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Cyanidin 3-(2″,3″-digalloylglucoside) from red leaves of *Acer platanoides*

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Abstract

The novel diacylated anthocyanin, cyanidin 3-(2″,3″-digalloyl-β-glucopyranoside), (3%) in addition to the known compounds cyanidin 3-(2″-galloyl-β-glucopyranoside) (37%) and cyanidin 3-β-glucopyranoside (60%) were isolated from the red leaves of *Acer platanoides* "Crimson King". The structures were determined mainly on the basis of homo- and heteronuclear two-dimensional nuclear magnetic resonance spectroscopy. This is the first report on a digalloylated anthocyanin.

Keywords: *Acer platanoides* "Crimson King"; Aceraceae; Red leaves; Anthocyanins; Galloylglucosides; Cyanidin 3-(2″,3″-digalloylglucoside); ^{13}C NMR

1. Introduction

The genus *Acer* (Aceraceae) consists of approx. 200 species widely distributed in the Northern hemisphere with a distribution centre in China (Ji, Yokoi, Saito & Mao, 1992a). Several cultivars with red leaves have ornamental importance. Robinson and Robinson (1932) have reported a cyanidin 3-monoside in the red autumn leaves of *Acer dissectum, A. palmatum* and *A. campestre*. Hattori and Hayashi (1937) identified cyanidin 3-glucoside as the main anthocyanin in autumn leaves of *A. ornatum, A. circumlobatum* and *A. sieboldianum*, while Ishikura (1973) found two anthocyanins, cyanidin 3-glucoside and cyanidin 3-rutinoside, as the major anthocyanins of red spring leaves of *A. palmatum* and *A. buergerianum*. More recently, Ji et al. (1992a) and Ji, Saito, Yokoi, Shigihara and Honda (1992b) in a comprehensive survey of spring sprouted and/or autumn coloured leaves of 119 *Acer* found in different proportions 3-(2″-galloylglucoside) and 3-(2″-galloylrutinoside) of cyanidin in addition to cyanidin 3,5-diglucoside and the 3-glucosides and 3-rutinosides of cyanidin and delphinidin. In *Dipteronia sinensis* the 3-glucoside, 3-rutinoside, 3,5-diglucoside and 3-galloylglucoside of cyanidin were found (Ji et al., 1992b), while cyanidin 3-(2″-xylosyl-6″-rhamnosylglucoside) was detected in brown-red spring leaves of *A. macrophyllum* (Ji, Yokoi, Saito, Ueda, Shigihara & Honda, 1995). In this paper we report the isolation and structure elucidation of a novel digalloylated anthocyanin in addition to two known anthocyanins from the red spring leaves of *A. platanoides* "Crimson King".

2. Results and discussion

The HPLC chromatogram of the methanolic extract of the red spring leaves of *A. platanoides* "Crimson King" detected in the visible spectral region, showed two major, **1**–**2**, and one minor anthocyanin, **3**. Pigments **1**–**3** were purified by partition against ethyl acetate followed by Amberlite XAD-7 column chromatography. The pigments were separated by Sephadex LH-20 column chromatography. The pure anthocya-

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Table 1
Chromatographic and spectral data of cyanidin 3-*O*-*β*-glucopyranoside (**1**), cyanidin 3-*O*-(2″-*O*-galloyl-*β*-glucopyranoside) (**2**) and cyanidin 3-*O*-(2″,3″-di-*O*-galloyl-*β*-glucopyranoside) (**3**)

	TLC (R_f)		UV-vis spectroscopy			
Compound	FHW	BAW	Vis-max (nm)	$A_{440}/A_{\text{Vis-max}}$ (%)	$A_{280}/A_{\text{Vis-max}}$ (%)	HPLC (t_R) (min)
1	0.32	0.28	528	24	69	8.72
2	0.57	0.63	530	22	109	9.79
3	0.46	0.72	531	29	196	10.90

nins were checked for homogeneity by analytical HPLC (Table 1). The UV-vis spectra of **1**–**3** showed visible maxima around 530 nm with A_{440}/A_{530} values ranging from 22 to 29% indicating 3-substituted cyanidin or peonidin derivatives, however there were significant differences in the $A_{\text{UV-max}}/A_{530}$ values of **1–3** (Table 1).

The downfield region of the ^{1}H NMR spectrum of **1** showed a singlet at δ 8.85 (H-4), a 3H AMX system at δ 8.09 (*dd*, 8.7 Hz, 2.3 Hz, H-6′), 7.86 (*d*, 2.3 Hz, H-2′) and 6.88 (*d*, 8.7 Hz, H-5′), and an AB system at δ 6.76 (*d*, 1.8 Hz, H-8) and 6.61 (*d*, 1.8 Hz, H-6), which is in accordance with a cyanidin derivative. The sugar region showed the presence of only one sugar unit. The anomeric coupling constant (7.7 Hz) and the six ^{13}C resonances in the sugar region of the ^{13}C SEFT spectrum of **1** were in accordance with *β*-glucopyranose. The ^{1}H sugar resonances (Table 2) were assigned by the 2D HSC spectrum. Pigment **1** co-chromatographed (TLC and HPLC) with authentic cyanidin 3-*O*-*β*-glucopyranoside.

The UV-vis spectrum of pigment **2** showed an A_{UV}/A_{530} value of 109% indicating cyanidin or peonidin 3-glycoside acylated with an aromatic acid. The ^{1}H NMR spectrum of **2** revealed, in addition to the signals of **1**, a 2H singlet at δ 7.10. The identification of the acyl moiety was determined to be gallic acid by the five additional ^{13}C resonances (SEFT spectrum) compared to the corresponding spectrum of **1** (Table 3). The pronounced downfield shift of H-2″ (δ 5.47) confirmed the linkage between the sugar and the acyl moiety, and thus confirming the identity of **2** to be cyanidin 3-*O*-(2″-*O*-galloyl-*β*-glucopyranoside).

The UV-vis spectrum of pigment **3** showed a very high A_{UV}/A_{531} of 196%. The relatively high mobility of **3** in both aqueous and in alcoholic TLC systems

Table 2
^{1}H NMR spectral data for cyanidin 3-*O*-*β*-glucopyranoside (**1**), cyanidin 3-*O*-(2″-*O*-galloyl-*β*-glucopyranoside) (**2**) and cyanidin 3-*O*-(2″,3″-di-*O*-galloyl-*β*-glucopyranoside) (**3**) in CD_3OD–CF_3COOD (19:1, v/v) at 25°C

	1, δ (ppm) *J* (Hz)	**2**, δ (ppm) *J* (Hz)	**3**, δ (ppm) *J* (Hz)
Cyanidin			
4	8.85 *s*	9.03 *s*	9.16 *s*
6	6.61 *d*, 1.8	6.71 *d*, 1.9	6.75 *d*, 2.0
8	6.75 *d*, 1.8	6.83 *d*, 1.9	6.94 *d*, 2.0
2′	7.86 *d*, 2.3	7.77 *d*, 2.3	7.86 *d*, 2.3
5′	6.88 *d*, 8.7	6.77 *d*, 8.7	6.83 *d*, 8.4
6′	8.09 *dd*, 8.7, 2.3	7.95 *dd*, 8.6, 2.3	8.04 *dd*, 8.4, 2.3
3-*O*-*β*-*glucoside*			
1″	5.35 *d*, 7.7	5.66 *d*, 7.9	5.87 *d*, 7.7
2″	3.78 *m*	5.47 *dd*, 7.9, 9.1	5.69 *dd*, 7.9, 9.7
3″	4.11 *m*	3.89 *t*, 9.2	5.64 *t*, 9.4
4″	4.15 *m*	3.69 *t*, 9.4	3.99 *t*, 9.4
5″	4.04 *m*	3.78 *m*, 9.7, 2.1	3.93 *m*
6A″	4.00 *m*	4.12 *dd*, 1.9, 12.2	4.09 *dd*, unresolved
6B″	3.93 *dd*, 5.5, 12.1	4.47 *dd*, 6.0, 12.2	3.92 *m*
2″-galloyl			
2‴, 6‴		7.10 *s*	6.97 *s*
3″-galloyl			
2⁗, 6⁗			7.12 *s*

Table 3
^{13}C NMR spectral data of cyanidin 3-*O*-*β*-glucopyranoside (**1**), cyanidin 3-*O*-(2″-*O*-galloyl-*β*-glucopyranoside) (**2**) and cyanidin 3-*O*-(2″,3″-di-*O*-galloyl-*β*-glucopyranoside) (**3**) in CD_3OD–CF_3COOD (19:1, v/v) at 25°C

	1, δ (ppm)	**2**, δ (ppm)	**3**, δ (ppm)
Aglycone			
2	162.95	163.73	164.42
3	145.33	145.08	145.10
4	135.94	136.19	136.76
5	159.05[a]	159.17[a]	159.37
6	103.46	103.40	103.62
7	170.42	170.45	170.65
8	95.17	95.14	94.93
9	157.13[a]	157.41[a]	157.78
10	113.10	113.12	113.15
1′	120.85	120.90	120.83
2′	118.15	117.59	117.71
3′	147.11	147.37	147.58
4′	155.65	155.74	155.88
5′	117.38	116.19	117.31
6′	128.17	128.48	128.50
3-*O*-*β*-glucopyranoside			
1″	103.46	101.68	101.61
2″	74.70	74.65	72.84
3″	78.09	76.11	76.61
4″	70.95	71.30	69.39
5″	78.63	79.02	79.06
6″	62.25	62.29	62.05
2″-galloyl			
1‴		120.71	120.27
2‴,6‴		110.57	110.57
3‴,5‴		146.32	146.28
4‴		140.07	140.29
7‴		167.50	167.11
3″-galloyl			
1⁗			120.94
2⁗,6⁗			110.50
3⁗,5⁗			146.37
4⁗			140.04
7⁗			167.81

[a] Assignments with the same superscript may be reversed.

and long retention time on ODS-HPLC column compared to pigment **1** (Table 1), were in accordance with an anthocyanin with aromatic acylation. The ^{1}H NMR spectrum of **3** showed, in addition to the signals of **1**, two 2H singlets at δ 7.12 and δ 6.97. Each of the 2H singlets showed in the long-range HMBC spectrum crosspeaks to 5 different carbons. The identification of the acyl moiety was confirmed to be gallic acid (two units) by the number and shifts of the ^{13}C resonances in the SEFT spectrum (Table 3). All the sugar ^{1}H resonances of **3** were assigned by the 2D COSY spectrum (Table 2). The pronounced downfield shifts of H-2″ (δ 5.69) and H-3″ (δ 5.64) indicated the linkages between the sugar and the acyl moieties. The crosspeaks at δ 5.69/166.80 (H-2″/C-7‴) and δ 5.64/167.63 (H-3″/C-7⁗) in the HMBC spectrum confirmed the linkages between the gallic acids and the sugar. The crosspeak at 5.90/144.8 (H-1″/C-3) showed the linkage between the sugar and the aglycone. Thus, **3** was determined to be the novel pigment cyanidin 3-*O*-(2″,3″-di-*O*-galloyl-*β*-glucopyranoside).

The occurrence of gallic acid as acyl moiety of anthocyanins has previously been recorded for the 3-(2″-galloylglucoside) and 3-(2″-galloylrutinoside) in *Aceraceae* (Ji et al., 1992a,b), and in six different anthocyanins from Nymphaeaceae containing 2″-galloylgalactoside (Strack, Wray, Metzger & Grosse, 1992; Fossen, Larsen & Andersen, 1998; Fossen & Andersen, 1999). Cyanidin 3-*O*-(2″,3″-di-*O*-galloyl-*β*-glucopyranoside), **3**, is the first identification of an anthocyanin diacylated with gallic acid. The flavonols kaempferol 3-(2″,6″-digalloylglucoside) and quercetin 3-(2″,6″-digalloylglucoside) have recently been isolated from *Loropetalum chinense* (Liu et al., 1997) and *A. okamotoanum* (Kim, Woo, Shin & Park, 1998), respectively. Pigment **3** contains two acyl groups on the same monosaccharide, however, with none connected to the sugar 6-position, contrary to reports on other diacylated anthocyanins.

3. Experimental

3.1. Plant material

Red spring leaves of *Acer* were collected in Bergen in May 1998. A voucher specimen has been deposited in Department of Chemistry, University of Bergen.

3.2. Isolation of pigments

Red leaves (400 g) were cut into pieces and extracted with 1% TFA in MeOH. The filtered extract was concd under red. pres., purified by partition (several times) against EtOAc and applied to an Amberlite XAD-7 column (Andersen, 1988). The anthocyanins were separated by Sephadex LH-20 column using stepwise gradient elution [from MeOH–TFA–H_2O; 19.8:0.2:80.0 (v/v) to MeOH–TFA–H_2O; 59.4:0.6:40.0 (v/v)].

3.3. Analytical chromatography

TLC was carried out on microcrystalline cellulose (F 5565, Merck) with the solvents BAW (1-BuOH–HOAc–H_2O; 4:1:5 v/v, upper phase) and FHW (HCO_2H–conc. HCl–H_2O; 1:1:2 v/v). Analyt. HPLC was performed with an ODS-Hypersil column (20 × 0.5 cm, 5 mm) using the solvents HCOOH–H_2O (1:9) (A) and HCOOH–H_2O–MeOH (1:4:5) (B). The elution profile consisted of a linear gradient from 10% B to 100% B for 17 min, followed by linear gradient

from 100% B to 10% B for 1 min. The flow rate was 1.2 ml min^{-1}, and aliquots of 15 μl were injected.

3.4. Spectroscopy

UV-vis absorption spectra were recorded in 0.1% conc. HCl in MeOH. The relative quantitative data were recorded on-line during HPLC analysis using a photodiode array detector (HP 1050, Hewlett–Packard). They were determined on the basis of average values of the absorptions on every second nanometer between 500 and 540 nm, without the different molar absorption coefficients of the pigments taking into account. The NMR experiments on pigment **3** (^{1}H NMR, DQF-COSY, HMBC, HSQC, SEFT) and **1** and **2** (^{1}H NMR, SEFT) were obtained at 600.13 and 150.92 MHz for ^{1}H and ^{13}C respectively, on a Bruker DRX-600 instrument at 25°C. The 2D HSC experiments on pigment **1** and **2** were obtained at 400.13 and 100.62 MHz for ^{1}H and ^{13}C respectively. The deuteriomethyl ^{13}C signal and the residual ^{1}H signal of the solvent (CF_3CO_2D–CD_3OD; 1:19, v/v) were used as secondary references (δ 49.0 and δ 3.4 from TMS, respectively).

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