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-C_5-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 $C_{23}H_{28}O_{11}$ was established from HRMS ((–)-FAB) ([M–H]$^-$, *m/z* = 479.153848, calcd. 479.155337) and ^{13}C NMR (DEPT) data, indicating 10 degrees of unsaturation.

Table 1. ^{1}H NMR (400.13 MHz) and ^{13}C NMR (100.62 MHz) data of **1** in CD_3OD[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 ^{1}H NMR spectrum (Table 1) exhib-

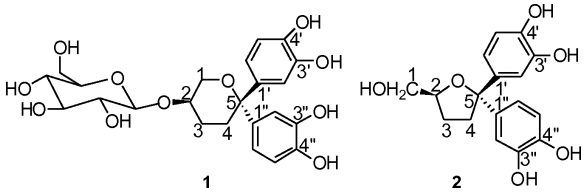

Fig. 1. The structures of compounds **1** and **2**.

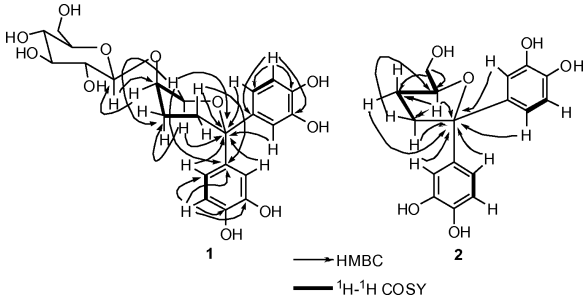

Fig. 2. The key HMBC and ^{1}H-^{1}H COSY correlations for compounds **1** and **2**.

ited signals for three methylene protons [δ_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 [δ_H = 3.84 (m, H-2)], and the protons of one glucosyl moiety [δ_H = 3.15 – 3.85 (6H, Glc. H-2 → H-6)] except for one anomeric proton [δ_H = 4.36 (d, J = 7.5 Hz, Glc H-1)]. The ^{1}H NMR spectrum of **1** also exhibited six aromatic protons assigned to H-2′ [δ_H = 6.701 (overlap)], H-5′ [δ_H = 6.78 (*br. s*)], H-6′ [δ_H = 6.656 (overlap)], H-2″ [δ_H = 6.661 (overlap)], H-5″ [δ_H = 6.82 (*br. s*)], and H-6″ [δ_H = 6.699 (overlap)]. Analysis of the HMBC and ^{1}H-^{1}H COSY spectra (Fig. 1) revealed that these were protons of two 1, 3, 4-trisubstituted aromatic rings. Analysis of the ^{1}H and ^{13}C NMR (Table 1) and HSQC spectra revealed that **1** contains two aromatic rings with four oxygen-bearing olefinic carbons [δ_C = 146.1 (C-3′), 145.8 (C-4′), 145.3 (C-3″), and 146.3 (C-4″)] and eight olefinic carbons [δ_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 [δ_C = 67.3 (C-1), 27.4 (C-3), and 33.9 (C-4)], one methine carbon [δ_C = 74.4 (C-2)], and one quaternary carbon [δ_C = 81.1 (C-5)].

The ^{1}H and ^{13}C NMR spectra indicated the presence of a glucosyl moiety. The anomeric proton signal appeared as a doublet at δ_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

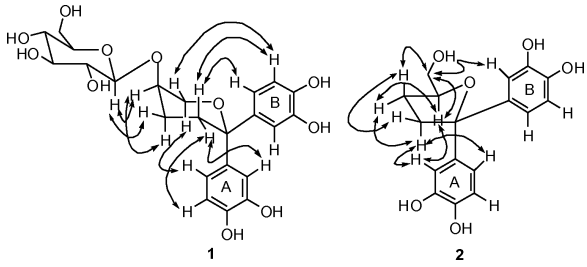

Fig. 3. The key NOESY correlations for compounds **1** and **2**.

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

The ^{1}H-^{1}H 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 δ_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 ^{1}H-^{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-C_5-Ph skeleton, named breviscaside A (Fig. 1).

Breviscapin B (**2**), a colorless powder, had the molecular formula $C_{17}H_{18}O_6$ with 9 degrees of unsaturation, as deduced by HRMS ((–)-FAB) ([M–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 ^{1}H NMR

spectrum (Table 1) exhibited signals for three methylene protons [δ_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 [δ_H = 4.11 (m, H-2)]. The ^{1}H NMR spectrum of **2** also exhibited six aromatic protons assigned to H-2′ [δ_H = 6.81 (*d*, J = 2.1 Hz)], H-5′ [δ_H = 6.62 (*d*, J = 8.3 Hz)], H-6′ [δ_H = 6.67 (overlap)], H-2″ [δ_H = 6.79 (*d*, J = 2.1 Hz)], H-5″ [δ_H = 6.66 (*d*, J = 8.3 Hz)], and H-6″ [δ_H = 6.68 (overlap)]. Incorporating the analysis of the ^{1}H-^{1}H COSY spectrum (Fig. 2) revealed that these were protons of two 1, 3, 4-trisubstituted aromatic rings. Analysis of the ^{1}H and ^{13}C NMR (Table 1) and HSQC spectra showed that **2** contains two aromatic rings with four oxygen-bearing olefinic carbons [δ_C = 145.5 (C-3′), 145.8 (C-4′), 144.88 (C-3″), and 144.90 (C-4″)] and eight olefinic carbons [δ_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 [δ_C = 66.3 (C-1), 29.0 (C-3), and 39.4 (C-4)], one methine carbon [δ_C = 80.4 (C-2)], and one quaternary carbon [δ_C = 89.8 (C-5)].

The ^{1}H-^{1}H 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 δ_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-C_5-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 (B_1–B_3). Fraction B_1 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} ($\lg \varepsilon_{max}$) = 190 (4.28), 206 (4.64), 289 (3.95). – $[\alpha]_D^{25}$ = −57.0 (c = 0.11, MeOH). – IR (KBr): ν = 3418 (OH), 2928, 1610, 1520, 1438, 1362, 1286, 1262, 1203, 1076, 1032, 813, 781 cm^{-1}. – ^{1}H (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 $C_{23}H_{27}O_{11}$, $[M–H]^-$).

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

−7.1 (c = 0.22, MeOH). – IR (KBr): ν = 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 $C_{17}H_{17}O_6$, $[M–H]^-$).

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