

Aglacins I–K, Three Highly Methoxylated Lignans from *Aglaiia cordata*

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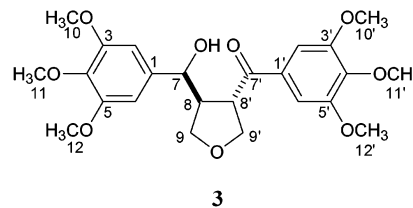
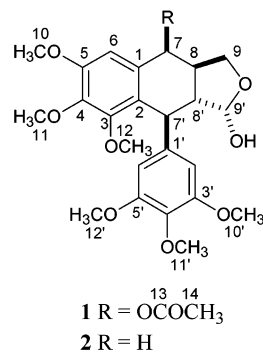
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Further chemical investigation of the stem bark of *Aglaiia cordata* has led to the isolation and identification of three new lignans, namely, aglacins I–K (**1**–**3**), all of which contain two contiguous trimethoxylated phenyl systems. Among them, aglacins I and J (**1** and **2**) are new members of the aryltetralin cyclic lactol class, while aglacin K (**3**) is a new example of tetrahydrofuran lignan. The structures of these compounds were established on the basis of spectroscopic data interpretation.

Naturally occurring lignans have attracted interest due to their widespread occurrence, diverse biological activity, and use as constituents of folk medicines.^{1–3} From the plant genus *Aglaiia* (Meliaceae), a wide variety of structurally unique and biologically active secondary metabolites, including the strongly insecticidal and cytotoxic 1*H*-cyclopenta[*b*]benzofuran lignans (the so-called rocaglamide derivatives, which to date have been isolated only from this genus), have been reported and reviewed.⁴ As part of our study within the genus *Aglaiia*,^{4–9} we have investigated the chemical constituents of *Aglaiia cordata* Hiern collected from Kalimantan, Indonesia, which resulted in the isolation and identification of aglacins A–H, a new class of aryltetralin cyclic ether lignans and norlignans from the stem bark of this plant.^{6,7} Further chemical work has led to the characterization of three further new minor lignans, namely, aglacins I–K (**1**–**3**), from the more polar fractions of the methanol extract of *A. cordata*. Of these compounds, aglacins I (**1**) and J (**2**) represent new examples of the aryltetralin cyclic lactol lignans, while aglacin K (**3**) is a new highly oxygenated tetrahydrofuran lignan. All of these metabolites contain two unusual contiguous trimethoxylated phenyl systems. The structures and relative stereochemistry of these metabolites were elucidated on the basis of comprehensive NMR spectral analysis as well as low- and high-resolution MS experiments. In the present paper, we report the isolation and structural elucidation of the new derivatives **1**–**3** from *A. cordata*.

An ethyl acetate-soluble fraction of the methanolic extract of the air-dried stem bark of *A. cordata* was subjected to silica gel vacuum-liquid chromatography (VLC), followed by further repeated column chromatography separation on silica gel and Sephadex LH-20, and by reversed-phase HPLC chromatographic steps, resulting in the isolation of three new compounds, aglacins I–K (**1**–**3**).

Aglacins I (**1**) and J (**2**), colorless waxy solid, were obtained as a mixture. Attempts to separate the two compounds by different column chromatographic steps, by



preparative TLC, and by reversed-phase HPLC with different solvent systems failed. On the basis of their ¹H NMR and ¹³C NMR spectra, the ratio of compounds **1** and **2** was deduced as 4:5 in the mixture. Most of the signals were well-resolved for both compounds. Aided by 2D NMR techniques, including ¹H–¹H COSY, HMQC, HMBC, and ROESY, as well as by comparison with previously reported data for the model compounds aglacins A and B,⁷ the structural elucidation and assignments of the chemical shifts for the individual metabolites were successfully carried out.

The molecular formula C₂₆H₃₂O₁₀ for aglacin I (**1**) was obtained from the high-resolution EIMS at *m/z* 504.1987 ([M]⁺, calcd 504.1995). In the ¹H–¹H COSY spectrum, a seven-proton spin system, including two low-field doublets at δ_H 6.13 (1H, d, *J* = 2.6 Hz, H-7) and 5.23 (1H, d, *J* = 4.4 Hz, H-9'), two one-proton triplets at δ_H 4.18 (1H, t, *J* = 8.2 Hz, H-9α) and 3.57 (1H, t, *J* = 8.9 Hz, H-9β), two multiplet methines at δ_H 2.64 (1H, m, H-8) and 2.46 (1H, m, H-8'), and finally, a one-proton methine occurring as a doublet at δ_H 4.24 (1H, d, *J* = 10.8 Hz, H-7'), was clearly discernible. On the other hand, the ROESY spectrum indicated a cross-peak between H-7 and H-6 and between H-7' and H-2'/H-6'. Starting from the above clearly distinguishable proton signals and proton spin system, in combination with careful inspection of the HMQC, HMBC, and ROESY spectra, the ¹H and ¹³C NMR data for **1** were fully assigned

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Table 1. ^1H and ^{13}C NMR Spectral Data of Aglacins I and J (1 and 2)^a

carbon	aglacin I (1)		aglacin J (2)	
	^1H (J in Hz)	^{13}C	^1H (J in Hz)	^{13}C
1		130.8 s		132.5 s
2		125.7 s		127.0 s
3		152.4 s		152.1 s
4		143.2 s		140.9 s
5		152.9 s		152.8 s
6	6.77 s	108.9 d	6.47 s	107.3 d
7	6.13 d (2.6)	68.8 d	2.96 dd (15.2, 4.2) 2.69 dd (15.5, 12.0)	34.3 t
8	2.64 m	40.2 d	2.43 m	36.7 d
9	α 4.18 t (8.2) β 3.57 t (8.9)	67.4 t	α 4.28 t (7.6) β 3.54 t (8.6)	72.5 t
10	3.87 s	55.9 q	3.86 s	55.8 q
11	3.76 s	60.4 q	3.74 s	60.4 q
12	3.18 s	59.4 q	3.18 s	59.4 q
13		170.8 s		
14	2.12 s	21.3 q		
1'		143.4 s		143.9 s
2'/6'	6.42 s	104.6 d	6.37 s	104.3 d
3'/5'		153.1 s		153.0 s
4'		136.2 s		136.0 s
7'	4.24 d (10.8)	42.2 d	4.30 d (7.9)	42.6 d
8'	2.46 m	49.0 d	1.83 m	56.6 d
9'	5.23 d (4.4)	96.7 d	5.19 d (4.1)	97.1 d
10'/12'	3.79 s	56.2 q	3.77 s	56.2 q
11'	3.82 s	60.9 q	3.81 s	60.9 q

^a ^1H and ^{13}C NMR spectra were measured in CDCl_3 at 500 and 125 MHz, respectively. All proton and carbon signals were assigned by detailed analysis of ^1H - ^1H COSY, HMQC, HMBC, and ROESY spectral data.

(Table 1). Detailed examination of the NMR spectral data and comparison with those reported for aglacin A⁷ showed that the structures of these two compounds are very similar. However, in the ^{13}C NMR spectrum, the oxygenated methylene carbon signal at δ_{C} 72.2 for C-9' in aglacin A was replaced by a significant downfield methine signal resonating at δ_{C} 96.7 for C-9' in **1**. This observation was strongly supported by the fact that the two one-proton signals appearing at δ_{H} 3.94 (br t, $J = 7.6$ Hz) and 3.59 (dd, $J = 10.4$ and 7.6 Hz) for H-9' α and H-9' β in aglacin A, respectively, were absent in the ^1H NMR spectrum of **1**. Instead, a downfield one-proton doublet signal at δ_{H} 5.23 (d, $J = 4.4$ Hz), derived from the acetalic proton H-9' in compound **1**, was observed.

The relative configurations of the stereocenters in compound **1** were determined using a combination of NMR methods (^1H - ^1H coupling constants and ROESY correlations) coupled with comparison of the NMR spectral data with those of aglacin A. The protons H-7 and H-8 were assigned as *cis* on the basis of a small coupling constant ($J = 2.6$ Hz) and a strong ROESY correlation observed between the proton pair. In contrast, no ROESY cross-peak was detected between H-7' and H-8', and the large coupling constant ($J = 10.8$ Hz) between the two protons indicated a *trans*-orientation of the proton pair. In addition, no cross-peak was observed between H-8 and H-8' in the ROESY experiment, which implied a *trans*-configuration for the two corresponding protons. The hydroxyl group at C-9' was assigned to have α -orientation by the observation of a characteristic ROESY correlation between H-8' and H-9'. The $J_{8',9'}$ value (4.4 Hz) supported this assignment.¹⁰ On the basis of the above data, the structure of **1** was determined as 9'-hydroxy-3,3',4,4',5,5'-hexamethoxy-7-acetoxy-9,9'-epoxy-2,7'-cycloglignan, which was named aglacin I.

Following the structure determination and assignment of chemical shifts for **1**, the remaining signals accounting for compound **2** were assigned. The low-resolution EIMS

Table 2. ^1H and ^{13}C NMR Spectral Data of Aglacin K (3)^a

carbon	δ_{H}	δ_{C}	carbon	δ_{H}	δ_{C}
1		137.8 s	1'		131.9 s
2/6	6.51 s	103.1 d	2'/6'	7.00 s	105.8 d
3/5		153.3 s	3'/5'		153.1 s
4		138.1 s	4'		143.0 s
7	4.65 dd (7.0, 3.3)	75.8 d	7'		198.2 s
8	3.28 m	50.2 d	8'	3.85 m	49.5 d
9	α 4.09 dd (9.0, 5.8) β 4.01 dd (8.7, 7.4)	70.8 t	9'	α 4.25 t (8.3) β 3.73 t (8.0)	72.2 t
10/12	3.78 s	56.1 q	10'/12'	3.87 s	56.3 q
11	3.74 s	60.9 q	11'	3.90 s	60.7 q
OH	2.17 s				

^a ^1H and ^{13}C NMR spectra were measured in CDCl_3 at 500 and 125 MHz, respectively. All proton and carbon signals were assigned by detailed analysis of ^1H - ^1H COSY, HMQC, HMBC, and ROESY spectral data.

of **2** displayed a molecular ion peak at m/z 446 $[\text{M}]^+$, and high-resolution MS measurement established its molecular formula as $\text{C}_{24}\text{H}_{30}\text{O}_8$ (determined at m/z 446.1929 $[\text{M}]^+$, calcd 446.1941). Analysis of the NMR data (Table 1) indicated that **2** shares many structural features with aglacin B.⁷ The only significant differences between the NMR spectra were the signals assigned to the oxymethylene protons H-9' at δ_{H} 3.91 (br t, $J = 7.6$ Hz, H-9' α) and 3.60 (dd, $J = 10.1$ and 7.6 Hz, H-9' β) of aglacin B, which were replaced by a downfield methine signal at δ_{H} 5.19 (d, $J = 4.1$ Hz) of **2** in the ^1H NMR spectra, and the oxymethylene carbon signal C-9' at δ_{C} 72.6 of aglacin B was replaced by a downfield oxymethine carbon signal C-9' at δ_{C} 97.1 of **2** in the ^{13}C NMR spectra. These differences indicated that an acetal hydroxyl substitution occurred at C-9'. The data from the ^1H - ^1H COSY spectrum, which indicated an eight-proton spin system comprising four methylene protons at H₂-7 and H₂-9 and four methine protons at H-7', H-8, H-8', and H-9', supported this assumption. Detailed analysis of the 1D and 2D NMR data enabled the assignment of all ^1H and ^{13}C NMR signals.

As performed for **1**, the relative stereochemistry of **2** was also determined by the analysis of J values obtained from the ^1H NMR spectrum as well as by a ROESY experiment. The large coupling constant observed for H-7' and H-8' ($J = 7.9$ Hz) indicated a *trans*-configuration of the two protons which showed no correlation in the ROESY spectrum. In contrast, the coupling constant between H-8' and H-9' ($J = 4.1$ Hz) suggested a *cis*-orientation of the proton pair.¹⁰ This assumption was corroborated by a cross-peak between H-8' and H-9' in the ROESY experiment. Additionally, cross-peaks were also observed between H-7' and H-8 and between H-8 and H-7 α and H-9 α in the ROESY spectrum. In contrast, there is no correlation between H-8 and H-8' (ROESY experiment), which suggested a *trans*-orientation for the two protons. Therefore, the relative stereochemistry of **2** was assigned as H-7' α , H-8 α , H-8' β , and H-9' β . Consequently, the structure of **2** was characterized as 9'-hydroxy-3,3',4,4',5,5'-hexamethoxy-9,9'-epoxy-2,7'-cycloglignan. This new compound was named aglacin J.

The EIMS of aglacin K (**3**), obtained as a colorless waxy solid, showed a molecular ion peak at m/z 462 $[\text{M}]^+$, corresponding to the molecular formula $\text{C}_{24}\text{H}_{30}\text{O}_9$, which was confirmed by HREIMS at m/z 462.1898 (calcd 462.1890). Its ^1H and ^{13}C NMR data (Table 2) indicated that the structure of **3** contains 13 sp^2 -hybridized carbon atoms corresponding to one keto group [δ_{C} 198.2 (s)], four methines [δ_{C} 105.8 (d \times 2) and 103.1 (d \times 2), δ_{H} 7.00 (s, 2H) and 6.51 (s, 2H)], eight quaternary carbons [δ_{C} 153.3 (s \times 2), 153.1 (s \times 2), 143.0 (s), 138.1 (s), 137.8 (s), and 131.9 (s)], six methoxyl groups [δ_{C} 60.9 (q), 60.7 (q), 56.3 (q \times 2),

and 56.1 (q × 2), δ_{H} 3.90 (3H), 3.87 (6H), 3.78 (6H), and 3.74 (3H)], and five sp³-hybridized carbon atoms, including one oxymethine [δ_{C} 75.8 (d), δ_{H} 4.65 (dd, 1H)], two methines [δ_{C} 50.2 (d) and 49.5 (d), δ_{H} 3.85 (m, 1H) and 3.28 (m, 1H)], and two oxymethylenes [δ_{C} 72.2 (t) and 70.8 (t), δ_{H} 4.25 (t, 1H), 4.09 (dd, 1H), 4.01 (dd, 1H), and 3.73 (t, 1H)]. The presence of two symmetrical substituted phenyl ring systems in **3** could be deduced from the above data. The ¹H–¹H COSY spectrum suggested that all aliphatic methine and methylene protons were part of a contiguous spin system comprising H-7, H-8, H-8', H₂-9, and H₂-9' in the molecule. Analysis of the 1D (¹H, ¹³C, and DEPT) and 2D (COSY, HMQC, and HMBC) NMR data indicated that, unlike aglacins I (**1**) and J (**2**), aglacin K (**3**) belongs to the tetrahydrofuran class of lignans of either the 9,9'-epoxy type or 7,9'-epoxy type.^{10–13} The chemical shift values for the oxymethine (δ_{C} 75.8) and oxymethylenes (δ_{C} 72.2 and 70.8) in the ¹³C NMR spectrum indicated that **3** is the tetrahydrofuran 9,9'-epoxy lignan,^{10–12} as the corresponding carbon signals for a tetrahydrofuran 7,9'-epoxy lignan (for example, magnone B isolated from *Magnolia fargesii*) should be detected at δ_{C} 83.9 for oxymethine and δ_{C} 70.9 and 61.4 for the oxymethylenes, respectively.¹³ The HMBC cross-peaks between the aromatic protons H-2/H-6 and the oxymethine C-7 indicated the presence of a hydroxyl group at position C-7. Similarly, the location of the keto group was confirmed to be at C-7' since the other aromatic protons H-2'/H-6' correlated with C-7' in the HMBC spectrum.

The relative stereochemistry of **3** was mainly determined from a ROESY experiment.¹² The H-8 signal showed cross-peaks with H-7, H-9 α , and H-9' α , while the H-8' signal exhibited correlations with H-9 β and H-9' β , and no cross-peak was observed between H-8 and H-8' in the ROESY spectrum, which suggested that H-8 and H-8' were in *trans*-orientation. On the basis of these results, the structure of **3** was determined as 7-hydroxy-3,3',4,4',5,5'-hexamethoxy-7'-keto-9,9'-epoxylignan, which was named aglacin K.

The current report together with our previous publications on *Aglaiia cordata* indicate that this plant is a rich source of highly methoxylated new lignans.^{6,7}

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a Perkin-Elmer model 341 LC polarimeter. UV spectra were run on a UNICO WFZ UV-2102 PCS spectrometer. IR spectra were recorded as KBr pellets on a Nexus 470 Nicolet spectrophotometer. ¹H, ¹³C, and DEPT NMR spectral data and ¹H–¹H COSY, HMQC, HMBC, and ROESY experiments were performed on a Bruker DRX-500 MHz NMR spectrometer. Low- and high-resolution MS were recorded with Finnigan MAT 311A and VG Auto Spec 3000 mass spectrometers. HPLC–UV analyses were conducted with a Dionex system coupled to a photodiode array detector using a 5 μ m Eurospher-100 C₁₈ column (4 mm i.d. × 150 mm; Knauer, Berlin, Germany). Routine detection was at 235 nm. Semipreparative HPLC was performed on a Merck-Hitachi instrument (pump L-7100, detector L-7400) using a 7 μ m Eurospher-100 C₁₈ column (8 mm × 300 mm; Knauer, Berlin, Germany). Vacuum-liquid chromatography (VLC) and column chromatography were performed on silica gel (0.040–0.063 mm; Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich, Steinheim, Germany), or RP-18 (Merck, Darmstadt, Germany), and TLC analyses were carried out using aluminum sheet precoated silica gel 60 F₂₅₄ (Merck, Darmstadt, Germany). Detection was by UV absorption at 254 nm. All solvents used were distilled prior to use.

Plant Material. The collection and identification of plant material were the same as reported previously.⁷

Extraction and Isolation. Solvent extraction and solvent–solvent partitioning procedures were the same as reported previously.⁷ The EtOAc-soluble fraction was pre-separated by VLC and eluted with a system of solvents of increasing polarity from *n*-hexane to ethyl acetate. The fraction that eluted with *n*-hexane–EtOAc (100:75) was subjected to repeated Sephadex LH-20 and silica gel column chromatographic steps and purified by reversed-phase semipreparative HPLC (MeOH–H₂O, 50:50) to afford a mixture of compounds **1** and **2** (12.0 mg). Efforts to use different methods for the chromatographic separation of these two compounds failed. Compound **3** (5 mg) was obtained from a more polar VLC fraction eluted with *n*-hexane–EtOAc (1:1) by repeated Sephadex LH-20 (MeOH) and reversed-phase semipreparative HPLC chromatographic separation (RP-18, with a gradient starting from 32% to 50% methanol in water).

Aglacin I (9'-hydroxy-3,3',4,4',5,5'-hexamethoxy-7-acetoxy-9,9'-epoxy-2,7'-cyclo lignan, 1): obtained together with aglacin J (**2**) as a mixture of colorless waxy solids; ¹H and ¹³C NMR data, see Table 1; EIMS *m/z* 504 [M]⁺ (30); HREIMS *m/z* 504.1987 (calcd for C₂₆H₃₂O₁₀ 504.1995).

Aglacin J (9'-hydroxy-3,3',4,4',5,5'-hexamethoxy-9,9'-epoxy-2,7'-cyclo lignan, 2): obtained together with aglacin I (**1**) as a mixture of colorless waxy solids; ¹H and ¹³C NMR data, see Table 1; EIMS *m/z* 446 [M]⁺ (100); HREIMS *m/z* 446.1929 (calcd for C₂₄H₃₀O₈ 446.1941).

Aglacin K (7-hydroxy-3,3',4,4',5,5'-hexamethoxy-7'-keto-9,9'-epoxylignan, 3): colorless waxy solid; [α]_D²⁰ –25.5° (c 0.62, CHCl₃); UV (CHCl₃) λ_{max} (log ϵ) 287 (4.06) nm; IR (KBr) ν_{max} 3417, 1733, 1671, 1590, 1506, 1462, 1416, 1327, 1235, 1189, 1158, 1128, 1063, 1004, 925, 836, 759, 724 cm^{–1}; ¹H and ¹³C NMR data, see Table 2; EIMS *m/z* 462 [M]⁺ (86), 240 (93), 195 (100), 181 (16), 169 (35), 139 (31); HREIMS *m/z* 462.1898 (calcd for C₂₄H₃₀O₉ 462.1890).

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Supporting Information Available: The ¹H NMR, ¹³C NMR, DEPT, ¹H–¹H COSY, HMQC, HMBC, ROESY, EIMS, and HREIMS spectra of aglacins I (**1**) and J (**2**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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