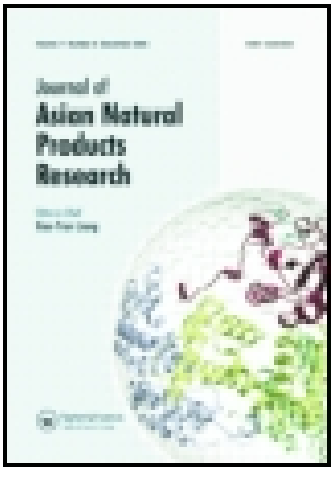


Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Two new lignans from twigs of Aglaia odorata

Lei Peng[ab], Wu-Xiang Fu[c], Chun-Xia Zeng[c], Lin Zhou[a], Mei-Fen Bao[b] & Xiang-Hai Cai[b]

[a] College of Horticulture and Landscape, Yunnan Agriculture University, Kunming 650201, China

[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

[c] Germplasm Bank of Wild Species in Southwest China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

Published online: 24 Jul 2015.

To cite this article: Lei Peng, Wu-Xiang Fu, Chun-Xia Zeng, Lin Zhou, Mei-Fen Bao & Xiang-Hai Cai (2015): Two new lignans from twigs of Aglaia odorata, Journal of Asian Natural Products Research, DOI: 10.1080/10286020.2015.1057575

To link to this article: http://dx.doi.org/10.1080/10286020.2015.1057575

Journal of Asian Natural Products Research, 2015
http://dx.doi.org/10.1080/10286020.2015.1057575

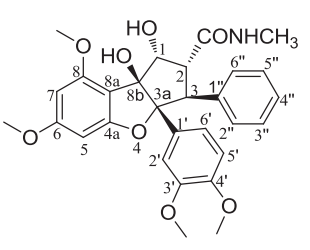
Two new lignans from twigs of *Aglaia odorata*

Lei Peng[a,b], Wu-Xiang Fu[c], Chun-Xia Zeng[c], Lin Zhou[a], Mei-Fen Bao[b] and Xiang-Hai Cai[b]*

[a]*College of Horticulture and Landscape, Yunnan Agriculture University, Kunming 650201, China;* [b]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China;* [c]*Germplasm Bank of Wild Species in Southwest China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China*

(*Received 2 March 2015; final version received 28 May 2015*)

HPLC-guided separation of twigs of *Aglaia odorata* led to the isolation of eight lignans, including two new ones, 3′-methoxy-*N*-demethylrocaglamide (**1**) and 4′-*O*-demethyl-deacetylaglaxiflorin A (**2**). Compound **1** showed excellent cytotoxicity against three human cancer cell lines, HeLa, SGC-7901 gastric cancer, and A-549 lung cancer with the values of 0.32, 0.12, and 0.25 μM, respectively.



1 **2**

Keywords: Meliaceae; *Aglaia odorata*; 3′-methoxy-*N*-demethylrocaglamide; 4′-*O*-demethyl-deacetylaglaxiflorin A; cytotoxicity

1. Introduction

Aglaia genus of the family Meliaceae, including approximately 120 species, is naturally distributed in tropical and subtropical Asia, Australia, and Pacific islands. Among them, eight species can be found in China and one of the plants, *A. odorata*, is cultivated as an ornamental plant [1]. The genus has been focused considerably because of its unique natural products, rocaglamides and aglains lignans [2]. Rocaglamides possess pesticidal and antitumor activity, in contrast, aglains appear to be devoid of any insecticidal or anticancer activity [3]. In the last several years, our phytochemical research on the cultivated species could not afford those typical constituents besides aglaxiflorin D. However, it disclosed a new distribution of the dolabellane diterpenoids [4,5]. The results are not consistent with the literature [2], which prompts us to

*Corresponding author. Email: xhcai@mail.kib.ac.cn

explore whether rocaglamides are distributed in the original plant. Subsequently, phytochemical analysis on the leaves of natural *A. odorata* could not detect the rocaglamides. After that, HPLC-guided separation on its twigs and trunks gave both types of chemicals. This paper will describe the isolation, structural elucidation, and cytotoxity of the two types of lignans.

2. Results and discussion

The MeOH extract of *A. odorata* twigs was partitioned between H_2O and EtOAc, and column chromatography (CC) over silica gel and C18 silica gel was used in the isolation of the EtOAc fraction to yield eight compounds with UV absorption maxima at 270–280 nm.

The IR spectrum of compound **1** showed absorption bands at 3500, 3405, 1726, 1626, and 1603 cm^{-1}, corresponding to OH/NH, an amide carbonyl, and benzene rings, respectively. Its UV spectrum indicated absorptions at 208 and 278 nm, consistent with the characteristic of lignans. In addition, the ^{1}H NMR spectrum of **1** showed four methoxyl groups, one phenyl group, one trisubstituted phenyl group, and one oxygenated benzene group with two meta protons, indicating typical signals of rocaglamides or aglains [6]. Its characteristic quaternary signals of δ_C 101.5 (s, C-3a) and 93.0 (s, C-8b) in the ^{13}C NMR spectrum (Table 1) further suggested it was a rocaglamide analog rather than aglains [7]. Molecular formula of **1** was determined as $C_{29}H_{31}O_8$ by HR-ESI-MS at *m/z* 544.1945 $[M + Na]^+$. Comparison of the NMR spectral data for **1** with those of *N*-demethylrocaglamide (**5**) (Figure 1) and 3′-methylrocaglamide [8] indicated the presence of 3′-methoxy and absence of *N*-methyl in compound **1**. The 3′-methoxyl could be confirmed by the HMBC correlations of H-5′/C-3′ and C-1′ and of 3′-OMe/C-3′. The stereo-configuration of **1** was identical to that of 3′-hydroxyrocaglamide (**6**) by comparison of their optical rotations (−89 for both **1** and **6**) and CD Cotton effect (− 10 for **1** and − 13 for **6** at ab. 212 nm) [9]. So, **1** was named as 3′-methoxy-*N*-demethylrocaglamide.

Compound **2** was isolated as colorless needles, and its molecular formula was determined as $C_{35}H_{40}N_2O_6$ by HR-ESI-MS. The IR spectrum showed absorption bands at 3426, 3401, 3372 (OH and NH), 1666 (amide carbonyl), and 1633, 1621, 1591 (benzene rings) cm^{-1}. Two methoxyl groups, three aromatic rings, one phenyl group, one parasubstituted phenyl, and one aromatic ring with meta positions unsubstituted were deduced from the ^{1}H NMR spectrum (Table 1). Analysis of the ^{1}H and ^{13}C NMR spectra of **2** indicated a similar skeleton to those of aglains [6]. However, the down-field carbon signal (δ_C 76.0, s) indicated that **2** had a new 2-hydroxy-2-methylbutyryl group, similar to aglaxiflorins A–D [10]. Its notable HMBC correlations of H-3/C-5 and C-1″, and H-2″ (6″) and H-4/C-3, and H-4/C-1″ could further determine the skeleton of **2** was identical to aglaxiflorin A rather than aglaxiflorin C [10]. Further comparison with NMR of aglaxiflorin A indicated that **2** was similar to aglaxiflorin A with exception for the absence of an acetyl and a methoxyl. A hydroxyl instead of methoxyl was located at C-4′ based on HMBC correlations of two methoxyls (δ_H 4.04 and 3.75) with C-6 (δ_C 157.7) and C-8 (δ_C 162.2), respectively, and of H-2′/6′ (δ_H 7.37) with C-4′ (δ_C 155.8). The relative stereochemical assignments were from ROESY correlations, e.g., H-3/H-10 and H-4/H-2″ (6″). Thus, there are still two skeleton isomers of **1** from orientations of H-3 and H-4 in consideration of the literature [10,11]. Chemical shift of C-4 was resonated at ab. δ_C 57.6 when H-3 and H-4 in aglains adopted β and α-orientation, respectively. In contrast, resonance at ab. δ_C 63.8 (C-4) was in H-3α, H-4β-isomer. In addition, small coupling

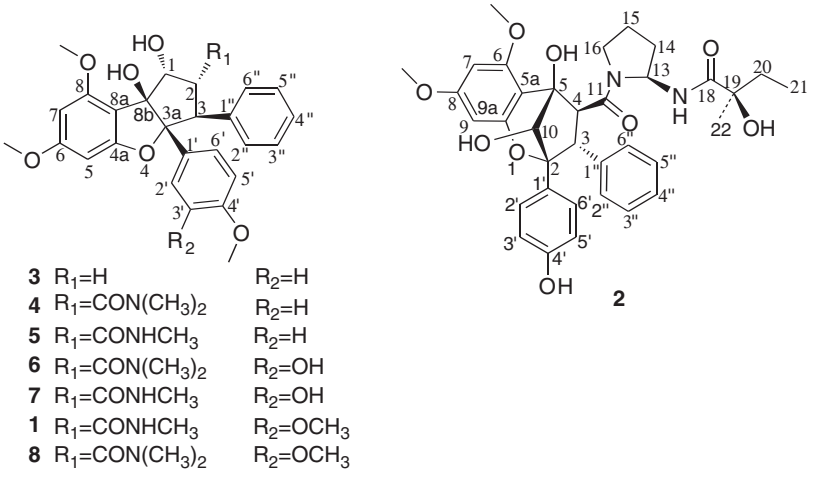
Table 1. ^{1}H and ^{13}C NMR spectral data of **1**–**2** (δ in ppm, J in Hz)[a].

No.	**1**[b]	**1**[c]	No.	**2**[d]	**2**[e]
1	4.87 (d, 5.2)	79.1 (d)	2		87.6 (s)
2	3.83 (dd, 14.0, 5.2)	51.5 (d)	3	4.25 (d, 5.0)	55.7 (d)
3	4.21 (d, 14.0)	55.5 (d)	4	3.94 (d, 5.0)	57.7 (d)
3a		101.5 (s)	5		81.1 (s)
4a		160.8 (s)	5a		108.3 (s)
5	6.23 (br.s)	89.1 (d)	6		157.7 (s)
6		163.9 (s)	7	6.31 (s)	93.4 (d)
7	6.10 (br.s)	92.4 (d)	8		162.2 (s)
8		157.2 (s)	9	6.01 (s)	94.8 (d)
8a		107.4 (s)	9a		159.8 (s)
8b		93.0 (s)	10	4.86 (s)	75.2 (d)
1′		126.8 s	11		173.0 (s)
2′	6.50 (br.s)	111.8 d	13	6.31–6.32 (m)	65.0 (d)
3′		147.7 s	14	2.23–2.24 (m)	35.0 (t)
				1.94–1.95 (m)	
4′		148.1 s	15	2.03–2.04 (m)	22.2 (t)
				1.98–2.00 (m)	
5′	6.63 (d, 8.4)	109.5 d	16	3.50–3.53 (m)	47.1 (t)
				3.60–3.62 (m)	
6′	6.87 (d, 8.4)	120.2 d	18		177.4 (s)
1″		136.4 s	19		76.0 (s)
2″/6″	6.94–6.96 (m)	128.4 d	20	1.63–1.66 (m)	34.0 (t)
				1.36–1.41 (m)	
3″/5″	7.04–7.05 (m)	128.0 d	21	0.74 (t, 7.0)	8.1 (q)
4″	7.05–7.06 (m)	126.9 d	22	0.93 (s)	26.4 (q)
11		171.0 s	1′		132.0 (s)
13		26.4 q	2′/6′	7.37 (d, 8.5)	128.3 (d)
6-OCH_3	3.83 (3H, s)	56.7 (q)	3′/5′	6.77 (d, 8.5)	115.7 (d)
8-OCH_3	3.82 (3H, s)	56.7 (q)	4′		155.8 (s)
3′-OCH_3	3.40 (3H, s)	56.7 (q)	1″		138.3 (s)
4′-OCH_3	3.75 (3H, s)	56.7 (q)	2″/6″	6.51 (d, 7.5)	130.5 (d)
			3″/5″	6.97 (t, 7.5)	128.7 (d)
			4″	7.03 (t, 7.5)	127.5 (d)
			6-OCH_3	4.04 (3H, s)	56.6 (q)
			8-OCH_3	3.75 (3H, s)	56.3 (q)

[a] **1** in $CDCl_3$ and **2** in DMSO-d_6.
[b] At 400 MHz.
[c] At 100 MHz.
[d] At 500 MHz.
[e] At 125 MHz.

constant (4.7 Hz) of H-3/4 was observed in the forth isomer, while the large one (9.3 Hz) in the latter. Thus, the configurations of H-3/4 of **2** were determined as H-3β and H-4α by both its coupling constant (5.0 Hz) and chemical shift δ_C 57.7 (C-4), and named as 4′-*O*-demethyldeacetylaglaxiflorin A.

The remaining compounds were determined as rocaglamide (**3**), rocaglanol (**4**), *N*-demethylrocaglamide (**5**), 3′-hydroxyrocaglamide (**6**), 3′-hydroxy-*N*-demethylrocaglamide (**7**), and 3′-methoxyrocaglamide (**8**), by comparison of their NMR spectroscopic data with the reported literature reference [6]. Compounds **1** and **2** were evaluated for their cytotoxicity against three human cancer cell lines. Only **1** showed cytotoxicity against HeLa, SGC7901 gastric cancer, and A549 lung cancer cells with IC_{50} values of 0.32,



3 R_1=H R_2=H
4 R_1=$CON(CH_3)_2$ R_2=H
5 R_1=$CONHCH_3$ R_2=H
6 R_1=$CON(CH_3)_2$ R_2=OH
7 R_1=$CONHCH_3$ R_2=OH
1 R_1=$CONHCH_3$ R_2=OCH_3
8 R_1=$CON(CH_3)_2$ R_2=OCH_3

Figure 1. Lignans (**1**–**8**) isolated from *A. odorata.*

0.12, and 0.25 μM, respectively, compared to cisplatin with IC_{50} values of 2.31, 1.54, and 7.25 μM.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter (Horiba Scientific, Kyoto, Japan). CD spectra were performed on the Applied Photophysics (Agilent Technologies, Santa Clara, CA, USA). UV spectra were recorded on a Shimadzu UV-2401A spectrophotometer (Shimadzu Corp., Kyoto, Japan). IR spectroscopy was performed on a *Tenor 27* spectrophotometer using KBr pellets (Bruker Optics GmbH, Ettlingen, Germany). NMR spectra were run on Bruker Avance-III 600, DRX-500, and AM-400 MHz spectrometers (Bruker BioSpin GmBH, Rheinstetten, Germany) with TMS as an internal standard. ESI-MS were performed on a Bruker HTC/Esquire spectrometer (Bruker, Rheinstetten, Germany), and HR-ESI-MS were recorded on an Agilent G6230 TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). CC was performed on silica gel (200–300 mesh, Qing-dao Haiyang Chemical Co., Ltd, Qingdao, China) and C_{18}-silica gel (20–45 μm, Fuji Silysia Chemical Ltd., Kasugai, Japan). Medium pressure liquid chromatography was employed using a Buchi pump system coupled with glass columns (15 mm × 230 mm and 26 mm × 460 mm, respectively) (Buchi Labortechnik AG, Flawil, Swissland). HPLC was performed using a Waters 1525EF pump (Waters Corp., Milford, USA) coupled with a Sunfire analytical (150 mm × 4.6 mm) and (semi-) preparative C_{18} column (150 mm × 10 mm and 250 mm × 19 mm, respectively). The HPLC system employed a Waters 2998 photodiode array detector and a Waters fraction collector III and detection was performed at 208 and 278 nm.

3.2 Plant material

Aglaia odorata was collected in Longzhou County, Guangxi province, China, in November 2011, and identified by Dr Chun-Xia Zeng, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. cai20111104) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

Air-dried twigs (5 kg) were crushed and extracted with MeOH (15 L × 3) at room temperature (48 h × 4). After removal of MeOH under reduced pressure, the viscous residue was partitioned with EtOAc (15 L × 4) to afford EtOAc and H_2O extract. The EtOAc (58 g) fraction was chromatographed on a silica gel (1.2 kg) column, using a mixture of $CHCl_3$–MeOH [from $CHCl_3$ to $CHCl_3$–MeOH (9:1)], to give nine fractions (I–IX). Fraction II (9.0 g) was chromatographed over prepacked Rp-18 silica gel (350 g), eluted by aqueous methanol (50%, 65%, and 75%) to give two subfractions II-1 and II-2. Subfraction II-1 (1.1 g) was separated by preparative HPLC column with gradient flow from 45% to 58% aqueous MeOH (10 ml/min) for 10 times to afford three fractions (A–C). Fraction A (37 mg) was purified by the same column with gradient flow from 30% to 38% aqueous acetonitrile (10 ml/min) to give **6** (25 mg, Rt 28 min). Fraction B (21 mg) was subjected to the same column eluted with aqueous acetonitrile (10 ml/min) from 32% to 38% to give **8** (11 mg, Rt 36 min). Fraction C (19 mg) was purified by the same column with gradient flow (10 ml/min) from 32% to 38% aqueous acetonitrile to give **1** (5 mg, Rt 29 min). Fraction II-2 (0.6 g) was loaded to preparative C18 HPLC eluting with 57% to 90% aqueous MeOH (10 ml/min) to afford two fractions (D and E). Fraction D (43 mg) was separated by preparative column with gradient flow (10 ml/min) from 38% to 50% aqueous acetonitrile to give **5** (21 mg, Rt 26 min) and **4** (5 mg, Rt 30 min). Fraction E (46 mg) was purified by the same column using gradient flow (10 ml/min) from 48% to 60% aqueous acetonitrile to give **3** (13 mg, Rt 29 min). Fraction III (0.6 g) was subjected to preparative C18 HPLC eluting with 57% to 90% aqueous MeOH (10 ml/min) to yield **7** (25 mg, Rt 32 min). Water layer was partitioned with *n*-butyl alcohol. Concentrated *n*-butyl alcohol layer (80 g) was loaded on silica gel CC (3.0 kg) eluted with $CHCl_3$–MeOH (from 9:1 to 2:1) to give five fractions (X–XIV). Fraction X (4 g) was subjected to RP-18 silica gel CC (100 g) eluted with MeOH–H_2O (from 4:6 to 6:4) and then purified by semi-preparative C18 HPLC with 25% to 35% aqueous acetonitrile (8 ml/min) to give **2** (12 mg, Rt 32 min).

3.3.1. 3′-Methoxy-N-demethylrocaglamide (*1*)

A white powder; $[\alpha]_D^{20}$ − 89 ($c = 0.13$, MeOH). CD (MeOH) $\Delta\epsilon_{208\,nm}$ − 10. UV (MeOH) λ_{max} (log ϵ): 208 (4.34), 278 (3.56) nm. IR (KBr) ν_{max}: 3500, 3405, 2941, 2842, 1726, 1626, 1603, 1514, 1271, 1148 cm^{-1}; for ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectral data, see Table 1; positive ESI-MS *m/z*: 544 $[M + Na]^+$; HR-ESI-MS *m/z*: 544.1945 $[M + Na]^+$ (calcd for $C_{29}H_{31}NO_8Na$, 544.1947).

3.3.2 4′-O-Demethyl-deacetylaglaxiflorin A (*2*)

A white powder; $[\alpha]_D^{20}$ + 19 ($c = 0.18$, MeOH). UV (MeOH) λ_{max} (log ϵ): 208 (4.32), 278 (3.70) nm. IR (KBr) ν_{max}: 3426, 3401, 3372, 2981, 2960, 1666, 1633, 1621, 1591, 1518, 1439, 1198, 1144 cm^{-1}; for ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectral data, see Table 1; Positive ESI-MS *m/z*: 655 $[M + Na]^+$; HR-ESI-MS *m/z*: 655.2625 $[M + Na]^+$ (calcd for $C_{35}H_{40}N_2O_9Na$, 655.2632).

3.4 Cytotoxicity assay

Three human cancer cell lines HeLa, SGC-7901, and A-549, were used for cytotoxic assays. Cells were cultured in Dulbecco's modified eagle medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA) in 5% CO_2 at 37°C. Cytotoxicity assays were performed according to the

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide method in 96-well microplates. In brief, 100 μl of adherent cell types was seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before the addition of test compounds. Suspended cell types were seeded with an initial density of 1×10^5 cells/ml just before drug addition. Each tumor cell line was exposed to a test compound at concentrations of 0.04, 0.2, 1.0, 5.0, and 25.0 μg/ml in triplicate for 48 h, with cisplatin (Sigma-Aldrich, St Louis, MO, USA) as the positive control. After treatment, cell viability was assessed, cell growth graphed, and IC_{50} values were calculated by Reed and Muench's method.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was financially supported by the National Natural Science Foundation of China [grant number 21172225], [grant number 31370377); the Young Academic and Technical Leader Raising Foundation of Yunnan Province [grant number 2010CI049]; and XiBuZhiGuang Project of the Chinese Academy of Sciences.

References

[1] H. Peng, D.J. Mabberley, C.M. Pannell, J. Edmonds, and B. Bartholomew, *Flora of China* (Science Press and Missouri Botanical Garden Press, Beijing and St. Louis, 2008), Vol. 11.

[2] P. Proksch, R. Edrada, R. Ebel, F.I. Bohnenstengel, and B.W. Nugroho, *Curr. Org. Chem.* **5**, 923 (2001).

[3] B. Baumann, F. Bohnenstengel, D. Siegmund, H. Wajant, C. Weber, I. Herr, K.M. Debatin, P. Proksch, and T. Wirth, *J. Biol. Chem.* **277**, 44791 (2002).

[4] X.H. Cai, X.D. Luo, J. Zhou, and X.J. Hao, *Helv. Chim. Acta* **88**, 2938 (2005).

[5] X.H. Cai, Y.Y. Wang, P.J. Zhao, Y. Li, and X.D. Luo, *Phytochemistry* **71**, 1020 (2010).

[6] W. H. Lin Chaidir, R. Ebel, R. Edrada, V. Wray, M. Nimtz, W. Sumaryono, and P. Proksch, *J. Nat. Prod.* **64**, 1216 (2001).

[7] S.K. Wang, Y.J. Cheng, and C.Y. Duh, *J. Nat. Prod.* **64**, 92 (2001).

[8] B.W. Nugroho, R.A. Edrada, B. Gussregen, V. Wray, L. Witte, and P. Proksch, *Phytochemistry* **44**, 1455 (1997).

[9] B.W. Nugroho, R.A. Edrada, V. Wray, L. Witte, G. Bringmann, M. Gehling, and P. Proksch, *Phytochemistry* **51**, 367 (1999).

[10] Y.J. Xu, X.H. Wu, B.K.H. Tan, Y.H. Lai, J.J. Vittal, Z. Imiyabir, L. Madani, K.S. Khozirah, and S.H. Goh, *J. Nat. Prod.* **63**, 473 (2000).

[11] B.W. Nugroho, R.A. Edrada, V. Gussrengen, V. Wary, L. Witte, and P. Proksch, *Phytochemistry* **44**, 1455 (1997).