*ORIGINAL ARTICLE*

A C G publications

*Rec. Nat. Prod.* 6:3 (2012) 237-241

records of natural products

# Two Cytotoxic Coumarin Glycosides from the Aerial Parts of *Diceratella elliptica* (DC.) Jonsell Growing in Egypt

**Mona M. Marzouk*[1], Ahmed Elkhateeb[*], Lamyaa F. Ibrahim, Sameh R. Hussein and Salwa A. Kawashty**

*Phytochemistry and Plant Systematic Department, National Research Centre, Dokki, Giza, Egypt, 12311*

*(Received March 12, 2011; Revised June 28, 2011; Accepted January 15, 2012)*

**Abstract:** Two new coumarin glycosides, 6-methoxy-5,7-dihydroxy-3,4-dihydrocoumarin-8-*C*-glucopyranoside (**1**) and 5-vinyl-6,7-dimethoxy-3,4-dihydrocoumarin-8-*C*-glucopyranoside (**2**), along with four known flavonoid compounds, were isolated from the aerial parts of *Diceratella elliptica* (DC.) Jonsell growing in Egypt. Their structures were established on the basis of detailed chromatographic and spectroscopic techniques (UV, 1D NMR, 2D NMR, and ESIMS). Compounds **1** and **2** were evaluated for their cytotoxic activity and showed relatively high activity against three human carcinoma cell lines; liver (HEPG2), cervix (HELA) and colon (HCT116).

**Keywords:** Brassicaceae; Coumarin glycosides; *Diceratella elliptica*; Flavonoids; Cytotoxic activity.

## 1. Introduction

Brassicaceae is one of the largest angiosperm families, comprising approximately 338 genera and more than 3709 species distributed worldwide [1]. It includes many economically important vegetable salad plants, crop species and ornamentals. The seeds of *Brassicaceae* are largely used as condiment and fertilizer [2]. The plants of this family are used in the treatment of many diseases due to their anticancer, antibacterial, antifungal, antirheumatic, and antidiabetic properties [3]. The genus *Diceratella* is represented by only one species; *D. elliptica* that grows in Egypt endemic in Gebel Elba [4,5]. The previous phytochemical studies on the chloroform extract of the aerial parts of *D. elliptica* resulted in isolation of three alkaloids; *N*-Me laurotetanine, corydine and isocorydine. In addition, five terpenoids were isolated from its petroleum ether extract; stigmast-7-en-3-ol, stigmastan-7-one, 4-methyl-5-ergosta-8,14,24(28)-triene-3,4-diol, 4-methyl-5-ergosta-8,14-diene-3,4-diol and $\alpha$-amyrin [6]. In this work, the phytochemical screening of the flowering aerial parts of *D. elliptica* indicated the presence of alkaloids, terpenoids, coumarins, and flavonoids.

* Corresponding author: E-Mail: monakhalil66@hotmail.com
† Current address: Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 03/25/2012 EISSN:1307-6167

Thus it was deemed of interest to study the phytochemical investigations of coumarins and flavonoids from the aqueous methanol extract of *D. elliptica.* The evaluation of two new coumarin glycosides (**1**&**2**) were reported and their cytotoxic activities were also tested against three carcinoma cell cancer.

## 2. Materials and Methods

### *2.1. General Experimental Procedures*

1D and 2D NMR experiments ($^1$H, $^{13}$C, COSY and HMBC) were recorded on a Jeol EX-500 spectrometer: 500 MHz ($^1$H NMR), 125 MHz ($^{13}$C NMR); and on Joel JNM-EX 270 spectrometer: 270 MHz ($^1$H NMR), 67.5 MHz ($^{13}$C NMR). UV spectrophotometer (Shimadzu UV-240), EIMS: Finnigan-Mat SSQ 7000 spectrometer, ESIMS: LCQ Advantage Thermo Finnigan spectrometer. CC Polyamide S6 (Riedel-De-Haen AG, Seelze Haen AG, Seelze Hanver, Germany) using solvent system of decreasing polarity starting with 100% water and ending with 100% MeOH. CC Silica gel 60 (Merck, 0.063-0.2 mm) using $CH_2Cl_2$-MeOH (2:3). TLC analyses were performed with Silica gel (Merck, Kieselgel) using $CH_2Cl_2$-MeOH (2:3). PC (descending) Whatman No. 1 and 3 MM papers, using solvent systems 1) $H_2O$, 2) 15% HOAc ($H_2O$–HOAc 85:15), 3) CAW ($CHCl_3$–HOAc–$H_2O$ 90:45:6), 4) BAW (*n*-BuOH–HOAc–$H_2O$ 4:1:5, upper layer), 5) ($C_6H_6$–*n*-BuOH–$H_2O$–pyridine 1:5:3:3, upper layer). Solvents 4 and 5 were used for sugar identification for flavonoids *O*- glycosides, Sephadex LH-20 (Pharmazia).

### *2.2. Plant Material*

A fresh sample of *D. elliptica* was collected from Wadi Yahmib, Gebel Elba, Egypt in 12 March 2004. The collected sample was identified by Prof. Dr. Salwa A. Kawashty. A voucher specimen (no. 902) was deposited in the herbarium of the National Research Centre (CAIRC).

### *2.3. Extraction and isolation*

Air-dried aerial parts of *D. elliptica* (500 g) were defatted with petroleum ether (40-60$^o$C) and extracted under reflux for three times with 70% ethanol/water. The aqueous ethanol extract was evaporated under reduced pressure affording 45 g residue, and then subjected to a polyamide column (80 × 3.5 cm) starting with water as eluent then decreasing the polarity by increasing the concentration of methanol. A total of 28 fractions were collected, each of about 500 ml. Similar fractions were combined according to their PC properties using $H_2O$, 15% HOAc, CAW and BAW as eluents and TLC properties using $CH_2Cl_2$-MeOH (2:3) to give four main fractions (I-IV). Fraction I (20% MeOH-$H_2O$) was applied to a Silica gel column (35 × 2.5 cm) using $CH_2Cl_2$: MeOH (2:3) giving two major fractions FI-1 and FI-2. Fractions FI-1 and 2 were chromatographed on PC using BAW two times and then purified on a Sephadex LH-20 column using methanol yielded compound **1** (22 mg) and compound **2** (12 mg). Fractions II-IV afforded compounds **3**-**6**. Their isolation and purification was achieved by a combination of Prep. PC and repeated Sephadex LH-20 column.

## 3. Results and Discussion

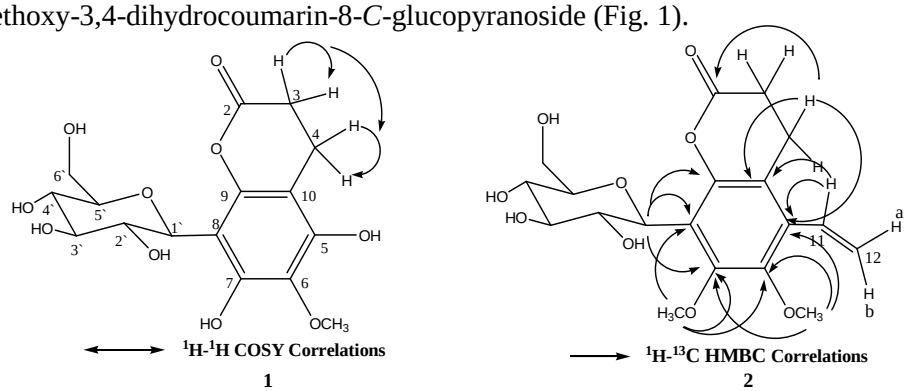
Two new naturally occurring coumarin glycosides; 6-methoxy-5,7-dihydroxy-3,4-dihydrocoumarin-8-*C*-glucopyranoside (**1**) and 5-vinyl-6,7-dimethoxy-3,4-dihydrocoumarin-8-*C*-glucopyranoside (**2**) were isolated, together with four known flavonoids; kaempferol-3,7-di-*O*-α-L-rhamnopyranoside (**3**), kaempferol-3-*O*-β-D-glucopyranoside (**4**), kaempferol-7-*O*-β-*D*-glucopyranoside (**5**), and kaempferol aglycone (**6**). The chemical structures of the isolated compounds

were elucidated by extensive UV, NMR, and MS spectral data [7-12]. Spectral data of the known flavonoids, **3**-**6**, were in a good accordance with those previously published ones [9, 13].

### *3.1. Structure elucidation*

Compound **1** was obtained as a yellow powder and showed a $[M+H]^+$ ion peak at *m/z* 372.9 in ESIMS spectrum corresponding to a molecular formula of $C_{16}H_{20}O_{10}$. The UV absorption spectrum showed maxima at 218 and 278 and shoulder at 230 nm indicating a dihydrocoumarin skeleton [7, 14]. The $^1$H NMR in DMSO-$d_6$ show two methylene multiplets at δ 2.32 and δ 2.60 in the aliphatic region assigned to two pairs of protons; one at position 3 and other one at position 4, respectively, one singlet at δ 3.79 indicated a methoxy group. In addition, the anomeric proton at δ 4.64 with *J*=9.2 Hz, indicating the *β*-configuration and the linkage of glucose moiety to the aglycone through C-C bond. The $^{13}$C NMR spectrum displayed sixteen carbon signals; nine for the dihydrocoumarin skeleton, six for *C*-β-glucopyranose unit and one methoxylated carbon [15]. The down field shift of the methoxy carbon at C6 (δ 60.87) indicate that oxygenation of aromatic ring carbons at C-5 and C-7 [16]. In the $^1$H-$^1$H COSY spectrum of **1,** the proton signal at δ 2.32 (H-3) showed correlation with that of δ 2.60 (H-4) and vice verse. In the HMBC spectrum of compound **1**, the doublet signal of H-1´(δ 4.64) showed a correlation with C-8 (δ 115.48), C-7 (δ 167.2) and C-9 (δ 156.1), and the signal of the methoxyl group at δ 3.79 was correlated with C-6 (δ 137), C-7 (δ 167.2) and C-5 (δ 156.1) confirming the attachment of the glucose unit and the methoxyl group to C-8 and C-6 of the aromatic ring, respectively. The correlation peaks between 5-OH (δ 12.5) and C-5 (δ 156.1), C-6 (δ 137) and C-10 (δ 115.5) were also observed. From the above data, compound **1** was identified as 6-methoxy-5,7-dihydroxy-3,4-dihydrocoumarin-8-*C*-glucopyranoside (Fig. 1).

Compound **2** was obtained as a yellow powder. The positive ESIMS spectrum of **2** showed $[M+H]^+$ at *m/z* 397.27, suggesting the molecular formula of $C_{19}H_{24}O_9$. The $^1$H NMR spectral data of compound **2** were closely related to those of **1**, except for methoxyl group at δ 3.70 (3H, s) and vinyl group. The vinylic protons (H-11, H-12a, b) resonate at δ 5.85 (1 H, dd *J*=11.5, 17.5 Hz), δ 4.96 (1H, dd, *J*=11.5, 2.0 Hz) and δ 5.08 (1H, dd, *J*=17.5, 2.0 Hz), respectively. The coupling constants of 11.5 Hz and 17.5 Hz confirmed the *cis* and *trans* relationship between the methine (H-11) and the methylene (H-12a, b) protons, indicating the presence of a vinyl group attached to the aromatic ring [15, 17]. In addition, comparison of $^{13}$C NMR chemical shifts of **2** with those of **1** supported methylation of the phenolic hydroxyl group at C-7 and the presence of a vinyl group (δ 116.2, C-11 and 138.3, C-12) at C-5, with down field shift of C-5 at 137 ppm [17, 18]. The HMBC spectral data showed correlations between H-4 (δ 2.66) and the carbonyl at (δ 175.4) which assigned to a dihydrocoumarin moiety. It also exhibited correlation with C-5 (δ 137), these correlations and chemical shift of C-5 were also confirmed its substitution with the vinyl group. More over the HMBC spectrum confirmed the attachments of two methoxyl groups (δ 3.79, δ 3.71) at C-6 (δ 137.2) and C-7 (δ 167.8), respectively. Based on the above mentioned data compound **2** was identified as 5-vinyl-6,7-dimethoxy-3,4-dihydrocoumarin-8-*C*-glucopyranoside (Fig. 1).




**1** **2**

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

### *3.2. Spectral Data*

*6-methoxy-5,7-dihydroxy-3,4-dihydrocoumarin-8-C-glucopyranoside* (**1**): Yellowish white powder, m.p. 178–179°C. UV spectral data, $\lambda_{max}$(nm) (MeOH): 218, 275, 316sh. $^{1}$H NMR (270 MHz, DMSO-$d_6$) δ, ppm, *J*/Hz: 4.64 (1H, d, *J* =9.2, H-1′); 3.79 (3H, s, $OCH_3$); 2.60 (2H, m, H-4); 2.32 (2H, m, H-3). $^{13}$C NMR (67.5 MHz, DMSO-$d_6$) δ, ppm: 175.7 (C-2), 167.2 (C-7); 156.1 (C-5), 156.1 (C-9), 137 (C-6), 115.5 (C-10), 115.48 (C-8), 82.05 (C-1′), 81.3 (C-3′), 78.1 (C-5′), 72.9 (C-4′), 69.8 (C-2′), 60.9 (C-6′), 60.87 ($OCH_3$), 31.2 (C-3), 25.1 (C-4). Positive ESIMS; *m/z* 372.9 $[M+H]^+$

*5-vinyl-6,7-dimethoxy-3,4-dihydrocoumarin-8-C-glucopyranoside* (**2**): Yellowish white powder, m.p. 170–172°C. UV spectral data, $\lambda_{max}$(nm) (MeOH): 220, 275, 314sh. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ, ppm, *J*/Hz: 5.85 (1 H, dd *J*=11.5, 17.5 Hz); 5.08 (1H, dd, *J*=17.5, 2.0, H-12a); 4.96 (1H, dd, *J*=11.5, 2.0, H-12b); 4.66 (1H, d, *J* =9.5, H-1′); 4.20 (1H, dd, *J* =9.1, 9.0, H-4′); 3.79 (3H, s, $OCH_3$); 3.71 (3H, s, $OCH_3$); 3.62 (1H, m, H-6′); 3.20 (1H, t, *J*=10 H-3′); 3.10 (1H, t, *J*=10 H-2′); 2.87 (1H, m, H-5′); 2.66 (2H, m H-4); 2.33 (2H, m, H-3). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ, ppm: 175.4 (C-2),167.8 (C-7); 156.2 (C-9), 138.3 (C-12), 137.2 (C-6), 137 (C-5), 116.2 (C-11), 115.5 (C-10), 115.4 (C-8), 82.6 (C-1′), 81.9 (C-3′), 78.5 (C-5′), 73.3 (C-4′), 70.1 (C-2′), 61.7 (C-6′), 56.6 ($OCH_3$), 56.5 ($OCH_3$), 31.2 (C-3), 24.7 (C-4). Positive ESIMS; *m/z* 397.27 $[M+H]^+$

### *3.3. Cytotoxic activity*

Potential cytotoxic activity of compounds **1** and **2** was tested using the method of Skehan [19]. Cells were placed in a 96-multi well plate (104 cells/well) for 24 h before treatment with the extract to allow attachment of cells to the wall of the plate. Different concentrations of the extracts (0, 5, 12.5, 25 and 50 µg/ml) were added to the cell monolayer in triplicate. Monolayer cells were incubated with the compounds for 48 h at 37°C and in an atmosphere of 5% $CO_2$. After 48 h, cells were fixed, washed and stained with sulforhodamine B. Excess stain was washed with acetic acid and attached stain was recovered with *tris*-EDTA buffer. Colour intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration was plotted to obtain the survival curve of each tumor cell line as compared with Doxorubcin; the control anticancer drug.

*In vitro* cytotoxic activity of compounds **1** and **2** revealed mild activity against three human carcinoma cell lines; liver (HEPG2), colon (HCT116) and cervix (HELA) with $IC_{50}$ of 13.5, 21.1 and 15.6 µg/ml for compound **1** and 17.9, 20.7 and 14.7 µg/ml for compound **2**, respectively, where the American National Cancer Institute assigns a significant cytotoxic effect of sample for future bioguided studies, if it exerts an $IC_{50}$ value ≤30 µg/ml [20]. The structure-activity relationships become very interesting when we compare the structure of the two tested compounds. They both have a methoxyl group and glucose moiety at C-6 and C-8, respectively, but compound **2** which exhibited relatively most activity against colon and cervix cell lines has additional methoxyl group at C-7 as well as a vinyl one at C-5. Compound **1** was also exhibiting most activity against liver carcinoma cell line; this activity may be due to the presence of two hydroxyl groups at C-5 and C-7.

## Acknowledgments

This work was financially supported by the *Phytochemistry and Plant Systematic Department*, National Research Centre, Giza, Egypt.

## Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

## References


[1] I. A. Al-Shehbaz, M. A. Beilstein and E. A. Kellogg (2006). Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview, *Plant Syst. Evol.* **259**, 89–120.

[2] W. S. Judd, C. S. Campbell, E. A. Kellogg and P. F. Stevens (1999). Plant Systematic; A Phylogenetic Approach, Sinauer Associates, Inc. Sunderland, Massachusetts U.S.A.

[3] R. Kirtikar and L. Basu (1975). Indian Medicinal Plants (2nd ed), Vol. 1, Bishen Singh Mahendra Pal Singh: Dehra Dun, India.

[4] L. Boulos (1999). Flora of Egypt, Vol. 1 (Azollaceae-Oxalidaceae), Al Hadara Publishing, Cairo, Egypt.

[5] V. Täckholm (1974) Student's Flora of Egypt, Cairo University, Cairo.

[6] S. A. El-Sawi and H. M. Motawe (2004). Cytotoxic alkaloids and terpenes from the aerial parts of *Diceratella elliptica* D.C., *Bull. N R C, Egypt*. **28:2**, 163-170.

[7] H. Liang and Y. Dequan (1988). The applications of UV Spectrum in organic chemistry, Vol. 2**,** Beijing: Scientific Publishing House. p. 227.

[8] T. J. Mabry, K. R. Markham and M. B. Thomas (1970). The systematic identification of flavonoids, Springer, Heidelberg.

[9] K. R. Markham and H. Geiger (1994). $^{1}$H-NMR spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulfoxide, In: The Flavonoids, Advances in Research since 1986, *ed*: J. B. Harborne, Chapman and Hall, London, pp.464–469.

[10] H. Duddeck and M. Kaiser (1982). $^{13}$C-NMR spectroscopy of coumarin derivatives, *Org. Magn. Resonance* **20:2**, 55-72.

[11] P. K. Agrawal and M. C. Bansal (1989). Flavonoid glycosides, In: Carbon-13 NMR of Flavonoids, *ed*: P. K. Agrawal, Elsevier, New York, pp. 283–363.

[12] T. J. Mabry, K. R. Markham and V. M. Chari (1982). Carbon-13 NMR Spectroscopy of the Flavonoids, In: The Flavonoids, Advances in Research, *eds*: J.B. Harborne, T. J. Mabry, Chapman and Hall, London, New York, pp.37-41.

[13] M. M. Marzouk, S. A. Kawashty, L. F. Ibrahim, N. A. M. Saleh, and A. Al-Nowehj (2008). Two New Kaempferol Glycosides from *Matthiola longipetala* (subsp. *livida*) (Delile) Maire and Carcinogenic Evaluation of its Extract, *Nat. Prod. Commun.* **3:8**, 1325-1328.

[14] M. Grifoll, M. Casellas J. M. Bayona and A. M. Solanas (1992). Isolation and characterization of fluorine-degrading bacterium: identification of ring oxidation and ring fission product, *Appl. Environ. Microbiol.* **2**, 910-917.

[15] B. Mikhova and H. Duddeck (1996). $^{13}$C-NMR Spectroscopy of Coumarins and their Derivatives: A Comprehensive Review, In: Studies in Natural Products Chemistry, *ed*: Atta-ur Rahman, Anton\ Rowe Ltd, Eastboume. **18**, 971-1071.

[16] S. A. M. Hussein, H. H. Barakat, M. A. Nawwar and G. Willuhn (1997). Flavonoids from *Ephedra aphylla*, *Phytochemistry* **45:7**, 1529-1532.

[17] D. L. Pavia, G. M. Lampman and G. S. Kriz (1996). Introduction to spectroscopy, Saunders Golden Sunburst Series.

[18] R. D. H. Murray, J. Mondez and S. A. Brown (1982). Chemistry and Biochemistry. Wiley, Bristol.

[19] P. Skehan, R. Storeng, D. A. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd (1990). New colorimetric cytotoxicity assay for anticancer-drug screening, *J. Natl. Cancer Inst.* **82**, 1107–1112.

[20] M. Stuffiness and J.M. Pezzuto (1991). Assays related to cancer drug discovery, In: Methods in plant biochemistry: Assays for bioactivity, *ed*: K. Hostettmann, Academic Press, London, pp. 71-153.