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Functional New Acylated Sophoroses and Deglucosylated Anthocyanins in a Fermented Red Vinegar

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The new acylated polyphenols were isolated from a red-colored vinegar produced via fermentation with purple-fleshed sweet potato storage roots, and identified mainly by MS and NMR. The three acylated sophoroses were determined as 6-*O*-(*E*)-caffeoyl-(2-*O*-(6-*O*-acyl)-β-D-glucopyranosyl)-D-glucopyranoses, where acyl was (*E*)-caffeoyl, *p*-hydroxybenzoyl, and (*E*)-feruloyl, respectively. The four acylated anthocyanins were also determined as cyanidin 3-*O*-(6-*O*-(*E*)-caffeoyl-(2-*O*-(6-*O*-(*E*)-feruloyl)-β-D-glucopyranosyl)-β-D-glucopyranoside), in addition to peonidin 3-*O*-(6-*O*-(*E*)-caffeoyl-(2-*O*-(6-*O*-acyl)-D-glucopyranosyl)-β-D-glucopyranosides), where acyl was (*E*)-caffeoyl, *p*-hydroxybenzoyl, and (*E*)-feruloyl, respectively. The diacylated sophoroses showed higher antioxidant capacity than that of monoacylated analogue 6-caffeoylsophorose, so the multiacylation established to enhance their antioxidant capacity. Similarly, 5-deglucosylated anthocyanins also gave somewhat stronger antioxidation than corresponding sweet potato anthocyanins. In rat intestinal α-glucosidase inhibition study, the diacylated sophoroses preferably inhibited maltase rather than sucrase with an IC_{50} value of <300 μM, indicating a potential role as antidiabetic phytochemicals. These acylated polyphenols in a red vinegar were expected to play important functional roles for health.

KEYWORDS: Red vinegar; purple-fleshed sweet potato; polyphenolics; diacylated sophoroses; 5-deglucosylated anthocyanins; radical scavenging activity; α-glucosidase inhibition

INTRODUCTION

A fermented red-colored vinegar (red vinegar) produced through acetic fermentation with storage roots of purple-fleshed sweet potato, *Ipomoea batatas* L. cv. Ayamurasaki (*1*), has been applied as healthy drinks, red seasonings and dressings in Japan. The red vinegar contains the constituents from purple-fleshed sweet potato like anthocyanins and caffeoylquinic acids etc. (*2*), and also newly generated compounds which probably derived from original purple-fleshed sweet potato substances during fermentation and/or storage processes.

As shown in **Figure 1**, the purple-fleshed sweet potato anthocyanins (named as "YGM" from the ancestral variety name "Yama Gawa Murasaki") have been identified as peonidin and cyanidin 3-sophoroside-5-glucosides (Pn3S5G and Cy3S5G) based and acylated with one or two phenolic acids such as caffeic acid, *p*-hydroxybenzoic acid and ferulic acid on the 3-sophorosyl moiety (*3*−*5*). Peonidin 3-caffeoylsophoroside-5-glucoside (YGM-5b), and peonidin 3-dicaffeoylsophoroside-5-glucoside (YGM-4b), peonidin 3-caffeoyl-*p*-hydroxybenzoylsophoroside-5-glucoside (YGM-5a) and peonidin 3-caffeoyl-feruloylsophoroside-5-glucoside (YGM-6) are major anthocyanins in the purple-fleshed sweet potato. The YGMs have not only high stability (*6*, *7*) but also multifaceted biological actions including antioxidant capacity (*6*−*9*) and other functions (*10*, *11*) as well as antidiabetic effect (*12*−*15*). In particular, the first finding that the acylated anthocyanins and their acylated moieties could lower the excess postprandial blood glucose level via α-glucosidase inhibition (*13*−*15*) led us to investigate antihyperglycemic potential of other acylated moieties from anthocyanins in this study. In addition, caffeoylquinic acids are also known to exhibit antioxidant capacity (*16*, *17*), and other physiological functions including antihepatotoxic effect (*18*−*20*). Moreover, newly generated compounds are likely to have similar functional roles for health. Thus, the red vinegar is expected to have additive or synergistic physiological effects of these functional components. In this paper, we describe the structural determination of the newly isolated polyphenols from the red vinegar, and their elucidation such DPPH radical scavenging activity and α-glucosidase inhibition activity as an index of antidiabetic potency.

MATERIALS AND METHODS

General Procedures. All experiments were carried out according a previous report (*21*). All reagents and solvents employed were of analytical grade and used without further purification. Analytical high performance

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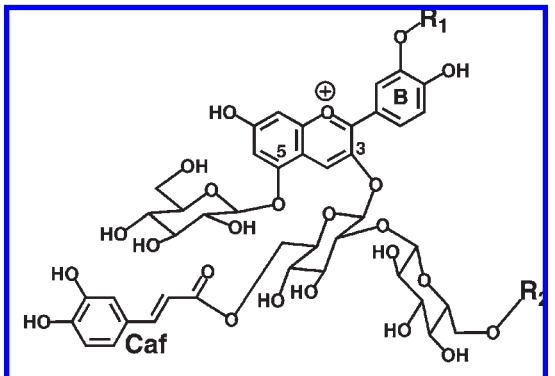

YGM	R_1	R_2
1a	H	*p*-Hydroxybenzoyl
1b	H	Caffeoyl
2	H	H
3	H	Feruloyl
4b	CH_3	Caffeoyl
5a	CH_3	*p*-Hydroxybenzoyl
5b	CH_3	H
6	CH_3	Feruloyl

Figure 1. Structures of the purple-fleshed sweet potato anthocyanins. Caf = caffeoyl residue.

liquid chromatography (HPLC) was run on a PU-2089 intelligent pump system (Jasco Co., Ltd., Tokyo, Japan) equipped with an MD-2010 plus multiwavelength (DAD) detector and a CO-2065 plus intelligent column oven. HPLC was eluted with linear gradient mode for 50 min from 25% to 60% solvent B (0.4% HCOOH, 50% MeCN in H_2O) in solvent A (0.4% HCOOH in H_2O) on a 250 mm × 4.6 mm i.d., 3 μm, Cadenza CD-C18 column (Imtakt Co. Ltd., Kyoto, Japan) at 30 °C with a flow rate of 0.6 mL/min monitoring at 520, 310, or 280 nm. Preparative HPLC was run on an L-7150 intelligent pump system (Hitachi Co., Ltd., Tokyo, Japan) with a UV-970 intelligent UV/vis detector (Jasco) with an isocratic elution of solvent A (0.4% HCOOH in H_2O):solvent B (0.4% HCOOH, 50% MeCN in H_2O) = (65: 35, v/v) on a 250 mm × 30 mm i.d., 5 μm, Inertsil ODS-3 column (GL Sciences Inc., Tokyo, Japan) at room temperature with a flow rate of 7.0 mL/min monitoring at 310 nm for acylated sophoroses and at 520 nm for anthocyanins. UV/vis spectra were recorded on a V-550 UV/vis spectrophotometer (Jasco Co., Ltd.) in MeOH for acylated sophoroses and for 0.01%HCl−MeOH for acylated anthocyanins. The melting point measurement for acylated sophoroses was carried out on a micro melting point apparatus MP-J3 (Yanaco New Science Inc., Kyoto, Japan). Electrospray ionization time-of-flight mass spectrometry (ESI-TOFMS) analysis was performed on a Mariner Workstation system (Applied Biosystems Co., Ltd., CA, USA) in 0.4% HCOOH−50% MeCN. The high-resolution mass spectra were measured using an electrospray ionization Fourier-transform ion-cyclotron resonance mass spectrometry (ESI/FT-ICRMS) on an APEX II 70e (Bruker Daltonics) in aqueous MeOH containing AcOH with a negative mode for acylated sophoroses and a positive mode for anthocyanins. ^{13}C (200 MHz)- and ^{1}H (800 MHz)-nuclear magnetic resonance (NMR) spectra were run on an Avance 800 spectrometer (Bruker BioSpin) in DMSO-d_6−CF_3COOD (9: 1, v/v) with tetramethylsilane as an internal standard at 30 °C.

Isolation of Acylated Polyphenols. Red vinegar produced by Miyazaki JA Food Research & Development Inc. (10 L) was applied on a 450 mm × 100 mm i.d. adsorbed resin, Amberlite XAD-2000 column (Rohm & Haas Co.), washed with 1% HCOOH (10 L), eluted with 1% HCOOH−70% MeOH (5 L), and evaporated *in vacuo* to give the red crude powder (9 g). HPLC analysis of the crude powder detected many UV-absorbing components such as anthocyanins, caffeoylquinic acids, acylated sophoroses etc. (**Figure 2A**), and the peak pattern was similar to the original red-vinegar (**Figure 2B**). The crude powder (8 g) was dissolved

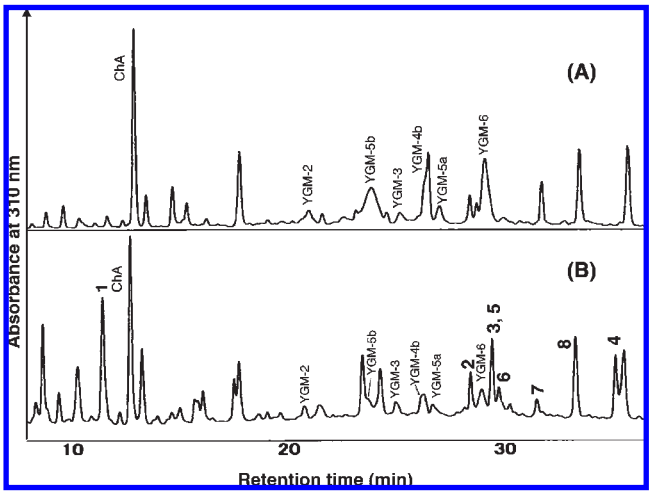

Figure 2. Typical HPLC profiles of a purple-fleshed sweet potato crude pigment (**A**) and a red vinegar (**B**). ChA = chlorogenic acid; YGMs: see Figure**1**.

in 1% HCOOH and applied on a 200 mm × 60 mm i.d., PVPP, Polyclar VT column (ISP Inc.) and fractionated with elution of 1% HCOOH−30% MeOH into seven fractions (frs 1−7). Preparative HPLC separation of the PVPP frs 2−4 and 7 gave four purified compounds **1**−**4**. The evaporation residues were dissolved in the smallest amount of MeOH and precipitated with excess diethyl ether, and then the precipitates were centrifuged and dried to obtain slightly reddish powders, **1** (89 mg), **2** (16 mg), **3** (3 mg) and **4** (11 mg). Similarly, four pigments **5**−**8** from PVPP frs 4−6 were isolated, and the residues were dissolved in the smallest amount of trifluoroacetic acid and precipitated with excess diethyl ether. Four anthocyanins were obtained as red powder of trifluoroacetic acid salts, **5** (6 mg), **6** (14 mg), **7** (26 mg) and **8** (59 mg).

Chemical Analyses. The alkaline hydrolysis of the each compound was done in 2 N NaOH deoxygenated with N_2 gas for 15 min with a sealed cap, and acidified with HCOOH. The components in the reaction mixture were identified by analytical HPLC for phenolic acids.

6-O-(E)-Caffeoyl-2-O- β*-D-glucopyranosyl-*α*-D-glucopyranose* (***1***). Mp 147−150 °C. UV/vis (MeOH) λ_{max} nm: 330 (ε = 17,100). ESI-TOFMS: *m*/*z* 503 $[M-H]^-$. High-resolution ESI/FT-ICRMS *m*/*z* 503.1406 ($[M-H]^-$, calcd for $C_{21}H_{27}O_{14}$, 503.1406 corresponding to caffeoyl-dihexose). ^{13}C and ^{1}H NMR data: see **Table 1**.

*6-O-(E)-Caffeoyl-(2-O-(6-O-(E)-caffeoyl)-*β*-D-glucopyranosyl)-*α*-D-glucopyranose* (***2***). Mp 162−165 °C. UV/vis (MeOH) λ_{max} nm: 330 (ε = 32,000). ESI-TOFMS: *m*/*z* 665 $[M-H]^-$. High-resolution ESI/FT-ICRMS: *m*/*z* 665.1726 ($[M-H]^-$, calcd for $C_{30}H_{33}O_{17}$, 665.1723 corresponding to dicaffeoyl-dihexose). ^{13}C and ^{1}H NMR data: see **Table 1**.

*6-O-(E)-Caffeoyl-(2-O-(6-O-p-hydroxybenzoyl)-*β*-D-glucopyranosyl)-*α*-D-glucopyranose* (***3***). Mp 158−161 °C. UV/vis (MeOH) λ_{max} nm: 330 (ε = 17,500), 254 (ε = 25,000). ESI-TOFMS: *m*/*z* 623 $[M-H]^-$. High-resolution ESI/FT-ICRMS: *m*/*z* 623.1617 ($[M-H]^-$, calcd for $C_{28}H_{31}O_{16}$, 623.1618 corresponding to caffeoyl-*p*-hydroxybenzoyl-dihexose). ^{13}C and ^{1}H NMR data: see **Table 1**.

*6-O-(E)-Caffeoyl-(2-O-(6-O-(E)-feruloyl)-*β*-D-glucopyranosyl)-*α*-D-glucopyranose* (***4***). Mp 165−168 °C. UV/vis (MeOH) λ_{max} nm: 330 (ε = 33,700). ESI-TOFMS: *m*/*z* 679 $[M-H]^-$. High-resolution ESI/FT-ICRMS: *m*/*z* 679.1877 ($[M-H]^-$, calcd for $C_{31}H_{35}O_{17}$, 679.1880 corresponding to caffeoyl-feruloyl-dihexose). ^{13}C and ^{1}H NMR data: see **Table 1**.

*Cyanidin 3-O-(6-O-(E)-Caffeoyl-(2-O-(6-O-(E)-feruloyl)-*β*-D-glucopyranosyl)-* β*-D-glucopyranoside*) (***5***). UV/vis (0.01% HCl−MeOH) λ_{max} nm: 528 (ε=28,300; bathochromic shift with $AlCl_3$ into +48 nm), 330 (ε=35,800), 287 (ε=35,400). $E_{440}/E_{vismax}=E_{440}/E_{528}=23\%$; $E_{acylmax}/E_{vismax}=E_{330}/E_{528}=127\%$; $E_{UVmax}/E_{vismax}=E_{287}/E_{528}=125\%$. ESI-TOFMS: *m*/*z* 949 $[M]^+$, 287 $[Cy]^+$. High-resolution ESI/FT-ICRMS: *m*/*z* 949.2040 ($[M]^+$, calcd for $C_{46}H_{45}O_{22}$, 949.2397 corresponding to caffeoyl-feruloyl-Cy3S5G). ^{13}C and ^{1}H NMR data: see **Table 2**.

Table 1. NMR Assigment Data of the Acylated Sophoroses **1**−**4** from a Red Vinegar[a]

	1		2		3		4	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
				Glucose a				
1	91.83	5.23 (*d*, 3.3)	91.97	5.20 (*d*, 3.2)	92.01	5.20 (*d*, 3.3)	91.99	5.20 (*d*, 3.3)
2	82.29	3.30 (*dd*, 3.3, 9.4)	81.72	3.30 (*dd*, 3.2, 9.3)	82.61	3.30 (*dd*, 3.3, 9.4)	82.67	3.30 (*dd*, 3.3, 9.4)
3	71.80	3.72 (*t*, 9.4)	71.65	3.72 (*t*, 9.3)	71.61	3.72 (*t*, 9.4)	71.66	3.72 (*t*, 9.4)
4	70.38	3.24 (*t*, 9.4)	70.44	3.20 (*brt*, 9.3)	70.40	3.20 (*t*, 9.4)	70.42	3.22 (*brt*, 9.4)
5	69.48	3.91 (*m*)	69.52	3.91 (*m*)	69.51	3.90 (*m*)	69.50	3.91 (*m*)
6a	64.03	4.21 (*dd*, 6.1, 11.6)	64.19	4.16 (*brt*, 6.6, 10.8)	65.15	4.18 (*brt*, 6.5, 11.5)	64.17	4.15 (*brdd*, 6.1,11.7)
6b	64.03	4.41 (*brdd*, 1.5, 11.6)	64.19	4.43 (*brd*, 10.8)	65.15	4.44 (*brdd*, 1.8, 11.5)	64.17	4.41 (*brd*, 11.7)
				Glucose b				
1	105.42	4.35 (*d*, 8.1)	105.57	4.41 (*d*, 8.3)	105.56	4.43 (*d*, 8.1)	105.57	4.42 (*d*, 8.3)
2	74.10	3.08 (*brt*, 8.5)	73.98	3.14 (*t*, 8.8)	73.99	3.15 (*brt*, 8.5)	73.98	3.14 (*brt*, 8.8)
3	76.57	3.20 (*t*, 8.5)	76.29	3.25 (*brt*, 8.8)	76.37	3.26 (*brt*, 8.5)	76.30	3.26 (*brt*, 8.8)
4	70.40	3.07 (*t*, 8.5)	70.25	3.22 (*brt*, 8.8)	70.53	3.25 (*brt*, 8.5)	70.20	3.22 (*brt*, 8.8)
5	77.12	3.17 (*m*)	74.01	3.48 (*m*)	74.04	3.52 (*m*)	74.01	3.49 (*m*)
6a	61.42	3.46 (*dd*, 6.3, 11.9)	63.84	4.15 (*brt*, 6.8, 11.0)	64.10	4.19 (*brt*, 6.7, 11.5)	63.85	4.19 (*brdd*, 6.3,11.0)
6b	61.42	3.71 (*brdd*, 2.2, 11.9)	63.84	4.47 (*brd*, 11.0)	64.10	4.59 (*brdd*, 1.8, 11.5)	63.85	4.46 (*brd*, 11.0)
				Caffeoyl (I)				
1	125.9		125.91		125.93		125.91	
2	115.15	7.10 (*brd*, 1.9)	115.13	7.08 (*d*, 1.6)	115.14	7.08 (*brd*, 2.0)	115.14	7.07 (*d*, 1.9)
3	148.54		148.50		148.49		148.50	
4	145.75		145.72		145.47		145.71	
5	116.03	6.81 (*d*, 8.2)	116.02	6.80 (*d*, 8.2)	116.01	6.80 (*d*, 8.2)	116.01	6.80 (*d*, 8.2)
6	121.62	7.03 (*dd*, 1.9, 8.2)	121.60	7.00 (*dd*, 1.6, 8.2)	121.58	7.01 (*dd*, 2.0, 8.2)	121.59	7.00 (*dd*, 1.9, 8.2)
α	114.30	6.29 (*d*, 15.9)	114.29	6.28 (*d*, 15.9)	114.30	6.29 (*d*, 15.8)	114.25	6.26 (*d*, 15.8)
β	145.48	7.51 (*d*, 15.9)	145.47	7.51 (*brd*, 15.9)	145.48	7.52 (*d*, 15.8)	145.48	7.50 (*d*, 15.8)
C═O	166.87		166.88		166.87		166.87	
				Acyl (II)				
			Caffeoyl		*p*-Hydroxybenzoyl		Feruloyl	
1			125.91		162.19		125.97	
2			114.94	7.10 (*d*, 1.8)	115.53	7.85 (*d*, 8.8)	111.16	7.34 (*d*, 1.7)
3			148.53		131.79	6.88 (*d*, 8.8)	145.24	
4			145.72		120.84		149.59	
5			115.92	6.78 (*d*, 8.2)	131.79	6.88 (*d*, 8.8)	115.68	6.81 (*d*, 8.1)
6			121.88	7.02 (*dd*, 1.8, 8.2)	115.53	7.85 (*d*, 8.8)	123.78	7.12 (*dd*,1.7, 8.1)
α			114.23	6.29 (*d*, 15.9)			114.69	6.50 (*d*, 15.9)
β			145.54	7.51 (*brd*, 15.9)			145.48	7.57 (*brd*, 15.9)
C═O			166.82		165.85		166.94	
OCH_3							55.90	3.83 (3H, s)

[a] δ_C and δ_H: ^{13}C (200 MHz)- and ^1H (800 MHz)-NMR chemical shifts (ppm), respectively; *s* = singlet, *d* = doublet, *t* = triplet, *br* = broad. Values in parentheses indicate coupling constants (*J* in Hz).

Peonidin 3-O-(6-O-(E)-Caffeoyl-(2-O-(6-O-(E)-caffeoyl)-β-D-glucopyranosyl)-β-D-glucopyranoside) (**6**). UV/vis (0.01% HCl−MeOH) λ_{max} nm: 528 (ε = 28,500; no bathochromic shift with $AlCl_3$), 330 (ε = 32,200), 287 (ε = 33,300); $E_{440}/E_{vismax} = E_{440}/E_{528} = 24\%$; $E_{acylmax}/E_{vismax} = E_{330}/E_{528} = 113\%$; $E_{UVmax}/E_{vismax} = E_{287}/E_{528} = 117\%$. ESI-TOFMS: *m*/*z* 949 $[M]^+$, 301 $[Pn]^+$. High-resolution ESI/FT-ICRMS: *m*/*z* 949.2385 ($[M]^+$, calcd for $C_{46}H_{45}O_{22}$, 949.2397 corresponding to dicaffeoyl-Pn3S5G). ^{13}C and ^1H NMR data: see **Table 2**.

Peonidin 3-O-(6-O-(E)-Caffeoyl-(2-O-(6-O-p-hydroxybenzoyl)-β-D-glucopyranosyl)- β-D-glucopyranoside) (**7**). UV/vis (0.01% HCl−MeOH) λ_{max} nm: 526 (ε = 29,500; no bathochromic shift with $AlCl_3$), 331 (ε = 19,900), 283 (ε = 28,400), 249 (ε = 29,700); $E_{440}/E_{vismax} = E_{440}/E_{526} = 25\%$; $E_{acylmax}/E_{vismax} = E_{331}/E_{526} = 68\%$ and $E_{249}/E_{526} = 100\%$; $E_{UVmax}/E_{vismax} = E_{283}/E_{526} = 96\%$; ESI-TOFMS: *m*/*z* 907 $[M]^+$, 301 $[Pn]^+$ High-resolution ESI/FT-ICRMS *m*/*z* 907.2289 ($[M]^+$, calcd for $C_{44}H_{43}O_{21}$, 907.2291 corresponding to caffeoyl-*p*-hydroxybenzoyl-Pn3S5G). ^{13}C and ^1H NMR data: see **Table 2**.

Peonidin 3-O-(6-O-(E)-Caffeoyl-(2-O-(6-O-(E)-feruloyl)-β-D-glucopyranosyl)-β-D-glucopyranoside) (**8**). UV/vis (0.01% HCl−MeOH) λ_{max} nm: 527 (ε = 30,200; no bathochromic shift with $AlCl_3$), 330 (ε = 34,300), 287 (ε = 34,400); $E_{440}/E_{vismax} = E_{440}/E_{527} = 25\%$; $E_{acylmax}/E_{vismax} = E_{330}/E_{527} = 114\%$; $E_{UVmax}/E_{vismax} = E_{287}/E_{527} = 114\%$. ESI-TOFMS: *m*/*z* 963 $[M]^+$, 301 $[Pn]^+$. High-resolution ESI/FT-ICRMS: *m*/*z* 963.2554 ($[M]^+$, calcd for $C_{47}H_{47}O_{22}$, 963.2553 corresponding to caffeoyl-feruloyl-Pn3S5G). ^{13}C and ^1H NMR data: see **Table 2**.

Radical Scavenging Activity Assay. Radical scavenging activity of each sample was tested according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) colorimetric method developed by Yamaguchi et al. (*22*) with some modifications. 6-Hydroxy-2,5,7,8-tetramethyl- chroman-2-carboxylic acid (Trolox) (Sigma-Aldrich Co.), 2,6-di-*t*-butyl-4-methylphenol (BHT) (Wako Pure Chemicals Co., Osaka, Japan), (−)-epigallocatechin 3-*O*-gallate (Wako Pure Chemicals), caffeic acid (Tokyo Chemical Industry Co., Tokyo, Japan) were used as authentic antioxidants. Each 500 μM sample EtOH solution (25 μL) was added to 375 μL of EtOH and 350 μL of 0.1 M Tris-HCl buffer (pH 7.4), respectively. To each mixture, 250 μL of 500 μM DPPH−EtOH solution was added, and immediately shaken and then kept for 20 min in the dark at room temperature. Initial and blank were measured without substrate and without DPPH,

Table 2. NMR Assigment Data of the Acylated Anthocyanins **5**−**8** from a Red Vinegar[a]

	5		**6**		**7**		**8**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
				Aglycon				
	Cyanidin		Peonidin		Peonidin		Peonidin	
2	162.34		162.18		162.14		162.23	
3	143.37		143.49		143.47		143.41	
4	136.38	8.81 *s*	138.38	8.90 *s*	138.60	8.92 *s*	138.67	8.89 *s*
5	155.95		156.13		156.00		156.12	
6	94.23	6.59 *brs*	94.55	6.65 *s*	94.59	6.65 *s*	94.58	6.62 *d* (2.0)
7	168.66		169.07		169.10		169.17	
8	102.44	6.68 *brs*	102.45	6.77 *brs*	102.31	6.77 *s*	102.40	6.72 *d* (2.0)
9	111.67		111.91		111.85		111.87	
10	157.61		157.80		157.75		157.90	
1′	154.39		155.16		155.11		155.18	
2′	117.32	7.89 *d* (2.2)	113.89	7.92 *d* (2.0)	113.96	7.96 *s*	113.81	7.88 *d* (2.1)
3′	146.20		148.33		148.27		148.29	
4′	119.59		119.39		119.34		119.34	
5′	117.28	7.02 *d* (8.7)	116.63	7.00 *d* (8.7)	116.54	7.06 *d* (8.8)	116.56	6.99 *d* (8.6)
6′	127.40	8.19 *dd* (2.2, 8.7)	128.62	8.22 *dd* (2.0, 8.7)	128.41	8.25 *d* (8.8)	128.66	8.21 *dd* (2.1, 8.6)
OCH_3			56.06	3.84 *s*	56.06	3.93 *s*	56.00	3.83 *s*
				Glucose a				
1	100.27	5.52 *d* (8.0)	101.65	5.52 *d* (8.3)	101.90	5.52 *d* (7.9)	101.75	5.48 d (8.0)
2	82.35	3.90 *t* (8.7)	82.61	3.83 *t* (8.6)	82.76	3.85 *t* (8.8)	82.74	3.82 *t* (8.8)
3	75.79	3.64 *t* (8.7)	75.77	3.65 *t* (8.6)	75.65	3.70 *t* (8.8)	75.77	3.65 *t* (8.8)
4	69.64	3.37 *t* (8.7)	69.49	3.40 *t* (8.6)	69.46	3.45 *t* (8.8)	69.42	3.41 *t* (8.8)
5	74.42	3.79 *m*	74.56	3.73 *m*	74.50	3.74 *m*	74.51	3.70 *m*
6a	63.39	4.11 *dd* (7.2, 11.2)	63.23	4.14 *dd* (6.8, 11.2)	63.21	4.18 *m*	63.14	4.10 *brd* (10.5)
6b	63.39	4.40 *brd* (11.2)	63.23	4.38 *brd* (11.2)	63.21	4.42 *brd* (11.2)	63.14	4.37 *brd* (10.5)
				Glucose b				
1	104.88	4.72 *d* (8.0)	104.87	4.69 *d* (8.3)	104.97	4.74 *d* (8.2)	104.92	4.68 *d* (8.3)
2	74.73	3.10 *t* (8.0)	74.72	3.12 *t* (8.8)	74.75	3.20 *t* (9.0)	74.68	3.13 *t* (8.8)
3	76.14	3.22 *m*	76.11	3.23 *t* (8.8)	76.12	3.29 *t* (9.0)	76.14	3.25 *t* (8.8)
4	69.64	3.22 *m*	69.93	3.15 *t* (8.8)	69.97	3.25 *t* (9.0)	69.99	3.17 *t* (8.8)
5	74.08	3.22 *m*	74.23	3.31 *m*	74.25	3.42 *m*	74.17	3.34 *m*
6a	62.79	3.94 brd (11.1)	63.47	3.97 *dd* (5.8, 10.9)	63.76	4.10 *m*	63.50	4.01 *dd* (6.1, 10.7)
6b	62.79	4.00 *brd* (11.1)	63.47	4.06 *brd* (10.9)	63.76	4.20 *brd* (11.2)	63.50	4.08 *brd* (10.7)
				Caffeoyl (I)				
1	125.73		125.54		125.70		125.69	
2	115.37	6.92 *d* (1.7)	115.25	6.91 brs	115.21	6.96 brs	115.21	6.90 *d* (2.0)
3	148.44		148.50		148.49		148.50	
4	145.61		145.65		145.64		145.65	
5	115.89	6.73 *d* (8.2)	115.84	6.73 *d* (8.4)	115.89	6.78 *d* (9.1)	115.95	6.73 *d* (8.3)
6	121.25	6.81 *dd* (1.7, 8.2)	121.38	6.77 *d* (8.4)	121.37	6.80 *d* (9.1)	121.36	6.77 *dd* (2.0, 8.3)
α	113.72	6.08 *d* (15.8)	113.49	6.03 *d* (15.8)	113.63	6.08 *d* (15.9)	113.60	6.00 *d* (15.8)
β	145.55	7.30 *d* (15.8)	145.55	7.27 *d* (15.8)	145.52	7.32 *d* (15.9)	145.49	7.25 *d* (15.8)
C═O	166.59		166.52		166.49		166.44	
				Acyl (II)				
	Feruloyl		Caffeoyl		*p*-Hydroxybenzoyl		Feruloyl	
1	125.61		125.43		161.92		125.50	
2	111.01	7.01 *d* (1.7)	115.00	6.78 *d* (1.9)	115.05	7.44 *d* (8.3)	110.89	6.92 *d* (2.0)
3	148.07		148.44		131.09	6.57 *d* (8.3)	148.03	
4	149.47		145.60		120.19		149.49	
5	115.60	6.70 *d* (8.2)	115.66	6.64 *d* (8.1)	131.09	6.57 *d* (8.3)	115.88	6.67 *d* (8.1)
6	123.10	6.79 *dd* (1.7, 8.2)	121.19	6.65 *dd* (1.9, 8.1)	115.05	7.44 *d* (8.3)	123.03	6.74 *dd* (2.0, 8.1)
α	114.13	6.06 *d* (15.8)	113.66	5.75 *d* (15.8)			113.90	5.95 *d* (15.8)
β	145.00	7.15 *d* (15.8)	145.06	7.06 *d* (15.8)			144.90	7.08 *d* (15.8)
C═O	166.48		166.36		165.32		166.45	
OCH_3	55.75	3.73 *s*					55.65	3.68 *s*

[a] δ_C and δ_H: ^{13}C (200 MHz) and ^1H (800 MHz) NMR chemical shifts (ppm), respectively; *s* = singlet, *d* = doublet, *t* = triplet, *br* = broad. Values in parentheses indicate coupling constants (*J* in Hz).

respectively. The DPPH radical scavenging ability (RS%) was calculated as RS% = $100(A_i - A_s + A_b)/A_i$, in which A_i, A_s and A_b were the absorbances at 520 nm of residual DPPH in initial, sample and blank solutions, respectively. The experiment was conducted with four replicates.

α-Glucosidase Inhibition Assay. The immobilization of α-glucosidase partially purified from rat acetone powder onto CNBr-activated Sepharose 4B has been described in detail previously (*23*). The iAGH assay was performed as follows: the immobilized α-glucosidase support (10 mg-wet gel) was placed in an end-capped mini-column with a 45−90 μm polyethylene filter CC-07, 5 mL (ASSIST, Tokyo, Japan). The assay was initiated after the addition of 100 μL of inhibitor solution and 900 μL of the model intestinal fluid, containing 10 mM maltose or 45 mM sucrose to the column. After incubation on a rotating 4 rpm, RT-5 cultivator (TAITEC, Saitama, Japan) at 37 °C for 30 min (maltase assay) or 60 min (sucrase assay), the reaction was terminated by filtration of the solution through the column. Maltase or sucrase activity was evaluated by determining the liberated glucose from substrate in the filtrate by an F-kit Glucose (Roche Diagnostics, Co., Tokyo, Japan). One unit of maltase or sucrase activity was defined as the amount of enzyme that hydrolyzed 1 μmol of substrate per min under the above-described assay conditions. The IC_{50} value was defined as the concentration of inhibitor required to inhibit 50% of the α-glucosidase activity under the assay conditions.

Statistical Analysis. Results are expressed as mean ± SD. Statistical difference between groups was analyzed using a Tukey−Kramer's *t*-test. Value of $P < 0.05$ was considered to be statistically significant. All analyses were performed with Stat View J 5.0 software (SAS Institute Inc.).

RESULTS AND DISCUSSION

Structure Determination of Polyphenols from a Red Vinegar. On HPLC analytical comparison with components of a purple-flashed sweet potato crude pigment and a red vinegar at 310 nm, the red vinegar contained some newly generated ingredients (**Figure 2**). Other than original purple-flashed sweet potato anthocyanins (YGMs) and chlorogenic acids, four new colorless components (**1**−**4**) and four new pigments (**5**−**8**) were detected as shown in **Figure 2B**. On MS and NMR analyses, and on comparison with UV data and HPLC coelution with an authentic compound, the most principal component **1** was identified to be 6-caffeoylsophorose already determined in our previous investigation of same red vinegar (*21*).

On alkaline hydrolysis, all of **1**−**4** gave caffeic acid, and **3** and **4** also yielded *p*-hydroxybenzoic acid and ferulic acid, respectively. The fact that all of **1**−**4** had caffeic acid was also supported by characteristic UV absorption (the wavelength of maximum absorption λ_{max} around 330 nm) which agreed closely with standard caffeic acid (λ_{max} = 332 nm). On ESI-TOFMS and the high-resolution ESI/FT-ICRMS negative mode measurements, **1**−**4** gave molecular mass ($[M-H]^-$) data corresponding to the compositions of caffeoyl-, dicaffeoyl-, caffeoyl-*p*-hydroxybenzoyl- and caffeoyl-feruloyl-dihexoses, respectively.

^{13}C and ^{1}H NMR analyses, including DQF-COSY, TOCSY, NOESY, HSQC and HMBC methods, determined the complete structures, and the assignment data were summarized in **Table 1**. All ^{1}H NMR spectra of **1**−**4** showed the presence of an (*E*)-caffeoyl group based on the 1,2,4-trisubstituted benzene ring and the (*E*)-olefinic proton signals with large coupling constant (*J*) values ca. 16 Hz, and with sugars to have a D-glucopyranosyl configuration due to the large *J* values (8.5−9.4 Hz) of the ring protons. Anomeric protons of glucose b (G_b-1H) of **1**−**4** were β-configuration due to having large *J* values (8.1−8.3 Hz), whereas G_a-1Hs were determined as α-configuration because the G_a-1Hs shifted to lower field (δ_H 5.2 ppm) than those (δ_H 4.4 ppm) of G_b-1Hs (β-anomer), and *J* values of G_a-1Hs were smaller (ca. 3.3 Hz) than those of G_b-1Hs. Moreover, the anomeric carbons of glucose a (G_a-1Cs) shifted to higher field (δ_C ca. 92 ppm) than those (δ_C ca. 106 ppm) of G_b-1Cs. Since

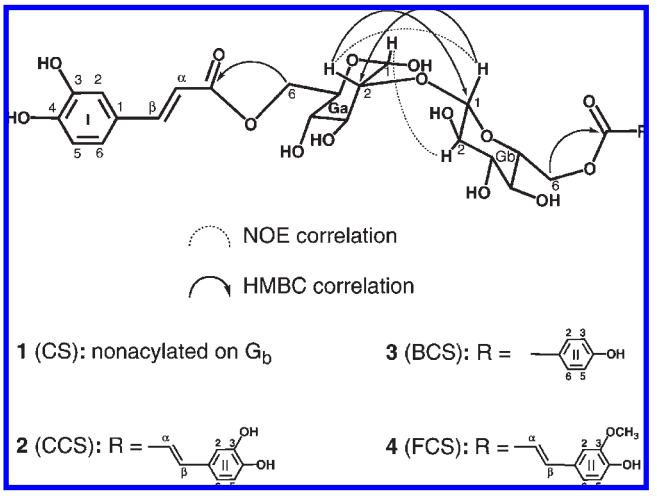

Figure 3. Structures of the acylated sophoroses **1**−**4** in a red vinegar.

G_b was linked on the 2-hydroxyl of G_a (G_a-2OH) based on the glycosylation shifts of $\delta_{Ga\text{-}2H}$ ca.3.3 and $\delta_{Ga\text{-}2C}$ ca. 83 ppm (**Table 1**), the interglycosidic linkage was β-G_b-(1→2)-G_a, meaning the sugar moiety to be sophorose. The connectivity was also supported by observation of the NOESY cross peaks between G_a-1H and G_b-2H, and G_b-1H and G_a-2H, in addition to HMBC correlations between G_a-2H and G_b-1C, and G_b-1H and G_a-2C as demonstrated in **Figure 3**. The deshielding shifts of G_a-6Hs (δ_H ca. 4.3 ppm) and G_a-6C (δ_C 64.0−65.2 ppm) suggested that the caffeoyl group was attached to G_a-6OH, that was confirmed with HMBC peak between G_a-6Hs and caffeoyl carbonyl carbons (δ_C ca.167 ppm). Similarly, in **2**−**4** the deshielding shifts of G_b-6Hs (δ_H 4.2−4.6 ppm) and G_b-6C (δ_C ca. 64 ppm) suggested that the second acyl group was attached to G_b-6OH, that was confirmed with HMBC peak between G_b-6Hs and the acyl carbonyl carbons (δ_C ca.167 ppm). These data proved that **1**−**4** all had a common 6-caffoylsophorosyl (**1**) structure. Moreover, **2**−**4** had one more acyl residue, caffeoyl, *p*-hydroxybenzoyl and feruloyl residue, respectively. On NMR spectra, **3** had a *p*-hydroxybenzoyl moiety with a 1,4-disubstituted benzene, and **2** and **4** respectively had an (*E*)-caffeoyl and a (*E*)-feruloyl moiety with the 1,2,4-trisubstituted benzene and the (*E*)-olefinic protons (*J* = ca.16 Hz). Moreover, **4** had a characteristic OCH_3 singlet signal at δ_H 3.8 ppm and δ_C 55.9 ppm. The acylation to G_b-6OH of **2**−**4** was confirmed by observation of the HMBC correlations between G_b-6Hs and carbonyl-carbons (δ_C ca. 167 ppm) of caffeoyl, *p*-hydroxybenzoyl and feruloyl residues, respectively, and the deshielding shifts of G_b-6Hs and G_b-6C compared with those of **1**. On the basis of all above analytical data, compounds **1**−**4** were determined as 6-*O*-(*E*)-caffeoyl-2-*O*-β-D-glucopyranosyl-α-D-glucopyranose, 6-*O*-(*E*)-caffeoyl-(2-*O*-(6-*O*-(*E*)-caffeoyl)-D-glucopyranosyl)-α-D-glucopyranose, 6-*O*-(*E*)-caffeoyl-(2-*O*-(6-*O*-*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-α-D-glucopyranose, and 6-*O*-(*E*)-caffeoyl-(2-*O*-(6-*O*-(*E*)-feruloyl)-β-D-glucopyranosyl)-α-D-glucopyranose, respectively (**Figure 3**). Thus **2**−**4** were corresponding to the 3-diacylated sophorosyl moieties of YGM-4b (CCS), of YGM-5a (BCS), and of YGM-3 and YGM-6 (FCS), respectively. As far as we know, these types of diacylated sophoroses were determined for the first time. Each pure acylated sophorose gave the two splitting HPLC peaks in the area ratio of about 82:18. These pairs of peaks were considered as the equilibrating anomeric isomers in the HPLC solvent system, because when the large peak was isolated from acylated sophorose solutions, the isolate converted into the original two peaks in a short time. Though the respective components in the HPLC solvent

system could not be identified, the *a*-anomer was predominant or exclusive in NMR solvent (DMSO-d_6 − CF_3COOD = 9:1, v/v).

On alkaline hydrolysis and followed by HPLC analysis, pigments **5**−**8** gave Cy3S5G + caffeic acid + ferulic acid, Pn3S5G + caffeic acid, Pn3S5G + caffeic acid + *p*-hydroxybenzoic acid, Pn3S5G + caffeic acid + ferulic acid, respectively. UV/vis spectra of **5**−**8** gave the absorptions $\lambda_{acylmax}$ around 330 nm and the absorption ratios ($E_{acylmax}$/ E_{vismax}) 68−127%, indicating the presence of one or two phenolic acids in each molecule. Moreover, the ratios (E_{440}/E_{vismax}) about 24% exhibited 3-monosubsutituted anthocyanins, not 3,5-disubustitutions such YGMs (*24*). On a positive mode MS measurement, **5**−**8** gave the molecular $[M]^+$ and fragment (aglycon) mass numbers *m*/*z* 949 and 287 $[Cy]^+$, 949 and 301 $[Pn]^+$, 907 and 301, and 963 and 301, respectively. Moreover, the HRMS data of **5**−**8** gave the $[M]^+$ corresponding to caffeoyl-feruloyl-Cy3S5G, dicaffeoyl-Pn3S5G, caffeoyl-*p*-hydroxybenzoyl-Pn3S5G, and caffeoyl-feruloyl-Pn3S5G, respectively, in which **5** and **6** were structural isomers of each other.

As summarized in **Table 2**, ^{1}H NMR spectra showed that all of **5**−**8** had an (*E*)-caffeoyl group with the 1,2,4-trisubstituted benzene ring and the (*E*)-olefinic protons with J = 16 Hz in addition to sugars (G_a and G_b) bearing two D-glucopyranosyl configurations due to the large J values (8.6−9.0 Hz) of the ring protons and the both anomeric protons were oriented in β-configurations with large J values (7.9−8.3 Hz). Since G_b was linked on the G_a-2OH based on the glycosylation shifts of $\delta_{Ga\text{-}2H}$ 3.8−3.9 and $\delta_{Ga\text{-}2C}$ 82.3−82.8 ppm (**Table 2**), the interglycosidic linkage was β-G_b-(1→2)-G_a of a sophorose. The connectivity was also supported by observation of the NOESY cross peak between G_a-1H and G_b-2H, and G_b-1H and G_a-2H, and HMBC correlations between G_a-2H and G_b-1C, and G_b-1H and G_a-2C as shown in **Figure 4**. The downfield shifts of G_a-6Hs (δ_H 4.1−4.4 ppm) and G_a-6C (δ_C ca. 63 ppm) suggested that the caffeoyl group was attached to G_a-6OH, and it was also confirmed with HMBC peak between G_a-6Hs and caffeoyl carbonyl carbons (δ_C ca. 167 ppm). Similarly, the deshielding shifts of G_b-6Hs (δ_H ca. 4 ppm) and G_b-6C (δ_C ca. 63 ppm) suggested that the second acyl group was attached to G_b-6OH, that was confirmed with HMBC peak between G_b-6Hs and the acyl carbonyl carbons (δ_C ca.166 ppm). Therefore, **5**−**8** were elucidated as having a common anthocyanidin 6-caffeoylsophoroside type structure.

Aglycons of **5** and **6**−**8** were respectively assigned as cyanidin (Cy) and peonidin (Pn) by ^{13}C and ^{1}H NMR analyses. Aglycons of **5** and **6**−**8** had the characteristic B-ring protons of Cy (3′, 4’-dihydroxybenzene structure), and those of Pn (3′-methoxy-4’-hydroxybenzene structure), respectively. The connecting position of the acylated sophorose chains to aglycons was confirmed to be aglycon-3OHs by correlations of NOE (G_a-1H ↔ aglycon-4H (δ_H 8.8−8.9 ppm)), and HMBC (G_a-1H ↔ aglycon-3C (δ_C 143−144 ppm)). On the basis of all the above analytical data, pigments **5**−**8** were unambiguously determined as cyanidin 3-*O*-(6-*O*-(*E*)-caffeoyl-(2-*O*-(6-*O*-(*E*)-feruloyl)-β-D-glucopyranosyl)-β-D-glucopyranoside), peonidin 3-*O*-(6-*O*-(*E*)-caffeoyl-(2-*O*-(6-*O*-(*E*)-caffeoyl)-β-D-glucopyranosyl)-β-D-glucopyranoside), peonidin 3-*O*-(6-*O*-(*E*)-caffeoyl-(2-*O*-(6-*O*-*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-β-D-glucopyranoside), and peonidin 3-*O*-(6-*O*-(*E*)-caffeoyl-(2-*O*-(6-*O*-(*E*)-feruloyl)-β-D-glucopyranosyl)-β-D-glucopyranoside), respectively (**Figure 4**). Thus **5**−**8** were corresponding to the 5-deglucosylated derivatives of YGM-3 (DGY-3), of YGM-4b (DGY-4b), of YGM-5a (DGY-5a), and of YGM-6 (DGY-6), respectively. As far as we know, these types of anthocyanins were determined for the first time.

Antioxidant Capacity of Acylated Polyphenols from a Red Vinegar. The antioxidant capacity of caffeoylated **1**−**8** from a red vinegar was evaluated with a DPPH-colorimetric method to check the radical scavenging activity (*22*). They showed significantly higher activity than authentic antioxidants (trolox, BHT and caffeic acid) other than EGCG (**Figure 5**). The diacylated sophoroses **2**−**4** gave higher activity than monocaffeoylated **1**, of which **2** with two caffeoyl residues had strongest antioxidant capacity (**2** > **4** > **3** > **1**). The second acyl residues in **2**−**4** strengthened the capacity (caffeoyl > feruloyl > *p*-hydroxybenzoyl). Caffeic acid derivatives are well-known to have potent antioxidant properties because the catechol structure donates the phenolic hydrogens or electrons to acceptors like reactive oxygen species or lipid peroxyl radicals (*25*, *26*). The antioxidant capacity of 5-deglucosylated YGM type **5**−**8** was observed in the same order (**6** > **5** > **8** > **7**) of corresponding acylation type YGMs (YGM-4b > -3 > -6 > -5a), and each intensity was higher than that of the corresponding YGM. Thus, 5-deglucosylation of YGMs is suggested to enhance their capacity. The more they had catechol moieties like caffeoyl residue and B-ring of cyanidin, the stronger their antioxidant power was, such as **6** (two caffeoyls), **5** (caffeoyl + cyanidin) > **8** (one caffeoyl), and **7** (one caffeoyl). The reason that the antioxidant capacity of **5**−**8** was superior to that of diacylated sophoroses **2**−**4** was considered due to the additive contribution of those of their aglycons.

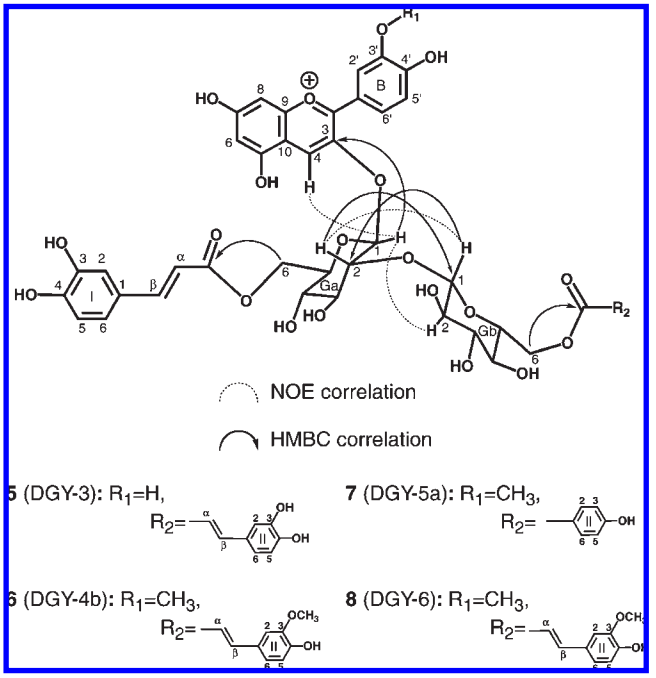

Figure 4. Structures of the acylated anthocyanins **5**−**8** from a red vinegar.

α-Glucosidase Inhibitory Actions of Acylated Polyphenols from a Red Vinegar. Table 3 summarizes α-glucosidase inhibitory activities of diacylated sophoroses **2**−**4** and their mother diacylated anthocyanins. **3** and its mother YGM-5a were not assayed due to quite low yields. As summarized in **Table 3**, newly identified **2** and **4** revealed a potential for retarding the action of intestinal maltase with an IC_{50} value of 214 and 289 μM, respectively. In addition, their maltase inhibitory powers were compatible with their mother acylated anthocyanins of YGM-4b for **2** and YGM-3 or YGM-6 for **4**, indicating that the anthocyanin-induced maltase inhibition would be caused by an acylated moiety. Poor inhibition of deacylated anthocyanins (IC_{50}; >1 mM (*13*)) also demonstrated the importance of acylated moiety responsible for the action of acylated anthocyanins. Hence, it seems likely that 5-deglucosylated YGM type **5**−**8** may have a compatible α-glucosidase inhibitory power with their YGMs, though

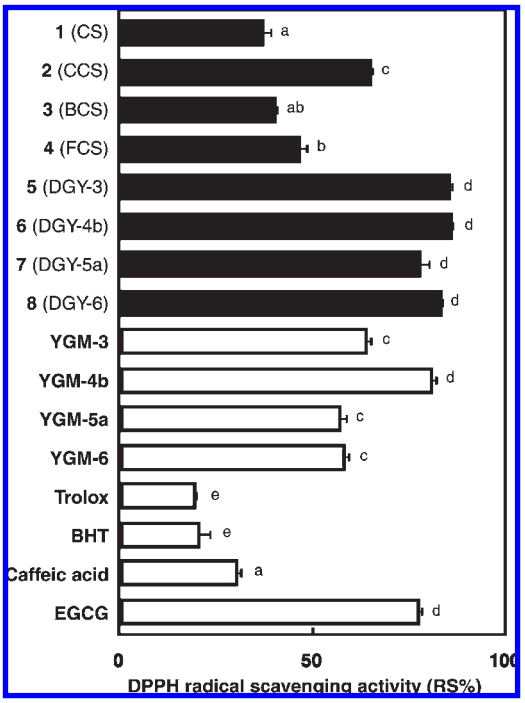

Figure 5. DPPH radical scavenging activity of the acylated polyphenols **1**−**8** from a red vinegar.

Black bars = acylated polyphenols **1**−**8**, white bars = authentic antioxidants; BHT = 2,6-di-*t*-butyl-4-methylphenol, EGCG = (−)-epigallocatechin 3-*O*-gallate. Values are means (RS %) ± SD (n = 4). Bars without a common letter differ at $P < 0.05$ by Tukey−Kramer's *t*-test.

additional experiments should be needed. Similar inhibition powers of **2** and **4** against sucrase with maltase were obtained in this study, while their mothers preferably inhibited maltase rather than sucrase (**Table 3**). As Hauri et al. reported, α-glucosidase enzyme in mammalian intestine is anchored in the membrane by the polypeptide chain spanning the bilayer only once in the manner of N(in)/C(out) orientation to form a sucrase−isomaltase (SI) complex (*27*). Although no crucial research on inhibitory actions of α-glucosidase inhibitor against the SI complex has been reported, the conflicting results between acylated anthocyanins and their acylated moieties suggested that their structural factors may affect the binding to the "S" or "I" complex of α-glucosidase and are now in progress for elucidating the inhibition mechanism(s). **Table 3** also revealed that diacylation of sophorose with caffeoyl residue and/or feruloyl residue enhanced the power of α-glucosidase inhibition rather than monoacylated sophorose (i.e., **1**), as similar with caffeoylquinic acids, in which an increasing number of caffeoyl groups attached with quinic acid enhanced maltase inhibitory activity (3,4,5-tricaffeoylquinic acid; IC_{50} value of 24 μM, (*28*)). The present and our previous findings that **1** elicited a 10% reduction in blood glucose level at a dose of 100 mg/kg in maltose-loaded rats (*15*), thus, led us to perform further rat experiments that the administration of **2** or **4** to rats may exert much more powerful antihyperglycemic action than **1**.

Thus, the red vinegar has been shown to have characteristic components such as high functional caffeoylated polyphenols **2**−**8** in addition to many functional anthocyanins and chlorogenic acids from original purple-fleshed sweet potato components. Probably, the lower molecular size of **1**−**8** might improve the gastrointestinal absorption over the larger molecular size mother anthocyanins YGMs that were found to absorb partially as the intact (glycoside) forms (*29*−*33*). Therefore, intake of red vinegar as seasoning or a healthy drink in our daily life is expected to be highly beneficial for the maintenance of health and prevention of diseases including diabetes. However, *in vivo* studies must be carried out to apply the vinegar to functional foods, since a huge intake of the vinegar due to weaker α-glucosidase inhibitory activity compared to that of the therapeutic drug acarbose (IC_{50}; 0.43 μM (*28*)) may be required to exert the antihyperglycemic effect in human.

Table 3. α-Glucosidase Inhibitory Activities of Diacylated Anthocyanins and Sophoroses Evaluated by Immobilized α-Glucosidase Assay System[a]

	IC_{50} (μM)	
inhibitor	maltase	sucrase
Anthocyanins		
YGM-3	193[c]	—[b]
YGM-4[b]	130	1796
YGM-5[b]	276	957
YGM-6	200[c]	—
Acylated Sophoroses		
1	699[c]	874[c]
2	214	257
4	289	314
Cinnamic Acids		
caffeic acid	17200[c]	3500[c]
feluric acid	NI[c]	NI[c]
Others		
sophorose	NI[c]	NI[c]

[a] Immobilized α-glucosidase assay was performed at 37 °C for 30 or 60 min for maltase or sucrase inhibitory assay, respectively. [b] Not measured. [c] Reported data from the ref *15*.

ABBREVIATIONS USED

ESI-TOFMS, electrospray ionization time-of-flight mass spectrometry; DPPH, 1,1-diphenyl-2-picrylhydrazyl; BHT, 2,6-di-*t*-butyl-4-methylphenol; DQF-COSY, homonuclear double quantum filtered correlation spectroscopy; NOESY, homonuclear nuclear Overhauser effect spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple-bond correlation spectroscopy.

LITERATURE CITED

(1) Yoshinaga, M. New cultivar "Ayamurasaki" for colorant production. *Sweetpotato Res. Front* (*KNAES*) **1995**, 2.

(2) Kojima, M.; Uritani, I. Studies on chlorogenic acid biosynthesis in sweetpotato root tissue in special reference to the isolation of a chlorogenic acid intermediate. *Plant Physiol.* **1973**, *51*, 768–771.

(3) Odake, K.; Terahara, N.; Saito, N.; Toki, K.; Honda, T. Chemical structure of two anthocyanins from purple sweetpotato, *Ipomoea batatas*. *Phytochemistry* **1992**, *31*, 2127–2130.

(4) Goda, Y.; Shimizu, T.; Kato, Y.; Nakamura, M.; Maitani, T.; Yamada, T.; Terahara, N.; Yamaguchi, M. Two acylated anthocyanins from purple sweetpotato, *Ipomoea batatas*. *Phytochemistry* **1997**, *44*, 183–186.

(5) Terahara, N.; Kato, Y.; Nakamura, M.; Maitani, T.; Yamaguchi, M.; Goda, Y. Six diacylated anthocyanins from purple sweetpotato, *Ipomoea batatas* cv Yamagawamurasaki. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 1420–1424.

(6) Terahara, N.; Matsui, T. Stability and functionality of acylated anthocyanins. In *Anthocyanins as Food Factor: Recent Progress in Bioavairability and Health Promoting Effects of Anthocyanins*;

Konishi, T., Ichiyanagi, T., Eds.; Research Signpost Review Books: India, 2008; pp 13−39.
(7) Terahara, N.; Matsui, T. Structures and functionalities of acylated anthocyanins. In *Functional Food and Health*, Shibamoto, T., Ho, C.-T., Kanazawa, K., Shahidi, F., Eds.; ACS Symposium Series 993; American Chemical Society: Washington, DC, 2008; pp 90−101.
(8) Furuta, S.; Suda, I.; Nishiba, Y.; Yamakawa, O. High *tert*-butylperoxyl radical scavenging activities of sweetpotato cultivars with purple flesh. *Food Sci. Technol. Int. Tokyo* **1998**, *4*, 33–35.
(9) Oki, T.; Masuda, M.; Furuta, S.; Nishiba, N.; Terahara, N.; Suda, I. Involvement of anthocyanins and other phenolic compounds in radical-scavenging activity of purple-fleshed sweetpotato cultivars. *J. Food Sci.* **2002**, *67*, 1752–1756.
(10) Yoshimoto, M.; Okuno, S.; Yoshinaga, M.; Yamaguchi, M.; Yamada, J. Antimutagenicity of sweetpotato (*Ipomoea batatas*) roots. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 537–541.
(11) Yoshimoto, M.; Okuno, S.; Yamaguchi, M.; Yamakawa, O. Antimutagenicity of deacylated anthocyanins in purple-fleshed sweetpotato. *Biosci., Biotechnol., Biochem.* **2001**, *65*, 1652–1655.
(12) Matsui, T.; Ueda, T.; Oki, T.; Sugita, K.; Terahara, N.; Matsumoto, K. α-Glucosidase inhibitory action of natural acylated anthocyanins. 1. Survey of natural pigments with potent inhibitory activity. *J. Agric. Food Chem.* **2001**, *49*, 1948–1951.
(13) Matsui, T.; Ueda, T.; Oki, T.; Sugita, K.; Terahara, N.; Matsumoto, K. α-Glucosidase inhibitory action of natural acylated anthocyanins. 2 α-Glucosidase inhibition by isolated acylated anthocyanins. *J. Agric. Food Chem.* **2001**, *49*, 1952–1956.
(14) Fujise, T.; Terahara, N.; Fukui, K.; Sugita, K.; Ohta, H.; Matsui, T.; Matsumoto, K. Durable antihyperglycemic effect of 6-*O*-caffeoylsophorose with α-glucosidase inhibitory activity in rats. *Food Sci. Technol. Res.* **2008**, *14*, 477–484.
(15) Matsui, T.; Ebuchi, S.; Fukui, K.; Matsugano, K.; Terahara, N.; Matsumoto, K.; Caffeoylsophorose, A new natural α-glucosidase inhibitor, from red vinegar by fermented purple-fleshed sweet potato. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 2239–2246.
(16) Kono, Y.; Kobayashi, K.; Tagawa, S.; Adachi, K.; Ueda, A.; Sawa, Y.; Shibata, H. Antioxidant activity of polyphenolics in diets. Rate constants of reactions of chlorogenic acid and caffeic acid with reactive species of oxygen and nitrogen. *Biochim. Biophys. Acta* **1997**, *1335*, 335–342.
(17) Lugasi, A.; Almedia, D. P. E.; Dworschak, E. Chlorogenic acid content and antioxidant properties of potato tubers as related to nitrogen fertilization. *Acta Aliment.* **1999**, *28*, 183–195.
(18) Kapil, A.; Koul, I. B.; Suri, O. P. Antihepatotoxic effects of chlorogenic acid from *Anthocephalus cadamba*. *Phytother. Res.* **1995**, *9*, 189–193.
(19) Yagasaki, K.; Miura, Y.; Okauchi, R.; Furuse, T. Inhibitory effects of chlorogenic acid and its related compounds on the invasion of hepatoma cells in culture. *Cytotechnology* **2000**, *33*, 229–235.
(20) Mahmood, N.; Moore, P. S.; Tommasi, N. D.; Simone, F. D.; Colman, S.; Hay, A. J.; Pizza, C. Inhibition of HIV infection by caffeoylquinic acid derivatives. *Antiviral Chem. Chemother.* **1993**, *4*, 235–240.
(21) Terahara, N.; Matsui, T.; Fukui, K.; Matsugano, K.; Sugita, K.; Matsumoto, K. Caffeoylsophorose in a red vinegar produced through fermentation with purple sweet potato. *J. Agric. Food Chem.* **2003**, *51*, 2539–2543.
(22) Yamaguchi, T.; Takamura, H.; Matoba, T.; Terao, J. HPLC method for evaluation of the radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 1201–1204.
(23) Oki, T.; Matsui, T.; Matsumoto, K. Evaluation of α-glucosidase inhibition by using an immobilized assay system. *Biol. Pharm. Bull.* **2000**, *23*, 1084–1087.
(24) Harborne, J. B. The anthocyanin pigments. In *Comparative biochemistry of the flavonoids*; Academic Press: London, 1967; p 17.
(25) Castelluccio, C.; Paganga, G.; Melikan, N.; Bolwell, G. P.; Pridham, J.; Sampson, J.; Rice-Evans, C. Antioxidant potential of intermediates in phenylpropanoid metabolism in higher plants. *FEBS Lett.* **1995**, *368*, 188–192.
(26) B.-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. -Technol.* **1995**, *28*, 25–30.
(27) Hauri, H. P.; Wacker, H.; Rickli, E. E.; Meier, B. B.; Quaroni, A.; Semenza, G. Biosynthesis of sucrase-isomaltase. *J. Biol. Chem.* **1982**, *257*, 4522–4528.
(28) Matsui, T.; Ebuchi, S.; Fujise, T.; Abesundara, K. J. M.; Doi, S.; Yamada, H.; Matsumoto, K. Strong antihyperglycemic effects of water-soluble fraction of Brazilian propolis and its bioactive constituent, 3, 4, 5-tri-*O*-caffeoylquinic acid. *Biol. Pharm. Bull.* **2004**, *27*, 1797–1803.
(29) Tsuda, T.; Horio, F.; Osawa, T. Absorption and metabolism of cyanidin 3-*O*-beta-D-glucoside in rats. *FEBS Lett.* **1999**, *449*, 179–182.
(30) Miyazawa, T.; Nakagawa, K.; Kudo, M.; Muraishi, K.; Someya, K. Direct intestinal absorption of red fruit anthocyanins, cyanidin 3-glucoside and cyanidin 3,5-diglucoside, into rats and humans. *J. Agric. Food Chem.* **1999**, *47*, 1083–1091.
(31) Matsumoto, H.; Inaba, H.; Kishi, M.; Tominaga, S.; Hirayama, M.; Tsuda, T. Orally administrered delphinidin 3-rutinoside and cyanidin 3-rutinoside are directly absorbed in rats and humans and appear in the blood as the intact forms. *J. Agric. Food Chem.* **2001**, *49*, 1546–1551.
(32) Suda, I.; Oki, T.; Masuda, M.; Nishiba, Y.; Furuta, S.; Matsugano, K.; Sugita, K.; Terahara, N. Direct absorption of acylated anthocyanin in purple-fleshed sweetpotato into rats. *J. Agric. Food Chem.* **2002**, *50*, 1672–1676.
(33) Ichiyanagi, T.; Terahara, N.; Rahman, M. M.; Konishi, T. Gastrointestinal uptake of nasunin, acylated anthocyanin in eggplant. *J. Agric. Food Chem.* **2006**, *54*, 5306–5312.

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