

Phenylvaleric Acid and Flavonoid Glycosides from *Polygonum salicifolium*

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(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.

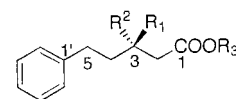
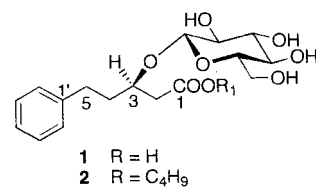
The genus *Polygonum* (Polygonaceae) is represented by thirty-three species in the flora of Turkey.^{1,2} Some of them are used in traditional medicine against kidney stones and as antidiabetic, diuretic, and antidiarrhoeal agents.³ Flavonoids,⁴ chalcones,^{5–8} anthraquinones,⁹ naphthoquinone,⁹ sesquiterpenoids,¹⁰ lignans,¹¹ coumarins,¹² stilbene glycoside,¹³ and acetophenone glycosides¹⁴ 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*-rhamnoglycoside, 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).

Results and Discussion

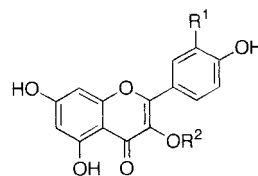
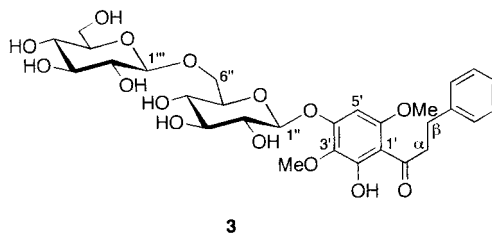
The methanolic extract of the aerial parts of *P. salicifolium* was concentrated and suspended in water and partitioned with solvents of increasing polarity (*n*-hexane, diethyl ether, ethyl acetate, and *n*-butanol). The *n*-BuOH residue was fractionated on polyamide and repeated column chromatography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

Compound **1** was obtained as a colorless powder, [α]_D²³ –7.5° (*c* 0.22, MeOH). The molecular formula of **1** was determined to be C₁₇H₂₄O₈ 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 ¹³C NMR spectrum of **1** (Table 1). The ¹H NMR and DQF-COSY spectrum of **1**

Chart 1



- 1a** R¹ = *O*-β-D-glucopyranose, R² = H, R³ = CH₃
1b R¹ = OH, R² = R³ = H
1c R¹ = OH, R² = H, R³ = CH₃
ent-**1c** R¹ = H, R² = OH, R³ = CH₃



- 4** R¹ = H, R² = β-D-glucopyranose
5 R¹ = H, R² = β-D-galactopyranose
6 R¹ = OH, R² = β-D-glucopyranose
7 R¹ = OH, R² = β-D-galactopyranose
8 R¹ = OH, R² = β-D-(2''-*O*-galloyl)-glucopyranose
9 R¹ = OH, R² = β-D-glucuronopyranose

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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

Table 1. ^{13}C and ^1H NMR Spectral Data for Compounds **1**, **1a**, **1b**, and **2** (^{13}C , 75.5 MHz; ^1H , 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 (1H, 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 ^1H 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**) (^1H 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-phenylvalerate with NaBH_4 to yield the racemic mixture ($k' = 2.24$ and 2.66) and enantioselective hydrogenation¹⁸ 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** ($[\alpha]_{\text{D}}^{25} -10.5^\circ$ (c 0.22, MeOH)) was determined to be $\text{C}_{21}\text{H}_{32}\text{O}_8$ on the basis of positive ion ESIMS (m/z 435 [$\text{M} + \text{Na}]^+$, 847 [$2\text{M} + \text{Na}]^+$). 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 ^1H 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, H₂-3'''); 1.60 (m, 2H, H₂-2'''); 4.08 (t, 2H, $J = 6.6$ Hz, H₂-1'''). 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)/H₂-1''' (δ 4.08) and C-1/H₂-2 (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 H₂-5 (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.

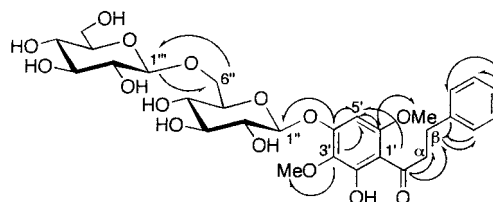
Compound **3** was obtained as an amorphous, pale yellow powder, $[\alpha]_{\text{D}}^{25} -20.0^\circ$ (c 0.12, MeOH). The elemental formula of **3** was determined as $\text{C}_{29}\text{H}_{38}\text{O}_5$ by negative and positive ion ESIMS (m/z 625 [$\text{M} - \text{H}]^-$, and 649 [$\text{M} + \text{Na}]^+$, respectively), ^1H and ^{13}C NMR spectral data. The complete ^1H and ^{13}C connectivity was established by extensive use and interpretation of 2D (^1H – ^1H) COSY, HMQC (one-bond ^{13}C – ^1H correlation), and HMBC (long range ^{13}C – ^1H 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 ^1H NMR Spectral Data for Compound **3** (^{13}C , 75.5 MHz; ^1H , 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) 3.80 ^b	H-1'''
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 ^1H 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 (H₂- α) and 2.96 (H₂- 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 (CH₂), and 2 methyl (CH₃). The ^1H 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 dihydropashanone¹⁹ except for the different substitution pattern, two methoxy and two hydroxy groups of which one of the latter was glycosylated with a disaccharide. The placement of all substituents on the A ring was established using 2D ^{13}C – ^1H 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 resonances at δ 70.3 (CH₂, 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 (H₂-6'') supported this proposal. The negative and positive ion ESI mass spectra of **3** exhibited ions at m/z 301 [aglycone (C₁₇H₁₈O₅) – H][–] and 303 [aglycone (C₁₇H₁₈O₅) + 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 \rightarrow 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** μ g) 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 ¹H and 75.5 MHz for ¹³C. Complete proton and carbon assignments are based on 1D (¹H, ¹³C, and DEPT) and 2D (¹H–¹H COSY, ¹H–¹³C HMQC, and ¹H–¹³C 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–H₂SO₄ reagent followed by heating at 100 °C for 5–10 min and/or exposure to NH₃ vapor. For enzymatic hydrolysis, β -glucosidase from almonds (Emulsin, Fluka, Nr. 49289) was used. HPLC analyses of the (*3R*)- and (*3S*)-isomers of 3-hydroxyphenylvaleric acid were performed on Chiralcel OD (250 \times 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 \times 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 H₂O (200 mL), followed by increasing concentrations of MeOH in H₂O (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 CHCl₃/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 CHCl₃ was rechromatographed using MPLC (column dimensions 18.5 \times 352 mm, LiChroprep RP-18) eluting with increasing amounts of MeOH in H₂O (H₂O, 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 CHCl₃/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/H₂O (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 CHCl₃/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 CHCl₃/MeOH/H₂O 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; [α]_D²³ –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}; ¹H NMR (CD₃OD, 300 MHz) see Table 1; ¹³C NMR (CD₃OD, 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 CH₂N₂/Et₂O to yield the ester **1a** (6 mg) which was purified on silica gel (10 g) using CHCl₃–MeOH–H₂O (80:20:2) as eluent (3 mL/fraction). ¹H 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 CH₂Cl₂/MeOH/H₂O (90:10:1) to afford **1b** (4 mg). ¹H NMR data, see Table 1.

The hydroxy-acid **1b** was methylated with CH₂N₂/Et₂O 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-phenylvalerate (188 mg) with (*R*)-BINAP–Ru/H₂ (30 bar, 100 °C, 24 h) in ethanol yielded after usual workup and chromatography on silica gel (hexane/Et₂O 2:1) **1c** (170 mg), [α]_D²³ = +1.5° (c 2.9, CH₂Cl₂). 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**):** [α]_D²³ –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}; ¹H NMR (CD₃OD, 300 MHz) see Table 1; ¹³C NMR (CD₃OD, 75.5 MHz) see Table 1; ESIMS *m/z* 435 [M + Na]⁺, 847 [2M + Na]⁺.

Salicifolioside A (3**):** amorphous, pale yellow powder; [α]_D²³ –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}; ¹H NMR (CD₃OD, 300 MHz) and ¹³C NMR (CD₃OD, 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): ¹H NMR (300 MHz) and ¹³C NMR (75.5 MHz) data superimposable with those reported in the literature.²⁰

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

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

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

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

Quercetin-3-O-β-D-glucuronopyranoside (9): ¹H NMR (300 MHz) and ¹³C 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

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