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Full Papers

Isolation and Structure Elucidation of an Isoflavone and a Sesterterpenoic Acid from *Henriettella fascicularis*

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A new isoflavone, 4',5,7-trihydroxy-6,8-dimethylisoflavone (**1**), and a new sesterterpenoic acid (**2**), together with five known compounds, lichexanthone (**3**), (–)-pinoresinol (**4**), betulinic acid, palmitic acid, and β -sitosterol, were isolated from a dichloromethane extract of the branches of *Henriettella fascicularis*. Their structures were established by extensive spectroscopic methods. An attempt to determine the absolute stereochemistry of (2*E*,6*S*)-6-[(1*R*,5*Z*,3*aS*,9*R*,10*Z*,12*aR*)-1,2,3,3*a*,4,7,8,9,12,12*a*-decahydro-9-hydroxy-3*a*,6,10-trimethylcyclopentanocycloundecen-1-yl]-2-methylhept-2-enoic acid (**2**) was performed by single-crystal X-ray analysis, using Cu K α radiation. Compound **1** showed significant competitive binding to estrogen receptor β and moderate antiestrogenic activity with cultured Ishikawa cells.

The genus *Henriettella* (Melastomataceae) comprises eight species in the exuberant Panamanian flora.¹ *Henriettella fascicularis* (Sw.) C. Wright, distributed mainly in the Canal Area, is also found in the eastern part of the province of Panama and in the province of Darien.² No previous phytochemical studies have been published on its chemical composition. Thus, the title plant was chemically investigated, and the new compounds 4',5,7-trihydroxy-6,8-dimethylisoflavone (**1**) and sesterterpenoic acid (**2**), as well as the known compounds lichexanthone, (–)-pinoresinol, betulinic acid, palmitic acid, and β -sitosterol, were characterized. The structure and the absolute configuration of **2** were confirmed by X-ray crystallographic analysis. In this report, we describe the isolation and structure determination of **1** and **2**. Estrogen receptor (ER) competitive binding

experiments revealed higher affinity of compound **1** for ER β than for ER α . In Ishikawa cells, when alkaline phosphatase was induced by treatment with estradiol, compound **1** mediated a decrease in activity, suggestive of an antiestrogenic effect.

Results and Discussion

The dried branches of *H. fascicularis* were extracted at room temperature with solvents of increasing polarity (dichloromethane and methanol). The dichloromethane extract was fractionated by a combination of silica gel column chromatography, RP-18 medium-pressure LC (MPLC), and gel filtration on Sephadex LH-20, to afford compounds **1**–**4**, together with the common compounds betulinic acid, palmitic acid, and β -sitosterol.

Compound **3** proved to be lichexanthone,^{3,4} by comparison of its spectral data with reported values. Although reports in the literature described the presence of lichexanthone in higher plants such as *Faramea cyanea* Muell. Arg. (Rubiaceae),⁵ *Zanthoxylum microcarpum* Griseb. (Ru-

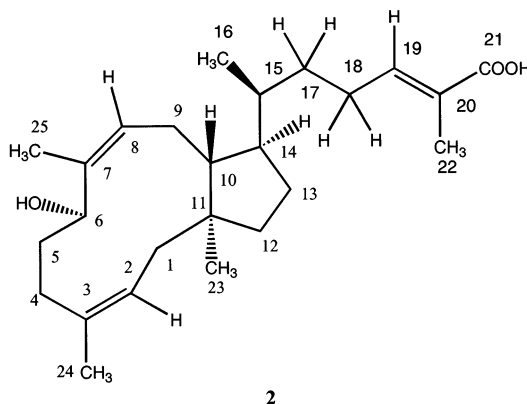
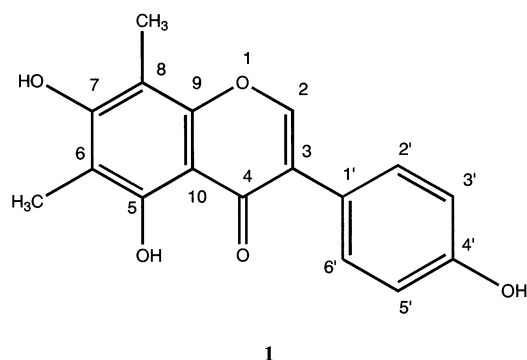
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taceae),⁴ *Zanthoxylum valens* (Macbr.) L. Williams (Rutaceae),⁴ *Zanthoxylum tetraspermun* Wight & Arn. (Rutaceae),⁶ and *Minquartia guianensis* Aubl. (Olacaceae),⁷ it is suspected that compound **3** originates from a lichen source, since epiphytes were observed on the branches of *H. fascicularis*.

The structure of compound **4** was established as (–)-pinoresinol, which has been isolated from only eight plant species (e.g., *Daphne tanguatica* Maxim. (Thymelaeaceae),⁸ *Zanthoxylum kellerianii* P. Wilson (Rutaceae),⁹ and *Festuca argentina* (Speg.) Par. (Gramineae)¹⁰). This is the first report of compound **4** in the Melastomataceae. Betulinic acid,¹¹ along with palmitic acid¹² and β -sitosterol,¹³ was identified by comparison of spectral data with published values.

The UV spectrum of **1** showed absorption maxima at 269 and 340 nm, suggesting an isoflavone type structure. The shift (269 \rightarrow 275 nm) of the maximum observed on addition of AlCl_3 indicated the presence of a 5-hydroxyl group.¹⁴ The molecular formula of **1** was established as $\text{C}_{17}\text{H}_{14}\text{O}_5$ by a combination of EIMS and ^1H and ^{13}C NMR spectra. The RDA fragments observed in the EI spectrum at m/z 180 and 118 indicated that two methyl and two hydroxyl groups were present on ring A and one hydroxyl group on ring B.^{15,16} The ^{13}C NMR spectrum showed 17 carbon signals, and a DEPT experiment indicated unequivocally the presence of nine quaternary carbons and five methine and two methyl groups (Table 1). A carbonyl function appeared at δ_{C} 180.5. This carbonyl group was hydrogen-bonded with a hydroxyl group at C-5, as evidenced by a proton signal at δ_{H} 13.13. Signals at δ_{H} 7.36, δ_{C} 130.1, δ_{H} 6.80, δ_{C} 115.0, and δ_{C} 156.5 indicated a disubstituted benzene ring (ring B). A characteristic singlet at δ_{H} 8.35 for H-2¹⁷ and chemical shifts at δ_{C} 153.8, 121.4, and 180.5, respectively, for C-2, C-3, and C-4 were attributed using HSQC¹⁸ and DEPT experiments. The position of the methyl groups of ring A was established through the observed HMBC

Table 1. ^1H and ^{13}C NMR Spectral Data for Compound **1**^a

position ^b	δ_{H} (ppm)	δ_{C} (ppm)
1		
2	8.35 (s, 1H)	
3		153.8
4		121.4
5		180.5
6		152.8
7		107.0
8		159.9
9		101.5
10		156.5
1'		104.3
2'	7.36 (d, 2H, J = 8 Hz)	121.8
3'	6.80 (d, 2H, J = 8 Hz)	130.1
4'		115.0
5'	6.80 (d, 2H, J = 8 Hz)	156.5
6'	7.36 (d, 2H, J = 8 Hz)	115.0
CH ₃	2.17	130.1
CH ₃	2.04	7.9
5-OH	13.13	8.0

^a Spectrum recorded at 500 MHz in $\text{DMSO}-d_6$ at 30 °C.

^b Numbering according to the name 4',5,7-trihydroxy-6,8-dimethylisoflavone.

correlations: CH_3 -8 and C-9, C-7, C-6; CH_3 -6 and C-7, C-5; H-2 and C-3, C-4, C-1'; H-3' and C-4'. On the basis of the EIMS and CIMS spectra, the methylation of **1** with CH_2N_2 in Et_2O suggested the formation of a trace amount of trimethylated product due to the presence of the ion peak at m/z 340 in both spectra. On the other hand, a ^1H NMR analysis of the methylate **1a** showed the appearance of two singlets at δ_{H} 3.76 and 3.79 corresponding to two methoxy groups located at positions C-7 and C-4', respectively. Moreover, these findings implied the presence of the third hydroxyl group at position C-5 which is hydrogen bonded to the carbonyl group at C-4. Finally, compound **1** was identified as 5,7-dihydroxy-3-(4-hydroxyphenyl)-6,8-dimethyl-4H-1-benzopyran-4-one or 4',5,7-trihydroxy-6,8-dimethylisoflavone and is a new natural product. This represents the first report of an isoflavone in the Melastomataceae.

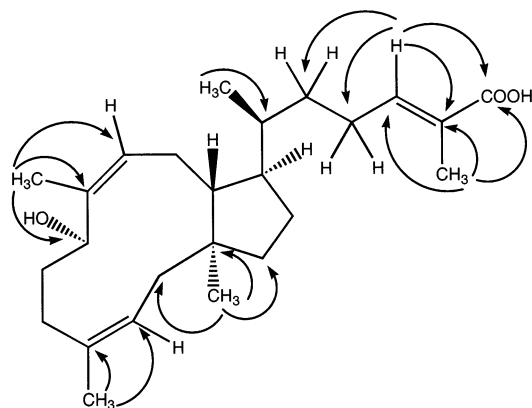
The HRESIMS of **2** displayed a molecular ion m/z 411.28754 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{25}\text{H}_{40}\text{O}_3 + \text{Na}^+$, 411.28697). Signals for all 25 carbons appeared in the ^{13}C NMR spectrum of **2**. The ^1H NMR spectrum of **2** displayed signals for three olefinic protons at δ_{H} 5.38 (H-8), 5.39 (H-2), and 6.88 (H-19); three methyl groups connected to an olefin at δ_{H} 1.65 (H₃-25), 1.78 (H₃-24), and 1.85 (H₃-22); one secondary methyl group at δ_{H} 0.89 (H₃-16); and one tertiary methyl group at δ_{H} 0.85 (H₃-23), while the other signals overlapped in the regions of δ_{H} 1.1–1.6 and δ_{H} 2.2–2.8. The assignments of the ^1H NMR and ^{13}C NMR signals summarized in Table 2 were attributed by HSQC and HMBC experiments. The methyl-bearing trisubstituted double bonds were readily positioned at C2–C3, C7–C8, and C19–C20 on the basis of HMBC experiments (Figure 1). The NOESY spectrum showed cross-peaks between H-2 and H₃-24, H₃-23, which indicated that the bridgehead methyl at C-11 and the methyl at the *Z*-trisubstituted olefin are oriented to one side of the molecule, while H-8 with H₃-25 are oriented to the opposite side of the molecule. The geometry of the trisubstituted olefin at C19–C20 was elucidated to be *E* from the NOESY correlations between H-18 and H₃-22.

To confirm the atomic arrangement and to establish other missing fragments of the macrocyclic ring of **2**, the complete structure and the absolute configuration were determined by a single-crystal X-ray diffraction analysis (Figure 2). Although from a purist's point of view the Flack parameter¹⁹ has an estimated standard deviation (esd) that

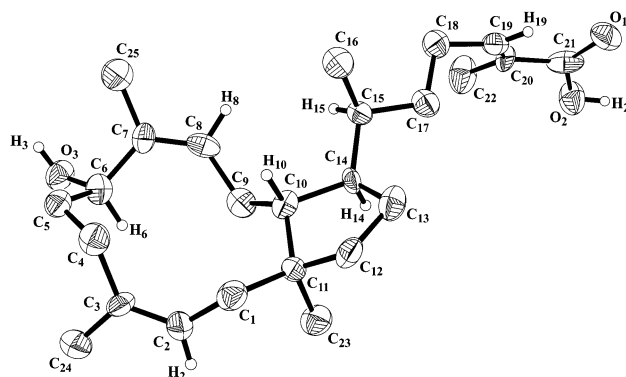
Table 2. ^1H and ^{13}C NMR Spectral Data for Compound **2**^a

position	δ_{H} (ppm) ^b	δ_{C} (ppm)
1	1.90 br s 2.25 m 5.39 t (8.3)	40.8
2		125.1
3		134.8
4	1.8 br s	22.7
5	1.75 m 1.85 m	31.4
6	4.41 d (10.8)	
7		68.0
8	5.38 t (14.2)	133.1
9	1.35 m 2.69 t (12.0)	129.8
10	1.30 m	28.2
11		51.0
12	1.25 m 1.35 m	53.3
13	1.35 m 1.50 m	41.6
14		20.5
15	1.75 br s	44.8
16	0.89 d (5.9)	32.9
17	1.35 m 1.35 m	13.2
18	2.15 m 2.25 m	35.5
19	6.88 ddd (1.5, 1.5, 1.0)	27.1
20		145.3
21		126.7
22	1.85 s	172.9
23	0.85 s	12.0
24	1.78 s	17.7
25	1.65 s	25.7
		16.9

^a Spectrum recorded at 500 MHz in CDCl_3 at 26 °C. ^b J values (in Hz) in parentheses.

**Figure 1.** HMBC correlations of compound **2**.

seems to be too large, the absolute configuration presented in Figure 2 is highly likely to be the right one. This compound possesses a very low enantiomorph discriminating¹⁹ capacity (as expressed in the rather high esd) and must therefore be considered as a limiting case. From the aforementioned data, **2** can be formulated as (2*E*,6*S*)-6-[(1*R*,5*Z*,3*aS*,9*R*,10*Z*,12*aR*)-1,2,3,3*a*,4,7,8,9,12,12*a*-decahydro-9-hydroxy-3*a*,6,10-trimethylcyclopentanocycloundecen-

**Figure 2.** ORTEP drawing of compound **2**.

1-yl]-2-methylhept-2-enoic acid. For clarity of data presentation, the numbering system shown in Figure 2 was used to assign the ^1H and ^{13}C NMR and crystallography data.

The basic bicyclic skeleton of compound **2** composed by a five- and an eleven-membered ring fused with a lateral side chain at C-14 was reported previously for albolineol, a compound isolated from the insect wax of *Ceroplastes albolineatus*.²⁰ Compound **2** and albolineol differ in the position of one double bond. In the structure of albolineol, the double bond is located at C-6, whereas compound **2** shows the double bond at C-7. Compound **2** represents the first report of this bicyclic sesterterpene in higher plants, particularly in the Melastomataceae. Unfortunately, a lack of information is presently in the literature regarding the taxonomic distribution of the sesterterpenes in plants and their role in the life cycle of a plant.

Due to epidemiological and experimental studies of isoflavones as phytoestrogens and their possible role in the prevention of breast and other estrogen-dependent cancers,^{21,22} compound **1** was tested for the ability to competitively bind to estrogen receptors α and β and to produce estrogenic or antiestrogenic activities in the Ishikawa (human endometrial adenocarcinoma) cell system. Compound **1** showed weak binding affinity to ER α , but with ER β , estradiol was displaced with an IC_{50} value of 0.88 μM . With Ishikawa cells, induction of alkaline phosphatase (AP), an estrogen-inducible marker enzyme, indicates an estrogenic response, whereas inhibition in the presence of estradiol represents an antiestrogenic effect. In this system, compound **1** mediated weak alkaline phosphatase (AP) induction, with an IC_{50} value of >20 μM . On the contrary, the compound appeared to be more efficient as a receptor antagonist, with an IC_{50} value of approximately 10 μM (Table 3). This response was greater than that observed with apigenin, which inhibited estrogen-induced alkaline phosphatase activity by 25% at a concentration of 20 μM .

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were determined on a Mettler FP 80/82 hot stage apparatus. UV spectra were recorded on a Perkin-Elmer Lambda 20 spectrophotometer. Optical rotations were determined on a Perkin-Elmer 241 MC polarimeter at 21° (Na, 589

Table 3. ER Binding and Alkaline Phosphatase Induction and Inhibition in Cultured Ishikawa Cells^a

compound	ER α binding IC_{50} , μM	ER β binding IC_{50} , μM	AP induction Ishikawa cells IC_{50} , μM	AP inhibition Ishikawa cells IC_{50} , μM
1	10.2 \pm 3.7	0.88 \pm 0.004	>20	10.5 \pm 5.0
genistein	0.3 \pm 0.01	0.018 \pm 0.002	0.51 \pm 0.1	
daidzein	17 \pm 2.5	1.2 \pm 0.0	1.2 \pm 0.6	

^a IC_{50} values are average values derived from triplicate experiments (\pm standard deviation).

nm). EIMS and DCIMS spectra were measured on a Finnigan-MAT/TSQ-700 triple-stage quadrupole instrument. HRESIMS in positive ion mode were recorded on a Bruker FTMS 4.7T. All NMR spectra were recorded at 499.87 MHz for ^1H and 125.7 MHz for ^{13}C on a Varian 500 Unity Inova spectrometer (Varian, Darmstadt, Germany); samples were dissolved in either DMSO- d_6 or CDCl_3 , and chemical shifts were given in ppm as δ relative to TMS (internal standard). Column chromatography was carried out with Si gel 60 (230–400 mesh; Merck) for normal-phase chromatography. Gel filtration chromatography was performed with Sephadex LH-20 (10–25 μM , Pharmacia), and medium-pressure liquid chromatography (MPLC) was run on a self-packed LiChroprep RP-18 column (15–25 μm , 450 mm \times 20 mm, Merck). TLC was performed on Si gel 60 F₂₅₄ Al sheets (Merck) using petroleum ether–EtOAc (1:1).

Plant Material. The first batch of branches of *Henriettella fascicularis* (Sw.) C. Wright was collected in June 1998, in Parque Nacional Soberanía, Panama, Republic of Panama, and identified by Prof. Mireya Correa. A voucher specimen (FLO-RPAN 3056) has been deposited in the Herbarium of the University of Panama, Panama, Republic of Panama. The second batch of *H. fascicularis* was collected in August 1999 in the same place. A voucher specimen (FLORPAN 4352) has also been deposited in the Herbarium of the University of Panama.

Extraction and Isolation. Dried branches of *H. fascicularis* (645 g) were ground and extracted (RT) with CH_2Cl_2 (3 \times 3 L) for 24 h. After solvent removal, 2.8 g of CH_2Cl_2 extract was obtained. The CH_2Cl_2 extract was chromatographed over Si gel 60 (60 \times 4.5 cm) using petroleum ether–EtOAc mixtures of increasing polarity. Elution by petroleum ether–EtOAc (90:10) afforded fractions containing compounds **3** and **6**, which were further purified by MPLC on a LiChroprep RP-18 column (450 \times 20 mm) using MeOH– H_2O (85:15) to yield compound **3** (2.8 mg, 0.00043% w/w) and 100% MeOH to obtain compound **6** (42.2 mg, 0.065% w/w), respectively. Elution by petroleum ether–EtOAc (80:20) afforded the fractions containing compound **5** and compound **7**. The separation of these compounds was carried out on a LiChroprep RP-18 column (450 \times 20 mm) by eluting with MeOH– H_2O (85:15) to afford compound **5** (25 mg, 0.0039% w/w) and 100% MeOH to afford compound **7**. Compound **7** (13 mg, 0.0020% w/w) was further purified by gel filtration on Sephadex LH-20 (45 \times 3 cm) using CHCl_3 –MeOH (50:50).

A second batch of branches of *H. fascicularis* (1.5 kg) was subjected to the same extraction procedure as described above. The CH_2Cl_2 extract (8 g) was subjected to Si gel open CC (60 \times 4.5 cm) using petroleum ether–EtOAc mixtures of increasing polarity. Elution by petroleum ether–EtOAc (60:40) afforded fractions containing compounds **1** and **2**, which were later separated on MPLC (450 \times 20 mm) with MeOH– H_2O (75:25) to obtain compound **1** (12.9 mg, 0.00086% w/w) and compound **2** (6 mg, 0.0004% w/w). Elution by petroleum ether–EtOAc (50:50) afforded a fraction containing compound **4**, which was purified by MPLC (450 \times 20 mm) with MeCN– H_2O (30:70) to yield compound **4** (7.3 mg, 0.00049% w/w).

4,5,7-Trihydroxy-6,8-dimethylisoflavone (1): pale yellow needles from MeOH; mp 253–254 $^\circ\text{C}$; UV λ_{max} (MeOH) nm (log ϵ) 269 (3.42) and 340 (3.54); + AlCl_3 275, 375; +NaOMe 284; +NaOAc 280, 345; ^1H and ^{13}C NMR data of **1**, see Table 1; EIMS m/z 298 [$\text{M}]^+$ (100), 268 (5), 180 (4), 151 (10), 149 (8), 118 (2); CIMS m/z 316 [$\text{M} + \text{NH}_4]^+$ (11), 299 (100); HRESIMS m/z 321.07340 [$\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{17}\text{H}_{14}\text{O}_5 + \text{Na}^+$, 321.07334).

Methylation of Compound 1. Compound **1** (3 mg) was dissolved in 5 mL of freshly prepared diazomethane (CH_2N_2 ; 35 mM in Et_2O). The mixture was shaken at room temperature for 24 h. The progress of the reaction was checked by TLC (petroleum ether–EtOAc, 50:50). After solvent removal, the methylate **1a** (3 mg) was dissolved in DMSO- d_6 to perform ^1H NMR experiments: EIMS m/z [$\text{M}]^+$ 340 (14), 326 (100), 310 (47), 307 (14), 296 (7), 163 (11); CIMS m/z 341 [$\text{M} + \text{H}]^+$ (18), 327 (100); ^1H NMR (500 MHz, DMSO- d_6) δ 13.0 (1H, s, OH-

5), δ 8.5 (1H, s, H-2), δ 7.5 (2H, d, J = 8 Hz, H-2', 6'), δ 7.0 (2H, d, J = 8 Hz, H-3', 5'), δ 3.79 (3H, s, OMe), δ 3.76 (3H, s, OMe), δ 2.2 (3H, s, Me), δ 2.1 (3H, s, Me); ^1H – ^1H NOESY correlations (500 MHz, DMSO- d_6) Me-6(8), OMe-7; H-3'(5'), OMe-4'.

Compound 2: colorless needles from MeOH; mp 95–96 $^\circ\text{C}$; [α]_D²⁵ –45.2 $^\circ$ (c 0.1, MeOH); UV λ_{max} (MeOH) nm (log ϵ) 210 (3.23); ^1H and ^{13}C NMR data of **2**, see Table 2; EIMS m/z 388.20 [$\text{M}]^+$ (25), 370.20 (82), 342.23 (25), 327.27 (12), 273.17 (12), 245.15 (14), 229.14 (27), 189.12 (23), 175.09 (26), 161.07 (42), 135.07 (51), 121.06 (65), 95.06 (93), 81.07 (100); HRESIMS m/z 411.28754 [$\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{25}\text{H}_{40}\text{O}_3 + \text{Na}^+$ 411.28697).

X-ray crystal structure analysis of compound 2: colorless joist-shaped crystals grown from MeOH; $\text{C}_{25}\text{H}_{40}\text{O}_3$, mol wt = 388.57, orthorhombic space group $P2_12_12_1$, a = 5.9941(12) Å, b = 14.864(3) Å, c = 26.194(5) Å, V = 2333.8(8) Å³, Z = 4, D_{calc} = 1.106 g cm^{–3}, Synchrotron radiation λ = 0.9002(5) Å. A specimen of 300 \times 40 \times 40 μm was used for the data collection that took place, at T = 293 K, on a MAR area detector at the Swiss-Norwegian Beamline at ESRF. A crystal–detector distance of 100 mm (θ_{max} = 23.25 $^\circ$) and a ϕ increment of 2 $^\circ$ were chosen. Using constant dose mode 180 images were exposed for 30 s each. A total of 23 867 measured reflections was reduced to 3144 unique ones (R_{int} = 0.0572). An absorption correction was deemed unnecessary. The structure was solved with the help of the SIR97 program²³ and refined by means of SHELXTL.²⁴ The final R_1 was 0.0585 (for 340 parameters). To obtain a more meaningful estimation of the absolute configuration, another data collection was carried out on a SMART 6000 system equipped with Cu K α radiation produced by a rotating anode. For technical reasons this measurement was limited to $2\theta_{\text{max}} \approx 70^\circ$. It yielded R_1 = 0.0299 and the extreme residuals were ± 0.10 e Å³. The values of Flack's parameter were 0.0(5) for one configuration and 1.3(5) for its mirror image. Crystallographic data of **2** reported in this paper have been deposited, under the CCDC number 194071, with the Cambridge Crystallographic Data Centre. Copies can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.ac.uk).

Lichexanthone (3): yellow crystals from MeOH; mp 165–166 $^\circ\text{C}$, lit. 187–190 $^\circ\text{C}$;³ UV λ_{max} (MeOH) nm (log ϵ) 250 (2.50) and 312 (2.28) nm; UV, ^1H NMR, and ^{13}C NMR data (recorded in CDCl_3), consistent with literature values.^{4,25}

(–)-Pinoresinol (4): white amorphous powder; [α]_D²⁵ –28.5 $^\circ$ (c 0.1, MeOH), lit. [α]_D²⁵ –34.7 (c 0.91, MeOH);⁸ UV λ_{max} (MeOH) nm (log ϵ) 230 (3.83) and 280 (3.41) nm; UV, ^1H NMR, and ^{13}C NMR data (recorded in CDCl_3), identical with the reported values.⁸

Estrogen Receptor (ER) Competitive Binding Assays.

The procedure of Obourn et al.²⁶ was used with minor modifications. Briefly, 24 h prior to the assay, a 50% v/v hydroxyapatite slurry was prepared using 10 g of hydroxyapatite in 60 mL of TE buffer (50 mM Tris-Cl, pH 7.4, 1 mM EDTA) and stored at 4 $^\circ\text{C}$. The ER binding buffer consisted of 10 mM Tris-Cl (pH 7.5), 10% glycerol, 2 mM dithiothreitol, and 1 mg/mL bovine serum albumin. The ER α wash buffer contained 40 mM Tris-Cl (pH 7.5), 100 mM KCl, and 1 mM EDTA, and the ER β buffer contained 40 mM Tris-Cl (pH 7.5). The reaction mixture consisted of 5 μL of test sample in DMSO, 5 μL of pure human recombinant diluted ER α or ER β (0.5 pmol) in ER binding buffer, 5 μL "Hot Mix" (400 nM, prepared fresh using 3.2 μL of 25 μM , 83 Ci/mmol [^3H] estradiol, 98.4 μL of ethanol, and 98.4 μL of ER binding buffer), and 85 μL of ER binding buffer. Following a 2 h incubation at room temperature, 100 μL of 50% hydroxyapatite slurry was added, and the tubes were incubated on ice for 15 min with vortexing every 5 min. ER wash buffer was added (1 mL), and the tubes were vortexed and then centrifuged at 2000g for 5 min. The supernatant was discarded, and this wash step was repeated three times. The hydroxyapatite pellet containing the ligand receptor complex was resuspended in 200 μL of ethanol and transferred to scintillation vials. Cytosint (4 mL/vial) was added, and the tubes were counted using a Beckman (Schaumburg, IL) LS 5801 liquid scintillation counter. The percent

inhibition of [^3H]estradiol bound to each ER was determined as follows: $[(\text{dpm}_{\text{sample}} - \text{dpm}_{\text{blank}})/(\text{dpm}_{\text{DMSO}} - \text{slope}_{\text{blank}}) - 1] \times 100$. The binding capability (percent) of the sample was calculated in comparison to that of estradiol (50 nM, 100%). The data represent the average \pm SD from triplicate experiments.

Cell Culture Conditions. The Ishikawa cell line was provided by R. B. Hochberg (Yale University, New Haven, CT). Ishikawa cells were maintained in Dulbecco's modified Eagle medium (DMEM)/F12 media with 10% heat inactivated FBS, sodium pyruvate (1%), penicillin-streptomycin (1%), and glutamax-1 (1%). One day prior to treating the cells, the medium was replaced with phenol red-free DMEM/F12 medium containing (10%) charcoal/dextran-stripped FBS to remove estrogens.

Induction and Inhibition of Alkaline Phosphatase (AP) with Cultured Ishikawa Cells. Ishikawa cells were used to evaluate the estrogenic and antiestrogenic properties of the isolated isoflavone as described previously by Pisha and Pezzuto.²⁷ Briefly, Ishikawa cells (5×10^4 /well) were incubated overnight with estrogen-free media in 96-well plates. Test samples in DMSO were added, and the cells in a total volume of 200 μL media/well were incubated at 37 $^{\circ}\text{C}$ for 4 days. For the determination of antiestrogenic activity, 2×10^{-8} M estradiol was added to the media. Enzyme activity was measured by reading the liberation of *p*-nitrophenol at 340 nm every 15 s for 16–20 readings with an ELISA reader (Power Wave 200 microplate scanning spectrophotometer, Bio-Tek Instrument, Winooski, VT). The maximum slope of the lines generated by the kinetic readings was calculated using a computer program. The percent induction for determination of estrogenic activity was calculated as $[(\text{slope}_{\text{sample}} - \text{slope}_{\text{cells}})/(\text{slope}_{\text{estrogen}} - \text{slope}_{\text{cells}})] \times 100$. For antiestrogenic activity, the percent induction was determined as $[(\text{slope}_{\text{sample}} - \text{slope}_{\text{cells}})/(\text{slope}_{\text{DMSO}} - \text{slope}_{\text{cells}})] \times 100$. The data represent the average \pm SD of triplicate determinations.

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Supporting Information Available: Tables of X-ray crystallographic data for compound **2** are available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- (1) D'Arcy, W. G. *Flora of Panama. Checklist and Index*; Missouri Botanical Garden: St. Louis, 1987.
- (2) Woodson, R. E.; Schery, R. W. S.; and collaborators. *Ann. Mo. Bot. Gard.* **1965**, *45*, 360–362.
- (3) Asahina, Y.; Nogami, H. *Bull. Chem. Soc. Jpn* **1942**, *17*, 202–207.
- (4) Jiménez, C.; Marcos, M.; Villaverde, M. C.; Riguera, R.; Castedo, L.; Stermitz, F. *Phytochemistry* **1989**, *28*, 1992–1993.
- (5) Ferrari, F.; Delle Monache, G.; Alves de Lima, R. *Phytochemistry* **1985**, *24*, 2753–2755.
- (6) Nissanka, A. P. K.; Karunarathne, V.; Ratnayake Bandara, B. M.; Kumar, V.; Nakanishi, T.; Nichi, M.; Inada, A.; Tillekeratne, L. M. V.; Wijesundara, D. S. A.; Gunatilaka, A. A. L. *Phytochemistry* **2001**, *56*, 857–861.
- (7) El-Seedi, H. R.; Hazell, A. C.; Torssell, K. B. G. *Phytochemistry* **1994**, *35*, 1297–1299.
- (8) Lin-gen, Z.; Seligmann, O.; Jurcic, K.; Wagner, H. *Planta Med.* **1982**, *45*, 172–176.
- (9) Marcos, M.; Jiménez, C.; Villaverde, M. C.; Riguera, R.; Castedo, L.; Stermitz, F. *Planta Med.* **1990**, *56*, 89–91.
- (10) Casabuono, A. C.; Pomilio, A. B. *Phytochemistry* **1994**, *35*, 479–483.
- (11) Mahato, S. B.; Kundu, A. P. *Phytochemistry* **1994**, *37*, 1517–1575.
- (12) Lide, D. R. *Handbook of Chemistry and Physics*; CRC Press LLC: Boca Raton, 1997; pp 3–183.
- (13) Iribarren, A. M.; Pomilio, A. B. *Phytochemistry* **1984**, *23*, 2087–2088.
- (14) Markham, K. R. *Techniques of Flavonoid Identification*; Academic Press: London, 1982; pp 37–39, 44.
- (15) Mabry, T. J.; Markham, K. R. In *The Flavonoids*; Harborne, J. B., Mabry, T. J., Mabry, E., Eds.; Academic Press: New York, 1975; Vol. 1, Chapter 3, pp 97–100.
- (16) Hedin, P. A.; Phillips, V. A. *J. Agric. Food Chem.* **1992**, *40*, 607–611.
- (17) Markham, K. R.; Mabry, T. J. In *The Flavonoids*; Harborne, J. B., Mabry, T. J., Mabry, E., Eds.; Academic Press: New York, 1975; Vol. 1, Chapter 2, p 68.
- (18) Sree Rama Murthy, M.; Venkata Rao, E.; Ward, R. S. *Magn. Reson. Chem.* **1986**, *24*, 225–230.
- (19) Flack, H. D.; Bernardinelli, G. *J. Appl. Crystallogr.* **2000**, *33*, 1143–1148.
- (20) Rios, T.; Quijano, L.; Calderón, J. *Chem. Commun.* **1974**, 728–729.
- (21) Zand, R. S.; Jenkins, D. J. A.; Diamandis, E. P. *Breast Cancer Res. Tr.* **2000**, *62*, 35–49.
- (22) Nikov, G. N.; Hopkins, N. E.; Boue, S.; Alworth, W. L. *Environ. Health Persp.* **2000**, *108*, 867–872.
- (23) Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Burla, M. C.; Polidori, G.; Camalli, M.; Spagna, R. *SIR97*, A Package for Crystal Structure Solution by Direct Methods and Refinement; 1997.
- (24) Sheldrick, G. M. *SHELXTL 5.05*; Bruker Analytical X-Ray Instruments, Inc.: Madison, WI, 1996.
- (25) García, R. F.; Brown, K. S. *Phytochemistry* **1976**, *15*, 1093–1095.
- (26) Obourn, J. D.; Koszewski, N. J.; Nortides, A. C. *Biochemistry* **1993**, *32*, 6229–6236.
- (27) Pisha, E.; Pezzuto, J. M. *Methods Cell Sci.* **1997**, *19*, 31–43.

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