

1-1-2004

Iridoid and Phenylethanoid Glycosides from *Verbascum lasianthum*

ZELİHA Ş. AKDEMİR

İ. İREM TATLI

ERDAL BEDİR

IKHLAS A. KHAN

Follow this and additional works at: <https://journals.tubitak.gov.tr/chem>

 Part of the [Chemistry Commons](#)

Recommended Citation

AKDEMİR, ZELİHA Ş.; TATLI, İ. İREM; BEDİR, ERDAL; and KHAN, IKHLAS A. (2004) "Iridoid and Phenylethanoid Glycosides from *Verbascum lasianthum*," *Turkish Journal of Chemistry*. Vol. 28: No. 2, Article 11. Available at: <https://journals.tubitak.gov.tr/chem/vol28/iss2/11>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Chemistry by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

Iridoid and Phenylethanoid Glycosides from *Verbascum lasianthum*

Zeliha Ş. AKDEMİR¹, İ. İrem TATLI¹, Erdal BEDİR², Ikhlas A. KHAN²

¹*Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy,
TR-06100, Ankara-TURKEY*

e-mail: zakdemir@hacettepe.edu.tr

²*The University of Mississippi, School of Pharmacy, National
Center for Natural Products Research, Research Institute of Pharmaceutical Sciences,
University MS 38677 USA*

Received 10.07.2003

Three iridoid glucosides, 8-*O*-acetylharpagide (**1**), harpagoside (**2**), and 6-*O*-vanilloylajugol (**3**), were isolated from the roots of *Verbascum lasianthum* Boiss. ex Benth. In addition, 2 phenylethanoid glycosides, verbascoside {=acteoside, [β -(3,4-dihydroxyphenyl)-ethyl]-(3'-*O*- α -L-rhamnopyranosyl)-(4'-*O*-caffeyl)- β -D-glucopyranoside} (**4**) and poliumoside {= [β -(3,4-dihydroxyphenyl)-ethyl]-(3',6'-*O*- α -L-dirhamnopyranosyl)-(4'-*O*-caffeyl)- β -D-glucopyranoside} (**5**), were also isolated. The structures of all compounds were established by spectroscopic evidence (UV, IR, 1D and 2D NMR, LC-ESIMS). Compounds **2-5** demonstrated scavenging properties toward the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in TLC autographic assays.

Key Words: *Verbascum lasianthum*, Scrophulariaceae, iridoid glucosides, 8-*O*-acetylharpagide, harpagoside, 6-*O*-vanilloylajugol, phenylethanoid glycosides, verbascoside (= acteoside), poliumoside, radical scavenging activity.

Introduction

The genus *Verbascum* L., known as “Mullein”, is represented by 228 species in the Flora of Turkey, including 185 species that are endemic to this area¹. Some *Verbascum* species are used as an expectorant and mucolytic in folk medicine². These species are also used externally for desiccating wounds, anal fistula and pruritic conditions in the urogenital organs³. Although the taxonomic and morphological aspects of the genus *Verbascum* appear more or less complex, the frequent occurrence of the iridoid and phenylethanoid glycosides in the Scrophulariaceae has been used in chemotaxonomic studies⁴. As part of our ongoing project, the chemical characterization of *Verbascum* species growing in Turkey¹, we have isolated 6 iridoid glycosides, 6-*O*-(α -L-rhamnopyranosyl)-catalpol, verbascoside A, pulverulentoside I, buddlejoside A₅, aucubin and unduloside III from *Verbascum lasianthum*⁵. Further investigation into the roots of this plant yielded 3 iridoid glucosides, 8-*O*-acetylharpagide (**1**), harpagoside (**2**), and 6-*O*-vanilloylajugol (**3**), along

with 2 phenylethanoid glycosides, verbascoside (= acteoside) (**4**) and poliumoside (**5**). The current study describes the structure elucidation of the isolated compounds. Radical scavenging activity of compounds **1-5** was also examined.

Experimental

General Experimental Procedures: The UV spectra (λ_{max}) were recorded on a Hitachi HP 8452 A spectrophotometer. The IR spectra (ν_{max}) were determined on an ATI Mattson Genesis Series FT-IR spectrophotometer. The 1D and 2D NMR spectra were obtained on a Bruker Avance DRX 500, 400 and 300 FT spectrometer operating at 500, 400 and 300 MHz for ^1H NMR, and at 125 and 75 MHz for ^{13}C NMR. 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 a distortionless enhancement by a polarization transfer (DEPT) experiment. LC-ESIMS data were obtained using a Bruker BioApex FT-MS instrument in the ESI mode. Polyamide (ICN) and Si gel (230-400 mesh) (Merck) were used for column chromatography (CC). Reverse-phase material (C-18, Sepralyte 40 μm) was used for vacuum liquid chromatography (VLC). Medium pressure liquid chromatography (MPLC) separations were performed on a Labomatic glass column packed with LiChroprep RP-18 (Merck), using a Lewa M5 peristaltic pump. Pre-coated silica gel 60 F₂₅₄ aluminum sheets (Merck) were used for TLC; developing system, $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (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 lasianthum* Boiss. ex Bentham was collected from İzmir, Urla (W. Anatolia, Turkey), Üçahırlar in August 1999. A voucher specimen has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 99139).

Extraction and Isolation. Air-dried and powdered roots of the plant (333.9 g) were extracted with MeOH (2 x 2 L) at 40 °C. After evaporation the extract was suspended in H_2O (400 mL), and then extracted with CHCl_3 . The water phase was concentrated to dryness and freeze-dried. The aqueous extract (16.0 g) was fractionated over polyamide (100 g) employing H_2O and MeOH/ H_2O mixtures (0-100%) to yield 5 main fractions (A-E). Fr. A (4.3 g) was subjected to vacuum liquid chromatography (VLC) using reversed-phase material (Sepralyte 40 μm , 350 g), employing MeOH/ H_2O mixtures (0-100%) to give 8-*O*-acetylharpagide (**1**) (13.0 mg). Fr. B (1.5 g) was chromatographed (MPLC= medium-pressure liquid chromatography) on a Sepralyte C18 reversed-phase column eluted with MeOH/ H_2O mixtures (2.5-90%) to yield 5 fractions (Frs. B₁-B₅). Fr. B₁ (112.8 mg) was applied to VLC using reversed-phase material (Sepralyte 40 μm , 25 g), employing MeOH/ H_2O mixtures (0-25%) to give 6-*O*-vanilloylajugol (**3**) (59.6 mg). Fr. B₄ (72.7 mg) was also subjected to VLC using reversed-phase material (Sepralyte 40 μm , 20 g), employing MeOH/ H_2O mixtures (20-35%) to give harpagoside (**2**) (67.8 mg). Fr. C (1.0 g) was chromatographed on a Si gel column (150 g) eluted with $\text{CHCl}_3\text{-MeOH}$ mixtures (95:5, 90:10, 85:15, 80:20) and $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ mixtures (80:20:2, 70:30:3) to yield 2 fractions (Frs. C₁-C₂). Fr. C₂ (26.3 mg) was further purified on a Si gel column (40 g) using $\text{CHCl}_3\text{-MeOH}$ mixtures (95:5, 90:10, 85:15, 80:20, 75:25) to give poliumoside (**5**) (3 mg). Fr. E (1.2 g) was subjected to VLC using reversed-phase material (Sepralyte 40 μm , 100 g), eluted

with MeOH/H₂O mixtures (10-100%) to give verbascoside (**4**) (100.1 mg).

Reduction of DPPH Radical

Methanolic solutions (0.1%) of compounds **1-5** were chromatographed on a Si gel TLC plate using CHCl₃-MeOH-H₂O (61:32:7). After drying, TLC plates were sprayed with a 0.2% DPPH (Sigma) solution in MeOH. Compounds showing a yellow-on-purple spot were regarded as antioxidants^{6,7}.

Results

8-O-acethylharpagide (1): UV (MeOH) λ_{max} 210 nm, IR (KBr) ν_{max} 3600 (OH), 1708 (C=O), 1670 (C=C) cm⁻¹, LC-ESIMS m/z 429 [M+Na]⁺ (calc. for C₁₇H₂₆O₁₁), ¹H NMR (300 MHz, DMSO-*d*₆) and ¹³C NMR (75 MHz, DMSO-*d*₆) data (Table 1).

Table 1. ¹H NMR (500 MHz, DMSO-*d*₆) and ¹³C NMR (125 MHz, DMSO-*d*₆) Data of Compounds **1-3**.

Position	C _{Atom}	1*			2			3**		
		δ_C (ppm)	δ_H (ppm)	<i>J</i> (Hz)	δ_C (ppm)	δ_H (ppm)	<i>J</i> (Hz) (Hz)	δ_C (ppm)	δ_H (ppm)	<i>J</i> (Hz)
Aglycone										
1	CH	93.5	6.06 brs	-	92.4	5.91 s	-	92.4	5.42 d	1.7
3	CH	142.7	6.40 d	6.3	141.2	6.32 d	6.3	140.0	6.13 dd	1.5/6.0
4	CH	106.3	4.93 d	6.3	107.4	4.84 d	6.3	103.8	4.91 m	-
5	CH (C) ξ	72.3	-	-	71.3	-	-	38.4	2.88 m	-
6	CH	76.1	3.71 d	3.9	77.1	3.74 d	7.5	79.4	4.98 m	-
7a	CH ₂	45.1	1.95 dd	4.4/15.2	44.6	1.79 dd	4.5/14.7	46.9	1.97 dd	3.7/14.0
7b	-	-	2.17 dd	6.5/15.1	-	2.13 dd	6.0/14.8	-	2.19 dd	6.3/14.0
8	C	87.6	-	-	86.8	-	-	78.2	-	-
9	CH	54.6	2.84 m	-	54.4	2.67 m	-	50.6	2.51 dd (t)	9.4
10	CH ₃	21.7	1.45 s	-	22.2	1.39 s	-	25.2	1.31 s	-
β - D-Glucose										
1'	CH	98.8	4.58 d	7.9	97.2	4.36 d	7.9	98.4	4.57 d	7.9
2'	CH	73.6	3.21 d	8.2	73.1	2.96 t	8.5	73.8	3.13 t	8.4
3'	CH	77.3	3.44-3.50 \dagger	-	76.2	3.13 t	8.8	77.0	3.30 t	8.9
4'	CH	70.7	3.38 d	9.1	70.1	3.05 d	9.0	70.7	3.21 t	8.9
5'	CH	76.1	3.45 m	-	75.7	3.30 m	-	77.2	3.30 m	-
6'a	CH ₂	61.9	3.72 dd	5.1/15.0	61.1	3.45 dd	5.6/11.2	61.9	3.57 dd	6.2/11.3
6'b	-	-	3.89 d	12.2	-	3.68 d	11.6	-	3.90 m	-
Acyl moiety										
1''	C	-	-	-	134.1	-	-	119.2	-	-
2''	CH	-	-	-	129.0	7.62 s	-	112.4	6.86 d	8.5
3''	C (CH) \aleph	-	-	-	128.3	7.35 s	-	152.0	-	-
4''	C (CH) \aleph	-	-	-	130.4	7.34 s	-	148.7	-	-
5''	CH	-	-	-	128.3	7.35 s	-	115.8	6.66 d	8.2
6''	CH	-	-	-	129.0	7.62 s	-	124.6	7.45 d	8.4
α	CH	-	-	-	119.5	6.47 d	16.0	-	-	-
β	CH	-	-	-	144.1	7.53 d	16.0	-	-	-
C=O	C	-	-	-	165.9	-	-	167.4	-	-
OCH ₃	CH ₃	-	-	-	-	-	-	55.3	3.73 s	-
OCOCH ₃	C	172.2	-	-	-	-	-	-	-	-
OCOCH ₃	CH ₃	21.4	2.02 s	-	-	-	-	-	-	-

\dagger unclear due to overlapping

ξ C for compounds **1** and **3**

\aleph CH for compound **3**

* 300 and 75 MHz

** 400 and 125 MHz

Harpagoside (2): UV (MeOH) λ_{max} 228 nm, IR (KBr) ν_{max} 3600 (OH), 1705 (C=O), 1637 (C=C), 1604, 1363 (aromatic ring) cm^{-1} , LC-ESIMS m/z 517 [M+Na]⁺ (calc. for C₂₄H₃₀O₁₁). ¹H NMR (500 MHz, DMSO-*d*₆) and ¹³C NMR (125 MHz, DMSO-*d*₆) data (Table 1) superimposable with those reported in the literature⁸.

Table 2. ¹H NMR (500 MHz, DMSO-*d*₆) and ¹³C NMR (125 MHz, DMSO-*d*₆) data of compounds **4** and **5**.

Position	4				5		
	C _{Atom}	δ_C (ppm)	δ_H (ppm)	<i>J</i> (Hz)	δ_C (ppm)	δ_H (ppm)	<i>J</i> (Hz)
Aglycone							
1	C	131.0	-	-	130.0	-	-
2	CH	116.7	6.66 s	-	117.1	6.63 s	-
3	C	145.0	-	-	144.4	-	-
4	C	144.0	-	-	144.4	-	-
5	CH	116.4	6.66 d	7.6	116.4	6.49 d	8.0
6	CH	120.5	6.52 s	-	120.4	6.64 d	8.0
α_a	CH ₂	71.0	3.67 brt	7.7	71.2	3.70 †	-
α_b	-	-	3.91 brt	7.7	-	3.88 †	-
β	CH ₂	35.9	2.73 m	-	35.9	2.70 m	-
β - D-Glucose							
1'	CH	103.3	4.37 d	7.7	103.2	4.38 d	7.8
2'	CH	75.4	3.26 t	8.2	75.2	3.32 m	-
3'	CH	80.1	3.68 †	-	79.7	3.83 m	7.9
4'	CH	69.6	4.75 t	9.5	69.7	4.74 t	9.6
5'	CH	75.4	3.45 m	-	73.8	3.69 m	-
6'a	CH ₂	61.7	3.45-3.70 †	-	66.8	3.36 m	10.0
6'b	-	-	3.45-3.70 †	-	-	3.60 †	-
α - L-Rhamnose							
1''	CH	102.0	5.08 brs	-	101.3	5.03 brs	-
2''	CH	71.5	3.72 †	-	71.2	3.60 d	1.6
3''	CH	70.1	3.35-3.50 †	-	71.1	3.29 dd	2.8/9.4
4''	CH	72.7	3.15 t	9.2	72.5	3.14 t	9.0
5''	CH	69.6	3.35-3.50 †	-	69.6	3.51 m	-
6''	CH ₃	19.0	1.00 d	5.8	19.0	0.96 d	6.1
α - L-Rhamnose							
1'''	CH	-	-	-	102.1	4.50 brs	-
2'''	CH	-	-	-	71.4	3.60 d	1.6
3'''	CH	-	-	-	71.3	3.40 dd	3.1/9.4
4'''	CH	-	-	-	72.7	3.22 t	9.0
5'''	CH	-	-	-	69.3	3.51 m	-
6'''	CH ₃	-	-	-	18.6	1.04 d	6.1
Acyl moiety							
1''''	C	127.0	-	-	126.0	-	-
2''''	CH	115.6	7.04 s	-	115.3	7.02 s	-
3''''	C	146.0	-	-	145.8	-	-
4''''	C	149.0	-	-	146.6	-	-
5''''	CH	114.7	6.79 d	7.7	113.8	6.75 d	8.0
6''''	CH	122.2	6.97 d	7.5	122.5	6.97 d	7.7
α'	CH	117.2	6.20 d	15.8	116.6	6.20 d	15.8
β'	CH	146.3	7.48 d	15.8	146.7	7.48 d	15.8
C=O	C	169.0	-	-	166.6	-	-

† unclear due to overlapping

(''') for compound **5**

6-*O*-vanilloylajugol (3): UV (MeOH) λ_{max} 208, 264 nm, IR (KBr) ν_{max} 3600 (OH), 1708 (C=O), 1654 (C=C), 1604, 1546, 1363 (aromatic ring) cm^{-1} , LC-ESIMS m/z 521 [M+Na]⁺ (calc. for C₂₃H₃₀O₁₂). ¹H NMR (400 MHz, DMSO-*d*₆) and ¹³C NMR (125 MHz, DMSO-*d*₆) data (Table 1).

Verbascoside {=acteoside, [β -(3,4-dihydroxyphenyl)-ethyl]-(3'-*O*- α -L-rhamnopyranosyl)-(4'-*O*-caffeoyl)- β -D-glucopyranoside} (**4**): UV (MeOH) λ_{max} 212, 332 nm, IR (KBr) ν_{max} 3689 (OH), 1708 (C=O), 1634 (C=C), 1604, 1515, 1385 (aromatic ring) cm^{-1} , LC-ESIMS m/z 647 [M+Na]⁺ (calc. for C₂₉H₃₆O₁₅). ¹H NMR (500 MHz, DMSO-*d*₆) and ¹³C NMR (125 MHz, DMSO-*d*₆) data (Table 2) superimposable with those reported in the literature⁹.

Poliumoside {= [β -(3,4-dihydroxyphenyl)-ethyl]-(3',6'-*O*- α -L-dirhamnopyranosyl)-(4'-*O*-caffeoyl)- β -D-glucopyranoside} (**5**): UV (MeOH) λ_{max} 206, 330 nm, IR (KBr) ν_{max} 3600 (OH), 1708 (C=O), 1640 (C=C), 1607, 1521, 1361 (aromatic ring) cm^{-1} , LC-ESIMS m/z 793 [M+Na]⁺ (calc. for C₃₅H₄₆O₁₉). ¹H NMR (500 MHz, DMSO-*d*₆) and ¹³C NMR (125 MHz, DMSO-*d*₆) data (Table 2).

Discussion

From the roots of *Verbascum lasianthum* Boiss. ex Bentham, 3 iridoid glucosides, 8-*O*-acethylharpagide (**1**), harpagoside (**2**) and 6-*O*-vanilloylajugol (**3**) (Figure 1) together with 2 phenylethanoid glycosides, verbascoside (=acteoside) (**4**) and poliumoside (**5**) (Figure 2), were isolated by fractionation of the methanolic extract through a polyamide column, followed by VLC, MPLC and silica gel CC. Compounds **1-5** were identified based on the evidence below.

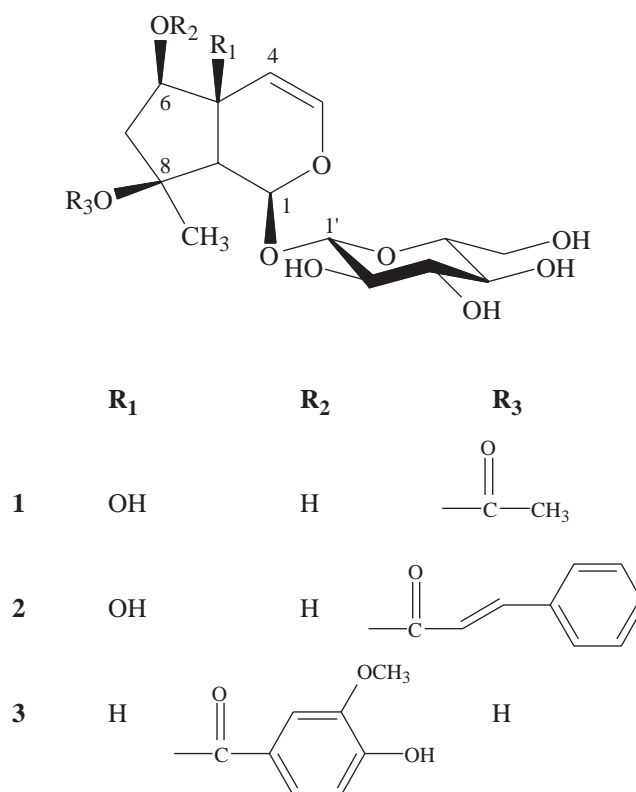


Figure 1. Iridoid glycosides isolated from *Verbascum lasianthum*.

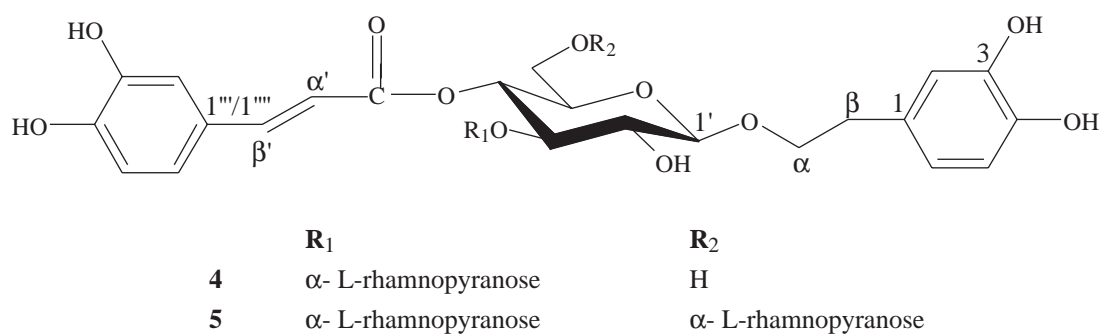


Figure 2. Phenylethanoid glycosides isolated from *Verbascum lasianthum*.

Compounds **1-3** were obtained as amorphous powders whose UV spectra indicated their non-conjugated enol-ether functional group. Their IR spectra showed absorption bands typical of conjugated carbonyl groups (see Results section).

The LC-ESIMS of compound **1** exhibited a pseudomolecular ion $[M+Na]^+$ at m/z 429, compatible with the molecular formula $C_{17}H_{26}O_{11}$, and in good agreement with the observation of 2 methyl, 2 methylene, 10 methine, and 3 quaternary carbon resonances in its ^{13}C NMR spectrum (Table 1). In addition, analysis of the 1H NMR spectrum of **1** (Table 1) revealed the feature of an iridoid glucoside with an acetyl moiety. The signal at δ_H 4.58 (d , $J = 7.9$ Hz), which was attributed to an anomeric proton, as well as the 1H NMR signal in the region of δ_H 3.21-3.89, suggested the presence of a β -glucopyranose unit. On the other hand the 1H NMR spectrum of **1** exhibited the signals arising from an oxymethine (δ_H 3.71, d , $J = 3.9$ Hz) and a tertiary methyl group (δ_H 1.45, s). The complete assignments of all proton and carbon resonances were based on the $^1H, ^1H$ COSY, HMQC and HMBC experiments. An HMBC correlation between H-1' and C-1 showed the attachment of the glucose unit at the C-1 position of the iridoid aglycone. Thus, the vicinally coupled olefinic protons at δ_H 6.40 (d , $J = 6.3$ Hz) and 4.93 (d , $J = 6.3$ Hz) were ascribed to H-3 and H-4, respectively, indicating the presence of an iridoid moiety with a non-conjugated enol-ether system. The chemical shift values and the multiplicities of H-3, H-4 and H-9 (δ_H 2.84, m) indicated an oxygen substitution at C-5. Therefore, the carbon resonance at δ_C 72.3, which showed heteronuclear long-range correlations with H-1 and H-9, was attributed to C-5. The geminally coupled C-7 methylene protons δ_H 1.95 (dd , $J = 15.2/4.4$ Hz) and 2.17 (dd , $J = 15.1/6.5$ Hz) were mutually coupled to an oxymethine proton at δ_H 3.71 (d , $J = 3.9$ Hz) consistent with the hydroxyl group being affixed to C-6 (δ_C 76.1, d). The HMBC cross-peak observed from H₃-10 (δ_H 1.45, s) to C-8 (δ_C 87.6, s) showed the attachment of the methyl group at C-8. On the other hand, the proton and the carbon signals at δ_H 2.02 (s) and δ_C 21.4 (q), 172.2 (s) suggested the presence of an acetyl group. Furthermore, the chemical shift values of both C-8 and H₃-10 indicated the attachment of the acetyl function at C-8. Based on the above results and comparison with the published data compound **1** was identified as 8-*O*-acetylharpagide¹⁰.

The molecular formula of compound **2** was determined by LC-ESIMS, which exhibited a pseudomolecular ion at m/z 517 $[M+Na]^+$, and 1H and ^{13}C NMR data (Table 1) as $C_{24}H_{30}O_{11}$. The 1H NMR spectrum of **2** revealed the resonances of 2 olefinic protons, observed as an AX system, at δ_H 6.47 and 7.53 (d , $J_{AX} = 16.0$ Hz) and 5 aromatic protons at δ_H 7.34 (1H), 7.35 (2H) and 7.62 (2H), consistent with the presence of a *trans*-cinnamoyl moiety. The chemical shift values of both C-8 and H₃-10 indicated that the acyl group was attached at C-8. From the above findings and comparison with the published data, compound **2** was

considered identical to harpagoside⁸.

Compound **3** proved to have the molecular formula C₂₃H₃₀O₁₂, as seen from the positive-ion ESIMS (m/z 521 [M+Na]⁺) combined with ¹H and ¹³C NMR data (Table 1). The UV and IR data of compound **3** showed that **3** consists of a non-conjugated enol-ether system. The ¹H NMR (Table 1) signals at δ_H 6.13 (*dd*, $J = 1.5/6.0$ Hz), 4.91 (*m*) were attributed to H-3 and H-4, respectively, whose chemical shift values and multiplicities indicated that C-5 was non-substituted. This assumption was also supported by the H-9 signal (δ_H 2.51, *dd* (*t*), $J = 9.4$ Hz). On the other hand, the multiplet signal at δ_H 4.98 was attributed to an oxymethine proton at C-6 (δ_C 79.4), which was coupled to H₂-7 (δ_H 1.97, *dd*, $J = 3.7/14.0$ and 2.19, *dd*, $J = 6.3/14.0$ Hz) methylene protons. In the ¹H NMR spectrum of **3**, an aromatic ABX signal pattern (δ_H 6.66, *d*, $J = 8.2$ Hz, 6.86, *d*, $J = 8.5$ Hz and 7.45, *d*, $J = 8.4$ Hz) together with a *O*-methyl signal (δ_H 3.73, *s*) implied the presence of a vanilloyl group¹¹. The vanilloyl group could be placed at C-6 based on the fact that the H-6 methine signal was shifted downfield ca. 1.08 ppm when compared with that of ajugol (δ_H 3.9)⁸. The final proof for this assumption came from the downfield shifted C-6 ($\Delta\delta$ 2.2) resonance comparing with that of ajugol (δ_C 77.2)⁸. Accordingly, the structure of **3** was determined to be 6-*O*-vanilloylajugol¹².

Compounds **4** and **5** were obtained as amorphous powders. The UV and IR spectra indicated their polyphenolic nature. Their IR spectra showed absorption bands typical of hydroxyls: α , β -unsaturated esters, olefinic double bonds, and aromatic rings (see Results section).

Compound **4** was identified as {[β -(3,4-dihydroxyphenyl)-ethyl]-(3'-*O*- α -L-rhamnopyranosyl)-(4'-*O*-caffeoyl)- β -D-glucopyranoside} = verbascoside (= acteoside)⁹ by comparing its ¹H, ¹³C (Table 2) and 2D NMR data with previously published data and by direct comparison with the authentic sample on a TLC plate.

Compound **5** was isolated as an amorphous powder, with the molecular formula C₃₅H₄₆O₁₉ as determined by LC-ESIMS and ¹H and ¹³C NMR data (Table 2). The LC-ESIMS of **5** exhibited a pseudomolecular ion at m/z 793 [M+Na]⁺. The ¹H and ¹³C NMR data indicated that **5** had most of the structural features of verbascoside (**4**). However, in the ¹H NMR spectrum of **5**, in addition to the anomeric proton resonances attributed to a β -glucose and a α -rhamnose moieties, an additional anomeric proton resonance was observed at δ_H 4.50 (*br s*). The corresponding carbon resonance at δ_C 102.1 suggested the presence of a second α -rhamnose moiety in the structure. Therefore, compound **5** was assumed to have a trisaccharide structure. In the ¹³C NMR spectrum of **5**, the C-6' resonance of the glucose moiety (δ_C 66.8) showed a marked downfield shift of ca. 5.0 ppm, suggesting the rhamnose moiety was attached to C-6' of the glucose unit. This suggestion was further verified by the heteronuclear long-range correlation observed from H-1''' of the rhamnose unit to C-6' of glucose moiety in the HMBC spectrum. Thus compound **5** was characterized as poliumoside {= [β -(3,4-dihydroxyphenyl)-ethyl]-(3',6'-*O*- α -L-dirhamnopyranosyl)-(4'-*O*-caffeoyl)- β -D-glucopyranoside}¹³.

Conclusion

In our continuing work on *Verbascum lasianthum*⁵, we now report the isolation and characterization of 8-*O*-acetylharpagide (**1**), harpagoside (**2**) and 6-*O*-vanilloylajugol (**3**), verbascoside (=acteoside) (**4**) and poliumoside (**5**) from the title plant. To our knowledge, 6-*O*-vanilloylajugol was for the first time reported from *Verbascum thapsus*¹⁴. To date, poliumoside has been reported from 6 different *Verbascum* species¹⁵.

Although 8-*O*-acetylharpagide has only previously been detected by TLC and paper chromatography in *V. phlomoides*¹⁰ and *V. thapsiforme*¹⁰, this study is the first report the isolation and structure elucidation of 8-*O*-acetylharpagide from *V. species*. In addition, this study is the first report of the isolation and characterization of phenylethanoid glycosides from *Verbascum lasianthum*.

Compounds **2-5** were found to have significant antioxidant properties, based on the experiments with 2,2-diphenyl-1-picrylhydrazyl (DPPH), which indicated their ability to efficiently scavenge free radicals^{6,7}.

Acknowledgments

The authors are grateful to Prof. Dr. Hayri Duman (Gazi University, Faculty of Science, Etiler, Ankara, Turkey) for the authentication of the plant specimen and to Dr. Chuck Dunbar for conducting the LC-ESIMS analysis. We also thank Mr. Frank Wiggers for his assistance in obtaining the 2D NMR spectra of compounds **1** and **4-5**. This work was supported by the Research Fund of Hacettepe University (Project no: 00 01 301 003). This work was also supported in part by the United States Department of Agriculture, ARS Specific Cooperative Research Agreement no. 58-6408-7-012.

References

1. A. Huber-Morath, "*Verbascum*" in: Flora of Turkey and the East Aegean Islands Vol. 6, ed. P.H. Davis, pp. 461-603, University Press, Edinburgh (1978).
2. T. Baytop, "Therapy with Medicinal Plants in Turkey (Past and Present)", 2nd ed. pp. 334-335, Nobel Tıp Kitabevleri Ltd., İstanbul (1999).
3. E. Sezik, E. Yeşilada, G. Honda, Y. Takaishi, Y. Takeda and T. Tanaka, **J. of Ethnopharm.**, **75**, 95-115 (2001).
4. C. Jiménez and R. Riguera, **Nat. Prod. Reports**, 591-606 (1994).
5. Z.Ş. Akdemir, İ.İ. Tatlı, E. Bedir and I. A. Khan, **Turk. J. Chem.**, **28**, 101-109 (2004).
6. M. Cuendet, K. Hostettman, O. Potterat and W. Dyatmiko, **Helv. Chim. Acta**, **80**, 1144- 1152 (1997).
7. T. Takao, F. Kitatani, N. Watanabe, A. Yagi and K. Sakata, **Biosci. Biotech. Biochem.**, **58**, 1780-1783 (1994).
8. F. Pardo, F. Perich, R. Torres and F. Delle Monache, **J. Chem. Ecol.**, **24**, 645-653 (1998).
9. O. Sticher and M.F. Lahloub, **Planta Med.**, **46**, 145-148 (1982).
10. L. Swiatek, S. Luczak and B. Grabias, **Farm. Pol.**, **40**, 415-418 (1984).
11. M. Özipek, İ. Saracoğlu, İ. Çalış, K. Kojima and Y. Ogihara, **Hacettepe University, Journal of Faculty of Pharmacy**, **20**, 1-6 (2000).
12. H. Lichti and A. Von Wartburg, **Helv. Chim. Acta**, **49**, 1552 (1966).
13. E. Bedir and İ. Çalış, **Hacettepe University, Journal of Faculty of Pharmacy**, **17**, 9-16 (1997).
14. T. Warashina, T. Miyase and A. Ueno, **Chem. Pharm. Bull.**, **39**, 3261-3264 (1991).
15. H. Abou Gazar, "**Phytochemical Studies on *Verbascum wiedenmannianum* Fisch. & Mey.**", PhD Thesis, Hacettepe University, Ankara, Turkey, 2001.