Flavonoid, Iridoid, and Lignan Glycosides from *Putoria calabrica*

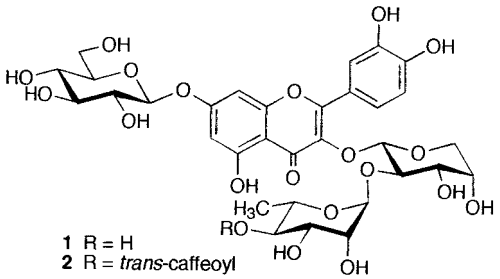
Ihsan Çalis,*,† Jörg Heilmann,‡ Deniz Tasdemir,⊥ Anthony Linden,§ Chris M. Ireland,⊥ and Otto Sticher‡

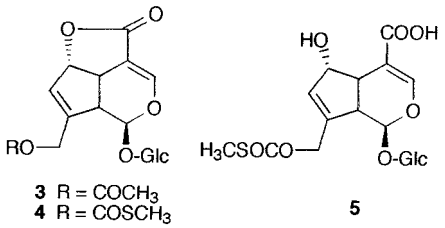
Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara, Turkey, Department of Applied BioSciences, Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology (ETH) Zurich, CH-8057 Zürich, Switzerland, Department of Medicinal Chemistry, University of Utah, Salt Lake City, Utah 84112, and Institute of Organic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Received December 19, 2000

From the aerial parts of *Putoria calabrica*, two new flavonol triglycosides were isolated and their structures were elucidated as quercetin-3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-*O*-β-D-glucopyranoside (**1**, calabricoside A) and quercetin-3-*O*-[4‴-*O*-caffeoyl-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-*O*-β-D-glucopyranoside (**2**, calabricoside B). Additionally, seven iridoid and three lignan glycosides were isolated and characterized. Radical scavenging activities of all compounds were determined by quantifying their effects on luminol-enhanced chemiluminescence in formyl-methionyl-leucyl-phenylalanine (FMLP) stimulated human polymorphonuclear neutrophils (PMNs). Calabricoside A and B showed strong radical scavenging activity with IC_{50} values of 0.25 and 0.3 μM, respectively.

In the flora of Turkey, the genus *Putoria* (Rubiaceae) is represented only by one species, *P. calabrica* (L. fil) DC.[1] Our previous research on this plant has resulted in the isolation of asperuloside (**3**), and its crystal structure has been determined in order to confirm the stereochemistry.[2] Further studies on the same plant material have resulted in the isolation of two new flavonoid glycosides (**1**, **2**), seven known iridoid glycosides, asperuloside, paederoside, deacetylasperuloside, paederosidic acid, asperulosidic acid, geniposidic acid, and scandoside, and three lignan glycosides, liriodendrin, dihydrodehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside, and 7*S*,8*R*,8′*R*-(−)-lariciresinol-4,4′-bis-*O*-β-D-glucopyranoside. All compounds have been tested for their effects on oxygen radical production by human PMNs stimulated with FMLP.





Compound **1** was obtained as an amorphous yellow powder. The positive and negative ion ESI mass spectra of **1** exhibited pseudomolecular ions, $[M + H]^+$, $[M + Na]^+$, and $[M-H]^-$ at *m*/*z* 743, 765, and 741, respectively, which were compatible with the molecular formula $C_{32}H_{38}O_{20}$. The UV spectrum of **1** shows absorption bands at λ_{max} 218, 253, 270 sh, 295 sh, and 344 nm, typical of a flavonol structure. The ^1H and ^{13}C NMR spectra of **1** display characteristic signals for a quercetin[3] moiety and three sugar residues. The complete assignments of the NMR data and the composition of the sugar moieties of **1** were achieved by 2D NMR experiments. The DQF-COSY experiment allowed the sequential assignment of all proton resonances within each sugar residue, starting from the well-separated anomeric proton signals at δ_H 5.56 (d, $J = 6.0$ Hz), 5.10 (br s), and 5.06 (d, $J = 7.8$ Hz). On the basis of chemical shifts, multiplicity, and the coupling constants values, the sugar residues were identified as α-arabinopyranose, α-rhamnopyranose, and β-glucopyranose, respectively (Table 1). In accordance with these data, the acid hydrolysis of **1** yielded quercetin, glucose, arabinose, and rhamnose. The HMBC experiment clarified all intermolecular connectivities, revealing **1** to be a disubstituted quercetin derivative. On the basis of long-range $^{13}C-^1H$ correlations observed from a carbon resonance at δ_C 164.5 (C-7) to H-6 (δ_H 6.73), H-8 (δ_H 6.48), and one of the anomeric protons (δ_H 5.06) which was assigned to H-1‴′ of β-glucose, it was obvious that C-7 was one of the two glycosydation sites on the quercetin aglycone. The absence of any ^{13}C glycosidation shift for the β-glucopyranosyl residue suggested that the β-glucopyranosyl was a terminal residue. The chemical shifts of C-2 (δ_C 159.0) and C-3 (δ_C 135.4) indicated the substitution at the C-3 of the quercetin moiety. The HMBC experiment, which displayed correlations between the anomeric proton of α-arabinose (δ_H 5.56, H-1″) and C-3 (δ_C 135.4) of the quercetin aglycone, confirmed the second glycosidation site to be at C-3. A 5 ppm downfield shift of C-2″ (δ_C 77.1) of the α-arabinopyranosyl residue implied that the third sugar unit, α-rhamnose, is attached to this position. Further evidence came from the HMBC spectrum of **1**, in which correlations between C-2″ (δ_C 77.1) of α-arabinose and H-1‴ (δ_H 5.10) of α-rhamnose, and vice versa, were observed, thus confirming the interglycosidic linkage in the disaccharide residue. On the basis of these results, the structure of **1** was elucidated as quercetin-3-*O*-[α-L-rham-

* To whom correspondence should be addressed. Tel: 90 312-3051089. Fax: 90 312-3114777. E-mail: icalis@hacettepe.edu.tr.
† Hacettepe University.
‡ ETH Zurich.
⊥ University of Utah.
§ University of Zurich.

10.1021/np000614h CCC: $20.00
Published on Web 06/22/2001

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Calabricosides A (**1**) and B (**2**) and HMBC Correlations for Calabricoside B (**2**) (^{1}H NMR, 500 MHz; ^{13}C NMR 75.5 MHz, δ ppm, J (Hz))[a]

C/H	DEPT	**1**	**1**	**2**	**2**	HMBC correlations **2**
2	C	159.0		159.2		H-2′, H-6′
3	C	135.4		135.3		H-1″
4	C	179.4		179.5		H-8
5	C	162.7		162.8		H-6
6	CH	95.7	6.73 d (1.4)	95.8	6.74 d (2.2)	H-8
7	C	164.5		164.6		H-1‴″, H-6, H-8
8	CH	100.6	6.48 d (1.4)	100.8	6.50 d (2.2)	H-6
9	C	157.8		157.9		H-8
10	C	107.5		107.6		H-6, H-8
1′	C	122.9		122.9		H-5′
2′	CH	117.3	7.65 d (1.7)	117.4	7.70 d (2.2)	H-6′
3′	C	146.0		146.1		H-2′, H-5′
4′	C	149.8		150.0		H-2′, H-5′, H-6′
5′	CH	116.2	6.89 d (8.4)	116.2	6.87 d (8.4)	
6′	CH	123.3	7.59 dd (8.4, 1.7)	123.2	7.59 dd (8.4, 2.2)	H-2′, H-5′
α-Ara						
1″	CH	101.1	5.56 d (6.0)	101.5	5.56 d (6.0)	H-2″, H_2-5″
2″	CH	77.1	4.11 dd (6.0, 7.0)	77.2	4.09 dd (6.0, 7.3)	H-1‴
3″	CH	72.9	3.84[b]	73.5	3.83[b]	
4″	CH	68.5	3.81[b]	69.2	3.82[b]	
5″	CH_2	65.4	3.82,[b] 3.40[b]	66.3	3.44 br d, 3.83[b]	
α-Rha						
1‴	CH	102.1	5.10 br s	102.1	5.19 d (0.8)	H-2″
2‴	CH	72.2	3.92[b]	70.4	4.00[b]	
3‴	CH	72.3	3.71[b]	72.5	4.01 dd (3.3, 9.6)	
4‴	CH	73.9	3.36[b]	75.5	5.00 t (9.6)	
5‴	CH	70.1	3.88[b]	68.0	4.21 dq (9.6, 6.3)	
6‴	CH_3	17.6	1.06 d (6.2)	17.4	0.92 d (6.3)	
β-Glc						
1⁗	CH	101.5	5.06 d (7.8)	101.5	5.07 d (7.8)	
2⁗	CH	74.6	3.50[b]	74.6	3.48[b]	
3⁗	CH	77.7	3.51[b]	77.7	3.51[b]	
4⁗	CH	71.2	3.41[b]	71.2	3.42 t (9.2)	
5⁗	CH	78.2	3.52[b]	78.2	3.54 m	
6⁗	CH_2	62.4	3.93[b] and 3.70[b]	62.4	3.90 dd (12.2, 2.2) 3.72 dd (12.2, 5.6)	
caffeoyl						
1⁗′	C			127.7		H-α, H-β, H-5⁗′
2⁗′	CH			114.9	7.00 d (2.0)	H-β, H-6⁗′
3⁗′	C			146.7		H-2⁗′, H-5⁗′
4⁗′	C			149.5		H-2⁗′, H-6⁗′
5⁗′	CH			116.4	6.87 d (8.4)	
6⁗′	CH			123.2	6.77 dd (8.4, 2.0)	H-β, H-2⁗′
α	CH			115.2	6.21 d (15.9)	H-β, H-6⁗′
β	CH			147.0	7.44 d (15.9)	H-6⁗′
C=O	C			169.0		H-α, H-β, H-4‴

[a] All proton and carbon assignments are based on 2D NMR (DQF-COSY, HSQC, and HMBC). [b] Signal patterns are unclear due to overlapping.

nopyranosyl-(1→2)-α-L-arabinopyranoside]-7-*O*-β-D-glucopyranoside. We propose the trivial name of calabricoside A for **1**.

Compound **2** was also isolated as an amorphous yellow solid with similar UV data (λ_{max} 218, 252, 270 sh, 301 sh, and 340 nm). The molecular formula of **2** ($C_{41}H_{44}O_{23}$) was derived from the positive and negative ion ESIMS (*m*/*z* 905 $[M + H]^+$, 927 $[M + Na]^+$, and 903 $[M - H]^-$) and ^{13}C NMR data. Detailed examination of its ^{1}H and ^{13}C NMR data (Table 1) indicated that **2** had structural features identical to those of **1**, except that it also contains a *trans*-caffeoyl function, which was evident from the observation of an ABX system at δ_H 7.00, 6.87, and 6.77 and two olefinic protons at δ_H 7.44 and 6.21 with a large coupling constant (J_{AX} = 15.9 Hz). Furthermore, mild alkaline hydrolysis of **2** afforded **1**. The 1.6 ppm downfield shift of the H-4‴ signal of the rhamnopyranose unit (δ_H 5.00, t, J = 9.6 Hz) indicated C-4‴ to be the acylation site. Actual connectivities between the quercetin moiety, sugars, and acyl unit were clearly identified from the results of a single HMBC experiment performed with **2** (Figure 1). A long-range ^{13}C–^{1}H correlation from the carbonyl group of the *trans*-caffeoyl moiety (δ_C 169.0) to a proton resonance at δ_H 5.00 (H-4‴) further supported the acylation site. Thus, compound **2** was identified as quercetin-3-*O*-[4‴-*O*-caffeoyl-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]-7-*O*-β-D-glucopyranoside. The trivial name of calabricoside B is proposed for **2**.

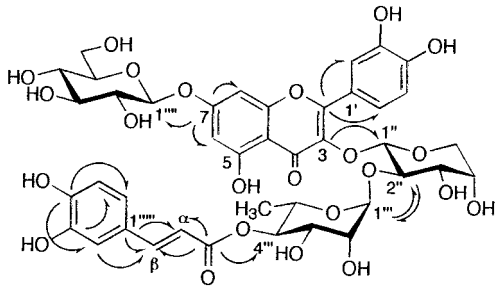


Figure 1. Heteronuclear multiple-bond correlations for **2**. Arrows point from carbon to proton.

Compounds **3** and **4** were isolated as a mixture from which the major component (**3**) was crystallized. Recrystallization gave a monohydrate, which was identified as asperuloside (**3**).[2] The spectroscopic data of the minor

component **4** (Experimental Section) were very similar to those of **3**. The negative and positive ESIMS indicated that the mass of **4** is 32 mass units higher than that of **3**. From a careful inspection of the ^{1}H and ^{13}C NMR data, **4** was identified as paederoside, a sulfur-containing iridoid glucoside. Paederoside was first reported as a component of an aphid's host plant, *Paederia scandens* (Rubiaceae).[4−7] The initial structure published for this compound has been revised by Kapadia and co-workers as an S-methyl thiocarbonate;[8] however, the full NMR assignments have not been reported in the literature. Therefore, we performed a complete 2D NMR study on **4** and include the complete ^{1}H and ^{13}C NMR assignments for this compound in the Experimental Section. Paederosidic acid (**5**) is also a sulfur-containing iridoid glucoside, also isolated from *Paederia scandens*.[4−6] Full NMR (^{1}H and ^{13}C NMR) assignments for paederosidic acid (**5**), which are also lacking in the literature,[4−8] are also presented in the Experimental Section.

1D and 2D NMR data as well as FABMS or ESIMS data of the iridoid glycosides deacetylasperuloside,[9,10] asperulosidic acid,[9,10] geniposidic acid,[11,12] and scandoside[9,11,13] and the lignan glycosides liriodendrin,[14] dihydrodehydrodiconiferyl alcohol-4-*O*-*β*-D-glucopyranoside[15,16] and 7*S*,8*R*,8′*R*-(−)-lariciresinol-4,4′-bis-*O*-*β*-D-glucopyranoside[17] were identical to published data.

The strong radical scavenging and antioxidant activity of 3′,4′-dihydroxyflavonols and 3′,4′-dihydroxyflavonol glycosides in different in vitro systems is well known.[18,19] In accordance, the calabricosides A (**1**) and B (**2**) showed high radical scavenging activity after stimulation of PMNs with the chemoattractant FMLP. The IC_{50} values 0.25 μM (**1**) and 0.3 μM (**2**) of the two compounds are nearly identical and are comparable to the aglycone quercetin (IC_{50} 0.5 μM), which was used as a positive control. Interestingly, the additional caffeic acid moiety of calabricoside B (**2**) did not enhance the radical scavenging activity of **2** in this cellular test system, despite the high activity of caffeic acid esters themselves.[20] Neither the lignan glycosides nor the iridoids showed any activity up to 30 μM.

Experimental Section

General Experimental Procedures. Optical rotations were measured on an AUTOPOL IV automatic polarimeter and a JASCO DIP-370 digital polarimeter using a sodium lamp operating at 589 nm. UV spectra were performed on Shimadzu UV-160A and Hitachi 220 spectrophotometers. IR spectra were measured on a Perkin-Elmer 2000 FT-IR spectrometer in KBr pellets. NMR measurements in CD_3OD were performed on Bruker AMX 300 and DRX 500 spectrometers operating at 300 and 500 MHz for ^{1}H and 75.5 and 125 MHz for ^{13}C, respectively, using the XWIN NMR software package for data acquisition and processing. Negative and positive mode ESIMS were recorded on a Finnigan TSQ 7000 instrument. TLC was carried out on silica gel 60 F_{254} precoated plates (Merck, Darmstadt), detection by 1% vanillin−H_2SO_4. For MPLC, a Lewa M5 pump, a LKB 17000 Minirac fraction collector, a Rheodyne injector, and a Büchi column (18.5 × 352 mm) were used. Silica gel 60 (0.063−0.200 mm; Merck, Darmstadt) was used for open column chromatography (CC) and vacuum liquid chromatography (VLC). Medium-pressure liquid chromatographic separations were performed over LiChroprep C-18 (Merck) material. Polyamid SC 6 (Macherey-Nagel, Düren) and Sephadex LH-20 (Fluka) were also utilized for further separations.

Plant Material. *Putoria calabrica* (L. fil) DC. (Rubiaceae) was collected from Antakya, Samandagi, South Anatolia, Turkey, in June 1999. Voucher specimens (99018) have been deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and Isolation. The air-dried and powdered plant material (90 g) was extracted with MeOH (500 mL × 2) for 6 h at 45 °C and filtered. The filtrate was concentrated to dryness in vacuo. The resulting residue was dissolved in H_2O and partitioned with $CHCl_3$. The H_2O phase was lyophilized (15 g, yield 15%) and preadsorbed onto SiO_2 (25 g). This mixture was subjected to a Si gel column (250 g) using the mixture of CH_2Cl_2−MeOH−H_2O (80:20:1, 80:20:2, 70:30:3) to give 150 fractions, which were combined into nine fractions, A−J. An aliquot (710 mg) of fraction D (1.91 g) was subjected to RP-MPLC employing MeOH gradients in H_2O (30% to 40% MeOH) to yield **3** (asperuloside, 168 mg) and **4** (paederoside, 284 mg). Fraction E (1.49 g) was applied to a Si gel (60 g) column using CH_2Cl_2−MeOH−H_2O (90:10:1, 85:15:1.5, 61:32:7) to afford **3** (27 mg), crude **4** (686 mg), and pure deacetylasperuloside (12 mg). Fraction G (1.5 g) was further chromatographed on a Si gel (60 g) column using EtOAc−MeOH−H_2O (100:15:10) to give five fractions, G_1 (70 mg), G_2 (390 mg), G_3 (354 mg), G_4 (55 mg), and G_5 (20 mg). Fractions G2, G3, and G5 were combined and applied to MPLC employing H_2O−MeOH mixtures with increasing amounts of MeOH in H_2O (5−70% MeOH) to yield geniposidic acid (7 mg), asperulosidic acid (29 mg), liriodendrin (11.5 mg), and a mixture of dihydrodehydroconiferyl alcohol-4′-*O*-*β*-D-glucopyranoside and **5** (paederosidic acid, 53 mg). This mixture was subjected to a Si gel (8 g) column using mixtures of CH_2Cl_2−MeOH (9:1) and CH_2Cl_2−MeOH−H_2O (90:10:0.5, 80:20:1, 61:32:7) to furnish pure dihydrodehydroconiferyl alcohol-4′-*O*-*β*-D-glucopyranoside (5 mg) and **5** (paederosidic acid, 27 mg). Fraction I (1.5 g) was first applied to a Sephadex LH-20 column using MeOH as eluent to yield four main fractions, I_1−I_4. Fraction I_2 (980 mg) was further chromatographed (RP-MPLC) using H_2O and MeOH gradients in H_2O (10−50% MeOH) to afford pure scandoside (122 mg) and crude 7*S*,8*R*,8′*R*-(−)-lariciresinol-4,4′-bis-*O*-*β*-D-glucopyranoside (19 mg). Final purification of 7*S*,8*R*,8′*R*-(−)-lariciresinol-4,4′-bis-*O*-*β*-D-glucopyranoside was achieved by a SiO_2 (7 g) CC using CH_2Cl_2−MeOH−H_2O mixtures (80:20:2 and 75:25:2.5). Fraction I_3 (159 mg) was applied to a Sephadex LH-20 column using MeOH as eluent to afford scandoside (45 mg) and **2** (calabricoside B, 24 mg). Fraction J (4.5 g) was fractionated by VLC using reversed-phase material (LiChroprep C18; column dimensions: 40 × 100 mm), employing H_2O, H_2O−MeOH mixtures, and MeOH as solvents to yield five fractions, J_1−J_5. Fraction J_3 (263 mg) was applied to a Sephadex LH-20 column using MeOH as eluent to furnish **1** (180 mg). Fractions J_4 (78 mg) and J_5 (267 mg) were combined and separated by RP-MPLC using MeOH gradients in H_2O (30−60% MeOH) to yield **1** (calabricoside A, 15 mg), liriodendrin (40 mg), and **2** (54 mg).

Calabricoside A (1): $[\alpha]^{20}_D$ −125° (*c* 0.1, MeOH); UV (MeOH) λ_{max} 218, 253, 270 (sh), 295 (sh), and 344 nm; IR (KBr) ν_{max} 3500−3400 (OH), 1695 (ester), 1654, 1601 (C=C−O), and 1495 cm^{-1} (aromatic ring); ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Table 1; negative and positive ion ESIMS *m*/*z* 741 $[M - H]^-$, 743 $[M + H]^+$, and 765 $[M + Na]^+$.

Calabricoside B (2): $[\alpha]^{20}_D$ −140° (*c* 0.11, MeOH); UV (MeOH) λ_{max} 218, 252, 301 (sh), 340 nm; IR (KBr) ν_{max} 3400 (OH), 1708 (ester), 1627 (C=C−O), and 1508 cm^{-1} (aromatic ring); ^{1}H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Table 1; negative and positive ion ESIMS *m*/*z* 903 $[M - H]^-$, 905 $[M + H]^+$, and 927 $[M + Na]^+$.

Paederoside (4): $[\alpha]^{20}_D$ −161° (*c* 0.17, MeOH); UV (MeOH) λ_{max} 234 nm; IR (KBr) ν_{max} 3385 (OH), 1747 (ester), 1714, and 1657 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 7.31 (1H, d, J = 2.0 Hz, H-3), 5.94 (1H, d, J = 1.2 Hz, H-1), 5.74 (1H, br s, H-7), 5.56 (1H, br d, J = 6.5 Hz, H-6), 4.92 (1H, dd, J = 14.3, 1.3 Hz, Ha-10), 4.83 (1H, dd, J = 14.3, 0.9 Hz, Hb-10), 4.67 (1H, d, J = 7.9 Hz, H-1′), 3.92 (1H, dd, J = 11.8, 1.9 Hz, Ha-6′), 3.68 (1H, dd, J = 11.8, 5.0 Hz, Hb-6′), 3.67 (1H, m, H-5), 3.40 (1H, t, J = 9.0 Hz, H-3′), 3.35 (1H, m, H-5′), 3.30 (1H, m, H-9), 3.28 (1H, t, J = 9.0 Hz, H-4′), 3.20 (1H, dd, J = 7.9, 9.0 Hz,

H-2′), 2.35 (3H, s, COS*CH*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ 172.6 (C, C-11), 172.5 (C, *C*OSCH$_3$), 150.3 (CH, C-3), 143.8 (C, C-8), 129.5 (CH, C-7), 106.1 (C, C-4), 100.0 (CH, C-1′), 93.3 (CH, C-1), 86.2 (CH, C-6), 78.3 (CH, C-5′), 77.9 (CH, C-3′), 74.6 (CH, C-2′), 71.5 (CH, C-4′), 64.3 (CH_2, C-10), 62.7 (CH_2, C-6′), 45.2 (CH, C-9), 37.5 (CH, C-5), 13.6 (CH_3, COS*CH*$_3$); negative and positive ion ESIMS *m*/*z* 445 $[M - H]^-$, 891 $[2M - H]^-$, 447 $[M + H]^+$, 469 $[M + Na]^+$, and 915 $[2M + Na]^+$.

Paederosidic acid (5): $[\alpha]^{20}_D$ +19° (*c* 0.13, MeOH); UV (MeOH) λ_{max} 232 nm; IR (KBr) ν_{max} 3380, 1696 and 1636 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 7.59 (1H, d, J = 2.0 Hz, H-3), 5.98 (1H, d, J = 1.0 Hz, H-7), 5.05 (1H, d, J = 15.0 Hz, Ha-10), 5.01 (1H, d, J = 1.2 Hz, H-1), 4.93 (1H, d, J = 15.0 Hz, Hb-10), 4.80 (1H, m, H-6), 3.81 (1H, dd, J = 11.6, 2.0 Hz, Ha-6′), 3.59 (1H, dd, J = 11.6, 5.0 Hz, Hb-6′), 3.40 (2H, m, H-4′, H-5′), 3.34 (1H, t, J = 9.0 Hz, H-3′), 3.21 (1H, dd, J = 7.8, 9.0 Hz, H-2′), 2.98 (1H, t, J = 7.3 Hz, H-5), 2.58 (1H, t, J = 7.3 Hz, H-9), 2.38 (3H, s, COS*CH*$_3$); ^{13}C NMR (125 MHz, CD_3OD) δ 172.9 (C, *C*OSCH$_3$), 172.3 (C, C-11), 155.0 (CH, C-3), 145.4 (C, C-8), 132.5 (CH, C-7), 108.9 (C, C-4), 101.2 (CH, C-1), 100.7 (CH, C-1′), 78.6 (CH, C-5′), 77.9 (CH, C-3′), 75.4 (CH, C-6), 75.0 (CH, C-2′), 71.6 (CH, C-4′), 66.3 (CH_2, C-10), 63.0 (CH_2, C-6′), 46.3 (CH, C-9), 42.6 (CH, C-5), 13.5 (CH_3, COS*CH*$_3$); positive ion ESIMS *m*/*z* 487 $[M + Na]^+$.

Chemiluminescence Assay with PMNs. Human PMN suspension (50 μL) in HBSS (5×10^6 cells/ml) was incubated for 30 min at 37 °C with 50 μL of luminol (0.2 mM), 50 μL of HBSS, and 50 μL of test solution. The latter solutions were prepared by dissolving 1 mg of the test compound in ethanol and dilution with HBSS to the different molar concentrations. The reaction was started by adding 50 μL of FMLP (0.5 μM) as stimulant. The luminol-enhanced chemiluminescence response was measured in a six-channel Biolumat LB 9505 (Berthold) compared to the medium control at 37 °C. Light emission was recorded continuously in counts per minute (cpm) for 15 min. For quantifying the chemiluminescence response, the area under the time−activity curve was calculated. All results were expressed as a percentage of the control response (chemiluminescence reaction without compounds). Five measurements were carried out at each concentration, and the means were calculated. Maximum observed standard deviation was about 15%. Positive control measurements were performed with quercetin (IC_{50} 0.5 μM).[18]

Acknowledgment. The authors thank Prof. Dr. Zeki Aytaç, Gazi University, Faculty of Science, Turkey, for the authentification of the plant specimen, Dr. Engelbert Zass, ETHZ, Switzerland, for performing computer-based literature searches, and Oswald Greter and Dr. Walter Amrein (ETHZ), for recording all mass spectra, and Dr. Oliver Zerbe (ETHZ) for 2D NMR measurements of flavonoid glycosides.

References and Notes

(1) Ehrendorfer, F. In *Flora of Turkey and the East Aegean Islands*; Davis, P. H., Ed.; Edinburgh University Press: 1982; Vol. 7, pp 723−724.
(2) Linden, A.; Çalis, I.; Neuburger, M.; Sticher, O. *Acta Crystallogr*. **2000**, *C56*, 616−618.
(3) Markham, K. R.; Chari, V. M.; Mabry, T. J. In *The Flavonoids: Advances in Research*; Harborne, J. B., Mabry, T. J., Eds.; Chapman & Hall: London, 1982; pp 19−134.
(4) Inouye, H.; Inouye, S.; Shimokawa, N.; Okikawa, M. *Tetrahedron Lett*. **1968**, 683−688.
(5) Inouye, H.; Inouye, S.; Shimokawa, N.; Okikawa, M. *Chem. Pharm. Bull*. **1969**, *17*, 1942−1948.
(6) Inouye, H.; Okikawa, M.; Shimokawa, N. *Chem. Pharm. Bull*. **1969**, *17*, 1949−1954.
(7) Nishida, R., Fukami, H. *J. Chem. Ecol*. **1989**, *15*, 1837−1845.
(8) Kapadia, G. J.; Shukla, Y. N.; Bose, A. K.; Fujiwara, H.; Lloyd, H. A. *Tetrahedron Lett*. **1979**, 1937−1938.
(9) Otsuka, H.; Yoshimura, K.; Yamasaki, K.; Cantoria, M. C. *Chem. Pharm. Bull*. **1991**, *39*, 2049−2052.
(10) Bianco, A.; Guiso, M.; Iavarone, C.; Passacantilli, P.; Trogolo, C. *Gazz. Chim. Ital*. **1978**, *108*, 13−16.
(11) Bianco, A.; Passacantilli, P.; Polidori, G.; Nicoletti, M.; Messana, I. *Gazz. Chim. Ital*. **1983**, *113*, 829−834.
(12) Akdemir, Z.; Calis, I. *Doga-Tr. J. Pharm*. **1991**, *1*, 67−75.
(13) Bianco, A.; Passacantilli, P.; Polidori, G.; Nicoletti, M. *Org. Magn. Reson*. **1983**, *21*, 460−461.
(14) Chaudhuri, R. K.; Sticher, O. *Helv. Chim. Acta* **1981**, *64*, 3−15.
(15) Franz, C. Isolierung und Strukturaufklaerung von Inhaltsstoffen aus der Rinde des Traubenholunders (*Sambucus racemosa* L.). Diss. ETH Nr. 8825, Zurich, 1989, pp 83−102.
(16) Abe, F.; Yamauchi, T. *Chem. Pharm. Bull*. **1986**, *34*, 4340−4345.
(17) El Gamal, A. A.; Takeya, K.; Itokawa, H.; Halim, A. F.; Amer, M. M.; Saad, H.-E. A. *Phytochemistry* **1997**, *45*, 597−600.
(18) Heilmann, J.; Merfort, I.; Weiss, M. *Planta Med*. **1995**, *61*, 435−438.
(19) Middleton, E.; Kandaswami, C. In *The Flavonoids, Advances in Research Since 1986*; Harborne, J. B., Ed.; Chapman and Hall: London, 1994; pp 619−645.
(20) Heilmann, J.; Çalis, I.; Kirmizibekmez, H.; Schühly, W.; Harput, S.; Sticher, O. *Planta Med*. **2000**, *66*, 746−748.

NP000614H