Ptercresions A-C: three new terpenes with hepatoprotective activity from *Pteris cretica* L.

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ABSTRACT

Three new terpenes were isolated from *Pteris cretica* L., namely ptercresions A (1), B (2) and C (3), together with two known terpenes and one terpene glycoside respectively identified as callisalignene D (4), berberiside A (5), creoside I (6). The structures of isolates were determined by HR-ESI-MS, ECD, NMR and acid hydrolysate analysis. The *in vitro* hepatoprotective activities of compounds 1-6 were studied against paracetamol-induced L-02 hepatocyte injury. The results revealed that compounds 2-4 showed moderate anti hepatocyte injury activity.

ARTICLE HISTORY

Received 17 October 2021
Accepted 30 December 2021

KEYWORDS

*Pteris cretica* L.; ptercresions A-C; terpenes; hepatoprotective; pteridaceae

1. Introduction

Drug induced liver injury (DILI) refers to liver injury or aggravation of original liver disease after drug administration, which is second only to viral hepatitis and fatty liver disease (Tang and Cheng 2019). A recent epidemiological survey in China shows that 26.81% of drug-induced liver injury is caused by Chinese herbal medicine, chemical drugs and dietary supplements (Cong et al. 2019). The development of drugs or lead compounds for the treatment of liver injury is particularly important.

*Pteris cretica* L., a perennial evergreen herb, belongs to the genus *Pteris* (Pteridaceae) (Hou et al. 2019). Previous pharmacology investigations of plants from this genus led to the isolation of sesquiterpenes, diterpenes and flavonoids from *Pteris* L. (Luo et al. 2016; Ge et al. 2008; Gong et al. 2007), which exhibit various...
activities, including anti-liver injury (Kaur et al. 2017) and anti-cancer activities (Yang et al. 2011).

To explore significant leading compounds for the treatment of DILI, the phytochemical investigation on *Pteris cretica* L. led to isolation of three new terpenes (Figure 1), and named ptercresion A (1), B (2) and C (3) which were identified by HR-ESI-MS, CD, NMR and acid hydrolysate analysis together with three known compounds callisalignene D (4) (Qin et al. 2017), berberiside A (5) (Dong et al. 2020), creosidel(6) (Nakamura et al. 2008). At the same time, the hepatoprotective activity against paracetamol-induced L-02 cells *in vitro* of compounds 1-6 were studied.

2. Results and discussion

2.1. Structural elucidation of compounds

Compound 1 was obtained as a yellow oil. Its molecular formula was determined as C₁₄H₂₇O₇ based on the HR-ESI-MS data (m/z: 327.1429 [M + Na]+, calcd. 327.1414), indicating 3 degrees of unsaturation in the structure.

The ¹H-NMR spectrum showed one double bond proton δₕ: 5.36 (1H, t, J = 7.2 Hz, H-5). The ¹H-NMR spectrum also displayed a methyleneoxl signal at δₕ: 4.08 (1H, d, J = 11.8 Hz, H-7a), 3.84 (1H, d, J = 11.8 Hz, H-7b), a β-D-glucopyranosyl moiety δₕ: 4.08(1H, d, J = 7.8 Hz, H-1'), 3.62 (1H, dd, J = 11.5, 5.6 Hz, H-6'), 3.39 (1H, dd, J = 11.5, 5.6 Hz, H-6'), 3.08 (2H, m, H-3', 5'), 3.00 (1H, m, H-4'), 2.93 (1H, m, H-2'). At the same time, two methyl groups δₕ: 2.05 (3H, s, H-1), 1.58 (3H, s, H-8) were observed.
The $^{13}$C-NMR, DEPT 135/90 and HSQC spectra showed one carbonyl group ($\delta_C$: 207.9 (C-2)), a double bond ($\delta_C$: 132.3 (C-6), 126.1 (C-5), one oxymethylene ($\delta_C$: 73.4 (C-7)). Meanwhile, a glucopyranosyl moiety ($\delta_C$: 101.6 (C-1'), 76.8 (C-5', C-3'), 73.4 (C-2'), 70.1 (C-4'), 61.1 (C-6')) and two groups of methyl signals ($\delta_C$: 29.7 (C-1), 13.8 (C-8)) were observed. The spectroscopic data of compound 1 were similar to creosideI. Therefore, we speculate that compound 1 and 7 are cis-trans isomers. The structure of compound 1 further confirmed by the $^1$H-$^1$H COSY and HMBC spectra (Figure S24).

The HMBC spectrum showed the cross-peaks from $\delta_H$: 4.08 (H-7a)/3.84 (H-7b) to $\delta_C$: 126.1 (C-5)/101.6 (C-1'), which indicated the bond of glucose at C-7. In the NOESY spectrum, the NOE correlations between H-5/H-7 and H-4/H-8 confirmed that the planar structure of the compound is trans, which is different from that of compound 6. Meanwhile, analysis of the acid hydrolysate (Figure S25) of 1 and the standard monosaccharides using HPLC method together with the NMR data ($J_{1/2'}$ = 7.8 Hz) proved the existences of $\beta$-D-glucopyranosyl. Then compound 1 was unequivocally established and named ptercresion A.

Compound 2 was isolated as an orangey oil. Its molecular formula C25H38O3 was deduced from the analysis of its HR-ESI-MS showing the pseudo molecular ion peak [M+H]$^+$ at m/z 387.2891 (calcd. 387.2899 for C25H39O3$^+$). The $^1$H and $^{13}$C-NMR spectrum of compound 2 showed two carbonyl groups [$\delta_C$: 213.8 (C-3), 198.8 (C-5)], a two-membered substituted double bond system [$\delta_H$: 5.93 (1H, dd, $J$ = 9.9, 4.4 Hz, H-3'), 5.71 (1H, dd, $J$ = 9.9, 1.9 Hz, H-2'); $\delta_C$: 134.8 (C-3'), 131.0 (C-4')] and a four-membered substituted double bond system [$\delta_C$: 169.0 (C-1), 110.0 (C-6)], nine methyl groups [$\delta_H$: 1.38 (3H, s, H-13), 1.36 (3H, s, H-15), 1.33 (3H, s, H-14), 1.30 (3H, s, H-12), 1.25 (3H, s, H-7'), 0.98 (3H, t, $J$ = 7.4 Hz, H-11), 0.97 (6H, d, $J$ = 6.7 Hz, H-9', 10'), 0.89 (3H, d, $J$ = 7.3 Hz, H-9); $\delta_C$: 26.8 (C-15), 25.8(C-12), 25.1(C-7'), 24.1(C-13), 23.0(C-14), 21.7(C-9'), 21.0(C-10'), 17.7(C-9), 13.0 (C-11)]. Comparing the above data with callisalignene D, it is proved that they have the same planar structure (Qin et al. 2017), and the structure of compound 2 was further confirmed by the $^1$H-$^1$H COSY, HMBC (Figure S24).

Further analysis showed that compound 2 has a different stereoconfiguration with callisalignene D. The ROESY correlations between H-7/H-7', H-6'/H-7', H-7/H-4' confirmed these protons were placed at the same side. To determine the absolute configuration of 2, the experimental ECD spectrum was compared with the calculated ECD spectrum by the TD-DFT method as shown in Figure S16. The assignment of the 8 S configuration could be explained in view of its proposed biosynthetic pathway (Qin et al. 2017), at the same time, the analysis of $J_{7/8}$ (5.6 Hz) can also determine that 7 and 8 are cis configuration. Therefore, the structure of compound 2 was completely confirmed and named ptercresion B.

Compound 3 was isolated as a yellowish oil substance, Its HREIMS exhibited a pseudomolecular ion peak [M+Na]$^+$ at m/z 261.1822 (calcd. for C_{15}H_{26}NaO_2$: 261.1830).

The $^1$H and $^{13}$C-NMR data (Table S1) analysis showed the presence of fifteen carbon signals suggesting that compound 3 might be a sesquiterpene (Hegazy et al. 2014). Based on the DEPT spectra they were assigned to four olefinic, and three quaternary carbons (one olefinic, one oxygen-bearing, and one free keto group), respectively. The $^1$H-NMR [$\delta_H$: 2.13 (1H, m, H-11), 0.92 (6H, d, $J$ = 6.6 Hz, H-12, 13)], $^1$H-$^1$H
COSY [δ_H: 2.13 (H-11) ↔ 0.92 (H-12/13)] and HMBC [δ_H: 2.13 (1H, m, H-11) → δ_C: 200.3 (C-9)] showed an isopropyl group linked with carbonyl. Two methyl groups appeared at δ_H: 2.11 (3H, s, H-14) with δ_C: 18.2 (C-14) and 1.28 (3H, s, H-15) with δ_C: 26.91 (C-15) and a quaternary oxygenated carbon at δ_C: 72.1 (C-3), respectively.

Further analysis of the ¹H and ¹³C-NMR spectra showed that compound 3 had a similar structure to chiladienol B. However, one singlet signal established the presence of olefin proton [δ_H: 6.03 (1H, s, H-8)], and is different from chiladienol B which has one triplet signal [δ_H: 5.24 (br t, J = 7.5 Hz)] (Hegazy et al. 2014). It is suggested that the main difference between compound 3 and chiladienol B is the position of double bond. The ¹H-¹H COSY which established the presence of 1, 3-disubstituted propyl [δ_H: 1.49 (H-6) ↔ 2.12 (H-5) ↔ 1.52 (H-4)] and HMBC (Figure S24) which showed the correlations between δ_H: 6.03 (H-8) and δ_C: 200.3 (C-9), 52.5 (C-10) determined the presence of double bond at C7-C8. The ROESY spectrum showed the cross-peaks from H-8 to H-14, which confirmed the double bond (C7 = C8) is cis configuration.

The above chemical data confirmed the structure of 3 as a previously undescribed compound, 3-hydroxy-3,7,11-trimethyl-1,7-dodecadien-9-one, named ptercresion C.

### 2.2. Bioactivity

The in vitro hepatoprotective activities of the purified compounds were evaluated on paracetamol-induced L-02 cells. The results revealed that compounds 2-4 exhibited anti hepatocyte injury effects on paracetamol-induced L-02 cells (Table S2) with EC₅₀ value from 107.18 ± 0.35 to 213 ± 1.15 μM. The increased ALT and AST levels revealed that compounds 2-4 showed anti hepatocyte injury activities (Table S3).

### 3. Experimental

#### 3.1. General

HR-ESI-MS were performed on a Xevo G2-S Q-TOF MS (Waters MS Technologies, Manchester, UK). NMR spectra were recorded on an Avance 400 (400 MHz for ¹H-NMR, 100 MHz for ¹³C-NMR, 298 K) spectrometer (Bruker, Bremen, Germany) in CDCl₃, or DMSO-d₆ with TMS as internal standard. UV spectra were recorded with a Shimadzu UV-1750 spectrometer. IR spectra were recorded on an Avatar 360 E.S.P spectrophotometer (Thermo Nicolet Co., USA) with a KBr disk. Column chromatography (CC) was carried out on silica gel (100-200 mesh or 200-300 mesh, Qingdao Marine Chemical Ltd., China), Sephadex LH-20 (GE Healthcare, USA), YMC 50 μm ODS-B (Milford, USA). HPLC isolation (Shimadzu LC-6AD, Shimadzu Crop., Japan) was conducted on an ODS silica column (YMC-Pack ODS-A, 5 μm, 10 × 250 mm). Cancer cell lines were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China).

#### 3.2. Plant material

The whole herb of Pteris cretica L. were collected in May, 2020 from Guiyang, Guizhou, People’s Republic of China (106° 47 e, 26° 35 n, 1017 m above sea level), which were
identified by plant character comparison and microscopic identification by doctor Zhi-Qiang Lu at Southern University of Science and Technology. The voucher specimen (No. 20200521013) was deposited at the Herbarium of College of Science, Southern University of Science and Technology, Shenzhen, P. R. China.

### 3.3. Extraction and isolation

The powdered and dry whole plant of *P. cretica* L. (1.0 kg) was extracted three times (24 h each time) with 95% ethanol at room temperature. After removal of the solvents in vacuo to give a brown gum (150.0 g), then the brown gum was then suspended in H$_2$O and extracted with dichloromethane.

The dichloromethane extract (70.0 g) was chromatographed directly on a silica gel column chromatography (CC) eluted with Petroleum ether-Ethyl acetate (100:1 to 80:20) to afford 7 fractions (Fr. A-G). Fr. B (5.2 g) was fractionated by Sephadex LH-20 gel chromatography which was separated with Petroleum ether-CH$_2$Cl$_2$-MeOH (50: 40: 10) as eluent to give 3 Sub-fractions (Fr. B.1-3). Fr. B.2 (1.1 g) was further purified on YMC ODS-B silica gel CC eluted with a gradient of MeOH-H$_2$O (70: 30 to 0: 100) to get 2 sub-fractions (Fr. B.3.1-2). Then Fr. B.3.1 (329.1 mg) was separated by a semi-preparative HPLC (MeOH: H$_2$O 82: 18, 2.5 mL/min, $\lambda = 210$, 254 nm) to yield compound 1 (1.7 mg, $t_R=24$ min), 3 (1.9 mg, $t_R=27$ min) and 6 (1.5 mg, $t_R=33$ min). Fr. C (4.3 g) was fractionated by Sephadex LH-20 gel chromatography (CHCl$_3$: MeOH 1: 3) to get 5 Sub-fractions (Fr. C.1-5), Fr. C-4 (350.9 mg) was fractionated by silica gel CC (Petroleum ether: Ethyl ether 100:0-50:50) to give compounds 5 (4.7 mg) and 2 Sub-fractions (Fr. C.4.1-2), Fr. C.4.2 (78.2 mg) was separated by a semi-preparative HPLC (MeOH: H$_2$O 92: 8, 3 mL/min, $\lambda = 210$, 254 nm) to yield compound 2 (5.8 mg, $t_R=20$ min) and 4 (10.7 mg, $t_R=25$ min).

**Ptercresion A:** yellow oil. HR-ESI-MS $m/z$: 327.1429 [M + Na]$^+$, calcd. C$_{14}$H$_{24}$NaO$_7$ 327.1414; $[\alpha]_{25}$D = + 53.5 (c 0.20 CHCl$_3$); $^1$H-NMR (DMSO-$d_6$, 400 MHz) and $^{13}$C-NMR (DMSO-$d_6$, 100 MHz) data see in Table S1.

**Ptercresion B:** orangey oil. HR-ESI-MS at $m/z$ 387.2891 [M + H]$^+$ calcd. for C$_{25}$H$_{39}$O$_3$ 387.2899; $^1$H-NMR (CDCl$_3$, 400 MHz) and $^{13}$C-NMR (CDCl$_3$, 100 MHz) data see in Table S1. CD data see in Figure S16.

**Ptercresion C:** yellowish oil. HR-ESI-MS at $m/z$ 261.1822 [M + Na]$^+$ calcd. for C$_{15}$H$_{26}$NaO$_2$ 261.1830; $^1$H-NMR (CDCl$_3$, 400 MHz) and $^{13}$C-NMR (CDCl$_3$, 100 MHz) data see in Table S1.

### 3.4. Acid hydrolysis

The procedure of acid hydrolysis was based on the literature method (Tanaka et al. 2007). Briefly, 1 (3 mg) were hydrolyzed in 2 mol/L HCl (10 mL) at 90°C for 5 h and then extracted with chloroform. The hydrotropic fraction was freeze-dried and dissolved in pyridine (1 mL) with L-cysteine methyl ester hydrochloride (3 mg). After heating at 60°C for 1 h, the solution was added o-torylisothiocyanate (3 mL) and blended in the condition of 60°C for 1 h. The mixture solution was analyzed by reversed-phase HPLC (YMC-Pack ODS-A (250 × 10 mm, 5 micron), the flow rate of 1 mL/min,
temperature 35°C, acetonitrile-0.05% formic acid water solution (25:75) column with the 250 nm wavelength. The peak was coincided with derivatives of D-glucopyranosyl at 11.75 min.

3.5. Hepatoprotective activity

The hepatoprotective effect of isolated 6 compounds against paracetamol-induced L-02 cells were determined by cell viability and the levels of AST and ALT. Cell viabilities were evaluated by MTT method. Log phase L-02 cells of \(8 \times 10^3\) cells/100 l/well were seeded in 96-well plates and control group, model group and 6 compounds groups (0, 20, 40, 80 and 160 lM) were set with 5 duplicate wells in each group. After incubation in CO\(_2\) incubator (37°C, 5% CO\(_2\)) for 24 h, the medium was removed and 100 l cell culture medium with 10 mM LPS was added into the wells to further incubated for 24 h. Then compounds and silymarin were added into the plates with incubation for another 24 h. Then, 10 l MTT (5 mg/ml) was added to each well for another 4 h. After removal of the supernatant, 100 lL of DMSO was added to dissolve the formazan crystals and the optical density (OD) were measured at a wavelength of 490 nm with multifunctional microplate reader. The inhibition rates of compounds were calculated as the follow formula, and the EC\(_{50}\) values were calculated from calibration curves according to the 5 inhibition rates.

In the pre-experiment, we studied the cytotoxicity of all compounds and found that there was no obvious cytotoxicity for L-02 cells when the concentration was below 320 lM. Then the aspartate transaminase (AST) and alanine transaminase (ALT) activities were measured by ELISA method. Cell viability experiments showed that there is no cell viability at concentrations lower than 40 lM, so three concentrations in the above groups were selected and set low, medium and high groups (40, 80, 160 lM). The cell supernatants were collected form the above experiment according to the instructions of the AST and ALT test kits. The results were expressed as “mean ± SD.” One-way analysis of variance (ANOVA) was used for significance analysis and multiple comparisons. \(P < 0.05\) was 5% significant level, and \(P < 0.01\) was 1% significant level.

4. Conclusions

The phytochemical investigations had led to isolation of one terpenes glycoside ptercresion A (1) and a monoterpene heterozygote ptercresions B (2), a sesquiterpene derivative ptercresions C (3), respectively, together with three known compounds callisalignene D (4), berberiside A (5), creosideI(6). Meanwhile, compounds 2–4 exhibited hepatoprotective activity against paracetamol-induced L-02 cells in vitro and the levels of AST or ALT significantly reduced at high dose groups. These compounds may serve as potential lead compounds for the treatment of drug induced liver injury.

Disclosure statement

No potential conflict of interest was reported by the authors.
Funding
The author(s) reported there is no funding associated with the work featured in this article.

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