ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 4:3 (2010) 141-148

records of natural products

Chemical Constituents of Two Endemic *Cephalaria* Species

Peyker Kayce and Süheyla Kırmızıgül*

Department of Chemistry, Faculty of Science, Ege University, 35100 Bornova, İzmir, Türkiye

(Received June15, 2010; Revised July 27, 2010; Accepted July 30, 2010)

Abstract: A new triterpene glycoside (**1**), isacoside, together with seven known glycosides and a common aglycone were isolated from *Cephalaria isaurica* and *Cephalaria stellipilis*. Two glycosides of the known compounds (vaniloloside and picein) were detected in this genus for the first time. The structures of all compounds were determined by spectroscopic analysis including 1D and 2D NMR, HRESIMS techniques and chemical evidence.

Keywords: Dipsacaceae; *Cephalaria isaurica,*;*Cephalaria stellipilis;* triterpene glycoside; isacoside.

1. Introduction

The genus *Cephalaria* (Dipsacaceae family) comprises about 93 species which are widespread in Europe, East Asia, East Mediterranean, North and Central Africa. Thirty-nine *Cephalaria* species are widely distributed in Turkey and 23 of them are endemic. *C. isaurica* Matthews and *C. stellipilis* Boiss. are two endemic *Cephalaria* species found in southwestern Anatolia [1]. Previous phytochemical studies on *Cephalaria* species have led to the identification of triterpenoids [2-11], iridoids [12], flavonoids [13-15], alkaloids [16] and lignan glycosides [17]. Some of these substances have exhibited antifungal [4], antimicrobial [4, 8], antioxidant [14] and cytotoxic [5] activities. For this reason, they are utilized for medical, agricultural and veterinary purposes.

In this work, we give an account of the phytochemical analysis of the *n*-BuOH extracts of the aerial parts of two *Cephalaria* species, describing the isolation of a new hederagenin-type triterpenoid glycoside **(1)** (isacoside) together with a known one (cilicicoside-I) **(2)** [9], two flavone C-glycosides swertiajaponin [18] **(3)** and isoorientin [19] **(9)**, loganin **(4)** [12], sweroside **(5)** [20], cyclopenta(c) piran-4-carboxylic acid, octahydro-3,6-dihydroxy-7-methyl-methyl ester **(6)** [12], vaniloloside **(7)** [21] and picein **(8)** [22]. Here, the structural elucidation of this new glycoside has been reported in detail.

* Corresponding author: E-mail: suheyla.kirmizigul@ege.edu.tr; Tel.: +90 232 3112816; Fax: +90 232 3888264.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 07/ 31/2010X EISSN:1307-6167

2. Materials and Methods

2.1. General procedures

Optical rotations were measured on a Rudolp Research Analitical Autopol I automatic polarimeter. IR measurements were obtained on an ATI Mattson Genesis Series Fourier transform infrared spectrophotometer. NMR experiments were performed on a Varian AS 400 MHz and Varian AS 600 MHz spectrometers using TMS as an internal standard in DMSO-d_6. Mass analyses were performed with a Bruker HCT Ultra ESIMS ion trap instrument in negative mode. HRESIMS measurements were run on a Bruker LC micro-Q-TOF in positive mode. GC-MS analysis was performed by a HP 6890-5973 instrument with a HP-5MS column. Medium Pressure Liquid Chromatography (MPLC) was carried out using a Buchi system (Buchi C-605 pumps, UV detector) with Buchi glass columns (15/460 and 49/230). Open CC and MPLC were carried out on silica gel 60 (0.063-0.200 mm) (Merck 7734) while Lichroprep RP–18 (25-40 μm) (Merck 9303) was used for Vacuum Liquid Chromatography (VLC). Silica gel 60F_{254} (Merck 5554), RP–18 F_{254S} (Merck 5560) and silica gel 60GF_{254} (Merck 5744) precoated plates were used for TLC and Prep. TLC, respectively. Compounds were detected under UV light (254 and 366 nm) and/or others were detected using 20% H_2SO_4 solution as spraying reagent followed by heating at 120 ^oC.

2.2. Plant materials

The aerial parts of *C. isaurica* was collected from Gündoğmuş, Antalya, Gündoğmuş-Söbüçimen plateau, and *C. stellipilis* was collected from Göksun-Kahramanmaraş, rocky places of Değirmendere village, in July 2007, and identified by Prof. Dr. Hüseyin Sümbül and Assoc. Prof. Dr. R. Süleyman Göktürk (Department of Biology, Faculty of Art and Science, Akdeniz University). Voucher specimens (No: R. S. Göktürk 6075 and R. S. Göktürk 6078) were kept in the Herbarium Research and Application Centre of Akdeniz University, Antalya in Turkey.

2.3. Extraction and Isolation

Air-dried and powdered plant *C. isaurica* (1.8 kg), was extracted with MeOH (4x2L) at room temperature overnight. The solvent was removed by rotary evaporator under reduced pressure at ~ 40 ^oC. The MeOH residue (100.3 g) was extracted (3 x 100 mL) with *n*-BuOH-H_2O (1:1) solvent system. The dried *n*-BuOH phase was extracted with *n*-hexane to distinguish apolar parts. Then, *n*-BuOH residue (38.3 g) was subjected to vacuum liquid chromatography (VLC) using Lichroprep RP–18 (25-40 μm) (Merck 9303) as an adsorbent by MeOH-H_2O solvent system eluting from 0% MeOH to 100% MeOH. Totally 12 fractions were obtained. Fraction 10 and 11 were combined (16.5 g) and exposed to MPLC over silica gel 60 (0.063-0.200 mm) (Merck 7734) using suitable Buchi column (49/230) and programme (max. pressure: 20 bar, flow rate: 50 mL/min, CH_2Cl_2-MeOH solvent system, from 0% MeOH to 100% MeOH, 10 segment, 25 min. per segment). Seven fractions were derived. Fractions 5-6-7 (4.3 g) were combined and fractionated by column chromatography over silica gel, using a solvent system of $CHCl_3$-MeOH-H_2O from 61:32:7 to 61:32:7+10% MeOH. Additional MeOH was used to increase the polarity of the solvent system. This process yielded 10 sub-fractions. Second (749.7 mg) of the 10 fractions was subjected to an open silica gel CC and eluted with a gradient of $CHCl_3$-MeOH-H_2O (90:10:1-70:30:3) solvent system to give 16 fractions and fraction 13 afforded compound **1** (185 mg). Sub-fractions 6-7 were combined (403.6 mg) and applied to an open silica gel CC with $CHCl_3$-MeOH-H_2O (80:20:2-70:30:3) solvent system to afford fraction 1 as compound **2** (32.8 mg). Compound **3** (194.0 mg) was obtained thank to MeOH-Acetone precipitation of 7th fraction from VLC

separation. The 6th fraction of VLC was exposed to MPLC over silica gel using suitable Buchi column (15/460) and programme (max. pressure: 50 bar, flow rate: 10 mL/min, CH_2Cl_2-MeOH solvent system, from 0% MeOH to 100% MeOH, 10 segment, 15 min. per segment), and 11 fractions were obtained. Fraction 6 was applied to an open silica gel CC with $CHCl_3$-MeOH-H_2O (90:10:1-61:32:7) solvent system to afford fraction 8 as compound **4** (103.2 mg) and fraction 4 as compound **5** (26.2 mg). Compound **6** (42.0 mg) was obtained by preparative thin layer chromatography (Prep. TLC) using suitable solvent system ($CHCl_3$-MeOH-H_2O 90:10:1) from 5th fraction of VLC.

To obtain the chemical constituents of *n*-BuOH extract (34.2 g) of *C. stellipilis,* the same procedures were followed as mentioned for *C. isaurica*. Totally 11 fractions were obtained from the *n*-BuOH extract by VLC using RP-18 silica gel. Firstly, fraction 3 (539.0 mg) was subjected to an open silica gel CC with $CHCl_3$-MeOH-H_2O (80:20:2-61:32:7) solvent system to afford fraction 11 as compound **7** (33.3 mg), fraction 6 as compound **8** (28.0 mg) and fraction 2 as compound **5** (36.7 mg). Secondly, 4th fraction (2.0 g) of VLC was applied to an open silica gel CC with $CHCl_3$-MeOH-H_2O (90:10:1-70:30:3) solvent system yielding compound **4** (77.0 mg). The last process was carried out for combined fraction 5 (6.3 g) and fraction 6 (1.9 g) which showed exact similarity and they were exposed to MPLC over silica gel using suitable Buchi column (49/230) and programme (max. pressure:20 bar, flow rate:40 mL/min, $CHCl_3$-MeOH-H_2O solvent system, from 90:10:1 to 100% MeOH, 9 segment, 20 min. per segment). This process gave 12 fractions and compound **9** (23.7 mg) was derived from the 9th fraction by MeOH-Acetone precipitation.

Compound **1**: A white amorphous powder, 185 mg, $[\alpha]_D^{25}$ -0.97 (*c*= 2.1 MeOH). IR (KBr) ν_{max} 3434, 1699, 1642, 1385, 1270, 1047 cm^{-1}. ^{1}H-NMR (600 MHz, DMSO-d_6) and ^{13}C-NMR (150 MHz, DMSO-d_6) spectral data, see Table 1. The negative-ion ESIMS *m/z*: 1352.7 $[M]^-$ and the sodiated HRESIMS *m/z*: 1375.6530 $[M+Na]^+$ (Calcd. for $C_{64}H_{104}O_{30}Na$: 1375.6505).

2.4. Alkaline Hydrolysis

Compound **1** (10 mg) was refluxed in 5% aqueous KOH solution for 1 h at 80 ºC. Then the reaction mixture was neutralized with 5% aqueous HCl solution [8]. After the evaporation process, the remaining residue was extracted with *n*-BuOH-H_2O (1:1, 5 mL) solvent system and the separated phase (*n*-BuOH) was examined by NMR. The NMR data of the hydrolyzed compound did not have any difference from the NMR findings of compound **1**.

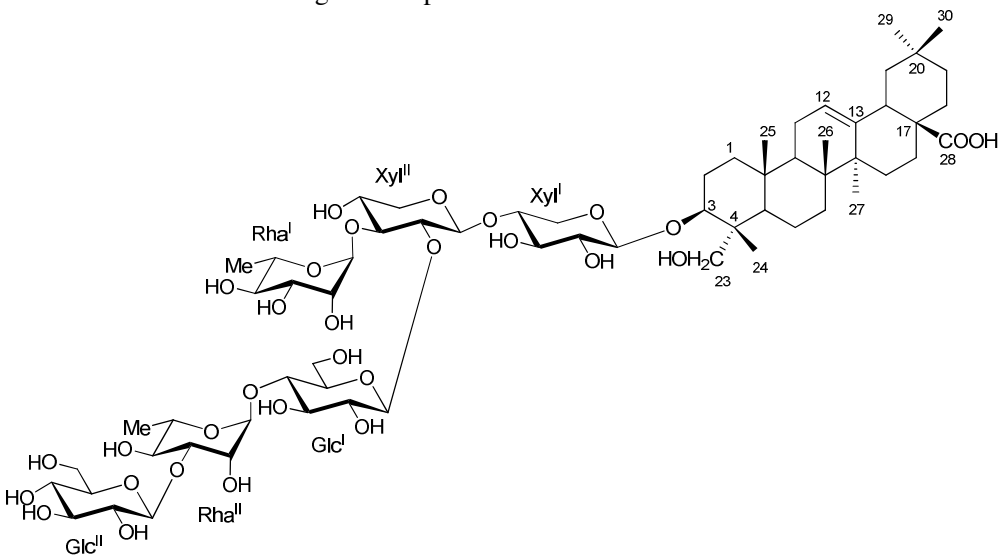

Figure 1. Isacoside (**1**) isolated from *C. isaurica.*

2.5. Sugar Analysis

The type of the carbohydrate units of **1** was determined firstly by micro-hydrolysis technique on a TLC plate [4] and then by GC-MS analysis [10]. For GC-MS analysis, compound **1** (7.0 mg) was refluxed with 1 N HCl (1.8 mL) in 80% MeOH-Benzene (1 : 1, 2.0 mL) solution for 6 h at 95 ^{o}C. After neutralizing with saturated Na_2CO_3 solution, the mixture was extracted with $CHCl_3$ (3 x 5 mL). The aqueous layer was evaporated to dryness. The dried sample was silylated with trimethylchlorosilane and hexamethyldisilazane (1:1, 1mL) in dry pyridine (1mL) under a $CaCl_2$ tube for 1h at 70 ºC. The silylated mixture was evaporated under N_2 stream in reduced pressure. The sugars were analyzed by GC-MS using a HP-5MS column and suitable temperature programme [10]. L-Rhamnose, D-xylose and D-glucose were detected by co-injection of the hydrolysate with standard silylated sugars. Identification of the D-glucose, D-xylose and L-rhamnose was carried out by GC-MS giving the peaks at 28.61, 16.68 and 14.02 min for **1**, respectively. The mass fragmentation of these sugar moieties were checked comparing these results with Wiley-Nist library data.

3. Results and Discussion

Isacoside was obtained as a white, amorphous powder from *n*-BuOH extract of *C. isaurica*. The molecular formula was determined as $C_{64}H_{104}O_{30}Na$ on the basis of the sodiated HRESIMS data (1375.6530 $[M+Na]^+$, calcd. 1375.6505). The IR spectrum showed specific absorption bands for hydroxyl (3434 cm^{-1}), carbonyl (1699 cm^{-1}) and an olefin (1642 cm^{-1}) groups. The ^{1}H-NMR data (Table 1) revealed that the aglycone of compound **1** possessed six methyl protons at δ_H 1.06 (H_3-27), 0.85 (H_3-25), 0.85 (H_3-30), 0.84 ppm (H_3-29), 0.70 (H_3-26), 0.55 (H_3-24), two CH_2O- protons at δ_H 3.08 and 3.32, (m, H-23), and an olefinic proton at 5.08 (br s, H-12) ppm. The HMQC spectrum exhibited that these protons were belonged to the carbons at δ_C 26.1 (C-27), 16.0 (C-25), 24.0 (C-30), 33.5 (C-29), 17.5 (C-26), 13.4 ppm (C-24), the CH_2O- carbon at δ_C 63.0 ppm (C-23) and a typical olefinic carbon at 121.3 (C-12) ppm. The literature data and the specific correlation between the H-24 (δ_H 0.55 ppm) proton and C-23 (δ_C 63.0 ppm), C-3 (δ_C 79.9 ppm) carbons in the HMBC spectrum indicated that the aglycon of compound **1** was a hederagenin [3]. The six anomeric proton signals of sugar units were observed at 4.24 (d, J = 7.2 Hz), 4.30 (d, J = 6.4 Hz), 4.35 (d, J = 6.8 Hz), 4.37 (d, J = 8.4 Hz), 5.08 (br s) and 5.08 (br s) ppm and they were correlated with carbons at 103.9, 103.7, 102.4, 104.4, 100.3 and 100.3 ppm, respectively in the HMQC spectrum. The HMBC spectrum exhibited the following specific correlations between C-3 (δ_C 79.9) and Xyl^I H-1 (δ_H 4.35), Xyl^I C-4 (δ_C 76.0) and Xyl^{II} H-1 (δ_H 4.30), Xyl^{II} C-3 (δ_C 78.0) and Rha^I H-1 (δ_H 5.08), Xyl^{II} C-2 (δ_C 78.8) and Glc^I H-1 (δ_H 4.24), Glc^I C-4 (δ_C 74.7) and Rha^{II} H-1 (δ_H 5.08), Rha^{II} C-3 (δ_C 82.0) and Glc^{II} H-1 (δ_H 4.37), indicating the aglycone-sugar and sugar-sugar connection points clearly (Table 1).

The acidic hydrolysis of compound **1** yielded three different types of sugar moiety indicating D-glucose, D-xylose and L-rhamnose which were confirmed by their co-chromatographic analysis on TLC and GC-MS results. The signals at 28.61, and 16.68 min in GC were confirmed by the coupling constants of the anomeric protons (J=8.4, 7.2, 6.8 and 6.4 Hz) indicating β- configurated pyranose (glucose) and furanose (xylose) moieties. Additionally, the configuration of two rhamnose moieties was determined as α- configuration by ^{1}H-NMR which was confirmed by GC giving the peak at 14.02 min. All these findings were also confirmed by the unaffected result of alkaline hydrolysis of compound **1** determining the connection point of the sugars at 3-O position of the aglycon.

Thus, the structure of compound **1** was established as 3-*O*-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→2)-[α-L-rhamnopyranosyl(1→3)]-xylopyranosyl (1→4)-β-D-xylopyranosyl hederagenin (isacoside) (Figure 1).

The eight known compounds were also established by spectroscopic analysis and by aid of literature data [9, 12, 18-22]. Although compounds namely vaniloloside and picein are known in other genera, this is the first report of isolation of them in *Cephalaria* species. Thus, these findings will be helpful for the chemotaxonomic profile of this genus for further investigations.

Table 1. ^{1}H-NMR and ^{13}C-NMR data of compound **1** in DMSO-d_6 [a,b]

Aglycone	^{1}H-NMR	^{13}C-NMR	C-3-*O*-sugars	^{1}H-NMR	^{13}C-NMR
1	1.46, nd, m	38.7	Xyl ^{I}		
2	0.88, 0.91, m	27.8	1	4.35, d, *J*=6.8 Hz	102.4
3	3.48, m	79.9	2	3.48, m	73.4
4	-	42.7	3	3.14, m	75.2
5	1.18, m	46.6	4	3.26, m	76.0
6	nd	17.6	5	3.32, 3.63, m	65.4
7	1.34, 1.37, m	32.5	Xyl ^{II}		
8	-	39.3	1	4.30, d, *J*=6.4 Hz	103.7
9	1.47, m	47.7	2	3.41, m	78.8
10	-	36.5	3	3.48, m	78.0
11	1.68, m	25.8	4	3.94, m	69.6
12	5.08, s	121.3	5	3.04, 3.72, m	66.3
13	-	145.2	Rha ^{I}		
14	-	41.9	1	5.08, s	100.3
15	1.21, nd, m	29.4	2	3.66, m	71.2
16	1.78, m	23.4	3	3.57, m	68.6
17	-	46.0	4	3.12, m	72.8
18	2.76, m	41.6	5	4.24, m	67.7
19	0.90, 1.95, m	47.0	6	1.03, d, *J*=6.0 Hz	18.3
20	-	30.9	Glc ^{I}		
21	1.05, 1.07, m	34.2	1	4.24, d, *J*=7.2 Hz	103.9
22	1.37, m	32.6	2	2.95, m	73.9
23	3.08, 3.32, m	63.0	3	3.21, m	75.7
24	0.55, s	13.4	4	3.48, m	74.7
25	0.85, s	16.0	5	3.26, m	76.0
26	0.70, s	17.5	6	3.66, 3.84, m	59.6
27	1.06, s	26.1	Rha ^{II}		
28	-	179.8	1	5.08, s	100.3
29	0.84, s	33.5	2	3.55, m	70.8
30	0.85, s	24.0	3	3.62, m	82.0
			4	3.38, m	71.1
			5	3.77, m	68.3
			6	1.08, d, *J*=4.2 Hz	18.3
			Glc ^{II}		
			1	4.37, d, *J*=8.4 Hz	104.4
			2	2.95, m	73.8
			3	3.26, m	75.0
			4	3.30, m	69.8
			5	3.09, m	76.9
			6	3.66, 3.76, m	60.4

[a]The assignments are based on DEPT, COSY, HMQC, TOCSY and HMBC experiments [b]nd: not detected

Acknowledgments

We thank Prof. Dr. H. Sümbül and Assoc. Prof. Dr. R. S. Göktürk for their valuable assistance for collection and identification of the plant materials. We also grateful to TUBITAK (107 T 028), EBILTEM (2008 BIL 003) and EUBAP (2009 FEN 043) for financial supports and would like to thank TUBITAK for running HRESIMS and NMR (600 MHz) spectra, EBILTEM for running NMR (400 MHz) spectra and Ege University Faculty of Science, Biology Department for ESIMS analysis.

Supporting Information:

The following Supporting Information is available for this article:
http://www.acgpubs.org/RNP/2010/Volume%204/2010_issue_4_3_list

References

[1] P. H. Davis (1972). Flora of Turkey and The East Aegean Islands, University Press, Edinburgh, Scotland, **4**, 585-597 pp.
[2] S. Kirmizigul and H. Anil (1994). Transsylvanoside A, an acidic triterpene glycoside from *Cephalaria transsylvanica*. *Phytochemistry* **35**, 1075-1076.
[3] S. Kirmizigul, H. Anil and M.E. Rose (1995). Triterpenoid glycoside from *Cephalaria transsylvanica. Phytochemistry* **39**, 1171-1174.
[4] S. Kirmizigul, H. Anil, F. Ucar and K. Akdemir (1996). Antimicrobial and antifungal activities of three new triterpenoid glycosides. *Phytother. Res.* **10**, 274-276.
[5] N. Tabatadze, R. Elias, R. Faure, P. Gerkens, M.C. Pauw-Gillet, E. Kemertelidze, A. Chea and E. Ollivier (2007). Cytotoxic triterpenoid saponins from the roots of *Cephalaria gigantea. Chem. Pharm. Bull.* **55**, 102-105.
[6] S. Capanlar and S. Kirmizigul (2010). Structural elucidations and spectral assigments of two novel Triterpene glycosides from *Cephalaria paphlagonica. Nat. Prod. Res.* in press.
[7] S. Kirmizigul and H. Anil (2002). New triterpenic saponins from *Cephalaria transsylvanica*. *Turk. J. Chem.* **26**, 947-954.
[8] N.B. Sarikahya and S. Kirmizigul (2010). Antimicrobial triterpenoid glycosides from *Cephalaria scoparia*. *J. Nat. Prod.* **73**, 825-830.
[9] E. Halay (2009). Chemical constituents and biological activities of two endemic *Cephalaria Species* from Dipsacaceae, M.Sc. Thesis, Ege University Press, Izmir, Turkey.
[10] S. Kirmizigul, H. Anil and M.E. Rose (1996). Triterpenoid saponins from *Cephalaria transsylvanica*. *J. Nat. Prod.* **59**, 415-418.
[11] S. Kirmizigul and M.E. Rose (1997). Hederagenin glycosides from the flowers of *Cephalaria transsylvanica*. *Planta Med.* **63**, 51-54.
[12] K. Mustafaeva, R. Elias, G. Balansard, T. Suleimanov, V. Mayu-Lede and Y. Kerimov (2008). Iridoid glycosides from *Cephalaria kotschyi* roots. *Chem. Nat. Compd.* **44**, 132-133.
[13] I. S. Movsumov and M.A. Aliev (1975). Flavonoids of some *Cephalaria* species. *Khim. Prir. Soedin*. **11**, 804-805.
[14] D. Godevac, V. Vajs, N. Menkovic, V. Tesevic, P. Janackovic and S. Milosavljevicv (2004). Flavonoids from flowers of *Cephalaria pastricensis* and their antiradical activity. *J. Serb. Chem. Soc*. **69**, 883-886.
[15] I. S. Movsumov, E. A. Garaev and M. I. Isaev (2006). Flavonoids from *Cephalaria gigantea* flowers. *Chem. Nat. Compd.* **42**, 677-680.
[16] A. M. Aliev, I. S. Movsumov and E. Bagirov (1975). Alkaloids from certain *Cephalaria* species. *Khim. Prir. Soedin.* **5**, 667.
[17] S. Pasi, N. Aligiannis, A.L. Skaltsounis and I.B. Chinou (2002). A new lignan glycoside and other constituents from *Cephalaria ambrosioides*. *Nat. Prod. Lett.* **16**, 365-370.

[18] M. L. Bouillant, J. Chopin and V. Plouvier (1972). Swertiajaponin from *Cephalaria leucantha*. *Phytochemistry* **11**, 1858.
[19] G.N. Zemtsova and V.A. Bandyukova (1977). C-Glycosides of species of dipsacaceae. *Khim. Prir. Soedin.*, **5**, 705-706.
[20] M. Xu, M. Zhang, Y. J. Zhang and C. R. Yang (2009). New acylated secoiridoid glycosides from *Gentiana straminea* (Gentianaceae). *Helv. Chim. Acta*. **92**, 321-327.

[21] A. Itoh, Y. Tanaka, N. Nagakura, T. Akita, T. Nishi and T. Tanahashi (2008). Phenolic and iridoid glycosides from *Strychnos axillaries*. *Phytochemistry* **69**, 1208–1214.
[22] I. Tatli, W. Schuhly, O. Kunert, E. Bedir, Z. S. Akdemir (2008). Secondary metabolites from the aerial parts of *Verbascum dudleyanum* and their biological activities. *Chem. Nat. Compd.* **44**, 292-295.

A C G
publications