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Anthocyanin 3-galactosides from *Cornus alba* 'Sibirica' with glucosidation of the B-ring

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Abstract

The three anthocyanins, delphinidin 3-*O*-β-galactopyranoside-3′,5′-di-*O*-β-glucopyranoside (**1**), delphinidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside (**2**) and cyanidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside (**3**), and the 3-*O*-β-galactopyranosides of delphinidin (**4**) and cyanidin (**5**) were isolated from the bluish white berries and compound umbel of Siberian dogwood, *Cornus alba* 'Sibirica'. The ornamental autumn leaves and the characteristic purplish red bark of this variety were found to contain only pigment **5**.

Keywords: *Cornus alba*; Siberian dogwood; Cornaceae; Anthocyanins; B-ring glucosylation; Delphinidin 3-*O*-β-galactopyranoside-3′,5′-di-*O*-β-glucopyranoside; Delphinidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside; Cyanidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside; Diagnostic UV–Vis spectra; NMR

1. Introduction

The genus *Cornus* (dogwoods) consists of more than fifty-five species, mostly shrubs and small trees. Around thirty of them belong to the subgroup Kraniopsis, the blue or white-fruited dogwoods (Xiang and Boufford, 2005). Siberian dogwood (*Cornus alba* 'Sibirica'), which belongs to the white dogwoods (*C. alba*), is native to both Asia and Europe. This up to three metre tall ornamental shrub (also known as 'Westonbirt') gains colourful autumn leaves, and its crimson bark is really outstanding in the winter season (Dean, 2001). Its flowers are white to yellowish white, and are succeeded by autumn berries with a bluish white metallic shine.

Some functions of anthocyanins in plants are still debated (Willson and Whelan, 1990; Gould and Lister, 2006), however, it is suggested that the masking of chlorophylls by anthocyanins of Redosier dogwood (*Cornus sericea*/*Cornus stolonifera*) and Pagoda dogwood (*Cornus alternifolia*) reduces risk of photo-oxidative damage to leaf cells as they senesce, which otherwise may lower the efficiency of nutrient retrieval from senescing autumn leaves (Feild et al., 2001; Hoch et al., 2003; Lee et al., 2003). In recent years there has been increased focus on health benefits from eating anthocyanin-rich diet including Cornus fruits. Anticancer, anti-inflammatory and antioxidant effects (Seeram et al., 2002; Vareed et al., 2006) as well as treatment of diabetes mellitus-related disorders (Jayaprakasam et al., 2006; Nair et al., 2006) by *Cornus* anthocyanins, have been indicated.

The 3-galactosides and 3-glucosides of pelargonidin, cyanidin and delphinidin are the most encountered anthocyanins in *Cornus* spp., although other mono- and disaccharides attached in the aglycone 3-position have been reported (Santamour and Lucente, 1967; Du and Francis, 1973a,b; Du et al., 1974a,b, 1975; Slimestad and Andersen, 1998; Vareed et al., 2006). There have been no reports of anthocyanins substituted with sugars in the 5- or 7-position, nor on the aglycone B-ring (Bhakuni et al., 2004). The red bark of *C. alba* 'Sibirica' has previously been reported to contain cyanidin-3-galactoside as the major pigment, with 'appreciable amounts' of cyanidin-3-arabinoside and traces of a delphinidin monoglycoside

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(Du et al., 1975). It has also been indicated that the anthocyanin content of leaves of *C. alba* is up to 50 nmol cm^{-2} (Gitelson et al., 2001).

With relevance to recent focus on potential health benefits and biological functions of anthocyanins from *Cornus*, the major aim of this paper is to report the identification of the anthocyanins isolated from various parts of Siberian dogwood (*C. alba* 'Sibirica'), including three new pigments from the bluish berries.

2. Results and discussion

HPLC profiles of the crude extracts of berries and compound umbel of Siberian dogwood (Fig. 1) revealed a total of five anthocyanins, **1**–**5** (Fig. 2). The structures of **4** and **5** were elucidated to be delphinidin 3-*O*-β-galactoside and cyanidin 3-*O*-β-galactoside, respectively, based on NMR data (see Section 3) in accordance with comparable literature data (Slimestad and Andersen, 1998; Reiersen et al., 2003). Anthocyanins **4** and **5** were also co-chromatographed (TLC and HPLC) with authentic pigments from *Cornus mas* L. (Du and Francis, 1973b).

Anthocyanins **1**–**3**, which had on-line λ_{max}-values of 511, 519 and 509 nm, respectively, revealed characteristic $A_{440}/A_{Vis.max}$ ratios (Table 1) in accordance with either pelargonidin as the aglycone, or alternatively sugar moieties attached to the anthocyanidin B-ring (Yoshitama and Abe, 1977). In the ^{1}H NMR spectrum of **2** the signal at δ 9.11 (H-4) having a 5-bond zig zag coupling to H-8 ($J = 0.9$ Hz), the 2H *meta*-coupled protons at δ 6.74 (H-6) and δ 7.06 (H-8), and the 2H *meta*-coupled AX system at δ 8.22 (H-2) and δ 8.01 (H-6) were in accordance with delphinidin aglycone with the B-ring protons as non-equivalent doublets indicating an asymmetrically substituted

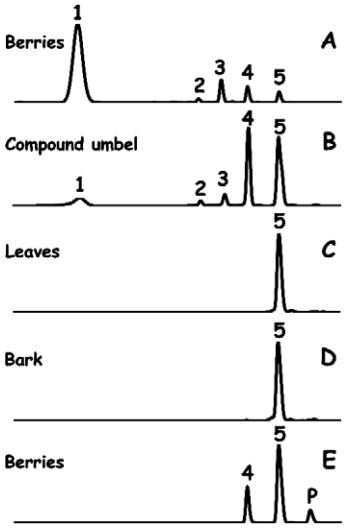

Fig. 1. HPLC profiles of *Cornus alba* 'Sibirica' (A–D) and *C. mas* (E). HPLC chromatograms were recorded at 500–540 nm in steps of 2 nm. The peak labelled P in (E) is assigned to pelargonidin 3-galactopyranoside (Du and Francis, 1973a).

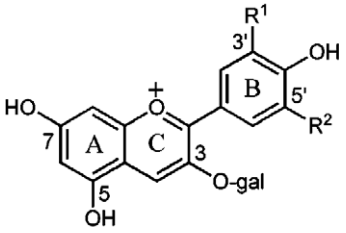

Fig. 2. Structures of the anthocyanins isolated from extracts of *Cornus alba* 'Sibirica'. **1** = delphinidin 3-*O*-β-galactopyranoside-3′,5′-di-*O*-β-glucopyranoside ($R^1 = R^2 =$ *O*-glc), **2** = delphinidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside ($R^1 =$ *O*-glc, $R^2 =$ OH), **3** = cyanidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside ($R^1 =$ *O*-glc, $R^2 =$ H), **4** = delphinidin 3-*O*-β-galactopyranoside ($R^1 = R^2 =$ OH), **5** = cyanidin 3-*O*-β-galactopyranoside ($R^1 =$ OH, $R^2 =$ H). gal = galactopyranoside; glc = glucopyranoside.

B-ring. The ^{1}H NMR spectrum of **2** showed two anomeric signals at δ 5.35 and δ 5.14 both with coupling constants of 7.7 Hz, indicating β-configurations of two monosaccharides. The 1-dimensional ^{1}H, ^{1}H–^{1}H COSY and ^{1}H–^{13}C HSQC NMR spectra of **2** showed that the chemical shifts and coupling constants (Tables 2 and 3) were in accordance with one galactosyl and one glucosyl unit. The axial position of H-4″ in the galactosyl was revealed by the small $^3J_{HH}$ coupling constants (3.4 and 0.7 Hz), due to the small dihedral angles between H-4″ and H-3″, and H-4″ and H-5″, respectively, in the cyclic chair conformation. In the glucosyl the equivalent dihedral angles of H-4″ will be greater (around 180°) resulting in larger coupling constants (9.8 and 9.0 Hz). The cross peak observed at δ 5.35/145.6 (H-1″/C-3) in the ^{1}H–^{13}C HMBC spectrum of **2** showed that the galactosyl was placed in the aglycone 3-position, while the crosspeak between H-1‴ and C-3′ at δ 5.14/147.4 confirmed the linkage position of the glucosyl to the B-ring. The molecular mass (*m*/*z* 627.1574) corresponding to $C_{27}H_{31}O_{17}$ in the ESI^+ high resolution mass spectrum of **2** and the fragment ion corresponding to delphinidin (*m*/*z* 303.05), confirmed the structure of **2** to be delphinidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside.

The NMR spectra of pigment **3** showed many similarities to the same spectra of **2** (Tables 2 and 3), including signals corresponding to the same two monosaccharides and the same linkage points. The difference between the two anthocyanins was revealed in the ^{1}H NMR spectrum of **3** by the 3H AMX system at δ 8.54 (*d*, 2.3 Hz, H-2′), 8.51 (*dd*, 8.8 Hz, 2.3 Hz, H-6′) and 7.21 (*d*, 8.8 Hz, H-5′) in agreement with the anthocyanidin, cyanidin. The molecular mass (*m*/*z* 611.1610) corresponding to $C_{27}H_{31}O_{16}$ in the ESI^+ high resolution mass spectrum of **3** and the fragment ion corresponding to cyanidin (*m*/*z* 287.06), confirmed the structure of **3** to be cyanidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside.

The NMR spectra of pigment **1** showed many similarities to the same spectra of **2** (Tables 2 and 3), including signals corresponding to delphinidin. However, in this latter case the singlet in the ^{1}H NMR spectrum at δ 8.35

Table 1
Chromatographic and spectral data for **1**–**5** isolated from extracts of *Cornus alba* 'Sibirica'

Anthocyanin	TLC on cellulose R_f values (100×)		On-line HPLC		
	FHW	BAW	$\lambda_{Vis.max.}$ (nm)	A_{440}/A_{max} (%)	R_t (min)
1	82	8	511	45	7.1
2	50	11	519	35	11.2
3	65	22	509	42	12.0
4	19	15	527	28	12.9
5	31	28	519	30	13.9

Table 2
^{1}H NMR spectral data for delphinidin 3-*O*-β-galactopyranoside-3′,5′-di-*O*-β-glucopyranoside (**1**), delphinidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside (**2**) and cyanidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside (**3**) in CF_3COOD-CD_3OD (5:95; v/v) at 25 °C

	^{1}H δ (ppm) J (Hz)		
	1	**2**	**3**
Aglycone			
4	9.14 *d* 0.9	9.11 *d* 0.9	9.16 *d* 0.9
6	6.74 *d* 2.0	6.74 *d* 2.0	6.75 *d* 2.0
8	7.13 *dd* 2.0, 0.9	7.06 *dd* 2.0, 0.9	7.09 *dd* 2.0, 0.9
2′	8.35 *s*	8.22 *d* 2.2	8.54 *d* 2.3
5′			7.21 *d* 8.8
6′	8.35 *s*	8.01 *d* 2.2	8.51 *dd* 8.8, 2.3
3-O-β-Galactopyranoside			
1″	5.35 *d* 7.7	5.35 *d* 7.7	5.34 *d* 7.7
2″	4.11 *dd* 9.6, 7.7	4.11 *dd* 9.7, 7.7	4.08 *dd* 9.6, 7.7
3″	3.76 *dd* 9.6, 3.4	3.76 *dd* 9.7, 3.4	3.75 *dd* 9.6, 3.4
4″	4.04 *dd* 3.4, 0.8	4.04 *dd* 3.4, 0.7	4.04 *dd* 3.4, 0.9
5″	3.93 m	3.91 *m*	3.91 *m*
6″	3.86 m	3.85 *m*	3.86 *m*
3′-O-β-Glucopyranoside			
1‴	5.19 *d* 7.7	5.14 *d* 7.7	5.15 *d* 7.7
2‴	3.67 *m*	3.66 *m*	3.67 *m*
3‴	3.66 *m*	3.66 *m*	3.66 *m*
4‴	3.47 *dd* 9.8, 8.9	3.47 *m*	3.46 *dd* 9.8, 9.0
5‴	3.69 *ddd* 9.8, 6.6, 2.2	3.69 *m*	3.71 *ddd* 9.8, 6.7, 2.2
6A‴	4.06 *dd* 12.3, 2.2	4.08 *dd* 12.3, 2.2	4.10 *dd* 12.3, 2.2
6B‴	3.78 *dd* 12.3, 6.6	3.80 *dd* 12.3, 6.6	3.81 *dd* 12.3, 6.7
5′-O-β-Glucopyranoside			
1′′	5.19 *d* 7.7		
2′′	3.67 *m*		
3′′	3.66 *m*		
4′′	3.47 *dd* 9.8, 8.9		
5′′	3.69 *ddd* 9.8, 6.6, 2.2		
6A′′	4.06 *dd* 12.3, 2.2		
6B′′	3.78 *dd* 12.3, 6.6		

See Fig. 2 for pigment identification.

integrating for two protons, showed that the aglycone had a symmetric substituted B-ring. The NMR signals of the sugars of **1** (Tables 2 and 3) were very similar to corresponding signals of **2**. However, some of the signals corresponded to two protons, in agreement with one galactosyl and two glucosyls. Integration values for the two anomeric signals of **1** (integration ratio 1:2) indicated in addition to the galactosyl two identical monosaccharides placed on each side of a

Table 3
^{13}C NMR spectral data for delphinidin 3-*O*-β-galactopyranoside-3′,5′-di-*O*-β-glucopyranoside (**1**), delphinidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside (**2**) and cyanidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside (**3**) in CF_3COOD–CD_3OD (5:95; v/v) at 25 °C

	^{13}C δ (ppm)		
	1	**2**	**3**
Aglycone			
2	163.16	163.8	163.85
3	145.83	145.6	145.72
4	137.02	136.6	137.26
5	158.8	159.1	159.2
6	103.57	103.3	103.47
7	171.03	170.6	170.7
8	95.57	95.2	95.36
9	158.2	157.9	157.9
10	114.24	113.8	113.95
1′	120.39	120.1	121.45
2′	116.68	114.1	120.82
3′	147.69	147.4	147.31
4′	146.67	145.4	156.40
5′	147.69	147.5	118.35
6′	116.68	115.0	130.57
3-O-β-Galactopyranoside			
1″	103.84	104.1	104.08
2″	72.19	72.0	72.12
3″	74.98	74.9	74.95[a]
4″	70.06	70.0	70.08
5″	77.81	77.8	77.82
6″	62.34	62.3	62.33
3′-O-β-Glucopyranoside			
1‴	103.84	104.1	103.92
2‴	74.98	75.0	75.00[a]
3‴	77.49	77.3	77.41
4‴	71.52	71.6	71.62
5‴	78.86	78.9	78.93
6‴	62.80	62.8	62.92
5′-O-β-Glucopyranoside			
1′′	103.84		
2′′	74.98		
3′′	77.49		
4′′	71.52		
5′′	78.86		
6′′	62.80		

See Fig. 2 for pigment identification. Chemical shifts given in two decimals are from CAPT spectra, while one decimal values are from HSQC/HMBC spectra.

[a] Signals may be reversed. Chemical shifts given in two decimals are from CAPT spectra, while one decimal values are from HSQC/HMBC spectra.

plane of symmetry, as possible only on the delphinidin B-ring. The cross peak at δ 5.19/147.7 (H-1‴, H-1⁗/C-3′, C-5′) in the ^{1}H–^{13}C HMBC spectrum of **1** showed indeed that the two glucosyls were connected to the delphinidin B-ring. The molecular mass (*m/z* 789.2095) corresponding to $C_{33}H_{41}O_{22}$ in the ESI^{+} high resolution mass spectrum of **1** and the fragment ion corresponding to delphinidin (*m/z* 303.05), confirmed the structure of **1** to be delphinidin 3-*O*-β-galactopyranoside-3′,5′-di-*O*-β-glucopyranoside.

UV–Vis spectra of **1**–**3** recorded on-line during HPLC analyses compared to analogous spectra of anthocyanin 3-galactosides without B-ring substitution, showed diagnostic hypsochromic shifts, about 8–10 nm, and increased $A_{440}/A_{Vis.max}$ ratios (45%, 35% and 42%) for pigments **1**–**3** compared to 28% and 30% for pigments **4** and **5**, respectively, in accordance with previous findings of 3′-substituted anthocyanins (Yoshitama and Abe, 1977). In the delphinidin series there is a decrease in λ_{max} of 8 nm (Table 1). For the cyanidin series there is a decrease in λ_{max} of 10 nm (Table 1). In 0.1% HCl–MeOH the λ_{max} of **1** and **3** are at 524 and 518 nm, respectively, and the A_{440}/A_{max} ratios are around 36%. The on-line UV–Vis data for cyanidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside (**3**) is quite similar to that of cyanidin 3,4′-di-*O*-β-glucopyranoside isolated from *Allium cepa* (Fossen et al., 2003). Thus, whether the glucosyl is located either in the anthocyanidin 3′-, 4′- or 5′-position seem to have the same characteristic additative hypsochromic shift effect on the UV–Vis maxima.

Pigments **1**–**3**, which have sugar moieties attached to the anthocyanidin B-rings in addition to galactosyl in their 3-positions, are new compounds. The major pigment, **1**, (Fig. 1) in the bluish berries has a glucosyl linked to both the 3′- and 5′-positions. Anthocyanins with sugars moieties in both the 3′- and 5′-positions have previously been isolated from Leguminosae, Lobeliaceae and Liliaceae (Andersen and Jordheim, 2006). The colourful autumn leaves and crimson bark contain only cyanidin 3-*O*-β-galactoside, **5** (Fig. 1).

3. Experimental

3.1. Plant material

Berries, compound umbel, bark and leaves of cultivated specimens of *C. alba* 'Sibirica' (identified by Professor Dag Olav Øvstedal, Department of Botany, University of Bergen) were collected near Realfagbygget in Bergen (Norway). Voucher specimens are deposited in BG (Bergen Herbarium, University of Bergen, accession number H/506).

3.2. Extraction and isolation

The plant material was extracted using methanol (MeOH) containing 0.2% trifluoroacetic acid (TFA) (v/v). After concentration under reduced pressure, the anthocyanins were purified by partition against ethyl acetate (EtOAc). The aqueous extracts (i.e. after removal of the EtOAc phase) of berries and compound umbel were combined and concentrated under reduced pressure. The preferred separation step using Amberlite XAD-7 cation-exchange resin (Goto et al., 1982; Andersen and Francis, 2004) was unsuccessful, possibly due to the high polarity of pigment **1**. The extracts were therefore applied to a Toyopearl HW-40F column (150 mm × 50 mm) and eluted using 20% MeOH in H_2O containing 0.2% TFA. Three red-coloured bands, containing pigment **1**, "**2/3**" and "**4/5**", respectively, were collected. The viscous (high sugar content) fraction containing **1** was further purified with a Bondesil C-18 column (300 mm × 25 mm, 140 μm) using 20% MeOH in H_2O containing 0.2% TFA as eluting solvent under medium pressure. Fractions containing **2/3** were concentrated under reduced pressure and applied to a Sephadex LH-20 column (800 mm × 20 mm). Two separated bands containing pigments **3** and **2**, respectively, were collected. Fractions containing **4/5** were purified similarly. All fractions were monitored by analytical HPLC (see below). Pigments **1** and **3** were prior to NMR-analyses purified by preparative HPLC (Econosil C18 column, 250 mm × 22 mm, 10.0 μm). Pigment **2** was purified by an Econosphere column (250 mm × 10 mm, 10.0 μm). A combination of two solvents were used for elution: A, H_2O–HCOOH (9:0.5, v/v) and B, H_2O–MeOH–HCOOH (4:5:0.5, v/v). The following solvent mixtures were used: from 10% B to 100% B in 0–45 min (linear gradient), 100% B during 45–58 min. The flow rate was 14.0 ml min^{-1} for the column with widest diameter, and 10.0 ml min^{-1} for the narrowest column. Prior to all HPLC injections the samples were filtered through a Whatman 0.45 μm syringe filter.

Analytical HPLC was performed using an ODS Hypersil column (200 mm × 5 mm, 5.0 μm) by injecting 20 μl of the filtered extracts. A combination of two solvents was used for elution: A, H_2O containing 0.5% TFA (v/v) and B, acetonitrile containing 0.5% TFA (v/v). The following solvent mixtures were used: 0–4 min 10% B (isocratic), 4–21 min 10–40% B (linear gradient), 21–28 min 40% B (isocratic), flow rate was 1.0 ml min^{-1}. TLC analyses were carried out on microcrystalline cellulose F (Merck) with the solvents BAW (*n*-BuOH–HOAc–H_2O, 4:5:1; upper phase) and FHW (HCO_2H–HCl (conc.)–H_2O, 1:1:2).

The aqueous extracts of bark and leaves were combined before application on an Amberlite XAD-7 column. Phenolic compounds were eluted using MeOH containing 0.2% TFA. The red-coloured fraction was collected and concentrated under reduced pressure before application on a Sephadex LH-20 column using 20% MeOH in H_2O containing 0.2% TFA as eluent.

3.3. 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 NMR experiments (^{1}H, ^{1}H–^{13}C HMBC, ^{1}H–^{13}C HSQC, ^{1}H–^{1}H COSY, ^{1}H–^{1}H TOCSY and ^{13}C CAPT) were obtained at 600.13 and 150.90 MHz for ^{1}H and ^{13}C, respectively, on a Bruker Biospin AV-600 MHz instrument equipped with a TCI ^{1}H–^{13}C/^{15}N CryoProbe at 25 °C. The deuterio-methyl ^{13}C signal and the residual ^{1}H signal of the solvent (CF_3COOD-CD_3OD; 5:95, v/v) were used as secondary references (δ 49.0 and δ 3.4 from TMS, respectively).

High-resolution LC–MS (ESI$^+$/TOF) spectra of **1**–**3** were recorded using a JMS-T100LC with an AccuTOF LP mass separator. The gradient used was equal to the one described for the analytical HPLC system with one exception; TFA was replaced with 0.5% formic acid (HCOOH) in both solvents A (water) and B (acetonitrile). A Develosil C18 (100 mm × 2.0 mm, 3.0 μm; particle size) column was used for separation. The retention times of **1**–**3** were 1.6, 2.8 and 3.6 min, respectively.

3.4. Anthocyanins

*Delphinidin 3-O-β-galactopyranoside-3′,5′-di-O-β-glucopyranoside (**1**)*. HPLC and TLC: see Table 1; ^{1}H NMR: see Table 2; ^{13}C NMR: see Table 3; LC–MS (ESI$^+$/TOF): *m/z* 789.2095, calc. for $C_{33}H_{41}O_{22}$, 789.2090, fragments: *m/z* 627.16 $[M-162)]^+$, *m/z* 465.10 $[M-2\times(162)]^+$, *m/z* 303.05 $[M-3\times(162)]^+$; UV–Vis (0.1% HCl-MeOH): $\lambda_{Vis.max}$ 524, $A_{440}/A_{Vis.max}$ 36%.

*Delphinidin 3-O-β-galactopyranoside-3′-O-β-glucopyranoside (**2**)*. HPLC and TLC: see Table 1; ^{1}H NMR: see Table 2; ^{13}C NMR: see Table 3; LC–MS (ESI$^+$/TOF): *m/z* 627.1574, calc. for $C_{27}H_{31}O_{17}$, 627.1561, fragments: *m/z* 465.10 $[M-162)]^+$, *m/z* 303.05 $[M-2\times(162)]^+$.

*Cyanidin 3-O-β-galactopyranoside-3′-O-β-glucopyranoside (**3**)*. HPLC and TLC: see Table 1; ^{1}H NMR: see Table 2; ^{13}C NMR: see Table 3; LC–MS (ESI$^+$/TOF): *m/z* 611.1610, calc. for $C_{27}H_{31}O_{16}$, 611.1612, fragments: *m/z* 449.11 $[M-(162)]^+$, *m/z* 287.06 $[M-2\times(162)]^+$; UV–Vis (0.1% HCl–MeOH): $\lambda_{Vis.max}$ 518, $A_{440}/A_{Vis.max}$ 35%.

*Delphinidin 3-O-β-galactopyranoside (**4**)*. HPLC and TLC: see Table 1; ^{1}H NMR (δ (ppm) *J* (Hz)): 9.07 *d* 0.9 (H-4), 6.73 *d* 2.0 (H-6), 6.95 *dd* 2.0, 0.9 (H-8), 7.89 *s* (H-2′/H-6′), 5.36 *d* 7.8 (H-1″), 4.11 *dd* 9.7, 7.8 (H-2″), 3.77 *dd* 9.7, 3.3 (H-3″), 4.05 *d* br. 3.3 (H-4″), 3.89 *m* (H-5″), 3.87 *m* (H-6″); ^{13}C NMR (δ (ppm)): 164.49 (C-2), 145.98 (C-3), 136.57 (C-4), 159.1 (C-5), 103.24 (C-6), 170.33 (C-7), 95.00 (C-8), 157.6 (C-9), 113.0 (C-10), 120.05 (C-1′), 112.59 (C-2′/6′), 147.56 (C-3′/5′), 144.73 (C-4′), 104.62 (C-1″), 72.14 (C-2″), 74.85 (C-3″), 70.12 (C-4″), 77.80 (C-5″), 62.34 (C-6″).

*Cyanidin 3-O-β-galactopyranoside (**5**)*. HPLC and TLC: see Table 1; ^{1}H NMR (δ (ppm) *J* (Hz)): 9.12 *d* 0.9 (H-4), 6.74 *d* 2.0 (H-6), 6.98 *dd* 2.0, 0.9 (H-8), 8.17 *d* 2.4 (H-2′), 7.11 *d* 8.8 (H-5′), 8.36 *dd* 8.8, 2.4 (H-6′), 5.34 *d* 7.9 (H-1″), 4.08 *dd* 9.7, 7.9 (H-2″), 3.76 *dd* 9.7, 3.3 (H-3″), 4.04 *d* br. 3.3 (H-4″), 3.89 *m* (H-5″), 3.87 *m* (H-6″); ^{13}C NMR (δ (ppm)): 164.53 (C-2), 145.77 (C-3), 137.03 (C-4), 159.3 (C-5), 103.28 (C-6), 170.45 (C-7), 95.07 (C-8), 157.8 (C-9), 113.40 (C-10), 121.30 (C-1′), 118.51 (C-2′), 147.42 (C-3′), 155.80 (C-4′), 117.43 (C-5′), 128.23 (C-6′), 104.45 (C-1″), 72.09 (C-2″), 74.93 (C-3″), 70.11 (C-4″), 77.81 (C-5″), 62.35 (C-6″); UV–Vis (0.1% HCl–MeOH): $\lambda_{Vis.max}$ 530, $A_{440}/A_{Vis.max}$ 21%.

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