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## A new alkaloid and two organic acids from *Portulaca oleracea* L. and their bioactivities

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### ABSTRACT

A new alkaloid, identified as 1-benzyl-2-nitroso-1,2,3,4-tetrahydroisoquinoline-6,7-diol, named oleraisoquinoline (**1**), and five organic acids and two esters, identified as 5-(hydroxymethyl)furan-2-carboxylic acid (**2**), 1*H*-pyrrole-2,5-dicarboxylic acid (**3**), (7*E*,10*E*)-octadeca-7,10-dienoic acid (**4**), (10*E*,13*E*)-octadeca-10,13-dienoic acid (**5**), (7*E*,10*E*)-hexadeca-7,10-dienoic acid (**6**), methyl tridecanoate (**7**) and methyl (9*E*,12*E*)-octadeca-9,12-dienoate (**8**), were isolated from *Portulaca oleracea* L., among which compounds **2** and **4–7** were isolated for the first time. Moreover, the anti-inflammatory activities of compounds **1–3** were studied, especially, compound **1** presented good inhibitory effects on the production of inflammatory factors IL-1 $\beta$  and TNF- $\alpha$ .

### KEYWORDS

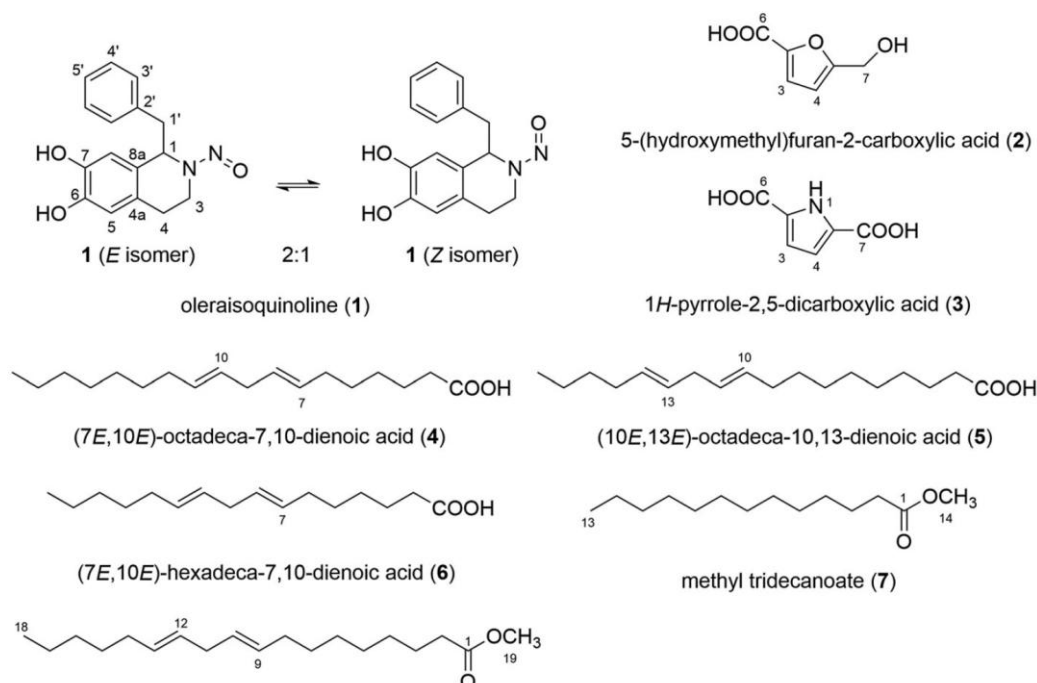
*Portulaca oleracea* L., alkaloid, organic acid, ester, anti-inflammatory activity

## 1. Introduction

*Portulaca oleracea* L., the species of Portulacaceae, is ubiquitous in the world and is a well-known traditional Chinese medicine with the function of clearing heat, cooling blood, stopping bleeding and diarrhea (The Pharmacopoeia Commission of PRC 2020). It is commonly known as purslane, rigla, pigweed, pourpier and Ma-Chi-Xian (Elkhayat et al. 2008). In many countries, *P. oleracea* is used as a folk medicine, as a diuretic, febrifuge, antiseptic, antispasmodic and vermifuge (Mohanapriya et al. 2006). In addition to its use as a medicine, it can be used as a vegetable because of its rich nutritional properties such as essential  $\omega$ -3 fatty acids,  $\alpha$ -linolenic acid,  $\alpha$ -tocopherol, ascorbic acid, linoleic acid,  $\beta$ -carotene and glutathione (Palaniswamy et al. 2001), while *P. oleracea* also contains essential trace elements such as K, Mg, P, Ca, and Fe, etc (Ali 2009). Modern studies have shown that *P. oleracea* possessed anti-inflammatory (Li et al. 2016; Jiang et al. 2018; Gu et al. 2022), antioxidant (Erkan 2012; Gu et al. 2015), anticholinesterase (Xiu et al. 2019; Ma et al. 2021; Xu et al. 2021), antitumor (Shen et al. 2013), hypolipidemic (El-Newary 2016) and antimicrobial (Iranshahy et al. 2017) effects, which are mainly attributed to its bioactive components such as alkaloids (Li et al. 2017; Xu et al. 2017; Zhao et al. 2018; Song et al. 2022), flavonoids (Duan et al. 2021), lignans (Ma et al. 2020), organic acids (Liu et al. 2021), terpenes (Xin et al. 2008), furans (Tian et al. 2022), polysaccharides (Chen et al. 2010) and phenolic acids (Siriamornpun and Suttajit 2010), especially, alkaloids, as one of the active substances, have been found to have significant anti-inflammatory effects (Meng et al. 2016; Lan et al. 2021; Liu et al. 2021).

Recent studies have shown that the inflammatory factors, interleukin 1 $\beta$  (IL-1 $\beta$ ) and tumor necrosis factor-alpha (TNF- $\alpha$ ) all play a pivotal role in the treatment of Alzheimer's disease (AD) and ulcerative colitis (UC), and both of them were significantly increased in AD and UC patients (Saresella

et al. [2016](#); Payao et al. [2018](#); Murakami et al. [2021](#)), so inhibition of IL-1 $\beta$  and TNF- $\alpha$  activities can be used as a treatment for AD and UC. In the study, a new alkaloid compounds **1** and two organic acids **2–3** were isolated from *P. oleracea* ([Figure 1](#)), and then the inflammatory factors of IL-1 $\beta$  and TNF- $\alpha$  were selected in order to investigate the anti-inflammatory activities of the three compounds.



**Figure 1.** Structures of compounds **1–8**.

## 2. Results and discussion

### 2.1. Structure clarification

Compound **1** was isolated in pure form showing up in NMR as a 2:1 mixture of isomers due to the slow rotation around the N-N partial double bond of the nitrosamine ([Figure 1](#)). The molecular formula was  $C_{16}H_{16}N_2O_3$  as deduced from the UHPLC-ESI-QTOF/MS deprotonated molecular ion at  $m/z$  283.1084 [ $M-H$ ] $^-$  (calcd. for  $C_{16}H_{15}N_2O_3^-$ , 283.1088), corresponding to a compound with 10 degrees of unsaturation. In the *E* isomer, it was possible to point out the presence of seven aromatic methines from  $\delta_H$  6.57 to 7.24 and  $\delta_C$  114.0 to 129.5, along with one methine at  $\delta_H$  5.78 and  $\delta_C$  62.6, three methylenes and five non-protonated carbons upon examination of the  $^1H$ ,  $^{13}C$ , DEPT and HSQC spectra ([Table S1](#) in [Supplementary material](#)). Five aromatic protons at  $\delta$  7.13 (H-3'/7'), 7.24 (H-4'/6') and 7.19 (H-5') were assigned to a monosubstituted phenyl ring based on the COSY correlation between H-3'/7' and H-4'/6' and the HMBC correlations of H-3'/7' with C-3'/7' and C-5', and of H-4'/6' with C-1' and C-4'/6' ([Figure S1](#) in [Supplementary material](#)). The allylic methylene in position 1' was revealed based on the HMBC correlations of H-3'/7' with C-1' and the HMBC correlations of H-1' with C-2' and C-3'/7'. C-1' was linked to C-1 based on the coupling between H-1' and H-1. Position 1 was placed next to a second aromatic ring thanks to the HMBC correlations of H-1 with C-4a, C-8 and C-8a, and the HMBC correlation of H-8 with C-1. The HMBC correlation between H-5 and C-4a revealed that H-5 was meta relatively to C-4a, i.e., para to position 8. The chemical shift of carbons 6 and 7 at  $\delta$  144.7 and 144.0, identified based on the HMBC correlations H-8/C-6 and H-5/C-7, leads to the conclusion that these carbons both bear hydroxyl groups. Finally, the COSY correlation between the methylene protons H-3 and H-4, and the H-5/C-4 and the H-1/C-3 correlations make it possible

to position the C-3–C-4 ethylene subunit and to demonstrate that the C-1 and C-3 carbons are linked by a heteroatom. Based on the raw formula of compound **1** and the chemical shift of the C-1 and C-3 carbons at  $\delta$  62.6 and 38.2, respectively, it could be stated that compound **1** was the nitrosamine 1-benzyl-2-nitroso-1,2,3,4-tetrahydroisoquinoline-6,7-diol. It was given the trivial name oleraisoquinoline.

The minor compound in the mixture was very similar to this one. The chemical displacements were close and the correlations, in particular in HMBC, were almost identical. Overall, the same analysis allowed us to assign the chemical shifts of protons and carbons in this compound and concluded that it was an isomer of the former. In nitrosamines, the N-N partial double bond has been demonstrated to rotate very slowly, and non-symmetrical nitrosamines usually appear as a mixture of rotamers in NMR (Chow and Colón [1968](#)). Steric hindrance should in principle favor the *E* isomer over the *Z*. It has been established in the literature that nitrosamine  $\alpha$ -methylene protons signal is shifted upfield when *cis* (1, *E* isomer) than when *trans* (1, *Z* isomer) to the nitroso oxygen (Karabatsos and Taller [1964](#)). On the contrary, methine protons should resonate at lower fields when *cis* to the nitroso oxygen (1, *Z* isomer). These data are consistent with the *E*-nitrosamine being the major rotamer in the NMR.

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data of the seven known compounds (**2–8**) were compared with literature and combined with the 2 D NMR spectra to identify these compounds as 5-(hydroxymethyl)furan-2-carboxylic acid (**2**) (Sahu and Dhepe [2014](#)), 1*H*-pyrrole-2,5-dicarboxylic acid (**3**) (Ding et al. [2009](#)), (7*E*,10*E*)-octadeca-7,10-dienoic acid (**4**) (Sprecher and Sankarappe [1981](#)), (10*E*,13*E*)-octadeca-10,13-dienoic acid (**5**) (Cheng et al. [2014](#)), (7*E*,10*E*)-hexadeca-7,10-dienoic acid (**6**) (Sampaio et al. [2006](#)), methyl tridecanoate (**7**) (Romeiro et al. [2012](#)) and methyl (9*E*,12*E*)-octadeca-9,12-dienoate (**8**) (Jiang et al. [2022](#)).

## 2.2. Anti-inflammatory activity

AD is a common degenerative disease of the central nervous system and a major killer of human health, and its pathogenesis is mainly attributed to the release of inflammatory factors in the brain (McCaulley and Grush [2015](#)). It has been reported that there is an extensive inflammatory response in the brain of AD patients, mainly due to the deposition of  $\beta$ -amyloid ( $\text{A}\beta$ ) that can further trigger the inflammatory response mediated by microglia and astrocytes (Lee et al. [2008](#)), releasing large amounts of inflammatory factors, such as IL-1 $\beta$ , TNF- $\alpha$ , which promote neuronal apoptosis and ultimately lead to the nervous system disorders (Ransohoff [2016](#); Dong et al. [2019](#)). As for another disease, UC, it is classified as one of the intractable diseases by the World Health Organization due to its long course, lingering disease, difficulty in healing, and susceptibility to recurrence (Ng et al. [2017](#)) but its pathogenesis remains unclear. Studies have shown that IL-1 $\beta$  is also a major inflammatory factor leading to UC and increased after the NLRP3 inflammatory signaling pathway was activated, also the activation of caspase-1 by the NLRP3 inflammasome will cause pro-IL-1 $\beta$  to cleavage and release IL-1 $\beta$  (Bauer et al. [2010](#); Zhong et al. [2018](#)). Meanwhile, clinical and experimental studies have shown that increased levels of TNF- $\alpha$  in serum and colonic mucosa of UC patients and experimental animals lead to a disruption of the body and the intestinal immune system, inflammatory reactions, and the formation of ulcers (Gustot et al. [2005](#)).

Therefore, the anti-inflammatory activities of the compounds **1–3** were tested via IL-1 $\beta$  and TNF- $\alpha$  production and ELISA assays after lipopolysaccharide-stimulated macrophages. Dexamethasone (DEX) was used as a positive control. The results showed that compound **1** could effectively inhibit the production of inflammatory factors IL-1 $\beta$  and TNF- $\alpha$  at a concentration of 1  $\mu\text{M}$  and 5  $\mu\text{M}$ ,

compound **2** could inhibit the production of two inflammatory factors at 20  $\mu$ M, and compound **3** could inhibit IL-1 $\beta$  and TNF- $\alpha$  at 10  $\mu$ M and 20  $\mu$ M, respectively.

### **3. Experimental**

#### **3.1. General experimental procedures, chemical and reagents**

The 1 D and 2 D NMR experiments were performed by an AVANCE 600 MHz instrument with cryogenic probe (Bruker Corporation, Switzerland). UHPLC-ESI-QTOF/MS were acquired in the positive or negative ion mode on a 6520 quadrupole-time-of-flight mass spectrometer (Agilent, Palo Alto, CA). RP-UHPLC separations were performed on a Shimadzu Nexera X2 UHPLC LC-30A system (Shimadzu, Kyoto, Japan), which was equipped with a solvent delivery pump (LC-30 AD), a vacuum degasser (DGU-20A), a UV spectrophotometric detector (SPD-20A) and LabStation software (Shimadzu), the column of analysis and separation was Kromasil C<sub>18</sub> column (150 mm  $\times$  4.6 mm, 5  $\mu$ m, Dalian Jiangshen Separation Science and Technology Co., flow rate 1.0 mL/min). In the separation process, column chromatography (CC) included silica-gel (100-200 mesh, Qingdao Marine Chemical Co., Qingdao, China), Sephadex LH-20 (GE Healthcare, Marlborough, MA), ODS (20-40  $\mu$ m, GE Healthcare, Marlborough, MA) and Bond Elut SI silica-gel (40  $\mu$ m, American Varian Technology China Co., Ltd., Beijing, China). TLC was performed on silica gel GF<sub>254</sub> (Qingdao Marine Chemical Co., Qingdao, China).

Methanol and formic acid were all high performance liquid chromatography (HPLC) grade produced in Kaixin Chemical Reagent Plant (Tianjin, China), and the water was WAHAHA purified water (Shenyang, China). The other reagents of analytical grade were from Jinfeng Chemical Factory (Tianjin, China). RAW 264.7 macrophage cells (ATCC TIB-71) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS) and penicillin-streptomycin solution were purchased from Hyclone (Logan, UT, USA). LPS (*Escherichia coli* strain 055:B5) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Interleukin 1 $\beta$  (IL-1 $\beta$ ) enzyme-linked immunosorbent assay (ELISA) kits and tumor necrosis factor-alpha (TNF- $\alpha$ ) ELISA kits were purchased from Solarbio Technology Co., Ltd. (Beijing, China). All other reagents were provided by Kaixin Chemical Co. (Tianjin, China).

#### **3.2. Plant material**

The whole herbs of *Portulaca oleracea* L. were collected in Shijiazhuang (Hebei, China) in June 2017, and identified by Prof. Xixiang Ying (School of Pharmacy, Liaoning University of Traditional Chinese Medicine). Voucher specimens (No. 20171001) were garnered at School of Pharmacy, Liaoning University of Traditional Chinese Medicine.

#### **3.3. Extraction and isolation**

The air-dried aerial parts of *P. oleracea* (250 kg) were extracted twice with 10 volumes of 50% ethanol for 2 h each time and then the ethanol extract was concentrated to obtain the crude extract (20 kg). The crude extract was subjected to a 100-200 mesh silica gel column (61  $\times$  55 cm, approximately, 150 kg) with a 2 volumes of ethyl acetate 3 times as the isocratic elution, and the distillate was concentrated to the extract (217 g). The above extract (217 g) was separated by a Bond Elut SI silica-gel column (40  $\mu$ m, 120  $\times$  8 cm, approximately, 1 kg) with 2 volumes of ethyl acetate, 2 volumes of ethyl acetate and methanol (5:1, 2:1, v/v), 2 volumes of methanol as the gradient eluant under moderate pressure to obtain 10 fractions (A-J, 1 L each) and evaporated these fractions to dryness. Fractions A eluted with ethyl acetate (20 g) and Fractions B eluted with ethyl acetate (23 g)

were processed by using a Bond Elut SI silica-gel column (40  $\mu\text{m}$ , 120  $\times$  8 cm, approximately, 500 g) that eluted with 2 volumes of cyclohexane and ethyl acetate (5:1, 1:1, v/v), 2 volumes of ethyl acetate, 2 volumes of ethyl acetate and methanol (5:1, v/v), 1 volume of methanol as the gradient eluant under moderate pressure to obtain 11 fractions (A-1 to A-11, 500 mL each) and 13 fractions (B-1 to B-13, 500 mL each), and then evaporated these fractions to dryness again. Fraction E eluted with ethyl acetate: methanol = 2:1 (35 g) was isolated by using ODS column chromatography that eluted with 2 volumes of each concentrations of methanol (60%, 70%, 80%, 90%, 100%, respectively) under medium pressure to obtain 5 fractions (E-1 to E-5, 500 mL each). A-3, A-6, B-8 and E-2 were isolated through a Sephadex LH-20 column (100 g,  $\phi$  2  $\times$  150 cm) with 3 volumes of methanol to get 12 fractions (A-3-1 to A-3-12, 120 mL each), 14 fractions (A-6-1 to A-6-14, 120 mL each), 16 fractions (B-8-1 to B-8-16, 120 mL each) and 9 fractions (E-2-1 to E-2-9, 120 mL each). Among the above fractions, fractions A-6-7 and B-8-9 were exposed to the Dragendorff's reagent on a TLC plate to give an orange. Fractions A-6-7 and A-3-5 were applied to a Bond Elut SI silica-gel column (40  $\mu\text{m}$ , 25  $\times$  3 cm, approximately, 150 g,) with a gradient of 1 volume of cyclohexane and ethyl acetate (5:1, 1:1, v/v), 1 volume of ethyl acetate, 1 volume of ethyl acetate and methanol (5:1, 1:1, v/v), 1 volume of methanol at moderate pressure to obtain 6 fractions (A-6-7-1 to A-6-7-6, 50 mL each) and 6 fractions (A-3-5-1 to A-3-5-6, 50 mL each). Fraction B-8-9 was chromatographed on an ODS column (20-40  $\mu\text{m}$ , 25  $\times$  3 cm, approximately, 150 g, Ultimate XB-C<sub>18</sub>), eluting with 1 volume of each concentrations of methanol reagent (20%, 40%, 60%, 80%, 100%, respectively) under medium pressure to give 5 fractions (B-8-9-1 to B-8-9-5, 50 mL each). Finally, fractions A-6-7-1, A-3-5-3, A-3-5-3 and E-2-4 were prepared by UHPLC at a flow rate of 1.0 mL/min and a column temperature of 40  $^{\circ}\text{C}$  to give compounds **1–8**, respectively. Obtain compound **1** from A-6-7-1 (3 mg, purity of > 99% with UHPLC,  $t_{\text{R}}$  4.212 min, methanol –0.1% formic acid, 70:30, v/v), compound **2** from A-3-5-3 (5 mg, purity of > 99% with UHPLC,  $t_{\text{R}}$  7.806 min, methanol –0.1% formic acid, 5:95, v/v), compound **3** from A-3-5-3 (3 mg, purity of > 99% with UHPLC,  $t_{\text{R}}$  7.845 min, methanol –0.1% formic acid, 15:85, v/v), compound **4** from E-2-4 (3 mg, purity of > 98%, with UHPLC,  $t_{\text{R}}$  5.580 min, MeOH-0.1% formic acid, 90:10, v/v), compound **5** from E-2-4 (2 mg, purity of > 98%, with UHPLC,  $t_{\text{R}}$  10.533 min, MeOH-0.1% formic acid, 90:10, v/v), compound **6** from E-2-4 (2 mg, purity of > 98%, with UHPLC,  $t_{\text{R}}$  8.053 min, MeOH-0.1% formic acid, 90:10, v/v), compound **7** from E-2-4 (2 mg, purity of > 98%, with UHPLC,  $t_{\text{R}}$  6.606 min, Acetonitrile-0.1% formic acid, 90:10, v/v), compound **8** from E-2-4 (2 mg, purity of > 98%, with UHPLC,  $t_{\text{R}}$  5.323 min, Acetonitrile-0.1% formic acid, 90:10, v/v).

### **3.4. Cell culture, treatment and MTT assay**

RAW 264.7 cell lines were cultured in DMEM containing 10% heat-inactivated fetal bovine serum (FBS) and antibiotics (100 units/mL penicillin and 100  $\mu\text{g}/\text{mL}$  streptomycin) in a humidified incubator at 37  $^{\circ}\text{C}$  with 5%  $\text{CO}_2$ . The cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Then the RAW 264.7 cells were seeded in 96-well plates at a density of  $1 \times 10^4$  cells/well, subsequently pre-incubated with or without various concentrations (5, 10, 25, 50 and 100  $\mu\text{M}$ ) of compounds **1–3** for 1 h in the incubator, then stimulated with 1  $\mu\text{g}/\text{mL}$  LPS for 24 h. After treatment removed the media and incubated with 5 mg/ml MTT solution for 4 h at 37  $^{\circ}\text{C}$ . The supernatants were discarded and the formazan was dissolved in 150  $\mu\text{L}$  DMSO. The absorbance was detected by BIO-TEK Microplate Reader at 570 nm contrasting with the untreated group as 100% viability. The cytotoxic effects of compounds **1–3** on RAW 264.7 cells were non-observed at a concentration of 25  $\mu\text{M}$ . However, because of the special structure of compound **1**, the concentrations between 0.1 and 10  $\mu\text{M}$  of compound **1** were considered non-cytotoxic and were used in the following experiments. And the concentrations between 1 and 20  $\mu\text{M}$  of compounds **2–3** were considered non-cytotoxic and were used in the following experiments.

### 3.5. Determination of IL-1 $\beta$ and TNF- $\alpha$ production

RAW 264.7 cells were plated in a 96-well plate ( $1 \times 10^4$  cells/well) in the presence or absence of tested compounds **1–3** (0.1, 1, 5 and 10  $\mu$ M for compound **1**; 1, 5, 10 and 20  $\mu$ M for compound **2–3**) for 1 h, followed by incubating with LPS (1  $\mu$ g/mL) for 24 h at 37 °C. The production of IL-1 $\beta$  and TNF- $\alpha$  in the culture media were determined by using ELISA kits. Dexamethasone (Dex, 10  $\mu$ M) was used as the positive IL-1 $\beta$  and TNF- $\alpha$  release inhibitor.

### 3.6. Statistical analysis

The data results were expressed in the form of mean  $\pm$  standard deviation (SD). Excel was used to analyze and process the obtained data. All data were evaluated and compared using analysis of Dunnett's t-test and variance (ANOVA).

## 4. Conclusion

A new alkaloid and seven known compounds were isolated from *P. oleracea*, among which compounds **2, 4–7** were isolated from *P. oleracea* for the first time. The results of anti-inflammatory activity showed that compounds **1–3** could inhibit the inflammatory factors IL-1 $\beta$  and TNF- $\alpha$ , among that, compound **1** showed better anti-inflammatory activity, could effectively inhibit the production of inflammatory factors IL-1 $\beta$  and TNF- $\alpha$  at a concentration of 1  $\mu$ M and 5  $\mu$ M, respectively.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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