-6′, C-1′, C-5′

[a] TMS was used as internal standard; chemical shifts are shown in the δ scale with J values in parentheses. [b] Data show carbon signals correlated to proton indicated.

A molecular formula of $C_{24}H_{24}O_7$ was determined by HREIMS (m/z 424.1512) for compound **2**. In the ^{1}H-NMR spectrum of this isolate (Table 2), matching doublets (J = 15.5 Hz) at δ_H 7.85 (H-α) and 7.78 (H-β) suggested that these protons were related to one another in a *trans*- arrangement. A carbonyl resonance at δ_C 193.0 (C-β′) could be assigned as the most downfield signal of the 24 carbon signals present in the ^{13}C-NMR spectrum of **2**. In the ^{1}H−^{13}C HMQC NMR

spectrum, the doublet at δ_H 7.78 (H-β) correlated to the carbon peak at δ_C 142.4, and a doublet at δ_H 7.85 (H-α) correlated to the carbon signal at δ_C 127.4. It was evident from both its ^{1}H- and ^{13}C-NMR spectra that a major structural unit of compound **2** was a chalcone unit in which ring B was unsubstituted.[7,19]

The remaining functional groups present in the molecule of **2** could be assigned as an OMe substituent (δ_C 56.2; δ_H 3.94), a hydroxyl group (δ_H 14.28), an acetate group (δ_C 169.8 and δ_C 20.9; δ_H 2.16) and a *gem*-dimethyl group (ν_{max} 1334−1234 cm^{-1}; δ_C 27.4, 23.2; δ_H 1.12, 1.27). The general structure of the remaining portion of the molecule of **2** was established as consisting of two substituted fused furan rings, by comparison of spectral data with literature values for (+)-purpurin (**3**),[7] a compound found together with **2** in the present investigation. Although (+)-purpurin (**3**) is a flavanone, two groups of investigators have reported that it undergoes deacetylation under mild alkaline conditions to produce a chalcone that retained the positive optical rotation ([α]$_D^{20}$ +157.4°) found in the parent compound ([α]$^{20}_D$ +20.0°).[7,28] The fact that **2** also exhibited a positive optical rotation ([α]$^{20}_D$ +61.0°) was suggestive that it exhibits the same stereochemistry as (+)-purpurin in the portion of the molecule constituted by the two fused furan rings.[7] The H-2″ and H-3″ protons were mutually coupled (J = 6.2 Hz), and experiments supported their occurrence in a *cis*- and α-orientation. Thus, a 15.7% NOE effect was observed at δ_H 4.03 (H-3″) with irradiation of H-2″ at δ_H 6.47, while irradiation at δ_H 4.03 (H-3″) gave a 20.2% NOE at δ_H 6.47 (H-2″). However, irradiation at δ_H 5.43 (H-4″) did not produce NOE effects, consistent with H-4″ being of β stereochemistry, as in the case of (+)-purpurin.[7]

HMBC and HMQC NMR experiments conducted in $CDCl_3$ (Table 2) allowed the complete assignment of the ^{1}H- and ^{13}C-NMR spectra of **2**. In addition, the presence of a *gem*-dimethyl group at C-5″ in one of the furan rings was supported by the HMBC spectrum. Thus, the resonances of the Me-5″ groups at δ_H 1.12 and 1.27 showed cross peaks with signals at δ_C 87.9 (C-5″), 79.8 (C-4″), 23.2 (Me-4″ or Me-5″), and 27.4 (Me-4″ or Me-5″), respectively. Furthermore, the H-2″ signal at δ_H 6.47 exhibited connectivities with δ_C 87.9 (C-5″), 165.5 (C-4′), and 52.3 (C-3″), while the H-3″ signal at δ_H 4.03 demonstrated cross peaks with δ_C 79.8 (C-4″) and 104.6 (C-5′). The HMBC data supported the location of the OH proton (δ_H 14.28) at C-6′, because correlations were observed with signals for δ 162.5 (C-6′), 106.9 (C-1′), and 104.6 (C-5′). In an 1D NOE NMR experiment, the location of the OMe group of compound **2** could be established at C-2′ from the following observations. Irradiation at δ_H 3.94 (OMe) gave a 10.4% NOE enhancement for H-3′ at δ_H 5.99, and, in a 2D NOESY experiment, H-β at δ 7.78 showed a NOE with a signal at δ_H 7.61 (H-2). Therefore, the structure of compound **2** was assigned as (+)-5″,5″-dimethyl-4″-α-acetoxytetrahydrofurano(2″,3″-*b*)-dihydrofurano(4′, 5′-*h*)- 2′-methoxy-6′-hydroxychalcone, to which we have accorded the trivial name (+)-tephropurpurin.

Nine constituents from *Tephrosia purpurea* of known structure were identified as (+)-purpurin (**3**)[7], pongamol (**4**)[21], lanceolatin B (**5**)[22], (−)-maackiain (**6**)[23], (−)-3-hydroxy-4-methoxy-8,9-methylenedioxypterocarpan (**7**),[20] (−)-medicarpin (**8**),[24] 3′-methoxydaidzein,[15] desmoxyphyllin B,[25,26] and 3,9-dihydroxy-8-methoxycoumestan,[27] by comparison of their physical and spectroscopic data with literature values. For pongamol (**4**), the presently assigned ^{1}H- and ^{13}C-NMR data were somewhat different from those published previously,[21] and corrected values have been made in this present investigation using HMBC and HETCOR NMR experiments.

Table 3. Quinone Reductase-Inducing Activity of Compounds **1**−**8** and Sulforaphane[a]

compound	CD (μM)[b]	IC_{50} (μM)[c]	CI[d]
1	17.2	>63.7	>3.7
2	0.15	13.4	89.0
3	5.6	>50.7	>9.0
4	6.1	18.7	3.1
5	22.9	>76.3	>3.3
6	8.8	>70.4	>8.0
7	14.7	>63.7	>4.4
8	13.7	>74.0	>5.4
sulforaphane	0.43	11.0	25.0

[a] Assays were carried out with the Hepa 1c1c7 mouse hepatoma cell line as described in the Experimental Section. [b] CD = concentration to double QR activity (μM). [c] 50% cell survival after two days of incubation (μM). [d] Chemopreventive index (CI) = IC_{50}/CD.

The potential of compounds **1**−**8** to induce QR activity in Hepa 1c1c7 cells is summarized in Table 3. The known compounds 3′-methoxydaidzein, desmoxyphyllin B, and 3,9-dihydroxy-8-methoxycoumestan were not significantly active in this test system. Compounds **1**−**8** all significantly induced QR activity, with the observed concentration to double induction (CD) values ranging from 0.15 to 17.2 μM. Compound **2** was approximately threefold more active than sulforaphane, the positive control compound used for this assay, and a superior chemopreventive index (CI) value was obtained as a result of limited cytotoxicity. The remaining isolates also demonstrated favorable CI values, but their CD values were relatively high. Compound **2** is therefore considered a promising lead for further exploration as a cancer chemopreventive agent.

Experimental Section

General Experimental Procedures. Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter. UV spectra were obtained with a Beckman DU-7 spectrometer. CD measurements were performed using a JASCO-600 CD spectrometer. IR spectra were obtained with a Midac Collegian FT-IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were measured with TMS as internal standard, using a Varian XL-300 instrument operating at 300 MHz and 75.6 MHz, respectively. 1D NOE, ^{1}H−^{1}H COSY, and ^{1}H−^{13}C HETCOR NMR experiments were also performed on this instrument, using standard pulse sequences. Selective INEPT NMR experiments were conducted on a Nicolet NMC-360 spectrometer operating at 90.8 MHz. ^{1}H−^{13}C HMBC NMR experiments were conducted on a GE Omega 500 MHz spectrometer. EIMS and HREIMS were obtained on a Finnigan MAT 90 instrument.

Biological Assays for the Induction of Quinone Reductase (QR) with Cultured Mouse Hepatoma Cells. For the evaluation of plant extracts, fractions, and pure isolates as inducers of QR, cultured mouse Hepa 1c1c7 cells were used as described previously.[12,14]

In brief, 4000 cells were added to the wells of 96-well plates (200 μL/well) and incubated for 24 h. Following replacement of the medium, test compounds, dissolved in 10 μL of 10% DMSO, were added (eight serial twofold dilutions in a final concentration range of 0.15−20 μg/mL), and the plates were incubated for an additional 48 h. QR activity was determined by measuring the NADPH-dependent menadiol-mediated reduction of MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide] to a blue formazan. Protein was determined by staining with crystal violet in an identical set of test plates. Induction of QR activity was calculated from the ratio of specific enzyme activities of compound-treated cells in comparison with a solvent control, and CD values (μg/mL), representing the concentration to double induction, were generated. Compounds with CD values of <10 μg/mL were considered active. IC_{50} values (μg/mL) (half maximal inhibitory concentration of cell viability) were divided by CD values to obtain a chemopreventive index, IC_{50}/CD.[12]

Plant Material. Whole plants (flowering and fruiting parts) of *Tephrosia purpurea* Pers. were collected in January 1977, in India, and dried. These were stored at room temperature at the University of Illinois Pharmacognosy Field Station until the present investigation. A voucher specimen (accession number PR48512) has been deposited in the Medicinal Plant Resources Laboratory, Plant Genetics and Germplasm Institute, USDA, Beltsville, MD 20705.

Extraction and Isolation. The dried plant material (15.0 kg) was ground and extracted with MeOH (2 × 40 L) by maceration. The resultant extracts were combined and concentrated to 4 L *in vacuo* at 37 °C. The concentrated extract was partitioned with petroleum ether (2 × 4 L) to yield a petroleum ether-soluble syrup (205.2 g). The extract was then concentrated and suspended in H_2O (1.5 L) and partitioned with EtOAc (2 × 4 L) to give, on drying, 294.5 g of an EtOAc-soluble residue. Both the petroleum ether- and EtOAc-soluble extracts significantly induced QR activity with cultured Hepa 1c1c7 mouse hepatoma cells (CD values of <0.3 μg/mL).

Fractionation of the petroleum ether extract was initiated by column chromatography over Si gel as stationary phase using petroleum ether- and ethyl acetate (0−100%) mixtures as eluents, with a final wash with 100% MeOH, to afford 21 pooled fractions. From fraction 6, using petroleum ether−EtOAc (12:1) as eluent, pongamol (**4**, 4.37 g, 0.029% w/w) was obtained as a major constituent. The QR biological activities were found to be concentrated in fractions 7−12, with CD values of <0.15 μg/mL. Fraction 7 from this first column fractionation afforded (+)-purpurin (**3**, 45.0 mg, 0.0003% w/w), by eluting with petroleum ether−EtOAc (10:1). Repeated column chromatography of fraction 8, eluted from petroleum ether-EtOAc (8:1), on Si gel with $CHCl_3$−EtOAc (10:1) as solvent, afforded lanceolatin B (**5**, 65.0 mg, 0.00043% w/w). Further purification of fraction 9, eluted from petroleum ether and EtOAc (5:1), by passage over Sephadex LH-20 eluted with MeOH, resulted in the crystallization of compound **2** (8.5 mg, 0.00005% w/w) from the mother liquor. Fractions 11 and 12, eluted from the first chromatographic column with petroleum ether−EtOAc (3:1 and 2:1, respectively) were combined and purified over a Si gel column eluted with $CHCl_3$-EtOAc (0−50%) to give (−)-maackiain (**6**, 50.0 mg, 0.00033% w/w), (−)-3-hydroxy-4-methoxy-8,9-methylenedioxypterocarpan (**7**, 38.6 mg, 0.00025% w/w) [eluted with $CHCl_3$−EtOAc (25:1)], and (−)-medicarpin (**8**, 9.5 mg, 0.00006% w/w) [eluted with $CHCl_3$−EtOAc (10:1)].

The EtOAc fraction was purified using Si gel as stationary phase and eluted with $CHCl_3$ and $CHCl_3$−MeOH mixtures of increasing polarity (0−50%), to afford 10 pooled fractions, of which fractions 6−9 were the most active subfractions in the QR assay, having CD values of <0.8 μg/mL. Fractions 6 and 7, eluted with $CHCl_3$−MeOH (15:1 and 10:1), were combined and chromatographed on another Si gel column eluted with $CHCl_3$−MeOH mixtures of increasing polarity (0−50%), to afford, in turn, compound **1** (8.0 mg, 0.00016% w/w) and 3,9-dihydroxy-8-methoxycoumestan (50.0 mg, 0.001% w/w), using $CHCl_3$−MeOH (25:1) as mobile phase. Repeated column chromatography of combined fractions 6 and 7, afforded sequentially 3′-methoxydaidzein (12.0 mg, 0.00024% w/w) and desmoxyphyllin B (9.0 mg, 0.00018% w/w), using $CHCl_3$−MeOH (30:1) as eluent.

7,4′-Dihydroxy-3′,5′-dimethoxyisoflavone (1): off-white needles; mp 260−262 °C; UV (MeOH) λ_{max} (log ϵ) 234 (4.36), 249 (4.34), 270 (4.32), 310 sh (3.98) nm; IR (film) ν_{max} 3600−3400 (br), 2924, 2853, 2359, 2342, 1616, 1456, 1302 cm^{-1}; ^1H NMR (DMSO-d_6 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75.4 MHz), see Table 1; EIMS (70 eV) m/z $[M]^+$ 314 (100), 271 (12), 200 (16), 137 (23), 117 (17); HREIMS m/z 314.0798, calcd for $C_{17}H_{14}O_6$, 314.0786.

(+)-Tephropurpurin [(+)-5″,5″-dimethyl-4″α-acetoxytetrahydrofurano(2″,3″-*b*)-dihydrofurano(4′,5′-*h*)-2′-methoxy-6′-hydroxychalcone] (2): yellow needles; mp 182−183 °C; $[\alpha]^{20}_D$ +61.0° (*c* 0.097, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ): 215 (4.52), 342 (4.40) nm; CD-$[\theta]_{305}$ +3089, $[\theta]_{252}$ −2179, $[\theta]_{233}$ +7631, $[\theta]_{227}$ +2722; IR (film) ν_{max} 3420, 2986, 2916, 2361, 1734, 1635, 1554, 1437, 1334, 1234, 1089 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75.4 MHz), see Table 2; EIMS (70 eV) m/z $[M]^+$ 424 (63), 364 (100), 349 (32), 321 (61), 260 (36), 245 (40), 217 (51), 131 (31), 103 (24); HREIMS m/z calcd for $C_{24}H_{24}O_7$, 424.1515, found 424.1512.

(+)-Purpurin (3): colorless needles; mp 147 °C (lit. 145−146 °C); $[\alpha]^{20}_D$ +23.3° (*c* 0.5, $CHCl_3$) [lit. $[\alpha]^{27}_D$ +20.3° (*c* 1.05, $CHCl_3$)]; CD $[\theta]_{327}$ +3871, $[\theta]_{302}$ −5769, $[\theta]_{251}$ −1055, $[\theta]_{242}$ −3298, $[\theta]_{230}$ +2634; UV, IR, ^1H- and ^{13}C-NMR data consistent with literature values.[7]

Pongamol (4): colorless crystals; mp 130−132 °C (lit. 135−136 °C); UV, IR, and EIMS consistent with literature values;[21] ^1H NMR ($CDCl_3$, 500 MHz) δ 4.13 (3H, s, OMe), 6.98 (1H, dd, J = 2.4, 1.0 Hz, H-3), 7.16 (1H, s, H-11), 7.29 (1H, dd, J = 8.8, 1.0 Hz, H-7), 7.48 (3H, m, H-15, H-16, and H-17), 7.60 (1H, d, J = 2.4 Hz, H-2), 7.86 (1H, d, J = 8.8 Hz, H-6), 7.97 (2H, m, H-14 and H-18), 16.90 (1H, s, OH-12); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 61.1 (OMe), 97.8 (C-11), 105.2 (C-3), 106.9 (C-7), 119.5 (C-9), 122.1 (C-5), 126.4 (C-6), 127.1 (C-15 and C-17), 128.6 (C-14 and C-18), 132.1 (C-16), 135.6 (C-13), 144.8 (C-2), 153.0 (C-4), 158.7 (C-8), 184.2 (C-12), 186.0 (C-10); corrected NMR assignments for **4** were made by analysis of HMBC and HETCOR spectra measured for this compound.

Lanceolatin B (5): amorphous powder from petroleum ether−EtOA, mp 136 °C (lit. 137 °C); UV, IR, ^{1}H and ^{13}C NMR, and EIMS, consistent with literature values.[22]

(−)-Maackiain (6): amorphous; mp 162 °C; $[\alpha]^{20}_D$ −175.0° (*c* 0.11, $CHCl_3$) [(lit. $[\alpha]^{21}_D$ −177.0° ($CHCl_3$)]; UV, IR, ^{1}H and ^{13}C NMR, and EIMS, consistent with literature values.[23]

(−)-3-Hydroxy-4-methoxy-8,9methylenedioxypterocarpan (7): white needles; mp 159−160 °C (lit. 156−159 °C); $[\alpha]^{20}_D$ −166.6° (*c* 0.12, $CHCl_3$); UV, IR, ^{1}H and ^{13}C NMR, and EIMS, consistent with literature values.[20]

(−)-Medicarpin (8): amorphous; mp 193 °C (lit. 195 °C); $[\alpha]^{20}_D$ −188.0° (*c* 0.106, $CHCl_3$) [lit. $[\alpha]^{21}_D$ −188.5° ($CHCl_3$)]; UV, IR, ^{1}H and ^{13}C NMR, and EIMS, consistent with literature values.[24]

3′-Methoxydaidzein: light yellowish needles; mp 244−246 °C (lit. 250−252 °C); UV, IR, ^{1}H and ^{13}C NMR, and EIMS, consistent with literature values.[15]

Desmoxyphyllin B: amorphous powder; mp >300 °C (lit. 285−288 °C); UV, IR, ^{1}H and ^{13}C NMR, and EIMS, consistent with literature values.[25,26]

3,9-Dihydroxy-8-coumestan: colorless needles; mp 290−293 °C; UV, IR, ^{1}H NMR, and EIMS, consistent with literature values.[27]

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