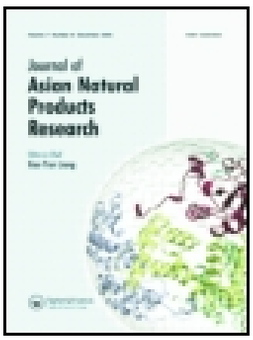

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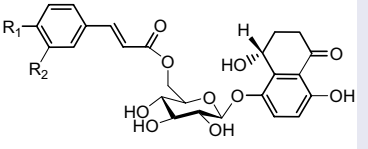
New tetralone derivatives from the leaves of *Cyclocarya paliurus*

Xian-Li Zhou[a§], Qin Luo[a§], Si-Xin Huang[a], Peng-Cheng Wang[a], Qin Xu[a,b], Xiao Huang[a], Cheng-Qin Liang[a] and Xu Chen[a]

[a]College of Pharmacy, Guilin Medical University, Guilin 541004, China; [b]Guangxi Key Laboratory of Molecular Medicine in Liver Injury and Repair, Guilin Medical University, Guilin 541001, China

ABSTRACT

Two new tetralone derivatives, named cyclopalosides A (**1**) and B (**2**), were isolated from the leaves of *Cyclocarya paliurus* by column chromatography on silica gel, reversed-phase C_{18} silica gel and preparative HPLC. Their chemical structures were established on the basis of extensive analyses of spectroscopic data. Their structural characteristic is tetralone glycoside with a caffeoyl unit. The antioxidant activities of compound **1** were evaluated by using hydroxyl, superoxide anion, and DPPH radical scavenging assay.



1 R_1 = OH R_2 = H
2 R_1 = OMe R_2 = OH

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KEYWORDS
Juglandaceae; *Cyclocarya paliurus*; tetralone derivatives; antioxidant

1. Introduction

Cyclocarya paliurus (Batal.) Ijinskaja (*C. paliurus*), a native plant from southern provinces of China belonging to a very distinctive genus of Juglandaceae, is the sole species in its genus [1–3]. For the special taste and functions of heat clearing and reducing blood pressure and blood glucose, the leaves of *C. paliurus* have been used as a tea in China [3–5]. Phytochemical and pharmacological studies on leaves revealed that the constituents are mainly lignans, flavonoids, and triterpenoids that possess various biological activities including antihyperglycemia, antihyperlipidemia, antihypertension, and antioxidant activities [6–8]. In order to identify new natural compounds with interesting bioactivities, the leaves of *C. paliurus*, indigenous to Ziyuan County of Guangxi Zhuang Autonomous Region, were

CONTACT Cheng-Qin Liang cqliang@glmc.edu.cn; Xu Chen chenxu@glmc.edu.cn
[§]These authors contribute to the paper equally.

phytochemically investigated. As a result, two new tetralone glycosides, cyclopalosides A (**1**) and B (**2**), with caffeoyl unit, were isolated from the leaves of this plant. This paper mainly reports the isolation and structure elucidation of the new compounds, and the antioxidant activities of compound **1**.

2. Results and discussion

Compound **1** was obtained as a yellow, amorphous solid. Its molecular formula $C_{25}H_{26}O_{11}$ was deduced from its HR-ESI-MS data ($[M + Na]^+$ *m/z* 525.1371, calcd 525.1367), with 13 indices of hydrogen deficiency. The IR spectrum showed absorption bands at 3528, 1627, 1647, 1630 and 1264 cm^{-1}, indicating the presence of hydroxy groups, carbonyl functionalities, and aromatic ring. The ^1H NMR spectrum (Table 1) showed signals for four aromatic protons (δ_H 7.43 (2H, d, J = 8.5 Hz, H-2″, 6″) and 6.82 (2H, d, J = 8.5 Hz, H-3″, 5″)), which constituted a classical AA′BB′ system and defined a *para*-substituted benzene ring, and other two aromatic protons (δ_H 7.44 (1H, d, J = 9.1 Hz, H-6) and 6.78 (1H, d, J = 9.1 Hz, H-7)), which constituted a classical AB system and defined a 1,2,3,4-tetrasubstituted aromatic ring. And the ^1H NMR spectrum (Table 1) also showed the presence of a pair of *trans*-olefinic protons (δ_H 7.59 (1H, d, J = 15.9 Hz, H-7″) and 6.32 (1H, d, J = 15.9 Hz,

Table 1. ^1H and ^{13}C NMR spectroscopic data for compounds **1** and **2** (CD_3OD, TMS, δ in ppm, J in Hz).

	1		**2**	
Position	$^1H^a$	$^{13}C^b$	$^1H^c$	$^{13}C^d$
1		206.4		206.4
2 a	3.00–3.08 (m)	33.5	2.94–3.04 (m)	33.5
2 b	2.47–2.52 (m)		2.42–2.48 (m)	
3	2.15–2.22 (m)	30.3	2.13–2.18 (m)	30.3
4	5.35 (t, 3.0)	61.3	5.30 (s)	61.3
5		148.5		148.4
6	7.44 (d, 9.1)	128.8	7.40 (d, 9.2)	128.8
7	6.78 (d, 9.1)	119.0	6.75 (d, 9.2)	119.0
8		159.2		159.3
9		116.2		116.2
10		135.3		135.3
1′	4.82 (d, 7.6)	104.4	4.77 (d, 7.5)	104.4
2′	3.55–3.58 (m)	75.2	3.48–3.52 (m)	75.2
3′	3.49–3.52 (m)	77.8	3.41–3.46 (m)	77.8
4′	3.42–3.46 (m),	71.7	3.35–3.40 (m)	71.7
5′	3.63–3.67 (m)	75.7	3.57–3.61 (m)	75.7
6′a	4.53 (dd, 11.9, 1.9)	64.5	4.46 (dd, 11.9, 1.9)	64.4
6′b	4.38 (dd, 11.9, 6.6)		4.31–4.39 (m)	
1″		127.0		127.5
2″	7.43(d, 8.5)	131.2	7.12 (d, 1.5)	111.5
3″	6.82 (d, 8.5)	116.9		149.4
4″		161.3		150.7
5″	6.82 (d, 8.5)	116.9	6.78 (d, 8.2)	116.5
6″	7.43 (d, 8.5)	131.2	7.01 (dd, 8.2, 1.5)	124.3
7″	7.59 (d, 15.9)	146.8	7.54 (d, 15.9)	147.1
8″	6.32 (d, 15.9)	114.9	6.31 (d, 15.9)	115.2
9″		168.9		168.8
$-OCH_3$			3.86 (s)	56.4

[a]Recorded at 600 MHz.
[b]Recorded at 150 MHz.
[c]Recorded at 400 MHz.
[d]Recorded at 100 MHz.

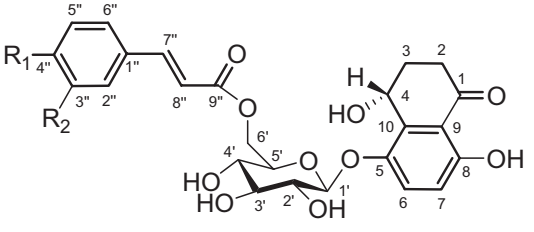
H-8″)), an oxygenated methine (δ 5.35 (1H, t, J = 3.0 Hz, H-4)), and an anomeric proton (δ 4.82 (1H, d, J = 7.6 Hz, H-1′)). The ^{13}C NMR and DEPT spectra (Table 1) exhibited 25 carbon signals, including 3 methylenes (1 oxygenated), 14 methines (6 aromatic, 2 olefinic and 6 oxygenated), and 8 quaternary carbons (2 carbonyl and 6 aromatic).

Careful investigation of the ^{1}H and ^{13}C NMR spectroscopic data of **1** established that it was quite similar to those reported for berchemiaside B [9], which was isolated from *Berchemia floribunda*. Analysis of HMBC and ^{1}H-^{1}H COSY spectra of **1** showed that two compounds have same carbon fragments, including a tetralone, a caffeoyl, and a glucosyl unit (Figure 1). The main difference between **1** and berchemiaside B was that the glucosyl group connected with a caffeoyl unit at δ_C 64.5 (t, C-6′) was located at δ_C 148.5 (s, C-5) in **1**, rather than C-4 in berchemiaside B. This was fully confirmed by the HMBC correlation of δ_H 4.82 (1H, d, J = 7.6 Hz, H-1′) with δ_C 148.5 (s, C-5), and the ROESY correlation of δ_H 4.82 (1H, d, J = 7.6 Hz, H-1′) with δ_H 7.44 (1H, d, J = 9.1 Hz, H-6).

To determine the absolute configuration of C-4 in **1**, the method of the reference [9] was used. After performing the acid hydrolysis of **1**, the hydrolysis product of 4,5,8-trihydroxy-α-tetralone exhibited a negative optical-rotation value ($[\alpha]_D^{24}$ – 34.7 (*c* 0.13, $CHCl_3$), indicating (*R*)-configuration at C-4 in comparison with the reported data of the tetralones from reference [9]. Therefore, the structure of **1** was unambiguously determined as shown in Figure 1 and named cyclopaloside A.

Compound **2** was isolated as an amorphous powder and had the molecular formula of $C_{26}H_{28}O_{12}$, which was determined by analysis of ^{13}C and DEPT NMR as well as HR-ESI-MS data. Careful analysis of 1D and 2D NMR spectroscopic data of **1** and **2** showed that the structure of **2** was strikingly similar to that of **1**, and the main differences between them only happened on the substituents of the caffeoyl unit. The structure of the caffeoyl unit of **2** was established unambiguously by the HMBC (Figure 2) correlations of δ 7.54 (1H, d, J = 15.9 Hz, H-7″) with C-1″, C-2″, C-6″, C-8″ and C-9″, δ 6.31 (1H, d, J = 15.9 Hz, H-8″) with C-1″, C-7″ and C-9″, δ 7.12 (1H, d, J = 1.5 Hz, H-2″) with C-3″ and C-4″, and δ 3.86 (3H, s, -OCH_3) with C-4″, together with the spin systems of H-5″/H-6″ and H-7″/H-8″ in the ^{1}H-^{1}H COSY spectrum. Acid hydrolysis of compound **2** also yielded a 4,5,8- trihydroxy-α-tetralone. It exhibited a negative optical-rotation value ($[\alpha]_D^{24}$ – 33.5 (*c* 0.20, $CHCl_3$)) which was similar as the value of **1**, indicating (*R*)-configuration at C-4 in **2** [9]. Thus, the structure of **2** was furnished as showed in Figure 1 and named cyclopaloside B.

Antioxidant activities have been attributed to various reactions and mechanisms, such as radical scavenging, reductive capacity, prevention of chain initiation, and binding of



1 R_1 = OH R_2 = H
2 R_1 = OMe R_2 = OH

Figure 1. Structures of compounds **1** and **2**.

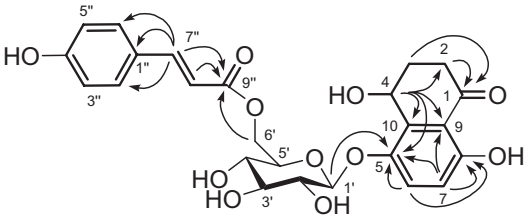


Figure 2. Selected HMBC correlations of compound **1**.

transition metal ion catalysts [10]. In addition, it has been reported that high antioxidant activity could be attributed to the phenolic hydroxyl groups [11]. So in this experiment, the *in vitro* antioxidant capacities of compound **1** were evaluated using different biochemical methods including DPPH, hydroxyl and superoxide anion radical scavenging assay. As illustrated in Figure 3(a), the DPPH radical scavenging rate of compound **1** at 80 μmol/L was 61.94%, with IC_{50} value of 57.50 μmol/L. In the superoxide test, the scavenging rate of compound **1** was 44.28% (Figure 3(c)) at 40 μmol/L, and the IC_{50} value was 61.23 μmol/L. These results indicated that compound **1** has potential antioxidant capacity, although the result of hydroxyl radical scavenging rate at 2–16 μmol/L was weak (Figure 3(b)).

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on a JASCO P-1020 digital polarimeter (Horiba, Kyoto, Japan). UV spectra were recorded on a UV-2401PC spectrometer (Shimadzu, Kyoto, Japan). IR spectra were measured with a Bruker Tensor 27 infrared spectrometer with KBr pellets (Bruker, Karlsruhe, Germany). 1D and 2D NMR spectra were performed on Bruker AM-400 and AVANCE III-600 MHz spectrometers (Bruker Optics, Ettlingen, Germany). High-resolution electrospray-ionization mass spectra (HR-ESI-MS) were obtained on an Agilent 6210 ESI/TOF mass spectrometer (Agilent, Santa Clara, CA, USA). Column chromatography (CC) was carried out on silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China), MCI gel (75–150 μM, Mitsubishi Chemical Corporation, Tokyo, Japan), Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden), and Lichroprep RP-18 gel (40–63 μM, Merck, Merck & Co Inc, Darmstadt, Germany). Semi-preparative HPLC was carried out on an Agilent 1260 liquid chromatography (LC) with a Zorbax SB-C18 column (5 μM, 9.4 mm × 25 cm) (Agilent, Santa Clara, CA, U.S.A.). Absorbance was recorded on an Infinite M200 Pro multifunctional microplate tester (Tecan, Grodig, Austria). TLC was carried out on silica gel 60 F_{254} on glass plates (Qingdao Marine Chemical, Inc., Qingdao, China) using various solvent systems and spots were visualized by heating the silica gel plates sprayed with 10% H_2SO_4 in EtOH (V/V = 10:90). Glucose was obtained from J&K Scientific Ltd. (Beijing, China). Vitamin C, $FeSO_4$, Ethanol, H_2O_2, Tris and HCl were obtained from XiLong Scientific Ltd. (Guangdong, China). DPPH and salicylic acid were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

3.2. Plant material

The leaves of *C. paliurus* (50.0 kg) were collected in Ziyuan County of Guangxi Province, China, in October 2013, and was identified by prof. Ding-Zhong Huang, Guilin Institute of Botany, Chinese Academy of Science. The voucher specimen (No. 201310001) has been deposited in the College of Pharmacy, Guilin Medical University, China.

3.3. Extraction and isolation

The air-dried and powdered leaves of *C. paliurus* (50 kg) were extracted three times with 70% aqueous EtOH (300 L × 3) at room temperature and concentrated *in vacuo* to yield a crude extract, which was dissolved in H_2O, and then extracted successively with EtOAc and n-BuOH. The EtOAc-soluble part was chromatographed by using a silica gel column (100–200 mesh, 20 × 150 cm, 8.0 kg), eluted with a $CHCl_3$–MeOH gradient system (9:1, 8:2, 2:1, 1:1, and 0:1, v/v), to yield four fractions (Fr.A–D). Fr.A (2.985 kg) was further purified by using silica gel column eluted with petroleum ether-EtOAc (1:2, 1:3, and 0:1, v/v) and MeOH to give four subfractions (Fr.A1–A4). After decolorized on MCI column, fraction Fr.A1 was chromatographed on Sephadex LH-20 eluted with MeOH to afford three subfractions (Fr.A1a–A1c). Fr.A1a was purified on silica gel column eluted with petroleum ether–acetone (5:1, 3:1, 1:1) to afford nine subfractions (Fr.A1a1–A1a9). Fr.A1a6 was subjected to semipreparative HPLC eluted with MeOH (65–75%, gradient system) to afford compound **1** (t_R 25.6 min, 25 mg) and **2** (t_R 28.2 min, 33 mg).

3.3.1. Cyclopaloside A (1)

Amorphous powder; $[\alpha]_D^{24}$ – 54.5 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε): 215 (4.19), 265 (3.82), 320 (4.08) nm; IR (KBr) ν_{max} 3528, 1627, 1647, 1630, 1264 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; ESI-MS: *m/z* 525 $[M + Na]^+$; HR-ESI-MS: *m/z* 525.1371 $[M + Na]^+$ (calcd for $C_{25}H_{26}O_{11}Na$, 525.1367).

3.3.2. Cyclopaloside B (2)

Amorphous powder; $[\alpha]_D^{18}$ –37.1 (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ε): 203 (3.63), 252 (3.37) nm; IR (KBr) ν_{max} 3432, 2955, 2925, 1697, 1638, 1606, 1515, 1468, 1175, 1075 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; positive ESI-MS: *m/z* 555 $[M + Na]^+$; HR-ESI-MS: *m/z* 555.1473 $[M + Na]^+$ (calcd for $C_{26}H_{28}O_{12}Na$, 555.1473).

3.4. Acid hydrolysis of compounds 1 and 2

Compounds **1** (5.0 mg) and **2** (5.0 mg) were each refluxed with 2 N HCl (5 ml) on a boiling water bath for 2 h. After neutralization with $NaHCO_3$ and extraction with ethyl acetate, the aqueous layer was concentrated, and glucose and aglycone were purified from it. The presence of glucose was confirmed by comparison with authentic samples by using TLC (silica gel, BuOH-AcOH-H_2O 5:1:5 upperlayer) [9]. And the D-form of glucose was determined by its positive optical rotation in water.

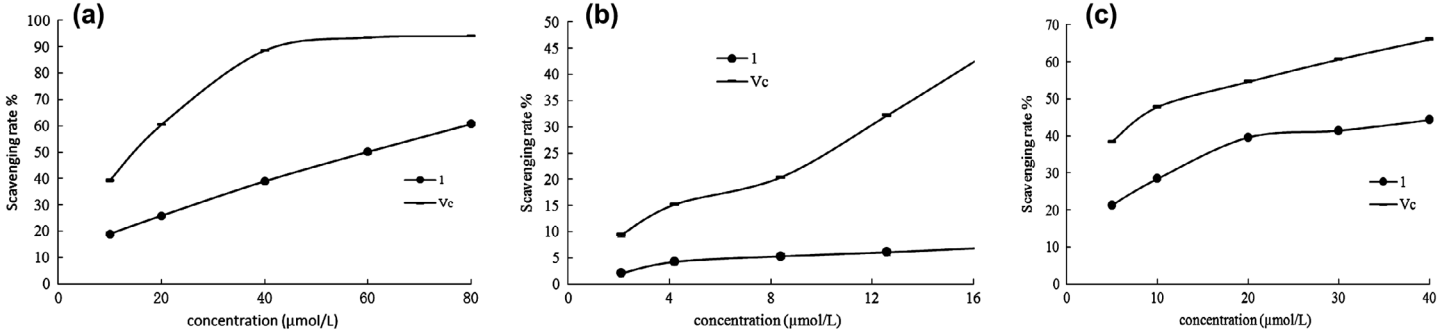


Figure 3. Antioxidant activity analysis of compound **1** with various methods: (a) DPPH radical scavenging assay; (b) Hydroxyl radical scavenging assay; (c) Superoxide radical scavenging assay.

3.5. Evaluation of antioxidant activity

3.5.1. DPPH radical scavenging activity

The radical scavenging activity was evaluated using an improved method of reference [11]. Vitamin C (Vc) was used as a positive control. The reaction mixture contained 100 µl of 0.2 mmol/L DPPH and 100 µl samples with different concentrations, The mixture was kept for 30 min in dark at room temperature for its reaction before the absorbance was measured at 517 nm. The antioxidant activity was estimated based on the percentage of DPPH radical scavenged using the equation: Antioxidant activity (%) = $[1 - (A_1 - A_2)/A_0] \times 100$. Where A_0 is the absorbance of DPPH solution without sample, A_1 is the absorbance of the test sample mixed with DPPH solution and A_2 is the absorbance of the sample without DPPH solution.

3.5.2. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging assay was performed by mixing the different concentrations (5, 10, 20, 30, and 40 µmol/L) of compounds and some reaction solutions such as $FeSO_4$, salicylic acid-ethanol and H_2O_2 together using the method from reference [12]. The reaction solutions were incubated at 37 °C for 30 min, and the absorbance was measured at 510 nm using vitamin C as a positive control. The hydroxyl radical scavenging activity was calculated by the formula: Hydroxyl radical scavenging rate (%) = $[1 - (A_1 - A_2)/A_0] \times 100$. Where A_0 is the absorbance of blank (water instead of sample), A_1 is the absorbance of sample, and A_2 is the absorbance of background (water instead of H_2O_2).

3.5.3. Superoxide radicals scavenging activity

Superoxide radical was generated with the method based on Pu et al. [13]. The reaction mixture contained 200 µl of Tris–HCl buffer (0.1 mol/L, pH 8.2), which contained 10 µl pyrogallol solution (0.025 mol/L) and the 10 µl samples with varying concentrations (2.1, 4.2, 8.4, 12.6, 16.8 µmol/L). And after being incubated at 25 °C for 20 min, the change speed of absorbance (A/min) of the reactive solution was measured at 320 nm. The superoxide radical scavenging activity was calculated by the formula: Antioxidant activity (%) = $[(\Delta A_0 - \Delta A_1)/\Delta A_0] \times 100$. Where ΔA_0 is the change speed of absorbance of the control group in the superoxide radical generation system and ΔA_1 is the change speed of absorbance of the test sample.

Disclosure statement

No potential conflict of interest was reported by the authors.

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