

Prenylated Flavonoids from the Root Bark of *Berchemia discolor*, a Tanzanian Medicinal Plant

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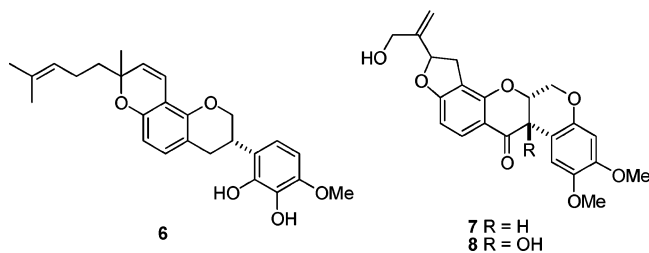
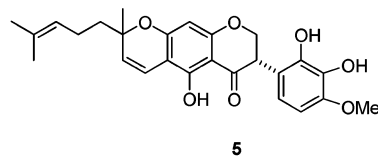
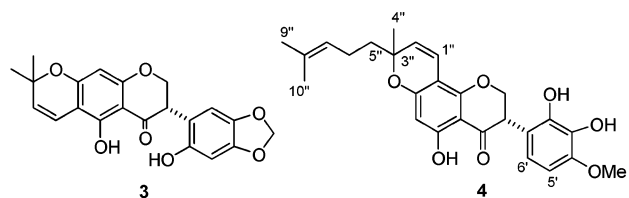
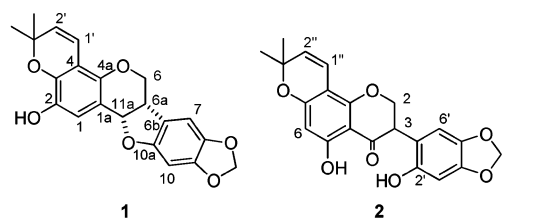
Five new prenylated flavonoids (**1–5**) were isolated from the root bark of *Berchemia discolor*, collected in Tanzania, along with 10 known compounds, by bioactivity-guided fractionation. The structures of compounds **1–5** were elucidated using various spectroscopic techniques. Of these isolates, compound **4**, and the known compounds nitidulin (**6**), amorphigenin (**7**), and dabinol (**8**), exhibited cytotoxic activity when evaluated against a small panel of human cancer cells. Nitidulin (**6**) was further tested in an in vivo hollow fiber assay and found to be active against LNCaP (human hormone-dependent prostate cancer) cells implanted intraperitoneally, at doses of 10, 20, and 40 mg/kg.

The species *Berchemia discolor* (Klotzsch) Hemsl. (Rhamnaceae), distributed in Africa and the Arabian peninsula, is a shrub or small tree.^{1,2} The fruits are edible, and the leaves are used to make beverages. Also, this plant is a good source of timber and of a dye material.³ Ethnobotanically, an aqueous extract of the stem bark of *B. discolor* is boiled with the whole roots of *Cordia crenata* Delile (Boraginaceae) and *Tamarindus indica* L. (Caesalpinaceae) and administered in divided doses to treat malaria in Tanzania.⁴

There have been no previous investigations on the bioactive secondary metabolites of *B. discolor*. As part of a systematic search for anticancer agents of plant origin,⁵ the root bark of this species, collected in Tanzania, where it is known as “mukuni”, was selected for activity-guided fractionation, following an initial screen of a CHCl₃-soluble extract using the LNCaP (hormone-dependent human prostate cancer) cell line. Bioactivity-guided fractionation of this extract using this same cell line led to the isolation of five new prenylated flavonoids (**1–5**) and 10 known compounds. Herein, the structure elucidation of these new substances and their biological evaluation as potential anticancer agents are described.

The structures of the known compounds were identified by physical and spectroscopic data measurement ([α]_D, CD, ¹H NMR, ¹³C NMR, 2D NMR, and MS) and by comparing the data obtained with published values, as nitidulin (**6**),⁶ amorphigenin (**7**),⁷ dabinol (**8**),⁷ heminitidulan,⁶ 3-hydroxy-4'-*O*-methylglabridin,⁸ 4'-hydroxy-cabenegegrin A-I,⁹ leiocarpin,⁶ leiocin,⁶ leiocinol,⁶ and nitidulan.⁶

Compound **1** was isolated as an amorphous solid, and its molecular formula of C₂₁H₁₈O₆ was deduced from a sodiated molecular ion peak observed at *m/z* 389.0974 (calcd for C₂₁H₁₈O₆-Na⁺, 389.0996). In the ¹H NMR spectrum of **1**, the signals at δ _H 3.40 (m, H-6a), 3.55 (t, *J* = 10.8 Hz, H-6), 4.14 (dd, *J* = 10.8, 4.8 Hz, H-6), and 5.35 (d, *J* = 6.9 Hz, H-11a) were assigned to H-6a, H₂-6, and H-11a of a pterocarpan skeleton.¹⁰ When compared to the ¹H NMR chemical shifts of leiocarpin,¹⁰ which was also isolated and identified in the present investigation, the ¹H NMR spectroscopic data of the two compounds were similar, except that there was a singlet aromatic proton peak at δ _H 6.85 and a hydroxy group signal at δ _H 5.08 in the A ring of **1** instead of two *ortho*-coupled protons in leiocarpin. The position of a hydroxy group in **1** was inferred as C-2 of ring A by the observed HMBC correlations of



H-1 to C-11a (δ _C 78.8), C-2 (δ _C 139.5), C-3 (δ _C 140.4), and C-4a (δ _C 144.5) (Figure 1). The absolute configurations of C-6a and C-11a were determined to be *S* and *S*, respectively, based on a comparison of the CD curve of compound **1** with literature data.¹⁰ Thus, the new compound **1** was assigned structurally as (6a*S*,11a*S*)-2-hydroxy leiocarpin.

The HRESIMS of **2** provided a sodiated molecular ion peak at *m/z* 405.0936, corresponding to an elemental formula of C₂₁H₁₈O₇-Na. The ¹H NMR spectrum (Table 1) showed signals at δ _H 4.90 (dd, *J* = 12.0, 3.1 Hz, H-2a), 4.77 (dd, *J* = 12.0, 4.6 Hz, H-2b), and 3.98 (brt, *J* = 3.6 Hz, H-3), assignable to the C ring of an isoflavanone.¹¹ Also observed were a singlet peak at δ _H 5.99 (H-

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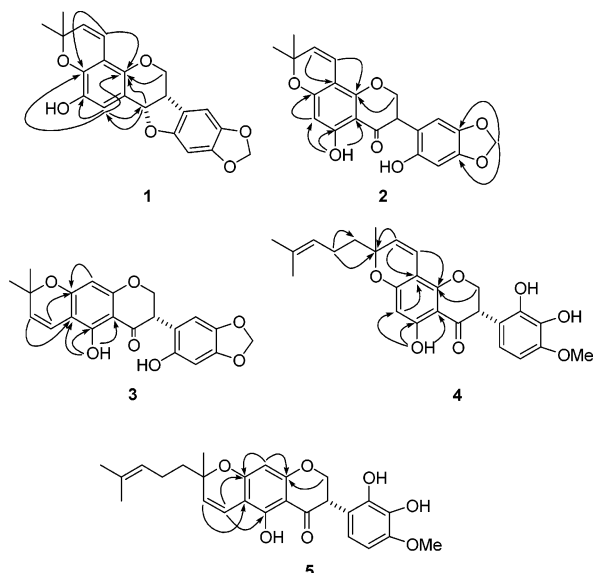


Figure 1. Selected HMBC correlations of 1–5.

Table 1. ^1H and ^{13}C NMR Chemical Shifts of Compounds 2 and 3 in CDCl_3

position	2		3	
	δ_{H} (J in Hz)	δ_{C} , mult. ^a	δ_{H} (J in Hz)	δ_{C} , mult. ^a
2	4.90, dd (12.0, 3.1)	69.8, CH ₂	4.83, dd (12.2, 3.1)	69.8, CH ₂
	4.77, dd (12.0, 4.6)		4.74, dd (12.2, 4.5)	
3	3.98, brt (3.6)	45.0, CH	3.96, brt (3.9)	45.1, CH
4		196.8, qC		196.8, qC
5		165.1, qC		159.5, qC
6	5.99, s	98.4, CH		103.6, qC
7		163.9, qC		163.7, qC
8		102.3, qC	5.99, s	96.7, CH
9		156.8, qC		162.5, qC
10		101.6, qC		101.5, qC
1'		114.9, qC		114.9, qC
2'		150.8, qC		150.7, qC
3'	6.57, s	100.8, CH	6.56, s	100.7, CH
4'		148.5, qC		148.5, qC
5'		142.5, qC		142.5, qC
6'	7.01, s	106.8, CH	7.02, s	106.8, CH
1''	6.63, d (10.1)	115.6, CH	6.58, d (10.1)	115.4, CH
2''	5.55, d (10.1)	127.1, CH	5.51, d (10.1)	126.8, CH
3''		79.0, qC		79.2, qC
4''	1.45, s	28.8, CH ₃	1.44, s	28.9, CH ₃
5''	1.47, s	29.0, CH ₃	1.46, s	28.9, CH ₃
OCH ₂ O	5.91, s	101.7, CH ₂	5.91, s	101.7, CH ₂
	5.93, s		5.92, s	
OH-5	11.72, s		11.89, s	

^a Multiplicity was deduced from the DEPT and HMQC spectra.

6), accounting for a pentasubstituted aromatic ring, and two singlet peaks at δ_{H} 6.57 (H-3') and 7.01 (H-6') of a 1,2,4,5-tetrasubstituted aromatic ring. Signals belonging to a 2,2-dimethylpyran ring were observed at δ_{H} 6.63 (1H, d, $J = 10.1$ Hz, H-1''), 5.55 (1H, d, $J = 10.1$ Hz, H-2''), 1.47 (3H, s, H-5''), and 1.45 (3H, s, H-4''), and two singlet peaks at δ_{H} 5.93 and 5.91 were assigned to a methylenedioxy group. An isoflavanone skeleton with a pyran ring was inferred from these data. The ^{13}C NMR, DEPT, and HMQC data supported the presence of an isoflavanone structure. The observed HMBC correlations from δ_{H} 11.72 (OH-5) to δ_{C} 98.4 (C-6), 101.6 (C-10), and 165.1 (C-5) enabled the pyran ring to be placed between C-7 and C-8. Furthermore, correlations of H-6 and C-7, H-2'' and C-8, and H-2 and C-9 supported the location of this pyran ring. The methylenedioxy group was positioned from the observed correlations between δ_{H} 5.93 and 5.91 to δ_{C} 142.5 (C-5') and 148.5 (C-4'). Since compound 2 exhibited a specific rotation of zero and no Cotton effects were observed in its CD spectrum, this compound was considered to be a racemate. Thus, the structure

Table 2. ^1H and ^{13}C NMR Chemical Shifts of Compounds 4 and 5 in CDCl_3

position	4		5	
	δ_{H} (J in Hz)	δ_{C} , mult. ^a	δ_{H} (J in Hz)	δ_{C} , mult. ^a
2	4.76, dd (11.1, 8.7)	70.1, CH ₂	4.72, dd (10.8, 8.4)	69.9, CH ₂
	4.62, dd (11.1, 5.7)		4.57, dd (10.8, 5.1)	
3	4.22, dd (8.7, 5.7)	45.9, CH	4.20, dd (8.4, 5.1)	46.1, CH
4		196.8, qC		196.8, qC
5		164.3, qC		158.9, qC
6	6.00, s	97.4, CH		102.9, qC
7		162.8, qC		162.7, qC
8		101.6, qC	5.94, s	95.8, CH
9		157.0, qC		162.7, qC
10		102.5, qC		102.5, qC
1'		115.0, qC		115.1, qC
2'		142.5, qC		142.5, qC
3'		133.4, qC		133.4, qC
4'		146.9, qC		146.9, qC
5'	6.50, d (8.1)	103.5, CH	6.49, d (8.7)	103.6, CH
6'	6.75, d (8.1)	119.3, CH	6.76, d (8.7)	119.3, CH
1''	6.63, d (10.2)	115.9, CH	6.65, d (10.2)	115.8, CH
2''	5.47, d (10.2)	125.2, CH	5.45, d (10.2)	124.9, CH
3''		80.7, qC		80.9, qC
4''	1.43, s	27.3, CH ₃	1.42, s	27.3, CH ₃
5''	1.68–1.72, m	41.8, CH ₂	1.68–1.72, m	41.8, CH ₂
6''	2.08, q (7.8)	22.7, CH ₂	2.09, q (7.7)	22.6, CH ₂
7''	5.11, brt (7.2)	123.7, CH	5.11, brt (7.4)	123.8, CH
8''		132.0, qC		131.9, qC
9''	1.60, s	17.7, CH ₃	1.59, s	17.6, CH ₃
10''	1.68, s	25.7, CH ₃	1.68, s	25.6, CH ₃
OH-5	12.12, s		12.30, s	
OCH ₃ -4'	3.89, s	56.2, CH ₃	3.88, s	56.2, CH ₃

^a Multiplicity was deduced from the DEPT and HMQC spectra.

of 2, named discoloranone A, was assigned as 5,2'-dihydroxy-3'',3''-methylenedioxy-3'',3''-dimethylpyrano[7,8]isoflavanone.

The molecular formula of 3 was found to be the same as 2 by the observed sodiated molecular ion peak at m/z 405.0950 (calcd for $\text{C}_{21}\text{H}_{18}\text{O}_7\text{Na}$, 405.0945) in the HRESIMS. The ^1H and ^{13}C NMR data of these two compounds were very similar, except for differences in the chemical shifts of C-5, C-6, C-8, and C-9 (Table 1). This implied that the pyran ring in 3 is located at a different position when compared to 2. The HMBC correlations of OH-5 to C-5, C-6, and C-9 and of H-2'' to C-6 were supportive of the attachment of the pyran ring to C-6 and C-7 instead of C-7 and C-8. Full assignments of ^1H and ^{13}C NMR chemical shifts were accomplished with the aid of DEPT, HMQC, and HMBC experiments. The absolute configuration of 3 at C-3 was determined to be *S* from the CD spectrum.¹⁰ All of these data helped finalize the structure of 3, named isodiscoloranone A, as (3*S*)-5,2'-dihydroxy-3'',4'-methylenedioxy-3'',3''-dimethylpyrano[6,7]isoflavanone.

A sodiated ion peak of 4 in the HRESIMS was observed at m/z 475.1719, accounting for an elemental formula of $\text{C}_{26}\text{H}_{28}\text{O}_7\text{Na}$. The ^1H NMR spectrum of 4 (Table 2) displayed characteristic signals of an isoflavanone at δ_{H} 4.76 (dd, $J = 11.1$, 8.7 Hz, H-2a), 4.62 (dd, $J = 11.1$, 5.7 Hz, H-2b), and 4.22 (dd, $J = 8.7$, 5.7 Hz, H-3) and gave evidence for the presence of a pyran ring and a prenyl group connected to the pyran ring. Two *ortho*-coupled signals at δ_{H} 6.50 (d, $J = 8.1$ Hz, H-5') and 6.75 (d, $J = 8.1$ Hz, H-6') were assigned to the B ring. Besides these signals, a hydroxy peak at δ_{H} 12.12 (OH-5) and an *O*-methyl peak at δ_{H} 3.89 were observed. The positions of the pyran ring and the prenyl group were confirmed by the HMBC correlations as shown in Figure 1. The 3J correlation of H-6'' (δ_{H} 2.08) to C-3'' (δ_{C} 80.7) suggested that the prenyl group was affixed to the pyran ring. The H-2 and H-1'' proton signals exhibited correlations with the same carbon signal (C-9) and enabled the pyran ring to be located between C-7 and C-8. The *O*-methyl resonance at δ_{H} 3.89 exhibited NOESY correlations with the proton at δ_{H} 6.50 (H-5'), which confirmed the position of the methoxy group at C-4' on the B ring. The absolute configuration of C-3 was determined to be *S* on the basis of the negative Cotton effect

Table 3. Cytotoxicity of Compounds from *B. discolor* against Cancer Cell Lines^{a,b}

compound	cell line ^c		
	Lu1	LNCaP	MCF-7
4	9.6	6.4	3.6
6	4.2	4.1	3.5
7	4.8	7.9	>20
8	3.1	5.4	13.1

^a Results are expressed as ED₅₀ values (μg/mL). ^b Compounds **1–3**, **5**, heminitidulan, 3-hydroxy-4'-*O*-methylglabridin, 4'-hydroxycabenegrin A-I, leiocarpin, leiocin, leiocinol, and nitidulan were inactive against all cell lines (ED₅₀ > 5.0 μg/mL). ^c Key: Lu1 (human lung carcinoma); LNCaP (hormone-dependent human prostate carcinoma); MCF-7 (human breast carcinoma).

at 312 nm in the CD spectrum.¹⁰ On the basis of the data obtained, the structure of compound **4** (discoloranone B) was elucidated as (3*S*)-5,2',3'-trihydroxy-4'-methoxy-3''-methyl-3''-(4-methylpent-3-enyl)pyrano[7,8]isoflavanone.

The molecular formula of compound **5** was assigned as the same as that of compound **4** by the observed sodiated ion peak in the HRESIMS, and the ¹H and ¹³C NMR data (Table 2) of these two compounds were almost identical. Differences were observed due to the different location of the pyran ring in an analogous manner to compounds **2** and **3**. The HMBC spectroscopic data displayed correlations of H-8 to C-9 and of H-2 to C-9 through two-bond and three-bond proton-carbon couplings, respectively. The *S* absolute configuration at C-3 was the same as that of compound **4** from the observed negative Cotton effect in the CD spectrum.¹⁰ Accordingly, the structure of compound **5** (isodiscoloranone B) was determined as (3*S*)-5,2',3'-trihydroxy-4'-methoxy-3''-methyl-3''-(4-methylpent-3-enyl)pyrano[6,7]isoflavanone.

All isolates obtained in this investigation from *B. discolor* root bark were evaluated for cytotoxicity against three human cancer cell lines (Lu1, LNCaP, and MCF-7). Among them, compounds **4** and **6–8** exhibited cytotoxic activity (ED₅₀ ≤ 5 μg/mL) for one or more cancer cell lines (Table 3). Since compound **6** was active in three human cancer cell lines and isolated in a reasonably large quantity, this compound was chosen for follow-up evaluation in an *in vivo* hollow fiber assay, which is used as a secondary discriminator bioassay in our program on the discovery of plant anticancer agents.¹² Hollow fibers containing either Lu1, LNCaP, or MCF-7 cells were propagated subcutaneously or within the peritoneum of immunodeficient mice. The animals were treated with vehicle or nitidulin (**6**) once daily by ip injection (10, 20, 40 mg/kg) from day 3–6 after implantation. On day 7, mice were sacrificed and fibers were retrieved and analyzed as described in the Experimental Section below. Nitidulin (**6**) inhibited the growth only of the LNCaP cell line (49–52%), propagated within the intraperitoneal site, at all doses tested. The compound was not active against any of the lines propagated subcutaneously (Figure S1, Supporting Information).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter. UV spectra were obtained with a Perkin-Elmer UV/vis spectrometer lambda 10. Circular dichroism (CD) spectra were recorded on a JASCO J-810 spectropolarimeter. IR spectra were run on an ATI Mattson Genesis Series FT-IR spectrophotometer. NMR spectroscopic data were recorded at room temperature on Bruker Avance DPX-300 and DRX-400 spectrometers with tetramethylsilane (TMS) as internal standard. Electrospray ionization (ESI) mass spectrometric analyses were performed with a 3-Tesla Finnigan FTMS-2000 Fourier transform mass spectrometer, and electron impact (EI) ionization was performed with a Kratos MS-25 mass spectrometer, using 70 eV ionization conditions. A SunFire PrepC₁₈-OBD column (5 μm, 150 × 19 mm i.d., Waters, Milford, MA) and a SunFire PrepC₁₈ guard column (5 μm, 10 × 19 mm i.d., Waters) were used for preparative HPLC, along with two Waters 515 HPLC pumps

and a Waters 2487 dual λ absorbance detector (Waters). Column chromatography was carried out with Purasil (230–400 mesh, Whatman, Clifton, NJ) and Sephadex LH-20 (Sigma, St. Louis, MO). Analytical thin-layer chromatography (TLC) was performed on pre-coated 250 μm thickness Partisil K6F (Whatman) glass plates, while preparative thin-layer chromatography was conducted on pre-coated 20 × 20 cm, 500 μm Partisil K6F (Whatman) glass plates.

Plant Material. The root bark of *B. discolor* was collected at Limbura Village, Urambo District, Tabora Region, Tanzania, in September 1999. The plant was identified by H. O. Suleiman, and a voucher specimen (collection number IMPP 002-0143) has been deposited at the Herbarium of the Institute of Traditional Medicine, Muhimbili University College of Health Science, Dar es Salaam, Tanzania.

Extraction and Isolation. The dried and milled root bark of *B. discolor* (1 kg) was extracted by maceration with MeOH three times at room temperature, for up to 2 days each, and then evaporated *in vacuo*. The dried MeOH extract (78 g) was suspended with a mixture of MeOH–H₂O (9:1, 400 mL) and partitioned sequentially with petroleum ether (3 × 200 mL) and CHCl₃ (3 × 200 mL).

The CHCl₃-soluble partition was washed with 1% saline solution and concentrated under a vacuum to yield a CHCl₃ extract (15.3 g), which exhibited cytotoxic activity (2.6 μg/mL) against the LNCaP cell line. The CHCl₃-soluble fraction (15.0 g) was chromatographed over a vacuum silica gel column, using a gradient of increasing polarity with CHCl₃ and acetone as solvents, and was fractionated into seven subfractions (F01–F07). Cytotoxic activity of these subfractions was monitored using the LNCaP cell line, and three fractions (F01, F02, and F03, 2.6, 3.9, and 3.5 μg/mL, respectively) were deemed to be active. Fraction F01 (323 mg) was subjected to column chromatography using Sephadex LH-20 with the solvent CH₂Cl₂–MeOH (2:1) and afforded seven subfractions (F0101–F0107). The constituents of subfraction F0107 were purified by HPLC. This separation was conducted with MeOH–H₂O (70:30), 7.0 mL/min, by isocratic elution for 20 min, then increasing from 70:30 to 100:0 for 30 min, and finally 100% MeOH for 15 min, to afford compounds **1** (*t*_R 27.7 min, 2.8 mg), 4'-hydroxycabenegrin A-I (*t*_R 36.8 min, 1.0 mg), leiocarpin (*t*_R 42.6 min, 70 mg), nitidulin (**6**, *t*_R 48.8 min, 45 mg), nitidulan (*t*_R 50.5 min), and heminitidulan (*t*_R 51.5 min, 0.8 mg). Fraction F02 (1.84 g) was subjected to column chromatography (55 × 150 mm) using Sephadex LH-20 with CH₂Cl₂–MeOH (2:1) as solvent to afford 13 subfractions (F0201–F0213). From subfraction F0212, leiocinol (*t*_R 20.7 min, 9 mg), **4** (*t*_R 46.7 min, 2.4 mg), **5** (*t*_R 47.7 min, 2.8 mg), a mixture of **2** and 3-hydroxy-4'-*O*-methylglabridin, and a mixture of **3** and leiocin were afforded. Compound **2** (*t*_R 63.0 min, 1.6 mg) and 3-hydroxy-4'-*O*-methylglabridin (*t*_R 60.0 min, 1.0 mg) were separated from this mixture by HPLC (MeCN–H₂O, 5:5, 7 mL/min). The mixture of compound **3** (*t*_R 28.1 min, 1.7 mg) and leiocin (*t*_R 31.8 min, 2.0 mg) was purified using HPLC (MeCN–MeOH–H₂O, 10:35:55, 7 mL/min). Fraction F03 (5.0 g) was subjected to a vacuum silica gel column using a gradient of increasing polarity with petroleum ether–acetone and gave seven subfractions (F0301–F0307). Amorphigenin (**7**, 126 mg) was precipitated from subfraction F0304. Subfraction F0306 was chromatographed over Sephadex LH-20 with MeOH and yielded dabinol (**8**, 280 mg).

(6a*S*,11a*S*)-2-Hydroxyleiocarpin (1): amorphous solid; [α]_D +111.4 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.36), 300 (3.85) nm; CD (MeOH, *c* 0.000 11 mol) [θ]₂₂₀ 112 780, [θ]₂₄₃ +121 164, [θ]₃₀₂ 0, [θ]₃₂₆ –47 100; IR (film) ν_{max} 3447, 2970, 2924, 1499, 1129 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ_H 6.85 (1H, s, H-1), 6.64 (1H, s, H-7), 6.54 (1H, d, *J* = 9.9 Hz, H-1'), 6.36 (1H, s, H-10), 5.83 (2H, s, OCH₂O), 5.51 (1H, d, *J* = 9.9 Hz, H-2'), 5.35 (1H, d, *J* = 6.9 Hz, H-11a), 5.08 (1H, s, OH), 4.14 (1H, dd, *J* = 10.8, 4.8 Hz, H-6), 3.55 (1H, t, *J* = 10.8 Hz, H-6), 3.40 (1H, m, H-6a), 1.38 (6H, s, H-4', H-5'); ¹³C NMR (CDCl₃, 75 Hz) δ_C 154.3 (C, C-10a), 148.1 (C, C-9), 144.5 (C, C-4a), 141.7 (C, C-8), 140.4 (C, C-3), 139.5 (C, C-2), 129.1 (CH, C-2'), 117.9 (C, C-6b), 116.8 (CH, C-1'), 115.1 (CH, C-1), 111.9 (C, C-1a), 110.3 (C, C-4), 104.7 (CH, C-7), 101.3 (CH₂, OCH₂O), 93.8 (CH, C-10), 78.8 (CH, C-11a), 77.0 (C, C-3'), 66.7 (CH₂, C-6), 40.5 (CH, C-6a), 27.9* (CH₃, C-4'), 27.8* (CH₃, C-5') (*assignments are interchangeable); HRESIMS *m/z* 389.0974 (calcd for C₂₂H₁₈O₆Na, 389.0996).

Discoloranone A (2): amorphous solid; [α]_D 0 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 271 (4.16), 297 (3.67) nm; CD (MeOH, *c* 0.000 14 mol) [θ] 0; IR (film) ν_{max} 3442, 2968, 2921, 1635, 1473, 1373,

1175 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) and ^{13}C NMR (CDCl_3 , 100 MHz), see Table 1; HRESIMS m/z 405.0936 (calcd for $\text{C}_{21}\text{H}_{18}\text{O}_7\text{Na}$, 405.0945).

(3S)-Isodiscoloranone A (3): amorphous solid; $[\alpha]_D -12.5$ (c 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 270 (4.17), 296 (3.81) nm; CD (MeOH, c 0.000 18 mol) $[\theta]_{214}$ 8919, $[\theta]_{241} +5230$, $[\theta]_{279}$ 0, $[\theta]_{307} -2677$; IR (film) ν_{max} 3442, 2964, 1637, 1483, 1175 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) and ^{13}C NMR (CDCl_3 , 100 MHz), see Table 1; HRESIMS m/z 405.0950 (calcd for $\text{C}_{21}\text{H}_{18}\text{O}_7\text{Na}$, 405.0945).

(3S)-Discoloranone B (4): amorphous solid; $[\alpha]_D +6.7$ (c 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 271 (4.14), 295 (sh) (3.77) nm; CD (MeOH, c 0.000 22 mol) $[\theta]_{269} +9356$, $[\theta]_{290}$ 0, $[\theta]_{312} -3739$; IR (film) ν_{max} 3442, 2968, 1647, 1457, 1374, 1162 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) and ^{13}C NMR (CDCl_3 , 100 MHz), see Table 2; HRESIMS m/z 475.1719 (calcd for $\text{C}_{26}\text{H}_{28}\text{O}_7\text{Na}$, 475.1727).

(3S)-Isodiscoloranone B (5): amorphous solid; $[\alpha]_D +13.2$ (c 0.19, MeOH); UV (MeOH) λ_{max} (log ϵ) 273 (4.35), 295 (sh) (3.96) nm; CD (MeOH, c 0.000 23 mol) $[\theta]_{270} +11\,633$, $[\theta]_{297}$ 0, $[\theta]_{311} -3882$; IR (film) ν_{max} 3442, 2968, 2924, 1644, 1456, 1386, 1164 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) and ^{13}C NMR (CDCl_3 , 100 MHz), see Table 2; HRESIMS m/z 475.1709 (calcd for $\text{C}_{26}\text{H}_{28}\text{O}_7\text{Na}$, 475.1727).

Cytotoxicity Assay. Fractions were tested in the LNCaP (hormone-dependent human prostate carcinoma) cell line, and all isolates were evaluated using the Lu1 (human lung carcinoma), LNCaP, and MCF-7 (human breast carcinoma) cancer cell lines using established protocols.^{13,14}

In Vivo Hollow Fiber Test. Compound **6** was evaluated in an in vivo hollow fiber model at doses of 10, 20, and 40 mg/kg, using Lu1, LNCaP, and MCF-7 cells.¹²

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Supporting Information Available: Data for compound **6** in an in vivo hollow fiber test. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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