SHORT REPORT

ACG publications

Rec. Nat. Prod. 19:5 (2025) 621-626

records of natural products

Cyclocarioside Z14, A New Dammarane Triterpenoid Glycoside from The Leaves of *Cyclocarya paliurus* with Cytotoxicity

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(Received May 15, 2025; Revised July 18, 2025; Accepted July 19, 2025)

Abstract: A new dammarane triterpenoid glycoside cyclocarioside Z14 (**1**) and four known compounds (**2**-**5**) were isolated from the dichloromethane extract of the leaves of *Cyclocarya paliurus*. The chemical structures were elucidated by the extensive spectroscopic data analysis of NMR, HR-ESI-MS, and acid hydrolysis. All isolated compounds were assayed on the cytotoxicity against seven human cancer cell lines. The compounds **1** and **3** showed moderate cytotoxicity against MCF-7 cells with an IC_{50} value of 29.51 μM and 33.88 μM.

Keywords: *Cyclocarya paliurus*; dammarane triterpenoid glycoside; structural elucidation; cytotoxicity.

1. Plant Source

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

2. Previous Studies

Cyclocarya paliurus (Batal.) Iljinsk, the only living species in the Cyclocarya genus of the Juglandaceae family, is mainly distributed in the south of China. Its leaves have been widely used as

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products September-October 2025 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.537.2505.3525
Available online: July 28, 2025

functional tea in folk due to its sweet taste and regulating effects on blood glucose, blood lipid and blood pressure [1, 2]. In our previous study, several novel dammarane triterpenoid glycosides with hypoglycemic and cytotoxic activities were isolated from the leaves of *C. paliurus* [3-6]. Ongoing phytochemical research to explore the chemical diversity of *C. paliurus*, a new dammarane triterpenoid glycoside named cyclocarioside Z14 (**1**) and four known compounds (**2**-**5**) isolate from the dichloromethane extract of the leaves of *C. paliurus* (Figure 1). During the latest 30 years, phytochemists home and abroad have carried out numerous investigations and obtained more than 100 compounds from the leaves of *C. paliurus* [7]. Of these, triterpenoids have always been the focus of research and attention over the years, because of their diverse structures and broad bioactivities, such as hypoglycemic, hypolipidemic, anti-inflammatory, anti-oxidant, anti-cancer and cytotoxic activities [8-15].

3. Present Study

The whole leaves of *C. paliurus* (45.0 kg) were exhaustively pulverised and extracted twice with 70% EtOH under reflux (450 L × 2h).The extract was concentrated to yield dried crude extract under reduced pressure. Then, the extract was suspended in water and successively partitioned with dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) (50 L × 4 times for each solvent). The CH_2Cl_2 extract (400.0 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 afford10 fractions (Fr. I-X) according TLC. The Fr.X (60.0 g) was gradient elution of H_2O/MeOH (v/v, 100:0 to 0:100) through a polyamide column to yield 5 fractions (Fr. A-E) according 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 C18 reversed-phase column (from 10% to 100% aqueous MeOH, stepwise) to obtain compound **1** (4.5 mg). Fr.D was performed on gel column chromatography and semi-preparative HPLC (3.0 mL/min, 230 nm, ACN-H_2O, 3.0:7.0, V/V) repeatedly to obtain compounds **2** (3.6 mg) and **3** (4.8 mg). Fr.C was subjected to gel column chromatography and further purified by semi-preparative HPLC (3.0 mL/min, 230 nm, ACN-H_2O, 3.0:7.0, V/V) repeatedly to obtain compounds **4** (2.5 mg) and **5** (4.5 mg).

Compound **1** was obtained as a colorless amorphous powder. Its molecular formula of $C_{41}H_{70}O_{12}$ was determined by the HR-ESI-MS ion peak at *m/z* 772.5215 $[M + NH_4]^+$, showing seven degrees of unsaturation. The NMR spectrum of **1** (Table 1) suggested that it was a triterpenoid glycoside with a dammarane triterpenoid aglycone skeleton and two sugar moieties. According to 1D NMR, compound **1** and the known compound cyclocarioside I have the same planar structure [16]. However, according to the NOESY spectrum, the stereostructure of them is different at the C-3 position. The configuration at C-3 was deduced to be *α*-positioned by NOESY correlation between H-3 and H-29 [17]. Similarly, the configuration at C-12 was deduced to be *β*-positioned by NOESY correlation between H-12 and H-30 (Figure 2). The sugar units of compound **1** were identified by acid hydrolysis and the comparison of the retention times with authentic standard D-quinovose and L-arabinofuranose, and the attachments were confirmed by the HMBC correlations from δ_H 4.93 (1H, br s, H-1') to δ_C 80.6 (C-3), and δ_H 4.34 (1H, d, J = 7.5 Hz, H-1") to δ_C 76.8 (C-12), respectively (Figure 2). The *α* configuration of L-arabinofuranose and the *β* configuration of D-quinovopyranose were based on the ^{13}C-NMR data [δ_C 106.3 (C-1'), δ_C 100.8 (C-1")]. The configurations at C-20 and C-24 were deduced to be *S* and *R*, respectively, by comparisons to the ^{13}C-NMR chemical shift data [δ_C 88.0 (C-20), 85.0 (C-24)] of analogous expoxydammaranes. Hence, the compound **1** was deduced as (20*S*, 24*R*)-(3*α*, 12*β*)-20, 24-epoxydammara-25-ol-12-*O*-*β*-D-quinovopyranoside-3-*O*-*α*-L-arabinofuranoside, and named cyclocarioside Z14.

*Cyclocarioside Z14 (**1**)* : colorless amorphous powder; [α]25 D – 21.9 (c 0.02, MeOH), HPLC-UV (ACN-H_2O) λmax: 230 nm, HR-ESI-MS: m/z 772.5215 $[M + NH_4]^+$ (calcd. for 772.5211), ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz) spectral data see Table 1.

The four known compounds (**2-5**) were identified by using the basis of spectroscopic experiments and comparing with published data as cyclocarioside D [18], cyclocarioside N [19], pterocaryoside A [20], cyclocarioside Z2 [12].

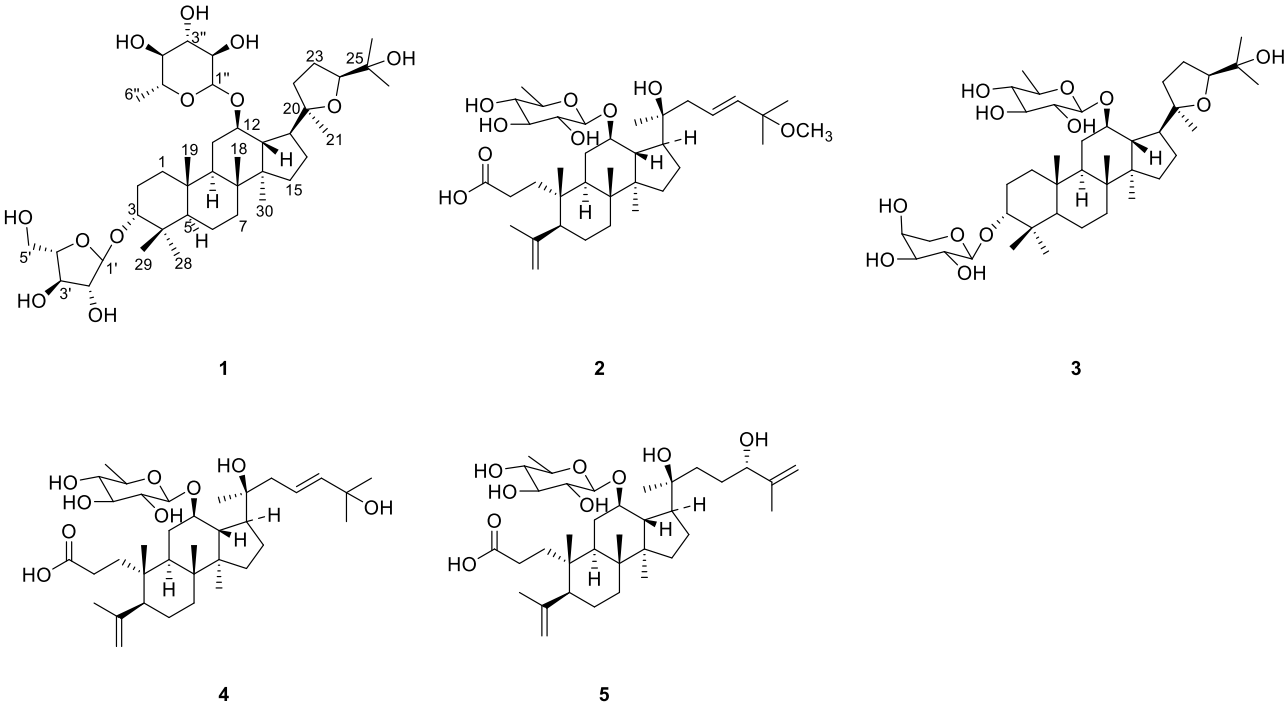

Figure 1. Structure of compounds **1-5**

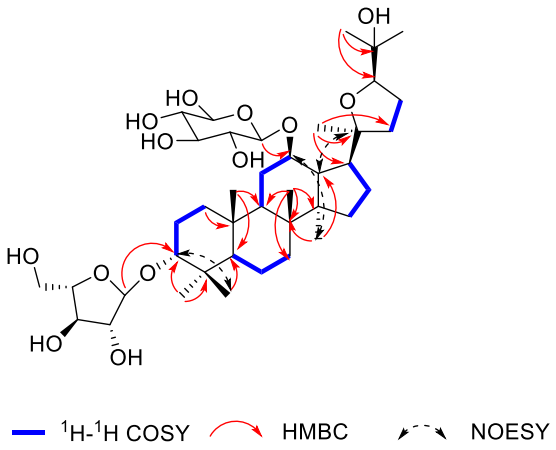

Figure 2. Key ^{1}H-^{1}H COSY, HMBC and NOESY correlations of compound **1**

Table 1. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data of compound **1** in CD_3OD

Position	δ_H (*J* in Hz)	δ_C	Position	δ_H (*J* in Hz)	δ_C
1	2.46, m 1.43, m	36.2	20		88.0
			21	1.18, s	24.6
2	1.86, m 1.46, m	27.0	22	2.42, m 1.71, m	34.7
3	3.34, overlapped	80.6	23	1.55, m 1.66, m	21.5
4		38.6			
5	1.35, m	51.5	24	3.81, m	84.9
6	1.86, overlapped 1.46, m	19.1	25		73.0
			26	1.17, s	25.2
7	1.59, m 1.23, overlapped	37.1	27	1.22, s	26.7
			28	0.96, s	30.0
8		42.4	29	0.90, s	23.0
9	1.76, m	54.9	30	1.01, s	17.3
10		40.7	1'	4.94, br s	106.3
11	2.42, m 1.36, m	34.7	2'	4.01, br d (1.5)	84.2
			3'	3.97, m	85.2
12	4.08, td(10.5, 4.5)	76.8	4'	3.84, m	79.3
13	1.70, m	41.8	5'	3.76, m	63.1
14		51.5	1"	3.65, m 4.35, d (7.5)	100.8
15	1.47, m 1.10, overlapped	32.3			
			2"	3.11, t (8.5)	75.7
16	1.99, m 1.86, m	27.0	3"	3.29, m	78.0
			4"	2.99, t (9.0)	77.2
17	1.80, m	50.2	5"	3.27, m	72.9
18	1.00, s	17.3	6"	1.27, d (6.0)	18.1
19	1.11, s	17.1			

All compounds were evaluated the cytotoxicity against seven human cancer cell lines (MCF-7, PC-3, Du145, NCI-1975, PC-9, SKVO3 and HepG2) by MTT method with positive control of STS. As shown in Table 2, the compounds **1** and **3** exhibited moderate cytotoxicity to MCF-7 cells with an IC_{50} value of 29.51 μM and 33.88 μM.

Table 2. Cytotoxicity of compounds **1-5**

Compound	IC_{50} (μM)						
	Du145	**PC-3**	**MCF-7**	**SKVO3**	**NCI-1975**	**PC-9**	**HepG2**
1	NA	72.44	29.51	67.60	67.60	NA	70.79
2	NA	NA	NA	NA	NA	NA	NA
3	NA	NA	33.88	NA	NA	NA	NA
4	NA	NA	NA	NA	NA	NA	NA
5	NA	NA	NA	NA	NA	NA	NA
STS	0.012	0.268	0.038	0.06	0.001	0.001	0.01

Note: NA: no active; STS: staurosporine (positive control).

Acknowledgments

This work was partially supported by the Furong Laboratory Programs for Science and Technology Development (No. 2023SK2102), Major Project of Health Research in Hunan Province (No. W20241006), Hainan Key Research Project (No. ZDYF2022SHFZ081; ZDYF2024SHFZ041),

Hunan Key Research Project (No. 2022SK2031; 2020SK2002), Hunan Natural Science Foundation (No. 2022JJ30788; 2025JJ50570).

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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