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Seco-dammarane triterpenoids from the leaves of *Cyclocarya paliurus*

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ABSTRACT

The leaves of *Cyclocarya paliurus* with sweet taste are often used as herbal tea in People's Republic of China. In this study eight previously undescribed *seco*-dammarane type triterpenoids, cyclocariols A-H along with seven known compounds were isolated and characterized from its leaves. A possible biogenetic pathway for *seco*-dammarane type triterpenoids formation has been discussed. Cyclocariols A-H were evaluated for their cytotoxicities against human liver (SMMC-7721) and breast cancer (BT-549) cell lines. Cyclocariols A, B, E, and H were also tested against human colon tumor (HCT-116) cell lines, where all four exhibited good activities with IC_{50} values of 6.53, 4.94, 8.24, and 6.48 μM, respectively.

1. Introduction

Cyclocarya paliurus (Batal.) Iljinsk, famous as “sweet tea tree”, belongs to the family Juglandaceae and is mainly distributed in southern parts of People's Republic of China (Wu et al., 2014). Due to the sweet taste of its leaves, it is often used as herbal tea (Kennelly et al., 1995). In addition it can act as a dietary supplement for trace elements, as well as a good food resource for maritime people. Furthermore, as an essential ingredient of Tujia ethnomedicine in People's Republic of China, the leaves of *C. paliurus* are often used to treat diabetes mellitus (Zhang et al., 2010; Wang et al., 2013). It has been reported that *C. paliurus* ethanol extract bared antihyperglycemic and ameliorated insulin resistance bioactivities (Ma et al., 2015). The literature review revealed that sequiterpenoids, *Seco*-dammarane triterpenoids and phenolic compounds have previously been isolated from *C. paliurus* (Shu et al., 1995; Li et al., 2011; Zhu et al., 2015).

Previous studies have revealed the cytotoxic capability of the ethanol extract of the leaves of *C. paliurus* (Xie et al., 2013). The present study is focused on the isolation of pure chemical constituents of *C. paliurus* and their cytotoxicity evaluation. During this project, eight previously undescribed *seco*-dammarane triterpenoids cyclocariol A-H (**1**–**8**), and seven known compounds (**9**–**15**) were isolated and characterized. Structures of the isolated compounds were elucidated using different spectroscopic techniques such as IR, UV, Mass, NMR (^{1}H, ^{13}C, 2D) and X-Ray crystallography. All compounds were evaluated for their cytotoxicities against human liver (SMMC-7721) and breast cancer (BT-549) cell lines, while **1**–**2**, **5**, and **8** were also tested against human colon tumor (HCT-116) cell lines (see Fig. 1).

2. Results and discussion

Cyclocariol A (**1**) was isolated as colorless crystals with $[\alpha]_D^{20}$ + 39.3 (*c* 0.21, MeOH). The molecular formula $C_{31}H_{52}O_5$ was determined by $[2M + H]^+$ ion at *m/z* 1009.7726 (calcd. 1009.7708)

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in HRESI-MS and ^{13}C NMR data. IR spectrum showed characteristic absorption peaks for hydroxyl (3233 cm^{-1}) and carbonyl (1740 cm^{-1}) groups. Analysis of the ^{1}H, ^{13}C (Table 1) and 2D-NMR spectroscopic data of **1** revealed high similarity to the aglycone moiety of cyclocarioside I (Cui and Li, 2015), suggesting that **1** contains the unusual *seco*-dammarane triterpenoid skeleton. The downfield chemical shifts of C-12, C-20, and C-24 at δ 70.3, 72.9, and 75.8, respectively, suggested the presence of OH groups at C-12, C-20, and C-24. Analysis of the ^{1}H-^{1}H COSY (Fig. 2a) spectrum of **1** suggested the presence of four fragments (H-1/H-2, H-5/H-6/H-7, H-9/H-11/H-12/H-13/H-17/H-16/H-15, H-22/H-23/H-24). These fragments were joined together to get a *seco*-dammarane triterpenoid skeleton for **1** based on the HMBCs (Fig. 2a). The position of methoxyl group was confirmed by the HMBC correlations between the protons at δ 3.62 (3-OCH_3) and the carbonyl carbon at δ 174.0 (C-3). The ROESY correlations (Fig. 2a) of H-12 with H-17 (α-orientation) suggested α-orientation for H-12, hence OH took β-orientation. Similarly ROESY correlations of H-21 with H-17, suggested α-orientation for H-21 and β-orientation for the OH group (Othman et al., 2016). The absolute configurations at different stereocenters were confirmed by X-ray crystallographic data (Table S1, Supporting Information). The absolute configuration at C-20 was found to be "*S*", while "*R*" configurations were confirmed at C-12 and C-24 by X-ray diffraction analysis (Fig. 2b). Therefore, the structure of **1** was assigned as 12*R*,20*S*,24*R*-trihydroxy-3,4-*seco*-dammara-4 (28),25-dien, 3-oic acid methyl ester.

Cyclocariol B (**2**) was obtained as colorless amorphous solid with specific rotation $[\alpha]_D^{20}$ +37.2 (*c* 0.23, MeOH). Its molecular formula $C_{31}H_{52}O_5$ was determined by $[2M + H]^+$ ion in HRESI-MS at *m/z* 1009.7709 (calcd. 1009.7708) and ^{13}C-NMR data. Comparison of the ^{1}H, ^{13}C (Table 1) and 2D-NMR spectra of **2** showed high similarity with those of **1**. The only difference observed was the slight variations of the chemical shifts of C-23 ($\Delta\delta_C$ +0.2), C-24 ($\Delta\delta_C$ +0.4), C-25 ($\Delta\delta_C$ −0.1), C-26 ($\Delta\delta_C$ +0.3), and C-27 ($\Delta\delta_C$ −0.3), due to the different configuration at C-24. Thus **2** was found to be a C-24 epimer of **1** according to spectroscopic data together with HPLC retention time results (Fig. S10, Supporting Information). These data led to the assignment of **2** as 12*R*,20*S*,24*S*-trihydroxy-3,4-*seco*-dammara-4 (28),25-dien, 3-oicacid methyl ester.

Cyclocariol C (**3**) was obtained as a colorless amorphous powder with $[\alpha]_D^{20}$ + 30.0 (*c* 0.20, MeOH). The molecular formula of $C_{30}H_{50}O_5$ was determined by $[2M + H]^+$ ion in HRESI-MS at *m/z* 981.7382 (calcd. 981.7395). ^{1}H, ^{13}C (Table 1) and 2D-NMR data of **3** was slightly different from that of **1**. Signals for methoxyl protons were not found in ^{1}H and ^{13}C-NMR spectra, and chemical shift for C-3 carbonyl carbon was observed at δ 176.2. This result suggested the presence of an acid group instead of ester at C-3, which was also supported by the molecular formula $C_{30}H_{50}O_5$. The Mosher's

Table 1
^{1}H and ^{13}C-NMR Spectroscopic Data of **1**–**4** (in pyridine-d_5, *J* in Hz at 500 MHz).

No.	1		2		3		4	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.74, m	34.8	1.74,m	34.9	1.91, m	35.3	1.91, m	35.3
2a	2.46, m	28.4	2.47, m	28.5	2.63, m	29.1	2.63, m	29.1
2b	2.30, m		2.30, m		2.48, m		2.49, m	
3		174.0		174.0		176.2		176.2
4		147.5		147.5		147.6		147.6
5	2.04, dd (12.5, 2.9)	50.3	2.04, dd (12.5, 2.9)	50.3	2.11, m	50.3	2.13, m	50.3
6a	1.79, m	24.7	1.79, m	24.8	1.80, m	24.8	1.80, m	24.8
6b	1.35, m		1.35, m		1.35, m		1.36, m	
7a	1.48, m	33.4	1.48, m	33.5	1.49, m	33.5	1.49, m	33.5
7b	1.16, dt (13.0, 3.0)		1.16, dt (13.0, 3.0)		1.15, dt (13.0, 3.0)		1.16, dt (13.0, 3.0)	
8		39.4		39.4		39.4		39.4
9	1.69, m	40.6	1.69, m	40.6	1.80, m	40.6	1.81, m	40.6
10		39.1		39.1		39.1		39.1
11a	2.01, m	32.4	2.01, m	32.4	2.16, m	32.5	2.16, m	32.5
11b	1.55, m		1.55, m		1.60, m		1.60, m	
12	3.88, td (10.4, 5.4)	70.3	3.88, td (10.3, 5.0)	70.3	3.92, td (10.4, 5.0)	70.4	3.92, td (10.4, 5.0)	70.4
13	2.09, m	48.3	2.09, m	48.4	2.12, m	48.3	2.11, m	48.3
14		52.0		52.0		52.0		52.0
15a	1.53, m	31.2	1.54, m	31.3	1.53, m	31.2	1.55, m	31.2
15b	1.02, m		1.03, m		1.01, m		1.03, m	
16a	1.90, m	26.7	1.90, m	26.8	1.88, m	26.7	1.89, m	26.7
16b	1.41, m		1.41, m		1.40, m		1.43, m	
17	2.36, m	54.6	2.37, m	54.4	2.35, m	54.6	2.35, m	54.4
18	1.00, s	15.4	1.01, s	15.4	1.01, s	15.4	1.01, s	15.4
19	0.82, s	20.1	0.82, s	20.2	0.85, s	20.2	0.85, s	20.2
20		72.9		72.8		72.8		72.7
21	1.43, s	27.2	1.43, s	27.2	1.43, s	27.1	1.43, s	27.2
22a	2.11, m	32.0	2.31, m	32.4	2.11, m	32.1	2.32, m	32.4
22b			1.78, m				1.78, m	
23a	2.30, m	30.4	2.31, m	30.6	2.33, m	30.4	2.31, m	30.6
23b	2.00, m		1.98, m		2.03, m		1.99, m	
24	4.40, dd (7.6, 4.1)	75.8	4.41, t (5.5)	76.2	4.42, dd (8.0, 4.2)	75.8	4.42, dd (7.2, 4.8)	76.2
25		149.9		149.8		150.0		149.8
26a	5.25, brs	109.6	5.25, brs	109.9	5.26, brs	109.6	5.25, brs	109.8
26b	4.93, brs		4.94, brs		4.93, brs		4.94, brs	
27	1.89, s	18.3	1.93, s	18.0	1.89, s	18.2	1.93, s	18.0
28a	4.93, brs	113.7	4.93, brs	113.7	4.94, brs	113.9	4.94, brs	113.6
28b	4.79, brs		4.79, brs		4.86, brs		4.86, brs	
29	1.74, s	23.2	1.74, s	23.2	1.77, s	23.4	1.77, s	23.4
30	0.94, s	16.8	0.94, s	16.8	0.94, s	16.8	0.94, s	16.8
OCH_3	3.62, s	51.2	3.62, s	51.3				