Diterpenoids and Limonoids from the Leaves and Twigs of Swietenia mahagoni

Wei-Ming Zhang · Jie-Qing Liu · Yuan-Yuan Deng · Jian-Jun Xia · Zhi-Run Zhang · Zhong-Rong Li · Ming-Hua Qiu

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Abstract Three new compounds, including two diterpenoids, nemoralisins H and I (1 and 2), and a limonoid, 2-methoxy khayseneganin E (3), along with four known constituents (4–7), were isolated from the leaves and twigs of Swietenia mahagoni. Their chemical structures were elucidated by means of spectroscopic analysis. The cytotoxities of these isolated constituents were assayed.

Keywords Meliaceae · Swietenia mahagoni · Diterpenoids · Limonoids

1 Introduction

Swietenia mahagoni, which is an economically important timber tree, has been used as a folk medicine for the treatment of hypertension, diabetes, and malaria [1, 2]. Chemical investigations conducted previously on S. mahagoni had led to the isolation of various B,D-seco limonoids such as mexicanolides, phragmalins, andirobins, gedunins, and rearranged phragmalins [3–7]. Modern pharmacological studies demonstrated that limonoids from Swietenia displayed insecticide, antitumor, antibacterial, antidiabetic, and antidyslipidemic activities [8–12].

With the aim of searching for structurally unique and bioactive chemical constituents, we isolated the leaves and twigs of S. mahagoni and gained two new diterpenoids (1 and 2), one new limonoid (3) and four known compounds (4–7) (Fig. 1). In this paper, we reported the isolation and structural elucidation of new compounds, as well as cytotoxicities of all the compounds against five human cancer cell lines.

2 Results and Discussion

The air-dried powder of leaves and twigs of S. mahagoni was extracted with 70% aqueous acetone at room temperature three times to give the residue, which was then partitioned between EtOAc and water to get the EtOAc-soluble fraction. Then, three new constituents together with four known compounds were acquired by a series of chromatographic methods. Herein, we described the isolation and structural elucidation of these new compounds.

Compound 1, colorless oil, was assigned a molecular formula C20H28O5 according to its positive HREIMS peak at m/z 348.1932 [M]⁺ (calcd for 348.1937), suggesting seven degrees of unsaturation. The IR spectrum revealed characteristic bands corresponding to hydroxyl (3,434 cm⁻¹), α,β-un satu rated-δ-lactone (1,761 cm⁻¹) and double-bond (1,639 cm⁻¹) groups. The ¹H NMR spectrum of 1 (Table 1)
showed the presence of three typical olefinic protons $\delta_H$ (5.80, s-like; 5.42, s; 5.26, dd, $J = 8.7, 1.2$ Hz), three methyl singlets at $\delta_H 1.35$ (6H), 2.05 (3H) and two methyl doublets at $\delta_H (1.23, J = 7.0$ Hz; 1.75, $J = 1.2$ Hz). The $^{13}$C DEPT displayed five methyls, three methylenes, six methines, and six quaternary carbons (Table 1). 1D-NMR data of 1
exhibited high resemblance with nemoralisin A isolated from Aphanamixis grandifolia (Meliaceae) [13], and they showed the same molecular formula and the similar skeletal structure (α,β-unsaturated ketone was connected with one α,β-unsaturated δ-lactone by an aliphatic chain). The only difference between 1 and nemoralisin A was that the hydroxyl group located at C-4 in 1 but at C-8 in nemoralisin A. And this was confirmed by HMBC correlations of H-4 (δH 4.04, d, J = 7.2 Hz) with C-2 (δC 117.1), C-3 (δC 162.6), C-5 (δC 80.5), C-6 (δC 121.9), and of Me-20 (δH 2.05) with C-4 (δC 89.3) (Fig. 2).

In the ROEY spectrum, the strong correlation from H-6 (δH 5.26, dd, J = 8.7, 1.2 Hz) to H-8 (δH 2.10, m) inferred E-geometry of the Δ6 double bond. This was confirmed by the upfield resonance of vinylic Me-19 group at δC 17.1 [14]. The ROEY correlations of H-4/H-6 and H-5/Me-19 (δH 1.75, d, J = 1.2 Hz) suggested that H-4 and H-5 was trans oriented. However, the absolute configuration was not determined on the basis of 1D and 2D NMR data. Accordingly, compound 1 was established and named as nemoralisin H.

Compound 2 was isolated as colorless oil. The molecular formula of C28H36O11 was determined by the positive ion peak at m/z 548.2250 [M]+ (calcd for 548.2258) in the HREIMS. Extensive analysis of the 1D-NMR spectroscopic data (Table 1) of 2 exhibited a close resemblance with nemoralisin [14]. However, downfield shifts of the lactone carbon (δC 176.3), the β-sp2 carbon (δC 170.7), and the oxygenated methine (δC 89.3) were observed in the 13C DEPT spectrum of 2, which implied the α,β-unsaturated δ-lactone (ring A) in nemoralisin was replaced by an α,β-unsaturated γ-lactone in 2. This deduction was confirmed by the observed HMBC correlations of H-4 (δH 4.89) with C-1 (δC 176.3), C-2 (δC 118.9), C-3 (δC 170.7), C-6 (δC 124.3), and of Me-20 (δH 2.13) with C-4 (δC 89.3) (Fig. 2). Meanwhile, on the basis of 1H-1H COSY correlations of H-4/H-5/H-6, a hydroxyl group at C-5 and nemoralisin A was that the hydroxyl group located at C-4 in 1 but at C-8 in nemoralisin A. And this was confirmed by HMBC correlations of H-4 (δH 4.04, d, J = 7.2 Hz) with C-2 (δC 117.1), C-3 (δC 162.6), C-5 (δC 80.5), C-6 (δC 121.9), and of Me-20 (δH 2.05) with C-4 (δC 89.3) (Fig. 2). Meanwhile, on the basis of 1H-1H COSY correlations of H-4/H-5/H-6, a hydroxyl group at C-5 and Δ6 double bond were established. The ROEY correlations of H-6 (δH 5.20, d, J = 8.8 Hz) with H-8 (δH 2.03, m) inferred E-geometry of the Δ6 double bond. Therefore, the structure of 2 was determined and gave the name nemoralisin I.

Compound 3, white amorphous powder, was found to possess the molecular formula of C28H36O11 as inferred by HREIMS peak at m/z 548.2250 [M]+ (calcd for 548.2258). The 1D NMR data (Table 2) of 3 indicated that it was a phragmalin-type limonoid and similar with khayseneganin E [15–17]. However, a methoxy in 3 replaced the hydroxyl group at C-2 in khayseneganin E, which was supported by

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**Table 1**  1H and 13C-NMR spectroscopic data of compounds 1 and 2 (δ in ppm)

| Pos. | 1 | 2 |
|------|---|---|
| δH (J in Hz) | δC | δH (J in Hz) | δC |
| 1 | 166.3 s | 176.3 s | 5.80 (s-like) | 111.7 d | 5.83 (s) | 118.9 d |
| 2 | 162.6 s | 170.7 s | 4.04 (d, 7.2) | 69.7 d | 4.89 (overlap) | 89.3 d |
| 3 | 4.99 (dd, 8.7, 7.2) | 80.5 d | 4.70 (dd, 8.8, 3.1) | 68.2 d |
| 4 | 5.26 (dd, 8.7, 1.2) | 121.9 d | 5.20 (d, 8.8) | 124.3 d |
| 7 | 145.4 s | 142.1 s | 1.64 (m, 1.52) | 34.7 t | 1.60 (m, 1.49) | 35.0 t |
| 8 | 2.10 (m, 2.10 (m) | 40.5 t | 2.03 (m) | 2.03 (m) | 40.7 t |
| 9 | 1.49 (m), 1.49 (m) | 25.9 t | 1.44 (m), 1.44 (m) | 26.5 t |
| 10 | 1.23 (d, 7.0) | 18.3 q | 1.20 (d, 6.9) | 18.4 q |
| 11 | 1.75 (d, 1.2) | 17.1 q | 1.68 (s) | 17.0 q |
| 12 | 2.05 (s) | 19.9 q | 2.13 (s) | 15.0 q |

**Table 2**  1H and 13C-NMR spectroscopic data of compound 3 (δ in ppm)

| Pos. | δH (J in Hz) | δC | Pos. | δH (J in Hz) | δC |
|------|-------------|----|------|-------------|----|
| 1 | 85.1 s | 15 | 3.82 (d, 18.3), 4.20 | 37.8 t |
| 2 | 106.9 s | 16 | 172.4 s |
| 3 | 3.78 (d, 5.9) | 84.7 d | 6.26 (s) | 81.7 d |
| 4 | 43.7 s | 18 | 1.39 (s) | 16.3 q |
| 5 | 3.50 (d, 8.4) | 41.4 d | 1.89 (s) | 19.2 q |
| 6 | 4.74 (d, 8.4) | 72.3 d | 20.2 s | 122.7 s |
| 7 | 176.7 s | 21 | 7.55 (br.s) | 142.1 d |
| 8 | 87.9 s | 22 | 6.54 (br.s) | 111.5 d |
| 9 | 2.96 (d, 9.2) | 57.3 d | 23 | 7.62 (br.s) | 143.7 d |
| 10 | 61.3 s | 28 | 1.39 (s) | 20.6 q |
| 11 | 2.11 (m), 2.51 (m) | 17.7 t | 29 | 1.94 (d, 11.8), 2.39 | 46.0 t |
| 12 | 1.22 (m), 2.51 (m) | 28.3 t | 30 | 3.55 (s) | 74.5 d |
| 13 | 83.9 s | 38.9 s | OMe-7 | 3.73 (s) | 52.4 q |
| 14 | 84.7 s | 38.9 s | OMe-2 | 3.68 (s) | 51.2 q |

Recorded in C6D6N; 1H and 13C-DEPT Recorded at 600, 150 MHz
HMBC correlation between OMe (δH 3.68) and the ketal carbon (δC 106.9) (Fig. 2). The relative configuration of 3 was determined by analysis of the ROESY spectrum, in which correlations of H-5/H-6, H-5/H-12β, H-12β/H-17, and H-6/Me-28 suggested that these groups are all β-oriented. In addition, Me-19, Me-18, H-9, and H-3 were assigned as α-oriented on the basis of the ROESY correlations between Me-19/H-9 and between H2-29/H-3, Me-18/H-3, suggesting that OH-3 is β-oriented. Consequently, the structure of 3 was determined as shown, named 2-methoxy khaysenganin E.

Four known constituents: nemoralisin C (4) [13], khayanolide B (5) [18], khaysenanegian G (6) [17], and deacetylkhayanolide E (7) [19], were identified by the comparison of their spectroscopic data with those reported in the literature.

Compounds 1–7 were tested for in vitro inhibitory activities against HL-60, SMMC-7721, A549, MCF-7 and SW480 human tumor cell lines. All the tested samples showed no activities against the mentioned cell lines with IC50 > 40 μM. Much to our delight, Liu J. et al. [20] reported that aphadilactones A–D, which can be considered as the dimers of diterpenoid compounds (1, 2 and 4) showed potent and selective inhibition against the diacylglycerol O-acyltransferase-1 (DGAT-1) enzyme, and are the strongest natural DGAT-1 inhibitors discovered to date. So emphasis can be laid on this class of compounds in our future search of potential DGAT-1 inhibitors.

3 Experiment Section

3.1 General Experimental Procedures

Optical rotations were obtained with a Jasco P-1020 polarimeter. UV (in MeOH) and IR (in CHCl3) spectra were measured on Shimadzu UV-2401 PC spectrophotometer and Bruker Tensor-27 infrared spectrophotometer, respectively. ESIMS spectra were recorded on an API QSTAR Pulsar spectrometer. EIMS and HREIMS were performed on a Waters Autospec Premier P776. 1D and 2D NMR spectra were recorded on Bruker DRX-500 and Bruker Avance III-600 MHz spectrometers. Chemical shifts (δ) were expressed in ppm with reference to the TMS resonance. Semi-preparative HPLC studies were carried out on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 (9.4 mm × 25 cm) column. Column chromatography was performed using Silica gel [(200–300) mesh, Qingdao Marine Chemical, Inc, Qingdao, China]. Fractions were monitored by TLC and spots were visualized by heating the silica gel plates sprayed with 10 % H2SO4 in EtOH. Lichroprep RP-18 [(40–63) μm, Merck] and Sephadex LH-20 [(20–150) μm, Pharmacia] were also used for column chromatography.

3.2 Plant Material

The leaves and twigs of S. mahagoni were collected from Xishuangbanna, Yunnan Province, China. A voucher sample has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and Isolation

The air-dried powdered leaves and twigs of S. mahagoni (10 kg) were extracted with 70 % aqueous acetone (each 30 L, 3 days) at room temperature three times to give a dark green residue (650 g), which was then partitioned between EtOAc and water to give the EtOAc-soluble fraction (120 g). The EtOAc extract was chromatographed by silica gel column eluted with CHCl3–MeOH as a gradient (100:1, 50:1, 20:1, 5:1) to afford four fractions. The CHCl3–MeOH (100:1) portion was evaporated to obtain a residue (20 g), which was subjected to silica gel chromatography column with petroleum ether–EtOAc (10:1, 6:1, 3:1, 1:1) as elution, to give fractions (A, B, C, D). Fraction B was further subjected to RP-18 chromatographic column, eluting with MeOH–H2O (50:50, 65:35, 80:20) to afford subfraction (E), which was then purified by semi-preparation HPLC with MeCN–H2O (50:50) to give compound 3 (6 mg), 5 (12 mg), 6 (10 mg), 7 (15 mg). Fraction C was subjected to silica gel chromatograph column with petroleum ether–EtOAc (8:1, 5:1, 3:1, 1:1) as elution, to give fractions F, which was successively subjected to RP-18, Sephadex LH-20 and semi-preparation HPLC, compound 1 (1.5 mg), 2 (1.3 mg), and 4 (6 mg) were obtained.

3.4 Nemoralisin H (1)

Colorless oil; [α]D 25 -0.48 (c 0.11, MeOH); UV (MeOH) λmax (log ε) 202 (3.75), 261(3.37) nm; IR (KBr) νmax 3434, 2976, 2930, 1761, 1639, 1585, 1457, 1383, 1270, 1177, 1088, and 1036 cm⁻¹, 1H and 13C-DEPT data see Table 1; EIMS m/z 371 [M + Na]⁺; HREIMS m/z 348.1932 (calcd for C20H28O5 [M]⁺, 348.1937).

3.5 Nemoralisin I (2)

Colorless oil; [α]D 25 -14.3 (c 0.18, MeOH); UV (MeOH) λmax (log ε) 204 (3.87), 261(3.70) nm; IR (KBr) νmax 3432, 2977, 2933, 1761, 1698, 1584, 1458, 1383, 1299, 1267,
1177, 1034 and 938 cm\(^{-1}\), \(^1\)H and \(^{13}\)C-DEPT data see Table 1; ESIMS \(m/z\) 371 [M + Na]\(^+\); HREIMS \(m/z\) 348.1972 (calcd for C\(_{20}\)H\(_{30}\)O\(_5\) [M]\(^+\), 348.1937).

3.6 2-Methoxy khayseneganin E (3)

White amorphous powder; \([\alpha]\)\(_D\)\(^{18}\) + 26.3 (c 0.03, MeOH); UV (MeOH) \(\lambda_{\text{max}}(\text{log } e)\) 209 (3.83) nm; IR (KBr) \(v_{\text{max}}\) 3440, 2952, 1727, 1632, 1462, 1441, 1387, 1280, 1245, 1159, 1053, 1024, and 600 cm\(^{-1}\), ESIMS \(m/z\) 571 [M + Na]\(^+\); HREIMS \(m/z\) 548.2250 (calcd for C\(_{28}\)H\(_{36}\)O\(_{11}\) [M]\(^+\), 548.2258).

3.7 Cytotoxicity Assay

The cytotoxic activities of compounds 1–7 against HL-60, SMMC-7721, A549, MCF-7 and SW480 cell lines were determined by the MTT method [21].

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