# Heterodimeric ent-Kauranoids from Isodon tenuifolius 

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## (S) Supporting Information


#### Abstract

Thirteen new heterodimeric ent-kauranoids, bistenuifolins $A-M(\mathbf{1}-\mathbf{1 3})$, were isolated from the aerial parts of Isodon tenuifolius. The constituent units of compounds 1-6 were linked by a six-membered dihydropyran ring, while those of compounds $7-11$ were joined by a rare single carbon-carbon bond $\left(\mathrm{C}-16 \rightarrow \mathrm{C}-17^{\prime}\right)$. The constituent units of 12 and 13 were linked via an unusual cyclobutane moiety. The  structures of these metabolites were established via spectrometric analyses, and the absolute configurations of 1 and 4 were defined by single-crystal X-ray diffraction. Selected compounds were evaluated for their cytotoxicity against a small panel of human tumor cell lines; bistenuifolin B (2) exhibited weak inhibitory effects.


The genus Isodon, which includes about 150 species, is one of the most widespread taxa of the family Labiatae and has attracted much attention due to the varied bioactivities of its major diterpenoid constituents and their chemical and structural diversity. ${ }^{1-3}$ Over the past 30 years, phytochemical studies on over 60 Isodon species from China resulted in the isolation of a series of new ent-kaurane, abietane, labdane, pimarane, isopimarane, gibberellane, clerodane, and atisane diterpenoids. However, only a few ent-kauranoid dimers have been isolated and characterized. Thus, far, eight different linkage patterns connecting their monomeric units have been exemplified by bisrubescensin $A,{ }^{4}$ bisrubescensin $B,{ }^{4}$ hebeiabinin $\mathrm{D},{ }^{5}$ bisjaponin $\mathrm{A},{ }^{6}$ iushanrubescensin $\mathrm{J},{ }^{7}$ diterp-complex-RA (DCRA), ${ }^{8}$ bispseurata F, ${ }^{9}$ and bisleuconin A. ${ }^{10}$

Isodon tenuifolius (W. W. Smith) Kudo is mainly distributed in the northwestern regions of Yunnan Province and the southwest of Sichuan Province. It is used in folk medicine to treat influenza, dysentery, jaundice, snake bites, and various types of inflammation. ${ }^{11,12}$ Previous phytochemical investigations of this plant have revealed the presence of ent-kaurane and abietane diterpenoids. ${ }^{13-15}$ In an ongoing search for bioactive diterpenoids from the genus Isodon, its chemical constituents were reinvestigated, which led to the identification of 13 new heterodimeric ent-kauranoids, bistenuifolins $\mathrm{A}-\mathrm{M}$ (1-13). Interestingly, these new dimers exhibit three different linkages between their two subunits. Dimers 1-6 have a six-membered dihydropyranyl linkage whose structure and absolute configuration were determined by single-crystal X-ray crystallographic diffraction, dimers $7-11$ possessed a unique $\mathrm{C}-16 \rightarrow \mathrm{C}-17^{\prime}$ single-bond linkage, and compounds 12 and 13 have a rare cyclobutane linkage. This paper describes the isolation and structure elucidation of these dimers along with preliminary assessment of their cytotoxicity.

## RESULTS AND DISCUSSION

A $70 \%$ aqueous acetone extract of the air-dried and powdered aerial parts of I. tenuifolius was partitioned between EtOAc and $\mathrm{H}_{2} \mathrm{O}$. The EtOAc layer was subjected to repeated column chromatography and HPLC to afford 13 new heterodimeric ent-kauranoids, bistenuifolins A-M (1-13).

Bistenuifolin A (1) was obtained as colorless crystals from $\mathrm{CH}_{3} \mathrm{OH}$ and yielded a pseudomolecular ion peak in the positive HRESIMS spectrum at $m / z 1003.4307[\mathrm{M}+\mathrm{Na}]^{+}$, which, in conjunction with ${ }^{13} \mathrm{C}$ NMR data, indicated a molecular formula of $\mathrm{C}_{52} \mathrm{H}_{68} \mathrm{O}_{18}$. Its ${ }^{13} \mathrm{C}$ NMR spectrum exhibited carbon signals from two diterpene units ( $\mathbf{1 a}$ and $\mathbf{1 b}$, Figure 1) bearing six acetoxy groups. Apart from the resonances of the six acetyl groups, its ${ }^{1} \mathrm{H},{ }^{13} \mathrm{C}$, and DEPT NMR data (Tables 1 and 2) showed 40 carbon resonances due to six tertiary methyl, eight methylene, 14 methine (eight oxygenated), eight quaternary carbons, an oxygenated tertiary carbon, and three ketocarbonyls. The carbon signals of 1 mostly appeared in pairs, suggesting that compound 1 was a diterpene dimer. The NMR data of $\mathbf{1}$ indicated that the monomeric moieties ( $\mathbf{1 a}$ and $\mathbf{1 b}$ ) had similar structures to $3 \beta, 7 \beta$-dihydroxy- $1 \alpha, 11 \beta$-diacetoxy-ent-kaur-16-ene-6,15-dione ${ }^{16}$ except that the conjugated double bond of $3 \beta, 7 \beta$-dihydroxy-1 $\alpha, 11 \beta$-diacetoxy-ent-kaur-16-ene-6,15-dione was replaced by a carbonyl carbon ( $\delta_{\mathrm{C}}$ 209.8, $\mathrm{C}-15$ ), an oxygenated tertiary carbon ( $\delta_{\mathrm{C}} 83.7, \mathrm{C}-16$ ), an olefinic bond ( $\delta_{\mathrm{C}} 151.9, \mathrm{C}-15^{\prime}$ and $\delta_{\mathrm{C}} 115.2, \mathrm{C}-16^{\prime}$ ), and two methylenes ( $\delta_{\mathrm{C}} 23.0, \mathrm{C}-17$, and $\delta_{\mathrm{C}} 17.8, \mathrm{C}-17^{\prime}$ ) in 1a and $1 \mathbf{b}$. These differences suggested that subunits $\mathbf{1 a}$ and $\mathbf{1 b}$ were linked by a dihydropyran ring, an assumption that was supported by the

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Chart 1


Figure 1. Key ${ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}$ COSY, HMBC , and ROESY correlations of $\mathbf{1}$.
presence of HMBC correlations between $\mathrm{H}_{2}-17$ and $\mathrm{C}-15$, $\mathrm{C}-16$, and $\mathrm{C}-16^{\prime}$ and between $\mathrm{H}_{2}-17^{\prime}$ and $\mathrm{C}-15^{\prime}, \mathrm{C}-16$, and $\mathrm{C}-16^{\prime}$. The substituents were accordingly assigned as AcO-1 $\alpha$, $\mathrm{AcO}-3 \beta, \mathrm{HO}-7 \beta$, $\mathrm{AcO}-11 \beta$, $\mathrm{AcO}-1^{\prime} \beta, \mathrm{HO}-3^{\prime} \alpha, \mathrm{AcO}^{\prime} 7^{\prime} \alpha$, and AcO-11' $\alpha$, respectively, based on the HMBC and ROESY correlations (Figure 1). A single-crystal X-ray diffraction analysis using the anomalous scattering of $\mathrm{Cu} \mathrm{K} \alpha$ radiation yielded a Flack parameter ${ }^{17}$ of -0.2 (3) (CCDC 1008727), which confirmed the structural assignment of 1 including its absolute configuration (Figure 2). This compound was named bistenuifolin A.

HRESIMS and ${ }^{13} \mathrm{C}$ NMR data indicated bistenuifolin B (2) was an isomer of $\mathbf{1}$. The dihydropyranyl linker was evident on the basis of its characteristic ${ }^{13} \mathrm{C}$ NMR signals: a carbonyl carbon ( $\delta_{\mathrm{C}}$ 206.0, C-15), an oxygenated tertiary carbon ( $\delta_{\mathrm{C}} 83.6$, C-16), an olefinic bond ( $\delta_{\mathrm{C}} 153.3, \mathrm{C}-15^{\prime}$, and $\delta_{\mathrm{C}} 114.6, \mathrm{C}-16^{\prime}$ ), and two methylenes ( $\delta_{\mathrm{C}} 23.3, \mathrm{C}-17$ and $\delta_{\mathrm{C}} 17.5, \mathrm{C}-17^{\prime}$ ). In contrast to $\mathbf{1},{ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}$ COSY and HMBC correlations indicated that compound 2 contained two hydroxy groups at C-3 and $\mathrm{C}-7^{\prime}$ and two acetoxy groups at $\mathrm{C}-3^{\prime}$ and $\mathrm{C}-7$. The structure of 2 (bistenuifolin B) was thus established as shown.

In the same way, bistenuifolin C (3) was shown to be an analogue of 2: a diterpene dimer with the molecular formula $\mathrm{C}_{48} \mathrm{H}_{64} \mathrm{O}_{16}$, indicating the presence of four acetoxy groups. The four acetoxy groups were located at C-1, C-1', C-11, and C-11 based on the HMBC correlations of the four acetate carbonyl resonances ( $\delta_{\mathrm{C}} 170.5,170.2,170.0$, and 169.2 ) with $\mathrm{H}-1\left(\delta_{\mathrm{H}} 5.97\right)$, $\mathrm{H}-1^{\prime}\left(\delta_{\mathrm{H}} 5.86\right), \mathrm{H}-11\left(\delta_{\mathrm{H}} 5.92\right)$, and $\mathrm{H}-11^{\prime}\left(\delta_{\mathrm{H}} 5.90\right)$, respectively. The remaining four oxymethines at $\mathrm{C}-3, \mathrm{C}-3^{\prime}, \mathrm{C}-7$, and $\mathrm{C}-7^{\prime}$ must therefore be substituted by four hydroxy groups based on the molecular formula. The correlations observed in the ROESY spectra of 2 and 3 indicated that the orientations of the substituents in 2 and 3 are the same as those in 1.

The NMR data of bistenuifolin $D(4)$ were similar to those for 3 except that the C-7 ( $\delta_{\mathrm{C}} 81.7$ ) and C-7' $\left(\delta_{\mathrm{C}} 83.8\right)$ oxymethine carbons of 3 were replaced by a methine carbon bound to an acetoxy group ( $\delta_{\mathrm{C}} 80.8, \mathrm{C}-7$ ) and a methylene carbon ( $\delta_{\mathrm{C}} 50.8, \mathrm{C}-7^{\prime}$ ), respectively, in 4 . This conclusion was confirmed by the HMBC correlations from H-7 ( $\delta_{\mathrm{H}} 5.37$ ) to the acetate carbonyl ( $\delta_{\mathrm{C}}$ 170.0) and from the methylene carbon ( $\delta_{\mathrm{C}} 50.8, \mathrm{C}-7^{\prime}$ ) to $\mathrm{H}-5^{\prime}, \mathrm{H}-9^{\prime}$, and $\mathrm{H}-14^{\prime}$. The structure of compound 4 was confirmed by single-crystal X-ray diffraction analysis, revealing its absolute configuration as shown in Figure 2.

Table 1. ${ }^{1} \mathrm{H}$ NMR Data of $1-3,12$, and 13 in Pyridine- $d_{5}, \delta$ in ppm ( $J$ in Hz)

| no. | $1 \mathrm{a}^{\text {a }}$ | $2 \mathrm{a}^{a}$ | $3 a^{b}$ | $12 a^{a}$ | $13 a^{b}$ | no. | $1 b^{a}$ | $2 b^{a}$ | $3 b^{b}$ | $12 b^{a}$ | $13 b^{\text {b }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | $\begin{aligned} & \text { 5.63, dd (11.2, } \\ & 4.2) \end{aligned}$ | 5.95 , overlap | 5.97, overlap | $\begin{aligned} & \text { 5.92, dd (11.1, } \\ & 5.1) \end{aligned}$ | $\begin{aligned} & 5.80, \mathrm{dd} \\ & (10.4,4.3) \end{aligned}$ | $1^{\prime}$ | $\begin{aligned} & \text { 5.93, dd } \\ & (10.6,3.8) \end{aligned}$ | $\begin{aligned} & 5.68, \mathrm{dd} \\ & (10.1,5.7) \end{aligned}$ | $5.86$ overlap | $\begin{aligned} & 5.63, \mathrm{dd} \\ & (8.7,4.4) \end{aligned}$ | $\begin{aligned} & 5.95, \\ & \text { overlap } \end{aligned}$ |
| $2 \alpha$ | 2.02, overlap | 2.06, m | 2.05, overlap | 2.00, overlap | 2.15 , overlap | $2^{\prime} \alpha$ | 2.50 , overlap | 2.25 , overlap | 2.37, m | 2.28 , overlap | $2.24$ overlap |
| $2 \beta$ | 2.33, overlap |  | 2.20, overlap | 2.30, overlap | 2.23 , overlap | $2^{\prime} \beta$ | 2.12, overlap | 2.04, overlap | 2.05, overlap | 2.00, overlap | $2.15$ overlap |
| 3 | 5.00, br s | 3.55, br s | 3.57, br s | 4.83, br s | 3.51, br s | $3^{\prime}$ | 3.51, br s | 4.80, br s | 3.60 , br s | 3.56, br s | 3.56 , br s |
| 5 | 4.44, s | 4.23, s | 4.62, s | 4.23, s | 3.43, s | 5 | 4.05, s | 4.48, s | 4.65, s | 4.43, s | 4.27, s |
| $7 \alpha$ | 4.18, s | 5.46, s | 4.47, s | 5.26, s | $\begin{aligned} & 3.44, \mathrm{~d} \\ & (10.8) \end{aligned}$ | $7^{\prime}$ | 4.47, s | 4.55, s | 3.81, s | 4.35, s | 5.27 , s |
| $7 \beta$ |  |  |  |  | 2.18, overlap |  |  |  |  |  |  |
| 9 | 3.20, br s | 3.00, br s | 3.06, br s | 2.83, br s | 2.42 , br s | $9^{\prime}$ | 2.87, br s | 3.10, br s | 3.00, br s | 3.03, br s | 2.87, br s |
| 11 | 5.88, br d (5.4) | 5.87 , br d (5.5) | 5.92 , overlap | 5.78, overlap | 5.85 , br s | $11^{\prime}$ | $\begin{gathered} 5.86, \text { br d } \\ (5.6) \end{gathered}$ | $\underset{(5.2)}{5.91, \mathrm{br} \mathrm{~d}}$ | 5.90, overlap | 5.81 , overlap | 5.84 , br s |
| $12 \alpha$ | 2.11, overlap | 2.17, overlap | 2.02, overlap | 1.98, m | 2.31 , overlap | $12^{\prime} \alpha$ | 2.18 , overlap | 1.80, overlap | 1.85, m | 2.12, m | 2.22 overlap |
| $12 \beta$ | 2.00, overlap | 2.30, overlap |  |  |  | $12 \beta$ | 2.37, overlap | 1.85, overlap |  |  |  |
| 13 | 2.36, br s | 2.16, br s | 2.48, br s | 3.00, br s | 3.08, br s | $13^{\prime}$ | 2.27, br s | 2.34, br s | 2.28, br s | 3.09, br s | 3.16 , br s |
| $14 \alpha$ | 2.39 , overlap | 2.81, overlap | 2.69, m | 1.28, m | 2.43 , overlap | $14^{\prime} \alpha$ | 1.32, overlap | 1.80, overlap | 1.48, overlap | 1.49, m | $\begin{aligned} & 1.21, \mathrm{~d} \\ & (13.8) \end{aligned}$ |
| $14 \beta$ | 2.18, overlap | 2.17, overlap | 2.18, overlap | 1.06, m | 1.50, overlap | $14^{\prime} \beta$ | $\begin{aligned} & 1.63, \mathrm{~d} \\ & (13.7) \end{aligned}$ | 1.82, overlap | 1.73, m | 2.23, overlap | $\begin{aligned} & 2.00, \mathrm{~d} \\ & (13.8) \end{aligned}$ |
| $17 \alpha$ | 2.29 , overlap | 2.22, overlap | 2.30 , overlap | 2.23, overlap | 2.22 , overlap | $17^{\prime} \alpha$ | 1.79, overlap | 1.81, overlap | 1.83, overlap | 1.42, overlap | 1.50, overlap |
| $17 \beta$ | 1.77, overlap | 1.83, overlap | $3.54, \mathrm{~d}$ (9.0) | 2.23, overlap | 2.22, overlap | $17^{\prime} \beta$ | 2.76, m | 2.82, overlap | 2.84, m | 1.42, overlap | $\begin{aligned} & \text { 1.50, } \\ & \text { overlap } \end{aligned}$ |
| 18 | 1.01, s | 1.02, s | 1.35, s | 1.20, s | 1.29, s | $18^{\prime}$ | 1.14, s | 1.18, s | 1.34, s | 1.09, s | 1.21 , s |
| 19 | 1.45, s | 1.46, s | 1.49, s | 1.51, s | 1.54, s | $19^{\prime}$ | 1.40, s | 1.43, s | 1.47, s | 1.48, s | 1.52, s |
| 20 | 1.41, s | 1.38, s | 1.36, s | 1.28, s | 1.27, s | $20^{\prime}$ | 1.32, s | 1.33, s | 1.43, s | 1.21, s | 1.44, s |
| AcO-1 | 2.20, s | 2.22, s | 2.20, s | 1.90, s | 2.17, s | AcO-1 ${ }^{\prime}$ | 2.17, s | 2.15, s | 2.18, s | 2.24, s | 2.12, s |
| AcO-3 | 2.03, s |  |  | 2.22, s |  | AcO-3' |  | 1.95, s |  |  |  |
| $\mathrm{AcO}-7$ |  | 1.95, s |  | 2.10, s |  | AcO-7' | 2.52, s |  |  |  | 2.00, s |
| AcO-11 | 1.84, s | 1.83, s | 1.92, s | 1.83, s | 1.85, s | AcO-11 ${ }^{\prime}$ | 1.88, s | 1.91, s | 1.84, s | 1.83, s | 1.87, s |
| ${ }^{a}$ Recorded at $500 \mathrm{MHz} .{ }^{b}$ Recorded at 400 MHz . |  |  |  |  |  |  |  |  |  |  |  |

Bistenuifolin E (5) was isolated as an amorphous powder. Its positive HRESIMS spectrum contained a pseudomolecular peak at $m / z 901.3978[\mathrm{M}+\mathrm{Na}]^{+}$, in conjunction with ${ }^{13} \mathrm{C}$ NMR data, corresponding to a molecular formula of $\mathrm{C}_{48} \mathrm{H}_{62} \mathrm{O}_{15}$ with 18 indices of hydrogen deficiency. The NMR data of 5 resembled those of $\mathbf{4}$. However, 5 appeared to have a carbonyl group at C-11', as was confirmed by the observation of HMBC correlations from $\mathrm{H}-9^{\prime}$ and $\mathrm{H}-13^{\prime}$ to the carbonyl carbon ( $\delta_{\mathrm{C}}$ 213.7, $\mathrm{C}-11^{\prime}$ ). The substituents were accordingly assigned as $\mathrm{AcO}-1 \alpha$, $\mathrm{HO}-3 \beta$, $\mathrm{AcO}-7 \beta$, $\mathrm{AcO}-11 \beta$, $\mathrm{HO}-1^{\prime} \beta$, and $\mathrm{AcO}-3^{\prime} \alpha$, respectively, based on ROESY correlations from $\mathrm{H}-1 \beta$ to $\mathrm{H}-5 \beta$ and $\mathrm{H}-9 \beta, \mathrm{H}-3 \alpha$ to $\mathrm{H}_{3}-18 \beta$ and $\mathrm{H}_{3}-19 \alpha, \mathrm{H}-7 \alpha$ to $\mathrm{H}-14 \beta, \mathrm{H}-11 \alpha$ to $\mathrm{H}_{3}-20 \alpha, \mathrm{H}-1^{\prime} \alpha$ to $\mathrm{H}-5^{\prime} \alpha$ and $\mathrm{H}-9^{\prime} \alpha$, and $\mathrm{H}-3^{\prime} \beta$ to $\mathrm{H}_{3}-18^{\prime} \alpha$ and $\mathrm{H}_{3}-19^{\prime} \beta$.

Comparison of the 2D NMR data for 6 and $\mathbf{3}$ indicated that the two compounds had similar structures but the carbonyl groups at C-6 and C-6' in 3 were replaced with two hydroxy groups in 6 . This deduction was verified by the observation of HMBC correlations from $\mathrm{H}-6\left(\delta_{\mathrm{H}} 4.54, \mathrm{~s}\right)$ to C-4, C-8, and $\mathrm{C}-10$ and from $\mathrm{H}-6^{\prime}\left(\delta 4.54\right.$, s) to $\mathrm{C}-4^{\prime}, \mathrm{C}-8^{\prime}$, and $\mathrm{C}-10^{\prime}$, respectively. The ROESY correlations from $\mathrm{H}-6$ to $\mathrm{H}-5 \beta$ and $\mathrm{CH}_{3}-18 \beta$, from $\mathrm{H}-6^{\prime}$ to $\mathrm{H}-5^{\prime} \alpha$ and $\mathrm{CH}_{3}-18^{\prime} \alpha$, from $\mathrm{H}-7$ to $\mathrm{H}-14 \beta$, and from $\mathrm{H}-7^{\prime}$ to $\mathrm{H}-14^{\prime} \alpha$ indicated that $\mathrm{H}-6$ and $\mathrm{H}-7^{\prime}$ are both $\beta$-oriented, while H-6' and H-7 are $\alpha$-oriented. The structure of bistenuifolin F (6) was thus determined as shown.

Bistenuifolin $G$ (7) was isolated as an amorphous powder. Its IR spectrum contained absorptions due to hydroxy ( $3447 \mathrm{~cm}^{-1}$ ) and carbonyl ( 1738 and $1728 \mathrm{~cm}^{-1}$ ) groups. A molecular formula of $\mathrm{C}_{48} \mathrm{H}_{66} \mathrm{O}_{17}$ was determined by HRESIMS for the $[\mathrm{M}+\mathrm{Na}]^{+}$
ion at $m / z 937.4200$ and ${ }^{13} \mathrm{C}$ NMR data, which requires 16 indices of hydrogen deficiency. Apart from the resonances of four acetyl groups, its ${ }^{1} \mathrm{H},{ }^{13} \mathrm{C}$ NMR, and DEPT data (Tables 4 and 5) showed 40 carbon resonances due to six tertiary methyl groups, eight methylenes (one oxygenated), 15 methine carbons (eight oxygenated), seven quaternary carbons, and four ketocarbonyls. The carbon signals mostly appeared in pairs, suggesting that 7 was a diterpene dimer. Its ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data suggested that both monomeric moieties ( 7 a and 7b, Figure 3) had similar structures to $3 \beta, 7 \beta$-dihydroxy- $1 \alpha, 11 \beta$ -diacetoxy-ent-kaur-16-ene-6,15-dione ${ }^{16}$ except that the conjugated double bond of $3 \beta, 7 \beta$-dihydroxy- $1 \alpha, 11 \beta$-diacetoxy-ent-kaur-16-ene-6,15-dione was replaced by a quaternary carbon ( $\delta_{\mathrm{C}} 58.3$, s, C-16), a methine carbon ( $\delta_{\mathrm{C}} 50.9$, d, C-16'), and two methylenes ( $\delta_{\mathrm{C}} 61.5, \mathrm{t}, \mathrm{C}-17 ; \delta_{\mathrm{C}} 32.0, \mathrm{t}, \mathrm{C}-17^{\prime}$ ). The HMBC correlations of $\mathrm{H}_{2}-17^{\prime}$ with $\mathrm{C}-15, \mathrm{C}-16$, and $\mathrm{C}-17$ and of $\mathrm{H}-16^{\prime}$ with $\mathrm{C}-16$ suggested that the subunits 7 a and $7 \mathbf{b}$ were connected by a single carbon - carbon bond ( $\mathrm{C}-16 \rightarrow \mathrm{C}-17^{\prime}$ ), rendering 7 a dimeric conjugate, a rare phenomenon among ent-kauranoid dimers. ${ }^{10}$ The relative configurations of the C-17 and C-17 carbons of 7 were established from the ROESY correlations of $\mathrm{H}-16^{\prime}$ with $\mathrm{H}-13^{\prime}$ and $\mathrm{H}-17 \beta$, as shown by the 3D drawing in Figure 3. Compound 7 was given the trivial name isoabietenin G.
Bistenuifolin $\mathrm{H}(8)$ was obtained as a white powder. It was assigned a molecular formula of $\mathrm{C}_{49} \mathrm{H}_{68} \mathrm{O}_{17}$ based on its positive HRESIMS spectrum ( $\left.m / z 951.4332[\mathrm{M}+\mathrm{Na}]^{+}\right)$and ${ }^{13} \mathrm{C}$ NMR data, and thus had 16 indices of hydrogen deficiency. Its ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data were similar to those of 7 except that it contained an additional signal due to a methoxy group. An HMBC
Table 2. ${ }^{13} \mathrm{C}$ NMR Data of $1-6$ in Pyridine- $d_{5}, \delta$ in ppm
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-
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 $\stackrel{N}{\text { Ni }}$





Figure 2. ORTEP plots for the molecular structures of 1 and 4 drawn with $30 \%$ probability displacement ellipsoids.
Table 3. ${ }^{1} \mathrm{H}$ NMR Data of $4-6$ in Pyridine- $d_{5}, \delta$ in ppm ( $400 \mathrm{MHz}, J$ in Hz )

| no. | 4a | 5a | 6a | no. | 4b | 5b | 6b |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | 5.90, overlap | 5.91, dd (10.9, 3.7) | 5.74, dd (11.0, 4.3) | $1^{\prime}$ | 5.90, overlap | 4.68, dd (8.4, 3.2) | 5.88, dd (11.1, 4.0) |
| $2 \alpha$ | 2.18, overlap | 2.18 , overlap | 2.21, overlap | $2^{\prime} \alpha$ | 2.18, overlap | 2.16, overlap | 2.21, m |
| $2 \beta$ | 2.25 , overlap | 2.30, overlap | 2.30, overlap | $2^{\prime} \beta$ | 2.04, overlap | 2.02, overlap | 2.16, overlap |
| 3 | 6.87, br s | 3.56 , br s | 3.72, br s | $3^{\prime}$ | 6.69 , br s | 4.84, br s | 3.73, br s |
| 5 | 4.23, s | 4.20, s | 2.91, s | $5^{\prime}$ | 2.54, s | 3.17, s | 2.86, s |
| 6 | 5.37, s | 5.27, s | 4.54, s | $6^{\prime}$ |  |  | 4.54, s |
| 7 |  |  | 4.31, s | $7^{\prime}$ | 3.42, overlap | 4.44, d (13.2) | 4.04, s |
|  |  |  |  |  | 2.57, overlap | $2.64, \mathrm{~d}(13.2)$ |  |
| 9 | 2.99, br s | 2.96, br s | 2.66, br s | $9^{\prime}$ | 3.43, br s | 3.27 , s | 2.61, br s |
| 11 | 5.89 , overlap | 5.83, br d (5.4) | 6.04, br d (4.9) | $11^{\prime}$ | 5.93, overlap | 2.62, m | 6.09, br d (5.7) |
| 12 | 2.26, overlap | 2.18, overlap | 2.40, overlap | $12^{\prime}$ | 1.84, overlap |  | 2.24, overlap |
|  | 2.19 , overlap | 2.02, overlap | 2.24, overlap |  | 1.80, overlap |  | 2.20, overlap |
| 13 | 2.40, br s | 2.32, br s | 2.38 , br s | $13^{\prime}$ | 2.14 , br s | 2.14, br s | 2.31, br s |
| $14 \alpha$ | 2.51, overlap | 1.91, overlap | 2.08, m | $14^{\prime} \alpha$ | 1.73, overlap | 1.78, m | 1.93, overlap |
| $14 \beta$ | 2.04, overlap | 2.18, overlap | 1.93, overlap | $14^{\prime} \beta$ | 1.95, overlap | 2.62, d (13.5) | 2.68, overlap |
| $17 \alpha$ | 2.27, overlap | 2.14 , overlap | 2.31, overlap | $17^{\prime} \alpha$ | 2.71, overlap | 2.62, overlap | 2.66, overlap |
| $17 \beta$ | 1.83 , overlap | 1.69, overlap | 1.90, overlap | $17^{\prime} \beta$ | 1.80 , overlap | 1.91, overlap | 1.90, overlap |
| 18 | 1.19, s | 1.21, s | 1.39, s | $18^{\prime}$ | 1.19, s | 1.01, s | 1.46, s |
| 19 | 1.44, s | 1.43, s | 1.59, s | $19^{\prime}$ | 1.45, s | 1.35, s | 160, s |
| 20 | 1.39, s | 1.35, s | 1.96, s | $20^{\prime}$ | 1.34, s | 1.44, s | 1.96, s |
| AcO-1 | 2.27, s | 2.10, s | 2.26, s | $\mathrm{AcO}-1^{\prime}$ | 2.22, s |  | 2.21, s |
| $\mathrm{AcO}-3$ |  |  |  | AcO-3' |  | 1.91, s |  |
| AcO-7 | 1.97, s | 1.99, s |  | $\mathrm{AcO}-7{ }^{\prime}$ |  |  |  |
| AcO-11 |  | 1.84, s | 2.02, s | AcO-11' | 1.91, s |  | 1.90, s |

experiment showed that the protons of the methoxy group ( $\delta_{\mathrm{H}}$ $3.22, \mathrm{~s}$ ) correlated with $\mathrm{C}-17\left(\delta_{\mathrm{C}} 72.0, \mathrm{t}\right)$, demonstrating that the methoxy group was located at $\mathrm{C}-17$. The relative configuration of 8 was determined to be the same as that of 7 by analysis of its ROESY spectrum and other spectroscopic data. The structure of $\mathbf{8}$ was thus determined unambiguously, and it was named bistenuifolin H .

The HRESIMS spectrum of bistenuifolin I (9) indicated that it had a molecular formula of $\mathrm{C}_{49} \mathrm{H}_{68} \mathrm{O}_{17}$ based on its $[\mathrm{M}+\mathrm{Na}]^{+}$ ion $m / z 951.4347$ and ${ }^{13} \mathrm{C}$ NMR data. It was thus isomeric to 8. The ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR spectroscopic data of 9 and 8 are similar except for the deshielded C-5 resonance ( $\Delta 5.6 \mathrm{ppm}$ ) in 9 , caused by the absence of the $\gamma$-steric compression effect between $7-\mathrm{OH}$ and $\mathrm{H}-5$. This indicated that the $7-\mathrm{OH}$ group of 9 was $\alpha$-oriented, while the ROESY correlations from $\mathrm{H}-7$ to $\mathrm{H}-5 \beta$ and $\mathrm{H}-9 \beta$ indicated that $\mathrm{H}-7$ was $\beta$-oriented. The remaining correlations observed in the ROESY spectra of 9 were in good agreement with those for $\mathbf{8}$. The structure of 9 (bistenuifolin I) was thus determined unambiguously.

Bistenuifolins J (10) and K (11) were assigned molecular formulas of $\mathrm{C}_{45} \mathrm{H}_{64} \mathrm{O}_{15}$ and $\mathrm{C}_{47} \mathrm{H}_{66} \mathrm{O}_{16}$ based on their HRESIMS
$\left(m / z 867.4147[\mathrm{M}+\mathrm{Na}]^{+}\right.$and $909.4231[\mathrm{M}+\mathrm{Na}]^{+}$, respectively) and ${ }^{13} \mathrm{C}$ NMR data. Their 1D NMR spectra indicated that they were heterodimeric ent-kauranoid dimers with structures similar to 8 . However, whereas 8 had three acetoxy groups at C-1, C-1', and C-11, 10 had two hydroxy groups at $\mathrm{C}-1$ and C-11, and 11 had a hydroxy group at C-1'. For compound 10, this conclusion was confirmed by (i) the HMBC correlations of $\mathrm{H}-1\left(\delta_{\mathrm{H}} 4.83\right)$ with $\mathrm{C}-3\left(\delta_{\mathrm{C}} 76.5\right)$, C-5 ( $\delta_{\mathrm{C}}$ 57.9), and C-9 ( $\delta_{\mathrm{C}} 60.5$ ); (ii) the HMBC correlations of $\mathrm{H}-11$ ( $\delta_{\mathrm{H}} 4.81$ ) with $\mathrm{C}-8\left(\delta_{\mathrm{C}} 55.9\right)$, C-10 ( $\delta_{\mathrm{C}} 45.9$ ), and C-13 ( $\delta_{\mathrm{C}}$ 38.1); and (iii) the significant shielding of C-1 ( $\Delta 6.6 \mathrm{ppm}$ ) and C-11 ( $\Delta 4.4 \mathrm{ppm}$ ) relative to the corresponding signals in the NMR spectrum of $\mathbf{8}$. Similarly, the proposed structure of $\mathbf{1 1}$ was confirmed by the HMBC correlations of $\mathrm{H}-1^{\prime}\left(\delta_{\mathrm{H}} 4.87\right)$ with C-3 ${ }^{\prime}\left(\delta_{\mathrm{C}} 75.3\right), \mathrm{C}-5^{\prime}\left(\delta_{\mathrm{C}} 50.3\right)$, and $\mathrm{C}-9^{\prime}\left(\delta_{\mathrm{C}} 51.2\right)$ as well as the shielding of $\mathrm{C}-1^{\prime}(\Delta 4.3 \mathrm{ppm})$ relative to the equivalent signal for $\mathbf{8}$. Compounds $\mathbf{1 0}$ (bistenuifolin J) and $\mathbf{1 1}$ (bistenuifolin K) were thus assigned the structures shown based on their 2D NMR, respectively.

Bistenuifolin L (12) was isolated as a white powder. Its HRESIMS spectrum exhibited an $[\mathrm{M}+\mathrm{Na}]^{+}$ion at $\mathrm{m} / \mathrm{z}$

Table 4. ${ }^{1} \mathrm{H}$ NMR Data of $7-11$ in Pyridine- $d_{5}, \delta$ in ppm ( $J$ in Hz)

| no. | $7 \mathrm{a}^{a}$ | $8 \mathrm{a}^{\text {a }}$ | $9 a^{a}$ | $10 a^{a}$ | $11 a^{b}$ | no. | $7 \mathrm{~b}^{\text {a }}$ | $8 \mathrm{~b}^{\text {a }}$ | $9 \mathrm{~b}^{\text {a }}$ | $10 b^{a}$ | $11 b^{\text {b }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | $\begin{aligned} & \text { 5.95, dd } \\ & (11.5,4.2) \end{aligned}$ | 5.86, overlap | 5.88, overlap | 4.83, overlap | $\begin{aligned} & \text { 5.94, dd } \\ & (11.5,4.9) \end{aligned}$ | $1^{\prime}$ | $\begin{aligned} & 5.98, \mathrm{dd} \\ & (10.9,4.9) \end{aligned}$ | 5.88, overlap | 5.87, overlap | $\begin{aligned} & \text { 5.91, dd } \\ & (11.1,4.7) \end{aligned}$ | 4.87, overlap |
| $2 \alpha$ | 2.16, m | 2.15, overlap | 2.05, overlap | 2.15, overlap | 2.05, overlap | $2^{\prime} \alpha$ | 2.16, m | 2.14, overlap | 2.22, overlap | 2.14, overlap | 2.22, overlap |
| $2 \beta$ | 2.30, m | 2.32, overlap | $\begin{aligned} & \text { 2.31, } \\ & \text { overlap } \end{aligned}$ | 2.32, overlap | 2.31 , overlap | $2^{\prime} \beta$ | 2.21, m | 2.20, overlap | $\begin{aligned} & \text { 2.02, } \\ & \text { overlap } \end{aligned}$ | 2.20, overlap | 2.02, overlap |
| 3 | 3.58, br s | $3.59, \mathrm{br} \mathrm{s}$ | 3.57 , br s | 3.66, br s | 3.59, br s | $3^{\prime}$ | 3.60, br s | 3.59, br s | 3.52 , br s | 3.58, br s | 3.68, br s |
| 5 | 4.69, s | 4.66, s | 3.45, s | 3.52, s | 4.73, s | $5^{\prime}$ | 4.76, s | 4.62, s | 4.67, s | 4.68, s | 4.60, s |
| 7 | 4.71, s | 4.58, s | 5.15, s | 4.70, s | 4.62 , s | 7' | 3.89, s | 3.86, s | 4.00, s | 3.99, s | 3.89, s |
| 9 | 2.84, br s | 3.01, br s | 2.70, br s | 2.38, br s | 3.08, br s | $9^{\prime}$ | 3.10, br s | 2.76, br s | 2.85, br s | 2.86, br s | 2.88, br s |
| 11 | 5.88 , br s | 5.86, overlap | $5.89$ overlap | 4.81, overlap | 5.85 , br s | $11^{\prime}$ | 5.91, br s | 5.83, br s | 5.86, overlap | 5.85, br s | 6.47, br s |
| 12 | 2.06 , overlap | $\begin{aligned} & \text { 2.06, } \\ & \text { overlap } \end{aligned}$ | $2.22$ overlap | 2.06, overlap | 2.22 , overlap | $12^{\prime}$ | 2.32, overlap | $\begin{aligned} & \text { 2.32, } \\ & \text { overlap } \end{aligned}$ | 2.31, overlap | 2.32, overlap | $\begin{aligned} & \text { 2.31, } \\ & \text { overlap } \end{aligned}$ |
| 13 | 2.52, overlap | 2.36, br s | 2.45, br s | 2.46, br s | 2.36, br s | $13^{\prime}$ | 2.80, br s | 2.54, br s | 2.65, br s | 2.64, br s | $2.52, \mathrm{br} \mathrm{s}$ |
| $14 \alpha$ | 2.35 , overlap | $\begin{aligned} & 2.35, \\ & \text { overlap } \end{aligned}$ | $\begin{aligned} & 2.71, \mathrm{~d} \\ & (13.8) \end{aligned}$ | $2.35$ overlap | $\begin{aligned} & 2.71, \mathrm{~d} \\ & (13.8) \end{aligned}$ | $14^{\prime} \alpha$ | 2.19, overlap | $\begin{aligned} & \text { 2.18, } \\ & \text { overlap } \end{aligned}$ | $\begin{aligned} & \text { 2.00, } \\ & \text { overlap } \end{aligned}$ | 2.18, overlap | $\begin{aligned} & \text { 2.00, } \\ & \text { overlap } \end{aligned}$ |
| $14 \beta$ | 2.52, overlap | $\begin{aligned} & 2.18, \\ & \text { overlap } \end{aligned}$ | $\begin{array}{r} 1.76, \mathrm{~d} \\ (13.8) \end{array}$ | $\begin{gathered} 2.18, \\ \text { overlap } \end{gathered}$ | $\begin{gathered} 1.60, \mathrm{~d} \\ (13.8) \end{gathered}$ | $14^{\prime} \beta$ | $\begin{gathered} 1.68, \mathrm{~d} \\ (14.2) \end{gathered}$ | $\begin{gathered} 1.60, \mathrm{~d} \\ (14.4) \end{gathered}$ | $\begin{gathered} 1.62, \mathrm{~d} \\ (14.3) \end{gathered}$ | 1.50, overlap | $\begin{gathered} 1.60, \mathrm{~d} \\ (13.8) \end{gathered}$ |
| 16 |  |  |  |  |  | $16^{\prime}$ | 3.05, m | 3.05, m | 3.04, m | 3.05, m | $\begin{aligned} & \text { 2.88, } \\ & \text { overlap } \end{aligned}$ |
| 17a | $\begin{aligned} & 4.22, \mathrm{~d} \\ & (10.3) \end{aligned}$ | $\begin{gathered} 3.65, \mathrm{~d} \\ (9.4) \end{gathered}$ | 3.83, s | $\begin{array}{r} 3.82, \mathrm{~d} \\ (9.0) \end{array}$ | 3.62, s | $17^{\prime} \alpha$ | 2.50, overlap | $\begin{aligned} & \text { 2.02, } \\ & \text { overlap } \end{aligned}$ | $\begin{aligned} & \text { 2.02, } \\ & \text { overlap } \end{aligned}$ | 2.02, overlap | 2.12, overlap |
| 17b | $\begin{aligned} & \text { 4.05, d } \\ & (10.3) \end{aligned}$ | $\begin{array}{r} 3.58, \mathrm{~d} \\ (9.4) \end{array}$ | 3.83, s | $\begin{array}{r} 3.54, \mathrm{~d} \\ (9.0) \end{array}$ | 3.59, s | $17^{\prime} \beta$ | 2.36, overlap | 2.36, overlap | 2.36, overlap | 2.36, overlap | 2.43, m |
| 18 | 1.30, s | 1.34, s | 1.29, s | 1.73, s | 1.30, s | $18^{\prime}$ | 1.37, s | 1.27, s | 1.19, s | 1.30, s | 1.33, s |
| 19 | 1.52, s | 1.47, s | 1.48, s | 1.20, s | 1.49, s | $19^{\prime}$ | 1.49, s | 1.48, s | 1.49, s | 1.48, s | 1.51, s |
| 20 | 1.49, s | 1.44, s | 1.41, s | 1.49, s | 1.42, s | $20^{\prime}$ | 1.38, s | 1.38, s | 1.37, s | 1.38, s | 1.46, s |
| $\mathrm{OCH}_{3}$ |  | 3.22, s | 3.17, s | 3.13, s | 3.19, s |  |  |  |  |  |  |
| AcO-1 | 2.23, s | 2.19, s | 2.22, s |  | 2.22, s | AcO-1' | 2.19, s | 2.19, s | 2.20, s | 2.16, s |  |
| AcO-11 | 1.92, s | 1.98, s | 2.00, s |  | 1.97, s | AcO-11' | 1.82, s | 1.88, s | 1.89, s | 1.73, s | 1.88, s |

${ }^{a}$ Recorded at $400 \mathrm{MHz} .{ }^{b}$ Recorded at 500 MHz .
1003.4296, in conjunction with ${ }^{13} \mathrm{C}$ NMR data, corresponding to a molecular formula of $\mathrm{C}_{52} \mathrm{H}_{68} \mathrm{O}_{18}$. This requires 19 indices of hydrogen deficiency. The 1D NMR spectra of its two subunits 12a and 12b (Figure 4) were identical to those of $3 \beta, 7 \beta$-dihydroxy- $1 \alpha, 11 \beta$-diacetoxy-ent-kaur-16-ene-6,15dione ${ }^{16}$ except that the signals of the olefinic bond between $\mathrm{C}-16$ and C -17 in $3 \beta, 7 \beta$-dihydroxy- $1 \alpha, 11 \beta$-diacetoxy-ent-kaur-16-ene-6,15-dione were replaced by two quaternary carbons (both $\delta_{\mathrm{C}} 62.5$ ) and two methylene carbons ( $\delta_{\mathrm{C}} 24.0$ and 24.1) in 12a and 12b. On the basis of these differences and the nine indices of hydrogen deficiency in its molecular skeleton, compound $\mathbf{1 2}$ was deduced to be an ent-kaurane dimer in which the monomers were linked by a rare cyclobutane moiety. Comparison of its NMR data with those for bisjaponin $A^{6}$ confirmed this conclusion. The HMBC correlations of H-12 and $\mathrm{H}-14$ with $\mathrm{C}-16$, of $\mathrm{H}-17$ with $\mathrm{C}-15$ and $\mathrm{C}-16^{\prime}$, and of $\mathrm{H}-12^{\prime}$ and $\mathrm{H}-14^{\prime}$ with $\mathrm{C}-16^{\prime}$ further confirmed the presence of the bond between $\mathrm{C}-17$ and $\mathrm{C}-17^{\prime}$ (Figure 4). The ROESY correlations from $\mathrm{H}-7 \alpha$ to $\mathrm{H}-7^{\prime} \beta$ and $\mathrm{H}-14^{\prime} \alpha$, from $\mathrm{H}-12 \beta$ to $\mathrm{H}-17 \alpha$, and from $\mathrm{H}-17 \beta$ to $\mathrm{H}-13^{\prime} \beta$ indicated that the relative configurations of C-16 and C-16 could both be assigned as $R^{*}$. The 3D structure of 12 was obtained by using a molecular modeling program with MM2 force-field calculations for energy minimization, the shortest interatomic distance of approximately $2.25 \AA$ between $\mathrm{H}-7 \alpha$ and $\mathrm{H}-14^{\prime} \alpha$ further supported the assignment of $16 R^{*}$ and $16^{\prime} R^{*}$ (Figure 4). The substituents were assigned as $\mathrm{AcO}-1 \alpha$, $\mathrm{AcO}-3 \beta$, $\mathrm{AcO}-7 \beta$, $\mathrm{AcO}-11 \beta$, $\mathrm{AcO}-$ $1^{\prime} \beta$, $\mathrm{HO}-3^{\prime} \alpha, \mathrm{HO}-7^{\prime} \alpha$, and $\mathrm{AcO}-11^{\prime} \alpha$, respectively, based on the HMBC and ROESY correlations. Therefore, the structure of bistenuifolin L (12) was determined as shown.

Bistenuifolin M (13) was obtained as a white powder. Its molecular formula was assigned as $\mathrm{C}_{50} \mathrm{H}_{66} \mathrm{O}_{16}$ based on its ${ }^{13} \mathrm{C}$ NMR data and the $[\mathrm{M}+\mathrm{Na}]^{+}$ion at $m / z 945.4249$ in its HRESIMS spectrum. Comparison of its NMR spectra with those of 12 revealed that the chemical shifts of C-16, C-16', $\mathrm{C}-17$, and $\mathrm{C}-17^{\prime}$ in both compounds were similar. The main difference between the two compounds was that 13 lacked a C7 substituent. This conclusion was supported by the HMBC correlations of $\mathrm{H}_{2}-7$ with $\mathrm{C}-5\left(\delta_{\mathrm{C}} 57.6, \mathrm{~d}\right)$, C-9 ( $\delta_{\mathrm{C}} 59.1, \mathrm{~d}$ ), and $\mathrm{C}-14\left(\delta_{\mathrm{C}} 41.0, \mathrm{t}\right)$. In addition, the $\mathrm{C}-5$ and C-9 signals in the ${ }^{13} \mathrm{C}$ NMR spectrum of 13 were deshielded (by $\Delta 6.3$ and 3.9 ppm , respectively) relative to the corresponding signals in the spectrum of 12 due to the removal of the $\gamma$-steric compression effect of AcO-7 on H-5 and H-9. This further supports the assigned structure of 13.

Compounds 1-10, 12, and 13 were evaluated for their cytotoxic activity against the HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human tumor cell lines by the MTT method. ${ }^{18}$ The results obtained are expressed as $\mathrm{IC}_{50}$ values in $\mu \mathrm{M}$; cisplatin and paclitaxel were used as positive controls. As shown in Table 6, compound 2 exhibited weak cytotoxicity against the HL-60, SMMC-7721, MCF-7, and SW-480 cell lines ( $\mathrm{IC}_{50}<10 \mu \mathrm{M}$ ). Compound 4 was not tested due to the limited amount of material available.

## EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on an XRC-1 apparatus and are uncorrected. X-ray data were collected using a Bruker APEX DUO instrument. Optical rotations were measured on a Horiba SEPA-300 polarimeter. IR spectra were



$\stackrel{3}{\square}$

 170．1，C
21．7， $\mathrm{CH}_{3}$




170．2，C
21．9， $\mathrm{CH}_{3}$

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$\mathrm{AcO}-3$
$\mathrm{AcO}-7$


Figure 3. Key ${ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}$ COSY, HMBC , and ROESY correlations of 7.


Figure 4. Key ${ }^{1} \mathrm{H}-{ }^{1} \mathrm{H}$ COSY, HMBC , and ROESY correlations of $\mathbf{1 2}$.

Table 6. Cytotoxic Activities of Diterpenoids from I. tenuifolius against Tumor Cell Lines ${ }^{a}$

| compound $^{b}$ | HL-60 | SMMC-7721 | A-549 | MCF-7 | SW-480 |
| :--- | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{2}$ | 4.6 | 9.9 | $>10$ | 4.2 | 4.0 |
| cisplatin $^{c}$ | 2.6 | $>10$ | $>10$ | $>10$ | $>10$ |
| paclitaxel $^{c}$ | $<0.008$ | $<0.008$ | $<0.008$ | $<0.008$ | 0.15 |

${ }^{a}$ Cell lines: HL-60, acute leukemia; SMMC-7721, hepatic cancer; A-549, lung cancer; MCF-7, breast cancer; SW-480, colon cancer.
${ }^{b}$ Compounds $\mathbf{1 , 3}$, and $\mathbf{5 - 1 3}$ were inactive for all cell lines $\left(\mathrm{IC}_{50}>10 \mu \mathrm{M}\right)$. ${ }^{c}$ Results are expressed as $\mathrm{IC}_{50}$ values in $\mu \mathrm{M}$.
obtained on a Tenor 27 spectrophotometer. UV spectra were measured on a Shimadzu UV-2401A spectrophotometer. ${ }^{1} \mathrm{H},{ }^{13} \mathrm{C}$, and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers using TMS as an internal standard. Mass spectra were acquired on an API QSTAR time-of-flight spectrometer. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C ${ }_{18}(9.4 \mathrm{~mm} \times 25 \mathrm{~cm})$ column. Column chromatography (CC) was performed on silica gel (100-200 and 200-300 mesh; Qingdao Marine Chemical Inc., Qingdao, People's Republic of China) and MCI gel (75-150 $\mu \mathrm{M}$, Mitsubishi Chemical Corporation, Tokyo, Japan).

Plant Material. Aerial parts of I. tenuifolius were collected from Shangri-la County of Yunnan Province, People's Republic of China, during August 2008 and identified by Prof. Xi-Wen Li, Kunming Institute of Botany. A voucher specimen (KIB 20080810) was deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. Aerial parts of I. tenuifolia ( 10 kg ) were crushed and extracted with $70 \%$ aqueous acetone $(3 \times 40 \mathrm{~L})$ under reflux. The extracts were concentrated to about 2 L and extracted with

EtOAc. The EtOAc extract ( 500 g ) was passed through a column containing MCI CHP 20P gel, eluting with $90 \% \mathrm{MeOH}-\mathrm{H}_{2} \mathrm{O}$. The $90 \% \mathrm{MeOH}$ fraction ( 430 g ) was chromatographed on silica gel (200-300 mesh) with a gradient of acetone in $\mathrm{CHCl}_{3}$ ranging from $0 \%$ to $100 \%$ to afford seven fractions (Fr. 1-7). Fr. 5 (46 g) was chromatographed over RP-18 CC with $30-80 \% \mathrm{MeOH}-\mathrm{H}_{2} \mathrm{O}$ as the eluent to give $\mathbf{1}(6 \mathrm{mg}), \mathbf{2}(7 \mathrm{mg})$, and six subfractions, $5 \mathrm{~A}-5 \mathrm{~F}$. Fr. 5B $(210 \mathrm{mg})$ was purified by semipreparative HPLC (solvent: $30 \%$ $\left.\mathrm{CH}_{3} \mathrm{CN}-\mathrm{H}_{2} \mathrm{O}\right)$ to give $4(2 \mathrm{mg})$ and $5(6 \mathrm{mg})$. Fr. $5 \mathrm{C}(8.3 \mathrm{~g})$ was chromatographed on silica gel, eluting with $20 \%, 50 \%, 70 \%$, and $100 \%$ $\mathrm{MeOH}-\mathrm{C} \mathrm{HCl}_{3}$, to give four subfractions, $5 \mathrm{C} 1-5 \mathrm{C} 4$. Fr. $5 \mathrm{C} 3(33 \mathrm{mg})$ was purified by semipreparative $\mathrm{HPLC}\left(50 \% \mathrm{MeOH}-\mathrm{H}_{2} \mathrm{O}\right)$ to afford $12(3 \mathrm{mg})$ and $13(4 \mathrm{mg})$. Fr. $6(42 \mathrm{~g})$ was separated by RP-18 $\left(20-100 \% \mathrm{MeOH}-\mathrm{H}_{2} \mathrm{O}\right)$ to give four subfractions, 6A-6D. Fr. 6A $(152 \mathrm{mg})$ was chromatographed on Sephadex LH-20 $(100 \% \mathrm{MeOH})$ and further purified by semipreparative $\mathrm{HPLC}\left(30 \% \mathrm{MeOH}-\mathrm{H}_{2} \mathrm{O}\right)$ to yield $3(11 \mathrm{mg})$ and $6(5 \mathrm{mg})$. Fr. 6C $(10.6 \mathrm{~g})$ was chromatographed on silica gel, eluting with a gradient of MeOH in $\mathrm{CHCl}_{3}$ ranging from $20 \%$ to $100 \%$, to give five subfractions, 6C1-6C5. Fr. 6C3 $(108.6 \mathrm{mg})$ yielded $7(9 \mathrm{mg})$ and $8(18 \mathrm{mg})$ after repeated purification on a Sephadex $\mathrm{LH}-20$ column $(100 \% \mathrm{MeOH})$ and by semipreparative HPLC $\left(35 \% \mathrm{MeOH}-\mathrm{H}_{2} \mathrm{O}\right)$. Finally, Fr. $6 \mathrm{C} 4(110 \mathrm{mg})$ was purified by semipreparative HPLC, eluting with $25 \% \mathrm{CH}_{3} \mathrm{CN}-\mathrm{H}_{2} \mathrm{O}$, to obtain 9 $(6 \mathrm{mg}), \mathbf{1 0}(19 \mathrm{mg})$, and $11(7 \mathrm{mg})$.

Bistenuifolin A (1): colorless crystals from $(\mathrm{MeOH})$; mp $256-257^{\circ} \mathrm{C}$; $[\alpha]_{\mathrm{D}}^{18}-27$ (c 0.3, acetone); UV (MeOH) $\lambda_{\text {max }}(\log \varepsilon) 203$ (3.62); IR (KBr) $\nu_{\max } 3453,2932,1742,1241 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data, see Tables 1 and 2; positive-ion ESIMS $\mathrm{m} / \mathrm{z} 1003(10)[\mathrm{M}+\mathrm{Na}]^{+}$; positive-ion HRESIMS $m / z 1003.4307[\mathrm{M}+\mathrm{Na}]^{+}$(calcd for $\mathrm{C}_{52} \mathrm{H}_{68} \mathrm{O}_{18} \mathrm{Na}$, 1003.4303).

Bistenuifolin $B$ (2): white, amorphous powder; $[\alpha]_{D}^{18}-32$ (c 0.1, acetone); UV (MeOH) $\lambda_{\max }(\log \varepsilon) 203(3.63) \mathrm{nm} ; \mathrm{IR}(\mathrm{KBr}) \nu_{\max }$ 3441, 2934, 1740, 1733, 1635, $1242 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data, see

Tables 1 and 2; positive-ion ESIMS $m / z 1003$ (8) $[\mathrm{M}+\mathrm{Na}]^{+}$; positive-ion HRESIMS $m / z 1003.4333[\mathrm{M}+\mathrm{Na}]^{+}$(calcd for $\mathrm{C}_{52} \mathrm{H}_{68} \mathrm{O}_{18} \mathrm{Na}, 1003.4303$ ).

Bistenuifolin C (3): white, amorphous powder; $[\alpha]_{\mathrm{D}}^{15}-71$ (c 0.1, acetone); UV (MeOH) $\lambda_{\text {max }}(\log \varepsilon) 203(3.61) \mathrm{nm} ; \mathrm{IR}(\mathrm{KBr}) \nu_{\text {max }}$ 3445, 2935, 1729, 1638, $1247 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data, see Tables 1 and 2; positive-ion ESIMS $m / z 919$ (90) $[\mathrm{M}+\mathrm{Na}]^{+}$; positive-ion HRESIMS $m / z 919.4073[\mathrm{M}+\mathrm{Na}]^{+}$(calcd for $\mathrm{C}_{48} \mathrm{H}_{64} \mathrm{O}_{16} \mathrm{Na}, 919.4092$ ).

Bistenuifolin $D$ (4): colorless crystals from ( MeOH ); mp 223$225{ }^{\circ} \mathrm{C}$; $[\alpha]_{\mathrm{D}}^{20}-40\left(c 0.1, \mathrm{CHCl}_{3}-\mathrm{MeOH}, 1: 1\right)$; UV ( MeOH$) \lambda_{\text {max }}$ $(\log \varepsilon) 203(3.70) \mathrm{nm}$; IR (KBr) $\nu_{\max } 3434,2947,1738,1730,1656$ $\mathrm{cm}^{-1}$; ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data, see Tables 2 and 3; positive-ion ESIMS $m / z 945$ (100) $[\mathrm{M}+\mathrm{Na}]^{+}$; positive-ion HRESIMS $\mathrm{m} / \mathrm{z} 945.4244$ $[\mathrm{M}+\mathrm{Na}]^{+}$(calcd for $\left.\mathrm{C}_{50} \mathrm{H}_{66} \mathrm{O}_{16} \mathrm{Na}, 945.4248\right)$.

Bistenuifolin $E$ (5): white, amorphous powder; $[\alpha]_{\mathrm{D}}^{21}-12$ (c 0.2, $\left.\mathrm{CHCl}_{3}-\mathrm{MeOH}, 1: 1\right) ; \mathrm{UV}(\mathrm{MeOH}) \lambda_{\max }(\log \varepsilon) 203$ (3.69), 195 (3.40) nm ; IR (KBr) $\nu_{\max } 3436,2953,1741,1239 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data, see Tables 2 and 3; positive-ion ESIMS $m / z 901$ (100) $[\mathrm{M}+\mathrm{Na}]^{+}$; positive-ion HRESIMS $m / z 901.3978[\mathrm{M}+\mathrm{Na}]^{+}$(calcd for $\mathrm{C}_{48} \mathrm{H}_{62} \mathrm{O}_{15} \mathrm{Na}$, 901.3986).

Bistenuifolin $F$ (6): colorless crystals from ( MeOH ); mp 177$178{ }^{\circ} \mathrm{C}$; $[\alpha]_{\mathrm{D}}^{17}-64\left(c 0.2\right.$, acetone); UV $(\mathrm{MeOH}) \lambda_{\text {max }}(\log \varepsilon) 205$ (3.53) nm; IR (KBr) $\nu_{\text {max }} 3453,2934,1733,1635,1256,1070 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data, see Tables 2 and 3; positive-ion ESIMS $\mathrm{m} / \mathrm{z}$ 923 (20) $\left[\mathrm{M}+\mathrm{Na}^{+}\right.$; positive-ion HRESIMS $\mathrm{m} / \mathrm{z} 923.4402[\mathrm{M}+$ $\mathrm{Na}]^{+}\left(\right.$calcd for $\left.\mathrm{C}_{48} \mathrm{H}_{68} \mathrm{O}_{16} \mathrm{Na}, 923.4405\right)$.

Bistenuifolin $G(7)$ : white, amorphous powder; $[\alpha]_{\mathrm{D}}^{15}-34$ (c 0.1, acetone); UV (MeOH) $\lambda_{\text {max }}(\log \varepsilon) 203(3.46) \mathrm{nm} ; \mathrm{IR}(\mathrm{KBr}) \nu_{\text {max }}$ 3447, 1738, 1728, 1630, $1243 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data, see Tables 4 and 5; positive-ion ESIMS $\mathrm{m} / \mathrm{z} 937$ (50) $[\mathrm{M}+\mathrm{Na}]^{+}$; positive-ion HRESIMS $m / z 937.4200[\mathrm{M}+\mathrm{Na}]^{+}$(calcd for $\mathrm{C}_{48} \mathrm{H}_{66} \mathrm{O}_{17} \mathrm{Na}, 937.4197$ ).

Bistenuifolin H (8): white, amorphous powder; $[\alpha]_{\mathrm{D}}^{15}-28$ (c 0.2, acetone) ; UV $(\mathrm{MeOH}) \lambda_{\max }(\log \varepsilon) 203$ (3.46), 191 (3.16) nm; IR $(\mathrm{KBr}) \nu_{\max } 3448,1740,1631,1242 \mathrm{~cm}^{-1} ;{ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data, see Tables 4 and 5; positive-ion ESIMS $m / z 951$ (100) $[\mathrm{M}+\mathrm{Na}]^{+}$; positive-ion HRESIMS $m / z 951.4332[\mathrm{M}+\mathrm{Na}]^{+}$(calcd for $\mathrm{C}_{49} \mathrm{H}_{68} \mathrm{O}_{17} \mathrm{Na}, 951.4354$ ).

Bistenuifolin I (9): white, amorphous powder; $[\alpha]_{\mathrm{D}}^{15}-46$ (c 0.1, acetone); UV (MeOH) $\lambda_{\text {max }}(\log \varepsilon) 206$ (3.41), 195 (3.20) nm; IR $(\mathrm{KBr}) \nu_{\max } 3441,2927,1740,1636,1243 \mathrm{~cm}^{-1} ;{ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data, see Tables 4 and 5 ; positive-ion ESIMS $m / z 951$ (40) $[\mathrm{M}+\mathrm{Na}]^{+}$; positive-ion HRESIMS $\mathrm{m} / \mathrm{z} 951.4347[\mathrm{M}+\mathrm{Na}]^{+}$(calcd for $\mathrm{C}_{49} \mathrm{H}_{68} \mathrm{O}_{17} \mathrm{Na}, 951.4354$ ).

Bistenuifolin J (10): white, amorphous powder; $[\alpha]_{\mathrm{D}}^{15}-36$ (c 0.1, acetone); UV (MeOH) $\lambda_{\max }(\log \varepsilon) 204$ (3.45), 193 (3.24) nm; IR $(\mathrm{KBr}) \nu_{\max } 3436,1726,1631,1245 \mathrm{~cm}^{-1}$; ${ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data, see Tables 4 and 5; positive-ion ESIMS $\mathrm{m} / \mathrm{z} 867$ (10) $[\mathrm{M}+\mathrm{Na}]^{+}$; positive-ion HRESIMS $m / z 867.4147[\mathrm{M}+\mathrm{Na}]^{+}$(calcd for $\mathrm{C}_{45} \mathrm{H}_{64} \mathrm{O}_{15} \mathrm{Na}, 867.4142$ ).

Bistenuifolin K (11): white, amorphous powder; $[\alpha]_{\mathrm{D}}^{18}-29$ (c 0.2, acetone); UV (MeOH) $\lambda_{\text {max }}(\log \varepsilon) 203$ (3.46) nm; IR (KBr) $\nu_{\text {max }}$ 3465, 2934, 1723, $1245 \mathrm{~cm}^{-1} ;{ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data, see Tables 4 and 5; positive-ion ESIMS $m / z 909(100)[\mathrm{M}+\mathrm{Na}]^{+}$; positive-ion HRESIMS $m / z 909.4231[\mathrm{M}+\mathrm{Na}]^{+}$(calcd for $\mathrm{C}_{47} \mathrm{H}_{66} \mathrm{O}_{16} \mathrm{Na}, 909.4248$ ).

Bistenuifolin $L$ (12): white, amorphous powder; $[\alpha]_{\mathrm{D}}^{21}+19$ (c 0.1, $\left.\mathrm{CHCl}_{3}-\mathrm{MeOH}, 1: 1\right) ; \mathrm{UV}(\mathrm{MeOH}) \lambda_{\text {max }}(\log \varepsilon) 202$ (3.78) nm; IR $(\mathrm{KBr}) \nu_{\max } 3448,2941,1740,1242 \mathrm{~cm}^{-1} ;{ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data, see Tables 1 and 5; positive-ion ESIMS $m / z 1003(100)[\mathrm{M}+\mathrm{Na}]^{+}$; positive-ion HRESIMS $m / z 1003.4296[\mathrm{M}+\mathrm{Na}]^{+}$(calcd for $\mathrm{C}_{52} \mathrm{H}_{68} \mathrm{O}_{18} \mathrm{Na}, 1003.4303$ ).

Bistenuifolin $M$ (13): white, amorphous powder; $[\alpha]_{\mathrm{D}}^{20}+22$ (c 0.2 , $\left.\mathrm{CHCl}_{3}-\mathrm{MeOH}, 1: 1\right) ; \mathrm{UV}(\mathrm{MeOH}) \lambda_{\text {max }}(\log \varepsilon) 203(3.67)$; IR (KBr) $\nu_{\max } 3524,2954,1735,1242 \mathrm{~cm}^{-1} ;{ }^{1} \mathrm{H}$ and ${ }^{13} \mathrm{C}$ NMR data, see Tables 1 and 5; positive-ion ESIMS $m / z 945$ (100) $[\mathrm{M}+\mathrm{Na}]^{+}$; positive-ion HRESIMS $m / z 945.4249[\mathrm{M}+\mathrm{Na}]^{+}$(calcd for $\mathrm{C}_{50} \mathrm{H}_{66} \mathrm{O}_{16} \mathrm{Na}, 945.4248$ ).

X-ray Crystal Structure Analysis. Colorless crystals of 1 and 4 were obtained from MeOH . Intensity data were collected at room
temperature on a Bruker APEX DUO diffractometer equipped with an APEX II CCD, using $\mathrm{Cu} \mathrm{K} \alpha$ radiation. Cell refinement and data reduction were performed with Bruker's SAINT program. The structures were solved by direct methods using SHELXS-97. ${ }^{19}$ Refinements were performed with SHELXL-97 using full-matrix least-squares, with anisotropic displacement parameters for all the non-hydrogen atoms. The H atoms were placed in calculated positions and refined using a riding model. Molecular graphics were computed with PLATON. Crystallographic data for the reported structures have been deposited with the Cambridge Crystallographic Data Center as supplementary publications no. CCDC 1008727 for 1 and CCDC 1008728 for 4. Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB 1EZ, UK [fax: Int. $+44(0)(1223) 336$ 033; e-mail: deposit@ccdc.cam.ac.uk].

Crystal data of bistenuifolin $A(1): \mathrm{C}_{52} \mathrm{H}_{68} \mathrm{O}_{18}, M=981.1$, monoclinic, $a=14.0764(4) \AA, b=10.8065(3) \AA, c=16.5499(5) \AA$, $\alpha=90.00^{\circ}, \beta=98.0850(10)^{\circ}, \gamma=90.00^{\circ}, V=2492.49(12) \AA^{3}, T=$ $100(2) \mathrm{K}$, space group $P 2_{1}, Z=2, \mu(\mathrm{Cu} \mathrm{K} \alpha)=0.818 \mathrm{~mm}^{-1}, 16342$ reflections measured, 7178 independent reflections $\left(R_{\mathrm{int}}=0.0600\right)$. The final $R_{1}$ values were $0.0895(I>2 \sigma(I))$. The final $w R\left(F^{2}\right)$ values were $0.2520(I>2 \sigma(I))$. The final $R_{1}$ values were 0.1010 (all data). The final $w R\left(F^{2}\right)$ values were 0.2734 (all data). The goodness of fit on $F^{2}$ was 1.156. Flack parameter $=-0.2(3)$.

Crystal data of bistenuifolin $D$ (4): $\mathrm{C}_{55} \mathrm{H}_{75} \mathrm{NO}_{18}\left(\mathrm{C}_{50} \mathrm{H}_{66} \mathrm{O}_{16}+\right.$ $\left.\mathrm{C}_{5} \mathrm{H}_{5} \mathrm{~N}+2 \mathrm{H}_{2} \mathrm{O}\right), M=1038.2$, orthorhombic, $a=10.7575(3) \AA$, $b=$ $11.2807(3) \AA, c=43.2752(11) \AA, \alpha=90.00^{\circ}, \beta=90.00^{\circ}, \gamma=90.00^{\circ}$, $V=5251.5(2) \AA^{3}, T=100(2) \mathrm{K}$, space group $P 2_{1} 2_{1} 2_{1}, Z=4, \mu(\mathrm{Cu}$ $\mathrm{K} \alpha)=0.810 \mathrm{~mm}^{-1}, 28423$ reflections measured, 9223 independent reflections $\left(R_{\text {int }}=0.0976\right)$. The final $R_{1}$ values were $0.0628(I>2 \sigma(I))$. The final $w R\left(F^{2}\right)$ values were $0.1457(I>2 \sigma(I))$. The final $R_{1}$ values were 0.1085 (all data). The final $w R\left(F^{2}\right)$ values were 0.1745 (all data). The goodness of fit on $F^{2}$ was 1.011. Flack parameter $=0.0(2)$.

Cytotoxicity Assay. The HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human tumor cell lines were used, all of which were obtained from the ATCC (Manassas, VA, USA). All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT, USA) supplemented with $10 \%$ fetal bovine serum (Hyclone) at $37{ }^{\circ} \mathrm{C}$ in a humidified atmosphere with $5 \% \mathrm{CO}_{2}$. Cell viability was assessed by colorimetric measurement of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO, USA). Briefly, $100 \mu \mathrm{~L}$ of adherent cells was seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition. Cells of both types were seeded at initial densities of $1 \times 10^{5}$ cells $/ \mathrm{mL}$ in $100 \mu \mathrm{~L}$ of media. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 h , using cisplatin and paclitaxel (Sigma) as a positive control. After the incubation, MTT $(100 \mu \mathrm{~g})$ was added to each well, and the incubation was maintained for 4 h at $37^{\circ} \mathrm{C}$. The cells were lysed with $100 \mu \mathrm{~L}$ of $20 \%$ SDS-50\% DMF after removal of $100 \mu \mathrm{~L}$ of medium. The optical density of the lysate was measured at 595 nm in a 96 -well microtiter plate reader (Bio-Rad 680). The $\mathrm{IC}_{50}$ value of each compound was calculated by Reed and Muench's method. ${ }^{18}$

## ASSOCIATED CONTENT

## (S) Supporting Information

This material $\left({ }^{1} \mathrm{H},{ }^{13} \mathrm{C}\right.$ NMR, DEPT, HSQC, HMBC, COSY, NOESY, HRESIMS, UV, and IR spectra of compounds 1-13; X-ray data of compounds 1 and 4 ) is available free of charge via the Internet at http://pubs.acs.org.

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## Notes

The authors declare no competing financial interest.

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