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CYTOTOXIC CYCLOARTANES FROM *AGLAIA ARGENTEA*

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Key Word Index—*Aglaia argentea*; Meliaceae; cycloartane; triterpenoid; cytotoxicity; structural elucidation.

Abstract—*Aglaia argentea* leaves yielded three new cycloartanes: argenteanones A and B, and argenteanol. The first two possess cytotoxic activity against KB cells (IC_{50}7.5 μg ml^{-1} and 6.5 μg ml^{-1} respectively). Structural elucidation was done by two-dimensional NMR spectroscopy.

INTRODUCTION

In a collaborative programme between the University of Malaya and CNRS, systematic bioassay screening on KB cells of Malaysian plant extracts was undertaken. This led to the chemical study of the ethanol extract from the leaves of *Aglaia argentea* Bl., a 30-m high tree which produces white latex. The plant material was collected at Dungun, Terengganu State on the east coast of the Malaysian Peninsula. The phytochemical study yielded three new triterpenoids, argenteanones A (**1**) and B (**2**), and argenteanol (**3**), of which two (**1**, **2**) possess cytotoxic activity. This paper reports on the isolation, structural identification and the cytotoxicity of these compounds.

RESULTS AND DISCUSSION

The EtOH extract was fractionated by column chromatography on silica gel and cytotoxic activity was detected in the 5% methanol–CH_2Cl_2 fraction. Further chromatography led to the isolation of compounds **1**–**3**.

Argenteanone (**1**) exhibited a $[M + Na]^+$ peak at *m/z* 493 in the FAB-mass spectrum corresponding to a molecular formula of $C_{30}H_{46}O_4$. The EI-mass spectrum gave fragmentation peaks at $[M - 18]^+$ and $[M - 36]^+$, suggesting the presence of two hydroxyls. The IR spectrum showed an absorption of a carbonyl at 1695 cm^{-1} (^{13}C NMR δ216.4). The ^{1}H NMR spectrum contained two doublets at δ0.68 and δ0.80 (J = 4 Hz), respectively, characteristic of a C-9, C-10 cyclopropyl methylene group of a cycloartanone type triterpenoid [1]. A vinyl proton (H-24) appeared at δ5.42 (*dt*, J = 8 Hz, J = 1 Hz) and resonances for two geminal vinyl methyls were observed at δ1.70 and δ1.80, respectively (^{13}C δ26.7 and δ19.2) thus indicating a Δ^{24} double bond in the side chain. In addition, a downfield signal of a methine was observed at δ_C 101.4 and δ_H, 5.30 implying that the carbon is flanked by two oxygens (C-21). Another two signals of oxymethines appeared at δ78.0 (C-22) and δ80.3 (C-23), respectively. The ^{1}H COSY spectrum showed couplings between H-17–H-20, H-20–H-21, H-20–H-22 and H-22–H-23. In addition, the HMBC spectrum exhibited a correlation between H-21 and C-23, suggesting an oxygen bridge between carbons 21 and 23 and thus a five-membered ring hemiacetal system. The vinyl proton correlated with C-23, which proved that the hemiacetal moiety and the gem dimethylvinyl group form the side chain.

The stereochemistry at C-21, C-22, and C-23 was resolved by NOESY correlations (Table 1). H-21 is α (21*S*) as it correlated with H-12. This hypothesis is supported by the lowfield chemical shift of C-21 (δ_C 101.4) which is typical of 1,2 antisubstituted furanosides [2, 3]. NOEs were observed for H-16/H-22, H-17/H-23 and H-22/H-23, suggesting that both H-22 and H-23 are α (22*S*, 23*S*). The remaining ^{1}H COSY, HMBC and NOESY spectra, together with the HMQC data, were consistent with the structure depicted in **1** for argenteanone A. In addition, the NOESY cross-peak H-11α–H-30 indicated that ring C has a boat conformation.

Argenteanone B (**2**) showed a $[M + Na]^+$ peak at *m/z* 495 (FAB mass spectrometry MS), in accordance with $C_{30}H_{48}O_4$. The EI-mass spectrum exhibited fragmentation peaks at *m/z* 314 [M – side chain]$^+$ and *m/z* 387 [M – fragment a]$^+$, suggesting that the five-membered hemiacetal ring was opened. This hypothesis was supported by the absence of the methine at C-21; instead, a methylene signal was observed (δ_C 59.9 and δ_H 4.00, 3.65). Furthermore, C-23 was shifted upfield to δ66.8. In addition, the C-21–H-23 and C-23–H-21 correlation peaks

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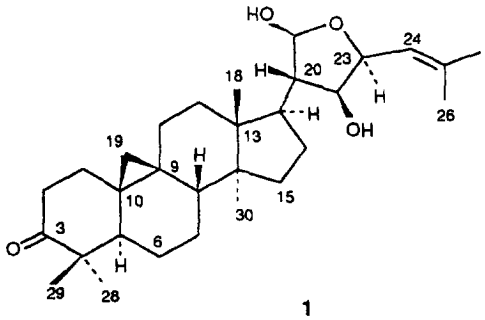

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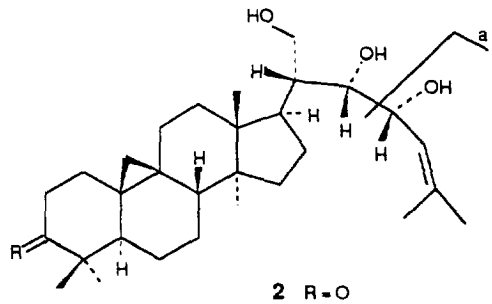

2 R = O
3 R = H, βOH

were absent in the HMBC spectrum. Despite the fact that free rotation is possible at C-20, C-22 and C-23, NOEs were observed, suggesting that the side-chain has a preferred conformation. NOESY correlations of CH_3-18–H-20, H-16–H-22, H-17–H-23 and H-22–H-23 indicated that C-22 and C-23 both have the same *S*-configuration as compound **1** (Fig. 1). The values of the ^{13}C and ^{1}H of the cycloartanone part of the molecule, based on ^{1}H COSY, HMQC and HMBC data, are similar to those of **1** (Table 1).

Argenteanol (**3**) gave a $[M + Na]^+$ peak in the FABMS at *m/z* 497 corresponding to $C_{30}H_{50}O_4$. The carbonyl absorption in the IR spectrum was absent which suggested that C-3 was a hydroxymethine (δ_C 78.9 and δ_H 3.28) instead of a carbonyl. The signal of C-2 was shifted about 7 ppm upfield to δ30.5 owing to the absence of the latter. The ring A and ring B ^{13}C chemical shift values are comparable to the normal values of a cycloartan-3β-ol [4] and the H-3α configuration is further confirmed by the diaxial coupling constant between H-3 and H-2β (J = 10 Hz). The ^{13}C and ^{1}H data for the side chain are similar to those of **2**. Thus, **2** and **3** differ only by the presence of a 3β-hydroxyl in **3** instead of a 3-ketone in **2**.

Compounds **1** and **2** exhibited moderate cytotoxicity against KB cells (IC_{50} 7.5 μg ml^{-1} and 6.5 μg ml^{-1}, respectively). Cycloartane derivatives have been isolated from A. *roxburghiana* [5, 6], but, to the knowledge of the authors, cycloartanones possessing a hemiacetal and a hydroxylated side chain, respectively, have never been reported in *Aglaia*. However, a Russian team found cycloartanol glycosides with a similar hemiacetal side chain but with a 21-methoxyl and a 23*R*-configuration in *Thalictrum squarrosum* [7]. No cytotoxic activity was reported for those compounds.

EXPERIMENTAL

General. Mp: uncorr.; optical rotation and IR: $CHCl_3$; EIMS: 70 eV; FABMS: glycerol matrix + NaCl or LiCl; ^{1}H NMR: 250 or 400 MHz; ^{13}C NMR: 62.5 or 75 MHz; chemical shifts are given in ppm with TMS as int. standard; 2D NMR: 400 MHz with standard pulse sequences.

Plant material. Bark material of *A. argentea* was collected in Dungun, Terengganu, on 22 March, 1993. Identification was made by one of us (G.P.). 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 of cycloartanes. Dried ground leaves (200 g) were extracted exhaustively with EtOH at room temp. The extract (19.6 g) was chromatographed on silica gel (70–200 mesh) with mixts of CH_2Cl_2–MeOH as eluent. Two successive frs (A and B) eluted with CH_2Cl_2–MeOH (19: 1) showing activity against KB cells (74% and 81% inhibition at 10 μg ml^{-1}) were subjected to further silica gel (Merck H 60) CC. **1** (40 mg) was obtained from fr. A using (1) CH_2Cl_2–MeOH (95.5:0.5), (2) hexane–AcOEt (4:1). **2** (40 mg) was also obtained from fr. A (1) CH_2Cl_2–MeOH (46:1), (2) hexane–AcOEt (4:1). **3** (14 mg) was obtained from fr. B using hexane–Me_2CO (4:1).

Argenteanone A (**1**). Recrystallized from hexane–EtOAc, mp 144–148°, $[\alpha]_D$ − 4.2° (*c* 1). IR ν_{max} cm^{-1}: 3410, 1695; FAB-MS *m/z* 493 $[M + Na]^+$; *m/z* 477 $[M + Li]^+$. EI-MS, *m/z* (rel. int.): 452 (1.3) (M-18), 434 (M-2 × 18) (10), 148 (100); ^{1}H and ^{13}C NMR: Table 1; found: C 76.32, H 9.81, O 13.57; $C_{30}H_{46}O_4$ requires C 76.54, H 9.86, O 13.60.

Argenteanone B (**2**). Amorphous powder, $[\alpha]_D$ + 12.6° (*c* 1). IR ν_{max} cm^{-1}: 3400, 1699; FAB-MS *m/z* 495 $[M + Na]^+$; *m/z* 479 $[M + Li]^+$. EI-MS, *m/z* (rel. int.): 387 (8), 314 (1), 85 (100); ^{1}H and ^{13}C NMR: Table 1.

Argenteanol (**3**). Amorphous powder, $[\alpha]_D$ − 1° (*c* 0.5). IR ν_{max} cm^{-1}: 3410; FAB-MS *m/z* 497 $[M + Na]^+$; *m/z* 481 $[M + Li]^+$. ^{1}H NMR ($CDCl_3$, 250 MHz): δ0.33 (1H, *d*, *J* = 4, H-19α), 0.56 (1H, *d*, *J* = 4, H-19β), 0.82 (3H, *s*, Me-28), 0.90 (3H, *s*, Me-30), 0.96 (3H, *s*, Me-29), 1.02 (3H, *s*, Me-18), 1.80 (6H, *s*, Me-26 and Me-27), 3.29 (1H, *dd*, *J* = 10, *J'* = 4, H-3α), 3.62 (1H, *brs* H-22), 3.68 (1H, *dd*, *J* = 11.5, *J'* = 6, H-21a), 4.00 (1H, *dd*, *J* = 11.5, *J'* = 2.5, H-21*b*), 4.58 (1H, *dd*, *J* = 9.5, *J'* = 3.5, H-23), 5.40 (1H, *br d*, *J* = 8.5, H-24); ^{13}C NMR ($CDCl_3$, 62.5 MHz): 14.1 (Me-29), 18.0 (Me-18), 18.5 (Me-27), 19.5 (Me-30), 20.0 (C-9), 21.5 (C-6), 25.6 (Me-26 and Me-28), 26.1 (C-7), 26.2 (C-10), 26.5 (C-11), 27.6 (C-16), 29.8 (C-19), 30.5 (C-2), 32.1, 32.3 (C-1 and C-12), 35.7 (C-15), 43.4

Table 1. ^{13}C (75 MHz) and ^{1}H NMR (400 MHz) data* for argenteanone A (**1**) and argenteanone B (**2**) ($CDCl_3$)

	1				**2**		
Position	δ_C	δ_H (*J* Hz)	HMBC	NOESY	δ_C	δ_H (*J* Hz)	HMBC
1	34.0	α 1.80 *m*		1β, 2β, 5	33.4	α 1.85 *m*	10, 19
		β 1.55 *m*	2, 3, 5, 10	2αβ, 11β, 19α		β 1.60 *m*	2, 3, 5, 10
2	38.0	α 2.25 *ddd* (14, 4, 2.5)	1, 3, 4, 10	2β	37.4	α 2.30 *ddd* (13, 4, 2.5)	1, 3, 10
		β 2.70 *ddd* (14, 7, 7)	1, 3, 10	19α, 29		β 2.68 *ddd* (13, 6.5, 6.5)	3, 10
3	216.4				216.4		
4	50.8				50.2		
5	48.9	1.75 *m*	1, 4, 19	28	47.7	1.70 *m*	1, 4, 9, 10, 19
6	22.0	α 1.55 *m*		6β, 28	21.5	α 1.55 *m*	
		β 0.95 *m*				β 0.90 *m*	
7	26.5	α 1.10 *m*		7β	25.8	α 1.10 *m*	
		β 1.40 *m*				β 1.35 *m*	9, 10, 14, 15, 19, 30
8	48.4	1.60 *m*	9, 14, 15, 19	15, 18, 19β	48.3	1.60 *m*	
9	21.5				20.9		
10	27.0				25.8		
11	27.1	α 2.05 *m*	8, 9, 12, 13, 19	11β, 12, 30	26.6	α 2.05 *m*	9
		β 1.20 *m*	9, 12, 19	18, 19α		β 1.15 *m*	
12	33.0	1.70 *m*	13, 18	21	32.0	α 1.60 *m*	
						β 1.75 *m*	
13	46.4				47.0		
14	49.4				48.5		
15	36.0	1.40 *m*		16α, 16β, 30	35.5	1.30 *m*	13, 14, 16, 30
16	28.2	α 1.90 *m*		16β, 22	27.4	α 1.70 *m*	
		β 1.55 *m*		22		β 1.45 *m*	
17	45.2	1.80 *m*		20, 23, 30	43.2	2.10 *m*	
18	19.9	1.10 *s*	12, 13, 17	20	18.1	1.02 *m*	12, 13, 17
19	30.2	α 0.68 *d* (4)	1, 5, 8, 9, 10	19β	29.3	α 0.55 *d* (4)	1, 5, 8, 9, 10
		β 0.80 *d* (4)	1, 4, 9, 10	29		β 0.75 *d* (4)	1, 5, 8, 9, 10
20	58.8	2.38 *d* (11.5)	16, 17, 22, 23	21	47.0	2.00 *m*	
21	101.4	5.30 *d* (7)	17, 20, 22, 23		59.9	a 4.00 *dd* (11, 2.5)	23
						b 3.65 *dd* (11, 4)	
22	78.0	4.00 *dd* (6, 4)	21, 23	23	75.8	3.60 *dd* (3.5, 3.5)	20, 23
23	80.3	4.75 *dd* (8, 4)	21, 24, 25	26	66.8	4.55 *dd* (9, 3.5)	24, 25
24	121.6	5.42 *dt* (8, 1)	26, 27		125.0	5.40 *br d* (9)	26, 27
25	138.9				136.3		
26	26.7	1.70 *s*	24, 25, 27		26.1	1.73 *s*	24, 25, 27
27	19.2	1.80 *s*	24, 25, 26		18.4	1.73 *s*	24, 25, 26
28	22.8	1.02 *s*	4, 5, 29		21.2	1.07 *s*	4, 5, 29
29	21.4	1.05 *s*	4, 5, 28		20.7	1.02 *s*	4, 5, 28
30	19.9	0.90 *s*	8, 13, 14, 15		19.4	0.88 *s*	8, 13, 14, 15
OH-21		3.70 *d* (7)					
OH-22		2.60 *d* (6)					

*Assignments based on 2D experiments.

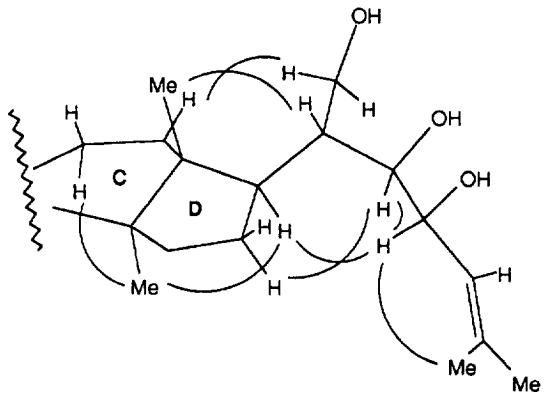

Fig. 1. Main NOESY correlations for argenteanone B (**2**)—rings C and D and side-chain.

(C-17), 44.9 (C-4), 46.1 (C-13), 47.2 (C-5 and C-20), 47.9 (C-8), 48.7 (C-14), 60.9 (C-21), 67.0 (C-22), 76.0 (C-23), 78.9 (C-3), 125.2 (C-24), 136.3 (C-25).

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