Antibacterial *C*-Geranylflavonoids from *Paulownia tomentosa* Fruits

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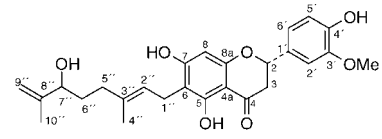
Received August 23, 2007

Eight *C*-6-geranylflavonoids (**1**−**8**) were isolated from an ethanol extract of *Paulownia tomentosa* fruits. Tomentodiplacone (**1**), 3′-*O*-methyl-5′-hydroxydiplacone (**2**), 3′-*O*-methyl-5′-*O*-methyldiplacone (**3**), and tomentodiplacone B (**4**) were obtained as new compounds, and 3′-*O*-methyldiplacol (**5**) was isolated for the first time from a natural source. The structures of these new compounds were determined by using mass spectrometry (including HRMS) and 1D and 2D NMR spectroscopy, and the absolute configurations of the compounds were determined by circular dichroism. The antibacterial activities of **1**−**8** against seven bacteria and yeast were evaluated.

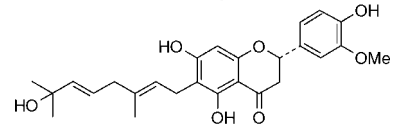
Antibacterial research is in crisis, as only a few major pharmaceutical companies are actively engaged in this field.[1] The crisis has coincided with a pressing need for new and improved antibacterial drugs due to the widespread nature of antibacterial drug resistance. Research on antibiotics must continue, and searching among medicinal plants is one way to find new compounds, either as lead compounds or as drugs per se for the treatment of bacterial, fungal, and viral diseases. A search of the accessible literature showed that *Paulownia tomentosa* Steud. (Scrophulariaceae) fruit extracts have antibacterial activity, and therefore we decided to identify the compounds responsible for this activity.[2] Previously published studies reported polyphenolic substances such as iridoids, phenolic glycosides, naphthoquinones, and phenylethanoids in MeOH and EtOH extracts from *P. tomentosa*.[3,4] Our previous work showed the presence of geranyl-substituted flavonoids.[4] In this paper, we report the isolation and the characterization of compounds **1**−**8**, including four new substances, **1**−**4**, obtained from an EtOH extract of *P. tomentosa* fruit and their antimicrobial activity.

The EtOH extract of *P. tomentosa* fruit was subjected to liquid–liquid fractionation. The $CHCl_3$-soluble fraction was then dissolved in 80% MeOH and treated with hexane to remove the lipid-soluble fraction. The MeOH-soluble portion was separated by column chromatography on silica gel. Selected fractions were further chromatographed by flash chromatography on silica gel and by preparative RP-HPLC (Figure S1, Supporting Information). This separation resulted in the isolation of tomentodiplacone (**1**), 3′-*O*-methyl-5′-hydroxydiplacone (**2**), 3′-*O*-methyl-5′-*O*-methyldiplacone (**3**), and tomentodiplacone B (**4**) as new compounds. 3′-*O*-Methyldiplacol (**5**) has been obtained previously from *Mimulus* species, but this was its first isolation from a plant in the genus *Paulownia*.[5,6] 3′-*O*-Methyldiplacone (**6**), mimulone (**7**), and diplacone (**8**) have been reported previously from the genus *Paulownia*.[4]

The basic characteristics of the structures of the compounds isolated were obtained by analyzing the UV and IR spectra. Similar UV absorptions were observed, with maximum values at λ ~230 (sh) nm, ~290 nm, and ~340 (sh) nm corresponding to the $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ electronic transitions of the flavanone skeletone.[7] A parallel series of absorption bands was evident from the analysis of the IR spectra: ν_{max} 3500–3400 cm^{-1}, corresponding to OH vibrations; 2950–2850 cm^{-1}, corresponding to CH vibrations, which are not usually so clearly visible in flavonoid spectra; and 1639–1622 cm^{-1}, assigned to the C═O vibrations of the carbonyl group.[7] We suggest that these are absorbances typical for *C*-geranylflavanones.



1



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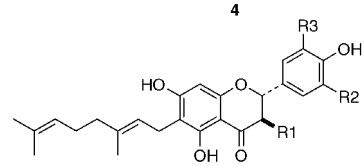

	R1	R2	R3
2	H	OMe	OH
3	H	OMe	OMe
5	OH	OMe	H
6	H	OMe	H
7	H	H	H
8	H	OH	H

The molecular formula of tomentodiplacone (**1**) was determined as $C_{26}H_{30}O_7$ by HRQTOFMS and supported by the presence of a

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10.1021/np070446u CCC: $40.75
Published on Web 02/23/2008

Table 1. ^{1}H NMR and ^{13}C Chemical Shifts (δ in ppm) and Spin–Spin Coupling Constants (J in Hz) of Compounds **1**–**4** at 303 K

	1 (DMSO-d_6)		**2** (DMSO-d_6)		**3** ($CDCl_3$)		**4** (DMSO-d_6)	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	78.4	5.38 dd (2.8, 12.9)	78.6	5.32 dd (2.9, 12.7)	79.3	5.31 dd (2.9, 12.7)	78.6	5.39 dd (2.9, 12.7)
3	42.1	2.66 dd (2.8[c], 17.1)	42.2	2.66 dd (2.8[c], 17.1)	43.5	2.66 dd (2.8[c], 17.1)	42.2	2.68 dd (2.9[c], 17.1)
		3.30 dd (12.9[d], 17.1)		3.20 dd (12.7[d], 17.1)		3.07 dd (12.7[d], 17.1)		3.28 dd (12.7[d], 17.1)
4	196.4		196.3		195.9		196.2	
4a	101.4		101.4		102.8		101.3	
5	160.5		160.5		161.2		160.5	
6	107.5		107.7		107.2		107.4	
7	164.4 CH_2		164.4		164.0		164.9	
8	94.4	5.96 s	94.3	5.97 s	95.6	6.02 s	94.5	5.97 s
8a	160.5		160.4		160.9		160.5	
1′	129.5		128.7		129.6		129.5	
2′	111.0	7.08 d (1.7)	102.2	6.60 d (1.7)	103.2	6.66 s	111.0	7.07 d (1.7)
3′	147.5		148.2		147.2		147.5	
4′	146.9		134.3		135.2		146.9	
5′	115.1	6.79 d (8.1)	146.6	-	147.2	-	115.1	6.79 d (8.1)
6′	119.6	6.89 dd (1.7, 8.1)	107.5	6.56 d (1.7)	103.2	6.66 s	119.6	6.89 dd (1.7, 8.1)
1″	20.5	3.12 d (6.8)	20.5	3.12 d (7.0)	21.0	3.35 d (7.1)	20.6	3.14 d (6.9)
2″	122.1	5.13 t (6.8)	122.4	5.13 t (7.0)	121.3	5.25 t (7.1)	123.1	5.17 t (6.9)
3″	134.0		133.7		138.9		133.1	
4″	16.0	1.69 s	15.8	1.70 s	16.1	1.80 s	15.9	1.68 s
5″	35.1	1.89 m	39.7	1.91 m	39.7	2.07 m	41.9	2.59 d (6.40)
6″	33.3	1.44 m	26.2	1.98 m	26.4	2.07 m	123.1	5.45 t (6.40)
7″	73.4	3.81 m	124.1	5.04 t (6.8)	123.8	5.05 t	140.6	5.51 t
8″	148.1		130.6		131.9		68.8	
9″	109.7	4.70 d	25.5	1.60 s	25.6	1.67 s	30.1	1.14 s
		4.80 d						
10″	17.6	1.61 s	17.5	1.53 s	17.7	1.59 s	30.1	1.14 s
MeO-3′	56.1	3.78 s	55.8	3.76 s	56.3	3.90 s	55.6	3.79 s
MeO-5′					56.3	3.90 s		
OH-5		12.44 s		12.42 s		12.35 s		12.42 s
OH-7		10.80 brs[a]		[b]		6.60 brs[a]		[b]
OH-4′		9.10 brs[a]		8–9 brs[a]		5.60 brs[a]		9.1 brs[a]
OH-5′				8–9 brs[a]				
OH-7″		4.65 brs[a]						
OH-8″								4.40 brs[a]

[a] Broad signal. [b] Not obtained. [c] *cis* relative to H-2. [d] *trans* relative to H-2.

pseudomolecular ion $[M + Na]^+$ at m/z 477.1890 (calcd 477.1889). The structure of compound **1** was completed using ^{1}H and ^{13}C NMR spectroscopy (Tables 1 and S1, Supporting Information). COSY, HSQC, and HMBC experiments were used to assign the observed resonances to individual atoms. The long-range ^{1}H−^{13}C interactions observed in gs-HMBC (adjusted for a long-range coupling of 7.5 Hz) are summarized in Table S1. The structure of compound **1** is very similar to the structure of tomentodiplacol described in our previous work; phenyl ring B is connected to C-2 and a modified geranyl-type side chain is bonded at C-6.[4] Signals at 12.44 (1H) and 10.80 (1H, broad signal) ppm were assigned to OH-5 and OH-7, respectively. A difference between tomentodiplacol and **1** was observed at positions C-2 and C-3 of the heterocyclic ring. The CH-CH_2 spin system detected in the ^{1}H NMR spectrum (δ 5.38, 2.66, and 3.30 ppm) clearly indicated the absence of the OH group at position C-3 in **1**. The H-3$_\alpha$ and H-3$_\beta$ protons were assigned stereochemically on the basis of their coupling constants with H-2 (see Table 1). The *trans* coupling usually ranges between 12.5 and 13.0 Hz,[8,9] whereas the *cis* coupling constant $J_{H2\text{-}H3}$ is reduced to 1–3 Hz.[10]

The molecular formula of **2** was determined to be $C_{26}H_{30}O_7$ on the basis of HRQTOFMS, and the presence of a pseudomolecular ion $[M + H]^+$ at m/z 455.2089 (calcd 455.2070) was observed. The molecular formula of **3** was determined to be $C_{27}H_{32}O_7$ by HRQTOFMS and supported by the presence of a pseudomolecular ion $[M + H]^+$ at m/z 469.2243 (calcd 469.2226). The structures of compounds **2** and **3** were determined by ^{1}H and ^{13}C NMR spectroscopy (Tables 1 and S1, Supporting Information) and 2D chemical shift correlation techniques. The chemical shifts of compounds **2** and **3** are very similar to those of diplacone (**8**),[4–6] and their structures differ only in the substitution pattern of ring B. For compound **2**, one methoxy group (δ 3.76, 3′-methoxy) and two OH groups (broad signals at δ ca. 8−9 ppm) observed in the ^{1}H NMR spectrum were assigned to the 3′,4′,5′-trisubstituted phenyl ring B. For compound **3**, the same phenyl ring is substituted by two methoxy groups at positions C-3′, C-5′ (δ 3.9, 6H) and an OH group at position C-4′ (δ 5.60, broad signal, 1H). The orientation of the two protons at C-3 relative to H-2 was characterized in a way similar to that used for **1**; the corresponding coupling constants are summarized in Table S1 (Supporting Information).

The molecular formula of **4**, tomentodiplacone B, was determined to be $C_{26}H_{30}O_7$ by HRQTOFMS and supported by the pseudomolecular ion $[M + H]^+$ at m/z 455.2079 (calcd 455.2070). NMR spectroscopy was used further to determine the structure of compound **4**. Singlets at δ 3.79 (3H) and δ 5.97 (1H) in the ^{1}H NMR spectrum were assigned to the methoxy group (MeO-3′) and to H-8, respectively. The signal at 12.42 (s, 1H) ppm was assigned to the OH-5. The missing signal of the proton at C-7 indicated the presence of OH at C-7, although the signal of OH was not observed. Resonances at 7.07, 6.89, and 6.79 indicated a 3′,4′-disubstituted phenyl ring B (MeO-3′, OH-4′). The long-range interactions of H-2′/C-2 and H-6′/C-2 in the HMBC experiments were used to identify the connectivity of the phenyl ring B to C-2. The ^{1}H NMR pattern of C(2)H−C(3)H_2 was very similar to those of compounds **1**−**3**. The geranyl-type side chain was assigned to C-6 (see interactions with C-6 in Table S1, Supporting Information). The quaternary carbon C-8″ with a resonance value of 68.8 ppm indicated the presence of an OH group in this position. The chemical shifts of C-7″ (140.6 ppm) and C-6″ (123.1 ppm) confirmed the presence of the double bond and the structure of compound **4**.

Circular dichroism (CD) spectra and their comparison with those of known flavanones were used for **1**−**4** to determine the absolute

Table 2. Minimum Inhibitory Concentrations (μg mL^{-1}) of *C*-Geranylflavonoids from *P. tomentosa* Fruit

	Gram-positive bacteria						Gram-negative bacteria			yeast
compound	*B.c.*	*B.s.*	*E.f.*	*L.m.*	*S.a.*	*S.e.*	*E.c.*	*P.a.*	*S.ent.*	*C.a.*
1	16			32	16	32	−	−	−	−
2	4	4	4	4	2	4	−	−	−	−
3	4	4	4	4	4	4	−	−	−	−
4	−	−	−	−	−	−	−	−	−	−
5	2	4	4	2	2	2	−	−	−	−
6	4	8	8	4	8	4	−	−	−	−
7	4	4	4	4	8	4	−	−	−	−
8	4	4	4	4	4	4	−	−	−	−
CIP/NYS[a]	1	2	1	1	0.5	1	0.015	0.25	0.015	4

[a] Ciprofloxacin (CIP) and nystatin (NYS) were used as positive controls for the bacteria and yeast, respectively; − not active (>32). *B.c., Bacillus cereus; B.s., Bacillus subtilis; E.f., Enterococcus faecalis; E.c., Escherichia coli; L.m., Listeria monocytogenes; P.a., Pseudomonas aeruginosa; S.ent., Salmonella enteritidis; S.a., Staphylococcus aureus; S.e., Staphylococcus epidermidis; C.a., Candida albicans*.

configuration at stereogenic centers C-2 and C-3.[11] A positive Cotton effect for n→π* electronic transitions at ca. 340 nm and a negative Cotton effect for π→π* electronic transitions at ca. 290 nm were observed in the spectra of **1**−**4**. Therefore, a 2*S* configuration was assigned to all these compounds.[11] A positive Cotton effect at ca. 330 nm (Θ +9020) and a negative Cotton effect at ca. 295 nm (Θ −39 500) were observed for **5**, and a 2*R*,3*R* configuration was assigned for compound **5** by comparison of its CD and NMR data with those of known flavanonols.[11]

The results of the antimicrobial activity assays are summarized in Table 2. All compounds, with the exception of **4**, exhibited some degree of activity in the range of the concentrations tested; however, none of the compounds were able to inhibit the growth of Gram-negative bacteria or the yeast. Compound **1** showed moderate activity, inhibiting only four out of six Gram-positive bacteria with minimum inhibitory concentrations (MICs) in the range 16–32 μg mL^{-1}. The other six compounds were active against all Gram-positive bacteria, with MICs from 2 to 8 μg mL^{-1}. Of these, **5** was the most active (MICs of 2–4 μg mL^{-1}).

Many present investigations have dealt with the antibacterial activity of flavonoids, but not all have described prenylated flavanones. Prenylflavanones obtained from *Erythrina subumbrans* showed weak activity against different *Streptococcus* and *Staphylococcus* strains,[12] *Eysenhardtia texana* gave compounds active against *S. aureus*,[13] and four antibacterial-active geranyl flavanones were isolated from *Macaranga pleiostemona* leaves.[14] Some structure–activity studies have also been published. It has been postulated that hydroxylation occurs at the C-5, C-7 and C-2′, C-4′ positions in antibacterial-active flavanones, whereas this pattern is absent for the nonactive species.[15,16] This corresponds partially with the present results. Some structure–activity experiments on the antibacterial activity of flavanones were carried out using *S. aureus*. The OH-5 group raises the activity of the flavanones tested, while the presence of methoxy groups diminishes the potential to inhibit bacterial growth. It was also found that substitution of a lipophilic functional group (e.g., geranyl) at position 6 or 8 increased the resultant antibacterial activity. The OH-5 group can form an intramolecular hydrogen bond with the C-4 carbonyl, and this can lead to a higher degree of electron delocalization within the molecule.[17,18] Compounds **2**, **3**, and **5** proved to be the most active of the compounds isolated. The 3′-methoxy-4′,5′-dihydroxyphenyl ring B of **2** and 3′,5′-methoxy-4-hydroxy substitution of **3** seem to be important for the activity due to an increase in the planar character of the molecule. The OH-3 group of **5** also increases the activity of the compound in comparison with the corresponding flavanone **6**. Some studies have reported greater activity against Gram-positive bacteria than Gram-negative.[19] This was demonstrated clearly with the *C*-geranyl flavonoids of *P. tomentosa*. None of the compounds tested showed activity against *E. coli, P. aeruginosa*, or *S. enteritidis*. This is probably due to the lipophilic character of the compounds tested caused by the presence of the geranyl side chain. Some of flavanones have been tested in a liposome membrane model, and it was shown that the presence of a lipophilic substituent on the flavonoid skeleton increased the alteration of membrane fluidity.[19] The resistance of Gram-negative bacteria to the compounds isolated is probably caused by the more complex structure and hydrophilic nature of their cell walls.

The results presented show that *P. tomentosa* is a rich source of geranyl-substituted flavanones. These flavanones are secreted on the surface of the fruits as protection against UV irradiation, desiccation, and microbial infection. The promising activity of the compounds isolated, which is comparable to that of standard antibiotics, offers an opportunity to find alternative drugs for fighting pathogenic bacteria, especially after functional synthetic derivatization.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage apparatus and were not corrected. UV spectra were recorded in MeOH on a Synergy HT multiplate reader. CD spectra were recorded on a JASCO J-810 spectrometer (MeOH; the molar elipticity Θ_λ values are presented). IR spectra were determined by the ATR method on a Nicolet Impact 400D FT-IR spectrophotometer. NMR spectra were recorded using a Bruker Avance 300 spectrometer operating at frequencies of 300.13 MHz (^{1}H) and 75.48 MHz (^{13}C). The spectra were measured in DMSO-d_6 or $CDCl_3$ at 303 K. The ^{1}H and ^{13}C NMR chemical shifts (δ in ppm) were referenced to the signal of the solvent [2.50 (^{1}H) and 39.43 (^{13}C) for DMSO-d_6 and 7.26 (^{1}H) and 77.00 (^{13}C) for $CDCl_3$]. The 2D NMR experiments, gs-COSY, gs-HSQC, and gs-HMBC were used to assign the individual ^{1}H and ^{13}C resonances. The HSQC experiment was adjusted for the coupling $^1J_{HC}$ = 150 Hz and the HMBC experiment for long-range couplings of 7.5 Hz. ESIMS were collected using an Agilent HP 1100 LC/MSD Trap VL Series in MeOH, using direct infusion with a linear pump (kd Electronics) at a flow rate of 300 μL min^{-1}. The spectra were collected in negative mode, with the nebulizing and drying gas N_2 (T = 300 °C) at a flow rate of 10 L min^{-1}, a nebulizer pressure of 80 psi, and a capillary voltage 3.5 kV. The full mass scan covered the range from *m*/*z* 200 to 1500. All spectra were collected in negative mode. A Q-TOF micro (Waters) with ESI was used to collect the HRMS. These spectra were collected in the positive mode.

Column chromatography and flash chromatography were performed on Merck silica gel 60 (particle size 0.040–0.063 mm). TLC analyses were performed using precoated silica gel 60 F254 (Merck) with detection under UV light at 254 and 366 nm and by spraying with Neu's reagent (1% diphenylaminoethylborate in MeOH) followed by heating to 110 °C for 10 min. Preparative HPLC was carried out on a LCP 4100 instrument, with loop injection of 500 μL, column block LCO 101, and UV detector LCD 2084 (Ecom, CR). Analytical and semipreparative HPLC were carried out on an Agilent 1100 apparatus equipped with a diode-array detector. Supelcosil ABZ+Plus columns were used: for preparative purposes, column length 250 × 21.2 mm i.d., particle size 5 μm; for semipreparative analyses, column length 250 × 10 mm i.d., particle size 5 μm; for analytical HPLC, column length 150 × 4.6 mm i.d., particle size 3 μm.

Plant Material. The fruit of *Paulownia tomentosa* was collected in the area of the University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, during October 2004. A voucher specimen (PT-04O) has been deposited at the herbarium of the Department of Natural

Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic.

Extraction and Isolation. The plant material (22.3 kg) was extracted with EtOH (20 L × 3). The EtOH extract (5254 g) was diluted to 60% with H_2O (30 L) and extracted with $CHCl_3$ (20 L × 3). After evaporation to dryness in vacuo, the $CHCl_3$ extract (562 g) was dissolved in 20% MeOH (10 L) and treated with hexane (5 L × 3) to give a MeOH fraction (462 g). After TLC analysis, the MeOH-soluble portion (200 g) was chromatographed on silica gel. Fractions of 150 mL were collected. On the basis of TLC analyses, the chosen combined fractions were further separated by flash chromatography or preparative RP-HPLC or both (Figure S1, Supporting Information). For flash chromatography on silica gel, the mobile phase was overpressurized with N_2 and fractions of about 150 mL were collected. The mobile phase compositions are shown in Figure S1. Based on TLC, combined fractions were used for further work. For preparative RP-HPLC, different ratios of MeCN and 0.2% HCOOH were used. Fractions were collected based on the UV-detector response (λ 280 nm). The solvent was evaporated from each fraction and the pure compounds were precipitated.

The physical and spectroscopic data used to identify **5**–**8** agreed with those reported in the literature.[4–6] All of these substances have been characterized by measurement of their ^{1}H and ^{13}C NMR, IR, UV, CD, and ESIMS data.

Tomentodiplacone (1): pale yellow powder; mp 76–79 °C; UV (MeOH) λ_{max} (log ϵ) 230 sh (4.5), 291 (4.38), 335.5 (3.65) nm; CD $\Theta_{330.5}$ +10 200, $\Theta_{292.5}$ −46 600, Θ_{219} +40 600; IR (ATR) ν_{max} 3515, 3458, 3396, 2966, 2913, 2850, 1609, 1515, 1495, 1445, 1370, 1268, 1213, 1157, 1117, 1078, 1025 cm^{-1}; ^{1}H and ^{13}C data, see Table 1; ESIMS [M − H]$^-$ *m*/*z* 453.5; HRQTOFMS [M + Na]$^+$ *m*/*z* 477.1890 (calcd for $C_{26}H_{30}O_7Na$ 477.1889).

3′-*O*-Methyl-5′-hydroxydiplacone (2): yellow powder; mp 105–108 °C; UV (MeOH) λ_{max} (log ϵ) 228 sh (7.55), 293 (4.36), 334 (3.73) nm; CD $\Theta_{332.5}$ +8810, Θ_{296} −33 700, $\Theta_{224.5}$ +30 200; IR (ATR) ν_{max} 3231, 2959, 2908, 2938, 1627, 1596, 1514, 1491, 1444, 1378, 1332, 1297, 1192, 1149, 1083, 1013 cm^{-1}; ^{1}H and ^{13}C data, see Table 1; ESIMS [M − H]$^-$ *m*/*z* 453.4; HRQTOFMS [M + H]$^+$ *m*/*z* 455.2089 (calcd for $C_{26}H_{31}O_7$ 455.2070).

3′-*O*-Methyl-5′-*O*-methyldiplacone (3): yellow powder; mp 75–78 °C; UV (MeOH) λ_{max} (log ϵ) 232 sh (4.36), 294 (4.24), 334 (3.5) nm; CD Θ_{331} +3790, Θ_{292} −17 600, Θ_{226} +14 800; IR (ATR) ν_{max} 3481, 3114, 2840, 2610, 1607, 1520, 1401, 1376, 1327, 1296, 1251, 1215, 1199, 1185, 1160, 1022 cm^{-1}; ^{1}H and ^{13}C data, see Table 1; ESIMS [M − H]$^-$ *m*/*z* 467.5; HRQTOFMS [M + H]$^+$ *m*/*z* 469.2243 (calcd for $C_{27}H_{33}O_7$ 469.2226).

Tomentodiplacone B (4): yellow powder; mp 146–150 °C; UV (MeOH) λ_{max} (log ϵ) 227 sh (4.49), 291.5 (4.34), 334 (3.59) nm; CD $\Theta_{338.5}$ +8330, $\Theta_{292.5}$ −40 600, $\Theta_{220.5}$ +35 900; IR (ATR) ν_{max} 3519, 3352, 2979, 2910, 1640, 1590, 1516, 1483, 1452, 1379, 1336, 1269, 1216, 1189, 1154, 1077 cm^{-1}; ^{1}H and ^{13}C data, see Table 1; ESIMS [M − H]$^-$ *m*/*z* 453.5; HRQTOFMS [M + H]$^+$ *m*/*z* 455.2079 (calcd for $C_{26}H_{31}O_7$ 455.2070).

3′-*O*-Methyldiplacol (5): yellow powder; mp 80–84 °C; CD $\Theta_{330.5}$ +9020, $\Theta_{295.5}$ −39 500, Θ_{223} +35 500; UV (MeOH), IR (ATR), ^{1}H and ^{13}C NMR in agreement with published data,[5,6] *trans* $J_{H2\text{-}H3}$ = 11.3 and 11.9 Hz in $CDCl_3$ and DMSO-d_6, respectively; ESIMS [M − H]$^-$ *m*/*z* 453.5.

3′-*O*-Methyldiplacone (6): yellow powder; mp 102–103 °C; UV (MeOH); IR (ATR), ^{1}H and ^{13}C NMR in agreement with published data;[4–6] ESIMS [M − H]$^-$ *m*/*z* 437.3.

Mimulone (7): yellow needles; mp 120–122 °C; UV (MeOH), IR (ATR), ^{1}H and ^{13}C NMR in agreement with published data;[4–6] ESIMS [M − H]$^-$ *m*/*z* 407.3.

Diplacone (8): light brown powder; mp 170–173 °C; UV (MeOH), IR (ATR), ^{1}H and ^{13}C NMR in agreement with published data;[4–6] ESIMS [M − H]$^-$ *m*/*z* 423.4.

Antimicrobial Activity Assay.[20] The isolated compounds were dissolved in a 7% (v/v) solution of dimethyl sulfoxide (DMSO) in Tris buffer saline at pH 7.6 to a final concentration of 64 μg mL^{-1} of the stock solution. The in vitro antimicrobial activity was determined by the broth microdilution method using 96-well microtiter plates. 2-Fold dilutions (seven) of each compound were prepared in appropriate broth media at concentrations from 32 to 0.5 μg mL^{-1}. Each well was inoculated with 5 μL of bacterial suspension at a density of 10^7 CFU mL^{-1}. The microtiter plates were incubated at 37 °C for 24 h (or 48 h for the yeast). The growth of microorganisms was observed as turbidity determined by the UV–vis spectrophotometer Helios ϵ (Spectronic Unicam, Cambridge, UK) at 600 nm. Minimum inhibitory concentrations (MIC) were calculated on the basis of the density of the growth control and were the lowest compound concentrations that resulted in an 80% reduction in growth compared with that of the compound-free growth control. The solution of DMSO (5% v/v) in TBS was assayed simultaneously as the negative control. All samples were tested in triplicate.

The following strains of microorganisms were used: *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* ATCC 13076, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, and *Candida albicans* ATCC 10231. All microorganisms were grown and tested in Mueller-Hinton broth. All microbial strains and cultivation media were purchased from Oxoid (Basingstoke, UK). Ciprofloxacin and nystatin (Sigma, St. Louis, MO) were used as positive controls (Table 2).

Acknowledgment. The financial support of this work by the IGA VFU (grant No. 22/2006 to K.Š.), by the Ministry of Education of the Czech Republic (MSM0021622413 and LC06030 to R.M.; MSM 6046137305 to P.K., MSM 6046137307 to M.U. and O.J.), and by the Ministry of Health (1A8666 to M.D.) is gratefully acknowledged.

Supporting Information Available: Table of HMBC NMR data for **1**−**4** and a scheme of separation. This information is available free of charge via the Internet at http://pubs.acs.org.

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NP070446U