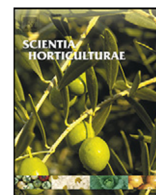
Scientia Horticulturae 129 (2011) 840–843

Contents lists available at ScienceDirect

Scientia Horticulturae

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



Anthocyanins from ornamental flowers of red frangipani, *Plumeria rubra*

Robert Byamukama[a,*], Jane Namukobe[a], Monica Jordheim[b], Øyvind M. Andersen[b], Bernard T. Kiremire[a]

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

ARTICLE INFO

Article history:
Received 13 December 2010
Received in revised form 15 March 2011
Accepted 18 March 2011

Keywords:
Red frangipani
Plumeria rubra L.
Apocynaceae
Ornamental colour
Anthocyanins
Cyanidin 3-O-β-(2″-glucopyranosyl-O-β-galactopyranoside)

ABSTRACT

Two anthocyanins were isolated from ornamental reddish flowers of *Plumeria rubra* L. (Apocynaceae) by a combination of chromatographic techniques. Their structures were elucidated mainly by the use of homo- and heteronuclear nuclear magnetic resonance spectroscopy and high-resolution electrospray mass spectrometry. The anthocyanin cyanidin 3-*O*-β-(2″-glucopyranosyl-*O*-β-galactopyranoside) (75%), has previously been isolated only from *Cornus suecica* (Cornaceae) fruits, while the other (20%) was identified as cyanidin-3-*O*-β-galactopyranoside. This is the first report of the anthocyanins responsible for the attractive colours of the flowers of red frangipani.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Plumeria rubra L. (Apocynaceae), also referred to as red frangipani (Gilman and Watson, 1994), is a gorgeous tropical tree with intensely fragrant flowers used for ornamental purposes. Its spiral-shaped, reddish blooms, which appear at the branch tips, are well known for their use in decorations such as leis and wreaths. Recently some iridoids have been isolated from flowers of *P. rubra* cv. *acutifolia* (Ye et al., 2008; Ye et al., 2009), and a non-characterized flavonoid from methanolic extract of flowers of *P. rubra* has shown antioxidant and hypolipidemic activity in alloxan induced hyperglycemic rats (Merina et al., 2010).

Anthocyanins give rise to most red to blue colours in plants (Andersen and Jordheim, 2006). No information has been reported about anthocyanins in *Plumeria*, except that they exist in flowers of this genus (Mell, 1928). In this paper we report isolation and structure elucidation of the pigments responsible for the attractive anthocyanic colours of the flowers of red frangipani (*P. rubra*).

2. Materials and methods

2.1. Plant material

Fresh flowers of *Plumeria rubra* were collected from the garden of Agakhan Secondary School compound in Kampala, Uganda in August 2009. The identification of the plants was carried out in the Botany department, Makerere University, and a voucher specimen has been deposited in the herbarium of the same Department, voucher number JN/02/2009. The flowers were weighed and kept in a freezer before extraction.

2.2. Extraction and isolation of the pigments

The flowers (120 g) were extracted with methanol (500 mL) containing trifluoroacetic acid, TFA (0.5%, v/v). After filtration and repeated extraction (250 mL), the combined extracts were concentrated under reduced pressure at 27 °C and purified by partition (several times) against ethyl acetate before application on an Amberlite XAD-7 column. The anthocyanins adsorbed to the column were washed with water, and eluted from the column with methanol containing TFA (1%, v/v). The anthocyanins were separated by Sephadex LH-20 column chromatography into two bands using H_2O–MeOH–TFA (49.5:50:0.5) as eluent. The two bands were further purified by Toyopearl HW-40F column chromatography using H_2O–MeOH–TFA (79.5:20:0.5) as eluent. The final clean up of pigment **1** was done by preparative HPLC (Gilson 305/306 pump equipped with a UV 6000LP detector) equipped with an ODS Hypersil column (25 cm × 2.2 cm; i.d. 5 μm). Two solvents were used for elution: A = HCO_2H–H_2O (1:9, v/v) and B = HCO_2H–H_2O–MeOH; (1:4:5, v/v/v). The elution profile consisted of a linear gradient from 10% to 100% B for 30 min, isocratic elution (100% B) for the next 10 min, followed by a linear gradient from 100% to 10% B for 1 min. The

* Corresponding author. Tel.: +256 392 548585; fax: +256 41 531061.
E-mail address: rbyamukama@chemistry.mak.ac.ug (R. Byamukama).

0304-4238/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2011.03.053

Table 1
Chromatographic (TLC and HPLC) and spectral (UV–Vis and MS) data recorded for the anthocyanins, **1** and **2**, isolated from red frangipani.

Compound	On-line (HPLC)			R_f (TLC)	ESI–MS		Molecular formula
	t_R (min)	Vis_{max} (nm)	$A_{440}/A_{Vis\text{-}max}$ (%)		M⁺ m/z observed	M⁺ m/z calculated	
1	15.14	520	33	0.65	611.1581	611.1612	$C_{27}H_{31}O_{16}$
2	19.41	521	36	0.46	449.1068	449.1084	$C_{21}H_{21}O_{11}$

See Fig. 1 for structures of **1** and **2**.

flow rate was 14 mL/min for 41 min, and aliquots of 250 μL were injected.

2.3. Chromatography

TLC was carried out on microcrystalline cellulose (F 5556, Merck) with the solvent FHW (HCO_2H–conc HCl–H_2O; 1:1:2, v/v/v) (Table 1). The HPLC instrument (HP-1050 module, system, Hewlett-Packard) was equipped with an ODS Hypersil column (25 cm × 0.46 cm, 5 μm). Two solvents; A, water with 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 elution for 10 min (14% B), isocratic elution 10–14 min, and the subsequent linear conditions; 18 min (16% B), 22 min (18% B), 26 min (23% B), 31 min (28% B) and 32 min (40% B), isocratic elution 32–40 min, and final linear elution 40–41 min (10% B). Aliquots of 15 μL were injected and the flow rate was 1 mL/min. Prior to injection, all samples were filtered through a 0.45 μm Millipore membrane filter.

2.4. Spectroscopy

The UV–Vis absorption spectra were recorded on-line during HPLC analysis, and the spectral measurements were made over the wavelength range 200–600 nm in steps of 2 nm (Table 1). Relative amounts of each anthocyanin are reported as percentages of total peak area in HPLC chromatograms based on absorptions recorded for every second nm between 500 and 540 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 3.40 ppm from TMS for ^{1}H and ^{13}C, respectively). The 1D ^{1}H NMR and the 2D Heteronuclear Single Quantum Coherence (^{1}H–^{13}C HSQC), Heteronuclear Multiple Bond Correlations (^{1}H–^{13}C HMBC), Double Quantum Filtered Correlation Spectroscopy (^{1}H–^{1}H DQF-COSY) and Total Correlation Spectroscopy (^{1}H–^{1}H TOCSY) experiments were recorded.

High-resolution LC-electrospray MS (ESI⁺/TOF) spectra of **1** and **2** were recorded using a JMS-T100LC with an AccuTOF LP mass separator (Table 1). 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 10–22% 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.

3. Results

The HPLC (detected at 520 ± 20 nm) and TLC chromatograms of the extract of red frangipani flowers showed two major anthocyanins, **1** and **2** (Table 1). Their UV–Vis spectra were similar showing λ_{max} around 520 nm with $A_{440}/A_{vis\text{-}max}$ ratios of 33% and 36%, respectively, indicating anthocyanins with 3-glycosylation based on anthocyanidins with two oxygen functions on their B-rings (Andersen, 1987).

The downfield part of the 1D ^{1}H NMR spectrum of **1** showed a doublet at 9.09 ppm (H-4), a 3H AMX system at 8.29 ppm (*dd*, 8.8 Hz, 2.4 Hz; H-6′), 8.17 ppm (*d*, 2.4 Hz; H-2′) and 7.15 ppm (*d*, 8.8 Hz; H-5′) and an unresolved 2H AB system at 6.98 ppm (H-8) and 6.75 ppm (H-6), respectively (Table 2), in accordance with the anthocyanidin, cyanidin. After the chemical shifts of the protons of the aglycone of **1** were assigned, the chemical shifts of the corresponding carbons were assigned from the HSQC experiment. The remaining quaternary C-atoms were assigned using the HMBC spectrum, which was optimized for $^2J_{CH}$ and $^3J_{CH}$ couplings (Table 2).

The sugar region of the 1D ^{1}H NMR of **1** showed the presence of two sugar units. Starting from H-1″ at δ 5.51 (*d*, J = 7.6 Hz), the observed cross-peak at 5.51/4.36 ppm in the DQF-COSY spectrum supported by corresponding cross peaks in the HSQC spectrum (Table 2), permitted the assignment of H-2″. The chain of coupled protons H-2″, H-3″, H-4″, H-5″ and H-6A″/H-6B″ were thereafter assigned similarly. In cases where several sugar protons showed similar chemical shifts, the assignment was assisted by the TOCSY experiment. The chemical shifts and the coupling constants of this glycosyl unit were in accordance with a β-galactopyranosyl (Table 2). A crosspeak at δ 5.51/145.1 in the HMBC spectrum between H-1″ and C-3 of the aglycone showed that the galactopyranosyl unit was connected to the 3-position of the aglycone (Fig. 1). By using the doublet at δ 4.78 (J = 7.7 Hz) as the starting point in the DQF-COSY spectrum together with the cross peaks in the TOCSY spectrum, it was likewise possible to assign all the chemical shifts for the second monosaccharide moiety, β-glucopyranosyl (Table 2). The linkage point between the two sugars was established to be at the galactosyl 2″-position by the cross peak between H-1‴ and C-2″ in the HMBC spectrum at δ 4.78/80.8 (Fig. 1). All the ^{13}C signals of the two sugar units were assigned from the HSQC experiment (Table 2). The most characteristic NMR values for this disaccharide are thus the downfield shifts of the 2-position of the galactosyl unit at 4.36 ppm (1H) and 80.82 ppm (13C), respectively. A molecular ion (M⁺) at m/z 611.1581 in the high-resolution ES-MS spectrum of **1** corresponding to the empirical formula $C_{27}H_{31}O_{16}$ (calc. 611.1612 amu) confirmed this structure to be cyanidin 3-*O*-β-(2″-glucopyranosyl-*O*-β-galactopyranoside) (Fig. 2).

The 1D and 2D NMR spectra of **2** showed many similarities with the corresponding spectra of **1**. However, the HSQC spectrum revealed only seven cross peaks in the sugar region in accordance with one monosaccharide. Based on assignments from homo- and heteronuclear NMR experiments (Table 2), pigment **2** was identified to be cyanidin-3-*O*-β-galactopyranoside (Fig. 2). A molecular ion (M⁺) at m/z 449.1068 in the high-resolution ES-MS spectrum of **2** corresponding to the empirical formula $C_{21}H_{21}O_{11}$ (calc. 449.1084 amu) confirmed this structure.

4. Discussion

The anthocyanin colouration of red frangipani flowers is based mainly on **1**, cyanidin 3-*O*-β-(2″-glucopyranosyl-*O*-β-galactopyranoside) (75%) and **2**, cyanidin-3-*O*-β-galactopyrano-

Table 2
^{1}H and ^{13}C NMR shift values (ppm) and proton–proton couplings (Hz) for cyanidin 3-*O*-β-(2″-glucopyranosyl-*O*-β-galactopyranoside) (**1**) and cyanidin 3-*O*-β-galactopyranoside (**2**), isolated from red flowers of *Plumeria rubra* and dissolved in CD_3OD–CF_3COOD (95:5, v/v) at 25 °C.

	1		**2**	
	^{1}H δ (ppm)	^{13}C δ (ppm)	^{1}H δ (ppm)	^{13}C δ (ppm)
Cyanidin				
2		164.12		164.10
3		145.04		145.04
4	9.09 *d* 0.9	136.63	9.11 *d* 0.9	136.70
5		158.66		158.98
6	6.75 *d* 1.9	103.29	6.74 *d* 1.9	103.34
7		169.88		170.02
8	6.98 *dd* 1.9, 0.9	95.08	6.98 *dd* 1.9, 0.9	95.01
9		157.29		157.37
10		112.77		113.08
1′		120.86		121.05
2′	8.17 *d* 2.4	118.75	8.17 *d* 2.4	118.70
3′		147.12		147.12
4′		155.42		155.41
5′	7.15 *d* 8.8	117.54	7.15 *d* 8.8	117.41
6′	8.29 *dd* 8.8, 2.4	128.04	8.30 *dd* 8.8, 2.4	128.04
3-*O*-β-galactopyranoside				
1″	5.51 *d* 7.6	102.33	5.35 *d* 7.6	104.45
2″	4.36 *dd* 7.6, 9.4	80.82	4.09 *dd* 7.6, 9.4	72.09
3″	3.97 *dd* 3.3, 9.4	74.69	3.98 *dd* 3.3, 9.4	74.67
4″	4.07 *dd* 3.3, 0.6	69.79	4.05 *dd* 3.3, 0.6	69.75
5″	3.89 *m*	77.43	3.90 *m*	77.43
6A″	3.87 *m* 1.9, 6.6, 12.3	62.23	3.87 *m* 1.9, 6.6, 12.3	62.24
6B″	3.87 *m*	62.23	3.87 *m*	62.23
2″-*O*-β-glucopyranosyl				
1‴	4.78 *d* 7.7	105.43		
2‴	3.27 *dd* 7.7, 9.3	75.09		
3‴	3.36 *t* 9.2	77.91		
4‴	3.30 *dd* 9.2, 9.7	71.02		
5‴	2.99 *m* 2.8, 4.9, 9.7, 11.6	77.88		
6A‴	3.48 *m* 2.8, 4.9, 11.8	62.19		
6B‴	3.48 *m*	62.19		

side (20%). Pigment **1** has previously been found only in scarlet fruits of *Cornus suecica* in Cornaceae (Slimestad and Andersen, 1998). When serological techniques have been employed in a systematic investigation of selected taxa of the Rubiaceae and selected putative relatives, it is interesting to note that presaturation tests using Rubiaceae antiserum revealed that the immunoprecipitating systems obtained with Apocynaceae, Asclepiadaceae and Gentianaceae were very similar to those systems obtained with the Cornaceae (Lee and Fairbrothers, 1978). However, pigment **1** has previously not been reported to occur in other *Cornus* species (Bjorøy et al., 2007). The disaccharide of **1**, 2″-glucopyranosyl-*O*-β-galactopyranose, has in fact a limited distribution as flavonoid moiety in nature. Apart from being present in pigment **1** in red frangipani flowers and *Cornus suecica* fruits, it has been found acylated in some anthocyanins isolated from flowers of *Pulsatilla cernua* (Yoshitama et al., 1998) and flowers of *Anemone coronaria* (Saito et al., 2002; Toki et al., 2003), both in Ranunculaceae.

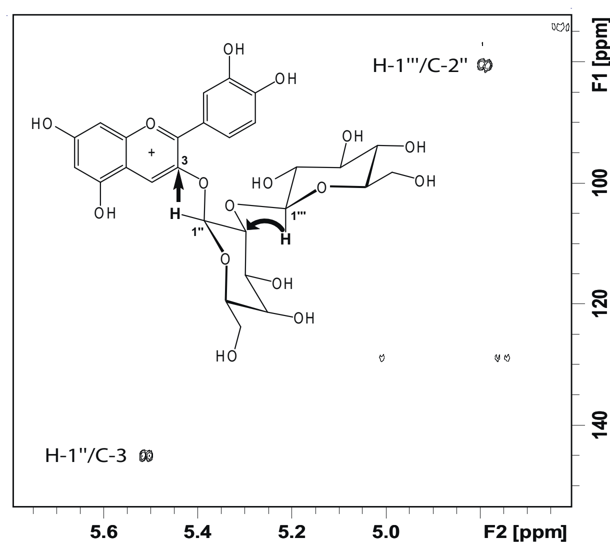


Fig. 1. Selected region of the ^{1}H–^{13}C HMBC NMR spectrum of **1**. The region shows the linkages between the galactosyl unit and cyanidin (H-1″/C-3), and between the glycosyl unit and galactosyl (H-1‴/C-2″). The structure highlights these correlations with black arrows.

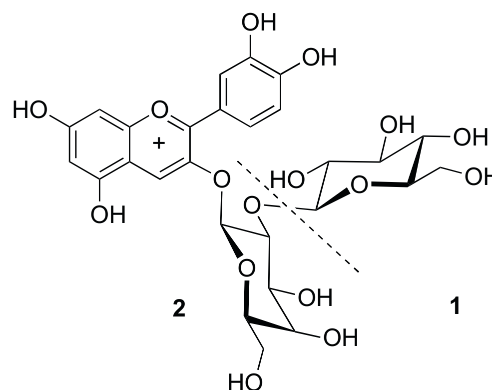


Fig. 2. The structures of the anthocyanins isolated from red frangipani flowers, cyanidin 3-*O*-β-(2″-glucopyranosyl-*O*-β-galactopyranoside) (**1**) and cyanidin 3-*O*-β-galactopyranoside (**2**).

Acknowledgements

We are grateful to sectional engineer Terje Lygre, Department of Chemistry, University of Bergen, for technical support during recording of the LC–MS spectra, and the Norwegian Council of Universities' Committee for Development Research and Education (NUFU) and Carnegie Corporation for financial support.

References

Andersen, Ø.M., 1987. Anthocyanins in fruits of *Vaccinium uliginosum* L. (Bog whortleberry). Journal of Food Sciences 665–666 (52), 680.

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. 471–553.

Bjorøy, Ø., Fossen, T., Andersen, Ø.M., 2007. Anthocyanin 3-galactosides from *Cornus alba* Sibirica with glucosidation of the B-ring. Phytochemistry 68, 640–645.

Gilman, E.F., Watson, D.G., 1994. Frangipani, Fact Sheet ST-491, A Series of the Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Lee, Y.S., Fairbrothers, D.E., 1978. Serological approaches to the systematics of the Rubiaceae and related families. Taxon 27, 159–185.

Mell, C.D., 1928. Interesting sources of natural dyestuffs. Textile Colorist 51, 453–455.

Merina, A.J., Sivanesan, D., Begum, V.H., Sulochana, N., 2010. Antioxidant and hypolipidemic effect of *Plumeria rubra* L. in alloxan induced hyperglycemic rats. E – Journal of Chemistry 7, 1–5.

Saito, N., Toki, K., Moriyama, H., Shigihara, A., Honda, T., 2002. Acylated anthocyanins from the blue-violet flowers of *Anemone coronaria*. Phytochemistry 60, 365–373.

Slimestad, R., Andersen, Ø.M., 1998. Cyanidin 3-(2-glucosylgalactoside) and other anthocyanins from fruits of *Cornus suecica*. Phytochemistry 49, 2163–2166.

Toki, K., Saito, N., Shigihara, A., Honda, T., 2003. Acylated cyanidin glycosides from the purple-red flowers of *Anemone coronaria*. Heterocycles 60, 345–350.

Ye, G., Li, Z.-X., Xia, G.-X., Peng, H., Sun, Z.-L., Huang, C.-G., 2009. A new iridoid alkaloid from the flowers of *Plumeria rubra* L. cv. acutifolia. Helvetica Chimica Acta 92, 2790–2794.

Ye, G., Yang, Y.-L., Xia, G.-X., Fan, M.-S., Huang, C.-G., 2008. Complete NMR spectral assignments of two new iridoid diastereoisomers from the flowers of *Plumeria rubra* L. cv. *acutifolia*. Magnetic Resonance in Chemistry 46, 1195–1197.

Yoshitama, K., Saeki, A., Iwata, T., Ishikura, N., Yahara, S., 1998. An acylated pelargonidin diglycoside from *Pulsatilla cernua*. Phytochemistry 47, 105–107.