

A new dichloro-substituted benzyl alcohol derivative from the rhizomes of *Curculigo capitulata* (Lour.) Kuntze

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Abstract:

Curculigo capitulata (Lour.) Kuntze's rhizomes have been utilised in traditional medicine for the treatment of various diseases, such as kidney stones, high blood pressure, nephritis, and cystitis. To date, there has been limited research on its chemical constituents in Vietnam. A phytochemical investigation of the ethyl acetate extract from *C. capitulata*'s rhizomes led to the isolation of five compounds, including a novel dichlorophenolic derivative, named capitulatol A (1), and four known compounds: *O*-orsellinaldehyde (2), cinnamic acid (3), orcinol (4), and orcinol glucoside (5). Their structures were elucidated using 1D and 2D nuclear magnetic resonance (NMR) spectroscopic and high-resolution electrospray ionisation-mass spectrometry (HRESI-MS) data, along with comparisons to previously published references. Compound 1 has not been described before, while compounds (2-4) have been isolated from this plant for the first time. These results indicate that phenolics and phenolic glucosides are the main components, which can serve as significant markers for the chemotaxonomy of the herb. Moreover, this chemical information will enhance our understanding of this plant, both in Vietnam and globally. Furthermore, the findings can be applied to further research in pharmacology and clinical medicine in the future.

Keywords: cinnamic acid, *Curculigo capitulata*, orcinol, orcinol glucoside, *O*-orsellinaldehyde.

Classification numbers: 2.2, 3.3

1. Introduction

Curculigo capitulata L. belongs to the *Curculigo* genus, within the Hypoxidaceae family, and widely distributed in tropical regions such as Asia, Africa, Australia, and America. The *Curculigo* genus comprises 15 species worldwide; however, only 8 species have been identified in Vietnam [1, 2]. In Vietnam, it has been utilised in traditional medicine for the treatment of various ailments, including as a diuretic, anti-inflammatory, menstrual regulator, and for blood stasis, kidney inflammation, cystitis, kidney stones, and high blood pressure [2]. In traditional Chinese medicine, this plant has been used to treat a variety of conditions, including asthma, jaundice, cough, kidney asthenia, haemorrhoids, diarrhoea, colic, impotence, and gonorrhoea, while in India, it has been used to treat bladder infections, nephritis-oedema, cystitis, nephrolithiasis, hypertension, acute renal pelvis and nephritis, and rheumatic arthritis [3, 4]. Secondary metabolites produced by the plants include triterpenes, diterpenes, phenolics, and polyphenols,

which possess various bioactivities such as antioxidant, anticancer, and anti-inflammatory properties, along with many different pharmacological effects [5-8]. Numerous previous studies have shown that the chemical constituents of the *Curculigo* genus are diverse, including tripterpenoids, steroids, lignin, lignan glycosides, norlignans, norlignan glucosides, curculigoside A, curculigoside B, curculigoside C, chlorophenolic derivatives, phenolic glycosides, and orcinosides [9-13]. Research has shown that the chemical constituents of *C. capitulata* L. closely resemble those of *C. orchioides* [2] and demonstrated that curculigoside is present in this plant. This prompted us to conduct a study on the chemical constituents of this species. However, we did not isolate curculigoside from this plant. In our research, we elucidated five compounds: 2,6-dichloro-5-hydroxy-3,4-dimethoxybenzyl alcohol (1), *O*-orsellinaldehyde (2), cinnamic acid (3), orcinol (4), and orcinol glucoside (5) using the column chromatography method from the methanol extract of *C. capitulata*'s rhizomes.

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2. Materials and methods

2.1. General experimental procedures

Column chromatography was performed using adsorption materials such as silica gel size 0.063-0.200 mm (Merck, 70-230 mesh), reverse phase powder RP-18 (ODS-A 75 μ m, YMC, Japan), and Diaion HP-20 resin (0.25-0.85 mm, Mitsubishi Chemical Corp., Japan). Thin layer chromatography (TLC) stains utilised ultraviolet (UV) illumination (254 and 365 nm), with visualisation by dipping in a 10% (v:v) aqueous solution of H₂SO₄ and heating. High-resolution electrospray ionisation mass spectrometry (HRESIMS) was conducted using a Waters QTOF micro mass spectrometer. NMR spectral data were obtained using a Bruker AM600 FT-NMR spectrometer. A JASCO P-2000 polarimeter was used for optical rotations, and an SMP3 was used for determining the melting point.

2.2. Plant materials

The rhizomes of *C. capitulata* were collected from Son Dong district, Bac Giang province (former address), Vietnam, in June 2024 and identified by Nguyen Quynh Nga and Dang Minh Tu, Centre for Medicinal Plant Resources. A voucher specimen was deposited in the herbarium of the National Institute of Medicinal Materials (code: DV-030624).

3. Extraction and isolation

Curculigo capitulata L. rhizomes powder (6.7 kg) was extracted with methanol at room temperature (3 times, 3×24 l). The methanol solvent was removed under reduced pressure to yield 658.8 g of extract, which was dissolved in 1,000 ml and then sequentially apportioned with *n*-hexane, ethyl acetate, respectively. Vacuum vaporisation of the organic solvent produced the corresponding extracts (421.1 g and 90.9 g, respectively). The ethyl acetate extract was subjected to silica gel column chromatography, rinsing with the solvent system CH₂Cl₂:CH₃OH (100:0-0:100; v:v) to give 46 fractions (CCE1-CCE46). Mixed fractions (CCE4-CCE7) were segregated on a silica gel column eluted (100:6; v:v) to give eighteen fractions (CCE4.1-CCE4.18). Orcinol (4) (77.6 mg) was obtained by further purifying fraction CCE4.10 using column chromatography (Sephadex LH-20) after washing with methanol. Mixed fractions (CCE4.4-CCE4.5) were detached on a Sephadex LH-20 column with CH₂Cl₂:CH₃OH (1:9) to yield *O*-orsellinaldehyde (2) (8.2 mg). Mixed fractions (CCE4.11-CCE4.15) were further separated on a YMC column and eluted with

CH₃OH:H₂O (2:3; v:v) to give nine fractions CCE8.1-CCE8.9. Mix fractions (CCE8.5-CCE8.6) were refined on a Sephadex LH-20 column eluting with methanol to obtain 2,6-dichloro-5-hydroxy-3,4-dimethoxybenzyl alcohol (1) (5.2 mg). Mix fractions (CCE16-CCE19) were separated on a YMC column with CH₃OH:H₂O (1:2; v:v) to yield CCE11.6. Fraction CCE11.6 was refined on a Sephadex LH-20 column with methanol to obtain cinnamic acid (3) (5.8 mg). Eighteen fractions (CCE12.1-CCE12.18) were obtained by silica gel chromatography, eluting with DCM:EtOAc:MeOH (100:1:0.1; v:v:v) from fractions CCE26-CCE34. Orcinol glucoside (29 mg) (5) was yielded by further purifying fraction CCE12.15 using a Sephadex LH-20 column, eluting with MeOH:DCM (9.5:0.5; v:v).

2,6-dichloro-5-hydroxy-3,4-dimethoxybenzyl alcohol (1): Yellow powder; molecular formula C₉H₁₀O₄Cl₂ (calculated: 253.075); HRESI-MS (*m/z* 250.9875, 252.9777, 254.9749; 9:6:1) [M-H]⁻; ¹H NMR (600 MHz, CD₃OD) δ_{H} : 3.89 (3H, s, OCH₃-3), 3.88 (3H, s, OCH₃-4), 4.86 (2H, s, H-7); ¹³C NMR (150 MHz, CD₃OD) δ_{C} : 132.7 (C-1), 120.9 (C-2), 149.9 (C-3), 143.6 (C-4), 147.9 (C-5), 119.4 (C-6), 60.1 (C-7), 61.5 (OCH₃-3), 61.3 (OCH₃-4).

O-orsellinaldehyde (2): White powder; melting point: 181-183°C; molecular formula C₈H₈O₃ (calculated: 152.149); HRESI-MS (*m/z* 191.0315) [M+K]⁺; ¹H NMR (600 MHz, CDCl₃) δ_{H} : 6.21 (1H, s, H-4), 6.21 (1H, s, H-6), 2.52 (3H, s, H-7), 10.0 (1H, s, H-8); ¹³C NMR (150 MHz, CDCl₃) δ_{C} : 144.9 (C-1), 113.6 (C-2), 166.3 (C-3), 101.3 (C-4), 163.4 (C-5), 110.6 (C-6), 18.2 (C-7), 192.9 (C-8).

trans-cinnamic acid (3): White powder; melting point: 133-134°C; molecular formula C₉H₈O₂ (calculated: 148.161); HRESI-MS (*m/z* 149.0590) [M+H]⁺; ¹H NMR (600 MHz, CD₃OD) δ_{H} : 7.61 (1H, m, H-2), 7.41 (1H, m, H-3), 7.41 (1H, m, H-4), 7.41 (1H, m, H-5), 7.61 (1H, m, H-6), 7.69 (1H, d, *J*=15.6 Hz, H-7), 6.51 (1H, d, *J*=15.6 Hz, H-8); ¹³C NMR (150 MHz, CD₃OD) δ_{C} : 135.9 (C-1), 129.1 (C-2), 130.0 (C-3), 131.3 (C-4), 130.0 (C-5), 129.1 (C-6), 146.0 (C-7), 120.0 (C-8), 171.0 (C-9).

Orcinol (4): White powder; melting point: 106-108°C; molecular formula C₇H₈O₂ (calculated: 124.139); HRESI-MS (*m/z* 157.0829) [M+CH₃OH+H]⁺; ¹H NMR (600 MHz, CDCl₃) δ_{H} : 4.83 (1H, s, OH-1), 6.16 (1H, s, H-2), 4.83 (1H, s, OH-3), 6.23 (1H, s, H-4), 6.23 (1H, s, H-6), 2.23 (3H, s, H-7); ¹³C NMR (150 MHz, CDCl₃) δ_{C} : 156.6 (C-1), 99.9 (C-2), 156.6 (C-3), 108.7 (C-4), 140.9 (C-5), 108.7 (C-6), 21.4 (C-7).

Orcinol glucoside (5): White powder; melting point: 131-132°C; molecular formula $C_{13}H_{18}O_7$ (calculated: 286.28); HRESI-MS (m/z 309.0931) $[M+Na]^+$; 1H NMR (600 MHz, CD_3OD) δ_H : 6.43 (1H, s, H-2), 6.38 (1H, t, $J=2.4$ Hz, H-4), 6.31 (1H, s, H-6), 2.23 (3H, s, H-7), 4.87 (1H, d, $J=7.2$ Hz; H-1'), 3.39-3.49 (1H, m, H-2'), 3.39-3.49 (1H, m, H-3'), 3.39-3.49 (1H, m, H-4'), 3.39-3.49 (1H, m, H-5'), 3.92 (1H, dd, $J=1.8, 12.0$ Hz, H-6a), 3.74 (1H, dd, $J=5.4, 12.0$ Hz, H-6b); ^{13}C NMR (150 MHz, CD_3OD) δ_C : 141.2 (C-1), 111.2 (C-2), 160.0 (C-3), 102.2 (C-4), 159.2 (C-5), 109.7 (C-6), 21.6 (C-7), 100.2 (C-1'), 74.8 (C-2'), 78.0 (C-3'), 71.4 (C-4'), 77.9 (C-5'), 62.5 (C-6').

4. Acid hydrolysis and sugar identification

Compound 5 (0.5 mg) was heated in 1N HCl (300 μ l) at 80°C for 2 hours, after which the solution was extracted with ethyl acetate (1 ml x3). The aqueous layer was neutralised with NH_4OH and then dried under reduced pressure. The resulting residue was dissolved in 100 μ l of pyridine containing 10 μ mol of L-cysteine methyl ester and heated at 80°C for 1 hour. Subsequently, 6 ml of *O*-tolyl isothiocyanate was added, and the solution was heated for an additional hour. The reaction solution was then analysed by HPLC using a Cosmosil 5C18-MS-II column (4.6x150 mm), with a mobile phase of 20% acetonitrile in 0.2% TFA water, and UV detection at 254 nm. The sugars were identified as D-glucose (t_R 9.05 min) [14].

5. Results and discussion

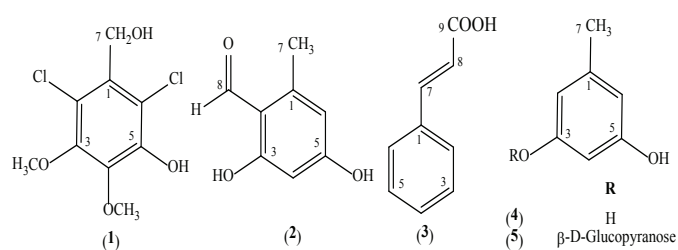


Fig. 1. Structures of compounds 1-5.

Structures of compounds 1-5 are described in Fig. 1. Compound 1 was obtained as a yellow powder from the ethyl acetate extract of *C. capitulata* L. rhizomes. The HRESIMS spectrum showed three molecular pseudo-ion peaks at (m/z 250.9875, 252.9777, 254.9749) $[M-H]^-$ with an approximation ratio 9:6:1, which were characteristic signals for the presence of two chlorines in the molecular formula and identified as $C_9H_7O_4Cl_2$. Combination 1H NMR and HSQC spectra of compound 1 exhibited a

singlet signal of oxymethylene at δ_H 4.86 (2H, s, H-7) and two singlet signals characteristic for the presence of two methoxyls at δ_H 3.89 (3H, s, OCH_3 -3), 3.88 (3H, s, OCH_3 -4). In addition, the ^{13}C -NMR and HSQC spectra of compound 1 displayed a total nine carbon signals, including one methylene ($1 \times CH_2$) at δ_C 60.1 (C-7), two methoxyls ($2 \times OCH_3$) at δ_C 61.5 (C-3), 61.3 (C-4), and six quaternary carbons (in which three carbons bonded with oxygen atoms) at δ_C 132.7 (C-1), 149.9 (C-3), 143.6 (C-4), 147.9 (C-5), 120.9 (C-2), 119.4 (C-6). The positions of functional groups were indicated by the correlations between H-7 and C-6, C-1, C-2 at δ_H 4.86 (2H, s, H-7)/ δ_C 119.4 (C-6), 132.7 (C-1), 120.9 (C-2), together with the correlations between two methoxyls (OCH_3 -3), (OCH_3 -4) and C-3, C-4 at δ_H 3.89 (3H, s, OCH_3 -3)/ δ_C 61.5 (C-3), 3.88 (3H, s, OCH_3 -4)/ δ_C 61.3 (C-4) on the HMBC spectrum. Spectral data (1D, 2D-NMR, HRESI-MS) of compound 1 was compared with chlorine-containing phenoloid derivatives, which were previously reported in the literature [15-19], which allowed the identification of the linkage positions of two methoxyls at C-3, C-4 but were not substituted at C-3, C-5. Therefore, compound 1 was determined to be 2,6-dichloro-5-hydroxy-3,4-dimethoxybenzyl alcohol.

Compound 2 was obtained from the ethyl acetate extract as a white powder. The 1H NMR spectrum of compound 2 exhibited characteristic resonance signals including: one singlet of proton in the aldehyde group (-CHO) at δ_H 10.0 (1H, s, H-8), one singlet of proton in the hydroxyl group at δ_H 12.37 (1H, s, H-3), and two singlet signals of two aromatic protons at δ_H 6.21 (1H, s, H-4), 6.21 (1H, s, H-6) together with one methyl singlet at δ_H 2.52 (3H, s, H-7). In addition, spectral analysis ^{13}C -NMR and HSQC spectra of compound 2 were exposed a total of eight carbon signals, including one aldehyde at δ_C 192.9 (C-8), one methyl ($1 \times CH_3$) at δ_C 18.2 (C-7), two methines at δ_C 101.3 (C-4), 110.6 (C-6), and four quaternary carbons (in which two carbons bonded to oxygen atoms) at δ_C 113.6 (C-2), 166.3 (C-3), 144.9 (C-1), and 163.4 (C-5). The positions of groups were indicated by the correlations on the HMBC spectrum between H-7 and C-1, C-2, C-6 at δ_H 2.52 (3H, s, H-7)/ δ_C 144.9 (C-1), 113.6 (C-2), 110.6 (C-6); between H-8 and C-2, C-3 at δ_H 10.0 (1H, s, H-8)/ δ_C 113.6 (C-2), 166.3 (C-3). These data demonstrated the linkage positions of methyl and aldehyde groups at C-1 and C-2, respectively. The molecular formula of compound 2 was suggested as $C_8H_8O_3$, which was inferred from the molecular positive ion peak at HRESI-MS (m/z 191.0315) $[M+K]^+$. Compound 2 was determined as

O-orsellinaldehyde through its spectrometric analysis and comparison with previously published data [20]. This is the first time compound 2 has been isolated from this plant.

Compound 3 was segregated from ethyl acetate as a white powder. The ^1H NMR spectrum of compound 3 revealed resonance signals of olefinic protons at δ_{H} 7.69 (1H, d, $J=15.6$ Hz), 6.51 (1H, d, $J=15.6$ Hz) with a large coupling constant ($J=15.6$ Hz). This data demonstrated the configuration of a double bond as *trans*. In addition, the ^1H NMR spectrum of 3 exhibited resonance signals of aromatic protons at δ_{H} 7.61 (2H, m, H-2, 6), 7.41 (3H, m, H-3, 4, 5). Combination ^{13}C -NMR and HSQC spectra of 3 displayed a total of nine carbon signals, including one carboxyl (-COOH) at δ_{C} 171.0 (C-9), seven methines at δ_{C} 129.1 (C-2), 130.0 (C-3), 131.3 (C-4), 130.0 (C-5), 129.1 (C-6), 146.0 (C-7), 120.0 (C-8), and one quaternary carbon at δ_{C} 135.9 (C-1). The correlations between H-8 and C-1, C-9 at δ_{H} 6.51 (1H, d, $J=15.6$ Hz)/ δ_{C} 135.9 (C-1), 171.0 (C-9); H-7 and C-1, C-2, C-6, C-8 at δ_{H} 7.69 (1H, d, $J=15.6$ Hz)/ δ_{C} 135.9 (C-1), 129.1 (C-2), 129.1 (C-6), 120.0 (C-8) in the HMBC were demonstrated linkage position of double bond as well as carboxyl group in the molecules structure of compound 3. Based on 1D, 2D-NMR spectra data, HRESIMS and compared with the literature [21], 3 was determined as *trans*-cinnamic acid. This is the first time compound 3 has been isolated from this plant.

Compound 4 was isolated from ethyl acetate extract as a white powder. Combination ^1H -NMR, and HSQC spectra of 4 appeared the signals of aromatic protons at δ_{H} 6.23 (1H, s, H-2), 6.16 (1H, s, H-4), 6.23 (2H, s, H-6), together with a methyl singlet at δ_{H} 2.23 (3H, s, H-7). In addition, the ^{13}C -NMR, and HSQC spectra of 4 were exhibited seven carbons including: one methyl ($1\times\text{CH}_3$) at δ_{C} 21.4 (C-7), three methines ($3\times\text{CH}$) at δ_{C} 108.7 (C-2), 99.9 (C-4), 108.7 (C-6), and three quaternary carbons (in which two carbons bonded to oxygen atoms) at δ_{C} 140.9 (C-1), 156.6 (C-5), 156.6 (C-3). The linkage position of groups was indicated by the correlations on the HMBC spectrum between H-7 and C-1, C-2, C-6 at δ_{H} 2.23 (3H, s, H-7)/ δ_{C} 140.9 (C-1), 108.7 (C-2), 108.7 (C-6). These data demonstrated that the linkage position of the methyl attached at the C-1 of the benzene ring. Moreover, the correlations between H-3 (OH-3) and C-2, C-3, C-4 at δ_{H} 4.83 (1H, s, OH-3)/ δ_{C} 108.7 (C-2), 156.6 (C-3), 99.9 (C-4), together with H-5 (OH-5) and C-2, C-3, C-4 at δ_{H} 4.83 (1H, s, OH-3)/ δ_{C} 108.7 (C-2), 156.6 (C-3), 99.9 (C-4) were confirmed the linkage positions of two hydroxy at C-3 and C-5. Based on 1D, 2D-NMR,

HRESIMS spectral data and comparison with the reference [22] allowed identifying 4 as orcinol. This is the first time compound 4 has been isolated from this plant.

Compound 5 was separated from the ethyl acetate extract as a white powder. The ^1H , and ^{13}C -NMR spectra of 5 were similar to those of compound 4, except for the appearance of one anomeric proton at δ_{H} 4.87 (1H, d, $J=7.2$ Hz; H-1')/ δ_{C} 100.2 (C-1'), which were the characteristic signals and demonstrated for the presence of sugar moiety. Spectral data indicated that 5 was a phenolic glycoside with a phenolic aglycone moiety linked to a β -glucopyranose unit. Based on 1D and 2D-NMR, HRESIMS data, and comparison with the literatures were reported [23], compound 5 was identified as orcinol glucoside.

6. Conclusions

From the ethyl acetate extract of *C. capitulata* L. rhizomes, five compounds were isolated, including a new dichlorine-containing phenoloid identified as 2,6-dichloro-5-hydroxy-3,4-dimethoxybenzyl alcohol, and four known compounds: *O*-orsellinaldehyde (2), cinnamic acid (3), orcinol (4), and orcinol glucoside (5). Their structures (1-5) were elucidated using HRESIMS and NMR. Compound 1 is a new natural product, while compounds (2-4) are known compounds reported for the first time from the rhizomes of this species.

CRedit author statement

Viet Hau Dang: Data curation, Principal investigator, Writing original draft; Thi Trang Phan: Investigation, Formal analysis; Thi Hue Nguyen: Conceptualisation, Methodology, Formal analysis; Thi Thu Trang Nguyen: Investigation, Formal analysis; Thi May Nguyen: Conceptualisation, Methodology, Formal analysis; Thi Loan Le: Conceptualisation, Formal analysis; Thi Hong Anh Nguyen: Methodology, Formal analysis; Minh Khoi Nguyen: Investigation, Formal analysis, Resources; Van Tai Nguyen: Supervision, Funding acquisition, Data curation, Principal investigator.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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