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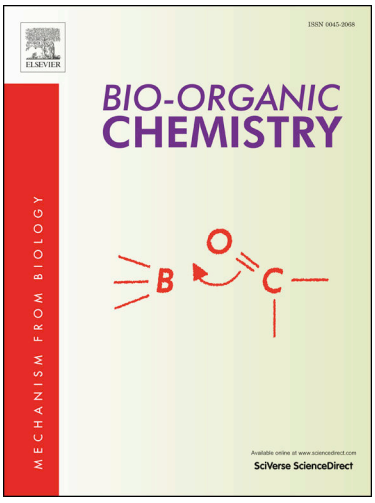

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Hypoglycemic Triterpenoid Glycosides from *Cyclocarya paliurus* (Sweet Tea Tree)

Huihui Sun[a], Jie Tan[a], Wenyan Lv[a], Jing Li[a], Jianping Wu[a], Jialing Xu[a], Hui Zhu[a], Zhichun Yang[a], Wenxuan Wang[a], Zijun Ye[b], Tongyao Xuan[b], Zhenxing Zou[a], Zuhui Chen[c], Kangping Xu[a,*]

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Abstract

Four new rarely occurred *seco*-dammarane triterpenoid glycosides (**1**-**4**) and four new dammarane triterpenoid glycosides (**5**-**8**), along with four known triterpenoids (**9**-**12**), were isolated from the 70% ethanol extract of the leaves of *Cyclocarya paliurus* (family Juglandaceae). Their structures were elucidated by extensive spectroscopic methods, including 1D/2D NMR and HRESIMS data, together with chemical analysis and DFT GIAO ^{13}C NMR calculation. In bioassay, compounds **5**-**8** significantly increased glucose consumption in 3T3-L1 adipocytes, which could be the bioactive constituents for the anti-diabetes effect of the traditional usage of *C. paliurus*.

Keywords: *Cyclocarya paliurus*; triterpenoid glycosides; glucose consumption; 3T3-L1 adipocytes

1. Introduction

Cyclocarya paliurus (Batal.) Iljinsk (family Juglandaceae), an endemic species in China, is widely distributed in south and southeast areas [1]. Its leaves are used as traditional tea, which have the effects of relieving diabetes, hypertension, coronary heart disease and neurasthenia [2, 3]. To date, various compounds including triterpenoids [4], flavonoids [5], phenolic acids [5], polysaccharides [6] and steroids [7] have been isolated from this plant. Especially, triterpenoids from *C. paliurus* have been the focus of research due to their interesting pharmacological activity on diabetes [8-13].

During our previous study, the dichloromethane, ethyl acetate and n-butanol portions of the total 70% ethanol extract of *C. paliurus* were evaluated for their effects on body weight, water intake, fasting blood glucose and fasting insulin values of type-II diabetic rats. As a result, dichloromethane portion showed significant effects and alleviated the insulin resistance symptoms of type-II diabetic rats. Further investigation on the dichloromethane portion led to the isolation of triterpenoid glycosides **1-12** (**Fig. 1**). Herein, their isolation, structure elucidation, and hypoglycemic activity assay were reported.

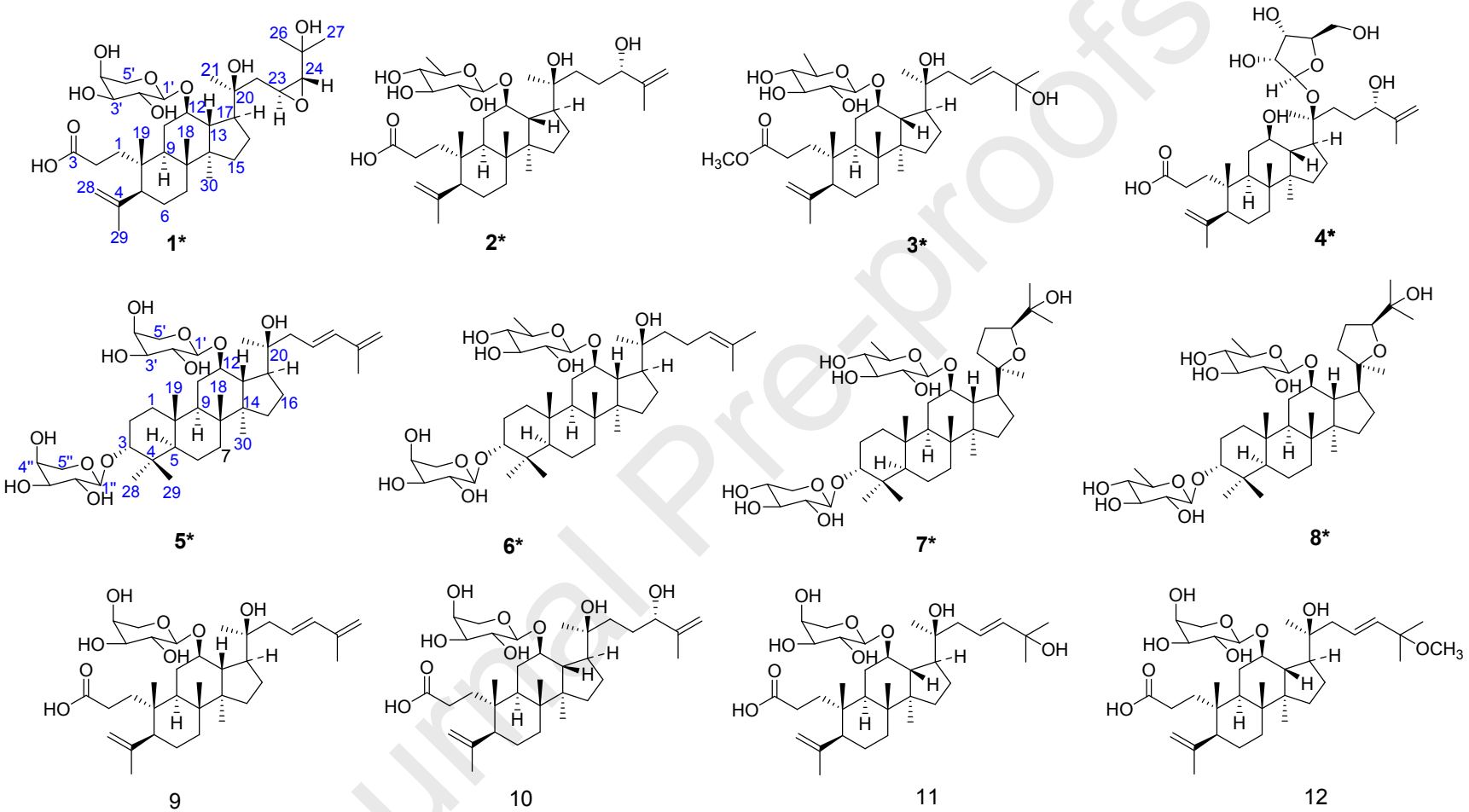

Fig. 1. Chemical structures of compounds **1-12**.

2. **Materials and methods**

2.1. General methods

Optical rotations were measured on a JASCO model 1020 polarimeter (Horiba, Tokyo, Japan) at room temperature. NMR spectra were measured on a Bruker AV-500 MHz spectrometer (Bruker, Karlsruhe, Germany) using acetone-d_6 or CD_3OD as solvent and tetramethylsilane (TMS) as an internal standard. The HRESIMS were measured on a LC-LTQ Orbitrap Velos Pro ETD (Thermo Fisher, MA, USA) in positive ion mode. Gas Chromatography-Mass Spectrometer (GC-MS) were measured on GCMS-QP2010 Ultra (SHIMADZU, Hongkong, China). Column chromatograph (CC) separations were carried out on silica gel (200-300 or 80-100 mesh; Qingdao Peremanent Sea Silica Ltd., Qingdao, China), polyamide (80-100 or 30-60 mesh, Taizhou Luqiao Sijia Biochemical Plastics Factory, Taizhou, China), MCI gel (75-150 μm, Mitsubishi Chemical Industries Ltd., Tokyo, Japan), C_{18} reverse-phased silica gel (40-75 μm, Fuji, Kasugai, Japan). Semi-preparative HPLC separations were conducted by using Agilent 1260 system with an YMC-peak ODS-A column (5 μm, 250 × 10 mm). All the analytic reagents were analytical grade and purchased from Cologne Chemical Co., Ltd., Chengdu, China.

2.2. Plant material

The leaves of *C. paliurus* (collected in Xinning County, Shaoyang City, Hunan Province) were provided by Hunan Heran Biotechnology Development Company, Hunan Province, People's Republic of China, in May 2016, identified by Prof. Kangping Xu (College of Xiangya Pharmacy, Central

South University). And its voucher specimen (No. 20160820) was deposited in the Xiangya School of Pharmaceutical Sciences, Central South University.

2.3. Extraction and isolation

The air-dried leaves of *C. paliurus* (10.0 kg) were extracted with 70% EtOH under reflux (100 L; 2 × 2 h) to get crude extracts, which were dried under reduced pressure afterwards. Then the residue was dispersed in water and partitioned with with CH_2Cl_2, EtOAc and n-BuOH, sequentially. The CH_2Cl_2 extract (400 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 obtain ten fractions (I-X) according to TLC patterns. The fraction IX was eluted with aqueous through Polyamide column (0-95%, stepwise) to yield five subfractions (A-E). IX_A (33.2 g) was further chromatographed by a silica gel column (CH_2Cl_2-MeOH, from 50:1 to 0:100), and followed by a C_{18} reversed-phase column (from 20% to 100% aqueous MeOH, stepwise) to obtain compound **1** (2.3 mg) and compound **2** (4.5 mg). Fr.IX_B-Fr.IX_C were merged together and then separated by a C_{18} reversed-phase column with a gradient system of MeOH/H_2O to get five fractions (IX_{BC-1}-IX_{BC-5}). IX_{BC-2} (3.5 g) was purified by an MCI gel column eluted with MeOH-H_2O system (20% to 100%, stepwise) to give six subfractions (IX_{BC-2-a}-IX_{BC-2-f}). Compound **3** (2.0 mg), compound **4** (3.2 mg) were isolated from IX_{BC-2-b} by semi-preparative HPLC (3.0 mL/min, 210 nm, ACN-H_2O, 7.0:3.0, V/V). Compound **6** (1.8 mg), compound **7** (1.5 mg) were isolated from IX_{BC-2-c} by semi-preparative HPLC (3.0 mL/min, 230 nm, ACN-H_2O, 7.0:3.0, V/V). Fraction IX_D (78.2 g) was applied to silica gel eluted with EtOAc-MeOH to give eight fractions (IX_{D-1}-IX_{D-8}). The fraction IX_{D-3} (18.4 g) was futher purified by silica gel eluted with CH_2Cl_2-MeOH to give four fractions (IX_{D-3-a}-IX_{D-3-d}). Subsequently, Compound **5** (3.8 mg) was

isolated from IX_{D-3-b} by semi-preparative HPLC (3.0 mL/min, 230 nm, ACN-H_2O, 8.0:2.0, V/V). Compound **8** (2.5 mg) and compound **9** (4.8mg) were isolated from IX_{D-3-c} by semi-preparative HPLC (3.0 mL/min, 230 nm, ACN-H_2O, 8.0:2.0, V/V). Next, IX_{D-4} (23.0 g) was purified by an MCI gel column eluted with aqueous MeOH (20% to 100%, stepwise) to give six subfractions (IX_{D-4-a}-IX_{D-4-e}). Compound **10** (8.4 mg) was isolated from IX_{D-4-c} by semi-preparative HPLC (3.0 mL/min, 210 nm, ACN-H_2O, 8.0:2.0, V/V). And Compound **11** (5.6 mg) and compound **12** were isolated from IX_{D-4-d} by semi-preparative HPLC (3.0 mL/min, 210 nm, ACN-H_2O, 8.0:2.0, V/V).

*2.3.1. Cyclocarioside Z1 (**1**)*

White amorphous powder; [α]25 D + 8.1 (*c* 0.06, MeOH); HPLC-UV (ACN-H_2O) λmax: 203 nm; ^{1}H NMR (500 MHz in acetone-d_6) data see Table 1, and ^{13}C NMR (125 MHz in acetone-d_6) data see Table 3. HRESIMS, m/z 637.3956 $[M - H]^-$ (calcd for $C_{35}H_{57}O_{10}$ 637.3952).

*2.3.2. Cyclocarioside Z2 (**2**)*

White amorphous powder; [α]25 D – 10.6 (*c* 0.10, MeOH); HPLC-UV (ACN-H_2O) λmax: 203 nm; ^{1}H NMR (500 MHz in CD_3OD) data see Table 1, and ^{13}C NMR (125 MHz in CD_3OD) data see Table 3. HRESIMS, m/z 635.4157 $[M - H]^-$ (calcd for $C_{36}H_{59}O_9$ 635.4159).

*2.3.3. Cyclocarioside Z3 (**3**)*

White amorphous powder; [α]25 D – 87.5 (*c* 0.056, MeOH); HPLC-UV (ACN-H_2O) λmax: 203 nm; ^{1}H NMR (500 MHz in CD_3OD) data see Table 1, and ^{13}C NMR (125 MHz in CD_3OD) data see Table 3. HRESIMS, m/z 673.4293 $[M + Na]^+$ (calcd for $C_{37}H_{62}O_9Na$ 673.4292).

*2.3.5. Cyclocarioside Z4 (**4**)*

White amorphous powder; [α]25 D + 32.0 (*c* 0.10, MeOH); HPLC-UV (ACN-H_2O) λmax: 203 nm; ^{1}H NMR (500 MHz in CD_3OD) data see Table 1, and ^{13}C NMR (125 MHz in CD_3OD) data see Table 3. HRESIMS, m/z 621.3997 $[M - H]^-$ (calcd for $C_{35}H_{57}O_9$ 622.4003).

*2.3.6. Cyclocarioside Z5 (**5**)*

White amorphous powder; [α]25 D – 87.6 (*c* 0.50, MeOH); HPLC-UV (ACN-H_2O) λmax: 230 nm; ^{1}H NMR (500 MHz in CD_3OD) data see Table 2, and ^{13}C NMR (125 MHz in CD_3OD) data see Table 3. HRESIMS, m/z 740.4947 $[M + NH_4]^+$ (calcd for $C_{40}H_{70}NO_{11}$ 740.4949).

*2.3.7. Cyclocarioside Z6 (**6**)*

Colorless powder; [α]25 D – 69.0 (*c* 0.10, MeOH); HPLC-UV (ACN-H_2O) λmax: 230 nm; ^{1}H NMR (500 MHz in CD_3OD) data see Table 2, and ^{13}C NMR (125 MHz in CD_3OD) data see Table 3. HRESIMS, m/z 756.5256 $[M + NH_4]^+$ (calcd for $C_{41}H_{74}NO_{11}$ 756.5262).

*2.3.8. Cyclocarioside Z7 (**7**)*

Colorless powder; [α]25 D + 51.9 (*c* 0.027, MeOH); HPLC-UV (ACN-H_2O) λmax: 230 nm; ^{1}H NMR (500 MHz in CD_3OD) data see Table 2, and ^{13}C NMR (125 MHz in CD_3OD) data see Table 3. HRESIMS, m/z 772.5215 $[M + NH_4]^+$ (calcd for $C_{41}H_{74}NO_{12}$ 772.5211).

*2.3.9. Cyclocarioside Z8 (**8**)*

Colorless powder; [α]25 D + 32.7 (*c* 0.07, MeOH); HPLC-UV (ACN-H_2O) λmax: 230 nm; ^{1}H NMR (500 MHz in CD_3OD) data see Table 2, and ^{13}C NMR (125 MHz in CD_3OD) data see Table 3. HRESIMS, m/z 786.5369 $[M + NH_4]^+$ (calcd for $C_{42}H_{76}NO_{12}$ 786.5368).

*2.3.10. Cyclocarioside I (**9**)*

White amorphous powder; HPLC-UV (ACN-H_2O) λmax: 230 nm; ^{1}H NMR (500 MHz in pyridine-d_5) δ_H 2.93 (H-1_a, 1H, m), 1.82 (H-1_b, 1H, m), 1.46 (H-2_a, 1H, m), 1.06 (H-2_b, 1H, m), 2.09 (H-5, 1H, m), 1.29 (H-6, 2H, m), 1.43 (H-7_a, 1H, m), 1.06 (H-7_b, 1H, m), 2.09 (H-9, 1H, m), 2.88 (H-11_a, 1H, m), 2.47 (H-11_b, 1H, m), 4.00 (H-12, 1H, d, 7.5), 2.09 (H-13, 1H, m), 1.49 (H-15_a, 1H, m), 1.06 (H-15_b, 1H, m), 1.99 (H-16, 2H, m), 1.98 (H-17, 1H, m), 0.88 (CH_3-18, 3H, s), 1.15 (CH_3-19, 3H, s), 1.47 (CH_3-21, 3H, s), 2.65 (H-22_a, 1H, m), 2.55 (H-22_b, 1H, m), 6.13 (H-23, 1H, m), 6.42 (H-24, 1H, m), 5.04 (H-26_a, 1H, br s), 4.96 (H-26_b, 1H, br s), 1.89 (CH_3-27, 3H, s), 4.90 (H-28_a, 1H, br s), 4.89 (H-28_b, 1H, br s), 1.80 (CH_3-29, 3H, s), 0.98 (CH_3-30, 3H, s), 4.78 (H-1', 1H, d, 7.0), 4.44 (H-2', 1H, m), 4.47 (H-3', 1H, m), 4.19 (H-4', 1H, m), 4.49 (H-$5'_a$, 1H, br s), 3.55 (H-$5'_b$, 1H, d, 15.5); ^{13}C NMR (125 MHz in pyridine-d_5) δ_C 38.9 (C-1), 29.5 (C-2), 148.3 (C-4), 51.4 (C-5), 25.3 (C-6), 34.6 (C-7), 40.5 (C-8), 40.4 (C-9), 39.8 (C-10), 32.3 (C-11), 74.2 (C-12), 43.9 (C-13), 50.7 (C-14), 31.1 (C-15), 25.2 (C-16), 50.1 (C-17), 16.4 (C-18), 20.3 (C-19), 73.9 (C-20), 27.2 (C-21), 45.0 (C-22), 128.1 (C-23), 135.4 (C-24), 142.4 (C-25), 114.6 (C-26), 18.6 (C-27), 113.4 (C-28), 24.0 (C-29), 16.3 (C-30), 99.6 (C-1'), 72.1 (C-2'), 73.8 (C-3'), 69.5 (C-4'), 67.5 (C-5').

*2.3.11. Cyclocarioside J (**10**)*

White amorphous powder; HPLC-UV (ACN-H_2O) λmax: 203 nm; ^{1}H NMR (500 MHz in acetone-d_6) δ_H 2.50 (H-1_a, 1H, m), 1.51 (H-1_b, 1H, m), 2.62 (H-2_a, 1H, m), 2.23 (H-2_b, 1H, m), 2.02 (H-5, 1H, m), 1.32 (H-6, 2H, m), 1.60 (H-7_a, 1H, m), 1.22 (H-7_b, 1H, m), 1.87 (H-9, 1H, m), 2.52 (H-11_a, 1H, m), 1.34 (H-11_b, 1H, m), 4.17 (H-12, 1H, m), 1.89 (H-13, 1H, m), 1.40 (H-15_a, 1H, m), 1.07 (H-15_b, 1H, m), 1.68 (H-16_a, 1H, m), 1.32 (H-16_b, 1H, m), 1.85 (H-17, 1H, m), 1.08 (CH_3-18, 3H, s), 1.11 (CH_3-19, 3H, s), 1.17 (CH_3-21, 3H, s), 1.57 (H-22_a, 1H, m), 1.44 (H-22_b, 1H, m), 1.68 (H-23_a, 1H, m), 1.57 (H-23_b, 1H, m), 3.91 (H-24, 1H, m), 4.95 (H-26_a, 1H, br s), 4.78 (H-26_b, 1H, br s), 1.82 (CH_3-27, 3H, s), 4.87 (H-28_a, 1H, br s), 4.78 (H-28_b, 1H, br s), 1.73 (CH_3-29, 3H, s), 1.02 (CH_3-30, 3H, s), 4.35 (H-1', 1H, d, 6.5), 3.54 (H-2', 1H, m), 3.54 (H-3', 1H, m), 3.89 (H-4', 1H, m), 4.03 (H-5'$_a$, 1H, br s), 3.54 (H-5'$_b$, 1H, m); ^{13}C NMR (125 MHz in acetone-d_6) δ_C 37.9 (C-1), 29.2 (C-2), 148.1 (C-4), 51.5 (C-5), 24.8 (C-6), 34.6 (C-7), 40.5 (C-8), 40.2 (C-9), 39.6 (C-10), 32.1 (C-11), 74.0 (C-12), 43.9 (C-13), 50.4 (C-14), 30.8 (C-15), 25.0 (C-16), 59.5 (C-17), 16.0 (C-18), 19.8 (C-19), 73.5 (C-20), 25.3 (C-21), 36.1 (C-22), 30.1 (C-23), 74.9 (C-24), 148.5 (C-25), 109.3 (C-26), 17.5 (C-27), 113.0 (C-28), 23.4 (C-29), 16.3 (C-30), 98.9 (C-1'), 71.2 (C-2'), 72.8 (C-3'), 68.7 (C-4'), 66.5 (C-5').

*2.3.12. Pterocaryoside B (**11**)*

White amorphous powder; HPLC-UV (ACN-H_2O) λmax: 203 nm; ^{1}H NMR (500 MHz in acetone-d_6) δ_H 2.53 (H-1_a, 1H, m), 1.57 (H-1_b, 1H, m), 2.65 (H-2_a, 1H, m), 2.27 (H-2_b, 1H, m), 1.83 (H-5, 1H, m), 1.33 (H-6, 2H, m), 1.42 (H-7_a, 1H, m), 1.07 (H-7_b, 1H, m), 1.88 (H-9, 1H, m), 2.47 (H-11_a, 1H, m), 1.33 (H-11_b, 1H, m), 4.19 (H-12, 1H, td, 4.5, 5.0, 4.5), 1.96 (H-13, 1H, m), 1.61 (H-15_a, 1H, m), 1.20 (H-15_b, 1H, dt, 3.2, 13.1), 1.88 (H-16_a, 1H, m), 1.70 (H-16_b, 1H, m), 2.10 (H-17,

1H, m), 1.11 (CH_3-18, 3H, s), 0.99 (CH_3-19, 3H, s), 1.17 (CH_3-21, 3H, s), 2.20 (H-22, 2H, m), 5.72 (H-23, 1H, m), 5.68 (H-24, 1H, d, 15.6), 1.26 (CH_3-26, 1H, s), 1.25 (CH_3-27, 3H, s), 4.86 (H-28_a, 1H, br s), 4.75 (H-28_b, 1H, d, 1.7), 1.80 (CH_3-29, 3H, s), 1.08 (CH_3-30, 3H, s), 4.33 (H-1', 1H, d, 7.0), 3.52 (H-2', 1H, m), 3.52 (H-3', 1H, m), 3.77 (H-4', 1H, m), 3.89 (H-$5'_a$, 1H, dd, 1.8, 10.7), 3.54 (H-$5'_b$, 1H, m); ^{13}C NMR (125 MHz in acetone-d_6) δ_C 38.2 (C-1), 30.2 (C-2), 177.6 (C-3), 148.9 (C-4), 49.8 (C-5), 25.4 (C-6), 31.5 (C-7), 40.3 (C-8), 40.8 (C-9), 41.3 (C-10), 33.1 (C-11), 74.9 (C-12), 44.7 (C-13), 51.2 (C-14), 35.3 (C-15), 25.7 (C-16), 52.3 (C-17), 20.4 (C-18), 16.7 (C-19), 74.5 (C-20), 26.9 (C-21), 44.8 (C-22), 123.0 (C-23), 142.4 (C-24), 70.1 (C-25), 30.1 (C-26), 30.2 (C-27), 113.8 (C-28), 24.0 (C-29), 16.8 (C-30), 100.0 (C-1'), 72.4 (C-2'), 73.8 (C-3'), 69.4 (C-4'), 67.1 (C-5').

*2.3.13. Cyclocarioside F (**12**)*

White amorphous powder; HPLC-UV (ACN-H_2O) λmax: 203 nm; ^{1}H NMR (500 MHz in acetone-d_6) δ_H 2.53 (H-1_a, 1H, m), 1.57 (H-1_b, 1H, m), 2.66 (H-2_a, 1H, m), 2.28 (H-2_b, 1H, m), 1.83 (H-5, 1H, m), 1.33 (H-6, 2H, m), 1.43 (H-7_a, 1H, m), 1.07 (H-7_b, 1H, m), 1.88 (H-9, 1H, m), 2.49 (H-11_a, 1H, m), 1.74 (H-11_b, 1H, m), 4.19 (H-12, 1H, td, 4.6, 9.6), 1.98 (H-13, 1H, d, 11.1), 1.61 (H-15_a, 1H, m), 1.24 (H-15_b, 1H, m), 1.88 (H-16_a, 1H, m), 1.74 (H-16_b, 1H, m), 2.10 (H-17, 1H, m), 1.11 (CH_3-18, 3H, s), 0.99 (CH_3-19, 3H, s), 1.17 (CH_3-21, 3H, s), 2.25 (H-22, 2H, d, 7.1), 5.71 (H-23, 1H, m), 5.46 (H-24, 1H, d, 15.8), 1.26 (CH_3-26, 3H, s), 1.25 (CH_3-27, 3H, s), 4.87 (H-28_a, 1H, br s), 4.76 (H-28_b, 1H, br s), 1.80 (CH_3-29, 3H, s), 1.08 (CH_3-30, 3H, s), 4.33 (H-1', 1H, d, 6.6), 3.52 (H-2', 1H, m), 3.52 (H-3', 1H, m), 3.77 (H-4', 1H, m), 3.89 (H-$5'_a$, 1H, d, 12.6), 3.54 (H-$5'_b$, 1H, m), 3.11 (OCH_3, 3H, s); ^{13}C NMR (125 MHz in acetone-d_6) δ_C 38.4 (C-1), 30.4 (C-2), 149.0 (C-4), 52.3 (C-5), 25.8 (C-6), 35.4 (C-7), 41.4 (C-8), 40.8 (C-9), 40.4 (C-10), 33.0 (C-11), 74.8 (C-12), 44.8 (C-13),

51.3 (C-14), 31.6 (C-15), 26.5 (C-16), 50.1 (C-17), 16.9 (C-18), 20.5 (C-19), 74.4 (C-20), 27.1 (C-21), 45.0 (C-22), 127.3 (C-23), 138.9 (C-24), 75.1 (C-25), 26.5 (C-26), 26.1 (C-27), 113.8 (C-28), 24.0 (C-29), 16.8 (C-30), 100.0 (C-1'), 72.4 (C-2'), 73.8 (C-3'), 69.5 (C-4'), 67.2 (C-5').

2.4. ^{13}C NMR calculation

Confab was used to search the conformational space of compound **1a** and **1b**. The obtained stable conformers were optimized on B3LYP-D3(BJ)/SVP (IEFPCM, acetone) level of theory, followed with frequency calculation. The conformers with Boltzmann population more than 1% were further subjected to GIAO ^{13}C NMR calculation on mPW1PW91/6-31G* (IEFPCM, acetone) level of theory. The calculated shielding tensors of **1a** and **1b** were linearly regressed with experimental data, respectively. The resulted linear formulas were used to convert shielding tensors into calculated chemical shifts.

2.5. Acid hydrolysis

The configurations of sugar moieties were established according to the published method with some modifications [14, 15]. Compounds **1**-**8** (each 0.5 mg) were hydrolyzed in 2 M HCl under reflux in the oil bath at 100 ℃ for 3 h. The reaction mixture was neutralized by Na_2CO_3 and extracted with $CHCl_3$. The aqueous layer was concentrated and dried to obtain the monosaccharide fraction. The residue was dissolved in pyridine (0.5 mL) containing 1 mg of L-cysteine methyl ester hydrochloride and heated in the water bath at 60 ℃ for 1 h. Then trimethylsilylimidazole (0.5 mL) was added and then heated at 60 ℃ for another 30 minutes. The reaction mixture was analyzed by GC-MS under the conditions: Column, Rxi-5Sil MS (0.25 μm × 30.0 mm, 0.32 mm); front inlet 300 ℃, column 150

℃-300 ℃ at 15 ℃/min. The sugar configurations of compounds **1-8** were identified by the comparison of the retention times with authentic standard treated by the same means.

2.6. Cell culture and treatment

The 3T3-L1 preadipocytes were gained from ATCC (Manassas, VA, USA). 3T3-L1 preadipocytes were grown in the complete medium containing DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37 ℃ under a humidified 5% CO_2 incubator. The cells were subcultured every 3-4 days to reach confluency, and then were seeded into 48-well plates at a density of 2 × 10^4 cells/well. After 90% confluency, the cells were incubated with DMEM supplied with 10% FBS and DMI (1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, and 10 μg/mL insulin) for 2 days. On the third day, differentiating media was replaced with a complete medium containing 10 μg/mL insulin and incubated for another 2 days (the fourth day). Thereafter, the cells were maintained in complete medium for additional 6 days (the tenth day) with medium changing every 2 days. On day 10, fully differentiated adipocytes were treated with compounds at indicated concentrations for 48 h.

2.7. Cell viability assay

Cell viability was determined by the MTT assay [16]. Briefly, 3T3-L1 adipocytes were seeded in 96-well plates at a density of 10^4 cells/well. And the cells were then treated with compounds at indicated concentrations (3.125 μm-100 μm) for 48 h, respectively. Subsequently, each well was added 20 μl MTT solution (5 mg/ml) and continued to culture at 37 ℃ for 4 h, followed by dissolving the violet-formazan crystals with 150 μL DMSO. The absorbance at 490 nm was then measured with

a microplate reader. Cell viability of each group was expressed as a percentage of the control group treated with DMSO.

2.8. Glucose consumption assay

Glucose consumption assay of 3T3-L1 adipocytes were measured by GOD-POD Kit as described previously [17]. Fully differentiated adipocytes were treated with compounds at indicated concentrations for 48 h. Then the glucose consumption of each well was obtained by subtracting the glucose concentration of the cells from the glucose in the blank wells.

2.9. Statistical analysis

All results and data were confirmed at least three separate experiments. SPSS (Statistical Package for the Social Sciences) software was used to determine significant differences via ANOVA followed by Student's t-test. The data were expressed as means ± SD, and values $P < 0.05$ were considered as statistically significant.

Table 1

^{1}H NMR data of **1**-**4** (δ in ppm, J in Hz).

Position	**1**[a]	**2**[b]	**3**[b]	**4**[b]
1	2.54, m	2.44, td (13.5, 4.5)	2.48, td (13.5, 5.5)	1.63, m
	1.58, m	1.60, m	1.46, m	
2	2.66, m	2.65, m	2.75, td (13.0, 5.0)	2.36, m
	2.28, td (12.0, 10.5)	2.23, t (12.5, 11.5)	2.29, m	2.22, m
5	2.09, overlapped	2.07, dd (13.0, 2.5)	2.03, d (12.5)	2.08, dd (13, 3.0)

6	1.78, m	1.61, m	1.90, m	1.90, m
	1.34, m	1.34, m	1.36, m	1.39, m
7	1.63, m	1.60, m	1.60, m	1.18, dd (11.5, 9.0)
	1.21, overlapped	1.23, m	1.22, m	
9	1.99, d (11.0)	1.95, overlapped	1.94, m	1.95, m
11	2.50, m	2.37, m	2.39, m	1.31, m
	1.37, m	1.42, m	1.41, m	
12	4.17,	4.12,	4.08,	3.51,
	td (10.5, 4.0)	td (10.5, 4.0)	td (10.5, 5.0)	td (10.5, 4.5)
13	1.91, m	1.80, overlapped	1.83, m	1.74, m
15	1.43, m	1.45, m	1.46, m	1.60, m
	1.09, overlapped	1.10, overlapped	1.09, overlapped	1.30, m
16	1.89, m	1.82, m	1.82, m	1.90, m
	1.64, m	1.62, m	1.64, m	1.56, m
17	1.88, m	1.82, m	1.80, m	2.55, m
18	1.09, s	1.08, s	1.10, s	0.97, s
19	1.12, s	1.11, s	1.08, s	0.90, s
21	1.31, s	1.17, s	1.19, s	1.22, s
22	1.89, m	1.62, m	2.23, m	1.70, m
	1.51, m	1.43, m		1.40, m
23	3.13, m	1.67, m	5.68, m	1.86, m
		1.60, m		1.72, overlapped
24	2.71, d (2.0)	4.00, t (6.0, 5.5)	5.67, m	3.99, t (5.5, 7.0)
26	1.22, s	4.96, overlapped	1.29, br s	4.91, overlapped
		4.85, d (7.5)		4.81, br s
27	1.20, s	1.75, s	1.29, br s	1.72, s

28	4.87, br s	4.86, d (7.5)	4.84, overlapped	4.87, overlapped
	4.77, br s	4.75, br s	4.70, br s	4.70, br s
29	1.80, s	1.80, s	1.77, s	1.76, s
30	1.01, s	1.01, s	1.01, s	1.09, s
1'	4.36, d (6.0)	4.38, d (8.0)	4.36, d (7.5)	4.97, d (7.0)
2'	3.53, m	3.23, m	3.21, m	3.71, m
3'	3.53, m	3.31, m	3.29, m	3.65, d (6.5)
4'	3.80, br s	3.02, t (9.0, 9.0)	3.02, t (9.5, 9.0)	3.68, m
5'	3.89, br d (12.5)	3.27, m	3.28, m	3.79, dd (11.5, 3.0)
	3.52, m			3.59, q (5.5)
6'		1.28, d (6.0)	1.28, d (6.1)	
-OCH_3			3.64, s	

[a]Measured in acetone-d_6 at 500 MHz.

[b]Measured in CD_3OD at 500 MHz.

Table 2

^{1}H NMR (500 MH_Z) data of **5-8** in CD_3OD (δ in ppm, J in Hz).

Position	**5**	**6**	**7**	**8**
1	2.50, m	2.44, m	2.44, m	2.43, m
	1.48, m	1.48, m	1.51, m	1.51, m
2	1.78, m	1.48, m	1.48, m	1.70, m
	1.62, m			1.58, m
3	3.34, overlapped	3.34, overlapped	3.33, overlapped	3.34, overlapped

5	1.32, m	1.35, m	1.35, m	1.35, m
6	1.86, overlapped	1.86, m	1.46, m	1.45, m
	1.45, m	1.46, m		
7	1.59, m	1.60, m	1.60, m	1.61, m
	1.23, m	1.23, m	1.23, m	1.23, m
9	1.80, m	1.80, m	1.82, m	1.81, m
11	2.39, m	2.34, m	2.42, m	2.44, m
	1.38, m	1.40, m	1.36, m	1.34, m
12	4.12, td (10.5, 5.0)	4.11, td (10.5, 5.0)	4.07, td (13.5, 6.5)	4.07, td (10.5, 5.0)
13	1.82, m	1.79, m	1.70, m	1.70, m
15	1.45, m	1.45, m	1.47, m	1.46, m
	1.07, m	1.07, m	1.09, overlapped	1.09, overlapped
16	1.79, m	1.79, m	1.86, m	1.99, m
	1.61, m	1.61, m		1.82, m
17	1.80, m	1.80, m	1.90, m	1.91, m
18	1.02, s	1.00, s	0.99, s	1.02, s
19	1.12, s	1.12, s	1.11, s	1.11, s
21	1.18, s	1.18, s	1.17, s	1.17, s
22	2.32, m	1.48, m	2.41, m	2.41, m
			1.70, m	1.70, m
23	5.77, m	2.07, m	1.47, m	1.46, m
24	6.20, d (15.5)	5.15, t (7.5, 7.5)	3.81, m	3.80, t (7.5, 7.0)
26	4.89, overlapped	1.70, s	1.17, s	1.17, s
27	1.86, s	1.65, s	1.22, s	1.22, s
28	0.98, s	0.98, s	0.94, s	0.94, s
29	0.90, s	0.90, s	0.89, s	0.89, s
30	0.99, s	1.00, s	1.01, s	1.01, s

1'	4.29, d (6.0)	4.29, d (6.0)	4.23, d (9.0)	4.25, d (8.0)
2'	3.61, m	3.60, m	3.21, m	3.22, m
3'	3.48, m	3.58, m	3.29, m	3.30, d (9.5)
4'	3.83, m	3.84, m	3.27, m	2.99, t(9.5, 9.0)
5'	3.89, m	3.86, m	3.87, m	3.26, m
	3.53, m	3.52, m	3.16, m	
6'				1.27, d (6.0)
1"	4.27, d (7.0)	4.33, d (7.5)	4.35, d (9.5)	4.35, d (8.0)
2"	3.46, m	3.11, m	3.11, m	3.10, t (9.5, 7.5)
3"	3.58, m	3.29, m	3.29, m	3.30, d (9.5)
4"	3.79, m	2.99, t (9.0, 9.0)	2.99, t (11.5, 11.5)	3.02, t (10.0, 9.0)
5"	3.86, m	3.27, m	3.27, m	3.26, m
	3.51, m			
6"		1.28, d (6.5)	1.27, d (7.5)	1.27, d (6.0)

Table 3

^{13}C NMR data of **1-8** (δ in ppm).

Position	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**7**[b]	**8**[b]
1	38.4	38.7	38.0	36.0	36.0	36.0	36.0	36.0

2	30.4	32.0	30.6	29.3	26.1	27.0	27.0	22.2
3	177.8		178.0	178.0	82.5	82.4	82.4	82.4
4	149.0	149.4	149.4	148.7	38.6	38.6	38.5	38.6
5	52.4	53.0	53.2	51.8	51.6	51.4	51.6	51.2
6	25.8	26.0	25.9	25.9	19.1	19.1	19.0	19.0
7	35.5	35.8	35.7	33.8	37.2	37.1	37.1	37.1
8	41.5	41.5	41.9	40.3	42.4	42.4	42.4	42.4
9	44.9	45.3	45.4	50.0	54.6	54.6	54.5	54.5
10	40.5	40.8	41.4	40.6	40.6	40.6	40.6	41.6
11	33.2	33.8	34.3	30.2	34.6	34.6	34.7	34.6
12	75.0	76.0	76.6	79.5	77.2	77.0	76.8	76.9
13	40.9	41.8	40.8	42.0	41.6	41.4	41.8	41.8
14	51.3	51.7	51.7	52.3	51.5	51.5	51.4	51.4
15	31.7	31.6	31.9	34.6	31.9	32.0	32.3	32.3
16	25.8	26.2	26.1	25.8	26.0	26.0	27.0	27.0
17	50.8	50.9	50.4	49.6	51.0	50.6	50.2	50.1
18	17.0	16.9	16.8	16.6	17.5	17.1	17.3	17.1
19	20.5	20.6	20.5	20.9	17.1	17.1	17.1	17.1
20	74.2	75.7	75.9	80.6	76.2	75.8	88.0	88.0
21	27.6	25.6	26.4	26.9	26.7	25.9	24.6	25.1
22	43.3	37.6	45.0	33.4	45.2	41.8	34.6	34.6
23	53.2	30.2	123.8	29.9	127.7	23.7	27.0	27.5
24	66.4	77.2	142.1	77.5	136.7	126.0	84.9	84.9
25	68.4	149.0	71.2	149.0	143.5	130.6	73.0	72.9
26	27.2	111.3	29.9	111.5	115.1	25.9	25.1	25.1
27	26.0	18.1	30.0	17.7	19.0	17.8	26.8	26.8
28	113.9	114.0	114.0	114.2	30.0	30.0	29.8	29.8

29	24.0	24.0	24.0	23.7	23.3	23.2	23.2	23.2
30	17.0	16.9	17.0	15.7	17.2	17.4	17.3	17.3
1'	100.1	99.8	100.6	96.5	101.5	101.5	102.2	101.4
2'	72.4	75.3	75.4	71.4	72.5	72.5	75.0	75.4
3'	73.9	77.7	77.8	73.1	74.7	74.3	78.1	78.1
4'	69.5	77.1	77.2	72.4	69.3	69.3	71.5	77.3
5'	67.3	72.6	73.0	65.0	67.6	66.1	66.9	73.0
6'		18.0	18.1					18.1
1"					102.0	100.8	100.8	100.7
2"					72.8	75.6	75.7	75.6
3"					74.4	78.0	78.0	78.0
4"					70.3	77.3	77.2	77.2
5"					66.1	73.0	72.9	73.0
6"						18.1	18.1	18.1
-OCH_3			52.0					

[a]Measured in acetone-d_6 at 125 MHz.

[b]Measured in CD_3OD at 125 MHz.

3. Result and discussion

Compound **1** was isolated as a white amorphous powder, which had a molecular formula of $C_{35}H_{58}O_{10}$ determined by the HRESIMS ion peak at 637.3956 $[M - H]^-$ (calcd for $C_{35}H_{57}O_{10}$ 637.3952). The ^1H NMR of compound **1** (**Table 1**) showed the presence of seven methyl proton signals at δ_H 1.01 (s, H-30), δ_H 1.80 (s, H-29), δ_H 1.20 (s, H-27), δ_H 1.22 (s, H-26), δ_H 1.30 (s, H-21), δ_H 1.11 (s, H-19), and δ_H 1.09 (s, H-18). Moreover, there were multiplets of methylene and methine signals ranging from δ_H 1.00 to 2.80, as well as the signals of one terminal methylene olefinic protons at δ_H 4.87 (br s, H-28_a) and δ_H 4.77 (br s, H-28_b), and an oxymethine proton at δ_H 4.17 (td, 1H, J = 10.5, 4.0 Hz, H-12). These data were used to assign the triterpenoid aglycone skeleton. The ^{13}C NMR (**Table 3**) showed 35 carbon signals, including signals of one terminal olefinic carbon at δ_C 149.0 and δ_C 113.9, as well as five glycosyl carbon signals at δ_C 100.1, 72.4, 73.9, 69.5, and 67.3 ppm. Therefore, an unusual 3,4-*seco*-dammarane type triterpenoid glycoside structure can be established for compound **1**, which is similar to known compound pterocaryoside B [19] except for the signals of side-chain. In the HMBC spectrum, key correlations from H_2-22 to C-23, H_2-24 to C-23 and C-25, from H_3-21 to C-17, C-20, and C-22, from H_3-26 to C-24 and C-25, together with chemical shifts of C-23 and C-24 moved to low field verified the 23, 24-epoxy linkage and an additional hydroxyl group linked to C-25. Furthermore, a pivotal HMBC correlation between the anomeric hydrogen H-1' and C-12 determined the position of glycosyl. Besides, the glycosyl moiety was identified as α-L-arabinopyranosyl based on the NMR data, as well as further acid hydrolysis and GC-MS analysis with L-arabinose standard (**Table 5**). The relative configurations of **1** were determined by NOESY spectrum (**Fig. 2**). Strong correlations of H-5/H-9, H-9/H_3-30, and H-12/H-17/H_3-30 indicated that

H-5, H-9, H-12, H-17, and H_3-30 are α-oriented. The β-orientation of H-13, H_3-18, and H_3-19 were then confirmed by NOESY correlations of H-13/H_3-18 and H_3-18/H_3-19. The configuration at C-20 was determined to be *S* based on NOESY correlations of H-17/H_3-21, respectively, on the basis of comparison to the ^{13}C NMR chemical shift for analogoues of 3,4-*seco*-dammaranes [18-20]. The key NOESY correlations between H_3-21 and H-23, H_3-26 and H-22_a, H_3-26 and H-23 indicated that H-23 and H-24 were *trans* oriented. Therefore, there were two possible configurations, namely 23*S*24*R* (**1a**) and 23*R*24*S* (**1b**). To determine the absolute configuration of the epoxy group, we performed GIAO DFT ^{13}C NMR calculations of structure **1a** and **1b**, and the mean absolute deviation (MAD) and sDP4+ values [21] of them showed that **1a** was the correct structure (**Table 4**). From all the evidence above, the chemical structure of **1** was established as (12*R*, 20*S*, 23*S*, 24*R*)-20,25-dihydroxy-23,24-epoxy-12-O-α-L-arabinopyranosyl-3,4-secodammara-4(28)-en-3-oic acid.

Table 4

GIAO DFT calculation of ^{13}C NMR data of structures of **1a** and **1b** (δ in ppm).

Position	Exptl.	Calcd. of **1a**	Deviation [a]	Calcd. of **1b**	Deviation [b]
1	38.4	37.3	1.1	36.5	1.9
2	30.4	31.0	0.6	29.4	1.0
3	177.8	176.4	1.4	174.5	3.3
4	149.0	149.4	0.4	148.2	0.8
5	52.4	49.8	2.7	49.4	3.1
6	25.8	25.5	0.5	25.3	0.7
7	35.5	35.1	0.4	34.0	1.5
8	41.5	41.4	0.1	40.5	1.0
9	44.9	40.3	0.6	40.5	0.4
10	40.5	42.5	2.0	41.4	0.9
11	33.2	27.2	6.0	28.0	5.2
12	75.0	73.0	2.0	73.2	1.8
13	40.9	48.5	3.6	48.6	3.7
14	51.3	53.7	2.4	54.4	3.1
15	31.7	32.4	0.6	34.3	2.5
16	25.8	26.1	0.3	28.7	2.9
17	50.8	52.0	1.2	45.9	4.9
18	17.0	17.3	0.4	16.2	0.7
19	20.5	21.9	1.4	21.8	1.3

20	74.2	74.2	0.0	74.6	0.4
21	27.6	25.3	2.3	26.3	1.3
22	43.3	43.8	0.5	44.6	1.3
23	53.2	54.2	0.9	55.8	2.5
24	66.4	62.9	3.6	68.0	1.5
25	68.4	71.5	3.1	67.3	1.1
26	27.2	26.7	0.5	28.0	0.8
27	26.0	26.7	0.7	28.0	2.0
28	113.9	116.7	2.8	115.7	1.8
29	24.0	25.1	1.0	24.7	0.6
30	17.0	18.0	1.1	19.2	2.3
1'	100.1	97.1	3.0	96.3	3.8
2'	72.4	71.9	0.5	70.9	1.5
3'	73.9	73.4	0.5	72.9	1.0
4'	69.5	70.8	1.3	69.0	0.5
5'	67.3	68.1	0.8	67.5	0.2
		MAD	1.5	MAD	1.8
		sDP4+	100%	sDP4+	0%

Compound **2** was obtained as a white amorphous powder. The molecular formula of **2** was determined to be $C_{36}H_{60}O_9$ by HRESIMS. The 1D, 2D NMR data were almost identical with cyclocarioside J [18] except for the replacement of the arabinopyranosyl moiety by a quinovopyranosyl unit in **2**. The HMBC (**Fig. 2**) correlations between δ_H 4.38 (d, J = 8.0 Hz, H-1') and δ_C 76.0 (C-12) indicated that the glycosyl was located at C-12. Moreover, further acid hydrolysis and GC-MS analysis with a D-quinovose standard (**Table 5**) suggested a quinovopyranosyl unit in **2**. Therefore, the structure of **2** was deduced as (12*R*, 20*S*, 24*S*)-20,24-dihydroxy-12-O-β-D-

quinovopyranosyl-3,4-secodammara-4(28),25-dien-3-oic acid.

Compound **3** was obtained as a white amorphous powder. The molecular formula of **3** was determined to be $C_{37}H_{62}O_9$ by HRESIMS. The 1D and 2D NMR data were almost identical to known compound pterocaryoside A [19] except for an additional methoxy group, as indicated a methyl proton signal at δ_H 3.64 (s, OCH_3) and a carbon signal at δ_C 52.0 (OCH_3). Moreover, the key correlation between OCH_3 and C-3 in the HMBC (**Fig. 2**) revealed that **3** was a 3-methyl ester derivative of pterocaryoside A. Besides, the glycosyl moiety was determined by acid hydrolysis and GC-MS analysis with D-quinovose standard (**Table 5**). Therefore, the structure of **3** was deduced as (23E)-(12*R*, 20*S*)-12,20,25-trihydroxy-12-O-β-D-quinovopyranosyl-3,4-secodammara-4(28),23-dien-3-oic acid methyl ester.

Compound **4** has the molecular formula $C_{35}H_{58}O_9$, as determined by HRESIMS analysis (m/z 621.3997 $[M - H]^-$). Detailed analysis of NMR spectra indicated that **4** was a 3,4-*seco*-dammarane triterpenoid glycoside similar to cyclocaroside O [22]. However, the 5-O-acetyl-α-L-arabinofuranosy at C-20 in cyclocaroside O was replaced by a ribopyranosyl moiety in **5**. And the linkage of glycosyl fragment was confirmed by a signficant HMBC correlation between δ_H 4.99 (d, J = 7.0 Hz, H-1') and δ_C 80.5 (C-20). Besides, the ribose was identified by acid hydrolysis and GC-MS analysis with standard (**Table 5**). Thus compound **4** was characterized as (12*R*, 20*S*, 24*S*)-12,20,24-trihydroxy-20-O-β-D-ribopyranosyl-3,4-secodammara-4(28),25-dien-3-oic acid.

Compound **5** was obtained as a white amorphous powder. The molecular formula of **5** was

determined to be $C_{40}H_{66}O_{11}$ by HRESIMS. Full NMR data anlysis indicated that **5** was a triterpenoid glycoside which similar to cyclocarioside K [18]. In the ^{1}H NMR (**Table 2**), there were seven methyl proton signals (δ_H 1.86, 1.18, 1.12, 1.02, 0.99, 0.98, 0.90) assigned to the aglycone moiety, together with two anomeric proton signals at δ_H 4.29 (d, 1H, J = 6.0 Hz) and δ_H 4.27 (d, 1H, J = 10.7 Hz) for sugar moieties in the molecule. Detailed analysis of the ^{13}C NMR (**Table 3**) data revealed that there were two anomeric carbon signals at δ_C 101.5 (C-1') and δ_C 102.0 (C-1"), which also proved the presence of two sugar moieties in **5**. The structure of two sugar moieties was determined to be arabinose by acid hydrolysis and GC-MS analysis with standard (**Table 5**). The correlations from H-1' to C-3, H-1" to C-12 in the HMBC spectrum (**Fig. 2**) confirmed the linkage of glycosyl fragment located at C-3 and C-12. In the NOESY spectrum (**Fig. 2**), correlations between H-3 (δ_H 3.34), H-1$_\beta$ (δ_H 1.48, β-orientation) and H-29 (δ_H 0.90), as well as correlations between H-12 (δ_H 4.12), H-11$_\alpha$ (δ_H 2.39, α-orientation), and H-9 (δ_H 1.80) indicated the H-3, H-12 were a-orientation and β-orientation. Furthermore, the side-chain contained one terminal methylene olefinic proton signals at δ_H 4.89 (m, H-26), together with a pair of olefinic proton signals at δ_H 5.77 (m, H-23) and 6.20 (d, 1H, J = 15.6 Hz, H-24), whose the coupling constant (J = 15.6 Hz) suggested that the configuration was E. The absolute configuration at C-20 was determined to be *S* based on the ^{13}C NMR spectra by comparing chemical shifts with analogous dammaranes [18, 23]. Consequently, the compound **5** was determined as (23E)-(20*S*)-(3α, 12β)-20-hydroxy-dammara-23,25-dien-12-O-α-L-arabinopyranoside-3-O-α-L-arabinopyranoside.

Compound **6** was isolated as colorless powder, which had a molecular formula of $C_{41}H_{70}O_{11}$ determined by the HRESIMS. Detailed analysis of 1D and 2D NMR data showed that **6** had a high

similarity to cyclocarioside K except for the only distinction in side-chain. Therefor, the structure of side-chain was futher confirmed by the correlations from H_3-21 to C-17, C-20, C-22 and C-23, H_3-26 to C-24, and C-25, H_3-27 to C-24, and C-25, and H_2-23 to C-24 in the HMBC spectrum. So the structure of **6** was deduced as (20*S*)-(3α, 12β)-20-hydroxy-dammara-24-dien-12-O-β-D-quinovopyranoside-3-O-α-L-arabinopyranoside.

Compound **7** was isolated as colorless powder, which had a molecular formula of $C_{41}H_{70}O_{12}$ determined by the HRESIMS. Detailed analysis of NMR data showed that **7** had a high similarity to cyclocarioside K except for replacement of arabinopyranosyl moiety at C-3 by a xylopyranosyl moiety at the same position in **7**. Moreover, xylopyranosyl moiety was determined by acid hydrolysis and GC-MS analysis with standard (**Table 5**). Therefore, the structure of **7** was deduced as (20*S*, 24*R*)-(3α, 12β)-20,24-epoxydammara-25-hydroxyl-12-O-β-D-quinovopyranoside-3-O-β-D-xylopyranoside.

Compound **8** was obtained as colorless powder. The molecular formula of **8** was determined to be $C_{42}H_{72}O_{12}$ by HRESIMS. The 1D and 2D NMR data were almost identical to known compound cyclocarioside K except for the only difference with the glycosyl moiety at C-3. There was a D-quinovose located in C-3, which was confirmed by acid hydrolysis and GC-MS analysis with standard (**Table 5**). Thus, the compound **8** was determined as (20*S*, 24*R*)-(3α, 12β)-20,24-epoxydammara-25-ol-12-O-β-D-quinovopyranoside-3-O-β-D-quinovopyranoside.

Cell viability was determined using MTT assay. The cell viability above 90% were considered as non-cytotoxicity. As shown in **Table 6**, the maximum safe dosages for most compounds were 50μM

except for compounds **2**, **10** were 100μM in 3T3-L1 adipocytes. Under maximum safe dosages, compounds **1-12** can increase glucose consumption with different degrees in 3T3-L1 adipocytes. Herein, compounds **5-8** signficantly enhanced glucose consumption by 46.74%, 57.55%, 62.38% and 62.27% in 50 μM. Compounds **9**, **10** may have potential hypoglycemic effects, which increased 27.32%, 28.94%, and 29.27% glucose consumption in 3.125 μM, respectively. Besides, other compounds have unobvious hypoglycemic activity in 3T3-L1 adipocytes. Metformin (1 mM) was used as positive control within a range [24] that was found to produce significant increase of adipocyte glucose consumption, which increased around 66.51%. Therefore, it was considered that the structure of the A ring at C-3 and C-4 may affect the hypoglycemic effect of the compounds.

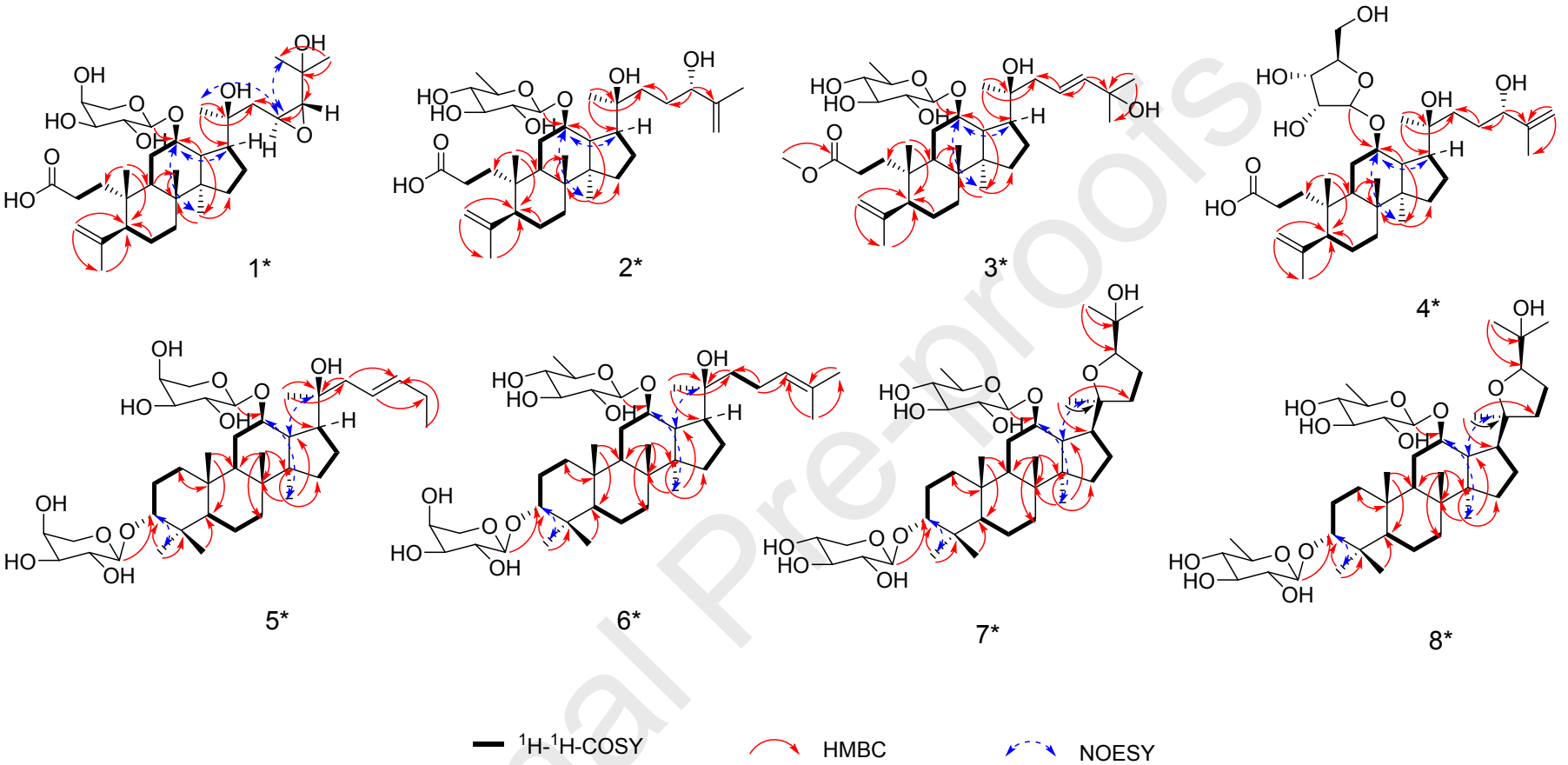

Fig. 2. Key ^{1}H-^{1}H COSY, HMBC and NOESY correlations of compounds **1-8**

Table 5

The GC retention time of compounds **1**-**8** and reference substances

Compounds	Retention Times (min)	Types of the monosaccharide
1*	6.477, 6.753	L-Ara
2*	7.573	D-Qui
3*	7.573	D-Qui
4*	6.820, 6.970	D-Rib
5*	6.465, 6.741	L-Ara, L-Ara
6*	6.474, 6.756, 7.572	L-Ara, D-Qui
7*	7.318, 7.575, 7.689	D-Xyl, D-Qui
8*	7.564	D-Qui, D-Qui
L-Ara	Peak 1: 6.460-6.495	
	Peak 2: 6.730-6.770	
D-Xyl	Peak 1: 7.300-7.350	
	Peak 2: 7.665-7.710	
D-Qui	Peak 1: 7.545-7.595	
D-Rib	Peak 1: 6.805-6.850	
	Peak 2: 6.950-6.995	

Table 6

Cell viability of compounds **1-12** in 3T3-L1 adipocytes. Data are shown as mean ± SD, n = 3. compound vs. Control (DMSO)

	Concentration (μM)								
Compound	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100
1*	100.18±3.87	98.00±3.55	99.36±4.02	101.36±6.07	98.73±3.14	94.64±2.94	103.09±2.58	105.18±1.25	87.81±5.63
2*	101.57±2.71	98.07±3.76	99.99±2.35	103.37±5.28	98.39±2.67	100.95±4.82	102.10±3.77	96.48±5.67	100.84±1.50
3*	100.18±3.87	98.00±3.55	101.36±6.07	101.21±6.04	98.73±3.14	95.97±0.73	101.09±1.09	100.85±0.41	87.82±5.63
4*	101.51±2.34	99.06±2.36	99.36±4.02	99.09±3.90	99.40±4.19	96.97±1.83	100.42±0.58	101.18±1.84	86.48±3.35
5*	100.84±3.84	95.99±6.38	97.35±1.74	97.40±1.38	95.02±1.21	97.44±9.19	94.03±6.96	94.02±3.70	82.93±2.64
6*	99.17±1.35	96.32±3.49	97.35±1.74	98.40±1.41	95.00±1.24	95.11±5.36	95.37±4.65	90.39±1.99	85.20±5.45
7*	100.64±4.11	97.32±3.46	97.65±1.32	97.40±1.98	96.26±1.02	96.77±3.44	97.37±1.18	92.02±6.61	83.55±2.28
8*	100.24±2.86	97.32±3.46	98.35±0.94	98.07±1.51	98.35±2.06	98.11±3.20	96.37±1.48	95.36±2.65	82.89±1.96
9	95.20±4.58	96.28±3.34	95.35±2.86	101.51±4.16	99.01±2.79	99.55±1.48	101.87±4.76	97.88±5.24	77.86±7.64
10	97.97±3.34	94.76±2.13	98.36±5.17	100.17±5.15	96.57±6.06	99.90±4.11	94.76±2.13	98.09±3.82	98.36±5.17
11	104.69±4.59	95.30±0.21	102.22±2.31	102.29±3.00	100.96±1.01	96.79±2.48	93.76±3.11	92.80±2.49	83.20±3.10
12	100.58±3.97	95.32±5.24	97.68±1.28	98.26±1.38	94.89±1.07	94.87±5.00	97.31±1.29	92.78±5.52	82.68±2.52

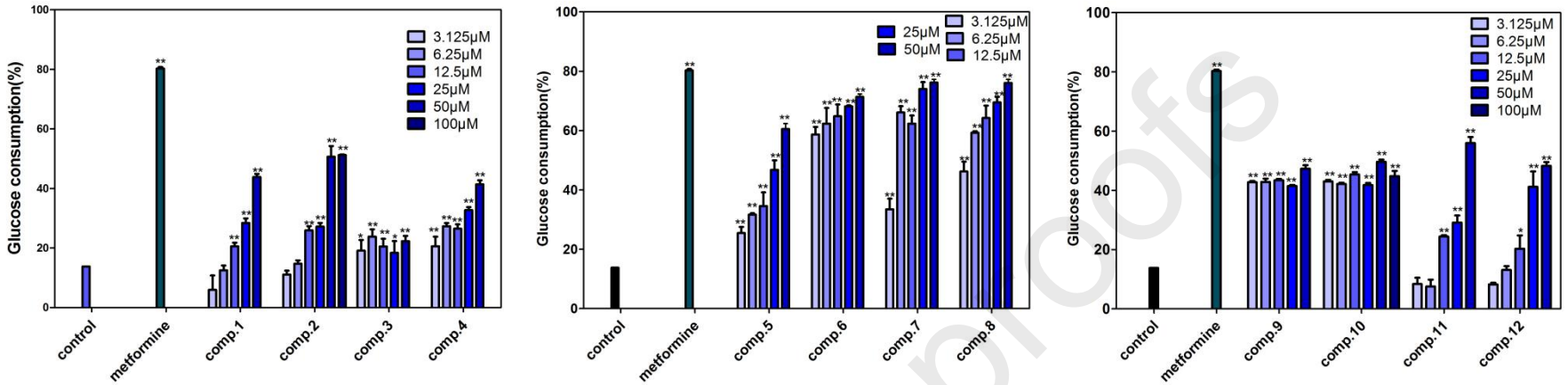

Fig. 4. Glucose consumption of compounds **1-12** in 3T3-L1 adipocytes. Data are shown as mean ± SD, n = 3. $^{*}p < 0.05$, $^{**}p < 0.01$, compound vs. control (DMSO)

4. Conclusion

Bioassay-guided fractionation of the CH_2Cl_2 extract led to the isolation of twelve triterpenoid glycosides including eight new compounds. In the glucose consumption assay, particularly, compounds **5-8** exhibited significant hypoglycemic activity. The discovery of these compounds enriched the chemical constituents of the triterpenoids of *C. paliurus*, and provided clues for clarifying the chemical basis of the physiological activities of *C. paliurus.*

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Conflict of interest

There are no conflicts of interest to declare.

Appendix A. Supplementary data

The HRESIMS, 1D and 2D NMR, and GC-MS chromatogram of compounds **1**-**8** are available in the supplementary data.

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Highlights:

1. Eight new dammarane triterpenoid glycosides were isolated from the leaves of Cyclocarya paliurus (family Juglandaceae)

2. The absolute configuration of the epoxy group of 1 was determined by DFT GIAO ^{13}C NMR calculation.

3. Dammarane triterpenoids showed good hypoglycemic effect in 3T3-L1 adipocytes.