ent-Kaurane and *ent*-Pimarane Diterpenoids from *Siegesbeckia pubescens*

Rui Wang,[†] Wen-Hao Chen,[†,‡] and Yan-Ping Shi*[,†,‡]

State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, People's Republic of China, and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, People's Republic of China

Received September 9, 2009

Phytochemical investigation of the above-ground parts of *Siegesbeckia pubescens* yielded 25 diterpenoids, including five new *ent*-pimarane diterpenoids (**1**−**3**, **11**, and **12**) and four new *ent*-kaurane diterpenoids (**13**−**16**). The structures and relative configurations of the new compounds were elucidated by comprehensive spectroscopic analysis and by comparison of their NMR data with those of related compounds. Single-crystal X-ray diffraction analysis confirmed the structures of **8**, **13**, and **14**. Seven of these diterpenoids were evaluated for cytotoxic activity against HSC-T6, HeLa, and B16 cell lines. Compounds **11** and **14** exhibited moderate cytotoxicity.

Plants of genus *Siegesbeckia* are annual herbs widely distributed throughout tropical, subtropical, and temperate regions of the world. Three species of *Siegesbeckia* grow in China, and their aerial parts have been used as the traditional Chinese medicine Xi-Xian to treat rheumatic arthritis, hypertension, malaria, neurasthenia, and snakebite.[1] Extracts of *Siegesbeckia* have been reported to exihibit antioxidant,[2] antiallergic,[3] and anti-infertility effects.[4] Pharmacological studies have suggested that diterpenoids are the main bioactive constituents in the treatment of rheumatic arthritis.[5,6] A series of *ent*-pimarane[1,7−12] and *ent*-kaurane[1] diterpenoids from *Siegesbeckia* species have been reported.

The medicinal importance and diverse activities of members of this genus prompted us to undertake further phytochemical investigation of *Siegesbeckia pubescens* Makino (Compositae) to identify new diterpenoid constituents and to evaluate their cytotoxic activities. As a result, five new *ent*-pimarane diterpenoids (**1**−**3**, **11**, and **12**), four new *ent*-kaurane diterpenoids (**13**−**16**), and 16 known diterpenoids were isolated from the dry aerial parts of *S. pubescens*. Structures of the new compounds were established by comprehensive spectroscopic analysis and by comparison of their NMR data with those of related compounds in the literature. The structures of **8**, **13**, and **14** were confirmed by single-crystal X-ray diffraction analysis. Cytotoxic evaluation of seven of the 25 diterpenoids showed that compounds **11** and **14** exhibited moderate activity against rat hepatic stellate cells (HSC-T6), human cervical carcinoma (HeLa), and mouse melanoma B16 cell lines.

Results and Discussion

Compound **1** was obtained as a white, amorphous powder. The IR spectrum showed the presence of OH groups (3422 cm^{-1}) and a double bond (1657 cm^{-1}). The molecular formula $C_{20}H_{34}O_4$ was deduced from its HRESIMS and ^{13}C NMR data. The ^1H NMR spectrum clearly showed four methyl groups on quaternary carbons (δ 0.66, 0.69, 0.79, 0.87, each 3H, s), a trisubstituted olefinic proton [δ 5.31 (1H, s)], one oxygenated methylene [δ 3.43 (1H, dd, J = 9.6, 3.6 Hz) and 3.23 (1H, m)], and three oxygenated methines [δ 3.95 (1H, d, J = 2.4 Hz), 3.28 (1H, m), and 3.04 (1H, m)]. The ^{13}C NMR (Table 1) and DEPT spectra indicated 20 carbon signals, including four methyl, six methylene (one oxygenated, at δ 62.6), six methine (three oxygenated, at δ 71.1, 75.2, 77.1, and one olefinic, at δ 131.9), and four quaternary carbons (one olefinic, at δ 139.8). The NMR data of **1** were similar to those of **6**[13] except for the chemical shifts of ring B atoms. Compared to the related *ent*-pimarane-type diterpenoid (**6**), CH_2-7 in **6** was replaced by an oxygenated methine at δ 71.1 in **1**, suggesting that an OH group was located at C-7. In its HMBC spectrum (Figure 1), this conclusion was supported by the cross-peak between H-7 (δ 3.95) and C-5, C-8, and C-14. The coupling constant of H-7 (J = 2.4 Hz) suggested that the 7-OH was β-oriented. Furthermore, the relative configuration at C-7 was deduced by a NOE experiment (Figure 1), which showed NOE enhancement at δ 4.28 (7-OH) when irradiating H-9β at δ 1.96. Therefore, compound **1** was determined to be *ent*-3α,7β,15,16-tetrahydroxypimar-8(14)-ene.

Compound **2** displayed a quasi molecular ion peak at m/z 576.3390 $[M + NH_4]^+$ consistent with the molecular formula

Table 1. ^{13}C NMR Spectroscopic Data (δ) of Compounds **1**−**3**, **2a**, **11**, and **12**

no.	**1**[a,c]	**2**[b,d]	**2a**[b,d]	**3**[b,c]	**11**[a,e]	**12**[b,e]
1	36.7 (t)	38.0 (t)	37.2 (t)	48.1 (t)	37.1 (t)	36.9 (t)
2	27.5 (t)	23.7 (t)	23.8 (t)	62.5 (d)	27.5 (t)	23.7 (t)
3	77.1 (d)	85.1 (d)	84.8 (d)	46.8 (t)	79.0 (d)	85.7 (d)
4	38.2 (s)	38.3 (s)	38.4 (s)	44.3 (s)	38.1 (s)	37.9 (s)
5	45.9 (d)	50.3 (d)	48.1 (d)	54.3 (d)	54.1 (d)	54.7 (d)
6	29.7 (t)	23.7 (t)	26.0 (t)	23.8 (t)	22.1 (t)	22.2 (t)
7	71.1 (d)	129.5 (d)	85.8 (d)	36.0 (t)	36.0 (t)	35.9 (t)
8	139.8 (s)	134.3 (s)	136.3 (s)	136.6 (s)	139.6 (s)	139.4 (s)
9	45.3 (d)	47.6 (d)	47.2 (d)	49.3 (d)	50.1 (d)	50.1 (d)
10	37.7 (s)	35.4 (s)	38.3 (s)	40.1 (s)	38.9 (s)	38.3 (s)
11	17.4 (t)	19.3 (t)	18.4 (t)	18.0 (t)	18.2 (t)	18.3 (t)
12	31.6 (t)	29.1 (t)	31.8 (t)	31.8 (t)	32.0 (t)	32.0 (t)
13	37.1 (s)	41.1 (s)	38.2 (s)	36.9 (s)	35.8 (s)	35.8 (s)
14	131.9 (d)	90.9 (d)	136.3 (d)	129.1 (d)	126.7 (d)	126.9 (d)
15	75.2 (d)	68.5 (d)	73.0 (d)	75.4 (d)	79.8 (d)	79.8 (d)
16	62.6 (t)	66.3 (t)	66.6 (t)	62.6 (t)	65.4 (t)	65.3 (t)
17	22.3 (q)	18.1 (q)	22.4 (q)	22.5 (q)	22.4 (q)	22.5 (q)
18	28.3 (q)	28.9 (q)	28.5 (q)	28.7 (q)	28.4 (q)	28.6 (q)
19	16.1 (q)	16.7 (q)	16.6 (q)	178.4 (s)	15.7 (q)	16.7 (q)
20	13.9 (q)	15.0 (q)	14.2 (q)	14.3 (q)	14.9 (q)	14.9 (q)
1′		101.2 (d)	101.2 (d)			100.4 (d)
2′		74.4 (d)	74.5 (d)			73.5 (d)
3′		77.6 (d)	77.6 (d)			76.3 (d)
4′		71.5 (d)	71.5 (d)			70.3 (d)
5′		76.8 (d)	76.9 (d)			75.1 (d)
6′		62.8 (t)	62.8 (t)			62.3 (t)
CH_3CO		20.7	20.6			
		171.1	171.1			
$C(Me)_2$					25.2 (q)	25.2 (q)
					26.2 (q)	26.2 (q)
					108.5 (s)	108.5 (s)

[a] Measured at 75 MHz. [b] Measured at 100 MHz. [c] Spectra recorded in DMSO-d_6. [d] Spectra recorded in acetone-d_6. [e] Spectra recorded in $CDCl_3$.

* To whom correspondence should be addressed. Tel: +86-931-4968208. Fax: +86-931-8277088. E-mail: shiyp@lzb.ac.cn.
[†] Lanzhou University.
[‡] Lanzhou Institute of Chemical Physics.

10.1021/np9005579
Published on Web 11/23/2009

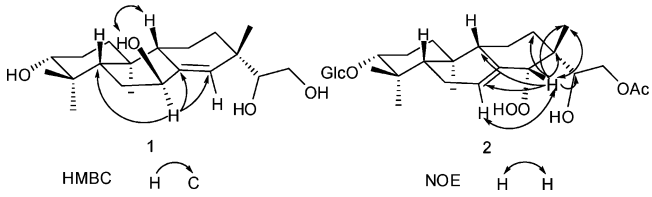
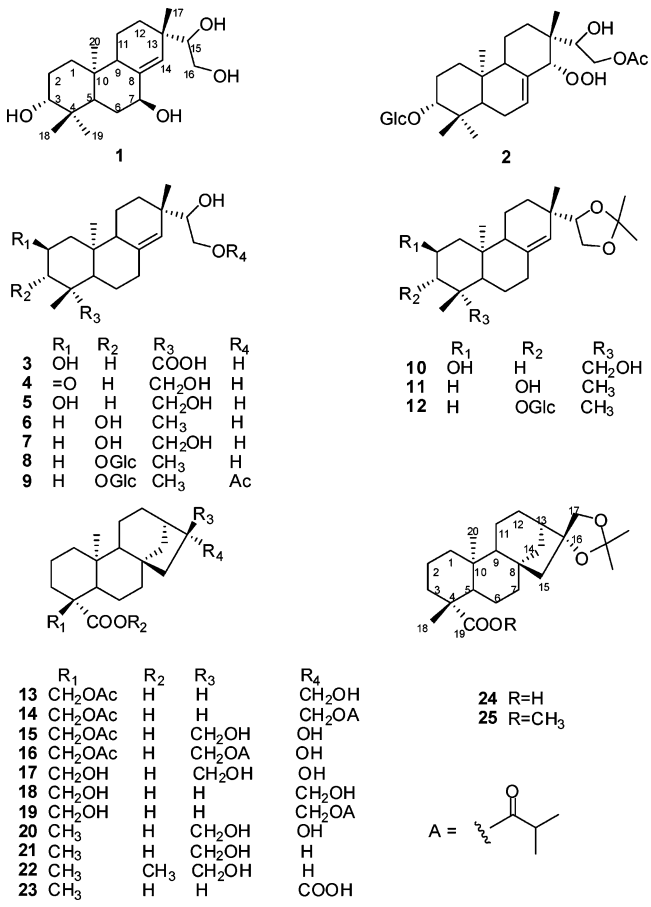


Figure 1. Selected HMBC and NOE correlations for **1** and **2**.



	R_1	R_2	R_3	R_4
3	OH	H	COOH	H
4	=O	H	CH_2OH	H
5	OH	H	CH_2OH	H
6	H	OH	CH_3	H
7	H	OH	CH_2OH	H
8	H	OGlc	CH_3	H
9	H	OGlc	CH_3	Ac

	R_1	R_2	R_3
10	OH	H	CH_2OH
11	H	OH	CH_3
12	H	OGlc	CH_3

	R_1	R_2	R_3	R_4
13	CH_2OAc	H	H	CH_2OH
14	CH_2OAc	H	H	CH_2OA
15	CH_2OAc	H	CH_2OH	OH
16	CH_2OAc	H	CH_2OA	OH
17	CH_2OH	H	CH_2OH	OH
18	CH_2OH	H	H	CH_2OH
19	CH_2OH	H	H	CH_2OA
20	CH_3	H	CH_2OH	OH
21	CH_3	H	CH_2OH	H
22	CH_3	CH_3	CH_2OH	H
23	CH_3	H	H	COOH

24 R=H
25 R=CH_3

A =

$C_{28}H_{64}O_{11}$, inferring the presence of six degrees of unsaturation. The ^{1}H NMR spectrum showed the presence of five methyl groups on quaternary carbons (δ 0.85, 0.88, 0.92, 1.00, 1.98, each 3H, s), one oxygenated methine (δ 3.88, 1H, s), one olefinic proton (δ 5.71, 1H, d, $J = 5.2$ Hz), and one anomeric proton (δ 4.37, 1H, d, $J = 7.6$ Hz). The ^{13}C NMR (Table 1) and DEPT spectra of **2** indicated the existence of five methyl, seven methylene (two oxygenated, at δ 62.8 and 66.3), 11 methine (three oxygenated, at δ 68.5, 85.1, and 90.9 in the aglycone, five oxygenated carbons in a sugar moiety, and one olefinic carbon at δ 129.5), and five quaternary carbons (one olefinic carbon at δ 134.3 and one carboxyl carbon at δ 171.1). The NMR spectra of **2** were similar to those of compound **8**,[9] whose structure was confirmed by X-ray crystallographic analysis (Figure 2), suggesting that **2** was also an *ent*-pimarane-type diterpenoid glycoside, and the degrees of unsaturation were consistent with the above analysis. The coupling constant of the anomeric proton at δ 4.37 (d, $J = 7.6$ Hz) and C-1′ signal at δ 101.2 indicated a β-glucopyranose. Compared with **8**, an extra oxygenated methine and an additional acetyl signal (δ_H 1.98, 3H, s; δ_C 177.1 and 20.7) were observed in **2**. The downfield shift of the oxymethine at δ 90.9 indicated **2** to be a hydroperoxylated compound, which was supported by analysis of its HRESIMS and ^{13}C NMR spectra. In the HMBC spectrum (Figure 1), correlation between the methyl at δ 1.98 and the oxygenated methylene at δ 66.3 (C-16) showed that the OAc group was located at C-16, and the correlations between H-14 and C-7, C-9, C-12, C-13, C-15, and C-17 confirmed that the hydroperoxy group was at C-14. HMBC cross-peaks between the olefinic CH-7 at δ 4.37 and C-5, C-6, C-9, and C-14 indicated that **2** possessed a double bond between C-7 and C-8. The relative configuration of 14-OOH was elucidated by an NOE experiment (Figure 1). On irradiating the proton H-14 at δ 3.88, significant NOE enhancements were obtained at δ 5.71 (H-7) and 0.92 (H_3-17), which showed that the 14-OOH was α-oriented. Thus, compound **2** was determined to be *ent*-16-acetoxy-3α,15-dihydroxy-14α-hydroperoxypimar-7-en-3α-*O*-β-glucopyranoside.

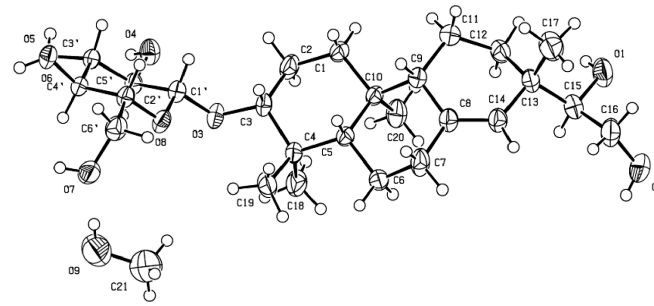


Figure 2. ORTEP drawing of **8**·CH_3OH.

The hydroperoxide (**2**) seemed to undergo an allylic rearrangement followed by a slower Smith epimerization[14,15] to another hydroperoxide (**2a**) (Scheme 1, Supporting Information) in acetone-d_6. Both types of rearrangement probably involve the corresponding allylperoxyl radicals. We proposed that the first rearrangement was nondissociative (Scheme 2, Supporting Information) and perhaps involved a pericyclic process with a transition state, but the epimerization would require a dissociative mechanism (Scheme 3, Supporting Information) involving free allyl radicals and oxygen.[16,17]

The main differences between the NMR spectra of **2a** and those of **2** were the locations of the double bond and the hydroperoxy groups. In the HMBC spectrum of **2a**, the olefinic proton was correlated with C-7, C-9, C-12, C-13, and C-17, indicating that the double bond was located between C-8 and C-14; the cross-peak between H-7 and C-5, C-9, and C-14 suggested that the hydroperoxy group was at C-7. The smaller coupling constant of H-7 (δ 4.24, br s), which was similar to that of **1**, indicated that the 7-OOH was β-oriented. Moreover, the sugar moieties in **2** and **2a** were confirmed to be β-glucose by an acid hydrolysis experiment of **2a**. Accordingly, compound **2a** was established as *ent*-16-acetoxy-3α,15-dihydroxy-7β-hydroperoxypimar-8(14)-en-3α-*O*-β-glucopyranoside.

Compound **3** was obtained as a white, amorphous powder. The IR spectrum indicated the presence of OH (3516 and 3413 cm^{-1}) and carboxyl (1713 cm^{-1}) groups. The molecular formula was deduced as $C_{20}H_{32}O_5$ from HRESIMS and ^{13}C NMR (Table 1) data. Comparison of the NMR spectra of **3** with those of **5**[10] showed that they were very similar expect for an additional carboxylic group (δ_H 12.11, 1H, s; δ_C 178.4, s), the absence of the oxygenated CH_2-19 (δ_C 40.7) in **5**, and the clearly downfield shifts of the C-4 signal from δ 40.6 in **5** to δ 44.3 in **3**. These observations indicated that the carboxylic group was at C-19, and this conclusion was supported by the HMBC spectrum. Therefore, compound **3** was established as *ent*-2β,15,16-trihydroxypimar-8(14)-en-19-oic acid.

Compound **11** was obtained as a pale gum. Its IR spectrum exhibited absorption bands at 3312 cm^{-1} (OH) and 1651 cm^{-1} (double bond). The HRESIMS of **11** gave a quasi-molecular ion peak at *m*/*z* 385.2711 $[M + NH_4]^+$, which suggested the molecular formula $C_{23}H_{38}O_3$. Compared with compound **6**,[13] the presence of extra dioxygenated CMe_2 signals [δ_H 1.39 and 1.34 (each 3H, s); δ_C 108.5 (s), 26.2 (q), and 25.2 (q)] and the downfield-shifted carbon signals of C-15 at δ 79.8 and C-16 at δ 65.4 in **11** showed that **11** was the acetonide of **6**. This conclusion was reinforced in its HMBC spectrum by the correlation between the oxygenated quaternary

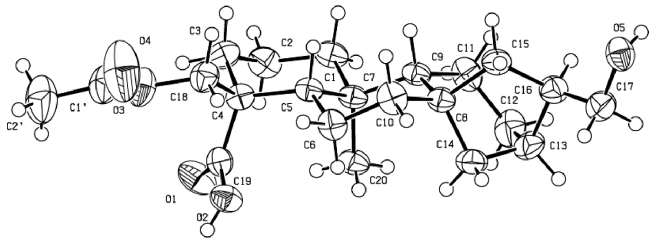


Figure 3. ORTEP drawing of **13**.

Table 2. ^{13}C NMR Spectroscopic Data (δ) of Compounds **13**−**16**

no.	**13**[a,c]	**14**[a,d]	**15**[b,c]	**16**[a,d]
1	40.3 (t)	40.0 (t)	39.7 (t)	39.9 (t)
2	18.4 (t)	18.2 (t)	18.0 (t)	18.1 (t)
3	32.2 (t)	32.2 (t)	31.9 (t)	32.7 (t)
4	46.9 (s)	47.4 (s)	46.6 (s)	47.4 (s)
5	51.2 (d)	52.2 (d)	50.8 (d)	52.0 (d)
6	22.4 (t)	22.2 (t)	21.8 (t)	22.0 (t)
7	41.1 (t)	41.0 (t)	41.4 (t)	41.2 (t)
8	44.2 (s)	44.5 (s)	43.7 (s)	44.4 (s)
9	55.0 (d)	55.2 (d)	55.3 (d)	55.6 (d)
10	39.1 (s)	39.3 (s)	38.9 (s)	39.4 (s)
11	18.7 (t)	18.6 (t)	18.1 (t)	18.4 (t)
12	31.3 (t)	31.1 (t)	25.8 (t)	26.1 (t)
13	37.7 (s)	38.4 (s)	44.5 (s)	45.8 (s)
14	36.8 (t)	37.0 (t)	36.7 (t)	36.9 (t)
15	45.0 (t)	44.7 (t)	52.7 (t)	52.6 (t)
16	43.2 (s)	39.5 (s)	80.5 (s)	80.2 (s)
17	65.8 (t)	68.2 (t)	65.3 (t)	68.2 (t)
18	71.7 (d)	72.1 (d)	71.3 (d)	71.9 (d)
19	176.2 (s)	181.2 (s)	175.8 (s)	179.7 (s)
20	15.5 (q)	15.3 (q)	15.2 (q)	15.4 (q)
CH_3CO	20.9 (q)	20.7 (q)	20.6 (q)	20.7 (q)
	170.4 (s)	170.8 (s)	170.1 (s)	170.8 (s)
$COCHMe_2$		177.4 (s)		177.2 (s)
		34.0 (d)		34.0 (d)
		18.9 (q)		18.9 (q)
		18.7 (q)		19.0 (q)

[a] Data recorded at 75 MHz. [b] Data recorded at 100 MHz. [c] Spectra recorded in DMSO-d_6. [d] Spectra recorded in $CDCl_3$.

carbon at δ 108.5 and H-15 at δ 3.88, H-16b at δ 3.75. Accordingly, compound **11** was determined to be *ent*-3α,15,16-trihydroxypimar-8(14)-en-15,16-acetonide.

Comparison of the NMR spectra of **12** with those of **11** suggested the existence of an extra β-glucose unit [δ_H 4.34 (1H, d, J = 7.6 Hz); δ_C 100.4 (s), 76.3 (d), 75.1 (d), 73.5 (d), 70.3 (d), 62.3 (t)] in **12**, which was confirmed by an acid hydrolysis experiment of **12**. The sugar moiety was at C-3, as judged from the downfield-shifted C-3 signal at δ 85.7 (ca. $\Delta\delta$ 6.6 ppm) resulting from glycosylation. In its HMBC spectrum, a cross-peak between the anomeric proton and C-3 verified the linkage between the aglycone and sugar moiety. Compound **12** was thus established as *ent*-3α,15,16-trihydroxypimar-8(14)-en-3α-*O*-*β*-glucopyranoside-15,16-acetonide.

Compound **13** showed IR absorption bands at 3405 cm^{-1} (OH) and at 1739 and 1711 cm^{-1} (carboxyl). The molecular formula was deduced as $C_{22}H_{34}O_5$ from its HRESIMS and ^{13}C NMR spectra (Table 2). The ^{1}H NMR and ^{13}C NMR spectra of **13** indicated an extra acetoxyl group [δ_H 1.98 (each 3H, s); δ_C 20.9 (q) and 170.4 (s)] in comparison with those of the related *ent*-kaurane-type diterpenoid **18**.[1] The acetoxyl group was linked to C-18 on the basis of the downfield-shifted C-18 signal at δ 71.7 (ca. $\Delta\delta$ 2.3 ppm). This was supported by an HMBC cross-peak between the carboxyl carbon (δ 170.4) and the oxygenated CH_2-18 (δ 4.24 and 3.89). X-ray crystallographic analysis (Figure 3) verified the *ent*-kaurane-type skeleton of **13** and its relative configuration. Compound **13** was thus established to be *ent*-18-acetoxy-17-hydroxy-16*βH*-kauran-19-oic acid.

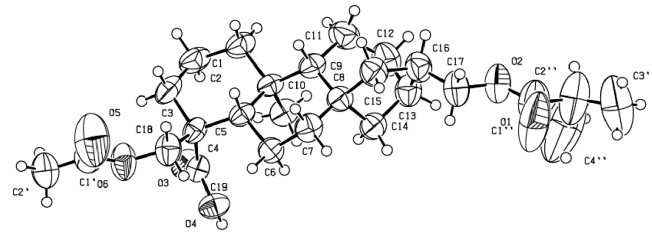


Figure 4. ORTEP drawing of **14**.

Compound **14** had the molecular formula $C_{26}H_{40}O_6$. Comparison of the NMR spectra of **14** with those of **13** indicated the presence of an extra isobutyryloxyl group [δ_H 1.13 and 1.15 (each 3H, s), 2.52 (1H, m); δ_C 177.4 (s), 34.0 (d), 18.9 (q), and 18.7 (q)] at C-17 in **14**. In the HMBC spectrum, this was supported by correlation between the carboxyl at δ 177.4 and the oxygenated CH_2-17 at δ 3.84. X-ray crystallographic analysis of compound **14** (Figure 4) confirmed the change at C-17 and showed the same relative configuration as compound **13**. Therefore, compound **14** was established as *ent*-18-acetoxy-17-isobutyryloxy-16*βH*- kauran-19-oic acid.

The molecular formula of **15** was deduced to be $C_{22}H_{34}O_6$. Its ^{1}H and ^{13}C NMR spectra were nearly superimposable with those of **17**[18] except for an additional acetyl moiety [δ_H 1.98 (3H, s); δ_C 170.2 (s) and 20.6 (q)]. The OAc group was attached to C-18, as was concluded from the variances in chemical shifts of C-18 (+2.7 ppm) and C-4 (−2.3 ppm). It was also supported by an HMBC cross-peak between the H_2-18 (δ 4.23 and 3.89) and the carboxyl carbon (δ 170.2). Therefore, compound **15** was determined to be *ent*-18-acetoxy-16α,17-dihydroxykauran-19-oic acid.

Compound **16** possessed the elemental composition $C_{26}H_{40}O_7$, and its ^{1}H and ^{13}C NMR spectra (Table 2) were nearly superimposable with those of **15** except for an additional isobutyryloxyl moiety [δ_H 1.19 and 1.17 (each 3H, s), 2.60 (1H, m); δ_C 177.2 (s), 34.0 (d), 19.0 (q), and 18.9 (q)], which was attached to C-17. This was verified by an HMBC cross-peak between the carboxyl carbon at δ 177.2 and the H_2-17 at δ 4.23. Therefore, compound **16** was determined to be *ent*-18-acetoxy-16α-hydroxy-17-isobutyryloxykauran-19-oic acid.

Other known diterpenoid compounds isolated from this plant were *ent*-2-oxo-15,16,19-trihydroxypimar-8(14)-ene (**4**),[7] kirenol (**5**),[10] darutigenol (**6**),[13] *ent*-3α,15,16,19-tetrahydroxypimar-8(14)-ene (**7**),[19] darutoside (**8**),[20] *ent*-16-acetoxy-15-hydroxypimar-8(14)-en-3α-*O*-*β*-glucopyranoside (**9**),[21] isopropylidenkirenol (**10**),[10] *ent*-16α,17,18-trihydroxykauran-19-oic acid (**17**),[18] *ent*-17,18-dihydroxy-16*βH*-kauran-19-oic acid (**18**),[1] *ent*-17-isobutyryloxy-18-hydroxykauran-19-oic acid (**19**),[22] *ent*-16α,17-dihydroxykauran-19-oic acid (**20**),[23] *ent*-17-hydroxy-16α*H*-kauran-19-oic acid (**21**),[24] *ent*-19-methyl-17-hydroxy-16α*H*-kauran-19-oic acid (**22**),[25] *ent*-16α*H*-kauran-17,19-dioic acid (**23**),[26] the 16α,17-acetonide of *ent*-16α,17-dihydroxykauran-19-oic acid (**24**),[1] and the 16α,17-acetonide of *ent*-19-methyl-16α,17-dihydroxykauran-19-oic acid (**25**).[27] Structures of the known compounds were confirmed by comparison with data reported in the literature.

The acetonide compounds **10**−**12**, **24**, and **25** were determined to be artifacts formed in the process of isolation, as confirmed by a series of HPLC experiments (see Supporting Information).

Seven compounds (**5**, **6**, **8**, **11**, **14**, **18**, and **20**) were evaluated for cytotoxic activity against the HSC-T6, HeLa, and B16 cell lines, using the MTT method, with cisplatin and paclitaxel as positive controls (Table, Supporting Information). Only compounds **11** and **14** showed moderate cytotoxicity (IC_{50} values, 34.7, 32.3, and 21.5 μM for **11** and 54.9, 54.8, and 50.9 μM for **14**).

Experimental Section

General Experimental Procedures. Melting points were measured on an X-5 Microscope Melting Point Inspect. Optical rotations were measured on a Perkin-Elmer model 341 polarimeter with a 1 dm cell.

IR spectra were obtained on a Nicolet NEXUS 670 FT-IR spectrometer. NMR spectra were recorded on Varian INOVA-300 and Bruker AVANCE III-400 spectrometers. Chemical shifts are given as δ (ppm) using TMS as internal standard. HRESIMS was carried out on a Bruker APEX II mass spectrometer. HPLC experiments were carried out on an Agilent Technologies 1200 series instrument. Silica gel (200−300 mesh) used for column chromatography (CC) and silica GF_{254} (10−40 μm) for TLC were both supplied by the Qingdao Marine Chemical Factory, Qingdao, People's Republic of China. TLC was detected at 254 nm, and spots were visualized by spraying with 5% H_2SO_4 in C_2H_5OH (v/v) followed by heating.

Plant Material. The aerial parts of *Siegesbeckia pubescens* were purchased from the Huanghe Medicinal Material Market in Gansu in 2008 and were identified by Prof. Huan-Yang Qi of the Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics. A voucher specimen (No. ZY2007S001) was deposited in our laboratory.

Extraction and Isolation. The air-dried and powdered *S. pubescens* plant material (9.0 kg) was extracted with EtOH (3 × 3 h) at ca. 65 °C. The crude extract was mixed with H_2O (2 L) to form a suspension and then partitioned successively with petroleum ether, EtOAc, and n-BuOH. The EtOAc-soluble part (120 g) was subjected to silica gel CC eluted with petroleum ether−acetone (30:1, 15:1, 8:1, 4:1, 2:1, 1:1, 0:100 and CH_3OH) to give eight fractions (A−H). Fractions D−F were separated by silica gel CC, using a $CHCl_3$−acetone gradient system, to give several subfractions. Further purification of each subfraction through repeated chromatography with $CHCl_3$−MeOH (30:1), $CHCl_3$−acetone (10:1), and petroleum ether−acetone−acetate acid (15:1:0.1) yielded compounds **11** (15.3 mg), **13** (25.5 mg), **14** (54.0 mg), **16** (16.9 mg), **18** (41.8 mg), **19** (120.0 mg), **20** (210.0 mg), **21** (150.0 mg), **22** (45.0 mg), **23** (36.0 mg), **24** (15.4 mg), and **25** (10.8 mg). Each subfraction of fraction E was separated by silica gel CC eluted with $CHCl_3$−MeOH (20:1, 10:1, and MeOH) and by recrystallization to give compounds **1** (15.0 mg), **4** (13.5 mg), **5** (340.0 mg), **6** (60.0 mg), **7** (25.9 mg), **8** (40.3 mg), **10** (33.3 mg), **15** (18.0 mg), and **17** (24.0 mg), respectively. A similar isolation procedure adopted for the subfractions of fraction F afforded **2** (9.3 mg), which almost completely changed into **2a** in the NMR tube at room temperature after 3 days, **3** (8.2 mg), **9** (1.8 g), and **12** (7.7 mg).

***ent*-3α,7*β*,15,16-Tetrahydroxypimar-8(14)-ene (1):** white, amorphous powder, $[\alpha]_D^{20}$ +20 (*c* 1.3 DMSO); IR (KBr) ν_{max} 3422, 2941, 1657, 1050, 1027, 1004, 825, 763 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 5.31 (1H, s, H-14), 3.95 (1H, d, J = 2.4 Hz, H-7), 3.43 (1H, dd, J = 9.6, 3.6 Hz, H-16a), 3.28 (1H, m, H-15), 3.23 (1H, m, H-16b), 3.04 (1H, m, H-3), 1.97 (1H, t, J = 8.0 Hz, H-9), 1.88 (1H, m, H-12), 0.87 (3H, s, H-18), 0.79 (3H, s, H-17), 0.69 (3H, s, H-19), 0.66 (3H, s, H-20); ^{13}C NMR (DMSO-d_6, 75 MHz) data, see Table 1; HRESIMS *m*/*z* 361.2344 $[C_{20}H_{34}O_4 + Na]^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2349).

***ent*-16-Acetoxy-3α,15-dihydroxy-14α-hydroperoxypimar-7-en-3α-*O*-*β*-glucopyranoside (2):** colorless gum; ^1H NMR (acetone-d_6, 400 MHz) δ 5.71 (1H, d, J = 5.6 Hz, H-7), 4.37 (1H, d, J = 7.6 Hz, H-1′), 4.11 (1H, dd, J = 11.2, 2.8 Hz, H-16a), 4.01 (1H, m, H-16b), 3.88 (1H, s, H-14), 3.73 (1H, m, H-15), 1.98 (3H, s, CH_3CO−), 1.00 (3H, s, H-18), 0.92 (3H, s, H-17), 0.88 (3H, s, H-19), 0.85 (3H, s, H-20); ^{13}C NMR (acetone-d_6, 100 MHz) data, see Table 1; HRESIMS *m*/*z* 576.3390 $[C_{28}H_{64}O_{11} + NH_4]^+$ (calcd for $C_{28}H_{68}O_{11}N$, 576.3378).

***ent*-16-Acetoxy-3α,15-dihydroxy-7*β*-hydroperoxypimar-8(14)-en-3α-*O*-*β*-glucopyranoside (2a):** colorless gum; $[\alpha]_D^{20}$ −13 (*c* 0.8 acetone); IR (KBr) ν_{max} 3377, 2936, 1722, 1654, 1077, 1039 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 5.59 (1H, s, H-14), 4.39 (1H, d, J = 7.6 Hz, H-1′), 4.24 (1H, br s, H-7), 4.17 (1H, dd, J = 11.2, 2.8 Hz, H-16a), 4.00 (1H, dd, J = 11.2, 8.8 Hz, H-16b), 3.70 (1H, dd, J = 8.8, 2.8 Hz, H-15); ^{13}C NMR (acetone-d_6, 100 MHz) data, see Table 1.

***ent*-2*β*,15,16-Trihydroxypimar-8(14)-en-19-oic acid (3):** white, amorphous powder; $[\alpha]_D^{20}$ −133 (*c* 0.1 CH_3OH); IR (KBr) ν_{max} 3516, 3413, 3004, 2921, 1713, 1422, 1362, 1222, 1091 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 12.11 (1H, br s, −COOH)5.17 (1H, s, H-14), 3.84 (1H, m, H-2), 3.46 (1H, d, J = 10.0 Hz, H-16a), 3.30 (1H, m, H-15), 3.25 (1H, m, H-16b), 1.15 (3H, s, H-18), 0.76 (3H, s, H-17), 0.61 (3H, s, H-20); ^{13}C NMR (DMSO-d_6, 100 MHz) data, see Table 1; HRESIMS *m*/*z* 370.2587 $[C_{20}H_{32}O_5 + NH_4]^+$ (calcd for $C_{20}H_{36}O_5N$, 370.2588).

X-ray Crystallography of 8. Colorless crystals were obtained from a solution of $CHCl_3$−CH_3OH (1:2), monoclinic space group $P2_1$, a = 13.551(6) Å, b = 6.242(3) Å, c = 16.767(7) Å, V = 1382.1(10) $Å^3$, Z = 2, D_x = 1.241 mg/m^3 refinement on F^2, R (gt) = 0.0421, wR (gt) = 0.0907, S (all) = 1.026.

***ent*-3α,15,16-Trihydroxypimar-8(14)-en-15,16-acetonide (11):** pale gum; $[\alpha]_D^{20}$ −36 (*c* 0.6 acetone); IR (KBr) ν_{max} 3312, 2939, 2907, 2878, 1651, 1454, 1374, 1264, 1064, 1036, 861 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.06 (1H, s, H-14), 4.01 (1H, t, J = 7.2 Hz, H-16a), 3.88 (1H, t, J = 8.1 Hz, H-15), 3.75 (1H, t, J = 8.1 Hz, H-16b), 3.25 (1H, dd, J = 11.7, 3.6 Hz, H-3), 2.25 (1H, br d, J = 14.7 Hz, H-7a), 2.02 (1H, ddd, J = 13.8, 5.7 Hz, H-7b), 1.39 (3H, s, CMe_2), 1.34 (3H, s, CMe_2), 1.00 (3H, s, H-18), 0.90 (3H, s, H-17), 0.81 (3H, s, H-19), 0.76 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 75 MHz) data, see Table 1; HRESIMS *m*/*z* 385.2711 $[C_{23}H_{38}O_3 + Na]^+$ (calcd for $C_{23}H_{38}O_3Na$, 385.2713).

***ent*-3α,15,16-Trihydroxypimar-8(14)-en-3α-*O*-*β*-glucopyranoside-15,16-acetonide (12):** colorless gum; $[\alpha]_D^{20}$ −53 (*c* 0.4 acetone); IR (KBr) ν_{max} 3387, 2938, 2875, 1657, 1455, 1374, 1209, 1071, 1041, 861 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.06 (1H, s, H-14), 4.34 (1H, d, J = 7.6 Hz, H-1′), 4.00 (1H, t, J = 6.8 Hz, H-16a), 3.88 (1H, t, J = 7.6 Hz, H-15), 3.75 (1H, t, J = 7.6 Hz, H-16b), 3.25 (1H, dd, J = 11.6, 3.2 Hz, H-3), 2.25 (1H, br d, J = 12.4 Hz, H-7a), 2.01(1H, m, H-7b), 1.40 (3H, s), 1.34 (3H, s, CMe_2), 0.99 (3H, s, H-18), 0.91 (3H, s, H-17), 0.82 (3H, s, H-19), 0.77 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; HRESIMS *m*/*z* 542.3692 $[C_{29}H_{48}O_8 + NH_4]^+$ (calcd for $C_{29}H_{52}O_8N$, 542.3687).

***ent*-18-Acetoxy-17-hydroxy-16*βH*-kauran-19-oic acid (13):** colorless needles; mp 180−182 °C; $[\alpha]_D^{20}$ −64 (*c* 0.4 CH_3OH); IR (KBr) ν_{max} 3405, 2929, 2854, 1739, 1711, 1446, 1370, 1235, 1038 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) 4.24 (1H, d, J = 10.5 Hz, H-18a), 3.89 (1H, d, J = 9.9 Hz, H-18b), 3.13 (2H, m, H-17), 2.12 (1H, m, H-3), 2.02 (1H, m, H-13), 1.98 (3H, s, CH_3CO−), 1.20 (1H, m, H-5), 0.89 (3H, s, H-20); ^{13}C NMR (DMSO-d_6, 75 MHz) data, see Table 2; HRESIMS *m*/*z* 396.2749 $[C_{22}H_{34}O_5 + NH_4]^+$ (calcd for $C_{22}H_{38}O_5N$, 396.2744).

X-ray Crystallography of 13. Colorless crystals were obtained from a solution of $CHCl_3$−CH_3COCH_3 (2:1), orthorhombic space group $P2_12_12_1$, a = 7.4147(8) Å, b = 10.7871(13) Å, c = 27.001(3) Å, V = 2159.6(4) $Å^3$, Z = 4, D_x = 1.219 mg/m^3, refinement on F^2, R (gt) = 0.0448, wR (gt) = 0.1189, S (all) = 1.252.

***ent*-18-Acetoxy-17-isobutyryloxy-16*βH*-kauran-19-oic acid (14):** colorless needles; mp 238−240 °C; $[\alpha]_D^{20}$ −68 (*c* 0.7 acetone); IR (KBr) ν_{max} 2934, 2858, 1742, 1699, 1469, 1386, 1248, 1192, 1156, 1034, 914 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.39 (1H, d, J = 9.9 Hz, H-18a), 3.97 (1H, d, J = 10.5 Hz, H-18b), 3.84 (2H, d, J = 7.5 Hz, H-17), 2.52 (1H, m, Me_2CHCO−), 2.04 (3H, s, CH_3CO−), 1.15 (3H, s, Me_2CHCO−), 1.13 (3H, s, Me_2CHCO−), 0.95 (3H, s, H-20), 0.80 (1H, m, H-1b); ^{13}C NMR ($CDCl_3$, 75 MHz) data, see Table 2; HRESIMS *m*/*z* 466.3160 $[C_{26}H_{40}O_6 + NH_4]^+$ (calcd for $C_{26}H_{44}O_6N$, 466.3163).

X-ray Crystallography of 14. Colorless crystals were obtained from a solution of $CHCl_3$−CH_3COCH_3 (2:1), orthorhombic space group $P2_12_12_1$, a = 21.962(3) Å, b = 7.6252(11) Å, c = 15.114(2) Å, V = 2531.0(6) $Å^3$, Z = 4, D_x = 1.177 mg/m^3, refinement on F^2, R (gt) = 0.0493, wR (gt) = 0.1278, S (all) = 1.075.

***ent*-18-Acetoxy-16α,17-dihydroxykauran-19-oic acid (15):** colorless needles; mp 178−180 °C; $[\alpha]_D^{20}$ −59 (*c* 0.7 CH_3OH); IR (KBr) ν_{max} 3459, 3385, 2952, 2868, 2840, 1742, 1701, 1376, 1232, 1036, 1019 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 4.23 (1H, d, J = 10 Hz, H-18a), 3.89 (1H, d, J = 10.8 Hz, H-18b), 3.49 (1H, d, J = 11.2 Hz, H-17a), 3.38 (1H, d, J = 11.2 Hz, H-17b), 2.10 (1H, br d, J = 12.8 Hz, H-3a), 1.98 (3H, s, CH_3CO−), 1.79 (1H, m, H-1a), 0.98 (1H, td, J = 13.2, 4.4 Hz, H-3b), 0.94 (1H, m, H-9), 0.89 (3H, s, H-20), 0.74 (1H, td, J = 13.2, 13.6, 3.6 Hz, H-1b); ^{13}C NMR (DMSO-d_6, 100 MHz) data, see Table 2; HRESIMS *m*/*z* 412.2693 $[C_{22}H_{34}O_6 + NH_4]^+$ (calcd for $C_{22}H_{38}O_6N$, 412.2694).

***ent*-18-Acetoxy-16α-hydroxy-17-isobutyryloxykauran-19-oic acid (16):** white, amorphous powder; $[\alpha]_D^{20}$ −54 (*c* 0.26 acetone); IR (KBr) ν_{max} 3465, 2925, 2871, 2852, 1728, 1462, 1382, 1245, 1159, 1035 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) 4.40 (1H, d, J = 10.5 Hz, H-18a), 4.23 (2H, br s, H-17), 3.98 (1H, d, J = 9.9 Hz, H-18b), 2.60 (1H, m, Me_2CHCO−), 2.29 (1H, br d, J = 14.4 Hz, H-3a), 2.05 (3H, s, CH_3CO−), 1.19 (3H, s, Me_2CHCO−), 1.17 (3H, s, Me_2CHCO−), 0.98 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 75 MHz) data, see Table 2; HRESIMS *m*/*z* 482.3122 $[C_{26}H_{40}O_7 + NH_4]^+$ (calcd for $C_{26}H_{44}O_7N$, 482.3112).

Acidic Hydrolysis of 2a and 12. Compound **2a** (4 mg) or **12** (6

mg) dissolved in 50% MeOH (10 mL) containing 7% HCl was heated in boiling water for 3 h. After cooling, the reaction mixture were neutralized and extracted with EtOAc three times (10 mL × 3) to obtain the aglycone. Glucose was identified as the sugar moiety by co-TLC of the aqueous solution compared with an authentic glucose sample.

Crystallographic Data of 8, 13, and 14. Crystallographic data of **8**, **13**, and **14** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 730006−730008. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44(0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

In Vitro Cytotoxicity Assay. The cytotoxicity of compounds **5**, **6**, **8**, **11**, **14**, **18**, and **20** against HSC-T6, HeLa, and B16 cell lines was evaluated by the MTT assay as described previously.[28] Briefly, cells at the exponential growth phase were harvested and seeded into a flat-bottom 96-well plate. A total of 100 μL containing 5×10^4 cells was added to each well of the plate. After 24 h incubation in a 5% humidified CO_2 incubator at 37 °C, the test agent was added (in triplicate experiments) to give final concentrations of 10, 50, and 100 μg/mL. After 44 h of incubation at 37 °C, 20 μL/well, MTT was then added and the plate was again incubated at 37 °C for 4 h. The absorbance of the MTT solution was measured at 490 nm. IC_{50} values were calculated on the basis of percentage inhibition using the linear regression method.

Acknowledgment. This work was financially supported by the Foundation for the National Natural Science Foundation of China (No. NSFC 20621091), the National 863 Program of China (No. 2007AA09Z403), and the National Key Technology Research and Development Program of China (No. 2007BAI37B05).

Supporting Information Available: The NMR spectra (^{1}H, ^{13}C NMR and DEPT135, HMBC of all new compounds, NOE spectra of compounds **1** and **2**, and HMQC of **1**, **2a**, and **15**), Schemes 1−3, a table, and CIF files of compounds **8**, **13**, and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Xiong, J.; Ma, Y. B.; Xu, Y. L. *Phytochemistry* **1992**, *31*, 917–921.
(2) Su, J. D.; Osawa, T.; Namiki, M. *Agric. Biol. Chem.* **1986**, *50*, 199–203.
(3) Kim, H. M.; Kim, C. Y.; Kwon, M. H.; Shin, T. Y.; Lee, E. J. *Arch. Pharmacol. Res.* **1997**, *20*, 122–127.
(4) Dong, X. Y.; Chen, M.; Jin, W.; Huang, D. X.; Shen, S. M.; Li, H. T. *Acta Pharm. Sin.* **1989**, *24*, 833–836.
(5) Hu, H. H.; Tang, L. X. *China J. Chin. Mat. Med.* **2004**, *29*, 542–545.
(6) Xin, H. L.; Liu, M.; Lin, W. H.; Qian, R. Q. *Chin. Trad. Herb Drugs* **2005**, *36*, 866–869.
(7) Xiang, Y.; Zhang, H.; Fan, C. Q.; Yue, J. M. *J. Nat. Prod.* **2004**, *67*, 1517–1521.
(8) Canonica, L.; Rindone, B.; Scolastico, C.; Han, K. D.; Kim, J. H. *Tetrahedron Lett.* **1969**, *10*, 4801–4804.
(9) Kim, J. H.; Han, K. D.; Yamasaki, K.; Tanaka, Q. *Phytochemistry* **1979**, *18*, 894–895.
(10) Liu, K.; Röder, E. *Planta Med.* **1991**, *57*, 395–396.
(11) Xiong, J.; Jin, Q. D.; Xu, Y. L. *Chin. Chem. Lett.* **2001**, *12*, 51–54.
(12) Fu, H. Z.; Feng, R.; Du, Z. H.; Miu, Z. C.; Yan, X. X.; Li, Y. G. *Zhongcaoyao* **1997**, *28*, 327–329.
(13) Barua, R. N.; Sharma, R. P.; Thyagarajan, G.; Werner, H.; Serengolam, V. G. *Phytochemistry* **1980**, *19*, 323–325.
(14) Avila, D.; Davies, A. G.; Davison, I. G. E. *J. Chem. Soc., Perkin Trans. 2* **1988**, *10*, 1847.
(15) Davies, A. G.; Davison, I. G. E. *J. Chem. Soc., Perkin Trans. 2* **1989**, 825–830.
(16) Beckwith, A. L, J.; Davies, A. G.; Davison, I. G. E.; Maccoll, A.; Mruzek, M. *J. Chem. Soc., Chem. Commun.* **1988**, *7*, 475–476.
(17) Beckwith, A. L. J. *J. Chem. Soc., Perkin Trans. 2* **1989**, *81*, 5–824.
(18) Gao, H.; Li, P. Y.; Li, D. K.; Du, X. P. *Zhongcaoyao* **2002**, *33*, 495–496.
(19) Cherkasov, A.; Shi, Z.; Li, Y.; Jones, S. J. M.; Fallahi, M.; Hammond, G. L. *J. Chem. Inform. Model.* **2005**, *45*, 1842–1853.
(20) Phan, T. S.; Le, K. N.; Phan, M. G.; Nguyen, V. D. *J. Viet. Pharm. Soc.* **2002**, *7*, 11–13.
(21) Chen, W. L.; Tang, W. D.; Zhang, R. J.; Lou, L. G.; Zhao, W. M. *J. Nat. Prod.* **2007**, *70*, 567–570.
(22) Fu, H. Z.; Lin, W. H. *Chin. J. Magn. Reson.* **1997**, *14*, 300–305.
(23) Tanaka, N.; Murakami, T.; Saiki, Y.; Chen, C. M. *Chem. Pharm. Bull.* **1985**, *33*, 152–157.
(24) Wu, Y. C.; Hung, Y. C.; Chang, F. R.; Cosentino, M.; Wang, H. K.; Lee, K. H. *J. Nat. Prod.* **1996**, *59*, 635–637.
(25) Croft, K. D.; Ghisalberti, E. L.; Jefferies, P. R. *Phytochemistry* **1978**, *17*, 695–699.
(26) Gao, F.; Miski, M.; Gage, D. A.; Norris, J. A.; Mabr, T. J. *J. Nat. Prod.* **1985**, *48*, 489–490.
(27) Delgado, G.; Cardenas, H.; Reláez, G.; Viver, A. R.; Rogelio, P. M. *J. Nat. Prod.* **1984**, *47*, 1042–1045.
(28) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.

NP9005579