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C-Geranyl Compounds from *Paulownia tomentosa* Fruits

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Five geranylflavonoids, one prenylated flavonoid, and a simple flavanone were isolated from an ethanolic extract of *Paulownia tomentosa* fruit. Tomentodiplacol (**1**), 3′-*O*-methyl-5′-methoxydiplacol (**2**), 6-isopentenyl-3′-*O*-methyltaxifolin (**3**), and dihydrotricin (**4**) are reported from a natural source for the first time and 3′-*O*-methyldiplacone (**6**) for the first time from the genus *Paulownia*. The structures of the compounds were determined by mass spectrometry, including HRMS, and by 1D and 2D NMR spectroscopy. The cytotoxicity and DPPH (2,2-diphenyl-1-picrylhydrazyl)-quenching activity of some of these compounds were tested, with diplacone proving to be the best antioxidant, although the most cytotoxic compound.

Paulownia tomentosa Steud. (Scrophulariaceae) is a large deciduous tree, usually 10−20 m tall, with strong branches that are dark felted when they are small. Paulownia trees bloom in May and June, before the leaves appear. The flowers are colored white to light purple. The fruit is a double-capsuled, red-brown ligneous seed ball, approximately 4 cm long. The fruit ripens in autumn and then bursts, and many winged seeds fly away.

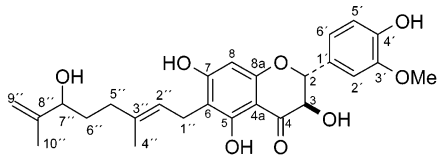
Extracts from *P. tomentosa* are used in traditional Chinese medicine. Extracts from the fruit, leaves, and wood are used in adjuvant therapy for bronchitis, and fruit extracts decrease the frequency of asthmatic attacks.[1] An aqueous extract of the fruit and leaves regenerates hair and stimulates the scalp.[2] Extracts from the fruit show a hypotensive effect, and extracts from the wood are used to treat some bacterial infections.[2]

Previous publications have reported polyphenolic substances, such as iridoids, phenolic glycosides, flavonoids, and phenylethanoids in the MeOH and EtOH extracts of *P. tomentosa*. In fact, most of the substances that have been isolated so far are polar, usually glycosides.[3−14]

This work is focused on the isolation and identification of partly polar and nonpolar compounds from the European paulownia tree and aims to find potentially bioactive substances with antiradical or cytotoxic activities. The screening test of an EtOH extract of *P. tomentosa* based on the DPPH (diphenylpicrylhydrazyl) assay showed significant antiradical activity (EC_{50} 7.928 mg/L). In this paper, we report the isolation and structural elucidation of seven compounds from an EtOH extract of *P. tomentosa* fruit and the cytotoxic and antiradical activities of some of them.

Results and Discussion

The EtOH extract of *P. tomentosa* fruit was subjected to liquid−liquid fractionation, and the antiradical-active $CHCl_3$-soluble fraction was repeatedly separated by column chromatography on silica. The fractions, selected by TLC analysis, were further separated by preparative RP-HPLC. This extensive separation process resulted in the isolation of tomentodiplacol (**1**), 3′-*O*-methyl-5′-methoxydiplacol (**2**), 6-isopentenyl-3′-*O*-methyltaxifolin (**3**), and dihydrotricin (**4**). 3′-*O*-Methyldiplacone (**6**) is reported from the genus *Paulownia* for the first time. Mimulone (**5**) and diplacone (**7**) have been isolated previously.[14]



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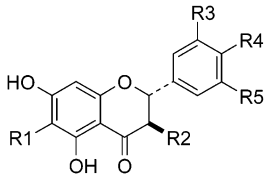

	R1	R2	R3	R4	R5
2	Geranyl	OH	OMe	OH	OMe
3	Prenyl	OH	OMe	OH	H
4	H	H	OMe	OH	OMe
5	Geranyl	H	H	OH	H
6	Geranyl	H	OMe	OH	H
7	Geranyl	H	OH	OH	H

The UV spectra of the compounds showed similar behavior, with maxima at λ 229−242 (sh), 289−296, and 335−345 (sh) nm, corresponding to the $\pi \rightarrow \pi^*$ and n $\rightarrow \pi^*$ electronic transitions matched to flavanone skeletons.[15] The IR spectra of the isolated compounds showed a similar series of absorption bands at ν_{max} 3500−3400 cm^{-1}, corresponding to OH vibrations; 2950−2850 cm^{-1}, corresponding to CH vibrations, which are not usually so apparent in flavonoid spectra; and 1639−1622 cm^{-1}, assigned to the C═O vibration of the carbonyl group.[15]

The molecular formula of tomentodiplacol (**1**) was determined on the basis of HRMS Q-TOF measurements that gave the pseudomolecular ion $[M - H]^-$ at *m*/*z* 469.1851 (calcd mass *m*/*z* 469.1862) as $C_{26}H_{30}O_8$. ESIMS spectra in the negative ionization

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Table 1. ^{1}H NMR Chemical Shifts (δ in ppm) and Indirect Spin−Spin Coupling Constants (J in Hz) of Compounds **1**−**4** in DMSO-d_6 at 303 K

	1	**2**	**3**	**4**
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
2	5.00 d (11.5)	4.94 d (11.4)	4.99 d (11.1)	5.41 dd (2.9; 13.0)
3	4.62 d (11.5)	4.61 d (11.4)	4.47 d (11.1)	2.68 dd (2.9; 17.1)[d]
				3.34 dd (13.0; 17.1)[e]
6				5.89 d
8	5.94 s	5.92 s	5.95 s	5.91 d
2′	7.08 d (1.8)	6.75 s	6.92[c]	6.79 s
5′	6.78 d (8.1)		6.93 d (8.2)	
6′	6.89 dd (1.8; 8.1)	6.75 s	6.88 dd (1.8; 8.2)	6.79 s
1″	3.13 d	3.14 d	3.12 d	
2″	5.13 t	5.09 t	5.13 t	
4″	1.70 s	1.66 s	1.62 s	
5″	1.85 m	1.87 t	1.70 s	
	1.94 m			
6″	1.45 m	1.96 t		
7″	3.80 q	5.00 t		
9″	1.61 s	1.56 s		
10″	4.70 s	1.49 s		
	4.81 s			
3′-OMe	3.78 s	3.73 s	3.78 s	3.77 s
5′-OMe		3.73 s		3.77 s
3-OH	5.71 d	5.71 s	5.74 d	
5-OH	12.19 s	12.16 s	12.16 s	12.15 s
7-OH	10.75 bs[a]	8.98[a]	*b*	*b*
4′-OH	9.07 s	8.47 bs	9.02 s	8.47 bs
7″-OH	4.60 s			

[a] Broad resonance. [b]Not obtained. [c]J not determined due to the overlap. [d]*cis* relative to H-2. [e]*trans* relative to H-2.

Table 2. ^{13}C NMR Chemical Shifts (δ_C in ppm) of Compounds **1**−**4** in DMSO-d_6 at 303 K

	1		**2**		**3**		**4**	
position	δ_C, mult.	HMBC[a]	δ_C, mult.	HMBC[a]	δ_C, mult.	HMBC[a]	δ_C, mult.	HMBC[a]
2	83.1, CH	3, 2′, 6′	83.5, CH	3, 2′, 6′	82.7, CH	3, 5′, 6′	78.9, CH	3a, 2′, 6′
3	71.4, CH	2	71.5, CH	2	71.5, CH	2	42.2, CH_2	
4	198.0, C_q	2, 3, 8	198.0, C_q	2, 8	197.6, C_q	2, 3	196.3, C_q	2, 3a, 3b, 6
4a	100.2, C_q	8	100.3, C_q	8	100.1, C_q	8	101.6, C_q	6
5	160.3, C_q	1″	160.5, C_q	1″	160.3, C_q	1″	163.2, C_q	6
6	107.8, C_q	8, 1″, 2″	107.9, C_q	8, 1″	107.7, C_q	8, 1″	95.8, CH	8
7	164.4, C_q	8, 1″	164.5, C_q	8, 1″	164.3, C_q	8, 1″	163.5, C_q	6, 8
8	94.4, CH		94.5, CH		94.3, CH		95.0, CH	6
8a	160.1, C_q	8	160.2, C_q	2, 8	160.0, C_q	2, 8	162.9, C_q	2
1′	128.2, C_q	2, 3, 2′, 5′	127.3, C_q	2, 3, 2′, 6′	129.8, C_q	2, 3, 2′	128.5, C_q	2, 3a, 2′, 6′
2′	112.2, CH	2, 5′, 6′	106.0, CH	2	111.8, CH		104.7, CH	2, 3b, 6′
3′	147.3, C_q	2′, 5′	147.7, C_q	2′, 6′	147.9, C_q	5′, 6′	147.8, C_q	2′, 6′
4′	147.0, C_q	6′	136.1, C_q	2′, 6′	146.1, C_q	2′	135.9, C_q	2′, 6′
5′	114.9, CH	2′	147.7, C_q	2′, 6′	114.9, CH	2, 6′	147.8, C_q	2′, 6′
6′	121.1, CH	2, 2′	106.0, CH	2	119.1,CH	2, 5′	104.7, CH	2, 2′
1″	20.5, CH_2	2″	20.6, CH_2	2″	20.6, CH_2	8, 2″		
2″	122.0, CH	1″, 4″, 5″	122.4, CH	1″, 4″, 5″	122.5, CH	1″, 4″, 5″		
3″	134.1, C_q	1″, 4″, 5″, 6″	133.9, C_q	1″, 4″, 5″	130.3, C_q	1″, 4″, 5″		
4″	16.0, CH_3	2″, 5″	15.9, CH_3	2″, 5″	25.4, CH_3	2″, 5″		
5″	35.2, CH_2	2″, 4″, 6″, 7″	39.3, CH_2	2″, 4″, 6″, 7″	17.6, CH_3	2″, 4″		
6″	33.4, CH_2	5″, 7″	26.3, CH_2	5″				
7″	73.4, CH	5″, 6″, 9″, 10″	124.2, CH	5″, 6″, 9″, 10″				
8″	148.2, C_q	6″, 7″, 9″, 10″	130.7, C_q	6″, 9″, 10″				
9″	17.5, CH_3	7″, 10″	25.5, CH_3	7″, 10″				
10″	109.8, CH_3	7″, 9″	17.5, CH_3	7″, 9″				
3′-OMe	55.7, CH_3		56.1, CH_3		55.7, CH_3		56.1, CH_3	
5′-OMe			56.1, CH_3				56.1, CH_3	

[a] ^{1}H−^{13}C HMBC (adjusted to 7.5 Hz) correlations are from the carbon(s) specified to the protons indicated.

mode showed the presence of a pseudomolecular ion $[M - H]^-$ at m/z 469. The constitution of compound **1** was determined by ^{1}H and ^{13}C NMR spectroscopy (Tables 1 and 2). COSY,[17] HSQC,[18] and HMBC[18] 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 2. The two singlets at δ 3.78 (3H) and 5.94 (1H) in the ^{1}H NMR spectrum of **1** were unequivocally assigned to the methyl group and H-8, respectively. Resonances at δ 6.78, 6.89, and 7.08 and their coupling patterns indicated a 3,4-disubstituted phenyl ring. The ^{1}H−^{13}C interactions observed in HMBC (Table 2) identified the connection of this substituted phenyl to C-2 (δ_C = 83.11 ppm). A modified geranyl-type side chain is bonded at C-6 (long-range interactions H1″−C6 and H2″−C6 were detected in the HMBC). A structure with a similar, unusual part of the side chain has been published recently.[19] The configuration at C_2−C_3 was determined to be *trans* on the basis of the magnitude of $^3J_{H2,H3}$ = 11.5 Hz. Generally, coupling constants for the *trans*

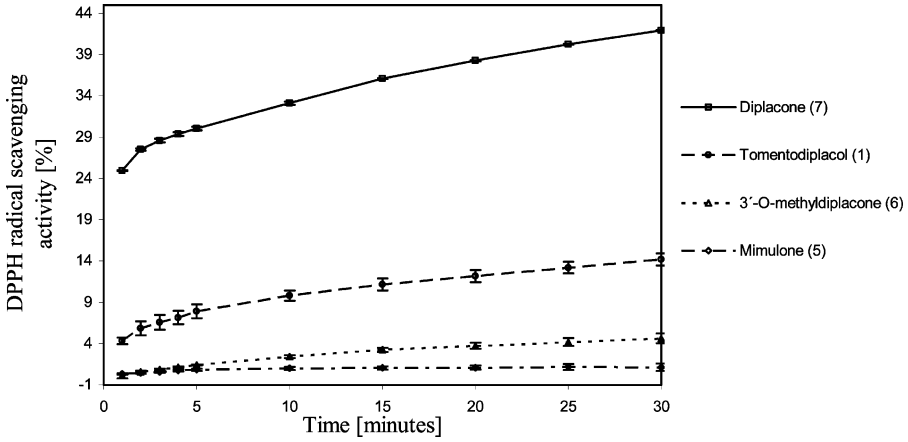

Figure 1. Time dependence of the antiradical activity of the compounds tested at a concentration of 10 μM.

arrangement[20,21] range between 11 and 12 Hz, whereas couplings for the *cis* configuration[22] are typically found in the range 1−3 Hz. A low amount of **1** prevented definition of the C-7″ configuration.

The molecular formula of 3′-*O*-methyl-5′-methoxydiplacol (**2**) was determined to be $C_{27}H_{32}O_8$ by HRMS Q-TOF and proved by the presence of the pseudomolecular ion $[M - H]^-$ at *m*/*z* 483.2015 (calcd mass *m*/*z* 483.2019). The ESIMS in the negative mode showed the presence of a pseudomolecular ion $[M - H]^-$ at *m*/*z* 483. The structure of compound **2** was determined by using 1D and 2D NMR spectroscopy (Tables 1 and 2) and the same methodology as for **1**. The ^1H NMR spectrum indicates a 3,4,5-trisubstituted phenyl group connected to carbon C-2. The ^1H and ^{13}C chemical shifts of the geranyl side chain attached to C-6 are similar to data of the known compounds mimulone (**5**)[23] and diplacone (**7**).[24] The *trans* configuration at C_2-C_3 was deduced from the coupling constant between H-2 and H-3. All observed long-range $^1H-^{13}C$ interactions are summarized in Table 2.

HRMS Q-TOF of 6-isopentenyl-3′-*O*-methyltaxifolin (**3**) gave a pseudomolecular ion $[M - H]^-$ at *m*/*z* 385.1297 (calcd mass *m*/*z* 385.1287). These data, together with the ^1H and ^{13}C NMR data, established the molecular formula of **3** as $C_{21}H_{22}O_7$. The ESIMS spectrum in the negative mode showed the presence of a pseudomolecular ion $[M - H]^-$ at *m*/*z* 385, which confirmed the deduced molecular weight of **3**, $M_r = 386$. The structure of compound **3** was determined by using 1D and 2D NMR (Tables 1 and 2). The important long-range $^1H-^{13}C$ interactions are summarized in Table 2. The substitution pattern of the phenyl ring of this compound is identical with that of compound **1**, as proved by the ^1H NMR spectrum. The prenyl chain is connected to C-6. The acquired data matched those found in the literature.[25] The configuration at C_2-C_3 was determined to be *trans* by the H2−H3 coupling constant.

The molecular formula of **4** was determined to be $C_{17}H_{16}O_7$ as proved by the HRMS Q-TOF and the pseudomolecular ion $[M - H]^-$ measured at *m*/*z* 331.0811 (calcd mass 331.0818). The ESIMS spectrum in the negative mode showed the presence of the pseudomolecular ion $[M - H]^-$ at *m*/*z* 331. Major fragments at *m*/*z* 177, 165, and 161 were obtained by MS/MS fragmentation of the parent ion *m*/*z* 331. All three of these fragments are linked to the cleavage of ring C of the flavonoid backbone.[16] The structure of compound **4** was elucidated by 1D and 2D NMR spectroscopy (Tables 1 and 2). The important long-range $^1H-^{13}C$ interactions are summarized in Table 2. The ^1H NMR spectrum shows that the substitution pattern of the phenyl ring is identical with that of **2**. Compound **4** was identified as dihydrotricin, a simple derivative of the flavone tricin, which is widely distributed in many plant species.[26]

Compounds **5**, **6**, and **7** were identified as the 6-geranylflavanones mimulone, 3′-*O*-methyldiplacone, and diplacone. These are major flavonoid constituents of the leaf resin of *Mimulus auranticus*,[23] and they have also been isolated from *M. clevelandii*.[24] The physical and spectroscopic data used to identify **5**, **6**, and **7** agreed with those reported previously.[23,24]

The absolute configurations at stereogenic centers C-2 and C-3 of the substances were determined by analyzing their circular dichroism spectra. A positive Cotton effect for the n → π^* electronic transition at 320−360 nm and negative Cotton effect for the $\pi \rightarrow \pi^*$ electronic transition at 280−310 nm were observed for compounds **1** and **3**−**7**. A 2*R*,3*R*-configuration was assigned to compounds **1** and **3** by comparison of CD and NMR data with those of flavanones.[27] The configuration for compounds **4**−**7** was determined as 2*S*.[27] No Cotton effect was observed in the CD spectrum of **2**. Since the arrangement at C-2/C-3 was determined as *trans* by NMR, compound **2** is a racemic mixture of 2*R*,3*R* and 2*S*,3*S* enantiomers.

The antiradical activity and cytotoxicity of some of the isolated compounds were tested using the DPPH and the NR cell-viability assays. The compounds tested showed significantly different antiradical activities at the concentration of 10 μM. The antioxidative capacity was expressed as Trolox equivalent antioxidant capacity, TEAC. The most effective scavenger was diplacone (**7**) (TEAC 5.2 ± 0.001). According to postulated theories, its antiradical activity corresponds with an *ortho*-dihydroxy functionality of the B ring, where the 4′-OH is the site of the donation of an electron and a proton to reduce diphenylpicrylhydrazil (DPPH•) radical to diphenylpicrylhydrazine (DPPH−H) and the 3′-OH assists in the formation of a stable flavonoid radical or in the termination reaction step.[28,29] In comparison, single 4′-OH substitution (**5**) (TEAC 0.4 ± 0.004) and 4′-OH substitution combined with 3′-OCH_3 (**1** and **6**) (TEAC 2.0 ± 0.007 and 0.8 ± 0.006) show significantly diminished activities. The geranyl side chain does not affect activity in a significant way, but it could modify the solubility of diplacone and eventually affects the reaction kinetics. The DPPH assay was performed using the modified method of Braca et al.;[30] the time dependence of the DPPH radical quenching, which effectively characterizes the antioxidants, was monitored. The compounds tested were found to be "slow scavengers", since the effectiveness of the DPPH quenching increased slowly during the 30 min of the assay. The time dependence of the quenching effect is shown in Figure 1.

The cytotoxicity of compounds **5**, **6**, and **7** was determined by using the NR cell-viability assay and by measuring of the capacity of cells (epithelioid cell line WB 344) to actively take up neutral red.[31] The calculated values of IC_{50} and the results are summarized in Table 3. A previous study (using HL-60 cells) showed that 4′-methoxy substitution or the loss of the 4′-hydroxy group significantly decreased the cytotoxicity of 4′-hydroxyflavanone,[32] while another study on flavanones showed no activity of taxifolin

Scheme 1

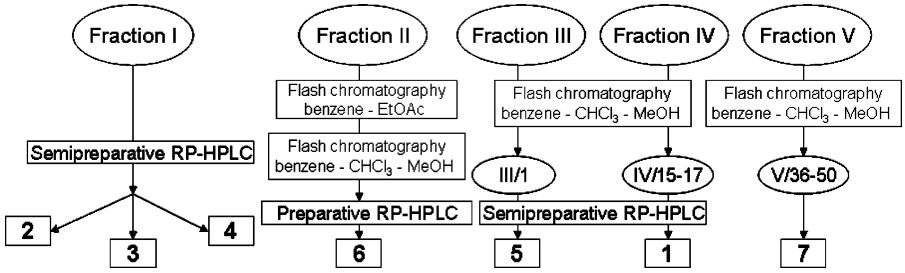

Table 3. Cytotoxicity of the Compounds Tested after a 24 h Treatment, as Determined by a Neutral Red Assay (expressed as % of the control − DMSO)[a]

	5 μM	25 μM	100 μM	IC_{50} [μM]
diplacone (**7**)	98 ± 0.03	57 ± 0.30	2 ± 0.00	14.3
3′-*O*-methyldiplacone (**6**)	100 ± 0.02	73 ± 0.05	2 ± 0.10	30.2
mimulone (**5**)	97 ± 0.03	95 ± 0.04	9± 0.01	*b*

[a] Each result is expressed as the mean ± SD of three independent measurements. [b] The IC_{50} could not be estimated because the cytotoxicity was less than 50% of that of the control.

(3,3′,4′,5,7-pentahydroxyflavanone) on colorectal carcinoma cells.[33] This agreed in part with our cytotoxicity data: diplacone (**7**) > mimulone (**5**) > tomentodiplacol (**1**). The *ortho*-dihydroxy substitution of ring B (**6**) enhances the cytotoxic effect, and 4′-methoxy substitution lowers the cytotoxicity on the WB 344 cell line. Insufficient amounts precluded testing of the other compounds.

The facts show that *P. tomentosa* is a medicinal plant with a high polyphenol secondary metabolism and with a terpenoid side chain incorporation into the flavonoid skeleton. The products of this metabolic pathway are liposoluble compounds with a high activity against UV radiation, which can be excreted onto the surface of the *P. tomentosa* fruits, where these flavonoids probably serve as antifeedants and as protection against excessive UV radiation.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage apparatus and are not corrected. UV spectra of MeOH solutions were recorded on a Hewlett-Packard 8453 spectrophotometer. CD spectra were recorded on a Jasco J-810 spectrometer (MeOH; molar elipticity Θ_λ values are presented). IR spectra were determined using the KBr disc method or ATR on a Nicolet Impact 400D FT-IR spectrophotometer.

NMR spectra were recorded using a Bruker Avance DRX 500 spectrometer operating at frequencies of 500.13 MHz (^{1}H) and 125.77 MHz (^{13}C). All NMR spectra were measured in DMSO-d_6 at 303 K. The ^{1}H and ^{13}C NMR chemical shifts (δ in ppm) were referenced to the resonance of the solvent [2.50 (^{1}H) for DMSO-d_5 and 39.50 (^{13}C) for DMSO-d_6]. The 2D NMR experiments, gradient-selected COSY, gs-HSQC, and gs-HMBC were used to assign the individual ^{13}C and ^{1}H resonances.[16,17] The gradient ratio for the ^{1}H−^{13}C HMBC experiment was 30:18:24 G cm^{-1}, and the experiment was adjusted for long-range couplings of 7.5 Hz.

ESIMS was done using an Agilent HP 1100 LC/MSD Trap VL Series and directly infusing MeOH solutions at a flow rate of 300 μL/min with a linear pump (kd Electronics). The spectra were collected in the negative mode; the nebulizing and drying gas was N_2 (t = 300 °C) flowing at a rate of 10 L/min; the nebulizer pressure was 80 psi and the capillary voltage 3.5 kV. The full mass scan covered the range from m/z 200 to 1500. Collision fragmentation experiments were performed in an ion trap, using helium as the collision gas. Full scan mass spectra, MS/MS of the selected pseudomolecular ion, and $(MS)^n$ (n = up to 3) were all collected in the negative mode.

The Q-TOF 2 instrument (Micromass/Waters, Manchester, UK) was calibrated in the mass range from m/z 80 to 600 using a 0.1% aqueous H_3PO_4/MeOH (50/50) solution. The same solution was added to the mobile phase at a flow rate of 0.5 μL/min through a T-piece just upstream from the ES interface. The phosphate cluster ions were used for internal mass reference in the acquired spectra. MeOH containing 0.1% HCOOH and flowing at a rate of 100 μL/min was used as the mobile phase. The precise masses of the compounds were obtained in the negative-ion mode by injecting 5 μL of a MeOH solution of the analyte and recording the spectra. The spectra were locked on a selected phosphate cluster ion near the deprotonated molecule. The accurate masses of the deprotonated molecules were compared with their calculated masses.

Column chromatography was performed using silica gel Lachema L 400/100. Flash chromatography was performed on silica gel Merck 60 (particle size 0.040−0.063 mm). Precoated silica gel 60 F254 (Merck) was used for the TLC analyses, with detection using UV light at 254 and 366 nm after spraying with Neu's reagent (1% diphenylaminoethylborate in MeOH) and heating to 110 °C for 10 min. Analytical and preparative HPLC were carried out on an Agilent 1100 instrument equipped with DAD. The columns used for analysis were filled with the stationary phase Supelcosil ABZ+Plus (column length 250 × 10 mm i.d., particle size 5 μm for the semipreparative analyses; column length 150 × 4.6 mm i.d., particle size 3 μm for the analytical HPLC). The mobile phase for the gradient elution was a mixture of MeCN and 40 mM HCOOH.

Plant Material. The fruits of *P. tomentosa* were collected in Brno (Czech Republic) during October of 2000. A voucher specimen (PT-02O) was deposited at the herbarium of the Department of Natural Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic.

Extraction and Isolation. The plant material (9.3 kg) was extracted with EtOH (4 L). The EtOH extract (500 g) was diluted to about 60% with H_2O (300 mL) and extracted with $CHCl_3$ (500 mL × 3). The $CHCl_3$ extract (84 g) was evaporated to dryness *in vacuo* at 45 °C and subjected to column chromatography on silica. It was eluted with benzene/acetone (95:5, 12 L) for fractions 1−83, benzene/acetone (9:1, 9 L) for fractions 84−147, benzene/acetone (8:2, 6.5 L) for fractions 148−193, benzene/acetone (7:3, 7 L) for fractions 194−240, and finally benzene/acetone (1:1, 5 L) for fractions 241−271. Fractions of about 150 mL were collected. Based on TLC analyses, the combined fractions I (26−30), II (55−70), III (109−112), IV (123−151), and V (180−189) were chosen for further work. Fractions III and IV were separated by FC on silica with benzene/$CHCl_3$/MeOH (7.4:2.5:0.1, 5 L), and subfractions of about 150 mL were collected. On the basis of the TLC analyses, the combined fractions III/1 and IV/15−17 were chosen for further work. Subfraction IV/15−17 (320 mg) was then separated by semipreparative RP-HPLC, using the stationary phase Supelcosil ABZ+Plus. The mobile phase consisted of (A) MeCN and (B) 40 mmol of HCOOH/MeCN (9:1). The gradient elution started with a mobile phase composition of A:B (65:35, v/v) and finished with a composition of A (100%). It required 15 min at a flow rate of 14.7 mL/min. The injection volume was 1 mL, and UV detection at λ 280 nm was used. Fractions were collected according to the detector response. Pure compound **1** (11 mg) was obtained from the subfraction IV/15−17. Subfraction III/1 (330 mg) was separated by the same method as described above for the subfraction IV/15−17 to give **5** (25 mg). Fraction II was repeatedly separated by flash chromatography with benzene/EtOAc (9:1, 1 L × 2) and benzene/$CHCl_3$/MeOH (7.4:2.5:0.1, 1 L × 2). The major compound **6** (120 mg) was then purified by preparative HPLC, using the method descibed above. Fraction V was separated by flash chromatography on silica with benzene/$CHCl_3$/MeOH (7.4:2.5:0.1, 2 L × 2, fractions of about 150 mL). Pure compound **7** (58 mg) precipitated from the subfraction V/36−50. Fraction I was separated directly by semipreparative chromatography, using a mobile phase consisting of (A) MeCN and (B) 40 mmol of HCOOH. The gradient elution started with a mobile phase composition of A:B (50:50, v/v) and finished with pure A (100%) after 20 min, at a flow rate

of 5 mL/min. The injection volume was 100 μL, and UV detection at λ 280 nm was used. Subfractions were collected according to the detector response, and compounds **2** (21 mg), **3** (15 mg), and **4** (12 mg) were obtained.

Tomentodiplacol (1): yellow powder; UV (MeOH) λ_{max} (log ϵ) 215 (4.12), 236 (sh), 295 (3.52), 341 (sh) nm; CD (MeOH) $\Theta_{332.5}$ +16 264, Θ_{296} −64 821.8, $\Theta_{224.5}$ 60 815.8; IR (KBr) ν_{max} 3419, 2929, 2853, 1636, 1517, 1463, 1276, 1164, 1118 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRMS Q-TOF [M − H]$^-$ *m*/*z* 469.1851 (calcd for $C_{26}H_{29}O_8^-$ 469.1862); ESIMS [M − H]$^-$ *m*/*z* 469.

3′-*O*-Methyl-5′-methoxydiplacol (2): yellow powder; UV (MeOH) λ_{max} (log ϵ) 214 (4.42), 229 (sh), 289 (4.23), 335 (sh) nm; IR (ATR) ν_{max} 3444−3328, 2922, 2909, 1634, 1604, 1515, 1457, 1381, 1330, 1157, 1002 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRMS Q-TOF [M − H]$^-$ *m*/*z* 483.2015 (calcd for $C_{27}H_{31}O_8^-$ 483.2019); ESIMS [M − H]$^-$ *m*/*z* 483.

6-Isopentenyl-3′-*O*-methyltaxifoline (3): yellow powder; UV (MeOH) (log ϵ) λ_{max} 212 (4.23), 229(sh), 289 (4.01), 335(sh) nm; CD (MeOH) $\Theta_{332.5}$ +8813.5, Θ_{296} −33 711.5, $\Theta_{224.5}$ +30 223.1; IR (ATR) ν_{max} 3444−3328, 2966, 2909, 1622, 1496, 1440, 1323, 1285, 1244, 1177, 1096 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRMS Q-TOF [M − H]$^-$ *m*/*z* 385.1297, (calcd for $C_{21}H_{21}O_7^-$ 385.1287); ESIMS [M − H]$^-$ *m*/*z* 385.

Dihydrotricin (4): yellow powder; UV (MeOH) λ_{max} (log ϵ) 218 (4.28), 242(sh), 296 (3.89), 345(sh) nm; CD (MeOH) Θ_{335} +2673.7, $\Theta_{295.5}$ −16 103.2, $\Theta_{224.5}$ +13 478.8; IR (ATR) ν_{max} 3361, 2919, 2850, 1625, 1515, 1452, 1338, 1270, 1211, 1155, 1109 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Tables 1 and 2.; HRMS Q-TOF [M − H]$^-$ *m*/*z* 331.0811 (calcd for $C_{17}H_{15}O_7^-$ 331.0818); ESIMS [M − H]$^-$ *m*/*z* 331.

Mimulone (5): yellow needles from MeOH; mp 120−122 °C; UV (MeOH) λ_{max} (log ϵ) 207 (4.38), 229 (sh), 295 (4.07), 336 (sh) nm; CD (MeOH) $\Theta_{331.5}$ +9065.3, Θ_{295} −30458.7, Θ_{221} +31649.7; IR (KBr) ν_{max} 3401, 2917, 2853, 1636, 1599, 1450, 1343, 1307, 1155, 1082 cm^{-1}; ^{1}H and ^{13}C NMR data in agreement with that published;[22,23] ESIMS [M − H]$^-$ *m*/*z* 407.

3′-*O*-Methyldiplacone (6): yellow powder; UV (MeOH) λ_{max} (log ϵ) 218 (4.26), 235 (sh), 290 (3.88), 345 (sh) nm; CD (MeOH) $\Theta_{331.5}$ +9132.7, $\Theta_{292.5}$ −43801.7, Θ_{220} +38376; IR (KBr) ν_{max} 3546, 2973, 2915, 1639, 1597, 1516, 1492, 1451, 1342, 1274, 1158, 1086 cm^{-1}; ^{1}H and ^{13}C NMR data in agreement with that published;[22,23] ESIMS [M − H]$^-$ *m*/*z* 437.

Diplacone (7): light brown powder; UV (MeOH) λ_{max} (log ϵ) 211 (4.20), 234 (sh), 290 (3.99), 345 (sh) nm; CD (MeOH) Θ_{331} +8058.3, Θ_{294} −29 856.6, Θ_{219} +32 587.5; IR (KBr) ν_{max} 3530, 3189, 2966, 2913, 1637, 1602, 1450, 1388, 1227, 1145, 1180 cm^{-1}; ^{1}H and ^{13}C NMR data in agreement with that published;[22,23] ESIMS [M − H]$^-$ *m*/*z* 423.

Cytotoxicity Assay. One hundred microliters of cells (BG/F) in Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, Prague, Czech Republic) was cultured in 96-well culture plates for approximately 24 h. The compounds to be tested were dissolved in DMSO and added to the wells (100 μL). Following a 24 h incubation, the solution was removed from all the plates and replaced with fresh DMEM containing neutral red (NR) at a concentration of 40 g/L. The viable cells were allowed to take up the supravital dye into lysosomes for 3 h. The cells were then washed twice with PBS, followed by fixation with 0.5% (v/v) formaldehyde supplemented with 1% (w/v) $CaCl_2$. After 15 min, the fixing solution was removed and the cells were washed with PBS and transferred to a 1% (v/v) HOAc, 50% (v/v) EtOH solution to extract the dye from the cells. The plates were then shaken gently on a plate shaker for 20 min at room temperature. The absorbance of the extracted dye was measured at 540 nm by means of a spectrophotometric microplate reader (Labsystems iEMS Reader, Labsystems, Turku, Finland). The percentage inhibition of cell growth was calculated as cell growth inhibition (%) = $(1 - T/C) \times 100$, where C was the OD_{540} value of the control and T was the OD_{540} value of the chemical tested. The 50% inhibitory concentrations (IC_{50} values) were obtained from dose−response curves.

DPPH-Quenching Assay. The modified method of Braca et al. was used to quantify the DPPH-quenching activity.[30] The MeOH DPPH (Sigma) solution was prepared at a concentration of 22 mg/L. The compounds to be tested were dissolved in MeOH at a concentration of 10 μM. A volume of 0.2 mL of the test solution was mixed with 1.8 mL of DPPH solution, and the absorbance of the mixture at 517 nm was measured every minute for the first 5 min of the experiment and then every 5 min for the next 25 min. The recorded data were used to draw the course of the increasing activity as a function of time, and the differences between the compounds tested were then compared. Results were expressed as Trolox equivalents.

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