Ether-Linked Biflavonoids from *Quintinia acutifolia*

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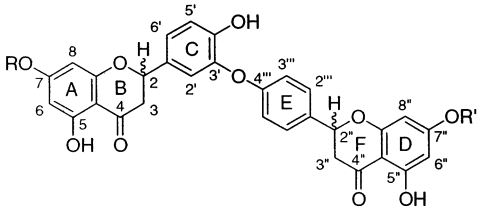
The New Zealand tree *Quintinia acutifolia* has yielded four biflavonoids, the new 2,3,2″,3″-tetrahydroochnaflavone (**3**), and its 7,7″-di-*O*-methyl derivative (**1**). The rare 7-*O*-methyl-2,3,2″,3″-tetrahydroochnaflavone (**2**) and 2″,3″-dihydroochnaflavone (**4**), both previously identified only from members of the Ochnaceae, were also isolated. Structures were determined by spectroscopic methods. This is the first report of biflavonoids from the Grossulariaceae.

The genus *Quintinia* A. DC. (family Grossulariaceae) contains around 15−20 species of trees or shrubs, found in New Zealand, Australia, the Philippines, and Papua New Guinea.[1] The three species endemic to New Zealand are *Q. acutifolia* Kirk, *Q. elliptica* Hook. f., and *Q. serrata* Cunn.[1,2] *Q. acutifolia* grows in lowland and higher mountain forests and is found in the North Island and northern South Island.[2] *Q. acutifolia* and *Q. serrata* have similar morphological features, but *Q. serrata* leaves are more coarsely serrated and are shorter and narrower (5−15 cm long, 1−3 cm wide).[1,3] However, some botanists believe that *Q. acutifolia* and *Q. serrata* are the same species and that the small changes in leaf form are due to geographical differences.[4] Further chemical studies of *Quintinia* species may help to clarify their taxonomic relationships.

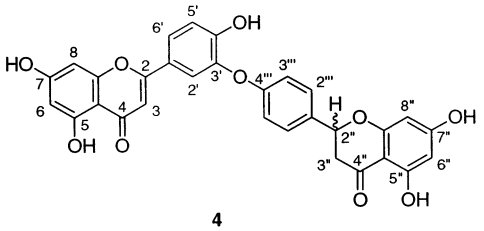
The chemistry of *Quintinia* has been explored very little. In 1918, *Q. serrata* bark extracts were examined in a survey of the tannin content of the bark of some New Zealand flora.[5] The first report of individual chemical constituents identified, by paper chromatography, a flavonoid glycoside, astilbin (dihydroquercetin 3-rhamnoside), and its aglycon, dihydroquercetin, in a methanol extract of the bark of *Q. serrata*.[6] Neither compound was found in the aqueous methanol leaf extract. More recently, flavones and flavonols and hydrocinnamates were noted during an investigation of the functional role of anthocyanins in the leaves of the same species.[7] The flavonols identified were glycosides of quercetin and its 3′-methyl derivative, isorhamnetin.

In the present investigation *Q. acutifolia* leaf extracts showed antimicrobial and cytotoxic activity. We have identified four biflavonoids from these extracts, including two that have not been reported previously.

Dried leaves of *Q. acutifolia* were extracted into ethanol, and the extract was fractionated on a C_{18} RP column. Three medium-polarity fractions had antibacterial activity against *B. subtilis* and cytotoxic activity against P388 leukemia. The ^{1}H NMR spectra of these fractions showed signals in the δ 12−13 ppm region, typical of the intramolecularly hydrogen-bonded OH-5 grouping of flavonoids.[8] Further separation on a Si gel column concentrated the flavonoids into the medium-polarity fractions. An early fraction was further separated by preparative TLC to give biflavanones **1** and **2**. The NMR sample of a later fraction, in $CDCl_3$, deposited crystals while being stored in a refrigerator. This solid material proved to be biflavanone **3**. Preparative TLC of a further fraction gave the flavone/flavanone **4**.



1. R = R' = OCH_3
2. R = OCH_3; R' = H
3. R = R' = H



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The paired signals in the ^{13}C NMR spectrum of **1** (Table 1) suggested either a dimer or a mixture of two closely related compounds. However, the HREIMS showed a molecular ion at 570.1526 Da, consistent with a dimer and corresponding to a molecular formula $C_{32}H_{26}O_{10}$. The UV spectrum in MeOH exhibited absorption maxima at 286 and 330 nm, typical of a biflavanone derivative,[9] while the IR spectrum exhibited a broad OH absorption band at 3416 cm^{-1} and a carbonyl band at 1640 cm^{-1}. The ^{1}H NMR spectrum (Table 1) revealed the signals of two methoxyl groups (δ 3.83, 3.84) and three hydroxyl groups (δ 8.92, 12.10, 12.11). Two of these were as expected for OH-5 groupings in a flavonoid.[8] Further support for a biflavonoid structure came from two carbonyl carbon signals in the ^{13}C NMR spectrum (δ 197.36, 197.39). The X patterns of two ABX systems were noted in the ^{1}H NMR spectrum (δ 5.51, dd, J = 3, 13 Hz and 5.55, dd, J = 3, 13 Hz, H-2″). These showed correlations in the COSY spectrum to two patterns of two overlapping double doublets (centered at δ 2.81 and 3.20, H-3/H-3″) associated with methylene carbon signals (δ 43.4, 43.5), suggesting that both of the flavonoid units were flavanones. These observations accounted for nine of

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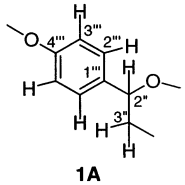
Table 1. NMR Data for Biflavanone **5**[a]

position	^{13}C[b]	^{1}H[c]	COSY	CIGAR[d]	NOESY[e]
2	79.5	5.51, dd, 3, 13	3A,3B	4,8a,1′,2′,6′	2′,6′
3,3″	43.4,43.5	*A* 2.81, 2.82, 2×dd, 3, 17	2/2″,3/3″*B*	4/4″,4a/4a″,1′,1‴	2,3*B*,2″,3″*B*
		B 3.20, 3.21, 2×dd, 13, 17	2/2″,3/3″*A*	2,2″,4/4″,1′,1‴	2,3*A*,2′,6′
4,4″	197.36,197.39				
4a,4a″	103.7,103.7				
5,5″	164.9,165.0				
OH-5,5″		12.10, 12.11, 2×s		4/4″,5/5″,6/6″	n.o.
6,6″	94.60,94.64	6.04, 6.05, 2×d, 2		5/5″,7/7″,8/8″	OMe-7, OMe-7″
7,7″	168.8,168.9				
OMe-7,7″	56.3,56.3	3.83, 3.84, 2×s		7/7″	6,8,6″,8″
8,8″	95.5,95.6	6.02, 6.04, 2×d, 2		5/5″,6/6″,7/7″,8a/8a″	OMe-7, OMe-7″
8a,8a″	163.9,164.0				
1′	131.8				
2′	121.1	7.25, d, 2		2,3′,4′,6′	3‴,5‴
3′	143.6				
4′	150.7				
OH-4′		8.92, s		3′,4′,5′	n.o.
5′	118.2	7.10,d, 8	6′	1′,3′,4′,6′	6′
6′	124.9	7.29, dd, 2, 8	5′	2,2′,3′,4′	2,3*B*,5′
2″	79.7	5.55, dd, 3, 13	3″*A*,3″*B*	4″,8a″,1‴,2‴	2‴,6‴
1‴	133.7				
2‴,6‴	129.0	7.52, dm, 8	3″,5‴	2″,2‴,6‴,4‴	2″,3″*B*,3‴,5‴
3‴,5‴	117.4	6.98, dm, 8	2″,6‴	1‴,3‴,5‴,4‴	2′,2‴,6‴
4‴	159.3				

[a] Recorded at 25 °C in acetone-d_6. ^{1}H NMR determined at 500 MHz referenced to solvent (δ 2.05) and ^{13}C NMR at 125 MHz referenced to solvent (δ 29.9). [b] Some peaks are quoted to 2 decimal places to distinguish distinct signals. [c] Quoted as chemical shift (ppm), multiplicity, coupling constant (Hz). *A* represents the C-3 proton *cis* to H-2 or H-2″ and *B* the *trans* one. [d] Correlation from proton signal to signal of numbered carbon. [e] n.o. = no correlation observed.

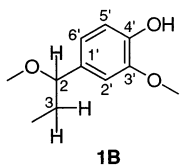
the oxygen atoms, suggesting that the remaining one was involved in the linkage between the two units. Full ^{1}H and ^{13}C NMR data are given in Table 1. One-bond $^{1}H-^{13}C$ correlations were determined by HSQC experiments.

A feature of the ^{1}H NMR spectrum was a coupled pair of two proton doublets of multiplets (δ 6.98, 7.52, J = 8 Hz, H-3‴/H-5‴ and H-2‴/H-6‴) consistent with a *p*-disubstituted benzene ring. The high-field doublet showed a correlation in the CIGAR−HMBC spectrum to an aromatic carbon signal at δ 133.7 (C-1‴), which also was correlated to the H-2″ signal (δ 5.55). The latter showed further correlation to the C-2‴/C-6‴ carbon signal (δ 129.0). This established the linkage between the B and C rings of one flavanone unit. Both the H-3‴/H-5‴ and the H-2‴/H-6‴ proton signals (δ 6.98, 7.52) showed correlations in the CIGAR spectrum to an oxygenated aromatic carbon signal at δ 159.3 (C-4‴), thereby establishing substructure **1A**.



1A

A *meta*-coupled, one-proton doublet (δ 7.25, J = 2 Hz, H-2′) in the ^{1}H NMR spectrum correlated to C-2 of the other flavanone unit (δ 79.54), to two oxygenated aromatic carbons (δ 143.6, 150.7; C-3′ and C-4′, respectively), and to one aromatic methine carbon (δ 124.9, C-6′). This established a 1,3,4-trisubstitution pattern. This unit proved to have a hydroxyl group at C-4′, as a CIGAR correlation was observed between the hydroxyl proton (δ 8.92) and the C-5′ methine carbon. These correlations established substructure **1B**.



1B

The remaining pairs of ^{1}H and ^{13}C NMR resonances were barely resolved (Table 1), and individual signals could not be assigned with confidence. CIGAR correlations from the carbonyl carbon pair (δ 197.36/197.39) to the previously assigned H-2, H-2″, H-3, and H-3″ were consistent with a bis-flavanone structure. The strongly hydrogen-bonded hydroxyl proton signals (δ 12.10, 12.11) suggested hydroxylation at C-5 and C-5″, and this was borne out by a correlation from this pair of peaks to the carbonyl carbon signals. The remaining aromatic proton signals showed *meta* couplings yielding a pattern of four resolved doublets (δ 6.02−6.05) each with coupling constants of 2 Hz. This demonstrated that both A rings had a common 5,7-pattern of dioxygenation. Methoxylation at C-7 and C-7″ was established by a CIGAR linkage between the pair of methoxyl proton peaks (δ 3.83, 3.84) and a pair of carbon signals at δ 168.8/168.9 that in turn correlated to the proton signals in the δ 6.02−6.05 range. This left two incomplete bonds to connect the flavanone units by an ether linkage from C-3′ to C-4‴. Support for this comes from the observation of NOESY correlations between H-2′ and both H-3‴ and H-5‴. Compound **1**, 7,7″-di-*O*-methyl-2,3,2″,3″-tetrahydroochnaflavone, has not been reported previously.

The EIMS of compound **2** showed a molecular ion at 556 Da, consistent with the molecular formula $C_{31}H_{24}O_{10}$, again indicating a dimeric flavonoid structure. ^{1}H and ^{13}C NMR, UV, IR, and optical rotation data matched those of 7-*O*-methyl-2,3,2″,3″-tetrahydroochnaflavone, which has been reported only once previously, from *Ochna beddomei* (Ochnaceae), a deciduous tree found widely in India.[10] The substitution pattern was confirmed by CIGAR and NOESY NMR correlations.

The negative-ion HRESIMS of compound **3** gave $[M - H]^-$ at *m*/*z* 541.1135, $[M - H_2]^{2-}$ at *m*/*z* 270.6, $[M + Cl]^-$ at *m*/*z* 577.1, and $[M_2 - H]^-$ at *m*/*z* 1083.3, which were all

consistent with the molecular formula $C_{30}H_{22}O_{10}$. Again the UV spectrum in MeOH exhibited absorption maxima typical of a flavanone (288, 329 nm).[9] The IR spectrum exhibited a broad OH absorption band at 3414 cm^{-1} and a sharp carbonyl band at 1641 cm^{-1}. The ^1H NMR spectrum closely resembled those of **1** and **2** but showed no methoxyl signals. Signals attributed to H-6, H-6′, H-8, and H-8′ were upfield of their positions in **1** and appeared as a multiplet in the range 5.94−5.97 ppm, as observed for H-6′ and H-8′ of **2** (δ 5.96, 5.97).

The ^{13}C NMR spectrum of **3** showed a twinned signal pattern similar to that of **1**, with considerable signal overlap, consistent with a relatively symmetrical structure. A set of resonances very similar to those for **2** could be assigned to the carbons of rings D, E, and F. Small, but significant differences between the spectra of **2** and **3** were noted in the chemical shifts of the A-ring carbons, particularly those around C-7. This implied that the sole difference between **2** and **3** was the lack of a methoxyl group in **3**. Correlations observed in the CIGAR spectrum paralleled those observed for **1**, in accordance with this structure. Thus, compound **3** is the previously unreported 2,3,2″,3″-tetrahydroochnaflavone.

Compound **4** exhibited a broad OH absorption band at 3411 cm^{-1} in the IR spectrum. There were two distinct carbonyl absorption bands at 1640 (consistent with a flavanone) and 1616 cm^{-1} (more typical of a flavone). Like the previous three compounds, **4** showed optical activity, consistent with at least one flavanone unit. The ^1H NMR spectrum showed no methoxyl proton signals, two intramolecular hydrogen-bonded hydroxyl proton signals (δ 12.14 and 12.95), and a broad three-proton peak at δ 9.64, indicating three further hydroxyl groups. A flavone/flavanone structure was further implicated by the presence of only one ABX system characteristic of a flavanone (δ 5.66, dd, J = 3, 13 Hz; 3.30, dd, J = 13, 17 Hz; 2.91, dd, J = 3, 17 Hz), along with a singlet at δ 6.71, consistent with a flavone. Thus compound was identified as 2″,3″-dihydroochnaflavone (**4**). This compound has been reported twice previously, once from *Ochna interrima*[11] and once from *Luxemburgia nobilis*,[12] both members of the Ochnaceae. Our ^1H NMR data (obtained in acetone-d_6) and ^{13}C NMR data (in DMSO-d_6) matched those of the compound reported from *Ochna interrima*.[11] An alternative structure, 2,3-dihydroochnaflavone, has been reported from *Ochna obtusata*.[13] Although there were some similarities between the ^1H NMR spectra of **4** and those reported for 2,3-dihydroochnaflavone, there were significant differences in the chemical shifts of the protons in the 1,3,4-trisubstituted benzene rings.

All four compounds isolated in this study showed optical activity, and the samples of **2** and **4** proved to have the same absolute stereochemistry as the compounds reported previously. Absolute stereochemistry has yet to be determined for any compound in this series, but most natural flavanones have the 2*S*-configuration.[14]

A quantitative HPLC method for analysis of these biflavonoids was developed. Examination of ethanol extracts of leaf samples from two specimens of *Q. acutifolia* (970128-14 and 010615-01) showed that **1**−**4** were present in ratios of 4.27:1:1.76:1.74 and 5.50:1:1.40:3.48, respectively. The level of **1** was approximately 70 mg per g dry leaves.

Crude ethanol leaf extracts of *Q. acutifolia* were active against *B. subtilis* at a concentration of 150 μg/disk, but biflavonoids **1**−**4** showed no antimicrobial activity at this concentration. Compounds **1**, **3**, and **4** showed some cytotoxicity, with IC_{50} values of 8.7, 8.2, and 3.1 μg/mL, respectively, against P388 murine lymphocytic leukemia cells.

The four biflavonoids identified from the leaves of *Q. acutifolia* in this investigation include two new compounds, 7-di-*O*-methyl-2,3,2″,3″-tetrahydroochnaflavone (**1**) and 2,3,2″,3″-tetrahydroochnaflavone (**3**). The remaining two are rare, with 7-*O*-methyl-2,3,2″,3″-tetrahydroochnaflavone (**2**) having been encountered once previously[10] and 2″,3″-dihydroochnaflavone (**4**) twice.[11,12] These previous records are from plants in the family Ochnaceae. This is the first report of ether-linked biflavonoids from a plant in the Grossulariaceae, although the C−C linked dimers, prodelphinidin B3 and B4, have been reported from *Ribes nigrum* along with a trimeric compound.[15] These uncommon biflavonoids may prove useful chemotaxonomic tools for further description of the Grossulariaceae family and for distinguishing species boundaries in *Quintinia*.

Experimental Section

General Experimental Procedures. Optical rotations were performed on a Perkin-Elmer 241 polarimeter using deuterium (Na, 589 nm) and mercury (Hg, 577, 546, 435, 405 nm) filters. The instrument was first calibrated using cholesterol (20 mg/mL). UV and IR spectra were recorded on Shimadzu UV 240 and Perkin-Elmer Spectrum BX FT-IR spectrometers, respectively. NMR spectra were recorded on a Varian INOVA-500 spectrometer operating at 500 MHz for ^1H and 125 MHz for ^{13}C. NMR spectra were recorded on ca. 0.075 M solutions in acetone-d_6 (referenced to solvent, δ 2.05 for ^1H and δ 29.9 for ^{13}C) at 25 °C. Spectra were assigned with the aid of double-quantum filtered COSY ($^1H-^1H$ correlations), HSQC (one-bond $^1H-^{13}C$ correlations), and HMBC−CIGAR experiments (two- and three-bond $^1H-^{13}C$ correlations).[16] High- and low-resolution electron impact (70 eV) mass spectra were recorded on a Kratos MS80 mass spectrometer. High-resolution, negative ion, electrospray mass spectra were recorded on a Micromass LCT instrument. Preparative TLC was performed using PSC-Platten, 20 × 20 cm, Kieselgel 60 F_{254} of 0.5 mm thickness (Merck). Analytical HPLC was performed on HPLC equipment consisting of a Waters 717 auto-sampler, 600 pump and controller, and a 490E UV programmable multiwavelength detector controlled by Millennium32 (version 3.05, 1998, Waters Corporation) software. Silica gel 60 (Merck 9385), 200−400 mesh, 40−63 μm, was used in the column chromatography, and octadecyl-functionalized Si gel (C_{18} Aldrich) was used for reversed-phase (RP) chromatography. All solvents were distilled before use.

Plant Material. Leaves of *Q. acutifolia* were collected in January 1997 from Charleston, New Zealand, and in June 2001 from the Botanic Gardens, Dunedin, New Zealand, and were identified by A. Evans. Voucher specimens (970128-14 and 010615-01, respectively) have been deposited in the Plant Extracts Research Unit Herbarium, Chemistry Department, University of Otago, Dunedin, New Zealand.

Extraction and Isolation. Air-dried *Q. acutifolia* leaves (71 g) were ground in a Waring blender with EtOH (2 × 400 mL, 300 mL, then 400 mL). The combined EtOH extracts were filtered, and the solvent was evaporated in vacuo. This afforded a brown-green solid mass (9.1 g). A subsample (8.0 g) of the crude extract was fractionated by RP chromatography over C_{18} Si gel [8 g precoated on C_{18} (16 g), loaded onto a C_{18} column (80 g)]. The column was eluted with solvent mixtures of H_2O−MeCN−$CHCl_3$ in decreasing polarity to give nine fractions (H_2O−MeCN−$CHCl_3$ ratio, solvent volume, mass): *A*, 1:0:0, 420 mL, 1.357 g; *B*, 9:1:0, 280 mL, 1.050 g; *C*, 3:1:0, 280 mL, 0.923 g; *D*, 1:1:0, 280 mL, 0.587 g; *E*, 1:3:0, 280 mL, 0.762 g; *F*, 1:9:0, 280 mL, 0.497 g; *G*, 0:1:0, 280 mL, 0.499 g; *H*, 0:1:1, 280 mL, 0.806 g; *I*, 0:0:1, 280 mL, 0.283 g. P388 and *B. subtilis* activity was noted in fractions *E*, *F*, and *G*, and these were chosen for further investigation. The separation was repeated to gain additional material.

Fraction *E* (0.626 g) was precoated onto Si gel (1.2 g) and further fractionated on Si gel (33 g). This column was eluted with hexanes (90 mL), followed by mixtures of hexanes−EtOAc (9:1, 90 mL; 4:1, 90 mL; 3:1, 180 mL; 7:3, 180 mL). Altogether, 10 fractions of progressively increasing polarity were subsequently collected (hexanes−EtOAc ratio, solvent volume, mass): *E1*, 13:7, 180 mL, 0.008 g; *E2*, 3:2, 180 mL, 0.026 g; *E3*, 11:9, 90 mL, 0.023 g; *E4*, 11:9, 90 mL, 0.025 g; *E5*, 1:1, 90 mL, 0.020 g; *E6*, 1:1, 90 mL, 0.025 g; *E7*, 9:11, 90 mL, 0.021 g; *E8*, 9:11, 90 mL, 0.009 g; *E9*, 2:3, 180 mL, 0.021 g; *E10*, 7:13, 180 mL, 0.013 g. Fraction *E4* was dissolved in $CDCl_3$ (0.8 mL) for NMR and stored at 4 °C. This yielded small crystals of 7-*O*-methyl-2,3,2″,3″-tetrahydroochnaflavone (**2**, 0.006 g), which were collected by removal of the supernatant liquid with a Pasteur pipet. Preparative TLC (benzene−EtOAc, 3:2) of the mother liquors gave 7,7″-di-*O*-methyl-2,3,2″,3″-tetrahydroochnaflavone (**1**, 0.008 g). Preparative TLC (benzene−EtOAc, 3:2) of fraction *E3* yielded both **1** (0.004 g) and **2** (0.002 g). Fraction *E2* was predominantly **1**. Evaporation of fraction *E5* gave **3** (0.020 g) as a creamy white solid, and *E7* likewise gave 2″,3″-tetrahydroochnaflavone **4** (0.021 g) as a cream-colored solid. Subsequently, pure samples of **1**−**4** were able to be obtained by semipreparative HPLC using the method outlined below.

7,7″Di-*O*-methyl-2,3,2″,3″-tetrahydroochnaflavone (1): white powder; $[\alpha]^{25.2}_{589}$ +26°, $[\alpha]^{27.4}_{577}$ −10°, $[\alpha]^{28.1}_{546}$ −63°, $[\alpha]^{28.4}_{435}$ −416°, $[\alpha]^{28.6}_{405}$ −502° (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.32), 286 (3.91), 330 (3.31), (MeOH + NaOH) 207 (4.79), 325 (3.65), 417 (3.06) nm; IR (KBr) ν_{max} 3416, 1640, 1508, 1458, 1300, 1157 cm^{-1}; ^1H NMR (Me_2CO-d_6) and ^{13}C NMR (Me_2CO-d_6), see Table 1; HREIMS *m*/*z* 570.1526 (100) $[M]^+$ (calcd for $C_{32}H_{26}O_{10}$, 570.1526); TLC R_f (Si gel, hexanes−EtOAc; 1:1) 0.80 (vis. vanillin−H_2SO_4).

7-*O*-Methyl-2,3,2″,3″-tetrahydroochnaflavone (2)[10] (CAS No. 270928-21-7): colorless crystals; $[\alpha]^{28.7}_{589}$ −6°, $[\alpha]^{28.7}_{577}$ −24°, $[\alpha]^{28.8}_{546}$ −53°, $[\alpha]^{28.9}_{435}$ −223°, $[\alpha]^{29.1}_{405}$ −237° (*c* 0.14, MeOH); ^1H and ^{13}C NMR, see Tables S1 and S2 (Supporting Information); EIMS *m*/*z* 556 $[M]^+$, 539 (2), 448 (1), 404 (3), 389 (15), 376 (2), 282 (1), 264, 240, 225, 207, 193 (5), 167 (18), 153 (11), 140 (100); TLC R_f (Si gel, hexanes−EtOAc, 1:1) 0.60 (vis. vanillin−H_2SO_4).

2,3,2″,3″-Tetrahydroochnaflavone (3): creamy white powder; $[\alpha]^{21.3}_{589}$ +451°, $[\alpha]^{21.9}_{577}$ +403°, $[\alpha]^{23.1}_{546}$ +323°, $[\alpha]^{22.8}_{435}$ −140°, $[\alpha]^{23.4}_{405}$ −236° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (4.67), 288 (4.38), 329 (3.91), (MeOH + NaOH) 209 (4.84), 323 (4.59), 396 (3.17) nm; IR (KBr) ν_{max} 3414, 1641, 1508, 1465, 1157 cm^{-1}; ^1H and ^{13}C NMR, see Tables S1 and S2 (Supporting Information); HRESIMS *m*/*z* 541.1140 $[M - H]^-$ (calcd for $C_{30}H_{21}O_{10}$, 541.1138), 270 $[M - H_2]^{2-}$, 577 $[M + Cl]^-$, 1083 $[M_2 - H]^-$; TLC R_f (Si gel, hexanes−EtOAc, 1:1) 0.52 (vis. vanillin−H_2SO_4).

2″, 3″-Dihydroochnaflavone (4)[11,12] (CAS No. 340997-02-6): cream-colored powder; $[\alpha]^{24.0}_{589}$ +426°, $[\alpha]^{24.2}_{577}$ +362°, $[\alpha]^{24.4}_{546}$ +294°, $[\alpha]^{24.7}_{435}$ −150°, $[\alpha]^{25.1}_{405}$ −297° (*c* 0.05, MeOH); ^1H and ^{13}C NMR, see Tables S1 and S2 (Supporting Information); TLC R_f (Si gel, hexanes−EtOAc, 1:1) 0.40 (vis. vanillin−H_2SO_4).

HPLC Method for Analysis of Biflavonoids 1−4. Analyses were carried at 25 °C on a C_{18} column (Phenomenex Prodigy ODS(3), 5 μm, 100A, 250 × 4.6 mm) with a 2 × 4 mm C_{18} guard column. The following gradient program was used (time {min}, ratio of MeCN−H_2O each containing 0.1% H_3PO_4): 0.0, 1:4; 30.0, 3:2; 40.0, 1:0; 50.0, 1:4. The flow rate was 1.0 mL/min, with an injection volume of 10 μL. Detection was at 280 nm, and the retention times (min) were as follows: **1**, 40.0; **2**, 36.6; **3**, 30.7; **4**, 30.9.

Biological Assays. Assays against *Bacillus subtilis* (ATCC 19659) and P388 murine leukaemia cells (ATCC CCL 46 P388D1) were conducted according to published protocols.[17] A crude ethanol extract of *Q. acutifolia* leaves showed activity against *B. subtilis* with a zone of growth inhibition of 3 mm at a dose level of 750 μg/disk [positive control: chloramphenicol (30 μg per disk), 12 mm inhibition zone]. In the P388 assay, the crude ethanol extract had an IC_{50} of 50 μg/mL, and IC_{50} values of 8.7, 8.2, and 3.1 μg/mL were determined for compounds **1**, **3**, and **4**, respectively [positive control: mitomycin C (0.06 μg/mL), which inhibited cell growth by 43−75%[1]].

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Supporting Information Available: Tables of ^1H and ^{13}C NMR data for compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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