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Laxiflorolides A and B, Epimeric Bishomoditerpene Lactones from *Isodon eriocalyx*

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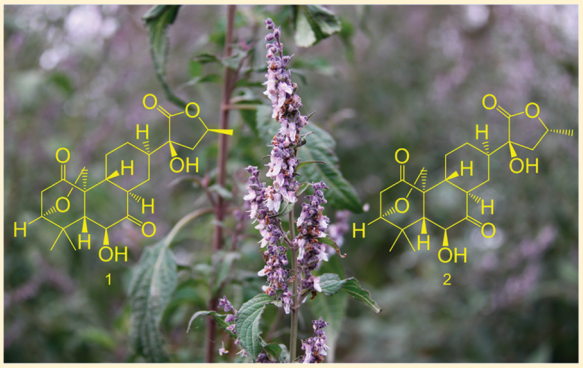
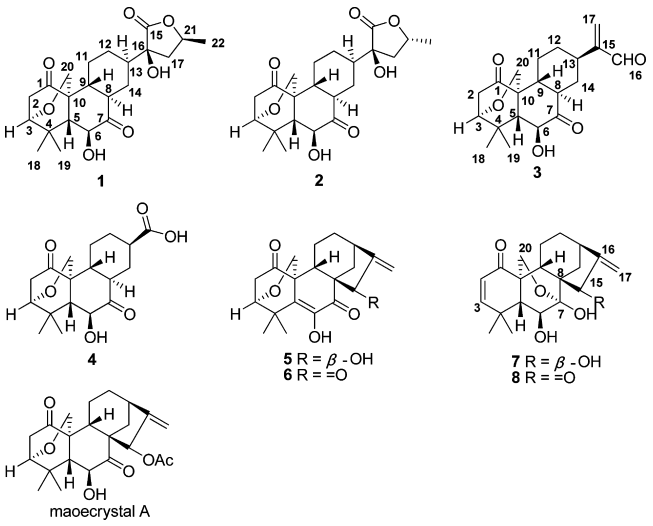
Supporting Information

ABSTRACT: Laxiflorolides A (**1**) and B (**2**), two unprecedented epimeric bishomoditerpene lactones with a unique C_{22} framework, along with laxiflorins P−R (**3**−**5**), maoecrystal P (**6**), maoecrystal C (**7**), and eriocalyxin B (**8**), were isolated from the leaves of *I. eriocalyx* var. *laxiflora*. The structures of **1** and **2**, including the absolute configurations, were determined by spectroscopic methods and single-crystal X-ray diffraction analysis. All of the compounds isolated were evaluated for their cytotoxicity against five tumor cell lines. Compounds **3**, **6**, and **8** showed remarkable cytotoxic activity against certain cell lines compared with the positive control.



Terpenoids are one of the largest group of natural products, and the chemodiversity of terpenoids is as important a characteristic as their biodiversity.[1] *ent*-Kaurane-type diterpenoids are excellent examples of natural products with diverse structural scaffolds[2] and important pharmaceutical activities.[3] *Isodon*, a genus of the Labiatae family, is well known for producing bioactive diterpenoids with diverse skeletons, including *ent*-kaurane diterpenoids. More than 600 new diterpenoids have been previously identified from this genus by our group.[2] Some new *ent*-kaurane diterpenoids have been recently isolated from the *Isodon* genus[4] and other genera[5] or obtained by microbial transformation.[6] Of the thousands of *ent*-kaurane diterpenoids previously identified, no compound has been reported to have a C_{22} carbon skeleton. Among the plants of southwest China, *I. eriocalyx* var. *laxiflora* was previously investigated phytochemically[2] and led to the isolation of more than 60 new diterpenoids, including 7,20-epoxy-*ent*-kauranoids (laxiflorins H and I),[7] 3,20-epoxy-*ent*-kauranoids (laxiflorins J−M),[8] 6,7-seco-*ent*-kauranoids (laxiflorins A−C),[9] 6,7:8,15-seco-*ent*-kauranoids (laxiflorins F and G),[10] *ent*-abietanoids (laxiflorin N),[11] and two unprecedented *ent*-kaurane diterpenoids (neolaxiflorins A and B).[12] Our further investigation of this plant led to the isolation of two new diterpenoids, laxiflorolides A (**1**) and B (**2**), which are the first examples of *ent*-kauranoids bearing a unique C_{22} carbon framework, along with laxiflorins P−R (**3**−**5**), and three other known compounds, maoecrystal P (**6**),[13] maoecrystal C (**7**),[8] and eriocalyxin B (**8**).[9] In this paper, we report the isolation, structural elucidation, and cytotoxic activities of compounds **1**−**8**.



RESULTS AND DISCUSSION

Laxiflorolide A (**1**) was obtained as colorless needles. The molecular formula $C_{22}H_{30}O_7$, with eight degrees of unsaturation, was established on the basis of HRESIMS ($[M + Na]^+$, 429.1878; calcd for $C_{22}H_{30}O_7Na$, 429.1889) and NMR spectroscopy (Tables 1 and 2). The IR spectrum of **1** indicated

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Table 1. ^{1}H NMR Data (Pyridine-d_5) of Compounds 1−5

position	1[a]	2[a]	3[b]	4[c]	5[b]
2α	2.78, dd (19.3, 2.3)	2.78, dd (19.4, 2,2)	2.77, dd (19.0, 2.7)	2.77, dd (19.0, 2.6)	3.01, dd (19.0, 3.1)
2β	2.72, dd (19.3, 3.0)	2.72, dd (19.4, 3.4)	2.72, dd (19.0, 3.4)	2.73, dd (19.0, 3.2)	2.85, d (19.0)
3α	3.74, br s	3.74, dd (3.4, 2.2)	3.74, br s	3.75, t (2.6)	3.85, br s
5β	1.85, d (11.0)	1.86, dd (11.3, 1.3)	1.83, d (11.0)	1.83, d (11.2)	
6α	4.77, d (11.0)	4.75, d (11.3)	4.79, d (11.0)	4.81, d (11.2)	
8α	2.25, m	2.33, m	2.38, m	2.44, m	
9β	2.27, m	2.28, m	2.19, dd (17.4,7.3)	2.26, m	4.03, d (7.6)
11α	0.94, m	0.97, m	1.02, m	1.02, ddd (15.4, 12.7, 3.2)	1.41, m
11β	1.98, m	2.02, m	1.88, dd (12.2, 3.1)	1.97, m	2.48, m
12α	1.31, m	1.13, m	1.27, m	2.19, m	1.50, m
12β	2.31, m	2.81, dt (13.0, 2.8)	1.63, m	1.63, dd (12.6, 3.0)	1.33, m
13α	2.01, m	2.14, m	2.48, m	2.46, m	2.74, br s
14α	2.14, dd (12.6, 2.2)	2.16, m	2.08, dd (13.4, 2.5)	2.59, m	1.77, d (12.0)
14β	1.62, br d (12.6)	1.51, m	1.44, m	2.03, m	1.58, dd (12.0, 4.6)
15					5.74, s
16			9.52, s		
17a	2.60, dd (13.7, 7.0)	1.80, dd (13.5, 9.6)	6.11, s		5.53, s
17b	1.94, dd (13.7, 7.5)	2.22, dd (13.5, 5.7)	5.86, s		5.12, s
18β	1.10, s	1.10, s	1.79, s	1.68, s	1.79, s
19α	1.68, s	1.68, s	1.68, s	1.10, s	1.27, s
20αa	4.39, d (9.5)	4.37, d (9.8)	4.46, d (10.0)	4.49, d (9.8)	4.78, d (8.9)
20αb	4.06, d (9.5)	4.05, dd (9.8, 1.3)	4.10, d (10.0)	4.12, d (9.8)	4.39, dd (8.9, 7.6)
21	4.49, m	4.87, m			
22	1.27, d (6.2)	1.21, d (6.2)			

[a]Recorded at 400 MHz. [b]Recorded at 500 MHz. [c]Recorded at 500 MHz.

Table 2. ^{13}C NMR Data (Pyridine-d_5) of Compounds 1−5

position	1[a]	2[a]	3[b]	4[c]	5[b]
1	209.6, C	209.6, C	209.5, C	209.4, C	206.6, C
2	41.2, CH_2	41.9, CH_2	41.9, CH_2	41.9, CH_2	42.0, CH_2
3	77.9, C	77.9, CH	77.9, CH	78.0, CH	77.9, CH
4	38.3, C	38.3, C	38.2, C	38.3, C	40.8, C
5	54.6, CH	54.6, CH	54.7, CH	54.6, CH	133.0, C
6	74.2, CH	74.1, CH	74.1, CH	74.2, CH	146.4, C
7	210.9, C	210.9, C	210.7, C	210.7, C	196.5, C
8	45.7, CH	45.7, CH	45.9, C	45.4, C	54.4, C
9	43.1, CH	43.3, CH	42.8, CH	42.8, CH	27.0, CH
10	50.0, C	50.0, C	50.0, C	50.1, C	54.5, C
11	28.6, CH_2	28.8, CH_2	28.8, CH_2	28.5, CH_2	19.9, CH_2
12	26.0, CH_2	26.1, CH_2	31.2, CH_2	29.4, CH_2	32.6, CH_2
13	43.2, CH	41.9, CH	34.4, CH	42.4, CH	41.3, CH
14	27.3, CH_2	27.9, CH_2	31.5, CH_2	29.3, CH_2	38.1, CH_2
15	179.5, C	178.0, C	154.5, C	177.6, C	76.5, CH
16	79.1, C	79.6, C	194.4, CH		157.4, C
17	39.9, CH_2	39.7, CH_2	133.1, CH_2		106.0, CH_2
18	29.5, CH_3	29.4, CH_3	29.4, CH_3	29.5, CH_3	23.6, CH_3
19	23.4, CH_3	23.4, CH_3	23.4, CH_3	23.4, CH_3	21.9, CH_3
20	60.2, CH_2	60.2, CH_2	60.2, CH_2	60.2, CH_2	67.7, CH_2
21	73.9, CH	74.7, CH			
22	22.2, CH_3	20.7, CH_3			

[a]Recorded at 100 MHz. [b]Recorded at 125 MHz. [c]Recorded at 150 MHz.

the presence of hydroxy groups (3442 cm^{-1}) and carbonyl groups (1723 cm^{-1}). The intense IR absorption band at 1766 cm^{-1} revealed the presence of a γ-lactone group,[14] and the broad C−O−C stretching band in the region of 1065 and 1086 cm^{-1} revealed the presence of an ether functional group.

The ^{1}H NMR (Table 1) spectrum showed resonances attributed to the characteristic AB methylene group at δ_H 4.39 and 4.06 (each 1H, d, 9.5 Hz), one secondary methyl at δ_H 1.27 (3H, d, 6.2 Hz), and two tertiary methyls at δ_H 1.10 (3H, s) and 1.68 (3H, s). In addition, the spectrum showed resonances due to an ABX spin system at δ_H 2.78 (1H, dd, 19.3, 2.3 Hz), 2.72 (1H, dd, 19.3, 3.0 Hz), and 3.74 (1H, br s) together with two oxygenated methines at δ_H 4.77 (1H, d, 11.0 Hz) and 4.49 (1H, m). The analysis of the ^{13}C NMR and DEPT spectra (Table 2)

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revealed the presence of 22 carbons, which were assigned as three methyl, six methylene (one oxygenated), seven methine (three oxygenated), and six quaternary carbons (one oxygenated, one ester, and two carbonyls), which suggested that **1** is a highly oxygenated bishomoditerpene with a C_{22} skeleton different from the *ent*-kaurane or *ent*-abietane skeletons reported previously. Importantly, the lack of any olefinic moieties required the presence of five rings to satisfy the degrees of unsaturation.

The HMBC spectrum of **1** showed correlations from the geminal methyls Me-18 (δ_H 1.10, 3H, s) and Me-19 (δ_H 1.68, 3H, s) to C-3, C-4, and C-5. Furthermore, the AB spin system of methylene H_2-20 showed HMBC correlations with C-1, C-3, C-5, and C-10. Other HMBC correlations were noted between the ABX spin system of methylene H_2-2 and C-1, C-3, and C-4 and between H-5 (δ_H 1.85, 1H, d, 11.0 Hz) and C-1, C-4, C-6, C-10, C-18, C-19, and C-20. These observed HMBC correlations, coupled with a spin system ($CHCH_2CH_2CHCH_2CH$, H-9/H_2-11/H_2-12/H-13/H_2-14/H-8) established by $^1H-^1H$ COSY correlations and HSQC spectra, gave rise to partial structure **1a** (Figure 1).

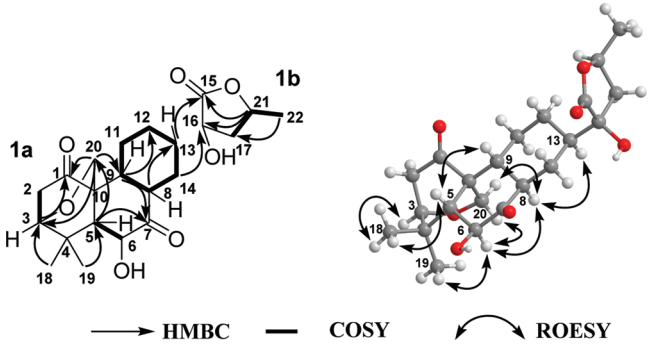

Figure 1. $^1H-^1H$ COSY (bold), selected HMBC (arrow), and key ROESY correlations of compound **1**.

The HMBC spectrum showed that Me-22 correlated with C-17 and C-21 and that H-21 correlated with C-15 (δ_C 179.5, s), C-16 (δ_C 79.1, s), and C-17. This evidence, along with the proton spin system deduced from the $^1H-^1H$ COSY correlations, H_2-17/H-21/H_3-22, suggested the partial structure **1b** (Figure 1). Moreover, the key HMBC correlations of H-13 (δ_H 2.01, 1H, m) with C-14, C-15, C-16, and C-17 and of H-17α (δ_H 2.60, 1H, dd, 13.7, 7.0 Hz) and H-17β (δ_H 1.94, 1H, dd, 13.7, 7.5 Hz) with C-13, C-15, C-16, C-21, and C-22 permitted the partial structures **1a** and **1b** to be connected through a carbon−carbon connection between C-13 and C-16. In the ROESY spectrum of **1**, the NOE correlations of Me-19/H-6, H-6/H-8/H_2-20, and H-8/H-13 suggested that H-6, H-8, H-13, Me-19, and C-20 all adopted an α-orientation. The cross-peaks between H-3/H-5, H-5/H-9, and H-5/Me-18 in the ROESY spectrum demonstrated that H-3, H-5, H-9, and Me-18 are β-oriented (Figure 1).

Single-crystal X-ray diffraction analysis using the anomalous scattering of Cu Kα radiation yielded a Flack parameter of 0.2 (3) (CCDC 837073),[15] confirming the above conjecture and indicating that the absolute configuration of compound **1** was 3*R*, 5*R*, 6*S*, 8*R*, 9*R*, 10*S*, 13*R*, 16*S*, 21*S* (Figure 2).

Laxiflorolide B (**2**) had the same molecular formula, $C_{22}H_{30}O_7$, as compound **1** according to the HRESIMS data (*m*/*z* 429.1883 for $[M + Na]^+$) and, thus, also has eight degrees

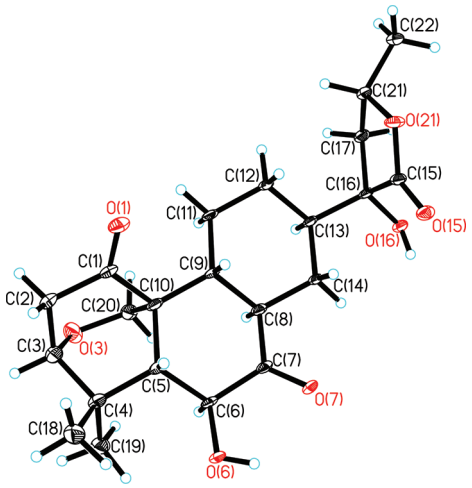

Figure 2. X-ray crystallographic structure of compound **1**.

of unsaturation. The chemical shift values in the ^1H and ^{13}C NMR spectra of compound **2** and compound **1** are similar, with a deviation of ~0.5 ppm, respectively. The NMR resonances for H-17α, H-21, C-15, C-21, and C-22 of compound **2** were shifted by $\Delta\delta_H$ −0.8, $\Delta\delta_H$ +0.38, $\Delta\delta_C$ −1.5, $\Delta\delta_C$ + 0.8, and $\Delta\delta_C$ −1.5 ppm, respectively, relative to the resonances of compound **1** (Table 1).

Extensive NMR and MS analyses showed that compounds **1** and **2** possessed the same gross structure and should be a pair of diastereomers. X-ray crystallographic analysis using a single crystal of compound **2** and Cu Kα radiation (CCDC 837074) confirmed the above deduced structure and revealed that compound **2** is the C-21 epimer of compound **1**. The absolute configuration of **2** is 3*R*, 5*R*, 6*S*, 8*R*, 9*R*, 10*S*, 13*R*, 16*S*, 21*R* (Figure 3).

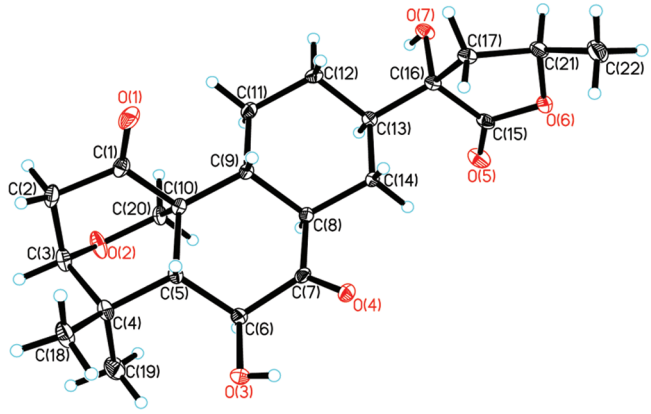

Figure 3. X-ray crystallographic structure of compound **2**.

Compound **3** was a new natural product that had been synthesized from maoecrystal A.[9,16] Because their spectroscopic data and melting points were consistent, compound **3** was determined to be 6β-hydroxy-3α,20-epoxy-15(17)-en-16-al-*ent*-kaur-1,7-dione and was named laxiflorin P.

Compound **4** had the molecular formula $C_{18}H_{24}O_6$. Comparison of the NMR data of **4** (Tables 1 and 2) and **3** revealed similarities. The principal differences between these compounds were the absence of two carbon signals (C-16 and C-17) for **3** and the appearance of a C-15 carboxylic group (δ_C 177.6, s) in compound **4**; this difference was supported by the

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Table 3. Cytotoxic (IC_{50}, μM, mean ± SD) Activity of Compounds 1−8[a,b] (n = 3)

	HL-60		SMMC-7721		MCF-7		A-549		SW480	
compound	IC_{50}	pIC_{50}	IC_{50}	pIC_{50}	IC_{50}	pIC_{50}	IC_{50}	pIC_{50}	IC_{50}	pIC_{50}
1	>40	<4.4	>40	<4.4	>40	<4.4	>40	<4.4	>40	<4.4
2	>40	<4.4	>40	<4.4	>40	<4.4	>40	<4.4	>40	<4.4
3	3.8 ± 0.2	5.4	5.6 ± 0.6	5.3	3.2 ± 0.03	5.5	11.7 ± 1.2	4.9	3.1 ± 0.1	5.5
4	>40	<4.4	>40	<4.4	>40	<4.4	>40	<4.4	>40	<4.4
5	9.9 ± 1.6	5.3	21.5 ± 0.4	4.7	12.9 ± 0.2	4.9	24.9 ± 2.4	4.6	15.4 ± 0.7	4.8
6	1.0 ± 0.04	6.0	2.8 ± 0.1	5.6	1.8 ± 0.1	5.7	6.3 ± 1.3	5.2	2.1 ± 0.04	5.7
7	18.9 ± 1.5	4.7	>40	<4.4	14.4 ± 0.2	4.8	>40	<4.4	14.5 ± 0.4	4.8
8	0.3 ± 0.03	6.5	0.8 ± 0.02	6.1	0.6 ± 0.04	6.2	3.1 ± 0.1	5.5	0.5 ± 0.1	6.3
cisplatin	2.0 ± 0.1	5.7	16.2 ± 0.6	4.8	17.8 ± 0.8	4.7	17.5 ± 1.1	4.8	12.8 ± 0.1	4.9

[a]Results were expressed as IC_{50} values in μM, data were obtained from triplicate experiments, and cisplatin was used as positive control. [b]Cytotoxic cutoff value was set at pIC_{50} = 5.5 ($-\log IC_{50}$).

HMBC correlations from H-12α (δ_H 2.19 m), H-12β (δ_H 1.63 dd, 12.6, 3.0 Hz), H-14α (δ_H 2.59 m), and H-14β (δ_H 2.03 m) to C-15. The correlations of H_2-20α/H-8α/H-13α in the ROESY spectrum showed that H-13α in compound **4** was α-oriented. Therefore, compound **4** was 6β-hydroxy-3α,20-epoxy-*ent*-abieta-16,17-bisnor-1,7-dion-15-oic acid, and it was named laxiflorin Q.

The HRESIMS of compound **5** suggested a molecular formula of $C_{20}H_{24}O_5$, with nine degrees of unsaturation. The ^1H and ^{13}C NMR data of **5** (Tables 1 and 2) were consistent with a 3α,20-epoxy-*ent*-kaurane, similar to maoecrystal P (**6**). The most notable difference is that the C-15 carbonyl group in maoecrystal P (**6**) was changed into a hydroxy group in **5**. This difference was supported by HMBC correlations from H-17a (δ_H 5.53) and H-17b (δ_H 5.12) to C-13, C-15, and C-16 and from H-15 to C-7, C-9, C-14, C-16, and C-17. The ROESY cross-peaks between H-13α/H-15 and other correlations indicated that compounds **5** and **6** had the same relative configuration. Consequently, compound **5** was named laxiflorin R.

Compounds **1**−**8** were tested for in vitro cytotoxicity against A-549, MCF-7, SMMC-7721, SW-480, and HL-60 human cancer cell lines using the MTT method;[17] cisplatin was used as the positive control. Compound **3** showed selective cytotoxic activity, with IC_{50} values of 3.2 ± 0.03 (pIC_{50} = 5.5) and 3.1 ± 0.1 μM (pIC_{50} = 5.5) against MCF-7 and SW-480 cells, respectively. Compound **6** exhibited cytotoxicity against SMMC-7721, SW-480, MCF-7, and HL-60 cells with IC_{50} values from 2.8 ± 0.1 to 1.0 ± 0.04 μM (pIC_{50} > 5.5). Compound **8** expressed inhibitory activity against all five cell lines, with IC_{50} values from 3.1 ± 0.1 to 0.3 ± 0.03 μM (pIC_{50} ≥ 5.5). The results are shown in Table 3. The cytotoxic cutoff value was set at pIC_{50} = 5.5 ($-\log IC_{50}$).[18]

In conclusion, laxiflorolides A (**1**) and B (**2**) are reported herein as the first example of unprecedented epimeric bishomoditerpene lactones with a unique C_{22} framework obtained from the *Isodon* genus. The absolute configurations of **1** and **2** were determined by single-crystal X-ray diffraction analysis using anomalous dispersion of Cu Kα radiation.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV data were obtained on a Shimadzu UV-2401A spectrophotometer. ECD spectra were measured on a Chirascan instrument. A BioRad FtS-135 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on DRX-400 spectrometers. Unless otherwise specified, chemical shifts (δ) are expressed in ppm with respect to the solvent signals. HRESIMS was performed on a VG Autospec-3000 spectrometer at 70 eV. Column chromatography was performed with silica gel (100−200 mesh; Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China). Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18} 9.4 mm × 25 cm column. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a Shimadzu PRC-ODS (K) column. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 8% H_2SO_4 in EtOH. All solvents including petroleum ether (60−90 °C) were distilled prior to use.

Plant Material. The leaves of *Isodon eriocalyx* var. *laxiflora* were collected from Yunnan Province, People's Republic of China, in September 2009. Voucher specimens (KIB20080028) were deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, and were identified by Prof. Xi-Wen Li.

Extraction and Isolation. The air-dried leaves *I. eriocalyx* var. *laxiflora* (10 kg) were extracted with 70% aqueous acetone (3 × 40 L, 2 days each) at room temperature. The solvent was evaporated in vacuo to afford a crude extract, which was suspended in H_2O and then extracted successively with EtOAc and *n*-BuOH. The EtOAc-soluble fraction (600 g) was decolorized on MCI gel with 90:10 MeOH/H_2O to obtain a yellow gum (427.5 g). The gum was purified by CC on SiO_2 with a $CHCl_3$/Me_2CO gradient system, 1:0, 9:1, 8:2, 7:3, 6:4, and 1:1, to yield six main fractions, A−F. Fraction B ($CHCl_3$/Me_2CO, 9:1; 80 g) was subjected to repeated chromatography over silica gel (petroleum ether/acetone, from 30:1 to 1:1) to give fractions B1−B4. Compounds **6** (20 mg) and **8** (1.0 g) were crystallized from fractions B1 (petroleum ether/acetone, 30:1) and B2 (petroleum ether/Me_2CO, 20:1). Fraction B3 (petroleum ether/Me_2CO, 10:1) was separated further by RP-18 CC (MeCN/H_2O, 30:70) to afford **3** (6 mg) and **7** (5 mg) and with MeCN/H_2O, 40:60, to afford **5** (5 mg). Fr. D ($CHCl_3$/Me_2CO 7:3, 50 g) was eluted with $CHCl_3$/MeOH (30:1, 20:1, and 10:1), yielding subfractions D1−D3. Subfraction D2 (10 g, $CHCl_3$/MeOH, 20:1) was fractionated by repeated CC, first on RP-18 with a gradient elution with MeOH/H_2O (2:8 to 1:0) to yield fractions D2/1−D2/5. Subsequently, fraction D2/2 (0.87 g) was purified using a silica gel column ($CHCl_3$/isopropyl alcohol, 30:1 to 10:1) to give subfractions D2/2/2 (105 mg) and D2/2/4 (120 mg). Subfraction D2/2/2 was purified by semipreparative HPLC (3 mL/min, UV detection at λ_{max} = 208 nm, MeCN/H_2O, 25:75) to yield **1** (12 mg). Subfraction D2/2/4 was purified by semipreparative HPLC (3 mL/min, UV detection at λ_{max} = 208 nm, MeCN/H_2O, 30:70) to yield **2** (3 mg). Fr. E ($CHCl_3$/Me_2CO, 6:4; 100 g) was eluted with MCI (30:70, 60:40, and 90:10 MeOH/H_2O) to afford fractions E1−E3. Compound **4** (5 mg) was separated further by RP-8 CC (MeCN/H_2O, 15:85) from fraction E1 (10 g).

Laxiflorolide A (1): colorless needles; mp 131.3−131.6 °C; $[\alpha]^{23}_D$ −34.7 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 208 (2.29), 280 (1.59) nm; IR (KBr) ν_{max} 3442, 2932, 2876, 1766, 1387, 1341, 1276, 1203, 1164, 1087 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2;

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positive ESIMS m/z 429 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 429.1878 (calcd for $C_{22}H_{30}O_7Na$, 429.1889).

Laxiflorolide B (2): colorless needles; mp 220.2−220.5 °C; $[\alpha]^{25.5}_D$ −72.7 (c 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.0); IR (KBr) ν_{max} 3425, 2938, 2874, 1766, 1448, 1389, 1240, 1204, 1132, 1089 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS m/z 429 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 429.1883 (calcd for $C_{22}H_{30}O_7Na$, 429.1889).

Laxiflorin P (3): colorless needles; mp 189.2−190.3 °C; $[\alpha]^{23.9}_D$ −62.5 (c 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 215 (3.2); IR (KBr) ν_{max} 3484, 2981, 2860, 1730, 1708, 1675, 1471 1382, 1204, 1157 1094 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS m/z 369 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 369.1319 (calcd for $C_{20}H_{26}O_5Na$, 369.1314).

Laxiflorin Q (4): white power; $[\alpha]^{23.4}_D$ −64.4 (c 0.31, MeOH); UV (MeOH) λ_{max} (log ε) 204 (2.7); IR (KBr) ν_{max} 3482, 3123, 2951, 2872, 1722, 1386, 1208, 1128, 1096 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1 and 2; positive ESIMS m/z 359 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 359.1460 (calcd for $C_{18}H_{24}O_6Na$, 359.1470).

Laxiflorin R (5): white power; $[\alpha]^{24.9}_D$ −48.7 (c 0.34, MeOH); UV (MeOH) λ_{max} (log ε) 360 (2.3), 306 (2.8), 279 (3.2), 203 (3.2); IR (KBr) ν_{max} 3422, 2967, 2878, 1729, 1665, 1462, 1366, 1207, 1178, 1077 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1 and 2; positive ESIMS m/z 367 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 367.1526 (calcd for $C_{20}H_{24}O_5Na$, 367.1521).

X-ray Crystal Structure Analysis. The intensity data for laxiflorolides A (**1**) and B (**2**) were collected on a Bruker APEX DUO diffractometer using graphite-monochromated Cu Kα radiation. The structures of these compounds were solved by direct methods (SHELXS97), expanded using difference Fourier techniques, and refined by the program and full-matrix least-squares calculations. The non-hydrogen atoms were refined anisotropically, and hydrogen atoms were fixed at calculated positions. Crystallographic data for the structures of laxiflorolides A (**1**) and B (**2**) have been deposited in the Cambridge Crystallographic Data Centre database (deposition numbers CCDC 837073 and CCDC 837074). Copies of the data can be obtained free of charge from the CCDC at www.ccdc.cam.ac.uk.

Crystallographic data for laxiflorolide A (1): $C_{22}H_{30}O_7$, M = 406.46, colorless needles, size 0.07 × 0.09 × 0.70 mm^3, orthorhombic, space group $P2_12_12_1$; a = 6.41970(10) Å, b = 11.1749(2) Å, c = 27.6949(5) Å, $\alpha = \beta = \gamma$ = 90.00°, V = 1986.82(6) $Å^3$, T = 100(2) K, Z = 4, ρ_{calcd} = 1.359 g/cm^3, μ(Cu Kα) = 0.831 mm^{-1}, $F(000)$ = 872, 10 092 reflections in $h(-6/7)$, $k(-13/10)$, $l(-30/33)$, measured in the range 3.19° ≤ θ ≤ 67.88°, completeness θ_{max} = 96.5%, 3400 independent reflections, R_{int} = 0.0395, 3237 reflections with $|F|^2 \geq 2\sigma|F|^2$, 268 parameters, 0 restraints, GOF = 1.090. Final R indices: R_1 = 0.0720, wR_2 = 0.2011. R indices (all data): R_1 = 0.0735, wR_2 = 0.2047. Flack parameter 0.2(3), largest difference peak and hole = 0.561 and −0.721 e $Å^{-3}$.

Crystallographic data for laxiflorolide B (2): $C_{22}H_{30}O_7$, M = 406.46, colorless needles, size 0.07 × 0.09 × 0.80 mm^3, orthorhombic, space group $P2_12_12_1$; a = 6.6090(1) Å, b = 15.7487(1) Å, c = 19.7887(2) Å, $\alpha = \beta = \gamma$ = 90.00°, V = 2059.67(4) $Å^3$, T = 100(2) K, Z = 4, ρ_{calcd} = 1.311 g/cm^3, μ(Cu Kα) = 0.801 mm^{-1}, $F(000)$ = 872, 10 234 reflections in $h(-6/7)$, $k(-18/16)$, $l(-23/20)$, measured in the range 3.59° ≤ θ ≤ 69.47°, completeness θ_{max} = 94.3%, 3524 independent reflections, R_{int} = 0.0255, 3489 reflections with $|F|^2 \geq 2\sigma|F|^2$, 268 parameters, 0 restraints, GOF = 1.067. Final R indices: R_1 = 0.0353, wR_2 = 0.0953. R indices (all data): R_1 = 0.0357, wR_2 = 0.0962. Flack parameter 0.07(17), largest difference peak and hole = 0.897 and −0.199 e $Å^{-3}$.

Cytotoxic Activity Assay. Colorimetric assays were performed to evaluate each compound's activity. The following human tumor cell lines were used: the A549 lung cancer cell line, the HL-60 human myeloid leukemia cell line, the MCF-7 breast cancer cell line, the SMMC-7721 human hepatocarcinoma cell line, and the SW-480 human pancreatic carcinoma. All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements 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 μL of suspended adherent cells was seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition. In addition, suspended cells were seeded just before drug addition, with an initial density of 1 × 10^5 cells/mL in 100 μL of medium. Each tumor cell line was exposed to each test compound at various concentrations in triplicate for 48 h; cisplatin (Sigma) was used as a positive control. After the incubation, MTT (100 μg) was added to each well, and the incubation was continued for 4 h at 37 °C. The cells were lysed with 100 μL of 20% SDS−50% DMF after removal of 100 μL of the medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_{50} value of each compound was calculated by Reed and Muench's method.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

This material (^1H, ^{13}C NMR, DEPT, HSQC, HMBC, COSY, NOESY, ESI, HRESIMS, IR, ORD, and UV spectroscopic data for compounds **1**−**5**, X-ray data for compounds **1** and **2**) 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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