Guaiane Sesquiterpene Lactones and Amino Acid-Sesquiterpene Lactone Conjugates from the Aerial Parts of *Saussurea pulchella*

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Received January 3, 2008

Two new guaiane sesquiterpene lactones (**1** and **2**) and seven new amino acid-sesquiterpene lactone conjugates (**3**−**9**), together with six known sesquiterpene lactones (**10**−**15**), were isolated from the methanol extract of the aerial parts of *Saussurea pulchella*. Their structures were determined on the basis of spectroscopic and chemical methods to be 8α-*O*-(3′-hydroxy-3′-methylbutyryl)desacylcynaropicrin (**1**), 8α-*O*-(2′, 3′-dihydroxyisobutyryl)11*β*,13-dihydrodesacylcynaropicrin (**2**), and pulchellamines A, B, C, D, E, F, and G (**3**−**9**). The structures of the new amino acid-sesquiterpene lactone conjugates, pulchellamines A, B, C, D, E, F, and G (**3**−**9**), were confirmed by synthesis. The isolated compounds were evaluated for cytotoxic activity against four human tumor cell lines. Compounds **11** and **12** exhibited cytotoxicity against skin melanoma (SK-MEL-2) and ovary malignant ascites (SK-OV-3) human tumor cell lines with ED_{50} values of 1.53 and 4.07 μM, and 2.49 and 7.42 μM, respectively.

In a continuing search for bioactive constituents from Korean Asteraceae medicinal plants,[1,2] the sesquiterpene lactones from the aerial parts of *Saussurea pulchella* Fisch (Asteraceae) were investigated. Sesquiterpenes,[3–7] lignans,[8–10]and flavonoids[11,12] have been reported as constituents of the genus *Saussurea* and have been found to possess a wide range of biological activities, including cytotoxic,[3–5] anti-inflammation,[6,7] and antioxidant activities.[11,12] Although there have been a number of studies on the chemical constituents and biological activities of the genus *Saussurea*, there have been few phytochemical investigations on *S. pulchella*, and to date only a sesquiterpene lactone[13,14] and four phenolics[15,16] have been reported. *S. pulchella* is widely distributed in Korea and has been used in Korean traditional medicine for the treatment of inflammation, hypertension, hepatitis, and arthritis.[17] We reported lignan and terpene constituents from the MeOH extract of the aerial parts of *S. pulchella*.[18] In our continuing study on this plant source, we have identified two new guaiane sesquiterpene lactones (**1** and **2**) and seven new amino acid-sesquiterpene lactone conjugates (**3**−**9**), together with six known sesquiterpene lactones (**10**−**15**), from the MeOH extract. The structures were determined on the basis of spectroscopic and chemical methods. Here we report the isolation, structural characterization, and cytotoxicity of the isolated sesquiterpene lactones.

Results and Discussion

Column chromatographic separation of the MeOH extract of *S. pulchella* afforded two new guaiane sesquiterpene lactones (**1** and **2**) and seven new amino acid-sesquiterpene lactone conjugates (**3**−**9**), as well as six known compounds, desacylcynaropicrin (**10**),[19,20] 8α-(4′-hydroxysenecioyloxy)dehydrozaluzanin C (**11**),[21] cynaropicrin (**12**),[22] 11*β*,13-dihydrodesacylcynaropicrin (**13**),[23,24] 3α-dihydro-4(15)-dehydrogrosshemin α,*β*-dihydroxyisoburyrate (**14**),[25] and 11*β*,13-dihydrodesacylcynaropicrin 8-*β*-D-glucoside (**15**).[26] (Figure 1).

Compound **1** was obtained as a colorless oil, $[\alpha]_D$ +23.0 (*c* 0.3, MeOH). The molecular formula of $C_{20}H_{26}O_6$ was determined from the molecular ion peak at *m*/*z* 362.1732 $[M]^+$ (calcd for $C_{20}H_{26}O_6$, 362.4228) obtained by HREIMS. The IR spectrum displayed absorption bands for hydroxy (3401 cm^{-1}), *γ*-lactone (1766 cm^{-1}), and ester functional groups (1732 cm^{-1}). The ^1H and ^{13}C NMR chemical shifts of H-13 at *δ* 6.25 (d, *J* = 3.5 Hz) and 5.66 (d, *J* = 3.0 Hz), H-6 at *δ* 4.23 (dd, *J* = 10.0, 9.0 Hz), H-7 at *δ* 3.12 (dddd, *J* = 10.0, 9.0, 3.5, 3.0 Hz), and C-12 at *δ* 168.9 indicated the presence of an α-exomethylene-6,7-*γ*-lactone moiety. Additionally, the ^1H and ^{13}C NMR spectra of **1** displayed resonances for two exomethylenes {*δ* 5.16 (s), 4.97 (d, *J* = 1.0 Hz); *δ* 141.6, 118.2, 5.50 (t, *J* = 2.0 Hz), 5.37 (t, *J* = 1.5 Hz); *δ* 152.2, 113.6}, two oxygenated methines {*δ* 4.57 (dddd, *J* = 7.5, 7.0, 2.0, 1.5 Hz); *δ* 73.7, 5.09 (m); *δ* 74.2}, and two methines {*δ* 2.98 (ddd, *J* = 10.0, 9.0, 7.5 Hz); *δ* 45.3, 2.84 (dd, *J* = 10.5, 9.0 Hz); *δ* 51.3}. These data were similar to those of **10**, which was also isolated from this plant source. The difference was only the addition of a 3-hydroxy-3-methylbutyryl group {*δ* 2.59 (2H, s, H-2′); *δ* 46.5 (C-2′), 1.36 (6H, d, *J* = 3.0 Hz, H-4′, 5′); *δ* 29.3 (C-4′), 29.2 (C-5′), 172.0 (C-1′), and 69.2 (C-3′)}.[27] In the HMBC spectrum, correlation of H-8 (*δ* 5.09, m) with C-1′ (*δ* 172.0) supported the connectivity of a 3-hydroxy-3-methylbutyrate group at C-8 (Figure 2). According to the *J* values in the ^1H NMR spectrum, the stereochemistry of **1** was expected to be the same as **10**. This was confirmed by the NOESY correlations (Figure 2). On the basis of these findings, the structure of **1** was determined to be 8α-*O*-(3′-hydroxy-3′-methylbutyryl)desacylcynaropicrin.

Compound **2** was obtained as a colorless oil, $[\alpha]_D$ +24.1 (*c* 0.1, MeOH). The molecular formula of $C_{19}H_{26}O_7$ was determined from the molecular ion peak at *m*/*z* 366.1679 $[M]^+$ (calcd for $C_{19}H_{26}O_7$, 366.4112) in the HREIMS. The IR spectrum showed a *γ*-lactone (1769 cm^{-1}) and an ester functional group (1735 cm^{-1}). The IR and ^1H and ^{13}C NMR data of **2** were very similar to those of **13**, which was isolated from this plant. The differences were due to the addition of an α,*β*-dihydroxyisobutyryl group {*δ* 3.61 (1H, d, *J* = 10.5 Hz, H-3′), 3.75 (1H, d, *J* = 10.5 Hz, H-3′); *δ* 69.3 (C-3′), 1.32 (3H, s, H-4′); *δ* 22.3 (C-4′), 175.6 (C-1′), and 76.9 (C-2′)}.[25] In the HMBC spectrum, correlation of the H-8 (*δ* 5.02, m) with C-1′ (*δ* 175.6) supported the connectivity of the α,*β*-dihydroxyisoburyrate at C-8. According to the ^1H and ^{13}C NMR data, the relative configuration of **2** is expected to be the same as **13**. This was confirmed from the correlations observed in the NOESY spectrum. The configuration at C-2′ was not determined, however.

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10.1021/np800005r CCC: $40.75
Published on Web 03/04/2008

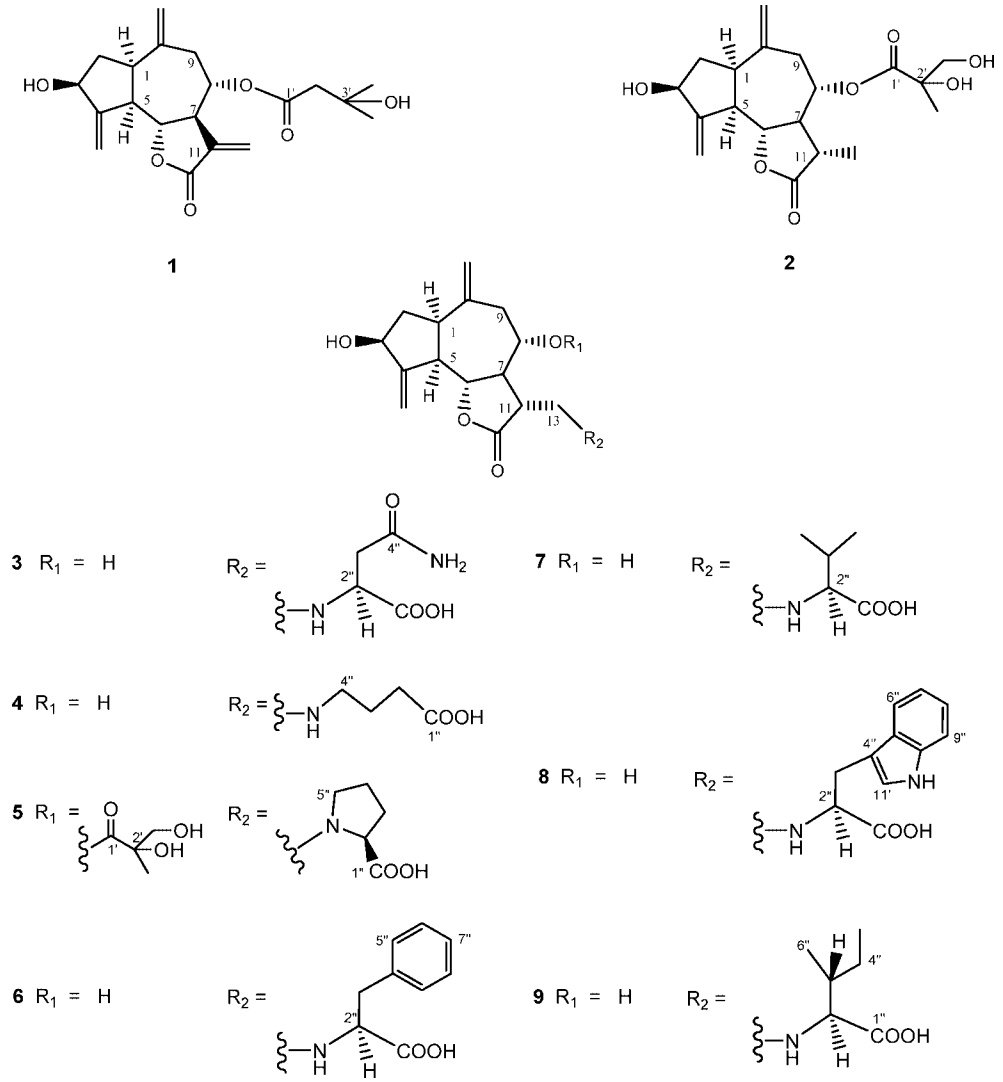

Figure 1. Structures of compounds **1**−**9** isolated from the MeOH extract of *S. pulchella*.

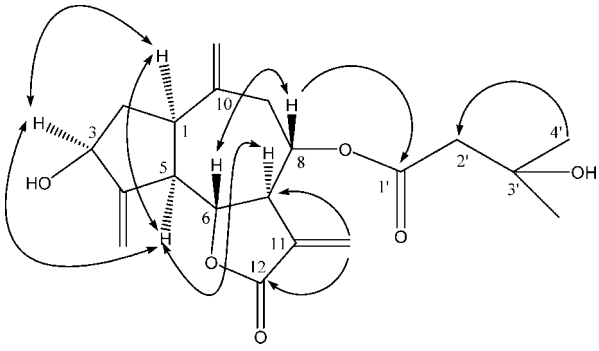

Figure 2. Key HMBC (→) and NOESY(↔) correlations of **1**.

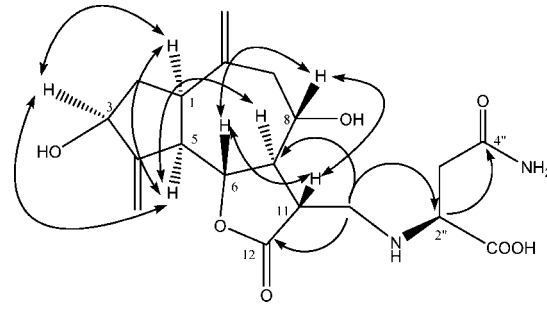

Figure 3. Key HMBC (→) and NOESY(↔) correlations of **3**.

Thus, the structure of **2** was determined to be 8α-*O*-(2′, 3′-dihydroxyisobutyryl)11β,13-dihydrodesacylcynaropicrin.

Compound **3**, a colorless gum, $[\alpha]_D$ +41.4 (*c* 0.3, MeOH), was deduced to possess a nitrogen function on the basis of the ninhydrin test. The molecular formula of $C_{19}H_{27}N_2O_7$ was determined from the molecular ion peak at *m*/*z* 395.1827 $[M + H]^+$ (calcd for $C_{19}H_{27}N_2O_7H$, 395.1812) obtained by HRESIMS. The IR spectrum exhibited absorption bands for hydroxy (3338 cm^{-1}) and γ-lactone functional groups (1762 cm^{-1}). The ^1H and ^{13}C NMR data were very similar to those of **13**, except for the additional appearance of the side chain moiety, an L-asparagine {^1H NMR δ 3.97 (dd, J = 6.1, 4.5 Hz, H-2″), 3.02 (dd, J = 17.1, 4.5 Hz, H-3″a), 2.96 (dd, J = 17.1, 6.1 Hz, H-3″b); ^{13}C NMR δ 174.5 (C-1″), 59.3 (C-2″), 33.9 (C-3″), 172.8 (C-4″)},[6,28] and the replacement of a CH_3 group {δ 1.42 (d, J = 7.0 Hz), 15.9} with a methylene moiety {δ 3.64 (dd, J = 12.6, 5.2 Hz), 3.42 (dd, J = 12.6, 8.1 Hz), 47.3}. The resonances of H-13a (δ 3.64, dd, J = 12.6, 5.2 Hz) and H-13b (δ 3.42, dd, J = 12.6, 8.1 Hz) showed correlations with the methine carbon (δ 59.3, C-2″) of the L-asparagine moiety in the HMBC spectrum (Figure 3), indicating the position of the L-asparagine moiety at C-13. On the basis of the J values of the ^1H NMR data, the stereochemistry of **3** was proposed to be the same as **13**. This was confirmed by the NOESY results (Figure 3). Thus, the structure of **3** was determined and it was named pulchellamine A. The structure of pulchellamine A was confirmed by synthesis from

desacylcynaropicrin (**10**) and L-asparagine using a Michael-type addition reaction.[6] The synthesized **3** was identified by comparison of its spectroscopic data (^{1}H NMR, ESIMS, and $[\alpha]_D$) with those of the isolated compound.

Compound **4**, a white crystal from MeOH−H_2O, mp 198 °C, $[\alpha]_D$ +52.0 (*c* 0.15, MeOH), showed a positive ninhydrin test. The molecular formula of $C_{19}H_{27}NO_6$ was determined from the molecular ion peak at *m/z* 366.1927 $[M + H]^+$ (calcd for $C_{19}H_{27}NO_6H$, 366.1911) obtained by HRESIMS. Its IR spectrum revealed absorption bands for hydroxy (3297 cm^{-1}) and γ-lactone functional groups (1764 cm^{-1}). The ^{1}H and ^{13}C NMR data were similar to those of **3**, except for a 4-aminobutanoic acid moiety {^{1}H NMR δ 2.45 (td, J = 11.4, 7.2 Hz, H-2″a), 2.60 (m, H-2″b), 1.62 (q, J = 7.2 Hz, H-3″), 2.22 (2H, t, J = 7.2 Hz, H-4″); ^{13}C NMR δ 174.4 (C-1″), 47.9 (C-2″), 24.3 (C-3″), 31.9 (C-4″)}.[29,30] The H-13a resonances (δ 3.01, dd, J = 12.6, 3.0 Hz) and H-13b (δ 2.53, dd, J = 12.6, 4.8 Hz) showed correlations with the methylene carbon (δ 31.9, C-4″) of the 4-aminobutanoic acid moiety in the HMBC spectrum, indicating the position of this moiety at C-13. The stereochemistry of **4** was proposed to be the same as **3**, on the basis of the *J* values. This was confirmed by a NOESY experiment. Thus, the structure of **4** was determined and it was named pulchellamine B. The structure of pulchellamine B was confirmed by synthesis from desacylcynaropicrin (**10**) and 4-aminobutanoic acid using a Michael-type addition reaction.[6] The synthesized **4** was identified by comparison of its spectroscopic data (^{1}H NMR, ESIMS, and $[\alpha]_D$) with those of the isolated compound.

Compound **5**, a colorless gum, $[\alpha]_D$ +22.0 (*c* 0.5, MeOH), showed a positive ninhydrin test. The molecular formula of $C_{24}H_{33}NO_9$was determined from the molecular ion peak at *m/z* 480.2249 $[M + H]^+$ (calcd for $C_{24}H_{33}NO_9H$, 480.2228) obtained by HRESIMS. The IR spectrum displayed absorption bands for hydroxy (3305 cm^{-1}) and γ-lactone functional groups (1755 cm^{-1}). The ^{1}H and ^{13}C NMR data were similar to those of **2**, except for the appearance of an L-proline moiety {^{1}H NMR δ 3.79 (t, J = 7.0 Hz, H-2″), 2.21 (2H, m, H-3″), 1.67, 1.84 (each m, H-4″a, 4″b), 3.46 (dt, J = 10.0, 5.0 Hz, H-5″a), 2.76 (q, J = 10.0 Hz, H-5″b); ^{13}C NMR δ 177.2 (C-1″), 68.8 (C-2″), 30.5 (C-3″), 25.1 (C-4″), 55.5 (C-5″)}[6,28] and the replacement of a CH_3 group {δ 1.42 (d, J = 7.0 Hz), 15.9} with methylene {δ 3.67 (dd, J = 13.5, 6.0 Hz), 2.53 (d, J = 13.5 Hz), 55.3}. The H-13a (δ 3.67, dd, J = 13.5, 6.0 Hz) and H-13b (δ 3.56, d, J = 13.5 Hz) resonances showed correlations with the methine carbon (δ 68.8, C-2″) of the L-proline moiety in the HMBC spectrum, indicating the position of the L-proline moiety at C-13. The stereochemistry of **5** was propsed to be the same as **2**, based on the *J* value of the ^{1}H NMR data. This was confirmed by a ROESY experiment. The configuration at C-2′ was not determined. Thus, the structure of **5** was determined and it was named pulchellamine C. The structure of pulchellamine C was confirmed by synthesis from 3α-dihydro-4(15)-dehydro-grosshemin α,β-dihydroxyisoburyrate (**14**) and L-proline using a Michael-type addition reaction.[6] The synthesized **5** was identified by comparison of its ^{1}H NMR, ESIMS, and $[\alpha]_D$ value with those of the isolated compound.

Compound **6**, a colorless gum, $[\alpha]_D$ +74.0 (*c* 0.3, MeOH), showed a positive ninhydrin test. The molecular formula of $C_{24}H_{29}NO_6$ was determined from the molecular ion peak at *m/z* 428.2080 $[M + H]^+$ (calcd for $C_{24}H_{29}NO_6H$, 428.2067) obtained by HRESIMS. The IR spectrum displayed absorption bands for hydroxy (3333 cm^{-1}) and γ-lactone groups (1763 cm^{-1}). The ^{1}H and ^{13}C NMR data were similar to those of **4**. The spectra showed the characteristic signals of an L-phenylalanine moiety {^{1}H NMR δ 3.98 (m, H-2″), 3.33 (2H, d, J = 6.1 Hz, H-3″), 7.29 (2H, t, J = 7.3 Hz, H-5″, 9″), 7.49 (2H, d, J = 7.3 Hz, H-6″, 8″), 7.22 (t, J = 8.0 Hz, H-7″); ^{13}C NMR δ 176.4 (C-1″), 64.3 (C-2″), 40.2 (C-3″), 138.8 (C-4″), 130.5 (C-5″, 9″), 129.1 (C-6″, 8″), 127.3 (C-7″)}.[6,28] The position of the L-phenylalanine moiety was confirmed by the HMBC spectrum. The H-13a (δ 3.98, m) and H-13b (δ 3.12, br t, J = 11.6 Hz) resonances showed correlations with the methine carbon (δ 64.3, C-2″) of the L-phenylalanine moiety. The stereochemistry of **6** was assumed to be the same as **4**, on the basis of the *J* values. This was confirmed by a NOESY experiment. Thus, the structure of **6** was determined and it was named pulchellamine D. Pulchellamine D was synthesized from desacylcynaropicrin (**10**) and L-phenylalanine.[6] The synthesized pulchellamine D was identified by comparison of its $[\alpha]_D$, ^{1}H NMR, and ESIMS data with those of the isolated compound (**6**).

Compound **7**, a white crystal from MeOH−H_2O, mp 220 °C, $[\alpha]_D$ +29.0 (*c* 0.2, MeOH), was deduced to possess a nitrogen function on the basis of the ninhydrin test. The molecular formula of $C_{20}H_{29}NO_6$ was determined from the molecular ion peak at *m/z* 380.2067 $[M + H]^+$ (calcd for $C_{20}H_{29}NO_6H$, 380.2068) obtained by HRESIMS. The IR spectrum revealed absorption bands for hydroxy (3333 cm^{-1}) and γ-lactone functional groups (1765 cm^{-1}). The ^{1}H and ^{13}C NMR data were similar to those of **6**. The ^{1}H and ^{13}C NMR spectra showed the characteristics of an L-valine moiety {^{1}H NMR δ 2.82 (m, H-2″), 1.88 (m, H-3″), 0.87 (3H, d, J = 6.6 Hz, H-4″), 0.90 (3H, d, J = 6.6 Hz, H-5″); ^{13}C NMR δ 173.6 (C-1″), 67.6 (C-2″), 22.0 (C-3″), 19.2 (C-4"), 18.3 (C-5″)}.[6,28] The H-13a (δ 3.18, m) and H-13b (δ 2.87, m) resonances showed HMBC correlations with the methine carbon (δ 67.6, C-2″) of the L-valine moiety. The stereochemistry of **7** was assumed to be the same as **13**, on the basis of the *J* values of the ^{1}H NMR data. This was confirmed from the correlations observed in the NOESY spectrum. Thus, the structure of **7** was determined and it was named pulchellamine E. The structure of pulchellamine E was confirmed by synthesis from desacylcynaropicrin (**10**) and L-valine using a Michael-type addition reaction.[6] The synthesized **7** was identified by comparison of its $[\alpha]_D$, ^{1}H NMR, and ESIMS data with those of the isolated compound.

Compound **8**, a colorless gum, $[\alpha]_D$ +40.4 (*c* 0.4, MeOH), showed a positive ninhydrin test. The molecular formula of $C_{26}H_{30}N_2O_6$ was determined from the molecular ion peak at *m/z* 467.2190 $[M + H]^+$ (calcd for $C_{26}H_{30}N_2O_6H$, 467.2176), obtained by HRESIMS. The IR spectrum showed absorption bands for hydroxy (3301 cm^{-1}), γ-lactone (1762 cm^{-1}), and C=C functional groups (1634 cm^{-1}). The ^{1}H and ^{13}C NMR data were similar to those of **6** and **7**. The differences were the amino acid (L-tryptophan) moiety {^{1}H NMR δ 3.87 (t, J = 5.5 Hz, H-2″), 3.41, 3.87 (each m, H-3″a, 3″b), 7.39 (d, J = 8.0 Hz, H-6″), 7.07 (dt, J = 7.0, 1.0 Hz, H-7″), 7.14 (dt, J = 7.0, 1.0 Hz, H-8″), 7.69 (d, J = 8.0 Hz, H-9″), 7.24 (s, H-11″); ^{13}C NMR δ 173.6 (C-1″), 63.7 (C-2″), 26.4 (C-3″), 108.3 (C-4″), 128.8 (C-5″), 122.9 (C-6″), 120.4 (C-7″), 119.5 (C-8″), 112.9 (C-9″), 138.3 (C-10″), 125.8 (C-11″)}.[6,28] The H-13a (δ 3.87, m) and H-13b (δ 2.99, dd, J = 12.5, 10.0 Hz) resonances showed HMBC correlations with the methine carbon (δ 63.7, C-2″) of the L-tryptophan moiety. The stereochemistry of **8** was assumed to be the same as **7**, on the basis of the *J* values of the ^{1}H NMR data and the data of the NOESY experiment. Thus, the structure of **8** was determined and it was named pulchellamine F. The structure of pulchellamine F was confirmed by synthesis from desacylcynaropicrin (**10**) and L-tryptophan using a Michael-type addition reaction.[6] The ^{1}H NMR, ESIMS, and $[\alpha]_D$ values of the synthetic **8** was identical to the data obtained for the isolated pulchellamine F.

Compound **9**, a white crystal from MeOH−H_2O, mp 199 °C, $[\alpha]_D$ +15.4 (*c* 0.1, MeOH), showed a positive ninhydrin test. The molecular formula of $C_{21}H_{31}NO_6$was determined from the molecular ion peak at *m/z* 394.2214 $[M + H]^+$ (calcd for $C_{21}H_{31}NO_6H$, 394.2224), obtained by HRESIMS. The IR spectrum displayed absorption bands for hydroxy (3372 cm^{-1}) and γ-lactone functional groups (1766 cm^{-1}). The ^{1}H NMR data of the sesquiterpene part in **9** is rather different from those of **7** and **8**. The differences were the amino acid (L-isoleucine) moiety {^{1}H NMR δ 2.96 (d, J = 5.0

Table 1. ^{1}H NMR Data of Compounds **1**−**4**

	1[a]	**2**[b]	**3**[c]	**4**[d]
H-1	2.98 (ddd, 10.5, 9.0, 7.5)	2.99 (ddd, 10.0, 9.0, 7.5)	3.04 (m)	2.80 (m)
H-2a	2.22 (ddd, 13.5, 7.5, 7.0,)	2.20 (ddd, 13.0, 7.5, 7.0)	2.36 (td, 13.1, 5.5)	2.07 (m)
H-2b	1.71 (ddd, 13.5, 10.5, 7.5,)	1.71 (ddd, 13.0, 10.0, 8.5)	1.71 (td, 13.1, 8.5)	1.52 (td, 12.6, 8.4)
H-3	4.57 (dddd, 7.5, 7.0, 2.0, 1.5)	4.52 (dddd, 8.5, 7.0, 2.5, 2.0)	4.62 (br t, 5.5)	4.31 (br t, 8.4)
H-5	2.84 (dd, 10.5, 9.0)	2.85 (m)	3.04 (m)	2.80 (m)
H-6	4.23 (dd, 10.0, 9.0)	4.25 (t, 10.0)	4.36 (br t, 10.0)	4.01 (br t, 10.0)
H-7	3.12 (dddd, 10.0, 9.0, 3.5, 3.0)	2.00 (ddd, 10.0, 10.0, 10.0)	2.49 (ddd, 10.0, 10.0, 10.0)	2.12 (ddd, 10.0, 10.0, 10.0)
H-8	5.09 (m)	5.02 (m)	3.89 (m)	3.50 (m)
H-9a	2.70 (dd, 14.5, 5.5)	2.77 (dd, 13.5, 5.0)	2.78 (dd, 13.0, 4.9)	2.56 (m)
H-9b	2.37 (dd, 14.5, 4.0)	2.22 (dd, 13.5, 6.0)	2.27 (dd, 13.0, 7.9)	2.04 (dd, 12.6, 7.8)
H-11		2.55 (dd, 10.0, 7.0)	3.29 (m)	2.71 (ddd, 10.0, 4.8, 3.0)
H-13	6.25 (d, 3.5)/5.66 (d, 3.0)	1.42 (d, 7.0, 3H)	3.64 (dd, 12.6, 5.2)/3.42 (dd, 12.6, 8.1)	3.01 (dd, 12.6, 3.0)/2.53 (dd, 12.6, 4.8)
H-14	5.16 (s)/4.97 (d, 1.0)	5.15 (s)/5.02 (s)	5.09 (2H, s)	4.92 (s)/4.87 (s)
H-15	5.50 (t, 2.0)/5.37 (t, 1.5)	5.43 (d, 2.5)/5.30 (d, 2.0)	5.33 (s)/5.30 (s)	5.14 (s)/5.10 (s)
H-2′	2.59 (s, 2H)			
H-3′		3.61 (d, 10.5)/3.75 (d, 10.5)		
H-4′	1.36 (d, 3.0, 3H)	1.32 (s, 3H)		
H-5′	1.36 (d, 3.0, 3H)			
H-2″			3.97 (dd, 6.1, 4.5)	2.45 (td, 11.4, 7.2)/2.60 (m)
H-3″			3.02 (dd, 17.1, 4.5)/2.96 (dd, 17.1, 6.1)	1.62 (2H, q, 7.2)
H-4″				2.22 (2H, t, 7.2)

[a] Measured in $CDCl_3$ at 500 MHz. [b] Measured in CD_3OD at 500 MHz. [c] Measured in D_2O at 500 MHz. [d] Measured in DMSO-d_6 at 500 MHz.

Hz, H-2″), 1.64 (m, H-3″), 1.14, 1.47 (m, H-4″a, 4″b), 0.86 (3H, d, J = 7.0 Hz, H-5″), 0.83 (3H, d, J = 7.0 Hz, H-6″); ^{13}C NMR δ 173.2 (C-1″), 66.4 (C-2″), 36.9 (C-3″), 24.9 (C-4″), 11.5 (C-5″), 15.5 (C-6″)}.[6,28] The H-13a (δ 3.17, dd, J = 12.0, 3.5 Hz) and H-13b (δ 2.55, m) resonances showed HMBC correlations with the methine carbon (δ 66.4, C-2″) of the L-isoleucine moiety. The stereochemistry of **9** was assumed to be the same as **8**, on the basis of the J values of the ^{1}H NMR data. This was confirmed by a NOESY experiment. Thus, the structure of **9** was determined and it was named pulchellamine G. The structure of pulchellamine G was confirmed by synthesis from deacylcynaropicrin (**10**) and L-isoleucine using a Michael-type addition reaction.[6] The synthesized **9** was identified by comparison of its $[\alpha]_D$, ^{1}H NMR, and ESIMS data with those of the isolated compound.

The isolated compounds were evaluated for cytotoxic activity against four human tumor cell lines (Table 4). Compounds **11** and **12** exhibited cytotoxicity against skin melanoma (SK-MEL-2) and ovary malignant ascites (SK-OV-3) human tumor cell lines with ED_{50} values of 1.53 and 4.07 μM, and 2.49 and 7.42 μM, respectively. Compounds **1**, **5**, and **10** were moderately cytotoxic against a skin melanoma (SK-MEL-2) human tumor cell line with ED_{50} values 6.78, 5.73, and 9.74 μM, respectively. The other compounds showed little cytotoxicity against the tested cell lines.

Considering structure–activity relationships for the cytotoxicity against four tested cancer cell lines of the isolated compounds, the presence of a side chain at C-8 increases the cytotoxicity and the α-exomethylene-γ-lactone ring is essential for cytotoxic activity.

Experimental Section

General Experimental Procedures. Melting points were determined on a Gallenkamp melting point apparatus and were uncorrected. Optical rotations were measured using a JASCO P-1020 polarimeter (JASCO Co., Japan). UV spectra were recorded on an Agilent 8453 UV spectrophotometer (Agilent Co.), and the IR spectra were recorded on a Bruker IFS-66/S instrument (Bruker Co., Germany). NMR spectra were obtained on either a Bruker Biospin Avance 500 (Bruker Co., Germany) or a Varian Unity INOVA 500 NB NMR spectrometer (Varian Co.). The EI, FAB, ESIMS, HREIMS, and HRESIMS data were obtained on a JEOL JMS 700 (JEOL Co., Japan) and Mariner (Perseptive Biosysystem Co.) mass spectrometer. A Gilson preparative HPLC (Gilson Co., France) with a refractive index detector (Shodex RI-101, Shodex Co., Japan) and Econosil C_{18} column (10 × 250 mm, 10 μm, Alltech Co.) was used for preparative HPLC. Low-pressure liquid chromatography was carried out using a Lobar-A glass prepacked column (Lichroprep Si 60, 240 × 10 mm, 40–63 μm, Merck Co., Germany) and a Lobar-A glass prepacked column (Lichroprep RP-18, 240 × 10 mm, 40–63 μm, Merck Co., Germany) with a FMI QSY-0 (Fluid Metering Inc.) pump. Open column chromatography was performed using Si gel (particle size 70–230 mesh and 230–400 mesh, Merck Co., Germany), Si gel 60 RP-18 (40–63 μm, Merck Co., Germany), and Sephadex LH-20 (Pharmacia Co., Sweden). Thin-layer chromatography (TLC) was performed on Si gel 60 F_{254} and RP-18 F_{254s} (Merck Co., Germany).

Plant Material. *S. pulchella* was collected at Mt. Odae, Korea, in August 2005. A voucher specimen (SKK-05-080) was deposited at the College of Pharmacy at Sungkyunkwan University.

Extraction and Isolation. The partially dried and chopped aerial parts of *S. pulchella* (3.5 kg) were extracted three times with MeOH at room temperature. The resulting MeOH extract (350 g) was subjected to successive solvent partition, giving *n*-hexane (70 g), $CHCl_3$ (33 g), EtOAc (21 g), and n-BuOH (40 g) soluble fractions. The $CHCl_3$ fraction (33 g) was chromatographed over a Si gel column using a gradient solvent system of $CHCl_3$−MeOH, 20:1–1:1, to give seven fractions (C1−C7). Fraction C3 (2.2 g) was subjected to Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1) and a Lichroprep Si 60 column ($CHCl_3$−MeOH, 8:1) and purified using preparative HPLC (25% CH_3CN) to afford **10** (90 mg, 0.0026% w/w), **11** (22 mg, 0.00063% w/w), and **1** (12 mg, 0.00034% w/w). Fraciton C4 (5.0 g) was subjected to Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1) and a Si gel column ($CHCl_3$−EtOAc−MeOH, 8:8:1) and purified using a Lichroprep RP-18 column (35% CH_3CN) to afford **12** (33 mg, 0.00094% w/w). Fraction C5 (3.0 g) was subjected to Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1) and purified using a Lichroprep RP-18 column (30% CH_3CN) to afford **13** (68 mg, 0.0019% w/w). Fraction C6 (4.5 g) was subjected to a Si gel column (CH_2Cl_2−EtOAc−MeOH, 8:7:1) and Sephadex LH-20 (CH_2Cl_2−MeOH, 1:1) and purified using a Lichroprep RP-18 column (25% CH_3CN) to afford **14** (115 mg, 0.0033% w/w) and **2** (18 mg, 0.00051% w/w). The EtOAc fraction (21 g) was chromatographed over a Si gel column using a gradient solvent system of $CHCl_3$−MeOH, 12:1–7:1, to give seven fractions (E1−E7). Fraction E6 (3.0 g) was subjected to Sephadex LH-20 (100% MeOH) and a Lichroprep RP-18 column (20% MeOH) and purified using preparative HPLC (20% MeOH) to afford **15** (118 mg, 0.0034% w/w).

The *n*-BuOH fraction (40 g) was chromatographed over a DIAION HP-20 resin column using a gradient solvent system of 100% distilled H_2O and 100% MeOH to give four fractions (B1−B4). Fraction B2 (22.0 g) was subjected to RP-18 column chromatography (40% MeOH) to give three subfractions (B21−B23). Subfraction B22 (13.0 g) was subjected to RP-18 column chromatography (15% CH_3CN) to give three subfractions (B221−B225). Subfraction B221 (3.5 g) was subjected to Sephadex LH-20 (70% MeOH) and a Si gel column (EtOAc−MeOH-H_2O, 9:3:1) and purified using preparative HPLC (EtOAc−MeOH−H_2O, 9:3:1) to afford **3** (15 mg, 0.00043% w/w). Subfraction B222 (2.0 g) was subjected to Sephadex LH-20 (70% MeOH) and a Si gel column (EtOAc−MeOH−H_2O, 9:3:1) and purified by Lichroprep

Table 2. ^{1}H NMR Data of Compounds **5–9**

	5[a]	**6**[a]	**7**[b]	**8**[c]	**9**[b]
H-1	3.02 (m)	2.94 (m)	2.79 (m)	2.69 (dd, 8.0, 8.5)	2.80 (m)
H-2a	2.27 (td, 13.5, 8.0)	2.35 (td, 13.1, 6.0)	2.06 (m)	2.21 (td, 13.5, 8.0)	2.08 (td, 13.0, 8.0)
H-2b	1.94 (td, 13.5, 9.0)	2.03 (td, 13.1, 8.5)	1.53 (m)	1.60 (td, 13.5, 8.0)	1.53 (td, 13.0, 8.5)
H-3	4.77 (br t, 8.0)	4.81 (br t, 6.0)	4.31 (br t, 7.8)	4.44 (br t, 8.0)	4.31 (br t, 8.5)
H-5	3.13 (t, 10.0)	2.94 (m)	2.79 (m)	2.60 (dd, 10.0, 8.5)	2.80 (m)
H-6	4.41 (br t, 10.0)	4.27 (br t, 10.0)	4.00 (br t, 10.0)	3.95 (br t, 10.0)	4.00 (br t, 10.0)
H-7	3.63 (m)	2.56 (ddd, 10.0, 10.0, 10.0)	2.19 (ddd, 10.0, 10.0, 10.0)	1.68 (m)	2.19 (ddd, 10.0, 10.0, 10.0)
H-8	5.34 (m)	3.91 (m)	3.58 (m)	3.41 (m)	3.57 (m)
H-9a	3.02 (m)	2.94 (m)	2.57 (m)	2.57 (dd, 12.0, 5.0)	2.55 (m)
H-9b	2.41 (dd, 12.6, 8.0)	2.47 (dd, 12.5, 7.3)	2.06 (m)	1.71 (m)	2.04 (m)
H-11	3.06 (m)	3.01 (m)	2.87 (m)	2.89 (dt, 10.0, 3.0)	2.89 (dt, 10.0, 3.5)
H-13	3.67 (dd, 13.5, 6.0)/3.56 (d, 13.5)	3.98 (m)/3.12 (br t, 11.6)	3.18 (m)/2.87 (m)	3.87 (m)/2.99 (dd, 12.5, 10.0)	3.17 (dd, 12.0, 3.5)/2.55 (m)
H-14	5.21 (s)/5.06 (s)	5.20 (s)/5.11 (s)	4.93 (s)/4.88 (s)	4.97 (s)/4.89 (s)	4.93 (s)/4.88 (s)
H-15	5.68 (s)/5.57 (s)	5.60 (s)/5.71 (s)	5.14 (s)/5.10 (s)	5.17 (s)/5.21 (s)	5.14 (d, 1.5)/5.10 (d, 1.5)
H-3′	4.32 (d, 10.5) /4.11 (d, 10.5)				
H-4′	1.70 (s)				
H-2″	3.79 (t, 7.0)	3.98 (m)	2.82 (m)	3.87 (t, 5.5)	2.96 (d, 5.0)
H-3″	2.21 (2H, m)	3.33 (2H, d, 6.1)	1.88 (m)	3.41 (m)/3.87 (m)	1.64 (m)
H-4″	1.84 (m)/1.67 (m)		0.87 (3H, d, 6.6)		1.47 (m)/1.14 (m)
H-5″	3.46 (dt, 10.0, 5.0) 2.76 (q, 10.0)	7.29 (2H, t, 7.3)	0.90 (3H, d, 6.6)		0.86 (d, 7.0)
H-6″		7.49 (2H, d, 7.3)		7.39 (d, 8.0)	0.83 (d, 7.0)
H-7″		7.22 (t, 8.0)		7.07 (dt, 7.0, 1.0)	
H-8″		7.49 (2H, d, 7.3)		7.14 (dt, 7.0, 1.0)	
H-9″		7.29 (2H, t, 7.3)		7.69 (d, 8.0)	
H-10″					
H-11″				7.24 (s)	

[a] Measured in pyridine-d_5 at 500 MHz. [b] Measured in DMSO-d_6 at 500 MHz. [c] Measured in CD_3OD at 500 MHz.

RP-18 column chromatography (20% CH_3CN) to afford **4** (22 mg, 0.00063% w/w). Subfraction B223 (3.0 g) was subjected to Sephadex LH-20 (70% MeOH) and a Si gel column (EtOAc−MeOH−H_2O, 9:3:1) and purified using preparative HPLC (EtOAc−MeOH−H_2O, 9:3:1) to afford **5** (60 mg, 0.0017% w/w). Subfraction B224 (4.0 g) was subjected to Sephadex LH-20 column chromatography (70% MeOH) and Lichroprep RP-18 column chromatography (50% MeOH) and purified using preparative HPLC (EtOAc−MeOH−H_2O, 9:3:0.5) to afford **6** (8 mg, 0.00023% w/w). Subfraction B225 (1.0 g) was subjected to Sephadex LH-20 column chromatography (70% MeOH), LichroprepRP-18 column chromatography (50% MeOH), and Lichroprep Si 60 column chromatography (EtOAc−MeOH−H_2O, 9:3:1) and purified using preparative HPLC (20% CH_3CN) to afford **7** (20 mg, 0.00057% w/w). Subfraction B23 (6.0 g) was subjected to RP-18 column chromatography (50% MeOH) to give three subfractions (B231−B232). Subfraction B231 (3.0 g) was subjected to Sephadex LH-20 (70% MeOH) and Lichroprep Si 60 column chromatography (EtOAc−MeOH−H_2O, 9:3:0.5) and purified using preparative HPLC (25% CH_3CN) to afford **8** (13 mg, 0.00037% w/w) and **9** (25 mg, 0.00071% w/w).

8α-*O*-(3′-Hydroxy-3′-methylbutyryl)desacylcynaropicrin (1): colorless oil; $[\alpha]_D$ +23.0 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (3.09) nm; IR (neat) ν_{max} 3401 (OH), 1766 (lactone), 1732 (ester) cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 3; HREIMS *m*/*z* 362.1732 [M]$^+$ (calcd for $C_{20}H_{26}O_6$, 362.4228).

8α-*O*-(2′,3′-Dihydroxyisobutyryl)11*β*,13-dihydrodesacylcynaropicrin (2): colorless oil; $[\alpha]_D$ +24.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (3.37) nm; IR (neat) ν_{max} 3399 (OH), 1769 (lactone), 1735 (ester), 1644 (C=C) cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 3; HREIMS *m*/*z* 366.1679 [M]$^+$ (calcd for $C_{19}H_{26}H_7$, 366.4112).

Pulchellamine A (3): colorless gum; ninhydrin positive; $[\alpha]_D$ +41.4 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 200 (3.61) nm; IR (neat) ν_{max} 3338 (OH, COOH), 1762 (lactone), 1640 (C=C) cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 3; HRESIMS *m*/*z* 395.1827 [M + H]$^+$ (calcd for $C_{19}H_{27}N_2O_7H$, 395. 1812).

Pulchellamine B (4): white crystals (obtained by recrystallization from MeOH−H_2O); mp 198 °C; ninhydrin positive; $[\alpha]_D$ +52.0 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 200 (3.80) nm; IR (neat) ν_{max} 3297 (OH, COOH), 1764 (lactone) cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 3; HRESIMS *m*/*z* 366.1927 [M + H]$^+$ (calcd for $C_{19}H_{27}NO_6H$, 366.1911).

Table 3. ^{13}C NMR Data of Compounds **1−9**

	1[a]	**2**[b]	**3**[c]	**4**[d]	**5**[e]	**6**[e]	**7**[d]	**8**[b]	**9**[d]
C-1	45.3	45.2	43.0	42.6	42.9	44.5	43.8	43.8	42.5
C-2	39.0	39.7	37.8	38.1	39.7	39.9	38.1	39.3	38.1
C-3	73.7	73.9	72.8	71.7	73.1	73.5	71.6	73.8	71.6
C-4	152.2	154.4	153.0	154.1	155.5	155.0	154.1	154.4	154.1
C-5	51.3	51.5	49.1	48.5	50.9	50.6	48.3	50.3	48.4
C-6	78.4	80.7	80.9	78.7	80.8	80.3	78.7	81.2	78.5
C-7	47.5	53.5	53.6	54.9	46.0	64.3	54.2	55.0	55.0
C-8	74.2	78.4	73.5	72.3	77.9	74.4	72.4	74.2	72.4
C-9	37.3	40.3	43.7	43.6	42.9	45.2	46.2	46.3	43.9
C-10	141.6	144.4	143.6	144.7	144.5	145.7	144.5	145.0	144.5
C-11	137.3	42.3	44.5	46.2	46.7	48.9	48.5	45.1	46.1
C-12	168.9	180.7	177.8	176.1	178.5	177.2	175.9	176.3	175.9
C-13	122.5	15.9	47.3	48.9	55.3	49.8	48.5	48.1	48.2
C-14	118.2	117.2	116.2	114.3	116.7	115.5	114.3	116.0	114.4
C-15	113.6	111.4	111.0	108.8	109.0	110.3	108.7	110.0	108.7
C-1′	172.0	175.6			176.2				
C-2′	46.5	76.9			77.2				
C-3′	69.2	69.3			69.8				
C-4′	29.3	22.3			23.2				
C-5′	29.2								
C-1″			174.5	174.4	177.2	176.4	173.6	173.6	173.2
C-2″			59.3	47.9	68.8	64.3	67.6	63.7	66.4
C-3″			33.9	24.3	30.5	40.2	22.0	26.4	36.9
C-4″			172.8	31.9	25.1	138.8	19.2	108.3	24.9
C-5″					55.5	130.5	18.3	128.8	11.5
C-6″						129.1		122.9	15.5
C-7″						127.3		120.4	
C-8″						129.1		119.5	
C-9″						130.5		112.9	
C-10″								138.3	
C-11″								125.8	

[a] Measured in $CDCl_3$ at 125 MHz. [b] Measured in CD_3OD at 125 MHz. [c] Measured in D_2O at 125 MHz. [d] Measured in DMSO-d_6 at 125 MHz. [e] Measured in pyridine-d_5 at 125 MHz.

Pulchellamine C (5): colorless gum; ninhydrin positive; $[\alpha]_D$ +22.0 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (3.94) nm; IR (neat) ν_{max} 3305 (OH, COOH), 1755 (lactone), 1632 (C=C) cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 480.2249 [M + H]$^+$ (calcd for $C_{24}H_{33}NO_9H$, 480.2228).

Table 4. Cytotoxicity of Compounds **1–15**

	ED_{50} values[a]			
compound	A 549	SK-OV-3	SK-MEL-2	HCT 15
1	>30.0	12.42	6.78	14.23
2	>30.0	>30.0	>30.0	>30.0
3	>30.0	>30.0	>30.0	>30.0
4	>30.0	>30.0	>30.0	>30.0
5	27.57	11.83	5.73	18.33
6	>30.0	>30.0	>30.0	>30.0
7	>30.0	>30.0	>30.0	>30.0
8	>30.0	24.19	18.56	>30.0
9	>30.0	>30.0	>30.0	>30.0
10	25.71	11.27	9.74	11.33
11	8.22	2.49	1.53	3.82
12	24.51	7.42	4.07	12.13
13	>30.0	>30.0	>30.0	>30.0
14	>30.0	18.04	10.52	28.76
15	>30.0	>30.0	>30.0	>30.0
doxorubicin	0.007	0.056	0.117	0.164

[a] ED_{50} value of compounds against each cancer cell line, which was defined as a concentration (μM) that caused 50% inhibition of cell growth *in vitro*.

Pulchellamine D (6): colorless gum; ninhydrin positive; $[\alpha]_D$ +74.0 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (3.97) nm; IR (neat) ν_{max} 3333 (OH, COOH), 1763 (lactone), 1638 (C=C) cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m/z* 428.2080 $[M + H]^+$ (calcd for $C_{24}H_{29}NO_6H$, 428.2067).

Pulchellamine E (7): white crystals (obtained by recrystallization from MeOH−H_2O); mp 220 °C; ninhydrin positive; $[\alpha]_D$ +29.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (3.89) nm; IR (neat) ν_{max} 3333 (OH, COOH), 1765 (lactone), 1636 (C=C) cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m/z* 380.2067 $[M + H]^+$(calcd for $C_{20}H_{29}NO_6H$, 380.2068).

Pulchellamine F (8): colorless gum; ninhydrin positive; $[\alpha]_D$ +40.4 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (3.45), 221 (3.49), 282 (2.77) nm; IR (neat) ν_{max} 3301 (OH, COOH), 1762 (lactone), 1634 (C=C) cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m/z* 467.2190 $[M + H]^+$ (calcd for $C_{26}H_{30}N_2O_6H$, 467.2176).

Pulchellamine G (9): white crystals (obtained by recrystallization from MeOH−H_2O); mp 199 °C; ninhydrin positive; $[\alpha]_D$ +15.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.00) nm; IR (neat) ν_{max} 3372 (OH, COOH), 1766 (lactone), 1633 (C=C) cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m/z* 394.2214 $[M + H]^+$(calcd. for $C_{21}H_{31}NO_6H$, 394.2224).

Synthesis of Pulchellamines A−G (3−9). [6] A solution of **10** (2.0 mg, 0076 mmol) in EtOH (1.0 mL) was treated with amino acid {L-asparagine (2.0 mg, 0.015 mmol: in **3**), 4-aminobutanoic acid (0.8 mg, 0.0076 mmol: in **4**), L-phenylalanine (3.8 mg, 0.023 mmol: in **6**), L-valine (1.8 mg, 0.015 mmol: in **7**), L-tryptophan (6 mg, 0.03 mmol: in **8**), and L-isoleucine (2.6 mg, 0.02 mmol: in **9**)} in the presence of Et_3N (0.05 mL), and the mixture was heated under reflux for 1 h. In the synthesis of **5**, a solution of **14** (2.0 mg, 0.0076 mmol) in EtOH (1.0 mL) was stirred at room temperature for 72 h with L-proline (1.7 mg, 0.015 mmol). After cooling, the reaction mixture was evaporated under reduced pressure and the residue purified by using preparative HPLC (EtOAc−MeOH−H_2O, 9:3:0.5, 9:3:1) to give compounds **3−9** (**3**: 0.4 mg, 13%, **4**: 0.4 mg, 14%, **5**: 1.5 mg, 57%, **6**: 2.0 mg, 61%, **7**: 0.7 mg, 24%, **8**: 2.2 mg, 62%, **9**: 2.0 mg, 67%).

Test for Cytotoxicity *in Vitro*. A sulforhodamin B bioassay (SRB) was used to determine the cytotoxicity of each compound against four cultured human cancer cell lines.[31] The assays were performed at the Korea Research Institute of Chemical Technology. The cell lines used were A549 (non small cell lungcarcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT (colon adenocarcinoma). Doxorubicin was used as a positive control. The cytotoxicities of doxorubicin against A549, SK-OV-3, SK-MEL-2, and HCT cell lines were ED_{50} 0.007, 0.056, 0.117, and 0.164 μM, respectively.

Acknowledgment. This research was supported by the Korea Science and Engineering Foundation (KRF-2004-202-E00227). The authors would like to thank Dr. E. K. Kwon, S. I. Lee, and Dr. J. J. Seo at the Korea Basic Science Institute for the measurements of NMR and MS spectra.

Supporting Information Available: ^1H, ^{13}C, and 2D NMR ($^1H-^1H$ COSY, HMBC, HMQC, HSQC, NOESY) data for compounds **1** and **3**. ^1H NMR data for compounds **2** and **4−9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP800005R