Cytotoxic Constituents of *Isodon rubescens* var. *lushiensis*

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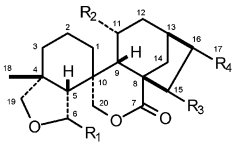
Five new *ent*-kaurane diterpenoids, ludongnins F−J (**1**−**5**), along with 10 known compounds, guidongnins A−C (**6**−**8**), angustifolin (**9**), 6-epiangustifolin (**10**), sculponeatin J (**11**), gardenin D, 5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone, pedalitin, and quercetin, were isolated from the leaves of *Isodon rubescens* var. *lushiensis*. The structures of **1**−**5** were determined by spectroscopic analysis, as well as X-ray crystallographic analysis of **1**. Compounds **1**−**11** were evaluated against K562 leukemia cells for their cytotoxic effects.

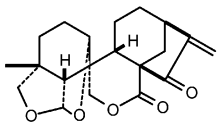
Isodon rubescens (Hemsl.) Hara (Labiatae), a perennial herb of the genus *Isodon* that is notable for being abundant in *ent*-kaurane diterpenoids,[1] is well-known in the People's Republic of China as a folk antitumor medicine. In 1977, rubescensins A and B (oridonin and ponicidin), which were isolated from *I. rubescens* as the major constituents,[2] were successfully developed in mainland China into a drug product used in treating sore throats and inflammation,[3] and since then, much attention has been focused on *I. rubescens* as a natural drug resource. Many phytochemical studies on *I. rubescens* and its varieties have been carried out, leading to the isolation of more than 20 *ent*-kaurane diterpenoids.[4] Among them, oridonin and ponicidin, which belong to 7,20-epoxy type *ent*-kauranoids, were regarded as the main diterpene constituents of this plant. However, there have been only two 6,7-seco-7,20-olide-*ent*-kaurane diterpenoids (ludongnins A and B) reported from *I. rubescens* var. *lushiensis* Gao et Li.[4] Our previous reinvestigation on the constituents of *I. rubescens* var. *lushiensis* yielded seven 6,7-*seco*-1,7-olide-*ent*-kauranoids besides ludongnins A and B (**12** and **13**).[5] The further search for additional bioactive substances from this plant revealed five new diterpenoids, ludongnins F−J (**1**−**5**), together with six known *ent*-kauranoids (**6**−**11**) and four known flavones. Diterpenoids **1**−**13** all possess a 6,7-*seco*-7,20-olide-*ent*-kaurane skeleton and were evaluated for their cytotoxicity against the K562 human leukemia cell line. In this paper, we report the structure elucidation of compounds **1**−**5** and the results of their cytotoxicity evaluation.

The 70% aqueous acetone extract of the leaves of *I. rubescens* var. *lushiensis* was partitioned successively between petroleum ether and water, then ethyl acetate and water. The ethyl acetate extract yielded ludongnins F−J (**1**−**5**), guidongnins A−C (**6**−**8**),[6,7] angustifolin (**9**),[8,9] 6-epiangustifolin (**10**),[8] sculponeatin J (**11**),[10] gardenin D,[11] 5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone,[12] pedalitin,[13] and quercetin[14] by repeated silica gel column chromatography, recrystallization, and preparative TLC.

Ludongnin F (**1**) was obtained as colorless cube crystals, with a molecular formula of $C_{21}H_{30}O_5$ indicated by the molecular ion peak at *m*/*z* 362.2090 in its HREIMS. The



	R_1	R_2	R_3	R_4
1	α-OCH_3	H	= O	β-CH_3
2	α-OCH_3	H	= O	α-CH_3
3	β-OCH_3	H	= O	α-CH_3
4	β-OCH_3	H	= O	β-CH_3
5	α-OCH_3	H	= O	= CH_2
6	= O	OH	β-OH	= CH_2
7	= O	H	= O	β-CH_3
8	= O	OH	= O	β-CH_3
9	β-OCH_3	OH	= O	= CH_2
10	α-OCH_3	OH	= O	= CH_2
12	= O	OH	= O	= CH_2
13	= O	H	= O	α-CH_3



11

^{13}C and DEPT NMR spectra of **1** exhibited 20 carbon signals besides a methoxy group, which were indicated to be composed of a ketone carbonyl carbon, a lactone carbonyl carbon, an acetal carbon, three quaternary carbons, four methines, eight methylenes including two oxygenated ones, and two methyls, suggesting **1** to be a functionalized diterpenoid. Considering the structures of the diterpenoids previously isolated from this plant, along with the characteristic lactone carbonyl signal at δ_C 172.4 (s) due to C-7 and two noticeable oxymethylenes [one appeared at δ_C 81.8 (t) and δ_H 3.66/3.54 (d, J = 8.0 Hz), attributable to C-19/H-19, and the other resonated at δ_C 71.6 (t) and δ_H 4.19/4.17 (d, J = 11.0 Hz), assignable to C-20/H-20], it was inferred that **1** is a 6,7-*seco*-7,20-olide-*ent*-kaurane diterpenoid similar to guidongnin B (**7**),[6] which was also isolated as one of the major constituents of this plant. By detailed comparison of the ^1H and ^{13}C NMR spectra of **1** with those of **7**, it was revealed that **1** was identical to **7** except for an acetal group at δ_C 107.3 (d) and δ_H 5.17 (d, J = 4.7 Hz) due to C-6/H-6 of **1**, which was linked to the methoxy group and replaced by a lactone group at δ_C 175.0 (s) in **7**. This deduction was supported by the $^1H-^{13}C$ long-range cor-

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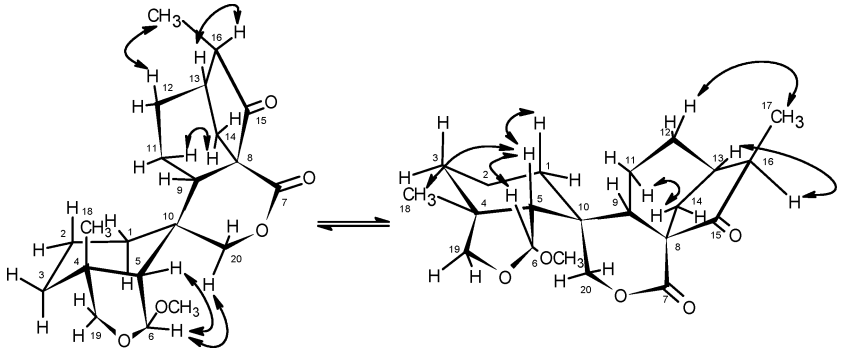

Figure 1. Configuration and key ROESY correlations of compound **1**.

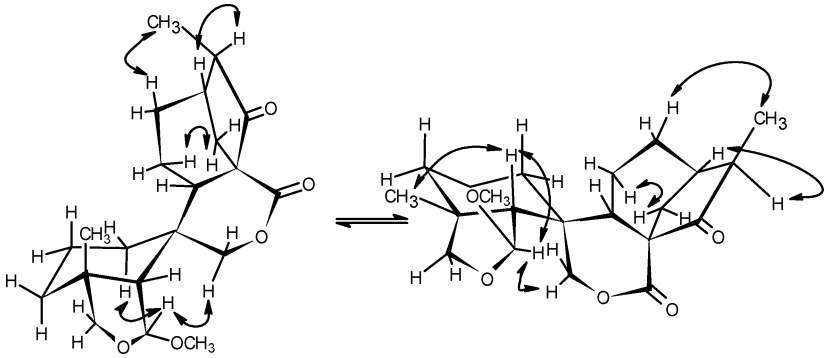

Figure 2. Configuration and key ROESY correlations of compound **4**.

relations of H-6 with C-19, C-10, and the methoxy carbon in the HMBC spectrum of **1**. Consequently, H-6 was suggested to be β-oriented by comparison of the NMR data of **1** and those of two analogues, angustifolin (**9**) and 6-epiangustifolin (**10**).[8]

However, there was no direct evidence to support the above suggestion in the ROESY spectrum of **1** (Figure 1), in which only three NOEs arising from H-6 (H-6/H-5β, H-6/H-20, and H-6/OCH_3) were evident. In fact, two conformations may exist in C_5D_5N for 6,7-*seco*-7,20-olide-*ent*-kaurane derivatives.[15,16] No matter what orientation H-6 took, there were always NOEs for H-6/H-5β and H-6/H-20 (Figures 1 and 2). Accordingly, the β-orientation of H-6 was confirmed by single-crystal X-ray analysis of **1** (Figure 3). Ludongnin F (**1**) was assigned therefore as (16*R*)-6α-methoxy-6,7-*seco*-6,19-epoxy-7,20-olide-*ent*-kaur-15-one.

Ludongnins F and G (**1** and **2**) were obtained initially as a mixture by silica gel column chromatography and then separated by recrystallization on the basis of their different crystal shapes (**1** as cubes and **2** as diamond-shaped crystals.). Both compounds have the same molecular formula, $C_{21}H_{30}O_5$, as determined by HREIMS. Analysis of their ^1H and ^{13}C NMR spectra (Table 1) disclosed that these two compounds were closely similar to one another, except for the configuration at C-16, indicating that **1** and **2** were C-16 epimers. Thus, ludongnin G was elucidated as (16*S*)-6α-methoxy-6,7-*seco*-6,19-epoxy-7,20-olide-*ent*-kaur-15-one (**2**) by the key NOE between Me-17 (δ_H 1.07) with H-13α (δ_H 1.97) observed in the ROESY spectrum of **2**.

Ludongnins H and I (**3** and **4**) were each found by HREIMS to possess the same molecular formula, $C_{21}H_{30}O_5$, the same as those of **1** and **2**. Detailed analysis of the NMR spectra of **3** and **4** made it clear that these two compounds, like **1** and **2**, were also C-16 epimers of each other. It was indicated further by the comparison of the NMR data of these two pairs that compounds **1** and **4** were epimeric at C-6, and a similar conclusion was made for compounds **2** and **3**. The above-mentioned inferences were supported by

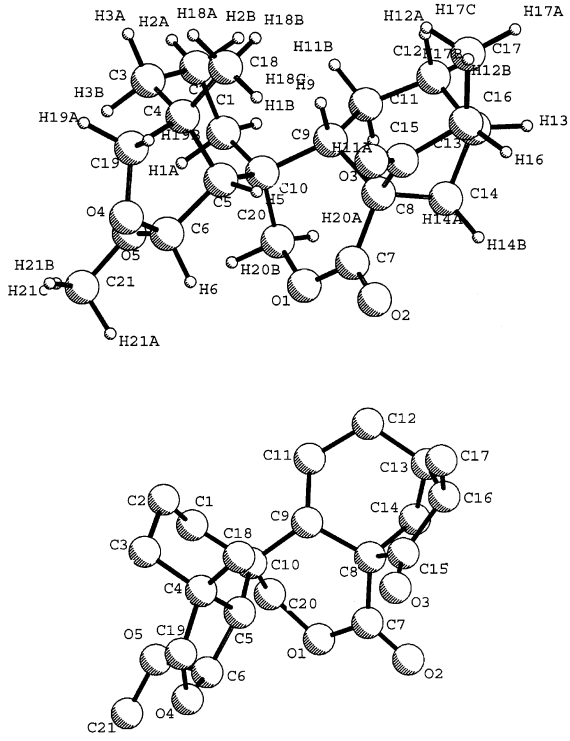

Figure 3. Crystal structure of compound **1**.

the ROESY spectra of **3** and **4**. The 6β-methoxy and 16α-methyl groups of **3** were confirmed by the NOEs between H-6α (δ_H 5.15) and H-1α (δ_H 1.50) and between Me-17 (δ_H 1.09) and H-13α (δ_H 2.01) in the ROESY spectrum of **3**. In turn, the 6β-methoxy and 16β-methyl groups of **4** were established on the basis of the ROESY interactions arising from H-6α (δ_H 5.17) with H-1α (δ_H 1.51), and H-16α (δ_H 2.45) with H-13α (δ_H 2.34) of **4** (Figure 2). Therefore, ludongnins H and I (**3** and **4**) were assigned as (16*S*)-6β-

Table 1. ^{13}C NMR Spectral Data of Compounds **1**−**5** (125 MHz, in C_5D_5N, δ in ppm

carbon	1	2	3	4	5
1	25.8 t	25.8 t	26.5 t	26.1 t	26.0 t
2	18.9 t	18.6 t	18.3 t	17.8 t	18.6 t
3	35.0 t	35.0 t	32.6 t	32.5 t	35.2 t
4	37.5 s	37.6 s	37.8 s	37.6 s	38.7 s
5	50.5 d	50.4 d	54.0 d	54.1 d	50.5 d
6	107.3 d	107.2 d	106.9 d	107.2 d	107.3 d
7	172.4 s	172.0 s	171.3 s	171.8 s	171.8 s
8	55.0 s	55.2 s	56.8 s	56.1 s	54.8 s
9	42.0 d	41.5 d	42.9 d	42.5 d	41.6 d
10	38.7 s	38.6 s	41.0 s	41.2 s	37.9 s
11	18.0 t	18.6 t	17.4 t	17.7 t	18.7 t
12	19.4 t	29.3 t	29.3 t	20.0 t	29.9 t
13	32.8 d	35.4 d	35.5 d	33.0 d	35.2 d
14	34.0 t	31.9 t	31.2 t	34.0 t	32.4 t
15	215.3 s	214.6 s	215.3 s	215.5 s	200.6 s
16	49.1 d	51.1 d	50.9 d	49.0 d	151.6 s
17	10.8 q	16.1 q	16.4 q	10.9 q	117.8 t
18	25.4 q	25.5 q	25.4 q	25.5 q	25.4 q
19	81.8 t	81.7 t	79.0 t	79.1 t	81.9 t
20	71.6 t	71.3 t	71.0 t	71.6 t	71.4 t
OMe	54.5 q	54.4 q	54.8 q	55.0 q	54.6 q

methoxy-6,7-*seco*-6,19-epoxy-7,20-olide-*ent*-kaur-15-one (**3**) and (16*R*)-6β-methoxy-6,7-*seco*-6,19-epoxy-7,20-olide-*ent*-kaur-15-one (**4**), respectively.

Ludongnin J (**5**) was also obtained as colorless cube crystals. The HREIMS of **5** exhibited a molecular ion peak at *m*/*z* 360.1922, in agreement with the molecular formula, $C_{21}H_{28}O_5$. Detailed comparison of the ^1H and ^{13}C NMR spectra of **5** with those of **1** indicated that compound **5** differed from **1** only at C-16/C-17. The methyl group at C-17 of **1** occurred as an exomethylene group in **5**. Therefore, on the basis of the HMBC correlations of H-17a (δ 6.07) with C-14, C-15, and C-16, and H-17b (δ 5.41) with C-14 and C-15, and the NOE between H-17a and H-13α of **5**, the structure of 6α-methoxy-6,7-*seco*-6,19-epoxy-7,20-olide-*ent*-kaur-16(17)-en-15-one was assigned for ludongnin J (**5**).

All the diterpenoids isolated (**1**−**13**) were evaluated for their inhibitory effects against K562 human leukemia cells using a previously described method.[17] Compounds **5**, **6**, and **9**−**12** exhibited significant inhibitory effects against K562 cells with IC_{50} values of 0.18, 0.30, 0.23, 0.87, 0.83, and 0.25 μg/mL, respectively, while the remaining compounds (**1**−**4**, **7**, and **8**) were inactive in this test system (IC_{50} > 50 μg/mL), which confirmed that the cyclopentanone conjugated with an exomethylene group was the active center of the inhibitory effect.[18] Compound **5** was also tested further for its cytotoxity against the CA liver cancer cell line and Hela uterine cervix cancer cells, with IC_{50} values of 0.09 and 0.70 μg/mL, respectively.[17]

Experimental Section

General Experimental Procedures. Melting points were obtained on an XRC-1 apparatus and are uncorrected. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were taken on a Shimadzu double-beam 210A spectrophotometer. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer as KBr pellets. 1D- and 2D-NMR spectra were run on Bruker AM-400 and DRX-500 instruments. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Mass spectra were recorded on a VG Auto Spec-3000 spectrometer. Column chromatography was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China) and silica gel H (10−40 μm, Qingdao Marine Chemical, Inc.). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The leaves of *I. rubescens* var. *lushiensis* Z. Y. Gao et Y. R. Li were collected in Lushi Prefecture, Henan Province, People's Republic of China, in August 2000. The plant material was identified by Prof. Zhong-Wen Lin at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (KIB-2000-10 Lin) was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The dried and powdered leaves (7.6 kg) were extracted with 70% Me_2CO (3 × 10 L) at room temperature for 72 h and filtered. The filtrate was evaporated, and the resulting residue was partitioned successively between H_2O and petroleum ether, then H_2O and EtOAc. The EtOAc extract (350 g) was applied to column chromatography over a silica gel (100−200 mesh, 3.0 kg) column, eluting with $CHCl_3$−Me_2CO (1:0, 9:1, 8:2, 7:3, and 0:1). The $CHCl_3$−Me_2CO (9:1) fraction (25 g) was chromatographed repeatedly over silica gel (cyclohexane−EtOAc, 10:1) to afford a mixture of **1** (11 mg) and **2** (8 mg), which was further separated by recrystallization in MeOH according to their different crystal shapes, as well as **3** (4 mg), **4** (3 mg), **5** (20 mg), and **6** (200 mg). The $CHCl_3$−Me_2CO (8:2) fraction (40 g) was chromatographed over silica gel (cyclohexane−EtOAc, 10:1) to obtain a mixture of several compounds, which was further chromatographed repeatedly over silica gel (CH_2Cl_2−2-propanol, 150:1) to afford **7** (16 mg), **8** (12 mg), gardenin **D** (1.0 g), and 5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone (400 mg). The $CHCl_3$−Me_2CO (7:3) fraction (23 g) was chromatographed on silica gel (CH_2Cl_2−2-propanol, from 150:1 to 25:1), then finally purified by preparative TLC (developed with CH_2Cl_2−2-propanol, 100:1, four times) and recrystallization to yield compounds **9** (22 mg), **10** (30 mg), **11** (17 mg), pedalitin (24 mg), and quercetin (20 mg).

Ludongnin F (1): colorless cube crystals (MeOH); mp 214−215 °C; $[\alpha]^{20}_D$ −281.9° (*c* 0.26, MeOH); IR ν_{max} 3446, 2954, 2929, 2871, 1754, 1715, 1460, 1270, 1045 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) δ 5.17 (1H, d, J = 4.7 Hz, H-6β), 4.19 and 4.17 (each 1H, d, J = 11.0 Hz, H_2-20), 3.66 and 3.54 (each 1H, d, J = 8.0 Hz, H_2-19), 3.24 (3H, s, OMe), 2.45 (1H, d, J = 12.0 Hz, H-14α), 2.42 (1H, m, H-16α), 2.35 (1H, m, H-13α), 2.05 (1H, d, J = 4.7 Hz, H-5β), 1.98 (1H, m, H-9β), 1.89 (1H, m, H-14β), 1.83 (1H, m, H-3α), 1.70 (1H, m, H-12α), 1.48 (1H, m, H-1α), 1.46 (1H, m, H-11α), 1.41 (1H, m, H-2α), 1.34 (1H, m, H-12β), 1.27 (1H, m, H-2β), 1.25 (1H, m, H-3β), 1.21 (1H, m, H-1β), 1.19 (1H, m, H-11β), 1.06 (3H, d, J = 6.5 Hz, Me-17), 1.05 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 125 MHz) spectral data, see Table 1; positive FABMS *m*/*z* 363 $[M + 1]^+$; EIMS *m*/*z* 362 $[M]^+$ (1), 346 (5), 331 (5), 302 (40), 284 (26), 274 (16), 136 (55), 122 (100); HREIMS *m*/*z* 362.2090 (calcd for $C_{21}H_{30}O_5$, 362.2093).

Ludongnin G (2): colorless diamond crystals (MeOH); mp 203−204 °C; $[\alpha]^{20}_D$ −183.6° (*c* 0.31, MeOH); IR ν_{max} 3445, 2955, 2929, 2870, 1755, 1716, 1478, 1370, 1113, 1043 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) δ 5.16 (1H, d, J = 4.8 Hz, H-6β), 4.20 and 4.16 (each 1H, d, J = 11.2 Hz, H_2-20), 3.66 and 3.53 (each 1H, d, J = 8.0 Hz, H_2-19), 3.22 (3H, s, OMe), 2.32 (1H, d, J = 11.4 Hz, H-14α), 2.18 (1H, m, H-16α), 2.03 (1H, d, J = 4.8 Hz, H-5β), 2.10 (3H, overlap, H-9β, H-12α, and H-14β), 1.97 (1H, m, H-13α), 1.81 (1H, m, H-3α), 1.52 (1H, m, H-1α), 1.47 (1H, m, H-11α), 1.42 (1H, m, H-2α), 1.30 (1H, m, H-3β), 1.28 (1H, m, H-2β), 1.24 (2H, overlap, H-1β and H-12β), 1.19 (1H, m, H-11β), 1.07 (3H, d, J = 8.0 Hz, Me-17), 1.04 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 125 MHz) spectral data, see Table 1; EIMS *m*/*z* 362 $[M]^+$ (1), 361 (3), 331 (5), 313 (38), 302 (40), 274 (60), 256 (30), 244 (60), 136 (65), 121 (100), 109 (72); HREIMS *m*/*z* 362.2092 (calcd for $C_{21}H_{30}O_5$, 362.2093).

Ludongnin H (3): white amorphous powder; $[\alpha]^{20}_D$ +2.7° (*c* 0.92, acetone); IR ν_{max} 3446, 2954, 2930, 2872, 1755, 1717, 1396, 1270, 1253, 1218, 1044, 973 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) δ 5.15 (1H, d, J = 3.2 Hz, H-6α), 4.38 and 4.19 (each 1H, d, J = 11.2 Hz, H_2-20), 3.64 and 3.48 (each 1H, d, J = 8.0 Hz, H_2-19), 3.36 (3H, s, OMe), 2.45 (1H, d, J = 12.0 Hz, H-14α), 2.18 (3H, overlap, H-5β, H-16α, and H-9β), 2.10 (1H, m, H-12α), 2.01 (1H, m, H-13α), 1.89 (1H, m, H-14β), 1.60 (1H, m, H-3α), 1.56 (1H, m, H-2α), 1.50 (1H, m, H-1α), 1.45 (2H, overlap, H-2β and H-11α), 1.24 (1H, m, H-12β), 1.30 (3H,

overlap, H-1β, H-3β, and H-11β), 1.13 (3H, s, Me-18), 1.09 (3H, d, J = 8.0 Hz, Me-17); ^{13}C NMR (C_5D_5N, 125 MHz) spectral data, see Table 1; EIMS m/z 362 $[M]^+$ (1), 331 (16), 313 (25), 302 (24), 284 (16), 274 (20), 272 (20), 245 (20), 136 (100), 122 (90), 107 (82), 93 (84); HREIMS m/z 362.2080 (calcd for $C_{21}H_{30}O_5$, 362.2093).

Ludongnin I (4): white amorphous powder; $[\alpha]^{20}_D$ −16.7° (c 0.12, acetone); IR ν_{max} 3444, 2952, 2928, 2868, 1754, 1716, 1394, 1272, 1248, 1215, 1046 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 5.17 (1H, d, J = 3.2 Hz, H-6α), 4.30 and 4.16 (each 1H, d, J = 11.2 Hz, H_2-20), 3.64 and 3.49 (each 1H, d, J = 8.0 Hz, H_2-19), 3.32 (3H, s, OMe), 2.45 (1H, m, H-16α), 2.43 (1H, d, J = 11.2 Hz, H-14α), 2.34 (1H, m, H-13α), 2.18 (1H, d, J = 3.2 Hz, H-5β), 1.96 (1H, m, H-9β), 1.90 (1H, m, H-14β), 1.74 (1H, m, H-12α), 1.58 (1H, m, H-3α), 1.54 (1H, m, H-2α), 1.51 (1H, m, H-1α), 1.47 (1H, m, H-11α), 1.42 (1H, m, H-2β), 1.34 (1H, m, H-12β), 1.30 (2H, overlap, H-1β and H-3β), 1.22 (1H, m, H-11β), 1.13 (3H, s, Me-18), 1.07 (3H, d, J = 7.2 Hz, Me-17); ^{13}C NMR (C_5D_5N, 125 MHz) spectral data, see Table 1; EIMS m/z 362 $[M]^+$ (1), 331 (8), 313 (9), 302 (14), 284 (6), 274 (8), 272 (8), 245 (6), 136 (55), 122 (70), 107 (62), 93 (72), 79 (100); HREIMS m/z 362.2111 (calcd for $C_{21}H_{30}O_5$, 362.2093).

Ludongnin J (5): colorless cube crystals (MeOH); mp 164−165 °C; $[\alpha]^{20}_D$ −233.9° (c 0.93, MeOH); IR ν_{max} 3442, 2949, 2930, 2875, 1753, 1715, 1645, 1394, 1340, 1270, 1193, 1117, 1035, 902 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 6.07 and 5.41 (each 1H, s, H_2-17), 5.19 (1H, d, J = 4.8 Hz, H-6β), 4.25 and 4.22 (each 1H, d, J = 8.5 Hz, H_2-20), 3.68 and 3.56 (each 1H, d, J = 10.0 Hz, H_2-19), 3.24 (3H, s, OMe), 2.93 (1H, m, H-13α), 2.47 (1H, d, J = 12.0 Hz, H-14α), 2.23 (1H, m, H-9β), 2.18 (1H, m, H-12α), 2.06 (1H, d, J = 4.8 Hz, H-5β), 1.98 (1H, dd, J = 4.0, 12.0 Hz, H-14β), 1.82 (1H, m, H-3α), 1.59 (1H, m, H-11α), 1.51 (1H, m, H-1α), 1.43 (1H, m, H-11β), 1.39 (1H, m, H-2α), 1.36 (1H, m, H-12β), 1.31 (1H, m, H-3β), 1.27 (1H, m, H-2β), 1.22 (1H, m, H-1β), 1.04 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 125 MHz) spectral data, see Table 1; EIMS m/z 360 $[M]^+$ (1), 345 (8), 328 (9), 316 (12), 300 (16), 286 (22), 270 (15), 136 (65), 121 (100), 107 (58), 93 (90), 79 (92); HREIMS m/z 360.1922 (calcd for $C_{21}H_{28}O_5$, 360.1937).

X-ray Diffraction of 1.[19] The mixture of **1** and **2** was recrystallized from MeOH, and **1** was separated from **2** with forceps and magnifier, on the basis of their different crystal shapes (**1** as cubes and **2** as diamond-shaped crystals.). The title compound is orthorhombic, space group $P2_12_12_1$, with unit cell parameters a = 10.077(4) Å, b = 11.598(3) Å, c = 16.125(6) Å, V = 1884.58(11) Å^3, Z = 4. A colorless crystal of $C_{21}H_{30}O_5$ having approximate dimensions 0.15 × 0.30 × 0.60 mm was mounted on a glass fiber. All measurements were made on a MAC DIP-2030K imaging plate area detector with graphite-monochromated Mo Kα radiation. The structure was solved by direct methods (SHELXS-86) and expanded using Fourier techniques. Cell constants and an orientation matrix for data collection corresponded to a primitive orthorhombic cell with dimensions a = 10.077(4) Å, b = 11.598(3) Å, c = 16.125(6) Å, V = 1884.58(11) Å^3, Z = 4. The calculated density is 1.278 g/cm^3. The space group was determined to be $P2_12_12_1$ (No. 19). A total of 1878 reflections were collected, and 1873 were observable and useful reflections ($|F|^2 \geq 3\sigma|F|^2$). The final R-factor is 0.057 with R_w = 0.055 ($w = 1/\sigma|F|^2$), S = 4.032, $\Delta\rho_{max}$ = 0.190 e Å^{-3}, and $\Delta\rho_{min}$ = −0.200 e Å^{-3}. The absolute configuration could not be determined reliably.

Bioassay of Diterpenoids 1−13. All the diterpenoids were evaluated for their cytotoxicity against K562 human leukemia cell line with the improved MTT method previously described,[17] using *cis*-platinum as the positive control (IC_{50} = 0.52 μg/mL). Compound **5** was also tested for its inhibitory effect on CA liver cancer and Hela uterine cervix cancer cells by the SRB method with *cis*-platinum being the positive control (IC_{50} = 0.88 and 3.60 μg/mL).[17] The OD data were recorded in X ± S, and the IC_{50} values were calculated by the software GWBASIC.

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(19) Crystallographic data for the structures reported in this paper have been deposited at the Cambridge Crystallographic Data Centre (deposition number CCDC 213983). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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