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•Original article•

Cyclocarysaponins A–J, dammarane-type triterpenoid glycosides from the leaves of *Cyclocarya paliurus*

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[ABSTRACT] Ten previously undescribed dammarane-type triterpenoid glycosides, cyclocarysaponins A–J (**1–10**), were isolated from the leaves of *Cyclocarya paliurus* (Batal.) Iljinskaja. The structures of these compounds were characterized through detailed spectroscopic analysis, including 1D and 2D nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS). The cytotoxic activities of all isolates were assessed against five human cancer cell lines (Bel-7402, Caski, BGC-823, A2780, and HCT-116). Of the tested compounds, compounds **1**, **7**, and **9** exhibited selective cytotoxicity against one or more human cancer cell lines.

[KEY WORDS] *Cyclocarya paliurus*; Juglandaceae; Triterpenoid glycosides; Cyclocarysaponin; Cytotoxic activities
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Introduction

Cyclocarya paliurus (Batal.) Iljinskaja, belonging to the Juglandaceae family, is widely distributed across Jiangxi, Guangxi, Guangdong, and Zhejiang Provinces of China. This species is classified as a medicinal and edible plant [1] and is commonly known as "sweet tea" due to its naturally sweet flavor [2]. The leaves are traditionally consumed either as green vegetables or brewed into tea [3]. In traditional Chinese medicine (TCM), *C. paliurus* is known for its therapeutic properties described as "Qingrejiedu (clearing heat and detoxification) and Shengjinzhike (promoting fluid production and relieving cough)," as documented in the ancient Chinese pharmacopeia, *Zhong Hua Ben Cao* [4]. In folk medicine, its leaves are widely used for managing conditions such as diabetes, hyperlipidemia, and hypertension [5-7], and these effects have been validated through both *in vivo* and *in vitro* studies. These pharmacological activities are attributed to the major bioactive constituents of *C. paliurus* leaves, including flavonoids, polysaccharides, triterpenoids, steroids, saponins, and phenolic acids [8]. CHEN *et al*. identified 210 distinct compounds within *C. paliurus*, of which triterpenoids accounted for 65.2% [9]. Therefore, research and development efforts focused on the triterpenoids from *C. paliurus* have received significant attention.

The triterpenoids isolated from *C. paliurus* include 3,4-*seco*-dammarane, dammarane, oleanane, ursane, lupinane, and taraxerane derivatives. Among these, 3,4-*seco*-dammarane triterpenoids are primarily unique to *C. paliurus*, suggesting that these distinctive compounds can serve as reliable chemotaxonomic markers for the species within the Juglandaceae family [10]. Dammarane-type and 3,4-*seco*-dammarane-type triterpenoids are also the primary contributors to the sweet taste of *C. paliurus*. For instance, the sweetness of cyclocariosides I and A has been reported to be approximately 250 and 200 times that of sucrose, respectively [11, 12]. Dammarane triterpenoids were first isolated from *C. paliurus* in 1992 [1, 12] and are considered key characteristic constituents of the plant. A recent study described two new dammarane triterpenoid saponins isolated from the leaves of *C. paliurus* [13], and subsequent studies have referred to these compounds as cypaliurusides [14, 15]. Dammarane triterpenoids exhibit hypoglycemic [14, 16], hypolipidemic [17, 18], cytotoxicity [14, 19, 20], and anti-inflammatory activities [21].

As part of an ongoing effort to identify bioactive dammarane triterpenoids from *C. paliurus*, we isolated ten previ-

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ously undescribed dammarane-type triterpenoid glycosides, cyclocarysaponins A–J (**1**–**10**, Fig. 1), from the leaves of *Cyclocarya paliurus* (Batal) Iljinskaja. Herein, we report the isolation and structure elucidation of these new compounds, as well as an evaluation of the *in vitro* cytotoxic activities of selected isolates against human cancer cell lines: Be1-7402 (hepatocarcinoma cell line), Caski (cervical cancer cell line), BGC-823 (gastric cancer cellline), A2780 (ovarian cancer cell line), and HCT-16 (colon cancer cell line).

Results and Discussion

Structure elucidation

Compound **1** was obtained as a white amorphous powder. High-resolution time-of-flight mass spectrometry (HR-TOF-MS) analysis revealed a molecular formula of $C_{40}H_{66}O_{11}$ at *m/z* 745.4489 $[M + Na]^+$. The ^1H nuclear magnetic resonance (NMR) spectrum of **1** in pyridine-d_5 exhibited seven methyl signals at δ_H 0.69, 0.97, 1.07, 1.24, 1.37, 1.46, and 1.84 (each 3H, s), a pair of terminal olefinic protons at δ_H 4.96 and 5.06 (each 1H, br s), two olefinic protons (δ_H 6.14 and 6.44) in a *trans* relationship (J = 16.2 Hz), and two sugar anomeric protons at δ_H 4.69 (1H, d, J = 6.0 Hz) and δ_H 4.81 (1H, d, J = 7.8 Hz). The ^{13}C NMR spectrum of **1** in pyridine-d_5 displayed 40 carbon signals, including four double bond signals at δ_C 143.1, 115.2, 128.8, 135.9 and two anomeric carbon signals at δ_C 102.0 and 103.3. These data demonstrated that **1** shared close structural similarity with cyclocarioside Z_{11}, differing primarily in the sugar types and the configuration of the hydroxyl group at C-3 [22]. Acid hydrolysis of **1** with 2 mol·L^{-1} HCl afforded a monosaccharide, which was identified as *α*-L-arabinopyranose by gas chromatography (GC) analysis of its trimethylsilyl L-cysteine derivatives and examination of the coupling constant of the anomeric protons. The ^1H–^1H correlation spectroscopy (COSY) correlations of H-9/H-11/H-12/H-13/H-17 and H-22/H-23/H-24 confirmed the presence of the two fragments –CHCHCH_2CHCH– and –CH_2CHCH– (Figs. 2 and S8). In the heteronuclear multiple bond correlation (HMBC) spectrum (Figs. 2 and S6), correlations from the anomeric protons Ara (p)-H-1' (δ_H 4.69) to C-3 (δ_C 81.7) and Ara (p)-H-1" (δ_H 4.81) to C-11 (δ_C 77.6) suggested that the two sugar units were attached at positions 3 and 11, respectively (Fig. 2). Additionally, HMBC cross-peaks from H-26 (δ_H 5.06, 4.96, br s) to C-24 (δ_C 135.9) and C-27 (δ_C 19.3) confirmed the attachment of the terminal double bond (–C=CH_2) at C-25. HMBCs from H-21-CH_3 (δ_H 1.46, s) to C-17 (δ_C 50.9) further validated the placement of the side chain at C-17. In the nuclear Overhauser effect spectroscopy (NOESY) spectrum, interactions between H-11 and both H_3-18 and H_3-19, along with correlations between H_3-29 and both H_3-18 and H_3-19, indicated an α-orientation for the hydroxyl group at C-11 and a β-orientation for the methyl group at C-29. Additional NOESY correlations between H-5/H_3-28, H_3-28/H-3, H-5/H_3-30, and H_3-30/H-17 suggested a *β*-orientation for the hydroxyl group at C-3 and an *α*-orientation for H-17 (Fig. 3). The *β*-orientation of the hydroxyl group at C-20 was confirmed by the similar chemical shifts of C-17, C-20, C-21, and C-22 between **1** and cypaliuruside L [14], further supported by the NOESY correlation between H-21 and H-17 (Fig. 3). Based on these analyses, the structure of **1** was determined to be (23*E*)-dammarane-11-*O*-*α*-L-arabinopyranosyl-3-*O*-*α*-L-arabinopyranoside and named cyclocarysaponins A.

Compound **2** was isolated as a white, amorphous powder. The HR-TOF-MS peak at *m/z* 787.4644 $[M + Na]^+$ indicated the molecular formula of **2** to be $C_{42}H_{68}O_{12}$ and is 42 Da more than that of **1**. The NMR spectroscopic data of **2** were almost identical to those of **1** (Table 1), except that one of the arabinopyranoses in **1** was replaced by an arabinofuranose moiety in **2** and an additional acetyl group [δ_H 1.99 (3H, s); δ_C 171.2 and 21.1] in the NMR spectrum of **2**. In the HMBC spectrum of **2**, a correlation between Ara (f)-H-1' (δ_H 5.45) and C-3 (δ_C 80.1) confirmed that the *α*-L-arabinofuranosyl unit is located at C-3. The long-range correlation was observed between Ara (f)-H-5' (δ_H 4.50 and 4.62) and acetyl carbonyl carbon (δ_C 171.2), indicating that the acetyl group was attached to the C-5' position of arabinose. Thus, compound **2** was determined to be (23*E*)-dammarane-11-*O*-*α*-L-arabinopyranosyl-3-*O*-(5'-*O*-acetyl)-*α*-L-arabinofuranoside and named cyclocarysaponins B.

Compound **3** was isolated as a white, amorphous powder. Its molecular formula, $C_{43}H_{70}O_{12}$, was established by HR-TOF-MS (*m/z* 801.4800 $[M + Na]^+$, Calcd. for 801.4765). The NMR spectroscopic data of **3** closely matched those of **2**, with the primary difference being the substitution of the arabinose unit in **2** with a quinovose in **3** (Tables 1 and 2). Acid hydrolysis of **3** with 2 mol·L^{-1} HCl yielded monosaccharides, which were identified as *β*-D-quinovose and *α*-L-arabinofuranose by GC analysis of their trimethylsilyl L-cysteine derivatives and the coupling constant of the anomeric protons. The quinovose unit was determined to be attached at C-11 of the aglycone, based on the HMBC between the H-1" (δ_H 4.94) of quinovose and C-11 (δ_C 76.9) of the aglycone. Consequently, compound **3** was determined as (23*E*)-dammarane-11-*O*-*β*-D-quinovopyranosyl-3-*O*-(5'-*O*-acetyl)-*α*-L-arabinofuranoside and named cyclocarysaponins C.

Compound **4** was a white amorphous powder. Its molecular formula, $C_{43}H_{72}O_{12}$, was determined by HR-TOF-MS at *m/z* 803.4945 $[M + Na]^+$, in agreement with the NMR spectroscopic data. The NMR spectra of compound **4** closely resembled those of **3**, with differences observed primarily in the C-23 to C-27 fragment. In the HMBC spectrum of **4**, cross-peaks from Me-26 and Me-27 to each other (δ_C 18.2 and δ_C 26.3, respectively), as well as to C-25 (δ_C 131.3) and C-24 (δ_C 126.5), indicated the presence of a $\triangle^{24(25)}$ double bond. In addition, HMBCs from Me-21 (δ_H 1.44) to C-17 (δ_C 50.6), C-20 (δ_C 74.4), and C-22 (δ_C 41.7) were observed. The remainder of compound **4** was confirmed by COSY, HMBC, and NOESY correlations. Thus, compound **4** was determined as dammarane-(3*β*,11*α*)-11-*O*-*β*-D-quinovopyranosyl-3-*O*-(5'-*O*-acetyl)-*α*-L-arabinofuranoside and named cyclocarysapon-

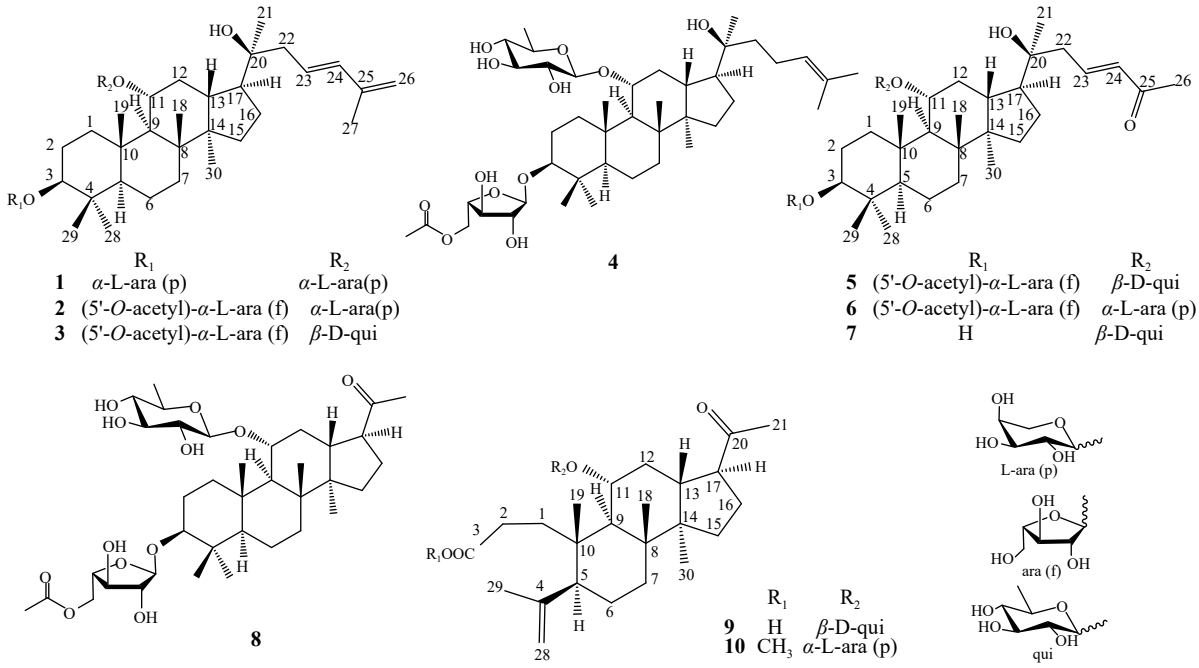

Fig. 1 Chemical structures of compounds 1–10.

ins D.

Compound **5** was obtained as a white, amorphous powder, with a molecular formula $C_{42}H_{68}O_{13}$, as determined by HR-TOF-MS with an $[M + Na]^+$ peak at *m/z* 803.4566. The NMR spectroscopic data of compound **5** closely matched those of **3**, with the primary difference being the conversion of the $\triangle^{25(26)}$ double bond in **3** to a carbonyl group (> C=O) in **5** (Tables 1 and 2). The structure of **5** was further confirmed by COSY, HMBC, and NOESY correlations. Thus, compound **5** was determined as (23*E*)-20-hydroxy-25-oxo-dammarane-11-*O*-*β*-D-quinovopyranosyl-3-*O*-(5'-*O*-acetyl)-*α*-L-arabinofuranoside and named cyclocarysaponins E.

Compound **6** was obtained as a white amorphous powder, and its molecular formula was deduced as $C_{41}H_{66}O_{13}$ by HR-TOF-MS. The NMR spectroscopic data of **6** closely resembled those of **5**, with the primary difference being the substitution of the quinovose unit in **5** with an arabinopyranose in **6** (Tables 1 and 2). The arabinofuranose and arabinopyranose units were linked to C-3 and C-11 of the aglycone, respectively, as confirmed by HMBCs of Ara (f)-H-1' (δ_H 5.45)/aglycone-C-3 (δ_C 80.2) and Ara (p)-H-1" (δ_H 4.84)/aglycone-C-11 (δ_C 77.1). In addition, an HMBC was observed between Ara (f)-H-5' (δ_H 4.61 and 4.81) and C=O (δ_C 171.2). Thus, compound **6** was elucidated as (23*E*)-20-hydroxy-25-oxo-dammarane-11-*O*-*α*-L-arabinopyranosyl-3-*O*-(5'-*O*-acetyl)-*α*-L-arabinofuranoside and named cyclocarysaponins F.

Compound **7** had the molecular formula $C_{35}H_{58}O_8$, as deduced from the HR-TOF-MS (*m/z* 629.4033 $[M + Na]^+$, Calcd. for 629.4029). The NMR spectroscopic data of **7** resembled those of **5**, except that **7** lacked one quinovose and one acetyl group present in **5**. In the HMBC spectrum of **7**, a correlation between Qui-H-1' (δ_H 4.94) and C-11 (δ_C 77.0) indicated that the quinovose was attached at the C-11 position of the aglycone. Thus, compound **7** was determined to be (23*E*)-20-hydroxy-25-oxo-dammarane-11-*O*-*β*-D-quinovopyranosyide and named cyclocarysaponins G.

Compound **8** was a white amorphous powder. The molecular formula $C_{37}H_{60}O_{12}$ was determined by HR-TOF-MS at *m/z* 719.4003 $[M + Na]^+$, in agreement with the NMR spectroscopic data. Its NMR data were similar to those of **7**, suggesting that **8** possessed a dammarane-type triterpenoid skeleton with the exception of the side chain resonances. The only significant changes observed were the presence of one isolated aceto-group on C-17, which was deduced by the HMBC correlation of H-17 (δ_H 2.56) with Me-21 (δ_C 30.4), and of H-13 (δ_H 2.21) and H-16 (δ_H 1.71 and 1.90) with the ketone of C-20 (δ_C 211.2) (Fig. 2). Thus, compound **8** was identified as 11-*O*-*β*-D-quinovopyranosyl-3-*O*-(5'-*O*-acetyl)-*α*-L-arabinofuranoside-23,24,25,26,27-hexanor-dammarane-20-one and named cyclocarysaponins H.

Compound **9** was obtained as a white powder, and its molecular formula was determined to be $C_{30}H_{48}O_8$ by HR-TOF-MS *m/z* 559.3244 $[M + Na]^+$ (Calcd. for $C_{30}H_{48}O_8Na$, 559.3247), indicating seven degrees of unsaturation. The ^1H NMR data of **9** exhibited six methyl signals at δ_H 0.91 (3H, s), 1.05 (3H, s), 1.37 (3H, s), 1.58 (3H, d, J = 6.0 Hz), 1.89 (3H, s), and 2.12 (3H, s), two terminal olefinic protons at δ_H 4.99 (2H, br s), an oxymethine proton at δ_H 4.46 (1H, m), and a glycosyl anomeric proton at δ_H 4.91 (1H, d, $J_{H\text{-}1'/H\text{-}2'}$ = 7.8 Hz). The ^{13}C NMR spectrum of **9** in pyridine-d_5 displayed the existence of 30 carbon signals, including those of a terminal olefinic carbon at δ_C 149.0 and 114.4, a carboxyl carbon sig-

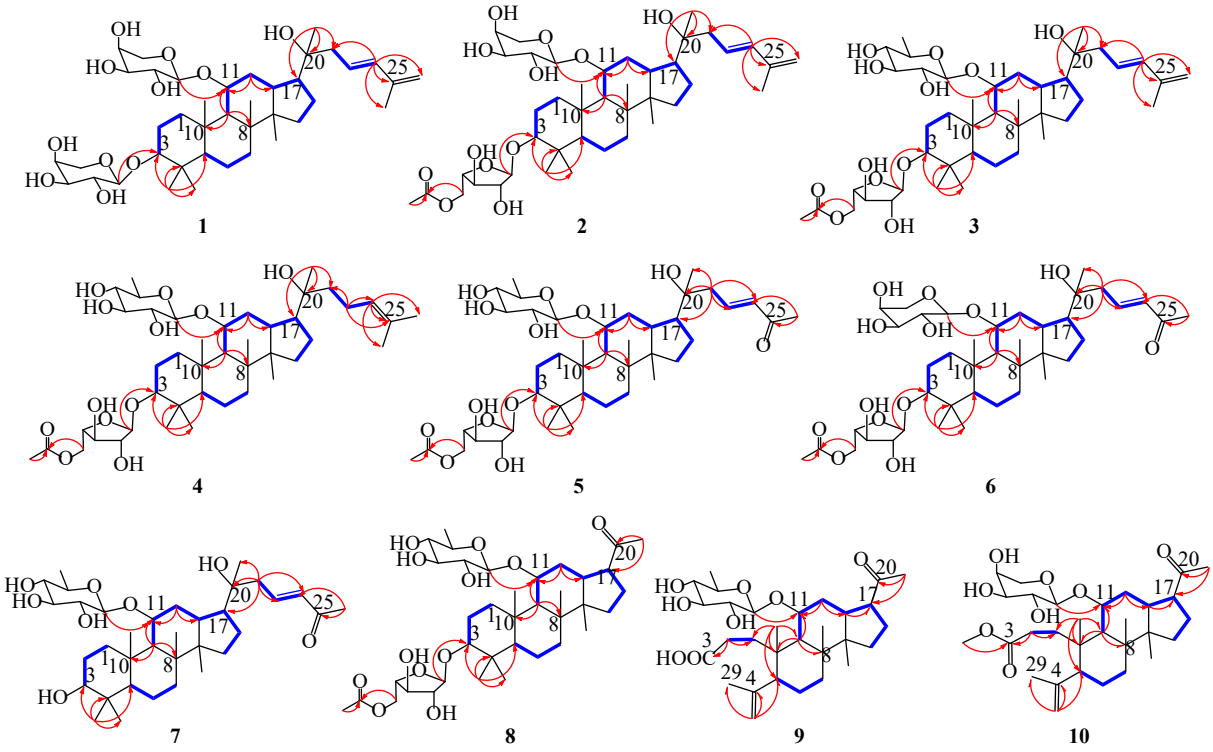

Fig. 2 Key HMBC (⟶), ^{1}H-^{1}H COSY (▬) correlations of compounds 1–10.

nals at δ_C 178.6, a ketone carbon signals at δ_C 211.4, as well as six glycosyl carbon signals at δ_C 101.0, 78.6, 77.2, 76.0, 73.3, and 19.0. All these data implied that **9** was structurally similar to the known compound cypaliuruside E [14], suggesting that it is a 3,4-*seco*-dammarane type triterpenoid glycoside with variations in the side chain. HMBCs from H-17 (δ_H 2.68) to Me-21 (δ_C 30.5) and from H-13 (δ_H 2.25) and H-16 (δ_H 1.74 and 1.97) to the ketone of C-20 (δ_C 211.4) (Fig. 2) suggested that the side chain contains an isolated aceto-group at C-17. HMBCs from H_2-28 (δ_H 4.99) to C-5 (δ_C 52.3) and C-29 (δ_C 24.6), from H_3-29 (δ_H 1.89) to C-4 (δ_C 149.0), C-5 (δ_C 52.3), and C-28 (δ_C 114.4), and from H_2-2 (δ_H 2.63 and 3.16) to C-1 (δ_C 38.3) and C-3 (δ_C 178.6), along with ^{1}H–^{1}H-COSY correlations between H_2-1 and H_2-2 and between H-5 and H_2-6 confirmed that the A-ring of triterpene aglycone of **9** was cleaved at positions 3 and 4, forming a carboxy group and a terminal double bond. In addition, The HMBC correlation from Qui-H-1' (δ_H 4.91) to C-11 (δ_C 75.5) indicated that the quinovose was linked at C-11. Its diagnostic NOESY correlations of H-5/H-9/H-17/H_3-30 suggested that H-5, H-9, H-17, and CH_3-30 are α-oriented (Figs. 3 and S72). The β-orientations of H-11, H-13, H_3-18, and H_3-19 were verified by the NOESY correlations of H-11/H-13/H_3-18/H_3-19. Thus, compound **9** was elucidated as 11-*O*-β-D-quinovopyranosyl-23,24,25,26,27-hexanor-3,4-secodammara-20-one-4(28)-en-3-oic acid and named cyclocarysaponins I.

Compound **10** was obtained as a white amorphous powder. The molecular formula of **10** was determined to be $C_{30}H_{48}O_8$ by HR-TOF-MS. The NMR spectroscopic data of compound **10** were similar to those of **9**, with the primary difference being the substitution of the quinovose unit in **9** with an arabinose unit in **10** and the presence of an additional methoxy group in **10**. In the HMBC spectrum, a correlation between OCH_3 (δ_H 3.60) and C-3 (δ_C 176.9) revealed that the methoxy group was connected to C-3 of the aglycone. An HMBC correlation from Ara-H-1 (δ_H 4.77) to C-11 (δ_C 75.9) confirmed that the arabinose was attached at C-11 of the aglycone. Therefore, compound **10** was deduced as 11-*O*-α-L-arabinopyranosyl-23,24,25,26,27-hexanor-3,4-secodammara-20-one-4(28)-en-3-oic acid methyl ester and named cyclocarysaponins J.

*Biological activity of cyclocarysaponins A–J (**1–10**)*

Compounds **1–10** were evaluated for their cytotoxic activities against five human tumor cell lines (Bel-7402, Caski, BGC-823, A2780, and HCT-116) with paclitaxel as a positive control. Among the tested compounds, compound **1** showed cytotoxic activities against Bel-7402 and BGC-823 cells, with IC_{50} values of 8.52 ± 0.63 and 21.14 ± 1.45 μmol·L^{-1}, respectively. Compound **7** exhibited cytotoxic activity against A2780 cells, with an IC_{50} value of 12.82 ± 1.86 μmol·L^{-1}. compound **9** showed cytotoxic activities against Bel-7402, A2780, and HCT-116 cells, with IC_{50} values of 13.46 ± 2.27, 10.33 ± 1.78, and 25.23 ± 3.57 μmol·L^{-1}, respectively. (Fig. 4). Additionally, the inhibitory activities of these compounds against α-glucosidase and PTP1B enzymes, as well as their inhibitory effects against nitric oxide (NO) production in RAW264.7 mouse macrophages induced by lipopolysaccharide (LPS) were also evaluated. However, none of the compounds exhibited significant inhibitory activities by these compounds at 10 μmol·L^{-1}.

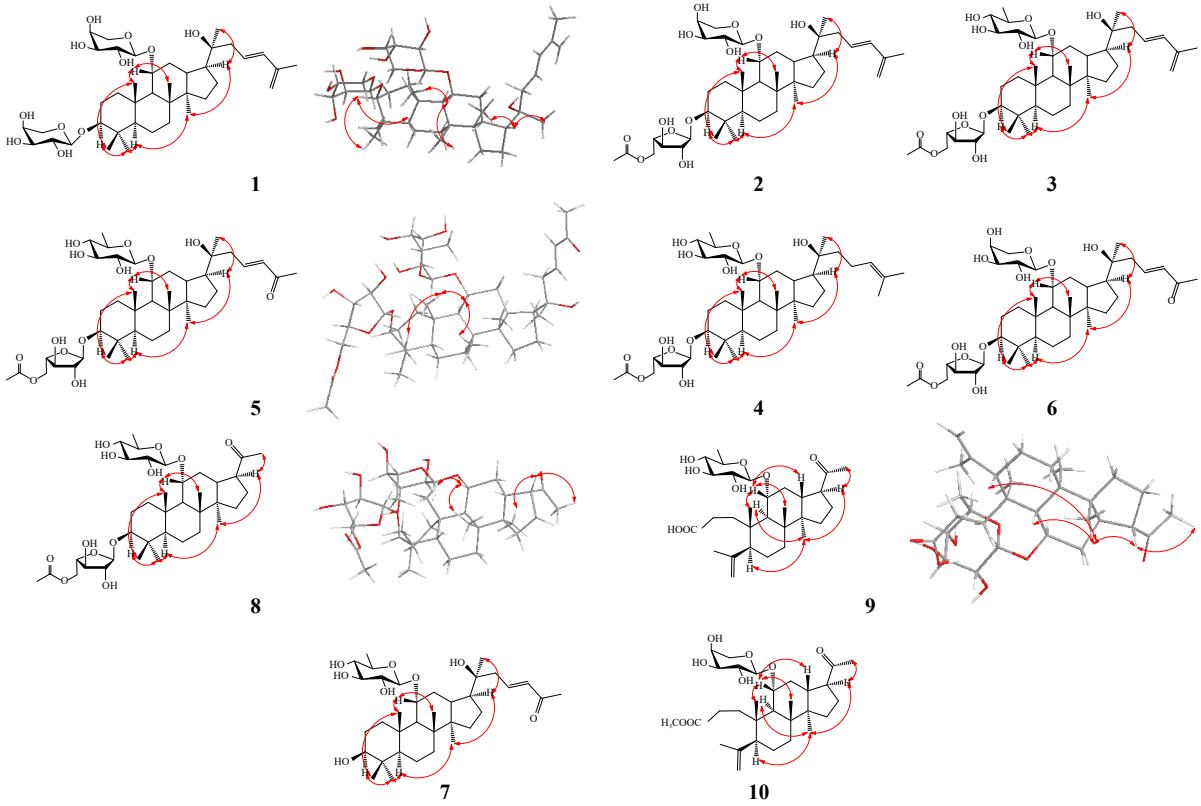

Fig. 3 NOESY correlations of compounds 1–10.

Table 1 ^{1}H NMR data for compounds 1–10 at 600 MHz, respectively (in pyridine-d_5, *J* in Hz)

No.	1	2	3	4	5	6	7	8	9	10
1	2.01, m 3.04, dt, 13.6, 4.8	1.80, m 3.05, m	1.81, m 3.02, m	1.82, m 3.01, m	1.81, m 3.00, dd, 3.6, 10.2	1.85, m 3.07, dt, 13.2, 4.8	2.14, m 3.10, dd, 4.2, 10.8	1.80, m 3.01, dd, 3.6, 10.2	2.01, m 3.13, m	1.75, m 3.00, m
2	1.77, m	1.68, m 1.54, m	1.66, m 1.81, m	1.67, m 1.81, m	1.66, m 1.81, m	1.54, m 1.80, m	1.77, m 2.14, m	1.66, m 1.81, m	2.63, m 3.16, m	2.43, m 3.00, m
3	3.58, t, 3.0	3.52, t, 3.0	3.57, t, 3.0	3.57, t, 3.0	3.57, br s	3.52, t, 3.0	3.62, br s	3.56, br s		
4										
5	1.58, m	1.52, m	1.54, m	1.56, m	1.55, m	1.53, m	1.81, m	1.53, m	2.21, m	2.10, m
6	1.46, m 1.51, m	1.44, m 1.52, m	1.50, m 1.56, m	1.47, m 1.57, m	1.49, m	1.48, m	1.54, m	1.43, m 1.51, m	1.36, m 1.85, m	1.33, m 1.81, m
7	1.19, m 1.51, m	1.19, m 1.54, m	1.21, m 1.56, m	1.22, m 1.57, m	1.21, m 1.55, m	1.21, m 1.61, m	1.25, m 1.68, m	1.14, m 1.52, m	1.08, m 1.52, m	1.11, m 1.54, m
8										
9	1.88, d, 10.8	1.88, d, 10.8	1.89, d, 10.2	1.92, d, 10.8	1.88, d, 11.4	1.87, d, 10.8	2.04, d, 10.8	1.84, d, 10.8	2.08, d, 10.8	2.02, d, 10.8
10										
11	4.45, td, 10.8, 4.8	4.47, td, 10.2, 4.8	4.47, td, 10.2, 4.8	4.45, td, 10.8, 4.8	4.44, td, 10.8, 4.8	4.45, td, 10.8, 4.8	4.49, td, 10.2, 4.8	4.41, td, 10.8, 4.8	4.46, td, 10.2, 4.8	4.42, m
12	1.61, m 2.99, dt, 13.2, 5.6	1.69, m 3.04, m	1.69, m 2.99, m	1.70, m 2.95, dt, 13.2, 5.6	1.67, m 2.95, dt, 13.2, 4.8	1.71, m 3.02, dt, 13.2, 4.8	1.71, m 2.97, dt, 13.2, 4.8	1.53, m 2.56, m	1.56, m 2.61, m	1.54, m 2.64, m
13	2.14, td, 10.2, 3.6	2.15, td, 10.2, 3.0	2.17, td, 10.2, 3.0	2.16, td, 10.2, 3.0	2.09, td, 10.8, 3.6	2.08, td, 10.2, 3.6	2.14, td, 11.4, 3.0	2.21, m	2.25, m	2.23, m
14										

Continued

No.	1	2	3	4	5	6	7	8	9	10
15	1.04, m 1.51, m	1.16, m 1.43, m	1.06, m 1.54, m	1.08, m 1.54, m	1.05, m 1.51, m	1.05, m 1.49, m	1.07, m 1.55, m	1.03, m 1.39, m	1.10, m 1.52, m	1.13, m 1.59, m
16	1.83, m 1.91, m	1.85, m 1.90, m	1.85, m 1.91, m	1.84, m 1.93, m	1.82, m	1.82, m	1.85, m	1.71, m 1.90, m	1.74, m 1.97, m	1.79, m 2.05, m
17	1.97, m	2.02, m	2.01, m	1.97, m	1.95, m	1.96, m	1.95, m	2.56, m	2.68, m	2.75, td, 6.6, 10.8
18	1.07, s	1.06, s	1.09, s	1.08, s	1.08, s	1.04, s	1.12, s	1.02, s	1.05, s	1.00, s
19	1.37, s	1.32, s	1.35, s	1.34, s	1.34, s	1.32, s	1.41, s	1.32, s	1.37, s	1.24, s
20										
21	1.46, s	1.47, s	1.43, s	1.44, s	1.41, s	1.43, s	1.42, s	2.08, s	2.12, s	2.25, s
22	2.53, dd, 7.8, 13.8 2.69, dd, 6.6, 13.8	2.54, dd, 7.8, 13.8 2.70, dd, 6.6, 13.8	2.51, dd, 7.8, 13.8 2.67, dd, 6.6, 13.8	1.77, m 1.89, m	2.50, dd, 8.4, 13.8 2.71, dd, 7.2, 13.8	2.54, dd, 7.8, 14.4 2.74, dd, 6.6, 13.8	2.53, dd, 7.8, 14.4 2.72, dd, 6.6, 13.8			
23	6.14, m	6.16, m	6.12, m	2.28, m 2.36, m	7.31, dd, 7.8, 16.2	7.32, dd, 7.8, 15.6	7.31, dd, 7.2, 15.6			
24	6.44, d, 16.2	6.45, d, 16.2	6.41, d, 15.6	5.31, t, 6.6, 13.8	6.36, d, 16.2	6.39, d, 16.2	6.36, d, 16.2			
25										
26	4.96, br s 5.06, br s	4.96, br s 5.07, br s	4.96, br s 5.05, br s	1.64, s	2.23, s	2.23, s	2.24, s			
27	1.84, s	1.85, s	1.84, s	1.67, s						
28	0.97, s	0.92, s	0.97, s	0.96, s	0.97, s	0.92, s	1.01, s	0.96, s	4.99, br s	4.87, br s 4.97, br s
29	1.24, s	1.24, s	1.26, s	1.26, s	1.27, s	1.24, s	1.27, s	1.26, s	1.89, s	1.80, s
30	0.69, s	0.79, s	0.80, s	0.83, s	0.78, s	0.76, s	0.87, s	0.71, s	0.91, s	0.95, s
1′	4.69, d, 6.0	5.45, d, 1.2	5.51, d, 1.2	5.52, d, 1.2	5.51, d, 1.8	5.45, d, 1.8		5.51, d, 1.8		
2′	4.25, dd, 3.6, 8.4	4.85, m	4.86, m	4.87, m	4.86, m	4.85, m		4.85, m		
3′	4.41, m	4.62, m	4.62, m	4.63, m	4.63, m	4.62, m		4.63, m		
4′	4.40, m	4.74, td, 6.6, 3.0	4.75, td, 6.6, 3.0	4.75, td, 6.6, 3.0	4.75, td, 7.2, 3.0	4.74, td, 6.6, 3.0		4.74, td, 7.2, 3.0		
5′	3.76, dd, 2.4, 12.0 4.31, m	4.81, dd, 3.6, 12.0 4.62, m	4.81, dd, 3.0, 12.0 4.62, m	4.82, dd, 3.6, 12.0 4.62, m	4.60, m 4.82, dd, 3.0, 5.4	4.61, d, 6.0 4.81, dd, 3.0, 6.0		4.61, m 4.81, dd, 3.0, 6.0		
1″	4.81, d, 7.8	4.84, d, 7.8	4.94, d, 7.8	4.91, d, 7.8	4.95, d, 7.8	4.84, d, 7.8	4.94, d, 7.8	4.84, d, 7.8	4.91, d, 7.8	4.77, d, 7.8
2″	4.06, dd, 3.6, 9.0	4.06, d, 9.0	3.94, t, 9.0	3.94, t, 8.4	3.94, t, 8.4	4.06, dd, 3.6, 9.0	3.98, dd, 8.4, 9.0	3.91, m	4.11, t, 5.4	4.12, dd, 3.0, 9.0
3″	4.37, m	4.35, t, 7.2	4.09, t, 8.4	4.10, t, 8.4	4.10, t, 8.4	4.36, t, 8.4	4.12, t, 9.0	4.09, t, 9.0	4.20, t, 9.0	4.37, m
4″	4.21, m	4.21, m	3.68, m	3.68, t, 8.4	3.67, t, 8.4	4.21, m	3.67, m	3.66, t, 9.0	3.69, t, 9.0	4.26, d, 1.8
5″	3.65, dd, 1.2, 12.6 4.29, m	3.64, d, 12.0 4.28, dd, 2.4, 12.0	3.63, m	3.61, m	3.64, m	3.66, dd, 1.8, 12.6 4.29, dd, 1.2, 12.6	3.64, m	3.59, m	3.63, m	3.63, d, 5.4 4.23, m
6″			1.61, d, 6.0	1.60, d, 6.0	1.61, d, 5.4		1.62, d, 5.4	1.60, d, 6.0	1.58, d, 6.0	
CH_3COO-										
CH_3COO-		1.99, s	1.98, s	1.99, s	1.98, s	1.98, s		1.99, s		
-OCH_3										3.60, s

Table 2 ^{13}C NMR data for compounds **1**–**10** at 150 MHz, respectively (in pyridine-d_5)

No.	Cyclocarioside Z_{11}	1	2	3	4	5	6	7	8	9	10
1	35.9	36.0	36.1	36.0	36.0	36.0	36.1	35.9	36.0	38.3	38.1
2	21.7	22.2	21.8	21.9	21.9	21.9	21.9	27.5	21.9	31.3	30.9
3	79.5	81.7	80.1	80.1	80.2	80.2	80.2	75.8	80.1	178.6	176.9
4	38.2	38.5	38.3	38.4	38.4	38.4	38.3	39.1	38.4	149.0	149.0
5	51.2	51.4	51.3	51.4	51.4	51.4	51.4	50.5	51.4	52.3	52.6
6	18.6	18.7	18.7	18.8	18.8	18.8	18.7	19.0	18.7	25.8	25.8
7	36.5	36.8	36.7	36.8	36.8	36.8	36.7	37.0	36.9	35.5	35.6
8	50.8	50.9	51.1	51.1	51.1	51.1	51.0	51.1	50.5	50.9	51.0
9	54.2	54.5	54.4	54.5	54.5	54.4	54.4	54.7	54.3	44.9	45.1
10	40.2	40.4	40.4	40.4	40.4	40.4	40.5	40.6	40.4	40.8	40.8
11	77.1	77.6	77.0	76.9	77.0	77.0	77.1	77.0	76.6	75.5	75.9
12	35.0	35.0	35.0	35.2	35.3	35.2	35.0	35.2	33.1	32.5	32.5
13	41.0	41.3	41.2	41.2	41.0	41.4	41.4	41.4	43.3	43.4	43.6
14	41.6	41.9	41.9	41.9	41.9	41.9	41.9	42.0	41.9	41.4	41.6
15	31.5	31.7	31.7	31.8	31.9	31.7	31.7	31.7	31.8	31.9	32.1
16	25.9	26.1	26.1	26.2	26.1	26.2	26.2	26.2	27.2	27.1	27.4
17	50.8	50.9	51.0	51.1	50.6	51.5	51.5	51.5	54.2	54.3	54.4
18	17.3	17.5	17.6	17.6	17.6	17.6	17.6	17.7	17.5	17.0	17.3
19	16.9	17.3	17.1	17.1	17.1	17.1	17.1	17.1	17.0	20.7	21.0
20	74.7	74.8	74.9	74.9	74.4	74.8	74.8	74.9	211.2	211.4	212.6
21	27.6	27.7	27.8	27.9	27.4	27.8	27.8	27.8	30.4	30.5	30.8
22	45.1	45.3	45.1	45.2	41.7	44.5	44.5	44.7			
23	128.6	128.8	128.8	128.8	23.9	146.8	146.7	146.9			
24	135.6	135.9	135.9	135.7	126.5	134.2	134.2	134.2			
25	142.9	143.1	143.1	143.1	131.3	198.5	198.3	198.7			
26	115.1	115.2	115.2	115.2	18.2	27.4	27.3	27.4			
27	19.1	19.3	19.3	19.3	26.3						
28	23.2	23.6	23.3	23.4	23.4	23.4	23.4	23.4	23.4	114.4	114.7
29	30.0	30.4	30.3	30.4	30.4	30.4	30.2	30.5	30.4	24.6	24.8
30	17.0	17.1	17.2	17.2	17.3	17.2	17.3	17.2	16.5	16.4	16.7
1′	106.6	102.0	107.0	107.1	107.1	107.1	107.1		107.1		
2′	84.4	75.0	84.6	84.6	84.6	84.7	84.7		84.7		
3′	79.7	72.8	80.3	80.3	80.3	80.3	80.4		80.3		
4′	85.8	69.4	81.9	81.9	82.0	82.0	82.0		82.0		
5′	63.2	66.5	65.5	65.6	65.6	65.6	65.6		65.6		
1″	102.2	103.3	102.9	101.9	101.9	102.1	103.0	102.1	101.9	101.0	102.0
2″	75.6	75.2	75.2	76.0	76.0	76.1	75.2	76.1	76.0	76.0	75.3
3″	78.7	73.1	73.2	78.7	78.7	78.7	73.2	78.7	78.7	78.6	73.3
4″	78.0	70.1	70.2	77.3	77.3	77.3	70.2	77.3	77.3	77.2	70.5
5″	72.6	67.9	68.0	73.1	73.1	73.1	68.0	73.2	73.1	73.3	68.2
6″	63.8			19.1	19.1	19.1		19.1	19.1	19.0	
CH_3COO-			171.2	171.3	171.3	171.3	171.2		171.3		
CH_3COO-			21.1	21.2	21.2	21.2	21.2		21.2		
-OCH_3											52.2

Conclusions

In summary, ten previously undescribed dammarane-type triterpenoid glycosides, cyclocarysaponins A–J (**1**–**10**), were isolated from the leaves of *C. paliurus*. Among these compounds, three triterpenoid saponins containing ketocarbonyl groups at position 25 and three triterpenoid saponins with cleavages at positions C_{22}-C_{27} were first found in *C. paliurus*. The results presented in the article contribute to ex-

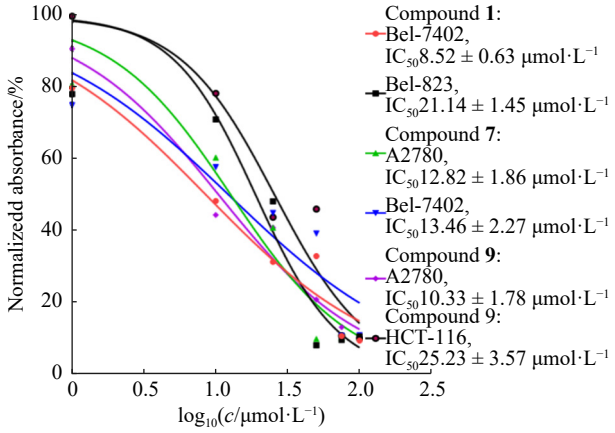

Fig. 4 Cytotoxic activities of compounds 1, 7, and 9 against Bel-7402, BGC-823, HCT-116, and A2780 cell lines.

panding our knowledge on the chemical diversity of dammarane-type triterpenoids. Although none of the tested compounds exhibited inhibitory activities against *α*-glucosidase, PPT1B, or LPS-induced NO production in mouse macrophages, compounds **1**, **7**, and **9** showed moderate cytotoxic activities against one to three human cancer cell lines.

Experimental

General experimental procedures

Optical rotations were measured on a Perkin-Elmer 241 automatic digital polarimeter (Waltham, Massachusetts, USA). UV spectra were obtained on a Shimadzu UV-260 spectrophotometer (Shimadzu, Tokyo, Japan). 1D and 2D NMR spectra were recorded on a Varian Unity INOVA 600 spectrometer using pyridine-d_5 as solvent and tetramethylsilane (TMS) as an internal standard (Varian Company, California, USA). The UPLC-Q-TOF-MS data were obtained on an Acquity UPLC/Xevo G2 QTOF (Waters Technologies, Massachusetts, USA). GC was conducted using an Agilent Technologies 7890A instrument (Agilent, USA). Preparative high-performance liquid chromatography (HPLC) was conducted on an Agilent 1260 infinity II Prep instrument with an MWD detector, using a YMC-Pack ODS (octadecylsilyl)-A column (5 μm, 250 mm × 20 mm). Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China) and ODS (50 μm, YMC, Japan). Fractions were visualized by silica gel plates sprayed with 10% H_2SO_4 ethanol solution, followed by heating. All analytical reagents were of analytical grade and purchased from Tianjin Fuyu Fine Chemical Co., Ltd., Tianjin, China.

Plant material

The leaves of *C. paliurus* (Batal.) Iljinskaja were collected from Shangrao City, Jiangxi Province, China, in September 2020 and identified by Prof. YANG Baiyun and deposited (No. YP20200911) in the herbarium of Nanchang University, China.

Extraction and isolation

The powdered dried leaves of *C. paliurus* (9.7 kg) were extracted four times with 70% EtOH under reflux (2 h each). The combined extract was concentrated under reduced pressure to yield a dark brown residue (2.9 kg). This residue was suspended in water (60 L) and then successively partitioned with petroleum ether (3 × 60 L), EtOAc (3 × 60 L), and *n*-BuOH (3 × 60 L). The solvent from the EtOAc-soluble fraction (530 g) was evaporated, and the residue was subjected to polyamide column chromatography, eluting with CH_3COCH_3 and then 70% EtOH. The 70% EtOH fraction (260 g) was further separated by silica gel CC, eluting with CH_2Cl_2–MeOH (30 : 1−50 : 50, *V*/*V*), yielding thirteen fractions (E_1–E_{13}). Fraction E_7 (23.5 g) was separated by ODS CC (30%–100%, MeOH–H_2O) into eight subfractions ($E_{7\text{-}1}$–$E_{7\text{-}8}$). Subfraction $E_{7\text{-}3}$ (284 mg) was further purified by preparative HPLC (YMC-ODS-A 5 μm, 250 mm × 20 mm, detection at 210 nm) using CH_3OH–H_2O containing 0.01% TFA (76 : 24, *V*/*V*, 7 mL·min^{-1}) as the mobile phase to yield compounds **7** (14.8 mg, t_R = 82.6 min) and **8** (40 mg, t_R = 143.8 min). Subfraction $E_{7\text{-}6}$ (550 mg) was purified by preparative HPLC (YMC-ODS-A 5 μm, 250 mm × 20 mm, detection at 210 nm) using CH_3CN–H_2O containing 0.01% TFA (51 : 49, *V*/*V*, 7 mL·min^{-1}) as the mobile phase, yielding compounds **4** (18.5 mg, t_R = 51.6 min), **9** (4.6 mg, t_R = 73.5 min), and **10** (15.2 mg, t_R = 126.2 min). Subfraction $E_{7\text{-}7}$ (485 mg) was subjected to preparative HPLC (YMC-ODS-A 5 μm, 250 mm × 20 mm, detection at 210 nm) using CH_3CN–H_2O containing 0.01% TFA (52 : 48, *V*/*V*, 7 mL·min^{-1}) as the mobile phase, yielding compounds **2** (22.4 mg, t_R = 48.9 min) and **3** (100 mg, t_R = 67.2 min). Fraction E_9 (18.2 g) was separated silica gel CC and eluted with CH_2Cl_2–MeOH (12 : 1−5 : 1, *V*/*V*), affording four subfractions ($E_{9\text{-}1}$–$E_{9\text{-}4}$). Subfraction $E_{9\text{-}3}$ (118 mg) was further purified by reversed-phase HPLC using 42% CH_3CN–H_2O containing 0.01% TFA (42 : 58, *V*/*V*) as the mobile phase, yielding compounds **5** (10.3 mg, t_R = 105.4 min) and **6** (8.7 mg, t_R = 127.8 min). Fraction E_{10} (10.4 g) was subjected to silica gel CC and eluted with CH_2Cl_2–MeOH (10 : 1−5 : 1, *V*/*V*), yielding three fractions ($E_{10\text{-}1}$–$E_{10\text{-}3}$). Subfraction $E_{10\text{-}3}$ (160 mg) was further separated by preparative HPLC (YMC-ODS-A 5 μm, 250 mm × 20 mm, detection at 210 nm) using CH_3OH–H_2O containing 0.01% TFA (76 : 24, *V*/*V*, 7 mL·min^{-1}) as the mobile phase, yielding compound **1** (12.3 mg, t_R = 134.6 min).

*Cyclocarysaponins A (**1**)*

White amorphous powder: $[\alpha]_D^{20}$ −14.8 (*c* 0.08, MeOH); UV (MeOH) *λ*max (log*ε*): 200.2 (0.63) nm; IR: 3 437.3, 2 959.9, 1 746.1, 1 644.4, 1 453.6, 1 387.9, 1 247.3, 1 166.6, 1 065.7, 980.3, 896.5, 668.3 cm^{-1}; ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) (Tables 1 and 2); HR-TOF-MS *m*/*z* 745.4489 $[M + Na]^+$ (Calcd. for $C_{40}H_{66}O_{11}Na$, 745.4503).

*Cyclocarysaponins B (**2**)*

White amorphous powder: $[\alpha]_D^{20}$ −19.3 (*c* 0.09, MeOH); UV (MeOH) *λ*max (log*ε*): 201.0 (0.93) nm; IR: 3 438.0, 2 943.7, 1 907.0, 1 452.0, 1 367.5, 1 245.0, 1 167.6, 1 065.9, 1 007.0, 980.2, 894.7, 668.4 cm^{-1}; ^{1}H NMR (600 MHz, pyrid-

ine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) (Tables 1 and 2); HR-TOF-MS *m/z* 787.4644 $[M + Na]^+$ (Calcd. for $C_{42}H_{68}O_{12}Na$, 787.4608).

Cyclocarysaponins C ***(3)***

White amorphous powder: $[\alpha]_D^{20}$ −16.4 (*c* 0.11, MeOH); UV (MeOH) *λ*max (log*ε*): 201.0 (0.57) nm; IR: 3 423.2, 2 960.0, 1 725.0, 1 453.4, 1 371.4, 1 246.3, 1 166.6, 1 065.4, 979.2, 885.5, 668.5 cm^{-1}; ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) (Tables 1 and 2); HR-TOF-MS *m/z* 801.4800 $[M + Na]^+$ (Calcd. for $C_{43}H_{70}O_{12}Na$, 801.4765).

Cyclocarysaponins D ***(4)***

White amorphous powder: $[\alpha]_D^{20}$ −34.8 (*c* 0.15, MeOH); UV (MeOH) *λ*max (log*ε*): 201.2 (1.40) nm; IR: 3 416.3, 2 960.7, 1 725.3, 1 674.6, 1 452.4, 1 381.1, 1 248.5, 1 204.6, 1 167.7, 1 065.3, 1 006.9, 980.5, 892.7, 802.5, 722.4, 598.0 cm^{-1}; ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) (Tables 1 and 2); HR-TOF-MS *m/z* 803.4945 $[M + Na]^+$ (Calcd. for $C_{43}H_{72}O_{12}Na$, 803.4921).

Cyclocarysaponins E ***(5)***

White amorphous powder: $[\alpha]_D^{20}$ −8.4 (*c* 0.06, MeOH); UV (MeOH) *λ*max (log*ε*): 201.2 (0.84) nm; IR: 3 416.0, 2 929.7, 1 726.2, 1 649.2, 1 451.7, 1 388.8, 1 308.2, 1 246.8, 1 166.6, 1 064.9, 980.9, 893.8, 804.9, 554.7 cm^{-1}; ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) (Tables 1 and 2); HR-TOF-MS *m/z* 803.4566 $[M + Na]^+$ (Calcd. for $C_{42}H_{68}O_{13}Na$, 803.4558).

Cyclocarysaponins F ***(6)***

White amorphous powder: $[\alpha]_D^{20}$ −4.8 (*c* 0.07, MeOH); UV (MeOH) *λ*max (log*ε*): 200.4 (0.62) nm; IR: 3 405.6, 2 957.6, 2 876.0, 1 724.8, 1 649.2, 1 451.7, 1 388.8, 1 308.2, 1 246.8, 1 167.0, 1 065.1, 1 007.0, 980.6, 896.2, 806.3, 668.5 cm^{-1}; ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) (Tables 1 and 2); HR-TOF-MS *m/z* 789.4410 $[M + Na]^+$ (Calcd. for $C_{41}H_{66}O_{13}Na$, 789.4401).

Cyclocarysaponins G ***(7)***

White amorphous powder: $[\alpha]_D^{20}$ +25.4 (*c* 0.06, MeOH); UV (MeOH) *λ*max (log*ε*): 201.0 (0.59) nm; IR: 3 416.5, 2 959.8, 1 725.8, 1 650.0, 1 452.0, 1 389.2, 1 247.7, 1 167.2, 1 065.1, 980.8, 896.2, 668.6 cm^{-1}; ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) (Tables 1 and 2); HR-TOF-MS *m/z* 629.4033 $[M + Na]^+$ (Calcd. for $C_{35}H_{58}O_8Na$, 629.4029).

Cyclocarysaponins H ***(8)***

White amorphous powder: $[\alpha]_D^{20}$ −11.7 (*c* 0.05, MeOH); UV (MeOH) *λ*max (log*ε*): 200.0 (0.28) nm; IR: 3 409.6, 2 966.7, 1 706.2, 1 453.3, 1 379.6, 1 279.7, 1 169.4, 1 066.8, 1 007.8, 971.7, 891.4, 635.7 cm^{-1}; ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) (Tables 1 and 2); HRTOFMS *m/z* 719.4003 $[M + Na]^+$ (Calcd. for $C_{37}H_{60}O_{12}Na$, 719.3982).

Cyclocarysaponins I ***(9)***

White amorphous powder: $[\alpha]_D^{20}$ +18.8 (*c* 0.08, MeOH); UV (MeOH) *λ*max (log*ε*): 200.8 (0.69) nm; IR: 3 404.6, 2 959.2, 1 657.6, 1 453.6, 1 389.0, 1 318.3, 1 255.8, 1 208.9, 1 166.7, 1 065.9, 978.5, 884.3, 808.1, 637.5 cm^{-1}; ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) (Tables 1 and 2); HR-TOF-MS *m/z* 559.3244 $[M + Na]^+$ (Calcd. for $C_{30}H_{48}O_8Na$, 559.3247).

Cyclocarysaponins J ***(10)***

White amorphous powder: $[\alpha]_D^{20}$ +21.6 (*c* 0.10, MeOH); UV (MeOH) *λ*max (log*ε*): 202.2 (1.45) nm; IR: 3 411.6, 2 967.2, 1 676.7, 1 455.6, 1 388.5, 1 203.2, 1 139.3, 1 067.1, 945.7, 893.8, 840.7, 800.8, 781.5, 721.4, 668.7 cm^{-1}; ^{1}H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) (Tables 1 and 2); HR-TOF-MS *m/z*: 559.3267 $[M + Na]^+$ (Calcd. for $C_{30}H_{48}O_8Na$, 559.3247).

Acid hydrolysis and sugar analysis

Based on the reported procedure [23-25], each (2 mg) of compounds **1**−**10** was dissolved in 2 mol·L^{-1} HCl (dioxane-H_2O, 1 ∶ 1, *V*/*V*) and refluxed for 10 h. After removal of the HCl by evaporation until dryness, the residue was suspended in water (2 mL) and then extracted with EtOAc. The remaining aqueous solution was evaporated and dried under reduced pressure to obtain a monosaccharide residue. The residue was dissolved in pyridine (1 mL), followed by the addition of 2 mg L-cysteine methyl ester hydrochloride. The mixture was heated at 60 °C for 2 h, evaporated under an N_2 stream, dried *in vacuo*, and then trimethylsilylated with *N*-trimethysilylimidazole (0.2 mL) for 2 h. The resulting mixture was partitioned between *n*-hexane and H_2O (2 mL each), and the *n*-hexane soluble layer was analyzed using GC under the following conditions: capillary column, HP-5 (30 m × 0.32 mm × 0.25 μm, Agilent); column temperature, 230 °C; injection temperature, 250 °C; flow rate, 1.0 mL·min^{-1}; carrier gas, N_2. The peaks corresponding to D-quinovose (12.8 min) were detected in the hydrolysate of compounds **3**−**5** and **7**−**9**, those corresponding to L-arabinofuranose (9.8 and 10.4 min) were observed in the hydrolysates of compounds **2**−**6**, **8**, and those corresponding to L-arabinopyranose (10.7 and 10.9 min) were detected in the hydrolysates of compounds **1**, **2**, **6**, **10**.

Evaluation of bioactivity

Cytotoxicity evaluation

The cytotoxicity assay was performed against five human tumor cell lines: Bel-7402 (human hepatoma cancer cell line), Caski (human cervical cancer cell line), BGC-823 (human gastric cancer cell line), A2780 (human ovarian cancer cell line), and HCT-116 (human colon cancer cell line). All the cell lines were purchased from FengHuiShengWu (Hunan, China). The cells were maintained in RPMI-1640 complete medium (WH1122G081, Yuchun Biolog, Shanghai, China) supplemented with 10% FBS and 1% P/S solution. Then the cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2. During the experiments, cells in the exponential growth phase were sub-cultured twice a week. Compounds **1**−**10** were dissolved in 0.5% DMSO and RPMI-1640 complete medium to prepare stock solutions, which were stored at 4 °C. Cells were cultured in 96-well plates at a density of 1 × 10^4 cells per well in 100 μL of the medium. After cultivation for 24 h, cells were treated with 100 μL of

test compounds and incubated for 48 h. Then, 10 μL CCK-8 (Cell Counting Kit-8) solution was added to each well, and the plates were incubated for 4 h. Absorbance was measured at 450 nm using a microplate reader [26-28].

Enzyme inhibition assay

The inhibitory activities of compounds **1**−**10** against the enzymatic activity of α-glucosidase and PTP1B were evaluated according to the previously described method [29, 30].

Anti-inflammatory activity

The anti-inflammatory activity of compounds **1**−**10** was assessed by measuring the NO production in LPS-induced RAW 264.7 mouse macrophages (positive control, dexamethasone), according to the previously described method [31].

Supporting Information

Supporting information can be requested by sending E-mail to the corresponding authors.

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