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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)-7methoxychroman-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-(2hydroxyphenyl)propan-1-one, oleracone E (**4**), together with one new natural product, 5,7dimethoxy-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 twodimensional nuclear magnetic resonance, high-resolution electrospray ionization time-offlight 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 IC₅₀ values of 23.27, 11.73, 13.17 μ M, respectively and anticholinesterase activities with IC₅₀ values ranging between 59.55 and 78.85 μ M.

Keywords

Portulaca oleracea L. Flavonoids Isolation Antioxidant activity Anticholinesterase activity

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

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, (2R, 4R)-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[•]) (purity \geq 99%) was purchased from Sigma Co. (USA). Butylated hydroxyanisole (BHA) (purity \geq 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 \geq 99.9%). All other reagents are of analytical grade purchased from Jinfeng Chemical Factory (Tianjin, China), and the water is WAHAHA purified water (Shenyang, China). Acetylthiocholine iodide (ATCI) (purity \geq 99%) and acetylcholinesterase (AChE) (vitality \geq 200 units/mg protein) were purchased from Dalian Meilun Biotechnology Co., Ltd. (Dalian, China). Eserine (purity \geq 98%) was purchased from Shanghai Hanxiang Biotechnology Co., Ltd. (Shanghai, China). 5,5dithiobis-2-nitrobenzoic acid (DTNB) (purity \geq 99%) was purchased from Shanghai Jinshui Biotechnology Co., Ltd. (Shanghai, China). Na₂HPO₄ and NaH₂PO₄ were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) (purity \geq 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 μ m, GE Healthcare, Marlborough, MA). TLC was performed on silica gel GF₂₅₄ (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 CDCl₃. 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 (CH₃OH) λ_{max} 288, 214 nm; IR (KBr) v_{max} 3425, 2928, 1650, 1603, 1506, 1456, 1303, 1231, 1193, 1162 cm⁻¹. ¹H and ¹³C NMR data (Table 1. and Table S1. in Supplementary material); HR-ESI-MS *m/z* 299.0930 [M – H]⁻ (calcd. for C₁₇H₁₅O₅, 299.0925).

2.5.2. Oleracone D (2)

Yellowish powder; UV (CH₃OH) λ max 286, 254 nm; IR (KBr) v_{max} 3428, 2925, 2853, 1659, 1634, 1596, 1384 cm⁻¹. ¹H and ¹³C NMR data (Table 1. and Table S2. in Supplementary material); HR-ES-IMS *m/z* 299.0911 [M + H]⁺ (calcd. for C₁₇H₁₅O₅, 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 ¹³C NMR data (Table 1. and Table S3. in Supplementary material); HR-ESI-MS *m/z* 285.1118 [M + H]⁺ (calcd. for C₁₇H₁₇O₄, 285.1121).

2.5.4. Oleracone E (4)

Yellowish powder; UV (CH₃OH) λ_{max} 290, 212 nm; IR (KBr) ν_{max} 3446, 2925, 2862 1653, 1559, 1540, 1506, 1459 cm⁻¹. ¹H and ¹³C NMR data (Table 1. and Table S4. in Supplementary material); HR-ESI-MS *m/z* 303.1229 [M + H]⁺ (calcd. for C₁₇H₁₉O₅, 303.1227).

2.6. Assay of scavenging DPPH radicals

An assay of DPPH radical scavenging activity was performed by the modified method described formerly [22]. For each compound, a dilution into five different concentrations in methanol was prepared and the methanol was used to zero the spectrophotometer. A

methanolic solution of the sample (1 mL) was mixed with a 126.80 μ M DPPH (1 mL) dissolved in methanol. The reaction mixture was allowed to stand for 10 min at room temperature, measured at 517 nm, and then followed at 30 min. The DPPH solution (1 mL) added to methanol (1 mL) were measured as the blank control. At the same time, the sample solution (1 mL), with methanol (1 mL) was operated as the control group. BHA (butylated hydroxyanisole) was tested as a positive control as the same series of concentrations as other samples. Each sample was measured three times in parallel, and the percentage of DPPH radical scavenging was calculated in the following way [23]:

DPPH radical scavenging effect $(\%) = (1 - (A_{sample} - A_{control})/A_{blank})$

imes 100%

where, A _{control} is the absorbance of the control group, A _{sample} is the absorbance of the test compound, and A _{blank} is the absorbance of the blank control. The antioxidant capacity was evaluated by the value of IC₅₀, and values are expressed as the means \pm SD for n = 3.

2.7. Assay of anticholinesterase activity

Anticholinesterase activity was determined against AChE, using the modified method according to Ellman [24]. The 20 μ L sample solutions in methanol diluted into five different concentrations and 15 μ L AChE solution in buffer of PBS (pH 8.0, containing 0.1 mol/L Na₂HPO₄ and NaH₂PO₄) were mixed in wells a 96-well microplate and incubated for 10 min at 37 °C, and 10 μ L DTNB in buffer was added. Then 10 μ L ATCI was added to initiate the reaction and the absorbance was monitored spectrophotometrically at 405 nm. Eserine, as the same concentration as samples, was used as the positive control. Instead of samples, methanol was included in the blank control. Each sample was measured three times in parallel, and the percentage of inhibition of AChE was calculated using the following formula [25]:Anticholinesterase activity%=Ablank–Asample/Ablank×100%where, A _{sample} is the absorbance of the test compound, and A _{blank} is the absorbance of the blank control. The anticholinesterase activity was evaluated by the value of IC₅₀, and values are expressed as the means ± SD for n = 3.

3. Results and discussion

Compound **1**, $[\alpha]_D^{20} + 0.8$ (c 0.1, MeOH), was obtained as yellowish-brown powder turning cyan upon spraying ferric chloride. Its molecular formula was deduced as $C_{17}H_{16}O_5$ based on a molecular ion peak at m/z 299.0930 $[M-H]^-$ in the HR-ESI-MS spectrum (calcd. for $C_{17}H_{15}O_5$, 299.0925). The degree of unsaturation of compound **1** is 10. The ¹H NMR spectrum exhibited six aromatic protons at δ_H 6.06 (1H, d, J = 2.3 Hz), 6.07 (1H, d, J = 2.3 Hz), 6.73 (1H, td, J = 7.4, 1.2 Hz), 6.82 (1H, dd, J = 8.4, 1.2 Hz), 7.05 (1H, brtd, J = 7.4, 1.6 Hz), and 7.06 (1H, brd, J = 7.4 Hz), a methoxy group at δ_H 3.79 (3H, s), and two hydroxyl groups at δ_H 9.59 (1H, brs) and 12.12 (1H, brs) (Table 1). According to the ¹³C NMR and DEPT 135 spectrum, 17 carbon signals were detected, including 7 quaternary carbons with one carbonyl at δ_C 198.7, 7 methines, two methylenes (δ_C 27.2, 69.4), and one methoxy group (δ_C 56.0).

The signals at $\delta_{\rm H}$ 6.06/ $\delta_{\rm C}$ 93.6 and $\delta_{\rm H}$ 6.07/ $\delta_{\rm C}$ 94.8 are typical of a tetrasubstituted aromatic A-ring of a flavonoid. Both protons correlate with the methoxyl at $\delta_{\rm H}$ 3.79 in NOESY, indicating that the methoxy substituent is in C-7 (at $\delta_{\rm C}$ 167.4). The relative position of these

two protons was difficult to ascertain. Both correlate with carbons at δ_{C} 102.2 (C-4a) and 167.4 (C-7). Eventually, it was found that proton at δ_{H} 6.06 correlates with carbon at δ_{C} 162.9 (C-8a) while proton at δ_{H} 6.07 correlates with carbon at δ_{C} 163.6 (C-5). These correlations allowed us to assign δ_{H} 6.06/ δ_{C} 93.6 at position 8 and δ_{H} 6.07/ δ_{C} 94.8 at position 6.

Compound 1 also contained a CH₂-CH-CH₂ system identified by the sequence of COSY correlations H-2a, b/H-3/H-9a, b and coupling constants in the system. Both protons H-2 correlate with C-8a at δ_c 162.9 through oxygen 1, and also correlate to carbonyl moiety at δ_c 198.7 which had to be linked to C-3, forming the C-ring of an isoflavonoid. However, in the isoflavonoids, an aromatic ring is usually linked to C-3. Here, C-9 is inserted between C-3 and an aromatic ring as shown by correlations of protons H-9 with nearby carbons C-1', C-6' (and C-2' for H-9a). Carbons C-1' to C-6' describe a disubstituted phenyl moiety as confirmed by the coupling figures and COSY correlations of protons H-3' to H-6', along with respective HMBC correlations (Fig. 2). It should be mentioned that H-6' also correlates to C-9 further confirming the C-9–C-1' link. The OH signals were attributed based on NOE correlation H-3'/HO-C2'. Finally, the absolute configuration at C-3 was assigned as *S* on the basis of the positive Cotton effect at 288 nm [26,27]. (Fig. S41–42, Supporting information).

Overall, compound **1** was identified as the (3S)-5-hydroxy-3-(2-hydroxybenzyl)-7methoxychroman-4-one (or (3S)-5,2'-dihydroxy-7-methoxyhomoisoflavanone) and was given the trivial name oleracone C.

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		2		З	
	$\delta_{\rm C}$	$\delta_{\rm H}(J \text{ in Hz})$	$\delta_{\rm C}$	δ _H (J in Hz)	Pos
((154.9	8.05, s	66.6	5.38, brq (2.5)	1
) , 5.3, 4.5)	121.0		26.0	a:2.11, dt (14.1, 2.8) b:2.18. dt (14.1, 2.8)	7
	181.8		61.2	5.56, brq (1.9)	ĉ
	105.1		103.0		1′
	161.3		158.7		2
		12.74, brs			2′-C
			55.6	3.79, s	3,
	97.9	6.38, d (1.9)	92.0	5.96, d (2.3)	4
	165.1		161.4		5
	56.0	3.84, s	55.1	3.65, s	6,
	92.3	6.59, d (1.9)	91.3	6.09, d (2.3)	1″
	157.7		154.4		2″
(?	24.4	3.61, s			2″-(
5)	124.5		121.4		3"
	155.2		153.3		4"
		9.65, brs			4"-(
	115.0	6.82, dd (7.9, 1.1)	116.6	6.78, brd (8.2)	5″
	127.4	7.03, brtd (7.6, 1.6)	130.3	7.19, ddd (8.2, 7.3, 1.7)	.9
	118.8	6.70, brtd (7.4, 1.1)	119.9	6.87, brtd (7.6, 1.6)	9"-(9
	129.8	7.08, dd (7.4, 1.6)	131.0	7.35, dd (7.7, 1.7)	



Fig. 2. ¹H-¹H COSY and HMBC correlations for compounds 1–4.

Compound 2 was obtained as yellowish powder, which turned cyan after spraying ferric chloride. Its molecular formula was inferred as C₁₇H₁₄O₅ based on a molecular ion peak at m/z 299.0911 [M + H]⁺ in positive-ion HR-ESI-MS (calcd. for C₁₇H₁₅O₅, 299.0914). The degree of unsaturation of compound **2** is 11. The ¹H NMR spectrum contains six aromatic proton signals including the two protons at $\delta_{\rm H}$ 6.38 (1H, d, J = 1.9 Hz) and 6.59 (1H, d, J = 1.9 Hz) attributable to H-6 and H-8 although they appear more downfield than in compound 1. Other aromatic signals are at $\delta_{\rm H}$ 6.82 (1H, dd, J = 7.9, 1.1 Hz), 6.70 (1H, brtd, J = 7.4, 1.1 Hz), 7.03 (1H, brtd, J = 7.6, 1.6 Hz), and 7.08 (1H, dd, J = 7.4, 1.6 Hz). These correspond to the sequence of protons H-3' to H-6', with chemical shifts almost identical to those of the same protons in **1**. An olefinic proton at $\delta_{\rm H}$ 8.05 (1H, s), a methylene proton at $\delta_{\rm H}$ 3.61 (2H, s), a methoxy group at $\delta_{\rm H}$ 3.84 (3H, s), and two hydroxyl groups at $\delta_{\rm H}$ 9.66 (1H, s) and 12.76 (1H, s) were also seen. Seventeen carbons were detected in the ¹³C NMR and DEPT 135 spectra. These include one carbonyl (δ_c 181.8) and 13 other sp² carbons, one methylene (δ_c 24.4,), one methine (δ_c 154.9), and one methoxy group (δ_c 56.0) (Table 1). Compound **2** was clearly related to **1** with 1 more double bond. The HMBC correlations from H-9 (δ_{H} 3.61) to C-1' (δ_{C} 124.5), C-2' (δ_c 155.2), C-6' (δ_c 129.8), C-2 (δ_c 154.9), C-3 (δ_c 121.0), C-4 (δ_c 181.8), and from H-2 (δ_H 8.05) to C-3 (δ_C 121.0), C-4 (δ_C 181.8) revealed direct connection of C-3 with C-4, and C-1' with C-9. HMBC correlations from the CH₃ at δ_H 3.84 (3H, s) to C-7 (δ_C 165.1) indicated that the methoxy group should be linked to C-7, like in 1 (Fig. 2). As a result, compound 2 was identified as the 5-hydroxy-3-(2-hydroxybenzyl)-7-methoxy-4H-chromen-4-one (or 5,2'dihydroxy-7-methoxy-homoisoflavone) and was given the trivial name oleracone D (2).

Compounds **3**, $[\alpha]_D^{20}$ -14 (c 0.01, MeOH), was obtained as yellowish powder. Its molecular formula was identified as C₁₇H₁₆O₄ based on a molecular ion peak at *m/z* 285.1118 [M + H]⁺ in its positive-ion HR-ESI-MS spectrum (calcd. for C₁₇H₁₇O₄, 285.1121). The degree of unsaturation of the compound **3** is 10. The ¹H NMR spectrum contained six aromatic proton signals at δ_H 5.96 (1H, d, *J* = 2.3 Hz), 6.09 (1H, d, *J* = 2.3 Hz), 6.78 (1H, brd, *J* = 8.2 Hz), 6.87 (1H, brtd, *J* = 7.6, 1.1 Hz), 7.19 (1H, ddd, *J* = 8.2, 7.3, 1.7 Hz) and 7.35 (1H, dd, *J* = 7.7, 1.7 Hz), one methylene with non-equivalent protons at δ_H 2.11 (1H, dt, *J* = 14.1, 2.8 Hz) and 2.18 (1H, dt, *J* = 14.1, 2.8 Hz), two methine protons at δ_H 5.38 (1H, brq, *J* = 2.5 Hz), 5.56 (1H, brq, *J* = 1.9 Hz), and two methoxy groups at δ_H 3.65 (3H, s) and 3.79 (3H, s). In the ¹³C NMR and DEPT 135 spectrum, there were 17 carbon signals, including 12 sp² carbons, one methylene carbon (δ_C 26.0), two methine carbons (δ_C 61.2, 66.6), two methoxy groups (δ_C 55.1, 55.6) (Table 1). The COSY and HMBC data, along with comparison with compounds **1** and **2** provide evidence that this compound also contain the same di- and tetrasubstituted aromatic rings (B- and A-ring, respectively, Fig. 2). The methoxy substituents were located based of HMBC correlations between the methoxy groups at δ_H 3.79 and 3.66 with C-5 (δ_C 158.7) and C-7 (δ_C 161.4), respectively.

A CH–CH₂–CH sequence was also identified based on $^{1}H-^{1}H$ couplings and COSY experiment. Unfortunately, it was not possible to detect any HMBC correlations for these protons. However, the methines chemical shifts at δ_{H} 5.38 (H-2) and 5.56 (H-4) indicate that both CH are deshielded presumably by an oxygen and an aromatic ring. Interestingly, H-2 and H-4 are coupled to each other ($J \approx 1.9-2.5$ Hz). This is specific to systems constrained in a Wshaped conformation, typical of the 4-O-2'-cycloflavan skeleton. In the closely related 3'hydroxy-5,7-dimethoxy-4-O-2'-cycloflavan isolated from Iris tenuifolia, H-2 and H-4 are reported at $\delta_{\rm H}$ 5.34 and 5.62 in dmso- d_6 , respectively [28]. These values are very similar to those of compound **3**. Overall, it can be concluded that **3** is the 5,7-dimethoxy-4-O-2'cycloflavan. This compound has been obtained in the past by synthesis but its analytical data have not been described [29,30]. It is isolated for the first time as a natural product. The circular dichroism spectrum of **3** was compared with the one reported for the (2R,4R)-4'-hydroxy-3',5'-methyl-6,7-methylenedioxy-4-O-2'-cycloflavan [31]. This compound is the only 4-O-2'-cycloflavan, not hydroxylated in C-3 as in the cyanomaclurin series, for which the CD spectrum has been reported. Compound 3 presented 3 positive maxima at 217, 242 and 281 nm (Fig. S41–42, Supporting information), while the reported CD spectrum had negative Cotton effect at these wavelengths. This indicated that the absolute configuration of compound **3** is 2S and 4S, as represented in Fig. 1.

Compound 4 was obtained as yellowish powder, which became cyan after spraying Ferric chloride. Its molecular formula was identified as C₁₇H₁₈O₅ based on a molecular ion peak at m/z 303.1229 [M + H]⁺ in its positive-ion HR-ESI-MS spectrum (calcd. for C₁₇H₁₉O₅, 303.1227). The degree of unsaturation of compound **4** is 9. The ¹H NMR spectrum exhibited six aromatic proton signals at $\delta_{\rm H}$ 6.08 (1H, d, J = 2.3 Hz), 6.09 (1H, d, J = 2.3 Hz), 6.68 (1H, td, J = 7.4, 1.0 Hz), 6.78 (1H, dd, J = 8.0, 1.0 Hz), 6.98 (1H, brtd, J = 7.6, 1.6 Hz) and 7.05 (1H, dd, J = 7.4, 1.6 Hz), two methylene protons at δ_{H} 2.80 (2H, brt, J = 7.7 Hz), 3.18 (2H, brt, J = 7.7 Hz), two methoxy groups at $\delta_{\rm H}$ 3.79 (3H, s), 3.81 (3H, s), and two hydroxyl groups at $\delta_{\rm H}$ 9.57 (1H, s) and 13.59 (1H, s). The 13 C NMR and DEPT 135 spectrum indicated that 17 carbon signals, including one carbonyl (δ_c 204.8) and 12 other sp² carbons, two methylene carbons (δ_c 25.1, 43.5), and two methoxy groups (δ_c 55.6, 56.0) (Table 1). Comparison with compounds 1-3 provided straightforward identification of the tetrasubstituted A-ring and the ortho-disubstituted B-ring. However in this case, it was eventually found that 4 was a dihydrochalcone, with both aromatic rings linked by a propane-1-one core. This linkage was identified based on the H-2–H-3 coupling and the HMBC correlations from H-3 (δ_{H} 2.80) to C-1' (δ_c 127.3), C-2' (δ_c 155.3), C-6' (δ_c 129.7), C-2 (δ_c 43.5), C-1 (δ_c 204.8), and from H-2 (δ_H 3.19) to C-1 (δ_c 204.8), C-3 (δ_c 25.1), and C-1' (δ_c 127.3). The HMBC correlations revealed that the methoxy groups (δ_{H} 3.79) was coupled to the C-4" (δ_{C} 165.4), and another methoxy groups (δ_H 3.82) was coupled to the C-6" (δ_C 162.3), which implied that two methoxy groups are affiliated with C-4" and C-6", respectively (Fig. 2). As a result, compound 4 was determined as the 1-(2-hydroxy-4,6-dimethoxyphenyl)-3-(2-hydroxyphenyl)propan-1-one and was given the trivial name oleracone E.

Compounds **5** and **6** had been described before and were identified as the (2*S*)-5,2'dihydroxy-7-methoxyflavanone and 2',4'-dihydroxy-4,6'-dimethoxychalcone respectively, according to the NMR data and comparison with the literature [32,33]. Previously, the antioxidant activity of extracts was assayed by using the DPPH radical scavenging method [34], which is now applied to evaluate the oxidation resistance of the three new compounds. Phenolic compounds are good hydrogen donors and are usually effective DPPH radical quenchers [35]. Indeed, oleracones C (1), D (2) and E (4) were found more effective than reference compound butylated hydroxyanisole (BHA) (Fig. S43–44, Supporting information). Their antioxidant potential was dose-dependent with IC₅₀ values of 11.7, 13.2 and 23.3 μ M, respectively (Table 2). The biological activity profiles were somewhat similar at 30 min, with IC₅₀ values of 12.0, 13.7 and 23.5 μ M, respectively (Table 2). Oleracone D (2) remained the most effective. Overall, the compared radical scavenging activities on DPPH were oleracone D (2)>E (4)>C (1)>BHA.

Acetylcholinesterase (AChE) inhibitors, inhibiting the production of choline, play an significant role in the treatment of Alzheimer disease (AD) [36]. As a result, antioxidant activities of phenolic compounds can be implicated in the neuroprotective effect at the same time [37], of which flavonoids are responsible to be the multipotent agents to fight against AD [38]. Consequently, the anticholinesterase activities of three new compounds, oleracone C (1), D (2) and E (3), were determined. All of three new compounds had different degree of inhibitory activities against the AChE (Fig. S45, Supporting Information). Table 2 indicated that although the anticholinesterase activity of compound 2 was lower than that of eserine at the same concentration, it had the highest anticholinesterase activity with IC₅₀, the lowest was that of compound 3.

Table 2

 $IC_{50}\ (\mu M)$ for antioxidant and acetylcholinesterase activities of compounds 1, 2 and 4 (n=3).

Compounds and standard inhibitors	DPPH IC ₅₀ (μM)		AChE IC ₅₀ (µM)
	10 min	30 min	
1 2 4 Eserine BHA	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$

Eserine and BHA as positive control, and values are expressed as the means \pm SD for n = 3.

Conflict of interest statement

The authors declare no competing financial interest.

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