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Cytotoxic Sesquiterpenoids from *Eupatorium chinense*

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Ten new sesquiterpenoids, namely, eupachinilides A–J (**1**–**10**), together with seven known sesquiterpenoids, eupachifolin D (**11**), budlein B (**12**), 8 β -(4'-hydroxytiglyloxy)-2 β -hydroxy-1 α H,5 α H,6 β H,7 α H-guai-3,10(14),11(13)-trien-6,12-olide (**13**), 1,10-hydrobahia (**14**), 2 α -hydroxyeupatolide (**15**), eupaserrin (**16**), and mollisorin B (**17**), were isolated from the whole plant of *Eupatorium chinense*. Their structures were elucidated mainly by spectral methods, especially 2D NMR techniques. Eupachinilides A (**1**), E (**5**), F (**6**), and I (**9**) exhibited moderate cytotoxic activities against several tumor cell lines. The structures assigned previously for eupachifolins B (**11a**), C (**13a**), and D (**11**) were revised by spectral analysis and 2D NMR techniques.

Plants of *Eupatorium chinense* L. (Compositae) and its two varieties, *E. chinense* var. *simplicifolium* and *E. chinense* var. *tripartitum*, are indigenous to China,¹ and its hot water extract is traditionally used for the treatments of cold, snakebite, and inflammation.² The methanolic extract of this plant showed anticancer activity against Hela cells *in vivo*.² The intriguing applications in traditional Chinese medicine and the reported anticancer activity of this plant have driven us to conduct the current investigation.

The *E. chinense* plant material used in the present study was collected from Songyang County of Zhejiang Province, China, where it is known as "Da-Fa-San", used for cold treatment by local residents and has a very bitter taste. Seven guaiane-type sesquiterpenoids were isolated previously from *E. chinense* L. var. *hakonense*,³ and a few sesquiterpenoids, eupachifolins A–E, were obtained from *E. chinense* var. *simplicifolium*.⁴ In the current research, 10 new sesquiterpenoids, namely, eupachinilides A–J (**1**–**10**), as well as seven known sesquiterpenoids, eupachifolin D (**11**), budlein B (**12**), 8 β -(4'-hydroxytiglyloxy)-2 β -hydroxy-1 α H,5 α H,6 β H,7 α H-guai-3,10(14),11(13)-trien-6,12-olide (**13**), 1,10-hydrobahia (**14**), 2 α -hydroxyeupatolide (**15**), eupaserrin (**16**), and mollisorin B (**17**), were isolated from the whole plant of *E. chinense*. Herein, we report the isolation and structural elucidation of these sesquiterpenoids and their cytotoxic activities. The revision of the structures assigned previously for eupachifolins B (**11a**), C (**13a**), and D (**11**) is also briefly discussed in this paper.

Results and Discussion

Eupachinilide A (**1**) was obtained as a white powder. The HREIMS spectrum of **1** showed the molecular ion at m/z 378.1633 to establish the molecular formula of C₂₀H₂₆O₇ (calcd 378.1679) with eight degrees of unsaturation. The ¹H and ¹³C NMR (Tables 1 and 2) revealed the presence of three methyls (δ_H 1.89, 3H, dd, J = 7.2, 1.0 Hz, δ_C 15.8; δ_H 1.78, 3H, s, δ_C 20.6; δ_H 1.59, 3H, s, δ_C 23.8), one hydroxymethylene (δ_H 4.18, 3.75; δ_C 65.9), three oxygenated methines (δ_H 3.87, δ_C 78.7; δ_H 4.40, δ_C 76.8; δ_H 5.70, δ_C 65.2), one exocyclic double bond (δ_H 6.24, 5.57, δ_C 120.7; δ_C 135.0), one trisubstituted double bond (δ_H 6.06, δ_C 138.9; δ_C 127.4), one persubstituted double bond (δ_C 141.3 and

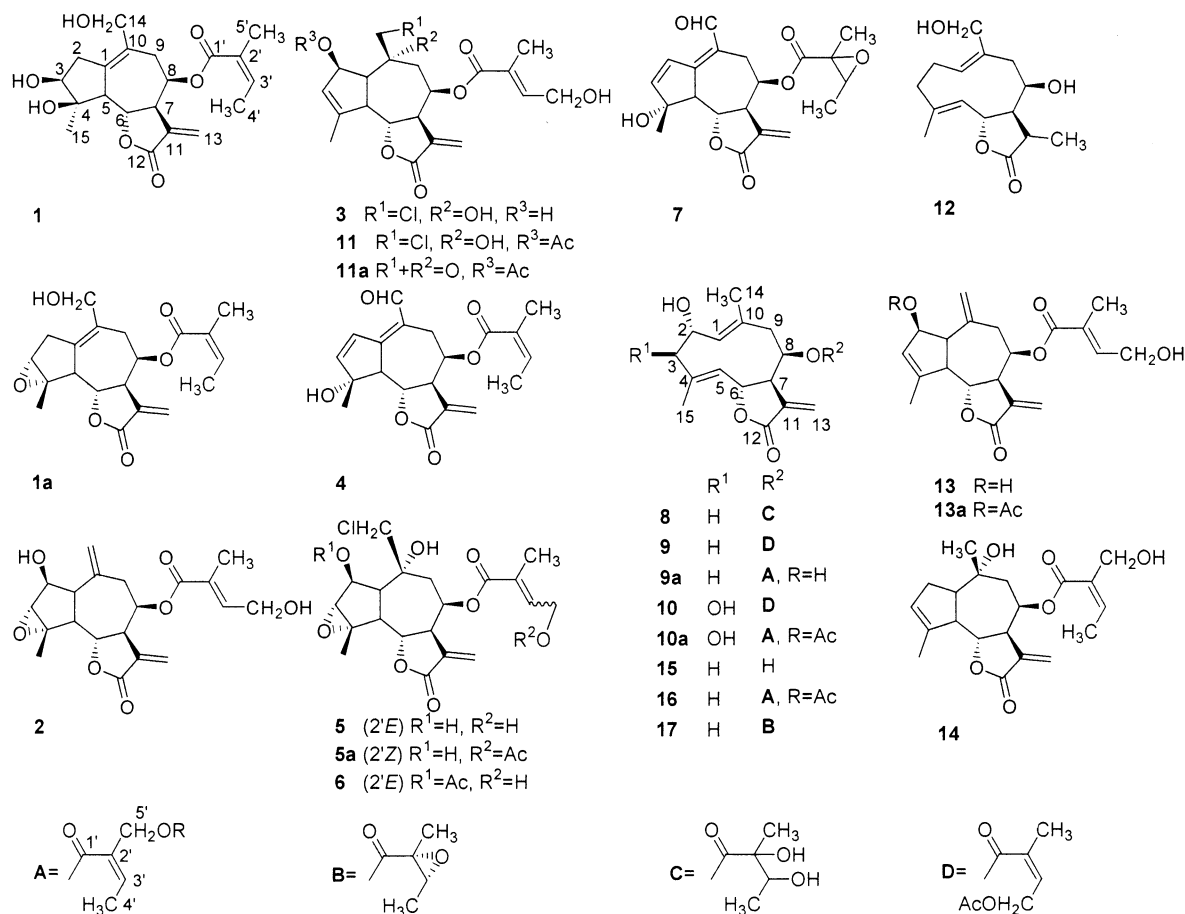
130.0), two carbonyls (δ_C 170.0 and 167.1), and one sp³ oxygenated quaternary carbon (δ_C 82.6). Three double bonds and two carbonyls accounted for five degrees of unsaturation. The remaining three degrees of unsaturation were attributed to a tricyclic ring system in compound **1**.

Comparison of the NMR data of **1** with those of euponin⁵ (**1a**), which was also isolated from this genus (*E. japonicum*), suggested that **1** was a tricyclic guaiane-type sesquiterpenoid. In the HMBC spectrum (Figure 1a), H₃-5' (δ 1.78, 3H, s) correlated with C-1' (δ 167.1), C-2' (δ 127.4), and C-3' (δ 138.9) and H₃-4' (δ 1.89, 3H, dd, J = 7.2, 1.0 Hz) correlated with C-2' and C-3', indicating the presence of a 2'-methyl-2'-butenoxyl moiety. In the NOESY spectrum (Figure 1b), H-3' showed cross-peaks with both H₃-4' and H₃-5', clearly inferring a 2'-methyl-2'-Z-butenoxyl moiety (angelyloxyl). The downfield shifted proton signal at δ 4.40 was assigned to H-6, and this inferred the presence of a five-membered lactone with an exocyclic double bond as in euponin,⁵ and this was supported by HMBC correlations. In the HMBC spectrum, the proton signal at δ 6.24 (H-13a) correlated with C-11 (δ_C 135.0) and C-12 (δ 170.0); H-6 correlated with C-5 (δ 54.6) and C-11; and H-7 at δ 3.13 correlated with the C-6 (δ 76.8). One oxygenated methine was assigned to CH-8 (δ_H 5.70; δ_C 65.2) bearing the 8-angelyloxyl moiety by HMBC. H-8 correlated with C-7 (δ 54.5), C-11, and C-1', and the downfield shifted proton signal of H-8 supported this connectivity. A $\Delta^{1(10)}$ double bond was assignable on the basis of the HMBC correlations, in which, H₂-2 (δ 2.91, 2.54), H-5 (δ 2.88), H₂-9 (δ 2.98, 2.45), and H₂-14 (δ 4.18, 3.75) showed correlations with both C-1 (δ 141.3) and C-10 (δ 130.0). The oxygenated methine (δ_H 3.87, δ_C 78.7) was attributable to C-3 bearing a hydroxyl judging from the HMBC correlations between H-3 and C-2 (δ 38.0) and between H-3 and C-4. The remaining hydroxyl was placed on the quaternary carbon C-4 by HMBC correlations with H-3, H-5, and H₃-15.

The relative stereochemistry of **1** was fixed by extensive analysis of NOESY correlations (Figure 1b), in which H-5 correlated with H-7, H-2b, H-3, and H₃-15, indicating that H-7, H-2b, H-3, and H₃-15 were in α -orientation since the H-5 of the guaiane-type sesquiterpenoid is defined in the α -configuration. The H-2a was assigned as a β -configuration, and it showed a key correlation with H-6, suggesting the β -orientation of H-6. The NOESY correlation between H-7 α and H-8 indicated that H-8 was α -orientated. A 3D structure (Figure 1c) of **1** was generated by computer

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Chart 1



modeling using the molecular modeling program CS Chem 3D Pro Version 6.0, and the MM2 force field calculation was applied for energy minimization. The relative stereochemistry and a favorable conformation of **1** offered by computer modeling were consistent with those of **1** assigned in the NOESY experiment. The structure of eupachinilide A (**1**) was thus unambiguously elucidated as 8 β -angelyloxy-3 β ,4 β ,14-trihydroxy-5 α H,6 β H,7 α H-guai-1(10),11(13)-diene-6,12-olide.

Eupachinilide B (**2**) was determined to have a molecular formula $C_{20}H_{24}O_7$ by HREIMS. The carbon signals (Table 2) assigned for the seven-membered ring, five-membered lactone, and the ester moiety were very close to those of eupachifolin C (**13a**)⁴ isolated from *E. chinense* var. *simplicifolium*, implying that the partial structure (the aforementioned three subunits) in both compounds was identical. This was consistent with the 2D NMR experiments (HMBC, HMQC, and NOESY; see Figure 2a,b) of **2**. A methine group attributable to CH-3 (δ_C 65.1, δ_H 3.33) and a quaternary carbon assigned for C-4 (δ_C 66.2) indicated the presence of an 3,4-epoxyl and was supported by the HMBC correlations of H₃-15 (δ 1.65, 3H, s) with C-4, C-3, and C-5 (δ 49.5). A carbon signal at δ 75.9 was allocated to the C-2 bearing a hydroxyl as judged by HMBC correlations between H-1 (δ 2.95) and C-2 and between H-3 (δ 3.33) and C-2.

The stereochemistry of **2** was determined via a NOESY experiment (Figure 2b,c) in which H₃-15 correlated with H-6 having been defined in the β -orientation, and as a consequence, the epoxyl was α -orientated; H-2 correlating with H-1 was assigned as α -orientated. The H₂-4' correlating with both H-3' (δ 6.68) and H₃-5' clearly revealed the

E geometry of the ester moiety. The *E* geometry of the ester moiety assigned for **2** was also supported by the relatively downfield shifted proton signal of H-3'. In similar compounds having *E* geometry, H-3' normally appears at δ 6.60–7.15,⁶ while for those with *Z* geometry, the chemical shift of H-3' usually appears around δ 6.04.^{6a} The structure of eupachinilide B (**2**) was therefore identified as 8 β -(4'-hydroxytyglyloxy)-3 α ,4 α -epoxy-2 β -hydroxy-1 α H,5 α H,6 β H,7 α H-guai-1(10),11(13)-diene-6,12-olide (**2**).

Eupachinilide C (**3**) showed a molecular formula $C_{20}H_{25}ClO_7$ as determined by HREIMS. The EIMS ions at m/z 396 (2%) and 394 (6%) [$M - H_2O$]⁺ supported the occurrence of an atom of chlorine in the molecule. Twenty carbon signals were resolved in its ¹³C NMR spectrum. A chloromethylene (δ_C 55.9, δ_H 3.87, and δ_H 3.64) was distinguished by its somewhat upfield shifted carbon signal^{6b,7} and nearly unchanged proton signals^{6–8} compared with those of an oxygenated methylene. A guaiane-type skeleton was elucidated by analysis of its ¹H NMR and ¹³C NMR data. Comparison of the EIMS, ¹H NMR, and ¹³C NMR data of **3** with those of a known compound, eupachifolin D⁴ (**11**), indicated that **3** was deacetyl eupachifolin D. The H-2 signal (δ 4.54) in **3** was upfield shifted about $\Delta\delta$ 0.84 ppm compared with that of **11**, supporting the structure of **3**. The 2D NMR spectra of **3**, including HMQC, HMBC, and NOESY, were applied to further confirm the structure of eupachinilide C (**3**).

Eupachinilide D (**4**) showed a molecular formula $C_{20}H_{22}O_6$ as determined by HREIMS. Analysis of 1D and 2D NMR spectra (¹H, ¹³C NMR, HMQC, HMBC, and NOESY) inferred that compound **4** had the same ester moiety, seven-membered ring, and five-membered lactone as that

Table 1. ¹H NMR Chemical Shifts (δ) for Compounds **1–10**

	1^a (multi, <i>J</i> (Hz))	2^a (multi, <i>J</i> (Hz))	3^b (multi, <i>J</i> (Hz))	4^a (multi, <i>J</i> (Hz))	5^c (multi, <i>J</i> (Hz))	6^a (multi, <i>J</i> (Hz))
1		2.95 (dd, 9.5, 5.2)	2.50 (t, 6.1)		2.31 (m)	2.64 (br d, 8.8)
2	2.91 (br d, 11.0) 2.54 (br d, 16.4)	4.31 (br d, 5.5)	4.54 (m)	7.10 (d, 5.8)	4.17 (br d, 4.8)	5.29 (br d, 3.2)
3	3.87 (d, 3.8)	3.33 (br s)	5.73 (br s)	6.59 (dd, 5.8, 0.7)	3.23 (s)	3.37 (d, 1.0)
5	2.88 (d, 9.2)	2.32 (dd, 11.3, 8.7)	2.73 (dd, 10.6, 7.3)	3.41 (dd, 11.3, 2.2)	2.47 (dd, 11.5, 8.0)	2.67 (d, 9.1)
6	4.40 (t, 10.5)	4.81 (dd, 11.3, 8.2)	4.68 (dd, 10.6, 8.5)	4.56 (t, 10.8)	4.76 (dd, 11.5, 8.8)	4.69 (dd, 10.7, 8.8)
7	3.13 (dd, 10.5, 1.6)	3.15 (m)	4.01 (m)	3.19 (m)	3.96 (m)	4.00 (m)
8	5.70 (d, 5.9)	5.44 (m)	5.67 (m)	5.91 (br d, 4.8)	5.54 (m)	5.67 (d, 9.1)
9	2.98 (dd, 15.6, 5.9)	2.89 (2H, br d, 7.2)	2.55 (dd, 14.7, 8.3)	3.76 (dd, 16.7, 5.1)	2.47 (dd, 14.3, 8.8)	2.51 (dd, 14.8, 8.9)
	2.45 (d, 15.6)		2.39 (ddd, 14.7, 8.4, 1.5)	2.21 (br d, 6.6)	2.32 (m)	1.96 (dd, 14.8, 7.3)
13	6.24 (d, 3.1) 5.57 (d, 2.8)	6.27 (d, 3.5) 5.48 (d, 3.1)	6.18 (d, 3.6) 5.45 (d, 3.3)	6.30 (d, 3.3) 5.73 (d, 3.0)	6.17 (d, 4.0) 5.38 (d, 3.3)	6.28 (d, 3.7) 5.46 (d, 3.3)
14	4.18 (d, 11.5) 3.75 (d, 11.5)	5.05 (d, 1.4) 5.03 (br s)	3.87 (d, 11.7) 3.64 (d, 11.7)	10.09 (s)	3.65 (d, 11.4) 3.52 (d, 11.4)	3.53 (d, 11.5) 3.49 (d, 11.5)
15	1.59 (3H, s)	1.65 (3H, s)	2.00 (3H, s)	1.57 (3H, s)	1.63 (3H, s)	1.69 (3H, s)
3'	6.06 (qd, 7.2, 1.0)	6.68 (br t, 6.0)	6.69 (br t, 5.9)	6.03 (br q, 7.2)	6.62 (br t, 5.9)	6.71 (br t, 5.9)
4'	1.89 (3H, dd, 7.2, 1.0)	4.29 (2H, dd, 6.0, 0.9)	4.21 (2H, br d, 5.9)	1.85 (3H, br.d, 7.3)	4.19 (dd, 5.9, 1.1)	4.33 (2H, dd, 6.0, 1.1)
5'	1.78 (3H, s)	1.76 (3H, d, 1.1)	1.74 (3H, d, 1.4)	1.72 (3H, br.s)	1.68 (3H, d, 1.1)	1.79 (3H, d, 1.1)
COMe						2.08 (3H, s)
	7^a (multi, <i>J</i> (Hz))	8^a (multi, <i>J</i> (Hz))	9^a (multi, <i>J</i> (Hz))	10^a (multi, <i>J</i> (Hz))	10^{a,d} (multi, <i>J</i> (Hz))	13^{a,d} (multi, <i>J</i> (Hz))
1		4.97 (d, 10.1)	5.03 (d, 9.9)	5.09 (br d, 9.9)	5.10 (br d, 9.5)	3.42 (dd, 8, 6)
2	7.09 (d, 5.9)	4.76 (dd, 9.7, 5.8)	4.71 (ddd, 9.9, 9.9, 5.9)	4.42 (dd, 9.9, 7.6)	4.45 (dd, 9.5, 8)	5.58 (m)
3	6.61 (dd, 5.8, 0.9)	2.74 (dd, 11.1, 5.8) 2.13 (t, 10.5)	2.70 (dd, 10.0, 5.8) 2.11 (t, 10.3)	4.10 (d, 7.6)	4.12 (d, 8)	5.64 (br s)
5	3.40 (dd, 11.3, 2.2)	5.08 (d, 9.9)	4.98 (d, 10.2)	5.04 (br d, 10.1)	5.04 (br d, 10)	2.72 (m)
6	4.53 (t, 10.8)	5.05 (t, 8.6)	5.06 (dd, 10.0, 8.7)	5.12 (dd, 10.1, 8.5)	5.13 (dd, 10, 9)	4.59 (dd, 11, 8.5)
7	3.17 (m)	2.99 (m)	2.97 (m)	2.96 (m)	2.96 (m)	3.18 (m)
8	5.90 (br d, 4.8)	5.81 (br s)	5.76 (br d, 4.0)	5.77 (br d, 3.3)	5.86 (br dd, 5, 2, <1)	5.56 (m)
9	3.74 (dd, 16.8, 5.2) 2.20 (br d, 6.8)	2.87 (dd, 14.7, 5.0) 2.42 (dd, 14.7, 2.5)	2.82 (dd, 14.4, 5.1) 2.34 (dd, 14.5, 2.3)	2.82 (dd, 14.5, 4.9) 2.34 (dd, 14.5, 2.5)	2.87 (dd, 15, 5) 2.39 (dd, 15, 2)	2.79 (dd, 14, 6.5) 2.53 (14, 8)
13	6.29 (d, 3.3) 5.65 (d, 3.0)	6.36 (d, 3.5) 5.64 (d, 2.9)	6.28 (d, 3.5) 5.58 (d, 3.1)	6.32 (d, 3.6) 5.62 (d, 3.0)	6.34 (d, 3.5) 5.64 (d, 3.0)	6.26 (d, 3.8) 5.48 (d, 3)
14	10.08 (s)	1.55 (3H, s)	1.50 (3H, s)	1.54 (3H, s)	1.60 (3H, br)	5.08 (br s) 5.03 (br s)
15	1.38 (3H, s)	1.80 (3H, d, 1.1)	1.87 (3H, dd, 3.3, 1.6)	1.89 (3H, m)	1.87 (3H, br)	1.99 (3H, s)
3'	2.96 (q, 5.4)	4.11 (q, 6.8)	6.05 (br t, 5.2)	6.08 (br t, 5.2)	6.56 (br q, 7)	6.67 (m)
4'	1.13 (3H, d, 5.4)	1.50 (3H, d, 6.8)	4.98 (2H, m)	5.02 (m)	2.15 (3H, d, 7)	4.32 (2H, br d, 5.8)
5'	1.61 (3H, s)	1.39 (3H, s)	1.75 (3H, d, 1.5) 2.05 (3H, s)	4.94 (m) 1.80 (3H, d, 1.1) 2.07 (3H, s)	4.69 (2H, AB spin) 2.01 (3H, s)	1.78 (3H, br s) 1.99 (3H)
COMe						

^a Measured in CDCl₃. ^b Measured in CD₃OD. ^c Measured in CDCl₃+CD₃OD. ^d Literature data.

of compound **1**. An aldehyde group was easily identified by the presence of a proton signal at δ 10.09 and a carbon signal at δ 190.1. The proton signal at δ 10.09 correlating with the C-10 at δ 128.6 in the HMBC (Figure 3a) was assigned to the H-14 of the aldehyde. The HMBC correlations indicated that a connectivity of two double bonds and the aldehyde group constructed a conjugated system, and the persubstituted double bond (δ 160.5, 128.6) and the disubstituted double bond (δ 151.4, 125.8) were assigned to Δ¹⁽¹⁰⁾ and Δ², respectively. A quaternary carbon signal at δ 82.6 was assigned to C-4 bearing a hydroxyl, and this was verified by the HMBC correlations of H₃-15 (δ 1.57, 3H, s) with C-3, C-4, and C-5 (δ 61.6). The stereochemistry of **4** was interpreted by NOESY (Figure 3b,c). The structure of **4** was thereby established as 8β-angelyloxy-4α-hydroxy-14-oxo-5α*H*,6β*H*,7α*H*-guai-2,10(14),11(13)-triene-6,12-olide. The complete assignments of ¹H NMR and ¹³C NMR data were achieved by a combination of 1D and 2D NMR spectra (¹H, ¹³C NMR, HMQC, HMBC, and NOESY).

Eupachinilide E (**5**) had the molecular formula C₂₀H₂₅ClO₈ as determined by HREIMS. Comparison of its ¹H and ¹³C NMR data (Tables 1 and 2) with those of compound **3** implied the structures of the two compounds were closely related; the only difference was the presence of one epoxy group in **5** rather than the double bond in compound **3**. An oxygenated methine group (δ_C 64.0, δ_H 2.47) and an oxygenated quaternary carbon at δ 65.6 suggested the presence of a 3,4-epoxyl group in **5**. Direct comparison of the ¹H NMR data of **5** with those of graminichlorin⁶ (**5a**) showed very good similarity of the sesquiterpenoid part, supporting the structural assignment for compound **5**. In comparison with H-3' (δ 6.03) of graminichlorin, H-3' (δ 6.62) of **5** was downfield shifted, indicating that the ester moiety of **5** also adopted *E* geometry. Hence, the structure of eupachinilide E (**5**) was elucidated to be 8β-(4'-hydroxy-tiglyloxy)-14-chlorine-3α,4α-epoxy-2β,10α-dihydroxy-1α*H*,5α*H*,6β*H*,7α*H*-guai-11(13)-ene-6,12-olide. The structure of **5** was

Table 2. ^{13}C NMR Data (δ) for Compounds **1**–**10**

	1 ^a	2 ^a	3 ^b	4 ^a	5 ^c	6 ^a	7 ^a	8 ^a	9 ^a	10 ^a	10a ^{a,d}	13a ^{a,d}
1	141.3	51.5	56.5	160.5	48.3	49.3	160.9	134.6	134.1	126.2	126.16	50.7
2	38.0	75.9	76.0	125.8	70.8	72.9	125.6	69.4	69.1	74.6	74.79	80.3
3	78.7	65.1	130.9	151.4	64.0	60.9	151.7	48.6	48.6	83.5	83.61	126.3
4	82.6	66.2	150.1	82.6	65.6	65.1	82.6	142.6	142.7	143.7	144.23	148.2
5	54.6	49.5	53.3	61.6	49.6	47.4	61.6	129.7	129.2	131.6	132.19	56.0
6	76.8	77.2	84.3	76.2	77.5	76.7	75.7	74.8	75.4	74.9	75.03	80.0
7	54.5	48.2	49.1	53.0	47.3	46.3	52.8	52.9	52.9	52.6	52.64	48.0
8	65.2	68.7	69.1	63.6	67.1	66.6	64.4	74.2	71.7	71.6	71.82	68.0
9	34.3	37.5	37.6	29.1	35.8	36.3	28.7	44.2	43.7	43.9	44.06	39.0
10	130.0	140.5	75.0	128.6	73.1	74.1	127.8	134.3	134.6	136.3	136.62	139.1
11	135.0	133.9	137.2	134.7	134.5	134.0	134.2	135.3	136.2	135.9	135.69	134.0
12	170.0	169.5	172.0	168.6	170.0	169.1	169.0	169.2	169.3	170.8	170.52	169.2
13	120.7	122.7	122.6	121.7	121.6	122.1	121.3	122.4	121.3	121.9	121.57	122.4
14	65.9	120.4	55.9	190.1	54.9	54.9	189.8	20.5	19.6	13.5	13.49	120.1
15	23.8	18.3	18.7	25.3	18.8	18.7	25.3	18.8	18.6	19.7	15.94	17.2
1'	167.1	166.8	168.7	166.4	167.0	166.4	160.9	173.6	165.3	165.3	164.44	166.5
2'	127.4	127.7	129.1	127.1	127.2	127.6	59.4	77.3	127.3	127.3	127.06	127.8
3'	138.9	141.2	143.5	139.0	141.6	141.4	59.7	62.1	140.9	141.0	146.89	141.1
4'	15.8	59.5	60.2	15.9	58.9	59.6	13.7	18.0	62.8	62.9	19.92	59.6
5'	20.6	12.7	13.2	20.4	12.3	12.8	19.1	22.7	19.7	20.1	65.41	12.7
COMe									170.7	169.4	169.56	ND
COMe									20.8	20.8	20.68	21.4

^a Measured in CDCl_3 . ^b Measured in CD_3OD . ^c Measured in $\text{CDCl}_3 + \text{CD}_3\text{OD}$. ^d Literature data. ND: No data were reported in the literature.

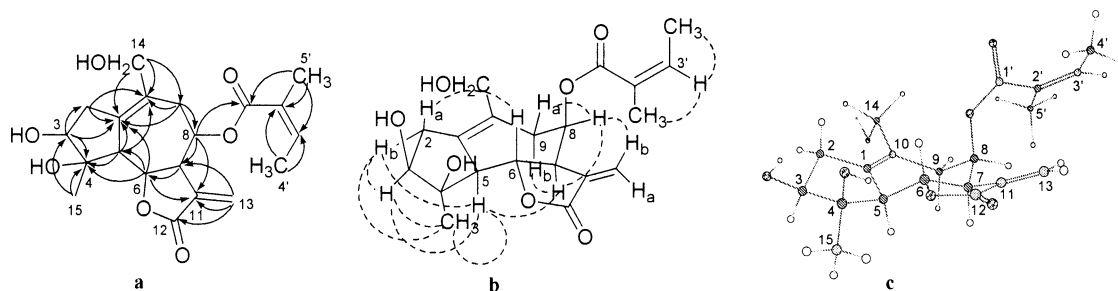


Figure 1. (a) Selected HMBC correlations (H–C) of compound **1**. (b) Key NOE interactions (---) of compound **1**. (c) Stereoview of compound **1** generated by computer modeling.

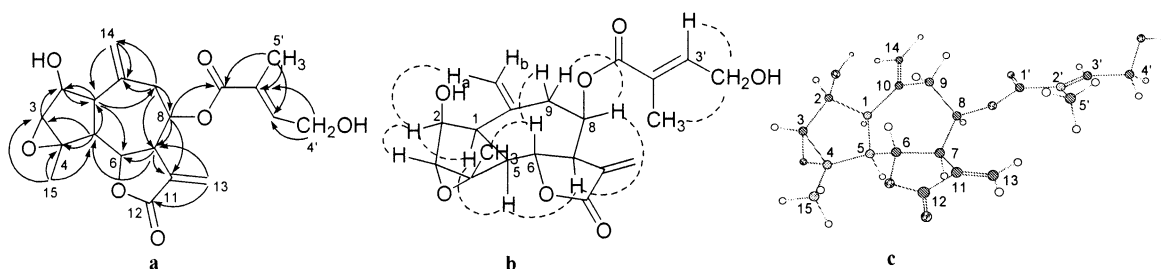


Figure 2. (a) Selected HMBC correlations (H–C) of compound **2**. (b) Key NOE interactions (---) of compound **2**. (c) Stereoview of compound **2** generated by computer modeling.

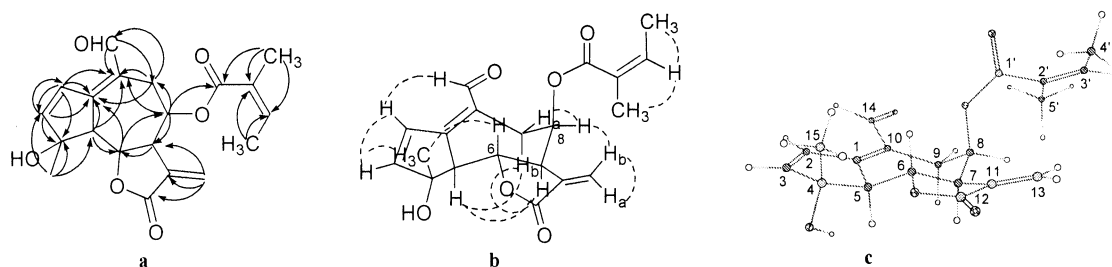


Figure 3. (a) Selected HMBC correlations (H–C) of compound **4**. (b) Key NOE interactions (---) of compound **4**. (c) Stereoview of compound **4** generated by computer modeling.

further confirmed by 2D NMR techniques (HMBC, HMQC, and NOESY).

Eupachinilide F (**6**) had a molecular formula $\text{C}_{22}\text{H}_{27}\text{ClO}_9$ as determined by HREIMS. The ^1H and ^{13}C NMR data of

eupachinilide F (**6**) were very similar to those of **5**, except for the presence of one more acetyl (δ_{C} 169.5, 21.2; δ_{H} 2.08, 3H, s). Compared with **5**, H-2 of **6** was downfield shifted, $\Delta\delta$ 1.12 ppm, indicating that the acetoxy group was

connected to C-2. The structure of eupachinilide F was thus elucidated to be 8 β -(4'-hydroxytiglyloxy)-2 β -acetoxy-14-chlorine-3 α ,4 α -epoxy-10 α -hydroxy-1 α H,5 α H,6 β H,7 α H-guai-11(13)-ene-6,12-olide (**6**).

Eupachinilide G (**7**) showed the molecular formula C₂₀H₂₂O₇ as determined by HREIMS. Extensive analysis of the ¹H and ¹³C NMR data of **7** established that the backbone of the sesquiterpenoid was identical with that of **4**. Compound **7** had an ester moiety (2',3'-epoxy-2'-methylbutanonyl) as judged from the ¹H and ¹³C NMR data, which was in good agreement with literature data.⁹ Thus, eupachinilide G (**7**) was identified as 8 β -(2',3'-epoxy-2'-methylbutanonyl)-4 α -hydroxy-14-oxo-5 α H,6 β H,7 α H-guai-1(10),2,11(13)-triene-6,12-olide.

Eupachinilides H (**8**) and I (**9**) were determined to have molecular formulas C₂₀H₂₈O₇ and C₂₂H₂₈O₇ by HREIMS, respectively. The ¹H and ¹³C NMR data of **8** and **9** indicated that both compounds were sesquiterpenoids, similar to eupaserrin (**16**), mollisorin B (**17**), and deacetyeupaserrin (**9a**).^{9,10}

The differences of these compounds (**8**–**10**, **15**–**17**, and **9a**) were in the ester moieties. For compound **8**, methyl signals at δ 1.50 (d, 3H, J = 6.8 Hz) and 1.39 (3H, s) and a proton signal at δ 4.11 (q, J = 6.8 Hz) indicated the ester moiety was 2',3'-dihydroxy-2'-methylbutanoxyl. The downfield shifted proton signal of H-8 (δ 5.76) placed the 2',3'-dihydroxy-2-methylbutanoxyl at C-8. The structure of eupachinilide H (**8**) was elucidated as 8 β -(2',3'-dihydroxy-2'-methylbutanoxyl)-2 α -hydroxy-6 β H,7 α H-germacra-1(10)-E,4E,11(13)-triene-6,12-olide. The ester moiety of eupachinilide I (**9**) was also determined by ¹H and ¹³C NMR data as 4'-acetoxy-2'-methylbutenoxyl with *Z* geometry as judged from the chemical shift of H-3' at δ 6.05.^{6a} The structure of eupachinilide I (**9**) was thus elucidated to be 8 β -(4'-acetoxyangeloxyl)-2 α -hydroxy-6 β H,7 α H-germacra-1(10)-E,4E,11(13)-triene-6,12-olide.

Eupachinilide J (**10**) had a molecular formula C₂₀H₂₈O₈ (HREIMS). The ¹H and ¹³C NMR data indicated that eupachinilide J (**10**) was a germacradiene-type sesquiterpenoid. The 2D NMR experiments, including HMQC, HMBC, and NOESY spectra, indicated that the sesquiterpenoid core of **10** was identical to that of compound **10a** (see Tables 1 and 2),¹¹ and the only difference between the two compounds was the ester moieties at C-8. The C-8 ester of eupachinilide J (**10**) was assigned as a 4'-acetoxyangeloxyl moiety by 1D and 2D NMR. Thus, the structure of eupachinilide J (**10**) was determined to be 8 β -(4'-acetoxyangeloxyl)-2 α ,3 β -dihydroxy-6 β H,7 α H-germacra-1(10)-E,4E,11(13)-triene-6,12-olide.

The C-15 signal of **10** was observed at δ 19.7 in the current research, and that of **10a** was given as δ 15.94 in the literature.¹¹ This chemical shift of C-15 at δ 15.94 assigned for compound **10a** in the literature was most likely a typographical error (δ 19.54, not δ 15.94).

Seven known sesquiterpenoids, eupachifolin D (**11**),⁴ budlein B (**12**),¹² 8 β -(4'-hydroxytiglyloxy)-2 β -hydroxy-1 α H,5 α H,6 β H,7 α H-guai-3,10(14),11(13)-trien-6,12-olide (**13**),¹³ 1,10-hydrobahia (**14**),¹⁴ 2 α -hydroxyeupatolide (**15**),⁹ eupaserrin (**16**),^{9,10} and deacetyeupaserrin (**17**),¹⁰ were identified mainly on comparison of NMR spectral data with literature values.

As shown in Tables 1 and 2, the ¹H and ¹³C NMR data of the ester moiety of **2** were very close to those of eupachifolins B (**11a**), C (**13a**), and D (**11**), indicating that the ester moieties in the four compounds adopted the same *E* geometry. The *E* geometry of the 4'-hydroxy-2'-methylbutenoxyl group assigned for **2** was mainly based on the

Table 3. Cytotoxic Activities of Compounds **1**, **5**, **6**, and **9** against Tumor Cell Lines

	IC ₅₀ (μg/mL)	
	HL-60	BEL-7402
1	10.8	72.2
5	1.30	18.0
6	0.87	3.7
9	0.94	3.6
hydroxycamptothecine ^a	0.024	0.62

^a Hydroxycamptothecine was used as positive control.

NOESY correlations and was supported by the relatively downfield shifted proton signal of H-3' due to the *E* geometry; H-3' normally appears at δ 6.60–7.15,⁶ while for the *Z* geometry, the chemical shift of H-3' usually appears around δ 6.04.^{6a} Therefore, the structures of eupachifolins B–D (**11a**, **13a**, and **11**) reported in the literature⁴ with *Z* geometry of the 4'-hydroxy-2'-methylbutenoxyl moiety should be revised as *E* geometry. Eupachifolin D (**11**) was isolated from the current plant material.

Compounds **1**, **5**, **6**, and **9** were evaluated for their cytotoxic activities according to standard protocols,¹⁵ and hydroxycamptothecine was used as positive control. Compounds **1**, **5**, **6**, and **9** showed cytotoxic activities against HL-60 and BEL-7402 tumor cell lines (see Table 3).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter (Na filter, λ = 589 nm). IR spectra were recorded on a Perkin-Elmer 577 spectrometer with KBr disk. ¹H NMR and ¹³C NMR spectra were obtained on a Bruker AM-400 spectrometer with TMS as internal standard. EIMS (70 eV) were carried out on a Finnigan MAT 95 instrument. All solvents used were of analytical grade (Shanghai Chemical Plant). Silica gel (200–300 mesh), silica gel H60, and Sephadex LH-20 were used for column chromatography, and precoated silica gel GF₂₅₄ plates (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) were used for TLC. C18 reversed-phase silica gel (250 mesh, Merck) and MCI gel (CHP20P, 75–150 μ , Mitsubishi Chemical Industries Ltd.) were also used for column chromatography.

Plant Material. *Eupatorium chinense* L. was collected from Songyang County of Zhejiang Province, the People's Republic of China, and was identified by Prof. Z.-T. Wang and Dr. M. Zhang of Shanghai Traditional Chinese Medical University. A voucher specimen has been deposited in the Herbarium of Shanghai Institute of Materia Medica (accession number Eup-2001-1Y).

Extraction and Isolation. The whole plant (2.2 kg) of *E. chinense* L. was ground and percolated with 95% EtOH. After removal of the EtOH under reduced pressure, a dark green residue (194.2 g) remained. The residue was subjected to silica gel column chromatography using a gradient solvent system of petroleum/acetone (10:0 to 0:10; v/v) to give 10 major fractions (1–10). Fraction 4 (3.22 g) was chromatographed sequentially over reversed-phase silica gel (aqueous MeOH, 50%) and silica gel columns (CHCl₃/MeOH, 100:1 to 10:1) to yield compounds **4** (80 mg), **6** (60 mg), **16** (30 mg), **9** (40 mg), and **17** (160 mg). Fraction 5 was recrystallized from aqueous MeOH (40%) to afford **15** (690 mg), and the liquid phase was subjected to a reversed-phase silica gel column (aqueous MeOH, 45%) to collect the major fractions. Each major fraction was purified by Sephadex LH-20 column chromatography (MeOH) and then a silica gel column chromatography (CHCl₃/MeOH, 10:1, v/v) to afford **3** (7 mg), **7** (5 mg), **10** (40 mg), **11** (50 mg), **12** (6 mg), **13** (30 mg), and **14** (80 mg). Fraction 6 was fractionated by a MCI gel column (aqueous MeOH, 45%) to give several fractions, each of which was further purified by RP-18 silica gel column chromatography (40% MeOH) and

then a silica gel column ($\text{CHCl}_3/\text{MeOH}$, 7.5:1) to afford **1** (80 mg), **2** (80 mg), **5** (70 mg), and **8** (20 mg), respectively.

Eupachinilide A (1): white powder; $[\alpha]_D^{20} -65.8^\circ$ (c 0.58, in CH_3OH); IR (KBr) ν_{max} 3408, 2929, 1767, 1705, 1647, 1458, 1387, 1234, 1155, 1084, 1051, 987, 864, 605 cm^{-1} ; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS m/z $[\text{M}]^+$ 378 (1), 278 (4), 260 (91), 242 (17), 227 (13), 217 (26), 199 (23), 186 (100), 173 (18), 143 (11), 91 (10), 83 (93), 55 (57); HREIMS m/z 378.1633 (calcd for $\text{C}_{20}\text{H}_{26}\text{O}_7$, 378.1679).

Eupachinilide B (2): white powder; $[\alpha]_D^{20} -67.1^\circ$ (c 0.50, CH_3OH); IR (KBr) ν_{max} 3450, 2937, 1755, 1713, 1637, 1385, 1336, 1255, 1140, 1013, 824, 589 cm^{-1} ; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS m/z 376 $[\text{M}]^+$ (7), 358 (16), 347 (28), 329 (11), 260 (21), 242 (23), 231 (28), 213 (30), 199 (33), 171 (38), 145 (30), 129 (37), 117 (26), 99 (100), 91 (43), 71 (80); HREIMS m/z 376.1526 (calcd for $\text{C}_{20}\text{H}_{24}\text{O}_7$, 376.1522).

Eupachinilide C (3): gum; $[\alpha]_D^{20} -66.9^\circ$ (c 0.50, CH_3OH); IR (KBr) ν_{max} 3435, 2924, 1751, 1713, 1653, 1402, 1321, 1259, 1140, 1063, 1013, 818, 739 cm^{-1} ; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS m/z 412 $[\text{M}]^+$ (3), 396 $[\text{C}_{20}\text{H}_{25}^{37}\text{ClO}_7 - \text{H}_2\text{O}]^+$ (2), 394 $[\text{M} - \text{H}_2\text{O}]^+$ (6), 376 (2), 361 (3), 345 (13), 328 (25), 278 (16), 229 (30), 199 (30), 99 (100), 87 (89), 69 (86); HREIMS m/z 412.1269 (calcd for $\text{C}_{20}\text{H}_{25}\text{ClO}_7$, 412.1289).

Eupachinilide D (4): white powder; $[\alpha]_D^{20} -204.0^\circ$ (c 1.53, CH_3OH); IR (KBr) ν_{max} 3447, 2929, 1770, 1716, 1662, 1603, 1385, 1230, 1151, 1078 cm^{-1} ; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS m/z 358 $[\text{M}]^+$ (6), 258 (53), 240 (8), 231 (28), 229 (39), 215 (31), 198 (12), 187 (12), 169 (13), 149 (13), 115 (10), 100 (15), 83 (100), 55 (76); HREIMS m/z 358.1426 (calcd for $\text{C}_{20}\text{H}_{22}\text{O}_6$, 358.1416).

Eupachinilide E (5): white powder; $[\alpha]_D^{20} -59.4^\circ$ (c 0.60, CH_3OH); IR (KBr) ν_{max} 3447, 2935, 1751, 1709, 1653, 1421, 1302, 1259, 1132, 1053, 1009, 825, 741, 501 cm^{-1} ; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS m/z 430 $[\text{C}_{20}\text{H}_{25}^{37}\text{ClO}_8]^+$ (1), 428 $[\text{M}]^+$ (3), 392 (7), 349 (7), 294 (8), 259 (16), 231 (10), 201 (12), 187 (15), 159 (13), 123 (15), 115 (10), 99 (100), 95 (95), 71 (74); HREIMS m/z 428.1231 (calcd for $\text{C}_{20}\text{H}_{25}\text{ClO}_8$, 428.1238).

Eupachinilide F (6): white powder; $[\alpha]_D^{20} -52.3^\circ$ (c 0.84, CH_3OH); IR (KBr) ν_{max} 3460, 2937, 1751, 1713, 1373, 1230, 1132, 1020, 824, 741, 511 cm^{-1} ; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS 472 $[\text{C}_{22}\text{H}_{27}^{37}\text{ClO}_9]^+$ (2), 470 $[\text{M}]^+$ (7), 452 (6), 434 (26), 417 (8), 405 (34), 375 (15), 272 (7), 259 (33), 245 (17), 215 (21), 187 (15), 165 (15), 123 (13), 99 (100), 95 (51), 82 (25), 71 (52); HREIMS m/z 470.1329 (calcd for $\text{C}_{22}\text{H}_{27}\text{ClO}_9$, 470.1344).

Eupachinilide G (7): gum; $[\alpha]_D^{20} -216.5^\circ$ (c 0.65, CH_3OH); IR (KBr) ν_{max} 3446, 2926, 1768, 1662, 1603, 1450, 1379, 1325, 1265, 1232, 1155, 1080, 1007, 968, 815, 563 cm^{-1} ; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS m/z 374 $[\text{M}]^+$ (58), 359 (5), 345 (23), 258 (87), 229 (100), 215 (83), 198 (33), 169 (36), 159 (21), 141 (24), 128 (19), 115 (24), 91 (23), 71 (16), 55 (19); HREIMS m/z 374.1359 (calcd for $\text{C}_{20}\text{H}_{22}\text{O}_7$, 374.1366).

Eupachinilide H (8): white powder; $[\alpha]_D^{20} +34.7^\circ$ (c 0.54, CH_3OH); IR (KBr) ν_{max} 3581, 3437, 2941, 1747, 1716, 1659, 1645, 1429, 1234, 1138, 1026, 960, 563 cm^{-1} ; ^1H NMR, see

Table 1; ^{13}C NMR, see Table 2; EIMS m/z 380 $[\text{M}]^+$ (16), 262 (9), 246 (29), 243 (41), 229 (41), 228 (72), 213 (46), 202 (35), 185 (31), 163 (33), 157 (38), 135 (34), 109 (43), 107 (100), 91 (70), 79 (36), 71 (40), 55 (34); HREIMS m/z 380.1858 (calcd for $\text{C}_{20}\text{H}_{28}\text{O}_7$, 380.1835).

Eupachinilide I (9): white powder; $[\alpha]_D^{20} +76.1^\circ$ (c 0.65, CH_3OH); IR (KBr) ν_{max} 3466, 2931, 1767, 1716, 1655, 1456, 1367, 1219, 1144, 1024, 966, 814, 554 cm^{-1} ; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS m/z 404 $[\text{M}]^+$ (2), 362 (3), 345 (4), 305 (5), 246 (15), 228 (10), 202 (11), 175 (7), 162 (16), 135 (12), 107 (11), 99 (100), 82 (21), 69 (10), 55 (11); HREIMS m/z 404.1829 (calcd for $\text{C}_{22}\text{H}_{28}\text{O}_7$, 404.1835).

Eupachinilide J (10): white powder; $[\alpha]_D^{20} +40.8^\circ$ (c 0.55, CH_3OH); IR (KBr) ν_{max} 3435, 2931, 1767, 1740, 1713, 1655, 1367, 1220, 1144, 1026, 972, 818, 569 cm^{-1} ; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS m/z 420 $[\text{M}]^+$ (1), 361 (2), 281 (3), 262 (8), 245 (7), 234 (11), 218 (16), 201 (10), 176 (8), 163 (8), 135 (8), 99 (100), 82 (41), 69 (15), 55 (8); HREIMS m/z 420.1806 (calcd for $\text{C}_{20}\text{H}_{28}\text{O}_8$, 420.1784).

Acknowledgment. Financial support of the National Natural Science Foundation (30025044) and the foundation from the Ministry of Science and Technology (2002CB512807) is gratefully acknowledged. We thank Prof. Z.-T. Wang and Dr. M. Zhang of Shanghai Traditional Chinese Medical University for the identification of the plant material.

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Additions and Corrections

2004, Volume 67

Sheng-Ping Yang, Jun Huo, Ying Wang, Li-Guang Lou, and Jian-Min Yue*: Cytotoxic Sesquiterpenoids from *Eupatorium chinense*.

Page 642, column 1, paragraph 7, line 5: “deacetye-upaserrin (**17**)¹⁰” should be revised as “mollisorin B (**17**)⁹”.

NP0402120

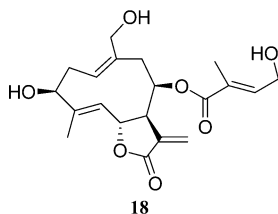
10.1021/np0402120

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2004, Volume 67

Jun Huo, Sheng-Ping Yang, Jian Ding, and Jian-Min Yue*: Cytotoxic Sesquiterpene Lactones from *Eupatorium lindleyanum*.

Page 1471, Chart 1: The structure of known compound **18** should be revised as follows, and its chemical name should be accordingly revised as 8 β -(4'-hydroxytigloyloxy)-3 β ,14-dihydroxy-6 β H,7 α H-germacra-1(10)Z,4E,11(13)-trien-6,12-olide (**18**) throughout the paper.



Page 1474, column 1, paragraph 2, line 7: “(**18**)⁷” should be revised as “(**18**)¹⁴”.

Page 1475, References and Notes: ref 7 “Hernandez, L. R. *Phytochemistry* **1994**, *37*, 1331–1335.” should be revised as “Hernández, L. R.; Catalán, C. A. N.; Cerda-García-Rojas, C. M.; Joseph-Nathan, P. *Phytochemistry* **1994**, *37*, 1331–1335.”

Page 1475, References and Notes: ref 14 “Hernandez, L. R. *Phytochemistry* **1996**, *42*, 1369–1373.” should be revised as “Hernández, L. R.; de Riscal, E. C.; Catalán, C. A. N.; Díaz, J. G.; Herz, W. *Phytochemistry* **1996**, *42*, 681–684.”

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