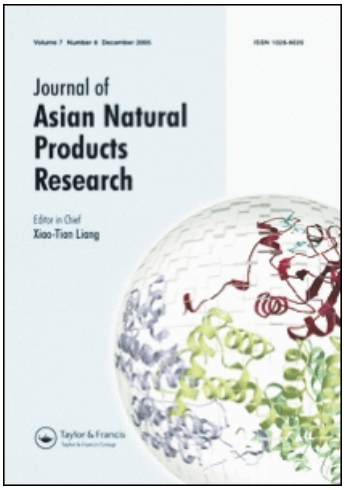

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ORIGINAL ARTICLE

New isoprenylated flavonoids and cytotoxic constituents from *Artocarpus tonkinensis*

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Two new isoprenylated flavonoids, artotonins A and B (**1** and **2**), along with 13 known compounds (**3**–**15**), were isolated from the roots of *Artocarpus tonkinensis* A. Chev. ex Gagnep. The structures were elucidated by spectroscopic methods. Cyclocommunol (**6**), isocyclomulberrin (**7**), cudraflavone C (**11**), and morusin (**13**) exhibited cytotoxicity against hepatocellular carcinoma (SMMC-7721) and gastric carcinoma (BGC-823 and SGC-7901) cell lines.

Keywords: *Artocarpus tonkinensis*; isoprenylated flavonoids; artotonins A and B; cytotoxicity

1. Introduction

Artocarpus species are rich in isoprenylated phenolic compounds, including flavonoids, 2-arylbenzofurans, and stilbenoids, some compounds showing antimycobacterial, antimalarial, and anti-inflammatory effects as well as cyclooxygenase- and tyrosinase-inhibitory activities [1–5]. Recently, our group reported a variety of cytotoxic prenylated flavonoids and 2-arylbenzofurans from *Artocarpus chama* and *Artocarpus petelotii* [6–8]. In our program searching for bioactive compounds from *Artocarpus* plants, the roots of *Artocarpus tonkinensis* A. Chev. ex Gagnep were collected in the Yunnan Province, China. The leaves and bark of *A. tonkinensis* are used as traditional medicine in Vietnam to treat backache and rheumatism. A few flavonoid glycosides with anti-inflammatory effects and benzofurans were isolated from this plant previously [9–11].

Our preliminary bioassay showed that the ethanol extract from the roots of *A. tonkinensis* exhibited cytotoxicity against hepatocellular carcinoma (SMMC-7721) and gastric carcinoma (BGC-823 and SGC-7901) cell lines. Further separation of this extract afforded two new isoprenylated flavonoids, artotonins A and B (**1** and **2**), along with 13 known compounds, i.e. artoheterophyllin D (**3**) [12], artoindonesianin X (**4**) [13], artonin A (**5**) [14], cyclocommunol (**6**) [15], isocyclomulberrin (**7**) [16], norartocarpetin (**8**) [17], kaempferol (**9**) [18], albanin A (**10**) [19], cudraflavone C (**11**) [19], 3″,3″-dimethylpyrano[3′,4′]2,4,2′-trihydroxychalcone (**12**) [3], morusin (**13**) [15], (+)-catechin (**14**) [20], and (+)-afzelechin-3-*O*-α-L-rhamnopyranoside (**15**) [21] (Figure 1). Compounds **4**–**7**, **9**, and **11**–**15** were screened for cytotoxicity against SMMC-7721, BGC-823, and

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Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2** (in $(CD_3)_2CO$, δ in ppm, J in Hz).

	1[a]		**2**[b]	
Position	δ_H	δ_C	δ_H	δ_C
2	5.76 (dd, $J = 2.6, 13.3$)	76.3		162.2
3	3.05 (dd, $J = 13.3, 16.7$), 2.74 (dd, $J = 2.6, 16.7$)	43.6		122.8
4		191.0		183.2
5	7.67 (d, $J = 8.6$)	128.3		163.4
6	6.49 (d, $J = 8.6$)	111.3	6.23 (br s)	99.1
7		159.9		164.6
8		110.3	6.31 (br s)	94.1
9		159.0		159.2
10		115.8		105.1
1′		117.8		113.0
2′		156.3		157.1
3′	6.49 (d, $J = 2.3$)	103.6	6.55 (br s)	103.9
4′		159.5		161.3
5′	6.45 (dd, $J = 2.3, 8.4$)	108.0	6.51 (br d, $J = 8.0$)	108.0
6′	7.36 (d, $J = 8.4$)	128.8	7.22 (d, $J = 8.0$)	132.1
11	6.63 (d, $J = 10.0$)	116.5	2.46 (t, $J = 8.4$)	21.1
12	5.72 (d, $J = 10.0$)	129.9	1.61 (t, $J = 8.4$)	43.0
13		78.1		70.1
14	1.43 (s)	28.2	1.06 (s)	29.3
15	1.45 (s)	28.5	1.06 (s)	29.3
5-OH			13.18 (s)	
7-OH			9.55 (br s)[c]	
2′-OH	8.60 (br s)[c]		8.75 (br s)[c]	
4′-OH	8.35 (br s)[c]		8.75 (br s)[c]	
13-OH			3.17 (s)	

Note: [a] 500 MHz.
[b] 400 MHz.
[c] Signals may be exchangeable.

SGC-7901 cell lines. In this paper, we have described the structural elucidation of the new compounds (**1** and **2**) and cytotoxicity evaluation.

2. Results and discussion

Artotonin A (**1**), an optically active compound ($[\alpha]_D^{20} = -19.2$), was isolated as a yellow amorphous powder. Its molecular formula was deduced as $C_{20}H_{18}O_5$ by HR-EI-MS at *m/z* 338.1160 $[M]^+$. The IR spectrum showed absorption bands for hydroxyl (3342 cm^{-1}), carbonyl (1660 cm^{-1}), and aromatic ring (1594, 1517, and 1462 cm^{-1}) moieties. The ^{1}H NMR spectrum exhibited signals of two hydroxyl groups at δ_H 8.60 and 8.35 (each 1H, br s), two *ortho*-coupled protons at δ_H 7.67 and 6.49 (each 1H, d, $J = 8.6$ Hz), an ABX spin aromatic system at δ_H 7.36 (1H, d, $J = 8.4$ Hz), 6.49 (1H, d, $J = 2.3$ Hz), and 6.45 (1H, dd, $J = 2.3, 8.4$ Hz), and a 2,2-dimethylpyran ring at δ_H 6.63, 5.72 (each 1H, d, $J = 10.0$ Hz) and 1.45, 1.43 (each 3H, s) (Table 1). Furthermore, signals of another ABX spin system at δ_H 5.76 (1H, dd, $J = 2.6, 13.3$ Hz, H-2), 3.05 (1H, dd, $J = 13.3, 16.7$ Hz, H-3), and 2.74 (1H, dd, $J = 2.6, 16.7$ Hz, H-3) were characteristic of ring C on a flavanone skeleton. The ^{13}C NMR and HMQC spectra showed 20 carbon signals attributable to one carbonyl group, seven quaternary sp^2, one quaternary sp^3, seven methine sp^2, one methine sp^3, one methylene sp^3,

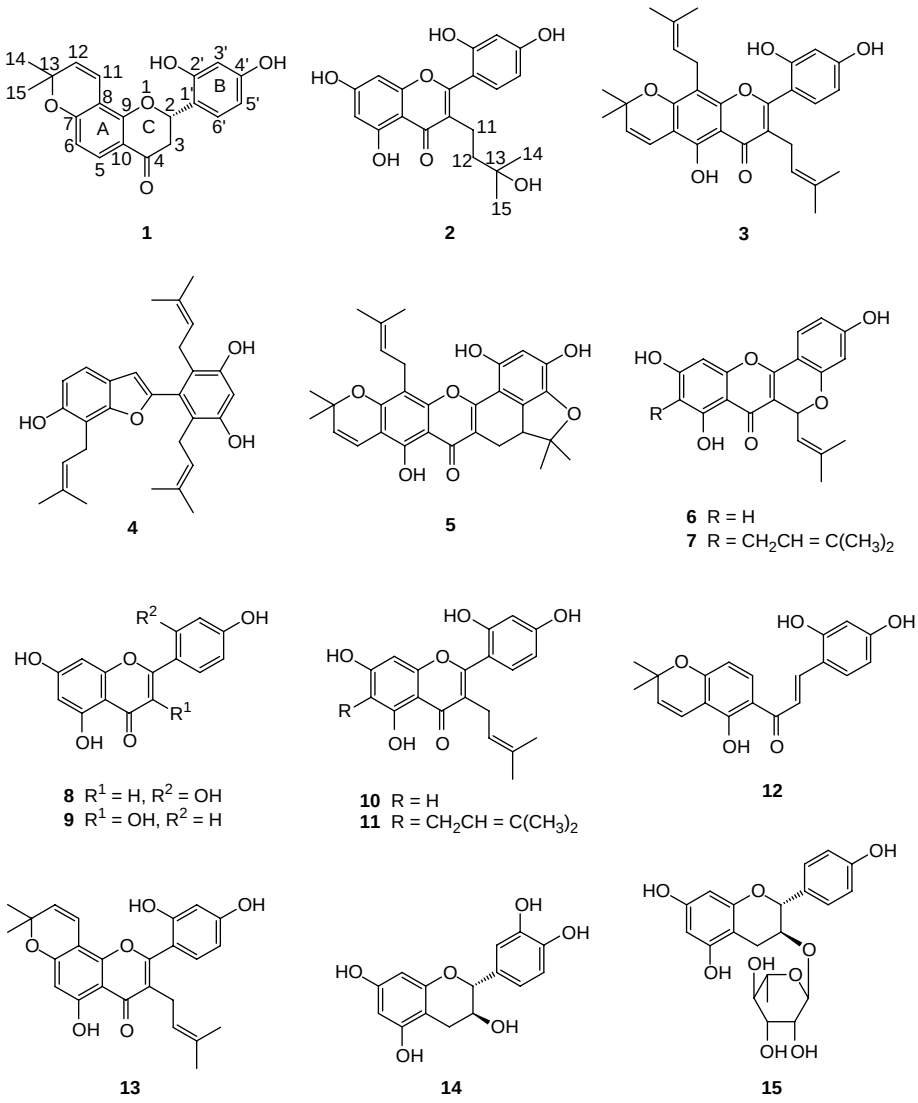

Figure 1. Structures of compounds **1**–**15**.

and two methyl carbon atoms. These NMR spectroscopic data suggest that **1** is a monoprenylated and dihydroxylated flavanone. The two *ortho*-coupled protons at δ_H 7.67 and 6.49 were assigned to H-5 and H-6, respectively, as established by the HMBC correlations from H-5 (δ_H 7.67) to C-4 (δ_C 191.0), C-7 (δ_C 159.9), and C-9 (δ_C 159.0), and from H-6 (δ_H 6.49) to C-8 (δ_C 110.3) and C-10 (δ_C 115.8) (Figure 2). The HMBC correlations from H-11 (δ_H 6.63) to C-7 and from H-12 (δ_H 5.72) to C-8 indicated that the 2,2-dimethylpyran ring was fused to C-7 and C-8. The 2′,4′-dihydroxy substitution in ring B was deduced by the HMBC correlations from H-2 (δ_H 5.76) to C-1′ (δ_C 117.8) and C-6′ (δ_C 128.8), from H-5′ (δ_H 6.45) to C-1′ and C-3′ (δ_C 103.6), and from H-6′ (δ_H 7.36) to C-2′ (δ_C 156.3) and C-4′ (δ_C 159.5). The configuration at C-2 was proposed as *S* according to the specific optical rotation of

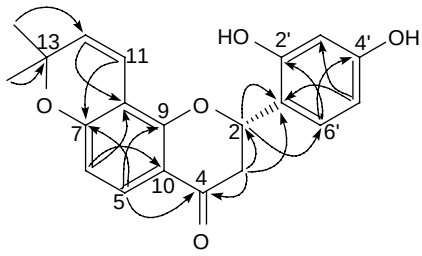

Figure 2. Selected HMBC (H → C) correlations of compound **1**.

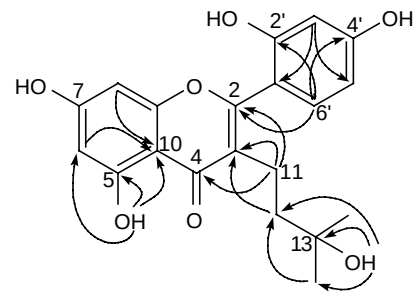

Figure 3. Selected HMBC (H → C) correlations of compound **2**.

1 ($[\alpha]_D^{20} = -19.2$) and the *trans*-diaxial coupling constant of H-2 and H-3 ($J = 13.3$ Hz) [22]. Thus, the structure of artotonin A was elucidated as (2*S*)-2′,4′-dihydroxy-2″,2″-dimethylpyrano-(7,8:6″,5″)flavanone (Figure 1).

Artotonin B (**2**), a yellow amorphous powder, was assigned a molecular formula of $C_{20}H_{20}O_7$ by HR-EI-MS at *m/z* 372.1215 $[M]^+$. The ^{1}H NMR spectrum contained signals of a hydrogen-bonded hydroxyl group at δ_H 13.18 (1H, s), three phenolic hydroxyl groups at δ_H 9.55 (1H, br s) and 8.75 (2H, br s), an ABX spin system at δ_H 7.22 (1H, d, $J = 8.0$ Hz), 6.55 (1H, br s), and 6.51 (1H, br d, $J = 8.0$ Hz), and two broad singlets at δ_H 6.31 and 6.23 (each 1H, br s), which were derived from a 5,7,2′,4′-tetrahydroxylated flavone. Moreover, a 3-hydroxy-3-methylbutyl group was inferred from the following ^{1}H and ^{13}C NMR spectral data at δ_H 3.17 (13-OH, s), 2.46 (2H, t, $J = 8.4$ Hz, H_2-11), 1.61 (2H, t, $J = 8.4$ Hz, H_2-12), and 1.06 (6H, s, H_3-14, 15), as well as δ_C 21.1 (C-11), 43.0 (C-12), 70.1 (C-13), and 29.3 (C-14, 15). This isoprenoid group was attached at C-3 according to the HMBC correlations from CH_2-11 to C-2 (δ_C 162.2), C-3 (δ_C 122.8), and C-4 (δ_C 183.2), and from CH_2-12 to C-3 (Figure 3). The substitution of rings A and B was further supported by the HMBC correlations shown in Figure 3. Thus, the structure of artotonin B was elucidated as 5,7,2′,4′-tetrahydroxy-3-(3-hydroxy-3-methylbutyl)flavone (Figure 1).

Compounds **4**–**7**, **9**, and **11**–**15** were evaluated for *in vitro* cytotoxicity against SMMC-7721, SGC-7901, and BGC-823 cell lines (Table 2). In this test, *cis*-platinum was used as a positive control. The results showed that isoprenylated flavones (**6**, **7**, **11**, and **13**), except **5**,

Table 2. IC_{50} (± SD) values (μM) of compounds against human carcinoma cells.

Compound	SMMC-7721	BGC-823	SGC-7901
4	NA	NA	NA
5	NA	NA	NA
6	16.1 ± 1.5	27.1 ± 2.8	79.7 ± 8.5
7	19.5 ± 1.4	33.1 ± 4.1	14.2 ± 2.3
9	NA	NA	NA
11	11.8 ± 0.9	24.9 ± 1.7	35.5 ± 3.7
12	NA	NA	NA
13	20.6 ± 2.4	12.6 ± 1.1	75.8 ± 4.4
14	NA	NA	NA
15	NA	NA	NA
cis-platinum	6.3 ± 0.6	7.3 ± 0.9	14.1 ± 1.3

Note: NA, not active.

exhibited inhibitory effects on three kinds of carcinoma cells. On the other hand, isoprenylated 2-arylbenzofuran (**4**) and chalcone (**12**), as well as nonprenylated flavone (**9**), flavanol, and its glycoside (**14** and **15**), were inactive.

3. Experimental

3.1 General experimental procedures

The UV spectra were determined on a Shimadzu UV-2401PC spectrophotometer, and IR spectra were measured on a Nicolet Avatar-360 spectrometer. Optical rotation was determined on a Jasco P1030 polarimeter. The 1D and 2D NMR spectra were recorded on Bruker DRX-500 and Varian Mercury Plus 400 instruments, using residual solvent peaks of $(CD_3)_2CO$ (δ_H 2.04, δ_C 206.0) or Me_4Si as an internal standard. Chemical shifts (δ) were reported in ppm and coupling constants (J) in Hz. EI-MS and HR-EI-MS measurements were carried out on a Finnigan MAT-95 mass spectrometer. Column chromatography (CC) was performed on silica gel H (10–40 μm and 200–300 mesh; Institute of Chemical Technology, Yantai, China), Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan), MCI gel CHP-20P (75–150 μm, Mitsubishi Chemical Co.), Chromatorex RP-18 gel (20–45 μm; Fuji Silysia Chemical Ltd, Kasugai, Japan), Toyopearl HW-40C gel (Tosoh Co., Tokyo, Japan), and Sephadex LH-20 gel (GE Healthcare Amersham Biosciences, Uppsala, Sweden). TLC analysis was run on precoated silica gel GF_{254} plates (10–40 μm; Institute of Chemical Technology, Yantai, China). Preparative HPLC was performed on Agilent 1200 (Agilent Technologies, Santa Clara, CA, USA) and YMC C_{18} column (10× 250 mm, 5 μm; YMC Co., Kyoto, Japan).

3.2 Plant material

The roots of *A. tonkinensis* A. Chev. ex Gagnep were collected in Xishuangbanna, Yunnan Province, China, in July 1998, and air-dried. The plant was identified by Prof. Han-Dong Sun, Kunming Institute of Botany, and a voucher specimen (TCM 98-07-03 Hou) has been deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Fudan University, China.

3.3 Extraction and isolation

The dried and powdered roots (6.35 kg) of *A. tonkinensis* were percolated with 95% EtOH (90 liters) at room temperature. The filtrate was evaporated *in vacuo* to give a residue (800 g), which was suspended in H_2O and extracted successively with petroleum ether and EtOAc. The petroleum ether extract (73 g) was subjected to CC on silica gel eluted with a gradient of petroleum ether–EtOAc (4:1, 7:3, 3:2, 1:1, 2:3) to yield fractions 1–12. Fraction 5 (3.19 g) was isolated by CC on a silica gel eluted with a gradient of $CHCl_3$–*i*-PrOH (150:1, 100:1, 60:1, 30:1) to provide fractions 5.1–5.8. Fraction 5.2 (580 mg) was purified by CC on silica gel ($CHCl_3$–EtOAc 25:1), followed by CC on RP-18 (MeOH–H_2O 7:3) to afford **4** (40 mg). Fraction 5.3 (274 mg) was separated by CC on MCI gel CHP-20P (MeOH–H_2O 3:2, 4:1, 9:1) to afford **5** (2 mg). Fraction 5.5 (491 mg) was fractionated by CC on silica gel (petroleum ether–Me_2CO 5:1), then by preparative HPLC (flow rate 1.0 ml/min, UV detector 254 nm) using 90% MeOH to yield **7** (5 mg, t_R 40 min) and **11** (8 mg, t_R 23 min). Fraction 5.8 (305 mg) was isolated by CC on silica gel (petroleum ether–Me_2CO 5:1), followed by CC on RP-18 (MeOH–H_2O 3:1) to yield **13** (24 mg) and **6** (12 mg). Fraction 6 (218 mg) was subjected to CC on silica gel (petroleum ether–EtOAc 3:1) to afford **3** (2 mg). Fraction 7 (512 mg) was purified by CC on silica gel (petroleum ether–Me_2CO 3:1), followed by preparative HPLC (flow rate 1.0 ml/min, UV detector 254 nm) using 90% MeOH to give **1** (2 mg,

t_R 20 min) and **12** (7 mg, t_R 23 min). Fraction 9 (296 mg) was isolated by CC on silica gel ($CHCl_3$–*i*-PrOH 20:1) to give **10** (1 mg).

The EtOAc extract (260 g) was separated by CC on Diaion HP-20 (MeOH–H_2O 1:4, 2:3, 3:2, 4:1) to yield fractions 13–17. Fraction 13 (8 g) was subjected to CC on silica gel ($CHCl_3$–MeOH–H_2O 90:20:1) to give fractions 13.1–13.14. Fraction 13.8 (1.5 g) was purified by CC on Toyopearl HW-40C (MeOH–H_2O 1:9, 1:4, 3:7, 2:3) to yield **14** (10 mg) and **15** (35 mg). Fraction 16 (13 g) was subjected to CC on silica gel ($CHCl_3$–MeOH–H_2O 85:15:1) to yield fractions 16.1–16.7. Fraction 16.2 (500 mg) was separated by CC on silica gel (petroleum ether–Me_2CO 3:1) to provide **9** (8 mg). Fraction 16.3 (320 mg) was purified by CC on silica gel (petroleum ether–Me_2CO 2:1), followed by CC on Sephadex LH-20 ($CHCl_3$–MeOH 2:1) to afford **2** (4 mg) and **8** (7 mg).

3.3.1 *Artotonin A (**1**)*

A yellow amorphous powder; $[\alpha]_D^{20} = -19.2$ ($c = 0.04$, MeOH); UV (MeOH) λ_{max} (log ε): 205 (3.96), 265 (4.25), 310 (3.77) nm; IR (KBr) ν_{max}: 3342, 2924, 1660, 1594, 1517, 1462, 1441, 1377, 1278, 1112 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; EI-MS *m/z* (%): 338 (7, $[M]^+$), 320 (19), 305 (53), 242 (100), 213 (15), 187 (44); HR-EI-MS *m/z*: 338.1160 $[M]^+$ (calcd for $C_{20}H_{18}O_5$, 338.1154).

3.3.2 *Artotonin B (**2**)*

A yellow amorphous powder; UV (MeOH) λ_{max} (log ε): 211 (4.19), 259 (4.34), 297 (3.99) nm; IR (film) ν_{max}: 3452, 2970, 2931, 1655, 1616, 1507, 1457, 1359, 1162 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; EI-MS *m/z* (%): 372 (4, $[M]^+$), 354 (51), 311 (100), 299 (24), 207 (15); HR-EI-MS *m/z*: 372.1215 $[M]^+$ (calcd for $C_{20}H_{20}O_7$, 372.1209).

3.4 *Cytotoxicity assay*

Compounds were tested for cytotoxicity against hepatocellular carcinoma (SMMC-7721) and gastric carcinoma (SGC-7901 and BGC-823) cell lines by the MTT assay according to a reported procedure [23].

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