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CYANIDIN 3-(2-GLUCOSYLGALACTOSIDE) AND OTHER ANTHOCYANINS FROM FRUITS OF *CORNUS SUECICA*

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Key Word Index—*Cornus suecica*; Cornaceae; fruits; anthocyanins; cyanidin 3-*O*-*β*-(2″-glucopyranosyl-*O*-*β*-galactopyranoside); 2D NMR.

Abstract—Four anthocyanins were isolated from the scarlet fruits of dwarf dogwood, *Cornus suecica*, by a combination of chromatographic techniques. Their structures were determined by means of chemical degradation, chromatography and spectroscopy, especially homo- and heteronuclear NMR techniques. The novel, major pigment (49%), cyanidin 3-*O*-*β*-(2″-glucopyranosyl-*O*-*β*-galactopyranoside), contains a very rare disaccharide. The other anthocyanins were identified as cyanidin 3-*O*-*β*-(2″-glucopyranosyl-*O*-*β*-glucopyranoside) (31%), cyanidin 3-galactoside (16%) and cyanidin 3-glucoside (4%). © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

The genus *Cornus* (dogwoods) contains about 40 species, including some used in preserves and sweets [1]. These plants often assume a brilliant fall colouring and attractive flower and fruit colours. The following anthocyanins have previously been found in Cornaceae: cyanidin 3-arabinoside, pelargonidin 3-sophoroside, the 3-rutinosides of delphinidin and petunidin, the 3-rhamnosylgalactosides of pelargonidin and cyanidin, the 3-monoglucosides and 3-monogalactosides of pelargonidin, cyanidin and delphinidin [2–8]. To our knowledge, only two reports on the phytochemistry of the dwarf dogwood (*Cornus suecica*), which is commonly distributed in Northern Europe, have appeared. These articles concern the characterisation of seed oils [9] and iridoid glycosides [10]. In this paper we report the isolation and structural elucidation of one novel and tree known anthocyanins from the scarlet fruits of this plant.

RESULTS AND DISCUSSION

The acidified methanolic extract of fruits of dwarf dogwood contained the anthocyanins **1**–**4**. Compounds **3** and **4** were identified as cyanidin 3-galactoside and cyanidin-3-glucoside, respectively, based on TLC, HPLC, acid hydrolysis and UV-Vis spectral data (Table 1). Compounds **3** and **4** were also co-chromatographed with authentic anthocyanins from *Vaccinium myrtillus* and *Ribes nigrum*.

After purification by partition against ethyl acetate followed by chromatography on Amberlite XAD-7 and Sephadex LH-20 columns, the on-line UV-Vis spectrum of **1** showed a visible maximum at 518 nm with an A_{440}/A_{518} of 30% in agreement with a cyanidin or peonidin 3-glycoside [12]. Partial acid hydrolysis of the major pigment, **1**, produced cyanidin 3-galactoside and cyanidin. The low-field part of the ^{1}H NMR spectrum of **1** showed 6 resonances (see Table 1). On the basis of the chemical shifts and coupling-patterns the signals of the AMX system at 8.27, 8.13 and 7.12 ppm were assigned to H6′, H2′ and H5′ of the B-ring, respectively, while the 3H AMX system at 6.95, 6.72 and 9.05 ppm were assigned to H-8, H-6 and H-4, respectively. The small coupling constant at 6.95 ppm (0.7 Hz) is caused by a long-range coupling between the H-4 and H-8 protons [13]. All these assignments confirmed the identity of the aglycone to be cyanidin.

The anomeric proton signals in the ^{1}H NMR spectrum of **1** appear considerably downfield for the other sugar resonances and thus the two doublets at 5.49 and 4.81 ppm together with the integration data defined the cyanidin:sugar:sugar ratio as 1:1:1. Starting from the doublet at 5.49 ppm (H-1″) the observed cross-peak with the signal at 4.36 ppm in the DQF-COSY permitted assignment of H-2″. This proton was connected to H-3″ and H-

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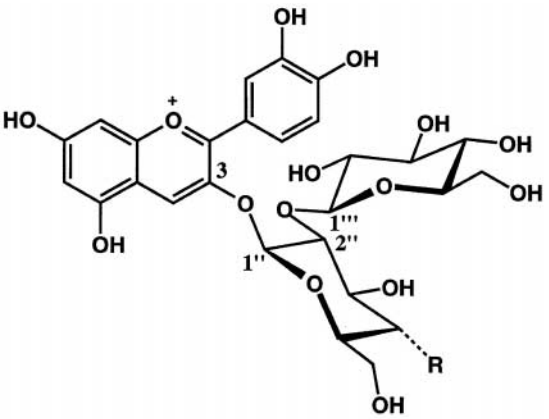

1: R = OH in axial position
2: R = OH in equatorial position

4″ via the H-2″/H-3″ and H-3″/H-4″ cross-peaks at 4.36/3.99 ppm and 3.99/4.07 ppm, respectively. The resonances around 3.90 ppm were assigned to H-5″, H-6A″ and H-6B″ based on integration data, cross-peaks in the TOCSY spectrum and the cross-peaks at 3.9/77.5 and 3.9/62.3 ppm in the HSC spectrum. The chemical shifts (Tables 2 and 3) and the coupling constants, including the small coupling constant of H-4 (3.3 Hz) which indicated an equatorial position for this proton, were in agreement with a β-galactopyranoside.

The sequential "walk" for the other sugar moiety protons in the DQF-COSY spectrum started at 4.81 ppm (H-1‴). The cross-peak at 4.81/3.30 ppm was used to assign H-2‴. The chain of coupled protons, H-2‴, H-3‴, H-4‴, H-5‴ and H-6‴ was more difficult to follow because of similarities between chemical shifts. However, by a combination with the SEFT, HSC and TOCSY NMR spectra it was possible to assign all the proton (Table 2) and carbon (Table 3) resonances of this sugar moiety, which was determined to be a β-glucopyranosyl.

The high-field position of the anomeric proton of the glucose unit (4.81 ppm) indicated a sugar–sugar linkage. In the HSC spectrum of **1** the carbon signal at 80.67 ppm was correlated to the proton signal at 4.36 ppm, which was assigned to H-2″ of the galactose moiety by means of the DQF-COSY experiment. The pronounced downfield shift of C-2″ (8.5 ppm) compared to the analogous C-2″ signal of delphinidin 3-β-galactopyranoside [14] showed that the glucosyl residue was connected to C-2″ of the galactose ring. The linkage between the two sugars was also supported by the observation that C-1″ of **1** was 2.2 ppm more shielded and H-1″, H-2″ and H-3″ were *ca.* 0.2 ppm more deshielded than the corresponding signals of delphinidin 3-β-galactopyranoside [14]. Thus the identity of **1** was found to be cyanidin 3-*O*-(2″-*O*-β-glucopyranosyl–β-galactopyranoside).

The on-line UV-Vis spectrum of **2** showed a visible maximum at 518 nm with an A_{440}/A_{518} of 30%. Partial acid hydrolysis gave cyanidin 3-glucoside and cyanidin. The NMR spectra of **2** showed many similarities with the analogous spectra of **1**. The ^{1}H–^{1}H coupling constants and assignments of all the proton and carbon resonances (Tables 2 and 3) of **2** were in agreement with cyanidin 3-*O*-(2″-*O*-β-glucopyranosyl–β-glucopyranoside).

The following disaccharides have previously been found as moieties of anthocyanins: sophoroside (2″-glucosylglucoside), laminariobioside (3″-glucosylglucoside), gentiobioside (6″-glucosylglucoside), sambubioside (2″-xylosylglucoside), neohesperidoside (2″-rhamnosylglucoside), rutinoside (6″-rhamnosylglucoside), (6″-glucosylgalactoside), lathyroside (2″-xylosylgalactoside), robinoside (6″-rhamnosylgalactoside), 6″-arabinofuranosylglucopyranoside and 2″-glucuronylglucoside [15–18]. In addition several anthocyanidin-disaccharides, including cyanidin 3-galactoglucoside from the fruits of *Arbutus*

Table 1. Chromatographic and spectral data on the anthocyanins in fruits of *Cornus suecica*: the 3-(2″-glucosylgalactoside) (**1**), 3-(2″-glucosylglucoside) (**2**), 3-galactoside (**3**) and 3-glucoside (**4**) of cyanidin, respectively

Anthocyanin	TLC (hR_F)		On-line HPLC		
	FHW	BAW	Vis. max. (nm)	A_{440}/A_{max} (%)	t_R (min)
1	82	37	518	30	8.3
2	82	38	518	30	8.6
3	32	36	518	29	9.3
4	30	37	519	30	9.8

Table 2. ^{1}H NMR spectral data for cyanidin 3-*O*-β-(2″-glucopyranosyl-*O*-β-galactopyranoside) (**1**), cyanidin 3-*O*-β-(2″-glucopyranosyl-*O*-β-glucopyranoside) (**2**), cyanidin 3-*O*-β-glucopyranoside (**4**), cyanidin 3-*O*-β-(2″-xylopyranosyl-*O*-β-glucopyranoside) (**5**) and delphinidin 3-*O*-β-galactopyranoside (**6**)

Positions	**1**, δ (ppm)	**2**, δ (ppm)	**5**, [13], δ (ppm)	**4** [13], δ (ppm)	**6** [14], δ (ppm)
4	9.05	9.06	8.96	9.10	9.07
6	6.72	6.74	6.73	6.76	6.74
8	6.95	6.98	6.94	6.98	6.95
2′	8.13	8.11	8.05	8.14	7.88
5′	7.12	7.12	7.05	7.11	–
6′	8.27	8.28	8.28	8.31	7.88
1″	5.49	5.54	5.53	5.38	5.36
2″	4.36	4.12	4.07	3.78	4.11
3″	3.99	3.85	3.90	3.65	3.78
4″	4.07	3.62	3.62	3.56	4.05
5″	3.90	3.65	3.71	3.67	3.85
6A″	3.90	4.00	4.03	4.02	3.85
6B″	3.90	3.81	3.85	3.82	3.85
1‴	4.81	4.85	4.87		
2‴	3.30	3.29	3.29		
3‴	3.40	3.36	3.43		
4‴	3.32	3.32	3.53		
5‴ (5A‴)	3.02	3.03	(3.80)		
6A‴ (5B‴)	3.50	3.54	(3.15)		
6B‴	3.50	3.54			

Table 3. ^{13}C NMR spectral data for cyanidin 3-*O*-*β*-(2″-glucopyranosyl-*O*-*β*-galactopyranoside) (**1**), cyanidin 3-*O*-*β*-(2″-glucopyranosyl-*O*-*β*-glucopyranoside) (**2**), cyanidin 3-*O*-*β*-glucopyranoside (**4**), cyanidin 3-*O*-*β*-(2″-xylopyranosyl-*O*-*β*-glucopyranoside) (**5**) and delphinidin 3-*O*-*β*-galactopyranoside (**6**)

Positions	**1**, δ (ppm)	**2**, δ (ppm)	**5** [13], δ (ppm)	**4** [13], δ (ppm)	**6** [14], δ (ppm)
2	164.20	164.34	164.29	164.36	164.49
3	145.35	145.33	145.40	145.64	145.96
4	136.58	136.80	136.34	137.03	136.61
5	159.16[a]	159.16[a]	159.56[a]	159.55[a]	159.03[a]
6	103.34	103.34	103.63	103.50	103.29
7	nd	170.23	170.71	170.56	170.38
8	95.10	95.15	95.23	95.19	95.03
9	157.58[a]	157.60[a]	157.75[a]	157.75[a]	157.72[a]
10	113.28	113.30	113.42	113.45	113.29
1′	121.28	121.27	121.36	121.31	120.07
2′	118.69	118.58	118.70	118.56	112.62
3′	147.36	147.40	147.79	147.41	147.56
4′	155.64	155.67	156.18	155.78	144.71
5′	117.53	117.45	117.55	117.48	147.56
6′	128.09	128.22	128.80	128.22	112.62
1″	102.40	101.98	101.69	103.79	104.63
2″	80.67	82.33	81.91	74.80	72.16
3″	74.75	77.84	78.47	78.13	74.87
4″	69.85	71.22	71.06	71.11	70.14
5″	77.53	77.69	78.90	78.79	77.80
6″	62.28	62.27	62.55	62.39	62.35
1‴	105.35	104.98	105.89		
2‴	75.97	75.87	75.98		
3‴	77.68	77.98	78.17		
4‴	71.14	70.82	71.25		
5‴	77.89	78.58	67.37		
6‴	62.27	62.27			

[a,b]Assignments with the same superscript may be reversed.
nd = not detected.

unedo [19], have been detected without proper determination of the linkage points between the sugar units. The sugar moiety of **1**, 2″-*O*-*β*-glucopyranosyl–*β*-galactopyranoside, has recently been found in pelargonidin 3-[2″-(2‴-*trans*-caffeoyl-*β*-D-glucopyranosyl)-*β*-D-galactopyranoside] isolated from *Pulsatilla cernua* (Ranunculaceae) [20].

EXPERIMENTAL

Plant material

Fruits of the dwarf dogwood, *Cornus suecica* L. [*Chamaepericlymenum suecicum* (L.) Aschers. and Graebn.] (Cornaceae), were collected at Mosvik, middle of Norway in August 1997. A voucher specimen has been deposited in the herbarium of the Chemistry Department, University of Bergen. The fruits (2.9 kg) were extracted with 3 l MeOH containing 0.05% trifluoracetic acid (TFA) (v/v). The filtered extracts were combined and concentrated under reduced pressure.

Chromatography

The concentrated extract was purified by partition against EtOAc (2 l) before application of the aqueous phase on an Amberlite XAD-7 column [11]. The anthocyanin coloured fractions were collected and subjected to a Sephadex LH-20 column (2.6 × 100 cm) using stepwise elution with 20% (360 ml), 40% (516 ml) and 70% (980 ml) MeOH in H_2O containing 0.1% TFA as eluents. The flow was adjusted to 0.86 ml min^{-1}. Eleven fractions were collected and monitored by HPLC and TLC. Fractions 1–3 contained **1** and **2**, whereas fractions 6–8 contained **3** and **4**. The combined fractions 1–3 were subjected to a Toyopearl HW-40F column (2.4 × 50 cm) and eluted using 20% (195 ml), 40% (230 ml) and 70% (440 ml) MeOH in H_2O containing 0.1% TFA as eluents. Eight fractions were collected and **1** was obtained in fraction 3, whereas **1** and **2** were obtained in fractions 4–5 and purified by repeated chromatography on the same column. Compound **3** was isolated in a pure state in fraction 7.

High performance liquid chromatography (HPLC) was performed on an HP-1050 module system (Hewlett Packard) using an ODS Hypersil column (20 × 0.5 cm, 5 μm). Two solvents were used for elution: A, HCO_2H–H_2O (1:9, v/v) and B, MeOH–HCO_2H–H_2O (5:1:4, v/v). The elution profile was 0–17 min, 10–100% B in A (linear gradient), 17–21 min 100% B (isocratic). The flow rate was 1.2 ml min^{-1}. Prior to injection all samples were filtered through a 0.45 μm Millipore membrane filter. The chromatogram was recorded as the average value of absorptions on every second nm between 240 and 600 nm using a photodiode array detector (HP 1050).

Thin layer chromatography was carried out on microcrystalline cellulose (F, Merck) with the solvents *n*-BuOH–HOAc–H_2O (4:1:5, upper phase) and HCO_2H–conc. HCl–H_2O (1:1:2).

Spectroscopy

UV-Vis absorption spectra were recorded on-line during HPLC analysis using a photodiode array detector (HP 1050, Hewlett-Packard). Spectral measurements were made over the wavelength range 240–600 nm in steps of 2 nm. The relative quantitative data were based on the average values of the absorptions on every second nm between 500 and 540 nm, without taking into account the different molar absorption coefficients of the pigments. The NMR experiments: double quantum filtered correlation spectroscopy (DQF-COSY), total correlation spectroscopy (TOCSY), spin echo Fourier transform (SEFT) and heteronuclear shift correlation (HSC) were obtained at 400.13 and 100.61 MHz for ^1H and ^{13}C, respectively, on a Bruker DMX-400 instrument 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 3.4 from TMS, respectively).

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