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Geranyl flavonoids from the leaves of *Artocarpus altilis*

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Abstract

Five geranyl dihydrochalcones, 1-(2,4-dihydroxyphenyl)-3-{4-hydroxy-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6H-dibenzo[*b*,*d*]pyran-5-yl}-1-propanone (**2**), 1-(2,4-dihydroxyphenyl)-3-[3,4-dihydro-3,8-dihydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone (**4**), 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(3,4-epoxy-4-methyl-1-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone (**5**), 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-hydroxy-4-methyl-2-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone (**8**), and 2-[6-hydroxy-3,7-dimethylocta-2(*E*),7-dienyl]-2′,3,4,4′-tetrahydroxydihydrochalcone (**9**), along with four known geranyl flavonoids (**1**, **3**, **6**, **7**), were isolated from the leaves of *Artocarpus altilis*. Their structures were established by spectroscopic means and by comparison with the literature values. Compounds **2**, **4**, and **9** exhibited moderate cytotoxicity against SPC-A-1, SW-480, and SMMC-7721 human cancer cells.

Keywords: *Artocarpus altilis*; Moraceae; Geranyl dihydrochalcones; Cytotoxicity

1. Introduction

The genus *Artocarpus* Moraceae, an exceptionally rich source of prenylated flavonoids, consists of approximately 50 species that are indigenous to South East Asia. *A. altilis* (Parkinson) Fosberg is native to Indonesia, and its leaves have been used traditionally there for treatment of liver cirrhosis, hypertension, and diabetes. Flavonoids from this and other species of *Artocarpus* have also been shown to have anti-inflammatory (Lu et al., 2002; Wei et al., 2005), antioxidative (Toshio et al., 2003), antiplatelet aggregation (Lin et al., 1996) and cytotoxicity (Tati et al., 2001) activities, as well as being able to inhibit cathepsin K (Patil et al., 2002) and 5α-reductase (Shimizu et al., 2000).

Regarding its chemical constituents, various triterpenes and flavonoids have previously been reported from *A. altilis* (Altman and Zito, 1976; Patil et al., 2002; Chan et al, 2003; Han et al., 2006; Wang et al., 2006; Weng et al., 2006). Within the scope of our continuous search for bioactive compounds from natural plants, the leaves of *A. altilis* were further investigated. In this paper, we report the isolation and structural elucidation of five new geranyl dihydrochalcones (**2**, **4**, **5**, **8**, **9**), along with four known geranyl flavonoids (**1**, **3**, **6**, **7**) (Fig. 1), and the presumed biosynthesis pathway to compounds **1**–**9**. In addition, the new compounds were evaluated for their *in vitro* cytotoxicity against a small panel of human cancer cell lines.

2. Results and discussion

The ethyl acetate soluble fraction of the methanol extract of the leaves of *A. altilis* was subjected to repeated silica gel column chromatography to yield compounds **1**–**9**. Compounds **1**, **3**, **6**, and **7** were identified as 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone (**1**), 2-geranyl-2′,3,4,4′-tetrahydroxydihydrochalcone (**3**) (McLean et al.,

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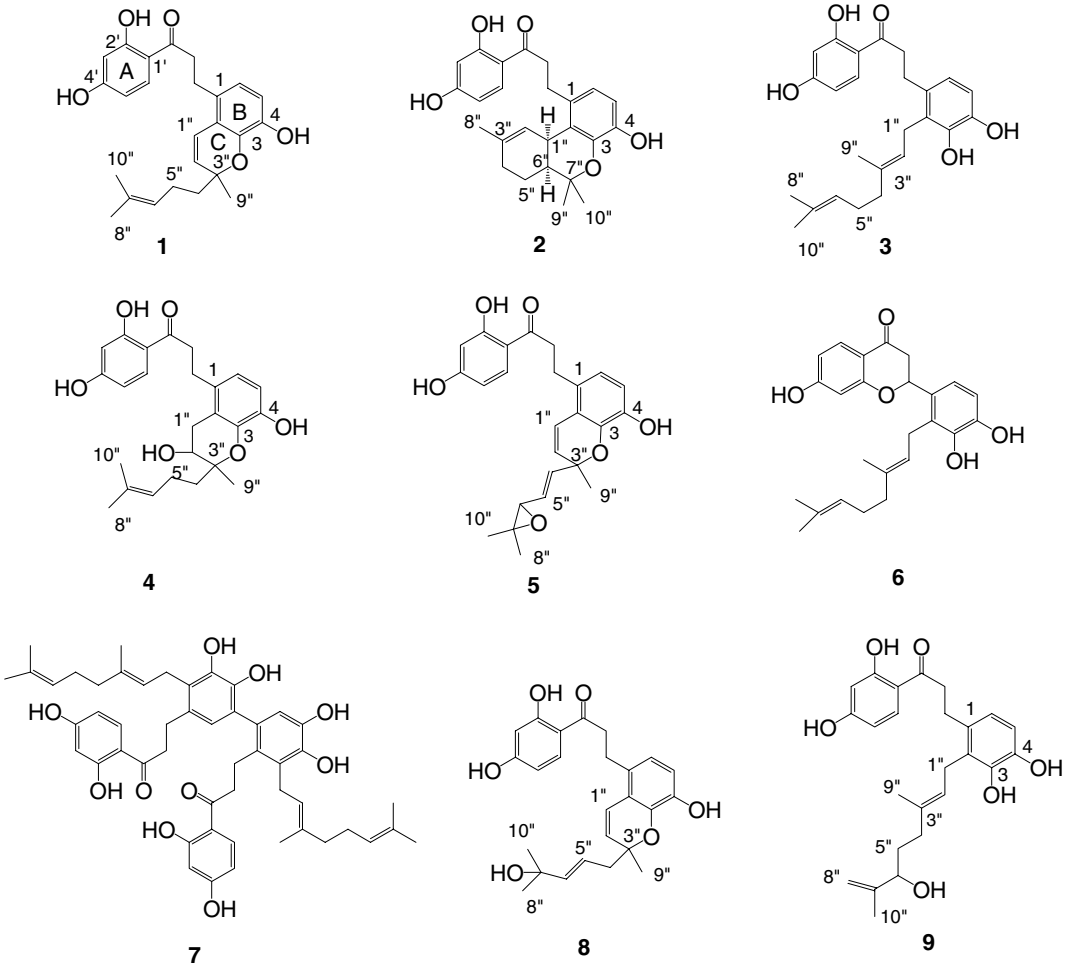

Fig. 1. Structures of compounds **1**–**9**.

1996), 2′-geranyl-3′,4′,7-trihydroxyflavanone (**6**) (Fujimoto et al., 1987), and cycloaltilisin 6 (**7**) (Patil et al., 2002), by comparison with published values. Compounds **2**, **4**, **5**, **8**, and **9** exhibited IR absorption peaks at ca. 3400 (O–H), 1630 (conj. C=O), and 1508 (C=C) cm^{-1}, as well as UV absorption peaks at ca. 341, 316, and 279 nm, which indicated their flavonoid character (McLean et al., 1996).

Compound **2** was isolated as a yellowish oil. Its molecular formula, $C_{25}H_{28}O_5$, was obtained from analysis of the HRESIMS ($[M+Na]^+$, *m/z* 431.1824) and NMR spectroscopic data. General analysis of the NMR spectroscopic data (Tables 1 and 2) showed that its spectrum resembled that of compound **1** except for the resonance of its geranyl group. The DEPT spectrum of this compound indicated that the geranyl group contained three methyl, two aliphatic methylene, and three methine carbon signals. The NMR spectra further confirmed the presence of the 6a,7,8,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo-[*b*,*d*]pyran ring (Botta et al., 2003). Additionally, a NOESY experiment showed an intense correlation between H-1″ and the signal at δ_H 2.01, but no correlation was observed between H-1″ and H_β-5″. After comparison with the literature data (Ganoi and Mechoulan, 1971; Onegi et al., 2002), the spatial proximity of H-1″ and H-6″ on the one face was established. Hence, compound **2** was determined to be 1-(2,4-dihydroxyphenyl)-3-{4-hydroxy-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6H-dibenzo[*b*,*d*]pyran-5-yl}-1-propanone.

Compound **4** was isolated as a brown gum. The molecular formula, $C_{25}H_{30}O_6$, was deduced from the HRESIMS ($[M+K]^+$, *m/z* 465.1679) and NMR spectroscopic data (Tables 1 and 2). Examination of the latter also indicated that **4** should be an analogue of compound **1**. The ^{13}C NMR and DEPT spectra of **4** showed new signals corresponding to one aliphatic methylene carbon at δ_C 29.8 and one oxymethine carbon at δ_C 67.9, with the disappearance of one inner double bond of the geranyl moiety. The oxymethine proton at δ_H 3.96 (1H, *dd*, $J = 7.2$, 5.6 Hz) was coupled to methylene protons at δ_H 3.05 (1H, *dd*, $J = 16.6$, 5.6 Hz) and 2.70 (1H, *dd*, $J = 16.6$, 7.2 Hz), thus assigning the oxymethine proton as H-2″ and the methylene protons as H_2-1″. The ^1H NMR spectrum displayed a methyl singlet signal at δ_H 1.23 (Me-9″) and a set of resonance due to a prenyl unit. The olefinic proton at δ_H 5.12 (1H, *t*, $J = 6.9$ Hz, H-6″) of the prenyl moiety was correlated via allylic coupling to two methyl singlets at δ_H 1.65 (3H, *s*, Me-8″) and 1.59 (3H, *s*, Me-10″) and methylene protons

Table 1
^{1}H NMR spectroscopic (500 MHz) data of compounds **2**, **4**, **5**, **8**, and **9** in acetone-d_6

H No.	**2**[a]	**4**	**5**	**8**	**9**
3′	6.39 (1H, *d*, *J* = 2.2 Hz)	6.33 (1H, *d*, *J* = 2.0 Hz)	6.31 (1H, *d*, *J* = 2.3 Hz)	6.33 (1H, *d*, *J* = 2.2 Hz)	6.33 (1H, *d*, *J* = 2.3 Hz)
5′	6.34 (1H, *dd*, *J* = 8.7, 2.2 Hz)	6.41 (1H, *dd*, *J* = 8.8, 2.0 Hz)	5.74 (1H, *dd*, *J* = 8.8, 2.3 Hz)	5.74 (1H, *dd*, *J* = 8.8, 2.2 Hz)	6.44 (1H, *dd*, *J* = 8.9, 2.3 Hz)
6′	7.63 (1H, *d*, *J* = 8.7 Hz)	7.81 (1H, *d*, *J* = 8.8 Hz)	6.86 (1H, *d*, *J* = 8.8 Hz)	7.73 (1H, *d*, *J* = 8.8 Hz)	7.77 (1H, *d*, *J* = 8.9 Hz)
β	3.02 (2H, *m*)	2.88 (2H, *m*)	2.89 (2H, *m*)	2.96 (2H, *m*)	2.91 (2H, *m*)
α	3.22 (2H, *m*)	3.21 (2H, *m*)	3.59 (2H, *m*)	3.16 (2H, *m*)	3.16 (2H, *m*)
5	6.75 (1H, *d*, *J* = 8.2 Hz)	6.60 (1H, *d*, *J* = 8.1 Hz)	6.72 (1H, *d*, *J* = 8.3 Hz)	6.64 (1H, *m*)[b]	6.65 (1H, *d*, *J* = 8.1 Hz)
6	6.67 (1H, *d*, *J* = 8.2 Hz)	6.66 (1H, *d*, *J* = 8.1 Hz)	6.82 (1H, *d*, *J* = 8.3 Hz)	6.64 (1H, *m*)[b]	6.58 (1H, *d*, *J* = 8.1 Hz)
1″	3.63 (1H, *m*)	2.70 (1H, *dd*, *J* = 16.6, 7.2 Hz) 3.05 (1H, *dd*, *J* = 16.6, 5.6 Hz)	6.06 (1H, *d*, *J* = 10.1 Hz)	6.64 (1H, *m*)[b]	3.44 (2H, *d*, *J* = 6.4 Hz)
2″	5.68 (1H, *d*, *J* = 2.0 Hz)	3.96 (1H, *dd*, *J* = 7.2, 5.6 Hz)	5.39 (1H, *d*, *J* = 10.1 Hz)	5.74 (1H, *d*, *J* = 10.0 Hz)	5.21 (1H, *t*, *J* = 6.4 Hz)
3″					
4″	2.01 (2H, *m*)[b]	1.75 (2H, *m*)	5.28 (1H, *m*)	2.38 (2H, *d*, *J* = 6.5 Hz)	1.98 (2H, *m*)
5″	1.71 (1H, *m*, H_β-5″) 2.01 (1H, *m*, H_α-5″)[b]	2.21 (2H, *m*)	5.35 (1H, *m*)	5.66 (1H, *m*)	1.55 (2H, *m*)
6″	2.01 (1H, *m*)[b]	5.12 (1H, *t*, *J* = 6.9 Hz)	4.49 (1H, *d*, *J* = 9.7 Hz)	5.71 (1H, *d*, *J* = 15.7 Hz)	3.96 (1H, *t*, *J* = 6.3 Hz)
7″					
8″	1.69 (3H, *s*)	1.65 (3H, *s*)	1.24 (3H, *s*)[c]	1.21 (3H, *s*)[b]	4.71 (1H, *s*) 4.86 (1H, *s*)
9″	1.36 (3H, *s*)[b]	1.23 (3H, *s*)	1.39 (3H, *s*)	1.38 (3H, *s*)	1.76 (3H, *s*)
10″	1.36 (3H, *s*)[b]	1.59 (3H, *s*)	1.26 (3H, *s*)[c]	1.21 (3H, *s*)[b]	1.65 (3H, *s*)
2′-OH	12.84 (1H, *s*)	12.83 (1H, *s*)	12.57 (1H, *s*)	12.81 (1H, *s*)	12.85 (1H, *s*)

[a] Measured in $CDCl_3$.
[b] Overlapping signals.
[c] Changeable with the corresponding carbon signal.

Table 2
^{13}C NMR (125 MHz) spectroscopic data for compounds **2**, **4**, **5**, **8**, and **9** in acetone-d_6

C No.[a]	**2**[b]	**4**	**5**	**8**	**9**
1′	113.8	113.8	116.2	113.2	113.2
2′	165.3	166.2	166.5	165.6	165.6
3′	103.8	103.5	109.8	102.9	102.9
4′	163.7	165.4	165.4	165.0	164.9
5′	108.4	108.7	114.3	108.3	108.2
6′	132.6	133.6	133.2	132.9	132.9
β	27.7	27.2	29.9	26.4	27.4
α	39.8	38.9	41.8	39.5	39.5
C=O	204.5	205.1	207.3	204.3	204.6
1	131.1	130.8	127.9	127.9	131.5
2	124.3	119.9	120.8	120.1	126.8
3	140.3	141.4	140.2	140.5	143.6
4	143.8	145.2	144.4	144.2	142.9
5	112.2	113.1	116.4	115.4	112.8
6	121.3	120.8	123.4	121.5	120.0
1″	33.3	29.8	119.1	119.9	25.2
2″	123.2	67.9	127.6	130.3	123.8
3″	135.9	79.2	77.4	77.7	134.5
4″	28.6	38.2	121.0	40.1	35.8
5″	21.9	22.2	141.5	120.7	34.0
6″	40.5	125.5	91.7	142.5	74.7
7″	77.9	131.6	71.6	69.9	148.7
8″	23.8	25.7	25.7[c]	29.7[c]	109.7
9″	25.0[c]	18.3	28.6	25.3	15.9
10″	26.8[c]	17.6	26.7[c]	29.8[c]	17.2

[a] Dihydrochalcone carbon numberings.
[b] Measured in $CDCl_3$.
[c] Interchangeable.

at δ_H 2.21 (2H, *m*, H-5″), which were further correlated with the other methylene protons at δ_H 1.75 (2H, *m*, H-4″). From the foregoing spectroscopic data, the structure of **4** was deduced to be 1-(2,4-dihydroxyphenyl)-3-[3,4-dihydro-3,8-dihydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone.

Compound **5** had the molecular formula $C_{25}H_{26}O_6$ as indicated by analysis of its HRESIMS ($[M+Na]^+$, *m*/*z* 445.1616) and NMR spectroscopic data (Tables 1 and 2), with the latter indicating 13 degrees of unsaturation. The NMR spectroscopic data of compound **5** also suggested the presence of A, B, and C rings as in compound **1**, and additional structural variations in the geranyl moiety. In the ^{1}H NMR spectrum, two proton signals at δ_H 5.28 (1H, *m*) and 5.35 (1H, *m*) were assigned to H-4″ and H-5″, respectively. The H-5″ exhibited a COSY correlation with the proton signal at δ_H 4.49 (1H, *d*, *J* = 9.7 Hz, H-6″), which displayed HMBC correlations with two methyl carbons at δ_C 25.7 (C-8″) and 26.7 (C-10″). In addition, two singlets at δ_H 1.24 and 1.26, assignable to two methyl protons (Me-8″ and Me-10″), showed HMBC correlations with two oxygenated carbon signals at δ_C 91.7 (C-6″) and 71.6 (C-7″). The foregoing spectroscopic data suggested the presence of 12 degrees of unsaturation. The remaining degree of unsaturation was ascribed to the existence of an epoxy ring at C-6″ and C-7″. These data thus established the structure of **5** as 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-

2-methyl-2-(3,4-epoxy-4-methyl-1-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone.

Compound **8** was assigned a molecular formula of $C_{25}H_{28}O_6$, as shown by analogues of the HRESIMS ($[M+K]^+$, *m/z* 463.1518) and NMR spectroscopic data. The NMR spectra (Tables 1 and 2) suggested that compound **8** should have identical A, B, and C rings to that of compound **1**, but differed in the prenyl unit. In the ^1H NMR spectrum, two proton signals at δ_H 5.71 (1H, *d*, $J = 15.7$ Hz) and 5.66 (1H, *m*) were assigned to H-6″ and H-5″, respectively. Analysis of the HMBC spectrum established that both H-6″ and H-5″ correlated with one aliphatic methylene carbon at δ_C 40.1 (C-4″), to which two protons at δ_H 2.38 (*d*, $J = 6.5$ Hz, H-4″) were attached; also H-4″ correlated with two carbon signals at δ_C 130.3 (C-2″) and 77.7 (C-3″). Moreover, one singlet at δ_H 1.21 (6H, *s*, Me-8″, 10″) exhibited correlations with an olefinic carbon signal at δ_C 142.5 (C-6″) and one oxygenated carbon resonance at δ_C 69.9 (C-7″). Based on the above evidence, compound **8** was determined to be 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-hydroxy-4-methyl-2-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone.

The HRESIMS ($[M+Na]^+$, *m/z* 449.1942) and NMR spectroscopic data of compound **9** suggested a molecular formula of $C_{25}H_{30}O_6$. Comparison of the NMR spectroscopic data of **9** (Tables 1 and 2) with those of compound **3** also indicated very similar structures. The most important point of difference with compound **3** in the NMR data was a $-CH_2CH(OH)C(CH_3)CH_2$ fragment from C-5″ to C-8″ (instead of a $-CH_2CHC(CH_3)CH_3$ fragment) (Stevenson et al., 2003). Two proton signals at δ_H 1.55 (2H, *m*) and 3.96 (1H, *t*, $J = 6.3$ Hz) were assigned to H_2-5″ and H-6″, respectively, and two singlets at δ_H 4.71 and 4.86 were assigned to a terminal olefinic methylene (H_2-8″). Moreover, the methyl singlet at δ_H 1.65 (*s*, Me-10″) exhibited HMBC correlations with carbon signals at δ_C 74.7 (C-6″), 109.7 (C-8″), and 148.7 (C-7″). On the basis of these observations, compound **9** was identified as 2-[6-hydroxy-3,7-dimethylocta-2(*E*),7-dienyl]-2′,3,4,4′-tetrahydroxydihydrochalcone.

In the presumed biogenetic pathway to the isolated compounds **1**–**9**, it is thought that 2′,3,4,4′-tetrahydroxychalcone is the precursor (Schijlen et al., 2004). Compounds **1**, **2**, **4**, **5**, and **7**–**9** might be biosynthesized from compound **3**, which was isolated as one major component. 2′,3,4,4′-tetrahydroxychalcone was converted to compound **3** by reduction of the olefin and introduction of the geranyl group at C-2 (Kuzuyama et al., 2005), although the sequence of the two steps was unclear. Compound **1** was derived from compound **3** via cyclization of the geranyl group with an ortho-phenolic hydroxyl group at C-3, and compound **2** was generated in a similar manner.

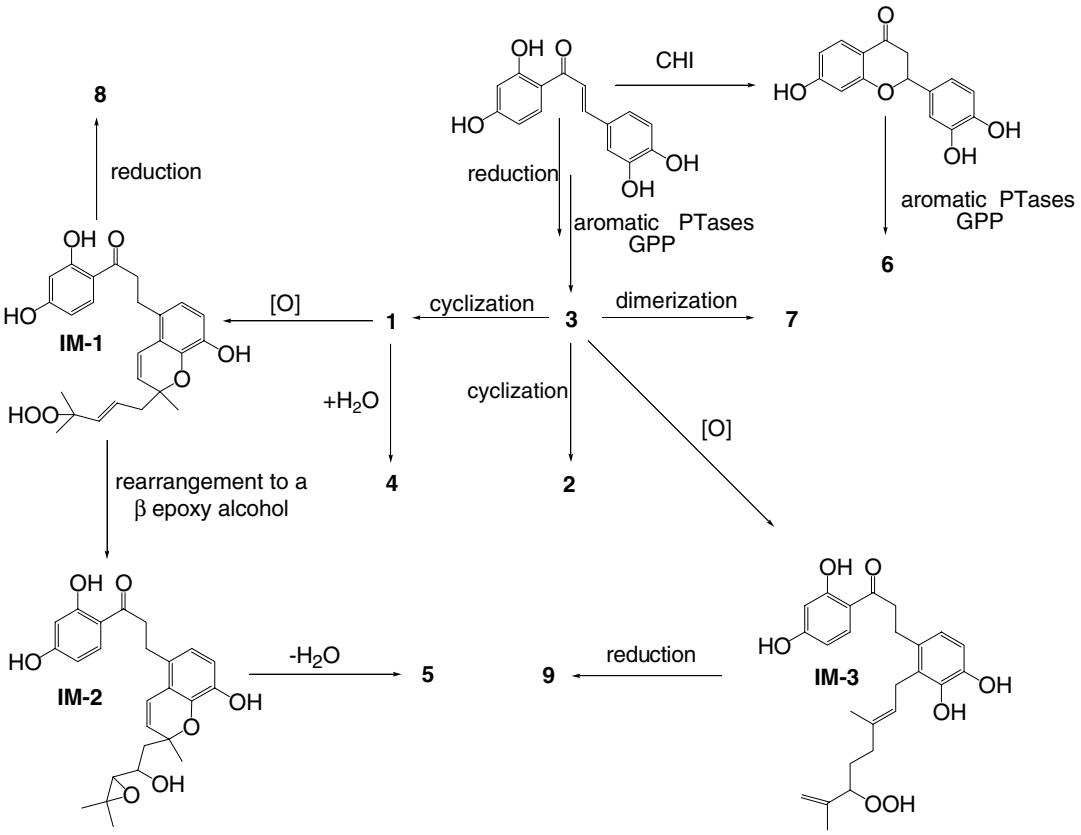

Fig. 2. The presumed biogenesis to geranyl flavonoids **1**–**9**. CHI, chalcone isomerase; Ptases, prenyltransferases; GPP, geranyl diphosphate; IM-1, intermediate 1; IM-2, intermediate 2; IM-3, intermediate 3.

Table 3
Cytotoxicity of compounds **2**, **4**, **5**, **8**, and **9** against human cancer cell lines

Compound	IC_{50} (μM)		
	SPC-A-1	SW-480	SMMC-7721
2	28.14	34.62	49.86
4	41.12	39.22	46.74
5	>80	>80	>80
8	>80	>80	>80
9	57.18	67.03	56.38
9-Fluorouracil[a]	31.67	52.67	26.39

[a] 9-Fluorouracil was used as a positive control.

Compound **4** might be derived from compound **1**, which was isolated as the other major component, i.e. by hydration of the inner olefin of the modified pyran ring. An allylic hydroperoxide, **IM-1**, from the oxidation of compound **1**, was proposed as the intermediate in generating compounds **5** and **8**. If this were the case, compound **3** afforded intermediate **IM-3**, which was then reduced to compound **9** (Brown et al., 2003). The presumed biosynthesis pathway to compounds **1**–**9** is shown in Fig. 2.

The new geranyl dihydrochalcones were tested for their cytotoxicity against cancer cell lines (human lung adenocarcinoma SPC-A-1 cells, human colon carcinoma SW-480 cells, and human hepatocellular carcinoma SMMC-7721 cells) using the MTT method with 9-fluorouracil as a positive control. The results are summarized in Table 3. Compounds **2**, **4**, and **9** exhibited moderate cytotoxicity against all three tested human cancer cell lines, while compounds **5** and **8** did not show any cytotoxicity at 80 μM. Among the tested compounds, compound **2** was the most potent against the SPC-A-1 and SW-480 cells, and was found to be relatively more active than standard fluorouracil. Further investigations of the anti-cancer activities of compound **2** are in progress.

3. Concluding remarks

According to our investigation and the data from the literature, all tissues of *A. altilis* are rich in flavonoids. In this paper, we add five new members to the list of compounds previously reported from this plant. It is noted that the leaves and bud covers mainly contain geranyl flavonoids (Patil et al., 2002; Wang et al., 2006), while the roots and stems afford various types of prenylated flavonoids (Lin and Shieh, 1991, 1992; Weng et al., 2006). So, continued investigation of the leaves of this species may represent a promising strategy for the discovery of other new geranyl flavonoids. It is interesting that *A. champeden*, *A. incisus*, and *A. nobilis* all produce geranyl flavonoids (Hakim et al., 1999; Shimizu et al., 2000; Jayasinghe et al., 2006), whereas geranyl dihydrochalcones are only detected in *A. altilis*. This feature appears to be characteristic of *A. altilis* and might be useful for further chemotaxonomic studies of the genus *Artocarpus*.

4. Experimental

4.1. General

Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. UV spectra were measured with a JASCO UV-2200 UV–vis recording spectrophotometer, and IR spectra were obtained with a Nicolet NEXUS-470 FT-IR spectrometer. The ^{1}H and ^{13}C NMR, as well as 2D spectra, were recorded on a Bruker AVANCE DRX-500 NMR (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) spectrometer; the chemical shifts are represented as ppm using tetramethylsilane as an internal standard. HRESIMS data (positive mode) were acquired with a Bruker Apex III mass spectrometer, whereas ESIMS spectra (positive mode or negative mode) were obtained with a Bruker Esquire 3000 ^{plus} mass spectrometer. CC was carried out on silica gel (200–300 mesh from Qingdao Marine Chemical Co. Ltd., China), Rp-18 (YMC Co. Ltd., Japan) and Sephadex LH-20 (Pharmacia Biotech, Sweden).

4.2. Plant material

The leaves of *A. altilis* were collected in September 2004 from Bandung (Indonesia) and authenticated by Dr. Lenny Sutedja from the Indonesian Institute of Sciences Research Center, Indonesia. A voucher specimen (No. 20040923) is deposited at the Department of Chemistry, Zhejiang University, PR China.

4.3. Extraction and isolation

The air dried powdered leaves of *A. altilis* (3.2 kg) were extracted with MeOH (3 × 10 L) at room temperature for seven months. The combined MeOH extracts were filtered and evaporated *in vacuo* to give a residue (260 g), will the latter suspended in H_2O and extracted successively with light petroleum and EtOAc. The EtOAc extract was next concentrated under reduced pressure at 35 °C to afford a dark residue (102 g), with this being subjected to fractionation by silica gel CC (1200 g, 5 cm diameter) eluted with light petroleum–EtOAc mixtures (20:1 → 1:3) to yield 12 fractions. Fractions 3 (7.6 g), 5 (4.2 g), and 7 (5.5 g) were submitted to Sephadex LH-20 CC (MeOH–H_2O, 80:20) to afford **1** (1600 mg), **2** (212 mg), and **3** (1100 mg), respectively. Fraction 8 (3.3 g) was applied to a Sephadex LH-20 column eluted with MeOH–H_2O, (70:30), then further purified by Rp-18 [MeOH–H_2O (65:35, 70:30)] to yield **4** (26 mg). Fraction 9 (3.9 g) was subjected to silica gel CC eluted with light petroleum–EtOAc (3:1), then further purified by Rp-18 [MeOH–H_2O (60:40, 65:35)] to give **5** (18 mg) and **6** (49 mg). Further Sephadex LH-20 column chromatography of fraction 11 (2.6 g), eluted with a MeOH–H_2O mixture (70:30), yielded **7** (86 mg), **8** (26 mg), and **9** (31 mg).

4.3.1. 1-(2,4-dihydroxyphenyl)-3-{4-hydroxy-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6H-dibenzo[b,d]pyran-5-yl}-1-propanone (2)

Yellowish oil; $[\alpha]_D^{20} + 11.3°$ (MeOH, *c* 0.40); UV λ_{max}^{MeOH} nm (log ε): 341 (3.62), 316 (3.83), 279 (4.16); IR υ_{max}^{KBr} cm^{-1}: 3422, 2977, 1631, 1508, 1487, 1447, 1370, 1239, 1136; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS *m/z*: 431 $[M+Na]^+$; HRESIMS *m/z*: 431.1824 $[M+Na]^+$ (calcd. for $C_{25}H_{28}O_5Na$, 431.1829).

4.3.2. 1-(2,4-dihydroxyphenyl)-3-[3,4-dihydro-3,8-dihydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone (4)

Brown gum; $[\alpha]_D^{20} - 2.1°$ (MeOH, *c* 0.21); UV λ_{max}^{MeOH} nm (log ε): 341 (3.40), 316 (3.74), 279 (3.97); IR υ_{max}^{KBr} cm^{-1}: 3422, 2971, 1632, 1511, 1493, 1452, 1376, 1209, 1135; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS *m/z*: 427 $[M+H]^+$; HRESIMS *m/z*: 465.1679 $[M+K]^+$ (calcd. for $C_{25}H_{30}O_6K$, 465.1677).

4.3.3. 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(3,4-epoxy-4-methyl-1-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone (5)

Yellow gum; $[\alpha]_D^{20} + 4.0°$ (MeOH, *c* 0.20); UV λ_{max}^{MeOH} nm (log ε): 341 (3.27), 316 (3.45), 279 (3.73); IR υ_{max}^{KBr} cm^{-1}: 3450, 2924, 1629, 1500, 1442, 1372, 1202, 1124; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS *m/z*: 423 $[M+H]^+$; HRESIMS *m/z*: 445.1616 $[M+Na]^+$ (calcd. for $C_{25}H_{26}O_6Na$, 445.1622).

4.3.4. 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-hydroxy-4-methyl-2-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone (8)

Brown gum; $[\alpha]_D^{20} + 1.1°$ (MeOH, *c* 0.45); UV λ_{max}^{MeOH} nm (log ε): 341 (3.47), 318 (3.65), 278 (4.05); IR υ_{max}^{KBr} cm^{-1}: 3395, 2974, 1632, 1508, 1495, 1446, 1369, 1209, 1140; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS *m/z*: 423 $[M-H]^-$; HRESIMS *m/z*: 463.1518 $[M+K]^+$ (calcd. for $C_{25}H_{28}O_6K$, 463.1520).

4.3.5. 2-[6-hydroxy-3,7-dimethylocta-2(E),7-dienyl]-2′,3,4,4′-tetrahydroxydihydrochalcone (9)

Brown gum; $[\alpha]_D^{20} - 1.8°$ (MeOH, *c* 0.17); UV λ_{max}^{MeOH} nm (log ε): 341 (3.25), 311 (3.44), 279 (3.71); IR υ_{max}^{KBr} cm^{-1}: 3422, 2937, 1630, 1508, 1499, 1449, 1375, 1210, 1137; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS *m/z*: 449 $[M+Na]^+$; HRESIMS *m/z*: 449.1942 $[M+Na]^+$ (calcd. for $C_{25}H_{30}O_6Na$, 449.1934).

4.4. Cytotoxicity assay

Cytotoxic studies were performed using the MTT assay (Mosmann, 1983). Cells (2×10^5/well) were continuously exposed to different concentrations of the investigated compounds in 96-well plates for 48 h at 37 °C. Controls were always treated with the same amount of DMSO as used in the corresponding experiments. Surviving cells were detected on the basis of their ability to metabolize 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) into formazan crystals. The absorbance was measured at 570 nm using a VersaMax microplate reader. IC_{50} concentration was defined as the concentration of a compound which inhibits cell survival by 50%, compared with a vehicle-treated control.

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