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Two new tetracyclic triterpenoids from the barks of *Melia azedarach*

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1. Introduction

*Melia azedarach* Linn. (Meliaceae), a high tree, enjoys a broad distribution in the most parts of China [1]. The plant is well known for its pharmacological properties, such as analgesic, anticancer, antiviral, antimalarial, antibacterial, and antifeedant activities [2,3]. However, in recent years, much attention has been paid to *M. azedarach* L. due to the powerful insect antifeedant azadirachtin from *Azadirachta indica*, which is already produced commercially [4,5]. In order to search for naturally occurring insecticides from the barks of *M. azedarach* L., we evaluated the antifeedant effect of the EtOAc extract against *Spodoptera litura*. The appreciable activity against different instars larvae of *S. litura* encouraged us to carry on further studies on chemical constituents of the title plant. In the present paper, we report two new tetracyclic triterpenoids, tirucalla-5(6), 7, 24(25)-triene-3-dion-21,16-olide (1) and 24-hydroperoxytirucalla-7, 25(26)-diene-3, 6-dion-21, 16-olide (2), together with 21 known compounds, isolated from the barks of *M. azedarach*. The structures of new compounds were elucidated by means of HRESIMS, 1D NMR, 2D NMR, and X-ray crystallography analysis.

2. Results and discussion

Compound 1 was obtained as a colorless oil, \([\alpha]_D^20 + 80\) (c 0.20, CH\(_2\)Cl\(_2\)). Its molecular formula, C\(_{30}\)H\(_{42}\)O\(_3\), with 10 degrees of unsaturation, was established on the basis of quasi-molecular ion peak at \(m/z\) 449.2702 [M − H]\(^−\) in the HRESIMS. The IR (KBr) absorptions were presented at 1776 (\(\gamma\)-lactone), 1712 (C=O), and 1640 (conjugated double bond) cm\(^−1\), respectively. The UV absorption at 274 nm (log \(e\) 4.66) suggested an intra-cyclic conjugated...
double bond. The $^1$H NMR spectrum of compound 1 exhibited seven methyl groups, two of which were geminal methyl groups (δ_H 1.55, 1.62) attached to an olefinic carbon, which could be readily assigned by the HMBC correlations from H-26 (δ_H 1.55, s), H-27 (δ_H 1.62, s) to C-25 (δ_C 132.7), and three olefinic protons (δ_H 5.04, 5.56, 5.78) (Table 1). The $^{13}$C NMR spectrum showed signals of two carbonyl groups (δ_C 214.4, 180.5), and three trisubstituted double bonds (δ_C 115.5, 118.2, 123.4, 132.7, 143.7, 151.0). The remaining degrees
of unsaturation were attributed to five rings. In the HMBC spectrum, correlations of H-22 (δ_H 1.41–1.43, 1.88–1.89), H-20 (δ_H 2.47–2.49) with C-21 (δ_C 180.5) suggested a 20, 16-olide moiety as in kulac tone (compound 3) [6,7] (Figure 1). The 1H and 13C NMR spectral data of 1 and 3 were almost the same except for the presence of a double bond (δ_C 151.0, 118.2) and the absence of two carbon signals at high field in 1. The double bond was assigned at C-5/C-6 on the basis of the correlations of H-19 (δ_H 0.79, s), H-28 (δ_H 1.21, s), H-29 (δ_H 1.25, s), and H-7 (δ_H 5.56, dd, J = 5.5 Hz, 3.0 Hz) with C-5 (δ_C 151.0), and of H-7 (δ_H 5.56, dd, J = 5.5 Hz) with C-6 (δ_C 115.5) in HMBC spectrum, which was further supported by the correlation of H-7 (δ_H 5.56, dd, J = 5.5, 3 Hz) with H-6 (δ_H 5.78, d, J = 5.5 Hz) in the 1H-1H COSY spectrum. Moreover, correlations of H-6 (δ_H 5.78, d, J = 5.5 Hz) with C-8 (δ_C 143.7) and C-7 (δ_C 115.5) indicated that the double bond at C-5/C-6 was conjugated with that at C-7/C-8 (Figure 2). On the basis of their almost identical NMR spectral data, the relative stereochemistry of 1 was the same as that of 3 whose structure was determined by the analysis of the X-ray diffraction experiment of 3. Thus, compound 1 was defined as tirucalla-5 (6), 7, 24 (25)-triene-3-dion-21, 16-olide.

Compound 2 was isolated as colorless oil, [α]_D^20 +35 (c 0.20, CH_2Cl_2) and possessed a pseudomolecular ion peak at m/z 521.2866 [M + Na]^+, corresponding to the molecular formula C_{30}H_{42}O_{6} by HRESIMS, suggesting 10 degrees of unsaturation. The IR (KBr) spectrum showed absorptions at 1772 (γ-lactone), 1706 (C=O), and 1650 (conjugated double bond) cm⁻¹. UV absorption at 243 nm (log ε 4.76) was consistent with an α,β-unsaturated ketone chromophore [8]. In the 1H NMR spectrum of 2, the signals for six methyl groups, one of which was attached to an olefinic carbon, a hydroperoxymethine (δ_H 4.36), an exomethylene (δ_H 5.05, 5.07), a trisubstituted double bond (δ_H 5.80), were observed (Table 1). The 13C NMR and DEPT spectra of 2 demonstrated three carbonyl groups (δ_C 214.2, 197.7, 179.5), a trisubstituted double bond (δ_C 167.3, 124.7), and a terminal double bond (δ_C 142.9, 114.6). The remaining degrees of unsaturation were attributed to five rings. In the HMBC spectrum, correlations of H-22 (δ_H 1.51–1.56, 1.98–2.00) and H-20 (δ_H 2.50–2.54) with C-21 (δ_C 179.5) indicated the presence of 20, 16-olide moiety as in sendanolactone [8]. The difference between 2 and sendanolactone was the signals of the side chain. A signal at δ_H 8.16 (1H, br s) in the 1H NMR spectrum and the corresponding tertiary carbon (δ_C 89.1) in the 13C NMR spectrum, together with two remaining oxygen atoms in compound 2, displayed that 2 must have a hydroperoxy group in the side chain. Detailed HMBC spectrum suggested that the terminal double bond in 2 was at C-25/C-26 and the hydroperoxy group was placed at

Figure 2. Selected HMBC (1H → 13C) and 1H-1H COSY (—) correlations of compounds 1 and 2.
C-24, which was revealed by the correlations of H-27 ($\delta_H$ 1.76, s) with C-26 ($\delta_C$ 114.6), C-25 ($\delta_C$ 142.9) and of H-27 ($\delta_H$ 1.76, s) and H-26 ($\delta_H$ 5.05–5.06, 5.06–5.07) with C-24 ($\delta_C$ 89.1), and of H-24 ($\delta_H$ 4.36, t, $J$ = 6.5 Hz) with C-23 ($\delta_C$ 28.3). The correlation of H-27 ($\delta_H$ 1.76, s) with H-24 ($\delta_H$ 4.36, t, $J$ = 6.5 Hz) in $^1$H-$^1$H COSY spectrum reinforced the position of the terminal double bond and the hydroperoxy group in the side chain (Figure 2). Compound 2 was elucidated as 24-hydroperoxytirucalla-7, 25(26)-diene-3, 6-dion-21, 16-olide.

The known compounds were identified as kulactone (3), meliasenin J [9], meliasenin W [9], meliasenin M [10], kulinone [11], 16-hydroxybutyropermol [11], aphanigranin G [12], mesendanin M [13], mesendanin L [13], mesendanin R [13], meliastatin 3 [14], dubione B [14], 1,2-benzenedicarboxylic acid dibutyl ester [15], 1,2-benzenedicarboxylic acid disobutyl ester [15], methyl kulonate [16], (−)-12β-hydroxykulactone [17], vanillin [18], 4-hydroxy-3-methoxycinnamaldehyde [18], 24,25-Dihydrokulinone [19], creosol [20], and veratraldehyde [20] by comparison of spectral data with those reported in the literatures.

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on a Bruker PolAAr-3005 polarimeter (Optical Activity Ltd., Huntingdon, England). The UV spectra were obtained on a Shimadzu UV 240 spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were recorded in KBr discs on a Thermo Nicolet 200 double beam spectrophotometer (Thermo Electron, Inc., Madison, WI, USA). NMR spectra were recorded on a Bruker Avance-500 spectrometer (Bruker, Karlsruhe, Germany), with TMS as an internal standard. HR-ESI-MS were measured on Bruker MicrOTOF-QII instrument (Bruker Daltonics, Bremen, Germany). Semi-preparative HPLC was performed on a Shimadzu LC-20AT equipped with DAD detector, using an Alltima (C$_{18}$, 250 × 10 mm i.d., 5 μm) (Shimadzu, Kyoto, Japan). X-ray crystallography analysis was performed on Bruker APEX2 instrument (Bruker, Karlsruhe, Germany). Column chromatography (CC) was performed using silica gel (200–300 mesh) from Qingdao Haiyang Chemical Group Co. and Sephadex LH-20 from Pharmacia Biotech AB, Uppsala, Sweden. Thin-layer chromatography (TLC) was performed on pre-coated silica gel GF254 plates (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). All solvents and materials were reagent grade and purified as required.

3.2. Plant material

The barks of Melia azedarah L. were collected in Wenzhou, Zhejiang Province of China in July 2012, and identified by prof. Xianxian Chen, College of Life and Environmental Science, Wenzhou University. A voucher specimen (No.20120701) is maintained in College of Chemistry & Materials Engineering, Wenzhou University.

3.3. Extraction and isolation

The air-dried and powdered barks of M. azedarach (10.6 kg) were percolated with 95% aqueous ethanol for 7 days at room temperature for three times. After evaporation of the solvent under reduced pressure, the gummy residue was suspended in water and then partitioned with EtOAc.
The EtOAc extract (145 g) was subjected to CC on silica gel eluting with petroleum ether-EtOAc (from 1:0 to 0:1, v/v) to give 15 fractions (1–15). Fraction 5 (22 g) was further separated on silica gel CC and eluted with petroleum-acetone from 20:1 to 3:1, yielding five sub-fractions (2a–2e). Sub-fraction 2b (3 g), subjected to a series of purification steps using silica gel CC, Sephadex LH-20, and semi-preparative HPLC (MeCN/H2O 90:10, λ = 254 nm, flow rate 3.8 ml/min) to afford compound 1 (11 mg, tR = 25 min), meliasenin M, kulinone. Fraction 8 (7 g) was repeatedly chromatographed over silica gel CC eluting with a mixture of petroleum ether-EtOAc with increasing polarity, followed by Sephadex LH-20 and preparative HPLC (MeCN/H2O 65:35, λ = 254 nm, flow rate 3.8 ml/min) to obtain compound 2 (2 mg, tR = 8 min). All fractions were investigated against S. litura larvae by Leaf disc method. Active fractions (Fr.5, Fr.7, Fr.8, and Fr.10) from the barks of M. azedarach L. and active fractions (Fr.3, Fr.4, Fr.6) from the fruits of M. azedarach L. were isolated and purified by silica gel column chromatography, Sephadex LH-20 column chromatography, semi-preparative HPLC to yield kulactone (3) (5.3 mg), meliasenin J (5.6 mg), meliasenin W (2.3 mg), meliasenin M (2.5 mg), kulonine (16.8 mg), 16-hydroxybutyroperospermol (2.3 mg), aphagranin G (1.2 mg), mesendanin M (4.2 mg), mesendanin L (2.0 mg), mesendanin R (1.0 mg), meliasenin 3 (3.4 mg), dubione B (10.0 mg), 1,2-benzenedicarboxylic acid dibutyl ester (15.0 mg), 1,2-benzenedicarboxylic acid diisobutyl ester (21.0 mg), methyl kulanate (3.0 mg), (−)-12β-hydroxykulanate (4.3 mg), vanillin (3.7 mg), 4-hydroxy-3-methoxycinnamaldehyde (4.5 mg), 24,25-dihydrokulanone (10.7 mg), creosol (2.3 mg), and veratraldehyde (2.3 mg). Take the isolation and purification process of dubione B as an example to show the specific procedure. Fr.8 (6.86 g) was chromatographed over a silica gel column eluted with increasing polarities of a mixture of dichloromethane-acetone (80:1–2:1) and petroleum ether-acetone (10:1–2:1) to yield six sub-fractions. The forth sub-fraction (348 mg) was applied to Sephadex LH-20 to give dubione B (see the detailed procedure in the supplementary data).

### 3.3.1. Tirucalla-5(6), 7, 24(25)-triene-3-oxo-21,16-olide (1)

Colorless oil. [α]D20 +80 (c 0.20, CH2Cl2); UV (C2H5OH) λ max (log ε): 274 (4.66) nm; IR (KBr) v max: 1776, 1712, 1640 cm⁻¹; for 1H NMR spectral data (500 MHz, in CDCl3) and 13C NMR spectral data (125 MHz, in CDCl3), see Table 1; positive HR-ESI-MS: m/z 449.2702 [M − H]⁻ (calcd for C30H41O3, 449.3056).

### 3.3.2. 24-Hydroperoxytirucalla-7, 25(26)-diene-3, 6-dion-21, 16-olide (2)

Colorless oil. [α]D20 +35 (c 0.20, CH2Cl2); UV (C2H5OH) λ max (log ε): 243 (4.76) nm; IR (KBr) v max: 1772, 1706, 1650 cm⁻¹; for 1H NMR spectral data (500 MHz, in CDCl3) and 13C NMR spectral data (125 MHz, in CDCl3), see Table 1; positive HR-ESI-MS: m/z 521.2866 [M + Na]⁺ (calcd for C30H42O6Na, 521.2879).

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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