New Triterpenoids from the Leaves of *Cyclocarya paliurus*

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Abstract

Six new triterpenoids including four new secodammarane triterpenoid glycosides (**1**–**4**), an epoxydammarane triterpenoid glycoside (**5**), and a new secodammarane triterpenoid (**6**) were isolated from the ethanolic extract of the leaves of *Cyclocarya paliurus*. The structures of these compounds were elucidated by spectroscopic analysis methods. Compounds **1**–**6** were evaluated for their inhibitory activities against α-glucosidase, lipase, DPP-IV, and aldose reductase.

Key words

Cyclocarya paliurus · Juglandaceae · triterpenoid · secodammarane type · biological activity

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Cyclocarya paliurus (Batal.) Iljinsk (Juglandaceae) is a Chinese endemic plant which has been used to treat hypertension and diabetes [1,2] and is known to have a hypolipemic effect [3]. Some dammarane-, oleanane-, and ursane-type triterpenes, flavonoids, and phenolic acids were reported from this plant [4–18]. Phytochemical investigation of its leaves was undertaken to assess the chemical and biological diversity. This report describes the isolation and characterization of six new triterpenoids from the leaves of *C. paliurus* (**Fig. 1**). Chemical and spectroscopic methods were used to elucidate the structures of the new compounds.

The HR-ESI-MS of **1** exhibited the molecular formula $C_{37}H_{62}O_9$. The ^{1}H NMR spectrum of **1** showed seven quaternary methyls, a pair of coupled olefinic protons at δ 5.69 and 5.44, whose coupling constant (J = 16.0 Hz) showed that the configuration of the double bond was *E*, terminal methylene olefinic protons at δ 4.83 and 4.72, and an oxymethine proton at δ 4.13, in addition to partially overlapped mutiplets of methylenes and methines protons between δ 0.78 and δ 2.70. These data were used to assign the triterpenoid aglycone skeleton. The presence of an anomeric proton of glycosyl at δ 4.40 as well as other oxymethine protons and one methyl doublet of glycosyl suggested that **1** was a triterpenoid saponin. The ^{13}C NMR spectra of **1** showed 37 carbon signals including a triterpenoid skeleton as well as glycosyl and methoxy moieties (**Table 1**). The seven degrees of unsaturation of **1** required by the molecular formula included one carboxyl, two double bonds, and one ring of glycosyl, so that three degrees of unsaturation remained, confirming that **1** possessed an unusual triterpenoid skeleton with a tricyclic parent nucleus, which was

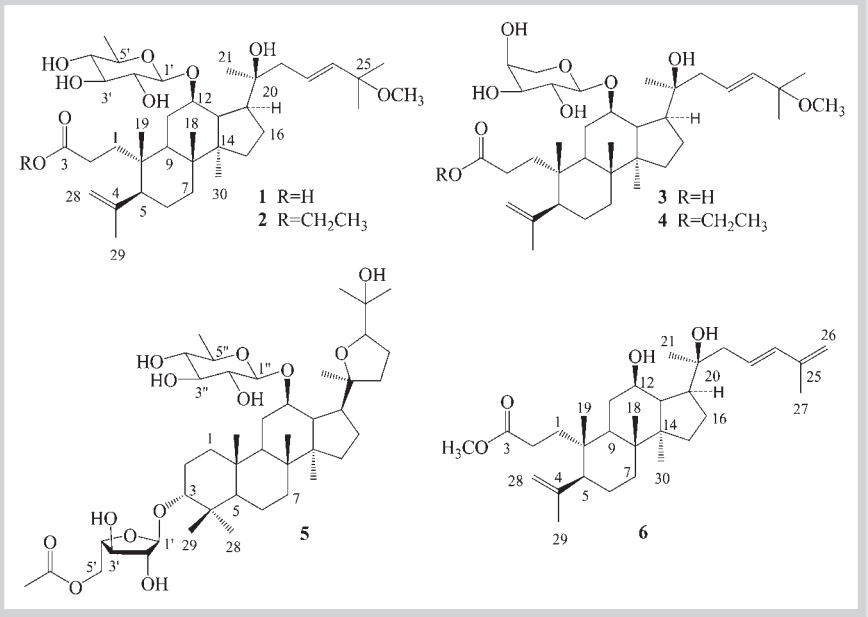

Fig. 1 Structures of compounds **1**–**6**.

finally established by detailed analyses of 2D NMR spectra of **1**. A further comparison of the NMR data of **1** with those of the related compounds in the literature [6] indicated that **1** was in good agreement with (23*E*)-(12*R*,20*S*)-20-hydroxy-3,4-secodammara-4(28),23-dien-3-oic acid 12-*O*-*β*-D-quinovopyranoside except for the data of C-25, which was methoxylated in **1**. HMBC correlations from H_3-OCH_3 (3.08) to C-25 (75.1) (**Fig. 2**) confirmed that the methoxy group was linked to position C-25. HMBC correlations from H-1′ (4.40) to C-12 (75.5) (**Fig. 2**) indicated that the glycosyl was located at C-12. The absolute configurations at C-12 and C-20 were determined to be *R* and *S*, respectively, on the basis of comparison to the ^{13}C NMR chemical shift and optical rotation data for analogous 3,4-secodammaranes [6, 19]. The stereochemistry of C-5 and C-10 were determined on the basis of the natural dammarane-type triterpene biosynthetic pathway [20]. Ultimately, the structure of **1** was deduced as (23*E*)-(12*R*,20*S*)-12,20-dihydroxy-25-methoxy-3,4-secodammara-4(28),23-dien-3-oic acid 12-*O*-*β*-D-quinovopyranoside, and **1** was named cyclocarioside D.

The HR-FAB-MS of **2** exhibited the molecular formula $C_{39}H_{66}O_9$. The NMR spectrum of **2** showed similarities to that of **1**, except that the NMR signals due to the proton of 3-carboxyl of **1** was replaced by signals attributable to an ethyl unit (δ_H 4.02 and 1.20; δ_C 60.4 and 14.6). This suggested that **2** was a derivative of **1** containing an ethyl ester unit at the 3-carboxyl. In the HMBC spectrum of **2**, long-range heteronuclear correlations from δ_H 4.02 to δ_C 175.6 (C-3) proved the location of the ethyl unit at C-3. Ultimately, the structure of **2** was deduced as (23*E*)-(12*R*,20*S*)-20-hydroxy-25-methoxy-3,4-secodammara-4(28),23-dien-3-oic acid ethyl ester 12-*O*-*β*-D-quinovopyranoside, and **2** was named cyclocarioside E.

The HR-ESI-MS of **3** exhibited the molecular formula $C_{36}H_{60}O_9$. The NMR spectrum of **3** showed similarities to those of **1** and related compounds in the literature [6], except that the NMR signals due to the quinovopyranosyl of **1** were replaced by signals attributable to an arabinopyranosyl unit (**Table 2**). This suggested that **3** was a triterpenoid arabinopyranoside. In the HMBC spectrum of **3**, long-range heteronuclear correlations from δ_H H-1′ (4.32) to δ_C C-12 (74.8) indicated that the arabinopyranosyl unit was also linked at a C-12 position. The stereochemistry at C-12 and C-20 were determined to be *R* and *S*, respectively, on the basis of comparison to the ^{13}C NMR chemical shift data and optical rotation for analogous 3,4-secodammaranes [6, 19]. The structure of **3** was deduced as (23*E*)-(12*R*,20*S*)-12,20-dihydroxy-25-methoxy-3,4-secodammara-4(28),23-dien-3-oic acid 12-*O*-*α*-L-arabinopyranoside, and **3** was named cyclocarioside F.

The HR-FAB-MS of **4** exhibited the molecular formula $C_{38}H_{64}O_9$. The NMR spectrum of **4** showed similarities to that of **3**, except that the NMR signals due to the proton of 3-carboxyl of **3** were replaced by signals attributable to an ethyl unit (δ_H 4.06 and 1.22; δ_C 60.6 and 14.5). This suggested that **4** was a derivative of **3** containing an ethyl ester unit. A comparison of the ^{13}C NMR data between **3** and **4** (**Table 2**) indicated that the C-3 of **4** was shielded by $\Delta\delta_C$ 1.1 ppm; this suggested that the location of the ethyl unit was also at the C-3-carboxyl of **4**. Therefore, the structure of **4** was deduced as (23*E*)-(12*R*,20*S*)-12,20 -dihydroxy-25-methoxy-3,4-secodammara-4(28),23-dien-3-oic acid ethyl ester 12-*O*-*α*-L-arabinopyranoside, and **4** was named cyclocarioside G.

The HR-FAB-MS of **5** exhibited the molecular formula $C_{43}H_{72}O_{13}$. The NMR spectrum of **5** showed that it was a triterpenoid glycoside with a dammarane triterpenoid aglycone skeleton and two sugars (**Table 3**). The configuration of the hydroxyl group at C-3 was deduced to be *α*-positioned by a small $^3J_{H,H}$ coupling constant. The NMR signals of protons and protonated carbons were assigned by the HSQC experiment. Further comparison of the NMR data of **5** with the related compound cyclocarioside I in the literature [4] indicated that the NMR data of **5** were in good agreement with those of (20*S*,24*R*)-epoxydammarane-(3*β*,12*β*)-25-hydroxyl-12-*O*-*β*-D-quinovopyranosyl-3-*O*-*α*-L-arabinofuranoside except for the data of C-5′ and C-3. Substitution patterns and position of C-5′ and C-3 were defined in the HMBC spectrum; the correlations from H-3 (δ_H 3.26) to C-1′ (106.0) and H-1′ (δ_H 4.93) to C-3 (79.6) revealed the linkage position of arabinofuranose at C-3; the correlations from H-5′ (4.10, 4.26) to C=O (170.8) of the acetyl group confirmed that the acetyl group was attached to H-5′ of the arabinofuranose. The correlations from H-1″ (δ_H 4.40) to C-12 (76.8) indicated that the quinovopyranose unit was linked at C-12 (**Fig. 3**). The stereochemistry at C-20

Table 1 NMR spectroscopic data for cyclocarioside D (**1**) and cyclocarioside E (**2**).

Position	Cyclocarioside D (1)		Cyclocarioside E (2)	
	δ_C, type	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	37. 9, CH_2	2.49, dt (5.0, 13.2)	37.9	2.49, dt (5.4, 13.2)
		1.51, m		1.50, dt (3.0, 13.2)
2	29.8 ca, CH_2	2.22, dt (3.0, 13.2)	30.4 ca	2.22, dt (3.0, 13.2)
		2.66, dt (5.0, 13.2)		2.69, dt (5.4, 13.2)
3	177.1, C		175.6	
4	149.0, C		149. 0	
5	52.3, CH	2.07, dd (12.9, 2.7)	52.3	2.08, dd (12.6, 2.4)
6	25.7, CH_2	1.87, m	25.7	1.87, m
		1.36, m		1.36, m
7	35.3, CH_2	1.59, dt (3.6, 13.2)	35.3	1.58, dt (3.3, 13.2)
		1.18 overlapping		1.18 overlapping
8	41.4, C		41.4	
9	40.8, CH	1.89, m	40.8	1.88, m
10	40.4, C		40.4	
11	33.6, CH_2	2.44, td (4.2, 13.2)	33.8	2.44, td (4.2, 12.6)
		1.40, m		1.40, m
12	75.5, CH	4.13, dt (4.8, 10.8)	75.6	4.12, dt (4.8, 10.8)
13	44.8, CH	1.94 br, d (10.8)	44.8	1.94 br, d (10.8)
14	51.3, C		51.3	
15	31.6, CH_2	1.06 overlapping	31.6	1.06 overlapping
		1.42, m		1.42, m
16	25.5, CH_2	1.72, m	25.5	1.72, m
		1.22 overlapping		1.22, overlapping
17	50.2, CH	1.80, dt (4.8, 9.6)	50.2	1.81, dt (6.0, 10.2)
18	16.8, CH_3	1.06, s	16.8	1.05, s
19	20.2, CH_3	1.11, s	20.2	1.10, s
20	74.6, C		74.6	
21	27.1, CH_3	1.17 br, s	27.1	1.17 br, s
22	45.1, CH_2	2.24 br, d (7.8)	45.1	2.23 br, d (7.8)
23	127.3, CH	5.69, dt (16.0, 7.8)	127.3	5.69, dt (16.0, 7.8)
24	138.9, CH	5.44, d (16.0)	138.9	5.44, d (16.0)
25	75.1, C		75.1	
26	26.5, CH_3	1.20, s	26.5	1.20, s
27	26.1, CH_3	1.21, s	26.1	1.21, s
28	113.7, CH_2	4.83 br, s	113.8	4.83 br, s
		4.72, d (1.2)		4.71, d (1.8)
29	24.0 ca, CH_3	1.76 br, s	24.1 ca	1.76 br, s
30	16.8, CH_3	0.97, s	16.7	0.96, s
1′	99.8, CH	4.40, d (7.2)	99.9	4.40, d (7.2)
2′	75.0, CH	3.21, dd (7.2, 9.0)	75.1	3.25, m
3′	77.6, CH	3.30, t (9.0)	77.6	3.30, m
4′	76.7, CH	3.01, t (9.0)	76.7	3.01, m
5′	72.5, CH	3.27, dd (6.6, 9.0)	72.5	3.27, m
6′	18.2, CH_3	1.23, d (6.6)	18.2	1.23, d (6.0)
O**CH_3**	50.2, CH_3	3.08, s	50.2	3.09, s
O**CH_2**CH_3			60.4	4.02, q (7.2)
OCH_2**CH_3**			14.6	1.20, t (7.2)

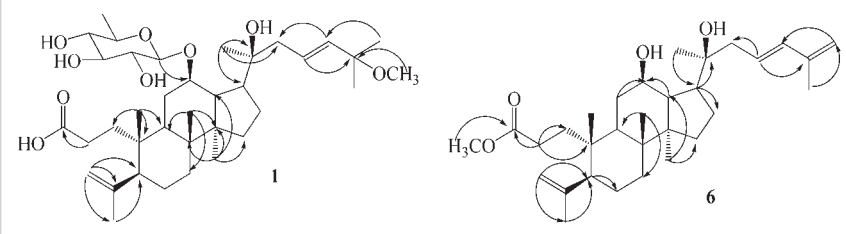

Fig. 2 HMBC (H→C) correlations of compounds **1** and **6**.

Table 2 NMR spectroscopic data for cyclocarioside F (**3**) and cyclocarioside G (**4**).

Position	Cyclocarioside F (3)		Cyclocarioside G (4)	
	δ_C, type	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	38.4, CH_2	2.50, dt (4.8, 13.2)	38.2	2.50, dt (4.8, 13.2)
		1.50, dt (3.6, 13.2)		1.50, dt (3.6, 13.2)
2	30.4, CH_2	2.24, m	30.7	2.24, m
		2.68, ddd (12.6, 5.4, 4.8)		2.68, ddd (12.6, 5.4, 4.8)
3	177.2 [a], C		176.1	
4	149.0, C		148.9	
5	52.3, CH	2.08, dd (11.7, 2.7)	52.2	2.08, dd (11.7, 2.7)
6	25.8, CH_2	1.88, m	25.7	1.88, m
		1.33, m		1.33, m
7	35.4, CH_2	1.60, dt (3.3, 13.2)	35.3	1.60, dt (3.3, 13.2)
		1.20 overlapping		1.20 overlapping
8	41.4, C		41.4	
9	40.8, CH	1.88, m	40.8	1.88, m
10	40.4, C		40.4	
11	33.0, CH_2	2.48, m	33.2	2.48, m
		1.34, m		1.34, m
12	74.8, CH	4.16, dt (4.8, 11.0)	75.0	4.16, ddd (4.8, 10.8, 10.8)
13	44.8, CH	1.94 br, d (11.0)	44.8	1.94 br, d (11.0)
14	51.3, C		51.3	
15	31.6, CH_2	1.08 overlapping	31.6	1.08, overlapping
		1.43, dt (10.8, 9.6)		1.43, dt (10.8, 9.6)
16	25.5, CH_2	1.72, m	25.5	1.70, m
		1.22 overlapping		1.22, overlapping
17	50.1, CH	1.81, dt (6.3, 10.5)	50.2	1.81, dt (6.3, 10.5)
18	16.9, CH_3	1.06, s	16.9	1.06, s
19	20.5, CH_3	1.10, s	20.4	1.10, s
20	74.4, C		74.5	
21	27.1, CH_3	1.18 br, s	27.1	1.18 br, s
22	45.0, CH_2	2.24 br, d (7.8)	45.1	2.24 br, d (7.8)
23	127.3, CH	5.70, dt (16.0, 7.8)	127.2	5.70, dt (16.0, 7.8)
24	138.9, CH	5.45, d (16.0)	138.9	5.45, d (16.0)
25	75.1, C		75.1	
26	26.5, CH_3	1.21, s	26.5	1.21, s
27	26.1, CH_3	1.22, s	26.1	1.22, s
28	113.8, CH_2	4.85 br, s	113.8	4.85 br, s
		4.73, d (1.8)		4.73, d (1.8)
29	24.0 ca, CH_3	1.78 br, s	24.0 ca	1.78 br, s
30	16.8, CH_3	0.97, s	16.8	0.97, s
1′	100.0, CH	4.32, d (7.2)	100.2	4.32, d (7.2)
2′	72.4, CH	3.54, m	72.6	3.54, m
3′	73.8, CH	3.52, m	74.0	3.52, m
4′	69.5, CH	3.77 br, s	69.6	3.77, m
5′	67.2, CH_2	3.89, dd (10.2, 2.4)	67.2	3.89, dd (10.2, 2.4)
		3.54, m		3.54, m
O**CH_3**	50.2, CH_3	3.09, s	50.1	3.09, s
O**CH_2**CH_3			60.6	4.06, q (6.9)
OCH_2**CH_3**			14.5	1.22, t (6.9)

[a] Determined from HMBC experiment

and C-24 of **5** were determined to be *S* and *R*, respectively, on the basis of comparison to the ^{13}C NMR chemical shift data (δ_C 87.0 and 84.4) of analogous expoxydammaranes, cyclocarioside I [4] and cyclocarioside A [8], B, and C [7]. In order to determine the stereochemistry of C-3 and C-12, a NOESY-experiment with a mixing time of 1.301 sec was carried out. The correlations between H-3 (3.26) and H-29 (0.88) as well as between H-12 (4.04) and H-30 (0.94) suggested that H-3 was present in *β*-orientation and H-12 in *α*-orientation. Therefore, the structure of **5** was deduced as (20*S*,24*R*)-epoxydammarane (3*α*,12*β*)-25-hydroxyl-12-*O*-*β*-D-quinovopyranosyl-3-*O*-(5′-*O*-acetyl)-*α*-L-arabinofuranoside, and **5** was named cyclocarioside H.

The HR-EI-MS of **6** exhibited the molecular formula $C_{31}H_{50}O_4$. The NMR spectrum of **6** showed a triterpenoid skeleton and a methoxy group. The seven degrees of unsaturation of **6** confirmed that it possessed a 3,4 secodammarane triterpene skeleton. The NMR signals of protons and protonated carbons (**Table 3**) were assigned by the HSQC and HMBC spectrum correlations (**Fig. 2**), together with resolvable homonuclear vicinal coupling correlations. Long-range heteronuclear correlations from δ_H 3.64 (-OCH_3) to δ_C 174.4 (C-3) proved that the carboxyl of C-3 was an acetate methyl ester in **6**. The stereochemistries at C-12 and C-20 of **6** were determined to be *R* and *S*, respectively, on the basis of comparison to the ^{13}C NMR chemical shift data for those corre-

Table 3 NMR spectroscopic data for cyclocarioside H (**5**) and cyclocarin A (**6**).

Position	Cyclocarioside H (5)		Cyclocarin A (6)	
	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1	35.7, CH_2	2.50, dt (13.8, 3.6)	34.3, CH_2	1.63, m
		1.30, m		
2	21.2, CH_2	1.54, m	28.4, CH_2	2.18, m
		1.70, m		2.33, m
3	79.6, CH	3.26, t (2.4)	174.4, C	
4	38.1, C		147.2, C	
5	51.2, CH	1.28, m	50.6, CH	1.97, br, dd (12.5, 2.5)
6	18.6, CH_2	1.42, m	24.6, CH_2	1.38, m
				1.79, t (10.5)
7	36.8, CH_2	1.25, m	33.4, CH_2	1.50, m
				1.24, br, d (13.0)
8	41.9, C		39.0, C	
9	54.5, CH	1.68, m	40.5, CH	1.63, m
10	40.2, C		39.3, C	
11	34.6, CH_2	2.46, dt (12.6, 4.8)	30.9, CH_2	1.06 br, d (8.8)
		1.32, m		1.32, m
12	76.8, CH	4.04, m	70.6, CH	3.60, m
13	41.4, CH	1.70, m	48.2, CH	1.79, t (10.5)
14	50.6, C		52.1, C	
15	32.0, CH_2	1.06, m	31.6, CH_2	1.38, m
		1.42, m		1.94, m
16	27.1, CH_2	1.43, m	26.4, CH_2	1.87, m
		1.84, m		
17	49.6, CH	1.84, m	52.5, CH	2.12, m
18	17.0, CH_3	1.00, s	15.4, CH_3	1.02, s
19	16.7, CH_3	1.10, s	20.1, CH_3	0.88, s
20	87.0, C		74.2, C	
21	24.8, CH_3	1.12, s	27.6, CH_3	1.19, s
22	34.2, CH_2	1.66, m	39.4, CH_2	2.27, dd (13.0, 8.5)
				2.45, dd (13.0, 8.0)
23	26.3, CH_2	1.84, m	125.7, CH	5.74, dt (16.0, 8.0)
24	84.4, CH	3.74, d (7.2)	136.5, CH	6.22, d (16.0)
25	71.7, C		141.8, C	
26	26.3, CH_3	1.16, s	115.2, CH_2	4.90, br, s
				4.89, br, s
27	26.9, CH_3	1.14, s	18.7, CH_3	1.85, s
28	29.8 ca, CH_3	0.92, s	113.6, CH_2	4.86, br, s
				4.66, br, s
29	22.9, CH_3	0.88, s	23.2, CH_3	1.73, s
30	17.2, CH_3	0.94, s	16.7, CH_3	0.89, s
1′	106.0, CH	4.93, br, s		
2′	83.8, CH	4.08, m	51.6, CH_3	3.64, s
3′	79.8, CH	3.84, dd (6.3, 6.3)		
4′	82.1, CH	4.08, m		
5′	65.0, CH_2	4.26, dd (11.4, 3.0)		
		4.10, dd (11.4, 6.6)		
CH_3**COO**	170.8, C			
CH_3COO	20.7, CH_3	2.00, s		
1′′	100.8, CH	4.40, d (7.8)		
2′′	75.3, CH	3.10, dd (9.0, 7.8)		
3′′	78.0, CH	3.31, dd (9.0, 9.0)		
4′′	76.8, CH	2.99, dd (9.0, 9.0)		
5′′	72.4, CH	3.28, dd (9.0, 6.0)		
6′′	18.2, CH_3	1.22, d (6.0)		

sponding to 3,4-secodammaranes compounds [19]. Therefore, the structure of **6** was deduced as (23*E*)(12*R*,20*S*)-12,20-dihydroxy-3,4-secodammarane-4(28),23,25-trien-3-oic acid methyl ester, and **6** was named cyclocarin A.

Compounds **1**–**6** were evaluated against several human cancer cell lines and were not active (IC_{50}>10 µM). They were screened for inhibitory activities against α-glucosidase (40 µM), lipase (5 µM), DPP-IV (10 µM), and aldose reductase (10 µM), respectively. These compounds showed weak activities at the same concentration as the positive control drugs (acarbose, orlistat, INDP-2, and epalrestat, see **Table 4**).

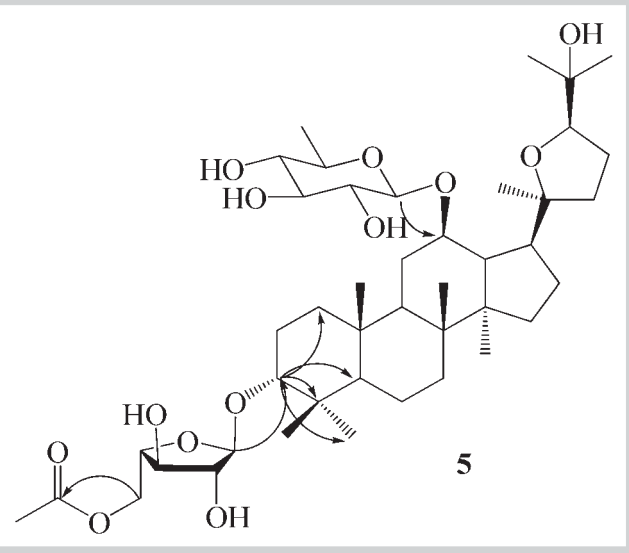

Fig. 3 Main HMBC (H→C) correlations of compound **5**.

Materials and Methods

The leaves of *C. paliurus* were collected at Qimen, Anhui Province, China, in September 2001, and were identified by Mr. Ma-Lin (Institute of Materia Medica, Chinese Academy of Medical Sciences). A voucher specimen (No. ZH02001) has been deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences.

The air-dried and powdered leaves of *C. paliurus* (1 kg) were extracted with 95% ethanol (6 L × 1 h × 3) by ultrasonication (230 W, 35 KHz) at room temperature. The extracts were combined and concentrated under reduced pressure (at < 60 °C) to give a dark brown residue (51 g). The residue was suspended in water (3 L) and then partitioned with ethyl acetate (3 L × 4, 29 g). The ethyl acetate fraction (28 g) was subjected to column chromatography (CC) [silica gel (mesh 45–75 µm; 500 g), column, 6 × 70 cm] eluted with a gradient of increasing methanol (0–20%) in chloroform and methanol to yield fourteen fractions (A–N) on the basis of TLC analyses. Fraction J [$CHCl_3$-MeOH (100 : 1, 6.0 L), 2.3 g] was applied to CC [silica gel (mesh 45–75 µm; 60 g), column, 2 × 45 cm] eluted with $CHCl_3$-MeOH (40 : 1, 3.5 L) to give the subfractions J_2 (651 mg) and J_3 (839 mg). The subfraction J_2 was purified by CC [Sephadex LH-20 (50 g), column, 1.5 × 60 cm] eluted with $CHCl_3$-MeOH and was applied to preparative HPLC [column, RP-18 (250 × 10 mm, 5 µm); MeOH-H_2O (70 : 30), 2.0 L, 2 mL/min flow rate] to obtain **2** (35 mg) and **4** (44 mg). Fraction K (0.9 g) was purified by CC [Sephadex LH-20 (50 g), column, 1.5 × 60 cm] eluted with petroleum ether-$CHCl_3$-MeOH (5 : 5 : 1, 450 mL) to give the subfraction K_2 (690 mg) which was applied to CC [silica gel (mesh 45–75 µm; 30 g), column, 1.5 × 30 cm] eluted with $CHCl_3$-MeOH (50 : 1, 1.9 L) to yield **6** (12 mg). Fraction L (2.5 g) was purified by CC [Sephadex LH-20 (50 g), column, 1.5 × 60 cm] eluted with petroleum ether-$CHCl_3$-MeOH (5 : 5 : 1, 1.6 L) to give six subfractions (L_1-L_6). The L_3 subfraction (1.2 g) was subjected to CC [silica gel (mesh 45–75 µm; 50 g), column, 2 × 45 cm] eluted with $CHCl_3$-MeOH (20 : 1, 3.0 L) and then applied to preparative HPLC [column, RP-18 (250 × 10 mm, 5 µm); MeOH-H_2O (70 : 30), 2.0 L, 2 mL/min flow rate] to obtain **1** (30 mg), **3** (80 mg), and **5** (221 mg).

Table 4 Assessment of α-glucosidase, lipase, DPP-IV, and aldose reductase inhibitory activity (%) of compounds **1**–**6**.

Compound	α-Glucosidase	Lipase	DPP-IV	Aldose reductase
1	11.4	8.9	11.1	9.5
2	10.7	14.7	11.4	6.8
3	8.6	9.5	23.2	5.3
4	3.6	3.7	11.1	10.0
5	1.5	5.7	17.1	9.1
6	2.9	8.4	15.7	9.1
Acarbose	100			
Orlistat		100		
INDP-2			97.50	
Epalrestat				100

Isolates: *Cyclocarioside D* (**1**): colorless needles (MeOH), m.p. 136.5–139.5 °C; R_f = ca. 0.55 ($CHCl_3$-MeOH-HAc, 5 : 1 : 0.1); $[\alpha]_D^{20}$ + 4.6 (*c* 0.09, MeOH); NMR data, see **Table 1**; FAB-MS *m/z* (%): 673 $[M + Na]^+$ (12); HR-ESI-MS *m/z* 673.4288 $[M + Na]^+$ (calcd. for $C_{37}H_{62}O_9Na$, 673.4286).

Cyclocarioside E (**2**): colorless powder (MeOH), m.p. 127.5–129.6 °C; R_f = ca. 0.63 ($CHCl_3$-MeOH, 10 : 1); $[\alpha]_D^{20}$ + 4.8 (*c* 0.10, MeOH). NMR data, see **Table 1**; FAB-MS *m/z* (%): 701 $[M + Na]^+$ (13); HR-ESI-MS 701.4616 $[M + Na]^+$ (calcd. for $C_{39}H_{66}O_9Na$ 701.4599).

Cyclocarioside F (**3**): colorless powder (MeOH), m.p. 133.0–136.5 °C; R_f = ca. 0.34 ($CHCl_3$-MeOH, 10 : 1); $[\alpha]_D^{20}$ + 12.4 (*c* 0.10, MeOH). NMR data, see **Table 2**; FAB-MS *m/z* (%): 659 $[M + Na]^+$ (4); HR-ESI-MS 659.4133 $[M + Na]^+$ (calcd. for $C_{36}H_{60}O_9Na$ 659.4135).

Cyclocarioside G (**4**): colorless powder (MeOH), m.p. 114.5–116.8 °C; R_f = ca. 0.60 ($CHCl_3$-MeOH, 10 : 1); $[\alpha]_D^{20}$ + 2.8 (*c* 0.11, MeOH); NMR data, see **Table 2**; FAB-MS *m/z* (%): 687$[M + Na]^+$ (3); HR-FAB-MS 687.4425 $[M + Na]^+$ (calcd. for $C_{38}H_{64}O_9Na$ 687.4448).

Cyclocarioside H (**5**): was obtained as colorless powder (MeOH), m.p. 129.5–133.4 °C; R_f = ca. 0.54 ($CHCl_3$-MeOH-HAc, 5 : 1 : 0.1); $[\alpha]_D^{20}$ − 31.4 (*c* 0.11, MeOH); NMR data, see **Table 3**; FAB-MS *m/z* (%): 797 $[M + H]^+$ (0.7); HR-ESI-MS 819.4867 $[M + Na]^+$ (calcd. for $C_{43}H_{72}O_{13}Na$ 819.4865).

Cyclocarin A (**6**): colorless powder (MeOH), m.p. 129.2–130.2 °C; R_f = ca. 0.43 ($CHCl_3$-MeOH, 20 : 1); $[\alpha]_D^{20}$ + 18.6 (*c* 0.06, MeOH). NMR data, see **Table 3**; EI-MS *m/z* (%): 486 (0.4), 468 (2.3), 405 (22), 387 (100), 355 (35), 85 (22), 71 (29), 57 (42); HR-EI-MS *m/z* 486.3702 (calcd. for $C_{31}H_{50}O_4$ 486.3709).

Acid hydrolysis: Solutions of **1, 2, 3, 4**, or **5** were mixed with 0.5 N HCl and refluxed for 1 h. Each reaction mixture was diluted with water and extracted exhaustively with $CHCl_3$. The $CHCl_3$ layers were dried over anhydrous Na_2SO4 and evaporated under reduced pressure. Examination of each $CHCl_3$ layer by TLC showed a number of products which were less polar than the starting materials. The aqueous layer was neutralized with sodium bicarbonate and allowed to dry at room temperature. TLC analysis of the residues using $CHCl_3$-MeOH-H_2O (6 : 4 : 1) showed the presence of quinovose or arabinose as the only sugars for **1, 2, 3**, and **4**, and both quinovose and arabinose for **5**.

Assessment of several human cancer cell lines inhibitory activity: See previous report [21].

Assessment of DPP-IV inhibitory activity: See previous report [22].

Supporting information

UV, IR, MS, ^{1}H NMR, and ^{13}C NMR spectra as well as 2D NMR correlation spectra of compounds **1**–**6**, the key chemical method of sugar moieties acid hydrolysis, and TLC identification are available as Supporting Information.

The detailed protocols for assaying α-glucosidase, lipase, and aldose reductase inhibition are also available as Supporting Information.

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Conflict of Interest

The corresponding authors declare that the manuscript is submitted on behalf of all authors. There are no conflicts of interest among all authors of the manuscript. Copyright belongs to the publisher upon acceptance of the manuscript.

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