Cytotoxic Germacranolides and Acyclic Diterpenoides from the Seeds of *Carpesium triste*

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Four new highly oxygenated germacranolides (**1**, **4**, **6**, and **7**) and four new acyclic diterpenes (**8**−**11**), along with three known germacranolides (**2**, **3**, and **5**), were isolated from the seeds of *Carpesium triste*. The structures of the new compounds were elucidated by spectroscopic methods including IR, HRESIMS, and 1D and 2D NMR experiments, and the absolute configurations of compounds **1** and **8**−**10** were established by CD and Mosher's methods, respectively. Compounds **1**, **2**, and **4**−**10** were evaluated for their *in vitro* cytotoxic activity against cultured SMMC-7721 (human hepatoma), HL-60 (human promyelocytic leukemia), and L02 (human hepatocyte) cell lines. Compounds **1**, **2**, and **4**−**7** exhibited significant cytotoxicity against HL-60 cells, and compound **10** exhibited cytotoxicity against SMMC-7721 cells.

The genus *Carpesium* has been reported to be a rich source of antifungal, antibacterial, and antitumor sesquiterpene lactones.[1,2] Only one cytotoxic acyclic diterpene, from *C. divaricatum*,[3] has been reported. In previous chemical and biological investigations of this genus, we found that sesquiterpenes[4−6] are widespread secondary metabolites in the genus *Carpesium*. *C. triste* Maxim (Compositae) is a Chinese herbal medicine that has been used to treat sore throat, toothache, urinary tract infection, diarrhea, and mastitis.[7] Two sesquiterpene lactones from *C. triste* var. *manshuricum* have been reported.[8] Continuing our search for biologically active compounds from this genus, we have investigated constituents of the seeds of *C. triste*, which led to the isolation and structure elucidation of seven highly oxygenated germacranolides (**1**−**7**) including four new ones (**1**, **4**, **6**, and **7**), and four new acyclic diterpenes (**8**−**11**). Cytotoxic activity of compounds **1**, **2**, and **4**−**10**, *in vitro*, was also examined.

Results and Discussion

Air-dried and pulverized seeds of *C. triste* Maxim were extracted with MeOH. After repeated chromatography of the extract using Si gel and different eluates, seven highly oxygenated germacranolides, including four new ones (**1**, **4**, **6**, and **7**), and four new acyclic diterpenes (**8**−**11**) were isolated. The known compounds incaspitolide A, B, and D (**2**, **3**, and **5**)[9] were identified by comparison of their spectroscopic data (MS, ^{1}H NMR) with those reported in the literature.

Compound **1** was obtained as colorless needles. The molecular formula was determined to be $C_{24}H_{34}O_8$ by HRESIMS. Its IR spectrum showed hydroxyl (3516 cm^{-1}), carbonyl (1776 and 1726 cm^{-1}), and double-bond (1645 cm^{-1}) absorptions. The ^{1}H NMR spectrum of **1** (Table 1) displayed the signals of isobutyryloxy and angeloyloxy groups. The EIMS of **1** showed fragment peaks at *m*/*z* 350 [M − HOAng]$^+$ and 262 [M − HOAng − HOiBu]$^+$, which further supported the above inferences. The ^{1}H and ^{13}C NMR (DEPT) spectra of **1** also showed an α-methylene γ-lactone, a ketone group, and an oxygenated quaternary carbon; the remaining signals indicated three methylenes, five methines including three oxygenated ones, and two methyl groups, in which one was tertiary and the other was secondary. These observations indicated that **1** was closely related to 8α-isobutyryloxyineupatorolide B (**12**),[10] but that the ester residue at C-5 was an isobutyryloxy group and that at C-8 there was an angeloyloxy group in **1**. The assignments were supported by 2D NMR experiments. The ^{1}H−^{1}H COSY spectrum showed two partial structure sequences for compound **1**: CH_2(3)-CH_2(2)CH_2(1)CH(10)CH_3(14) and CH(5)CH(6)CH(7)CH(8). The C−C interconnectivity of all fragments was established from the HMBC spectrum as correlations of H-15 with C-3, C-5, and C-4, H-14 with C-1, C-9, and C-10, H-13 with C-7, C-12, and C-11, H-6 with C-8, C-4, and C-12; H-5 with δ_C 176.7 (ester carbonyl of isobutyryloxy); and H-8 with δ_C 165.3 (ester carbonyl of angeloyloxy). The relative configuration of **1** was deduced from NOE difference spectra; irradiation of H-8 produced NOE enhancement of the H-6 (4.31%) resonance, while irradiation of H-7 produced NOE enhancement of the H-5 (15.10%) and H-10 (8.31%) resonances. Coupling constants similar to those of **12** further indicated that **1** was 8α-angeloyloxy-4β-hydroxy-5β-isobutyryloxy-9-oxo-germacran-7β,12α-olide.

The CD curve of **1** exhibited two positive Cotton effects at 252 nm (α-methylene γ-lactone region) and 294 nm (ketone n, π* region), which corresponded to those of epoxyineupatorolide B (**13**),[11,12] indicating that the absolute configuration of **1** was the same as that of **13** (4*S*, 5*R*, 6*S*, 7*S*, 8*R*, 10*R*) as shown.

Compound **4** was isolated as colorless needles, $C_{24}H_{36}O_9$, by HRESIMS. Its IR spectrum indicated the presence of OH (3452 cm^{-1}), carbonyl groups (1753 and 1740 cm^{-1}), and a double bond (1662 cm^{-1}). The ^{1}H NMR spectrum indicated an exocyclic methylene, four oxygenated methines, a tertiary methyl group, and a secondary methyl group and closely resembled that of incaspitolide D (**5**)[9] except that the isobutyryloxy signals of the latter compound were replaced by signals for a 3-methylbutyryloxy group in **4**. The positions of the functional groups were established by 2D NMR spectra run in CD_3OD, as it was difficult to distinguish some of the signals in $CDCl_3$. It was possible to distinguish signals in the similar compound **5** [9] in $CDCl_3$ when the spectrum was run at 60 °C. The ^{1}H NMR signals of **4** were easier to assign when the spectra were run in CD_3OD rather than $CDCl_3$. The ^{1}H−^{1}H COSY and HMBC spectra (in CD_3OD) gave the following sequence: CH_2-(2)CH_2(1)CH(10)CH(9)CH(8)CH(7)CH(6)CH(5). Connected correlations were as follows: H-13/C-7, C-12, C-11; H-15/C-3, C-5, C-4; H-14/C-1, C-9, C-10; H-6/C-12, C-8, C-5, C-7; H-9/carbonyl of 3-methylbutyryloxy and H-5/carbonyl of isobutyryloxy. The similar coupling constants of **4** (Table 1) and **5** also indicated that **4** was 4β,8α-dihydroxy-5β-isobutyryloxy-9β-3-methylbutyryloxy-3-oxo-germacran-7β,12α-olide.

The molecular formula ($C_{25}H_{38}O_9$) of compound **6** was assigned by HRESIMS. The IR spectrum and the ^{1}H and ^{13}C NMR data of **6** (Tables 1 and 2) implied that **6** was closely related to **4** except

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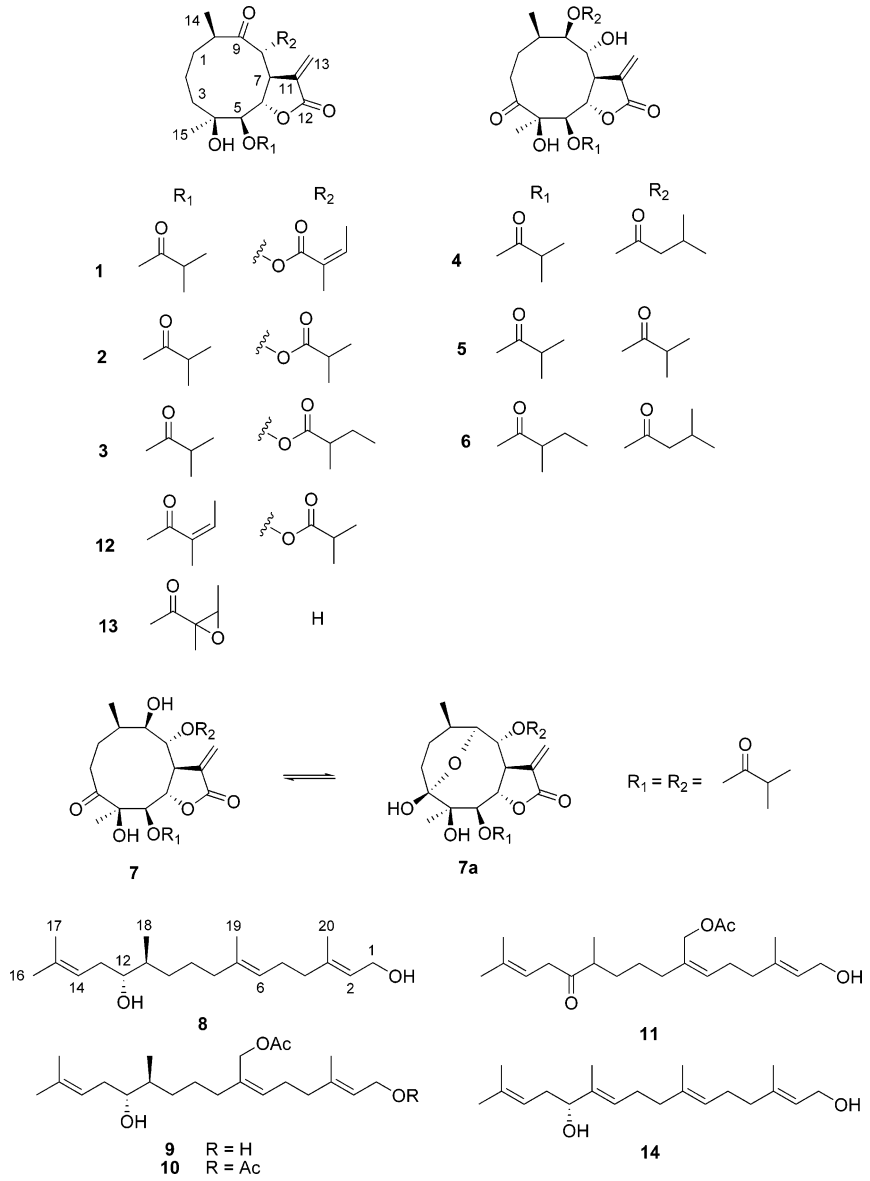
Chart 1



for the ester residue at C-5. A 2-methylbutyryloxy group appeared in **6** instead of the isobutyryloxy group in **4**. Hence, the structure of **6** was assigned as 4β,8α-dihydroxy-5β-2-methylbutyryloxy-9β-3-methylbutyryloxy-3-oxo-germacran-7β,12α-olide.

The HRESIMS (*m*/*z* 472.2548 $[M + NH_4]^+$) of compound **7** provided the molecular formula $C_{23}H_{34}O_9$. The IR spectrum displayed absorptions at 3475, 1739, and 1662 cm^{-1}. The ^1H NMR data of compound **7** (in CD_3OD) resembled those of **5**, but there were differences in placement of functional groups. The structure of **7** was determined from the 2D NMR spectra in CD_3OD (COSY and HMBC assignments, see Tables 1 and 2). The relative stereochemistry was similar to that of **5** as established from NOE difference spectra. Irradiating H-6 enhanced the signal of H-8 (7.13%), and irradiating H-7 showed NOE effects on H-5, H-9, and H-10 (8.80%, 3.28%, and 10.78%). Thus, compound **7** was assigned as 4β,9β-dihydroxy-5β,8α-di(isobutyryloxy)-3-oxo-germacran-7β,12α-olide.

When **7** was dissolved in $CDCl_3$, an equilibrium was observed between **7** and **7a** (about 2:1) due to the weak acidity in the solvent; a similar equilibrium had been observed with incaspitolide E in $CDCl_3$.[9] The equilibrium did not occur and **7** remained unchanged in CD_3OD.

Compound **8**, $C_{20}H_{36}O_2$ (HRESIMS), was an optically active oil. Its IR spectrum displayed hydroxyl (3357 cm^{-1}) and double-bond (1667 cm^{-1}) absorptions. The ^1H NMR spectrum showed three olefinic protons [δ_H 5.40 (brt, J = 7.2 Hz), 5.16 (brt, J = 7.2 Hz), and 5.10 (brt, J = 6.8 Hz)], an oxygenated methylene [δ_H 4.14 (d, J = 7.2 Hz)], an oxygenated methine [δ_H 3.40 (dt, J = 7.6, 5.2 Hz)], and five methyl signals including four broad singlets [δ_H 1.74, 1.68, 1.64, and 1.59] and one doublet [δ_H 0.90 (d, J = 6.8 Hz)], in combination with the ^{13}C NMR signals (Table 3). Considering the above information, **8** was an acyclic geranylgeraniol-derived diterpene, with a structure similar to that of bifurcadiol (**14**),[13] and containing three double bonds. The difference between **8** and **14** was one less double bond in the third isoprenic unit in **8**. Correlations by gHMBC were as follows: H-1/C-2, C-3; H-20/C-2, C-4, C-3; H-19/C-6, C-8, C-7; H-18/C-10, C-12, C-11; H-12/C-10, C-14, C-18; and H-16(17)/C-14, C-15, C-17(16). The *E* configuration of double bonds was consistent with the position of the C-19 and C-20 methyl signals observed at approximately δ 16.3 in the ^{13}C NMR spectrum.[14]

To confirm the configuration of C-12, a modified Mosher's method[15,16] was applied to metabolite **8**. Calculation of the $\Delta\delta^{SR} = \delta_H(S) - \delta_H(R)$ from ^1H NMR spectra of the derivatives of **8** allowed assignment of the *R* configuration to the secondary alcohol at C-12 (Figure 1). In a NOE difference spectrum, irradiation at H-12 produced positive NOE effects on H-18 (4.71%); hence H-12 and H-18 were in the same orientation. Thus, the structure of **8**

Table 1. ^{1}H NMR Data for Compounds **1**, **4**, **6**, and **7** (400 MHz, δ in ppm, J in Hz)

pos.	**1** ($CDCl_3$)	**4** ($CDCl_3$)	**4** (CD_3OD)	**6** ($CDCl_3$)	**6** (CD_3OD)	**7** ($CDCl_3$)	**7** (CD_3OD)
1a	1.68 m	1.75 m	1.70 m	1.75 m	1.70 m	1.82 m	1.72 m
1b		1.87 m	1.85 m	1.87 m	1.85 m		1.89 m
2a	1.45 m	3.73 brt (15.0)	3.84 brt (15.0)	3.73 brt (15.0)	3.84 brt (15.0)	3.74 m	3.73 brt (15.0)
2b		2.21 m	2.21 m	2.21 m	2.21 m	2.34 brd, (15.0)	2.32 dt (15.0, 4.8)
3	1.58 m						
5	4.62 d (6.0)	5.38 m	5.39 d (9.6)	5.39 m	5.40 d (9.6)	5.27 d (9.9)	5.15 d (9.8)
6	4.65 dd (6.0, 1.6)	4.68 m	4.65 dd (9.6, 6.4)	4.67 m	4.65 dd (9.6, 6.4)	4.74 m	4.88 dd (9.8, 4.8)
7	3.50 brd, (11.2)	2.99 m	3.02 m	2.98 m	3.02 m	3.07 m	3.11 m
8	4.93 d (11.2)	4.30 m	4.40 brd (10.2)	4.30 m	4.40 brd (10.2)	5.49 m	5.50 brd (10.2)
9		5.03 brd (10.2)	5.15 brd (10.2)	5.03 brd (10.2)	5.15 brd (10.2)	3.76 m	3.71 brd (10.2)
10	3.05 m	2.20 m	2.21 m	2.20 m	2.21 m	2.11 m	2.07 m
13a	6.37 brd (1.2)	6.42 brd (2.4)	6.31 brd (2.4)	6.42 brd (2.4)	6.30 brd (2.4)	6.39 brd (1.2)	6.34 brd (1.2)
13b	5.91 brd (1.2)	5.62 brd (2.4)	5.66 brd (2.4)	5.62 brd (2.4)	5.67 brd (2.4)	5.79 brd (1.2)	5.69 brd (1.2)
14	1.02 d (6.8)	0.99 d (6.8)	0.98 d (7.2)	0.99 d (6.8)	0.98 d (7.2)	1.10 d (7.0)	1.10 d (6.8)
15	1.14 s	1.29 s	1.22 s	1.29 s	1.22 s	1.28 s	1.22 s
R_1	2.65 hept. (7.2)	2.68 hept. (7.2)	2.70 hept. (7.2)	2.43 sext. (7.2)	2.53 sext. (7.2)	2.66 hept. (7.2)	2.69 hept. (7.2)
	1.20 d (7.2)	1.25 d (7.2)	1.22 d (7.2)	1.73 m	1.74 m	1.22 d (7.2)	1.22 d (7.2)
	1.19 d (7.2)	1.23 d (7.2)	1.21 d (7.2)	1.47 m	1.54 m	1.09 d (7.2)	1.06 d (7.2)
				1.20 d (7.2)	1.20 d (7.2)		
				0.97 t (7.2)	0.95 t (7.2)		
R_2	6.24 qq (7.2, 1.6)	2.26 d (7.2)	2.29 d (7.2)	2.26 d (7.2)	2.29 d (7.2)	2.52 hept. (7.2)	2.58 hept. (7.2)
	2.00 dq (7.2, 1.2)	2.12 m	2.11 m	2.11 m	2.11 m	1.21 d (7.2)	1.21 d (7.2)
	1.98 dq (1.6, 1.2)	0.98, 0.99 d (7.2)	0.97, 0.98 d (7.2)	0.98, 0.99 d (7.2)	0.97, 0.98 d (7.2)	1.08 d (7.2)	1.09 d (7.2)

Table 2. ^{13}C NMR (DEPT) Data for Compounds **1**, **4**, **6**, and **7** (100 MHz)

pos.	**1** ($CDCl_3$)	**4** (CD_3OD)	**6** (CD_3OD)	**7** (CD_3OD)
1	25.0 t	26.6 t	26.6 t	24.2 t
2	32.8 t	33.4 t	33.4 t	35.2 t
3	35.3 t	217.8 s	217.8 s	217.8 s
4	73.1 s	80.6 s	80.6 s	80.4 s
5	77.2 d	78.9 d	78.9 d	78.2 d
6	71.0 d	80.1 d	80.1 d	80.2 d
7	45.3 d	41.3 d	41.3 d	40.4 d
8	78.1 d	70.6 d	70.6 d	76.4 d
9	211.2 s	78.3 d	78.3 d	75.3 d
10	41.9 d	30.0 d	30.0 d	31.0 d
11	132.6 s	133.0 s	133.0 s	132.7 s
12	168.3 s	169.8 s	169.8 s	169.6 s
13	126.6 t	124.0 t	124.0 t	125.1 t
14	20.3 q	20.3 q	20.3 q	19.9 q
15	22.1 q	23.6 q	23.6 q	23.3 q
R_1	176.7 s	176.7 s	176.2 s	177.3 s
	33.7 d	34.2 d	41.8 d	34.1 d
	18.7 q	18.2 q	25.6 t	18.4 q
	18.7 q	18.2 q	16.0 q	18.2 q
			10.8 q	
R_2	165.3 s	173.5 s	173.4 s	176.8 s
	125.5 s	43.2 t	43.2 t	34.0 d
	142.4 d	25.6 d	25.6 d	18.2 q
	20.3 q	21.7 q	21.7 q	17.8 q
	15.8 q	21.7 q	21.7 q	

was assigned as (2*E*,6*E*,11*S*,12*R*)-3,7,11,15-tetramethylhexadeca-2,6,14-triene-1,12-diol.

Compound **9**, optically active oil, $C_{22}H_{38}O_4$ by HRESIMS, displayed IR absorptions at 3408, 1738, and 1670 cm^{-1}. The NMR data of **9** were very similar to those of **8** except that a methyl (C-19) was absent, and an oxygenated methylene [δ_H 4.57 (brs); δ_C 61.5 (CH_2)] and a corresponding acetoxy [δ_H 2.05 (s); δ_C 170.8 (C), 20.5 (CH_3)] were present. The configurations of C-11 and C-12 (*S* and *R*) were deduced by the same method as above for **8**. The *Z* configuration of the double bond of the second isoprenoid unit was deduced from the NOE difference spectrum; irradiation of H-6 enhanced the signal of H-8 (2.56%). Hence compound **9** was assigned as (2*E*,6*Z*,11*S*,12*R*)-3,7,11,15-tetramethylhexadeca-2,6,-14-triene-7-[(acetyloxy)methyl]-1,12,19-triol.

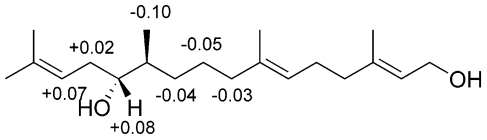
The molecular formula $C_{24}H_{40}O_5$ of compound **10**, optically active oil, was determined by HRESIMS. The IR spectrum displayed absorptions at 3404, 1738, and 1664 cm^{-1}. Comparing the data with those of **9**, one more acetoxy group [δ_H 2.04 (s); δ_C 171.0 (C), 21.0 (CH_3)] appeared in **10**. As a consequence, downfield shifts ($\Delta\delta_H$ 0.45 and $\Delta\delta_C$ 3.4 ppm) of the H-1 and C-1 signals were observed in the NMR spectra of **10**. Thus, **10** was assigned as (2*E*,6*Z*,11*S*,12*R*)-3,7,11,15-tetramethylhexadeca-2,6,14-triene-7-[(acetyloxy)methyl]-12,19-diol-1-acetate.

HRESIMS provided the molecular formula ($C_{22}H_{36}O_4$) of compound **11**, and the IR spectrum displayed absorptions at 3406, 1708, and 1668 cm^{-1}. The NMR data of **11** closely resembled those of **9**, but a ketone group appeared instead of the oxymethine at C-12 in **9**; for that reason, H-13 and H-11 were downfield shifted to δ 3.13 and 2.56, respectively. Hence **11** was assigned as (2*E*,6*Z*)-3,7,11,15-tetramethylhexadeca-2,6,14-trien-7-[(acetyloxy)methyl]-12-oxo-1,19-diol, the isomer of 19-acetoxy-12-oxo-10,11-dihydrogeranylnerol.[17] The difference between the two isomers was the configuration of the double bond in the first isoprene unit. The *E* configuration of the first double bond in **11** was further supported by an NOE effect observed between H-1 and H-20 (6.37%).

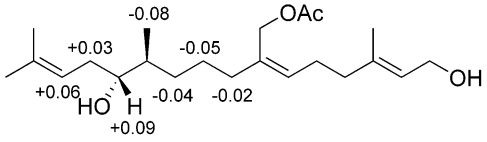
It was found that **1**, **2**, and **4**−**7** showed significant cytotoxic activity against the HL-60 cell line and that **10** showed significant cytotoxic activity against SMMC-7721 cells; IC_{50} values are

Table 3. ^{1}H NMR (400 MHz, δ in ppm, J in Hz) and ^{13}C NMR (DEPT, 100 MHz) Data for Compounds **8**−**11** ($CDCl_3$)

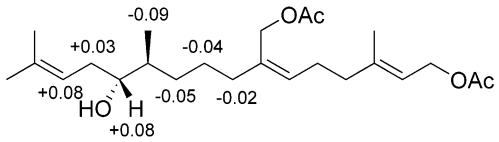
	8		**9**		**10**		**11**	
pos.	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	4.14 d (7.2)	59.1 t	4.13 d (7.2)	58.5 t	4.58 d (7.2)	61.9 t	4.13 d (7.2)	59.1 t
2	5.40 brt (7.2)	123.7 d	5.40 brt (7.2)	124.2 d	5.34 brt (7.2)	118.8 d	5.39 brt (7.2)	124.2 d
3		139.0 s		137.0 s		141.4 s		138.3 s
4	2.07 m	39.4 t	2.08 m	39.0 t	2.06 m	39.5 t	2.06 m	39.3 t
5	2.20 m	26.0 t	2.22 m	25.6 t	2.21 m	25.9 t	2.21 m	25.8 t
6	5.10 brt (6.8)	123.4 d	5.38 brt (6.8)	129.9 d	5.38 brt (6.8)	130.0 d	5.37 brt (6.8)	130.4 d
7		135.3 s		133.6 s		134.3 s		133.5 s
8	2.00 m	39.7 t	2.00 m	35.1 t	2.01 m	35.6 t	2.01 m	35.1 t
9	1.31 m, 1.98 m	25.3 t	1.30 m, 1.99 m	25.2 t	1.31 m, 1.98 m	25.7 t	1.27 m, 1.97 m	25.6 t
10	1.12 m, 1.49 m	31.4 t	1.13 m, 1.49 m	31.0 t	1.14 m, 1.50 m	31.8 t	1.16 m, 1.30 m	32.4 t
11	1.55 m	37.9 d	1.55 m	37.6 d	1.55 m	38.1 d	2.56 m	45.5 d
12	3.40 dt (7.6,5.2)	75.4 d	3.40 dt (7.6,5.2)	75.1 d	3.39 dt (7.6,5.2)	75.4 d		213.0 s
13	2.12 m	32.2 t	2.12 m	32.1 t	2.12 m	32.5 t	3.13 d (7.2)	41.0 t
14	5.16 brt (7.2)	120.6 d	5.17 brt (7.2)	120.6 d	5.16 brt (7.2)	120.6 d	5.28 brt (7.2)	115.9 d
15		135.0 s		135.6 s		135.3 s		135.4 s
16	1.74 brs	25.9 q	1.74 brs	25.5 q	1.74 brs	25.9 q	1.74 brs	25.6 q
17	1.64 brs	17.9 q	1.65 brs	17.5 q	1.64 brs	18.0 q	1.62 brs	18.0 q
18	0.90 d (6.8)	15.3 q	0.90 d (6.8)	15.1 q	0.90 d (6.8)	15.3 q	1.06 d (6.8)	16.1 q
19	1.59 brs	15.7 q	4.57 brs	61.5 t	4.57 brs	61.3 t	4.55 brs	61.7 t
20	1.68 brs	16.1 q	1.64 brs	15.7 q	1.69 brs	16.4 q	1.65 brs	16.4 q
19-OAc			2.05 s	20.5 q 170.8 s	2.05 s	21.0 q 171.0 s	2.05 s	20.8 q 171.0 s
1-OAc					2.04 s	21.0 q 171.0 s		



8a R = (-)-MTPA
8b R = (+)-MTPA



9a R = (-)-MTPA
9b R = (+)-MTPA



10a R = (-)-MTPA
10b R = (+)-MTPA

Figure 1. $\Delta\delta^{SR} = \delta_H(S) - \delta_H(R)$ for MTPA esters of compound **8**−**10** and spatial consequences.

summarized in Table 4. The α,β-unsaturated lactone is apparently the key active center in these sesquiterpenoids, and the number of acetoxy groups may also effect the cytotoxicity against SMMC-7721 cells in 12-hydroxygeranylgeraniol-derived diterpenes. Only very small quantities of **3** and **11** were obtained, and it was not possible to screen these for cytotoxicity.

Table 4. Cytotoxicity Data for Compounds **1**, **2**, and **4**−**10** (IC_{50}, μg/mL)

compound	SMMC-7721	HL-60	L02
1	46.3 ± 4.7	3.4 ± 0.4	72.4 ± 8.9
2	97.1 ± 13.3	11.8 ± 0.7	86.4 ± 15.8
4	44.8 ± 4.7	1.7 ± 0.2	23.1 ± 3.9
5	62.5 ± 9.3	14.3 ± 3.0	95.7 ± 10.1
6	29.7 ± 2.8	10.3 ± 1.2	57.5 ± 10.0
7	33.5 ± 4.9	3.2 ± 0.5	42.3 ± 5.4
8	>100	42.4 ± 4.6	>100
9	>100	>100	>100
10	12.5 ± 1.6	20.2 ± 3.1	31.3 ± 5.9
vincristine sulfate	26.7 ± 4.1	11.2 ± 1.9	28.4 ± 4.2

Experimental Section

General Experimental Procedures. Melting points were determined on an X-4 digital display micromelting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were taken on a Nicolet NEXUS 670 FT-IR TU-1901 spectrometer. The CD spectrum was recorded on an Olis RSM 1000 CD. NMR spectra were recorded on a Varian Mercury plus-400 NMR spectrometer with TMS as an internal standard. HRESIMS data were measured on a Bruker Daltonics APEX II 47e spectrometer. EIMS data were obtained on an HP5988 AGCMS spectrometer. Silica gel (200−300 mesh) used for column chromatography and silica gel GF_{254} (10−40 μm) used for TLC were supplied by the Qingdao Marine Chemical Factory, Qingdao, People's Republic of China. Spots were detected on TLC under UV light or by heating after spraying with 5% H_2SO_4 in C_2H_5OH.

Plant Material. The seeds of *C. triste* were collected from Chongqing, China, during August 2005. They were identified by Prof.

Guo-Liang Zhang, School of Life Sciences, Lanzhou University. A voucher specimen (No. 20050915) was deposited in the College of Chemistry and Chemical Engineering, Lanzhou University.

Extraction and Isolation. The air-dried seeds of *C. triste* (3.7 kg) were pulverized and extracted with CH_3OH three times (7 days each time) at room temperature. The extract was concentrated under reduced pressure, and the residue (130 g) was subjected to silica gel column chromatography (CC) (200−300 mesh, 1300 g) using a gradient of petroleum ether (bp 60−90 °C)−acetone (30:1, 15:1, 8:1, and 2:1) as eluent. Four fractions were collected according to TLC analysis. Fraction 2 (petroleum ether−acetone, 15:1, 20 g) was separated by silica gel CC (200−300 mesh, 200 g) with petroleum ether−EtOAc (10:1 and 8:1) as eluent to give fractions Fr2-1 and Fr2-2. Fraction Fr2-1 (petroleum ether−EtOAc, 10:1, 7.8 g) was purified by chromatography over a silica gel column (200−300 mesh, 78 g) with petroleum ether−EtOAc (18:1) as eluent to give fractions Fr2-1-1−Fr2-1-4, and **10** (15 mg) was obtained from Fr2-1-2. Fraction Fr2-1-4 (2.2 g) was further separated by repeated silica gel CC (200−300 mesh, 22 g) with petroleum ether−EtOAc (15:1) as eluent to obtain **11** (2 mg) and **9** (28 mg). Fraction Fr2-2 (petroleum ether−EtOAc, 8:1, 9.2 g) after chromatography on silica gel, with petroleum ether−EtOAc (12:1) as eluent, gave **8** (160 mg). Fraction 3 (petroleum ether−acetone, 8:1, 32 g) was further chromatographed on a silica gel column eluted with $CHCl_3$−EtOAc (1:0, 15:1, and 10:1) to afford fractions Fr3-1−Fr3-3. Fraction Fr3-2 ($CHCl_3$−EtOAc, 15:1, 26.1 g) was separated by silica gel CC using petroleum ether−EtOAc−MeOH (10:2:0.1) as eluent to obtain a mixture of **1** and **3** (203 mg) and **2** (55 mg). Compounds **3** (2 mg) and **1** (42 mg) were obtained by repeated silica gel CC of the mixture of **1** and **3** with petroleum ether−EtOAc−MeOH (10:2:0.1). Fraction 4 (petroleum ether−acetone, 2:1, 41 g) was separated by silica gel CC with $CHCl_3$−EtOAc (8:1 and 5:1) to give fractions Fr4-1 and Fr4-2. Fraction Fr4-1 ($CHCl_3$−EtOAc, 8:1, 15.6 g) was purified by repeated chromatography over silica gel with petroleum ether−EtOAc−MeOH (10:3:0.1) to afford a mixture of **6** and **4** (137 mg) and **5** (59 mg). The mixture of **6** and **4** was further chromatographed on silica gel eluted with petroleum ether−EtOAc−MeOH (10:2:0.1) to afford **6** (45 mg) and **4** (33 mg). Fraction 4-2 ($CHCl_3$−EtOAc, 5:1, 19.8 g) was chromatographed over silica gel with petroleum ether−EtOAc−MeOH (10:5:0.1) as eluent to give fractions Fr4-2-1−Fr4-1-4. Compound **7** was isolated (42 mg) by repeated silica gel CC of fraction Fr4-2-2 (3.3 g) with petroleum ether−EtOAc−MeOH (10:4:0.1).

8α-Angeloyloxy-4β-hydroxy-5β-isobutyryloxy-9-oxo-germacran-7β,12α-olide (1): colorless needles from EtOH; mp 160−161 °C; $[\alpha]^{20}_D$ +7.5 (*c* 0.80, $CHCl_3$); IR (KBr) ν_{max} 3516, 1776, 1726, 1645, 1127 cm^{-1}; CD in MeOH (*c*, 1.1 gL^{-1}) $[\theta]_{252}$ +7245, $[\theta]_{294}$ +14242; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 450 $[M]^+$ (11), 432 $[M - H_2O]^+$ (17), 350 $[M - HOAng]^+$ (31), 262 $[M - HOAng - HOiBu]^+$ (49), 268 (48), 250 (62), 207 (83), 194 (75), 179 (56), 165 (87), 153 (100), 123 (33), 97 (46); HRESIMS *m/z* 473.2141 $[M + Na]^+$ (calcd for $C_{24}H_{34}O_8Na$ 473.2146).

4β,8α-Dihydroxy-5β-isobutyryloxy-9β-3-methylbutyryloxy-3-oxo-germacran-7β,12α- olide (4): colorless needles from EtOH; mp 230−231 °C; $[\alpha]^{20}_D$ −88.0 (*c* 0.40, $CHCl_3$); IR (KBr) ν_{max} 3452, 1753, 1740, 1709, 1662, 1148 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 468 $[M]^+$ (10), 450 $[M - H_2O]^+$ (15), 366 $[M - HOMeBu]^+$ (24), 278 $[M - HOMeBu - HOiBu]^+$ (41), 268 (46), 250 (59), 207 (87), 194 (80), 179 (55), 165 (83), 153 (100), 123 (36), 97 (44); HRESIMS *m/z* 486.2694 $[M + NH_4]^+$ (calcd for $C_{24}H_{36}O_9NH_4$ 486.2698).

4β,8α-Dihydroxy-5β-2-methylbutyryloxy-9β-3-methylbutyryloxy-3-oxo-germacran-7β,12α-olide (6): colorless needles from EtOH; mp 225−226 °C; $[\alpha]^{20}_D$ −94.0 (*c* 0.50, $CHCl_3$); IR (KBr) ν_{max} 3431, 1751, 1740, 1661, 1146 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 482 $[M]^+$ (7), 464 $[M - H_2O]^+$ (12), 380 $[M - HOMeBu]^+$ (37), 278 $[M - 2\times HOMeBu]^+$ (42), 268 (61), 250 (60), 207 (80), 194 (93), 179 (61), 165 (100), 153 (73), 123 (40), 97 (49); HRESIMS *m/z* 500.2861 $[M + NH_4]^+$ (calcd for $C_{25}H_{38}O_9NH_4$ 500.2854).

4β,9β-Dihydroxy-5β,8α-di(isobutyryloxy)-3-oxo-germacran-7β,-12α-olide (7): colorless needles from EtOH; mp 220−221 °C; $[\alpha]^{20}_D$ −147.0 (*c* 0.40, $CHCl_3$); IR (KBr) ν_{max} 3475, 1739, 1662, 1142 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 454 $[M]^+$ (10), 436 $[M - H_2O]^+$ (15), 366 $[M - HOiBu]^+$ (24), 278 $[M - 2HOiBu]^+$ (36), 268 (42), 250 (63), 207 (79), 194 (84), 179 (52), 165 (88), 153 (100), 123 (37), 97 (41); HRESIMS *m/z* 472.2548 $[M + NH_4]^+$ (calcd for $C_{23}H_{34}O_9NH_4$ 472.2541).

(2*E*,6*E*,11*S*,12*R*)-3,7,11,15-Tetramethylhexadeca-2,6,14-triene-1,-12-diol (8): colorless oil; $[\alpha]^{20}_D$ −2.0 (*c* 0.30, $CHCl_3$); IR (KBr) ν_{max} 3357, 2964, 2927, 1667 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 3; HRESIMS *m/z* 331.2605 $[M + Na]^+$ (calcd for $C_{20}H_{36}O_2Na$ 331.2608).

(2*E*,6*Z*,11*S*,12*R*)-3,7,11,15-Tetramethylhexadeca-2,6,14-trien-7-[(acetyloxy)methyl]-1,12,19-triol (9): colorless oil; $[\alpha]^{20}_D$ −2.3 (*c* 0.15, $CHCl_3$); IR (KBr) ν_{max} 3408, 2963, 2929, 1738, 1670 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 3; HRESIMS *m/z* 389.2658 $[M + Na]^+$ (calcd for $C_{22}H_{38}O_4Na$ 389.2662).

(2*E*,6*Z*,11*S*,12*R*)-3,7,11,15-Tetramethylhexadeca-2,6,14-trien-7-[(acetyloxy)methyl]-12,19-diol-1-acetate (10): colorless oil; $[\alpha]^{20}_D$ −1.9 (*c* 0.60, $CHCl_3$); IR (KBr) ν_{max} 3404, 2924, 1738, 1664 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 3; HRESIMS *m/z* 431.2763 $[M + Na]^+$ (calcd for $C_{24}H_{40}O_5Na$ 431.2768).

(2*E*,6*Z*)-3,7,11,15-Tetramethylhexadeca-2,6,14-trien-7-[(acetyloxy)-methyl]-12-oxo-1,19-diol (11): colorless oil; $[\alpha]^{20}_D$ −1.2 (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 3406, 2927, 1708, 1668 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 3; HRESIMS *m/z* 387.2500 $[M + Na]^+$ (calcd for $C_{22}H_{36}O_4Na$ 387.2506).

Cytotoxicity Bioassays. Testing for *in vitro* cytotoxic activities of compounds **1**, **2**, and **4**−**10** against SMMC-7721 (human hepatoma), HL-60 (human promyelocytic leukemia), and L02 (human hepatocytes) cells was carried out according to the sulforhodamine B (SRB) method.[18] Briefly, exponentially growing cells were harvested and seeded in 96-well plates with the final volume 100 μL containing 5×10^3 cells per well. After 24 h incubation, cells were treated with various concentrations of those compounds (and vincristine sulfate used as a positive control) for 48 h. The absorbency of extracted sulforhodamine B at 515 nm was measured on a microplate reader (Bio-Rad). The experiments were carried out in triplicate. Each run entailed 5−6 concentrations of the compounds being tested. The percentage survival rates of cells exposed to the compounds were calculated by assuming the survival rate of untreated cells to be 100%.

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