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NOTE

A new phenolic glycoside and cytotoxic constituents from *Celosia argentea*

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A new phenolic glycoside, 4-*O*-β-D-apifuranosyl-(1 → 2)-β-D-glucopyranosyl-2-hydroxy-6-methoxyacetophenone (**2**) and 11 known compounds were isolated from the MeOH extract of the plant *Celosia argentea*. The structures of the compounds were elucidated on the basis of spectroscopic analysis and chemical methods. Among the isolated compounds, stigmasterol (**10**) showed moderate inhibitory activities against SGC-7901 and BEL-7404 cells.

Keywords: Amaranthaceae; *Celosia argentea*; phenolic glycoside; cytotoxicity; SGC-7901 cells; BEL-7404 cells

1. Introduction

Celosia argentea (Amaranthaceae) is a kind of annual herbaceous plant. Its seeds are used as a Chinese herbal medicine [1]. Early researches on *C. argentea* indicated that its seeds were pharmaceutically active on eyes and displayed hepatoprotective [2], protecting oxidative injury of lens [3], anti-hyperglycemic [4], and anti-metastatic effects [5]. The water and alcoholic extracts of the leaves of the title plant also exhibited antimicrobial activities [6]. Betalains [7] and peptides [8] are found from the seeds of *C. argentea*. Betalains [9], indole derivatives, were suggested to have antioxidant activity *in vivo* and radioprotective activity *in vivo* and *in vitro*. Cyclic peptides were reported to display antimitotic activity, which played important roles in mitosis, cell signaling, and motility in eucaryotes [8].

In this paper, we describe the isolation and structural elucidation of a new phenolic glycoside, 4-*O*-β-D-apifuranosyl-(1 → 2)-β-D-glucopyranosyl-2-hydroxy-6-methoxyacetophenone (**2**), along with 11 known compounds: 4-*O*-β-D-glucopyranosyl-2-hydroxy-6-methoxyacetophenone (**1**) [10], eugenyl-*O*-β-D-glucopyranoside (**3**) [11], sucrose (**4**) [12], quercetin-3-*O*-β-D-glucopyranoside (**5**) [13], isorhamnetin-3-*O*-β-D-glucopyranoside (**6**) [14], rhamnazin-3-*O*-β-D-glucopyranoside (**7**) [15], isorhamnetin-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (**8**) [16], β-sitosterol (**9**) [17], stigmasterol (**10**) [18], stigmasterol-3-*O*-β-D-glucopyranoside (**11**) [19], and

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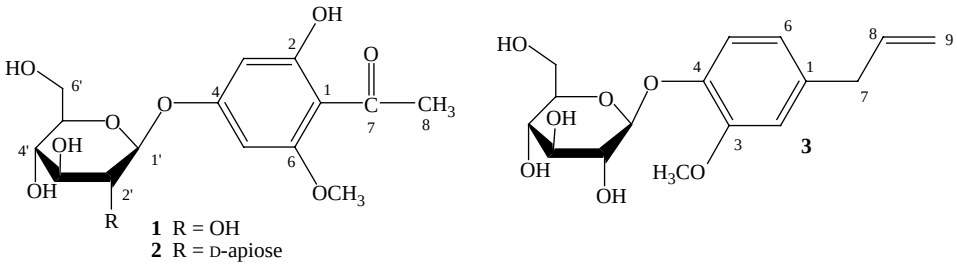

Figure 1. Structures of compounds **1**–**3**.

oleanolic acid (**12**) [20] (Figure 1). Then, we test their cytotoxicities against SGC-7901 and BEL-7404 cells. It was found that compound **10** had moderate cytotoxicity against both cells at the measured concentration.

2. Results and discussion

Compound **2** was obtained as a colorless amorphous powder, whose molecular formula was determined to be $C_{20}H_{28}O_{13}$ (seven unsaturation degrees) by observation of a quasi-molecular ion peak at *m/z* 475.1450 $[M - H]^-$ in the positive ion HR-FAB mass spectrum. The IR spectrum indicated the presence of a hydroxyl group (3390 cm^{-1}), a conjugated ketone (1693 cm^{-1}), and a benzene ring (1601 cm^{-1}). Acid hydrolysis afforded a mixture of sugars, which were identified as D-glucose and D-apiose detected by HPTLC. The ^1H NMR spectrum of **2** showed aromatic protons at δ_H 6.75 (1H, d, $J = 1.8$ Hz) and 6.51 (1H, d, $J = 1.8$ Hz), a methyl signal at δ_H 2.50 (3H, s), a methoxyl group at δ_H 3.76 (3H, s), and two anomeric protons at δ_H 6.57 (1H, s) and 5.59 (1H, d, $J = 7.8$ Hz). The ^1H NMR spectrum of **2** also revealed the presence of an intramolecularly hydrogen-bonded, phenolic hydroxyl group (δ_H 14.29) and permitted the assignment of a tetra-substituted benzene ring. The ^{13}C NMR spectrum of **2** gave a carbonyl resonance at δ_C 203.7, a methyl signal at δ_C 33.1, two anomeric carbons at δ_C 110.5, 99.9, and an aromatic ring signal at δ_C 167.4, 164.9, 163.6, 107.3, 97.5, and 92.4 (Table 1). Because of the intramolecular hydrogen bond, the ketone position should be presumed to be located *ortho* to the hydroxyl group of the aromatic ring. Comparison of ^1H and ^{13}C NMR spectral data of **2** with those reported for compound **1** indicated that a D-apiose moiety was located at C-2′ in **2** instead of the hydroxy group in **1** (Figure 1). In the HMBC experiment, the hydrogen-bonded phenolic hydroxyl proton afforded all of the possible two- and three-bond correlations, leading to the assignment of the carbon signals for C-1, C-2, and C-3. Furthermore, the aryl proton H-3 correlated with C-1, C-2, C-4, and C-5; the aryl proton H-5 with C-1, C-3, C-4, and C-6; the methyl proton H-8 with C-1 and C-7; the methoxy protons with C-6; the anomeric proton of Glc with C-4; and the anomeric proton of Api with C-2 of Glc (Figure 2). The above HMBC correlations revealed the structure of **2** as 4-*O*-β-D-apifuranosyl-(1 → 2)-β-D-glucopyranosyl-2-hydroxy-6-methoxy-acetophenone, named as argentoside A. In the NOESY experiment (Figure 2), the key correlations between H-5 and OCH_3 at C-6, between H-1 of Glc and H-3 and H-5 were observed. Thus, the structure of **2** was determined unambiguously as shown in Figure 1.

Table 2 showed the cytotoxic activities of all compounds. Among these, compound **10** displayed significant inhibition, 86.96% against SGC-7901 cells and 82.74% against BEL-7404 cells, respectively.

Table 1. ^{1}H and ^{13}C NMR spectral data (400 and 100 MHz) of compounds **1** and **2**, in MeOD (*J*, Hz).

		1		**2**		
No.		C	H	C	H	No.
1	C	107.1	–	107.3	–	1
2	C	167.4	–	167.4	–	2
2-OH	–	–	14.31, s	–	14.29, s	3
3	CH	97.5	6.70, d, $J = 1.7$	97.5	6.75, d, $J = 1.8$	3-OCH_3
4	C	165.0	–	164.9	–	4
5	CH	92.3	6.35, d, $J = 1.7$	92.4	6.51, d, $J = 1.8$	5
6	C	163.5	–	163.6	–	6
6-OCH_3	–	55.7	3.68, s	55.9	3.76, s	7
7	C	203.7	–	203.7	–	8
8	CH_3	33.0	2.51, s	33.1	2.50, s	9
4-Glc						Glc
1′	CH	101.5	5.74, d, $J = 7.0$	99.9	5.59, d, $J = 7.8$	1
2′	CH	74.8	4.34, overlap	77.2	4.53, overlap	2
3′	CH	79.3	4.40, t, $J = 9.0$	78.9	4.38, t, $J = 9.2$	3
4′	CH	71.2	4.29, t, $J = 9.0$	71.4	4.19, t, $J = 9.2$	4
5′	CH	78.5	4.15, m	79.2	4.06, m	5
6′	CH_2	62.3	4.55, br d, $J = 12.0$ 4.36, overlap	62.3	4.50, overlap 4.29, dd, $J = 12.0, 6.0$	6
D-Apiose						
1″	CH	–	–	110.5	6.57, s	
2″	CH	–	–	78.1	4.78, br s	
3″	C	–	–	81.1	–	
4″	CH_2	–	–	75.9	4.82, d, $J = 9.2$ 4.60, d, $J = 9.2$	
5″	CH_2	–	–	66.5	4.23, overlap	

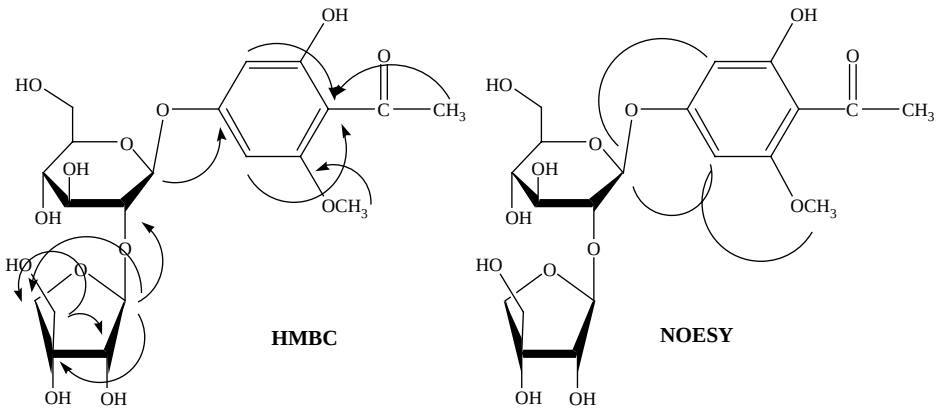

Figure 2. Key HMBC and NOESY correlations of compound **2**.

Table 2. Cytotoxic activities of compounds **1**–**12**.

Compound no.	Inhibition rate[a] (%)	Inhibition rate[b] (%)
1	–[c]	–
2	–	14.73 ± 0.05
3	–	14.82 ± 0.25
4	10.80 ± 0.19	–
5	19.36 ± 0.17	21.65 ± 0.04
6	27.00 ± 0.14	16.26 ± 0.03
7	–	18.51 ± 0.07
8	14.44 ± 0.13	29.20 ± 0.06
9	–	28.48 ± 0.01
10	86.96 ± 0.02	82.74 ± 0.11
11	–	–
12	49.05 ± 0.07	31.09 ± 0.06
5-FU[d]	85.59 ± 0.41	83.45 ± 3.28
CDDP[d]	84.77 ± 0.98	89.79 ± 1.78

Notes: Compounds' concentration is 200 μg/ml.
[a] Inhibition rate of compounds against SGC-7901 cells.
[b] Inhibition rate of compounds against BEL-7404 cells.
[c] No inhibitory activity.
[d] 5-FU diluted in 50 μg/ml and CDDP diluted in 20 μg/ml, served as positive controls.

Table 3. The cytotoxic activity of compound **10** (IC_{50}).

Compound no.	IC_{50}[a] (μg/ml ± SD)	IC_{50}[b] (μg/ml ± SD)
10	44.00 ± 0.16	52.06 ± 3.28
5-FU[c]	1.01 ± 0.04	1.45 ± 0.21
CDDP[c]	1.37 ± 0.26	0.72 ± 0.11

Notes: [a]Cytotoxicity against SGC-7901 cells.
[b] Cytotoxicity against BEL-7404 cells.
[c] Positive controls.

But compounds **9** and **11**, possessing similar structures of their aglycones, were hardly active against both cells. The reason should be due to a glucose linking at position C-3 in compound **11**, and a C=C double bond at the position C-20 in compound **9**. Compounds **5**–**8**, flavonoid glycosides, with the same skeleton but differing mainly in their substituted groups, showed inhibitory activities from 14.44 to 29.20%, except for compound **7**, which showed no inhibitory activity against SGC-7901 cells. The IC_{50} values of compound **10** were 44.00 μg/ml against SGC-7901 cells and 52.06 μg/ml against BEL-7404 cells (see Table 3).

3. Experimental

3.1 General experimental procedures

The ^{1}H, ^{13}C, DEPT, $^{1}H-^{1}H$ COSY, NOESY, HMQC, and HMBC NMR spectra were performed using a Bruker AM-400 and DRX-500 spectrometer. FAB mass spectra were recorded on a Jeol JMS-HX 110 instrument. IR spectra were recorded with a Perkin-Elmer 1750 FT-IR spectrometer and the films of all the samples were measured on KBr disks. Optical rotations were measured with a Jasco DIP-180 digital polarimeter spectrophotometer. Chromatographic stationary phase used RP-18 (40–60 μm; Merck & Co. Inc., Darmstadt, Germany), silica gel (160–200 mesh; Qingdao Oceanic Chemical Co., Qingdao, China), Sephadex LH-20 (25–100 μm; Pharmacia Fine Chemical Co. Ltd, Tokyo, Japan), and MCI-gel CHP20P (75–150 μm; Mitsubishi Chemical Industries, Ltd, Tokyo, Japan). HPLC was performed on a P-230-UV-230 (Dalian Elite Analytical Instruments Co., Ltd, Dalian, China) and HPLC column (YMC-Pack ODS-A, 250–10 μm; Kyoto, Japan). The following solvent systems were used: (a) $CHCl_3$–MeOH–H_2O (80:20:3), $CHCl_3$–MeOH–H_2O (70:30:5), and MeOH–H_2O (0–100%) for the glycosides and (b) $CHCl_3$–MeOH–H_2O (7:3:1) lower-layer 9 + 1 ml HOAc for sugars. Compounds on TLC were detected by spraying with 5% H_2SO_4, followed by heating. Sugars were detected by spraying with aniline–phthalate reagent.

5′-Fluorouracial (5-FU, ≥ 99%) (Sigma-Aldrich, Inc., St Louis, MO, USA), *cis*-Platin (CDDP, ≥ 99%) (Sigma-Aldrich, Inc.), RPMI-1640 medium (Hyclone Biochemical Products Co. Ltd, Logan, UT, USA), penicillin–streptomycin solution (Hyclone Biochemical Products Co. Ltd), Hepes (Sigma-Aldrich, Inc.), methylthiazolyldiphenyl-tetrazolium bromide (MTT) (Sigma-Aldrich, Inc.), and fetal bovine serum (Hyclone, Thermo Scientific, Tauranga, New Zealand) were all purchased from Xiamen Lulong Biotech Co. Ltd, Xiamen, China.

3.2 Plant material

C. argentea was collected from the suburb of Quanzhou, Fujian in 2003. The specimen was identified by Prof. Ke-Cuo He, College of Plant Protection, Fujian Agriculture and Forestry University. A voucher specimen (No. 4316) has been deposited in the College of Plant Protection, Fujian Agriculture and Forestry University.

3.3 Extraction and isolation

The fresh *C. argentea* (10 kg) was extracted with MeOH (2 × 15 liters) at room temperature. The extract was evaporated *in vacuo* to yield a residue, which was dissolved in water and filtered. The water soluble fraction was passed through a Diaion column and eluted with water (4 × 3 liters) and methanol (4 × 1 liters). Evaporation of the methanol eluate yielded 386 g of a brown fraction (A). Fraction A was subjected to dry column chromatography (DCC) on silica gel (3.0 kg), eluted with $CHCl_3$–MeOH–H_2O (10:2:0.2) to afford 13 fractions. Each fraction was purified by Sephadex LH-20 (MeOH–H_2O, 5–50%), RP-18 gel column chromatography (MeOH–H_2O,

10–70%), then purified by a silica gel column with $CHCl_3$–MeOH–H_2O (100:10:1–70:30:5), and finally repeatedly purified by RP-HPLC with MeOH–H_2O (60–80%) as the solvent to yield **1** (300 mg), **2** (50 mg), **3** (120 mg), **4** (31 mg), **5** (38 mg), **6** (24 mg), **7** (30 mg), and **8** (47 mg). Water insoluble fraction was treated by DCC on silica gel (1.0 kg), eluted with $CHCl_3$–MeOH (30:1–10:2) to afford **9** (23 mg), **10** (41 mg), **11** (23 mg), and **12** (19 mg).

3.3.1 Compound (2)

A colorless amorphous powder, $[\alpha]_D^{21} - 17$ ($c = 0.22$, MeOH); IR ν_{max} (liquid)/cm^{-1}: 3390, 1693, 1601, 1450, 1012, 903; ^1H and ^{13}C NMR spectral data (see Table 1); FAB-MS: *m/z* 475 $[M - H]^-$; HR-FAB-MS: *m/z* 475.1450 $[M - H]^-$ (calcd for $C_{20}H_{27}O_{13}$, 475.1452).

3.4 Acid hydrolysis

A solution of compound **2** (50 mg) was heated under reflux at 100°C in 2 M aqueous CF_3COOH (5 ml) on a water bath for 3 h. The reaction mixture was then diluted with H_2O (15 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The aqueous layer was neutralized with Amberlite IR-45 and concentrated *in vacuo* to dryness. The sugars were analyzed by comparison with authentic sample (solvent system b, $CHCl_3$–MeOH–H_2O (7:3:1) lower-layer 9 + 1 ml HOAc) on silica gel HPTLC.

3.5 Cell lines and culture

Human gastric cancer cells SGC-7901 and human hepatoma cells BEL-7404 were provided by Fujian Medical University, Fujian, China. These cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum and 10% penicillin–streptomycin solution in an atmosphere of 5% CO_2 at 37°C.

Compounds were dissolved in DMSO and diluted with the medium to the required concentrations before use. Cells grown in media containing equivalent amounts of DMSO without compound treatment served as a negative control.

3.6 MTT assay

Cancer cells were plated onto 96-well culture dishes at a density of 1×10^3 cells/well in a 180 μl medium. After plating for 24 h, cells were treated with compounds ranging from 12.5 to 200 μg/ml (using DMSO as the vehicle at a maximum concentration of 0.1%). Cells were incubated with various concentrations of the agents for 72 h, at which time 20 μl of 2 mg/ml MTT was added, and the absorbance at 570 nm was determined by a microtiter plate reader [21]. 5-FU at a concentration of 50 μg/ml and CDDP at a concentration of 20 μg/ml were used as the positive controls. Experiments were conducted in triplicate. Cytotoxicity was expressed as IC_{50} (concentration of 50% cytotoxicity, which was extrapolated from linear regression analysis of the experimental data).

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References

[1] Q. Xue, M.L. Guo, and G. Zhang, *Pharm. Care Res.* **6**, 345 (2006).
[2] K. Hase, S. Kadota, P. Basnet, T. Namba, and T. Takahashi, *Phytother. Res.* **10**, 387 (1996).
[3] X.R. Huang, M.X. Qi, Z.Y. Wang, and Y. Wang, *Chin. J. Clin. Pharm. Ther.* **9**, 441 (2004).
[4] J.J. Shan, J.W. Ren, J. Yang, and Y.M. Zhao, *Chin. Pharm. J.* **40**, 1230 (2005).
[5] Y. Hayakawa, H. Fujii, K. Hase, Y. Ohnishi, R. Sakukawa, S. Kadota, T. Namba, and I. Saiki, *Biol. Pharm. Bull.* **21**, 1154 (1998).

[6] A. Gnanamani, K.P. Shanmuga, N. Radhakrishnan, and M. Babu, *J. Ethnopharmacol.* **86**, 59 (2003).
[7] T. Degenkolb, Y. Cai, J. Schmidt, H. Corke, and W. Schliemann, *Phytochemistry* **58**, 159 (2001).
[8] H. Suzuki, H. Morita, S. Iwasaki, and J.I. Kobayashi, *Tetrahedron* **59**, 5307 (2003).
[9] T. Frank, F.C. Stintzing, R. Carle, I. Bitsch, D. Quaas, G. Strass, R. Bitsch, and M. Netzel, *Pharmacol. Res.* **52**, 290 (2005).
[10] D. Prasad, *Fitoterapia* **70**, 266 (1999).
[11] T. Fujita, M. Nakayama, and A. Perilloside, *Phytochemistry* **31**, 3265 (1992).
[12] D.M. Grant, J.C. Facelli, D.W. Alderman, and M.H. Sherwood, *Nuclear Magnetic Shieldings and Molecular Structure J.A* (Kluwer, Dordrecht, 1993), p. 367.
[13] C. Morand, C. Manach, V. Crespy, and C. Remesy, *Free Radic. Res.* **33**, 667 (2000).
[14] W. Mullen, B. Aurelie, A.J. Stewart, and A. Crozier, *J. Chromatogr. A* **1058**, 163 (2004).
[15] H.Y. Hsu, S.F. Tsang, K.W. Lin, S.C. Yang, and C.N. Lin, *Food Chem. Toxicol.* **46**, 2394 (2008).
[16] S. Frison-Norrie and P. Sporns, *J. Agric. Food Chem.* **50**, 2782 (2002).
[17] L. Christiansen, M. Karjalainen, T. Seppänen-Laakso, R. Hiltunen, and J. Yliruusi, *Int. J. Pharm.* **254**, 155 (2003).
[18] V.D.P. Nair, I. Kanfer, and J. Hoogmartens, *J. Pharm. Biomed.* **41**, 731 (2006).
[19] K. Murofushi-Murakami, K. Nakamura, J. Ohta, A. Suzuki, and H. Murofushi, *J. Biol. Chem.* **34**, 16719 (1987).
[20] J. Liu, *J. Ethnopharmacol.* **49**, 57 (1995).
[21] T. Miyase, H. Saitoh, and K. Shiokawa, *Chem. Pharm. Bull.* **43**, 466 (1995).