Isolation and Structure Elucidation of Carotenoid−Glycosyl Esters in Gardenia Fruits (*Gardenia jasminoides* Ellis) and Saffron (*Crocus sativus* Linne)

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From the fruits of *Gardenia jasminoides* several carotenoids were isolated and identified. Crocetin **(1)** was characterized by UV/vis, cochromatography, and LC-MS. The following glycosyl esters of crocetin **(1)** were identified: crocin (crocetin di(β-gentiobiosyl) ester) **(6)** by UV/vis, ^{1}H- and ^{13}C-NMR, and FAB-MS and crocetin mono(β-gentiobiosyl) ester **(4)** by UV/vis, ^{1}H-NMR, and LC-MS. There are strong indications for the presence of 13*Z*-crocin **(9)** (UV/vis, ^{1}H-NMR, and LC-MS). From saffron (*Crocus sativus* L.), another glycosyl ester of crocetin **(1)** was isolated. It was identified as crocetin (β-gentiobiosyl) (β-neapolitanosyl) ester **(7)** by UV/vis, ^{1}H-NMR, and MS.

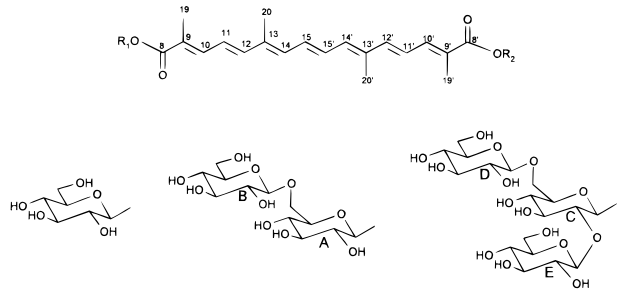
Keywords: *Carotenoids; glycosyl esters; isolation; structure elucidation; Gardenia jasminoides; saffron; Crocus sativus*

INTRODUCTION

Glycosyl esters are compounds containing cyclic carbohydrates which are esterified with a carboxylic acid group at the anomeric carbon atom. On the one hand, these compounds can be classified as esters, on the other hand they can also be regarded as cyclic acetals. In contrast to glycosides which are stable toward bases, glycosyl esters can easily be hydrolyzed both under basic and acidic conditions. Glycosyl esters are very widespread in nature; one of the first isolated representatives of this class of natural products was tannin, a glycosyl ester of glucose and gallic acid (Strecker, 1852). The most prominent carotenoid glycosyl esters which have been isolated from saffron (*Crocus sativus* L. Iridaceae) are derivatives of the C_{20}-dicarboxylic acid crocetin (8,8′-diapocarotene-8,8′-dicarboxylic acid) **(1)**. The main pigment of saffron is crocin **(6)** and was identified by the schools of Karrer and Kuhn as the digentiobiosyl ester of crocetin **(1)** (Karrer and Miki, 1929; Kuhn and L'Orsa, 1931). Later additional carotenoid glycosyl esters have been isolated (Wittwer and Pfander, 1975a,b), and on the basis of UV/vis, IR, ^{1}H-NMR, and mass spectra they were identified as crocetin mono(β-D-glucosyl) ester **(2)**, crocetin di(β-D-glucosyl) ester **(3)**, crocetin mono(β-gentiobiosyl) ester **(4)** and crocetin (β-D-glucosyl) (β-gentiobiosyl) ester **(5)**. Furthermore, 13*Z*-crocin **(9)** has been isolated from saffron and characterized by spectroscopic methods (UV/vis, NMR) and elemental analysis (Speranza et al., 1984). In the course of our investigations of garden crocusses, two new crocetin glycosyl esters with a hitherto unknown trisaccharide have been isolated. On the basis of spectroscopic data the trisaccharide was identified as *O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→6)]-D-glucose, for which the name "neapolitanose" was suggested. The structures of the two new glycosyl esters were established as crocetin (β-gentiobiosyl) (β-neapolitanosyl) ester **(7)** and crocetin di(β-neapolitanosyl) ester **(8)**.

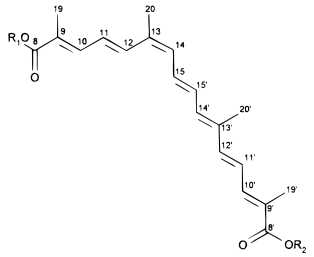
Recently the pigments in the fruits of *Gardenia jasminoides* have been investigated. It was demonstrated that the color of these fruits is mainly due to the presence of carotenoids. On the basis of HPLC and UV/vis data it was postulated that crocetin glycosyl esters, especially crocin **(6)**, occur as the main pigments (Nishizawa et al., 1983; Kamikura and Nakazato, 1985). Since a detailed spectroscopic investigation, especially high-resolution NMR, was not carried out, these postulated structures remain uncertain.

G. jasminoides is either an evergreen bush or a small tree and belongs to the botanical family of the Rubiaceae. The plant has its origin in the southeast of Asia, China, Japan, and India. It is used for several purposes. The flowers have a strong scent; thus, the essential oil is used in perfumery. The fruits are yellow or red husks



X Y Z

1: $R_1 = R_2 =$ H, Crocetin
2: R_1 = H, R_2 = X, Crocetin-mono-(β-D-glucosyl)-ester
3: $R_1 = R_2$ = X, Crocetin-di-(β-D-glucosyl)-ester
4: R_1 = H, R_2 = Y, Crocetin-mono-(β-gentiobiosyl)-ester
5: R_1 = X, R_2 = Y, Crocetin-(β-D-glucosyl)-(β-gentiobiosyl)-ester
6: $R_1 = R_2$ = Y, Crocin [Crocetin-di-(β-gentiobiosyl)-ester]
7: R_1 = Y, R_2 = Z, Crocetin-(β-gentiobiosyl)-(β-neapolitanosyl)-ester
8: $R_1 = R_2$ = Z, Crocetin-di-(β-neapolitanosyl)-ester



9: $R_1 = R_2$ = Y, 13Z-crocin

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and are used as colorants for textiles and especially for food, e.g. noodles and sweets, which makes the identification of these carotenoids highly desirable. In the present paper we describe the isolation and identification of glycosyl esters of carotenoids from the fruits of *G. jasminoides*, as well as the identification of a further glycosyl ester from saffron.

MATERIALS AND METHODS

1. Gardenia. *Apparatus.* High-performance liquid chromatography (HPLC) was performed with a Waters photodiode-array system PDA 991 with two Kontron LC pumps T 414. UV/vis spectra were measured by using the detector PDA 991 of the HPLC apparatus. FAB mass spectrometry was performed with an AutoSpec Q mass spectrometer. For the ionization with a Cs^+ beam the substance was dissolved in dithiothreitol/dithioerythritol as matrix. The LC-MS was measured with a Fisons VG platform. The substances were ionized by APCI (atmospheric pressure chemical ionization), in negative or positive mode. The measuring conditions for compounds **1** and **9** are as follows: corona voltage, negative mode −2 kV; cone voltage, −20 V; probe temperature, 400 °C; source temperature: 120 °C. The measuring conditions for compound **4** are as follows: corona voltage, negative mode −2 kV, positive mode +3.2 kV; cone voltage, −40 V and +20 and +70 V, respectively; probe temperature, 400 °C; source temperature, 120 °C. The NMR spectra were recorded on a Bruker AC 300 or Bruker DRX 400 instrument.

Solvents. All solvents were freshly distilled. For HPLC, solvents with HPLC quality were used (Romil Ltd., Cambridge U.K.).

Plant Material. Dried and cut gardenia fruits were purchased from Haenseler AG, Herisau, Switzerland. The sample preparation was as follows: The fruits were ground with an electric mill. The resulting powder was lyophilized for 4 days to remove the remaining H_2O (about 8%).

Extraction of Gardenia Fruits. The lyophilized powder was extracted three times with diethyl ether to remove the lipids and other nonpolar substances. After the solvent was evaporated, a brown, oily substance was obtained in a 13% yield (extract 1). The remaining gardenia powder was extracted three times with ethanol (EtOH) (70%) to dissolve the polar substances. After the solvent was evaporated, a red-orange solid material was obtained in a 33% yield (extract 2).

*Isolation of Crocetin **(1)**.* Extract 1 was extracted with methanol (MeOH) by shaking in a centrifugation tube. The phase separation was achieved by centrifugation. The yellow MeOH extract was decanted and separated on silica (TLC) with the eluent hexane/ethyl acetate (EtOAc)/MeOH (3:1:1). The most intense yellow zone at R_f = 0.31 was isolated, followed by a further TLC separation on silica with 94% EtOH/triethylamine (TEA) (4:1) as solvent. Afterward the substance was submitted to HPLC. A separation from impurities was achieved on a nitrile column (250 × 10 mm Nucleosil 5 CN, Macherey-Nagel, Switzerland) with the solvent isopropyl alcohol (IPA)/MeOH/tetrahydrofuran (THF) (2:3:1). For cochromatography with authentic crocetin **(1)**, the TLC system on silica with the eluent hexane/EtOAc/MeOH (3:1:1) (R_f = 0.31) and the described HPLC system (t_R = 11.5 min) were used.

*Isolation of Crocin (Crocetin Di(β-gentiobiosyl) Ester) **(6)**.* Extract 2 was separated on silica (TLC) with the solvent EtOAc/IPA/H_2O (56:34:10). Six yellow zones were obtained from R_f = 0.1 to R_f = 0.6 (TLC1). The most intense yellow zone at R_f = 0.19 was isolated from TLC and peracetylated in pyridine/acetic anhydride (2:1) with 4-(dimethylamino)pyridine as catalyst. The reaction was performed at room temperature during 24 h under argon atmosphere. In order to remove the pyridine, the mixture was washed very shortly in a separatory funnel with 1 N HCl and diethyl ether. The ether phase was quickly washed with H_2O and a saturated $NaHCO_3$ solution and dried over anhydrous Na_2SO_4. The resulting substance was purified on silica (TLC) with the eluent toluene/EtOAc (3:7), where the yellow substance exhibited a R_f = 0.6. After a second purification on silica (TLC) with hexane/EtOAc/MeOH (3:1:1) (R_f = 0.35) a pure substance was obtained which was submitted to spectroscopic analysis.

*Isolation of 13Z-Crocin (13Z-Crocetin Di(β-gentiobiosyl) Ester) **(9)**.* From TLC1 the yellow substance at R_f = 0.1 was isolated. It was peracetylated and worked up as described above for crocin **(6)**. The substance was purified on silica (TLC) with the solvent toluene/EtOAc (3:7). Three yellow not-well-separated zones from R_f = 0.45 to R_f = 0.65 were isolated together. A further separation on silica (TLC) was carried out with the solvent hexane/EtOAc/MeOH (3:1:1). Three yellow zones at R_f = 0.48, 0.64, and 0.75 were isolated separately. The substances with R_f = 0.64 and 0.75 were not submitted to further investigation. The substance with R_f = 0.48 was further purified with HPLC on a reversed-phase column (250 × 10 mm Nucleosil 5 C_{18}, Macherey-Nagel) with the solvent MeOH/H_2O (9:1). The purified substance was submitted to spectroscopic analysis.

*Isolation of Crocetin Mono(β-gentiobiosyl) Ester **(4)**.* Extract 2 was peracetylated as described above for crocin. The reaction mixture was separated on silica with a flash column into four fractions with the solvent toluene/EtOAc (3:2). The first eluted fraction was purified on silica (TLC) with the solvent hexane/EtOAc/MeOH (3:1:1). With another separation on silica (TLC) with the solvent *tert*-butyl methyl ether (TBME)/acetone (1:1), two yellow zones were obtained (R_f = 0.35 and 0.7). The zone with R_f = 0.35 was again purified on silica (TLC) with the solvent TBME/acetone/THF (1:1:1) and afterward purified with HPLC on a reversed phase column (250 × 4.6 mm, Nucleosil 120-3 C_{18}, Macherey-Nagel) with the solvent MeOH/H_2O/TEA (90:10:1) and submitted to spectroscopic analysis.

2. Saffron. *Apparatus.* HPLC was performed with a Waters photodiode-array system PDA 996, a multisolvent delivery system pump Waters 600 E. UV/vis spectra were measured with the detector PDA 996 of the HPLC apparatus. MALDI-TOF mass spectrometry was performed with a linear time of flight spectrometer (built in our laboratories) which contained a turbomolecular pump of 300 L s^{-1}, a roughing pump of 16 m^3 h^{-1}, a two-stage microchannel plate detector, a vacuum of 2 × 10^{-7} mbar, a nitrogen laser, a pulse width of 3 ns, a wavelength of 337 nm, a field-free drift tube of 1.5 m, and acceleration by two stages to 28 keV. In order to prevent a photochemical degradation of the analyte, 2,5-dihydroxybenzoic acid was used as matrix. The NMR spectra were recorded on Bruker DRX 400 or Bruker DRX 500 instruments.

Plant Material. Whole stigmas of *C. sativus* L. (from Spain) were bought from Siegfried AG, Zofingen, Switzerland.

Extraction. In order to remove lipids and other nonpolar compounds, the pulverized stigmas were stirred two times with diethyl ether, and afterward the solvent was discarded. The remaining deep red powder was extracted twice with 70% EtOH . After the solvent was evaporated under reduced pressure at a temperature below 35 °C, a dark red shiny powder was obtained in a 44% yield.

*Isolation of Crocetin (β-Gentiobiosyl) (β-Neapolitanosyl) Ester **(7)**.* The EtOH extract was separated on silica (TLC) with EtOAc/IPA/H_2O (45:30:20) as mobile phase. Seven zones were obtained, six of them have been identified before by Wittwer and Pfander (1975a,b) as crocetin **(1)** and mono- and diester, respectively, of crocetin with glucose and gentiobiose. The most polar zone (yellow-orange) R_f = 0.29 was isolated from TLC and peracetylated in pyridine/acetic anhydride (2:1) with 4-(dimethylamino)pyridine as catalyst. The reaction was performed at room temperature during 24 h under argon atmosphere. In order to remove the pyridine, the mixture was treated very quickly in a separatory funnel with 1 M HCl and diethyl ether. The diethyl ether phase was washed twice with saturated $NaHCO_3$ solution and four times with H_2O and then dried over anhydrous Na_2SO_4. The resulting compound was purified on silica (TLC) with toluene/EtOAc (3:7) as mobile phase. Three zones were obtained, the major and most polar one (R_f = 0.35) was desorbed with EtOAc. The isolated acetylated carotenoid−glycosyl ester was further purified by HPLC on a reversed-phase column (250 × 10 mm Nucleosil 5 C_{18}, Macherey-Nagel) with MeOH/EtOAc/H_2O (2:1:1) as mobile

phase, flow rate of 2 mL/min. The purified compound was submitted to MALDI-TOF MS and NMR spectroscopy.

RESULTS AND DISCUSSION

1. Gardenia. *Crocetin **(1)***. On the basis of the cochromatography in two different chromatographic systems (described before), the UV/vis spectrum, and the LC-MS data, the substance was identified as crocetin **(1)**. It displayed UV/vis maxima at $\lambda = 417$ and 442 nm in MeOH/H_2O (9:1). LC-MS (negative mode): m/z 328 [M^-], 284 [$M - 44]^-$. The peak at m/z 284 is characteristic of loss of a carboxy group.

*Crocin (Crocetin Di(β-gentiobiosyl) Ester) **(6)***. On the basis of spectroscopic data (UV/vis, ^{1}H- and ^{13}C-NMR, FAB-MS) the substance was identified as peracetylated crocin **(6)**. It displayed UV/vis maxima at $\lambda = 437$ and 461 nm in MeOH/H_2O (9:1). Compared to crocetin **(1)**, the maxima exhibited a bathochromic shift of about 20 nm, which is also visible by the orange color of the solutions of crocin **(6)**, while solutions of crocetin are yellow. ^{1}H-NMR (300 MHz, $CDCl_3$): carotenoid part, δ 1.98 [s, $H_3C(19)$], 2.00 [s, $H_3C(20)$], 6.42 [AA′XX′, H−C(14)], 6.54 [dd, $J_{11/10} = 11.5$, $J_{11/12} = 14.9$, H−C(11)], 6.68 [d, $J_{12/11} = 14.9$, H−C(12)], 6.74 [AA′XX′, H−C(15)], 7.33 [d, $J_{10/11} = 11.5$, H−C(10)]; carbohydrate part (A1 means proton of C1 in sugar moiety A, etc.; A6.1 means proton 1 of C6 in sugar moiety A, etc.), sugar moiety A, δ 3.60 [dd, $J_{A6.1/A6.2} = 11.0$, $J_{A6.1/A5} = 1.3$, H(A6.1)], 3.84 [ddd, $J_{A5/A4} = 9.4$, $J_{A5/A6.1} = 1.3$, $J_{A5/A6.2} = 4.9$, H(A5)], 3.94 [dd, $J_{A6.2/A6.1} = 11.0$, $J_{A6.2/A5} = 4.9$, H(A6.2)], 5.02 [dd, $J_{A4/A3} = 9.7$, $J_{A4/A5} = 9.4$, H(A4)], 5.20 [dd, $J_{A2/A1} = 8.0$, $J_{A2/A3} = 11.9$, H(A2)], 5.28 [dd, $J_{A3/A2} = 11.9$, $J_{A3/A4} = 9.7$, H(A3)], 5.74 [d, $J_{A1/A2} = 8.0$, H(A1)], carbohydrate part, sugar moiety B, δ 3.64 [ddd, $J_{B5/B4} = 9.4$, $J_{B5/B6.1} = 2.0$, $J_{B5/B6.2} = 4.9$, H(B5)], 4.09 [dd, $J_{B6.1/B6.2} = 12.4$, $J_{B6.1/B5} = 2.0$, H(B6.1)], 4.25 [dd, $J_{B6.2/B6.1} = 12.4$, $J_{B6.2/B5} = 4.9$, H(B6.2)], 4.56 [d, $J_{B1/B2} = 7.9$, H(B1)], 4.99 [dd, $J_{B2/B1} = 7.9$, $J_{B2/B3} = 9.4$, H(B2)], 5.05 [dd, $J_{B4/B3} = 9.8$, $J_{B4/B5} = 9.4$, H(B4)], 5.18 [dd, $J_{B3/B2} = 9.4$, $J_{B3/B4} = 9.8$, H(B3)]. For supplementary information a T-ROESY experiment (Hwang and Shaka, 1992) was performed. Cross-peaks were found between the following proton signals: H-A1 ↔ H-A5; H-A3 ↔ H-A5; H-A4 ↔ H-A6.1; H-A4 ↔ H-A6.2; H-A6.1 ↔ H-A6.2; H-B1 ↔ H-B5; H-B3 ↔ H-B5; H-B4 ↔ H-B6.1; H-B4 ↔ H-B6.2. ^{13}C-NMR (100 MHz, $CDCl_3$): carotenoid part, δ 12.60 [C(20)], 12.75 [C(19)], 123.55 [C(11)], 124.42 [C(9)], 131.72 [C15)], 136.24 [C(14)], 136.83 [C(13)], 141.36 [C(10)], 145.33 [C(12)], 165.98 [C(8)]; carbohydrate part, sugar moiety A, δ 67.55 [C(A6)], 68.64 [C(A4)], 70.15 [C(A2)], 72.77 [C(A3)], 73.87 [C(A5)], 91.95 [C(A1)]; sugar moiety B, δ 61.75 [C(B6)], 68.28 [C(B4)], 70.86 [C(B2)], 71.83 [C(B5)], 72.77 [C(B3)], 100.52 [C(B1)]. FAB-MS: m/z 1604 $[M + H + K]^+$, 1588 $[M + H + Na]^+$, 1565 $[M + H]^+$, 619 $[M - 945]^+$ (peracetylated gentiobiose residue), 331 $[M - 1233]^+$ (peracetylated glucose residue). The coupling constants (J) of all vicinal protons within the sugar moieties are ≥8 Hz. This fact proves clearly that the positions of the protons in the pyrane rings are axial. The proof of the C6→C1 connection between the sugar moieties is the following: the proton(s) of the C-atom to which the anomeric C-atom is bound must exhibit a shift to higher field (Rychener et al., 1984). This is the case for A6.1 and A6.2. The fact that no ROE signal between any protons of moieties A and B are visible is an additional argument for the C6→C1 connection.

*13Z-Crocin (13Z-Crocetin Di(β-gentiobiosyl) Ester) **(9)***. Under the NMR measuring conditions a partial *E*/*Z*-isomerization of the isolated compound **9** could be noticed, due to the presence of very weak corresponding signals in the proton spectrum. This fact prevented the realization of further experiments than COSY for proving the 13*Z*-configuration clearly. However, characterisitic chemical shift differences $\Delta\delta = \delta_z - \delta_{allE}$ (ppm) between selected proton resonances of any *Z*-carotenoid and its corresponding all-*E* isomer usually allow one to determine the sort of isomer (Englert, 1995); in the case of an isomerization at C(13) the proton chemical shifts of H−C(10) {+0.05}, H−C(12) {+0.52}, H−C(14) {−0.13}, H−C(15) {+0.16}, and H−C(15′) {−0.08} are most and significantly influenced, with expected $\Delta\delta$ values according to Englert (1995) shown in braces. The resulting analogous proton shift differences $\Delta\delta = \delta_{13z-crocin} - \delta_{allE-crocin}$ values of +0.05, +0.49, −0.16, +0.13, and −0.12 ppm were nearly identical with the expected ones. Therefore, the 13*Z*-configuration of **9** can be taken as correct. Considering also the LC-MS and UV/vis data, the existence of crocin in its 13*Z*-configuration can be regarded as true. The substance displayed UV/vis maxima at $\lambda = 324$, 432 and 456 nm in MeOH/H_2O (9:1). Compared to all-*E*-crocin, the maxima exhibited a hypsochromic shift of 5 nm, which is characteristic for *Z*-isomers, as well as the "cis-peak" (at 324 nm), which appeared about 140 nm below the maximum with the highest wavelength. ^{1}H-NMR (400 MHz, $CDCl_3$): carotenoid part, δ 2.00 [$H_3C(19)$, $H_3C(20)$, $H_3C(19')$, $H_3C(20')$], 6.26 [H−C(14)], 6.35 [H−C(14′)], 5.56 [H−C(11)], 6.57 [H−C(11′)], 6.62 [H−C(15′)], 6.70 [H−C(12′)], 6.87 [H−C(15)], 7.17 [H−C(12)], 7.38 [H−C(10)]; carbohydrate part, sugar moiety A, δ 3.85 [H(A6.1)], 3.86 [H(A5)], 3.94 [H(A6.2)], 5.02 [H(A4)], 5.21 [H(A2)], 5.29 [H(A3)], 5.74 [H(A1)]; sugar moiety B, δ 3.63 [H(B5)], 4.11 [H(B6.1)], 4.26 [H(B6.2)], 4.57 [H(B1)], 4.99 [H(B2)], 5.04 [H(B4)], 5.19 [H(B3)]. LC-MS (negative mode): 1564 [M^-].

*Crocetin Mono(β-gentiobiosyl) Ester **(4)***. On the basis of spectroscopic data (UV/vis, ^{1}H-NMR, LC-MS) compound **4** was identified as peracetylated crocetin mono-(β-gentiobiosyl) ester. It displayed UV/vis maxima at $\lambda = 430$ and 452 nm in MeOH/H_2O (9:1). Compared to crocetin **(1)**, the maxima exhibited a bathochromic shift of about 12 nm. ^{1}H-NMR (400 MHz): carotenoid part (methanol-d_4), δ 2.00 [s, $H_3C(19)$, $H_3C(20)$, $H_3C(19')$, $H_3C(20')$], 6.37 [d, $J_{14'/15'} = 11.4$, H−C(14′)], 6.50 [m, $J_{14/15} = 11.8$, H−C(14)], 6.54 [d, $J_{12/11} = 15.5$, H−C(12)], 6.63 [dd, $J_{11'/10'} = 11.7$, $J_{11'/12'} = 14.8$, H−C(11′)], 6.64 [dd, $J_{11/10} = 10.2$, $J_{11/12} = 15.5$, H−C(11)], 6.75 [dd, $J_{15/14} = 11.8$, $J_{15/15'} = 14.2$, H−C(15)], 6.75 [d, $J_{12'/11'} = 14.8$, H−C(12′)], 6.84 [dd, $J_{15'/14'} = 11.4$, $J_{15'/15} = 14.2$, H−C(15′)], 7.06 [d, $J_{10/11} = 10.2$, H−C(10)], 7.34 [d, $J_{10'/11'} = 11.7$, H−C(10′)]; carbohydrate part ($CDCl_3$), sugar moiety A, δ 3.61 [H(A6.1)], 3.84 [H(A5)], 3.93 [H(A6.2)], 5.02 [dd, $J_{A4/A3} = 9.5$, H(A4)], 5.21 [dd, $J_{A2/A1} = 8.1$, H(A2)], 5.29 [dd, $J_{A3/A4} = 9.5$ H(A3)], 5.74 [d, $J_{A1/A2} = 8.1$, H(A1)]; sugar moiety B, δ 3.65 [H(B5)], 4.09 [dd, $J_{B6.1/B6.2} = 12.0$, $J_{B6.1/B5} = 2.6$, H(B6.1)], 4.25 [dd, $J_{B6.2/B6.1} = 12.0$, $J_{B6.2/B5} = 4.5$, H(B6.2)], 4.56 [d, $J_{B1/B2} = 7.9$, H(B1)], 4.99 [dd, $J_{B2/B1} = 7.9$, $J_{B2/B3} = 9.7$, H(B2)], 5.05 [dd, $J_{B4/B3} \cong 9.5$, $J_{B4/B5} = 9.8$, H(B4)], 5.18 [dd, $J_{B3/B2} = 9.7$, $J_{B3/B4} \cong 9.5$, H(B3)], 4.56 [d, $J_{B1/B2} = 7.9$, H(B1)). LC-MS: negative mode, m/z 946 [M^-]; positive mode, m/z 969 $[M + Na]^+$, 331 $[M - 615]^+$ (peracetylated glucose residue).

2. Saffron. *Crocetin (β-Gentiobiosyl) (β-Neapolitanosyl) Ester **(7)***. On the basis of spectroscopic data (UV/vis, ^{1}H-NMR, MALDI-TOF-MS) compound **7** was iden-

tified as peracetylated crocetin (β-gentiobiosyl) (β-neapolitanosyl) ester). The substance displayed UV/vis maxima at λ = 441 and 465 nm in MeOH/H_2O (9:1). Compared to crocetin **(1)**, the maxima exhibited a bathochromic shift of about 24 nm. ^{1}H-NMR (500 MHz, $CDCl_3$): carotenoid part, δ 2.00 [s, $H_3C(19)$, $H_3C(19')$, $H_3C(20)$, $H_3C(20')$], 6.42 [m, H−C(14), H−C(14′)], 6.54 [dd, $J_{11'/10'} \cong 11$, $J_{11'/12'} = 15.35$, H−C(11′)], 6.55 [dd, $J_{11/10} \cong 11$, $J_{11/12} = 14.7$, H−C(11)], 6.63 [d, $J_{12/11} = 14.7$, H−C(12)], 6.69 [d, $J_{12'/11'} = 15.35$, H−C(12′)], 6.73 [m, H−C(15′)], 6.74 [m, H−C(15)], 7.34 [d, $J_{10'/11'} \cong 11$, H−C(10′)],7.38 [d, $J_{10/11} \cong 11$, H−C(10)]; carbohydrate part, sugar moiety A, δ 3.56 [H(A6.1)], 3.80 [H(A5)], 3.99 [H(A6.2)], 5.02 [H(A4)], 5.21 [H(A2)], 5.28 [H(A3)], 5.75 [d, $J_{A1/A2} = 8.1$, H(A1)]; sugar moiety B, δ 3.62 [H(B5)], 4.10 [H(B6.1)], 4.24 [H(B6.2)], 4.69 [$J_{B1/B2} = 8.2$, H(B1)], 4.92 [$J_{B2/B1} = 8.2$, $J_{B2/B3} = 9.4$, H(B2)], 5.02 [H(B4)], 5.12 [H(B3)]; sugar moiety C, δ 3.59 [H(C6.1)], 3.83 [H(C5)], 3.94 [H(C6.2)], 3.95 [dd, $J_{C2/C1} = 8.0$, $J_{C2/C3} = 9.3$, H(C2)], 5.01 [H(C4)], 5.24 [dd, $J_{C3/C2} = 9.3$, $J_{C3/C4} = 9.1$, H(C3)], 5.72 [d, $J_{C1/C2} = 8.0$, H(C1)]; sugar moiety D, δ 3.64 [H(D5)], 4.10 [H(D6.1)], 4.21 [H(D6.2)], 4.56 [d, $J_{D1/D2} = 8.0$, H(D1)], 4.99 [H(D2)], 5.06 [H(D4)], 5.18 [H(D3)]; sugar moiety E, δ 3.66 [H(E5)], 4.04 [H(E6.1)], 4.28 [H(E6.2)], 4.52 [d, $J_{E1/E2} = 8.0$, H(E1)], 5.00 [H(E2)], 5.03 [H(E4)], 5.19 [H(E3)]. MALDI-TOF: m/z 1876 $[M + Na]^+$.

A comparison between gardenia fruits and saffron shows that all the carotenoid−glycosyl esters which have been found in gardenia are also present in saffron. Crocetin (β-gentiobiosyl) (β-neapolitanosyl) ester **(7)** but also a few less polar glycosyl esters of saffron have not yet been isolated from gardenia. Due to this similar pigment composition of gardenia fruits and saffron, it is evident that gardenia extracts can be used as food colorant. As gardenia fruits are very cheap, they could even replace expensive saffron in cases in which only the color and not the taste of saffron is desired.

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