Stability of Anthocyanins of *Sambucus canadensis* and *Sambucus nigra*

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Elderberry anthocyanins (*Sambucus nigra*) including acylated derivatives (*Sambucus canadensis*) were studied for use as beverage pigments. Cyanidin 3-*O*-(6-*O*-*E*-*p*-coumaroyl-2-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside-5-*O*-*β*-D-glucopyranoside from *S. canadensis* was more stable than cyanidin 3-sambubioside from *S. nigra*. Acylation improved both heat and light stability, whereas glycosidation only stabilized anthocyanins in the presence of light. Cyanidin 3-(*E*)-*p*-coumaroyl-sambubioside-5-glucoside changed to three new anthocyanins under light irradiation. These three anthocyanins were isolated, and their structures were identified to be cyanidin 3-*O*-(6-*O*-*Z*-*p*-coumaroyl-2-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside-5-*O*-*β*-D-glucopyranoside, cyanidin 3-*O*-(6-*O*-*E*-*p*-coumaroyl-2-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside, and cyanidin 3-*O*-(6-*O*-*Z*-*p*-coumaroyl-2-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside.

Keywords: *Sambucus nigra; S. canadensis; Caprifoliaceae; elderberry; color stability; acylated anthocyanin*

INTRODUCTION

Anthocyanins are alternatives to synthetic dyes that have been controlled by governmental agencies controlling foods. These natural, largely red pigments often have reasonably high molar extinction coefficients and appealing colors and are considered safe to consume. However, anthocyanins have not been used in many foods and beverages because they are not as stable as synthetic dyes to heat, light, and high pH (Palamidis, 1975; Clydesdale, 1978). Therefore, for coloring foods, anthocyanins that are highly stable against heat, light, metal, and so on are required. Many efforts have been made to search for highly stable anthocyanins from fruit and vegetable sources (Robinson, 1966; Van Buren, 1968; Brouillard, 1981; Bass, 1987; Teh, 1988; Francis, 1989) and to improve stability of anthocyanins (Sweeny, 1981).

Elderberry has been used to color jams, jellies, and wines in Europe. Elderberry varieties include ~20 species belonging to the genus *Sambucus* in the family Caprifoliaceae. Among these species, *S. nigra* and *S. canadensis* are presently used in foods as coloring materials because of their high yields and anthocyanin content. Recently, we found that the color of *S. canadensis* is more stable to heat and light than that of *S. nigra* in model beverages, which prompted us to find the reason for the difference in pigment stability between *S. nigra* and *S. canadensis*. The stability of the anthocyanin pigment of *S. nigra* was investigated under various conditions of temperature and light (Brønnum-Hansen, 1983, 1985; Drdak, 1985), but there is no report on the stability of the anthocyanin pigment of *S. canadensis*. Several anthocyanins were isolated from both of these species (Reichel, 1977; Harborne, 1967; Hovlíková, 1987; Johansen, 1991; Nakatani, 1995), indicating the difference in anthocyanin composition between the two species.

Our objective was to compare the stability of pigments in *S. nigra* and *S. canadensis* as well as the stability of their major anthocyanins used in model beverages and to investigate how the structural difference of anthocyanins is associated with their stability.

MATERIALS AND METHODS

Extraction of Elderberry Anthocyanins. Ripe fruits of *S. nigra* (500 g), collected in Poland in 1991, and *S. canadensis* (500 g), collected in Toronto, Canada, in 1989, were individually extracted with 2 L of 0.1% HCl in MeOH at room temperature. After five repeated extractions, the combined extracts were filtered under reduced pressure through Whatman No. 2 filter paper and the filtrate was then evaporated under reduced pressure. The resulting residue was adsorbed onto highly porous polymer resin made from styrene divinylbenzene (SEPABEADS SP-207, Mitubishi Kasei Company, Ltd., Japan), packed in a glass column (50 × 240 mm), and then washed with 1 L of distilled water and eluted from the column with 600 mL of 1% TFA in MeOH solution. The eluted solutions were concentrated to dryness on a rotary evaporator at 40−50 °C, yielding crude pigments *S. nigra* (SN-extract; 15.3 g; $E^{1\%}_{1cm}$, 103) and *S. canadensis* (SC-extract; 7.3 g; $E^{1\%}_{1cm}$, 50.2).

HPLC Analysis. A modular HPLC system (Waters, Milford, MA) consisting of a pump with a system controller (600E), a photodiode array detector (991J), and computing integrator (PC-9801RA, NEC, Japan) was used. The column (4.6 × 250 mm) was a 5-*μ*m Capcell Pak C18 (Shiseido, Japan), and 0.5% (v/v) phosphoric acid in water (A) and 0.5% phosphoric acid in 60% tetrahydrofuran (B) were used as solvents. A linear gradient between 10% B and 100% B over a period of 30 min at a flow of 1.0 mL/min was established. Detection was made at 520 nm because of the highest sensitivity of the SC-extract at 520 nm. SN-extract and SC-extract (100 mg each) were dissolved in 50 mL of 1% TFA MeOH and filtered through a 0.45-*μ*m membrane filter (Ekicrodisc 13, Gelman Science, Germany). Then, 10 *μ*L of the filtrate was injected into the analytical HPLC system.

Isolation of Elderberry Anthocyanins. Preparative HPLC was performed with a Capcell Pak C18 20 × 250 mm

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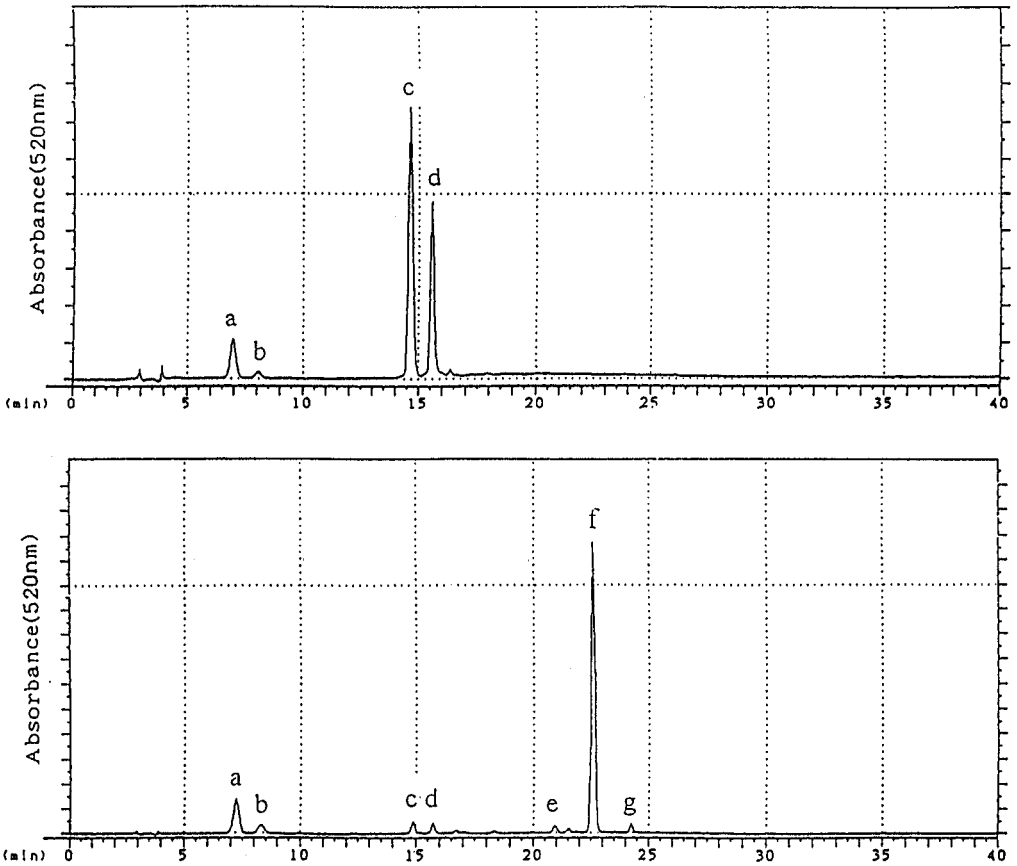

Figure 1. HPLC chromatograms of SN-extract (top) and SC-extract (bottom): (a) cyanidin 3-sambubioside-5-glucoside (**III**); (b) cyanidin 3,5-diglucoside; (c) cyanidin 3-sambubioside (**I**); (d) cyanidin 3-glucoside; (e) cyanidin 3-*O*-(6-*O*-*Z*-*p*-coumaroyl-2-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside-5-*O*-*β*-D-glucopyranoside; (f) cyanidin 3-*O*-(6-*O*-*E*-*p*-coumaroyl-2-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside-5-*O*-*β*-D-glucopyranoside (**II**); (g) cyanidin 3-*O*-(6-*O*-*E*-*p*-coumaroyl-2-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside.

(Shiseido, Japan) column. Solvent A was 0.5% (v/v) phosphoric acid in water and solvent B was 0.5% phosphoric acid in 60% tetrahydrofuran. The separation consisted of a linear gradient between 10% B and 100% B over a period of 50 min at a flow rate of 9.0 mL/min. The SN-extract and SC-extract (1.2 g of each) were individually dissolved with 25 mL of 1% TFA MeOH and filtered through a 0.45-μm membrane filter. Then 300 μL of the filtrate was injected into the preparative HPLC system. Four anthocyanins, corresponding to peaks a−d, and seven anthocyanins, corresponding to peaks a−g, were isolated from SN-extract and SC-extract, respectively (Figure 1).

Heat Stability Test on Anthocyanins. The absorbance of the model beverage [12% sugar, 0.15% citric acid, and 0.017% sodium citrate (w/v)] at pH 3.0 was adjusted to 1.0 at 520 nm with SN-extract (97 mg/L), SC-extract (199 mg/L), and compounds **I** (153 mg/L; $E^{1\%}_{1cm}$, 65), **II** (120 mg/L; $E^{1\%}_{1cm}$, 83), and **III** (187 mg/L; $E^{1\%}_{1cm}$, 53), respectively. These colored model beverages were sterilized at 90 °C and 30 s and hot packed in a transparent bottle. After cooling, the bottles were kept in dark at 50 °C. The absorbance of the beverage was measured at 520 nm with a JASCO Ubest-50 spectrophotometer (Tokyo, Japan) at two intervals during the storage period. Pigment retention was calculated as a percentage of absorption at zero storage time.

Light Stability Test. The model beverages just mentioned were exposed to direct sun rays for several days of fine weather from June to October in 1993, at our laboratory in Japan. To measure the energy of sunlight, a pyranometer (MS-801; Eko Instrument, Japan) was used. A pyranometer is an instrument for measuring radiation energy in the ultraviolet, visible, and infrared spectral band (300−2800 nm). The absorbance of the samples was read at 520 nm at two intervals during the storage period. Pigment retention was calculated as a percentage of absorption at zero storage time.

Isolation of Compounds IV−VI. After the model beverage colored by **II** (120 mg/L) was exposed to direct sun rays at 32 MJ/m^2, one portion of it was analyzed by HPLC (Figure 5), and the remainder (1 L) was adsorbed on SEPABEADS SP-207, washed with water, then eluted from the column with 1% trifluoroacetic acid in MeOH (v/v). The eluted solution was evaporated to dryness, yielding 72 mg of residue. This residue was dissolved in 2 mL of methanol and subjected to preparative HPLC under the same condition as just mentioned to yield three compounds (**IV**, 8.3 mg; **V**, 5.8 mg; and **VI**, 18.4 mg).

UV−Vis Spectra of Compounds II and IV at pH 3.0. Dried 6.0-mg samples of **II** and **IV** were dissolved in 50 mL of 0.05 M Michaelis buffer solution (sodium tartrate−tartaric acid, pH 3.0). The absorbance of the sample was read at 520 nm with a spectrophotometer. UV−vis spectra of these solution at pH 3.0 were measured with the same instrument and sample cells of 1 cm length (Figure 8). All measurements were done at room temperature.

Instruments for Structure Determination. UV−vis absorption spectra were determined with a Hitachi 220 spectrophotometer after each sample was dissolved in 0.1% HCl in MeOH. The ^{1}H NMR spectra of anthocyanins dissolved with DMSO-d_6:TFA-d_1 (9:1) were measured with a JEOL GX-400 spectrometer (400 MHz) with tetramethylsilane (TMS) as an internal standard. Secondary-ionization mass spectra (SIMS) in the negative ion mode were obtained with a Hitachi M-2000 mass spectrometer. The matrix used was a *m*-nitrobenzyl alcohol.

RESULTS AND DISCUSSION

As shown in Figure 1, SN-extract contains four anthocyanins and SC-extract contains seven. The anthocyanins were isolated by preparative HPLC and their

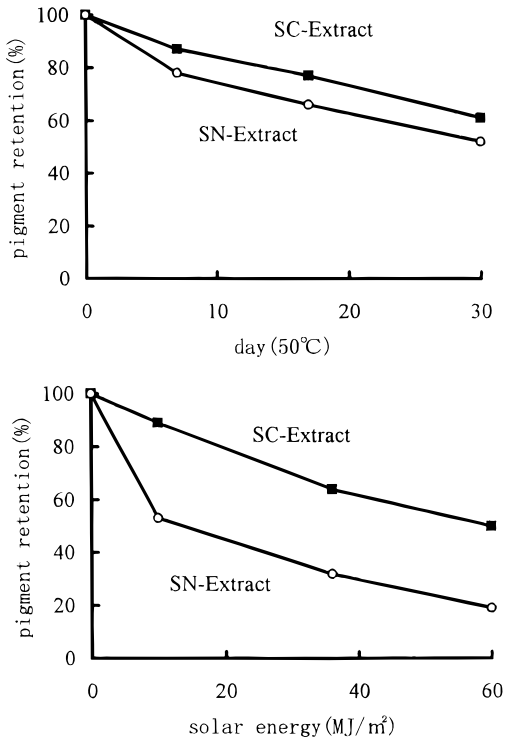

Figure 2. Color stability of elderberries: (○) SN-extract; (■) SC-extract. (Top) Heat stability: the absorbance of the beverage was measured at 520 nm on the 7th, 17th, and 30th days. (Bottom) Light stability: measured at 10, 35, and 60 MJ/m^2 solar energy.

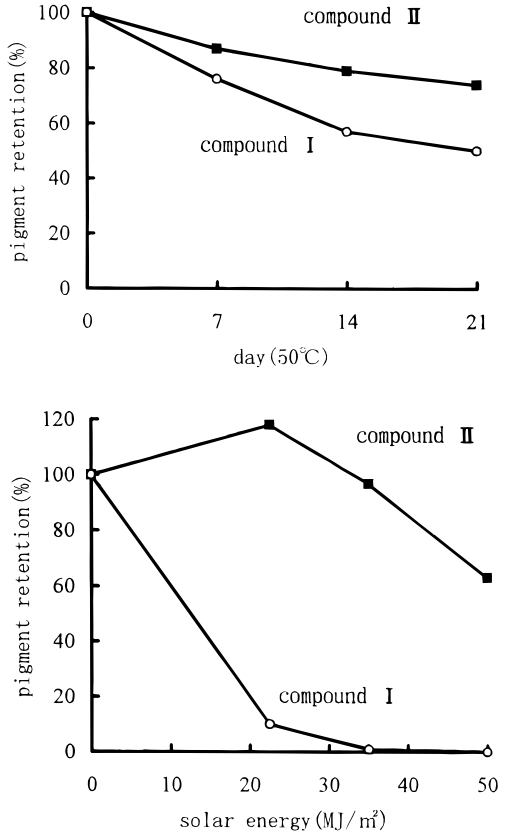

Figure 3. Color stability of **I** and **II**: (○) **I**; (■) **II**. (Top) Heat stability: the absorbance of the beverage was measured at 520 nm on the 7th, 14th, and 21st days. (Bottom) Light stability: measured at 23, 35, and 50 MJ/m^2 solar energy.

structures were identified by spectral analysis and comparison of spectra with those of authentic samples (Johansen et al., 1991; Nakatani et al., 1995). The main anthocyanins of SN-extract were cyanidin 3-sambubioside (**I**; peak c) and cyanidin 3-*O*-*β*-D-glucopyranoside (peak d). A major anthocyanin of SC-extract was cyanidin 3-*O*-(6-*O*-*E*-*p*-coumaroyl-2-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside-5-*O*-*β*-D-glucopyranoside (**II**; peak f), which was not detected at all in SN-extract. In general, most anthocyanins of *S. nigra* have a free hydroxyl group on C-5 and no acyl group in their molecules, whereas a major anthocyanin of *S. canadensis* has a glucosyl residue on C-5 and is acylated by a *p*-coumaroyl group.

To measure color stability, the model beverages (pH 3.0) with SN- and SC-extracts were prepared (mentioned under Materials and Methods). The color stability against heat of each model beverage was measured after the beverage had been kept at 50 °C in the dark for a specific number of days. After 30 days, the pigment retention of SN- and SC-extracts decreased to 50 and 60%, respectively (Figure 2). Regarding stability to sunlight, the pigment retention of SN-extract fell to 20% after a total of 60 MJ/m^2 of irradiation, whereas that of SC-extract remained as much as 50% under the same conditions (Figure 2). These observations indicate that SC-extract is more stable against both heat and sunlight than SN-extract and that SC-extract displays particularly remarkable stability against sunlight. It is possible that the difference in stability to heat and light between SN- and SC-extracts may depend on the structure of their individual anthocyanins.

To clarify this possibility, stability was evaluated with **I** and **II**, major anthocyanins of *S. nigra* and *S. canadensis*, respectively. The pigment retention in the heat test of **I** was 50% and that of **II** was 80% at the 21st day. Compound **I** was extremely unstable to sunlight. When irradiated with sunlight to a total of 20 MJ/m^2, the pigment retention of **I** fell to 20%, and at a total of 35 MJ/m^2, pigment faded out completely. In the case of **II**, even after irradiation to a total of 50 MJ/m^2, the pigment still remained at the concentration of 60% (Figure 3). These results indicate that the difference of stability to heat and light between SN- and SC-extracts is due to the structural characteristics of anthocyanins. It was suggested that acylation by a coumaroyl group onto the anthocyanin molecule and/or glycosidation on C-5 improved the stability of the color of SC-extract.

Compound **III**, a component of both *S. nigra* and *S. canadensis*, has a glucose moiety on C-5 and no acyl group. To investigate the effect of glycosidation of C-5, the color stability of **III** to heat and light was measured and compared with that of **I**. As shown in Figure 4, very little difference was observed between **I** and **III** in heat stability. This observation suggests that glycosidation on C-5 is not a crucial factor for heat stability but acylation plays an important role in heat stability. Unlike the case of heat stability, **III** was more stable than **I** to sunlight, suggesting that glycosidation on C-5, as well as acylation, has some influence on color stability to light.

Van Buren and co-workers reported the stability of anthocyanins in wine exposed to light (Van Buren et al., 1968). The acylated diglucosides were the most stable, the monoglucosides were the least stable, and the nonacylated diglucosides were intermediate. Our results are in good agreement with theirs. In the UV−

Table 1. UV−Vis and SIMS Spectral Data for IV, V, and VI

spectrum	IV	V	VI
UV−vis λ max. (nm)	532 (lot $\epsilon = 4.49$)	528 (log $\epsilon = 4.62$)	530 (log $\epsilon = 4.40$)
	296 (log $\epsilon = 4.29$)	314 (log $\epsilon = 4.43$)	311 (log $\epsilon = 4.35$)
	281 (log $\epsilon = 4.33$)	294 (log $\epsilon = 4.57$)	285 (log $\epsilon = 4.45$)
		282 (log $\epsilon = 4.62$)	
SIMS	887[M-2]$^-$	725[M-2]$^-$	725[M-2]$^-$

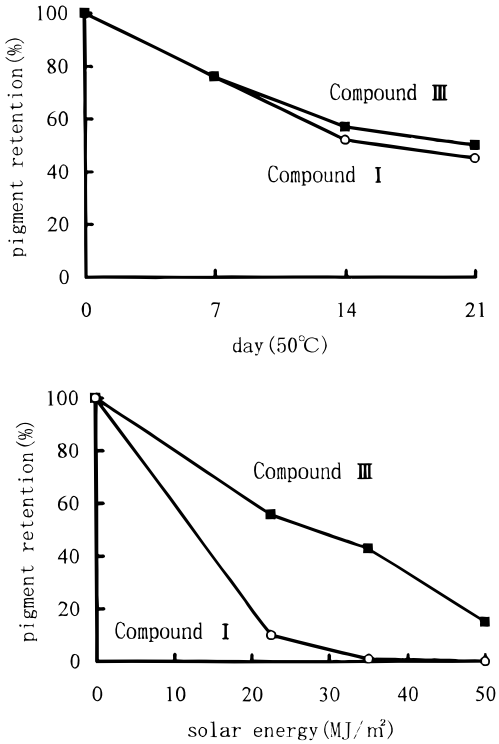

Figure 4. Color stability of **I** and **III**: (○) **I**; (■) **III**. (Top) Heat stability: the absorbance of the beverage was measured at 520 nm on the 7th, 14th, and 21st days. (Bottom) Light stability: measured at 23, 35, and 50 MJ/m^2 of solar energy.

vis spectra of diluted wine, the absorbance of Ives wine (anthocyanins of Ives wine are malvidin diglucoside and acylated malvidin diglucoside) at 530 nm increased slightly after exposure to light, but it was not made clear why this occurred. A similar phenomenon was observed in our stability test. As shown in Figure 3, the optical intensity of **II** increased over 100% in the early stages of sunlight exposure. This finding suggests some chemical changes might have occurred in the molecule.

The HPLC chromatogram shown in Figure 5 (monitored at 520 nm) indicates the appearance of three new anthocyanins (**IV**−**VI**) after sunlight irradiation (32 MJ/m^2) of the model beverage colored with **II**. Variation of the peak area at 520 nm of **II** and **IV**−**VI** up to total 9 MJ/m^2 of solar energy is shown in Figure 6. At the very early stages, **II** decreased rapidly, whereas **IV** was dramatically produced. Concurrently, the residual color reached 130%. Then, both **II** and **IV** gradually decreased, and **V** and **VI** appeared progressively.

To elucidate the structures of **IV**−**VI**, these three compounds were purified by repeated preparative HPLC. The structures of the isolated compounds were determined with spectroscopic evidence.

The spectral data of UV−vis, SIMS, and ^{1}H NMR of **IV**−**VI** are shown in Tables 1 and 2. From these data, **IV** and **VI** were identified as 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, respectively (Nakatani et al., 1995).

Compound **V** revealed a visible maximum at 528 nm with 25% of E_{440}/E_{528} in the UV−vis spectrum, which indicated that **V** is a 3-glycosylated anthocyanin (Harborne, 1958). Negative SIMS measurement gave a molecular size of 727 *m*/*z*, corresponding to $C_{35}H_{35}O_{17}$. In the less shielded region in the ^{1}H NMR spectrum, a singlet at δ 8.78 was assigned to H-4 on anthocyanidin skeleton. Two doublets coupled to each other with a coupling constant of 1.8 Hz were observed at δ 6.75 and 6.83. In double-resonance experiments, a long-range coupling between H-4 and the doublet at δ 6.83 indicated that the signal of δ 6.83 is attributable to H-8 and that of δ 6.75 is attributable to H-6. Three aromatic proton signals at δ 7.06 (1H, d, $J = 8.5$ Hz), 8.00 (1H, d, $J = 1.8$ Hz) and 8.30 (1H, dd, $J = 8.5$, 1.8 Hz) showed the presence of a 1,2,4-trisubstituted benzene ring, indicating the aglycon moiety to be cyanidin. The upfield shifts of H-6 and H-8 compared with those of **IV** confirmed that C-5 hydroxyl group of cyanidin is free. Two 2H ortho-coupling proton signals at δ 7.53 and δ

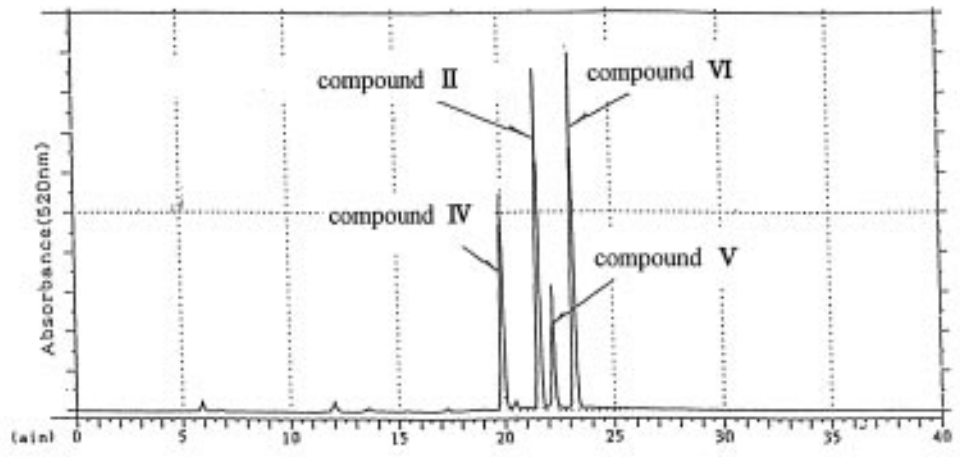

Figure 5. HPLC chromatogram monitored at 520 nm after sunlight irradiation (32 MJ/m^2) to a model beverage of **II**.

Table 2. ^{1}H-NMR Spectral Data[a]

	II	IV	V	VI
cyanidin				
4	8.78 s	8.65 s	8.78 s	8.82 s
6	7.01 d 1.8	6.97 d 1.8	6.75 d 1.8	6.70 d 1.8
8	7.04 d 1.8	7.00 d 1.8	6.83 d 1.8	6.88 d 1.8
2′	8.05 d 2.5	8.03 d 1.8	8.00 d 1.8	7.98 d 2.4
5′	7.07 d 9.2	7.06 d 8.6	7.06 d 8.5	7.03 d 9.2
6′	8.31 dd 9.2, 2.5	8.33 dd 8.6, 1.8	8.30 dd 8.5, 1.8	8.33 dd 9.2, 2.4
glucose-A				
1	5.73 d 7.3	5.72 d 7.3	5.69 d 7.9	5.71 d 7.3
2	4.02 dd 9.2, 7.3	4.03 dd 8.5, 7.3	3.96 dd 8.5, 7.9	3.94 dd 8.5, 7.3
3	3.77 t 9.2	3.72 dd 9.8, 8.5	3.71 dd 9.4, 8.5	3.72 dd 9.2, 8.5
4	3.47 t 9.2	3.37 t 9.8	3.38 t 9.4	3.39 t 9.2
5	4.0 m	3.97 br dd 9.8, 8.6	3.9 m	3.97 ddd 9.2, 7.9, 1.4
6a	4.33 dd 12.2, 9.2	4.21 dd 12.2, 8.6	4.26 dd 12.2, 7.9	4.18 dd 12.2, 7.9
6b	4.42 br d 12.2	4.43 br d 12.2	4.45 br d 12.2	4.48 br d 12.2
glucose-B				
1	5.09 d 7.9	5.13 d 7.9		
2	3.5−3.6 m	3.55 dd 9.2, 7.9		
3	3.40 t 8.5	3.40 t 9.2		
4	3.33 dd 9.8, 8.5	3.30 t 9.2		
5	3.5−3.6 m	3.55 dd 9.2, 4.9		
6a	3.5−3.6 m	3.66 dd 11.6, 4.9		
6b	3.81 br d 10.4	3.85 br d 11.6		
xylose-C				
1	4.73 d 7.3	4.72 d 7.9	4.71 d 7.3	4.70 d 7.9
2	3.04 dd 8.5, 7.3	3.00 dd 8.5, 7.9	3.03 dd 8.6, 7.3	3.04 dd 8.5, 7.9
3	3.17 t 8.5	3.14 dd 9.8, 8.5	3.16 dd 9.8, 8.6	3.15 dd 9.8, 8.5
4	3.28 m	3.23 td 9.8, 5.5	3.28 td 9.8, 5.5	3.28 td 9.8, 5.5
5a	3.5−3.6 m	3.52 dd 11.4, 5.5	3.52 dd 11.0, 5.5	3.56 dd 11.6, 5.5
5b	2.99 dd 11.0, 9.8	2.93 dd 11.4, 9.8	2.95 dd 11.0, 9.8	2.98 dd 11.6, 9.8
p-coumaroyl				
2″,6″	7.40 d 8.6	7.52 d 9.2	7.53 d 9.2	7.39 d 8.5
3″,5″	6.79 d 8.6	6.62 d 9.2	6.66 d 9.2	6.80 d 8.5
7″	7.39 d 15.9	6.85 d 12.8	6.72 d 12.8	7.45 d 15.9
8″	6.29 d 15.9	5.79 d 12.8	5.76 d 12.8	6.28 d 15.9

[a] [$(CD_3)_2SO$:CF_3COOD = 9:1; 400 MHz; room temp.; $\delta_H J$(ppm) (Hz)].

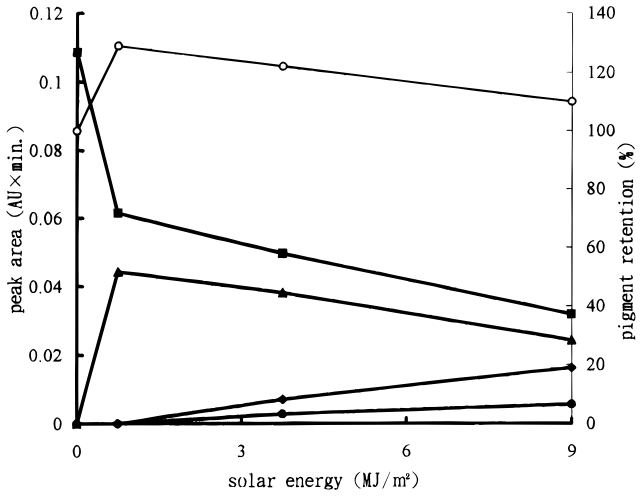

Figure 6. Conversion profile of **II** by sunlight irradiation recorded at 520 nm: (■) **II**; (▲) **IV**; (●) **V**; (◆) **VI**; (○) pigment retention at 520 nm.

6.66, and two doublets at δ 6.72 and 5.76 with a coupling constant of 12.8 Hz revealed the presence of a *Z*-*p*-coumaroyl group as an acyl moiety. As to a sugar part, two anomeric protons were observed at δ 5.69 (d, J = 7.9 Hz) and 4.71 (d, J = 7.3 Hz), which showed that two sugar residues are present in the molecule. The NOE between the signal at δ 5.69 and H-4 (δ 8.78) allowed the doublet at δ 5.69 to be assigned to H-1 of sugar A attached to C-3 of cyanidin. Assignment of proton of sugar A [δ 5.69 (1H, d, J = 7.9 Hz, H-1), 3.96 (1H, dd, J = 8.5, 7.9 Hz, H-2), 3.71 (1H, dd, J = 9.4, 8.5 Hz, H-3), 3.38 (1H, t, J = 9.4 Hz, H-4), 3.9 (1H, m, H-5), 4.26 (1H, dd, J = 12.2, 7.9 Hz, H-6a), and 4.45 (1H, br d, J = 12.2 Hz, H-6b)] was based on H-H COSY experiment, indicating that sugar A is β-glucopyranose. The NOE between another anomeric proton (δ 4.71) and H-2 of sugar A showed that another sugar moiety (sugar C) is bonded to C-2 of sugar A. H-H COSY data indicated that sugar C is xylopyranose [δ 4.71 (1H, d, J = 7.3 Hz, H-1), 3.03 (1H, dd, J = 8.6, 7.3 Hz, H-2), 3.16 (1H, dd, J = 9.8, 8.6 Hz, H-3), 3.28 (1H, td, J = 9.8, 5.5 Hz, H-4), 3.52 (1H, dd, J = 11.0, 5.5 Hz, H-5a), and 2.95 (1H, dd, J = 11.0, 9.8 Hz, H-5b)]. Thus, the sugar part of **V** is 2-O-β-D-xylopyranosyl-β-D-glucopyranose. The observation of methylene signals at δ 4.26 and 4.45 revealed that the C-6 hydroxyl group of sugar A is acylated by *Z*-*p*-coumaroyl group. From these spectral data, **V** was determined to be cyanidin 3-*O*-(6-*O*-*Z*-*p*-coumaroyl-2-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside (Figure 7).

Based on this finding, light irradiation results in rapid isomerization from *E* form (**II**) to *Z* form (**IV**) of *p*-coumaroyl group and successive deglucosidation on C-5 from these two isomers. The UV−vis spectra for **II** and **IV** in a buffer solution at pH 3.0 are shown in Figure 8. Compound **IV** (*Z* form) had 2.4 times as much absorbance as **II** (*E* form) at 520 nm. This finding might explain why the absorbance increased in the early stages of light exposure.

It is concluded from the results of this study that

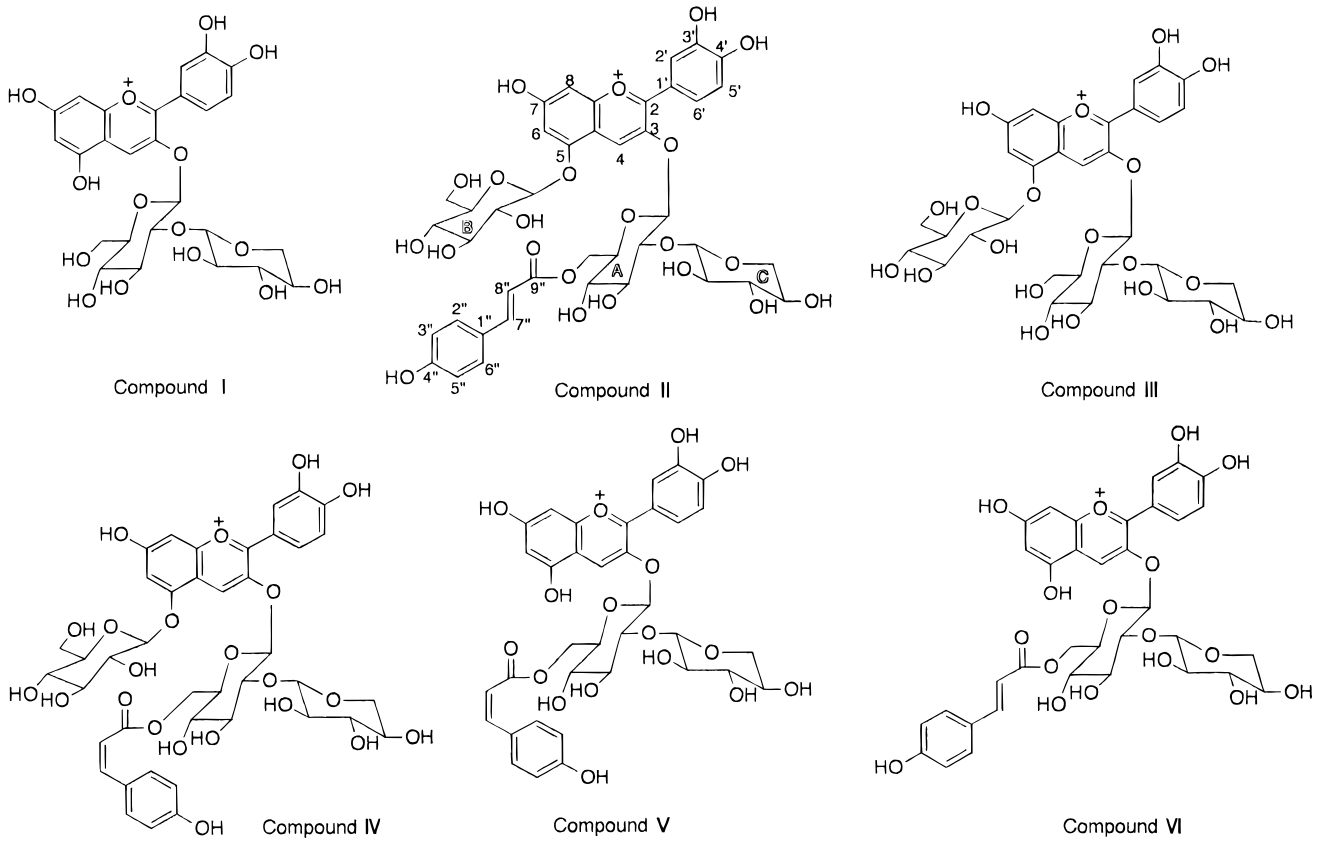

Figure 7. Chemical structures of **I**–**VI**.

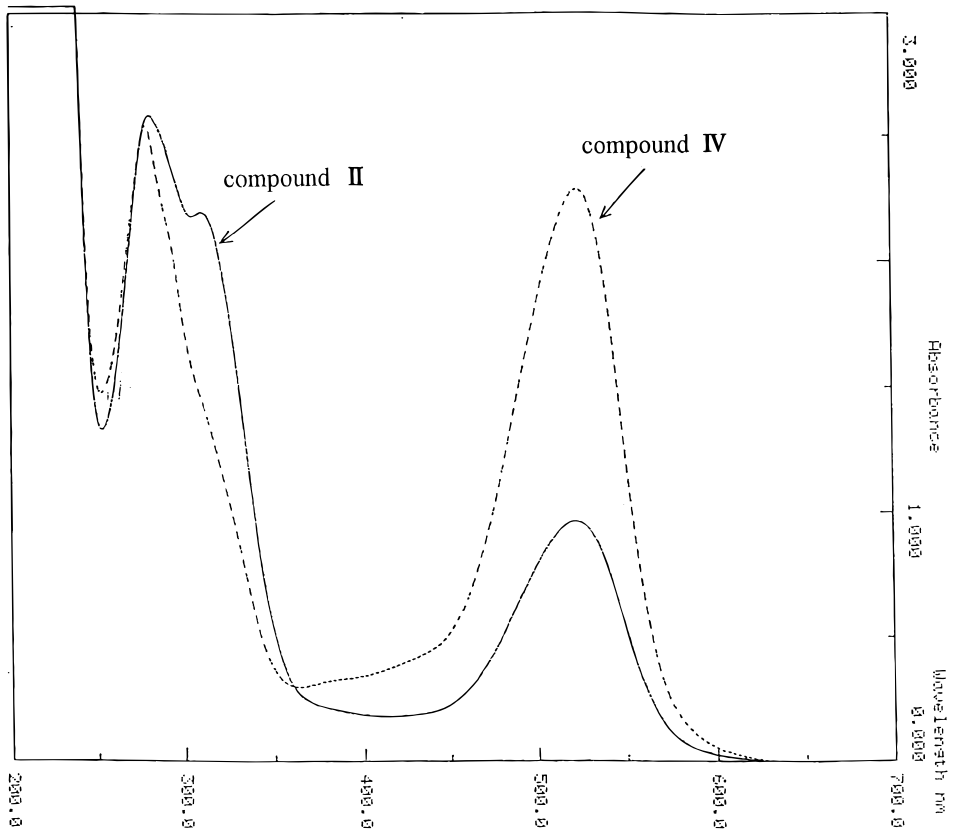

Figure 8. UV–vis spectra of **II** (—) and **IV** (···).

acylated anthocyanins play an important role in maintaining color in food system and the isomerization from the *E*-form to the *Z*-form of *p*-coumaroyl group contributes to the color value.

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