Two novel amide alkaloids from *Portulaca oleracea* L. and their anti-inflammatory activities

Xiujuan Lan\textsuperscript{a*}, Ziyue Ying\textsuperscript{b*}, Shengnan Guo\textsuperscript{a}, Yang Duan\textsuperscript{a}, Xinyu Cui\textsuperscript{a}, Aijing Leng\textsuperscript{c} and Xixiang Ying\textsuperscript{a}

\textsuperscript{a}School of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian, China; \textsuperscript{b}Department of Clinical Laboratory, The Second Affiliated Hospital of Dalian Medical University, Dalian, China; \textsuperscript{c}Department of Pharmacy, First Affiliated Hospital of Dalian Medical University, Dalian, China

**ABSTRACT**

In this article, two novel amide alkaloids were identified as (E)-3-(4-hydroxy-3-methoxyphenyl)-1-(5-hydroxy-6-((3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2\textsubscript{H}-pyran-2-yl)oxy)-1\textsubscript{H}-indol-1-yl)prop-2-en-1-one (1) and (E)-1-(5-hydroxy-6-((3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2\textsubscript{H}-pyran-2-yl)oxy)-1\textsubscript{H}-indol-1-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one (2), the two compounds were named oleraindole E and oleraindole F, respectively. The structures were elucidated using 1D and 2D NMR and HR-ESI-TOF-MS spectra. Additionally, the anti-inflammatory activities were evaluated on RAW264.7 cells induced by LPS, compounds 1 and 2 exhibited anti-inflammatory activities at 20 \textmu M.

**ARTICLE HISTORY**

Received 26 July 2021
Accepted 12 December 2021

**KEYWORDS**

*Portulaca oleracea* L.; amide alkaloids; structure elucidation; anti-inflammatory activity

**CONTACT**

Xixiang Ying \textsuperscript{a} yingxixiang@163.com; Aijing Leng \textsuperscript{c} L18098877517@163.com

\*These authors contributed equally to this work.

Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2021.2021519.
1. Introduction

*Portulaca oleracea* L. is an annual grassy plant belonging to the Portulacaceae family, which is the homology of medicine and food (Tian et al. 2015). It is listed by the World Health Organization (WHO) as one of the most widely used medicinal plants, and it has been given the term ‘Global Panacea’ (Xu et al. 2006). *P. oleracea* is distributed widely in the world and especially the tropical and subtropical areas (Zhou et al. 2015). It has a reputation for ‘vegetable of long life’ in China, and it has been used as traditional Chinese Medicine for thousands of years (Chen et al. 2009; Jin et al. 2013). *P. oleracea* is sour in taste and cold in nature, and it is used to clear heat, resolve toxins, cool the blood and stanch bleeding. The constituents of *P. oleracea* have been isolated, including alkaloids (Xu et al. 2020; Liu et al. 2019), flavonoids (Yan et al. 2012; Duan et al. 2020), organic acids (Uddin et al. 2014), terpenoids (Xin et al. 2008), and so forth. Additionally, modern pharmacology studies showed that *P. oleracea* has many biological activities, such as anti-inflammatory (Lee et al. 2012), antiulcerogenic (Karimi et al. 2004), antimicrobial (Zhu et al. 2006), antioxidant (Karimi et al. 2011; Duan et al. 2020), anticancer (Yan et al. 2012; Zheng et al. 2014), antidiabetic (Lan and Lu 2003; El-Sayed 2011), neuroprotective (Yang et al. 2012; Abdel Moneim 2013) and hepatoprotective (Elkhayat et al. 2008) activity. Particularly in terms of anti-inflammatory and antimicrobial activity, *P. oleracea* is known as ‘natural antibiotic’, which is used for the treatment of carbuncle, dysentery, diarrhoea and hematochezia. In a previous study, alkaloids of the plant exhibited marked anti-inflammatory activities, such as Oleracimine (Li et al. 2016) and Oleracone (Meng et al. 2016). Therefore, it is of significance to further study alkaloids and bioactivities of *P. oleracea*. In this study, two novel amide alkaloids, oleraindole E (1) and oleraindole F (2) (Figure 1) were isolated from *P. oleracea* and their anti-inflammatory activities were further evaluated on RAW264.7 cells stimulated by lipopolysaccharide (LPS).

2. Results and discussion

2.1. Structure elucidation

Compound 1 was obtained as yellow powder and it turned orange when sprayed with Dragendorff’s reagent. Its molecular formula was identified as C_{24}H_{25}NO_{10} based on 13C NMR data and HR-ESI-TOF-MS spectrum. The degrees of unsaturation of compound 1 were 13, and the molecular ion peak was m/z 486.1406 [M – H]⁻ in the HR-ESI-TOF-MS spectrum (calcd. for C_{24}H_{25}NO_{10}, 486.1405) (Figure S17, supplementary material). The data of 1H NMR (600 MHz, methanol-d_4) and 13C NMR (150 MHz, methanol-d_4) are listed in Table S1 (supplementary material). According to the data of 13C NMR (C-3a 128.31, C-7a 131.02, H-3 6.59, d, J = 3.6 Hz) to C-3a (δC 109.73), C-6 (δC 145.57), C-7a, H-7 to C-5 (δC 146.32), C-3a, indicated that there is a
5,6-disubstituted indole ring. Then, the HMBC spectrum showed that H-2" correlate with C-6; therefore the β-D-glucose linked to C-6. Additionally, the chemical shift of C-5 was in the low field, so the hydroxyl group is substituted at C-5. The 1H NMR displayed signals of δH 7.39 (1H, d, J = 15.6 Hz, H-2') and δH 7.89 (1H, d, J = 15.6 Hz, H-3'), and the coupling constant of 15.6 Hz demonstrated that there is no doubt about the existence of a trans double bond. In addition, the HMBC correlations from H-2' to C-1' (δC 166.68), H-3' to C-1' and C-2' (δC 115.06) showed that C-2' linked to C-1'. Furthermore, a correlation from H-2 to H-2' in the ROESY spectrum suggested that C-1' linked to N-1. The peaks of δH 7.41 (1H, d, J = 1.8 Hz, H-2'), δH 6.86 (1H, d, J = 7.8 Hz, H-5"), δH 7.24 (1H, dd, J = 1.8, 8.4 Hz, H-6") were the sign of 1,3,4-trisubstituted benzene ring, and the peaks of δC 56.78/δH 3.95 (3H, s) must be a methoxy group. The HMBC spectrum showed that H-2" correlate with C-4" (δC 151.23), C-6" (δC 125.15), H-5" correlate with C-1" (δC 128.37), C-3" (δC 149.69), H-6" correlate with C-2" (δC 112.33), C-4", and the hydrogen of methoxy group correlate with δC 149.69, therefore, the methoxy group is substituted at C-3" and a hydroxyl group is substituted at C-4". Meanwhile, H-2' correlate with C-1" and H-3' correlate with C-2", C-6", we can know that C-1" linked to C-3' of the trans double bond. Taken together, compound 1 was identified as (E)-3-(4-hydroxy-3-methoxyphenyl)-1-(5-hydroxy-6-(3,4,5-trihydroxy-6-}

---

**Figure 1.** Structures of compounds 1 and 2.
Compound 2 was identified as white powder and it became orange with spraying Dragendorff’s reagent. Its molecular formula was identified as C_{23}H_{23}NO_{9} based on $^{13}$C NMR data and HR-ESI-TOF-MS spectrum. The degrees of unsaturation of compound 2 were 13 and the molecular ion peak was $m/z$ 456.1295 [M$^{+}$/C_{23}H_{23}NO_{9}] in the HR-ESI-TOF-MS spectrum (calcd. for C_{23}H_{23}NO_{9}, 456.1295) (Figure S31, in supplementary material). The 1H NMR spectrum showed two single peaks, they are H-7 ($\delta_H$ 8.34, 1H, s) and H-4 ($\delta_H$ 6.99, 1H, s), and the HMBC spectrum showed that H-3 ($\delta_H$ 6.45, d, $J = 3.6$ Hz) correlate with C-7a ($\delta_C$ 130.25), H-4 correlate with C-3 ($\delta_C$ 109.95), C-6 ($\delta_C$ 145.62), C-7a ($\delta_C$ 130.25), H-7 correlate with C-3a ($\delta_C$ 128.47), C-5 ($\delta_C$ 146.43), these signals indicated that a 5,6-disubstituted indole ring exit in the structure. We could find that H-2 correlate with C-6, so the $\beta$-D-galactose linked to C-6. In addition, C-5 ($\delta_C$ 146.43) was substituted by the hydroxyl group because of its increased chemical shift. A trans double bond also could be identified in view of $\delta_H$ 6.36 (1H, d, $J = 12.6$ Hz, H-2) and $\delta_H$ 6.97 (1H, d, $J = 12.6$ Hz, H-3), and it was obvious that the chemical shift of C-2' ($\delta_C$ 119.11) was decreased and the chemical shift of C-3' ($\delta_C$ 140.85) was increased, so we can conclude that C-2' linked to a carbonyl group (C-1', $\delta_C$ 168.64). In the ROESY spectrum, H-2 correlated with H-2', that is to say C-1' linked to N-1. The signals of $\delta_H$ 7.31 (2H, d, $J = 9.0$ Hz, H-2', H-6''), $\delta_H$ 6.66 (2H, d, $J = 8.4$ Hz, H-3'', H-5'') indicated that there is a AA'BB' spin system in the structure, in the meantime, there are two groups of overlapping peaks ($\delta_C$ 132.31, C-2'', C-6''); $\delta_C$ 116.52, C-3'', C-5'') in the $^{13}$C NMR, and they are typical signals of AA'BB' spin system. Moreover, C-4'' was substituted by the hydroxyl group due to its increased chemical shift. The HMBC spectrum showed that H-2' correlate with C-1'' ($\delta_C$ 127.72), H-3' correlate with C-2'', C-6'', it can be seen that C-1'' linked to C-3' of the trans double bond. In summary, compound 2 was identified as (E)-1-(5-hydroxy-6-((3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-1H-indol-1-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one, named oleraindole F.

### 2.2. Anti-inflammatory activity

*P. oleracea* has various kinds of biological activity, and it is well known for its anti-inflammatory effect, so *P. oleracea* is used for the treatment of carbuncle, dysentery and ulcerative colitis. In addition, it is well known that IL-1$\beta$ plays a key role in inflammatory diseases (Bauer et al. 2010). Thereby, the anti-inflammatory activity could be evaluated by testing the concentration of IL-1$\beta$. In this article, cytotoxicity test of compounds was measured by the method of CCK-8 at first, then the inflammation model of RAW264.7 cells was constructed using LPS (Zhong et al. 2018), and the activity was evaluated by testing the concentration of IL-1$\beta$. The results showed that the two compounds are non-cytotoxic when the concentration was 50$\mu$M (Figure S32, in
supplementary material), compound 1 and compound 2 displayed anti-inflammatory activities at 20 μM (Figure S33, supplementary material).

3. Experimental

3.1. General instruments

The structures of the compounds were identified by $^1$H and $^{13}$C NMR spectroscopy (Bruker Avance 600 MHz spectrometer, Switzerland), with methanol-$d_4$ used as the solvent. The molecular weights of the compounds were measured by a 6520 quadrupole-time-of-flight mass spectrometer (Agilent, Palo Alto, CA). In the separation process, silica-gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Co., Qingdao, China), polyamide resin (80–100 mesh, Taizhou Luqiao Sijia Biochemical Plastic Factory, Zhejiang, China) and ODS (20–40 μm, GE Healthcare, Marlborough, MA) were used for the separation of compounds. A Nexera X2 UHPLC LC-30A system (Shimadzu, Kyoto, Japan) with a Kromasil C18 column (150 mm × 4.6 mm, 5 μm, Dalian Jiangshen Separation Science and Technology Corporation) was used for the purification of compounds.

3.2. Plant materials and chemicals

The plants were collected in Shijiazhuang (Hebei, China), and the herbs were identified as Portulaca oleracea L. by Prof. Xixiang Ying. The voucher specimens (No. 20171001) were deposited at School of Pharmacy, Liaoning University of Traditional Chinese Medicine. Methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from Damao Chemical Reagent Plant (Tianjin, China), the purified water was provided by WAHAHA Co. (Shenyang, China). RAW 264.7 macrophage cells (ATCC TIB-71) were provided by American Type Culture Collection (ATCC, Manassas, VA, USA), Lipopolysaccharides (LPS) (Escherichia coli strain 055:B5) was from Sigma-Aldrich Co. (Santa Clara, CA, USA), Dulbecco’s modified Eagle’s medium (DMEM) and mouse interleukin 1β (IL-1β) enzyme-linked immunosorbent assay (ELISA) kit were obtained from Solarbio Technology Co., Ltd. (Beijing, China).

3.3. Isolation and identification

The dried P. oleracea (250 kg) was refluxed and extracted with 10 times of 50% ethanol twice, and two hours each time, then, the ethanol extract was concentrated to gain crude extract (25 kg). The crudes were purified using a silica gel column (100–200 mesh, 60 × 55 cm, 150 kg) and eluted with ethyl acetate three times, the ethyl acetate eluent was concentrated to get extract (850 g). The extract was isolated using a polyamide resin column (80–100 mesh, 8 × 120 cm, 2.5 kg) and eluted with water and 95% ethanol, the two fractions were concentrated to gain extracts (Fr.1 and Fr.2). Fr.2 (116 g) was further isolated using a silica gel column (200–300 mesh) and eluted with ethyl acetate, ethyl acetate/methanol (v:v = 1:1), ethyl acetate/methanol (v:v = 2:1), the three fractions were concentrated to get extracts (A1–A3). A1 (68 g) was isolated using ODS column chromatography and eluted with 60%, 70%, 80%, 90%, 100% methanol,
respectively, the five fractions were concentrated to gain extracts (B1–B5). B1 was further isolated using a Sephadex LH-20 column (33 g, Φ 2 × 150 cm) and eluted with methanol, four fractions (C1–C4) were obtained. C4 was purified using UHPLC, the flow rate of the mobile phase is 1 mL/min, the temperature is 40°C. Compound 1 (2 mg) was obtained with purity of >99% (tR 8.212 min, MeOH-0.1% formic acid, 43:57, v/v), compound 2 (1 mg) was obtained with purity of >90% (tR 9.028 min, acetonitrile-0.1% formic acid, 28:72, v/v).

3.4. Cell culture and cytotoxicity test

The RAW264.7 cells were cultured in DMEM, and the medium consists of 10% heat-inactivated fetal bovine serum and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin), the 96-well microplates incubated in a humidified incubator at 37°C for 24 hours.

The WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium) assay was used for evaluating cell viability. The cells were added to the 96-well plates and cultured for 24 h, the initial density is 10^4 cells/mL. Then, compounds were dispensed into the wells of the microwell plates and incubated for 1 h, the concentrations of compounds were 0, 5, 10, 25, 50 μM. Next, 1 μg/mL LPS was added to the 96-well plates and incubated for 24 h. After that, the CCK-8 solution (10 μL) was added to the wells of the microwell plates, and the absorbances were detected using a microplate reader at 450 nm.

3.5. Anti-inflammatory activity

As described above, the inflammation model was constructed by LPS, and the RAW 264.7 cells were cultured in DMEM. First, the cells were added to the 48-well plates and cultured for 24 h, the initial density is 10^5 cells/mL. Then, compounds were dispensed into the wells of the microwell plates and incubated for 1 h, the concentrations of compounds were 0, 1, 5, 10, 20 μM. Next, 1 μg/mL LPS was added to the plates and incubated for 24 h. After that, the activity was evaluated by testing the concentrations of IL-1β in the supernatant according to the IL-1β ELISA kit.

4. Conclusions

Two novel amide alkaloids, oleraindole E (1) and oleraindole F (2), were isolated from P. oleracea. What’s more, the results of anti-inflammatory experiments showed that both oleraindole E and oleraindole F could inhibit the production of IL-1β at 20 μM, and there was a dose-dependent effect on IL-1β inhibition. We could find that the activities of two compounds were similar, and the results may due to their same core structure. These results could provide some valuable information for further investigation on P. oleracea.

Disclosure statement

No potential conflict of interest was reported by the authors.
Funding

This work was supported by the National Natural Science Foundation of China under Grant (82073990).

References

Abdel Moneim AE. 2013. The neuroprotective effects of purslane (*Portulaca oleracea*) on rotenone-induced biochemical changes and apoptosis in brain of rat. CNS Neurol Disord Drug Targets. 12(6):830–841.

Bauer C, Duewell P, Mayer C, Lehr HA, Fitzgerald KA, Dauer M, Tschopp J, Endres S, Latz E, Schnurr M. 2010. Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. Gut. 59(9):1192–1199.

Chen CJ, Wang WY, Wang XL, Dong LW, Yue YT, Xin HL, Ling CQ, Li M. 2009. Anti-hypoxic activity of the ethanol extract from *Portulaca oleracea* in mice. J Ethnopharmacol. 124(2):246–250.

Duan Y, Ying ZM, Zhang MB, Ying XX, Yang GL. 2020. Two new homoisoflavones from *Portulaca oleracea* and their activities. Nat Prod Res. 1–9. DOI: 10.1080/14786419.2020.1815742.

Elkhayat ES, Ibrahim SRM, Aziz MA. 2008. Portulene, a new diterpene from *Portulaca oleracea* L. J Asian Nat Prod Res. 10(11–12):1039–1043.

El-Sayed M-IK. 2011. Effects of *Portulaca oleracea* L. seeds in treatment of type-2 diabetes mellitus patients as adjunctive and alternative therapy. J Ethnopharmacol. 137(1):643–651.

Fujitaka Y, Hamada H, Uesugi D, Kuboki A, Shimoda K, Iwaki T, Kiriake Y, Saikawa T. 2019. Synthesis of daidzein glycosides, α-Tocopherol glycosides, hesperetin glycosides by bioconversion and their potential for anti-allergic functional-foods and cosmetics. Molecules. 24(16): 2975.

GuVenalp Z, Demirezer LO. 2005. Flavonol glycosides from *Asperula arvensis* L. Turk J Chem. 29(2):163–169.

Jin R, Lin ZJ, Xue CM, Zhang B. 2013. An improved association-mining research for exploring Chinese herbal property theory: based on data of the Shennong’s Classic of Materia Medica. J Integ Med. 11(5):352–365.

Karimi G, Aghasizadeh M, Razavi M, Taghiabadi E. 2011. Protective effects of aqueous and ethanolic extracts of *Nigella sativa* L. and *Portulaca oleracea* L. on free radical induced hemolysis of RBCs. DARU. 19(4):295–300.

Karimi G, Hosseinzadeh H, Ettehad N. 2004. Evaluation of the gastric antiulcerogenic effects of *Portulaca oleracea* L. extracts in mice. Phytother Res. 18(6):484–487.

Lee AS, Kim JS, Lee YJ, Kang DG, Lee HS. 2012. Anti-TNF-α activity of *Portulaca oleracea* in vascular endothelial cells. Int J Mol Sci. 13(5):5628–5644.

Li CY, Meng YH, Ying ZM, Xu N, Hao D, Gao MZ, Zhang WJ, Xu L, Gao YC, Ying XX. 2016. Three novel alkaloids from *Portulaca oleracea* L. and their anti-inflammatory effects. J Agric Food Chem. 64(29):5837–5844.

Liu XL, Wu HB, Tao XJ, Ying XX, Stien D. 2019. Two amide glycosides from *Portulaca oleracea* and its bioactivities. Nat Prod Res. 35:2655–2659.

Meng YH, Ying ZM, Xiang Z, Hao D, Zhang WJ, Zheng Y, Gao YC, Ying XX. 2016. The anti-inflammation and pharmacokinetics of a novel alkaloid from *Portulaca oleracea* L. J Pharm Pharmacol. 68(3):397–405.

Tian J-L, Liang X, Gao P-Y, Li L-Z, Song S-J. 2015. Chemical constituents of *Portulaca oleracea*. Chem Nat Compd. 51(4):760–761.

Uddin MK, Juraimi AS, Hossain MS, Nahar MA, Ali ME, Rahman MM. 2014. Purslane weed (*Portulaca oleracea*): a prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes. Sci. World J. 2014:951019.
Xin HL, Xu YF, Hou YH, Zhang YN, Yue XQ, Lu JC, Ling CQ. 2008. Two novel triterpenoids from *Portulaca oleracea* L. HCA. 91(11):2075–2080.

Xu W, Ying ZM, Tao XJ, Ying XX, Yang GL. 2020. Two new amide alkaloids from *Portulaca oleracea* L. and their anticholinesterase activities. Nat Prod Res.:1–7. DOI:10.1080/14786419.2020.1739040.

Xu XQ, Yu LS, Chen GN. 2006. Determination of flavonoids in *Portulaca oleracea* L. by capillary electrophoresis with electrochemical detection. J Pharm Biomed Anal. 41(2):493–499.

Yan J, Sun LR, Zhou ZY, Chen YC, Zhang WM, Dai HF, Tan JW. 2012. Homoisoflavonoids from the medicinal plant *Portulaca oleracea*. Phytochemistry. 80:37–41.

Yang ZD, Zhang DB, Ren J, Yang MJ, Li S. 2012. Acetyl-cholinesterase inhibitory activity of the total alkaloid from traditional Chinese herbal medicine for treating Alzheimer’s disease. Med Chem Res. 21(6):734–738.

Zheng GY, Qu LP, Yue XQ, Gu W, Zhang H, Xin HL. 2014. Portulacerebroside A induces apoptosis via activation of the mitochondrial death pathway in human liver cancer HCCLM3 cells. Phytochem Lett. 7(1):77–84.

Zhong ZY, Liang S, Sanchez-Lopez E, He F, Shalapour S, Lin XJ, Wong J, Ding SY, Seki E, Schnabl B, et al. 2018. New mitochondrial DNA synthesis enables NLRP3 inflammasome activation. Nature. 560(7717):198–203.

Zhou YX, Xin HL, Rahman K, Wang SJ, Peng C, Zhang H. 2015. *Portulaca oleracea* L.: a review of phytochemistry and pharmacological effects. Biomed Res Int. 2015:925631.

Zhu D, Niu GC, Sun XY, Meng XJ. 2006. Study on antimicrobial effect of flavonoids from *Portulace oleracea* L. J Anhui Agric Sci. 34(1):7–8.