

# Chemical Constituents from the Leaves of *Aglaia odorata*

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A new dammarane triterpene, 3-acetoxy aglinin C (**1**), and a new aglain, 10-oxo-aglaxiflorin D (**2**), along with five known compounds, **3–7**, were isolated from the leaves of *Aglaia odorata* using chromatographic methods. The structures of **1** and **2** were determined on the basis of spectroscopic analyses. Bioactivities of **1–7** against AGZY 83-a (human lung cancer cell line) and SMMC-7721 (human liver cancer cell line) cells were determined.

**Key words:** *Aglaia odorata*, 3-Acetoxy Aglinin C, 10-Oxo-aglaxiflorin D

## Introduction

The genus *Aglaia* (Meliaceae) comprises nearly 120 species and is distributed mainly in the tropical forest of Southeast Asia (Pannell, 1992). Previous phytochemical investigation has revealed the presence of unique secondary metabolites such as bisamides, lignans, and triterpenes (Fuzzati *et al.*, 1996; Xie *et al.*, 2007; Kim *et al.*, 2006). Some of these compounds exhibited insecticidal, antifungal, anti-inflammatory, and antiproliferative activities against cancer cell lines (Bacher *et al.*, 1999; Saifah *et al.*, 1999; Puripattavong *et al.*, 2000; Proksch *et al.*, 2005). We have previously also reported on dammaranes and pregnanes from the genus *Aglaia* (Yang *et al.*, 2008a, b). Here we present a new dammarane triterpene, 3-acetoxy aglinin C (**1**), and a new aglain, 10-oxo-aglaxiflorin D (**2**), together with five known compounds, aglain C (**3**), aglaxiflorin D (**4**), 10-*O*-acetylglain C (**5**), rocaglaol (**6**), and odorinol (**7**), obtained from the leaves of *Aglaia odorata*. The bioactivities of **1–7** against AGZY 83-a (human lung cancer cell line) and SMMC-7721 (human liver cancer cell line) cells were assessed. Among them, compound **6** exhibited remarkable cytotoxicity towards the two cell lines with IC<sub>50</sub> values of 0.03 and 3.62 μM, respectively.

## Results and Discussion

Compound **1**, a white powder, was found to possess a molecular formula of C<sub>32</sub>H<sub>54</sub>O<sub>5</sub> as evidenced

by HR-ESI-MS (*m/z* 541.3859 [M+Na]<sup>+</sup>). The <sup>13</sup>C NMR (DEPT) spectrum of **1** displayed signals for 32 carbon atoms, eight tertiary methyl groups ( $\delta_C$  24.9, 24.5, 27.9, 21.4, 16.0, 24.2/24.0, 16.6/16.5, 15.5 ppm), ten methylene groups ( $\delta_C$  34.3, 22.9, 18.1, 35.2, 21.4/21.2, 25.9/25.2, 31.6, 27.3/26.9, 36.8/34.6, 31.5/31.1 ppm), five methane groups ( $\delta_C$  78.4, 50.6, 50.9, 43.3/42.8, 50.6/50.4 ppm), seven quaternary carbon atoms ( $\delta_C$  37.2, 40.6, 36.8, 50.1, 88.7/88.0, 108.6, 74.7/74.1 ppm), and an acetyl group at  $\delta_C$  170.8 (s, CH<sub>3</sub>COO) and 21.7 ppm (q, CH<sub>3</sub>COO). The <sup>1</sup>H and <sup>13</sup>C NMR spectral data were quite similar to those of aglinin C with the exception of additional signals for an acetyl group (Mohamad *et al.*, 1999). The downshift (1.23 ppm) of H-3 in **1** compared to aglinin C and the signals of the additional acetyl group at  $\delta_C$  170.8 (s), 21.7 ppm (q) and  $\delta_H$  2.10 ppm (s, 3H) revealed an acetyl group instead of a hydroxy group attached to C-3. This was proven by the HMBC correlations between  $\delta_H$  4.83 ppm (1H, brs, H-3) and  $\delta_C$  78.4 (d, C-3), 37.2 ppm (s, C-4) (see Fig. 1). So **1** was named 3-acetoxy aglinin C. The observed ‘peak doubling’ in the <sup>13</sup>C NMR spectrum suggested that **1** was a mixture of C-24 epimers which were probably interconvertible just as aglinins A, B, and C (Mohamad *et al.*, 1999). Attempts to separate these isomers were not successful.

Compound **2** was obtained as a colourless gum. The molecular formula was determined as C<sub>36</sub>H<sub>40</sub>N<sub>2</sub>O<sub>9</sub> by HR-ESI-MS (*m/z* 667.2623 [M+Na]<sup>+</sup>). The <sup>1</sup>H NMR spectrum disclosed three methoxy groups at  $\delta_H$  3.84 (3H, s, MeO-8),

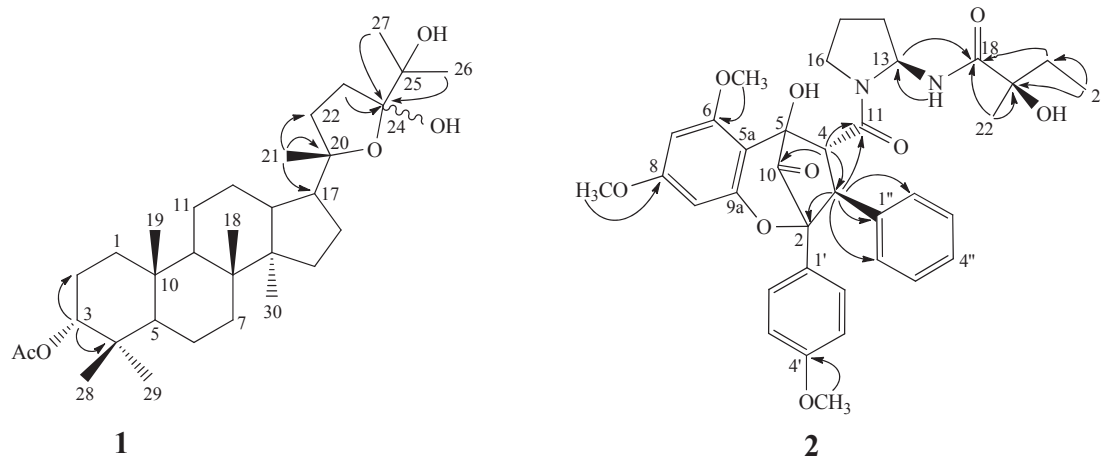


Fig. 1. Key HMBC correlations of 3-acetoxy aglinin (**1**) and 10-oxo-aglaxiflorin D (**2**).

3.79 (3H, s, MeO-6), 3.74 ppm (3H, s, MeO-4'). Three aromatic rings related to those observed for rocaglaol (**6**) were deduced to be one mono-substituted phenyl group:  $\delta_{\text{H}}$  7.04 (2H, dd,  $J = 7.7, 1.8$  Hz, H-2'', 6''), 7.11–7.15 ppm (3H, m, H-3'', 4'', 5''), one *p*-substituted phenyl group:  $\delta_{\text{H}}$  6.97 (2H, d,  $J = 8.8$  Hz, H-2', 6'), 6.73 ppm (2H, d,  $J = 8.8$  Hz, H-3', 5'), and two *m*-coupled aromatic protons:  $\delta_{\text{H}}$  6.35 (1H, d,  $J = 1.5$  Hz, H-9), 6.11 ppm (1H, d,  $J = 1.5$  Hz, H-7). In addition, resonances for a methane pair appeared at  $\delta_{\text{H}}$  4.59 (1H, d,  $J = 12.9$  Hz, H-4), 4.38 ppm (1H, d,  $J = 12.9$  Hz, H-3), and were mutually coupled in the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum. Based on the observed HMQC, these two signals were found to correspond to the  $^{13}\text{C}$  NMR signals at  $\delta_{\text{C}}$  53.1 (C-3) and 55.5 ppm (C-4), respectively. Characteristic signals of a pyrrolidine-type bisamide unit in **2** were apparent, with two carbonyl groups at  $\delta_{\text{C}}$  166.4 (C-11) and 175.5 ppm (C-18) (Kim *et al.*, 2005).

The HMBC spectrum showed the following long-range correlations:  $\delta_{\text{H}}$  3.74 ppm (3H, s, MeO-4') to  $\delta_{\text{C}}$  158.9 ppm (s, C-4');  $\delta_{\text{H}}$  3.84 ppm (3H, s, MeO-8) to  $\delta_{\text{C}}$  164.8 ppm (s, C-8);  $\delta_{\text{H}}$  3.79 ppm (3H, s, MeO-6) to  $\delta_{\text{C}}$  158.6 ppm (s, C-6);  $\delta_{\text{H}}$  4.38 ppm (1H, d,  $J = 12.9$  Hz, H-3) to  $\delta_{\text{C}}$  99.9 (s, C-2), 55.5 (d, C-4), 166.4 (s, C-11), 125.4 (s, C-1'), 135.8 (s, C-1''), 128.4 ppm (d, C-2'', 6'');  $\delta_{\text{H}}$  4.59 ppm (1H, d,  $J = 12.9$  Hz, H-4) to  $\delta_{\text{C}}$  53.1 (d, C-3), 166.4 (s, C-11), 135.8 ppm (s, C-1''). Therefore, it was deduced that a *p*-substituted phenyl group was located at C-2, and an unsubstituted phenyl group at C-3.

All above-mentioned observations in the NMR spectra suggested that compound **2** is a cyclopenta[*bc*]benzopyran derivative (Xu *et al.*, 2000; Inada *et al.*, 2000; Joycharat *et al.*, 2008; Salim *et al.*, 2007).

In the HMBC spectrum, the correlations between  $\delta_{\text{H}}$  0.97 ppm (3H, t,  $J = 7.5$  Hz, Me-21) with  $\delta_{\text{C}}$  76.3 (s, C-19) and 33.4 ppm (t, C-20),  $\delta_{\text{H}}$  1.58 ppm (3H, s, Me-22) with  $\delta_{\text{C}}$  175.5 (s, C-18) and 76.3 ppm (s, C-19) indicated the presence of a 2-hydroxy-2-methylbutyryl group located at C-18.

The molecular formula of **2** comprised two hydrogen atoms less than that of aglaxiflorin D (**4**) (Xu *et al.*, 2000), and the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data of **2** were in good agreement with those of **4**, except that the methylene carbon atom at  $\delta_{\text{C}}$  79.7 ppm (d, C-10) was replaced by a ketonyl carbon atom at  $\delta_{\text{C}}$  207.5 ppm (C-10). In the HMBC spectrum, the correlation of H-4 ( $\delta_{\text{H}}$  4.59 ppm, 1H, d,  $J = 12.9$  Hz) to C-10 ( $\delta_{\text{C}}$  207.5 ppm, s) was observed (see Fig. 1). So **2** was determined as 10-oxo-aglaxiflorin D.

To date, tetracyclic triterpenes of the dammarane, tirucallane, or cycloartane series have been found in all *Aglaia* species studied. Cyclopentatetrahydrobenzofurans of the rocaglaol type are also frequently encountered (Mohamad *et al.*, 1999; Wang *et al.*, 2004). Bioactivity investigations revealed that the cyclopentatetrahydrobenzofurans were apparently the active components responsible for the cytotoxicity, while they had lower cytotoxic activity and even no cytotoxic activity at all (Bohnenstengel *et al.*, 1999a, b; Proksch *et*

*al.*, 2001). In our study, Compounds **1–7** were assayed for their cytotoxic activity towards AGZY 83-a (human lung cancer cell line) and SMMC-7721 (human liver cancer cell line) cells; the result are given in Table I. We found that compound **6**, rocaglaol, exhibited distinctive antiproliferative activities against the two cell lines with IC<sub>50</sub> values of 0.03  $\mu\text{M}$  and 3.62  $\mu\text{M}$ , respectively. Compound **7** was strongly active against SMMC-7721 cells with an IC<sub>50</sub> value of 10.69  $\mu\text{M}$ . Compound **1**, a new dammarane triterpene, and the four cyclopentatetrahydrobenzopyrans **2–5** were inactive.

## Experimental

### General

Silica gel (200–300 mesh) for column chromatography (CC) and silica gel GF<sub>254</sub> for thin layer chromatography (TLC) were obtained from Qingdao Marine Chemical Factory, Qingdao, P. R. China. The XRC-1 apparatus for determination of melting points was provided by Sichuan University, Sichuan, P. R. China. The SEAP-300 polarimeter for determination of optical rotation was the product of Horiba (Kyoto, Japan). IR spectra were recorded on an FTS-135 spectrophotometer (Bio-Rad, Richmond, CA, USA). The AM-400 or DRX-500 NMR spectrometers were from Bruker, Karlsruhe, Germany. Mass spectra were recorded on a VG Autospec-3000 spectrometer (Manchester, UK).

### Plant material

The leaves of *A. odorata* were collected in Xishuangbanna County of Yunnan Province, P. R. China, in January 2006. The plant material was identified by Prof. Jing-Yun Cui, Xishuangbanna Tropical Botanical Garden, the Chinese Academy of Science, Mengla County, P. R. China.

### Extraction and isolation

The air-dried leaves of *A. odorata* (15 kg) were crushed and extracted with 95% EtOH at reflux temperature to yield an EtOH extract. After removal of EtOH *in vacuo*, the remaining viscous concentrate was successively partitioned between H<sub>2</sub>O and petroleum ether, CHCl<sub>3</sub>, and *n*-BuOH, respectively. The CHCl<sub>3</sub> extract (230 g) was subjected to CC (SiO<sub>2</sub>; petroleum ether/Me<sub>2</sub>CO, 1:0 → 0:1, v/v) to give 9 fractions (Fr. 1–Fr. 9), as judged by TLC. Fr. 4 (9 g) was repeatedly chromatographed on silica gel (petroleum ether/EtOAc, 97:3 → 8:2) to give **1** (210 mg). Fr. 5 (6 g) was repeatedly chromatographed over silica gel (CHCl<sub>3</sub>/Me<sub>2</sub>CO, 98:2 → 9:1) and RP-18 (MeOH/H<sub>2</sub>O, 1:1 → 1:0) to yield **6** (133 mg). Fr. 6 (17 g) was chromatographed on silica gel (CHCl<sub>3</sub>/Me<sub>2</sub>CO, 9:1 → 7:3) to obtain nine subfractions, A–F. Subfraction A was recrystallized to obtain **7** (325 mg), subfraction C was purified repeatedly by CC on silica gel (CHCl<sub>3</sub>/Me<sub>2</sub>CO, 5:1) and RP-18 (CH<sub>3</sub>OH/H<sub>2</sub>O, 1:1 → 1:0) to give **2** (85 mg), **3** (170 mg), **5** (11 mg). Fr. 7 (5 g) was repeatedly chromatographed over silica gel (CHCl<sub>3</sub>/Me<sub>2</sub>CO, 9:1 → 1:1) and RP-18 (CH<sub>3</sub>OH/H<sub>2</sub>O, 1:1 → 1:0) to yield **4** (134 mg).

*3-Acetoxy aglinin C (1)*: White powder. – [ $\alpha$ ]<sub>D</sub><sup>26</sup> = –2.8° (c 0.395, MeOH). – IR (KBr):  $\nu$  = 3512, 2945, 1720, 1462, 1388, 1249, 1180, 1037, 883 cm<sup>-1</sup>. – <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 4.83 (1H, brs, H-3), 2.10 (3H, s, OAc), 1.29/1.27 (3H, s, Me-27), 1.26 (3H, s, Me-26), 1.13 (3H, s, Me-21), 1.00/0.97 (3H, s, Me-18), 0.98 (3H, s, Me-28), 0.93/0.92 (3H, s, Me-30), 0.87 (3H, s, Me-29), 0.85 (3H, s, Me-19). – <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  = 34.3 (t, C-1), 22.9 (t, C-2), 78.4 (d, C-3), 37.2 (s, C-4), 50.6 (d, C-5), 18.1 (t, C-6), 35.2 (t, C-7), 40.6 (s, C-8), 50.9 (d, C-9), 36.8 (s, C-10), 21.4/21.2 (t, C-11), 25.9/25.2

Table I. Cytotoxicity of compounds **1–7**.

Cell line <sup>a</sup>	IC <sub>50</sub> [ $\mu\text{M}$ ] <sup>b</sup>							
	<i>cis</i> -Platin <sup>c</sup>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>
AGZY 83-a	5.67±0.37	n.a. <sup>d</sup>	n.a.	53.80±3.07	n.a.	n.a.	0.03±0.002	n.a.
SMMC-7721	3.95±0.07	n.a.	47.00±3.85	63.06±1.34	n.a.	39.25±1.20	3.62±0.13	10.69±0.82

<sup>a</sup> AGZY 83-a, human lung cancer cells; SMMC-7721, human liver cancer cells.

<sup>b</sup> The IC<sub>50</sub> values are presented as means ± SD.

<sup>c</sup> Positive control.

<sup>d</sup> n.a., no activity.

(t, C-12), 43.3/42.8 (d, C-13), 50.1 (s, C-14), 31.6 (t, C-15), 27.3/26.9 (t, C-16), 50.6/50.4 (d, C-17), 15.5 (q, C-18), 16.6/16.5 (q, C-19), 88.7/88.0 (s, C-20), 24.2/24.0 (q, C-21), 36.8/34.6 (t, C-22), 31.5/31.1 (t, C-23), 108.6 (s, C-24), 74.7/74.1 (s, C-25), 24.9 (q, C-26), 24.5 (q, C-27), 27.9 (q, C-28), 21.4 (q, C-29), 16.0 (q, C-30), 170.8 (s, CH<sub>3</sub>COO), 21.7 (q, CH<sub>3</sub>COO). – ESI-MS:  $m/z$  = 1059 [2M+Na]<sup>+</sup>, 541 [M+Na]<sup>+</sup>. – HR-ESI-MS:  $m/z$  = 541.3859 [M+Na]<sup>+</sup> (calcd. 541.3869 for C<sub>32</sub>H<sub>54</sub>O<sub>5</sub>Na).

**10-Oxo-aglaxiflorin D (2):** Colourless gum. –  $[\alpha]_D^{26}$  = +54.8° (c 0.345, MeOH). – IR (KBr):  $\nu$  = 3396, 2968, 2937, 2840, 1751, 1619, 1517, 1425, 1251, 1149, 815 cm<sup>-1</sup>. – <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 7.74 (1H, d,  $J$  = 9.4 Hz, NH), 7.04 (2H, dd,  $J$  = 7.7, 1.8 Hz, H-2'', 6''), 7.11 – 7.15 (3H, m, H-3'', 4'', 5''), 6.97 (2H, d,  $J$  = 8.8 Hz, H-2', 6'), 6.73 (2H, d,  $J$  = 8.8 Hz, H-3', 5'), 6.35 (1H, d,  $J$  = 1.5 Hz, H-9), 6.11 (1H, d,  $J$  = 1.5 Hz, H-7), 5.90 (1H, dd,  $J$  = 9.4, 6.2 Hz, H-13), 4.59 (1H, d,  $J$  = 12.9 Hz, H-4), 4.38 (1H, d,  $J$  = 12.9 Hz, H-3), 3.84 (3H, s, MeO-8), 3.79 (3H, s, MeO-6), 3.74 (3H, s, MeO-4'), 3.62 (1H, m, H-16a), 3.20 (1H, m, H-16b), 2.07 (1H, m, H-14a), 1.90 (1H, m, H-20a), 1.85 (1H, m, H-14b), 1.88 (2H, m, H-15), 1.71 (1H, m, H-20b), 1.58 (3H, s, Me-22), 0.97 (3H, t,  $J$  = 7.5 Hz, Me-21). – <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  = 99.9 (s, C-2), 53.1 (d, C-3), 55.5 (d, C-4), 88.8 (s, C-5), 105.7 (s, C-5a), 158.6 (s, C-6), 88.9 (d, C-7), 164.8 (s, C-8), 93.0 (d, C-9), 161.1 (s, C-9a), 207.5 (s, C-10), 166.4 (s, C-11), 62.6 (d, C-13), 34.5 (t, C-14), 21.8 (t, C-15), 46.4 (t, C-16), 175.5 (s, C-18), 76.3 (s, C-19), 33.4 (t, C-20), 8.0 (q, C-21), 25.3 (q, C-22), 125.4 (s, C-1'),

127.8 (d, C-2', 6'), 113.2 (d, C-3', 5'), 158.9 (s, C-4'), 135.8 (s, C-1''), 128.4 (d, C-2'', 6''), 128.0 (d, C-3'', 5''), 127.0 (d, C-4''), 55.1 (q, OCH<sub>3</sub>-4'), 55.6 (q, OCH<sub>3</sub>-8), 55.7 (q, OCH<sub>3</sub>-6). – ESI-MS:  $m/z$  = 1311 [2M+Na]<sup>+</sup>, 645 [M+H]<sup>+</sup>. – EI-MS:  $m/z$  = 626 (8), 557 (9), 458 (7), 414 (16), 311 (12), 300 (100), 285 (28), 135 (6). – HR-ESI-MS:  $m/z$  = 667.2623 [M+Na]<sup>+</sup> (calcd. 667.2632 for C<sub>36</sub>H<sub>40</sub>N<sub>2</sub>O<sub>9</sub>Na).

The structures of **3–7** were elucidated on the basis of their spectral data and comparison with published data (Dumontet *et al.*, 1996; Xu *et al.*, 2000; Ishibashi *et al.*, 1993; Shienhthong *et al.*, 1979).

### Bioassays

An improved MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay was performed in 96-well plates. The experimental details have been reported previously (Niu *et al.*, 2002)

Compounds **1–7** were assayed for their cytotoxic activity towards AGZY 83-a (human lung cancer cell line) and SMMC-7721 (human liver cancer cell line) cells. *cis*-Platin was used as the positive control.

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