

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 xanthoangelol (**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 G₄ (**41**),²¹ phaseollin (**42**),¹⁸ gangetinin (**43**),²² lespeflorin I₂ (**44**),²¹ and lespeflorin J₃ (**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 ¹H NMR spectrum of lespecyrtin A₁ (**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 laevorotatory optical activity,²⁵ and a positive Cotton effect at the n→π* absorption band in the CD spectrum²⁵ of lespecyrtin A₁.

Lespecyrtins B₁ (**2**), B₂ (**3**), and B₃ (**4**) were assigned with a chalcone skeleton having a 1,4-disubstituted benzene ring. The ¹H 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 B₁ (**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 ¹³C 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 B₂ (**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 ¹³C NMR spectrum of lespecyrtin B₃ (**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 C₁ (**5**) gave similar ¹H NMR data to those of lespecyrtin B₁ (**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 ¹H NMR spectrum of lespecyrtin D₁ (**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

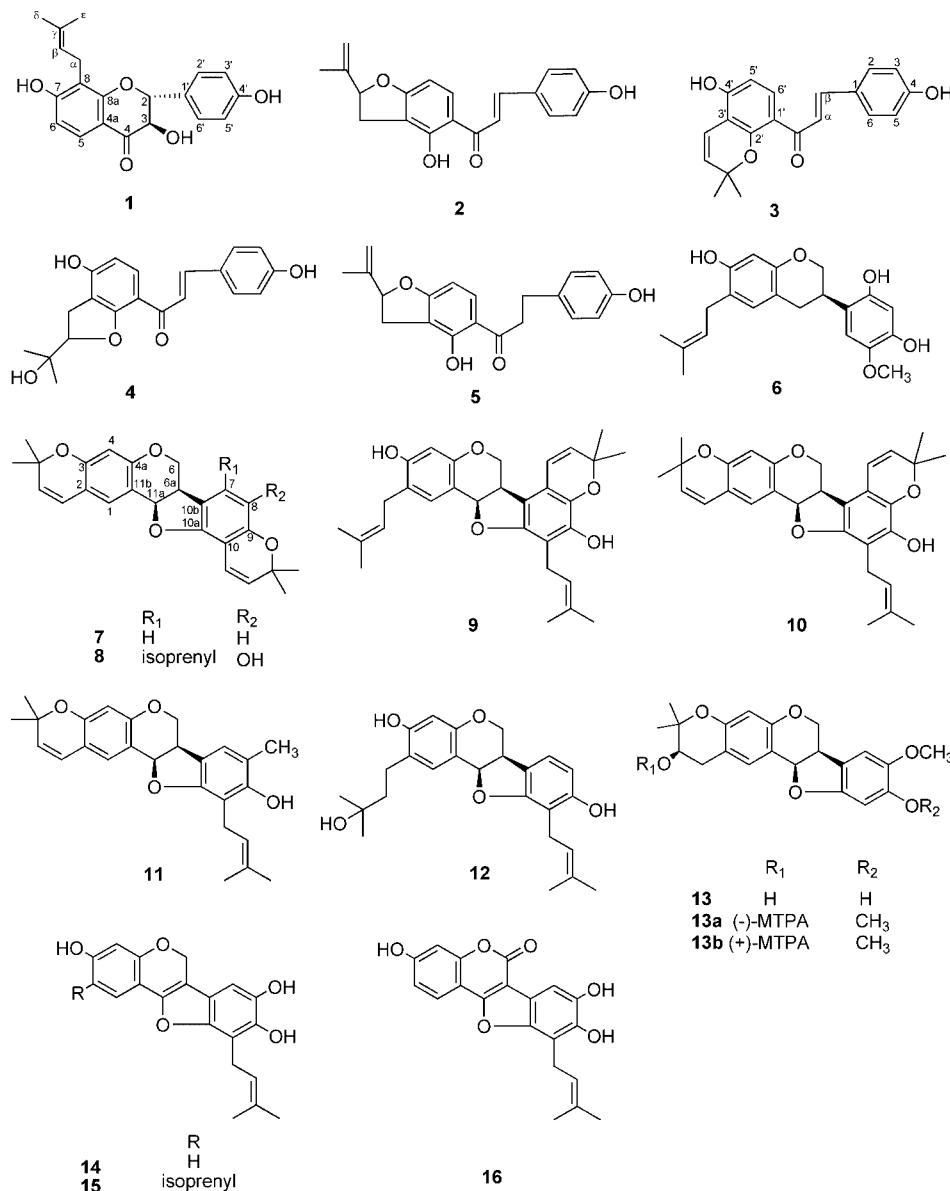
Lespecyrtins E₁ (**7**), E₂ (**8**), E₃ (**9**), E₄ (**10**), E₅ (**11**), E₆ (**12**), and E₇ (**13**) were assigned with a pterocarpan skeleton from the characteristic ¹H NMR spectroscopic data in the aliphatic proton region, and in the aromatic proton region two singlet proton signals due to H-1 and H-4 were observed. The former five compounds also had one or two 2,2-dimethyl-2*H*-chromene rings. The ¹H NMR spectrum of lespecyrtin E₁ (**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 ¹H NMR spectrum of lespecyrtin E₂ (**8**) was similar to that of lespecyrtin E₁ (**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 C₃₀H₃₂O₅. The ¹³C

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NMR spectrum showed a specifically hindered α -methylene carbon signal of an isoprenyl residue at δ 27.2 and *ortho* dioxxygenated aromatic carbon signals at δ 137.8 and 140.0.²¹ From these NMR data, the structure of lespecrytin E₂ was deduced as shown. Lespecrytins E₃ (9) and E₄ (10) gave similar ¹H NMR spectra to that of lespecrytin E₂ (8). In the ¹H NMR spectrum of lespecrytin E₃ (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 ¹H NMR spectrum of lespecrytin E₄ (10) exhibited two 2,2-dimethyl-2H-chromene structures as well as a set of isoprenyl proton signals that was specifically hindered as determined from the ¹³C 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 lespecrytin E₄ was determined to be 10. Lespecrytin E₅ (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

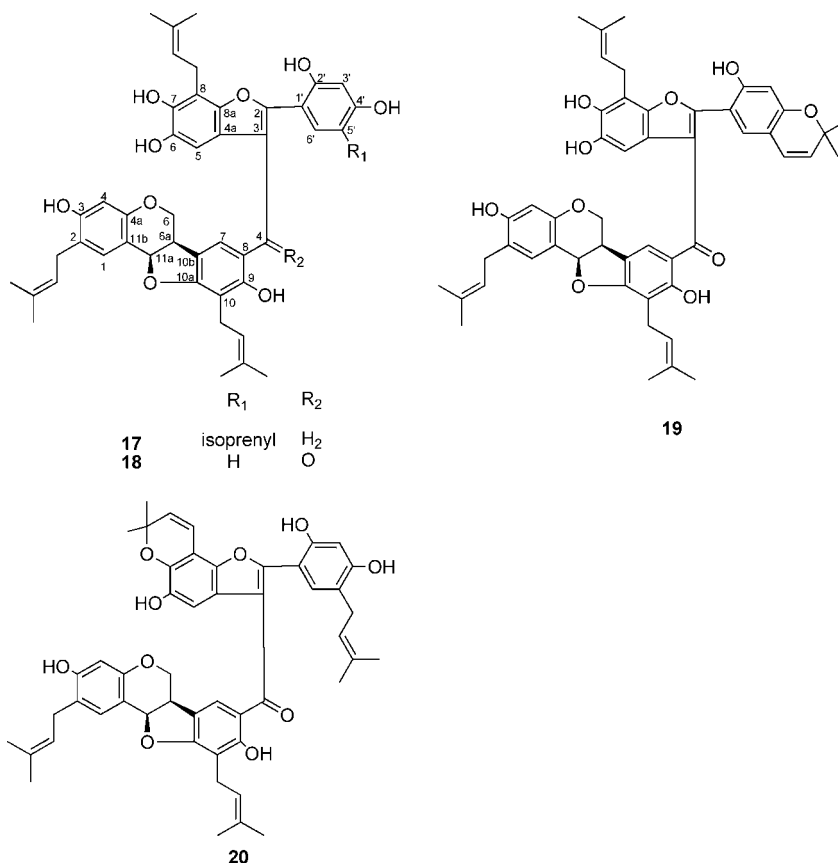
116.8), and C-9 (δ 154.1), and the α -methylene proton signal at δ 3.29 (2H, brd, J = 7 Hz) was long-range coupled to C-9 (δ 154.1), C-10 (δ 112.2), and C-10a (δ 157.7). The HRFABMS of lespecrytin E₆ (12) showed a molecular ion peak at m/z 410.2097, suggesting a molecular formula of C₂₅H₃₀O₅. The ¹H 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 proton 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 ¹³C 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 E₆ to be 12. The ¹H NMR spectrum of lespecrytin E₇ (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 ¹³C NMR spectrum indicated the presence of a 2,2-dimethyl-3-hydroxy-2H-chromene 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 E₁–E₇ were determined to be 6a*R* and 11a*R* from their levoratory optical activities³⁵ and positive Cotton effects at the region 260–310 nm in their CD spectra.³¹

Lespecyrtrins **F**₁ (**14**) and **F**₂ (**15**) showed a characteristic singlet proton signal (H₂-6) of a pterocarpene skeleton near 5.5 ppm in their ¹H NMR spectra.³⁰ In the ¹H 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 H₂-6, and a set of isoprenyl proton signals. In the ¹H 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 lespecyrtrins **F**₁ and **F**₂, respectively.

Lespecyrtin G₁ (**16**) was assumed to have a coumestan skeleton from the characteristic carbon signal at δ 158.8.³⁰ The ¹H NMR spectrum of lespecyrtin G₁ 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 G₁.

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 B₂ and B₃, which were isolated from *Lepedeza homoloba* Nakai.³⁷ Lespecyrtin H₂ (**18**) showed similar NMR data to those of lespeflorin J₄,³⁶ but lacked a methoxyl signal. The NMR spectra of lespecyrtins H₃ and H₄ showed three sets of isoprenyl proton signals and a dimethylbenzopyrene structure. In a ROE experiment on lespecyrtin H₃, 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 H₄, 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 H₄, 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



Lespecyrtins H₁ (**17**), H₂ (**18**), H₃ (**19**), and H₄ (**20**) were assumed to be dimeric flavonoids composed of a pterocarpin and a 2-arylbenzofuran derivative, from their molecular formulas and ¹³C NMR spectra like lespeflorins J₁–J₄, which were isolated from *Lespedeza floribunda* Bunge.²¹ The ¹H NMR spectra of lespecyrtin H₁ (**17**) showed a characteristic spin system of a pterocarpin 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

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 (IC₅₀) 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**

position	1^a		6^a	
	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) 4.19 (ddd, 10, 4, 2.5)	70.5
3	4.53 (d, 12)	73.9	3.47 (m)	33.0
4		193.6	2.75 (ddd, 15.5, 5, 2.5) 2.95 (dd, 15.5, 11)	31.3
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 IC₅₀ 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.

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

position	2^a		3^a		4^a		5^a	
	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) 3.37 (dd, 16, 10)	31.1	6.73 (d, 10)	117.6	3.14 (dd, 15.5, 9.5) 3.18 (dd, 15.5, 8)	27.6	2.93 (dd, 15.5, 7.5) 3.34 (dd, 15.5, 10)	31.6
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) 5.10 (m)	112.5	1.53 (s)	20.8	1.24 (s)	25.9	4.92 (m) 5.08 (m)	112.4

^a Measured in acetone-*d*₆.

Some flavonoid derivatives from plants, such as quercetin and artocarpone, 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. ¹H (400 MHz) and ¹³C (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. crytobotrya* 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 (Fr. 1–33). Fr. 3 (584 mg) was subjected to semipreparative HPLC [column, Cosmosil 5C₁₈-AR-II 2 $\times$ 25 cm; solvent, H₂O–CH₃CN (20:80), UV 280 nm] to give **7** (27 mg). Fr. 4 (105 mg) was subjected to semipreparative HPLC [column, Inertsil ODS-3, 3 $\times$ 50 cm; solvent, H₂O–CH₃CN (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 $\times$ 50 cm; solvent, H₂O–CH₃CN (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 $\times$ 50 cm; solvent, H₂O–CH₃CN (15:85), UV 280 nm] to give **11** (3.1 mg). Fr. 12 (731 mg) was subjected to semipreparative HPLC

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

position	7^a		8^b		9^a		10^a	
	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
position	11^b		12^b		13^a		13a^a	
	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)
β	5.65 (d, 10)	129.8	1.77 (m)	45.0	2.99 (dd, 16.5, 5.5)	69.8	3.23 (dd, 17, 5)	3.22 (dd, 16.5, 5)
γ		77.1		70.3	3.77 (dd, 8.5, 5.5)	78.1	5.14 (dd, 7, 5)	5.14 (dd, 8, 5)
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 CDCl₃. ^b Measured in acetone-*d*₆.

[column, Inertsil ODS-3, 3 × 50 cm; solvent, H₂O–CH₃CN (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, H₂O–CH₃CN (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, H₂O–CH₃CN (32.5:67.5), UV 280 nm] to give **2** (12

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

position	14^a		15^a		16^b	
	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, H₂O–CH₃CN (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 5C₁₈-AR-II 2 × 25 cm; solvent, H₂O–CH₃CN (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, H₂O–CH₃CN (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, H₂O–CH₃CN (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, H₂O–CH₃CN (20:80), UV 280 nm] to give **19** (15 mg). Fr. 24 (3.27 g) was subjected to semipreparative HPLC [column, Cosmosil 5C₁₈-AR-II 2 × 25 cm; solvent, H₂O–CH₃CN (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, H₂O–CH₃CN (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, H₂O–CH₃CN (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, H₂O–CH₃CN (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, H₂O–CH₃CN (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, H₂O–CH₃CN (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, H₂O–CH₃CN (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, H₂O–CH₃CN (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, H₂O–CH₃CN (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, H₂O–CH₃CN (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, H₂O–CH₃CN (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 A₁ (1): colorless, amorphous solid; $[\alpha]_{\text{D}}^{25}$ –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); ¹H NMR and ¹³C NMR, Table 1; HRFABMS *m/z* 341.1417 (calcd for C₂₀H₂₀O₅ + H, 341.1389).

Lespecyrtin B₁ (2): colorless, amorphous solid; $[\alpha]_{\text{D}}^{25}$ +34.1 (*c* 1.48, MeOH); UV (MeOH) λ_{max} (log ϵ) 370 (4.47) nm; ¹H NMR and ¹³C NMR, Table 2; HRFABMS *m/z* 323.1320 (calcd for C₂₀H₁₈O₄ + H, 323.1284).

Lespecyrtin B₂ (3): colorless, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 226 (4.05), 284 (3.89), 347 (3.96) nm; ¹H NMR and ¹³C NMR, Table 2; HRFABMS *m/z* 323.1311 (calcd for C₂₀H₁₈O₄ + H, 323.1284).

Lespecyrtin B₃ (4): colorless, amorphous solid; $[\alpha]_{\text{D}}^{25}$ –11.7 (*c* 0.75, MeOH); UV (MeOH) λ_{max} (log ϵ) 369 (4.35) nm; ¹H NMR and ¹³C NMR, Table 2; HRFABMS *m/z* 341.1393 (calcd for C₂₀H₂₀O₅ + H, 341.1389).

Lespecyrtin C₁ (5): colorless, amorphous solid; $[\alpha]_{\text{D}}^{25}$ +59.4 (*c* 1.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 sh (4.44), 241 sh (4.05), 288 (4.27) nm; ¹H NMR and ¹³C NMR, Table 2; HRFABMS *m/z* 325.1457 (calcd for C₂₀H₂₀O₄ + H, 325.1440).

Lespecyrtin D₁ (6): colorless, amorphous solid; $[\alpha]_{\text{D}}^{25}$ –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); ¹H NMR and ¹³C NMR, Table 1; HRFABMS *m/z* 356.1609 (calcd for C₂₁H₂₄O₅, 356.1624).

Lespecyrtin E₁ (7): colorless, amorphous solid; $[\alpha]_{\text{D}}^{25}$ –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); ¹H NMR and ¹³C NMR, Table 3; HRFABMS *m/z* 388.1668 (calcd for C₂₅H₂₄O₄, 388.1675).

Lespecyrtin E₂ (8): colorless, amorphous solid; $[\alpha]_{\text{D}}^{25}$ –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); ¹H NMR and ¹³C NMR, Table 3; HRFABMS *m/z* 472.2258 (calcd for C₃₀H₃₂O₅, 472.2251).

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

position	17^a		18^a		19^a		20^a	
	δ_{H} (<i>J</i> in Hz)	δ_{C}	δ_{H} (<i>J</i> in Hz)	δ_{C}	δ_{H} (<i>J</i> in Hz)	δ_{C}	δ_{H} (<i>J</i> 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) ^j	17.8 ⁿ
ϵ'	1.75 (brs) ^c	17.8			1.44 (s)	28.7	1.65 (brs) ^j	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		
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) ^j	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) ^j	25.9
ϵ'''	1.74 (brs) ^c	17.9	1.74 (brs) ⁱ	17.9 ^k	1.78 (brs)	17.9	1.73 (brs) ^j	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 E₃ (9): colorless, amorphous solid; $[\alpha]_{\text{D}}^{25} -191.1$ (*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); ¹H NMR and ¹³C NMR, Table 3; HRFABMS *m/z* 474.2405 (calcd for C₃₀H₃₄O₅, 474.2407).

Lespecyrtin E₄ (10): colorless, amorphous solid; $[\alpha]_{\text{D}}^{25} -84.0$ (*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); ¹H NMR and ¹³C NMR, Table 3; HRFABMS *m/z* 472.2225 (calcd for C₃₀H₃₂O₅, 472.2251).

Lespecyrtin E₅ (11): colorless, amorphous solid; $[\alpha]_{\text{D}}^{25} -93.9$ (*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); ¹H NMR and ¹³C NMR, Table 3; HRFABMS *m/z* 404.1992 (calcd for C₂₆H₂₈O₄, 404.1988).

Lespecyrtin E₆ (12): colorless, amorphous solid; $[\alpha]_{\text{D}}^{25} -110.3$ (*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); ¹H NMR and ¹³C NMR, Table 3; HRFABMS *m/z* 410.20974 (calcd for C₂₅H₃₀O₅, 410.2094).

Lespecyrtin E₇ (13): colorless, amorphous solid; $[\alpha]_{\text{D}}^{25} -359.6$ (*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); ¹H NMR and ¹³C NMR, Table 3; HRFABMS *m/z* 370.1423 (calcd for C₂₁H₂₂O₆, 370.1417).

Lespecyrtin F₁ (14): colorless, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 220 sh (4.46), 241 (4.06), 288 (4.29) nm; ¹H NMR and ¹³C NMR, Table 4; HRFABMS *m/z* 338.1173 (calcd for C₂₆H₁₈O₅, 338.1154).

Table 6. Melanin Synthesis Inhibitory Effects (IC₅₀ μM) of Isolated Flavonoids

compound	IC ₅₀ (μM)	compound	IC ₅₀ (μM)
flavanone	21	14.7	pterocarpan
	22	18.0	7
	23	^a	8
	24	2.0	9
	25	73.5	10
	26	^a	11
flavanonol			12
	1	^a	13
	27	^a	38
isoflavone			39
	32	29.2	40
	33	^a	41
	34	9.4	42
isoflavanone			43
	35	5.4	pterocarpen
	36	38.4	14
chalcone			15
	2	1.3	coumestan
	3	1.0	16
	4	9.1	44
	28	1.4	dimer
	29	3.2	17
dihydrochalcone	30	0.98	18
	31	2.4	19
			20
			20
isoflavan	5	3.0	
	37	2.0	
			hydroquinone
	6	2.1	arbutin
			103

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

Lespecyrtn F₂ (15): colorless, amorphous solid; UV (MeOH) λ_{max} (log ε) 211 (4.54), 254 (4.12), 308 (3.79), 354 (4.18) nm; ¹H NMR and ¹³C NMR, Table 4; HRFABMS *m/z* 406.1808 (calcd for C₂₅H₂₆O₅, 406.1781).

Lespecyrtn G₁ (16): colorless, amorphous solid; UV (MeOH) λ_{max} (log ε) 247 (4.04), 342 (4.11) nm; ¹H NMR and ¹³C NMR, Table 4; HRFABMS *m/z* 353.1030 (calcd for C₂₀H₁₆O₆ + H, 353.1025).

Lespecyrtn H₁ (17): colorless, amorphous solid; [α]_D²³ −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); ¹H NMR and ¹³C NMR, Table 5; HRFABMS *m/z* 798.3726 (calcd for C₅₀H₅₄O₉, 798.3762).

Lespecyrtn H₂ (18): colorless, amorphous solid; [α]_D²³ −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); ¹H NMR and ¹³C NMR, Table 5; HRFABMS *m/z* 745.3022 (calcd for C₄₅H₄₄O₁₀ + H, 745.3014).

Lespecyrtn H₃ (19): colorless, amorphous solid; [α]_D²³ −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); ¹H NMR and ¹³C NMR, Table 5; HRFABMS *m/z* 810.3428 (calcd for C₅₀H₅₀O₁₀, 810.3405).

Lespecyrtn H₄ (20): colorless, amorphous solid; [α]_D²³ −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); ¹H NMR and ¹³C NMR, Table 5; HRFABMS *m/z* 810.3396 (calcd for C₅₀H₅₀O₁₀, 810.3405).

Methylation and MTPA Esters of Compound 13. Compound 13 (1 mg) was methylated with CH₂N₂ 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)propyl]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). ¹H 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-[2-¹⁴C]-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 IC₅₀ 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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