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Spectroscopic, stability and radical-scavenging properties of a novel pigment from gardenia

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Abstract

A novel pigment, named gardecin, has been isolated from gardenia fruits, together with another five known crocins. The pigment, which possessed a structure which is unique among crocins, was characterised using spectrometric techniques, particularly 1D and 2D NMR. The NMR assignments were based on data from ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H–^{1}H COSY, NOESY, HMQC and HMBC measurements. The five known crocins were identified on the basis of MS, UV/visible and 1D NMR data. Chemical stability and antioxidant ability of gardecin in comparison with the other five crocins were studied. The stronger DPPH free radical-scavenging ability of gardecin compared, with the other crocins, was observed. Kinetic studies have shown that all crocins were unstable under various conditions, but surprisingly gardecin was fairly stable.

Keywords: Gardecin; *Gardenia jasminoides*; Pigment; Spectroscopic; Structure elucidation; Crocin; Antioxidant; Stability

1. Introduction

Crocins are some of the few carotenoids found in nature that are freely soluble in water, which is the reason for their widespread application as a food colourant. The light absorption spectra of the main crocins from gardenia fruits have a fine-structure typical of carotenoids; they are glycosyl ester derivatives of the C_{20}-dicarboxylic acid crocetin (8,8′-diapocarotene-8,8′-dicarboxylic acid) (Fig. 1).

Numerous studies have dealt with the yellow pigments isolated from gardenia fruits and their spectroscopic characterisation (Carmona, Zalacain, Sanchez, Novella, & Alonso, 2006; Choi et al., 2001; Ichi et al., 1995; Pfister, Meyer, Steck, & Pfander, 1996; Van Calsteren et al., 1997). On the other hand, although stability of saffron pigments was well investigated and summarised by Tsimidou and Tsatsaroni (1993), comparative kinetic data of crocins is still relatively scarce.

In the course of our screening for antioxidants from gardenia fruits, we previously obtained a series of major crocins from the herb. A further search for additional minor agents from gardenia fruits has resulted in the isolation of a novel pigment, named gardecin, whose structure probably derived from the modification of a similar precursor to crocins. With one ester group substituted by a ketoneic bond, gardecin featured a structure which is unique among crocins. The properties of a carotenoid molecule are primarily dependent upon its structure and hence its chemistry. Therefore, we were interested in the chemical stability and radical-scavenging ability of the pigment, in comparison with the main crocins isolated from gardenia fruits.

2. Materials and methods

2.1. Apparatus

Medium pressure liquid chromatography (MPLC) separations were performed using an MCI (CHP20P, 75–150 μm, Mitsubishi, Tokyo, Japan) column MPLC system

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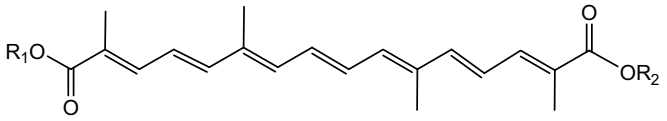
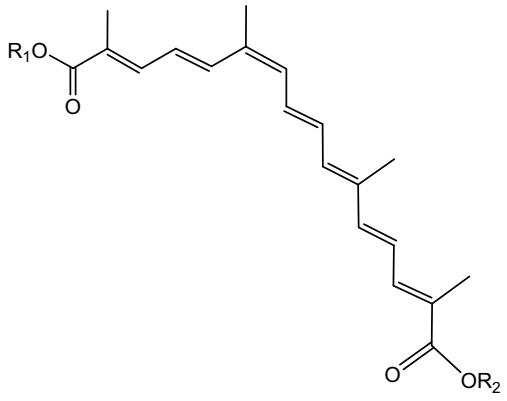
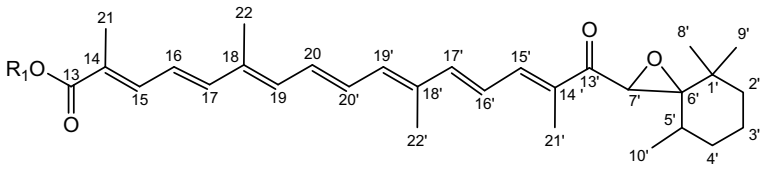


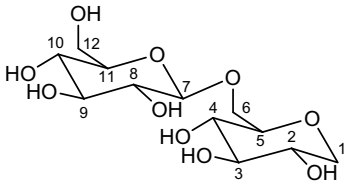
crocin-1: R_1=R_2=Gen (2)
crocin-2: R_1=Gen, R_2=Glu (3)
crocin-3: R_1=Gen, R_2=H (4)
crocin-4: R_1=Glu, R_2=H (5)
crocetin: R_1=R_2=H (7)



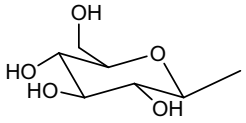
cis crocin-1: R_1=R_2=Gen (6)



gardecin:R_1=Gen(1)



gentiobiosyl(Gen)



glucosyl(Glu)

Fig. 1. Structures of gardecin and crocins.

(Buchi, Flamil, Switzerland). High performance liquid chromatography (HPLC) was performed on a Shimadzu HPLC system, equipped with two LC-10AT VP pumps, CTO-10AS VP column oven, UV–vis SPD-10A VP detector and fitted with a ODS column (150 × 4 mm; Shimadzu, Tokyo, Japan). ESI–MS spectra were recorded on a Finnigan LCQ^{DECA} spectrometer, and high resolution ESI–MS spectra were measured on a VG Autospec 3000 mass spectrometer. IR spectra were obtained on a Nicolet FT-IR spectrophotometer. ^{1}H, ^{13}C NMR and 2D NMR experiments were measured in DMSO-D_6, with TMS as internal standard, on a Varian Unity INOVA 400/54 NMR spectrometer. Visible spectra were recorded on the Cintra 10_e UV/visible spectrometer (GBC, Dandenong, Australia). pH measurements were recorded using the PHS-3C digital pH meter (Rex, Shen Zen, China).

2.2. Materials

Dried gardenia fruits (*Gardenia jasminoides* Ellis) were purchased from Chengdu, Sichuan province, in August 2004, and identified by Hao Zhang at West China School Of Pharmacy, Sichuan University, China. Methanol (Sigma, St. Louis, MO) was of chromatographic purity and water was double distilled for HPLC. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical (Sigma) was used for testing the radical-scavenging activity of gardecin and crocins, and α-tocopherol (Sigma) was used as a reference. All other

reagents and solvents were analytical grade and purchased from local firms.

2.3. Extraction and isolation

The dried gardenia fruits (40 kg) were ground to a coarse powder and extracted with ethanol:water (40:60) by cold percolation (4 × 40 l). The alcohol extract was concentrated, suspended in water, and then partitioned with ethyl acetate. The ethyl acetate layer extract was subjected to column chromatography (CC) on silica gel, eluting with CH_2Cl_2, containing increasing amounts (3%, 5%, 7%, 10%) of methanol. Upon concentration of the fraction eluted with 3% MeOH, crocetin (7, 40 mg) crystallised. The fraction eluted with 5% MeOH was purified by CC on silica gel, which yielded crocin-4 (5, 60 mg). The fraction eluted with 10% MeOH was separated by CC on Sephadex LH-20 (Amersham, Uppsala, Sweden) (CH_2Cl_2:methanol, 1:1) and purified by medium pressure preparative liquid chromatography on MCI (ethanol:water, 30–60%), which yielded analytically pure orange amorphous gardecin (1, 45 mg). The water layer, further diluted with water, was subjected to HPD-100 resin (Cangzhou bon, Hebei, China), and eluted with water containing increasing amounts (0%, 25%, 60%) of ethanol. The 60% ethanol fraction was evaporated to dryness and separated by CC on

Table 1
1D and 2D NMR data of gardecin (DMSO, δ in ppm, J in Hz)

Position	^{13}C NMR	^{1}H NMR	HMBC	^{1}H–^{1}H COSY	NOESY
1	94.8	5.43 d(8.0)	C5,13	H-2	H-3, H-5
2	72.6	3.27 m	C1,3	H-1, H-3	H-4
3	76.51	3.28 m	C1,2,4,5	H-2, H-4	H-1, H-5
4	69.41	3.29 m	C2,3,5	H-3, H-5	H-2, H-6(β)
5	76.35	3.44 m	C3,4	H-4, H-6(α), H-6(β)	H-1, H-3
6	68.18	3.61 m(α)	C5,7	H-5, H-6(β)	H-6(β), H-7
		4.00 d(β)(10.4)	C5,7	H-5, H-6(α)	H-4, H-6(α)
7	103.3	4.18 d(8.0)	C6,8,9,11	H-8	H-6(α),H-9, H-11
8	73.61	2.97 t(8.4)	C7,9	H-7, H-9	–
9	76.84	3.16 m	C8,10	H-8, H-10	H-7, H-11
10	70.16	3.08 m	C9,11	H-9, H-11	H-12(α)
11	77.04	3.09 m	C7,9,10,12	H-10, H-12(α), H-12(β)	H-7, H-9
12	61.18	3.48 m(α)	–	H-11, H-12(β)	H-10
	–	3.68 m(β)	C10	H-11, H-12(α)	–
13	166.49	–	–	–	–
14	125.63	–	–	–	–
15	140.17	7.37 d(11.2)	C13,17,21	H-16	H-17, H-19
16	124.29	6.69 m	C14,17,18	H-15, H-17	H-21, H-22
17	145.48	6.81 m	C15,16,18,19,22	H-16	H-15, H-19
18	137.42	–	–	–	–
19	136.19	6.55 d (10)	C17,20,20′,22	H-20	H-15, H-17
20	132.22	6.90 m	C18,19,19′	H-19, H-20′	H-22
21	12.92	1.98 s	C13,14,15	–	H-16
22	12.78	2.01 s	C17,18,19	–	H-16, H-20
13′	195.57	–	–	–	–
14′	133.98	–	–	–	–
15′	141.12	7.31 d(9.6)	C13′,17′,21′	H-16′	H-2′(α), H-5′, H-7′, H-8′, H-10′, H-17′, H-19′
16′	124.29	6.77 m	C14′,15′,18′	H-15′, H-17′	H-7′, H-8′, H-21′, H-22′
17′	144.85	6.84 m	C15′,16′,18′,19′,22′	H-16′	H-15′, H-19′
18′	137.06	–	–	–	–
19′	137.06	6.65 m	C17′,18′,22′,20′	H-20′	H-15′, H-17′
20′	132.66	6.90 m	C19,18′,19′	H-19′, H-20	H-22′
21′	11.5	1.90 s	C13′,14′,15′	–	H-7′, H-16′
22′	12.78	1.99 s	C17′,18′,19′	–	H-16′, H-20′
1′	35.63	–	–	–	–
2′	39.28	1.42 m(α)	C1′,6′,9′	H-2′(β), H-3′	H-5′, H-15′
	–	1.55 m(β)	C1′,6′	H-2′(α), H-3′	H-9′, H-10′
3′	19.32	1.53 m	C1′	H-2′(α), H-2′ (β), H-4′(α), H-4′(β)	H-9′, H-10′
4′	32.93	1.32 br(α)	–	H-3′, H-4′(β), H-5′	H-10′
	–	1.46 m(β)	–	H-3′, H-4′(α), H-5′	–
5′	30.92	1.78 m	C6′,10′,4′	H-4′(α), H-4′(β), H-10′	H-2′ (α),H-8′, H-10′, H-15′
6′	70.41	–	–	–	–
7′	62.47	3.94 s	C1′,6′,13′,14′	–	H-8′, H-9′, H-15′, H-16′, H-21′
8′	26.28	1.09 s	C1′,2′,6′,9′	–	H-5′, H-7′, H-15′, H-16′
9′	24.79	0.94 s	C1′,2′,6′,8′	–	H-3′, H-7′
10′	17.48	0.66 d(6.8)	C4′,5′,6′	H-5′	H-2′(β), H-4′(α), H-5′, H-15′

silica gel, eluting with ethyl acetate containing increasing amounts (5%, 10%, 15%, 20%) of methanol:water (16:13), the 5% methanol:water, (16:13) fraction was further purified by a preparative ODS column to yield crocin-3 (4, 3 g). Similar treatment of the fractions eluted with 10%, 15% and 20% methanol:water, (16:13) yielded crocin-2 (3, 1.5 g), *cis*-crocin-1 (6, 200 mg) and crocin-1 (2, 3 g), respectively. Gardecin aglycone was obtained by saponifying gardecin, following the method described by Asai, Nakano, Takahashi, and Nagao (2005). The purity of each isolated compound was confirmed by TLC, HPLC, and NMR analyses.

Gardecin (1): Amorphous reddish-yellow powder. IR (KBr) cm^{-1}: 3426 (O–H), 1706 (C=O) and 1073 (C–O). UV–visible λ_{max}: 450 nm. ESI–MS: *m/z* 811 $[M+Na]^+$, 787 $[M-H]^-$. HRESIMS: 811.3882 ($C_{42}H_{60}Na_1O_{14}$ Calcd. 811.3875). CD (gardecin aglycone) $\Delta\varepsilon$(nm): −2.95(265), +2.30(445), ($C = 1.49 * 10^{-5}$ M, ethanol). ^{1}H NMR and ^{13}C NMR spectra of gardecin are given in Table 1.

2.4. DPPH free radical-scavenging assay

The DPPH free radical-scavenging activity of gardecin and crocins was determined using the Cintra 10_e UV/visible spectrometer, according to the method described by Leong and Shui (2002) with modification. Briefly, a 0.06 mM solution of DPPH in ethanol was prepared. The initial absorbance of the DPPH in ethanol was measured at 517 nm and did not change throughout the period of assay. Various concentrations of pigments (diluted to 0.5 ml with ethanol) were added to 3.5 ml of ethanolic DPPH solution. The change in absorbance at 517 nm was measured at 30 min and converted into the percentage of antioxidative activity (AA), using the following formula:

$$AA\% = ((A_{control} - A_{sample} - A_0)/A_{control})100$$

where $A_{control}$ is the absorbance of DPPH solution, A_{sample} is the absorbance of DPPH solution in the presence of sample and A_0 is the absorbance of sample at the corresponding concentration without DPPH. The absorbance change at 517 nm was used to calculate the amount of DPPH reduced; the percentage of DPPH reduced was plotted against the concentration of gardecin and crocins, and an EC_{50} value was calculated from the graph. This is defined as the amount of antioxidant necessary to reduce concentration of DPPH by 50%. α-Tocopherol was used as a reference. All measurements were the means of three determinations. The SD was estimated to be below 10%.

2.5. Kinetic studies

The chemical stability of gardecin and crocins was studied at pH 3, light (1200 lux) and temperature (60 °C) according to a described procedure (Tsimidou & Tsatsaroni, 1993) with modification. The absorbance(absorption peaks, crocin-1 and crocin-2 at 439 nm, crocin-3 and crocin-4 at 432 nm, ethanol–water (60%) fraction at 440 nm and gardecin at 450 nm, respectively) was measured and the colouring power of gardecin and crocins was expressed as $E^{1\%}_{\lambda_{max}}$ according to the International Standard ISO 3632-1980, where $E^{1\%}_{\lambda_{max}} = A_{\lambda_{max}}/C_{(1\ g/100\ ml)}$. The loss of colouring power of gardecin and crocins was determined by measuring absorbance of the solutions at various time intervals. The values are expressed as the mean values of three determinations, the ln $E^{1\%}_{\lambda_{max}}$ of each pigment was plotted against time, k (h^{-1}) value and $t_{1/2}$ were calculated. $t_{1/2}$=0.693/k, where k was calculated by the negative slope of each straight line.

3. Results and discussion

3.1. Structures elucidation

3.1.1. Gardecin

Compound 1 was assigned a molecular formula of $C_{42}H_{60}O_{14}$, as deduced from the $[M+Na]^+$ ion at *m/z* 811 in the positive ESI–MS, the $[M-H]^-$ ion at *m/z* 787 in the negative ESI–MS and HR–ESI–MS $[M+Na]^+$ (Calcd. for $C_{42}H_{60}Na_1O_{14}$: 811.3875 and found: 811.3882). The visible spectrum (Fig. 5) exhibited bathochromic shift (λ_{max} = 450 nm) in comparison with that of crocin-1 (λ_{max} = 439 nm). The IR spectrum showed absorptions at 3426, 1706 and 1073 cm^{-1} for O–H, C=O and C–O, and these values slightly shift to higher frequency, if compared with those of crocin-1 (3423, 1700 and 1068 cm^{-1}, respectively).

The ^{1}H NMR and ^{13}C NMR spectra of 1 were similar to those of crocin-1, and suggested the presence of a disaccharide moiety (δ_C 61.18–103.3; δ_H 2.97–4.18, 5.43), a conjugated polyene moiety (δ_C 124.29–144.85; δ_H 6.55–7.37), four methyl groups (δ_C 11.5–12.92; δ_H 1.90–2.01), and two conjugated carbonyl carbons (δ_C 166.49 and 195.57). Changes of carbon chemical shift of conjugated polyene moiety in comparison with those of crocin-1, as well as overlapping of some proton signals in the same unit, indicated asymmetric structure of the conjugated polyene moiety.

Since some proton signals of the conjugated polyene moiety, carbohydrate groups and a additional moiety overlapped, 2D NMR was performed to assign the proton and carbon chemical shifts. Heteronuclear multiple bond correlation (HMBC) (Fig. 2), one bond ^{1}H–^{13}C HMQC (Fig. 3), ^{1}H–^{1}H COSY (Fig. 4) and ^{1}H–^{1}H NOESY spectra were recorded, and the data are listed in Table 1. When compared to crocin-1, additional signals of 10 carbons were observed in the ^{13}C NMR spectrum, and some upfield proton signals and a singlet signal (δ 3.94) were detected in the ^{1}H-NMR spectrum. With the aid of ^{1}H NMR, ^{13}C NMR, DEPT and HMQC spectra, the additional moiety structure is suggested by a composition of three methyls (δ_C; δ_H: 26.28; 1.09 (s), 24.79; 0.94 (s) and 17.48; 0.66 (d), respectively), three methylenes (δ_C; δ_H: 39.28; 1.42 (m) and 1.55 (m), 19.32; 1.53 (m), 32.93; 1.32 (br) and 1.46 (m), respectively), two quaternary carbons (δ_C : 35.63 and 70.41

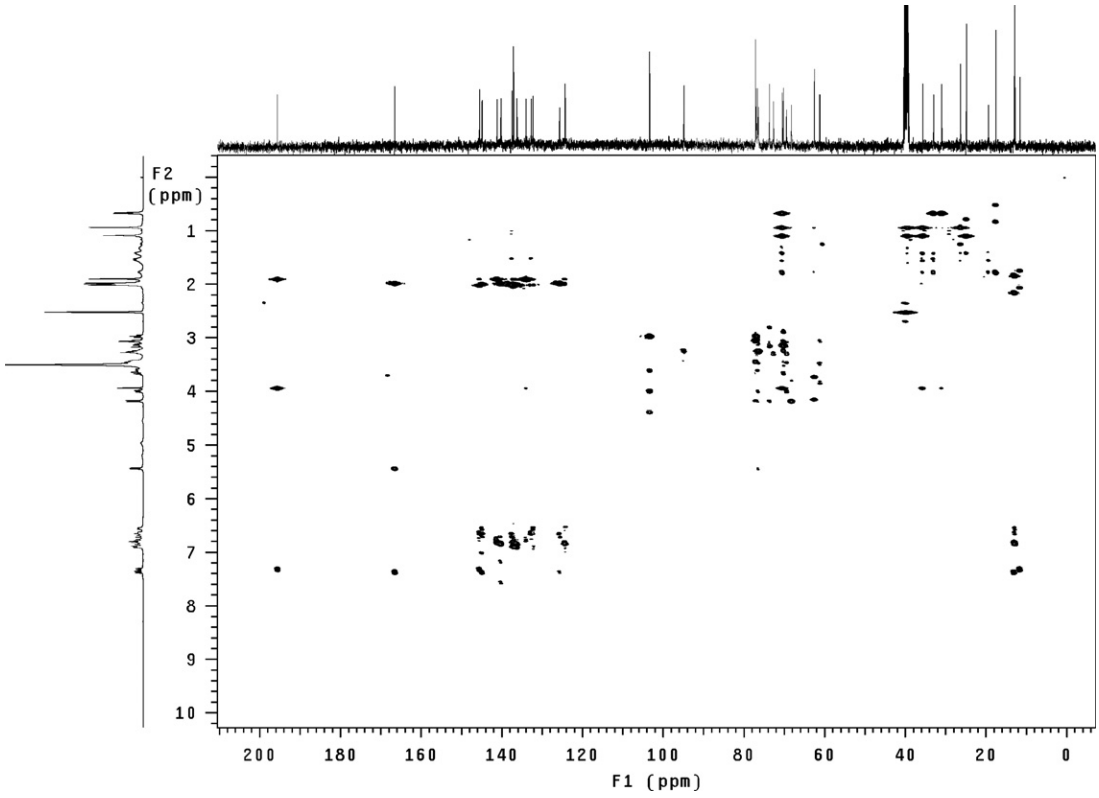


Fig. 2. HMBC spectrum.

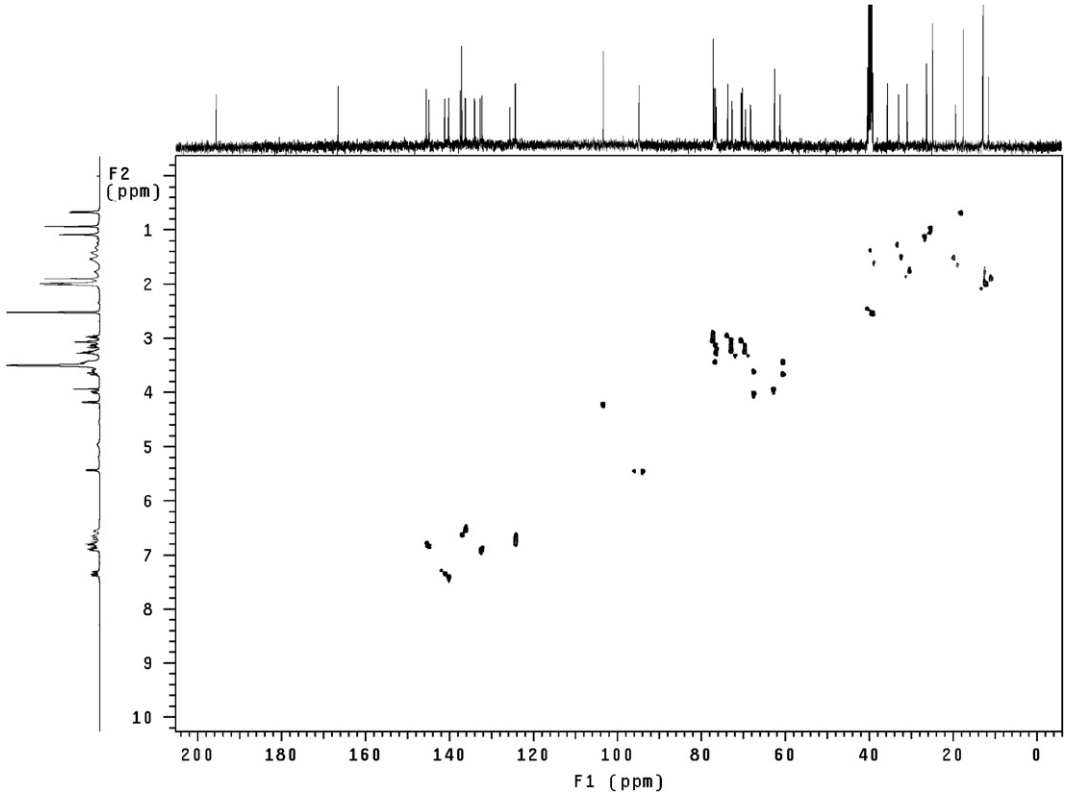


Fig. 3. HMQC spectrum.

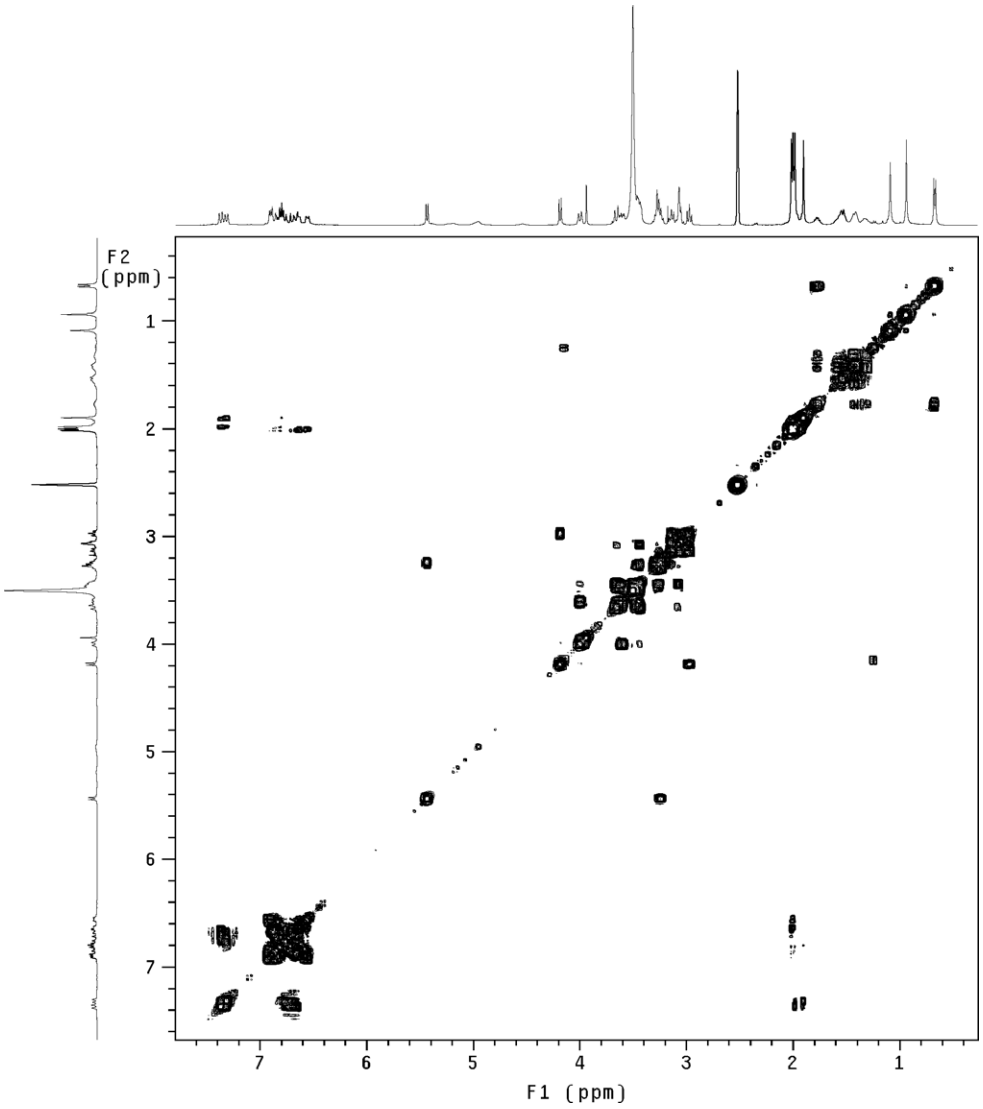


Fig. 4. ^{1}H–^{1}H COSY spectrum.

(oxidised)) and two methine (δ_C; δ_H: 30.92; 1.78 (m), 62.47 (oxymethine); 3.94 (s)). In HMBC spectrum, correlations between H-8′ (1.09, s), H-9′ (0.94, s) and C-1′ (35.63), C-2′ (39.28), C-6′ (70.41), as well as mutual correlation between these two methyls, indicated the link position of two methyls with one quaternary carbon. Neighbouring positions of three methylenes were suggested in ^{1}H–^{1}H COSY spectrum by the following correlations: H-2′ (δ 1.42, 1.55) and H-3′ (1.53), H-3′ (1.53) and H-4′ (1.32, 1.46). The correlation between H-10′ (δ 0.66(d)) and H-5′ (δ 1.78) in ^{1}H–^{1}H COSY spectrum implied the position of 10′-methyl (δ_C; δ_H 17.48; 0.66 (d)) to 5′-C. With the aid of HMQC, HMBC and ^{1}H–^{1}H COSY, as well as information mentioned above, the moiety was hypothesised to be a safranal-like skeleton (2,6,6-trimethyl-3-cyclohexadiene-1-carboxaldehyde) (Tarantilis, Tsoupras, & Polissiou, 1995). In addition, the attachment of the moiety directly to C-13′, suggested by the downfield chemical shift δ 195.57 (C-13′), was confirmed by the HMBC correlations: H-7′ (δ 3.94) with C-13′, 14′. These assignments were additionally confirmed by spectroscopic similarities of the known compound fortunate A which was isolated from *Cryptomerica fortunei* (Zheng, Hu, & Liang, 2005).

The relative stereostructure of gardecin was disclosed in the NOESY spectrum. Correlation between H-5′ and H-8′ indicated that H-5′ and CH_3-8′ are located on the same side of cyclohexane, and the location of CH_3-9′ and CH_3-10′ on the other side of cyclohexane was implied by the correlation between H-2′β and H-9′, H-10′. Similarly, the location of H-7′, CH_3-8′, CH_3-9′ on the same side of the epoxy groups was confirmed by the correlation between H-7′, H-8′ and H-9′. The absolute stereochemistry of C-6′ and C-7′ was determined by a circular dichroism (CD) spectrum of gardecin aglycone and the empirical reversed octant rule was applied to elucidate the absolute configuration of an epoxy-ketone system (Djerassi, Klyne, Norin,

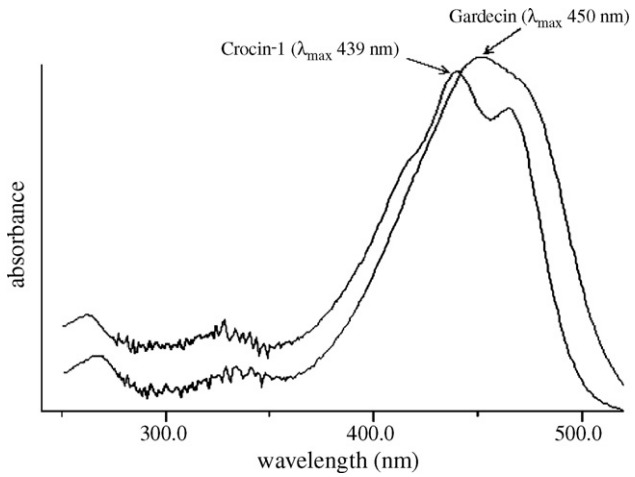


Fig. 5. UV-Vis spectra of crocin-1 and gardecin.

Ohloff, & Klein, 1965). The CD spectrum of gardecin aglycone exhibited a positive cotton effect (+2.30, 445 nm) from the C-13′ carbonyl group, which, as well as the data obtained in the NOESY spectrum, indicated that the absolute configurations at C-6′ and C-7′ were S and R, respectively. However, the absolute stereochemistry of C-5′ remained to be determined. The structure of gardecin was established as shown in Fig. 1.

3.1.2. Crocins and gardecin aglycone

With the aid of MS, UV/visible and 1D NMR data, five other known crocins have been identified as *trans*-crocetin di-(D-gentibiosyl) ester (2, crocin-1), *cis*-crocetin di-(D-gentibiosyl) ester (6, *cis*-crocin-1), *trans*-crocetin (D-glucosyl)-(D-gentibiosyl) ester (3, crocin-2), *trans*-crocetin (D-gentibiosyl) ester (4, crocin-3) and *trans*-crocetin (D-glucosyl) ester (5, crocin-4), respectively, as well as crocetin (7) (data not listed). All spectroscopic data were in good agreement with data reported in the literature (Carmona et al., 2006; Choi et al., 2001; Pfister et al., 1996; Van Calsteren et al., 1997).

TLC, MS, ^{1}H NMR and ^{13}C NMR spectra of the compound obtained by saponifying gardecin were compared with the corresponding spectra of gardecin and the aglycone of gardecin was confirmed.

3.2. UV–vis spectrophotometry

As shown in Fig. 5, gardecin was characterised by bathochromic shifts in comparison with crocin-1 ($\lambda_{max} = 439$ nm). The main structural difference between gardecin and crocin, that could interfere in the chromophore features, lies in the substitutive groups of C-13 (C-13′). Due to the stronger delocalisation effect of the α,β-epoxyketone group compared to that of the ester group, substituting one ester group by a ketoneic bond to give gardecin causes a bathochromic shift ($\lambda_{max} = 450$ nm). Therefore, the intenser orange colour of gardecin, in comparison with other crocins, might be attributed primarily to its bathochromic shift.

3.3. Radical-scavenging ability

Crocins and their aglycone crocetin, the water-soluble carotenoids isolated from saffron and gardenia, were shown to be capable of a variety of pharmacological effects, such as protection from cardiovascular diseases (He et al., 2005; Shen & Qian, 2006; Zhou, Qian, Zheng, & Xiang, 2006), inhibition of tumour cell proliferation (Magesh, Singh, Selvendiran, Ekambaram, & Sakthisekaran, 2006) and hepatocyte protection (Wang, Shiow, & Lin, 1991). Among the mechanisms underlying its various protective actions, the antioxidant property was hypothesised to be responsible for the various pharmacological effects of the carotenoids. Thanh reported crocin-1 exhibits strong antioxidant capacity, evaluated by the thiocyanate method (Pham, Cormier, Farnworth, Tong, & Van Calsteren, 2000).

DPPH is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples. When a hydrogen atom or electron was transferred to the odd electron in DPPH, the absorbance at 515–517 nm decreased proportionally to the increases of non-radical forms of DPPH. Conventionally, high free radical-scavenging ability is regarded as high antioxidant activity and DPPH method has been used as one of the basic screening steps for searching new antioxidant compounds in organic solvent extracts from natural resources including spices, herbs, fruits, and vegetables (Lee, Chung, Chang, & Lee, 2007). The present investigation demonstrated that gardecin and all crocins possess a potent activity tested by the DPPH radical-scavenging assay, exhibiting a concentration dependence. As shown in Fig. 6, the EC_{50} concentrations of α-tocopherol, gardecin, crocin-1, crocin-2, crocin-3 and *cis*-crocin-1 were approximately 0.014, 0.035, 0.062, 0.08, 0.27 and 0.12 μmol/ml, respectively.

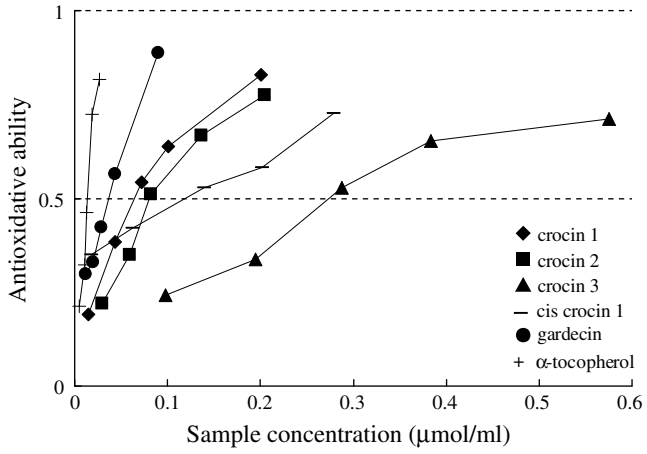


Fig. 6. DPPH free radical scavenging activity of crocins and gardecin.

The structure–activity relationships of crocins have been investigated and the comparative evaluation among several crocins indicated that sugars attached to the acid moiety might play a key role when crocins exert biological activity (Abe, Sugiura, Shoyama, & Saito, 1998; Sugiura, Shoyama, Saito, & Abe, 1994). Current DPPH data clearly suggested that the more sugars the derivatives contain, the stronger radical-scavenging activity the pigments exhibited. However, the activity of gardecin, which exhibited approximately 40% of the effect of α-tocopherol, remained higher than all crocins. This study pointed to the evidence that different electronic charge distribution along the chromophore, which occurred between gardecin and the other crocins, probably accounted for different DPPH free radical-scavenging ability.

3.4. Kinetic studies

Carotenoids are unstable and their degradation can be triggered by light, temperature or extreme pH (Aman, Schieber, & Carle, 2005; Chen & Tang, 1998; Gouveia & Empis, 2003). Stability of saffron pigments under various conditions was investigated by Tsimidou and Tsatsaroni (1993) and experimental data indicated that the degradation of crocins increases with light, decreasing pH and increasing temperature. In the present work, light (1200 lux) seemed the most destructive agent, as evidenced by the shorter $t_{1/2}$ for crocin-1, crocin-2, crocin-3 and ethanol–water (60%) fraction, in comparison with corresponding values for the other two conditions. Regression of $\ln E^{1\%}_{\lambda_{max}}$ vs time of all samples gave straight lines with negative slope which indicated that the degradation of all crocins followed first order kinetics under all experimental conditions. Degradation rate constants and half-life periods of each crocin at all experimental conditions were compared (Tables 2–4). Generally, experimental results indicated less polar carotenoids were more stable in all conditions, which was in agreement with results reported in literature (Ramakrishnan & Francis, 1980) which revealed more polar carotenoids had a depressing effect on the ease of the oxidation of carotenoids. Data showed all crocins were unstable under the various conditions described above, but surprisingly gardecin was fairly stable. Under 60 °C, pH 3 and light (1200 lux), gardecin exhibited 5.7, 13.2 and 46.2-fold higher values of $t_{1/2}$, respectively, in comparison with those of crocin-1, the major crocetin derivative of gardecin and saffron. Kinetic results indicated that the occurrence of the ketone terminal group in the conjugated polyene moiety, in which the electron density profile differs from crocins, probably underlies the unique stability of the gardecin-like skeleton.

Table 2
First-order reaction rate constants and half-life periods for degradation of crocins at 60 °C

Crocins	$K \times 10^{-3}$ (h^{-1})	Correlation coefficient	$t_{1/2}$ (h)
Crocin-1	9.1	−0.996	76
Crocin-2	7.2	−0.992	96
Crocin-3	6.3	−0.995	110
Crocin-4	5.9	−0.994	117
Gardecin	1.6	−0.992	433
Ethanol–water (60%) fraction	2.8	−0.989	247

Table 3
First-order reaction rate constants and half-life periods for degradation of crocins at pH 3

Crocins	$K \times 10^{-3}$ (h^{-1})	Correlation coefficient	$t_{1/2}$ (h)
Crocin-1	27.2	−0.996	25
Crocin-2	30.8	−0.981	22
Crocin-3	21.3	−0.985	32
Crocin-4	15.0	−0.967	46
Gardecin	2.1	−0.991	330
Ethanol–water (60%) fraction	7.8	−0.995	89

Table 4
First-order reaction rate constants and half-life periods for degradation of crocins under light

Crocins	$K \times 10^{-3}$ (h^{-1})	Correlation coefficient	$t_{1/2}$ (h)
Crocin-1	70.5	−0.945	10
Crocin-2	45.5	−0.987	15
Crocin-3	31.0	−0.991	22
Crocin-4	4.0	−0.987	173
Gardecin	1.5	−0.984	462
Ethanol–water (60%) fraction	17.8	−0.994	39

4. Conclusion

From gardenia fruits a novel pigment gardecin was isolated and characterised. With the aid of 1D NMR and 2D NMR, structure elucidation of gardecin was achieved unambiguously and a novel structure, in comparison with other known crocins was reported for the first time. Current data revealed the ketone terminal group in the conjugated polyene moiety probably influenced the antioxidant ability and stability of these water-soluble carotenoids.

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