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Two new phenolic glucosides from *Curculigo orchioides* and their cytotoxic effects

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ABSTRACT

Two new phenolic glucosides, curculigoside J (**1**) and K (**2**), and seven known compounds, curculigoside B (**3**), curculigoside (**4**), curculigoside C (**5**), orchioside B (**6**), 3''-dehydroxynyasicoside (**7**), curculigine J (**8**), and curculigine B (**9**) were isolated from a methanolic extract of the roots of *Curculigo orchioides*. The structures of these compounds were elucidated using spectroscopic methods and comparison of their NMR data with those reported in the literature. In addition, all the isolated compounds were evaluated for their cytotoxic effects. Compounds **1**, **2**, and **7** were characterized by moderate inhibition of the HepG2 and MCF7 cancer cell lines with IC_{50} values ranging from 50.0 to 94.4 μM.

1. Introduction

The genus *Curculigo* (Hypoxidaceae) comprises 24 globally distributed species with accepted names, several of which, including C. *orchioides, C. capitulate,* and *C. pilosa* hold significance in herbalism on account of their medicinal properties. *Curculigo orchioides* Gaertn., in particular, has a long-standing history of medicinal usage dating back to the Tang Dynasty, with well-attested effects such as sustaining robust energy levels and supporting the health of the liver and kidneys (Nie et al., 2013). Phytochemical analyses of C. *orchioides* revealed the presence of a range of active compounds, including phenolic glycosides (Lakshmi et al., 2003; Wang et al., 2014), cycloartane glycosides (Yokosuka et al., 2010a, 2010b), lignans (Gupta et al., 2005), and alkaloids (Xu et al., 1992). Some of secondary metabolites and extracts derived from this plant have been demonstrated to have diverse beneficial effects, such as anticancer (Yokosuka et al., 2010b), immunostimulatory (Bafna and Mishra, 2006), antiosteoporotic (Jiao et al., 2009), and hepatoprotective activities (Venukumar and Latha, 2002). Herein, we report the isolation and structural elucidation of two new phenolic glucosides, along with seven known compounds from C. *orchioides*. Furthermore, the cytotoxic effects of all compounds were also evaluated.

2. Results and discussion

Compound **1** was obtained as a white powder. A molecular formula of $C_{23}H_{26}O_{10}$ was established based on HR-ESI-MS analysis, which revealed a pseudomolecular ion peak at *m/z* 463.1568 $[M+H]^+$ (calcd. for $[C_{23}H_{27}O_{10}]^+$, 463.1599). The ^{1}H NMR spectrum of **1** showed proton signals of one olefinic proton at δ_H 6.28 (1 H, dd, $J = 6.6$, 7.8 Hz), one *p*-disubstituted aromatic ring at δ_H 6.74 (2 H, d, $J = 9.0$ Hz) and 7.11 (2 H, d, $J = 9.0$ Hz), one tetrasubstituted aromatic ring at δ_H 6.46 (1 H, s) and 7.22 (1 H, s), and one anomeric proton at δ_H 4.46 (1 H, d, $J = 7.8$ Hz). The ^{13}C NMR and HSQC spectra of **1** showed the signals of 23 carbons, among which were 7 non-protonated carbons at δ_C 130.2, 134.2, 134.6, 144.2, 144.8, 145.7, and 158.0; 14 methines at δ_C 71.7, 74.8, 75.0, 78.0 × 2, 94.2, 102.7, 112.5, 115.9 × 2, 117.1, 123.6, and 130.2 × 2; and 2 methylene carbons at 31.5 and 62.7 (Table 1). Analysis of the ^{1}H- and ^{13}C NMR data suggested that the structure of **1** was similar to crassifoside D (Li et al., 2006), a compound previously reported from *Curculigo crassifolia* with the exception of an absence of the hydroxy group at C-3′ (Fig. 1). HMBC correlations (Fig. 2) from H-2 (δ_H 6.28) to C-1 (δ_C 144.2)/C-10 (δ_C 130.2), H-3 (δ_H 1.95 and 2.46) to C-1 (δ_C 144.2)/C-5 (δ_C 75.0), H-5 (δ_H 4.62) to C-3 (δ_C 31.5)/C-6 (δ_C 112.5)/C-10 (δ_C 130.2)/C-11 (δ_C 134.2), and from H-9 (δ_H 6.46) to C-1 (δ_C 144.2)/C-11

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Table 1
^{1}H- and ^{13}C NMR spectroscopic data for compounds **1** and **2** in CD_3OD.

	1		**2**	
C	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)
1	144.2	-	144.9	-
2	123.6	6.28 (dd, 6.6, 7.8)	122.3	6.05 (dd, 7.2, 7.2)
3	31.5	1.95 (m)	32.4	2.36 (m)
		2.46 (m)		2.46 (m)
4	94.2	4.11 (m)	82.2	3.82 (m)
5	75.0	4.62 (d, 8.4)	65.8	3.56 (dd, 6.0, 12.0)
				3.61 (dd, 3.0, 12.0)
6	112.5	7.22 (s)	116.2	6.80 (d, 8.4)
7	145.7	-	145.4	-
8	144.8	-	146.1	-
9	117.1	6.46 (s)	118.1	6.60 (d, 1.8)
10	130.2	-	135.8	-
11	134.2	-	122.5	6.50 (dd, 1.8, 8.4)
1′	134.6	-	135.8	-
2′	130.2	7.11 (d, 9.0)	129.4	7.09 (d, 8.4)
3′	115.9	6.74 (d, 9.0)	115.7	6.68 (d, 8.4)
4′	158.0	-	157.7	-
5′	115.9	6.74 (d, 9.0)	115.7	6.68 (d, 8.4)
6′	130.2	7.11 (d, 9.0)	129.4	7.09 (d, 8.4)
4-*O*-Glc				
1″	102.7	4.46 (d, 7.8)	103.3	4.31 (d, 7.8)
2″	74.8	3.27 (t, 7.8)	75.1	3.22 (dd, 7.8, 9.0)
3″	78.0	3.40 (m)	78.0	3.36 (t, 9.0)
4″	71.7	3.32 (m)	71.6	3.30 (m)
5″	78.0	3.34 (m)	77.8	3.27 (m)
6″	62.7	3.69 (dd, 5.4, 12.0)	62.6	3.67 (dd, 6.0, 12.0)
		3.91 (dd, 1.8, 12.0)		3.88 (dd, 2.4, 12.0)

(δ_C 134.2), and also COSY correlations of H-2 (δ_H 6.28)/H-3 (δ_H 1.95 and 2.46)/H-4 (δ_H 4.11)/H-5 (δ_H 4.62), indicated the presence of a benzo [10,11] cycloheptene (Li et al., 2006). In addition, the position of the *p*-hydroxyphenyl group at C-1 of benzo [10,11] cycloheptene was confirmed by HMBC correlations between H-2′/H-6′ (δ_H 7.11) and C-1 (δ_C 144.2). The multiplicity of an anomeric proton [δ_H 4.46 (1 H, d, J = 7.8 Hz)] and ^{13}C NMR chemical shifts of the sugar unit at δ_C 102.7, 78.0 × 2, 74.8, 71.7, and 62.7) are similar to those of the monosaccharide of *β*-glucopyranosyl. Moreover, the sugar unit of compounds from the same *Curculigo* genus was identified as D-glucopyranosyl (Wu et al., 2005). Thus, on the basis of NMR evidence and investigations of compounds containing a sugar unit from *Curculigo* genus, we tentatively identified the sugar unit as *β*-D-glucopyranosyl. The position of *β*-D-glucopyranosyl at C-4 of benzo [10,11] cycloheptene was confirmed by HMBC correlations from glc H-1′ (δ_H 4.46) to C-4 (δ_C 94.2). Furthermore, the large coupling constant between H-4 and H-5 (J = 8.4 Hz) indicated the configuration of substitutions at 4-*β*-D-glucopyranosyl and the 5-hydroxy group to be *trans*. Consequently, the structure of **1** was elucidated and named as curculigoside J.

HR-ESI-MS analysis of **2** revealed an ion peak at *m/z* 465.1711 $[M+H]^+$, thereby indicating a molecular formula of $C_{23}H_{28}O_{10}$. The ^{1}H NMR spectrum of **2** showed proton signals of one olefinic proton at δ_H 6.05 (1 H, dd, J = 7.2, 7.2 Hz), one *p*-disubstituted aromatic ring at δ_H 6.68 (2 H, d, J = 8.4 Hz) and 7.09 (2 H, d, J = 8.4 Hz), one 1,3,4-trisubstituted of aromatic ring at δ_H 6.50 (1 H, dd, J = 1.8, 8.4 Hz), 6.80 (1 H, d, J = 8.4 Hz), and 6.60 (1 H, d, J = 1.8 Hz), and one anomeric proton at δ_H 4.31 (1 H, d, J = 7.8 Hz). The ^{13}C NMR and HSQC spectra of **2** were characterized by the signals of 23 carbons, among which there were 6 non-protonated carbons, 14 methines, and 3 methylenes (Table 1). Analysis of ^{1}H- and ^{13}C NMR data of **2** and comparison with the structure of **1,** indicated a cleavage of the bond between C-5 and C-11. The HMBC correlations (Fig. 2) from H-4 (δ_H 3.82) to C-2 (δ_C 122.3)/C-3 (δ_C 32.4)/C-5 (δ_C 65.8), and from H-2 (δ_H 6.05) to C-1 (δ_C 144.9)/C-3 (δ_C 32.4)/C-4 (δ_C 82.2) and COSY correlations of H-2 (δ_H 6.05)/H-3 (δ_H 2.36 and 2.46)/H-4 (δ_H 3.82)/H-5 (δ_H 3.56 and 3.61), indicated the presence of a 4-oxygenated-5-hydroxypent-1-enyl group. The positions of both the 3,4-dihydroxyphenyl and *p*-hydroxyphenyl groups at C-1 were confirmed by HMBC correlations from H-2 (δ_H 6.05) to C-1 (δ_C 144.9)/C-10 (δ_C 133.4)/C-1′ (δ_C 135.8). The NOESY correlations between H-2 (δ_H 6.05) and H-2′/H-6′ (δ_H 7.09) and H-3 (δ_H 2.36 and 2.46) and H-9 (δ_H 6.60) were observed, thereby indicating the configuration of the double bond at C-1/C-2 to be *Z*. The multiplicity and ^{13}C NMR chemical shifts of the sugar unit were similar to those of **1**, thus enabling us to identify the sugar unit as *β*-D-glucopyranosyl. Furthermore, the position of the sugar unit at C-4 was confirmed by HMBC correlation from glc H-1″ (δ_H 4.31) to C-4 (δ_C 82.2). Consequently, the structure of **2** was elucidated as a new compound and named curculigoside K.

The known compounds were identified as curculigoside B (**3**) (Valls et al., 2006), curculigoside (**4**) (Palazzino et al., 2000), curculigoside C (**5**) (Valls et al., 2006), orchioside B (**6**) (Gupta et al., 2005), 3″-dehydroxynyasicoside (**7**) (Chang et al., 1997), **c**urculigine J (**8**) (Wang et al., 2014), and curculigine B (**9**) (Kanchanapoom et al., 2021). Their chemical structures were elucidated by spectroscopic methods and comparison with literature-reported data.

All compounds were screened for cytotoxic effects against the breast cancer (MCF7) and liver cancer (HepG2) cell lines at a concentration of 100 μM (Fig. 3) (Anh et al., 2023). Compounds **1**, **2**, and **7** were found to inhibit cell growth by more than > 50%, and on the basis of this finding, we further evaluated these compounds. Consequently, these compounds were further evaluated at concentrations of 20, 4, and 0.8 μM to determine the corresponding IC_{50} values. As a positive control, we used the anticancer agent ellipticine, which exhibited cytotoxic activity against the HepG2 and MCF7 cancer cell lines with IC_{50} values of 0.38 ± 0.02 and 0.34 ± 0.03 μM, respectively. Comparatively, compounds **1**-**3** were characterized by moderate inhibition of these cell lines, with IC_{50} values ranging from 49.96 to 94.35 μM (Table 2).

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded on a Bruker 600 MHz spectrometer. HR-ESI-MS spectra were obtained from the Korea Basic Science Institute (KBSI, Chuncheon Center). Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70 – 230 mesh and 230 – 400 mesh, Merck) or RP-18 gel (30 – 50 μm, Fuji Silysia Chemical Ltd.). For thin

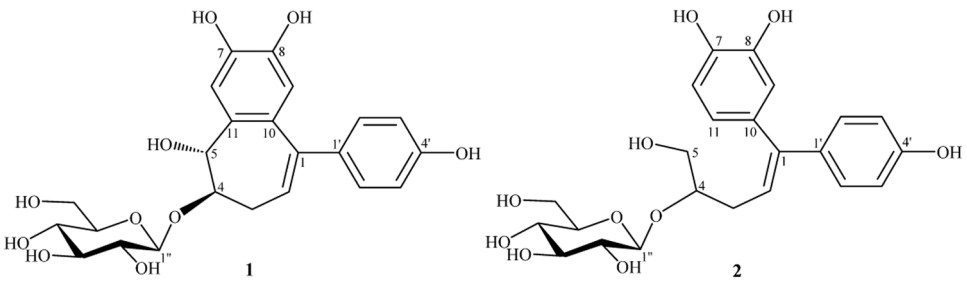

Fig. 1. Chemical structures of compounds **1** and **2**.

layer chromatography (TLC), pre-coated silica gel 60 F_{254} (0.25 mm, Merck) and RP-18 $F_{254}S$ (0.25 mm, Merck) plates were used. The HPLC system was an Agilent HPLC 1100 (General condition: J′sphere H-80 column: 250 mm length × 20 mm ID and a flow rate of 3 mL/min), detector DAD. Spots were visualized by spraying with 10% aqueous H_2SO_4, followed by heating.

3.2. Plant material

The roots of *Curculigo orchioides* Gaertn. were collected in Mau Son, Cao Loc, Lang Son, province, Vietnam in May, 2022, and identified by Dr. Do Van Hai, Institute of Ecology and Biological Resources. A voucher specimen (ACO22.05) was deposited at the Institute of Marine Biochemistry, VAST.

3.3. Extraction and isolation

The dried powder of *C. orchioides* roots (5.0 kg) was sonicated three times with hot MeOH (50 C, 11 L, each) to yield a MeOH extract (ACO, 609 g) after removal of the solvent. The ACO extract was suspended in water (1.5 L), then successively partitioned with *n*-hexane and EtOAc to obtain an *n*-hexane extract (ACO1, 54.5 g), EtOAc extract (ACO2, 39.5 g), and an aqueous layer (ACO3).

The aqueous layer (ACO3) was subjected to chromatographic separation on a Diaion HP-20 column eluting with water (2 L) to remove the polar components, followed by MeOH in water (50%, 75%, and 100%, each 0.8 L) to give three fractions, ACO3A (54.0 g), ACO3B (24.5 g), and ACO3C (2.5 g), respectively. ACO3A was separated on a silica gel column eluting with CH_2Cl_2/MeOH (20/1, 10/1, 5/1, 2.5/1, 1/1, v/v) to give five fractions, ACO3A1 (4.9 g), ACO3A2 (7.0 g), ACO3A3 (6.5 g), ACO3A4 (4.8 g), and ACO3A5 (5.0 g). From among these, ACO3A1 (4.9 g) was subjected to chromatographic separation on a silica gel column and eluted with CH_2Cl_2/acetone/water (1/1.5/0.1, v/v/v) to give six fractions, ACO3A1A (0.7 g), ACO3A1B (0.7 g), ACO3A1C (0.6 g), ACO3A1D (0.6 g), ACO3A1A (0.5 g), and ACO3A1F (0.6 g). The ACO3A1D fraction was subsequently underwent chromatographic separation on an RP-18 column, eluted with MeOH/water (1/1.5, v/v). Further HPLC using a mobile phase of 25% acetonitrile in water yielded compounds **3** (6.5 mg, t_R 37.1 min) and **4** (21.0 mg, t_R 46.8 min). Similarly, ACO3A1F was chromatographed on an RP-18 column, eluted with MeOH/water (1/2, v/v) following by HPLC with 25% acetonitrile in water as the mobile phase yielded compounds **5** (37.0 mg, t_R 23.3 min) and **6** (20.0 mg, t_R 33.0 min). The ACO3A5 fraction was chromatographed on a silica gel column eluting with CH_2Cl_2/acetone/water (1/2.5/0.1, v/v/v) to give four fractions, ACO3A5A (0.8 g), ACO3A5B (0.5 g), ACO3A5C (0.7 g), and ACO3A5D (0.6 g), among which, ACO3A5C was separated on an RP-18 column eluting with acetone/water (1/4, v/v) to give two fractions, ACO3A5A1 and ACO3A5A2. Of these, ACO3A5A1 was chromatographed on an HPLC with 15% acetonitrile in water as the mobile phase to yield compounds **1** (4.5 mg, t_R 36.9 min) and **7** (12.0 mg, t_R 34.8 min), whereas ACO3A5A2 was purified on an HPLC with a mobile phase of 16% acetonitrile in water to yield compound **2** (4.0 mg, t_R 44.9 min).

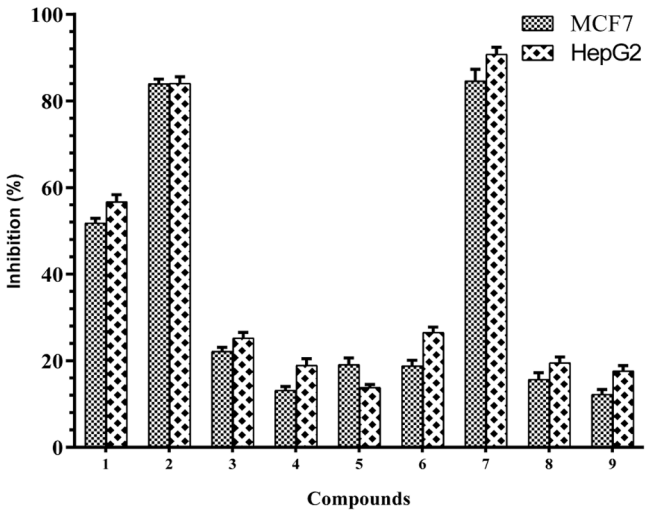

Fig. 3. Cell inhibition of compounds **1**-**9** on MCF7 and HepG2 cancer cell lines at the concentration of 100 μM.

Table 2
Effects of compounds **1**, **2**, and **7** on the growth of human cancer cells.

Compound	IC_{50} (μM)	
	MCF7	HepG2
1	94.4 ± 3.3	80.9 ± 4.7
2	59.0 ± 2.5	50.0 ± 3.0
7	61.6 ± 2.3	66.82 ± 1.6
Ellipticine	0.38 ± 0.1	0.34 ± 0.1

Ellipticine was used as a positive control.

ACO3B was separated on a silica gel column and eluted with CH_2Cl_2/MeOH (20/1, 10/1, 5/1, 2.5/1, 1/1, v/v) to give five fractions, ACO3B1 (2.8 g), ACO3B2 (5.9 g), ACO3B3 (4.5 g), ACO3B4 (5.8 g), and ACO3B5 (6.0 g). ACO3B3 (4.5 g) was chromatographed on a silica gel column eluting with CH_2Cl_2/acetone/water (1/1.5/0.1, v/v/v) to give five fractions, ACO3B3A-ACO3B3E. ACO3B3E was chromatographed on an RP-18 column with MeOH/water (1/1, v/v) to give five fractions, ACO3B3E1-ACO3B3E5. The ACO3B3E2 and ACO3B3E3 fractions were successively purified on an HPLC using a mobile phase of 30% acetonitrile in water to yield compounds **8** (14.5 mg, t_R 31.0 min) and **9** (8.5 mg, t_R 37.8 min), respectively.

3.3.1. Curculigoside J (1)

White powder. $[\alpha]_D^{25}$: − 56.0 (c = 0.05, MeOH); HR-ESI-MS m/z: 463.1568 $[M + H]^+$ (Calcd. for $[C_{23}H_{27}O_{10}]^+$, 463.1599). ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz): see Table 1.

3.3.2. Curculigoside K (2)

White powder. $[\alpha]_D^{25}$: − 144.0 (c = 0.05, MeOH); HR-ESI-MS m/z:

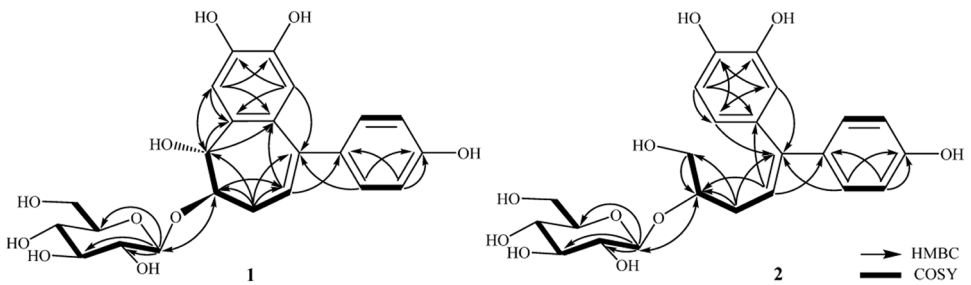

Fig. 2. Key HMBC and COSY correlations of compounds **1** and **2**.

465.1711 $[M + H]^+$ (Calcd. for $[C_{23}H_{29}O_{10}]^+$, 465.1755). ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz): see Table 1.

3.4. Cytotoxic assay

Human cancer cell lines, including breast cancer (MCF7) and liver cancer (HepG2), were obtained from Milan University, Italy and Long Island University, USA. The cells were maintained and cultured in DMEM supplemented with FBS, trypsin-EDTA, L-glutamine, sodium pyruvate, $NaHCO_3$, and penicillin/streptomycin at 37 °C in a humidified atmosphere (5% CO_2 and 95% air). Cytotoxic effects of compounds were determined using the sulforhodamine B (SRB) assay as previously described (Kiem et al., 2018). In brief, the cells were incubated with/-without compounds for three days in a 96-well culture plate. After incubation, cells were stained with sulforhodamine B and optical density (OD) was measured at 540 nm. The difference in OD between samples and vehicle well during experiments indicated the cell situation induced by the compounds. Results are expressed as the percentage of cell death in comparison with the vehicle as well. The dose–response curves of compounds were generated to determine IC_{50} values of the compounds corresponding to each cell line. Ellipticine was used as a positive control throughout the experiments.

Declaration of Competing Interest

The authors declared no conflict of interest.

Data availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phytol.2024.01.004.

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