

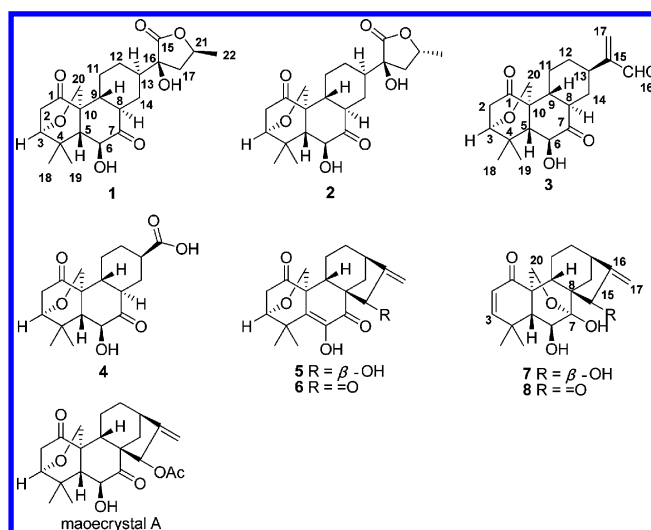
Laxiflorolides A and B, Epimeric Bishomoditerpene Lactones from *Isodon eriocalyx*Wei-Guang Wang,^{†,‡} Xiao-Nian Li,[†] Xue Du,[†] Hai-Yan Wu,[†] Xu Liu,[†] Jia Su,[†] Yan Li,[†] Jian-Xin Pu,^{*,†} and Han-Dong Sun^{*,†}[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China[‡]Graduate University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

S Supporting Information

ABSTRACT: Laxiflorolides A (1) and B (2), two unprecedented epimeric bishomoditerpene lactones with a unique C₂₂ 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 scaffolds² 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* genus⁴ and other genera⁵ or obtained by microbial transformation.⁶ Of the thousands of *ent*-kaurane diterpenoids previously identified, no compound has been reported to have a C₂₂ carbon skeleton. Among the plants of southwest China, *I. eriocalyx* var. *laxiflora* was previously investigated phytochemically² 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 C₂₂ 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 C₂₂H₃₀O₇, with eight degrees of unsaturation, was established on the basis of HRESIMS ([M + Na]⁺, 429.1878; calcd for C₂₂H₃₀O₇Na, 429.1889) and NMR spectroscopy (Tables 1 and 2). The IR spectrum of 1 indicated

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Table 1. ^1H NMR Data (Pyridine- d_5) of Compounds 1–5

position	1 ^a	2 ^a	3 ^b	4 ^c	5 ^b
2 α	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)
2 β	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)
3 α	3.74, br s	3.74, dd (3.4, 2.2)	3.74, br s	3.75, t (2.6)	3.85, br s
5 β	1.85, d (11.0)	1.86, dd (11.3, 1.3)	1.83, d (11.0)	1.83, d (11.2)	
6 α	4.77, d (11.0)	4.75, d (11.3)	4.79, d (11.0)	4.81, d (11.2)	
8 α	2.25, m	2.33, m	2.38, m	2.44, m	
9 β	2.27, m	2.28, m	2.19, dd (17.4, 7.3)	2.26, m	4.03, d (7.6)
11 α	0.94, m	0.97, m	1.02, m	1.02, ddd (15.4, 12.7, 3.2)	1.41, m
11 β	1.98, m	2.02, m	1.88, dd (12.2, 3.1)	1.97, m	2.48, m
12 α	1.31, m	1.13, m	1.27, m	2.19, m	1.50, m
12 β	2.31, m	2.81, dt (13.0, 2.8)	1.63, m	1.63, dd (12.6, 3.0)	1.33, m
13 α	2.01, m	2.14, m	2.48, m	2.46, m	2.74, br s
14 α	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)
14 β	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
18 β	1.10, s	1.10, s	1.79, s	1.68, s	1.79, s
19 α	1.68, s	1.68, s	1.68, s	1.10, s	1.27, s
20aa	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)
20ab	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)			

^aRecorded at 400 MHz. ^bRecorded at 500 MHz. ^cRecorded 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			

^aRecorded at 100 MHz. ^bRecorded at 125 MHz. ^cRecorded 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 ^1H 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)

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 H₂-20 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 H₂-2 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 (CHCH₂CH₂CHCH₂CH, H-9/H₂-11/H₂-12/H-13/H₂-14/H-8) established by ¹H–¹H COSY correlations and HSQC spectra, gave rise to partial structure **1a** (Figure 1).

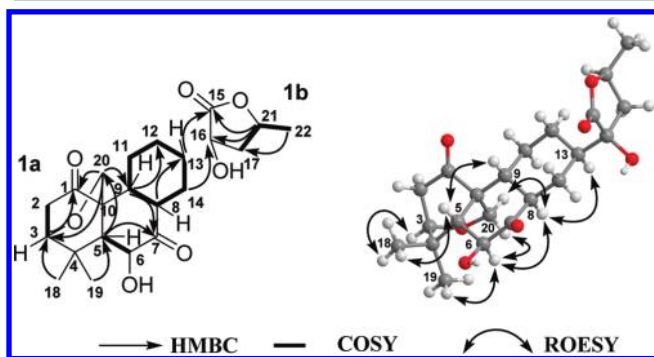


Figure 1. ¹H–¹H COSY (bold), selected HMBC (arrow), and key ROESY correlations of compound **1**.

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, H₂-17/H-21/H₃-22, 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-17 α (δ_H 2.60, 1H, dd, 13.7, 7.0 Hz) and H-17 β (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/H₂-20, 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 K α 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

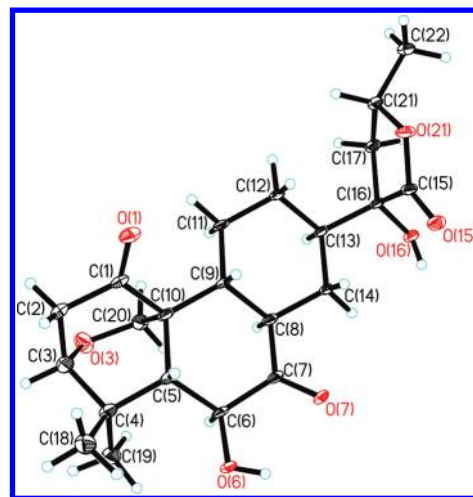


Figure 2. X-ray crystallographic structure of compound **1**.

of unsaturation. The chemical shift values in the ¹H and ¹³C NMR spectra of compound **2** and compound **1** are similar, with a deviation of ~ 0.5 ppm, respectively. The NMR resonances for H-17 α , 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 K α 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).

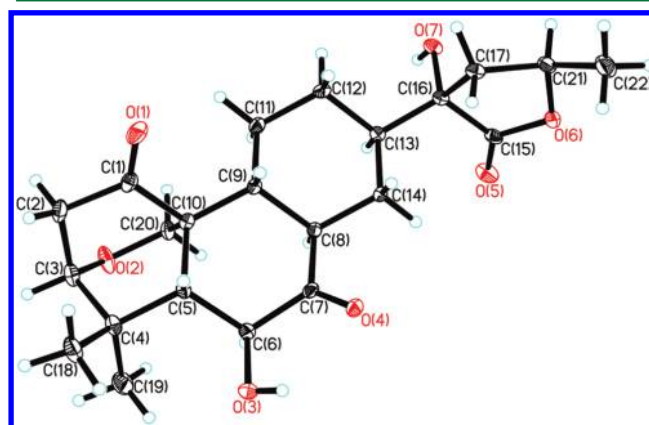


Figure 3. X-ray crystallographic structure of compound **2**.

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 6 β -hydroxy-3 α ,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

Table 3. Cytotoxic (IC_{50} , μM , mean \pm SD) Activity of Compounds 1–8^{a,b} ($n = 3$)

compound	HL-60		SMMC-7721		MCF-7		A-549		SW480	
	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

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

HMBC correlations from H-12 α (δ_H 2.19 m), H-12 β (δ_H 1.63 dd, 12.6, 3.0 Hz), H-14 α (δ_H 2.59 m), and H-14 β (δ_H 2.03 m) to C-15. The correlations of H₂-20 α /H-8 α /H-13 α in the ROESY spectrum showed that H-13 α in compound 4 was α -oriented. Therefore, compound 4 was 6 β -hydroxy-3 α ,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 C₂₀H₂₄O₅, with nine degrees of unsaturation. The ¹H and ¹³C NMR data of 5 (Tables 1 and 2) were consistent with a 3 α ,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-13 α /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 ($pIC_{50} \geq 5.5$). 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 C₂₂ 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 K α 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-C₁₈ 9.4 mm \times 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% H₂SO₄ 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 \times 40 L, 2 days each) at room temperature. The solvent was evaporated in vacuo to afford a crude extract, which was suspended in H₂O and then extracted successively with EtOAc and *n*-BuOH. The EtOAc-soluble fraction (600 g) was decolorized on MCI gel with 90:10 MeOH/H₂O to obtain a yellow gum (427.5 g). The gum was purified by CC on SiO₂ with a CHCl₃/Me₂CO gradient system, 1:0, 9:1, 8:2, 7:3, 6:4, and 1:1, to yield six main fractions, A–F. Fraction B (CHCl₃/Me₂CO, 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/Me₂CO, 20:1). Fraction B3 (petroleum ether/Me₂CO, 10:1) was separated further by RP-18 CC (MeCN/H₂O, 30:70) to afford 3 (6 mg) and 7 (5 mg) and with MeCN/H₂O, 40:60, to afford 5 (5 mg). Fr. D (CHCl₃/Me₂CO 7:3, 50 g) was eluted with CHCl₃/MeOH (30:1, 20:1, and 10:1), yielding subfractions D1–D3. Subfraction D2 (10 g, CHCl₃/MeOH, 20:1) was fractionated by repeated CC, first on RP-18 with a gradient elution with MeOH/H₂O (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 (CHCl₃/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 $\lambda_{max} = 208$ nm, MeCN/H₂O, 25:75) to yield 1 (12 mg). Subfraction D2/2/4 was purified by semipreparative HPLC (3 mL/min, UV detection at $\lambda_{max} = 208$ nm, MeCN/H₂O, 30:70) to yield 2 (3 mg). Fr. E (CHCl₃/Me₂CO, 6:4; 100 g) was eluted with MCI (30:70, 60:40, and 90:10 MeOH/H₂O) to afford fractions E1–E3. Compound 4 (5 mg) was separated further by RP-8 CC (MeCN/H₂O, 15:85) from fraction E1 (10 g).

Laxiflorolide A (1): colorless needles; mp 131.3–131.6 °C; $[\alpha]_D^{23}$ -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 ¹³C NMR data, see Tables 1 and 2;

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 $K\alpha$ 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 $0.07 \times 0.09 \times 0.70$ mm³, orthorhombic, space group $P2_12_12_1$; $a = 6.41970(10)$ Å, $b = 11.1749(2)$ Å, $c = 27.6949(5)$ Å, $\alpha = \beta = \gamma = 90.00^\circ$, $V = 1986.82(6)$ Å³, $T = 100(2)$ K, $Z = 4$, $\rho_{calcd} = 1.359$ g/cm³, $\mu(Cu K\alpha) = 0.831$ mm^{–1}, $F(000) = 872$, 10 092 reflections in $h(-6/7)$, $k(-13/10)$, $l(-30/33)$, measured in the range $3.19^\circ \leq \theta \leq 67.88^\circ$, completeness $\theta_{max} = 96.5\%$, 3400 independent reflections, $R_{int} = 0.0395$, 3237 reflections with $|I| \geq 2\sigma$ I , 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 $0.07 \times 0.09 \times 0.80$ mm³, orthorhombic, space group $P2_12_12_1$; $a = 6.6090(1)$ Å, $b = 15.7487(1)$ Å, $c = 19.7887(2)$ Å, $\alpha = \beta = \gamma = 90.00^\circ$, $V = 2059.67(4)$ Å³, $T = 100(2)$ K, $Z = 4$, $\rho_{calcd} = 1.311$ g/cm³, $\mu(Cu K\alpha) = 0.801$ mm^{–1}, $F(000) = 872$, 10 234 reflections in $h(-6/7)$, $k(-18/16)$, $l(-23/20)$, measured in the range $3.59^\circ \leq \theta \leq 69.47^\circ$, completeness $\theta_{max} = 94.3\%$, 3524 independent reflections, $R_{int} = 0.0255$, 3489 reflections with $|I| \geq 2\sigma$ I , 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% CO₂. 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 μ L 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 μ L 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 μ g) was added to each well, and the incubation was continued for 4 h at 37 °C. The cells were lysed with 100 μ L of 20% SDS–50% DMF after removal of 100 μ L 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 IC₅₀ 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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