New Cycloartane-type Triterpenoids from *Artocarpus nobilis*

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Recent phytochemical studies on the ethanolic extract of *Artocarpus nobilis*, collected from Sri Lanka, have resulted in the isolation of two new cycloartane-type triterpenoids, artocarpuate A (**1**) and artocarpuate B (**2**). Structures of these new compounds were established with the aid of extensive NMR spectroscopic studies. Compounds **1** and **2** were found to exhibit weak acetylcholinesterase inhibitory activity.

Key words: Artocarpus nobilis, Acetylcholinesterase, Cycloartane-type Triterpenoids

Introduction

Artocarpus nobilis is a tree of moderate size and the only endemic species of the genus *Artocarpus* found in Sri Lanka [1]. Previous chemical investigations on the crude extract of *A. nobilis* have shown that it contains cycloartane-type triterpenoids, flavonoids, benzofurans, and stilbene derivatives [1 – 3]. A few of them have shown antifungal and anti-oxidant activities. For instance, 2′,4′,4-trihydroxy-3′-[6-hydroxy-3,7-dimethyl-2(E),7-octadienyl]chalcone and 2′,4′,4-trihydroxy-3′-[2-hydroxy-7-methyl-3-methylene-6-octaenyl]chalcone were reported to exhibit antifungal activity against *Cladosporium cladosporioides* and radical scavenging activity [3].

The crude ethanolic extract of *A. nobilis* exhibited antibacterial activity against *Bacillus cereus*, *Corynebacterium xerosis*, *Streptococcus agalactiae, Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis* at the concentration of 150 μg mL^{-1} in our preliminary screening. Our recent phytochemical studies on the crude ethanolic extract of this plant resulted in the isolation of two new cycloartane-type triterpenoids, artocarpuate A (**1**) and artocarpuate B (**2**). Spectroscopic methods were used to establish the structures of compounds **1** and **2**, which were found to be inactive in our antibacterial bioassay against the aforementioned bacteria but exhibited moderate acetylcholinesterase (AChE) inhibitory activity in our bioassay. In this paper, we report the isolation and structure elucidation of compounds **1** and **2**.

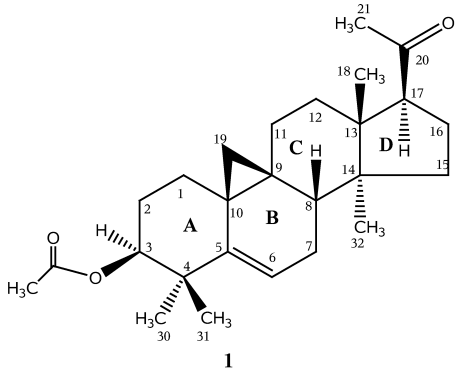

Fig. 1. Structures of compounds **1** and **2**.

Results and Discussion

The first compound, artocarpuate A (**1**), was isolated as a colorless amorphous solid. Its UV spectrum displayed terminal absorption, indicating the lack of any conjugated π bond. The IR spectrum showed intense absorption bands at 1734 (C=O) and 1636 (C=C) cm^{-1}. The chemical-ionization mass spectrum (CI-MS) of **1** showed the $[M+H]^+$ ion at *m/z* = 399 while its high-resolution electron-impact mass spectrum (HREIMS) exhibited the molecular ion peak at *m/z* = 398.2824 corresponding to the molecular formula $C_{26}H_{38}O_3$ (calcd. 398.2821). This indicated the presence of eight degrees of unsaturation in compound **1** and these were accounted for by the cycloartane triterpenoidal skeleton having a double bond in ring B and two carbonyl groups (Fig. 1).

The ^1H NMR spectrum ($CDCl_3$, 300 MHz) of **1** showed a set of AB doublets at δ = 0.33 and 0.57 (*J* = 4.0 Hz) due to the C-19 cyclopropyl methylene protons [4, 5]. Four three-proton singlets at δ = 0.90, 0.94, 0.84 and 1.04 were assigned to the C-30, C-31, C-32 and C-18 methyl protons, respectively. The C-21 methyl protons resonated as a three-proton singlet at δ = 2.17. Another three-proton singlet at δ = 2.05 was ascribed to the acetyl protons of an ester group, substituted at C-3. The C-3 methine proton resonated as a double doublet at δ = 4.56 (*J* = 11.4, 6.0 Hz). Its downfield chemical shift value was indicative of the presence of a geminal ester functionality. An olefinic signal at δ = 5.12 was ascribed to the C-6 methine proton.

The COSY-45° and TOCSY spectra manifested the presence of four isolated spin systems in compound **1**. The first spin system "a" was traced from the C-3 methine proton (δ = 4.56), which showed cross peaks with

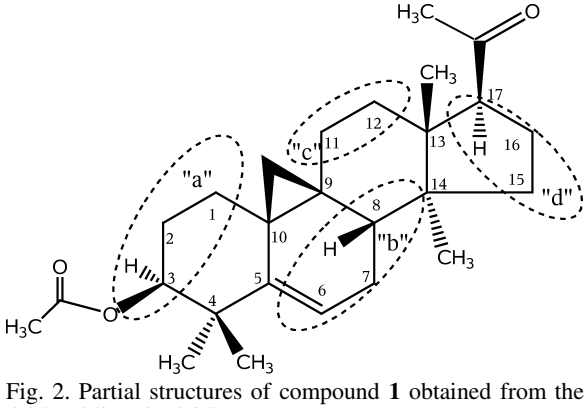

Fig. 2. Partial structures of compound **1** obtained from the COSY-45° and TOCSY spectra.

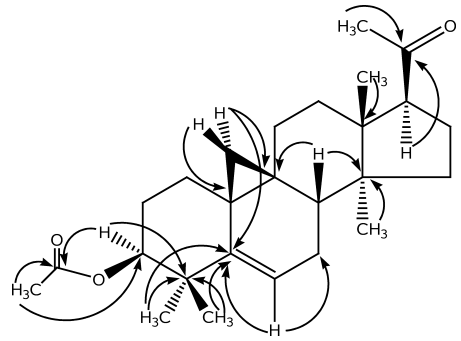

Fig. 3. Important HMBC interactions in compound **1**.

the C-2 methylene protons (δ = 2.02 and 2.15), and the C-2 methylene protons in turn exhibited vicinal couplings with the C-1 methylene protons (δ = 1.30 and 1.55). The second spin system "b" consisted of a fragment starting from the C-6 methine proton and ending at the C-8 methine proton (δ = 1.49). The C-6 olefinic proton (δ = 5.12) displayed ^1H-^1H spin correlations with the C-7 methylene proton (δ = 1.38 and 1.63) which in turn showed COSY-45° interactions with the C-8 methine proton (δ = 1.49). The third partial structure was traced from the vicinal couplings of the C-11 methylene protons (δ = 1.13 and 2.01) with the C-12 methylene protons (δ = 1.68 and 2.10). The fourth spin system "d" consisted of ring D. The C-17 methine proton (δ = 1.59) showed vicinal couplings with the C-16 methylene protons (δ = 1.35 and 1.90). The latter in turn showed cross-peaks with the C-15 methylene protons (δ = 1.25 and 1.41). All of these four spin systems "a-d" present in compound **1** are shown in Fig. 2.

The ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of **1** showed the resonances of all twenty six carbon atoms, and an attached proton test (APT) experiment was performed to establish the multiplicity of each signal in the broadband ^{13}C NMR spectrum. The APT spectrum revealed the presence of six methyl, eight methylene, four methine and eight quaternary carbon atoms in compound **1**. The HSQC spectrum was used to establish $^1H/^{13}C$ one-bond connectivities of all protonated carbon atoms. Complete ^{13}C NMR chemical shift assignments of compound **1** and $^1H/^{13}C$ one-bond shift correlations of all hydrogen-bearing carbon atoms, as determined from the HSQC spectrum, are presented in Table 1. A combination of ^1H, ^{13}C, COSY, HSQC, HMBC and mass spectral data suggested that compound **1** had a cycloartane-type structure with a degraded C-20 side chain and an ester moiety at C-3, as most of the signals in the ^1H and ^{13}C NMR spec-

	1		**2**	
Carbon	^{1}H	^{13}C†	^{1}H	^{13}C†
1	1.30, 1.55 (m)	32.0 (t)	1.31, 1.52 (m)	32.1 (t)
2	2.02, 2.15 (m)	29.6 (t)	1.52, 2.17 (m)	29.8 (t)
3	4.56 (dd, *J* = 11.4, 6.0 Hz)	80.6 (d)	3.60 (dd, *J* = 9.8, 6.2 Hz)	77.3 (d)
4	–	39.4 (s)	–	40.6 (s)
5	–	134.4 (s)	1.89 (m)	47.2 (d)
6	5.12 (br. s)	119.3 (d)	0.82, 1.68 (m)	20.8 (t)
7	1.38, 1.63 (m)	26.4 (t)	1.16, 1.35 (m)	26.2 (t)
8	1.49 (m)	47.8 (d)	1.50 (m)	48.2 (d)
9	–	20.2 (s)	–	20.5 (s)
10	–	26.8 (s)	–	26.5 (s)
11	1.13, 2.01	25.9 (t)	1.12, 2.05 (m)	25.8 (t)
12	1.68, 2.10 (m)	32.6 (t)	1.60, 2.09 (m)	34.9 (t)
13	–	40.6 (s)	–	40.5 (s)
14	–	48.7 (s)	–	48.5 (s)
15	1.25, 1.41 (m)	35.5 (t)	1.22, 1.37 (m)	34.7 (t)
16	1.35, 1.90 (m)	29.1 (t)	4.56 (m)	80.1 (d)
17	1.59 (m)	52.2 (d)	1.62 (m)	51.5 (d)
18	1.04 (s)	18.0 (q)	1.04 (s)	18.6 (q)
19	0.33, 0.57 (d, *J* = 4.0 Hz)	30.8 (t)	0.34, 0.55 (d, *J* = 4.1 Hz)	30.4 (t)
20	–	207.0 (s)	1.91 (m)	35.7 (d)
21	2.17 (s)	19.2 (q)	0.98 (d, *J* = 6.0 Hz)	20.1 (q)
22	–	–	1.19, 1.26 (m)	33.8 (t)
23	–	–	1.02, 1.30 (m)	23.9 (t)
24	–	–	1.28, 1.34 (m)	29.4 (t)
25	–	–	–	77.4 (s)
26	–	–	1.38 (s)	18.8 (q)
27	–	–	1.58 (s)	19.5 (q)
30	0.90 (s)	15.1 (q)	0.91(s)	16.3 (q)
31	0.94 (s)	14.1 (q)	0.84 (s)	14.6 (q)
32	0.84 (s)	20.9 (q)	0.88 (s)	17.4 (q)
$OCOCH_3$	2.05	20.9 (q)	2.03 (s)	21.3 (q)
$OCOCH_3$	–	171.2 (s)	–	173.5 (s)

Table 1. ^{1}H and ^{13}C NMR chemical shift assignments and ^{1}H/^{13}C one-bond shift correlations determined from HSQC spectra of compounds **1** and **2**.

† Multiplicities were determined by an APT spectrum.

tra were identical to those of cycloartane-type triterpenoids [5–7]. The HMBC spectral data were used to build the gross structure of compound **1** from the partial structures "a-d", derived from the COSY-45° and TOCSY spectra. Important HMBC interactions are shown around structure **1a** in Fig. 3.

After establishing a gross structure for compound **1**, the NOESY spectrum was used to determine the relative stereochemistry at all chiral centers present in this compound. Proton 3-H (δ = 4.56) showed a NOE with the C-30 methyl protons (δ = 0.90). Proton 17-H (δ = 1.59) showed a NOE with the C-32 methyl protons (δ = 0.84). The C-31 methyl protons (δ = 0.94) exhibited cross-peaks with the C-8 methine proton (δ = 1.49), which further showed a NOE with the C-18 methyl protons (δ = 1.04). It has been reported that 3-H, 30-H_3 and 32-H_3 protons have invariably α orientations while C-8 methine, C-18 and C-31 methyl protons have β-orientation in this class of natural products [8]. These NOESY spectral observations led us to assume α-stereochemistry for 3-H, 17-H-, 30-H_3 and 32-H_3, and β-stereochemistry for 8-H, 18-H_3 and 31-H_3. Based on these spectroscopic studies, structure **1** was established for this new natural product.

The second compound, artocarpuate B (**2**), was purified as an amorphous solid. The CI-MS showed a $[M+H]^+$ ion peak at *m/z* = 503. The HREIMS also exhibited an ion peak M^+ at *m/z* = 502.3999 (calcd. 502.4022), corresponding to the molecular formula $C_{32}H_{54}O_4$ and indicating the presence of six double bond equivalents. The UV and IR spectra of **2** were nearly identical to those of **1** except that the IR spectrum of the former exhibited an intense absorption band at 3421 cm^{-1} due to a hydroxyl group. The ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of compound **2** showed the resonances of the C-3 and C-16 methine protons at δ = 3.60 and 4.56, respectively. The downfield chemical shift values of C-3 and C-16 methine protons were indicative of the presence of geminal hydroxyl and acetoxy functionalities at C-3 and C-16, respectively. The rest of the ^{1}H NMR spectrum of **2** was similar to that of compound **1**. The C-3 methine proton

(δ = 3.60) showed cross peaks with the C-2 methylene protons (δ = 1.50 and 2.17) in the COSY-45° spectrum. A doublet, integrating for three protons, resonating at δ = 0.98 (J = 6.0 Hz) was assigned to the C-21 methyl protons. Additionally, two singlets, integrating for three protons each, at δ = 1.38 and 1.58 due to the C-26 and C-27 methyl protons were also observed in the ^{1}H NMR spectrum of **2**.

The analysis of ^{1}H-^{1}H COSY and TOSCY plots of **2** revealed the presence of a side chain at C-20 in this molecule. The C-21 methyl protons (δ = 0.98) showed ^{1}H-^{1}H spin couplings with the C-20 methine proton (δ = 1.91). The latter exhibited cross peaks with the C-22 methylene (δ = 1.19 and 1.26) and C-17 methine (δ = 1.62) protons. Proton 17-H showed cross-peaks with the C-16 methine proton (δ = 4.56), which in turn exhibited cross peaks with the C-15 methylene protons (δ = 1.22 and 1.37). Protons 22-H_2 further showed vicinal couplings with the C-23 methylene protons (δ = 1.02 and 1.30), which further showed COSY-45° interactions with the C-24 methylene protons (δ = 1.28 and 1.34). The remaining ^{1}H-^{1}H spin correlations in the COSY-45° and TOCSY spectra were the same as observed for compound **1**. The ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) of **2** was similar to that of **1** except for the additional signals for the C-20 side chain and the difference in the chemical shift values for C-3, C-5, C-6, C-16, and C-20 which resonated at δ = 77.3, 47.2, 20.8, 80.1, and 35.7, respectively. Another aliphatic downfield signal at δ = 77.4 was assigned to C-25, and its downfield resonance was due to the presence of hydroxyl functionality. The presence of a hydroxyl moiety at C-25 was also confirmed from the HMBC spectrum of **2**, in which long-range heteronuclear couplings of the C-26 (δ = 1.38) and C-27 (δ = 1.58) methyl protons with C-25 (δ = 77.4) were observed. Important HMBC interactions are shown around structure **2a** in Fig. 3. Complete ^{13}C NMR chemical shift assignments of **2** and ^{1}H/^{13}C one-bond shift correlations of compound **2** are given in Table 1.

The NOESY spectrum of **2** indicated a stereochemistry at C-3, C-5, C-10, C-8, C-13, C-14, and C-20 similar to that of compound **1**, as previously discussed. The C-16 methine proton (δ = 4.56) showed a NOE with the C-18 methyl protons (δ = 1.04), suggesting a β-orientation of 16-H and an α-orientation for C-16/OAc. The stereochemistry at C-20 was established by the comparison of ^{1}H and ^{13}C NMR chemical shift values of C-20 with data reported for other compounds of this class [9]. Based on these spectroscopic studies, structure **2** was established for this new natural product.

Acetylcholinesterase inhibition assay: Compounds **1** and **2** were evaluated for acetylcholinesterase inhibitory activity, and were found to exhibit IC_{50} values of 0.195 and 0.146 mM, respectively. Compounds exhibiting this bioactivity may have applications in the treatment of Alzheimer's [10, 11].

Experimental Section

General

All ACS grade solvents (methanol, ethyl acetate, chloroform and hexane) were purchased from VWR, Canada. Acetylthiocholine iodide, acetylcholinesterase, sodium phosphate and 5, 5′-dithiobis[2-nitrobenzoic acid] were purchased from Sigma-Aldrich. EI and CI mass spectra were recorded on a Hewlett Packard Series II spectrometer using the direct insertion probe method. The ^{1}H NMR spectra were recorded in $CDCl_3$ on an AM 300 Bruker NMR spectrometer at 300 MHz while ^{13}C NMR spectra were recorded on the same instrument at 75 MHz with TMS as internal standard. The IR and UV spectra were recorded on a Michelson Bomem Hartmann and Braun MB-series spectrometer and a Shimadzu UV 250 spectrophotometer, respectively. The optical rotations were measured on a Polatronic D polarimeter (Hitachi). The column chromatography was carried out using silica gel (mesh size 200 – 400, purchased from Merck). The purities of the samples were checked by TLC (silica gel, GF 254 pre-coated plates purchased from Merck).

Plant material

The bark of *Artocarpus nobilis* (2.2 kg) was collected from Malwana, Sri Lanka. This plant was identified by Dr. Radhika Samarasekera, Natural Products Development Group, Industrial Technology Institute, Colombo-7, Sri Lanka, and a voucher specimen was deposited at the Industrial Technology Institute, Sri Lanka.

*Extraction and isolation of compounds **1** and **2***

The air dried bark (2.2 kg) of *A. nobilis* was extracted with 95 % ethanol at r. t.. Filtration and evaporation of the solvent *in vacuo* afforded a gum (68.32 g). This gum was loaded onto a silica gel column, which was eluted with hexane-ethyl acetate (0 – 100 %) and ethyl acetate-methanol (0 – 100 %) to afford several fractions. Fractions containing similar compounds, as indicated by analytical TLC, were pooled. A primary fraction (6.43 g) was rechromatographed over a silica gel column using gradient elution with hexane-ethyl acetate (0 – 100 %). From these sub-fractions, a fraction (89 mg), obtained by the elution from a silica gel column with hexane-ethyl acetate (75 : 25) was subjected to preparative TLC. These silica gel TLC plates were eluted with hexane-ethyl

acetate (90 : 10) to afford compound **1** (5.6 mg, R_f = 0.48). Compound **2** was purified from another fraction (25 mg), obtained by elution from a silica gel column with hexane-ethyl acetate (90 : 10) that was also rechromatographed over a silica gel column by using hexane-dichloromethane-ethyl acetate (8 : 1 : 1) as a mobile phase. This afforded compound **2** as a greenish oily liquid (4.8 mg, R_f = 0.45) along with a number of minor constituents. These minor constituents were not obtained in a quantity sufficient to do NMR spectroscopic studies.

*Artocarpuate A (**1**)*

$[\alpha]_D^{25}$ = +98° (*c* = 0.26, $CHCl_3$). – UV/vis (MeOH): λ_{max} = 226 nm. – IR ($CHCl_3$): $\tilde{\nu}$ = 2925 (CH), 1734 (carbonyl) and 1636 (C=C) cm^{-1}. – ^{1}H NMR (300 MHz, $CDCl_3$): see Table 1. – ^{13}C NMR (75 MHz, $CDCl_3$): see Table 1. – MS (EI, 70 eV): *m/z* (%) = 398 (5) [M^+], 383 (12), 339 (25), 260 (7), 95 (100). – MS (HREI): *m/z* = 398.2824 (calcd. 398.2821 for $C_{36}H_{38}O_3$, [M^+]). – MS (CI): *m/z* = 399 [M^++H].

*Artocarpuate B (**2**)*

$[\alpha]_D^{25}$ = +65° (*c* = 0.33, $CHCl_3$). – UV/vis (MeOH): λ_{max} = 225 nm. – IR ($CHCl_3$): $\tilde{\nu}$ = 3421 (OH) and 2931 (CH) cm^{-1}. – ^{1}H NMR (300 MHz, $CDCl_3$): see Table 1. – ^{13}C NMR (75 MHz, $CDCl_3$): see Table 1. – MS (EI, 70 eV): *m/z* (%) = 502 (2.5) [M^+], 487 (19), 443 (18), 373 (9), 314 (11), 95 (100). – MS (HREI): *m/z* = 502.3999 (calcd. 502.4022 for $C_{32}H_{54}O_4$, M^+). – MS (CI): *m/z* = 503 [M^++H].

AChE inhibition assay

The acetylcholinesterase activity of **1** and **2** was determined by using modified Ellman's assay [12, 13].

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