# Two Antioxidant Alkaloids from *Portulaca oleracea* L.

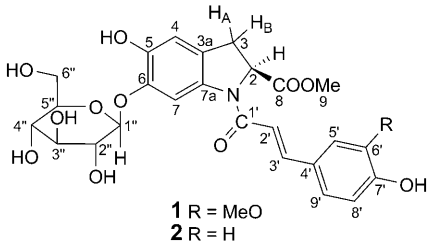
by **Dianyu Liu**, **Tao Shen**, and **Lan Xiang***

School of Pharmacy, Shandong University, Jinan 250012, P. R. China
(phone: +86-531-88382028; fax: +86-531-88382548; e-mail: xianglan02@sdu.edu.cn)

Two alkaloids, oleraceins F and G, were isolated from *Portulaca oleracea* L., and their structures were determined as methyl (2*S*)-6-[(*β*-D-glucopyranosyl)oxy]-2,3-dihydro-5-hydroxy-1-[(2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]-1*H*-indole-2-carboxylate and methyl (2*S*)-6-[(*β*-D-glucopyranosyl)oxy]-2,3-dihydro-5-hydroxy-1-[(2*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]-1*H*-indole-2-carboxylate, based on their spectroscopic data. Oleraceins F and G exhibited scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, with $EC_{50}$ values of 21.00 and 37.69 μM, respectively.

**Introduction.** – *Portulaca oleracea* L. is widely distributed all over the world. This plant is edible and also used in folk medicine in several countries as a diuretic, antiseptic, antispasmodic, and vermifuge. It has a wide range of pharmacological effects, such as antibacterial [1], hypolipidemic, anti-aging, anti-inflammatory [2], antioxidative [3], analgesic, and wound-healing activities [4]. A variety of constituents have been isolated from *P. oleracea*, including unsaturated fatty acids [5], terpenoids [6], coumarins, flavonoids [7], and alkaloids such as dopa, dopamine, noradrenaline, betalain, *N-trans*-feruloyltyramine, cyclodipeptide [8], *etc*.

In our previous study, five alkaloids named oleraceins A – E were isolated from this plant [9], and some of them exhibited potent antioxidant activities [10]. Further screening for this kind of alkaloid in the EtOH extract of *P. oleracea* using LC/MS/MS has furnished two new analogs, whose molecular weights are higher than those of oleracein A or B [11], respectively, by 14. These two alkaloids were named oleraceins F (**1**) and G (**2**). Here, we describe the isolation, purification, structure elucidation of these two compounds, as well as their scavenging activity against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.




**Results and Discussion.** – Oleracein F (**1**) was isolated as a yellow powder. It showed a yellow-brown fluorescence under UV 365 nm and turned bright yellow when

treated with $NH_3$ vapor. Its molecular formula was determined as $C_{26}H_{29}NO_{12}$ based on HR-ESI-MS (*m/z* 548.1748 $[M + H]^+$, 570.1571 $[M + Na]^+$). The $^1$H-NMR spectrum (*Table 1*) revealed that compound **1** contained a cyclo-dopa moiety similar to oleraceins A and B with two aromatic H-atoms ($\delta$(H) 6.73 (*s*) and 8.11 (*s*)), one N-connected CH group ($\delta$(H) 5.66 (*d*, $J = 10.8$)), and two $CH_2$ H-atoms ($\delta$(H) 3.19 (*d*, $J = 15.6$) and 3.52–3.56 (*m*)). The $^1$H-NMR spectrum also displayed signals for *ABX*-type aromatic H-atoms at $\delta$(H) 7.12 (*d*, $J = 7.8$), 6.81 (*d*, $J = 7.8$), and 7.26 (br. *s*), as well as for two (*E*)-olefinic H-atoms at $\delta$(H) 6.73 (*d*, $J = 15.0$) and 7.52 (*d*, $J = 15.0$). In addition, the HMBCs of the aromatic H-atom signal at $\delta$(H) 7.12 with those of C-atoms at $\delta$(C) 112.3 and 149.2, correlations of the aromatic H-atom signal at $\delta$(H) 6.81 with C-atom signals at $\delta$(C) 126.8 and 148.2, and the correlation of the MeO H-atom signals at $\delta$(H) 3.82 (*s*) with that of the aromatic C-atom at $\delta$(C) 148.2 evidenced that compound **1** also contained one feruloyl group similar to oleracein B.

Table 1. *$^1$H- and $^{13}$C-NMR Spectral Data* (600 and 150 MHz, resp.; ($D_6$)DMSO) *of* **1** *and* **2**. $\delta$ in ppm, $J$ in Hz.

| | Oleracein F (**1**) | | Oleracein G (**2**) | |
|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) |
| H–C(2) | 5.66 (*d*, $J = 10.2$) | 60.7 | 5.63 (*d*, $J = 10.2$) | 60.7 |
| $CH_2$(3) | 3.52–3.56 (*m*, $H_A$), 3.19 (*d*, $J = 15.6$, $H_B$) | 32.8 | 3.52–3.56 (*m*, $H_A$), 3.16 (*d*, $J = 15.6$, $H_B$) | 32.8 |
| C(3a) | | 124.7 | | 124.7 |
| H–C(4) | 6.74 (*s*) | 112.3 | 6.74 (*s*) | 112.3 |
| C(5) | | 144.1 | | 144.1 |
| HO–C(5) | 8.55 (*s*) | | 8.52 (*s*) | |
| C(6) | | 144.4 | | 144.4 |
| H–C(7) | 8.11 (*s*) | 108.1 | 8.09 (*s*) | 108.1 |
| C(7a) | | 135.6 | | 135.6 |
| C(8) | | 172.7 | | 172.7 |
| Me(9) | 3.67 (*s*) | 53.2 | 3.71 (*s*) | 53.2 |
| C(1′) | | 164.2 | | 164.2 |
| H–C(2′) | 6.73 (*d*, $J = 15.0$) | 116.3 | 6.73 (*d*, $J = 15.0$) | 116.2 |
| H–C(3′) | 7.52 (*d*, $J = 15.0$) | 143.0 | 7.52 (*d*, $J = 15.0$) | 142.7 |
| C(4′) | | 126.8 | | 126.3 |
| H–C(5′) | 7.26 (br. *s*) | 112.1 | 7.53 (*d*, $J = 7.8$) | 130.5 |
| C(6′) or H–C(6′) | | 148.2 | 6.81 (*d*, $J = 7.8$) | 116.0 |
| *Me*O–C(6′) | 3.82 (*s*) | 56.2 | | |
| C(7′) | | 149.2 | | 159.8 |
| HO–C(7′) | 9.55 (*s*) | | 9.9 (*s*) | |
| H–C(8′) | 6.81 (*d*, $J = 7.8$) | 116.0 | 6.81 (*d*, $J = 7.8$) | 116.0 |
| H–C(9′) | 7.12 (br. *d*, $J = 7.8$) | 122.8 | 7.53 (*d*, $J = 7.8$) | 130.5 |
| H–C(1″) | 4.62 (*d*, $J = 4.8$) | 103.9 | 4.62 (*d*, $J = 4.8$) | 103.9 |
| H–C(2″) | 3.28–3.42 (*m*) | 73.8 | 3.28–3.42 (*m*) | 73.8 |
| H–C(3″) | 3.28–3.42 (*m*) | 77.4 | 3.28–3.42 (*m*) | 77.4 |
| H–C(4″) | 3.28–3.42 (*m*) | 69.6 | 3.28–3.42 (*m*) | 69.6 |
| H–C(5″) | 3.28–3.42 (*m*) | 76.3 | 3.28–3.42 (*m*) | 76.3 |
| $CH_2$(6″) | 3.71–3.74 (*m*), 3.61–3.63 (*m*) | 60.7 | 3.71–3.74 (*m*), 3.61–3.63 (*m*) | 60.7 |

The signals of a sugar moiety were clearly discernable in $^{13}$C-NMR spectrum, and $\beta$-D-glucose was determined as the sugar part according to the coupling constant of the anomeric H-atom ($d$, $J = 4.8$) and by comparison with literature data [12]. Similar to oleracein B, the signal of this anomeric H-atom at $\delta$(H) 4.62 has a HMBC with that of C(6) at $\delta$(C) 144.4.

Comparison of the NMR spectra of **1** and oleracein B revealed that **1** possesses an additional MeO group, as indicated by the signals at $\delta$(H) 3.67 ($s$) in the $^1$H-NMR and $\delta$(C) 53.2 in the $^{13}$C-NMR spectrum. The HMBC spectrum (*Fig.*) showed that this MeO signal at $\delta$(H) 3.67, $CH_2$(3) signals at $\delta$(H) 3.19 and 3.52–3.56, and H–C(2) signal at $\delta$(H) 5.66 all correlated with the carboxy C-atom signal at $\delta$(C) 172.7, demonstrating that the COOH in oleracein B was replaced by COOMe in **1**. Compound **1** was inferred to have the ($S$)-configuration at C(2) on the basis of a negative *Cotton* effect at 238 nm and a positive *Cotton* effect at 260 nm, with tendency similar to that of ($S$)-indoline-2-carboxylic acid, which displayed a negative *Cotton* effect at 210 nm and positive *Cotton* effect at 250 nm. Based on these evidences, compound **1** was determined to be methyl (2$S$)-6-[($\beta$-D-glucopyranosyl)oxy]-2,3-dihydro-5-hydroxy-1-[(2$E$)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]-1$H$-indole-2-carboxylate.

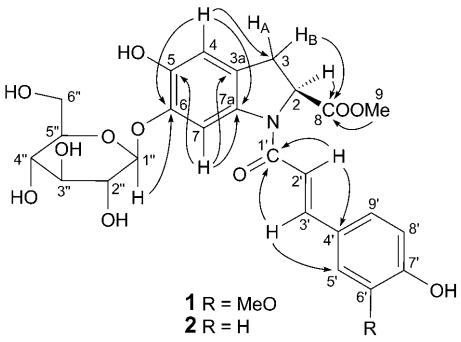



Figure. *Key HMBCs in* **1** *and* **2**

Oleracein G (**2**), isolated as a yellow powder, displayed a yellow-brown fluorescence under UV 365 nm and retained this color when exposed to $NH_3$ vapor. The molecular formula was determined as $C_{25}H_{27}NO_{11}$ based on peaks at $m/z$ 518.1651 ($[M + H]^+$) and 540.1469 ($[M + Na]^+$) in HR-ESI-MS spectrum, which is lower than the corresponding values of oleracein F by 30. Accordingly, the $^1$H-NMR spectrum (*Table 1*) of **2** indicated $AA'BB'$-type aromatic H-atoms ($\delta$(H) 7.53 ($d$, $J = 7.8$) and 6.81 ($d$, $J = 7.8$)) as well as two ($E$)-olefinic H-atoms ($\delta$(H) 6.70 ($d$, $J = 15.0$) and 7.52 ($d$, $J = 15.0$)), implying that compound **2** contained a $p$-coumaroyl moiety, instead of a feruloyl group. The CD spectrum of **2** showed a negative *Cotton* effect at 245 nm and a positive effect at 265 nm, indicating that **2** has the ($S$)-configuration at C(2) similar to compound **1**. From the above analysis, compound **2** was elucidated as methyl (2$S$)-6-[($\beta$-D-glucopyranosyl)oxy]-2,3-dihydro-5-hydroxy-1-[(2$E$)-3-(4-hydroxyphenyl)prop-2-enoyl]-1$H$-indole-2-carboxylate.

The scavenging activities against DPPH radical of oleracein F (**1**) and oleracein G (**2**) were evaluated, using butylated hydroxy anisole (BHA) and vitamin C (VC) as the positive controls. The $EC_{50}$ values of oleracein F and G are lower than those of BHA and VC. Compared with DPPH scavenging activities of oleraceins A and B, which were higher than those of BHA and VC as reported in [10], it can be concluded that the DPPH scavenging activities are reduced when the COOH group is replaced by its methyl ester, as in the cases of oleraceins F and G, **1** and **2**, respectively.

Table 2. *DPPH Free Radical-Scavenging Activity* ($n = 3$)

| | $EC_{50}$ [μM] |
|---|---|
| Oleracein F (**1**) | $21.00 \pm 0.10$ |
| Oleracein G (**2**) | $37.69 \pm 0.75$ |
| BHA[a]) | $15.26 \pm 0.72$ |
| VC[a]) | $16.44 \pm 0.44$ |

[a]) Butylated hydroxy anisole (BHA) and vitamin C (VC) were used as positive controls.

## Experimental Part

*General.* (*R*)- and (*S*)-indoline-2-carboxylic acids were kindly provided by *Ningbo Wusheng Chem Co. Ltd.* (Zhejiang, P. R. China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and butylated hydroxy anisole (BHA) produced by *Sigma–Aldrich* were purchased from *Beijing Huamei Scientific Company* (Beijing, P. R. China). All other reagents and chemicals used were of anal. grade and purchased from *Chengyan Chemical Reagent Co., Ltd.* (Jinan, P. R. China). Column chromatography (CC): *AB-8* resin (*Changzhou Bao'en Chemical Co.*, Hebei, P. R. China); silica gel ($SiO_2$, 200–300 mesh; *Marine Chemical Group Co.*, Qingdao, P. R. China); *Sephadex LH-20* (*Pharmacia Fine Chemicals*, Piscataway, NJ, USA); polyamide (*Zhejiang Siqing Chemical Co.*, Taizhou, P. R. China); and *ODS-C18* (*YMC Co.*, Japan). TLC: *Merck* silica gel *60 F 254*, *RP-18 F 254*, or polyamide membrane; visualization under UV 365 nm and by spraying with 5% $H_2SO_4$ or 5% $FeCl_3$ reagent, or by exposing the sample to $NH_3$ or $I_2$ vapor. UV Spectra: *TU-1800* UV spectrophotometer (*Beijing Purkinje General Instrument Co., Ltd.*, P. R. China). CD Spectra: *Applied Photophysics Chirascan* CD spectrophotometer (*Applied Photophysics Ltd.*, UK). NMR Spectra: *Bruker AV-600*, $\delta$ in ppm, with TMS as an internal standard, *J* in Hz. HR-MS Spectra: *ThermoFinnigan LTQ-Orbitrap XL* mass spectrometer (*ThermoFinnigan*, D-Bremen), in *m*/*z*.

*Plant Material.* Dried plants of *P. oleracea* were purchased from *Jinan Dahong Drug Co.* (Shandong, P. R. China) in January 2006 and authenticated by Prof. *Y.-L. Chen*. A voucher specimen (No. MCX-06-1-4) was deposited with the Institute of Pharmacognosy, School of Pharmacy, Shandong University.

*Extraction and Isolation.* The dried plants (50 kg) of *P. oleracea* were refluxed three times (1 h each time) with EtOH/$H_2O$ 7:3 (250 l, 200 l, and 200 l). The combined extract was condensed to a concentration of 0.5 g/ml. The supernatants were applied to *AB-8* resin and eluted sequentially with $H_2O$, $H_2O$/EtOH 7:3, $H_2O$/EtOH 4:6, and $H_2O$/EtOH 5:95. The fractions obtained with $H_2O$/EtOH 7:3 were concentrated and then extracted sequentially with AcOEt and BuOH. The BuOH extract (64 g) was subjected to CC ($SiO_2$ (1.35 kg); AcOEt/MeOH 9:1, 8:2, 7:3, 6:4, 5:5, and MeOH) to afford *Eluates 1–148*. *Eluate 26–68* (24.43 g) was subjected to CC (polyamide (400 g); EtOH/$H_2O$ 45:55 to 95:5). The resulting *Eluate 7–80* (0.73 g) was fractionated by CC (*ODS* (20 g); MeOH/$H_2O$ 2:8, 4:6, 6:4, 7:3) at reduced pressure. The combined fractions obtained with MeOH/$H_2O$ 4:6 and 7:3 were subjected to CC (*ODS* (100 g) EtOH/$H_2O$ 35:65, 60:40, 80:20). The resulting and combined *Frs. 2–16* (200 mg) were further fractionated by CC (*ODS* (130 g); EtOH/$H_2O$ 15:85 to 35:65). The resulting *Eluate 213–218* was then purified by CC (*Sephadex LH-20* (20 g); EtOH/$H_2O$ 65:35). Yellow crystals (mixture of **1** and **2**) were obtained from *Frs. 18–28*. Then, the mixture was subjected to prep. HPLC (a *Lumtech* LC instrument with a UV detector, monitored at 336 nm using a *YMC-Pack ODS-A* column (250 mm × 10 mm, 5 μm); MeOH/$H_2O$ 35:65) to afford **1** (8.3 mg) and **2** (2.5 mg).

*DPPH Free Radical-Scavenging Activity.* The method reported by *Termentzi et al.* [13] was slightly modified and used in our experiment to evaluate the scavenging activities of oleracein F and G (**1** and **2**, resp.) against DPPH free radical. A natural antioxidant, vitamin C (VC), and a well-known synthetic antioxidant, butylated hydroxy anisole (BHA), were used as the positive controls. Briefly, 0.1 ml of different concentrations of test samples was mixed vigorously with 3.9 ml of DPPH soln. (25 μg/ml). The mixture was kept at r.t. in the dark for 30 min prior to the absorbance determination at 515 nm with a *TU-1800* spectrophotometer. The apparatus was zeroed by the MeOH or MeOH/DMSO 39:1 (*v*/*v*). The DPPH radical-scavenging activity was determined by measuring the content of remaining DPPH•, which was calculated according to the following equation:

$$\%\,\mathrm{DPPH}^{\bullet}_{R} = [(\mathrm{DPPH}^{\bullet})_{T=30}/(\mathrm{DPPH}^{\bullet})_{T=0}] \times 100,$$

where $\%\,\mathrm{DPPH}^{\bullet}_{R}$ is the percentage of remaining DPPH•, $\mathrm{DPPH}^{\bullet}_{T=0}$ is the initial concentration of DPPH•, and $\mathrm{DPPH}^{\bullet}_{T=30}$ is the concentration of DPPH• at 30 min. The scavenging activity of each sample was expressed as $EC_{50}$, which represented the concentration of sample necessary to decrease the initial DPPH• concentration by 50%. This value was obtained by interpolation from the linear regression analysis.

*Methyl (2*S*)-6-[(β-*D*-Glucopyranosyl)oxy]-2,3-dihydro-5-hydroxy-1-[(2*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]-1*H*-indole-2-carboxylate* (**1**). Yellow powder. UV (MeOH): 304 (3.62), 345 (3.88). CD ($c = 0.04$, MeOH): 238 (−1.0), 260 (+0.7). $^1$H- and $^{13}$C-NMR: see *Table 1*. HR-ESI-MS (pos.): 548.1748 ($[M + H]^+$, $C_{26}H_{30}NO_{12}^+$; calc. 548.1763), 570.1571 ($[M + Na]^+$, $C_{26}H_{29}NNaO_{12}^+$; calc. 570.1582).

*Methyl (2*S*)-6-[(β-*D*-Glucopyranosyl)oxy]-2,3-dihydro-5-hydroxy-1-[(2*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]-1*H*-indole-2-carboxylate* (**2**). Yellow powder. UV (MeOH): 304 (3.67), 335 (3.75). CD ($c = 0.008$, MeOH): 245 (+0.17), 265 (+0.38). $^1$H- and $^{13}$C-NMR: see *Table 1*. HR-ESI-MS (pos.): 518.1651 ($[M + H]^+$, $C_{25}H_{28}NO_{11}^+$; calc. 518.1657), 540.1469 ($[M + Na]^+$, $C_{25}H_{27}NNaO_{11}^+$; calc. 540.1476).

This work was supported by *National Natural Science Foundation of China* (Project No. 30500651).

## REFERENCES

[1] X.-J. Zhang, Y.-B. Ji, Z.-Y. Qu, J.-C. Xia, L. Wang, *Chin. J. Microecol.* **2002**, *14*, 277.
[2] K. Chan, M. W. Islam, M. Kamil, R. Radhakrishnan, M. N. M. Zakaria, M. Habibullah, A. Attas, *J. Ethnopharmacol.* **2000**, *73*, 445.
[3] I. Oliveira, P. Valentão, R. Lopes, P. B. Andrade, A. Bento, J. A. Pereira, *Microchem. J.* **2009**, *92*, 129.
[4] A. N. Rashed, F. U. Afifi, A. M. Disi, *J. Ethnopharmacol.* **2003**, *88*, 131.
[5] L. Liu, P. Howe, Y.-F. Zhou, Z.-Q. Xu, C. Hocart, R. Zhan, *J. Chromatogr., A* **2000**, *893*, 207.
[6] E. S. Elkhayat, S. R. M. Ibrahim, M. A. Aziz, *J. Asian Nat. Prod. Res.* **2008**, *10*, 1039; N. Sakai, K. Inada, M. Okamoto, Y. Shizuri, Y. Fukuyama, *Phytochemistry* **1996**, *42*, 1625; H.-L. Xin, Y.-F. Xu, Y.-H. Hou, Y.-N. Zhang, X.-Q. Yue, J.-C. Lu, C.-Q. Ling, *Helv. Chim. Acta* **2008**, *91*, 2075.
[7] N. E. Awad, *Bull. Fac. Pharm. Cairo Univ.* ***1994****, 32*, 137.
[8] P. C. Feng, L. J. Haynes, K. E. Magnus, *Nature* **1961**, *191*, 1108; J.-Y. Zhang, X.-G. Chen, Z.-D. Hu, X. Ma, *Anal. Chim. Acta* **2002**, *471*, 203; F. Imperato, *Phytochemistry* **1975**, *14*, 2091; M. Mizutani, Y. Hashidoko, S. Tahara, *FEBS Lett.* **1998**, *438*, 236; L. Xiang, D. Guo, R. Ju, B. Ma, Y. Chen, L. Du, *Chin. Tradit. Herbal Drugs* **2007**, *38*, 1622.
[9] L. Xiang, D. Xing, W. Wang, R. Wang, Y. Ding, L. Du, *Phytochemistry* **2005**, *66*, 2595.
[10] Z. Yang, C. Liu, L. Xiang, Y. Zheng, *Phytother. Res.* **2009**, *23*, 1032.
[11] J. Xing, Z. Yang, B. Lv, L. Xiang, *Rapid Commun. Mass Spectrom.* **2008**, *22*, 1415.
[12] D. Q. Yu, J. S. Yang, 'Analytical Chemistry Guidance, second ed. NMR Spectroscopic Analysis', Chemical Industry Press, Beijing, Vol. 7, p. 901.
[13] A. Termentzi, P. Kefalas, E. Kokkalou, *Food Chem.* **2006**, *98*, 599.

*Received June 30, 2010*