

Two New Iridoid Glucosides from *Verbascum salviifolium* Boiss.

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From the aerial parts of the plant *Verbascum salviifolium*, two new iridoid glucosides, 6-*O*- β -D-glucopyranosylcatalpol (**1**) and 6-*O*-(6'-*O*-*trans*-*p*-hydroxycinnamoyl)- β -D-glucopyranosylaucubin (**2**) along with five known iridoid glycosides, 6-*O*- β -D-glucopyranosylaucubin (**3**), 6-*O*- α -L-rhamnopyranosylcatalpol (**4**), verbaspinoside [= 6-*O*-(2''-*O*-*trans*-cinnamoyl)- α -L-rhamnopyranosylcatalpol] (**5**), pulverulentoside I [= 6-*O*-(2''-*O*-*trans*-*p*-methoxycinnamoyl)-3''-*O*-acetyl)- α -L-rhamnopyranosylcatalpol] (**6**), and buddlejoside A₈ [= 6-*O*-(4''-*O*-*trans*-3,4-dimethoxycinnamoyl)- α -L-rhamnopyranosylcatalpol] (**7**) were isolated. The structures of the new compounds were established on the basis of spectroscopic evidence.

Key words: *Verbascum*, Scrophulariaceae, Iridoid Glycosides, 6-*O*- β -D-Glucopyranosylcatalpol, 6-*O*-(6''-*O*-*trans*-*p*-Hydroxycinnamoyl)- β -D-glucopyranosylaucubin

Introduction

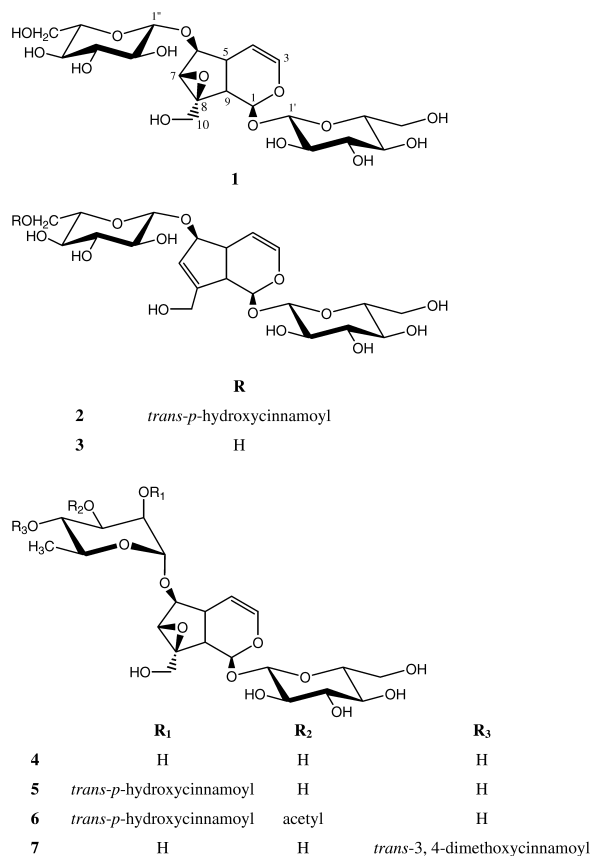
Verbascum, commonly known as “Mullein”, is a widespread genus of the family Scrophulariaceae. This taxon is represented by 228 species, 185 of which are endemic, in the flora of Turkey [1]. Various preparations of some plants of this genus have been used as expectorant, mucolytic, sudorific, sedative, diuretic and constipate in traditional Turkish medicine [2]. *Verbascum* species are also used externally for desiccating wounds, anal fistula and pruritic conditions in urogenital organs [3]. Iridoid glycosides are widely distributed in the genus *Verbascum* which is well known for its variety of iridoids being of value for taxonomic evaluation of this genus [4].

Our previous studies have resulted in the isolation of iridoid, phenylethanoid and monoterpene glycosides as well as saponins from *V. lasianthum* [5], *V. cilicicum* [6] and *V. pterocalycinum* var. *mutense* [7]. In the course of an investigation on *Verbascum* species, growing in Turkey, we here report the isolation and structure elucidation of the iridoid compounds **1–7** from *V. salviifolium* Boiss., an endemic species distributed in South Anatolia [1].

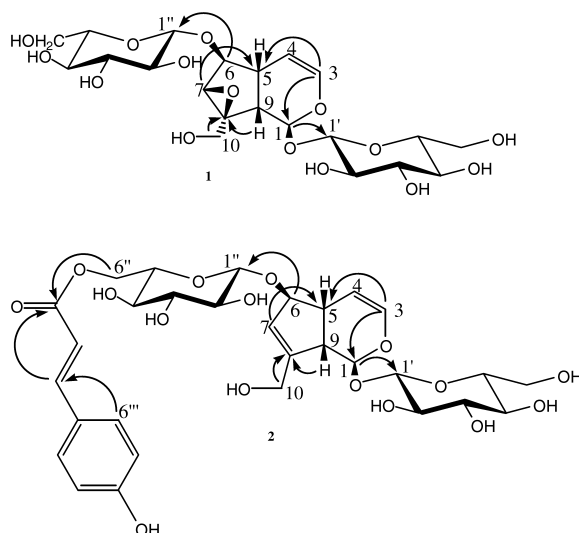
Results and Discussion

The methanol extract of *V. salviifolium* was suspended in water and partitioned with CHCl₃. Removing the chloroform layer, the aqueous layer was fractionated over polyamide VLC followed by C₁₈-MPLC, C₁₈-VLC and Si gel CC to yield compounds **1–7** (see Fig. 1).

Compound **1** was isolated as an amorphous powder, [α]_D²⁰ + 8.1 (*c* 2.3, CHCl₃). The molecular formula was determined as C₂₁H₃₂O₁₅ by using a combination of positive-ion HR-ESIMS (*m/z* 547.1630 [M+Na]⁺), LC-ESIMS (*m/z* 547 [M+Na]⁺) and NMR data (see Table 1). The UV spectra of **1** exhibited maxima at 206 nm, suggesting the presence of an iridoid enol ether system. Similarly, the IR absorptions [3470 (OH), 1650 (C=C-O) cm⁻¹] were in accordance with the presence of a non-conjugated enol ether system. In addition, ¹H, ¹³C NMR and DEPT 135 data of **1** (see Table 1) indicated the presence of a C-4 non-substituted iridoid skeleton. In the ¹H NMR spectrum (see Table 1) of compound **1**, the proton signals of H-3 (δ _H 6.35, d, *J* = 5.0 Hz) and H-4 (δ _H 5.16, d, *J* = 5.5 Hz) were observed as a doublet implying no

Fig. 1. Iridoid glycosides (**1**–**7**) from *V. salviifolium*.

substitution at C-3 and C-4 positions. The chemical shift value and splitting pattern of H-5 (δ_{H} 2.30, m) and H-9 (δ_{H} 2.36, m) indicated the presence of fully substituted C-8 atom. This conclusion was confirmed by the signal at δ_{C} 65.3 (s, C-8) in the ^{13}C NMR spectrum. Proton signals at δ_{H} 4.00 and 3.59 assigned to H-6 and H-7, suggested C-6 and C-7 to be oxygenated. Additional signals at δ_{H} 3.68 (d, $J = 13.0$ Hz) and 3.90 (dd, $J = 13.0/3.0$ Hz) belong to an AB system which were assigned to the protons of a $-\text{CH}_2\text{-O-}$ group located at C-8. In the ^{13}C NMR spectrum, the signal at δ_{C} 84.6 pointed that an OH group was located at C-6, while the chemical shifts of C-7, C-8 and C-9 (δ_{C} 59.1, 65.3 and 42.9, resp.) indicated that an epoxy function was present between C-7 and C-8. On the other hand, in the ^1H NMR spectrum of **1**, two anomeric proton signals were observed at δ_{H} 4.59 (d, $J = 7.5$ Hz) and δ_{H} 4.30 (d, $J = 7.5$ Hz) attributed to two β -glucopyranosyl moieties. In the ^{13}C NMR spectrum, 21 carbon resonances, 12 of which could be assigned to the two glu-

Fig. 2. Selected HMBC correlations for **1** and **2**.

cose units, were observed. The complete assignment of proton and carbon resonances were based on the ^1H - ^1H COSY, ^1H - ^{13}C HMQC and HMBC (see Table 1). Moreover, ^1H , ^{13}C NMR and DEPT 135 data of compound **1** showed signals very similar to those of catalpol [8] with additional signals arising from the second glucose moiety. The attachment of the glucose moiety was determined to be C-6 (O) of the aglycone due to the downfield shift of C-6 atom (δ_{C} 84.6, $\Delta\delta +9.3$) by comparison to that of reported for the catalpol (δ_{C} 75.3) [8]. This assumption was also supported by the HMBC correlation observed between C-6 (δ_{C} 84.6) and H-1'' (δ_{H} 4.30, d, $J = 7.5$ Hz) (see Fig. 2). Consequently, compound **1** was established as 6-*O*- β -D-glucopyranosylcatalpol which was isolated for the first time from nature. We propose the trivial name salviifolioside I for this compound.

Compound **2** was isolated as an amorphous powder, $[\alpha]_{\text{D}}^{20} -101.9$ (c 0.1, MeOH), with the molecular formula $\text{C}_{30}\text{H}_{38}\text{O}_{16}$ as determined by HR-ESIMS, LC-ESIMS together with ^1H and ^{13}C NMR data. The HR-ESIMS of **2** showed the $[\text{M}+\text{Na}]^+$ peak at m/z 677.1939 and LC-ESIMS of **2** exhibited a pseudomolecular ion at m/z 677 $[\text{M}+\text{Na}]^+$. Its UV spectrum suggested the presence of an iridoid enol ether system (205 nm) and an aromatic acyl moiety (316 nm). Moreover, its IR spectra absorption bands were typical for a hydroxyl group (3430 cm^{-1}), a conjugated ester carbonyl (1705 cm^{-1}), a double bond (1643 cm^{-1}) and an aromatic ring ($1604, 1546, 1363\text{ cm}^{-1}$). Analysis of the ^1H NMR spectrum (see Table 2) revealed

Position	Connectivity	¹³ C δ [ppm]	¹ H δ [ppm]	(HMQC) J [Hz]	HMBC	Table 1. ¹³ C NMR (125 MHz, DMSO- <i>d</i> ₆), ¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) Data and HMBC of 1 .
1	CH	93.7	4.93 d	9.5	C-8, C-9, C-1'	† Unclear due to overlapping.
3	CH	140.6	6.35 d	5.0	C-1, C-4, C-5	
4	CH	103.5	5.16 d	5.5	C-1, C-5, C-9	
5	CH	36.4	2.30 m	–	–	
6	CH	84.6	4.00 d	8.0	C-5, C-7, C-1''	
7	CH	59.1	3.59 br s	–	C-5, C-6, C-9	
8	C	65.3	–	–	C-1, C-9, C-10	
9	CH	42.9	2.36 m	–	–	
10a	CH ₂	59.5	3.90 dd	13.0/3.0	C-8	
10b			3.68 d	13.0	C-8	
β-D-Glucose						
1'	CH	98.3	4.59 d	7.5	C-1, C-3'	
2'	CH	73.9	2.99–3.05 †	–	–	
3'	CH	77.0	3.08–3.25 †	–	–	
4'	CH	70.6	3.04–3.18 †	–	–	
5'	CH	77.5	3.08–3.25 †	–	–	
6'a	CH ₂	61.7	3.66 d–3.69 †	6.0	C-1'	
6'b			3.44 †	–	C-1'	
β-D-Glucose						
1''	CH	102.7	4.30 d	7.5	C-6	
2''	CH	74.0	2.99–3.05 †	–	–	
3''	CH	77.3	3.08–3.25 †	–	–	
4''	CH	70.8	3.04–3.18 †	–	–	
5''	CH	78.0	3.08–3.25 †	–	–	
6''a	CH ₂	61.9	3.66 d	6.0	C-1''	
6''b			3.44 †	–	C-1''	

2 to be an iridoid glycoside with an acyl moiety. The olefinic proton signals at δ_{H} 5.76 (br s, H-7), 6.18 (dd, $J = 6.0/1.5$ Hz, H-3), 5.18 (d, $J = 5.5$ Hz, H-4) and oxymethine signal at δ_{H} 4.38 (m, H-6) indicated that the aglycone was aucubin-type [5], suggesting a different aglycone from compound **1**. The two anomeric proton signals were observed at δ_{H} 4.48 (d, $J = 8.0$ Hz) and δ_{H} 4.29 (d, $J = 7.5$ Hz) together with the signals in the region 2.99–4.43 suggested the presence of two β -glucopyranosyl moieties. The ¹H and ¹³C NMR data indicated that **2** had most of the structural features of compound **1** with additional signals arising from an aromatic acid. The signals of two *trans* olefinic protons (δ_{H} 6.39 and 7.58, d, $J = 16.0$ Hz), as well as two pairs of *ortho*-coupled aromatic protons (δ_{H} 6.81 and 7.54, d, $J = 8.5$ Hz) in the ¹H NMR spectrum, showed clearly that the acyl moiety was *trans*-*p*-hydroxycinnamoyl [6]. ¹³C NMR and DEPT-135 spectra of **2** confirmed the presence of the *p*-hydroxycinnamic acid. The site of esterification was determined to be C-6'' position of the glucopyranosyl moiety based on the chemical shift of C-6'' ($\Delta\delta + 2.6$ ppm, δ_{C} 64.5 in **2**; δ_{C} 61.9 in **1**). This assumption was supported by downfield shift of H-6'' (ca. 0.7 ppm; δ_{H} 4.16 and 4.43 in **2**; δ_{H} 3.44 and 3.66 in **1**). Moreover, the site of the esterification was also confirmed

on the basis of long-range correlations observed in the HMBC spectrum, between the signal at δ_{C} 166.8 (carbonyl carbon of *trans*-*p*-hydroxycinnamoyl group) and the signals at δ_{H} 4.43 and 4.16 (H-6''a and H-6''b) (see Fig. 2). Consequently, compound **2** was established as 6-*O*-(6''-*O*-*trans*-*p*-hydroxycinnamoyl)- β -D-glucopyranosylaucubin which was also isolated for the first time from nature. We propose the trivial name salviifolioside II for this compound.

The known compounds **3**–**7** were identified as 6-*O*- β -D-glucopyranosylaucubin (**3**) [9], 6-*O*- α -L-rhamnopyranosylcatalpol (**4**) [5], verbaspinoside [= 6-*O*-(2''-*O*-*trans*-cinnamoyl)- α -L-rhamnopyranosylcatalpol] (**5**) [6], pulverulentoside I [= 6-*O*-(2''-*O*-*trans*-*p*-methoxycinnamoyl-3''-*O*-acetyl)- α -L-rhamnopyranosylcatalpol] (**6**) [5], buddlejoside A₈ [= 6-*O*-(4''-*O*-*trans*-3,4-dimethoxycinnamoyl)- α -L-rhamnopyranosylcatalpol] (**7**) [10] by comparing their ¹H and ¹³C NMR and ESIMS data with previously published data.

In addition to the new compounds **1** and **2**, this is the first demonstration of the occurrence of buddlejoside A₈ [= 6-*O*-(4''-*O*-*trans*-3,4-dimethoxycinnamoyl)- α -L-rhamnopyranosylcatalpol] (**7**) the second report for the isolation of 6-*O*- β -D-glucopyranosylaucubin (**3**) and verbaspinoside (**5**) in the genus *Verbascum*.

Position	Connectivity	¹³ C δ [ppm]	¹ H δ [ppm]	(HMQC) J [Hz]	HMBC
1	CH	96.5	4.71 d	6.5	C-3, C-5, C-1'
3	CH	140.4	6.18 dd	6.0/1.5	C-1, C-4, C-5
4	CH	105.4	5.18 d	5.5	C-3, C-9
5	CH	44.0	2.67 m	—	—
6	CH	90.3	4.38 m	—	—
7	CH	126.4	5.76 br s	—	C-5, C-9, C-10
8	C	148.5	—	—	—
9	CH	47.6	2.67 m	—	—
10a	CH ₂	60.2	4.17 d	15.0	C-7, C-8
10b			3.97 d	16.5	C-7, C-8
β-D-Glucose					
1'	CH	98.6	4.48 d	8.0	C-1
2'	CH	74.0	2.99 †	—	C-3', C-4'
3'	CH	77.1	3.20 †	—	C-4'
4'	CH	70.6	3.10 †	—	C-5'
5'	CH	77.6	3.06 †	—	C-4', C-6'
6'a	CH ₂	61.6	3.63 d	11.0	C-5'
6'b			3.44 †	—	C-5'
β-D-Glucose					
1''	CH	103.5	4.29 d	7.5	C-6, C-5'
2''	CH	74.3	3.48 †	—	C-3', C-4'
3''	CH	77.2	3.22 †	—	C-4'
4''	CH	70.9	3.12 †	—	C-5'
5''	CH	77.6	3.08 †	—	C-2', C-4'
6''a	CH ₂	64.5	4.43 d	10.5	C-5', C=O
6''b			4.16 †	—	C-5', C=O
Acyl moiety					
1'''	C	125.2	—	—	—
2'''	CH	130.6	7.54 d	8.5	C-4''', C-6''', C-α, C-β, C=O
3'''	CH	116.3	6.81 d	8.5	C-1''', C-5'''
4'''	C	160.0	—	—	—
5'''	CH	116.3	7.54 d	8.5	C-1''', C-3'''
6'''	CH	130.6	6.81 d	8.5	C-2''', C-4''', C-α, C-β, C=O
α	CH	115.4	6.39 d	15.5	C-1''', C=O
β	CH	145.2	7.58 d	16.0	C-1''', C-2''', C-6''', C-α, C=O
C=O	C	166.8	—	—	—

Table 2. ¹³C NMR (125 MHz, DMSO-*d*₆), ¹H NMR (500 MHz, DMSO-*d*₆) Data and HMBC of **2**.

† Unclear due to overlapping.

Our investigation of *V. salviifolium* demonstrated that rhamnopyranosyl catalpol esters are the main iridoid constituents present in this species. Although, similar compounds have been isolated from several *Verbascum* species [5,6], the isolation of the acylated 6-glucosyl aucubin from *Verbascum* spec. in this study was recorded for the first time. Thus, the significance of acyl rhamnopyranosyl catalpol derivatives as taxonomic markers is limited since they obviously evolved several times independently in different families [11]. However at the level of genera the substitution pattern of these iridoids like the 7,8-oxido group and the 10-hydroxyl group, acylation of the iridoids with unsubstituted or substituted cinnamic acids as well as the esterification sites are different than those reported in the literature, which might be useful from the chemotaxonomy point of view in the genus *Verbascum*.

Experimental Section

General experimental procedures

Optical rotations were measured on a JASCO DIP-370 polarimeter using a sodium lamp operating at 589 nm. The UV spectra (λ_{\max}) were recorded on a Hitachi HP 8452 A spectrophotometer. The IR spectra (ν_{\max}) was determined on ATI Mattson Genesis Series FT-IR spectrophotometer. The ¹H and ¹³C NMR spectra were obtained on a Bruker Avance DRX 500 FT spectrometer operating at 500 MHz for ¹H NMR, 125 MHz for ¹³C 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 ¹³C NMR spectra, multiplicities were determined by DEPT experiment. HR-ESIMS and LC-ESIMS FT data were obtained using a Bruker BioApex FT-MS instrument in the ESI mode. Polyamide (ICN) and reverse-phase material (C-18, sepralyte 40 μ m) were 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. Si gel (230–400 mesh) (Merck) was used for column chromatography (CC). Pre-coated silica gel 60 F₂₅₄ aluminum sheets (Merck) were used for TLC with developing solvent-system, CHCl₃-MeOH-H₂O (61:32:7). Plates were examined by UV fluorescence and sprayed with 1% vanillin in conc. H₂SO₄, followed by heating at 105 °C for 1–2 min.

Plant material

Verbascum salviifolium Boiss. (Scrophulariaceae) was collected from Burdur, Yesilova, Southwest of Burdur Lake, 880 m, in June 2002. A voucher specimen has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 02003).

Extraction and isolation

The air-dried and powdered aerial parts of *Verbascum salviifolium* (339.08 g) were extracted twice with MeOH (2 × 2000 ml) at 40 °C. After evaporation of the combined extract *in vacuo*, 40.84 g MeOH extract was obtained. The crude extract was dissolved in water and partitioned in CHCl₃. The lyophilized H₂O phase (29.49 g) was fractionated over polyamide column (VLC, 250 g), eluting with H₂O (400 ml) and gradient MeOH-H₂O mixtures (25–100%) to afford eight main fractions (A–H). Fraction B (264.76 mg) was subjected to C₁₈ medium pressure liquid chromatography (C₁₈-MPLC) using H₂O (250 ml) and MeOH-H₂O gradients (10–40% MeOH) to yield **1** (4.7 mg). Fraction C (855.06 mg) was fractionated over LiChroprep C₁₈ (VLC). Employment of H₂O (0–75% MeOH) and MeOH afforded **4** (8.2 mg) and additional four fractions C_{2–5}. Purifica-

tion of fr. C₃ (244.8 mg) by C₁₈-MPLC (20–70% MeOH) furnished two fractions (C_{3a–b}). Fraction C_{3b} (106.1 mg) rechromatographed on silica gel column (CHCl₃-MeOH, 90:10 to 80:20 v/v) to give **5** (4.8 mg) and **2** (5.4 mg). Fraction C₅ (51.0 mg) was applied to a silica gel column. Elution with CHCl₃-MeOH mixtures (90:10 to 87.5:12.5 v/v) yielded **7** (6.1 mg). Fraction D (1.74603 g) was likewise subjected to C₁₈-MPLC using stepwise gradients of MeOH (0–70%) in H₂O to yield **3** (4.4 mg) and additional four fractions (Frs. D_{2–5}). Repeated chromatography of fr. D₄ (107.9 mg) on silica gel column using CHCl₃-MeOH mixtures (90:10 to 85:15 v/v) afforded **6** (36.7 mg).

6-O-β-D-glucopyranosylcatalpol (1): Amorphous powder; $[\alpha]_D^{20} + 8.1$ (c 2.3, CHCl₃); UV λ_{max} (MeOH): 206 nm; IR ν_{max} (KBr) 3470 (OH), 1650 (C=C-O) cm⁻¹; HR-ESIMS m/z : 547.1630 [M+Na]⁺ (calcd. for C₂₁H₃₂O₁₅Na: 547.1639), LC-ESIMS m/z 547 [M+Na]⁺ (C₂₁H₃₂O₁₅Na); ¹H NMR (500 MHz, DMSO-*d*₆), ¹³C NMR (125 MHz, DMSO-*d*₆) and HMBC: Table 1.

6-O-(6''-O-trans-p-hydroxycinnamoyl)-β-D-glucopyranosylaucubin (2): Amorphous powder; $[\alpha]_D^{20} - 101.9$ (c 0.1, MeOH); UV λ_{max} (MeOH): 205, 316 nm; IR ν_{max} (KBr) 3430 (OH), 1705 (ester C=O), 1643 (C=C-O), 1604, 1546, 1363 (aromatic ring) cm⁻¹; HR-ESIMS m/z 677.1939 [M+Na]⁺ (calcd. for C₃₀H₃₈O₁₆Na: 677.2058), LC-ESIMS m/z 677 [M+Na]⁺ (C₃₀H₃₈O₁₆Na); ¹H NMR (500 MHz, DMSO-*d*₆), ¹³C NMR (125 MHz, DMSO-*d*₆) and HMBC: Table 2.

Acknowledgments

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