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The Flavonoids of Tomatoes

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Tomatoes (*Lycopersicon esculentum* Mill.) have been recognized as an important source of dietary flavonoids because of a high consumption worldwide. The qualitative and quantitative flavonoid compositions of assorted tomato cultivars including individual quantitative contributions of the five most significant flavonoids have been determined in this work. The dihydrochalcone phloretin 3′,5′-di-*C*-β-glucopyranoside and the flavonol quercetin 3-*O*-(2″-*O*-β-apiofuranosyl-6″-*O*-α-rhamnopyranosyl-β-glucopyranoside) were identified for the first time in *Solanaceae* spp. and found to be among the main flavonoids in all cultivars. Phloretin 3′,5′-di-*C*-glc is the first *C*-glycoside identified in tomatoes and also the first dihydrochalcone from this species. In addition, chalconaringenin, kaempferol 3-rutinoside, and quercetin 3-rutinoside (rutin), though previously reported to occur in tomato, were fully characterized by extensive use of 2D NMR techniques and high-resolution LCMS. The total flavonoid content of different tomato types varied from 4 to 26 mg 100^{-1} g FW with chalconaringenin as the predominant compound comprising 35 to 71% of the total flavonoid content. The individual quantities of quercetin 3-*O*-(2″-*O*-β-apiofuranosyl-6″-*O*-α-rhamnopyranosyl-β-glucopyranoside) and phloretin 3′,5′-di-*C*-β-glucopyranoside was similar to that of rutin in several cultivars.

KEYWORDS: Tomato; *Lycopersicon esculentum* Mill.; flavonoids; dihydrochalcone; chalcone; flavonol; phloretin 3′,5′-di-*C*-β-glucopyranoside; quercetin 3-*O*-(2″-*O*-β-apiofuranosyl-6″-*O*-α-rhamnopyranosyl)-β-glucopyranoside; rutin; kaempferol 3-rutinoside; chalconaringenin; NMR; MS

INTRODUCTION

Flavonoids are considered as potentially health-promoting substances, and efforts are made to produce tomatoes with a standardized content of flavonoids and other compounds (*1, 2*), or to elevate the concentration of especially quercetin 3-rutinoside (rutin). Such work is based upon either GMO strategies (*3–5*), non-GMO strategies (*6*), or combinations thereof. Commercial tomatoes belong to the family of *Solanaceae* and are most frequently referred to as *Lycopersicon esculentum* Miller. Alternative names (*Solanum lycopersicum* L. or *Lycopersicon lycopersicum* L. (Karsten)) have appeared in previous literature. The large and diverse *Solanacea* family comprising more than 3000 species, is extensively used by humans as important sources of food, spice, and medicine. *Solanacea* includes important food plants like tomato, potato, pepper, and eggplant, plants of high horticultural value like petunia, as well as poisonous genera.

Tomatoes are worldwide among the most consumed vegetables and play an important role in the human diet. In previous literature, however, flavonoids of tomatoes have scarcely been completely characterized. The occurrence of flavonoids in fruits of tomatoes is almost exclusively restricted to their skin, leaving only negligible quantities in the remaining parts of the fruit (*7*). The main flavonoids in fruits of tomatoes identified in previous literature have been reported to be rutin (*8–10*), naringenin (*8, 11, 12*), and chalconaringenin (*1, 13, 14*). Moreover, some minor flavonoids have been identified from tomato fruits as reviewed by Moco and co-workers (2006) (*15*), among them kaempferol 3-rutinoside (*9, 12*) and naringenin 7-glucoside (*13, 16*). The

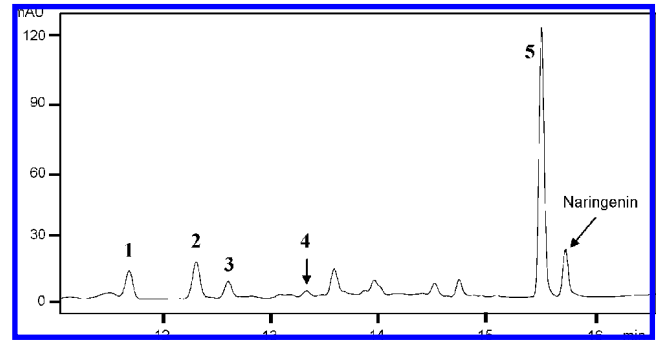

Figure 1. HPLC chromatogram (280 ± 10 nm) of crude extract of peel of tomato ('Dometica') revealing the presence of **1–5** together with the artifact naringenin formed by modification of chalconaringenin.

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Table 1. Chromatographic (HPLC) and Spectral (UV and High-Resolution MS) Data Recorded for Quercetin 3-(2″-Apiosyl-6″-rhamnosylglucoside) (**1**), Quercetin 3-(6″-Rhamnosylglucoside) (Rutin) (**2**), Kaempferol 3-(6″-Rhamnosylglucoside) (**3**), Phloretin 3′,5′-Di-*C*-glucoside (**4**), and Chalconaringenin (**5**)

compd	t_R, min	UV_{max}, nm (online HPLC)	$[M+H]^+$, *m/z*	fragment ions, *m/z*
1	11.66	356, 256	743.2047	611.1646; 597.1628; 465.1125; 303.0470
2	12.29	355, 257	611.1602	465.1033; 303.0495
3	13.30	349, 266	595.1603	449.1184; 287.0538
4	12.59	286, 330 sh	599.1896	581.1801; 563.1680
5	15.52	366	273.0708	

Table 2. ^1H and ^{13}C NMR Chemical Shifts (ppm) and Coupling Constants (Hz) of Quercetin 3-(2″-β-Apiofuranosyl-6″-α-rhamnopyranosyl-β-glucopyranoside) (**1**), Quercetin 3-(6″-α-Rhamnopyranosyl-β-glucopyranoside) (**2**) and Kaempferol 3-(6″-α-Rhamnopyranosyl-β-glucopyranoside) (**3**)

	1 δ H	2 δ H	3 δ H	1 δ C	2 δ C	3 δ C
2				156.33	156.67	156.93
3				133.04	133.37	133.29
4				177.34	177.46	177.47
5				161.34	161.29	161.27
6	6.16 d 2.1	6.18 d 2.1	6.19 d 2.1	98.71	98.74	98.80
7				164.20	164.15	164.20
8	6.35 d 2.1	6.38 d 2.1	6.40 d 2.1	93.58	93.65	93.82
9				156.41	156.48	156.56
10				103.97	104.03	104.07
1′				121.29	121.24	120.96
2′	7.48 d 2.2	7.52 d 2.3	7.97 'd' 8.9	116.07	116.33	130.96
3′			6.87 'd' 8.9	148.48	148.49	115.17
4′				144.88	144.83	159.99
5′	6.81 d 8.4	6.83 d 8.2	6.87 'd'8.9	115.25	115.30	115.17
6′	7.57 dd 2.2, 8.4	7.53 dd 2.3, 8.2	7.97 'd'8.9	121.89	122.66	130.96
			3-*O*-glc			
1″	5.47 d 7.7	5.33 d 7.6	5.30 d 7.6	98.82	101.25	101.40
2″	3.46 dd 7.7, 9.0	3.21 m	3.20 m	76.95	74.14	74.25
3″	3.37 t 9.0	3.26 m	3.31 m	77.03	75.98	75.82
4″	3.04 t 9.0	3.04 m	3.04 m	70.36	70.07	70.00
5″	3.21 m	3.22 m	3.21 m	75.80	76.52	76.44
6A″	3.66 m	3.69 dd 1.8, 11.4	3.67 dd 1.8, 11.4	66.90	67.06	66.96
6B″	3.20 m	3.27 m	3.26 m			
			6″-*O*-rha			
1‴	4.31 d 1.7	4.37 d 1.8	4.36 d 1.8	100.71	101.82	101.84
2‴	3.33 dd 1.7, 3.4	3.37 dd 1.8, 3.6	3.37 m	70.41	70.44	70.37
3‴	3.23 dd 3.4, 9.4	3.26 m	3.33 m	70.60	70.63	70.67
4‴	3.04 t 9.4	3.06 t 9.4	3.07 t 9.4	71.88	71.91	71.91
5‴	3.21 m	3.25 m	3.25 m	68.30	68.31	68.34
6‴	0.95 d 6.2	0.98 d 6.3	0.97 d 6.3	17.76	17.81	17.81
			2″-*O*-apiosyl			
1⁗	5.32 d 1.6			108.68		
2⁗	3.78 d 1.6			76.23		
3⁗				79.31		
4A⁗	3.80 d 9.3			74.03		
4B⁗	3.47 d 9.3					
5A⁗	3.43 d 11.2			64.37		
5B⁗	3.35 d 11.2					

pre-1980 structure determinations were mainly based on paper or thin layer chromatographic methods and from the 1980s on hyphenated high-performance liquid chromatography (HPLC) with UV and/or MS detection (*15*). Nuclear magnetic resonance (NMR) is the most important technique for complete structure determination of flavonoids (*17*). However, the 3,7-di-*O*-glucoside and the 3-*O*-rutinoside-7-*O*-glucoside of kaempferol, identified as minor constituents in tomatoes (*18*), are the only flavonoids from tomatoes characterized by NMR spectroscopic methods in previous literature.

In this paper, complete NMR spectroscopic characterization of the major flavonoids isolated from tomatoes is presented, followed by quantification of individual pigments, leading to a major qualitative and quantitative revision of the main flavonoids from this source.

MATERIALS AND METHODS

Plant Material. Samples of different types of tomatoes including round ('Elanto'), cluster ('Dometica'), big plum ('Romana'), cherry ('Favorita' and 'Sebra'), cocktail ('Aranca'), beaf ('Buffalo'), small plum ('Ministar'), and marmande ('Eugenia') were received from commercial greenhouses in close proximity to Særheim Research Centre (58° 47′ N, 5° 41′ E). All tomato cultivars were grown on rockwool and harvested during the 2007 season at ripening stage III (*19*). The average mass of the tomatoes used in this paper is provided in **Table 4**.

Chemicals. Technical grade methanol was obtained from Statoil, Stavanger, Norway. Rutin and chalconaringenin were isolated and purified as in-house reference compounds by PlantChem according to methods described below. Naringin (naringenin 7-*O*-β-neohesperidoside), naringenin, acetonitrile, ethyl acetate, and trifluoroacetic acid were obtained from Sigma-Aldrich, Oslo, Norway.

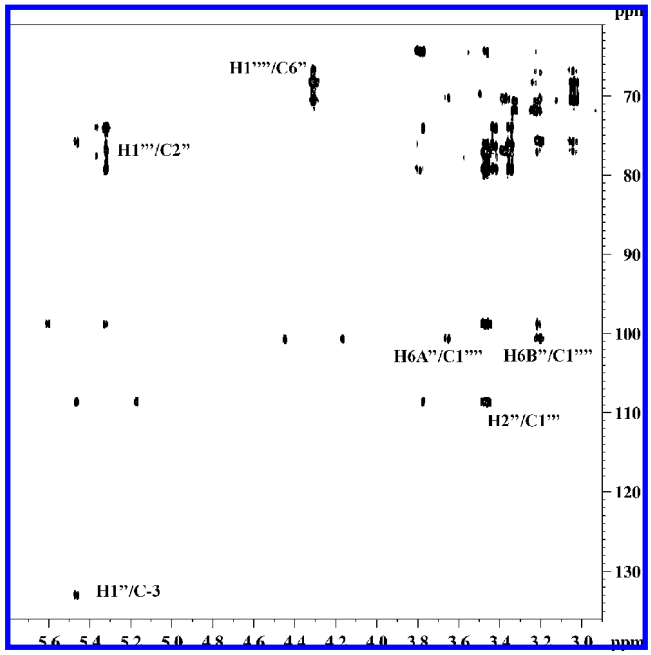

Figure 2. Expanded region of the 2D ^{1}H–^{13}C HMBC NMR spectrum of quercetin 3-*O*-(2″-*O*-β-apiofuranosyl-6″-*O*-α-rhamnopyranosyl-β-glucopyranoside) (**1**). Cross peaks vital for determination of the inter-residual connections are highlighted.

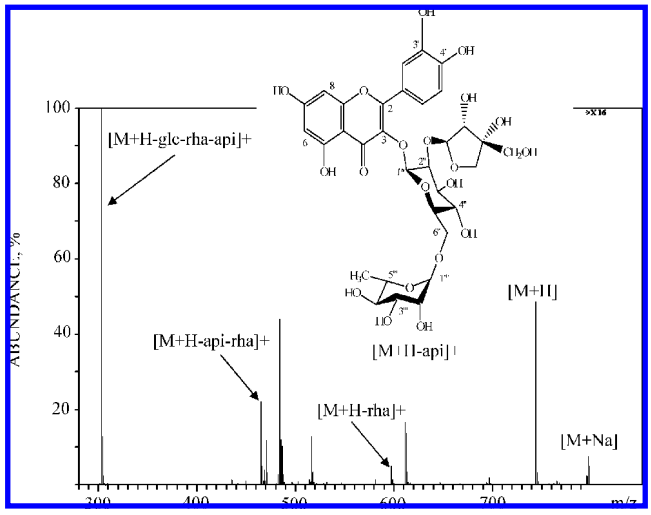

Figure 3. High-resolution mass spectrum of quercetin 3-*O*-(2″-*O*-β-apiofuranosyl-6″-*O*-α-rhamnopyranosyl-β-glucopyranoside) (**1**). Pseudomolecular ions and fragment ions resulting from subsequent loss of glycosyl units are annotated.

Extraction and Isolation. About 7 kg FW frozen tomatoes ('Dometica') were soaked in temperated water (around 10 °C) for about 2 min and then peeled. The skin was extracted in darkness at ambient temperature for 24 h with 2 × 2 L methanol. After concentration to about 0.5 L on a rotary evaporator, the combined extracts were partitioned against 3 × 0.5 L ethyl acetate. The pale yellow water (lower) phase was further concentrated to 10 mL, subjected to a 50 × 5 cm bed of Amberlite XAD-7, and washed with water. The adsorbed part of the sample was eluted with methanol, concentrated under reduced pressure, and then subjected to an 80 × 1.5 cm bed of Sephadex LH-20 (Amersham Bioscience, Uppsala, Sweden), and eluted using a 15 to 30% stepwise gradient of methanol in water. The ethyl acetate (upper) phase from the partitioning of the crude extract was concentrated and subjected to an 80 × 1.5 cm bed of Sephadex LH-20, and the main phenolic band representing chalconaringenin was eluted using 70% of methanol in water.

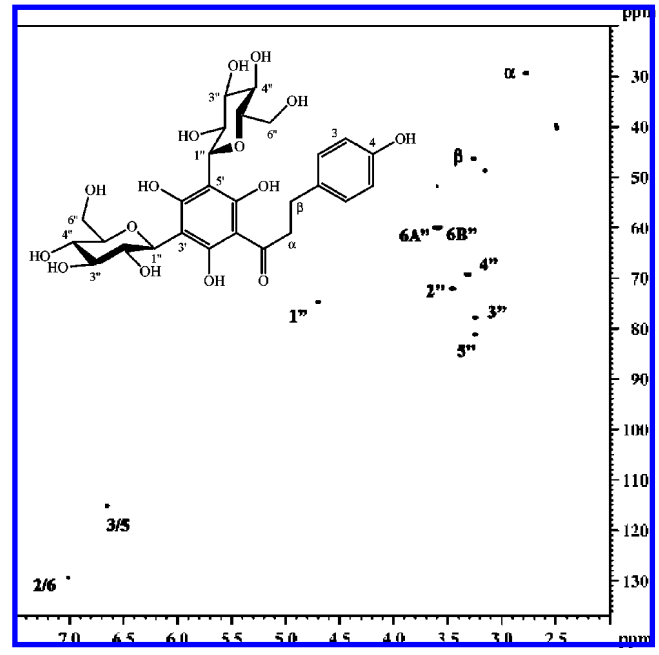

Figure 4. 2D ^{1}H–^{13}C HSQC NMR spectrum of phloretin 3′,5′-di-*C*-β-glucopyranoside (**4**) showing assignment of ^{13}C resonances important for verification of the symmetrically di-*C*-glycosylated dihydrochalcone.

Four tomatoes within each variety were measured by weight and size and submerged in liquid N_2 for 1 min in order to make the fruits fragile. Thereafter, the plant material originating from each cultivar was homogenized in a coffee grinder (Bosch, Germany) for 15 s, giving a white, farinaceous powder. About 1 g of each sample was exactly weighed, transferred to sample tubes (24 × 150 mm), and mixed with 10 mL of acidic methanol (0.05% TFA, v/v) for extraction of flavonoids. The extracts were vortexed for 10 s; then the sample tubes were sealed and the extracts were kept in darkness at ambient temperature for 24 h. Aliquots of these extracts were analyzed directly by HPLC.

HPLC. HPLC analyses were performed on an Agilent1100-system, Agilent Technologies, equipped with an autosampler and a photodiode array detector. Separation of individual compounds was achieved on an Eclipse XDB-C8 (4.6 × 150 mm, 5 μm) column (Agilent Technologies). The flavonoids were separated by use of a binary solvent system consisting of (A) 0.05% TFA in water and (B) 0.05% TFA in acetonitrile. The gradient elution profile (%B in A) consisted of linear gradient from 5% B to 10% B (0–5 min), a linear gradient (10% B to 25% B) for the next 5 min, a linear gradient from 25% B to 85% B during the next 6 min, followed by linear gradient from 85% B to 5% B in 2 min. Prior to each injection, the column was reconditioned (5% B, 95% A) for 2 min. The flow rate was 0.8 mL/min. Aliquots of 10 μL were injected and separation was monitored at 30 °C. To cover individual variations of maximum absorption wavelengths of the structurally diverse flavonoids encountered, chromatograms were recorded at 280 ± 10 nm and 370 ± 20 nm. Prior to injection, individual samples were filtered through a 13 mm syringe filter (Nylon 0.45 μm, VWR International). Standard curves were made for individual compounds analyzed by HPLC. Depending on their molecular structure and hence their UV absorption properties, the individual compounds were quantified as equivalents to rutin (**1**–**3**), naringin (**4**) and authentic chalconaringenin (**5**).

UV. Absorption spectra were recorded online during HPLC analysis over the wavelength range 230–450 nm in steps of 2 nm.

MS. Mass spectral analyses were performed on a Q-TOF Micro (Waters) instrument with Lockspray function. Individual samples, each consisting of 0.5 to 1.0 mg dry weight, were dissolved in 1 mL of 50% acetonitrile, 49.9% water, and 0.1% acetic acid, and aliquots of 20 μL were administered to the ion source through a HotSep PLRP-S 5 μm 1000 Å 0.5 × 225 mm column (G&T Septech, Norway) using an Acquity UPLC chromatograph (Waters). The samples were isocratically eluted by a 1:1 mixture of 0.1% aqueous acetic acid and acetonitrile as mobile phase at flow rate 15 μL/min. Electrospray

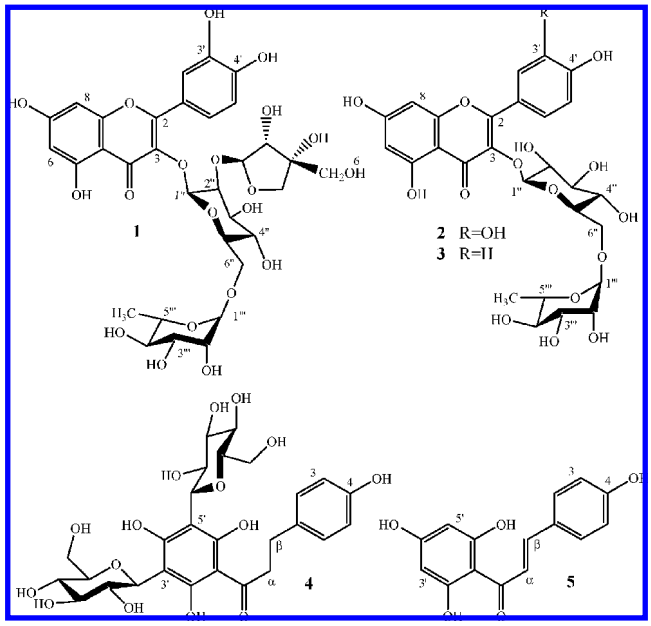

Figure 5. Structures of quercetin 3-*O*-(2″-*O*-β-apiofuranosyl-6″-*O*-α-rhamnopyranosyl)-β-glucopyranoside (**1**), quercetin 3-*O*-(6″-*O*-α-rhamnopyranosyl-β-glucopyranoside) (**2**), kaempferol 3-*O*-(6″-*O*-α-rhamnopyranosyl-β-glucopyranoside) (**3**), phloretin 3′,5′-di-*C*-β-glucopyranoside (**4**), and chalconaringenin (**5**).

ionization in the positive mode (ESI+) was used with a capillary charge 2 kV and cone voltage 35 V. Desolvation temperature was set to 300 °C and the source temperature to 100 °C.

NMR. The 1D ^{1}H and the 2D ^{1}H–^{13}C HSQC, ^{1}H–^{13}C HSQC-TOCSY, ^{1}H–^{13}C HMBC, ^{1}H–^{1}H TOCSY, and ^{1}H–^{1}H DQF-COSY NMR experiments were obtained at 600.13 and 150.90 MHz for ^{1}H and ^{13}C, respectively, on a Bruker Avance 600 instrument (Fällanden, Switzerland) equipped with a 600 MHz Ultrashield Plus magnet (Bruker Biospin AG) and a triple resonance cryogenic probe (5 mm CPTCI ^{1}H–^{13}C/^{15}N/D Z-gradient coil). Wilmad 528-PP NMR sample tubes (Sigma-Aldrich, Germany) were used for all samples. Sample temperatures were stabilized at 298 K. The deuteriomethyl ^{13}C signal and the residual ^{1}H signal of the solvent (DMSO-d_6) were used as secondary references (δ 39.6 and δ 2.49 from TMS, respectively).

RESULTS AND DISCUSSION

The HPLC chromatograms of the methanolic extract of tomato, *L. esculentum* cv. 'Dometica', detected in the UV spectral region (280 ± 10nm), revealed five flavonoids (**1**–**5**) in addition to naringenin as an artifact, formed from chalconaringenin (*20*) (**Figure 1**). The compounds were fractionated by partition against ethyl acetate followed by purification of the aqueous phase containing compounds **1**–**4** on an Amberlite XAD-7 resin. Compounds **1**–**4** were further purified and isolated on a Sephadex LH-20 column, whereas the concentrated ethyl acetate phase derived from the initial partitioning step, containing **5** as the main flavonoid, was purified on a separate Sephadex column. The pure flavonoids were analyzed by analytical HPLC (**Table 1**). The retention order of the compounds on the Sephadex column was phloretin 3′,5′-di-*C*-β-glucopyranoside < quercetin 3-*O*-(2″-apiosyl-6″-rhamnosyl-glucoside) < kaempferol 3-rutinoside < rutin.

The UV spectrum of **1** exhibited absorption maxima at 356 and 256 nm, respectively, in accordance with a flavonol derivative (*21*). The aromatic region of the 1D ^{1}H NMR spectrum showed a 3H AMX system at δ 7.57 (*dd,* 2.2 Hz, 8.4 Hz; H-6′), δ 7.48 (*d,* 2.2 Hz; H-2′) and δ 6.81 (*d,* 8.4 Hz; H-5′) and a 2H AX system at δ 6.35 (*d,* 2.1 Hz; H-8) and δ 6.16 (*d,* 2.1 Hz; H-6) in accordance to quercetin aglycone. The sugar regions of the 1D ^{1}H and 1D ^{13}C CAPT spectra of **1** showed the presence of three sugar units. All the ^{1}H sugar resonances were assigned by the 2D DQF-COSY and the TOCSY experiments, and the corresponding ^{13}C resonances were then assigned by the 2D ^{1}H–^{13}C HSQC and the 2D ^{1}H–^{13}C HSQC-TOCSY experiments. The ^{1}H coupling constants, the 17 ^{13}C (**Table 2**) resonances belonging to the sugar units together with the three anomeric ^{1}J(CH) at 169, 175, and 168 Hz, were in accordance to a β-glucopyranose unit, a β-apiofuranose unit, and an α-rhamnopyranose unit, respectively (*22*). Assignments of the ^{13}C resonances belonging to the aglycone, as well as the inter-residual connections, were determined by the 2D ^{1}H–^{13}C HMBC experiment (**Figure 2**). The downfield chemical shift of C-2″ (δ 76.95) and C-6″ (δ 66.90) indicated glycosyl substitution at these positions (*17*). The crosspeak at δ 5.46/133.0 (H-1″/C-3) confirmed the linkage between the glucopyranose unit and the aglycone at the 3-hydroxyl. Moreover, the crosspeaks at δ 5.32/76.9 (H-1‴/C-2″), δ 3.46/108.7 (H-2″/C-1‴) confirmed the interglycosidic linkage between the apiofuranosyl and the glucopyranose at the 2″-hydroxyl. The interglycosidic linkage between the α-rhamnopyranosyl and the glucopyranose was determined to be at the 6″-hydroxyl by the crosspeaks at δ 4.31/66.9 (H-1⁗/C-6″), δ 3.66/100.7 (H-6A″/C-1⁗) and δ 3.20/100.7 (H-6B″/C-1⁗) observed in the HMBC spectrum (**Figure 2**). A pseudomolecular ion ($M+H^+$) at *m/z* 743, corresponding to quercetin–hexose–deoxyhexose–pentose, and fragment ions ($F+H^+$) at *m/z* 611, *m/z* 597, *m/z* 465, and *m/z* 303 in the positive ion ESI mass spectrum of **1** (**Figure 3**, **Table 1**) corresponding to quercetin–hexose–deoxyhexose, quercetin–hexose–pentose, quercetin hexose, and quercetin, respectively, confirmed the identity of **1** to be quercetin 3-(2″-β-apiofuranosyl-6″-α-rhamnopyranosyl-β-glucopyranoside) (**Figure 5**). This compound has not previously been identified in tomatoes. A quercetin-3-*O*-trisaccharide (*3*) and a derivative of quercetin substituted with a hexose, a deoxyhexose, and a pentose has previously been indicated to occur in tomatoes (*15*) on the basis of LC-MS analyses of tomato extracts, however, without proper determination of neither the identities of the sugar units nor the inter-residual linkages.

The UV spectrum of **4** exhibited absorption maximum at 286 nm together with a shoulder at 330 nm, which is similar to spectra of dihydroflavonoids (*21*). The downfield spectral region of the 1D ^{1}H NMR spectrum showed a 4H AA'XX′ system at

Table 3. ^{1}H and ^{13}C chemical shift values (ppm) and coupling constants (Hz) of phloretin 3′,5′-di-*C*-β-glucopyranoside (**4**) and chalconaringenin (**5**).

	4 δ H	5 δ H	4 δ C	5 δ C
1			131.65	126.21
2/6	7.02 'd' 8.5	7.50 'd' 8.7	129.35	130.43
3/5	6.65 'd' 8.5	6.83 'd' 8.7	115.20	116.08
4			155.51	159.96
1'			104.69	104.33
2'/6'			161.23	164.53
3′/5′		5.83 *s*	104.15	94.98
4'			161.19	164.86
α	3.26 m	7.64 d 15.6	46.32	142.39
β	2.77 t 7.9	7.96 d 15.6	29.37	123.92
C=O			205.25	191.80
3′/5′-di-*C*-glc				
1''	4.70 d 9.8			74.74
2''	3.46 dd 9.8, 9.0		72.14	
3''	3.25 t 9.0		77.89	
4''	3.31 dd 9.0, 9.5		69.27	
5''	3.24 m		81.19	
6A''	3.60 dd 12.1, 2.0		60.04	
6B''	3.58 dd 12.1, 4.3			

Table 4. Quantities (mg 100^{-1} g Fresh Weight and Percentage of Total Content (%)) of Individual Flavonoids (**1–5**) in Different Tomato Types

type	cultivar	mass, g	dia, mm	1	2	3	4	5	sum
beaf	'Buffalo'	159	68	0.47 (12.7)	0.54 (14.6)	nd[a]	0.20 (5.4)	2.51 (67.8)	3.7
cherry	'Favorita'	10	26	0.75 (2.9)	4.45 (17.4)	0.75 (2.9)	1.45 (5.7)	18.18 (71.0)	25.6
cherry	'Sebra'	22	34	0.63 (7.3)	1.03 (12.0)	0.47 (5.5)	0.77 (9.0)	5.70 (66.3)	8.6
cluster	'Dometica'	99	57	0.45 (13.2)	0.87 (25.6)	nd	0.41 (12.1)	1.68 (49.4)	3.4
cocktail	'Aranca'	36	40	0.57 (6.2)	1.64 (17.6)	0.50 (5.4)	0.50 (5.4)	6.04 (64.9)	9.3
marmande	'Eugenia'	127	61	0.46 (10.5)	0.71 (16.1)	0.20 (4.5)	0.22 (5.0)	2.81 (63.9)	4.4
plum	'Romana'	81	48	0.56 (17.5)	0.48 (15.0)	nd	0.45 (14.1)	1.69 (52.8)	3.2
round	'Elanto'	65	50	0.35 (13.5)	0.82 (31.5)	0.18 (6.9)	0.36 (13.8)	0.92 (35.4)	2.6
small plum	'Ministar'	23	30	0.56 (4.4)	2.60 (20.5)	0.32 (2.5)	0.74 (5.8)	8.46 (66.6)	12.7
Contribution to the overall sum of **1–5** (%)				6.5	17.9	3.3	6.9	65.3	

[a] nd = not detected.

δ 7.02 ('d' 8.5 Hz; H-2,6) and δ 6.65 ('d' 8.5 Hz; H-3,5). The additional ^{1}H signals at δ 3.26 (H-α) and δ 2.77 (t 7.9 Hz; H-β) and the 11 ^{13}C signals observed in the 1D ^{13}C CAPT spectrum belonging to the aglycone, was in accordance to 3′,5′-di-*C*-substituted dihydrochalconaringenin aglycone (**Table 3**). In the aliphatic region of the 1D ^{1}H NMR spectrum of **4** seven ^{1}H signals each integrating for two protons were observed, in accordance to two identical sugar units and showing that the A-ring of **4** is symmetrically substituted. All the ^{1}H sugar resonances were assigned by the 2D DQF-COSY experiments and the corresponding ^{13}C resonances were then assigned by the 2D ^{1}H-^{13}C HSQC experiment (**Figure 4**). The ^{1}H coupling constants observed in the 1D ^{1}H NMR spectrum of **4** and the 6 ^{13}C resonances observed in the 1D ^{13}C CAPT spectrum belonging to the sugar units (**Table 3**) were typical for C-β-glucopyranose units. The large anomeric coupling constant (9,8 Hz), and the upfield shift of the anomeric carbon (δ 74.74) confirmed *C*-glycosylation (*17*). The crosspeaks at δ 4.70/161.2 (H-1″/C-2′,6′) and δ 4.70/104.2 (H-1″/C-3′,5′) confirmed the *C*-*C* linkage between the glucopyranose units and the aglycone at the 3′ and 5′ positions (**Figure 4**). Thus, **4** was identified as dihydrochalconaringenin (phloretin) 3′,5′-di-*C*-β-glucopyranoside (**Figure 5**). A pseudomolecular ion at *m*/*z* 599.1896, corresponding to $C_{27}H_{22}O_{15}$ + H and fragment ions indicating loss of two water molecules (**Table 1**), which are indicative for *C*-glycosylflavonoids (*23, 24*), confirmed this structure.

Compounds **2**, **3**, and **5** (**Figure 5**) were identified as quercetin 3-*O*-(6-*O*-α-rhamnopyranosyl-β-glucopyranoside), kaempferol 3-*O*-(6-*O*-α-rhamnopyranosyl-β-glucopyranoside), and chalconaringenin, respectively (**Figure 5**) by UV and NMR spectroscopy and electrospray MS (**Tables 1**, **2**, and **3**). These compounds have previously been identified or indicated to occur in tomatoes (*15*), however, their structures have not previously been confirmed by NMR spectral verification.

From 7 kg FW tomatoes, about 5 mg of phloretin 3′,5′-di-*C*-β-glucopyranoside, 6 mg of quercetin 3-*O*-(2″-apiosyl-6″-rhamnosyl-glucoside), 2 mg of kaempferol 3-rutinoside, 8 mg of quercetin 3-rutinoside (rutin), and 20 mg of chalconaringenin were isolated. Naringenin was detected in minute amounts in all cultivars analyzed. The occurrence of naringenin as a native compound in fresh plant material is questionable due to the ability of the main flavonoid chalconaringenin to isomerize to form naringenin (*20*). During sample preparation, increasing concentration of naringenin, apparently due to isomerization of chalconaringenin, was indeed observed.

Integration of HPLC-DAD chromatograms of crude extracts of several tomato cultivars revealed that **1** was one of the major flavonols occurring in similar or lower quantities than rutin. The interglycosidic bond between the terminal apiofuranosyl and the glucose unit proved to be susceptible to hydrolysis even at weakly acidic solution conditions. This may explain why **1** has escaped detection previously, and the fact that the consequential hydrolysis product (rutin, **2**) has been assumed to be the predominating flavonol in tomatoes. Quercetin 3-(2″-β-apiofuranosyl-6″-α-rhamnopyranosyl-β-glucopyranoside) isolated from cottonseeds has been associated with an antidepressant effect in mice (*25*).

Phloretin 3′,5′-di-*C*-β-glucopyranoside (**4**) represents the first dihydrochalcone reported from the genus *Lycopersicon* and the first demonstration of dihydrochalcones in the family Solanaceae. Moreover, **4** is the first report on a flavonoid *C*-glycoside identified from tomato. The compound has previously been reported as a characteristic compound of species of *Fortunella* (Rutaceae) (*26*) and has been recognized to accumulate in the fruits and leaves of *Fortunella* spp. Recently, a synthetic route of phloretin 3′,5′-di-*C*-β-glucopyranoside from di-*C*-β-D-glucopyranosylphloroacetophenone has been demonstrated (*27*).

We have previously found that the flavonoid content of tomato varies during a production season due to both abiotic (e.g., light) and agronomic (e.g., fertilization) factors (*1*). Therefore, samples of nine cultivars representing quite different types of tomatoes were randomly sampled at the same date, and the tomato plants were at a similar developmental stage and same ripening stage. The tomatoes were analyzed with respect to their content of flavonoids (**Table 4**). These tentative assessments revealed, not surprisingly, that chalconaringenin (**5**) is the major flavonoid in all nine cultivars, followed by rutin (**2**) (*1, 28*). However, both quercetin 3-(2″-apiosyl-6″-rhamnosyl-glucoside) (**1**) and phloretin 3′,5′-di-*C*-glucoside (**4**) were present at similar levels as rutin in some cultivars and were found to contribute 3–18% and 5–14%, respectively, to the total flavonoid content (**Table 4**). Kaempferol 3-rutinoside (**3**) comprised about 3–7% of the total flavonoid content in some cultivars (**Table 4**); however, the absence of this compound in detectable quantities in three of the cultivars investigated (**Table 4**) may indicate a more limited distribution of kaempferol 3-rutinoside in tomatoes. The fact that all compounds except **3** are present in the nine diverse tomato cultivars indicates a widespread occurrence of these compounds in tomatoes.

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