# Antioxidant Chalcone Glycosides and Flavanones from *Maclura* (*Chlorophora*) *tinctoria*

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Four chalcone glycosides (**1**−**4**), including three new natural products, and three flavanones (**5**−**7**) were isolated from the methanol extract of stem bark of *Maclura tinctoria*. The new compounds have been characterized as 4′-*O*-*β*-D-(2″-*p*-coumaroyl)glucopyranosyl-4,2′,3′-trihydroxychalcone (**1**), 4′-*O*-*β*-D-(2″-*p*-coumaroyl-6″-acetyl)glucopyranosyl-4,2′,3′-trihydroxychalcone (**2**), and 3′-(3-methyl-2-butenyl)-4′-*O*-*β*-D-glucopyranosyl-4,2′-dihydroxychalcone (**3**); the known derivatives were elucidated as 4′-*O*-*β*-D-(2″-acetyl-6″-cinnamoyl)glucopyranosyl-4,2′,3′-trihydroxychalcone (**4**), eriodictyol 7-*O*-*β*-D-glucopyranoside (**5**), naringenin (**6**), and naringenin 4′-*O*-*β*-D-glucopyranoside (**7**). Their structures were determined by 1D and 2D NMR and ESIMS. The antioxidant activity of all the isolated compounds was determined by measuring free-radical-scavenging effects using two different assays, namely, the Trolox Equivalent Antioxidant Capacity (TEAC) assay and the coupled oxidation of *β*-carotene and linoleic acid (autoxidation assay). The results showed that compound **3** was the most active in both antioxidant assays.

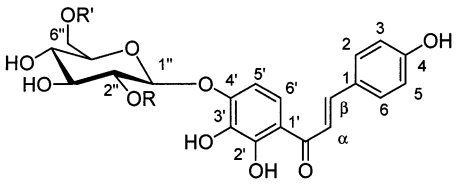
*Maclura* (*Chlorophora*) *tinctoria* L. (Gaud.) (Moraceae) is a tree growing in the humid and dry tropical forest of Central and South America, where it is known as “mora”, “moral”, and “palo de mora” and widely used by natives as medicine, material, and food. Its resin is used against toothache and also as colorant; its fruits are edible and eaten by the native hunters in the jungle.[1]

The isolation of flavonoids[2] and prenylated xanthones with anti-HIV activity[3] from the stem bark of *M. tinctoria* was recently reported, while antifungal chalcones were isolated from the leaves.[4] Morin, a pigment extractable from the wood of the plant, showed antioxidant activity protecting myocytes, endothelial cells, and erythrocytes against oxyradical damage.[5]

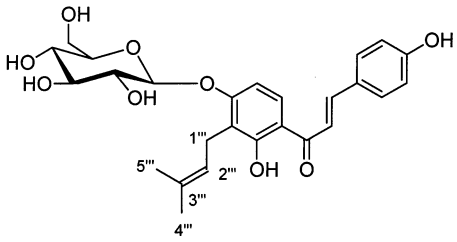
Recently, our research was directed toward the study of flavonoids in medicinal or food plants with antioxidant activity to isolate new or known secondary metabolites that could confer significant health benefits as bioactive non-nutrients, playing an important role in cardiovascular disease, aging, cancer, and inflammatory disorders.[6,7] Consequently, the present work deals with the isolation, structural characterization, and antioxidant evaluation by radical-scavenging activity in the Trolox Equivalent Antioxidant Capacity (TEAC) assay[8,9] and the coupled oxidation of *β*-carotene and linoleic acid[10,11] of four chalcones, including three new natural products and three flavanones from the stem bark of *M. tinctoria*.

## Results and Discussion

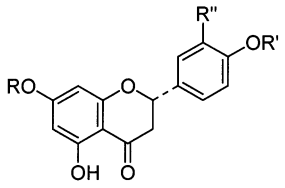
The methanol extract of *M. tinctoria* stem bark showed antioxidant activity in one preliminary assay conducted to determine free-radical-scavenging activity of some South American medicinal and food plants. This prompted us to search for antioxidant compounds from this plant. Fractionation of the methanol extract by Sephadex LH-20 and RP-HPLC led to the isolation of three new chalcone glycosides, compounds **1**−**3**. Their structures were determined by analysis of their spectral data (ESIMS, 1D and 2D NMR).



**1** R = *p*-coumaroyl R′ = H
**2** R = *p*-coumaroyl R′ = Ac
**4** R = Ac R′ = cinnamoyl



**3**



**5** R = β-D-glc R′ = H R″ = OH
**6** R = R′ = R″ = H
**7** R = R″ = H R′ = β-D-glc

Compound **1** was assigned a molecular formula $C_{30}H_{28}O_{12}$. Its ESIMS exhibited a peak at *m*/*z* 581 $[M + H]^+$ together with other ion fragments at *m*/*z* 435 $[(M + H) - 146]^+$ due

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to the loss of a *p*-coumaroyl moiety and 273 [(M + H) − 146 − 162]$^+$ ascribable to the loss of an additional glucopyranosyl moiety. The chalcone structure of **1** was indicated by the UV bands at 293, 316, and 380 nm. Analysis of its MS, $^{13}$C, and $^{13}$C DEPT NMR data indicated the presence of 30 carbon signals, of which 15 carbon atoms were assigned to the aglycon, six carbons to the sugar moiety, and nine carbons to the acyl residue. The 600 MHz $^1$H NMR spectrum indicated the characteristic pattern of a chalcone with two *ortho*-H atoms in the A-ring at $\delta$ 6.95 (d, $J$ = 8.0 Hz) and 7.25 (d, $J$ = 8.0 Hz) and a 4-substituted B-ring, permitting the identification of the aglycone as 4,2′,3′,4′-tetrahydroxychalcone, commonly known as okanin.[12] The four doublets at $\delta$ 6.38 ($J$ = 16.0 Hz), 6.73 ($J$ = 8.0 Hz), 7.41 ($J$ = 16.0 Hz), and 7.46 ($J$ = 8.0 Hz) were attibuted to a *p*-coumaroyl group, whereas the anomeric proton at $\delta$ 5.15 (d, $J$ = 7.5 Hz) was assigned, with the help of 1D-TOCSY and HSQC experiments, to a $\beta$-D-glucopyranose moiety. A signal at $\delta$ 4.45 (1H, t, $J$ = 8.6 Hz) indicated acylation of the glucose.[13] The $^1$H−$^1$H COSY spectra together with the 1D-TOCSY experiments permitted complete sequential assignment for all proton resonances of the glucose unit starting from the anomeric proton and showing that the hydroxyl group at C-2″ was acylated. A comprehensive analysis of the HSQC and HMBC spectra of **1** permitted the complete assignments of its proton and carbon signals and of the substitution sites. The HMBC experiment showed correlations between $\delta$ 5.15 (H-1″) and 151.0 ppm (C-4′), $\delta$ 4.45 (H-2″) and 169.0 ppm (C-9‴), and $\delta$ 6.38 (H-8‴) and 169.0 (C-9‴), thus indicating that the $\beta$-D-glucose was linked at C-4′, while the *p*-coumaroyl group was placed at C-2″ of the glucosyl moiety. The structure of **1** was thus established as 4′-*O*-$\beta$-D-(2″-*p*-coumaroyl)glucopyranosyl-4,2′,3′-trihydroxychalcone.

The molecular formula of $C_{32}H_{30}O_{13}$ for **2** was provided by ESIMS (*m*/*z* 623 [M + H]$^+$) and elemental analysis. Its positive ESIMS showed, together with the quasi-molecular ion at *m*/*z* 623 [M + H]$^+$, peaks at *m*/*z* 477 [(M + H) − 146]$^+$, due to the loss of a *p*-coumaroyl moiety, and at *m*/*z* 435 [(M + H) − 146 − 42]$^+$ and 273 [(M + H) − 146 − 42 − 162]$^+$, ascribable to the loss of *p*-coumaroyl, acetyl, and glucopyranosyl moieties. The $^{13}$C NMR spectrum confirmed the presence of 32 carbons, and both the $^1$H and $^{13}$C NMR closely resembled those of compound **1**, except for the presence of a singlet at $\delta$ 1.98 in the $^1$H NMR and two carbon signals at $\delta$ 22.4 and 179.0, readily attributable to an acetyl group. The deshielding of H-2″ ($\delta$ 4.48) and C-2″ ($\delta$ 75.6), and H-6″ ($\delta$ 4.05, 4.68) and C-6″ ($\delta$ 64.8), of the glucosyl unit suggested the substitution sites of the *p*-coumaroyl and acetyl residues. The HMBC cross-peaks between $\delta$ 4.48 (H-2″) and 170.0 (C-9‴), $\delta$ 4.05 and 4.68 (H-6″) and 179.0 (*C*OCH$_3$), and $\delta$ 1.98 (CO*C*H$_3$) and 179.0 (*C*OCH$_3$) confirmed the location of the *p*-coumaroyl group at C-2″ and the acetyl moiety at C-6″, respectively. Compound **2** was therefore identified as 4′-*O*-$\beta$-D-(2″-*p*-coumaroyl-6″-acetyl)glucopyranosyl-4,2′,3′-trihydroxychalcone.

Compound **3** showed a molecular formula $C_{26}H_{30}O_9$ from ESIMS and elemental analysis. The ESIMS spectrum with peaks at *m*/*z* 487 [M + H]$^+$, 444 [(M + H) − 43]$^+$, 432 [(M + H) − 55]$^+$, and 325 [(M + H) − 162]$^+$ suggested the presence of a prenylated chalcone glycoside that was confirmed also by UV bands (256, 296, and 385 nm). The presence in the $^1$H NMR spectrum of **3** of the signals for two *ortho*-coupled aromatic protons at $\delta$ 6.78 (d, $J$ = 8.0 Hz) and 7.10 (d, $J$ = 8.0 Hz) required the placement of the prenyl group at C-3′, thus establishing the substitution pattern of the A-ring. Conversely, the 4-hydroxy substitution of the B-ring was supported by the multiplicities of the proton signals, which showed an *ortho*-coupled AA′XX′ system at $\delta$ 6.92 (d, $J$ = 8.0 Hz) and 7.28 (d, $J$ = 8.0 Hz). The side chain was determined to be a 3-methyl-2-butenyl group by the following $^1$H NMR and 1D-TOCSY spectral data: irradiation of the proton triplet at $\delta$ 5.09, assigned to the methine proton at C-2‴, showed connectivities to the split double doublet at $\delta$ 3.51 ($J$ = 12.0, 6.5 Hz) and 3.58 ($J$ = 12.0, 7.0 Hz), assigned to the methylene protons H-1‴, and a weak coupling with two methyl signals at $\delta$ 1.68 and 1.75, attributable to H-4‴ and H-5‴. Selected 1D-TOCSY data yielded the subspectrum of the sugar residue that was identified as glucose with a $\beta$-configuration at the anomeric proton by means of $^{13}$C NMR and HSQC experiments. The relative positions of the $\beta$-D-glucopyranose unit and the prenyl chain were established from HMBC correlations [$\delta$ 5.18 (H-1″) with 159.0 ppm (C-4′) and $\delta$ 3.50 and 3.58 (H-1‴) with 115.0 ppm (C-3′) and 159.0 (C-4′)]. Thus, compound **3** was identified as 3′-(3-methyl-2-butenyl)-4′-*O*-$\beta$-D-glucopyranosyl-4,2′-dihydroxychalcone.

Four known derivatives were also isolated from the methanol extract by Sephadex LH-20 column chromatography followed by HPLC and were identified as 4′-*O*-$\beta$-D-(2″-acetyl-6″-cinnamoyl)glucopyranosyl-4,2′,3′-trihydroxychalcone (**4**),[14] (2*S*)-eriodictyol 7-*O*-$\beta$-D-glucopyranoside (**5**),[15] (2*S*)-naringenin (**6**),[15] and (2*S*)-naringenin 4′-*O*-$\beta$-D-glucopyranoside (**7**),[16] by comparison with literature data.

Phenolic derivatives in the human diet may exert a beneficial health effect via protecting against some diseases, including coronary heart disease and some cancers. Their antioxidant activity is mainly due to their redox properties, which can play an important role in neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. The antioxidant activity of compounds **1**−**7** isolated from *M. tinctoria* stem bark 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-ethylbenzothiazoline-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. TEAC results for compounds **1**−**7** and results for quercetin, used as reference compound, are summarized in Table 2. Compounds **1** and **3** had free-radical-scavenging activity of less potency with respect to the reference antioxidant quercetin,[8] compounds **2** and **4** had moderate activity, while compounds **5**−**7** were weakly active. The antioxidative effect of pure compounds **1**−**7** on the autoxidation of linoleic acid was also determined. The values of antioxidant activity (AA) measured at $t$ = 60 and 120 min, employing bleaching of $\beta$-carotene as model system, are reported in Table 2. Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Especially, linoleic acid and arachidonic acid are the targets of lipid peroxidation. It is generally thought that the inhibition of lipid peroxidation by antioxidants may be due to their free-radical-scavenging activities. The data obtained showed that compounds **1**−**4** had a good antioxidant activity; chalcone **3** was the most active compound

**Table 1.** $^{13}C$ NMR Data (150 MHz, $CD_3OD$) of Compounds **1**−**3**

| | $\delta_C$ | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| 1 | 127.0 | 127.0 | 127.8 |
| 2 | 130.8 | 130.3 | 130.0 |
| 3 | 118.0 | 118.2 | 119.0 |
| 4 | 162.0 | 160.9 | 162.0 |
| 5 | 118.0 | 118.2 | 119.0 |
| 6 | 130.8 | 130.3 | 130.0 |
| α | 118.0 | 118.2 | 118.0 |
| *β* | 147.3 | 144.8 | 147.5 |
| CO | 198.0 | 198.3 | 198.0 |
| 1′ | 118.0 | 117.2 | 120.0 |
| 2′ | 155.0 | 154.0 | 163.0 |
| 3′ | 137.0 | 137.0 | 115.0 |
| 4′ | 151.0 | 151.0 | 159.0 |
| 5′ | 110.0 | 109.0 | 108.0 |
| 6′ | 121.1 | 121.2 | 120.0 |
| 1″ | 99.4 | 99.8 | 99.4 |
| 2″ | 75.8 | 75.6 | 74.0 |
| 3″ | 76.8 | 76.9 | 77.9 |
| 4″ | 71.5 | 71.0 | 71.2 |
| 5″ | 77.8 | 76.4 | 78.0 |
| 6″ | 61.5 | 64.8 | 61.5 |
| 1‴ | 124.0 | 127.3 | 28.2 |
| 2‴ | 130.0 | 130.9 | 127.0 |
| 3‴ | 116.7 | 116.4 | 135.5 |
| 4‴ | 161.2 | 161.0 | 17.5 |
| 5‴ | 116.7 | 116.4 | 21.5 |
| 6‴ | 130.0 | 130.9 | |
| 7‴ | 146.8 | 147.3 | |
| 8‴ | 118.0 | 115.0 | |
| 9‴ | 169.0 | 170.0 | |
| $CO\mathit{CH}_3$ | | 22.4 | |
| $\mathit{CO}CH_3$ | | 179.0 | |

**Table 2.** Antioxidant Activities of Compounds **1**−**7** in the TEAC and Autoxidation Assay[a]

| compound | TEAC assay (mM) ± SD[b] | autoxidation assay | |
|---|---|---|---|
| | | $t = 60$ min | $t = 120$ min |
| **1** | 1.930 ± 0.05 | 52.3 | 41.1 |
| **2** | 1.710 ± 0.04 | 48.2 | 33.3 |
| **3** | 2.263 ± 0.03 | 58.0 | 45.6 |
| **4** | 1.600 ± 0.03 | 46.2 | 29.8 |
| **5** | 1.263 ± 0.05 | 22.3 | 8.2 |
| **6** | 0.770 ± 0.05 | 21.0 | 5.7 |
| **7** | 0.782 ± 0.04 | 20.3 | 4.5 |
| quercetin | 2.60 ± 0.03 | | |
| 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.

compared with the standard phenolic antioxidant 2,6-di-*tert*-butyl-4-methoxyphenol (BHT).

From our results for the antioxidant activity of compounds **1**−**7** it seems that chalcones are more active than flavanones. Chalcones are unique in the flavonoid family in lacking a heterocyclic C-ring. There are only a few literature reports on the antioxidant activity of chalcones. Studies on structure−activity relationship reported that the presence of an α,*β*-double bond, hydroxyl, and methoxyl groups in the A- and B-rings appear to be important in the antioxidant activity.[17] Chalcone **3** is the most active compound in both antioxidant assays: prenylation may increase the antioxidant activity of hydroxylated chalcones. Flavanones **5**−**7**, due to the lack of conjugation provided by the 2,3-double bond with the 4-oxo group, are weak antioxidants. Nevertheless, compound **5**, having a cathecol group in ring B, is more active than flavanones **6** and **7**.[18]

## 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 Beckman DU 670 spectrophotometer. A Bruker DRX-600 NMR spectrometer, operating at 599.19 MHz for $^1H$ and 150.86 MHz for $^{13}C$, using the UXNMR software package was used for NMR experiments; chemical shifts are expressed in $\delta$ (ppm) referring to the solvent peaks $\delta_H$ 3.34 and $\delta_C$ 49.0 for $CD_3OD$. DEPT $^{13}C$, 1D-TOCSY, $^1H-^1H$ DQF-COSY, $^1H-^{13}C$ 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 Xcalibur 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 $\mu$-Bondapak $C_{18}$ column and U6K injector.

**Plant Material.** The stem bark of *M. tinctoria* L. (Gaud.) was collected at Santa Cruz, Bolivia, in September 1998 and identified by Lourdes Vargas and Michel Rossy, Herbario Nacional de Bolivia, Universidad Mayor de San Andres, La Paz, Bolivia, where a voucher specimen was deposited.

**Extraction and Isolation.** The air-dried powdered stem bark of *M. tinctoria* (80 g) was extracted by accelerated solvent extraction (ASE) with the following order of solvents: *n*-hexane, $CHCl_3$, $CHCl_3$−MeOH (9:1), and MeOH. Part of the methanol residue (2.5 g) was chromatographed on Sephadex LH-20 (100 × 5 cm, flow rate 1.2 mL $min^{-1}$), using MeOH as eluent, to obtain 70 fractions of 12 mL that were grouped in four groups, A, B, C, and D, by TLC results on silica gel plates [eluents: *n*-BuOH−AcOH−$H_2O$ (60:15:25) and $CHCl_3$−MeOH−$H_2O$ (40:9:1)]. Fraction A (120 mg) was purified by RP-HPLC on a $C_{18}$ $\mu$-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL $min^{-1}$) with MeOH−$H_2O$ (4.5:5.5) to give pure compounds **5** ($t_R$ = 9 min, 8.0 mg) and **7** ($t_R$ = 13 min, 5.0 mg). Fraction B (570 mg) was partitioned between *n*-BuOH and $H_2O$, and the organic phase (80 mg) was fractionated by RP-HPLC on a $C_{18}$ $\mu$-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL $min^{-1}$) with MeOH−$H_2O$ (8.5:1.5) to yield pure compounds **1** ($t_R$ = 12 min, 7.0 mg) and **2** ($t_R$ = 15 min, 11.0 mg). In the same way, fraction C (296 mg) was partitioned between *n*-BuOH−$H_2O$, and the 1-butanol fraction (65 mg) was chromatographed over RP-HPLC on a $C_{18}$ $\mu$-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL $min^{-1}$) with MeOH−$H_2O$ (8.5:1.5) to give pure compounds **1** ($t_R$ = 12 min, 3.0 mg), **3** ($t_R$ = 14 min, 6.0 mg), and **4** ($t_R$ = 14 min, 6.5 mg). Fraction D contained pure compound **6** (15.0 mg).

**4′-*O*-*β*-D-(2″-*p*-Coumaroyl)glucopyranosyl-4,2′,3′-trihydroxychalcone (1):** yellow amorphous powder; [α] +62° (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 293 (5.71), 316 (4.75), 380 (3.42) nm; $^1H$ NMR (600 MHz, $CD_3OD$) $\delta$ 3.42 (1H, t, $J$ = 9.0 Hz, H-4″), 3.48 (1H, t, $J$ = 9.0 Hz, H-3″), 3.50 (1H, m, H-5″), 3.72 (1H, dd, $J$ = 12.0, 3.5 Hz, H-6″a), 3.93 (1H, dd, $J$ = 12.0, 5.5 Hz, H-6″b), 4.45 (1H, br t, $J$ = 8.6 Hz, H-2″), 5.15 (1H, d, $J$ = 7.5 Hz, H-1″), 6.38 (1H, d, $J$ = 16.0 Hz, H-8‴), 6.73 (2H, d, $J$ = 8.0 Hz, H-3‴, H-5‴), 6.80 (1H, d, $J$ = 8.0 Hz, H-5′), 6.95 (2H, d, $J$ = 8.0 Hz, H-2, H-6), 7.25 (2H, d, $J$ = 8.0 Hz, H-3, H-5), 7.41 (1H, d, $J$ = 16.0 Hz, H-7‴), 7.45 (1H, d, $J$ = 15.0 Hz, H-α), 7.46 (2H, d, $J$ = 8.0 Hz, H-2‴, H-6‴), 7.55 (1H, d, $J$ = 8.0 Hz, H-6′), 7.97 (1H, d, $J$ = 15.0 Hz, H-*β*); $^{13}C$ NMR (600 MHz, $CD_3OD$), see Table 1; ESIMS *m*/*z* 581 $[M + H]^+$, 435 $[(M + H) - 146]^+$, 273 $[(M + H) - 146 - 162]^+$; *anal.* C 62.02%, H 4.89%, calcd for $C_{30}H_{28}O_{12}$, C 62.07%, H 4.86%.

**4′-*O*-*β*-D-(2″-*p*-Coumaroyl-6″-acetyl)glucopyranosyl-4,2′,3′-trihydroxychalcone (2):** yellow amorphous powder; [α] +70° (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 295 (4.76), 320 (4.12), 384 (3.51) nm; $^1H$ NMR (600 MHz, $CD_3OD$) $\delta$ 1.98 (3H, s, $CH_3CO$), 3.48 (1H, t, $J$ = 9.0 Hz, H-3″), 3.49 (1H, t, $J$ = 9.0 Hz, H-4″), 3.62 (1H, m, H-5″), 4.05 (1H, dd, $J$ = 12.0, 3.5 Hz, H-6″a), 4.48 (1H, br t, $J$ = 8.5 Hz, H-2″), 4.68 (1H, dd, $J$ = 12.0, 5.5 Hz, H-6″b), 5.12 (1H, d, $J$ = 7.6 Hz, H-1″), 6.20 (1H, d, $J$ = 15.5 Hz, H-8‴), 6.70 (2H, d, $J$ = 8.0 Hz, H-3‴, H-5‴), 6.81 (1H, d, $J$ = 8.0 Hz, H-5′), 6.89 (2H, d, $J$ = 8.0 Hz, H-2, H-6), 7.13 (1H, d, $J$ = 8.0 Hz, H-6′), 7.30 (1H, d, $J$ = 16.0 Hz, H-*β*), 7.33 (2H, d, $J$ = 8.0 Hz, H-3, H-5), 7.40 (1H, d, $J$ = 16.0 Hz, H-α), 7.46 (2H, d, $J$ = 8.0 Hz, H-2‴, H-6‴), 7.50 (1H,

d, $J$ = 15.5 Hz, H-7‴); $^{13}$C NMR (600 MHz, $CD_3OD$), see Table 1; ESIMS $m/z$ 623 $[M + H]^+$, 477 $[(M + H) - 146]^+$, 435 $[(M + H) - 146 - 42]^+$, 273 $[(M + H) - 146 - 42 - 162]^+$; *anal.* C 61.70%, H 4.87%, calcd for $C_{32}H_{30}O_{13}$, C 61.74%, H 4.86%.

**3′-(3-Methyl-2-butenyl)-4′-*O*-*β*-D-glucopyranosyl-4,2′-dihydroxychalcone (3):** yellow amorphous powder; [α] +80.5° (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 256 (4.25), 296 (3.68), 385 (3.04) nm; $^1$H NMR (600 MHz, $CD_3OD$) $\delta$ 1.68 (3H, d, $J$ = 1.5 Hz, H-5‴), 1.75 (3H, d, $J$ = 1.5 Hz, H-4‴), 3.38 (1H, m, H-5″), 3.41 (1H, t, $J$ = 9.0 Hz, H-2″), 3.47 (1H, t, $J$ = 9.0 Hz, H-3″), 3.50 (1H, t, $J$ = 9.0 Hz, H-4″), 3.51 (1H, dd, $J$ = 12.0, 6.5 Hz, H-1‴a), 3.58 (1H, dd, $J$ = 12.0, 7.0 Hz, H-1‴b), 3.62 (1H, dd, $J$ = 12.0, 2.5 Hz, H-6″a), 3.95 (1H, dd, $J$ = 12.0, 5.5 Hz, H-6″b), 5.09 (1H, br t, $J$ = 6.8 Hz, H-2‴), 5.18 (1H, d, $J$ = 7.5 Hz, H-1″), 6.78 (1H, d, $J$ = 8.0 Hz, H-5′), 6.92 (2H, d, $J$ = 8.0 Hz, H-2, H-6), 7.10 (1H, d, $J$ = 8.0 Hz, H-6′), 7.28 (2H, d, $J$ = 8.0 Hz, H-3, H-5), 7.40 (1H, d, $J$ = 16.0 Hz, H-α), 7.82 (1H, d, $J$ = 16.0 Hz, H-β); $^{13}$C NMR (600 MHz, $CD_3OD$), see Table 1; ESIMS $m/z$ 487 $[M + H]^+$, 444 $[(M + H) - 43]^+$, 432 $[(M + H) - 55]^+$, 325 $[(M + H) - 162]^+$; *anal.* C 64.15%, H 6.21%, calcd for $C_{26}H_{30}O_9$, C 64.19%, H 6.22%.

**TEAC Test.** Pure compounds were tested by using the TEAC assay. The TEAC value is based on the ability of the antioxidant to scavenge the radical cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$) with spectrophotometric analysis.[8] 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 phosphate-buffered saline (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 of 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**−**7** are expressed as TEAC (Trolox Equivalent Antioxidant Capacity) values in comparison with TEAC activity of reported reference compound quercetin.[8] Compounds are considered active when their TEAC is near to that of quercetin. 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.[11] 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 time $t$, with test compound; $A_{00}$ = absorbance at beginning of the incubation, without test compound; $A_{0t}$ = absorbance at time $t$, without test compound. Compounds are considered active when their AA is close to that of BHT, the control substance.

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## References and Notes

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