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Two new triterpenoid glycosides from leaves of *Cyclocarya paliurus*

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ABSTRACT

Two dammarane glycosides (**1-2**) were isolated from the leaves of *Cyclocarya paliurus*. The structures of new compounds were established by application of spectroscopic methods, including one-dimensional and two-dimensional NMR, HRESIMS, and chemical hydrolysis. When evaluated against seven human cancer cell lines, the two compounds exhibited selective cytotoxicity to MCF-7 cells.

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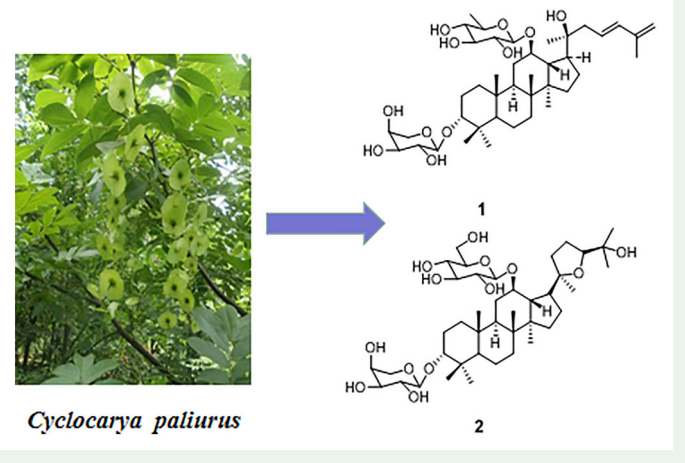

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1. Introduction

Cyclocarya paliurus (Batal.) Iljinskaja, a Chinese indigenous plant, is the sole species in the genus *Cyclocarya* Iljinskaja of the Juglandaceae family. They have been used in folk medicine to treat diabetes, hypertension, coronary heart disease and neurasthenia (The Editorial Committee of the Administration Bureau of Traditional Chinese Medicine 1999). Previous phytochemical investigations on the leaves of *C. paliurus* have led to the isolation of various chemical constituents including triterpenoid (Kennelly et al. 1995; Li et al. 2012; Cui & Li 2015; Chen et al. 2018), flavonoids (Xie et al. 2010) and phenolic acids (Zhang et al. 2010), of which some exhibited hypoglycemic (Kurihara et al. 2003; Shi et al. 2009; Zhang et al. 2010; Li et al. 2011), antihyperlipidemic (Ma et al. 2015; Yao et al. 2015), antioxidant (Yang et al. 2018), antimicrobial (Xie et al. 2012), anti-inflammatory (Jiang et al. 2014) and cytotoxic effects (Liu et al. 2007; Cheng et al. 2009).

And in our previous study, several novel triterpenoids(Xuan et al. 2019; Sun et al. 2020a, 2020b) and flavonoids (Ye et al. 2020a, 2020b) were found in this plant. To further explore the chemical diversity of triterpenoids, two undescribed triterpenoiids, cyclocarioside Z12-Z13 (**1-2**), were isolated and identified. Herein, the isolation, structure elucidation, and biological evaluations were reported.

2. Results and discussion

2.1. Chemistry

Compound **1** was isolated as colorless amorphous powder. Its molecular formula $C_{41}H_{68}O_{11}$ was identified by the HRESIMS m/z 754.5123 $[M+NH_4]^+$ (calcd. 754.5105) and ^{13}C NMR data (supplementary material Table S1). Full NMR data analysis indicated that **1** was a triterpenoid glycoside which was similar to cyclocarioside Z5 (Sun et al. 2020a). The ^1H and ^{13}C-NMR data (supplementary material Table S1) for the side chain suggested two double bond between C-23, C-24, C-25 and C-26, and a methyl at C-27. Analysis of the coupling constants for olefinic protons H-23 and H-24 ($J_{23,24} = 15.5$ Hz) in the side chain supported *E* stereochemistry for the double bond. And there were two anomeric proton signals at δ_H 4.29 (d, 1H, $J = 6.0$ Hz) and δ_H 4.34 (d, 1H, $J = 7.5$ Hz) for sugar moieties in the ^1H-NMR spectrum. The monosaccharides obtained by acid hydrolysis were identified by comparison on GC-MS analysis with authentic sample as L-arabinose and D-quinovose. Furthermore, the relative configurations of **1** were determined by NOESY spectrum. Key correlations (supplementary material Figure S2) between H-3 (δ_H 3.34), H-1$_\beta$ (δ_H 1.47, β-orientation) and H-29 (δ_H 0.90), as well as correlations between H-12 (δ_H 4.12), H-11$_\alpha$ (δ_H 2.36, α-orientation), and H-9 (δ_H 1.80) indicated the H-3, H-12 were β-orientation and a-orientation. Besides, the configuration at C-20 was deduced as "*S*" on the basis of optical rotation and ^{13}C-NMR chemical shift data comparison with reported analogous dammaranes (Sun et al. 2020a). Thus, the compound **1** was determined as (20*S*,23*E*)-3α,12β,20-trihydroxy-12-O-β-D-qunovopyranosyldammara-23,25-diene 3-O-α-L-arabinopyranoside.

Compound **2** was isolated as colorless amorphous powder, which had a molecular formula of $C_{41}H_{70}O_{13}$ determined by the HRESIMS. Detailed analysis of NMR data

showed that **2** had a high similarity to cyclocarioside K (Cui & Li 2015) except for replacement of arabinopyranosyl moiety at C-12 by a glucopyranosyl moiety at the same position in **2**. Moreover, glucopyranosyl moiety was identified by acid hydrolysis and GC-MS analysis with standard. Therefore, the structure of **2** was deduced as (20S,24R)-20,24-epoxy-3α,12β,25-trihydoxy-12-O-β-D-glucopyranosyldammarane 3-O-α-L-arabinopyranoside.

2.2. Cytotoxicity assay

The inhibitory effects of compound **1** and compound **2** against seven human cancer cells (MCF-7, PC-3, Du145, NCI-1975, PC-9, SKVO3 and HepG2) were evaluated by the MTT assay with positive control of STS. As shown in supplementary material Table S2, all compounds displayed inhibitory activities on MCF-7 cells with IC_{50} values <35 μM.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a JASCO model 1020 polarimeter (Horiba, Tokyo, Japan) at room temperature. Gas chromatography-mass spectrometer (GC-MS) were measured on GCMS-QP2010 Ultra (SHIMADZU, Hongkong, China). NMR spectra were measured on a Bruker AV-500 MHz spectrometer (Bruker, Karlsruhe, Germany) using CD_3OD as solvent and tetramethylsilane (TMS) as an internal standard. The HRESIMS were measured on a LC-LTQ Orbitrap Velos Pro ETD (Thermo Fisher, MA, USA) in positive ion mode. Column chromatography (CC) was performed on silica gel (200–300 or 80–100 mesh; Qingdao Peremanent Sea Silica Ltd., Qingdao, China), polyamide (80–100 or 30–60 mesh, Taizhou Luqiao Sijia Biochemical Plastics Factory, Taizhou, China), C_{18} reverse-phased silica gel (40–75 μm, Fuji, Kasugai, Japan). Analytical HPLC experiments were conducted with a YMC Pack ODS-A column (5 μm, 250 mm × 4.6 mm i.d.; Tokyo, Japan) in Agilent 1100 (Agilent Technologies, Ltd) equipped with a diode array detector (DAD) under reversed-phase. And semipreparative HPLC separations were carried out on an Agilent 1200 with YMC Pack ODS-A column (5 μm, 250 × 10 mm, YMC Co. Ltd., Kyoto, Japan). UV spectra were detected with Waters Acquity UPLC equipped 2998 PDA Detector (America). All the analytic reagents were analytical grade and purchased from Cologne Chemical Co., Ltd., Chengdu, China.

3.2. Plant material

The leaves of *C. paliurus* (collected in Xinning County, Shaoyang City, Hunan Province) were provided by Hunan Heran Biotechnology Development Company, Hunan Province, People's Republic of China, in May 2016, identified by Prof. Kangping Xu (Xiangya School of Pharmaceutical Sciences, Central South University). And its voucher specimen (No. 20160820) was deposited in the Xiangya School of Pharmaceutical Sciences, Central South University.

3.3. Extraction and isolation

Whole leaves of *C. paliurus* (45.0 kg) were exhaustively extracted with 75% EtOH twice under reflux (450 L × 2 h), and were dried under reduced pressure to get crude extract. The crude extract was suspended in water and successively partitioned with CH_2Cl_2, EtOAc and n-BuOH(50L × 4 times for each solvent). The CH_2Cl_2 extract (400 g) was subjected to column chromatography on silica gel and eluted with a gradient mixture of CH_2Cl_2/MeOH (from 100:0 to 0:100) to obtain ten Fr. (I-X) according to TLC patterns. The Fr. X (60 g) was gradient elution of H_2O/MeOH (v/v, 100:0 to 0:100) through Polyamide column to yield 5 fractions (Fr. A-Fr. E) according to analytical HPLC. Fr. B (13.2 g) was further chromatographed by a silica gel column with CH_2Cl_2/MeOH (10:0 to 0:10), and followed by a C_{18} reversed-phase column (from 10% to 100% aqueous MeOH, stepwise). One of the fraction was found to develop crystal and compound **1** (3.5 mg) was isolated from the crystal (supplementary material Figure S24). Fr. C was separated by a C_{18} reversed-phase column with a gradient system of MeOH/H_2O to get five fractions (Fr. C1–Fr. C5). Compound **2** (2.4 mg) was isolated from Fr. C2 by semi-preparative HPLC (2.5 mL/min, 230 nm, ACN-H_2O, 7.5:2.5, V/V).

3.3.1. Cyclocarioside Z12 (1)

Colorless amorphous powder; [α]25 D − 7.6 (*c* 0.10, MeOH); HPLC-UV (ACN-H_2O) λ_{max}: 230 nm; ^{1}H NMR (500 MHz in CD_3OD) data see supplementary material Table S1, and ^{13}C NMR (125 MHz in CD_3OD) data see supplementary material Table S1. HRESIMS, *m/z* 754.5123 $[M + NH_4]^+$ (calcd for $C_{41}H_{72}NO_{11}$ 754.5105).

3.3.2. Cyclocarioside Z13 (2)

Colorless amorphous powder; [α]25 D − 29.6 (*c* 0.10, MeOH); HPLC-UV (ACN-H_2O) λmax: 230 nm; ^{1}H NMR (500 MHz in CD_3OD) data see supplementary material Table S1, and ^{13}C NMR (125 MHz in CD_3OD) data see supplementary material Table S1. HRESIMS, *m/z* 788.5159 $[M + NH_4]^+$ (calcd for $C_{41}H_{74}NO_{13}$ 788.5160).

3.4. Acid hydrolysis

The configurations of sugar moieties were established according to the published method(Gan et al. 2015) with some modifications. Compounds **1-2** (each 1 mg) were refluxed with 2 M HCl in the oil bath at 100 °C for 2 h. The reaction mixture was neutralized with Na_2CO_3 and extracted with $CHCl_3$ for three times. The aqueous layer was concentrated and dried to obtain monosaccharide fraction. The mixture was dissolved in 1 mL pyridine followed by 2 mg of L-cysteine methyl ester hydrochloride and heated at 60 °C for 1 h. Then trimethylsilylimidazole (1 mL) was added and heated at 60 °C for another 0.5 h. The reaction mixture was analyzed by GC–MS under the conditions: Column, Rxi-5Sil MS (0.25 μm × 30.0 mm, 0.32 mm);front inlet 300 °C, column 150–300 °C at 15 °C/min. The sugar configurations of compounds 1-2 were identified by the comparison of the retention times with authentic standard treated in the same means.

3.5. Cytotoxic activity assay

All human cancer cell lines were gained from ATCC (Manassas, VA, USA). Cytotoxic activities of all isolates (**1-2**) was tested by MTT assay with positive control of staurosporine (STS). And specific experimental operations refer to published methods(Sun et al. 2020b).

4. Conclusion

Two previously undescribed dammarane glycosides from *Cyclocarya paliurus*. were isolated in this study. The cytotoxic activity assay suggested that compounds **1** and **2** have no broad-spectrum cytotoxicity against human cancer cells but showed a selective inhibitory activity on MCF-7.

Disclosure statement

No potential conflict of interest was reported by the authors.

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