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Novel octulosonic acid derivatives in the composite *Smallanthus sonchifolius*

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Abstract—Two novel octulosonic acid derivatives with a 6,8-dioxabicyclo[3.2.1]octane skeleton that are major water-soluble phenolic compounds were found in the roots of *Smallanthus sonchifolius*. The structures of these compounds were determined to be (1*R*,2*S*,3*S*,4*R*,5*S*,7*R*)-4-hydroxy-7-hydroxymethyl-3-[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyloxy]-6,8-dioxabicyclo[3.2.1]-octan-5-carboxylic acid (4-*O*-caffeoyl-2,7-anhydro-D-*glycero*-β-D-*galacto*-oct-2-ulopyranosonic acid) and (1*R*,2*S*,3*R*,4*R*,5*S*,7*R*)-2,4-dihydroxy-7-hydroxymethyl-2,3-bis[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyloxy]-6,8-dioxabicyclo[3.2.1]octan-5-carboxylic acid (4,5-di-*O*-caffeoyl-2,7-anhydro-D-*glycero*-β-D-*galacto*-oct-2-ulopyranosonic acid) by MS, NMR and CD spectral analyses.

Smallanthus sonchifolius is a plant originally cultivated in South America and has gradually received more attention due to its abundant content of fructooligosaccharide and phenolic compounds.[1] We previously reported that *S. sonchifolius* has three types of caffeoyl esters of altraric acid as major polyphenol compounds.[2] In this study, we report two other types of caffeoyl esters of octulosonic acid derivative that have a 6,8-dioxabicyclo[3.2.1]octane skeleton.

Tuberous roots of *S. sonchifolius* were harvested at the National Agricultural Research Center for Western Region in Japan. A whole root (160 g) was homogenized in 0.2 M L-ascorbic acid aqueous solution (160 mL). The homogenate was centrifuged, and the supernatant was subjected to chromatography (ODS) using H_2O, H_2O/MeOH (9/1, 8/2, 7/3) and MeOH as eluents. Compound **1** (12 mg) was isolated from H_2O/MeOH (9/1), and compound **2** (7 mg), from H_2O/MeOH (8/2) fractions after preparative HPLC (ODS) separations.

From the analyses of 2D NMR and high-resolution MS spectra, we determined compound **1** was a monocaffeoyl ester and compound **2** a dicaffeoyl ester of a 6,8-dioxabicyclo[3.2.1]octane derivative, which was an intramolecular acetal of oct-2-ulosonic acid (Fig. 1). Their similar coupling pattern in ^{1}H NMR indicated that compounds **1** and **2** had the same stereochemical substitution of the bicyclo structure. We then investigated their stereochemistry, including the absolute configuration, in more detail as follows.

The result of the H/D exchange experiment using FTICR-MS (ESI, negative) obtained for compound **2**

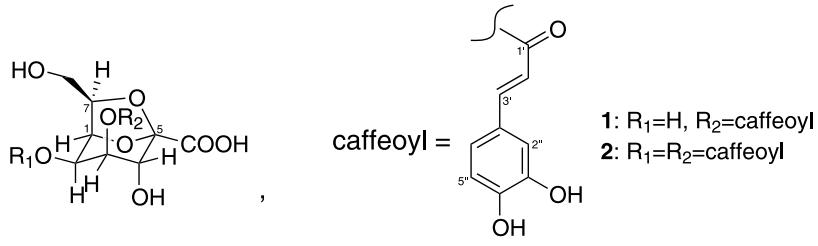

Figure 1. Structures of compounds **1** and **2**.

Keywords: *Smallanthus sonchifolius*; octulosonic acid; 6,8-dioxabicyclo[3.2.1]octane; CD; absolute stereochemistry.
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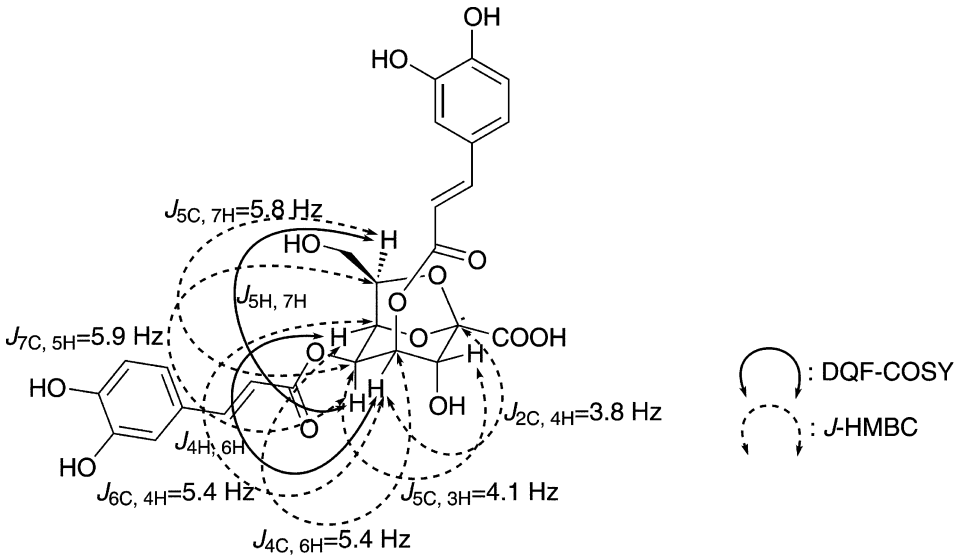

Figure 2. Key correlations observed in the NMR experiments for compound **2**. The values of $^{3}J_{C,H}$ were determined by *J*-HMBC experiment, and the W-type correlations were observed in DQF-COSY experiment.

showed that the two *m*/*z* values in H_2O/CH_3OH (1/1) and D_2O/CD_3OD (1/1) differed by 6. Hence, the number of deuterium-exchangeable H atoms was determined to be 7, which was consistent with the proposed structure. Subsequently, compound **2** was methylated by diazomethane–diethyl ether to yield its partially methylated derivative.[3] Its NMR spectrum gave additional information of the structure. It was proved that four phenolic hydroxyl groups and one carboxyl group of octulosonic acid moiety were methylated. In addition, non-methylated hydroxyl groups could be observed by the NMR measurement in $CDCl_3$, demonstrating that two hydroxyl groups bonded to 3-C and 8-C. These were also consistent with the estimated structure.

To clarify the relative stereochemistry of the substituents attached to 6,8-dioxabicyclo[3.2.1]octane, we investigated some $^{3}J_{H,H}$ and $^{4}J_{H,H}$ (W-type coupling) values of compounds **1** and **2** by ^{1}H 1D and DQF-COSY NMR experiments. Furthermore, we calculated some $^{3}J_{C,H}$ of compound **2** by ^{1}H–^{13}C *J*-HMBC method[4] to find the ^{1}H–^{13}C pairs of the antiperiplanar relationship that had a dihedral angle of nearly 180° and exhibited a sufficiently large coupling constant.[5] Figure 2 describes the key correlations and the coupling constants of $^{3}J_{C,H}$ obtained in octulosonic acid moiety. Some $^{3}J_{C,H}$ values of compound **2** were close to those of corresponding parts of 1,6-anhydro-β-D-galactopyranose that had the same skeleton as compound **2**.[6]

To determine the absolute configuration of **2**, the CD exciton chirality method was employed. The compound had two caffeoyl chromophores that bonded to the rigid bicyclo skeleton, and its UV spectrum exhibited a strong $\pi\rightarrow\pi^*$ transition band around 330 nm. In that region, the CD spectrum of compound **2** had an exciton coupling CD Cotton curve with negative first (λ_{ext} 348 nm, $\Delta\varepsilon$ −14.9) and positive second (λ_{ext} 292 nm, $\Delta\varepsilon$ +8.1) signs, i.e. negative exciton chirality (Fig. 3).[7] The two caffeoyl groups therefore constitute a counterclockwise screw sense. Thus, we unambiguously determined the structure of compound **2** to be 4,5-di-*O*-caffeoyl-2,7-anhydro-D-*glycero*-β-D-*galacto*-oct-2-ulopyranosonic acid as shown in Figure 3.

It was implied that compound **1** had the same absolute configuration as compound **2** in octulosonic acid moiety since both compounds were isolated from identical

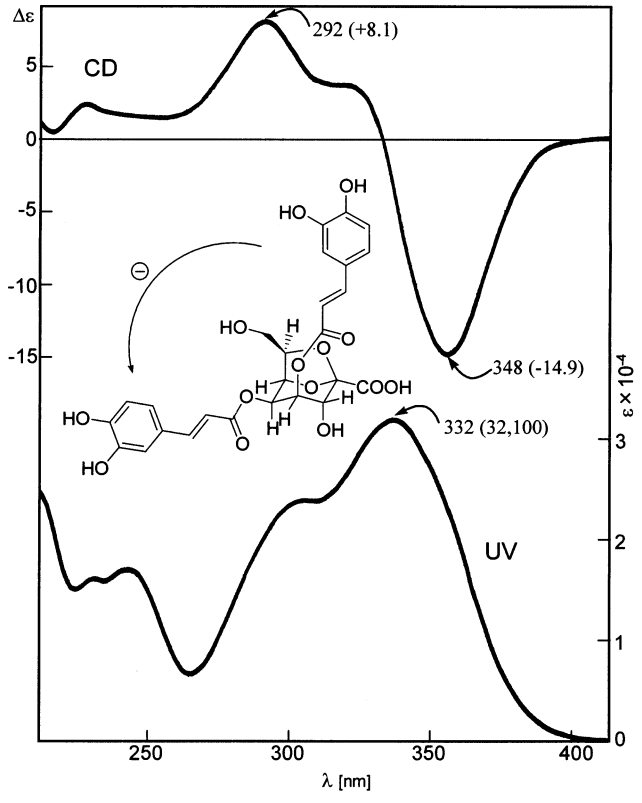

Figure 3. CD and UV spectra of compound **2**.

Table 1. ^{1}H NMR (800 MHz) spectroscopic data of octulosonic acid derivatives **1** and **2** in CD_3OD

	1			**2**		
H	δ (ppm)	Multiplicity	J (Hz)	δ (ppm)	Multiplicity	J (Hz)
6,8-Dioxabicyclo[3.2.1]octane						
1	4.463	dd	4.4, 3.7	4.699	dd	4.8, 3.7
2	4.343	dd	4.4, 6.3	5.551	dd	4.8, 6.3
3	5.247	d	6.3	5.425	d	6.3
4	4.019	br s		4.114	br s	
7	4.283	ddd	3.7, 4.2, 8.2	4.327	ddd	3.7, 4.4, 8.0
2-*O*-Caffeoyl						
2′	6.361	d	15.8	6.379	d	15.9
3′	7.626	d	15.8	7.610	d	15.9
2″	7.088	d	2.0	7.072	d	2.1
5″	6.782	d	8.2	6.765	d	8.1
6″	6.993	dd	2.0, 8.2	6.949	dd	2.1, 8.1
3-*O*-Caffeoyl						
2′				6.140	d	15.9
3′				7.464	d	15.9
2″				6.975	d	2.1
5″				6.646	d	8.2
6″				6.767	dd	2.1, 8.2
7-Hydroxymethyl						
	4.367	dd	8.2, 12.1	4.402	dd	8.0, 11.6
	4.039	dd	4.2, 12.1	4.057	dd	4.4, 11.6

plant tissue. We therefore characterized the structure of compound **1** as 4-*O*-caffeoyl-2,7-anhydro-D-*glycero*-β-D-*galacto*-oct-2-ulopyranosonic acid. Octulosonic acid is classified as ketoaldonic acid, which is seldom reported as a natural product. A few compounds analogous with compounds **1** and **2** have been reported, such as 4,8-di-*O*-caffeoyl-2,7-anhydro-3-deoxy-β-*altro*-oct-2-ulopyranosonic acid methyl ester from *Erigeron breviscapus*, though the absolute configuration of the compound has not been determined.[8]

(1*R*,2*S*,3*S*,4*R*,5*S*,7*R*)-4-Hydroxy-7-hydroxymethyl-3-[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyloxy]-6,8-dioxabicyclo[3.2.1]octan-5-carboxylic acid (4-*O*-caffeoyl-2,7-anhydro-D-*glycero*-β-D-*galacto*-oct-2-ulopyranosonic acid) (1): $C_{17}H_{18}O_{11}$, colorless amorphous solid, FAB-MS (glycerol) m/z 397 [M–H]$^-$, 235 [M–$C_9H_7O_3$ (1 caffeoyl group)]$^-$; high-resolution FTICR-MS (methanol/H_2O/AcOH = 1/1/0.002) calcd for $C_{17}H_{17}O_{11}$ ([M–H]$^-$) 397.07654, found 397.07710. NMR assignments are shown in Tables 1 and 2.

(1*R*,2*S*,3*R*,4*R*,5*S*,7*R*)-2,4-Dihydroxy-7-hydroxymethyl-2,3-bis[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenyloxy]-6,8-dioxabicyclo[3.2.1]octan-5-carboxylic acid (4,5-di-*O*-caffeoyl-2,7-anhydro-D-*glycero*-β-D-*galacto*-oct-2-ulopyranosonic acid) (2): $C_{26}H_{24}O_{14}$, colorless amorphous solid, UV (ethanol) λ_{max} 332 nm (ε 3.21×10^4), 248 (1.60×10^4), 219 (2.33×10^4); CD (ethanol) λ_{ext} 348 nm ($\Delta\varepsilon$ −14.9), 292 (+8.1); FAB-MS (glycerol) m/z 559 [M–H]$^-$, 397 [M–$C_9H_7O_3$ (1 caffeoyl group)]$^-$, 235 [M–$C_{18}H_{13}O_6$ (2 caffeoyl groups)]$^-$; High-resolution FTICR-MS (methanol/H_2O = 1/1) calcd for $C_{26}H_{23}O_{14}$ ([M–H]$^-$) 559.10933, found 559.10917. NMR assignments are shown in Tables 1 and 2.

Table 2. ^{13}C NMR (150.9 MHz) spectroscopic data of octulosonic acid derivatives **1** and **2** in CD_3OD

	1	**2**
	δ (ppm)	δ (ppm)
6,8-Dioxabicyclo[3.2.1]octane		
1	78.60	76.30
2	65.44	66.36
3	73.17	71.02
4	73.17	73.24
5	104.88	115.07
7	82.55	82.54
2-*O*-Caffeoyl		
1′	168.42	167.82
2′	115.17	114.36
3′	147.45	148.40
1″	127.79	127.56
2″	115.27	115.27
3″	146.82	146.86
4″	149.67	149.92
5″	116.48	116.51
6″	123.13	123.45
3-*O*-Caffeoyl		
1′		167.03
2′		113.86
3′		148.10
1″		127.38
2″		114.79
3″		146.86
4″		149.90
5″		116.39
6″		123.68
5-Carboxy	170.58	170.19
7-Hydroxymethyl	60.72	60.56

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3. Compound **2** (1 mg) was dissolved in a small amount of MeOH, and cooled in an ice bath. After addition of diazomethane–diethyl ether (3 mL), the flask of the mixture was stood for 20 min and then concentrated in vacuo. This procedure was repeated three more times. The concentrated mixture was dissolved in a small amount of $CHCl_3$ and applied to a silica gel column (1 cm ϕ×6 cm) prepared with $CHCl_3$. The column was developed with $CHCl_3$/MeOH (10:0.8) and fractionated by 3 mL. The major reactant was included in the third fraction. This fraction was dried and dissolved in $CDCl_3$ for NMR measurement. Data for methylated **2**, methyl (1*R*,2*S*,3*R*,4*R*,5*S*,7*R*)-4-hydroxy-7-hydroxymethyl-2,3-bis[3-(3,4-dimethoxyphenyl)-1-oxo-2-propenyloxy]-6,8-dioxabicyclo[3.2.1]octan-5-carboxylate: (600.13 MHz, $CDCl_3$) δ 7.379 (1H, d, J=15.9 Hz, H-3′ of O-2), 7.577 (1H, d, J=15.9 Hz, H-3′ of O-3), 7.105 (1H, d, J=2.0 Hz, H-2″ of O-2), 7.032 (1H, dd, J=2.0, 8.4 Hz, H-6″ of O-2), 7.013 (1H, d, J=2.0 Hz, H-2″ of O-3), 6.947 (1H, dd, J'=2.0, 8.3 Hz, H-6″ of O-3), 6.863 (1H, d, J=8.4 Hz, H-5″ of O-2), 6.784 (1H, d, J=8.3 Hz, H-5″ of O-3), 6.352 (1H, d, J=15.9 Hz, H-2′ of O-2), 6.169 (1H, d, J=15.9 Hz, H-2′ of O-3), 5.615 (1H, dd, J=5.0, 6.4 Hz, H-2), 5.580 (1H, d, J=6.4 Hz, H-3), 4.786 (1H, dd, J=5.0, 2.2 Hz, H-1), 4.529–4.508 (2H, m, H-7 and C$\underline{H}_2$OH), 4.246 (1H, d, J=7.8 Hz, H-4), 4.165–4.152 (1H, m, C$\underline{H}_2$OH), 3.930 and 3.894 (6H, s, 4″-OMe×2), 3.916 and 3.819 (6H, s, 3″-OMe×2), 3.901 (3H, s, $COOCH_3$), 2.764 (1H, d, J=7.8 Hz, 4-OH), 1.852–1.831 (1H, m, $CH_2O\underline{H}$).
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