Fitoterapia 81 (2010) 869–872

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

New norlignan derivatives from *Curculigo capitulata*

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ARTICLE INFO

Article history:
Received 6 February 2010
Received in revised form 17 May 2010
Accepted 19 May 2010
Available online 23 May 2010

Keywords:
Curculigo capitulata
Norlignan derivatives
Crassifoside I
Sinensigenin C

ABSTRACT

Two new norlignan derivatives, crassifoside I (**1**) and sinensigenin C (**2**), were isolated from the rhizomes of *Curculigo capitulate*, along with six known norlignan derivatives, 1,1-bis(3,4-dihydroxyphenyl)-1-(2-furan)-methane (**3**), crassifogenin B (**4**), crassifoside A (**5**), breviscaside A (**6**), crassifoside D (**7**), and curcapital (**8**). Their structures were elucidated on the basis of spectral evidence and comparisons with literature data. The ^{1}H and ^{13}C NMR data of compound **3** was first assigned. Compounds **3**–**7** were isolated from this plant for the first time.

1. Introduction

The genus *Curculigo* belongs to the Hypoxidaceae. More than 30 new norlignan derivatives have been isolated from the genus *Curculigo* by our group [1–9], including some interesting novel norlignans, such as crassifosides D–F [6,7], breviscaside A [2], breviscapin B [2], and sinensigenin A [1]. The herb *C. capitulata* (Lour.) Ktze, used as a tonic and a medicine for treating dysmenorrhea and rheumatism [10], is widely distributed in Southern and Southwestern China. Previous chemical studies of the rhizomes of *C. capitulata* collected in Yunnan Province evidenced the presence of norlignans [8]. As a continuation of a program directed toward the isolation of new and biologically active constituents, we reinvestigated this plant collected in Guangxi Province. Two new norlignan derivatives, named crassifoside I (**1**) and sinensigenin C (**2**), were isolated from the rhizomes of *C. capitulata*, along with six known norlignan derivatives, 1,1-bis(3,4-dihydroxyphenyl)-1-(2-furan)-methane (**3**) [11], crassifogenin B (**4**) [9], crassifoside A (**5**) [9], breviscaside A (**6**) [2], crassifoside D (**7**) [6], and curcapital (**8**) [12] (Fig. 1). The new structures were identified by extensive NMR spectroscopic means including ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY techniques. Herein, details of the isolation and structure elucidation of compounds **1** and **2** are described.

2. Experimental

2.1. General

Optical rotation was measured on a Horiba SEPA-300 polarimeter. A UV-2401PC spectrometer was used to obtain the UV spectrum in methanol (MeOH). IR spectra were taken on a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were measured on a Bruker AM-400 spectrometer with TMS as an internal standard. FAB-MS and HR-TOF-MS were performed on a VG Autospec-3000 spectrometer and API-QSTAR-Pulsar-1 spectrometer, respectively. 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.), and spots were detected by spraying with 5% H_2SO_4 in EtOH followed by heating.

2.2. Plant material

The rhizomes of *C. capitulata* were collected in Napo, Guangxi Province, China, in August 2007 and identified by Prof. Dr. Kai-Jin

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doi:10.1016/j.fitote.2010.05.012

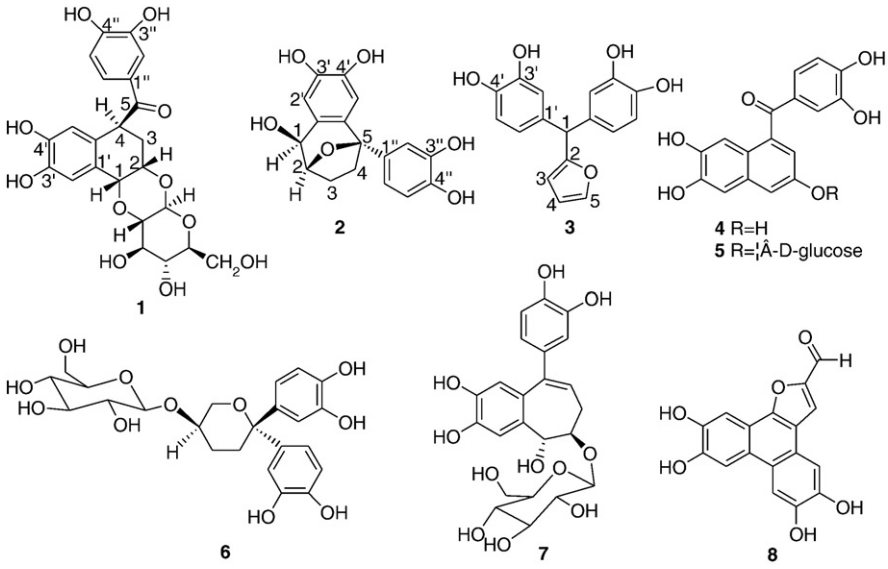

Fig. 1. Structures of compounds **1**–**8**.

Wang from the School of Life Sciences, Anhui University, where a voucher specimen (No. 20070803) was deposited.

2.3. Extraction and isolation

The air-dried and powdered rhizomes of *C. capitulata* (1.25 kg) were extracted with 85% EtOH (3×6 L) under reflux for 3 h. The combined organic layer was concentrated *in vacuo* to achieve a residue (55 g). The residue was suspended in H_2O and then passed though a D101 resin column eluted sequentially with water followed by 30%, 60%, and 90% aqueous MeOH. The fraction (5.3 g) eluted from 30% MeOH was purified by Sephadex LH-20 (MeOH–H_2O, 0:1–1:0) to yield two fractions (A_1 and A_2). Fraction A_1 was subjected to further separation on Sephadex LH-20 (EtOH–acetone, 1:1) to afford **3** (24 mg) and **5** (62 mg). Fraction A_2 was purified by Sephadex LH-20 (EtOH) and then ODS (EtOH–H_2O, 0:1–1:0) to yield **2** (35 mg) and **6** (56 mg). The fraction (5.8 g) eluted from 60% MeOH was purified by Sephadex LH-20 chromatography (MeOH-H_2O, 0:1–1:0) to yield three fractions (B_1–B_3). Fraction B_1 was subjected on ODS (MeOH–H_2O, 0:1–1:0) and then Sephadex LH-20 (EtOH–acetone, 1:1) to afford compound **1** (22 mg). Compound **4** (33 mg) was obtained from fraction B_2 by column chromatography on Sephadex LH-20 (EtOH). Compound **7** (19 mg) was obtained from fraction B_3 by column chromatography on Sephadex LH-20 (EtOH–acetone, 1:1). The fraction (3.8 g) eluted from 90% MeOH was subjected to chromatography on Sephadex LH-20 (MeOH–H_2O, 0:1–1:0, then EtOH) to yield compound **8** (102 mg).

2.4. Acidic hydrolysis of compound **1**

Compound **1** (15 mg) was refluxed with 2 mol L^{-1} HBr-dioxane (1:1, v/v, 2 mL) on a water bath for 6 h. The reaction mixture was evaporated to dryness. The dry reaction mixture was extracted with $CHCl_3$ and H_2O four times. The H_2O-souble fraction was evaporated to dryness. The dried sugar residue was diluted in 1 mL pyridine without water and treated with 0.5 mL trimethyl-chlorsilan (TMCS) and stirred at 60 °C for 5 min. After drying the solution with a stream of N_2, the residue was extracted with ether (1 mL). The ether layer was analyzed by GC with the following conditions: HP AC-5 quartz capillary column (30 m×0.32 mm); detector: FID (250 °C); injection temperature: 250 °C; column temperature: 180–280 °C; rate: 3 °C/min; and retention times (min): the derivative of D-glucose (7.22).

Crassifoside I (**1**), white powder; $[\alpha]_D^{17} = -18.6$ ($c = 0.11$, Acetone); UV λ_{max} (MeOH): 207 (logε 4.82), 233 (logε 4.44), 284 (logε 4.24), 314 (logε 4.10) nm; IR (KBr) cm^{-1}: 3406 (OH), 2930, 1655 (C O), 1596, 1526, 1444, 1373, 1293, 1181, 1112, 1024, 991, 880, 796; ^1H and ^{13}C NMR data: see Table 1; FAB-MS (pos.) m/z: 477 $[M+H]^+$; HR-TOF-MS (pos.) m/z: 499.1210 $[M+Na]^+$ ($C_{23}H_{24}O_{11}Na$, calcd. 499.1216).

Sinensigenin C (**2**), white powder; $[\alpha]_D^{14} = -12.0$ ($c = 0.20$, MeOH); UV λ_{max} (MeOH): 205 (logε 4.55), 285 (logε 3.66) nm; IR (KBr) cm^{-1}: 3417 (OH), 2924, 1611, 1517, 1442, 1294, 1043, 868, 810, 781, 638; ^1H and ^{13}C NMR data: see Table 1; FAB-MS (pos.) m/z: 317 $[M+H]^+$; HR-TOF-MS (pos.) m/z: 339.0836 $[M+Na]^+$ ($C_{17}H_{16}O_6$ Na, calcd. 339.0844).

1,1-bis(3,4-dihydroxyphenyl)-1-(2-furan)-methane (**3**), black powder; UV λ_{max} (MeOH): 194 (logε 4.31), 206 (logε 4.67), 286 (logε 3.85) nm; IR (KBr) cm^{-1}:3405 (OH), 1608, 1517, 1442, 1352, 1283, 1191, 1109, 1072, 1011, 967, 923, 874, 823, 758, 737, 644, 598; ^1H and ^{13}C NMR data: see Table 1. FAB-MS (pos.) m/z: 299 $[M+H]^+$; HR-TOF-MS (pos.) m/z: 299.0920 $[M+1]^+$ ($C_{17}H_{15}O_5$, calcd. 299.0919).

3. Results and discussion

Compound **1**, obtained as white powder, has a molecular formula of $C_{23}H_{24}O_{11}$ based on HR-TOF-MS (pos.), showing a quasi-molecular ion peak at m/z 499.1210 ($C_{23}H_{24}O_{11}Na$, calcd. 499.1216), and corresponding to an unsaturation index of 12. The IR spectrum showed absorption bands of hydroxyl (3406 cm^{-1}) and conjugated carbonyl (1655 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) exhibited signals for one methylene group at 1.93 (m), 2.84 (m), and three methine

Table 1
^{1}H NMR and ^{13}C NMR data for **1**–**3** (400 and 100 MHz, CD_3OD, *J* in Hz and δ in ppm).

No.	**1**		**2**		**3**	
	δ (C)	δ (H)	δ (C)	δ (H)	δ (C)	δ (H)
1	75.1 (d)	4.59 (d, 2.8)	70.3 (d)	5.07 (d, 5.1)	52.2 (d)	5.09 (s)
2	75.0 (d)	4.36 (m)	79.4 (d)	4.55 (t, 6.4, 6.2)	159.8 (s)	
3	28.3 (t)	1.93 (m)	23.4 (t)	2.03 (m)	109.2 (d)	5.81 (d, J = 3.2)
		2.84 (m)		2.26 (m)		
4	48.1 (d)	4.69 (dd, 12.2, 4.9)	39.7 (t)	2.23 (m)	111.8 (d)	6.22 (dd, J = 3.2, 1.6)
5	202.5 (s)		87.6 (s)		143.4 (d)	7.33 (d, J = 1.2)
1′	126.5 (s)		128.4 (s)		136.3 (s)	
2′	119.3 (d)	6.86 (s)	114.7 (d)	6.89 (s)	121.9 (d)	6.54 (d, J = 2.0)
3′	147.4 (s)		145.5 (s)		145.5 (s)	
4′	145.7 (s)		144.9 (s)		146.6 (s)	
5′	114.9 (d)	6.30 (s)	113.1 (d)	5.99 (s)	117.6 (d)	6.64 (d, J = 8.0)
6′	128.8 (s)		136.6 (s)		116.8 (d)	6.41 (dd, J = 8.0, 2.0)
1″	130.3 (s)		135.7 (s)		136.3 (s)	
2″	116.9 (d)	7.48 (d, 2.0)	116.3 (d)	6.86 (d, 1.7)	121.9 (d)	6.54 (d, J = 2.0)
3″	146.7 (s)		145.94 (s) [a]		145.5 (s)	
4″	152.8 (s)		145.96 (s) [a]		146.6 (s)	
5″	116.1 (d)	6.85 (d, 8.4)	115.7 (d)	6.77 (d, 8.0)	117.6 (d)	6.64 (d, J = 8.0)
6″	124.4 (d)	7.52 (dd, 8.4, 2.0)	120.2 (d)	6.79 (dd, 7.9, 1.7)	116.8 (d)	6.41 (dd, J = 8.0, 2.0)
Glucose						
1	94.6 (d)	4.66 (d, 7.9)				
2	81.6 (d)	3.23 (dd, 9.4, 8.1)				
3	75.2 (d)	3.55 (m)				
4	72.1 (d)	3.39 (m)				
5	79.8 (d)	3.40 (m)				
6	62.6 (t)	3.70 (dd, 12.0, 4.7)				
		3.86 (d, 11.8)				

[a] Values may be interchangeable.

protons at 4.59 (d, J = 2.8 Hz), 4.36 (m), and 4.69 (dd, J = 12.2, 4.9 Hz). The ^{1}H NMR spectrum of **1** also appeared five aromatic protons, three of them were assigned to H-2″ at δ 7.48 (d, J = 2.0 Hz), H-5″ at δ 6.85 (d, J = 8.4 Hz), and H-6″ at δ 7.52 (dd, J = 8.4, 2.0 Hz), which suggested the existence of 1,3,4-trisubstituted benzene ring, in which H-2″ and H-6″ were shifted downfield due to an *ortho* carbonyl group (IR ν_{CO} 1655 cm^{-1} and δ_{CO} 202.5); the remaining two aromatic protons were assigned to H-2′ at δ 6.86 (s), and H-5′ at δ 6.30 (s) in another 1,3,4,6-tetrasubstituted benzene ring. The ^{13}C NMR (DEPT) spectrum (Table 1) showed one methylene carbon at δ 28.3 (C-3), three methine carbons at δ 75.1 (C-1), 75.0 (C-2), and 48.1 (C-4), one conjugated carbonyl carbon at δ 202.5 (C-5), four oxygen-bearing aromatic carbons at δ 145.7 (C-4′), 146.7 (C-3″), 147.4 (C-3′), and 152.8 (C-4″), five aromatic CH at δ 114.9 (C-5′), 116.1 (C-5″), 116.9 (C-2″), 119.3 (C-2′), 124.4 (C-6″), three aromatic quaternary carbons at δ 126.5 (C-1′), 128.8 (C-6′) and 130.3 (C-1″) together with six carbons of one glucosyl moiety. 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 4.66 (d, J = 7.9 Hz) suggested a β-configured glucose unit. Acid hydrolysis of **1** with 2 mol L^{-1} HBr under refluxing produced D-glucose as sugar residue determined by GC analysis. 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 situated between δ 62.6 and 81.6 except for that at the anomeric position, which was assigned to the signal at δ 94.6.

In the ^{1}H–^{1}H COSY spectrum, the C-2 methine proton [δ 4.36 (m)] displayed strong correlation with the C-1 methine proton [δ 4.59 (d, J = 2.8 Hz)] and C-3 methylene protons [δ 1.93 (m), 2.84 (m)], and the C-4 methine proton [δ 4.69 (dd, J = 12.2, 4.9 Hz)] displayed strong correlation with the C-3 methylene protons, suggesting a –CH(O)CH(O)CH_2CH– structural fragment. In the HMBC experiment (Fig. 2), long-range correlations were observed between the 1-proton and the 1′-, 2′-, and 6′-carbons, and between the 4-proton and the 5-, 1′- and 5′-carbons, which showed the presence of a benzo [1′, 6′] cyclohexatane linked to the carbonyl at 4-carbon. In the HMQC spectrum, two singlet signals at δ = 6.86 (H-2′) and 6.30 (H-5′) had connectivities with carbon atoms at δ = 119.3 (C-2′) and 114.9 (C-5′). The benzoyl was established by the HMBC correlations between the 2″-, and 6″-protons and the 5-carbon. The long-range ^{1}H–^{13}C correlations between the proton of GlcH-1 and 2-carbon, and between the proton of GlcH-2 and 1-carbon, confirmed that the fused glucosyl moiety was GlcH-1 ether-linked to C-2 and GlcH-2 to C-1.

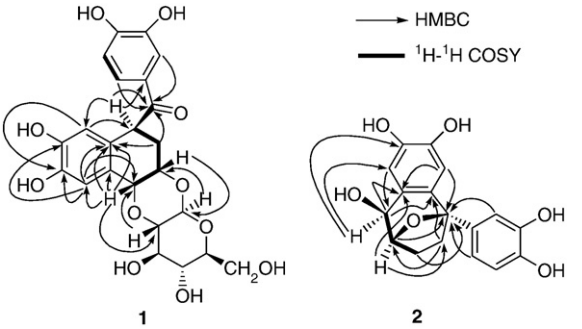

Fig. 2. The key HMBC correlations of compounds **1** and **2**.

NOESY correlations of H-1 with H-2, H-1 and H-2 with Glc. H-2, H-1 and H-2 with H-3a [δ 1.93 (m)], and H-4 with H-3b [δ 2.84 (m)], indicated the *cis* relationship of H-1, H-2 and the benzoyl. Incorporating the known stereochemistry of the β-D-glucosyl unit would require 1*S*, 2*S* and 4*S* stereochemistry in **1**. Therefore, the structure of **1** was deduced as a glucosyl-fused norlignan derivative, named crassifoside I (Fig. 1).

Compound **2**, obtained as white powder, has a molecular formula of $C_{17}H_{16}O_6$ based on HR-TOF-MS (pos.), showing a quasi-molecular ion peak at m/z 339.0836 ($C_{17}H_{16}O_6Na$, calcd. 339.0844), and corresponding to an unsaturation index of 10. The IR spectrum showed the presence of hydroxyl groups (3417 cm^{-1}). The ^1H NMR spectrum (Table 1) showed the following clear signals: two methylene groups at δ 2.03 (m), 2.26 (m), and 2.23 (m), two oxygenated methine protons at δ 5.07 (d, J = 5.1 Hz), and 4.55 (t, J = 6.4, 6.2 Hz). The ^1H NMR spectrum of **2** also exhibited five low-field aromatic protons. Three of them were assigned to H-2″ at δ 6.86 (d, J = 1.7 Hz), H-5″ at δ 6.77 (d, J = 8.0 Hz), and H-6″ at δ 6.79 (dd, J = 7.9, 1.7 Hz), which suggested the existence of 1,3,4-trisubstituted benzene ring. The remaining two aromatic protons were assigned to H-2′ at δ 6.89 (s), and H-5′ at δ 5.99 (s) in a 1, 3, 4, 6-tetrasubstituted aromatic ring. Analysis of the ^1H and ^{13}C NMR (Table 1) and HSQC spectra revealed that **2** contains two aromatic rings, including four oxygen-bearing olefinic carbons at δ 144.9 (C-4′), 145.5 (C-3′), 145.94 (C-3″), and 145.96 (C-4″), five aromatic CH at δ 113.1 (C-5′), 114.7 (C-2′), 115.7 (C-5″), 116.3 (C-2″), and 120.2 (C-6″), three aromatic quaternary carbons at δ 128.4 (C-1′), 135.7 (C-1″) and 136.6 (C-6′), as well as five aliphatic carbons including one oxygenated quaternary carbon at δ 87.6 (C-5), two oxymethine carbons at δ 70.3 (C-1), and 79.4 (C-2), and two methylene carbons at δ 23.4 (C-3), and 39.7 (C-4).

The connectivity –CH(O)CH(O)CH_2CH_2C(O)– was deduced from the ^1H, ^1H-COSY correlations of the C-2 methine proton with the C-1 methine proton and C-3 methylene protons, and the HMBC correlations of 2-proton with 4-carbon, and 3-protons with 5-carbon. The HMBC experiments (Fig. 2) showed the long-range couplings of 2″- and 6″-protons with 5-carbon, which suggested that the 1, 3, 4-trisubstituted aromatic ring was connected with 5-carbon. Long-range correlations observed between the 1-proton and the 1′-, 2′-, 3′- and 6′-carbons, between the 4-protons and the 6′-carbon, and between the 5′-proton and the 5-carbon, indicated the linkage of C-1 to C-1′ and C-5 to C-6′. The linkage of C-2 and C-5 to an O-atom was established by the HMBC correlations of the 2-proton and the 5-carbon, and the low-field chemical shift of C-2 and C-5, at δ 79.4 and 87.6, respectively (Table 1). The clear NOESY correlations of H-1 with H-2 and H-3a (δ 2.26), H-3a (δ 2.26) with H-2″ and H-6″, but not between H-3b (δ 2.03) with H-1 and H-2, indicated the *cis* relationship of H-1, H-2, and the 1, 3, 4-trisubstituted aromatic ring. Thus, the structure of sinensigenin C was deduced as shown in Fig. 1.

Comparison of the spectroscopic and physical data with those published allowed us to establish the structures of known norlignan derivatives 1,1-bis(3,4-dihydroxyphenyl)-1-(2-furan)-methane (**3**) [11], crassifogenin B (**4**) [9], crassifoside A (**5**) [9], breviscaside A (**6**) [2], crassifoside D (**7**) [6], and curcapital (**8**) [12], respectively. Compounds **3–7** were isolated from this plant for the first time.

Acknowledgement

This work was financially 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).

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