

## Two New Monoterpene Diglycosides from *Winchia calophylla* A. DC

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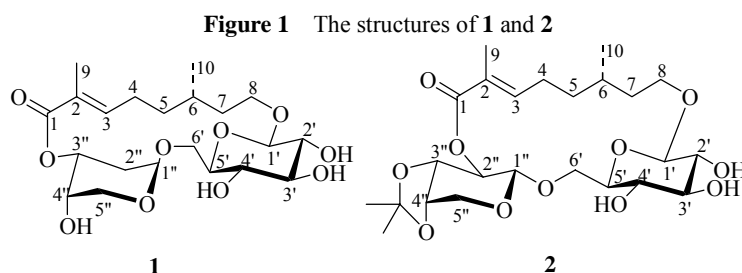
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**Abstract:** Two new monoterpene diglycosides, wincaloside A (**1**) and wincaloside B (**2**), along with loganin (**3**) were isolated from the stem barks of *Winchia calophylla* A. DC. The structures of **1** and **2** were established by spectroscopic and chemical methods.

**Keywords:** *Winchia calophylla*, Apocynaceae, monoterpene diglycosides, wincaloside.

*Winchia calophylla* A. DC. (Apocynaceae) is a traditional medicinal plant, distributed in Yunnan and Hainan Provinces of China, India, Burma and Indonesia<sup>1</sup>. In Xishuangbanna, Yunnan Province, its stem barks were used in the treatment of chronic tracheitis in Dai Nationality<sup>2</sup>. In order to search its relative bioactive components, we investigated the chemical constituents of this medicinal plant. The ethanol extracts of the stem barks of *W. calophylla* were subjected to repeated column chromatography over silica gel H, two new monoterpene diglycosides, wincaloside A (**1**) and wincaloside B (**2**) were obtained.



Compound (**1**) was obtained as a white powder,  $[\alpha]_D^{22} -29.5$  ( $c$  2.00,  $C_5H_5N$ ). The high resolution FAB-MS exhibited the molecular ion peak at  $m/z$  461.1939 ( $M^+$ -1) corresponding to the molecular formula  $C_{21}H_{34}O_{11}$  (calcd. 461.2022 for  $M^+$ -H). The molecular and fragment ion peaks at  $m/z$  462 ( $M^+$ ), 330 (462-132), 301 (462-161) and 169 (301-132 = 330-161) showed by EIMS implied there were a pentose and a hexose

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moieties in this molecule, and the fragment ion peaks at  $m/z$  197 (330-161+18=301-132+18) indicated the two glucose moieties formed a cycle with the

**Table 1** The NMR Spectra Data of Compound (**1**)<sup>a</sup>

C	<sup>1</sup> H	<sup>13</sup> C	HMBC <sup>b</sup>	<sup>1</sup> H- <sup>1</sup> H COSY
1	/	169.2	/	/
2	/	128.5	/	/
3	6.82 (t, J=7.3 Hz)	145.1	1, 2, 4, 5, 9	4
4	2.35 (ddd, 2H, J=6.7, 7.3, 13.0 Hz)	26.7	2, 3, 6, 7	3, 5
5	1.39 (m, 2H)	35.8	3, 4, 6, 7, 10	4, 6
6	1.52 (m, J=6.5 Hz)	28.9	4, 5, 8, 10	5, 7, 10
7	1.64 (ddd, 2H, J=4.1, 6.4, 6.5 Hz)	36.6	5, 6, 8, 10	6, 8
8	3.71 (dd, J=4.9, 6.4 Hz), 3.76 (dd, J=4.1, 6.4 Hz)	68.2	6, 7, 1'	7
9	1.90 (s, 3H)	12.8	1, 2, 3	/
10	0.91 (d, 3H, J=6.5 Hz)	20.5	5, 6, 7	6
1'	4.25 (d, J=7.5 Hz)	103.7	8, 3'	2'
2'	3.14 (dd, J=7.5, 8.4 Hz)	75.2	1', 3'	1', 3'
3'	3.30 (dd, J=8.4, 8.9 Hz)	78.3	1', 4'	2', 4'
4'	3.28 (dd, J=4.6, 8.9 Hz)	71.1	5', 6'	3', 5'
5'	3.32 (t, J=4.6 Hz)	76.8	1', 4'	4', 6'
6'	3.96 (dd, 2H, J=4.6, 11.0 Hz)	67.2	1'', 4', 5'	5'
1''	4.68 (s)	101.7	6', 3'', 5''	2''
2''	3.85 (d, J=2.8 Hz)	69.0	3'', 4''	1'', 3''
3''	5.07 (br.s)	73.1	2'', 5''	2'', 4''
4''	4.12 (m)	63.9	3''	3'', 5''
5''	3.50 (dd, J=4.9, 5.7 Hz), 3.94 (dd, J=4.8 Hz)	60.4	1'', 3'', 4''	4''

<sup>a</sup> <sup>1</sup>H, <sup>13</sup>C NMR and HMBC, <sup>1</sup>H-<sup>1</sup>H COSY spectra were obtained at 400 MHz, 100 MHz and 500 MHz, and recorded in CD<sub>3</sub>OD at room temperature, respectively. Unless otherwise indicated, all proton signals integrated to 1 H.

<sup>b</sup> Carbon atoms coupled with proton

aglycone whose composition was C<sub>10</sub>H<sub>18</sub>O<sub>3</sub> (M 196). And the fragment ion peaks at  $m/z$  169 (197-18), 151 (169-18) and 123 (169-46) implied a hydroxyl and a carboxyl group in the aglycone. Except for the characteristic signals of glycoses, its <sup>1</sup>H-NMR spectra showed a vinyl proton ( $\delta$  6.82, dd, 1H, J=7.3 Hz), a methylene bearing oxygen ( $\delta$  3.71, dd, 1H, J=4.1, 4.9 Hz and  $\delta$  3.76, dd, 1H, J=4.1, 7.9 Hz), a tertiary methyl ( $\delta$  1.90, s, 3H) and a secondary methyl ( $\delta$  0.91, d, 3H, J=6.5 Hz) which indicated **1** belonged to the lonitioside- type compounds<sup>3</sup>. Also the <sup>13</sup>C-NMR spectra showed the signals of two methyls, four ethylenes one of which bore an oxygen, a methine, a vinyl methane, a vinyl quaternary carbon and an ester carbonyl which indicated the aglycone of **1** was a carboxylic acid of monoterpene bearing a hydroxyl (**Table 1**). In HMBC experiments of **1**, the following long-range correlations between <sup>1</sup>H and <sup>13</sup>C were observed: between H-3 ( $\delta$  6.82, t, 1H, J=7.3 Hz) and C-1 ( $\delta$  169.2), C-2 ( $\delta$  128.5), C-4 ( $\delta$  26.7), C-5 ( $\delta$  35.8) and C-9 ( $\delta$  12.8); between H-9 ( $\delta$  1.19, s, 1H) and C-1, C-2 and C-3 ( $\delta$  145.1); between H-10 ( $\delta$  0.91, d, 1H, J=6.5 Hz) and C-5, C-6 ( $\delta$  28.9), and C-7 ( $\delta$  36.7); between H-6 ( $\delta$  1.52, m, 1H, J=6.5 Hz) and C-4, C-6, C-7 and C-8 ( $\delta$  68.2). Besides, <sup>1</sup>H-<sup>1</sup>H COSY spectra showed the connectivities between H-4 and H-5, between H-5 and H-6, between H-6 and H-7 and H-10, between H-7 and H-8. And its NOESY spectra did not show the long-range correlation between H-3 with H-9. All above mentioned supported the aglycone of **1** was 2,6-dimethyl-8-hydroxy-2*E*-octenoic acid. The anomeric protons at

$\delta$  4.25 (d, 1H,  $J=7.5$  Hz) and  $\delta$  4.68 (s, 1H), and anomeric carbons at  $\delta$  103.7 and  $\delta$  101.7, indicated the hexose was  $\beta$ -D-glucopyranose and pentose was  $\beta$ -L-arabinopyranose. Its HMBC experiments showed the  $^1\text{H}$ - $^{13}\text{C}$  long-range correlations between H-1' ( $\delta$  4.25, d, 1H,  $J=7.5$  Hz) and C-8 ( $\delta$  68.2), between H-8 ( $\delta$  3.71, dd, 1H,  $J=4.1, 4.9$  Hz / 3.76, dd, 1H,  $J=4.1, 7.9$  Hz) and C-1' ( $\delta$  103.7), between H-6' ( $\delta$  3.96, dd, 1H,  $J=5.2, 5.8$  Hz) and C-1'' ( $\delta$  101.7), between H-1'' ( $\delta$  4.68, s, 1H) and C-6' ( $\delta$  67.4) (**Table 1**), which indicated the biose was 6'-*O*- $\beta$ -L-arabinopyranosyl- $\beta$ -D-glucopyranose that formed the glucoside with 8-OH of the monoterpene acid. Hence, the H-3'' and C-3'' of the biose ( $\delta$  5.07 and  $\delta$  73.1, respectively) had obvious down-field shifts than those of  $\beta$ -L-arabinopyranose<sup>4</sup> and methyl  $\beta$ -L-arabinopyranose<sup>5</sup> ( $\delta$  3.6 and  $\delta$  70.7, respectively), which showed the 3''-OH formed ester with -COOH of the monoterpene acid. The absolute configuration of monoterpene acid was determined by the T. I wagawa's method<sup>14</sup>. On hydrolysis with 10% HCl, 2,6-dimethyl-8-hydroxy-2*E*-octenoic acid was produced, which showed negative optical rotation ( $[\alpha]_{\text{D}}^{22}$  -6.7) that was agreement with that of 2*E*, 6*S*-dimethyl-8-hydroxy-2*E*-octenoic acid<sup>6</sup> and opposite to that of 2*E*, 6*R*-dimethyl-8-hydroxy-2*E*-octenoic acid<sup>7</sup>. Thus, the monoterpene acid in compound (**1**) was 2*E*, 6*S*-dimethyl-8-hydroxy-2*E*-octenoic acid. And compound (**1**) named as wincaloside A whose structure was elucidated as to be shown (**Figure 1**).

Compound (**2**), obtained as a white powder,  $[\alpha]_{\text{D}}^{22}$  -34.0 ( $c$  1.00,  $\text{C}_3\text{H}_5\text{N}$ ). The high resolution EIMS exhibited the molecular ion peak at  $m/z$  502.2420 corresponding to the molecular formula  $\text{C}_{24}\text{H}_{38}\text{O}_{11}$  (calcd. 502.2414). Except for more 40 units than **1** in molecular weight, its EIMS,  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR, HMBC,  $^1\text{H}$ - $^1\text{H}$  COSY (**Table 2**) and NOSEY spectra were very similar to those of **1**, implying they belonged to the same kind of compounds. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of the anomeric protons and carbons at  $\delta$  4.11 (d, 1H,  $J = 7.8$  Hz) and  $\delta$  103.5,  $\delta$  4.41 (d, 1H,  $J = 7.6$  Hz) and  $\delta$  102.3 indicated the hexose was  $\beta$ -D-glucopyranose and pentose was  $\alpha$ -L-arabinopyranose. Comparing with those of **1**, the following fragment ion signals EIMS of **2** at  $m/z$  369 (502-133), 340 (502-162), 329 (502-132-40-1), 312 (502-150-40) and 169 (340-132-40+1 = 329-161+1) showed a isopropylidene unit bore in the pentose moiety on the framework of **2**; the  $^1\text{H}$ -NMR spectra showed two signals of the tertiary methyls at  $\delta$  1.32 (s, 3H) and  $\delta$  1.52 (s, 3H) in high-field; the  $^{13}\text{C}$ -NMR (DEPT) spectra showed the signals of two methyls at  $\delta$  26.3 and  $\delta$  26.5 as well as a quaternary carbon at  $\delta$  111.3. HMBC spectra showed the  $^1\text{H}$ - $^{13}\text{C}$  long-range correlations between H-3'' ( $\delta$  4.26, m, 1H), H-4'' ( $\delta$  4.25, m, 1H) and C-2''' ( $\delta$  111.3), and all of which indicated the 2-OH and 3-OH formed acetal with acetone in  $\alpha$ -L-arabinopyranose. Besides, the  $^1\text{H}$ - $^{13}\text{C}$  long-range correlations of HMBC between H-6' (3.86, dd, 1H,  $J = 2.0, 13.2$  Hz, and 4.24, dd, 1H,  $J=4.5, 13.2$  Hz) and C-1'' ( $\delta$  102.3), between H-1'' ( $\delta$  4.41, d, 1H,  $J = 7.6$  Hz) and C-6' ( $\delta$  70.9) showed the biose was 6'-*O*- $\alpha$ -L-arabinopyranosyl- $\beta$ -D-glucopyranose. And the  $^1\text{H}$ - $^{13}\text{C}$  long-range correlations between H-1' ( $\delta$  4.11, d, 1H,  $J = 7.8$  Hz) and C-8 ( $\delta$  70.2), between H-8 (3.59, m, 1H,  $J = 5.7, 6.8$  Hz) and C-1' ( $\delta$  103.5), between H-2'' ( $\delta$  5.00, t, 1H,  $J = 7.6$  Hz) and C-1 ( $\delta$  168.6) indicated the 8-OH and carboxyl of 2*E*, 6*S*-dimethyl-8-hydroxy-2*E*-octenoic acid formed the glycoside and ester with the anomeric hydroxyl and 2''-OH of the biose, respectively. Thus, compound (**2**) named wincaloside B whose structure was elucidated as shown in **Figure 1**.

**Table 2** The NMR spectra data of compound **2**<sup>a</sup>

C	<sup>1</sup> H	<sup>13</sup> C	HMBC <sup>b</sup>	<sup>1</sup> H- <sup>1</sup> H COSY
1	/	168.7	/	/
2	/	128.5	/	/
3	6.85 (t, J=7.2 Hz)	144.7	1, 2, 4, 5, 9	4
4	2.18 (m), 2.37 (m)	26.3	2, 3, 6, 7	3, 5
5	1.41 (m), 1.54 (m)	36.0	3, 4, 6, 7, 10	4, 6
6	1.50 (m, J=5.9 Hz)	31.0	4, 5, 8, 10	5, 7, 10
7	1.45 (m)	36.4	5, 6, 8, 10	6, 8
8	3.59 (m, J=5.7, 6.8 Hz)	70.2	6, 7, 1'	7
9	1.83 (s, 3H)	12.7	1, 2, 3	/
10	0.98 (d, 3H, J=5.9 Hz)	22.7	5, 6, 7	6
1'	4.11 (d, J=7.8 Hz)	103.5	8, 2', 3'	2'
2'	3.10 (t, J=7.8, 9.2 Hz)	75.3	1', 3', 4'	1', 3'
3'	3.29 (dd, J=9.2 Hz)	78.2	1', 4'	2', 4'
4'	3.00 (t, J=9.2 Hz)	72.4	3', 6'	3', 5'
5'	3.33 (t, J=4.5, 9.2 Hz)	76.9	1', 3', 6'	4', 6'
6'	3.86 (dd, J=2.0, 13.2 Hz), 4.24 (dd, J=4.5, 13.2 Hz)	70.9	1'', 4''	5''
1''	4.41 (d, J=7.6 Hz)	102.3	6', 3'', 5''	2''
2''	5.00 (t, J=7.6 Hz)	75.0	1, 1'', 3''	1'', 3''
3''	4.26 (m)	78.1	1'', 2'''	2'', 4''
4''	4.25 (m)	75.2	3''	3'', 5''
5''	3.87 (d, J=13.0 Hz), 4.19 (d, J=13.0 Hz)	64.3	1'', 4''	4''
1'''	1.32 (s, 3H)	26.3	2''', 3'''	/
2'''	/	111.3	/	/
3'''	1.52 (s, 3H)	28.1	1''', 2'''	/

<sup>a</sup> <sup>1</sup>H, <sup>13</sup>C NMR and HMBC, <sup>1</sup>H-<sup>1</sup>H COSY spectra were obtained at 400 MHz, 100 MHz and 500 MHz, and recorded in CD<sub>3</sub>OD at room temperature, respectively. Unless otherwise indicated, all proton signals integrated to 1 H.

<sup>b</sup> Carbon atoms coupled with proton.

### Acknowledgment

This work was financially supported by the National Natural Science Foundation for Outstanding Young Scientists to Prof. Xiao Jiang Hao (No. 39525025).

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Received 21 October, 2002