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Seven compounds from *Portulaca oleracea* L. and their anticholinesterase activities

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Abstract

A novel lignan, identified as 4-(3,4-dihydroxyphenyl)-6,7-dimethoxy-3a,4-dihydronaphtho[2,3-c]furan-1(3H)-one, named oleralignan A (**1**), together with six known compounds, loliolide (**2**), isolololide (**3**), dehydrolololide (**4**), daphnetin (**5**), esculetin (**6**), and trans-coumaric acid methyl ester (**7**) was obtained from *Portulaca oleracea* L., while compounds **3**, **4**, and **6-7** were isolated from the plant for the first time. Their structures were elucidated using spectroscopic methods, including one- and two-dimensional NMR and high-resolution electrospray ionization time-of-flight mass spectrometry. In addition, the results of activity assay demonstrated that compounds **1-7** have anticholinesterase activities.

Keywords: *Portulaca oleracea* L.; lignan; isolation; anticholinesterase activity

1. Introduction

Portulaca oleracea L., an annual green herb, belonging to the family of Portulacaceae, with branched and succulent stems, is edible and has a slightly acidic and salty taste similar to spinach (Lim and Quah 2007). It is consumed as a vegetable and has been reported to provide better nutrition than the major cultivated vegetables (Yazici et al. 2007). It is widely distributed in Africa, China, India, Australia, Middle East, Europe and America (Rashed et al. 2003). It has been listed by the World Health Organization as one of the most used medicinal plants and has been given the term ‘Global Panacea’ (Zheng et al. 2017). As a traditional Chinese medicine, it is well-known as diuretic, febrifuge, antiseptic, antispasmodic and vermifuge (Shen et al. 2013). At the same time, *P. oleracea* L. has been reported to possess a wide variety of pharmacological effects, such as anti-inflammatory (Meng et al. 2016), antibacterial (Nayaka et al. 2014), antiulcerogenic (Karimi et al. 2004), antitumor (Shen et al. 2013), antioxidant (Yang et al. 2019), antidiabetic (Bai et al. 2016), or anticholinesterase (Xiu et al. 2019), among others. These activities have been related to its chemical constituents including alkaloids (Zhao et al. 2019), flavonoids (Yang et al. 2018), lignans (Ma et al. 2018), fatty acids (Teixeira et al. 2010), or minerals (Uddin et al. 2012). In the present work, we isolated 7 interesting compounds from *P. oleracea* L. including 1 new aryl-naphthalene lignan containing 2 methoxy groups. At the same time, some lignans have been reported to have anticholinesterase activity, and the methoxy group in lignans can effectively enhance its anticholinesterase effect (Salleh et al. 2016). However, study on the anticholinesterase activity of aryl-naphthalene lignans has not been found. Therefore, we undertook to evaluate their anticholinesterase activities following the modified Ellman’s method.

2. Results and discussion

2.1. Structure elucidation

Compound **1**, a yellowish powder, has the molecular formula $C_{20}H_{18}O_6$, as deduced from the HR-ESI-TOF-MS deprotonated molecular ion at m/z 353.1021 $[M-H]^-$ (calcd. for $C_{20}H_{17}O_6^-$, 353.1031), corresponding to 12 degrees of unsaturation. The examination of the 1H , ^{13}C , and HSQC spectra of **1** demonstrated that several signals were very broad in 1H and ^{13}C NMR spectra. Some signals were also duplicated. This eventually turned out to originate from the slow rotation of the unsymmetrical phenyl

ring (1'-6'). This phenyl ring appears in ^1H NMR in the form of a very broad signal between 6.70 and 6.94 ppm integrating for 3 protons. The signals of the methoxy group in C-6 (δ_{H} 3.73, δ_{C} 55.6) and the methine 3a (δ_{H} 3.55, δ_{C} 39.7) are also very broad due to the proximity of the rotating phenyl ring.

Figure 1

With regard to the structural determination itself, it was possible to establish the presence of a 1,2,4,5-tetrasubstituted benzene ring based on the signals of protons H-5 (δ_{H} 6.16) and H-8 (δ_{H} 7.16) together with their respective carbons at δ_{C} 114.9 and 113.9 and their HMBC correlations with non-protonated carbons of the benzene ring (**Table S1** in supplementary material, **Figure 2**). Since protons H-5 and H-8 correlated with both C-6 and C-7, the assignment of both methoxy groups was based on the COSY and ROESY correlations of MeO-C7 with H-8, but also the ROESY correlation of MeO-C6 with H-2'. The COSY and ROESY spectra also provided key information regarding the substituents in positions 4a and 8a. Thanks to these data, it

was possible to position H-4 close to H-5 and H-9 close to H-8. This was confirmed by the HMBC correlations of H-4 with C-4a and C-8a and of H-9 with C-4a, C-8a and C-8. The COSY spectrum defined a proton sequence H-4/H-3a/H-3, and the proton and carbon chemical shifts in position 3 (δ_{H} 3.97/4.33, δ_{C} 71.5) demonstrated that C-3 was link to an oxygen. The HMBC correlations H-9/C-3a, H-9/C-1, and H-3-y/C-1 completed the 3a,4-dihydroisobenzofuran-1-one subunit.

Figure 2

Additional correlations of H-4 with C-1', C-2' and C-6' demonstrated that an aromatic ring was linked to C-4 and that both *ortho* positions were protonated. The fact that the signals are very wide and are split does not prevent the structural determination because protons H-2' and H-6' also correlated in ROESY with H-3a, H-4 and for H-2', MeO-C6. As the lateral aromatic ring could not be symmetrical, two hydroxy groups had to be positioned on the C-3' and C-4' carbons. The hydroxy protons are visible in ^1H NMR at δ_{H} 8.97 and 9.45. The remaining methine was assigned to position 5'. The relative configuration of compound **1** was ascertained based on the 8.6 Hz coupling between H-3a and H-4 along with the ROESY correlations H-4/H-3-x and H-3a/H-3-y which were consistent with an anti-periplanar configuration of protons H-3a and H-4. Coupling constants of 8-10 Hz are found in analogous compounds described in the literature, including conidendrin (Barton 1968; Davies and Jin 2003). Eventually, compound **1** was identified as a conidendrin-related lignan and was given the trivial name oleralignan A.

By comparison of the ^1H and ^{13}C NMR spectral data with literature, the 6 known compounds (**2-7**) were identified, respectively, as loliolide (**2**) (Mori and Khlebnikov 1993), isolololide (**3**) (Zheng et al. 2014), dehydrolololide (**4**) (Zheng et al. 2014),

daphnetin (**5**) (Xu et al. 2008), esculetin (**6**) (Zhou et al. 2009), trans-coumaric acid methyl ester (**7**) (Wan et al. 2012).

2.2. Anticholinesterase activity

Alzheimer's disease (AD) is the most common neurodegenerative disorder of aging, accounting approximately for two-third of all cases of senile dementia (Tandon et al. 2000). AD is neurochemically characterised by a consistent deficit in cholinergic neurotransmission, particularly affecting cholinergic neurons in the basal forebrain. Symptoms of AD and other forms of dementia can be treated by the use of agents which restore the level of acetylcholine through inhibition of both major cholinesterases: acetylcholinesterase and butyrylcholinesterase (Loizzo et al. 2009). Studies have shown that some lignans exhibit neuroprotective effects, increasing choline acetyltransferase activity, inhibiting acetylcholinesterase (AChE) activity *in vitro*, and increasing hippocampal acetylcholine release *in vivo* (Howes and Houghton 2003). Consequently, the anticholinesterase activities of the 7 compounds were evaluated using a microplate assay. Oleralignan A (**1**) inhibited enzyme activity at $58.31 \pm 0.23 \mu\text{M}$. Compounds **2-7** activity ranged from $71.50 \pm 0.39 \mu\text{M}$ to $93.88 \pm 0.39 \mu\text{M}$. Overall, oleralignan A exhibited higher anticholinesterase activity than compounds **2-7** (Table S2, in supplementary material).

3. Experimental

3.1. General experimental procedures

The NMR spectra were obtained using an AVANCE 600 MHz instrument (Bruker Corporation, Switzerland). HRMS was determined using a 6520 quadrupole-time-of-flight mass spectrometer (Agilent, Palo Alto, CA). The UV data was obtained from a HITACHI U-3010 spectrophotometer (Hitachi Ltd, Tokyo, Japan), the IR spectrum and data obtained from IR200 spectrophotometer (Thermo Electron Corporation, Waltham, MA). In the separation process, the GF₂₅₄ (Qingdao Marine Chemical Co., Qingdao, China) was being as TLC filling, the 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 as column chromatographic filling. The 96-well microplate reader (HBS-1096A) was from Nanjing Detie Experimental Equipment Co., Ltd. (Nanjing, China).

3.2. Plant materials and chemicals

The whole herbs of *P. oleracea* L. were collected in Shijiazhuang (Hebei, China) in June 2017, and identified by Prof. Xixiang Ying. The voucher specimens (No. 20171001) were deposited at School of Pharmacy, Liaoning University of Traditional Chinese Medicine. Acetylthiocholine iodide (ATCI) (purity $\geq 99\%$) and AChE (vitality ≥ 200 units/mg protein) were from Dalian Meilun Biotechnology Co., Ltd. (Dalian, China), 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) (purity $\geq 99\%$) was from Shanghai Jinsui Bio-Technology Co., Ltd. (Shanghai, China) and eserine (purity $\geq 98\%$) was from Shanghai Hanxiang Biotechnology Co., Ltd. (Shanghai, China). The HPLC grade formic acid, acetonitrile and methanol were purchased from Damao Chemical Reagent Plant (Tianjin, China, purity $\geq 99.9\%$). All other reagents were analytical grade reagents supplied by Jinfeng Chemical Factory (Tianjin, China), and the purified water was from WAHAHA Company (Shenyang, China).

3.3. Isolation and identification

The dried whole herb of *P. oleracea* L. (250 kg) were extracted twice with 10 volumes of water for 2 hours each time, and then the water extract was concentrated to obtain a crude extract (21 kg), which was subjected to a 100-200 mesh silica-gel column chromatography (61×55 cm, approximately, 70 kg) with a 2 volumes of ethyl acetate 3 times as the isocratic elution, affording the fraction evaporated (550 g). Then, the fraction was subjected to the 80-100 mesh polyamide resin column chromatography (120×8 cm, approximately, 1 kg), eluting with water, 30%, 50%, 70% and 100% ethanol to obtain five fractions (4 L each). The fraction of 50% extracting (121 g) was condensed then subjected to a 200-300 mesh silica-gel column (120×8 cm, approximately, 1 kg) for further purification with ethyl acetate, ethyl acetate and methanol (5:1, 2:1, v/v) as the gradient eluant, obtaining three fractions (Frs. 1-3, 4 L each). The fraction of ethyl acetate (69 g) was subjected to the 20-40 μ m octadecylsilyl (ODS) column chromatography (25×3 cm, approximately, 150 g, Ultimate XB-C18) with methanol and water (60:40, 70:30, 80:20, 90:10, 100:0, v/v) as the gradient eluant under medium pressure, acquiring five fractions (A1-A5, 500 mL each). Among that, A1 was purified on a Sephadex LH-20 column (100 g, $\phi 2 \times 150$ cm) using methanol solvent to obtain seven fractions B1-B7, and then these fractions were further prepared by UHPLC, using MeOH-0.1% formic acid as the mobile phase, with flow rate of 1.0 mL/min, and obtained compound **1** (2 mg, purity of $> 98\%$ with UHPLC, t_R 13.82 min, MeOH-0.1% formic acid, 61:39, v/v),

compound **2** (2 mg, purity of > 98% with UHPLC, t_R 25.32 min, MeOH-0.1% formic acid, 20:80, v/v), compound **3** (1 mg, purity of > 98% with UHPLC, t_R 18.14 min, MeOH-0.1% formic acid, 20:80, v/v), compound **4** (3 mg, purity of > 97% with UHPLC, t_R 17.67 min, MeOH-0.1% formic acid, 20:80, v/v), compound **5** (1 mg, purity of > 98% with UHPLC, t_R 15.32 min, MeOH-0.1% formic acid, 12:88, v/v), compound **6** (2 mg, purity of > 98% with UHPLC, t_R 19.14 min, MeOH-0.1% formic acid, 10:90, v/v), compound **7** (3 mg, purity of > 98% with UHPLC, t_R 9.76 min, MeOH-0.1% formic acid, 45:65, v/v).

Oleralignan A (**1**): Yellowish powder; UV (CH₃OH) λ_{max} 348, 254 nm; IR (KBr) ν_{max} 2934, 2850, 1740, 1565, 1518, 1452, 1212 cm⁻¹; ¹H NMR (600 MHz, DMSO-*d*₆) and ¹³C NMR (150 MHz, DMSO-*d*₆), see **Table S1** (in supplementary material); HR-ESI-MS m/z 353.1021 [M-H]⁻ (calcd. for C₂₀H₁₇O₆⁻, 353.1031).

3.4. Anticholinesterase activity assay

Acetylcholinesterase activities of compounds were assessed by a modified Ellman's method (Ellman et al. 1961). In a 96-well plate, 140 μ L PBS (0.1 M, pH = 8.0, containing 0.1 mol/L NaH₂PO₄ and Na₂HPO₄), 10 μ L DTNB (15 mmol/L), 15 μ L AChE (0.2 U/mL) and 20 μ L of sample solution serially diluted with methanol were mixed and incubated at 37°C for 10 minutes, then 10 μ L ATCI (15 mmol/L) was added and incubated at 20°C for 10 minutes. The absorbance was measured at 405 nm. Eserine and methanol were used as positive and blank controls, respectively. The inhibition rate (%) of each sample AChE was calculated according to the following formula (Aktumsek et al. 2013): Inhibition activity (%) = [(A_{blank} - A_{sample})/A_{blank}] × 100%, in which, A_{sample} and A_{blank} were respectively the absorbance of the test compound and the blank control. The anticholinesterase activity was evaluated by the value of IC₅₀ (means ± SD, n = 3).

4. Conclusion

In the study, a novel lignan named oleralignan A and six known compounds were isolated from the water extract of *P. oleracea* L. The results of anticholinesterase activities assay showed that oleralignan A presented a higher anticholinesterase activity than others, with IC₅₀ value of 58.31 ± 0.23 μ M.

Supplementary material

Supporting information can be found in the online version of this article.

Disclosure statement

The authors have no conflicts of interest to disclose.

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