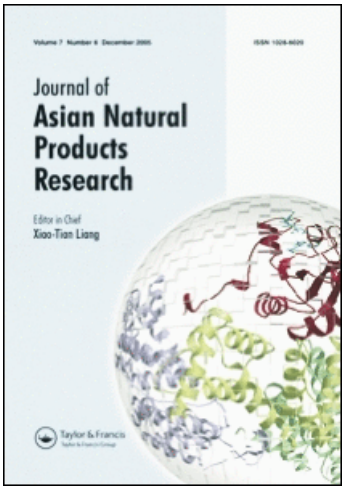

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Cytotoxic sesquiterpene lactones from *Eupatorium lindleyanum*

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Three new germacrane sesquiterpenes, eupalinolides C–E (**1**–**3**), along with three known germacrane sesquiterpenes, eupalinolide A (**4**), eupalinolide B (**5**), and 3β-acetoxy-8β-(4′-hydroxytigloyloxy)-14-hydroxycostunolide (**6**), were isolated from *Eupatorium lindleyanum*. They were tested for cytotoxicity against A-549, BGC-823, SMMC-7721, and HL-60 tumour cell lines. The results showed that these compounds demonstrated potent cytotoxicity. The structures of the compounds were elucidated by means of ^{1}H and ^{13}C NMR spectroscopic analysis, including 2D NMR experiments.

Keywords: *Eupatorium lindleyanum*; Compositae; Sesquiterpene; Eupalinolide C; Eupalinolide D; Eupalinolide E

1. Introduction

Various germacrane sesquiterpenes have been isolated from many species of genus *Eupatorium*. In recent years these compounds have been of increasing interest due to their insecticidal, cytotoxic, anti-tumour-promoting and insect-anti-feedant activities [1]. The plant *Eupatorium lindleyanum* DC, which has shown antihistamine and antibacterial activities, is geo-authentic to Jiangsu province in China. The whole plant, called "Ye-Ma-Zhui" by local residents, is used for the treatment of cough and tracheitis, and has a bitter and acerbic taste [2]. Chemical studies on this plant have been mostly limited to its volatile constituents. In order to clarify its active constituents, we have investigated its involatile constituents and reported flavonoids, triterpenoids, sesquiterpenes isolated from the aerial parts of this plant [3–5]. In this paper, we deal with the structural elucidation of three new germacrane sesquiterpenes, eupalinolides C–E (**1**–**3**), and another three known germacrane sesquiterpenes (figure 1), and report some of their cytotoxic activities against

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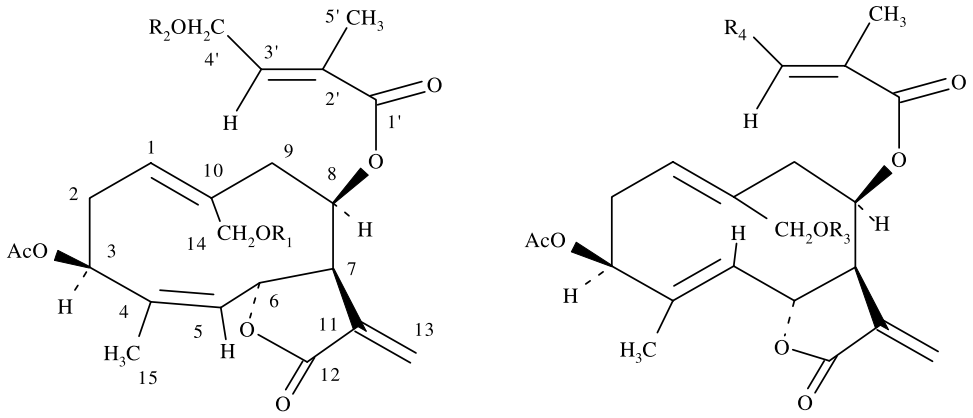

Figure 1. The structure of compounds **1**–**6**.

lung cancer cell A549, gastric gland cancer cell BGC-823, liver cancer cell SMMC-7721, and leukaemia cell HL-60 tumour cell lines. Their structures were elucidated by spectral and chemical means.

2. Results and discussion

Compound **1** was obtained as a colourless gum, $[\alpha]_D^{20}$ − 133.1 (*c* 0.06, $CHCl_3$). Its molecular formula was established as $C_{22}H_{28}O_8$ by HRESI-MS at *m/z* 443.1683 $[M + Na]^+$. The IR

Table 1. ^1H NMR (500 MHz) data of **1**–**3** (in $CDCl_3$, δ ppm).

Position	***1** (multi, J (Hz))*	***2** (multi, J (Hz))*	***3** (multi, J (Hz))*
1	5.25 (1H, dd, 12, 5.5)	5.26 (1H, dd, 12, 5)	5.42 (1H, dd, 12, 5)
2a	2.42 (1H, m)	2.43 (1H, m)	2.35 (1H, m)
2b	2.74 (1H, m)	2.77 (1H, m)	3.01 (1H, m)
3	5.28 (1H, dd, 12, 6)	5.30 (1H, dd, 12, 6)	5.31 (1H, dd, 12.5, 6)
5	5.20 (1H, brd, 10)	5.22 (1H, brd, 10.5)	5.35 (1H, brd, 10.5)
6	5.87 (1H, dd, 12, 9)	5.82 (1H, dd, 12, 9)	5.28 (1H, dd, 10, 9)
7	2.99 (1H, m)	2.98 (1H, m)	2.98 (1H, m)
8	5.37 (1H, m)	5.47 (1H, m)	5.50 (1H, m)
9a	2.30 (1H, m)	2.31 (1H, m)	2.28 (1H, m)
9b	3.16 (1H, m)	3.15 (1H, m)	2.90 (1H, m)
13a	6.36 (1H, d, 3)	6.37 (1H, d, 3.5)	6.41 (1H, d, 3.5)
13b	5.79 (1H, d, 3)	5.82 (1H, d, 3.5)	5.80 (1H, d, 3.5)
14a	4.48 (1H, d, 13)	4.98 (1H, d, 13)	4.98 (1H, d, 13)
14b	4.10 (1H, d, 13)	4.57 (1H, d, 13)	4.64 (1H, d, 13)
15	1.84 (3H, brs)	1.85 (3H, brs)	1.82 (3H, brs)
3′	6.75 (1H, t, 6)	6.68 (1H, t, 6)	6.74 (1H, t, 6)
4′	4.28 (2H, d, 6)	4.74 (2H, d, 6)	10.14 (2H, d, 6)
5′	1.80 (3H, brs)	1.84 (3H, brs)	2.28 (3H, brs)
CH_3COO	2.10 (3H, s)	2.15 (3H, s)	2.10 (3H, s)
CH_3COO		2.10 (3H, s)	2.02 (3H, s)
CH_3COO		2.06 (3H, s)	

spectrum of **1** indicated the presence of carbonyl and hydroxyl groups. By comparison of the ^{1}H NMR and ^{13}C NMR spectral data of **1** with those of eupalinolide A (**4**) [5] (tables 1 and 2), their spectral data were very similar, except for that H-14a, H-14b and C-14 of **1** had an up-field shift, which suggested that C-14 with the signal at δ 61.2 bears a hydroxy group, an AB quartet at δ 4.10, and 4.48 was assigned to H-14a and H-14b. HMBC and NOESY (figure 2) experiments were run to support these assignments. HMBC experiments allowed us to assign all the other proton and carbon signals for **1**. The signal at δ 4.28 (d, $J = 6$ Hz) was assigned to H-4′ attached in the carbon bearing a hydroxy group, and the signal at δ 6.75 (t, $J = 6$ Hz) was assigned to H-3′. NOESY of **1** showed a correlation between H-4′ and a methyl proton with the signal at δ 1.80. HMBC of **1** showed a correlation between the methyl proton and the carboxylic carbon (δ_C 166.1), and a correlation between H-3′ and carbon of the methyl group (δ_C 12.6). These results together with the NMR data (tables 1 and 2) provided the evidence of presence of a 2-methyl-4-hydroxyl-2 (E)-butenoyloxyl group in the side chain. The signals at δ 5.20 (brd, $J = 10$ Hz), 5.25 (dd, $J = 12$, 5.5 Hz) and 5.28 (dd, $J = 12$, 6 Hz) were assigned to H-5, H-1 and H-3 respectively; the signal at δ5.87 (dd, $J = 12$, 9 Hz) was assigned to H-6. The assignments of H-2, H-8, H-9 and H-13 are more likely. The HMBC spectrum of **1** showed correlations between H-3 with the carboxylic carbon (δ_C 169.5) of one acetate group, H-8 has a correlation with the carboxylic carbon (δ_C 166.1) of α-methyl butenoate. NOESY experiments also allowed us to establish the stereochemistry for **1**. The lactone ring closed to C-6 was *trans* and the substituents at C-3 and C-8 were both β-oriented. Thus, compound **1** was identified as 3β,8β,14-trihydroxy-1(10)*E*,4Z,11(13)-germacratrien-12,6-olide,8-(4′-hydroxytigloyloxy),3-acetoxy, named eupalinolide C.

Table 2. ^{13}C NMR (125 MHz) data of **1**–**3** (in $CDCl_3$, δ ppm).

C	***1***	***2***	***3***
1	127.6	129.7	130.7
2	29.2	29.4	29.5
3	71.3	71.4	77.0
4	136.6	136.3	136.3
5	127.3	126.4	125.2
6	78.1	78.2	78.6
7	48.4	48.5	48.2
8	74.3	74.0	74.4
9	37.5	37.5	38.4
10	138.6	134.5	134.1
11	137.2	137.0	136.9
12	170.0	169.5	168.7
13	125.0	124.7	125.1
14	61.2	62.8	62.5
15	23.1	23.0	18.0
1′	166.1	165.6	166.2
2′	127.8	129.7	144.4
3′	141.9	139.3	134.2
4′	59.5	60.8	191.0
5′	12.6	12.6	13.0
CH_3COO	21.2	20.9	21.0
	169.5	169.3	169.9
CH_3COO		20.6	20.7
		170.4	170.4
CH_3COO		20.5	
		170.3	

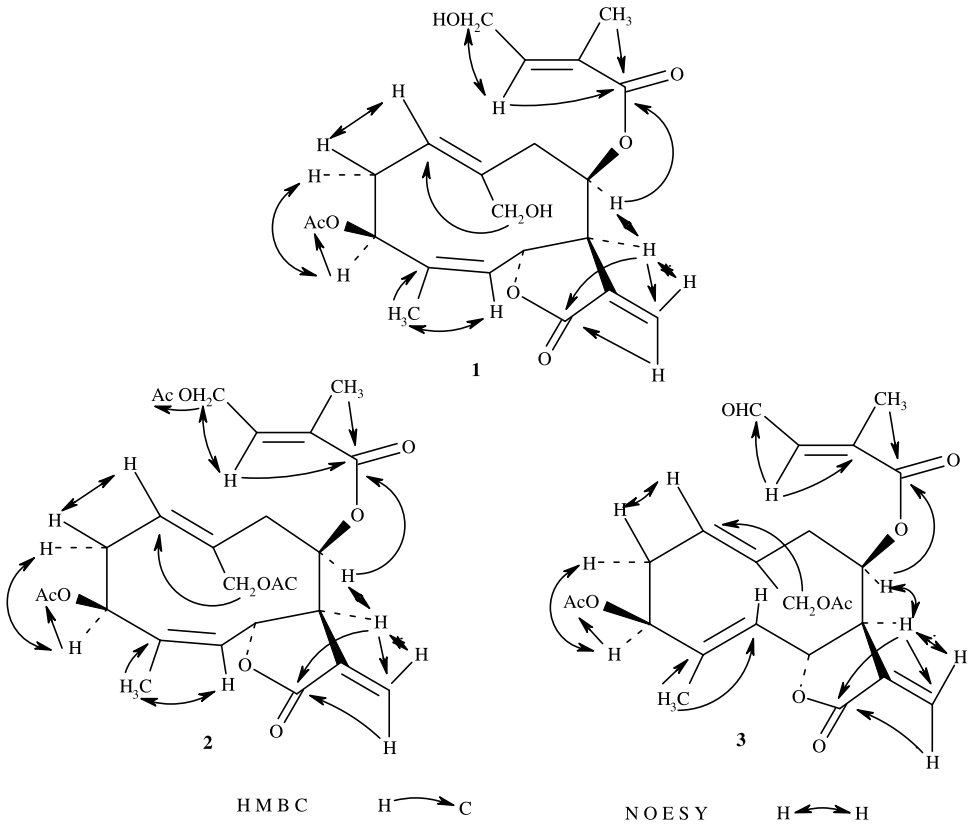

Figure 2. The key HMBC and NOESY correlations of **1**–**3**.

Compound **2** was obtained as colourless needles, mp 86–88°C. $[\alpha]_D^{20}$ −137.3 (*c* 0.08, $CHCl_3$). Its molecular formula was determined as $C_{26}H_{32}O_{10}$ by HRESI-MS at *m/z* 527.1904 $[M + Na]^+$. By comparison of the ^1H NMR and ^{13}C NMR data of **2** with eupalinolide A (**4**) [5] (tables 1 and 2), their spectral data was very similar, except for that H-4′ and C-4′ of **2** showed a down-field shift, which suggested that C-4′ with the signal at δ 60.8 bears an ester group; a proton signal at δ 4.74 (d, $J = 6$ Hz) was assigned to H-4′. HMBC and NOESY experiments were run to assign all proton and carbon signals and the relative stereochemistry for **2**. Compound **2** was identified as 3β,8β,14-trihydroxy-1(10)*E*,4*Z*,11(13)-germacratrien-12,6-olide,8-(4′-acetoxytigloyloxy),3,14-diacetoxy, named eupalinolide D.

Compound **3** was obtained as a colourless gum, $[\alpha]_D^{20}$ −111.4 (*c* 0.06, $CHCl_3$), Its molecular formula was determined as $C_{24}H_{28}O_9$ by HRESI-MS at *m/z* 483.1639 $[M + Na]^+$. By comparison of the ^1H NMR and ^{13}C NMR data of **3** with eupalinolide B (**5**) [5] (tables 1 and 2), their spectral data were similar, except for that H-4′ and C-4′ of **3** showed an apparent down-field shift, which suggested that C-4′ with the signal at δ 191.0 is an aldehydic carbon; a proton signal at δ10.14(d, $J = 6$ Hz) was assigned to H-4′, the presence of aldehyde function in **3** being obvious from the IR (1682 cm^{-1}). This is in agreement with previous observations that the chemical shift of aldehydic protons in *trans*-α,β-unsaturated system (*E*-form) generally appear at lower field ($\geq$10 ppm) than the same proton in *cis*-α,β-unsaturated system, which usually appear at 9.26–9.4 [6]. HMBC and NOESY experiments were run to assign all proton and carbon signals and the relative

stereochemistry for **3**. Compound **3** was identified as 3β,8β,14-trihydroxy-1(10)*E*,4*E*, 11(13)-germacratrien-12,6-olide,8-(4′-dehydrotigloyloxy),3,14-diacetoxy, named eupalinolide E.

The three known sesquiterpenoids were identified as eupalinolide A (**4**) [5], eupalinolide B (**5**) [5], and 3β-acetoxy-8β-(4′-hydroxytigloyloxy)-14-hydroxycostunolide (**6**) [7] by comparison of their spectroscopic data with those reported in the literature.

3. Experimental

3.1 General experiment procedures

Optical rotations were measured on a Perkin-Elmer 341 polarimeter (Na filter, $\lambda = 589$ nm). IR spectra were obtained on a Perkin-Elmer 577spectrameter with KBr disk. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-500 spectrometer with TMS as internal standard. ESI-MS were measured on a Finnigan MAT 95 instrument. All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai). Silica gel (200–300 mesh) and C18 reversed-phase silica gel (250 mesh, Merck) were used for column chromatography, and precoated silica gel GF254 plates (Qingdao Haiyang Chemical Plant, Qingdao, China) used for TLC.

3.2 Plant material

The aerial parts of *Eupatorium lindleyanum* DC were collected from Xuyi County of Jiangsu Province, China, and identified by Professor Shi-hui Qian. A voucher specimen (No. Eup-2001-01) has been deposited in the Herbarium of Jiangsu Academy of Traditional Chinese Medicine.

3.3 Extraction and isolation

The dried powder of the aerial parts (3.5 kg) of *E. lindleyanum* was percolated with 95% EtOH. After removal of the EtOH under reduced pressure, a dark green residue (480 g) was obtained, and the residue was dissolved in hot water, then successively extracted with petroleum ether, $CHCl_3$, EtOAc, and *n*-butanol. The $CHCl_3$ extract was evaporated *in vacuo* to give a residue (50 g), which was chromatographed over silica gel column eluting with petroleum ether/EtOAc (in gradient, 10:1 to 0:100; v/v) to yield 7 major fractions, combined on the basis of TLC. Fraction 5 (petroleum ether/EtOAc 2:1) was separated by silica gel column chromatography eluted by a gradient of MeOH in $CHCl_3$ ($CHCl_3$/MeOH, 100:1 to 20:1) to give subfractions 5a–5p, compound **2** (20 mg) was isolated from subfractions 5a–5b, sequentially. Subfractions 5c–5d were purified by reverse-phased C-18 silica gel column eluted with 25% H_2O in MeOH to afford **3** (10 mg). Subfractions 5e–5i were purified by reverse-phased C-18 silica gel column eluted with 35% H_2O in MeOH to afford **4** (32 mg), **5** (31 mg). Subfractions 5k–5n were purified by reverse-phased C-18 silica gel column eluted with 45% H_2O in MeOH to afford **1** (15 mg), **6** (12 mg).

Compound **1** was obtained as a colourless gum, $[\alpha]_D^{20}$ −133.1 (*c* 0.06, $CHCl_3$). UV λ_{max}^{EtOH} (log ε): 219 (3.92). IR (KBr) cm^{-1}: 3431, 2924, 1751, 1713, 1647, 1448, 1379, 1252, 1130,

1011. ^{1}H NMR ($CDCl_3$, 500 MHz), see table 1. ^{13}C NMR ($CDCl_3$, 125 MHz), see table 2. HRESI-MS: *m/z* 443.1683 $[M + Na]^+$ (calcd for $C_{22}H_{28}O_8Na$, 443.1682).

Compound **2** was obtained as colourless needles, mp 86–88°C. $[\alpha]_D^{20}$ −137.3 (*c* 0.08, $CHCl_3$). UV λ_{max}^{EtOH} (log ε): 220 (3.78). IR (KBr) cm^{-1}: 3430, 2926, 1753, 1710, 1645, 1446, 1378, 1249, 1134, 1009. ^{1}H NMR ($CDCl_3$, 500 MHz), see table 1. ^{13}C NMR ($CDCl_3$, 500 MHz), see table 2. HRESI-MS: *m/z* 527.1904 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{10}Na$, 527.1893).

Compound **3** was obtained as a colourless gum, $[\alpha]_D^{20}$ −111.4 (*c* 0.06, $CHCl_3$). UV λ_{max}^{EtOH} (log ε): 221 (4.02). IR (KBr) cm^{-1}: 3429, 2922, 1752, 1720, 1682, 1636, 1450, 1370, 1240, 1132, 1020. ^{1}H NMR ($CDCl_3$, 500 MHz), see table 1. ^{13}C NMR ($CDCl_3$, 500 MHz), see table 2. HRESI-MS: *m/z* 483.1639 $[M + Na]^+$ (calcd for $C_{24}H_{28}O_9Na$, 483.1631).

Compound **4** was obtained as a colourless gum, $[\alpha]_D^{20}$ −167.4 (*c* 0.20, $CHCl_3$). UV λ_{max}^{EtOH} (log ε): 220 (3.85). IR (KBr) cm^{-1}: 3425, 2919, 1750, 1718, 1640, 1451, 1371, 1245, 1134, 1016. ESI-MS: *m/z* 463.2 $[M + H]^+$(100).

Compound **5** was obtained as a colourless gum, $[\alpha]_D^{20}$ −93.1 (*c* 0.18, $CHCl_3$). UV λ_{max}^{EtOH} (log ε): 219 (3.80). IR (KBr) cm^{-1}: 3427, 2916, 1751, 1716, 1639, 1449, 1370, 1246, 1139, 1019. ESI-MS: *m/z* 463.2 $[M + H]^+$(100).

Compound **6** was obtained as a colourless gum, $[\alpha]_D^{20}$ +13.0 (*c* 0.25, $CHCl_3$). IR (KBr) cm^{-1}: 3421, 2915, 1747, 1712, 1634, 1447, 1365, 1244, 1137, 1011. ESI-MS: *m/z* 421 $[M + H]^+$(100).

3.4 Cytotoxcity evaluation of compounds 1–6

All these compounds were tested for cytotoxicity against lung cancer cell A549, gastric gland cancer cell BGC-823, liver cancer cell SMMC-7721 and leukaemia cell HL-60 tumour cell

Table 3. Cytotoxic activities of compounds **1–6** against A-549, BGC-823, SMMC-7721 and HL-60 tumour cell lines.

Sample	*C (mol/l)*	*A-549*		*BGC-823*		*SMMC-7721*		*HL-60*	
		RCI%	*Effect*	*RCI%*	*Effect*	*RCI%*	*Effect*	*RCI%*	*Effect*
1	1×10^{-7}	0.00	−	0.00	−	0.00	+	11.25	+
	1×10^{-6}	0.00		0.24		29.72		21.31	
	1×10^{-5}	0.00		5.84		52.36		56.11	
2	1×10^{-7}	1.80	−	6.49	−	20.17	−	14.23	+
	1×10^{-6}	21.34		14.84		11.16		28.43	
	1×10^{-5}	11.05		20.68		31.97		55.84	
3	1×10^{-7}	4.63	+	10.14	−	0.00	−	0.23	+
	1×10^{-6}	25.96		12.98		0.00		11.52	
	1×10^{-5}	50.49		31.55		0.00		84.61	
4	1×10^{-7}	2.75	+	21.45	+	8.79	−	0.00	+
	1×10^{-6}	4.28		25.09		0.00		15.57	
	1×10^{-5}	66.86		66.00		0.00		86.30	
5	1×10^{-7}	0.00	+	13.27	+	0.00	−	0.00	+
	1×10^{-6}	0.00		19.45		0.00		22.33	
	1×10^{-5}	69.26		67.82		1.12		93.43	
6	1×10^{-7}	11.70	−	0.00	−	0.00	−	0.00	+
	1×10^{-6}	12.08		14.76		23.69		6.73	
	1×10^{-5}	14.14		20.68		23.84		79.04	
ADR	1×10^{-7}	30.41	+	17.39	+	19.35	+	15.98	++
	1×10^{-6}	30.78		39.76		49.11		74.69	
	1×10^{-5}	62.98		80.72		86.04		95.80	

Adriamycin was used as positive control. RCI, rate of cell inhibition; −, no effect; +, weak effect; ++, medium effect.

lines according to standard protocols [8] and adriamycin was used as positive control. The results showed that these compounds demonstrated potent cytotoxicity (table 3).

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