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### Two New Germacranolides from *Magnolia Grandiflora*

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## TWO NEW GERMACRANOLIDES FROM *MAGNOLIA GRANDIFLORA*

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Two new sesquiterpenoids, 4,5-epoxy-13-methoxy-1(10)-germacren-12,6-olide and 4,5-epoxy-13-acetoxy-1(10)-germacren-12,6-olide, were isolated from the leaves of *Magnolia grandiflora*, together with six known compounds, 2 $\alpha$ -hydroxy-dihydroparthenolide, parthenolide, costunolide, syringaresinol, (+) medioresinol and 6,7-dimethoxycoumarin. The structures of the new compounds were elucidated on the basis of spectroscopic methods and X-ray diffraction.

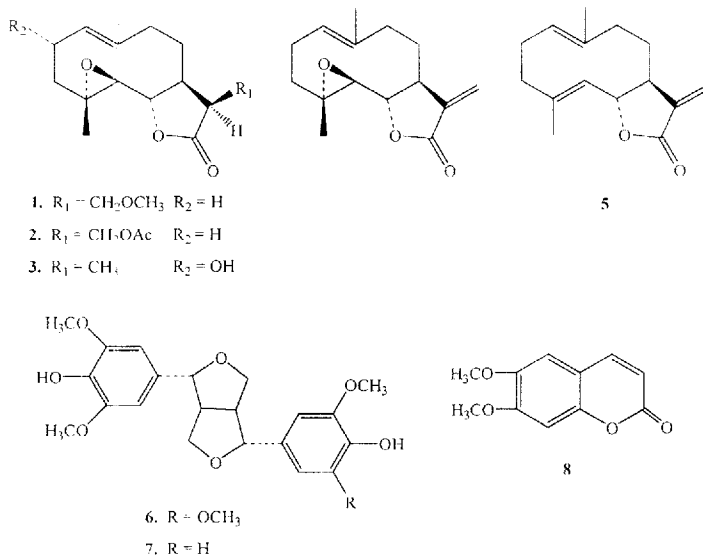
**Keywords:** *Magnolia grandiflora* Linn.; Magnoliaceae; Sesquiterpenoids; Germacranolides

### INTRODUCTION

*Magnolia grandiflora* Linn., has been used for the treatment of cold, headache, and stomach-ache in traditional Chinese medicine for a long time [1]. Much work has been done about this species. Our investigation on the leaves of this plant has resulted in the isolation of two new germacranolides, 4,5-epoxy-13-methoxy-1(10)-germacren-12,6-olide (**1**) and 4,5-epoxy-13-acetoxy-1(10)-germacren-12,6-olide (**2**), along with six known compounds, 2 $\alpha$ -hydroxy-dihydroparthenolide (**3**) [2], parthenolide (**4**) [3], costunolide (**5**) [3], syringaresinol (**6**) [4], (+) medioresinol (**7**) [5], and

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6,7-dimethoxycoumarin (**8**) [6]. In this paper, we report the isolation and structural elucidation of these constituents.



## RESULTS AND DISCUSSION

The molecular formula of compound **1** was determined to be  $\text{C}_{16}\text{H}_{24}\text{O}_4$  based on positive-ion HRFABMS at  $m/z$  281.1776  $[\text{M}+1]^+$  and the  $^{13}\text{C}$  NMR spectrum. The IR spectrum showed strong absorption of  $\gamma$ -lactone ( $1762\text{ cm}^{-1}$ ). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra exhibited signals for three tertiary methyls, five methylenes, four methines, a quaternary carbon, two olefinic carbons [ $\delta_{\text{C}}$  125.1 (d), 134.5 (s)], and a carboxyl group ( $\delta_{\text{C}}$  175.0). Comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra with reference data [3] indicated that **1** is a germacran-12,6-olide derivative. The NMR spectra of **1** were similar to those of parthenolide (**4**) except for the presence of a methoxyl group ( $\delta_{\text{C}}$  59.3) and an oxymethylene group ( $\delta_{\text{C}}$  68.4), instead of the exocyclic methylene group. The above information suggested that **1** should be a 4,5-epoxy-13-methoxy-1(10)-germacren-12,6-olide. The configuration of C-11 in **1** was confirmed by X-ray crystallographic analysis (see Fig. 1), which indicated that the hydrogen at C-11 was in  $\alpha$  position.

The conjugate addition of parthenolide (**4**) with  $\text{CH}_3\text{OH} - \text{CH}_3\text{ONa}$  also afforded **1** (see Experimental section). The methoxyl anion attack on the  $\beta$

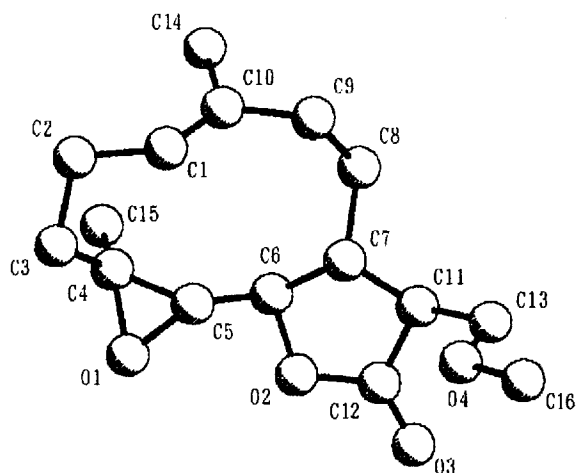


FIGURE 1 X-ray crystallographic structure of compound 1.

position (C-13) in  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone resulted in the formation of a carbanion at C-11. The carbanion conjugated with the carboxyl group forming the plane configuration.  $H^-$  was then added to C-11, and the  $CH_2OCH_3$  was in  $\beta$  position attributed to the less steric hindrance.

Compound 2 possessed a molecular formula of  $C_{17}H_{24}O_5$  determined by positive-ion HRFABMS at  $m/z$  309.1780  $[M+1]^+$  and  $^{13}C$  NMR spectrum. The  $^1H$  and  $^{13}C$  NMR of 2 showed similarities to those of 1 except that the methoxyl group at C-13 in 1 was replaced by an acetoxy group [ $\delta_C$  170.3 (s), 20.4 (q)] in 2. Thus, compound 2 was determined as 4,5-epoxy-13-acetoxy-1(10)-germacren-12,6-olide.

## EXPERIMENTAL SECTION

### General Experimental Procedures

Melting points were obtained on a Kofler apparatus and are uncorrected. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were taken on a Shimadzu double-beam 210A spectrophotometer. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets.  $^1H$  NMR and  $^{13}C$  NMR spectra were recorded on a Bruker AM-400 MHz spectrometer with TMS as internal standard. MS data were recorded on a VG Autospec-3000 spectrometer. X-ray analysis was done with a MAC DIP-2030K diffractometer.

## Plant Material

The leaves of *M. grandiflora* were collected in Kunming, Yunnan province, in November, 1995, where the plant is widely cultivated. The plant material was identified by Prof. Tian Lu Ming, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming, Yunnan, where a voucher specimen was deposited.

## Extraction and Isolation

The air-dried and powdered leaves of *M. grandiflora* (7.2 kg) were extracted with 70% MeOH three times at room temperature. The combined extract was concentrated *in vacuo*. Then, the residue was suspended in H<sub>2</sub>O, and partitioned with CHCl<sub>3</sub>. The CHCl<sub>3</sub> extract was concentrated *in vacuo* to give a residue (167 g), which was chromatographed on a silica gel column (200–300 mesh, 2.0 kg) and eluted with petroleum ether-Me<sub>2</sub>CO (9:1–3:1) to give 30 fractions. Fractions 2–13 were repeatedly subjected to column chromatography over silica gel eluted with various eluent systems to afford 4,5-epoxy-13-methoxy-1(10)-germacren-12,6-olide (**1**, 22 mg), 4,5-epoxy-13-acetoxy-1(10)-germacren-12,6-olide (**2**, 42 mg), 2 $\alpha$ -hydroxy-dihydro-parthenolide (**3**, 110 mg), parthenolide (**4**, 11.2 g), costunolide (**5**, 1.2 g), syringaresinol (**6**, 840 mg), (+) medioresinol (**7**, 82 mg), 6,7-dimethoxy-coumarin (**8**, 10 mg).

**4,5-Epoxy-13-methoxy-1(10)-germacren-12,6-olide (1)** Prisms (Me<sub>2</sub>CO): mp 101–103°C;  $[\alpha]_D^{26} -23.9$  (*c* 0.35, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3512, 2935, 1762, 1446, 1386, 1344, 1315, 1214, 1188, 1083, 1040 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  1.20 (3H, s, H-15), 1.66 (3H, s, H-14), 2.70 (1H, d, *J* = 8.9 Hz, H-5), 3.32 (3H, s, OCH<sub>3</sub>), 3.60 (1H, dd, *J* = 8.4, 3.0 Hz, H-3), 3.79 (1H, t, *J* = 8.8 Hz, H-6), 4.12 (2H, d, *J* = 6.0 Hz, H-13), 5.15 (1H, dd, *J* = 12.0, 2.6 Hz, H-1); <sup>13</sup>C NMR data, see Table 1; EIMS *m/z* [M]<sup>+</sup> 280 (12), [M-CH<sub>3</sub>]<sup>+</sup> 265 (8), [M-CH<sub>3</sub>OH]<sup>+</sup> 248 (5), [M-CH<sub>2</sub>OCH<sub>3</sub>]<sup>+</sup> 235 (16), 177 (75), 163 (60), 149 (51), 133 (45), 107 (51), 95 (71), 81 (69), 67 (63), 55 (100); HRFAB+MS *m/z* [M+1]<sup>+</sup> 281.1776 (calcd. for C<sub>16</sub>H<sub>25</sub>O<sub>4</sub>, 281.1753).

**4,5-Epoxy-13-acetoxy-1(10)-germacren-12,6-olide (2)** Viscous oil;  $[\alpha]_D^{26} -22.0$  (*c* 0.58, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3524, 2927, 1788, 1731, 1681, 1634, 1453, 1386, 1231, 1075 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  1.18 (3H, s, H-15), 1.63 (3H, s, H-14), 2.00 (3H, s, OAc), 2.63 (1H, dd, *J* = 8.9, 2.2 Hz, H-5), 3.78 (1H, dd, *J* = 9.1, 9.0 Hz, H-6), 4.29 (2H, d, *J* = 5.8 Hz, H-13), 5.08

TABLE I  $^{13}\text{C}$  NMR spectral data for compounds 1–3 (100 MHz)<sup>a</sup>

C	1	2	3
1	124.9 d	125.1 d	130.1 d
2	23.8 t	24.0 t	67.8 d
3	36.3 t	36.6 t	46.6 t
4	61.2 s	61.3 s	62.2 s
5	65.7 d	66.3 d	67.0 d
6	82.1 d	82.2 d	83.5 d
7	47.0 d	48.6 d	52.9 d
8	29.5 t	29.9 t	30.2 t
9	40.7 t	41.0 t	42.2 t
10	134.2 s	134.5 s	137.2 s
11	45.7 d	45.5 d	43.4 d
12	173.7 s	175.0 s	180.3 s
13	59.7 t	68.4 t	13.3 q
14	16.9 q	17.1 q	18.6 q
15	16.6 q	16.8 q	17.6 q
CH <sub>3</sub> COO	170.3 s		
CH <sub>3</sub> C=O	20.4 q		
ÖCH <sub>3</sub>		59.3 q	

<sup>a</sup> Compounds 1, 2 was measured in CDCl<sub>3</sub>, 3 in CD<sub>3</sub>OD.

(1H, brd,  $J = 11.8$  Hz, H-1);  $^{13}\text{C}$  NMR data, see Table I; EIMS  $m/z$  [M]<sup>+</sup> 308 (18), [M-CH<sub>3</sub>]<sup>+</sup> 293 (3), [M-Ac]<sup>+</sup> 265 (8), [M-HOAc]<sup>+</sup> 248 (20), 233 (13), 205 (30), 190 (52), 177 (55), 163 (54), 149 (55), 133 (67), 107 (62), 81 (84), 67 (68), 55 (100); HRFAB+MS  $m/z$  [M+1]<sup>+</sup> 309.1780 (calcd. for C<sub>17</sub>H<sub>25</sub>O<sub>5</sub>, 309.1702).

*2α-Hydroxy-dihydroparthenolide* (3) Prisms (Me<sub>2</sub>CO); mp 198–201°C; IR (KBr)  $\nu_{\text{max}}$  3498, 2947, 1766, 1670, 1458, 1444, 1391, 1315, 1231, 1186, 1081 cm<sup>-1</sup>;  $^1\text{H}$  NMR (CD<sub>3</sub>OD, 400 MHz)  $\delta$  1.14 (3H, d,  $J = 6.8$  Hz, H-13), 1.28 (3H, s, H-15), 1.78 (3H, s, H-14), 2.89 (1H, d,  $J = 9.1$  Hz, H-5), 3.97 (1H, t,  $J = 9.2$  Hz, H-6), 4.62 (1H, ddd,  $J = 10.4, 10.8, 5.8$  Hz, H-2), 5.26 (1H, dd,  $J = 10.4, 1.2$  Hz, H-1);  $^{13}\text{C}$  NMR data, see Table I; EIMS  $m/z$  [M]<sup>+</sup> 266 (2), [M-CH<sub>3</sub>]<sup>+</sup> 251 (2), [251-H<sub>2</sub>O]<sup>+</sup> 233 (3), 208 (4), 196 (13), 167 (15), 150 (21), 135 (42), 123 (53), 107 (47), 95 (53), 81 (48), 55 (100).

*Parthenolide* (4) Prisms (Me<sub>2</sub>CO); mp 115–117°C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 207 (4.12) nm; IR (KBr)  $\nu_{\text{max}}$  3498, 2940, 1758, 1658, 1443, 1387, 1325, 1291, 1077 cm<sup>-1</sup>;  $^1\text{H}$  NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  1.30 (3H, s, H-15), 1.72 (3H, s, H-14), 2.78 (1H, d,  $J = 9.0$  Hz, H-5), 3.88 (1H, t,  $J = 8.2$  Hz, H-6), 5.25 (1H, m, H-1), 5.58 (1H, d,  $J = 3.0$  Hz, H-13a), 6.32 (1H, d,  $J = 3.0$  Hz, H-13b); EIMS  $m/z$  [M]<sup>+</sup> 248 (4), [M-H<sub>2</sub>O]<sup>+</sup> 230 (2), 217 (38), 204 (17), 189 (25), 175 (34), 161 (36), 150 (38), 133 (35), 123 (66), 109 (83), 91 (75), 81 (100), 67 (68), 55 (85).

*Costunolide* (**5**) Prisms (Me<sub>2</sub>CO); mp 103–105°C; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 208 (4.08) nm; IR (KBr)  $\nu_{\max}$  3503, 2937, 1765, 1661, 1440, 1289 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  1.42 (3H, s, H-15), 1.70 (3H, s, H-14), 4.55 (1H, t,  $J=10.0$  Hz, H-6), 4.79 (1H, m, H-5), 4.92 (1H, m, H-1), 5.54 (1H, d,  $J=3.0$  Hz, H-13a), 6.27 (1H, d,  $J=3.4$  Hz, H-13b); EIMS  $m/z$  [M]<sup>+</sup> 232 (55), [M-CH<sub>3</sub>]<sup>+</sup> 217 (42), 204 (15), 189 (23), 175 (31), 161 (33), 150 (36), 123 (63), 109 (83), 91 (72), 81 (100), 55 (85).

*Syringaresinol* (**6**) Prisms (Me<sub>2</sub>CO); mp 172–175°C; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 224 (4.42), 230.5 (4.35), 235.5 (4.05), 248 (3.42), 269.5 (3.12) nm; IR (KBr)  $\nu_{\max}$  3441, 3005, 2943, 2872, 1612, 1520, 1456, 1426, 1379, 1319, 1247, 1204, 1156, 1063, 986 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  3.03 (2H, m, H-8, H-8'), 3.76 (12H, s, OCH<sub>3</sub>), 3.83 (2H, dd,  $J=9.2, 3.4$  Hz, H-9 $\alpha$ , H-9' $\alpha$ ), 4.21 (2H, dd,  $J=9.0, 6.8$  Hz, H-9 $\beta$ , H-9' $\beta$ ), 4.66 (2H, d,  $J=4.2$  Hz, H-7, H-7'), 5.84 (2H, s, OH), 6.51 (4H, s, H-2, H-2', H-6, H-6'); <sup>13</sup>C NMR data, see Table II; EIMS  $m/z$  [M]<sup>+</sup> 418, 388 (16), 280 (13), 236 (26), 221 (29), 210 (41), 193 (57), 181 (100), 167 (89), 154 (57), 123 (43), 84 (67), 55 (92).

(+)-*Medioresinol* (**7**) Prisms (Me<sub>2</sub>CO); mp 181–183°C;  $[\alpha]_D^{23} +17.8$  (c 0.49, CH<sub>3</sub>OH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 207 (4.35), 213.5 (4.23), 235.5 (4.08), 251 (3.42), 261.5 (3.32) nm; IR (KBr)  $\nu_{\max}$  3454, 3097, 2944, 2866, 1612, 1519, 1457, 1425, 1368, 1326, 1281, 1160, 1061, 982 cm<sup>-1</sup>; <sup>1</sup>H NMR

TABLE II <sup>13</sup>C NMR spectral data for compounds **6–8** (CDCl<sub>3</sub>, 100 MHz)

C	6	7	8
1	131.7 s	133.0 s	
2	102.5 d	108.7 d	161.2 s
3	146.7 s	146.8 s	113.6 d
4	134.1 s	145.3 s	143.1 d
5	146.7 s	114.3 d	108.3 d
6	102.5 d	118.9 d	146.5 s
7	85.7 d	86.1 d	153.1 s
8	56.0 d	54.2 d	100.2 d
9	71.4 t	71.9 t	150.2 s
10			111.5 s
1'	131.7 s	132.2 s	
2'	102.5 d	103.0 d	
3'	146.5 s	147.3 s	
4'	134.1 s	134.5 s	
5'	146.7 s	147.3 s	
6'	102.5 d	103.0 d	
7'	85.7 d	85.8 d	
8'	56.0 d	54.4 d	
9'	71.4 t	71.6 t	
OCH <sub>3</sub>	53.7 q (4 ×)	56.4 q (2 ×)	56.4 q
		55.0 q	56.5 q

(CDCl<sub>3</sub>, 400 MHz)  $\delta$  3.07 (2H, m, H-8, H-8'), 3.87 (9H, s, OCH<sub>3</sub>), 4.23 (2H, dd,  $J=9.2, 3.4$  Hz, H-9 $\alpha$ , H-9' $\alpha$ ), 4.27 (2H, dd,  $J=9.2, 6.8$  Hz, H-9 $\beta$ , H-9' $\beta$ ), 4.69 (1H, d,  $J=4.7$  Hz, H-7'), 4.72 (1H, d,  $J=4.5$  Hz, H-7), 5.52 (1H, s, OH), 5.64 (1H, s, OH), 6.56 (2H, d,  $J=2.0$  Hz, H-2', H-6'), 6.80 (1H, dd,  $J=8.1, 1.6$  Hz, H-6), 6.86 (1H, s, H-2), 6.88 (1H, dd,  $J=8.1, 1.6$  Hz, H-5); <sup>13</sup>C NMR data, see Table II; EIMS  $m/z$  [M]<sup>+</sup> 388 (100), 357 (54), 210 (31), 193 (47), 182 (75), 181 (67), 167 (84), 151 (67), 137 (25), 123 (51), 93 (52), 81 (78), 65 (89).

**6,7-Dimethoxycoumarin (8)** Colorless needles (Me<sub>2</sub>CO); mp 131–132°C; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 204 (4.22), 251.5 (3.35), 255 (3.12), 344 (3.30) nm; IR (KBr)  $\nu_{\max}$  3061, 2934, 1708, 1615, 1558, 1517, 1437, 1424, 1384, 1282, 1250, 1172, 1096, 927 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  3.89 (3H, s, OCH<sub>3</sub>), 3.92 (3H, s, OCH<sub>3</sub>), 6.26 (1H, d,  $J=9.5$  Hz, H-3), 6.82 (1H, s, H-8), 6.83 (1H, s, H-5), 7.60 (1H, d,  $J=9.5$  Hz, H-4); <sup>13</sup>C NMR data, see Table II; EIMS  $m/z$  [M]<sup>+</sup> 206 (100), [M-CH<sub>3</sub>]<sup>+</sup> 191 (52), [M-43]<sup>+</sup> 163 (58), 148 (12), 135 (43), 120 (27), 107 (41), 92 (33), 79 (52), 69 (46).

The conjugate addition of parthenolide: Parthenolide (200 mg) was dissolved in methanol (10 ml), which was treated with the methanol solution containing 0.1 mol/L NaOCH<sub>3</sub> (5 ml) at room temperature for 5 h. The reaction mixture was acidified with diluted HCl to pH = 4. Then the reaction product was crystallized in the solution by adding H<sub>2</sub>O. The crystals were recrystallized in MeOH-H<sub>2</sub>O to give colorless needles (117 mg).

X-ray crystallographic analysis [7]: Compound 1 crystallized in the monoclinic space group *P*2<sub>1</sub> with molecules of composition C<sub>16</sub>H<sub>24</sub>O<sub>4</sub> ( $Z = 2$ ), accurate cell constants of  $a = 8.404$  (2) Å,  $b = 7.734$  (2) Å,  $c = 12.371$  (2) Å,  $\beta = 104.76$  (1) Å,  $V = 777.5$  (3) Å<sup>3</sup>. All reflections were collected on the Nonius CAD-4 diffractometer, with graphite-monochromator, Mo-K $\alpha$  radiation, maximum  $2\theta$  value of 50°, independent reflections: 1424, observed number of reflections: 1319 [ $|F|^2 \geq 3.0 \sigma|F|^2$ ]. The structure was solved by the direct method SHELXS-86 and expanded using difference Fourier techniques, refined by full-matrix least-squares calculations. Hydrogen atoms were fixed at calculated positions. The final indices were  $R = 0.046$ ,  $R_w = 0.044$ .

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