Cytotoxic *ent*-Kaurane Diterpenoids from *Isodon rubescens* var. *lushiensis*

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Received February 25, 2010

Ten new *ent*-kaurane diterpenoids, isolushinins A−J (**1**−**10**), together with 20 known compounds, were isolated from the aerial parts of *Isodon rubescens* var. *lushiensis*. The structures of **1**−**10** were elucidated by spectroscopic analysis. Several of the compounds isolated were evaluated for their cytotoxicity against the HL-60, SMMC-7721, A-549, SK-BR-3, and PANC-1 human cancer cell lines, and some exhibited quite potent inhibitory activities.

The genus *Isodon*, consisting of over 150 species, is the largest genus in the family Lamiaceae and has attracted considerable attention as a rich source of diterpenoids with diverse structures and interesting biological properties.[1] *Isodon rubescens* (Hemsl.) Hara, popularly known as "dong-ling-cao" in Henan Province of mainland China, is used for the treatment of sore throats and inflammation in folk medicine and was developed into a drug in 1977.[2] Pharmacological study has shown that oridonin and ponicidin, the main constituents of *I. rubescens*, have significant antiangiogenic activity.[3] Recently, these two compounds were found to be potent inhibitors of NF-κB transcription activity and the expression of its downstream targets, COX-2 and inducible nitric-oxide synthase.[4] Previous phytochemical investigations have demonstrated the secondary metabolites of this species to exhibit biological variation attributed to different ecological environments.[5] As a continuation of a search for further new bioactive constituents, a sample of this plant collected in Lushi County of Henan Province was investigated. Further study has led to the isolation of a series of *ent*-kaurane diterpenoids with miscellaneous carbon skeletons, including 7,20-epoxy-*ent*-kauranoids (**1**−**6**, **11**−**13**, **18**, **19**), C-20-oxygenated-*ent*-kauranoids (**7**−**10**, **14**, **15**), C-20-nonoxygenated-*ent*-kauranoids (**16**, **17**), and 6,7-*seco*-*ent*-kauranoids (**20**−**24**). The structures of isolushinins A−J (**1**−**10**) were established by spectroscopic evidence and by comparison with reported data. Most of these diterpenoids were tested for cytotoxicity against the HL-60, SMMC-7721, A-549, SK-BR-3, and PANC-1 human cancer cell lines, and compounds **4**, **8**, **11**, **12**, **13**, **14**, and **16** exhibited inhibitory activities.

Results and Discussion

The 70% aqueous acetone extract of the aerial parts of *I. rubescens* var. *lushiensis* was partitioned between ethyl acetate and water. The ethyl acetate extract was subjected repeatedly to column chromatography on silica gel, MCI gel, Sephadex LH-20, and RP-18 and then purified by HPLC to afford 10 new *ent*-kaurane diterpenoids, isolushinins A−J (**1**−**10**), and 20 known compounds, namely, (20*S*)-11β,14β,20-trihydroxy-7α,20-epoxy-*ent*-kaur-16-en-15-one (**11**),[6] (20*S*)-11β,14β-dihydroxy-20-methoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one (**12**),[6] (20*S*)-11β,14β-dihydroxy-20-ethoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one (**13**),[6] flexicanlin A (**14**),[7] kamebakaurinin (**15**),[8] phyllostachysin F (**16**),[9] phyllostachysin H (**17**),[9] oridonin (**18**),[10] enmenol (**19**),[11] ludongnin A (**20**),[12] ludongnin G (**21**),[5f] guidongnin E (**22**),[13] guidongnin F (**23**),[13] sculponeatin B (**24**),[14] 5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone,[15] (+)-1-hydroxypinoresinol,[16] oleanolic acid,[17] ursolic acid,[18] methyl rosmarinate,[19] and blumenol.[20]

Isolushinin A (**1**) was isolated as a white powder. HRESIMS analysis gave the molecular formula $C_{20}H_{28}O_3$ (*m*/*z* 339.1924 [M + Na]$^+$, calcd 339.1936), requiring seven degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra (Table 1) of **1** displayed 20 carbon signals corresponding to two singlet methyls, seven methylenes, seven methines (four oxygenated), and four quaternary carbons, consistent with a skeleton of an *ent*-kauranoid. Apart from an exocyclic double bond and rings A−D, two additional rings were necessary to satisfy the unsaturation of **1**. As shown in Figure 1, HMBC correlations of H-20 (δ_H 5.31) with C-1, C-3, C-5, and C-7, of H-7 (δ_H 3.94) with C-5, C-6, C-8, C-9, C-15, and C-20, of H-3 (δ_H 3.44) with C-2 and C-20, and of H$_3$-18 and H$_3$-19 with C-3 (δ_C 77.8) suggested that C-20 is connected with C-3 and C-7 through oxygen, respectively, forming two six-membered rings between C-20 and C-3 with C-7. The hydroxy group at C-15 was evident from the chemical shift value of C-15 (δ_C 74.4, d), as well as HMBC correlations of H-15 with C-14 and C-16. The β-orientation of the C-15 hydroxy group was determined by the upfield shift of C-9 (δ_C 48.3) in **1**, which was caused by a γ-gauche steric compression effect between HO-15 and H-9β.[1b] ROESY correlations of H-15 with H-7 and H-13 confirmed the above deduction. Therefore, **1** was determined as 15β-hydroxy-20,3;20,7-diepoxy-*ent*-kaur-16-ene.

Isolushinin B (**2**) exhibited the molecular formula $C_{22}H_{32}O_6$, as determined by its HRESIMS [M + Na]$^+$ ion at *m*/*z* 415.2096, indicating seven degrees of unsaturation. Typical ^{13}C NMR spectroscopic signals, such as a hemiketal quaternary carbon (δ_C 97.4, C-7), an oxygenated methylene (δ_C 66.3, C-20), and an exocyclic double bond [δ_C 162.1 (s), C-16; 107.3 (t), C-17], suggested that the gross structure of **2** is 7-hydroxy-7,20-epoxy-kaur-16-ene. As shown in Figure 2, HMBC correlations of H$_2$-19 with C=O (δ_C 170.9), of H-15 with C-9 and C-16, and of H-6 with C-5, C-7, C-8, and C-10, combined with ^{1}H−^{1}H COSY correlations of H-5/H-6/HO-6 and H-15/HO-15, suggested an acetoxy group is located at C-19 and two hydroxy groups occur at C-6 and C-15, respectively. The significant downfield chemical shift of C-5 (δ_C 59.2; δ_H 1.80, d, J = 5.8 Hz) along with H-19 gave a correlation with H-6 in the ROESY spectrum and indicated that HO-6 adopts a β-orientation. The upfield shift of C-9 (δ_C 42.1) and ROESY correlations of H-15 with H-13 suggested a β-oriented HO-15. Thus, the structure of **2** was elucidated as 6β,15β-dihydroxy-19-acetoxy-7α,20-epoxy-*ent*-kaur-16-ene.

The molecular formula of isolushinin C (**3**) was established as $C_{20}H_{30}O_5$ by HRESIMS (*m*/*z* 373.1974 [M + Na]$^+$, calcd 373.1990). The ^{1}H and ^{13}C NMR spectra of **3** were similar to those of **2** except that a C-18 hydroxy group was present, rather than a C-19 acetate, and a β-oriented hydroxy group was positioned at C-14 (δ_C 75.3)

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10.1021/np100110u
Published on Web 06/03/2010

Table 1. ^{13}C NMR Data of Compounds **1**−**10** (δ in ppm)

carbon	**1**[a,c]	**2**[b,e]	**3**[a,e]	**4**[a,c]	**5**[b,c]	**6**[a,e]	**7**[a,e]	**8**[b,c]	**9**[a,c,c]	**10**[a,d]
1	22.5, CH_2	35.9, CH_2	31.7, CH_2	29.0, CH_2	25.6, CH_2	29.6, CH_2	37.3, CH_2	34.3, CH_2	33.4, CH_2	34.2, CH_2
2	23.1, CH_2	18.7, CH_2	18.8, CH_2	18.2, CH_2	18.4, CH_2	28.1, CH_2	19.6, CH_2	17.8, CH_2	17.5, CH_2	18.9, CH_2
3	77.8, CH	30.7, CH_2	35.7, CH_2	40.5, CH_2	40.7, CH_2	77.9, CH	41.7, CH_2	34.5, CH_2	34.2, CH_2	42.3, CH_2
4	35.8, C	36.2, C	35.1, C	34.0, C	34.0, C	40.6, C	33.4, C	37.3, C	37.7, C	34.2, C
5	41.4, CH	59.2, CH	42.6, CH	48.3, CH	48.1, CH	49.2, CH	53.4, CH	45.7, CH	44.9, CH	52.9, CH
6	24.8, CH_2	73.5, CH	33.5, CH_2	24.5, CH_2	29.4, CH_2	25.2, CH_2	30.1, CH_2	27.8, CH_2	27.7, CH_2	27.8, CH_2
7	69.1, CH	97.4, C	99.3, C	66.2, CH	69.4, CH	67.2, CH	75.1, CH	74.5, CH	74.0, CH	71.8, CH
8	50.9, C	52.3, C	53.7, C	57.5, C	52.7, C	58.5, C	59.8, C	61.5, C	58.9, C	61.2, C
9	48.3, CH	42.1, CH	45.0, CH	52.5, CH	46.1, CH	57.6, CH	67.0, CH	53.6, CH	64.6, CH	63.2, CH
10	34.5, C	37.5, C	39.1, C	39.8, C	39.1, C	40.5, C	40.4, C	41.2, C	41.1, C	43.2, C
11	16.8, CH_2	15.6, CH_2	15.1, CH_2	68.2, CH	68.3, CH	64.6, CH	70.3, CH	17.7, CH_2	65.2, CH	68.6, CH
12	41.7, CH_2	32.7, CH_2	32.9, CH_2	37.8, CH_2	39.9, CH_2	43.3, CH_2	78.7, CH	30.0, CH_2	37.2, CH_2	78.8, CH
13	46.0, CH	36.9, CH	45.6, CH	41.8, CH	43.8, CH	44.0, CH	54.3, CH	46.2, CH	44.9, CH	51.1, CH
14	31.3, CH_2	27.0, CH_2	75.3, CH	71.6, CH	73.6, CH	70.8, CH	71.6, CH	76.1, CH	76.2, CH	67.7, CH
15	74.4, CH	75.2, CH	73.2, CH	204.9, C	74.3, CH	206.7, C	208.1, C	208.6, C	208.3, C	209.1, C
16	156.4, C	162.1, C	163.6, C	150.3, C	160.3, C	153.9, C	147.8, C	147.3, C	148.4, C	145.7, C
17	107.7, CH_2	107.3, CH_2	109.1, CH_2	118.0, CH_2	110.0, CH_2	115.9, CH_2	115.9, CH_2	118.6, CH_2	116.2, CH_2	115.5, CH_2
18	30.4, CH_3	27.2, CH_3	71.3, CH_2	32.7, CH_3	32.8, CH_3	28.9, CH_3	33.5, CH_3	70.7, CH_2	70.0, CH_2	34.2, CH_3
19	26.4, CH_3	66.7, CH_2	17.3, CH_3	20.5, CH_3	21.0, CH_3	15.6, CH_3	21.3, CH_3	18.5, CH_3	18.6, CH_3	22.9, CH_3
20	94.8, CH	66.3, CH_2	67.0, CH_2	101.2, CH	101.7, CH	101.9, CH	67.4, CH_2	64.0, CH_2	63.6, CH_2	61.2, CH_2
OAc		170.9, C		170.0, C	170.3, C		170.6, C	170.8, C	170.8, C	
		20.7, CH_3		21.4, CH_3	21.6, CH_3		21.3, CH_3	21.2, CH_3	21.0, CH_3	
OMe				55.9, CH_3	55.8, CH_3	55.4, CH_3				

[a] Recorded at 125 MHz. [b] Recorded at 100 MHz. [c] Recorded in $CDCl_3$. [d] Recorded in $(CD_3)_2CO$. [e] Recorded in C_5D_5N. [f] Recorded in MeOD.

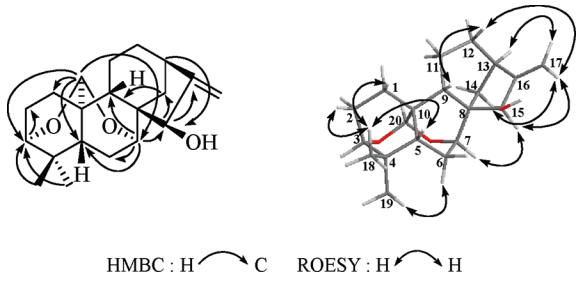


Figure 1. Key HMBC and ROESY correlations of **1**.

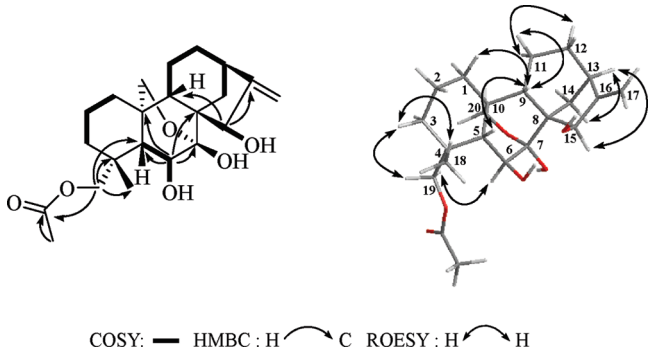


Figure 2. Key COSY, HMBC, and ROESY correlations of **2**.

instead of at C-6 in **2**. These proposals were confirmed by the correlations of H_2-18 with H-5, and of H-11α with H-14α, in the ROESY experiment. Accordingly, **3** was assigned as 14β,15β,18-trihydroxy-7α,20-epoxy-*ent*-kaur-16-ene.

Isolushinin D (**4**) was obtained as a white, amorphous powder and found to exhibit a molecular formula of $C_{23}H_{32}O_6$, as deduced from its HRESIMS at *m*/*z* 427.2088 $[M + Na]^+$ (calcd 427.2096). Analysis of the ^1H and ^{13}C NMR and HSQC spectra revealed that **4** possesses five quaternary carbons (one α,β-unsaturated carbonyl and one OAc), seven methines (four oxygenated), six methylenes, three methyls, and one methoxy group. The IR absorptions indicated the presence of OH (3440 cm^{-1}), C=O (1727 cm^{-1}), and C=C (1641 cm^{-1}) groups. The NMR spectroscopic features of **4** were similar to those of the known compound **12** and differed only in the appearance of a β-oriented AcO-11, which was confirmed by the chemical shift value of C-11 (δ_C 68.2) and the HMBC correlation of H-11 (δ_H 5.65) with C=O (δ_C 170.0), as well as the ROESY correlation of H-11α with H-14α. On considering that all the substituents have the same orientations as those in **12**, compound **4** was established as (20*S*)-14β-hydroxy-11β-acetoxy-20-methoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one.

Both isolushinins E (**5**) and F (**6**) were isolated as white, amorphous powders. Their molecular formulas, $C_{23}H_{34}O_6$ and $C_{21}H_{30}O_6$, were determined by HRESIMS (*m*/*z* 429.2234 $[M + Na]^+$ and 401.1939 $[M + Na]^+$, respectively). Comparison of the spectroscopic data of **5** and **6** with those of **4** disclosed that the main structural differences between these compounds are in the substituent group patterns. The ^{13}C NMR spectra of **5** and **6** were quite similar to those of **4**, except that the carbonyl is reduced to a β-oriented C-15 hydroxy group in **5**, and there is an α-oriented C-3 hydroxy group and a C-11β hydroxy group in **6**. The upfield chemical shift of C-15 (δ_C 74.3) and the ROESY correlation of H-15 with H-7 implied the C-15 hydroxy group possesses a β-orientation in **5**. The C-9 (δ_C 46.1) upfield shift was supported by the γ-steric compression effect between H-9 and HO-15. However, an α-oriented C-3 hydroxy group in **6** was deduced from the observation of HMBC correlations of H-3 with C-4, Me-18, and Me-19 and ROESY correlations of H-3 with H_3-18 and H-5. By further ROESY NMR spectroscopic comparison, the orientations of all substituents in **5** and **6** were found to be identical to those of **4**. Consequently, these two compounds were elucidated as (20*S*)-14β,15β-dihydroxy-11β-acetoxy-20-methoxy-7α,20-epoxy-*ent*-kaur-16-ene (**5**) and (20*S*)-3α,11β,14β-trihydroxy-20-methoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one, respectively (**6**).

Isolushinin G (**7**), obtained as a pale yellow, amorphous powder, was assigned with the molecular formula $C_{22}H_{32}O_7$, as determined by its HRESIMS data, *m*/*z* 431.2040 $[M + Na]^+$ (calcd 431.2045). The NMR spectra of **7** resembled those of the known compound **14**, except for the presence of an α-oriented C-12 hydroxy group. HMBC correlations of H-12 (δ_H 4.72) with C-9 (δ_C 67.0), C-11 (δ_C 70.3), and C-13 (δ_C 54.3) as well as the correlation of H-17a with H-12β and the absence of correlations of H-12 with H-11α and H-14α observed in the ROESY spectrum indicated the C-12 hydroxy group to be α-oriented. Further ROESY correlations demonstrated the relative configurations of the other stereocenters to be consistent with those of **14**. Thus, the structure of compound **7** was established as 7α,11β,12α,14β-tetrahydroxy-20-acetoxy-*ent*-kaur-16-en-15-one.

Table 2. Cytotoxic Activities of Compounds **1**, **2**, **4**, **6**−**8**, **11**−**21**, and **24** against Selected Tumor Cell Lines[a]

compound	HL-60	SMMC-7721	A-549	SK-BR-3	PANC-1
4	1.1	4.7	6.4	6.4	7.0
8	2.5	>10	>10	6.1	6.6
11	1.2	6.2	5.9	4.4	8.6
12	0.95	4.4	5.6	3.3	9.8
13	0.64	3.5	4.2	2.4	5.2
14	0.64	9.9	9.8	>10	5.7
15	1.8	>10	>10	>10	>10
16	0.85	5.5	3.3	3.3	4.5
17	6.4	>10	>10	>10	>10
18	3.9	>10	>10	>10	>10
19	6.7	>10	>10	>10	>10
20	3.1	>10	>10	>10	>10
cisplatin	1.3	>10	>10	>10	>10
paclitaxel	<0.008	<0.008	1.4	0.11	<0.008

[a] Results are expressed as IC_{50} values in μM. Cell lines: HL-60 acute leukemia; SMMC-7721 liver cancer; A-549 lung cancer; SK-BR-3 colon cancer; PANC-1 pancreatic cancer. Compounds **1**, **2**, **6**, **7**, **21**, and **24** were inactive for all cell lines (IC_{50} > 10 μM).

Comparison of the UV, IR, and NMR data of compounds **8**−**10** with those of related compounds disclosed that the main structural differences between them were the positions of the functional groups. Isolushinin H (**8**) was obtained as a white, amorphous powder and assigned a molecular formula of $C_{22}H_{32}O_6$, established from its HRESIMS at *m/z* 415.2078 $[M + Na]^+$ (calcd 415.2096) and NMR data. When compared with **14**, the 1D and 2D NMR spectra of **8** showed a β-oriented hydroxy group located at C-18 instead of at C-11, which was confirmed by the HMBC correlations of H_2-18 with C-3, C-4, C-5, and Me-19 and ROESY correlations of H_2-18 with H-5. The NMR spectra of **9** were quite similar to those of **14** except that Me-18 is oxygenated, which was verified by the downfield chemical shift of C-18 (δ_C 70.0) and a ROESY correlation of H_2-18 with H-5. Similarly, the NMR spectra of **10** were very close to those of **15**. The only difference was the appearance of an α-oriented C-12 hydroxy group, which was clarified by the correlations from H-12 (δ_H 3.89) to C-9 (δ_C 63.2), C-11 (δ_C 68.6), and C-13 (δ_C 51.1) in the HMBC experiment. The relative configurations of **10** and **7** were identical, according to the ROESY correlations observed. Since all the other relative configurations of the stereocenters in these three compounds were the same as those in **14**, isolushinins H−J were assigned as 7α,14β,18-trihydroxy-20-acetoxy-*ent*-kaur-16-en-15-one (**8**), 7α,11β,14β,18-tetrahydroxy-20-acetoxy-*ent*-kaur-16-en-15-one (**9**), and 7α,11β,12α,14β,20-pentahydroxy-*ent*-kaur-16-en-15-one (**10**), respectively.

Due to the limited amounts available, only some of the compounds obtained were assayed for their cytotoxicity against five human tumor cell lines (HL-60, SMMC-7721, A-549, SK-BR-3, PANC-1) by the MTT method (Table 2).[21] Compounds **4**, **8**, **11**, **12**, **13**, **14**, and **16** exhibited more potent cytotoxicity than oridonin (**18**) against all five cell lines. The results suggested that a cyclopentanone conjugated with an exomethylene group is necessary for more potent activity among the *ent*-kauranoids tested and that an ethoxy substituent increases activity more than hydroxy and methoxy substituents at C-20.[22] In addition, it was of interest that **7**, which possesses an α,β-unsaturated carbonyl group, was inactive in these bioassays (IC_{50} > 10 μM), and this result was similar to that observed for parvifoline P.[23]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectropolarimeter. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. NMR spectra were recorded on a Bruker AM-400 and a DRX-500 spectrometer with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. Mass spectra were performed on an API QSTAR time-of-flight spectrometer. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 (9.4 mm ×25 cm) column. Column chromatography was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40−63 μm, Merck, Darmstadt, Germany), and MCI gel CHP 20P (75−150 μm, Mitsubishi Chemical Corp., Tokyo, Japan). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

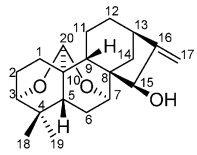
Plant Material. The aerial parts of *I. rubescens* var. *lushiensis* were collected in Lushi County, Henan Province, People's Republic of China, in August 2006. The sample was identified by Prof. Xi-Wen Li, and a voucher specimen (KIB 200608014) has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered aerial parts of *I. rubescens* var. *lushiensis* (7.5 kg) were extracted with 70% aqueous acetone (20 L × 3) at room temperature overnight and filtered, with the filtrate evaporated under reduced pressure and partitioned successively with EtOAc (3 L × 3). The EtOAc extract (200 g) was applied to silica gel (200−300 mesh, 1.2 kg) column, eluting with a $CHCl_3$−Me_2CO gradient system (1:0 to 0:1), to give five fractions, A−E. The separation of fraction A (70 g) by repeated silica gel chromatography, eluted with petroleum ether−EtOAc (10:1 to 1:1), yielded 5,3′,4′-trihydroxy-6,7,8- trimethoxyflavone (20 mg), oleanolic acid (10 mg), ursolic acid (8 mg), and blumenol (5 mg). Fraction B (33 g) was decolorized on MCI gel, eluted with 90% MeOH−H_2O, to yield a brown gum. The gum (27 g) was applied to a Sephadex LH-20 column, using $CHCl_3$−MeOH (1:1) as eluant, to afford two fractions, B1 and B2. Fraction B1 (21 g) was then chromatographed over RP-18 (20%−80% MeOH−H_2O gradient system) to obtain four fractions, B11−B14. Fractions B12 and B13 were subjected to silica gel column chromatography, eluted with petroleum ether−EtOAc (9:1 to 1:1), then crystallized from MeOH, and compounds **11** (310 mg), **12** (400 mg), and **13** (220 mg) were obtained. The mother liquor was further chromatographed over a silica gel column repeatedly and then purified by semipreparative HPLC, eluting with 50% MeOH−H_2O, to yield compounds **4** (5 mg), **5** (1.5 mg), and **6** (3 mg). Fractions B11 and 14 were separated over normal-phase silica gel (petroleum ether−EtOAc, 10:1−1:1, gradient system), and further repeated purification by semipreparative HPLC with 60% MeOH−H_2O afforded compounds **1** (5 mg), **8** (9 mg), **14** (300 mg), **16** (5 mg), **20** (10 mg), **21** (8 mg), **22** (1.5 mg), and **23** (1 mg). Fraction C (5.2 g) was applied to RP-18 (30%−80% MeOH−H_2O), then purified by silica gel (petroleum ether−acetone, 6:1−1:1) to give compounds **3** (1 mg), **15** (200 mg), **17** (4 mg), **18** (230 mg), and **19** (3 mg). Fraction D (3.1 g) was passaged over a Sephadex LH-20 column, eluting with MeOH, and the diterpenoid portion was chromatographed over silica gel, then further purified by semipreparative HPLC to obtain **2** (12 mg), **7** (5 mg), **9** (1.5 mg), **10** (1 mg), and **24** (4 mg). Fraction E (30 g) was purified by repeated silica gel chromatography, eluted with $CHCl_3$−MeOH (10:1−5:1), to yield (+)-1-hydroxypinoresinol (8 mg) and methyl rosmarinate (20 mg).

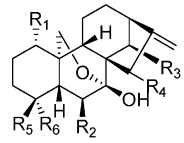
Isolushinin A (1): white powder; $[\alpha]_D^{27}$ −26.6 (*c* 0.29, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.33) nm; IR (KBr) ν_{max} 3432, 2951, 2871, 1736, 1657, 1446, 1386, 1364, 1253, 1056, 1004, 914, 877, 785 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.31 (1H, s, H-20), 5.04 (1H, s, H-17a), 4.88 (1H, s, H-17b), 4.12 (1H, s, H-15α), 3.94 (1H, d, J = 4.9 Hz, H-7β), 3.44 (1H, m, H-3β), 2.55 (1H, d, J = 9.5 Hz, H-13α), 2.38 (1H, overlap, H-12α), 2.32 (1H, overlap, H-14β), 2.32 (1H, overlap, H-6α), 1.86 (1H, overlap, H-12β), 1.81 (1H, overlap, H-2β), 1.70 (1H, m, H-2α), 1.57 (1H, overlap, H-9β), 1.57 (1H, overlap, H-6β), 1.57 (1H, overlap, H-1α), 1.46 (1H, m, H-14α), 1.39 (1H, m, H-5β), 1.28 (2H, overlap, H_2-11), 1.28 (1H, overlap, H-1β), 0.94 (3H, s, Me-19), 0.87 (3H, s, Me-18); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; positive ESIMS *m/z* 339 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 339.1924 (calcd 339.1936).

Isolushinin B (2): white powder; $[\alpha]_D^{26}$ −68.0 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.56) nm; IR (KBr) ν_{max} 3394, 3221, 2935, 2879, 1739, 1659, 1629, 1503, 1464, 1398, 1374, 1337, 1235, 1161, 1118, 1064, 1038, 1029, 986, 874, 766 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.46 (s, OH-6), 6.89 (s, OH-15), 5.48 (1H, s, H-17a), 5.21 (1H, s, H-17b), 5.16 (1H, s, H-15α), 4.69 (1H, d, J = 11.1 Hz, H-19a), 4.47 (1H, d, J = 11.1 Hz, H-19b), 4.38 (1H, brs, H-6), 4.05 (2H, brs, H_2-20), 2.65 (1H, m, H-13α), 2.41 (1H, m, H-9β), 2.11 (1H, m, H-12α), 2.08 (1H, m, H-14β), 1.95 (3H, s, H_3-21), 1.87 (1H, overlap, H-14α),

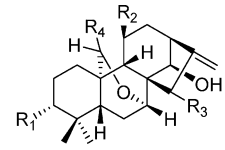
Chart 1



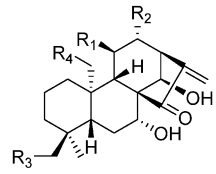
1



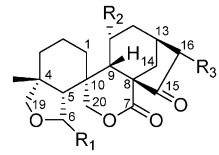
	R_1	R_2	R_3	R_4	R_5	R_6
2	H	OH	H	β-OH	CH_3	CH_2OAc
3	H	H	OH	β-OH	CH_2OH	CH_3
18	OH	OH	OH	=O	CH_3	CH_3
19	OH	OH	H	β-OH	CH_3	CH_3



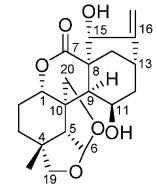
	R_1	R_2	R_3	R_4
4	H	OAc	=O	OMe
5	H	OAc	β-OH	OMe
6	OH	OH	=O	OMe
11	H	OH	=O	OH
12	H	OH	=O	OMe
13	H	OH	=O	OEt



	R_1	R_2	R_3	R_4
7	OH	OH	H	OAc
8	H	H	OH	OAc
9	OH	H	OH	OAc
10	OH	OH	H	OH
14	OH	H	H	OAc
15	OH	H	H	OH
16	OH	H	H	H
17	OH	OH	H	H



	R_1	R_2	R_3
20	=O	OH	$=CH_2$
21	OMe	H	α-CH_3
22	α-OH	H	β-CH_3
23	β-OH	H	β-CH_3



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1.84 (1H, overlap, H-1α), 1.80 (1H, d, J = 5.8 Hz, H-5α), 1.54 (1H, m, H-12β), 1.43 (1H, m, H-11α), 1.32 (2H, overlap, H_2-2), 1.32 (1H, overlap, H-3α), 1.32 (3H, s, Me-18), 1.22 (1H, m, H-11β), 0.98 (1H, m, H-1β), 0.95 (1H, m, H-3β); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive ESIMS m/z 415 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 415.2096 (calcd 415.2096).

Isolushinin C (3): yellow powder; $[\alpha]_D^{27}$ −17.5 (c 0.37, C_5H_5N); UV (MeOH) λ_{max} (log ε) 205 (3.43) nm; IR (KBr) ν_{max} 3384, 3316, 2950, 2921, 2867, 1731, 1631, 1449, 1387, 1199, 1050, 895, 739 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 5.69 (1H, s, H-15α), 5.66 (1H, s, H-17a), 5.35 (1H, s, H-17b), 4.95 (1H, s, H-14α), 4.41 (1H, d, J = 8.6 Hz, H-20a), 4.09 (1H, d, J = 8.6 Hz, H-20b), 3.52 (1H, d, J = 10.5 Hz, H-18a), 3.39 (1H, d, J = 10.5 Hz, H-18b), 3.11 (1H, t, J = 12.3 Hz, H-6β), 2.81 (1H, d, J = 8.8 Hz, H-13α), 2.50 (1H, m, H-9β), 2.30 (1H, overlap, H-5α), 2.28 (1H, overlap, H-12α), 2.11 (1H, m, H-6α), 1.58 (1H, overlap, H-12β), 1.57 (2H, overlap, H_2-3), 1.48 (1H, m, H-11α), 1.36 (2H, brs, H_2-2), 1.36 (3H, s, Me-19), 1.18 (1H, d, J = 9.6 Hz, H-1α), 1.05 (1H, m, H-11β), 0.84 (1H, m, H-1β);^{13}C NMR (C_5D_5N, 125 MHz), see Table 1; positive ESIMS m/z 373 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 373.1974 (calcd 373.1990).

Isolushinin D (4): white, amorphous powder; $[\alpha]_D^{25}$ −5.5 (c 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 238 (3.50) nm; IR (KBr) ν_{max} 3440, 2951, 2933, 2872, 1727, 1641, 1631, 1450, 1366, 1254, 1244, 1105, 1075, 1030, 936, 877, 793 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.06 (1H, s, H-17a), 5.65 (1H, m, H-11α), 5.64 (1H, s, H-17b), 5.07 (1H, s, H-20), 4.84 (1H, s, H-14α), 4.11 (1H, m, H-7β), 3.41 (3H, s, OMe-20), 3.00 (1H, m, H-12α), 2.95 (1H, s, H-13α), 2.78 (1H, t, J = 12.9 Hz, H-6β), 1.95 (3H, s, Me-22), 1.75 (1H, overlap, H-1α), 1.73 (1H, overlap, H-6α), 1.66 (1H, d, J = 9.1 Hz, H-9β), 1.48 (1H, d, J = 12.6 Hz, H-3β), 1.40 (2H, brs, H_2-2), 1.32 (1H, m, H-5β), 1.31 (1H, m, H-12β), 1.09 (1H, overlap, H-3α), 1.07 (1H, overlap, H-1β), 0.95 (3H, s, Me-19), 0.86 (3H, s, Me-18); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; positive ESIMS m/z 427 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 427.2088 (calcd 427.2096).

Isolushinin E (5): white, amorphous powder; $[\alpha]_D^{26}$ +13.1 (c 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.43) nm; IR (KBr) ν_{max} 3436, 2950, 2931, 2871, 1729, 1633, 1449, 1366, 1257, 1102, 1029, 964, 877 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.57 (1H, m, H-11α), 5.24 (1H, s, H-17a), 5.22 (1H, s, H-17b), 5.06 (1H, s, H-20), 4.52 (1H, s, H-14α), 4.39 (1H, brd, J = 5.9 Hz, H-15β), 4.09 (1H, brd, J = 3.3 Hz, H-7β), 3.39 (3H, s, OMe-20), 2.89 (1H, m, H-12α), 2.56 (1H, d, J = 8.2 Hz, H-13α), 2.07 (1H, t, J = 12.8 Hz, H-6β), 1.99 (1H, d, J = 9.0 Hz, H-9β), 1.97 (3H, s, Me-22), 1.69 (1H, brs, H-1α), 1.67 (1H, m, H-6α), 1.58 (1H, m, 3β), 1.42 (2H, brs, H_2-2), 1.32 (1H, m, H-5β), 1.25 (1H, m, H-12β), 1.13 (1H, overlap, H-1β), 1.07 (1H, overlap, H-3α), 0.95 (3H, s, Me-19), 0.84 (3H, s, Me-18); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive ESIMS m/z 429 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 429.2234 (calcd 429.2253).

Isolushinin F (6): white, amorphous powder; $[\alpha]_D^{26}$ −47.1 (c 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.41) nm; IR (KBr) ν_{max} 3423, 2954, 2936, 2873, 1723, 1643, 1447, 1366, 1268, 1194, 1054, 1030, 937, 876 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.24 (1H, s, H-17a), 5.48 (1H, s, H-20), 5.42 (1H, s, H-17b), 5.25 (1H, s, H-14α), 5.17 (1H, m, H-11α), 4.81 (1H, brd, J = 3.8 Hz, H-7β), 3.57 (1H, m, H-3β), 3.43 (3H, s, OMe-20), 3.28 (1H, overlap, H-13α), 3.26 (1H, overlap, H-6β), 3.01 (1H, m, H-12α), 2.63 (1H, m, H-1α), 2.17 (1H, m, H-1β), 2.02 (1H, m, H-6α), 1.80−2.00 (2H, overlap, H_2-2), 1.80−2.00 (1H, overlap, H-12β), 1.80−2.00 (1H, overlap, H-9β), 1.65 (1H, m, H-5β), 1.29 (3H, s, Me-19), 1.15 (3H, s, Me-18); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; positive ESIMS m/z 401 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 401.1939 (calcd 401.1940).

Isolushinin G (7): pale yellow, amorphous powder; $[\alpha]_D^{26}$ −50.3 (c 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 238 (3.39) nm; IR (KBr) ν_{max} 3432, 2929, 2874, 1725, 1630, 1460, 1376, 1254, 1166, 1089, 1075, 938, 877, 793 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 6.40 (1H, s, H-17a), 6.12 (1H, s, H-14α), 5.50 (1H, s, H-17b), 5.05 (1H, d, J = 12.8 Hz, H-20a), 5.01 (1H, m, H-7β), 4.90 (1H, d, J = 12.8 Hz, H-20b), 4.72 (1H, brs, H-12β), 4.44 (1H, s, H-11α), 3.75 (1H, s, H-13α), 2.62 (1H, m, H-6α), 2.25 (1H, m, H-6β), 2.24 (1H, s, H-9β), 2.20 (3H, s, Me-22), 1.96 (1H, overlap, H-1α), 1.56 (1H, m, H-2α), 1.33 (1H, m, H-3α), 1.20−1.30 (1H, overlap, H-2β), 1.20−1.30 (1H, overlap, H-5β), 1.00−1.10 (1H, overlap, H-3β), 1.00−1.10 (1H, overlap, H-1β), 0.89 (3H, s, Me-19), 0.84 (3H, s, Me-18); ^{13}C NMR (C_5D_5N, 125 MHz), see Table 1; positive ESIMS m/z 431 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ m/z 431.2040 (calcd 431.2045).

Isolushinin H (8): white, amorphous powder; $[\alpha]_D^{26}$ −88.6 (c 0.22, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.47) nm; IR (KBr) ν_{max} 3407, 2935, 2873, 1733, 1648, 1459, 1375, 1237, 1154, 1095, 1037, 939, 798 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.22 (1H, s, H-17a), 5.47 (1H, s, H-17b), 4.79 (1H, s, H-14α), 4.70 (1H, d, J = 12.7 Hz, H-20a), 4.58 (1H, m, H-7β), 4.31 (1H, d, J = 12.7 Hz, H-20b), 3.57 (1H, d, J = 11.2 Hz, H-18a), 3.05 (1H, overlap, H-18b), 3.05 (1H, overlap, H-13α), 2.13 (3H, s, Me-22), 2.05 (1H, brs, H-12α), 2.00 (1H, m, H-1α), 1.90 (1H, m, H-6β), 1.79 (1H, overlap, H-6α), 1.70−1.80 (1H,

overlap, H-2α), 1.70−1.80 (1H, overlap, H-12β), 1.50−1.70 (1H, overlap, H-5β), 1.50−1.70 (1H, overlap, H-3β), 1.50−1.70 (1H, overlap, H-2β), 1.50−1.70 (1H, overlap, H-11β), 1.50−1.60 (1H, overlap, H-9β), 1.40 (1H, m, H-11α), 1.27 (1H, m, H-3α), 0.83 (3H, s, Me-19), 0.70 (1H, m, H-1β); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive ESIMS *m/z* 415 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 415.2078 (calcd 415.2096).

Isolushinin I (9): yellow powder; $[\alpha]_D^{26}$ −80.5 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 232 (3.48) nm; IR (KBr) ν_{max} 3420, 2930, 2873, 1730, 1640, 1460, 1375, 1247, 1160, 1091, 1037, 939, 793 cm^{-1}; ^{1}H NMR ($CDCl_3$+MeOD, 500 MHz) δ 5.99 (1H, s, H-17a), 5.37 (1H, s, H-17b), 4.78 (1H, d, *J* = 12.6 Hz, H-20a), 4.66 (1H, s, H-14α), 4.36 (1H, m, H-7α), 4.18 (1H, overlap, H-20b), 4.17−4.21 (1H, overlap, H-9β), 3.43 (1H, d, *J* = 11.4 Hz, H-18a), 2.93 (1H, s, H-13α), 2.82 (1H, d, *J* = 11.4 Hz, H-18b), 2.37 (1H, m, H-12α), 2.07 (3H, s, Me-22), 2.00 (1H, m, H-1α), 1.91(1H, m, H-12β), 1.77 (1H, m, H-6β), 1.67 (1H, overlap, H-11α), 1.62 (1H, overlap, H-5β), 1.50−1.62 (1H, overlap, H-6α), 1.50−1.62 (1H, overlap, H-3β), 1.50 (2H, overlap, H_2-2), 1.18 (1H, m, H-3α), 1.02 (1H, m, H-1β), 0.76 (3H, s, Me-19); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; positive ESIMS *m/z* 431 $[M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 431.2014 (calcd for 431.1998).

Isolushinin J (10): yellow powder; $[\alpha]_D^{26}$ −53.2 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 238 (3.40) nm; IR (KBr) ν_{max} 3407, 1735, 1650, 1461, 1376, 1239, 1153, 1096, 1037, 937, 796, 630 cm^{-1}; ^{1}H NMR (acetone-d_6, 500 MHz) δ 5.92 (1H, s, H-17a), 5.27 (1H, s, H-17b), 5.04 (1H, s, H-14α), 4.25 (1H, s, H-11α), 4.20 (1H, m, H-7β), 3.96 (2H, brs, H_2-20), 3.10 (2H, s, H-13α), 2.41 (1H, m, H-1α), 1.90 (1H, m, H-6β), 1.79 (1H, m, H-6α), 1.54 (1H, m, H-2α), 1.42 (1H, overlap, H-3β), 1.41 (1H, overlap, H-9β), 1.40 (1H, overlap, H-2β), 1.20 (1H, m, H-3α), 0.88 (3H, s, Me-18), 0.87 (3H, s, H-5β), 0.88 (3H, overlap, Me-19), 0.71 (1H, m, H-1β); ^{13}C NMR (acetone-d_6, 125 MHz), see Table 1; positive ESIMS *m/z* 389 $[M + Na]^+$, positive HRESIMS $[M + Na]^+$ *m/z* 389.2078 (calcd 389.2096).

Cytotoxicity Assay. The following human tumor cell lines were used: HL-60, SMMC-7721, A-549, SK-BR-3, and PANC-1. All the 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).[21] Briefly, 100 μL of adherent cells was seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 1×10^5 cells/mL in 100 μL of medium. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with cisplatin and paclitaxel (Sigma) as positive controls. After the incubation, MTT (100 μg) was added to each well, and the incubation 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 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.[21]

Acknowledgment. This work was supported financially by the NSFC (Nos. 30772637 to H.-D.S. and 30830119 to S.-J.C.), the NSFC-Joint Foundation of Yunnan Province (No. U0832602 to H.-D.S.), the Major State Basic Research Development Program of China (Nos. 2009CB-522300 and 2009CB940900), the Natural Science Foundation of Yunnan Province (No. 2008CD162), and the Key Project of Knowledge Innovation Project of CAS (KSCX2-YW-R-25).

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Fujita, E.; Node, M. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Tamm, Ch., Eds.; Springer: Vienna, 1984; Vol. 46, pp 77−157. (b) Sun, H. D.; Xu, Y. L.; Jiang, B. *Diterpenoids from Isodon Species*; Science Press: Beijing, 2001. (c) Sun, H. D.; Huang, S. X.; Han, Q. B. *Nat. Prod. Rep.* **2006**, *23*, 673–698.
(2) *The Pharmacopoeia of the People's Republic of China*; People's Health Press: Beijing, 1977; p 186.
(3) (a) Henan Institute of Medical Science, Henan Medical College, Yunnan Institute of Botany, and Zhengzhou Chemicopharmaceutical Plant. *Chin. Sci. Bull.* **1978**, *23*, 53−58. (b) Meade-Tollin, L. C.; Wijeratne, E. M. K.; Cooper, D.; Guild, M.; Jon, E.; Fritz, A.; Zhou, G. X.; Whitesell, L.; Liang, J. Y.; Gunatilaka, A. A. L. *J. Nat. Prod.* **2004**, *67*, 2–4.
(4) Leung, C. H.; Grill, S. P.; Lam, W.; Han, Q. B.; Sun, H. D.; Cheng, Y. C. *Mol. Pharmacol.* **2005**, *68*, 286–297.
(5) (a) Han, Q. B.; Li, S. H.; Peng, L. Y.; Sun, H. D. *Heterocycles* **2003**, *60*, 933–938. (b) Han, Q. B.; Zhang, J. X.; Zhao, A. H.; Sun, H. D.; Lu, Y.; Wu, Y. S.; Zheng, Q. T. *Tetrahedron* **2004**, *60*, 2373–2377. (c) Han, Q. B.; Lu, Y.; Zhang, L. L.; Zheng, Q. T.; Sun, H. D. *Tetrahedron Lett.* **2004**, *45*, 2833–2837. (d) Han, Q. B.; Jiang, B.; Zhang, J. X.; Niu, X. M.; Sun, H. D. *Helv. Chim. Acta* **2003**, *86*, 773–777. (e) Han, Q. B.; Li, R. T.; Zhang, J. X.; Sun, H. D. *Helv. Chim. Acta* **2004**, *87*, 1119–1124. (f) Han, Q. B.; Zhao, A. H.; Zhang, J. X.; Lu, Y.; Zhang, L. L.; Zheng, Q. T.; Sun, H. D. *J. Nat. Prod.* **2003**, *66*, 1391–1394. (g) Huang, S. X.; Xiao, W. L.; Li, L. M.; Li, S. H.; Zhou, Y.; Ding, L. S.; Lou, L. G.; Sun, H. D. *Org. Lett.* **2006**, *8*, 1157–1160.
(6) Liu, H. M.; Zhu, W. C.; Zhu, C. G.; Wang, Q. D.; Ke, Y.; Liu, J. Z. Z.; Yan, X. B.; Zhang, J. Y.; Qu, H. L. WO 2007/082475 A1, 2007, 07, 26.
(7) Zhang, H. J.; Sun, H. D. *Phytochemistry* **1989**, *28*, 3534–3536.
(8) Takeda, Y.; Ichihara, T.; Takaishi, Y.; Fujita, T.; Shingu, T.; Kusano, G. *J. Chem. Soc., Perkin Trans.* **1987**, 2403–2409.
(9) Li, X.; Xiao, W. L.; Ban, L. L.; Shen, Y. H.; Weng, Z. Y.; Li, S. H.; Sun, H. D. *Phytochemistry* **2006**, *67*, 1336–1340.
(10) Fujita, E.; Fujita, T.; Katayama, H.; Shibuya, M.; Shingu, T. *J. Chem. Soc. (C)* **1970**, *12*, 1674–1681.
(11) Mori, S.; Shudo, K.; Ageta, T.; Koizumi, T.; Okamoto, T. *Chem. Pharm. Bull.* **1970**, *18*, 884–889.
(12) Zheng, X. R.; Gao, Z. Y.; Sun, H. D.; Lin, Z. W. *Acta Bot. Yunnan.* **1984**, *6*, 316–320.
(13) Han, Q. B.; Zhao, Q. S.; Li, S. H.; Peng, L. Y.; Sun, H. D. *Acta Chim. Sin.* **2003**, *61*, 1077–1082.
(14) Sun, H. D.; Lin, Z. W.; Xu, Y. L.; Minami, Y.; Marunaka, T.; Togo, T.; Takeda, Y.; Fujita, T. *Heterocycles* **1986**, *24*, 1–4.
(15) Tomas, F.; Ferreres, F.; Guirado, A. *Phytochemistry* **1979**, *18*, 185–186.
(16) Tsukamoto, H.; Hisada, S.; Nishibe, S. *Chem. Pharm. Bull.* **1984**, *32*, 2730–2735.
(17) Yu, Z. H.; Li, G. P.; Zhao, J. F.; Mei, S. X.; Li, L. *Nat. Prod. Dev.* **2003**, *15*, 27–28.
(18) Hu, Y. J.; Lai, X. P.; Liu, Z. Q.; Zhu, Y. T.; Deng, X. L.; Liu, N.; Chen, J. N. *Chin. Tradit. Herb. Drugs* **2005**, *36*, 1612–1615.
(19) Zheng, X. K.; Li, Q.; Feng, W. S. *Chin. Pharm. J.* **2004**, *39*, 335–336.
(20) González, A. G.; Guillermo, J. A.; Ravelo, A. G.; Jimenez, J. A. *J. Nat. Prod.* **1994**, *57*, 400–402.
(21) Reed, L. J.; Muench, H. *Am. J. Hyg.* **1938**, *27*, 493–497.
(22) Huang, S. X.; Zhou, Y.; Pu, J. X.; Li, R. T.; Li, X.; Xiao, W. L.; Lou, L. G.; Han, Q. B.; Ding, L. S.; Peng, S. L.; Sun, H. D. *Tetrahedron* **2006**, *62*, 4941–4947.
(23) Li, L. M.; Li, G. Y.; Huang, S. X.; Xiao, W. L.; Liao, X.; Lou, L. G.; Ding, L. S.; Sun, H. D. *Planta Med.* **2006**, *72*, 1–6.

NP100110U