A New Tricyclic Alkaloid from *Portulaca oleracea* L.

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A new tricyclic alkaloid named portulacatone (**1**), *i.e.*, 5,6-dihydro-8,9-dihydroxy-11*H*-pyrrolo[2,1-*b*][3]benzazepin-11-one, together with eight known compounds, methyl 4-hydroxyphenylacetate (**2**), *p*-hydroxybenzaldehyde (**3**), vanillin (**4**), protocatechualdehyde (**5**), *p*-hydroxybenzoic acid (**6**), iseluxine (**7**), oleracein E (**8**), and (+)-(*R*)-feruloyl malate (**9**) were isolated from aerial parts of *Portulaca oleracea* L. Their structures were elucidated based on spectroscopic analyses. Among them, compounds **1**–**7** and **9** were isolated from this medicinal plant for the first time. Compounds **1** and **7** showed dose-dependent scavenging activities against DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical, with EC_{50} values of 14.36 μм and 9.98 μм, respectively, more potent than the natural antioxidant vitamin C (EC_{50} 20.72 μм).

Introduction. – *Portulaca oleracea* L. (Purslane in English; Machixian in Chinese; Portulacacea) is an edible and medicinal plant which is widely spread throughout the world [1]. The aerial part of this plant is recorded officially in the Chinese Pharmacopoeia [2]. Pharmacological research has indicated that *P. oleracea* possesses a variety of pharmacological effects, such as anti-bacterial [3], anti-flammatory [4], antioxidant [5], neuroprotection [6], antidiabetic [7], and antitumor [8] activities. Up to now, various kinds of constituents have been isolated from this plant, including organic acids [9], terpenoids [10], alkaloids [11–13], homoisoflavonoids [14], steroids, and phenolic compounds [15]. In our continuous search for bioactive constituents from *P. oleracea*, one new phenolic tricyclic alkaoid bearing a rare seven-membered ring, *i.e.*, 5,6-dihydro-8,9-dihydroxy-11*H*-pyrrolo[2,1-*b*][3]benzazepin-11-one (**1**), along with eight known compounds, methyl 4-hydroxyphenylacetate (**2**) [16], *p*-hydroxybenzaldehyde (**3**) [17], vanillin (**4**) [17], protocatechualdehyde (**5**) [18], *p*-hydroxybenzoic acid (**6**) [19], iseluxine (**7**) [20], oleracein E (**8**) [11], and (+)-(*R*)-feruloyl malate (**9**) [21][22] (*Fig. 1*) were isolated. Among them, compounds **1**–**7** and **9** were isolated from this medicinal plant for the first time. Herein, we report the structure elucidation of compound **1**, and DPPH free radical scavenging activities of **1** and **7**.

Results and Discussion. – Compound **1** was obtained as brown solid with a molecular formula as $C_{13}H_{11}NO_3$, according to the signals of *m*/*z* 230.0812 ($[M+H]^+$) and *m*/*z* 252.0632 ($[M+Na]^+$) in the HR-ESI-MS. It showed blue fluorescence under UV at 365 nm and brown-red color when sprayed with $FeCl_3$ solution, revealing that **1** was a phenolic compound. In the ^{1}H-NMR spectrum of compound **1** (*Table*), five unsaturated H-atoms can be observed, among which two were isolated aromatic H-atoms (δ(H) 6.62 (*s*, H–C(7)), 7.46 (*s*, H–C(10))), the other three were *cis*-coupled

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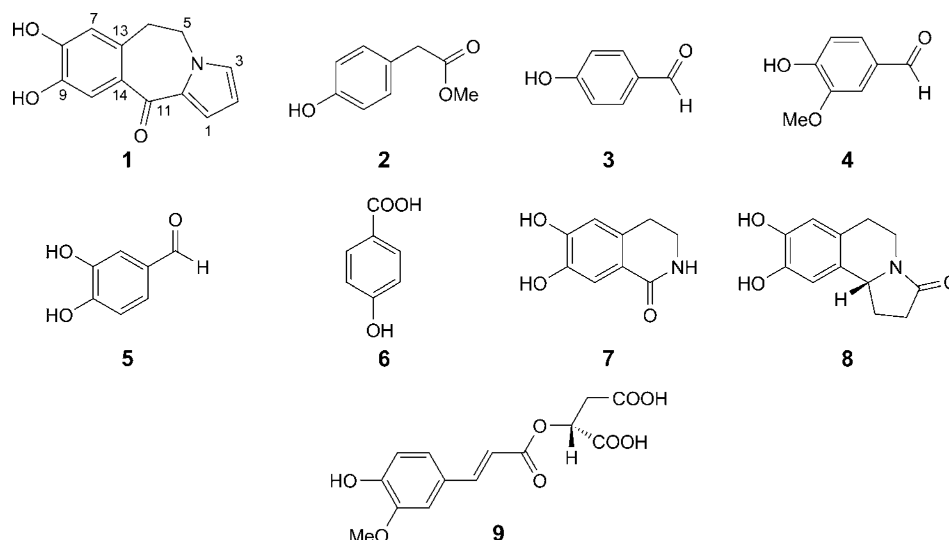


Fig. 1. *Structures of compounds* **1**–**9**, *isolated from* P. oleracea

olefinic H-atoms (δ(H) 7.09 (*dd*, $J = 2.4$, 1.8, H–C(3)), 7.02 (*dd*, $J = 4.2$, 1.8, H–C(2)), 6.12 (*dd*, $J = 4.2$, 2.4, H–C(1))). In addition to the unsaturated H-atoms, the signals of a moiety linked to an N-atom were observed at δ(H) 4.27 (*t*, $J = 4.8$, CH_2(5)) and 3.05 (*t*, $J = 4.8$, CH_2(6)), respectively. The ^{13}C-NMR spectrum (*Table*) and HMQCs displayed signals of 13 C-atoms, including one C=O group at δ(C) 178.9, two CH_2 groups at δ(C) 50.0, 35.2, three olefinic CH groups at δ(C) 109.0, 120.1, and 128.8, two aromatic CH groups at δ(C) 117.0, 118.3, two O-bearing aromatic C-atoms at δ(C) 150.3, and 144.6, and other three unsaturated quaternary C-atoms at δ(C) 133.61, 133.63, and 127.9. The presence of a benzene ring with two OH groups located at C(8) and C(9) was confirmed by HMBCs (*Fig. 2*) of H–C(7)/C(8,9,14) and H–C(10)/C(8,9,13,14). In addition, the HMBCs H–C(7)/C(6), and H–C(10)/C(11) demonstrated that the benzene ring was connected with one C=O group and one CH_2 group, respectively. Furthermore, the N-connected CH_2 group at δ(H) 4.27 (CH_2(5)) showed HMBCs with C(3), C(6), C(12), and C(13), whereas the three olefinic CH groups showed HMBCs with the corresponding C-atoms as indicated in *Fig. 2*. The HMBCs further demonstrated that the CH_2CH_2 fragmemt is connected to a pyrrole ring, and with a benzene ring. Therefore, a seven-membered ring was established containing C(13) and C(14) of the benzene ring, the C=O C-atom, C(12) and N from the pyrole ring, and the CH_2CH_2 moiety. From the above analysis, the structure of compound **1** was elucidated

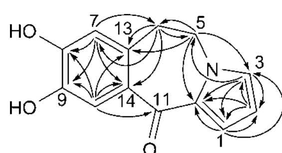


Fig. 2. *Key HMBCs* (H → C) *of* **1**

Table. *^{1}H- and ^{13}C-NMR Data* (600 and 125 MHz, resp.; DMSO(D_6)) *of Compound* **1**. δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1*.

	δ(H)	δ(C)	HMBC (H → C)
1	6.12 (*dd*, J = 4.2, 2.4)	109.0	2, 3, 12
2	7.02 (*dd*, J = 4.2, 1.8)	120.1	1, 3, 12
3	7.09 (*dd*, J = 2.4, 1.8)	128.8	1, 2, 5, 12
5	4.27 (*t*, J = 4.8)	50.0	3, 6, 12, 13
6	3.05 (*t*, J = 4.8)	35.2	5, 7, 13, 14
7	6.62 (*s*)	117.0	6, 8, 9, 14
8		144.6	
9		150.3	
10	7.46 (*s*)	118.3	8, 9, 11, 13, 14
11		178.9	
12		133.63	
13		133.61	
14		127.9	

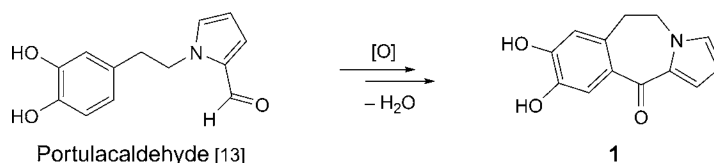
as 5,6-dihydro-8,9-dihydroxy-11*H*-pyrrolo[2,1-*b*][3]benzazepin-11-one, and named portulacatone.

In 2012, a new pyrrole alkaloid named portulacaldehyde was isolated from *P. oleracea* [13]. From a biosynthetic point of view, compound **1** can be considered as an intramolecular *Friedel–Crafts* acylation product of portulacaldehyde after oxidation to the corresponding carboxylic acid (*Scheme 1*). Moreover, the strategy for a chemical synthesis of tricyclic alkaloid derivatives of 5*H*-benzo[*d*]pyrrolo[1,2-*a*]-azepin-11(6*H*)-one was tentatively described by *Girard et al.* in 1983 [23], since these compounds were heteroanalogues of the dibenzo[*a*,*d*]cycloheptene ring system, a rich source of very useful drugs, particularly for treatment of diseases implicating in the central nervous system, such as cyclobenzaprine, a skeletal muscle relaxant.

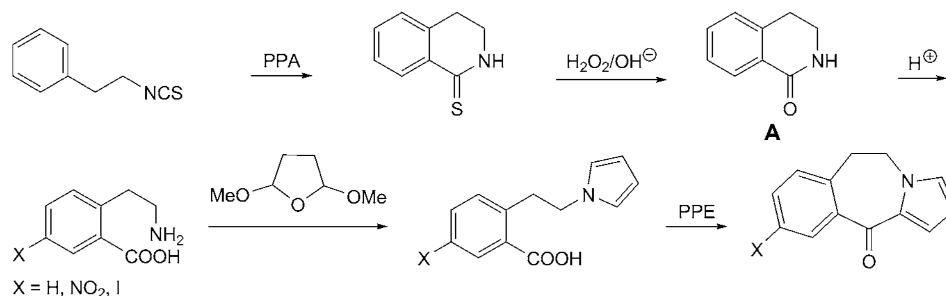
Additionally, it should be noted that compound **7** was elucidated as 6,7-dihydroxy-3,4-dihydroisoquinolinone, *i.e.*, iseluxine, based on analysis of NMR spectra (^{1}H-, ^{13}C-, HMQC, and HMBC). This amide was firstly isolated in 2000 from the epigeal part of *Iseia luxurians* (MORIC.) O'DONELL (Convolvulaceae), a climber indigenous to the tropical Americans [20]. Interestingly, 3,4-dihydroisoquinolinone (**A**) without 6,7-dihydroxy group was an intermediate product in the chemical synthesis of substituted tricyclic alkaloid of 5*H*-benzo[*d*]pyrrolo[1,2-*a*]-azepin-11(6*H*)-one (*Scheme 2*) [23].

Compounds **1** and **7** showed dose-dependent scavenging activities against the DPPH free radical with EC_{50} values of 14.36 μM and 9.98 μM, respectively, as shown in *Fig. 3*, more potent than natural antioxidant vitamin C (EC_{50} 20.72 μM). Considering

Scheme 1. *Possible Biosynthetic Pathway to Compound* **1**



Scheme 2. *3,4-Dihydroisoquinolinone* (**A**) *as an Intermediate in a Synthesis of the Tricyclic Alkaloid* 5H-*Benzo[*d*]pyrrolo[1,2-*a*]-azepin-11(*6H*)-one*



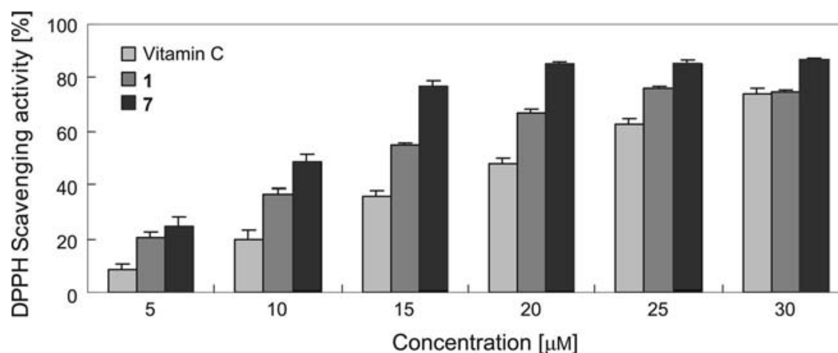


Fig. 3. *DPPH Scavenging activities of compounds* **1**, **7**, *and positive control vitamin C* ($n = 3$)

that alkaloids commonly possess a variety of biological activities, further chemical syntheses and in-depth bioactivity research on compounds **1** and **7** need to be addressed in the future.

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Experimental Part

General. TLC: Silica gel *GF* 254 (*Qingdao Haiyang Chemical Group Co. Ltd.*, P. R. China); polyamide film (*Taizhou Luqiao Sijia Biochemical Plastics Factory*, P. R. China); visualization under UV 365 nm and 254 nm or by heating the plates sprayed with 10% H_2SO_4/EtOH, or 5% $FeCl_3$/EtOH, or by iodine staining. Column chromatography(CC): polyamide gel (100–200 mesh; *Taizhou Luqiao Siqing biochemical Factory*, P. R. China), silica gel (SiO_2, 200–300 mesh; *Qingdao Haiyang Chemical Group Co. Ltd.*, P. R. China), *MCI* gel (*CHP-20P*, 75–150 μm, *Mitsubishi Chemical Co.*, Japan), *Sephadex LH-20* (*Pharmacia Fine Chemicals*, USA), and *ODS-C_{18}* (75 μm, *YMC Co.*, Japan). Semi-prep. HPLC: *Shimadzu Prominence LC-20A* liquid chromatography, with *LC-20AT* pumps, *SPD-20A* UV detector (*Shimadzu Co.*, Japan), and a *YMC-Pack ODS-A* column (250 mm × 10 mm, 5 μm; *YMC Co.*, Japan). Optical rotations: digital automatic polarimeter (*Kernchen Co.*, Germany). UV Spectra: *UV-2450* spectrophotometer (*Shimadzu Co.*, Japan); λ_{max} (log ε) in nm. NMR Spectra: *Agilent 600 MHz DD2*;

δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-ESI-MS: *LTQ-Orbitrap* mass spectrometer (*Thermo Fisher Co.*, USA); in m/z. Microplate reader (model *680 UV*, *Bio-Rad Co.*, USA) was used in the microplate assay.

Plant Material. The dried aerial parts of *P. oleracea* were purchased from *Jianlian Pharmacy* (Jinan, P.R. China) in May 2012 and were identified by Prof. *L. Xiang*, School of Pharmaceutical Sciences, Shandong University, P.R. China. A voucher specimen (No. 20120501) has been deposited with the Laboratory of Pharmacognosy, School of Pharmaceutical Sciences, Shandong University.

Extraction and Isolation. The dried sliced aerial parts (4 kg) of *P. oleracea* were refluxed with 60% EtOH (3 × 24 l). The combined extracts were concentrated under reduced pressure to the concentration of 0.5 g crude drug/ml (8 l). The supernatant (6 l) was subjected twice to polyamide CC (10 × 100 cm) eluted with gradient EtOH/H_2O (0 : 100 – 90 : 10) then $NH_3 \cdot H_2O$ soln. to afford nine fractions (*Frs. 1 – 9*). *Fr. 3*, *Fr. 4*, and *Fr. 6* were partitioned by AcOEt to obtain the AcOEt fractions (*Fr. 3.1* (1.2476 g), *Fr. 4.1* (1.7642 g), and *Fr. 6.1* (0.873 g)), and the H_2O fractions (*Fr. 3.2*, *Fr. 4.2*, and *Fr. 6.2*). *Fr. 6.1* was subjected to CC (*Sephadex LH-20* (4 × 90 cm); MeOH/H_2O 80 : 20) to give 14 fractions (*Frs. 6.1.1 – 6.1.14*). *Fr. 6.1.8* (89.5 mg) was then purified by CC (*Sephadex LH-20* (2 × 85 cm); MeOH/H_2O 80 : 20) to afford three fractions *Frs. 6.1.8.1 – 6.1.8.3*. *Fr. 6.1.8.2* (26 mg) was further purified by semi-prep. HPLC (MeOH/0.1% HCOOH 40 : 60) to obtain compound **1** (3.1 mg). *Fr. 3.1* was subjected to CC (*Sephadex LH-20* (4 × 90 cm); MeOH/H_2O 80 : 20) to give *Frs. 3.1.1 – 3.1.18*. *Fr. 3.1.11* (217 mg) was then subjected to CC (SiO_2, PE/AcOEt 8 : 2 to 5 : 5) to yield six fractions. *Frs. 3.1.11.1 – 3.1.11.6*. *Fr. 3.1.11.1* was further purified by semi-prep. HPLC (MeOH/0.1% HCOOH 40 : 60) to obtain compound **2** (2 mg). *Fr. 3.1.11.2* was further purified by semi-prep. HPLC (MeOH/0.1% HCOOH 32 : 68) to obtain compounds **3** (1.6 mg) and **4** (3.5 mg). *Fr. 3.1.12* (88 mg) was repurified by CC (*Sephadex LH-20*; MeOH/H_2O 80 : 20; followed by *MCI* (2.5 × 22 cm); MeOH/H_2O 0 : 100 – 80 : 20) and semi-prep. HPLC (MeOH/0.1% HCOOH 20 : 80) to provide compound **7** (5 mg). *Fr. 4.1* was subjected to CC (*Sephadex LH-20* (4 × 90 cm); MeOH/H_2O 80 : 20) to give 14 fractions: *Frs. 4.1.1 – 4.1.14*. *Fr. 4.1.6* (237 mg) was then purified by CC (*Sephadex LH-20* (2 × 85 cm); MeOH/H_2O 80 : 20) to afford five fractions: *Frs. 4.1.6.1 – 4.1.6.5*. *Fr. 4.1.6.4* (70 mg) was further purified by CC (*Sephadex LH-20* (2 × 85 cm); MeOH/H_2O 80 : 20) to yield four fractions *Frs. 4.1.6.4.1 – 4.1.6.4.4*. *Fr. 4.1.6.4.3* (26 mg) was then purified by semi-prep. HPLC (MeOH/0.1% HCOOH 37 : 63) to obtain compound **5** (2.5 mg). *Fr. 4.1.6.4.4* (12.4 mg) was purified by semi-prep. HPLC (MeOH/0.1% HCOOH 28 : 72) to obtain compound **6** (6 mg). Compound **8** (15 mg) was precipated and purified from *Fr. 4.1.7*. *Fr. 9* (3.0405 g) was first subjected to CC (*Sephadex LH-20* (6 × 59 cm), MeOH/H_2O 80 : 20) to obtain *Frs. 9.1 – 9.9*. *Fr. 9.5* was then separated by CC (*Sephadex LH-20* (6 × 59 cm), MeOH/H_2O 80 : 20) to give *Frs. 9.5.1 – 9.5.17*. *Fr. 9.5.6* was subjected to CC (*ODS-C_{18}* (3.5 × 8.5 cm), EtOH/H_2O 0 : 10 to 10 : 0) to yield *Frs. 9.5.6.1 – 9.5.6.11*. *Fr. 9.5.6.1* was repurified by CC (*ODS-C_{18}* (2 × 25 cm), EtOH/H_2O 0 : 10 to 10 : 0) to afford *Frs. 9.5.6.1.1 – 9.5.6.1.3*. *Fr. 9.5.6.1.1* was last purified by semi-prep. HPLC (MeOH/H_2O 43 : 57) to provide compound **9** (5 mg) with $[\alpha]^{20}_{D}$ value of + 7.40 (c = 0.1, MeOH).

Portulacatone (= *5,6-Dihydro-8,9-dihydroxy-11*H*-pyrrolo[2,1-*b*][3]benzazepin-11-one*; **1**). Brown solid. UV (MeOH): 346.1 (4.07), 317.6 (3.99), 246.9 (3.89). ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 230.0812 ($[M + H]^+$, $C_{13}H_{12}NO_3^+$; calc. 230.0817), 252.0632 ($[M + Na]^+$, $C_{13}H_{11}NNaO_3^+$; calc. 252.0637).

DPPH Scavenging Activity Assay. The experiment was carried out according to the reported method [24] with a little modification. Briefly, 25 µl sample soln. (dissolved in MeOH) and 200 µl DPPH (100 µM, dissolved in MeOH) were mixed in 96-well plates. After shaking for 30 min at r.t. in the dark, the absorbance was measured at 490 nm by microplate reader and recorded as $A_{\text{sample+DPPH}}$. The absorbance of 25 µl sample mixed with 200 µl MeOH was recorded as A_{sample}. The absorbance of 25 µl MeOH mixed with 200 µl DPPH was recorded as A_{DPPH}, with the absorbance of 225 µl MeOH as the blank and recorded as A_{blank}. Each sample was examinated for three times. The percentage inhibition was calculated according to the following equation:

$$\text{DPPH scavenging activity (\%)} = [1 - (A_{\text{sample+DPPH}} - A_{\text{sample}})/(A_{\text{DPPH}} - A_{\text{blank}})] \times 100$$

The EC_{50} value was obtained by interpolation from linear regression analysis, which represented the concentration of sample that decreased the DPPH free radical by 50%.

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