

Two Novel Norlignan Derivatives from *Curculigo breviscapa*

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Two novel skeleton-rearranged norlignans, breviscaside A (**1**) and breviscapin B (**2**), have been isolated from the rhizomes of *Curculigo breviscapa*. Their structures were established on the basis of spectroscopic techniques, especially the 2D NMR spectral analysis.

Key words: *Curculigo breviscapa*, Hypoxidaceae, Norlignan Derivatives, Breviscaside A, Breviscapin B

Introduction

The family Hypoxidaceae consists of the genera *Curculigo* and *Hypoxis* in China. Several species of both genera have been reported to contain mainly phenols, norlignans, cycloartane triterpene alcohols and their glycosides [1–8]. Plants of the genus *Curculigo* are known to be a rich source of norlignans possessing the Ph-C₅-Ph skeleton [2, 3, 7–11]. In our recent phytochemical studies on some species of the genus *Curculigo*, we also reported the isolation and structure elucidation of several skeleton-rearranged norlignans, including crassifogenin A–C, crassifoside A–F, crassifoside H, and curcapitoside [7, 12–15]. Aiming at finding novel secondary metabolites from another species of the genus *Curculigo*, we investigated the rhizomes of *Curculigo breviscapa*. From this plant two novel norlignan derivatives, breviscaside A (**1**) and breviscapin B (**2**), were isolated and identified to have rearranged skeletons derived from norlignan. In this paper, we present the isolation and structural elucidation of the two novel compounds.

Results and Discussion

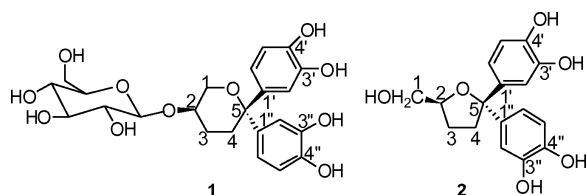
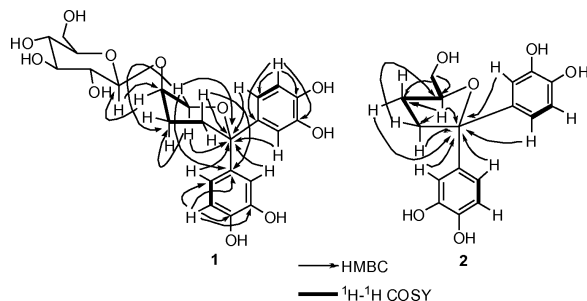
Breviscaside A (**1**) was obtained as a colorless powder. Its molecular formula of C₂₃H₂₈O₁₁ was established from HRMS ((–)-FAB) ([M–H][–], *m/z* = 479.153848, calcd. 479.155337) and ¹³C NMR (DEPT) data, indicating 10 degrees of unsaturation.

Table 1. ¹H NMR (400.13 MHz) and ¹³C NMR (100.62 MHz) data of **1** in CD₃OD^a.

Position	— 1 —		— 2 —	
	δ (C)	δ (H)	δ (C)	δ (H)
1	67.3 t	3.57 (m), 3.83 (m)	66.3 t	3.52 (dd, 11.2, 5.1) 3.58 (dd, 11.2, 5.9)
2	74.4 d	3.84 (m)	80.4 d	4.11 (m)
3	27.4 t	1.82 (m), 1.97 (m)	29.0 t	1.74 (m), 1.90 (m)
4	33.9 t	2.05 (m), 2.53 (m)	39.4 t	2.32 (m), 2.48 (m)
5	81.1 s		89.8 s	
1'	139.3 s		140.1 s	
2'	116.1 d	6.701 (overlap)	114.8 d	6.81 (d, 2.1)
3'	146.1 s		145.5 s	
4'	145.1 s		145.8 s	
5'	115.2 d	6.78 (br. s)	115.6 d	6.62 (d, 8.3)
6'	119.1 d	6.656 (overlap)	118.7 d	6.67 (overlap)
1''	138.3 s		139.3 s	
2''	116.0 d	6.661 (overlap)	115.1 d	6.79 (d, 2.1)
3''	145.3 s		144.88 s	
4''	146.3 s		144.90 s	
5''	115.7 d	6.82 (br. s)	115.7 d	6.66 (d, 8.3)
6''	119.8 d	6.699 (overlap)	118.5 d	6.68 (overlap)
Glucose				
1	103.1 d	4.36 (d, 7.5)		
2	75.3 d	3.15 (t, 9.0, 8.0)		
3	78.3 d	3.34 (m)		
4	71.9 d	3.26 (m)		
5	78.2 d	3.27 (m)		
6	63.0 t	3.64 (dd, 12.0, 5.0) 3.85 (overlap)		

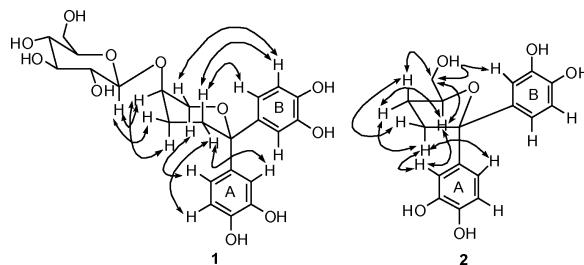
^a Chemical shift values δ in ppm, coupling constants *J* in Hz.

The IR spectrum showed absorptions of OH groups at 3418 cm^{–1}. The ¹H NMR spectrum (Table 1) exhib-

Fig. 1. The structures of compounds **1** and **2**.Fig. 2. The key HMBC and ^1H - ^1H COSY correlations for compounds **1** and **2**.

ited signals for three methylene protons [$\delta_{\text{H}} = 3.57$ (m, H-1b), 3.83 (m, H-1a), 1.82 (m, H-3b), 1.97 (m, H-3a), 2.05 (m, H-4b), 2.53 (m, H-4a)], one oxymethine proton [$\delta_{\text{H}} = 3.84$ (m, H-2)], and the protons of one glucosyl moiety [$\delta_{\text{H}} = 3.15\text{--}3.85$ (6H, Glc. H-2 $\rightarrow$ H-6)] except for one anomeric proton [$\delta_{\text{H}} = 4.36$ (d, $J = 7.5$ Hz, Glc H-1)]. The ^1H NMR spectrum of **1** also exhibited six aromatic protons assigned to H-2' [$\delta_{\text{H}} = 6.701$ (overlap)], H-5' [$\delta_{\text{H}} = 6.78$ (*br. s*)], H-6' [$\delta_{\text{H}} = 6.656$ (overlap)], H-2'' [$\delta_{\text{H}} = 6.661$ (overlap)], H-5'' [$\delta_{\text{H}} = 6.82$ (*br. s*)], and H-6'' [$\delta_{\text{H}} = 6.699$ (overlap)]. Analysis of the HMBC and ^1H - ^1H COSY spectra (Fig. 1) revealed that these were protons of two 1, 3, 4-trisubstituted aromatic rings. Analysis of the ^1H and ^{13}C NMR (Table 1) and HSQC spectra revealed that **1** contains two aromatic rings with four oxygen-bearing olefinic carbons [$\delta_{\text{C}} = 146.1$ (C-3'), 145.8 (C-4'), 145.3 (C-3''), and 146.3 (C-4'')] and eight olefinic carbons [$\delta_{\text{C}} = 115.2$ (C-5'), 115.7 (C-5''), 116.0 (C-2''), 116.1 (C-2'), 119.1 (C-6'), 119.8 (C-6''), 138.3 (C-1''), and 139.3 (C-1')], as well as three methylene carbons [$\delta_{\text{C}} = 67.3$ (C-1), 27.4 (C-3), and 33.9 (C-4)], one methine carbon [$\delta_{\text{C}} = 74.4$ (C-2)], and one quaternary carbon [$\delta_{\text{C}} = 81.1$ (C-5)].

The ^1H and ^{13}C NMR spectra indicated the presence of a glucosyl moiety. The anomeric proton signal appeared as a doublet at $\delta_{\text{H}} = 4.36$ ($J = 7.5$ Hz). Incorporating ^{13}C NMR chemical shifts it showed the presence of a β -D-glucosyl unit. All the carbons of

Fig. 3. The key NOESY correlations for compounds **1** and **2**.

the glucosyl moiety were assigned through direct ^1H - ^{13}C correlations in the HMQC spectrum and were located between $\delta_{\text{C}} = 63.0$ and 78.3 except for that at the anomeric position, which was assigned to the signal at $\delta_{\text{C}} = 103.1$.

The ^1H - ^1H COSY correlations (Fig. 2) of H-1/H-2, H-2/H-3, and H-3/H-4 showed the connectivity C(1)-C(2)-C(3)-C(4), which was further confirmed by HMBC correlations of H-1/C-3, and H-2/C-4. The HMBC correlations (Fig. 2) of H-3/C-5 and H-4/C-5 showed the linkage of C-4 to C-5. The linkage of C-2 and C-5 to an O-atom was established by the HMBC correlations of H-1/C-5 and the low-field chemical shift of C-1 and C-5, at $\delta_{\text{C}} = 67.3$ and 81.1, respectively (Table 1). Thus, a tetrahydro-2*H*-pyran ring moiety was established. The HMBC experiments showed the long-range couplings of H-2'/C-5, H-6'/C-5, H-2''/C-5, and H-6''/C-5, which suggested that the two 1, 3, 4-trisubstituted aromatic rings were connected with C-5. The long-range ^1H - ^{13}C correlations of Glc. H-1/C-2 and H-2/Glc. C-1 confirmed that the glucosyl moiety was linked to C-2.

By NOESY correlations (Fig. 3) of H-2/Glc. H-1, H-3b/Glc. H-1, H-3a/H-2, H-1b/H-5', H-4b/H-5', H-4b/H-6', H-1a/H-6'', H-4a/H-2'', and H-4a/H-5'', the β -D-glucosyl unit, aromatic ring A and aromatic ring B were assigned axial, axial, and equatorial positions, respectively. Thus, the stereochemistry of the β -D-glucosyl unit and the aromatic ring B were the β -orientation, and that of the aromatic ring A was the α -orientation. Therefore, the structure of **1** was deduced as a norlignan derivative, with a rearranged Ph-C₅-Ph skeleton, named breviscaside A (Fig. 1).

Breviscapin B (**2**), a colorless powder, had the molecular formula $\text{C}_{17}\text{H}_{18}\text{O}_6$ with 9 degrees of unsaturation, as deduced by HRMS ((-)-FAB) ($[\text{M}-\text{H}]^-$ at $m/z = 317.103155$; calcd. 317.102514) and ^{13}C NMR experiments (DEPT, Table 1). The IR spectrum showed the presence of OH groups (3384 cm^{-1}). The ^1H NMR

spectrum (Table 1) exhibited signals for three methylene protons [$\delta_{\text{H}} = 3.52$ (1H, dd, $J = 11.2, 5.1$ Hz, H-1a), 3.58 (1H, dd, $J = 11.2, 5.9$ Hz, H-1b), 1.74 (m, H-3b), 1.90 (m, H-3a), 2.32 (m, H-4b), 2.48 (m, H-4a)] and for one oxymethine proton [$\delta_{\text{H}} = 4.11$ (m, H-2)]. The ^1H NMR spectrum of **2** also exhibited six aromatic protons assigned to H-2' [$\delta_{\text{H}} = 6.81$ (d, $J = 2.1$ Hz)], H-5' [$\delta_{\text{H}} = 6.62$ (d, $J = 8.3$ Hz)], H-6' [$\delta_{\text{H}} = 6.67$ (overlap)], H-2'' [$\delta_{\text{H}} = 6.79$ (d, $J = 2.1$ Hz)], H-5'' [$\delta_{\text{H}} = 6.66$ (d, $J = 8.3$ Hz)], and H-6'' [$\delta_{\text{H}} = 6.68$ (overlap)]. Incorporating the analysis of the ^1H - ^1H COSY spectrum (Fig. 2) revealed that these were protons of two 1, 3, 4-trisubstituted aromatic rings. Analysis of the ^1H and ^{13}C NMR (Table 1) and HSQC spectra showed that **2** contains two aromatic rings with four oxygen-bearing olefinic carbons [$\delta_{\text{C}} = 145.5$ (C-3'), 145.8 (C-4'), 144.88 (C-3''), and 144.90 (C-4'')] and eight olefinic carbons [$\delta_{\text{C}} = 114.8$ (C-2'), 115.1 (C-2''), 115.6 (C-5'), 115.7 (C-5''), 118.5 (C-6''), 118.7 (C-6'), 139.3 (C-1''), and 140.1 (C-1')], as well as three methylene carbons [$\delta_{\text{C}} = 66.3$ (C-1), 29.0 (C-3), and 39.4 (C-4)], one methine carbon [$\delta_{\text{C}} = 80.4$ (C-2)], and one quaternary carbon [$\delta_{\text{C}} = 89.8$ (C-5)].

The ^1H - ^1H COSY correlations (Fig. 2) of H-1/H-2, H-2/H-3, and H-3/H-4 showed the connectivity C(1)-C(2)-C(3)-C(4), which was further confirmed by HMBC correlations of H-1/C-2, H-1/C-3, H-2/C-3, and H-2/C-4. The HMBC correlations (Fig. 2) of H-3/C-5 and H-4/C-5 showed the linkage of C-4 to C-5. The linkage of C-2 and C-5 to an O atom was established by the HMBC correlations of H-2/C-5 and the low-field chemical shift of C-2 and C-5, at $\delta_{\text{C}} = 80.4$ and 89.8 , respectively (Table 1). Thus, a tetrahydrofuran ring moiety was established. The HMBC experiments showed the long-range couplings of H-2'/C-5, H-6'/C-5, H-2''/C-5, and H-6''/C-5, which suggested that the two 3, 4-disubstituted aromatic rings were connected with C-5.

By NOESY correlations (Fig. 3) of H-1/H-2', H-1/H-3b, H-2/H-3a, H-2/H-2'', H-4a/H-6'', and H-4a/H-2'', the hydroxymethyl group, aromatic ring A and aromatic ring B were assigned equatorial, axial, and equatorial positions, respectively. Thus, the stereochemistry of the hydroxymethyl group and of the aromatic ring B were the β -orientation, and that of the aromatic ring A was the α -orientation. Therefore, the structure of **2** was deduced as a norlignan derivative, with a rearranged Ph-C₅-Ph skeleton, named breviscapin B (Fig. 1).

Experimental Section

General experimental procedures

Optical rotation was measured on a Horiba SEPA-300 polarimeter. A UV-2401PC spectrometer was used to obtain the UV spectrum in methanol (MeOH). The IR spectra were recorded on a Nexus 870-FT-IR spectrophotometer with KBr pellets. FAB-MS and FAB-HRMS were performed on a VG Autospec-3000 spectrometer. NMR spectra were measured on a Bruker AM-400 spectrometer with TMS as an internal standard. Column chromatography was carried out on Sephadex LH-20 gel (25–100 μm , Pharmacia Fine Chemical Co. Ltd.) and Chromatorex ODS (30–50 μm , Fuji Silysia Chemical Co. Ltd.). Thin-layer chromatography (TLC) was carried out on silica gel G precoated plates (Qingdao Haiyang Chemical Co. Ltd.). Spots were detected by spraying with 10 % H_2SO_4 in EtOH followed by heating.

Plant material

The plant material was collected in Napo, Guangxi Province, China, in August 2007 and identified by Prof. Kai-Jin Wang from the School of Life Sciences, Anhui University, where a voucher specimen (No. 20070801) was deposited.

Extraction and isolation

The air-dried and powdered rhizomes of *C. breviscapa* (1.3 kg) were extracted with 85 % EtOH (3×7 L) under reflux for 3 h. The combined organic layer was concentrated *in vacuo* to achieve a residue (52 g). The residue was suspended in H_2O and then passed through a D101 resin column eluted sequentially with water followed by 20 %, 40 %, 60 %, 80 %, and 95 % aqueous MeOH. The fraction eluted with 40 % MeOH (4.8 g) was purified by Sephadex LH-20 (MeOH- H_2O , 0:1–1:0) to yield three fractions (B₁–B₃). Fraction B₁ was subjected to further separation on ODS (MeOH- H_2O , 0:1–1:0) and then Sephadex LH-20 (EtOH- CH_3COCH_3 , 1:1) to afford **1** (27 mg) and **2** (14 mg).

Physical and spectroscopic data

Breviscaside A (1), colorless powder. – UV (MeOH): λ_{max} ($\lg \epsilon_{\text{max}}$) = 190 (4.28), 206 (4.64), 289 (3.95). – $[\alpha]_{\text{D}}^{25} = -57.0$ ($c = 0.11$, MeOH). – IR (KBr): $\nu = 3418$ (OH), 2928, 1610, 1520, 1438, 1362, 1286, 1262, 1203, 1076, 1032, 813, 781 cm^{-1} . – ^1H (400.13 MHz, CD_3OD , TMS) and ^{13}C NMR (100.62 MHz, CD_3OD): see Table 1. – MS ((–)-FAB): m/z (%) = 405 (30), 479 (100). – HRMS ((–)-FAB): m/z = 479.153848 (calcd. 479.155337 for $\text{C}_{23}\text{H}_{27}\text{O}_{11}$, $[\text{M}-\text{H}]^-$).

Breviscapin B (2), colorless powder. – UV (MeOH): λ_{max} ($\lg \epsilon_{\text{max}}$) = 195 (4.19), 206 (4.59), 288 (3.85) nm. – $[\alpha]_{\text{D}}^{27} =$

–7.1 ($c = 0.22$, MeOH). – IR (KBr): $\nu = 3384$ (OH), 2952, 1606, 1522, 1440, 1368, 1284, 1258, 1198, 1115, 1049, 879, 813, 780 cm^{-1} . – ^1H (400.13 MHz, CD_3OD , TMS) and ^{13}C NMR (100.62 MHz, CD_3OD): see Table 1. – MS ((–)-FAB): m/z (%) = 80 (6), 127 (7), 205 (7), 297 (10), 317 (100). – HRMS ((–)-FAB): $m/z = 317.103155$ (calcd. 317.102514 for $\text{C}_{17}\text{H}_{17}\text{O}_6$, $[\text{M}-\text{H}]^-$).

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