Complete Assignment of the ^{1}H and ^{13}C NMR Spectra of the Camelliagenin A and A_1-Barrigenol from the seed of *Barringtonia asiatica*

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Abstract: The ^{1}H and ^{13}C NMR spectra of camelliagenin A and A_1-barrigenol from the seeds of *Barringtonia asiatica* were completely assigned for the first time by one- and two-dimensional homo- and heteronuclear studies (^{1}H, ^{13}C, DQCOSY, TOCSY, HMQC, HMBC) at 600 and 150.89 MHz. The article reports standard data that may be important for potential authors needing such information.

Keywords: NMR, ^{1}H NMR, ^{13}C NMR, triterpenes, camelliagenin A, A_1-barrigenol.

INTRODUCTION

Barringtonia asiatica also known as "fish killer tree" is one of the mangrove plants that grows in tropical Asia and Pacific, including Northern Australia, the seeds of which are used as fish poison [1,2]. In previous paper,[1,2] we reported that the triterpene moieties of saponins from the seeds of *B. asiatica* were camelliagenin A (**1**) [1] and A_1-barrigenol (**2**). [2] Both camelliagenin A (**1**), $C_{30}H_{50}O_4$ and A_1-barrigenol (**2**), $C_{30}H_{50}O_5$ have been isolated from *B. asiatica*, [3] *Pittosporum undulatum*, [4,5] and *Harpullia cupaniodes*[6,7]. Beside that, **1** has also been isolated from *Maesa chisia*, [8] while from *Camellia* species. The structures of **1** and **2** had earlier been deduced by ^{1}H NMR spectroscopy to be 3β,16α,22α,28-tetrahydroxyoelan-12-ene [6,9] and 3β,15α, 16α,22α,28-pentahydroxyolean-12-ene, [10] respectively. However, there has not been an extensive high-field NMR study on these compounds. In this paper, we wish to report complete assignments of the signals of the ^{1}H and ^{13}C NMR spectra **1** and **2**, as well as their 2D data of homo- and heteronuclear correlations including DQCOSY, TOCSY, HMQC, HMBC experiments. These assignments allowed us to determine the chemical shifts, positions, and configurations of hydroxyl groups of **1** and **2**. The resulting proton and carbon assignments should serve as a basis for structural and spectral assignments of other members of the family of triterpenoidal sapogenins and for derivatives of these compounds.

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EXPERIMENTAL SECTION

NMR Spectra

^{1}H and ^{13}C NMR were recorded using a Varian INOVA instrument at 600 MHz (^{1}H) and 150.89 MHz (^{13}C). All the NMR data were measured in d_5-pyridine, and chemical shifts were expressed in δ (ppm), and were carried out at 25 ^{0}C. 2D experiments were performed using standard INOVA programs.

Isolation

Extraction of a brown residue (4.35 g) containing crude saponin was described previously. [1,2] A portion of the crude saponin (100 mg) containing material was first heated with 5% aqueous HCl in ethanol at 100 ^{0}C for 3 hours and then rotary evaporated to remove ethanol. The residue was extracted with ethyl acetate and the ethyl acetate extractives evaporated and heated at 100 ^{0}C for 16 hours with 5% KOH in ethanol. The reaction mixture was then rotary evaporated to remove ethanol and this residue was extracted with ethyl acetate. These ethyl acetate extractives were then purified by HPLC (YMC ODS-AQ 5 μm 120 Å 250 mm column of 10 mm internal diameter), thermostated at 40 ^{0}C, mobile phase was 65% acetonitrile in water, flow rate was 4 mL min^{-1} and UV (210 nm) detection afforded camelliagenin A (1, 4.7 mg) and A_1-barrigenol (2, 8.4 mg).

RESULTS AND DISCUSSION

The EI-MS spectra of **1** and **2** showed the presence of molecular ions $[M]^{+}$ at m/z 474 and 490 respectively. Moreover, **1** and **2** from EI-MS showed four and five successive

losses of 18 mass units from $[M]^+$ ions, indicated the presence of four hydroxyl and five hydroxyl groups in **1** and **2** respectively. The ^1H and ^{13}C NMR spectra of **1** and **2** which are presented in Table **1**, each showed seven signals for methyl groups, one olefinic proton, and two olefinic carbons. Four and five hydroxyl substituents were also observed (Table **1**) indicated tetra and pentahydroxyolean-12-ene skeletons respectively, with further structural information being provided by 2-D NMR techniques (DQCOSY, TOCSY, HMBC, and HMQC; see Table **2** for correlations).

Structure of Camelliagenin A

(**1**). Structure elucidation of **1** was begun from the position assignment of a methine proton doublet of triplets signal at 3.46 ppm. From HMQC, the methine proton signal showed a directly connected to a carbon at 78.0 ppm. Comparison with literature data for triterpenolean-12-ene [6, 9] indicated that the carbon at 78.0 ppm very likely belong to C-3, thus the methine proton at 3,46 ppm was designated as H-3. DQCOSY spectrum showed that H-3 coupled with a methylene group at 1.87/1.89 ppm (H-2) and a hydroxyl group at 5.77 ppm (OH-3). Based on the vicinal coupling constant of H-3 (td 5.4; 10.8 Hz) can be assigned the 3-hydroxyl configuration is β equatorial. In HMBC spectrum, C-3 showed long-range correlations to two methyl groups at 1.22 (H-23) and 1.04 ppm (H-24), then the methyls, from HMBC, showed long-range correlations to carbons at 39.4 (C-4) and 55.6 ppm (C-5). C-5 by HMQC connected to a proton at 0.87 ppm (H-5), which was coupled, from TOCSY, to two methylene groups at 1.53/1.55 ppm (H-6) and 1.30/1.64 ppm (H-7). While H-5 in HMBC showed long-range correlation to carbons at 18,7 (C-6), 37.1 (C-10), 16.6 (C-24), and 15.9 ppm (C-25). From HMQC, H-7 showed a directly connected to a carbon at 33.0 ppm, which in turn showed a long-range correlation by HMBC to a methyl group at 0.95 ppm (H-26), and H-26 showed long-range correlations to carbons at 40.2 (C-8), 47.3 (C-9), and 42.7 ppm (C-14). From HMBC, C-9 showed a long-range correlation to olefinic proton at 5.38 ppm (H-12), and the latter from TOCSY was coupled to a methylene group at 1.90/1.98 ppm (H-11), while H-12 in HMQC was directly connected to a carbon at 123.9 ppm (C-12). H-12 by HMBC showed a long-range correlation to a carbon at 42.7 ppm (C-14), and H-11 in HMBC showed a long-range correlation to a quaternary carbon at 143.1 ppm (C-13).

From HMBC, C-14 showed long-range correlations to two methyl groups at 0.95 ppm (H-26) and 1.84 ppm (H-27), and to an ABX system signal of methylene at 1.67/2.17 ppm (H-15). The latter, from HMBC showed a long-range correlation to carbon at 27.6 ppm (C-27), which in turn directly connected to H-27. From DQCOSY, H-15 was coupled to a broad singlet signal of methine proton at 5.17 ppm (H-16), and the latter showed coupling to a hydroxyl group at 5.89 ppm (OH-16). H-16 from HMQC was directly connected to a carbon at 66.9 ppm (C-16). The low-field shift of H-16 indicated the proton attached to a oxygen-bearing carbon. The multiplicity of H-16 was not used to determine the configuration of 16-hydroxyl, but the low-field shift of methyl-27 at 1.84 ppm indicated the present of 1,3 diaxial interaction with hydroxyl group, thus 16-hydroxyl configuration is α axial.

From HMBC, proton methylene at 1.67/2.17 ppm (H-15) showed a long-range correlation to a carbon at 45.1 ppm (C-17), and the latter, also from HMBC, showed long-range correlations to protons at 2.52 ppm (H-18), 1.28/2.89 ppm (H-19), and an isolated AB system signal at 3.70/4.08 ppm (H-28). The low-field shift of H-18 indicated there is not γ-gaus effect with H-27, and this is characteristic of the cis geometry of D/E rings in triterpenoleananes. H-19 from HMBC showed a long-range correlation to a quaternary carbon at 32.2 ppm (C-20), which in turn showed long-range correlation to two methyl groups at 1.04 ppm (H-29) and 1.15 ppm (H-30), and to a methylene group at 1.92/2.75 ppm (H-21). The latter, in DQCOSY showed coupling to methine proton at 4.67 ppm (H-22), which was directly connected, from HMQC, to a carbon at 69.5 ppm (C-22). H-22 from DQCOSY showed coupling to a hydroxyl group at 5.59 ppm (OH-22). Configuration of 22-hydroxyl was assigned as α equatorial based on the vicinal coupling constant of H-22 (br d 10.2 Hz). H-22 from HMBC also showed a long-range correlation to a methylene carbon at 70.1 ppm (C-28), which was directly connected, from HMQC, to an isolated AB system signal of hydroxymethylene group at 3.70/4.08 ppm (H-28), and H-28 by HMBC showed long-range correlations to carbons at 66.9 ppm (C-16) and 45.1 ppm (C-17), which completed the circumnavigation of the camelliagenin A (**1**) structure. This NMR analysis enabled us to assign the structure of **1** as 3β,16α,22α,28-tetrahydroxyoelan-12-ene.

Structure of A_1-barrigenol

(**2**). Since **2** has clearly identified to be a hydroxy-camelliagenin A (**1**), [10] only the position and configuration of the additional hydroxyl group remains to be determined. The chemical shifts of the 27-methyl signals of **2**, when compared with those of **1**, suggested that the additional hydroxyl group is probably attached to the ring D (Table **1**). The high-field shift of C-27 (21.2 ppm) indicating that there is γ-gaus effect of 15-hydroxyl substituent. The ABX system due to H-15 and a broad signal due to H-16 in **1** are absent, and instead the doublet of doublets at δ 4.48 (1H axial, dd, 4.2; 9.0 Hz) and 5.05 ppm (1H equatorial, dd, 4.2; 9.0 Hz), which were latter determined to be H-15 and H-16 respectively. These doublet of doublets were occurred by the coupling of H-16 to H-15 or *vice-versa* together with the coupling of their hydroxyl groups from which it can be concluded that the additional hydroxyl group is at C-15. Since the configuration of 16-hydroxyl was assigned to be α axial like in **1**, which was supported by the high-field shift of protons attached to the 26-methyl (δ 1.08), indicating that there is not 1,3-diaxial relationship to hydroxyl, so the 15-hydroxyl has an α equatorial configuration based on the coupling constants of H-15 (dd, 4.2; 9.0 Hz). Moreover, the low-field shifts of C-15 (δ 67.4 ppm) and C-16 (δ 72.7 ppm) are characteristic of carbons which are respectively attached by the α(eq) and α(ax) configurations of hydroxyl groups. The assignments of H-15, H-16, C-15, and C-16, as well as the other protons and carbons of **2** were unambiguously assigned by supporting the 2D NMR techniques (see Table **2** for correlations). Thus, the structure of **2** can be assigned as 3β,15α,16α,22α,28-pentahydroxyolean-12-ene.

Table 1. NMR Data (in d_5-pyridine) for 1 and 2

Position	δC (ppm)		DEPT		δH (ppm), multiplicity, *J* (Hz)	
	1	**2**	**1**	**2**	**1**	**2**
1	39.1	39.287	CH_2	CH_2	1.02, m; 1.59, m	1.06, m; 1.57, m
2	28.1	28.2	CH_2	CH_2	1.87, m; 1.89, m	1.82a, m; 1.89, m
3	78.0	78	CH	CH	3.46, td, 10.2,10.8	3.47, td, 10.8, 10.8
4	39.4	39.348	C	C		
5	55.6	55.6	CH	CH	0.87 br d, 11.4	0.93, br d, 12.0
6	18.7	19.1	CH_2	CH_2	1.53, m; 1.55, m	1.83a, m; 1.92, m
7	33.0	36.7	CH_2	CH_2	1.30, m; 1.64, m	2.09, m; 2.14, m
8	40.2	41.5	C	C		
9	47.3	48.2	CH	CH	1.78, dd, 4.8, 10.8	1.78, dd, 4.8; 10.8
10	37.1	37.4	C	C		
11	23.9	24	CH_2	CH_2	1.90, m; 1.98, m	1.80, m; 1.98, m
12	123.9	124	CH	CH	5.38, br t, 3.0	5.47, br t, 3.6
13	143.1	144.8	C	C		
14	42.7	47.4*	C	C		
15	34.7	67.4	CH_2	CH	1.67, dd, 1.2, 14.4; 2.17, dd, 4.2, 14.4	4.48, dd, 4.2, 9.0
16	66.9	72.7	CH	CH	5.17, br s	5.05, dd, 4.2, 9.0
17	45.1	44.9	C	C		
18	43.1	43.2	CH	CH	2.52, dd, 4.2, 13.8	2.50, dd, 4.2, 14.4
19	46.6	47.4*	CH_2	CH_2	1.28, m; 2.89, t, 13.8	2.87, t, 13.8, 1.28, m
20	32.2	31.7	C	C		
21	44.3	45.5	CH_2	CH_2	1.92, dd, 4.2, 13.8; 2.75, t, 12.6	1.86, m; 2.78, t, 11.4
22	69.5	74.2	CH	CH	4.67, br d, 10.2	4.64, dd, 4.8, 11.4
23	28.8	28.7	CH_3	CH_3	1.22, s	1.21, s
24	16.6	16.6	CH_3	CH_3	1.04, s	1.04, s
25	15.9	15.9	CH_3	CH_3	0.94, s	0.96, s
26	17.0	17.5	CH_3	CH_3	0.95, s	1.08, s
27	27.6	21.2	CH_3	CH_3	1.84, s	1.85, s
28	70.1	69.4	CH_2	CH_2	3.70, dd, 3.0, 10.8; 4.08, dd, 6.0, 10.8	3.75, dd, 3.0, 10.8; 4.15, dd, 5.4, 10.8
29	33.5	33.7	CH_3	CH_3	1.04, s	1.05, s
30	25.0	25.4	CH_3	CH_3	1.15, s	1.14, s
3-OH					5.77, d, 4,2	5.87, d, 5.4
15-OH						5.77, br d, 4.8

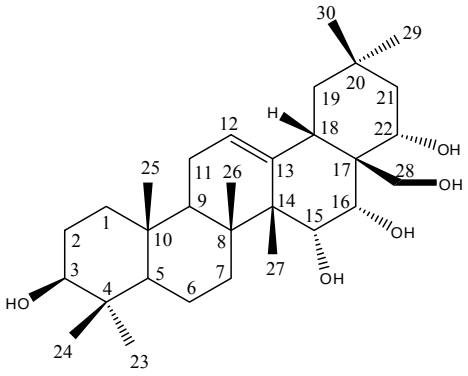
Table 1. Contd…..

Position	δC (ppm)		DEPT		δH (ppm), multiplicity, *J* (Hz)	
	1	2	1	2	1	2
16-OH					5.89, br d, 5.4	6.18, d, 4.8
22-OH					5.59, d, 3.6	5.62, br s
28-OH					6.52, br t, 4.8	6.65, t, 4.2

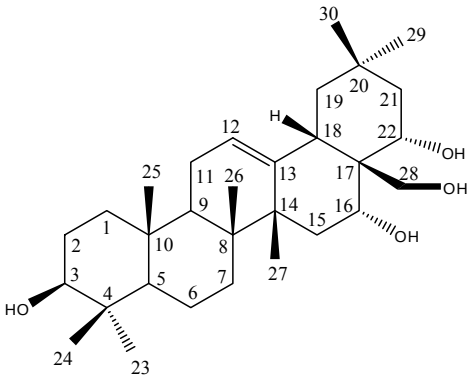
* Overlapping signals

Table 2. NMR Correlations Observed in 1 and 2

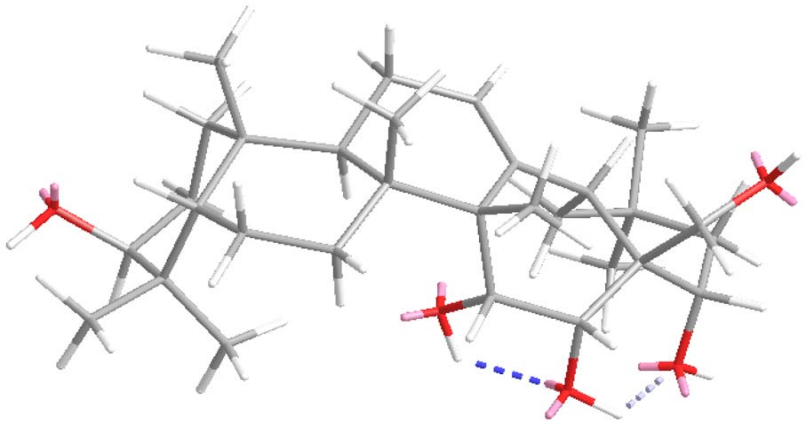
Proton	^{1}H-^{1}H DQCOSY		^{1}H-^{1}H TOCSY		^{1}H-^{13}C HMQC		^{1}H-^{13}C HMBC	
	1	2	1	2	1	2	1	2
1	H-2	H-2	H-2, H-3, OH-3	H-2, H-3, OH-3	C-1	C-1	C-2, C-3, C-5, C-10, C-25	C-2, C-10, C-25
2	H-1, H-3	H-1, H-3	H-1, H-3, OH-3	H-1, H-3, OH-3	C-2	C-2	C-1, C-3	C-3, C-10
3	H-2, OH-3	H-2, OH-3	H-1, H-2, OH-3	H-1, H-2, OH-3	C-3	C-3	C-2, C-23, C-24	C-1, C-2, C-4, C-23, C-24
5	H-6, H-7	H-6	H-6, H-7	H-6, H-7	C-5	C-5	C-6, C-10, C-24, C-25	C-1, C-3, C-4, C-7, C-8, C-10, C-23, C-25
6	H-5, H-7	H-5, H-7	H-5, H-7	H-5, H-7	C-6	C-6	C-7	C-7
7	H-5, H-6	H-6	H-5, H-6	H-5, H-6	C-7	C-7	C-5, C-6, C-10, C-26	C-6, C-26, C-27
9	H-11, H-12	H-11	H-11, H-12	H-11, H-12	C-9	C-9	C-8, C-10, C-25, C-26	C-25, C-26, C-11
11	H-9, H-12	H-9, H-12	H-9, H-12	H-9, H-12	C-11	C-11	C-12, C-13	C-12, C-9, C-13
12	H-9, H-11	H-11	H-9, H-11	H-9, H-11	C-12	C-12	C-9, C-11, C-14	C-9, C-11, C-14, C-18, C-19
15	H-16	H-16	H-16, OH-16	H-16, OH-16	C-15	C-15	C-13, C-14, C-16, C-17, C-27	C-8, C-14, C-16, C-18, C-27
16	H-15, OH-16	H-15, OH-16	H-15, OH-16	H-15, OH-16	C-16	C-16		C-15, C-17, C-18, C-19, C-21, C-28
18	H-19	H-19	H-19	H-19	C-18	C-18	C-12, C-13, C-14, C-16, C-17, C-19, C-28	C-12, C-13, C-14, C-16, C-17, C-28
19	H-18	H-18	H-18	H-18	C-19	C-19	C-17, C-18, C-20, C-21, C-30	C-13, C-17, C-18, C-20, C-29, C-30
21	H-22	H-22	H-22, OH-22	H-22, OH-22	C-21	C-21	C-20, C-22, C-29, C-30	C-17, C-20, C-22, C-29, C-30
22	H-21, OH-22	H-21, OH-22	H-21, OH-22	H-21, OH-22	C-22	C-22	C-16, C-20	C-16, C-17, C-18, C-21, C-28
23					C-23	C-23	C-3, C-4, C-5, C-24	C-24, C-3, C-4, C-5
24					C-24	C-24	C-3, C-4, C-23	C-23, C-3, C-4, C-5
25					C-25	C-25	C-5, C-9, C-10	C-9, C-10, C-5
26					C-26	C-26	C-8, C-9, C-14	C-7, C-8, C-9, C-14, C-5
27					C-27	C-27	C-8, C-13, C-14, C-15	C-8, C-13, C-14, C-15
28	OH-28	OH-28	OH-28	OH-28	C-28	C-28	C-16, C-17, C-18, C-22	C-16, C-17, C-18
29					C-29	C-29	C-20, C-21, C-30	C-19, C-20, C-21
30					C-30	C-30	C-19, C-20, C-29	C-19, C-20, C-21



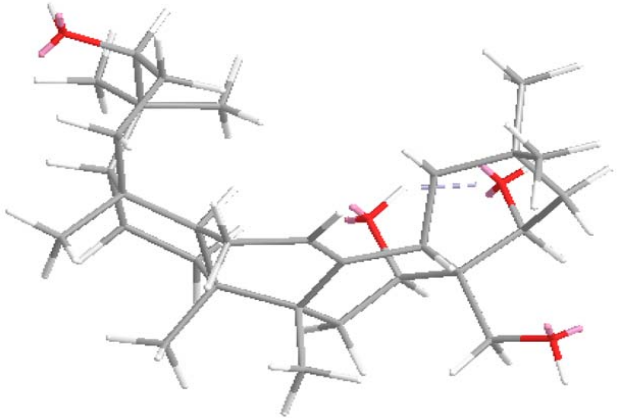
Structure of cameliagenin A



Structure of A_1-Barrigenol



MM2 minimized energy conformation of the compound showing hyrogen bondings (Chem Office) Camelliagenin



Barrigenol

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENT

None declared.

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