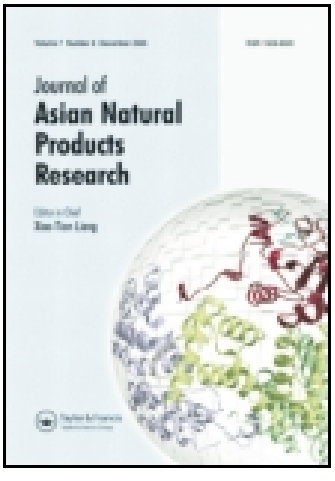

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

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Published online: 29 Nov 2013.

To cite this article: Yue Liu, Man-Qi Zhang, Xia-Lei Li, Tun-Hai Xu, Sheng-Xu Xie, Ya-Juan Xu & Dong-Ming Xu (2014) Study on chemical constituents of Cyclocarya paliurus, Journal of Asian Natural Products Research, 16:2, 206-209, DOI: 10.1080/10286020.2013.825254

To link to this article: http://dx.doi.org/10.1080/10286020.2013.825254

Journal of Asian Natural Products Research, 2014
Vol. 16, No. 2, 206–209, http://dx.doi.org/10.1080/10286020.2013.825254

Study on chemical constituents of *Cyclocarya paliurus*

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(*Received 14 December 2012; final version received 11 July 2013*)

A new dammarane triterpenoid glycoside named cyclocarioside J (**1**) and other three known triterpenoid glycosides were isolated from the leaves of *Cyclocarya paliurus*. Based on ESI-MS, HR-ESI-MS, ^{1}H NMR, ^{13}C NMR, and 2D NMR techniques including ^{1}H–^{1}H COSY, HMBC, HMQC, and NOESY correlations, the structure of cyclocarioside J was elucidated as (20*S*,24*R*)-epoxydammarane 3β,12β,25-trihydroxy-12-*O*-β-D-quinovopyranosyl-3-*O*-α-L-arabinopyranoside.

Keywords: *Cyclocarya paliurus*; dammarane; triterpenoid glycosides; cyclocarioside J

1. Introduction

Cyclocarya paliurus (Batal.) Iljinsk (Juglandaceae) is an endemic species growing in southern China [1]. Its leaves possess an intensive sweet taste and are used as a tea which is reported to have beneficial effects of lowering blood sugar [2,3]. Previously, several sweet dammarane triterpenoid glycosides had been reported from *C. paliurus* [4–6]. In order to obtain a large amount of triterpenoid glycosides for pharmacological study and quality control, the leaves of *C. paliurus* were investigated to afford one new dammarane triterpenoid glycoside and three known triterpenoid glycosides. This study deals with the isolation and characterization of cyclocarioside J (**1**).

2. Results and discussion

Compound **1** was obtained as a white amorphous solid with a molecular formula of $C_{41}H_{70}O_{12}$, as determined by the data of the positive-ion HR-ESI-MS at *m/z* 777.4753 $[M + Na]^+$. ESI-MS showed the major ion peak at *m/z* 777 which was assigned to $[M + Na]^+$. MS/MS of **1** showed fragment ion peaks at *m/z* 613 $[M + Na\text{-}164]^+$, corresponding to the loss of a deoxyhexose unit, and at *m/z* 481 $[M + Na\text{-}164\text{-}132]^+$, corresponding to the loss of a pentose unit. The ^{13}C NMR spectrum of **1** exhibited 41 carbon signals, of which two anomeric C-atoms at δ_C 102.0 (C-1′) and δ_C 102.0 (C-1″) supported the presence of two sugar moieties in **1**. By comparing the ^{13}C NMR data of **1** with those of cyclocarioside C [7] and cyclocarioside I [8], it was suggested that **1** has a similar aglycone as those of cyclocarioside C and cyclocarioside I. The almost identical ^{13}C NMR data ascribable to C-3 (δ_C 81.5), C-12 (δ_C 77.4), C-20 (δ_C 86.6), and C-24 (δ_C 84.3) in **1** with those of cyclocarioside C and cyclocarioside I proposed that the stereochemistry of C-12, C-20, and C-24 in compound **1** should be *R*-, *S*-, and *R*-configurations, respectively. In addition,

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Table 1. ^{1}H and ^{13}C NMR spectral data of **1** (400/100 MHz in pyridine-d_5; δ in ppm, J in Hz).

Aglycone of **1**			Sugar moieties of **1**		
No.	δ_C	δ_H (J, Hz)	No.	δ_C	δ_H (J, Hz)
1	35.7	3.09 (br d, 13.2), 2.00 (m)	3-*O*-Ara (*p*)		
2	26.8	2.00 (m), 1.72 (m)	1′	102.0	4.70 (d, 6.4)
3	81.5	3.60 (m)	2′	72.5	4.37 (m)
4	38.2	–	3′	74.8	4.17 (m)
5	51.1	1.56 (m)	4′	69.2	4.34 (m)
6	18.4	1.46 (m)	5′	66.3	4.28 (dd, 12.0, 3.6), 3.73 (br d, 12.0)
7	36.5	1.51 (m), 1.17 (m)			
8	41.6	–	12-*O*-Qui		
9	54.1	1.86 (br d, J = 10.0 Hz)	1″	102.0	4.99 (d, 7.6)
10	40.1	–	2″	75.6	3.93 (m)
11	34.6	2.85 (br d, 12.0), 1.46 (m)	3″	78.5	4.14 (t-like, 8.8)
12	77.4	4.38 (m)	4″	77.0	3.65 (m)
13	41.3	1.78 (m)	5″	72.9	3.79 (m)
14	50.2	–	6″	18.7	1.59 (d, 6.0)
15	31.7	1.37 (m), 0.96 (m)			
16	22.0	1.86 (m), 0.98 (m)			
17	49.3	1.84 (m)			
18	17.1	1.06 (s)			
19	16.9	1.39 (s)			
20	86.6	–			
21	24.6	1.14 (s)			
22	34.2	1.73 (m), 1.54 (m)			
23	26.4	1.93 (m), overlapped			
24	84.3	3.92 (dd, 13.2, 7.6)			
25	71.3	–			
26	26.2	1.42 (s)			
27	27.8	1.37 (s)			
28	23.3	0.98 (s)			
29	30.1	1.23 (s)			
30	16.9	0.62 (s)			

Note: Assignments based on ^{1}H–^{1}H COSY, HMQC, and HMBC experiments.

carbon signals for D-quinovopyranose and L-arabinopyranose were observed in the ^{13}C NMR spectrum of **1** (Table 1). Acidic hydrolysis of **1** with mineral acid afforded quinovose and arabinose, which were identified by comparison with authentic samples.

In the ^{1}H NMR spectrum of **1**, eight methyl signals (δ 1.42, 1.39, 1.37, 1.23, 1.14, 1.06, 0.98, and 0.62, each s) assignable to the aglycone moiety were observed. There were also two anomeric proton signals at δ_H 4.99 (d, J = 7.6 Hz) for the quinovose and at δ_H 4.70 (d, J = 6.4 Hz) for arabinose units. In the NOESY spectrum, correlations between H-3 (δ_H 3.60) and H-5 (δ_H 1.56), H-28 (δ_H 0.98), correlations between H-12 (δ_H 4.38) and H-11a (δ_H 2.85, α-orientation), H-9 (δ_H 1.86), and correlations between H-21 (δ_H 1.14) and H-24 (δ_H 3.92), H-17 (δ_H 1.84) indicated the α-orientations of H-3, H-12, and H-24. All ^{1}H and ^{13}C NMR data (Table 1) were assigned by ^{1}H–^{1}H COSY, HMQC, and HMBC experiments. In the HMBC spectrum of **1** (Figure 1), the correlations between H-1′ (δ_H 4.70) and C-3 (δ_C 81.5) suggested that the arabinopyranose was linked at C-3, while the correlations between H-1″ (δ_H 4.99)

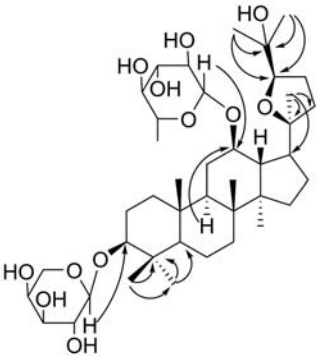

Figure 1. Structure and key HMBC correlations (H → C) of compound **1**.

and C-12 (δ_C 77.4) indicated that the quinovopyranose was present at C-12. Consequently, the structure of **1** was determined as (20*S*,24*R*)-epoxydammarane 3β,12β,25-trihydroxy-12-*O*-β-D-quinovopyranosyl-3-*O*-α-L-arabinopyranoside.

The other compounds were identified as cyclocarioside I (**2**) [8], arjunglucoside II (**3**) [9], and quadranoside IV (**4**) [10].

3. Experimental

3.1 General experimental procedures

Melting points were determined on an X-6 microscope apparatus and are uncorrected (Beijing Fukai Instrument Co., Ltd., Beijing, China). Optical rotations were determined on a WZZ-2SS auto-polarimeter (Shanghai Jingke Scientific Instrument Co., Ltd., Shanghai, China). IR spectrum was measured as film on KBr pellet using a Bruker VERTEX70 spectrometer (Bruker Corporation, Billerica, MA, USA). NMR spectra were obtained on a Bruker AV400 instrument (Bruker Corporation, Rheinstetten, Switzerland), using tetramethylsilane as internal standard. The HR-MS was recorded on an autoflex III MALDI/TOF/TOF-MS instrument (Bruker Corporation, Ettlingen, Germany). ESI-MS were recorded on a Finnigan Mat LCQ mass spectrometer (Thermo Fisher Scientific Inc., West Palm Beach, FL, USA). High-performance liquid chromatography (HPLC) (Shimadzu Company, Kyoto, Japan) was carried out using an octadecylsilane-bonded silica column (HC-ODS, 250 mm × 4.6 mm, 5 μm). Prep-HPLC (Waters 600E, Waters Company, Billerica, MA, USA) was carried out using an octadecylsilane-bonded silica column (Agilent-ZORBAX PrepHT Eclipse XDB-C18, 250 mm × 21.2 mm, 7 μm). Column chromatography (CC) was carried out on silica gel (200–300 mesh, Qingdao Oceanic Chemical Industry, Qingdao, China), reversed silica gel (50 μm, YMC Co., Ltd., Kyoto, Japan), and macroporous resin D_{21} made in Shandong Lu Neng Gel Co. (Shandong, China). TLC was carried out on aluminium sheets (20 × 20 cm, RP-18$F_{254\,S}$, MERCK KGaA, Darmstadt, Germany). Spots were detected after spraying 10% H_2SO_4.

3.2 Plant material

The leaves used in this experiment were collected from Xiushui County, Jiangxi Province, China, in September 2009 and identified by Professor Minglu Deng, Changchun College of Traditional Chinese Medicine. A voucher specimen has been deposited in the phytochemistry laboratory of Jilin Academy of Chinese Medicine Sciences.

3.3 Extraction and isolation

The dried leaves (10 kg) were extracted three times with 60% EtOH for 2 h under reflux. The extract was concentrated under vacuum to give a residue, which was suspended in H_2O and extracted with $CHCl_3$ and *n*-BuOH, respectively. The *n*-BuOH fraction (205 g) was chromatographed on 1.5 kg D_{21} porous resin, eluting with water until the eluate was colorless and then with 70% EtOH (12 liters). The 70% EtOH eluate was further subjected to neutral resin to remove most of the color material and then evaporated to dryness to

give crude glycosides (74 g). Part of crude glycosides were chromatographed on silica gel (200–300 mesh) with $CHCl_3$:MeOH (100:1 to 1:1) to give Fr. 1–Fr. 14. Fr. 11 (6.7 g) was subjected to reversed-phase silica gel CC [$CHCl_3$:MeOH:H_2O: *n*-BuOH (25:4:2:0.5)] to give 91 fractions. Fr. 64–Fr. 67 were separated by HPLC [MeOH:H_2O (85:15, v/v)] to give compound **1** (82 mg). Fr. 45– Fr. 55 (41 mg) were recrystallized to give compound **2** (32 mg). Fr. 9–Fr. 11 were separated by HPLC [MeOH:H_2O (82:18, v/v)] to give compounds **3** (66 mg) and **4** (34 mg).

3.3.1 *Compound **1***

Amorphous powder; m.p. 148.0–148.7°C; $[\alpha]_D^{20}$: −1.29 ($c = 0.98$, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3428, 2969, 1055. ^1H NMR (400 MHz, pyridine-d_5) and ^{13}C NMR (100 MHz, pyridine-d_5) spectral data are given in Table 1. ESI-MS: *m/z* 777 $[M + Na]^+$, 613 $[M + Na\text{-}164]^+$, 481 $[M + Na\text{-}164\text{-}132]^+$. HR-ESI-MS: *m/z* 777.4753 $[M + Na]^+$ (calcd for $C_{41}H_{70}O_{12}Na$, 777.4759).

3.4 *Acid hydrolysis*

Compound **1** (15 mg) in a mixture of MeOH (5 ml) and 5% H_2SO_4 (5.0 ml) was refluxed for 2 h. After cooling, the reaction mixture was diluted twofold with H_2O, and extracted with $CHCl_3$ (3 × 30 ml). The aqueous layer was neutralized with aq. $Ba(OH)_2$ and concentrated *in vacuo* to give a residue, then chromatographed on silica gel with $CHCl_3$:MeOH:H_2O (70:30:5) to give two monosaccharides with R_f [$CHCl_3$:MeOH:H_2O (6:4:1) and (EtOAc:$CHCl_3$:MeOH:H_2O, 3:2:2:1)] and specific rotation ($[\alpha]_D^{20}$ + 110.9 ($c = 0.11$, H_2O) corresponding to those of L-arabinose and with R_f [$CHCl_3$:MeOH:H_2O (6:4:1) and (EtOAc:$CHCl_3$:MeOH:H_2O, 3:2:2:1)] and specific rotation ($[\alpha]_D^{20}$ + 69.2 ($c = 0.13$, H_2O) corresponding to those of D-quinovose, respectively.

Acknowledgments

The authors were financially supported by the International Science – Technology Cooperation Program of China (2010DFB33260) and the Cooperation Program of Beijing Municipal Education Commission and the Innovative team of Beijing TCM University (No. 2011-CXTD-19).

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