Cytotoxic Prenylated Flavonoids from the Stem Bark of *Maackia amurensis*

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Five new prenylated flavonoids, maackiaflavanone A (1), maackiaflavanone B (2), maackiapentone (3), maackiapterocarpan A (4), maackiapterocarpan B (5) along with eleven known flavonoids were isolated from the stem bark of *Maackia amurensis*. The structures of the new compounds were elucidated by spectroscopic methods. The cytotoxicities of compounds 1—4, 6, 8—12 and 14—16 against four human cancer cell lines, A375S2, HeLa, MCF-7 and HepG2, were tested by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Among the compounds tested, compound 2 showed the strongest cytotoxic activity with IC_{50} value of 7.8 μM against A375S2 and euchrenone b_1 showed the most potent cytotoxicity with IC_{50} value of 4.5 μM against HeLa.

Key words *Maackia amurensis*; Leguminosae; prenylated flavonoid; cytotoxicity

Maackia amurensis Rupr. *et* Maxim. (Leguminosae) is a deciduous tree distributed widely in the northeast of China as well as in the southern part of the Russian Far East and North Korea. The dried stem bark of this plant has been used as folk medicine for the treatment of cancer, cholecystitis and arthritis. Flavonoids and alkaloids have been reported as main constituents of this genus.[1)] Cytotoxicity of prenylated flavonoids against certain human cancer cell lines has also been reported.[2,3)] As a part of our continuing study on cytotoxic compounds from natural sources, we herein report the isolation and structure elucidation of five new prenylated flavonoids (**1**—**5**) (Fig. 1), along with nine known flavonoids as the cytotoxic constituents against the four human cancer lines, A375S2, HeLa, MCF-7 and HepG2.

Result and Discussion

Two new prenylated flavanones (**1**, **2**), one new prenylated isoflavone (**3**) and two new prenylated pterocarpans (**4**, **5**), together with eleven known flavonoid constituents (**6**—**16**), were isolated from the petroleum ether-soluble extract of the stem bark of *M. amurensis* by column chromatography on silica gel, octadecyl silica (ODS), Sephadex LH-20, and semi-preparative HPLC. The structures of the new compounds were elucidated on the basis of spectroscopic methods, including 2D NMR spectral techniques. The structures of new compounds were determined as follows:

Compound **1** was obtained as a white amorphous powder and its molecular formula was determined as $C_{26}H_{28}O_6$ by HR-ESI-MS (*m*/*z* 437.1968 $[M+H]^+$). The presence of a flavanone skeleton was evident from the UV (λ_{max} 289 nm), ^{1}H-[δ 2.80 (1H, dd, J=17.1, 3.0 Hz, H-3α), 3.12 (1H, dd, J=17.1, 13.2 Hz, H-3β) and 5.67 (1H, dd, J=13.2, 3.0 Hz, H-2)] and ^{13}C- [δ 74.49 (C-2), 42.1 (C-3), and 197.7 (C-4)] NMR spectra (Table 1). The ^{1}H-NMR spectra further showed two hydroxyl groups [δ 12.26, 8.91 (each 1H, s)], three singlet aromatic protons [δ 6.14, 7.21, 6.37 (each 1H, s)], a methoxy group [δ 3.90 (3H, s)], an isoprenyl group [δ 1.62 (3H, s), 1.60 (3H, s), 3.22 (2H, d, J=7.2 Hz), 5.15 (1H, br t)] and a 2,2-dimethylpyran ring unit [δ 1.39 (6H, s), 5.56 (1H, d, J=9.6 Hz), 6.35 (1H, d, J=9.6 Hz)]. The locations of these substituents were determined by the heteronuclear multiple quantum correlation (HMQC) and heteronuclear multiple bonding correlation (HMBC) experiments (Fig. 2). Cross peaks between δ 12.26 and C-6 (δ 92.3), C-10 (δ 102.9) in the HMBC spectrum and the correlation of δ 6.14 to C-6 in the HMQC spectrum indicated that the hydroxyl group was attached to C-5 while the singlet aromatic proton was assigned to H-6. HMBC correlations of H-1″ (δ 3.22) to C-7 (δ 164.9), C-9 (δ 161.0), and δ 3.90 (3H, s) to C-7 implied that the isoprenyl group was attached to C-8 while the methoxy was located at C-7, thus the A-ring substituents were identical with maackiaflavanone.[4)] The correlations of δ 8.91 (1H, s) to C-2′ (δ 155.4) in the HMBC spectrum and δ 7.21 (1H, s) to C-6′ (δ 125.1) in the HMQC spectrum together with HMBC correlations from H-2 (δ 5.68) to C-2′ and C-6′ indi-

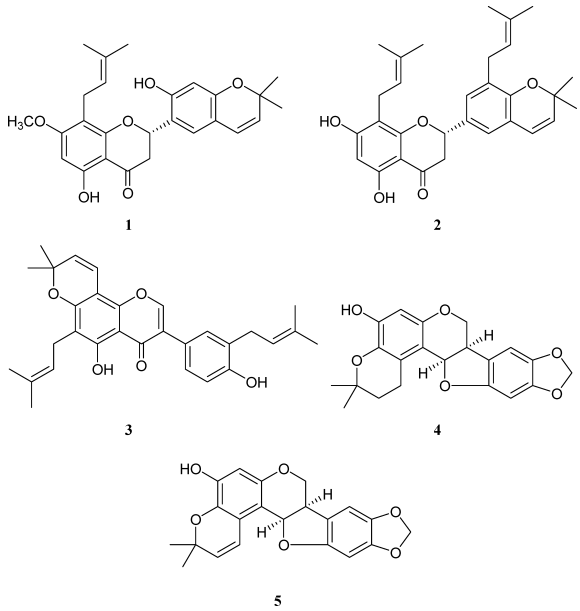

Fig. 1. Structures of Compounds **1**—**5** Isolated from *M. amurensis*

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Table 1. NMR Spectroscopic Data (600 MHz) for Maackiaflavanone A (**1**), Maackiaflavanone B (**2**) and Maackiapentone (**3**)

Position	**1**[a]		**2**[a]		**3**[b]	
	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
2	74.4	5.67, dd (13.2, 3.0)	79.6	5.40, dd (12.6, 3.0)	151.9	7.86, s
3		3.12, dd (17.1, 13.2)		3.11, dd (17.1, 12.6)	123.3	
	42.1	2.80, dd (17.1, 3.0)	43.4	2.66, dd (17.1, 3.0)		
4	197.7		197.4		180.7	
5	163.0		162.9		159.1	
6	92.3	6.14, s	96.4	6.03, s	112.5	
7	165.7		164.9		156.8	
8	108.6		108.2		100.4	
9	159.7		161.0		150.2	
10	102.9		103.3		105.3	
1′	118.4		129.8		122.9	
2′	155.4		128.5	7.21, d (1.8)	130.3	7.25 (m)
3′	103.5	6.37, s	131.2		126.7	
4′	154.4		151.4		154.4	
5′	114.1		121.9		115.6	6.87, d (8.7)
6′	125.1	7.21, s	123.2	7.08, d (1.8)	127.9	7.27 (m)
1″	21.6	3.22, d (7.2)	22.3	3.23, d (6.9)	21.0	3.35, d (7.2)
2″	123.0	5.15, br t	123.7	5.20, br t	121.7	5.24, br t
3″	130.6		132.4		131.3	
4″	25.3	1.62, s	25.9	1.61, s	25.5	1.81, s
5″	17.2	1.60, s	17.8	1.61, s	17.6	1.68, s
2‴	76.5		77.1		77.5	
3‴	128.0	5.56, d (9.6)	131.9	5.76, d (9.9)	126.8	5.58, d (9.6)
4‴	122.0	6.35, d (9.6)	123.1	6.42, d (9.9)	114.7	6.70, d (9.6)
5‴	27.7	1.39, s	28.2	1.43, s	27.8	1.48, s
6‴	27.7	1.39, s	28.2	1.43, s	27.8	1.48, s
1⁗			22.3	3.30, d (7.2)	21.0	3.40, d (7.2)
2⁗			123.5	5.29, br t	121.2	5.35, br t
3⁗			132.0		134.8	
4⁗			25.9	1.70, s	25.5	1.80, s
5⁗			17.9	1.74, s	17.6	1.79, s
5-OH		12.26, s		12.14, s		13.16, s
7-OH				9.55, s		
2′-OH		8.91, s				
7-OMe		3.90, s				

a) In acetone-d_6. *b*) In $CDCl_3$.

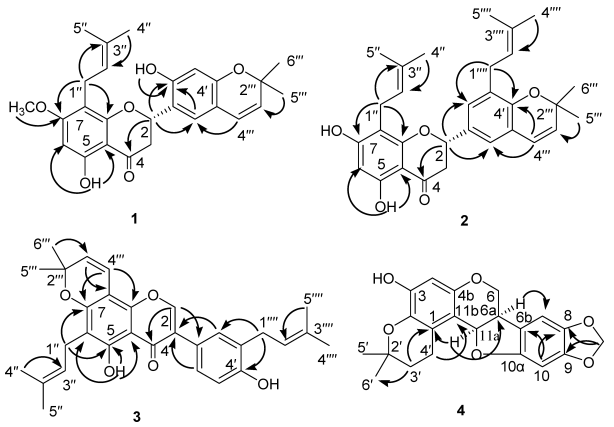

Fig. 2. Selected HMBC Correlations of Compounds **1**—**4**

cated that there were a hydroxyl group and an aromatic proton at the position of C-2′ and C-6′ of B-ring, respectively. HMBC correlations of δ 6.37 to C-2′ and C-4′ (δ 155.4), and the carbon signal at δ 103.5 showed HMQC correlation with δ 6.37 indicated that the singlet aromatic proton was between two oxygenated aromatic carbons (C-2′ and 4′). Combined with HMBC correlations of H-4‴ (δ 6.35) to C-4′ and C-6′, indicated that the dimethylpyran ring was fused to C-5′ and 4′. The circular dichroism (CD) spectrum showed a positive Cotton effect at 350 nm and a negative one at 290 nm, consistent with the *S*-configuration at C-2.[5—7] On the basis of the data obtained, compound **1** was assigned as (2*S*)-5,2′-dihydroxy-7-methoxy-8-(3-methylbut-2-enyl)-2‴,2‴-dimethylpyrano[5‴,6‴: 5′,4′] flavanone, named maackiaflavanone A.

Compound **2** was isolated as a pale yellow gum. A molecular formula of $C_{30}H_{34}O_5$ was determined for this compound from the quasimolecular ion peak at *m*/*z* 475.2480 $[M+H]^+$. The UV (λ_{max} 293 nm), ^{1}H- and ^{13}C-NMR spectra (Table 1) indicated that **2** was also a flavanone. The ^{1}H- and ^{13}C-NMR spectra (Table 1) further showed the presence of two hydroxyl groups, two isoprenyl groups and a 2,2-dimethylpyran ring unit. The locations of the substituents were arranged by the HMQC and HMBC experiments (Fig. 2). The correlations of the proton of –OH (δ 12.14) to C-6 (δ 96.4), and C-10 (δ 103.3) in the HMBC spectrum and δ 6.03 to C-6 in the HMQC spectrum confirmed that the hydroxyl group was attached to C-5 while the singlet aromatic proton was assigned to H-6. HMBC correlations of H-1″ (δ 3.23) to C-7 (δ 164.9) and C-9 (δ 161.0) indicated one isoprenyl group was located at C-8. HMQC correlations of δ 7.21 (1H, d, J=1.8 Hz) to δ

128.5 and δ 7.08 (1H, d, J=1.8 Hz) to δ 123.2, together with long-range correlations from H-2 (δ 5.40) to C-2′ (δ 128.5) and C-6′ (δ 123.2), indicated that the two meta-coupled protons were at the positions of C-2′ and 6′ of B-ring separately. The correlations of H-1‴ (δ 3.30) to C-2′ (δ 128.5) and C-4′ (δ 151.4) in the HMBC spectrum indicated another isoprenyl group was at C-3′, whereas the long range correlations of H-4‴ (δ 6.42) to C-4′ and C-6′ (δ 123.2) suggested that the dimethylpyran ring was fused to C-5′ and C-4′. The left hydroxyl group (δ 9.55, 1H, s) could only be attached at C-7 (δ 164.9) of A-ring. The absolute stereochemistry of **2** ($[\alpha]_D^{20}$ −34.7°) was deduced to be 2*S* because of its almost same specific rotation value with that of (2*S*)-5,7-dihydroxy-3′-(3-methylbut-2-enyl)-2″,2″-dimethylpyrano[5″,6″ : 5′,4′] flavanone.[8] Thus **2** was assigned as (2*S*)-5,7-dihydroxy-3′,8-di(3-methylbut-2-enyl)-2″,2″-dimethylpyrano[5″,6″ : 5′,4′] flavanone, named maackiaflavanone B.

Compound **3** was isolated as a pale yellow solid. The molecular formula of $C_{30}H_{32}O_5$ was determined from the quasi-molecular ion peak at *m/z* 473.2326 $[M+H]^+$. The IR spectrum showed a broad band of O–H stretching at 3444 cm^{-1} and a sharp band of C=O stretching at 1650 cm^{-1}. The ^{1}H-NMR spectrum (Table 1) showed the characteristic signal of H-2 of an isoflavone at δ 7.86 and a hydrogen-bonded hydroxy group at δ 13.16, together with typical signals due to two sets of isoprenyl groups and a 2,2-dimethylpyran ring unit. The ^{13}C-NMR spectrum further showed five oxygenated aromatic carbons at δ 159.1, 156.8, 151.9, 150.2 and 154.4. The locations of the substituents were elucidated on the basis of the HMQC and HMBC experiments (Fig. 2). In the HMBC spectrum, correlations from δ 13.16 to C-6 (δ 112.5) and C-10 (δ 105.3) confirmed that the hydroxyl group was attached to C-5. Cross peaks between H-1″ (δ 3.35) and C-5, C-7 (δ 156.8) indicated that the isoprenyl group was located at C-6. HMBC correlations of H-4‴ (δ 6.70) to C-7, C-9 (δ 150.2) and H-3‴ (δ 5.58) to C-8 (δ 100.4) indicated that the 2,2-dimethylpyran ring was fused to C-7 and C-8. The B-ring should then contain a hydroxyl and an isoprenyl group. In addition to the biogenentically expected oxygenation at C-4′ (δ 154.4), the placement of the other isoprenyl at C-3′ was confirmed from HMBC spectrum where H-1‴ (δ 3.40) correlates with C-2′ (δ 130.3) and C-4′. Therefore, the structure of compound **3** was elucidated as 5,4′-dihydroxy-3′,6-di(3-methylbut-2-enyl)-2‴,2‴-dimethylpyrano[6‴,5‴ : 7,8] isoflavone, named maackiapentone.

Compound **4**, obtained as a white amorphous powder, was assigned the molecular formula as $C_{21}H_{20}O_6$ by HR-ESI-MS *m/z* 369.1327 $[M+H]^+$. The IR spectrum showed a broad band of O–H stretching at 3454 cm^{-1}. In the ^{1}H-NMR spectrum (Table 2), a characteristic set of signals at δ 3.38 (1H, m, H-6a), 3.53 (1H, t-like, J=11.2 Hz, H-6β), 4.16 (1H, dd, J=10.8, 5.0 Hz, H-6α) and 5.45 (1H, d, J=6.6 Hz, H-11a), together with the characteristic carbon signals at δ 66.1 (C-6), 40.2 (C-6a) and 77.3 (C-11a) in the ^{13}C-NMR suggested that **4** had a pterocarpan skeleton. A methylenedioxy group (δ 5.90, 5.93, each 1H) and three singlet aromatic protons (δ 6.42, 6.73, 6.43) were also observed in the downfield of the ^{1}H-NMR spectrum. The ^{1}H-NMR spectrum signals at δ 3.06, 2.76 (2H, each m), 1.93, 1.85 (2H, each m), 1.38 (3H, s) and 1.37 (3H, s), combined with the ^{13}C-NMR (δ 74.8, 32.8, 19.6, 26.2, 27.2), HMQC and HMBC (Fig. 2) spectrum, indicated the presence of a 2,2-dimethyl-3,4-dihydro-2*H*-pyran group in **4**. The positions of the pyran ring and the methylenedioxy group were established by the HMQC and HMBC experiments (Fig. 2) and by comparison NMR data with those of related (−)-2,3-dihydroxy-8,9-methylenedioxy pterocarpan.[9] HMBC correlations of H-4′ (δ 3.06, 2.76) to C-11b (δ 108.6) and C-2 (δ 135.9), and H-3′ (δ 1.93, 1.85) to C-1 indicated the pyran ring was fused to C-1 and C-2. The methylenedioxy group was positioned to C-8 (δ 141.5) and C-9 (δ 147.9) from the HMBC correlations between δ 5.90 and 5.93 to C-8 and C-9. According to the literature,[10,11] the absolute configuration at C-6a and C-11a was assigned as 6a*R*,11a*R* because of the coupling constant $J_{6a/11a}$ (6.6 Hz) and the highly negative optical rotation value (−240°), therefore, **4** was determined to be 3-hydroxy-8,9-methylenedioxy-[2′,2′-dimethyl-3′,4′-dihydropyrano-(5′,6′ : 1,2)][6a*R*,11a*R*]-pterocarpan, named maackiapterocarpan A.

Table 2. NMR Spectroscopic Data (600 MHz) for Maackiapterocarpan A (**4**) and Maackiapterocarpan B (**5**) in $CDCl_3$

Position	**4**		**5**	
	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
1	118.0		117.9	
2	135.9		134.3	
3	146.7		146.1	
4	101.2	6.42, s	103.1	6.43, s
4a	149.3		149.9	
6	66.1	4.16, dd (10.8, 5.0) 3.53, t-like (11.2)	66.2	4.16, dd (10.8, 5.0) 3.55, t-like (11.2)
6a	40.2	3.38, m	40.1	3.40, m
6b	121.4		120.8	
7	104.7	6.73, s	104.7	6.72, s
8	141.5		141.6	
9	147.9		148.0	
10	93.7	6.43, s	93.8	6.45, s
10a	154.1		154.2	
11a	77.3	5.45, d (6.6)	77.2	5.52, d (6.6)
11b	108.6		106.7	
2′	74.8		76.8	
3′	32.8	1.93, 1.85, each m	132.0	5.76, d (10.2)
4′	19.6	3.06, 2.76, each m	119.2	6.74, d (10.2)
7′	26.2	1.38, s	27.6	1.48, s
8′	27.1	1.37, s	27.7	1.46, s
OCH_2O	101.0	5.93, 5.90, each d (1.3)	101.2	5.93, 5.90, each d (1.3)

Compound **5** was also obtained as a white amorphous powder. The HR-ESI-MS *m/z* 367.1182 $[M+H]^+$ confirmed the molecular formula as $C_{21}H_{18}O_6$. The ^{1}H- and ^{13}C-NMR data of **5** were very similar to those of **4** (Table 2). Comparing with **4**, only did the chemical shifts of 3′ and 4′ move to downfield, from δ 32.8 to δ 132.0 for C-3′ and from δ 19.6 to δ 119.2 for C-4′ in ^{13}C-NMR spectrum, and the others were almost the same. It demonstrated that the 2,2-dimethyl-3,4-dihydro-2*H*-pyran ring was replaced by a 2,2-dimethyl-2*H*-pyran ring, which was also proved by the proton signals at δ 6.74 (1H, d, J=10.2 Hz, H-3′) and δ 5.76 (1H, d, J=10.2 Hz, H-4′). The absolute configuration at C-6a and C-11a was assigned likewise as 6a*R*,11a*R* for the coupling constant $J_{6a/11a}$ (6.6 Hz) and the highly negative optical rotation value (−233°), therefore, **5** was determined to be 3-hydroxy-8,9-methylenedioxy-2′,2′-dimethyl-pyrano[5′,6′ : 1,2]-[6a*R*,11a*R*]pterocarpan, named maackiapterocarpan B.

The known prenylated flavonoids, euchrenone b_1 (**6**),[12]

Table 3. Growth Inhibition Effects of Different Samples on A375S2, HeLa, MCF-7 and HepG2 Cells[a,b]

Compound	IC_{50} (μM)[a]			
	A375S2	Hela	MCF-7	HepG2
Maackiaflavanone A (**1**)	35.1±0.8	27.7±1.1	39.2±0.9	40.3±0.7
Maakiaflavanone B (**2**)	7.8±1.4	36.8±1.0	16.8±1.2	37.4±0.9
(−)-Sigmoidin E (**12**)	9.2±1.8	28.8±1.6	74.2±2.0	91.8±2.1
Abyssinone IV (**11**)	15.5±1.7	14.3±2.2	>100	91.0±2.4
Abyssinone V (**10**)	29.3±1.9	36.2±2.5	95.9±2.1	>100
5-Hydroxysophoranone (**9**)	20.8±2.1	39.8±1.8	80.3±1.5	90.2±1.4
Maackiaflavanone (**14**)	58.4±1.6	42.1±1.3	>100	>100
Euchrenone b_1 (**6**)	14.8±1.2	4.5±0.9	13.1±1.1	32.9±1.5
Maackiapentone (**3**)	67.1±1.5	25.5±1.1	47.6±1.7	47.0±2.0
Ulexone A (**8**)	63.7±1.8	33.2±2.2	>100	>100
Maackiapterocarpan A (**4**)	12.2±1.3	7.6±1.0	66.2±1.7	85.6±1.4
(−)-Maackiain (**16**)	25.8±2.1	15.6±1.5	>100	>100
(−)-Medicarpin (**15**)	93.8±1.6	78.5±2.4	>100	>100
5-FU[b]	9.6±0.2	50.9±0.4	48.2±0.2	33.6±0.5

a) Each value represents the mean±S.D. of three replicates. *b*) Positive control.

ulexone B (**7**),[13] ulexone A (**8**),[13,14] 5-hydroxysophoranone (**9**),[4] abyssinone V (**10**),[7] abyssinone IV (**11**),[15] (−)-sigmoidin E (**12**),[8] abyssinone II (**13**),[5] maackiaflavanone (**14**),[4] (−)-medicarpin (**15**),[16] (−)-maackiain (**16**),[17] were identified by comparison of their physical and spectroscopic data reported in the literature. Compounds **6**—**8**, **10**—**13** were isolated from this genus for the first time.

The cytotoxic activitiy was shown in Table 3. All the three kinds of prenylated flavonoids demonstrated cytotoxicity against the four human cancer cell lines and their inhibitory activities against A375S2 and HeLa cells were generally more potent than those against MCF-7 and HepG2 cells. Among seven tested prenylated flavanones, compound **2**, with double isoprenyl groups at 8-position of the A-ring and 3′-position of the B-ring separately and a dimethylpyran ring fused to B-ring, was proved to be the most active against A375S2, MCF-7 and HepG2 cells, with IC_{50} values of 7.8, 16.8 and 37.4 μM, respectively. Among the three tested prenylated isoflavones, euchrenone b_1, which has genistein nucleus and three free isoprenyl groups at 6- and 8-positons of A-ring and 3′-position of B-ring, exhibited the most potent inhibitory activities with IC_{50} values of 14.8 μM for A375S2, 4.5 μM for Hela, 13.1 μM for MCF-7 and 32.9 μM for HepG2, respectively. Otherwise, compound **3** exhibited a significantly lower inhibitory activity than euchrenone b_1, suggesting that cyclization of isoprenyl groups in the A or B-ring may be responsible for the reduction of *in vitro* activity for prenylated isoflavones. The prenylated pterocarpan, compound **4**, showed more potent cytotoxicity than non-prenylated pterocarpans, such as (−)-maackiain and (−)-medicarpin.

Experimental

General Experimental Procedures Optical rotations were measured in MeOH at 25 °C on a Perkin 241 polarimeter. UV spectra were taken in MeOH using a SHIMADZU UV-2201 spectrometer. The CD spectrum was recorded in MeOH on a CD-2095 (JASCO) and IR was measured on a Bruker IFS55 spectrometer. NMR spectra were recorded on Bruker-ARX-300 or ARX-600 NMR spectrometer, and TMS was used as an internal standard. High resolution FTICR-MS was performed on Fourier transform ion cyclotron resonance mass spectrometry (Varian 7.0T) and ESI-MS was performed on Finnigan LCQ mass spectrometer. Column chromatography was conducted using silica gel (100—200 mesh, Qingdao Marine Chemical Co., China), Sephadex LH-20 (Sigma) and ODS (75 μm, YMC). Preparative TLC was carried out on Kieselgel 60 GF_{254} (Merck). HPLC runs were carried out using a Shimadzu system LC-10AD pump equipped with a model SPD-10Avp UV detector and a Phenomenex C_{18} column (10×250 mm, 5 μm) for semipreparation.

Plant Material The stem bark of *M. amurensis* was collected in Benxi, Liaoning province, China, in May 2004, and identified by Weining Wang, Deputy director of pharmacist, Liaoning Provincial Institute Control of Food and Drug Products. A voucher specimen (No. 2004510) is deposited at the Department of Pharmacognosy, Shenyang Pharmaceutical University, Shenyang, China.

Extraction and Isolation The air-dried stem bark (9.0 kg) of *M. amurensis* was refluxed with 70% ethanol (81×4) and the extract was concentrated under vacuum to afford a viscous residue (1800 g). The residue was suspended in water and partitioned successively with petroleum ether, EtOAc and *n*-BuOH in the same volume for three times. The petroleum ether extract (120 g) was chromatographed on a silica gel column (800 g, 100—200 mesh), using a gradient of petroleum ether–CH_2Cl_2 (10 : 1, 10 : 4, 1 : 1), then CH_2Cl_2–EtOAc (100 : 1, 50 : 1, 30 : 1, 10 : 1, 10 : 3, 1 : 1), to yield nine fractions (Fr. 1—9). Fr. 3 (2.4 g) was subjected to Sephadex LH-20 column chromatography eluting with CH_2Cl_2–MeOH (1 : 1) repeatedly to reduce the mass of oil. All the subfractions contained flavonoids (according to TLC behavior) were combined together, then subjected to Sephadex LH-20 column chromatography again, eluted with MeOH, and finally afforded 5 subfrations (Fr. 3-1—3-5). Fr. 3-1 (0.1 g) was prepared with preparative TLC (hexane–EtOAc, 10 : 1) to give ulexone A (**8**, 1.5 mg), ulexone B (**7**, 3 mg). Fr.3-2 (0.3 g) was purified by ODS C_{18} chromatography (MeOH–H_2O, 80 : 20) to give 5-hydroxysophoranone (**9**, 30 mg). Fr. 3-3 (0.2 g) was prepared with high performance TLC (hexane–acetone, 10 : 3) to obtain compound **3** (5 mg), euchrenone b_1 (**6**, 3 mg). Fr. 3-4 (0.4 g) was purified by preparative TLC (CH_2Cl_2–EtOAc, 50 : 1) and semipreparative HPLC (MeOH–H_2O, 75 : 25) to obtain compound **4** (4.5 mg) and compound **5** (1.5 mg). Fr. 4 (6.4 g) was chromatographed on a Sephadex LH-20 column, eluted with CH_2Cl_2–MeOH (1 : 1) repeatedly, to reduce the mass of oil, then eluted with MeOH and finally afforded 6 subfractions (Fr. 4-1—4-6). Fr. 4-2 (1.3 g) was purified by ODS C_{18} chromatography, using MeOH–H_2O (80 : 20 to 90 : 10) to give compound **2** (20 mg). Fr. 4-3 (1.5 g) and Fr. 4-4 (2.2 g) were combined together, then chromatographed on a Sephadex LH-20 column (MeOH) and on a ODS C_{18} column (MeOH–H_2O, 80 : 20 to 85 : 15), to yield compound **1** (15 mg), abyssinone V (**10**, 20 mg), abyssinone II (**13**, 2 mg), abyssinone IV (**11**, 8 mg), (−)-sigmoidin E (**12**, 30 mg), (−)-maackiain (**16**, 11 mg) and (−)-medicarpin (**15**, 25 mg). Fr. 5 (4.8 g) was repeatedly chromatographed on a Sephadex LH-20 column (MeOH) to give maackiaflavanone (**14**, 40 mg).

Maackiaflavanone A (**1**): White amorphous solid ($CHCl_3$); $[\alpha]_D^{20}$ −67.3° (c=0.1, MeOH); UV (MeOH) λ_{max} 289 nm; IR (KBr) ν_{max} cm^{-1} 3443 (broad), 2925, 1634, 1502, 1445, 1383, 1206, 1167, 1106, 1073; CD (c=0.05, MeOH; nm): $[\theta]_{350}$ +0.92, $[\theta]_{290}$ −6.20; ^{1}H- and ^{13}C-NMR data, see Table 1; HR-ESI-MS *m/z* 437.1968 $[M+H]^+$ (Calcd for $C_{26}H_{28}O_6H$, 437.1959); ESI-MS *m/z*: 437.3 $[M+1]^+$, 435.3 $[M-1]^-$, 873.8 $[2M+1]^+$, 871.6 $[2M-1]^-$.

Maackiaflavanone B (**2**): Pale yellow gum ($CHCl_3$); $[\alpha]_D^{20}$ −34.7° (c=0.2, MeOH), UV (MeOH) λ_{max} 293 nm; IR (KBr) ν_{max} cm^{-1} 3420, 2973, 2923, 1637, 1505, 1435, 1383, 1170, 1148, 1077; ^{1}H- and ^{13}C-NMR data, see Table 1; ESI-MS *m/z*: 475.4 $[M+1]^+$, 473.4 $[M-1]^-$; HR-ESI-MS *m/z* 475.2480 $[M+H]^+$ (Calcd for $C_{30}H_{34}O_5H$, 475.2476).

Maackiapentone (**3**): Pale yellow amorphous solid ($CHCl_3$); UV (MeOH) λ_{max} 273 nm; IR (KBr) ν_{max} cm^{-1} 3444, 2922, 1650, 1560, 1507, 1434, 1384, 1254, 1117; ^{1}H- and ^{13}C-NMR data, see Table 1; HR-ESI-MS *m/z*: 473.2326 $[M+H]^+$ (Calcd for $C_{30}H_{32}O_5H$, 473.2322).

Maackiapterocarpan A (**4**): White amorphous powder (MeOH); $[\alpha]_D^{20}$ −240° (c=1.5, MeOH); UV (MeOH) λ_{max} 304 nm; IR (KBr) ν_{max} cm^{-1} 3454, 2925, 1612, 1473, 1384, 1135, 1035, 925, 847; ^{1}H- and ^{13}C-NMR data see Table 2; ESI-MS *m/z*: 369.3 $[M+1]^+$, 368.3 $[M-1]^-$; HR-ESI-MS *m/z* 369.1327 $[M+H]^+$ (Calcd for $C_{21}H_{20}O_6H$, 369.1333).

Maackiapterocarpan B (**5**): White amorphous powder (MeOH); $[\alpha]_D^{20}$ −233° (c=1.1, MeOH); UV (MeOH) λ_{max} 311 nm; ^{1}H- and ^{13}C-NMR data, see Table 2; ESI-MS *m/z*: 367.3 $[M+1]^+$, 365.3 $[M-1]^-$; HR-ESI-MS *m/z* 367.1182 $[M+H]^+$ (Calcd for $C_{21}H_{18}O_6H$, 367.1176).

***In Vitro* Cytotoxicity Assay** The cytotoxic activities of the compounds were evaluated *in vitro* by measuring cell viability by the MTT assay, with 5-FU as the positive control. The Hela, A375S2, MCF-7 and HepG2 cells were seeded in RPMI 1640 medium (100 μl) in a 96 well plate at a concen-

tration of 5×10^4 cells per well, after cultured for 24 h at 37 °C in 5% CO_2, cells were incubated with various concentrations of the samples for 48 h, then MTT was added at a terminal concentration of 5 μg/ml and incubated for 4 h. The formazan crystals were dissolved in DMSO (150 μl) in each well and the optical density was mearsured at 492 nm (for the absorbance of MTT formazan) and 630 nm (for the reference wavelength). Each compound was tested in triplicate at every concentration.

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