# Antioxidant and Anti-inflammatory Phenylpropanoid Derivatives from *Calamus quiquesetinervius*

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*Received March 16, 2010*

Eight new phenylpropanoid derivatives [quiquesetinerviusides A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), as well as quiquesetinerviusins A (**6**), B (**7**), and C (**8**)] and seven known compounds (**8**−**15**), were isolated from an EtOAc extract of *Calamus quiquesetinervius* stems. The structures of **1**−**8** were elucidated on the basis of 1D- and 2D-NMR spectroscopic data analysis. Bioassay results showed that **1**−**5** possess weak DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity, but potent ·OH radical scavenging activity ($IC_{50}$ 3.6−8.4 $\mu$M). Of the tested isolates, compounds **4**−**6** and **9** showed potent inhibition ($IC_{50}$ 9.2−29.5 $\mu$M) of LPS-stimulated NO production when compared with a positive control substance, quercetin ($IC_{50}$ 34.5 $\mu$M).

*Calamus quiquesetinervius* Burret (Arecaceae), an endemic rattan, is distributed at low elevations throughout the island of Taiwan. It has been an important industrial crop since the mid-20th century.[1,2] This species not only serves as an essential food plant of *Stichophthalma howqua formosana* Fruhstorfer (Nymphalidae) in the natural ecosystem but also is an important foodstuff for indigenous ethnic groups in Taiwan. The stems and roots have been used in traditional herbal medicine for treating hypertension, hepatitis, and skin disease; however, the constituents of *C. quiquesetinervius* have not been studied previously. Preliminary results showed that the total phenolic content of the EtOAc extract of *C. quiquesetinervius* correlated with the antioxidant potency. Spectroscopic analysis revealed that an EtOAc-soluble subfraction is rich in phenylpropanoid-type constituents. Phenylpropanoids have been found to play crucial roles in plant biochemistry and physiology, such as regulating plant growth and resisting environmental stress.[3] These compounds are also widespread in vegetables, fruits, and herbs. Their biological effects as antioxidants in repairing oxidatively damaged DNA, as well as their antihypertensive, hepatoprotective, antitumor, anti-inflammatory, and antiplatelet aggregation effects, have been studied over the past few years.[4,5] Therefore, this investigation was focused on the phenylpropanoid antioxidants from *C. quiquesetinervius*.

Eight new compounds, including five phenylpropanoid glycosides (**1**−**5**) and three lignans with a $\beta$-5 (**6**−**7**) or a $\beta$-*O*-4 (**8**) interunit linkage, were isolated, along with seven known compounds, from *C. quiquesetinervius* stems. The known compounds included tricin 4′-*O*-(*erythro*-$\beta$-guaiacylglyceryl) ether (salcolin B) (**9**),[6] 5,7-dihydroxychromone (**10**),[7] $\beta$-sitosteryl 3-*O*-$\beta$-D-glucoside (**11**),[8] 4-hydroxybenzoic acid 2-hydroxyethyl ester (**12**),[9] *p*-methoxybenzoic acid (**13**),[10] 3-hydroxy-1-(3,5-dimethoxy-4-hydroxypheny)propan-1-one (**14**),[11] and 2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-*p*-hydroxybenzoyloxymethyl-5-(2-formylvinyl)-7-methoxybenzofuran (**15**).[12] Scavenging activities for **1**−**9** and **15** against DPPH (2,2-dipheny1-1-picrylhydrazyl) radicals, ·OH and $\cdot O_2^-$, and inhibitory effects on the production of nitric oxide (NO) by a macrophage-mediated bioassay are reported.

## Results and Discussion

Dried stems of *C. quiquesetinervius* (15.4 kg) were extracted with 95% EtOH and then in turn with *n*-hexane, EtOAc, *n*-butanol, and water, to give four partitioned fractions, as described in the Experimental Section. The total phenolic content and antioxidant activities (DPPH radical scavenging) of these fractions are shown in Table S1, Supporting Information. By using bioassay-guided fractionation together with repeated chromatographic and spectroscopic methods,[13] eight new antioxidant phenylpropanoid derivatives (**1**−**8**) were obtained, along with the known compounds **9**−**15**.

The HRESIMS of compound **1** revealed the molecular formula $C_{42}H_{46}O_{20}$ from the pseudomolecular ion at *m*/*z* 893.2477 $[M + Na]^+$. The UV and IR spectra displayed absorption bands for hydroxy, $\alpha,\beta$-unsaturated carbonyl ester, and aromatic ring moieties. The $^1$H NMR spectrum (Table 1) exhibited characteristic signals that could be attributed to three benzyl moieties with an ABX spin system [$\delta_H$ 7.22 (d, $J$ = 1.6 Hz, H-2″), 7.13 (dd, $J$ = 8.4, 1.6 Hz, H-6″), 6.73 (d, $J$ = 8.4 Hz, H-5″), 7.18 (d, $J$ = 2.0 Hz, H-2‴), 7.07 (dd, $J$ = 8.0, 2.0 Hz, H-6‴), 6.80 (d, $J$ = 8.0 Hz, H-5‴), 7.02 (d, $J$ = 2.0 Hz, H-2⁗), 6.86 (dd, $J$ = 8.0, 2.0 Hz, H-6⁗), and 6.80 (d, $J$ = 8.0 Hz, H-5⁗)]. $^{13}$C NMR signals for 27 $sp^2$ carbons, together with three methoxy groups ($\delta_H$ 3.82−3.88) on aromatic moieties, as determined from the HMBC spectrum, suggested the presence of three feruloyl moieties. Furthermore, the large coupling constants (16.0 Hz) of H-$\alpha$″ and H-$\beta$″ ($\delta_H$ 7.76 and 6.46), H-$\alpha$‴ and H-$\beta$‴ ($\delta_H$ 7.66 and 6.42), and H-$\alpha$⁗ and H-$\beta$⁗ ($\delta_H$ 7.54 and 6.12) were in good accordance with three pairs of *trans*-olefinic protons in the $\alpha,\beta$-unsaturated carbonyl systems of the feruloyl units in **1**.[14,15] The $^1$H and $^{13}$C NMR spectrum (Tables 1 and 2) of **1** showed an $\alpha$-anomeric resonance at $\delta_H$ 5.53 with a small coupling constant (d, $J$ = 3.6 Hz, H-1′) due to a *gauche* conformation, according to the Karplus relation,[16] together with

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10.1021/np100181c 
Published on Web 09/08/2010

**Table 1.** $^1$H NMR Spectroscopic Data of **1**−**5**[a] ($\delta_H$ in $CD_3OD$, *J* in Hz)

| position | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 3.71, d (12.0) | 3.72, d (12.0) | 3.71, d (12.0) | 3.72, d (12.0) | 3.72, d (11.2) |
| | 3.63, d (12.4) | 3.65, d (12.4) | 3.54, d (11.6) | 3.65, d (12.4) | 3.52, d (11.6) |
| 3 | 5.49, d (6.8) | 5.48, d (7.2) | 5.56, d (8.0) | 5.45, d (7.2) | 5.52, d (7.6) |
| 4 | 4.50, t (7.2) | 4.50 overlapped | 4.52, t (8.0) | 4.50, t (6.4) | 4.52, t (7.6) |
| 5 | 4.20 m | 4.18 m | 4.12 m | 4.19 m | 4.18 m |
| 6 | 4.53 overlapped | 4.54 overlapped | 4.49 overlapped | 4.58, dd (12.0, 4.0) | 4.48 overlapped |
| | | | | 4.55, dd (12.0, 3.6) | |
| 1′ | 5.53, d (3.6) | 5.66, d (3.6) | 5.72, d (4.0) | 5.58, d (3.6) | 5.71, d (3.2) |
| 2′ | 3.54, dd (10.0, 4.0) | 3.55, dd (10.0, 3.6) | 4.70, dd (10.0, 3.6) | 3.55, dd (10.0, 3.6) | 4.69, dd (10.0, 3.6) |
| 3′ | 3.92 overlapped | 3.86 overlapped | 4.09, t (9.6) | 3.86 overlapped | 4.08, t (10.0) |
| 4′ | 4.93, t (10.4) | 4.88 overlapped | 5.0, t (10.0) | 4.87 overlapped | 4.98, t (9.6) |
| 5′ | 4.21 overlapped | 4.43 m | 4.24 m | 4.43 overlapped | 4.21 m |
| 6′ | 3.75 overlapped | 4.32, dd (12.4, 2.0) | 3.75, dd (12.4, 2.0) | 4.32, br d (10.8) | 3.80, br d (11.6) |
| | 3.60 overlapped | 4.09, dd (12.4, 6.8) | 3.63, dd (12.0, 4.8) | 4.08, dd (12.0, 6.4) | 3.60, dd (12.8, 5.2) |
| OAc-2′ | | | 2.06 s | | 2.06 s |
| OAc-6′ | | 2.04 s | | 2.04 s | |
| X or X′-3 | | | | | |
| α″ | 7.76, d (16.0) | 7.74, d (16.0) | 7.74, d (16.0) | 7.75, d (15.6) | 7.75, d (15.6) |
| *β*″ | 6.46, d (16.0) | 6.45, d (15.6) | 6.50, d (16.0) | 6.46, d (17.2) | 6.50, d (16.0) |
| 2″ | 7.22, d (1.6) | 7.24, d (1.6) | 7.04, d (1.6) | 7.27, br s | 7.19, br s |
| 5″ | 6.73, d (8.4) | 6.73, d (8.0) | 6.78, d (8.0) | 6.78, d (8.4) | 6.79, d (7.6) |
| 6″ | 7.13, dd (8.4, 1.6) | 7.12, dd (8.4, 1.6) | 7.12, dd (8.4, 1.6) | 7.12, br d (9.2) | 7.19, br d (8.4) |
| OMe | 3.82 s | 3.80 s | 3.81 s | 3.84 s | 3.81s |
| X or X′-6 | | | | | |
| α‴ | 7.66, d (16.0) | 7.66, d (15.6) | 7.66, d (16.0) | 7.66, d (15.6) | 7.67, d (16.0) |
| *β*‴ | 6.42, d (16.4) | 6.41, d (16.0 | 6.40, d (16.0) | 6.42, d (16.4) | 6.43, d (16.0) |
| 2‴ | 7.18, d (2.0) | 7.17, d (1.6) | 7.24, d (1.6) | 7.18, d (8.0) | 7.26 (d, 8.4) |
| 3‴ | | | | | |
| 5‴ | 6.80, d (8.0) | 6.80, d (7.6) | 6.80, d (8.0) | 6.82, d (8.0) | 6.80, d (8.4) |
| 6‴ | 7.07, dd (8.0, 2.0) | 7.07, dd (8.4, 1.6) | 7.08, dd (8.4, 2.0) | 7.08, br d (8.4) | 7.09, dd (8.0, 1.6) |
| OMe | 3.83 s | 3.90 s | 3.89 s | 3.87 s | 3.89 s |
| X or X′-4′ | | | | | |
| α⁗ | 7.54, d (16.0) | 7.52, d (15.6) | 7.57, d (16.0) | 7.50, d (15.6) | 7.55, d (16.0) |
| *β*⁗ | 6.12, d (16.0) | 6.08, d (16.0) | 6.19, d (16.0) | 5.96, d (15.6) | 6.08, d (16.0) |
| 2⁗ | 7.02, d (2.0) | 6.97, d (1.6) | 7.18, d (2.0) | 7.18, d (8.0) | 7.26, d (8.4) |
| 3⁗ | | | | 6.77, d (8.8) | 6.77, (d, 8.8) |
| 5⁗ | 6.80, d (8.0) | 6.82, d (8.4) | 6.74, d (8.0) | 6.77, d (8.8) | 6.77, d (8.8) |
| 6⁗ | 6.86, dd (8.0, 2.0) | 7.12, dd (8.4, 1.6) | 6.90, dd (8.4, 2.0) | 7.18, d (8.0) | 7.26, d (8.4) |
| OMe | 3.88 s | 3.81 s | 3.89 s | | |

[a] Assignments were confirmed from the $^1$H−$^1$H COSY, HMQC, and HMBC spectra. Data were measured at 400 MHz.

12 oxygenated carbon signals at $\delta_C$ 62.4−105.8. These data implied that **1** is a disaccharide containing both a pentose and a hexose moiety. On the basis of chemical evidence and comparison of spectroscopic data with those of authentic samples,[14,17] the sugar moiety was confirmed to contain 1→2 linked α-D-glucopyranosyl and *β*-D-fructofuranosyl units. These results indicated that **1** is a phenylpropanoid sucroside. According to the foregoing evidence, compound **1** was concluded to be a sucrose derivative acylated by three feruloyl moieties. Upon inspection of the HMBC spectrum, long-range correlations were observed between C-*γ*″ ($\delta_C$ 167.9)/H-3 ($\delta_H$ 5.49) and C-*γ*‴ ($\delta_C$ 169.0)/H-6 ($\delta_H$ 4.53) in the fructofuranosyl moiety and between C-*γ*⁗ ($\delta_C$ 168.6)/H-4′ ($\delta_H$ 4.93) in the glucopyranosyl moiety. From these data, the positions of the acyl residues were determined as shown. Accordingly, the structure of **1** was characterized as (3,6-*O*-diferuloyl)-*β*-D-fructofuranosyl-(2→1)-(4-*O*-feruloyl)-α-D-glucopyranoside and has been named quiquesetinerviuside A.

Compound **2** exhibited a quasimolecular ion at *m*/*z* 911.2569 $[M - H]^-$, corresponding to the molecular formula $C_{44}H_{48}O_{21}$, in the negative-ion HRESIMS. The UV and IR spectra of **2** were very similar to those of **1**. Likewise, the $^1$H and $^{13}$C NMR spectra of **1** and **2** (Tables 1 and 2) were similar, except for additional signals for an acetyl group [$\delta_H$ 2.04 (3H, s, OAc-6′), $\delta_C$ 172.8 and 20.9 (OAc-6′)] in **2**. Moreover, the carbon signals for C-6′ ($\delta_C$ 64.7) and C-5′ ($\delta_C$ 70.3) in **2** were shifted downfield and upfield, respectively, relative to those in **1**. These data, together with a long-range correlation between $H_2$-6′ and the ester carbonyl group in the HMBC spectrum, implied that the acetyl group is present at the C-6′ position of the glucopyranosyl moiety in **2**. Consequently, the structure of **2** was elucidated as (3,6-*O*-diferuloyl)-*β*-D-fructofuranosyl-(2→1)-(4-*O*-feruloyl-6-*O*-acetyl)-α-D-glucopyranoside (quiquesetinerviuside B).

Compound **3** was isolated as a light yellow powder. The positive HRESIMS gave a sodiated molecular ion peak at *m*/*z* 935.2707 $[M + Na]^+$, consistent with the same molecular formula as **2**, $C_{44}H_{48}O_{21}$. A comparison of the $^1$H and $^{13}$C NMR spectroscopic data of **2** and **3** showed that the latter compound also possesses a *β*-D-fructofuranosyl-(2→1)-α-D-glucopyranosyl unit, an acetyl group, and three feruloyl moieties. The major difference between the $^{13}$C NMR spectra (Table 3) of **2** and **3** involved the *β*-D-glucopyranosyl moiety. In **3**, the C-2′ signal shifted to lower field ($\delta_C$ 73.1 to 74.4), while the C-1′ and C-3′ signals shifted to higher fields ($\delta_C$ 92.5 to 90.7 and $\delta_C$ 72.6 to 70.1, respectively). Furthermore, C-5′ was shifted from $\delta_C$ 70.3 to 72.3, and C-6′ was shifted from $\delta_C$ 64.7 to 62.3. These data indicated that the acetyl group is linked at the C-2′ position in **3**, rather than at the C-6′ position in **2**. Moreover, in **3**, H-2′ exhibited a long-range HMBC correlation with the acetyl ester carbon, which supported the above conclusion. According to the above data, **3** (quiquesetinerviuside C) was determined as (3,6-*O*-diferuloyl)-*β*-D-fructofuranosyl-(2→1)-(4-*O*-feruloyl-2-*O*-acetyl)-α-D-glucopyranoside.

Compound **4** was obtained as a light yellow, amorphous powder, and its HRESIMS indicated a molecular formula of $C_{43}H_{46}O_{20}$, as determined by a quasimolecular ion peak at *m*/*z* 905.2605 $[M + Na]^+$. The IR spectrum resembled that of **2**, with absorption bands at 3352 (OH), 1704, and 1634 (α,*β*-unsaturated carbonyl group), and 1600 and 1515 (aromatic rings) $cm^{-1}$. The $^1$H NMR, $^{13}$C NMR, HMQC, and $^1$H−$^1$H COSY spectra revealed that the skeletons of

**Table 2.** $^{13}C$ NMR Spectroscopic Data of **1**−**5** ($\delta_C$ in $CD_3OD$)[a]

| position | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 65.3 | 65.8 | 64.8 | 65.8 | 64.9 |
| 2 | 105.8 | 105.5 | 105.8 | 105.7 | 105.8 |
| 3 | 79.4 | 79.2 | 78.8 | 79.4 | 79.0 |
| 4 | 75.0 | 74.2 | 74.2 | 74.4 | 74.4 |
| 5 | 81.9 | 81.8 | 81.4 | 82.0 | 81.5 |
| 6 | 65.6 | 64.7 | 65.3 | 64.7 | 65.2 |
| 1′ | 93.0 | 92.5 | 90.7 | 92.6 | 90.7 |
| 2′ | 73.3 | 73.1 | 74.4 | 73.1 | 74.4 |
| 3′ | 72.9 | 72.6 | 70.1 | 72.6 | 70.2 |
| 4′ | 72.7 | 72.6 | 72.5 | 72.5 | 72.4 |
| 5′ | 72.6 | 70.3 | 72.3 | 70.2 | 72.3 |
| 6′ | 62.4 | 64.7 | 62.3 | 64.7 | 62.3 |
| OAc-2′ | | | 172.3 | | 172.3 |
| | | | 21.0 | | 21.0 |
| OAc-6′ | | 172.8 | | 172.8 | |
| | | 20.9 | | 20.9 | |
| X or X′-3 | | | | | |
| α″ | 147.9 | 148.0 | 148.0 | 148.0 | 148.0 |
| β″ | 114.9 | 114.6 | 114. 8 | 114.7 | 114.7 |
| γ″ | 167.9 | 167.9 | 168.2 | 167.9 | 168.2 |
| 1″ | 127.6 | 127.5 | 127.5 | 127.6 | 127.5 |
| 2″ | 111.7 | 111.9 | 111.6 | 111.6 | 111.8 |
| 3″ | 149.4 | 149.4 | 149.3 | 149.4 | 149.4 |
| 4″ | 150.9 | 150.9 | 151.1 | 151.0 | 151.0 |
| 5″ | 116.6 | 116.6 | 116.6 | 116.6 | 116.6 |
| 6″ | 124.4 | 124.5 | 124.6 | 124.5 | 124.6 |
| $OCH_3$ | 56.44 | 56.4 | 56.4 | 56.5 | 56.4 |
| X or X-6 | | | | | |
| α‴ | 147.3 | 147.2 | 147.3 | 147.2 | 147.1 |
| β‴ | 115.1 | 115.2 | 115.1 | 115.2 | 115.1 |
| γ‴ | 169.0 | 168.9 | 169.0 | 168.9 | 169.0 |
| 1‴ | 127.6 | 127.4 | 127.6 | 127.7 | 127.6 |
| 2‴ | 111.6 | 111.5 | 111.6 | 111.5 | 111.6 |
| 3‴ | 149.4 | 149.2 | 149.4 | 149.4 | 149.4 |
| 4‴ | 150.8 | 150.7 | 150.8 | 150.7 | 150.8 |
| 5‴ | 116.6 | 116.5 | 116.5 | 116.5 | 116.5 |
| 6‴ | 124.3 | 124.3 | 124.4 | 124.3 | 124.4 |
| $OCH_3$ | 56.5 | 56.5 | 56.5 | 56.5 | 56.4 |
| X or X′-4′ | | | | | |
| α⁗ | 147.5 | 147.7 | 147.6 | 147.4 | 147.3 |
| β⁗ | 114.9 | 114.6 | 114.7 | 114.3 | 114.4 |
| γ⁗ | 168.6 | 168.3 | 168.4 | 168.4 | 168.4 |
| 1⁗ | 127.5 | 127.7 | 127.4 | 126.8 | 126.9 |
| 2⁗ | 111.9 | 111.5 | 111.9 | 131.2 | 131.3 |
| 3⁗ | 149.3 | 149.4 | 149.4 | 116.9 | 116.9 |
| 4⁗ | 150.7 | 150.7 | 150.8 | 161.3 | 161.5 |
| 5⁗ | 116.59 | 116.5 | 116.5 | 116.9 | 116.9 |
| 6⁗ | 123.9 | 123.9 | 124.1 | 131.2 | 131.3 |
| $OCH_3$ | 56.4 | 56.4 | 56.4 | | |

[a] Assignments were confirmed from the HMQC and HMBC spectra. Data were measured at 100 MHz.

**2** and **4** contain a sucrose unit with one acetyl and three phenylpropanoid esters. In the $^1H$ NMR spectrum of compound **4** (Table 1), the signals for $A_2B_2$ protons [($\delta_H$ 7.18 for H-2⁗, H-6⁗ (d, $J$ = 8.0 Hz) and $\delta_H$ 6.77 for H-3⁗, H-5⁗ (d, $J$ = 8.8 Hz)] and *trans*-olefinic protons ($\delta_H$ 7.50 and 5.96 for α⁗, β⁗ (d, $J$ = 15.6 Hz)) indicated that this compound contains a *trans*-*p*-coumaroyl moiety. Moreover, the chemical shifts, including two sets of ABX pattern protons and two pairs of *trans*-olefinic protons, were the same as those found in **2**. Detailed analysis of the HMBC spectrum of **4** confirmed the presence of two *trans*-*O*-feruloyl moieties at C-3 and C-6 on the fructofuranosyl unit, one *trans*-*p*-coumaroyl substituent at C-4′, and an acetyl group at C-6′ on the α-glucopyranosyl unit. These data supported the conclusion that **4** and **2** differ only in their C-4′ substituent, which is a *trans*-*O*-feruloyl moiety in **2** and a *trans*-*p*-coumaroyl moiety in **4**. Therefore, the structure of **4** (quiquesetinerviuside D) was assigned as (3,6-*O*-diferuloyl)-β-D-fructofuranosyl-(2→1)-(4-*O*-*p*-coumaroyl-6-*O*-acetyl)-α-D-glucopyranoside.

According to the HRESIMS peak at *m*/*z* 881.2469 [M − H]$^-$, compound **5** could be assigned with the same molecular formula, $C_{43}H_{46}O_{20}$, as **4**. Their structural similarities were verified from their comparable UV, IR, and $^1H$ and $^{13}C$ NMR spectra. Interestingly, the differences in the $^{13}C$ NMR and HMBC signals between **4** and **5** were the same as those between **2** and **3**. Thus, the acetyl group was present at C-6′ in **4** and at C-2′ in **5**. Accordingly, **5** (quiquesetinerviuside E) was elucidated as (3,6-*O*-diferuloyl)-β-D-fructofuranosyl-(2→1)-(4-*O*-*p*-coumaroyl-2-*O*-acetyl)-α-D-glucopyranoside.

Compound **6** was isolated as a white, amorphous powder. Its molecular formula was determined to be $C_{34}H_{30}O_{10}$, with 20 degrees of unsaturation, on the basis of the HRESIMS data (*m*/*z* 597.1756 [M − H]$^-$). The IR spectrum indicated the presence of hydroxy (3366 cm$^{-1}$), carbonyl ester (1694 cm$^{-1}$), aromatic (1607, 1515, and 1453 cm$^{-1}$), methoxy (1286 cm$^{-1}$), and ether (1128 cm$^{-1}$) groups. Both the UV and IR data were consistent with the presence of an oxygenated aromatic ring. Thirteen aryl proton signals [$\delta_H$ 6.78 (d, $J$ = 8.4 Hz, H-3‴, H-5‴) and 7.74 (1H, d, $J$ = 8.8 Hz, H-2‴, H-6‴), $\delta_H$ 6.82 (d, $J$ = 8.8 Hz, H-3″, H-5″) and 7.89 (1H, d, $J$ = 8.8 Hz, H-2″, H-6″), $\delta_H$ 6.75 (d, $J$ = 8.0 Hz, H-5), 6.82 (dd, $J$ = 8.8, 2.0 Hz, H-6), and 6.90 (d, $J$ = 2.0 Hz, H-2), $\delta_H$ 6.99 (s, H-2′) and 7.00 (s, H-6′)] found in the $^1H$ NMR spectrum of **6** (Table 3) were attributed to two 1,4-disubstituted ($A_2B_2$ system) moieties, one 1,3,4-trisubstituted (ABX system) moiety, and one 1,3,4,5-tetrasubstituted (AX system) phenyl moiety. In addition, signals for an AB set of (*E*)-vinylic protons [$\delta_H$ 6.68 (d, $J$ = 15.6 Hz, H-7′) and 6.27 (m, H-8′)], two aliphatic methine protons [$\delta_H$ 5.53 (d, $J$ = 6.8 Hz, H-7) and 3.88 (m, H-8)], two methoxy protons [$\delta_H$ 3.70 (3H, s, OC$H_3$-3) and 3.89 (3H, s, OC$H_3$-3′)], and two sets of oxygenated methylene protons [$\delta_H$ 4.45 (dd, $J$ = 11.2, 8.0 Hz, $H_a$-9) and 4.67 (dd, $J$ = 11.2, 4.8 Hz, $H_b$-9), $\delta_H$ 4.89 (2H, overlapped, H-9′)] were observed. Analysis of the $^1H-^1H$ COSY spectrum of **6** was used to determine the linkage of the aliphatic protons at H-7′, H-8′, and H-9′ in ring A and H-7, H-8, and H-9 in ring C. The $^{13}C$ NMR spectrum (Table 3) showed 34 signals, characteristic of two methoxy carbons, 24 phenyl carbons, two olefinic carbons, four aliphatic carbons, and two ester carbonyl groups. In addition to the above spectroscopic findings, the HMBC spectrum of **6** showed cross-peaks between H-8′/C-1′ ($\delta_C$ 132.2), H-2′, 6′/C-7′ ($\delta_C$ 135.3), H-2, 6/C-7 ($\delta_C$ 90.6), H-7/C-2 ($\delta_C$ 110.9), C-6 ($\delta_C$ 120.1), C-8 ($\delta_C$ 51.8), C-9 ($\delta_C$ 66.5), and H-8/C-1 ($\delta_C$ 133.6), C-9 (Figure 1). Moreover, other long-range correlations, including H-7/C-4′ ($\delta_C$ 149.6), H-8/C-5′ ($\delta_C$ 129.3), H-9/C-5′, H-9′/C-8′ ($\delta_C$ 122.3), and H-2′/C-3′, C-4′, C-6′, were also found. Thus, the postulated structure of **6** contains two phenylpropanoid moieties and two benzoyl units. Subtracting 15 double bonds (13 C═C, 2 C═O) from the 20 degrees of unsaturation suggested five rings were remaining in **6**. Together with the above findings, **6** was proposed as possessing a dihydrobenzo[*b*]furan neolignan skeleton, which is similar to that of the coniferaldehyde derivative, dehydrodiconiferyl alcohol,[18,19] a guaiacyl-type lignan precursor with a β-5 interunit linkage.[19] Furthermore, long-range correlations between H-9/C-7‴ and H-9′/C-7″, as well as the other correlated cross-peaks found in the HMBC spectrum, supported the presence of ester linkages between the dehydrodiconiferyl moieties and both of the 4-hydroxybenzonate moieties (Figure 1). In addition, the coupling constant between H-7 and H-8 was 6.8 Hz ($CD_3OD$) in the $^1H$ NMR spectrum, and cross-peaks of H-7 to $H_2$-9 and H-8 to H-6 were observed in the NOESY spectrum (Figure 1) in **6**, which suggested the relative conformation of H-7 and H-8 as *trans*.[20−22] A computer-generated 3D chemical model for **6** using MM2 force field calculations also agreed with these assignments. However, due to the lack of an available signal in the CD spectrum of **6**, the absolute configurations of C-7 and C-8 remain to be determined. On the basis of the above, **6** (quiquesetinerviusin A) was assigned as (*E*)-7-(4-hydroxy-3-methoxyphenyl)-1′-[3-(4-hydroxybenzoyl-

**Table 3.** NMR Spectroscopic Data of **6**−**8** ($\delta_H$ and $\delta_C$ in $CD_3OD$, $J$ in Hz)[a]

| | **6** | | **7** | | **8** | |
|---|---|---|---|---|---|---|
| position | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | | 133.6 | | 132.8 | | 131.0 |
| 2 | 6.90 d (2.0) | 110.9 | 6.63 s | 104.4 | 6.98 d (1.6) | 112.1 |
| 3 | | 149.1 | | 149.4 | | 149.1 |
| 4 | | 147.8 | | 136.6 | | 147.6 |
| 5 | 6.75 d (8.0) | 116.2 | | 149.4 | 6.78 d (8.0) | 116.2 |
| 6 | 6.82 dd (8.8, 2.0) | 120.1 | 6.63 s | 104.4 | 6.85 dd (8.8, 2.4) | 121.4 |
| 7 | 5.53 d (6.8) | 90.6 | 5.53 d (7.2) | 90.9 | 4.61 d (6.0) | 83.1 |
| 8 | 3.88 m | 51.8 | 3.90 m | 51.6 | 4.70 m | 83.3 |
| 9 | 4.67 dd (11.2, 4.8) | 66.5 | 4.70 dd (11.2, 4.8) | 66.5 | 4.20 dd (11.6, 6.4) | 65.5 |
| | 4.45 dd (11.2, 8.0) | | 4.51 br d (8.4) | | 4.31 dd (11.6, 3.2) | |
| 10 | | | | | 3.44 m | 65.7 |
| 11 | | | | | 1.15 t (7.2) | 15.6 |
| 1′ | | 132.2 | | 132.3 | | 132.5 |
| 2′ | 6.99 s | 112.6 | 7.01 s | 112.6 | 7.00 d (1.6) | 111.5 |
| 3′ | | 145.7 | | 145.7 | | 152.1 |
| 4′ | | 149.6 | | 149.6 | | 150.0 |
| 5′ | | 129.3 | | 129.1 | 6.95 d (8.4) | 119.1 |
| 6′ | 7.00 s | 116.5 | 7.01 s | 116.5 | 6.91 dd (8.4, 1.6) | 120.9 |
| 7′ | 6.68 d (15.6) | 135.3 | 6.67 d (16.4) | 135.3 | 6.66 d (15.6) | 135.0 |
| 8′ | 6.27 m | 122.3 | 6.29 m | 122.5 | 6.31 m | 123.1 |
| 9′ | 4.89 overlapped | 67.1 | 4.92 overlapped | 67.2 | 4.88 overlapped | 66.4 |
| 1″ | | 122.5 | | 122.3 | | 122.3 |
| 2″ | 7.89 d (8.8) | 132.8 | 7.89 d (8.8) | 132.8 | 7.91 d (8.8) | 132.9 |
| 3″ | 6.82 d (8.8) | 116.2 | 6.81 d (8.8) | 116.2 | 6.83 d (8.8) | 116.1 |
| 4″ | | 163.7 | | 163.8 | | 163.6 |
| 5″ | 6.82 d (8.8) | 116.2 | 6.81 d (8.8) | 116.2 | 6.83 d (8.8) | 116.1 |
| 6″ | 7.89 d (8.8) | 132.8 | 7.89 d (8.8) | 132.9 | 7.91 d (8.8) | 132.9 |
| 7″ | | 168.1 | | 168.0 | | 168.1 |
| 1‴ | | 121.8 | | 121.8 | | 121.9 |
| 2‴ | 7.74 d (8.8) | 132.9 | 7.74 d (8.8) | 132.9 | 7.66 d (8.8) | 132.9 |
| 3‴ | 6.78 d (8.4) | 116.2 | 6.78 d (8.4) | 116.2 | 6.75 d (8.4) | 116.1 |
| 4‴ | | 163.5 | | 163.6 | | 163.6 |
| 5‴ | 6.78 d (8.4) | 116.2 | 6.78 d (8.4) | 116.2 | 6.75 d (8.4) | 116.1 |
| 6‴ | 7.74 d (8.8) | 132.9 | 7.74 d (8.8) | 132.8 | 7.66 d (8.8) | 132.9 |
| 7‴ | | 167.8 | | 167.8 | | 167.8 |
| $OCH_3$-3 | 3.70 s | 56.3 | 3.69 s | 56.7 | 3.71 s | 56.3 |
| $OCH_3$-5 | | | 3.69 s | 56.7 | | |
| $OCH_3$-3′ | 3.89 s | 56.8 | 3.89 s | 56.8 | 3.76 s | 56.4 |
| $OCH_3$-5′ | | | | | | |

[a] Assignments were confirmed from the $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra. $^1H$ NMR and $^{13}C$ NMR spectroscopic data were measured at 400 and 100 MHz.

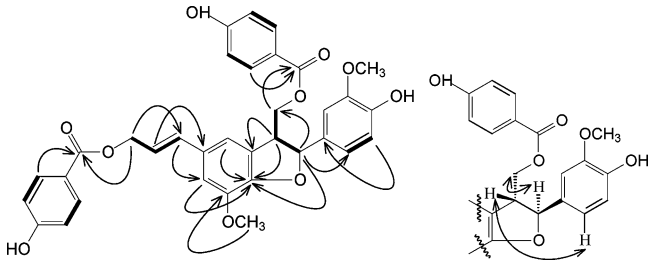



**Figure 1.** Key COSY (—), HMBC (→), and NOESY (↔) correlations of compound **6**.

oxymethyl)-1-propenyl]-8-(4-hydroxybenzoyloxymethyl)-3′-methoxybenzodihydrofuran.

Compound **7** was obtained as a white powder. Its molecular formula was assigned as $C_{35}H_{32}O_{11}$ (20 degrees of unsaturation) by HRESIMS (*m*/*z* 651.1915 $[M + Na]^+$). The $^1H$ and $^{13}C$ NMR spectra of **7** showed similarities to those of **6**, except for the presence of a proton at C-5, which was replaced by a methoxy group ($\delta_H$ 3.69, s, OC$\underline{H}_3$-5; $\delta_C$ 56.7, C-5) in **7**. These minor structural changes were further supported by HMQC correlations (OC$\underline{H}_3$-5/O$\underline{C}$H$_3$-5) and HMBC correlations (OC$\underline{H}_3$-5/O$\underline{C}$H$_3$-3) and confirmed as 1,3,4,5-tetrasubstituted phenyl moieties on the B ring. Thus, **7** (quiquesetinerviusin B) was determined as (*E*)-7-(2,6-dimethoxyphenyl)-1′-[3-(4-hydroxybenzoyloxymethyl)-1-propenyl]-8-(4-hydroxybenzoyloxymethyl)-3′-methoxybenzodihydrofuran.

Compound **8**, a pale yellow, amorphous powder, showed a $[M + Na]^+$ ion peak at *m*/*z* 667.2224 by HRESIMS, indicating a molecular formula of $C_{36}H_{36}O_{11}$, with 19 degrees of unsaturation. The UV absorption maxima at 261 and 275 (sh) nm and the presence of hydroxy (3366 $cm^{-1}$), carbonyl (1695 $cm^{-1}$), aromatic (1600, 1509, and 1446 $cm^{-1}$), and ester (1267, 1160, and 1113 $cm^{-1}$) absorption bands in the IR spectrum suggested the presence of oxygenated aromatic rings and ester substitutions in the structure. In the $^1H$ NMR spectrum of **8** (Table 3), two sets of ABX coupled signals at $\delta_H$ 6.98 (d, $J$ = 1.6 Hz, H-2), 6.85 (dd, $J$ = 8.8, 2.4 Hz, H-6), and 6.78 (d, $J$ = 8.0 Hz, H-5) and $\delta_H$ 7.00 (d, $J$ = 1.6 Hz, H-2′), 6.95 (d, $J$ = 8.4 Hz, H-5′), and 6.91 (dd, $J$ = 8.4, 1.6 Hz, H-6′) were attributed to two 1,3,4-trisubstituted aromatic rings. Two sets of *ortho*-coupled doublets at $\delta_H$ 7.66 (d, $J$ = 8.8 Hz, H-2‴, H-6‴) and 6.75 (d, $J$ = 8.4 Hz, H-3‴, H-5‴) and $\delta_H$ 7.91 (d, $J$ = 8.8 Hz, H-2″, H-6″) and 6.83 (d, $J$ = 8.8 Hz, H-3″, H-5″) were consistent with the aromatic proton signals of an $A_2B_2$ system. The $^1H$ NMR spectrum also showed two aliphatic oxygenated methines at $\delta_H$ 4.61 (d, $J$ = 6.0 Hz, H-7) and 4.70 (m, H-8), two olefinic methines at $\delta_H$ 6.66 (d, $J$ = 15.6 Hz, H-7′) and 6.31 (m, H-8′), two sets of oxygenated methylenes at $\delta_H$ 4.20 (dd, $J$ = 11.6, 6.4 Hz, $H_a$-9) and 4.31 (dd, $J$ = 11.6, 3.2 Hz, $H_b$-9) and $\delta_H$ 4.88 (overlapped, $H_2$-9′), two methoxy groups at $\delta_H$ 3.71 (s, OC$\underline{H}_3$-3′) and 3.76 (s, OC$\underline{H}_3$-3), and one ethoxy group at $\delta_H$ 1.15 (t, $J$ = 7.2 Hz, C$\underline{H}_3$-11) and 3.44 (m, $H_2$-10). These data, together with the cross-peaks of H-7/H-8, H-8/$H_2$-9, H-7′/H-8′, and H-8′/$H_2$-9′ in the $^1H-^1H$ COSY spectrum, as well as data from the $^{13}C$ NMR (Table

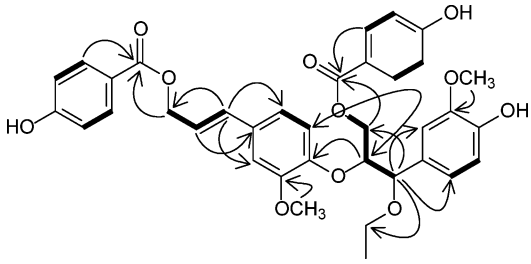


**Figure 2.** Key COSY (—), HMBC (→), and NOESY (↔) correlations of compound **8**.

**Table 4.** Scavenging Activity toward DPPH Radicals, Hydroxyl Radicals ($^{-}$OH), and Superoxide Anions ($^{\cdot}O_2^{-}$) and Anti-inflammatory Activities of **1**−**9** and **15**

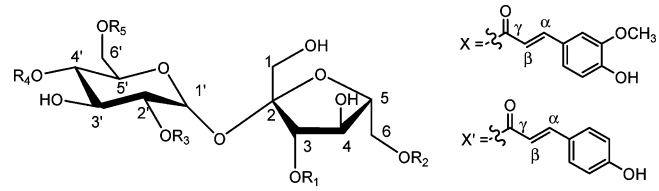
| | $IC_{50}$ ($\mu$M)[a] | | | |
|---|---|---|---|---|
| | DPPH | $OH^-$ | $^{\cdot}O_2^{-}$[b] | NO |
| **1** | 62.5 ± 2.1 | 6.8 ± 1.0 | >200 | 78.1 ± 4.9 |
| **2** | 99.6 ± 2.2 | 7.4 ± 1.0 | >200 | 60.3 ± 4.7 |
| **3** | 69.1 ± 1.7 | 3.6 ± 0.8 | 184.3 ± 3.9 | 42.1 ± 7.7 |
| **4** | 64.4 ± 1.8 | 8.4 ± 1.1 | >200 | 9.5 ± 1.9 |
| **5** | 68.4 ± 1.4 | 5.5 ± 0.9 | >200 | 9.2 ± 2.3 |
| **6** | —[c] | 23.7 ± 2.3 | >200 | 23.4 ± 3.7 |
| **7** | —[c] | 25.7 ± 2.8 | >200 | 223.2 ± 7.0 |
| **8** | >100 | 16.6 ± 1.7 | >200 | 95.7 ± 4.5 |
| **9** | >100 | 13.3 ± 1.8 | >200 | 29.5 ± 1.2 |
| **15** | >100 | 19.1 ± 2.5 | >200 | 69.0 ± 3.2 |
| Trolox[d] | 32.9 ± 1.8 | 4.31 ± 0.2 | 25.3 ± 1.0 | —[c] |
| quercetin[d] | | | | 34.5 ± 2.9 |

[a] All values were measured with thee replicates and are presented as means ± SD. [b] A compound is regarded as inactive with $IC_{50}$ > 200 $\mu$M for $^{\cdot}O_2^{-}$. [c] Not detected. [d] Trolox and quercetin were used as positive controls.

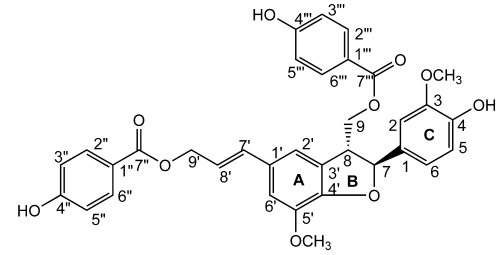
3) and HMQC spectra, suggested the presence of 1,2,3-propanetriol and *trans*-1-propenol moieties. Moreover, correlations between H-7/C-1 ($\delta_C$ 131.0), C-2 ($\delta_C$ 112.1), C-6 ($\delta_C$ 121.4), C-8 ($\delta_C$ 83.3), C-9 ($\delta_C$ 65.5), H-8/C-7 ($\delta_C$ 83.1), H-9/C-7, C-8, H-7′/C-1′ ($\delta_C$ 132.5), C-2′ ($\delta_C$ 111.5), C-6′ ($\delta_C$ 120.9), C-9′ ($\delta_C$ 66.4), H-8′/C-1′, C-9′, and H-9′/C-7′ ($\delta_C$ 135.0), C-8′ ($\delta_C$ 123.1) were observed in the HMBC spectrum. The H-7 doublet signal also correlated with C-10 at $\delta_C$ 65.7 in the HMBC and NOESY spectra, suggesting that the ethoxy group should be placed at the C-7 position. Together, the evidence above indicated a partial structure of **8** consisting of two phenylpropanoid units, including a 7-*O*-ethylguaiacylglycerol moiety and a *trans*-coniferyl alcohol moiety. Cross-peaks between H-8/C-4′ ($\delta_C$ 150.0), C-7 ($\delta_C$ 83.1), H-7/C-8 ($\delta_C$ 83.3), and H-9/C-8 also were observed in the HMBC spectrum, combined with correlations between H-8/H-3′, H-7, H-9 in the NOESY spectrum (Figure 2), confirming that **8** is a guaiacyl-type lignan with a $\beta$-*O*-4 interunit linkage.[17] On the basis of correlations between H-9/C-7‴ ($\delta_C$ 167.8) and H-9′/C-7″ ($\delta_C$ 168.1) in the HMBC spectrum, two additional 4-hydroxybenzoate moieties were determined. The HMBC and NOESY correlations also supported the assignment of the two methoxy groups at C-3 and C-3′. Furthermore, the coupling constant between H-7 and H-8 ($J_{7,8}$) was 6.0 Hz, indicating a *threo*-type relative configuration between C-7 and C-8, based on literature data[22−24] for 8-*O*-4′ neolignans. Consequently, the structure of **8** (quiquesetinerviusin C) was elucidated as *threo*-1-(4-hydroxy-3-methoxyphenyl)-1-ethoxy-2-{4-hydroxy-3-methoxyphenyl-4-[(1*E*)-3-(4-hydroxybenzoyl)-1-propenyl]phenoxy}-3-(4-hydroxybenzoyl)-propane.

Compounds **1**−**9** and **15** were evaluated for antioxidant activity by measuring their free radical scavenging activities. As shown in Table 4, only compound **3** showed marginal $^{\cdot}O_2^{-}$ scavenging activity (<200 $\mu$M). However, the bioassay results also showed that phenylpropanoid glycosides **1**−**5** had better DPPH or $^{\cdot}$OH free radical scavenging potencies than the guaiacyl-type lignans **6**−**8** and **15** and the flavonolignan **9** (Table 4).

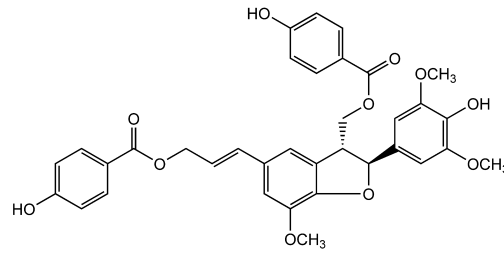
Compounds **1**−**9** and **15** were evaluated for anti-inflammatory activities in RAW 264.7 cells after a 24 h incubation. The MTS assay did not indicate any discernible cytotoxicity (cell viability >90%) in the absence or presence of LPS in the culture medium at various concentrations (up to 100 $\mu$M; data not shown) for these isolates. As shown in Table 4, compounds **4**−**6** and **9** exhibited potent activities against LPS-stimulated NO production compared with a positive control, quercetin ($IC_{50}$ 34.5 $\mu$M).



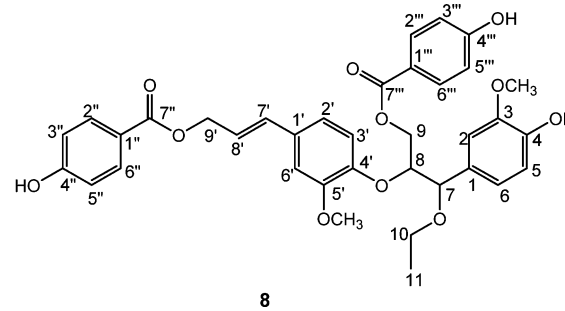
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **1** | X | X | H | X | H |
| **2** | X | X | H | X | $COCH_3$ |
| **3** | X | X | $COCH_3$ | X | H |
| **4** | X | X | H | X' | $COCH_3$ |
| **5** | X | X | $COCH_3$ | X' | H |



**6**



**7**



**8**

## Experimental Section

**General Experimental Procedures.** Melting points were determined by using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained with a JASCO P-1020 polarimeter. UV spectra were measured using a Hitachi U-3200 spectrophotometer. Infrared spectra were recorded with KBr disks, using a Nicolet Avatar 320 FT-IR spectrometer. NMR spectra were performed on a Bruker NMR spectrometer (Unity Plus 400 MHz). Mass spectra were measured with a Finnigan MAT95S mass spectrometer and a Shimadzu LCMS-

IT-TOF mass spectrometer. Materials for column chromatography were Sephadex LH-20 (GE Healthcare Bio-Sciences AB) and silica gel 60 (70−230 mesh and 230−400 mesh, Merck, Darmstadt, Germany). Thin-layer chromatography was performed on analytical TLC plates [silica gel 60 $F_{254}$, 20 × 20 cm (Merck, Darmstadt, Germany)] and preparative glass TLC plates [silica gel 60 $GF_{254}$, 20 × 20 cm (Merck, Darmstadt, Germany)]. Spots were visualized under UV light (254 or 356 nm) or by spraying with an anisaldehyde−sulfuric acid reagent after heating at 75 °C for ca. 1 min on a hot plate equipped with a temperature controller (Mandarin Scientific Co. Ltd., Taipei, Taiwan). The Büchi MPLC equipment (Labortechnik AG, Flawil, Switzerland) comprised a pump (B-688) and a column (50 × 230 mm i.d., Borosilicat 3.3) packed with silica gel 60. The semipreparative HPLC system used consisted of a Hitachi L-2130 pump, a Hitachi L-2450 diode array detector, and a Cosmosil 5C18-AR-II column (10 × 250 mm i.d.). A Shimadzu preparative recycle HPLC system was equipped with a CBM-20A system controller, a Shimadzu preparative LC-8A pump, a SPD-20A UV−vis absorbance detector, and a Cosmosil 5C18-AR-II column (20 × 250 mm i.d.). A BJL ultraweak chemiluminescence analyzer with a high-sensitivity detector ($3.3 \times 10^{-15}$ W/cm$^2$•count from Jye Horn Co. (Taipei, Taiwan)) was used for assessing the hydroxyl radical ($^{\bullet}OH$) and superoxide ($^{\bullet}O_2^-$) scavenging activities. Absorbances were measured by ELISA (Molecular Dynamics Spectra Max, GMI Inc., Ramsey, MN) for DPPH radical scavenging activity and anti-inflammatory activity. All of the analytical reagents were purchased from Sigma-Aldrich (St. Louis, MO).

**Plant Material.** Stems of *C. quiquesetinervius*, 5−10 cm in diameter, were collected from the mountains in Yunlin County, Taiwan, in September 2005 and authenticated by Professor Muh-Tsuen Kao of the National Institute of Chinese Medicine, Taipei, Taiwan, and Assistant Professor Yen-Hsueh Tseng of the Department of Forestry, National Chung Hsing University, Taichung, Taiwan. A voucher specimen of *C. quiquesetinervius* (no. NRICM20050916A) was deposited in the National Research Institute of Chinese Medicine, Taipei, Taiwan.

**Extraction and Isolation.** The stems, after removal of the leaves, were dried in an oven (Channel, Channel Business Co. Ltd., Taipei, Taiwan) at 40 °C for 72 h. The dried stems (15.4 kg) were extracted five times with 100 L of 95% EtOH (50 °C) for 72 h. The EtOH extract was concentrated continuously at reduced pressure at 45 °C to yield a brown syrup (ca. 1.0 kg). This extract was suspended in 10% alcohol (1 L) and partitioned sequentially using *n*-hexane (5 × 1 L), EtOAc (5 × 1 L), and *n*-butanol (3 × 1 L) to obtain four solvent-soluble fractions, comprising a *n*-hexane fraction (CQSH, 90 g), an EtOAc fraction (CQSE, 85 g), a *n*-butanol fraction (CQSB, 462 g), and a water fraction (CQSW, 343 g), respectively. Using the bioactivity-guided fractionation method,[13] the EtOAc fraction, which showed potent antioxidant activity, was eluted with *n*-hexane (3 L), EtOAc (6 L), EtOAc−MeOH [1:1 (v/v), 6 L], and MeOH (1.5 L), through an open column (Celite 535, 35 × 8.0 cm), to yield four corresponding fractions, Frs A (5.3 g), B (48.0 g), C (25.8 g), and D (3.4 g), respectively. Fr B was applied to MPLC over silica gel 60 by gradually increasing the amount of MeOH in $CH_2Cl_2$ (flow rate: 10 mL/min) to give six fractions (Frs $B_1$−$B_6$). Fr $B_2$ (3.6 g) was chromatographed over a Sephadex LH-20 column using $CH_2Cl_2$−MeOH (1:1) to yield six fractions (Frs $B_{2\text{-}1}$−$B_{2\text{-}6}$). Fr $B_{2\text{-}2}$ (3.6 g) was chromatographed over silica gel 60 in a gradient system by increasing the ratio of MeOH in $CH_2Cl_2$, to yield six fractions, Frs $B_{2\text{-}2\text{-}1}$−$B_{2\text{-}2\text{-}6}$. Compound **14** (1.4 mg) precipitated from Fr $B_{2\text{-}2\text{-}1}$(34.2 mg). Fr $B_{2\text{-}2\text{-}6}$ (1.0 g) was further subjected to column chromatography with silica gel 60, employing a gradient system ($CH_2Cl_2$−MeOH, 50:1−4:1) as eluent, to afford eight fractions (Frs $B_{2\text{-}2\text{-}6\text{-}1}$−$B_{2\text{-}2\text{-}6\text{-}8}$). Fr $B_{2\text{-}2\text{-}6\text{-}6}$ (394.3 mg) was chromatographed on silica gel 60 by a gradient $CH_2Cl_2$−MeOH mixture (30:1−4:1), and compound **10** (1.6 mg) was obtained from fraction $B_{2\text{-}2\text{-}6\text{-}6\text{-}4}$ (268.9 mg) by eluting with MeOH over a Sephadex LH-20 column.

Fr $B_3$ (22.9 g) was subjected to Sephadex LH-20 column chromatography with $CH_2Cl_2$−MeOH (1:1) to yield Frs $B_{3\text{-}1}$−$B_{3\text{-}6}$. Compound **11** (56.7 mg) precipitated from fraction $B_{3\text{-}2}$ (19.9 g). Fraction $B_{3\text{-}2}$ was further chromatographed over silica gel 60 ($CHCl_3$−MeOH, 25:1−4:1) to give six fractions (Frs $B_{3\text{-}2\text{-}1}$−$B_{3\text{-}2\text{-}6}$). Fr $B_{3\text{-}2\text{-}5}$ (4.87 g) was chromatographed over a column of Sephadex LH-20 eluting with $CHCl_3$−MeOH (2:1) to give eight fractions (Frs $B_{3\text{-}2\text{-}5\text{-}1}$−$B_{3\text{-}2\text{-}5\text{-}8}$). Fr $B_{3\text{-}2\text{-}5\text{-}5}$ (2.68 g) was purified over a column of Sephadex LH-20, with $CHCl_3$−MeOH (1:3) as eluent, to afford five fractions (Frs $B_{3\text{-}2\text{-}5\text{-}5\text{-}1}$−$B_{3\text{-}2\text{-}5\text{-}5\text{-}5}$). Fr $B_{3\text{-}2\text{-}5\text{-}5\text{-}4}$ (250 mg) was purified by preparative recycling-HPLC (20 × 250 mm i.d., Cosmosil 5$C_{18}$ AR-II column), using 50% acetonitrile as eluent, at a flow rate of 10 mL/min for 60 min, to obtain **6** (4.7 mg), **7** (6.3 mg), **8** (5.7 mg), and **15** (2.3 mg). Fr $B_{3\text{-}3}$ (5.6 g) was chromatographed on silica gel 60, with a gradient solvent system $CH_2Cl_2$−MeOH (25:1−4:1), to give seven fractions (Frs $B_{3\text{-}3\text{-}1}$−$B_{3\text{-}3\text{-}7}$). Fr $B_{3\text{-}3\text{-}5}$ (2.7 g) was separated into four fractions with $CHCl_3$−MeOH (1:1) using Sephadex LH-20, and then Fr $B_{3\text{-}3\text{-}5\text{-}3}$ (226.7 mg) was eluted with $CHCl_3$−acetone−MeOH (20:1:1) over Sephadex LH-20 to give four further fractions (Frs $B_{3\text{-}3\text{-}5\text{-}3\text{-}1}$−$B_{3\text{-}3\text{-}5\text{-}3\text{-}4}$). Finally, **9** (2.1 mg) was purified from $B_{3\text{-}3\text{-}5\text{-}3\text{-}4}$ by semipreparative HPLC (10 × 250 mm i.d., Cosmosil 5$C_{18}$ AR-II column), eluting with 35% MeOH as eluent A and 65% $H_2O$ as eluent B, at a flow rate of 2.5 mL/min for 40 min. This compound was purified finally by preparative TLC, with $CH_2Cl_2$−acetone−MeOH (8:1:0.6) as solvent. Fr $B_{3\text{-}4}$ was separated by silica gel 60 using column chromatography using a gradient system ($CH_2Cl_2$−MeOH, 35:1 → 1:1) to yield Frs $B_{3\text{-}4\text{-}1}$−$B_{3\text{-}4\text{-}6}$. Compounds **12** (1.4 mg) and **13** (0.7 mg) were obtained by Sephadex LH-20 column chromatography eluting with MeOH−$CHCl_3$ (1:1) (1.5 L) from Fr $B_{344}$ (820 mg).

Fr $B_4$ (19.0 g) was subjected to column chromatography over Sephadex LH-20 with $CH_2Cl_2$−MeOH (1:1) to give four subfractions (Frs $B_{4\text{-}1}$−$B_{4\text{-}4}$). Fr $B_{4\text{-}2}$ (15.45 g) was separated over Sephadex LH-20, eluting with $CHCl_3$−MeOH (1:3), with fractions Fr $B_{4\text{-}2\text{-}1}$−$B_{4\text{-}2\text{-}3}$ obtained. Fr $B_{4\text{-}2\text{-}3}$ (3.72 g) was chromatographed over Sephadex LH-20 with MeOH again, to give three subfractions, Frs $B_{4\text{-}2\text{-}3\text{-}1}$−$B_{4\text{-}2\text{-}3\text{-}3}$. Of these, Fr $B_{4\text{-}2\text{-}3\text{-}1}$ (1.02 g) was further fractionated via reversed-phase semipreparative HPLC, with 35% MeOH as the mobile phase, at a flow rate of 10 mL/min for 40 min, to afford Frs $B_{4\text{-}2\text{-}3\text{-}1\text{-}5}$−$B_{4\text{-}2\text{-}3\text{-}1\text{-}8}$. Fr $B_{4\text{-}2\text{-}3\text{-}1\text{-}5}$ (516.10 mg) was eluted at 13.5 min and further separated by HPLC (10 × 250 mm i.d., Cosmosil 5$C_{18}$ AR-II column), at a flow rate of 2.5 mL/min with acetonitrile as eluent A and $H_2O$ as eluent B ($A$ = 26% at $t$ = 0 min, $A$ = 31% at $t$ = 50 min; $A$ = 80% at $t$ = 50.1 min for washing), and eight subfractions (Frs $B_{4\text{-}2\text{-}3\text{-}1\text{-}5\text{-}1}$−$B_{4\text{-}2\text{-}3\text{-}1\text{-}8}$) were obtained. Finally, compounds **1** (7.8 mg), **2** (5.2 mg), **3** (3.8 mg), **4** (12 mg), and **5** (4.1 mg) were purified from Fr $B_{4\text{-}2\text{-}3\text{-}1\text{-}5\text{-}3}$ by preparative TLC with $CH_2Cl_2$−acetone−MeOH (8:1:0.4).

**Quiquesetinerviuside A (1):** light yellow powder; mp 158 °C; $[\alpha]^{25}_D$ +70.5 (*c* 0.78, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 327 (sh) (4.55), 236 (4.39), 203 (4.61) nm; IR (KBr) $\nu_{max}$ 3382, 2938, 2839, 1701, 1629, 1595, 1519, 1454, 1032 cm$^{-1}$; $^1$H (400 MHz) and $^{13}$C (100 MHz) NMR, Tables 1 and 2, respectively; ESIMS *m/z* 893 [M + Na]$^+$; HRESIMS *m/z* 893.2477 [M + Na]$^+$ (calcd for $C_{42}H_{46}O_{20}Na$, 893.2480).

**Quiquesetinerviuside B (2):** light yellow powder; mp 224 °C; $[\alpha]^{25}_D$ +67.3 (*c* 0.52, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 327 (sh) (4.80), 236 (4.63), 202 (4.83) nm; IR (KBr) $\nu_{max}$ 3376, 2945, 2838, 1706, 1633, 1595, 1510, 1453 cm$^{-1}$; $^1$H (400 MHz) and $^{13}$C (100 MHz) NMR, Tables 1 and 2, respectively; ESIMS *m/z* 935 [M + Na]$^+$; HRESIMS *m/z* 911.2569 [M − H]$^-$ (calcd for $C_{44}H_{47}O_{21}$, 911.2609).

**Quiquesetinerviuside C (3):** light yellow powder; mp 245 °C; $[\alpha]^{25}_D$ +29.0 (*c* 0.38, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 326 (4.66), 300 (sh) (4.21), 235 (4.49), 201 (4.67) nm; IR (KBr) $\nu_{max}$ 3386, 2938, 2839, 1699, 1627, 1596, 1516, 1453 cm$^{-1}$; $^1$H (400 MHz) and $^{13}$C (100 MHz) NMR, Tables 1 and 2, respectively; ESIMS *m/z* 935 [M + Na]$^+$; HRESIMS *m/z* 935.2707 [M + Na]$^+$ (calcd for $C_{44}H_{48}O_{21}Na$, 935.2586).

**Quiquesetinerviuside D (4):** light yellow powder; mp 146 °C; $[\alpha]^{25}_D$ +26.7 (*c* 1.2, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 321 (sh) (4.67), 234 (4.37), 219 (4.37) nm; IR (KBr) $\nu_{max}$ 3352, 2938, 2831, 1704, 1634, 1600, 1515, 1454 cm$^{-1}$; $^1$H (400 MHz) and $^{13}$C (100 MHz) NMR, Tables 1 and 2, respectively; ESIMS *m/z* 905 [M + Na]$^+$; HRESIMS *m/z* 905.2605 [M + Na]$^+$ (calcd for $C_{43}H_{46}O_{20}Na$, 905.3079).

**Quiquesetinerviuside E (5):** light yellow powder; mp 163 °C; $[\alpha]^{25}_D$ +24.4 (*c* 0.41, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 322 (4.66), 300 (sh) (4.12), 234 (4.44), 205 (4.51) nm; IR (KBr) $\nu_{max}$ 3376, 2942, 2838, 1700, 1631, 1604, 1516, 1453 cm$^{-1}$; $^1$H (400 MHz) and $^{13}$C (100 MHz) NMR, Tables 1 and 2, respectively; ESIMS *m/z* 905 [M + Na]$^+$; HRESIMS *m/z* 881.2469 [M − H]$^-$ (calcd for $C_{43}H_{45}O_{20}$, 881.2504).

**Quiquesetinerviusin A (6):** white, amorphous powder; mp 110 °C; $[\alpha]^{25}_D$ −4.3 (*c* 0.47, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 308 (sh) (3.95), 262 (4.52), 203 (4.80) nm; IR (KBr) $\nu_{max}$ 3366, 2943, 1694, 1607, 1515, 1453, 1379, 1286, 1128 cm$^{-1}$; $^1$H (400 MHz) and $^{13}$C (100 MHz) NMR, Table 3; ESIMS *m/z* 597 [M − H]$^-$; HRTOFMS *m/z* 597.1756 [M − H]$^-$ (calcd for $C_{34}H_{29}O_{10}$, 597.1761).

**Quiquesetinerviusin B (7):** white, amorphous powder; mp 175 °C; $[\alpha]^{25}_D$ +4.8 (*c* 0.63, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 305 (sh) (3.97), 267 (3.71), 210 (4.06) nm; IR (KBr) $\nu_{max}$ 3368, 2939, 1701, 1607, 1515,

1459, 1271, 1165, 1115 $cm^{-1}$; $^1H$ (400 MHz) and $^{13}C$ (100 MHz) NMR, Table 3; ESIMS *m/z* 650 $[M + Na]^+$; HRESIMS *m/z* 651.1915 $[M + Na]^+$ (calcd for $C_{35}H_{32}O_{11}Na$, 651.1842).

**Quiquesetinerviusin C (8):** pale yellow, amorphous powder; mp 92 °C; $[\alpha]^{25}_D$ −3.4 (*c* 0.58, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 275 (sh) (4.63), 261 (4.71), 203 (4.75) nm; IR (KBr) $\nu_{max}$ 3366, 2966, 1695, 1600, 1509, 1446, 1370,1267, 1160, 1113 $cm^{-1}$; $^1H$ (400 MHz) and $^{13}C$ (100 MHz) NMR, Table 3; ESIMS *m/z* 667 $[M + Na]^+$; HRESIMS *m/z* 667.2224 $[M + Na]^+$ (calcd for $C_{36}H_{36}O_{11}Na$, 667.2155).

**Determination of Total Phenolic Content.**[25] To 0.1 mL of the test sample, CQSH, CQSE, CQSB, and CQSW were added 0.5 mL of 0.2 N Folin−Ciocalteu's phenol reagent and 0.4 mL of 7.5% $Na_2CO_3$ solution in succession. After mixing well, the mixture was placed in the dark at room temperature for 60 min. Absorbance was read at 765 nm by an ELISA reader. A standard curve of gallic acid (10−100 $\mu$g/mL) was prepared, and the polyphenol concentration was expressed as $\mu$g gallic acid equivalent per g of sample on a dry weight basis.

**DPPH Radical Scavenging Activity.**[14] An aliquot of each test sample (120 $\mu$L, 10−200 $\mu$g/mL) was mixed with 30 $\mu$L of 0.75 mM DPPH [2,2-dipheny1-1-picrylhydrazyl] dissolved in MeOH in a 96-well microplate. The mixture was shaken vigorously on an orbital shaker in the dark at room temperature for 30 min, and then the absorbance was measured at 517 nm using an ELISA reader. The DPPH radical scavenging activity was calculated according to the following equation: $\% = [1 - (A_s - A_0/A)] \times 100$, where $A$ is the absorbance of the solution used (MeOH) to replace the sample solution, $A_s$ is the absorbance of sample with DPPH solution, and $A_0$ is the absorbance of sample dissolved in MeOH. Trolox (5−100 $\mu$M) was used as positive control. The final result was reported as the $IC_{50}$ concentration, which is the concentration of sample required to cause 50% inhibition against DPPH radical in reaction solution.

**Hydroxy Radical and Superoxide Anion Scavenging Ability.**[26,27] The reaction mixture used in the $^{\cdot}OH$-scavenging activity assay contained 0.05 mL of 10 mM EDTA, 1.0 mL of 3 $\mu$M indoxyl-$\beta$-glucuronide, 1.6 mL of 3% $H_2O_2$, and 0.1 mL of 1.0 mM $FeSO_4$. Moreover, the reaction mixture used for the $^{\cdot}O_2^-$-scavenging activity assay contained 1.0 mL of 2.0 mM lucugenin, 0.05 mL of 1.4 $\mu$M methylglyoxal, 0.05 mL of 1.0 M arginine, and 1.0 mL of PBS (pH 7.4). The ultraweak photons were measured using a BJL-ultraweak chemiluminescence analyzer with a high-sensitivity detector ($3.3 \times 10^{-15}$ W/cm$^2$·count). When the chemiluminescence of the reaction mixture in the chemiluminescence analyzer did not reach a plateau, various concentrations of test samples were added to the reaction mixture. As a result, varying degrees of sudden drop in chemiluminescence counts could be observed. These data represent the different degrees of $^{\cdot}OH$ or $^{\cdot}O_2^-$ scavenging abilities. The concentration of test sample that inhibited 50% of the generated $^{\cdot}OH$ or $^{\cdot}O_2^-$ in the systems was defined as $IC_{50}$. Trolox was used as the positive control in these ROS assay system.

**Measurement of Nitric Oxide Production in RAW 264.7 Macrophage Cells.**[28,29] Murine RAW 264.7 macrophage cells obtained from the Bioresource Collection and Research Center (Food Industry Research and Development Institute, Hsinchu, Taiwan) were maintained in 96-well plates ($1 \times 10^5$ cells/well) containing Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO) with 10% fetal bovine serum and $NaHCO_3$ (3.7 g/L) under an atmosphere of 5% $CO_2$ at 37 °C. After overnight preincubation, cells were incubated with vehicle or compounds **1**−**9** and **15** at various concentrations or with quercetin (1−66.2 $\mu$M) in the absence or presence of 1 $\mu$g/mL lipopolysaccharide (LPS) from *Escherichia coli* serotype 0127:B8 (Sigma-Aldrich) for 24 h. All compounds tested were dissolved in DMSO, and the final concentration was adjusted to 0.2% (v/v). Nitric oxide concentration was determined by measuring the accumulation of nitrite ($NO_2^-$) in the RAW 264.7 cell culture supernatant. The aforementioned supernatant medium (100 $\mu$L) was aliquoted and mixed with 100 $\mu$L of Griess reagent (1:1, v/v, 1% sulfanilamide and 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride in 2.5% $H_3PO_4$) in the 96-well plates. After a 5 min incubation, the optical density was measured at 540 nm with an ELISA reader (Molecular Dynamics Spectra Max, GMI Inc., Ramsey, MN).

For further evaluation of cell viability, the MTS assay was also carried out in the presence or absence of LPS and tested compounds. MTS solution (Promega Corporation, Madison, WI) (16.7% MTS in RPMI 1640) was added to each well and incubated for 10 min. Absorbance was measured at 490 nm with an ELISA reader.

**Acknowledgment.** This research was supported financially by the National Science Council, Taiwan, Republic of China (NSC95-2816-B-077-001 and NSC 95-2313-B-077-002- MY2). The authors would like to thank Dr. G.-R. Her and Ms. S.-Y. Sun, Instrumentation Center, National Taiwan University, for mass spectrometric measurements.

**Supporting Information Available:** Tables of biological activities of crude fractions, structures of the known compounds, **8**−**15**, and NMR data of compounds **1**−**8**. This information is available free of charge via the Internet at http://pubs.acs.org.

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NP100181C