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Acylated anthocyanins from red radish (*Raphanus sativus* L.)

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Abstract

Twelve acylated anthocyanins were isolated from the red radish (*Raphanus sativus* L.) and their structures were determined by spectroscopic analyses. Six of these were identified as pelargonidin 3-*O*-[6-*O*-(*E*)-feruloyl-2-*O*-β-D-glucopyranosyl]-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside), pelargonidin 3-*O*-[6-*O*-(*E*)-caffeoyl-2-*O*-(6-(*E*)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside), pelargonidin 3-*O*-[6-*O*-(*E*)-*p*-coumaroyl-2-*O*-(6-(*E*)-caffeoyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside), pelargonidin 3-*O*-[6-*O*-(*E*)-feruloyl-2-*O*-(6-(*E*)-caffeoyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside), pelargonidin 3-*O*-[6-*O*-(*E*)-*p*-coumaroyl-2-*O*-(6-(E)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside), and pelargonidin 3-*O*-[6-*O*-(*E*)-feruloyl-2-*O*-(2-(*E*)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside). © 2002 Published by Elsevier Science Ltd.

Keywords: *Raphanus sativus* L.; Cruciferae; Red radish; Acylated anthocyanins; Pelargonidin; Cyanidin

1. Introduction

The red radish (*Raphanus sativus* L.) contain significant amounts of anthocyanins, which are widely used as natural food colors, because of the high stability and characteristics similar to synthetic Food Red No. 40 (Rodriguez-Saona et al., 1999). It is known that the major anthocyanidin is pelargonidin (Ishikura and Hayashi, 1962, 1963; Harborne, 1963; Fuleki, 1969). Recently, Shimizu et al. (1996) reported that two major anthocyanins in the red radish pigment were the acylated pelargonidin glycosides with one or two ferulic acids. Also, Giusti et al. (1998) reported that four anthocyanins in the red radish were identified as acylated pelargonidin glycosides with a combination of *p*-coumaric acid, ferulic acid, and malonic acid. Although they mentioned that there were many unknown anthocyanins in the red radish, were not investigated furthur. In this study, we report the isolation and identification of twelve acylated anthocyanins including six new compounds.

2. Results and discussion

Anthocyanins in the red radish were extracted with diluted H_2SO_4 and partitioned on polymeric resin. The HPLC analysis revealed that the anthocyanin extract contained 12 components (**1**–**12**), of which six were major compounds (**5**, **6**, **8**, **9**, **10** and **11**) and six were the minor compounds (**1**, **2**, **3**, **4**, **7** and **12**), and they were successively purified by column chromatography and subsequent prep. HPLC. We then obtained the six known compounds (**2**, **3**, **4**, **6**, **8** and **11**) and six new compounds (**1**, **5**, **7**, **9**, **10** and **12**).

The six known compounds were identified by spectral analyses and comparison with reported data (Lu et al., 1992; Saito et al., 1996; Shimizu et al., 1996; Giusti et al., 1998; Terahara et al., 1999); (**2**) cyanidin 3-*O*-[6-*O*-(*E*)-caffeoyl-2-*O*-(6-(*E*)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside), (**3**) pelargonidin 3-*O*-[6-*O*-(*E*)-caffeoyl-2-*O*-β-D-glucopyranosyl]-(1→2)-β-D-glucopyranoside)-5-*O*-(β-D-glucopyranoside), (**4**) pelargonidin 3-*O*-[6-*O*-(*E*)-caffeoyl-2-*O*-(6-(*E*)-caffeoyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside), (**6**) pelargonidin 3-*O*-[6-*O*-(*E*)-*p*-coumaroyl-2-*O*-β-D-glucopyranosyl]-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside),

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(**8**) pelargonidin 3-*O*-[6-*O*-(*E*)-feruloyl-2-*O*-β-D-glucopyranosyl]-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-gluco pyranoside) and (**11**) pelargonidin 3-*O*-[6-*O*-(*E*)-feruloyl-2-*O*-(6-(*E*)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside).

In the UV-vis spectra of the six new compounds, maximum absorpta were observed for each at a 509 nm in the visible region. Also, the percentages of the absorbance ratios at 440 nm and at the visible maximum ($E_{440}/E_{\text{vis, max}}$) were 22.6–23.6%; Harborne (1958) reported the characterization of the anthocyanins based on the maximum absorption and the ratio ($E_{440}/ E_{\text{vis, max}}$). Therefore, these data suggested that the new compounds may have a pelargonidin 3,5-diglycosyl type structure. This assumption was additionally supported by NMR measurements, since in the ^{1}H NMR spectra of each new compound, the seven proton signals of pelargonidin and the three anomeric proton signals of glucose were observed. The coupling constants (7.0–9.0 Hz) of the three anomeric proton signals suggested that the three glucose units (A, B, and C) were of the β-D-glucopyranoside type. Also, in the HMBC spectra, correlations between the anomeric proton signal of glucose B and the carbon signal at the 2-position of glucose A, and between the proton signal at the 2-position of glucose A and the carbon signal at the 1-position of glucose B were observed. Moreover, the anomeric proton signals of glucose A and glucose C correlated to the carbon signals at the 3-position and the 5-position of pelargonidin, respectively. Since these correlations were observed in the HMBC spectra for all new compounds, this confirmed that they (**1**, **5**, **7**, **9**, **10** and **12**) had a pelargonidin 3-*O*-sophoroside 5-*O*-glucoside basic structure.

The FAB mass spectrometry analysis of compound **1** showed a M^+ peak at *m*/*z* 933 and the high-resolution (HR) FABMS spectrum of the peak indicated that the molecular formula was $C_{43}H_{49}O_{23}$. The percentage ratio of the absorbance at the maximum of the acyl unit and the absorbance at the visible maximum ($E_{\text{acyl}}/ E_{\text{vis, max}}$) of **1** was 81.8%. This implied the presence of one acyl residue in **1**. In the ^{1}H NMR spectrum, proton signals from one acyl residue were observed (δ 7.31, 7.08, 6.91, 6.79, 6.18 and 3.81) corresponding to the proton. The acyl residue was elucidated as the *trans*-feruolyl group, based on the methoxy proton (δ 3.81) and the large coupling constants (16.0 Hz) of the α-and β-positions (δ 6.18 and 7.31, respectively) and the HMBC analysis. The ^{13}C NMR spectral data also supported their elucidation. The structure was finally confirmed by the HMBC technique. In the HMBC spectrum, a correlation was observed between the methoxy proton signal and the carbon signal at the 3-position of feruloyl group, and between the proton signals (δ 4.16 and 4.18) at the 6-position of glucose B and the carbonyl carbon signal (δ 166.8) of the feruloyl group. Therefore, compound **1** was determined to pelargonidin 3-*O*-[6-*O*-(*E*)-feruloyl-2-*O*-β-D-glucopyranosyl]-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside).

Compound **5** had a M^+ peak at *m*/*z* 1095 in the FABMS analysis, and its molecular formula was determined as $C_{52}H_{55}O_{26}$ based on HR–FABMS analysis. The percentage $E_{\text{acyl}}/E_{\text{vis, max}}$ ratio of **5** was 106.6%, suggesting that **5** had two acyl units. The ^{1}H and ^{13}C NMR spectra also showed the presence of two acyl units. One acyl unit was elucidated as a *trans*-feruloyl group, because the ^{1}H and ^{13}C NMR data of an acyl unit and a methoxy group were very similar to those of **1**. Another acyl unit was elucidated as a *trans*-caffeoyl group based on 1,2,4-trisubstituted benzene signals and *trans*-olefinic proton signals with a large coupling constant ($J = 16.0$ Hz). Moreover, a correlation was observed between the proton signals (δ 4.30 and 4.44) at the 6-position of glucose A and the carbonyl carbon signal (δ 166.8) of the caffeoyl group, and between the proton signals (δ 4.12 and 4.18) at the 6-position of glucose B and the carbonyl carbon signal (δ 166.6) of the feruloyl group in the HMBC spectrum. Therefore, compound **5** was determined as pelargonidin 3-*O*-[6-*O*-(*E*)-caffeoyl-2-*O*-(6-(*E*)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside).

Compound **9** gave a M^+ peak at *m*/*z* 1095 in the FABMS analysis, and the HR–FABMS analysis revealed that **9** was isomeric with **5**. The UV, ^{1}H and ^{13}C NMR spectral data of **9** were similar to those of **5**. Based on analysis a the HMBC spectrum, the structure of compound **9** was determined to be pelargonidin 3-*O*-[6-*O*-(*E*)-feruloyl-2-*O*-(6-(*E*)-caffeoyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside).

The spectral data of compound **7** were similar to those of **10**. The ^{1}H and ^{13}C NMR spectra revealed that **7** and **10** have *p*-coumaroyl and caffeoyl groups, and *p*-coumaroyl and feruloyl groups, respectively. The connectivities of the sugar and two acyl groups in **7** and **10** were confirmed by the HMBC spectra, and the structures of **7** and **10** were determined to be pelargonidin 3-*O*-[6-*O*-(*E*)-*p*-coumaroyl-2-*O*-(6-(*E*)-caffeoyl-β-D-gluco pyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-gluco pyranoside) and pelargonidin 3-*O*-[6-*O*-(*E*)-*p*-coumaroyl-2-*O*-(6-(*E*)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside), respectively.

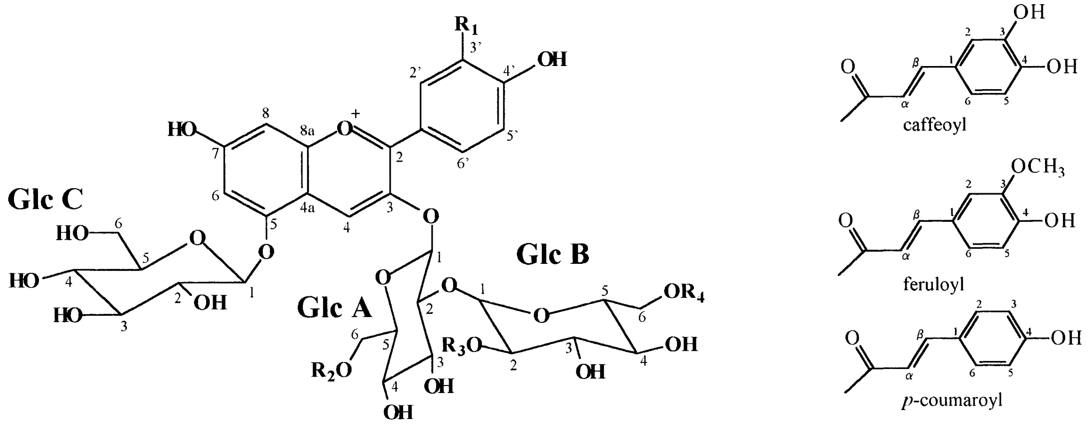
The molecular formula of **12** was determined to be $C_{52}H_{55}O_{26}$ by FABMS and HR–FABMS analysis. The percentage $E_{\text{acyl}}/E_{\text{vis, max}}$ ratio of **12** was 119.0%, suggesting that **12** had two acyl units. Also, the ^{1}H and ^{13}C NMR spectra revealed the presence of two *trans*-feruloyl groups as well as having others similar to **11**; however, the chemical shifts of glucose B were different from those of **11**. This suggested that the connecting positions of the acyl group would be different from those of **11**. In the HMBC spectrum, a correlation was observed between the proton signals (δ 4.28 and 4.40) at the 6-position of glucose A and the carbonyl carbon signal (δ 166.8) of the feruloyl group, and between the proton signal (δ 4.70) at the 2-position of glucose B and the carbonyl carbon signal (δ 166.1) of the feruloyl group. Therefore, **12** was identified as pelargonidin 3-*O*-[6-*O*-(*E*)-feruloyl-2-*O*-(2-(*E*)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside). The structures of isolated anthocyanins were shown in Fig. 1

The acylating processes during the anthocyanin biosynthesis steps is under enzyme control (Hrazdina et al., 1977). However, while some anthocyanin aromatic acyltransferases have been isolated and characterized from *Matthiola incana* flowers (Teusch et al., 1987), *Daucus carota* cell cultures (Glaessgen and Seitz, 1992), *Ajuga reptans* cell cultures (Callebaut et al., 1996), *Gentiana triflora* (Fujiwara et al., 1997), *Perilla frutescens* (Fujiwara et al., 1998, Yonekura-Sakakibara et al., 2000), and *Iris ensata* (Yabuya et al., 2001), there are no data regarding acyltransferases from red radish. We found that the major anthocyanins from red radish (compounds **5**–**11**) all had acyl groups attached to glucose A. The only new anthocyanin with acylation at glucose B (compound **1**) was a minor compound. These findings suggest that acylation at glucose B was a rate-determining step, in other words, the transfer rate of the acyl group to the glucose A would be much faster than that to glucose B. We also found that a variety of acyl groups were connected to glucose A, while most of the acyl groups connected to glucose B were feruloyl groups. This indicated that the acyltransferase responsible for the acylation of glucose B would have a high specificity, though further studies are needed to clarify them.

3. Experimental

3.1. General experimental procedures

All reagents were of HPLC or special grade and purchased from Wako Chemical Ind., Japan, and were used without further purification. Analytical HPLC was performed on an L-column ODS (4.6×250 mm, Chemical Evaluation and Research Institute) column at 40 °C at a flow rate of 1.5 ml min^{-1} with monitoring at 520 nm. Solvents used for the gradient elution were as follows:



Compound	R_1	R_2	R_3	R_4
1	H	H	H	feruloyl
2	OH	caffeoyl	H	feruloyl
3	H	caffeoyl	H	H
4	H	caffeoyl	H	caffeoyl
5	H	caffeoyl	H	feruloyl
6	H	*p*-coumaroyl	H	H
7	H	*p*-coumaroyl	H	caffeoyl
8	H	feruloyl	H	H
9	H	feruloyl	H	caffeoyl
10	H	*p*-coumaroyl	H	feruloyl
11	H	feruloyl	H	feruloyl
12	H	feruloyl	feruloyl	H

Fig. 1. Structures of anthocyanins from red radish.

solvents A (1.5% aq H_3PO_4) and B (1.5% aq H_3PO_4, 20% aq HOAc, 25% aq MeCN). The gradient conditions were as follows: 0–80 min, 30% solvent B in solvent A; 80–200 min, linear gradient from 30% to 45% solvent B in solvent A; 200–270 min, 45% solvent B in solvent A. Prep. HPLC was run on C. I. G. pre-packed column C_{18}-20 (22×100 mm, Kusano) column at 40 °C at a flow rate of 5 ml min^{-1} using HOAc–MeCN–H_2O (15:6:79) mixed solvents as the mobile phase at 520 nm, followed by using a SUPELCOSIL ABZ + Plus column (10×250 mm, Sigma-Aldrich) column under the same conditions. The UV-vis spectra were measured on a Hitachi U-2000A spectrophotometer with MeOH containing 0.01% HCl. The FABMS and HR–FABMS were analyzed by a Jeol JMS-700 spectrometer using *m*-nitrobenzyl alcohol and/or glycerol as the matrix in the positive mode. The ^1H (600 MHz) and ^{13}C (150 MHz) NMR spectra were measured in a mixed solvent of DMSO-d_6–TFA-d_1 (9:1) with a Jeol A-600 spectrometer using TMS as the internal standard. The signals in the ^1H and ^{13}C NMR spectra of the isolated anthocyanins were assigned on the basis of their chemical shifts and the results of the correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) studies.

3.2. Materials

Red radish (*Raphanus sativus* L.) from Yunnan (China) was obtained from San-ei-gen FFI, Ltd. (Osaka, Japan).

3.3. Isolation of red radish pigments

The pigments in the red radish (20 kg) were extracted with 0.05% H_2SO_4, and applied to a polymeric resin (Diaion SP207) and eluted with 70% aq MeOH containing 1% citric acid. The HPLC analysis revealed that the pigments contained 12 or more compounds, and that the relative peak areas of compounds **1**–**12** were 0.88% (R_t, 25.1 min), 0.30% (R_t, 59.1 min), 2.33% (R_t, 63.3 min), 2.54% (R_t, 70.4 min), 7.06% (R_t, 114.7 min), 10.21% (R_t, 118.1 min), 3.36% (R_t, 124.6 min), 17.76% (R_t, 128.1 min), 10.76% (R_t, 133.7 min), 8.58% (R_t, 159.7 min), 24.46% (R_t, 166.8 min), and 2.17% (R_t, 189.0 min), respectively. The pigments (131.7 g) were applied to a Diaion HP-20 column at ambient temperature, washed with 0.1% aq TFA and eluted stepwise with 5, 10, 20, 30, 50 and 70% aq MeOH containing 0.1% TFA, and then 100% MeOH containing 0.1% TFA. The 70% aq MeOH fraction containing large amounts of the anthocyanins (17.4 g) was collected and evaporated to dryness in vacuo. A part of the pigment fraction (ca 5 g) was subjected to prep. HPLC to purify the anthocyanins. During the first step, prep. HPLC was run on a C. I. G. pre-packed column C_{18}-20 column to obtain several fractions. Furthermore, their fractions were purified by using SUPELCOSIL ABZ + Plus column. Finally, compounds **1** (12.0 mg), **2** (1.4 mg), **3** (10.3 mg), **4** (5.1 mg), **5** (16.8 mg), **6** (1.3 mg), **7** (1.8 mg), **8** (17.8 mg), **9** (18.5 mg), **10** (27.8 mg), **11** (38.1 mg), and **12** (2.3 mg) were obtained.

*3.4. Pelargonidin 3-O-[6-O-(E)-feruloyl-2-O-β-D-glucopyranosyl]-(1→2)-β-D-glucopyranoside)-5-O-β-D-glucopyranoside) (**1**)*

Red amorphous powder. UV-vis λ_{max} (0.01% HCl–MeOH) nm: 505, 327, 283, $E_{440}/E_{vis,\ max} = E_{440}/E_{505} = 0.234/1.009 = 0.232$, $E_{acyl}/E_{vis,\ max} = E_{327}/E_{505} = 0.825/1.009 = 0.818$; FABMS and HR–FABMS data: see Table 1; ^1H NMR data: see Table 2; ^{13}C NMR data: see Table 3.

*3.5. Cyanidin 3-O-[6-O-(E)-caffeoyl-2-O-(6-(E)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-O-(β-D-glucopyranoside) (**2**)*

Purple amorphous powder. UV-vis λ_{max} (0.01% HCl–MeOH) nm: 513, 323, 288, $E_{440}/E_{vis,\ max} = E_{440}/E_{513} = 0.123/0.444 = 0.277$, $E_{acyl}/E_{vis,\ max} = E_{323}/E_{513} = 1.025/0.444 = 2.308$; FABMS and HR–FABMS data: see Table 1; ^1H NMR data: see Table 2; ^{13}C NMR data: see Table 3.

*3.6. Pelargonidin 3-O-[6-O-(E)-caffeoyl-2-O-β-D-glucopyranosyl]-(1→2)-β-D-glucopyranoside)-5-O-(β-D-glucopyranoside) (**3**)*

Red amorphous powder. UV-vis λ_{max} (0.01% HCl–MeOH) nm: 508, 331, 286, $E_{440}/E_{vis,\ max} = E_{440}/E_{508} = 0.235/1.139 = 0.206$, $E_{acyl}/E_{vis,\ max} = E_{331}/E_{508} = 0.593/1.139 = 0.521$; FABMS *m*/*z*: 919 $[M]^+$; HR–FABMS *m*/*z*:

Table 1
FABMS and HR–FABMS data for red radish anthocyanin

Compound	FABMS	HR–FABMS of molecular ion		
	$[M]^+$ (*m*/*z*)	Observed	Calculated	Molecular formula
1	933	933.2637	933.2664	$C_{43}H_{49}O_{23}$
2	1111	1111.2822	1111.2930	$C_{52}H_{55}O_{27}$
3	919	919.2450	919.2508	$C_{42}H_{47}O_{23}$
4	1081	1081.2823	1081.2825	$C_{51}H_{53}O_{26}$
5	1095	1095.3030	1095.2981	$C_{52}H_{55}O_{26}$
6	903	903.2546	903.2559	$C_{42}H_{47}O_{22}$
7	1065	1065.2592	1065.2981	$C_{51}H_{52}O_{25}$
8	933	933.2584	933.2664	$C_{43}H_{49}O_{23}$
9	1095	1095.2936	1095.2981	$C_{52}H_{55}O_{26}$
10	1079	1079.3062	1079.3032	$C_{52}H_{55}O_{25}$
11	1109	1109.3153	1109.3138	$C_{53}H_{57}O_{26}$
12	1109	1109.3141	1109.3138	$C_{53}H_{57}O_{26}$

Table 2
1H NMR chemical shifts for red radish anthocyanins in DMSO-d_6–TFA-d_1 (9:1)[a]

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Aglycon												
4	9.02 *s*	8.73 *s*	8.87 *s*	8.92 *s*	8.93 *s*	8.87 *s*	8.92 *s*	8.84 *s*	8.94 *s*	8.92 *s*	8.88 *s*	8.86 *s*
6	6.98 *brs*	6.84 *s*	7.03 *brs*	6.97 *d* (1.5)	6.95 *brs*	7.03 *brs*	6.98 *d* (2.0)	6.96 *d* (1.5)	6.96 *brs*	6.96 *brs*	6.90 *d* (1.5)	6.99 *brs*
8	7.10 *brs*	6.84 *s*	7.12 *brs*	7.01 *brs*	6.97 *brs*	7.10 *brs*	7.01 *d* (2.0)	7.06 *d* (1.5)	*6.99 brs*	6.97 *brs*	6.94 *d* (1.5)	7.03 *brs*
2′	8.63 *d* (9.0)	7.92 *d* (2.5)	8.62 *d* (9.0)	8.57 *d* (9.0)	8.54 *d* (9.0)	8.63 *d* (9.0)	8.59 *d* (9.0)	8.57 *d* (9.0)	8.58 *d* (9.0)	8.54 *d* (9.0)	8.52 *d* (9.0)	8.62 *d* (9.0)
3′	7.13 *d* (9.0)		7.14 *d* (9.0)	7.09 *d* (9.0)	7.07 *d* (9.0)	7.13 *d* (9.0)	7.10 *d* (9.0)	7.07 *d* (9.0)	7.08 *d* (9.0)	7.08 *d* (9.0)	7.03 *d* (9.0)	7.16 *d* (9.0)
5′	7.13 *d* (9.0)	7.01 *d* (9.0)	7.14 *d* (9.0)	7.09 *d* (9.0)	7.07 *d* (9.0)	7.13 *d* (9.0)	7.10 *d* (9.0)	7.07 *d* (9.0)	7.08 *d* (9.0)	7.08 *d* (9.0)	7.03 *d* (9.0)	7.16 *d* (9.0)
6′	8.63 *d* (9.0)	8.16 *dd* (2.5, 9.0)	8.62 *d* (9.0)	8.57 *d* (9.0)	8.54 *d* (9.0)	8.63 *d* (9.0)	8.59 *d* (9.0)	8.57 *d* (9.0)	8.58 *d* (9.0)	8.54 *d* (9.0)	8.52 *d* (9.0)	8.62 *d* (9.0)
Glc A												
1	5.43 *d* (8.0)	5.55 *d* (7.5)	5.69 *d* (7.5)	5.61 *d* (7.0)	5.57 *d* (7.5)	5.69 *d* (7.5)	5.66 *d* (7.5)	5.64 *d* (7.0)	5.60 *d* (8.0)	5.58 *d* (7.5)	5.53 *d* (7.0)	5.70 *d* (7.5)
2	3.94 *dd* (8.0, 9.0)	3.95 *dd* (7.0, 9.0)	4.09 *brt* (9.0)	3.99 *dd* (7.0, 9.0)	3.98 *dd* (7.5, 9.0)	4.10 *dd* (7.5, 8.5)	4.01 *brt* (8.5)	4.05 *dd* (7.0, 9.0)	3.99 *brt* (8.0)	3.98 *dd* (7.5, 8.0)	3.93 *dd* (7.0, 9.0)	4.16 *dd* (7.5, 8.5)
3	3.67 *t* (9.0)	3.65 *t* (9.0)	3.75 *t* (9.0)	3.74 *t* (9.0)	3.75 *t* (9.0)	3.77 *t* (8.5)	3.76 *t* (8.5)	3.72 *t* (9.0)	3.75 *t* (9.0)	3.75 *ol**	3.66 *t* (9.0)	3.69 *t* (8.5)
4	3.37 *t* (9.0)	3.39 *t* (9.0)	3.56 *t* (9.0)	3.48 *t* (9.0)	3.49 *t* (9.0)	3.54 *t* (8.5)	3.47 *t* (8.5)	3.48 *ol**	3.53 *ol**	3.45 *ol**	3.47 *t* (9.0)	3.54 *ol**
5	3.36 *m*	3.85 *m*	3.99 *m*	3.89 *m*	3.86 *m*	4.01 *m*	3.92 *m*	3.94 *m*	3.88 *m*	3.87 *m*	3.80 *m*	3.95 *m*
6a	3.53 *m*	4.22 *dd* (7.0, 12.5)	4.35 *dd* (6.0, 11.5)	4.31 *dd* (6.0, 11.0)	4.30 *dd* (6.0, 11.0)	4.34 *dd* (6.0, 11.0)	4.31 *dd* (6.5. 12.5)	4.28 *dd* (6.0, 12.0)	4.30 *dd* (6.0, 11.0)	4.28 *dd* (6.5, 11.0)	4.24 *dd* (6.0, 12.0)	4.28 *m*
6b	3.74 *brd* (11.0)	4.31 *dd* (2.5, 12.5)	4.44 *brd* (11.5)	4.42 *brd* (11.0)	4.44 *brd* (11.0)	4.45 *brd* (11.0)	*4.44 brd* (12.5)	*4.40 brd* (12.0)	4.44 *brd* (11.0)	4.44 *brd* (11.0)	4.38 *brd* (12.0)	4.40 *brd* (11.0)
Glc B												
1	4.90 *d* (8.0)	4.71 *d* (8.0)	4.76 *d* (7.5)	4.84 *d* (8.0)	4.85 *d* (8.0)	4.76 *d* (7.5)	4.86 *d* (8.0)	4.71 *d* (8.0)	4.85 *d (7.5)*	4.85 *d* (8.0)	4.80 *d* (8.0)	5.27 *d* (7.5)
2	3.14 *brt* (8.5)	3.06 *dd* (8.0, 9.0)	3.04 *brt* (9.0)	3.16 *brt* (8.0)	3.22 *brt* (8.5)	3.02 *brt* (8.5)	3.17 *brt* (8.0)	2.96 *dd* (8.0, 9.0)	3.17 *m*	3.18 *dd* (8.0. 9.0)	3.12 *dd* (8.0. 9.0)	4.70 *dd* (7.5, 8.5)
3	3.32 *ol**	3.19 *tm*	3.28 *t* (9.0)	3.28 *ol* *	3.36 *t* (8.5)	3.16 *t* (8.5)	3.30 *ol**	3.121 *t* (9.0)	3.281(9.5)	3.32 *ol*	3.251 *t* (9.0)	3.46 *t* (8.5)
4	3.33 *ol**	3.17 *m*	3.08 *t* (9.0)	3.31 *ol* *	3.33 *ol* *	3.08 *t* (8.5)	3.30 *ol**	3.02 ***t*** (9.0)	3.30 *ol* *	3.31 *ol*	3.27 *o1*	3.23 *m*
5	3.33 *m*	3.07 *m*	2.89 *m*	3.31 *ol* *	3.36 *m*	2.88 *m*	3.30 *ol**	2.84 *m*	3.30 *oF* *	3.32 *m*	3.27 *m*	3.20 *m*
6a	4.16 *m*	3.91 *dd* (4.0, 12.0)	3.31 *brd* (10.0)	4.07 *brd* (12.0)	4.12 *brd* (11.5)	3.30 *m*	4,08 *brd* (12.5)	3.35 *dd* (6.0, 12.0)	4.07 *brd* (11.5)	4.12, *m*	4.05 *m*	3.50 *ol**
6b	4.18 *m*	3.84 *dd* (12.0)	3.39 *brd* (10.0)	4.08 *brd* (12.0)	4.18 *brd* (11.5)	3.39 *m*	4.11 *brd* (12.5)	3.24 *m*	4.12 *brd* (11.5)	4.16 *brd* (11.5)	4.03 *m*	3.53 *ol**
Glc C												
1	5.11 *d* (8.0)	4.95 *d* (8.0)	5.13 *d* (7.5)	5.12 *d* (8.0)	5.11 *d* (8.0)	5.11 *d* (7.5)	5.11 *d* (7.5)	5.07 *d* (8.0)	5.11 *d* (7.5)	5.08 *d* (8.0)	5.04 *d* (8.0)	5.11 *d* (7.5)
2	3.51 *dd* (8.0, 9.0)	3.46 *dd* (8.0, 9.0)	3.57 *brt* (8.5)	3.56 *dd* (8.0, 9.0)	3.59 brt (9.0)	3.55 *dd* (7.5, 8.5)	3.54 *brt* (7.5)	3.49 *dd* (8.0, 9.0)	3.55 *brt* (7.5)	3.55 *dd* (8.0. 9.0)	3.50 *dd* (8.0, 9.0)	3.54 *ol**
3	3.42 *t* (9.0)	3.31 *t* (9.0)	3.41 *t* (8.5)	3.42 *t* (9.0)	3.44 *t* (9.0)	3.41 *t* (8.5)	3.42 ***t*** (9.0)	3.31 *t* (9.0)	3.42 *t* (9.0)	3.42 *t* (9.0)	3.35 *t* (9.0)	3.41 *t* (9.0)
4	3.30 *t* (9.0)	3.19 *t* (9.0)	3.29 *t* (8.5)	3.31*ol* *	3.31 *t* (9.0)	3.29 *t* (8.5)	3.30 *ol**	3.22 *t* (9.0)	3.30 *ol**	3.29 *ol**	3.22 *t* (9.0)	3.28 *t* (9.0)
5	3.49 *m*	3.39 *m*	3.53 *m*	3.50 *m*	3.51 *m*	3.50 *m*	3.50 *m*	3.45 *m*	3.50 *m*	3.47 *m*	3.42 *m*	3.53 *ol**
6a	3.58 *dd* (6.0, 12.0)	3.48 *dd* (6.0, 11.0)	3.58 *brd* (10.5)	3.58 *dd* (6.0, 11.5)	3.60 *m*	3.57 *brd* (11.0)	3.57 *dd* (5.5, 10.5)	3.48 *ol**	*3.56 m*	3.59 *m*	3.50 *m*	3.51 *ol**
6b	3.77 *m*	3.73 *brd* (11.0)	3.81 *brd* (10.5)	3.82 *brd* (11.5)	3.82 *brd* (11.0)	3.81 *brd* (11.0)	3.81 *brd* (10.5)	3.74 *brd* (12.0)	3.76 *m*	3.80 *m*	3.70 *brd* (12.0)	3.81 *ol**

(*continued on next page*)

Table 2 (*continued*)

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Acyl A												
2		6.86 *d* (2.0)	6.97 *d* (2.0)	6.96 *d* (1.5)	6.96 *brs*	7.40 *d* (8.5)	7.39 *d* (9.0)	7.08 *d* (2.0)	7.09 *brs*	7.38 *d* (8.5)	7.07 *d* (2.0)	7.08 *brs*
3						6.79 *d* (8.5)	6.80 *d* (9.0)			6.80 *d* (8.5)		
5		6.67 *d* (8.5)	6.77 *d* (8.0)	6.77 *d* (8.0)	6.77 *d* (8.5)	6.79 *d* (8.5)	6.80 *d* (9.0)	6.76 *d* (8.5)	6.81 *d* (8.0)	6.50 *d* (8.5)	6.76 *d* (8.5)	6.80 *d* (8.5)
6		6.76 *dd* (2.0, 8.5)	6.87 *dd* (2.0, 8.0)	6.86 *d* (8.0)	6.83 *d* (8.5)	7.40 *d* (8.5)	7.39 *d* (9.0)	6.97 *dd* (2.0, 8.5)	7.00 *brs*	7.38 *d* (8.5)	6.94 *dd* (2.0, 8.5)	7.01 *brs*
a		6.07 *d* (16.0)	6.20 *d* (16.0)	6.15 *d* (16.0)	6.13 *d* (16.0)	6.27 *d* (16.0)	6.26 *d* (16.0)	6.29 *d* (16.0)	6.28 *d* (16.0)	6.18 *d* (16.0)	6.23 *d* (15.5)	6.31 *d* (15.5)
b		7.22 *d* (16.0)	7.32 *d* (16.0)	7.32 *d* (16.0)	7.32 *d* (16.0)	7.38 *d* (16.0)	7.38 *d* (16.0)	7.37 *d* (16.0)	7.40 *d* (16.0)	7.35 *d* (16.0)	7.48 *d* (15.5)	7.38 *d* (16.0)
OMe								3.75 *s*	3.79 *s*		3.74 *s*	3.78 *s*
Acyl B												
2	7.08 *brs*	7.00 *d* (2.0)		6.89 *d* (1.5)	7.02 *brs*		6.91 *d* (2.0)		6.88 *brs*	7.04 *brs*	7.03 *d* (2.0)	7.24 *brs*
5	6.79 *d* (8.0)	6.68 *d* (8.5)		6.74 *d* (8.0)	6.75 *d* (8.5)		6.75 *d* (8.0)		6.74 *d* (8.0)	6.75 *d* (8.0)	6.71 *d* (8.0)	6.83 *d* (8.5)
6	6.91 *brd* (8.0)	6.81 *dd* (2.0, 8.5)		6.79 *d* (8.0)	6.85 *brd* (8.5)		6.80 *d* (8.0)		6.77 *d* (8.0)	6.86 *brd* (8.0)	6.84 *dd* (2.0, 8.0)	7.06 *brs*
a	6.18 *d* (16.0)	6.02 *d* (16.0)		5.94 *d* (16.0)	6.08 *d* (15.5)		5.93 *d* (16.0)		5.94 *d* (16.0)	6.10 *d* (16.0)	6.08 *d* (15.5)	6.44 *d* (15.5)
b	7.31 *d* (16.0)	7.13 *d* (16.0)		7.21 *d* (16.0)	7.23 *d* (15.5)		7.23 *d* (16.0)		7.20 *d* (16.0)	7.24 *d* (16.0)	7.20 *d* (15.5)	7.51 *d* (16.0)
OMe	3.81 *s*	3.70 *s*			3.77 *s*					3.80 *s*	3.72 *s*	3.84 *s*

[a] Values in parentheses indicate coupling constants (*J* in Hz).

* Overlapped.

Table 3
^{13}C NMR chemical shifts for red radish anthocyanins in DMSO-d_6–TFA-d_1 (9:1)

Position	1	2	3	4	5	6	7	8	9	10	11	12
Aglycon												
2	163.3	162.3	162.8	162.8	163.0	163.0	163.0	163.0	162.9	163.0	163.0	162.7
3	144.4	144.1	144.4	144.0	144.0	144.4	144.1	144.5	144.1	144.0	144.1	144.3
4	136.3	132.5	132.9	135.1	136.0	133.0[a]	135.3	133.0	135.3	135.5	135.5	133.2
4a	112.0	111.6	112.0	111.9	112.0	112.0[a]	112.1	112.0	112.0	112.0	112.0	112.0
5	155.6	155.1	155.3	155.3	155.5	155.4	155.5	155.3	155.4	155.5	155.4	155.3
6	104.5	104.8	104.9	104.8	104.9	105.3	105.1	104.9	104.8	105.0	104.8	104.8
7	168.5	167.8	168.0	168.3	168.6	167.8	168.5	167.7	168.4	168.2	168.2	168.0
8	96.5	96.2	96.4	96.4	96.5	96.5[a]	96.6	96.5	96.4	96.5	96.4	96.4
8a	155.9	155.1	155.4	155.5	155.7	155.4	155.5	155.4	155.6	155.7	155.6	155.4
1′	119.4	119.3	119.4	119.1	119.1	119.5	119.2	119.4	119.1	119.2	119.2	119.5
2′	135.5	117.5	135.3	135.1	135.2	135.4	135.2	135.3	135.1	135.2	135.2	133.2
3′	117.3	146.3	117.2	117.1	117.2	117.2	117.2	117.1	117.1	117.2	117.0	117.3
4′	164.4	155.1	165.6	165.5	165.6	165.8	165.6	165.5	165.5	165.6	165.4	165.7
5′	117.3	116.9	117.2	117.1	117.2	117.2	117.2	117.1	117.1	117.2	117.2	117.3
6′	135.5	127.6	135.3	135.1	135.2	135.4	135.2	135.3	135.1	135.2	135.2	133.2
Glc A												
1	100.8	99.5	99.4	100.0	100.6	99.3	100.1	99.5	100.2	100.3	100.3	98.9
2	80.5	81.6	79.6	80.9	81.2	81.1	81.0	79.7	80.9	81.1	81.0	76.9
3	76.9	75.9	76.2	76.1	76.2	76.2	76.2	76.2	76.1	76.2	76.0	76.6
4	69.7	69.6	69.7	69.7	69.8	70.0	69.9	69.5	69.7	69.9	69.5	70.1
5	77.7	74.0	74.0	74.2	74.4	74.0[a]	74.3	74.0	74.2	74.3	74.2	74.0
6	60.9	62.9	62.9	62.9	63.0	63.0[a]	63.1	62.9	62.9	63.0	62.8	62.8
Glc B												
1	100.4	104.5	103.2	104.2	104.3	103.4	104.3	103.3	104.2	104.3	104.2	99.6
2	74.7	74.7	74.5	74.6	74.7	74.6	74.7	74.4	74.6	74.7	74.5	74.2
3	76.7	76.1	76.3	76.3	76.5	76.3	76.4	76.5	76.3	76.5	76.2	74.9
4	70.0	69.4	70.1	69.7	69.9	70.1	70.0	70.0	69.7	69.9	69.7	71.0
5	74.3	74.0	77.2	74.2	74.2	77.2	74.3	77.2	74.2	74.2	74.1	77.8
6	63.1	62.3	61.1	62.9	63.0	61.0	63.1	61.0	62.9	63.0	62.9	61.5
Glc C												
1	101.5	101.9	102.0	101.8	101.8	102.4	102.1	102.1	101.8	102.0	101.8	102.2
2	73.3	73.2	73.4	73.3	73.4	73.4	73.4	73.3	73.3	73.4	73.2	73.4
3	76.3	76.1	76.5	76.2	76.5	76.6	76.4	76.2	76.3	76.4	76.3	76.2
4	69.8	69.8	69.9	69.7	69.9	69.9	70.0	69.8	69.7	69.9	69.7	70.0
5	77.8	77.6	77.7	77.7	77.8	77.8	77.8	77.7	77.7	77.8	77.7	77.8
6	60.9	60.9	61.1	60.9	61.0	61.0	61.0	60.9	60.8	61.0	60.8	61.0
Acyl A[b]												
1		125.5	125.6	125.5	125.6	125.1	125.2	125.6	125.6	125.1	125.6	125.7
2		115.2	115.2	115.2	115.2	130.7	130.6	111.7	111.6	130.5	111.6	111.8
3		145.4	145.7	145.6	145.7	116.0[a]	116.1	148.0	148.0	116.0	148.0	148.1
4		148.4	148.6	148.5	148.7	160.1	160.1	149.5	149.5	160.1	149.5	149.6
5		115.9	115.9	115.9	116.0	116.0[a]	116.1	115.7	115.8	116.0	115.7	115.8
6		121.3	121.6	121.5	121.7	130.7	130.6	123.1	123.0	130.5	123.0	123.1
α		113.6	113.7	113.6	113.8	114.0[a]	113.8	114.1	114.1	113.8	114.1	114.2
β		145.5	145.6	145.5	145.7	145.4	145.3	145.6	145.6	145.2	145.5	145.6
Carbonyl		166.5	166.8	166.7	166.8	166.7	166.8	166.7	166.7	166.7	166.7	166.8
OMe								55.8	55.9		55.8	55.9
Acyl B[c]												
1	125.8	125.5		125.5	125.7		125.7		125.5	125.6	125.6	126.0
2	111.2	111.1		115.0	111.1		115.1		115.0	111.1	111.1	111.5
3	148.3	148.0		145.6	148.2		145.8		145.7	148.1	148.0	148.2
4	149.7	149.4		148.5	149.6		148.6		148.5	149.6	149.5	149.6
5	115.9	115.6		115.8	115.8		116.1		115.9	115.7	115.6	115.8
6	123.3	123.0		121.3	123.2		121.4		121.3	123.2	123.2	123.2
α	114.7	114.0		113.6	114.1		113.8		113.6	114.1	114.1	115.2
β	145.3	144.9		145.2	145.1		145.3		145.2	145.1	145.1	145.1
Carbonyl	166.8	166.3		166.4	166.6		166.5		166.5	166.6	166.5	161.1
OMe	55.9	55.7			55.8					55.8	55.8	56.0

[a] Signals were detected by HMQC or HMBC analysis.
[b] Acyl A: acyl group attaching to glucose A.
[c] Acyl B: acyl group attaching to glucose B.

919.2450 $[M]^+$, calcd for $[C_{42}H_{47}O_{23}]^+$ 919.2508; ^{1}H NMR data; ^{13}C NMR data: see Table 1.

3.7. *Pelargonidin 3-O-[6-O-(E)-caffeoyl-2-O-(6-(E)-caffeoyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-O-(β-D-glucopyranoside) (**4**)*

Red amorphous powder. UV-vis λ_{max} (0.01% HCl–MeOH) nm: 509, 331, 287, $E_{440}/E_{vis,\ max} = E_{440}/E_{509} = 0.208/0.910 = 0.229$, $E_{acyl}/E_{vis,\ max} = E_{331}/E_{509} = 0.919/0.910 = 1.010$; FABMS and HR–FABMS data: see Table 1; ^{1}H NMR data: see Table 2; ^{13}C NMR data: see Table 3.

3.8. *Pelargonidin 3-O-[6-O-(E)-caffeoyl-2-O-(6-(E)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-O-(β-D-glucopyranoside) (**5**)*

Red amorphous powder. UV-vis λ_{max} (0.01% HCl–MeOH) nm: 509, 329, 286, $E_{440}/E_{vis,\ max} = E_{440}/E_{509} = 0.197/0.854 = 0.231$, $E_{acyl}/E_{vis,\ max} = E_{329}/E_{509} = 0.910/0.854 = 1.066$; FABMS and HR–FABMS data: see Table 1; ^{1}H NMR data: see Table 2; ^{13}C NMR data: see Table 3.

3.9. *Pelargonidin 3-O-[6-O-(E)-p-coumaroyl-2-O-β-D-glucopyranosyl]-(1→2)-β-D-glucopyranoside]-5-O-(β-D-glucopyranoside) (**6**)*

Red amorphous powder. UV-vis λ_{max}(0.01% HCl–MeOH) nm: 507, 316, 286, $E_{440}/E_{vis,\ max} = E_{440}/E_{505} = 0.153/0.706 = 0.217$, $E_{acyl}/E_{vis,\ max} = E_{316}/E_{507} = 0.468/0.706 = 0.663$; FABMS and HR–FABMS data: see Table 1; ^{1}H NMR data: see Table 2; ^{13}C NMR data: see Table 3.

3.10. *Pelargonidin 3-O-[6-O-(E)-p-coumaroyl-2-O-(6-(E)-caffeoyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-O-(β-D-glucopyranoside) (7)*

Red amorphous powder. UV-vis λ_{max} (0.01% HCl–MeOH) nm: 508, 319, 287, $E_{440}/E_{vis,\ max} = E_{440}/E_{508} = 0.283/1.254 = 0.226$, $E_{acyl}/E_{vis,\ max} = E_{319}/E_{508} = 1.340/1.254 = 1.069$; FABMS and HR–FABMS data: see Table 1; ^{1}H NMR data: see Table 2; ^{13}C NMR data: see Table 3.

3.11. *Pelargonidin 3-O-[6-O-(E)-feruloyl-2-O-β-D-glucopyranosyl]-(1→2)-β-D-glucopyranoside]-5-O-(β-D-glucopyranoside) (**8**)*

Red amorphous powder. UV-vis λ_{max} (0.01% HCl–MeOH) nm: 507, 328, 285, $E_{440}/E_{vis,\ max} = E_{440}/E_{507} = 0.251/\ 1.202 = 0.209$, $E_{acyl}/E_{vis,\ max} = E_{328}/E_{507} = 0.710/\ 1.202 = 0.591$; FABMS and HR–FABMS data: see Table 1; ^{1}H NMR data: see Table 2; ^{13}C NMR data: see Table 3.

3.12. *Pelargonidin 3-O-[6-O-(E)-feruloyl-2-O-(6-(E)-caffeoyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-O-(β-D-glucopyranoside) (**9**)*

Red amorphous powder. UV-vis λ_{max} (0.01% HCl–MeOH) nm: 508, 329, 286, $E_{440}/E_{vis,\ max} = E_{440}/E_{508} = 0.242/1.062 = 0.228$, $E_{acyl}/E_{vis,\ max} = E_{329}/E_{508} = 1.065/1.062 = 1.003$; FABMS and HR–FABMS data: see Table 1; ^{1}H NMR data: see Table 2; ^{13}C NMR data: see Table 3.

3.13. *Pelargonidin 3-O-[6-O-(E)-p-coumaroyl-2-O-(6-(E)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-O-(β-D-glucopyranoside) (**10**)*

Red amorphous powder. UV-vis λ_{max} (0.01% HCl–MeOH) nm: 507, 320, 287, $E_{440}/E_{vis,\ max} = E_{440}/E_{507} = 0.202/0.878 = 0.230$, $E_{acyl}/E_{vis,\ max} = E_{320}/E_{507} = 0.960/0.878 = 1.093$; FABMS and HR–FABMS data: see Table 1; ^{1}H NMR data: see Table 2; ^{13}C NMR data: see Table 3.

3.14. *Pelargonidin 3-O-[6-O-(E)-feruloyl-2-O-(6-(E)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-O-(β-D-glucopyranoside)(**11**)*

Red amorphous powder. UV-vis λ_{max} (0.01% HCl–MeOH) nm: 507, 326, 286, $E_{440}/E_{vis,\ max} = E_{440}/E_{507} = 0.209/0.939 = 0.223$, $E_{acyl}/E_{vis,\ max} = E_{326}/E_{507} = 1.027/0.939 = 1.094$; FABMS and HR–FABMS data: see Table 1; ^{1}H NMR data: see Table 2; ^{13}C NMR data: see Table 3.

3.15. *Pelargonidin 3-O-[6-O-(E)-feruloyl-2-O-(2-(E)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-O-(β-D-glucopyranoside) (**12**)*

Red amorphous powder. UV-vis λ_{max} (0.01% HCl–MeOH) nm: 509, 327, 289, $E_{440}/E_{vis,\ max} = E_{440}/E_{509} = 0.206/0.872 = 0.236$, $E_{acyl}/E_{vis,\ max} = E_{327}/E_{509} = 1.038/0.872 = 1.190$; FABMS and HR–FABMS data: see Table 1; ^{1}H NMR data: see Table 2; ^{13}C NMR data: see Table 3.

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