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Phytochemistry 66 (2005) 2595–2601
PHYTOCHEMISTRY
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Alkaloids from *Portulaca oleracea* L.

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Received 5 April 2004; received in revised form 11 July 2005
Available online 3 October 2005

Abstract

Five alkaloids (oleraceins A, B, C, D and E) were isolated from *Portulaca oleracea* L., and their structures determined by spectroscopic methods as 5-hydroxy-1-*p*-coumaric acyl-2,3-dihydro-1*H*-indole-2-carboxylic acid-6-*O*-β-D-glucopyranoside, 5-hydroxy-1-ferulic acyl-2,3-dihydro-1*H*-indole-2-carboxylic acid-6-*O*-β-D-glucopyranoside, 5-hydroxy-1-(*p*-coumaric acyl-7′-*O*-β-D-glucopyranose)-2,3-dihydro-1*H*-indole-2-carboxylic acid-6-*O*-β-D-glucopyranoside, 5-hydroxy-1-(ferulic acyl-7′-*O*-β-D-glucopyranose)-2,3-dihydro-1*H*-indole-2-carboxylic acid-6-*O*-β-D-glucopyranoside and 8,9-dihydroxy-1,5,6,10b-tetrahydro-2*H*-pyrrolo[2,1-a]isoquinolin-3-one, respectively.

Keywords: *Portulaca oleracea* L.; Portulacaceae; Alkaloids; Oleracein A–E

1. Introduction

Portulaca oleracea L. (Portulacaceae) is a widely distributed weed. It has been used as a folk medicine in many countries as a diuretic, febrifuge, antiseptic, antispasmodic and vermifuge. It exhibits a wide range of pharmacological effects, including antibacterial (Zhang et al., 2002), analgesic, anti-inflammatory (Chan et al., 2000), skeletal muscle-relaxant (Parry et al., 1987; Parry et al., 1993) and wound-healing (Rashed et al., 2003) activities. It is also consumed as a vegetable and has been reported to be rich in α-linolenic acid and β-carotene (Liu et al., 2000). In addition to flavonoids, coumarins (Awad, 1994) and a monoterpene glycoside (Sakai et al., 1996), alkaloids have also been reported to be important chemical constituents of this plant. In particular, it contains *N-trans*-feruloyltyramine (Mizutani et al., 1998), dopamine, dopa and a high concentration of noradrenaline (Feng et al., 1961). As in many other members of the Caryophyllales, *P. oleracea* contains betalains rather than anthocyanins. Betalains are water-soluble nitrogen-containing pigments, which consist of the red- violet betacyanins and the yellow betaxanthins (Strack et al., 2003). To date, two acylated betacyanins, oleracins I and II, have been isolated from stems of *P. oleracea* (Piattelli and Minale, 1964; Imperato, 1975).

In the present study, we isolated five new alkaloids, called oleraceins A (**1**), B (**2**), C (**3**), D (**4**) and E (**5**), from *P. oleracea* L., together with some known constituents including *p*-coumaric acid (**6**) (Fang et al., 1989; Ding and Chen, 1990), ferulic acid (**7**) (Chen, 2000) and adenosine (**8**) (Meng et al., 1981). In this paper, we report the isolation and structural elucidation of the five new alkaloids.

2. Results and discussion

Compound **1** was a water-soluble yellow powder and showed a yellow-brown fluorescence under UV light at 365 nm and retained this color when exposed to ammonia vapor. The molecular formula of **1** was determined to be $C_{24}H_{25}O_{11}N$ according to ion peaks at 504.1500 $[M+H]^+$ (calc. for $C_{24}H_{26}O_{11}N$: 504.1500) and 526.1319 $[M+Na]^+$ (calc. for $C_{24}H_{25}O_{11}NNa$: 526.1319) in the HR-P-FAB-MS spectrum. The ^1H NMR spectrum (data

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doi:10.1016/j.phytochem.2005.08.011

shown in Table 1) revealed that compound **1** contained a moiety with signals that were similar to those of *cyclo*-dopa: two isolated aromatic protons at δ 6.70 (1H, *s*) and δ 8.31(1H, *s*), one nitrogen-connected methine at δ 5.32, and two methylene protons at δ 3.12 (1H, *d*, $J = 16.5$ Hz) and δ 3.45 (1H, *m*), respectively. ^{1}H–^{1}HCOSY spectra indicated that the methine was coupled with the methylene. In addition to these signals, the ^{1}H NMR spectrum also showed AA′BB′-type aromatic protons at δ 7.49 (2H, *d*, $J = 9.0$ Hz) and δ 6.48 (2H, *d*, $J = 9.0$ Hz) as well as two *trans*-olefinic protons at δ 6.73 (1H, *d*, $J = 15.0$ Hz) and δ 7.48 (1H, *d*, $J = 15.0$ Hz), which demonstrated that compound **1** contained one acylated *p*-coumaric moiety. The HMBC spectra showed that the methylene protons at δ 3.12 and δ 3.45 were correlated with a carboxyl carbon at δ 173.8, a methine carbon at δ 60.9 and aromatic carbons at δ 125.1 and δ 135.5, respectively. The aromatic proton at δ 6.70 was assigned as H-4 because it showed connectivity with the methylene carbon at δ 32.8 and aromatic carbons at δ 135.5 and δ 143.8, and another proton at δ 8.31 was assigned as H-7 based on its correlation with aromatic carbons at δ 125.1, δ 135.5 and δ 143.5. These findings further supported the presence of a *cyclo*-dopa moiety. At the same time, correlation from the olefinic protons to a carbonyl carbon at δ 163.8 and aromatic carbons at δ 126.0 and δ 129.8 also confirmed the existence of an acylated *p*-coumaric moiety. The formation of acylamide from *cyclo*-dopa with *p*-coumaric acyl groups made the carbonyl signal shift slightly upfield (δ 163.8). The signals of a sugar moiety were clearly seen in the ^{13}C NMR spectrum, and it was determined to be glucose with a β configuration by comparison with reference data (Yu and Yang, 1999) and the coupling constant (5.5 Hz) of the anomeric proton. In the NOESY spectrum, the anomeric proton (δ 4.58) only correlated with H-7 (δ 8.31), which shows that the glucosyl moiety was connected to C-6. Based on the above analysis, compound **1** was determined to be 5-hydroxy-1-*p*-coumaric acyl-2,3-dihydro-1*H*-indole-2-carboxylic acid-6-*O*-β-D-glucopyranoside, and was called oleracein A. The hydrogen and carbon signals were assigned in detail according to analysis of the DEPT, ^{1}H–^{1}HCOSY, HMQC, HMBC and NOESY spectra.

Compounds **1**–**4** were all highly water-soluble yellow powders and contained a *cyclo*-dopa moiety (Fig. 1). Their ^{1}H NMR and ^{13}C NMR spectroscopic data are shown in Tables 1 and 2, respectively. Compound **2** displayed a yellow-brown fluorescence under UV 365 nm and turned bright yellow when treated with ammonia vapor. Thus, it could be easily distinguished from compound **1**. Based on a careful analysis of the HR-P-ESI-MS spectrum, the molecular formula of compound **2** was confirmed to be $C_{25}H_{27}O_{12}N$ based on ion peaks at 534.1607 $[M + H]^+$ (calc. for $C_{25}H_{28}O_{12}N$: 534.1606) and 556.1424 $[M + Na]^+$ (calc. for $C_{25}H_{27}O_{12}NNa$: 556.1425), which is 30 more than the molecular weight of compound **1**. The only difference between the structures of compounds **2** and **1** was that the former contained an acylated ferulic acid group, instead of the acylated *p*-coumaric moiety. This was also evident from the signals of a methoxy group in the ^{13}C NMR spectrum. The HMBC spectrum further

Table 1
^{1}H NMR spectroscopic data for oleraceins A–D (500 MHz, DMSO-d_6)

No.	Oleracein A	Oleracein B	Oleracein C	Oleracein D
2	5.32 (1H, *d*, $J_{2-3A} = 10.0$ Hz)	5.35 (1H)	5.39 (1H, *d*, $J_{2-3A} = 9.0$ Hz)	5.05 (1H)
3A	3.45 (1H, *m*)	3.48 (1H, *m*)	3.45 (1H, *m*)	3.45 (1H, *m*)
3B	3.12 (1H, *d*, $J_{3A-3B} = 16.5$ Hz)	3.11 (1H, *d*, $J_{3A-3B} = 17.0$ Hz)	3.15 (1H, *d*, $J_{3A-3B} = 17.0$ Hz)	3.15 (1H, *d*, $J_{3A-3B} = 16.0$ Hz)
4	6.70 (1H, *s*)	6.57 (1H, *s*)	6.72 (1H, *s*)	6.67 (1H, *s*)
5-OH	8.51 (1H, *s*)	8.49 (1H, *s*)	8.54 (1H, *s*)	8.50 (1H, *s*)
7	8.11 (1H, *s*)	8.11 (1H, *s*)	8.12 (1H, *s*)	8.13 (1H, *s*)
2′	6.73 (1H, *d*, $J = 15.0$ Hz)	6.71 (1H, *d*, $J = 14.0$ Hz)	6.83 (1H, *d*, $J = 15.0$ Hz)	6.84 (1H, *d*, $J = 15.0$ Hz)
3′	7.48 (1H, *d*, $J = 15.0$ Hz)	7.46 (1H, *d*, $J = 14.0$ Hz)	7.53 (1H, *d*, $J = 15.0$ Hz)	7.46 (1H, *d*, $J = 15.0$ Hz)
5′	7.49 (1H, *d*, $J = 9.0$ Hz)	7.26 (1H, *br.s*)	7.62 (1H, *d*, $J = 8.0$ Hz)	7.26 (1H, *br.s*)
6′	6.84 (1H, *d*, $J = 9.0$ Hz)		7.06 (1H, *d*, $J = 8.0$ Hz)	
6′-OCH_3		3.79 (3H, *s*)		3.84 (3H, *s*)
7′-OH	9.9 (1H, *s*)	9.55 (1H, *s*)		
8′	6.84 (1H, *d*, $J = 9.0$ Hz)	6.78 (1H, *d*, $J = 8.0$ Hz)	7.06 (1H, *d*, $J = 8.0$ Hz)	7.08 (1H, *d*, $J = 7.5$ Hz)
9′	7.49 (1H, *d*, $J = 9.0$ Hz)	7.07 (1H, *d*, $J = 8.0$ Hz)	7.62 (1H, *d*, $J = 8.0$ Hz)	7.15 (1H, *d*, $J = 7.5$ Hz)
1″	4.58 (1H, *d*, $J = 5.5$ Hz)	4.56 (1H, *d*, $J = 5.5$ Hz)	4.60 (1H, *d*, $J = 4.5$ Hz)	4.55 (1H, *d*, $J = 4.5$ Hz)
2″	3.28–3.42 (1H, *m*)	3.26–3.47 (1H, *m*)	3.2–3.8 (1H, *m*)	3.2–3.8 (1H, *m*)
3″	3.28–3.42 (1H, *m*)	3.26–3.47 (1H, *m*)	3.2–3.8 (1H, *m*)	3.2–3.8 (1H, *m*)
4″	3.28–3.42 (1H, *m*)	3.26–3.47 (1H, *m*)	3.2–3.8 (1H, *m*)	3.2–3.8 (1H, *m*)
5″	3.28–3.42 (1H, *m*)	3.26–3.47 (1H, *m*)	3.2–3.8 (1H, *m*)	3.2–3.8 (1H, *m*)
6″	3.71–3.82 (2H, *m*)	3.59–3.69 (2H, *m*)	3.82 (2H, *m*)	3.82 (2H, *m*)
1‴			4.92 (1H, *d*, $J = 7.5$ Hz)	4.94 (1H, *d*, $J = 6.0$ Hz)
2‴			3.2–3.8 (1H, *m*)	3.2–3.8 (1H, *m*)
3‴			3.2–3.8 (1H, *m*)	3.2–3.8 (1H, *m*)
4‴			3.2–3.8 (1H, *m*)	3.2–3.8 (1H, *m*)
5‴			3.2–3.8 (1H, *m*)	3.2–3.8 (1H, *m*)
6‴			3.82 (2H, *m*)	3.82 (2H, *m*)

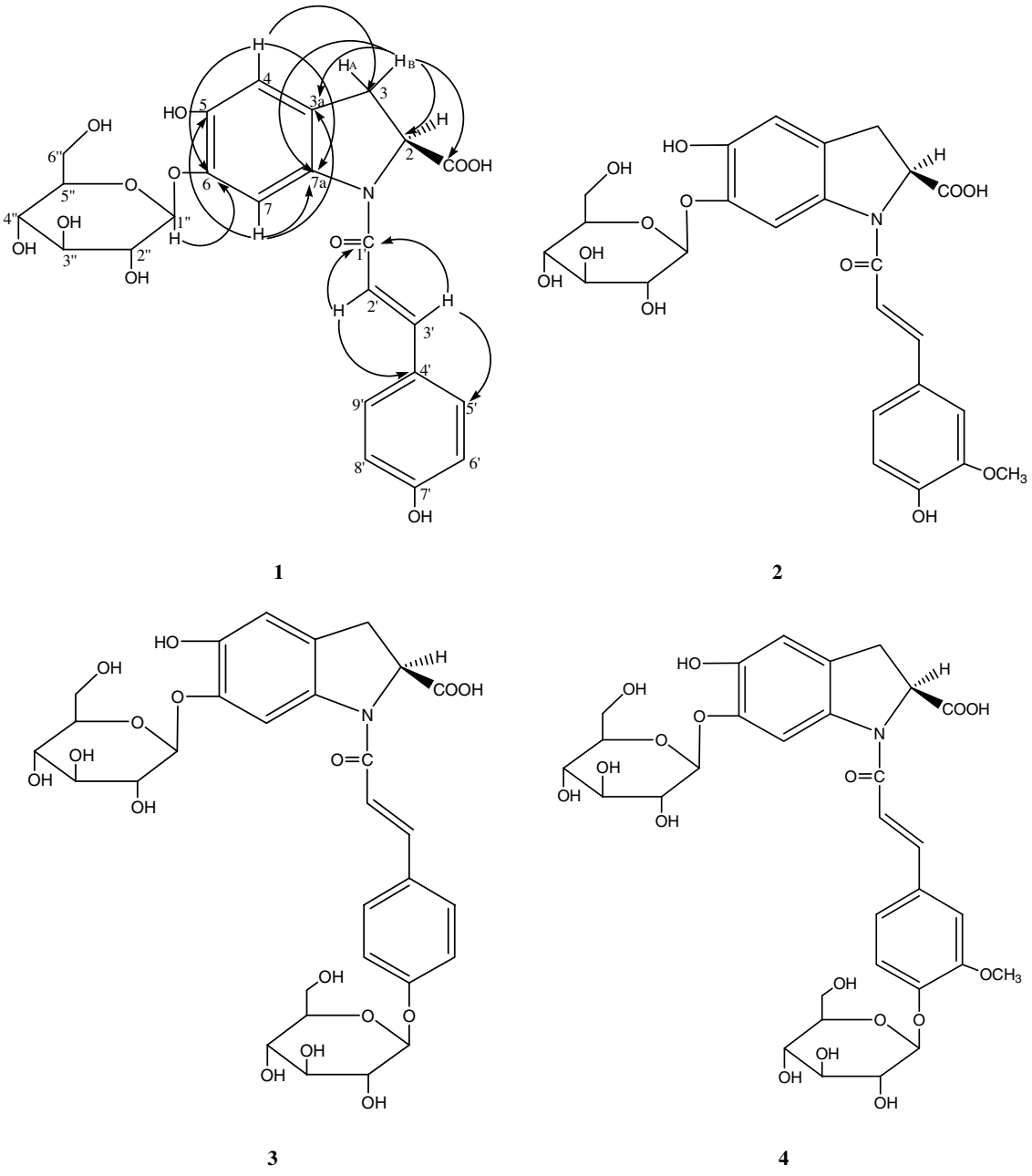

Fig. 1. Structures of **1**–**4** and key HMBC correlations of **1**.

confirmed the presence of an acylated ferulic moiety based on correlations from the aromatic proton at δ 7.07 (1H, *d*, $J = 8.0$ Hz) to carbons at δ 111.7 and δ 148.5, correlations from an aromatic proton at δ 6.78 (1H, *d*, $J = 8.0$ Hz) to carbons at δ 126.5 and δ 147.7, and a correlation from the methoxy protons to an aromatic carbon at δ 147.7. As in compound **1**, the NOESY spectrum also showed the correlation of the anomeric proton (δ 4.56) with H-7 (δ 8.11), which showed that the glucosyl moiety was connected to C-6. Therefore, compound **2** was elucidated to be 5-hydroxy-1-ferulic acyl-2,3-dihydro-1*H*-indole-2-carboxylic acid-6-*O*-β-D-glucopyranoside, and was called oleracein B.

Compound **3** possessed one more sugar moiety than compound **1**, as indicated by analysis of its ^{13}C NMR spectroscopic data, as well as by the ion signals at 666.2034 $[M + H]^+$ (calc. for $C_{30}H_{36}O_{16}N$: 666.2028) and 688.1851 $[M + Na]^+$ (calc. for $C_{30}H_{35}O_{16}NNa$: 688.1848) in the HR-P-ESI-MS spectrum. Based on the literature (Yu and Yang, 1999) and the coupling constants of the anomeric protons (4.5 and 7.5 Hz), these two sugar moieties were both determined to be glucose with a β configuration. In the HMBC spectrum, the anomeric protons at δ 4.60 and δ 4.92 correlated with the carbons at δ 144.6 and δ 159.2, respectively. In addition, the NOESY spectrum showed that the anomeric protons at δ 4.60 only correlated with

Table 2
^{13}C NMR spectroscopic data for oleraceins A–D (125 MHz, DMSO-d_6)

No.	Oleracein A	Oleracein B	Oleracein C	Oleracein D
2	60.9	61.1	61.5	63.2
2-COOH	173.8	173.6	174.4	175.0
3	32.8	32.7	33.4	33.9
4	111.8	111.7	112.6	112.4
5	143.5	143.3	144.2	143.9
6	143.8	143.7	144.6	144.4
7	108	108.1	108.7	109.2
7a	135.5	135.6	136.1	136.5
3a	125.1	125.2	125.7	127.0
1′	163.8	163.7	164.3	164.3
2′	116.4	116.9	118.7	119.9
3′	141.4	141.3	141.5	140.7
4′	126.0	126.5	129.4	129.9
5′	129.8	111.7	130.2	112.4
6′	115.7	147.7	117.2	149.7
OCH_3		55.7		56.5
7′	159.2	148.5	159.3	148.5
8′	115.7	115.6	117.2	115.9
9′	129.8	121.8	130.2	121.9
1″	103.8	103.8	104.4	104.8
2″	73.4	73.4	74.1	74.2
3″	77.0	76.9	77.8	77.7
4″	69.2	69.3	70.3	70.3
5″	75.9	75.9	76.7	76.7
6″	60.4	60.4	61.3	61.3
1‴			100.8	100.6
2‴			73.8	73.8
3‴			77.7	77.7
4‴			69.9	70.0
5‴			77.3	77.5
6‴			61.0	61.1

H-7 (δ 8.12). This indicated that these two glucosyl moieties were connected with C-6 and C-7′, respectively. Thus, compound **3** was elucidated to be 5-hydroxy-1-(*p*-coumaric acyl-7′-*O*-β-D-glucopyranose)-2,3-dihydro-1*H*-indole-2-carboxylic acid-6-*O*-β-D-glucopyranoside, and was called oleracein C.

HR-P-ESI-MS indicated that the molecular formula of compound **4** was $C_{31}H_{37}O_{17}N$ based on ion peaks at 696.2140 $[M+H]^+$ (calc. for $C_{31}H_{38}O_{17}N$: 696.2134) and 718.1949 $[M+Na]^+$ (calc. for $C_{31}H_{37}O_{17}NNa$: 718.1953). It contained one more sugar moiety than compound **2**. Both sugar moieties were determined to be glucose with a β configuration based on the literature (Yu and Yang, 1999) and the coupling constants (4.5 and 6.0 Hz) of anomeric protons. The anomeric protons at δ 4.55 and δ 4.94 were correlated with the aromatic carbons at δ 144.4 and δ 148.4 in the HMBC spectrum, and the NOESY spectrum showed that the anomeric protons at δ 4.55 only correlated with H-7 at δ 8.13, which indicated that these two glucosyl moieties connected to C-6 and C-7′. Therefore, compound **4** was elucidated to be 5-hydroxy-1-(ferulic acyl-7′-*O*-β-D-glucopyranose)-2,3-dihydro-1*H*-indole-2-carboxylic acid-6-*O*-β-D-glucopyranoside, and was called oleracein D.

Compound **5** was a pale-white powder. It was colorless under natural light and turned pink when exposed to iodine vapor. The HR-EI-MS spectrum indicated that the molecular formula of compound **5** was $C_{12}H_{13}O_3N$ based on a molecular ion peak at 219.0875 (calc. for $C_{12}H_{13}O_3N$: 219.0895). The ^1H NMR spectrum showed two isolated aromatic protons at δ 6.48 (1H, *s*) and δ 6.49 (1H, *s*), two phenol protons at δ 8.77 and δ 8.81, one methine proton at δ 4.56 (1H, *t*, $J = 8.0$, 8.0 Hz), and eight methylene protons at δ 3.95 (1H, *m*), δ 2.89 (1H, *m*), δ 2.20 (1H, *m*), δ 2.40 (1H, *m*), δ 1.57 (1H, *m*), δ 2.57 (1H, *m*) and δ 2.58 (2H, *m*), respectively. The methine (δ 4.56) and methylene (δ 3.95, δ 2.89) resonances were shifted downfield due to their connection to nitrogen. The ^1H–^1HCOSY spectrum revealed that the methine (δ 4.56) was coupled with the methylene protons at δ 2.57 and δ 1.57. The latter proton also had correlations with the methylene protons at δ 2.20 and δ 2.40, suggesting the presence of an N–CH–CH_2–CH_2 fragment. The correlations from the methylene protons at δ 3.95 and δ 2.89 to another methylene proton at δ 2.58 indicated the existence of an N–CH_2–CH_2 fragment. In the HMBC spectrum, the aromatic proton at δ 6.48 was correlated with aromatic carbons at δ 128.4 and δ 144.2, as well as a methylene carbon at δ 27.4, which showed that the terminal methylene group in the N–CH_2–CH_2 fragment was connected to the benzene ring. The correlations from the aromatic proton at δ 6.49 to carbons at δ 123.7 and δ 143.9 and a methine carbon at δ 55.5 indicated that the methine in the N–CH–CH_2–CH_2 fragment was connected to the benzene ring. The HMBC spectrum also showed that the methylene protons at δ 2.20 and δ 2.40 were correlated with the methine carbon at δ 55.5, the carbonyl carbon at δ 171.9, and the methylene carbon at δ 27.3, which indicated that the terminus of the N–CH–CH_2–CH_2 fragment was connected to a carbonyl carbon. Based on the above analysis, the structure of compound **5** was elucidated to be 8,9-dihydroxy-1,5,6,10b-tetrahydro-2*H*-pyrrolo[2,1-a]isoquinolin-3-one (Fig. 2), and was called oleracein E. Its ^1H NMR and ^{13}C NMR spectroscopic data are shown in Table 3.

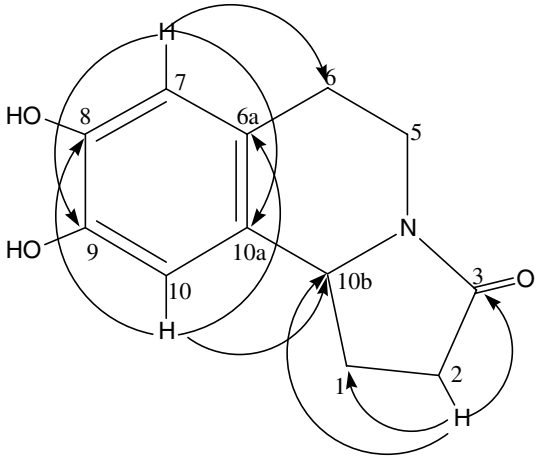

Fig. 2. Structure of **5** and key HMBC correlations.

Table 3
^{1}H NMR and ^{13}C NMR spectroscopic data for oleracein E (DMSO-d_6)

No.	C	H
1	27.3	1.57 (1H, *m*), 2.57 (1H, *m*)
2	31.2	2.20 (1H, *m*), 2.40 (1H, *m*)
3	171.9	
5	36.6	3.95 (1H, *m*), 2.89 (1H, *m*)
6	27.4	2.58 (2H, *m*)
6a	123.7	
7	115.3	6.48 (1H, *s*)
8	143.9	
8-OH		8.81
9	144.2	
9-OH		8.77
10	111.6	6.49 (1H, *s*)
l0a	128.4	
l0b	55.5	4.56(1H, *t*, $J = 8.0$, 8.0 Hz)

Betalains have attracted the interest of researchers in various fields because of their potential for use as food-coloring agents and their antioxidant and radical-scavenging properties, which could help to protect against certain oxidative stress-related disorders. Since they show some structures similar to those of betalains, we were very interested in the *cyclo*-dopa derivatives oleraceins A–D (**1**–**4**), particularly with regard to their biosynthetic pathways and chemotaxonomic significance. Betalains are conjugates of betalamic acid with *cyclo*-dopa and amino acids or amines, respectively. It has been shown that L-[*S*]-dopa serves as a precursor for betalamic acid and L-[*S*]-*cyclo*-dopa (Chang et al., 1974), and the decisive steps in betalain biosynthesis, i.e., condensation of the betalain chromophore betalamic acid with *cyclo*-dopa and amino acid or amine in the respective aldimine formation, are most likely non-enzymatic (Strack et al., 2003). Acylated betacyanins such as oleracins I and II have been isolated from *P. oleracea*, and upon alkaline hydrolysis they gave ferulic acid and two new pigments that were shown to be 5-*O*-β-cellobiosides of betanidin and isobetanidin (Imperato, 1975). Oleraceins A–D (**1**–**4**) isolated in this study are highly water-soluble yellow pigments that all possess a *cyclo*-dopa moiety. However, in contrast to oleracins I and II, they lack betalamic acid. Based on biosynthetic pathway of betalains, it is reasonable to suggest that oleraceins A–D may be derived from the condensation of *cyclo*-dopa with ferulic acid or *p*-coumaric acid, and the *cyclo*-dopa moiety in oleraceins A–D (**1**–**4**) should be the 2*S* configuration, as confirmed by the signals of H-2, H-3A and H-3B in ^{1}H NMR spectrum similar to those of betalains (Schliemann et al., 1996; Kobayashi et al., 2000; Wybraniec et al., 2001).

Moreover, the Caryophyllales-specific occurrence of betalains is a prominent example of the chemotaxonomic relevance of plant secondary products (Strack et al., 2003). In the genus *Portulaca*, different species show distinct patterns of betacyanins. Only oleracin I and oleracin II were detected in stems of *P. oleracea*, while the betacyanins detected in flowers and stems of *Portulaca grandiflora* included betanidin, isobetanidin, betanin, isobetanin, mesembryanthemin II and mesembryanthemin III (Piattelli and Minale, 1964). The yellow pigments in *P. grandiflora* mainly consisted of betaxanthins, including portulacaxanthin, portulacaxanthin II and portulacaxanthin III, vulgaxanthin I, vulgaxanthin II, indicaxanthin, dopaxanthin, miraxanthin (Adachi and Nakatsukasa, 1983; Trezzini and Zryd, 1991) and humilixanthin (Strack et al., 1987). In contrast, so far only the *cyclo*-dopa derivatives oleraceins A–D (**1**–**4**) have been isolated from *P. oleracea* as highly water-soluble yellow pigments. Furthermore, feruloyl, coumaroyl and other phenolic acyl fragments are often found in the structures of betacyanins isolated from other plants in Caryophyllales, usually connected to a sugar moiety, e.g., gomphrenin II [betanidin 6-*O*-(6′-*O*-*E*-4-coumaroyl)-β-D-glucopyranoside] and gomphrenin III [betanidin 6-*O*-(6′-*O*-*E*-feruloyl)-β-D-glucopyranoside] from *Gomphrena globosa* (Heuer et al., 1992). With regard to biosynthesis, the condensation of *cyclo*-dopa with ferulic acid or *p*-coumaric acid could also occur in other Caryophyllales species. It may be worthwhile, therefore, to determine if other related plants also contain the newly isolated yellow pigments oleraceins A–D (**1**–**4**), and thus further studies are needed to determine whether these *cyclo*-dopa derivatives show chemotaxonomical significance within the genus *Portulaca* or even in the Caryophyllales.

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded on a Bruker DRX-500 instrument, using TMS as an internal standard. MS spectra were obtained on APEXII and GCT-MS UK instruments. IR spectra were recorded on a NICOLET 5DX-FTIR spectrophotometer. Column chromatography was performed on AB-8 resin (Tianjin Nankai University Chemical Co., Tianjin, China), silica gel (Qingdao Haiyang Chemical Group Co., Qingdao, China), Sephadex LH-20 (Pharmacia Co.), polyamide (Zhejiang Siqing Chemical Co., Taizhou, China) or ODS (Jinouya Co., Beijing, China), respectively. TLC was conducted on Merck Si gel 60 F254 or with polyamide on a plastic plate. Spots on the TLC plate were visualized under UV light (365 nm) and by spraying with 5% H_2SO_4 or 5% $FeCl_3$ reagent, or by exposing the sample to ammonia or iodine vapor.

3.2. Plant material

Dried plants of *P. oleracea* were purchased from Beijing Songlan Drug Co. (Beijing, China) in September, 2002. A voucher specimen (No. M200209) was kept in Laboratory of Pharmacy, Department of Biological Science and Biotechnology, Tsinghua University.

3.3. Extraction and isolation

The dried plants (9 kg) of *P. oleracea* were refluxed three times (1 h each time) with EtOH–H_2O (7:3, 45 L, 36 L and

36 L). The combined extract was condensed in vacuo to give a concentration of 0.5 g/ml. The concentrated extract(15 L) was applied to AB-8 resin and eluted sequentially with H_2O (27 L), H_2O–EtOH (7:3) (18 L), H_2O–EtOH (4:6) (18 L) and EtOH–H_2O (95:5) (12 L). The combined fractions (200 g) obtained with H_2O–EtOH(7:3 and 4:6) were subjected to silica gel(1.2 kg) CC and eluted sequentially with $CHCl_3$–MeOH (9:1, 8:2, 7:3, 6:4, 5:5) and MeOH (each fraction 500 ml), to obtain eluates 1–76.

The eluate l–4 ($CHCl_3$–MeOH (9:1), 6.2 g) was refractionated on silica gel (360 g), eluting sequentially with petroleum ether (60–90 °C)–EtOAc (95:5, 9:1, 8:2, 7:3, 6:4, 5:5), EtOAc and EtOAc–95% EtOH (9:1, 4:1, 2:l) (each fraction 100 ml). The resulting Fr. 67–78 (petroleum ether–EtOAc (5:5), 0.46 g) was applied to a polyamide (20 g) column, eluting with H_2O–EtOH (7:3) (each fraction 20 ml) to give compounds **6** (8 mg) and **7** (8 mg). The resulting Fr. l00–122 (EtOAc–95% EtOH (4:1), 1.52 g) was subjected to polyamide (20 g) CC and eluted with EtOH–H_2O (1:9) (each fraction 20 ml) to give compound **5** (60 mg).

The eluate 31–38 ($CHCl_3$–MeOH (7:3), 7.2 g) was applied to Sephadex LH-20 (150 g) CC and eluted with MeOH (each 20 ml). The resulting eluate 25–34 (0.93 g) was subjected to Sephadex LH-20 (150 g) CC again and eluted with MeOH (each fraction 20 ml). The resulting Fr. 7–15 (0.14 g) was fractionated by ODS (l00 g) CC and eluted with MeOH–H_2O (1:1) (each fraction 20 ml) to give **1** (6 mg) and **2** (3 mg). Recrystallization of Fr. 17–18 gave compound **8** (6 mg).

The eluate 65–68 ($CHCl_3$–MeOH (5:5), 4.18 g) was applied to a Sephadex LH-20 column(150 g) and eluted with MeOH–H_2O (4:1) (each fraction 20 ml). The resulting Fr. 7–16 (1.02 g) was further fractionated by ODS (l00 g) CC and eluted with EtOH–H_2O (1:9 and 1:4) (each fraction 20 ml). The resultant Fr. 41–49 was then purified using Sephadex LH-20 (100 g) and eluted with MeOH–H_2O (6:4) (each 20 ml) to give compound **3** (27 mg). Fr. 50–56 was purified by this same method and gave compound **4** (9 mg).

3.4. 5-Hydroxy-1-p-coumaric acyl-2,3-dihydro-1H-indole-2-carboxylic acid-6-O-β-D-glucopyranoside (*1*)

Yellow powder. UV $\lambda^{max}_{MeOH}(\log\varepsilon)$: 304 nm (4.01, sh), 336.5 nm (4.07). IR ν^{KBr}_{max} (cm^{-1}): 3342, 2924, 2854, 1731, 1644, 1603, 1512, 1489, 1446, 1336, 1270, 1172, 1072, 1033, 860, 828. HR-P-FAB-MS (*m/z*): 526.1319 $[M+Na]^+$ (calc. for $C_{24}H_{25}O_{11}NNa$: 526.1319), 504.1500 $[M+H]^+$ (calc. for $C_{24}H_{26}O_{11}N$: 504.1500). For ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

3.5. 5-Hydroxy-1-ferulic acyl-2,3-dihydro-1H-indole-2-carboxylic acid-6-O-β-D-glucopyranoside (*2*)

Yellow powder. HR-P-ESI-MS (*m/z*): 534.1607 $[M+H]^+$ (calc. for $C_{25}H_{28}O_{12}N$: 534.1606), 556.1424 $[M+Na]^+$ (calc. for $C_{25}H_{27}O_{12}NNa$: 556.1425). For ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

3.6. 5-Hydroxy-1-(p-coumaric acyl-7′-O-β-D-glucopyranose)-2,3-dihydro-1H-indole-2-carboxylic acid-6-O-β-D-glucopyranoside (*3*)

Yellow powder. $[\alpha]^{26}_D$: −83.70 (*c* 0.35, H_2O). UV $\lambda^{max}_{MeOH}(\log\varepsilon)$: 306.5 nm (4.32), 335 nm (4.28). IR ν^{KBr}_{max} (cm^{-1}): 3350, 2926, 1739, 1641, 1602, 1509, 1490, 1431, 1395, 1303, 1237, 1178, 1072, 1040, 826. P-FAB-MS (*m/z*): 666 $[M+H]^+$. HR-P-ESI-MS (*m/z*): 666.2034 $[M+H]^+$ (calc. for $C_{30}H_{36}O_{16}N$: 666.2028), 688.1851 $[M+Na]^+$ (calc. for $C_{30}H_{35}O_{16}NNa$: 688.1848). For ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

3.7. 5-Hydroxy-1-(ferulic acyl-7′-O-β-D-glucopyranose)-2,3-dihydro-1H-indole-2-carboxylic acid-6-O-β-D-glucopyranoside (*4*)

Yellow powder, $[\alpha]^{26}_D$: +263.85 (*c* 0.15, H_2O). UV $\lambda^{max}_{H_2O}(\log\varepsilon)$ 239 nm (4.13), 295 nm (4.13), 335.5 nm (4.20). IR ν^{KBr}_{max} (cm^{-1}): 3335, 2922, 1644, 1601, 1508, 1498, 1427, 1272, 1072, 1043, 825. P-FAB-MS (*m/z*): 696 $[M+H]^+$, 718 $[M+Na]^+$. HR-P-ESI-MS (*m/z*): 696.2140 $[M+H]^+$ (calc. for $C_{31}H_{38}O_{17}N$: 696.2134), 718.1949 $[M+Na]^+$ (calc. for $C_{31}H_{37}O_{17}NNa$: 718.1953). For ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

3.8. 8,9-Dihydroxy-1,5,6,10b-tetrahydro-2H-pyrrolo[2,1-a]-isoquinolin-3-one (*5*)

Pale-white powder (MeOH); m.p. 238–240 °C. $[\alpha]^{26}_D$: +61.12 (*c* 0.32, MeOH). UV $\lambda^{max}_{MeOH}(\log\varepsilon)$: 318.5 (4.43), 290 (4.44). IR ν^{KBr}_{max} (cm^{-1}): 3176, 2931, 1652, 1524, 1463, 1361, 1337, 1311, 1273, 1236, 1188, 1108, 1068, 1021, 875, 781, 739. HR-EI-MS (*m/z*): 219.0875 $[M]^+$ (calc. for $C_{12}H_{13}O_3N$: 219.0895), 218.0800 $[M-1]^+$ (100%) (calc. for $C_{12}H_{12}O_3N$: 218.0817), 163.0624, 162.0555, 157.0656, 136.1123, 122.0970, 97.0940, 91.0535. For ^1H NMR and ^{13}C NMR spectroscopic data, see Table 3.

References

Adachi, T., Nakatsukasa, M., 1983. High-performance liquid chromatographic separation of betalains and their distribution in *Portulaca grandiflora* and related species. Z. Pflanzenphysiol. 109 (2), 155–162.

Awad, N.E., 1994. Lipid content and antimicrobial activity of phenolic constituents of cultivated *Portulaca oleracea* L.. Bull. Fac. Pharm. Cairo Univ. 32 (1), 137–142.

Chan, K., Islam, M.W., Kamil, M., Radhakrishnan, R., Zakaria, M.N.M., Habibullah, M., Attas, A., 2000. The analgesic and anti-inflammatory effects of *Portulaca oleracea* L. subsp. sativa (Haw.) Celak. J. Ethnopharmacol. 73 (3), 445–451.

Chang, C., Kimler, L., Mabry, T.J., 1974. Biogenesis of betalamic acid. Phytochemistry 13, 2771–2775.

Chen, D.Ch., 2000. Manual of Standard Compounds of Traditional Chinese Medicine. China Medico-Pharmaceutical Science and Technology Press, Beijing, p.109.

Ding, L.S., Chen, W.X., 1990. Studies on the chemical constituents of *Aconitum campylorrhynchum*. Acta Pharm. Sin. 25 (6), 441–444.

Fang, Sh.D., Zhang, Zh.D., Chen, Y., He, Zh.Sh., Cai, G.Q., Du, Ch.J., 1989. Studies on the analgesic constituents from the hyphae of Anluo Xiaopisan (*Marasmius androsaceus*). Chinese Traditional Herbal Drugs 20 (10), 2–4.

Feng, P.C., Haynes, L.J., Magnus, K.E., 1961. High concentration of (−)-noradrenaline in *Portulaca oleracea* L.. Nature 191, 1108.

Heuer, S., Wray, V., Metzger, J.W., Strack, D., 1992. Betacyanins from flowers of *Gomphrena globosa*. Phytochemistry 31, 1801–1807.

Imperato, F., 1975. Acylated betacyanins of *Portulaca oleracea*. Phytochemistry 14 (9), 2091–2092.

Kobayashi, N., Schmidt, J., Nimtz, M., Wray, V., Schliemann, W., 2000. Betalains from *Christmas cactus*. Phytochemistry 54, 419–426.

Liu, L.X., Howe, P., Zhou, Y.F., Xu, Zh.Q., Hocart, C., Zhang, R., 2000. Fatty acids and β-carotene in Australian purslane (*Portulaca oleracea*) varieties. J. Chromatogr. A 893, 207–213.

Meng, X.Zh., Ni, S.F., Suo, H.X., 1981. Studies on *Ixeris sonchifolia* Hance used for coronary heart disease 6. Isolation and identification of the active constituents for dilating coronary blood vessel. J. Shenyang Coll. Pharm. 14, 32–35.

Mizutani, M., Hashidoko, Y., Tahara, S., 1998. Factors responsible for inhibiting the motility of zoospores of the phytopathogenic fungus *Aphanomyces cochlioides* isolated from the non-host plant *Portulaca oleracea*. FEBS Lett. 438 (3), 236–240.

Parry, O., Marks, J.A., Okwuasaba, F., 1993. The skeletal muscle relaxant action of *Portulaca oleracea*: role of potassium ions. J. Ethnopharmacol. 40 (3), 187–194.

Parry, O., Okwuasaba, F., Ejike, C., 1987. Skeletal muscle relaxant action of an aqueous extract of *Portulaca oleracea* in the rat. J. Ethnopharmacol. 19 (3), 247–253.

Piattelli, M., Minale, L., 1964. Pigments of centrospermae-II, distribution of betacyanins. Phytochemistry 3, 547–557.

Rashed, A.N., Afifi, F.U., Disi, A.M., 2003. Simple evaluation of the wound healing activity of a crude extract of *Portulaca oleracea* L. (growing in Jordan) in *Mus musculus* JVI-1. J. Ethnopharmacol. 88 (2–3), 131–136.

Sakai, N., Inada, K., Okamoto, M., Shizuri, Y., Fukuyama, Y., 1996. Portuloside A, a monoterpene glucoside from *Portulaca oleracea*. Phytochemistry 42 (6), 1625–1628.

Schliemann, W., Joy IV, R.W., Komamine, A., Metzger, J.W., Nimiz, M., Wray, V., Strack, D., 1996. Betacyanins from plants and cell cultures of *Phytolacca americana*. Phytochemistry 42 (4), 1039–1046.

Strack, D., Schmitt, D., Reznik, H., Boland, W., Grotjahn, L., Wray, V., 1987. Humilixanthin, a new betaxanthin from *Rivina humilis*. Phytochemistry 26 (8), 2285–2287.

Strack, D., Vogt, T., Schliemann, W., 2003. Recent advances in betalain research. Phytochemistry 62, 247–269.

Trezzini, G.F., Zryd, J.P., 1991. Two betalains from *Portulaca grandiflora*. Phytochemistry 30 (6), 1897–1899.

Yu, D.Q., Yang, J.Sh., 1999. Analytical Chemistry Guidance, second ed. NMR Spectroscopic Analysis, vol. 7, Chemical Industry Press, Beijing, p. 901.

Wybraniec, S., Platzner, I., Geresh, S., Gottlieb, H.E., Haimberg, M., Mogilnitzki, M., Mizrahi, Y., 2001. Betacyanins from vine cactus *Hylocereus polyrhizus*. Phytochemistry 58, 1209–1212.

Zhang, X.J., Ji, Y.B., Qu, Zh.Y., Xia, J.Ch., Wang, L., 2002. Experimental studies on antibiotic functions of *Portulaca oleracea* L. in vitro. Chinese J. Microecol. 14 (5), 277–280.