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 A_8 [= 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

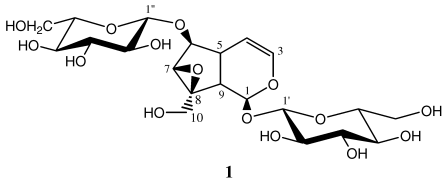
Verbascum, commonly known as "Mullein", is a widespread genus of the family Scrophulariaceae. This taxon is represented by 228 species, 185 of which are endemic, in the flora of Turkey [1]. Various preparations of some plants of this genus have been used as expectorant, mucolytic, sudorific, sedative, 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 $CHCl_3$. Removing the chloroform layer, the aqueous layer was fractionated over polyamide VLC followed by C_{18}-MPLC, C_{18}-VLC and Si gel CC to yield compounds **1** – **7** (*see* Fig. 1).

Compound **1** was isolated as an amorphous powder, $[\alpha]_D^{20} + 8.1$ (c 2.3, $CHCl_3$). The molecular formula was determined as $C_{21}H_{32}O_{15}$ 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, ^{13}C 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



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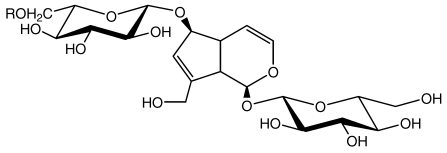
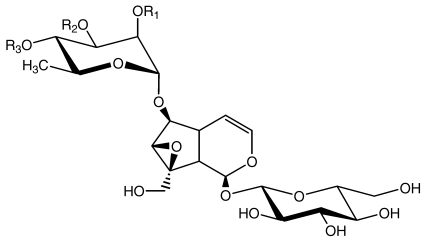


R

2	*trans-p*-hydroxycinnamoyl
3	H



	R_1	R_2	R_3
4	H	H	H
5	*trans-p*-hydroxycinnamoyl	H	H
6	*trans-p*-hydroxycinnamoyl	acetyl	H
7	H	H	*trans*-3, 4-dimethoxycinnamoyl

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 -CH_2-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 ^{1}H 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 glucose units, were observed. The complete assignment of proton and carbon resonances were based on the ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC and HMBC (*see* Table 1). Moreover, ^{1}H, ^{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.

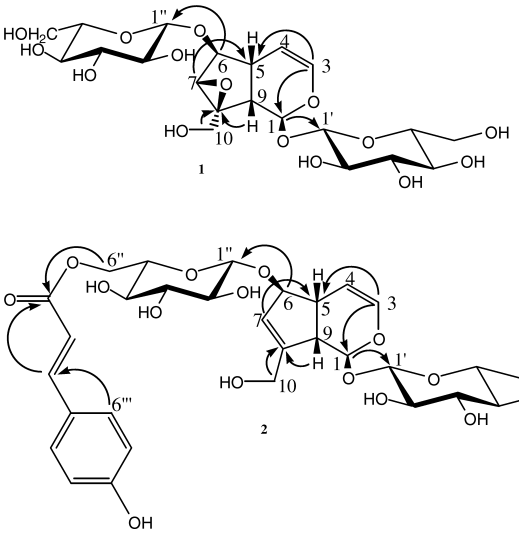


Fig. 2. Selected HMBC correlations for **1** and **2**.

Compound **2** was isolated as an amorphous powder, $[\alpha]_D^{20} - 101.9$ (c 0.1, MeOH), with the molecular formula $C_{30}H_{38}O_{16}$ as determined by HR-ESIMS, LC-ESIMS together with ^{1}H and ^{13}C NMR data. The HR-ESIMS of **2** showed the $[M+Na]^+$ peak at *m/z* 677.1939 and LC-ESIMS of **2** exhibited a pseudomolecular ion at *m/z* 677 $[M+Na]^+$. 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 cm^{-1}). Analysis of the ^{1}H NMR spectrum (*see* Table 2) revealed

Position	Connectivity	^{13}C δ [ppm]	^{1}H δ [ppm]	(HMQC) J [Hz]	HMBC
1	CH	93.7	4.93 d	9.5	C-8, C-9, C-1′
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″

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

† Unclear due to overlapping.

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 ^{1}H and ^{13}C 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 ^{1}H NMR spectrum, showed clearly that the acyl moiety was *trans*-*p*-hydroxycinnamoyl [6]. ^{13}C 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 A_8 [= 6-*O*-(4″-*O*-*trans*-3,4-dimethoxycinnamoyl)-α-L-rhamnopyranosylcatalpol] (**7**) [10] by comparing their ^{1}H and ^{13}C 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 A_8 [= 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*.

Table 2. ^{13}C NMR (125 MHz, DMSO-d_6), ^{1}H NMR (500 MHz, DMSO-d_6) Data and HMBC of **2**.

† Unclear due to overlapping.

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	–	–	–

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 ^{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. 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 μm) 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 F_{254} aluminum sheets (Merck) were used for TLC with 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 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 $CHCl_3$. The lyophilized H_2O phase (29.49 g) was fractionated over polyamide column (VLC, 250 g), eluting with H_2O (400 ml) and gradient MeOH-H_2O mixtures (25 – 100%) to afford eight main fractions (A-H). Fraction B (264.76 mg) was subjected to C_{18} medium pressure liquid chromatography (C_{18}-MPLC) using H_2O (250 ml) and MeOH-H_2O gradients (10 – 40% MeOH) to yield **1** (4.7 mg). Fraction C (855.06 mg) was fractionated over LiChroprep C_{18} (VLC). Employment of H_2O (0 – 75% MeOH) and MeOH afforded **4** (8.2 mg) and additional four fractions C_{2-5}. Purification of fr. C_3 (244.8 mg) by C_{18}-MPLC (20 – 70% MeOH) furnished two fractions (C_{3a-b}). Fraction C_{3b} (106.1 mg) rechromatographed on silica gel column ($CHCl_3$-MeOH, 90:10 to 80:20 v/v) to give **5** (4.8 mg) and **2** (5.4 mg). Fraction C_5 (51.0 mg) was applied to a silica gel column. Elution with $CHCl_3$-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 C_{18}-MPLC using stepwise gradients of MeOH (0 – 70%) in H_2O to yield **3** (4.4 mg) and additional four fractions (Frs. D_{2-5}). Repeated chromatography of fr. D_4 (107.9 mg) on silica gel column using $CHCl_3$-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, $CHCl_3$); 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 $C_{21}H_{32}O_{15}Na$: 547.1639), LC-ESIMS *m/z* 547 $[M+Na]^+$ ($C_{21}H_{32}O_{15}Na$); ^1H NMR (500 MHz, DMSO-d_6), ^{13}C 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 $C_{30}H_{38}O_{16}Na$: 677.2058), LC-ESIMS *m/z* 677 $[M+Na]^+$ ($C_{30}H_{38}O_{16}Na$); ^1H NMR (500 MHz, DMSO-d_6), ^{13}C NMR (125 MHz, DMSO-d_6) and HMBC: Table 2.

Acknowledgments

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