Phenylethanoid and Iridoid Glycosides from *Veronica persica*

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A new phenylethanoid glycoside, persicoside (1) and three known phenylethanoid glycosides, acteoside (2), isoacteoside (3) and lavandulifolioside (4) were isolated from the aerial parts of *Veronica persica*. On the basis of spectral analyses, the structure of the new compound was elucidated to be 3,4-dihydroxy-β-phenylethoxy-*O*-[β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→3)]-4-*O*-caffeoyl-β-D-glucopyranoside. Persicoside (1) and acteoside (2) exhibited radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Beside phenylethanoid glycosides, a hexitol, dulcitol (5) and seven known iridoid glucosides, aucubin (6), veronicoside (7), amphicoside (8), 6-*O*-veratroyl-catalpol (9), catalposide (10), verproside (11) and verminoside (12) were isolated.

Key words *Veronica persica*; Scrophulariaceae; phenylethanoid glycoside; iridoid glucoside; persicoside; 2,2-diphenyl-1-picrylhydrazyl (DPPH)

In the flora of Turkey, the genus *Veronica* L. (Scrophulariaceae) is represented by 79 species, 26 of which are endemic.[1)] Some *Veronica* species are used as folk medicines in Turkey and in several other countries.[2—5)] The genus *Veronica* is known to contain mainly iridoid glucosides, especially benzoic and cinnamic acid esters of catalpol, some phenylethanoid and flavonoid glycosides.[6—10)] In previous studies, iridoid glucosides, phenylethanoid glycosides and alkaloids have been reported from *Veronica persica* POIRET.[11—13)] Our previous research on this plant has demonstrated that the water soluble portion of MeOH extract has a suppressive effect on nitric oxide production in lipopolysaccharide-stimulated mouse peritoneal macrophages due to its free radical scavenging activity, and the chloroform soluble portion of MeOH extract shows cytotoxicity against KB and B16 cells.[14)] Continuing our studies on bioactive constituents of *Veronica* species, we here examine *V. persica* and isolate a new phenylethanoid glycoside, termed persicoside (**1**) together with eleven known compounds: acteoside (**2**),[15)] isoacteoside (**3**),[16)] lavandulifolioside (**4**),[17)] dulcitol (**5**), aucubin (**6**),[18)] veronicoside (**7**),[19)] amphicoside (**8**),[20)] 6-*O*-veratroyl-catalpol (**9**),[21)] catalposide (**10**),[20)] verproside (**11**)[22)] and verminoside (**12**).[23)] This paper describes the isolation and the structure determination of the new phenylethanoid along with its free radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Dulcitol was isolated as a crystal, and therefore its structure was determined by X-ray crystallographic analysis. The structures of the other known compounds were identified by comparison of their spectral data with those reported in the literature.

Results and Discussion

Compound **1** was isolated as an amorphous powder, $[\alpha]_D^{23}$ −63° (EtOH), whose UV spectra showed λ_{max} at 344 (sh), 325 and 304 (sh) nm indicating its polyphenolic nature. The molecular formula of **1**, $C_{35}H_{46}O_{21}$, was established by high-resolution (HR)-FAB-MS and was in good agreement with the observation of the five methylene, 23 methine and seven quaternary carbon resonances in its ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra. The ^{1}H-NMR spectrum of **1** showed the presence of (*E*)-caffeic acid and 3,4-dihydroxy phenylethanol moieties confirmed by the six aromatic proton signals between δ 6.58—7.07 for 2 ABX systems, two olefinic protons (AB system, d, J_{AB}=16.1 Hz) at δ 6.34 and 7.56, a benzylic methylene at δ 2.79 (2H, t, J=7.4 Hz) and two non-equivalent protons at δ 3.76 and 4.07 (each 1H, m). Additionally, three doublets of anomeric protons were observed at δ 4.54 (d, J=7.6 Hz), δ 4.73 (d, J=7.9 Hz) and δ 4.65 (d, J=7.6 Hz) indicating its triglycosidic structure. This triglycosidic structure was also confirmed by the ^{13}C-NMR spectrum of **1**, where three anomeric carbons at δ 103.33, 104.05 and 104.24 were observed. All protons of the three sugar units were assigned unambiguously from the shift correlation spectroscopy (COSY) spectrum, and a heteronuclear multiple quantum coherence (HMQC) experiment correlated all proton resonances with those of the corresponding carbons in each sugar unit; and all the sugars were found to be β-D-glucose. The significant deshielding of C-4′ of the glucose (δ 4.92 t, J=9.4 Hz) and the heteronuclear multiple bond correlation (HMBC) cross-peak between this proton and the carbonyl carbon at 168.59 ppm confirmed that the caffeoyl residue was attached to C-4′ of the inner glucose. A downfield shift of C-2′ (δ 81.45 d)

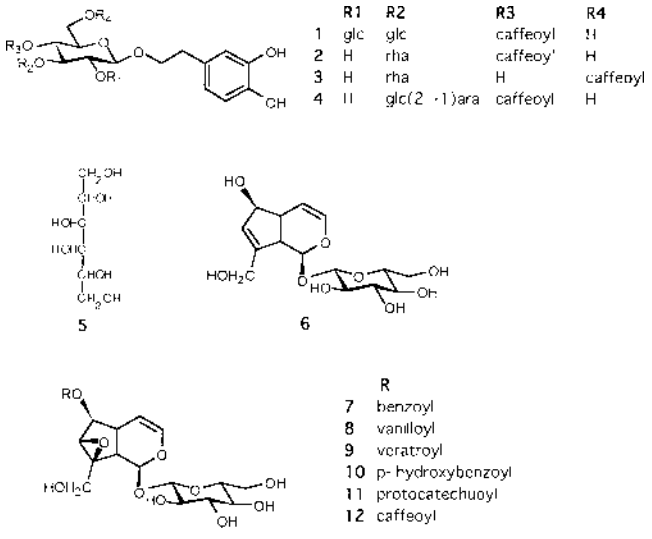

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Table 1. ^{13}C- and ^{1}H-NMR Spectral Data and Significant HMBC Correlations for Persicoside (**1**) (CD_3OD; ^{13}C, 125 MHz; ^{1}H, 500 MHz)

C	DEPT	δ_C	δ_H	J (Hz)	HMBC (C→H)
Aglycon					
1	C	131.69			H-5
2	CH	117.38	6.72 d	(2.2)	H-6
3	C	146.17			H-2, H-5
4	C	144.72			H-2, H-6
5	CH	116.39	6.67 d	(8.0)	H-2
6	CH	121.43	6.58 dd	(8.0/2.2)	H-2, H_2-β
α	CH_2	72.28	3.76 m		H-1′, H_2-β
			4.07 m		
β	CH_2	36.64	2.79 t	(7.4)	H_2-α, H-2, H-6
Glucose-1					
1′	CH	103.33	4.54 d	(7.6)	H_2-α, H-2′
2′	CH	81.45	3.74 d	(9.0)	H-1‴
3′	CH	82.09	4.13 t	(9.3)	H-1″
4′	CH	70.60	4.92 t	(9.4)	H-3′
5′	CH	75.78	3.59 m		
6′	CH_2	62.37	3.54 dd	(12.0/5.5)	
			3.66[a]		
Glucose-2					
1″	CH	104.05	4.73 d	(7.9)	H-3′, H-2″
2″	CH	75.85	3.20 t	(9.0)	
3″	CH	77.83	3.32[a]		
4″	CH	71.58	3.27 t	(9.7)	
5″	CH	78.74	3.42 t	(6.7)	
6″	CH_2	63.23	3.66[a]		
			3.80 dd	(12.0/2.0)	
Glucose-3					
1‴	CH	104.24	4.65 d	(7.6)	H-2′, H-2‴
2‴	CH	75.48	3.09 dd	(9.4/1.5)	
3‴	CH	77.95	3.27 t	(9.7)	
4‴	CH	70.60	3.02 t	(9.3)	
5‴	CH	78.09	3.20 t	(9.0)	
6‴	CH_2	62.84	3.44 dd	(12.0/6.0)	
			3.78 m		
Acyl moiety					
1⁗	C	127.67			H-α′, H-5⁗
2⁗	CH	115.28	7.07 d	(1.8)	H-β′, H-6⁗
3⁗	C	146.92			H-2⁗, H-5⁗
4⁗	C	149.73			H-2⁗, H-5⁗
5⁗	CH	116.57	6.78 d	(8.2)	
6⁗	CH	123.06	6.98 dd	(8.2/1.8)	H-2⁗
α′	CH	115.45	6.34 d	(16.1)	H-β′
β′	CH	147.24	7.56 d	(16.1)	H-α′, H-2⁗, H-6⁗
C=O	C	168.59			H-4′, H-β′

a) Signal patterns are unclear due to overlapping.

and C-3′ (δ 82.09 d) in the ^{13}C-NMR indicated that two other glucose units were terminal and attached to C-2′ and C-3′ of the inner glucose. This suggestion was confirmed by the HMBC experiment at which long-range correlations were observed between the following protons and carbons: H-1′ of inner glucose (δ 4.54, d) and C-α of the aglycon (δ 72.28), H-1″ of glucose-2 (δ 4.73, d) and C-2′ of inner glucose (δ 81.45), H-1‴ of glucose-3 (δ 4.65, d) and C-3′ of inner glucose (δ 82.09). The structure of compound **1** was also confirmed by comparison of the data published for triglycosidic phenylethanoids, which contain three glucose units as sugar moiety, previously isolated from *V. persica*.[12] From the above results, compound **1** was determined as 3,4-dihydroxy-β-phenylethoxy-*O*-[β-D-glucopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→3)]-4-*O*-caffeoyl-β-D-glucopyranoside for which the trivial name Persicoside is proposed.

Phenylethanoid glycosides are natural polyphenol constituents of plants, widely distributed in many dicotyledon families.[24] Many phenylethanoid glycosides were shown to have a wide range of biological properties, including anti-oxidant and anti-tumor effects.[25—28] Recently some phenylethanoids have been reported to be good anti-oxidants and free radical scavengers in some *in vitro* assay models.[29—31] These free radicals play a major role in the pathogenesis of various disorders, including atherosclerosis, cancer, aging, rheumatoid arthritis and inflammation.[32] As shown in Table 2, persicoside and acteoside have potent radical scavenging activity which is more than that of 3-*tert*-butly-4-hydroxyanisole (BHA) and *dl*-α-tocopherol known as natural anti-oxidants. Their IC_{50} values are as follows: persicoside 3.2×10^{-4} M; acteoside 2.4×10^{-4} M; *dl*-α-tocopherol 4.8×10^{-4} M; BHA 6.3×10^{-4} M. Inhibitory activity of acteoside was higher than that of persicoside. This may be due to the substitution of the third sugar moiety at the 2′ position in persicoside. The radical scavenging effects of anti-oxidants on DPPH radical are thought to be due to their hydrogen donating ability.[33,34] Both compounds have four phenolic hydroxyl groups in the structure, and phenolic hydroxyls have been recognized to function as electron or hydrogen donors. Thus, the DPPH radical scavenging activity of these compounds may be mostly related to their phenolic hydroxyl groups.

Table 2. Scavenging Activity of Compounds **1** and **2**, BHA and *dl*-α-Tocopherol on DPPH Radical (1.5×10^{-4} M)

	1×10^{-5} M	1×10^{-4} M	2.5×10^{-4} M	5×10^{-4} M	IC_{50} (M)
Persicoside (**1**)	4.0	12.9	45.6	74.8	3.2×10^{-4}
Acteoside (**2**)	8.4	40.2	62.2	71.2	2.4×10^{-4}
BHA	3.2	13.5	25.5	39.1	6.3×10^{-4}
dl-α-Tocopherol	4.2	17.1	31.6	55.0	4.8×10^{-4}

Each value is the average of duplicate determinations. Inhibitory ratio of each compound is expressed as follow: inhibition %=10×[$Abs_{blank}-Abs_{samp.}$)/Abs_{blank}). Blank: in the absence of sample.

Experimental

General Experimental Procedures Optical rotations were measured on a JASCO DIP 140 digital spectrometer using a sodium lamp operating at 589 nm. UV (λ_{max}) was 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 a NBA matrix in the positive ion mode on a JEOL JMS-DX300 spectrometer. TLC plates Silica gel 60 F_{254} and RP18 F_{254s} were obtained from Merck (Darmstadt, Germany). DPPH was purchased from Aldrich Chem. Co. (Milw., WI, U.S.A.). BHA and *dl*-α-tocopherol were obtained from Nacalai Tesque Co. (Kyoto, Japan).

Plant Material *Veronica persica* POIRET was collected from the central campus of Hacettepe University, Ankara-Turkey. A voucher specimen (HUEF 99015) has been deposited in the herbarium of the Faculty of Pharmacy, Hacettepe University.

Extraction and Isolation The air-dried aerial parts of the plant (220 g) were extracted twice with 2 l of MeOH at 40 °C for 12 h. The MeOH solution was evaporated under vacuum to give MeOH extract (35.5 g), this extract was dissolved in 0.1 l of H_2O and H_2O insoluble material was removed by filtration. The filtrate was fractionated with $CHCl_3$ (×5, 100 ml) to give 18.3 g of water fraction and 8.7 g of $CHCl_3$ fraction. The water fraction (16 g) was subjected to polyamide column chromatography eluting with H_2O, followed by increasing concentrations of MeOH to yield seven fractions: Frs. A–G (Fr. A, 12 g; Fr. B, 0.21 g; Fr. C, 0.85 g; Fr. D, 2.23 g; Fr. E, 0.15 g; Fr. F, 0.59 g; Fr. G, 0.85 g). Aliquots of Fr. A (500 mg) and Fr. C (150 mg) were chromatographed over silica gel separately by stepwise elution with $CHCl_3$: MeOH : H_2O (90 : 10 : 1→60 : 40 : 4) to give compounds **5**—**8**. Fraction D (200 mg) was subjected to silica gel column chromatography eluting

with $CHCl_3$: MeOH (95 : 5→70 : 30) to yield compounds **9**—**11**. Fraction D (45 mg) was also chromatographed over medium pressure liquid chromatography (MPLC) using reversed-phase material (Lichroprep RP-18, 40—63 μm) eluting with increasing amounts of MeOH (30→50%) to give compound **1**. Compounds **12** and **2** from Fr. E and compounds **3** and **4** from Fr. F were isolated using RP-MPLC (20→60%). Fraction G, rich in flavonoid glycosides, is still under investigation.

Persicoside (**1**): Amorphous powder, $[\alpha]_D^{23}$ −63° (c=0.17, EtOH). UV λ_{max} (H_2O) nm (log ε): 344 (sh, 3.3), 325 (3.1) and 304 (sh, 3.1). ^{1}H- and ^{13}C-NMR: see Table 1. FAB-MS *m/z*: 825 $[M+Na]^+$. HR-FAB-MS *m/z*: 825.2671 (Calcd for $C_{35}H_{46}O_{21}Na$: 825.2637).

Measurement of DPPH Radical Scavenging Activity Each EtOH solution (100 μl) of compounds **1** and **2** at various concentrations was added to 1.5×10^{-5} M DPPH/EtOH solution. The reaction mixture was shaken vigorously and the absorbance of remaining DPPH was measured at 520 nm after 30 min. The radical scavenging activity was determined by subtracting the absorbance with that of blank (100%) containing only DPPH and solvent. BHA and *dl*-α-tocopherol were used as standards and samples were prepared using the same dilution procedures.[35,36]

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