Phytochemistry 71 (2010) 1558–1563

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Anthocyanins with unusual furanose sugar (apiose) from leaves of *Synadenium grantii* (Euphorbiaceae)

Øyvind M. Andersen [a,*], Monica Jordheim [a], Robert Byamukama [b], Angella Mbabazi [b], George Ogweng [b], Irene Skaar [a], Bernard Kiremire [b]

[a] Department of Chemistry, University of Bergen, Allégt. 41, 5007 Bergen, Norway
[b] Chemistry Department, Makerere University, P.O. Box 7062, Kampala, Uganda

ARTICLE INFO

Article history:
Received 20 March 2010
Received in revised form 7 May 2010
Available online 30 June 2010

Keywords:
Synadenium grantii
African milk bush
Anthocyanins
Apiose
β-apiofuranosyl-β-xylopyranoside
2D NMR
High resolution electrospray MS

ABSTRACT

Four anthocyanins, cyanidin 3-*O*-(2″-(5‴-(*E*-*p*-coumaroyl)-β-apiofuranosyl)-β-xylopyranoside)-5-*O*-β-glucopyranoside, cyanidin 3-*O*-(2″-(5‴-(*E*-*p*-coumaroyl)-β-apiofuranosyl)-β-xylopyranoside), cyanidin 3-*O*-(2″-(5‴-(*E*-caffeoyl)-β-apiofuranosyl)-β-xylopyranoside) and cyanidin 3-*O*-(2″-(5‴-(*E*-feroyl)-β-apiofuranosyl)-β-xylopyranoside) were isolated from leaves of African milk bush, (*Synadenium grantii* Hook, Euphorbiaceae) together with the known cyanidin 3-*O*-β-xylopyranoside-5-*O*-β-glucopyranoside and cyanidin 3-*O*-β-xyloside. The four former pigments are the first reported anthocyanins containing the monosaccharide apiose, and the three 5‴-cinnamoyl derivative-2″-(β-apiosyl)-β-xyloside subunits have previously not been reported for any compound.

1. Introduction

Anthocyanins have covalent linkage(s) between aglycone (anthocyanidin) and sugar(s), and in many cases between sugar(s) and acyl group(s) (Andersen and Jordheim, 2006). Some anthocyanins are associated with metal ions, co-pigments, etc. Most of the 650 anthocyanins, which previously have been reported, contain one, two or three monosaccharide units (Andersen and Jordheim, 2010). However, as many as seven units have been found in ternatin A1 (*Clitoria ternatea*) (Terahara et al., 1990) and cyanodelphin (*Delphinium hybridum*) (Kondo et al., 1991). The monosaccharides are represented by the hexoses glucose, galactose, rhamnose and glucuronic acid, and the pentoses arabinose and xylose connected through an *O*-linkage to the aglycone 3- and sometimes also to the 5-, 7-, 3′-, 4′- or 5′-hydroxyl groups. Two cyanidin 3-*O*-glucosides having an 8-*C*-β-glucopyranosyl unit have been isolated from purple flowers of *Tricyrtis formosana* cultivar Fujimusume (Liliaceae) (Saito et al., 2003; Tatsuzawa et al., 2004), and eight 3-deoxyanthocyanidin 6-*C*/8-*C* glycosides have recently been made from their respective flavone 6-*C*-glycosides (Bjorøy et al., 2009). Glucosyl moieties are widely distributed occurring in more than 90% of the various anthocyanins, while the pentose moieties, xylosyl and arabinosyl, are restricted to 75 and 12 anthocyanins, respectively (Andersen and Jordheim, unpublished database).

In our continuing survey of anthocyanins in the East-African flora, an investigation of the anthocyanin content of *Synadenium grantii* 'Hook' (Euphorbiaceae), called African milk bush, a shrub commonly found growing as hedges, was carried out. To our knowledge there is no report of the anthocyanin content of this species, however, immunoregulatory (Rogerio et al., 2007), fibrinolytic (Rajesha et al., 2006), and antitumoral (Premaratna et al., 1981) properties have been reported in association with chemical constituents of the genus *Synadenium*. In this paper, we report isolation and structural elucidation of six anthocyanins of the leaves, of which four were found to contain the pentose sugar apiose on its furanosyl form. This is the first report on anthocyanins containing the monosaccharide apiosyl.

2. Results and discussion

The HPLC analysis revealed the presence of two major (**3**, **5**) and several minor anthocyanins (**1**, **2**, **4**, **6**) in extracts of leaves of the African milk bush. The individual anthocyanins (Fig. 1) were isolated using combinations of Sephadex LH-20 column chromatography and reversed phase preparative HPLC, and further purified on small scale Toyopearl HW-40F columns. Compounds **1** and **2** showed UV-visible spectra (Table 1) typical for cyanidin 3,5-diglycoside and cyanidin 3-glycoside, respectively, with molecular ions

* Corresponding author. Tel.: +47 55 583 460; fax: +47 55 589 490.
E-mail address: oyvind.andersen@kj.uib.no (Ø.M. Andersen).

0031-9422/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2010.05.025

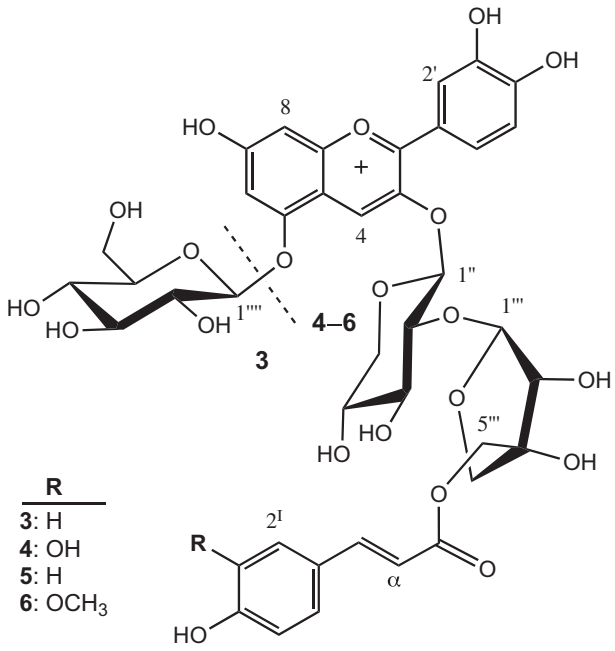

Fig. 1. Structure of anthocyanins **3**–**6**.

at *m*/*z* 581.1523 and 419.0984 (Table 1) in their high resolution ESI–MS spectra, in accordance with cyanidin 3-xyloside-5-glucoside (**1**) and cyanidin 3-xyloside (**2**). Their NMR assignments are shown in 4.4 and 4.5. The identity of **2** was confirmed by co-chromatography with authentic pigment from chokeberry (Strigl et al., 1995). Pigment **1** has previously been reported to occur in castor, *Ricinus communis* (Byamukama et al., 2008), also family Euphorbiaceae.

The UV-visible spectra of **3**–**6** showed local $\lambda_{UV\text{-}max}$ around 320 nm, and bathochromic shifts of the $\lambda_{Vis\text{-}max}$ to 529–531 nm compared to corresponding values of **1** and **2** (515 and 519 nm), indicating aromatic acylation of the former. The 1D ^{1}H NMR spectrum of **5** showed in the downfield area ten proton signals. Three of these at δ 8.87 (d, J = 0.6 Hz, H-4), δ 6.73 (d, J = 1.9 Hz, H-6), and δ 6.79 (dd, J = 0.6, 1.9 Hz, H-8) belonged to the AMX-system of the flavylium A- and C-rings of the aglycone cyanidin, while the three protons at δ 8.22 (dd, J = 2.3, 8.7 Hz, H-6′), δ 8.01 (d, J = 2.3 Hz, H-2′) and δ 7.07 (d, J = 8.7 Hz, H-5′) were caused by the AMX-system of the flavylium B-ring. The rest of the downfield ^{1}H NMR signals were due to coupled aromatic proton resonances at δ 7.38 (2H, d, J = 8.6 Hz) and δ 6.87 (2H, d, J = 8.6 Hz), and the coupled olefinic resonances at δ 7.32 (1H, d, J = 15.9 Hz) and δ 6.12 (1H, d, J = 15.7 Hz). These latter signals together with resonances for the two quaternary carbons at δ 126.5 (C-1^I) and δ 161.4 (C-4^I), and the ester carbonyl carbon at δ 168.6 showed the presence of one *p*-coumaroyl moiety. The *trans*-configuration of the double bond of this acyl moiety was established by the coupling constant (15.9 Hz) of the olefinic protons.

The NMR spectra showed the presence of two sugar moieties. Using results from the DQF-COSY, TOCSY and HSQC NMR spectra, the anomeric proton at δ 5.55 (d, J = 7.1 Hz, H-1″) was found to belong to a sugar unit with six protons and five carbons. The HMBC correlation peak between H-5A″ and C-1″ at δ 4.08/101.7, the relative large axial-axial coupling constants of neighbouring protons, and the chemical shifts of the carbons of this pentose (Table 2) were in agreement with a β-xylopyranosyl. A cross-peak at δ 5.55/144.9 in the HMBC spectrum showed that this pentose was connected to the 3-position of the aglycone. This linkage was further supported by the NOESY cross-peak observed from the anomeric proton at δ 5.55 to the cyanidin H-4 proton at δ 8.87.

The second sugar residue showed NMR signals (Table 2) corresponding to two methine and two methylene groups, in addition to a quaternary carbon, in agreement with the pentose moiety, apiosyl (Wang et al., 2006; Stochmal et al., 2009). The HMBC correlation peaks of H-1‴ with C-3‴ and C-4‴ indicated a pentofuranosyl substituent. In addition, the high-field chemical shifts of H-5A‴ (δ 4.31) and H-5B‴ (δ 4.10) along with the HMBC correlation peaks of these protons (Fig. 2), showed that the apiofuranosyl was further substituted at position 5‴. The β-anomeric configuration for the apiofuranosyl was supported by the anomeric signal at δ_C 109.9 with $^3J_{HH}$ = 1.2 Hz (Kitagawa et al., 1993; Ishii and Yanagisawa, 1998; Wang et al., 2006; Costantino et al., 2008; Stochmal et al., 2009) and $^1J_{CH}$ = 177.9 Hz. The NOESY spectrum of **5** exhibited correlation between the anomeric proton signal of apiosyl at δ 5.65 and H-2″ of xylosyl at δ 4.05, and the cross-peak at δ 5.65/77.5 in the HMBC spectrum showed that the apiosyl was connected to the 2″-position of the xylosyl. Similarly, cross-peaks in the HMBC spectrum at δ 4.31/168.6 (H-5A‴/C=O^I) and 4.10/168.6 (H-5B‴/C=O^I) revealed the linkage of the *p*-coumaroyl group to be the 5‴-position of the apiosyl. There are very good correlations between the chemical shifts and coupling constants of **5** (Table 2) and corresponding signals recorded recently in a similar solvent (CD_3OD) for an analogous 5‴-acyl-β-apiofuranosyl moiety linked in the 2″-position of xylose (Stochmal et al., 2009). The HR–ESI–MS spectrum of **5** showed a molecular ion at *m*/*z* 697.1785 corresponding to the empirical formula $C_{34}H_{33}O_{16}$ (calc. 697.1763 amu) in agreement with the new compound cyanidin 3-*O*-(2″-(5‴-(*E*-*p*-coumaroyl)-β-apiofuranosyl)-β-xylopyranoside) (Fig. 1).

The NMR resonances of pigment **3** shared many similarities with the corresponding resonances of **5** (Table 2). However, the additional presence of a third sugar with an anomeric signal at δ 5.30 (d, J = 7.8 Hz, H-1⁗) and seven protons and six carbons were in accordance with an extra hexose. The ^{13}C NMR chemical shifts, the large axial–axial coupling constants (Table 2), and the HMBC correlation peak between H-1⁗ and C-5⁗ at δ 5.30/78.3 indentified this sugar moiety as a β-glucopyranosyl found to be connected to the 5-position of the aglycone by the HMBC cross-peak at δ 5.30/158.9 between H-1⁗ and C-5. An $A_{440}/A_{vis\text{-}max}$ ratio of only 14%

Table 1
Chromatographic (HPLC) and spectral (UV–vis and MS) data recorded for anthocyanin **1–6** isolated from *Synadenium grantii*.

Compound	On-line HPLC					ESI–MS		Molecular formula
	Vis_{max} (nm)	Local UV_{max} (nm)	A_{440} $A_{vis\text{-}max}$ (%)	A_{320} $A_{vis\text{-}max}$ (%)	t_R (min)	M^+ *m*/*z* (observed)	M^+ *m*/*z* (calculated)	
1	515	277	17	7	19.86	581.1523	581.1506	$C_{26}H_{29}O_{15}^+$
2	519	291	30	8	28.66	419.0978	419.0984	$C_{20}H_{19}O_{10}^+$
3	530	281, 320	14	63	29.31	859.2297	859.2312	$C_{40}H_{43}O_{21}^+$
4	531	285, 333	30	63	31.15	713.1718	713.1699	$C_{34}H_{33}O_{17}^+$
5	530	285, 317	27	76	33.63	697.1785	697.1763	$C_{34}H_{33}O_{16}^+$
6	529	283, 319sh	30	82	34.02	727.1874	727.1857	$C_{35}H_{35}O_{17}^+$

sh = shoulder.

(Table 1) for **3** was in accordance with a 3,5-diglycoside. The HR–ESI–MS spectrum of **3** showed a molecular ion at *m/z* 859.2312 corresponding to the empirical formula $C_{40}H_{43}O_{21}$ (calc. 859.2291 amu) in agreement with the new compound cyanidin 3-*O*-(2″-(5‴-(*E*-*p*-coumaroyl)-β-apiofuranosyl)-β-xylopyranoside)-5-*O*-β-glucopyranoside (Fig. 1).

Table 2
^{1}H NMR spectral data for **3–6** isolated from *Synadenium grantii* recorded in CF_3COOD–CD_3OD (5:95; v/v) at 25 °C. See Fig. 1 for structures.

	3	4	5	6
Aglycone				
4	8.97 d 0.6	8.88 d 0.7	8.87 d 0.6	8.86 d 0.8
6	7.11 d 1.9	6.73 d 2.0	6.73 d 1.9	6.74 d 1.9
8	6.97 dd 0.6, 1.9	6.81 dd 0.7, 2.0	6.79 dd 0.6, 1.9	6.80 dd 0.8, 1.9
2′	8.05 d 2.4	8.03 d 2.3	8.01 d 2.3	8.02 d 2.3
5′	7.09 d 8.7	7.08 d 8.7	7.07 d 8.7	7.08 d 8.7
6′	8.28 dd 2.4, 8.7	8.20 dd 2.3, 8.7	8.22 dd 2.3, 8.7	8.22 dd 2.3, 8.7
3-O-β-xylopyranoside				
1″	5.68 d 7.2	5.56 d 7.1	5.55 d 7.1	5.56 d 7.1
2″	4.08 dd 7.3, 8.9	4.06 dd 7.1, 8.8	4.05 dd 7.1, 8.6	4.06 dd 7.1, 8.6
3″	3.81 dd 8.6, 13.2	3.78 dd 8.8, 13.4	3.79 dd 8.6, 13.5	3.79 dd 8.6, 13.7
4″	3.75 ddd 4.4, 8.2, 13.2	3.74 ddd 4.2, 8.7, 13.4	3.74 ddd 4.8, 8.3, 13.5	3.74 ddd 4.7, 8.6, 13.7
5A″	4.09 dd 8.2, 11.2	4.08 dd 8.7, 11.5	4.08 dd 4.8, 11.4	4.09 m
5B″	3.58 dd 4.4, 11.2	3.58 dd 4.2, 11.5	3.57 dd 8.3, 11.4	3.57 dd 4.7, 11.3
2″-O-β-apiofuranosyl				
1‴	5.67 d 1.2	5.65 d 1.3	5.65 d 1.2	5.63 d 1.2
2‴	3.96 d 1.2	3.98 d 1.3	3.98 d 1.2	3.96 d 1.2
4A‴	3.86 d 9.7	3.82 d 9.7	3.82 d 9.7	3.83 d 9.5
4B‴	3.61 d 9.7	3.64 d 9.7	3.63 d 9.7	3.63 d 9.5
5A‴	4.31 d 11.6	4.31 d 11.5	4.31 d 11.5	4.30 d 11.5
5B‴	4.09 d 11.6	4.11 d 11.5	4.10 d 11.5	4.10 d 11.5
5‴-O-acyl				
2^I	7.36 d 8.7	6.99 d 2.0	7.38 d 8.6	7.08 d 1.8
3^I	6.87 d 8.7		6.87 d 8.6	
5^I	6.87 d 8.7	6.84 m	6.87 d 8.6	6.69 d
6^I	7.36 d 8.7	6.86 m	7.38 d 8.6	6.97 dd
α	6.09 d 15.9	6.06 d 15.9	6.12 d 15.9	6.46 d 15.9
β	7.27 d 15.9	7.26 d 15.9	7.32 d 15.9	7.75 d 15.9
OMe				3.98
5-O-β-glucopyranoside				
1⁗	5.30 d 7.8			
2⁗	3.76 dd 7.8, 9.1			
3⁗	3.67 dd 8.3, 9.1			
4⁗	3.56 dd 8.3, 9.2			
5⁗	3.69 m			
6A⁗	4.04 dd 2.5, 12.3			
6B⁗	3.83 dd 6.1, 12.3			

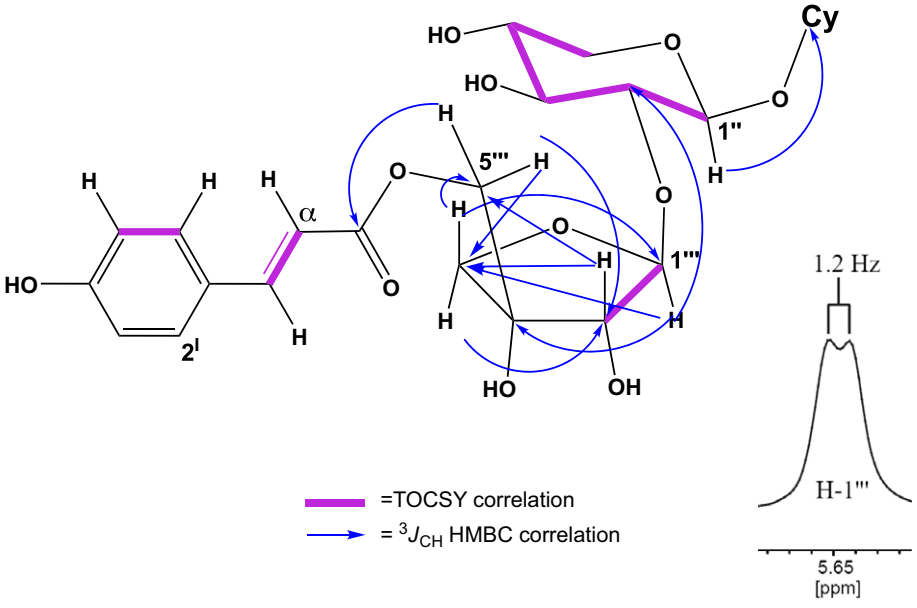

Fig. 2. *Left:* Highlighted NMR correlations observed in the ^{1}H–^{13}C HMBC and ^{1}H–^{1}H TOCSY spectra of pigment **5**. *Right*: Anomeric ^{1}H signal of the apiofuranosyl moiety of **5** showing $^3J_{H\text{-}1/H\text{-}2}$.

The NMR resonances of pigments **4** and **6** shared many similarities with the corresponding resonances of **5** (Tables 2 and 3). However, the cinnamic acyl moiety was identified to be *E*-caffeoyl and *E*-feroyl for **4** and **6**, respectively. The molecular ions in the respective HR–ESI–MS spectre (Table 1) confirmed the structures of the new anthocyanins, cyanidin 3-*O*-(2″-(5‴-(*E*-caffeoyl)-β-apiofuranosyl)-β-xylopyranoside) (**4**) and cyanidin 3-*O*-(2″-(5‴-(*E*-feroyl)-β-apiofuranosyl)-β-xylopyranoside) (**6**).

3. Concluding remarks

Cyanidin 3-*O*-(2″-(5‴-(*E*-*p*-coumaroyl)-β-apiofuranosyl)-β-xylopyranoside)-5-*O*-β-glucopyranoside (**3**), cyanidin 3-*O*-(2″-(5‴-(*E*-caffeoyl)-β-apiofuranosyl)-β-xylopyranoside) (**4**), cyanidin 3-*O*-(2″-(5‴-(*E*-*p*-coumaroyl)-β-apiofuranosyl)-β-xylopyranoside) (**5**), and cyanidin 3-*O*-(2″-(5‴-(*E*-feroyl)-β-apiofuranosyl)-β-xylopyranoside) (**6**) are the first reported anthocyanins containing the monosaccharide apiose. This pentose, which occurred in its furanosyl configuration, was linked as a disaccharide to xylose in its pyranosyl form. When the ring size of the monosaccharides of anthocyanins has been reported, the sugars have been identified in their pyranosyl forms, even for the pentoses. The only previous reported exemptions are three anthocyanins with similar structures isolated from the family Commelinaceae, where the arabinosyl moieties have been reported as furanosyls (Idaka et al., 1987; Baublis and Berber-Jimenez, 1995). Twelve different disaccharides have been identified in the 287 anthocyanins, which have been reported to contain a disaccharide moiety (Andersen and Jordheim, 2010). None of these disaccharides contain two pentose units, which were identified in pigments **3**–**6**. To the best of our knowledge, this is the first report of the three 5‴-cinnamoyl derivative-2″-(β-apiosyl)-β-xyloside subunit of any compound.

Table 3
^{13}C NMR spectral data for **3**–**6** isolated from *Synadenium grantii* recorded in CF_3COOD–CD_3OD (5:95; v/v) at 25 °C. See Fig. 1 for structures.

	3	4	5	6
Aglycone				
2	164.6	163.8	163.7	163.8
3	145.7	144.8	144.9	144.9
4	133.3	134.9	134.8	134.5
5	157.1	158.8	158.9	158.7
6	105.7	103.4	103.4	103.3
7	169.3	170.1	170.1	170.3
8	97.4	95.1	94.9	95.1
9	156.6	157.6	157.4	157.5
10	113.3	112.9	113.1	113.2
1′	121.1	121.2	121.1	121.1
2′	118.7	118.5	118.4	118.5
3′	147.5	147.2	147.5	147.4
4′	156.5	155.7	155.8	155.7
5′	117.4	117.2	117.2	117.3
6′	128.9	128.2	128.3	128.2
3-O-β-xylopyranoside				
1″	101.3	101.6	101.7	101.5
2″	77.1	77.2	77.5	77.5
3″	78.2	78.0	77.9	77.9
4″	70.6	70.9	70.9	70.8
5″	67.3	67.3	67.2	67.2
2″-O-β-apiofuranosyl				
1‴	109.8	109.9	109.9	110.3
2‴	78.3	78.4	78.2	78.4
3‴	79.0	78.7	78.9	78.9
4‴	74.7	74.6	74.7	74.6
5‴	67.8	67.5	67.7	67.6
5‴-O-acyl				
1^{I}	126.6	127.5	126.5	126.9
2^{I}	131.3	116.2	131.2	111.4
3^{I}	116.9	147.1	116.9	149.4
4^{I}	161.2	149.4	161.4	150.9
5^{I}	116.9	116.3	116.9	115.8
6^{I}	131.3	122.6	131.2	124.4
α	114.1	114.8	114.2	114.7
β	146.9	146.9	146.8	147.1
C=O	168.7	170.2	168.6	168.6
OMe				56.4
5-O-β-glucopyranoside				
1⁗	102.5			
2⁗	74.6			
3⁗	77.7			
4⁗	71.1			
5⁗	78.3			
6⁗	62.4			

4. Experimental

4.1. Isolation of anthocyanins

Leaves of *S. grantii* 'Hook' were collected in the Entomology department gardens in Kampala (Uganda) in July 2008. The identification of the plant was carried out in the Botany Department at Makerere University, and voucher specimen has been deposited in the herbarium of that Department, voucher No.RB15/2008. The leaves were weighed (900 g) and extracted for 8 h in 2 l of methanol containing TFA (0.5% v/v). The filtered extract was concentrated under reduced pressure at 27 °C, purified by partition (several times) against ethyl acetate and applied to an Amberlite XAD-7 column. The anthocyanins adsorbed to the column were washed with water, and eluted from the column with methanol containing 0.5% TFA. The concentrated anthocyanin extract was purified by Sephadex LH-20 chromatography using H_2O–MeOH–TFA (79.5:20:0.5, v/v) as eluent. Fifty-four fractions were collected, and fractions with similar qualitative anthocyanin contents revealed by HPLC were combined. In order to isolate higher quantities of the minor anthocyanins, more leaves (2.5 kg) of the same sample were collected in September 2009, and treated as described above.

Individual anthocyanins were separated by preparative HPLC using a Gilson 321 pump equipped with an Ultimate 3000 Variable Wavelength Detector, a 25 × 2.2 cm (10 μm) Econosphere C18 column (Grace, USA), and the solvents; A, water (0.5% TFA) and B, acetonitrile (0.5% TFA). 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 15 ml min^{-1}, and aliquots of 250 μl were injected.

Some of the separated anthocyanins were further purified before NMR analyses with a micro glass column (200 × 10 mm i.d.) packed with Toyopearl HW-40F (TOSHO) material, using H_2O–MeOH (90:10) containing 0.5% TFA.

4.2. Analytical HPLC

The Agilent 1100 HPLC system was equipped with a HP 1050 diode-array detector, and a 200 × 4.6 mm i.d., 5 μm ODS Hypersil column, (Supelco, Bellefonte, USA). 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^{-1}, and aliquots of 15 μl were injected with an Agilent 1100 Series, Micro Autosampler. Prior to injection, all samples were filtered through a 0.45 μm Millipore membrane filter. Anthocyanins isolated from blackcurrant (*Ribes nigrum*) (Frøytlog et al., 1998), black beans (*Phaseolus vulgaris* L.) (Takeoka et al., 1997) and gooseberries (*Ribes grossularia* L.) (Jordheim et al., 2007) were used as references.

4.3. Spectroscopy

UV–visible absorption spectra were recorded on-line during HPLC analysis (Table 1), and the spectral measurements were made over the wavelength range 200–600 nm in steps of 2 nm.

The NMR experiments were obtained at 600.13 MHz and 150.92 MHz for ^{1}H and ^{13}C, respectively, on a Bruker Biospin Ultrashield Plus AV-600 MHz instrument equipped with a TCI $^{1}H–^{13}C/^{15}N$ CryoProbehead at 298 K. The deuteriomethyl ^{13}C signal and the residual ^{1}H signal of the solvent, $CF_3COOD–CD_3OD$ (95:5; v/v), were used as secondary references (δ 49.0 and δ 3.4 ppm from TMS for ^{1}H and ^{13}C, respectively) (Andersen and Fossen, 2005). The NMR experiments ^{1}H, $^{1}H–^{13}C$ HSQC, $^{1}H–^{13}C$ HMBC, $^{1}H–^{1}H$ DQF–COSY, $^{1}H–^{1}H$ TOCSY and $^{1}H–^{1}H$ NOESY were recorded.

High resolution LC-electrospray mass spectrometry (ESI^{+}/TOF), spectra of **1**–**6** were recorded using a JEOL AccuTOF JMS-T100LC in combination with an Agilent Technologies 1200 Series HPLC system. A Zorbax SB-C18 (50 mm × 2.1 mm, length × i.d., 1.8 μm) column was used for separation, and combinations of two solvents were used for elution: A, H_2O containing 0.5% TFA (v/v) and B, acetonitrile containing 0.5% TFA (v/v). The following solvent composition was used: 0–1.25 min 10 to 22% B (linear gradient), 1.25–5 min 22–30% B (linear gradient), 5–7 min 30% B (isocratic), 7–8 min 30–40% B (linear gradient), 8–14 min 40% B (isocratic) and 14–15 min 40–10% B (linear gradient). The flow rate was 0.4 ml min^{-1}.

4.4. NMR data – cyanidin 3-O-β-xylopyranoside-5-O-β-glucopyranoside (*1*)

$^{1}H/^{13}C$ NMR chemical shifts (ppm) and coupling constants, *J* (Hz): H-4, 9.06 (d, 0.7)/134.9; H-6′, 8.42 (dd, 2.4, 8.8)/129.2; H-2′, 8.13 (d, 2.4)/118.5; H-5′, 7.11 (d, 8.8)/117.5; H-8, 7.16 (dd, 0.7, 2.0)/97.4; H-6, 7.13 (d, 2.0)/105.6; H-1″, 5.48 (d, 7.0)/103.9; H-2″, 3.79 (dd, 7.1, 8.6)/74.1; H-3″, 3.65 (dd, 8.6, 12.9)/77.8; H-4″, 3.75 (ddd, 4.9, 9.3, 12.9)/70.6; H-5A″, 4.11 (dd, 4.9, 11.5)/66.9; H-5B″, 3.60 (dd, 9.3, 11.5)/66.9; H-1‴, 5.28 (d, 7.8)/102.6; H-2‴, 3.74 (dd, 7.8, 9.2)/74.6; H-3‴, 3.66 (t, 9.2)/78.7; H-4‴, 3.55 (t, 9.5)/71.1; H-5‴, 3.65 (ddd, 2.2, 6.3, 8.9)/77.7; H-6A‴, 4.04 (dd, 2.2, 12.1)/62.4; H-6B‴, 3.84 (dd, 6.3, 12.1)/62.4. Quaternary C-atom: C-2, 164.9; C-3, 146.2; C-5, 156.9; C-7, 169.3; C-9, 156.5; C-10, 112.8; C-1′, 120.8; C-3′, 147.4; C-4′, 156.3. Aglycone-sugar linkages confirmed by cross-peaks in HMBC spectrum: H-1″/C-3, 5.34/145.3 and H-1‴/C-5, 5.28/156.9.

4.5. NMR data – cyanidin 3-O-β-xylopyranoside (*2*)

$^{1}H/^{13}C$ NMR chemical shifts (ppm) and coupling constants, *J* (Hz): H-4, 9.02 (d, 0.7)/136.8; H-6′, 8.37 (dd, 2.4, 8.7)/128.5; H-2′, 8.11 (d, 2.4)/118.4; H-5′, 7.10 (d, 8.7)/117.4; H-8, 6.98 (dd, 0.7, 1.9)/95.1; H-6, 6.75 (d, 1.9)/103.4; H-1″, 5.34 (d, 7.1)/104.5; H-2″, 3.77 (dd, 7.1, 8.7)/74.3; H-3″, 3.62 (dd, 8.7, 14.4)/77.3; H-4″, 3.74 (ddd, 5.1, 9.5, 14.4)/70.6; H-5A″, 4.12 (dd, 5.1, 11.5)/67.1; H-5B″, 3.54 (dd, 9.5, 11.5)/67.1. Quaternary C-atom: C-2, 164.5; C-3, 145.3; C-5, 158.8; C-7, 170.3; C-9, 157.5; C-10, 113.2; C-1′, 121.2; C-3′, 147.4; C-4′, 155.9. Aglycone-sugar linkage confirmed by cross-peak in HMBC spectrum: H-1″/C-3: 5.34/145.3.

Acknowledgements

The authors are grateful to Sectional Engineer Terje Lygre, Department of Chemistry, University of Bergen, for technical support during recording of the LC–MS spectra. The team at Makerere University gratefully acknowledges Carnegie Cooperation of New York for a fellowship.

References

Andersen, Ø.M., Fossen, T., 2005. Characterization of anthocyanins by NMR. In: Wrolstad, R.E. (Ed.), Handbook of Food Analytical Chemistry: Pigments, Colorants, Flavors, Texture, and Bioactive Food Components. John Wiley and Sons Inc., Hoboken, NJ, pp. 47–69.

Andersen, Ø.M., Jordheim, M., 2006. The anthocyanins. In: Andersen, Ø.M., Markham, K.R. (Eds.), Flavonoids: Chemistry, Biochemistry and Applications. CRC Press, Boca Raton, pp. 471–551.

Andersen, Ø.M., Jordheim, M., 2010. Chemistry of flavonoid-based colors in plants. In: Mander, L.N., Liu, H.W. (Eds.), Comprehensive Natural Products II: Chemistry and Biology, vol. 3. Elsevier, Oxford, pp. 547–614.

Baublis, A.J., Berber-Jimenez, M.D., 1995. Structural and conformational characterization of a stable anthocyanin from *Tradescantia pallida*. J. Agric. Food Chem. 43, 640–646.

Bjorøy, Ø., Rayyan, S., Fossen, T., Kalberg, K., Andersen, Ø.M., 2009. *C*-glycosylanthocyanidins synthesized from *C*-glycosylflavones. Phytochemistry 70, 278–287.

Byamukama, R., Jordheim, M., Kiremire, B., Andersen, Ø.M., 2008. New anthocyanins from stem bark of castor, *Ricinus communis*. Nat. Prod. Commun. 3, 1497–1500.

Costantino, V., Fattorusso, E., Imperatore, C., Mangoni, A., 2008. Glycolipids from Sponges. 20. (1) J-coupling analysis for stereochemical assignments in furanosides: structure elucidation of vesparioside B, a glycosphingolipid from the marine sponge Spheciospongia vesparia. J. Org. Chem 73, 6158–6165.

Frøytlog, C., Slimestad, R., Andersen, Ø.M., 1998. Combination of chromatographic techniques for the preparative isolation of anthocyanins - applied on blackcurrant (*Ribes nigrum*) fruits. J. Chromatogr. A 825, 89–95.

Idaka, E., Ohashi, Y., Ogawa, T., Kondo, T., Goto, T., 1987. Structure of zebrinin, a novel acylated anthocyanin isolated from *Zebrina pendula*. Tetrahedron Lett. 28, 1901–1904.

Ishii, T., Yanagisawa, M., 1998. Synthesis, separation and NMR spectral analysis of methyl apiofuranosides. Carbohydr. Res. 313, 189–192.

Jordheim, M., Måge, F., Andersen, Ø.M., 2007. Anthocyanins in berries of *Ribes* including gooseberry cultivars with a high content of acylated pigments. J. Agric. Food Chem. 55, 5529–5535.

Kitagawa, I., Hori, K., Uchida, E., Chen, W.Z., Yoshikawa, M., Ren, J., 1993. Saponin and sapogenol. L. On the constituents of the roots of *Glycyrrhiza uralensis* Fischer from Xinjiang, China. Chemical structures of licorice-saponin L3 and isoliquiritin apioside. Chem. Pharm. Bull 41, 1567–1572.

Kondo, T., Suzuki, K., Yoshida, K., Oki, K., Ueda, M., Isobe, M., Goto, T., 1991. Structure of cyanodelphin, a tetra-*p*-hydroxybenzoated anthocyanin from the blue flower of *Delphinium hybridum*. Tetrahedron Lett. 32, 6375–6378.

Premaratna, A., Shadaksharaswamy, M., Nanjappa, S., 1981. Isolation, purification and properties of a lectin from the latex of *Synadenium grantii* 'Hook' f. Ind. J. Biochem. Biophys. 18, 32–35.

Rajesha, R., Natarajua, A., Gowdaa, C.D.R., Freyb, B.M., Freyb, F.J., Vishwanath, B.S., 2006. Purification and characterization of 34-kDa, heat stable glycoprotein from *Synadenium grantii* latex: action on human fibrinogen and fibrin clot. Biochimie 88, 1313–1322.

Rogerio, A.P., Cardoso, C.R., Fontanari, C., Souza, M.A., Afonso-Cardoso, S.R., Silva, E.V.G., Koyama, N.S., Basei, F.L., Soares, E.G., Calixto, J.B., Stowell, S.R., Dias-Baruffi, M., Faccioli, L.H., 2007. Anti-asthmatic potential of a D-galactose-binding lectin from *Synadenium carinatum* latex. Glycobiology 17, 795–804.

Saito, N., Tatsuzawa, F., Miyoshi, K., Shigihara, A., Honda, T., 2003. The first isolation of *C*-glycosylanthocyanin from the flowers of *Tricyrtis formosana*. Tetrahedron Lett. 44, 6821–6823.

Stochmal, A., Kowalska, I., Janda, B., Perrone, A., Piacente, S., Oleszek, W., 2009. Gentisic acid conjugates of *Medicago truncatula* roots. Phytochemistry 70, 1272–1276.

Strigl, A.W., Leitner, E., Pfannhauser, W., 1995. Qualitative and quantitative analysis of the anthocyanins in black chokeberries (*Aronia melanocarpa* Michx. Ell.) by TLC, HPLC and UV/VIS-spectrometry. Z. Lebensm. Unters. For. 91, 177–180.

Takeoka, G.R., Dao, L.T., Full, G.H., Wong, R.Y., Harden, L.A., Edwards, R.H., Berrios, J.D., 1997. Characterization of black bean (*Phaseolus vulgaris* L.) anthocyanins. J. Agric. Food Chem 45, 3395–3400.

Tatsuzawa, F., Saito, N., Miyoshi, K., Shinoda, K., Shigihara, A., Honda, T., 2004. Diacylated 8-C-glucosylcyanidin 3-glucoside from the flowers of *Tricyrtis formosana*. Chem. Pharm. Bull. 52, 631–633.

Terahara, N., Saito, N., Honda, T., Toki, K., Osajima, Y., 1990. Structure of ternatin A1, the largest ternatin in the major blue anthocyanins from *Clitoria ternatea* flowers. Tetrahedron Lett. 31, 2921–2924.

Wang, J., Di, Y., Yang, X., Li, S., Wang, Y., Hao, X., 2006. Hydroquinone diglycoside acyl esters from the stems of *Glycosmis pentaphylla*. Phytochemistry 67, 486–491.