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ent-Kaurane Diterpenoids from *Isodon lungshengensis*

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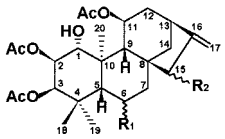
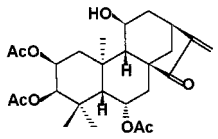
Six new *ent*-kaurane diterpenoids, lungshengenins B−G (**1**−**6**), together with three known diterpenoids, lungshengenin A (**7**), inflexin (**8**), and lushanrubescinsin C (**9**), were isolated from the leaves and tender branches of *Isodon lungshengensis*. Their structures were elucidated by means of spectroscopy, mainly 1D and 2D NMR techniques. Lungshengenins A (**7**), C (**2**), and G (**6**) were cytotoxic toward K562 cells, having IC_{50} values equal to or less than 10 μg/mL.

The genus *Isodon* is rich in *ent*-kaurane diterpenoids, which have been verified as the main biologically active constituents.[1−4] *Isodon lungshengensis* (Labiatae), a perennial herb, is found only in Longsheng Prefecture, Guangxi Zhuang Autonomous Region, and has been used in local folk medicine as an agent for reducing swelling and dissolving lumps.[5] Previous studies on this plant led to the isolation of two diterpenoids, lungshengenin A and lushanrubescinsin C.[6] As a continuation of a program directed toward the isolation of biologically active metabolites from the *Isodon* genus, we have reinvestigated the diterpenoids of *I. lungshengensis*. An extract of the leaves and tender branches of *I. lungshengensis* has yielded a series of new *ent*-kaurane diterpenoids, lungshengenins B−G (**1**−**6**), along with the known diterpenoids, lungshengenin A (**7**), inflexin (**8**), and lushanrubescinsin C (**9**). Varied NMR experiments led to revision of assignments of a few NMR signals of **7** and **8**, and the orientation of the acetoxy substituent at C-11 of **9** in a previous report.[7] Lungshengenins A (**7**), B (**1**), F (**5**), and G (**6**) are the only *ent*-kaurane diterpenoids known in this genus with three oxy-groups on the A ring. In this paper, we report the isolation and structure elucidation of **1**−**6** and structure revision of **7**−**9**.

Compounds **2**, **6**, and **7** showed potent antitumor activity against K562 cells.



1. R_1 = =O, R_2 = β–OAc
5. R_1 = H, R_2 = =O
6. R_1 = =O, R_2 = =O
7. R_1 = α–OH, R_2 = =O



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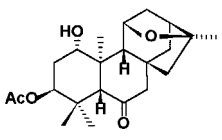

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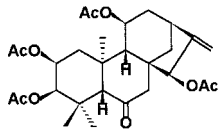

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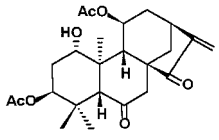

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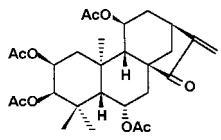

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Results and Discussion

An ethyl acetate extract of the leaves and tender branches of *I. lungshengensis* was subjected to column

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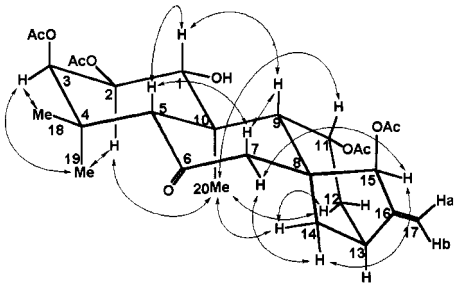

Figure 1. Significant NOESY correlations of **1**.

chromatography on Si gel, followed by further repeated column chromatography and recrystallization to give diterpenoids **1**−**9**. The IR, MS, and NMR of lungshengenin B (**1**) indicated three methyls, three methylenes, eight methines, three quaternary carbons, one ketonic carbon, two olefinic carbons, and four acetoxy groups. The molecular formula of **1** ($C_{28}H_{38}O_{10}$) suggested that it possessed 10 degrees of unsaturation, which indicated that **1** had four rings, five carbonyl groups, and one olefinic bond. Absence of characteristic UV and IR absorptions for an α,β-unsaturated *exo*-methylene ketone[1−3] indicated that the *exo*-methylene group (δ 151.5, 106.9) and the ketone (δ 209.4) were unconjugated. Based on the spectral characteristics of lungshengenin A (**7**)[6] and other related compounds isolated from this genus,[1−4] compound **1** was presumed to possess a polysubstituted *ent*-kaurane diterpenoid skeleton.

From the ^{1}H−^{1}H COSY and ^{13}C−^{1}H COSY spectra of **1**, the existence of fragments −CHCHCH− (C-1 to C-3) and −CHCHCH$_2$CHCH$_2$− (C-9, C-11 to C-14) were clearly evident. Fragment −CHCHCH− was a structure in which each carbon bore an acetoxy or hydroxy group (the carbon signals at δ 76.3, 72.9, and 78.0 ppm, respectively). This presumption was confirmed by the three protons resonating at low field (δ 4.79, 5.59, and 5.25) in the ^{1}H NMR and the coupling constants of H-1β with H-2α (J = 9.9 Hz) and H-2α with H-3α (J = 2.8 Hz). Comparing NMR spectra of **1** with those of lungshengenin A (**7**), this fragment was most likely in the A ring. These partial structures were further determined using an HMBC experiment.

The HMBC spectrum contained the *ent*-kauranoid correlation signals characteristic of two methyl groups at δ 1.00 (Me-18) and 1.56 (Me-19) with one quaternary carbon (δ 36.8, C-4) and two methines (δ 78.0 and 58.3, C-3 and C-5), and the methyl at δ 1.37 (Me-20) correlated with a quaternary carbon (δ 49.5, C-10). There were also cross-peaks between H-5β (δ 3.21, s) and a carbonyl group (δ 209.4). The carbonyl function was assigned to C-6. The Me-20 signal showed correlations with two other methine carbons (C-1 and C-9). H-9β was correlated with two other quaternary carbons (δ 49.5 and 49.5), one methine carbon (δ 70.0), and three methylene carbons (δ 54.1, 38.9, and 37.0), which were assigned to C-8, C-10, C-11, C-7, C-12, and C-14, respectively. Based on the correlation of the signals of δ 2.56 (^{13}C δ 39.1) and H-15α (δ 5.49) with a quaternary carbon (δ 151.5) and H-17 with a methine (δ 39.1), the methine and quaternary carbons were C-13 and C-16. Thus, the remaining methine carbon (δ 72.9) was not assigned, and this had to be C-2. Assignments of all carbons are given in Table 1. Assignments of the four acetoxy groups were also determined by the HMBC spectrum, and the correlation peaks among H-2α, H-3α, H-11α, and H-15α with ester carbonyls were also observed.

Unambiguous assignments of stereochemistry in **1** were achieved using a NOESY experiment; most of the NOESY correlations are indicated by arrows in Figure 1. NOESY correlations between the H-1β with H-5β and H-9β, H-2α with Me-19 and Me-20, H-3α with Me-18 and Me-19, H-7α with H-15α and H-14β, H-11α with Me-20, and H-14β with H-15α protons, confirmed that the C-1 hydroxy group and the C-2, C-3, C-11, and C-15 acetoxy groups had the 1α, 2β, 3β, 11β, and 15β orientations, respectively. Therefore, compound **1** was elucidated as 1α-hydroxy-2β,3β,11β,15β-tetraacetoxy-*ent*-kaur-16-en-6-one.[8]

Table 1. ^{13}C NMR Data of Lungshengenins B−G (**1**−**6**) (100.6 or 125.8 Hz, δ in ppm with Reference to the Signal of C_5D_5N)

carbon	1	2	3	4	5	6
1	76.4 (d)	40.6 (t)	74.3 (d)	39.1 (t)	76.7 (d)	75.6 (d)
2	72.9 (d)	67.8 (d)	33.0 (t)	67.9 (d)	72.7 (d)	73.0 (d)
3	78.0 (d)	77.7 (d)	79.3 (d)	77.1 (d)	77.9 (d)	78.0 (d)
4	36.8 (s)	38.5 (s)	35.9 (s)	37.5 (s)	37.6 (s)	36.9 (s)
5	58.3 (d)	48.7 (d)	60.6 (d)	58.6 (d)	49.0 (d)	59.0 (d)
6	209.4 (s)	68.8 (d)	210.2 (s)	209.3 (s)	18.2 (t)	209.9 (s)
7	54.1 (t)	38.7 (t)	57.7 (t)	53.1 (t)	34.6 (t)	50.4 (t)
8	49.5 (s)	49.2 (s)	48.3 (s)	48.9 (s)	51.1 (s)	54.6 (s)
9	54.5 (d)	63.5 (d)	59.8 (d)	53.5 (d)	60.4 (d)	60.0 (d)
10	49.5 (s)	39.9 (s)	48.7 (s)	44.7 (s)	45.3 (s)	51.0 (s)
11	70.0 (d)	65.0 (d)	79.4 (d)	67.9 (d)	71.5 (d)	70.5 (d)
12	38.9 (t)	41.3 (t)	44.6 (t)	39.6 (t)	38.2 (t)	37.4 (t)
13	39.1 (d)	38.0 (d)	46.1 (d)	38.8 (d)	37.3 (d)	36.9 (d)
14	37.0 (t)	37.9 (t)	40.3 (t)	36.5 (t)	37.1 (t)	36.9 (t)
15	81.4 (d)	207.9 (s)	53.5 (t)	81.4 (d)	208.4 (s)	205.0 (s)
16	151.5 (s)	150.8 (s)	85.1 (s)	151.4 (s)	151.3 (s)	150.1 (s)
17	106.8 (t)	111.7 (t)	23.4 (q)	107.8 (t)	111.9 (t)	113.6 (t)
18	26.7 (q)	28.0 (q)	27.0 (q)	27.0 (q)	28.1 (q)	26.7 (q)
19	21.9 (q)	22.7 (q)	22.4 (q)	21.9 (q)	21.4 (q)	21.6 (q)
20	16.8 (q)	20.0 (q)	14.5 (q)	19.8 (q)	15.7 (q)	17.2 (q)
OAc	171.1 (s)	170.5 (s)	170.3 (s)	171.1 (s)	170.8 (s)	170.8 (s)
	170.7 (s)	170.3 (s)	20.9 (q)	170.5 (s)	170.7 (s)	170.6 (s)
	170.4 (s)	170.0 (s)		170.4 (s)	169.5 (s)	169.4 (s)
	170.3 (s)	21.5 (q)		169.6 (s)	21.3 (q)	21.3 (q)
	21.7 (q)	21.0 (q)		21.5 (q)	20.9 (q)	20.8 (q)
	20.8 (q)	20.6 (q)		20.9 (q)	20.6 (q)	20.5 (q)
	20.8 (q)			20.8 (q)		
	20.4 (q)			20.4 (q)		

The IR, MS, and NMR indicated that lungshengenin C (**2**), $C_{26}H_{36}O_8$, possessed three methyls, five methylenes (including one olefinic carbon), seven methines, five quaternary carbons (including one olefinic carbon and one ketonic carbon), and three acetoxy groups. The *exo*-methylene group and the ketone were conjugated as evidenced by characteristic UV and IR absorptions. A comparison of the ^{1}H and ^{13}C NMR data of **2** with those of the known *ent*-kaurane diterpenoid, lushanrubescensin C (**9**),[7] showed that they were similar except for ring C. The upfield shift of H-11α from δ 5.88 in **9** to δ 4.35 in **2** indicated that the C-11β hydroxy group in **2** had been replaced by an acetoxy group in **9**. This conclusion was confirmed by the downfield shift of C-12 in **2** due to a β-downfield effect of the 11β-hydroxy group[7,9] and by inspection of the ^{1}H−^{1}H COSY and HMBC spectra of **2**.

The relative configuration of **2** was achieved using the NOESY experiment, in which correlations between H-2α with Me-19 and Me-20, H-3α with Me-18 and Me-19, H-6β with Me-18, and H-11α with H-1α and Me-20 were evident. Therefore, the substituents possessed the C-2β, C-3β, C-6α, and C-11β orientations, and **2** was elucidated as 11β-hydroxy-2β,3β,6α-triacetoxyl-*ent*-kaur-16-en-15-one.[8]

HREIMS of lungshengenin D (**3**) indicated it has a molecular formula of $C_{22}H_{32}O_5$, suggesting seven degrees of unsaturation. ^{13}C NMR and DEPT spectra of **3** indicated five methyls, five methylenes, six methines, four quaternary carbons, and two ketonic carbons (δ 210.2 and 170.3). The IR, MS, and NMR indicated one acetoxy group and one hydroxy group. Analysis of ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, and HMBC spectra suggested that **3** possessed an *ent*-kaurane diterpenoid skeleton with a ketonic oxygen at

Table 2. The NOESY and HMBC Correlations Observed for Lungshengenin D (**3**)

proton	NOESY	HMBC[a]
1*β*	2*β*, 5*β*, 9*β*, 11α	n.o.
2α	3α, 19, 20	n.o.
2*β*	1*β*, 3α	n.o.
3α	2α, 2*β*, 18, 19	5, OAc
5*β*	1*β*, 9*β*, 18	(4), (6), 9, (10), 18, 19, 20
7α	14*β*, 15α	5, (8), 9
7*β*	9*β*, 15*β*	n.o.
9*β*	1*β*, 5*β*, 7*β*, 11α	5, (8, 10, 11), 12, 14, 20
11α	1*β*, 9*β*, 12α, 20	8, 10, 16
13α	12α, 12*β*, 17	8, 11, (12, 14, 16), 15
14α	12α, 20	12, 16
14*β*	7α, 15α	(8), 15
15α	7α, 14*β*, 17	6, 14
15*β*	7*β*	6, 10, 12,
17	13α, 15α	8, (16)
18	3α, 5*β*, 19	3, (4), 5, 19
19	2α, 3α, 18, 20	3, (4), 5, 18
20	2α, 11α, 12α, 14α, 19	5, 9, (10)

[a] Two-bond correlations are indicated in parentheses; n.o. indicate no clear correlation with this proton.

C-6 and with no unsaturated functionalities on ring D. However, the further degrees of unsaturation required by the molecular formula indicated an additional ring.

The *δ* 70−90 region of the ^{13}C NMR spectrum of **3** exhibited four signals, suggesting that one of the three oxygenated substituents in **3** was connected with two carbons to form an epoxy unit. This was supported by IR absorptions characteristic of an ether bond at 1105 and 1045 cm^{-1}. Comparing NMR spectra of **3** with those of isodoglutinosin B,[10] liangshanin G,[11] and euphoranginol C,[12] all *ent*-kaurane diterpenoids possessing an ether bond between rings C and D to form an additional ring, indicated that **3** was very similar. The linkage of this additional ring through an ether bridge from C-11 to C-16 in **3** was established unambiguously by analysis of the ^2J and ^3J heteronuclear couplings visualized through an HMBC experiment (Table 2). Placement of the other substituents was determined by HMBC and ^{1}H−^{1}H COSY.

The relative stereochemistry of **3** was assigned on the basis of NOESY correlations (Table 2). These assignments were confirmed by the coupling constants of H-11α with H-9*β* and H-12*β*, and Me-18 and Me-20 in the ^{13}C NMR shifted upfield due to the *γ*-steric compression effects between 1α-OH and Me-20, 3*β*-AcO and Me-18. Therefore, the structure of **3** was deduced to be 1α-hydroxy-3*β*-acetoxy-11*β*,16*β*-epoxy-*ent*-kaur-6-one.

Lungshengenin E (**4**) differed from **1** only in the lack of a hydroxy group. Comparison of the ^{1}H and ^{13}C NMR spectra revealed that the signals at *δ* 4.79 and *δ* 76.4 in **1** replaced the signals at *δ* 2.35 and 1.85, and *δ* 39.1 in **4**, respectively. This indicated that **4** retained most of the structural features of **1**, with the exception of a hydroxy group at C-1. Other noticeable differences between **1** and **4** were the two signals at *δ* 72.9 and 49.5 in **1** that were shifted upfield to *δ* 67.9 and 44.7 in **4** due to absence of an α-oriented hydroxy group at C-1. Thus, **4** was characterized as 2*β*,3*β*,11*β*,15*β*-tetraacetoxy-*ent*-kaur-16-en-6-one.

IR, MS, and NMR of lungshengenin F (**5**), $C_{26}H_{36}O_8$, indicated three methyls, four methylenes, seven methines, three quaternary carbons, one ketonic carbon, one *exo*-methylene group, three acetoxy groups, and one hydroxy group. ^{1}H−^{1}H COSY, HMQC, and HMBC indicated that **5** was also an *ent*-kaurane diterpenoid. The ketonic bond and the *exo*-methylene group were obviously conjugated[1−3] as evidenced by UV and IR. ^{1}H−^{1}H COSY and HMBC demonstrated that the hydroxy and acetoxy substituents

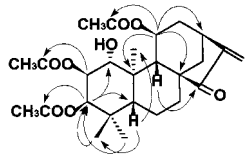

Figure 2. Key HMBC correlations of **5**.

were at C-1, C-2, C-3, and C-11 (Figure 2). All substituents were further determined to be C-1α, C-2*β*, C-3*β*, and C-11*β* by a NOESY experiment. Thus, **5** was elucidated as 1α-hydroxy-2*β*,3*β*,11*β*-triacetoxy-*ent*-kaur-16-en-15-one.

UV, IR, MS, and NMR of lungshengenin G (**6**) ($C_{26}H_{34}O_9$) suggested an *ent*-kaur-16-en-15-one skeleton with three acetoxy groups. MS showed a molecular ion 2 amu less than that of **7**, which was presumed to be a result of one of the hydroxy groups in **7** being replaced by a carbonyl group in **6**. Comparison of the ^{1}H and ^{13}C NMR data of **6** with those of **7** showed that **6** was similar to **7** except for ring B. Inspection of the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra in **6** indicated that the C-6α hydroxy group in **7** had been replaced by a carbonyl group at C-6. This conclusion was confirmed by the downfield shift of signals of C-5 (*δ* 59.0, d) and C-7 (*δ* 50.4, t) in **6** due to deshielding by the C-6 carbonyl group.[9] A NOESY experiment showed the substituents of **6** to have the same orientations with those of **7**. Therefore, the structure of **6** was deduced to be 1α-hydroxyl-2*β*,3*β*,11*β*-triacetoxy-*ent*-kaur-16-en-6,15-dione.

Compound **7** possessed a molecular formula of $C_{28}H_{38}O_9$. Based on comparison of **7** with authentic samples, it was verified to be lungshengenin A, which was further confirmed by comparison of NMR spectra. Most of spectral data of **7** were in agreement with those of lungshengenin A except for the ^{13}C NMR data about C-6 and C-11. A previous report had assigned the signals at *δ* 71.9 to C-6 and at *δ* 65.8 to C-11.[6] According to the ^{1}H−^{1}H COSY, HMQC, and HMBC experiments, such assignments were obviously incorrect because the correlations between H-11α (*δ* 6.97) with H_2-12; H-11α with the carbon at *δ* 71.9; and H-11α with 11*β*-OAc, C-8, C-9, C-10, C-12, and C-13 were clearly observed. Correlations between H-6*β* (*δ* 4.64) and the carbon at *δ* 66.0 and between H-6*β* and C-4, C-5, C-7, C-8, and C-10 were also observed. Therefore, the signal at *δ* 66.0 should be assigned to C-6, and the signal at *δ* 71.9 should be assigned to C-11.

Compound **8** was determined to have molecular formula $C_{24}H_{32}O_7$ by HREIMS and was identical to a comparison sample of inflexin. Several NMR assignments were different from those reported in the previous papers,[13,14] and the NMR signals of inflexin were reassigned here according to 1D and 2D NMR experiments.

Compound **9** had a molecular formula of $C_{28}H_{38}O_9$ by HREIMS. The UV, IR, MS, and NMR data were identical to those of lushanrubescensin C.[7] Based on correlations of H-11α with H-1α and Me-20 in the NOESY spectrum and the signal of H-11 at *δ* 5.88 in the ^{1}H NMR spectrum, the relative configuration of the acetoxy at C-11 in **9** was clearly *β*-oriented. The α-orientation of acetoxy at C-11 in lushanrubescensin C should be revised to *β*. Thus, the revised structure of lushanrubescensin C is 2*β*,3*β*,6α,11*β*-tetraacetoxy-*ent*-kaur-16-en-15-one (**9**).

Compounds **1**, **2**, **3**, **6**, and **7** were assayed for cytotoxicity against K562 cells by the method of MTT.[15] Only **2**, **6**, and **7** showed significant antitumor activity, and their IC_{50} values are 1.14, 3.29, and 11.7 *μ*g/mL, respectively.

Experimental Section

General Experimental Procedures. All melting points

were obtained on a Koffler apparatus and are uncorrected. IR spectra were obtained on a Perkin−Elmer 577 or Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were recorded on a Bruker AM-400 or DRX-500 instrument with TMS as internal standard and pyridine-d_5 as solvent. ^{1}H NMR, ^{1}H−^{1}H COSY, NOESY spectra were measured at 400.13 or 500.13 MHz; ^{13}C NMR and DEPT spectra were recorded at 100.6 or 125.8 MHz; HMBC spectrum was obtained at 400.13 MHz/100.6 or 500.13 MHz/125.8 MHz. ^{13}C NMR assignments were determined by ^{13}C−^{1}H COSY and HMQC spectra. The EIMS, FABMS, and HRMS were carried out on a VG Auto Spec-3000 spectrometer at 70 eV.

Plant Material. The leaves and tender branches of *I. lungshengensis* were collected in the Huaping Natural Reserve Area, Longsheng Prefecture of the Guangxi Zhuang Autonomous Region, in September and November 1996, and identified by Professor J. H. Shi, who was working in Guangxi Institute of Botany. A voucher specimen (KIB 96−11−08 Lin) is deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Academia Sinica, Kunming, Yunnan, People's Republic of China.

Extraction and Isolation. Dried and powdered leaves and tender branches of *I. lungshengensis* (2.6 kg) were extracted with EtOH (7000 mL × 3) under reflux for 2 h each time. The solution was treated with activated charcoal (100.0 g × 3) and then filtered. The filtrate was concentrated in vacuo to give a crude extract (173 g). The extract was dissolved in EtOH and then diluted with H_2O to give a solution containing 20% alcohol. The solution was partitioned with petroleum ether (60−90 °C) and EtOAc (2000 mL × 3), respectively. The EtOAc extract was evaporated under vacuum to give a residue (56.0 g) that was chromatographed on a Si gel column (200−300 mesh, 1.2 kg) and eluted with $CHCl_3$ containing increasing amounts of Me_2CO. The fractions were combined after monitoring by TLC. Fractions 2−7 (obtained from $CHCl_3$−Me_2CO, 9:1) and 8 (obtained from $CHCl_3$−Me_2CO, 8:2) were submitted repeatedly to Si gel or RP-18 gel column chromatography and eluted with solvents of increasing polarity (cyclohexane, isopropyl alcohol, and H_2O−MeOH), and appropriate fractions were crystallized yielding nine diterpenoids: **1** (124 mg), **2** (98 mg), **3** (38 mg), **4** (10 mg), **5** (70 mg), **6** (34 mg), **7** (1.38 g), **8** (183 mg), and **9** (195 mg).

Lungshengenin B (1): colorless cubes (cyclohexane); mp 166−167 °C; $[\alpha]^{22}_D$ −65.5° (*c* 0.57, $CHCl_3$); UV ($CHCl_3$) λ_{max} no absorption; IR (KBr) ν_{max} 3450 (br), 2965, 2925, 2860, 1745−1705 (br), 1695, 1425, 1360, 1265, 1230, 1105, 1055, 1025, 960, 875 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 4.79 (1H, d, J = 9.9 Hz, H-1β), 5.59 (1H, dd, J = 9.9, 2.8 Hz, H-2α), 5.25 (1H, d, J = 2.8 Hz, H-3α), 3.21 (1H, s, H-5β), 3.17 (1H, d, J = 12.6 Hz, H-7β), 2.21 (1H, d, J = 12.6 Hz, H-7α), 3.00 (1H, s, H-9β), 6.91 (1H, d, J = 4.6 Hz, H-11α), 2.00 (2H, overlapped, H-12α and 12β), 2.56 (1H, br s H-13α), 1.99 (1H, overlapped, H-14α), 1.29 (1H, dd, J = 12.2, 4.0 Hz, H-14β), 5.49 (1H, br s, H-15α), 5.00 (2H, s, H-17a and 17b), 1.00 (3H, s, Me-18), 1.56 (3H, s, Me-19), 1.37 (3H, Me-20), 2.01, 1.94, 1.90, 1.87 (each 3H, s, 4 × Ac); ^{13}C NMR, see Table 1; EIMS *m*/*z* 534 [M]$^+$ (17), 516 (35), 474 (61), 456 (10), 432 (32), 414 (100), 372 (35), 354 (51), 312 (85), 294 (88); HREIMS *m*/*z* 534.2453 (calcd for $C_{28}H_{38}O_{10}$, 534.2464).

Lungshengenin C (2): colorless needles (Me_2CO); mp 199−201 °C; $[\alpha]^{22}_D$ −53.7° (*c* 0.39, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 241.5 (3.77) nm; IR (KBr) ν_{max} 3480 (br), 2960, 2920, 2880, 1740−1695 (br), 1631, 1420, 1360, 1240, 1220, 1160, 1040, 1020, 940, 915 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 2.03 (1H, overlapped, H-1α), 1.69 (1H, overlapped, H-1β), 5.48 (1H, ddd, J = 12.1, 3.8, 2.1 Hz, H-2α), 5.21 (1H, d, J = 2.1 Hz, H-3α), 1.72 (1H, s, H-5β), 5.65 (1H, br s, H-6β), 2.47 (1H, dd, J = 15.2, 3.2 Hz, H-7β), 1.73 (1H, overlapped, H-7α), 2.06 (1H, s, H-9β), 4.35 (1H, d, J = 3.8 Hz, H-11α), 2.20 (2H, m, H-12α and 12β), 3.05 (1H, br s, H-13α), 2.62 (1H, d, J = 12.2 Hz, H-14α), 1.33 (1H, overlapped, H-14β), 6.01 (1H, s, H-17a), 5.29 (1H, s, H-17b), 0.98 (3H, s, Me-18), 1.12 (3H, s, Me-19), 1.50 (3H, s, Me-20), 2.09, 1.99, 1.98 (each 3H, s, 3 × Ac); ^{13}C NMR, see Table 1; EIMS *m*/*z* 476 [M]$^+$ (5), 458 (1), 434 (2), 416 (6), 356 (7), 341 (3), 314 (10), 296 (70), 281 (56), 257 (100); HREIMS *m*/*z* 476.2405 (calcd for $C_{26}H_{36}O_8$, 476.2410).

Lungshengenin D (3): colorless prisms (from Me_2CO); mp 199−200 °C; $[\alpha]^{22}_D$ +26.4° (*c* 0.27, $CHCl_3$); UV ($CHCl_3$) λ_{max} no absorption; IR (KBr) ν_{max} 3365 (br), 2945, 2920, 2860, 1725, 1690, 1380, 1230, 1175, 1105, 1045, 980 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) δ 6.35 (1H, d, J = 5.8 Hz, H-1α-OH), 4.32 (1H, ddd, J = 12.0, 5.8, 3.0 Hz, H-1β), 2.22 (2H, m, H-2α and 2β), 4.83 (1H, t, J = 3.0 Hz, H-3α), 2.96 (1H, s, H-5β), 1.83 (1H, d, J = 11.0 Hz, H-7β), 1.38 (1H, d, J = 11.0 Hz, H-7α), 2.55 (1H, br d, J = 3.4 Hz, H-9β), 5.71 (1H, t, J = 3.4 Hz, H-11α), 1.94 (1H, dt-like, J = 11.8, 3.4 Hz, H-12α), 1.27 (1H, m, H-12β), 2.13 (1H, overlapped, H-13α), 2.15 (1H, overlapped, H-14α), 2.09 (1H, overlapped, H-14β), 2.75 (1H, d, J = 13.3 Hz, H-15α), 2.10 (1H, d, J = 13.3 Hz, H-15β), 1.38 (3H, s, Me-17), 1.08 (3H, s, Me-18), 1.50 (3H, s, Me-19), 1.34 (3H, s, Me-20), 2.00 (3H, s, Ac); ^{13}C NMR, see Table 1; EIMS *m*/*z* 376 [M]$^+$ (49), 333 (32), 316 (56), 298 (26), 288 (9), 273 (28), 219 (100), 201 (31); HREIMS *m*/*z* 376.2236 (calcd for $C_{22}H_{32}O_5$, 376.2250).

Lungshengenin E (4): colorless prisms (from cyclohexane); mp 241−243 °C; $[\alpha]^{22}_D$ −44.5° (*c* 0.25, MeOH); UV ($CHCl_3$) λ_{max} no absorption; IR (KBr) ν_{max} 2987, 2943, 1745−1705 (br), 1700, 1433, 1372, 1253, 1229, 1113, 1040, 959, 894 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 2.35 (1H, overlapped, H-1α), 1.85 (1H, overlapped, H-1β), 5.50 (1H, ddd, J = 12.4, 4.2, 2.8 Hz, H-2α), 5.22 (1H, d, J = 2.8 Hz, H-3α), 3.04 (1H, s, H-5β), 3.12 (1H, d, J = 13.4 Hz, H-7β), 2.34 (1H, dd, J = 13.4, 4.4 Hz, H-7α), 2.68 (1H, s, H-9β), 5.33 (1H, d, J = 4.3 Hz, H-11α), 2.00 (2H, overlapped, H− 12α and 12β), 2.57 (1H, br s, H-13α), 1.99 (1H, overlapped, H-14α), 1.29 (1H, dd, J = 12.2, 4.4 Hz, H-14β), 5.22 (1H, br s, H-15α), 5.08 (2H, s, H-17a and 17b), 1.04 (3H, s, Me-18), 1.10 (3H, s, Me-19), 1.48 (3H, s, Me-20), 2.12, 2.02, 2.01, 1.94 (each 3H, s, 4 × Ac); ^{13}C NMR, see Table 1; EIMS *m*/*z* 518 [M]$^+$ (9), 476 (1), 458 (86), 416 (36), 398 (28), 383 (7), 356 (35), 338 (31), 323 (20), 314 (26), 296 (100), 281 (65), 278 (37), 263 (46), 253 (21); HREIMS *m*/*z* 518.2529 (calcd for $C_{28}H_{38}O_9$, 518.2516); .

Lungshengenin F (5): colorless cubes; mp 95−96 °C; $[\alpha]^{22}_D$ −94.9° (*c* 0.47, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 240 (3.91) nm; IR (KBr) ν_{max} 3473 (br), 2945, 2881, 1745−1700 (br), 1647, 1456, 1435, 1372, 1258, 1240, 1035, 963 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 4.31 (1H, d, J = 10.0 Hz, H-1β), 5.68 (1H, dd, J = 10.0, 2.4 Hz, H-2α), 5.37 (1H, d, J = 2.4 Hz, H-3α), 1.61 (1H, dd, J = 11.7 and 2.8 Hz, H-5β), 1.49 (1H, br d, J = 11.7 Hz, H-6β), 1.33 (1H, overlapped, H-6α), 2.20 (1H, overlapped, H-7β), 1.39 (1H, overlapped, H-7α), 2.11 (1H, br s, H-9β), 6.94 (1H, t, J = 2.7 Hz, H-11α), 2.07 (2H, overlapped, H-12α and 12β), 2.91 (1H, br d, J = 3.0 Hz, H-13α), 2.38 (1H, d, J = 12.1 Hz, H-14α), 1.34 (1H, overlapped, H-14β), 5.97 (1H, s, H-17a), 5.18 (1H, s, H-17b), 0.84 (3H, s, Me-18), 0.95 (3H, s, Me-19), 1.36 (3H, s, Me-20), 2.04, 1.86, 1.73 (each 3H, s, 3 × Ac); ^{13}C NMR, see Table 1; EIMS *m*/*z* 476 [M]$^+$ (14), 433 (52), 416 (61), 373 (9), 356 (42), 331 (23), 313 (78), 296 (100), 278 (24), 253 (20), 241 (26), 200 (60); HREIMS *m*/*z* 476.2410 (calcd for $C_{26}H_{36}O_8$, 476.2393).

Lungshengenin G (6): amorphous powder; $[\alpha]^{27}_D$ −80.4° (*c* 0.34, MeOH); UV ($CHCl_3$) λ_{max} (log ϵ) 237 (3.87) nm; IR (KBr) ν_{max} 3502 (br), 2992, 2955, 2886, 1750−1695 (br), 1648, 1437, 1372, 1245, 1961, 1035, 970, 950 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 4.70 (1H, d, J = 9.2 Hz, H-1β), 5.58 (1H, dd, J = 9.2, 2.3 Hz, H-2α), 5.28 (1H, d, J = 2.3 Hz, H-3α), 3.22 (1H, s, H-5β), 3.54 (1H, d, J = 12.5 Hz, H-7β), 2.42 (1H, d, J = 12.5 Hz, H-7α), 2.72 (1H, s, H-9β), 6.97 (1H, t, J = 3.2 Hz, H-11α), 2.05 (2H, overlapped, H-12α and H-12β), 2.91 (1H, br s, H-13α), 2.42 (1H, d, J = 12.1 Hz, H-14α), 1.53 (1H, overlapped, H-14β), 5.99 (1H, s, H-17a), 5.24 (1H, s, H-17b), 1.06 (3H, s, Me-18), 1.56 (3H, s, Me-19), 1.45 (3H, s, Me-20), 2.00, 1.82, 1.78 (each 3H, s, 3 × Ac); ^{13}C NMR, see Table 1; EIMS *m*/*z* 490 [M]$^+$ (13), 472 (2), 447 (18), 430 (16), 412 (3), 388 (6), 370 (20), 342 (25), 328 (21), 310 (33), 295 (15), 282 (12), 215 (100); HREIMS *m*/*z* 490.2222 (calcd for $C_{26}H_{34}O_9$, 490.2203).

Lungshengenin A (7): ^{1}H NMR (C_5D_5N, 500 MHz) δ 4.23 (1H, d, J = 10.0 Hz, H-1β), 5.69 (1H, dd, J = 10.0, 2.2 Hz, H-2α), 5.35 (1H, d, J = 2.2 Hz, H-3α), 1.69 (1H, s, H-5β), 4.64

(1H, br s, H-6β), 2.44 (1H, d, J = 13.8 Hz, H-7β), 1.73 (1H, d, J = 13.8 Hz, H-7α), 2.20 (1H, s, H-9β), 6.97 (1H, d, J = 3.4 Hz, H-11α), 2.14−2.22 (2H, overlapped, H-12α and H-12β), 2.98 (1H, br s, H-13α), 3.09 (1H, d, J = 12.6 Hz, H-14α), 1.63 (1H, overlapped, H-14β), 5.98 (1H, s, H-17a), 5.23 (1H, s, H-17b), 1.05 (3H, s, Me-18), 1.61 (3H, s, Me-19), 1.97 (3H, s, Me-20), 2.09, 1.92, 1.78 (each 3H, s, 3 × Ac); ^{13}C NMR (C_5D_5N, 125 MHz) δ 78.2 (d, C-1), 72.7 (d, C-2), 79.0 (d, C-3), 38.4 (s, C-4), 49.1 (d, C-5), 66.0 (d, C-6), 43.2 (t, C-7), 49.8 (s, C-8), 60.6 (d, C-9), 45.3 (s, C-10), 72.1 (d, C-11), 39.0 (t, C-12), 37.9 (d, C-13), 38.6 (t, C-14), 209.4 (s, C-15), 151.2 (s, C-16), 111.9 (t, C-17), 28.6 (q, C-18), 23.6 (q, C-19), 16.5 (q, C-20), 171.0 (s, OAc), 170.9 (s, OAc), 169.9 (s, OAc), 21.6 (q, OAc), 21.1 (q, OAc), 20.9 (q, OAc).

Inflexin (8): ^{1}H NMR (C_5D_5N, 500 MHz) δ 4.54 (1H, dd, J = 11.1, 4.7 Hz, H-1β), 2.24 (2H, overlapped, H-2α and H-2β), 4.81 (1H, t, J = 2.5 Hz, H-3α), 3.11 (1H, s, H-5β), 3.52 (1H, d, J = 12.6 Hz, H-7β), 2.42 (1H, d, J = 12.6 Hz, H-7α), 2.65 (1H, s, H-9β), 6.98 (1H, d, J = 4.0 Hz, H-11α), 2.18 (1H, overlapped, H-12α), 2.09 (1H, overlapped, H-12β), 2.91 (1H, br s, H-13α), 2.20 (1H, overlapped, H-14α), 1.49 (1H, overlapped, H-14β), 6.00 (1H, s, H-17a), 5.24 (1H, s, H-17b), 1.06 (3H, s, Me-18), 1.45 (3H, s, Me-19), 1.36 (3H, s, Me-20), 1.90, 1.80 (each 3H, s, 2 × Ac); ^{13}C NMR (C_5D_5N, 125 MHz) δ 74.2 (d, C-1), 33.3 (t, C-2), 78.7 (d, C-3), 36.2 (s, C-4), 59.3 (d, C-5), 210.6 (s, C-6), 50.5 (t, C-7), 54.8 (s, C-8), 60.0 (d, C-9), 50.8 (s, C-10), 70.9 (d, C-11), 37.5 (t, C-12), 37.0 (d, C-13), 36.8 (t, C-14), 205.1 (s, C-15), 150.0 (s, C-16), 113.4 (t, C-17), 26.6 (q, C-18), 22.0 (q, C-19), 15.7 (q, C-20), 170.2 (s, OAc), 169.5 (s, OAc), 21.4 (q, OAc), 20.9 (q, OAc).

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