Bioactive Constituents of the Root Bark of *Artocarpus rigidus* subsp. *rigidus*

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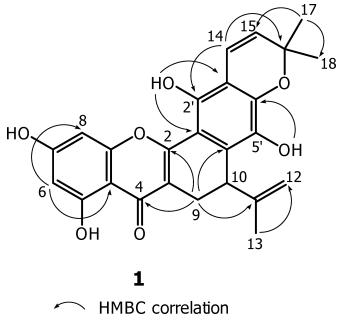
Investigation of the chemical constituents of the root bark of *Artocarpus rigidus* Blume subsp. *rigidus* has led to the isolation of six, structurally diverse phenolic compounds. These included two new compounds with modified skeletons, the flavonoid 7-demethylartonol E (1) and the chromone artorigidusin (2), together with four known phenolic compounds, the xanthone artonol B (3), the flavonoid artonin F (4), the flavonoid cycloartobiloxanthone (5), and the xanthone artoindonesianin C (6). Compounds 1, 4, and 5 exhibited antiplasmodial activity against *Plasmodium falciparum*. All compounds showed antimycobacterial activity against *Mycobacterium tuberculosis*, with 4 being the most active compound (MIC 6.25 μg/ml). Compounds 5 and 6 were active against KB cells, whereas 2, 5, and 6 showed varying toxicity to BC cells. Compounds 1—3, 5, and 6 were active in the NCI-H187 cytotoxicity assay, with 3 being the most active compound (IC_{50} 1.26 μg/ml).

Key words *Artocarpus rigidus* subsp. *rigidus*; 7-demethylartonol E; artorigidusin; antiplasmodial activity; antimycobacterial activity; cytotoxicity

The genus *Artocarpus* belongs to the Moraceae family, consisting of about 47 species distributed in south-eastern Asia and the Pacific, and some species are cultivated throughout the tropics.[1)] A number of *Artocarpus* species, including *A. rigidus* Blume subsp. *rigidus* are found wild in the southern part of Thailand. No phytochemical investigation of this plant species has been reported to date. It should be noted that this plant species is different from *A. rigida*, the latter of which has been extensively investigated.[2—8)] As part of an ongoing project on bioactive compounds from Thai plants for the treatment of tropical diseases, the plant species was investigated and it was found that the $CHCl_3$ and MeOH extracts exhibited antiplasmodial and antimycobacterial activities. Both crude extracts also showed cytotoxic activity against human epidermoid carcinoma of the nasopharynx (KB), human breast cancer (BC), and human small cell lung cancer (NCI-H187) cells. The present report deals with the isolation and antiplasmodial, antimycobacterial, and cytotoxic activities of the isolated new compounds **1** and **2**, and the known compounds **3**—**6**.

Investigation of the chemical constituents of the root bark of *A. rigidus* subsp. *rigidus* resulted in the isolation of six compounds. These included two new phenolics with modified skeletons, the flavonoid 7-demethylartonol E (**1**) and the chromone artorigidusin (**2**), together with four, known, structurally modified phenolics, the xanthone artonol B (**3**), the flavonoid artonin F (**4**), the flavonoid cycloartobiloxanthone (**5**), and the xanthone artoindonesianin C (**6**). Compounds **3** and **4** were identified as artonol B and artonin F isolated previously from *A. communis* on the basis of spectroscopic (^{1}H- and ^{13}C-NMR spectral) comparisons.[9,10)] On the same basis, compounds **5** and **6** were identified as cycloartobiloxanthone and artoindonesianin C isolated from *A. nobilis*[11)] and *A. teysmanii*[12)] respectively.

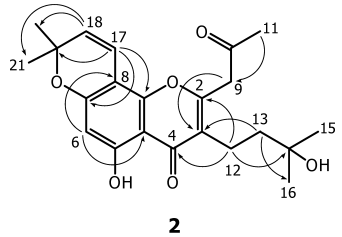
Compound **1** was obtained as a pale brownish yellow powder, mp 224—226 °C. The IR spectrum showed absorptions for the hydroxyl (3535, 3434 cm^{-1}) and conjugated keto (1662 cm^{-1}) groups. The HR-FAB-MS (positive ion mode) gave an $[M+H]^+$ ion at *m/z* 435.1446, corresponding to a molecular formula of $C_{25}H_{22}O_7$. The ^{1}H-NMR spectrum of **1** (Table 1) revealed two doublets (J=1.9 Hz) at δ 6.24 and 6.56 corresponding, respectively, to the characteristic H-6 and H-8 signals of a flavonoid. The 5,7-dihydroxyl substituted pattern was confirmed by HMBC correlations as shown



1

HMBC correlation

Fig. 1. Chemical Structure and Selected HMBC Correlations of Compound **1**



2

HMBC correlation

Fig. 2. Chemical Structure and Selected HMBC Correlations of Compound **2**

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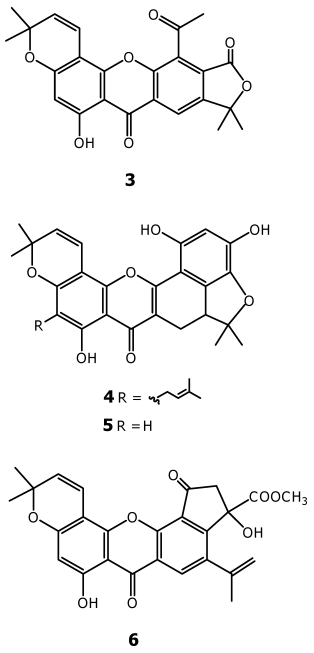

Fig. 3. Chemical Structures of Compounds **3**—**6**

in Fig. 1. Placement of the 5-hydroxyl group was confirmed by the presence of a chelated signal at δ 13.14 in the ^{1}H-NMR spectrum of **1**. The ^{1}H-NMR spectrum of **1** revealed an ABX system of signals as two double doublets at δ 2.44 (J=16.0, 6.6 Hz) and 3.35 (J=16.0, 2.0 Hz) and a broad doublet at δ 3.97 (J=6.6 Hz) assignable to two H-9 and the H-10 signals, respectively. The presence of an isopropenyl group was evident from the singlet signal at δ 1.75 and two broad singlet signals at δ 4.30 and 4.63. The point of attachment of this moiety is at the 10-position as indicated by the HMBC correlations (see Fig. 1).

The presence of the 2,2-dimethylpyran ring system was evident from two singlet signals at δ 1.44 (17-Me) and 1.46 (18-Me), and two olefinic resonances at δ 5.74 (H-15) and 6.75 (H-14). The orientation of this moiety in the molecule, as well as the placement of the phenolic hydroxyl groups at the 2′- and 5′-positions, were established by HMBC experiments (Fig. 1). Assignments of the ^{1}H- and ^{13}C-NMR spectral data of **1** were confirmed by COSY, DEPT, HMQC, and HMBC experiments. The ^{1}H- and ^{13}C-NMR data of **1** were similar to those of artonol E,[9] except for the absence of a methoxyl signal of the former at the 7-position. The structure of this flavonoid was thus concluded as 7-demethylartonol E (**1**). Compound **1** possesses one chiral center at the 10-position, with the $[\alpha]_D^{26}$ value in MeOH of $-7.6°$. However, the existing data did not permit the assignment of the configuration at this position.

Compound **2** was a pale brownish orange gum, the IR absorption bands of which exhibited the hydroxyl (3383 cm^{-1}), conjugated keto (1659 cm^{-1}), and nonconjugated keto (1727 cm^{-1}) functional groups. The HR-FAB-MS showed the $[M+H]^+$ peak at m/z 387.1802, corresponding to a molecular formula of $C_{22}H_{26}O_6$. The broad singlet signal at δ 12.75 in the ^{1}H-NMR spectrum of **2** suggested the presence of a chelated 5-hydroxyl group. The only aromatic resonance appeared as a singlet signal for H-6 at δ 6.22. The broad singlet signal of two methyl groups at δ 1.43 and two doublets

Table 1. ^{1}H- (300 MHz) and ^{13}C-NMR (100 MHz) Data for Compounds **1** (in Acetone-d_6) and **2** (in $CDCl_3$)[a]

Position	**1**		**2**	
	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)
2		161.0		157.9
3		112.1		122.2
4		180.9		181.8
4a		104.9		104.8
5	13.14 s	163.2	12.75 br s	161.9
6	6.24 d (1.9)	99.8	6.22 s	100.1
7	9.80 br s	164.5		159.5
8	6.56 d (1.9)	94.9		100.7
8a		157.5		152.0
9	2.44 dd (16.0, 6.6)	22.3	3.81 s	46.7
	3.35 dd (16.0, 2.0)			
10	3.97 br d (6.6)	37.7		201.0
11		145.4	2.30 s	30.0
12	4.30 br s	111.7	2.52 m	19.1
	4.63 br s			
13	1.75 s	21.8	1.60 m	42.2
14	6.75 d (10.0)	117.2		70.5
15	5.74 d (10.0)	129.7	1.26 s	29.3
16		78.3	1.26 s	29.3
17	1.44 s	28.0	6.55 d (10.0)	114.6
18	1.46 s	28.0	5.51 d (10.0)	127.2
19				78.0
20			1.43 s	28.2
21			1.43 s	28.2
1′		107.1		
2′	8.02 br s	145.3		
3′		110.4		
4′		145.4		
5′	7.59 s	137.3		
6′		128.6		

Assignments were confirmed by COSY, HMQC, HMBC and DEPT experiments. *a*) δ in ppm, value in parentheses is coupling constant in Hz.

(J=10.0 Hz) of olefinic resonances at δ 5.51 and 6.55 revealed the presence of a 2,2-dimethylpyran ring. HMBC experiments (see Fig. 2) showed long-range correlations consistent with the partial structure of this part of the molecule.

The presence of the 1,1-dimethylpropanol moiety at the 3-position was evident from two sets of two-proton multiplets at δ 2.52 (H-12) and 1.60 (H-13), and a singlet of two methyl groups (15-Me and 16-Me) at δ 1.26. The two singlet signals at δ 3.81 (H-9) and 2.30 (H-11) were attributable to the $-CH_2COCH_3$ substituent at the 2-position. The presence of the saturated keto group at C-10 was confirmed by the ^{13}C-NMR resonance at δ 201.0. HMBC experiments (see Fig. 2) of these two substituents were in agreement with the structure. The structure of this chromone was thus concluded to be **2** and named artorigidusin.

Compounds **1**, **4** and **5** exhibited antiplasmodial activity against *Plasmodium falciparum*, whereas compounds **2**, **3**, and **6** were inactive (see Table 2). All compounds showed antimycobacterial activity against *Mycobacterium tuberculosis*, with **4** being the most active compound (MIC 6.25 μg/ml). For cytotoxic activity, compounds **5** and **6** were active against the KB cells, whereas **2**, **5**, and **6** showed varying toxicity to the BC cells. Compounds **1**—**3**, **5**, and **6** were active against the NCI-H187 cytotoxicity assay, with artonol B (**3**) being the most active compound (IC_{50} 1.26 μg/ml).

Table 2. Antiplasmodial, Antimycobacterial and Cytotoxic Activities of Compounds **1**—**6**

Compound	Antiplasmodial (IC_{50}, μg/ml)	Antimycobacterial (MIC, μg/ml)	Cytotoxicity (IC_{50}, μg/ml)		
			KB	BC	NCI-H187
1	7.9	50	Inactive[b]	Inactive[b]	5.7
2	Inactive[a]	12.5	Inactive[b]	12.10	15.63
3	Inactive[a]	100	Inactive[b]	Inactive[b]	1.26
4	2.4	6.25	Inactive[b]	Inactive[b]	Inactive[b]
5	3.7	25	8.56	4.23	11.83
6	Inactive[a]	12.5	8.4	7.7	7.1

a) Inactive at 10 μg/ml. *b*) Inactive at 20 μg/ml.

Experimental

General Procedures Melting points were determined with an Electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Spectrum BX spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AVANCE 400 FT-NMR spectrometer, operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). For spectra taken in acetone-d_6 and $CDCl_3$, the residual nondeuterated solvent signals at δ 2.04 and 7.24, and the solvent signals at δ 29.80 and 77.00, were used as references for ^{1}H- and ^{13}C-NMR spectra, respectively. FAB-MS and ES-MS spectra were measured with a Finnigan MAT 90 and a Finnigan LC-Q mass spectrometers. Unless indicated otherwise, column chromatography and TLC were carried out using Merck silica gel 60 (finer than 0.063 mm) and precoated silica gel 60 F_{254} plates, respectively. Spots on TLC were visualized under UV light and by spraying with anisaldehyde–H_2SO_4 reagent, followed by heating.

Plant Material The root bark of *A. rigidus* subsp. *rigidus* was collected from Jana district, Songkhla Province in 2001. A voucher specimen is deposited at the Faculty of Science, Chiang Mai University, Thailand (Wisit Arjchomphu No. 011) and was identified by James F. Maxwell.

Extraction and Isolation The dried root bark (1.5 kg) was milled and extracted successively with *n*-hexane, $CHCl_3$ and MeOH in a Soxhlet extraction apparatus. The extracts were evaporated to dryness under reduced pressure at about 40 °C. The hexane extract (brownish syrup, 8.4 g), the $CHCl_3$ extract (dark brownish sticky solid, 16.1 g) and the methanolic extract (dark brownish mass, 45.6 g) were obtained, respectively.

The $CHCl_3$ extract (14.9 g) was fractionated by quick column chromatography[13] (Merck silica gel 60 PF_{254}, 250 g), eluting with *n*-hexane–$CHCl_3$, $CHCl_3$, and $CHCl_3$–MeOH with increasing amounts of the more polar solvent. The eluates were examined by TLC and 12 combined fractions (C1—C12) were obtained. Fraction C10 was rechromatographed over silica gel (0.063—0.200 mm, 125 g) with *n*-hexane–CH_2Cl_2, CH_2Cl_2, and CH_2Cl_2–MeOH as eluting solvent to give 11 subfractions. Subfraction 3 was chromatograped using CH_2Cl_2 and CH_2Cl_2–MeOH as eluents, with an increasing amount of the more polar solvent, followed by column chromatography eluted under isocratic conditions (0.9% MeOH in CH_2Cl_2) to yield compound **1** (7 mg).

Fractions C11 and C12 were combined and chromatographed over silica gel (0.063—0.200 mm, 150 g) using *n*-hexane–$CHCl_3$, $CHCl_3$ and $CHCl_3$–MeOH as eluents, with an increasing amount of the more polar solvent to give 15 subfractions. Subfraction 6 was subjected to repeated column chromatography (3×) with similar eluting solvent systems to give artonol B (**3**) as pale orange needles (14 mg), mp 267—270 °C.

Subfraction 5 was rechromatographed twice using CH_2Cl_2 and CH_2Cl_2–MeOH as eluents, with an increasing amount of the more polar solvent to afford three selected subgroups. Subgroup 1 was similarly chromatographed, followed by column chromatography eluted under isocratic conditions (0.9% MeOH in $CHCl_3$) to yield artonin F (**4**) (2 mg) as yellow needles, mp 251—253 °C. Subgroup 2 was similarly chromatographed twice to afford dark yellow needles (15 mg), mp 287—289 °C, which was identified as cycloartobiloxanthone (**5**). The selected subgroup 3 was rechromatographed under isocratic conditions (0.9% MeOH in CH_2Cl_2), followed by another column chromatography using isocratic elution (0.8% MeOH in CH_2Cl_2) to give compound **2** (7 mg).

The MeOH extract (40.3 g) was fractionated by quick column chromatography (Merck silica gel 60 PF_{254}, 250 g) eluting with CH_2Cl_2, CH_2Cl_2–EtOAc, EtOAc, EtOAc–MeOH, and MeOH with an increasing amount of the more polar solvent. The eluates were examined by TLC and 17 groups of eluting fractions (fractions M1—M17) were obtained. Fraction M7 was rechromatographed twice eluting with CH_2Cl_2 and CH_2Cl_2–MeOH with an increasing amount of the more polar solvent to yield a pale yellow solid of artoindonesianin C (**6**) (2 mg).

7-Demethylartonol E (**1**): Pale brownish yellow powder, mp 224—226 °C, $[\alpha]_D^{26}$ −7.6° (c=0.29, MeOH). IR (KBr) cm^{-1}: 3535, 3434, 3159, 2964, 2913, 2855, 1662, 1615, 1560, 1515, 1451, 1401, 1370, 1262, 1183, 1095, 1028, 863, 802, ^{1}H- and ^{13}C-NMR data: see Table 1. ES-MS *m*/*z* (rel. int.): 435 $[M+H]^+$ (100), HR-FAB-MS (positive ion mode) *m*/*z*: 435.1446 $[M+H]^+$ (Calcd for $C_{25}H_{22}O_7$+H: 435.1444).

Artorigidusin (**2**): Pale brownish orange gum, IR (KBr) cm^{-1}: 3383, 2968, 2924, 2852, 1727, 1659, 1578, 1486, 1435, 1350, 1267, 1176, 1159, 1113, 1085, 822, ^{1}H- and ^{13}C-NMR data: see Table 1. ES-MS *m*/*z* (rel. int.): 409 $[M+Na]^+$ (100), HR-FAB-MS (positive ion mode) *m*/*z*: 387.1802 $[M+H]^+$ (Calcd for $C_{22}H_{26}O_6$+H: 387.1808).

Antiplasmodial Assay Antiplasmodial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain) that was cultured continuously according to the method of Trager and Jensen.[14] Quantitative assessment of antiplasmodial activity *in vitro* was determined by means of the microculture radioisotope technique based upon the method described by Desjardins *et al.*[15] The inhibitory concentration that caused a 50% reduction in parasite growth as indicated by *in vitro* uptake of [^{3}H]-hypoxanthine by *P. falciparum* was determined. An IC_{50} value of 1 ng/ml was observed for the standard drug, artemisinin, in the same test system. The assay results are shown in Table 2.

Antimycobacterial Assay Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H_{37}Ra using the Microplate Alamar Blue Assay.[16] In our system, the standard drugs, rifampicin, isoniazid, and kanamycin sulfate showed MIC values of 0.004, 0.06, and 2.5 μg/ml, respectively. The assay results are presented in Table 2.

Cytotoxicity Assays The cytotoxicity assays against human epidermoid carcinoma of the nasopharynx (KB), human breast cancer (BC) and human small cell lung cancer (NCI-H187) cells were performed employing colorimetric method[17] and are shown in Table 2. The standard drug ellipticine exhibited IC_{50} values against these cell lines at 1.33, 1.46 and 0.39 μg/ml, respectively.

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