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FIVE NEW COMPOUNDS FROM *ARENGA PINNATA* (WURMB.) MERR. FRUITS

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Abstract – Phytochemical investigation of *Arenga pinnata* (Wurmb.) Merr. fruits led to the isolation of 5 new compounds, designated A. pinnata A-E (**1–5**), and 13 known compounds (**6–18**). All compounds were isolated from *A. pinnata* for the first time. Their chemical structures were identified based on extensive spectroscopic methods, including HR-ESI-MS, 1D and 2D-NMR. To the best of our knowledge, this is the first systematic scientific study on the chemical composition of the *Arenga* genus.

INTRODUCTION

Arenga pinnata (Wurmb.) Merr. are tall evergreen trees in the genus *Arenga* of the family Palmae. They are widely distributed in Southern China and Southeast Asia.[1] They are widely used as folk remedies and were first found in "Kai Bao Ben Cao" of the Song Dynasty and "Ben Cao Hui Yan" of the Ming Dynasty.[2,3] In folk medicines, people used to soak *A. pinnata* in wine, which has a remarkable effect on the painful parts of the body.[4] They are abundant, and there is a huge development space.[5] However, there is almost no current research on *A. pinnata*, and its main effective medicinal component remains unclear. Palm plants contain terpenes, alcohols, alkanes, esters, phenols, quinones, aldehydes and alkaloids.[6] This research is the first systematic study of *A. pinnata* fruit using modern spectroscopy and chromatographic techniques. The crude product was separated and identified to include a new flavonoid, a new lignin compound, 2 new glycosides, a new *O*-containing heterocycle compound and 13 known compounds

(**6–18**) (Figure 1). This study plays an important role in expanding the diversity of *A. pinnata* chemical components.

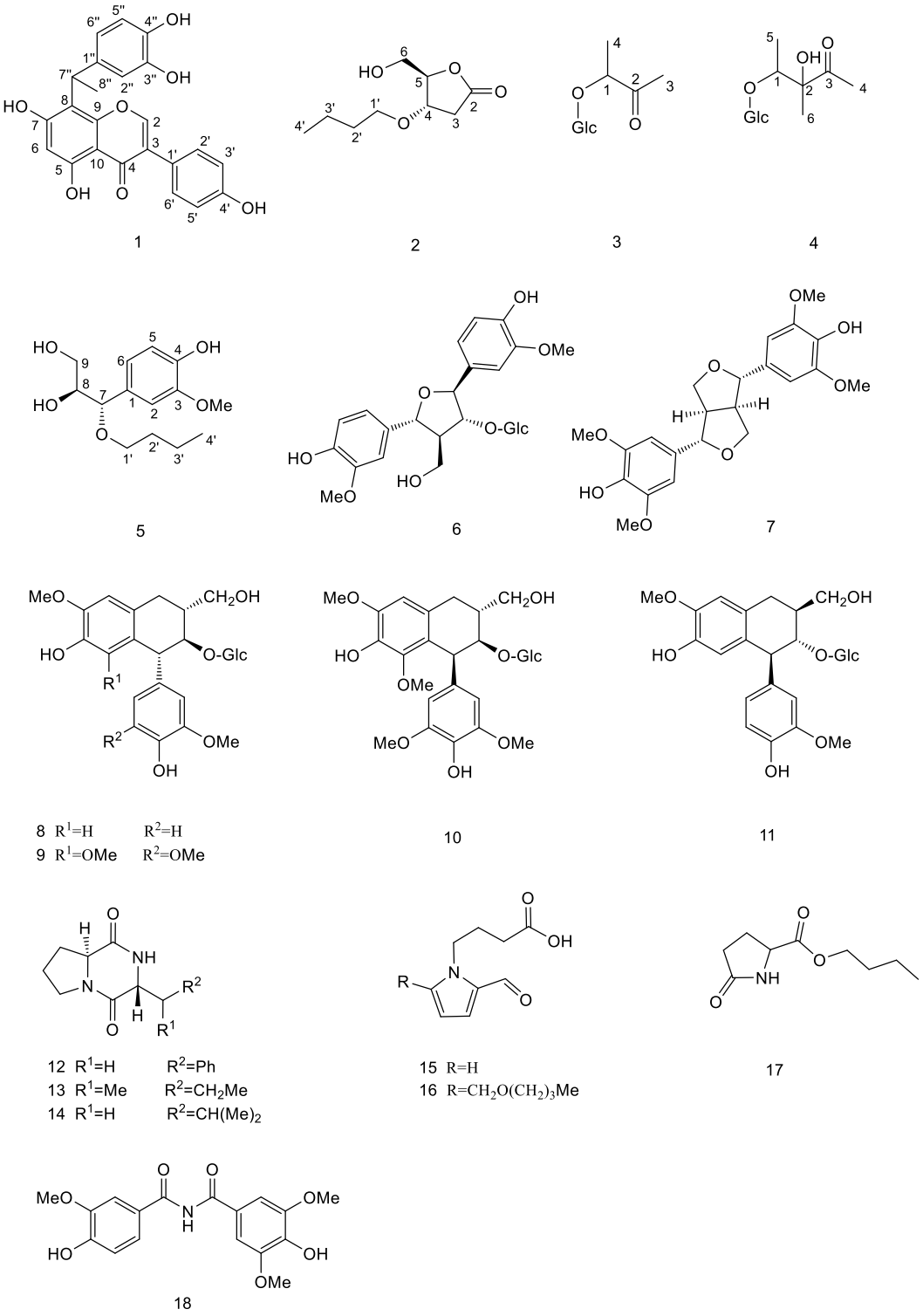

Figure 1. Structures of compounds **1**–**18** from *A. pinnata* fruits

RESULTS AND DISCUSSION

Compound **1** has the molecular formula of $C_{23}H_{18}O_7$ according to HR-ESI-MS at *m/z* 407.1125 $[M+H]^+$. The ^{1}H-NMR data of **1** displayed 1 group of ABX coupling systems with proton signals at δ_H 6.76 (1H, br. s), 6.65 (1H, s), 6.64 (1H, br. s) and 1 group of AABB coupling systems with proton signals at δ_H 7.34 (2H, d, *J* = 8.6 Hz), 6.82 (2H, d, *J* = 8.6 Hz). All proton signals were assigned by HSQC analysis. A proton at δ_H 8.0 (1H, s) was ascribed to δ_C 154.5 (C-2) for the HMBC correlation from δ_H 8.0 (1H, s) to δ_C 182.6 (C-4), and a proton at δ_H 6.29 (1H, s) was ascribed to δ_C 99.9 (C-6) for the HMBC correlation from δ_H 6.29 (1H, s) to δ_C 163.4 (C-7), 161.6 (C-5), so it was considered to be located at the flavonoid skeleton. The HMBC correlation from δ_H 1.68 (3H, d, *J* = 7.2 Hz) to δ_C 113.0 (C-8) led to the connection of 7''-phenylethyl to δ_C 113.0 (C-8) of the flavonoid skeleton. Combined with DEPT-135 and ^{13}C-NMR data, compound **1** presented signals for 23 carbons, including 21 aromatic carbons (δ_C 182.6, 163.4, 161.6, 158.8, 157.2, 154.5, 145.7, 144.0, 138.3, 131.4, 131.4, 124.3, 123.4, 119.5, 116.3, 116.3, 115.9, 115.7, 113.0, 106.5, 99.9), 1 methyl carbon (δ_C 18.4), and 1 tertiary carbon signal (δ_C 33.6). The connectivity of these partial structures and their functional groups was investigated by an analysis of HMBC. As shown in Figure 2, long-range correlations were observed between the following: H-7'' (CH) and C-7, C-8, C-9, C-1'', C-2'', C-6''; H-2 (CH) and C-3, C-4, C-9, C-1'; H-8'' (Me) and C-1'', C-7'', C-8.

Table 1. ^{1}H- and ^{13}C-NMR Data of **1**–**5** (δ_H 400 MHz, δ_C 100 MHz in CD_3OD)

NO.	1		5		
	δ_H (*J*, Hz)	δ_C		δ_H (*J*, Hz)	δ_C
2	8.0 (s)	154.5	1		132.3
3		124.3	2	6.94 (s)	111.8
4		182.6	3		149.1
5		161.6	4		147.4
6	6.29 (s)	99.9	5	6.78 (m)	116.0
7		163.4	6	6.78 (m)	121.5
8		113.0	7	4.19 (d, 6.6)	83.8
9		157.2	8	3.66 (m)	77.2
10		106.5	9	3.47 (dd, 11.3, 3.8) 3.30 (m)	64.0
1'		123.4	1'	3.35 (m)	69.7
2',6'	7.34 (d, 8.6)	131.4	2'	1.54 (m)	33.0

3',5'	6.82 (d, 8.6)	116.3	3'	1.33 (m)	20.4
4'		158.8	4'	0.90 (t, 6.6)	14.2
1"		138.3	OMe	3.84 (s)	56.4
2"	6.64 (s)	115.9			
3"		145.7			
4"		144.0			
5"	6.76 (br. s)	115.7			
6"	6.65 (br. s)	119.5			
7"	4.70 (q, 7.2)	33.6			
8"	1.68 (d, 7.2)	18.4			

Based on the obtained data, the structure was established as 8-(7″-(3″,4″-dihydroxyphenyl)-ethyl)-4′,5,7-trihydroxyisoflavone and named A. pinnata A (Table 1; Figure 1).

Compound **2** has the molecular formula of $C_9H_{16}O_4$ according to HR-ESI-MS at *m/z* 189.1117 $[M+H]^+$. The ^{1}H-NMR data of **2** displayed 1 methyl group at δ_H 0.93 (3H, t, J = 7.5 Hz), 5 methylene groups at δ_H 3.86 (dd, J = 12.3, 2.9 Hz), 3.84 (br d, J = 12.3 Hz); 3.37 (1H, m), 3.51 (1H, m); 2.78 (1H, dd, J = 2.1, 17.6 Hz), 2.58 (1H, dd, J = 5.8, 17.6 Hz); 1.65 (2H, m) and 1.35 (2H, m), respectively. Combined with DEPT-135, HSQC and ^{13}C-NMR data, compound **2** presented signals for 9 carbons, including 1 methyl carbon (δ_C 14.2), 5 methylene carbon signals (δ_C 70.6, 36.7, 61.1, 32.9, 20.4), 2 tertiary carbon signals (δ_C 85.9, 76.8), and 1 quaternary carbon signal (δ_C 178.0). In the HMBC spectrum,[7] the correlations between δ_C 76.8 (C-4) and H-1' and between δ_C 70.6 (C-1') and δ_H 4.26 (1H, q, J = 4.7 Hz) suggest that the butoxy group must be attached to C-4. As shown in Figure 2, long-range correlations were observed between the following: H-3 (CH_2) and C-2 (C=O), C-4, C-5; H-4 (CH) and C-3, C-2(C=O), C-5, C-6, C-1'; H-5 (CH) and C-3, C-4, C-6; H-6 (CH_2) and C-4, C-5. The observed coupling constants of H-4 ($J_{3,4}$ =17.6 Hz, $J_{3,4}$ = 17.6, 2.1 Hz and $J_{4,5}$ = 4.7 Hz), H-5 ($J_{4,5}$ = 4.6 Hz) and H-6 ($J_{5,6}$ = 12.3, 2.9 Hz, $J_{5,6}$ = 12.3 Hz) in the ^{1}H-NMR spectrum were identical to those of H-4 ($J_{3,4}$ = 17.1, $J_{3,4}$ = 17.1, 2.9 Hz and $J_{4,5}$ = 5 Hz), H-5 ($J_{4,5}$ = 5 Hz) and H-6 ($J_{5,6}$ = 12.7, 2.9 Hz, $J_{5,6}$ = 12.7 Hz) in *trans*-4-methoxy-5-methoxymethyloxolan-2-one.[8-10] Based on the obtained data, the structure was established as *trans*-4-butoxyl-5-methoxymethyloxolan-2-one and named A. pinnata B (Table 2; Figure 1).

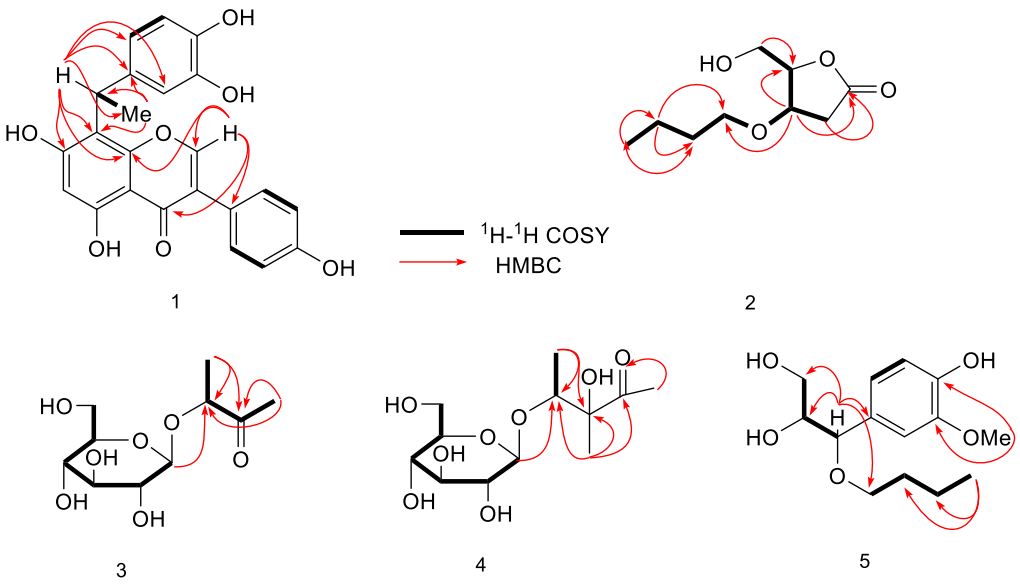

Figure 2. Key HMBC and ^{1}H- ^{1}H COSY correlations of Compounds **1**–**5**

Compound **3** has the molecular formula of $C_{10}H_{18}O_7$ according to HR-ESI-MS at *m/z* 251.1125 $[M+H]^+$. The ^{1}H-NMR spectrum of **3** showed the characteristic signals of 2 methyl at δ_H 2.22 (3H, s) and 1.31 (3H, d, J = 6.9 Hz), 2 methine signals at δ_H 4.33 (1H, d, J = 8.5 Hz) and 4.18 (1H, q, J = 6.8 Hz), respectively. Combined with DEPT-135, HSQC and ^{13}C-NMR data, compound **3** exhibited signals for 10 carbons, including 2 methyl carbons (δ_C 25.8, 17.0), 1 tertiary carbon signal (δ_C 81.7), 1 quaternary carbon signal (δ_C 213.2), and 1 glucose group (δ_C 103.7, 78.1, 75.0, 71.5, 78.0, 62.7). The ^{1}H-^{1}H COSY and HSQC analysis of **3** is shown in Figure 2. In the HMBC spectrum, the correlations between δ_C 81.7 (C-1) and δ_H 4.33 (1H, d, J = 8.5 Hz) and between δ_C 103.7 (C-1') and δ_H 4.18 (1H, q, J = 6.8 Hz) suggest that the glucose group must be attached to C-1. As shown in Figure 2, long-range correlations were observed between the following: H-4 (CH_3) and C-1, C-2 (C=O); H-3 (Me) and C-1, C-2 (C=O). According to the literature,[11,12] the structure was established as 3-oxobutan-2-yl-beta-D-glucoside and named A. pinnata C (Table 2; Figure 1).

Compound **4** has the molecular formula of $C_{12}H_{22}O_8$ according to HR-ESI-MS at *m/z* 295.1385 $[M+H]^+$. The ^{1}H-NMR data of **4** displayed 3 methyl groups at δ_H 2.25 (3H, s), 1.29 (3H, s) and 1.13 (3H, d, J = 6.4 Hz), 2 methine protons at δ_H 4.31 (1H, d, J = 7.7 Hz) and 4.04 (1H, q, J = 6.4 Hz). Combined with DEPT-135, HSQC and ^{13}C-NMR data, compound **4** exhibited signals for 12 carbons, including 3 methyl carbons (δ_C 26.7, 21.8, 14.4), 1 signal of tertiary carbon (δ_C 79.1), 2 quaternary carbon signals (δ_C 215.1, 82.3), and 1 glucose group (δ_C 101.7, 78.0, 77.9, 74.9, 71.7, 62.8). The ^{1}H-^{1}H COSY and HSQC analysis of **4** is shown in Figure 2. In the HMBC spectrum, the correlations between δ_C 79.7 (C-1) and δ_H 4.31 (1H, d, J = 7.7 Hz) and between δ_C 101.7 (C-1') and δ_H 4.04 (1H, q, J = 6.4 Hz) suggest that the glucose group

must be attached to C-1. As shown in Figure 2, long-range correlations were observed between the following: H-4 (Me) and C-2, C-3 (C=O); H-5 (CH_3) and C-1, C-2; H-6 (CH_3) and C-1, C-2, C-3. According to the literature, it was named A. pinnata D (Table 2; Figure 1).

Compound **5** has the molecular formula of $C_{14}H_{22}O_5$ according to HR-ESI-MS at *m/z* 270.1693 $[M+H]^+$. The ^{1}H-NMR data of **5** displayed one group of ABX coupling systems with proton signals at 6.94 (1H, s), 6.78 (1H, m) and 6.78 (1H, m), 2 methyl groups δ_H 3.84 (3H, s), 0.90 (3H, t, *J* = 6.6 Hz), 4 methylene protons at δ_H 3.47 (1H, dd, *J* = 11.3, 3.8 Hz), 3.35 (2H, m), 3.30 (1H, m), 1.54 (2H, m), 1.33 (2H, m), 2 methine protons at δ_H 4.19 (1H, d, *J* = 6.6 Hz), 3.66 (1H, m). Combined with DEPT-135, HSQC and ^{13}C-NMR data, compound **5** presented signals for 14 carbons, including 6 aromatic carbons (δ_C 149.1, 147.4, 132.3, 121.5, 111.8, 116.0), 2 methyl carbons (δ_C 56.4, 14.2), 2 tertiary carbon signals (δ_C 83.8, 77.2), and 4 methylene carbon signals (δ_C 69.7, 64.0, 33.0, 20.4). In the HMBC spectrum,[13] the correlations between δ_C 83.8 (C-7) and H-1' and between δ_C 69.7 (C-1') and δ_H 4.19 (1H, d, *J* = 6.6 Hz) suggest that the butoxy group must be attached to C-7. As shown in Figure 2, long-range correlations were observed between the following: H-7 (CH) and C-8, C-9, C-1', C-1; H-4' (Me) and C-3', C-2'; H-2' (CH_2) and C-1', C-3', C-4'; OCH_3 and C-3, C-4. This evidence indicates that compound **5** can be identified as 7-*O*-butylguaiacylglycerol.

It has been reported[14] that in the case of syringoylglycerol and guaiacylglycerol derivatives, the coupling constant between H-7 and H-8 was approximately 5 Hz for the *erythro* isomer and 7 Hz for the *threo* isomer. Based on the obtained data, the structure was established as threo-7-*O*-butylguaiacylglycerol and named A. pinnata E (Table 1; Figure 1).

Table 2. ^{1}H- and ^{13}C-NMR Data of **2**–**4** (δ_H 400 MHz, δ_C 100 MH in CD_3OD)

NO.	2		3		4	
	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C
1			4.18 (q, 6.8)	81.7	4.04 (q, 6.4)	79.1
2		178.0		213.2		82.3
3	2.58 (br. d, 17.6) 2.78 (dd, 2.1, 17.6)	36.7	2.22 (3H, s)	25.8		215.1
4	4.26 (q-like, 4.7)	76.8	1.31 (d, 6.9)	17.0	2.25 (s)	26.7
5	4.56 (q-like, 4.6)	85.9			1.13 (d, 6.4)	21.8
6	3.86 (dd, 12.3, 2.9) 3.84 (br. d, 12.3)	61.1			1.29 (s)	14.4

1'	3.51 (m) 3.37 (m)	70.6	4.33 (d, 8.5)	103.7	4.31 (d, 7.7)	101.7
2'	1.65 (m)	32.9	3.25 (m)	75.0	3.16 (dd, 7.7, 9.0)	74.9
3'	1.35 (m)	20.4	3.27 (m)	78.1	3.35 (m)	77.9
4'	0.93 (t, 7.5)	14.2	3.27 (m)	71.5	3.29 (m)	71.7
5'			3.34 (m)	78.0	3.27 (m)	78.0
6'			3.82 (dd, 2.1, 2.3) 3.62 (dd, 5.6, 5.4)	62.7	3.87 (dd, 1.2, 11.7) 3.65 (m)	62.8

The 13 known compounds were identified by comparing their spectroscopic data with those reported in the literatures as (7*R*,8*S*,7'*R*,8'*S*)-4,9,4',9'-tetrahydroxy-3,3'-dimethoxy-7,7'-epoxylignan 9-*O*-*β*-D-glucopyranoside (**6**),[15] (-)-syringaresinol (**7**),[16] (-)-isolarisiresinol 3α-*O*-*β*-D-glucopyranoside (**8**),[17] (-)-lyonirenisol-3a-*O*-*β*-D-glucopyranoside (**9**),[18] (+)-lyonirenisol-3a-*O*-*β*-glucopyranoside (**10**),[19] (+)-isolariciresinol 9'-*O*-*β*-glucopyranoside (**11**),[20] cyclo(*L*-Pro-*L*-Phe) (**12**),[21] cyclo(*L*-Pro-*D*-Ile) (**13**),[21] cyclo((*S*)-Pro-(*R*)-Leu) (**14**),[22] 4-(2-formyl-1*H*-pyrrol-1-yl)butanoic acid (**15**),[23] 2-formyl-5-(butoxylmethyl)-1*H*-pyrrole-1-butanoic acid (**16**),[24] butyl 2-pyrrolidone-5-carboxylate (**17**),[25] 4'-hydroxy-*N*-(4-hydroxy-3-methoxybenzoyl)-3',5'-dimethoxybenzamide (**18**).[26]

CONCLUSIONS

In recent years, Feng et al. found the cytoprotective effect of phenethylflavone (A. pinnata A) on the oxidative damage of Caco-2 cells induced by hydrogen peroxide (H_2O_2).[27] Through literature research, it has been found that these nitrogen-containing compounds have a synergistic effect on the intestinal flora of mice[28] and display neuroprotective effects against hydrogen peroxide (H_2O_2)-induced neuronal cell damage in human neuroblastoma SH-SY5Y cells.[29] Therefore, these known compounds may have potential biological research value and great chemical taxonomic value for *A. pinnata*.

EXPERIMENTAL

General experimental procedures. NMR spectra were measured on a Bruker AV-400 spectrometer (Bruker Company, Waltham MA, USA) with TMS as an internal standard. High-resolution ESI-MS mass spectra were obtained on an AB SCEIX Triple-TOFTM 5600^+ instrument (A.B. Company, Milwaukee, WI, USA). UV spectra were recorded on a PerkinElmer Lambda UV-365 instrument (PE Company, Waltham MA, USA). IR spectra were recorded on a PerkinElmer Spectrum Two spectrometer (PE Company, Waltham MA, USA) with KBr disks. Preparative HPLC (515–2414, Waters, Milford, CT,

USA) was performed on a 5C18 MS-II (10 μm, 20×250 mm, cat. no.: 38024–01, COSMOSIL, Tokyo, Japan). Silica gel (200–300 mesh, Haiyang Co, Qingdao, China) and ODS (50 μm, AAG12S50, YMC Company, Kyoto, Japan) were used for column chromatography. Detectors (2424, ELS, Waters and 2998, PDA, Waters) were used in the HPLC. Precoated silica GF 254 plates (Haiyang Company, Qingdao, China) were used for the TLC analysis. All solvents were of analytical grade (TianJinfuyu Company Ltd., TianJin, China).

Plant Material. *A. pinnata* fruits were collected from Guangxi in China during September 2017 and authenticated by Prof. Weiming Wang of the HeilongJiang Research Institute of Chinese Medicine. The fruitage (Guangxi-201709001) was deposited at the HeilongJiang Research Institute of Chinese Medicine.

Extraction and Isolation. Fresh *A. pinnata* fruits (30.0 kg) were extracted with 70% EtOH (200 L×3 h×3 times). The combined extract was concentrated in vacuum to obtain the residue (3.0 kg), which was dissolved in H_2O (12 L) and extracted with petroleum, $CHCl_3$, EtoAc and *n*-butanol (12 L×3 h×5 times) in sequence. The 4 eluents were concentrated in vacuo to obtain 109.0 g, 123.0 g, 205.0 g, and 380.0 g. In this research, we chose the *n*-butanol layer as the follow-up research object. The *n*-butanol extract was subjected to column chromatography on silica gel (4460.0 g) using CH_2Cl_2/MeOH (20:1 (80.0 L), 10:1 (110.0 L), 5:1 (120.0 L), 3:1 (100.0 L), 2:1 (80.0 L) and 1:1 (60.0 L), v/v) elution to obtain 6 fractions: A (36.0 g), B (96.7 g), C (90.2 g), D (40.1 g), E (21.2 g), and F (20.1 g). Each fraction was analyzed using TLC and HPLC, and similar fractions were combined to obtain A1-A6, B1-B5, C1-C6, D1-D6, E1-E10, and F1-F6. Fraction A2 (21.7 g) was eluted by Rp-18 (600.0 g) (MeOH/H_2O 2:8 (1.4 L)→3:7 (2.0 L)→4:6 (2.7 L)→5:5 (2.0 L)→6:4 (2.0 L)→7:3 (1.4 L)→8:2 (1.4 L)→9:1 (0.8 L)→1:0 (1.0 L), v/v) to afford 9 subfractions (subfractions A2–1–A2–9). Subfractions A2–1, A2–2, and A2–5 were further purified by preparative RP-HPLC (55% MeOH/H_2O, flow rate 5 mL/min) to obtain **1** (16.6 mg, t_R = 28 min), **2** (17.1 mg, t_R = 45 min) and **3** (18.0 mg, t_R = 23 min), respectively. Fraction B5 (8.0 g) was eluted by Rp-18 (600.0 g) (MeOH/H_2O 2:8 (1.4 L)→3:7 (2.0 L)→4:6 (2.7 L)→5:5 (2.7 L)→6:4 (2.0 L)→7:3 (1.4 L)→8:2 (1.4 L)→9:1 (0.8 L)→1:0 (1.0 L), v/v) to afford 9 subfractions (subfractions B5-1–B5-9). Subfractions B5–1 were further purified by preparative RP-HPLC (20% MeOH/H_2O, flow rate 5 mL/min) to give **4** (16.1 mg, t_R = 30 min). Subfraction B5–2 was further purified by preparative RP-HPLC (45% MeOH/H_2O, flow rate 5 mL/min) to give **5** (15.9 mg, t_R = 35 min).

A. pinnata A (**1**) Light yellow oil. UV (MeOH) λmax 205, 272, 351 nm; IR (KBr) 3412, 2920, 2851, 1654, 1607, 1577, 1512, 1447, 1384, 1260, 1178, 1121, 1046 cm^{-1}; HR-ESI-MS *m/z* 407.1125 $[M+H]^+$ (calcd for $C_{23}H_{18}O_7$, 407.1125).

A. pinnata B (**2**) Yellow amorphous powder. IR (KBr) 3426, 2937, 2834, 1770, 1634, 1368, 1181, 1094, 935 cm^{-1}; HR-ESI-MS *m/z* 189.1117 $[M+H]^+$ (calcd for $C_9H_{16}O_4$, 189.1117).

A. pinnata C (**3**) White amorphous powder. IR (KBr) 3398, 3340, 1705, 1627, 1172, 898 cm^{-1}; HR-ESI-MS *m/z* 251.1125 $[M+H]^+$ (calcd for $C_{10}H_{18}O_7$, 251.1125).

A. pinnata D (**4**). Yellow amorphous powder. IR (KBr) 3411, 3305, 1710, 1163, 1156, 1132, 927 cm^{-1}; HR-ESI-MS *m/z* 295.1385 $[M+H]^+$ (calcd for $C_{12}H_{22}O_8$, 295.1385).

A. pinnata E (**5**) Colorless oil. UV (MeOH) λ_{max} 204, 229, 280 nm; IR (KBr) 3421, 2929, 1631, 1612, 1518, 1454, 1432, 1372, 1356, 1279, 1156, 1122, 1094, 1035, 853, 773 cm^{-1}; HR-ESI-MS *m/z* 270.1693 $[M+H]^+$ (calcd for $C_{14}H_{22}O_5$, 270.1693).

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