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Four new prenylated flavonoids and xanthones from the root bark of *Artocarpus nobilis*

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Abstract

Chemical investigation of the *n*-butanol extract from the methanol extract of the root bark of *Artocarpus nobilis* furnished four new prenylated flavonoids together with artonin E 2′-methylether (**4**), isoartonin E 2′-methylether (**5**), dihydroisoartonin E 2′-methylether (**6**), artonin V 2′-methylether (**7**), artobiloxanthone (**1**), artonin E (**2**) and cycloartobiloxanthone (**3**). All these compounds showed strong radical scavenging properties towards DPPH radical.

Keywords: *Artocarpus nobilis*; Prenylated flavonoids; Xanthones; Radical scavenging; DPPH

1. Introduction

Artocarpus nobilis is the only endemic species of *Artocarpus* available in Sri Lanka. Seeds of the *A. nobilis* are edible. Several pyranodihydrobenzoxanthones, chromenoflavonoids, triterpenes, chalcones and stilbenes have been reported from the bark of the plant [1–6]. In a search for bioactive secondary metabolites from Sri Lankan plants, we carried out a chemical investigation of methanol extract of the root bark of *A. nobilis*. In this paper we report the isolation, structure elucidation and radical scavenging properties towards 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical of four new prenylated flavonoids artonin E 2′-methylether (**4**), isoartonin E 2′-methylether (**5**), dihydroisoartonin E 2′-methylether (**6**), artonin V 2′-methylether (**7**), together with artonin E (**2**), artobiloxanthone (**1**) and cycloartobiloxanthone (**3**) from the root bark of the plant.

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2. Experimental

2.1. General

Mp. Gallenkamp apparatus. UV: UV-160 A. NMR: Bruker DRX500. EIMS (70eV) and FABMS: JEOL JMS-AX700. HPLC: Shimadzu LC-6A apparatus equipped with UV detector under a reversed phase C_{18} column under isocratic solvent condition.

2.2. Plant

A. nobilis Thw. (Moraceae), root bark was collected from the central province of Sri Lanka in December 2002 and identified by comparison with the Herbarium specimen (No. 57333) available at the National Herbarium, Peradeniy, Sri Lanka. A voucher specimen is deposited in the Institute of Fundamental Studies Hantana Road, Kandy, Sri Lanka.

2.3. Extraction and isolation

Dried, ground root bark of *A. nobilis* (300g) defatted with *n*-hexane, extracted with MeOH at r.t. after evaporation gave a dark brown solid (35g). The dried extract (20g) was partitioned with *n*-BuOH and water. Evaporation of *n*-BuOH gave a dark brown solid (13g). A portion of *n*-BuOH extract (12g) was Si-gel CC with *n*-hexane–EtOAc–MeOH to give six major fractions. Each fraction was further purified by a combination of Si-gel, Sephadex LH-20, RP Si-gel and RP-HPLC [STR Prep ODS 20 × 250mm column, 5ml/min, 20% H_2O–MeOH, UV detection 254nm] to give compounds **1** (26mg), **2** (6mg), **3** (60mg), **4** (70mg), **5** (70mg), **6** (36mg), and **7** (34mg).

Artobiloxanthone (**1**). Gummy solid; UV max (EtOH): 220, 257, 382nm; ^{1}H NMR ($CDCl_3$, 500MHz): δ 1.46 (3H, *s*, 17-Me), 1.48 (3H, *s*, 18-Me) 1.79 (3H, *brs*, 13-Me), 2.63 (1H, *dd*, *J* 16.6, 7.8Hz, Ha-9), 3.36 (1H, *dd*, *J* 16.6, 1.7Hz, Hb-9), 3.86 (1H, *brd*, *J* 7.0Hz, H-10), 4.51 (1H, *brs*, H_a-12), 4.80 (1H, *brs*, H_b-12), 5.64 (1H, *d*, *J* 10.0Hz, H-15), 6.39 (1H, *s*, H-6), 6.51 (1H, *s*, H-3′), 6.54 (1H, *d*, *J* 10.0Hz, H-14), 13.01 (1H, *s*, 5-OH); FABMS *m*/*z*: 433 $[M–H]^-$, $C_{25}H_{22}O_7$.

^{13}C NMR: see Table 1.

Artonin E (**2**). Gummy solid; UV max (EtOH): 233, 274nm; ^{1}H NMR (CD_3OD, 500MHz): δ 1.41 (3H, *brs*, 13-Me), 1.43 (6H, *s*, 17-Me and 18-Me), 1.59 (3H, *brs*, 12-Me), 3.11 (2H, *brd*, *J* 7.0Hz, H-9), 5.10 (1H, *m*, H-10), 5.59 (1H, *d*, *J* 10.0Hz, H-15), 6.14 (1H, *s*, H-6), 6.45 (1H, *s*, H-3′), 6.61 (1H, *d*, *J* 10.0Hz, H-14), 6.69 (1H, *s*, H-6′); FABMS *m*/*z*: 435$[M–H]^-$, $C_{25}H_{24}O_7$.

^{13}C NMR: see Table 1.

Cycloartobiloxanthone (**3**). Mp. 270°C; UV max (EtOH): 233, 274nm; ^{1}H NMR ($CDCl_3$–CD_3OD 10:1, 500MHz): δ 1.35 (3H, *s*, 13-Me), 1.47 (3H, *s*, 17-Me), 1.47(3H, *s*, 18-Me), 1.67 (3H, *s*, Me-12), 2.41 (1H, *t*, *J* 15.2Hz, H-9a), 3.20 (1H, *dd*, *J* 15.2, 7.2Hz, H-10), 3.40 (1H, *dd*, *J* 15.2, 7.2Hz, H-9b), 5.58 (1H, *d*, *J* 10.0Hz, H-15), 6.25 (1H, *s*, H-6), 6.26 (1H, *s*, H-3′), 6.78 (1H, *d*, *J* 10.0Hz, H-14); EIMS *m*/*z*: 434 $[M]^+$, 419, 391, 377, 347. $C_{25}H_{22}O_7$.

^{13}C NMR: see Table 1.

Artonin E 2′-methylether (**4**). Mp. 97°C; UV max (EtOH): 252, 290, 349nm; ^{1}H NMR ($CDCl_3$, 500MHz): δ 1.41 (3H, *brs,* 13-Me), 1.43 (6H, *s*, 17-Me and 18-Me), 1.58 (3H, *brs*,12-Me), 3.04 (2H, *brd*, *J* 6.6Hz, H-9), 3.72 (3H, *s*, $–OCH_3$), 5.07 (1H, *m*, H-10), 5.46 (1H, *d*, *J* 10.1Hz, H-15), 6.24 (1H, *s*, H-6), 6.57 (1H, *d*, *J* 10.1Hz, H-14), 6.62 (1H, *s*, H-3′), 6.84 (1H, *s*, H-6′), 13.07 (1H, *s*, 5-OH); FABMS *m*/*z*: 449 $[M–H]^-$; HREIMS *m*/*z*: [M] 450.1667, calc. for $C_{26}H_{26}O_7$ 450.1679.

^{13}C NMR: see Table 1.

Isoartonin E 2′-methylether (**5**). Mp. 97°C; UV max (EtOH): 225, 273, 333nm; ^{1}H NMR ($CDCl_3$, 500MHz): δ 1.39 (3H, *brs*, 13-Me), 1.44 (6H, *s*, 17-Me and 18-Me), 1.58 (3H, *brs*, 12-Me), 3.02 (2H, *brd*, *J* 6.6Hz, H-9), 3.70 (3H, *s*, OCH_3), 5.06 (1H, *m*, H-10), 5.59 (1H, *d*, *J* 10.1Hz, H-15), 6.25 (1H, *s*, H-8), 6.61 (1H, *s*, H-3′), 6.70 (1H, *d*, *J* 10.1Hz, H-14), 6.79 (1H, *s*, H-6′), 13.2 (1H, *s*, 5-OH); EIMS *m*/*z*: $[M]^+$ 450 (95), 435 (100), 407 (50), 366 (30), 203 (35), 97 (12), 69 (20), 55 (20), 44 (30), 18 (18); FABMS *m*/*z*: 449 $[M–H]^-$; HREIMS (*m*/*z*): 450.1695, calc. for $C_{26}H_{26}O_7$ 450.1679.

^{13}C NMR: see Table 1.

Table 1
^{13}C NMR data for compounds **1**–**7** (125MHz, δ in ppm)[a]

C	δ_C						
	1	2	3	4	5	6	7
2	161.7	163.2	160.5	160.6	160.8	162.7	160.8
3	110.8	122.0	111.7	121.1	121.0	121.7	120.2
4	180.1	183.9	180.6	182.6	182.5	183.5	182.7
4a	104.8	105.9	104.0	105.1	105.1	105.1	104.5
5	159.5	162.7	158.7	159.3	156.1	159.9	158.8
6	100.6	100.1	99.9	99.7	105.2	112.5	106.3
7	159.2	160.5	160.9	161.4	159.3	163.4	161.0
8	100.4	102.2	101.1	101.0	94.8	93.6	97.9
8a	151.0	153.8	150.9	152.4	157.5	157.6	155.5
9	21.7	24.9	19.7	24.2	24.2	24.9	24.0
10	38.1	122.6	46.4	121.2	121.2	122.8	121.5
11	149.8	133.0	93.7	132.2	132.2	132.6	131.8
12	112.8	25.9	28.0	25.6	25.6	26.0	25.5
13	20.9	17.6	22.5	17.5	17.5	17.6	17.3
14	113.9	115.8	114.8	115.0	115.5	22.3	22.8
15	128.7	128.2	127.3	126.8	128.0	123.6	122.0
16	77.9	79.1	77.8	78.0	77.8	131.9	131.6
17	27.9	28.4	27.9	28.1	28.2	25.9	25.4
18	28.1	28.4	27.9	28.1	28.2	17.9	17.3
1′	105.2	111.7	104.6	113.1	113.0	113.8	112.9
2′	150.4	150.1	150.2	152.1	151.9	152.5	151.4
3′	103.0	104.7	103.6	100.0	100.2	101.4	99.8
4′	144.8	150.0	145.9	147.3	147.3	149.6	147.3
5′	134.7	139.4	136.8	136.7	136.7	139.9	137.5
6′	127.7	117.2	127.3	117.0	116.9	117.8	116.8
OMe	–	–	–	56.1	56.1	56.7	55.8

[a]Compounds **1**, **4** and **5** were recorded in $CDCl_3$, compounds **2** and **6** in CD_3OD, and compounds **3** and **7** in $CDCl_3$–CD_3OD (10:1).

Dihydroisoartonin E 2′-methylether (**6**). Mp. 131°C; UV max (EtOH): 230, 254, 305, 330nm; ^{1}H NMR (CD_3OD, 500MHz): δ 1.36 (3H, *brs*, 13-Me), 1.58 (3H, *brs*, 12-Me), 1.66 (3H, *brs*, 17-Me), 1.77 (3H, *brs*, 18-Me), 3.00 (2H, *brd*, *J* 6.8Hz, H_2-9), 3.31 (2H, *m*, H-14), 3.70 (3H, *s*, OCH_3), 5.06 (1H, *m*, H-10), 5.23 (1H, *m*, H-15), 6.27 (1H, *s*, H-8), 6.59 (1H, *s*, H-3′), 6.70 (1H, *s*, H-6′); FABMS(−) *m*/*z*: 451 $[M–H]^-$; EIMS *m*/*z*: 452 $[M]^+$, 409, 397, 365, 353; HREIMS *m*/*z*: 452.1827 $[M]^+$, calc. for $C_{26}H_{28}O_7$ 452.1835.

^{13}C NMR: see Table 1.

Artonin V 2′-methylether (**7**). Gummy solid; UV max (EtOH): 230, 254, 305, 330nm; ^{1}H NMR ($CDCl_3$–CD_3OD, 10:1, 500MHz): δ 1.44 (3H, *brs*, 13-Me), 1.58 (3H, *brs*, 12-Me), 1.66 (6H, *brs*, 17-Me and 18-Me), 3.05 (2H, *brd*, *J* 6.0Hz, H_2-9), 3.33 (2H, *brd*, *J* 7.0Hz, H-14), 3.72 (3H, *s*, OCH_3), 5.11 (1H, *m*, H-10), 5.18 (1H, *m*, H-15), 6.25 (1H, *s*, H-6), 6.58 (1H, *s*, H-3′), 6.81 (1H, *s*, H-6′); FABMS *m*/*z*: 451 $[M–H]^-$; EIMS *m*/*z*: 452 $[M]^+$, 421, 409, 397, 365, 353. HREIMS *m*/*z*: 452.1877 $[M]^+$, calc. for $C_{26}H_{28}O_7$ 452.1835.

^{13}C NMR: see Table 1.

3. Results and discussion

A. nobilis root bark extracts furnished seven yellow colored compounds **1**–**7** (Fig. 1) Compounds **1**–**3** were identified as artobiloxanthone (**1**) [4,7], artonin E (**2**) [8] and cycloartobiloxanthone (**3**) [4] by comparison of their spectral data with the reported values. Compounds **4–7** were found to be new natural prenylated flavones. Moreover, accurate ^{13}C NMR data of **3** are reported in this paper.

Compound **4** was assigned the molecular formula $C_{26}H_{26}O_7$ {EIMS *m*/*z* 450; FABMS(−) *m*/*z* 449 $[M–H]^-$} which is different from that of **2** by CH_2 unit. The ^{1}H NMR spectrum of **4** resembled that of **2**, except for the presence of a methoxy signal at δ 3.72, suggesting that **4** is a mono-methylether of **2**. The ^{13}C NMR spectrum showed 26 signals including the signal assignable to the methoxy group at δ 56.1. The location of the methoxy group was settled as

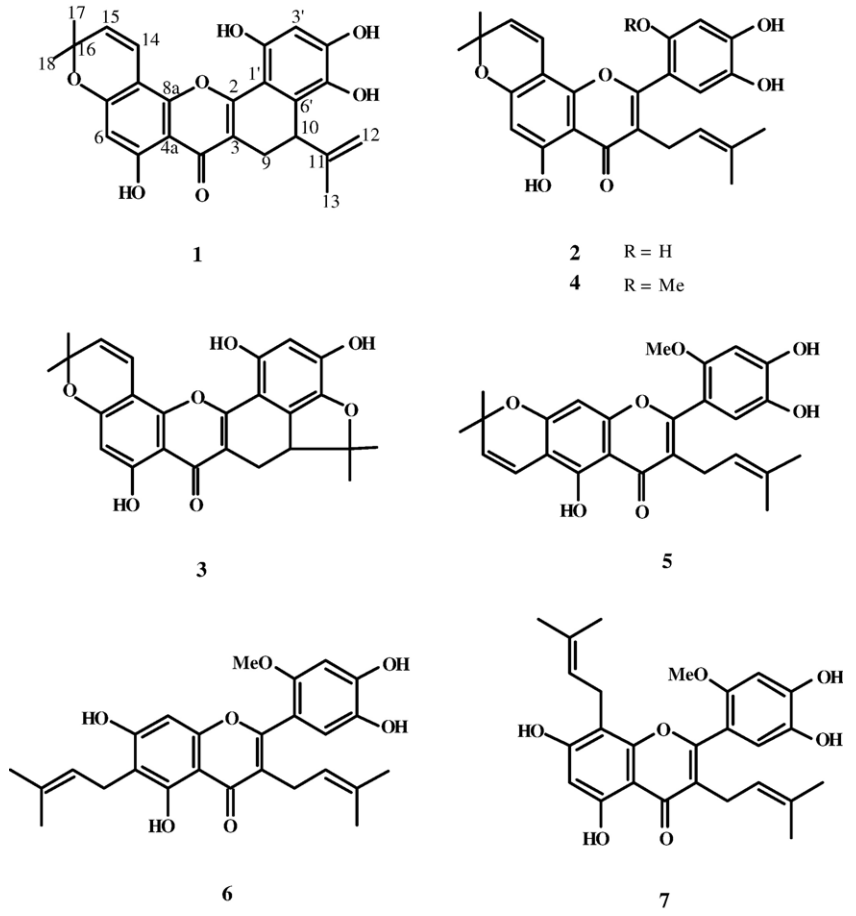

Fig. 1. Structures of compounds **1**–**7**.

follows. A characteristic chelated 5-OH signal was observed at δ 13.07 to which NOE correlation was observed from H-6 (δ 6.25), confirming that the chromene moiety exists through C-8 and C-7 on a flavone skeleton. Irradiation of the methoxy signal (δ 3.72) caused NOE enhancement toward an aromatic proton at δ 6.62 (H-3′) and CH_2 (δ 3.04) of the prenyl group, but not the other aromatic proton at δ 6.84 (H-6′). Thus, the methoxy group can be placed at either C-2′ or C-4′ position. The position of the methoxy group was deduced from analogy of compound **5** for which extensive 2D-NMR studies were performed. Hence, compound **4** was determined to be a new natural product, artonin E 2′-methylether.

The MS and NMR spectral data of compound **5** ($C_{26}H_{26}O_7$) suggested that **5** is an isomer of **4**. Comparison of the ^1H and ^{13}C NMR data of **5** and **4** showed the diagnostic difference for the signals for the chromene rings, whereas the data for 2′-methoxy-4′,5′-dihydroxybenzene ring were essentially identical. The present study and literature survey revealed that 6-alkylated flavones show the C-8 signal upper field (< 95ppm) while 8-alkylated flavones show the C-6 signal lower field (> 98ppm). The empirical rule suggested that compound **5** (C-6 of **4** at δ 99.7; C-8 of **5** at δ 94.8) is an isomer with a chromene ring through C-6 and C-7. This was unambiguously proved by the HMBC spectrum of **5**, which also gave direct evidence for the position of the methoxy group (Fig. 2). NOE correlation from OCH_3 (δ 3.70) to H-3′ (δ 6.61) and CH_2 (δ 3.02) of the prenyl group were observed. Hence, the structure of **5** was established as a new natural product. This compound is named isoartonin E 2′-methylether, although the parent isoartonin E has not yet been isolated as a natural compound. Instead, its higher homologue, 3-geranyl derivative (artoindonesianin) was reported previously[9]. The NMR data of **5** are in good agreement with those of artoindonesianin L except for the additional prenyl unit.

Compound **6** has two hydrogens more than **4** and **5**. Comparison of its spectra with **5** spectra revealed a second prenyl unit (δ 1.66 and 1.77 for two methyl singlets, δ 5.23 for an olefinic proton, δ 3.31 a CH_2) and the absence of chromene

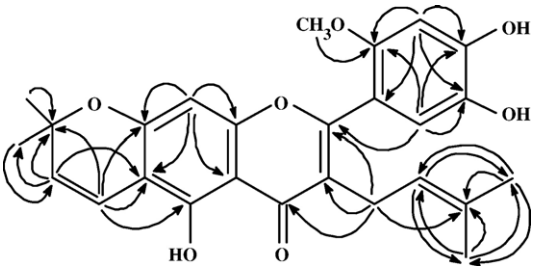

Fig. 2. HMBC correlations (H to C) of compound **5**.

ring. The HMBC spectrum of **6** exhibited clear correlations among the prenyl methylene protons H-14 to C-5 and C-7 and olefinic H to C-6, which supported the location of the prenyl unit at C-6. Compound **6** was named as dihydroisoartonin E 2′-methylether, the isolation of dihydroisoartonin E from *A. rigida* was described in a patent without any data [10].

The molecular formula of **7** was identical with that of **6**. Comparison of the ^{1}H and ^{13}C NMR spectra of **7** with **6** suggested that **7** is a C-8 prenylated structural isomer of **6**. Careful assignments of ^{13}C signals supported this formulation. Hence, **7** was established as the new natural compound 2′-methylether of artonin V. Artonin V was isolated from *A. altilis* [11].

Finally, antioxidant properties of **1**–**7** were evaluated against the DPPH radical by TLC bio-autography method [12]. *Artocarpus* species are known to be a rich source of prenylflavones and radical scavenging/antioxidant properties of prenylflavones are documented previously [13–15].

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