Cytotoxic 3,4-Secoapotirucallanes from *Aglaia argentea* Bark

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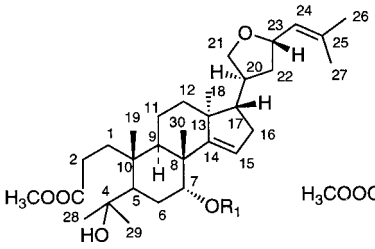
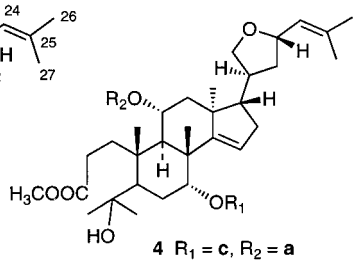
Nine 3,4-secoapotirucallanes, argentinic acids A−I, were isolated from the bark of *Aglaia argentea* and transformed to their methyl esters **1**−**9**. The structures were determined by spectral and chemical means. Compounds **1**−**8** showed moderate cytotoxic activity against KB cells (IC_{50} 1.0−3.5 μg/mL).

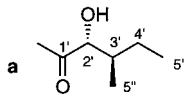
The genus *Aglaia* is a rich source of compounds of different kinds with often interesting biological activities: bisamides, lignans, aromatic derivatives of the benzofuran series, and tetracyclic triterpenes.[1] From the seeds of *A. argentea* Bl. (Meliaceae) collected in Malaysia,[2] we have previously isolated five apotirucallane-type triterpenes, gentinones A−D and gentinin.[3] We report here the isolation, from an ethanolic bark extract, of apotirucallane triterpenes characterized by a seco-A ring, argentinic acids A−I, which were purified in the form of their methyl esters **1**−**9**. Compounds **1**−**8** possess α-hydroxy acid ester chains with five or six carbons at position 7 or at positions 7 and 11. They showed moderate cytotoxic activity against KB cells, while the naturally occurring acids were inactive.



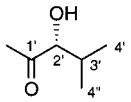
1 R_1 = **a**
2 R_1 = **b**
3 R_1 = **c**



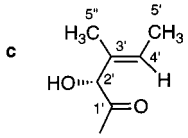
4 R_1 = **c**, R_2 = **a**
5 R_1 = **c**, R_2 = **b**
6 R_1 = **b**, R_2 = **a**
7 R_1 = R_2 = **a**
8 R_1 = R_2 = **c**



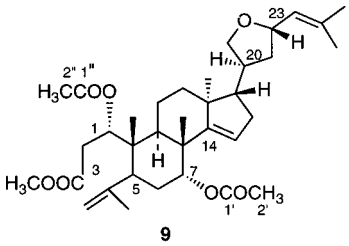
a



b



c



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Results and Discussion

Argentinic acid A methyl ester (**1**) gave a $[M + H]^+$ peak at *m/z* 617.4417 in the HRCIMS corresponding to the molecular formula $C_{37}H_{60}O_7$. The IR spectrum showed an absorption for a methyl ester group at 1735 cm^{-1}. In the ^{13}C NMR spectrum the signal of the carbonyl at position 3 resonated at δ 175.4, while the quaternary COH-4 appeared at δ 75.3. The apotirucallane triterpene skeleton was based on the presence of seven tertiary methyls (δ_H between 1.8 and 0.9) and an olefin with chemical shifts typical of a Δ^{14} double bond (δ_C 159.1 and 118.5). In addition, the NMR spectra (Table 1) showed signals of a C-7α oxymethine at δ_C 76.9 and δ_H 5.18 (br s). The latter downfield proton shift indicated the presence of an ester function at position 7. The corresponding CO resonance was observed at δ 174.6. In addition, two methyl groups appeared at highfield in the ^{13}C NMR spectrum (δ 12.1 and 13.0) and finally an oxymethine at δ 73.3 suggested the presence of an aliphatic hydroxylated acid ester chain. The 2D NMR spectra (COSY, HMQC, and HMBC) confirmed the preceding 1D NMR data of the triterpene fused rings and permitted the assignment of the hydroxylated acid moiety as 2-hydroxy-3-methylpentanoic acid (also named isoleucic acid). The ^1H COSY spectrum, aided by the HMQC experiment, revealed the spin system $H_{5'}$-$H_{4'}$-$H_{3'}$-($H_{5''}$)-$H_{2'}$, while the HMBC spectrum showed the typical correlations H-2′/C-1′, C-3′, H-3′/C-2′, H-4′/C-2′, C-3′, C-5′; and H-5′, H-5″/C-3′, C-4′. For the side chain at C-17 in **1**, the 1D and 2D NMR spectra indicated a tetrahydrofuran ring bearing a dimethylallyl group, as already found in several tetracyclic triterpenes.[4] The NOESY spectrum showed the correlations H-18/H-20, H-18/H-21α, H-20/H-21α, and H-21β/H-23 diagnostic of 20*S* and 23*S* configurations. The stereochemistry of the isoleucic acid moiety was determined as D-*allo* by chiral TLC analysis of an acid hydrolysate of **1** using a method previously described for depsipeptides.[5]

Argentinic acid B methyl ester (**2**) gave a $[M + H]^+$ peak in the HRCIMS at *m/z* 603.4256 corresponding to the molecular formula $C_{36}H_{58}O_7$. The ^1H and ^{13}C NMR spectra (Table 1) were similar to those of **1,** except for the ester chain at position 7, which, as deduced from the mass spectrum, possessed one less carbon atom. Analysis of the COSY spectrum revealed a diastereotopic isopropyl group (δ_H 2.02, δ_{Me} 0.79 and 1.07, d, J = 7 Hz), which was α to a hydroxymethine (δ_H 3.96), thus suggesting the presence of a 2-hydroxy-3-methylbutyric acid moiety. Finally, the 2D NMR spectra fully supported the structure and the 20*S*,23*S*

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Table 1. ^{13}C (75 MHz) and ^{1}H NMR (400 MHz) Data for Argentinic Acid Methyl Esters **1** and **2** ($CDCl_3$)[a]

	1				**2**			
position	δ_C	δ_H (*J* Hz)	HMBC	NOESY	δ_C	δ_H (*J* Hz)	HMBC	NOESY
1	34.2	α 1.64 m	2,3,5	1*β*	34.2	α 1.70 m	2, 3, 5, 10	1*β*
		β 2.45 m	2,3	19, 28, 29		*β* 2.50 m	2, 3, 10	19, 28, 29
2	28.9	α 2.47 m	1, 3	2*β*, 5	29.0	α 2.52 m	1, 3, 10	2*β*, 5
		β 2.14 m	1, 3, 10			*β* 2.18 m	1, 3	
3	175.4					175.4		
4		75.3					75.3	
5	44.4	1.71 m	7, 10		44.5	1.73 m	7, 10	
6	26.7	α 1.76 m	5, 8	6*β*, 7, 29	26.8	α 1.75 m	5, 8	6*β*, 7, 29
		β 1.85 m	5, 8	7, 19, 28, 30		*β* 1.92 m	5, 8	7, 19, 28, 30
7	76.9	5.18 br s	5, 9, 1′	30	76.8	5.19 br s	5, 9, 1′	30
8	41.6				41.6			
9	35.0	2.10 m	8, 10, 11, 14, 19, 30		35.1	2.18 m	14	
10	41.6				41.6			
11	16.2	1.53 m	9, 12, 13, 18		16.3	1.60 m	9, 12, 13	
12	33.4	α 1.60 m	13, 14	18	33.3	α 1.63 m	13, 14	18
		β 1.35 m				*β* 1.40 m		
13	46.9				46.9			
14	159.1				159.1			
15	118.5	5.22 m	8, 13, 14, 16, 17	16α*β*, 30, 5″	118.6	5.27 m	8, 13, 14, 16, 17	16α*β*, 30, 4″
16	35.5	α 2.02 m	14, 15, 17	16*β*, 18, 5″	35.5	α 2.02 m	13, 14, 15, 17	16*β*, 18, 20
		β 2.12 m	14, 15, 17, 20	17		*β* 2.12 m	14, 15, 17, 20	17′
17	58.6	1.55 m	13, 20, 21, 22	21*β*, 22	58.5	1.60 m	13, 16, 20	21*β*
18	18.6	0.95 s	12, 13, 14, 17	20, 21α	18.6	0.97 s	12, 13, 14, 17	20, 21α
19	19.8	1.08 s	1, 5, 9, 10		19.9	1.11 s	1, 5, 9, 10	
20	40.6	2.32 m		21α, 22, 24	40.6	2.36 m	21α, 22	
21	72.4	α 4.05 m	22, 23	21*β*, 22	72.4	α 4.08dd (8, 8)	22, 23	21*β*, 22
		β 3.21 dd (8, 9)	17	23		*β* 3.24dd (8, 9)	17	22, 23
22	38.4	1.70 m	17, 23	23	38.4	1.73 m	23	23
23	74.6	4.57 m	25		74.6	4.60 m	25	
24	126.8	5.22 m	26, 27	26	126.8	5.24 m	26, 27	26
25	135.4				135.3			
26	25.9	1.69 s	24, 25, 27		25.9	1.70 s	24, 25, 27	
27	18.2	1.68 s	24, 25, 26		18.2	1.69 s	24, 25, 26	
28	27.4	1.20 s	4, 5, 29		27.4	1.23 s	4, 5, 29	
29	34.1	1.18 s	4, 5, 28		34.1	1.20 s	4, 5, 28	
30	27.7	1.10 s	7, 8, 9, 14		27.8	1.14 s	7, 8, 9, 14	
CO_2Me	52.0	3.66 s	3		52.0	3.70 s	3	
1′	174.6				174.2			
2′	73.3	4.05 m	1′, 3′, 4′, 5″	3′, 5′, 4′a	75.4	3.96 d (3)	1′, 4″	3′, 4′, 4″
3′	38.4	1.70 m	2′	4′ab, 5′, 5″	31.7	2.02 m	4′, 4″	4′, 4″
4′	26.4	a 1.51 m	2′, 3′, 5′	5′, 5″	19.5	1.07 d (7)	4″	4″
		b 1.30 m	2′, 3′, 5′	5′, 5″				
4″					15.2	0.79 d (7)	4′	
5′	12.1	0.93 t (7)	3′, 4′	5″				
5″	13.0	0.73 d (7)	3′, 4′					

[a] Assignments based on 2D experiments (COSY, HMQC, HMBC, NOESY).

stereochemistry of **2** in the same way as for **1**. Chiral TLC analysis of the acid hydrolysate by the method used for **1** indicated the (2*R*)-hydroxy-3-methylbutyric acid configuration.

Argentinic acid C methyl ester (**3**) exhibited a $[M + H]^+$ peak at *m*/*z* 615.4249 in the HRCIMS, which matched the molecular formula $C_{37}H_{58}O_7$. The ^{1}H and ^{13}C NMR spectra (Table 2) were again similar to those of **1** and **2** except for the ester chain at position 7, which possessed a trisubstituted double bond. The olefinic CH appeared as a quartet (δ_H 5.58, $J = 6.5$ Hz) and was thus coupled to a methyl group (δ_H 1.62, d, $J = 6.5$ Hz). A second methyl was located on the double bond (δ_H 1.51, s), and a hydroxymethine singlet appeared at δ_H 4.38. The above data suggested a 2-hydroxy-3-methylpenten-3-oic acid moiety. The highfield ^{13}C NMR shift of the methyls (δ 11.3 and 13.7) indicated *E* geometry for the olefin.[6] The 2D experiments confirmed the structure and the stereochemistry of **3**. The NOESY spectrum especially showed the cross peaks H-2′/H-4′, Me-5″ and H-4′/Me-5′, which supported the *E* configuration of the double bond. The absolute stereochemistry of the ester moiety in **3** was established using catalytic hydrogenation followed by acid hydrolysis and chiral TLC analysis of the hydrolysate. The latter contained a mixture of (2*R*)-*allo*- and (2*R*)-*iso*-hydroxy-3-methylpentanoic acids, showing the C-2′*R*- configuration for compound **3**.

Argentinic acid methyl ester D (**4**) revealed a $[M + Na]^+$ peak at *m*/*z* 767.4870 in the HRCIMS corresponding to the molecular formula $C_{43}H_{68}O_{10}$. The NMR spectra (Table 2) showed a 3,4-secoapotirucallane skeleton with the C-17 side chain similar to the preceding compounds. There was again an ester chain at C-7α, as shown by the downfield shift of H-7 (δ_H 5.18). However, an additional oxymethine signal was observed at δ_H 5.50, and analysis of the ^{13}C and 2D NMR spectra revealed the presence of a second ester chain, which was located at C-11α. The two chains were assigned the structures of 2-hydroxy-3-methylpentanoyl and 2-hydroxy-3-methylpenten-3-oyl moieties, respectively, as deduced from the NMR data in comparison with the data of compounds **1** and **3**. The HMBC correlations H-7/C-1′ and H-11/C-1‴ indicated that the unsaturated chain was attached at C-7, while the saturated chain was at C-11.[7] This was also supported by the NOESY cross peaks Me-5″/H-15, Me-5″/H-16, and Me-5⁴′/H-21.[7]

The four other related triterpenes from the plant, argentinic acids E−H, isolated as their methyl esters **5**−**8**,

Table 2. ^{13}C (75 MHz) and ^{1}H NMR (400 MHz) Data for Argentinic Acid Methyl Esters **3** and **4** ($CDCl_3$)[a]

	3				**4**			
position[b]	δ_C	δ_H (*J* Hz)	HMBC	NOESY	δ_C	δ_H (*J* Hz)	HMBC	NOESY
1	34.1	α 1.62 m	2, 3, 5, 10	1*β*	36.8	α 1.42 m		1*β*
		β 2.44 m	2, 3, 10	19, 28, 29		*β* 2.52 m	2, 3	28, 29
2	28.7	α 2.47 m	1, 3, 10	2*β*, 5	29.8	2.50 m	1, 3	
		β 2.12m	1, 3, 10					
3	175.5				177.0			
4	75.2				75.5			
5	44.2	1.73 m	1, 7, 10		43.4	1.85 m	6, 19, 28, 29	
6	26.7	α 1.73 m	5,8	6*β*, 7, 29	26.2	α 1.80 m		7
		β 1.88 m	5,8	7, 19, 28, 30		*β* 1.88 m	5,7	7, 28, 30
7	77.1	5.21 br s	5, 9, 10, 1′	30	76.2	5.18 br s	5, 9, 1′	30, 5′
8	41.5				41.0			
9	34.7	2.15 m	14		39.7	2.48 d (9)	1, 8, 10, 11, 14, 19, 30	18
10	41.4				42.4			
11	16.2	1.56 m	9,1 2, 13		71.6	5.50 m	9, 10, 13, 1‴	12*β*, 19, 30
12	33.3	α 1.64 m	13, 14	18	43.5	α 1.60 m	9, 11, 13, 14, 18	12*β*
		β 1.35 m				*β* 1.85 m	13, 17	30
13	46.8				45.7			
14	159.2				158.4			
15	118.1	5.20 m	8, 13, 14, 16, 17	16α*β*, 30, 5′	118.2	5.25 m	13, 16, 17	5″
16	35.6	α 2.00 m	13, 14, 15, 17	16*β*, 18, 20, 5′, 5″	35.4	α 2.05 m	14, 15	16*β*, 18, 5"
		β 2.10 m	14, 15, 17, 20	17, 5′, 5″		*β* 2.12 m	14	17
17	58.5	1.60 m	13, 20, 21	21*β*	58.5	1.53 m	12	
18	18.5	0.87 s	12, 13, 14	20, 21α	18.8	0.98 m	12, 13, 14, 17	20, 21α
19	19.8	1.08 s	1, 5, 9, 10		21.2	1.23 s	1, 5, 9, 10	
20	40.7	2.30 m		21α, 22	40.5	2.30 m		21α ,22
21	72.4	α 4.05 dd (8,8)	17, 20, 22	21*β*, 22	72.2	α 3.93 dd (8,8)	22, 23	21*β*, 5[4′]
		β 3.21 dd (8,9)	20, 22	22, 23		*β* 3.13dd (8,9)	17	
22	38.4	1.73 m	23	23	38.3	1.68 m	17, 20, 21, 23	23
23	74.6	4.58 m	24	27	74.7	4.53 m	25	24
24	126.8	5.21 m	26, 27	26	126.5	5.18 m	26, 27	26
25	135.4				135.6			
26	25.9	1.71 s	24, 25, 27		25.9	1.70 s	24, 25, 27	
27	18.2	1.68 s	24, 25, 26		18.2	1.65 s	24, 25, 26	
28	27.5	1.21 s	4, 5, 29		27.8	1.23 s	4, 5, 29	
29	34.0	1.19 s	4, 5, 28		34.5	1.23 s	4, 5, 28	
30	27.5	1.10 s	7, 8, 9, 14		29.3	1.17 s	7, 8, 9, 14	
CO_2Me	51.9	3.68 s	3		52.3	3.66 s		
1′	173.4				173.3			
2′	77.5	4.38 s	1′, 3′, 4′, 5′	4′, 5″	77.5	4.40 s	3′, 4′, 5″	4′, 5″
3′	132.9				132.7			
4′	125.9	5.58 q (6.5)	5′	5′	126.2	5.60 q (6.5)	2′, 5′, 5″	5′
5′	13.7	1.62 d (6.5)	3′, 4′	5″	13.6	1.62 (6.5)	3′, 4′	
5″	11.3	1.51 s	2′, 3′, 4′		11.3	1.50 s	2′, 3′, 4′	
1‴					174.4			
2‴					73.8	4.12 m	1‴, 3‴	3‴, 5‴
3‴					38.9	1.88 m		
4‴					26.4	a 1.55 m	3‴, 5‴, 5[4′]	4‴b, 5‴
						b 1.35 m	3‴, 5‴	5‴
5‴					11.9	0.92 t (7)	3‴,4‴	
5[4′]					14.1	0.90 d (7)	2‴, 3‴, 4‴	

[a] Assignments based on 2D experiments (COSY, HMQC, HMBC, NOESY). [b] See note.[7]

all possessed two ester chains attached to C-7 and C-11, respectively, as indicated by their mass and NMR spectra (Experimental Section and Table 3). Compound **5** differed from **4** only by the nature of the chain attached to C-11, which was a 2-hydroxy-3-methylbutyric acid ester, while **6** had a 2-hydroxy-3-methylbutyroyl moiety at C-7 and a 2-hydroxy-3-methylpentanoyl moiety at C-11. Compounds **7** and **8** showed symmetrical chains, two 2-hydroxy-3-methylpentanoyl moieties and two 2-hydroxy-3-methylpenten-3-oyl moieties, respectively. The NOESY experiment confirmed the 20*S*,23*S* configurations and allowed assignment of the chains at C-11 and C-7 for **5** and **6** by the observation of the cross peaks H-15/Me-5″ and H-15/Me-4″, respectively. The C-2*R*,C-3*R* and C-2*R* configuration of the symmetrical ester residues of compounds **7** and **8**, respectively, was determined by the same method as for derivatives **1**−**3**. By comparison of the ^{13}C NMR resonances of the side chains, and/or from biogenetic arguments, the same chain configurations as for **1**−**3**, **7**, **8** were tentatively assigned to **4**−**6**.

Argentinic acid I methyl ester (**9**) gave a $[M + H]^+$ peak in the HRCIMS at *m*/*z* 585.3832, which corresponded to the molecular formula $C_{35}H_{52}O_7$. As in known 3,4-seco-terpenoids, the dimethyl hydroxy group at C-4 was replaced by an isopropenyl group with typical ^{13}C NMR resonances for C-28 (δ_C 116.4) and C-4 (δ_C 144.9) (Table 3). The 1D and 2D NMR signals of the fused rings and of the side chain at C-17 were similar to those of compounds **1**−**8**, thus indicating an apotirucallane skeleton with a 23*S* stereochemistry. However, the usual C-1 resonance was absent. Two oxymethines were observed, one (δ_H 5.17, d, brs) corresponding to CH-7, while the second one (δ_H 5.45, dd) was assigned to CH-1. Both C-1 and C-7 bore acetyl groups, which gave the characteristic signals for the C═O (δ_C 170.0

Table 3. ^{13}C (75 MHz) and ^{1}H NMR (400 MHz) Data for Argentinic Acid Methyl Esters **5**−**9** ($CDCl_3$)

	5		**6**		**7**		**8**		**9**[a]	
position[b]	δ_C	δ_H (*J* Hz)	δ_C	δ_H (*J* Hz)	δ_C	δ_H (*J* Hz)	δ_C	δ_H (*J* Hz)	δ_C	δ_H(*J* Hz)
1	36.7		36.7		36.7		35.8		76.7	5.45dd (10, 1)
2	29.7		29.8		29.7		29.3		35.3	a 2.76 brd (13) b 2.40 m
3	177.1		176.9		176.9		177.2		171.7	
4	74.7		75.3		75.3		75.3		144.9	
5	43.4		43.1		42.3		43.2		44.1	
6	26.2		26.1		26.2		26.3		29.1	
7	76.1	5.19 br s	75.7	5.19 br s	75.8	5.18 br s	76.2	5.19 br s	74.5	5.17 br s
8	40.5		41.0		40.2		40.9		42.3	
9	39.7		39.9		39.8	2.52 d (9)	39.3		34.4	
10	42.4		42.4		42.3		42.2		44.1	
11	71.4	5.52 ddd (6, 9, 9)	71.3	5.52 ddd (6, 9, 9)	71.3	5.53 ddd (6, 9, 9)	72.3	5.51 ddd (6, 9, 9)	18.2	
12	43.5		43.4		43.3		43.3		33.7	
13	45.7		45.5		45.6		45.5		46.4	
14	158.4		158.3		158.1		158.2		159.0	
15	118.1	5.26 m	118.3	5.27 m	118.4	5.27 m	118.1	5.28 m	119.0	5.25 m
16	35.5		35.1		35.2		35.4		35.4	
17	58.5		58.2		58.3		58.4		58.6	
18	18.8	0.99 s	18.8	1.07 s	18.8	1.07 s	18.9	0.97 s	19.9	0.98 s
19	21.1	1.23 s	21.1	1.23 s	21.1	1.23 s	20.9	1.22 s	15.0	0.92 s
20	40.5		40.2		40.2		40.4		40.6	
21	72.1	α 3.93 m *β* 3.13 dd	71.9	α 3.92 m *β* 3.14 dd (8, 9)	72.0	α 3.94 dd (8, 8) *β* 3.14 dd (8, 9)	72.2	α 3.92 dd (8, 8) *β* 3.13 m	72.3	α 4.05 m *β* 3.22 dd (8, 9)
22	38.3		38.1		38.1		38.2		38.3	
23	74.6	4.53 m	74.4	4.53 m	74.5	4.53 m	74.7	4.55 m	74.5	4.58 m
24	126.5	5.18 m	126.3	5.17 m	126.4	5.18 m	126.6	5.18 m	126.8	5.20 m
25	135.6		135.5		135.3		135.5		135.2	
26	25.9	1.70 s	25.7	1.68 s	25.7	1.66 s	25.9	1.70 s	25.8	1.68 d (1.3)
27	18.2	1.68 s	18.0	1.68 s	18.0	1.65 s	18.2	1.68	18.1	1.66 d (1.3)
28	27.8	1.23 s	27.6	1.23	27.6	1.23 s	27.4	1.23 s	116.4	a 5.00 br s b 4.80 br s
29	34.4	1.23 s	34.2	1.23 s	34.3	1.23 s	34.3	1.23 s	22.8	1.75 s
30	29.3	1.15 s	29.4	1.16 s	29.3	1.16 s	29.0	1.14 s	26.8	1.10 s
CO_2Me	52.3	3.67 s	52.1	3.64 s	52.1	3.64 s	52.3	3.68 s	52.0	3.63 s
1′	173.3		174.1		174.5		173.3		170.0	
2′	77.5	4.40 s	75.1	3.94 d (3)	73.0	4.09 m	77.5	4.40 d (4)[c]	21.0	1.94 s
3′	132.9		31.6		38.3		133.0			
4′	126.1	5.62 q (6.5)	19.3	1.04 d (6)	26.0		126.2	5.62 q(6.5)		
5′	13.7	1.64 d (6.5)			11.7	0.94 t (7)	13.7	1.66 d(6.5)		
1″									170.3	
2″									21.3	2.00 s
4″			15.3	0.75 d (6)						
5″	11.3	1.51 s			12.9	0.72 d (7)	11.3	1.51 s		
1‴	173.7		174.5		174.1		172.8			
2‴	76.2	3.96 m	73.6	4.13 m	73.7	4.14 m	77.5	4.51 d (4)[c]		
3‴	32.3		31.6		38.7		132.6			
4‴	19.4	1.05 d (7)	26.0		26.2		125.1	5.66 q (6.5)		
$4^{4'}$	17.0	0.95 d (7)								
5‴			11.6	0.92 t (7)	11.9	0.94 t (7)	13.4	1.65 d (6.5)		
$5^{4'}$			14.0	0.91 d (7)	14.0	0.91 d (7)	12.3	1.64 s		

[a] Assignments based on 2D experiments (COSY, HMQC, HMBC, NOESY). [b] See note.[7] [c] Coupling with OH.

and 170.3) and methyl groups (δ_C 21.3 and 21.1, δ_H 2.00 and 1.94). The HMBC correlations observed between H-1,H-7 and the ester carbonyls confirmed the above assignments. Finally, in the NOESY spectrum (see Experimental Section), the cross peaks H-1/Me-19, Me-29 and H-2b/H-28b indicated a C-1*S* configuration.

Compounds **1**−**8** exhibited moderate cytotoxicity against KB cells (IC_{50} 2.0, 2.0, 2.0, 1.0, 3.5, 2.0, 2.5, 2.0 μg/mL, respectively). The corresponding acids were inactive. However, the crude extract showed mild cytotoxic properties (100% inhibition at 10 μg/mL). One possibility for this is that other compounds are present in the extract, which allow a better penetration of the argentinic acids across the KB cell membrane.

A. argentea bark was found to contain none of the highly cytotoxic cyclopentatetrahydrobenzofuran-type compounds, which were isolated previously from the seeds of this plant[8] in minute quantities and from a number of other *Aglaia* species.[8,9]

Experimental Section

General Experimental Procedures. Optical rotations at 20 °C were taken on a Perkin−Elmer 241 polarimeter. Spectra were recorded as follows: IR ($CHCl_3$), Nicolet 205 FT-IR spectrometer; NMR, Bruker AC 300 (^1H and ^{13}C NMR spectra) and AMX 400 (2D NMR spectra); HRCIMS (Reagent gas CH_4), Kratos MS 9; HRFABMS, ZAB spectrometer TOF. Column chromatography Si gel Merck 60 H. Semipreparative HPLC, column Ultrasphere C_{18} (10 × 250 mm), MeCN−H_2O−HOAc (65:35:1), flow rate 4 mL/min, RI detection.

Plant Material. Bark material of *Aglaia argentea* Bl. was collected in Dungun, Terengganu, Malaysia, in March 1993. The identification was made by G. Perromat (Institut de Chimie des Substances Naturelles, C.N.R.S., Gif-sur-Yvette, France). Voucher specimens (KL 4347) are deposited at the Laboratoire de Phanérogamie, Muséum National d'Histoire Naturelle in Paris, 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. Dried ground bark material (200 g) of *A. argentea* was extracted exhaustively with EtOH at room temperature. The extract (10 g) was chromatographed on Si gel with mixtures of CH_2Cl_2−MeOH. A fraction eluted with CH_2Cl_2−MeOH 9:1 (1.32 g) was submitted to semipreparative HPLC yielding the crude argentinic acid I, a mixture of argentinic acids B and C, and crude argentinic acid A. The acids were esterified in a MeOH solution with CH_2N_2 and purified by chromatography on Si gel (heptane−EtOAc, 8:2), yielding **9** (23 mg), **2** (8 mg), **3** (9 mg), and **1** (13 mg). A fraction from the original EtOH extract eluted with CH_2Cl_2−MeOH 8:2 (0.5 g) was submitted to semipreparative HPLC yielding the crude argentinic acids H, E, D, F, and G. The acids were esterified as above and purified by column chromatography or preparative TLC (heptane−EtOAc, 6:4), affording **8** (20 mg), **5** (27 mg), **4** (95 mg), **6** (24 mg), and **7** (20 mg).

Argentinic acid A methyl ester (1): $[\alpha]_D$ −41° (*c* 1, $CHCl_3$); IR ν_{max} 3415, 1735 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; HRCIMS *m/z* 617.4417, a $[M + H]^+$ ($C_{37}H_{61}O_7$, Δ 0 mmu).

Argentinic acid B methyl ester (2): $[\alpha]_D$ −42° (*c* 1, $CHCl_3$); IR ν_{max} 3415, 1735 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; HRCIMS *m/z* 603.4256 $[M + H]^+$ ($C_{36}H_{59}O_7$, Δ −0.5 mmu).

Argentinic acid C methyl ester (3): $[\alpha]_D$ −77° (*c* 1, $CHCl_3$); IR ν_{max} 3415, 1735 cm-^{1}; ^1H NMR and ^{13}C NMR, see Table 2; HRCIMS *m/z* 615.4249 $[M + H]^+$ ($C_{37}H_{59}O_7$, Δ −1.2 mmu).

Argentinic acid D methyl ester (4): $[\alpha]_D$ −50° (*c* 1, $CHCl_3$); IR ν_{max} 3415, 1735 cm-^{1}; ^1H NMR and ^{13}C NMR, see Table 2; HRFABMS *m/z* 767.4712 $[M + Na]^+$ ($C_{43}H_{68}O_{10}Na$, Δ 0.2 mmu).

Argentinic acid E methyl ester (5): $[\alpha]_D$ −87° (*c* 1, $CHCl_3$); IR ν_{max} 3415, 1735 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 3; HRFABMS *m/z* 753.4554 $[M + Na]^+$ ($C_{42}H_{66}O_{10}Na$, Δ 0.0 mmu); main NOESY correlations: H-7/Me-30, H-18/H-21α, H-20/H-21α, H-21β/H-23, H-15/Me-5″, H-2′/Me-5″, H-4′/Me-5′.

Argentinic acid F methyl ester (6): $[\alpha]_D$ −42° (*c* 1, $CHCl_3$); IR ν_{max} 3415, 1735 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 3; HRFABMS *m/z* 755.4709 $[M + Na]^+$ ($C_{42}H_{68}O_{10}Na$, Δ 0.1 mmu); main NOESY correlations: H-7/Me-30, H-18/H-21α, H-20/H-21α, H-21β/H-23, H-15/Me-4″.

Argentinic acid G methyl ester (7): $[\alpha]_D$ −36° (*c* 1, $CHCl_3$); IR ν_{max} 3415, 1735 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 3; HRFABMS *m/z* 769.4870 $[M + Na]^+$ ($C_{43}H_{70}O_{10}Na$, Δ 0.3 mmu); main NOESY correlations: H-7/Me-30, H-18/H-21α, H-20/H-21α, H-21β/H-23, H-15/Me-5″.

Argentinic acid H methyl ester (8): $[\alpha]_D$ −66° (*c* 1, $CHCl_3$); IR ν_{max} 3415, 1735 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 3; HRFABMS *m/z* 765.4579 $[M + Na]^+$ ($C_{43}H_{66}O_{10}Na$, Δ 2.5 mmu); main NOESY correlations: H-7/Me-30, H-18/H-21α, H-20/H-21α, H-21β/H-23, H-15/Me-5″, H-2′/H-4′, H-5″, H-2‴/H-4‴,H-5$^{4'}$, H-4′/H-5′, H-4‴/H-5‴.[7]

Argentinic acid I methyl ester (9): $[\alpha]_D$ −44° (*c* 1, $CHCl_3$); IR ν_{max} 3415, 1735 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 2; HRCIMS *m/z* 585.3832 $[M + H]^+$ ($C_{35}H_{53}O_7$, Δ 4.1 mmu); main HMBC correlations: H-1/C-3,C-5,C-9,C-1″, H-7/C-5, C-9, C-2′; main NOESY correlations: H-1/H-2α*β*, Me-19, H-28b, Me-29, H-2b/H-28b, H-7/H-15, Me-30, H-18/H-21α, H-20/H-21α, H-21β/H-23.

Acid Hydrolysis of Compounds 1, 2, and 7. To a solution of the compound (8 mg) in HOAc (0.2 mL) was added H_2O (0.2 mL) and concentrated HCl (0.4 mL). The mixture was refluxed for 12 h and evaporated to dryness.

Acid Hydrolysis of Compounds 3 and 8. A MeOH solution (3 mL) of the compound (14 mg) and PtO_2 (ca. 5 mg) were shaken for 4 h under an atmosphere of hydrogen. After removal of the catalyst and the solvent, the hydrogenated derivatives, which showed the disappearance of the signal of H-4′ and H-4‴ in the ^1H NMR spectrum,[7] were hydrolyzed using the same method as above.

Chiral TLC Analysis. Each acid hydrolysate was analyzed as described previously.[5] Comparison with authentic commercial standards of D- and L-isoleucic acids and D-*allo*-isoleucic acid showed that the hydrolysate of compounds **1** and **7** contained D-*allo*-isoleucic acid (R_f 0.56), while the hydrolysate of compound **3** and **8** contained a mixture of D-isoleucic (R_f 0.53) acid and d-*allo*-isoleucic acid. D-Isoleucic acid was not commercially available. However, the R_f value previously described[5] is 0.64 greater than that of L-isoleucic acid (0.61). The 2-hydroxy-3-methylbutyric acid from compound **2** showed a spot with the same R_f value as a commercial standard of (2*R*)-hydroxy-3-methylbutyric acid (0.56).

References and Notes

(1) Brader, G.; Varodaya, S.; Gregner, H.; Bacher, M.; Kalchauser, H.; Hofer, O. *J. Nat. Prod.* **1998**, *61*, 1482−1490, and references therein.
(2) This work was performed in the framework of a collaborative program between the University of Malaya and C. N. R. S.
(3) Omobuwajo, O. R.; Martin, M.-T.; Perromat, G.; Sévenet, T.; Awang, K.; Païs, M. *J. Nat. Prod.* **1996**, *59* , 614−617.
(4) Connolly, J. D.; Hill, R. A. *Nat. Prod. Rep.* **1997,** *14*, 661−679, and previous reviews in this series.
(5) Golakoti, T.; Ogino, J.; Helze, C. E.; Husebo, T. E., Jensen C. M.; Larsen, L. K.; Patterson, G. M. L.; Moore, R. E.; Moobery, S. L.; Corbett, T. H.; Valeriote, F. *J. Am. Chem. Soc.* **1995**, *117*, 12030−12049.
(6) Stothers, J. B. *Carbon-13 NMR Spectroscopy*; Academic: New York 1972, pp 434−436, 453.
(7) Positions (or protons) 1‴ to 5$^{4'}$ refer to moieties **a**, **b**, **c** attached to C-11 in formulas **5**−**8**.
(8) Dumontet, V.; Thoison, O.; Omobuwajo, O. R.; Martin, M.-T.; Perromat, G.; Chiaroni, A.; Riche, C.; Païs, M.; Sévenet, T.; Hadi, A. H. A. *Tetrahedron* **1996**, *52*, 6931−6942, and references therein.
(9) Oshe, T.; Ohba, S.; Yamamoto, T.; Koyano, T.; Umezawa, K. *J. Nat. Prod.* **1996**, *59*, 650−652.

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