A New Chlorine-containing Glucosyl-fused Compound from *Curculigo glabrescens*

Cui-Cui Zhu, Tian-Ming Wang, Kai-Jin Wang, and Ning Li

Anhui Province Key Laboratory of Research and Development of Chinese Medicine, School of Life Sciences, Anhui University, Hefei 230039, P. R. China

Reprint requests to Prof. Kai-Jin Wang. E-mail: wkjahla@sina.com, or Prof. Ning Li. Fax: +86-551-5107354. E-mail: ln0110@sina.com

Z. Naturforsch. **2009**, *64b,* 1077 – 1080; received April 2, 2009

A new chlorine-containing glucosyl-fused compound, crassifoside H (**1**), was isolated from the EtOH extract of the rhizomes of *Curculigo glabrescens*. The structure was established on the basis of MS, IR, 1D and 2D NMR experiments. In addition, seven known compounds (**2** – **8**) were isolated and identified by spectroscopic analysis and comparison of their spectral data with those reported previously. All the compounds were isolated from this plant for the first time. The free radical scavenging activity of the isolated compounds was evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Compounds **1** and **2** showed strong radical scavenging activities. The primary structure-activity relationship is also discussed.

Key words: *Curculigo glabrescens*, Hypoxidaceae, Chlorine-containing Glucosyl-fused Compound, DPPH Radical Scavenging Activity

Introduction

We have studied several species of the genus *Curculigo*, well known for their use in Chinese folk medicine, on their phytochemical and pharmacological characteristics [1 – 6]. *Curculigo glabrescens* (Ridl.) Merr. (Hypoxidaceae) is distributed in Hannan Province of China, in Malaysia and Indonesia [7]. However, the chemical constituents and bioactivities of this plant have not been reported as yet. The interesting immense medicinal importance of this genus encouraged us to undertake the phytochemical and pharmacological investigation on *C. glabrescens* and led to the isolation and identification of a new chlorine-containing glucosyl-fused compound, crassifoside H (**1**), together with the seven known compounds curcapital (**2**) [8], 4-ethoxy-3-hydroxymethylphenol (**3**) [11], orcinol-1-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**4**) [9], ocinol glucoside (**5**) [10], curculigoside I (**6**) [10], 3,5-dihydroxy toluene (**7**), and 2,6-dimethoxy-benzoic acid (**8**), from the rhizomes of *C. breviscapa* (Fig. 1). Their structures were established by spectroscopic analyses and comparisons with literature data. All the compounds were isolated from this plant for the first time. The structure of a new chlorine-containing glucosyl-fused compound **1** has been elucidated, and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of the isolated compounds was also determined.

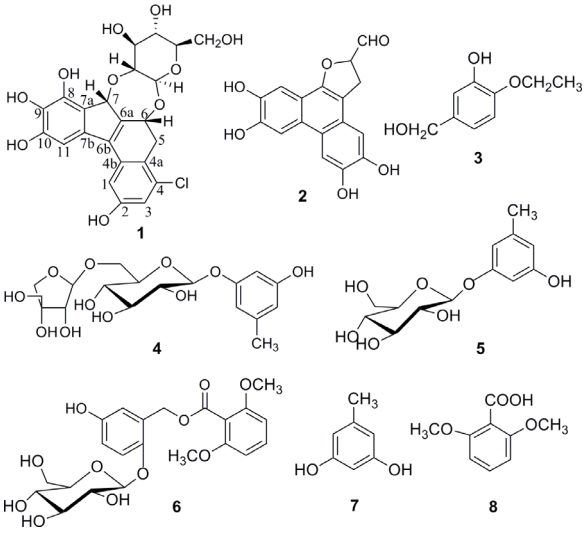

Fig. 1. The structures of compounds **1** – **8**.

Results and Discussion

Compound **1** was obtained as a light-brown powder. Its HRESI-MS gave a quasimolecular ion at

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

Position	^{13}C	^{1}H	HMBC(H→C)
1	107.8 d	7.77 (s)	2, 3, 4a, 4b, 6b
2	147.5 s[c]		
3	109.8 d	7.63 (s)	1, 2, 4, 4a
4	126.2 d[b]		
4a	126.4 s[b]		
4b	124.3 s		
5	27.7 t	3.66 (m)	4, 4a, 4b, 6, 6a
		3.57 (m)	
6	74.3 d	4.36 (m)	4a, 5, 6a, 6b, 7, Glc.1
6a	147.6 s[c]		
6b	122.7 s		
7	71.1 d	5.27 (d, 2.4)	6, 6b, 7b, 8, Glc.2
7a	117.6 s		
7b	124.7 s		
8	146.2 s		
9	125.5 s		
10	147.4 s		
11	108.3 d	7.83 (s)	8, 9, 7a, 7b, 6b
Glc.			
1	94.4 d	4.95 (d, 8.0)	6
2	81.8 d	3.39 (dd, 9.6, 8.0)	
3	74.9 d	3.59 (m)	
4	72.2 d	3.46 (m)	
5	80.0 d	3.49 (m)	
6	62.6 t	3.92 (dd, 12.0, 2.0)	
		3.75 (dd, 12.0, 5.2)	

Chemical shift values δ in ppm, coupling constants J in Hz; [b/c] values labeled [b] and [c] may be interchangeable.

m/z = 491.0765 $[M-1]^-$ and a fragment ion at *m/z* = 493.0752 $[M-1+2]^-$, in which the relative abundance ratio for [M–1]:[M–1+2] was 3 : 1, indicating that **1** contains one chlorine and has the molecular formula $C_{23}H_{21}O_{10}Cl$, which was confirmed by the ^{13}C NMR data. Thus, the structure of **1** contains 13 degrees of unsaturation. The IR spectrum showed absorptions of OH groups at 3424 cm^{-1}. The ^{1}H NMR spectrum (Table 1) exhibited signals for two methylene protons [δ_H = 3.57, 3.66 (each 1H, m, H_a-5, H_b-5, respectively)], two oxymethine protons [δ_H = 4.36 (m, H-6), 5.27 (d, J = 2.0 Hz, H-7)], three low-field aromatic protons [δ_H = 7.77, 7.63, 7.83 (each 1H, s, H-1, H-3 and H-11, respectively)] and for the protons of one glucosyl moiety [δ_H = 3.39 – 3.92 (6H, Glc.H-2→H-6)] except for one anomeric proton [δ_H = 4.95 (d, J = 8.10 Hz, Glc H-1)]. The ^{13}C NMR and DEPT data showed one CH_2, five CH, and eleven C signals together with six carbons of a glucosyl moiety, including those for two oxygen-bearing carbons [δ_C = 71.1 (C-7), and 74.3 (C-6)], four oxygen-bearing olefinic carbons [δ_C = 125.5 (C-9), 146.2 (C-8), 147.4 (C-10), and 147.5 (C-2)], one chlorine-bearing carbon [δ_C = 126.2 (C-4)], and nine olefinic carbons [δ_C = 107.8 (C-1), 108.3 (C-11), 109.8 (C-3), 117.6 (C-7a), 122.7 (C-6b), 124.3 (C-4b), 124.7 (C-7b), 126.4 (C-4a) and 147.6 (C-6a)] (Table 1).

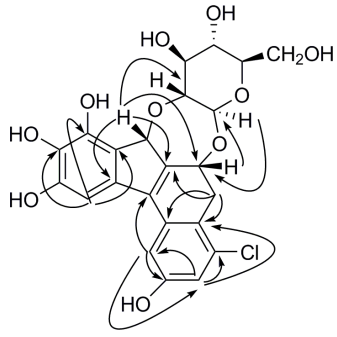

Fig. 2. Key HMBC correlations of compound **1**.

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 = 4.95 (J = 8.0 Hz). Incorporating ^{13}C NMR chemical shifts it showed the presence of a β-D-glucosyl unit. All the carbons of the glucosyl moiety were assigned through direct ^{1}H-^{13}C correlations in the HMQC spectrum and were located between δ = 62.6 and 81.8 except for that at the anomeric position, which was assigned to the signal at δ =94.4.

The ^{1}H and ^{13}C NMR data of **1** were similar to those of crassifoside E [4], suggesting that it possesses the same skeleton. This was confirmed by the HMBC correlations (Fig. 2) of H-5/C-4a, C-4, C-4b, C-6, C-6a; H-7/C-6, C-6a, C-7a, C-7b; and H-11/C-6b, C-7a, C-7b, C-9, confirming the presence of the 5, 6-2H-benzo[4a,4b]fluorene unit in **1**. The distinct difference in the ^{13}C NMR spectra of **1** and crassifoside E was that the signal at δ = 148.7 (C-4) of crassifoside E was replaced by one resonance at δ = 126.4 in the case of **1**, the OCH_2CH_3-4(C) being replaced by chlorine in **1**.

The long-range ^{1}H-^{13}C correlations of Glc.H-1/C-6 and Glc.H-2/C-7 confirmed that the fused glucosyl moiety was Glc.C-1 ether-linked to C-6 and Glc.C-2 to C-7. So compound **1** possessed the structural feature of a glucosyl-fused 5,6-2H-benzo[4a,4b]fluorene.

NOESY correlations of H-6/H-7, H-6/Glc.H-2 indicated the *cis* relationship of H-6 and H-7, and incorporating the known stereochemistry of the β-D-glucosyl unit this would require a 6*R* and 7*R* stereochemistry in **1**. Therefore, the structure of crassifoside H was deduced, as shown in Fig. 1, with a new chlorine-containing glucosyl-fused skeleton.

Compound	IC_{50} (μM)
L-Ascorbic acid (positive control)	36.80
1	33.31
2	22.94
3	76.91
4	> 100
5	> 100
6	> 100
7	> 100
8	> 100

Table 2. DPPH free radical scavenging activity of compounds **1**–**8**[a].

[a] IC_{50}: radical scavenging activity (concentration in μM required for 50 % inhibition of DPPH radical).

As is shown in Table 2, the antioxidant activites of the isolated compounds were evaluated by the DPPH radical test. Among the tested compounds, compounds **1** and **2** exhibited marked scavenging activities, with respective IC_{50} values of 33.31 and 22.94 μM comparable to that of the positive control ascorbic acid (IC_{50} = 36.80 μM). Compound **3** showed weak activity with an IC_{50} value of 76.91. Compounds **4**–**8** evidenced no scavenging activity. Antioxidant potency is related to the structure of a compound. Compounds **1** and **2** are phenolic norlignan compounds and possess the same Ph-C_5-Ph skeleton, with the addition of three adjacent phenolic hydroxyl groups in **1** and two pairs of adjacent phenolic hydroxyl groups in **2**. Compounds **3**–**8** possess less phenolic hydroxyl groups with no adjacent phenolic hydroxyl groups. This result suggested that the number of phenolic hydroxyl groups and the adjacence of phenolic hydroxyl groups are all important factors in the enhancement of the antioxidant activity of these compounds. The above results show that the phenolic norlignans are the main activity constituents of this plant suggesting the usage of this herb in the treatment of oxidative damage.

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 spectra in methanol (MeOH). The IR spectra were recorded on a Nexus 870-FT-IR spectrophotometer with KBr pellets. FAB-MS was performed on VG Autospec-3000 spectrometer, and the HRMS ((–)-ESI) was recorded with an API-QSTAR-Pulsar-1 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.). 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 10 % H_2SO_4 in EtOH followed by heating.

Plant material

The plant material was collected in Lingshui, Hainan 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. 20070802) was deposited.

Extraction and isolation

The air-dried and powered rhizomes of *C. glabrescens* (1.45 kg) were extracted with 85 % EtOH (3 × 7 L) under reflux for 3 h. The combined organic layers were concentrated *in vacuo*, the deep-brown gum (180 g) suspended in H_2O and then passed though a D101 resin column eluted sequentially with water followed by 20 %, 40 %, 60 %, 80 %, and 95 % aqueous MeOH. The fraction eluted with 20 % MeOH (7.2 g) was repeatedly purified by Sephadex LH-20 (MeOH-H_2O, 0 : 1 – 1 : 0, then MeOH) to afford compounds **4** (88 mg) and **5** (126 mg). The fraction eluted with 40 % MeOH (4.1 g), eluted further with MeOH-H_2O (0 : 1 – 1 : 0) over Sephadex LH-20, was further purified repeatedly over a Sephadex LH-20 column with EtOH to give compound **7** (17 mg). The fraction eluted with 60 % MeOH (10.3 g), further eluted with $CHCl_3$-MeOH (8 : 1 and 8 : 2), was purified repeatedly over Sephadex LH-20 (MeOH, then EtOH-CH_3COCH_3, 1 : 1) to give compounds **6** (74 mg), **8** (192 mg) and **3** (8 mg). The fraction eluted with 80 % EtOH (4.3 g), eluted further with $CHCl_3$-MeOH (9 : 1), was purified over Sephadex LH-20 with MeOH to yield compound **1** (16 mg). Compound **2** (21 mg) was obtained from the eluate with 95 % MeOH (2.6 g) by refractionation over Sephadex LH-20 (EtOH-H_2O, 0 : 1 – 1 : 0, then EtOH).

Physical and spectroscopic data

Crassifoside H (**1**), black powder. – UV (MeOH): λ_{max} ($\lg \varepsilon_{max}$) = 203 (4.22), 224 (4.11), 261 (4.33), 296 (4.11) nm. – $[\alpha]_D^{28}$ = +20.9 (c = 0.17, MeOH). – IR (KBr): ν = 3424 (OH), 2963, 2926, 2255, 2127, 1626, 1512, 1448, 1396, 1341, 1262, 1185, 1114, 1043, 1024, 994, 863, 823, 765, 607 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* (%) = 493 (12), 491(30), 447 (82), 265 (100), 223 (41), 127 (25), 97 (38), 80 (52). – HRMS ((–)-ESI): *m/z* = 491.0765 (calcd. 491.0744 for $C_{23}H_{21}O_{10}Cl$, $[M–H]^-$).

DPPH radical scavenging assay

The DPPH assay was performed as previously described [12]. L-Ascorbic acid was used as a positive control, and reaction mixtures containing an ethanolic solution of 200 μM DPPH (100 μL) and two-fold serial dilutions of the sample (dissolved in 100 μL ethanol, with amounts of sample ranging from 2 to 1000 μg mL^{-1}) were placed in a 96 well

microplate and incubated at 37 °C for 30 min. After incubation the absorbance was read at 517 nm by an E_{max} precision microplate reader, and the mean of three readings was obtained. The scavenging activity was determined by the following equation:

$$\%\,Scavenging\ activity = 100 \times \frac{Absorbance_{\text{control}} - Absorbance_{\text{sample}}}{Absorbance_{\text{control}}}$$

The IC_{50} value was obtained through extrapolation from a linear regression analysis and denotes the concentration of the sample required to scavenge 50 % of DPPH radicals.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (30670217), the International Foundation for Science (F/4340-1), the Science and Technology Foundation of Distinguished Young Scholars of Anhui Province (08040106812), and the Foundation of personnel developing of Anhui Province (2008Z020).

[1] N. Li, A. Q. Jia, Y. Q. Liu, J. Zhou, *Acta Bot. Yunna.* **2003**, *25*, 241 – 244.
[2] N. Li, J. J. Chen, J. Zhou, *Helv. Chim. Acta* **2004**, *87*, 845 – 850.
[3] N. Li, J. J. Chen, Y. X. Zhao, J. Zhou, *J. Asian Nat. Prod. Res*. **2005**, *7*, 189 – 195.
[4] N. Li, K. J. Wang, J. J. Chen, J. Zhou, *Tetrahedron Lett*. **2005**, *46*, 6445 – 6447.
[5] N. Li, J. J. Chen, J. Zhou, *Z. Naturforsch.* **2006**, *61b*, 611 – 614.
[6] K. J. Wang, N. Li, *Arch. Pharm. Res*. **2008**, *31*, 1313 – 1316.
[7] *Delectis Florae Reipublicae Popularis Sinicae Agendae Academiae Sinicae Edita. Flora Reipublaris Sinicae*, Vol.16, Science Press, Beijing, **1985**, pp. 33 – 39.
[8] W. L. Chang, C. H. Chen, S. S. Lee, *J. Nat. Prod*. **1999**, *62*, 734 – 739.
[9] Q. Wu, D. X. Fu, A. J. Hou, G. Q. Lei, Z. J. Liu, J. K. Chen, T. S. Zhou, *Chem. Pharm. Bull*. **2005**, *53*, 1065 – 1067.
[10] C. H. Chen, W. Ni, W. L. Mei, *Acta Bot. Yunna*. **1999**, *21*, 521 – 524.
[11] S. S. Lee, W. L. Chang, C. H. Chen, *Tetrahedron Lett*. **1996**, *37*, 4405 – 4408.
[12] K. J. Wang, C. R. Yang, Y. J. Zhang, *Food Chemistry* **2007**, *101*, 365 – 371.