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FLAVONOIDS FROM RED ONION (*ALLIUM CEPA*)

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Key Word Index—*Allium cepa*; Alliaceae; red onion; flavonol; quercetin 3,7,4′-*O*-*β*-triglucopyranoside; dihydroflavonol; taxifolin-4′-*O*-*β*-glucopyranoside; ^{13}C NMR.

Abstract—Quercetin 3,7,4′-*O*-*β*-triglucopyranoside together with quercetin, quercetin 4′-*O*-*β*-glucopyranoside and quercetin 3,4′-*O*-*β*-diglucopyranoside were isolated from the pigmented scales of *Allium cepa* var. 'Red Baron'. The former flavonol has previously been reported to be formed when cell cultures of a *Vitis* hybrid is fed with quercetin. Minor amount of taxifolin 4′-*O*-*β*-glucopyranoside, a rare dihydroflavonol, was also detected. The structures were established on the basis of acid hydrolysis, chromatography (TLC and HPLC) and homo- and heteronuclear NMR spectroscopic techniques. © 1997 Published by Elsevier Science Ltd

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INTRODUCTION

Knowledge of plant flavonoid effects on mammalian biology is steadily increasing [1]. Quercetin is known to reduce the carcinogenic activity of several cooked food mutagens, inhibit enzymatic activities associated with several types of tumour cells [2] as well as to have antioxidative properties [3]. Hertog *et al.* [4] have examined hydrolysed samples from numerous fruits and vegetables for total aglycone content and have found the highest concentration of quercetin in onions (284–486 mg kg^{-1}).

Earlier studies [5–12] on the flavonoid content of onions (*Allium cepa* L.) have shown that the main flavonoids are quercetin, quercetin-4′-glucoside, quercetin-3,4′-diglucoside, quercetin-7,4′-diglucoside and isorhamnetin glycoside. Koeppen and Van der Spuy [8] and Brandwein [9] have identified some of the minor flavonoids to be quercetin-3-glucoside and quercetin-5-glucoside. Recently, we have identified anthocyanins with unusual aliphatic acyl substitution in red onion cultivars [13] as well as in other *Allium* species [14, 15]. In this paper the flavonoids of red onion, var. 'Red Baron', including a new glycoside are reported.

RESULTS AND DISCUSSION

The HPLC chromatogram of the crude extract of the pigmented scales of red onion showed two major (**2**, **3**) and several minor flavonoids. The UV spectra of **1**–**4** indicated in all instances a flavonol nucleus (band I at 344–372 nm, band II at 253–267 nm), while the UV spectrum of **5** was in accordance with a dihydroflavonol having band I as a shoulder at 338 nm on band II (287 nm). The chromatographic properties of pigment **1** indicated a non-glycosylated flavonoid (Table 1). The ^{1}H and ^{13}C NMR data (Table 2 and 3) confirmed **1** to be quercetin. Total acid hydrolysis of **2**–**4** gave in all instances quercetin and glucose. In order to determine unambiguously the structures of **2**–**5**, especially the linkage points of the glucose moieties, it was necessary to apply homo- and heteronuclear NMR techniques in tandem.

The low-field part of the ^{1}H NMR spectrum of **3** showed 5 resonances. On the basis of the chemical shifts and coupling-patterns the signals at δ 7.64, 7.57 and 7.20 were assigned to H2′, H6′ and H5′ of the B-ring, respectively. The remaining signals of the aglycone at δ 6.35 and δ 6.14 were attributed to the H8 and H6 protons, respectively [21]. The spin echo Fourier transform (SEFT) spectrum of **3** showed in addition to solvent signals 12 positive resonances originating from 10 quaternary carbons and 2 methylene carbons, and 15 negative resonances attributed to 15 methine carbons. Once the ^{1}H spectrum had been completely assigned, the protonated carbons were assigned using the one-bond heteronuclear shift correlation (HSC) experiment (see Table 3). The assignment of the 10 quaternary carbon resonances of the aglycone was accomplished by comparison with quercetin (**3** and ref. 21).

The anomeric carbon signals appear considerable downfield of the other sugar resonances, and thus the two crosspeaks at δ 5.48/101.23 and δ 4.85/101.86 in the HSC spectrum of **3** together with integration data indicated two sugar rings. The spectral region between

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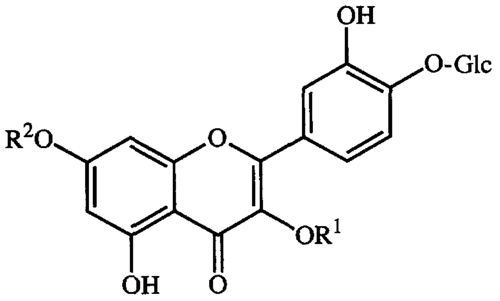
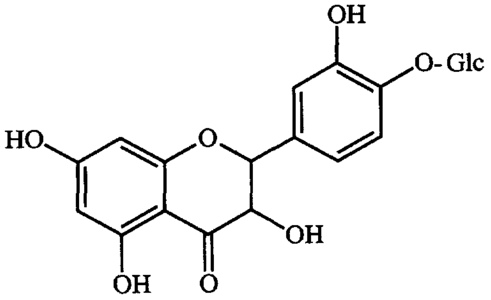
Table 1. Chromatographic and spectral data for the flavonols (1–4) from red onion

Compound	TLC (R_f)			On-line HPLC		
	AW	FHW	BAW	UV (Band II, nm)	UV (Band I, nm)	t_r (min)
1	0.03	0.03	0.69	256	372	20.54
2	0.11	0.17	0.42	253	366	17.25
3	0.54	0.77	0.24	265	344	13.00
4	0.71	0.87	0.14	267	345	7.67



2: $R^1 = H$, $R^2 = H$
3: $R^1 = Glc$, $R^2 = H$
4: $R^1 = Glc$, $R^2 = Glc$



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δ 80 and δ 60 in the SEFT spectrum showed 10 resonances which, together with the two anomeric carbon resonances, were in agreement with two hexoses. Starting from the anomeric protons, the sequential 'walk' through the crosspeaks in the double quantum filtered ^1H-^1H shift correlated (DQF-COSY) spectrum assigned all the protons in the two sugar rings (Table 2). In cases where several protons had similar chemical shifts, the relationships were supported by the total correlation spectroscopy (TOCSY) experiment which gave cross-peaks between the anomeric protons and all the protons in the same sugar unit. Thereafter the chemical shifts of the corresponding sugar carbons were assigned from the HSC experiment. The chemical shifts (Table 2) and the large ^1H-^1H coupling constants of the anomeric protons (7.7 Hz) and the sugar ring protons (around 9 Hz) agreed with two β-linked D-glucopyranoses.

The binding sites of the sugars of **3** on the aglycone were derived from the ROESY (Rotating Frame Overhauser Enhancement Spectroscopy) experiment. Strong cross-peaks between the anomeric proton at δ 4.85 and H5′ (δ 7.20), H6′ (δ 7.57) and H2′ (δ 7.64) showed that one glucose ring was connected to either the 3′- or 4′-position of the B-ring. The attachment of this sugar unit to the 4′-position was confirmed by the pronounced downfield shift of H5′ (δ 7.20). Based on the downfield shift of C2 (δ 155.96) and that no strong cross-peaks were observed in the ROESY spectrum between the anomeric proton at δ 5.48 and the aglycone protons, the other glucose ring was determined to be in the 3-position on the aglycone. Thus, **3** was identified as quercetin 3,4′-*O*-β-diglucopyranoside.

The ^1H and ^{13}C NMR resonances of **2**, **4** and **5** (Tables 2 and 3) were assigned in a similar way as for **3**. The attachment of the glucopyranose unit of **2** to the quercetin 4′-hydroxyl was confirmed by the pronounced downfield shift of H5′ (δ 7.35) in comparison with the same signal of quercetin (δ 6.97) (Table 2). In the ROESY spectrum of **4** strong cross-peaks between the anomeric proton at δ 5.09 and H6 (δ 6.43) and H8 (δ 6.80) showed that one glucose ring was connected to the 7-position. Strong cross-peaks between the anomeric proton at δ 4.87 and H5′ (δ 7.21), H6′ (δ 7.62) and H2′ (δ 7.67), no cross-peaks from the anomeric proton at δ 5.51, the downfield shifts of H5′ (δ 7.21), H6 (δ 6.43) and H8 (δ 6.80), and the upfield shift of C7 (δ 162.99) confirmed the identity of **4** as the 3,7,4′-*O*-β-triglucopyranoside of quercetin. The downfield shift of H5′ and the large coupling constant (11.9 Hz) between the signals at δ 5.08 (H2) and δ 4.59 (H3) [22] contributed to the characterisation of, **5**, the rare dihydroflavonol taxifolin 4′-*O*-β-glucopyranoside, with H2 and H3 in the *trans* relationship. Taxifolin 4′-*O*-β-glucoside has previously been found in *Urginea maritima* [23]. Notice that both *Urginea* and *Allium* belong to Liliaceae. Quercetin 3,7,4′-*O*-β-triglucopyranoside (**4**) has previously been reported to be formed from quercetin when cell cultures of a *Vitis* hybrid is fed with quercetin, phytohormones, etc. [24]. There exist no reports

Table 2. ^{1}H NMR spectral data for the flavonols (**1**–**4**) and the dihydroflavonol (**5**) from red onion in CD_3OD (**1**, **2**, **5**) and DMSO-d_6 (**3**, **4**)

	1 δ (ppm)	**2** δ (ppm)	**3** δ (ppm)	**4** δ (ppm)	**5** δ (ppm)
Aglycone					
2					5.08
3					4.59
6	6.27	6.25	6.14	6.43	6.02*
8	6.47	6.44	6.35	6.80	5.99*
2′	7.82	7.81	7.64	7.67	7.14
5′	6.97	7.35	7.20	7.21	7.33
6′	7.72	7.75	7.57	7.62	7.06
4′-*O*-β-glucopyranoside					
1″		5.00	4.85	5.09	4.91
2″		3.61	3.34	3.31	3.60
3″		3.60	3.32	3.26	3.52
4″		3.53	3.21	3.16	3.50
5″		3.57	3.39	3.41	3.56
6A″		4.02	3.72	3.69	3.99
6B″		3.85	3.50	3.46	3.82
3-*O*-β-glucopyranoside					
1‴			5.48	5.51	
2‴			3.21	3.19	
3‴			3.23	3.22	
4‴			3.10	3.03	
5‴			3.11	3.07	
6A‴			3.58	3.54	
6B‴			3.33	3.30	
7-*O*-β-glucopyranoside					
1⁗				4.87	
2⁗				3.23	
3⁗				3.26	
4⁗				3.12	
5⁗				3.37	
6A⁗				3.69	
6B⁗				3.46	

* Assignment with the same superscript may be reversed.

on this flavonol from any plant growing outside the laboratory.

EXPERIMENTAL

Extraction and separation. Red onion (*Allium cepa* var. 'Red Baron') were obtained from the local food market. A voucher specimen has been deposited in the herbarium of ARBOHA, University of Bergen. The inner pigmented scale leaves were collected, cut with a pair of scissors and extracted (×2) with MeOH–HOAc (19:1). The filtered extracts were combined, concd under red. pres. and applied on an Amberlite XAD-7 column [16]. The pigments were thereafter sepd on a Sephadex LH-20 column (100×1.6 cm, Pharmacia) using a gradient of MeOH–HOAc–H_2O starting with the mixt. 47.5:5:47.5 ending at 14:1:5.

Chromatography. Analyt. TLC was carried out on microcrystalline cellulose (F 1440, Schleicher and Schüll) with the solvents BAW (1-BuOH–HOAc–H_2O; 4:1:5 upper phase), FHW (HCO_2H–HCl–H_2O; 1:1:2) and AW (HOAc–H_2O; 3:17). HPLC was carried out on an HP-1050 module system (Hewlett Packard) using an ODS-Hypersil column (20×0.5 cm, 5 mm). Two solvents were used for elution: HCO_2H–H_2O (1:9) (A) and HCO_2H–H_2O–MeOH (1:4:5) (B). the elution profile was 0–4 min, 10% B in A (isocratic); 4–21 min, 10–100% B in A (linear gradient), 21–25 min, 100% B in A (isocratic). The flow rate was 1.2 ml min^{-1} and aliquots of 10 μl were injected.

Spectroscopy. UV spectra were recorded on-line during HPLC-analysis using a photodiode array detector (HP 1050), and spectral measurements were made over the wavelength range 210–600 nm in steps of 2 nm. Most of the NMR experiments were obtained at 400.13 MHz and 100.62 MHz for ^{1}H and ^{13}C,

Table 3. ^1H NMR spectral data for the flavonols (**1**–**4**) and the dihydroflavonol (**5**) from red onion in CD_3OD (**1**, **2**, **5**) and DMSO-d_6 (**3**, **4**)

	1 δ (ppm)	**2** δ (ppm)	**3** δ (ppm)	**4** δ (ppm)	**5** δ (ppm)
Aglycone					
2	148.00	148.04	155.96	156.27	85.55
3	137.21	137.89	134.26	134.20	73.70
4	177.33	177.36	174.82	177.62	198.22
5	162.50	162.47	161.64	160.90	168.69
6	99.25	99.31	99.16	99.48	97.39*
7	165.34	165.66	164.65	162.99	165.72
8	94.40	94.47	94.08	94.49	96.32*
9	158.22	158.19	156.83	156.12	164.35
10	104.52	104.54	104.53	105.61	102.19
1′	124.15	127.60	124.92	124.42	133.96
2′	115.99	116.49	115.86	116.73	116.57
3′	148.75	147.83	147.67	147.72	147.22
4′	146.21	146.79	146.52	146.22	148.37
5′	116.22	117.62	116.97	115.52	118.51
6′	121.67	121.26	121.42	120.40	120.70
4′-*O*-β-glucopyranoside					
1″		103.42	101.86	101.56	104.24
2″		74.82	73.66	73.55	74.87
3″		77.54	76.22	76.49†	77.63
4″		71.32	70.13	69.88‡	71.33
5″		78.36	77.56	77.19§	78.37
6″		62.44	61.10	61.04¶	62.01
3-*O*-β-glucopyranoside					
1‴			101.23	100.69	
2‴			74.55	74.19	
3‴			76.66	76.49	
4‴			70.36	70.19	
5‴			77.92	77.76	
6‴			61.36	61.46	
7-*O*-β-glucopyranoside					
1⁗				99.73	
2⁗				73.15	
3⁗				76.50†	
4⁗				69.83‡	
5⁗				77.83§	
6⁗				60.79¶	

*,†,‡,§,¶ Assignments with the same superscript may be reversed.

respectively. The ^{13}C detected NMR experiments (one-bond Heteronuclear Shift Correlation, HSC) [17] and Spin Echo Fourier Transform, SEFT, were obtained using a 5 mm ^1H-^{13}C dual probe. The ROESY [18] (Rotating Frame Overhauser Enhancement Spectroscopy) experiments on **3** and **4**, and the DQF-COSY [19] (Double Quantum Filtered Correlation Spectroscopy) experiments on **3** were obtained using a 5 mm inverse probe on a Bruker DMX 400 spectrometer. The COSY experiment on **4** and the TOCSY [20] (Total Correlation Spectroscopy) experiments on **3** and **4** were obtained at 600.13 MHz (^1H) using a 5 mm inverse gradient probe. All the NMR experiments were performed at 25° using the ^{13}C and residual ^1H signal of the solvents as secondary references (δ 39.50/2.49 for $(CD_3)_2SO$ and δ 49.00/3.40 for CD_3OD).

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