Bisabolane-Type Sesquiterpenes from the Aerial Parts of *Lippia dulcis*

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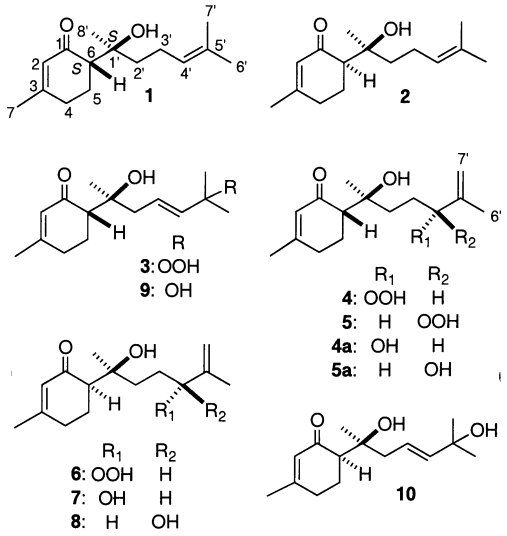
Six new bisabolane-type sesquiterpenes, peroxylippidulcines A−C (**3**−**5**), peroxyepilippidulcine B (**6**), and epilippidulcines B (**7**) and C (**8**), have been isolated from the aerial parts of *Lippia dulcis*, along with two known bisabolane-type sesquiterpenes, seven known flavonoids, and a known triterpenoid. The structures of **3**−**8** were characterized on the basis of NMR, MS, specific rotation, and X-ray crystallographic analysis data and chemical evidence.

Lippia dulcis Trev. (Verbenaceae) is an intensely sweet herb endemic to tropical America, and the leaves are used as a traditional medicine for the treatment of cough and bronchitis.[1] With regard to the chemical constituents of the leaves and flowers of this herb, Compadre et al.[2] and Kaneda et al.[3] reported the isolation and structure elucidation of two sweet bisabolane-type sesquiterpenes, (+)-hernandulcin (**1**) and (+)-4β-hydroxyhernandulcin, a nonsweet bisabolane-type sesquiterpene, (−)-epihernandulcin (**2**), a monoterpene, and a phenylethanoid glycoside ester. The absolute configurations of **1** and **2** were defined as 6*S*, 1′*S* and 6*R*, 1′*S*, respectively.[4] The major constituents of the volatile oil of the leaves were investigated by using GC/MS analysis.[5] Recently, the antiproliferative activity of three bisabolane-type sesquiterpenes and four phenylethanoid glycoside esters from the aerial parts of *L. dulcis* against cancer cells, B16F10, MK-1, and HeLa, was reported.[6] We have recently reported the isolation and structure elucidation of two bisabolane-type sesquiterpenes, five flavonoids, three phenylethanoid glycosides, and two iridoid glucosides from the aerial parts of *L. dulcis* and, further, that three phenolic compounds among these compounds had a stronger antioxidative activity than α-tocopherol.[7] Here we report the isolation and identification of six new bisabolane-type sesquiterpenes, designated peroxylippidulcines A−C (**3**−**5**), peroxyepilippidulcine B (**6**), and epilippidulcines B (**7**) and C (**8**), along with two known bisabolane-type sesquiterpenes, lippidulcine A (**9**)[7] and epilippidulcine A (**10**),[7] seven known flavonoids, sakuranetin,[8] cirsimaritin,[9] pectolinarigenin,[10] salvigenin,[10] eupatorin,[11] eupatilin,[12] and 5,3′-dihydroxy-6,7,4′,5′-tetramethoxyflavone,[13] and a known triterpenoid, betulinic acid.[14]



Results and Discussion

Peroxylippidulcine A (**3**) was obtained as a colorless syrup. The positive and negative FABMS of **3** gave an $[M + H]^+$ ion at *m*/*z* 269, which was 16 mass units higher than that of **9**, and an $[M - H]^-$ ion at *m*/*z* 267, respectively. The molecular formula of **3** was thus defined as $C_{15}H_{24}O_4$. The ^{1}H NMR spectrum of **3**, which was quite similar to that of **9**, showed signals due to four tertiary methyl groups (δ 1.96, 1.35, 1.33, 1.20) and three olefinic protons [δ 5.87 (s), 5.86 (ddd, J = 6.0, 8.5, 16.0 Hz), 5.62 (d, J = 16.0 Hz)]. The ^{13}C NMR spectrum of **3** gave 15 carbon signals, comprising one carbonyl carbon (δ 204.1), four olefinic carbons (δ 163.8, 136.8, 127.4, 126.6), two oxygenated quaternary carbons (δ 82.0, 74.3), one methine carbon (δ 51.9), three methylene carbons (δ 43.2, 31.3, 24.8), and four methyl carbons (δ 24.4, 24.1, 24.0, 23.7). These ^{1}H and ^{13}C NMR signals (Tables 1 and 2, respectively) were assigned with the aid of ^{1}H−^{1}H COSY, HMQC, and HMBC spectra, and the planar structure of **3**, a bisabolane-type sesquiterpene possessing the same framework as **9**, could be defined. In comparing the ^{13}C NMR data of **3** and **9**, the resonances due to C-1−C-7, C-1′, C-2′, and C-8 in **3** were superimposable on those of **9**; in contrast, the signals due to C-3′−C-7′ were shifted by +4.2, −4.3, +11.3, −5.5, and −5.8 ppm, respectively. It was reported that the ^{13}C NMR chemical shifts of C-6, C-2′, and C-8′ in **1** and **2** differed because of differences in the intramolecular steric interactions.[2] Therefore, the relative configurations at C-6 and C-1′ in **3** are likely to be the same as those of **9**. From these data, **3** was considered to be the peroxide at C-5′ in **9**. This assumption was confirmed by the reduction of the hydroperoxy group in **3** with triphenylphosphine, giving **9**.[15] Consequently, **3** was elucidated as (6*S*,1′*S*)-6-(1′-hydroxy-5′-hydroperoxy-1′,5′-dimethyl-3′-hexenyl)-3-methyl-2-cyclohexenone.

Peroxylippidulcine B (**4**) and peroxylippidulcine C (**5**) were obtained as colorless needles and a colorless syrup, respectively. The HRFABMS indicated the molecular formula of both compounds to be the same as that of **3**. The ^{1}H and ^{13}C NMR spectra of **4** and **5** were similar. The ^{1}H NMR spectra of both **4** and **5** showed signals due to three tertiary methyl groups (δ 1.97, 1.77, 1.17 in **4**; δ 1.97, 1.76, 1.18 in **5**), two *exo*-methylene protons [δ 5.00 (s) × 2 in **4**; δ 5.00 (s) × 2 in **5**], and one oxygenated methine proton [δ 4.30 (dd, J = 6.5, 6.5 Hz) in **4**; δ 4.29 (dd, J = 6.5, 6.5

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Table 1. ^{1}H NMR Data (500 MHz, $CDCl_3$) for Compounds **3**−**7**[a]

position	3	4	5	6	7
2	5.87 s	5.89 s	5.89 s	5.87 s	5.87 s
4a	ca. 2.34	ca. 2.40	ca. 2.41	ca. 2.39	ca. 2.40
4b	ca. 2.31	2.31 ddd (2.5,5.0,18.5)	2.31 ddd (2.5,5.0,18.5)	2.32 ddd (2.5,5.0,18.5)	2.32 ddd (2.5,5.0,18.5)
5a	2.03 dddd (2.5,5.0,5.0,13.0)[b]	ca. 2.00	2.01 dddd (2.5,5.0,5.0,13.0)	2.05 dddd (2.5,4.5,4.5,13.5)	2.07 dddd (2.5,4.5,4.5,13.0)
5b	1.66 dddd (5.5,11.0,13.0,14.0)	ca. 1.68	ca. 1.69	ca. 1.79	ca. 1.78
6	2.40 dd (4.5,14.0)	2.39 dd (4.5,14.0)	2.39 dd (4.5,14.0)	2.37 dd (4.5,14.0)	2.38 ddd (4.5,14.0)
7	1.96 s	1.97 s	1.97 s	1.97 s	1.97 s
2′a	2.27 dd (6.0,14.0)	1.60 ddd (4.5,13.5,13.5)	ca. 1.62	ca. 1.80	ca. 1.67
2′b	2.21 dd (8.5,14.0)	1.46 ddd (4.5,11.0,13.5)	1.49 ddd (4.5,10.5,13.0)	ca. 1.56	ca. 1.67
3′a	5.86 ddd (6.0,8.5,16.0)	1.82 m	1.80 m	ca. 1.80	ca. 1.78
3′b		ca. 1.68	ca. 1.65	ca. 1.56	ca. 1.63
4′	5.62 d (16.0)	4.30 dd (6.5,6.5)	4.29 dd (6.5,6.5)	4.26 dd (6.5,6.5)	4.03 dd (5.0,5.0)
6′a	1.33 s	5.00 s	5.00 s	4.99 s	4.96 s
6′b		5.00 s	5.00 s	4.99 s	4.84 s
7′	1.35 s	1.77 s	1.76 s	1.74 s	1.73 s
8′	1.20 s	1.17 s	1.18 s	1.16 s	1.20 s
OH	8.90 br s	8.97 br s	8.90 br s	8.28 br s	5.15 br s
OH	5.65 br s	5.55 br s	5.49 br s	5.08 s	

[a] Chemical shifts (δ) are in ppm relative to TMS. [b]Coupling constants (J) in Hz are given in parentheses.

Table 2. ^{13}C NMR Data ($CDCl_3$) for Compounds **1**−**10**, **4a**, and **5a**

	1[a]	2[b]	3	4	5	6	7	8	4a	5a	9	10
position	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.
1	204.0, qC	203.4, qC	204.1, qC	203.9, qC	204.0, qC	203.6, qC	203.5, qC	203.0, qC	204.0, qC	204.0, qC	204.1, qC	203.3, qC
2	127.4, CH	127.4, CH	127.4, CH	127.5, CH	127.5, CH	127.4, CH	127.5, CH	127.6, CH	127.6, CH	127.5, CH	127.6, CH	127.5, CH
3	163.6, qC	163.6, qC	163.8, qC	163.8, qC	163.8, qC	163.8, qC	163.6, qC	162.9, qC	163.7, qC	163.7, qC	163.4, qC	163.4, qC
4	31.2, CH_2	31.5, CH_2	31.3, CH_2	31.3, CH_2	31.3, CH_2	31.5, CH_2	31.6, CH_2	31.5, CH_2	31.3, CH_2	31.3, CH_2	31.3, CH_2	31.6, CH_2
5	25.0, CH_2	25.0, CH_2	24.8, CH_2	25.0, CH_2	25.0, CH_2	25.0, CH_2	25.1, CH_2	25.0, CH_2	25.0, CH_2	25.0, CH_2	24.8, CH_2	24.9, CH_2
6	52.0, CH	55.3, CH	51.9, CH	52.6, CH	51.8, CH	55.4, CH	55.5, CH	55.3, CH	52.1, CH	52.0, CH	51.9, CH	54.6, CH
7	24.1, CH_3	24.1, CH_3	24.1, CH_3	24.1, CH_3	24.1, CH_3	24.1, CH_3	24.1, CH_3	24.0, CH_3	24.1, CH_3	24.1, CH_3	23.8, CH_3	24.1, CH_3
1′	73.9, qC	74.3, qC	74.3, qC	74.3, qC	74.2, qC	74.2, qC	74.3, qC	74.2, qC	74.2, qC	74.0, qC	74.3, qC	74.6, qC
2′	40.1, CH_2	37.1, CH_2	43.2, CH_2	35.3, CH_2	35.5, CH_2	32.8, CH_2	32.7, CH_2	33.0, CH_2	36.5, CH_2	36.0, CH_2	43.1, CH_2	40.7, CH_2
3′	21.5, CH_2	22.1, CH_2	126.6, CH	23.7, CH_2	24.0, CH_2	25.0, CH_2	29.0, CH_2	29.4, CH_2	29.1, CH_2	28.7, CH_2	122.4, CH	123.0, CH
4′	124.4, CH	124.8, CH	136.8, CH	88.9, CH	89.1, CH	90.0, CH	76.1, CH	76.1, CH	75.8, CH	75.6, CH	141.1, CH	140.9, CH
5′	131.4, qC	131.1, qC	82.0, qC	143.8, qC	143.8, qC	143.8, qC	147.8, qC	147.5, qC	147.7, qC	147.8, qC	70.7, qC	70.7, qC
6′	25.7, CH_3	25.7, CH_3	24.4, CH_3	18.1, CH_3	18.0, CH_3	17.4, CH_3	18.1, CH_3	17.8, CH_3	18.2, CH_3	18.2, CH_3	29.9, CH_3	29.7, CH_3
7′	17.6, CH_3	17.6, CH_3	24.0, CH_3	113.6, CH_2	113.6, CH_2	113.9, CH_2	110.7, CH_2	110.7, CH_2	110.5, CH_2	110.5, CH_2	29.8, CH_3	29.7, CH_3
8′	23.6, CH_3	25.4, CH_3	23.7, CH_3	23.1, CH_3	23.8, CH_3	25.2, CH_3	25.4, CH_3	25.4, CH_3	23.7, CH_3	23.7, CH_3	23.8, CH_3	26.2, CH_3

[a] Values (90.8 MHz) from ref 2. [b]Values (75.6 MHz) from ref 3. Values of **3**−**10**, **4a**, and **5a** are recorded at 125 MHz.

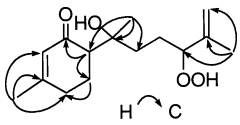

Figure 1. ^{1}H−^{13}C long-range correlations observed for **4** in the HMBC spectrum.

Hz) in **5**]. The ^{13}C NMR spectra of both **4** and **5** showed 15 carbon signals, including one carbonyl carbon (δ 203.9 in **4**; δ 204.0 in **5**), four olefinic carbons (δ 163.8, 143.8, 127.5, 113.6 in **4**; δ 163.8, 143.8, 127.5, 113.6 in **5**), one oxygenated quaternary carbon (δ 74.3 in **4**; δ 74.2 in **5**), and one oxygenated methine carbon (δ 88.9 in **4**; δ 89.1 in **5**). These ^{1}H and ^{13}C NMR signals (Tables 1 and 2, respectively) were assigned by using techniques similar to those used for **3**. From the assignments, it was deduced that **4** and **5** have a common planar structure, a bisabolane-type sesquiterpene, with a hydroperoxy group at C-4′ and an *exo*-methylene group at C-5′ (Figure 1). The location of the hydroperoxy group in **4** and **5** was confirmed by the following evidence. Treatment of **4** and **5** with triphenylphosphine afforded **4a** and **5a**, designated lippidulcines B and C, respectively. In comparing the ^{13}C NMR data of **4** and **4a**, and **5** and **5a**, the signals due to C-3′, C-4′, C-5′, and C-7′ in **4** were shifted by −5.4, +13.1, −3.9, and +3.1 ppm, respectively, and those in **5** were shifted by −4.7, +13.5, −4.0, and +3.1 ppm, respectively. Further, the resonances due to C-6 and C-8′ in **4** and **5** were almost the same as those of **1**; this indicated that both the relative configurations at C-6 and C-1′ in **4** and **5** were identical with those of **1**. Thus, **4** and **5** were inferred to be 4′-epimers. To define the relative configuration at C-4′, an X-ray analysis (Figure 2, Table 3) of **4** was undertaken. From the analysis, the relative configurations at C-4′ in **4** and **5** were determined to be *R** and *S**, respectively. Therefore, the structures of **4** and **5** were defined as (*rel*-6*S*,1′*S*,4′*R*)-6-(1′-hydroxy-4′-hydroperoxy-1′,5′-dimethyl-5′-hexenyl)-3-methyl-2-cyclohexenone and (*rel*-6*S*,1′*S*,4′*S*)-6-(1′-hydroxy-4′-hydroperoxy-1′,5′-dimethyl-5′-hexenyl)-3-methyl-2-cyclohexenone, respectively.

Peroxyepilippidulcine B (**6**) was obtained as colorless needles, and its molecular formula was the same as those of **3**−**5**. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2, respectively) of **6** were also similar to those of **4** and **5**, although the chemical shifts of the signals due to C-6 and C-8′ were superimposable on those of **2**. From these data, **6** was considered to be the 6-epimer of **4** or **5**. Finally, the relative configurations of **6** were elucidated by X-ray crystallography (Figure 2, Table 3). Consequently, **6** was identified as (*rel*-6*R*,1′*S*,4′*R*)-6-(1′-hydroxy-4′-hydroperoxy-1′,5′-dimethyl-5′-hexenyl)-3-methyl-2-cyclohexenone.

Epilippidulcine B (**7**) was obtained as a colorless syrup and analyzed for the molecular formula $C_{15}H_{24}O_3$, which contained one fewer oxygen atom than **6** by HRFABMS. The ^{1}H and ^{13}C NMR

Table 3. Crystal Data and Intensity Measurement of **4** and **6**

	4	**6**
formula	$C_{15}H_{24}O_4$	$C_{15}H_{24}O_4$
fw	268.35	268.35
cryst syst	monoclinic	orthorhombic
lattice params		
a (Å)	9.236(7)	6.599(2)
b (Å)	6.525(4)	9.997(5)
c (Å)	12.959(7)	23.190(8)
V (A^3)	764.4(9)	1529(1)
space group	$P2_1$ (No. 4)	$P2_12_12_1$ (No. 19)
Z	2	4
D_c (g/cm^3)	1.166	1.165
no. of reflns collected	3656	3523
no. of unique data collected	3378	3501
no. of unique data used ($I > 3.0\sigma I$)	2190	2814
R	0.062	0.036
R_w	0.098	0.109

spectra (Tables 1 and 2, respectively) of **7** were almost the same as those of **4a**, apart from the signals due to C-6, C-2′, and C-8′. Further, **7** was obtained by the reduction of the hydroperoxy group in **6** with triphenylphosphine. Thus, **7** was elucidated as (*rel*-6*R*,1′*S*,4′*R*)-6-(1′,4′-dihydroxy-1′,5′-dimethyl-5′-hexenyl)-3-methyl-2-cyclohexenone.

Epilippidulcine C (**8**) was obtained as a colorless syrup. The molecular formula of **8** was the same as that of **7**. Further, the ^{1}H and ^{13}C NMR spectra (Tables 4 and 2, respectively) of **8** were very similar to those of **7**, including the chemical shifts of the signals due to C-6, C-2′, and C-8′. Therefore, **8** was defined as (*rel*-6*R*,1′*S*,4′*S*)-6-(1′,4′-dihydroxy-1′,5′-dimethyl-5′-hexenyl)-3-methyl-2-cyclohexenone.

Although **1** and **2** were not isolated in this study, **3**−**6** probably formed via ene reaction of **1** or **2** with singlet oxygen ($^1O_2$).[16,17] Since compounds **3**−**5** were detected in the EtOAc-soluble fraction by HPLC analysis, compounds **3**−**6** were probably produced in the plant. In a previous paper, one of the present authors reported the isolation of **1** and **2** from the materials that were obtained from the same clone as that used in this study.[6] However, the materials were grown in a hothouse and harvested in April; in contrast, those used in this study were grown outdoors and harvested in October. Therefore, the difference in the constituents might be derived from a seasonal variation and/or difference in the culture conditions. The sweetness of compounds **3**−**10** was not tested because only a small amount was isolated.

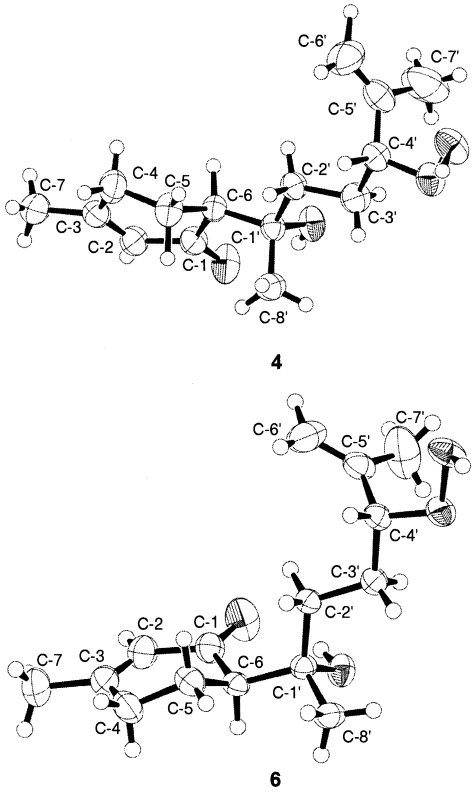

Figure 2. ORTEP[20] drawings of **4** and **6**. The ellipsoid probability level of **4** and **6** is 50%.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting-point apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-1000 KUY digital polarimeter. UV spectra were measured with a JASCO V-530 UV/vis spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded by using a JEOL alpha 500 spectrometer at 500 and 125 MHz, respectively, and chemical shifts were specified on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. FABMS data were collected using a JEOL JMS-DX-303HF mass spectrometer, and HRFABMS data were obtained using a JEOL JMS-700T mass spectrometer. Column chromatography was carried out over silica gel 60 (Merck, Art. 9385) and Chromatorex ODS (Fuji Silysia Chemical Ltd.). HPLC separation was performed on a Shimadzu LC-10AS pump with a Shimadzu RID-10A

Table 4. ^{1}H NMR Data (500 MHz, $CDCl_3$) for Compounds **8**−**10**, **4a**, and **5a**[a]

position	**8**	**4a**	**5a**	**9**	**10**
2	5.86 s	5.89 s	5.89 s	5.87 s	5.86 s
4a	ca. 2.40	2.40 ddd (1.0,11.0,18.5)	2.40 br dd (12.0,18.0)	ca. 2.32	ca. 2.38
4b	2.31 ddd (2.5,4.5,18.5)[b]	2.30 ddd (1.5, 5.0,18.5)	2.31 br d (18.0)	ca. 2.30	ca. 2.32
5a	2.06 dddd (2,5,4.5,4.5,13.0)	ca. 2.00	2.03 dddd (2.5,4.0,4.0,13.0)	2.02 dddd (2.5,4.5,4.5,13.5)	2.07 dddd (3.0,3.0,4.5,13.5)
5b	ca. 1.78	ca. 1.68	ca. 1.69	1.66 dddd (5.5,11.0,13.5,14.0)	1.79 dddd (5.5,11.5,13.5,14.0)
6	2.36 dd (5.0,14.0)	2.48 dd (5.0,14.0)	2.44 dd (5.0,14.0)	2.39 dd (4.5,14.0)	2.36 dd (5.0,14.0)
7	1.97 s	1.97 s	1.97 s	1.96 s	1.97 s
2′a	ca. 1.76	ca. 1.60	ca. 1.62	2.24 dd (5.5,14.0)	ca. 2.36
2′b	ca. 1.67	ca. 1.60	ca. 1.55	2.17 dd (8.5,14.0)	2.15 dd (8.0,14.0)
3′a	ca. 1.74	ca. 1.74	ca. 1.80	5.82 ddd (6.0,8.0,15.5)	5.74 ddd (7.0,8.0,15.5)
3′b	ca. 1.62	ca. 1.64	ca. 1.63		
4′	4.03 dd (5.0,8.0)	4.06 dd (5.0,6.5)	4.06 dd (4.5,4.5)	5.68 d (15.5)	5.62 d (15.5)
6′a	4.92 s	4.98 s	4.99 s	1.33 s	1.30 s
6′b	4.80 s	4.83 s	4.85 s		
7′	1.73 s	1.74 s	1.74 s	1.33 s	1.30 s
8′	1.20 s	1.20 s	1.19 s	1.18 s	1.18 s
OH		5.66 br s	5.57 br s	5.29 br s	5.20 br s

[a] Chemical shifts (δ) are in ppm relative to TMS. [b]Coupling constants (*J*) in Hz are given in parentheses.

RI-detector. For HPLC separation, COSMOSIL 5C_{18}-AR-II (Nacalai Tesque, 20 mm i.d. × 250 mm) and COSMOSIL 5SL-II (Nacalai Tesque, 20 mm i.d. × 250 mm) were used. Analytical HPLC was performed on a Jasco PU-2080 Plus pump with a Jasco MD2015 photodiode array detector and a Jasco CO-2060 plus column thermostat.

Plant Material. The aerial parts of *L. dulcis* were collected in October 2004 at the Medical Plant Garden of the Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka Prefecture, Japan. A voucher specimen (F2004) is deposited in the Laboratory of Natural Products Chemistry, School of Agriculture, Kyushu Tokai University.

Extraction and Isolation. The freshly cut aerial parts of *L. dulcis* (721.4 g) were extracted with MeOH (× 3) at room temperature, and the solvent was removed under reduced pressure to yield a syrup (107.8 g). The MeOH extract was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (17.8 g) was chromatographed over silica gel by using hexane−acetone (20:1, 15:1, 10:1, 5:1, 1:1, 0:1) and MeOH as eluents to yield fractions 1−12. The chromatography of fraction 2 (2449 mg) over silica gel that was eluted with hexane−EtOAc (10:1, 5:1, 3:1, 1:1, 1:3, 0:1) and MeOH furnished fractions 2.1−2.14. Fractions 2.10 (292 mg), 2.11 (427 mg), and 2.12 (324 mg) were each subjected to HPLC (COSMOSIL 5SL-II) by using hexane−acetone (10:1) as the eluent to yield **5** (24 mg) from fraction 2.10; **3** (91 mg), **5** (40 mg), and **4** (16 mg) from fraction 2.11; and **6** (8 mg) and fractions 2.12.1−2.12.4 from fraction 2.12. HPLC (COSMOSIL 5C_{18}-AR-II) of fraction 2.13 (120 mg) that was eluted with 60% MeOH afforded **7** (7 mg), **8** (6 mg), **10** (7 mg), **9** (16 mg), and fraction 2.13.1 (2 mg); this fraction was elucidated as a mixture of **4a** and **5a** by the ^{1}H and ^{13}C NMR spectral data. However, the separation of fraction 2.13.1 could not be achieved. Fraction 3 (1313 mg) was chromatographed over Chromatorex ODS, eluted with MeOH−H_2O mixtures (70% MeOH, 80% MeOH, 90% MeOH, 100% MeOH), to afford betulinic acid (52 mg) and fractions 3.1−3.10. Fractions 3.3 (25 mg) and 3.5 (126 mg) were each subjected to HPLC (COSMOSIL 5C_{18}-AR-II) by using 70% MeOH as the eluent to yield sakuranetin (7 mg) from fraction 3.3 and pectolinaringenin (8 mg) and salvigenin (47 mg) from fraction 3.5. Chromatography of fraction 7 (1376 mg) over Chromatorex ODS by using MeOH−H_2O mixtures (70% MeOH, 80% MeOH, 90% MeOH, 100% MeOH) as the eluent yielded fractions 7.1−7.4. Fraction 7.3 (71 mg) was subjected to HPLC (COSMOSIL 5C_{18}-AR-II) by elution with 60% MeOH to yield cirsimaritin (9 mg), eupatorin (11 mg), 5,3′-dihydroxy-6,7,4′,5′-tetramethoxyflavone (11 mg), and eupatilin (10 mg).

Peroxylippidulcine A (3): colorless syrup; $[\alpha]^{13}_D$ +42.0 (*c* 3.2, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 234 (4.12) nm; ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS (positive mode) *m*/*z* 269 $[M + H]^+$; FABMS (negative mode) *m*/*z* 267 $[M - H]^-$; HRFABMS *m*/*z* 269.1752 (calcd for $C_{15}H_{25}O_4$, 269.1753).

Peroxylippidulcine B (4): colorless needles (MeOH); mp 129−130 °C; $[\alpha]^{29}_D$ +72.5 (*c* 2.4, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 234 (4.15) nm; ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS (positive mode) *m*/*z* 269 $[M + H]^+$; HRFABMS *m*/*z* 269.1758 (calcd for $C_{15}H_{25}O_4$, 269.1753).

Peroxylippidulcine C (5): colorless syrup; $[\alpha]^{29}_D$ +64.3 (*c* 6.1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 234 (4.22) nm; ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS (positive mode) *m*/*z* 269 $[M + H]^+$; HRFABMS *m*/*z* 269.1751 (calcd for $C_{15}H_{25}O_4$, 269.1753).

Peroxyepilippidulcine B (6): colorless needles (MeOH); mp 168−169 °C; $[\alpha]^{31}_D$ −86.8 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 234 (4.19) nm; ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS (positive mode) *m*/*z* 269 $[M + H]^+$; HRFABMS *m*/*z* 269.1753 (calcd for $C_{15}H_{25}O_4$, 269.1753).

Epilippidulcine B (7): colorless syrup: $[\alpha]^{29}_D$ −118.9 (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 234 (4.25) nm; ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS (positive mode) *m*/*z* 275 $[M + Na]^+$; HRFABMS *m*/*z* 275.1617 (calcd for $C_{15}H_{24}O_3Na$, 275.1624).

Epilippidulcine C (8): colorless syrup; $[\alpha]^{13}_D$ −90.1 (*c* 0.8, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 235 (4.19) nm; ^{1}H and ^{13}C NMR, see Tables 4 and 2; FABMS (positive mode) *m*/*z* 275 $[M + Na]^+$; HRFABMS *m*/*z* 275.1624 (calcd for $C_{15}H_{24}O_3Na$, 275.1624).

Reduction of 3−6 with Triphenylphosphine. Triphenylphosphine (15 mg) was added to a solution of **3** (15 mg) in benzene (15 mL). The mixture was left to stand at room temperature for 2 h and then evaporated in vacuo to yield a residue. The residue was subjected to HPLC (Cosmosil 5C_{18}-AR-II, 55% MeOH) to afford **3a** (5 mg). The reduction of **4** (10 mg), **5** (20 mg), and **6** (5 mg) was carried out in the same manner as for **3**, affording **4a** (4 mg) from **4**, **5a** (6 mg) from **5**, and **6a** (1.5 mg) from **6**.

Compound 3a: colorless syrup; $[\alpha]^{29}_D$ −130.1 (*c* 0.1, $CHCl_3$). The ^{1}H NMR spectrum of **3a** was superimposable on that of **9**.

Lippidulcine B (4a): colorless syrup; $[\alpha]^{29}_D$ +113.3 (*c* 0.4, $CHCl_3$); ^{1}H and ^{13}C NMR, see Tables 4 and 2.

Lippidulcine C (5a): colorless syrup; $[\alpha]^{29}_D$ +119.8 (*c* 0.7, $CHCl_3$); ^{1}H and ^{13}C NMR, see Tables 4 and 2.

Compound 6a: colorless syrup; $[\alpha]^{29}_D$ −148.5 (*c* 0.2, $CHCl_3$). The ^{1}H NMR spectrum of **6a** was superimposable on that of **7**.

X-ray Crystallographic Analysis of 4 and 6. The single crystals of **4** and **6** were prepared by the slow evaporation of an MeOH solution at room temperature. All measurements were performed on a Rigaku R-AXIS RAPID II imaging plate area detector with graphite-monochromated Mo Kα radiation (λ = 0.71075 Å). The reflection data were collected at room temperature up to a maximum 2θ value of 54.9°. The crystal structures were solved by the direct method by using the SIR92 program.[18] The non-hydrogen atoms were refined anisotropically, while the hydrogen atoms were refined isotropically. All atomic parameters were refined by a full-matrix least-square method. All calculations were performed by using the Crystal Structure crystallographic software package.[19]

HPLC Analysis of EtOAc-Soluble Fraction. The EtOAc-soluble fraction was subjected to HPLC analysis [column: COSMOSIL 5C_{18}-AR-II (Nacalai Tesque, 4.6 mm i.d. × 250 mm); eluent: 40% MeOH; flow rate: 0.8 mL/min; column temperature: 30 °C; detector: photodiode array detector] and exhibited the presence of **3** (t_R, 15.1 min; UV λ_{max}, 234 nm), **4** (t_R, 15.1 min; UV λ_{max}, 234 nm), and **5** (t_R, 18.2 min; UV λ_{max}, 234 nm). However, **6** was not identified under these conditions owing to the overlapping of some signals.

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