

Apotirucallane Triterpenes from *Aglaia argentea*

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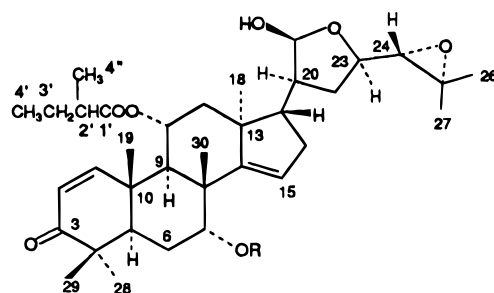
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Five new apotirucallane triterpenes were isolated from the seeds of *Aglaia argentea*: gentinones A (**1**), B (**2**), C (**3**), D (**4**), and gentinin (**5**). Their structures were established using spectroscopic and chemical means.

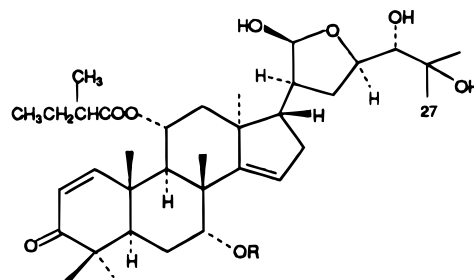
Tirucallane triterpenes are well known in the family Meliaceae.^{2,3} Such compounds have been found very recently in the genus *Aglaia*.^{4,5} We have isolated from the seeds of *Aglaia argentea* Bl., grown in Malaysia, five new apotirucallane triterpenes named gentinones A (**1**), B (**2**), C (**3**), D (**4**) and gentinin (**5**). The crude ethanolic extract possessed cytotoxic properties against KB cells. However, compounds **1–5** were inactive. The cytotoxic component of the extract, an aromatic derivative of the benzofuran series, will be reported separately.⁶

The EtOH extract was fractionated by repeated column chromatography on Si gel using CH₂Cl₂–MeOH and heptane–EtOAc or heptane–Me₂CO mixtures to give compounds **1–5**.

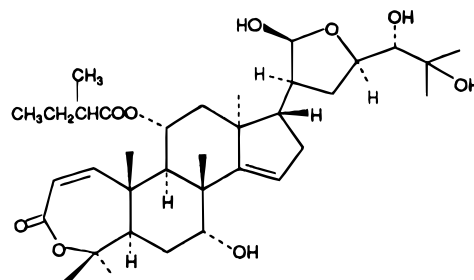
Gentinone (**1**), [α]_D²⁰ –24°, exhibited a [M + Na]⁺ peak in the FABMS at *m/z* 607. The molecular formula C₃₅H₅₂O₇ was established by HRFABMS (607.3592, Δ –1.9 mmu). The IR spectrum showed the absorptions of an ester and an α,β -unsaturated ketone at 1722 and 1669 cm⁻¹, respectively. In the ¹³C NMR the keto group resonance appeared at δ 204.7, whereas the conjugated double bond resonated at δ 124.0 and δ 158.6. In the ¹H NMR, the corresponding signals (δ 5.65 and 6.90, respectively) showed a coupling of 11 Hz, suggesting a triterpene Δ^1 -3-ketone. Another double bond was present with chemical shift values of δ_C 161.3 and 120.4 and δ_H 5.46, indicative of the Δ^{14} bond of an apotirucallane triterpene. The apotirucallane skeleton was confirmed by the signals of seven tertiary methyl groups (Table 1) and further supported by detailed analysis of the HMBC and NOESY data (Table 1). The 2D spectra confirmed the presence of a C_{7 α} -OH group with typical chemical shifts and splitting pattern (δ_C 71.6 and δ_H 4.00, br s) and indicated that a second oxymethine, which resonated at δ_C 70.7 and δ_H 5.44, corresponded to a CH-11 bearing a 2-methylbutyric ester. The value of the coupling ³*J*_{9,11} = 9 Hz showed that H-11 was quasi-axial and hence in a β position. Ring C probably adopted a deformed boat conformation, inasmuch as a weak NOE was observed between H-9 and H-11 in the NOESY experiment. Such a conformation has been reported for a related apotirucallane triterpene by X-ray.⁷ The H-11 β configuration was confirmed by the NOESY relationships H-11/CH₃-19 and H-11/H12 β (Table 1).



1 R = H
2 R = Ac



3 R = H
4 R = Ac



5

In addition, the NMR spectra exhibited the characteristic resonances of a five-membered hemiacetal ring and a 24,25 epoxide (Table 1) located in the chain as depicted in **1**. A similar chain has been reported previously in various triterpenes, especially of the apotirucallane type.^{8–10} Thus, gentinone was assigned structure **1**. However **1** was, like other known compounds possessing the same side chain, an epimeric mixture with respect to the hemiacetal carbon atom, containing the C-21 α epimer as a minor component^{8,10} (see Table 1). The stereochemistry of the chain was firmly established by comparison of the ¹³C NMR reso-

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Table 1. ^{13}C -NMR (75 MHz) and ^1H -NMR (400 MHz) Data^a for Gentinone A (**1**) and Gentinin (**5**) (CDCl_3)

position ^b	1				5			
	δ_{C}	δ_{H} (JHz)	HMBC	NOESY	δ_{C}	δ_{H} (JHz)	HMBC	NOESY
1	158.6	6.90 d (11)	3, 5	2,9,11	153.2	6.05 d (11)	5, 19	2, 9, 11, 19
2	124.0	5.65 d (11)	4, 10		116.9	5.70 d (11)	1, 3, 10, 19	
3	204.7				167.8			
4	44.8 ^c				84.8			
5	44.4	2.43 m	4, 6, 10, 19, 28, 29	6,28	47.3	2.82 dd (1,12)	4, 6, 10, 19	6 α β , 9, 28
6	24.4	1.90 m		7,19,28,29,30	27.7	α 2.05 m β 1.85 m		7, 28, 29 7, 19, 30
7	71.6	4.00 brs	9	15, 30	71.4	3.95 br s	5	15, 30
8	44.6 ^c				44.3			
9	43.8	2.52 d (9)	8, 10, 11, 19, 30	11, 18	46.1	2.52 d (9)	1, 8, 10, 11, 14, 30	18
10	41.2				45.4			
11	7.07 [70.6]	5.44 [5.42] m	9, 10, 13	12a, 19	70.1	5.45 br s	13, 1'	19
12	42.9 [42.0]	α 1.85 m β 2.00 m	9, 11, 13, 14 9, 11, 13, 14	18 30	42.6	α 1.74 m β 1.95 m	11, 13, 14, 18 11, 13, 14, 18	12 β , 18, 21 30
13	46.0				45.8			
14	161.3 [161.8]				160.6			
15	120.4 [119.7]	5.46 m	16, 17	16, 30	120.8	5.55 m	13, 14, 16, 17	16, 30
16	35.3 [35.0]	2.20 m	13, 14, 15	17, 18	35.2	2.20 m	13, 14, 15	17, 18
17	52.9 [57.4]	2.05 m			52.8	2.00 m		21
18	20.3	1.08 s	12, 13, 14, 17	20, 21	20.5	1.05 s	12, 13, 17	20, 21
19	20.5	1.27 s	1, 5, 9, 10	29	18.9	1.28 s	1, 5, 10	
20	45.6 [47.5]	2.20 [2.35] m		21	44.8	2.14 m		20, 22a
21	97.6 [103.0]	5.22 m		22a	96.5	5.20 br s		
22	31.8 [35.3]	a 1.70 [1.38] m b 2.00 [2.10] m	23 23	22b	30.5	a 1.87 m b 2.00 m	21, 23, 24 21, 24	22b, 23
23	78.0 [77.7]	3.80 [3.55] m	24	24, 26 or 27	79.1	4.55 t (8)	21, 24	24, 27
24	68.0 [65.7]	2.80 [2.62] d (8)	23, 25, 26	26 or 27	75.2	3.15 d (6) ^d		27
25	58.3				74.4			
26	25.3	1.28 s	25		27.0	1.28 s	24, 25, 27	
27	19.6	1.28 s	25		27.0	1.28 s	24, 25, 26	
28	26.4	1.17 s	3, 4, 29		25.6	1.42 s	4, 5, 29	
29	21.9	1.02 s	3, 4, 28		32.2	1.44 s	4, 5, 28	
30	30.4	1.17 s	7, 9, 14		30.0	1.15 s	7, 8, 9, 14	
1'	176.6 [176.3]				176.3			
2'	42.5	2.30 m	1', 3', 4', 4''	4''	42.0	2.30 m	1, 3, 4, 4'	4', 4''
3'	26.8	1.42 m	2', 4', 4''	4'	26.4	1.40 m	1', 2', 4', 4''	4''
		1.70 m	2', 4', 4''	4'		1.70 m	1', 2', 4', 4''	
4'	12.4	0.86 m	2', 3'		12.4	0.90 m	2', 3'	
4''	17.1	1.12 d (7)	1', 2', 3'		17.3	1.15 d (7)	1', 2', 3'	

^a Assignments based on 2D experiments; δ_{C} and δ_{H} Values under Brackets are for the $\text{C}_{21\alpha}$ -OH epimer of **1**. ^b The oxygen atom in the A ring of compound **5** has been omitted in the numbering scheme. ^c Values for position 4 and 8 may be reversed. ^d $^3\text{J}_{24\text{-OH}}$.

nances with compounds of known stereochemistry.⁷ The absolute configuration of the 2-methylbutyric acid moiety was determined as *R* after alkaline hydrolysis. However, the acid was partially racemized. This was shown by ^1H NMR using the chiral solvating agent 1,2-diphenylethane-1,2-diamine.¹¹ The ratio *R*:*S* was about 60:40.

Gentinone B (**2**), $[\alpha]_{\text{D}}^{20} -24^\circ$, showed a $[\text{M} + \text{Na}]^+$ peak at m/z 649.3738 in the HRFABMS ($\Delta -3.2$ mmu) corresponding to molecular formula of $\text{C}_{37}\text{H}_{54}\text{O}_8$. The IR spectrum exhibited two carbonyl absorptions at 1722 and 1669 cm^{-1} . The NMR spectra (Table 1) were similar to those of compound **1**, except for the signal of CH-7, which was shifted to δ_{H} 5.24 and δ_{C} 74.1. In addition, the resonances of an acetate group, obviously located at C-7, were observed (Table 1). Thus, gentinone B was assigned the structure depicted in **2**.

Gentinone C (**3**), $[\alpha]_{\text{D}}^{20} -18^\circ$, exhibited a $[\text{M} + \text{Na}]^+$ peak at m/z 625. The molecular formula $\text{C}_{35}\text{H}_{54}\text{O}_8$, which corresponded to the formula of compound **1** with an additional water molecule, was determined by HRFABMS (625.3721, $\Delta +0.5$ mmu). The IR spectrum exhibited the absorptions of an ester at 1715 cm^{-1} and a conjugated carbonyl at 1662 cm^{-1} . The NMR data of the fused rings (Table 1) were close to those of compound **1**. The signals of the 24,25 epoxide of the side chain were absent and replaced by those of 24,25 diol. The

resonances observed for the side-chain carbons and protons were similar to the ones reported for apotirucallane triterpenes possessing the same chain^{8,10} but with unknown C-24 configuration. Acid opening of the epoxide ring of gentinone A (**1**) at C-25 using HClO_4 in DMF¹² afforded gentinone C. Thus, the latter has the structure depicted in **3** (24*S*-configuration).

Gentinone D (**4**), $[\alpha]_{\text{D}}^{20} -24^\circ$, revealed a $[\text{M} + \text{Na}]^+$ peak at m/z 667.3795 in the HRFABMS ($\Delta +3.7$ mmu), which matched the molecular formula of $\text{C}_{37}\text{H}_{56}\text{O}_9$. The IR spectrum exhibited two carbonyl absorptions at 1722 and 1669 cm^{-1} . The NMR spectra (Table 1) were similar to those of compound **3**, except for the signal of H-7, which was shifted downfield by 1.30 ppm in the ^1H NMR and the presence of an acetyl group (Table 1). Thus, gentinone D was assigned structure **4**.

Gentinin (**5**), $[\alpha]_{\text{D}}^{20} +15^\circ$, showed a $[\text{M} + \text{Na}]^+$ peak at m/z 641.3679 in the HRFABMS ($\Delta -1.3$ mmu) corresponding to the molecular formula of $\text{C}_{35}\text{H}_{54}\text{O}_9$. In addition to the ester band at 1720 cm^{-1} , the IR spectrum exhibited an absorption at 1687 cm^{-1} , suggesting the presence of a α,β -unsaturated lactone. In the ^{13}C NMR the signals of the conjugated double bond were shifted upfield to δ_{C} 116.9 and 153.2 as compared to compound **1**, and the conjugated carbonyl resonance was observed at δ_{C} 167.8. The remaining ^{13}C signals of the fused rings were similar to the ones of **1**, except that C-4 was

shifted to δ 84.8, indicating that this carbon bore an oxygen. These data suggested that the fused ring moiety of gentinine has the structure depicted in **2**, which was entirely supported by COSY, HMQC, HMBC, and NOESY experiments (see Table 1). The side-chain resonances were similar to those of gentinone C (**3**). Thus, gentinin (**5**) differed from **3** only by the presence of an unsaturated lactone instead of Δ^1 -3-ketone in ring A.

Compounds **1**–**5**, which exhibited classical apotirucallane skeleton and side chains of known type, possess, however, a unique 2-methylbutyric ester function at C-11 α . The C-24*S* configuration in the hydroxylated side chain of gentinone C (**3**) and D (**4**) and gentinin (**5**) is established for the first time in this paper.

Experimental Section

General Experimental Procedures. Optical rotations at 20° were taken on a Perkin-Elmer 241 polarimeter. UV spectra were recorded in MeOH on a Shimadzu UV-161 UV-vis spectrophotometer; IR, on a Nicolet 205 FT-IR spectrometer; FABMS, on a Kratos MS 80; HRFABMS, on a VG-Zab-Seq; and NMR, on a Bruker AC 250, AC 300, or AM 400 spectrometer. The HMBC spectra were obtained using a INVDR2LP in Bruker program with an evolution delay for CH long-range coupling of 70 ms. The NOESY (phase sensitive) values were recorded using the NOESYPH Bruker program with a mixing time of 0.6 s. Column chromatography was performed using Si gel Merck H60.

Plant Material. Seeds of *A. argentea* Bl. were collected in Dungun, Terengganu, on 22 March 1993. Identification was made by one of us (GP.). Voucher specimens (KL 4347) are deposited at the Laboratoire de Phanérogamie, Muséum National d'Histoire Naturelle in Paris, and at the Herbarium of Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia, and at the Herbarium of the Forest Research Institute, Kepong, Malaysia.

Extraction and Isolation. The dried, ground seeds (250 g) were extracted exhaustively with EtOH at room temperature. The extract (7.6 g) was chromatographed on Si gel with mixtures of CH₂Cl₂–MeOH as eluent, yielding four main fractions. Fraction I eluted with CH₂Cl₂–MeOH 98:2 (0.50 g) was chromatographed again using *n*-heptane–EtOAc (9:3), yielding gentinone B (142 mg). Fraction II (CH₂Cl₂–MeOH 98:2, 1.14 g) was gentinone A. Fraction III (CH₂Cl₂–MeOH 98:2, 0.50 g) was chromatographed using *n*-heptane–acetone (9:2), yielding successively gentinone D (150 mg) and gentinone C (200 mg). Fraction III (CH₂Cl₂–MeOH 95:5, 0.34 g) was chromatographed using *n*-heptane–Me₂CO (9:3), yielding gentinin (30 mg).

Gentinone A (1): amorphous solid; $[\alpha]_D^{20}$ –34° (*c* 1, CHCl₃); UV λ max nm 227 ($\log \epsilon$ 3.96); IR ν max (CHCl₃) 3555, 3400, 1722, 1669 cm⁻¹; HRFABMS *m/z* 607.3592 [M + Na]⁺ (Δ –1.9 mmu); NMR see Table 1.

Gentinone B (2): amorphous solid; $[\alpha]_D^{20}$ –24° (*c* 1, CHCl₃); UV λ max nm 227 ($\log \epsilon$ 3.96); IR ν max (CHCl₃) 3555, 3400, 1722, 1669 cm⁻¹; HRFABMS *m/z* 649.3738 [M + Na]⁺ (Δ –3.2 mmu); NMR (main C_{21 β} -epimer) see Table 2.

Gentinone C (3): amorphous solid; $[\alpha]_D^{20}$ –24° (*c* 1, CHCl₃); UV λ max nm 227 ($\log \epsilon$ 3.96); IR ν max (CHCl₃)

Table 2. ¹³C-NMR (75 MHz) and ¹H-NMR (250 MHz) Data for Gentinone B (**2**), C (**3**), and D (**4**) (CDCl₃)

position	2		3		4	
	δ_C	δ_H (JHz)	δ_C	δ_H (JHz)	δ_C	δ_H (JHz)
1	158.5	6.97 d (11)	158.2	6.90 (11)	157.6	6.92 (11)
2	126.9	5.72 d (11)	123.6	5.72 d (11)	123.1	5.72 (11)
3	204.2		204.4		203.4	
4	44.5		44.3		43.8	
5	45.7		44.5		45.4	
6	23.9		24.1		23.1	
7	74.1	5.24 br s	71.2	3.85 br s	73.5	5.30 br s
8	44.5		44.3		45.2	
9	45.2	2.52 (9)	43.9	2.52 d (9)	44.5	2.45 d (8)
10	42.0		40.9		40.2	
11	70.6	5.50 m	70.3	5.58 m	69.8	5.50 m
12	42.1		42.1		42.1	
13	45.6		45.6		45.9	
14	159.5		160.9		158.1	
15	119.1	5.24 m	120.2	5.58 m	118.5	5.30 m
16	35.9		34.9		34.5	
17	52.7		52.4		51.9	
18	20.1	1.08 s	20.0	1.12 s	19.4	1.05 s
19	20.5	1.31 s	20.1	1.28 s	19.9	1.27 s
20	46.1		43.6		44.2	
21	97.6	5.24 m	96.2	5.22 br s	95.9	5.22 br s
22	31.7		30.2		29.8	
23	78.9	3.84 m	79.0	4.55 t (8)	78.3	4.50 t (8)
24	68.0	2.82 d (8)	75.4	3.17 br s	76.6	3.10 br s
25	57.6		74.0		76.2	
26	25.3	1.30 s	26.6	1.30 s	26.0	1.32 s
27	19.8	1.30 s	26.6	1.30 s	26.0	1.32 s
28	26.3	1.10 s	26.0	1.08 s	25.6	1.10 s
29	21.5	1.05 s	21.5	1.20 s	21.0	1.03 s
30	30.3	1.23 s	30.4	1.20 s	29.6	1.27 s
1'	176.6		176.2		175.4	
2'	42.0		42.1	2.20 m	41.8	2.25 m
3'	26.8	1.40 m	26.4	1.40 m	25.9	1.40 m
		1.70 m		1.70 m		1.70 m
4'	12.4	0.92 m	12.1	0.95 m	11.7	0.95 m
4''	17.2	1.17 d (7)	16.8	1.18 d (7)	16.5	1.18 d (7)
CO	170.4				169.2	
Me	20.1	1.98 s			20.8	1.95 s

3515, 1715, 1662 cm⁻¹; HRFABMS *m/z* 625.3721 [M + Na]⁺ (Δ –3.2 mmu); NMR (main C_{21 β} -epimer) see Table 2.

Gentinone D (4): amorphous solid; $[\alpha]_D^{20}$ –18° (*c* 1, CHCl₃); UV λ max nm 227 ($\log \epsilon$ 3.96); IR ν max (CHCl₃) 3515, 1722, 1669 cm⁻¹; HRFABMS *m/z* 667.3795 [M + Na]⁺ (Δ +3.7 mmu); NMR (main C_{21 β} -epimer) see Table 2.

Gentinin (5): amorphous solid; $[\alpha]_D^{20}$ +14° (*c* 1, CHCl₃); IR ν max (CHCl₃) 3530, 1687 cm⁻¹; HRFABMS *m/z* 641.3679 [M + Na]⁺ (Δ –1.3 mmu); NMR (main C_{21 β} -epimer) see Table 1.

Alkaline Hydrolysis of Gentinone A. A solution of gentinone A (500 mg) in EtOH containing 5% KOH (30 mL) was refluxed for 6 h. After removal of the solvent *in vacuo*, the residue was acidified with 5 N HCl and extracted with CH₂Cl₂. The CH₂Cl₂ extract was distilled off. The fraction boiling at 180° was chromatographed on Si gel with CH₂Cl₂–MeOH mixtures yielding 2-methylbutyric acid (CH₂Cl₂–MeOH 98:2, 30 mg), $[\alpha]_D^{20}$ –5° (*c* 1, CHCl₃). [(*S*)-2-Methylbutyric acid Aldrich has $[\alpha]_D^{20}$ +20° (*c* 1, CHCl₃).] ¹H-NMR (CDCl₃) of a mixture of the acid (1.4 g %) and 1,2-diphenylethane-1,2-diamine (1.4 g %): δ 1.109, 1.106, (2d, Me-4''), 0.900, 0.897 (2t, Me-4').

Acid Opening of the Epoxide Ring of Gentinone A. A solution of gentinone A (30 mg) in DMF/HClO₄ was kept at room temperature for 18 h. The reaction mixture was diluted with NH₃ and extracted with Et₂O.

The solvent was evaporated and the residue was purified using preparative TLC (eluent CH₂Cl₂-MeOH 95:5), yielding gentinone C (20 mg), ¹H- and ¹³C-NMR consistent with those of the natural product.

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References and Notes

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