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Two new triterpenoid glycosides from *Cyclocarya paliurus*

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Two new dammarane triterpenoid glycosides named cyclocarosides B (1) and C (2) were isolated from the leaves of *Cyclocarya paliurus*. Based on FAB-MS, HRESI-MS, IR, ^{1}H NMR, ^{13}C NMR, and 2D-NMR (HMQC, HMBC, COSY, ROESY) data, the structures of cyclocarosides B (1) and C (2) were elucidated as (20*S*,24*R*)-epoxydammarane (3β,12β)-25-hydroxyl-12-*O*-β-D-quinovopyranosyl-3-*O*-β-D-quinovopyranoside (1), and (20*S*,24*R*)-epoxydammarane (3β, 12β)-25-hydroxyl-12-*O*-α-L-arabinopyranosyl-3-*O*-(5′-*O*-acetyl)-α-L-arabinofuranoside (2).

Keywords: *Cyclocarya paliurus*; Dammarane; Triterpenoid glycosides; Cyclocarioside B; Cyclocarioside C

1. Introduction

Cyclocarya paliurus (Batal.) Iljinsk (Juglandaceae), also known as *Pterocarya paliurus*, is an endemic species growing in Southern China [1]. It has been reputed as a natural "sweetener" as implied by its Chinese trivial name "tian (sweet) cha (tea) shu (tree)". The leaves of *C. paliurus* have traditionally been used by indigenous people for the treatment of hypertension and diabetes, but the constituents responsible and the molecular mechanism underlying these biological activities are unknown. Previously, several triterpenoids, flavonoids, steroids and some other compounds had been reported from *C. paliurus* [2–10]. In order to obtain a large amount of compounds for the pharmacological study and quality control, the leaves of *C. paliurus* were investigated to afford two new dammarane triterpenoid glycosides, cyclocarioside B (1) and cyclocarioside C (2). The present paper deals with the isolation and structure elucidation of cyclocarioside B (1) and cyclocarioside C (2).

2. Results and discussion

Compound 1 (figure 1) was obtained as a white amorphous powder. The IR spectrum of 1 showed absorptions ascribable to hydroxyl (3429 cm^{-1}) groups. The negative FAB-MS gave

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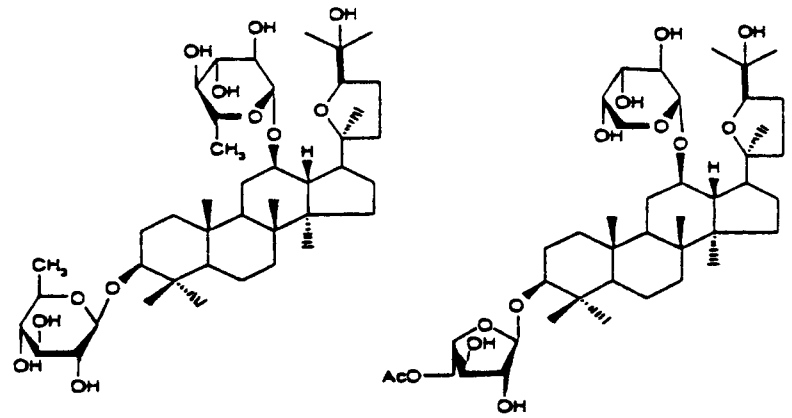

1 2

Figure 1. Structures of compounds 1 and 2.

a quasi-molecular ion peak at *m/z* 767. The HRESI-MS analysis suggested the molecular formula of 1 to be $C_{42}H_{71}O_{12}$ (*m/z* 767.4955 $[M - H]^-$). Analysing the ^1H NMR and ^{13}C NMR data of 1 revealed to be a dammarane triterpenoid glycoside. In the ^1H NMR spectrum of 1 (table 1), eight Me singlets assignable to an aglycone were observed, together with two Me doublets at δ_H 1.60

Table 1. ^1H NMR (500 MHz) and ^{13}C NMR (100 MHz) data for the aglycone moieties of compounds 1 and 2 in C_5D_5N* (δ in ppm. *J* in Hz).

	1		2	
	^{13}C (DEPT)	^1H	^{13}C (DEPT)	^1H
1	35.7 (CH_2)	3.16 (brd, 13.6), 2.12 (m)	35.7 (CH_2)	3.03 (brd, 13.5), 1.78 (m)
2	26.7 (CH_2)	Overlapped	26.4 (CH_2)	Overlapped
3	81.2 (CH)	3.59 (m)	79.9 (CH)	3.47 (m)
4	38.1 (C)	–	37.9 (C)	–
5	51.0 (CH)	1.66 (m)	51.0 (CH)	1.56 (m)
6	18.4 (CH_2)	1.54 (m)	18.4 (CH_2)	1.50 (m)
7	36.5 (CH_2)	1.50, 1.16 (m)	36.4 (CH_2)	1.50 (m), 1.14 (brd, 12.6)
8	41.6 (C)	–	41.6 (C)	–
9	53.9 (CH)	1.91 (dd, 10.8, 4.3)	54.2 (CH)	1.88 (dd, 10.1, 4.2)
10	40.0 (C)	–	40.1 (C)	–
11	34.6 (CH_2)	2.87 (brd, 12.4), 1.48 (m)	34.5 (CH_2)	2.97 (brd, 12.3), 1.56 (m)
12	77.5 (CH)	4.39 (ddd, 10.4, 10.4, 4.7)	77.2 (CH)	4.38 (ddd, 10.6, 10.6, 5.2)
13	41.3 (CH)	1.83 (brd, 10.4)	41.2 (CH)	1.83 (brd, 10.7)
14	50.1 (C)	–	50.2 (C)	–
15	31.6 (CH_2)	1.36–1.41, 0.94 (m),	31.6 (CH_2)	1.36–1.39, 0.98 (m),
16	22.2 (CH_2)	2.02, 1.92 (m)	21.4 (CH_2)	Overlapped
17	49.2 (CH)	1.87–1.95 (m)	49.3 (CH)	1.96 (m)
18	17.0 (Me)	1.07 (s)	17.1 (Me)	1.02 (s)
19	16.7 (Me)	1.42 (s)	16.9 (Me)	1.46 (s)
20	86.5 (C)	–	86.5 (C)	–
21	24.5 (Me)	1.14 (s)	24.8(Me)	1.18 (s)
22	34.2 (CH_2)	1.71–1.75, 1.55 (m)	34.1 (CH_2)	Overlapped
23	26.3 (CH_2)	2.02 (m)	26.8 (CH_2)	2.03 (m)
24	84.2 (CH)	3.94 (t, 7.2)	84.3 (CH)	3.96 (t, 7.2)
25	71.2 (C)	–	71.2 (C)	–
26	26.1 (Me)	1.44 (s)	26.4 (Me)	1.50 (s)
27	27.7 (Me)	1.39 (s)	27.5 (Me)	1.30 (s)
28	23.2 (Me)	1.00 (s)	22.9 (Me)	0.88 (s)
29	30.0 (Me)	1.30 (s)	29.8 (Me)	1.20 (s)
30	16.7 (Me)	0.56 (s)	16.9 (Me)	0.68 (s)

* Assignment based on HMQC, HMBC, and COSY correlations.

Table 2. ^1H (500 MHz) and ^{13}C NMR (100 MHz) data for the sugar moieties of compounds 1 and 2 in C_5D_5N[a] (δ in ppm, J in Hz).

	1			2	
	^{13}C (DEPT)	^1H		^{13}C (DEPT)	^1H
3-O-Qui			3-O-Ara		
1′	101.6 (CH)	4.74 (d, 7.8)	1′	106.6 (CH)	5.41 (d, 4.1)
2′	75.5 (CH)[b]	4.01 (t, 8.5)	2′	81.6 (CH)	4.71 (m)
3′	78.4 (CH)[c]	3.72–3.76 (m)	3′	79.8 (CH)	4.56 (brd, 6.4)
4′	77.0 (CH)[d]	4.17 (t, 8.9)	4′	84.2 (CH)	4.82 (m)
5′	72.8 (CH)[e]	3.82 (m)	5′	65.2 (CH_2)	4.78 (dd, 3.0, 11.7)
					4.59 (dd, 4.0, 11.4)
6′	18.7 (Me)[f]	1.60 (d, 5.6)	MeC=O	170.8 (C)	–
				20.7 (Me)	1.94 (s)
12-O-Qui			12-O-Ara		
1″	101.9 (CH)	5.02 (d, 7.8)	1″	102.6 (CH)	4.92 (d, 7.6)
2″	75.5 (CH)[b]	3.98 (t, 9.0)	2″	72.8 (CH)	4.37 (m)
3″	78.6 (CH)[c]	3.72–3.76 (m)	3″	74.9 (CH)	4.15 (dd, 9.2, 3.5)
4″	76.8 (CH)[d]	4.14 (t, 8.8)	4″	69.8 (CH)	4.22 (m)
5″	72.8 (CH)[e]	3.72 (m)	5″	67.6 (CH_2)	4.29 (dd, 11.7, 1.7)
					3.80 (brd, 11.7)
6″	18.6 (Me)[f]	1.59 (d, 5.9)			

[a] Assignment based on HMQC, HMBC, and ^1H–^1H COSY correlations.
[b–f] ^{13}C NMR data might be interchangeable.

(d, J = 5.6 Hz) and 1.59 (d, J = 5.9 Hz) due to the sugar moieties. The two anomeric protons H-1′ at δ_H 4.74 (d, J = 7.8 Hz) and H-1″ at δ_H 5.02 (d, J = 7.8 Hz) proposed there might be two β-linkaged sugar moieties in the molecule. The ^{13}C NMR spectrum of 1 exhibited 42 carbon signals, of which two anomeric C-atoms at δ_C 101.6 (C-1′) and 101.9 (C-1″) supported the presence of two sugar moieties in the molecule. The oxygenated methine ascribable to H-12 at δ_H 4.39 (*ddd*, J = 10.4, 10.4, 4.7 Hz) indicated H-12 to be α-orientated. The almost identical ^{13}C NMR data ascribable to C-12 (δ_C 77.5), C-20 (δ_C 86.5) and C-24 (δ_C 84.2) in 1 compared with those of cyclocarioside A (3) [2] and cyclocarioside I (4) [3] proposed that the stereochemistry of C-12, C-20 and C-24 in compound 1 should be *R*-, *S*-, and *R*-configurations, respectively. The two sets of ^{13}C NMR data (table 2) due to sugar moieties were identical with those of methyl-β-D-quinovopyranoside [11]. Acidic hydrolysis of 1 with 5% H_2SO_4 in MeOH liberated quinovose, which was identified by comparison with an authentic sample on paper chromatography. In the HMBC experiment of 1, the long-range correlations between H-1′ (δ_H 4.74) and C-3 (δ_C 81.2), H-1″ (δ_H 5.02) and C-12 (δ_C 77.5) were observed (figure 2), showing the two sugar moieties were linked at C-3 and C-12 respectively. The correlations in ROESY spectrum between H-3 (δ_H 3.59) and H_α-1 (δ_H 2.12), H-5 (δ_H 1.66), H-28 (δ_H 1.00) indicated H-3 was α-orientated. The other correlations (figure 2) in HMBC confirmed the structure of 1. Consequently, the structure of 1 was determined as (20*S*,24*R*)-epoxydammarane (3β,12β)-25-hydroxyl-12-*O*-β-D-quinovopyranosyl-3-*O*-β-D-quinovopyranoside.

Compound 2 was obtained as a white amorphous powder. In the IR spectrum of 2, absorptions for OH (3427 cm^{-1}) and ester-carbonyl (1732 cm^{-1}) functions were observed. The negative FAB-MS exhibited a quasi-molecular ion peak at *m/z* 781, consistent with a molecular formula $C_{42}H_{69}O_{13}$ (*m/z* 781.4751 [M – 1]$^-$) shown in the HRESI-MS experiment. And a fragment ion at *m/z* 143 attributable to the 20, 24-epoxyl partial structure indicated the presence of *O*-bearing C-atoms at C-20, C-24, and C-25 [12,13]. This was supported by the C-atoms signals at δ_C 86.5 (C-20), 84.3 (C-24), and 71.2 (C-25) in the ^{13}C NMR spectrum (table 1). By comparing the ^{13}C NMR data of 2 with those of cyclocarioside I (4) [3] and 1, it was suggested that 2 has a similar

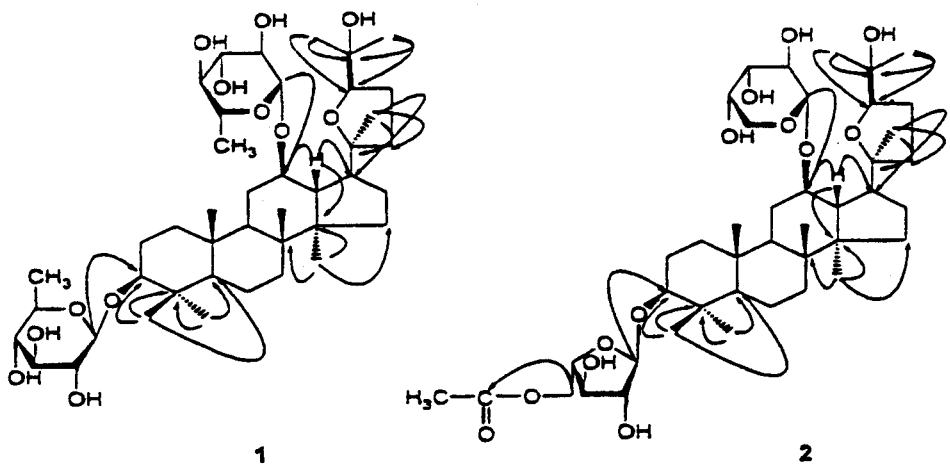

Figure 2. Selected HMBC correlations of compounds 1 and 2.

aglycone as that of cyclocarioside I (4) and 1. The ^{13}C NMR spectrum (tables 1 and 2) of 2 showed two signals of anomeric C-atoms at δ_C 106.6 and 102.6, indicating two sugar moieties in the molecule. Additionally, one set of C-signals for L-arabinofuranose and one set of C-signals for L-arabinopyranose (table 2) were observed in the ^{13}C NMR spectrum of 2. Acidic hydrolysis of 2 furnished arabinose, which was identified by comparison with the authentic sample on paper chromatography. In the HMBC spectrum of 2 (figure 2), the correlations between H-5′ (δ_H 4.78, 4.59) and C=O of the Ac group (δ_C 170.8), H-1′ (δ_H 5.41) and C-3 (δ_C 79.9) suggested the arabinofuranose was linked at C-3 and the Ac group attached to H-5′ of the arabinofuranose, the correlations between H-1″ (δ_H 4.92) and C-12 (δ_C 77.2) indicated the arabinopyranose was present at C-12.

In the ^1H NMR spectrum (table 1) of 2, 8 Me signals (each *s*) assignable to the aglycone moiety were observed, together with an oxygenated methine signal for H-12 (δ_H 4.38 (*ddd*, J = 10.6, 10.6, 5.2 Hz)) inferring H-12 in α-orientation, and a Me singlet (δ_H 1.94) for Ac group. There were also two anomeric-proton signals (table 2) for the two arabinose units. One signal for H-1″ at δ_H 4.92 (*d*, J = 7.6 Hz) meant the L-arabinopyranose attached at C-12 and should be present in α-linkage. The other signal for H-1′ with a smaller J value (δ_H 5.41 (*d*, J = 4.1 Hz)) was also assigned to be α-linkage because the correlation between H-1′ (δ_H 5.41) and H-5′ (δ_H 4.78, 4.59) was observed in the ROESY spectrum, which was identical with that of the α-L-arabinose in cyclocarioside A [2] and monepaloside C [14]. The correlations between H-3 (δ_H 3.47) and H_α-1 (δ_H 1.78), H-5 (δ_H 1.56), H-28 (δ_H 0.88) in the ROESY spectrum suggested that H-3 was presented in α-orientation.

Based on the discussion above, compound 2 was deduced as (20*S*,24*R*)-epoxydammarane (3β,12β)-25-hydroxyl-12-*O*-α-L-arabinopyranosyl-3-*O*-(5′-*O*-acetyl)-α-L-arabinofuranoside.

3. Experimental

3.1 General experimental procedures

Melting points were measured on an XRC-1 apparatus and are uncorrected. Silica gel (200–300 mesh) for column chromatography were obtained from Qingdao Marine Chemical

Factory, China; D_{101} macroreticular resins were obtained from Tianjing Pesticide Chemical Company, Tianjing, China; ODS-Q3, MCI gel CHP-20P (70–150 μ) were bought from Mitsubishi Chemical Corporation, Tokyo, Japan; Lichrospher Rp-8 gel (40–63 μ) was obtained from Merck Company, Germany. Detection was performed by TLC on silica gel sprayed with 10% H_2SO_4 in EtOH, followed by heating. Optical rotations were measured on a Horiba SEPA-300 High Sensitive Polarimeter. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets, ν in cm^{-1}. 1D- and 2D-NMR experiments were run on a Bruker-AM-400 (^1H and ^{13}C, at 400 and 100 MHz, respectively) or DRX-500 (^1H and ^{13}C, at 500 and 125 MHz, respectively) spectrometer with TMS as internal reference, J in Hz. Mass spectra were recorded on a VG-Auto-Spec-3000 instrument.

3.2 Plant material

The plant used in this experiment was collected in Xiushui County, Jiangxi Province, P.R. China, in July 2003, and was identified as *C. paliurus* (Batal.) Iljinsk by Dr. Li-Gong Lei. The voucher specimen is deposited in the Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The dried leaves (10 kg) were extracted three times with 80% 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 BuOH, respectively. The BuOH fraction (216.0 g) was submitted to column chromatography (D_{101} macroreticular resins), gradient elution with H_2O, 20% EtOH/H_2O, 70% EtOH/H_2O and 90% EtOH/H_2O to afford four fractions: Fractions I–IV. The Fraction III (70% EtOH/H_2O eluted, 280 g) was subjected to column chromatography (MCI gel, CHP-20P, MeOH/H_2O 70:30 → 90:10, 500 ml each) to provide four fractions: Frs. III.A–D. Fraction III.B (50.0 g) was chromatographed on silica gel column chromatography (500 g, $CHCl_3$/MeOH/H_2O 90:10:1 → 70:30:5) to give five fractions: Frs. III.B1–5. Fraction III.B2 was submitted to column chromatography (Rp-8 gel, MeOH/H_2O 75:25) to afford three fractions: Frs. III.B2.1–3. Fraction III.B2.2 was purified successively by column chromatography (Rp-8 gel, Me_2CO/H_2O 60:40) to yield compound **1** (115 mg). Fraction III.B3 was submitted to column chromatography (silica gel, EtOAc/MeOH/H_2O 90:10:0.5) to provide four fractions: Frs. III.B3.1–4. Fraction III.B3.2 underwent column chromatography (Rp-8 gel, MeOH/H_2O 75:25, followed by silica gel, $CHCl_3$/MeOH/H_2O 85:15:1) to give compound **2** (84 mg).

3.3.1 (20*S*,24*R*)-epoxydammarane (3β,12β)-25-hydroxyl-12-*O*-β-D-quinovopyranosyl-3-*O*-β-D-quinovopyranoside (1). White amorphous powder, mp 165–167.5°C; $[\alpha]_D^{22}$ −3.4 (*c* 0.31, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3429, 2969, 1067; FAB-MS (−), *m/z*: 767 $[M - H]^-$ (100), 143 (15). HRESI-MS (−): 767.4955 $[M - H]^-$ (calcd for $C_{42}H_{71}O_{12}$, 767.4945). ^1H NMR and ^{13}C NMR data are shown in tables 1 and 2.

3.3.2 (20*S*,24*R*)-epoxydammarane(3β,12β)-25-hydroxyl-12-*O*-α-L-arabinopyranosyl-3-*O*-(5′-*O*-acetyl)-α-L-arabinofuranoside (2). White amorphous powder, mp 158.5–161°C;

$[\alpha]_D^{22.3}$ −20.5 (*c* 0.20, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3427, 2969, 2936, 1732, 1045; FAB-MS (−), *m/z*: 781 $[M - H]^-$ (85), 143 (10). HRESI-MS (−): 781.4751 $[M - H]^-$ (calcd for $C_{42}H_{69}O_{13}$, 781.4738). ^1H NMR and ^{13}C NMR data are shown in tables 1 and 2.

3.4 Acidic hydrolysis

Each solution of compounds 1, 2 (each 5 mg) in a mixture of MeOH (2.0 ml) and 5% H_2SO_4 (2.0 ml) was refluxed for 2 h. The hydrolysate was allowed to cool, diluted 2-fold with H_2O, and extracted with EtOAc. The aqueous layer was neutralised with aq. $Ba(OH)_2$ and concentrated *in vacuo* to give a residue, in which quinovose (from 1) or arabinose (from 2) were identified by comparison with authentic samples (BuOH/EtOAc/H_2O 4:1:5, upper layer; PhOH/H_2O, 4:1) on paper chromatography.

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