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DELPHINIDIN 3-XYLOSYLRUTINOSIDE IN PETALS OF *LINUM GRANDIFLORUM*

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Key Word Index—*Linum grandiflorum*; Linaceae; anthocyanidin triglycoside; delphinidin 3-[2-(xylosyl)-6-(rhamnosyl)-glucoside]; scarlet flower colour.

Abstract—A new anthocyanidin triglycoside was isolated from the scarlet flowers of *Linum grandiflorum* cv Scarlet Flax as a major anthocyanin. The structure of this pigment was determined to be delphinidin 3-*O*-[2-*O*-(β-D-(xylopyranosyl)-6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside].

INTRODUCTION

Linum grandiflorum cv Scarlet Flax bears characteristic scarlet blooms. As no information on this species is available [1–4], we started to investigate the anthocyanins in the flowers of this cultivar, and unexpectedly isolated a new delphinidin triglycoside as the main anthocyanin.

Dubois and Harborne [4] isolated five anthocyanidin triglycosides; 3-glucosylrutinosides of pelargonidin, cyanidin and delphinidin, triglucosides of delphinidin and cyanidin, along with four biosides and three monosides from several colour mutants of *Linum usitatissimum*. However, no xylosylrutinosides of anthocyanidins were observed [3].

This paper deals with the structure elucidation of the delphinidin xylosylrutinoside from flowers of *Linum grandiflorum* cv Scarlet Flax.

RESULT AND DISCUSSION

We isolated the main anthocyanin, **1**, and two minor ones, **2** and **3**, from the scarlet flowers of *Linum grandiflorum* cv Scarlet Flax using a process similar to that described previously [5].

On acid hydrolysis, **1** yielded delphinidin, glucose, rhamnose and xylose, and alkaline hydrolysis of **1** gave no acyl group. The FAB-mass spectrum of **1** showed $[M]^+$ *m/z* 743, corresponding to $C_{32}H_{39}O_{20}$, which was composed of one molecule each of delphinidin, glucose, rhamnose and xylose. On partial acid hydrolysis of **1**, three intermediate anthocyanins were detected on converting to delphinidin. These three intermediates were isolated with PC and HPLC, and identified as delphinidin 3-glucoside, 3-rhamosylglucoside and 3-xylosylglucoside by standard analysis (Table 1) [6]. These data show that the structure of **1** is delphinidin 3-xylosylrhamnosylglucoside.

The detailed structure of **1** was elucidated by ^1H NMR spectral analysis as shown in Table 2. The proton signals of **1** were mainly assigned by ^1H–^1H COSY, and sugar linkages were confirmed by difference NOE. The characteristic signals in the low magnetic region were easily correlated to delphinidin nucleus protons. The signals of the sugar moieties were observed in the region of δ5.64–2.96. The signals of three anomeric protons appeared at δ5.64 (*d*, $J = 8.1$ Hz, glucose), 4.55 (*d*, $J = 8.1$ Hz, xylose) and 4.51 (*br s*, rhamnose). The observed vicinal coupling constants of the glucose and xylose units were J = ca 7–11 (Table 2). Therefore, both glucose and xylose units must be in the β-D-pyranose forms [7]. The rhamnose moiety was identified as the α-L form by proton analysis [7–9]. The position of attachment was confirmed by difference NOE (Fig. 1). The appearance of an NOE signal at H-4 of delphinidin by irradiation at H-1 of glucose indicated glucose to be

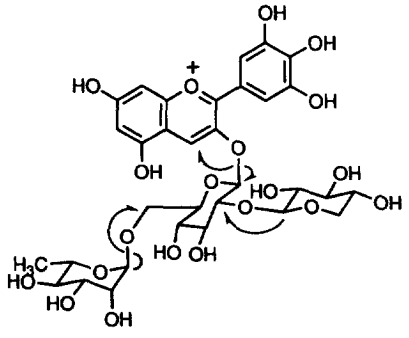

Fig. 1. Anthocyanin of scarlet flowers of *Linum grandiflorum*. Observed NOEs are indicated by arrows.

Table 1. Chromatographic and spectral properties of anthocyanins from scarlet flowers of *Linum grandiflorum*

Anthocyanin*	R_f values (×100)†				R_t† (min)	Spectral data in 0.1% HCl–MeOH			FAB-MS† $[M]^+$
	BAW	BuH	1% HCl	AHW		λ_{max}(nm)	E_{440}/E_{max}(%)	$AlCl_3$	
(1)	28	14	42	68	6.5	279, 544	17	+	743
Dp3GX‡	27	12	16	40	7.4	279, 542	17	+	—
(2)‡	24	9	6	22	9.1	279, 544	17	+	—
D_p3G	20	6	2	9	8.6	279, 542	17	+	—
(3)	32	17	11	33	13.5	282, 531	22	+	—

*Dp3GX, delphinidin 3-xylosylglucoside; Dp3G, delphinidin 3-glucoside.
†For key to abbreviations and analytical conditions, see Experimental.
‡Partial acid hydrolosis product of **1**.

Table 2. ^{1}H NMR spectral data of **1** (in TFA-*d*–DMSO-d_6, 1:9)

	Glucose*	Rhamnose*	Xylose*		Delphinidin
H-1	5.64 *d* (8.1)†	4.51 *br s*†	4.55 *d* (8.1)†	H-4	8.67 *s*†
H-2	3.98 *t* (8.1)†	3.55 *dd* (1.3, 3.4)	2.96 *t* (8.1)	H-6	6.69 *d* (2.1)
H-3	3.65 *t* (8.6)	3.42 *dd* (3.4, 9.4)	3.05 *t* (8.6)	H-8	6.80 *d* (1.7)
H-4	3.34 *m*	3.12 *m*	2.71 *t* (10.7)	H-2′, H-6′	7.73 *s*
H-5	3.47 *dd* (6.8, 11.6)†	3.32 *m*	3.11 *m*		
H-6a	3.78 *m*		3.22 *dd* (4.9, 10.7)		
H-6b	3.88 *d* (11.6)†				
Me		1.03 *d* (16)			

*Assigned by ^{1}H-^{1}H COSY.
†Assigned by DIFNOE.
Coupling constants (*J* in Hz) in parentheses.

attached to OH-3 of delphinidin through a glucosidic bond. Also, the presence of NOEs was observed between H-1 of rhamnose and H-6 of glucose, and H-1 of xylose and H-2 of glucose. Thus, rhamnose and xylose are attached to the OH-6 and OH-2 of glucose, respectively. Therefore, **1** is delphinidin 3-*O*-[2-*O*-(β-D-xylopyranosyl)-6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside]. The minor anthocyanins, **2** and **3**, were identified as the 3-rutinosides of delphinidin and cyanidin, respectively, by direct comparison with authentic samples (Table 1).

EXPERIMENTAL

Plant material. We obtained the seeds of *Linum grandiflorum* cv Scarlet Flax from Sakata Seed Co. Ltd, Yokohama, Japan and they were cultivated in a greenhouse of the experimental farm of Minami-Kyushu University. Fresh petals were collected and air-dried at 45°.

Extraction and isolation. The dried petals (22 g) were extracted with 5% HOAc at room temp. overnight. The filtered extract was adsorbed on Diaion HP-20, washed with 1% HOAc and eluted with HOAc–MeOH–H_2O (1:14:6). The eluate was concd, and fractionated over Sephadex LH-20 CC using HOAc–EtOH–H_2O (1:6:12). The frs containing anthocyanins were further purified by PC (*n*-BuOH–HOAc–H_2O, 4:1:2) and HPLC. Prep. HPLC was performed on Hitachi 6200 system, using Inertsil ODS-2 (20φ × 250 mm) column and HOAc solvent system. Pigment **1** (*ca* 380 mg), **2** (*ca* 21 mg) and **3** (*ca* 20 mg) were obtained.

Analysis. Charcterization of these three anthocyanins and three intermediate anthocyanins obtained by partial acid hydrolysis (2M HCl, 100°, 4 min) was carried out with the standard procedures involving deacylation with alkaline and acid hydrolysis [5, 6]. Furthermore, pigment **1** was analysed by FAB-MS and ^{1}H NMR spectra containing ^{1}H–^{1}H COSY and difference NOE [5, 7–9]. TLC was carried out on microcrystalline cellulose (Avicel SF, Funakoshi) using BAW (*n*-BuOH–HOAc–H_2O, 4:1:5), BuH (*n*-BuOH–2M HCl, 1:1), 1% HCl and AHW (HOAc–HCl–H_2O, 15:3:82) for anthocyanins, BAW, PBW (*iso*-PrOH–*n*-BuOH–H_2O, 7:1:2) and PhW (PhOH–H_2O, 4:1) for sugars. HPLC was run on Inertsil ODS-2 column (4.6φ × 250 mm, at 35°, flow rate 0.8 ml min^{-1}, monitoring at 530 nm). Solvent systems used were as follows; a linear gradient elution for 40 min from 25 to 85% solvent B (1.5% H_3PO_4, 20% HOAc, 25% MeCN) in solvent A (1.5% H_3PO_4). ^{1}H NMR

spectra (400 MHz) were obtained with a JOEL JNM-GX 400 spectrometer in (TFA-*d*–DMSO-d_6, 1:9).

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