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Glucosides from *Curculigo orchioides*

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Abstract

From the rhizomes of *Curculigo orchioides* two phenolic glucosides named orchiosides A and B were isolated besides four known compounds and their structures were elucidated by the combination of 2D-NMR analysis, mass spectrometry and chemical evidences.

Keywords: *Curculigo orchioides*; Amaryllidaceae; Orchioside A; Orchioside B

1. Introduction

The rhizomes of *Curculigo orchioides* Gaerten (Amaryllidaceae) are reported to be used for the treatment of decline in strength, jaundice, asthma and to enhance phagocytic activity of macrophages (Lakshmi et al., 2003). A literature survey revealed the plant to possess cycloartane saponins (Misra et al., 1990; Mehta and Gawarikar, 1991; Xu et al., 1992a,b; Xu and Xu, 1992c), phenolic glycosides and chlorophenyl glucosides (Kubo et al., 1983; Garg et al., 1989; Xu and Xu, 1992d). In the present communication, we report the isolation of two new glucosides and four known compounds and the elucidation of their structures using ^{1}H NMR, ^{13}C NMR, DQF COSY, HMBC and HMQC techniques.

2. Results and discussion

Orchioside A (**1**), a light yellow amorphous solid, was deduced to have a molecular formula of $C_{22}H_{26}O_{11}$ from Q-TOF MS $[M-H]^-$ ion peak at *m/z* 465 and ^{13}C NMR (Table 1). It exhibited an UV absorption maximum at 294 nm, which implied the presence of a highly conjugated double bond system. The IR spectrum showed the presence of hydroxyl group(s) (3393 cm^{-1}), carbonyl group(s) (1724 cm^{-1}) and aromatic ring(s) (1602, 1495 cm^{-1}). The ^{1}H NMR spectrum of **1** (Table 1) showed the presence of one 1,2,3,4-tetra substituted aromatic ring, one 1,2-disubstituted aromatic ring and one benzyl ester group. A characteristic doublet signal at δ 4.85 with coupling constant of 7.5 Hz was ascribed to the anomeric proton in a β-glucopyranose unit. The ^{13}C NMR spectrum had signals for one carbonyl carbon, two aromatic rings, one benzylic carbon, one hexose unit and two aromatic methyl ethers. The sugar moiety was identified as D-glucose by TLC comparison of the acid hydrolysis product of **1** with authentic sugar samples.

An HMBC experiment determined the linkage between the benzyl group, aromatic acyl moiety and sugar unit. The carbonyl carbon signal (δ 166.3) ascribed to the aromatic acyl part showed a cross-peak with benzylic protons (δ 5.41 and 5.38), establishing the linkage between the two aromatic moieties. The benzylic proton signals showed cross-peaks also with carbon signals of δ 125.8 (*q*), 155.7 (*q*) and 129.0 (*d*), signifying that this aromatic ring carries an *ortho*-oxy substitution.

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Table 1
NMR data of orchioside A (**1**) (DMSO-d_6, ^{1}H: 500 MHz, ^{13}C: 125 MHz)

Carbon no.	δ_c	HMQC (δ_H)	HMBC
1	125.8	–	H-7, H-3, H-5
2	155.7	–	H-7, H-6, H-4, H-3, H-1″
3	115.7	7.15(1H, *d*, *J* = 8 Hz)	H-5
4	130.0	7.29(1H, *t*, *J* = 8 Hz)	H-6
5	122.6	7.04(1H, *t*, *J* = 8 Hz)	H-3
6	129.0	7.40(1H, *d*, *J* = 6.5 Hz)	H-4, H-7
7	62.5	5.41,5.38(1H × 2, AB *d*, *J* = 13.5 Hz)	H-6
1′	119.5	–	H-5′
2′	145.5	–	H-4′, 2′ (OMe)
3′	144.7	–	H-5′
4′	118.6	6.88 (1H, *d*, *J* = 9 Hz)	–
5′	108.3	6.66 (1H, *d*, *J* = 9 Hz)	–
6′	149.4	–	H-4′, H-5′, 6′ (OMe)
7′	166.3	–	H-7
1″	101.9	4.85(1H, *d*, *J* = 7.5 Hz)	H-2″ and/or H-3″
2″	74.2	3.29*(1H)	–
3″	77.4	3.29*(1 H)	–
4″	70.6	3.18(1H, *t*, *J* = 9.0 Hz)	H-2″ and/or H-3″
5″	77.9	3.33(1H, *m*)	H-1″, H-6″
6″	61.6	3.48* (1 H), 3.70*(1H)	–
OMe (2′)	61.4	3.70(3H, *s*)	–
OMe (6′)	57.1	3.70(3H, *s*)	–

* Overlapped by other signals.

Examination of HMQC spectrum showed that the carbon signal of δ 129.0 corresponds to the proton signal of δ 7.40; the proton was in turn a part of 4 continuous aromatic protons signals, the other three being located at δ 7.04, 7.29 and 7.15 (DQF-COSY). The carbon signal at δ 155.7 showed cross-peak with the anomeric proton signal (δ 4.85), establishing the point of attachment of the glucose unit. For the acyl substituted aromatic ring, structures having *ortho*-hydroxy carbonyl substitution pattern could be rejected based on the absence of downfield signal (~10 ppm). The downfield shift of one methoxy carbon (δ 61.4) suggested that it must have two *ortho* substituents. Of the two possible structures, the one having 2,3,4-trioxygenation pattern could be ruled out based on the absence of any carbon signal in δ 130–135 ppm region. This allowed us to settle for the other possible structure, which was fully in agreement with the HMBC spectroscopic results. Hence, the structure of orchioside A (**1**) was established as 2-β-D-glucopyranosyloxy benzyl 3-hydroxy-2,6-dimethoxy benzoate (see Fig. 1).

Orchioside B (**2**), a white amorphous solid was deduced to have a molecular formula $C_{23}H_{26}O_{10}$ from Q-TOF MS $[M-H]^-$ ion peak at *m*/*z* 461 and ^{13}C NMR (Table 2). The UV absorption maximum at 279 nm and IR absorptions at 3394, 1663, 1601 and 1516 cm^{-1} indicated the presence of an aryl ketone moiety having hydroxyl group(s). The ^{1}H NMR spectrum showed signals for seven aromatic protons, seven sugar

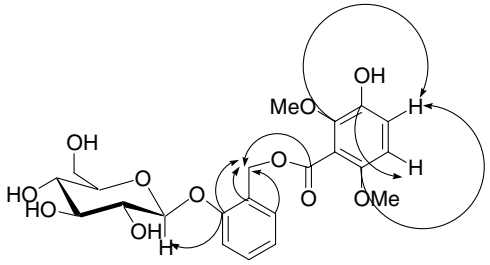


Fig. 1. Selected HMBC (C → H) of orchioside A (**1**).

Table 2
NMR data of orchioside B (**2**) (DMSO-d_6, ^{1}H: 500 MHz, ^{13}C: 125 MHz)

Carbon no.	δ_c	HMQC (δ_H)	HMBC
1	78.0	4.50 (1H, *d*, *J* = 6 Hz)	H-2, H-2′, H-6′
2	74.4	4.36 (1H, *m*)	H-1, H-3, H-4
3	26.8	1.86, 1.72 (2H, *m*)	H-1, H-4
4	34.0	2.92 (2H, *m*)	H-3
5	198.3	–	H-3, H-4, H-2″, H-6″
1′	131.2	–	H-1, H-5′, H-6′
2′	116.2	6.84 (1H, *s*)	–
3′	146.0	–	H-5′
4′	145.9	–	H-2′
5′	116.1	6.70 (1H, *s*)	–
6′	119.8	6.70 (1H, *s*)	H-2′
1″	128.6		H-3″, H-5″
2″	131.2	7.78 (1H, *d*, *J* = 8.5 Hz)	–
3″	116.3	6.80 (1H, *d*, *J* = 8.5 Hz)	–
4″	163.6	–	H-2″, H-6″
5″	116.3	6.80 (1H, *d*, *J* = 8.5 Hz)	–
6″	131.2	7.78 (1H, *d*, *J* = 8.5 Hz)	–
1‴	96.5	4.67 (1H, *d*, *J* = 8.5 Hz)	H-2
2‴	73.4	3.40 (1H, *t*, *J* = 9 Hz)	H-3‴, H-1
3‴	75.0	3.30 (1H, *t*, *J* = 9 Hz)	H-2‴
4‴	71.3	3.11*(1H)	H-3‴, H-6‴
5‴	79.3	3.23 (1H, *m*)	H-1‴
6‴	61.7	3.66 (1H, *d*, *J* = 11.5 Hz), 3.46 (1H, *dd*, *J* = 12.0, 5.5 Hz)	–

* Overlapped by other signals.

protons and six aliphatic protons, corroborated by DQF-COSY spectrum. The ^{1}H signals at δ 7.78 and 6.80 (each 2H *d*, *J* = 8.5 Hz) along with appropriate carbon signals and additional ^{13}C NMR signals at δ 198.3, 163.6 and 128.6 indicated the presence of a 4-hydroxylbenzoyl group. The DEPT ^{13}C NMR spectrum showed three other substituted aromatic carbon signals at δ 131.2, 145.9 and 146.0. From chemical shift consideration the last two carbon signals should be oxygenated carbons *ortho* to each other. The HMQC study showed the remaining three carbons were unsubstituted. The appearance of the proton signals as apparent 1H singlet and 2H singlets suggested that a 3,4-dihydroxyphenyl grouping might be present. Indeed the HMBC experiment showed cross-peaks for carbon signal of δ 131.2 with the three proton signals at δ 6.84 (1H, *s*) and 6.70

(2H, s), supporting the above consideration. ^{1}H NMR signals in the aliphatic region showed the presence of two protons [δ 4.50 (1H, *d*, $J = 6$ Hz) and 4.36 (1H, *m*)], which from HMQC consideration must be attached to two oxygenated carbon atoms (δ 78.0 and 74.4). The remaining four protons exist as two methylene groups, as deduced from HMQC and DEPT experiments. From the DQF-COSY experiment, one could arrive at the partial structure of the aliphatic portion as –CH (OR_1)–CH (OR_2)–CH_2–CH_2–. In the HMBC experiment, the carbon signal at δ 78.0 (δ_H 4.50) showed cross-peak with H-2′ (δ 6.84) and most likely H-6′ (δ 6.70) of the aromatic ring. Hence, this oxygenated carbon was directly attached to the aromatic ring. Similarly one of the two methylene groups was attached to the carbonyl carbon (δ 198.3) as the protons [δ 2.92 (2H, *m*)] are deshielded compared to the other methylene protons and gave cross-peaks with the carbonyl carbon in HMBC spectrum. These evidences helped us to identify the linkage in the aliphatic moiety. The ^{1}H NMR signals of sugar protons showed the presence of a β-D-glucosyl unit. The linkage between the sugar and the aglycone parts was established from HMBC spectrum. As the H-1 signal showed cross-peak with C-2‴ and H-2 with C-1‴ albeit weakly, the glucose unit must be fused to C-1 and C-2 of aglycone through its 1- and 2-OH groups. Thus, the structure of **2** is similar to that of curcapicycloside (Chang et al., 1999) except for the absence of one hydroxyl group at C-3‴ of the aromatic ring. Indeed the ^{13}C NMR values for **2** compare well with the values reported for curcapicycloside tetramethyl ether except for those of the aromatic moieties. This new compound is named as orchioside B (see Fig. 2).

The other constituent (**3**) was deduced to have a molecular formula $C_{22}H_{26}O_{12}$ from Q-TOF MS $[M - H]^-$ ion peak at *m/z* 481 and ^{13}C NMR. The UV absorption maximum at 292 nm and IR absorptions at 3392, 1724, 1644 and 1498 cm^{-1} indicated its similarity to **1**. ^{1}H and ^{13}C NMR spectra showed it to possess all the proton and carbon signals of the tetra substituted aromatic acyl moiety similar to those of **1**. The sugar moiety in **3** was demonstrated to be D-glucose through hydrolysis experiment. The ^{1}H NMR spectrum of **3** indicated the presence of an ABX pattern in the benzylic component. This along with molecular weight consideration showed the presence of one additional hydroxyl group either at C-4 or C-5 in **3**. A ^{13}C NMR signal at δ 153.20 supported this conclusion. DQF-COSY and HMBC examination then established that the hydroxyl group is present at C-5. Thus, structure of **3** was determined to be 2-β-D-glucopyranosyloxy-5-hydroxy benzyl 3-hydroxy-2,6-dimethoxy benzoate. During the preparation of the manuscript identical spectral data was reported for the compound curculigoside C (Fu et al., 2004).

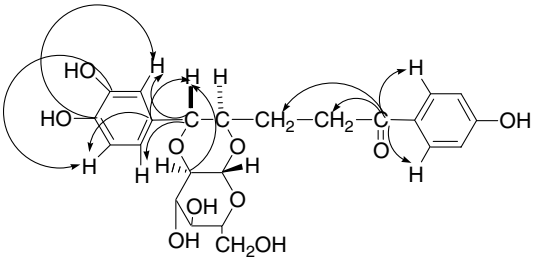


Fig. 2. Selected HMBC (C → H) of orchioside B (**2**).

3. Experimental

3.1. General

Mps: uncorr. Optical rotations were measured on a JASCO P-1020 polarimeter; IR spectra were taken on a JASCO-FT-IR-model 410 spectrometer; UV spectra were taken on CARY|1E| UV–vis spectrometer. NMR spectrum including DQF-COSY, HMBC and HMQC spectra were recorded using Bruker DRX (500 MHz) in DMSO-d_6. Q-TOF-MS was performed on a Q-TOF-Micromass spectrometer. TLC was carried out on silica gel 60 F_{254} (Merck) using $CHCl_3$–MeOH–H_2O (13:7:1) as developing solvent and the spots were visualized by spraying with Liebermann-Burchard reagent followed by heating at 120 °C. For HPLC, X-Terra preparative reverse phase C_{18} column (10 μm, 19 × 300 mm) was used.

3.2. Plant materials

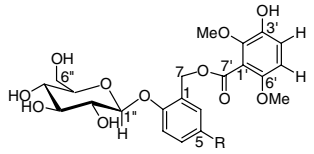
Rhizomes of *Curculigo orchioides* were supplied by United Chemicals Ltd, Kolkata who maintain a voucher specimen at their Herbarium. Dr. N.D. Paria, Department of Botany, University of Calcutta identified the plant materials.

3.3. Extraction and isolation

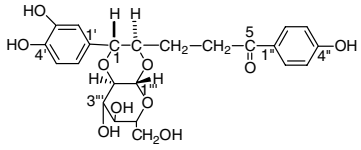
Powdered air-dried rhizomes of *C. orchioides* (10 kg) were extracted with MeOH (5 L × 3) at room temperature for a total of 10 days. After filtration and removal of solvent by evaporation in vacuum, a residue (300 g) was obtained, which was suspended in H_2O (1 L). The suspension was washed with EtOAc (1 L × 2) to remove less polar materials. It was then extracted with *n*-BuOH (1 L × 3) to separate the more polar materials. Evaporation of the solvent gave the *n*-BuOH soluble residue (120 g).

The *n*-BuOH soluble residue was subjected to Diaion HP-20 column chromatography eluting with increasing concentration of MeOH in H_2O (1:1 → 100%) to give three fractions (1a–3a). Fr. 1a (16 g) was subjected to

silica gel cc eluting with a $CHCl_3$–MeOH gradient (1:0 → 3:2) to give four fractions (Fr.1b– 4b). Fr. 1b (100 mg) was purified by preparative ODS-HPLC with MeOH:H_2O = 2:3 furnishing orchioside A (**1**) (32 mg). Fr. 2b (250 mg) was further separated by preparative ODS-HPLC with MeOH:H_2O = 7:13 to give orchioside B (**2**) (41 mg) and curculigoside C (**3**) (34 mg). Crystallization of Fr. 3b (200 mg) with a mixture of $CHCl_3$ and MeOH gave orcinol-3-*O*-β-D glucoside (**4**) (120 mg) (Li et al., 2003). Repeated cc of Fr. 4b (300 mg) over silica gel using $CHCl_3$–MeOH (7:3) afforded corchioside A (**5**) (180 mg) (Garg et al., 1989) which was crystallized from MeOH. Another fraction obtained in the impure state was further purified by preparative ODS-HPLC with MeOH:H_2O = 3:7 to afford anacardoside (41 mg) (**6**) (Gil et al., 1995).



1. R = H
3. R = OH



2.

3.4. Orchioside A (*1*)

Light yellow amorphous solid, $[\alpha]_D^{26}$ −27.9 (*c* 1.22, MeOH); Q-TOF-MS *m/z* 465 $[M-H]^-$; UV λ^{MeOH} nm (log ε): 205 (4.6), 220 (4.3), 294 (3.5); IR ν^{KBr} cm^{-1} 3393, 1724, 1602 and 1495. ^1H and ^{13}C NMR (Table 1).

3.5. Orchioside B (*2*)

White amorphous solid, m.p. 65–68 °C, $[\alpha]_D^{26}$ +38.8 (*c* 0.75, MeOH); Q-TOF-MS *m/z* 461 $[M-H]^-$; UV λ^{MeOH} nm (log ε): 205 (4.7), 220 (4.3), 279 (4.3); IR $\nu^{KBr}cm^{-1}$ 3394, 1663, 1601 and 1516. ^1H and ^{13}C NMR (Table 2).

3.6. Curculigoside C (*3*)

White solid, m.p. 162–165 °C, $[\alpha]_D^{26}$ −21.8 (*c* 1.8 pyridine); Q-TOF-MS *m/z* 481 $[M-H]^-$; UV λ^{MeOH} nm (log ε): 205 (4.7), 220 (4.3), 292 (3.8); IR ν^{KBr} cm^{-1} 3392, 1724, 1644 and 1498. ^1H NMR δ 6.99 (1H, *d*, *J* = 9 Hz), 6.88 (1H, *d*, *J* = 9 Hz), 6.82 (1H, *d*, *J* = 3 Hz), 6.66 (2H, overlapping signals), 5.33 (2H, *s*), 4.64 (1H, *d*, *J* = 7 Hz), 3.70 (3H, *s*), 3.48 (1H, *dd*, *J* = 5.5, 12 Hz), 3.24 (3H, *m*), 3.16 (1H, *t*, *J* = 9 Hz). ^{13}C NMR δ 127.38 (C-1), 148.51 (C-2), 118.12 (C-3), 115.89 (C-4), 153.20 (C-5), 115.54 (C-6), 62.42 (C-7), 119.53 (C-1′), 145.50 (C-2′), 144.79 (C-3′), 118.60 (C-4′), 108.20 (C-5′), 149.37 (C-6′), 166.25 (C-7′), 103.42 (C-1″), 74.25 (C-2″), 77.86 (C-3″), 70.68 (C-4″), 77.43 (C-5″), 61.72 (C-6″), 57.01 (OMe, 6′), 61.38 (OMe, 2′).

3.7. Orcinol-3-O-β-D glucoside (*4*)

White crystalline solid, m.p. 115–118 °C. Spectral data was same as reported (Li et al., 2003).

3.8. Corchioside A (*5*)

White crystalline solid, m.p. 210–212 °C. Spectral data was same as reported (Garg et al., 1989).

3.9. Anacardoside (*6*)

Crystalline solid, m.p. 117–120 °C. Spectral data was same as reported (Gil et al., 1995).

3.10. Acid hydrolysis of *1* and *2*

Each compound (5 mg) was refluxed in 1 ml of 2 N aqueous HCl (with addition of some MeOH to ensure dissolution) for 3 h on a water bath. MeOH was then evaporated under reduced pressure and the product after dilution with H_2O was neutralized with Ag_2CO_3, filtered and concentrated. The concentrated part was subjected to TLC on silica gel plates using EtOAc:-MeOH:H_2O:AcOH (13:4:3:3) as developing solvent against standard sugar samples.

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