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Lignan Dicarboxylates and Terpenoids from the Flower Buds of *Cananga odorata* and Their Inhibitory Effects on Melanogenesis

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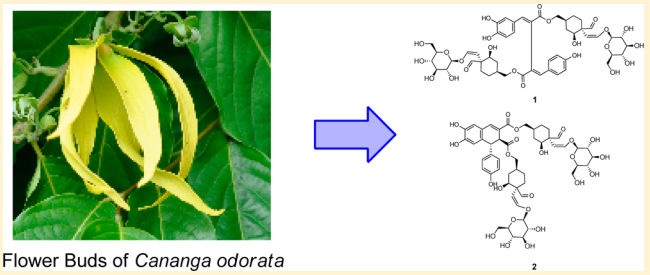
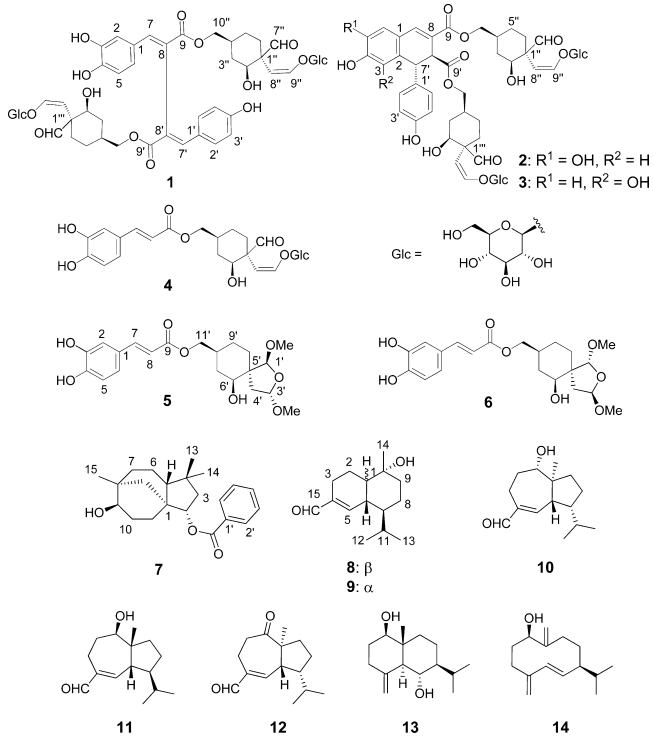
Supporting Information

ABSTRACT: The methanolic extract from the flower buds of *Cananga odorata* showed an inhibitory effect on melanogenesis in theophylline-stimulated murine B16 melanoma 4A5 cells. From the methanolic extract, two new lignan dicarboxylates, canangalignans I and II, three new terpenoids, canangaterpenes I, II, and III, and eight known compounds were isolated. The structures of these compounds were elucidated on the basis of chemical/physicochemical evidence. Several mono- and sesquiterpene analogues significantly inhibited melanogenesis. In particular, canangaterpene I and (3*R*,3a*R*,8a*S*)-3-isopropyl-8a-methyl-8-oxo-1,2,3,3a,6,7,8,8a-octahydroazulene-5-carbaldehyde exhibited a potent inhibitory effect on melanogenesis [inhibition (%): 34.7 ± 4.2 ($p < 0.01$), 45.5 ± 5.7 ($p < 0.01$) at 1 μM, respectively] without inducing cytotoxicity. Moreover, the biological effect of these compounds was much stronger than that of the reference compound, arbutin. Thus, these isolated terpenoid derivatives may be promising therapeutic agents for the treatment of several skin disorders.



Cananga odorata Hook. f. and Thomson (Annonaceae) is widely distributed in tropical and subtropical regions. The flowers have been used as a tonic for the heart and for the treatment of dizziness and fainting spells. The essential oil from the flowers has also been used in the field of aromatherapy. Previous studies have focused on constituents such as sesquiterpenes,[1] monoterpenes,[2] and lignans[3] from the fruit, stem, or bark of *C. odorata*. However, the chemical constituents of the flowers have not been characterized. In the course of our studies on bioactive constituents from medicinal flowers.[4−14] A methanolic extract from the flower buds of *C. odorata* cultivated in Thailand was shown to have inhibitory effects on melanogenesis. From the MeOH extract, we have isolated two new lignan dicarboxylates, canangalignans I (**1**) and II (**2**), three new terpenoid derivatives, canangaterpenes I (**5**), II (**7**), and III (**8**), and eight known compounds. Herein, we describe the isolation and structural elucidation of the five new compounds as well as the inhibitory effects of the isolated compounds on melanogenesis in theophylline-stimulated B16 melanoma 4A5 cells.



RESULTS AND DISCUSSION

A MeOH extract of the dried flower buds of *C. odorata* (cultivated in Thailand) showed melanogenesis inhibitory activity [inhibition (%): 43.8 ± 1.9 ($p < 0.01$) at 3 μg/mL]. The MeOH extract was partitioned into an EtOAc−H_2O (1:1, v/v) mixture to furnish an EtOAc-soluble fraction (9.8%) and an aqueous layer. The aqueous layer was extracted with 1-butanol to give 1-butanol (18.8%) and H_2O (10.9%) soluble fractions. The EtOAc- and the 1-butanol-soluble fractions

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Table 1. ^{13}C NMR (125 MHz) Spectroscopic Data[a] for 1 and 2

position	1	position	1	position	2	position	2
1	128.1	9″	146.1	1	131.3	9″	146.2
2	117.5	10″	70.2	2	124.9	10″	69.8
3	146.4	1‴	56.1	3	117.1	1‴	56.2
4	148.7	2‴	75.8	4	149.3	2‴	75.7
5	116.4	3‴	35.7	5	145.7	3‴	35.8
6	124.7	4‴	37.3	6	117.1	4‴	37.3
7	144.4	5‴	25.9	7	139.6	5‴	26.0
8	124.9	6‴	31.2	8	124.9	6‴	31.2
9	169.0	7‴	206.8	9	168.5	7‴	206.8
1′	127.6	8‴	110.1	1′	134.8	8‴	110.1
2′	133.1	9‴	146.1	2′	129.7	9‴	146.3
3′	116.6	10‴	70.2	3′	116.2	10‴	70.1
4′	160.6	1⁗	104.1	4′	157.2	1⁗	104.2
5′	116.6	2⁗	74.5	5′	116.2	2⁗	74.5
6′	133.1	3⁗	78.4	6′	129.7	3⁗	78.5
7′	144.1	4⁗	71.1	7′	47.0	4⁗	71.2
8′	124.9	5⁗	77.8	8′	49.6	5⁗	77.9
9′	169.0	6⁗	62.5	9′	174.5	6⁗	62.6
1″	56.1	1⁗′	104.1	1″	56.2	1⁗′	104.2
2″	75.8	2⁗′	74.5	2″	75.8	2⁗′	74.5
3″	35.7	3⁗′	78.4	3″	35.6	3⁗′	78.5
4″	37.3	4⁗′	71.1	4″	37.3	4⁗′	71.2
5″	26.0	5⁗′	77.8	5″	25.9	5⁗′	77.9
6″	31.2	6⁗′	62.5	6″	31.2	6⁗′	62.6
7″	206.8			7″	206.8		
8″	110.1			8″	110.1		

[a]Measured in methanol-d_4.

significantly inhibited melanogenesis [inhibition (%): 37.5 ± 3.4 ($p < 0.01$), 46.9 ± 6.8 ($p < 0.01$), respectively, at 3 μg/mL], but the H_2O-soluble fraction showed no detectable biological effect at 3 μg/mL. The EtOAc- and the 1-butanol-soluble fractions were subjected to normal- and reversed-phase silica gel column chromatography and repeated HPLC to give two new lignan dicarboxylates, canangalignans I (**1**, 0.052%) and II (**2**, 0.0015%), three new terpenoid derivatives, canangaterpenes I (**5**, 0.0014%), II (**7**, 0.00087%), and III (**8**, 0.00061%), the known lignan (1*R*,2*S*)-1,2-dihydro-5,6-dihydroxy-1-(4-hydroxyphenyl)-2,3-naphthalenedicarboxylic acid 2,3-bis(10-cananga-fruiticoside A) ester (**3**, 0.0038%),[3] and eight known terpenoid derivatives, canangafruiticoside E (**4**, 0.0039%),[3] (*E*)-{(1*R*,3*R*,5*S*,6*S*,8*S*)-6-hydroxy-1,3-dimethoxy-2-oxaspiro[4.5]-decan-8-ylmethyl} caffeate (**6**, 0.0015%),[3] 15-oxo-α-cadinol (**9**, 0.0029%),[15,16] 10β-hydroxyisodauc-6-en-14-al (**10**, 0.0028%),[17] aphanamol II (**11**, 0.0017%),[18] (3*R*,3a*R*,8a*S*)-3-isopropyl-8a-methyl-8-oxo-1,2,3,3a,6,7,8,8a-octahydroazulene-5-carbaldehyde (**12**, 0.0042%),[19] voleneol (**13**, 0.0019%),[20] and (1*R*,5*E*,7*S*)-4,10-bis(methylene)-7-(1-methylethyl)-5-cyclodecen-1-ol (**14**, 0.0055%).[21]

Canangalignan I (**1**) was isolated as an amorphous powder with a positive specific rotation ($[\alpha]^{25}_D$ +25, in MeOH). Its IR spectrum showed absorption bands at 3400, 1703, 1682, 1601, 1512, and 1075 cm^{-1} ascribable to hydroxy, ester, formyl, aromatic ring, and ether functionalities, respectively. FABMS in the positive-ion mode revealed a quasimolecular ion $[M + Na]^+$ at *m/z* 1053, from which the molecular formula $C_{50}H_{62}O_{23}$ was determined via HRMS and ^{13}C NMR data. Acid hydrolysis of **1** with 5% aqueous H_2SO_4−1,4-dioxane yielded D-(+)-glucose, which was identified by HPLC of the chiral tolylthiocarbamoyl thiazolidine derivative.[22] The ^{1}H (methanol-d_4) and ^{13}C NMR data (Table 1) of **1**, assigned via various NMR experiments,[23] showed resonances assignable to two phenylpropanoid moieties {two olefinic protons [δ 7.73 (s, H-7), 7.80 (s, H-7′)], seven aromatic protons [δ 6.68 (d, *J* = 8.6 Hz, H-5), 6.71 (d, *J* = 8.6 Hz, H-3′, -5′), 6.92 (dd, *J* = 8.6, 1.9 Hz, H-6), 7.07 (d, *J* = 1.9 Hz, H-2), 7.41 (d, *J* = 8.6 Hz, H-2′, -6′)], and two carbonyl carbons [δ_C 169.0, 169.0]}, two monoterpene units {two oxymethylenes [δ 3.86 (2H, m, H-10″a, -10‴a), 3.96 (2H, m, H-10″b, -10‴b)], two oxymethines [δ 3.70 (2H, m, H-2″, 2‴)], four olefinic protons [δ 4.56 (2H, d, *J* = 6.2 Hz, H-8″, -8‴), 6.42 (2H, d, *J* = 6.2 Hz, H-9″, -9‴)], two formyl protons [δ 9.76 (2H, s, H-7″, -7‴)]}, and two β-D-glucopyranosyl moieties [δ 4.46 (2H, d, *J* = 7.8 Hz, H-1⁗, 1⁗′)]. The DQF COSY and HMBC experiments indicated **1** to be a lignan derivative with two glucosylated monoterpenoid moieties. The linkages of the phenylpropenoic acid moieties as well as the locations of the monoterpene and glucopyranosyl moieties were confirmed by HMBC spectroscopy. Correlations were observed between H-7 and C-8′, 9; H-7′ and C-8, 9′; H-10″ and C-9; H-10‴ and C-9′; H-1⁗ and C-9″; and H-1⁗′ and C-9‴. The geometry of the olefinic bonds of **1** was characterized by NOESY experiments of lignan **1e** derived from **1** (Scheme 1). Specifically, catalytic reduction of **1** yielded **1a**, which upon treatment with $NaBH_4$ afforded **1b**. Enzymatic hydrolysis of **1b** with hesperidinase gave **1c**, which was treated with trimethylsilyldiazomethane ($TMSCH_2N_2$) followed by ester hydrolysis with NaOMe to give lignan **1e**. NOESY experiments of **1e** showed correlations between H-7 and H-2′, -6′ and between H-7′ and H-2, -6. Therefore, the geometries of both olefinic groups were *E*. In addition, the known monoterpene glucoside **1h**[3] was obtained from **1b** by methylation and ester hydrolysis, hence defining the structure of the monoterpenoid moiety. Collectively, the data

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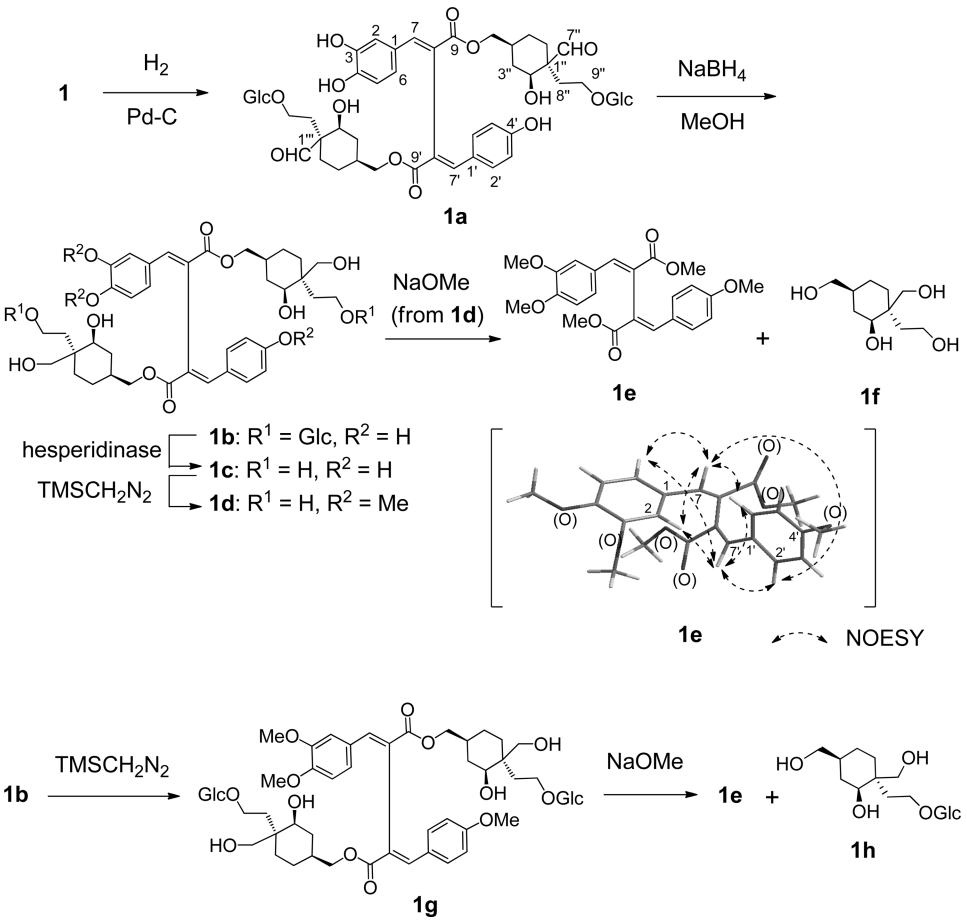
Scheme 1



permitted assignment of the structure of canangalignan I (**1**) as shown.

Canangalignan II (**2**), obtained as an amorphous powder with a positive specific rotation ($[\alpha]^{25}_{D}$ +33 in MeOH), showed absorption bands due to hydroxy, ester, formyl, aromatic ring, and ether functionalities in the IR spectrum. Positive-ion FABMS revealed a quasimolecular ion $[M + Na]^+$ at m/z 1053, from which the molecular formula $C_{50}H_{62}O_{23}$ was determined via HRMS and ^{13}C NMR data. Acid hydrolysis of **2** yielded D-(+)-glucose.[22] The ^1H (methanol-d_4) and ^{13}C NMR data (Table 1) of **2**, assigned via various NMR experiments,[23] showed resonances assignable to a lignan moiety {two methines [δ 3.80 (d, J = 2.2 Hz, H-8′), 4.37 (d, J = 2.2 Hz, H-7′)], an olefinic proton [δ 7.52 (s, H-7)], and six aromatic protons [δ 6.46 (s, H-3), 6.59 (d, J = 8.5 Hz, H-3′, -5′), 6.78 (d, J = 8.5 Hz, H-2′, -6′), 6.80 (s, H-6)], and two carbonyl carbons [δ_C 168.5, 174.5]} together with a monoterpene glucoside unit. The proton and carbon resonances in the ^1H and ^{13}C NMR data of **2** were superimposable on those of **3**, except for the resonances around C-3 and C-5 of **2**. The DQF COSY and HMBC experiments indicated **2** to be a lignan derivative with the same monoterpene glucoside moieties as **1**. The absolute configurations at C-7′ and C-8′ in **2** were confirmed by electronic circular dichroism (ECD). The ECD spectrum (MeOH) of **2** was identical to that of the known compound **3**.[24] Both **2** and **3** showed a positive Cotton effect at 257 nm indicative of a (7′*R*, 8′*S*) absolute configuration. Thus, the structure of canangaligan II (**2**) was defined as shown.

Canangaterpene I (**5**) was isolated as an amorphous powder with a negative specific rotation ($[\alpha]^{25}_{D}$ −2, in MeOH). Its IR spectrum showed absorption bands at 3400, 1716, and 1508 cm^{-1} ascribable to hydroxy, ester, and aromatic ring functionalities, respectively. EIMS revealed a molecular ion $[M]^+$ at m/z 408, from which the molecular formula $C_{21}H_{28}O_8$ was determined via HRMS and ^{13}C NMR data. The ^1H NMR (methanol-d_4) and ^{13}C NMR data (Table 2) of **5**[23] showed resonances assignable to a caffeoyl group [δ 6.23 (d, J = 15.5 Hz, H-8), 6.76 (d, J = 8.3 Hz, H-5), 6.92 (d, J = 8.3 Hz, H-6), 7.03 (s, H-2), 7.51 (d, J = 15.5 Hz, H-7)] and a monoterpenoid moiety {an oxymethylene [δ 4.03 (2H, m, H_2-11′)], three oxymethines [δ 3.39 (m, H-6′), 4.89 (s, H-1′), 5.07 (dd, J = 4.2, 6.2 Hz, H-3′)], and two methoxy groups [δ 3.37 (s, 3′-OMe), 3.38 (s, 1′-OMe)]}. As shown in Figure 1 the DQF COSY experiments indicated the presence of partial structures (written in bold), and in the HMBC experiments, correlations were observed between H-2 and C-7, -3; H-6 and C-7, -1, -4; H-7 and C-2; H-1′ and C-5′; H-3′ and C-5′; H-6′ and C-8′; H-7′ and C-9′, -11′; H-8′ and C-6′; H-9′ and C-11′; H-10′ and C-5′; H-11′ and C-9; and $OC\underline{H}_3 \times 2$ and C-1′, -3′. The molecular structure was the same as that of the known compound **6**. The relative configuration of the monoterpenoid moiety **5** was characterized by NOESY (Figure 2). Correlations were observed between H-4′β and H-3′; H-6′ and H-4′β, -7′α, -8′; and H-8′ and H-7′α, -9′α. On the basis of all this evidence, canangaterpene I (**5**) was characterized to be a monoterpene analogue possessing a rare oxaspiro[4.5]decane skeleton similar to **6**. Compound **6** might be formed from an oxaspiro[4.5]-

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Table 2. ^{13}C NMR (125 MHz) Spectroscopic Data for 5, 7, and 8

position	5[a]	position	7[b]	position	8[b]
1	127.7	1	45.1	1	45.8
2	115.1	2	82.8	2	19.6
3	146.8	3	44.5	3	22.5
4	149.6	4	38.4	4	140.7
5	116.6	5	50.4	5	154.8
6	123.0	6	20.9	6	35.9
7	146.9	7	33.1	7	43.8
8	115.2	8	34.6	8	19.6
9	169.2	9	74.8	9	34.5
1′	106.7	10	27.5	10	71.9
3′	107.6	11	26.6	11	27.3
4′	44.8	12	35.5	12	21.4
5′	53.2	13	31.6	13	15.6
6′	76.8	14	25.4	14	29.5
7′	36.3	15	28.2	15	194.5
8′	37.5	1′	130.8		
9′	27.3	2′	129.7		
10′	32.4	3′	128.3		
11′	69.6	4′	132.7		
7′-OMe	55.5	5′	128.3		
9′-OMe	55.7	6′	129.7		
		1′-COO	166.3		

[a]Measured in methanol-d_4. [b]$CDCl_3$.

decane with hydroxy groups at C-1′ and C-3′ during extraction with MeOH.[3]

Canangaterpene II (**7**), obtained as colorless crystals with a positive specific rotation ($[\alpha]^{25}_D$ +25 in MeOH), showed absorption bands due to hydroxy, ester, and aromatic ring functionalities in the IR spectrum. EIMS revealed a molecular ion $[M]^+$ at m/z 342, from which the molecular formula $C_{22}H_{30}O_3$ was determined via HRMS and ^{13}C NMR data. The ^{1}H ($CDCl_3$) and ^{13}C NMR (Table 2) data of **7**[23] showed resonances assignable to three methyl groups [δ 0.96 (3H, s, H-15), 0.98 (3H, s, H-14), 1.11 (3H, s, H-13)], two oxymethines [δ 3.34 (1H, m, H-9), 5.09 (1H, dd, J = 6.2, 8.2 Hz, H-2)], and five aromatic protons [δ 7.43 (2H, dd, J = 7.6, 7.6 Hz, H-3′, -5′), 7.55 (1H, t, J = 7.6 Hz, H-4′), 8.03 (2H, d, J = 7.6 Hz, H-2′, -6′)]. The DQF COSY and HMBC experiments indicated **7** to be a clovane-type sesquiterpene with a benzoyl group. The C-2 location of the benzoate group was confirmed by the HMBC correlations between H-2 and the carbonyl carbon. The relative configuration was characterized by the observed NOE correlations between H-2 and H-3β, -5; H-3α and H_3-14; H-5 and H-11β, -13; H-9 and H-11α, -12β, -15; and H-11α and H-12β (Figure 2). The C-9 absolute configuration was determined by application of the modified Mosher's method. Treatment of **7** with (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(−)-MTPA-Cl] in pyridine yielded the (*S*)-MTPA ester **7a**. The (*R*)-MTPA ester **7b** was derived from **7** by treatment with (+)-MTPA-Cl in pyridine. As shown in Figure 2, the resonances due to the C-10 and C-11 protons in **7a** were deshielded compared to those of **7b** [$\Delta\delta$: positive], while the resonances due to the protons C-6, C-7, C-12, and C-15 of the (*S*)-MTPA ester (**7a**) were shielded compared to those of the (*R*)-MTPA ester (**7b**) [$\Delta\delta$: negative]. Thus, the C-9 absolute configuration in **7** was defined as *R*. Thus, the structure of canangaterpene II (**7**) was defined as (1*R*,2*S*,5*R*,8*S*,9*R*)-2-benzoylclovan-2,9-diol.

Canangaterpene III (**8**), obtained as a white powder with a positive specific rotation ($[\alpha]^{25}_D$ +25 in MeOH), showed absorption bands due to hydroxy and formyl functionalities in the IR spectrum. EIMS revealed a molecular ion $[M]^+$ at m/z 236, from which the molecular formula $C_{15}H_{24}O_2$ was determined via HRMS and ^{13}C NMR data. The ^{1}H ($CDCl_3$) and ^{13}C NMR data (Table 2) of **8**[23] showed resonances

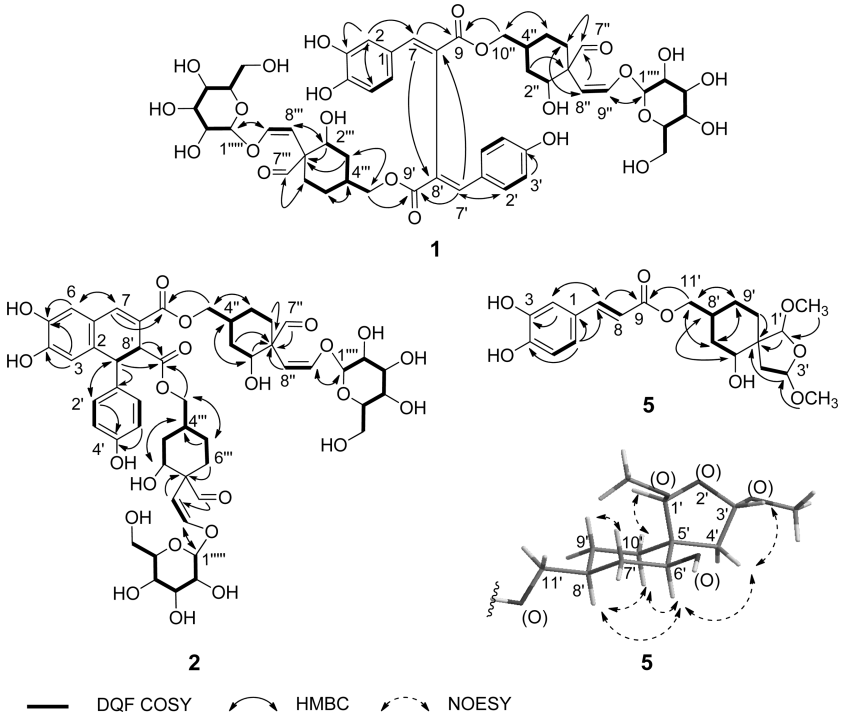

Figure 1.

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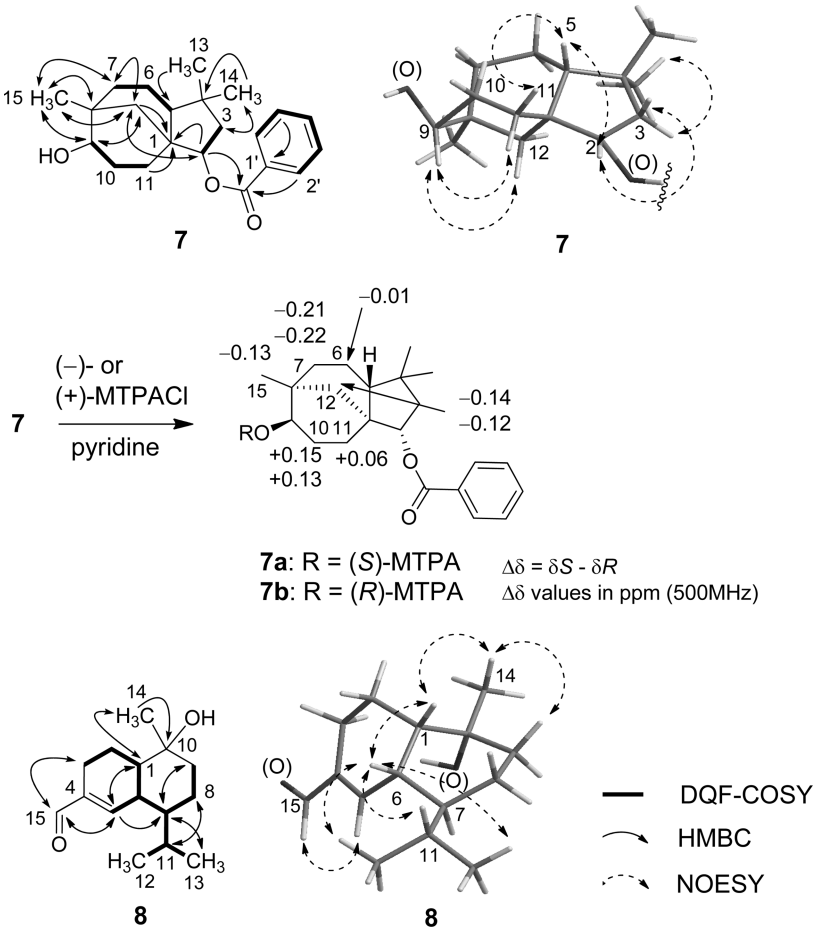

Figure 2.

assignable to three methyl groups [δ 0.91, 0.95 (3H each, both d, J = 6.7 Hz, H_3-12, -13), 1.25 (3H, s, H_3-14)], an olefinic proton [δ 6.96 (1H, d, J = 5.7 Hz, H-5)], and a formyl proton [δ 9.44 (1H, s, H-15)]. DQF COSY and HMBC experiments indicated **8** to be similar to the cadinane-type sesquiterpenes 15-oxo-α-cadinol (**9**) and 15-oxo-T-muurolol. The relative configuration of **8** was characterized by NOESY correlations between H-1 and H-6, H_3-14 and between H-6 and H-11, H_3-12, -13 (Figure 2). Therefore, the structure of canangaterpene III (**8**) was defined as shown.

Melanin production, which is principally responsible for skin color, is a major defense mechanism against the harmful ultraviolet rays in sunlight. However, excess production of melanin after long periods of exposure to the sun can cause dermatological disorders such as melasma, freckles, post-inflammatory melanoderma, and solar lentigo. We previously reported that several constituents from natural medicines and medicinal foods inhibited melanogenesis in theophylline-stimulated B16 melanoma 4A5 cells.[8,9,11−13,25−29] As a continuation of these studies, the inhibitory effects of constituents from the flower buds of *C. odorata* on melanogenesis were examined (Table 3). Terpenoid derivatives **5**, **6**, **8**, **9**, and **11**−**14** inhibited melanogenesis [inhibition: 28.9−45.5% (p < 0.01) at 1 μM]. In particular, compounds **5** (IC_{50} = 3.6 μM) and **12** (IC_{50} = 2.5 μM) exhibited considerable inhibitory effects on melanogenesis without inducing cytotoxicity [cell viability: more than 95.2% at 30 μM]. Indeed, **5** and **12** displayed greater potency for inhibiting melanogenesis than the control, arbutin (IC_{50} = 174 μM). Compounds **9** and **12**, with a *trans*-fused ring junction, showed stronger inhibitory effects than compounds **8** and **11**, with a *cis*-fused junction. Compound **12**, with a C-2 carbonyl, displayed a stronger inhibitory effect than compound **10**, with a C-2 hydroxy group. By contrast, lignan **1c**, derived from **1**, showed moderate inhibitory activity. However, lignans **1a**, **1b**, **1d**, and **1e** displayed either no inhibitory action or only a very weak inhibitory effect on melanogenesis. These findings indicate that a catechol moiety and structures lacking glucosyl moieties are important for the melanogenesis inhibitory activity of the lignans studied here.

In conclusion, two new lignan carboxylates, canangalignans I (**1**) and II (**2**), and three new terpenoids, canangaterpenes I (**5**), II (**7**), and III (**8**), were isolated from the dried flower buds of *C. odorata* cultivated in Thailand. Compounds **5** and **12** exhibited significant inhibitory effects on melanogenesis without inducing cytotoxicity. Therefore, compounds **5** and **12** are potential therapeutic agents for the treatment of several skin disorders. Further structure activity relationship studies and elucidation of the inhibitory mechanism are warranted.

EXPERIMENTAL SECTION

General Experimental Procedures. The following instruments were used to obtain physical data: optical rotations, a Horiba SEPA-300 digital polarimeter (l = 5 cm); IR spectra, a Shimadzu FTIR-8100 spectrometer; ECD spectra, a JASCO J-720WI spectrometer; FABMS and HRFABMS, a JEOL JMS-SX 102A mass spectrometer; EIMS and HREIMS, a JEOL JMS-GCMATE mass spectrometer; ^{1}H NMR spectra, a JEOL JNM-LA 500 (500 MHz); ^{13}C NMR spectra, a JEOL

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Table 3. Inhibitory Effects of Constituents and Their Derivatives from the Flower Buds of *Cananga odorata* on Melanogenesis in B16 Melanoma 4A5 Cells[a]

	inhibition (%)						IC_{50}
	control	1 μM	3 μM	10 μM	30 μM	100 μM	(μM)
1[b]	0.0 ± 2.1	28.6 ± 1.6**	30.2 ± 2.2**	31.8 ± 1.1**	34.8 ± 1.8**	16.7 ± 1.9**	>100
2[c]	0.0 ± 4.0	9.2 ± 3.8	8.7 ± 2.1	3.6 ± 5.8	2.9 ± 4.1	8.7 ± 4.6	>100
3[c]	0.0 ± 5.2	12.8 ± 3.7	14.9 ± 2.8	9.4 ± 5.0	8.4 ± 3.7	−27.9 ± 5.9	>100
4[b]	0.0 ± 4.2	−3.8 ± 3.0	−8.5 ± 4.1	9.3 ± 5.5	11.4 ± 3.4	12.2 ± 5.6	>100
5[b]	0.0 ± 6.8	34.7 ± 4.2**	42.8 ± 2.2**	65.2 ± 2.6**	62.8 ± 0.8**	66.8 ± 0.5**	3.6
6[c]	0.0 ± 5.4	13.5 ± 2.3*	16.9 ± 3.7**	36.4 ± 2.6**	60.0 ± 0.8**	81.2 ± 0.6**	19.3
7[e]	0.0 ± 1.3	45.3 ± 1.1**	40.2 ± 1.4**	66.7 ± 0.8**			ca. 3
8[c]	0.0 ± 4.4	19.2 ± 3.8**	29.9 ± 4.1**	35.8 ± 1.0**	50.8 ± 1.7**	66.3 ± 1.5**	26.2
9[c]	0.0 ± 5.0	25.0 ± 3.1**	45.5 ± 1.0**	64.0 ± 2.7**	65.9 ± 3.0**	48.8 ± 1.4**	5.1
10[c]	0.0 ± 3.5	−4.4 ± 11.3	9.2 ± 5.6	14.7 ± 3.1	37.7 ± 4.2**	58.8 ± 1.3**	57.5
11[d]	0.0 ± 6.9	37.6 ± 6.3**	34.3 ± 6.9**	32.9 ± 7.7**	28.3 ± 7.8**	42.1 ± 2.6**	>100
12[b]	0.0 ± 7.8	45.5 ± 5.7**	50.3 ± 2.1**	57.6 ± 3.7**	68.5 ± 1.9**	80.6 ± 2.6**	2.5
13[d]	0.0 ± 4.8	42.3 ± 4.5**	39.7 ± 4.2**	53.8 ± 2.7**	54.7 ± 1.9**	59.2 ± 2.4**	8.4
14[b]	0.0 ± 7.3	28.9 ± 2.5**	30.5 ± 3.0**	40.6 ± 2.3**	61.2 ± 5.2**	62.2 ± 1.6**	17.3
1a[b]	0.0 ± 4.9	8.1 ± 5.1	18.0 ± 5.0*	18.4 ± 1.9*	24.2 ± 1.0**	29.8 ± 1.9**	>100
1b[c]	0.0 ± 4.7	22.3 ± 1.7**	27.2 ± 3.9*	27.3 ± 2.1*	32.3 ± 4.7**	25.3 ± 3.7**	>100
1c[b]	0.0 ± 2.5	36.1 ± 3.8**	45.0 ± 3.2*	59.0 ± 1.7**	49.8 ± 2.0**	44.2 ± 1.3**	ca. 5
1d[e]	0.0 ± 1.5	34.4 ± 2.5**	24.1 ± 2.3*	8.1 ± 5.4	−19.9 ± 6.7	−15.7 ± 5.0	
1e[d]	0.0 ± 5.1	49.0 ± 4.6**	48.4 ± 12.3	43.6 ± 1.4**	35.9 ± 2.5**	11.5 ± 2.8	
1f[b]	0.0 ± 4.1	45.0 ± 1.5**	55.1 ± 1.8**	55.9 ± 2.5**	51.6 ± 1.2**	25.6 ± 5.5	ca. 2
1g[b]	0.0 ± 1.3	14.8 ± 5.6	20.2 ± 3.1*	8.9 ± 2.6	−9.2 ± 7.2	−21.8 ± 4.7	
1h[c]	0.0 ± 2.1	16.1 ± 7.1	17.9 ± 3.2*	15.3 ± 5.0	−2.5 ± 6.5	−9.4 ± 3.8	

	inhibition (%)						IC_{50}
	control	10 μM	30 μM	100 μM	300 μM	1000 μM	(μM)
arbutin[f]	0.0 ± 1.4	10.6 ± 0.6**	20.4 ± 0.5**	38.1 ± 0.9**	61.5 ± 0.6**	83.7 ± 0.5**	174

[a]Each value represents the mean ± SEM (n = 4). Significantly different from the control group, *p < 0.05, **p < 0.01. IC_{50} values were determined graphically. [b]The cell viabilities at 30 μM are more than 95.2%. [c]The cell viabilities at 30 μM are more than 80.3%. [d]The cell viabilities at 30 μM are more than 74.5%. [e]Cytotoxicity was observed [cell viabilities: **7**: 75.9% at 10 μM, 7.9% at 30 μM; **1d**: 82.0% at 10 μM, 68.8% at 30 μM]. [f]Reference compound.

JNM-LA (125 MHz) spectrometer with TMS as internal standard; ^{1}HNMR spectra, JEOL JNM-ECA600 (600 MHz) spectrometers; and HPLC, SPD-10A*vp* fitted with UV−vis detectors. The following columns from Nacalai Tesque (Kyoto, Japan) were used: (i) for analytical purposes, a COSMOSIL 5C_{18}-MS-II 250 × 4.6 mm (5 μm i.d.) and (ii) for preparative purposes, a COSMOSIL 5C_{18}-MS-II 250 × 20 mm (5 μm i.d.). The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography (CC), silica gel BW-200 (Fuji Silysia Chemical, Ltd., Kasugai, Japan; 150−350 mesh); reversed-phase silica gel CC, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100−200 mesh); TLC, precoated TLC plates with silica gel 60F_{254} (Merck, Darmstadt, Germany; 0.25 mm) (ordinary phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, precoated TLC plates with silica gel RP-18 WF_{254S} (Merck, 0.25 mm). Detection was achieved by spraying with 1% $Ce(SO_4)_2$−10% aqueous H_2SO_4 followed by heating.

Plant Material. Flower buds of *C. odorata* cultivated in Thailand were purchased from Mae Chu Co. Ltd. (Nara, Japan) in 2012 and identified by one of the authors (M.Y.). A voucher specimen is on file in our laboratory (KPU CO-2012-1).

Extraction and Isolation. The dried flower buds (4 kg) were extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a MeOH extract (1575 g, 39.4%). A part of the MeOH extract (210 g) was partitioned into an EtOAc−H_2O (1:1, v/v) mixture to furnish an EtOAc-soluble fraction (52 g, 9.8%) and an aqueous phase. The aqueous phase was further extracted with 1-butanol to give a 1-butanol-soluble fraction (100 g, 18.8%) and an H_2O-soluble fraction (58 g, 10.9%). The 1-butanol-soluble fraction (100 g) was subjected to normal-phase silica gel column chromatography [2.5 kg, $CHCl_3$−MeOH (1:0 → 50:1 → 10:1 → 5:1 → 1:1 → 1:2, v/v) → MeOH] to give nine fractions [Fr.B1 (1.4 g), Fr.B2 (6.2 g), Fr.B3 (1.2 g), Fr.B4 (6.6 g), Fr.B5 (8.4 g), Fr.B6 (6.2 g), Fr.B7 (30.1 g), Fr.B8 (6.8 g), Fr.B9 (2.3 g)]. Fraction B7 (30.1 g) was further separated by reversed-phase silica gel column chromatography [1 kg, MeOH−H_2O (3:7 → 4:6 → 5:5 → 6:4 → 7:3, v/v) → MeOH] to give eight fractions [Fr.B7-1, Fr.B7-2, Fr.B7-3 (6.2 g), Fr.B7-4 (573 mg), Fr.B7-5, Fr.B7-6, Fr.B7-7, Fr.B7-8]. Fraction B7-3 (6.2 g) was purified by reversed-phase silica gel column chromatography [180.0 g, MeOH−H_2O (1:9 → 2:8 → 3:7 → 4:6 → 5:5, v/v) → MeOH] to give six fractions [Fr.B7-3-1, Fr.B7-3-2, Fr.B7-3-3, Fr.B7-3-4, Fr.B7-3-5 (1.5 g), Fr.B7-3-6]. Fraction B7-3-5 (1.5 g) was purified by HPLC [mobile phase: H_2O−MeOH (80:20, v/v)] to give **1** (275.5 mg), **2** (8.1 mg), **3** (20.5 mg), and **4** (21.3 mg). A part of the EtOAc-soluble fraction (50.0 g) was subjected to normal-phase silica gel column chromatography [3.0 kg, *n*-hexane → *n*-hexane−$CHCl_3$ (5:1 → 2:1, v/v) → $CHCl_3$−MeOH (200:1 → 100:1 → 50:1 → 10:1 → 3:1, v/v) → MeOH] to give seven fractions [Fr.E1 (7.5 g), Fr.E2 (3.9 g), Fr.E3 (3.6 g), Fr.E4 (27.6 g), Fr.E5 (6.6 g), Fr.E6 (16.8 g), Fr.E7 (10.0 g)]. Fraction E4 (27.6 g) was separated by reversed-phase silica gel column chromatography [900.0 g, MeCN−H_2O (4:6 → 5:5 → 6:4 → 7:3 → 8:2 → 9:1, v/v) → MeCN] to give seven fractions [Fr.E4-1, Fr.E4-2 (0.73g), Fr.E4-3 (1.0 g), Fr.E4-4 (0.84g), Fr.E4-5, Fr.E4-6 (1.5 g), Fr.E4-7]. Fraction E4-2 (0.73 g) was purified by HPLC [mobile phase: H_2O−MeCN−HOAc (650: 350: 3, v/v/v)] to give **13** (10.0 mg). Fraction E4-3 (1.0 g) was purified by HPLC [mobile phase: H_2O−MeOH−HOAc (800:200:3, v/v/v)] to give **8** (6.3 mg), **9** (15.2 mg), **10** (14.8 mg), and **11** (8.9 mg). Fraction E4-4 (0.84 g) was purified by HPLC [mobile phase: H_2O−MeOH−HOAc (500:500:3, v/v/v)] to give **12** (22.3 mg) and **14** (29.2 mg). Fraction E4-6 (1.5 g) was purified by HPLC [mobile phase: H_2O−MeOH−HOAc (320:680:3, v/v/v)] to give **7** (8.9 mg). Fraction E5 (6.6 g) was

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separated by reversed-phase silica gel column chromatography [500.0 g, MeCN−H_2O (1:9 → 2:8 → 3:7 → 4:6 → 5:5 → 6:4 → 7:3 → 8:2 → 9:1, v/v) → MeCN] to give seven fractions [Fr.E5-1, Fr.E5-2, Fr.E5-3, Fr.E5-4, Fr.E5-5 (40.7 mg), Fr.E5-6, Fr.E5-7]. Fraction E6 (16.8 g) was separated by reversed-phase silica gel column chromatography [500 g, MeOH−H_2O (1:9 → 2:8 → 3:7 → 4:6 → 5:5 → 6:4 → 7:3 → 8:2 → 9:1, v/v) → MeOH] to give seven fractions [Fr.E6-1, Fr.E6-2, Fr.E6-3, Fr.E6-4 (1.2 g), Fr.E6-5, Fr.E6-6 (1.6 g), Fr.E6-7]. Fraction E6-4 (1.2 g) was separated by normal-phase silica gel column chromatography [1.2 g, *n*-hexane−EtOAc (2:1 → 1:1 → 1:2, v/v) → EtOAc−MeOH (1:1, v/v) → MeOH] to give five fractions [Fr.E6-4-1, Fr.E6-4-2, Fr.E6-4-3 (130 mg), Fr.E6-4-4 (225 mg), Fr.E6-4-5 (399 mg)]. Fraction E6-4-5 (399 mg) was purified by HPLC [H_2O−MeCN−HOAc (670:330:3, v/v/v)] to give **5** (14.0 mg) and **6** (8.2 mg).

*Canangalignan I (**1**):* amorphous powder; $[\alpha]^{25}_D$ +25 (*c* 0.6, MeOH); UV (MeOH) λ_{max} 200.0 nm (log ε 4.20), 314.0 nm (log ε 4.22); IR (KBr) ν_{max} 3400, 1703, 1682, 1601, 1512, 1075 cm^{-1}; ^1H NMR (methanol-d_4, 600 MHz) δ 1.21 (2H, m, H-6″α, -6‴α), 1.28 (2H, m, H-5″β, -5‴β), 1.62 (2H, m, H-4″, -4‴), 1.76 (2H, m, H-3″α, -3‴α), 2.29 (2H, dd-like, H-6″β, -6‴β), 3.24 (2H, m, H-2⁗, -2⁗′), 3.30 (4H, m, H-5″α, -5‴α, -4⁗, -4⁗′), 3.34 (4H, m, H-3″β, -3‴β, -5⁗, -5⁗′), 3.70 (2H, m, H-2″, -2‴), 3.84 (4H, d, *J* = 12.4 Hz, H-6⁗, -6⁗′), 3.86 (2H, m, H-10″a, -10‴a), 3.96 (2H, m, H-10″b, -10‴b), 4.46 (2H, d, *J* = 7.8, H-1⁗, -1⁗′), 4.56 (2H, d, *J* = 6.2 Hz, H-8″, -8‴), 6.42 (2H, d, *J* = 6.2, H-9″, -9‴), 6.68 (1H, d, *J* = 8.6 Hz, H-5), 6.71 (2H, d, *J* = 8.6 Hz, H-3′, -5′), 6.92 (1H, dd, *J* = 1.9, 8.6 Hz, H-6), 7.07 (1H, d, *J* = 1.9 Hz, H-2), 7.41 (2H, d, *J* = 8.6 Hz, H-2′, -6′), 7.73 (1H, s, H-1), 7.80 (1H, s, H-7′), 9.76 (2H, s, H-7″, -7‴); ^{13}C NMR (methanol-d_4, 125 MHz) δ given in Table 1; positive-ion FABMS *m*/*z* 1053 [M + Na]$^+$; HRFABMS *m*/*z* 1053.3580 (calcd for $C_{50}H_{62}O_{23}Na$ [M + Na]$^+$, 1053.3574).

*Canangalignan II (**2**):* amorphous powder; $[\alpha]^{25}_D$ +33 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 226.0 nm (log ε 4.20), 331.0 nm (log ε 4.33); IR (KBr) ν_{max} 3400, 1701, 1685, 1508, 1075 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz) δ 1.21 (2H, m, H-6″α, -6‴α), 1.27 (2H, m, H-5″β, -5‴β), 1.62 (2H, m, H-4″, -4‴), 1.87 (2H, m, H-3″α, -3‴α), 2.27 (2H, m, H-6″β, -6‴β), 3.22 (2H, m, H-2⁗, -2⁗′), 3.27 (4H, m, H-5″α, -5‴α, -4⁗, -4⁗′), 3.30 (4H, m, H-3″β, -3‴β, -5⁗, -5⁗′), 3.60 (2H, m, H-2″, -2‴), 3.80 (4H, d, *J* = 10.4 Hz, H-6⁗, -6⁗′), 3.82 (2H, m, H-10″b, -10‴b), 3.86 (2H, m, H-10″a, -10‴a), 4.42 (2H, d, *J* = 7.8, H-1⁗, -1⁗′), 4.56 (2H, d, *J* = 6.5 Hz, H-8″, -8‴), 6.38 (2H, d, *J* = 6.5, H-9″, -9‴), 6.46 (1H, s, H-3), 6.59 (2H, d, *J* = 8.5 Hz, H-3′, -5′), 6.78 (2H, d, *J* = 8.5 Hz, H-2′, -6′), 6.80 (1H, s, H-6), 7.52 (1H, s, H-7), 9.74, 9.77 (2H, s, H-7″, -7‴); ^{13}C NMR (methanol-d_4, 125 MHz) δ given in Table 1; ECD $\Delta\varepsilon$ (nm) −1.6 (203), −2.1 (230), +2.2 (257), −0.2 (293), +0.1 (311), −2.7 (340) (*c* 8.00 × 10^{-5} *m*, MeOH); positive-ion FABMS *m*/*z* 1053 [M + Na]$^+$; HRFABMS *m*/*z* 1053.3573 (calcd for $C_{50}H_{62}O_{23}Na$ [M + Na]$^+$, 1053.3574).

*Canangaterpene I (**5**):* amorphous powder; $[\alpha]^{25}_D$ −2 (*c* 0.4, MeOH); IR (film) ν_{max} 3400, 1716, 1508, 1456 cm^{-1}; ^1H NMR (methanol-d_4, 500 MHz) δ 1.11 (m, H-9′β), 1.26 (m, H-10′α), 1.33 (m, H-7′β), 1.52 (m, H-9′α), 1.68 (dd, *J* = 4.2, 6.2 Hz, H-4′α), 1.84 (m, H-8′), 1.91 (m, H-7′α), 2.12 (m, H-10′β), 2.22 (dd, *J* = 4.2, 6.2 Hz, H-4′β), 3.37 (s, 3′-OMe), 3.38 (s, 1′-OMe), 3.39 (m, H-6′), 4.03 (dd, *J* = 4.2, 6.2 Hz, H-11′), 4.89 (s, H-1′), 5.07 (dd, *J* = 4.2, 6.2 Hz, H-3′), 6.23 (d, *J* = 15.5 Hz, H-8), 6.76 (d, *J* = 8.3 Hz, H-5), 6.92 (d, *J* = 8.3 Hz, H-6), 7.03 (s, H-2), 7.51 (d, *J* = 15.5 Hz, H-7); ^{13}C NMR given in Table 1; EIMS *m*/*z* 408 [M]$^+$; HREIMS *m*/*z* 408.1788 (calcd for $C_{21}H_{28}O_8$ [M]$^+$: *m*/*z* 408.1784).

*Canangaterpene II (**7**):* colorless crystals (*n*-hexane−$CHCl_3$); mp 181−185 °C; $[\alpha]^{25}_D$ +25 (*c* 0.5, MeOH); IR (film) ν_{max} 3400, 1716, 1508 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.96 (3H, s, H-15), 0.98 (3H, s, H-14), 1.11 (3H, s, H-13), 1.26 (1H, m, H-10β), 1.40 (1H, m, H-6β), 1.46 (1H, m, H-7β), 1.53 (1H, m, H-6α), 1.58 (1H, m, H-12α), 1.59 (1H, m, H-5), 1.64 (1H, m, H-11α), 1.65 (1H, m, H-12β), 1.68 (1H, m, H-3α), 1.69 (1H, m, H-10α), 1.92 (1H, dd, *J* = 6.2, 8.2 Hz, H-3β), 2.03 (1H, m, H-11β), 2.30 (1H, m, H-7α), 3.34 (1H, m, H-9), 5.09 (1H, dd, *J* = 6.2, 8.2, H-2), 7.43 (2H, dd, *J* = 7.6, 7.6 Hz, H-3′, -5′), 7.55 (1H, dd, *J* = 7.6, 7.6, H-4′), 8.03 (2H, t, *J* = 7.6 Hz, H-2′, -6′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ given in Table 2; EIMS *m*/*z* 342 [M]$^+$; HREIMS *m*/*z* 342.21981 (calcd for $C_{22}H_{30}O_3$ [M]$^+$, 342.21948).

*Canangaterpene III (**8**):* white powder; $[\alpha]^{25}_D$ −21 (*c* 0.5, MeOH); IR (film) ν_{max} 3410, 1684, 1509 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.91 (3H, d, *J* = 6.7 Hz H-12), 0.95 (3H, d, *J* = 6.7, H-13), 1.25 (3H, s, H-14), 1.39 (1H, m, H-7), 1.46 (2H, m, H-2β, -8β), 1.51 (1H, m, H-9α), 1.52 (2H, m, H-2α, -8α), 1.63 (1H, m, H-9β), 1.75 (1H, m, H-1), 1.96 (1H, m, H-11), 2.43 (1H, dd, *J* = 5.7, 17.6 Hz, H-3α), 2.47 (1H, m, H-3β), 2.76 (1H, m, H-6), 6.96 (1H, d, *J* = 5.7 Hz, H-5), 9.44 (1H, s, H-15); ^{13}C NMR ($CDCl_3$, 125 MHz) δ given in Table 2; EIMS *m*/*z* 236 [M]$^+$; HREIMS *m*/*z* 236.1780 (calcd for $C_{15}H_{24}O_2$ [M]$^+$, 236.1776).

Catalytic Hydrogenation of Canangalignan I (1). Canangalignan I (**1**) (178 mg, 0.17 mmol) was dissolved in MeOH (16 mL) and reduced with 10% Pd−C (10 mg) under a H_2 atmosphere for 18 h at room temperature. The mixture was filtered, and the filtrate evaporated to give **1a** (178 mg, quant.).

1a: amorphous powder; $[\alpha]^{25}_D$ −27 (*c* 0.2, MeOH); UV (MeOH) λ_{max} 207.0 nm (log ε 4.22), 316.0 nm (log ε 4.33); ^1H NMR (methanol-d_4, 500 MHz) δ 1.02 (2H, m, H-5″β, -5‴β), 1.06 (2H, m, H-8″a, -8‴a), 1.08 (2H, m, H-6″α, -6‴α), 1.18 (2H, m, H-5″α, -5‴α), 1.48 (2H, m, H-4″, -4‴), 1.69−1.72 (4H, m, H-3″α, -3‴α, -H-8″b, -8‴b), 1.90 (2H, m, H-6″β, -6‴β), 2.12 (2H, m, H-3″β, -3‴β), 3.05 (2H, t, *J* = 9.0 Hz, H-2⁗, -2⁗′), 3.16−3.18 (4H, m, H-3⁗, -3⁗′, -4⁗, -4⁗′), 3.25 (2H, m, H-5⁗, -5⁗′), 3.46 (2H, m, H-9″a, -9‴a), 3.51 (2H, m, H-2″, -2‴), 3.55 (2H, m, H-6⁗a, -6⁗′a), 3.76 (4H, m, H-6⁗b, -6⁗′b, -10″a, -10‴a), 3.84−3.90 (4H, m, H-9″b, -9‴b, -10″b, -10‴b), 4.12 (2H, d, *J* = 7.75 Hz, H-1⁗, -1⁗′), 6.61 (1H, d, *J* = 8.6 Hz, H-5), 6.62 (2H, d, *J* = 8.6 Hz, H-3′, -5′), 6.84 (1H, dd, *J* = 2.1, 8.6 Hz, H-6), 6.97 (1H, d, *J* = 2.1 Hz, H-2), 7.33 (2H, d, *J* = 8.6 Hz, H-2′, -6′), 7.63 (1H, s, H-7), 7.70 (1H, s, H-7′), 9.71 (2H, s, H-7″, -7‴); ^{13}C NMR (methanol-d_4, 125 MHz) δ 128.1 (C-1), 117.5 (C-2), 146.5 (C-3), 149.3 (C-4), 116.4 (C-5), 124.7 (C-6), 144.5 (C-7), 124.9 (C-8, -8′), 169.0 (C-9, -9′), 127.6 (C-1′), 133.1 (C-2′, -6′), 116.6 (C-3′, -5′), 160.7 (C-4′), 144.1 (C-7′), 59.3 (C-1″, -1‴), 75.1 (C-2″, -2‴), 35.6 (C-3″, -3‴), 37.5 (C-4″, -4‴), 26.2 (C-5″, -5‴), 30.1 (C-6″, -6‴), 210.0 (C-7″, -7‴), 36.6 (C-8″, -8‴), 66.5 (C-9″, -9‴), 70.1 (C-10″, -10‴), 104.4 (C-1⁗, -1⁗′), 75.1 (C-2⁗, -2⁗′), 78.1 (C-3⁗, -3⁗′), 71.6 (C-4⁗, -4⁗′), 77.9 (C-5⁗, -5⁗′), 62.8 (C-6⁗, -6⁗′); positive-ion FABMS *m*/*z* 1057 [M + Na]$^+$; HRFABMS *m*/*z* 1057.3885 (calcd for $C_{50}H_{66}O_{23}Na$ [M + Na]$^+$, 1057.3893).

$NaBH_4$ Reduction of 1a. To a solution of **1a** (174 mg, 0.17 mmol) in MeOH (16 mL) was added $NaBH_4$ (13 mg, 0.34 mmol), and the mixture was stirred for 2 h at room temperature. Saturated aqueous NH_4Cl was added, and the solution was evaporated. The residue was subjected to normal-phase silica gel column chromatography [$CHCl_3$−MeOH] and purified by HPLC [mobile phase: H_2O−MeOH (50:50, v/v)] to give **1b** (115 mg, 66%).

1b: amorphous powder; $[\alpha]^{25}_D$ −13 (*c* 0.1, M.eOH); UV (MeOH) λ_{max} 204.2 nm (log ε 4.26),316.2 nm (log ε 4.36); ^1H NMR (methanol-d_4, 500 MHz) δ 1.02 (2H, m, H-5″β, -5‴β), 1.07 (2H, m, H-6″α, -6‴α), 1.25 (4H, m, H-3″α, -3‴α, -5″α, -5‴α), 1.27 (2H, m, H-6″β, -6‴β), 1.28 (2H, m, H-4″, -4‴), 1.65 (2H, m, H-3″β, -3‴β), 1.81 (2H, m, H-8″a, -8‴a), 1.88 (H-8″b, -8‴b), 3.16 (2H, t, *J* = 7.7 Hz, H-2⁗, -2⁗′), 3.28−3.29 (4H, m, H-3⁗, -3⁗′, -4⁗, -4⁗′), 3.34 (2H, m, H-5⁗, -5⁗′), 3.54 (2H, m H-9″b, -9‴b), 3.54−3.58 (4H, m, H-7″, -7‴), 3.56 (2H, m, H-2″, -2‴), 3.65 (2H, d-like, *J* = 11.6 Hz, H-6⁗a, -6⁗′a), 3.81 (2H, m H-9″a, -9‴a), 3.86 (2H, d-like, *J* = 11.6 Hz, H-6⁗b, -6⁗′b), 3.91 (2H, m, H-10″a, -10‴a), 3.96 (2H, m, H-10″b, -10‴b), 4.28 (2H, d, *J* = 7.75 Hz, H-1⁗, -1⁗′), 6.70 (1H, d, *J* = 8.0 Hz, H-5), 6.71 (2H, d, *J* = 8.0 Hz, H-3′, -5′), 6.92 (1H, dd, *J* = 2.1, 8.0 Hz, H-6), 7.07 (1H, d, *J* = 2.1 Hz, H-2), 7.41 (2H, d, *J* = 8.0 Hz, H-2′, -6′), 7.74 (1H, s, H-7), 7.81 (1H, s, H-7′); ^{13}C NMR (methanol-d_4, 125 MHz) δ 128.2 (C-1), 117.5 (C-2), 146.5 (C-3), 149.1 (C-4), 116.4 (C-5), 124.9 (C-6), 144.5 (C-7), 124.9 (C-8, -8′), 169.0 (C-9, -9′), 127.6 (C-1′), 133.1 (C-2′, -6′), 116.7 (C-3′, -5′), 160.7 (C-4′), 144.1 (C-7′), 42.0 (C-1″, -1‴), 76.4 (C-2″, -2‴), 34.5 (C-3″, -3‴), 37.8 (C-4″, -4‴), 25.1 (C-5″, -5‴), 31.6 (C-6″, -6‴), 76.4 (C-7″, -7‴), 37.1 (C-8″, -8‴), 63.4 (C-9″, -9‴), 70.3 (C-10″, -10‴), 104.2 (C-1⁗, -1⁗′),

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75.1 (C-2‴, -2‴′), 78.2 (C-3‴, -3‴′), 71.7 (C-4‴, -4‴′), 78.0 (C-5‴, -5‴′), 62.9 (C-6‴, -6‴′); positive-ion FABMS *m/z* 1061 $[M + Na]^+$; HRFABMS *m/z* 1061.4202 (calcd for $C_{50}H_{70}O_{23}Na$ $[M + Na]^+$, 1061.4206).

Enzymatic Hydrolysis of 1b. To a solution of **1b** (75 mg, 0.072 mmol) in 20 mM acetate buffer (20 mL, pH = 3.70) was added hesperidinase (10 mg, from *Penicillium* sp. Sigma-Aldrich), and the mixture was stirred for 12 h at 37 °C. EtOH was the added to each mixture, and the solution was centrifuged at 4000 rpm for 10 min. The supernatant solution was concentrated under vacuum to give a residue, which was subjected to HPLC [mobile phase: H_2O−MeCN (70:30, v/v)] to give **1c** (13.5 mg).

1c: amorphous powder; $[\alpha]^{25}_D$ −5 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 208.4 nm (log ε 4.19),316.4 nm (log ε 4.36); ^{1}H NMR (methanol-d_4, 500 MHz) δ 0.95 (2H, m, H-6″α, -6‴α), 1.06 (2H, m, H-5″β, -5‴β), 1.22 (4H, m, H-3″α, -3‴α, -5″α, -5‴α), 1.57 (2H, m, H-4″, -4‴), 1.64 (2H, m, H-8″a, -8‴a), 1.65 (2H, m, H-3″β, -3‴β), 1.71 (2H, m, H-6″β, -6‴β), 1.78 (H-8″b, -8‴b), 3.43 (2H, m, H-2″, -2‴), 3.57 (2H, d-like, *J* = 11.6 Hz, H-7″a, -7‴a), 3.65 (2H, d-like, *J* = 11.6 Hz, H-6″a, -6‴a), 3.67 (2H, m H-9″a, -9‴a), 3.72 (2H, m H-9″b, -9‴b), 3.79 (2H, d-like, *J* = 11.6 Hz, H-7″b, -7‴b), 3.94 (2H, m, H-10″a, -10‴a), 4.00 (2H, m, H-10″b, -10‴b), 6.68 (1H, d, *J* = 8.3 Hz, H-5), 6.70 (2H, d, *J* = 8.7 Hz, H-3′, -5′), 6.92 (1H, dd, *J* = 2.1, 8.3 Hz, H-6), 7.07 (1H, d, *J* = 2.1 Hz, H-2), 7.41 (2H, d, *J* = 8.7 Hz, H-2′, -6′), 7.74 (1H, s, H-7), 7.81 (1H, s, H-7′); ^{13}C NMR (methanol-d_4, 125 MHz) δ 128.0 (C-1), 117.5 (C-2), 146.6 (C-3), 149.4 (C-4), 116.4 (C-5), 124.8 (C-6), 144.4 (C-7), 124.9 (C-8, -8′), 169.2 (C-9, -9′), 127.6 (C-1′), 133.1 (C-2′, -6′), 116.7 (C-3′, -5′), 160.8 (C-4′), 144.1 (C-7′), 42.4 (C-1″, -1‴), 77.2 (C-2″, -2‴), 34.4 (C-3″, -3‴), 37.8 (C-4″, -4‴), 25.1 (C-5″, -5‴), 32.2 (C-6″, -6‴), 62.1 (C-7″, -7‴), 41.7 (C-8″, -8‴), 58.9 (C-9″, -9‴), 70.4 (C-10″, -10‴); positive-ion FABMS *m/z* 737 $[M + Na]^+$; HRFABMS *m/z* 737.3145 (calcd for $C_{38}H_{50}O_{13}Na$ $[M + Na]^+$, 737.3149).

Methylation of 1c. A solution of **1c** (12.0 mg) in dry MeOH (1.0 mL) was treated with trimethylsilyldiazomethane (2 M in diethyl ether, 0.3 mL), and the mixture was stirred for 15 h at room temperature. Removal of the solvent under reduced pressure gave a residue, which was purified by HPLC [mobile phase: H_2O−MeCN (70:30, v/v)] to give **1d** (11.5 mg).

1d: amorphous powder; $[\alpha]^{25}_D$ −3 (*c* 0.2, MeOH); UV (MeOH) λ_{max} 207.0 nm (log ε 4.14),312.8 nm (log ε 4.19); ^{1}H NMR (methanol-d_4, 500 MHz) δ 0.91 (2H, m, H-6″α, -6‴α), 1.01 (2H, m, H-5″β, -5‴β), 1.21 (2H, m, H-5″α, -5‴α), 1.24 (2H, m, H-3″α, -3‴α), 1.57 (2H, m, H-4″, -4‴), 1.65 (2H, m, H-8″a, -8‴a), 1.65 (2H, m, H-3″β, -3‴β), 1.71 (2H, m, H-6″β, -6‴β), 1.78 (H-8″b, -8‴b), 3.39 (2H, m, H-2″, -2‴), 3.59 (2H, m, H-7″a, -7‴a), 3.60 (2H, m H-9″b, -9‴b), 3.62 (3H, s, 3-OCH_3), 3.69 (2H, m H-9″a, -9‴a), 3.71 (3H, s, 4′-OCH_3), 3.74 (3H, s, 4-OCH_3), 3.81 (2H, d-like, *J* = 11.6 Hz, H-7″b, -7‴b), 3.94 (2H, m, H-10″a, -10‴a), 3.97 (2H, m, H-10″b, -10‴b), 6.79 (2H, d, *J* = 8.7 Hz, H-3′, -5′), 6.83 (1H, d, *J* = 8.7 Hz, H-5), 7.06 (1H, dd, *J* = 2.0, 8.7 Hz, H-6), 7.12 (1H, d, *J* = 2.0 Hz, H-2), 7.43 (2H, d, *J* = 8.7 Hz, H-2′, -6′), 7.77 (1H, s, H-7), 7.80 (1H, s, H-7′); ^{13}C NMR (methanol-d_4, 125 MHz) δ 128.6 (C-1), 113.5 (C-2), 150.3 (C-3), 152.3 (C-4), 112.6 (C-5), 125.9 (C-6), 143.7 (C-7), 125.9 (C-8), 168.9 (C-9), 129.0 (C-1′), 132.9 (C-2′, -6′), 115.3 (C-3′, -5′), 162.7 (C-4′), 143.5 (C-7′), 126.1 (C-8′), 168.7 (C-9′), 42.4 (C-1″, -1‴), 77.1 (C-2″, -2‴), 34.5 (C-3″, -3‴), 37.8 (C-4″, -4‴), 25.0 (C-5″, -5‴), 32.2 (C-6″, -6‴), 61.9 (C-7″, -7‴), 41.7 (C-8″, -8‴), 58.9 (C-9″, -9‴), 70.5 (C-10″, -10‴), 56.3 (3-OCH_3), 56.4 (4-OCH_3), 55.9 (4′-OCH_3); positive-ion FABMS *m/z* 779 $[M + Na]^+$; HRFABMS *m/z* 779.3615 (calcd for $C_{41}H_{56}O_{13}Na$ $[M + Na]^+$, 779.3619).

Hydrolysis of 1d. A solution of **1d** (9.3 mg) in dry MeOH (1.0 mL) was treated with $NaOCH_3$ (0.1 M in MeOH, 3 mL), and the mixture was stirred for 3 h at room temperature. Removal of the solvent under reduced pressure gave a residue, which was subjected to normal-phase silica gel column chromatography [1.0 g, EtOAc−MeOH (2:1 → 1:1 → 1:2 → 0:1, v/v)] to give **1e** (2.7 mg) and **1f** (2.0 mg).

1e: amorphous powder; UV (MeOH) λ_{max} 207.0 nm (log ε 4.16), 311.6 nm (log ε 4.32); ^{1}H NMR (methanol-d_4, 600 MHz) δ 3.67 (3H, s, 3-OCH_3), 3.70 (6H, s, H-9-OCH_3, 9′-OCH_3), 3.77 (3H, s, 4′-OCH_3), 3.80 (3H, s, 4-OCH_3), 6.84 (2H, d, *J* = 8.7 Hz, H-3′, -5′), 6.88 (1H, d, *J* = 8.7 Hz, H-5), 7.08 (1H, dd, *J* = 2.0, 8.7 Hz, H-6), 7.14 (1H, d, *J* = 2.0 Hz, H-2), 7.45 (2H, d, *J* = 8.7 Hz, H-2′, -6′), 7.81 (1H, s, H-7), 7.85 (1H, s, H-7′); ^{13}C NMR (methanol-d_4, 150 MHz) δ 128.9 (C-1), 113.3 (C-2), 150.2 (C-3), 152.2 (C-4), 112.5 (C-5), 125.9 (C-6), 143.8 (C-7), 125.6 (C-8, -8′), 169.3 (C-9), 128.5 (C-1′), 132.8 (C-2′, -6′), 115.2 (C-3′, -5′), 162.7 (C-4′), 143.6 (C-7′), 168.4 (C-9′), 56.2 (3-OCH_3), 56.3 (4-OCH_3), 55.8 (4′-OCH_3), 52.8 (9, 9′-O-CH_3); positive-ion EIMS *m/z* 412 $[M]^+$; HREIMS *m/z* 412.1525 (calcd for $C_{23}H_{24}O_7$ $[M]^+$, 412.1522). **1f**: amorphous powder; $[\alpha]^{25}_D$ −34 (*c* 0.1, MeOH); ^{13}C NMR (methanol-d_4, 125 MHz) δ 42.6 (C-1), 77.6 (C-2), 34.7 (C-3), 40.9 (C-4), 25.2 (C-5), 32.4 (C-6), 62.0 (C-7), 41.9 (C-8), 58.9 (C-9), 68.1 (C-10); positive-ion CIMS *m/z* 205 $[M + H]^+$; HRCIMS *m/z* 205.1442 (calcd for $C_{10}H_{22}O_4$ $[M + H]^+$, 205.1440).

Methylation of 1b. The procedure was the same as that used for the preparation of **1d**. Compound **1g** (14.2 mg) was obtained from **1b** (20 mg) using trimethylsilyldiazomethane (2 M in diethyl ether, 0.2 mL). The crude product was purified by reversed-phase ODS open column chromatography [1.0 g, MeOH−H_2O (2:8 → 3:7 → 4:6 → 1:0, v/v)].

1g: amorphous powder; $[\alpha]^{25}_D$ −21 (*c* 0.2, MeOH); UV (MeOH) λ_{max} 204.2 nm (log ε 4.31), 313.0 nm (log 4.41); ^{1}H NMR (methanol-d_4, 500 MHz) δ 1.05 (2H, m, H-5″β, -5‴β), 1.08 (2H, m, H-6″α, -6‴α), 1.25 (2H, m, H-3″α, -3‴α), 1.26 (2H, m, H-5″α, -5‴α), 1.29 (2H, m, H-4″, -4‴), 1.29 (2H, m, H-6″β, -6‴β), 1.62 (2H, m, H-3″β, -3‴β), 1.81 (2H, m, H-8″a, -8‴a), 1.88 (H-8″b, -8‴b), 3.16 (2H, t, *J* = 7.7 Hz, H-2‴, -2‴′), 3.27−3.28 (4H, m, H-3‴, -3‴′, -4‴, -4‴′), 3.34 (2H, m, H-5‴, -5‴′), 3.55 (2H, m H-9″b, -9‴b), 3.55−3.57 (4H, m, H-7″, -7‴), 3.56 (2H, m, H-2″, -2‴), 3.69 (3H, s, 3-OCH_3), 3.66 (2H, d-like, *J* = 11.6 Hz, H-6‴a, -6‴′a), 3.77 (3H, s, 4′-OCH_3), 3.83 (3H, s, 4-OCH_3), 3.81 (2H, m H-9″a, -9‴a), 3.85 (2H, d-like, *J* = 11.6 Hz, H-6‴b, -6‴′b), 3.91 (2H, m, H-10″a, -10‴a), 3.96 (2H, m, H-10″b, -10‴b), 4.27 (2H, d, *J* = 8.0 Hz, H-1‴, -1‴′), 6.90 (1H, d, *J* = 9.0 Hz, H-5), 7.49 (2H, d, *J* = 9.0 Hz, H-3′, -5′), 7.12 (1H, dd, *J* = 2.0, 9.0 Hz, H-6), 7.18 (1H, d, *J* = 2.0 Hz, H-2), 6.86 (2H, d, *J* = 9.0 Hz, H-2′, -6′), 7.83 (1H, s, H-7), 7.87 (1H, s, H-7′); ^{13}C NMR (methanol-d_4, 125 MHz) δ 143.5 (C-7), 126.0 (C-8), 126.1 (C-8′), 143.5 (C-7′), 168.8 (C-9), 168.9 (C-9′), 129.0 (C-1), 113.5 (C-2), 150.3 (C-3), 152.3 (C-4), 112.6 (C-5), 125.9 (C-6), 128.6 (C-1′), 132.9 (C-2′, -6′), 115.3 (C-3′, -5′), 162.7 (C-4′), 42.0 (C-1″, -1‴), 76.4 (C-2″, -2‴), 34.6 (C-3″, -3‴), 37.8 (C-4″, -4‴), 25.1 (C-5″, -5‴), 31.6 (C-6″, -6‴), 76.5 (C-7″, -7‴), 37.2 (C-8″, -8‴), 63.3 (C-9″, -9‴), 70.5 (C-10″, -10‴), 104.2 (C-1‴, -1‴′), 75.1 (C-2‴, -2‴′), 78.2 (C-3‴, -3‴′), 71.7 (C-4‴, -4‴′), 78.1 (C-5‴, -5‴′), 62.9 (C-6‴, -6‴′), 56.3 (3-OCH_3), 56.4 (4-OCH_3), 55.9 (4′-OCH_3); positive-ion FABMS *m/z* 1103 $[M + Na]^+$; HRFABMS *m/z* 1103.4668 (calcd for $C_{53}H_{76}O_{23}Na$ $[M + Na]^+$, 1103.4675).

Hydrolysis of 1g. The procedure was the same as that used for the preparation of **1e** and **1f**. Compound **1e** (2.2 mg) and the known dihydrocanangafruticoside A 7-ol (**1h**, 3.7 mg) were obtained from **1g** (11.2 mg) using $NaOCH_3$ (0.1 M in MeOH, 4 mL). The crude product was purified by reversed-phase ODS open column chromatography [1.0 g, MeOH−H_2O (1:9 → 2:8 → 5:5 → 7:3, v/v)]. Compound **1h** was identified by comparison of ^{1}H NMR data with the reported data.

1h: amorphous powder; $[\alpha]^{25}_D$ −2 (*c* 0.7, MeOH); ^{1}H NMR data were the same as the reported data;[3] ^{13}C NMR data (methanol-d_4, 125 MHz) δ 42.3 (C-1), 76.9 (C-2), 34.9 (C-3), 40.9 (C-4), 25.3 (C-5), 31.9 (C-6), 76.9 (C-7), 37.4 (C-8), 63.4 (C-9), 68.1 (C-10), 104.3 (C-1′), 75.2 (C-2′), 78.2 (C-3′), 71.8 (C-4′), 78.1 (C-5′), 62.9 (C-6′); positive-ion FABMS *m/z* 389 $[M]^+$; HRFABMS *m/z* 389.1795 (calcd for $C_{16}H_{30}O_9Na$ $[M + Na]^+$, 389.1788).

Preparation of the (*S*)- and (*R*)-MTPA Esters (7a and 7b) of 7. A solution of **7** (2.0 mg, 0.0059 mmol) in pyridine (0.5 mL) was treated with (−)-MTPA-Cl (0.02 mL), and the mixture was stirred at rt for 12 h. Removal of the solvent from the reaction mixture under reduced pressure furnished a residue, which was subjected to reversed-phase silica gel column chromatography [H_2O → MeOH] to give the

(*S*)-MTPA ester (**7a**, 1.7 mg). Employing a similar procedure, the (*R*)-MTPA ester (**7b**, 1.3 mg) was obtained from **7** (2.0 mg, 0.0059 mmol) using (+)-MTPA-Cl.

*(S)-MTPA ester (**7a**):* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.75 (s, H-15), 1.26 (m, H-6β), 1.27 (m, H-10α), 1.36 (m, H-7α), 1.45 (m, H-10β), 1.56 (m, H-12b), 1.69 (m, H-12a), 2.09 (m, H-7β), 2.10 (m, H-11α); FABMS *m*/*z* 581 $[M + Na]^+$; HRFABMS *m*/*z* 581.2498 (calcd for $C_{32}H_{37}O_5F_3Na$ $[M + Na]^+$, *m*/*z* 581.2491).

*(R)-MTPA ester (**7b**):* ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (s, H-15), 1.27 (m, H-6β), 1.10 (m, H-10α), 1.57 (m, H-7α), 1.32 (m, H-10β), 1.68 (m, H-12b), 1.83 (m, H-12a), 2.31 (m, H-7β), 2.04 (m, H-11α); FABMS *m*/*z* 581 $[M + Na]^+$; HRFABMS *m*/*z* 581.2498 (calcd for $C_{32}H_{37}O_5F_3Na$ $[M + Na]^+$, *m*/*z* 581.2491).

Acid Hydrolysis and Monosaccharide Composition of Canangalignans I (1) and II (2). To determine the absolute configurations of the constituent monosaccharide of **1** and **2**, the reported method[22] by Tanaka et al. was used with slight modifications as follows. Compounds **1** and **2** (each 1.0 mg) were dissolved in 5% aqueous H_2SO_4−1,4-dioxane (1:1, v/v, 1.0 mL), and each solution was heated at 90 °C for 3 h. After extraction with EtOAc (3 × 3 mL), the aqueous layer was neutralized by dropwise addition of 5% $NaHCO_3$. After drying in vacuo, the residue was dissolved in pyridine (0.1 mL) containing L-cysteine methyl ester hydrochloride (0.5 mg) and heated at 60 °C for 1 h. A solution of *o*-tolylisothiocyanate (0.5 mg) in pyridine (0.1 mL) was added, and the mixture heated at 60 °C for 1 h. The mixture was analyzed by reversed-phase HPLC [column: COSMOSIL 5C18-AR-II (Nacalai Tesque), 250 × 4.6 mm i.d. (5 μm); mobile phase: MeCN−H_2O in 1% AcOH (18:82, v/v); detection: UV (250 nm); flow rate: 0.8 mL/min; column temperature: 35 °C] to identify the derivatives of constituent D-glucose in **1** and **2** by comparison of their retention times with those of authentic samples (t_R: D-glucose, 56.0 min; L-glucose, 50.3 min).

Reagents for Bioassays. Dulbecco's modified Eagle's medium (DMEM, 1 or 4.5 g/L glucose) was purchased from Sigma-Aldrich (St. Louis, MO, USA); the Cell Counting Kit-8 was from Dojindo Lab. (Kumamoto, Japan); fetal bovine serum (FBS), amphotericin B, penicillin, and streptomycin were purchased from Gibco (Invitrogen, Carlsbad, CA, USA); the Pierce BCA protein assay kit was from Takara Bio Inc. (Shiga, Japan); all other chemicals were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan).

Cell Culture. Murine B16 melanoma 4A5 cells (RCB0557)[30] were obtained from Riken Cell Bank (Tsukuba, Japan). The cells were grown in DMEM (4.5 g/L glucose) supplemented with 10% FBS, streptomycin (100 μg/mL), and penicillin (100 units/mL) at 37 °C in 5% CO_2/air and harvested by incubation in phosphate-buffered saline (PBS) containing 1 mM EDTA and 0.25% trypsin for ca. 2 min at 37 °C. The cells were used for the bioassays.

Melanogenesis. Screening for melanogenesis with B16 melanoma 4A5 cells was performed as described previously[27] with slight modifications. The melanoma cells were seeded into 24-well multiplates (2.0 × 10^4 cells/400 μL/well). After 24 h of culture, a test compound and 1 mM theophylline were added and incubated for 72 h. The cells were harvested by treatment with PBS containing 1 mM EDTA and 0.25% trypsin and washed with PBS. The cells were treated with 1 M NaOH (120 μL/tube, 80 °C, 30 min) to obtain a lysate. An aliquot (100 μL) of the lysate was transferred to a 96-well microplate. The optical density of each well was measured with a microplate reader (SH-1000, Corona Electric Co., Hitachinaka, Japan) at 405 nm. The test compound was dissolved in DMSO, and the final concentration of DMSO in the medium was 0.1%. Inhibition (%) was calculated using the following formula, where *A* and *B* indicate the optical density of vehicle- and test compound-treated groups, respectively, and *C* indicates cell viability (%). IC_{50} values were determined graphically.

$$\text{Inhibition (\%)} = [(A - 100 \times B/C)/A] \times 100$$

Viability of Melanoma Cells. Cell viability was assessed as described previously[27] with slight modifications. B16 melanoma 4A5 cells were seeded into 96-well microplates (5.0 × 10^4 cells/100 μL/well) and incubated for 24 h. After incubation with 1 mM theophylline and a test compound for 70 h, the cells were washed with PBS. After 2 h further in culture with 100 μL of DMEM (4.5 g/L glucose) and 10 μL of WST-8 solution (Cell Counting Kit-8), the optical density of the water-soluble formazan produced by the cells was measured with a microplate reader (SH-1000, Corona Electric Co.) at 450 nm (reference: 655 nm). The test compound was dissolved in DMSO. The final concentration of DMSO in the medium was 0.1%. Inhibition of proliferation was calculated using the following formula, where *A* and *B* indicate the optical density of vehicle- and test compound-treated groups, respectively.

$$\text{Inhibition (\%)} = [(A - B)/A] \times 100$$

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The NMR spectra [^{1}H (solvent: methanol-d_4, $CDCl_3$), ^{13}C NMR (solvent: methanol-d_4, $CDCl_3$)] for the new compounds are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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