Melanin Synthesis Inhibitors from *Lespedeza cyrtobotrya*

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From the roots of *Lespedeza cyrtobotrya*, 45 flavonoids were isolated along with 20 new and 25 known compounds. Lipophilic flavonoids **2**, **3**, **7**, **9**, **11**, **28**, **30**, and **39** exhibited strong inhibitory activities on melanin synthesis in normal human epidermal melanocytes.

We have reported chalcone and isoflavene derivatives from the aerial parts of *Lespedeza cyrtobotrya* Miq. (Leguminosae),[1,2] and these isoflavene derivatives showed strong inhibitory activity on melanin synthesis.[3] In the course of further research for new melanogenesis inhibitors from plants, 20 new and 25 known flavonoids were isolated from the roots of this plant. The structures of the new compounds were elucidated by NMR analysis, while the absolute configurations were determined from the CD spectra. The known compounds were identified as liquiritigenin (**21**),[4] bavachin (**22**),[5] isobavachin (**23**),[6] abyssinone II (**24**),[7] naringenin (**25**),[8] leachianone G (**26**),[9] 6-methylaromadendrin (**27**),[10] isobavachalcone (**28**),[11] licoagrochalcone A (**29**),[12] xanthoangerol (**39**),[1] lespeol (**31**),[1] genistein (**32**),[1] 6-methylgenistein (**33**),[13] neobavaisoflavone (**34**),[14] uncinanone A (**35**),[15] bavaisoflavanone (**36**),[16] 1-(5-hydroxy-2,2-dimethyl-2*H*-chromen-6-yl)-3-(4-hydroxyphenyl-)propanone (**37**),[17] phaseolidin (**38**),[18] erybraedin A (**39**),[19] erybraedin C (**40**),[20] lespeflorin G_4 (**41**),[21] phaseollin (**42**),[18] gangetinin (**43**),[22] lespeflorin I_2 (**44**),[21] and lespeflorin J_3 (**45**),[21] by comparison with reported NMR data. We measured inhibitory activities of these compounds on the production of melanin in normal human epidermal melanocytes and found that many compounds showed inhibitory activities, with compounds **2**, **3**, **7**, **9**, **11**, **28**, **30**, and **39** being more potent than hydroquinone, which is a major skin-lightening drug.

Results and Discussion

The ^{1}H NMR spectrum of lespecyrtin A_1 (**1**) showed a typical AB-type proton signal pattern at δ 4.53 and 5.03 (each 1H, d, J = 12 Hz), suggesting this compound to be a flavanonol.[23] Also observed were one set of isoprenyl proton signals, AA′BB′-type proton signals at δ 6.91 and 7.46 (each 2H, d, J = 8.5 Hz), and AX-type proton signals at δ 6.69 and 7.59 (each 1H, d, J = 8.5 Hz). The absolute stereochemistry was decided as 2*R*,3*R* from the large coupling constant (12 Hz) between H-2 and H-3,[24] a laevoratory optical activity,[25] and a positive Cotton effect at the n→π* absorption band in the CD spectrum[25] of lespecyrtin A_1.

Lespecyrtins B_1 (**2**), B_2 (**3**), and B_3 (**4**) were assigned with a chalcone skeleton having a 1,4-disubstituted benzene ring. The ^{1}H NMR spectra of these chalcones showed a set of AX-type proton signals in the aromatic proton region due to H-5′ and H-6′ and an ether-ring side chain. Lespecyrtin B_1 (**2**) showed a vinyl methyl proton at δ 1.78 (brs), two exocyclic methylene protons at δ 4.94 (m) and 5.10 (m), a set of methylene protons at δ 3.03 (dd, J = 16, 8 Hz) and 3.37 (dd, J = 16, 10 Hz), a methine proton at δ 5.33 (dd, J = 10, 8 Hz), and a hydrogen-bonded hydroxyl proton at δ 13.50 (s). In the ^{13}C NMR spectrum, an ether-linked methine carbon signal was observed at δ 88.1.[15,26] Sheng et al.[27] reported the same structure for artonin ZA from *Artocarpus heterophyllus* Lamk. (Moraceae), having an oxygenated carbon signal at δ 77.1. This carbon should be assigned to a carbon bearing a hydroxyl group.[28] Lespecyrtin B_2 (**3**) has a benzpyrene structure [δ 6.73 and 5.75 (each 1H, d, J = 10 Hz)] and two tertiary methyl groups at δ 1.53 (6H, s). The ^{13}C NMR spectrum of lespecyrtin B_3 (**4**) showed two oxygenated carbon signals at δ 71.4 and 92.6. The former was assigned to the γ-carbon and the latter to the β-carbon of a side chain from the HMQC and HMBC spectra.[29]

Lespecyrtin C_1 (**5**) gave similar ^{1}H NMR data to those of lespecyrtin B_1 (**2**) except for a set of AA′BB′-type aliphatic proton signals at δ 2.93 and 3.26 (each 2H, d, J = 7.5 Hz), which were characteristic of a dihydrochalcone.[17]

The ^{1}H NMR spectrum of lespecyrtin D_1 (**6**) showed five aliphatic proton signals at δ 2.75 (ddd, J = 15.5, 5, 2.5 Hz), 2.95 (dd, J = 15.5, 10 Hz), 3.47 (m), 3.95 (dd, J = 10, 10 Hz), and 4.19 (ddd, J = 10, 4, 2.5 Hz), which were characteristic of an isoflavan.[30] In the aromatic proton region, four singlet proton signals were observed at δ 6.35, 6.47, 6.76, and 6.77. The aromatic proton signal at δ 6.76 showed ROEs between the proton signals at δ 3.23 (2H, brd, J = 7.5 Hz), 5.32 (1H, tsept, J = 7.5, 1 Hz), and 2.95 (1H, dd, J = 15.5, 10 Hz), due to the α-methylene protons, the β-olefinic proton, and the H-4 proton signal, respectively. The singlet proton signal at δ 6.77 showed ROEs between the proton signals at δ 3.75 (3H, s), 2.75 (1H, ddd, J = 15.5, 5, 2.5 Hz), 3.47 (1H, m), and 3.95 (1H, dd, J = 10, 10 Hz), due to the methoxyl protons, the H-4 proton, the H-3 proton, and the H-2 proton, respectively. The absolute stereochemistry at C-3 was deduced as *R* from the negative Cotton effect at 233 nm (−6648) and the positive Cotton effect at 290 nm (+1583).[31]

Lespecrytins E_1 (**7**), E_2 (**8**), E_3 (**9**), E_4 (**10**), E_5 (**11**), E_6 (**12**), and E_7 (**13**) were assigned with a pterocarpan skeleton from the characteristic ^{1}H NMR spectroscopic data in the aliphatic proton region, and in the aromatic proton region two singlet proton signals due to H-1 and H-4 were observed. The former five compounds also had one or two 2,2-dimethyl-2*H*-chromene rings. The ^{1}H NMR spectrum of lespecrytin E_1 (**7**) showed two sets of 2,2-dimethylpyrene ring protons at δ 1.39, 1.41, 1.43, and 1.44 (each 3H, s) and 5.54, 5.57, 6.32, and 6.51 (each 1H, d, J = 10 Hz) and one set of AX-type aromatic proton signals at δ 6.33 and 6.94 (each 1H, d, J = 8 Hz). The aromatic proton at δ 6.94 showed ROEs between the proton signals at δ 3.48 (m), 3.59 (1H, dd, J = 11, 11 Hz), and 4.21 (1H, dd, J = 11, 5 Hz) due to H-6a, H-6β, and H-6α, respectively. The ^{1}H NMR spectrum of lespecrytin E_2 (**8**) was similar to that of lespecrytin E_1 (**7**) except for one set of isoprenyl proton signals. The HRFABMS showed a molecular ion peak at *m*/*z* 472.2258, suggesting the molecular formula $C_{30}H_{32}O_5$. The ^{13}C

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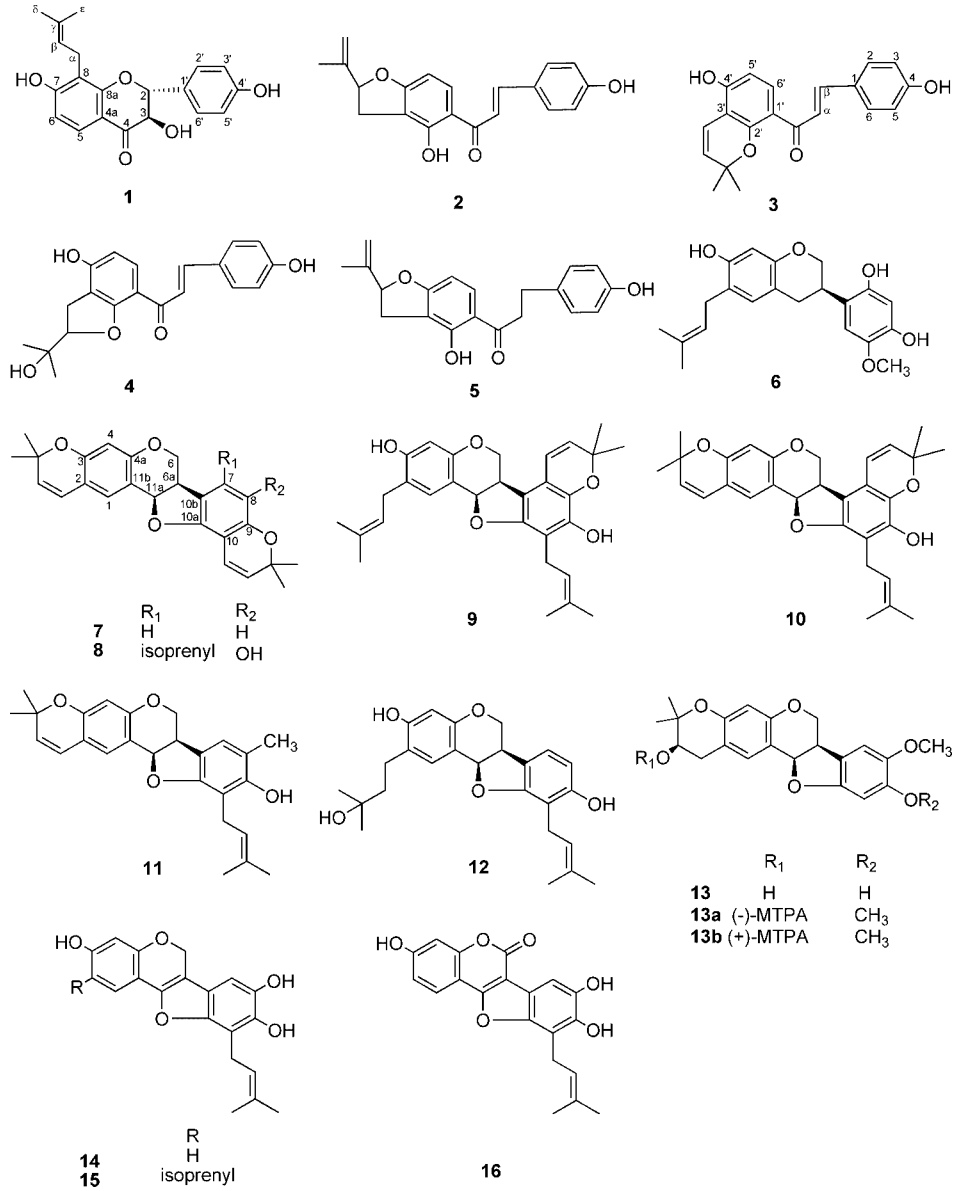
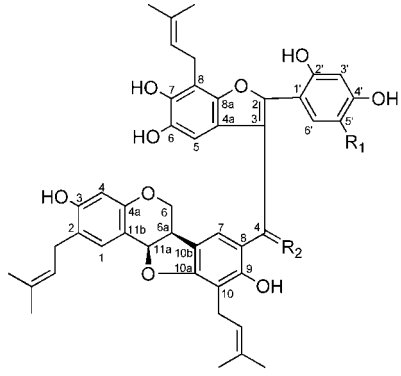


NMR spectrum showed a specifically hindered α-methylene carbon signal of an isoprenyl residue at δ 27.2 and *ortho* dioxygenated aromatic carbon signals at δ 137.8 and 140.0.[21] From these NMR data, the structure of lespecrytin E_2 was deduced as shown. Lespecrytins E_3 (**9**) and E_4 (**10**) gave similar ^{1}H NMR spectra to that of lespecrytin E_2 (**8**). In the ^{1}H NMR spectrum of lespecrytin E_3 (**9**), two sets of isoprenyl proton signals were evident, and ROEs were observed between the proton signal at δ 3.34 (1H, brd, J = 7.5 Hz) and an aromatic proton signal at δ 7.24 and between an olefinic proton signal at δ 6.30 (1H, d, J = 10 Hz) and one of the methylene protons at δ 3.52 (1H, dd, J = 11, 11 Hz) and an olefinic proton signal at δ 5.66 (1H, d, J = 10 Hz). The ^{1}H NMR spectrum of lespecyrtin E_4 (**10**) exhibited two 2,2-dimethyl-2*H*-chromene structures as well as a set of isoprenyl proton signals that was specifically hindered as determined from the ^{13}C NMR spectroscopic data for the α-carbon (δ 23.9). ROEs were observed between an aromatic proton at δ 7.18 (s) and an olefinic proton signal at δ 6.42 (1H, d, J = 10 Hz) and a methine proton signal at δ 5.33 (1H, d, J = 7 Hz), and between an olefinic proton signal at δ 6.51 (1H, d, J = 10 Hz) and one methylene proton signal at δ 4.27 (1H, dd, J = 11.5, 5 Hz) and a methine proton signal at δ 3.60 (1H, m). From these data, the structure of lespecyrtin E_4 was determined to be **10**. Lespecrytin E_5 (**11**) had one methyl group resonance at δ 2.17, which was attached to C-8. In the HMBC spectrum, this methyl proton was long-range coupled to C-7 (δ 124.1), C-8 (δ 116.8), and C-9 (δ 154.1), and the α-methylene proton signal at δ 3.29 (2H, brd, J = 7 Hz) was long-range coupled to C-9 (δ 154.1), C-10 (δ 112.2), and C-10a (δ 157.7). The HRFABMS of lespecrytin E_6 (**12**) showed a molecular ion peak at m/z 410.2097, suggesting a molecular formula of $C_{25}H_{30}O_5$. The ^{1}H NMR spectrum was similar to that of erybraedin C (**40**)[20] except for one set of isoprenyl proton signals. In the aliphatic proton region, isolated ethylene proton signals at δ 1.77 and 2.70 (each 2H, m) and two singlet methyl proton signals at δ 1.25 (6H, s) occurred. ROEs were observed in the methylene ptoton signal at δ 2.70 and the methine proton signal at δ 5.45 (1H, d, J = 7 Hz) due to the α-methylene proton and H-11a, on irradiation of the aromatic proton signal at δ 7.23 (1H, s). In the ^{13}C NMR spectrum, an oxygenated carbon signal was observed at δ 70.3, which was long-range coupled to the carbon signals due to the methyl proton signal at δ 1.25 and the α-methylene proton signal at δ 2.70.[32] These NMR data showed the structure of lespecrytin E_6 to be **12**. The ^{1}H NMR spectrum of lespecrytin E_7 (**13**) showed a methoxyl proton signal at δ 3.80 (s), four singlet aromatic proton signals at δ 6.22, 6.33, 7.01, and 7.15, and ABX-type proton signals at δ 2.70 (1H, dd, J = 16.5, 8.5 Hz), 2.99 (1H, dd, J = 16.5, 5.5 Hz), and 3.77 (1H, dd, J = 8.5, 5.5 Hz). The ^{13}C NMR spectrum indicated the presence of a 2,2-dimethyl-3-hydroxy-2*H*-chromane skeleton[33,34] at δ 31.5, 69.8, and 78.1. ROEs were observed between the aromatic proton at δ 7.01 (1H, s) and two methylene protons at δ 3.57 (1H, dd, J = 10, 10

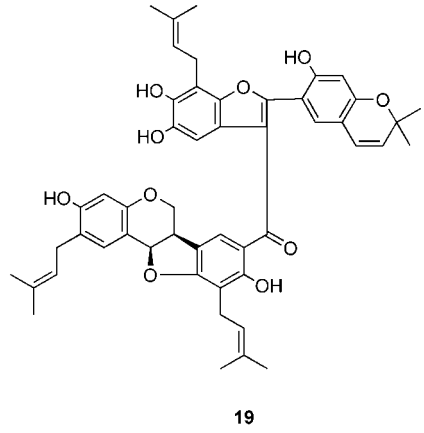
Hz) and 4.23 (1H, dd, $J = 10$, 10 Hz), a methine proton signal at δ 3.55 (1H, m), and a methoxyl proton signal at δ 3.80. The absolute stereochemistry of the β-carbon was determined to be *R* using the Mosher ester method for its methyl ether derivative. The absolute stereochemistries at C-6a and C-11a of lespecrytins E_1-E_7 were determined to be 6a*R* and 11a*R* from their levoratory optical activities[35] and positive Cotton effects at the region 260−310 nm in their CD spectra.[31]

Lespecyrtins F_1 (**14**) and F_2 (**15**) showed a characteristic singlet proton signal (H_2-6) of a pterocarpene skelton near 5.5 ppm in their ^{1}H NMR spectra.[30] In the ^{1}H NMR spectrum of **14**, a set of ABX-type aromatic proton signals at δ 6.44 (1H, d, $J = 2$ Hz), 6.51 (1H, dd, $J = 8.5$, 2 Hz), and 7.31 (1H, d, $J = 8.5$ Hz) and a singlet aromatic proton signal at δ 6.78 (1H, brs) were seen. The latter showed ROEs between a methylene proton signal at δ 5.49 (2H, s), which was assigned to H_2-6, and a set of isoprenyl proton signals. In the ^{1}H NMR spectrum of **15**, three singlet aromatic proton signals at δ 6.45, 6.75, and 7.19 and two sets of isoprenyl proton signals were observed. These NMR data led to the structures **14** and **15** for lespecyrtins F_1 and F_2, respectively.

Lespecyrtin G_1 (**16**) was assumed to have a coumestan skeleton from the characteristic carbon signal at δ 158.8.[30] The ^{1}H NMR spectrum of lespecyrtin G_1 showed a set of ABX-type aromatic proton signals at δ 6.91 (1H, d, $J = 2$ Hz), 6.96 (1H, dd, $J = 8.5$, 2 Hz), and 7.80 (1H, d, $J = 8.5$ Hz) and a set of isoprenyl proton signals. These data led to the assignment of structure **16** for lespecyrtin G_1.



	R_1	R_2
17	isoprenyl	H_2
18	H	O



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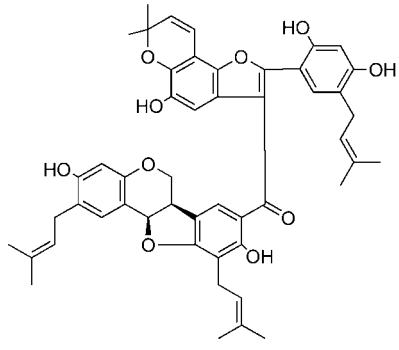

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Lespecyrtins H_1 (**17**), H_2 (**18**), H_3 (**19**), and H_4 (**20**) were assumed to be dimeric flavonoids composed of a pterocarpan and a 2-arylbenzofuran derivative, from their molecular formulas and ^{13}C NMR spectra like lespeflorins J_1-J_4, which were isolated from *Lespedeza floribunda* Bunge.[21] The ^{1}H NMR spectra of lespecyrtin H_1 (**17**) showed a characteristic spin system of a pterocarpan at δ 3.48 (1H, dd, $J = 10.5$, 10.5 Hz), 4.12 (1H, m), 3.40 (1H, m), and 5.39 (1H, d, $J = 7$ Hz), six singlet aromatic proton signals at δ 6.36, 6.53, 6.59, 6.87, 7.12, and 7.19, and four sets of isoprenyl proton signals and isolated methylene proton signals at δ 3.92 and 3.96 (each 1H, d, $J = 16.5$ Hz), which showed long-range coupling to C-2, C-3, and C-4a (upper moiety) and C-7, C-8, and C-9 (lower moiety) in the HMBC spectrum. ROE experiments on irradiating at δ 6.87 suggested that this proton could be assigned to H-7 of the lower moiety, by showing ROEs at the proton signals at δ 3.92, 3.44, 3.48, 3.96, 6.53, and 7.12. Irradiating at δ 7.12 showed ROEs at δ 3.29 (2H, brd, $J = 7.5$ Hz) and 5.36 (1H, tsept, $J = 7.5$, 1 Hz), and irradiating at δ 7.19 showed ROEs at δ 3.25 (2H, brd, $J = 7.5$ Hz) and 5.30 (1H, tsept, $J = 7.5$, 1 Hz). From these data, the structure of lespecyrtin J_1 was assigned as **17** like lespedezols B_2 and B_3, which were isolated from *Lespedeza homoloba* Nakai.[37] Lespecyrtin H_2 (**18**) showed similar NMR data to those of lespeflorin J_4,[36] but lacked a methoxyl signal. The NMR spectra of lespecyrtins H_3 and H_4 showed three sets of isoprenyl proton signals and a dimethylbenzopyrene structure. In a ROE experiment on lespecyrtin H_3, irradiation at δ 7.33 showed ROEs at δ 6.37 (α proton) and δ 7.41 (1H, brs). In a ROE experiment of lespecyrtin H_4, on irradiating at δ 7.27 (1H, s), a ROE occurred at δ 5.30 (1H, tsept, $J = 7.5$, 1 Hz), and on irradiation at δ 7.18 (1H, s) ROEs occurred at δ 3.27 (2H, brd, $J = 7.5$ Hz) and 5.35 (1H, t sept, $J = 7.5$, 1 Hz). In the HMBC spectrum of lespecyrtin H_4, long-range couplings were observed between H-α at δ 6.84 (1H, d, $J = 10$ Hz) and the carbon signals at δ 107.0, 139.3, and 144.3, which were assigned to C-8, C-9, and C-8a of the upper moiety, respectively, from the upfield shifted oxygenated aromatic carbon signals. The absolute stereochemistry of the pterocarpan skeleton of these compounds was deduced as 6a*R*, 11a*R* from their levoratory optical activities.[35]

To evaluate the inhibitory activities on melanin synthesis of the isolated flavonoids, we measured 50% inhibition concentration (IC_{50}) values of melanin synthesis in normal human epidermal melanocytes (NHEM).[37] As a result, 37 compounds showed inhibitory activities among 45 compounds from this plant (Table

Table 1. NMR Spectroscopic Data (400 MHz) for Compounds **1** and **6**

	1[a]		**6**[a]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
2	5.03 (d, 12)	84.8	3.95 (dd, 10, 10)	70.5
			4.19 (ddd, 10, 4, 2.5)	
3	4.53 (d, 12)	73.9	3.47 (m)	33.0
4		193.6	2.75 (ddd, 15.5, 5, 2.5)	31.3
			2.95 (dd, 15.5, 11)	
4a		113.3		110.3
5	7.59 (d, 8.5)	126.6	6.76 (s)	130.9
6	6.69 (d, 8.5)	111.0		121.0
7		162.8		154.6
8		116.6	6.30 (s)	103.5
8a		162.1		154.0
1′		129.7		118.0
2′	7.46 (d, 8.5)	130.1		150.2
3′	6.91 (d, 8.5)	115.8	6.47 (s)	104.3
4′		158.6		146.9
5′	6.91 (d, 8.5)	115.8		142.0
6′	7.46 (d, 8.5)	130.1	6.77 (s)	113.1
side chain at 6				
α			3.23 (brd, 7.5)	28.4
β			5.32 (tsept, 7.5, 1)	124.6
γ				131.6
δ			1.70 (brs)	25.9
ε			1.70 (brs)	17.8
at 8				
α	3.30 (m)	22.7		
β	5.21 (tsept, 7.5, 1)	122.9		
γ		131.9		
δ	1.61 (brs)	25.9		
ε	1.58 (brs)	17.9		
OMe			3.75 (s)	57.4

[a] Measured in acetone-d_6.

6), and none affected cell viability (data not shown). In particular, compounds **2**, **3**, **7**, **9**, **11**, **28**, **30**, and **39**, which have more lipophilic structures than the other compounds, showed strong inhibitory activities. The IC_{50} values of these compounds were lower than 2 μM, while that of hydroquinone, a positive control, was 2.2 μM. Thus, these compounds were more potent in cell culture than hydroquinone, which is widely used as a skin lightening agent. Some flavonoid derivatives from plants, such as quercetin and artocarpanone, were demonstrated previously to have inhibitory activities against mushroom tyrosinase, the rate-limiting enzyme of melanin synthesis.[38] We are now studying the inhibitory mechanisms of these active compounds.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. UV spectra were measured in methanol on a JASCO V-630 spectrophotometer. Circular dichroism spectra were measured on JASCO J-20A spectrometer. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a JEOL JNM α-400 FT-NMR spectrometer, and chemical shifts are given as δ values with TMS as an internal standard at 35 °C. Inverse-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{C-H} = 145$ Hz) and HMBC (optimized for $^nJ_{C-H} = 8$ Hz) pulse sequences with a pulse field gradient. HRFABMS data were obtained on a JEOL JMS 700 mass spectrometer in the positive mode using a *m*-nitrobenzyl alcohol matrix. Preparative HPLC was performed on a JASCO 800 instrument.

Plant Material. The roots of *Lespedeza crytobotrya* were harvested from our medicinal plant garden in April 2007. The plant was authenticated by Prof. Akira Ueno, University of Shizuoka, and a voucher specimen (200704023) has been deposited at the Herbarium of the University of Shizuoka.

Extraction and Isolation. Air-dried roots (4.0 kg) of *L. cyrtobotrya* were extracted with methanol (10 L) under reflux for 3 h twice. The methanol extract was concentrated under reduced pressure to give a brown residue (345 g). The residue was suspended in hot water (2 L) and extracted with ether continuously for 3 h. The ether layer was concentrated under reduced pressure to give a brown residue (85 g). The ether extract was chromatographed on a silica gel (Fiji Silysia PSQ-100B, 800 g) column using mixture of hexane−ethyl acetate (9:1−5:5) as solvents to give 33 fractions (Frs. 1−33). Fr. 3 (584 mg) was subjected to semipreparative HPLC [column, Cosmosil 5C_{18}-AR-II 2 × 25 cm; solvent, H_2O-CH_3CN (20:80), UV 280 nm] to give **7** (27 mg). Fr. 4 (105 mg) was subjected to semipreparative HPLC [column, Inertsil ODS-3, 3 × 50 cm; solvent, H_2O-CH_3CN (15:85), UV 280 nm] to give **43** (2.1 mg). Fr. 5 (101 mg) was subjected to semipreparative HPLC [column, Inertsil ODS-3, 3 × 50 cm; solvent, H_2O-CH_3CN (15:85), UV 280 nm] to give **10** (1.8 mg) and **8** (2.3 mg). Fr. 6 (221 mg) was subjected to semipreparative HPLC [column, Inertsil ODS-3, 3 × 50 cm; solvent, H_2O-CH_3CN (15:85), UV 280 nm] to give **11** (3.1 mg). Fr. 12 (731 mg) was subjected to semipreparative HPLC

Table 2. NMR Spectroscopic Data (400 MHz) for Compounds **2**−**5**

	2[a]		**3**[a]		**4**[a]		**5**[a]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1′		115.0		121.8		115.5		115.1
2′		161.5		157.7		168.1		161.0
3′		113.3		110.1		114.6		113.6
4′		166.9		154.8		162.1		167.6
5′	6.44 (d, 9)	101.8	6.54 (d, 8.5)	109.2	6.40 (d, 8.5)	102.3	6.39 (d, 9)	102.4
6′	7.79 (d, 9)	131.9	7.52 (d, 8.5)	132.1	8.09 (d, 8.5)	133.1	7.83 (d, 9)	132.7
C=O		192.1		189.3		193.1		205.3
α	7.44 (d, 17)	118.3	7.57 (d, 15.5)	126.3	7.78 (d, 15.5)	118.7	2.93 (dd, 7.5, 7.5)	30.4
β	7.82 (d, 17)	144.0	7.63 (d, 15.5)	141.7	7.83 (d, 15.5)	145.1	3.26 (dd, 7.5, 7.5)	40.6
1		127.9		128.7		127.7		133.8
2	7.55 (d, 8.5)	130.5	7.58 (d, 8.5)	130.8	7.74 (d, 8.5)	131.7	7.10 (d, 8.5)	130.2
3	6.88 (d, 9)	116.0	6.93 (d, 8.5)	116.8	6.93 (d, 8.5)	116.7	6.75 (d, 8.5)	116.0
4		158.0		160.2		160.9		156.5
5	6.88 (d, 9)	116.0	6.93 (d, 8.5)	116.8	6.93 (d, 8.5)	116.7	6.75 (d, 8.5)	116.0
6	7.55 (d, 8.5)	130.5	7.58 (d, 8.5)	130.8	7.74 (d, 8.5)	131.7	7.10 (d, 8.5)	130.2
side chain at 3′								
α	3.03 (dd, 16, 8)	31.1	6.73 (d, 10)	117.6	3.14 (dd, 15.5, 9.5)	27.6	2.93 (dd, 15.5, 7.5)	31.6
	3.37 (dd, 16, 10)				3.18 (dd, 15.5, 8)		3.34 (dd, 15.5, 10)	
β	5.33 (dd, 10, 8)	88.1	5.75 (d, 10)	129.3	4.81 (dd, 9.5, 8)	92.6	5.38 (dd, 10, 7.5)	88.5
γ		143.3		77.7		71.4		144.8
δ	1.78 (brs)	17.0	1.53 (s)	20.8	1.29 (s)	25.4	1.77 (brs)	17.1
ε	4.94 (m)	112.5	1.53 (s)	20.8	1.24 (s)	25.9	4.92 (m)	112.4
	5.10 (m)						5.08 (m)	

[a] Measured in acetone-d_6.

Table 3. NMR Spectroscopic Data (400 MHz) for Compounds **7**−**13**

	7[a]		**8**[b]		**9**[a]		**10**[a]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	7.14 (s)	128.5	7.17 (s)	129.8	7.24 (s)	132.1	7.18 (s)	129.7
2		116.3		117.5		122.0		116.9
3		154.6		155.4		155.6		155.3
4	6.37 (s)	104.7	6.29 (s)	104.9	6.41 (s)	103.9	6.28 (s)	104.9
4a		156.5		157.3		154.9		157.3
6	3.59 (dd, 11, 11)	66.6	3.38 (dd, 11, 11)	66.6	3.52 (dd, 11, 11.5)	66.1	3.45 (dd, 11.5, 11.5)	66.8
6	4.21 (dd, 11, 5)		4.28 (dd, 11, 5)		4.19 (dd, 11, 3)		4.27 (dd, 11.5, 5)	
6a	3.48 (m)	39.8	3.52 (ddd, 11, 6.5, 5)	39.9	3.49 (m)	38.8	3.60 (m)	39.5
11a	5.45 (d, 7)	78.9	5.31 (d, 6.5)	78.8	5.31 (d, 6)	77.5	5.33 (d, 7)	78.3
11b		112.3		113.6		112.6		114.0
10b		119.1		118.4		112.6		113.1
7	6.94 (d, 8)	123.8		126.0		115.1		116.6
8	6.33 (d, 8)	108.6		137.8		133.1		134.7
9		153.8		140.0		143.0		144.4
10		106.2		116.9		111.6		112.2
10a		155.5		148.4		151.8		152.7
Me								
OMe								
side chain at 2								
α	6.32 (d, 10)	121.7	6.42 (d, 10)	122.4	3.34 (brd, 7.5)	29.2	6.42 (d, 10)	122.4
β	5.54 (d, 10)	129.1	5.67 (d, 10)	129.9	5.34 (tsept., 7.5, 1)	120.9	5.66 (d, 10)	129.9
γ		76.7		77.3		134.7		77.2
δ	1.43 (s)	28.0	1.40 (s)	27.9	1.79 (brs)	25.8	1.45 (s)	27.6
ε	1.44 (s)	28.3	1.42 (s)	28.4	1.79 (brs)	17.9	1.45 (s)	28.3
at 7								
α′			3.35 (brd, 7.5)	27.2	6.30 (d, 10)	118.9	6.51 (d, 10)	119.9
β'			5.27 (tsept., 7.5, 1)	124.0	5.66 (d, 10)	131.8	5.79 (d, 10)	133.3
γ'				131.7		76.5		76.6
δ'			1.70 (d, 1)	25.8	1.44 (s)	27.8	1.45 (s)	27.3
ε'			1.80 (brs)	18.0	1.45 (s)	27.8	1.45 (s)	27.5
at 10								
α″	6.51 (d, 10)	116.8	6.44 (d, 10)	117.5	3.30 (m)	23.3	3.26 (brd, 7.5)	23.9
β''	5.57 (d, 10)	129.6	5.65 (d, 10)	129.9	5.29 (tsept., 7.5, 1)	122.0	5.27 (tsept., 7.5, 1)	123.4
γ''		76.1		77.2		131.9		131.4
δ''	1.39 (s)	27.8	1.40 (s)	27.8	1.69 (brs)	25.8	1.63 (brs)	25.9
ε''	1.41 (s)	27.9	1.42 (s)	28.2	1.79 (brs)	17.7	1.74 (brs)	17.9

	11[b]		**12**[b]		**13**[a]		**13a**[a]	**13b**[a]
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_H (J in Hz)
1	7.16 (s)	129.7	7.23 (s)	132.8	7.15 (s)	132.9	7.19 (s)	7.16 (s)
2		116.9		124.4		115.2		
3		155.2		157.0		155.0		
4	6.23 (s)	104.9	6.35 (s)	103.7	6.22 (s)	104.7	6.40 (s)	6.38 (s)
4a		157.5		155.6		155.9		
6	3.63 (dd, 11, 11)	67.2	3.55 (dd, 10, 10)	67.2	3.57 (dd, 10, 10)	67.2	3.66 (dd, 11, 11)	3.61 (dd, 11, 11)
6	4.25 (dd, 11, 4.5)		4.20 (dd, 10, 4)		4.23 (dd, 10, 6)		4.24 (dd, 11, 5)	4.23 (dd, 11, 5)
6a	3.55 (m)	41.2	3.53 (m)	41.1	3.55 (m)	41.2	3.55 (m)	3.53 (m)
11a	5.42 (d, 7)	78.4	5.45 (d, 7)	79.1	5.42 (d, 7)	78.8	5.47 (d, 7)	5.45 (d, 7)
11b		114.5		112.0		114.0		
10b		118.7		119.1		117.8		
7	6.89 (s)	124.1	6.94 (d, 8)	122.6	7.01 (s)	110.4	6.83 (s)	6.81 (s)
8		116.9	6.38 (d, 8)	108.0		142.6		
9		154.1		159.9		148.4		
10		112.2		113.0	6.33 (s)	98.6	6.50 (s)	6.49 (s)
10a		157.7		156.7		155.1		
Me	2.17 (s)	16.5						
OMe					3.80 (s)	57.4	3.83 (s)	3.82 (s)
OMe							3.85 (s)	3.84 (s)
side chain at 2								
α	6.41 (d, 10)	122.4	2.70 (m)	25.2	2.70 (dd, 16.5, 8.5)	31.5	2.90 (dd, 17, 7)	2.76 (dd, 16.5, 8)
					2.99 (dd, 16.5, 5.5)		3.23 (dd, 17, 5)	3.22 (dd, 16.5, 5)
β	5.65 (d, 10)	129.8	1.77 (m)	45.0	3.77 (dd, 8.5, 5.5)	69.8	5.14 (dd, 7, 5)	5.14 (dd, 8, 5)
γ		77.1		70.3		78.1		
δ	1.38 (s)	28.4	1.25 (s)	29.2	1.23 (s)	20.6	1.21 (s)	1.24 (s)
ε	1.41 (s)	28.2	1.25 (s)	29.4	1.34 (s)	26.1	1.30 (s)	1.36 (s)
at 7								
α′								
β'								
γ'								
δ'								
ε'								
at 10								
α″	3.29 (brd, 7.5)	23.8	3.27 (brd, 7.5)	23.5				
β''	5.23 (tsept., 7.5, 1)	123.5	5.26 (tsept., 7.5, 1)	123.6				
γ''		131.8		131.3				
δ''	1.61 (brs)	25.9	1.62 (brs)	25.9				
ε''	1.73 (brs)	17.9	1.74 (brs)	17.9				

[a] Measured in $CDCl_3$. [b] Measured in acetone-d_6.

[column, Inertsil ODS-3, 3 × 50 cm; solvent, H_2O−CH_3CN (40:60−30:70) linear gradient, UV 280 nm] to give **42** (1.8 mg), **5** (16 mg), **41** (50 mg), **9** (18 mg), and **31** (123 mg), and fr. 12-1 (35 mg). Fr. 12-1 was subjected to semipreparative HPLC [column, Develosil C30-UG-5, 2 × 25 cm; solvent, H_2O−CH_3CN (35:65), UV 280 nm] to give **39** (9.3 mg) and **37** (14 mg). A 70 mg portion of fr. 15 (1.161 g) was subjected to semipreparative HPLC [column, Capcell Pak ODS, 2 × 25 cm; solvent, H_2O−CH_3CN (32.5:67.5), UV 280 nm] to give **2** (12

Table 4. NMR Spectroscopic Data (400 MHz) for Compounds **14**−**16**

	14[a]		**15**[a]		**16**[b]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	7.31 (d, 8.5)	121.5	7.19 (s)	121.9	7.80 (d, 8.5)	122.3
2	6.51 (dd, 8.5, 2)	109.3		121.3	6.96 (dd, 8.5, 2)	113.4
3		159.4		156.5		160.9
4	6.44 (d, 2)	104.5	6.45 (s)	104.2	6.91 (d, 2)	104.5
4a		155.9		153.8		154.4
6	5.49 (s)	66.0	5.43 (s)	65.9		158.8
6a		106.8		106.7		102.4
11a		147.3		147.6		157.9
11b		110.0		109.9		103.1
10b		117.8		117.9		113.7
7	6.78 (s)	101.6	6.75 (s)	101.5	7.17 (s)	102.0
8		142.9		142.9		144.0
9		142.2		142.1		143.0
10		113.0		112.9		112.5
10a		149.6		149.6		148.0
side chain						
at 2						
α			3.30 (brd, 7.5)	28.1		
β			5.38 (m)	123.7		
γ				132.6		
δ			1.77 (brs)	25.9		
ε			1.74 (brs)	17.9		
at 10						
α′	3.65 (brd, 7)	23.8	3.64 (brd, 7.5)	23.8	3.59 (brd, 7.5)	22.8
β'	5.43 (tsept., 7, 1)	122.9	5.38 (m)	122.9	5.35 (brt, 7.5)	121.6
γ'		132.1		132.0		131.5
δ'	1.68 (brs)	25.9	1.69 (brs)	25.9	1.67 (brs)	25.5
ε'	1.89 (brs)	17.9	1.92 (brs)	17.9	1.87 (brs)	17.7

[a] Measured in acetone-d_6. [b] Measured in DMSO-d_6.

mg). Then, 50 mg of fr. 17 (564 mg) was subjected to preparative HPLC [column, TSKgel ODS-80TS, 5.5 × 60 cm; solvent, H_2O−CH_3CN (60:40), UV 280 nm] to give **40** (18 mg). Next, 50 mg of fr. 20 (371 mg) was subjected to semipreparative HPLC [column, Cosmosil 5C_{18}-AR-II 2 × 25 cm; solvent, H_2O−CH_3CN (57.5:42.5), UV 280 nm] to give **38** (24 mg). Fr. 23 (4.05 g) was subjected to preparative HPLC [column, TSKgel ODS-80TS, 5.5 × 60 cm; solvent, H_2O−CH_3CN (60:40−40:60) linear gradient, UV 280 nm] to give **25** (15 mg), **36** (65 mg), **24** (16 mg), **29** (108 mg), and **28** (834 mg), and frs. 23-1−23-2. A 30 mg portion of fr. 23-1 (211 mg) was subjected to semipreparative HPLC [column, Develosil C30-UG-5, 2 × 25 cm; solvent, H_2O−CH_3CN (25:75), UV 280 nm] to give **30** (20 mg). Fr. 23-2 (46 mg) was subjected to semipreparative HPLC [column, Develosil C30-UG-5, 2 × 25 cm; solvent, H_2O−CH_3CN (20:80), UV 280 nm] to give **19** (15 mg). Fr. 24 (3.27 g) was subjected to semipreparative HPLC [column, Cosmosil 5C_{18}-AR-II 2 × 25 cm; solvent, H_2O−CH_3CN (20:80), UV 280 nm] to give **32** (316 mg), **23** (47 mg), and **14** (229 mg), and frs. 24-1−24-5. Fr. 24-1 (17 mg) was subjected to semipreparative HPLC [column, Develosil C30-UG-5, 2 × 25 cm; solvent, H_2O−CH_3CN (35:65), UV 280 nm] to give **32** (1.2 mg) and **33** (1.6 mg). Fr. 24-2 (37 mg) was subjected to semipreparative HPLC [column, Develosil C30-UG-5, 2 × 25 cm; solvent, H_2O−CH_3CN (52.5:47.5), UV 280 nm] to give **6** (10 mg). Fr. 24-3 (14 mg) was subjected to semipreparative HPLC [column, Develosil C30-UG-5, 2 × 25 cm; solvent, H_2O−CH_3CN (52.5:47.5), UV 280 nm] to give **22** (4.5 mg). Fr. 24-4 (43 mg) was subjected to semipreparative HPLC [column, Develosil C30-UG-5, 2 × 25 cm; solvent, H_2O−CH_3CN (40:60), UV 280 nm] to give **28** (33 mg). Fr. 24-5 (162 mg) was subjected to semipreparative HPLC [column, Inertsil ODS-3, 3 × 50 cm; solvent, H_2O−CH_3CN (40:60−30:70) linear gradient, UV 280 nm] to give **15** (36 mg) and **44** (4.3 mg). A 100 mg sample of fr. 25 (651 mg) was chromatographed on a Sephadex LH-20 column (2.5 × 100 cm) using methanol as solvent to give fr. 25-1 (24 mg). Fr. 25-1 was subjected to semipreparative HPLC [column, Develosil C30-UG-5, 2 × 25 cm; solvent, H_2O−CH_3CN (20:80), UV 280 nm] to give **45** (6.3 mg). Fr. 26 (594 mg) was subjected to semipreparative HPLC [column, Inertsil ODS-3, 3 × 50 cm; solvent, H_2O−CH_3CN (15:85), UV 280 nm] to give fr. 26-1 (176 mg). Fr. 26-1 was subjected to semipreparative HPLC [column, Develosil C30-UG-5, 2 × 25 cm; solvent, H_2O−CH_3CN (15:85), UV 280 nm] to give **45** (36 mg). Fr. 27 (209 mg) was subjected to semipreparative HPLC [column, Inertsil ODS-3, 3 × 50 cm; solvent, H_2O−CH_3CN (15:85), UV 280 nm] to give **18** (77 mg), **17** (39 mg), **45** (36 mg), **20** (11 mg), and fr. 27-1 (266 mg). Fr. 27-1 was subjected to semipreparative HPLC [column, Inertsil ODS-3, 3 × 50 cm; solvent, H_2O−CH_3CN (60:40), UV 280 nm] to give **21** (3.9 mg), **27** (3.1 mg), **1** (5.7 mg), **13** (6.2 mg), **26** (12 mg), **3** (3.3 mg), **23** (1.0 mg), **4** (6.5 mg), **35** (40 mg), **34** (6.8 mg), **12** (2.5 mg), and **16** (25 mg).

Lespecyrtin A_1 (1): colorless, amorphous solid; $[\alpha]_D^{23}$ −30.6 (*c* 0.73, MeOH); UV (MeOH) λ_{max} (log ϵ) 285 (4.08) nm; CD (MeOH) λ_{max} nm ([θ]) 261 (+4082), 305 (+9070), 332 (+12 698); ^{1}H NMR and ^{13}C NMR, Table 1; HRFABMS *m*/*z* 341.1417 (calcd for $C_{20}H_{20}O_5$ + H, 341.1389).

Lespecyrtin B_1 (2): colorless, amorphous solid; $[\alpha]_D^{23}$ +34.1 (*c* 1.48, MeOH); UV (MeOH) λ_{max} (log ϵ) 370 (4.47) nm; ^{1}H NMR and ^{13}C NMR, Table 2; HRFABMS *m*/*z* 323.1320 (calcd for $C_{20}H_{18}O_4$ + H, 323.1284).

Lespecyrtin B_2 (3): colorless, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 226 (4.05), 284 (3.89), 347 (3.96) nm; ^{1}H NMR and ^{13}C NMR, Table 2; HRFABMS *m*/*z* 323.1311 (calcd for $C_{20}H_{18}O_4$ + H, 323.1284).

Lespecyrtin B_3 (4): colorless, amorphous solid; $[\alpha]_D^{23}$ −11.7 (*c* 0.75, MeOH); UV (MeOH) λ_{max} (log ϵ) 369 (4.35) nm; ^{1}H NMR and ^{13}C NMR, Table 2; HRFABMS *m*/*z* 341.1393 (calcd for $C_{20}H_{20}O_5$ + H, 341.1389).

Lespecyrtin C_1 (5): colorless, amorphous solid; $[\alpha]_D^{23}$ +59.4 (*c* 1.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 sh (4.44), 241 sh (4.05), 288 (4.27) nm; ^{1}H NMR and ^{13}C NMR, Table 2; HRFABMS *m*/*z* 325.1457 (calcd for $C_{20}H_{20}O_4$ + H, 325.1440).

Lespecyrtin D_1 (6): colorless, amorphous solid; $[\alpha]_D^{23}$ −42.8 (*c* 1.02, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 sh (4.24), 292 (3.93) nm; CD (MeOH) λ_{max} nm ([θ]) 233 (−6648), 257 (+1108), 290 (−1583); ^{1}H NMR and ^{13}C NMR, Table 1; HRFABMS *m*/*z* 356.1609 (calcd for $C_{21}H_{24}O_5$, 356.1624).

Lespecyrtin E_1 (7): colorless, amorphous solid; $[\alpha]_D^{23}$ −59.9 (*c* 0.46, MeOH); UV (MeOH) λ_{max} (log ϵ) 230 (4.84), 279 (4.23), 308 (4.01), 319 (4.02) nm; CD (MeOH) λ_{max} nm ([θ]) 318 (+18977); ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m*/*z* 388.1668 (calcd for $C_{25}H_{24}O_4$, 388.1675).

Lespecyrtin E_2 (8): colorless, amorphous solid; $[\alpha]_D^{23}$ −108.2 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (4.62), 283 (4.09), 319 (3.88) nm; CD (MeOH) λ_{max} nm ([θ]) 270 (+3148), 303 (+3779), 320 (+5247); ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m*/*z* 472.2258 (calcd for $C_{30}H_{32}O_5$, 472.2251).

Table 5. NMR Spectroscopic Data (400 MHz) for Compounds **17**−**20**

	17[a]		**18**[a]		**19**[a]		**20**[a]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
upper moiety								
2		149.9		153.9		153.4		155.1
3		118.8		120.3		120.3		121.9
4	3.92 (d, 16.5)	26.3		196.4		196.3		196.0
	3.96 (d, 16.5)							
4a		114.8		116.4		116.8		116.3
5	6.53 (s)	103.1	6.93 (s)	103.1	6.93 (s)	103.1	6.98 (s)	105.5
6		142.2		143.2		143.3		144.5
7		142.1		142.8		142.9		139.3
8		112.0		112.2		112.2		107.0
8a		148.8		148.3		148.3		144.3
1′		110.6		111.2		112.0		110.4
2′		154.2		156.9		156.7		155.4
3′	6.59 (s)	103.6	6.37 (d, 2.5)	103.6	6.35 (s)	103.6	6.40 (s)	103.8
4′		157.3		160.9		156.4		158.2
5′		120.9	6.51 (dd, 8.5, 2.5)	108.6		114.9		121.1
6′	7.12 (s)	131.8	7.49 (d, 8.5)	131.7	7.33 (s)	128.2	7.27 (s)	131.7
isoprenyl at 8								
α	3.61 (brd, 7.5)	23.8	3.67 (brd, 7.5)	23.8	3.67 (brd, 7.5)	23.8	6.84 (d, 10)	116.6
β	5.44 (tsept., 7.5, 1)	124.0	5.48 (overlapped)	122.7	5.48 (overlapped)	122.9	5.90 (d, 10)	132.0
γ		132.4		132.0		132.5		77.8
δ	1.74 (brs)[b]	25.8	1.66 (brs)[i]	25.9	1.74 (brs)	25.9	1.50 (s)	27.6
ε	1.83 (brs)	17.7	1.87 (brs)	18.0	1.88 (brs)	18.0	1.50 (s)	27.6
at 5′								
α′	3.29 (brd, 7.5)	28.1			6.39 (d, 10)	122.1	3.25 (brd, 7.5)	28.3
β′	5.36 (tsept., 7.5, 1)	123.7[d]			5.62 (d, 10)	129.0	5.30 (tsept., 7.5, 1)	123.8
γ′		132.0				77.7		132.5[m]
δ′	1.73 (brs)[b]	25.9			1.39 (s)	28.7	1.69 (brs)[l]	17.8[n]
ε′	1.75 (brs)[c]	17.8			1.44 (s)	28.7	1.65 (brs)[l]	25.9
lower moiety								
1	7.19 (s)	132.4	7.19 (s)	132.3	7.19 (s)	132.3	7.18 (s)	132.2
2		122.8		123.8		123.1	123.1	
3		156.8		157.2		157.1		157.2
4	6.36 (s)	103.9	6.36 (s)	103.8	6.24 (s)	104.3	6.37 (s)	103.7
4a		155.5		155.6		155.6		155.7
6	3.48 (dd, 10.5, 10.5)	67.3	3.25 (dd, 11, 11)	66.8	3.25 (dd, 10.5, 10.5)	66.9	3.20 (dd, 11, 11)	66.8
6	4.12 (m)		3.90 (dd, 11, 5)		3.96 (dd, 10.5, 5.5)		3.87 (dd, 11, 5)	
6a	3.44 (m)	41.2	3.40 (m)	39.9	3.41 (m)	40.0	3.35 (m)	40.0
11a	5.39 (d, 7)	78.8	5.48 (d, 7)	80.3	5.46 (d, 7)	80.3	5.46 (d, 7)	80.4
11b		113.0		111.6		112.0		111.9
10b		118.9		119.3		119.3		119.3
7	6.87 (s)	123.4	7.42 (s)	127.9	7.41 (s)	127.9	7.39 (s)	127.9
8		122.0		115.2		115.3		115.0
9		154.3		164.5		164.4		164.6
10		112.0		111.9		111.6		111.6
10a		158.2		164.4		164.4		164.5
isoprenyl at 2								
α″	3.25 (brd, 7.5)	28.4	3.29 (brd, 7.5)	28.4	3.29 (brd, 7.5)	28.4	3.27 (brd, 7.5)	28.4
β″	5.30 (tsept., 7.5, 1)	123.2[d]	5.35 (tsept., 7.5, 1)	122.9	5.35 (tsept., 7.5, 1)	123.8	5.35 (tsept., 7.5, 1)	123.8
γ″		131.8		132.3[j]		132.2		132.4[m]
δ″	1.63 (brs)[b]	25.9	1.78 (brs)[i]	25.9	1.67 (brs)	25.9	1.74 (brs)[l]	25.9
ε″	1.63 (brs)[c]	18.0	1.70 (brs)[i]	17.8[k]	1.75 (brs)	17.9	1.78 (brs)	17.8[n]
at 10								
α‴	3.29 (brd, 7.5)	23.8	3.27 (brd, 7.5)	22.8	3.28 (brd, 7.5)	22.9	3.29 (brd, 7.5)	22.9
β‴	5.24 (tsept., 7.5, 1)	123.4[d]	5.26 (tsept., 7.5, 1)	122.9	5.26 (tsept., 7.5, 1)	122.7	5.26 (tsept., 7.5, 1)	122.7
γ‴		131.8		132.2[j]		132.0		132.6
δ‴	1.63 (brs)[c]	25.9	1.73 (brs)[i]	25.9	1.67 (brs)	25.9	1.70 (brs)[l]	25.9
ε‴	1.74 (brs)[c]	17.9	1.74 (brs)[i]	17.9[k]	1.78 (brs)	17.9	1.73 (brs)[l]	17.9[n]
OH at 9			13.44 (s)		13.35 (s)		13.40 (s)	

[a] Measured in acetone-d_6.[b−n]Assignments are interchangeable in each column.

Lespecyrtin E_3 (9): colorless, amorphous solid; $[\alpha]_D^{23}$ −191.1 (*c* 0.67, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 sh (4.55), 276 (4.27), 285 (4.28), 342 (3.73) nm; CD (MeOH) λ_{max} nm ([θ]) 236 (−68 501), 270 (+17 916), 288 (−24 239), 345 (−2347); ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m*/*z* 474.2405 (calcd for $C_{30}H_{34}O_5$, 474.2407).

Lespecyrtin E_4 (10): colorless, amorphous solid; $[\alpha]_D^{23}$ −84.0 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ϵ) 228 (4.50), 273 (4.08), 307 (3.89) nm; CD (MeOH) λ_{max} nm ([θ]) 270 (+5457), 315 (+1049); ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m*/*z* 472.2225 (calcd for $C_{30}H_{32}O_5$, 472.2251).

Lespecyrtin E_5 (11): colorless, amorphous solid; $[\alpha]_D^{23}$ −93.9 (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (4.64), 274 (4.06), 306 (3.88), 319 (3.85) nm; CD (MeOH) λ_{max} nm ([θ]) 279 (+7186), 304 (+10 779), 315 (+7725); ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m*/*z* 404.1992 (calcd for $C_{26}H_{28}O_4$, 404.1988).

Lespecyrtin E_6 (12): colorless, amorphous solid; $[\alpha]_D^{23}$ −110.3 (*c* 0.37, MeOH); UV (MeOH) λ_{max} (log ϵ) 290 (3.86), 363 (3.53) nm; CD (MeOH) λ_{max} nm ([θ]) 239 (−17 320), 290 (+29 626); ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m*/*z* 410.20974 (calcd for $C_{25}H_{30}O_5$, 410.2094).

Lespecyrtin E_7 (13): colorless, amorphous solid; $[\alpha]_D^{23}$ −359.6 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ϵ) 295 (4.19) nm; CD (MeOH) λ_{max} nm ([θ]) 236 (−106 930), 282 (−77 318), 296 (+190 829); ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m*/*z* 370.1423 (calcd for $C_{21}H_{22}O_6$, 370.1417).

Lespecyrtin F_1 (14): colorless, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 220 sh (4.46), 241 (4.06), 288 (4.29) nm; ^{1}H NMR and ^{13}C NMR, Table 4; HRFABMS *m*/*z* 338.1173 (calcd for $C_{20}H_{18}O_5$, 338.1154).

Table 6. Melanin Synthesis Inhibitory Effects (IC_{50} μM) of Isolated Flavonoids

compound		IC_{50} (μM)	compound		IC_{50} (μM)
flavanone			pterocarpan		
	21	14.7		**7**	
	22	18.0		**8**	3.7
	23	[a]		**9**	0.40
	24	2.0		**10**	4.5
	25	73.5		**11**	1.5
	26	[a]		**12**	3.4
flavanonol				**13**	16.0
	1	[a]		**38**	8.7
	27	[a]		**39**	2.0
isoflavone				**40**	5.5
	32	29.2		**41**	3.2
	33	[a]		**42**	7.5
	34	9.4		**43**	4.6
isoflavanone			pterocarpen		
	35	5.4		**14**	[a]
	36	38.4		15	15.9
chalcone			coumestan		
	2	1.3		**16**	[a]
	3	1.0		44	25.7
	4	9.1	dimer		
	28	1.4		**17**	8.9
	29	3.2		**18**	[a]
	30	0.98		**19**	10.1
	31	2.4		**20**	6.2
dihydrochalcone				**20**	37.7
	5	3.0			
	37	2.0			
isoflavan			hydroquinone		2.2
	6	2.1	arbutin		103

[a] Inhibitory activity not shown within the range of the concentration where the cell viability is unaffected.

Lespecyrtin F_2 (15): colorless, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 211 (4.54), 254 (4.12), 308 (3.79), 354 (4.18) nm; ^{1}H NMR and ^{13}C NMR, Table 4; HRFABMS *m/z* 406.1808 (calcd for $C_{25}H_{26}O_5$, 406.1781).

Lespecyrtin G_1 (16): colorless, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 247 (4.04), 342 (4.11) nm; ^{1}H NMR and ^{13}C NMR, Table 4; HRFABMS *m/z* 353.1030 (calcd for $C_{20}H_{16}O_6$ + H, 353.1025).

Lespecyrtin H_1 (17): colorless, amorphous solid; $[\alpha]_D^{23}$ −105.7 (*c* 0.38, MeOH); UV (MeOH) λ_{max} (log ϵ) 292 (4.31), 311 (4.17) nm; CD (MeOH) λ_{max} nm ([θ]) 233 (−55 886), 290 (+35 483); ^{1}H NMR and ^{13}C NMR, Table 5; HRFABMS *m/z* 798.3726 (calcd for $C_{50}H_{54}O_9$, 798.3762).

Lespecyrtin H_2 (18): colorless, amorphous solid; $[\alpha]_D^{23}$ −62.9 (*c* 0.75, MeOH); UV (MeOH) λ_{max} (log ϵ) 309 (4.33) nm; CD (MeOH) λ_{max} nm ([θ]) 235 (−31 426), 270 (−13 232), 329 (−12 239), 393 (+860); ^{1}H NMR and ^{13}C NMR, Table 5; HRFABMS *m/z* 745.3022 (calcd for $C_{45}H_{44}O_{10}$ + H, 745.3014).

Lespecyrtin H_3 (19): colorless, amorphous solid; $[\alpha]_D^{23}$ −48.3 (*c* 1.65, MeOH); UV (MeOH) λ_{max} (log ϵ) 264 (4.44), 294 (4.35), 310 (4.36) nm; CD (MeOH) λ_{max} nm ([θ]) 235 (−36 015), 258 (+45 019), 285 (+7203), 326 (−5867), 394 (+28 812); ^{1}H NMR and ^{13}C NMR, Table 5; HRFABMS *m/z* 810.3428 (calcd for $C_{50}H_{50}O_{10}$, 810.3405).

Lespecyrtin H_4 (20): colorless, amorphous solid; $[\alpha]_D^{23}$ −80.8 (*c* 1.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 278 (4.40), 348 (4.26) nm; CD (MeOH) λ_{max} nm ([θ]) 275 (−27 732), 302 (−13 686), 342 (−15 487), 393 (+11 525); ^{1}H NMR and ^{13}C NMR, Table 5; HRFABMS *m/z* 810.3396 (calcd for $C_{50}H_{50}O_{10}$, 810.3405).

Methylation and MTPA Esters of Compound 13. Compound **13** (1 mg) was methylated with CH_2N_2 in diethyl ether solution for 1 h to give a methyl ether (1 mg). To a solution of the methyl ether of **13** (each ca. 0.5 mg) in pyridine (30 μL) was added (+)-MTPA chloride or (−)-MTPA chloride (3 μL), and the solution was left overnight at room temperature. 3-[(Dimethylamino)prolyl]amine (3 μL) was added to the reaction mixture, and the mixture was left for 1 h. The solvent was evaporated and the mixture was subjected to preparative HPLC [column, YMC ODS, 4.6 mm × 25 cm; solvent, acetonitrile−water (6:4); detector UV, 280 nm] to give the (−)-MTPA ester (**13a**, 0.3 mg) and the (+)-MTPA ester (**13b**, 0.3 mg). ^{1}H NMR: Table 3.

Assay of Melanin Synthesis in Normal Human Epidermal Melanocytes. Normal human epidermal melanocytes were purchased from Kurabo Industries Ltd. (Osaka, Japan) and were maintained in M254 calcium-free medium supplemented with human melanocyte growth supplement (HMGS), 50 μg/mL streptomycin, and 50 U/mL penicillin. NHEM were seeded in a 24-well culture plate. After incubation for 24 h, the medium was exchanged for that containing the sample compound dissolved in DMSO and 0.25 μCi 2-[2-^{14}C]-thiouracil (Amersham, Freiburg, Germany). After incubation for 72 h, the cells were washed twice with phosphate-buffered saline and lysed with trichloroacetic acid (TCA). On centrifugation, the pellets were washed twice with 10% TCA. The pellets were mixed with scintillation fluid and the incorporated radioactivity was determined with a liquid-scintillation counter (LSC6100; Aloka, Tokyo, Japan). Cell viability was examined by a colorimetric assay using a WST-8 cell-counting kit (Dojindo, Kumamoto, Japan) according to the manufacturer's protocol. The IC_{50} value of melanin synthesis was determined using SAS software version 9.1.3 (SAS Institute Inc., Cary, NC) within the range of the concentration where cell viability remains unaffected.

Supporting Information Available: Structures of known compounds and ROE and HMBC data. This material is available free of charge via the Internet at http://pubs.acs.org.

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