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Iridoid and Phenylethanoid Glycosides from *Verbascum lasianthum*

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Three iridoid glucosides, 8-*O*-acethylharpagide (**1**), harpagoside (**2**), and 6-*O*-vanilloylajugol (**3**), were isolated from the roots of *Verbascum lasianthum* Boiss. ex Bentham. In addition, 2 phenylethanoid glycosides, verbascoside {=acteoside, [β-(3,4-dihydroxyphenyl)-ethyl]-(3′-*O*-α-L-rhamnopyranosyl)-(4′-*O*-caffeoyl)-β-D-glucopyranoside} (**4**) and poliumoside {= [β-(3,4-dihydroxyphenyl)-ethyl]-(3′,6′-*O*-α-L-dirhamnopyranosyl)-(4′-*O*-caffeoyl)-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*-acethylharpagide, 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 area[1]. Some *Verbascum* species are used as an expectorant and mucolytic in folk medicine[2]. These species are also used externally for desiccating wounds, anal fistula and pruritic conditions in the urogenital organs[3]. 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 studies[4]. As part of our ongoing project, the chemical characterization of *Verbascum* species growing in Turkey[1], we have isolated 6 iridoid glycosides, 6-*O*-(α-L-rhamnopyranosyl)-catalpol, verbascoside A, pulverulentoside I, buddlejoside A_5, aucubin and unduloside III from *Verbascum lasianthum*[5]. Further investigation into the roots of this plant yielded 3 iridoid glucosides, 8-*O*-acethylharpagide (**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 (υ_{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 ^{1}H 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 F_{254} aluminum sheets (Merck) were used for TLC; developing 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 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*-acethylharpagide (**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. B_1-B_5). Fr. B_1 (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. B_4 (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 $CHCl_3$-MeOH mixtures (95:5, 90:10, 85:15, 80:20) and $CHCl_3$-MeOH-H_2O mixtures (80:20:2, 70:30.3) to yield 2 fractions (Frs. C_1-C_2). Fr. C_2 (26.3 mg) was further purified on a Si gel column (40 g) using $CHCl_3$-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/H_2O 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 $CHCl_3$-MeOH-H_2O (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) υ_{max} 3600 (OH), 1708 (C=O), 1670 (C=C) cm^{-1}, LC-ESIMS m/z 429 $[M+Na]^+$ (calc. for $C_{17}H_{26}O_{11}$), ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6) data (Table 1).

Table 1. ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) Data of Compounds **1-3**.

		1*			**2**			**3****		
Position	$\mathbf{C}_{\text{Atom}}$	δ_C (ppm)	δ_H (ppm)	J (Hz)	δ_C (ppm)	δ_H (ppm)	J (Hz)	δ_C (ppm)	δ_H (ppm)	J (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 †	-	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) ℵ	-	-	-	128.3	7.35 s	-	152.0	-	-
4″	C (CH) ℵ	-	-	-	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	-	-	-	-	-	-	

† unclear due to overlapping
ξ C for compounds **1** and **3**
ℵ CH for compound **3**
* 300 and 75 MHz
** 400 and 125 MHz

Harpagoside (**2**): UV (MeOH) λ_{max}228 nm, IR (KBr) υ_{max} 3600 (OH), 1705 (C=O), 1637 (C=C), 1604, 1363 (aromatic ring) cm^{-1}, LC-ESIMS m/z 517 $[M+Na]^+$ (calc. for $C_{24}H_{30}O_{11}$). ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data (Table 1) superimposable with those reported in the literature[8].

Table 2. ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data of compounds **4** and **5**.

		4			**5**		
Position	C_{Atom}	δ_C (ppm)	δ_H (ppm)	J (Hz)	δ_C (ppm)	δ_H (ppm)	J (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**

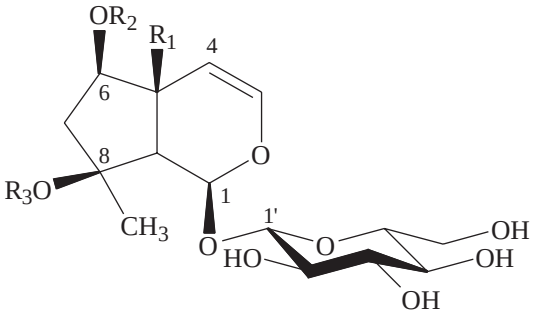
6-*O*-vanilloylajugol (**3**): UV (MeOH) λ_{max}208, 264 nm, IR (KBr) υ_{max} 3600 (OH), 1708 (C=O), 1654 (C=C), 1604, 1546, 1363 (aromatic ring) cm^{-1}, LC-ESIMS m/z 521 $[M+Na]^+$ (calc. for $C_{23}H_{30}O_{12}$). ^1H NMR (400 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data (Table 1).

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) υ_{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$C_{29}H_{36}O_{15}$).^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data (Table 2) superimposable with those reported in the literature[9].

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) υ_{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$C_{35}H_{46}O_{19}$). ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C 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.



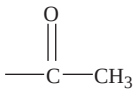
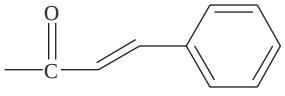
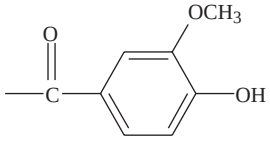
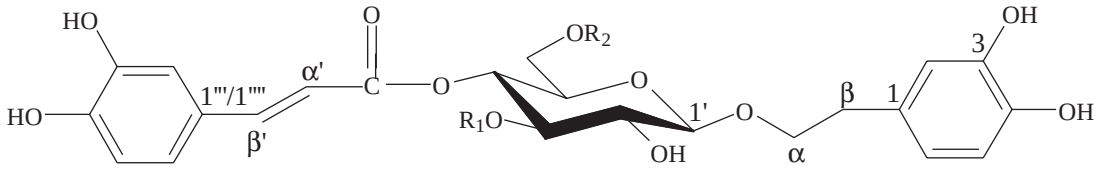
	R_1	R_2	R_3
1	OH	H	
2	OH	H	
3	H		H

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



	$\mathbf{R}_1$	$\mathbf{R}_2$
4	α- L-rhamnopyranose	H
5	α- L-rhamnopyranose	α- L-rhamnopyranose

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_H3.71 (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 H_3-10 (δ_H1.45, 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_H2.02 (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 H_3-10 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*-acethylharpagide[10].

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 H_3-10 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 harpagoside[8].

Compound **3** proved to have the molecular formula $C_{23}H_{30}O_{12}$, as seen from the positive-ion ESIMS (m/z 521 $[M+Na]^+$) combined with ^1H and ^{13}C 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_H6.13 (*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_H2.51, *dd* (*t*), J= 9.4 Hz). On the other hand, the multiplet signal at δ_H4.98 was attributed to an oxymetine proton at C-6 (δ_c79.4), which was coupled to H_2-7 (δ_H1.97, *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_H6.66, *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_H3.73, *s*) implied the presence of a vanilloyl group[11]. 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*-vanilloylajugol[12].

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, ^{13}C (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 $C_{35}H_{46}O_{19}$ as determined by LC-ESIMS and ^1H and ^{13}C NMR data (Table 2). The LC-ESIMS of **5** exhibited a pseudomolecular ion at m/z 793 $[M+Na]^+$. The ^1H and ^{13}C 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 ^{13}C 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 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*-acethylharpagide (**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* species[15].

Although 8-*O*-acethylharpagide 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*-acethylharpagide 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].

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