# Iridoid Glucosides from *Veronica hederifolia*

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**A new iridoid glucoside, urphoside A, and six known iridoid glucosides, pikuroside, aucubin, veronicoside, catalposide, amphicoside, and verminoside, were isolated from *Veronica hederifolia* together with a known megastigmane glucoside, 3-hydroxy-5,6-epoxy-β-ionol-9-*O*-β-D-glucopyranoside, and a hexitol, dulcitol. The structures of the isolated compounds were established by the extensive 1D- and 2D-NMR spectroscopy.**

**Key words** *Veronica hederifolia*; Scrophulariaceae; iridoid glucoside; urphoside A; pikuroside; megastigmane glucoside

The genus *Veronica* (Scrophulariaceae), which is widely distributed in Europe and Asia, especially in the Mediterranean area, is represented by 79 species in Turkey, 26 of which are endemic.[1—3] Iridoid glucosides, phenylethanoid, and flavonoid glycosides have been mainly reported from different *Veronica* species.[4—10] Our previous research on *Veronica hederifolia* L. has showed that the water-soluble portion of the MeOH extract suppresses nitric oxide production in lipopolysaccharide-stimulated mouse peritoneal macrophages due to its free radical scavenging activity, while the chloroform-soluble portion of the MeOH extract is cytotoxic against KB and B16 cells.[11] In a continuation of our studies on the bioactive constituents of *Veronica* species, we report here the isolation and structure elucidation of one new urphoside A (**1**) and six known iridoid glucosides, pikuroside (**2**), aucubin (**3**), veronicoside (**4**), catalposide (**5**), amphicoside (**6**), and verminoside (**7**), as well as a known megastigmane glucoside (**8**) and a hexitol, dulcitol (**9**).

## Results and Discussion

The water-soluble portion of the methanolic extract of *V. hederifolia* was subjected to polyamide column chromatography to afford six main fractions. Repeated chromatography of the polyamide fractions resulted in the isolation of nine compounds (**1**—**9**) in pure form. Compound **1** was isolated as an amorphous powder with negative optical rotation ($[\alpha]_D^{23}$ −22°, MeOH). The molecular formula of **1** was determined to be $C_{23}H_{30}O_{14}$ by high-resolution (HR)-FAB-MS. Its UV spectrum showed $\lambda_{max}$ at 210, 299, and 331 (sh) nm, indicating the presence of a nonconjugated enol–ether system and an aromatic acid.[12] The $^{13}$C-NMR spectral data revealed the presence of one glucopyranosyl unit, one tri-substituted aromatic ring with one methoxy group, and one carbonyl function, in addition to the aglycone moiety containing nine carbon signals (Table 1). The gross structure was determined from $^1$H-NMR and $^1$H–$^1$H shift correlation spectroscopy ($^1$H–$^1$H COSY) experiments. Construction of the iridoid skeleton started with the carbon at $\delta$ 93.1 (C-1), which has an acetal proton at $\delta$ 5.55 (d, $J$=4.3 Hz). This acetal proton was coupled to the methine proton at $\delta$ 2.52 (dd, $J$=10.1, 4.3 Hz, H-9), which in turn was coupled to the second methine proton at $\delta$ 2.81 (dddd, $J$=10.1, 4.9, 3.5, 2.1 Hz, H-5). H-5 was further coupled to an olefinic proton at $\delta$ 5.27 (dd, $J$=6.3, 3.5 Hz), which in turn was coupled to another olefinic proton $\delta$ 6.27 (dd, $J$=6.3, 2.1 Hz). These vicinally coupled olefinic protons were ascribed to H-4 and H-3, respectively, confirming the presence of an iridoid moiety with a nonconjugated enol–ether system. These assignments were confirmed by the $^2J_{CH}$ and $^3J_{CH}$ correlations in the heteronuclear multiple bond correlation (HMBC) spectrum of **1** (Table 1).

In the other direction, the proton at C-5 was correlated to the oxymethine proton at $\delta$ 4.85 (dd, $J$=5.8, 4.9 Hz, H-6), which in turn was coupled to another oxymethine proton at $\delta$ 4.20 (d, $J$=5.8 Hz, H-7). The absence of any other homonuclear coupling observed for H-7 and H-9 indicated a totally substituted C-8 ($\delta$ 80.8, s). HMBC correlations between C-8/H$_2$-10, C-10/H-9, and C-10/H-7 showed the attachment of a hydroxymethyl group at C-8. The chemical shift value and coupling constant of C-10 ($\delta_C$ 64.4; $\delta_H$ 4.04, 4.85 d, $J$=11.9 Hz) required a tertiary hydroxyl function at the C-8 position. Signals in the region of $\delta$ 3.19—4.66 suggested the presence of a glucopyranose unit. The β-anomeric configuration of the glucose was judged based on the large $^3J_{H1,H2}$ coupling constants of the anomeric proton ($\delta$ 4.66, d, $J$=7.9 Hz).

Table 1. $^{13}$C- and $^1$H-NMR Spectral Data and Selected HMBC Correlations for Compound **1** ($CD_3OD$; $^{13}$C, 125 MHz; $^1$H, 500 MHz)

| C/H | DEPT | $\delta_C$ | $\delta_H$ | $J$ (Hz) | HMBC (C→H) |
|---|---|---|---|---|---|
| 1 | CH | 93.1 | 5.55 d | (4.3) | H-1′, H-3, H-9 |
| 3 | CH | 140.9 | 6.27 dd | (6.3, 2.1) | H-1, H-4 |
| 4 | CH | 106.0 | 5.27 dd | (6.3, 3.5) | H-3, H-6, H-9 |
| 5 | CH | 36.8 | 2.81 dddd | (10.1, 4.9, 3.5, 2.1) | H-1, H-3, H-6, H-9 |
| 6 | CH | 86.2 | 4.85 dd | (5.8, 4.9) | H-4, H-7, H-9 |
| 7 | CH | 84.4 | 4.20 d | (5.8) | H-6, H-9, H$_2$-10 |
| 8 | C | 80.8 | | | H-1, H-7, H-9, H$_2$-10 |
| 9 | CH | 48.5 | 2.52 dd | (10.1, 4.3) | H-4, H$_2$-10 |
| 10 | CH$_2$ | 64.4 | 4.04 d | (11.9) | H-7, H-9 |
| | | | 4.85 d | (11.9) | |
| 1′ | CH | 99.6 | 4.66 d | (7.9) | H-1, H-2′ |
| 2′ | CH | 74.8 | 3.19 t | (7.9) | H-3′ |
| 3′ | CH | 78.0 | 3.36 t | (8.9) | H-4′, H-2′ |
| 4′ | CH | 71.7 | 3.28[a)] | | H-3′ |
| 5′ | CH | 78.2 | 3.28[a)] | | H-4′, H2-6′ |
| 6′ | CH$_2$ | 62.9 | 3.66 dd | (12.0, 5.2) | H-5′ |
| | | | 3.87 dd | (12.0, 2.4) | |
| 1″ | C | 122.5 | | | H-2″, H-5″ |
| 2″ | CH | 113.8 | 7.58 d | (1.8) | H-6″ |
| 3″ | C | 148.8 | | | H-2″, H-5″, OCH$_3$ |
| 4″ | C | 153.1 | | | H-2″, H-5″, H-6″ |
| 5″ | CH | 116.0 | 6.85 d | (8.2) | H-6″ |
| 6″ | CH | 125.3 | 7.60 dd | (8.2, 1.8) | H-2″ |
| C=O | C | 168.1 | | | H-6, H-2″, H-6″ |
| OCH$_3$ | CH$_3$ | 56.5 | 3.90 s | | |

*a*) Overlapping with each other.

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HMBC and nuclear Overhouser effect (NOE) correlations between C-1/H-1′ and H-1/H-1′ showed that a $\beta$-D-glucopyranose unit was attached at the C-1 position of the aglycon. In the $^1$H-NMR spectrum, the H-6 proton signal ($\delta$ 4.85, dd) showed a downfield shift, suggesting the attachment of an acyl group to this position. The signals at $\delta$ 6.85 (d, $J$=8.2 Hz, H-5″), $\delta$ 7.60 (dd, $J$=8.2, 1.8 Hz, H-6″), and $\delta$ 7.58 (d, $J$=1.8 Hz, H-2″) were assignable to three aromatic protons of an ABX system. The signal at $\delta$ 3.90 (3H, s, $OCH_3$), which correlated to the C-3 signal of the aromatic ring in the HMBC spectrum, indicated the presence of a vanilloyl group. The HMBC correlation of the ester carbonyl ($\delta$ 168.1) to the H-6 signal of the aglycon also confirmed the attachment of a vanilloyl group to C-6.

To determine the relative stereochemistry of the chiral centers in **1**, NOE and $^1$H-NMR decoupling experiments were performed. The $\alpha$-positioned C–H bond at C-1 was established by its coupling ($J$=4.3 Hz) with only one proton at $\delta$ 2.52 (H-9) indicating a $\beta$ H-9, where the dihedral angle was close to 45°. The large coupling between H-5 and H-9 (10.1 Hz), indicating a dihedral angle near 0°, demonstrated that the stereochemistry of the catalpol ring fusion was *cis*. The H-9 proton signal also showed cross-peaks with H-5 and H-7 in NOE spectroscopy (NOESY) spectrum. Furthermore, NOE correlations were observed between H-9 and H-5, H-5 and H-7, and H-7 and H-10 (Fig. 2). Since no interactions were found between H-6 and H-7, H-5, or H-9, the $\alpha$-orientation was suggested for H-6. The middle coupling constants of H-6/H-5 ($J$=4.9 Hz) and H-6/H-7 ($J$=5.8 Hz) supported *trans* interactions between these protons, because in the case of *cis* interaction both coupling constants should have been large. The stereochemistry of the C-8 center was determined to be $\beta$ OH and $\alpha$ $CH_2OH$ since there was good agreement between the C-9 resonance value and reported data.[13] These data confirmed the stereostructure of **1** as shown in the Fig. 1. To the best of our knowledge, compound **1** is described here for the first time and named urphoside A, after the district name Urpha (Urfa), where the materials were collected.

In addition to this compound, six known iridoid glucosides, pikuroside (**2**),[14] aucubin (**3**),[15] veronicoside (**4**),[16] catalposide (**5**), amphicoside (**6**),[1] and verminoside (**7**),[17] a known megastigmane glucoside, 3-hydroxy-5,6-epoxy-$\beta$-ionol-9-*O*-$\beta$-D-glucopyranoside (**8**),[18] and a hexitol, dulcitol (**9**) were isolated and their structures were identified by the comparison of their spectral data with those reported in the literature. Pikuroside (**2**), which has a rigid three-ring skeleton, and the megastigmane glucoside (**8**) were isolated from the genus *Veronica* for the first time in this study and this is the second report of the isolation of pikuroside from natural sources. A range of iridoid glucosides, especially aucubin, catalpol, benzoic, and cinnamic acid esters of catalpol have been identified in nearly all investigated *Veronica* species.[4—7] In addition to catalpol derivatives, we have reported here three different compounds (**1**, **2**, **8**) for the genus *Veronica*. On the other hand, previous studies also resulted in the isolation of some phenylethanoid or flavonoid glycosides, although until now we have not observed any phenolic compounds during the isolation studies. Therefore *V. hederifolia* has shown different chemical composition from the other *Veronica* species. Our continuing studies will be of assistance in clarifying the chemotaxonomical classification of the genus *Veronica*, and bioactivity studies of the isolated compounds are in progress.

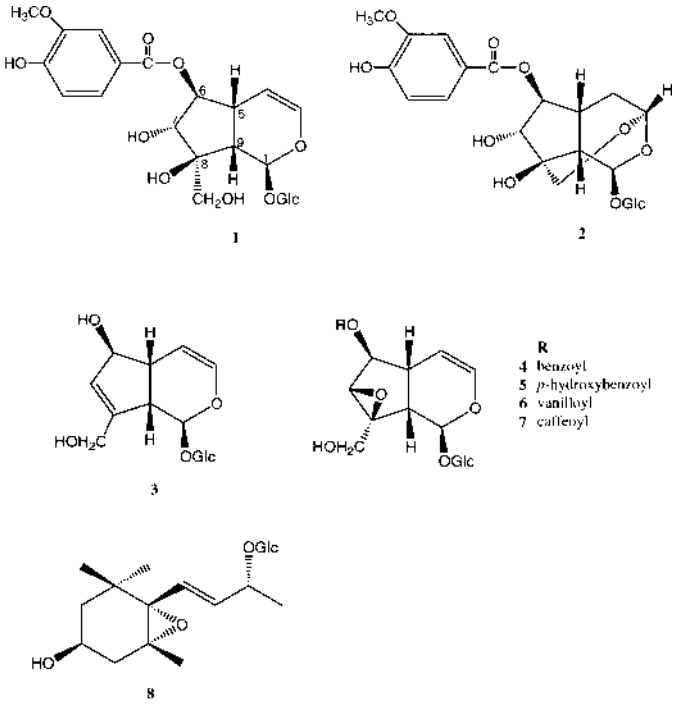


Fig. 1. The Chemical Structures of Isolated Compounds (**1**—**8**)

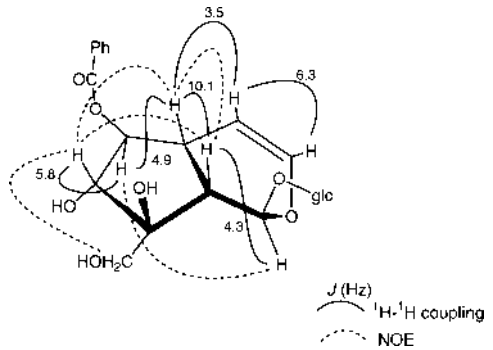


Fig. 2. Selected $^1$H–$^1$H Coupling Constants and NOEs Detected for Compound **1**

## Experimental

**General Experimental Procedures** Optical rotations were measured on a JASCO DIP 140 digital spectrometer using a sodium lamp operating at 589 nm. The UV spectra ($\lambda_{max}$) were recorded on a Shimadzu UV-240 spectrometer. NMR spectra were recorded on a JEOL JNM-A 500 spectrometer in methanol-$d_4$ with tetramethylsilane (TMS) as an internal standard. FAB-MS and HR-FAB-MS were recorded in an NBA matrix in the positive-ion mode on a JEOL JMS-DX300 spectrometer. TLC plates using Silica gel 60 $F_{254}$ and RP18 $F_{254}$ were obtained from Merck (Darmstadt, Germany).

**Plant Material** *V. hederifolia* L. was collected from Urfa, Turkey. A voucher specimen (HUEF 99016) is deposited in the Herbarium of the Faculty of Pharmacy, Hacettepe University.

**Extraction and Isolation** The air-dried aerial parts of *V. hederifolia* (270 g) were extracted with MeOH at 40 °C for 12 h (×2, 2 l). The combined extracts were evaporated under vacuum to give 31 g of crude extract. The MeOH extract was dissolved in $H_2O$ (0.1 l), and $H_2O$-insoluble material was removed by filtration. The filtrate was fractionated with $CHCl_3$ (×5, 100 ml), and the water fraction was lyophilized to yield 22 g dry weight. The water fraction (9 g) was subjected to polyamide column chromatography eluted with $H_2O$, followed by increasing concentrations of MeOH to give six fractions: frs. A—F (fr. A, 4.37 g; fr. B, 1.66 g; fr. C, 0.16 g; fr. D, 0.27 g; fr. E,

0.28 g; fr. F, 0.15 g). The polyamide fractions were found to be rich in iridoid glucosides as a result of TLC controls eluted with 5% $H_2SO_4$ in EtOH. An aliquot of fr. B (500 mg), was chromatographed over silica gel by stepwise elution with $CHCl_3$ : MeOH : $H_2O$ (90 : 10 : 1→60 : 40 : 4) to give compounds **8** (2 mg), **3** (3 mg), and **9** (3 mg). Fr. C (45 mg) was subjected to medium pressure liquid chromatography (MPLC) using reversed-phase material (Lichroprep RP-18, 40—63 $\mu$m). Elution with increasing amounts of MeOH (30→50%) yielded compounds **1** (5.2 mg), **2** (3.3 mg), **4** (2.2 mg) and **6** (2 mg). Fr. D and fr. F (45 mg each) were chromatographed over reverse phase (RP)-MPLC (15→50% and 30→50% MeOH, respectively) and compounds **7** (2.5 mg) and **5** (3.6 mg) were isolated. Isolation studies of *V. hederifolia* are continuing.

Urphoside A (**1**): Amorphous powder, $[\alpha]_D^{23}$ −22° ($c$=0.15, MeOH). UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 331 (sh, 2.3), 299 (2.5), and 210 (2.3). $^1$H- and $^{13}$C-NMR: see Table 1. FAB-MS $m/z$: 553 $[M+Na]^+$. HR-FAB-MS $m/z$: 553.1514 (Calcd for $C_{23}H_{30}O_{14}Na$: 553.1533).

Pikuroside (**2**): Amorphous powder, $[\alpha]_D^{23}$ −285° ($c$=0.20, MeOH). UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 419 (sh, 2.0), 333 (sh, 2.5), 299 (2.5), 217 (2.3). FAB-MS $m/z$: 553 $[M+Na]^+$. $^1$H-NMR ($CD_3OD$) $\delta$: 2.13 (1H, dd, $J$=3.2, 13.8 Hz, H-4), 2.29 (1H, t, $J$=7.9 Hz, H-5), 2.46 (1H, dd, $J$=8.4, 13.2 Hz, H-4), 2.55 (1H, d, $J$=10.1 Hz, H-9), 3.14 t (1H, $J$=9.1 Hz, H-2′), 3.28 (2H, t, $J$=7.6 Hz, H-3′, 4′), 3.35 (1H, t, $J$=9.4 Hz, H-5′), 3.59 (1H, d, $J$=11.5 Hz, H-10), 3.67 (1H, dd, $J$=5.2, 12.0 Hz, H-6′), 3.87 (1H, dd, $J$=1.8, 12.0 Hz, H-6′), 3.90 (3H, s, $OCH_3$), 4.02 (1H, d, $J$=11.3 Hz, H-10), 4.28 (1H, d, $J$=7.3 Hz, H-7), 4.69 (1H, d, $J$=7.9 Hz, H-1′), 4.93 (1H, dd, $J$=2.7, 7.3 Hz, H-6), 5.31 (1H, d, $J$=2.7 Hz, H-3), 5.65 (1H, d, $J$=2.2 Hz, H-1), 6.85 (1H, d, $J$=8.2 Hz, H-5″), 7.57 (1H, d, $J$=1.8 Hz, H-2″), 7.59 (1H, dd, $J$=1.8, 8.2 Hz, H-6″). $^{13}$C-NMR ($CD_3OD$) $\delta$: 33.4 (C-5), 34.9 (C-4), 47.7 (C-9), 56.5 ($O\underline{C}H_3$), 61.0 (C-10), 62.8 (C-6′), 71.7 (C-4′), 74.8 (C-2′), 78.2 (C-3′, 5′), 79.7 (C-8), 82.4 (C-7), 88.2 (C-6), 93.4 (C-1), 96.0 (C-3), 99.0 (C-1′), 113.7 (C-2″), 116.0 (C-5″), 122.7 (C-1″), 125.2 (C-6″), 148.8 (C-3″), 153.0 (C-4″).[19]

3-Hydroxy-5,6-epoxy-$\beta$-ionol-9-*O*-$\beta$-D-glucopyranoside (**8**): Amorphous powder, $[\alpha]_D^{23}$ −141.8° ($c$=0.10, MeOH). UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 332 (sh, 2.1), 266 (2.4). FAB-MS $m/z$: 389 $[M+1]^+$. $^1$H-NMR ($CD_3OD$) $\delta$: 0.96 (3H, s, H-12), 1.12 (3H, s, H-11), 1.18 (3H, s, H-13), 1.23 (1H, dd, $J$=10.7, 14.3 Hz, H-2), 1.27 (3H, d, $J$=6.4 Hz, H-10), 1.55 (1H, dd, $J$=3.5, 14.3 Hz, H-2), 1.60 (1H, dd, $J$=9.1, 14.2 Hz, H-4), 2.26 (1H, dd, $J$=6.2, 14.2 Hz, H-4), 3.17 (1H, dd, $J$=7.8, 10.3 Hz, H-2′), 3.22 (1H, t, $J$=9.3 Hz, H-5′), 3.33 (1H, d, $J$=7.6 Hz, H-4′), 3.34 (1H, br s, H-3′), 3.67 (1H, dd, $J$=5.1, 11.8 Hz, H-6′), 3.73 (1H, m, H-3), 3.82 (1H, dd, $J$=2.1, 11.8 Hz, H-6′), 4.35 (1H, d, $J$=7.8 Hz, H-1′), 4.41 (1H, t, $J$=6.3 Hz, H-9), 5.72 (1H, dd, $J$=6.4, 15.6 Hz, H-8), 5.96 (1H, d, $J$=15.6 Hz, H-7). $^{13}$C-NMR ($CD_3OD$) $\delta$: 20.3 (C-13), 21.0 (C-10), 25.2 (C-12), 30.2 (C-11), 36.0 (C-1), 41.6 (C-4), 48.0 (C-2), 62.6 (C-6′), 64.6 (C-3), 68.1 (C-5), 71.4 (C-4′), 71.2 (C-6), 75.3 (C-2′), 77.0 (C-9), 78.0 (C-5′), 78.2 (C-3′), 102.7 (C-1′), 127.8 (C-7), 137.2 (C-8).[19]

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19) $^1$H- and $^{13}$C-NMR data described here have been corrected to the previous data for compounds **2** and **8**.