Antioxidant and Free-Radical Scavenging Activity of Constituents of the Leaves of *Tachigalia paniculata*

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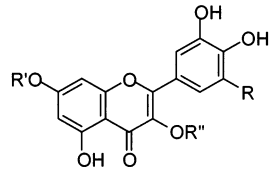
Two new myricetin glycosides, myricetin 7-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**1**) and myricetin 7-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**2**), together with the known compounds quercetin 3-*O*-*β*-D-glucopyranoside (**3**), quercetin 3-*O*-α-L-rhamnopyranoside (**4**), quercetin 3-*O*-*β*-D-galactopyranoside (**5**), methyl gallate (**6**), isovanillin (**7**), 4-hydroxymethylbenzoate (**8**), 3,4-dihydroxymethylbenzoate (**9**), and caffeoyl aldehyde (**10**) were isolated from the leaves of *Tachigalia paniculata*. The structures of these compounds were determined by spectroscopic methods. Their antioxidant activity was determined by measuring free-radical scavenging effects using three different assays, namely, the Trolox Equivalent Antioxidant Capacity (TEAC) assay, the coupled oxidation of *β*-carotene and linoleic acid (autoxidation assay), and the inhibition of xanthine oxidase activity. Compounds **1**, **2**, and **6** showed activity in the TEAC test, compounds **5**−**7** and **10** were moderately active in the autoxidation assay, while compounds **1** and **2** were the most potent of the isolates in the xanthine oxidase test.

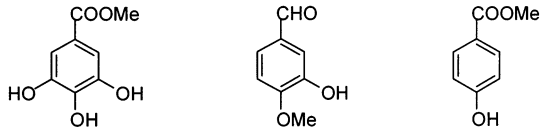
In the course of a systematic biochemical study on Latin American medicinal plants, we wish to report the phytochemical investigation of *Tachigalia paniculata* Aubl. (Leguminosae) leaves. This species is a perennial tree native to South America (Brazil, Colombia, Guyana, Peru, Venezuela),[1] where it is commonly known as "tachy" or "bergantin",[2] and its wood is used for commercial purposes. Previous studies on this species led to the isolation of 3-indoleacetic acid from the wood[3] and indole alkylamines from the inflorescence[4] of the plant.

In a series of preliminary screening for different biological activities, it was found that the chloroform−methanol and methanol extracts of the leaves of this plant possess interesting antioxidant activity in a bioautographic TLC assay.[5] Fractionation of these active extracts, using Sephadex LH-20 and HPLC, led to the isolation of two new flavonol glycosides, myricetin 7-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**1**) and myricetin 7-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**2**), along with the known compounds **3**−**10**.

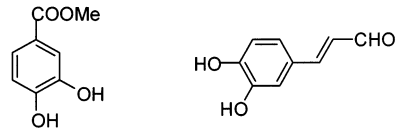
Phenolic natural products such as flavonoids are of particular interest because of their antioxidant activity through scavenging oxygen radicals and inhibiting peroxidation.[6] Antioxidants that scavenge free radicals play an important role in cardiovascular disease, aging, cancer, and inflammatory disorders. These observations have accelerated the search for potential antioxidant principles from traditional medicinal plants. The present paper deals with the structure elucidation of compounds **1** and **2**, as well as the antioxidant evaluation of all isolated compounds by radical-scavenging activity in the Trolox Equivalent Antioxidant Capacity (TEAC) assay,[7,8] the coupled oxidation of *β*-carotene and linoleic acid,[9,10] and a xanthine oxidase (XOD) activity assay.[11]



1	R=OH	R'=β-D-glc-(1→6)-β-D-glc	R''=H
2	R=OH	R'=α-L-rha-(1→6)-β-D-glc	R''=H
3	R=H	R'=H	R''=β-D-glc
4	R=H	R'=H	R''=α-L-rha
5	R=H	R'=H	R''=β-D-gal



6 **7** **8**



9 **10**

Results and Discussion

The dried leaves of *T. paniculata* were extracted successively with petroleum ether, $CHCl_3$, $CHCl_3$−MeOH (9:1), and MeOH. The methanolic extract was fractionated by a combination of gel filtration chromatography on Sephadex LH-20 and reversed-phase HPLC, to yield two new compounds (**1** and **2**). After spraying the TLC with Natural Products (Naturstoff) Reagent (NTS), they gave red spots typical of a 3′,4′,5′-trihydroxyflavonol. The structures of compounds **1** and **2** were determined by analysis of their spectral data (ESIMS, 1D and 2D NMR).

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Table 1. ^{13}C NMR Data (150 MHz, CD_3OD) and HMBC Correlations of Compounds **1** and **2**

	1		**2**	
	δ_C	HMBC	δ_C	HMBC
2	158.1	6.75, 7.00	157.9	6.73, 7.00
3	136.5		136.3	
4	177.0	6.49 weak, 6.75	178.0	6.51 weak, 6.73
5	164.5	6.49	164.3	6.51
6	100.0		100.1	
7	165.0	5.25, 6.75	165.2	5.10, 6.73
8	94.5	6.49	95.0	6.51
9	159.0	6.75	158.8	6.73
10	103.5	6.49, 6.75	103.8	6.51, 6.73
1′	123.0	7.00	123.0	7.00
2′	109.6		109.8	
3′	147.0		147.2	
4′	137.5	7.00	137.9	7.00
5′	147.0		147.2	
6′	109.6		109.8	
7-*O*-glc-1″	99.7		101.2	
2″	73.3		73.9	
3″	76.2		78.2	
4″	69.8		71.4	
5″	77.8		77.2	
6″	67.0	4.72	67.4	4.80
glc-1‴	100.3			
2‴	74.0			
3‴	78.1			
4‴	70.1			
5‴	78.2			
6‴	61.5			
rha-1‴			102.4	
2‴			72.1	
3‴			72.4	
4‴			74.0	
5‴			69.7	
6‴			17.8	

Compound **1** was assigned a molecular formula of $C_{27}H_{30}O_{18}$. Analysis of its MS, ^{13}C, and ^{13}C DEPT NMR data indicated that it was a flavonoid with 15 carbon atoms assigned to the aglycon and 12 carbons to the sugar moieties. The 600 MHz ^1H NMR spectrum indicated a 5,7-dihydroxylated pattern for ring A (two *meta*-coupled doublets at δ 6.49 and 6.75, J = 1.8 Hz) and a 3′,4′,5′-trihydroxylation pattern for ring B (two-proton singlet at δ 7.00), allowing the aglycon to be recognized as myricetin.[12] Two anomeric protons were also identified in this spectrum (see Experimental Section) and resonated at δ 5.25 (d, J = 7.8 Hz) and 4.72 (d, J = 8.0 Hz) and correlated, respectively, with signals at δ 99.7 and 100.3 in the HSQC spectrum. Analysis of the chemical shifts, signal multiplicities, the absolute values of the coupling constants, and their magnitude in the ^1H NMR spectrum, as well as ^{13}C NMR data (Table 1), indicated the presence of two glucopyranosyl residues with β-configuration at the anomeric carbons.[12] The $^1H-^1H$ COSY spectra together with the 1D TOCSY experiment allowed the assignment of the spin systems of each sugar residue. In particular, the lower field shift of H-6″ (δ 3.76, 3.98) and C-6″ (δ 67.0) of one glucosyl unit suggested the substitution site of the other glucosyl residue. Unequivocal assignments were obtained from the 2D NMR spectra; the HMBC experiment showed correlations between δ 4.72 (H-1‴) and 67.0 ppm (C-6″) and δ 5.25 (H-1″) and 165.0 ppm (C-7). Therefore, compound **1** was determined to be myricetin 7-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Compound **2** was assigned the molecular formula $C_{27}H_{30}O_{17}$ from its ESIMS, ^{13}C NMR (Table 1), and DEPT data. When **1** was used as a reference compound in the spectral analysis of compound **2**, close similarities were observed in the spectral data of the aglycon portion of both compounds, while the sugar moieties provided the points of difference. Except for the aglycon signals, the ^1H NMR spectrum of **2** revealed the presence of two one-proton doublets at δ 5.10 (J = 7.8 Hz) and 4.80 (J = 1.5 Hz), representative of two anomeric protons, together with a methyl doublet at δ 1.10 (J = 6.0 Hz) (see Experimental Section). Selected 1D TOCSY data obtained by irradiating the anomeric proton signals and the methyl doublet yielded a subspectrum of the sugar residues with high digital resolution. These 1D TOCSY data, when compared with those obtained from the ^{13}C NMR and HSQC experiments, allowed the identification of the sugars as glucose with a β-configuration and rhamnose with an α-configuration. The relative positions of the β-D-glucopyranose and α-L-rhamnopyranose units were established from HMBC correlations [δ 4.80 (H-1‴) with 67.4 ppm (C-6″) and δ 5.10 (H-1″) with 165.2 ppm (C-7)]. Compound **2** was therefore identified as myricetin 7-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

Table 2. Antioxidant Activities of Compounds **1**−**10** in the TEAC, Autoxidation Assay, and Inhibition of Xanthine Oxidase Assays[a]

	TEAC assay	autoxidation assay		xanthine oxidase assay
compd	(mM) ± SD[b]	t = 60 min	t = 120 min	IC_{50} (μM) ± SD[b]
1	1.620 ± 0.01	0	0	6.52 ± 0.1
2	1.420 ± 0.05	0	0	6.13 ± 1.3
3	0.757 ± 0.03	26.6	4.0	>50
4	0.854 ± 0.05	26.0	4.8	14.74 ± 1.0
5	1.100 ± 0.05	36.2	24.6	26.31 ± 1.1
6	1.154 ± 0.05	39.9	10.0	15.52 ± 1.4
7	0.144 ± 0.03	43.8	21.1	>50
8	0.147 ± 0.03	0	0	>50
9	0.855 ± 0.03	4.5	0	29.64 ± 2.4
10	0.713 ± 0.02	35.5	24.5	31.5 ± 1.2
quercetin	2.60 ± 0.02			3.00 ± 0.13
BHT[c]		71.3	64.1	

[a] For protocols used, see Experimental Section. [b] n = 3. [c] BHT = 2,6-di-*tert*-butyl-4-methoxyphenol; standard control substance.

Eight known derivatives were also isolated from the methanol and chloroform−methanol extracts of the plant by Sephadex LH-20 column chromatography followed by HPLC and were identified as quercetin 3-*O*-β-D-glucopyranoside (**3**),[12] quercetin 3-*O*-α-L-rhamnopyranoside (**4**),[13] quercetin 3-*O*-β-D-galactopyranoside (**5**),[14] methyl gallate (**6**),[15] isovanillin (**7**),[16] 4-hydroxymethylbenzoate (**8**),[17] 3,4-dihydroxymethylbenzoate (**9**),[18] and caffeoyl aldehyde (**10**),[19] by comparison with literature data.

Mechanisms of antioxidant action can include suppressing reactive oxygen species formation either by inhibition of enzymes or by chelating trace elements involved in free-radical production; scavenging reactive species; and upregulating or protecting antioxidant defenses. The antioxidant activity of compounds **1**−**10** was first studied in the TEAC assay. This method measures the relative ability of antioxidant substances to scavenge the radical cation 2,2′-azinobis(3-ethylbenzothiozoline-6-sulfonate) ($ABTS^{\bullet+}$) as compared to a standard amount of the synthetic antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), an aqueous soluble vitamin E analogue. The activity of the tested compounds was expressed as TEAC (Trolox Equivalent Antioxidant Capacity) values; TEAC value is defined as the concentration of standard Trolox with the same antioxidant capacity as a 1 mM concentration of the antioxidant compound under investigation. The results (Table 2) showed that compounds **1** and **2** exhibited free-radical scavenging activity of lesser potency than the reference antioxidant compound quercetin,[7] while compounds **3**−**6**, **9**, and **10** had weaker activities, and **7** and **8**

were inactive. The data obtained suggested that the major determinants for radical-scavenging capability are the presence of a catechol group in ring B of the flavonoid skeleton and a 2,3-double bond conjugated with the 4-oxo group, which is responsible for electron delocalization. The presence of a 3-hydroxyl group also increases the radical-scavenging activity, but the glycosidation of this group greatly reduces the radical-scavenging capacity. The results of the antioxidative effect of compounds **1**−**10** on the autoxidation of linoleic acid are reported in Table 2. Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. In particular, linoleic acid and arachidonic acid are the target of lipid peroxidation. It is generally thought that the inhibition of lipid peroxidation by antioxidants may be due to their free-radical scavenging activities. Superoxide indirectly initiates lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and hydroxyl.[20] Hydroxyl radical eliminates hydrogen atoms from the membrane lipid, which results in lipid peroxidation. The data obtained showed that all compounds tested had only weak antioxidant activity (AA) or were not active compared with that of the standard phenolic antioxidant 2,6-di-*tert*-butyl-4-methoxyphenol (BHT). Finally, the activity of the 10 compounds as inhibitors against the production of uric acid from xanthine in the oxidation reaction catalyzed by xanthine oxidase (XOD) was studied (Table 2). Inhibition of xanthine oxidase resulted in a decreased production of uric acid, which could be measured spectrophotometrically. The enzymatic oxidation of xanthine to uric acid was inhibited by four out of the 10 compounds investigated, namely, compounds **1**, **2**, **4**, and **6**. The potency of their inhibitory action was encompassed within a narrow range of IC_{50} values of 6−7 μM for myricetin derivatives **1** and **2** and IC_{50} values of 14−20 μM for quercetin glycosides **4** and **6**. Quercetin, the positive control, showed an IC_{50} value of 3 μM. Except for **6**, the phenolic compounds showed weak activity.[21] Our results for the antioxidant activity of flavonol glycosides **1**−**5** agreed with those reported in the literature: a free 3-hydroxyl group enhanced the activity compared with *O*-glycosylation at C-3 (compounds **1** and **2** were more active than **3**−**5**), while *ortho*-dihydroxylation contributed markedly to the antioxidant activity in all compounds tested.[22]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. UV spectra were recorded on a Perkin-Elmer-Lambda 12 spectrophotometer. A Bruker DRX-600 NMR spectrometer, operating at 599.19 MHz for ^{1}H and 150.86 MHz for ^{13}C, using the UXNMR software package, was used for NMR experiments; chemical shifts are expressed in δ (ppm) referring to the solvent peaks δ_H 3.34 and δ_C 49.0 for CD_3OD. DEPT ^{13}C, 1D TOCSY, ^{1}H−^{1}H DQF-COSY, ^{1}H−^{13}C HSQC, and HMBC NMR experiments were carried out using the conventional pulse sequences as described in the literature. ESIMS (positive mode) were obtained from a Finningan LC-Q Deca Termoquest spectrometer, equipped with an Excalibur software. Column chromatography was performed over Sephadex LH-20 (Pharmacia); HPLC separations were conducted on a Waters 590 series pumping system equipped with a Waters R401 refractive index detector and with a Waters μ-Bondapak C_{18} column and U6K injector.

Plant Material. Leaves of *T. paniculata* Aubl. were collected from the Amazonas region of Venezuela, near the Cuao River, in August 1998. The plant material was identified by Prof. Anibal Castillo, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela, where a voucher specimen was deposited (voucher number VEN 315048).

Extraction and Isolation. The air-dried powdered leaves of *T. paniculata* (200 g) were defatted with petroleum ether and successively extracted for 48 h with $CHCl_3$, $CHCl_3$−MeOH (9:1), and MeOH, by exhaustive maceration (3 × 2 L), to give 7.0, 3.2, 3.0, and 10.0 g of the respective residues. Part of the methanol extract (2.0 g) was chromatographed on Sephadex LH-20, using MeOH as eluent, to obtain 15 major fractions. Fractions 9 (100 mg) and 11 (65.2 mg) were separately purified by RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL min^{-1}) with MeOH−H_2O (1:1) to yield pure compounds **1** (t_R = 13 min, 2.0 mg), **5** (t_R = 17 min, 6.0 mg), **2** (t_R = 18 min, 1.0 mg), and **4** (t_R = 20 min, 2.0 mg) from fraction 9 and compounds **1** (t_R = 13 min 10.0 mg), **2** (t_R = 18 min 6.0 mg), and **3** (t_R = 19 min, 2.0 mg) from fraction 11, respectively. Fraction 12 (70.0 mg), under the same conditions, yielded compounds **3** (t_R = 19 min, 2.0 mg) and **4** (t_R = 20 min, 2.0 mg). Part of the chloroform−methanol residue was submitted to a Sephadex LH-20 column using MeOH as eluent to obtain 10 major fractions. Fractions 8 (57 mg), 9 (130 mg), and 10 (97 mg) were separately purified by RP-HPLC on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL min^{-1}) with MeOH−H_2O (3:7) to give pure compound **8** (t_R = 12 min, 3.0 mg) from fraction 8, compounds **6** (t_R = 8 min, 10.0 mg) and **7** (t_R = 28 min, 8.0 mg) from fraction 9, and compounds **9** (t_R = 15 min, 4.0 mg) and **10** (t_R = 25 min, 3.0 mg) from fraction 10, respectively.

Myricetin 7-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (1): yellow amorphous powder; $[\alpha]_D^{25}$ −72° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 270 (4.31), 360 (3.75) nm; IR (KBr) ν_{max} 3400, 3020, 1650, 1610, 1570 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ 3.26 (1H, t, *J* = 9.0 Hz, H-3‴), 3.35 (1H, t, *J* = 9.0 Hz, H-4‴), 3.38 (1H, br t, *J* = 9.0 Hz, H-2‴), 3.42 (1H, m, H-5‴), 3.48 (1H, m, H-5″), 3.58 (1H, t, *J* = 9.0 Hz, H-3″), 3.68 (1H, dd, *J* = 12.2, 2.0 Hz, H-6‴a), 3.76 (1H, dd, *J* = 12.0, 2.0 Hz, H-6″a), 3.83 (1H, br t, *J* = 9.0 Hz, H-2″), 3.86 (1H, t, *J* = 9.0 Hz, H-4″), 3.92 (1H, dd, *J* = 12.2, 5.0 Hz, H-6‴b), 3.98 (1H, dd, *J* = 12.0, 5.0 Hz, H-6″b), 4.72 (1H, d, *J* = 8.0 Hz, H-1‴), 5.25 (1H, d, *J* = 7.8 Hz, H-1″), 6.49 (1H, d, *J* = 1.8 Hz, H-6), 6.75 (1H, d, *J* = 1.8 Hz, H-8), 7.00 (2H, s, H-2′, H-6′); ^{13}C NMR (600 MHz, CD_3OD), see Table 1; ESIMS *m*/*z* 643 [M + 1]$^+$; *anal.* C 50.39%, H 4.73%, O 44.88%, calcd for $C_{27}H_{30}O_{18}$, C 50.47%, H 4.71%, O 44.82%.

Myricetin 7-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (2): yellow amorphous powder; $[\alpha]_D^{25}$ −121° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 272 (4.03), 365 (3.67) nm; IR (KBr) ν_{max} 3450, 3000, 1660, 1605, 1555 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ 1.10 (3H, d, *J* = 6.0 Hz, H-6‴), 3.45 (1H, t, *J* = 9.0 Hz, H-3″), 3.46 (1H, t, *J* = 9.0 Hz, H-4″), 3.50 (1H, br t, *J* = 9.0 Hz, H-2″), 3.52 (1H, m, H-5″), 3.55 (1H, t, *J* = 8.5 Hz, H-4‴), 3.70 (1H, dd, *J* = 12.0, 2.0 Hz, H-6″a), 3.84 (1H, m, H-2‴), 3.88 (1H, dd, *J* = 8.5, 3.5 Hz, H-3‴), 4.00 (1H, dd, *J* = 12.0, 5.0 Hz, H-6″b), 4.15 (1H, m, H-5‴), 4.80 (1H, d, *J* = 1.5 Hz, H-1‴), 5.10 (1H, d, *J* = 7.8 Hz, H-1″), 6.51 (1H, d, *J* = 1.8 Hz, H-6), 6.73 (1H, d, *J* = 1.8 Hz, H-8), 7.00 (2H, s, H-2′, H-6′); ^{13}C NMR (600 MHz, CD_3OD), see Table 1; ESIMS *m*/*z* 627 [M + 1]$^+$; *anal.* C 51.71%, H 4.84%, O 43.45%, calcd for $C_{27}H_{30}O_{17}$, C 51.76%, H 4.83%, O 43.41%.

TEAC Test. Pure compounds were tested by using the Trolox Equivalent Antioxidant Capacity (TEAC) assay. The TEAC value is based on the ability of the antioxidant to scavenge the radical cation 2,2′-azinobis(3-ethylbenzothiozoline-6-sulfonate) ($ABTS^{\bullet+}$) with spectrophotometric analysis.[7] The $ABTS^{\bullet+}$ cation radical was produced by the reaction between 7 mM ABTS in H_2O and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. The $ABTS^{\bullet+}$ solution was then diluted with PBS (pH = 7.4) to an absorbance of 0.70 at 734 nm and equilibrated at 30 °C. Samples were diluted with methanol to produce solutions of 0.3, 0.5, 1, 1.5, and 2 mM concentration. The reaction was initiated by the addition of 1 mL of diluted ABTS to 10 μL of each sample solution. Determinations were repeated three times for each sample solution. The percentage inhibition of

absorbance at 734 nm was calculated for each concentration relative to a blank absorbance (methanol) and was plotted as a function of concentration compound or standard 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Aldrich Chemical Co., Gillingham, Dorset, U.K.). The antioxidant activities of compounds **1**−**10** are expressed as TEAC (Trolox Equivalent Antioxidant Capacity) values in comparison with TEAC activity of the reported reference compound quercetin.[7] Compounds are considered active when their TEAC is near that of quercetin, the reference compound. The TEAC value is defined as the concentration of standard Trolox with the same antioxidant capacity as a 1 mM concentration of the antioxidant compound under investigation.

Autoxidation of β-Carotene. Oxidation of linoleic acid was measured by the method described by Pratt.[10] Quantities of linoleic acid (20 mg) and Tween 20 (200 mg) were placed in a flask, and a solution of 2 mg of β-carotene in 10 mL of $CHCl_3$ was added. After removal of $CHCl_3$, 50 mL of distilled water saturated with oxygen for 30 min was added. Aliquots (200 μL) of each compound, dissolved in ethanol to a 15 μg/mL solution, were added to each flask with shaking. Samples without test compounds were used as blanks, and a sample with 2,6-di-*tert*-butyl-4-methoxyphenol (BHT, Aldrich Chemical Co., Gillingham, Dorset, U.K.) was used as a control substance. Samples were subjected to oxidation by placing in an oven at 50 °C for 3 h. The absorbance was read at 470 nm at regular intervals. The antioxidant activity was expressed as AA and calculated with the equation

$$AA = [1 - (A_0 - A_t)/(A_{00} - A_{0t})] \times 100$$

A_0 = absorbance at the beginning of the incubation, with test compound; A_t = absorbance at the time t, with test compound; A_{00} = absorbance at beginning of the incubation, without test compound; A_{0t} = absorbance at the time t, without test compound. Compounds are considered active when their AA is close to that of BHT, the control substance.

Xanthine Oxidase Activity Assay. Xanthine oxidase (XOD) activity was evaluated by the spectrophotometric measurement of the formation of uric acid by xanthine. An aliquot of a 100 μM solution of xanthine in 0.1 M phosphate buffer pH 7.8 with 0.04 units/mL of XOD was incubated for 10 min at room temperature (total volume 1 mL) and read at 295 nm against a blank sample that did not contain the enzyme. Different concentrations of test compounds were added to samples before the enzyme had been added, and their effect on the generation of uric acid was used to calculate regression lines and IC_{50} values.[11] Quercetin was used as reference compound: compounds were considered active when their IC_{50} is close to that of quercetin. Xanthine and XOD were obtained from Sigma Aldrich (Gillingham, Dorset, U.K.).

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Note Added after ASAP. In the version posted on the Web on Sept. 12, 2002, the paragraph in the Experimental Section headed Autoxidation of β-Carotene mentions linolenic acid. This has been corrected to linoleic acid in the version posted on Nov. 5, 2002.

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