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6,7-Seco-*ent*-kaurane-type diterpenoids from *Isodon eriocalyx* var. *laxiflora*

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ABSTRACT

Twenty nine 6,7-seco-*ent*-kaurane-type diterpenoids including 18 new ones, laxiflorolides C–T (**1**–**18**), along with 21 known ones were obtained from *Isodon eriocalyx* var. *laxiflora*. Laxiflorolides E–G (**3**–**5**) are the first identified naturally occurring 6,7-seco-*ent*-kauranoids that feature a 3,6-epoxy unit, and laxiflorolide M (**11**) is the first identified naturally occurring 6-nor-6,7-seco-*ent*-kauranoid. The absolute configurations of compounds **1**, **3**, **6**, and **11** were determined by single-crystal X-ray diffraction analyses. The cytotoxic activity of the isolates was evaluated by an MTT assay.

1. Introduction

ent-Kaurane-type diterpenoids have been recognized as interesting natural products with diverse structural scaffolds[1] and important pharmaceutical activities,[2] which are mainly found in *Isodon* genus[1e] and also reported from other source.[3] More than 700 new diterpenoids have been previously identified from *Isodon* genus by our group, and all of these compounds could be classified into 11 groups, including C-20 non-oxygenated *ent*-kauranes, C-20 oxygenated *ent*-kauranes, 6,7-seco-*ent*-kauranes, and so on.[1e] 6,7-seco-*ent*-Kauranes are excellent examples of natural products with highly oxygenated functionality, complicated cyclic connectivity, highly packed ring system, and multiple stereogenic centers, and therefore attracted great attention of synthetic chemists due to the challenge posed by the unusual skeleton. Some 6,7-seco-kauranoids have been totally synthesized, such as maoecrystal V,[4] maoecrystal Z,[5] sculponeatin N,[6] trichorabdal A,[5b,7] and longikaurin E.[5b,7] *Isodon eriocalyx* var. *laxiflora*, an *Isodon* species distributed in southwest China, produced dozens of 6,7-seco-*ent*-kauranoids.[1e] In our program to construct *an ent*-kaurane diterpenoids library,[8] we continued[1c,8,9] to investigate the 6,7-seco-*ent*-kauranoids produced by *I. eriocalyx* var. *laxiflora* collected in Xishuangbanna prefecture. Twenty nine 6,7-seco-*ent*-kauranoids including 18 new ones, laxiflorolides C–T (**1**–**18**) (Fig. 1), were isolated from this species. In these isolates, laxiflorolides E–G (**3**–**5**) are the first identified naturally occurring a 3,6-epoxy unit; only two ones, laxiflorins F[10] and G (**19**), have been reported to have the 15,16-seco-*ent*-kaurane diterpenoid motif previously, while compounds **1** and **2** also belong to 6,7:8,15-diseco-*ent*-kauranoids; compound **11** is the first example of 6-nor-6,7-seco-*ent*-kauranoid. In this paper, we report the isolation, structural elucidation, bioevaluation of compounds **1**–**29**.

2. Results and discussion

The air-dried leaves of *I. eriocalyx* var. *laxiflora* (10 kg) were extracted with 70% aqueous $(CH_3)_2CO$ (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 successively extracted with EtOAc and *n*-BuOH. The EtOAc-soluble fraction (600 g) was subjected to column chromatography over silica gel, MCI CHP-20 gel, ODS, and Lichroprep RP-18, after which it was further purified by MPLC and PHPLC to afford 18 new *ent*-kauranoids that have been named laxiflorolides C–T (**1**–**18**) (Fig. 1), and 11 known compounds including laxiflorin G (**19**),[10] laxiflorin B (**20**),[11] laxiflorin C (**21**),[11] laxiflorin E (**22**),[12] laxiflorin D (**23**),[12a,13] maoecrystal L (**24**),[14] eriocalyxin A (**25**),[15] *epi*-eriocalyxin A (**26**),[14b] laxiflorin A (**27**),[11] *epi*-maoecrystal N (**28**),[14a] and

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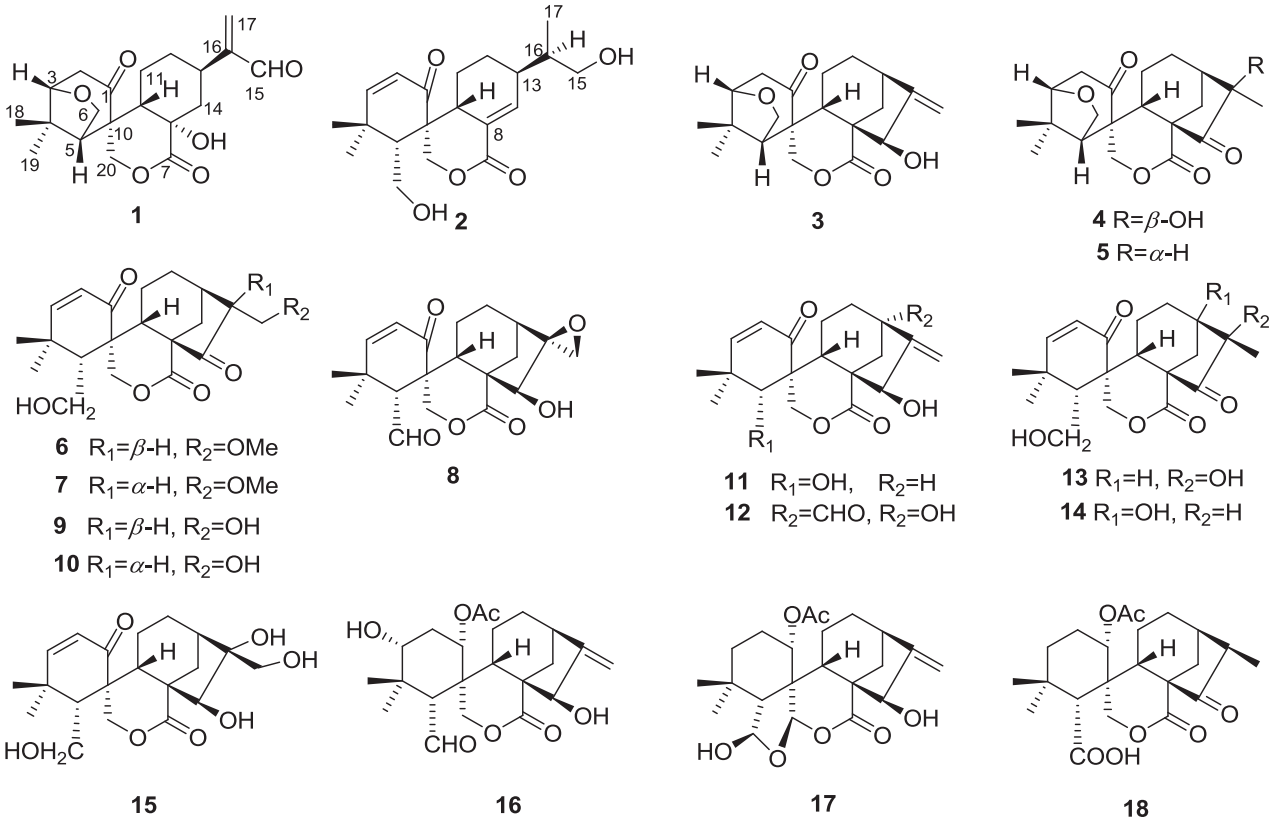

Fig. 1. Structures of compounds **1**–**18**.

maoecrystal N (**29**).[14b] The structures of the known compounds were determined by comparing their spectroscopic data to literature values.

Laxiflorolide C (**1**) was obtained as colorless needles crystallized from a mixed solvent system (CH_3OH/H_2O, 3:1). Its molecular formula of $C_{20}H_{24}O_5$ was determined by HRESIMS ($[M]^+$ *m*/*z* 362.1727, calcd 362.1729). Assignments of the ^1H and ^{13}C NMR spectra of **1** (Tables 1 and 3) were supported by a series of 2D NMR (^1H–^1H COSY, HSQC, and HMBC) experiments. In the ^{13}C NMR and DEPT spectra (Table 1), 20 carbon signals were observed, which were assigned as two methyls, seven methylenes (including one olefinic and two oxygenated), five methines (including one oxygenated and an aldehyde carbonyl), and six quaternary carbons (including two carbonyls for lactone and saturated ketone, one olefinic and one oxygenated). These data indicated that **1** is a 6,7:8,15-diseco-*ent*-kauranoid, similar to compound laxiflorin G (**19**).[16]

Table 1
^{13}C NMR data (δ in ppm, C_5D_5N) of compounds **1**–**9**

No.	1[a]	2[a]	3[a]	4[a]	5[a]	6[b]	7[a]	8[a]	9[a]
1	213.7 s	200.5 s	211.6 s	213.3 s	210.0 s	201.1 s	200.9 s	199.5 s	200.9 s
2	48.1 t	124.6 d	47.4 t	47.9 t	46.9 t	124.4 d	124.5 d	125.3 d	124.5 d
3	81.9 d	158.3 d	81.2 d	81.8 d	80.8 d	159.0 d	159.0 d	157.3 d	158.7 d
4	42.9 s	36.6 s	42.7 s	42.8 s	42.3 s	36.8 s	36.7 s	36.3 s	36.7 s
5	53.5 d	47.4 d	45.4 d	53.5 d	46.8 d	47.4 d	47.4 d	58.6 d	47.6 d
6	67.9 t	58.5 t	67.6 t	67.8 t	67.5 t	58.1 t	58.2 t	202.0 d	58.2 t
7	172.1 s	164.7 s	175.7 s	172.0 s	172.0 s	170.3 s	170.2 s	174.6 s	170.2 s
8	75.1 s	130.6 s	51.3 s	54.8 s	55.2 s	60.1 s	59.7 s	54.3 s	60.3 s
9	48.0 d	39.9 d	37.3 d	47.7 d	44.3 d	41.5 d	40.6 d	37.0 d	41.7 d
10	54.9 s	50.7 s	55.5 s	54.8 s	54.7 s	52.7 s	52.4 s	49.7 s	52.7 s
11	29.5 t	23.9 t	19.4 t	28.2 t	19.1 t	18.4 t	17.9 t	17.4 t	18.5 t
12	31.5 t	27.2 t	32.9 t	28.8 t	19.4 t	30.6 t	20.9 t	28.0 t	30.8 t
13	34.2 d	39.5 d	37.1 d	48.3 d	49.5 d	32.1 d	31.0 d	37.9 d	31.9 d
14	41.4 t	142.0 d	32.7 t	38.5 t	33.6 t	30.6 t	32.5 t	31.0 t	31.0 t
15	194.7 d	65.1 t	79.4 d	209.3 s	216.1 s	214.1 s	213.8 s	81.6 d	214.8 s
16	153.6 s	40.7 d	159.3 s	74.8 s	33.1 d	57.2 d	54.4 d	73.3 s	60.2 d
17	134.1 t	14.0 q	108.7 t	28.1 q	10.4 q	72.3 t	69.5 t	48.8 t	62.0 t
18	26.6 q	31.9 q	26.8 q	26.5 q	26.8 q	31.7 q	31.8 q	31.1 q	31.8 q
19	22.5 q	23.8 q	22.3 q	22.3 q	22.2 q	23.7 q	23.8 q	24.0 q	23.7 q
20	74.3 t	71.8 t	68.0 t	74.3 t	68.4 t	70.7 t	70.8 t	69.0 t	70.6 t
CH_3O						58.7 q	58.5 q		

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

Table 2
^{13}C NMR data (δ in ppm, C_5D_5N) of compounds **10**–**18**

No.	**10**[a]	**11**[a]	**12**[a]	**13**[b]	**14**[a]	**15**[c]	**16**[a]	**17**[a]	**18**[b]
1	200.9 s	201.6 s	198.7 s	200.9 s	200.8 s	202.3 s	72.9 d	68.6 d	75.6 d
2	124.5 d	126.1 d	125.5 d	124.5 d	124.5 d	125.2 d	33.6 t	30.1 t	24.6 t
3	159.0 d	158.3 d	157.3 d	158.9 d	159.0 d	159.4 d	74.6 d	27.1 t	39.3 t
4	36.7 s	38.9 s	36.3 s	36.6 s	36.6 s	37.0 s	39.6 s	30.7 s	33.8 s
5	47.5 d	73.4 d	59.0 d	47.6 d	47.8 d	48.9 d	61.4 d	60.3 d	58.2 d
6	58.3 t	—	201.7 d	58.3 t	58.2 t	59.2 t	203.4 d	102.0 d	174.0 s
7	170.4 s	175.5 s	174.8 s	170.5 s	169.9 s	176.4 s	175.6 s	174.2 s	170.4 s
8	60.0 s	52.9 s	53.1 s	59.5 s	61.7 s	56.3 s	52.6 s	51.0 s	58.8 s
9	40.6 d	34.4 d	37.0 d	42.5 d	41.4 d	35.5 d	37.7 d	36.1 d	45.2 d
10	52.4 s	54.7 s	50.0 s	52.5 s	52.1 s	52.1 s	43.6 s	52.2 s	42.7 s
11	18.0 t	17.5 t	18.7 t	17.9 t	19.8 t	18.3 t	16.4 t	19.7 t	17.1 t
12	21.0 t	33.1 t	41.1 t	23.4 t	30.2 t	24.9 t	32.8 t	34.1 t	19.5 t
13	31.1 d	36.6 d	76.0 s	41.5 d	75.2 s	44.3 d	36.3 d	40.3 d	32.3 d
14	32.6 t	31.5 t	40.6 t	30.0 t	41.6 t	31.2 t	29.9 t	33.2 t	31.2 t
15	214.6 s	82.7 d	80.4 d	215.0 s	215.5 s	90.2 s	83.0 d	77.7 d	216.7 s
16	57.2 d	160.5 s	162.3 s	79.3 s	56.1 d	84.8 s	159.7 s	156.8 s	48.6 d
17	59.2 t	108.8 t	109.2 t	21.6 t	9.8 q	53.0 t	109.6 t	106.1 t	11.2 q
18	31.9 q	30.5 q	31.2 q	31.7 q	31.6 q	32.5 q	29.2 q	29.6 q	33.3 q
19	23.9 q	22.6 q	24.1 q	23.7 q	23.7 q	24.2 q	18.0 q	28.7 q	23.1 q
20	70.7 t	68.8 t	69.0 t	70.5 t	70.9 t	70.4 t	67.8 t	104.7 d	69.0 t
AcO							170.2 s	171.1 s	170.2 s
							21.3 q	21.3 q	21.4 q

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

Analysis of its ^{1}H–^{1}H COSY, HSQC, and HMBC spectra confirmed this assumption and the planar structure of compound **1**. The HMBC spectrum of **1** showed correlations from the geminal methyls Me-18 (δ_H 1.16, 3H, s) and Me-19 (δ_H 1.41, 3H, s) to C-3, C-4, and C-5, from H-9 (δ_H 2.78) to C-1, C-5, C-7, C-8, C-10, C-12, C-14, and C-20, and from H-15 (δ_H 9.55, s) to C-13, C-16, and C-17 (Fig. 2). Furthermore, one of an AB spin system of methylene H_2-20 (δ_H 4.92, d, 10.5 Hz; δ_H 4.74, d, 10.5 Hz) showed HMBC correlations with C-1, C-5, C-7, C-9, and C-10; another AB spin system of methylene H_2-17 (δ_H 6.25, d, 3.0 Hz; δ_H 5.94, d, 3.0 Hz) showed HMBC correlations with C-13, C-16, and C-15. Other HMBC correlations were noted between the ABX spin system of methylene H_2-6 (δ_H 3.94, dd, 10.0, 5.0 Hz; δ_H 3.73, br d, 10.0 Hz) and C-3, C-4, C-5, and C-10 and between H-5 (δ_H 2.85, br d, 5.0 Hz) and C-1, C-3, C-4, C-6, C-10, C-18, C-19, and C-20. These observed HMBC correlations, coupled with three spin system (i: CH_2CH, H_2-2/H-3, ii: CH/CH_2, H-5/H_2-6, and iii: $CHCH_2CH_2CHCH_2$, H-9/H_2-11/H_2-12/H-13/H_2-14) established by ^{1}H–^{1}H COSY correlations and HSQC spectra, gave rise to the gross structure of **1** (Fig. 2).

In the ROESY spectrum of **1**, the NOE correlations of H-5/H-3/H-9/Me-18, Me-19/H-6, and H-6/H_2-20 suggested that C-6, Me-19, and C-20 all adopted an α-orientation, and H-3, H-5, H-9, and Me-18 are β-oriented, respectively (Fig. 2). The absence for 8-OH signal in the ^{1}H NMR is difficult to confirm the orientation of 8-OH and H-13. Single-crystal X-ray diffraction analysis using the anomalous scattering of Cu Kα radiation (CCDC 1007902),[17] confirming the above conjecture and indicating that the structure of **1** was 3*R*, 5*R*, 8*S*, 9*S*, 10*R*, and 13*R* (Fig. 2). Thus, compound **1** was elucidated as 8α-hydroxy-3,6-epoxy-6,7:8,15-diseco-7,20-olide-*ent*-kaur-16-en-1-on-15-al and named as laxiflorolide C.

Laxiflorolide D (**2**) was isolated as a white amorphous powder and its molecular formula was established to be $C_{20}H_{28}O_5$ by HREIMS and ^{13}C NMR data (Table 1). The ^{1}H–^{1}H COSY correlations of H-2/H-3, H-5/H_2-6, and H-9/H_2-11/H_2-12/H-13(H-16(H_3-17)/H_2-15)/H-14, along with the HMBC correlations of H-9 with C-1, C-5, C-7, C-8, C-10, C-12, C-14, and C-20, of H_2-15 and H_3-17 with C-13 and C-16, and of H_2-20 (δ_H 5.24, d, 9.2 Hz; δ_H 4.83, 9.2 Hz) with C-1, C-5, C-7, C-9, and C-10 suggest that **2** is also a 6,7:8,15-diseco-7,20-olide-*ent*-kauranoid (Fig. 2).

Further analysis of its 2D NMR data and comparison with those of laxiflorin C[11] indicate that compound **2** could be considered as the 8,15-seco-analogs of laxiflorin C. This assumption was confirmed by the HMBC correlations of H-14 with C-7, C-8, C-9, C-12, C-13, C-15, and C-16. The ROESY spectrum of **2** indicates that the relative configurations of the stereogenic centers in **2** are identical to those of **1** except for the undetermined orientation of H-16. Compound **2** was thus identified as 6,15-dihydroxy-6,7:8,15-diseco-7,20-olide-*ent*-kaur-2,8-dien-1-one and was named as laxiflorolide D.

Similar laxiflorolides E–G (**3**–**5**) are the first identified naturally occurring 6,7-seco-*ent*-kauranoids that feature a 3,6-epoxy unit supported by the HMBC correlation from H-3 to C-6. The principal differences between these compounds were the substituent groups of C-15, C-16, and C-17, e.g.: 15-hydroxy-16-en-**3**, 15-on-16-hydroxy-**4**, and 15-on-16-hydrogen-**5**. On the basis of careful analyses of 1D NMR, 2D NMR (Tables 1 and 3), and single-crystal X-ray diffraction using anomalous scattering of Cu Kα radiation (CCDC 1007903)[17] data (Fig. 3), the absolute configuration of compound **3** was assigned and can be described according to the following nomenclature: (3*R*, 5*R*, 8*S*, 9*S*, 10*S*, 13*R*, 15*R*)-3,6-epoxy-15β-hydroxy-6,7-seco-7,20-olide-*ent*-kaur-16-en-1-one. The difference between 15-on-16-hydroxy-**4** with that of 15-hydroxy-16-en-**3** was supported by the HMBC correlations from Me-17 (δ_H 2.11, s) to C-13, C-15 (δ_C 209.3), and C-16 (δ_C 74.8) in **4** (Tables 1 and 3). Then, the difference between 15-on-16-hydrogen-**5** with that of 15-hydroxy-16-en-**3** was supported by the HMBC correlations from Me-17 (δ_H 1.04, br s) to C-13, C-15 (δ_C 216.1), and C-16 (δ_C 33.1) in **5**. The ROESY cross peaks between H-13α/Me-17 in **4**, H-9β/Me-17 in **4** and other correlations indicated that compounds **4** and **5** had the same relative configuration with **3** except for the Me-17α-oriented in **4** but Me-17β-oriented in **5**.

The molecular formula of laxiflorolide H (**6**) was found to be $C_{21}H_{28}O_6$ as determined by positive ion HREIMS (*m*/*z* 376.1892, $[M]^+$, calcd for 376.1886). Comparisons of the ^{1}H and ^{13}C NMR data of **6** (Tables 3 and 1) with those of laxiflorin E (**22**)[12a] indicate that both compounds have identical skeletons and substitution patterns, differing only in that the hydrogen at Me-17 in **22** is substituted by a methoxy group in **6**. The planar structure of compound **6** was discovered by analyzing HMBC correlations between H_2-17 (δ_H 3.49, dd, 10.0, 7.2 Hz; δ_H 3.45, dd, 10.0, 5.0 Hz) with C-13, C-14, C-15 (δ_C 214.1), and the methoxyl group (δ_C 58.7) and ^{1}H–^{1}H COSY correlations (for the observed proton spin system, H-

Table 3
^{1}H NMR data (δ in ppm, J in Hz, C_5D_5N) of compounds **1**–**10**

No.	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]	**6**[b]	**7**[a]	8	**9**[a]	**10**[a]
2	2.78 (overlap)	5.90 (d, 10.2)	2.64 (overlap)	3.00 (br t, 10.0)	2.60 (overlap)	5.90 (d, 11.5)	5.92 (d, 10.0)	5.96 (d, 10.0)	5.91 (d, 10.1)	5.89 (d, 10.1)
3	3.91 (br s)	6.44 (d,10.2)	3.84 (br s)	3.94 (br s)	3.81 (br s)	6.49 (d, 11.5)	6.46 (d,10.0)	6.49 (d,10.0)	6.46 (d, 10.1)	6.46 (d, 10.1)
5	2.85 (br d, 5.0)	2.40 (d, 3.7)	2.47 (br s)	2.90 (br d, 4.0)	2.23 (br s)	2.26 (br t, 3.7)	2.26 (br s)	3.24 (br s)	2.27 (br s)	2.26 (br s)
6	3.94 (dd, 10.0, 5.0)	4.17 (br d, 10.0)	4.14 (br d, 10.0)	3.99 (dd, 10.0, 4.0)	4.14 (br s)	4.05 (br s)	4.04 (br t, 3.6)	10.28 (br s)	4.07 (overlap)	4.03 (br s)
	3.73 (br d, 10.0)	4.12 (br d, 10.0)	3.91(dd, 10.0, 5.0)	3.80(d, 10.0)	3.91 (br d, 4.5)					
9	2.78 (overlap)	3.36 (m)	3.21 (br d, 8.0)	2.76 (br d, 8.0)	2.37 (overlap)	2.97 (br d, 8.8)	2.96 (overlap)	3.00 (br t, 6.7)	3.00 (br d, 11.1)	3.07 (overlap)
11	1.55 (m)	2.10 (m)	1.95 (m)	1.95 (m)	1.15 (br s)	1.80 (m)	1.68 (m)	1.61 (m)	1.84 (m)	1.73 (m)
	1.45 (m)	1.71 (m)	1.08 (m)	1.31 (m)	0.95 (br s)	1.67 (m)	1.59 (m)	1.82 (m)	1.67 (m)	1.62 (m)
12	1.78 (m)	1.84 (overlap)	2.01 (overlap)	1.64 (m)	1.92 (m)	2.03 (m)	1.74 (m)	1.45 (m)	2.09 (m)	2.03 (m)
	1.22 (m)	1.49 (m)	1.38 (m)	1.54 (m)	1.63 (m)	1.36 (m)			1.38 (m)	1.77 (m)
13	3.17 (m)	2.82 (br s)	2.64 (overlap)	3.67 (br s)	2.45 (br s)	3.67 (br d, 4.8)	2.66 (br s)	1.98 (br s)	2.80 (br s)	2.83 (br s)
14	2.93 (d, 12.7)	7.54 (br s)	2.01 (overlap)	3.09 (br d, 14.0)	2.60 (overlap)	2.93 (dd, 12.3, 4.4)	2.98 (overlap)	2.70 (br t, 12.4)	3.14 (br d, 12.3)	3.05 (overlap)
	1.94 (br t, 12.7)	—	1.64 (br t, 6.0)	2.00 (br t, 14.0)	1.89 (m)	2.84 (br d, 12.3)	2.71 (dd, 12.3, 4.2)	2.58 (d, 12.4)	2.92 (br d, 12.3)	2.76 (dd, 12.3, 3.8)
15	9.55 (s)	3.80 (m)	5.47 (s)	—	—	—	—	4.80 (s)	—	—
	—	3.72 (m)	—	—	—					
16	—	1.84 (overlap)	—	—	2.37 (overlap)	2.43 (br t. 7.2)	2.96 (overlap)	—	2.50 (br s)	3.05 (overlap)
17	6.25 (d, 3.0)	0.96 (d, 6.9)	5.45 (br s)	2.11 (s)	1.04 (br s)	3.49 (dd, 10.0, 7.2)	3.60 (br t, 10.0)	3.26 (d, 6.5)	4.07 (overlap)	4.22 (dd, 11.2, 4.0)
	5.94 (d, 3.0)		5.17 (br s)	—		3.45 (10.0, 5.0)	—	2.76 (d, 6.5)		4.15 (d, 11.2)
18	1.16 (s)	1.19 (s)	1.11(s)	1.19(s)	1.08 (s)	1.13 (s)	1.12 (s)	1.21 (s)	1.13(s)	1.12 (s)
19	1.41 (s)	1.20 (s)	1.42 (s)	1.42 (s)	1.08 (s)	1.15 (s)	1.14 (s)	1.21 (s)	1.17 (s)	1.12 (s)
20	4.92 (d, 10.5)	5.24 (d, 9.2)	4.74 (d, 12.0)	5.00 (d, 10.5)	4.60 (br s)	5.23 (d, 11.1)	5.29 (d, 11.5)	5.55 (d, 10.7)	5.31 (d, 11.0)	5.29 (d, 11.2)
	4.74 (d, 10.5)	4.83 (d, 9.2)	4.36 (d, 12.0)	4.84 (d, 10.5)	4.43 (br s)	4.82 (d, 11.1)	4.82 (d, 11.5)	4.99 (d, 10.7)	4.85 (d, 11.0)	4.81 (d, 11.2)
CH_3O						3.12 (s)	3.11 (s)			

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

Fig. 2. Key HMBC, ^{1}H−^{1}H COSY, and ROESY correlations, and X-ray crystallographic structure of **1** [(HMBC, H→C in blue), ^{1}H−^{1}H COSY (—), and (⟷) in ROESY)].

Fig. 3. Key HMBC and ^{1}H−^{1}H COSY correlations, and X-ray crystallographic structure of **3** [(HMBC, H→C in blue) and ^{1}H−^{1}H COSY (—)].

2/H-3, H-5/H_2-6, H-9/H_2-11/H_2-12/H-13 (H-16/H_2-17)/H_2-14). The relative configurations of the stereogenic centers in compound **6** were determined to be the same as those in laxiflorin E, based on detailed analyses of ROESY data. Single-crystal X-ray diffraction using anomalous scattering of Cu Kα radiation (CCDC 1007904)[17] indicated the absolute stereochemistry of compound **6** to be (5*R*, 8*S*, 9*S*, 10*S*, 13*R*, 16*R*)-17-methoxy-6,7-seco-7,20-olide-*ent*-kaur-2-en-1,15-dione (Fig. 4).

Fig. 4. Key ROESY correlations and X-ray crystallographic structure of **6** (⟷ in ROESY).

Comparison of the 1D and 2D NMR data for laxiflorolide I (**7**) with those of **6** (Tables 1 and 3) revealed that laxiflorolide H was the 16-epimeride of **6**, which was supported by HMBC correlations from H_2-17 (δ_H 3.60, br t, 10.0 Hz) to C-13, C-14, C-15 (δ_C 213.8), and the methoxy group (δ_C 58.5) and the NOE correlations between H-13α/H-16 and H-9β/H_2-17.

Laxiflorolide J (**8**) was obtained as a white amorphous powder whose molecular formula was determined to be $C_{20}H_{24}O_6$ by

HREIMS and ^{13}C NMR data (Table 1). The ^{1}H and ^{13}C NMR data of **8** are similar to those of laxiflorin D (**23**)[12a] except that the carbon–carbon double bond between C-16 and C-17 is oxidized to become a three member oxirane ring. The 16,17-epoxy group was demonstrated by the HMBC correlations of H_2-17 (δ_H 3.26, d, 6.5 Hz; δ_H 2.76, d, 6.5 Hz) with C-13 (δ_C 37.0), C-15 (δ_C 81.6), and C-16 (δ_C 73.3), and of H-15 (δ_H 4.80) with C-7, C-8, C-9, C-13, C-14, C-16, and C-17 confirm this conclusion. The ROESY correlations from H_2-17 to H-13α and H-15 in **8** indicate that the C-17 and H-15 in **8** are α-oriented, respectively. Therefore, the structure of compound **6** was assigned as 15β-hydroxy-16,17-epoxy-6,7-seco-7,20-olide-*ent*-kaur-2-en-1,15-dion-6-al.

Laxiflorolides K (**9**) and L (**10**) were determined to be an epimeric pair with the same molecular formula, $C_{20}H_{26}O_6$, by HREIMS (**9**, *m*/*z* 362.1724 for $[M]^+$, calcd 362.1729; **10**, *m*/*z* 362.1730 for $[M]^+$, calcd 362.1729). The planar structure and relative configurations of these two compounds were obtained by analysis of 1D and 2D NMR data (Tables 1–3). These two compounds are structurally similar to laxiflorolides H and I (**6** and **7**) and the only difference is that the methoxy group at C-17 in **6** and **7** is replaced by a hydroxy group in **9** and **10**, respectively. The only difference between the epimers was that the relative configurations of H-16 was deduced to be β-oriented in **9**, but α-oriented in **10** (Fig. 1), on the basis of the ROESY correlation data for H-13α/H_2-17 in **9** and H-9β/H_2-17 in **10**. Thus, the relative configurations of the epimeric pair can be described by the following nomenclature: 16β (H)-6,17-dihydroxy-6,7-seco-7,20-olide-*ent*-kaur-2-en-1,15-dione (**9**) and 16α(H)-6,17-dihydroxy-6,7-seco-7,20-olide-*ent*-kaur-2-en-1,15-dione (**10**).

Laxiflorolide M (**11**) is the first identified naturally occurring 6-nor-6,7-seco-*ent*-kauranoid. On the basis of careful analyses of 1D NMR, 2D NMR, and single-crystal X-ray diffraction using anomalous scattering of Cu Kα radiation (CCDC 1007905)[17] data (Fig. 5), the structure of compound **11** was assigned and can be described according to the following nomenclature: 6-nor-5,15β-dihydroxy-6,7-seco-7,20-olide-*ent*-kaur-1-en-1-one.

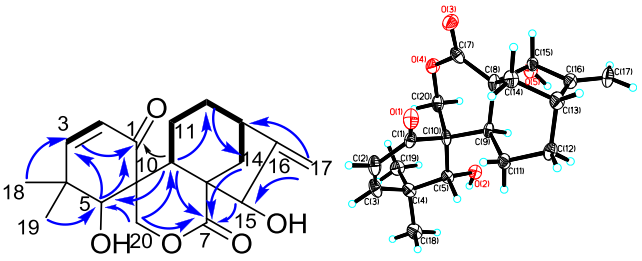

Fig. 5. Key HMBC and $^{1}H-^{1}H$ COSY correlations, and X-ray crystallographic structure of **11** [(HMBC, H→C in blue) and $^{1}H-^{1}H$ COSY (—)].

Laxiflorolide N (**12**) was obtained as a white, amorphous powder with a molecular formula of $C_{20}H_{24}O_6$ based on HREIMS and ^{13}C NMR data (Table 2). Its spectroscopic data indicate that its structure is similar to that of known laxiflorin D (**23**)[12a] but for the location of the α-hydroxy group at C-13 (δ_C 76.0, s) in **12**. This assignment was verified by the HMBC correlations from H_2-11 (δ_H 1.67, m; δ_H 1.42, m), H_2-14 (δ_H 3.03, d, 11.5 Hz; δ_H 2.74, d, 11.5 Hz), H-15 (δ_H 5.22, s), and H_2-17 (δ_H 5.78, br s; δ_H 5.58, br s) to C-13. The relative configurations of the stereogenic centers in **12** were determined to be the same as those in **23**, based on detailed analyses of ROESY data. Therefore, the structure of compound **12** was assigned as 13α,15β-hydroxy-6,7-seco-7,20-olide-*ent*-kaur-2,16-dien-1-on-6-al.

Laxiflorolides O (**13**) and P (**14**) were determined to be the same molecular formula, $C_{20}H_{26}O_6$, by HREIMS (**13**, *m*/*z* 362.1723 for $[M]^+$, calcd 362.1729; **14**, *m*/*z* 362.1750 for $[M]^+$, calcd 362.1729). The planar structure and relative configurations of these two compounds were obtained by analysis of 1D and 2D NMR data (Tables 2 and 4). These two compounds are structurally similar to *epi*-maoecrystal N (**28**)[14a] and the only difference is that the H-16 and H-13 in **28** is replaced by a hydroxy group in **12** (16-OH) and **13** (13-OH), respectively.

The assignment of 16-OH was verified by the HMBC correlations from H_2-12 (δ_H 1.90, m; δ_H 1.68, overlap), H-13 (δ_H 2.53, dd, 9.5, 3.9 Hz), H_2-14 (δ_H 3.56, dd, 12.2, 3.9 Hz; δ_H 2.94, br d, 12.2 Hz), and Me-17 (δ_H 1.55, s) to C-16 (δ_C 79.3, s), and the location of 13-OH was supported by the HMBC correlations from H_2-11 (δ_H 2.03, m; δ_H 1.80, m), H_2-14 (δ_H 3.54, br d, 11.2 Hz; δ_H 3.13, br d, 11.2 Hz), H-16 (δ_H 2.96, q, 7.1 Hz), and Me-17 (δ_H 1.27, d, 7.1 Hz) to C-13 (δ_C 75.2, s). In addition, correlations observed in the ROESY spectrum indicate that the orientations of the substituents in **13** and **14** are the same as in *epi*-maoecrystal N. Thus, the relative configurations of them can be described by the following nomenclature: 6,16-dihydroxy-6,7-seco-7,20-olide-*ent*-kaur-2-en-1,15-dione (**13**). and 6,13-dihydroxy-6,7-seco-7,20-olide-*ent*-kaur-2-en-1,15-dione (**14**).

The HREIMS and ^{13}C NMR data for laxiflorolide Q (**15**) indicated a molecular formula of $C_{20}H_{28}O_7$. A comparison of the NMR data for **15** and **13** (Tables 2 and 4) revealed two differences. One difference was absence of a carbonyl group in **13** where there is a hydroxy group in **15** at C-15, which was supported by the HMBC correlations between H-15 (δ_H 5.16, s) and C-7, C-8, C-9, C-13, C-14, C-16, and C-17. The other one was the hydroxymethyl instead of a methyl group at C-16 in **15**, and this was confirmed by the HMBC correlations between H_2-17 (δ_H 4.33, d, 12.0 Hz; δ_H 4.27, d, 12.0 Hz) with C-13 (δ_C 44.3, d), C-15 (δ_C 90.2, s), and C-16 (δ_C 84.8, s) and between H-13 (δ_H 2.66, dd, 9.0, 4.8 Hz) with C-17 (δ_C 53.0, t). The correlations observed in the ROESY spectrum indicate that the orientations of the substituents in **15** are the same as in **13**. The structure of **15** was thus defined as 6,15β,16α,17-tetrahydroxy-6,7-seco-7,20-olide-*ent*-kaur-2-en-1-one.

Laxiflorolide R (**16**) was shown to have the molecular formula $C_{22}H_{30}O_7$ by HREIMS and ^{13}C NMR data (Table 2). Its NMR data indicate that its structure is similar to that of **30**,[18] but the hydroxy group at C-11 in **30** shifted to C-3 in **16**. These replacements are verified by the HMBC correlations of Me-18 (δ_H 1.25, s) and Me-19 (δ_H 1.35, s) with C-3 (δ_C 74.6, d), and the $^{1}H-^{1}H$ COSY correlations (for the observed proton spin system, H-9/H_2-11/H_2-12/H-13/H_2-14). A ROESY experiment confirmed that **16** has the same configurations as **30** and the H-3 is β-oriented based on the correlation of H-3 with Me-18β. The structure of **16** was thus defined as 3,15β-dihydroxy-1-acetoxy-6,7-seco-7,20-olide-*ent*-kaur-16-en-6-al.

Laxiflorolide S (**17**) was assigned the molecular formula $C_{22}H_{30}O_7$ based on its HREIMS and ^{13}C NMR data (Table 2). Its spectroscopic data indicate that its structure resembles that of macrocalyxoformin E.[19] The only difference is that the methylene at C-1 is replaced by an acetoxy group in **17**. This conclusion was supported by the HMBC correlations of H-1 (δ_H 5.77, br t, 4.0 Hz) with C-3, C-5, C-9, C-10, C-20, and the carboxyl carbon (δ_C 171.1, s). The relative configuration of **17** was identical to that of macrocalyxoformin E[19] based on the observed ROESY correlations including the correlation between H-1β and H-5β. Therefore, the structure of compound **17** was assigned as 6β,15β-dihydroxy-1α-acetoxy-6,20-epoxy-6,7-seco-7,20-olide-*ent*-kaur-16-ene.

Laxiflorolide T (**18**) and maoecrystal O[14b] were determined to be an epimeric pair with the same molecular formula $C_{22}H_{30}O_7$. The only difference between the epimers was that the relative configurations of H-16 were deduced to be α-oriented in **18**, but β-oriented in maoecrystal O (Fig. 1), on the basis of the ROESY correlation data for H-9β/H_2-17 in **18**. Thus, the relative configurations of the epimeric pair can be described by the following nomenclature: 1α-acetoxy-6,7-seco-7,20-olide-*ent*-kaur-6-ac.

Considering the interesting cytotoxicity of the *ent*-kauranoids,[1e] compounds **1**–**29** were tested for in vitro cytotoxicity against A-549, MCF-7, SMMC-7721, SW-480, and HL-60 human cancer cell

Table 4
^{1}H NMR data (δ in ppm, J in Hz, C_5D_5N) of compounds **11**–**18**

No.	**11**[a]	**12**[a]	**13**[a]	**14**[a]	**15**[c]	**16**[a]	**17**[a]	**18**[b]
1	—	—	—	—	—	5.23 (dd, 11.4, 3.2)	5.77 (br t, 4.0)	5.14 (dd, 8.0, 3.1)
2	5.99 (d, 10.3)	5.96 (d, 10.0)	5.90 (d, 10.2)	5.90 (d, 11.5)	5.96 (d, 10.2)	2.52 (m)	1.72 (m)	2.00 (m)
							1.16 (m)	1.94 (m)
3	6.68 (d,10.3)	6.48 (d,10.0)	6.46 (d, 10.2)	6.48 (d, 11.5)	6.49 (d, 10.2)	3.82 (br t, 5.6)	2.11 (m)	1.42 (m)
							1.67 (m)	
5	4.19 (d, 6.9)	3.21 (br d, 4.5)	2.29 (br s)	2.28 (br t, 4.5)	2.51 (br t, 4.2)	2.57 (d, 5.2)	2.22 (d, 5.1)	2.79 (br s)
6	—	10.25 (d, 4.5)	4.03 (br d, 3.0)	4.05 (br t, 4.5)	4.37 (dd, 11.4, 4.2)	10.30 (d, 5.2)	5.85 (d, 5.1)	—
					4.18 (dd, 11.4, 3.6)			
9	3.59 (dd, 14.0, 4.4)	2.89 (dd, 12.8, 5.0)	3.01 (dd, 15.7, 3.5)	2.91 (dd, 12.1, 4.9)	3.70 (dd, 12.6, 4.8)	2.81 (br d, 12.0)	3.23 (br d, 7.4)	2.27 (overlap)
11	1.45 (m)	1.67 (m)	1.79 (m)	2.03 (m)	1.77 (m)	1.58 (m)	2.39 (m)	1.85 (m)
	1.36 (m)	1.42 (m)	1.68 (overlap)	1.80 (m)		1.37 (m)	2.00 (m)	1.56 (m)
12	1.85 (m)	2.37 (m)	1.90 (m)	2.12 (m)	1.96 (m)	1.93 (m)	2.09 (m)	1.58 (m)
	1.11 (m)	2.03 (m)	1.68 (overlap)	2.08 (m)		1.36(m)	1.64 (m)	1.38 (m)
13	2.65 (br s)	—	2.53 (dd, 9.5, 3.9)	—	2.66 (dd, 9.0, 4.8)	2.63 (br d, 9.0)	2.77 (br s)	2.30 (m)
14	2.47 (br s)	3.03 (d, 11.5)	3.56 (dd, 12.2, 3.9)	3.54 (br d, 11.2)	3.19 (dd, 12.6, 4.8)	2.40 (dd, 12.2, 2.4)	2.13 (overlap)	2.49 (dd, 12.2, 3.4)
		2.74 (d, 11.5)	2.94 (br d, 12.2)	3.13 (br d, 11.2)	2.75 (br d, 12.6)	1.72 (br d, 12.3)	1.66 (overlap)	2.10 (br d, 12.2)
15	4.99 (overlap)	5.22 (s)	—	—	5.16 (s)	4.90 (br s)	5.67 (br s)	—
16	—	—	—	2.96 (q, 7.1)	—	—	—	2.43 (m)
17	5.53 (br s)	5.78 (br s)	1.55 (s)	1.27 (d, 7.1)	4.33 (d, 12.0)	5.41 (br s)	5.47 (br s)	0.90 (d, 7.2)
	5.17 (br s)	5.58 (br s)		—	4.27 (d, 12.0)	5.16 (br s)	5.10 (br s)	
18	1.30 (s)	1.18 (s)	1.10(s)	1.13 (s)	1.29 (s)	1.25(s)	1.14 (s)	1.07(s)
19	1.28 (s)	1.18 (s)	1.12 (s)	1.14 (s)	1.16 (s)	1.35 (s)	0.93 (s)	1.24 (s)
20	5.46 (d, 10.8)	5.54 (d, 10.5)	5.27 (d, 11.1)	5.34 (d, 11.2)	5.42 (d, 10.8)	5.55 (d, 11.5)	6.10 (s)	5.51 (d, 12.4)
	4.85 (d, 10.8)	5.03 (overlap)	4.84 (d, 11.1)	4.86 (d, 11.2)	4.74 (d, 10.8)	5.52 (d, 11.5)		5.38 (d, 12.4)
AcO						2.15 (s)	2.09 (s)	2.16 (s)

[a] Recorded at 500 MHz.
[b] Recorded at 400 MHz.
[c] Recorded at 600 MHz.

lines using the MTT method;[20] cisplatin was used as the positive control. Compound **1** showed selective cytotoxic activity, with IC_{50} values of 4.8±0.1 and 7.9±2.4 μM against HL-60 and SW-480 cells, respectively, while **20** showed a significant cytotoxic activity against all of the five cell lines above with IC_{50} values ranging from 0.6±0.1 to 2.0±0.2 μM[9b] (Table 5). In our previous research, *ent*-kauranoids not only displayed the potential for tumor inhibition[2f,4e] but also had anti-inflammatory effects,[1e] Therefore, these two cytotoxic *ent*-kauranoids (**1** and **20**) were tested for their inhibitory activity against NO production in LPS stimulated RAW264.7 cells. Compounds **1** and **20** exhibited a strong inhibitory activity against NO production with IC_{50} values of 1.5±0.3 and 0.5±0.1 μM, respectively. The viability of RAW264.7 cells was simultaneously monitored using the MTS assay. None of the tested compounds showed any obvious cytotoxicity towards RAW264.7 cells, which suggested that the inhibitory activities against NO production in LPS stimulated RAW264.7 cells were not induced by the cytotoxicity of the tested compounds.

Table 5
IC_{50} values (μM) of diterpenoids from *I. eriocalyx* var. *laxiflora* for human tumor cell lines[a,b]

No.	HL-60	SMMC-7721	A-549	MCF-7	SW-480
1	4.8±0.1	14.9±0.3	18.8±1.0	15.1±0.5	7.9±2.4
20	0.8±0.03	1.0±0.01	2.0±0.2	1.3±0.1	0.6±0.1
Cisplatin	1.3±0.3	16.2±1.0	14.1±0.9	17.0±0.8	18.1±0.9

[a] Results were expressed as IC_{50} values in μM, and data were obtained from triplicate experiments, and cisplatin was used as positive control.
[b] Cytotoxic cut off value was set at IC_{50} values=40 μM. The other compounds showed no cytotoxic activity.

3. Experimental section

3.1. 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 noted, the chemical shifts (δ) are expressed in parts per million with respect to the solvent signals. HREIMS 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). Semi-preparative 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. The fractions were monitored by TLC, and the spots were visualized by heating silica gel plates sprayed with 8% H_2SO_4 in EtOH. All of the solvents including petroleum ether (60–90 °C) were distilled prior to use.

3.2. 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.

3.3. Extraction and isolation

The air-dried leaves of *Isodon eriocalyx* var. *laxiflora* (10 kg) were extracted with 70% aqueous $(CH_3)_2CO$ (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 successively extracted with EtOAc and *n*-BuOH. The EtOAc-soluble fraction (600 g) was decolorized on an MCI gel with 90:10 CH_3OH/H_2O to obtain a yellow gum (427.5 g). The gum was purified by CC on SiO_2 with a $CHCl_3/(CH_3)_2CO$ gradient system consisting of 1:0, 9:1, 8:2, 7:3, 6:4, and 1:1 to yield six main fractions (A–F). Fraction C ($CHCl_3$/$(CH_3)_2CO$, 8:2; 30 g) was subjected to repeated chromatography over silica gel ($CHCl_3/CH_3OH$, from 90:1, 60:1, to 30:1) to yield fractions C1–C3. Fraction C1 ($CHCl_3/CH_3OH$, 90:1; 10 g) was

fractionated by RP-18 CC (CH_3OH/H_2O, from 20:80 to 100:0) to afford fractions C1/1–C1/5. Fraction C1/3 (5.6 g) was subjected to repeated chromatography over silica gel ($CHCl_3$/isopropyl alcohol, from 90:1, 60:1, to 30:1) to yield fractions C1/3/1–C1/3/5. Fraction C1/3/2 was purified using ODS (CH_3OH/H_2O, 60:40) to afford compounds **1** (15 mg), **5** (16 mg), and **8** (7 mg). Compounds **25** (62 mg) and **26** (65 mg) were isolated from fraction C1/3/3 (306 mg) using MPLC (35 ml/min, CH_3OH/H_2O, 40:60) to achieve this separation. Fraction C2 ($CHCl_3/CH_3OH$, 60:1; 15 g) was eluted with RP-18 (CH_3OH/H_2O, 15:85 to 100:0) yielding subfractions C2/1-C2/4. Compounds **21** (4 g) and **22** (6 g) were crystallized from fraction C2/1 (12 g), and the remaining mother liquors were fractionated by MPLC (30 ml/min, CH_3OH/H_2O, 40:60) to afford compounds **23** (12 mg), **3** (5 mg), and **24** (6 mg). Fraction C2/3 (500 mg) was further separated by PHPLC (15 ml/min, CH_3CN/H_2O, 35:65) to afford compounds **10** (8 mg), **11** (9 mg), and **12** (7 mg). Subfraction C2/4 (300 mg) was eluted with PHPLC (15 ml/min, CH_3CN/H_2O, 25:75) yielding compounds **13** (8 mg), **14** (3 mg), **15** (6 mg), and **16** (6.0 mg). Fraction C3 ($CHCl_3/CH_3OH$, 30:1; 5 g) was eluted with RP-18 CC (CH_3OH/H_2O, 10:90 to 1:0) yielding subfractions C3/1–C3/3. Subfraction C3/1 (1.06 g) was fractionated by repeated CC, first on silica gel column with a gradient elution with $CHCl_3$/isopropyl alcohol (60:1 to 20:1) to yield fractions C3/1/1–C3/1/3. Subsequently, fraction C3/1/1 (250 mg) was purified using RP-18 CC (CH_3OH/H_2O, 45:60) to give **9** (3 mg), **17** (9 mg), and **18** (5 mg). Compounds **6** (6 mg) and **7** (5 mg) were precipitated from subfraction C3/1/2 (175 mg) by subsequent silica gel CC and RP-18 (45:55 CH_3OH/H_2O).

Subfraction D ($CHCl_3/(CH_3)_2CO$, 7:3; 50 g) was fractionated by using a silica gel column ($CHCl_3/CH_3OH$, 30:1, 20:1, and 10:1) to afford subfractions D1–D3. Subfraction D1 ($CHCl_3/CH_3OH$, 30:1; 20 g) was fractionated by repeated CC, first on RP-18 with gradient CH_3OH/H_2O (20:80 to 100:0) to yield fractions D1/1–D1/8. Subsequently, fraction D1/3 (2.27 g) was purified using a silica gel column ($CHCl_3/CH_3OH$, 50:1 to 10:1) to afford subfractions D1/3/1–D1/3/8. Subfraction D1/3/4 (80 mg) was purified by PHPLC (20 ml/min, CH_3OH/H_2O, 40:60) to yield **20** (25 mg). Subfraction D3 ($CHCl_3/CH_3OH$, 10:1; 6 g) was purified by CC on RP-18 (CH_3OH/H_2O, 15:85 to 100:0) to yield fractions D3/1–D3/6. Subsequently, fraction D3/3 (1.26 g) was purified by CC on SiO_2 ($CHCl_3$/isopropyl alcohol, 30:1 to 10:1) to yield subfractions D3/3/1 (750 mg), D3/3/2 (85 mg), and D3/3/3 (120 mg). Compounds **27** (650 mg) and **4** (5 mg) were precipitated from subfraction D3/3/1 by subsequent silica gel CC ($CHCl_3/CH_3OH$, 20:1) and RP-18 (CH_3OH/H_2O, 40:60).

Fraction E ($CHCl_3/(CH_3)_2CO$ 6:4; 100 g) was eluted with MCI (CH_3OH/H_2O, 30:70, 60:40, and 90:10) yielding subfractions E1–E3. Subfraction E1 (10 g) was purified by RP-18 CC (CH_3CN/H_2O, 15:85) to yield compound **2** (5 mg). Fraction E2 (13 g) was purified by ODS CC (CH_3CN/H_2O, 22:78) to yield compounds **19** (6 mg), **28** (150 mg), and **29** (28 mg).

*3.3.1. Laxiflorolide C (**1**).* Colorless needle crystals. $[\alpha]_D^{24.9}$ +26.9 (*c* 0.10, MeOH). UV (MeOH) λ_{max} (log ε): 216.8 (3.2), 309.6 (1.7) nm; IR (KBr) ν_{max} 3385, 2955, 2932, 1742, 1686, 1675, 1466, 1408, 1390, 1209, 1121, 1058 cm^{-1}; For ^{13}C and ^1H spectroscopic data, see Tables 1 and 3; Positive ESIMS: *m/z* 385 $[M+Na]^+$; HREIMS $[M]^+$ *m/z* 362.1727 (calcd for $C_{20}H_{26}O_6$, 362.1729).

*3.3.2. Laxiflorolide D (**2**).* White powder. $[\alpha]_D^{20.3}$ +5.3 (*c* 0.10, MeOH). UV (MeOH) λ_{max} (log ε): 230.4 (2.8) nm; IR (KBr) ν_{max} 3441, 2966, 2936, 1738, 1709, 1663, 1641, 1387, 1368, 1265, 1221, 1110, 1042 cm^{-1}; For ^{13}C and ^1H spectroscopic data, see Tables 1 and 3; Positive ESIMS: *m/z* 371 $[M+Na]^+$; HREIMS $[M]^+$ *m/z* 348.1923 (calcd for $C_{20}H_{28}O_5$, 348.1937).

*3.3.3. Laxiflorolide E (**3**).* Colorless needle crystals. $[\alpha]_D^{25.3}$ −109.3 (*c* 0.22, MeOH). UV (MeOH) λ_{max} (log ε): 204.2 (3.2) nm; IR (KBr) ν_{max} 3373, 2949, 2907, 2866, 1724, 1694, 1486, 1380, 1260, 1187, 1047 cm^{-1}; Positive ESIMS: *m/z* 369 $[M+Na]^+$; For ^{13}C and ^1H spectroscopic data, see Tables 1 and 3; Positive ESIMS: *m/z* 369 $[M+Na]^+$; HRESIMS $[M+H]^+$ *m/z* 347.1867 (calcd for $C_{20}H_{27}O_5$, 347.1858).

*3.3.4. Laxiflorolide F (**4**).* White power. $[\alpha]_D^{25.6}$ +6.4 (*c* 0.18, MeOH). UV (MeOH) λ_{max} (log ε): 209.4 (3.0) nm; IR (KBr) ν_{max} 3433, 2956, 2940, 1749, 1706, 1659, 1451, 1359, 1245, 1132, 1053 cm^{-1}; For ^{13}C and ^1H spectroscopic data, see Tables 1 and 3; EIMS: *m/z* 362 $[M]^+$; HREIMS $[M]^+$ *m/z* 362.1730 (calcd for $C_{20}H_{26}O_6$, 362.1729).

*3.3.5. Laxiflorolide G (**5**).* White powder. $[\alpha]_D^{25.1}$ −563.0 (*c* 0.10, MeOH). UV (MeOH) λ_{max} (log ε): 204.8 (3.3), 297.8 (2.0) nm; IR (KBr) ν_{max} 2961, 2924, 1763, 1725, 1695, 1452, 1392, 1376, 1273, 1193, 1053 cm^{-1}; For ^{13}C and ^1H spectroscopic data, see Tables 1 and 3; EIMS: *m/z* 346 $[M]^+$; HREIMS $[M]^+$ *m/z* 346.1774 (calcd for $C_{20}H_{26}O_5$, 346.1780).

*3.3.6. Laxiflorolide H (**6**).* White powder. $[\alpha]_D^{20.4}$ +121.9 (*c* 0.10, MeOH). UV (MeOH) λ_{max} (log ε): 203.0 (3.0), 228.6 (3.3), 294.8 (1.2) nm; IR (KBr) ν_{max} 3393, 2972, 2950, 2939, 1750, 1725, 1678, 1479, 1375, 1363, 1241, 1218, 1108, 1093 cm^{-1}; For ^{13}C and ^1H spectroscopic data, see Tables 1 and 3; Positive ESIMS: *m/z* 399 $[M+Na]^+$; HREIMS $[M]^+$ *m/z* 376.1892 (calcd for $C_{21}H_{28}O_6$, 376.1886).

*3.3.7. Laxiflorolide I (**7**).* White powder. $[\alpha]_D^{24.9}$ +25.8 (*c* 0.24, MeOH). UV (MeOH) λ_{max} (log ε): 227.2 (3.0) nm; IR (KBr) ν_{max} 3434, 2957, 2933, 1720, 1665, 1640, 1464, 1377, 1293, 1121, 1098 cm^{-1}; For ^{13}C and ^1H spectroscopic data, see Tables 1 and 3; Positive ESIMS: *m/z* 399 $[M+Na]^+$; HREIMS $[M]^+$ *m/z* 376.1887 (calcd for $C_{21}H_{28}O_6$, 376.1886).

*3.3.8. Laxiflorolide J (**8**).* White powder. $[\alpha]_D^{23.6}$ +5.3 (*c* 0.10, MeOH). UV (MeOH) λ_{max} (log ε): 201.6 (2.8), 226.6 (2.9) nm; IR (KBr) ν_{max} 3434, 2957, 2933, 1720, 1665, 1640, 1464, 1377, 1239, 1098 cm^{-1}; For ^{13}C and ^1H spectroscopic data, see Tables 1 and 3; Positive ESIMS: *m/z* 383 $[M+Na]^+$; HREIMS $[M]^+$ *m/z* 360.1581 (calcd for $C_{20}H_{24}O_6$, 360.1573).

*3.3.9. Laxiflorolide K (**9**).* White powder. $[\alpha]_D^{18.9}$ +54.4 (*c* 0.10, MeOH). UV (MeOH) λ_{max} (log ε): 201.4 (2.7), 228.6 (2.9), 361.8 (0.9) nm; IR (KBr) ν_{max} 3430, 2962, 2915, 1746, 1716, 1658, 1475, 1386, 1371, 1244, 1207, 1193, 1092, 1050 cm^{-1}; For ^{13}C and ^1H spectroscopic data, see Tables 1 and 3; Positive ESIMS: *m/z* 385 $[M+Na]^+$; HREIMS $[M]^+$ *m/z* 362.1724 (calcd for $C_{20}H_{26}O_6$, 362.1729).

*3.3.10. Laxiflorolide L (**10**).* White powder. $[\alpha]_D^{18.9}$ +20.4 (*c* 0.10, MeOH). UV (MeOH) λ_{max} (log ε): 197.2 (2.7), 230.4 (2.9), 362.2 (1.1) nm; IR (KBr) ν_{max} 3441, 2963, 2933, 1743, 1712, 1657, 1469, 1369, 1264, 1130, 1005 cm^{-1}; For ^{13}C and ^1H spectroscopic data, see Tables 2 and 3; Positive ESIMS: *m/z* 385 $[M+Na]^+$; HREIMS $[M]^+$ *m/z* 362.1730 (calcd for $C_{20}H_{26}O_6$, 362.1729).

*3.3.11. Laxiflorolide M (**11**).* Colorless needle crystals. $[\alpha]_D^{20.4}$ +29.6 (*c* 0.10, MeOH). UV (MeOH) λ_{max} (log ε): 203.4 (3.1), 225.4 (3.1) nm; IR (KBr) ν_{max} 3444, 2966, 2933, 1738, 1709, 1660, 1452, 1388, 1265, 1111, 1042 cm^{-1}; For ^{13}C and ^1H spectroscopic data, see Tables 2 and 4; Positive ESIMS: *m/z* 355 $[M+Na]^+$; HREIMS $[M]^+$ *m/z* 332.1625 (calcd for $C_{19}H_{24}O_5$, 332.1624).

*3.3.12. Laxiflorolide N (**12**).* White powder. $[\alpha]_D^{18.1}$ +1.8 (*c* 0.10, MeOH). UV (MeOH) λ_{max} (log ε): 201.6 (2.6), 227.0 (2.5), 361.8 (1.0) nm; IR (KBr) ν_{max} 3437, 2962, 2927, 1715, 1660, 1632, 1454,

1378, 1237, 1155, 1065 cm^{-1}; For ^{13}C and ^{1}H spectroscopic data, see Tables 2 and 4; Positive ESIMS: *m/z* 383 $[M+Na]^+$; HREIMS $[M]^+$ *m/z* 360.1576 (calcd for $C_{20}H_{24}O_6$, 360.1573).

*3.3.13. Laxiflorolide O (**13**).* White powder. $[\alpha]_D^{20.5}$ +41.9 (*c* 0.10, MeOH). UV (MeOH) λ_{max} (log ε): 228.2 (3.0) nm; IR (KBr) ν_{max} 3440, 2966, 2940, 1754, 1724, 1665, 1450, 1381, 1368, 1243, 1113, 1037 cm^{-1}; For ^{13}C and ^{1}H spectroscopic data, see Tables 2 and 4; Positive ESIMS: *m/z* 385 $[M+Na]^+$; HREIMS $[M]^+$ *m/z* 362.1723 (calcd for $C_{20}H_{26}O_6$, 362.1729).

*3.3.14. Laxiflorolide P (**14**).* White powder. $[\alpha]_D^{18.5}$ +93.1 (*c* 0.10, MeOH). UV (MeOH) λ_{max} (log ε): 202.0 (3.2), 228.2 (3.4), 282.0 (2.0), 362.0 (1.3) nm; IR (KBr) ν_{max} 3480, 2973, 2941, 1734, 1705, 1669, 1452, 1389, 1248, 1107, 1063 cm^{-1}; For ^{13}C and ^{1}H spectroscopic data, see Tables 2 and 4; Positive ESIMS: *m/z* 385 $[M+Na]^+$; HREIMS $[M]^+$ *m/z* 362.1750 (calcd for $C_{20}H_{26}O_6$, 362.1729).

*3.3.15. Laxiflorolide Q (**15**).* White powder. $[\alpha]_D^{20.4}$ +5.6 (*c* 0.10, MeOH). UV (MeOH) λ_{max} (log ε): 199.6 (2.4), 228.0 (2.6) nm; IR (KBr) ν_{max} 3429, 2963, 2933, 1713, 1661, 1466, 1369, 1236, 1099, 1044 cm^{-1}; For ^{13}C and ^{1}H spectroscopic data, see Tables 2 and 4; EIMS: *m/z* 380 $[M]^+$; HREIMS $[M]^+$ *m/z* 380.1841 (calcd for $C_{20}H_{28}O_7$, 380.1835).

*3.3.16. Laxiflorolide R (**16**).* White powder. $[\alpha]_D^{24.8}$ +17.0 (*c* 0.12, MeOH). UV (MeOH) λ_{max} (log ε): 204.0 (3.2), 253.6 (2.5) nm; IR (KBr) ν_{max} 3437, 2956, 2940, 1714, 1640, 1458, 1371, 1234, 1122, 1087, 1025 cm^{-1}; For ^{13}C and ^{1}H spectroscopic data, see Tables 2 and 4; Positive ESIMS: *m/z* 429 $[M+Na]^+$; HREIMS $[M]^+$ *m/z* 406.1998 (calcd for $C_{22}H_{30}O_7$, 406.1992).

*3.3.17. Laxiflorolide S (**17**).* White powder. $[\alpha]_D^{25.2}$ −12.6 (*c* 0.11, MeOH). UV (MeOH) λ_{max} (log ε): 204.4 (2.0), 296.6 (0.8) nm; IR (KBr) ν_{max} 3388, 2948, 2933, 1738, 1730, 1454, 1367, 1258, 1242, 1110, 1081 cm^{-1}; For ^{13}C and ^{1}H spectroscopic data, see Tables 2 and 4; Positive ESIMS: *m/z* 406 $[M]^+$; HREIMS $[M]^+$ *m/z* 406.1993 (calcd for $C_{22}H_{30}O_7$, 406.1992).

*3.3.18. Laxiflorolide T (**18**).* White powder. $[\alpha]_D^{25.2}$ −12.6 (*c* 0.11, MeOH). UV (MeOH) λ_{max} (log ε): 204.4 (2.0), 296.6 (0.8) nm; IR (KBr) ν_{max} 3440, 2968, 2950, 2933, 1731, 1697, 1632, 1465, 1393, 1369, 1231, 1141, 1044 cm^{-1}; For ^{13}C and ^{1}H spectroscopic data, see Tables 2 and 4; Positive ESIMS: *m/z* 429 $[M+Na]^+$; HREIMS $[M]^+$ *m/z* 406.2000 (calcd for $C_{22}H_{30}O_7$, 406.1992).

3.4. X-ray crystal structure analysis

The intensity data for compounds **1**, **3**, **6**, and **11** 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 compounds **1**, **3**, **6**, and **11** have been deposited in the Cambridge Crystallographic Data Centre database (deposition number **1**, CCDC 1007902; **3**, CCDC 1007903; **6**, CCDC 1007904; **11**, CCDC 1007905). Copies of the data can be obtained free of charge from the CCDC at www.ccdc.cam.ac.uk.

*3.4.1. Crystal data for **1**.* $C_{20}H_{26}O_6$, *M*=362.41, orthorhombic, *a*=7.6592(2) Å, *b*=11.8666(3) Å, *c*=20.7746(5) Å, *α*=90.00°, *β*=90.00°, *γ*=90.00°, *V*=1888.18(8) $Å^3$, *T*=296(2) K, space group *P*212121, *Z*=4, 7341 reflections measured, 3170 independent reflections (R_{int}=0.0439). The final R_1 values were 0.1415 ($I>2\sigma(I)$). The final $wR(F^2)$ values were 0.3158 ($I>2\sigma(I)$). The final R_1 values were 0.1447 (all data). The final $wR(F^2)$ values were 0.3275 (all data). The Hooft parameter is 0.01(13) for 1222 Bijvoet pairs.

*3.4.2. Crystal data for **3**.* $C_{20}H_{26}O_5$, *M*=346.41, orthorhombic, *a*=10.9250(4) Å, *b*=11.6275(4) Å, *c*=14.0233(5) Å, *α*=90.00°, *β*=90.00°, *γ*=90.00°, *V*=1781.39(11) $Å^3$, *T*=100(2) K, space group *P*212121, *Z*=4, 14,429 reflections measured, 2985 independent reflections (R_{int}=0.0439). The final R_1 values were 0.0354 ($I>2\sigma(I)$). The final $wR(F^2)$ values were 0.0961 ($I>2\sigma(I)$). The final R_1 values were 0.0356 (all data). The final $wR(F^2)$ values were 0.0962 (all data). Flack parameter=0.08(19). The Hooft parameter is 0.11(5) for 1226 Bijvoet pairs.

*3.4.3. Crystal data for **6**.* $C_{21}H_{28}O_6$, *M*=376.43, monoclinic, *a*=10.0462(2) Å, *b*=6.55770(10) Å, *c*=14.8542(2) Å, *α*=90.00°, *β*=107.97°, *γ*=90.00°, *V*=930.86(3) $Å^3$, *T*=100(2) K, space group *P*21, *Z*=2, 9674 reflections measured, 3072 independent reflections (R_{int}=0.0406). The final R_1 values were 0.0327 ($I>2\sigma(I)$). The final $wR(F^2)$ values were 0.0848 ($I>2\sigma(I)$). The final R_1 values were 0.0327 (all data). The final $wR(F^2)$ values were 0.0848 (all data). Flack parameter=0.06(14). The Hooft parameter is 0.12(5) for 1220 Bijvoet pairs.

*3.4.4. Crystal data for **11**.* $C_{19}H_{24}O_5$, *M*=332.38, hexagonal, *a*=7.3862(2) Å, *b*=7.3862(2) Å, *c*=52.0680(14) Å, *α*=90.00°, *β*=90.00°, *γ*=120.00°, *V*=2460.05(12) $Å^3$, *T*=100(2) K, space group *P*65, *Z*=6, 10,713 reflections measured, 2535 independent reflections (R_{int}=0.0555). The final R_1 values were 0.0905 ($I>2\sigma(I)$). The final $wR(F^2)$ values were 0.2643 ($I>2\sigma(I)$). The final R_1 values were 0.0959 (all data). The final $wR(F^2)$ values were 0.2683 (all data). The Hooft parameter is −0.24(18) for 1065 Bijvoet pairs.

3.5. Cytotoxic activity assay

Colorimetric assays were performed to evaluate each compound's activity. The following human tumor cell lines were used: the A-549 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) 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). Briefly, 100 μL of suspended adherent cells were 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.

3.6. NO production assay

The Murine monocytic RAW264.7 macrophages were dispensed into 96-well plates (2×10^5 cells/well) containing RPMI-1640

medium (Hyclone) with 10% FBS under a humidified atmosphere of 5% CO_2 at 37 °C. After 24 h pre-incubation, cells were treated with serial dilutions of the compounds with the maximum concentration of 25 μM in the presence of 1 μg/mL LPS for 18 h. Each compound was dissolved in DMSO and further diluted in medium to produce different concentrations. NO production in each well was assessed by adding 100 μL of Griess reagent (Reagent A & Reagent B, respectively, Sigma) to 100 μL of each supernatant from LPS (Sigma) treated or LPS and compound-treated cells in triplicate. After 5 min incubation, the absorbance was measured at 570 nm with 2104 Envision Multilabel Plate Reader (Perkin–Elmer Life Sciences, Inc., Boston, Ma, USA). MG-132 was used as a positive control (IC_{50}: 0.1±0.002 μM).[21]

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Supplementary data

Supplementary data associated with this article (^{1}H, ^{13}C NMR, DEPT, HSQC, COSY, HMBC, ROESY, MS, IR, and UV spectra of compounds **1**, **3**, and **11**; ^{1}H, ^{13}C NMR, DEPT, and HRMS spectra of compounds **2**, **4**–**10**, and **15**–**18**; X-ray structures of compounds **1**, **3**, **6**, and **11**) is available free of charge via the Internet at http://www.sciencedirect.com. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2014.08.018. These data include MOL files and InChiKeys of the most important compounds described in this article.

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