Asian Journal of Chemistry Vol. 17, No. 2 (2005), 725–729

Separation and Purification of Some Components of Iranian Saffron

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The isolation and purification of several components of the dried stigmas of *Crocus Sativus* L. from Iran were performed by different methods including organic solvent extraction, adsorption column chromatography, acid and alkaline hydrolysis. The progresses in the purification steps were monitored by TLC, HPLC and UV-Vis spectroscopy. IR spectroscopy was used for the characterization of purified compounds. Also the melting point of the purified compounds was determined. The separated components are crocetin, crocin (s), safranal, picrocrocin (s) and dimethylcrocetin. For the first time, the existence of three types of picrocrocin in Iranian saffron has been shown using TLC and HPLC techniques.

Key Words: Saffron, Crocin, Crocetin, Safranal, Picrocrocin, Dimethylcrocetin.

INTRODUCTION

Saffron, the dried stigmas of *Crocus Sativus* L., is a very expensive spice that is used mainly as a herbal medicine or food colouring and flavouring agent in different parts of the world. *Crocus Sativus* L. had originally been grown in Iran, Spain, India, Greece and some other countries. It has also been successfully cultivated in various places in China, especially Tibet[1–4]. The value of saffron is determined by the existence of three main secondary metabolites: (1) crocin and its derivatives, which are responsible for color; (2) picrocrocin, responsible for taste and (3) safranal, responsible for odour[3–8]. The structures of these compounds are shown in Fig. 1. There are some reports about the purification and recognition

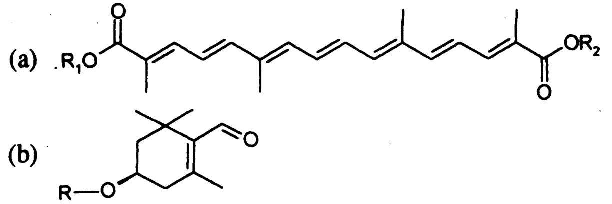

Fig. 1. Structures of different saffron components: (a) crocin: $R_1{=}R_2{=}\beta$-D-gentiobiosyl; crocetin: $R_1{=}R_2{=}H$; dimethylcrocetin: $R_1{=}R_2{=}CH_3$, (b) picrocrocin. Safranal is the aglycone dehydrated derivative of picrocrocin

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of the mentioned components of saffron from Spain[1-3] and Greece[4-6, 9] in literature, but there is no report about Iranian saffron.

The crude extract of saffron has been used clinically to relieve the symptoms of angina pectoris in China[1]. Also, the antioxidant effect of saffron has been studied[10, 11]. The studies have indicated its potential as an anti-cancer agent in leukemia, neuroblastoma, adenocarcinoma, colon and skin cancer[12-16]. It has been demonstrated that crocin derivatives and the crude extract of saffron suppress tumour growth and increase the diffusion of oxygen to the capillary endothelial cells[1]. Also, the apoptosis of the cells treated with crocin has been shown[15]. Therefore, separation and purification of saffron components seem to be important for their biological effects.

In this study, we isolated and purified all the three essential groups of Iranian saffron components responsible for colour, odour and taste (from Iranian saffron) using different techniques and the characteristic features of the purified compounds were compared with those reported previously.

EXPERIMENTAL

Dry stigmas of pure 'Ghaenat' Saffron (*Crocus sativus* L.) were purchased locally in Ghaenat, Iran and stored in dark at 4°C until further use. Crocetin as a standard component was obtained from Sigma Chemical Company.

All compounds were analyzed using a reversed-phase C-18 column HPLC (Shimadzu). The solvent flow rate was 1 mL/min and UV detector was used in all experiments but in their own related wavelengths. A stationary phase was silicagel G-60 plate from Merck company and mobile phase was *n*-butanol : acetic acid : water (4 : 1 : 1). Spectrophotometric measurements were performed by a Shimadzu UV-Vis double beam spectrophotometer, model-3101. IR spectra were measured on a Shimadzu IR-460 spectrometer.

Purification Methods

Picrocrocin (s) and crocin (s): 4.0 g of dried stigma powder mixed with 20 mL of *n*-hexane for 10 min in shaker incubator, at room temperature in the dark. The extract was centrifuged at 4000 rpm for 10 min. The process was repeated three times. The pellet was finally extracted twice with 10 mL of 50% (v/v) ethanol and centrifuged at 4000 rpm for 5 min. The supernatant applied on a glass column (2 × 80 cm) was packed with neutral aluminum oxide 90-active (Merck) and was eluted with 500 mL of 50% ethanol, followed by 500 mL of 50% ethanol containing acetic acid (4 : 1 v/v). Fractions of 4 mL were collected and their absorbance at 250 and 440 nm was monitored. Picrocrocins were the first fractions which were eluted and then crocins were separated.

Safranal and crocetin: 3 g of saffron was extracted twice using water at 70°C and then centrifuged at 4000 rpm for 20 min. The pH of the supernatant was adjusted to 1 with HCl and incubated at 70°C with shaking for 1 h. The resulting material was concentrated under vacuum using a rotary evaporator.

The volatile fraction containing safranal was collected in a cold trap at –78°C. The desiccated non-volatile fraction containing crocetin was washed twice with

10 mL of water, adjusted to pH 1.0 with HCl and washed once with 10 mL of water. The sediment was stored in dark[2] at 4°C.

Dimethylcrocetin: Saffron was extracted using *n*-hexane, diethyl ether (Et_2O) and methanol 80% by succylation system. Dimethylcrocetin (DMC) was synthesized by alkaline hydrolysis of the methanolic extract and purified by extraction with dichloromethane (DCM) and recrystallized in the solved system of DCM : Et_2O (50 : 50)[4].

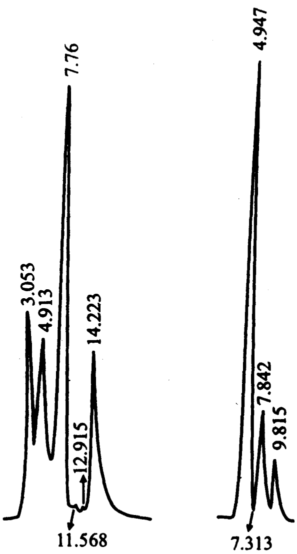

Fig. 2. Chromatographic analysis of crocins and picrocrocins isolated from Iranian saffron stigmas. Both of them were analyzed using a reversed-phase HPLC with C-18 column. Crocins (left) and picrocrocins (right) were eluted using acetonitrile 26% in 25 min and monitored at 250 nm and 261 nm, respectively.

RESULTS AND DISCUSSION

Table-1 demonstrates the results obtained for purification and characterization of Iranian saffron components. The λ_{max} for all compounds are in agreement with the previously reported data[17]. Figs. 2a and 2b show the HPLC picks for crocins and picrocrocins, respectively. Our results indicate the presence of four types of crocin ingredients, such as the saffron from Spain[1]. The main fraction (di-gentibiose crocetin) was the third peak according to the HPLC chromatogram (Fig. 2a and Table-1). The crocin-4 and crocetin have not been observed in Tibet saffron[1]. Furthermore, TLC and HPLC showed three types of picrocrocin in saffron from Iran, which have not been reported previously. The major ingredient of picrocrocins, $t_R = 4.947$, has higher concentration than the two others (Fig. 2b and Table-1). The only picrocrocin which was reported in the saffron from other countries was monoglycosilated safranal with some characterisitc features[2] similar to the major ingredient of picrocrocin from Iran. Also the melting point of picrocrocin reported in Merck Index was equal to 155–156°C [17], which is in accordance with that obtained by us, *i.e.*, 155°C.

TABLE-1
THE RESULTS OBTAINED FROM SEPARATION AND PURIFICATION OF DIFFERENT COMPONENTS OF SAFFRON FROM IRAN
(t_R = retention time and R_f = resolution factor)

Components	UV-Vis (λ_{max})	m.p. (°C)	HPLC (t_R)	TLC (R_f)	IR (cm^{-1})					
					ν(O—H)	ν(C—H)	ν(C═O)	ν(C═C)	ν(C—O)	ν(—CH_3)
Picrocrocin-1	In water: 250	155	4.947* (89%)	0.96	3390	2930	1659	1610	1041–1074	1374
Picrocrocin-2			7.842 (7%)	0.90						
Picrocrocin-3			9.815 (4%)	0.83						
Crocin-1	In water: 437, 463	186	3.353* ( )	0.34	3405	2925	1705	1580 1466	1070	1422
Crocin-2			4.913 ( )	0.40						
Crocin-3			7.760 (80%)	0.50						
Crocin-4			14.223 ( )	0.65						
Safranal	In water: 315	—	4.172†	—	—	—	—	—	—	—
Dimethyl-crocetin	In ethanol: 431, 458	222	5.185‡	0.91	—	2925	1692	1574	1227	1429
Crocetin	In ethanol: 436, 463	285	2.367‡	0.98	3400-3500	2800-3000	1661	1573	1285	1420

* Picrocrocins and crocins were eluted by acetonitrile (26%) and monitored at 250 nm and 261 nm, respectively.

† Safranal was eluted by acetonitrile (100%) and monitored at 315 nm.

‡ For crocetin and dimethylcrocetin analysis, the HPLC column was equilibrated and eluted with 100% methanol and monitored at 320 nm.

Our results show that the m.p. of DMC is 222°C, which is also in accordance with 221.5°C that was reported elsewhere[17].

All parameters reported in Table-1 for crocetin are the same as those obtained for crocetin from Sigma Chemical Co. as a standard. Table-1 also shows the peaks observed in the IR spectra of all compounds. The same IR spectra were reported previously for crocin[9]. Other data in this table are reported for the first time.

ACKNOWLEDGEMENTS

The financial support by the Research Council of the University of Tehran is gratefully acknowledged. Also we are very thankful to the Research Council of Tarbiat Modarres University.

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(*Received*: 10 March 2004; *Accepted*: 15 October 2004) AJC-4000