Phenylvaleric Acid and Flavonoid Glycosides from *Polygonum salicifolium*

Ihsan Calis,*,† Ayse Kuruüzüm,† L. Ömür Demirezer,† Otto Sticher,‡ Walter Ganci,§ and Peter Rüedi§

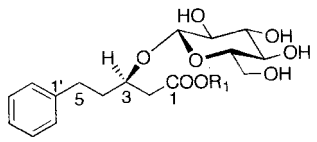
Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, TR-061000 Ankara, Turkey, Swiss Federal Institute of Technology (ETH) Zurich, Department of Pharmacy, CH-8057 Zurich, Switzerland, and University of Zurich, Institute of Organic Chemistry, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

Received February 22, 1999

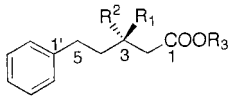
(3*R*)-*O*-*β*-D-Glucopyranosyloxy-5-phenylvaleric acid (**1**), (3*R*)-*O*-*β*-D-glucopyranosyloxy-5-phenylvaleric acid *n*-butyl ester (**2**), and a new dihydrochalcone diglycoside 4′-*O*-[*β*-D-glucopyranosyl-(1→6)-glucopyranosyl]-oxy-2′-hydroxy-3′,6′-dimethoxydihydrochalcone (**3**), together with six known flavonoid glycosides [kaempferol-3-*O*-*β*-D-glucopyranoside (= astragalin) (**4**), kaempferol-3-*O*-*β*-D-galactopyranoside (**5**), quercetin-3-*O*-*β*-D-glucopyranoside (= isoquercitrin) (**6**), quercetin-3-*O*-*β*-D-galactopyranoside (= hyperoside) (**7**), quercetin-3-*O*-(2″-*O*-galloyl)-*β*-D-glucopyranoside (**8**), and quercetin-3-*O*-*β*-D-glucuronopyranoside (**9**)] were isolated from the aerial parts of *Polygonum salicifolium*. The structure elucidation of the isolated compounds was performed by spectroscopic (UV, IR, ESI-MS, 1D- and 2D-NMR), chemical (methylation, enzymatic hydrolysis, partial synthesis), and chromatographic methods (HPLC, Chiralcel OD). The flavonoid glycosides (**4**−**9**) demonstrated scavenging properties toward the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in TLC autographic assays.

The genus *Polygonum* (Polygonaceae) is represented by thirty-three species in the flora of Turkey.[1,2] Some of them are used in traditional medicine against kidney stones and as antidiabetic, diuretic, and antidiarrhoeal agents.[3] Flavonoids,[4] chalcones,[5−8] anthraquinones,[9] naphthoquinone,[9] sesquiterpenoids,[10] lignans,[11] coumarins,[12] stilbene glycoside,[13] and acetophenone glycosides[14] are some of the secondary metabolites isolated from *Polygonum* species. There is only one paper reported on *Polygonum salicifolium,* showing the presence of kaempferol-7-*O*-rhamnoglucoside, quercetin-7-*O*-galactoside, orobol-7-*O*-glucoside, and isorhamnetin-3-*O*-galactoside in the leaves, stems, and flowers of *P. salicifolium*.[15] We now report on the isolation and structure elucidation of the novel compounds **1**−**3**, in addition to six known flavonoid glycosides (**4**−**9**) from the aerial parts *of P. salicifolium* Brouss. ex Willd (Chart 1).

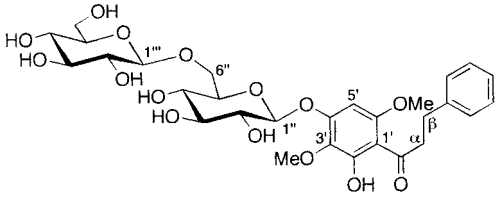
Chart 1



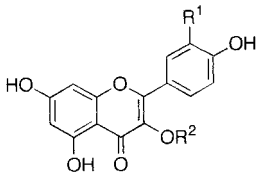
1 R = H
2 R = C_4H_9



1a R^1 = *O*-β-D-glucopyranose, R^2 = H, R^3 = CH_3
1b R^1 = OH, R^2 = R^3 = H
1c R^1 = OH, R^2 = H, R^3 = CH_3
ent-**1c** R^1 = H, R^2 = OH, R^3 = CH_3



3



4 R^1 = H, R^2 = β-D-glucopyranose
5 R^1 = H, R^2 = β-D-galactopyranose
6 R^1 = OH, R^2 = β-D-glucopyranose
7 R^1 = OH, R^2 = β-D-galactopyranose
8 R^1 = OH, R^2 = β-D-(2″-*O*-galloyl)-glucopyranose
9 R^1 = OH, R^2 = β-D-glucuronopyranose

Results and Discussion

The methanolic extract of the aerial parts of *P. salicifolium* was concentrated and suspended in water and partitioned with solvents of increasing polarity (*n*-hexane, diethyl ether, ethyl acetate, and *n*-butanol). The *n*-BuOH residue was fractionated on polyamide and repeated column chromatography (silica gel, RP-18, Sephadex LH-20) to yield compounds **1**−**9**.

Compound **1** was obtained as a colorless powder, $[\alpha]^{23}_D$ −7.5° (*c* 0.22, MeOH). The molecular formula of **1** was determined to be $C_{17}H_{24}O_8$ on the basis of negative-ion ESIMS (*m*/*z* 355 $[M - H]^-$, 711 $[2M - H]^-$). In the UV spectrum of **1**, the maximum bands are at 268, 261, 252, and 248 nm. Its IR spectrum showed absorption bands due to hydroxy (3369 cm^{-1}), carboxyl (1713 cm^{-1}), aromatic (1567 cm^{-1}), and ether (1077 and 1033 cm^{-1}) groups. Sixteen carbons comprising six aromatic carbons, one hydroxymethine, three methylenes (except for the −COOH carbon due to an aglycone), and six carbons due to glucopyranose were observed in the ^{13}C NMR spectrum of **1** (Table 1). The ^1H NMR and DQF−COSY spectrum of **1** revealed the presence of a monosubstituted benzene ring [*δ* 7.22 (4H, m, H-2′,3′,5′,6′), 7.12 (1 H, m, H-4′)] and a

* To whom correspondence should be addressed. Fax: +90 312 3114777. E-mail: acalis@dominet.in.com.tr.
† Hacettepe University.
‡ Swiss Federal Institute of Technology.
§ University of Zurich.

10.1021/np9900674 CCC: $18.00
Published on Web 07/14/1999

Table 1. ^{13}C and ^{1}H NMR Spectral Data for Compounds **1**, **1a**, **1b**, and **2** (^{13}C, 75.5 MHz; ^{1}H, 300 MHz, MeOD) and HMBC Correlations for **1**[a]

		1			1a	1b		2
position	C atom	δ_C (ppm)	δ_H (ppm), *J* (Hz)	HMBC (from C to H)	δ_H (ppm), *J* (Hz)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm), *J* (Hz)
1	C	181.7		H-2, H-3			173.7	
2	CH_2	45.2	2.41 dd (14.7, 5.5)	H-3, H-4	2.56 dd (15.5, 5.7)	2.66 m	42.7	2.41 dd (15.3, 6.0)
			2.64 dd(14.7, 7.0)		2.80 dd (15.5, 6.8)	2.79 m		2.81 dd (15.3, 6.5)
3	CH	78.3	4.17 m	H-1″, H-2, H-4	4.11 m	4.22 m	78.0	4.08[b]
4	CH_2	37.9	1.92 m	H-2, H-5	1.89 m	1.78 m	37.9	1.90 m
5	CH_2	32.4	2.78 m	H-3, H-4, H-2′, H-6′	2.79 m	2.44 m	32.1	2.80[b]
$COOCH_3$					3.66 s			
1′	C	143.0		H-5			143.3	
2′	CH	129.3	7.22 m	H-3′, H-4′, H-5	7.23 m	7.20 m	129.5	7.23 m
3′	CH	129.5	7.22 m	H-4′	7.23 m	7.20 m	129.3	7.23 m
4′	CH	126.7	7.12 m	H-2′, H-3′, H-5′, H-6′	7.15 m	7.20 m	126.7	7.17 m
5′	CH	129.5	7.22 m	H-4′	7.23 m	7.20 m	129.3	7.23 m
6′	CH	129.3	7.22 m	H-4′	7.23 m	7.20 m	129.5	7.23 m
glucose								
1″	CH	103.8	4.41 d (7.7)	H-3, H-5″	4.34 d (7.8)		104.4	4.35 d (7.8)
2″	CH	75.2	3.22[b]	H-3″	3.18 dd (7.8, 8.5)		75.2	3.18 dd (7.8, 8.8)
3″	CH	78.1	3.42[b]	H-5″	3.32[b]		77.6	3.36 t (8.8)
4″	CH	71.6	3.30[b]	H-3″	3.27[b]		71.7	3.28[b]
5″	CH	77.9	3.28[b]		3.19[b]		77.4	3.22[b]
6″	CH_2	62.8	3.86 dd (12.0, 2.0)		3.80 dd (11.8, 2.0)		62.9	3.81 dd (12.0, 2.0)
			3.66 dd (12.0, 4.5)		3.60 dd (11.8, 5.0)			3.63 dd (12.0, 5.5)
butyl								
1‴	CH_2						65.5	4.08 m
2‴	CH_2						20.1	1.60 m
3‴	CH_2						31.7	1.38 m
4‴	CH_3						14.0	0.94 m

[a] The assignments were based on DEPT, DQF-COSY, HMQC, and HMBC. [b] Signal patterns are unclear due to overlapping.

β-glucopyranosyl moiety [*δ* 4.41 (d, *J* =7.7 Hz)]. On the other hand, three sets of methylene protons [*δ* 1.92 (2H, m, H-4], 2.41 (1H, dd, *J* = 4.7, 5.5 Hz, H-2a), 2.64 (1 H, dd, *J* = 14.7, 7.0 Hz, H-2b), 2.78 (2H, m, H-5)] and one oxymethine proton at *δ* 4.17 (1H, m, H-3) were successively coupled. The ^{13}C NMR signals of **1** were assigned with the help of an HMQC experiment, establishing direct C−H bonding. The connectivities of the molecular fragments were established by a heteronuclear multiple bond correlation experiment (HMBC), where the long-range correlations were observed between C-1, H-2′; C-1, H-3; C-1′, H-5; C-5, H-2′; and C-5, H-6′. This experiment also clarified the site of glycosidation showing a long-range correlation between the anomeric proton of glucose at δ_H 4.41 (d, *J* = 7.7 Hz, H-1″) and the oxygenated carbon atom at δ_C 78.3 (C-3).

Methylation of **1** with CH_2N_2 yielded the ester **1a**. The ^{1}H NMR spectrum of **1a** revealed a methyl resonance (3H) at *δ* 3.66 arising from the carbomethoxy group. This result supported that the presence of a free carboxyl group in **1**. Thus, the constitution of **1** was determined to be 3-*O*-*β*-glucopyranosyloxy-5-phenylvaleric acid. Such a compound has been isolated recently from *Perilla frutescens* (Labiatae); however, its absolute configuration remained open.[16]

The absolute configuration at C-3 of **1** was determined as follows.[17] Enzymatic hydrolysis with *β*-glucosidase yielded 3-hydroxyphenylvaleric acid (**1b**) (^{1}H NMR data, see Table 1). Esterification of **1b** with CH_2N_2 (Et_2O) yielded the hydroxy-ester **1c** which was identified unambiguously as the (*R*)-enantiomer by HPLC (Chiralcel OD, hexane/2-propanol 9:1, *k*′ = 2.66, ee > 99%). The respective reference compounds **1c** ((+)-(*R*), *k*′ = 2.66), *ent*-**1c** ((−)-(*S*), *k*′ = 2.24) have been prepared by reduction of methyl 3-oxo-5-phenylvaleroate with $NaBH_4$ to yield the racemic mixture (*k*′ = 2.24 and 2.66) and enantioselective hydrogenation[18] with (*R*)-BINAP−Ru/H_2 afforded **1c** and (*S*)-BINAP−Ru/H_2 yielded *ent*-**1c**.[17] Moreover, the substrate specificity of *β*-glucosidase confirms the expected D-series of the glucose moiety. Consequently, the structure of **1** was established as (3*R*)-*O*-*β*-D-glucopyranosyloxy-5-phenylvaleric acid.

The molecular formula of compound **2** ($[\alpha]^{23}_D$ −10.5° (*c* 0.22, MeOH)) was determined to be $C_{21}H_{32}O_8$ on the basis of positive ion ESIMS (*m*/*z* 435 $[M + Na]^+$, 847 $[2M + Na]^+$). In the UV spectrum of **2**, the maximum bands were observed at 268, 259, 253, and 247 nm. The IR spectrum showed absorption bands due to hydroxy (3402 cm^{-1}), ester (1732 cm^{-1}), aromatic (1567 cm^{-1}), and ether groups (1078 and 1033 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) were similar to those of **1** except for the presence of signals due to a butyl moiety. A COSY experiment showed the proton resonances in the same spin system which were assigned to a *n*-butyl moiety: *δ* 0.94 (t, 3H *J* = 7.3 Hz, Me-4‴); 1.38 (m, 2H, H_2-3‴); 1.60 (m, 2H, H_2-2‴); 4.08 (t, 2H, *J* = 6.6 Hz, H_2-1‴). The HMQC experiment correlated all proton resonances with those of the corresponding carbons of the butyl moiety (*δ* 65.5 t, 20.1 t, 31.7 t, and 14.0 q; C-1‴−C-4‴, respectively). The information concerning the linkage of the *n*-butyl moiety was obtained from the HMBC spectrum. The long-range correlations were observed from the following pairs: C-1 (*δ* 173.7)/H_2-1‴ (*δ* 4.08) and C-1/H_2-2 (*δ* 2.81 and 2.55). Furthermore, the long-range correlations between C-3 (*δ* 78.0) and H-1″ (*δ* 4.35), and C-1′ (*δ* 143.3) and H_2-5 (*δ* 2.80) supported the proposed assignments. Therefore, the structure of **2** was established to be (3*R*)-*O*-*β*-D-glucopyranosyloxy-5-phenylvaleric acid butyl ester. Since we used *n*-BuOH during the extraction procedure, compound **2** is considered to be an artifact.

Compound **3** was obtained as an amorphous, pale yellow powder, $[\alpha]^{23}_D$ −20.0° (*c* 0.12, MeOH). The elemental formula of **3** was determined as $C_{29}H_{38}O_5$ by negative and positive ion ESIMS (*m*/*z* 625 $[M - H]^-$, and 649 $[M + Na]^+$, respectively), ^{1}H and ^{13}C NMR spectral data. The complete ^{1}H and ^{13}C connectivity was established by extensive use and interpretation of 2D ($^{1}H-^{1}H$) COSY, HMQC (one-bond $^{13}C-^{1}H$ correlation), and HMBC (long range $^{13}C-^{1}H$ correlation) NMR spectra. This provided unequivocally the atomic network for **3** (Table 2). The UV spectrum of **3** showed absorption bands at 333, 287, 233 (sh), and 208 nm. The IR spectrum of **3** showed absorbances for hydroxy

Table 2. ^{13}C and ^{1}H NMR Spectral Data for Compound **3** (^{13}C, 75.5 MHz; ^{1}H, 300 MHz, MeOD)[a]

position	C atom	δ_C (ppm)	δ_H (ppm), *J* (Hz)	HMBC (form C to H)
1	C	142.9		H-2, H-6, H_2-α, H_2-*β*
2	CH	129.4	7.20 m	H_2-*β*
3	CH	129.4	7.20 m	
4	CH	127.0	7.14 m	H-3, H-5
5	CH	129.4	7.20 m	
6	CH	129.4	7.20 m	H_2-*β*
α	CH_2	47.4	3.31[b]	
β	CH_2	31.7	2.96 t (7.8)	
C═O	C	207.0		H_2-α, H_2-*β*
1′	C	108.3		H-5′
2′	C	162.8		
3′	C	132.3		H-5′, OMe (C-3′)
4′	C	157.3		H-1″, H−5′
5′	CH	91.3	6.37 s	
6′	C	159.9		OMe (C-6′), H−5′
OMe (C-3′)	CH_3	61.5	3.79 s	
OMe (C-6′)	CH_3	56.8	3.90 s	
glucose				
1″	CH	101.1	5.07 d (7.2)	
2″	CH	74.7	3.52[b]	
3″	CH	78.1	3.30[b]	
4″	CH	71.3	3.38[b]	
5″	CH	77.6	3.77[b]	
6″	CH_2	70.3	4.16 br d (11.8)	H-1‴
			3.80[b]	
terminal glucose				
1‴	CH	105.1	4.31 d (7.7)	H_2-6″
2‴	CH	75.1	3.16 dd (7.7, 8.5)	
3‴	CH	78.0	3.22[b]	
4‴	CH	71.5	3.24[b]	
5‴	CH	77.8	3.50[b]	
6‴	CH_2	62.7	3.82 dd (11.8, 2.0)	
			3.63 dd (11.8, 5.2)	

[a] The assignments were based on DEPT, DQF-COSY, HMQC, and HMBC. [b] Signal patterns are unclear due to overlapping.

(3436 and 3400 cm^{-1}), a conjugated ketone (1625 cm^{-1}), aromatic (1457 and 1420 cm^{-1}), and ether groups (1068 and 1053 cm^{-1}). The ^{1}H NMR spectrum displayed the characteristic ABX pattern of a monosubstituted benzyl moiety, five proton multiplets (*δ* 7.20−7.14, m, H-2,3,4,5,6) characteristic of an unsubstituted B ring, two methoxy resonances at *δ* 3.90 and 3.79, an aromatic proton at *δ* 6.37 (1H, s, H-5′), and two methylene signals linked to each other at *δ* 3.31 (H_2-α) and 2.96 (H_2-*β*) (each 2H, the former overlapped, the latter t, $J = 7.8$ Hz). Additionally, two anomeric proton resonances were observed at *δ* 5.07 (d, $J = 7.2$ Hz) and 4.31 (d, $J = 7.7$ Hz), indicating its diglycosidic dihydrochalcone structure. The ^{13}C NMR spectrum of **3** exhibited 29 carbon resonances: 7 quaternary carbons (C), 16 methine (CH), 4 methylene (CH_2), and 2 methyl (CH_3). The ^{1}H and ^{13}C NMR spectral data together with ESIMS supported the presence of two hexose units in **3**. From the chemical shift values and the coupling constants of the anomeric protons, their glycosidations were found to be on a phenolic and an aliphatic hydroxyl group, respectively, and *β*-linkages for both. The remaining carbon resonances for the aglycone moiety supported that the aglycone moiety of **3** has a pentasubstituted A ring as in dihydropashanone[19] except for the different substitution pattern, two methoxy and two hydroxy groups of which one of the latters was glycosylated with a disaccharide. The placement of all substituents on the A ring was established using 2D $^{13}C-^{1}H$ long-range correlation (HMBC) and ROESY NMR experiments. The long-range correlation between the quaternary carbon atom at *δ* 157.3 (C-4′) and the anomeric proton (*δ* 5.07) of the inner glucose moiety showed the site of glycosidation. In addition, to the carbon resonance assigned as C-4′ (*δ* 157.3), the resonances at *δ* 108.3 (C-1′), 132.3 (C-3′), 159.9 (C-6′) showed also the long-range correlations to the aromatic proton at *δ* 6.37 (H-5′).

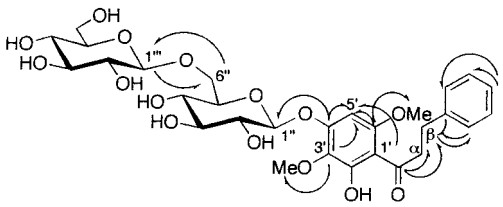


Figure 1. Heteronuclear multiple-bond correlations for **3**. Arrows point from carbon to proton.

The carbon resonances at *δ* 132.3 (C-3′) and 159.9 (C-6′) exhibited long-range correlations to the methoxy signals at *δ* 3.79 and 3.90, respectively, showing the site of these groups on the A ring. The other significant correlations are shown on Figure 1. Additionally, in the ROESY experiment, the anomeric proton (H-1″) of the inner glucose and the methoxyl signal (δ_H 3.90) located at C-6′ exhibited correlations to the aromatic proton at *δ* 6.37 (H-5′), supporting the proposed substitution in the A ring. Apart from the anomeric protons, 2D NMR experiments (COSY and HMQC) indicated the presence of two glucose units. A HMBC experiment performed with **3**, showed a correlation between the anomeric proton at *δ* 4.31 (H-1‴) and the carbon resonans at *δ* 70.3 (CH_2, H-6″), indicating the presence of a 6-*O*-*β*-D-glucopyranosyl-glucose moiety as the disaccharidic sugar chain. Reversed correlations between the anomeric carbon of the terminal glucose moiety at *δ* 105.1 (C-1‴) and the methylene protons of the inner glucose moiety at *δ* 4.16 and 3.80 (H_2-6″) supported this proposal. The negative and positive ion ESI mass spectra of **3** exhibited ions at *m*/*z* 301 [aglycone ($C_{17}H_{18}O_5$) − H]$^-$ and 303 [aglycone ($C_{17}H_{18}O_5$) + H]$^+$ were in good agreement for the proposed structure of the dihydrochalcone moiety. Consequently, the structure of compound **3** was established as 4′-*O*-[*β*-D-glucopyranosyl-(1→6)-glucopyranosyl]oxy-2′-

hydroxy-3′,6′-dimethoxydihydrochalcone for which salicifolioside A is proposed as the trivial name.

The presence of dihydrochalcones as well as their glycosides in nature are very rare. Although there are some studies reporting methoxylated β-hydroxychalcone derivatives from *Polygonum nepalense*,[5] dihydrochalcone and chalcone derivatives from *P. lapathifolium*,[6,7] there is only one report on dihydrochalcone monoglycosides from *Polygonum* species, *Polygonum senegalense*.[8]

The structures of the six known flavonoids, kaempferol-3-*O*-β-D-glucopyranoside (= astragalin) (**4**),[20] kaempferol-3-*O*-β-D-galactopyranoside (**5**),[21] quercetin-3-*O*-β-D-glucopyranoside (=isoquercitrin) (**6**),[20] quercetin-3-*O*-β-D-galactopyranoside (= hyperoside) (**7**),[20] quercetin-3-*O*-(2″-*O*-galloyl)-β-D-glucopyranoside (**8**),[22] and quercetin-3-*O*-β-D-glucuronopyranoside (**9**),[23] were identified on the basis of comparison of their spectroscopic (NMR, FABMS) data in comparison with literature values.

Radical-scavenging properties of the compounds (**1**−**9**) were evaluated against the DPPH radical.[24,25] By using DPPH as a TLC spray reagent, compounds **4**−**9** (2, 4, 6, 8 μg) appeared as yellow spots against a purple background, while compounds **1**−**3** did not react with the radical. Compounds **6**−**9**, the quercetin glycosides, were more active in all concentrations applied, while the kaempferol glycosides **4** and **5** showed lower activity. These results indicate that *ortho*-hydroxyl groups are an essential feature for the antioxidant properties of the flavonoid type compounds.

Experimental Section

General Experimental Procedures. UV spectra were determined in spectroscopic grade MeOH on a Shimadzu UV-160A spectrophotometer. IR spectra were determined on a Perkin-Elmer 2000 FT-IR spectrometer as pressed KBr disks. NMR spectra were recorded using a Bruker AMX300 instrument at 300 MHz for ^{1}H and 75.5 MHz for ^{13}C. Complete proton and carbon assignments are based on 1D (^{1}H, ^{13}C, and DEPT) and 2D (^{1}H−^{1}H COSY, ^{1}H−^{13}C HMQC, and ^{1}H−^{13}C HMBC) NMR experiments. ESI-MS was recorded on Hitachi-Perkin-Elmer-RMUGM mass spectrometer. TLC was carried out on precoated silica gel 60F-254 aluminum sheets (Merck). For column chromatography (CC), normal phase silica gel 60 (0.063−0.200 mm, Merck), reversed phase silica gel (LiChroprep RP-18, Merck), Sephadex LH-20 (Fluka), and polyamide (Polyamid-MN−Polyamid SC 6, Macherey-Nagel, Düren) were used. Compounds were detected by UV fluorescence and/or spraying with vanillin−H_2SO_4 reagent followed by heating at 100 °C for 5−10 min and/or exposure to NH_3 vapor. For enzymatic hydrolysis, β-glucosidase from almonds (Emulsin, Fluka, Nr. 49289) was used. HPLC analyses of the (3*R*)- and (3*S*)-isomers of 3-hydroxyphenylvaleric acid were performed on Chiralcel OD (250 × 4.6 mm) using hexane/2-propanol (9:1) as eluent, flow rate 1 mL/min. For the radical-scavenging TLC autographic assays, 2,2-diphenyl-1-picrylhydrazyl (DPPH, Fluka) was used as spray reagent.

Plant Material. *P. salicifolium* Brouss. ex Willd. was collected from Trabzon-Uzungöl (North Anatolia) in July, 1994. A voucher specimen has been deposited in the Herbarium of Pharmaceutical Botany, Faculty of Pharmacy, Hacettepe University (HUEF 94-105).

Extraction and Isolation. The dried powdered aerial parts of *P. salicifolium* (300 g) were extracted twice with MeOH (2 × 3.5 L) at 40 °C. The MeOH extracts were combined and evaporated to dryness *in vacuo*. The crude extract (42 g) was suspended in water and partitioned with *n*-hexane, diethyl ether, ethyl acetate, and *n*-butanol, respectively. The *n*-BuOH extract (7 g) was chromatographed over polyamide (100 g), eluting with H_2O (200 mL), followed by increasing concentrations of MeOH in H_2O (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% MeOH; each mixture 200 mL; fraction volume 100 mL) to yield 22 fractions which were combined into seven main fractions (A−G).

The fraction eluted with 10% MeOH (fraction A, 2.1 g) was chromatographed over CC using normal-phase silica gel (200 g) as stationary phase eluting with $CHCl_3$/MeOH mixtures, 80:20 (200 mL), 70:30 (200 mL), 60:40 (200 mL), 50:50 (200 mL), 40:60 (200 mL), 30:70 (200 mL), and 10:90 (500 mL) to give 17 fractions (100 mL/fraction) which were combined to seven main groups on the basis of their TLC profiles. The fractions eluted with 60% MeOH in $CHCl_3$ was rechromatographed using MPLC (column dimensions 18.5 × 352 mm, LiChroprep RP-18) eluting with increasing amounts of MeOH in H_2O (H_2O, 200 mL; 10% MeOH, 200 mL; 20% MeOH, 200 mL; 30% MeOH, 200 mL; 40% MeOH, 200 mL; MeOH, 200 mL) to give 80 fractions (15 mL/fraction). Fractions 31−41 gave compound **1** (177 mg). The fractions eluted with 20−30% MeOH (fraction B, 320 mg) were purified by repeated open CC (normal-phase silica gel) using $CHCl_3$/MeOH (90:10) solvent system to yield compound **2** (31 mg). The fraction eluted with 40−50% MeOH (fraction C, 178 mg) was chromatographed on a normal-phase silica gel column (20 g) eluting with EtOAc (500 mL); EtOAc/MeOH (100:5; 500 mL); EtOAc/MeOH/H_2O (100:5:1; 400 mL); and MeOH (100 mL), respectively (10−15 mL/fraction). The combined fractions 51−91 (54 mg) were further fractionated by normal phase silica gel (10 g) CC using $CHCl_3$/MeOH (90:10; 400 mL; 8 mL/fraction) to give compound **3** (fractions 26−52; 17 mg).

Fraction E eluted with 70% MeOH (607 mg) was repeatedly chromatographed over Sephadex LH-20 open CC using MeOH and normal-phase silica gel open CC using $CHCl_3$/MeOH/H_2O mixtures (90:10:1, 80:20:2, 70:30:3) to yield four flavonoid glycosides (**4**−**7**). Chromatography of fraction G eluted with 90% MeOH (604 mg) using similar conditions yielded compounds **8** and **9**.

(3*R*)-*O*-β-D-Glucopyranosyloxy-5-phenylvaleric acid (1): colorless powder; $[\alpha]^{23}_D$ −7.5° (*c* 0.22, MeOH); UV (MeOH) λ_{max} 248, 252, 261, 268 nm; IR (KBr) ν_{max} 1033, 1077, 1567, 1713, 3369 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) see Table 1; ^{13}C NMR (CD_3OD, 75.5 MHz) see Table 1; ESIMS *m*/*z* 355 $[M - H]^-$, 711 $[2M - H]^-$.

Methylation of 1. Compound **1** (10 mg) was treated with CH_2N_2/Et_2O to yield the ester **1a** (6 mg) which was purified on silica gel (10 g) using $CHCl_3$−MeOH−H_2O (80:20:2) as eluent (3 mL/fraction). ^{1}H NMR data, see Table 1.

Enzymatic Hydrolysis and Determination of the Absolute Configuration of 1. A solution of **1** (9 mg) in acetate buffer (pH 4.4, 10 mL) was treated with β-glucosidase (20 mg), and the solution was left at 37 °C for 48 h. The reaction solution was evaporated to dryness, and the residue was chromatographed on silica gel (10 g), using CH_2Cl_2/MeOH/H_2O (90:10:1) to afford **1b** (4 mg). ^{1}H NMR data, see Table 1.

The hydroxy-acid **1b** was methylated with CH_2N_2/Et_2O and purified on silica gel (hexane/AcOEt 2:1) to yield **1c** (3 mg). HPLC on Chiralcel OD, with hexane/2-propanol (9:1) exhibited only one peak (k' = 2.66, ee >99%).

Enantioselective hydrogenation of methyl 3-oxo-5-phenylvaleroate (188 mg) with (*R*)-BINAP−Ru/H_2 (30 bar, 100 °C, 24 h) in ethanol yielded after usual workup and chromatography on silica gel (hexane/Et_2O 2:1) **1c** (170 mg), $[\alpha]^{23}_D$ = +1.5° (*c* 2.9, CH_2Cl_2). HPLC on Chiralcel OD as above eluted the major enantiomer at k' = 2.66 and the minor *ent*-**1c** at k' = 2.24 (ee = 72%). For a full account on these transformations that unambiguously established the absolute configuration, see ref 17.

(3*R*)-*O*-β-D-Glucopyranosyloxy-5-phenylvaleric acid *n*-butyl ester (2): $[\alpha]^{23}_D$ −10.5° (*c* 0.22, MeOH); UV (MeOH) λ_{max} 247, 253, 259, 268 nm; IR (KBr) ν_{max} 1033, 1078, 1567, 1713, 3402 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) see Table 1; ^{13}C NMR (CD_3OD, 75.5 MHz) see Table 1; ESIMS *m*/*z* 435 $[M + Na]^+$, 847 $[2M + Na]^+$.

Salicifolioside A (3): amorphous, pale yellow powder; $[\alpha]^{23}_D$ −20.0° (*c* 0.12, MeOH); UV (MeOH) λ_{max} 208, 233 (sh), 287, 333 nm; IR (KBr) ν_{max} 1053, 1068, 1420, 1457, 1625, 3400, 3436 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) and ^{13}C NMR (CD_3OD, 75 MHz), see Table 2; Negative ion ESIMS *m*/*z* 625 $[M - H]^-$,

301 [aglycone ($C_{17}H_{18}O_5$) − H]$^-$; Positive ion ESIMS *m*/*z* 649 [M + Na]$^+$, 303 [aglycone ($C_{17}H_{18}O_5$) + H]$^+$.

Kaempferol-3-*O*-*β*-D-glucopyranoside (= astragalin) (4): ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz) data superimposable with those reported in the literature.[20]

Kaempferol-3-*O*-*β*-D-galactopyranoside (5): ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz) data superimposable with those reported in the literature.[21]

Quercetin-3-*O*-*β*-D-glucopyranoside (= isoquercitrin) (6): ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz) data superimposable with those reported in the literature.[20]

Quercetin-3-*O*-*β*-D-galactopyranoside (= hyperoside) (7): ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz) data superimposable with those reported in the literature.[20]

Quercetin-3-*O*-(2″-*O*-galloyl)-*β*-D-glucopyranoside (8): ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz) data superimposable with those reported in the literature.[22]

Quercetin-3-*O*-*β*-D-glucuronopyranoside (9): ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz) data superimposable with those reported in the literature.[23]

Reaction of DPPH Radical. TLC Autographic Assay. After developing and drying, TLC plates were sprayed with a 0.2% DPPH solution in MeOH. The plates were examined 30 min after spraying. Active compounds appear as yellow spots against a purple background.[24,25] Quercetin was used as reference compound.

References and Notes

(1) Davis, P. H. *Flora of Turkey and the East Aegean Islands;* University Press: Edinburgh, 1967; Vol. 2.
(2) Davis, P. H. *Flora of Turkey and the East Aegean Islands;* University Press: Edinburgh, 1988; Vol 10.
(3) Baytop, T. *Therapy with Medicinal Plants (Past and Present)*; Istanbul University Publications: Istanbul, 1984; p 313.
(4) Isobe, T.; Noda, Y. *Yakugaku Zasshi* **1987**, *107*, 1001−1004.
(5) Rathore, A.; Sharma, S. C.; Tandon, J. S. *J. Nat. Prod.* **1987**, *50*, 357−359.
(6) Ahmed, M.; Khaleduzzaman, M.; Rashid, M. A. *Phytochemistry* **1988**, *27*, 2359−2360.
(7) Ahmed, M.; Khaleduzzaman, M.; Islam, M. S. *Phytochemistry* **1990**, *20*, 2009−2011.
(8) Maradufu, A.; Ouma, J. H. *Phytochemistry* **1978**, *17*, 823−824.
(9) Kimura, Y.; Kozawa, M.; Baba, K.; Hata, K. *Planta Medica* **1983**, *48*, 164−168.
(10) Fukuyama, Y.; Sato, T.; Miura, I.; Asakawa, Y. *Phytochemistry* **1985**, *24*, 1521−1524.
(11) Kim, H. J.; Woo, E.-R.; Park, H. *J. Nat. Prod.* **1994**, *57*, 581−586.
(12) Petrescu, A. D. *Plant. Med. Phytother.* **1974**, *8*, 224−227.
(13) Layatilake, G. S.; Jayastiriya, H.; Lee, E. S.; Koonchanok, N. M.; Geahlen, R. L.; Ashendel, C. L.; McLaughlin, J. L.; Chang, C. J. *J. Nat. Prod.* **1993**, *56*, 1805−1810.
(14) Yoshizaki, M.; Fujino, H.; Arise, A.; Ohmiira, K.; Arisawa, M.; Morita, N. *Planta Medica* **1987**, *52*, 273−275.
(15) Balbaa, S. I.; Afifi, M. S. *Bull. Pharm. Sci., Assiut Univ.* **1988**, *11*, 176−184.
(16) Fujita, T.; Terato, K.; Nakayama, M. *Biosci. Biotech. Biochem* **1996**, *60*, 732−735.
(17) Calis, I.; Kuruüzüm, A.; Ganci, W.; Rüedi, P. *Tetrahedron Asymmetry* **1999**, *10* (submitted).
(18) Kitamura, M.; Ohkuma, T.; Inoue, S.; Sayo, N.; Kumobayashi, H.; Akutagawa, S.; Ohta, T.; Takaya, H.; Noyori, R. *J. Am. Chem. Soc.* **1988**, *110*, 629−631 and references therein.
(19) Ichino, K.; Tanaka, H.; Ito, K.; Tanaka, T.; Mizuno, M. *J. Nat. Prod.* **1988**, *51*, 915−917.
(20) Markham, K. R.; Geiger, H. In *The flavonoids: Advances in research since 1986*; Harborne, J. B., Ed.; Chapman & Hall: London, 1993; Chapter 10, pp 441−447.
(21) Agrawal, P. K.; Rastogi, R. P. *Heterocycles* **1981**, *16*, 2181−2236.
(22) Kuroyanagi, M.; Yamamoto, Y.; Fukushima, S.; Ueno, A.; Noro, T.; Miyase, T. *Chem. Pharm. Bull.* **1982**, *30*, 1602−1608.
(23) Nawwar, M. A. M.; Souleman, A. M. A.; Buddrus, J.; Linscheid, M. *Phytochemistry* **1984**, *23*, 2347−2349.
(24) Takao, T.; Kitatani, F.; Watanabe, N.; Yagi, A.; Sakata, K. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 1780−1783.
(25) Cuendet, M.; Hostettmann, K.; Potterat, O.; Dyatmiko, W. *Helv. Chim. Acta* **1997**, *80*, 1144−1152.

NP9900674