Sesquiterpenoids and Norsesquiterpenoids from the Formosan Soft Coral *Lemnalia laevis*

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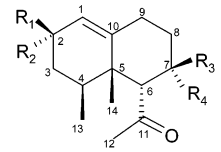
Received September 1, 2005

Eight new nornardosinane sequiterpenoids, laevinols A−H (**1**−**8**), a new neolemnane sesquiterpenoid, laevinone A (**9**), and the previously known 6β-acetyl-4β,5β-dimethyl-1(10)α-epoxy-2β-hydroxy-7-oxodecalin (**10**) and 11,12-dihydroxy-6,10-eremophiladiene (**11**) were isolated from the methylene chloride solubles of the Formosan soft coral *Lemnalia laevis.* Their structures were elucidated by extensive spectroscopic analysis, and their cytotoxicity against selected cancer cells was measured in vitro.

The genus *Lemnalia* has afforded a number of bioactive sesquiterpenes.[1−10] As part of our search for bioactive substances from marine organisms, the Formosan soft coral *Lemnalia laevis* Thomson and Dean (Nephtheidae) was studied because the CH_2Cl_2 extract showed significant cytotoxicity to A-549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), and P-388 (mouse lymphocytic leukemia) cell cultures, as determined by standard procedures.[11,12] Bioassay-guided fractionation of the methylene chloride solubles of *L. laevis* resulted in the isolation and characterization of eight new nornardosinane sequiterpenoids, laevinols A−H (**1**−**8**), a new neolemnane sesquiterpenoid, laevinone A (**9**), and the previously known 6β-acetyl-4β,5β-dimethyl-1(10)α-epoxy-2β-hydroxy-7-oxodecalin (**10**)[2] and 11,12-dihydroxy-6,10-eremophiladiene (**11**).[3]



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3 R_1 = H, R_2 = OH, R_3 = OH, R_4 = H
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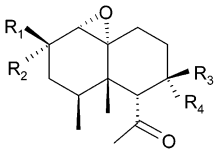
5 R_1 = H, R_2 = OH, R_3, R_4 = O



6 R_1 = H, R_2 = OH, R_3 = OH, R_4 = H
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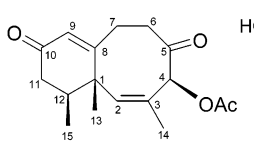

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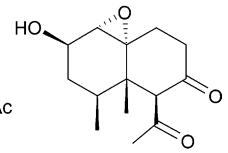

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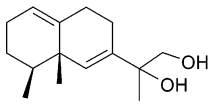

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Results and Discussion

The molecular formula of laevinol A (**1**) was found to be $C_{15}H_{22}O_4$ from its HREIMS and ^{13}C NMR data. The DEPT spectrum showed signals for three methyls, three sp^3 methylenes, four sp^3 methines, one sp^3 quaternary carbon, one sp^2 methine, and two sp^2 quaternary carbons. The ^1H and ^{13}C NMR spectra indicated the presence of a secondary methyl at δ_H 0.95 (3H, d, Me-13) and δ_C 15.6 (CH_3, Me-13); a tertiary methyl at δ_H 1.02 (3H, s, Me-14) and δ_C 18.0 (CH_3, Me-14); a secondary hydroxyl at δ_H 3.99 (1H, br s, H-2) and δ_C 63.7 (CH, C-2); a secondary formyloxy group at δ_H 5.44 (1H, dt, J = 12.0, 5.4 Hz, H-7), 8.04 (1H, s, OC*H*O) and δ_C 71.6 (CH, C-7), 160.2 (CH, O*C*HO); a methyl ketone at δ_H 2.19 (3H, s, H_3-12) and δ_C 35.4 (CH_3, C-12), 210.5 (qC, C-11); and a trisubstituted olefin at δ_H 5.69 (1H, d, J = 4.8 Hz, H-1) and δ_C 123.9 (CH, C-1), 143.0 (qC, C-10). These spectroscopic data, coupled with the determined number of degrees of unsaturation (five), suggested that compound **1** is a bicyclic norsesquiterpenoid with secondary hydroxyl, secondary formyloxyl, and methyl ketone groups.

After assignments of all the direct $^1H-^{13}C$ bondings were made on the basis of the HMQC spectrum, the gross structure of **1** was determined by $^1H-^1H$ COSY and HMBC NMR spectroscopic analysis (Figure 1). The $^1H-^1H$ COSY spectrum revealed two partial structures, **a** and **b** (Figure 1). The connectivity between C-11 and C-6 was indicated by the HMBC correlations from H-12 [δ_H 0.88 (3H, s)] to C-11 [δ_C 213.1 (qC)] and C-6 [60.8 (CH)] and from H-7 [δ_H 5.44 (1H, dt, J = 11.4, 5.1 Hz)] to C-11. The HMBC correlation from H-13 to C-5 [δ_C 35.9 (CH)] comfirmed the connectivity between C-4 and C-5. The HMBC correlations from the proton signal at δ 8.04 to C-7 and from H-7 to the carbon signal at δ 160.2 revealed the location of the secondary formyloxyl group.[1] The connectivity between partial structures **a** and **b** was exhibited by the HMBC correlations as shown in Figure 1.

The relative stereochemistry of **1** was deduced from a 2D NOESY NMR experiment (Table S1, Supporting Infor-

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10.1021/np050326r CCC: $30.25
Published on Web 12/07/2005

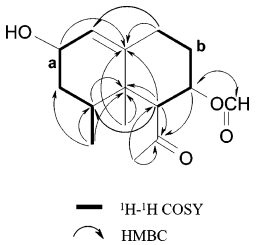


Figure 1. Key COSY and HMBC correlations of **1**.

mation), which showed that Me-14 (axial), Me-13 (equatorial), H-6 (equatorial), and H-7 (axial) are on β face of the molecule, while H-4 (axial) and Me-12 are on the opposite, α face. The α-configuration of hydroxy at C-2 was determined by comparison with the $J_{1,2}$ value of lemnacarnol ($J_{1,2} = 0$ Hz) and 2-*epi*-lemnacarnol ($J_{1,2} = 4.5$ Hz).[1] From the aforementioned data, laevinol A could be formulated as 6α-acetyl-4β,5β-dimethyl-1(10)-ene-2α-hydroxy-7α-formyloxydecalin.

The HREIMS and ^{13}C NMR data revealed laevinol B (**2**) to have a molecular formula of $C_{14}H_{22}O_3$. The DEPT spectrum showed resonances for three methyls, three sp^3 methylenes, four sp^3 methines, one sp^3 quaternary carbon, one sp^2 methine, and two sp^2 quaternary carbons. The IR absorptions at 3520 and 1722 cm^{-1} indicated the presence of hydroxyl and carbonyl groups. The ^{1}H and ^{13}C NMR spectra showed the presence of a secondary methyl at δ_H 0.95 (3H, d, $J = 6.6$ Hz, Me-13) and δ_C 15.6 (CH_3, Me-13); a tertiary methyl at δ_H 1.00 (3H, s, Me-14) and δ_C 18.2 (CH_3, Me-14); two secondary hydroxyls at δ_H 3.97 (1H, br s, H-2), 4.25 (1H, dt, $J = 11.4$, 5.1 Hz, H-7) and δ_C 63.9 (CH, C-2), 69.0 (CH, C-7); a methyl ketone at δ_H 2.34 (3H, s, H_3-12) and δ_C 36.2 (CH_3, C-12), 213.1 (qC, C-11); and a trisubstituted olefin at δ_H 5.66 (1H, d, $J = 4.5$ Hz, H-1) and δ_C 123.3 (CH, C-1), 144.0 (qC, C-10). These spectroscopic data suggested that compound **2** is the deformylated derivative of **1**. The COSY NMR correlations from H-7 to H-6 and H-8 revealed the location of the secondary hydroxyl group at C-7. The relative stereochemistry of **2** was established from a 2D NOESY experiment (Table S1, Supporting Information), which showed results similar to those determined from **1**. Therefore, laevinol B was established as 6α-acetyl-4β,5β-dimethyl-1(10)-ene-2α,7α-dihydroxydecalin.

Laevinol C (**3**) was assigned a molecular formula of $C_{14}H_{22}O_3$, as indicated by its HREIMS and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectroscopic data resembled those of **2** except for the resonances and splitting patterns in the vicinity of C-7. The relative stereochemistry of **3** was deduced from a 2D NOESY experiment (Table S1, Supporting Information). NOESY correlations from Me-14 to H-6, H-9β, H-8β, and Me-13 indicated that Me-14 (axial), Me-13 (equatorial), and H-6 (equatorial) are on the β face of the molecule, while NOESY correlations from Me-12 to H-4 and from H-7 to H-8α suggested that H-4 (axial), H-7 (equatorial), and Me-12 (axial) are on the opposite, α face. Therefore, laevinol C was determined as 6α-acetyl-4β,5β-dimethyl-1(10)-ene-2α,7β-dihydroxydecalin.

Laevinol D (**4**) was shown to have the molecular formula $C_{14}H_{22}O_3$ by HREIMS and from its ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectra of **4** were quite similar to those of **2** with the exception of the resonances and splitting patterns in the vicinity of C-2. The configuration of the hydroxy group at C-2 was determined as β by comparison with the $J_{1,2}$ value of lemnacarnol ($J_{1,2} = 0$ Hz) and 2-*epi*-lemnacarnol ($J_{1,2} = 4.5$ Hz).[1] The relative stereochemistry of **4** was deduced from a 2D NOESY experiment (Table S1, Supporting Information), which exhibited similar results as determined from **1**. From the aforementioned data, laevinol D was formulated as 6α-acetyl-4β,5β-dimethyl-1(10)-ene-2β,7α-dihydroxydecalin.

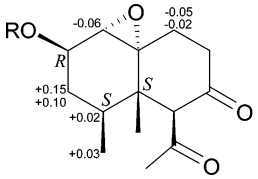
Laevinol E (**5**) gave a molecular formula of $C_{14}H_{20}O_3$ from the interpretation of its HREIMS and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectra of **5** were analogous to those of **2** and established that the secondary hydroxyl at C-7 in **2** was replaced by a ketone in **5**. The HMBC correlations from H-6 to C-7 and from H-8 to C-7 helped position the ketone carbonyl at C-7. Therefore, laevinol E was determined as 6α-acetyl-4β,5β-dimethyl-1(10)-ene-2α-hydroxy-7-oxodecalin.

The molecular formula of laevinol F (**6**) was found to be $C_{14}H_{22}O_4$ from its HREIMS and ^{13}C NMR data. The DEPT spectrum showed the presence of signals for three methyls, three sp^3 methylenes, five sp^3 methines, two sp^3 quaternary carbons, and one sp^2 quaternary carbon. The ^{1}H and ^{13}C NMR spectra supported the presence of two secondary hydroxyls at δ_H 3.98 (1H, t, $J = 4.2$ Hz, H-2), 4.18 (1H, br s, H-7) and δ_C 63.6 (CH, C-2), 67.4 (CH, C-7); a methyl ketone at δ_H 2.31 (3H, s, H_3-12) and δ_C 31.8 (CH_3, C-12), 210.0 (qC, C-11); and a trisubstituted epoxide ring at δ_H 3.14 (1H, d, $J = 4.2$ Hz, H-1) and δ_C 61.1 (CH, C-1), 67.4 (qC, C-10). These data were similar to those of 6α-acetyl-4β,5β-dimethyl-1(10)α-epoxy-2α-hydroxy-7-oxodecalin, but were consistent with the replacement of a ketone at C-7 by a secondary hydroxyl.[6] The HMBC correlations from H-6 to C-7 and from H-8 to C-7 enabled the correct positioning of the secondary hydroxyl at C-7. The configurations of the epoxide ring and hydroxyl at C-2 were determined by comparison with the $J_{1,2}$ and $J_{2,3}$ values of 6α-acetyl-4β,5β-dimethyl-1(10)α-epoxy-2α-hydroxy-7-oxodecalin ($J_{1,2} = J_{2,3\beta} = 4.5$ Hz, $J_{2,3\alpha} = 0$ Hz) and its 2-epimer.[2,13] The relative stereochemistry of **6** was deduced from a 2D NOESY experiment (Table S1, Supporting Information), which exhibited results similar to those determined from **3**. From these data, laevinol F could be formulated as 6α-acetyl-4β,5β-dimethyl-1(10)α-epoxy-2α,7β-dihydroxydecalin.

Laevinol G (**7**) was assigned a molecular formula of $C_{14}H_{22}O_4$, as indicated by its HREIMS and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectra of **7** were quite similar to those of **6** except for the resonances and splitting patterns in the vicinity of the secondary hydroxyl methine at C-2. The configurations of the epoxy and hydroxy group at C-2/C-3 were determined by comparison with $J_{1,2}$ and $J_{2,3}$ values of **6** and its 2-epimer.[2,13] The relative stereochemistry of **7** was established from a 2D NOESY experiment (Table S1, Supporting Information), which showed results similar to those determined from **6**. Therefore, laevinol G was formulated as 6α-acetyl-4β,5β-dimethyl-1(10)α-epoxy-2β,7β-dihydroxydecalin.

Laevinol H (**8**) was shown to have a molecular formula of $C_{14}H_{22}O_3$ by HREIMS and from its ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectroscopic data were similar to those of **7** except for the absence of the C-2 OH. HMBC correlations from H-1 to C-2/C-3 and from H-4 to C-2/C-3 confirmed the absence of the secondary hydroxyl at C-2. Therefore, laevinol H was assigned as 6α-acetyl-4β,5β-dimethyl-1(10)α-epoxy-7β-hydroxydecalin.

Laevinone A (**9**) gave a molecular formula of $C_{17}H_{22}O_4$, from its HREIMS and ^{13}C NMR data. The DEPT spectrum showed signals for four methyls, three sp^3 methylenes, two sp^3 methines, one sp^3 quaternary carbon, two sp^2 methines, and five sp^2 quaternary carbons. Analysis of its ^{1}H, ^{13}C, ^{1}H−^{1}H COSY, HMQC, and HMBC NMR spectral data revealed that **9** is a neolemnane sesquiterpene[3] containing



10a R = (*S*)-MTPA
10b R = (*R*)-MTPA

Δδ = δ(*S*)-MTPA − δ(*R*)-MTPA (ppm)

Figure 2. ^{1}H NMR chemical shift differences [δ(*S*)-MTPA − δ(*R*)-MTPA] of the MTPA esters.

a secondary acetoxyl at δ_H 5.98 (1H, s, H-4), 2.11 (3H, s) and δ_C 76.1 (CH, C-4), 20.5 (CH_3), 170.0 (qC); a methyl-bearing trisubstituted olefin at δ_H 5.55 (1H, s, H-2), 1.75 (3H, s, Me-14) and δ_C 136.9 (CH, C-2), 129.0 (qC, C-3), 18.1 (CH_3, C-14); a β,β-substituted enone at δ_H 6.13 (1H, s, H-9) and δ_C 128.7 (CH, C-9), 172.9 (qC, C-8), 197.6 (qC, C-10); and a secured ketone at δ_C 200.6 (qC, C-5). HMBC NMR correlations from H-9 [δ_H 6.13 (1H, s)] to C-10 [δ_C 197.6 (qC)] and C-11 [δ_C 39.1 (CH_2)] and from H_2-11 [δ_H 2.38 (2H, m)] to C-10 were used to position the enone at C-8 through C-10. The location of the secondary acetoxy and ketone at C-4, C-5 was demonstrated by HMBC correlations from H-4 [δ_H 5.98 (1H, s)] to C-5 [δ_C 200.6 (qC)], from H-14 [δ_H 1.75 (3H, s)] to C-4 [δ_C 76.1 (CH)], and from H_2-6 [δ_H 2.73 (2H, m)] to C-5. The relative configuration of **9** was deduced from a 2D NOESY experiment. NOESY correlations between H-2 and Me-13, Me-14, and Me-15 indicated that Me-13, Me-14, Me-15, and H-2 are on the β face of the molecule. In turn, a NOESY correlation between H-4 and H-12 suggested that H-12 and H-4 are on the opposite side of the molecule. From the aforementioned data, laevinone A was established as 4(*S**)-acetoxy-5,10-dioxo,1(*S**),12(*S**)-neolemna-2*Z*,8-diene.

The spectroscopic and physical data of **10** were identical with those of 6β-acetyl-4β,5β-dimethyl-1(10)α-epoxy-2β-hydroxy-7-oxodecalin[2] isolated from a soft coral, *Lemnalia africana*. To determine the absolute configuration, compound **10** was treated with (*R*)- or (*S*)-α-methoxy-α-trifluoromethylphenylacetyl chloride [(*R*)- or (*S*)-MTPA-Cl] in the presence of pyridine to yield the (*R*)- and (*S*)-MTPA esters (**10b** and **10a**), respectively.[14] The MTPA esters formed at C-2 were elucidated from the ^{1}H NMR chemical shifts and coupling constants of H-2 in **10a** and **10b** (**10a**, δ 5.33, 1H, t, J = 5.0 Hz, H-2; **10b**, δ 5.32, 1H, t, J = 5.0 Hz, H-2). Comparison of the ^{1}H NMR chemical shifts for **10a** and **10b** (Δ values shown in Figure 2) led to the assignment of the *R*-configuration at C-2. Therefore, the absolute structure of this compound was determined as shown in formula **10**.

Compound **11** exhibited cytotoxicity against P-388 and HT-29 cell lines with ED_{50} values of 0.21 and 0.33 μg/mL, respectively. The other isolates were not cytotoxic against P-388 and HT-29 cell lines (ED_{50} > 5 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO DIP-181 polarimeter. IR spectra were recorded on a Hitachi 26-30 spectrophotometer. The NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer at 300 MHz for ^{1}H and 75 for ^{13}C using $CDCl_3$ with TMS as internal standard. EIMS were obtained with a JEOL JMS-SX/SX 102A mass spectrometer at 70 eV. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography; precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

Animal Material. The soft coral *L. laevis* was collected at Green Island, off Taiwan, in March 2003, at a depth of 3 m and was stored for 2 weeks in a freezer until extraction. A voucher specimen, NSUGN-062, was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Taiwan.

Extraction and Isolation. The bodies of the soft coral *L. laevis* were freeze-dried to give 1.2 kg of a solid, which was extracted with CH_2Cl_2 (3.0 L × 3, overnight for each cycle) at room temperature. After removal of solvent in vacuo, the residue (40 g) was chromatographed over a column containing silica gel 60, using *n*-hexane−EtOAc and MeOH−EtOAc mixtures as eluting solvents. Elution by *n*-hexane−EtOAc (65:35) afforded fractions containing **1** and **9**. Elution by *n*-hexane−EtOAc (50:50) afforded fractions containing **2**. Elution by *n*-hexane−EtOAc (30:70) afforded fractions containing **7**, **10**, and **11**. Elution by *n*-hexane−EtOAc (1:3) afforded fractions containing **5** and **8**. Elution by EtOAc afforded fractions containing **3**, **4**, and **6**. Compound **1** (4 mg, 0.01%) was further purified using a RP-C_{18} HPLC column, eluting with MeOH−H_2O (85:15). Compound **2** (5 mg, 0.01%) was further purified by silica gel column chromatography, eluting with MeOH−CH_2Cl_2 (98:2). Compound **3** (2 mg, 0.005%) was further purified by passage over a RP-C_{18} HPLC column, eluting with MeOH−H_2O (52:48). Compound **4** (3 mg, 0.075%) was further purified by RP-C_{18} HPLC separation, eluting with MeOH−H_2O (70:30). Compound **5** (1 mg, 0.0025%) was further purified using a RP-C_{18} HPLC column, eluting with MeOH−H_2O (63:37). Compound **6** (3 mg, 0.0075%) was also purified further by RP-C_{18} HPLC separation, eluting with MeOH−H_2O (52:48). Compound **7** (1 mg, 0.0025%) was further purified by silica gel column chromatography, eluting with MeOH−CH_2Cl_2 (95:5). Compound **8** (3 mg, 0.0075%) was further purified by RP-C_{18} HPLC separation, eluting with MeOH−H_2O (63:37). Compound **9** (5 mg, 0.01%) was further purified by silica gel column chromatography, eluting with MeOH−CH_2Cl_2 (85:15). Compound **10** (4 mg, 0.01%) was further purified by silica gel column chromatography, eluting with MeOH−CH_2Cl_2 (99:1). Finally, compound **11** (25 mg, 0.05%) was further purified by silica gel column chromatography, eluting with *n*-hexane−acetone (85:15).

Laevinol A (1): $[\alpha]^{25}_D$ −173 (*c* 0.4, $CHCl_3$); IR (neat) ν_{max} 3449, 1720 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 266 $[M]^+$ (5), 248 (9), 202 (10), 176 (15), 232 (10), 201 (12), 173 (18), 160 (100); HREIMS *m/z* 266.1566 (calcd for $C_{15}H_{22}O_4$, 266.1569).

Laevinol B (2): $[\alpha]^{25}_D$ −132 (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3520, 1722 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 238 $[M]^+$ (3), 220 (28), 202 (10), 160 (32), 120 (56), 55 (100); HREIMS *m/z* 238.1568 (calcd for $C_{14}H_{22}O_3$, 238.1563).

Laevinol C (3): $[\alpha]^{25}_D$ −98 (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3480, 1718 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 238 $[M]^+$ (4), 220 (18), 202 (12); HREIMS *m/z* 238.1566 (calcd for $C_{14}H_{22}O_3$, 238.1563).

Laevinol D (4): $[\alpha]^{25}_D$ −136 (*c* 0.3, $CHCl_3$); IR (neat) ν_{max} 3448, 1723 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 238 $[M]^+$ (6), 220 (23), 202 (7), 160 (22); HREIMS *m/z* 238.1560 (calcd for $C_{14}H_{22}O_3$, 238.1563).

Laevinol E (5): $[\alpha]^{25}_D$ −145 (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3520,1725 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 236 $[M]^+$ (8), 218 (12), 200 (10); HREIMS *m/z* 236.1403 (calcd for $C_{14}H_{20}O_3$, 236.1407).

Laevinol F (6): $[\alpha]^{25}_D$ −76 (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3410, 1718 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 254 $[M]^+$ (6), 236 (10), 218 (8), 190 (30), 174 (52), 106 (100); HREIMS *m/z* 254.1515 (calcd for $C_{14}H_{22}O_4$, 254.1512).

Laevinol G (7): $[\alpha]^{25}_D$ −166 (*c* 0.3, $CHCl_3$); IR (neat) ν_{max} 3509, 1722 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 254 $[M]^+$ (4), 236 (11), 218 (7), 190 (33), 106 (100); HREIMS *m/z* 254.1516 (calcd for $C_{14}H_{22}O_4$, 254.1512).

Laevinol H (8): $[\alpha]^{25}_D$ −82 (*c* 0.1, $CHCl_3$); IR (neat) ν_{max} 3430, 1725 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 238 $[M]^+$ (8), 220 (12), 202 (9); HREIMS *m/z* 238.1569 (calcd for $C_{14}H_{22}O_3$, 238.1563).

Table 1. ^{1}H NMR Spectroscopic Data of Compounds **1**−**8** in $CDCl_3$[a]

position	1	2	3	4	5	6	7	8
1	5.69 d (4.8)[b]	5.66 d (4.5)	5.68 d (4.8)[b]	5.48 br s	5.86 d (5.1)	3.14 d (4.2)	2.86 br s	2.88 d (3.6)
2	3.99 br s	3.97 br s	3.99 br s	4.25 m	4.08 br s	3.98 t (4.2)	4.11 t (7.8)	1.88 m, 2.30 m
3	1.52 m	1.53 m	1.60 m	1.35 m, 1.74 m	1.65 m	1.36 m 1.58 m	1.31 m 1.78 m	1.28 m
4	1.85 m	1.84 m	1.90 m	1.65 m	2.15 m	1.99 m	1.86 m	1.97 m
6	3.42 d (5.4)	3.45 d (5.1)	3.28 br s	3.40 d (5.4)	3.93 s	3.07 br s	3.00 br s	2.97 br s
7	5.44 dt (12.0, 5.4)	4.25 dt (11.4, 5.1)	4.11 br s	4.25 m		4.18 br s	4.19 br s	4.18 m
8	1.89 m, 2.03 m	1.77 m, 2.39 m	1.72 m, 1.91 m	1.77 m, 1.95 m	2.48 m, 2.85 m	1.79 m, 2.26 m	1.83 m, 2.29 m	1.75 m, 2.32 m
9	2.29 m, 2.45 m	1.90 m, 2.24 m	2.08 m, 2.20 m	2.30 m, 2.40 m	2.65 m	2.54 dt (13.5, 4.2) 1.05 dt (13.5, 1.5)	2.53 dt (14.4, 4.8) 1.15 m	1.01 m, 2.53 m
12	2.19 s	2.34 s	2.24 s	2.21 s	2.22 s	2.31 s	2.31 s	2.33 s
13	0.95 d (6.9)	0.95 d (6.6)	0.92 d (6.9)	0.96 d (6.6)	0.93 d (6.9)	0.75 d (6.9)	0.78 d (6.9)	0.73 d (6.6)
14	1.02 s	1.00 s	1.17 s	1.05 s	0.91 s	1.31 s	1.34 s	1.27 s
OCHO	8.04 s							

[a] Recorded at 300 MHz (assigned by COSY, HSQC, and HMBC experiments). [b] J values (in Hz) in parentheses.

Table 2. ^{13}C NMR Spectroscopic Data of Compounds **1**−**9** in $CDCl_3$[a]

	1	2	3	4	5	6	7	8	9
1	123.9	123.3	122.5	126.6	124.7	61.1	61.8	58.6	45.1
2	63.7	63.9	63.9	67.4	63.7	63.6	64.8	21.8	136.9
3	35.1	35.2	35.3	36.6	35.5	35.8	34.9	24.4	129.0
4	29.7	29.8	29.7	34.3	28.7	27.1	26.1	29.9	76.1
5	42.6	41.9	40.3	42.0	46.6	37.7	37.1	38.2	200.6
6	57.6	60.8	63.1	60.7	74.8	62.0	62.6	63.4	43.3
7	71.6	69.0	67.0	69.3	202.9	67.4	67.2	67.9	29.5
8	26.2	30.2	29.7	30.3	37.6	27.2	27.4	27.4	172.9
9	29.7	30.1	26.2	29.9	31.3	25.1	24.8	25.4	128.7
10	143.0	144.0	145.8	140.8	141.8	67.4	63.2	63.8	197.6
11	210.5	213.1	210.5	212.0	205.0	210.0	207.2	208.7	42.1
12	35.4	36.2	32.8	35.8	33.3	31.8	30.8	31.3	39.1
13	15.6	15.6	15.9	15.7	15.6	15.7	15.1	16.0	20.6
14	18.0	18.2	20.0	19.8	18.3	18.4	18.4	18.6	18.1
15									16.8
OCHO	160.2								
OAc									170.0
									20.5

[a] Recorded at 75 MHz (assigned by DEPT, COSY, HSQC, and HMBC experiments).

Laevinone A (9): $[\alpha]^{25}_D$ +165 (*c* 0.4, $CHCl_3$); UV λ_{max} (log ϵ) 232 (3.2) nm; IR (neat) ν_{max} 1724, 1667 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (3H, d, J = 6.6 Hz, Me-15), 1.20 (3H, s, Me-13), 1.75 (3H, s, Me-14), 2.11 (3H, s, $OCOCH_3$), 2.38 (2H, m, H_2-11), 2.40 (1H, m, H-7β), 2.54 (1H, dq, J = 7.0, 2.0 Hz, H-12), 2.62 (1H, m, H-7α), 2.73 (2H, m, H_2-6), 5.55 (1H, s, H-2), 5.98 (1H, s, H-4), 6.13 (1H, s, H-9); ^{13}C NMR, see Table 2; EIMS *m/z* 290 $[M]^+$ (4), 272 (5), 230 (18), 188 (16), 91 (100); HREIMS *m/z* 290.1599 (calcd for $C_{17}H_{22}O_4$, 290.1596).

(*R*)- and (*S*)-MTPA Derivatives of 10. To a solution of compound **10** (1.0 mg, 4.0 × 10^{-3} mmol) in pyridine (0.5 mL) at room temperature was added (*R*)-MTPA-Cl (2.4 mg, 1.0 × 10^{-2} mmol), and the resultant mixture was stirred for 24 h at room temperature. The reaction mixture was worked up by adding 2 mL of water. Further purification was performed with a short silica gel column with CH_2Cl_2 to give **10b** (0.6 mg) as a colorless oil. The (*S*)-MTPA ester **10a** (0.5 mg) was prepared in the same way. Selected $\Delta\delta$ values [δ (*S*) − δ (*R*)] are as follows: H-1 = −0.06, H-3α = +0.10, H-3β = +0.15, H-4 = +0.02, H-9α = −0.02, H-9β = −0.05, H_3-13 = + 0.03.

(*S*)-MTPA ester of 10: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.70 (3H, d, J = 6.9 Hz, H_3-13), 1.24 (3H, s, H_3-14), 1.45 (1H, m, H-3α), 1.46 (1H, m, H-9α), 1.70 (1H, m, H-3β), 2.15 (1H, m, H-4), 2.22 (3H, s, H_3-12), 2.47 (1H, s, H-9β), 2.50 (1H, m, H-8β), 2.76 (1H, m, H-8α), 3.58 (3H, OMe), 3.57 (1H, s, H-1), 3.74 (1H, s, H-6), 5.33 (1H, t, J = 5.0 Hz, H-2), 7.40−7.65 (5H, aromatic H).

(*R*)-MTPA ester of 10: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.67 (3H, d, J = 6.9 Hz, H_3-13), 1.24 (3H, s, H_3-14), 1.35 (1H, m, H-3α), 1.48 (1H, m, H-9α), 1.60 (1H, m, H-3β), 2.13 (1H, m, H-4), 2.23 (3H, s, H_3-12), 2.52 (1H, s, H-9β), 2.53 (1H, m, H-8β), 2.72 (1H, m, H-8α), 3.63 (1H, s, H-1), 3.66 (3H, OMe), 3.74 (1H, s, H-6), 5.32 (1H, t, J = 5.0 Hz, H-2), 7.39−7.66 (5H, aromatic H).

Cytotoxicity Testing. P-388 (mouse lymphocytic leukemia) cells were kindly supplied by J. M. Pezzuto, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago; A-549 (human lung adenocarcinoma) and HT-29 (human colon adenocarcinoma) were purchased from the American Type Culture Collection. Cytotoxic assays were carried out according to the procedure described previously.[3] Three concentrations (50, 5, and 0.5 μg/mL) of the tested compounds were used in the cytotoxicity assays.

Acknowledgment. We thank J. M. Pezzuto, formerly of the Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, for the provision of P-388 cell lines. This work was supported by grants from the National Science Council and Ministry of Education of Taiwan awarded to C.-Y.D.

Supporting Information Available: NOESY correlations of **1**−**9** are available free of charge via the Internet at http://pubs.acs.org.

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NP050326R