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Anthocyanin from strawberry (*Fragaria ananassa*) with the novel aglycone, 5-carboxypyranopelargonidin

Øyvind M. Andersen*, Torgils Fossen, Kjell Torskangerpoll, Arve Fossen, Unni Hauge

Department of Chemistry, University of Bergen, Allégt. 41, N-5007 Bergen, Norway

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Abstract

An anthocyanin, **1**, with the novel 4-substituted aglycone, 5-carboxypyranopelargonidin, was isolated in small amounts from the acidified, methanolic extract of strawberries, *Fragaria ananassa* Duch., by preparative HPLC after purification by partition against ethyl acetate, Amberlite XAD-7 and Sephadex LH-20 column chromatography. It was identified mainly by 2D NMR spectroscopy and electrospray LC-MS as the 3-*O*-β-glucopyranoside of 5-carboxy-2-(4-hydroxyphenyl)-3,8-dihydroxy-pyrano[4,3,2-*de*]-1-benzopyrylium, an anthocyanidin which is homologous to 5-carboxypyranomalvidin (vitisidin A) reported in red wines and 5-carboxypyranocyanidin recently isolated from red onions. By comparison of UV–Vis absorption spectra, **1** showed in contrast to **2**, pelargonidin 3-*O*-β-glucopyranoside, a local absorption peak around 360 nm, a hypsochromic shift (8 nm) of the visible absorption maximum, and lack of a distinct UV absorption peak around 280 nm. The similarities between the absorption spectra of **1** in various acidic and neutral buffer solutions implied restricted formation of the instable colourless equilibrium forms, which are typical for most anthocyanins in weakly acidic solutions. The molar absorptivity (ϵ) of **1** varied little with pH contrary to similar values of for instance the major anthocyanin in strawberry, **2**. However, **2** revealed higher ϵ-values than **1** at all pH values except 5.1. At pH 5.1, the ϵ-value of **1** (6250) was nearly four times the corresponding value of **2** (1720), which showed the potential of 5-carboxypyranopelargonidin derivatives as colorants in solutions with pH around 5. The colours of **1** and **2** in buffered solutions with pH 1.1 and pH 6.9 have been described by the CIELAB coordinates h_{ab} (hue angle), C^* (chroma), and L^* (lightness).

Keywords: Strawberries; *Fragaria ananassa*; Anthocyanin; 5-Carboxypyranopelargonidin 3-*O*-β-glucopyranoside; UV–Vis spectrum; Colours; CIELAB; 2D NMR

1. Introduction

In recent years flavonoids in fruits have achieved much attention due to their potential antioxidant properties and their putative role in the prevention of chronic diseases like cancer and heart disease (Middleton et al., 2000). In animal experiments, strawberry has been found to inhibit oesophageal cancer and to reverse the course of neuronal and behavioural aging in rats (Joseph et al., 2000; Torronen and Maatta, 2002). The antioxidant activity of strawberries in vitro oxidation assays has been correlated with anthocyanin content in the fruits (Heinonen et al., 1998; Wang and Lin, 2000).

The main anthocyanins of strawberries have previously been identified as the 3-glucoside (Robinson and Robinson, 1932), the 3-arabinoside (Fiorini, 1995), the 3-(6″-malonylglucoside) (Tamura et al., 1995) of pelargonidin, in addition to the 3-(6″-rhamnosylglucoside) of pelargonidin and cyanidin (Lopes da Silva et al., 2002) and the 3-glucoside of cyanidin (Lukton et al., 1955).

In this paper we report isolation and characterization of an anthocyanin, **1**, with the novel 4-substituted anthocyanidin, 5-carboxypyranopelargonidin, isolated as a minor compound from strawberries, *Fragaria ananassa* Duch. The colours of **1** and the major anthocyanin in strawberries, pelargonidin 3-*O*-β-glucopyranoside, **2**, in buffered solutions with pH 1.1 and pH 6.9 have been compared by CIELAB coordinates. Their UV–Vis

* Corresponding author. Tel.: +47-555-834-60; fax: +47-555-894-90.
E-mail address: oyvind.andersen@kj.uib.no (Ø. M. Andersen).

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absorption spectra in eight buffer solutions with pH ranging from 1.1 to 8.9 have been compared. In recent years, 4-substituted malvidin derivatives have been discovered in small amounts in red wine and grape pomace (Bakker et al., 1997; Bakker and Timberlake, 1997; Fulcrand et al., 1998; Asenstorfer et al., 2001; Vivar-Quintana et al., 2002), and 4-substituted cyanidin derivatives in petals of *Rosa hybrida* cv. 'M'me Violet' (Fukui et al., 2002) and in pigmented scales of red onion, *Allium cepa* (Fossen and Andersen, 2003).

2. Results and discussion

The aqueous concentrate of the acidified methanolic extract of strawberries, *Fragaria ananassa*, was purified by partition against ethyl acetate followed by Amberlite XAD-7 column chromatography. The anthocyanins in the purified extract were fractionated by Sephadex LH-20 column chromatography. Individual anthocyanins, **1** and **2**, were separated by preparative HPLC.

2.1. Identification

The main pigment in strawberries, pelargonidin 3-*O*-β-glucopyranoside, **2**, (Fig. 1) has previously been characterized by NMR (Pedersen et al., 1993) (Table 1). The downfield part of the 1D ^{1}H NMR spectrum of **1** showed a 4H AA'XX' system at δ 8.52 ('*d*', 8.9 Hz; H-2′,6′) and δ 7.10 ('*d*', 8.9 Hz; H-3′,5′), a 1H singlet at δ 8.11 (H-4) and an AX system at δ 7.37 (*d*, 1.9 Hz; H-9) and δ 7.27 (*d*, 1.9 Hz; H-7), revealing a 4-substituted anthocyanin having a symmetrically substituted B-ring. The ^{13}C resonances belonging to the aglycone were assigned by the HMBC and HSQC spectra, respectively, and the ^{13}C chemical shift values were then established by the 1D ^{13}C spectrum (Table 1). The crosspeaks at *δ* 8.11/161.1 (H-4/COOH), *δ* 8.11/135.9 (H-4/C-3), and δ 8.11/111.1 (H-4/C-9b) in the HMBC spectrum of **1** were used to assign COOH, C-3 and C-9b. Furthermore, C-2 was identified by its long-range range correlation with H-2′,6′ (δ 8.52/166.4). The anthocyanidin A-ring carbon signals were identified by the crosspeaks at *δ* 7.37/169.6 (H-9/C-8), *δ* 7.37/154.5 (H-9/C-9a), *δ* 7.37/111.1 (H-9/C-9b), δ 7.37/101.8 (H-9/C-7), *δ* 7.27/169.6 (H-7/C-8), *δ* 7.27/154.5 (H-7/C-6a), *δ* 7.27/111.1 (H-7/C-9b), *δ* 7.27/101.6 (H-7/C-9) corresponding to the long-range correlations of H-9 and H-7, respectively. However, the assignments of H-7 and H-9 may be reversed. The carbons belonging to the anthocyanidin B-ring (Table 1) were assigned by the crosspeaks at *δ* 8.52/165.8 (H-2′,6′/C-4′), *δ* 8.52/117.4 (H-2′,6′/C-3′,5′), *δ* 7.10/165.8 (H-3′,5′/C-4′), *δ* 7.10/135.0 (H-3′,5′/C-2′,6′) and *δ* 7.10/120.9 (H-3′,5′/C-1′). The 16 ^{13}C resonances belonging to the aglycone were thus in agreement with 5-carboxy-2-(4-hydroxyphenyl)-3,8-dihydroxy-pyrano[4,3,2-*de*]-1-benzopyrylium. According to previously suggested nomenclature for carboxypyranoanthocyanins (Fossen and Andersen, 2003), we suggest to use 5-carboxypyranopelargonidin as trivial name for this aglycone.

The sugar region showed the presence of only one sugar unit. All the ^{1}H sugar resonances were assigned by the DQF-COSY experiment, and the corresponding ^{13}C resonances were then assigned by the ^{1}H–^{13}C HSQC experiment. The ^{1}H–^{1}H coupling constants and the six ^{13}C resonances in the sugar region of the ^{13}C spectrum of **1** (Table 1) were in accordance with β-glucopyranose (Pedersen et al., 1993). The crosspeak at *δ* 4.75/135.95 (H-1″/C-3) in the HMBC spectrum confirmed the linkage between the aglycone and the sugar unit to be at the 3-hydroxyl. A molecular ion at *m/z* 501 in the ES-MS spectrum of **1**, and the fragment ion at *m/z* 339 corresponding to the aglycone, confirmed the identity of **1** to be 5-carboxypyranopelargonidin 3-*O*-β-glucopyranoside (Fig. 1).

Due to overlap with the major anthocyanin, **2**, it was difficult to detect the minor pigment **1** in the HPLC chromatogram (using Gradient 1) of the crude extract. A new HPLC profile (Gradient 2), which improved separation of **1** and **2**, was developed. The peak area ratio between **1** and **2** (0.3%) remained constant when the HPLC chromatograms of the fresh and one week old extract were compared, indicating no conversion from **2** to **1** in the extract.

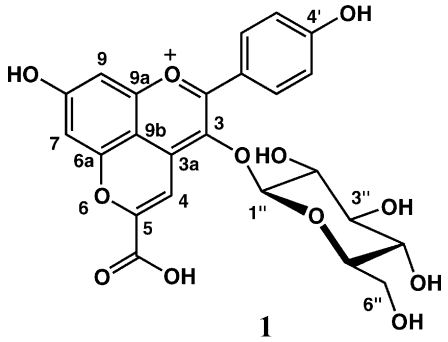

Fig. 1. The structure of 5-carboxypyranopelargonidin 3-*O*-β-glucopyranoside (**1**) and pelargonidin 3-*O*-β-glucopyranoside (**2**) isolated from strawberries.

2.2. Colours

It is relative easy to recognize that **1** was different from a normal pelargonidin derivative (like **2**) by its characteristic UV–Vis spectrum (Fig. 2). When this spectrum was recorded on-line during HPLC, it showed a visible maximum at 496 nm. Compared to the analogous spectrum of **2**, the "normal UV absorption band" of **1** also showed a hypsochromic shift, leading to lack of a distinct absorption peak near 280 nm (Table 2). This spectrum also revealed a characteristic local UV band at 360 nm ($A_{360}/A_{496}=47\%$). Similar characteristics have previously been reported for 4-substituted malvidin derivatives (Bakker and Timberlake, 1997) and 4-substituted cyanidin derivatives (Fukui et al., 2002; Fossen and Andersen, 2003).

The colours of **1** and **2** in buffered solutions (0.10 mM) with pH 1.1 and 6.9 expressed by the CIELAB parameters, h_{ab} (hue angle), L^* (lightness), and C^* (chroma) are presented in part 3.4. The hue angle of **1** at pH 1.1 (61.1°) decreased to 34.8° at pH 6.9, going from orange-yellow hue to a more reddish colour, consistent with the bathochromic shift (13 nm) of the visible band (Table 3). Pigment **2** having the same molar concen-

Table 1
^{1}H and ^{13}C NMR data for 5-carboxypyranopelargonidin 3-*O*-β-glucopyranoside (**1**) and pelargonidin 3-*O*-β-glucopyranoside (**2**) in CF_3COOD–CD_3OD (5:95, v/v (**1**) and 10:90, v/v (**2**)) at 25 °C

	1 ^{1}H δ (ppm) *J* (Hz)	**2**[a] ^{1}H δ (ppm) *J* (Hz)	**1** ^{13}C δ (ppm)	**2**[a] ^{13}C δ (ppm)
Aglycone				
2			166.46	163.85
3			135.95	145.31
3a (4)[b]		9.10 *s*	150.44	137.08
4	8.11 *s*		107.48	
5			154.55	
COOH			161.17	
6a (5)[b]			154.48	157.48
7 (6)[b]	7.27[c] *d*, 1.9 Hz	6.74 *d*, 1.2 Hz	101.77[c]	103.49
8 (7)[b]			169.33	170.59
9 (8)[b]	7.37[c] *d*, 1.9 Hz	6.96 *d*, 1.2 Hz	101.53[c]	95.28
9a (9)[b]			154.48	157.44
9b (10)[b]			111.00	113.39
1′			120.95	120.61
2′,6′	8.52 *′d′*, 8.9 Hz	8.61 *′d′*, 9.1 Hz	135.04	135.67
3′,5′	7.10 *′d′*, 8.9 Hz	7.10 *′d′*, 9.1 Hz	117.40	117.91
4′			165.75	166.57
3-*O*-β-glucopyranoside				
1″	4.75 *d*, 7.8 Hz	5.37 *d*, 8.0 Hz	105.69	103.70
2″	3.71 *dd*, 7.8 Hz, 9.3 Hz	3.76 *dd*, 8.0 Hz, 9.1 Hz	75.38	74.77
3″	3.43 *t*, 9.1 Hz	3.69 *dd*, 9.1 Hz, 8.9 Hz	77.56	78.13
4″	3.31 *dd*, 9.1 Hz, 9.3 Hz	3.58 *dd*, 8.9 Hz, 9.9 Hz	71.52	71.06
5″	3.22 *ddd*, 9.3 Hz, 7.0 Hz, 1.9 Hz	3.22 *ddd*, 9.9 Hz, 6.0 Hz, 2.3 Hz	79.01	78.73
6A″	3.82 *dd*, 11.7 Hz, 1.9 Hz	4.05 *dd*, 12.3 Hz, 2.3 Hz	62.86	62.33
6B″	3.46 *dd*, 11.7 Hz, 7.0 Hz	3.46 *dd*, 12.3 Hz, 6.0 Hz		

[a] Pedersen et al. (1993).
[b] The number in brackets refer to skeleton positions of the common anthocyanidins (for instance pelargonidin in Fig. 1).
[c] Assignments may be reversed.

Table 2
Chromatographic (HPLC, gradient 1) and spectral (UV–Vis and MS) data recorded for 5-carboxypyranopelargonidin 3-*O*-β-glucopyranoside (**1**), pelargonidin 3-*O*-β-glucopyranoside (**2**), and 5-carboxypyranocyanidin 3-*O*-*β*-glucopyranoside (**3**)[a]

Compound	On-line HPLC							ES-MS	
	Vis_{max} (nm)	Local UV_{max} (nm)	UV_{max} (nm)	$A_{LocUV-max}/A_{vis-max}$ (%)	$A_{280}/A_{vis-max}$ (%)	$A_{440}/A_{vis-max}$ (%)	t_R (min)	$[M]^+$ *m*/*z*	$[A]^+$ *m*/*z*
1	496	360	[b]	47	40	51	19.98	501	339
2	504	331[c]	278	13	67	43	19.45	433	271
3	505	351	278	35	54	38	18.60	517	355

[a] $[M]^+$ = molecular ion; $[A]^+$ = aglycone fragment ion
[b] Not a discrete peak around 280 nm, which is characteristic for most anthocyanin without the carboxypyrano unit.
[c] Weak.

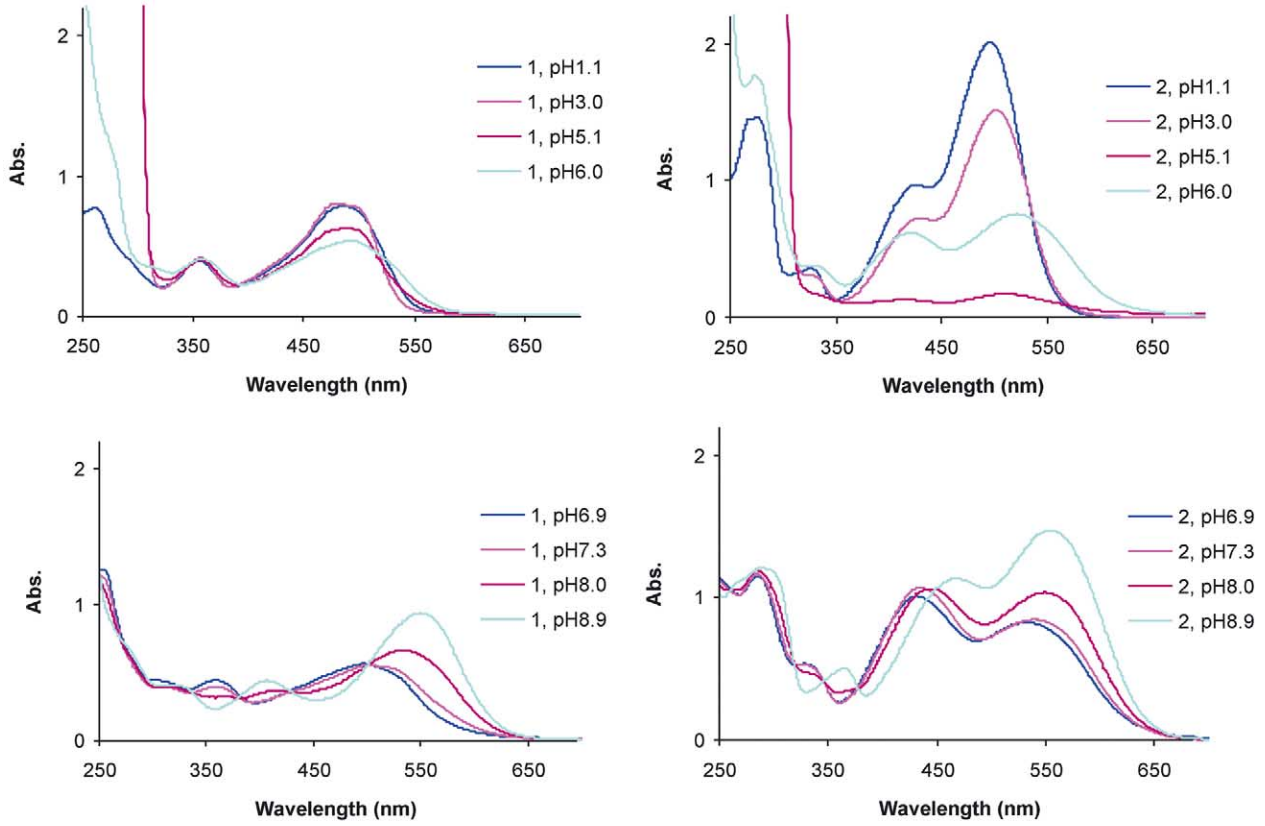

Fig. 2. UV–Vis absorption spectra of 5-carboxypyranopelargonidin 3-*O*-β-glucopyranoside (**1**) and pelargonidin 3-*O*-β-glucopyranoside (**2**) in eight 0.10 mM buffered solutions with pH ranging from 1.1 to 8.9.

tration (0.10 mM), showed rather similar hue angles at these pH values (58.7° and 37.3°, respectively). Compared to **1**, this pigment had its visible λ_{max} at higher wavelengths at all pH values between 1.1 and 8.9 (Table 3). However, **1** showed much less variation than **2** when the absorption spectra of these compounds in the different buffer solutions (pH 1.1 to 8.9) were compared (Fig. 2). The similarities between the various absorption spectra of **1** throughout the pH region including weakly acidic solutions, proves restricted formation of the colourless equilibrium forms reported for "normal" anthocyanins in weakly acidic solutions. These colourless carbinol bases are instable (Brouillard and Dangles, 1994), and **1** may thus have higher stability than **2** in weakly acidic solutions.

The molar absorptivity (ϵ) of **1** varied little with pH contrary to similar values for **2** in the pH range 1.1 to 8.9 (Fig. 3). However, **2** revealed higher colour intensity than **1** at all pH values except 5.1. The intensity difference was especially noticeable at low pH values. Bakker and Timberlake (1997) have contrary to this reported that the homologous carboxypyranomalvidins (vitisin derivatives) express more colour up to pH 7 than malvidin 3-glucoside. At pH 5.1, the ϵ-value of **1** (6250) was nearly four times the corresponding value of **2** (1720), which indicated that 5-carboxypyranopelargonidin derivatives may be beneficial as colorants of solutions with pH around 5. Similarly, Vivar-Quintana et al. (2002) have recently reported that vitisin-like pigments made the major contribution to the colour of wine at pH 4, even though these anthocyanins were hardly detected in the chromatograms, where the "normal" anthocyanins greatly predominated. The explanation was that the chromatograms were obtained under very acidic conditions at which the "normal" anthocyanins existed in their highly coloured flavylium forms; how-

Table 3
Visible absorption maxima (λ_{max}) of 5-carboxypyranopelargonidin 3-*O*-β-glucopyranoside (**1**) and pelargonidin 3-*O*-β-glucopyranoside (**2**) in 0.10 mM buffer solutions with pH from 1.1 to 8.9

pH	**1** λ_{max} (nm)	**2** λ_{max} (nm)
1.1	484.0	496.5
3.0	480.0	502.5
5.1	490.5	510.0
6.0	493.5	521.5
6.9	496.5	532.0
7.3	503.5	540.0
8.0	533.0	549.5
8.9	549.5	553.0

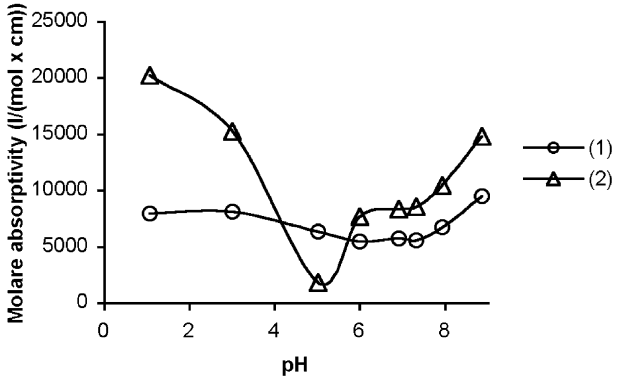

Fig. 3. Absolute molar absorptivity as a function of pH for 5-carboxypyranopelargonidin 3-*O*-β-glucopyranoside (**1**) (o) and pelargonidin 3-*O*-β-glucopyranoside (**2**) (Δ) in 0.10 mM buffered solutions with pH ranging from 1.1 to 8.9.

ever, at the usual pH value of the wine (3.2–4.0) the ''normal'' anthocyanins existed mainly as colourless hemiacetal structures.

3. Experimental

3.1. Isolation of pigments

Strawberries (5.0 kg) bought on the local food market were cut into pieces and extracted (2 times) with 0.5% TFA in MeOH at 4 °C. The filtered extract was concentrated under reduced pressure, purified by partition against EtOAc (equal volume), and then subjected to Amberlite XAD-7 column chromatography (Andersen, 1988). The anthocyanins were further purified on a Sephadex LH-20 column (100×5 cm) using MeOH–H_2O–TFA (29.7:70.0:0.3; v/v) to MeOH–H_2O–TFA (69.3:30.0:0.7; v/v) (gradient elution). The flow rate was 2.5 ml min^{-1}. Pure anthocyanins were then isolated by preparative HPLC.

Preparative HPLC (Gilson 305/306 pump equipped with an HP-1040A detector) was performed with an ODS-Hypersil column (25×2.2 cm, 5 μm) using the solvents HCOOH–H_2O (1:19; v/v) (A) and HCOOH–H_2O–MeOH (1:9:10; v/v) (B). The elution profile consisted of a linear gradient from 10% B to 100% B for 45 min, isocratic elution (100% B) for the next 13 min, followed by linear gradient from 100% B to 10% B for 1 min. The flow rate was 14 ml min^{-1}, and aliquots of 300 μl were injected. Altogether 5.9 mg of pigment **1** was isolated.

Analytical HPLC was performed with an ODS-Hypersil column (25×0.3 cm, 5 μm) using the solvents HCOOH–H_2O (1:18; v/v) (A) and HCOOH–H_2O–MeOH (1:9:10; v/v) (B). Gradient 1 consisted of a linear gradient from 10% B to 100% B for 23 min, 100% B for the next 5 min, followed by linear gradient from 100% B to 10% B for 1 min. The flow rate was 0.75 ml min^{-1}, and aliquots of 10 μl were injected. To improve separation between **1** and **2**, an extended gradient (Gradient 2) consisting of a linear gradient from 10% B to 30% B for 3 min, isocratic elution for the next 42 min, linear gradient from 30% B to 100% B for 7 min, isocratic elution for the next 5 min, followed by linear gradient from 100% B to 10% B for 1 min was used. The flow rate was 0.75 ml min^{-1}, and aliquots of 10 μl were injected.

3.2. Buffer solutions

Pure anthocyanins, **1** and **2**, were dissolved in eight buffer solutions with different pH values. The solvents used for the buffer solutions were 0.2 M KCl (A), 0.2 M HCl (B), 0.1 M $KHC_8O_4H_4$ (C), 0.1 M HCl (D), 0.1 M NaOH (E), 0.1 M KH_2PO_4 (F), and 0.025 M borax (G). The following solvent proportions were used for pH 1.1: A–B, 271.7:728.3; pH 3.0: C–D, 691.6:308.4; pH 5.1: C–E, 688.7:311.3; pH 6.0: E–F, 100.7:899.3; pH 6.9: E–F, 367.9:632.1; pH 7.3: E–F, 438.8:561.2; pH 8.0: D–G, 290.8:709.2; pH 8.9: D–G, 84.2:915.8. The accurate pH values were measured with a Hanna HI 9224 pH-meter equipped with a Hanna HI 1330B pH electrode.

3.3. Spectroscopy

UV–Vis absorption spectra were recorded on-line during HPLC analysis over the wavelength range 240–600 nm in steps of 2 nm. UV–Vis absorption spectra of 0.10 mM solutions of **1** and **2** at the different pH-values (Fig. 2) were recorded between 240 and 700 nm by a Varian Cary3 UV–Visible Spectrophotometer. As references the respective buffer solutions were used. In fresh buffer solutions at pH 1.1 the following spectral characteristics were measured for **1** and **2**, respectively: Vis_{max} (nm): 484 and 497, localUV_{max} (nm): 356 and 327sh, UV_{max} (nm): 261 and 276, $A_{440}/A_{vis\text{-}max}$ (%): 58 and 47.

The NMR experiments were obtained at 600.13 MHz and 150.90 MHz for ^1H and ^{13}C respectively, on a Bruker DRX-600 instrument equipped with a multinuclear inverse probe for the 1D ^1H and the 2D Heteronuclear Single Quantum Coherence (HSQC), Heteronuclear Multiple Bond Correlations (HMBC) and Double Quantum Filtered Correlation Spectroscopy (DQF-COSY) experiments. The ^{13}C 1D experiment was performed on a $^1H/^{13}C$ BBO probe. Sample temperatures were stabilised at 25 °C. The deuteriomethyl ^{13}C signal and the residual ^1H signal of the solvent (CF_3CO_2D–CD_3OD; 5:95, v/v) were used as secondary references (δ 49.0 and δ 3.40 from TMS, respectively). See Fossen et al. (2001) for more experimental details.

Mass spectral data were achieved by a LCMS system (Waters 2690 HPLC-system connected to Micromass LCZ mass spectrometer) with electrospray ionisation in positive mode (ESP+). The following ion optics were

used: Capillary 3 kV, cone 30 V and 60 V, and extractor 7 V. The source block temperature was 120 °C and the desolvation temperature was 150 °C. The electrospray probe-flow was adjusted to 100 μl/min. Continuous mass spectra were recorded over the range m/z 150–800 with scan time 1 s and interscan delay 0.1 s.

3.4. Colour measurements by CIELAB parameters

The colours have also been measured using an Ultra Scan XE Hunter colorimeter (Hunter Associates Laboratories Inc., Reston Va., USA) giving the following CIEL*C*h_{ab} parameters for **1** and **2**, respectively (for the CIE D65/10° illuminant/observer condition): C* (chroma = colour saturation) = 48.7 and 86.9 (pH 1.1), 37.5 and 43.09 (pH 6.9); L* (lightness) = 87.5 and 76.6 (pH 1.1), 81.2 and 58.0 (pH 6.9); and h_{ab} (hue angle = chromatic tonality) = 61.1 and 58.7 (pH 1.1), 34.8 and 37.3 (pH 6.9). Red colours are characterized by h_{ab} values around 0°, while yellow are described by values close to 90. Thus, orange colours have h_{ab} values around 45°.

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References

Andersen, Ø.M., 1988. Semipreparative isolation and structure determination of pelargonidin 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside and other anthocyanins from the tree *Dacrycarpus dacrydioides*. Acta Chem. Scand. 42, 462–468.

Asenstorfer, R.E., Hayasaka, Y., Jones, G.P., 2001. Isolation and structures of oligomeric wine pigments by bisulfite-mediated ion-exchange chromatography. J. Agric. Food Chem. 49, 5957–5963.

Bakker, J., Timberlake, C.F., 1997. Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines. J. Agric. Food Chem. 45, 35–43.

Bakker, J., Bridle, P., Honda, T., Kuwano, H., Saito, N., Terahara, N., Timberlake, C.F., 1997. Identification of an anthocyanin occurring in some red wines. Phytochemistry 44, 1375–1382.

Brouillard, R., Dangles, O., 1994. Flavonoids and flower colour. In: Harborne, J.B. (Ed.), The Flavonoids. Advances in Research Since 1986. Chapman and Hall, London, pp. 565–588.

Fiorini, M., 1995. Preparative high-performance liquid chromatography for the purification of natural anthocyanins. Journal of Chromatography A 692, 213–219.

Fossen, T., Slimestad, R., Andersen, Ø.M., 2001. Anthocyanins from maize, *Zea mays*, and reed canarygrass, *Phalaris arundinacea*. J. Agric. Food Chem. 49, 2318–2321.

Fossen, T., Andersen, Ø.M., 2003. Anthocyanins from red onion, *Allium cepa*, with novel aglycone. Phytochemistry 62, 1217–1220.

Fukui, Y., Kusumi, T., Masuda, K., Iwashita, T., Nomoto, K., 2002. Structure of rosacyanin B, a novel pigment from the petals of *Rosa hybrida*. Tetrahedron Lett. 43, 2637–2639.

Fulcrand, H., Benabdeljalil, C., Rigaud, J., Cheynier, V., Moutounet, M., 1998. A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. Phytochemistry 47, 1401–1407.

Heinonen, M., Meyer, A.S., Frankel, E.N., 1998. Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. J. Agric. Food Chem. 46, 4107–4112.

Joseph, J.A., Denisova, N.A., Bielinski, D., Fisher, D.R., Shukitt-Hale, B., 2000. Oxidative stress protection and vulnerability in aging: putative nutritional implications for intervention. Mechanisms of Ageing and Development 116, 141–153.

Lopes da Silva, F., de Pascual-Teresa, S., Rivas-Gonzalo, J., Santos-Buelga, C., 2002. Identification of anthocyanin pigments in strawberry (cv Camarosa) by LC using DAD and ESI-MS detection. European Food Research and Technology 214, 248–253.

Lukton, A., Chichester, C.O., Mackinney, G., 1955. Characterisation of a second pigment in strawberries (*Fragaria vesca*). Nature (London) 176, 790.

Middleton Jr., E., Kandaswami, C., Theoharides, T.C., 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological Reviews 52, 673–751.

Pedersen, A.T., Andersen, Ø.M., Aksnes, D.W., Nerdal, W., 1993. NMR on anthocyanins, assignments and effects of exchanging aromatic protons. Magn. Reson. Chem. 31, 972–976.

Robinson, G.M., Robinson, R., 1932. A survey of anthocyanins. II. Biochemical Journal 26, 1647–1664.

Tamura, H., Takada, M., Yoshida, Y., 1995. Pelargonidin 3-*O*-(6-*O*-malonyl-beta-D-glucopyranoside) in *Fragaria×ananassa* Duch cv Nyoho. Bioscience, Biotechnology and Biochemistry 59, 1157–1158.

Torronen, R., Maatta, K., 2002. Bioactive substances and health benefits of strawberries. Acta Horticulturae 567, 797–803.

Vivar-Quintana, A.M., Santos-Buelga, C., Rivas-Gonzalo, J.C., 2002. Anthocyanin-derived pigments and color of red wines. Anal. Chim. Acta 458, 147–155.

Wang, S.Y., Lin, H.S., 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. J. Agric. Food Chem. 48, 140–146.