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Anthocyanins in Berries of *Ribes* Including Gooseberry Cultivars with a High Content of Acylated Pigments

MONICA JORDHEIM,[†] FINN MÅGE,[‡] AND ØYVIND M. ANDERSEN*,[†]

Department of Chemistry, University of Bergen, Allégt. 41, N-5007 Bergen, Norway, and Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, P.O. Box 5003, N-1432 Aas, Norway

Consumption of berries from various sources including the genus *Ribes* has been associated with diverse potential health benefits. The 14 examined cultivars of European gooseberry (*R. grossularia* L.) contained in various proportions the 3-glucoside (**3**), 3-rutinoside (**4**), 3-xyloside (**7**), 3-*O*-β-(6″-*E*-caffeoylglucopyranoside) (**8**), and 3-*O*-β-(6″-*E*-*p*-coumaroylglucopyranoside) (**10**) of cyanidin and the 3-rutinoside (**6**) and 3-glucoside of peonidin (**5**). Pigments **3**, **4**, delphinidin 3-rutinoside (**2**), delphinidin 3-glucoside (**1**), and minor amounts of **6**, **7**, and **10** were found in red flowering currant (*R. sanguineum* Pursh). Golden currant (*R. aureum* Pursh) contained **3**, **4**, and trace amounts of **1**, **6**, and **7**, while alpine currant (*R. alpinum* L.) contained **3**, **4**, and trace amounts of **10**. The major anthocyanins in two cultivars of jostaberries (*R.* × *nidigrolaria* Bauer), **1**−**4**, **8**, and **10**, reflected that this hybrid contained the major anthocyanins of both parents, black currant and gooseberry. This is the first complete identification of **8** and the ring size of the sugar of **10**. Pigment **9** was tentatively identified as cyanidin 3-(6″-*Z*-*p*-coumaroylglucoside). This new pigment occurred in minor amounts (<2%) in all *R. grosssularia* and *R.* × *nidigrolaria* cultivars. No commercially available berries have been reported to contain such high proportions of aromatic acylated anthocyanins as found in the gooseberry cultivars "Samsø", "Hinnomäki Red", "Taastrup", "Lofthus", and "Glendal", which are in this context the most obvious candidates for consumption, colorant, and breeding programs.

KEYWORDS: Anthocyanins; aromatic acylation; cyanidin 3-*O*-β-(6″-*E*-caffeoylglucopyranoside); cyanidin 3-*O*-β-(6″-*E*-*p*-coumaroylglucopyranoside); cyanidin 3-(6″-*Z*-*p*-coumaroylglucoside); ^{13}C NMR; *Ribes*; gooseberry; alpine currant; golden currant; red flowering currant; jostaberry

INTRODUCTION

Anthocyanins, being part of the flavonoid group, are widely distributed among fruits, berries, and flowers, providing attractive colors ranging from orange through red and blue to black (*1*). These polyphenolic compounds are water-soluble, which facilitates their incorporation into numerous aqueous food systems. They impart vital biological functions to plants, and recent interests in anthocyanins in fruits and vegetables focus on their antioxidant capacity (free radical scavenging and metal chelating activities) and possible beneficial roles in human health, such as reducing the risk of cancer, cardiovascular diseases, improvement of vision, and other pathologies (*3*−*5*).

Around 600 naturally occurring anthocyanins have hitherto been reported (*1*). They occur primarily as glycosides of their respective aglycones (anthocyanidins), and more than half of these are acylated with aromatic acyl groups, including various hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic, sinapic, and 3,5-dihydroxycinnamic acids) and two hydroxybenzoic acids (*p*-hydroxybenzoic and gallic acids) (*1*). Anthocyanin diversity is thus highly associated with the nature, number, and linkage positions of the aromatic acyl groups. Recent research has shown that anthocyanins with different aglycones and glycosyl moieties may have quite different responses in terms of their stability, bioavailability, and potential health effects (*6*−*12*). Acylated anthocyanins in particular may behave quite differently from nonacylated analogues (*6*−*9*, *11*, *12*). Increased stability of anthocyanins with aromatic acylation has been achieved by inter- and intramolecular copigmentation involving stacking between the anthocyanidin and the aromatic acyl groups (*13*−*15*). In slightly acidic to neutral aqueous solutions, most anthocyanins without aromatic acylation occur in their most unstable equilibrium forms (*16*). Some acylated anthocyanins have been reported to have unique physiological functions (*17*, *18*). However, the extent of absorption and bioavailability of anthocyanins with aromatic acylation in humans are discussed in the literature (*8*−*11*, *19*−*21*).

Following the success of the North Karelian intervention program in Finland (*22*, *23*), where increased dietary intake of berries was associated with the reduction of heart disease and strokes by 60%, promotion of research in related government-

* To whom correspondence should be addressed. Telephone: +47-5558-3460. Fax +47-5558-9490. E-mail: oyvind.andersen@kj.uib.no.
[†] University of Bergen.
[‡] Norwegian University of Life Sciences.

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funded projects has been encouraged in many Western countries. Several species belonging to the genus *Ribes*, such as black currants (*R. nigrum* L.) and red currants (*R. rubrum* L.), are commonly consumed in the Western diet. Anthocyanin containing extracts of black currant have been used to investigate anthocyanin absorption in humans as well as in rats and rabbits (*24*−*27*).

The gooseberry in the family Saxifragaceae is native to Europe, northwestern Africa, and southwestern Asia. Its subspecies *R. grossularia* L. (European gooseberry) grows in Central Europe and Scandinavia. Gooseberries are cultivated in many private gardens; however, they have been of limited commercial value. Some of the cultivars with red to dark blue pigmented berries have been reported to contain the 3-rutinosides and 3-glucoside of cyanidin and some unidentified acylated anthocyanins (*26*−*30*). According to Nilsson and Trajkovski (*31*), the European gooseberry differed from the American species by having no delphinidin glycosides. In a more recent paper, the 3-galactoside, 3-glucoside, 3-rutinoside, 3-xyloside, 3-caffeoylglucoside, and 3-*p*-coumaroylglucoside of cyanidin and the 3-glucoside and 3-rutinoside of peonidin were tentatively identified in "Whinham", "Lancashine", and "Dan's Mistake" based on the comparison of their mass spectrometry (MS) data and retention times with those of standards (*32*). Similarly, the 3-galactoside and 3-rutinoside of cyanidin were assigned in "Careless". The anthocyanins of berries of golden currant (*R. aureum* Pursh) have been reported to be the glucoside and rutinoside of cyanidin without determination of the linkage positions of the sugar moieties (*29*, *33*). The anthocyanin content of jostaberry (*Ribes* × *nidigrolaria* Bauer), the hybrid of black currant and gooseberry, has been reported to be intermediate between the glucosides and rutinosides of delphinidin and cyanidin content of black currant and the glucoside and rutinoside of cyanidin content from gooseberry, without presenting data for determination of the linkage positions of the sugar moieties of these pigments (*29*). The flowers of flowering currant (*R. sanguineum* Pursh) have been reported to contain cyanidin 3-rhamnoglucoside (*34*); however, there are no reports on the anthocyanin content of the berries. Alpine currant (*R. alpinum* L.) is a shrub widespread in mountainous areas across Western Europe. This species is not cultivated today, although its berries collected in the wild were traditionally used to prepare a wine. There is no report on the anthocyanin content of this species.

As far as we know, there exists no report on cultivated berries of commercial value used in the human diet containing anthocyanins acylated with aromatic acyl groups as the major pigments. The aim of this study was thus to look for potential sources among various gooseberry varieties, to determine the structure of the involved acylated anthocyanins properly, including new pigments, and to determine the qualitative and quantitative anthocyanin content of other *Ribes* species such as golden currant, flowering currant, jostaberry, and alpine currant.

MATERIALS AND MEHTODS

Plant Material. Alpine currant (*Ribes alpinum* L.), golden currant (*R. aureum* Pursh), the European gooseberry (*R. grossularia* L.) cultivars "Martlet", "Rokula", "Larell", "Rolanda", "Rosko", "Scania", "John's", "Glendale", "Agro", "Taastrup", "Pax", "Samsø", "Lofthus", and "Hinnonmäki Red", and two jostaberry (*Ribes* × *nidigrolaria* Bauer, originally hybrids between gooseberry and black currant) cultivars "Josta" and "Jostine" were collected at the experimental fields at the Norwegian University of Life Sciences in August 2006 and stored at −20 °C. Red flowering currant, *R. sanguineum* Pursh, was collected at Foldøy in Ryfylke (Norway) in October 2006.

Isolation of Anthocyanins. The various samples were extracted with acidified (0.5% trifluoroacetic acid, TFA) methanol and analyzed by high-performance liquid chromatography (HPLC). Prior to injection, the samples were filtered through a 0.45 μm Millipore membrane filter. For preparative isolation of pigments **8**−**10**, a combined lot (2 kg) of various gooseberry samples were extracted repeatedly (six times) with 0.5% TFA in MeOH. The combined filtered extract was concentrated under reduced pressure, purified by partition against EtOAc (three times), and applied to an Amberlite XAD-7 column (*35*). The eluate was concentrated under reduced pressure before 80% of the sample was applied on to a Toyopearl HW-40F column (100 × 2.6 cm). The column was eluted with MeOH−water (20:80) containing 0.5% TFA, and pigment **10** was isolated as a distinct band. Pigment **8** on the other hand was isolated by subjecting the remaining 20% of the concentrated eluate to preparative HPLC using a Gilson 305/306 pump equipped with an HP-1040 A detector on a 25 × 2.2 cm, 5 μm ODS-Hypersil column (Supelco, Bellefonte, PA) using the solvents HCOOH−H_2O (1:19, v/v) (A) and HCOOH−H_2O−MeOH (1:4:5, 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 a linear gradient from 100% B to 10% B for 1 min. The flow rate was 14 mL/min, and aliquots of 250 μL were injected. Pigment **9** occurred in trace amounts in the purified fraction of **8**.

Analytical HPLC-DAD System. The Agilent 1100 HPLC system was equipped with a HP 1050 diode-array detector and a 200 × 4.6 mm inside diameter, 5 μm ODS Hypersil column (Supelco, Bellefonte, PA). Two solvents, A, water (0.5% TFA), and B, acetonitrile (0.5% TFA), were used for elution. The elution profile for HPLC consisted of initial conditions with 90% A and 10% B followed by linear gradient elution for the next 10 min to 14% B, isocratic elution (10−14 min), and the subsequent linear gradient conditions: 14−18 min (to 16% B), 18−22 min (to 18% B), 22−26 min (to 23% B), 26−31 min (to 28% B), and 31−32 min (to 40% B), isocratic elution 32−40 min (40% B), and final linear gradient elution 43−46 min (to 10% B). The flow rate was 1.0 mL/min, and aliquots of 15 μL were injected with an Agilent 1100 series micro autosampler. The UV/vis absorption spectra were recorded on-line during HPLC analysis over the wavelength range 240−600 nm in steps of 2 nm. The following data were recorded on-line for **8**−**10** during HPLC analyses. **8**: Vis_{max} 523 nm, UV_{max} 283 nm, 329 nm, t_R 33.1 min. **9**: Vis_{max} 525 nm, UV_{max} 283 nm, 319 nm, t_R 34.8 min.**10**: Vis_{max} 522 nm, UV_{max} 283 nm, 314 nm, t_R 35.6 min.

NMR Spectroscopy. One-dimensional ^1H, compensated attached proton test (CAPT), 2D heteronuclear single quantum coherence (^1H−^{13}C HSQC), heteronuclear multiple bond correlation (^1H−^{13}C HMBC), double quantum filtered correlation (^1H−^1H DQF-COSY), and total correlation spectroscopy (^1H−^1H TOCSY) experiments were obtained at 600.13 and 150.90 MHz for ^1H and ^{13}C respectively, on a Bruker 600 MHz instrument (Fallanden, Switzerland) equipped with a cryogenic probe. Sample temperatures were stabilized at 298 K. The deuteriomethyl ^{13}C signal and the residual ^1H signal of the solvent (CF_3-CO_2D−CD_3OD; 5:95, v/v) were used as secondary references (δ 49.0 and δ 3.40 from TMS, respectively).

LC−**MS.** High-resolution LC−MS (ESI^+/TOF) spectra were recorded using a JMS-T100LC instrument with an AccuTOF LP mass separator (Jeol, Tokyo, Japan). The gradient used was identical 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 100 mm × 2.0 mm internal diameter, 3.0 μm Develosil C18 column (Phenomenex, Torrance, CA) was used for separation. **3**: *m*/*z* 449.1106 $[M]^+$, *m*/*z* 287.0582 $[M\text{-}162]^+$. **4**: *m*/*z* 595.1688 $[M]^+$, *m*/*z* 287.0578. **5**: *m*/*z* 463.1259 $[M]^+$, *m*/*z* 301.0735. **8**: *m*/*z* 611.1379 $[M]^+$. **9**: *m*/*z* 595.1405 $[M]^+$. **10**: *m*/*z* 595.1418 $[M]^+$.

Quantitative Determination. The berries were freeze-dried and ground. Each pulverized sample (1 g) was weighed and placed into a 15 mL screw-cap glass container and extracted with 5 mL of acidified methanol (0.5% TFA) with magnetic stirring for 2 h followed by centrifugation at 3000*g* for 5 min. The supernatant was removed and stored in a sealed glass tube in the freezer (−20 °C). This procedure was repeated twice. The combined supernatant was transferred into a volumetric flask to determine the total volume followed by HPLC

Table 1. Qualitative and Quantitative Anthocyanin Content of Berries in Different *Ribes* Species and Cultivars

	anthocyanins[a]											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	total An[b] (DW)	total AAn[c] (DW)	total An[d] (FW)
R. grosssularia												
"Samsø"			0.29	0.58	0.02	0.03	t	0.43	0.56	1.91	0.99	22.92
			(15.1)	(30.4)	(1.1)	(1.6)		(22.5)	(29.3)		(51.8)	
"Hinnonmäki Red"			0.40	0.47	0.85	0.01	0.01	0.42	0.43	1.74	0.85	20.88
			(23.0)	(27.0)	(48.9)	(0.6)	(0.6)	(24.1)	(24.7)		(48.9)	
"Taastrup"			0.45	0.38	0.02	0.04	t	0.20	0.64	1.73	0.84	20.76
			(26.0)	(21.9)	(1.2)	(2.3)		(11.6)	(37.0)		(48.6)	
"Lofthus"			0.23	0.32	0.01	0.03	t	0.40	0.39	1.38	0.78	16.56
			(16.7)	(23.2)	(0.7)	(2.2)		(28.9)	(28.3)		(57.3)	
"Glendale"			1.10	0.41	0.02	t	0.01	0.13	0.56	2.23	0.69	26.76
			(49.3)	(18.4)	(0.9)		(0.5)	(5.8)	(25.1)		(30.9)	
"Larell"			0.46	0.60	0.02	0.04	t	0.22	0.31	1.65	0.53	19.80
			(27.9)	(36.4)	(1.2)	(2.4)		(13.3)	(18.8)		(32.1)	
"Rosko"			0.95	0.44	t	t	t	0.06	0.42	1.87	0.48	22.44
			(50.8)	(23.5)				(3.2)	(22.5)		(25.7)	
"Scania"			0.24	0.56		t	t	0.03	0.31	1.14	0.34	13.68
			(21.1)	(49.1)				(2.6)	(27.2)		(29.8)	
"Rolanda"			0.22	1.21		0.16	t	0.12	0.17	1.88	0.29	22.56
			(11.7)	(64.4)		(8.5)		(6.4)	(9.0)		(15.4)	
"Agro"			0.24	0.09	0.02	0.02	t	0.04	0.25	0.66	0.29	7.92
			(36.4)	(13.6)	(3.0)	(3.0)		(6.1)	(37.9)		(43.9)	
"John's"			0.17	0.14	0.03	0.04	t	0.03	0.11	0.52	0.14	6.24
			(32.6)	(26.9)	(5.8)	(7.7)		(5.8)	(21.2)		(26.9)	
"Martlet"	t	t	0.17	0.07	0.01	0.01	t	0.10	0.18	0.54	0.28	6.48
			(31.5)	(12.9)	(1.9)	(1.9)		(18.5)	(33.3)		(51.9)	
"Rokula"			0.26	1.00		0.13	t	0.09	0.13	1.61	0.22	19.32
			(16.2)	(62.0)		(8.1)		(5.6)	(8.1)		(13.7)	
"Pax"			0.08	0.12	0.01	0.02	t	0.01	0.06	0.30	0.07	3.60
			(26.7)	(40.0)	(3.3)	(6.7)		(3.3)	(20.0)		(23.3)	
R. × nidigrolaria												
"Josta"	0.30	0.58	0.44	2.20		0.01		0.06	0.22	3.81	0.28	45.72
	(7.9)	(15.2)	(11.5)	(57.7)		(0.3)	t	(1.6)	(5.8)		(7.4)	
"Jostine"	0.24	0.51	0.37	2.07		t	t	0.01	0.13	3.33	0.14	39.96
	(7.2)	(15.3)	(11.1)	(62.2)				(0.3)	(3.9)		(4.2)	
R. sanguineum	1.27	2.17	4.68	17.25		0.02	t		0.30	25.69	0.30	308.28
	(5.0)	(8.5)	(18.2)	(67.0)		(0.1)			(1.2)		(1.2)	
R. aureum	t		4.61	9.55		t	t			14.16		169.92
			(33.0)	(67.0)								
R. alpinum			0.34	0.04					t	0.38		4.56
			(89.0)	(11.0)								

[a] Anthocyanins (An) (**1**–**8** and **10**) in mg An/g dry weight (DW); relative proportions (%) are given in brackets. **9** has tentatively been identified in minor amounts (<2%) in all *R. grosssularia* and *R. × nidigrolaria* cultivars. See **Figure 1** for structures. [b] Total amount in mg An/g DW. [c] Acylated anthocyanins (AAn) in mg AAn/g DW; relative proportions (%) are given in brackets. [d] Total amount in mg An/100 g fresh weight (FW) based on 88% water content in samples (*67*); *t* = trace amount ≤ 0.009 mg/g DW.

analysis. Prior to injection, the solutions were filtered through a 0.45 μm Millipore membrane filter.

The quantitative amounts of each of the anthocyanins, **1**−**10**, were determined from a HPLC calibration curve of pure (>95%, determined by HPLC−DAD/NMR standardization) cyanidin 3-galactoside isolated from *Aronia melanocarpa*, without taking into account the variation of molar absorption coefficients for individual pigments. The calibration curve ($C_{anthocyanin}$ = 1.16 (± 0.02) × 10^{-4} area, R-Sq = 99.8%) was based on HPLC profiles recorded for four different pigment concentrations and three parallel injections of each concentration. A Statistical significance of 5% ($p < 0.05$) was chosen, and a Student's *t* test (Minitab) was performed. The results are presented as milligrams of cyanidin 3-*O*-β-galactoside equivalents per gram of dried weight (DW) (**Table 1**).

RESULTS AND DISCUSSION

Structure Elucidation of Pigments 8 and 10. In the ^{1}H NMR spectrum of **8**, eleven signals were located in the aromatic region; a singlet at δ 8.99 (H-4), two 2H metacoupled protons at δ 6.63 (*d*, 1.9 Hz; H-6) and δ 6.89 (*d*, 1.9 Hz; H-8), and an AMX system at δ 8.09 (*d*, 2.3 Hz; H-2′), δ 8.32 (*dd*, 2.3 Hz, 8.7 Hz; H-6′), and δ 7.09 (*d*, 8.7 Hz; H-5′) were in accordance with a cyanidin derivative (**Table 2**). The doublets at δ 6.99 (*d*, 1.8 Hz; H-2‴), δ 6.26 (*d*, 15.9 Hz; H-α), and δ 7.45 (*d*, 15.9 Hz; H-β) together with the multiplets at δ 6.84 (H-5‴) and δ 6.86 (H-6‴) were in accordance with a caffeoyl unit. The coupling constants of H-α and H-β (15.9 Hz) of the caffeoyl moiety revealed the *E*-configuration. The cross-peaks at δ 8.99/95.2 (H-4/C-8), δ 8.99/112.8 (H-4/C-10), δ 8.99/121.2 (H-4/C-1′), δ 8.99/145.1 (H-4/C-3), δ 8.99/157.7 (H-4/C-9), δ 8.99/158.6 (H-4/C-5), and δ 8.99/164.5 (H-4/C-2) and the $^1J_{CH}$ correlation δ 8.99/137.1 (H-4/C-4) in the HMBC spectrum of **8** were among the cross-peaks used to assign C-8, C-10, C-1′, C-3, C-9, C-5, C-2, and C-4. The other carbons belonging to the anthocyanidin B-ring were assigned by the cross-peaks at δ 8.32/118.4 (H-6′/C-2′), δ 8.32/155.8 (H-6′/C-4′), δ 8.32/147.5 (H-6′/C-3′), δ 8.32/117.4 (H-6′/C-5′), δ 8.09/121.2 (H-2′/C-1′), δ 8.09/128.3 (H-2′/C-6′), δ 8.09/147.5 (H-2′/C-3′), δ 8.09/155.8 (H-2′/C-4′), and the $^1J_{CH}$ correlation δ 8.07/164.5 (H-2′/C-2′).

The sugar region of **8** showed the presence of one sugar unit. The ^{1}H and ^{13}C resonances of the monosaccharide were assigned

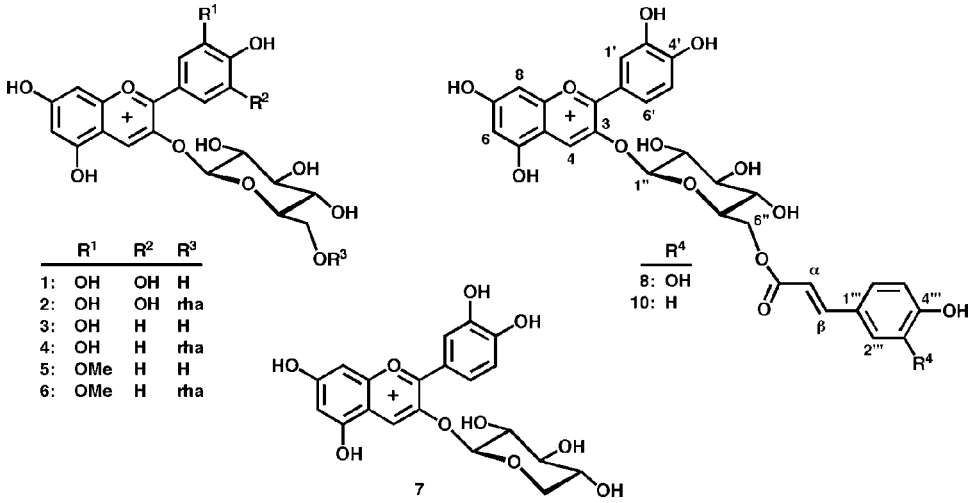

Figure 1. Structures of the anthocyanins identified in the examined *Ribes* species. **1**: delphinidin 3-*O*-β-glucopyranoside, **2**: delphinidin 3-*O*-β-(6″-*O*-α-rhamnosylglucopyranoside), **3**: cyanidin 3-*O*-β-glucopyranoside, **4**: cyanidin 3-*O*-β-(6″-*O*-α-rhamnosylglucopyranoside), **5**: peonidin 3-*O*-β-glucopyranoside, **6**: peonidin 3-*O*-β-(6″-*O*-α-rhamnosylglucopyranoside), **7**: cyanidin 3-*O*-β-xylopyranoside, **8**: cyanidin 3-*O*-β-(6″-*E*-caffeoylglucopyranoside), **10**: cyanidin 3-*O*-β-(6″-*E*-*p*-coumaroylglucopyranoside). **9** has tentatively been identified as cyanidin 3-*O*-β-(6″-*Z*-*p*-coumaroylglucopyranoside).

Table 2. ^{1}H (600.13 MHz) and ^{13}C (150.90 MHz) NMR Data for Cyanidin 3-*O*-β-(6″-*E*-Caffeoylglucopyranoside) (**8**) and Cyanidin 3-*O*-β-(6″-*E*-*p*-Coumaroylglucopyranoside) (**10**) in CF_3CO_2D–CD_3OD (5:95, v/v) at 25 °C

	^{1}H δ (ppm), *J* (Hz)		^{13}C δ (ppm)	
	8	**10**	**8**	**10**
2			164.54	164.03
3			145.08	144.71
4	8.99	8.94	137.11	136.71
5			158.60[a]	158.34
6	6.63 *d* 1.9	6.60 *d* 1.9	103.64	103.41
7			170.62	170.41
8	6.89 *d* 1.9	6.82 *d* 1.9	95.18	95.04
9			157.73	157.61
10			112.81[a]	112.99
1′			121.22	120.99
2′	8.09 *d* 2.3	8.07 *d* 2.3	118.36	118.27
3′			147.48	147.09
4′			155.82	155.54
5′	7.09 *d* 8.7	7.07 *d* 8.7	117.39	115.34
6′	8.32 *dd* 2.3; 8.7	8.27 *dd* 2.3; 8.7	128.31	128.09
1″	5.41 *d* 7.7	5.39[b]	103.18	102.91
2″	3.79	3.82 *dd* 2.1; 12.1	74.70	74.61
3″	3.66	3.69	77.88	77.72
4″	3.56	3.58 *dd* 9.8; 9.2	71.70	71.55
5″	3.92	3.92	76.06	75.84
6A″	4.61	4.61 *dd* 2.1; 12.1	64.42	64.53
6B″	4.45	4.45 *dd* 7.7; 12.1	64.42	64.53
	6″-*E*-caffeoyl	6″-*E*-*p*-coumaroyl		
1‴			127.57	126.60
2‴	6.99 *d* 1.8	7.36 *d* 8.6	115.39	
3‴		6.86 *d* 8.6	146.75	
4‴			149.62	131.00
5‴	6.84 *m*	6.86 *d* 8.6	116.46	116.64
6‴	6.86 *m*	7.36 *d* 8.6	122.83	161.06
α	6.26 *d* 15.9	6.31 *d* 15.9	114.63	114.20
β	7.45 *d* 15.9	7.51 *d* 15.9	147.19	146.85
C=O			168.96	168.8

[a] ^{13}C chemical shift value from the HMBC spectrum. [b] Chemical shift value from the COSY spectrum. Anomeric signal was overlapped by the water signal.

by a combination of the 1D ^{1}H NMR, DQF-COSY, TOCSY, and HSQC (**Figure 2**) experiments (**Table 2**). The ^{1}H−^{1}H coupling constants and the six ^{13}C resonances in the sugar region of the ^{13}C spectrum of **8** were in accordance with β-glucopyranose (*36*). The cross-peak at 5.41/145.1 (H-1″/C-3) in the HMBC spectrum confirmed the linkage between the aglycone and the sugar unit to be at the 3-hydroxyl. The downfield chemical shift values of H-6A″ (δ 4.61), H-6B″ (δ 4.45), and C-6″ (δ 64.4) indicated that the caffeoyl group was attached to the 6″-hydroxyl. The cross-peaks at δ 4.61/169.0 (H-6A″/C=O caffeoyl) and δ 4.45/169.0 (H-6B″/C=O caffeoyl) confirming the linkage between the 3-glucose and caffeoyl moiety to be at the 6″-hydroxyl and the molecular ion at *m*/*z* 611.1379 in the high-resolution MS spectrum were in accordance with cyanidin 3-*O*-β-(6″-*E*-caffeoylglucopyranoside). Even though this pigment has been partially characterized in *Orobanche minor* (*37*), *Mimulus cardinalis* (*38*), grapes (*39*), *Chrysanthemum corollas* (*40*), *Ligustrum* spp (*41*), *Ipomoea nil* (*42*), and gooseberry (*32*), the anomeric configuration, the ring size of the β-glucopyranoside, and the 6″-linkage position of the caffeoyl moiety have not previously been properly determined.

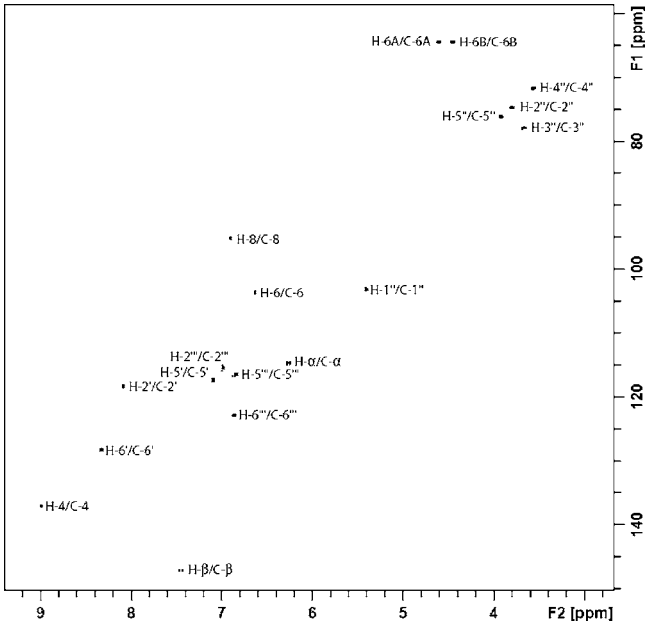

Figure 2. HSQC NMR spectrum of cyanidin 3-*O*-β-(6″-*E*-caffeoylglucopyranoside) (**8**) isolated from gooseberry showing labeled $^1J_{CH}$ cross-peaks.

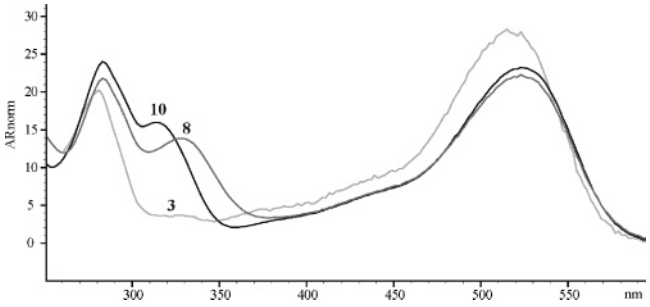

Figure 3. UV/vis spectrum of cyanidin 3-*O*-*β*-glucopyranoside (**3**), cyanidin 3-*O*-*β*-(6″-*E*-caffeoylglucopyranoside) (**8**), and cyanidin 3-*O*-*β*-(6″-*E*-*p*-coumaroylglucopyranoside) (**10**) recorded on-line during HPLC. ARnorm = spectra are presented in the area normalized scale.

The NMR resonances of pigment **10** shared many similarities with the corresponding resonances of **8** (**Table 2**). However, the chemical shift values at *δ* 7.36 (*d*, 8.6 Hz; H-2‴,6‴), *δ* 6.86 (*d*, 8.6 Hz; H-3‴,5‴), *δ* 6.31 (*d*, 15.9 Hz; H-α), and *δ* 7.51 (*d*, 15.9 Hz; H-*β*) were in accordance with a *p*-coumaroyl unit and not a caffeoyl unit as for pigment **8**. The coupling constants of H-α and H-*β* (15.9 Hz) of the *p*-coumaroyl also revealed the *E*-configuration of the aromatic acid, and the cross-peaks at *δ* 4.61/168.8 (H-6A″/C═O *p*-coumaroyl) and *δ* 4.45/168.8 (H-6B″/C═O *p*-coumaroyl) confirmed the linkage between the 3-glucose and coumaroyl moiety to be at the 6″-hydroxyl. A molecular ion at *m*/*z* 595.1418 in the high-resolution MS spectrum confirmed the identity of **10** to be cyanidin 3-*O*-*β*-(6″-*E*-*p*-coumaroylglucopyranoside). Besides being present in several grapes and grape products, this pigment has also been isolated from *Camellia* species (*43*) and black currant (*44*). However, **10**, as far as we know, has not previously been supplied with ^{13}C NMR data to support the determination of the pyranose form of the sugar moiety.

Anthocyanins in Alpine Currant, Golden Currant, and Red Flowering Currant. The HPLC profiles of the acidified methanolic extracts of alpine currant (*R. alpinum*) (**Figure 4**), golden currant (*R. aureum*), and red flowering currant (*R. sanguineum*) revealed **3** and **4** (**Figure 1**) as the major anthocyanins (**Table 1**). The pigments were co-chromatographed with the standards cyanidin 3-glucoside and cyanidin 3-rutinoside purified from black currant (*44*, *45*). The identities of these pigments were confirmed by UV/vis absorption spectra (**Figure 3**) and by their molecular ions and fragment ions in the electrospray ionization (ESI)−MS spectra.

Red flowering currant contained in addition smaller amounts of pigments **1**, **2**, **6**, **7**, and **10 (Table 1)**. The structures of **1** and **2** were characterized as delphinidin 3-glucoside and delphinidin 3-rutinoside, respectively, by co-chromatography with authentic pigments from black currant (*44*, *45*). Pigments **6** and **7** were determined to be peonidin 3-rutinoside and cyanidin 3-xyloside by UV/vis spectra and co-chromatography with authentic pigments from black currant (*44*, *45*) and chokeberry (*46*), respectively. Pigment **10** was identified as cyanidin 3-(6″-*E*-*p*-coumaroylglucoside) by co-chromatography (HPLC) with authentic pigment isolated from gooseberries. Golden currant contained in addition trace amounts of **1**, **6**, and **7**, while trace amounts of **10** were detected in alpine currant (**Table 1**).

Anthocyanins in Gooseberry and Jostaberry Cultivars. All the examined European gooseberry (*R. grossularia*) cultivars showed nearly the same qualitative anthocyanin content including pigments **3**−**10** (**Table 1**). Pigment **5,** peonidin 3-glucoside, was not detected in "Rokula", "Rolanda", and "Scania", while trace amounts of the 3-glucoside and 3-rutinoside of delphinidin (**1** and **2**) were found in "Martlet". Peonidin 3-glucoside, **5**, cyanidin 3-*O*-*β*-(6″-*E*-caffeoylglucopyranoside), **8**, and cyanidin 3-*O*-*β*-(6″-*E*-*p*-coumaroylglucopyranoside), **10**, have previously only tentatively been assigned in gooseberries (*30*, *32*). Pigment **9** was tentatively identified as cyanidin 3-(6″-*Z*-*p*-coumaroylglucoside) based on high-resolution MS data (*m*/*z* 595.1405) and on-line HPLC. This new pigment occurred in minor amounts (<2%) in all *R. grosssularia* and *R.* × *nidigrolaria* cultivars. Elucidation of the structures of **8** and **10** have been described

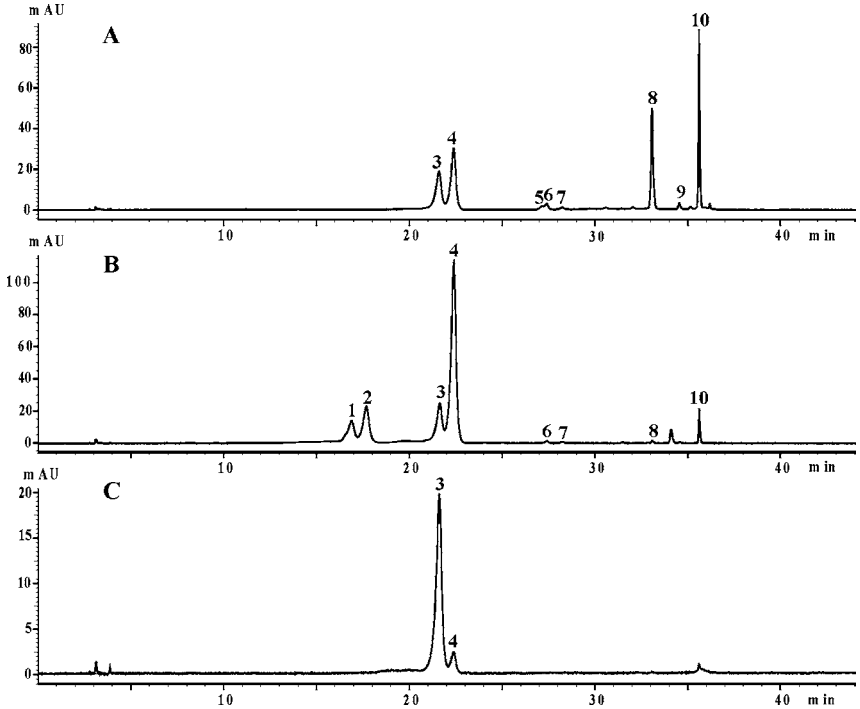

Figure 4. HPLC chromatograms detected at 520 ± 20 nm showing the anthocyanin content in (**A**) gooseberry cultivar "Samsø", (**B**) jostaberry cultivar "Josta", and (**C**) alpine currant. See **Figure 1** for peak identification.

earlier in this study, while **5** co-chromatographed with authentic peonidin 3-glucoside isolated from black rice (*47*).

The major anthocyanins of the two jostaberry (*Ribes* × *nidigrolaria*) cultivars "Josta" (**Figure 4**) and "Jostine" (**1**−**4**, **8**, and **10**) (**Table 1**) reflected the major anthocyanins of both of their original parents, gooseberry and black currant (*44*, *45*). The gooseberry cultivar "Martlet" showed a similar qualitative anthocyanin content as the two jostaberry cultivars; however, the quantitative content is somewhat different (**Table 2**).

Quantitative Content of Examined *Ribes* Species and Cultivars. The total anthocyanin content in the examined gooseberry cultivars varied from 0.30 mg/g DW in "Pax" to 2.23 mg/g DW in "Glendale" (**Table 1**). Assuming the water content of the berries to be 88% (*48*), the anthocyanin amounts in "Pax" and "Glendale" corresponded to 3.6 and 26.8 mg/100 g fresh weight (FW), respectively. Wu et al. (*48*) have previously reported that the total anthocyanin content in the gooseberry varieties "Whinham", "Lancashine", "Dan's Mistake", and "Careless" ranged from 0.7 to 10.2 mg/100 g FW, while Moyer et al. (*49*) similarly reported 14 mg/100 g FW in berries of "Captivator". When considering the other *Ribes* species examined in our survey, both *R. aureum* and *R. sanguineum* had a relatively high anthocyanin content of 169.9 and 308.3 mg/100 g FW, respectively, while *R. alpinum* contained only 4.6 mg/100 g FW (**Table 1**). The two jostaberry cultivars, which are hybrids between gooseberry and black currant, had higher total anthocyanin content (40.0 and 45.7 mg/100 g FW) than the gooseberriesbut considerably lower content than that previously reported for black currant (*48*, *50*, *51*).

The anthocyanin content in commercially available berries such as strawberry, red currant, and black currant have been reported to be 15−41, 13−18, and 130−500 mg/100 g FW, respectively (*48*, *50*, *51*). The gooseberry cultivars with the highest anthocyanin content (**Table 1**) are thus in the range of red currant and strawberry. However, it is not just the total anthocyanin level that may have significance for health benefits, as individual anthocyanins vary in their biological activity. When considering the amounts of individual anthocyanins in the samples examined (**Table 1**), several of the gooseberry cultivars contained relatively high amounts of the aromatic acylated pigments **8** and **10**. In "Lofthus", "Samsø" (**Figure 4**), "Martlet", "Hinnonmäki Red", and "Taastrup", these pigments together constituted as much as 57%, 52%, 52%, 49%, and 49% of the total anthocyanin content, respectively. The relative proportions of aromatic acylated anthocyanins in the two jostaberry cultivars were, however, rather small (4% and 7%) (**Table 1**).

Gooseberries have less commercial importance compared to, for instance, their relatives black currants and red currants. However, aromatic acylated anthocyanins are considered to be more stable than nonacylated anthocyanins (*13*−*15*, *17*, *18*). As far as we know, no commercially available berries have previously been reported to contain such high proportions of anthocyanins acylated with aromatic acids as reported in **Table 1**. When the total amount of acylated anthocyanins in the various cultivars are considered, "Samsø", "Hinnomäki Red", "Taastrup", "Lofthus", and "Glendal" are the most obvious candidates for consumption, colorant, and breeding programs.

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