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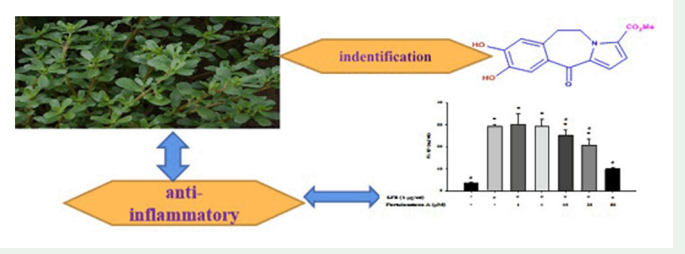
A novel alkaloid from *Portulaca oleracea* L. and its anti-inflammatory activity

Yingying Gu[a], Aijing Leng[b], Wenjie Zhang[a], Xixiang Ying[a] and Didier Stien[c]

[a]School of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian, P.R. China;
[b]Department of Pharmacy, First Affiliated Hospital of Dalian Medical University, Dalian, P.R. China;
[c]CNRS, Laboratoire de Biodiversité et Biotechnologie Microbienne, Observatoire Océanologique, Banyuls-sur-mer, France

ABSTRACT

A novel alkaloid identified as methyl 8,9-dihydroxy-11-oxo-6,11-dihydro-5H-benzo[d]pyrrolo[1,2-a]azepine-3-carboxylate, named portulacatone A (**1**) with six known compounds Oleracein E (**2**), 6,7-dihdroxy-3,4-dihydro-2H-isoquinolin-1-one (**3**), *N-trans-p*-coumaroytyramine (**4**), 9H-carbazole (**5**), isoaspergin (**6**) and flavoglaucin (**7**) were obtained from *Portulaca oleracea* L., while compounds (**5–7**) were isolated from the plant for the first time. The new structure was identified by using UHPLC-ESI-Q-TOF/MS, 1D, 2D NMR and the others were proved by ^{1}H-NMR and ^{13}C NMR that comparing with previous reports. It was suggested that the portulacatone A (**1**) can significantly inhibit the inflammatory factor, interleukin-1β (IL-1β) in the RAW 264.7 cells induced by LPS.



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1. Introduction

Portulaca oleracea L. is an annual herbaceous plant with reddish stems and alternate leaves from family Portulacaceae that distributed in many parts of the world and specifically the tropical and subtropical areas (Zhou et al. 2015). It is called khorfeh in Persian language and grows in warm climates of Iran with a fleshy stem and succulent leaves, yellow or white small flowers and small black seeds (Changizi-Ashtiyani et al.

CONTACT Xixiang Ying yingxixiang@163.com; Didier Stien didier.stien@cnrs.fr
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2013). As a traditional Chinese medicine, it has been used for the treatment of dysentery with bloody stools, externally for boils and sores, eczema, erysipelas, and insect, snake bites (Xu et al. 2006). *P. oleracea* were listed in the World Health Organization as one of the most used medicinal plants and it has been given the term 'Global Panacea' (Lim and Quah 2007) owing to flavonoid (Yang et al. 2018), lignans (Ma et al. 2018), fatty acids (Liu et al. 2000) terpenes (Elkhayat et al. 2008), alkaloid (Zhao et al. 2017, 2019; Xu et al. 2020), etc. In recent years, our laboratory found that many alkaloids presented significantly anti-inflammatory bioactivities such as oleracone (Meng et al. 2016) and oleracimine (Li et al. 2016) . In this study, seven compounds were isolated from *P. oleracea*, including a new alkaloid, portulacatone A (**1**) and six known compounds **2–7**. And portulacatone A (**1**) can significantly inhibited the inflammatory factor of interleukin-1β (IL-1β) via the RAW 264.7 macrophage cells model stimulated by lipopolysaccharide (LPS).

2. Results and discussion

Compound **1** was obtained as yellowish-brown powder that sprayed with Dragendor reagent it turned orange. Compound **1** has the molecular formula $C_{15}H_{13}NO_5$, as deduced from the UHPLC-ESI-Q-TOF/MS protonated molecular ion at *m/z* 288.0860 $[M+H]^+$ (calcd. for $C_{15}H_{14}NO_5^+$ 288.0866), requiring 10 degrees of unsaturation (Figure 1). The examination of the proton, ^{13}C and HSQC spectra demonstrated the presence of 1 methyl, 2 aliphatic methylenes and 4 aromatic or olefinic methines. The ^{13}C and HMBC spectra showed that compound **1** also had 8 non-protonated carbons.

Examination of the HMBC spectrum demonstrated that protons H-7 (δ_H 6.64) and H-10 (δ_H 7.46) belonged to the same 1,2,4,5-tetrasubstituted aromatic ring. As a matter of fact, H-7 correlated with C-9 and C-10a while H-10 correlated with C-6a, C-8 but also C-9 (Figure S1). The chemical shifts of C-8 (δ_C 151.2) and C-9 (δ_C 144.5) correspond to those of carbons linked to a hydroxyl group. The presence of exchangeable protons next to H-7 and H-10 was further confirmed by the ROE correlation of these two protons with water (δ_H 3.32). On the other side of the aromatic ring, the additional HMBC correlations H-7/C-5 and H-10/C-11 allowed to link the C-5–C-6 ethylene moiety to C-6a and a carbonyl group to C-10a. Further confirmation was obtained from the HMBC correlation H-6/C-7 and from the ROE correlation H-6/H-7. The proton and carbon chemical shifts in position 5 (δ_H 4.78, δ_C 46.7) indicated that carbon 5 should be connected to a nitrogen.

On the other side of the molecule, the H-1/H-2 chemical shifts and coupling constant (4.3 Hz) were typical of two protons in positions β and β′ of a pyrrole subunit. The α and α′ quaternary carbons were assigned based on the HMBC correlations of both H-1 and H-2 with C-3 and C-11a. A methyl ester was identified in compound **1** thanks to the correlation of a methyl group at δ_H 3.80/δ_C 51.7 with a carbonyl a δ_C 160.9. This methoxycarbonyl group was linked to C-3 based on the ROE correlation of H-2 with the methyl group. Although the C-11–C-11a bond was not supported by any correlation, no other solution would meet the molecular formula as all atoms had been placed at this stage. Therefore, compound **1** was identified as the methyl 8,9-dihydroxy-11-oxo-6,11-dihydro-5H-benzo[d]pyrrolo[1,2-a]azepine-3-carboxylateand named portulacatone A.

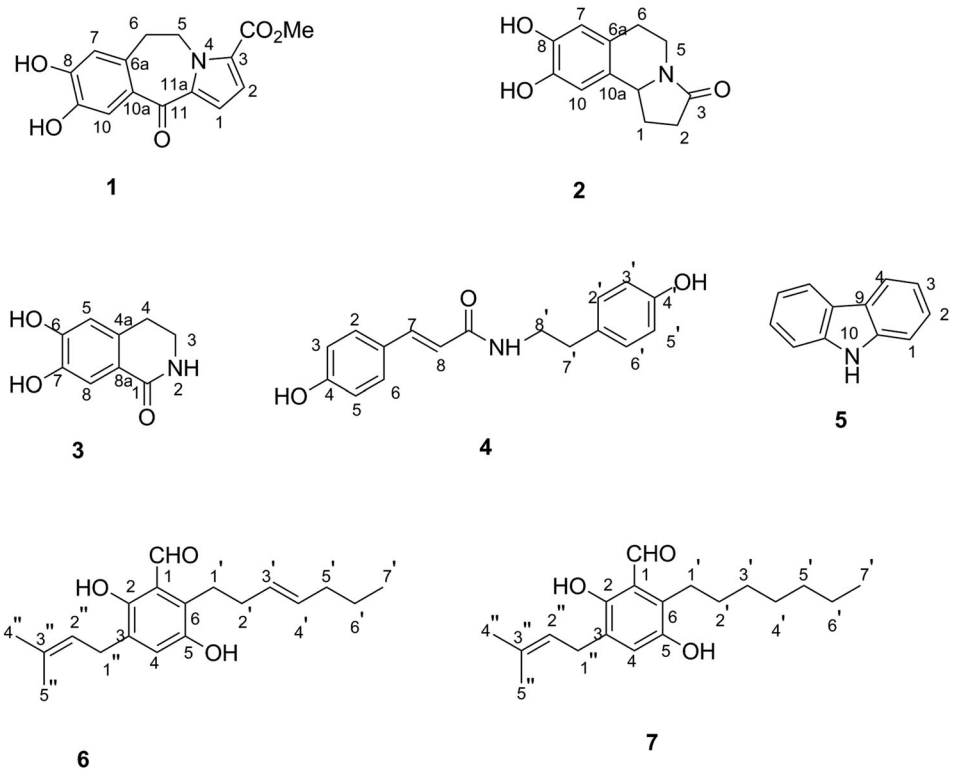

Figure 1. Structure of compounds **1**–**7**.

According to the NMR data shown and some literature reported, compounds **2–7** had been identified respectively as Oleracein E (**2**) (Xiang et al. 2005), 6,7-dihdroxy-3,4-dihydro-2H-isoquinolin-1-one (**3**) (Schimming et al. 2000), *N-trans-p*-coumaroytyramine (**4**) (Wu et al. 1995), 9H-carbazole (**5**) (Xu et al. 1980), isoaspergin (**6**) (Huang et al. 2012) and flavoglaucin (**7**) (Miyake et al. 2009). We drew a conclusion according to the MTT assay (Figure S20) that portulacatone A shown the cytotoxicity at the concentration of 50 μM, then the concentrations of 1 to 50 μM were detected for the inflammatory factor of IL-1β. Portulacatone A can significantly inhibit IL-1β at 50 μM (Figure S21).

3. Experimental

3.1. Main instruments and chemicals

The NMR spectra were obtained by an AVANCE 600 MHz instrument while the compounds were dissolved in DMSO-d 6 (Bruker Corporation, Switzerland). Relative molecular mass was recorded using a 6520 quadrupole-time-of-flight mass spectrometer (Agilent, Palo Alto, CA). The column chromatography (CC) through the whole separation progress including silica gel (100–220 and 200–300 mesh, Qingdao Marine Chemical Co., Qingdao, China), polyamide resin (80–100 mesh, Taizhou Luqiao Sijia Biochemical Plastic Factory, Taizhou, Zhejiang, China) and ODS (20–40 μm, GE

Healthcare, Marlborough, MA). The TLC was carried out by silica gel GF 254 (Qingdao Marine Chemical Co., Qingdao, China). RAW 264.7 cells (TIB-71) were collected from American Type Culture Collection (ATCC, Manassas, VA, USA). Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin solution and fetal bovine serum (FBS) purchased from Hyclone (Logan, UT, USA). LPS (Escherichia coli strain 0111:B4). IL-1β ELISA kit was from Quanzhou jiubang Biotechnology Co., Ltd (Fujian, China). All other chemicals were provided by Sinopharm (Shanghai, China) Chemical Reagent Co., Ltd.

3.2. Plant materials

The whole herbs of *P. oleracea* were harvested in Shijiazhuang (Hebei, China) in June 2017, and identified by Prof. Xixiang Ying. The specimens (No. 20171001) were stored at Liaoning University of TCM.

3.3. Isolation and identification

The dried parts of *P. oleracea* 250 kg were extracted and enriched, the part (116 g) was dealed with on the basis of previous research (Yang et al. 2019) in our laboratory has been reported that ODS column chromatography eluted with methanol (60%, 70%, 80%, 90%, 100%, respectively) with a medium pressure. Five fractions (A1–A5) were acquired and A3 purified on a Sephadex LH-20 column (100 g, φ2 × 150cm) with methanol solvent for getting fractions B1-B7, then B4 was purified by ultra-high performance liquid chromatography (UHPLC), with the flow rate of 1 mL/min that the whole processes carried out at a temperature of 40 °C. Acquired compound **1** (2 mg, purity of >99% with UHPLC, t_R 8.860 min, MeOH-0.1% formic acid, 65:35, v/v), compound **2** (1 mg, purity of >98% with UHPLC, t_R 11.400 min, MeOH-0.1% formic acid, 25:75, v/v), compound **3** (2 mg, purity of >98% with UHPLC, t_R 9.700 min, MeOH-0.1% formic acid 20:80, v/v), compound **4** (2 mg, purity of >99% with UHPLC, t_R 9.606 min, Acetonitrile-0.1% formic acid 22:78, v/v), compound **5** (2 mg, purity of >98% with UHPLC, t_R 18.700 min, MeOH-0.1% formic acid 15:85, v/v), compound **6** (1 mg, purity of >98% with UHPLC, t_R 5.795 min, Acetonitrile-0.1% formic acid 80:20, v/v), compound **7** (2 mg, purity of >98% with UHPLC, t_R 5.479 min, Acetonitrile-0.1% formic acid 85:15, v/v).

3.4. Cell culture, treatment and MTT assay

The macrophage cell line RAW 264.7 was collected in DMEM that was composed by 10% heat inactivated fetal bovine serum and antibiotics (100 U/ml penicillin and 100 ug/ml streptomycin) and maintained at 37 °C in a 5% CO_2 humidified incubator. The cell viability was identified by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Pre-incubating with or without different concentrations (1, 4, 10, 20, 50 μM) of the portulacatone A for 1 hour in the incubator that the cells have been seeded in 96-well plates with initial density of 5×10^4 cells/ml, then treated with 1 μg/ml LPS for 48 h. The media was removed and incubated 5 mg/ml MTT solution in the cells for 4 h at 37 °C. The formazan was dissolved in 150 μL DMSO while the

supernatants were removed, and the absorbance was detected at 570 nm by a microplate reader that the normal group was considered as 100% viable.

Cultivated RAW 264.7 cells according to the above method, and established an inflammation model with LPS (1 ug/ml). In short, after the portulacatone A of different concentrations (1, 4, 10, 20, 50 μM) was added into the 96-well plates and incubated the cells for 6 h, the LPS was added and co-incubated for 12 h. The supernatant was taken for determination according to the IL-1β ELISA kit on the basis of the instruction.

4. Conclusion

A novel alkaloid named portulacatone A and six known compounds isolated and characterized from *P. oleracea,* which significantly inhibit the inflammatory factor IL-1β in RAW 264.7 cells induced by LPS at 50 μM.

Disclosure statement

The authors have no conflicts of interest to disclose.

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