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.

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 l–6 linkage between the two glucose moieties (Tables 1 and 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 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 ^1H 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 emerged 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 ^{1}H 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.

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; ^{1}H and ^{13}C NMR spectra: JEOL α-400 spectrometer (400 MHz and 100 MHz, respectively) with TMS as internal standard; ESI-TOF-MS: Applied Biosystems QSTAR® XL NanoSpray™ System. Parts of the general experimental procedures were described in previous papers [1].

Plant material

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 (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 column chromatography 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 (*c* 0.54, MeOH); IR ν_{max} (film) cm^{-1}: 3367, 2927, 2874, 1650, 1370, 1165, 1072, 1040; ^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}: 3368, 2932, 2879, 1650, 1512, 1368, 1162, 1053; ^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}: 3371, 2931, 2883, 1716, 1654, 1512, 1369, 1164, 1078, 1032; ^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).

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148 Analyses of the sugar moiety

149 About 500 μg each of **3**, **6** and **7** was hydrolyzed with 1N HCl (0.1 ml) at 100 ℃ for 2

150 h. The reaction mixtures were partitioned with an equal amount of EtOAc (0.1 ml), and

151 the water layers were analyzed with a chiral detector (JASCO OR-2090plus) on an

152 amino column [Asahipak NH2P-50 4E, CH_3CN-H_2O (4:1), 1 ml/min]. Hydrolyzates of

153 **3**, **6** and **7** gave the peak for D-glucose at the retention time of 14.4 min (positive optical

154 rotation sign). Peaks were identified by co-chromatography with authentic D-glucose.

155

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160 Spray)-TOF-MS at the Analytical Center of Molecular Medicine and the Analysis

161 Center of Life Science, respectively, of the Hiroshima University Faculty of Medicine.

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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		

236 Figure Legend

237 Figure 1 Structures

238 Figure 2 H-H COSY correlations (———) and HMBC correlations (H—→C) of **7**

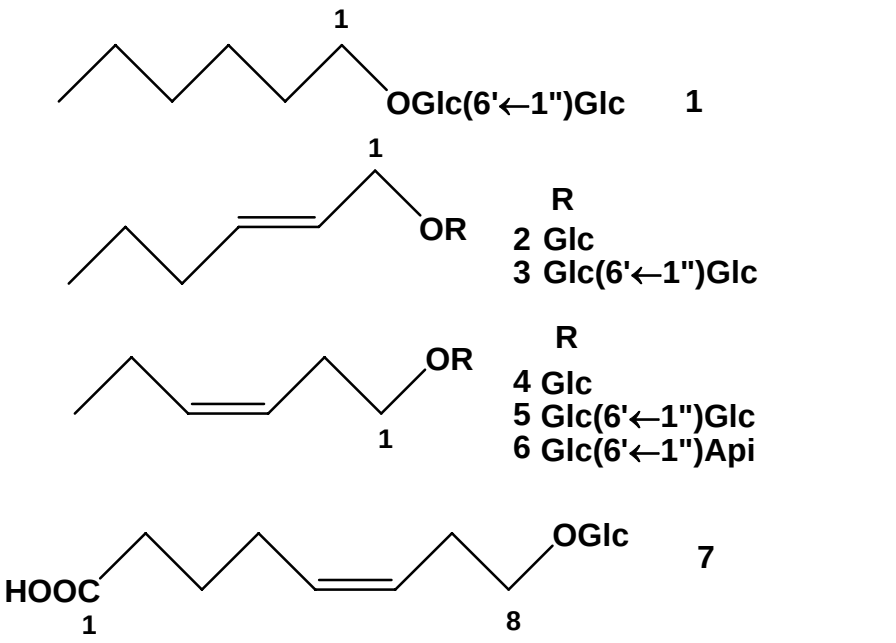

Fig. 1 Structures

Glc: β-D-glucopyranosyl
Api: β-apiofuranosyl

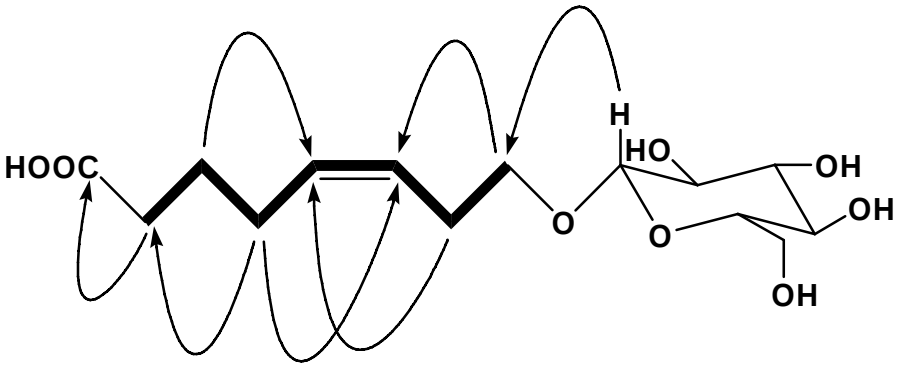

Fig. 2 H-H COSY correlations (▬) and HMBC correlations (H⟶C) of **7**