Chemistry of Natural Compounds, Vol. 44, No. 3, 2008

SECONDARY METABOLITES FROM THE AERIAL PARTS OF *Verbascum dudleyanum* AND THEIR BIOLOGICAL ACTIVITIES

I. Irem Tatli,[1*] Wolfgang Schuhly,[2] Olaf Kunert,[3] Erdal Bedir,[4] and Zeliha S. Akdemir[5]

UDC 547.972

From the overground parts of Verbascum dudleyanum*, six iridoid glycosides, aucubin, ajugol, catalpol, 6-*O*-α-L-rhamnopyranosylcatalpol, saccatoside, and 6-*O*-(3″-*O*-trans-p-coumaroyl)-α-L-rhamnopyranosylcatalpol, and two saponins, ilwensisaponin A and C, as well as a flavonoid, luteolin-7-*O*-β-glucopyranoside, together with an acetophenone glucoside, picein, were isolated. The structures of isolated compounds were elucidated by spectroscopic methods. These compounds showed biological acitivites.*

Key words: *Verbascum dudleyanum*, Scrophulariaceae, iridoid glycosides, saponins, flavonoid, acetophenone glucoside.

The genus *Verbascum* (Scrophulariaceae) is represented by 228 species in Turkey. *Verbascum dudleyanum* is reported to be an endemic species [1]. Previous investigations on Turkish *Verbascum* species by our research group led to the isolation and characterization of a number of secondary metabolites such as iridoids, monoterpene glucoside, saponins, phenylethanoid, neolignan, and flavonoid glycosides [2-10]. As a part of our ongoing studies on the secondary metabolites of *Verbascum* species, we have now investigated the methanolic extract of the aerial parts of *V. dudleyanum* and isolated six iridoid glycosides, aucubin (**1**) [3], ajugol (**2**) [6], catalpol (**3**) [5], and 6-*O*-rhamnopyranosylcatalpol (**4**) [8], saccatoside (**5**) [5], and 6-*O*-(3″-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosylcatalpol (**6**) [5], as well as two saponins, ilwensisaponin A (**7**) [6, 11] and C (**8**) [6, 11], along with a flavonoid, luteolin-7-*O*-β-glucopyranoside (**9**) [10] and an acetophenone glucoside, picein (**10**) [12, 13] by means of various chromatographic techniques (Fig. 1). The current paper deals with the isolation and structure elucidation of compounds **1**-**10** from the title plant. Compounds **1**-**9** were identified by comparing their spectroscopic data with those published in the literature, whereas the structure of compound **10** was identified based on the following evidence.

Compound **10** was obtained as a colorless amorphous powder. The LC-ESIMS spectrum of **10** exhibited a molecular ion peak $[M+Na]^+$ at *m/z* 321, suggesting a molecular formula of $C_{14}H_{18}O_7$ together with ^1H and ^{13}C NMR data. Its UV spectrum suggested the presence of an aromatic ring (λ_{max} 265 nm), and the IR spectrum exhibited absorption bands for a hydroxyl group (3383 cm^{-1}), a conjugated ketone carbonyl (1660 cm^{-1}), and an aromatic ring (1605, 1590, 1510 cm^{-1}). The ^1H NMR spectrum of **10** showed typical resonances of the AA′BB′ system of the *para*-disubstituted phenyl ring observed at δ_H 7.00 (2H, d, J = 8.8 Hz, H-2/6) and 6.65 (2H, d, J = 8.8 Hz, H-3/5) and an anomeric proton signal observed at δ_H 4.20 (1H, d, J = 8.0 Hz) as well as a methyl signal at δ_H 2.50 (3H, s). The chemical shift of the methyl signal (3H, s) indicated that the methyl group was connected to the carbonyl function. Moreover, the anomeric carbon appearing at δ_C 102.1 together with the signals in the region of δ_C 61.6-77.8 suggested the presence of a glucopyranosyl moiety. The coupling constant (J = 8.0 Hz) of the anomeric proton indicated that the sugar moiety was in the β-configuration. Furthermore, the quaternary carbon resonance at δ_C 199.0 was attributable to a carbonyl function in the ^{13}C NMR spectrum.

1) Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Syhhiye, TR-06100 Ankara, Turkey, tel: 90 312 3051089; fax: 90 312 3114777, e-mail: itatli@hacettepe.edu.tr; 2) University of Graz, Institut fur Pharmakognosie, A-8010 Graz, Austria; 3) Institution of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Karl-Franzens-University, Universitaetsplatz 1, 8010 Graz, Austria; 4) Ege University, Faculty of Engineering, Department of Bioengineering, Bornova, TR-35100, Izmir, Turkey; 5) Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, Syhhiye, TR-06100 Ankara, Turkey. Published in Khimiya Prirodnykh Soedinenii, No. 3, pp. 232-234, May-June, 2008. Original article submitted January 15, 2007.

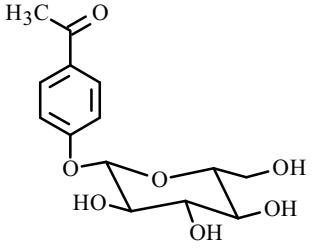
 0009-3130/08/4403-0292

TABLE 1. ^{13}C and ^{1}H NMR (^{13}C; 125 and ^{1}H; 500 MHz, DMSO-d_6, ppm) Data of Compound **10**

C atom		δ_C	δ_H	J (Hz)
Aglycone				
1	C	131.7		
2/6	CH	130.4	7.00 d	J = 8.8
3/5	CH	115.7	6.65 d	J = 8.8
4	C	161.8		
7	C	199.0		
8	CH_3	25.5	2.50 s	
Glucose				
1′	CH	102.1	4.20 d	J = 8.0
2′	CH	74.7	3.00-3.50*	
3′	CH	77.4	3.00-3.50*	
4′	CH	70.9	3.00-3.50*	
5′	CH	77.8	3.00-3.50*	
6′	CH_2	61.6	3.64*	
			3.86 dd	J = 12.0, 1.7

*Signal patterns are unclear due to overlapping.

In the ^{13}C NMR and DEPT-135 spectra of **10**, eight signals were identical with ketone carbonyl, glucose, and methyl (δ_C 25.5) groups. The rest of the ^{13}C NMR signals showed the presence of two quaternary carbon resonances (δ_C 161.8 and 131.7) and an equivalent two pairs of methine signals (δ_C 130.4 and 115.7, each 2H) arising from a *para*-disubstituted aromatic ring. From the chemical shift of C-4 (δ_C 161.8) in **10**, it can be deduced that the hydroxyl group was at C-4 and the β-glucose unit was connected at C-4 through an *O*-linkage. Consequently, the ketone carbonyl was linked at C-1. Based on its NMR data and a comparison of the data given in the literature, compound **10** was identified as an acetophenone glucoside, picein (**10**) [12, 13].



10

The isolated compounds, ilwensisaponin A (**7**), ilwensisaponin C (**8**), and luteolin 7-*O*-β-glucopyranoside (**9**), exhibited a dose-dependent inhibition of bioautographic and spectrophotometric DPPH activities [10, 14]. Aucubin (**1**) and ilwensisaponin A (**7**) were found to possess significant antinociceptive and anti-inflammatory activities per os without inducing any apparent acute toxicity or gastric damage [15]. The antimicrobial activity of ilwensisaponin A and C was detected from the potent growth inhibition against *Aspergillus fumigatus* ATCC 90906 [16]. In the test for fungicidal activity, both saponins were found to exhibit potent *in vitro* activity against *Colletotrichum acutatum*, *C. fragariae*, and *C.* gloeosporioides [17].

Concerning the iridoid glycosides of the genus *Verbascum*, the isolation of aucubin (**1**) [2, 3, 9], ajugol (**2**) [2, 6, 9], catalpol (**3**) [2, 5, 9], 6-*O*-L-rhamnopyranosylcatalpol (**4**) [2, 3, 8, 9], saccatoside (**5**) [2, 5], and 6-*O*-(3″-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosylcatalpol (**6**) [2, 5] from several other *Verbascum* species has been reported previously. It is well known that aucubin, ajugol, and catalpol are common iridoid glycosides, and acylated 6-*O*-L-rhamnopyranosylcatalpol derivatives are taxonomic markers in the genus *Verbascum* and family Scrophulariaceae. The occurrence of saponin glycosides in the genus *Verbascum* is well documented in the literature [2]. To the best of our knowledge, ilwensisaponin C (**8**) has been isolated from *Verbascum* species for the third time. This compound was earlier reported from *V. nigrum* [2] and *V. pterocalycinum* var. *mutense* [2, 6]. Picein (**10**) was previously reported from *Veronica bellidioides* [18] and *Penstemon acuminatus* [19] from the Scrophulariaceae family, and this is the first report from *Verbascum* genus. Additionally, this is the first report on the isolation

and characterization of all these compounds from *V. dudleyanum* as well as a *Verbascum* species from Group A of the genus [1]. Our continuing studies will be of assistance in clarifying the chemotaxonomic classification of the genus *Verbascum*.

EXPERIMENTAL

General Experimental Procedures: The UV spectra (λ_{max}) was recorded on a Hitachi HP 8452 A spectrophotometer. The IR spectra (ν_{max}) was determined on an ATI Mattson Genesis Series FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance DRX 500 FT spectrometer operating at 500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR spectra. The chemical shift values are reported as parts per million (ppm) relative to tetramethylsilane (TMS), and the coupling constants are in Hertz (Hz, in parentheses). For the ^{13}C NMR spectra, multiplicities were determined by DEPT experiment. LC-ESIMS FT data were obtained using a Bruker BioApex FT-MS instrument in the ESI mode. Polyamide (ICN) was used for vacuum liquid chromatography (VLC), and Sephadex LH-20 was used for column chromatography (CC). MPLC was performed on Labomatic (1.8×35.2 cm and 1.3×38 cm) glass columns packed with LiChroprep RP-18 (Merck), using a Lewa M5 (peristaltic) pump. Pre-coated silica gel 60 F_{254} aluminum sheets (Merck) were used for TLC with the developing solvent system $CHCl_3$–MeOH–H_2O (61:32:7). Plates were examined by UV fluorescence and sprayed with 1% vanillin in conc. H_2SO_4, followed by heating at 105°C for 1-2 min.

Plant Material. *Verbascum dudleyanum* (Hub.-Mor.) Hub.-Mor. was collected from Burdur, south shore of Lake Salda, wet places by the lake, 1170 m, in July 2000. A voucher specimen has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 02001).

Extraction and Isolation. Dried and powdered aerial parts of *V. dudleyanum* (231.9 g) were extracted with MeOH (2×2000 mL) at 40°C, and the combined MeOH extracts were concentrated under reduced pressure (20 g). The resultant residue was then dissolved in H_2O (150 mL), and the water–soluble portion was partitioned between $CHCl_3$ (3×100 mL) and *n*-BuOH (5×100 mL). An aliquot of the *n*-BuOH extract (8.4 g) was chromatographed over polyamide (250 g) using the VLC (Vacuum liquid chromatography) technique eluting with H_2O, followed by increasing concentrations of MeOH in H_2O (25%, 50%, 75% and 100%, each 250 mL) to yield six main fractions (A-F). Fraction A (858 mg) was carried out on C_{18}-MPLC using gradient H_2O-MeOH mixtures (0-100%) to give compounds **1** (25 mg), **2** (3 mg), **3** (15 mg), **4** (10 mg), and **10** (7 mg). Fraction B (635 mg) was subjected to a C_{18}-column using gradient H_2O–MeOH mixtures (20-100%) to afford compounds **5** (25 mg), **6** (14.5 mg), **7** (7 mg), and **8** (6 mg). Fraction F (192 mg) was further purified on a Sephadex LH-20 (20 g) column using MeOH to give compound **9** (9 mg).

Picein (10). UV (MeOH, λ_{max}): 265nm. IR (KBr, ν_{max}, cm^{-1}): 3383 (OH), 1660 (C=O), 1605, 1590, 1510 (aromatic ring). Positive ion: LC-ESIMS *m/z* 321 ($[M+Na]^+$, calc. for $C_{14}H_{18}O_7$). ^{1}H (500 MHz, DMSO-d_6) and ^{13}C (125 MHz, DMSO-d_6) NMR data were given in Table 1.

Reduction of DPPH Radical by Bioautographic Assay. Methanolic solutions (0.1%) of compounds were chromatographed on a Si gel TLC plate using $CHCl_3$-MeOH-H_2O (61:32:7). After drying, TLC plates were sprayed with a 0.2% DPPH solution in MeOH. Compounds showing a yellow-on purple spot were regarded as antioxidants [20].

Reduction of DPPH Radical by Spectrophotometric Assay. Methanolic solutions of the compounds at various concentrations were added to 1.5×10^{-5} M DPPH in MeOH. The absorbance of the remaining DPPH was measured at 520 nm, after 30 min incubation at room temperature. A decrease in the DPPH solution absorbance indicates an increase in DPPH radical-scavenging activity. This activity is given as % DPPH radical scavenging, which is calculated from the equation: (Control absorbance–sample absorbance)/Control absorbance × 100. (±)-α-Tocopherol, ascorbic acid, and 3-BHA, as well as DPPH solution without sample solution, were used as control [21].

Antinociceptive Activity (*p*-Benzoquinone-Induced Abdominal Constriction Test in Mice). Sixty minutes after oral administration of test samples, the mice were intraperitoneally injected with 0.1 mL/10 g body weight of 2.5% (v/v) *p*-benzoquinone (PBQ) solution in distilled H_2O. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the 5th min after the PBQ injection. The data represent the average of the total number of writhes observed. The antinociceptive activity was expressed as percentage change from writhing controls; 100 mg/kg and 200 mg/kg aspirin (ASA) were used as reference drug [22].

Anti-Inflammatory Activity (Carrageenan-Induced Hind Paw Edema). Sixty min after the oral administration of test sample or dosing vehicle, each mouse was injected with a freshly prepared (0.5 mg/25 μL) suspension of carrageenan in physiological saline (154 nM NaCl) into subplantar tissue of the right hind paw. As the control, 25 μL saline solution was injected into that of the left hind paw. Paw edema was measured in every 90 min during 6 h after induction of inflammation. The difference in footpad thickness was measured by a gauge calipers. Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. Indomethacin (10 mg/kg) was used as a reference drug [23].

Antimicrobial Assay. Susceptibility testing was performed using a modified version of the National Committee for Clinical Laboratory Standards (NCCLS) methods [24, 25]. Inhibition of fungal growth on chromatographic plates was evaluated by modifications of TLC bioautographic assays [26, 27].

ACKNOWLEDGMENT

The authors thank Prof. Dr. Hayri Duman, Gazi University, Faculty of Science, Department of Botany, Etiler, Ankara, Turkey for authenticating the plant specimen. This work was financially supported by the Research Fund of Hacettepe University (Project No. 04 D05 301 002).

REFERENCES

1. A. Huber-Morath, *Verbascum*, in "*Flora of Turkey and the East Aegean Islands,*" Vol. 6, ed. P.H. Davis, University Press, Edinburgh, 1978, 461.
2. I. I. Tatli and Z. S. Akdemir, *J. Pharm. Sci. (FABAD)*, **29**, 93 (2004).
3. Z. S. Akdemir, I. I. Tatli, E. Bedir, and I. A. Khan, *Turk. J. Chem.,* **28**, 101 (2004a).
4. Z. S. Akdemir, I. I. Tatli, E. Bedir, and I. A. Khan, *Turk. J. Chem.,* **28**, 227 (2004b).
5. I. I. Tatli, Z. S. Akdemir, E. Bedir, and I. A. Khan, *Turk. J. Chem.,* **27**, 765 (2003).
6. I. I. Tatli, Z. S. Akdemir, E. Bedir, and I. A. Khan, *Turk. J. Chem.*, **28**, 111 (2004).
7. Z. S. Akdemir, I. I. Tatli, E. Bedir, and I. A. Khan, *Turk. J. Chem.*, **28**, 621 (2004c).
8. Z. S. Akdemir, I. I. Tatli, E. Bedir, and I. A. Khan, *Z. Naturforsch.*, **60b**, 113 (2005).
9. I. I. Tatli, I. A. Khan, and Z. S. Akdemir, *Z. Naturforsch.*, **61b**, 1183 (2006).
10. Z. S. Akdemir, I. I. Tatli, E. Bedir, and I. A. Khan, *J. Pharm. Sci. (FABAD)*, **28**, 71 (2003).
11. I. Calis, M. Zor, A. A. Basaran, A. D. Wright, and O. Sticher, *Helv. Chim. Acta*, **76**, 1352 (1993).
12. P. Junior, *Planta Med.,* **50**, 444 (1984).
13. H. Kirmizibekmez, Pharmacognostical Studies on *Globularia* Species, PhD Thesis, Hacettepe University, Institute of Health Sciences, Ankara, Turkey, 2002.
14. I. I. Tatli, S. Takamatsu, I. A. Khan, and Z. S. Akdemir, *Z. Naturforsch. C,* **62c**, 673 (2007).
15. E. Kupeli, I. I. Tatli, Z. S. Akdemir, and E. Yesilada, *J. Ethnopharmacol*, **110**, 444 (2006).
16. I. I. Tatli and Z. S. Akdemir, *J. Pharm. Sci. (FABAD)*, **30**, 84 (2006).
17. I. I. Tatli, Z. S. Akdemir, E. Bedir, and I. A. Khan, *J. Pharm. Sci. (FABAD),* **28**, 137 (2003).
18. R. Taskova, N. Handjieva, D. Peev, and S. Popov, *Phytochemistry*, **49** (5), 1323 (1998).
19. T. Vesper and K. Seifert, *Phytochemistry*, **37** (4), 1087 (1994).
20. T. Takao, F. Kitatani, N. Watanabe, A. Yagi, and K. Sakata, *Biosci. Biotech. Biochem.*, **58**, 1780 (1994).
21. T. Hatano, R. Edamatsu, M. Hiramatsu, A. Mori, Y. Fujita, and T. Yasuhara, *Chem Pharm Bull,* **37**, 2016 (1989).
22. R. Okun, S. C. Liddon, and L. Lasagnal, *J. Pharmacol. Exp. Ther.*, **139**, 107 (1963).
23. E. Yesilada and E. Küpeli, *J. Ethnopharmacol.,* **79**, 237 (2002).
24. National Committee for Clinical Laboratory Standards (NCCLS), *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. Approved Standard. M7-A, 4th ed., National Committee for Clinical Laboratory Standards: Wayne, P.A.1997.
25. National Committee for Clinical Laboratory Standards (NCCLS), *Methods for Dilution Antifungal Susceptibility Tests for Yeasts*. Approved Standard. M27-A. Wayne, P.A. 1997.
26. A. L. Homans and A. Fuchs, *J. Chromatogr.,* **51**, 327 (1970).
27. A. E. Osborne, B. R. Chase, P. Lunness, P. R. Scott, and M. J. Daniels, *Physiol. Mol. Plant Pathol.*, **45**, 457 (1994).