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STAPHYLOSIDES A AND B: TWO NEW CHROMONE DIGLUCOSIDES FROM LEAVES OF *STAPHYLEA BUMALDA* DC.

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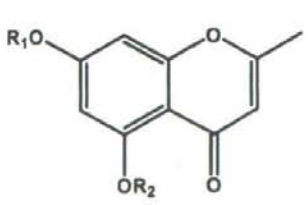
Abstract – Two new chromone diglucosides, staphylosides A and B (**4** and **5**), were isolated from the leaves of *Staphylea bumalda* DC., along with three known chromone glycosides. Their structures were elucidated by detailed inspection of NMR spectral data.

INTRODUCTION

Staphylea bumalda DC. grows wild in China, Japan and Korea. It is a deciduous shrub and blooms from May to June. Previously, we reported the isolation of 11 new and two known megastigmane glucosides from the title plant.[1] This paper describes the isolation and structural investigation of two new chromone diglucosides; staphylosides A and B (**4** and **5**), and three known chromone mono- and diglycosides, staphylin (**1**),[2] 5,7-dihydroxy-2-methylchromone 7-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside (**2**),[3] and schumanniofioside A (**3**) (Figure 1).[4]



	R_1	R_2
1	Glc	H
2	Glc(6'←1")Api	H
3	H	Glc
4	H	β-Glc(6'←1")β-Glc
5	H	β-Glc(6'←1")α-Glc

Figure 1. Structures of 5,7-dihydroxy-2-methylchromone glycosides.

RESULTS AND DISCUSSION

Staphyloside A (**4**), $[\alpha]_D^{29}$ –70, was isolated as an amorphous powder and its elemental composition was

determined to be $C_{22}H_{28}O_{14}$ by HR-ESI-MS. The IR spectrum showed hydroxyl and α,β-unsaturated carbonyl absorptions at 3362 and 1650 cm^{-1}, respectively. The ^1H and ^{13}C NMR spectra indicated the presence of 12 signals assignable to two β-glucopyranoses, which are expected to comprise the β-gentiobiose [β-(6-*O*-β-D-glucopyranosyl)-D-glucopyranose] forming the 1–6 linkage between the two glucose moieties (Table 1). The remaining 10 carbon signals, representing four aromatic carbons without a hydrogen atom and two aromatic carbons with a hydrogen atom, a trisubstituted double bond possessing an oxygen atom, and a ketonic and a methyl carbon, which must form a γ-benzopyrone skeleton termed a chromone. Upon closer inspection of the 2D NMR spectra, the structure of the aglycone portion was confirmed to be a 5,7-dihydroxy-2-methylchromone because HMBC correlations were observed between H-3 (δ_H 6.03), and C-2, 10 and 11 (δ_C 167.1, 109.2 and 19.9, respectively), between H-6 (δ_H 6.92) and C-5, 7, 8 and 10 (δ_C 160.0, 164.8, 99.3 and 109.2, respectively), and between H-8 (δ_H 6.56) and C-6, 7, 9 and 10 (δ_C 105.0, 164.8, 161.2 and 109.2, respectively). Moreover, the anomeric proton (δ_H 4.82) of the inner glucose exhibits a HMBC correlation with C-5 (δ_C 160.0). The absolute configuration of glucose was determined to be of the D-series on HPLC analysis of the hydrolyzate of **4** using an optical rotation detector. Therefore, the structure of staphyloside A (**4**) was established to be 5,7-dihydroxy-2-methylchromone 5-*O*-β-(6-*O*-β-D-glucopyranosyl)-D-glucopyranoside.

Staphyloside B (**5**), $[\alpha]_D^{24}$ –27, was isolated as an amorphous powder and its elemental composition was determined to be $C_{22}H_{28}O_{14}$ by HR-FAB-MS. The IR spectra of **4** and **5** showed that compound **5** exhibited the essentially identical absorptions to compound **4**, and the ^1H and ^{13}C NMR spectra indicated the presence of a 5,7-dihydroxy-2-methylchromone, and α- and β-glucopyranose units, as suggested by the different coupling constants of 4 Hz and 8 Hz for two anomeric protons. These glucoses were deduced to form a β-(6-*O*-α-D-glucopyranosyl)-D-glucopyranose (β-isomaltose), based on the HMBC correlation observed between the anomeric proton of the outer α-glucopyranose and C-6' of the inner β-glucopyranose, as shown in Figure 2. Moreover, the anomeric proton (δ_H 4.82) of the inner glucose exhibits a HMBC correlation with C-5 (δ_C 159.9). Therefore, the structure of staphyloside B (**5**) was established to be 5,7-dihydroxy-2-methylchromone 5-*O*-β-(6-*O*-α-D-glucopyranosyl)-D-glucopyranoside.

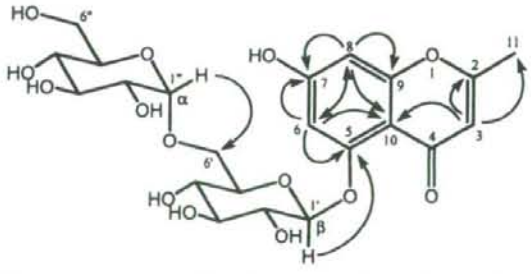


Figure 2. The important HMBC correlations of staphyloside B (**5**). Arrowheads denote carbons and arrow tails protons.

To confirm the sugar linkage, staphyloside B (**5**) was derivatized to its octaacetate (**5a**) using acetic anhydride and pyridine, since the sugar protons were overlapped each other. Each proton signal of glucoses of **5a** was assigned on inspection of the ^{1}H-^{1}H COSY spectrum. Although the α-linked D-glucoses frequently found in polymers, such as glycogen and amylose, β-linkage is a general form in plant secondary metabolites.

Table 1. ^{1}H and ^{13}C NMR data for staphylosides A and B (**4** and **5**), and staphyloside B octaacetate (**5a**).

	4 (CD_3OD)		**5** (CD_3OD)		**5a** ($CDCl_3$)
	C	**H**	**C**	**H**	**H**
2	167.1		167.2		
3	111.7	6.03 (1H, *s*)	111.7	6.02 (1H, *s*)	6.02 (1H, *d*, *J*=1 Hz)
4	180.3		180.2		
5	160.0		159.9		
6	105.0	6.92 (1H, *d*, *J*=2 Hz)	105.1	6.83 (1H, *d*, *J*=2 Hz)	6.97 (1H, *d*, *J*=2 Hz)
7	164.8		164.6		
8	99.3	6.56 (1H, *d*, *J*=2 Hz)	99.6	6.56 (1H, *d*, *J*=2 Hz)	6.83 (1H, *d*, *J*=2 Hz)
9	161.2		161.1		
10	109.2		109.3		
11	19.9	2.33 (3H, *s*)	19.9	2.32 (3H, *s*)	2.28 (1H, *d*, *J*=1 Hz)
1'	104.9	4.82 (1H, *d*, *J*=8 Hz)	105.0	4.82 (1H, *d*, *J*=8 Hz)	5.18 (1H, *d*, *J*=8 Hz)
2'	75.4	3.59 (1H, *dd*, *J*=9, 8 Hz)	74.8	3.59 (1H, *dd*, *J*=10, 8 Hz)	5.35 (1H, *dd*, *J*=10, 8 Hz)
3'	78.0	3.49 (1H, *dd*, *J*=9, 9 Hz)	77.4		5.18 (1H, *dd*, *J*=10, 10 Hz)
4'	71.8	3.42 (1H, *dd*, *J*=10, 9 Hz)	71.5	3.49–3.71 (overlapped)	5.31 (1H, *dd*, *J*=10, 10 Hz)
5'	77.3	3.49 (1H, overlapped)	76.9		3.85 (1H, *ddd*, *J*=10, 5, 3 Hz)
6'	70.0	3.86 (1H, *dd*, *J*=12, 6 Hz)	67.6	3.84 (1H, *dd*, *J*=11, 2 Hz)	3.67 (1H, *dd*, *J*=11, 3 Hz)
		4.20 (1H, *dd*, *J*=12, 2 Hz)		3.98 (1H, *dd*, *J*=11, 6 Hz)	3.79 (1H, *dd*, *J*=11, 5 Hz)
1"	104.8	4.45 (1H, *d*, *J*=, 8 Hz)	99.9	4.89 (1H, *d*, *J*=4 Hz)	5.07 (1H, *d*, *J*=4 Hz)
2"	74.8	3.27 (1H, *dd*, *J*=9, 8 Hz)	73.8	3.43 (1H, *dd*, *J*=10, 4 Hz)	4.84 (1H, *dd*, *J*=10, 4 Hz)
3"	78.0	3.38 (1H, *dd*, *J*=9, 9 Hz)	75.1	3.72 (1H, *dd*, *J*=10, 9 Hz)	5.40 (1H, *dd*, *J*=10, 10 Hz)
4"	71.5	3.31 (1H, *dd*, *J*=10, 9 Hz)	71.8	3.35 (1H, *dd*, *J*=9, 9 Hz)	5.00 (1H, *dd*, *J*=10, 10 Hz)
5"	77.9	3.26 (1H, *ddd*, *J*=10, 6, 2 Hz)	73.7	3.49–3.71 (overlapped)	3.99 (1H, *ddd*, *J*=10, 5, 3 Hz)
6"	62.8	3.68 (1H, *dd*, *J*=12, 6 Hz)	62.6	3.68 (1H, *dd*, *J*=14, 5 Hz)	4.00 (1H, *dd*, *J*=13, 3 Hz)
		3.87 (1H, *dd*, *J*=12, 2 Hz)		3.81 (1H, *dd*, *J*=14, 5 Hz)	4.10 (1H, *dd*, *J*=13, 5 Hz)

EXPERIMENTAL

General experimental procedures Optical rotations were measured on a JASCO P-1030 digital polarimeter. The FT–IR spectra were recorded on a Horiba FT-710 spectrophotometer. The UV spectra were recorded on a Shimadzu UV-160A spectrophotometer. The ^{1}H and ^{13}C NMR spectra were taken on a JEOL α-400 spectrometer (400 and 100 MHz, respectively) with TMS as the internal standard. HR-FAB-MS was performed on a JEOL SX-102 spectrometer with PEG-400 as the calibration matrix. NanoSpray™ ESI–TOF–MS was carried out on an Applied Biosystems QSTAR® XL System. The absolute configurations of sugars were determined on a JASCO OR-2090plus chiral detector. Parts of the general experimental procedures were described in a previous paper.[1]

Plant materials Leaves of *Staphylea bumalda* DC. were collected in the suburbs of Hiroshima City, Japan, in June 2000, and a voucher specimen was deposited in the Herbarium of the Department of Pharmacognosy, Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima University (00-SB-Hiroshima-0618).

Extraction and isolation The air-dried leaves of *S. bumalda* (5.71 kg) were extracted with MeOH (45 l) three times for one week. Parts of the extraction and isolation procedures were described in the previous paper.[1]

The residue (12.3 g in fractions 9–11) of the 40% MeOH eluate obtained on Diaion HP-20 column chromatography was subjected to silica gel (300 g) CC, with elution with $CHCl_3$ (2 l) and $CHCl_3$–MeOH [(99:1, 3 L), (39:1, 3 L), (19:1, 3 L), (37:3, 3 lL), (9:1, 3 Ll), (7:1, 3 L), (17:3, 3 L), (33:7, 3 L), (4:1, 3 L), (3:1, 3 L) and (7:3, 3 L)], and $CHCl_3$–MeOH–H_2O (70:30:4, 3 L), 500 mL fractions being collected. Combined fractions 41–51 (1.86 g) of the 15–17.5% MeOH eluate were separated by reversed-phase open CC (RPCC). The residue (228 mg in fractions 83–90) was subjected to droplet counter-current chromatography (DCCC) to give a **4**-enriched fraction, which was purified by HPLC (ODS, H_2O-MeOH) to give 7.2 mg of pure **4**. The residue (224 mg in fractions 91–100) was subjected to DCCC to give 22.3 mg of **2**.

The residue (24.0 g in fractions 12–15) of the 40–60% MeOH eluate was filtrated by suction to remove the precipitates, which were washed thoroughly in MeOH and then dried under vacuum to give 1.11 g of **1**. The mother liquid was subjected to silica gel (500 g) CC, with elution with $CHCl_3$ (2 l) and $CHCl_3$–MeOH [(99:1, 3 L), (39:1, 3 L), (19:1, 3 L), (37:3, 3 L), (9:1, 3 L), (7:1, 3 L), (17:3, 3 L), (33:7, 3 L), (4:1, 3 L), (3:1, 3 L) and (7:3, 3 L)] , and $CHCl_3$–MeOH–H_2O (70:30:4, 3 L), 500 mL fractions being collected. Combined fractions 29–37 (3.00 g) of the 10–12.5% MeOH eluate were filtrated by suction to remove the precipitates of **1** from the mother liquid. The mother liquid was separated by RPCC. The residue (125 mg) of fractions 88–91 was subjected to DCCC to give 16.7 mg of **3** in fractions 62–79. Combined fractions 57–76 (3.80 g) of the 20–30% MeOH eluate were separated by RPCC. The residue (62.9 mg) of fractions 83–90 was subjected to DCCC to give 29.8 mg of **5** in fractions 26–38.

Staphylin (1): Colorless needles; mp 252–254°C; $[\alpha]_D^{26}$ –60.2 (*c* 0.88, pyridine).

5,7-Dihydroxy-2-methylchromone 7-*O*-β-D-apiofuranosyl(1"→6')-β-D-glucopyranoside (2): Amorphous powder; $[\alpha]_D^{24}$ –90.1 (*c* 1.49, MeOH).

Schumanniofioside A (3): Amorphous powder; $[\alpha]_D^{24}$ –80.8 (*c* = 1.11, MeOH).

Staphyloside A (4): Amorphous powder; $[\alpha]_D^{29}$ –70.4 (*c* = 0.48, MeOH); IR υ_{max} (film) cm^{-1}: 3362, 1650, 1583, 1072, 1032; UV λ_{max} (MeOH) nm (log ε): 249 (4.04), 290 (3.96); ^1H NMR and ^{13}C NMR (CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) *m/z*: 539.1392 $[M+Na]^+$ (calc. for $C_{22}H_{28}O_{14}Na$: 539.1371).

Staphyloside B (5): Amorphous powder; $[\alpha]_D^{24}$ –26.7 (*c* 1.99, MeOH); IR υ_{max} (film) cm^{-1}: 3366, 1650, 1585, 1075, 1028; UV λ_{max} (MeOH) nm (log ε): 250 (4.00), 290 (3.88); ^1H NMR and ^{13}C NMR (C_5D_5N–CD_3OD): Table 1; HR-FAB-MS (negative-ion mode) *m/z*: 515.1415 $[M-H]^-$ (calc. for $C_{22}H_{27}O_{14}$: 515.1401).

Acetylation of 5 to staphyloside B octaacetate (5a)

Staphyloside B (**5**) (5.0 mg) was acetylated with acetic anhydride (0.5 mL) in the presence of a drop of pyridine, the mixture being occasionally stirred at 60°C for 15 min. After leaving overnight at rt, the reagents were removed under a N_2 stream to give staphyloside B octaacetate (**5a**). Staphyloside B octaacetate (**5a**): amorphous powder, ^1H NMR ($CDCl_3$, 400 MHz) δ:1.97 (3H, *s*), 2.00 (3H, *s*), 2.04 (3H, *s*), 2.06 (6H, *s*), 2.09 (3H, *s*), 2.11 (3H, *s*) (CH_3CO- × 7 on alcoholic OH), 2.34 (3H, *s*, CH_3CO- on phenolic OH), other signals are shown in Table 1; HR-ESI-MS (positive-ion mode) *m/z*: 875.2232 $[M+Na]^+$ (calc. for $C_{38}H_{44}O_{22}Na$: 875.2222).

Acid hydrolyses of 4 and 5

Staphyloside A (**4**) (1.0 mg) was hydrolyzed with 0.1 mL of 1N HCl under reflux for 2 h. The reaction mixture was extracted with 0.1 mL of EtOAc and then centrifuged at 1000 rpm for 2 min. Ten μL of the water layer was injected into the HPLC system under the following conditions: column, Shodex Asahipak NH2P-50 4E (25 cm × 4.6 mm, i.d.); solvent, MeCN–H_2O (4:1); flow rate, 1 mL/min; and detection, optical rotation (JASCO OR-2090*plus*). Identification of the sugar afforded was performed based on its retention time (t_R 13.8 min) and positive sign of optical rotation compared with those of authentic D-glucose.

Through a similar procedure, D-glucose was also liberated from staphyloside B (**5**).

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Three new olefinic acetogenin glycosides from leaves of *Staphylea bumalda* DC.

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Abstract Three new olefinic acetogenin glycosides (**3**, **6** and **7**) have been isolated from *Staphylea bumalda* DC., together with four known congeners (**1**, **2** and **4**, **5**). Their structures were determined on the bases of spectral data.

Keywords *Staphylea bumalda* · Staphyleaceae · Olefinic glycosides

Introduction

Staphylea bumalda DC. (Staphyleaceae) is a deciduous shrub distributed in China, Japan and Korea. Previously, we dealt with the isolation and structural investigation of 11 new megastigmane glucosides from the leaves of the title plant [1]. The present paper describes the isolation and structure determination of three new olefinic acetogenin glycosides (**3**, **6** and **7**) and four known C_6 aliphatic glycosides; *n*-hexyl β-D-gentiobioside (**1**) [2], (*E*)-2- and (*Z*)-3-hexenyl β-D-glucosides (**2** and **4**) [3] and (*Z*)-3-hexenyl *O*-β-D-glucopyranosyl-(1″ → 6′)-β-D-glucopyranoside (**5**) [4], from the leaves of the title plant.

Experimental

General experimental procedures

The following instruments were used to record physical data. Optical rotations: JASCO P-1030 digital polarimeter; FT-IR spectra: Horiba FT-710 spectrophotometer; ^1H and ^{13}C NMR spectra: JEOL α-400 spectrometer (400 and 100 MHz, respectively), with TMS as internal standard; ESI-TOF-MS: Applied Biosystems QSTAR® XL Nano-Spray™ System. Parts of the general experimental procedures were described in previous papers [1].

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Plant material

Leaves of *S. bumalda* DC. were collected in the suburbs of Hiroshima City, Japan, in June 2000, and a voucher specimen was deposited in the Herbarium of the Department of Pharmacognosy, Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima University (00-SB-Hiroshima-0618).

Extraction and isolation

The air-dried leaves of *S. bumalda* (5.71 kg) were extracted with MeOH (15 l × 3). Parts of the extraction and isolation procedures were described in the previous paper [1].

The 40% MeOH eluate (12.3 g) of obtained on Diaion HP-20 column chromatography (CC) was subjected to silica gel (300 g) CC, with elution with $CHCl_3$ (2 l) and $CHCl_3$–MeOH [(99:1, 3 l), (97:3, 3 l), (19:1, 3 l), (37:3, 3 l), (9:1, 3 l), (7:1, 3 l), (17:3, 3 l), (33:7, 3 l), (4:1, 3 l), (3:1, 3 l) and (7:3, 3 l)], 500 ml fractions being collected. Combined fractions 41–51 (1.86 g) were separated by reversed-phase open CC (H_2O–MeOH). The residues (228 mg in fractions 83–90, 224 mg in fractions 91–100 and 214 mg in fractions 101–113) were subjected to droplet counter-current chromatography (DCCC) ($CHCl_3$–MeOH–H_2O–1-PrOH) and HPLC (ODS, H_2O–MeOH) to give 17.8 mg of **7** from the first, 92.9 mg of **5** from the second and 30.0 mg of **3** from the third residues.

The 40–60% MeOH eluate (24.0 g) of obtained on Diaion HP-20 CC was subjected to silica gel (500 g) CC, with elution with $CHCl_3$ (2 l) and $CHCl_3$–MeOH [(99:1, 3 l), (97:3, 3 l), (19:1, 3 l), (37:3, 3 l), (9:1, 3 l), (7:1, 3 l), (17:3, 3 l), (33:7, 3 l), (4:1, 3 l), (3:1, 3 l) and (7:3, 3 l)], 500 ml fractions being collected. Combined fractions 29–37 (3.00 g) of the 10–12.5% MeOH eluate were filtrated in a vacuum filtrator to remove the precipitates from the mother liquid, which (1.71 g) were separated by reversed-phase open CC (H_2O–MeOH). The residue (90.4 mg in fractions 112–123) were subjected to DCCC ($CHCl_3$–MeOH–H_2O–1-PrOH) and HPLC (ODS, H_2O–MeOH) to give 19 mg of **2**. Combined silica gel CC fractions 42–49 (2.16 g) of the 15–17.5% MeOH eluate were filtrated in a vacuum filtrator to remove the precipitate from the mother liquid, which (810 mg) were separated by reversed-phase open CC (H_2O–MeOH). The residue (283 mg in fractions 92–101, 69.5 mg in fractions 102–106, 110 mg in fractions 114–120) were subjected to DCCC ($CHCl_3$–MeOH–H_2O–1-PrOH) and HPLC (ODS, H_2O–MeOH) to give 9.8 mg of **4** from the first, 23.1 mg of **6** from the second and 20.9 mg of **1** from the third residue.

Known compounds isolated

n-Hexyl *O*-β-D-glucopyranosyl-(1″ → 6′)-β-D-glucopyranoside (**1**): amorphous powder; $[\alpha]_D^{26}$ −32.6 (*c* 1.4, MeOH) [2]. (*E*)-2-Hexenyl β-D-glucopyranoside (**2**), amorphous powder; $[\alpha]_D^{25}$ −32.6 (*c* 1.4, MeOH) [3]. (*Z*)-3-Hexenyl β-D-glucopyranoside (**4**), amorphous powder; $[\alpha]_D^{25}$ −33.4 (*c* 0.65, MeOH) [3]. (*Z*)-3-Hexenyl *O*-β-D-glucopyranosyl-(1″ → 6′)-β-D-glucopyranoside (**5**), amorphous powder; $[\alpha]_D^{23}$ −41.0 (*c* 4.6, MeOH) [4].

(E)-Hex-2-en-1-ol O-β-D-glucopyranosyl-(1″ → 6′)-β-D-glucopyranoside (3)

Amorphous powder; $[\alpha]_D^{25}$ −43.9 (ç 0.54, MeOH); IR ν_{max} (film) cm^{-1}: 3,367, 2,927, 2,874, 1,650, 1,370, 1,165, 1,072, 1,040; ^1H NMR (400 MHz, CD_3OD): Table 1; ^{13}C NMR (100 MHz, CD_3OD): Table 2; HR-ESI-MS (positive-ion mode) *m/z*: 447.1819 $[M + Na]^+$ (Calcd for $C_{18}H_{32}O_{11}Na$: 447.1836).

(Z)-Hex-3-en-1-ol O-β-apiofuranosyl-(1″ → 6′)-β-D-glucopyranoside (6)

Amorphous powder; $[\alpha]_D^{23}$ −63.3 (*c* 1.54, MeOH); IR ν_{max} (film) cm^{-1}: 3,368, 2,932, 2,879, 1,650, 1,512, 1,368, 1,162, 1,053; ^1H NMR (400 MHz, CD_3OD): Table 1; ^{13}C NMR (100 MHz, CD_3OD): Table 2; HR-ESI-MS (positive-ion mode) *m/z*: 417.1728 $[M + Na]^+$ (Calcd for $C_{17}H_{30}O_{10}Na$: 417.1731).

(Z)-8-Hydroxyoct-5-enoic acid O-β-D-glucopyranoside (7)

Amorphous powder; $[\alpha]_D^{23}$ −23.8 (*c* 1.19, MeOH); IR ν_{max} (film) cm^{-1}: 3,371, 2,931, 2,883, 1,716, 1,654, 1,512, 1,369, 1,164, 1,078, 1,032; ^1H NMR (400 MHz, CD_3OD): Table 1; ^{13}C NMR (100 MHz, CD_3OD): Table 2; HR-ESI-MS (positive-ion mode) *m/z*: 343.1364 $[M + Na]^+$ (Calcd for $C_{14}H_{28}O_8Na$: 343.1363).

Analyses of the sugar moiety

About 500 μg each of **3**, **6** and **7** was hydrolyzed with 1 N HCl (0.1 ml) at 100°C for 2 h. The reaction mixtures were partitioned with an equal amount of EtOAc (0.1 ml), and the water layers were analyzed with a chiral detector (JASCO OR-2090plus) on an amino column [Asahipak NH2P-50 4E, CH_3CN–H_2O (4:1), 1 ml/min]. Hydrolyzates of **3**, **6** and **7** gave the peak for D-glucose at the retention time of 14.4 min (positive optical rotation sign). Peaks were identified by co-chromatography with authentic D-glucose.

Results and discussion

(*E*)-Hex-2-en-1-ol *O*-β-D-glucopyranosyl-(1″ → 6′)-β-D-glucopyranoside (**3**), $[\alpha]_D^{25}$ −43.9, was isolated as an amorphous powder and its elemental composition was determined to be $C_{18}H_{32}O_{11}$ by HR-ESI-MS. The ^1H and ^{13}C NMR spectra showed the presence of 12 signals assignable to two β-glucopyranoses, which are expected to comprise a β-gentiobiose forming the 1–6 linkage between the two glucose moieties (Tables 1, 2) and this was confirmed by the HMBC experiment, in which correlation peaks between δ_H 3.79 (H-6′a) and 4.14 (H-6′b), and δ_C 104 (C-1″) were observed. The remaining six carbon signals, representing a disubstituted double bond, three methylenes, one of which possessed an oxygen atom, and a methyl carbon. The coupling patterns of proton signals in the ^1H NMR spectrum showed the existence of a *trans*-double bond [δ_H 5.60 (1H, *dddt*, J = 15, 7, 6 and 1 Hz) and 5.76 (1H, *dtt*, J = 15, 7 and 1 Hz)] and other NMR spectral data were essentially the same as those of **2**. The absolute configuration of glucose was determined to be of the D-series on HPLC analysis of the hydrolyzate of **3** using an optical rotation detector. Therefore, the structure of **3** was elucidated as shown in Fig. 1.

(*Z*)-Hex-3-en-1-ol *O*-β-apiofuranosyl-(1″ → 6′)-β-D-glucopyranoside (**6**), $[\alpha]_D^{23}$ −63.3, was isolated as an amorphous powder and its elemental composition was determined to be $C_{17}H_{30}O_{10}$ by HR-ESI-MS. The ^1H and ^{13}C NMR spectra showed the presence of 11 signals assignable to 6-substituted β-glucopyranose and outer

Table 1 The ^{1}H NMR spectroscopic data for compounds **3**, **6** and **7** (CD_3OD, 400 MHz)

	3	6	7
1	4.09 (1H, *ddd*, J = 12, 7, 1 Hz)	3.54 (1H, *dt*, J = 10, 7 Hz)	
	4.30 (1H, *ddd*, J = 12, 6, 1 Hz)	3.83 (1H, *dt*, J = 10, 7 Hz)	
2	5.60 (1H, *dddt*, J = 15, 7, 6, 1 Hz)	2.38 (2H, *q*, J = 7 Hz)	2.30 (2H, *t*, J = 7 Hz)
3	5.76 (1H, *dtt*, J = 15, 7, 1 Hz)	5.38 (1H, *dtt*, J = 11, 7, 1 Hz)	1.67 (2H, *quint.*, J = 7 Hz)
4	2.04 (2H, *qd*, J = 7, 1 Hz)	5.45 (1H, *dtt*, J = 11, 7, 1 Hz)	2.12 (2H, *q*, J = 7 Hz)
5	1.42 (2H, *sextet*, J = 7 Hz)	2.08 (2H, *quint.d*, J = 7, 1 Hz)	5.42 (1H, *dt*, J = 11, 7 Hz)
6	0.92 (3H, *t*, J = 7 Hz)	0.97 (3H, *t*, J = 7 Hz)	5.47 (1H, *dt*, J = 11, 7 Hz)
7			2.38 (2H, *q*, J = 7 Hz)
8			3.56 (1H, *dt*, J = 10, 7 Hz)
			3.87 (1H, *dt*, J = 10, 7 Hz)
1′	4.30 (1H, *d*, J = 8 Hz)	4.25 (1H, *d*, J = 8 Hz)	4.27 (1H, *d*, J = 8 Hz)
2′	3.19 (1H, *dd*, J = 9, 8 Hz)	3.17 (1H, *dd*, J = 9, 8 Hz)	3.17 (1H, *dd*, J = 9, 8 Hz)
3′	3.27–3.38 (overlapped)	3.27 (1H, *t*, J = 9 Hz)	3.35 (1H, *t*, J = 9 Hz)
4′	3.27–3.38 (overlapped)	3.35 (1H, *t*, J = 9 Hz)	3.27–3.38 (overlapped)
5′	3.43 (1H, *ddd*, J = 10, 6, 2 Hz)	3.39 (1H, *ddd*, J = 9, 6, 2 Hz)	3.27–3.38 (overlapped)
6′	3.79 (1H, *dd*, J = 12, 6 Hz)	3.61 (1H, *dd*, J = 11, 6 Hz)	3.67 (1H, *dd*, J = 12, 6 Hz)
	4.14 (1H, *dd*, J = 12, 2 Hz)	3.98 (1H, *dd*, J = 11, 2 Hz)	3.87 (1H, *dd*, J = 12, 2 Hz)
1″	4.38 (1H, *d*, J = , 8 Hz)	5.00 (1H, *d*, J = 2 Hz)	
2″	3.22 (1H, *dd*, J = 9, 8 Hz)	3.89 (1H, *d*, J = 2 Hz)	
3″	3.27–3.38 (overlapped)		
4″	3.27–3.38 (overlapped)	3.75 (1H, *d*, J = 10 Hz)	
		3.96 (1H, *d*, J = 10 Hz)	
5″	3.27–3.38 (overlapped)	3.58 (2H, *s*)	
6″	3.67 (1H, *dd*, J = 12, 5 Hz)		
	3.87 (1H, *dd*, J = 12, 2 Hz)		

Table 2 The ^{13}C NMR spectroscopic data for compounds **3**, **6** and **7** (CD_3OD, 100 MHz)

	3	6	7
1	71.1	70.6	177.6
2	127.4	28.9	34.4
3	135.9	126.0	26.0
4	35.5	134.6	27.6
5	23.4	21.6	131.8
6	14.0	14.7	127.7
7			29.0
8			70.4
1′	103.2	104.4	104.4
2′	75.1	75.1	75.2
3′	78.1	78.1	78.0
4′	71.7	71.8	71.7
5′	77.1	78.1	78.2
6′	69.9	68.7	62.9
1″	104.9	111.0	
2″	75.2	76.9	
3″	78.1	80.6	
4″	71.6	75.1	
5″	78.0	65.8	
6″	62.9		

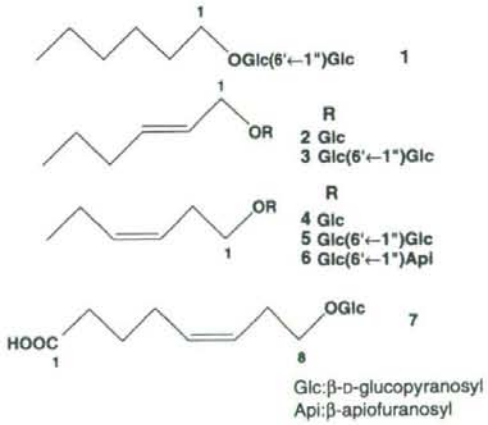

Fig. 1 Structures

β-apiofuranose moieties [5], which were expected to be linked through 1″–6′ positions and this was confirmed by the HMBC experiment. The remaining six carbon signals analogous to those of compound **3** must form *n*-hexenol. The coupling patterns of proton signals in the ^{1}H NMR spectrum showed the existence of a *cis*-double bond [δ_H 5.38 (1H, *dtt*, J = 11, 7 and 1 Hz) and 5.45 (1H, *dtt*,

$J = 11$, 7 and 1 Hz)], which must be located on the 3-position from the fact that the methyl protons appeared as a triplet signal [δ_H 0.97 (1H, *t*, $J = 7$ Hz)] and the carbinol proton signals coupled as triplet with the adjacent methylene protons (Table 1). The absolute configuration of glucose was determined to be of the D-series on HPLC analysis of the hydrolyzate of **6** using an optical rotation detector. Therefore, the structure of **6** was elucidated as shown in Fig. 1.

(*Z*)-8-Hydroxyoct-5-enoic acid *O*-β-D-glucopyranoside (**7**), $[\alpha]_D^{23}$ −23.8, was isolated as an amorphous powder and its elemental composition was determined to be $C_{14}H_{28}O_8$ by HR-ESI-MS. The ^1H and ^{13}C NMR spectra showed the presence of six signals assignable to a β-glucopyranose and the remaining eight carbon signals, including the merged three methylenes and one methylene, a carboxylic acid instead of the methyl group, compared with compound **6**, a primary alcohol, and a disubstituted double bond, must form a 8-hydroxyoct-5-enoic acid. Judging from the coupling patterns of olefinic proton signals in the ^1H NMR spectrum [δ_H 5.42 (1H, *dtt*, $J = 11$, 7 and 1 Hz) and 5.47 (1H, *dtt*, $J = 11$, 7 and 1 Hz)], the geometry of the double bond was determined to be in a cisoid form. The two-dimensional NMR spectra were closely inspected in order to determine the position of the double bond. In the H–H COSY spectrum, all the proton signals were correlated as shown in Fig. 2 and thus, the structure of 7 was elucidated as shown in Fig. 1. The correlation peaks, observed from the anomeric proton (δ_H 4.27) of glucose to C-8 (δ_C 70.4) and from the proton (δ_H 3.56 and 3.87) of C-8 to the olefinic carbon (δ_C 127.7) in the HMBC spectrum also supported the structure (Fig. 2). The absolute configuration of glucose was determined to be of the D-series on HPLC analysis of the hydrolyzate of **7** using an optical rotation detector.

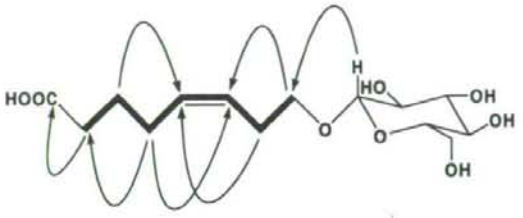

Fig. 2 H–H COSY correlations (*horizontal bar*) and HMBC correlations (H → C) of **7**

Acknowledgments The authors are grateful for access to the superconducting NMR instrument at the Analytical Center of Molecular Medicine of Graduate School of Biomedical Sciences, Hiroshima University and an Applied Biosystem QSTAR XL system ESI (Nano Spray)-TOF-MS at the Analytical Center of Molecular Medicine and the Analysis Center of Life Science, respectively, of the Hiroshima University Faculty of Medicine.

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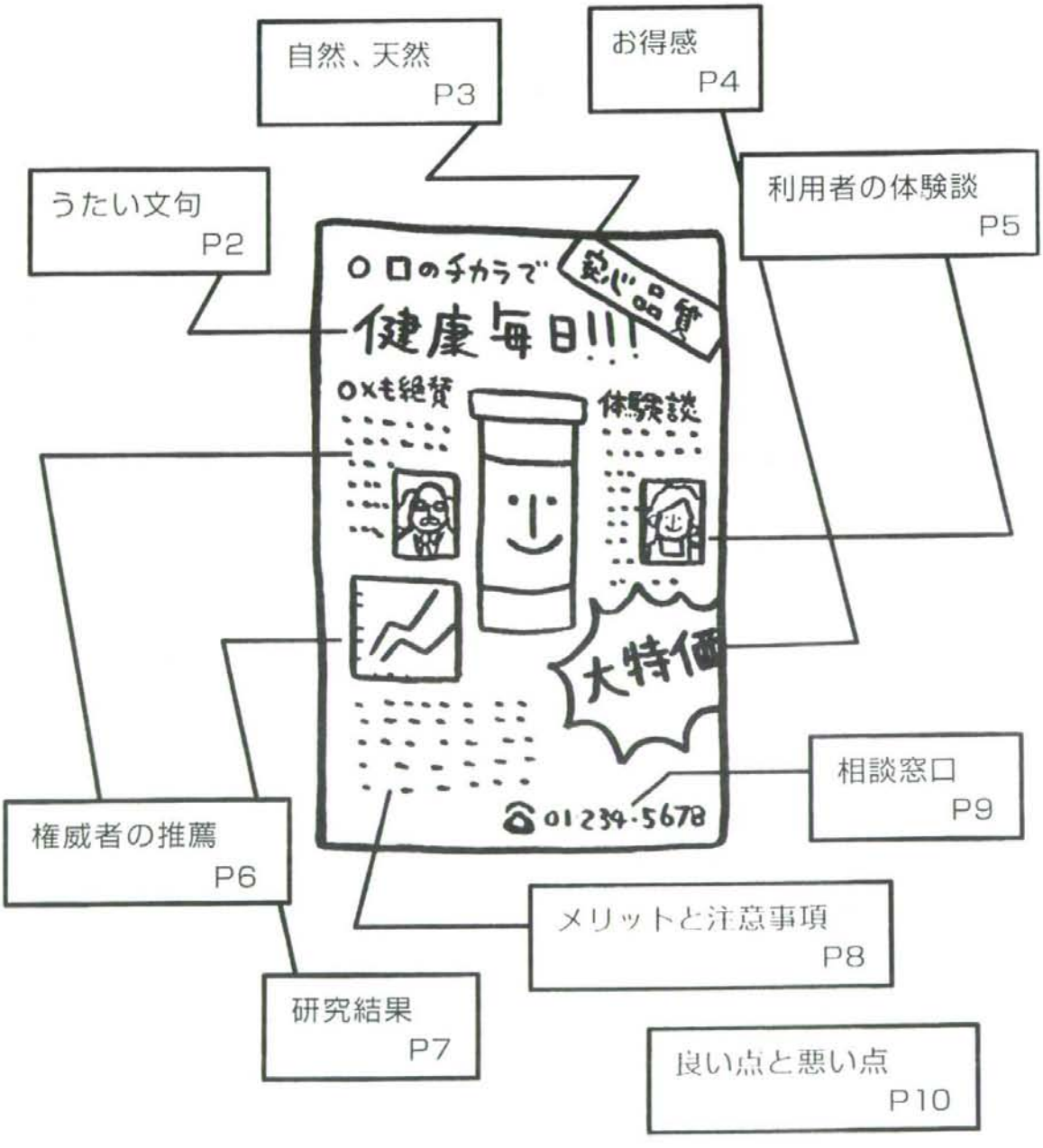
これから
健康食品を利用しよう
と考えているあなたへ

あなたが買おうとしているその健康食品は、本当にあなたに必要なものですか？



健康食品の広告を、もう一度みてみましょう。

私たちはうたい文句から推測して、「健康に良い」と勝手に解釈していることが多くあります。

でも、よく読んでみると、健康に対する効果をはっきりとは述べていないことがほとんどです。

本当はどのくらい効果があるのか、考えてみましょう。

「自然・天然由来の成分です」と聞くと、いかにも安全で、安心できる商品のような気がします。

でも、たとえば、天然のキノコには、シイタケやシメジなどの無毒なキノコがある一方で、有毒なキノコもあります。

「自然」だから、「天然」だから、本当に安心できますか？

受賞に関する表示について

例えば、"モンドセレクション受賞"などと、記載されていることがあります。モンドセレクションは、ベルギーにおける、主に食品を対象とした国際品評会です。

しかし、評価の基準は、一般に公開されていません。

3

割引された商品は、果たして本当に「お得」なのでしょうか？

その商品の効き目が現れるまでに、あなたはどれくらい利用し続ける必要があるのでしょうか？

健康食品は、利用したからといってすぐに効果が現れるものではありません。一定期間買い続けた場合、いったい総額でいくら必要になるのか計算してみましょう。

1商品 [　　] 円 ÷ [　　] 日分 ＝ 1日あたり [　　] 円

↓ × 30日

＝ 1ヶ月では [　　] 円

↓ × 12ヶ月

＝ 1年では [　　] 円

たとえば・・・

6000円の商品、20日分の場合

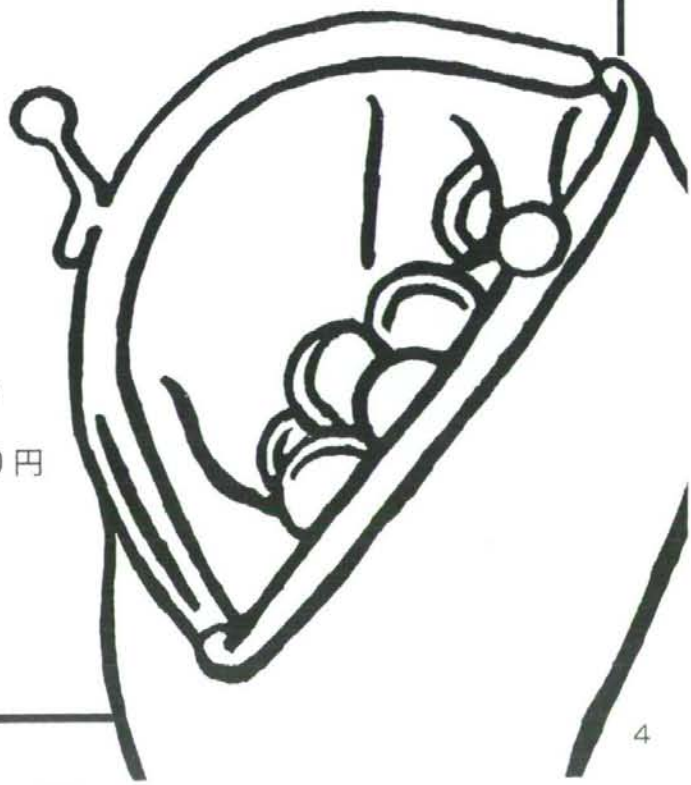
6000円÷20日分＝1日300円

×30日

＝1ヶ月9000円

×12ヶ月

＝1年108,000円



4

広告には、実際に利用した人の体験談が書かれていることがあります。

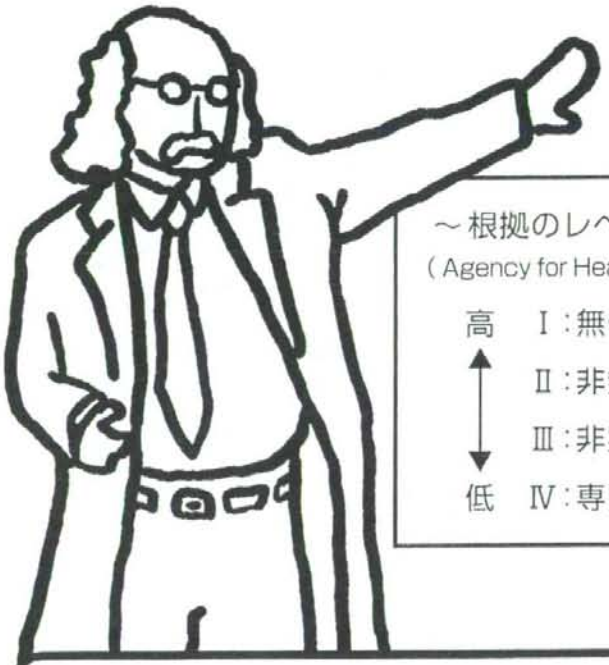
それが有名人の体験談だったり、体験談がいくつもあると、説得力のある商品に見えます。

しかし、体格や体質が人によって異なるように、健康食品の効果の現れ方にも個人差があります。

あなたが利用した場合にも「その商品の効果が現れる」という保証はありません。

健康成分の効果を示す、科学的な根拠のレベルは４段階あります。

そのうち、「権威者（博士や専門家など）による推薦」は、４段階の中で一番低いレベルにあたります。つまり、権威者の推薦があっても、「必ず効果がある」、「安全だ」という保証にはなりません。



～根拠のレベル～

（Agency for HealthCare Policy and Research の４段階）

高　Ⅰ：無作為化比較試験による結果

　　Ⅱ：非無作為化比較試験による結果

　　Ⅲ：非実験的・記述的研究による結果

低　Ⅳ：専門家の意見や権威者の臨床経験

健康食品ってなに？

健康食品は、保健機能食品とその他に分類されます。

健康食品

保健機能食品　　**その他**

厚生労働省によって平成１３年に創設された、保健機能食品制度に基づいています。保健機能食品には、「個別に審査を受けたもの」と「審査はないが、規格基準を満たしているもの」の２つがあります。

6

広告には、研究結果を記載している場合があります。

研究結果は、商品の効果をアピールするために用いられているということを、きちんとふまえた上で、広告を見るようにしましょう。

保健機能食品ってなに？

保健機能食品

特定保健用食品　　栄養機能食品

保健機能食品には、個別に審査を受け、承認を得ている特定保健用食品（トクホ）と、審査はありませんが、規格基準を満たしている栄養機能食品があります。これらに該当する食品だけが、この名称を表示できます。

保健機能食品は他に比べて、比較的信用のおけるものですが、「100％安全」、「100％効果がある」とは言えません。

健康食品は健康効果が期待できると同時に、効果の現れ方に個人差があったり、使い方によっては健康に悪影響を及ぼす可能性もあります。

商品のメリットばかりでなく、利用するときの注意事項について、きちんと書かれているか、チェックしましょう。

たとえば・・・

個人差に関する表示

「効果の感じ方には個人差があります」

リスクに関する表示

「アレルギーのある方、高齢の方は、使用前に医師などにご相談ください」

食事を基本とすることの表示

「食生活は主食、主菜、副菜を基本にバランスのとれた食事を」

私たち消費者には、商品について分からないことを聞く権利があります。
広告には多くの場合、問い合わせ先が書かれています。利用してみましょう。

下記の情報も参考にしてみましょう。

「健康食品」の安全性・有効性について

「健康食品」の安全性・有効性情報：http://hfnet.nih.go.jp/

（独立行政法人 国立健康・栄養研究所）

9