Melanin Synthesis Inhibitors from *Lespedeza floribunda*

Maya Mori-Hongo,[†,‡] Hiroyuki Takimoto,[‡] Takayuki Katagiri,[‡] Makoto Kimura,[‡] Yu Ikeda,[‡] and Toshio Miyase*[,†]

Cutaneous Drug Research Department, POLA Chemical Industries, Inc., 560 Kashio-cho, Totsuka-ku, Yokohama 244-0812, Japan, and School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan

Received July 1, 2008

In the course of our search for new melanin synthesis inhibitors from plants, 40 new flavonoids and 11 known flavonoids were isolated from the roots of *Lespedeza floribunda* Bunge. The structures of the new compounds were determined by MS and NMR analyses, and the absolute configurations by CD spectra. Many of the compounds inhibited melanin synthesis in normal human epidermal melanocytes (NHEM), and compounds **3**, **7**, **8**, **11**, **16**, **24**, **27**, **29**, **33**, **43**, **45**, and **51** were particularly inhibitory. Their activities were stronger than that of hydroquinone, which is known as a major skin-lightening drug.

Skin pigmentations, such as melasma, freckles, and solar lentigo, can be serious aesthetic problems. They result from increased production and accumulation of melanin. Consequently, inhibition of melanin synthesis is very important for the treatment of a variety skin pigmentations. Melanin synthesis inhibitors have been of interest as target molecules of natural product chemistry. Prenyl flavanones from aerial parts of *Lespedeza buergeri* Miq. (Leguminosae) showed strong inhibition of melanin synthesis in normal human epidermal melanocytes (NHEM).[1] In the course of our search for active compounds from natural sources, we examined the diethyl ether layer of a methanol extract of roots of *L. floribunda* Bunge and isolated 51 flavonoids. Among these, **1**−**40** were new compounds and 11 were identified as bavachin (coryfolin) (**41**),[2] 6,8-di(γ,γ-dimethylallyl)-4′,7-dihydroxyflavanone (**42**),[3] prostratol (**43**),[4] 3′,6-di(γ,γ-dimethylallyl)-4′,5,7-trihydroxyflavanone (**44**),[5] euchrenone a_3 (**45**),[6] cajaflavanone (erythrisenegalone) (**46**),[7] genistein (**47**),[8] erythrabyssin II (**48**),[9] erybraedin A (**49**),[10] erybraedin B (**50**),[10] and folitenol (**51**)[11] by comparison of reported NMR data. The inhibitory activities of these compounds on melanin synthesis in NHEM were also determined. The inhibitory activities of compounds **3**, **7**, **8**, **11**, **16**, **24**, **27**, **29**, **33**, **43**, **45**, and **51** were all greater than that of hydroquinone, which is a major skin-lightening drug.

Results and Discussion

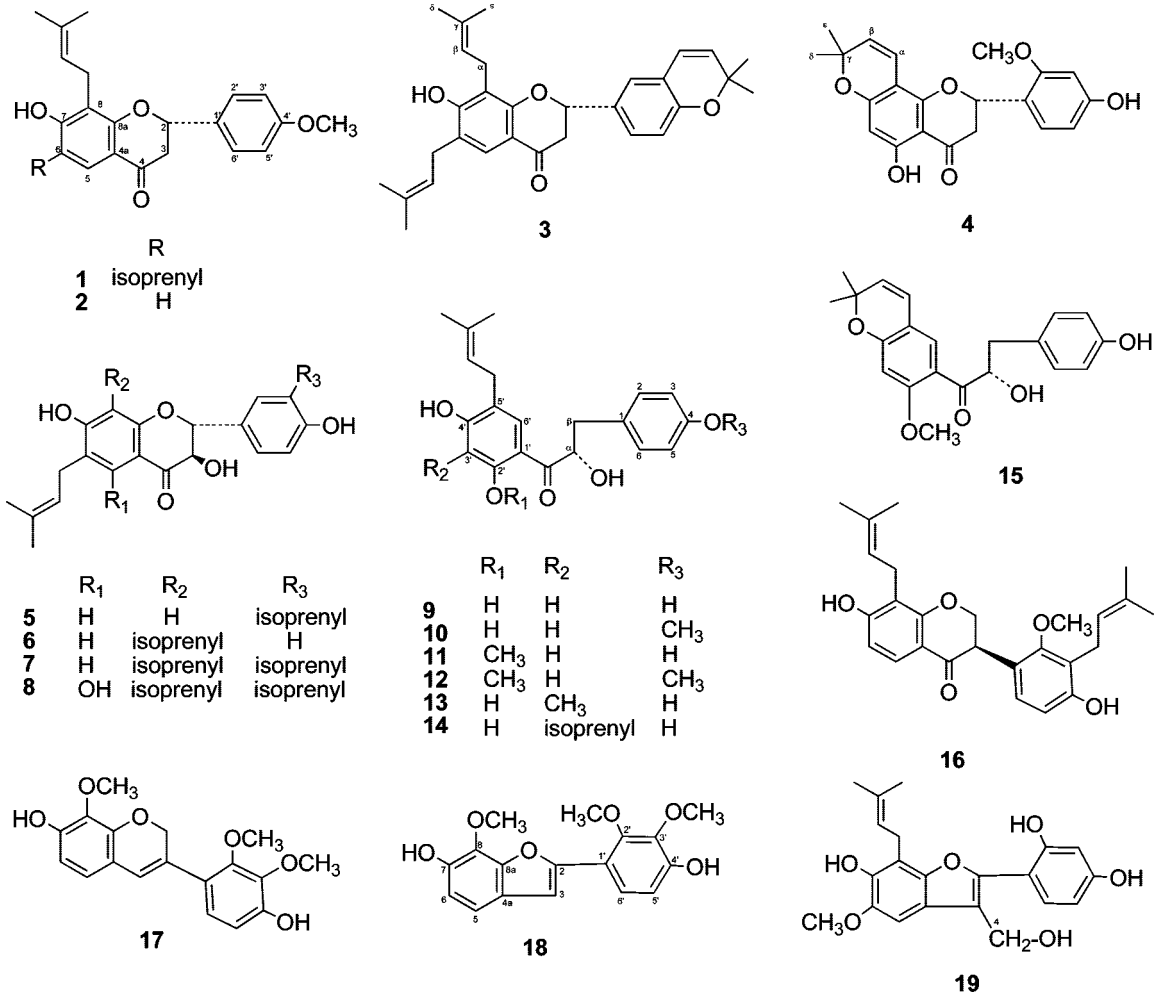
The ^{1}H NMR spectra of compounds **1**−**4**, named lespeflorins A_1, A_2, A_3, and A_4, showed a set of ABX-type proton signals in the aliphatic proton region, and the ^{13}C NMR spectrum of these compounds each showed a carbonyl carbon signal, suggesting that they were flavanones.[12] In the ^{1}H NMR spectrum of **1**, a singlet aromatic proton signal at δ 7.61, a set of AA′XX′ proton signals at δ 6.94 (2H, d, J = 8.5 Hz) and δ 7.38 (2H, d, J = 8.5 Hz), a methoxy singlet at δ 3.83, and two sets of isoprenyl proton signals at δ 3.30 (2H, d, J = 7 Hz), 3.40 (2H, d, J = 7 Hz) and δ 5.23 (t sept, J = 7, 1 Hz), 5.30 (t sept, J = 7, 1 Hz), 1.74 (3H, brs), 1.74 (3H, brs), 1.76 (3H, d, J = 1 Hz), 1.78 (3H, brs) were observed except for three proton signals for H-2 and H_2-3. In the ROE difference spectrum, an ROE was observed with the proton signal at δ 6.94 on irradiation at the methoxy signal, suggesting the presence of a 4′-OMe. In the HMBC spectrum, C−H long-range couplings were observed between the proton signal at δ 7.41 and the carbon signals at δ 29.1 (C-α of the isoprenyl at C-6), 114.5 (C-4a), 159.8 (C-8a), and 191.5 (C-4). The ^{1}H NMR spectrum of **2** was similar to that of **1**, lacking one isoprenyl residue. In the aromatic proton region, a set of AX-type proton signals at δ 6.53, 7.75 (J = 8.5 Hz) and a set of AA′XX′-type proton signals at δ 6.95, 7.39 (J = 8.5 Hz) were observed. Lespeflorin A_3 (**3**) had a ^{1}H NMR spectrum similar to that of **1**. Three proton signals due to a 1,3,4-trisubstituted benzene ring were observed at δ 6.80 (d, J = 8.5 Hz), 7.05 (d, J = 2.5 Hz), and 7.18 (dd, J = 8.5, 2.5 Hz). The proton signal at δ 7.05 was C−H long-range coupled with the carbon signals at δ 79.4 (C-2) and 122.0 in the HMBC spectrum. The latter carbon signal was assigned to an α-carbon of the dimethylchromene ring because of the chemical shift and coupling constant of the attached proton signal at δ 6.32 (d, J = 10 Hz). The ^{1}H NMR spectrum of **4** showed a methoxy singlet at δ 3.81, a hydrogen-bonded OH group at δ 12.14 (s), a 1,2,4-trisubstituted benzene ring [δ 6.45 (d, J = 2 Hz), 6.48 (dd, J = 8, 2 Hz), 7.40 (d, J = 8 Hz)], a pentasubstituted benzene ring [δ (5.99 (s)], and a dimethylchromene ring [δ 1.42 (3H, s), 1.44 (3H, s), 5.45 (d, J = 10 Hz), 6.54 (d, J = 10 Hz)]. The location of the OCH_3 group was decided to be 2′ due to the ROE effect on H-2 and H-3′ on irradiating at the signal at δ 3.81. The singlet aromatic proton at δ 5.99 was assigned to H-6 because that proton was attached to the carbon at δ 97.4, which was long-range coupled with the hydrogen-bonded OH at δ 12.14 in the HMBC spectrum. The absolute configuration at C-2 of these flavanones was *S* because of their levorotatory optical activity[13] and negative Cotton effects at the $\pi\rightarrow\pi^*$ absorption band.[14] The structures of these four new compounds were determined as shown from the NMR, UV, and HRMS spectral data.

Lespeflorins B_1 (**5**), B_2 (**6**), B_3 (**7**), and B_4 (**8**) had a flavanonol skeleton showing typical AB-type proton signals in the aliphatic region of the ^{1}H NMR spectrum.[15] The ^{1}H NMR spectrum of **5** had two singlet aromatic proton signals at δ 6.40 (s), 7.67 (s), a set of 1,3,4-trisubstituted benzene ring proton signals at δ 6.86 (d, J = 8 Hz), 7.28 (brs), 7.30 (dd, J = 8, 2 Hz), two sets of isoprenyl proton signals at δ 3.33 (2H, brd, J = 7 Hz), 3.39 (2H, brd, J = 7 Hz) and 5.29 (t sept, J = 7, 1 Hz), 5.34 (t sept, J = 7, 1 Hz), 1.77 (9H, brs), 1.79 (3H, d, J = 1 Hz), and a set of AB-type proton signals at δ 4.55 (d, J = 12 Hz), 4.98 (d, J = 12 Hz) due to H-3 and H-2. In the HMBC spectrum, the proton signal at δ 7.67 was C−H long-range coupled with the carbon signals at δ 28.9 (C-α of isoprenyl at C-6) and 162.3 (C-7), 192.9 (C-4) and was assigned to H-5. Another proton singlet at δ 6.40 was assigned to H-8, as it showed C−H long-range couplings with the carbon signals at δ 112.5 (C-4a), 122.5 (C-6), 162.3 (C-7), 192.9 (C-4). The aromatic proton at δ 7.28 was C−H long-range coupled with the carbon signals at δ 29.9 (C-α of the isoprenyl at C-3′), 84.0 (C-2), 127.0 (C-6′), 155.3 (C-4′). Lespeflorin B_2 (**6**) had a ^{1}H NMR spectrum similar to that of **5**. In the aromatic proton region, a proton singlet

* To whom correspondence should be addressed. Tel: +81-54-264-5661. Fax: +81-54-264-5661. E-mail: miyase@ys7.u-shizuoka-ken.ac.jp.
† University of Shizuoka.
‡ POLA Chemical Industries, Inc.

10.1021/np800395j CCC: $40.75
Published on Web 01/08/2009

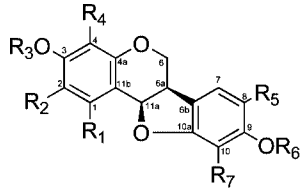
Chart 1



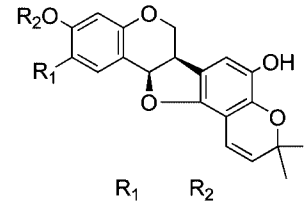
at δ 7.58 and a set of AA′XX′-type signals at δ 6.85, 7.41 (each 2H, d, J = 8.5 Hz) were observed, indicating two sets of isoprenyl proton signals. The singlet was assigned to H-5 from its chemical shift and the C−H long-range couplings with C-4 (δ 193.4), C-7 (δ 160.8), C-8a (δ 159.6), and C-α of the isoprenyl residue at C-6 (δ 29.0). In the ^{1}H NMR spectrum of lespeflorin B_3 (**7**), there were three sets of isoprenyl proton signals and a set of 1,3,4-trisubstituted benzene ring proton signals at δ 6.79 (d, J = 8.5 Hz), 7.27 (dd, J = 8.5, 2 Hz), 7.28 (brs), except for two aliphatic proton signals due to H-2 and H-3. The signal at δ 7.28 showed C−H long-range couplings with C-2 (δ 83.8), C-3′ (δ 127.1), C-4′ (δ 155.0) and then was assigned to H-2′. The ^{1}H NMR spectrum of lespeflorin B_4 (**8**) was similar to that of **7** and showed the presence of a hydrogen-bonded OH group (δ 11.49) instead of a singlet aromatic proton. The absolute configuration of these four new compounds was determined to be 2*R*, 3*R* from the large coupling constant (12−12.5 Hz) between H-2 and H-3,[12] a levorotatory optical activity,[16] and a positive Cotton effect at the n→π* absorption band in the CD spectrum.[14] These data determined the structures of lespeflorins B_1−B_4 (**5**−**8**) to be as shown.

Lespeflorins C_1 (**9**), C_2 (**10**), C_3 (**11**), C_4 (**12**), C_5 (**13**), C_6 (**14**), and C_7 (**15**) had α-hydroxydihydrochalcone skeletons with one or two isoprenyl groups, as determined from their ^{1}H NMR spectra, showing ABX-type signals in the aliphatic region.[17] The ^{1}H NMR spectrum of **9** showed resonances for the ABX-type protons at δ 2.82 (dd, J = 14, 8 Hz), 3.04 (dd, J = 14, 4.5 Hz), 5.15 (m), two singlet aromatic protons at δ 6.41 (s), 7.66 (s), a set of AA′XX′-type aromatic protons at δ 6.74, 7.06 (each 2H, d, J = 8 Hz), an isoprenyl group at δ 3.27 (2H, brd, J = 7 Hz), 5.36 (brt, J = 7 Hz), 1.72 (3H, brs), 1.77 (3H, brs), a hydrogen-bonded OH at δ 12.19 (s), an OH at δ 4.13 (brd, J = 7.5 Hz), and two phenolic OH protons at δ 8.04 (brs), 9.56 (s). In the HMBC spectrum, C−H long-range couplings were observed between the OH signal at δ 4.13 and the carbonyl carbon signal at δ 205.0, between the aromatic proton signal at δ 6.41 and the carbon signals at δ 111.5 (C-1′), 121.6 (C-5′), 163.9 (C-4′), 165.0 (C-2′), between the aromatic proton signal at δ 7.66 and the carbon signals at δ 111.5 (C-1′), 121.6 (C-5′), 163.9 (C-4′), 165.0 (C-2′), and between the aromatic proton signal at δ 7.06 and the carbon signals at δ 42.5 (C-β), 115.9 (C-3, C-5), 131.3 (C-2, C-6), 157.0 (C-4). Lespeflorins C_2 (**10**) and C_3 (**11**) showed NMR spectra similar to those of **9** except for methoxy proton signals at δ 3.78 (s) and 3.87 (s), respectively. The methoxy group was placed at C-4 in **10** and at C-2′ in **11** from the ROE experiment. In the ^{1}H NMR spectrum of **12**, there were two methoxy singlets (δ 3.78 and 3.89) that were placed at C-2′ and C-4 using ROE experiments. The ^{1}H NMR spectrum of **13** showed a methyl singlet at δ 2.14 instead of a singlet aromatic proton signal at δ 6.41 in the spectrum of **9**. Long-range C−H correlations were observed between the methyl proton signal and the carbon signals at δ 112.0 (C-3′), 160.3 (C-4′), 162.1 (C-2′). The ^{1}H NMR spectrum of **14** showed two isoprenyl groups and lacked a singlet aromatic proton signal at δ 6.41, as in the spectrum of **9**. Lespeflorin C_7 (**15**) showed a methoxy singlet at δ 3.91 and a set of dimethylchromeme ring proton signals at δ 1.46 (3H, s), 1.48 (3H, s), 5.57 (d, J = 10 Hz), 6.32 (d, J = 10 Hz) in the ^{1}H NMR spectrum. The methoxy group was located at C-2′ from the ROE experiment, and the dimethylchromene ring was placed as shown from the C−H long-range correlations between the singlet aromatic proton at δ 7.60 and the carbon signals at δ 121.1 (C-α of the chromene ring), 159.7 (C-4′), 161.0 (C-2′), 200.0 (C=O). The absolute configuration

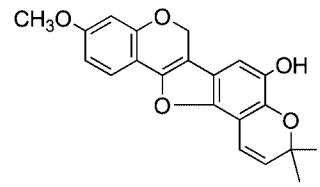
Chart 2



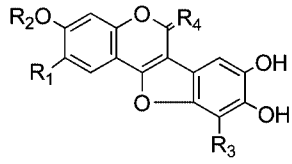
	R_1	R_2	R_3	R_4	R_5	R_6	R_7
20	H	H	H	isoprenyl	OCH_3	H	H
21	H	isoprenyl	H	H	OH	CH_3	isoprenyl
22	OCH_3	isoprenyl	H	H	OH	CH_3	isoprenyl
23	H	isoprenyl	H	H	CH_3	H	isoprenyl
24	H	H	H	isoprenyl	CH_3	H	isoprenyl
25	H	H	H	H	OH	CH_3	isoprenyl
26	H	H	H	H	OCH_3	H	isoprenyl
27	H	H	CH_3	H	OH	CH_3	isoprenyl
28	H	H	CH_3	H	OCH_3	H	isoprenyl
29	H	H	H	H	CH_3	H	isoprenyl



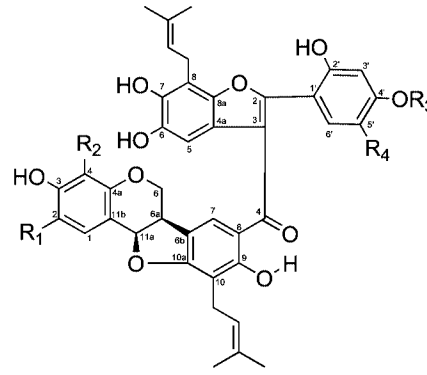
	R_1	R_2
30	isoprenyl	H
31	H	CH_3



33



	R_1	R_2	R_3	R_4
32	H	CH_3	isoprenyl	H_2
34	isoprenyl	H	H	O
35	isoprenyl	H	isoprenyl	O
36	H	CH_3	isoprenyl	O



	R_1	R_2	R_3	R_4
37	H	isoprenyl	H	isoprenyl
38	H	isoprenyl	CH_3	H
39	isoprenyl	H	H	isoprenyl
40	isoprenyl	H	CH_3	H

at C-α of these compounds was *S*, as the CD spectra showed a positive Cotton effect at 330−332 nm in **9**, **10**, **13**, and **14** (C-2′ OH type) and a positive Cotton effect at 270−288 nm and a negative Cotton effect at 326−340 nm in **11**, **12**, and **15** (C-2′ OMe type).[18]

The ^{1}H NMR spectrum of lespeflorin D_1 (**16**) showed a set of ABX-type proton signals at δ 4.50 (dd, J = 11, 11 Hz), 4.54 (dd, J = 11, 6 Hz), 4.29 (dd, J = 11, 6 Hz), which were characteristic of an isoflavanone,[19] a methoxy proton signal at δ 3.72 (s), two AX-type signals in the aromatic proton region, and two set of isoprenyl proton signals. The aromatic proton signal at δ 6.83 (d, J = 8.5 Hz) was C−H long-range coupled with signals at δ 46.5 (C-3), 155.6 (C-4′), 157.5 (C-6′), and the aromatic proton at δ 7.79 was C−H long-range coupled with the carbons at δ 161.1 (C-7), 192.8 (C-4) in the HMBC spectrum. On irradiation at the methoxy proton signal, ROEs were observed at H-3 (δ 4.29), H-α (δ 3.42), H-β (δ 5.26). The CD spectrum of **16** showed a positive Cotton effect (+11389) at 325 nm (n→π*), suggesting the absolute configuration at C-3 to be *R*.[14]

The ^{1}H NMR spectrum of lespeflorin E_1 (**17**) showed signals at δ 5.05 (2H, d, J = 1.5 Hz) and 6.55 (1H, t, J = 1.5 Hz), which were characteristic of an isoflavene,[20] except for three methoxy signals and two aromatic AX-type proton signals. The lower field proton of the AX-type signal at δ 6.72 (δ, J = 8 Hz) showed C−H long-range couplings with signals at δ 121.9 (C-3), 124.9 (C-4a), 145.5 (C-8a), 149.3 (C-7), and another lower field proton at δ 6.94 (d, J = 8 Hz) showed C−H long-range couplings with signals at δ 110.8 (C-5′), 128.4 (C-3), 149.6 (C-4′). The ^{13}C NMR chemical shifts (δ 60.5, 60.9, 61.0) suggested that the three OCH_3 groups were all hindered spatially.

Lespeflorin F_1 (**18**) also had three hindered OCH_3 groups (δ 59.8, 60.6, 61.0). In the ^{1}H NMR spectrum, a singlet olefinic proton signal (δ 7.11) was observed except for three OCH_3 and two AX-type proton signals in the lower field. The singlet proton was C−H long-range coupled with signals at δ 117.2 (C-1′), 125.1 (C-4a), 131.8 (C-8) in the HMBC spectrum and was assigned to H-3 of the 2-arylbebzofuran skelton.[20] The ^{1}H NMR spectrum of lespeflorin F_2 (**19**) showed a OCH_3 signal at δ 3.91 (s), a set of 1,2,4-trisubstituted benzene ring proton signals at δ 5.65 (d, J = 2.5 Hz), 6.51 (dd, J = 8, 2.5 Hz), 7.35 (d, J = 8 Hz), a singlet aromatic proton signal at δ 7.12 (s), an isolated methylene signal at δ 4.70 (2H, s), and a set of isoprenyl proton signals. A ROE was observed between the singlet aromatic proton and the OCH_3 signal. The C−H long-range correlations were observed between the isolated methylene proton and the carbons at δ 117.4 (C-4a), 121.0 (C-3), 150.2 (C-2), between the singlet aromatic proton and the carbons at δ 117.4 (C-4a), 143.2 (C-8a), 145.8 (C-6), 149.1 (C-7), and between the doublet aromatic proton at δ 7.35 (d, J = 8 Hz) and the carbons at δ 150.2 (C-2), 157.1 (C-2′), 160.4 (C-4′).

The ^{1}H NMR spectra of lespeflorins G_{1-12} (**20**−**31**) showed signals characteristic of pterocarpans in the aliphatic region.[12] Lespeflorin G_1 (**20**) had a OCH_3 singlet at δ 3.86, two singlet aromatic proton signals at δ 6.50 (s), 6.67 (s), a set of AX-type aromatic proton signals (δ 6.55, 7.26), and a set of isoprenyl proton signals except for four aliphatic proton signals for H_2-6, H-6a, and H-11a. In the ROE experiment irradiating at the OCH_3 signal, a ROE was observed at the singlet aromatic proton signal at δ 6.79, which had C−H long-range couplings with the carbon signals at δ 40.3 (C-6a), 141.1 (C-8), 146.7 (C-9), 154.1 (C-10a) in the HMBC spectrum. The singlet aromatic proton signal at δ 6.50 had C−H long-range couplings with the carbon signals at δ 117.1 (C-6b), 141.1 (C-8), 146.7 (C-9), 154.1 (C-10), and the doublet proton signal at δ 7.26 had C−H long-range couplings with the carbon signals at δ 78.8 (C-11a), 153.9 (C-4a), 155.7 (C-3) in the HMBC spectrum. Lespeflorin G_2 (**21**) had a OCH_3 signal at δ 3.77 (s), three singlet aromatic protons (δ 6.39, 6.73, 7.22), and two sets of isoprenyl proton signals except for four aliphatic proton signals due to H_2-6, H-6a, H-11a. The singlet protons at δ 6.39, 6.73, and 7.22 were assigned to H-4, H-7, and H-1, respectively, from their chemical shifts and HMBC correlations. The OCH_3 group was placed at C-9 from the ROE effects between the proton at δ 6.73 and H_2-6 and H-6a and comparison of the ^{13}C NMR data with those of **20**. Lespeflorin G_3 (**22**) showed a ^{1}H NMR spectrum similar to that of **21**, with one more OCH_3 signal at δ 3.97 (s) instead of the singlet aromatic proton signal at δ 7.22. Lespeflorin G_4 (**23**) had NMR data similar to those of **21**, and a methyl group at δ 2.16 (s) was observed instead of a OCH_3 signal of **21**. In the ROE experiment on irradiating this methyl signal, a ROE was observed at the aromatic proton signal at δ 6.85, which was assigned to H-7 because of the HMBC correlation to C-6a (δ 40.3) and C-10a (δ 156.5). The ^{1}H NMR spectrum of lespeflorin G_5 (**24**) was similar to that of **23**, and a set of AX-type aromatic proton signals were observed at δ 6.55, 7.28 (d, J = 8,5 Hz) instead of two singlet aromatic proton signals. The ^{1}H NMR spectrum of lespeflorin G_6 (**25**) was similar to that of **21**, and a set of 1,2,4-trisubstituted benzene ring proton signals were observed at δ 6.40 (d, J = 2.5 Hz), 6.53 (dd, J = 8.5, 2.5 Hz), 7.37 (d, J = 8.5 Hz) instead of a set of isoprenyl proton signals. The ^{1}H NMR spectrum of lespeflorin G_7 (**26**) was similar to that of **25** and showed the same signal pattern. The location of the OCH_3 group was decided to be C-8 from the ROE experiment. Lespeflorin G_8 (**27**) showed NMR data similar to those of **25** and two OCH_3 signals at δ 3.77 (s), 3.79 (s). The OCH_3 groups were indicated to be at C-3 and C-9 from the ROE experiments. The NMR data of lespeflorin G_9 (**28**) were very similar to those of **27**, having two OCH_3 signals at δ 3.79 (s), 3.85 (s). The OCH_3 groups were placed at C-3 and C-8 from the ROE experiments. The ^{1}H NMR spectrum pattern of lespeflorin G_{10} (**29**) was similar to that of **25** and **26**. The signal at δ 2.26 (s) was assigned to the C-8 methyl group based on the ROE experiment. Lespeflorin G_{11} (**30**) had an isoprenyl group and a dimethylchromene ring. The locations of these residues were decided from the HMBC correlations. Lespeflorin G_{12} (**31**) had a OCH_3 group and a dimethylchromene ring. The locations of these residues were decided to be as shown from the ROE experiment and the HMBC correlations. The absolute configurations of these pterocarpans were all 6a*R* and 11a*R* based on their levorotatory optical activities[21] and positive Cotton effects at 260−310 nm in the CD spectrum (see Experimental Section).[14]

Lespeflorins H_1 (**32**) and H_2 (**33**) showed a singlet proton signal (H_2-6) characteristic of pterocarpenes near δ 5.5 in the ^{1}H NMR spectrum.[19] In **32**, a OCH_3 group (δ 3.80) and an isoprenyl group were attached to the pterocarpene skeleton at C-3 and C-10, respectively, from the ROE and HMBC data. In **33**, the isoprenyl group appeared as a dimethylchromene ring.

Lespeflorins I_1 (**34**), I_2 (**35**), and I_3 (**36**) were assumed to have coumestan skeletons from their characteristic carbon signals (δ 158−159).[20] The ^{1}H NMR spectrum of lespeflorin I_1 (**34**) showed four singlet aromatic proton signals and a set of isoprenyl proton signals. The aromatic protons were assigned to be H-4, H-10, H-7, and H-1, respectively, from their HMBC correlations. Lespeflorin I_2 (**35**) had three aromatic proton singlets (δ 6.87, 7.12, 7.49) and two sets of isoprenyl group signals. The isoprenyl groups were located at C-2 and C-10 based on HMBC correlations. Lespeflorin I_3 (**36**) had a OCH_3 group and an isoprenyl group. The ROE experiment and the HMBC correlations decided the position of these residues.

Lespeflorins J_{1-4} (**37**−**40**) were assumed to be dimeric flavonoids composed of a pterocarpan and a 2-arylbenzofuran from their molecular formulas and ^{13}C NMR spectra.[19] The ^{1}H NMR spectrum of **37** showed a characteristic pterocarpan spin system at δ 3.16 (dd, J = 11, 11 Hz), 3.37 (dddd, J = 11, 7, 5, 1 Hz), 3.95 (dd, J = 11, 5 Hz), 5.48 (d, J = 7 Hz), three singlet aromatic proton signals at δ 6.39, 6.97, 7.32, a doublet aromatic proton signal at δ 7.39 (d, J = 1 Hz), AX-type aromatic proton signals at δ 6.60, 7.18 (d, J = 8.5 Hz), a hydrogen-bonded OH signal at δ 13.42, and four sets of isoprenyl signals. The ROE experiment on irradiating at a doublet proton signal at δ 7.39 suggested that this proton was H-7, which was coupled with H-6a in the COSY spectrum, and irradiating the doublet aromatic proton signal at δ 7.18 suggested that this proton was H-1 of the pterocarpan skeleton. In the HMBC spectrum, the aromatic proton at δ 7.39 had a C−H long-range coupling with the carbonyl carbon at δ 196.4 and the carbon at δ 164.5, which was assigned to be the carbon with a hydrogen-bonded OH group in the HMBC spectrum. Lespeflorin J_2 (**38**) showed NMR data similar to those of **37**, lacking a set of isoprenyl proton signals and adding a OCH_3 signal at δ 3.79 (s). In the ROE experiment, irradiating at the OCH_3 signal led to the conclusion that the B ring of the 2-arylbenzofuran skeleton was 2′-hydroxy-4′-methoxyphenyl. The ^{1}H NMR spectrum of **39** was similar to that of **37**, showing two more aromatic proton singlets (δ 6.37, 7.19) instead of a set of AX-type signals. The aromatic proton signal at δ 7.19 had C−H long-range couplings with the carbon signals at δ 28.3 (C-α of isoprenyl at C-2), 80.4 (C-11a), 112.0 (C-11b), 155.6 (C-4a), 157.2 (C-3). In the ^{1}H NMR spectrum of **40**, the substitution pattern of the A ring of the pterocarpan skeleton was the same as that of **39**, and the substitution pattern of the B ring of 2-arylbenzofuran was the same as that of **38**. The absolute configurations of the pterocarpan skeletons of these compounds were indicated to be 6a*R*, 11a*R* from the levorotatory optical activities.[21]

To evaluate the inhibitory activities on melanin synthesis of the 51 isolated flavonoids, we measured IC_{50} values of melanin synthesis in NHEM.[22] Forty-six compounds showed inhibitory activity (Table 8), and none of them affected cell viability (data not shown). Compounds **3**, **7**, **8**, **11**, **16**, **24**, **27**, **29**, **33**, **43**, **45**, and **51**, which have more lipophilic structures than the other compounds, showed the greatest activity. The IC_{50} of each of these compounds was lower than 2 μM, while that of hydroquinone, a positive control, was 2.2 μM. Thus, these compounds were more potent than hydroquinone, a compound widely used as a skin-lightening agent, at least in cell cultures. We are currently investigating the mechanism of inhibitory activity of these 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 Hitachi U-2010 spectrophotometer. Circular dichroism spectra were measured on a JASCO J-20A spectrometer. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL JNM α-400 FT-NMR spectrometer, and chemical shifts are give in δ 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

Table 1. NMR Spectroscopic Data (400 MHz) for Compounds **1**−**4**

	1[a]		**2**[a]		**3**[a]		**4**[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
2	5.38 (dd, 13, 3)	79.3	5.41 (dd, 13, 3)	79.4	5.32 (dd, 13, 3)	79.4	5.69 (dd, 12.5, 3.5)	74.3
3	2.81 (dd, 17, 3.5)	44.2	2.82 (dd, 17, 3)	44.0	2.80 (dd, 17, 3)	44.1	2.85 (dd, 17, 3.5)	42.5
	2.98 (dd, 17, 13)		3.00 (dd, 17, 13)		2.97 (dd, 17, 13)		2.90 (dd, 17, 12.5)	
4		191.5		191.3		191.5		196.7
4a		114.8		115.1		114.5		103.0
5	7.61 (s)	125.7	7.75 (d, 8.5)	126.5	7.60 (s)	125.7		163.9
6		121.8	6.53 (d, 8.5)	110.6		121.8	5.99 (s)	97.4
7		159.3		161.3		159.8		162.1
8		114.5		114.5		114.7		101.7
8a		159.8		160.7		159.3		
1′		131.5		131.3		131.5		119.8
2′	7.38 (d, 8.5)	127.5	7.39 (d, 8.5)	127.5	7.05 (d, 2.5)	124.2		157.4
3′	6.94 (d, 8.5)	114.1	6.95 (d, 8.5)	114.1		121.2	6.45 (d, 2)	99.0
4′		159.9		159.8		153.2		157.0
5′	6.94 (d, 8.5)	114.1	6.95 (d, 8.5)	114.1	6.80 (d, 8.5)	116.4	6.48 (dd, 8, 2)	107.2
6′	7.38 (d, 8.5)	127.5	7.39 (d, 8.5)	127.5	7.18 (dd, 8.5, 2.5)	127.0	7.40 (d, 8)	127.4
α					6.32 (d, 10)	122.0		
β					5.64 (d, 10)	131.2		
γ						76.5		
δ					1.45 (s)	28.1		
OH at 5							12.14 (s)	
OMe at 2′							3.81 (s)	55.5
at 4′	3.83 (s)	55.3	3.84 (s)	55.4				

[a] Measured in $CDCl_3$. Isoprenyl groups were observed; two in **1**, one in **2**, **4**, and three in **3**.

Table 2. NMR Spectroscopic Data (400 MHz) for Compounds **5**−**8**

	5[a]		**6**[a]		**7**[a]		**8**[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
position								
2	4.98 (d, 12)	84.0	4.98 (d, 12.5)	83.6	4.96 (d, 12)	83.8	4.94 (d, 12)	83.1
3	4.55 (d, 12)	73.2	4.50 (d, 12.5)	73.3	4.51 (d, 12)	73.2	4.50 (d, 12)	72.5
4		192.9		193.2		193.4		196.5
4a		112.2		111.7		111.7		100.5
5	7.67 (s)	128.6	7.58 (s)	125.8	7.58 (s)	125.7		158.8
6		122.5		122.8		122.6		107.8
7		162.3		160.8		160.7		163.2
8	6.40 (s)	103.9		114.9		114.9		107.0
8a		162.5		159.6		159.7		157.9
1′		129.0		129.0		128.8		128.8
2′	7.28 (brs)	129.5	7.41 (d, 8.5)	129.0	7.28 (brs)	129.2	7.27 (brs)	129.3
3′		127.2	6.85 (d, 8.5)	115.5		127.1		127.0
4′		155.3		156.3		155.0		155.1
5′	6.86 (d, 8)	116.0	6.85 (d, 8.5)	115.5	6.79 (d, 8.5)	115.7	6.86 (d, 8)	115.9
6′	7.30 (dd, 8, 2)	127.0	7.41 (d, 8.5)	129.0	7.27 (dd, 8.5, 2)	126.7	7.28 (dd, 8, 2)	126.8
OH at 5							11.49 (s)	
OH at 7			6.22 (brs)				6.43 (brs)	

[a] Measured in $CDCl_3$. Isoprenyl groups were observed; two in **5**, **6** and three in **7**, **8**.

mass spectrometer in a positive mode and on a JEOL JMS SX 102 mass spectrometer in a negative mode using a *m*-nitrobenzyl alcohol matrix. Preparative HPLC was performed on a JASCO 800 instrument.

Plant Material. Roots of *Lespedeza floribunda* Bunge were purchased from Maruzen Pharmaceuticals Co., Onomichi, Hiroshima, Japan. The plant was authenticated by Dr. Bing-Hui Che, Herbarium, South China Botanical Garden, The Chinese Academy of Sciences (IBSC), Guizhou Province, China, and a voucher specimen (7183) has been deposited at IBSC.

Extraction and Isolation. The roots of *L. floribunda* (500 g) were extracted twice with MeOH (2 L) under reflux for 2 h. The combined MeOH extract was concentrated under reduced pressure to give brown residue (38 g). The MeOH extract was suspended in hot H_2O (500 mL) and extracted with ether. The ether layer gave a brown residue (20.2 g) after evaporation of ether under reduced pressure. The H_2O layer was extracted with ethyl acetate to give a brown residue (1.15 g). The ether layer was subjected to silica gel (400 g) column chromatography (CC) using hexane−ethyl acetate (99:1−97:3) to give 13 fractions (1A−1M). Fraction 1C (461 mg) was subjected to preparative HPLC [Inertsil ODS-3 3 × 50 cm; H_2O−CH_3CN (35:65−15:85) linear gradient, UV, 280 nm] to give **1** (7.4 mg), **3** (6.5 mg), **24** (17.0 mg), **28** (7.7 mg), **31** (13.6 mg), **33** (68.5 mg), **49** (2.6 mg), and **50** (12.7 mg). Fraction 1D (2924 mg), preparative HPLC (PHPLC) [TSKgel ODS-80TS 5.5 × 120 cm; solvent, H_2O−CH_3CN (35:65−19:81) linear gradient, UV, 280 nm] gave 26 fractions (3A−3Z). Fraction 1E (652 mg) [PHPLC, Inertsil ODS-3 3 × 50 cm; solvent, H_2O−CH_3CN (40:60−30:70) linear gradient, UV, 280 nm] gave **7** (14.0 mg), **29** (6.2 mg), **30** (11.6 mg), and four fractions (4A−4D). Fraction 1F (620 mg) [PHPLC, column, Inertsil ODS-3 3 × 50 cm; solvent, H_2O−CH_3CN (40:60−25:75) linear gradient, UV, 280 nm] yielded **2** (2.3 mg), **4** (1.7 mg), **7** (40.6 mg), **8** (2.9 mg), **10** (39.1 mg), **17** (31.2 mg), **21** (13.5 mg), **22** (15.5 mg), **26** (1.8 mg), **29** (4.2 mg), **30** (7.2 mg), **42** (0.7 mg), **44** (2.4 g), **46** (1.4 mg), **48** (2.1 mg), and one fraction (5A). Fraction 1G (2078 mg) [PHPLC, column, TSKgel ODS-80TS 5.5 × 120 cm; solvent, H_2O−CH_3CN (40:60−8:92) linear gradient, UV, 280 nm] gave **21** (46.1 mg) and two fractions (6A and 6B). Fraction 1H (2114 mg) was subjected to silica gel (70 g) (silica gel PSQ-100B) CC using hexane−ethyl acetate (75:25) to give fractions 7A and 7B. Fraction 1J (3578 mg) [PHPLC, TSKgel ODS-80TS 5.5 × 120 cm; solvent, H_2O−CH_3CN (35:65−19:81) linear gradient, UV, 280 nm] gave **35** (82.5 mg), **47** (26.5 mg), and three fractions (8A−8E). Fraction 1K (990 mg) [PHPLC, TSKgel ODS-80TS 5.5 × 120 cm; solvent, H_2O−CH_3CN (40:60 −24:76) linear gradient, UV, 280 nm] yielded **19** (9.1 mg) and **34** (9.7 mg). Fraction 3C (49.9

Table 3. NMR Spectroscopic Data (400 MHz) for Compounds **9**–**15**

	9[a]		**10**[a]		**11**[a]		**12**[a]		**13**[a]		**14**[a]		**15**[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	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		129.3		128.5		129.8		130.1		128.4		128.7		128.8
2	7.06 (d, 8)	131.3	7.06 (d, 8.5)	130.4	7.01 (d, 8.5)	130.5	7.08 (d, 8.5)	130.4	6.99 (d, 8.5)	130.5	7.01 (d, 8.5)	130.5	7.03 (d, 8)	130.5
3	6.74 (d, 8)	115.9	6.81 (d, 8.5)	114.0	6.68 (d, 8.5)	115.1	6.80 (d, 8.5)	113.7	6.70 (d, 8.5)	115.3	6.71 (d, 8.5)	115.5	6.70 (d, 8)	115.1
4		157.0		158.6		154.3		158.3		154.6		155.9		154.3
5	6.74 (d, 8)	115.9	6.81 (d, 8.5)	114.0	6.68 (d, 8.5)	115.1	6.80 (d, 8.5)	113.7	6.70 (d, 8.5)	115.3	6.71 (d 8.5)	115.5	6.70 (d, 8)	115.1
6	7.06 (d, 8)	131.3	7.06 (d, 8.5)	130.4	7.01 (d, 8.5)	130.5	7.08 (d, 8.5)	130.4	6.99 (d, 8.5)	130.5	7.01 (d, 8.5)	130.5	7.03 (d, 8)	130.5
α	5.15 (m)	74.2	5.17 (ddd, 8, 7, 4.5)	72.9	5.31 (m)	77.2	5.26 (ddd, 7.5, 7, 3.5)	77.2	5.20 (brdd, 7, 4)	72.7	5.18 (ddd, 7.5, 7, 4)	72.9	5.27 (m)	77.2
β	2.82 (dd, 14, 8)	42.5	2.88 (dd, 14, 7)	42.4	2.62 (dd, 14, 7.5)	40.2	2.63 (dd, 14, 7.5)	40.3	2.86 (dd, 14, 7)	42.4	2.81 (dd, 14.5, 7)	42.5	2.62 (dd, 14, 7.5)	40.3
β	3.04 (dd, 14, 4.5)		3.10 (dd, 14, 4.5)		3.07 (dd, 14, 3.5)		3.07 (dd, 14, 3.5)		3.10 (dd, 14, 4)		3.10 (dd, 14.5, 4)		3.07 (dd, 14, 3)	
C=O		205.0		202.9		200.2		200.3		202.9		203.0		200.0
1′		111.5		110.8		116.6		116.8		110.0		110.1		116.7
2′		165.0		164.1		159.9		159.9		162.1		161.5		161.0
3′	6.41 (s)	103.5	6.38 (s)	104.0	6.44 (s)	99.6	6.44 (s)	99.6		112.0		114.4	6.41 (s)	99.8
4′		163.9		162.3		161.1		161.0		160.3		160.9		159.7
5′		121.6		119.7		119.9		119.6		118.4		119.8		114.7
6′	7.66 (s)	132.1	7.33 (s)	130.7	7.70 (s)	133.6	7.68 (s)	133.7	7.24 (s)	127.7	7.29 (s)	128.0	7.60 (s)	130.0
γ		133.5		135.7		135.9		136.1		136.4		135.7		78.1
δ	1.77 (brs)	25.9	1.82 (brs)	25.8	1.79 (brs)	25.8	1.80 (s)	25.8	1.82 (brs)	25.8	1.77 (d, 1)[b]	25.8	1.48 (s)[d]	28.6
Me at 3′									2.14 (s)	7.5				
OMe at 4			3.78 (s)	55.2			3.78 (s)	55.2					3.91 (s)	55.8
OMe at 2′					3.87 (s)	55.7	3.89 (s)	55.7						
OH at 2′	12.19 (s)		11.87 (s)						12.22 (s)		12.27 (s)			
OH at 4′	9.56 (s)		6.28 (brs)		6.15 (brs)		5.94 (brs)		6.37 (s)		6.37 (s)			
OH at 4	8.04 (brs)													
OH at α	4.13 (brd, 7.5)		3.62 (brd, 8)		4.08 (brd, 6.5)		3.92 (d, 7)				3.68 (d, 7.5)			

[a] Measured in $CDCl_3$. Isoprenyl groups were observed; one in **9**–**13**, **15** and two in **14**.

Table 4. NMR Spectroscopic Data (400 MHz) for Compounds **16** and **17**

	16[a]		**17**[a]	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
position				
2	4.50 (dd, 11, 11)	71.9	5.05 (d, 1.5)	68.3
2	4.54 (dd, 11, 6)			
3	4.29 (dd, 11, 6)	46.5		128.4
4		192.8	6.55 (t, 1.5)	121.9
4a		115.7		124.9
5	7.79 (d, 8.5)	127.1	6.72 (d, 8)	121.9
6	6.52 (d, 8.5)	110.5	6.54 (d, 8)	107.7
7		161.1		149.3
8		114.4		134.8
8a		161.1		145.5
1′		120.9		117.8
2′		157.5		150.7
3′		120.7		139.9
4′		155.6		149.6
5′	6.58 (d, 8.5)	112.4	6.72 (d, 8)	110.8
6′	6.83 (d, 8.5)	127.8	6.96 (d, 8)	123.5
OMe at 2′	3.72 (s)	62.2	3.82 (s)	60.5
OMe at 3′			3.94 (s)	60.9
OMe at 8			3.97 (s)	61.0

[a] Measured in $CDCl_3$. Two isoprenyl groups were observed in **16**.

Table 5. NMR Spectroscopic Data (400 MHz) for Compounds **18** and **19**

	18[a]		**19**[b]	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
2		151.4		150.2
3	7.11 (s)	104.1		121.0
4			4.70 (s)	55.8
4a		125.1		117.4
5	7.11 (d, 8.5)	114.4	7.12 (s)	99.8
6	6.89 (d, 8.5)	111.6		145.8
7		144.6		149.1
8		131.8		111.9
8a		144.3		143.2
1′		117.2		110.7
2′		150.0		157.1
3′		140.4	5.65 (d, 2.5)	104.4
4′		149.8		160.4
5′	6.83 (d, 8.5)	110.9	6.51 (dd, 8, 2.5)	108.6
6′	7.58 (d, 8.5)	121.9	7.35 (d, 8)	132.5
OMe at 2′	3.95 (s)	59.8		
OMe at 3′	4.00 (s)	61.0		
OMe at 6			3.91 (s)	57.0
OMe at 8	4.30 (s)	60.6		

[a] Measured in $CDCl_3$. [b] Measured in acetone-d_6. One isoprenyl group was observed in **19**.

mg) [preparative TLC (PTLC), silica gel PF_{254}; hexane−ethyl acetate (65:35)] gave **18** (22.6 mg). Fraction 3H (36 mg of 417 mg) [PTLC, hexane−ethyl acetate (65:35)] gave **27** (12.1 mg). Fraction 3O (23.0 mg) [PTLC, hexane−ethyl acetate (65:35)] gave **23** (11.5 mg). Fraction 3R (17.6 mg) [PTLC, hexane−ethyl acetate (7:3)] gave **51** (7.1 mg). Fraction 3T (50.4 mg) [PTLC, hexane−ethyl acetate (7:3)] gave **43** (34.7 mg). Fraction 3W (17.0 mg) [PTLC, hexane−ethyl acetate (7:3)] yielded **45** (9.7 mg). Fraction 4B (22.2 mg) [PTLC, hexane−ethyl acetate (6:4)] gave **20** (11.0 mg). Fraction 4D (10.6 mg) [PTLC, hexane−ethyl acetate (7:3)] provided **33** (2.9 mg). Fraction 5A (39.1 mg) [PTLC, Develosil 60−10 2 × 25 cm; hexane−ethyl acetate (75:25), UV, 280 mn] gave **32** (7.4 mg). Fraction 6A (38.3 mg) [PTLC, hexane−ethyl acetate (7:3)] provided **10** (14.6 mg) and **12** (5.6 mg). Fraction 6B (68.9 mg) [PHPLC, Capcellpak phenyl 2 × 25 cm; H_2O−CH_3CN (42:58), UV, 280 nm] gave **14** (26.2 mg). Fraction 7A (562 mg) [PHPLC, Inertsil ODS-3 3 × 50 cm; H_2O−CH_3OH (20:80), UV, 280 nm] yielded **5** (6.2 mg), **6** (10.4 mg), **16** (8.8 mg), **36** (12.0 mg), and **38** (23.1 mg). Fraction 7B (264 mg) [preparative HPLC, Inertsil ODS-3 3 × 50 cm; H_2O−CH_3CN (35:65−20:80), UV, 280 nm] provided **15** (6.6 mg), **36** (19.6 mg), and **40** (27.0 mg). Fraction 8A (37.6 mg) [PTLC, hexane−ethyl acetate (6:4)] gave **9** (14.3 mg). Fraction 8B (51.7 mg) [PTLC, hexane−ethyl acetate (55:45)] yielded

Table 6. NMR Spectroscopic Data (400 MHz) for Compounds **20**−**36**

	20[a]		**21**[a]		**22**[a]		**23**[a]		**24**[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	δ_H (*J* in Hz)	δ_C
1	7.26 (d, 8.5)	129.3	7.22 (s)	132.0		159.7	7.24 (s)	132.0	7.28 (d, 8.5)	129.4
2	6.55 (d, 8.5)	109.9		120.9		114.2		120.9	6.55 (d, 8.5)	109.7
3		155.7		155.5		157.1		155.6		155.6
4		115.0	6.39 (s)	103.9	6.28 (s)	100.4	6.39 (s)	103.9		114.9
4a		153.9		155.0		155.3		155.1		154.0
6	3.62 (dd, 11, 11)	66.9	3.66 (dd, 11, 11)	66.2	3.63 (dd, 11, 11)	66.0	3.58 (dd, 11, 11)	66.7	3.59 (dd, 11, 11)	67.0
6	4.27 (dd, 11, 5)		4.21 (dd, 11, 5)		4.17 (dd, 11, 5)		4.19 (dd, 11, 5)		4.26 (dd, 11, 5)	
6a	3.48 (ddd, 11, 7, 5)	40.3	3.48 (ddd, 11, 7, 5)	40.8	3.33 (m)	40.1	3.45 (ddd, 11, 7, 5)	40.3	3.46 (m)	40.3
11a	5.46 (d, 7)	78.8	5.39 (d, 7)	77.5	5.57 (d, 6.5)	75.0	5.39 (d, 7)	77.9	5.42 (d, 7)	78.5
11b		112.6		112.8		103.3		112.6		112.9
10b		117.1		122.0		122.2		118.0		118.0
7	6.79 (s)	108.0	6.73 (s)	108.8	6.75 (s)	108.9	6.85 (s)	123.6	6.84 (s)	123.6
8		141.1		143.2		143.1		116.4		116.3
9		146.7		145.3		145.3		153.6		153.6
10	6.50 (s)	98.1		117.9		117.8		109.6		109.5
10a		154.1		151.7		151.7		156.5		156.5
OMe-at 1					3.97 (s)	63.3				
OMe-at 3										
OMe-at 8	3.86 (s)	57.3								
OMe-at 9			3.77 (s)	61.4	3.77 (s)	61.4				
Me at 8							2.16 (s)	15.7	2.16 (s)	15.7
OH at 3			5.32 (brs)							
OH at 8										
OH at 9			5.66 (brs)							

	25[a]		**26**[a]		**27**[a]		**28**[a]		**29**[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	δ_H (*J* in Hz)	δ_C
1	7.37 (d, 8.5)	132.3	7.40 (d, 8.5)	132.4	7.42 (d, 8.5)	132.0	7.43 (d, 8.5)	132.0	7.39 (d, 8.5)	132.4
2	6.53 (dd, 8.5, 2.5)	109.6	6.54 (dd, 8.5, 2.5)	109.6	6.63 (dd, 8.5, 2.5)	109.1	6.64 (dd, 8.5, 2.5)	109.0	6.54 (dd, 8.5, 2.5)	109.6
3		156.9		156.8		161.0		160.9		156.9
4	6.40 (d, 2.5)	103.5	6.40 (d 2.5)	103.8	6.47 (d, 2.5)	101.6	6.46 (d, 2.5)	101.6	6.41 (d, 2.5)	103.8
4a		156.5		156.7		156.6		156.6		156.7
6	3.69 (dd, 11, 11)	66.2	3.67 (dd, 11, 11)	66.6	3.71 (dd, 11, 11)	66.2	3.68 (dd, 11, 11)	66.6	3.62 (dd, 11, 11)	66.7
6	4.22 (dd, 11, 5)		4.22 (dd, 11, 5)		4.25 (dd, 11, 5)		4.24 (dd, 11, 5)		4.21 (dd, 11, 5)	
6a	3.49 (ddd, 11, 7, 5)	40.6	3.49 (ddd, 11, 7, 5)	40.7	3.52 (ddd, 11, 7, 5)	40.8	3.49 (ddd, 11, 7, 5)	40.8	3.36 (m)	40.3
11a	5.41 (d, 7)	77.4	5.41 (d, 7)	77.2	5.44 (d, 7)	77.2	5.43 (d, 7)	77.5	5.41 (d, 7)	77.9
11b		113.0		113.4		112.9		113.1		113.1
10b		122.0		115.7		122.1		115.8		117.8
7	6.73 (s)	108.8	6.66 (s)	105.1	6.74 (s)	108.7	6.66 (s)	105.1	6.85 (s)	123.6
8		143.1		141.1		143.2		141.1		116.4
9		145.3		144.4		145.4		144.4		153.4
10		118.0		111.8		118.0		111.8		109.6
10a		151.6		152.5		151.7		152.5		156.4
OMe-at 1										
OMe-at 3					3.79 (s)	55.4	3.79 (s)	55.5		
OMe-at 8			3.85 (s)	57.0			3.85 (s)	57.0		
OMe-at 9	3.76 (s)	61.5			3.77 (s)	61.4				
Me at 8									2.16 (s)	15.7
OH at 3										
OH at 8										
OH at 9			5.69 (brs)							

	30[a]		**31**[a]		**32**[a]		**33**[a]		**34**[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	δ_H (*J* in Hz)	δ_C
1	7.24 (s)	132.0	7.42 (d, 8.5)	131.9	7.37 (d, 8.5)	121.3	7.38 (d, 8.5)	121.0	7.58 (s)	120.7
2		121.0	6.63 (dd, 8.5, 2.5)	109.1	6.58 (dd, 8.5, 2.5)	107.9	6.52 (dd, 8.5, 2.5)	107.2		126.3
3		155.7		161.1		161.8		160.9		158.6
4	6.40 (s)	104.0	6.47 (d, 2.5)	101.6	6.48 (d, 2.5)	103.3	6.50 (d, 2.5)	102.4	6.92 (s)	102.3
4a		155.1		156.7		155.9		155.1		152.5
6	3.58 (dd, 11, 11)	66.5	3.63 (dd, 11, 11)	66.6	5.51 (s)	66.2	5.51 (s)	65.6		159.0
6	4.20 (dd, 11, 5)		4.24 (dd, 11, 5)							
6a	3.43 (ddd, 11, 7, 5)	40.3	3.46 (ddd, 11, 7, 5)	40.2		107.5		106.6		102.1
11a	5.41 (d, 7)	78.3	5.44 (d, 7)	78.2		147.0		147.4		157.8
11b		112.3		112.5		111.1		110.0		104.0
10b		118.3		118.1		117.7		118.6		114.1
7	6.70 (s)	110.4	6.71 (s)	110.4	6.78 (s)	101.7	6.73 (s)	102.4	7.26 (s)	104.8
8		138.7		139.0		143.1		136.8		144.3
9		139.0		138.7		142.5		141.9		145.5
10		106.0		106.0		113.1		106.2	7.19 (s)	98.9
10a		148.2		148.4		149.8		145.2		148.8
OMe-at 1										
OMe-at 3			3.79 (s)	55.4	3.80 (s)	55.7	3.80 (s)	55.4		
OMe-at 8										
OMe-at 9										
Me at 8										
OH at 3										
OH at 8							5.39 (brs)			
OH at 9										

	35[a]		**36**[a]	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	7.49 (s)	120.3	7.86 (d, 9)	121.9
2		126.0	7.08 (dd, 9, 2.5)	112.8
3		158.5		161.9
4	6.87 (s)	102.3	7.16 (d, 2.5)	101.4
4a		152.5		154.1

Table 6. Continued

	35[a]		**36**[a]	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
6		158.8		158.2
6a		102.2		103.0
11a		157.8		157.6
11b		104.1		105.6
6b		113.5		113.2
7	7.12 (s)	102.0	7.17 (s)	102.0
8		143.9		144.0
9		142.8		143.1
10		112.3		112.4
10a		148.0		148.1
OMe-at 1				
OMe-at 3			3.89 (s)	55.8
OMe-at 8				
OMe-at 9				
Me at 8				
OH at 3				
OH at 8				
OH at 9				

[a] Measured in $CDCl_3$. [b] Measured in acetone-d_6. [c] Measured in DMSO-d_6. [d] Isoprenyl groups were observed; one in **20**, **25**−**29**, **31**−**34**, **36** and two in **21**−**24**, **30**, **35**.

Table 7. NMR Spectroscopic Data (400 MHz) for Compounds **37**−**40**

	37[a]		**38**[a]		**39**[a]		**40**[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		154.6		153.5		154.5		153.4
3		120.4		120.3		120.4		120.3
4		196.4		196.3		196.3		196.3
4a		116.3		116.8		116.3		116.8
5	6.97 (s)	103.2	6.94 (s)	103.2	6.96 (s)	103.2	6.91 (s)	103.1
6		143.3		143.0		143.2		143.0
7		142.8		143.3		142.8		143.3
8		112.2		115.2		112.2		112.3
8a		148.4		148.4		148.3		148.4
1′		110.8		112.4		110.7		112.2
2′		154.9		156.8		154.9		156.8
3′	6.39 (s)	103.7	6.43 (d, 2)	102.7	6.40 (s)	103.7	6.42 (d, 2.5)	102.6
4′		158.2		163.0		158.2		163.0
5′		121.1	6.62 (dd, 8, 2)	106.8		121.0	6.61 (dd, 8.5, 2.5)	106.9
6′	7.32 (s)	131.2	7.59 (d, 8)	131.7	7.27 (s)	131.4	7.58 (d, 8.5)	131.6
OMe at 4′			3.79 (s)	55.7			3.78 (s)	
OH at 6								
OH at 7	8.35 (brs)							
OH at 2′	8.41 (brs)							
OH at 4′	8.46 (brs)							
lower moiety								
1	7.18 (d, 8.5)	129.7	7.17 (d, 8.5)	129.1	7.19 (s)	132.4	7.18 (s)	132.4
2	6.60 (d, 8.5)	110.1	6.61 (d, 8.5)	110.1		123.1		123.1
3		157.0		157.0		157.2		157.2
4		116.9		116.8	6.37 (s)	103.7	6.35 (s)	103.7
4a		155.4		155.4		155.6		155.7
6	3.16 (dd, 11, 11)	67.2	3.20 (dd, 10.5, 10.5)	67.3	3.21 (dd, 11, 10.5)	66.8	3.25 (dd, 10.5, 10.5)	66.9
6	3.95 (dd, 11, 5)				3.87 (dd, 11, 5)		3.89 (dd, 10.5, 5)	
6a	3.37 (dddd, 11, 7, 5, 1)	39.8		39.8	3.37 (dddd, 10.5, 7, 5, 1)	39.9	3.42 (dddd, 10.5, 7, 5, 1)	40.0
11a	5.48 (d, 7)	80.9		80.9	5.46 (d, 7)	80.4	5.50 (d, 7)	80.4
11b		112.4		112.2		112.0		112.1
10b		119.3		119.4		119.2		119.4
7	7.39 (d, 1)	128.1		128.0	7.38 (d, 1)	128.0	7.43 (d, 1)	127.9
8		115.1		115.2		115.1		115.2
9		164.5		164.5		164.5		164.5
10		111.5		111.6		111.6		111.6
10a		164.3		164.3		164.4		164.5
OH at 3	8.35 (brs)							
OH at 9	13.42 (s)				13.41 (s)		13.43 (s)	

[a] Measured in acetone-d_6. Isoprenyl groups were observed; four in **37**, **38** and three in **38**, **40**.

11 (11.1 mg). Fraction 8C (26.6 mg) was subjected to PTLC [hexane−ethyl acetate (6:4)] to give **41** (10.2 mg). Fraction 8D (83.8 mg) [PTLC, hexane−ethyl acetate (6:4)] gave **37** (17.0 mg). Fraction 8E (71.7 mg) [PTLC, hexane−ethyl acetate (6:4)] yielded **39** (25.7 mg). Fraction 9A (54.3 mg) [PHPLC, Capcellpak phenyl 2 × 25 cm; H_2O−CH_3CN−trifluoroacetic acid (67.4:32.5:0.1), UV, 280 nm] gave **13** (19.9 mg) and **25** (24.0 mg).

Lespeflorin A_1 (1): colorless, amorphous solid; $[\alpha]_D^{23}$ −43.0 (*c* 0.74, MeOH); UV λ_{max} (log ϵ) 275 (4.10), 315 (3.76) nm; CD (MeOH) λ_{max} nm ($[\theta]$) 240 (+22 736), 305 (−40 015), 337 (+28 192); ^{1}H NMR and ^{13}C NMR, Table 1; HRFABMS *m*/*z* 405.2059 (calcd for $C_{26}H_{30}O_4$ − H, 405.2067).

Lespeflorin A_2 (2): colorless, amorphous solid; $[\alpha]_D^{23}$ −89.8 (*c* 0.23, MeOH); UV λ_{max} (log ϵ) 275 (3.95), 310 sh (3.76) nm; CD (MeOH)

Table 8. Melanin Synthesis Inhibitory Effects (IC_{50} μM) of Isolated Flavonoids

compd	IC_{50} (μM)	compd	IC_{50} (μM)
hydroquinone	2.20	isoflavone	
arbutin	103	**47**	29.2
euchrenone a_6	1.02	benzofuran	
amorilin	1.21	**18**	10.8
amorisin	3.60	**19**	8.27
lespedezaflavanone H	7.80	pterocarpan	
		20	3.73
flavanone		**21**	2.07
1	3.96	**22**	2.03
2	3.86	**23**	3.24
3	1.50	**24**	1.52
4	4.93	**25**	6.16
41	2.70	**26**	6.02
42	2.52	**27**	1.63
43	0.98	**28**	8.38
44	2.59	**29**	1.44
45	1.51	**30**	2.90
46	8.41	**31**	8.34
flavanonol		**48**	2.00
5	2.87	**49**	3.84
6	2.19	**50**	2.93
7	0.68	**51**	1.84
8	1.23	pterocarpene	
hydroxydihydrochalcone		**32**	27.0
9	>146	**33**	1.48
10	28.1	coumestan	
11	1.68	**34**	3.21
12	>135	**35**	28.3
13	>135	**36**	27.3
14	22.0	dimer	
15	3.78	**37**	39.6
isoflavanone		**38**	>52.8
16	1.47	**39**	37.7
isoflavene		**40**	>52.8
17	4.29		

λ_{max} nm ([θ]) 300 (−12 882), 332 (+43 074); ^{1}H NMR and ^{13}C NMR, Table 1; HRFABMS *m/z* 337.1432 (calcd for $C_{21}H_{22}O_4$ − H, 337.1440).

Lespeflorin A_3 (3): colorless, amorphous solid; $[\alpha]_D^{23}$ −35.6 (*c* 0.65, MeOH); UV λ_{max} (log ϵ) 279 (4.10), 321 sh (3.88) nm; CD (MeOH) λ_{max} nm ([θ]) 302 (−21 663), 340 (+24 995); ^{1}H NMR and ^{13}C NMR, Table 1; HRFABMS *m/z* 457.2394 (calcd for $C_{30}H_{34}O_4$ − H, 457.2380).

Lespeflorin A_4 (4): colorless, amorphous solid; $[\alpha]_D^{23}$ −13.6 (*c* 0.18, MeOH); UV λ_{max} (log ϵ) 270 (4.76), 350 sh (4.02) nm; CD (MeOH) λ_{max} nm ([θ]) 300 (−860); ^{1}H NMR and ^{13}C NMR, Table 1; HRFABMS *m/z* 367.1209 (calcd for $C_{21}H_{20}O_6$ − H, 367.1182).

Lespeflorin B_1 (5): colorless, amorphous solid; $[\alpha]_D^{23}$ −11.5 (*c* 0.62, MeOH); UV λ_{max} (log ϵ) 279 (4.11), 325 (3.85) nm; CD (MeOH) λ_{max} nm ([θ]) 302 (−17 521), 333 (+12 302); ^{1}H NMR and ^{13}C NMR, Table 2; HRFABMS *m/z* 407.1827 (calcd for $C_{25}H_{28}O_5$ − H, 407.1859).

Lespeflorin B_2 (6): colorless, amorphous solid; $[\alpha]_D^{23}$ −2.5 (*c* 1.04, MeOH); UV λ_{max} (log ϵ) 283 (4.10), 325 sh (3.76) nm; CD (MeOH) λ_{max} nm ([θ]) 307 (−23 203), 338 (+16 543); ^{1}H NMR and ^{13}C NMR, Table 2; HRFABMS *m/z* 407.1882 (calcd for $C_{25}H_{28}O_5$ − H, 407.1859).

Lespeflorin B_3 (7): colorless, amorphous solid; $[\alpha]_D^{23}$ −10.2 (*c* 4.06, MeOH); UV λ_{max} (log ϵ) 283 (4.15), 319 sh (3.80) nm; CD (MeOH) λ_{max} nm ([θ]) 305 (−22 860), 338 (+23 495); ^{1}H NMR and ^{13}C NMR, Table 2; HRFABMS *m/z* 475.2458 (calcd for $C_{30}H_{36}O_5$ − H, 475.2486).

Lespeflorin B_4 (8): colorless, amorphous solid; $[\alpha]_D^{23}$ −27.9 (*c* 0.29, MeOH); UV λ_{max} (log ϵ) 297 (4.12), 339 sh (3.73) nm; CD (MeOH) λ_{max} nm ([θ]) 298 (−22 628), 340 (+4764); ^{1}H NMR and ^{13}C NMR, Table 2; HRFABMS *m/z* 491.2407 (calcd for $C_{30}H_{36}O_6$ − H, 491.2435).

Lespeflorin C_1 (9): colorless, amorphous solid; $[\alpha]_D^{23}$ +76.7 (*c* 1.00, MeOH); UV λ_{max} (log ϵ) 282 (4.20), 330 (3.95) nm; CD (MeOH) λ_{max} nm ([θ]) 250 (−5702), 287 (+11 405), 330 (+13 230); ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m/z* 341.1367 (calcd for $C_{20}H_{22}O_5$ − H, 341.1389).

Lespeflorin C_2 (10): colorless, amorphous solid; $[\alpha]_D^{23}$ +68.4 (*c* 1.00, MeOH); UV λ_{max} (log ϵ) 274 (4.07), 316 (3.93) nm; CD (MeOH) λ_{max} nm ([θ]) 250 (−5936), 287 (+11 872), 330 (+13 772); ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m/z* 355.1522 (calcd for $C_{21}H_{24}O_5$ − H, 355.1546).

Lespeflorin C_3 (11): colorless, amorphous solid; $[\alpha]_D^{23}$ −2.9 (*c* 1.00, MeOH); UV λ_{max} (log ϵ) 274 (4.06), 316 (3.92) nm; CD (MeOH) λ_{max} nm ([θ]) 230 (−9203), 271 (+23 008), 297 (+10 123), 327 (−2956); ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m/z* 355.1517 (calcd for $C_{21}H_{24}O_5$ − H, 355.1546).

Lespeflorin C_4 (12): colorless, amorphous solid; $[\alpha]_D^{23}$ −7.0 (*c* 0.56, MeOH); UV λ_{max} (log ϵ) 277 (4.18), 320 sh (3.87) nm; CD (MeOH) λ_{max} nm ([θ]) 235 (−9307), 270 (+23 268), 326 (−15 653); ^{1}H NMR and ^{13}C NMR Table 3; HRFABMS *m/z* 369.1727 (calcd for $C_{22}H_{26}O_5$ − H, 369.1703).

Lespeflorin C_5 (13): colorless, amorphous solid; $[\alpha]_D^{23}$ +72.5 (*c* 1.00, MeOH); UV λ_{max} (log ϵ) 287 (3.60), 331 (3.31) nm; CD (MeOH) λ_{max} nm ([θ]) 255 (−5406), 290 (+12 720), 332 (+9540); ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m/z* 355.1533 (calcd for $C_{21}H_{24}O_5$ − H, 355.1546).

Lespeflorin C_6 (14): colorless, amorphous solid; $[\alpha]_D^{23}$ +89.5 (*c* 1.00, MeOH); UV λ_{max} (log ϵ) 287 (4.12), 330 sh (3.83) nm; CD (MeOH) λ_{max} nm ([θ]) 256 (−4280), 291 (+15 338), 332 (+9631); ^{1}H NMR and ^{13}C NMR Table 3; HRFABMS *m/z* 409.2005 (calcd for $C_{25}H_{30}O_5$ − H, 409.2016).

Lespeflorin C_7 (15): colorless, amorphous solid; $[\alpha]_D^{23}$ −43.3 (*c* 0.66, MeOH); UV λ_{max} (log ϵ) 257 (4.39), 336 sh (3.85) nm; CD (MeOH) λ_{max} nm ([θ]) 288 (+10 821), 318 (+393), 340 (−4919); ^{1}H NMR and ^{13}C NMR, Table 3; HRFABMS *m/z* 353.1376 (calcd for $C_{21}H_{22}O_5$ − H, 353.1389).

Lespeflorin D_1 (16): colorless, amorphous solid; $[\alpha]_D^{23}$ +25.1 (*c* 0.88, MeOH); UV λ_{max} (log ϵ) 282 (4.16) nm; CD (MeOH) λ_{max} nm ([θ]) 290 (−8649), 325 (+11 380); ^{1}H NMR and ^{13}C NMR, Table 4; HRFABMS *m/z* 421.2010 (calcd for $C_{26}H_{30}O_5$ − H, 421.2016).

Lespeflorin E_1 (17): colorless, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 242 (4.14), 312 (4.13) nm; ^{1}H NMR and ^{13}C NMR, Table 4; HRFABMS *m/z* 330.1095 (calcd for $C_{18}H_{18}O_6$, 330.1103).

Lespeflorin F_1 (18): colorless, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 312 (4.50), 326 (4.41) nm; ^{1}H NMR and ^{13}C NMR, Table 5; HRFABMS *m/z* 315.0893 (calcd for $C_{17}H_{16}O_6$ − H, 315.0869).

Lespeflorin F_2 (19): colorless, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 312 (4.16) nm; ^{1}H NMR and ^{13}C NMR, Table 5; HRFABMS *m/z* 369.1353 (calcd for $C_{21}H_{22}O_6$ − H, 369.1353).

Lespeflorin G_1 (20): colorless, amorphous solid; $[\alpha]_D^{23}$ −110.0 (*c* 1.00, MeOH); UV λ_{max} (log ϵ) 300 (3.88), 341 (3.51) nm; CD (MeOH) λ_{max} nm ([θ]) 295 (+11 736); ^{1}H NMR and ^{13}C NMR, Table 6; HRFABMS *m/z* 353.1370 (calcd for $C_{21}H_{22}O_5$ − H, 353.1389).

Lespeflorin G_2 (21): colorless, amorphous solid; $[\alpha]_D^{23}$ −50.2 (*c* 1.35, MeOH); UV λ_{max} (log ϵ) 285 (3.98), 337 (3.33) nm; CD (MeOH) λ_{max} nm ([θ]) 295 (+4979); ^{1}H NMR and ^{13}C NMR, Table 6; HRFABMS *m/z* 422.2060 (calcd for $C_{26}H_{30}O_5$, 422.2094).

Lespeflorin G_3 (22). colorless, amorphous solid; $[\alpha]_D^{23}$ −178.1 (*c* 1.55, MeOH); UV λ_{max} (log ϵ) 287 (3.94), 340 (3.84) nm; CD (MeOH) λ_{max} nm ([θ]) 288 (+3211); ^{1}H NMR and ^{13}C NMR, Table 6; HRFABMS *m/z* 452.2180 (calcd for $C_{27}H_{32}O_6$, 452.2200).

Lespeflorin G_4 (23). colorless, amorphous solid; $[\alpha]_D^{23}$ −177.1 (*c* 0.50, MeOH); UV λ_{max} (log ϵ) 290 (4.04), 345 (3.56) nm; CD (MeOH) λ_{max} nm ([θ]) 292 (+15 607); ^{1}H NMR and ^{13}C NMR, Table 6; HRFABMS *m/z* 406.2166 (calcd for $C_{26}H_{30}O_4$, 406.2145).

Lespeflorin G_5 (24): colorless, amorphous solid; $[\alpha]_D^{23}$ −55.6 (*c* 1.70, MeOH); UV λ_{max} (log ϵ) 278 (3.83), 326 (3.36) nm; CD (MeOH) λ_{max} nm ([θ]) 286 (+13160); ^{1}H NMR and ^{13}C NMR, Table 6; HRFABMS *m/z* 406.2176 (calcd for $C_{26}H_{30}O$, 406.2145).

Lespeflorin G_6 (25): colorless, amorphous solid; $[\alpha]_D^{23}$ −118.1 (*c* 1.00, MeOH); UV λ_{max} (log ϵ) 300 (3.75), 340 (3.19) nm; CD (MeOH) λ_{max} nm ([θ]) 290 (+10 624); ^{1}H NMR and ^{13}C NMR, Table 6; HRFABMS *m/z* 353.1373 (calcd for $C_{21}H_{22}O_5$ − H, 353.1389).

Lespeflorin G_7 (26): colorless, amorphous solid; $[\alpha]_D^{23}$ −84.7 (*c* 0.18, MeOH); UV λ_{max} (log ϵ) 281 (3.87), 330 (3.70) nm; CD (MeOH) λ_{max} nm ([θ]) 289 (+5407); ^{1}H NMR and ^{13}C NMR, Table 6; HRFABMS *m/z* 354.1482 (calcd for $C_{21}H_{22}O_5$, 354.1468).

Lespeflorin G_8 (27): colorless, amorphous solid; $[\alpha]_D^{23}$ −121.8 (*c* 0.74, MeOH); UV λ_{max} (log ϵ) 300 (3.66), 340 sh (3.30) nm; CD (MeOH) λ_{max} nm ([θ]) 290 (+5647); ^{1}H NMR and ^{13}C NMR, Table 6; HRFABMS *m/z* 367.1550 (calcd for $C_{22}H_{24}O_5$ − H, 367.1546).

Lespeflorin G_9 (28): colorless, amorphous solid; $[\alpha]_D^{23}$ −109.3 (*c* 0.77, MeOH); UV λ_{max} (log ϵ) 279 (3.91), 333 (3.58) nm; CD (MeOH) λ_{max} nm ([θ]) 289 (+8516); ^{1}H NMR and ^{13}C NMR, Table 6; HRFABMS *m/z* 368.1616 (calcd for $C_{22}H_{24}O_5$, 368.1624).

Lespeflorin G_{10} (29): colorless, amorphous solid; $[\alpha]_D^{23}$ −91.7 (*c* 0.42, MeOH); UV λ_{max} (log ϵ) 282 (3.94), 334 (3.84) nm; CD (MeOH) λ_{max} nm ([θ]) 287 (+7546); ^{1}H NMR and ^{13}C NMR, Table 6; HRFABMS *m*/*z* 338.1534 (calcd for $C_{21}H_{22}O_4$, 338.1519).

Lespeflorin G_{11} (30): colorless, amorphous solid; $[\alpha]_D^{23}$ −233.7 (*c* 0.72, MeOH); UV λ_{max} (log ϵ) 271 (4.02), 344 (3.66) nm; CD (MeOH) λ_{max} nm ([θ]) 289 (+3450); ^{1}H NMR and ^{13}C NMR, Table 1; HRFABMS *m*/*z* 406.1747 (calcd for $C_{25}H_{26}O_5$, 406.1781).

Lespeflorin G_{12} (31): colorless, amorphous solid; $[\alpha]_D^{23}$ −110.0 (*c* 0.45, MeOH); UV λ_{max} (log ϵ) 271 (3.99), 341 (3.66) nm; CD (MeOH) λ_{max} nm ([θ]) 284 (+8200); ^{1}H NMR and ^{13}C NMR, Table 6; HRFABMS *m*/*z* 351.1247 (calcd for $C_{21}H_{20}O_5$ − H, 351.1233).

Lespeflorin H_1 (32): colorless, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 339 (4.32), 355 (4.29) nm; ^{1}H NMR and ^{13}C NMR, Table 6; HRFABMS *m*/*z* 351.1247 (calcd for $C_{21}H_{20}O_5$ − H, 351.1233).

Lespeflorin H_2 (33): colorless, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 358 (4.01), 367 sh (3.99) nm; ^{1}H NMR and ^{13}C NMR, Table 6; HRFABMS *m*/*z* 349.1082 (calcd for $C_{21}H_{18}O_5$ − H, 349.1076).

Lespeflorin I_1 (34): colorless, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 353 (4.24), 366 sh (4.20) nm; ^{1}H NMR and ^{13}C NMR, Table 6; HRFABMS *m*/*z* 351.0898 (calcd for $C_{20}H_{16}O_6$ − H, 351.0869).

Lespeflorin I_2 (35): colorless, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 355 (4.22), 367 sh (4.18) nm; ^{1}H NMR and ^{13}C NMR Table 6; HRFABMS *m*/*z* 419.1494 (calcd for $C_{25}H_{24}O_6$ − H, 419.1495).

Lespeflorin I_3 (36): colorless, amorphous solid; UV (MeOH) λ_{max} (log ϵ) 352 (4.26), 363 sh (4.23) nm; ^{1}H NMR and ^{13}C NMR Table 6; HRFABMS *m*/*z* 365.1028 (calcd for $C_{21}H_{18}O_6$ − H, 365.1025).

Lespeflorin J_1 (37): colorless, amorphous solid; $[\alpha]_D^{23}$ −17.8 (*c* 1.00, MeOH); UV λ_{max} (log ϵ) 315 (439) nm; CD (MeOH) λ_{max} nm ([θ]) 232 (−48 741), 254 (+8124), 272 (−34 931), 329 (−2464), 394 (+14 622); ^{1}H NMR and ^{13}C NMR, Table 7; HRFABMS *m*/*z* 811.3492 (calcd for $C_{50}H_{52}O_{10}$ − H, 811.3483).

Lespeflorin J_2 (38): colorless, amorphous solid; $[\alpha]_D^{23}$ −9.0 (*c* 1.00, MeOH); UV λ_{max} (log ϵ) 314 (4.39) nm; CD (MeOH) λ_{max} nm ([θ]) 231 (−31 862), 254 (+9559), 270 (−25 489), 329 (−2218), 385 (+10 833); ^{1}H NMR and ^{13}C NMR, Table 7; HRFABMS *m*/*z* 757.3016 (calcd for $C_{46}H_{46}O_{10}$ − H, 757.3014).

Lespeflorin J_3 (39): colorless, amorphous solid; $[\alpha]_D^{23}$ −67.4 (*c* 1.00, MeOH); UV λ_{max} (log ϵ) 320 (4.42) nm; CD (MeOH) λ_{max} nm ([θ]) 233 (−40 618), 255 (+1310), 272 (−32 756), 325 (−2271), 393 (+13 103); ^{1}H NMR and ^{13}C NMR, Table 7; HRFABMS *m*/*z* 811.3492 (calcd for $C_{50}H_{52}O_{10}$ − H, 811.3483).

Lespeflorin J_4 (40): colorless, amorphous solid; $[\alpha]_D^{23}$ −55.0 (*c* 1.00, MeOH); UV, λ_{max} (log ϵ) 316 (4.43) nm; CD (MeOH) λ_{max} nm ([θ]) 233 (−41 665), 255 (+4167), 270 (−25 833), 329 (−2321), 390 (+12 500); ^{1}H NMR and ^{13}C NMR, Table 7; HRFABMS *m*/*z* 757.3016 (calcd for $C_{46}H_{46}O_{10}$ − H, 757.3014).

Assay of Melanin Synthesis in NHEM. NHEM 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 the sample compound dissolved in DMSO and 0.25 μCi ^{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). After 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. A WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt] solution was added after the 72 h treatment and then incubated for another 3 h at 37 °C. The absorbance of each well was measured at 450 nm with a reference wavelength at 630 nm. The mean absorbance of control wells (cells without compound) represented 100% cell viability. Viability of compound-treated cells was determined in triplicate and related to the absorbance of control cells. The 50% inhibition concentration (IC_{50}) value of melanin synthesis was determined using SAS software version 9.1.3 (SAS Institute Inc., Cary, NC).

Acknowledgment. The authors would like to thank Prof. S. Kidou, Cryobiosystem Research Center, Iwate University, Morioka, Iwate, Japan, for partial support of this work.

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

References and Notes

(1) Katagiri, T.; Hongo, M.; Miyase, T. *Jpn. Kokai Tokkyo Koho*JP2007186441, 2007.
(2) Bhalla, V. K.; Nayak, U. R. *Tetrahedron Lett.* **1968**, 2401–2406.
(3) Kyogoku, K.; Hatayama, K.; Suzuki, K.; Yokomori, S.; Maejima, K.; Komatsu, M. *Chem. Pharm. Bull.* **1973**, *21*, 1777–1782.
(4) Iinuma, M.; Ohyama, M.; Tanaka, T. *Phytochemistry* **1995**, *38*, 539–543.
(5) (a) Dawidar, A. M.; Jakupovic, J.; Abdel-Mogib, M.; Mashaly, I. A. *Phytochemistry* **1994**, *36*, 803–806. (b) Schütz, B. A.; Wrig, A. D.; Rali, T.; Sticher, O. *Phytochemistry* **1995**, *40*, 1273–1277.
(6) Mizuno, M.; Tamura, K.; Tanaka, T.; Iinuma, M. *Phytochemistry* **1988**, *27*, 1831–1834.
(7) (a) Bhanumati, S.; Chhabra, S. C.; Gupta, S. R.; Krishnamoorthy, V. *Phytochemistry* **1978**, *17*, 2045. (b) Fomum, Z. T.; Ayafor, J. F.; Wandji, J. *Phytochemistry* **1985**, *24*, 3075–3076.
(8) Miyase, T.; Ueno, A.; Noro, T.; Fukushima, S. *Chem. Pharm. Bull.* **1980**, *28*, 1172–1177.
(9) Iinuma, M.; Tanaka, T.; Mizuno, M.; Yamamoto, H.; Kobayashi, Y.; Yonemori, S. *Chem. Pharm. Bull.* **1992**, *40*, 2749–2752.
(10) Mitscher, L. A.; Okwute, S. K.; Gollapudi, S. R.; Drake, S. R.; Avona, E. *Phytochemistry* **1988**, *27*, 3449–3452.
(11) Tanaka, H.; Tanaka, T.; Etho, H. *Phytochemistry* **1998**, *47*, 475–477.
(12) Andersen, Ø. M.; Markham, K. R. *Flavonoids*; Taylor & Francis: New York, 2006; p 56.
(13) Schütz, B. A.; Wright, A. D.; Pali, T.; Sticher, O. *Phytochemistry* **1995**, *40*, 1273–1277.
(14) Slade, D.; Ferreira, D.; Marais, J. P. J. *Phytochemistry* **2005**, *66*, 2177–2215.
(15) Harborne, J. B. *The Flavonoids*; Chapman & Hall: New York, 1986; p 462.
(16) Harborne, J. B. *The Flavonoids*; Chapman & Hall: New York, 1986; p 421.
(17) Fukai, T.; Sheng, C.-B.; Horikoshi, T.; Nomura, T. *Phytochemistry* **1996**, *43*, 1119–1124.
(18) Augustyn, J. A. N.; Bezuidenhoudt, B. C. B.; Swanepoel, A.; Ferreira, D. *Tetrahedron* **1990**, *46*, 4429–4442.
(19) Miyase, T.; Sano, M.; Yoshino, K.; Nonaka, K. *Phytochemistry* **1999**, *52*, 311–319.
(20) Miyase, T.; Sano, M.; Nakai, H.; Muraoka, M.; Nakazawa, M.; Suzuki, M.; Yoshino, K.; Nishihara, Y. *Phytochemistry* **1999**, *52*, 303–310.
(21) Harborne, J. B. *The Flavonoids*; Chapman & Hall: New York, 1986; p 167.
(22) (a) Napolitano, A.; Palumbo, A.; d'Ischia, M.; Prota, G. *J. Med. Chem.* **1996**, *39*, 5192–5201. (b) Yada, Y.; Higuchi, K.; Imokawa, G. *J. Biol. Chem.* **1991**, *266*, 18352–18357. (c) Whittaker, J. R. *J. Biol. Chem.* **1971**, *246*, 6217–6226.

NP800395J