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

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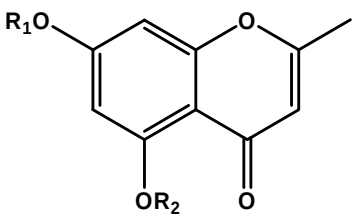
Abstract – Two new chromone diglucosides, staphylosides A and B (**4** and **5**), were isolated from the leaves of *Staphylea bumalda* DC., along with three known chromone glycosides. Their structures were elucidated by detailed inspection of NMR spectral data.

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 ^{1}H 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 l–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-methyl-chromone 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 ^{1}H 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.

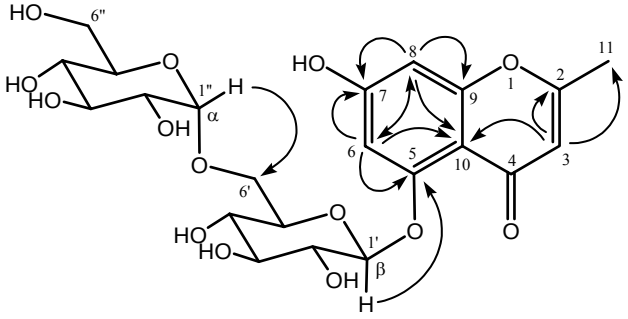


Figure 2. The important HMBC correlations of staphyloside B (**5**). Arrowheads denote carbons and arrow tails protons.

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 (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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