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New flavonoids from *Portulaca oleracea* L. and their activities

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ABSTRACT

Three new compounds, identified as (3*S*)-5-hydroxy-3-(2-hydroxybenzyl)-7-methoxychroman-4-one, oleracone C (**1**), 5-hydroxy-3-(2-hydroxybenzyl)-7-methoxy-4*H*-chromen-4-one, oleracone D (**2**), and 1-(2-hydroxy-4,6-dimethoxyphenyl)-3-(2-hydroxyphenyl)propan-1-one, oleracone E (**4**), together with one new natural product, 5,7-dimethoxy-4-*O*-2′-cycloflavan (**3**) already known from synthesis, and two known compounds, (2*S*)-5,2′-dihydroxy-7-methoxyflavanone (**5**) and 2′,4′-dihydroxy-4,6′-dimethoxychalcone (**6**) were isolated from the *Portulaca oleracea* L. for the first time. Their structures were elucidated using spectroscopic methods, including one- and two-dimensional nuclear magnetic resonance, high-resolution electrospray ionization time-of-flight mass spectrometry and circular dichroism spectrometry. Oleracone C (**1**), D (**2**) and E (**4**) presented scavenging activities in 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical quenching assay, with IC_{50} values of 23.27, 11.73, 13.17 μM, respectively and anticholinesterase activities with IC_{50} values ranging between 59.55 and 78.85 μM.

1. Introduction

Portulaca oleracea L. a herbaceous succulent annual plant, of which stems and leaves are edible with a slightly acidic and salty taste [1], belonging to the family of Portulacaceae, is widely distributed in tropical and subtropical areas [2]. *P. oleracea*, cold in nature and acid in flavor [3], is a nutritious vegetable crop, rich in essential ω-3 fatty acids, α-linolenic acid, α-tocopherol, ascorbic acid, linoleic acid, β-carotene, glutathione [4], and highest dietary minerals, such as K, Ca, Mg, P, Fe and Zn [5], which are essential for normal human healthy growth. As a result, the vigorous, invasive, yet edible plant *P. oleracea* is widely used in folk medicine or for other applications [6]. In view of its popularity, more and more pharmacological effects have been studied, including anti-inflammatory [7], analgesic [8], antibacterial [9], antioxidant [10], and neuroprotective [11] ones. More recently, *P. oleracea* being a crassulacean acid metabolism (CAM) photosynthetic plant [12], many scholars also concentrated on its constituents, such as phenolic acids [13], terpenes [14], coumarins [15], flavonoids [16] and alkaloids [17], in which flavonoids can be produced via its photosynthesis [18] that can also effectively prevent oxidative damage to the plant cells to enhance its antioxidant constituents [19]. In the study, three new flavonoids including oleracone C (**1**), oleracone D (**2**) and oleracone E (**3**) were found, especially, the absolute configuration of oleracone C was elucidated, (2*R*, 4*R*)-5,7-dimethoxy-4-*O*-2′-cycloflavan being the new natural product whose absolute configuration was also determined for the first time (Fig. 1). Flavonoids, with high antioxidation was related to many pharmacological effects, such as cardiovascular disease, senile dementia, cancer, etc. [20]. At the same time, flavonoids as acetylcholinesterase (AChE) inhibitor, can promote the production of acetylcholine effectively [21]. Therefore, the antioxidant and anticholinesterase activities of three new compounds isolated from *P. oleracea*, including oleracone C (**1**), D (**2**) and E (**3**) were studied.

2. Experimental

2.1. Chemical and reagents

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$) (purity ≥ 99%) was purchased from Sigma Co. (USA). Butylated hydroxyanisole (BHA) (purity ≥ 98%) was purchased from Shanghai Xiangrui Biological Technology Co., Ltd. (Shanghai, China). Methanol and formic acid are all of HPLC grade provided by Damao Chemical Reagent Plant (Tianjin, China, purity ≥ 99.9%). All other reagents are of analytical grade purchased from Jinfeng Chemical Factory (Tianjin, China), and the

Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; BHA, butylated hydroxyl anisole; ATCI, acetylthiocholine iodide; AChE, acetylcholinesterase; DTNB, 5,5-dithiobis-2-nitrobenzoic acid; PBS, phosphate buffer saline; AD, Alzheimer disease; CAM, crassulacean acid metabolism

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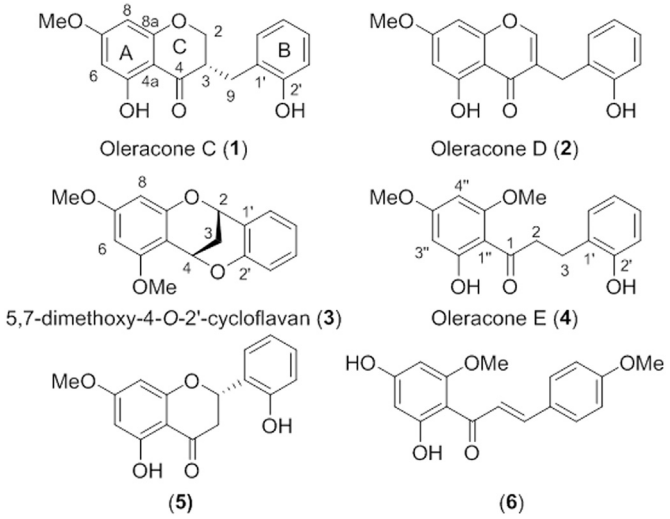

Fig. 1. Chemical structures of compounds isolated from *P. oleracea* L.

water is WAHAHA purified water (Shenyang, China). Acetylthiocholine iodide (ATCI) (purity ≥ 99%) and acetylcholinesterase (AChE) (vitality ≥ 200 units/mg protein) were purchased from Dalian Meilun Biotechnology Co., Ltd. (Dalian, China). Eserine (purity ≥ 98%) was purchased from Shanghai Hanxiang Biotechnology Co., Ltd. (Shanghai, China). 5,5-dithiobis-2-nitrobenzoic acid (DTNB) (purity ≥ 99%) was purchased from Shanghai Jinshui Biotechnology Co., Ltd. (Shanghai, China). Na_2HPO_4 and NaH_2PO_4 were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) (purity ≥ 99.0%).

2.2. Plant material

The whole herbs of *P. oleracea* were collected in Shijiazhuang (Hebei, China) in June 2014, and identified by Prof. Xixiang Ying. Voucher specimens (No. 20140312) were deposited at School of Pharmacy, Liaoning University of Traditional Chinese Medicine.

2.3. General experimental procedures

In the separation process, column chromatography (CC) included 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, Zhejiang, China) and ODS (20–40 μm, GE Healthcare, Marlborough, MA). TLC was performed on silica gel GF_{254} (Qingdao Marine Chemical Co., Qingdao, China).

2.4. Equipment

The UV spectrum and data of absorbance were determined by using a HITACHI U-3010 spectrophotometer (Hitachi Ltd., Tokyo, Japan). The IR spectrum was obtained by using an IR200 spectrophotometer (Thermo Electron Corporation, Waltham, MA). The Circular dichroism spectra were obtained using J-810 Circular Dichroism spectrometer (JASCO Corporation, Japan). Optical rotations were measured with Autopol I automatic polarmeter (Rudolph Research Analytical, Hackettstown, NJ). The NMR spectra were recorded using an AVANCE 500 MHz instrument (Bruker Corporation, Switzerland). All compounds were dissolved in DMSO-d_6 or $CDCl_3$. Relative molecular mass were recorded by using a 6520 quadrupole-time-of-flight mass spectrometer (Agilent, Palo Alto, CA). Purity was detected on a Nexera X2 UHPLC LC-30A system (Shimadzu, Kyoto, Japan), using a Kromasil C18 column (150 mm × 4.6 mm, 5 μm, Dalian Johnsson Separation Science and Technology Corporation). The oven temperature was maintained at 40 °C. Water containing 0.1% formic acid served as solvent system A, and methanol served as solvent system B. The flow rate was 1.0 mL/min, and injection volume was 5 μL. 96-well microplate reader (HBS-1096A) was purchased from Nanjing Detie Experimental Equipment Co., Ltd. (Nanjing, China).

2.5. Extraction and isolation

The dried whole herb of *P. oleracea* (150 kg) were extracted twice with a 10-fold amount 50% ethanol for 2 h each time, and then the ethanol extract was concentrated under reduced pressure reflux, obtaining the extract (15 kg), which was latter absorbed on a 100–200 mesh silica-gel column chromatography (82 × 59 cm, approximately, 118.5 kg) with a 2-fold amount ethyl acetate added aqueous ammonia (100:1, v/v) 3 times as the isocratic elution, affording the fraction evaporated (510 g). Then, the fraction, via the a 80–100 mesh polyamide resin column chromatography (120 × 8 cm, approximately, 2.5 kg), was eluted with water, 30%, 50%, 70% and pure ethanol to obtain 5 fractions (4 L each). The fraction of 50% extracting was condensed under the reflux to obtain the *P. oleracea* extract of 100 g, which was subjected to a 200–300 mesh silica-gel column (120 × 8 cm, approximately, 2.5 kg) chromatography with ethyl acetate, ethyl acetate and methanol (5:1, 2:1, 1:2, v/v) as the gradient eluent in proper order, obtaining 4 fractions (Frs. 1–4, 500 mL each). Then the fractions were spotted on a thin-layer chromatography (TLC) plate and sprayed with ferric chloride reagent. Frs. 1 (62 g) turned cyan and afterwards passed through the 20–40 μm ODS (25 × 3 cm, approx. 150 g, Ultimate XB-C18) under medium pressure, with a gradient of methanol/water (84:16, 93:7, 97:3, v/v), acquiring 3 fractions (G1-G3, 200 mL each). Then the fractions were repeatedly spotted on a thin-layer chromatography (TLC) plate and sprayed with ferric chloride reagent. G1 (15 g) turned cyan and then was prepared by with ultra-high-performance liquid chromatography (UHPLC), using MeOH - 0.1% formic acid different ratio as the mobile phase, with flow rate of 1.0 mL/min, and resulted in compound **1** (40 mg, purity of > 99% with UHPLC, MeOH - 0.1% formic acid, 61:39, v/v), compound **2** (30 mg, purity of > 98% with UHPLC, MeOH - 0.1% formic acid, 58:42, v/v), compound **3** (3 mg, purity of > 98% with UHPLC, MeOH - 0.1% formic acid, 52:48, v/v), compound **4** (10 mg, purity of > 98% with UHPLC, MeOH - 0.1% formic acid, 57:43, v/v), compound **5** (4 mg, purity of > 97% with UHPLC, MeOH - 0.1% formic acid, 52:48, v/v), compound **6** (3 mg, purity of > 96% with UHPLC, MeOH - 0.1% formic acid, 56:44, v/v).

2.5.1. Oleracone C (1)

Yellowish-brown powder; $[\alpha]_D^{20}$ + 0.8 (c 0.1, MeOH); UV (CH_3OH) λ_{max} 288, 214 nm; IR (KBr) ν_{max} 3425, 2928, 1650, 1603, 1506, 1456, 1303, 1231, 1193, 1162 cm^{-1}. ^1H and ^{13}C NMR data (Table 1. and Table S1. in Supplementary material); HR-ESI-MS *m/z* 299.0930 $[M - H]^-$ (calcd. for $C_{17}H_{15}O_5$, 299.0925).

2.5.2. Oleracone D (2)

Yellowish powder; UV (CH_3OH) λmax 286, 254 nm; IR (KBr) ν_{max} 3428, 2925, 2853, 1659, 1634, 1596, 1384 cm^{-1}. ^1H and ^{13}C NMR data (Table 1. and Table S2. in Supplementary material); HR-ES-IMS *m/z* 299.0911 $[M + H]^+$ (calcd. for $C_{17}H_{15}O_5$, 299.0914).

2.5.3. 5,7-Dimethoxy-4-O-2′-cycloflavan (3)

Yellowish powder; $[\alpha]_D^{20}$–14 (c 0.01, MeOH); ^1H and ^{13}C NMR data (Table 1. and Table S3. in Supplementary material); HR-ESI-MS *m/z* 285.1118 $[M + H]^+$ (calcd. for $C_{17}H_{17}O_4$, 285.1121).

2.5.4. Oleracone E (4)

Yellowish powder; UV (CH_3OH) λ_{max} 290, 212 nm; IR (KBr) ν_{max} 3446, 2925, 2862 1653, 1559, 1540, 1506, 1459 cm^{-1}. ^1H and ^{13}C NMR data (Table 1. and Table S4. in Supplementary material); HR-ESI-MS *m/z* 303.1229 $[M + H]^+$ (calcd. for $C_{17}H_{19}O_5$, 303.1227).