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ACYLATED ANTHOCYANINS FROM FRUITS OF *SAMBUCUS CANADENSIS*

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Key Word Index—*Sambucus canadensis*; Caprifoliaceae; fruits; acylated anthocyanin.

Abstract—Two new acylated anthocyanin glycosides were isolated from fruits of *Sambucus canadensis*. The structures were determined to be cyanidin 3-*O*-(6-*O*-*Z*-*p*-coumaroyl-2-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside)-5-*O*-*β*-D-glucopyranoside and cyanidin 3-*O*-(6-*O*-*E*-*p*-coumaroyl-2-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside.

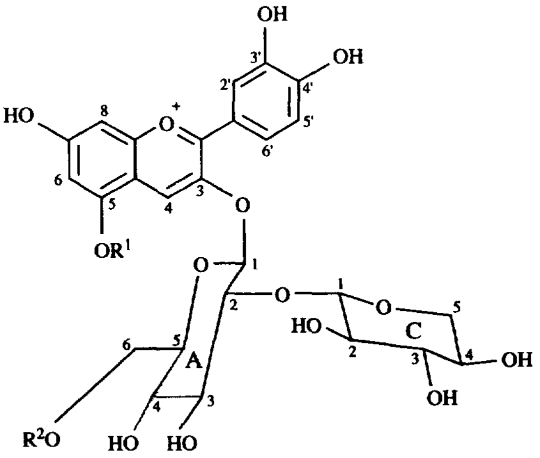
INTRODUCTION

The elderberry has been used to colour jams, jellies and wines in Europe. Elderberry species include *Sambucus nigra* and *S. canadensis*. Recently, the latter has been proposed as a natural food colour, because it was found that the colour of *S. canadensis* is more stable to light and heat than that of *S. nigra*. This prompted us to investigate anthocyanins contained in the fruits of *S. canadensis*. Four anthocyanins have already been found from this fruit [1]. In the present paper, we report the isolation and structure determination of two new anthocyanins.

RESULTS AND DISCUSSION

Fruits of *Sambucus canadensis* were extracted with acidic methanol and the extract was purified by column chromatography with MCI gel to give crude pigments. Seven anthocyanins were detected by HPLC analysis of this crude sample and were isolated by repeated column chromatographies using ODS and MCI gels. The major anthocyanin was identified as cyanidin 3-*O*-(6-*O*-*E*-*p*-coumaroyl-2-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside-5-*O*-*β*-D-glucopyranoside (**1**) by means of spectroscopy and chemical degradation [1]. The 3-sambubioside-5-glucoside, 3-sambubioside, 3-glucoside and 3,5-diglucoside of cyanidin were also identified. Among them, cyanidin 3,5-diglucoside was isolated from *S. canadensis* for the first time. The remaining two minor anthocyanins (**2** and **3**) were found to be new compounds.

The UV-vis spectrum of **2** showed a visible maximum at 532 nm. The ratio of E_{440}/E_{532} (14%) indicated **2** to be glycosylated on both C-3 and C-5. The ratio of E_{310}/E_{532} (51%) suggested the presence of a cinnamoyl moiety in the molecule [2]. Negative SIMS measurement of **2** gave a molecular size of 889 *m/z*, corresponding to $C_{41}H_{45}O_{22}$.



	R^1	R^2
1	β-D-glucopyranosyl (B)	*E*-*p*-coumaroyl
2	β-D-glucopyranosyl (B)	*Z*-*p*-coumaroyl
3	H	*E*-*p*-coumaroyl

The ^{1}H NMR of this pigment was measured in CF_3COOD–DMSO-d_6 (1:9). In the less shielded region, the signals at δ8.33 (1H, *dd*, J = 8.6, 1.8 Hz), 8.03 (1H, *d*, J = 1.8 Hz) and 7.06 (1H, *d*, J = 8.6 Hz) revealed the aglycone moiety to be cyanidin, which was supported by the bathochromic shift of the visible maximum after adding aluminium chloride. Two 2H *ortho*-coupling proton signals at δ7.52 and 6.62, and two doublets at δ6.85 and 5.79 with coupling constant of 12.8 Hz indicated an acyl group to be *Z*-*p*-coumaroyl moiety. Concerning the proton NMR signals of the sugar part, three anomeric protons were observed at δ5.72 (*d*, J = 7.3 Hz), 5.13 (*d*, J = 7.9 Hz) and 4.72 (*d*, J = 7.9 Hz), which showed that **2** had 3 mol of sugar residues (A–C). Irradiation of the signal at δ5.72 yielded a negative NOE for the signal at

δ8.65 (H-4), which allowed the doublet at δ5.72 to be assigned to H-1 of sugar A attached to C-3 of cyanidin. Similarly, irradiation of the signal at δ5.13 caused a negative NOE on H-6 (δ6.97), indicating that this anomeric proton signal was assigned at H-1 of sugar B. The chemical shift of H-1 (δ4.72) of sugar C was associated with a sugar–sugar linkage. All assignment of sugar moieties was based upon ^{1}H–^{1}H COSY and double resonance experiments, which allowed sugars A–C to be corresponding to β-D-glucopyranoside, β-D-glucopyranoside and β-D-xylopyranoside, respectively. The observation of downfield shift of H-2 of glucose A and a negative NOE between H-2 (glucose A) and H-1 (xylose C) confirmed that the xylose ring was bonded to C-2 of glucose A. Furthermore, the methylene peaks at δ4.21 (1H, *dd*, *J* = 12.2, 8.6 Hz) and 4.43 (1H, *br d*, *J* = 12.2 Hz) assigned to H-6 of glucose A showed that a *Z*-*p*-coumaroyl group is attached to the C-6 hydroxyl group of glucose A. From these data, the structure of **2** was confirmed to be cyanidin 3-*O*-(6-*O*-*Z*-*p*-coumaroyl-2-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside)-5-*O*-β-D-glucopyranoside.

Table 1. ^{1}H NMR spectral data of **2** and **3** [CF_3COOD: $(CD_3)_2SO$ = 1:9; δ_H *J* (ppm) (Hz)]

	2	**3**
Cyanidin		
4	8.65 *s*	8.82 *s*
6	6.97 *d* 1.8	6.70 *d* 1.8
8	7.00 *d* 1.8	6.88 *d* 1.8
2′	8.03 *d* 1.8	7.98 *d* 2.4
5′	7.06 *d* 8.6	7.03 *d* 9.2
6′	8.33 *dd* 8.6, 1.8	8.33 *dd* 9.2, 2.4
Glucose-A		
1	5.72 *d* 7.3	5.71 *d* 7.3
2	4.03 *dd* 8.5, 7.3	3.94 *dd* 8.5, 7.3
3	3.72 *dd* 9.8, 8.5	3.72 *dd* 9.2, 8.5
4	3.37 *dd* 9.8, 9.8	3.39 *dd* 9.2, 9.2
5	3.97 *br dd* 9.8, 8.6	3.97 *ddd* 9.2, 7.9, 1.4
6a	4.21 *dd* 12.2, 8.6	4.18 *dd* 12.2, 7.9
6b	4.43 *br d* 12.2	4.48 *br d* 12.2
Glucose-B		
1	5.13 *d* 7.9	
2	3.55 *dd* 9.2, 7.9	
3	3.40 *dd* 9.2, 9.2	
4	3.30 *dd* 9.2, 9.2	
5	3.55 *dd* 9.2, 4.9	
6a	3.66 *dd* 11.6, 4.9	
6b	3.85 *br d* 11.6	
Xylose-C		
1	4.72 *d* 7.9	4.70 *d* 7.9
2	3.00 *dd* 8.5, 7.9	3.04 *dd* 8.5, 7.9
3	3.14 *dd* 9.8, 8.5	3.15 *dd* 9.8, 5.5
4	3.23 *ddd* 9.8, 9.8, 5.5	3.28 *ddd* 9.8, 9.8, 8.5
5a	3.52 *dd* 11.4, 5.5	3.56 *dd* 11.6, 5.5
5b	2.93 *dd* 11.4, 9.8	2.98 *dd* 11.6, 9.8
p-Coumaroyl		
2,6	7.52 *d* 9.2	7.39 *d* 8.5
3,5	6.62 *d* 9.2	6.80 *d* 8.5
7	6.85 *d* 12.8	7.45 *d* 15.9
8	5.79 *d* 12.8	6.28 *d* 15.9

Compound **3** revealed a visible maximum at 530 nm with 22% of E_{440}/E_{530} and 88% of E_{310}/E_{530} in the UV-vis spectrum, which suggested that **3** was a 3-glycosylated anthocyanin with monoacyl moiety. SIMS measurement gave a molecular size of 727 *m/z* in agreement with $C_{35}H_{35}O_{17}$. The ^{1}H NMR measurement showed the presence of cyanidin as an aglycone and *E*-*p*-coumaroyl group as an acyl residue in the molecule. As to a sugar part, the signals corresponding to 2-*O*-β-D-xylopyranosyl-β-D-glucopyranosyl (sambubiosyl) moiety were observed in the ^{1}H NMR spectrum (Table 1). The presence of the methylene signals at δ4.18 (1H, *dd*, *J* = 12.2, 7.9 Hz) and 4.48 (1H, *br d*, *J* = 12.2 Hz) indicated that the C-6 hydroxyl group of glucose A was acylated by *E*-*p*-coumaroyl group. In the less shielded resonance, the upfield shifts of H-6 (δ6.70, *d*, *J* = 1.8 Hz) and H-8 (δ6.88, *d*, *J* = 1.8 Hz) compared with those of **1** and **2** confirmed that the C-5 hydroxyl group of cyanidin was free. Thus, **3** was cyanidin 3-*O*-(6-*O*-*E*-*p*-coumaroyl-2-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside.

The major anthocyanin in *S. canadensis* is **1**, having a glucosyl residue on C-5 and a *p*-coumaroyl group. As regards *S. nigra*, cyanidin 3-sambubioside and 3-glucoside, having free hydroxyl groups on C-5 and no acyl groups, are the main pigments [1, 3]. It is deduced that the difference of stability to light and heat between *S. canadensis* and *S. nigra* results from the structures of the major anthocyanins.

EXPERIMENTAL

General. ^{1}H NMR: 400 MHz, CF_3COOD–DMSO-d_6 (1:9), TMS as int. standard; SIMS in the negative ion mode: *m*-nitrobenzyl alcohol (matrix); CC: MCI gel (Mitsubishi Chem.), ODS (Fuji Silysia); HPLC: Develosil ODS-5 (4.6 × 250 mm) with a flow rate of 1.0 ml min^{-1} monitoring at 525 nm, solvent system, a linear gradient elution for 20 min from 10 to 25% MeCN in 4% H_3PO_4 in H_2O and for 10 min 25% MeCN in 4% H_3PO_4 in H_2O.

Extraction and isolation. Ripe fruits of *S. canadensis* (100 g), collected in Toronto, Canada, in 1989, were extracted (× 5) with 0.1% conc. HCl in MeOH at room temp. After filtration and evapn of the combined extracts, the residue was washed with EtOAc and then subjected to MCI gel CC eluted with H_2O and subsequently with MeOH. The fr. eluted with MeOH was analysed by HPLC to detect 7 anthocyanins. This fr. was chromatographed with MCI gel to give 3 frs eluting with mixts of H_2O–MeOH. Fr. 2, eluted with H_2O–MeOH (6:4), was chromatographed repeatedly with ODS gel (4% H_3PO_4 in H_2O–MeCN) to give **1** and **2**. Fr. 3, eluted with MeOH, was purified by rechromatography with ODS column using MeCN: 1% TFA in H_2O to give **3**.

Compound **2**. Yield 7 mg. UV-vis $\lambda^{0.1\%\ \mathrm{HCl-MeOH}}$ nm (log ε): 281.0 (4.33), 296.0 (4.29), 532.0 (4.49). SIMS: 887 $[M - 2]^-$.

Compound **3**. Yield 8 mg. UV-vis $\lambda_{\max}^{0.1\%\ \mathrm{HCl-MeOH}}$ nm (log ε): 284.5 (4.45), 310.5 (4.35), 529.5 (4.40). SIMS: 725 $[M-2]^-$.

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