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İ. İREM TATLI

ZELİHA Ş. AKDEMİR

ERDAL BEDİR

IKHLAS A. KHAN

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İ. İrem TATLI, Zeliha Ş. AKDEMİR
Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, TR-06100, Ankara-TURKEY
e-mail: itatli@hacettepe.edu.tr
Erdal BEDİR, Ikhlas A. KHAN
The University of Mississippi, School of Pharmacy, National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, University MS 38677 USA

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Ilwensisaponin C (= 3-*O*-{[α-L-rhamnosyl-(1→4)-(β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl]-(1→2)-β-D-fucopyranosyl}-11-methoxy-olean-12-ene-3β, 23, 28-triol) (**1**), ilwensisaponin A (= mimengoside A = 3-*O*-{[α-L-rhamnosyl-(1→4)-(β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl]-(1→2)-β-D-fucop- yranosyl}-13β, 28-epoxyolean-11-ene-3β, 23-diol) (**2**), ajugol (**3**), picroside IV (= 6′-*O*-*trans*-*p*-hydroxycin- namoylcatalpol) (**4**), verbascoside {= acteoside, [β-(3,4-dihydroxyphenyl)-ethyl]-(3′-*O*-α-L-rhamnopyra- nosyl)-(4′-*O*-caffeoyl)-β-D-glucopyranoside} (**5**) and 1-(β-D-glucopyranosyl)-8-hydroxy-3, 7-dimethyl-oct-2(*E*), 6(*E*)- dienoate (**6**) were isolated from the flowers of *Verbascum pterocalycinum* var. *mutense* Hub.-Mor. The structures of the compounds were determined primarily from 1D and 2D NMR experiments. This is the first phytochemical study performed on *V. pterocalycinum* var. *mutense* and the first report of the presence of 1-(β-D-glucopyranosyl)-8-hydroxy-3, 7-dimethyl-oct-2(*E*), 6(*E*)- dienoate (**5**) as a monoterpene glycoside along with picroside IV (= 6′-*O*-*trans*-*p*-hydroxycinnamoylcatalpol) (**4**) from the genus *Verbascum*.

Key Words: *Verbascum pterocalycinum* var. *mutense*, Scrophulariaceae, saponin glycosides, ilwensisaponin C and ilwensisaponin A, iridoid glucosides, ajugol and picroside IV, phenylethanoid glycoside, verbascoside, monoterpene glucoside, 1-(β-D-glucopyranosyl)-8-hydroxy-3, 7-dimethyl-oct-2(*E*), 6(*E*)-dienoate.

Introduction

Verbascum, commonly known as mullein, is a widespread genus of the family Scrophulariaceae, which comprises more than 300 species in the world's flora[1]. This taxon is represented by 185 endemic species in the flora of Turkey[2]. Various preparations of some species of this genus have been used as an expectorant and mucolytic in folk medicine[3]. The treatment of chills, coughs, etc. is known due to the mild expectorant action of the saponins[4].

In the framework of our research program on the constituents of *Verbascum* species, we initiated a phytochemical study on *Verbascum pterocalycinum* var. *mutense*. We present here the isolation and structure elucidation of ilwensisaponin C (**1**), ilwensisaponin A (= mimengoside A) (**2**), ajugol (**3**), picroside IV (**4**), verbascoside (**5**), and 1-(β-D-glucopyranosyl)-8-hydroxy-3, 7-dimethyl-oct-2(E), 6(E)- dienoate (**6**) from the flowers of *V. pterocalycinum* var. *mutense*, which is an endemic species in Anatolia[2].

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 a ATI Mattson Genesis Series FT-IR spectrophotometer. The 1D and 2D NMR spectra were obtained on a Bruker Avance DRX 500 and 300 FT spectrometer operating at 500 and 300 MHz for ^{1}H NMR, and 125 and 75 MHz for ^{13}C NMR. 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. Reversed-phase material (C-18, Sepralyte 40 μm) was used for vacuum liquid chromatography (VLC). Si gel (230-400 mesh) (Merck) and Sephadex LH-20 were used for column chromatography (CC). Pre-coated silica gel 60 F_{254} aluminum sheets (Merck) were used for thin layer chromatography (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 pterocalycinum* var. *mutense* Hub.-Mor. was collected from İçel, between Mut and Karaman, 930-1100 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 00184).

Extraction and Isolation. Air-dried and powdered flowers of the plant (485.6 g) were extracted with MeOH (2 x 2 L) under reflux. The MeOH extract was evaporated to dryness in vacuo to yield 43.5 g of crude extract. The methanol extract was fractionated by CC on silica gel (500 g) using hexane, ethylacetate, chloroform, acetone and methanol (each 4 L), respectively, to yield 5 fractions (Frs. A-E). Fraction E (11.5 g), eluted with methanol, was subjected to VLC using reversed-phase material (Sepralyte 40 μm, 750 g), using MeOH-H_2O mixtures (0-100%) to give ilwensisaponin C (**1**) (260.1 mg), ilwensisaponin A (= mimengoside A) (**2**) (283. 1 mg), and fraction E_3 (80.9 mg). Fr. E_3 was rechromatographed on a silica gel column (65 g) and eluted with $CHCl_3$-MeOH mixtures (90:10, 85:15, 80:20) to yield ajugol (**3**) (3.4 mg). Fr. D (1.6 g), eluted with acetone, was applied to VLC using reversed-phase material (Sepralyte 40 μm, 150 g) and MeOH-H_2O mixtures (0-100%) to give 3 fractions (Frs. D_1-D_3). Fr. D_2 (945.6 mg) was subjected to a silica gel column (57 g) using $CHCl_3$ and $CHCl_3$-MeOH mixtures (95:5, 90:10, 85:15, 80:20, 70:30) to yield picroside IV (**4**) (6.3 mg), verbascoside (**5**) (27.7 mg) and Fr. D_{2a}. Fr. D_{2a} (12 mg) was further purified on a Sephadex LH-20 (15 g) column using MeOH to give picroside IV (**4**) (2.8 mg) and 1-(β-D-glucopyranosyl)-8-hydroxy-3, 7-dimethyl-oct-2(E), 6(E)- dienoate (**6**) (3 mg).

Acetylation of 1 and 2: 20 mg of compounds **1** and **2** were separately dissolved in pyridin (1 mL) and acetic anhydride (1 mL) and the solutions were left at room temperature overnight. The reaction mixtures were diluted with cold water and filtered through an RP-18 cartridge. The cartridges were then washed with cold water (10 mL). The acetylated products in the cartridges were eluted with $CHCl_3$ (20

mL). The $CHCl_3$ extracts were concentrated in vacuo to give tridecaacetate (**1a**) and dodecaacetate (**2a**) derivatives.

Ilwensisaponin C (= 3-*O*-{[α-L-rhamnopyranosyl-(1→4)-(β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl]-(1→2)-β-D-fucopyranosyl}-11-methoxy-olean-12-ene-3β, 23, 28-triol) (**1**): IR (KBr) υ_{max} 3428 (OH), 1644 (C=C) cm^{-1}, positive ion- LC-ESIMS m/z 1072 $[M+Na]^+$ (calc. for $C_{54}H_{88}O2_1$). ^1H NMR (500 MHz, DMSO-d_6) of **1**: δ_H 5.78 (1H, *br s*, H-1‴), 5.54 (1H, *d*, *J*= 7.0 Hz, H-1⁗), 5.46 (1H, *br s*, H-12), 5.21 (1H, *d*, *J*= 7.0 Hz, H-1″), 4.91 (1H, *d*, *J*= 6.6 Hz, H-1′), 4.33 (1H, *overlapped*, H-23b), 4.10 (1H, *overlapped*, H-3), 3.82 (1H, *br d*, *J* = 8.2 Hz, H-11), 3.81 (1H, *d*, *J*= 11.7 Hz, H-28b), 3.69 (1H, *d*, *J*= 8.3 Hz, H-23a), 3.57 (1H, *d*, *J*= 10.2 Hz, H-28a), 3.21 (3H, *s*, OMe), 1.68 (3H, *d*, *J*= 5.5 Hz, H-6‴), 1.35 (3H, *d*, *J*= 4.8 Hz, H-6′), 1.30 (3H, *s*, H-27), 1.08 (3H, *s*, H-24), 1.07 (3H, *s*, H-25), 0.96 (3H, *s*, H-26), 0.95 (3H, *s*, H-30), 0.88 (3H, *s*, H-29). ^1H NMR (500 MHz, DMSO-d_6) of **1a**: aglycone moiety: δ_H 5.51 (1H, *br s*, H-12), 4.66 (1H, *d*, *J*= 11.5 Hz, H-23b), 4.49 (1H, *d*, *J*= 11.5 Hz, H-23a), 4.18 (1H, *d*, *J*= 10.0 Hz, H-28b), 3.98 (1H, *d*, *J*= 10.0 Hz, H-28a), 3.84 (1H, *overlapped*, H-11), 1.32 (3H, *s*, H-26), 1.08 (3H, *s*, H-25), 1.06 (3H, *s*, H-24), 0.98 (3H, *s*, H-29), 0.98 (3H, *s*, H-30), 0.97 (3H, *s*, H-27); sugar moieties; fucose: δ_H 5.74 (1H, *overlapped*, H-4′), 4.75 (1H, *d*, *J*= 6.6 Hz, H-1′), 4.44 (1H, *overlapped*, H-2′), 4.41 (1H, *overlapped*, H-3′), 4.01 (1H, *overlapped*, H-5′), 1.32 (3H, *d*, *J* = 4.8 Hz, H-6′), glucose (inner): 5.62 (1H, *overlapped*, H-3″), 5.39 (1H, *t*, *J* = 7.7 Hz, H-2″), 5.27 (1H, *d*, *J*= 7.0 Hz, H-1″), 5.06 (1H, *overlapped*, H-6″b), 4.58 (1H, *overlapped*, H-6″a), 4.27 (1H, *t*, *J* = 9.4 Hz, H-4″), 3.97 (1H, *overlapped*, H-5″), rhamnose: 5.72 (1H, *overlapped*, H-3‴), 5.66 (1H, *overlapped*, H-2‴), 5.50 (1H, *overlapped*, H-4‴), 5.30 (1H, *br s*, H-1‴), 4.14 (1H, *m*, H-5‴), 1.33 (3H, *d*, *J*= 5.5 Hz, H-6‴), glucose (terminal): 5.74 (1H, *t*, *J* = 9.4 Hz, H-3⁗), 5.70 (1H, *overlapped*, H-4⁗), 5.54 (1H, *overlapped*, H-2⁗), 5.33 (1H, *d*, *J*= 7.0 Hz, H-1⁗), 4.68 (1H, *dd*, *J* = 12.3/4.4 Hz, H-6⁗b), 4.37 (1H, *dd*, *J* = 12.3/2.3 Hz, H-6⁗a), 3.89 (1H, *overlapped*, H-5⁗), $\underline{CH_3}O$: 2.38, 2.27, 2.25, 2.24, 2.20, 2.19, 2.16, 2.10, 2.07, 2.05, 2.04, 2.03, 2.01 (each 3H, *s*).

Ilwensisaponin A (=mimengoside A=3-*O*-{[α-L-rhamnopyranosyl-(1→4)-(β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl]-(1→2)-β-D-fucopyranosyl}-13β, 28 epoxy-olean-11-ene-3β, 23-diol) (**2**): IR (KBr) υ_{max} 3434 (OH), 1645 (C=C) cm^{-1}, positive ion- LC-ESIMS m/z 1127 $[M+Na]^+$ (calc. for $C_{55}H_{92}O_{22}$). ^1H NMR (500 MHz, DMSO-d_6) of **2**: δ_H 5.94 (1H, *br d*, *J*= 10.4 Hz, H-11), 5.77 (1H, *br s*, H-1‴), 5.53 (1H, *overlapped*, H-12), 5.53 (1H, *d*, *J*= 7.9 Hz, H-1⁗), 5.20 (1H, *d*, *J*= 7.6 Hz, H-1″), 4.91 (1H, *d*, *J*= 7.7 Hz, H-1′), 4.34 (1H, *overlapped*, H-23b), 4.11 (1H, *overlapped*, H-3), 3.72 (1H, *overlapped*, H-28b), 3.70 (1H, *overlapped*, H-23a), 3.33 (1H, *d*, *J*= 6.2 Hz, H-28a), 1.68 (3H, *d*, *J*= 6.1 Hz, H-6‴), 1.38 (3H, *br s*, H-6′), 1.31 (3H, *s*, H-26), 1.04 (3H, *s*, H-24), 0.98 (3H, *s*, H-27), 0.96 (3H, *s*, H-25), 0.87 (3H, *s*, H-29), 0.82 (3H, *s*, H-30). ^1H NMR (500 MHz, DMSO-d_6) of **2a**: aglycone moiety: δ_H 5.96 (1H, *br d*, *J*= 10.4 Hz, H-11), 5.58 (1H, *overlapped*, H-12), 4.72 (1H, *d*, *J*= 11.5 Hz, H-23b), 4.50 (1H, *d*, *J*= 11.4 Hz, H-23a), 3.75 (1H, *d*, *J*= 6.6 Hz, H-28b), 3.35 (1H, *d*, *J*= 6.5 Hz, H-28a), 1.32 (3H, *s*, H-26), 1.02 (3H, *s*, H-24), 1.00 (3H, *s*, H-27), 0.97 (3H, *s*, H-25), 0.97 (3H, *s*, H-29), 0.85 (3H, *s*, H-30); sugar moieties; fucose: δ_H 5.73 (1H, *overlapped*, H-4′), 4.75 (1H, *d*, *J*= 6.8 Hz, H-1′), 4.43 (1H, *overlapped*, H-2′), 4.40 (1H, *overlapped*, H-3′), 4.00 (1H, *overlapped*, H-5′), 1.35 (3H, *br s*, H-6′), glucose (inner): 5.64 (1H, *overlapped*, H-3″), 5.39 (1H, *t*, *J* = 7.7 Hz, H-2″), 5.26 (1H, *d*, *J*= 7.3 Hz, H-1″), 5.07 (1H, *overlapped*, H-6″b), 4.57 (1H, *dd*, *J* = 12.2/2.5 Hz, H-6″a), 4.28 (1H, *t*, *J* = 9.5 Hz, H-4″), 3.99 (1H, *overlapped*, H-5″), rhamnose: 5.63 (1H, *overlapped*, H-2‴), 5.58 (1H, *overlapped*, H-3‴), 5.52 (1H, *overlapped*, H-4‴), 5.29 (1H, *br s*, H-1‴), 4.10 (1H, *m*, H-5‴), 1.33 (3H,

d, *J*= 5.1 Hz, H-6‴), glucose (terminal): 5.75 (1H, *t*, *J* = 9.4 Hz, H-3⁗), 5.70 (1H, *overlapped*, H-4⁗), 5.54 (1H, *overlapped*, H-2⁗), 5.32 (1H, *d*, *J*= 7.9 Hz, H-1⁗), 4.68 (1H, *dd*, *J* = 12.4/4.4 Hz, H-6⁗b), 4.35 (1H, *dd*, *J* = 12.2/2.3 Hz, H-6⁗a), 4.02 (1H, *overlapped*, H-5⁗), $\underline{CH}_3O$: 2.48, 2.31, 2.28, 2.27, 2.19, 2.16, 2.11, 2.06, 2.04, 2.03, 2.01, 2.00 (each 3H, *s*).

Ajugol (**3**): UV (MeOH) λ_{max} 220 nm, IR (KBr) υ_{max} 3416 (OH), 1656 (C=C) cm^{-1}, positive ion-LC-ESIMS m/z 370.9 $[M+Na]^+$ (calc. for $C_{15}H_{24}O_9$). ^1H NMR (500 MHz, DMSO-d_6): δ_H 6.15 (1H, *d*, *J*= 5.1 Hz, H-3), 5.45 (1H, *s*, H-1), 4.85 (1H, *overlapped*, H-4), 4.64 (1H, *d*, *J*= 7.9 Hz, H-1′), 3.90 (1H, *overlapped*, H-6), 3.89 (1H, *overlapped*, H-6′b), 3.66 (1H, *dd*, *J*= 11.6/4.8 Hz, H-6′a), 3.40-3.15 (*overlapped*, H-2′, H-3′, H-5′), 3.19 (1H, *t*, *J*= 8.7 Hz, H-4′), 2.72 (1H, *m*, H-5), 2.54 (1H, *d*, *J*= 9.4 Hz, H-9), 2.04 (1H, *dd*, *J*= 13.4/5.6 Hz, H-7′b), 1.79 (1H, *dd*, *J*= 13.4/4.5 Hz, H-7′a), 1.31 (1H, *s*, H-10) and ^{13}C NMR (125 MHz, DMSO-d_6) (see Table 2) data superimposable with those reported in the literature[5].

Picroside IV (= 6′-O-*trans*-*p*-hydroxycinnamoylcatalpol) (**4**): UV (MeOH) λ_{max} 206, 250 (sh), 312 nm, IR (KBr) υ_{max} 3413 (OH), 1705 (C=O), 1642 (C=C), 1604, 1546, 1363 (aromatic ring) cm^{-1}, positive ion- LC-ESIMS m/z 531 $[M+Na]^+$ (calc. for $C_{24}H_{28}O_{12}$). ^1H NMR (300 MHz, DMSO-d_6): δ_H 7.51 (1H, *d*, *J*= 16.0 Hz, H-β), 7.49 (2H, *d*, *J*= 8.8 Hz, H-2″/6″), 6.76 (2H, *d*, *J*= 8.2 Hz, H-3″/5″), 6.35 (1H, *d*, *J*= 16.0 Hz, H-α), 6.31 (1H, *d*, *J*= 5.5 Hz, H-3) 4.95 (1H, *dd*, *J*= 9.9/5.0 Hz, H-4), 4.94 (1H, *overlapped*, H-6′b), 4.73 (1H, *d*, *J*= 9.7 Hz, H-1), 4.60 (1H, *d*, *J*= 7.7 Hz, H-1′), 4.37 (1H, *d*, *J*= 11.3 Hz, H-10b), 4.23 (1H, *dd*, *J*= 11.6/5.6 Hz, H-6′a), 3.93 (1H, *d*, *J*= 13.0 Hz, H-10a), 3.70-3.00 (*overlapped*, H-2′-H-5′), 3.64 (1H, *d*, *J*= 8.0 Hz, H-6), 3.40 (1H, *br s*, H-7), 2.32 (1H, *t*, *J*= 8.3 Hz, H-9), 2.1 (1H, *m*, H-5) and ^{13}C NMR (75 MHz, DMSO-d_6) (see Table 2).

Verbascoside {= acteoside, [β-(3, 4-dihydroxyphenyl)-ethyl]-(3′-*O*-α-L-rhamnopyranosyl)-(4′-*O*-caffeoyl)-β-D-glucopyranoside} (**5**): 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}, positive ion-LC-ESIMS m/z 647 $[M+Na]^+$ (calc. for $C_{29}H_{36}O_{15}$).^1H NMR (300 MHz, DMSO-d_6) and DEPT-135 NMR (75 MHz, DMSO-d_6) data superimposable with those reported in the literature[6].

1-(β-D-glucopyranosyl)-8-hydroxy-3, 7-dimethyl-oct-2(*E*), 6(*E*)- dienoate (**6**): UV (MeOH) λ_{max} 218 nm, IR (KBr) υ_{max} 3416 (OH), 1705 (C=O), 1642 (C=C) cm^{-1}, positive ion-LC-ESIMS m/z 368.9 $[M+Na]^+$ (calc. for $C_{16}H_{26}O_8$). ^1H NMR (500 MHz, DMSO-d_6): δ_H 5.67 (1H, *s*, H-2), 5.34 (1H, *d*, *J*= 8.1 Hz, H-1′), 5.27 (1H, *br t*, H-6), 3.74 (2H, *br s*, H-8a, H-8b), 3.62-3.08 (*overlapped*, H-2′-H-6′), 2.17 (4H, *m*, H-4a, H-4b, H-5a, H-5b), 2.11 (3H, *s*, H-9), 1.52 (3H, *br s*, H-10) and ^{13}C NMR (125 MHz, DMSO-d_6) (see Table 3).

Results and Discussion

Compounds **1** and **2** (see Figure 1) were obtained as amorphous compounds with the molecular weights 1104 {LC-ESIMS: m/z 1127 ($[M+Na]^+$)}, and 1072 {LC-ESIMS: m/z 1095 ($[M+Na]^+$)}, as calculated for $C_{55}H_{92}O_{22}$ and $C_{54}H_{88}O_{21}$, respectively. Upon acetylation, **1** yielded a tridecaacetate **1a** {LC-ESIMS: m/z 1650 ($[M]^+$), 1673 ($[M+Na]^+$), calc. for $C_{81}H_{118}O_{35}$}, and **2** yielded a dodecaacetate **2a** {LC-ESIMS: m/z 1576 ($[M]^+$), 1599 ($[M+Na]^+$), calc. for $C_{78}H_{112}O_{33}$}.

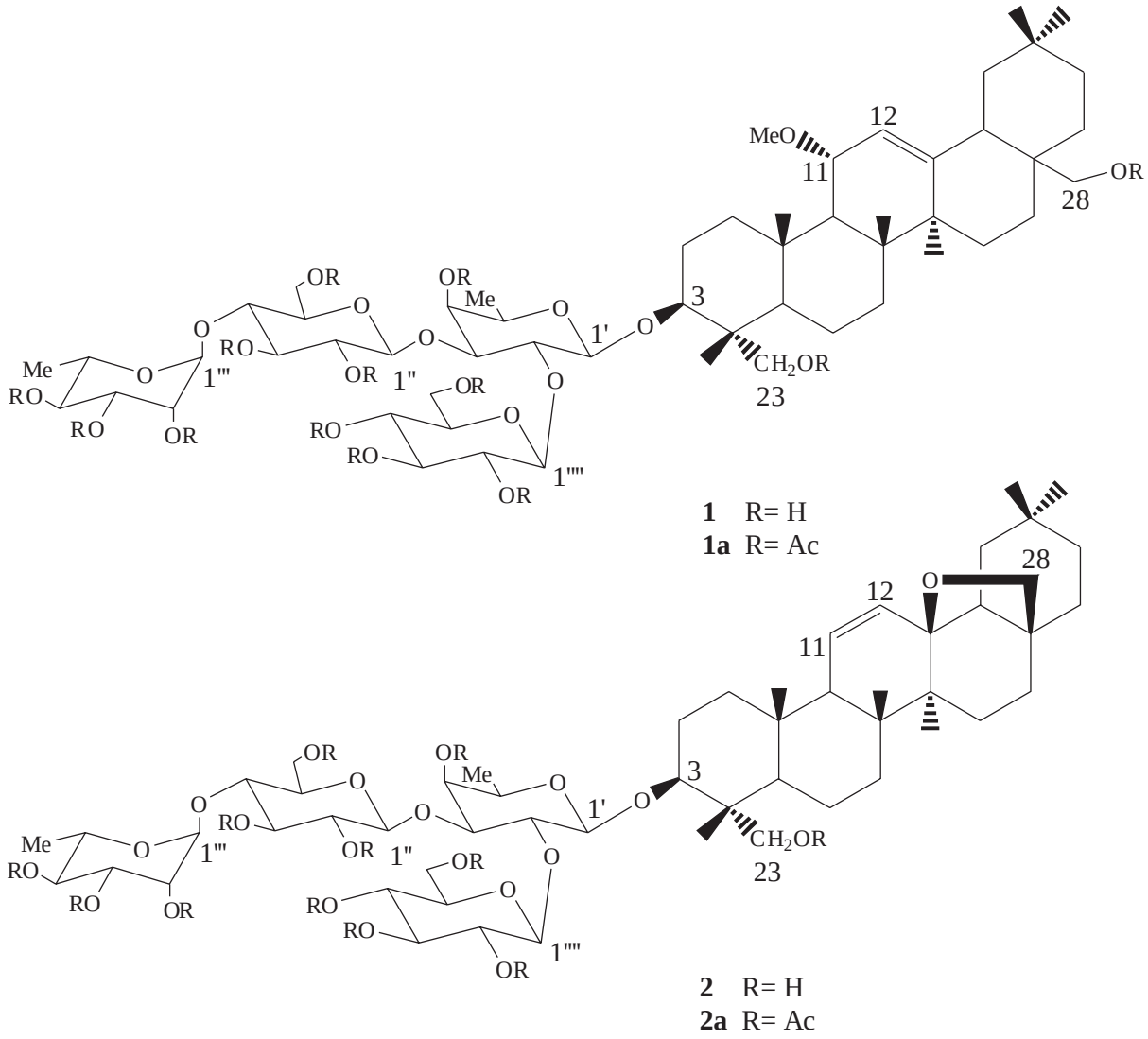

Figure 1. Saponin glycosides isolated from *Verbascum pterocalycinum* var. *mutense.*

In their IR spectra, the observed absorbances were consistent with the presence of olefinic double bonds.

The ^{1}H and ^{13}C NMR data (see Experimental and Table 1) of **1** and **2** suggested that they had similar structures, possessing the same sugar moieties but differing in their aglycones. Each showed glucose, rhamnose and fucose and the molar ratio of the sugars from each compound was 2:1:1 as determined by ^{1}H and ^{13}C NMR data (see Experimental and Table 1). The sugar sequence was determined by 2D NMR experiments on acetylated derivatives **1a** and **2a**.

In the ^{1}H NMR spectrum of **1**, 4 characteristic resonances for anomeric protons were observed at δ_H 4.91 (*d*, *J*= 6.6 Hz), 5.21 (*d*, *J*= 7.0 Hz), 5.54 (*d*, *J*= 7.0 Hz) and 5.78 (*br s*), as well as 2 proton signals at δ_H 1.35 (*d*, *J*= 4.8 Hz) and 1.68 (*d*, *J* = 5.5 Hz), arising from the secondary methyl groups in the sugar moiety. The spin systems were analyzed by means of a DQF-COSY experiment of **1a**. Therefore, the anomeric proton signals, given above, were assigned to β-D-fucopyranose, β-D-glucopyranose (inner), β-D-glucopyranose (terminal) and α-L-rhamnopyranose, respectively. The ^{13}C NMR spectrum of **1** also

proved the tetraglycosidic structure by the existence of anomeric carbon resonances at δ_C103.8 (fucose), 104.2 (glucose-inner), 102.7 (glucose-terminal) and 101.4 (rhamnose). The observation of HMBC cross peaks between H-1‴ (δ_H 5.78) of rhamnose and C-4″ (δ_C 78.4) of glucose (inner), H-1″ (δ_H 5.21) of glucose (inner) and C-3′ (δ_C 85.0) of fucose, H-1⁗ (δ_H 5.54) of glucose (terminal) and C-2′ (δ_C 77.0) of fucose, and H-1′ (δ_H 4.91) of fucose and C-3 (δ_C 83.0) of sapogenin allowed sequencing of the chain as [α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→2)]-β-D-fucopyranoside.

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

		1	**2**			**1**	**2**
Position	C_{Atom}	δ (ppm)	δ (ppm)	Position	C_{Atom}	δ (ppm)	δ (ppm)
Aglycone				Sugar units			
1	CH_2	40.2	38.6	β- D-fucose			
2	CH_2	22.9	26.1	1′	CH	104.2	103.8
3	CH	83.0	81.4	2′	CH	77.0	77.2
4	C	44.1	41.9	3′	CH	85.0	83.8
5	CH	48.1	47.0	4′	CH	72.2	71.5
6	CH_2	18.5	17.5	5′	CH	70.6	70.1
7	CH_2	31.9	31.4	6′	CH_3	17.3	17.5
8	C	37.6	41.9	β- D-glucose (inner)			
9	C	52.8	53.6	1″	CH	105.1	104.2
10	C	35.8	36.2	2″	CH	75.6	75.2
11	CH	76.2	132.2	3″	CH	77.8	76.3
12	CH	122.6	131.7	4″	CH	78.4	77.7
13	C	148.1	84.8	5″	CH	77.2	76.3
14	C	43.6	43.5	6″	CH_2	61.4	62.6
15	CH_2	26.7	25.7	α- L-rhamnose			
16	CH_2	26.4	26.1	1‴	CH	102.8	101.4
17	C	42.2	44.2	2‴	CH	72.8	72.7
18	CH	42.5	51.5	3‴	CH	72.6	71.7
19	CH_2	47.1	37.6	4‴	CH	74.0	74.9
20	C	31.4	31.4	5‴	CH	70.5	69.6
21	CH_2	33.3	35.2	6‴	CH_3	18.5	18.6
22	CH_2	34.8	31.2	β- D-glucose (terminal)			
23	CH_2	64.8	63.3	1⁗	CH	104.0	102.7
24	CH_3	13.4	12.6	2⁗	CH	76.2	75.9
25	CH_3	18.0	18.9	3⁗	CH	78.8	77.4
26	CH_3	18.7	20.1	4⁗	CH	72.2	72.7
27	CH_3	26.4	19.9	5⁗	CH	76.5	76.9
28	CH_2	68.9	76.9	6⁗	CH_2	63.3	61.1
29	CH_3	33.5	34.2				
30	CH_3	24.0	24.2				
OCH_3	CH_3	54.1	-				

The ^{1}H NMR of **1** showed 6 tertiary methyl signals at δ_H 0.88, 0.95, 0.96, 1.07, 1.08 and 1.30. The proton singlet at δ_H 3.21 (3H) was assigned to methoxyl protons. The signal at δ_H 5.46 (*br s*) was attributed to the olefinic proton of the aglycone. The signals at δ_C122.6 and 148.1 in the ^{13}C NMR spectrum confirmed the presence of an olefinic double bond, implying that the aglycone was an oleanane-Δ^{12} type. The assignment of the remaining NMR signals was achieved by means of ^{1}H-^{1}H COSY and HMQC and HMBC experiments.

The location of the methoxyl group was determined from homo- and heteronuclear COSY of **1**, establishing the assigments of H-11 (δ 3.82, *br d*, J= 8.2 Hz) and C-11 (δ_C 76.2). The former showed correlations with H-12 (δ 5.46, *br s*) and H-9 (δ 1.95, *overlapped*), indicating the methoxyl group to be at C-11. From the chemical shift of C-11 (δ_C 76.2) in **1**, it can be deduced that the methoxyl group has an α-configuration as reported for saikosaponin-b$_4$[7].

The H-3 methine proton at δ_H4.10 and the diastereotype H-23 methylene protons (δ_H3.69, *d*, J= 8.3 Hz, H-23a – 4.33, H-23b) and H-28 (δ_H3.57, *d*, J= 10.2 Hz, H-28a – 3.81, *d*, J= 11.7 Hz, H-28b) showed the expected downfield shifts due to oxygen substitutions. Upon acetylation, of the 13 acetyl groups in the ^{1}H NMR of **1a**, 11 were attributed to the sugar moieties and 2 belonged to the aglycone, confirming the presence of 2 primary alcohol units (-CH_2OH; 23 and 28) in the aglycone.

Consequently, the structure was elucidated to be 3-*O*-{[α-L-rhamnosyl-(1→4)-(β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl]-(1→2)-β-D-fucopyranosyl}-11-methoxy-olean-12-ene-3β, 23, 28-triol (= ilwensisaponin C)[8].

Compound **2** showed very similar ^{1}H and ^{13}C NMR spectra (see Experimental and Table 1) to those of **1**. The ^{1}H NMR, the ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY experiments revealed that both saponins (**1** and **2**) possess the same sugar chain sequence. The major differences between the ^{1}H NMR spectra of **1** and **2** arise from the aglycone parts.

Concerning the most representative signals, the ^{1}H NMR spectrum of **2** showed 6 tertiary methyl groups characterized by the singlets at δ_H 0.82, 0.87, 0.96, 0.98, 1.04 and 1.31. The signals at δ_H 5.53 (*overlapped*) and 5.94 (*br d*, J= 10.4 Hz) were assigned to the olefinic protons H-12 and H-11, respectively. These data showed that the aglycone was an oleanane-Δ^{11} type. From NMR data as well as mass spectral data, it was deduced that the main structural difference between **1** and **2** was the absence of the methoxy group in **2**.

The ^{1}H NMR of **2** showed 2 AB systems at δ_H 3.70–4.34 [-CH_2 (23)] and 3.33 (*d*, J= 6.2 Hz)–3.72 [-CH_2 (28)]. Signals of C-28 methylene protons appeared at a much higher field (δ_H 3.57-3.81 in **1**), indicating the presence of an oxo-bridge between C-28 and C-13, while signals of C-23 methylene protons appeared at δ_H 3.70-4.34 in the ^{1}H NMR spectrum. Besides these, upon acetylation, of the 12 acetyl groups in the ^{1}H NMR of **2a**, 11 were attributed to the sugar moieties and only 1 belonged to the aglycone, confirming the presence of 1 primary alcohol unit (-CH_2OH; 23), indicating that **2** was hexacyclic. Furthermore, the ^{13}C NMR data for the aglycone of **2** were in good agreement with those published for 13β, 28-epoxyolean-11-ene-3β, 23-diol[9].

As a result, the structure of **2** was determined to be 3-*O*-{[α-L-rhamnosyl-(1→4)-(β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl]-(1→2)-β-D-fucopyranosyl}-13β, 28-epoxyolean-11-ene-3β, 23-diol (=ilwensisaponin A[8]= mimengoside A[10]).

Compound **3** (see Figure 2) was isolated as a yellow amorphous powder with the molecular formula $C_{15}H_{24}O_9$ (LC-ESIMS m/z 370.9 $[M+Na]^+$). Its UV spectrum suggested the presence of an iridoid enol-ether system (220 nm) and in its IR spectra absorption bands were typical for a hydroxyl group (3416 cm^{-1}) and a double bond (1656 cm^{-1}). The ^{1}H and ^{13}C NMR spectra (see Experimental and Table 2) of **3** were superimposible with those of ajugol. Based on this evidence, compound **3** was identified as ajugol[5].

Table 2. ^{13}C NMR (125 MHz, DMSO-d_6) data of compounds **3** and **4**.

		3	**4***
Position	C_{Atom}	δ (ppm)	δ (ppm)
Aglycone			
1	CH	92.7	94.6
3	CH	139.4	141.2
4	CH	104.9	104.2
5	CH	40.3	38.3
6	CH	77.2	78.2
7	CH_2 $(CH)^{\xi}$	49.0	61.4
8	C	78.5	66.0
9	CH	50.8	42.7
10	CH_3 $(CH_2)^{\aleph}$	24.2	60.2
β- D-glucose			
1′	CH	98.4	99.1
2′	CH	73.8	74.1
3′	CH	76.8	77.0
4′	CH	70.7	70.8
5′	CH	77.0	74.8
6′	CH_2	61.9	63.6
Acyl moiety			
1″	C		126.1
2″	CH		131.2
3″	CH		116.7
4″	C		161.4
5″	CH		116.7
6″	CH		131.2
α	CH		114.8
β	CH		145.7
C=O	C		167.4

ξ CH for compound **4**
$\aleph$ CH_2 for compound **4**
$*$ 75 MHz

Compound **4** (see Figure 2) was isolated as a yellow amorphous powder with the molecular formula $C_{24}H_{28}O_{12}$ (LC-ESIMS m/z 531 $[M+Na]^+$). The IR spectrum showed absorption bonds for a hydroxyl group (3413 cm^{-1}), a conjugated ester carbonyl (1705 cm^{-1}) and a double bond (1642 cm^{-1}). The^{1}H and ^{13}C NMR spectra (see Experimental and Table 2) of **4** showed signals very similar to those of catalpol[11], with additional signal arising from an aromatic acid. The signals of 2 *trans* olefinic protons (δ_H 6.35 and 7.51, *d*, AB system, J_{AB} = 16.0 Hz), as well as 2 pairs of *ortho*-coupled aromatic protons (δ_H 6.76 and 7.49, *d*,J= 8.8 Hz) in the ^{1}H NMR spectrum of **4,** showed clearly the presence of a *trans-p*-hydroxycinnamoyl unit[11]. A comparison of the ^{1}H, ^{13}C and DEPT-135 NMR data of **4** with those of catalpol[11] indicated that **4** was a monoacyl derivative of catalpol (see Experimental and Table 2). The position of the acyl moiety was determined by a comparison of ^{1}H and ^{13}C NMR spectra with those of unsubstitued catalpol[11]. The H_2-6′ and C-6′ signals of **4** were shifted downfield ca. 1.37 and 1.60 ppm, respectively. These features were only compatible with the attachment of the acyl group to the C-6′ (OH). This assignment was also confirmed by the comparison of the NMR data of **4** with those of 6′-acyl iridoid derivative[12]. Therefore, **4** was determined

to be picroside IV (= 6′-*O*-*trans*-*p*-hydoxycinnamoylcatalpol)[13].

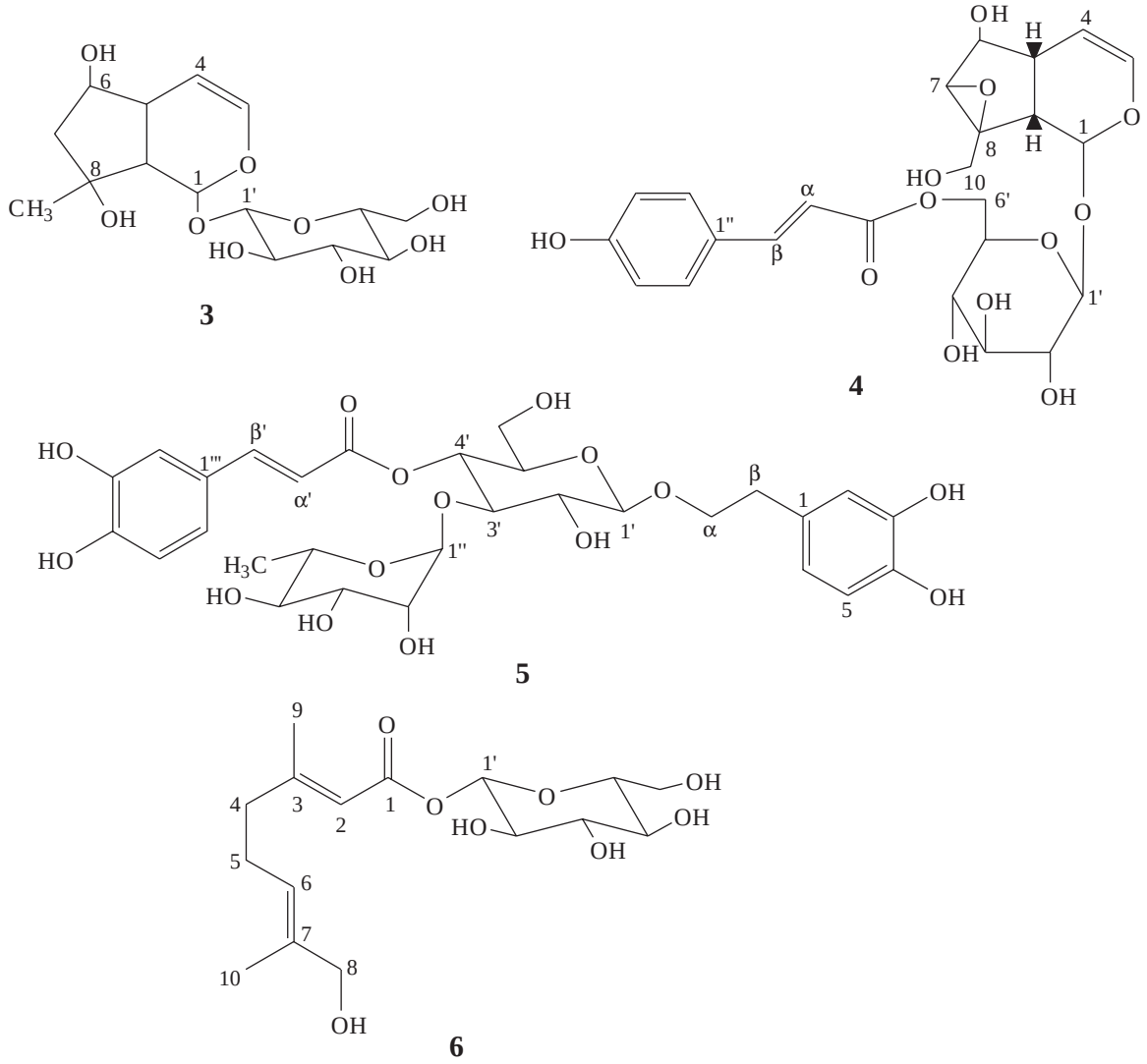

Figure 2. Iridoid, phenylethanoid and monoterpene glycosides isolated from *Verbascum pterocalycinum* var. *mutense.*

Compound **5** (see Figure 2) was obtained as an amorphous powder. Its structure was identified as verbascoside[6] by comparing its ^{1}H and DEPT-135 NMR data with previously published data and by direct comparison with the authentic sample on a TLC plate.

Compound **6** (see Figure 2) was isolated as a colorless, amorphous compound with the molecular formula $C_{16}H_{26}O_8$. The IR spectrum showed characteristic absorption bands at 3416 (OH), 1705 (an α, β-unsaturated ester) and 1642 (C=C) cm^{-1}. The UV spectrum showed a maximum at 218 nm. The ^{1}H NMR spectrum (see Experimental) showed an anomeric proton resonance at δ_H 5.34 (*d*, *J*= 8.1 Hz), indicating its monoglycosidic structure. This was confirmed by the ^{13}C NMR resonance at δ_C 94.5 assigned for an anomeric carbon atom of a β-D-glucopyranose unit. The ^{1}H and ^{13}C NMR spectra of **6** contained signals belonging to acyclic monoterpene moiety. All ^{13}C NMR multiplicities of compound **6** were confirmed by

DEPT-135 measurements, and signal connectivities were determined by HMQC and HMBC. Signals at δ_H 1.52 (*br s*, H-10) and 2.11 (*s*, H-9) were assigned to 2 olefinic methyl groups. The resonances at δ_H 2.17 were related to allylic-type protons (*m*, H_2-4 and H_2-5). In the ^{13}C NMR spectrum (see Table 3) the carbon resonances at δ_C 163.0 (C-2) and 115.6 (C-3) were assumed to arise from a double bond in conjugation with the carboxyl function (δ_C 165.1). The signals at δ_C 137.2 (C-7) and 122.7 (C-6) were attributed to a second double bond. The ^{1}H NMR spectrum of **6** also showed the signals of 2 olefinic protons at δ_H 5.67 (*s*, H-2) and 5.27 (*br t*, H-6). Compound **6** was then assumed to be derived from a geranic acid due to the chemical shifts of C-1 to C-5 (δ_C 167.0, 115.4, 159.7, 41.0 and 26.2, respectively)[14]. The configuration, 2(*E*), could be assigned in relation to the ^{13}C NMR data published for methylgeranoate and methylneroate[14]. The resonance at δ_C 67.0 resulted from a vinylic hydroxy-methyl group (C-8). The chemical shift of C-8 confirmed the *trans*-configuration, 6(*E*), at this center (*cis*-configuration gives a signal at 60.5 ppm) and the position of the second methyl group at C-7[15]. The assignment of the signals of C-7 (δ_C 137.2), C-8 (δ_C67.0) and C-10 (δ_C 14.4) was in good agreement with the reported ^{13}C NMR data for 8-hydroxy-3, 7-dimethyl-2, 6-octadienoic acid[16]. The chemical shift of the anomeric proton at δ_H 5.34 in the ^{1}H NMR spectrum and the C-1′ chemical shift at δ 94.5 in the ^{13}C-NMR spectrum suggested that the monoterpene acid was linked in ester linkage at the C-1′ hydroxyl group in glucose.

Table 3. ^{13}C NMR (125 MHz, DMSO-d_6) data of compound **6**.

		6
Position	C_{Atom}	δ (ppm)
Aglycone		
2	CH	115.6
3	C	163.0
4	CH_2	40.9
5	CH_2	25.8
6	CH	122.7
7	C	137.2
8	CH_2	67.0
9	CH_3	19.5
10	CH_3	14.4
C=O (1)	C	165.1
β- D-glucose		
1′	CH	94.5
2′	CH	73.2
3′	CH	78.7
4′	CH	70.3
5′	CH	77.4
6′	CH_2	61.4

These data were identical with those of 1-β-D-glucopyranosyl-8-hydroxy-3, 7-dimethyl-2(*E*), 6(*E*)-octadienoate [17].

Conclusion

The occurrence of saponin glycosides in several species from Scrophulariaceae within the genus *Verbascum* is well documented in the literature[18]. To the best of our knowledge, ilwensisaponin C (**1**) has been isolated

from *Verbascum* species for the second time. This compound has been earlier reported from *Verbascum nigrum*[18], which is not represented in the Turkish flora[2]. During the preparation of this manuscript, we became aware of a preliminary report on the isolation and structural elucidation of saponins **1** and **2** as ilwensisaponin C and A from *Scrophularia ilwensis*[8], suggesting a taxonomically interesting relationship between plants of the genera *Verbascum* and *Scrophularia*.

It has been reported that *Verbascum* species contain diverse iridoid glycosides such as catalpol[11] and ajugol[5]. This paper is the first report of the presence of picroside IV (**4**) from the genus *Verbascum*. In addition, it is interesting that the acyclic monoterpene glycoside (**6**) has also been isolated and characterized for the first time in the genus *Verbascum*. Our continuing studies will be of assistance in clarifying the chemotaxonomical classification of the genus *Verbascum*. Bioactivity studies of the isolated compounds are in progress.

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References

1. T.G. Tutin, Flora Europaea Vol. 3, p. 461, University Press, Cambridge, UK 1972.
2. 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.
3. T. Baytop, "Therapy with Medicinal Plants in Turkey (Past and Present)", 2^{nd} ed. pp. 334-335, Nobel Tıp Kitabevleri Ltd., İstanbul 1999.
4. K. Kraus and G. Franz, **Dtsch. Apoth. Ztg. 127**, 665 (1987).
5. F. Pardo, F. Perich, R. Torres and F. Delle Monache, **J. Chem. Ecol. 24**, 645-653 (1998).
6. Z.Ş. Akdemir, İ.İ. Tatlı, E. Bedir and I.A. Khan, **Turk. J. Chem.**, (2003). (*submitted*).
7. H. Ishii, S. Seo, K. Tori, T. Tozyo and Y. Yoshimura, **Tetrahedron Lett.** 1227-1230 (1977).
8. İ. Çalış, M. Zor, A.A. Başaran, A.D. Wright and O. Sticher, **Helv. Chim. Acta 76**, 1352-1360 (1993).
9. K. Tori, Y. Yoshimura, S. Seo, K. Sakurawi, Y. Tomita and H. Ishii, **Tetrahedron Lett.** 4163-4166 (1976).
10. N. Ding, S. Yahara and T. Nohara, **Chem. Pharm. Bull. 40**, 780-782 (1992).
11. İ.İ. Tatlı, Z.Ş. Akdemir, E. Bedir and I.A. Khan, **Turk. J. Chem.**, **27**, 765-772 (2003).
12. T. Miyase, C. Akahori, H. Kohsaka and A. Ueno, **Chem. Pharm. Bull. 39**, 2944-2951 (1991).
13. J.X. Li, P. Li, Y. Tezuka, T. Namba and S. Kadota, **Phytochemistry 48**, 537-542 (1998).
14. L. Crombie, R.W. King and D.A. Whiting, **J. Chem. Soc., Perkin Trans. 1**, 913-915 (1975).

15. J.B. Stothers, "Carbon-13 nmr Spectroscopy", Academic Press, New York and London 1972.
16. F. Bohlmann, R. Zeisberg and E. Klein, **Org. Magn. Reson. 7**, 426-432 (1975).
17. B. Gering and M. Wichtl, **J. Nat. Prod. 50**, 1048-1054 (1987).
18. B. Klimek, C. Lavaud and G. Massiot, **Phytochemistry 31**, 4368-4370 (1992).