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Diacylglycerol Acyltransferase-inhibitory Prenylated Flavonoids from *Maackia amurensis* Rupr

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The *Maackia amurensis* RUPR. *et* MAXIM. (Leguminosae) is a deciduous tree distributed widely in the northeast of China and Korea. The dried stem bark of this plant has been used as folk medicine for the treatment of cancer, cholecystitis and arthritis. Its constituents have been shown for antimicrobial, antiviral, hypolipidemic, antithrombogenic, and antiplatelet activities.[1-3] Flavonoids, alkaloids and polyphenolic have been reported as main constituents of this genus.[4] As a part of our exploration on the medicinal plant, the MeOH extract of the stem bark of this plant was studied. One new prenylated flavanone, (5,4',7-trihydroxy-3'-prenyl-5'-formyl flavanone), along with six known compounds (Fig. 1) were isolated.

This paper describes the isolation and structure elucidation of a new prenylated flavanone based on spectroscopic techniques including MS, 1D and 2D NMR, and evaluation of DGAT inhibitory properties of these compounds.

Compound **1** was obtained as a white powder with $[\alpha]_D^{25}$ −28.10 (c 0.1, MeOH). Its UV spectrum exhibited absorption maxima at 224, 289, and 326 nm. A molecular formula of $C_{21}H_{20}O_6$ was determined from the molecular ion peak at *m/z* 368.1261 $[M]^+$ obtained by HREIMS. The ^{1}H NMR spectrum of **1** showed three signals at δ 5.56 (1H, dd, J = 2.4, 12.4 Hz), 3.21 (1H, dd, J = 16.8, 12.4 Hz), and 2.81 (1H, dd, J = 2.4, 16.8 Hz) characteristic of H-2, H-3ax and H-3eq, respectively of a flavanone moiety.[13] The carbon resonances at δ_C 79.2, 43.1, and 196.7 in the ^{13}C NMR spectrum supported this observation. In addition, the ^{1}H and ^{13}C NMR spectra suggested the presence of an aldehyde group at δ_H 10.04 (1H, s) and the corresponding carbon signal at δ_C 198.3, and also a prenyl group from signaling assignable as H-1" (δ_H 3.40, br d, J = 7.2 Hz, 2H), H-2" (δ_H 5.35, m, 1H), H-4" (δ_H 1.72, br s, 3H) and H-5" (δ_H 1.72, br s, 3H). The HMBC correlations from the aldehyde proton to C-6' (δ_C 131.0), C-5' (δ_C 121.1) and C-4' (δ_C 160.1), and from proton signal of H-6' at δ_H 7.83 (1H, br s) to the aldehyde carbon (δ_C 198.3) indicated that the aldehyde group is located at C-5' (Fig. 2). The HMBC correlations from proton H-1" (δ_H 3.40, br d, J = 7.2 Hz) to C-2' (δ_C 135.9), C-3' (δ_C 131.3), C-4' (δ_C 160.1), and from H-2' (δ_H 7.70, br s) to C-1" (δ_C 28.1) indicated that the prenyl group was attached to C-3'. The configuration at C-2 was inferred to be *S* by the CD spectrum, which had a positive Cotton effect near 329 nm and a negative Cotton effect near 301 nm. These observations were indicative of a 2(*S*)-flavanone skeleton.[5] Thus, compound **1** was identified as a new compound, 2(*S*)-5,4',7-trihydroxy-3'-prenyl-5'-formyl flavanone. In addition, six compounds of previously known structures were elucidated as warangalone (**2**),[6] maackiaflavanone B (**3**),[7] maackiapentone (**4**),[7] warangalone4'-methyl ether (**5**),[8] abyssinin II (**6**),[9] and sigmoidin B (**7**),[10] respectively, by comparing their physical and spectroscopic data with those reported in the literature.

Triglycerides (TG) are the major storage form of energy in mammalian cells. However, excessive deposition of TG in various tissues leads to obesity and is associated with tissue dysfunction referred to as lipotoxicity.[11] Two DGAT enzymes, DGAT1 and DGAT2 are known to catalyze the final and only committed step in triglyceride synthesis. Studies using DGAT1-knockout mice showed that DGAT1 could be a more potent target for the treatment of obesity and type 2

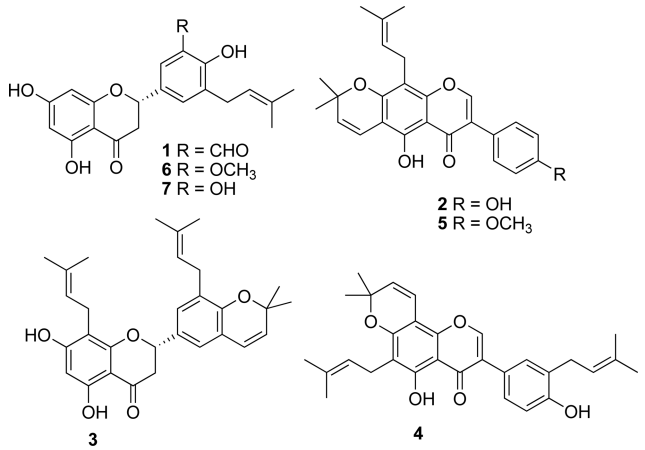

Figure 1. Structures of compounds **1**-**7**.

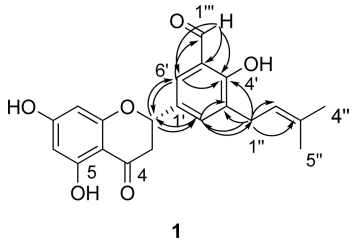

Figure 2. Key HMBC correlations of compound **1**.

diabetes. Bioactivity-guided fractionation of the MeOH extract of *M. amurensis*, using an *in vitro* DGAT1 inhibitory assay, yielded one new prenylated flavanone, three known flavanones (**3**, **6**, **7**) and three known isoflavones (**2**, **4**, **5**). All the isolates were assayed for their inhibitory activity against DGAT1, and the DGAT1 assay was accomplished in this study in the presence of 100 mM of $MgCl_2$, as $MgCl_2$ at this concentration did not detectably affect the increase in the DGAT2 activity.[12] The results are presented in Table 2. The known DGAT1 inhibitors, kuraridine (IC_{50} = 9.8 ± 0.3 mM),[12] were used as positive controls in this assay. Among the isolates, except for compounds **6**, **7**, inhibited DGAT1 activity in a dose-dependent manner with IC_{50} values ranging from 84.2 ± 1.3 to 131.7 ± 1.5 μM. Compounds **6**, **7**, which was fused as methoxy and hydroxyl group at C-5' in the B ring exhibited a significantly lower DGAT1 inhibitory activity than compound **1**, on which similar position was attached as aldehyde group.

Experimental Section

General Experimental Procedures. UV spectra were taken in MeOH using a Shimadzu spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained from a Varian Unity Inova 400 MHz spectrometer using acetone-d_6 as solvents, with TMS as the internal standard. All accurate mass experiments were performed on a Micromass QTOF2 (Micromass, UK) mass spectrometer. Column chromatography was conducted using silica gel 60, Sephadex LH-20 and RP-18 For thin-layer chromatography, precoated TLC silica gel 60 F_{254} plates from Merck were used. HPLC runs were carried out using a Shimadzu System LC-10AD pump equipped with a model SPD-10Avp UV detector, and an Optima Pak® C_{18} column (10 × 250 mm, 10 m particle size, RS Tech Korea).

Bovine serum albumin and Trizma-base were obtained from Sigma Chemical Co (ST. Louis, MO, USA). [1-^{14}C] oleoyl-CoA (250 μCi) was purchased from Amersham. For the cell culture, Dulbecco's modified eagle medium (DMEM), L-glutamine, kanamycin sulfate, and fetal bovine serum (FBS) were purchased from GIBCO-BRL (Gaithersburg, MD, USA).

Plant Material. The stem bark of *M. amurensis* (3.0 kg) was collected in Yanji, Jilin province, China, in May 2009. A voucher specimen of the plant (No. 2009510) was deposited at the College of Pharmacy, Beihua University, Jilin, China.

Extraction and Isolation. The stem bark (3.0 kg) of *M. amurensis* was extracted with MeOH at room temperature for 2 weeks and the solution was concentrated to obtain a crude extract (165.0 g, 66% inhibition at 30 μg/mL). This extract was suspended in H_2O, partitioned successively with $CHCl_3$. EtOAc and BuOH, and then the organic solvents were removed. A portion of the EtOAc-soluble fraction (10.0 g, 74% inhibition at 30 μg/mL) was chromatographed over a silica gel column using a gradient of $CHCl_3$–MeOH (from 20:1, 19:1 to 0:1), and was separated into 10 fractions (Fr.1–Fr.10). Fr.5 ($CHCl_3$–MeOH 10:1, 1.0 g, 77% inhibition at 30 μg/mL) was chromatographed over Silica gel, eluted with a stepwise gradient of CH_2Cl_2/MeOH (from 100:1, 80:1, 50:1 to 0:1) to afford 10 subfractions (Fr.5A–Fr.5J). Purification of Fr.5D (110.0 mg) by semipreparative HPLC using an gradient solvent system of 70-80% MeCN in H_2O over 60 min to yield compounds **2** (4.4 mg) and **7** (6.4 mg). Fr.5F (123.0 mg) was purified by preparative HPLC using an isocratic solvent system of 50% MeCN in H_2O over 30 min followed by 60% MeCN in H_2O over 70 min to obtain compounds **5** (5.9 mg), **6** (5.1 mg), and **3** (3.1 mg). Fr.6 (84% inhibition at 30 μg/mL) was subjected to an RP-18 column and was eluted with MeOH-H_2O (1:10, 1:2, to 10:1) to yield six fractions (F.6A-F.6F). The most active

Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data of compound **1** (acetone-d_6, δ, ppm, *J*/Hz)

Position	δ_H (*J* in Hz)	δ_C
2	5.56 dd (2.4 12.4)	79.2
3*eq*	2.81 dd (2.4 16.8)	
3*ax*	3.21 dd (12.4 16.8)	43.1
4		196.7
5		164.1
6	5.97 br s	96.1
7		165.1
8	5.99 br s	97.1
9		167.9
10		103.0
1′		131.5
2′	7.70 br s	135.9
3′		131.3
4′		160.1
5′		121.1
6′	7.83 br s	131.0
1″	3.40 br d (7.2)	28.1
2″	5.35 m	122.2
3″		134.0
4″	1.72 br s	17.9
5″	1.72 br s	25.9
1‴	10.04 s	198.3

[a]Chemical shifts in ppm relative to TMS; coupling constants (*J*) in Hz.

Table 2. The inhibitory activity of the isolated compounds **1**-**7** and related compounds against DGAT1

Compounds	DGAT1 inhibitory activity IC_{50} (μM)[a]
5,4′,7-trihydroxy-3-prenyl-5′-formyl flavanone (**1**)	84.2 ± 1.3
warangalone (**2**)	94.2 ± 1.1
maackiaflavanone B (**3**)	131.7 ± 1.5
maackiapentone (**4**)	122.4 ± 2.1
warangalone4'-methyl ether (**5**)	98.2 ± 1.7
abyssinin II (**6**)	> 200
sigmoidin B (**7**)	> 200
kuraridine[b]	9.8 ± 0.3

[a]IC_{50} values were determined by regression analyses and expressed as mean ± SD of three replicates. [b]Positive control.

fraction, Fr.6C (171.0 mg), was further separated by a Silica gel column eluted with $CHCl_3$-MeOH (40:1, 35:1, to 10:1) to yield 10 subfractions (Fr.6C1-Fr.6C10). Fr.6C8 was separated by HPLC, using a gradient of 20-30% MeCN in H_2O as the mobile phase to produce compounds **1** (3.1 mg) and **4** (4.9 mg).

Compound 1: White amorphous powder; $[\alpha]_D^{25}$ −28.10 (*c* 0.1, MeOH); UV (MeOH): λ_{max} (log ε) 224 (4.29), 289 (4.21), 326 (3.43) nm; CD (*c* 0.55 MeOH): $[\theta]_{329}$ +3.25, $[\theta]_{301}$ −5.30, $[\theta]_{215}$ +15.74; ^{1}H NMR (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, acetone-d_6) spectral data see Table 1; HR-EI-MS *m*/*z*: 368.1261 $[M]^+$ (calcd for $C_{21}H_{20}O_6$, 368.1260).

DGAT1 Assay. Preparation of microsomes from rat liver and measurement of the *in vitro* DGAT1 activity was measured as reported previously.[12]

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Supporting Information. The UV, CD and NMR spectral data of compound **1** are available as Supporting Information.

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