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

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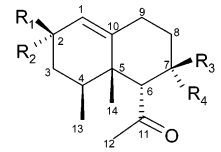
Eight new nornardosinane sequiterpenoids, laevinols A−H (**1**−**8**), a new neolemnane sesquiterpenoid, laevinone A (**9**), and the previously known 6β-acetyl-4β,5β-dimethyl-1(10)α-epoxy-2β-hydroxy-7-oxodecalin (**10**) and 11,12-dihydroxy-6,10-eremophiladiene (**11**) were isolated from the methylene 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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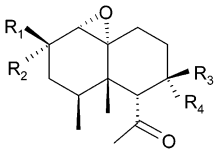
3 R_1 = H, R_2 = OH, R_3 = OH, R_4 = H
4 R_1 = OH, R_2 = H, R_3 = H, R_4 = OH
5 R_1 = H, R_2 = OH, R_3, R_4 = O



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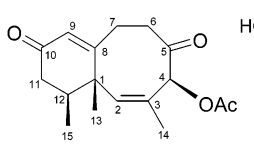

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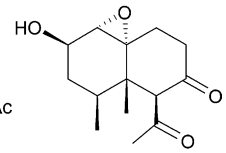

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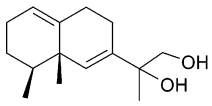

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Results and Discussion

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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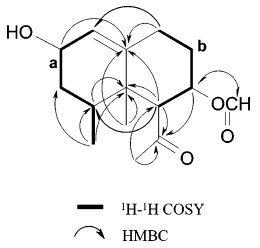


Figure 1. Key COSY and HMBC correlations of **1**.

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.

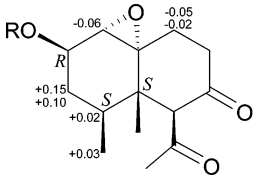
Laevinol E (**5**) gave a molecular formula of $C_{14}H_{20}O_3$ from the interpretation of its HREIMS and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectra of **5** were analogous to those of **2** and established that the secondary hydroxyl at C-7 in **2** was replaced by a ketone in **5**. The HMBC 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.

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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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