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A new alkaloid from *Portulaca oleracea* L. and its antiacetylcholinesterase activity

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ABSTRACT

A new alkaloid, (10*E*, 12*E*)-9-ureidooctadeca-10, 12-dienoic acid, named oleraurea (**1**) and 10 known compounds, *p*-hydroxybenzaldehyde (**2**), *p*-hydroxybenzoic acid (**3**), *p*-hydroxyacetophenone (**4**), benzamide (**5**), (*E*)-*p*-coumaramide (**6**), (*E*)-ferulamide (**7**), soyalkaloid A (**8**), β -carboline-3-carboxylic acid (**9**), 2, 3, 4, 9-tetrahydro-1*H*-pyrido [3, 4-*b*] indole-3-carboxylic acid (**10**), (1*S*, 3*S*)-1-methyl-1, 2, 3, 4-tetrahydro- β -carboline-3-carboxylic acid (**11**) were obtained from *Portulaca oleracea* L., in which, compounds **4**, **5**, **8–11** were isolated from the plant for the first time. The structure of the compound **1** was identified using spectroscopic methods including 1D and 2D NMR, HR-ESI-TOFMS. The compounds **1**, **5–11** presented anticholinesterase activities, but the *P. oleracea* extract (POE) presented very low anticholinesterase activity.

KEYWORDS

Portulaca oleracea L.; alkaloid; NMR; anticholinesterase activity

1. Introduction

Portulaca oleracea L. is succulent annual herb belonging to the family Portulacaceae, order Caryophyllales in phytochemical taxonomy (Lei et al. 2015), which is widely distributed in the tropical and subtropical areas in the world (Zhou et al. 2015). *P. oleracea* is named 'Ma Chi Xian' because its leaves seem like teeth of horse and taste likes amaranth, also, named 'Chang Shou Cai' because of its cold resistance, tenacious vitality and prolonging lifespan.

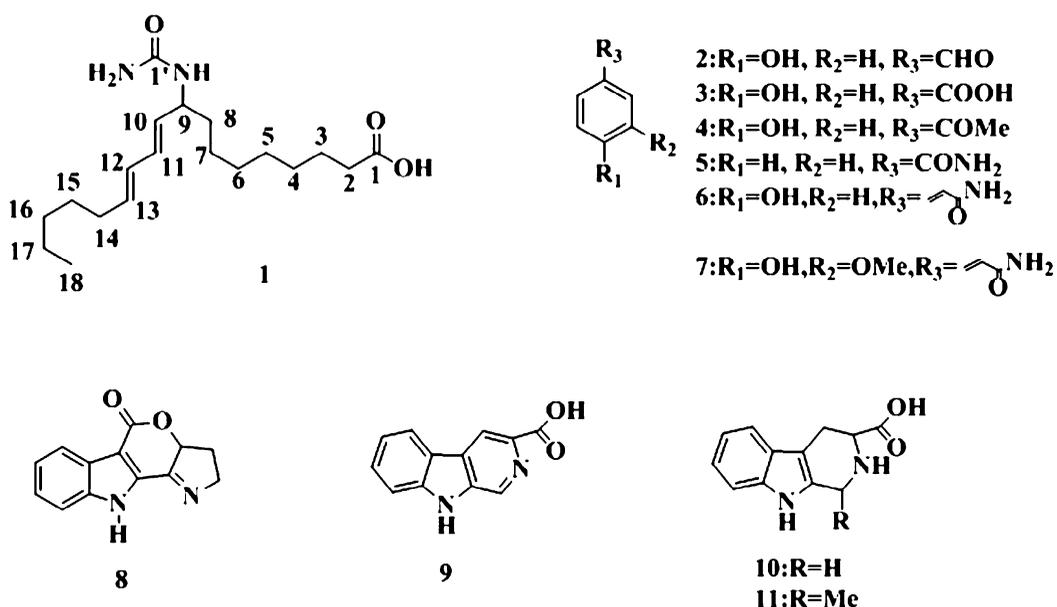


Figure 1. Chemical structures of compounds isolated from *P. oleracea*

P. oleracea is regarded as the plant of 'homology of food and medicine' (Liang et al. 2014) because of its safety for daily consumption as a vegetable (Yen et al. 2001) and its many health effects, i.e. anti-inflammatory (Li et al. 2016), antioxidant (Yang et al. 2009), antimicrobia (Nayaka et al. 2014), antidiabetics

(Li et al. 2009), anti-tumor (Zhao et al. 2013), and the effects should mainly attribute to the constituents in *P. oleracea*, such as alkaloids (Li et al. 2016; Xu et al. 2016; Li et al. 2017; Zhao et al. 2017), flavonoids (Xu et al. 2006), terpenoids (Xin et al. 2010), organic acids (Wang et al. 2017), phenolic compounds (Erkan 2012), cerebrosides (Zheng et al. 2014).

Concerning alkaloids have a wide range of structural diversity and biological activities, for examples, some alkaloids are anticholinesterase (AChE) inhibitors, which are the useful pathway for curing the Alzheimer's disease (AD) (Richard et al. 2010), in this paper we investigated the alkaloids in *P. oleracea* extract (POE) and evaluated their AChE inhibitory activities. A new alkaloid, (10*E*, 12*E*)-9-ureidooctadeca-10, 12-dienoic acid, named oleraurea, together with 10 known compounds were finally obtained (Figure 1). In addition, the anticholinesterase activities of the 11 compounds and the POE were studied by Ellman's colorimetric method with some modifications. The compounds **1**, **8–11** presented higher anticholinesterase activities among the pure compounds with IC_{50} between 62.22–67.65 μ M, conversely, the POE with IC_{50} of 257.09 ± 5.90 μ M nearly presented no anticholinesterase activity, and eserine with IC_{50} of 29.74 ± 0.14 μ M was to be the positive control in the study.

2. Results and discussion

2.1. Structure elucidation

Compound **1** was a white power, which presented a dark spot at UV 254 nm and

a blue fluoresce at UV 365 nm. The UV (MeOH) λ_{max} : 226 nm (Figure S4) and IR: 3442, 1650, 1606 cm^{-1} (Figure S5). Its molecular formula was $\text{C}_{19}\text{H}_{34}\text{N}_2\text{O}_3$ with 4 degrees of unsaturation which was deduced from the ^1H NMR, ^{13}C NMR spectrum data (Table S1) and the HR-ESI-TOF-MS ion peaks at m/z 339.2650 $[\text{M} + \text{H}]^+$ (calc for: 339.2643), m/z 699.5030 $(2\text{M} + \text{Na})^+$ (calc for: 699.5032), m/z 337.2512 $[\text{M}-\text{H}]^-$ (calc for: 337.2496). The HR-ESI-TOF-MS showed a strong ion peak at m/z 279.2518 $(\text{M}^+ - \text{HNCONH}_2 + \text{H})^+$ (Figure S3.1), belonging to octadecadienoic acid (Huang et al. 2004). Therefore, δ_{C} 175.3 in ^{13}C NMR spectrum of compound **1** was assigned to carboxyl group. The ^1H NMR spectrum (Figure S6) proved the presence of the methyl that gave rise to signals at δ_{H} 0.84 (t, 3H, $J = 6.7$ Hz); the methylenes that gave rise to signals at δ_{H} 1.22 (m, 10H, overlapped), 1.26 (m, 2H), 1.33 (m, 2H), 1.36 (m, 2H), 1.46 (m, 2H), 2.01 (q, 2H, $J = 14.4$ Hz) and 2.14 (m, 2H); the methine group that gave rise to signals at δ_{H} 4.02 (m, 1H); the ' - HNCONH_2 ' that gave rise to signals at δ_{H} 5.32 (2H, br) and 5.90 (d, 1H, $J = 8.5$ Hz); the olefinic methines that gave rise to signals at δ_{H} 5.47 (m, 1H), 5.60 (m, 1H) and 5.99 (d, 2H, $J = 14.1$ Hz, overlapped). The ^{13}C NMR spectrum (Table S1) (Figure S7) and DEPT spectrum (Figure S8) revealed 19 carbon resonances, which include one methyl (δ_{C} 13.9), eleven methylenes (δ_{C} 21.9, 24.6, 25.3, 28.4, 28.6, 28.7, 28.8, 30.8, 31.9, 34.1, 35.2), one methine group (δ_{C} 50.2), four

olefinic methines (δ_c 128.6, 129.9, 133.3, 134.0) and two quaternary carbons (δ_c 158.2, 175.3). The ^1H - ^1H COSY (Figure S2, S9) showed the strong correlations of H-2/H-3/H-4/H-5/H-6/H-7/H-8/H-9/H-10/H-11/H-12/H-13/H-14/H-15/H-16/H-17/H-18, at the same time, the HMBC correlation (Figure S1, S10) showed correlations from H-2 to C-1, 3; H-8 to C-7; H-9 to C-10, 11; H-10 to C-12; H-13 to C-11; H-14 to C-12, 13, 15, 16; H-15 to C-13, 14, 16, 17; H-16 to C-17; H-17 to C-16, 18; H-18 to C-16, 17. Therefore, both ^1H - ^1H COSY and HMBC confirmed the existence of long carbon chain. There is an obvious correlation signal of δ_H 2.14 to δ_c 175.3 in the HMBC spectrum, indicating that a signal at δ_c 175.3 existed in the carbon spectrum. In addition, the downfield chemical shift of methylene protons happened because of δ_H 2.14 connecting with the carboxyl carbon of δ_c 175.3, and this allowed us to assign δ_H 2.14/ δ_c 34.1 at C-2. The ^1H - ^1H COSY correlation suggested the presence of ' - HNCONH_2 ' because of the downfield chemical shifts of H-9 and the correlation from H-9 to NH, and the NOESY correlation (Figure S2, S12) from NH to NH_2 , H-8, 9, 10; NH_2 to NH, H-9 also. NOESY spectrum showed that the four olefinic carbons belong to two double bonds and were respectively assigned to C-10, 11, 12 and 13, the intensive signals leading the adjacent methylene protons shifting to downfield at 31.9 (position 14) and methine protons at 50.2 (position 9), furthermore, the coupling constant (14.4 Hz) confirmed the trans-configuration of two double

bonds. Thus, the structure of compound **1** was elucidated as (10*E*, 12*E*)-9-ureidooctadeca-10, 12-dienoic acid, named oleraurea.

By comparison of the ^1H and ^{13}C NMR spectral data with literature, the 10 known compounds (**2–11**) were identified, respectively, as *p*-hydroxybenzaldehyde (**2**) (Kim et al. 2003), *p*-hydroxybenzoic acid (**3**) (Wang et al. 2000), *p*-hydroxyacetophenone (**4**) (Liu et al. 2013), benzamide (**5**) (Cheng et al. 2017), (*E*)-*p*-coumaramide (**6**) (Kokubun et al. 2012), (*E*)-ferulamide (**7**) (Kokubun et al. 2012), soyalkaloid A (**8**) (Wang et al. 2016), β -carboline-3-carboxylic acid (**9**) (Tran et al. 2015), 2, 3, 4, 9-tetrahydro-1*H*-pyrido [3,4-*b*]indole-3-carboxylic acid (**10**) (Goda et al. 1999), (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid (**11**) (Brossi et al. 1973).

2.2. Anticholinesterase activity

AD is the most common form of neurodegenerative disease in the aged and the patient have loss of memory, cognitive abilities and obstacle in behavior (Mohamed et al. 2011). Currently, the more accepted AD pathology is the cholinergic hypothesis and the effective treatment strategy is to inhibit the activity of AChE and improve the level of acetylcholine in the brain (Kurt et al. 2015). Our experiment herein was devoted to search for the effective cholinesterase inhibitors. The anticholinesterase activities of the 11 compounds and the POE were determined using a microplate assay, and IC_{50} values of the

11 compounds and the POE were presented in Table S2. The compounds **1**, **5–11** isolated from *P. oleracea* ranged 1–35 μM exhibited different degree of activities against the AChE with IC_{50} values of 62.22 ± 1.02 – 121.77 ± 0.72 μM far less than that of POE with IC_{50} of 257.09 ± 5.90 μM , meaning that the POE presented very low anticholinesterase activity which was similar with the results of the literature (Boga, et al. 2011). IC_{50} value of positive control eserine was 29.74 ± 0.14 μM .

3. Experimental

3.1. General experimental procedures

The further separation and purity of compounds **1–11** used a Shimadzu Nexera X2 UHPLC LC-30A system (Shimadzu, Kyoto, Japan) with a UV spectrophotometric detector (SPD-20A), a vacuum degasser (DGPU-20A), a solvent delivery pump (LC-30AD) and Lab Station software (Shimadzu). The analytical column was a Diamonsil C_{18} column (150 mm \times 4.6 mm i.d., 5 μm) (Dikma Technologies, Beijing, China). The UV spectrum was detected using a HITACHI U-3010 spectrophotometer (Hitachi Ltd, Tokyo, Japan). The IR spectrum was recorded on an IR 200 spectrophotometer (Thermo Electron Corporation, Waltham, MA). The optical rotation was detected on an autopolarimeter (Rudolph Research Analytical, Hackettstown, NJ). The ^1H (1D, 2D COSY) and ^{13}C (1D, 2D HMBC) NMR spectra were determined using an

AVANCE 500 MHz instrument (Bruker Corporation, Switzerland) in MeOD and DMSO, using TMS as an internal standard. An Agilent 1290 UHPLC system coupled to an Agilent 6520 quadrupole-time of flight mass spectrometer (Agilent, Waldbronn, Germany) was carried out to reference UHPLC-Q-TOF/MS spectra. The acetylcholinesterase inhibitory assay was performed in 96-well microplates and determined using an Emax precision microplate reader (Nanjing defe experiment equipment co. LTD).

3.2. Plant materials and reagents

The air-dried aerial parts *P. oleracea* was collected from Shijiazhuang, Hebei, China in June 2014. The plant material was kindly identified by Professor Dr. Xixiang Ying. Voucher specimens (No. 20140312) were deposited at School of Pharmacy, Liaoning University of Traditional Chinese Medicine. Methanol and acetonitrile were all of HPLC grade purchased from Damao Chemical Reagent Plant (Tianjin, China). All other reagents were analytical grade provided by Jinfeng Chemical Factory (Tianjin, China) and the water was WAHAHA purified water (Shenyang, China). Sodium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Sinopharm Chemical Reagent Co., Ltd. Eserine was provided by Biochempartner. 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) was purchased from Shanghai Jinsui Bio-Technology Co., Ltd. Acetylthiocholine iodide (ATCI) and AChE were provided by Dalian Meilun Biotech Co., Ltd. Polyamide and silica gel (Qingdao Marine Chemical Co.,

Qingdao, China). Sephadex LH-20 and ODS (GE Healthcare, Marlborough, MA) were used for column chromatography (CC). TLC was performed on TLC plates pre-coated with silica gel GF254 (Qingdao Marine Chemical Co., Qingdao, China).

3.3. Extraction and isolation

A sample (150 kg) of the air-dried aerial parts *P. oleracea* was extracted with 50% ethanol twice under reflux for 2 h each time. The ethanol POE was concentrated under reduced pressure to yield a crude POE (21 kg) and then subjected to chromatography on a silica-gel (80–100 mesh) column eluted sequentially with ethyl acetate and ethanol (1:0, 4:1, 2:1, v/v). The part of 2:1 was provided (1 kg) after concentrated, then the POE was divided into two equal parts, adsorbed on a polyamide resin (80–100 mesh, Taizhou Luqiao Sijia Biochemical Plastic Factory, Zhejiang, China) column eluted with equal distilled water, 30, 50 and 70% ethanol, respectively. The fractions of 30% ethanol (150 g) were evaporated and applied to a silica-gel (200–300 mesh, approximately 2.5 kg) column (8 × 120 cm), eluting sequentially with isocratic ethyl acetate and methanol (5:1, 2:1, 1:2, 1:3, v/v) for further purification to obtain eluates 1–4. The resulting Fr. 1 (ethyl acetate and methanol 5:1, 14 g) was applied to 20–40 μm ODS-C₁₈ (150 g) column (φ3 × 70 cm) under medium pressure and eluted with MeOH-H₂O (20:80, 40:60, 60:40, 80:20, 100:0, v/v) to obtain 5 fractions (200 mL each). The fourth was applied to Sephadex LH-20 (150 g)

column ($\varphi 2 \times 150$ cm) using methanol as eluant to obtain a pure mixture. Final separation was performed on a UHPLC equipped with an autosampler and eluted with the mobile phase consisted of acetonitrile-0.1% formic acid water (49:51, v/v), yielding compound **1** (5 mg, purity > 98% with UHPLC, t_R 13.45 min).

The eluate 3 was purified with Sephadex LH-20 (150 g) column ($\varphi 2 \times 150$ cm) and eluted with MeOH, followed by UHPLC preparation using a mobile phase of MeOH-H₂O (15:85), yielding six known compounds, *p*-hydroxybenzaldehyde (**2**) (18 mg, purity > 98% with UHPLC, t_R 18.34 min), *p*-hydroxybenzoic acid (**3**) (8 mg, purity > 98% with UHPLC, t_R 8.17 min), *p*-hydroxyacetophenone (**4**) (5 mg, purity > 98% with UHPLC, t_R 17.59 min), benzamide (**5**) (10 mg, purity > 98% with UHPLC, t_R 11.61 min), (*E*)-*p*-coumaramide (**6**) (7 mg, purity > 98% with UHPLC, t_R 8.04 min) and (*E*)-ferulamide (**7**) (4 mg, purity > 98% with UHPLC, t_R 7.38 min). The eluate 1 was separated by UHPLC and eluted with the mobile phase consisted of acetonitrile -0.1% formic acid water (20:80, v/v), yielding five known compounds, soyalkaloid A (**8**) (15 mg, purity > 98% with UHPLC, t_R 18.12 min), β -carboline-3-carboxylic acid (**9**) (6 mg, purity > 98% with UHPLC, t_R 7.32 min), 2,3,4,9-tetrahydro-1*H*-pyrido[3, 4-*b*]indole-3-carboxylic acid (**10**) (10 mg, purity > 98% with UHPLC, t_R 5.09 min), (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid (**11**) (15 mg, purity > 98% with UHPLC, t_R 6.87 min). The flow rate was 1.0 mL/min and the

column temperature was 40 °C.

oleraurea (**1**): white power; $[\alpha]_{20}^D + 9.8$ (c 0.22, CHCl_3); UV (MeOH) λ_{max} 226

nm; IR (KBr) $\nu_{\text{N-H}}$ 3442 cm^{-1} , ν_{CH} 2932, 2861 cm^{-1} , $\nu_{\text{C=O}}$ 1650, 1606 cm^{-1} , ν_{CH} 1355 cm^{-1} ; ^1H and ^{13}C NMR spectroscopic data (DMSO), see Table S1;

UHPLC-Q-TOF/MS m/z 339.2650 $[\text{M} + \text{H}]^+$ (calcd: 339.2643), m/z 699.5030 (2 M + Na) $^+$ (calc for: 699.5032), m/z 337.2512 $[\text{M} - \text{H}]^-$ (calc for: 337.2496)

3.4. Acetylcholinesterase activity assay

Acetylcholinesterase activity was tested by Ellman's colorimetric method (Ellman et al. 1961) with some modifications. In the 96-well plates, 140 μL PBS (0.1 M, pH = 8.0, containing 0.1 mol/L NaH_2PO_4 and Na_2HPO_4), 10 μL DTNB (15 mmol/L), 15 μL AChE (0.2 U/mL) and 20 μL of a serially diluted solution of the tested samples were mixed and incubated for 10 min, then added with 10 μL ATCI (15 mmol/L) and incubated at 20 °C for 10 min. The absorbance of each well was determined at 405 nm using a microplate reader. In the blank group, the tested sample was replaced by methanol. The percentage inhibition was calculated according to the following formula: inhibition activity (%) = [(blank group - test group)/blank group] \times 100%. Anticholinesterase activities were evaluated by the value of IC_{50} .

4. Conclusion

In the study, a new alkaloid, (10*E*, 12*E*)-9-ureidooctadeca-10, 12-dienoic acid, along with 10 known compounds were isolated from *P. oleracea*, in which, compounds **4, 5, 8, 9, 10, 11** were isolated from the plant for the first time, and compounds **1, 5, 6, 7, 8, 9, 10, 11** exhibited higher anticholinesterase activities than that of the POE, suggested that the natural compounds in the *P. oleracea* presented a better pharmacological activities than that the extract, and therefore it is interesting to isolate more natural bioactive compounds from *P. oleracea* in the further study.

Disclosure statement

No potential conflict of interest was reported by the authors.

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