Phytochemical Constituents and Biological Activities of *Origanum syriacum*

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Z. Naturforsch. **2009**, *64b,* 447 – 451; received September 11, 2008

The cytotoxicity of the methanol extract of *Origanum syriacum* against human cerivical adenocarcinoma, Hela cells, was evaluated by the MTT assay. From the crude extract, a new flavone glycoside, acacetin-7-O-[2″-O-α-L-rhamnopyranosyl-6″-O-β-D-glucopyranosyl]-β-D-glucopyranoside (**1**), together with the ten known flavonoids luteolin (**2**), apigenin (**3**), luteolin-6-C-glucoside (**4**), luteolin-3′-methylether-6-C-glucoside (**5**), luteolin-7,4′-dimethyether-6-C-glucoside (**6**), apigenin-7-methylether-6-C-glucoside (**7**), apigenin-7-O-glucoside (**8**), diosmetin-7-O-glucoside (**9**), acacetin-7-O-glucoside (**10**) and acacetin-7-O-rutinoside (**11**), was isolated from the methanol extract of the aerial parts of *Origanum syriacum*. The structure elucidation of the isolated compounds was performed by spectroscopic and chromatographic methods.

Key words: *Origanum syriacum*, Lamiaceae, Flavonoids, Acacetin Triglycoside, Hela Cells, Cytotoxicity

Introduction

Origanum syriacum L., a very popular Arab spice, is native to the eastern Mediterranean, southern Europe and western Asia, and is cultivated in many parts of the world. The leaves of *O. syriacum* have been used as a herbal traditional medicine, flavor and fragrance, and for aromatherapy in the form of bath, massage, steam inhalation and vaporization. It is used in teas and cooked or baked foods [1].

Many studies were carried out on the plant species of the genus Origanum in view of their antimicrobial, cytotoxic, antioxidant and antihyperglycaemic activities [2 – 6].

The majority of the flavonoids occurring in the Lamiaceae family are flavones, while flavonols are found only in a few instances [7]. However, the previous phytochemical investigations on the plants of this genus led to the isolation of flavonoids, phenolic acids and terpenoids [8 – 12].

We previously reported the isolation and characterization of a novel prenylated biflavone and four flavone C-glucosides obtained from the aerial parts of *O. syriacum* [13].

This work describes the isolation and structure elucidation of one new flavonoid glycoside together with 10 known flavonoids from the methanol extract of *O. syriacum*. Furthermore, the present study is also assessing the cell cytotoxicity of the methanol extract as well as of the new acacetin glycoside.

Results and Discussion

Hela cells incubated with DMSO in the corresponding concentration were used as a control. To further assess the cytotoxic effects of the MeOH extract on Hela cells, the cells were treated with various concentrations (20 – 400 μg mL^{-1}) and incubated for 12 – 48 h. The results indicate that the extract-induced growth inhibition acts in a time- and dose-dependent manner on the Hela cells. The IC_{50} value of the MeOH extract on Hela cells was 474.92 μg mL^{-1} after treatment for 12 h. The detailed investigation of the methanol extract of the aerial parts of *O. syriacum* led to the isolation of eleven flavonoid compounds. Ten of them were identified as the known luteolin [14], apigenin [14], luteolin-6-C-glucoside [15], luteolin-3′-methylether-6-C-glucoside [16],

luteolin-7,4′-dimethylether-6-C-glucoside [17], apigenin-7-methylether-6-C-glucoside [18], apigenin-7-O-glucoside [19], diosmetin-7-O-glucoside [20], acacetin-7-O-glucoside [20, 21], and acacetin-7-O-rutinoside [19, 22]. The chemical structures of the known compounds (**2** – **11**) were elucidated by extensive UV analysis, FABMS and NMR spectroscopic data and also by comparing experimental data with respective literature data [14 – 22].

Compound **1** was obtained as an amorphous yellow powder, $[\alpha]_D = -57°$ (c = 0.05, MeOH). Its molecular formula $C_{34}H_{42}O_{19}$ was established by positive-ion HR-FABMS. The spectrum displayed the molecular ion peak $[M+H]^+$ at m/z = 755.2301 in agreement with the molecular formula $C_{34}H_{43}O_{19}$ (calcd. 755.2319).

Acid hydrolysis of **1** gave acacetin, β-glucose and α-rhamnose which were identified by TLC, and comparison with authentic samples.

The IR spectrum of **1** showed absorption bands typical of hydroxyl (3380 cm^{-1}) and carbonyl groups (1706 cm^{-1}) and aromatic rings (1635, 1600 and 1520 cm^{-1}).

Compound **1** displayed UV absorption maxima at 271 and 327 nm characteristic for a substituted flavone skeleton [23]. The results of the UV analysis using various shift reagents were in good agreement with the substitution pattern depicted in the structure of **1** [23].

The identity of **1** was confirmed unambiguously by a series of 1D and 2D NMR spectral data, including ^{1}H and ^{13}C NMR, DEPT, COSY, HSQC and HMBC.

The ^{1}H and ^{13}C NMR spectra (including DEPT 135) of **1** showed characteristic shift values and multiplicities of a 7-O-glycosylated acacetin derivative [24].

The ^{1}H NMR spectrum (Table 1) displayed signals due to *ortho*-coupled A_2B_2-type protons at δ = 8.08 and 7.19 (each 2H, d, J = 8.6 Hz) assignable to H-2′,6′ and H-3′,5′, respectively, and *meta*-coupled protons at δ = 6.87 and 6.53 (each 1H, d, J = 2.3 Hz) assigned to H-8 and H-6, respectively.

The spectrum further revealed the presence of a methoxyl group at δ = 3.88, an isolated proton at δ = 6.95 (1H, s) assigned to H-3, and three signals assignable to anomeric sugar protons, which were identified to be due to a terminal β-glucopyranose and a terminal α-rhamnopyranose connected to a core β-glucopyranose unit. This was further confirmed by the presence of 18 carbon signals in the ^{13}C NMR and DEPT spectra (Table 1), of which 3 are anomeric carbon signals at δ = 99.9, 101.1 and 105.1, 12 methine carbon signals, 2 oxymethylene carbons at δ = 62.7 and 70.8, and 1 methyl carbon at δ =17.7.

Table 1. ^{1}H and ^{13}C NMR spectral data for compound **1**[a].

Position	δ_C	DEPT	δ_H
Acacetin			
2	164.5	C	
3	103.8	CH	6.95 s
4	182.2	C	
5	162.3	C	
6	99.4	CH	6.53 d (2.3)
7	163.4	C	
8	94.8	CH	6.87 d (2.3)
9	158.7	C	
10	105.1	C	
1′	123.3	C	
2′	128.7	CH	8.08 d (8.6)
3′	116.1	CH	7.19 d (8.6)
4′	164.3	C	
5′	116.1	CH	7.19 d (8.6)
6′	128.7	CH	8.08 d (8.6)
OCH_3	55.6	CH_3	3.88 s
Inner glucose			
1″	99.9	CH	5.24 d (7.2)
2″	80.7	CH	3.56[b]
3″	76.3	CH	3.43[b]
4″	71.3	CH	3.38[b]
5″	75.9	CH	3.76[b]
6″	70.8	CH_2	3.74 dd (11.6, 2.2) 3.38[b]
Rhamnose			
1‴	105.1	CH	4.57 d (1.2)
2‴	70.9	CH	3.84[b]
3‴	71.1	CH	3.37 m
4‴	72.2	CH	3.19 t (9.8)
5‴	68.4	CH	3.63 dd (9.8, 6.1)
6‴	17.7	CH_3	1.09 d (6.1)
Terminal glucose			
1⁗	101.1	CH	4.52 d (7.2)
2⁗	74.7	CH	3.37 dd (7.2, 8.5)
3⁗	76.5	CH	3.22[b]
4⁗	71.5	CH	3.31[b]
5⁗	76.8	CH	3.50[b]
6⁗	62.7	CH_2	3.82 dd (11.8, 2.1) 3.61 dd (11.8, 5.3)

[a] 600 MHz in [D_6]DMSO, J in Hz; the assignments are based on DEPT, HSQC and HMBC data; [b] signal patterns are unclear due to overlap.

Although the signals of some of the glycosidic protons overlapped in the ^{1}H NMR spectrum, the COSY spectrum and HSQC correlations allowed the assignment of corresponding signals and the remaining NMR signals, as summarized in Table 1.

In the ^{13}C NMR spectrum of **1**, the carbon resonances assigned to the outer β-glucose and α-rhamnose units showed no unusual chemical shifts, suggesting their terminal positions.

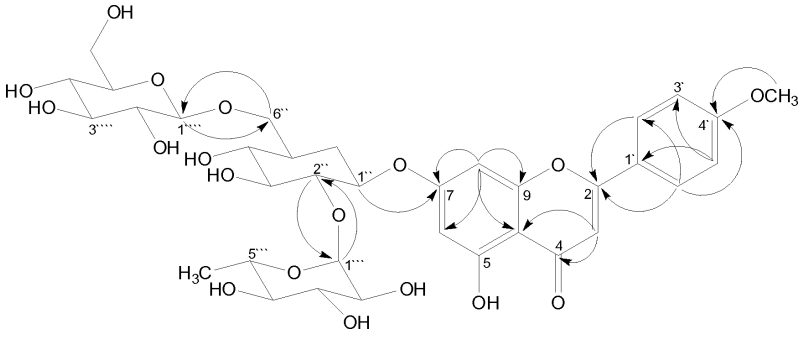

Fig. 1. Heteronuclear multiple bond correlations for **1**. Arrows point from proton to carbon.

In the HMBC spectrum of **1**, the three-bond correlations between the anomeric C-1′′′′ at δ = 101.1 and H_2-6′′ at δ = 3.82 and between H-1′′′′ at δ = 4.52 and C-6′′ at δ = 70.8 demonstrated that the outer glucosyl moiety was connected to C-6′′ of the inner glucose unit which, in turn, was linked to the acacetin moiety. In addition, the anomeric C-1′′′ (rhamnosyl) at δ = 105.1 correlated to both H-2′′ at δ = 3.65 and H-5′′′ at δ = 3.63, and consequently, the rhamnosyl moiety was glycosidically linked to C-2′′ of the inner glucose unit.

It was also proved by the HMBC spectrum that the inner β-glucopyranosyl moiety is attached to C-7, showing correlations between the anomeric proton H-1′′ at δ = 5.24 and C-7 at δ = 163.4.

The position of the methoxyl group was assigned to C-4′ on the basis of HMBC correlations between C-4′ at δ = 164.3 and the protons of the methoxyl group at δ = 3.88. The complete assignment of all proton and carbon resonances was achieved after careful analysis of ^{1}H-^{1}H COSY, HSQC and HMBC experiments. Some significant HMB correlations are shown in Fig. 1.

Compound **1** is closely related to the previously reported acylated acacetin triglycosides from *Peganum harmala* and *Calamintha glandulosa* [25, 26], while **1** lacked the acetyl residue, and the sequence of the sugar moieties provided the points of difference.

On the basis of these data, compound **1** was determined to be acacetin-7-O-[2′′-O-α-L-rhamnopyranosyl-6′′-O-β-D-glucopyranosyl]-β-D-glucopyranoside, a new acacetin triglycoside. This new compound has no cytotoxicity on Hela cells even in higher concentration (500 μg mL^{-1}).

Experimental Section

Reagents

Penicilin, streptomycin, DMEM, and FBS were obtained from Welgene Bioscience (Daegu, Korea). Silica gel 60, 0.040 – 0.063 mm, and TLC plates, silica gel 60 F_{254}, were obtained from Merck (Darmstadt, Germany). Octadecyl silica gel (ODS) was obtained from Waters (Massachusetts, USA). Polyamide 6, 50 – 160 μm was obtained from Fluka (Saint Louis, USA), and Sephadex LH-20 was obtained from Pharmacia (Upsala, Sweden). Other reagents were obtained from Sigma-Aldrich (Saint Louis, USA).

General experimental procedures

TLC analysis was carried out using silica gel 60 F_{254} plates; chromatograms were visualized under UV light at 254 and 366 nm, then sprayed with anisaldehyde reagent (Aldrich). UV spectra were recorded on a Shimadzu UV-240 spectrophotometer. IR spectra were obtained on a Bruker IFS 113v instrument. Optical rotations were measured on a Jasco P-1020 polarimeter. ^{1}H and ^{13}C NMR spectra were obtained using a Varian Unit-INOVA 600 spectrometer in [D_6]DMSO. The chemical shifts are given in δ (ppm) relative to $SiMe_4$. HR-FABMS spectra were recorded on a Jeol JMS-SX 102A spectrometer.

Cell culture and sample treatment

The human cervical asenocarcinoma (Hela) cells used in this work were purchased from the Korean Cell Line Bank (KCLB). Hela cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10 % (v/v) fetal bovine serum (FBS), 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin in a humidified incubator containing 5 % CO_2 at 37 °C. The methanol extract was dissolved in dimethyl sulfoxide (DMSO), and the final DMSO concentration in all cultures was 0.5 %.

Plant material

Aerial parts of *O. syriacum* were collected from Saint Kathrine, South Sinai, Egypt in April 2007. The plant was identified by Prof. Salwa A. Kawashty, Department of Phytochemistry and Plant Systematic, National Research Centre, Cairo, Egypt. A voucher specimen (SKOR-4-08) has been kept in the Herbarium of National Research Centre, Egypt.

Extraction and isolation

The air-dried aerial parts of *O. syriacum* (800 g) were extracted three times with MeOH at 70 °C. The combined solutions were then evaporated under reduced pressure at 45 °C to give a MeOH extract (45.3 g). The MeOH extract was placed on a polyamide column and eluted sequentially with H_2O/EtOH mixtures (1 : 0, 8 : 1, 4 : 1, 3 : 2, 1 : 1, 1 : 4, 0 : 1) to give seven fractions.

Fraction 2 was subjected to polyamide column chromatography using 50 % MeOH, and the eluate was separated into three fractions (frs. 2.1 – 2.3). Fraction 2.2 was chromatographed on a Sephadex LH-20 column using MeOH-CH_2Cl_2 (4 : 1) to afford **1** (28 mg). Repeated chromatography of fraction 2.3 on a Sephadex LH-20 column using MeOH yielded **11** (33 mg). Fraction 3 was further applied to polyamide column chromatography, eluted with H_2O-EtOH (5 : 1) to give **8** (40 mg), **9** (22 mg) and **10** (18 mg) which were purified using Sephadex LH-20 column chromatography with MeOH.

Repeated purification of fraction 5 by Sephadex LH-20 column chromatography using MeOH–H_2O furnished **5** (23 mg), **6** (28 mg) and **7** (18 mg). Fraction 6 was subjected to silica gel column chromatography using CH_2Cl_2-MeOH (3 : 1) to afford **4** (41 mg).

Fraction 7 was separated by silica gel column chromatography using CH_2Cl_2-MeOH (15 : 1) to give **2** (34 mg) and **3** (50 mg).

*Acid hydrolysis of compound **1***

A solution of compound **1** (8 mg) in 2 M HCl (2 mL) was refluxed for 3 h. The reaction mixture was diluted with H_2O and then extracted with EtOAc. The aqueous phase was neutralized with $BaCO_3$ and evaporated to dryness. The residue was identified by comparison with authentic samples of rhamnose and glucose by TLC on silica gel with pyridine-EtOAc-AcOH-H_2O (36 : 36 : 7 : 21) and aniline phthalate as spray reagent.

Cell cytotoxicity and viability analysis

Viabilities of sample-treated Hela cells compared to control were evaluated using the MTT assay in triplicate. Briefly, exponentially growing cells were seeded at 1×10^4 cells per well in 96-well plates and exposed to various concentrations of the extract of *O. syriacum*. At the end of the sample expose period, cells in each well then were incubated in 100 μL fresh medium containing 10 μL of an MTT solution (5 mg mL^{-1} in PBS) at 37 °C for 4 h. After the medium and MTT were removed from the wells, and the crystals, purple MTT-formazan, were dissolved by adding 100 μL of DMSO to all wells. Absorbance at 570 nm of the liquid was detected using a microplate ELISA reader (Opsys MR^{TM}, DYNEX Technologies, Chantilly, VA, USA). The 50 % inhibitory concentration (IC_{50}) of the extract of *O. syruacum* on cells was calculated using the MTT assay. The results were determined by three independent experiments.

*Acacetin-7-O-[2″-O-α-L-rhamnopyranosyl-6″-O-β-D-glucopyranosyl]-β-D-glucopyranoside (**1**)*

Amorphous yellow powder. – $[\alpha]_D = -57$ (c = 0.05, MeOH). – HR-FABMS: m/z = 755.2301, (calcd. 755.2319 for $C_{34}H_{43}O_{19}$, $[M+H]^+$). – UV (MeOH): λ_{max} = 271, 327 nm. – IR (KBr): ν_{max} = 3380 (OH), 1706 (C=O), 1625 (C=C), 1635, 1600, 1520 (aromatic rings) cm^{-1}. – ^1H and ^{13}C NMR spectra: see Table 1.

Acknowledgement

This study was carried out with the support of the Forest Science & Technology Projects (Project No. S210707L010 110 and S120808L1101104) by the Korea Forest Service and a 2007 Kookmin University research grant.

[1] M. H. Alam, A. Mavi, A. Yildirim, M. Digrak, T. Hirata, *Biol. Pharm. Bull*. **2003**, *26*, 1725 – 1729.
[2] B. Aslim, N. Yucel, *Food Chemistry* **2008**, *107*, 602 – 606.
[3] M. Bendahou, A. Muselli, M. Grignon-Dubois, M. Benyoucef, J. M. Desjobert, A. F. Bernardini, J. Costa, *Food Chemistry* **2008**, *106*, 132 – 139.
[4] I. Chinou, C. Liolios, D. Moreau, C. Roussakis, *Fitoterapia* **2007**, *78*, 342 – 344.
[5] H. Matsuura, H. Chiji, C. Asakawa, M. Amano, T. Yoshihara, J. Mizutani, *Biosci. Biotech. Biochem.* **2003**, *67*, 2311 – 2316.
[6] A. Lemhadri, N.-A. Zeggwagh, M. Maghrani, H. Jouad, M. Eddouks, *J. Ethnopharmcol*. **2004**, *92*, 251 – 256.
[7] F. A. Tomas-Barberan, R. Grayer-Barkmeijer, M. I. Gil, J. B. Harborne, *Phytochemistry* **1988**, *27*, 2631 – 2645.
[8] A. Bosabalidis, C. Gabrieli, I. Niopas, *Phytochemistry* **1998**, *49*, 1549 – 1553.
[9] J. Kawabata, K. Mizuhata, E. Sato, T. Nishioka, Y. Aoyama, T. Kasai, *Biosci. Biotechnol. Biochem.* **2003**, *67*, 445 – 447.
[10] S.-S. Chun, D. A. Vattem, Y.-T. Lin, K. Shetty, *Process Biochemistry* **2005**, *40*, 809 – 816.
[11] E. Vági, E. Rapavi, M. Hadolin, K. Vásárhelyiné Perédi, A. Balázs, A. Blázovics, B. Simándi, *J. Agri. Food Chem.* **2005**, *53*, 17 – 21.
[12] M. S. Kamel, M. H. Assaf, H. A. Hasanean, K. Ohtani, R. Kasai, K. Yamasaki, *Phytochemistry* **2001**, *58*, 1149 – 1152.

[13] S. K. El-Desouky, M. Sharaf, *Rev. Latinoamer. Quim.* **2004**, *32*, 21 – 24.
[14] R. W. Owen, R. Haubner, W. Mier, A. Giacosa, W. E. Hull, B. Spiegelhalder, H. Bartsch, *Food Chem. Toxicol*. **2003**, *41*, 703 – 717.
[15] J. Peng, G. Fan, Z. Hong, Y. Chai, Y. Wu, *J. Chromatogr*. **2005**, *1047*, 111 – 115.
[16] G. T. Maatooq, S. H. El-Sharkawy, M. S. Afifi, P. N. Rosazza, *Phytochemistry* **1997**, *44*, 184 – 190.
[17] N. H. El-Sayed, A. A. Ahmed, M. S. Ishak, E. F. Kandil, *Phytochemistry* **1991**, *30*, 2442.
[18] C. A. Williams, J. B. Harborne, M. Colasante, *Biochem. System. Ecology* **1997**, *25*, 309 – 325.
[19] V. M. Chari, M. Jordan, H. Wagner, P. W. Thies, *Phytochemistry* **1997**, *16*, 1110 – 1112.
[20] S. A. Basudan, M. Ilyas, M. Parveen, H. M. Muhisen, R. Kumar, *J. Asian Nat. Prod. Res*. **2005**, *7*, 81 – 85.
[21] S. Asen, R. N. Stewart, K. H. Norris, *Phytochemistry* **1975**, *14*, 1443 – 1444.
[22] H. Ina, H. Lida, *Phytochemistry* **1981**, *20*, 1176 – 1177.
[23] T. J. Mabry, K. R. Markham, M. B. Thomas, *The Systematic Identification of Flavonoids*, Springer-Verlag, New York, Heidelberg, Berlin, **1970**, pp. 41 – 61.
[24] K. R. Markham, V. M. Chari, T. J. Mabry, in *The Flavonoids: Advances in Research*, (Eds.: J. B. Harborne, T. J. Mabry), Chapman & Hall, New York **1982**, pp. 19 – 134.
[25] M. Sharaf, M. A. El-Ansari, S. A. Matlin, N. A. M. Saleh, *Phytochemistry* **1997**, *44*, 533 – 536.
[26] P. D. Marin, R. J. Grayer, N. C. Veitch, G. C. Kite, J. B. Harborne, *Phytochemistry* **2001**, *58*, 943 – 947.