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

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Abstract

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. (Caesalpiniaceae), 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 $CHCl_3$-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 ($[\alpha]_D$, CD, ^{1}H NMR, ^{13}C NMR, 2D NMR, and MS) and by comparing the data obtained with published values, as nitidulin (**6**),[6] amorphigenin (**7**),[7] dabinol (**8**),[7]

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heminitidulan,[6] 3-hydroxy-4′-*O*-methylglabridin,[8] 4′-hydroxycabenegrin 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 $C_{21}H_{18}O_6$ was deduced from a sodiated molecular ion peak observed at *m/z* 389.0974 (calcd for $C_{21}H_{18}O_6Na^+$, 389.0996). In the ^{1}H 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, H_2-6, and H-11a of a pterocarpan skeleton.[10] When compared to the ^{1}H NMR chemical shifts of leiocarpin,[10] which was also isolated and identified in the present investigation, the ^{1}H 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 (*6aS*, 11a*S*)-1-hydroxyleiocarpin.

The HRESIMS of **2** provided a sodiated molecular ion peak at *ml*z 405.0936, corresponding to an elemental formula of $C_{21}H_{18}O_6$Na. The ^{1}H 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-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 0to 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 of **2**, named discoloranone A, was assigned as 5,2′-dihydroxy-3′,4′-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 $C_{21}H_{18}O_7$Na, 405.0945) in the HRESIMS. The ^{1}H 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 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 ^{1}H 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 $C_{26}H_{18}O_7$Na. The ^{1}H 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″ (δc 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* based on the negative Cotton effect at 312 nm in the CD spectrum.[10] Based on 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 ^{1}H and ^{13}C 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 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 ($ED5_0 \leq 5$ 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, 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 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™ PrepC_{18}OBD™ column (5 μm, 150×19 mm i.d., Waters, Milford, MA) and a SunFire™ PrepC_{18} 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 precoated 250 μm thickness Partisil® K6F (Whatman) glass plates, while preparative thin-layer chromatography was conducted on precoated 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-H_2O (9:1, 400 mL) and partitioned sequentially with petroleum ether (3×200 mL) and $CHCl_3$ (3×200 mL).

The $CHCl_3$-soluble partition was washed with 1% saline solution and concentrated under a vacuum to yield a $CHCl_3$ extract (15.3 g), which exhibited cytotoxic activity (2.6 μg/ml) against the LNCaP cell line. The $CHCl_3$-soluble fraction (15.0 g) was chromatographed over a vacuum silica gel column, using a gradient of increasing polarity with $CHCl_3$ and acetone as solvents and was fractionated into seven sub-fractions (F01-F07). Cytotoxic activity of these sub-fractions 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 of CH_2Cl_2-MeOH (2:1) and afforded seven sub-fractions (F0101-F0107). The constituents of sub-fraction F0107 were purified by HPLC. This separation was conducted with MeOH-H_2O (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 CH_2Cl_2-MeOH (2:1) as solvent, and to afford 13 sub-fractions (F0201-F0213). From sub-fraction 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-H_2O, 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) were purified using HPLC (MeCN-MeOH-H_2O, 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 sub-fractions (F0301–F0307). Amorphigenin (**7**, 126 mg) was precipitated from sub-fraction F0304. Sub-fraction F0306 was chromatographed over Sephadex LH-20 with MeOH and yielded dabinol (**8**, 280 mg).

(6aS, 11aS)-2-hydroxyleiocarpin (**1**) was obtained as an amorphous solid: $[\alpha]_D$ +111.4 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.36), 300 (3.85) nm; CD (MeOH, *c* 0.00011 mol) $[\theta]_{220}$ 112780, $[\theta]_{243}$ +121164, $[\theta]_{302}$ 0, $[\theta]_{326}$ −47100; IR (film) ν_{max} 3447, 2970, 2924, 1499, 1129 cm^{-1}; ^1H NMR ($CDCl_3$, 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, OCH_2O), 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′); ^{13}C NMR ($CDCl_3$, 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 (CH_2, OCH_2O), 93.8 (CH, C-10), 78.8 (CH, C-11a), 77.0 (C, C-3′), 66.7 (CH_2, C-6), 40.5 (CH, C-6a), 27.9* (CH_3, C-4′), 27.8* (CH_3, C-5′) (*assignments are interchangeable); HRESIMS *m/z* 389.0974 (calcd for $C_{21}H_{18}O_6Na$, 389.0996).

Discoloranone A (2) was obtained as an amorphous solid: $[\alpha]_D$ 0 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 271 (4.16), 297 (3.67) nm; CD (MeOH, *c* 0.00014 mol) [θ] 0; IR (film) ν_{max} 3442, 2968, 2921, 1635, 1473, 1373, 1175 $cm^{-1;}$ ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; HRESIMS *m/z* 405.0936 (calcd for $C_{21}H_{18}O_7Na$, 405.0945).

(3*S*)-isodiscoloranone A (3) was obtained as an 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* 0.00018 mol) $[\theta]_{241}$ 8919, $[\theta]_{241}$ +5230, $[\theta]_{279}$ 0, $[\theta]_{307}$ − 2677; IR (film) ν_{max} 3442, 2964, 1637, 1483, 1175 $cm^{-1;}$ ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; HRESIMS *m/z* 405.0950 (calcd for $C_{21}H_{18}O_7Na$, 405.0945).

(3*S*)-discoloranone B (4) was obtained as an 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* 0.00022 mol) $[\theta]_{269}$ +9356, $[\theta]_{290}$ 0, $[\theta]_{312}$ − 3739; IR (film) ν_{max} 3442, 2968, 1647, 1457, 1374, 1162 $cm^{-1;}$ ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HRESIMS *m/z* 475.1719 (calcd for $C_{26}H_{28}O_7Na$, 475.1727).

(3*S*)-isodiscoloranone (5) was obtained as an 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* 0.00023 mol) $[\theta]_{270}$ +11633, $[\theta]_{297}$ 0, $[\theta]_{311}$ − 3882; IR (film) ν_{max} 3442, 2968, 2924, 1644, 1456, 1386, 1164 $cm^{-1;}$ ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HRESIMS *m/z* 475.1709 (calcd for $C_{26}H_{28}O_7Na$, 475.1727).

Cytotoxicity Assay

Fractions were tested in the LNCaP (hormone-dependent human prostate carcinoma) cell line and all isolates were evaluated in using the Lul (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 the in vivo hollow fiber model at doses of 10, 20, and 40 mg/kg, using Lu1, LNCaP, and MCF-7 cells.[12]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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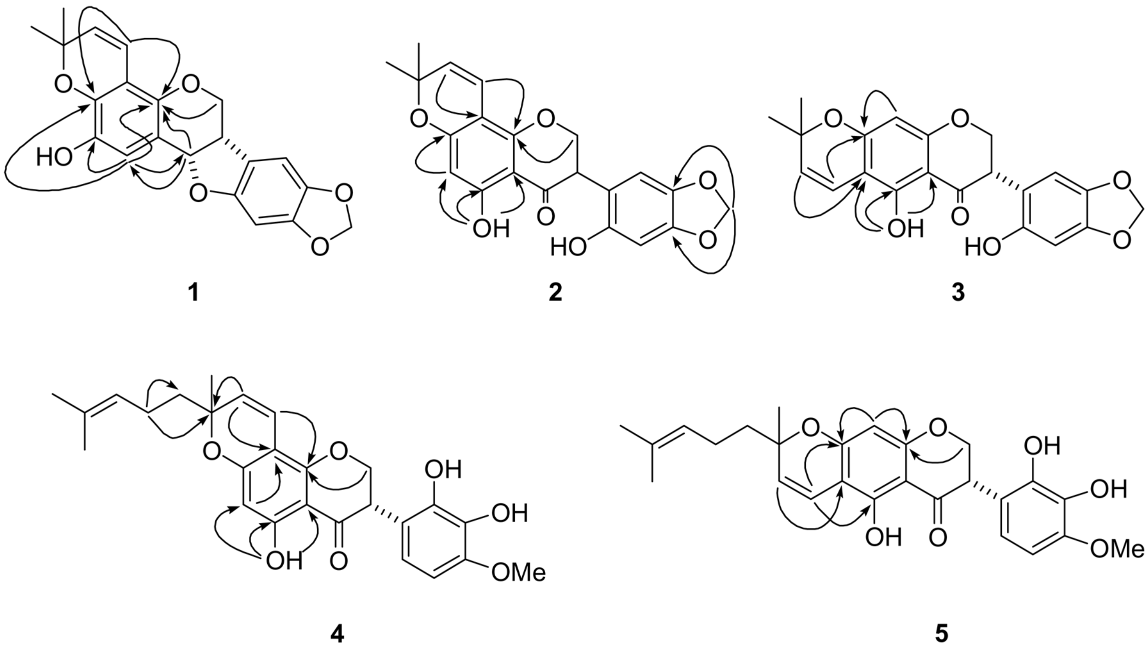
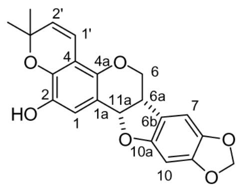


Figure 1.
Selected HMBC correlations of **1–5**.



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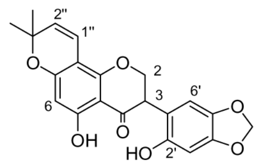

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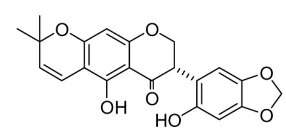

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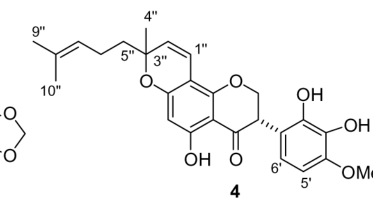

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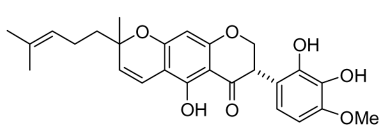

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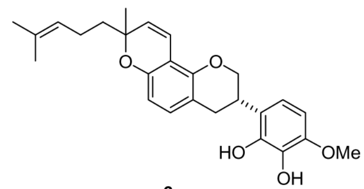

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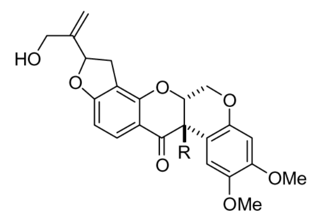

7 R = H
8 R = OH

Table 1

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

	2		**3**	
position	**δ_H(*J* inHz)**	**δ_C, mult.[a]**	**δ_H(*J* inHz)**	**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_2O	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.

Table 2

^{1}H and ^{13}C NMR Chemical Shifts of Compounds **4** and **5** in $CDCl_3$

	4		**5**	
position	**δ_H(*J* inHz)**	**δ_C, mult.[a]**	**δ_H(*J* inHz)**	**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.

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 ED_{50} 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 (ED_{50} >5.0 μg/mL).

[c]Key: Lu1 (human lung carcinoma); LNCaP (hormone-dependent human prostate carcinoma); MCF-7 (human breast carcinoma).