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Crocetin Esters, Picrocrocin and Its Related Compounds Present in *Crocus sativus* Stigmas and *Gardenia jasminoides* Fruits. Tentative Identification of Seven New Compounds by LC-ESI-MS

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Crocetin esters present in saffron (*Crocus sativus* L.) stigmas and in *Gardenia jasminoides* Ellis fruit are the compounds responsible for their color. Of the fifteen crocetin esters identified in this study, five new compounds were tentatively identified: *trans* and *cis* isomers of crocetin (β -D-triglucoside)-(β -D-gentibiosyl) ester, *trans* and *cis* isomers of crocetin (β -D-neapolitanose)-(β -D-glucosyl) ester, and *cis* crocetin (β -D-neapolitanose)-(β -D-gentibiosyl) ester. The most relevant differences between both species were a low content of the *trans* crocetin (β -D-glucosyl)-(β -D-gentibiosyl) ester, the absence of *trans* crocetin di-(β -D-glucosyl) ester in gardenia, and its higher content of *trans* crocetin (β -Dgentibiosyl) ester and *cis* crocetin di-(β -D-gentibiosyl) ester. With the same chromatographic method it was possible to identify, in a single run, ten glycosidic compounds in saffron extracts with a UV/vis pattern similar to that of picrocrocin; among them, 5-hydroxy-7,7-dimethyl-4,5,6,7-tetrahydro-3*H*isobenzofuranone 5-*O*- β -D-gentibioside and 4-hydroxymethyl-3,5,5-trimethyl-cyclohexen-2-one 4-*O*- β -D-gentibiosyl ester of 2-methyl-6-oxo-2,4-hepta-2,4-dienoic acid was found in gardenia samples, but it was possible to identify the iridoid glycoside, geniposide.

KEYWORDS: Crocetin esters; gardenia; geniposide; glycosidic aroma precursors; picrocrocin; saffron

INTRODUCTION

An unusual apocarotenoid, called crocetin ($C_{44}H_{64}O_{24}$, 8,8'diapo- Ψ, Ψ' -carotenedioic acid), which is esterified with one or two glucose, gentibiose, or neapolitanose sugar moieties, is present in *Crocus sativus* L. stigmas and *Gardenia jasminoides* Ellis fruit. These compounds are known for their coloring properties owing to their peculiar water soluble behavior, in contrast to most families of carotenoids.

The bibliography on crocetin ester elucidation and analysis for both spices is quite abundant (1-5), but there is no consensus as to which compounds are present in each species (**Table 1**). Accurate knowledge about the presence of these compounds may provide important information on their generation pathway and will clarify if the presence of one of them in saffron and not in gardenia may be related to the glycoside and volatile compounds present in saffron only. It is known that, by thermal treatment applied to fresh saffron stigmas to obtain the spice, volatile compounds are generated from its precursors (6-8). Although several authors have proposed analytical methods such as liquid chromatography to simultaneously detect in saffron the compounds responsible for its color (crocetin glycosides), bitterness (picrocrocin), and aroma (mainly safranal) (9-12), recently Gregory et al. (7) have demonstrated that this is not possible with a single-polarity extraction method and only one detection technique. Safranal and other volatiles should be extracted with nonpolar solvents and analyzed by gas chromatography. The glycosidic compounds related to picrocrocin have been studied by countercurrent chromatography (MLCCC) (13-15). However, the acid form of picrocrocin was detected in saffron when crocetin ester identification was carried out by RP-chromatography (11, 12). Evidently, it is of interest to use a unique methodology to determine both families of compounds, crocetin esters and glycosides related to picrocrocin, and to determine if the quantification of the glycosides could be used to estimate the aromatic potential of the samples. The aroma precursors described in gardenia are linalool and borneol, 1-linalyl 6-O- α -L-arabinopyranosyl- β -D-glucopyranoside and bornyl 6-O- β -D-xylopyranosyl- β -D-glucopyranoside (16), which are different from the ones found in saffron. Iridoid glycosides, such as geniposide, gardenoside, gardoside, and scandoside methyl ester, among others, are also present in gardenia (17-19). Geniposide, the major component, is reported to be present

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					Crocins identified in									Results				
Crocins structure $OR' OR' OR' OR' OR' OR' OR' OR' OR' OR'$					Tarantilis <i>et al.</i> (23)	Pfister <i>et al.</i> (12) Straubinger <i>et al.</i> (21)		Straubinger et al. (21)	Van Calsteren <i>et al.</i> (13)		Knapp <i>et al.</i> (10)	Carmona <i>et al.</i> (14)	Carinoua et ul. (1+) Tentatively identified		Chromatographic and spectrometric behavior			
n° glucose moieties (R + R')	moiety distribution (R/R')	isomer	named as	Gj	Cs	Gj	Cs	Cs	Gj	Cs	Gj	Cs	Cs	Gj	RT (min)	Fragmentation pattern ES+	UV/Vis (λmax)	
5	trig/gen	trans	trans-5-tG										X	X	8.2	592(100), 675(35), 837(21),1161(40)	258,444, 467	
		cis	cis-5-tG										X	Χ	10.0	592(100), 675(12), 1161(25)	263, 349, 444, 468	
	nea/gen	trans	trans-5-nG		X		X					Χ	X	Χ	9.7	592(62), 797(100), 1161(30)	264, 422sh, 467sh, 440	
		cis	cis-5-nG		X								X	Χ	12.2	592(65), 635(100), 1161(15)	263,327, 412, 434	
4	nea/glu	trans	trans-4-ng										X	Х	10.8	635 (100), 817(21), 999(28)	252, 422sh, 440	
		cis	cis-4-ng										X	Х	12.9	635(100), 797(18), 999(52)	246, 326, 424, 437sh	
	gen/gen	trans	trans-4-GG		Х	X		X	Х	X	X	X	X	Х	10.3	511(22), 999(100)	262, 442, 465	
		cis	cis-4-GG		Х	X				Х	X	Χ	X	Х	12.6	511(32), 999(100)	264, 327,434, 457	
3	gen/glu	trans	trans-3-Gg	Х	X			X	Х	X		X	X	X	11.0	329(17), 675(14), 837(100)	262, 442, 465	
		cis	cis-3-Gg		X					X		X	X	Χ	13.4	543(24), 675(15), 837(100)	259, 327,434, 457	
2	gen/-	trans	trans-2-G	Χ	X	X		X	X	X	X	X	X	Χ	13.7	513(25), 675(100)	260, 434, 457	
		cis	cis-2-G	Х				X					X	Х	15.9	513(100)	260, 324,432, 452	
	glu/glu	trans	trans-2-gg	X	X			X		X		X	X		11.8	675(100)	262, 44, 464	
		cis	cis-2-gg		X			X					X	X	15.7	675(100)	260, 326, 430, 452	
1	glu	trans	trans-1-g	Χ	X			X	X				X	X	15.0	675(100)	261,433, 458	
		cis	cis-1-g	X														

^a Abbreviations: (trig) triglucoside; (nea) neapolitanoside; (gen) gentibioside; (glu) glucoside; (Cs) Crocus sativus L.; (G) Gardenia jasminoides Ellis; (RT) retention time.

up to 35.9% in commercial gardenia preparations (20, 21) and can be hydrolyzed to its aglycone genipin, which possesses genotoxicity. With this in mind, re-evaluation of the safety of gardenia must be carried out before using it as a food colorant (22).

The aim of this study was to establish which crocetin esters are present in saffron and in gardenia, comparing the results with those found in the literature, and to determine if it is possible to detect and identify aroma glycoside precursors reported in both species in the same run.

MATERIALS AND METHODS

Plant Materials. *Saffron (Crocus sativus L.).* Harvesting, removal, and dehydration of the stigmas took place in Motilla del Palancar (Cuenca, Spain) using traditional procedures which adhered to the trade standard of the Protected Denomination of Origin "Azafrán de la Mancha" (23, 24). Two samples from the 2003 harvest plus two samples from 2004 were analyzed.

Gardenia (Gardenia jasminoides Ellis). Two samples of gardenia were characterized. One sample was a commercial standard supplied by Chromadex (Santa Ana, CA), and the second one was a commercial extract supplied by Verdú-Cantó Saffron Spain (Novelda, Alicante, Spain).

LC-DAD-MS Conditions. Twenty milligrams of the sample was macerated for 1 h in 8 mL of milliQ water previously bubbled with helium. The entire process was carried out in darkness and at room temperature. Twenty microliters of the extract filtered through a PVDF filter of 0.45 μ m (Millipore) was injected into an Agilent 1100 HPLC chromatograph (Palo Alto, CA) equipped with a 150 mm × 4.6 mm i.d., 5 μ m Phenomenex Luna C18 column thermostated at 30 °C. Two elution systems were assayed, the unique difference being that the aqueous phase was acidified. The solvents were water (or acidified with 0.25% formic acid) (A) and acetonitrile (B), using the following gradient: 80% A for 5 min to 20% A in 15 min, at a flow rate of 0.8 mL/min. Dual on-line detection was carried out by a diode array spectrophotometer and a quadrupole mass spectrometer with electrospray ionization (ESI) (Agilent 1100). The probe of the mass

spectrometer was connected to the UV cell outlet. The DAD detector was set at 250, 330, and 440 nm. Both the auxiliary and the sheath gases were nitrogen with a flow rate of 12 L/min. The drying gas temperature was set at 350 °C, and the nebulizer pressure, at 30 psi. The capillary voltage was ± 2500 V, and the capillary temperature, 195 °C. Spectra were recorded in positive and negative ion modes between m/z 100 and 1500. Identification was carried out with Agilent Chemstation software for LC/MS.

Nomenclature for Crocetin Esters. To abbreviate the names of crocetin esters in this paper, they have been labeled as follows: first, the nomenclature which refers to the isomeric *cis* and *trans* forms has been written with a hyphen separating the total number of glucose moieties at both extremes of the base molecule. Then, the glucose moiety distribution was indicated as (t) triglucoside, (n) neapolitanoside, (G) gentibioside, or (g) glucoside. The name of the base structure, crocetin esters, was removed, since it is the same in all compounds.

RESULTS AND DISCUSSION

Crocetin Ester Identification. During routine assays with the mobile phase, it was observed that the addition of formic acid to the aqueous phase to facilitate compound ionization scarcely improved the sensitivity. However, on the contrary, it produced wider peaks and a different chromatographic behavior in the case of the *trans* crocetin (β -D-gentibiosyl) ester (*trans*-2-G), since it was eluted earlier due to the protonation of its acidic terminus. This elution behavior made the identification of the preceding *cis* isomers difficult. Next, all samples were analyzed by both methods, taking advantage of these acidic properties plus a better chromatographic resolution and higher fragmentation degree.

Although Pfander and Schurtenberger (25) were the first to isolate six glycosides of crocetin in saffron, Tarantilis *et al.* (11, 12) identified a greater number of crocetin esters and their *trans* and *cis* isomers (**Table 1**), by high performance liquid chromatography with UV/vis photodiode array detection coupled to mass spectrometry. The compounds with higher molecular

weight found were the crocetin esters with five glucose units. Unexpectedly, it seems that a crocetin di-(β -D-neapolitanose) ester exists, but it has not been possible to detect its presence in saffron spice extracts. Dufresne *et al.* (26) found that this was the pigment preponderantly produced by a *Crocus sativus* L. culture in suspension when crocetin was the substrate.

The results present in this paper confirmed what has been found by these authors, except for trans-5-tG, whose structure was not in accordance with the one proposed by Pfister (4) as trans-5-nG and corroborated later by Carmona et al. (6). In this study, its corresponding cis isomer (cis-5-nG), which eluted at 12.2 min, was tentatively identified for the first time, with a fragmentation pattern of m/z 1161, 797, and 592, corresponding to $[M + Na]^+$, $[M - gentibiose]^+$, and $[M + 2Na]^{+/2}$, respectively. A signal at m/z 635 was also observed and corresponded to the loss of an additional glucose moiety (162 amu) from the structure responsible for m/z 797. This situation was probably due to the gentibiose loss of one extreme and the glucose molecule in position 2 (179 amu) from the trisaccharide end. Furthermore, in relation to their trans isomers, cis-crocetin esters present an additional absorption band around 324 nm in their UV/vis spectra and their maximum absorption at 440 nm presents a hypsochromic effect of some 5 nm (27) that facilitates their identification.

A new crocetin ester present in both species, which would correspond to the structure proposed by Tarantilis *et al.* (*12*), *trans*-5-tG, was identified at $t_R = 8.2 \text{ min}$ (**Figure 1**). The mass spectrum of this new crocetin ester denoted the presence of five glucose residues, m/z 1161, corresponding to $[M + Na]^+$, as well as the previous one, but with a different disposition. Its shorter retention time implied a more unfolded structure, as crocetin esters of a larger size are the first to elute from the chromatographic column. Its fragmentation pattern coincided with this structure proposal with m/z 1161 and 592 and two other signals 837 and 675 which correspond to the loss of two and three glucoses, respectively. Also, a tentative identification of its corresponding *cis* isomer (*cis*-5-tG) at 10.1 min was possible. Using LC-MS, it is not possible to determine the positions of the sugar moieties in relation to the C₁₃ bond.

The higher crocetin ester content in both spices was trans-4-GG at $t_{\rm R} = 10.3$ min (Figure 1), whose signals at m/z 511 and 999 correspond to $[M + Na]^+$ and $[M + 2Na]^+/2$, respectively. Its *cis* isomer (*cis*-4-GG) at $t_{\rm R} = 12.6$ min was found in higher proportion in gardenia than in saffron (Figure 1). In addition, for the first time, it was possible to tentatively identify two new compounds ($t_{\rm R} = 10.8$ and 12.9 min) with four glucose molecules, neapolitanose at one end and a glucose at the other end of the molecule, the *trans*-4-ng and *cis*-4-ng. Next to the m/z 999 signal corresponding to $[M + Na]^+$, a predominant m/z 635 peak appears in both cases that again corresponds to $[M - glucose (162 \text{ uma}) - glucose (179 \text{ uma})]^+$. Its UV/vis spectrum also revealed the structure proposed, as it was identical to that of trans-5-nG (Figure 2a) and different from the characteristic spectrum of the other crocetin esters (Figure 2b). Nevertheless, in its *cis* isomer (*cis*-4-ng), spectroscopic differences were not so evident, but the signal at m/z797 and 635 allowed us to differentiate it easily from the crocetin ester (cis-4-GG) that eluted next to it (Figure 1).

The crocetin structure with three glucoses had a gentibiose moiety in one extreme and glucose in the other (*trans*-3-Gg, *cis*-3-Gg). Both isomers, *trans* ($t_R = 11.0$ min) and *cis* ($t_R = 13.4$ min) showed signals at m/z 837 and 675 corresponding to $[M + Na]^+$ and $[M - glucose + Na]^+$, respectively. As can be observed in **Figure 1**, the content of *trans*-3-Gg was much



Figure 1. Crocetin esters identified in saffron and gardenia extracts, based on UV/vis spectra and mass fragmentation patterns.



Figure 2. Characteristic UV/vis spectra of (a) a *trans*-crocetin ester including the neapolitanose moiety and (b) *trans*-crocetin esters including glucose and gentibiose but without neapolitanose.

higher in saffron than in gardenia in relation to the *trans*-4-GG content, which in both cases is the most important compound.

The crocetin ester with two glucose units $(m/z 675, [M + Na]^+)$ had two possible compositions, one with one glucose at each extreme (trans-2-gg, $t_R = 11.8$ min; cis-2-gg, $t_R = 14.9$ min) and one with both as a gentiobiose unit in one extreme (trans-2-G, $t_{\rm R} = 13.7$ min; *cis*-2-G, $t_{\rm R} = 15.1$ min). The *trans*-2-G ester was possible to identify since its chromatographic behavior changed when formic acid was added to the mobile phase, showing an additional m/z 513 peak which corresponded to the loss of one of the two glucoses that form the gentibiose unit and which was not observed for *trans*-2-gg. This m/z 513 ion was the one which allowed us to identify *trans*-1-g ($t_{\rm R} = 14.4$ min). In the case of gardenia, trans-2-gg was not detected and the peak at retention time 11.9 min (Figure 1) was a compound with a fragmentation pattern at m/z 828 (100), 635 (22), 386 (54), and 289 (61). Its m/z 635 ion and UV/vis spectra were similar to the ones found in trans-5-nG and trans-4-ng (Figure **2b**), confirming the presence of a neapolitanose moiety. The m/z 828 fragment may indicate an additional methyl group [M + H]⁺ in the structure. It is possible, as happens in commercial food grade extracts, that the use of methanol might re-esterify trans-5-nG and trans-4-ng, as there is evidence that methanol in the chromatographic system or during extraction produces this effect (25, 28). It is also important to point out that, in relation to saffron, crocetin esters cis-2-G, cis-2-gg, and trans-1-g were found in higher proportions in old saffron samples, probably due to the degradation of higher structures over time, as several authors suggest that this happens during storage (29-33).

In general, the differences found between the saffron samples were few, probably due to the fact that the dehydration process used was the same. In addition, the coincidence of the results with those reported when Greek saffron was analyzed (12) indicates that it is possible to identify the same crocetin ester structures independently of their geographical origin, while the important differences would be the ratio between them. Nowa-days, it is assumed that the differences found are due to the plant. Since it is a sterile triploid, all the world's vegetable material could come from a unique starting point (34), although some genetic variations could occur in diverse populations and they could not be genetically identical.

In relation to the crocetin ester analysis in gardenia extracts, we noted the similar behavior of both gardenia samples, an extract acquired on the international market as a coloring material, and a commercial standard. They contained the same compounds, and the relationship between them hardly varied. How these pigments are extracted from the fruit and whether the extraction techniques used may affect the crocetin ester profile remain to be studied. Previous papers that have compared both profiles (4, 5, 35) always identified a larger number of crocetin esters in saffron than in gardenia, which has not been corroborated in this paper, with the exception of *trans*-2-gg. To sum up, the most relevant differences between both species were the absence of *trans*-2-gg in gardenia, the low presence of *trans*-3-Gg, and the high content of *trans*-2-G and *cis*-4-GG.

Glycosidic Compounds: Aroma Precursors. In contrast to what occurred with crocetin esters, when the detector was set at 250 nm, the differences found between both species were very important (**Figure 3**). In the case of saffron, seven different compounds with a maximum wavelength at 250 nm, characteristic of picrocrocin, were found. Only peak **a** showed a UV spectrum significantly different from that of picrocrocin, showing a λ_{max} at 290 nm, probably due to the linear configuration chain of this compound. The signals at m/z 501 and 367 of peak

a corresponded respectively to $[M + Na]^+$ and the loss of a linear fragment of the molecule, $[M - C_7OH_{10}]^+$ (**Figure 3a**). The compound was identified as the *O*- β -D-gentibiosyl ester of 2-methyl-6-oxo-2,4-hepta-2,4-dienoic acid, previously described in saffron (*13*-*15*). When the aqueous mobile phase was acidified with formic acid to facilitate the substances ionization, peak **a** was observed to move from a retention time of 2.5 min to 3.5 min. It would be the same compound in an ionic form but with a glucose residue loss (*m*/*z* 361 [M + 2Na]⁺ in the positive ion mode and *m*/*z* 337 [M - H + Na]⁻ in the negative ion mode), confirming this structure with *m*/*z* 177 [M - glucose + Na]⁺ and the UV spectrum previously described.

Peak **b** was identified as 4-hydroxy-2,6,6-trimethyl-cyclohexen-1-carbaldehyde 4-O- β -D-gentibioside, where the m/z 515 and 339 values corresponded to $[M + Na]^+$ and the loss of the cyclohexene moiety $[M - C_{10}H_{16}O]^+$ (**Figure 3b**). Peak **c** was identified as 5-hydroxy-7,7-dimethyl-4,5,6,7-tetrahydro-3*H*isobenzo-furanone5-O- β -D-gentibioside, after assigning the signals at m/z 527, 369, and 185 to their respective ions $[M + Na]^+$, $[M - C_5H_{12}O_4]^+$, and $[M - C_7H_{12}O_7]^+$ (**Figure 3c**). The signals 369 and 185 corresponded to different sugar losses.

The compound assigned to peak **d** matched one found by Tarantilis et al. (12) and Winterhalter (36), which was identified as the 4-hydroxy-2,6,6-trimethyl-1-cyclohexene carboxylic acid 4-O- β -D-glucopyranoside. The mass fragmentation signals at m/z369 and 167 corresponded to $[M + Na]^+$ and the loss of a sugar moiety $[M - C_6H_{11}O_5]^+$, respectively (Figure 3d). The mass fragmentation pattern of peak e (Figure 3e) was consistent with a compound whose molecular formula was C10O4H14 plus a glucose moiety. Careful examination of the signals at m/z 383 and 367, which corresponded to $[M + Na]^+$ and [M - OH +Na]⁺, identified the peak as 5-hydroxy-7,7-dimethyl-4,5,6,7tetrahydro-3*H*-isobenzo-furanone 5-O- β -D-glucopyranoside, which is the hydrated structure of the compound assigned to peak c but with a glucose residue loss. Peak f showed m/z 515 [M + Na]⁺ and 357 $[M - C_5H_{12}O_4]^+$, which means that a part of the gentibiose had been cleaved. This compound was identified as 4-hydroxymethyl-3,5,5-trimethyl-cyclohex-2-en-1-one 4-*O*-β-D-gentibioside (Figure 3f).

The most abundant compound, peak **g**, found at 250 nm, had m/z 353, corresponding to $[M + Na]^+$, while m/z 169 represented a glucose residue loss $[M - \text{glucose} + Na]^+$ which confirmed the picrocrocin identification (**Figure 3g**). Finally, peak **h**, which showed two shoulders, was assigned to the two isomers, *R* and *S*, of 4-hydroxy-3,5,5-trimethyl-cyclohex-2-en-1-one 4-*O*- β -D-glucopyranoside (m/z 337 $[M + Na]^+$) (**Figure 3h**).

Besides the clear benefit of using the LC technique to identify at the same time crocetin esters and glycoside aroma precursors, it should be pointed out that this methodology could reproduce the real composition of saffron more precisely. The important differences from the results found by other authors are that when they employed MLCCC, compounds **c**, **e**, and **f** were not found (14, 15), even though they were able to identify similar ones but with one glucose molecule less in the structure, as happened here when the mobile phase was acidified and peak **a** determined. This might have been the result of using an exhaustive Soxhlet extraction instead of the water extraction and direct injection as proposed in this paper.

In the case of gardenia, only two compounds were detected, the first with the same chromographic behavior, fragmentation pattern, and UV/vis spectra as compound **a** (Figure 3), previously identified in saffron. The second compound, peak **i** (Figure 3i), showed a fragmentation pattern (m/z 411) corre-



Figure 3. Chromatogram (250 nm) for saffron and gardenia extracts with their respective mass fragmentation patterns.

sponding to $[M + Na]^+$ and tentatively identified as 1,4,5,7tetrahydro-7-(acetomethyl)-cyclopentapyran-4-carboxylic acid methyl ester, commonly known as geniposide (20). This last identification is of great importance, because the ionization of this iridoid glycoside via electrospray is difficult (37). According to Wang and co-workers (19), this identification is an analytical challenge since there are no LC-MS methods published. The absence of picrocrocin in gardenia extract makes the authors doubt about the biosynthesis of crocetin and its glycosylated forms, related with the oxidative degradation of zeaxanthin (25). This oxidative degradation takes place at both ends of zeaxanthin, (7, 8) and (7', 8'), by means of a carotenase action liberating crocetinaldehyde and picrocrocin (38). The crocetindialdehyde is oxidized to crocetin and subsequently glycosylated by different glycosyltransferases (39, 40), generating the different crocetin glycosides, mainly crocin, trans-crocetin di- $(\beta$ -Dgentibiosyl) ester. This hypothesis explains what happens with saffron, as both zeaxanthin extremes produce picrocrocin (4-(β -D-glucopyranosyl)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde), the compound responsible for saffron bitterness as described in the bibliography (9, 41, 42). Degradation of picrocrocin and other glycosidic compounds, which results from the different chain breaks due to the action of other carotenases, produces different volatile saffron compounds (36). In contrast, in gardenia fruits, none of these glycosidic compounds have been identified. If the biosynthetic pathway described for crocetin esters in both cases is assumed to be the same, then, due to the economy principle for unusual compounds, gardenia should have an extraordinarily efficient enzymatic system in order to eliminate both chain extremes that are produced in considerable amounts, e.g. picrocrocin. To our knowledge, this mechanism has not yet been described.

The chromatographic method proposed in this study enabled us to identify the crocetin esters present in saffron and gardenia. The differences between both species are, except for *trans*-2gg, a question of proportions, as the same compounds are identified. At the same time, it is possible to identify the glycosides present in both species that would permit the authentification of saffron powder, avoiding the adulterations with cheaper gardenia extracts. Further studies could determine the possibility of relating the glycosidic compound content to the aromatic potential determined by gas chromatography.

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