

## Chemical Constituents from Rhizomes of *Curculigo breviscapa*

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We have studied several species of the genus *Curculigo*, well known for their use in Chinese folk medicine, on their phytochemical and pharmacological characteristics.<sup>1-6</sup> *Curculigo breviscapa* S. C. Chen (Hypoxidaceae) is distributed in southwest region of Guangxi Province of China.<sup>7</sup> However, so far, the chemical constituents of this plant have not been reported. The interesting medicinal properties of this genus encouraged us to undertake the phytochemical investigation on *C. breviscapa*, which led to the isolation and identification of one novel phenolic compound, breviscapin A (**1**), together with five known compounds, crassifogenin B (**2**),<sup>2</sup> crassifoside A (**3**),<sup>2</sup> 3-hydroxy-5-methylphenol-1-*O*-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranoside] (**4**),<sup>8</sup> ocinol glucoside (**5**),<sup>9</sup> and 3,4-dihydroxybenzoic acid (**6**) from its rhizomes as shown in Figure 1. Their structures were established by spectroscopic analysis, especially using 2D-NMR techniques (<sup>1</sup>H-<sup>1</sup>H COSY, HMQC, HMBC) and comparisons of their data with literature values. All the compounds in Figure 1 were isolated for the first time from this plant. Herein, the structural elucidation of a novel phenolic compound **1** was provided.

### Experimental

**General experimental procedures.** UV-2401PC spectrometer was used to obtain the UV spectra in methanol (MeOH). IR spectra were recorded on Nexus 870-FT-IR spectrophotometer with potassium bromide (KBr) pellets. Mass spectrometry (MS) was performed on VG Autospec-3000 spectrometer and API-QSTAR-Pulsar-1 spectrometer using a positive ion model. 1D and 2D NMR spectra were measured on Bruker AM-400 spectrometer with TMS as an internal standard. Column chromatography was carried out on Sephadex LH-20 gel (25 - 100  $\mu$ m, Pharmacia Fine Chemical Co. Ltd.). Thin layer chromatography

(TLC) was carried out on silica gel G precoated plates (Qingdao Haiyang Chemical Co. Ltd.) and spots were detected by spraying with 5% H<sub>2</sub>SO<sub>4</sub> 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  $\times$  7 L) under reflux for 3 h. The combined organic layer was concentrated in *vacuo* to afford a residue (52 g). The residue was suspended in H<sub>2</sub>O and then passed through a *D101* resin column eluting sequentially with water followed by 20%, 40%, 60%, 80% and 95% aqueous MeOH. The fraction (8.5 g) eluted from 20% MeOH was repeatedly purified by Sephadex LH-20 chromatography (EtOH-H<sub>2</sub>O, 0:1-1:0) to yield four fractions (A<sub>1</sub>-A<sub>4</sub>). Fraction A<sub>1</sub> was subjected on Sephadex LH-20 chromatography (MeOH-H<sub>2</sub>O, 0:1-1:0, then EtOH-acetone, 1:1) to afford compounds **4** (18 mg) and **5** (13 mg). Compound **6** (17 mg) was obtained from fraction A<sub>2</sub> by column chromatography on Sephadex LH-20 (MeOH). Compounds **1** (5 mg) and **3** (9 mg) were obtained from fraction A<sub>3</sub> by column chromatography on Sephadex LH-20 (EtOH). The fraction A<sub>4</sub> was subjected to chromatography on Sephadex LH-20 (EtOH) repeatedly to yield compound **2** (11 mg).

**Breviscapin A (1).** Black powder, IR (KBr)  $\nu_{\max}$  3415 (OH), 1676 (C=O), 1605 (C=C), 1519, 1437, 1365, 1290, 1263, 1115, 818, 780, 761 cm<sup>-1</sup>; UV (MeOH)  $\lambda_{\max}$  (log $\epsilon$ ) 205 (4.60), 283 (3.87) nm. <sup>1</sup>H- and <sup>13</sup>C-NMR data see Table 1. HR-TOF-MS (positive mode)  $m/z$  337.0679 [M+Na]<sup>+</sup> (calcd. 337.0688 for C<sub>17</sub>H<sub>14</sub>O<sub>6</sub>Na).

**Crassifogenin B (2).** Pale yellow powder, <sup>1</sup>H-NMR (400 MHz, acetone-*d*<sub>6</sub>)  $\delta$  6.90 (1H, d,  $J$  = 2.8 Hz, H-2), 7.09 (1H, d,

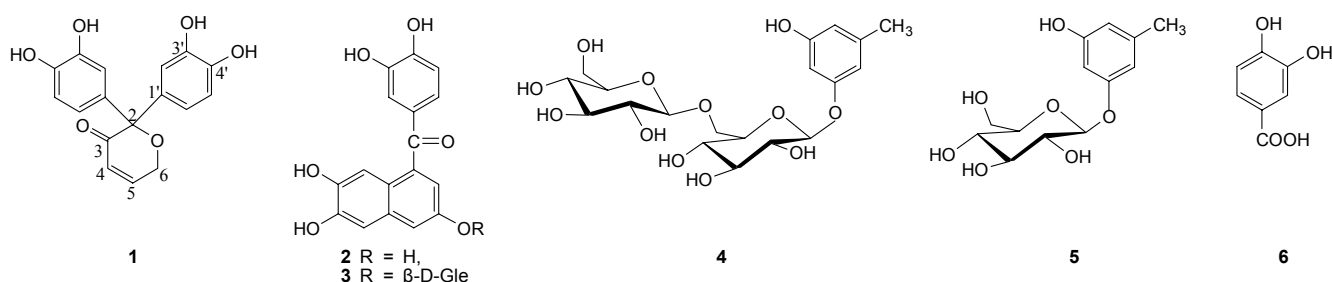


Figure 1. Structures of compounds 1-6.

$J = 2.4$  Hz, H-4), 7.07 (1H, s, H-5), 7.11 (1H, s, H-8), 7.33 (1H, d,  $J = 2.0$  Hz, H-2'), 6.87 (1H, d,  $J = 8.0$  Hz, H-5'), 7.18 (1H, dd,  $J = 8.0, 2.0$  Hz, H-6');  $^{13}\text{C-NMR}$  (100 MHz, acetone- $d_6$ )  $\delta$  197.5 (s, C=O), 139.0 (s, C-1), 117.6 (d, C-2), 154.0 (s, C-3), 112.1 (d, C-4), 110.5 (d, C-5), 146.7 (s, C-6), 148.9 (s, C-7), 109.8 (d, C-8), 123.2 (s, C-9), 133.5 (s, C-10), 132.5 (s, C-1'), 118.5 (d, C-2'), 146.3 (s, C-3'), 152.3 (s, C-4'), 116.7 (d, C-5'), 125.9 (d, C-6'); FAB-MS (+)  $m/z$  313 [M+H] $^+$ .

**Crassifoside A (3).** White amorphous powder,  $^1\text{H-NMR}$  (400 MHz, acetone- $d_6$ )  $\delta$  7.07 (1H, d,  $J = 2.4$  Hz, H-2), 7.40 (1H, d,  $J = 2.4$  Hz, H-4), 7.21 (1H, s, H-5), 7.17 (1H, s, H-8), 7.34 (1H, d,  $J = 2.0$  Hz, H-2'), 6.88 (1H, d,  $J = 8.4$  Hz, H-5'), 7.22 (1H, dd,  $J = 8.4, 2.0$  Hz, H-6'), 5.07 (1H, d,  $J = 7.2$  Hz, H-1''), 3.43-3.57 (4H, m, glc.H), 3.69 (1H, dd,  $J = 11.6, 5.6$  Hz, Ha-6'), 3.90 (1H, dd,  $J = 11.6, 1.6$  Hz, Hb-6');  $^{13}\text{C-NMR}$  (100 MHz, acetone- $d_6$ )  $\delta$  197.5 (s, C=O), 138.6 (s, C-1), 118.9 (d, C-2), 154.6 (s, C-3), 114.7 (d, C-4), 111.6 (d, C-5), 147.6 (s, C-6), 149.2 (s, C-7), 109.9 (d, C-8), 124.8 (s, C-9), 133.1 (s, C-10), 132.4 (s, C-1'), 118.9 (d, C-2'), 146.8 (s, C-3'), 152.7 (s, C-4'), 117.1 (d, C-5'), 126.1 (d, C-6'), 103.4 (d, C-1''), 72.6 (d, C-2''), 79.1 (d, C-3''), 75.9 (d, C-4''), 78.8 (d, C-5''), 63.9 (t, C-6''); FAB-MS (-)  $m/z$  473 [M-H] $^-$ .

**3-Hydroxy-5-methylphenol-1-O- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranoside (4).** White amorphous powder,  $^1\text{H-NMR}$  (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  2.26 (3H, s,  $\text{CH}_3$ ), 6.49 (1H, s, H-2), 6.35 (1H, s, H-4), 6.45 (1H, s, H-6), 4.89 (1H, d,  $J = 7.2$  Hz, H-1''), 4.46 (1H, d,  $J = 7.6$  Hz, H-1'');  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  21.7 (q,  $\text{CH}_3$ ), 160.0 (s, C-1), 102.0 (d, C-2), 159.1 (s, C-3), 111.3 (d, C-4), 141.3 (s, C-5), 110.0 (d, C-6), 102.0 (d, C-1'), 74.8 (d, C-2'), 77.8 (d, C-3'), 71.3 (d, C-4'), 77.3 (d, C-5'), 69.6 (t, C-6'), 104.5 (d, C-1''), 75.2 (d, C-2''), 77.3 (d, C-3''), 71.5 (d, C-4''), 77.8 (d, C-5''), 62.6 (t, C-6''); FAB-MS (-)  $m/z$  447 [M-H] $^-$ .

**Orcinol glucoside (5).** White amorphous powder,  $^1\text{H-NMR}$  (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  2.12 (3H, s,  $\text{CH}_3$ ), 6.33 (1H, s, H-2), 6.29 (1H, s, H-4), 6.21 (1H, s, H-6), 4.76 (1H, d,  $J = 6.8$  Hz, H-1'), 3.33-3.46 (4H, m, glc.H), 3.63 (1H, dd,  $J = 11.6, 2.4$  Hz, Ha-6'), 3.80 (1H, d,  $J = 12.0$  Hz, Hb-6');  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  21.7 (q,  $\text{CH}_3$ ), 160.0 (s, C-1), 102.2 (d, C-2), 159.1 (s, C-3), 109.8 (d, C-4), 141.2 (s, C-5), 111.2 (d, C-6), 102.1 (d, C-1'), 74.8 (d, C-2'), 77.9 (d, C-3'), 71.3 (d, C-4'), 77.9 (d, C-5'), 62.4 (t, C-6'); FAB-MS (-)  $m/z$  285 [M-H] $^-$ .

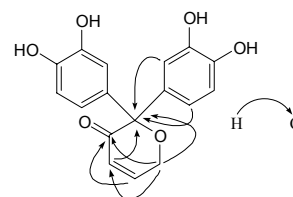
**3,4-Dihydroxy-benzoic acid (6).** Colorless needle,  $^1\text{H-NMR}$  (400 MHz,  $\text{CD}_3\text{OCD}_3$ )  $\delta$  7.54 (1H, d,  $J = 2.0$  Hz, H-2), 6.90 (1H, d,  $J = 8.4$  Hz, H-5), 7.48 (1H, dd,  $J = 8.4, 2.0$  Hz, H-6);  $^{13}\text{C-NMR}$  (100 MHz,  $\text{CD}_3\text{OCD}_3$ )  $\delta$  169.0 (s, C=O), 124.1 (s, C-1), 118.4 (d, C-2), 146.4 (s, C-3), 151.6 (s, C-4), 116.6 (d, C-5), 124.6 (d, C-6); EI-MS  $m/z$  154 [M] $^+$ .

## Results and Discussion

Breviscapin A (**1**) was obtained as black powder. Its molecular formula was determined as  $\text{C}_{17}\text{H}_{14}\text{O}_6$ , with 11 degrees of unsaturation, on the basis of  $^{13}\text{C-NMR}$  (DEPT) data and the pseudomolecular ion [M+Na] $^+$  at  $m/z$  337.0679 in HR-TOF $^+$ -MS (calc. 337.0688). The IR spectrum indicated absorptions of OH groups at 3415, a carbonyl group at 1676 and a conjugated olefinic bond at 1605  $\text{cm}^{-1}$ . The carbonyl and olefinic IR bands were

**Table 1.**  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  Data of **1** (400 MHz, in  $\text{CD}_3\text{OCD}_3$ ,  $\delta$  ppm,  $J$  in Hz)

position	$\delta$ (C)	$\delta$ (H)	HMBC (H $\rightarrow$ C)
2	88.3 (s)		
3	196.2 (s)		
4	128.1 (d)	6.14 (dt, $J = 10.4, 2.4$ )	C-2, C-6
5	150.2 (d)	7.13 (dt, $J = 10.4, 2.8$ )	C-3, C-4, C-6
6	62.8 (t)	4.34 (t, $J = 2.4$ )	C-2, C-3, C-4, C-5
1'	133.5 (s)		
2'	117.3 (d)	6.72 (d, $J = 2.0$ )	C-2, C-4', C-6'
3'	146.2 (s)		
4'	146.6 (s)		
5'	116.2 (d)	6.76 (d, $J = 8.0$ )	C-1', C-3'
6'	121.3 (d)	6.60 (dd, $J = 8.0, 2.0$ )	C-1, C-2', C-4'
1''	133.5 (s)		
2''	117.3 (d)	6.72 (d, $J = 2.0$ )	C-2, C-4', C-6'
3''	146.2 (s)		
4''	146.6 (s)		
5''	116.2 (d)	6.76 (d, $J = 8.0$ )	C-1', C-3'
6''	121.3 (d)	6.60 (dd, $J = 8.0, 2.0$ )	C-1, C-2', C-4'



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

confirmed by the  $^{13}\text{C-NMR}$  signals at  $\delta_{\text{C}}$  196.2, 128.1 and 150.2 ppm (Table 1). The  $^1\text{H-NMR}$  spectrum showed the presence of two symmetrical 3,4-disubstituted aromatic rings. 1D-NMR and 2D-NMR ( $^1\text{H-}^1\text{H}$  COSY, HMQC, HMBC) experiments suggested the presence of a six-membered ring containing oxygen and  $\alpha,\beta$ -unsaturated ketone. Thus, the structure of **1** was elucidated as 2,2-bis(3,4-dihydroxyphenyl)-2H-pyran-3(6H)-one based on further analysis of 2D-NMR experiments.

The  $^1\text{H-}^1\text{H}$  COSY correlations of H-4/H-5, and H-5/H-6 showed the connectivity of C(4)-C(5)-C(6). The linkage of C-2 and C-6 to an O-atom was established by the HMBC correlations of H-6/C-2 as shown in Figure 2 and the low-field chemical shifts of C-2 and C-6 at  $\delta_{\text{C}}$  88.3 and 62.8 ppm, respectively (Table 1). The HMBC correlations of H-5/C-3 and H-4/C-2 indicated the presence of C(4)-C(3)-C(2). Thus, a six-membered ring containing oxygen and  $\alpha,\beta$ -unsaturated ketone was established. The HMBC experiments also showed the long-range couplings of H-2'/C-2 and H-6'/C-2, which suggested that two symmetrical 3,4-disubstituted aromatic rings were connected with C-2, respectively (Figure 2). Acetylation of **1** with  $\text{Ac}_2\text{O}$  in pyridine led to the preparation of tetraacetyl analogue, which indicated that four free OH groups are present in **1**. This was confirmed by its FAB-MS spectrum showing a peak at  $m/z$  483 corresponding to the analogue's protonated ion peak.

Five known compounds, crassifogenin B (**2**),<sup>2</sup> crassifoside A

(**3**),<sup>2</sup> 3-hydroxy-5-methylphenol-1-*O*-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranoside] (**4**),<sup>8</sup> ocinol glucoside (**5**),<sup>9</sup> and 3,4-dihydroxy-benzioc acid (**6**), were also isolated from the Rhizomes of *C. breviscapa*. Their structures were elucidated by spectral data and their comparison with literature values. All of these compounds were isolated for the first time from this plant.

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